AMIDES OF DE-ACETYLGLUCOSAMINYL-DEOXY TEICOPLANIN ACTIVE AGAINST HIGHLY GLYCOPEPTIDE-RESISTANT ENTEROCOCCI

SYNTHESIS AND ANTIBACTERIAL ACTIVITY

ADRIANO MALABARBA, ROMEO CIABATTI, JÜRGEN KETTENRING, PIETRO FERRARI, ROBERTO SCOTTI, BETH P. GOLDSTEIN and MAURIZIO DENARO

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

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Removal, by selective reduction, of the acetylglucosamine from teicoplanin A2-2 (CTA/2) produced the 34-de(acetylglucosaminyl)-34-deoxy pseudoaglycone (**II**). This compound was more active *in vitro* than CTA/2 against coagulase-negative staphylococci (CNS).

Amide derivatives obtained by condensation of the carboxyl group of **II** with primary amines were particularly active against *Streptococcus pyogenes* and had some *in vitro* activity against VanA enterococci highly resistant to both teicoplanin and vancomycin. Among them, a carboxamide (**VII**) with a branched tetramine also had better activity than the corresponding amide of teicoplanin against CNS. In contrast, the dimethylamide (**VIII**) of **II** had little activity against VanA enterococci.

While the overall structure of the heptapeptide backbone of the secondary carboxamides of **II** is the same as in CTA/2 and its amide derivatives, in deoxy pseudoaglycone **II** and its tertiary amide **VIII** the 51,52-peptide bond undergoes a conformational change from the original *cisoid* to the *transoid* orientation. This difference between the secondary amides of **II** and dimethylamide **VIII** is reflected in their different antibacterial spectrum.

The direct synthesis of the amides of deoxy pseudoaglycone **II** from parent CTA/2-amides by reaction with sodium borohydride is also described.

The influence of the sugars on the *in vitro* and *in vivo* activity of teicoplanin has been matter of investigation for years in order to establish structure-activity relationships among derivatives of teicoplanin, its pseudoaglycones and aglycone.[1] In particular teicoplanin derivatives were studied for their activities against coagulase-negative staphylococci (CNS), and Gram-negative bacteria (which are generally resistant to glycopeptide antibiotics of the dalbaheptide group).[2] A current challenge for glycopeptides is the emerging resistance in enterococci,[3] which are often resistant to other first-line antibiotics.

By selective removal of the three sugars of teicoplanin A2 complex (CTA), or of component A2-2 (CTA/2, Fig. 1a), four of the six possible pseudoaglycones and the aglycone were obtained (Fig. 1b). In particular, the stepwise acidic hydrolysis of teicoplanin gave the 56-de(acylglucosaminyl) (TB) and 34-acetylglucosaminyl (TC) pseudoaglycones, and the aglycone (TD). Demannosylation of CTA, to give the 42-demannosyl pseudoaglycone (DMCTA), and mannosylation of TD, to give the 42-mannosyl pseudoaglycone (MTD), were achieved by biotransformation methods.[4]

The differences in the antibacterial *in vitro* and *in vivo* properties between teicoplanin derivatives and their available sugar-deficient pseudo-aglycones and aglycone suggested it was possible to improve the anti-CNS activity with the synthesis of CTA-amides and to pursue the anti-Gram-negative activity with the synthesis of TD amides.[5,6] However, no such indication emerged from the activity of the above compounds against highly glycopeptide-resistant enterococci. To complete this investigation, only the de(acetylglucosaminyl) pseudoaglycones (GMTD and GTD) were missing.

Fig. 1a. Structure of teicoplanin A2-2 (CTA/2).

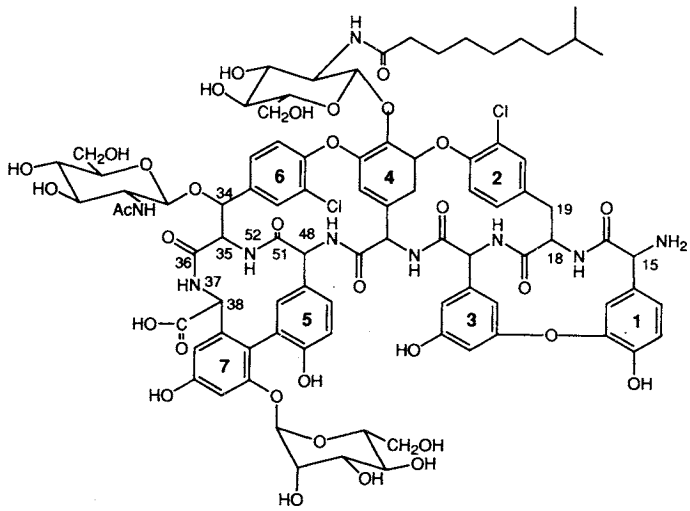
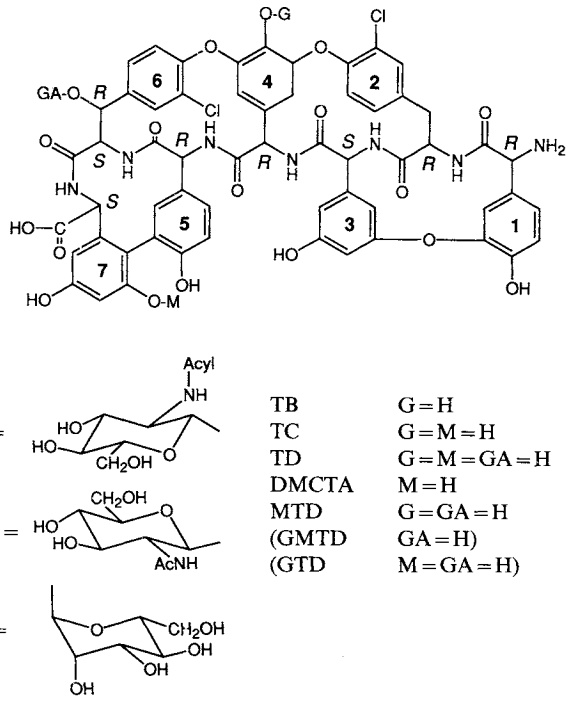


Fig. 1b. Structures of teicoplanin pseudoaglycones and aglycone (with absolute configurations of the heptapeptide backbone).



All attempts to selectively remove the acetylglucosamine from CTA were unsuccessful due to the resistance of this sugar to enzymatic hydrolysis and its higher acidic stability than that of the other two sugars. A result was achieved under basic conditions, but through a β-elimination mechanism which produced unsaturated de(acetylglucosaminyl)-didehydrodeoxy pseudoaglycone **I** (Fig. 2a).[7] Its antibacterial

Fig. 2. Structures of the didehydro-deoxy (**I**) and deoxy (**II**) derivatives of de(acetylglucosaminyl) CTA/2.

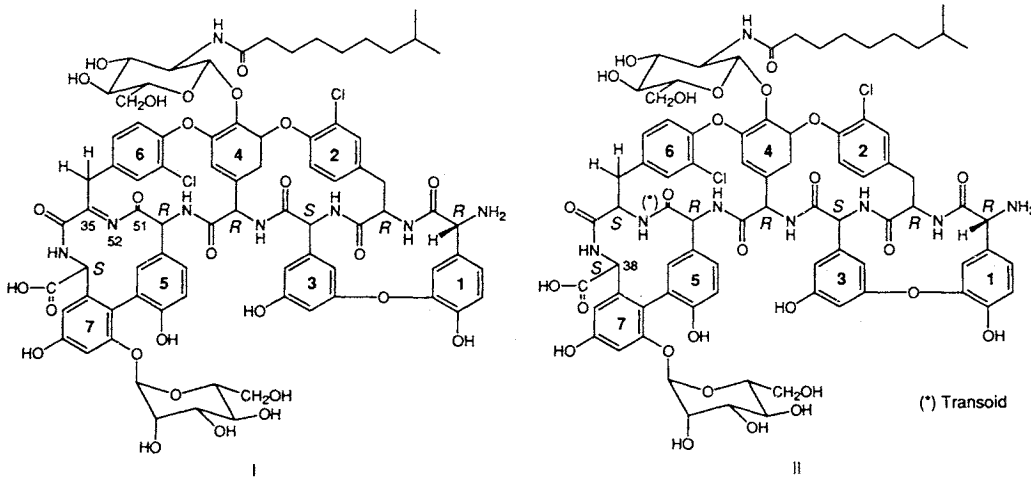
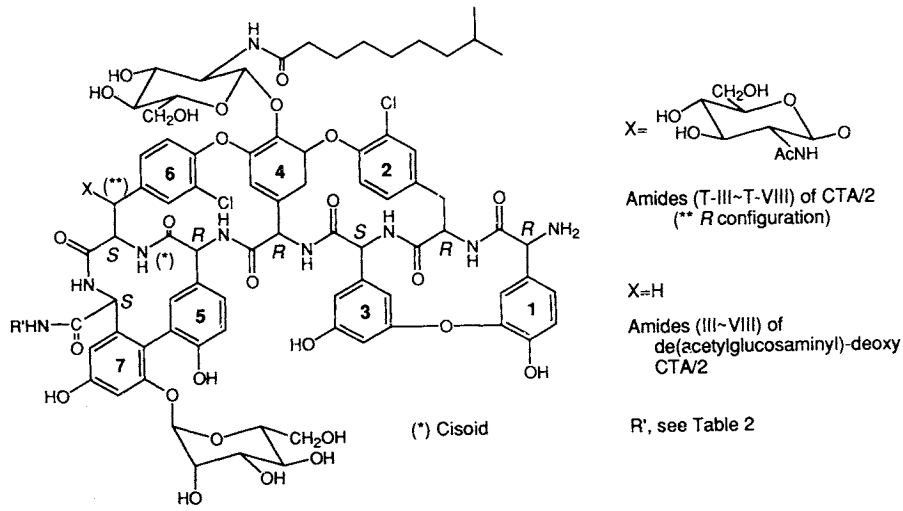


Fig. 3a. Structures of the secondary amides of CTA/2 and its 34-de(acetylglucosaminyl)-34-deoxy pseudoaglycone, and dimethylamide (T-VIII) of CTA/2.



spectrum of activity was comparable to that of teicoplanin.

In this paper the synthesis and activity of the saturated de(acetylglucosaminyl)-deoxy pseudoaglycone **II** (Fig. 2b), its secondary amide derivatives **III**~**VII** (Fig. 3a) and dimethylamide **VIII** (Fig. 3b) are described.

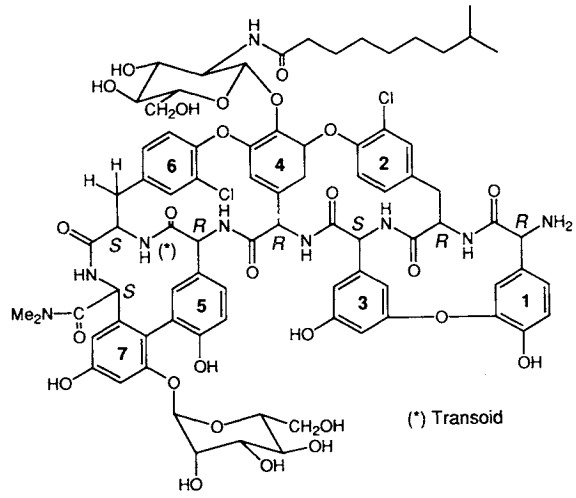
Chemistry

Synthesis of deoxy-pseudoaglycone II

The 34-de(acetylglucosaminyl)-34-deoxy pseudoaglycone **II** was prepared from CTA/2 by two different synthetic pathways (Methods a and b, Scheme 1).

Method a is based on the catalytic hydrogenation (5% Pd/C, 1 atmosphere, room temperature) of the 35,52-C=N bond of unsaturated pseudoaglycone **I**, which was obtained according to a procedure

Fig. 3b. Structure of dimethylamide **VIII**.



Scheme 1.

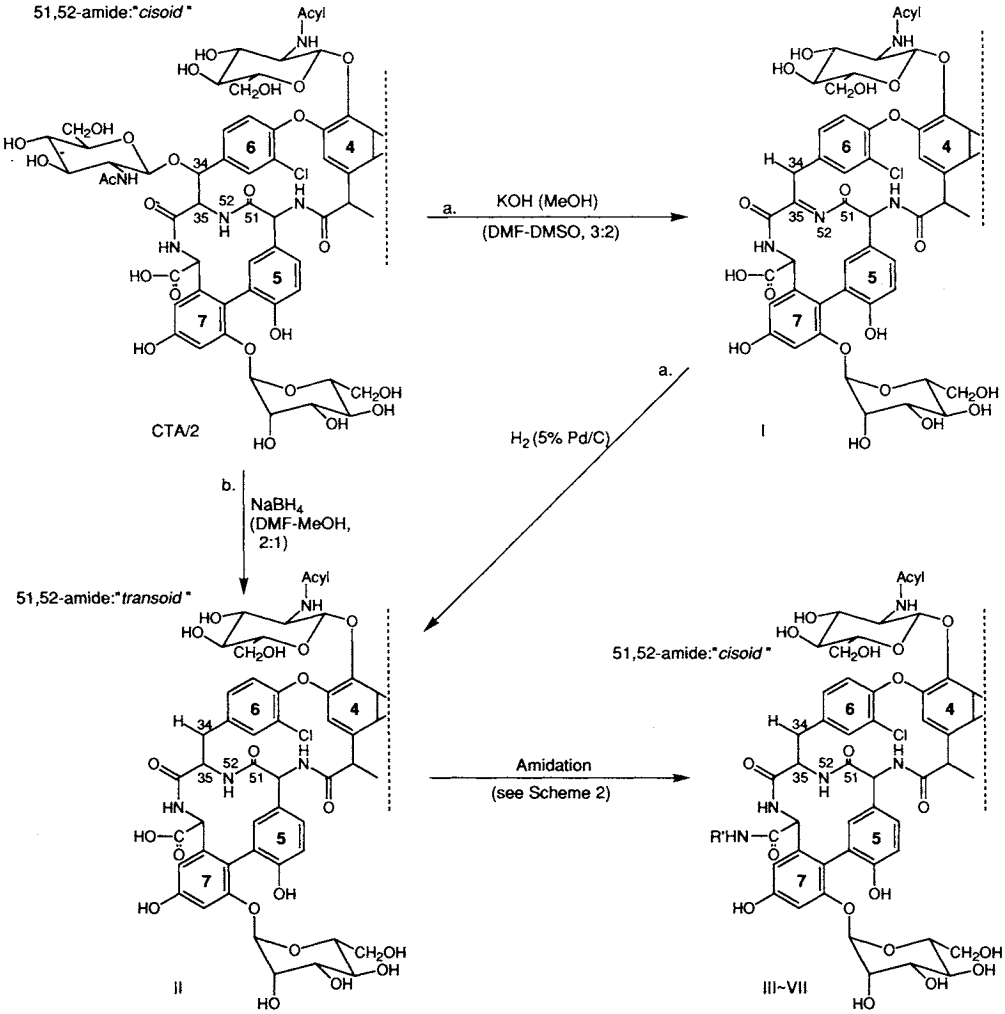


Table 1. Analytical data of 34-de(acetylglucosaminyl)-34-deoxy pseudoaglycone **II** (Fig. 2b) and its dimethylamide **VIII** (Fig. 3b).

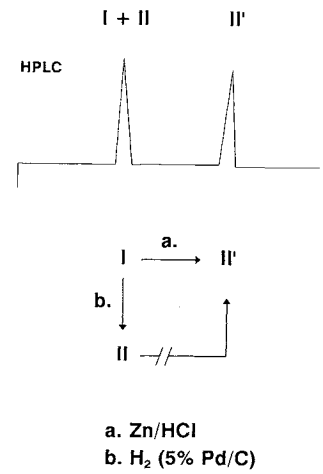
Compound	Starting material (Method)	Yield (%)	HPLC (Rt, minutes)	FAB-MS $(MH)^+$	MW	Formula
II	I (a) / CTA/2 (b)	73.5 / 28.5	8.1	1660	1660.5	$C_{80}H_{84}N_8O_{27}Cl_2$
VIII	T-VIII (b)	15.7	12.9	1687	1687.6	$C_{82}H_{89}N_9O_{26}Cl_2$

(treatment of CTA/2 with methanolic KOH in a DMF-DMSO 3:2 solution) previously reported.[7]

HPLC was unsuitable for following the reduction of compound **I** to compound **II** since **I** and **II** have the same retention time. Therefore, the progress of the reaction was monitored by ^1H NMR analysis (decrease of signals, d, at δ 4.13 and 4.76 ppm, due to the 34-CH_2 of **I**, and related increase of signals, dd, at 3.00 and 3.25 ppm of the 34-CH_2 protons of **II**).

The discovery of a selective reaction for unsaturated compound **I** provided a suitable method for the isolation and purification of **II** (Chart 1). Reduction of the C=N bond of **I** with Zn at room temperature in concentrated HCl produced a more lipophilic molecular isomer **II'** (MW 1660)[a] of **II** whose structure was not further investigated. As expected, **II** was resistant to these reduction conditions and was then purified by chromatographic resolution of the reaction mixture. The overall transformation yield of CTA/2 into pure **II** by way of unsaturated compound **I** was about 60%.

Chart 1. Chemical separation of derivative **II** from unsaturated compound **I**.



Method b. A more convenient, although less efficient (yield <30%, Table 1) one-step procedure to obtain **II** involved direct reductive removal of the acetylglucosamine from CTA/2 with sodium borohydride at room temperature in a DMF-MeOH (2:1) solution. Also with this reaction a small amount of unsaturated compound **I** formed which was separated as described above.

Amidation of the 38-COOH (Scheme 2)

Preliminary protection of the terminal amino group of **II** was necessary to avoid undesired intermolecular side reactions. Benzylcarbamate derivative N^{15}-CBZ-II was obtained by treatment of **II** with benzyl chloroformate (CBZ-Cl) in DMF at room temperature in the presence of triethylamine (TEA). The N^{15}-CBZ derivatives of amides **III**~**VII** were prepared by either direct reaction of the carboxyl group of N^{15}-CBZ-II with the appropriate amine[b], using diphenyl phosphorazidate (DPPA) as the condensing agent in the presence of TEA (Method c), or by amidation *via* the activated cyanomethyl ester

[a] By FAB-MS.

[b] N^{15}-CBZ-amide VII was prepared (Method c') by reaction of N^{15}-CBZ-II with $[BOC\text{-}NH(CH_2)_3]_2N(CH_2)_3NH_2$, in DMSO in the presence of DPPA and TEA, followed by removal of the BOC protective groups with dry TFA.

Scheme 2.

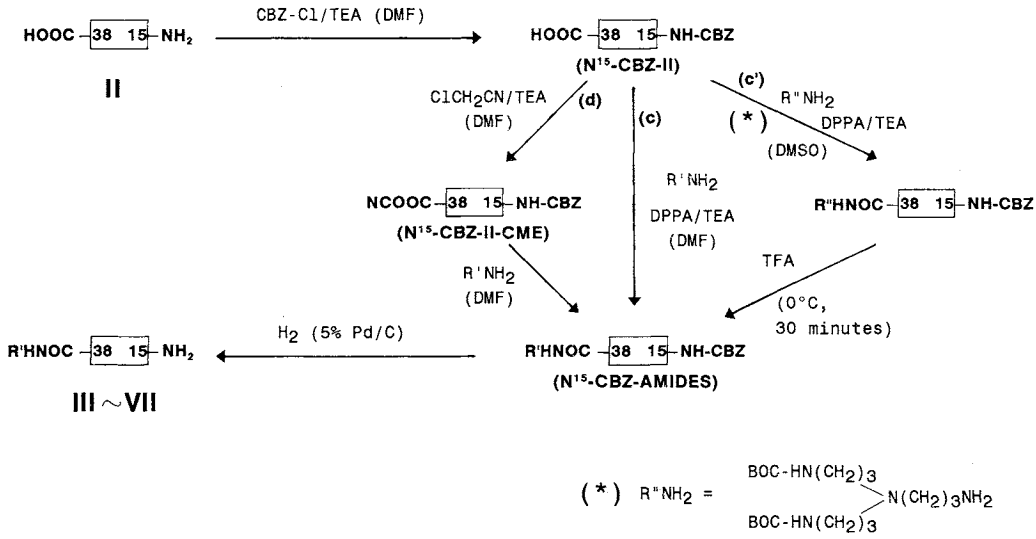


Table 2. Analytical data of the secondary amides of 34-de(acetylglucosaminyl)-34-deoxy pseudoaglycone **II**.

Compound	R' (see Fig. 3a)	Starting material (Method)	Yield (%)	HPLC (Rt, minutes)	FAB-MS $(MH)^+$	MW	Formula
III	$-(CH_2)_3N(CH_3)_2$	II (c) 47 II (d) 52 T-III (b) 37		13.8	1744	1744.6	$C_{85}H_{96}N_{10}O_{26}Cl_2$
IV	$-(CH_2)_3NH(CH_2)_2OH$	II (c) 41 II (d) 39		13.9	1782*	1760.6	$C_{85}H_{96}N_{10}O_{27}Cl_2$
V	$-(CH_2)_3NH(CH_2)_4NH_2$	II (d)	27	15.7	1787	1787.7	$C_{87}H_{101}N_{11}O_{26}Cl_2$
VI	$-(CH_2)_3NH(CH_2)_4NH(CH_2)_3NH_2$	II (d)	23	16.6	nd	1844.8	$C_{90}H_{108}N_{12}O_{26}Cl_2$
VII	$-(CH_2)_3N[(CH_2)_3NH_2]_2$	II (c')	36	16.2	1830	1830.8	$C_{89}H_{106}N_{12}O_{26}Cl_2$

nd: Not determined.
* [MNa].

(N^{15}-CBZ-II-CME; Method d), in DMF at room temperature. Amides **III**~**VII** (Table 2, Fig. 3a) were then obtained from corresponding N^{15}-CBZ-amides by removing the CBZ protective group under hydrogenolytic conditions (5% Pd/C, 1 atmosphere, room temperature).

Amides of deoxy-pseudoaglycone II from CTA-amides (Scheme 3)

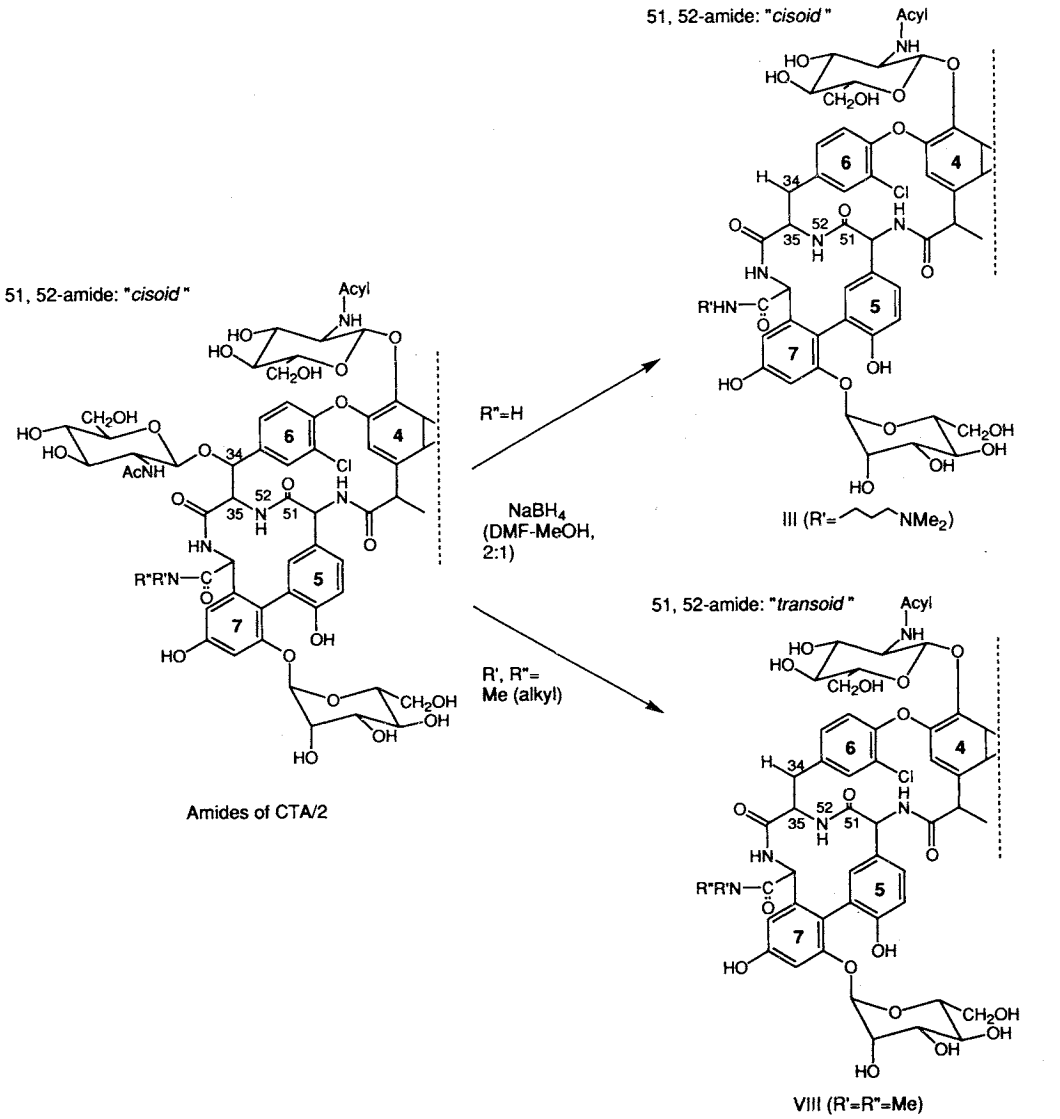
Method b was also applied to the preparation of dimethyl amide **VIII** (Table 1, Fig. 3b) from the corresponding amide (T-VIII) of CTA/2 and as an alternative synthesis of dimethylaminopropyl amide **III** from parent CTA/2-amide (T-III).

Structure Elucidation

The structure of compound **II** was elucidated by ^1H NMR spectroscopy and FAB-MS spectrometry.

Compound **II**. From NMR data (Table 3) it appears that, in addition to the absence of the 34-acetylglucosamine and the presence of the 34-CH_2, pseudoaglycone **II** differs from CTA/2 in the conformation of the 51,52-peptide bond which is *cisoid* in CTA/2 (52-NH, δ 6.09 ppm) and TD (52-NH,

Scheme 3.



δ 6.72 ppm) while it is very likely in the *transoid* conformation in **II** (downfield shift of the 52-NH proton to δ 7.90 ppm).[7] Reduction in **II** of the C=N double bond present in **I** is confirmed by the FAB-MS spectrum of **II** (MW 1660, 2 mass units higher than that of **I**).

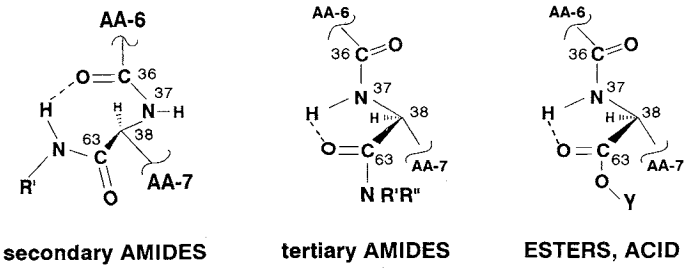
Amides of **II**. The ^{1}H NMR spectra of the secondary amides of **II** show that the amidic moieties are present and that the conformation of the 51,52-peptide bond is *cisoid* (52-NH, δ 6.80 ppm) as in the teicoplanin amides. In contrast, the downfield shift of the 52-NH proton (δ 7.85 ppm) in the spectrum

Table 3. Significant ^{1}H NMR signals (δ, ppm) for compounds **II**, **III** and **VIII**, in comparison with deglucoteicoplanin (TD).

Protons*	Compound			
	II	**III**	**VIII**	TD
35-C–H	5.10	5.05	5.10	4.12
34-C–H	3.25	3.25	3.30	5.10
34-C–H′	3.00	2.95	2.95	—
52-N–H	7.90	6.80	7.90	6.72

* See Fig. 1a.

Chart 2. Proposed hydrogen bonding systems resulting from different modifications at the 38-COOH.



of the intermediate cyanomethyl ester N^{15}-CBZ-II-CME indicates that the conformation of that linkage is probably *transoid* as in **II**. The same possible *transoid* conformation of the 51,52-peptide bond is shown by the tertiary amide **VIII**, as it results by comparing the ^{1}H NMR spectrum of **VIII** with that of the secondary amide **III** (Table 3).

Remarks. In "natural" teicoplanins, their ester and amide derivatives, as well as in the other glycopeptides of the dalbaheptide family, the 51,52-peptide linkage is always *cisoid*. The presence of the 34-benzylic hydroxyl group or sugars is hypothesized to stabilize the macrocyclic ring formed by amino acids, 5, 6 and 7 in the original conformation, by hindering any twisting in the left hand side of the molecule and thus preventing the 51,52-peptide bond from assuming the *transoid* orientation.

In deoxy pseudoaglycone **II**, the rearrangement of the 5,6,7-macrocyclic ring towards a thermodynamically more stable conformation, in which the 51,52-peptide bond is likely *transoid*, is permitted because of the absence of the above benzylic functions at position 34.

The conformational differences among the de(acetylglucosaminyl)-deoxy derivatives of teicoplanin might be explained by the formation of different hydrogen bonding systems (Chart 2) which would constrain the 5,6,7-macrocyclic ring in two different arrangements in which the 51,52-peptide bond is alternatively *cisoid* or *transoid*.

In deoxy pseudoaglycone **II**, its ester derivatives and tertiary amides, the *transoid* orientation of the 51,52-linkage might be stabilized by the only possible hydrogen bonding system involving the 37-NH proton and the 63-C=O oxygen in a five-membered ring.

In the secondary amides of **II**, there might be an alternative hydrogen bond between the amidic N-H proton and the carbonyl oxygen of adjacent 36,37-peptide linkage. The resulting seven-membered hydrogen bonding system might be favoured in this particular case by the proximity in the space of the amidic proton and the 36-carbonyl oxygen.[c] The consequent opposite orientation of the *transoid* 36,37-peptide bond in the two different hydrogen bonding systems is reflected in the different conformation of their 5,6,7-macrocyclic ring and 51,52-peptide linkage between the secondary amides and the other derivatives of **II**.

Antibacterial Activity

The compounds were first tested against a panel of Gram-positive bacteria, including a CNS strain of *Staphylococcus haemolyticus* for which the MIC of teicoplanin (CTA/2) is relatively high (8 μg/ml).

As shown in Table 4, deoxy pseudoaglycone **II** was more active than CTA/2 against the *S. haemolyticus* strain and had similar activity against other Gram-positive bacteria. Though less active against

[c] Deduced from studies with molecular models.

Table 4. *In vitro* activity of de(acetylglucosaminyl)-deoxy derivatives of CTA/2 in comparison with CTA/2 (and its amide T-VII).

Organism	MIC (μg/ml)								
	II	**III**	**IV**	**V**	**VI**	**VII**	(T-VII)	**VIII**	CTA/2
Staphylococcus aureus L165	0.13	4	2	2	2	0.13	0.13	0.5	0.13
S. haemolyticus L602	1	2	2	0.5	1	0.03	2	2	8
S. epidermidis ATCC 12228	0.06	0.25	0.13	0.13	0.13	0.004	0.13	0.25	0.13
Streptococcus pyogenes L49	0.06	0.13	0.06	0.016	0.03	0.008	0.06	0.06	0.06
S. pneumoniae L44	0.06	0.5	0.25	0.25	1	0.13	0.06	0.13	0.06
Enterococcus faecalis ATCC 7080	0.13	2	2	2	4	0.13	0.13	1	0.13

Table 5. *In vitro* activity of the secondary amides (**III**～**VII**) of **II** and corresponding CTA/2-amides (T-III～T-VII) against VanA enterococci.

Organism	MIC (μg/ml)					
	III	**IV**	**V**	**VI**	**VII**	T-III
E. faecalis L562	32	16	16	32	8	128
E. faecalis L560	16	16	16	32	16	128
E. faecalis L563	32	32	32	64	16	>128
E. faecium L564	16	16	8	16	8	128
E. faecium L565	16	16	16	16	8	>128
E. faecium L1666	>128	>128	128	128	64	>128
E. hirae L1683	16	16	8	8	8	64

Organism	MIC (μg/ml)					
	T-IV	T-V	T-VI	T-VII	CTA/2	Vanco
E. faecalis L562	128	32	16	32	128	>128
E. faecalis L560	128	64	64	128	>128	>128
E. faecalis L563	>128	128	128	64	128	>128
E. faecium L564	128	64	32	32	64	>128
E. faecium L565	128	64	32	16	64	>128
E. faecium L1666	>128	>128	>128	>128	>128	>128
E. hirae L1683	64	16	8	32	>128	>128

Staphylococcus aureus and *Enterococcus faecalis*, dimethylamide **VIII** had almost the same spectrum of activity as compound **II**.

Antibacterial Activity

The compounds were first tested against a panel of Gram-positive bacteria, including a CNS strain of *Staphylococcus haemolyticus* for which the MIC of teicoplanin (CTA/2) is relatively high (8 μg/ml).

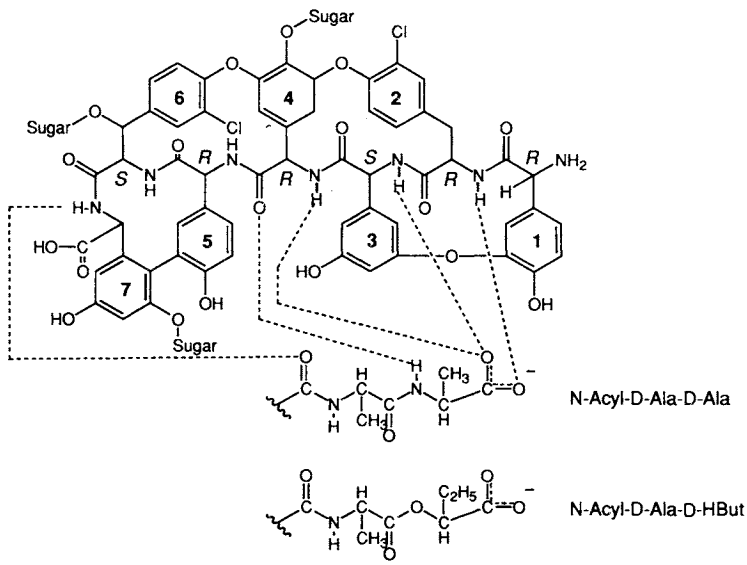
As shown in Table 4, deoxy pseudoaglycone **II** was more active than CTA/2 against the *S. haemolyticus* strain and had similar activity against other Gram-positive bacteria. Though less active against *Staphylococcus aureus* and *Enterococcus faecalis*, dimethylamide **VIII** had almost the same spectrum of activity as compound **II**.

Secondary amides **III**～**VI** had activity similar to that of **II** against *Streptococcus pyogenes* and *S. haemolyticus*, but they were less active than either **II** or CTA/2 against *Staphylococcus aureus* and *Enterococcus faecalis*. The most active compound of this family was amide **VII**, which was very active against all of the isolates and was significantly more active than the other compounds, including

Table 6. *In vitro* activity of amide **VII** in comparison with teicoplanin against *Streptococcus pyogenes*.

S. pyogenes Strain No.	MIC (μg/ml)		*S. pyogenes* Strain No.	MIC (μg/ml)	
	VII	CTA/2		**VII**	CTA/2
L33	0.004	0.06	L1303	0.004	0.06
L317	0.008	0.06	L1304	0.004	0.06
L800	0.008	0.06	L1306	0.004	0.06
L801	0.004	0.06	L1315	0.008	0.06
L802	0.016	0.06	L1316	0.004	0.06
L803	0.008	0.06	L1318	0.008	0.06
L804	0.008	0.06	L1319	0.008	0.06
L805	0.004	0.06			

Fig. 4. Peptide binding interaction in teicoplanin-like dalbaheptides.



CTA/2-amide T-VII, against *S. pyogenes*, *S. haemolyticus*, and *Staphylococcus epidermidis*.

Preliminary tests with a highly glycopeptide-resistant enterococcus (L562, VanA) prompted investigation of the activity of amides **III**～**VII** against 7 isolates of VanA enterococci from various sources. As shown in Table 5, amides **III**～**VII** had moderate activity against these isolates.

Compound **VII** was also tested against 15 isolates of *S. pyogenes*. As shown in Table 6, compound **VII** was 4 to 16 times as active as CTA/2 against these strains.

Conclusions

The reductive removal of the 34-acetylglucosamine from CTA/2 and its tertiary dimethylamide (T-VIII) produced 34-deoxy pseudoaglycone derivatives **II** and **VIII**, respectively, which were more active than teicoplanin against *S. haemolyticus*. In the structure of these compounds, the orientation of the 51, 52-peptide bond appears changed from *cisoid* to *transoid*. This is probably due to the absence of a 34-hydroxyl group or sugar, one of which is always present in natural glycopeptides of the dalbaheptide group. It follows a different conformation of the 5,6,7-macrocyclic ring between teicoplanin and these 34-deoxy derivatives which does not allow a direct comparison of the two classes of compounds such as to establish

the exact role of the acetylglucosamine in the activity of "natural" teicoplanin antibiotics.

The effect of this sugar on the activity of teicoplanin amides can be deduced by comparing the activity of the secondary amides (**III**~**VII**) of deoxy pseudoaglycone **II** with that of the corresponding amides (T-III~T-VIII) of CTA/2, as these compounds all have the same overall conformation of the heptapeptide backbone. The most evident result is that the presence of the acetylglucosamine has a negative influence on the activity of teicoplanin amides against VanA enterococci.

Studies are in progress to assess whether the activity of the acetylglucosamine-missing secondary amides of **II** against these highly glycopeptide-resistant enterococci is due to increased affinity for the depsipeptide, a D-Alanyl-D-hydroxy acid (Fig. 4),[d] which replaces the normal antibiotic's target peptide D-Alanyl-D-Alanine in the peptidoglycan biosynthesis,[8] or to a different mechanism.

Experimental

The progress of the reactions and the homogeneity of the final products were checked by HPLC.

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

FAB-MS positive ion spectra were obtained on a Kratos MS-50 instrument fitted with a standard FAB source and a high-field magnet. The sample (~10 nmol) was dispersed in few microliters of α-thioglycerol - diglycerol 1 : 1 matrix and bombarded with a 6~9 keV beam on Xe atoms.

The products were purified by reversed-phase column chromatography on silanized silica gel (0.063~0.2 mm; Merck). Reactions, column eluates, and final products were checked by HPLC performed on a column Hibar (120 × 4.5 mm; Merck) prepacked with LiChrosorb RP-8 (10 μm), using a Varian Model 5500 LC pump equipped with a 20-μl loop injector Rheodyne Model 7125 and a Varian Model 2050 UV variable detector. Chromatograms were recorded at 254 nm. Elutions were carried out at a flow rate of 2ml/minute according to a linear step gradient from 20% to 60% of CH_3CN in 0.2% aqueous HCO_2NH_4 in 30 minutes.

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

Most of the intermediate di- and polyamines are commercially available products which were purchased from Fluka-Chemie AG or Aldrich-Chemie Gmbh & Co. KG, with the exception of the branched tetramine $N[(CH_2)_3NH_2]_3$, used in the synthesis of amide **VII**, which was prepared according to a procedure previously described.[6] The preparations of the intermediate unsaturated pseudoaglycone **I**[7] and amides T-III~T-VIII[5,6] of CTA/2 were also already reported.

Preparation of 34-De(acetylglucosaminyl)-34-deoxy CTA/2 (**II**)

Method a. A solution of 3.4 g (*ca.* 2 mmol) of 34-de(acetylglucosaminyl)-35,52-didehydro-34-deoxy CTA/2 (**I**) in 300 ml of a MeOH - 0.04 N HCl (7 : 3) was hydrogenated (1 atm, room temperature) in the presence of 1.5 g of 5% Pd-C for 30 minutes, while absorbing 57 ml of H_2. After adding additional 2 g of the same catalyst, hydrogenation was continued under the above conditions for 2 hours (further 70 ml of H_2 was absorbed). The catalyst was filtered off and, after adding 600 ml of a BuOH - H_2O (2 : 1) mixture, the filtrate was concentrated at 25°C under reduced pressure to remove most of MeOH. The organic layer was separated and concentrated at 25°C under reduced pressure to a small (*ca.* 50 ml) volume. On adding ethyl acetate (350 ml), the precipitated solid was collected, washed with Et_2O and dried *in vacuo* at 40°C

[d] The mechanism of resistance to glycopeptides in VanA enterococci is related to a modification in the structure of peptidoglycan precursors in which terminal D-Ala-D-Ala is substituted by a D-Ala-D-HBut residue. The peptide binding interaction in teicoplanin-like antibiotics is outlined in Fig. 4.

overnight, yielding 2.5 g of the title compound, as the hydrochloride.

Method b. To a stirred solution of 10 g (*ca.* 5 mmol) of CTA/2 in 200 ml of a DMF-MeOH (2:1) mixture, 75 g (*ca.* 2 mol) of $NaBH_4$ (pellets of *ca.* 0.4 g each) was added at 0~5°C. The reaction mixture was stirred at room temperature for 24 hours and then it was poured into 1 liter of a MeOH-AcOH (85:15) mixture while cooling to 10~15°C. The resulting solution was concentrated at 45°C under reduced pressure to a volume of *ca.* 200 ml and the precipitated solid was filtered off. Then, the filtered solution was concentrated at 50°C under reduced pressure to a small (*ca.* 20 ml) volume. On adding Et_2O (300 ml), the precipitated solid (*ca.* 19 g, containing inorganic salts and a 15:85 mixture[e] of compounds **I** and **II**, respectively) was collected and re-dissolved in 500 ml of a 10 N HCl-DMF (1:4) mixture at 0~5°C. Afterwards 25 g of Zn (powder) was added under vigorous stirring. The formation of a more lipophilic compound (**II'**: HPLC, Rt 11.7 minutes) was observed within few minutes which derived from reaction by-product **I**. The transformation of **I** into **II'** was completed in 1 hour. Then, the insoluble matter was filtered off and 1.5 liter of H_2O was added to the filtered solution. The aqueous mixture was extracted with BuOH (1.5 liter) and the organic layer was washed with H_2O several times (10×500 ml) until the pH of final aqueous layer was about 4. The butanolic phase was separated and concentrated at 45°C under reduced pressure to a small (*ca.* 50 ml) volume. The resulting suspension was poured into 1 liter of a H_2O-MeCN (8:2) mixture obtaining a solution which was loaded on a column of 1.5 kg of silanized silica gel in the same solvent mixture. The column was developed eluting with a linear gradient from 20 to 70% of MeCN in 0.001 N HCl in 20 hours at a flow rate of 400 ml/hour, while collecting 20 ml-fractions. Those containing pure title compound were pooled and, after adding 2 volumes of BuOH, they were concentrated at 35°C under reduced pressure to a final volume of about 50 ml. On adding Et_2O (200 ml), the precipitated solid was collected and dried *in vacuo* at room temperature overnight, yielding 2.37 g of pure compound **II**, as the internal salt.

Preparation of Secondary Amides **III**~**VI** (Method d)

General procedure. A solution of 4 g (*ca.* 2.5 mmol) of compound **II** and 0.36 ml (*ca.* 2.6 mmol) of TEA in 20 ml of DMF was stirred at room temperature for 30 minutes while adding 0.4 ml (*ca.* 2.8 mmol) of benzyl chloroformate.[f] Then, 0.4 ml (*ca.* 3.3 mmol) of TEA and 4 ml (*ca.* 65 mmol) of chloroaceto-nitrile were added and stirring was continued at room temperature for additional 20 hours. The reaction mixture was poured into 300 ml of ethyl acetate. The precipitated solid was collected and dried at room temperature *in vacuo* overnight, yielding 4.3 g of crude N^{15}-CBZ-II-CME (HPLC, Rt 19.5 minutes). A 30 mg-pure sample of this compound (from 100 mg of the crude product) was obtained by reversed-phase column chromatography under the same conditions described above in the purification of compound **II**.

To a stirred solution of 4.2 g of crude N^{15}-CBZ-II-CME in 30 ml of DMF, 35 mmol of the appropriate amine was added and the resulting solution was stirred at room temperature overnight. Then, 25 ml of absolute ethanol was added, followed by 250 ml of ethyl acetate. The precipitated solid was collected, washed with 100 ml of Et_2O, and then dried at room temperature *in vacuo* overnight, obtaining crude corresponding N^{15}-CBZ-amide.

This product was dissolved in 350 ml of a MeOH-0.01 N HCl (7:3) mixture and the resulting solution was hydrogenated (1 atm, room temperature) in the presence of 4 g of 5% Pd-C for 2 hours. while absorbing 120~130 ml of H_2. The catalyst was filtered off and the filtrated solution was adjusted at pH 6.5 with 1 N NaOH. After adding 300 ml of BuOH and 15 g of silanized silica gel, solvents were evaporated at 40°C under reduced pressure. The solid residue was suspended in 200 ml of H_2O and the resulting suspension was loaded on a column of 400 g of the same silanized silica gel in H_2O. The column was developed eluting with a linear gradient from 10 to 80% of MeCN in H_2O in 20 hours at a flow rate of 250 ml/hour, while collecting 25 ml-fractions. Those containing pure title compounds were pooled and, after adding 2 volumes of BuOH, they were concentrated at 35°C under reduced pressure to a small volume (*ca.* 50 ml). The solid which separated was collected by centrifugation, washed with 10 ml of H_2O, then with 250 ml of ethyl

[e] The composition was determined by ^1H NMR spectroscopy.

[f] Intermediate benzyl carbamate N^{15}-CBZ-II was not isolated. Its formation, deduced from HPLC analysis of the reaction mixture (disappearance of peak at Rt 8.1 minutes, corresponding to compound **II**, and formation of a new peak at Rt 13.2 minutes, corresponding to its benzyl carbamate) was then confirmed by the structure of cyanomethyl ester N^{15}-CBZ-II-CME which was isolated in a pure state after treatment of N^{15}-CBZ-II with chloroacetonitrile.

acetate and 100 ml of Et_2O. After drying *in vacuo* at room temperature overnight, pure title compounds were obtained as the free bases.

Preparation of 3,3-Dimethylamino-1-propylamide **III**

From compound **II** (Method c).[g] To a stirred solution of 8.3 g (5 mmol) of compound **II** and 1.4 ml (*ca.* 10 mmol) of TEA in 100 ml of DMF, 0.71 ml (*ca.* 5 mmol) of benzyl chloroformate was added at room temperature in 30 minutes.[h] Then, 0.7 ml (*ca.* 5.5 mmol) of 3,3-dimethylamino-1-propylamine was added followed by 1.55 g (*ca.* 5.5 mmol) of diphenylphosphorazidate (DPPA), and stirring was continued at room temperature for 5 hours. On adding 500 ml of Et_2O, the precipitated solid (crude N^{15}-CBZ-amide III) was collected and redissolved in 500 ml of a MeOH - 0.01 N HCl (7 : 3) mixture. Hydrogenation to remove the CBZ-group and purification of crude amide **III** were then performed as described previously, yielding 4.1 g of pure title compound.

From parent CTA/2-amide (T-III) (Method b). To a stirred solution of 20 g (*ca.* 10 mmol) of the 3,3-dimethylamino-1-propylamide of CTA/2 (T-III) in 3 liters of a DMF - MeOH (2 : 1) mixture, 200 g (*ca.* 5.5 mol) of $NaBH_4$ is added in 90 minutes while cooling at 5 ~ 10°C. Stirring was continued at room temperature for 7 days and then the reaction mixture was poured into a solution of 320 ml of glacial AcOH in 6 liters of MeOH, while cooling at 5 ~ 10°C. The resulting solution was concentrated at 35°C under reduced pressure until most methanol was evaporated. The solid which separated was filtered off and the filtered solution was concentrated at 50°C under reduced pressure to a final volume of about 300 ml. On adding 1 liter of Et_2O, the precipitated solid was collected and purified by reversed-phase column chromatography under usual conditions, yielding 6.5 g of the title compound.

Preparation of Amide **VII** (Method c')

To a stirred solution of 16.6 g (10 mmol) of compound **II** and 2.8 ml (*ca.* 20 mmol) of TEA in 150 ml of DMSO, 1.4 ml (*ca.* 10 mmol) of benzyl chloro-formate was added at room temperature in 30 minutes. Then, (also in this case, intermediate N^{15}-CBZ-II was not isolated) 2 g (*ca.* 10.5 mmol) of N^3,N^3-di-[3-[(*tert*-butyloxycarbonyl)amino]propyl]-1,3-diaminopropane[6] was added. The resulting solution was stirred at 10°C while adding dropwise, in 30 minutes, a solution of 2.5 ml (*ca.* 10 mmol) of DPPA in 5 ml of DMSO. After stirring at room temperature overnight, the reaction mixture was poured into 500 ml of ethyl acetate. The precipitated solid was collected and re-dissolved in 100 ml of cold (0°C) TFA. After 30 minutes, 1 liter of ice-cold Et_2O was added and the oil which separated was slurried with ethyl acetate (3 × 250 ml), obtaining a solid which was collected and washed with 250 ml of Et_2O, yielding 21 g of crude N^{15}-CBZ-amide VII. This product was not purified for the next hydrogenation step, which was run as described previously. Purification by reversed-phase column chromatography under usual conditions yielded 6.6 g of the title compound.

Preparation of Dimethyl Amide **VIII** (Method b)

To a stirred solution of 19.1 g (10 mmol) of CTA/2-dimethylamide T-VIII in 3 liter of a DMF - MeOH (2 : 1) mixture, 260 g (*ca.* 7 mol) of $NaBH_4$ was added portionwise in 2 hours while cooling at 5 ~ 10°C. Stirring was continued at room temperature for 5 days and then the reaction mixture was poured dropwise into a ice-cold solution of 500 ml of glacial AcOH in 6 liters of MeOH, while maintaining the temperature at 0 ~ 5°C. Most MeOH was evaporated at 40°C under reduced pressure and the precipitated solid was filtered off. The filtered solution was concentrated at 55°C under reduced pressure to a final volume of about 500 ml, then Et_2O (1.5 liters) was added. The precipitated solid was collected and purified by reversed-phase column chromatography under usual conditions, obtaining 2.7 g of the title compound.

Determination of Antibacterial Activity

MICs were determined by microdilution methodology. With the exception of the study of VanA enterococci (Table 6), in which inocula were 5×10^5 CFU/ml, the inocula were 10^4 CFU/ml. Iso-Sensitest broth (Oxoid) was used for staphylococci and enterococci; Todd-Hewitt broth (Difco) was used for streptococci. Incubation was at 37°C for 24 hours.

[g] Amide **IV** (10.8 g) was also prepared from **II** (24 g) according to Method c.

[h] The benzyl carbamate N^{15}-CBZ-II was not isolated. See Method d, general procedure.

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