were dried, and the radioactivity bound to the filters was measured by liquid scintillation spectrometry. Specific [$^3$H]-8-OH-DPAT binding was defined as the difference between binding in the absence and presence of 10 $\mu$M 5-HT. $IC_{50}$ and slope values from the competition assays were determined by nonlinear regression analysis by using the program PCNONLIN and the four-parameter logistic function described by De Lean et al.[18]

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**Supplementary Material Available**: Effects of the enantiomers of 1–4 on the NSD 1015 induced accumulation of 5-HTP (brain stem, hemispheres) and DOPA (striatum, limbic system, brain stem, hemispheres) in rats (2 pages). Ordering information is given on any current masthead page.

(18) De Lean, A.; Muson, P. J.; Rodbard, D. *Am. J. Physiol.* **1978**, *255*, E97–E102.

# Synthesis and Biological Evaluation of De(acetylglucosaminyl)didehydrodeoxy Derivatives of Teicoplanin Antibiotics

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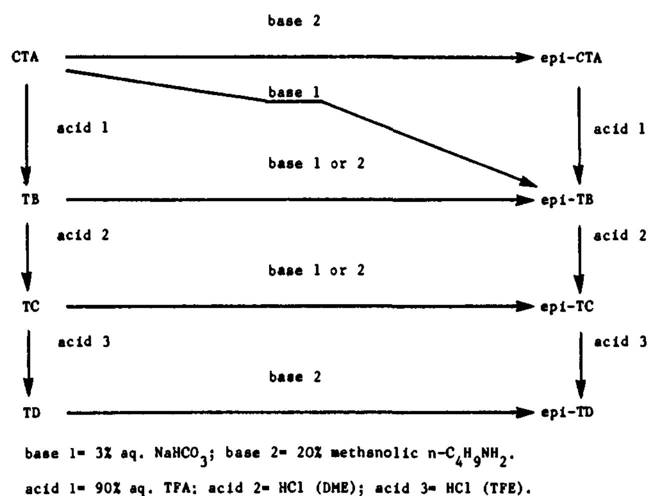
A series of 34-de(acetylglucosaminyl)-34-deoxy derivatives of 34,35- and 35,52-didehydro teicoplanin antibiotics have been synthesized from teicoplanin and its N-acetylglucosamine containing pseudoaglycons under basic conditions. The structures of these compounds have been determined by $^1$H NMR, UV, and FAB-MS. 35,52-Unsaturated derivatives maintained in vitro and in vivo antimicrobial activity to a different extent as well as the ability for binding to $Ac_2$-L-Lys-D-Ala-D-Ala, a bacterial cell-wall model for the site of action of glycopeptide antibiotics. In contrast, 34,35-unsaturated compounds were markedly less active and possessed a negligible affinity for the synthetic tripeptide.

Teicoplanin[1] is a glycopeptide antibiotic produced by *Actinoplanes teichomyceticus* ATCC 31121.[2] It belongs to the vancomycin–ristocetin family and it is active against Gram-positive bacteria.[3] Teicoplanin is a complex consisting (Figure 1)[4] of five major closely related factors (CTA) differing in the *N*-acyl aliphatic chain linked with $\beta$-D-glucosamine at position 56 and of one pseudoaglycon (TB) deriving from CTA by loss of the above N-acylated amino sugar. Both CTA and TB contain one $\alpha$-D-mannose and one *N*-acetyl-$\beta$-D-glucosamine at the 42- and 34-positions,[5] respectively, which are removed in that order by selective acidic hydrolysis, thus obtaining a second pseudoaglycon (TC) and the aglycon (TD).[6,7]

In the course of structural studies of teicoplanin, basic transformation products were also investigated. In particular, reaction with aqueous bicarbonate or methanolic amines resulted in the epimerization at C-3 (epiteicoplanins).[8] The relationship between the species involved in the acidic and basic treatments is outlined in Scheme I.

**Scheme I**




The glycosidic linkages of carbohydrates are generally hydrolyzed under acidic conditions but are relatively stable in diluted alkali,[9] with the exception of $\alpha$-D-mannosyl phenyl glycosides.[10] Sometimes, sugars attached to the hydroxyl group of serine can be removed by alkaline treatment through a $\beta$-elimination mechanism.[11] The 34-(*N*-acetyl-$\beta$-D-glucosamine) of teicoplanin is, in principle, amenable to undergo $\beta$-elimination on treatment with alkalies to produce new pseudoaglycons or aglycon.

(1) Teicoplanin is the recommended INN of teichomycin.
(2) (a) Parenti, F.; Beretta, G.; Berti, M.; Arioli, V. *J. Antibiot.* **1978**, *31*, 276–283. (b) Bardone, M. R.; Paternoster, M.; Coronelli, C. *Ibid.* **1978**, *31*, 170–177.
(3) (a) Pallanza, R.; Berti, M.; Goldstein, B. P.; Mapelli, E.; Randisi, E.; Arioli, V. *J. Antimicrob. Chemother.* **1983**, *11*, 419–425. (b) Somma, S.; Gastaldo, L.; Corti, A. *Antimicrob. Agents Chemother.* **1984**, *26*, 917–923.
(4) Teicoplanin and its acidic hydrolysis products were formerly named teichomycin $A_2$ complex, i.e. T-A2-1 to T-A2-5 (CTA) and T-A3-1 (TB), and T-A3-2 (TC), and T-aglycon (TD).
(5) Barna, J. C. J.; Williams, D. H.; Stone, D. J. M.; Leung, T.-W. C.; Doddrell, D. M. *J. Am. Chem. Soc.* **1984**, *106*, 4895–4902.
(6) Malabarba, A.; Strazzolini, P.; DePaoli, A.; Landi, M.; Berti, M.; Cavalleri, B. *J. Antibiot.* **1984**, *37*, 988–999.
(7) Malabarba, A.; Ferrari, P.; Gallo, G. G.; Kettenring, J.; Cavalleri, B. *J. Antibiot.* **1986**, *39*, 1430–1442.
(8) Barna, J. C. J.; Williams, D. H.; Strazzolini, P.; Malabarba, A.; Leung, T.-W. C. *J. Antibiot.* **1984**, *37*, 1204–1208.
(9) Overend, W. G. In *Chemistry and Biochemistry of Carbohydrates*; Academic Press: New York, 1972; Vol. 1A, p 332.
(10) Kyosaka, S.; Murata, S.; Tanaka, M. *Chem. Pharm. Bull.* **1983**, *31*, 3902–3905.
(11) Neurberger, A.; Gottschalk, A.; Marshall, R. D.; Spiro, G. In *Glycoproteins; Their Composition, Structure, and Function*, 2nd ed.; Gottschalk, A., Ed.; Elsevier: Amsterdam, 1972; p 450.

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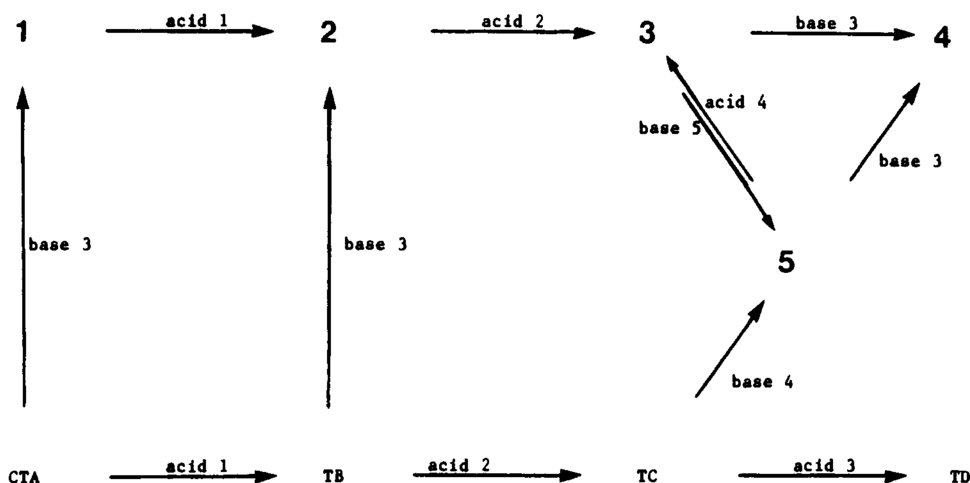
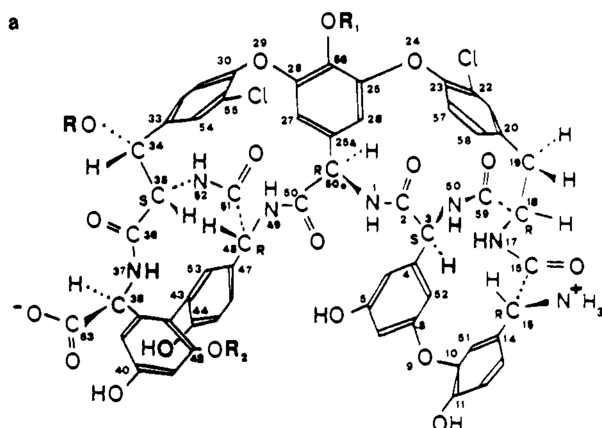
**Scheme II**



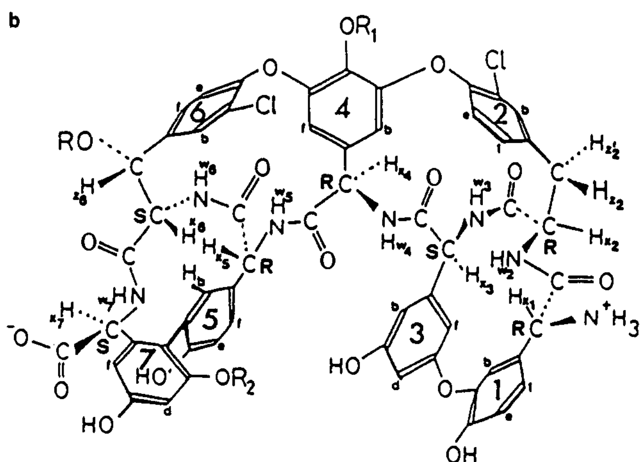

base 3 = methanolic KOH (DMF/DMSO, 3/2); base 4 = 2% methanolic $NaOCH_3$ (DMF); base 5 = 0.1% $NaHCO_3$ ($MeOH/H_2O$, 1/1).

acid 1 = 90% aq. TFA; acid 2 = $H_2SO_4$ or HCl (DME or THF); acid 3 = HCl (TFE); acid 4 = 1N HCl.

a



b



CTA R = N-acetyl-β-D-glucosaminyl; $R_1$ = N-($C_{10}$-$C_{11}$)acyl-β-D-glucosaminyl; $R_2$ = α-D-mannosyl.

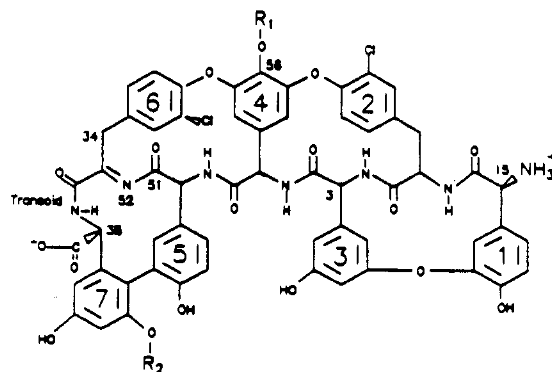
TB R = N-acetyl-β-D-glucosaminyl; $R_1$ = H; $R_2$ = α-D-mannosyl.

TC R = N-acetyl-β-D-glucosaminyl; $R_1$ = $R_2$ = H.

TD R = $R_1$ = $R_2$ = H.

**Figure 1.** The structures of teicoplanin $A_2$ (CTA) and its pseudoaglycons T-A3-1 (TB), T-A3-2 (TC), and aglycon (TD). (a) IUPAC nomenclature. (b) Nomenclature adopted by Williams et al.[5,7]

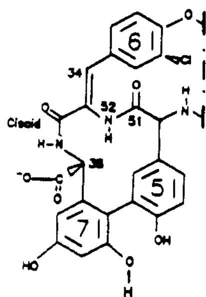
Therefore, we decided to investigate this possibility by submitting teicoplanin antibiotics to basic treatment under conditions different from those leading to C-3 epimerization.

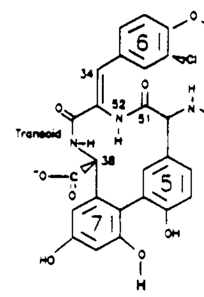


1 $R_1$ = N-($C_{10}$-$C_{11}$)acyl-β-D-glucosaminyl; $R_2$ = α-D-mannosyl.

2 $R_1$ = H; $R_2$ = α-D-mannosyl.

3 $R_1$ = $R_2$ = H.





4 5

**Figure 2.** The structures of unsaturated derivatives.

**Chemistry.** Teicoplanin $A_2$ (CTA) and its pseudoaglycon TB were respectively transformed (Scheme II, Figure 2) into the corresponding 35,52-unsaturated derivatives 1 and 2 at room temperature in the presence of 5% methanolic KOH in DMF/DMSO (3/2). In contrast, the removal of *N*-acetyl-β-D-glucosamine from pseudoaglycon TC under the above conditions produced 34,35-unsaturated compound 4 through an intermediate isomer 5. The formation of 5 and its subsequent transformation into 4 was observed while the course of the reaction was monitored by HPLC. Compound 5 was the main product when sugar elimination was carried out with 2% methanolic NaOMe in DMF/DMSO (4/1). Unsaturated aglycons 4 and 5 differ in the structure of the peptidic linkage at position

**Table I**

| compd | yield, % | HPLC: $t_R$, min | potentiometry,[a] $pK_{MCS}$ (EW) | FAB-MS | formula (MW) | anal.[b] |
|---|---|---|---|---|---|---|
| 1 | 81 | *d* | 5.0, 6.9 (935) | 1680 (M + Na) | (1656.5, 1658.5, 1672.5) | |
| 2 | 64 (98)[c] | 7.74 | 4.8, 6.9 (720) | 1342 | $C_{64}H_{53}Cl_2N_7O_{22}\cdot HCl$ (1343.1 + 36.5) | C, H, Cl, N |
| 3 | 80 | 10.06 | 4.7, 7.0 (670) | 1180 | $C_{58}H_{43}Cl_2N_7O_{17}\cdot HCl$ (1180.9 + 36.5) | C, H, Cl, N |
| 4 | 41 | 11.18 | 4.8, 7.1 (650) | 1180 | $C_{58}H_{43}Cl_2N_7O_{17}$ (1180.9) | C, H, Cl, N |
| 5 | 9 | 12.24 | 4.9, 6.9 (655) | 1180 | $C_{58}H_{43}Cl_2N_7O_{17}$ (1180.9) | C, H, Cl, N |

[a] Equivalent weights (EW) were corrected for solvent content and inorganic residue. Data for CTA were 5.0, 7.1 (965); for TD, 4.9, 6.9 (704). [b] Analytical results were within 0.4% of the theoretical values. Inorganic residue, determined in $O_2$ atmosphere at 900 °C, was always <0.3%. [c] The value in parentheses refers to reaction of 1 with 90% aqueous TFA. [d] $t_R$ of the five components: (I) 15.7, (II) 17.5, (III) 18.1, (IV) 20.4, (V) 21.2 min.

**Table II.** Significant $^1$H NMR Signals for Compounds 1–5, in Comparison with TD ($\delta$, ppm; m, multiplicity)[d]

| protons | 1 (m) | 2 (m) | 3 (m) | 4 (m) | 5 (m) | TD (m) |
|---|---|---|---|---|---|---|
| H-15 ($x_1$)[a] | 4.69 (s)[c] | 4.66 s) | 4.67 (s) | 4.67 (s) | 4.56 (s) | 4.62 (s) |
| H-18 ($x_2$) | 4.93 (ddd) | 4.94 (ddd) | 4.93 (ddd) | 4.90 (ddd) | 4.91 (d) | 4.97 (d) |
| H-3 ($x_3$) | 5.33 (d)[c] | 5.29 (d) | 5.28 (d) | 5.69 (d) | 5.29 (d) | 5.34 (d) |
| H-50a ($x_4$) | 5.67 (d) | 5.65 (d) | 5.55 (d) | 5.67 (d) | 5.56 (d) | 5.67 (d) |
| H-48 ($x_5$) | 4.53 (d) | 4.57 (d) | 4.58 (d) | 5.57 (d) | 5.34 (d) | 4.34 (d) |
| H-35 ($x_6$) | | | | | | 4.12 (dd) |
| H-38 ($x_7$) | 4.13 (d) | 4.15 (d) | 4.00 (d) | 5.04 (d) | 4.90 (d) | 4.39 (d) |
| H-19 ($z_2$) | 2.86 (dd) | 2.86 (dd) | 2.82 (dd) | 2.84 (dd) | 2.88 (dd) | 2.85 (dd) |
| H-34 ($z_6$) | 4.13 (d) | 4.15 (d) | 4.16 (d) | *b* (s) | *b* (s) | 5.10 (d) |
| H′-34 ($z_6'$) | 4.76 (d) | 4.83 (d) | 4.93 (d) | | | |
| H-26 (4b) | 5.59 (s) | 5.58 (s) | 5.40 (s) | | 5.57 (s) | 5.51 (s) |
| H-27 (4f) | 4.72 (s) | 4.71 (s) | 4.68 (s) | | 4.87 (s) | 5.11 (s) |
| $N_{52}$-H ($w_6$) | | | | 9.41 (s) | 9.32 (s) | 6.72 (d) |
| $N_{37}$-H ($w_7$) | nd[c] | nd | nd | 6.76 (d) | 7.54 (d) | 8.36 (d) |

[a] Nomenclature adopted by Williams et al.[5,7] is given as reference. [b] Shifted in the aromatic region. [c] s = singlet; d = doublet; nd = not determined. [d] CH of mannose: $\delta$ 3.48 ppm (m) in both 1 and 2. Anomeric H of mannose: $\delta$ 5.48 ppm (s, 1); 5.44 ppm (s, 2).

36,37, which is "transoid" in **5**, as in teicoplanin, while it is "cisoid" in **4**. It follows that the conformation of the two molecules is remarkably different in this region. In addition, in compound **4** aromatic rings 5 and 7 tend to be more coplanar, as gathered from UV spectra.

The 35,52-unsaturated aglycon **3** was prepared by acidic hydrolysis of derivatives **1** or **2** with HCl in DME at room temperature. **3** isomerized to **5** upon treatment with 0.1% aqueous $NaHCO_3$, and in turn, **5** reverted to **3** with 1 N HCl at room temperature, but these compounds irreversibly transformed into **4** with KOH under the conditions previously described for the preparation of **4** from TC.

Compound **2** was also obtained from **1** by acidic hydrolysis in the presence of 90% aqueous TFA at room temperature or with 0.5 N HCl at 80 °C for 45 min.[6]

**Structure Elucidation.** FAB-MS spectrometry showed that the MW's of compounds **1–5** (Table I) were 221 $\mu$ less than those of the corresponding teicoplanin starting materials.[6] This indicated the loss of the acetylglucosamine unit and the presence of a double bond, as expected from $\beta$-elimination mechanism.

A possible tautomerism $C_{34}{=}C_{35} \leftrightarrow C_{35}{=}N_{52}$ was suggested for isomers **3–5**, in particular for **3** and **5**, based on their interconversion under acidic–basic conditions.

The structures of the compounds shown in Figure 2 were determined by $^1$H NMR. The most significant $^1$H chemical shifts, given in Table II, were assigned by comparison of the spectra of compounds **3–5** with that of TD. The $^1$H NMR spectrum of TD[7] shows signals of 10 protons in the 4–6 ppm region which are due to the one H-34, the two high field aromatic H-26 and H-27, and the seven $\alpha$-CH's of the peptide backbone. Only eight protons were found in the same region of $^1$H NMR spectra of **4** and **5**, the two absent ones being H-34, which is shifted in the field of aromatic protons, and H-35. Moreover, in **4** and **5** H-38, H-48, and H-27 move downfield. These results can be explained by a remarkable modification around C-34 and C-35, as expected from the change of hybridization of C-34 and C-35 from $sp^3$ to $sp^2$.

$^1$H NMR spectra of teicoplanins show the signal of NH-52[5,7] at higher field than those of the other NH's, as a consequence of the unusual "cisoid" conformation of 51,52-amide. This is a characteristic of the structures of these glycopeptide antibiotics, and the attribution is based on the spin coupling of NH-52 with H-35. In particular, in TD the chemical shift of NH-52 is at $\delta$ 6.72 ppm.

The $^1$H NMR spectrum of compound **4** shows one doublet at $\delta$ 6.76 ppm that is attributable to NH-37, as results from decoupling of H-38 by irradiation in the 6.6–6.8 ppm region. Hence, in **4** the amide at position 36,37 is hypothesized in the "cisoid" conformation. In **5**, all amides are present in the "transoid" conformation, as gathered from the absence of high field NH signals and of CH–NH "cisoid" coupling. The NH-52 shows a singlet at low field chemical shift in both derivatives **4** ($\delta$ 9.41 ppm) and **5** ($\delta$ 9.32 ppm), and this confirms the absence of adjacent protons and the "transoid" conformation of 51,52-amide. It follows that **4** and **5** should differ in the orientation of 36,37-peptide linkage and hence in the arrangement of the macrocycle containing aromatic rings 5 and 7.

The $^1$H NMR spectrum of **3** shows nine $^1$H signals in the 4–6 ppm region. In this case, two doublets are present at $\delta$ 4.16 and 4.93 ppm, geminally coupled, which are due to $CH_2$-34. This, compared with **5**, is justified by the migration of one proton from N-52 to C-34, leading to the formation of $C_{35}{=}N_{52}$. The similarity in their $^1$H NMR spectra allowed the same structural features as **3** to be assigned to **1** and **2**, since a direct complete study of the

Table III. UV Data [$\lambda_{max}$, nm ($\epsilon$)] of Compounds 1–5 in Comparison with CTA and TD

| solvent | 1 | 2 | 3 | 4 | 5 | CTA | TD |
|---|---|---|---|---|---|---|---|
| 0.1 N HCl | 279<br>(17600) | 279<br>(18200) | 280<br>(18100) | 278<br>(19600) | 278<br>nd[a] | 278<br>(11200) | 278<br>(11100) |
| 0.1 N NaOH | 296<br>(21700) | 296<br>(26500) | 296<br>nd | 296<br>(27700) | 296<br>(23500) | 296<br>(17100) | 297<br>(20800) |
| $H_2O$, pH 7.4 (buffer) | 279<br>(18300) | 279<br>(20200) | 280<br>(20000) | nd | 278<br>(17600) | nd | 280<br>(15600) |

[a] nd = not determined.

Table IV. In Vitro (MIC) and in Vivo ($ED_{50}$) Activity

| test organisms | | 1 | 2 | 3 | 4 | 5 | teicoplanin | TB | TC | TD |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | MIC, μg/mL | | | | | | |
| *Staph. aureus* | TOUR | 0.5 | 0.25 | 0.25 | 1 | 2 | 0.125 | 0.25 | 0.5 | 0.063 |
| *Staph. epidermidis* | ATCC 12228 | 0.25 | 0.5 | 0.125 | 0.125 | 0.5 | 0.25 | 0.25 | 0.125 | 0.016 |
| *Strepto. pyogenes* | C 203 | 0.063 | 2 | 1 | 4 | 8 | 0.063 | 0.5 | 0.5 | 0.125 |
| *Strepto. pneumoniae* | UC 41 | 0.063 | 2 | 1 | 4 | 8 | 0.063 | 0.5 | 1 | 0.125 |
| *Strepto. faecalis* | ATCC 7080 | 1 | 1 | 1 | 8 | 16 | 0.125 | 2 | 1 | 0.125 |
| | | | | Mouse $ED_{50}$, mg/kg sc[b] | | | | | | |
| *Strepto. pyogenes* C203 | | 0.35 | 5.8 | 5.0 | nd[a] | nd | 0.18 | 2.64 | 2.46 | 0.95 |

[a] nd = not determined. [b] sc = subcutaneous administration.

Table V. In Vitro Activity against Coagulase-Negative Staphylococci, Clinical Isolates

| organism | | MIC, μg/mL | | |
|---|---|---|---|---|
| | | 3 | TD | teicoplanin |
| *S. epidermidis* | L 1378 | 0.125 | 0.063 | 0.5 |
| *S. simulans*[a] | L 785 | 0.125 | 0.063 | 0.25 |
| *S. simulans*[a] | L 1142 | 0.125 | 0.063 | 0.125 |
| *S. hominis* | L 1070 | 0.125 | 0.063 | 0.25 |
| *S. warneri* | L 1375 | 1 | 0.25 | 2 |
| *S. haemolyticus*[a] | L 1520 | 0.5 | 0.125 | 4 |

[a] Methicillin-resistant.

Table VI. Association Constants with $Ac_2$-L-Lys-D-Ala-D-Ala

| compd | $K_a$, $M^{-1}$ | |
|---|---|---|
| | pH 5 | pH 9 |
| 1 | $3.9 \times 10^5$ | $6.0 \times 10^4$ |
| 2 | $3.8 \times 10^5$ | $6.1 \times 10^4$ |
| 3 | $1.0 \times 10^5$ | nd |
| 4 | $<10^2$ | $<10^2$ |
| 5 | nd[a] | $<10^2$ |
| CTA | $1.6 \times 10^6$ | $2.1 \times 10^5$ |
| TB | $1.2 \times 10^6$ | $0.9 \times 10^5$ |
| TC | $2.6 \times 10^5$ | $3.2 \times 10^4$ |
| TD | $2.5 \times 10^5$ | $3.0 \times 10^4$ |

[a] nd = not determined.

spectra of 1 and 2 was difficult for the presence of additional signals due to α-D-mannose (in 2) and also to N-acyl-β-D-glucosamine (in 1).

The UV spectra of derivatives 1–5 show the same pattern as teicoplanin, but the intensities of the absorption maxima of the unsaturated compounds are higher (Table III). This effect is due to a strengthening of the interaction among π orbitals of the diphenyl moiety that must derive from the enhanced coplanarity of rings 5 and 7.

IR spectroscopy and acid–base titration data were in agreement with the structures given.

**Biological Activity.** The antibacterial activity of compounds 1–5 was determined against Gram-positive (staphylococci and streptococci) and Gram-negative (*Escherichia coli* SKF 12140, *Pseudomonas aeruginosa* ATCC 10145, and *Proteus vulgaris* X19H ATCC 881) organisms in comparison with teicoplanin, its pseudoaglycons and aglycon (Table IV). C=C containing aglycons 4 and 5 showed markedly reduced activity against test organisms, while C=N compounds 1 and 2 possessed in vitro activities comparable to those of parent teicoplanins CTA and TB, respectively. 1 was less active than teicoplanin only against *Streptococcus faecalis*, but it was the most active unsaturated derivative against *Streptococcus pyogenes* and *Streptococcus pneumoniae*. The activity of 3 was lower than that of TD but comparable to that of TC. Against six clinical isolates of coagulase-negative staphylococci (Table V), 3 was still less active than TD but more active than teicoplanin against *Staphylococcus epidermidis* and *Staphylococcus haemolyticus*. All the above unsaturated teicoplanin derivatives were found to be inactive (MIC > 128 μg/mL) against the Gram-negative bacteria tested.

The efficacy of compounds 1, 2, and 3 was lower than that of CTA, TB, and TC, respectively, in curing *Strepto. pyogenes* experimental septicemia in the mouse.

**Binding Studies.** To verify whether the above microbiological results are connected with different abilities of derivatives 1–3 in comparison with 4 and 5 to complex with bacterial precursor containing peptide D-alanyl-D-alanine, we measured the binding of these compounds to the synthetic peptidoglycane analogue $Ac_2$-L-Lys-D-Ala-D-Ala, using a differential UV assay.[12] The values of association constants ($K_a$'s, Table VI) were determined at pH 5 and 9 for unsaturated derivatives and unmodified teicoplanin antibiotics. It emerges that, as for CTA and TB compared to TC and TD, the presence of mannose at C-42 plays a certain role in binding formation in 1 and 2, in comparison with 3. This behavior confirms that the mannose residue is involved in forming part of the binding pocket.[13] The $K_a$ values[14] for 3 and 5 indicate that the different structural features of these compounds strongly influence their ability to complex with the tripeptide. In particular, the simultaneous conformational changes occurring in the region of the active site in going from structure $C_{35}$=$N_{52}$ to $C_{34}$=$C_{35}$ cause a significant drop in binding strength. Instead, a less marked effect is seen with teicoplanin and its $C_{35}$=$N_{52}$ unsaturated derivatives, as a consequence of the minor change of the overall structures of these compounds.

## Conclusions

The treatment of teicoplanin and its acidic hydrolysis pseudoaglycons with strong alkali in polar organic solvents

(12) Nieto, M.; Perkins, H. R. *Biochem. J.* 1971, *124*, 845.
(13) Barna, J. C. J.; Williams, D. H.; Williamson, M. P. *J. Chem. Soc., Chem. Commun.* 1985, 254–256.
(14) $K_a$ values were determined at pH 5 for 3 and at pH 9 for 5 to avoid the transformation of 3 to 5 under basic conditions and that of 5 to 3 in acidic media.

leads to the selective removal by $\beta$-elimination of *N*-acetyl-$\beta$-D-glucosamine and the introduction of a double bond in position 34,35, which is in equilibrium with position 35,52.

The unsaturation position seems to depend on mannose at C-42. When this sugar is present, the isolated products possess the double bond in $C_{35}$=$N_{52}$ while, in its absence, the double bond is in $C_{34}$=$C_{35}$. This is hypothesized as due to a limited twisting of aromatic rings 5 and 7 in the presence of mannose, a situation that would favour the tautomerism C=C → C=N and cause the formation of compounds **1** and **2**. Compound **4** has the C=C double bond in the same position as **5**, but it is hypothesized to be an isomer of **5** bearing the 36,37-amidic bond in "cisoid" conformation instead of transoid. Unexpectedly, compound **4** does not revert to **5**, and the "cisoid" conformation of the 36,37-amide might be stabilized by steric requirements of the peptidic ring.

The position of the double bond modifies the overall conformation of these molecules, with respect to unmodified teicoplanin antibiotics, to a different extent, hence influencing their microbiological properties. In the case of $C_{35}$=$N_{52}$ (compounds **1**–**3**) this modification is a minor one and consequently is not critical for the ability to complex with $Ac_2$-L-Lys-D-Ala-D-Ala and for the in vitro antimicrobial activity. Actually, the in vitro activity of **3** is much lower than that of TD with respect to that of **1** and **2** in comparison with CTA and TB, respectively. This apparent contradiction is most probably due to the instability of **3** under the in vitro test conditions (pH 7.4). In fact, though at a slower rate, the transformation of **3** into the less active compound **5** also occurs at neutral pH. In the case of $C_{34}$=$C_{35}$ (derivatives **4** and **5**), the shape of the molecules affects the active site and reduces the binding strength. It follows that the in vitro activities of compounds **4** and **5** are negligible compared to those of **1**–**3** and of unmodified teicoplanin antibiotics.

## Experimental Section

Evaporation was done at 45–50 °C (bath temperature) in vacuo. Reactions and final products were monitored by HPLC with a Varian 5000 LC pump equipped with a Rheodyne 7125 injector (20-$\mu$L loop) and a UV detector (254 nm), using a precolumn (5 cm) packed with Perisorb RP-8 (30 $\mu$m Merck) followed by a column (25 cm, Hibar RT 250-4 Merck) prepacked with LiChrosorb RP-8 (10 $\mu$m). The chromatograms were obtained by injecting solutions of each compound or samples of reaction mixtures in $CH_3CN/H_2O$ (1/1) at pH 3 (1 mg/mL) and eluting with a linear gradient from 15 to 30% of $CH_3CN$ in 0.2% aqueous $HCO_2NH_4$ in 30 min at a flow rate of 2 mL/min. Preparative column chromatography was performed on silanized silica gel (0.063–0.2 mm, Merck).

IR spectra (Nujol) were recorded on a Perkin-Elmer 580 spectrometer. UV spectra were obtained with a Unicam SP 800 spectrophotometer. $^1$H NMR spectra were recorded on a Bruker WH-250 cryospectrometer at 250 MHz using $Me_4Si$ ($\delta$ 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 $\alpha$-thioglycerol/diglycerol (1/1) matrix and bombarded with a 6–9-keV beam of Xe atoms.

Acid–base titrations were carried out as follows: the sample was dissolved in $MCS/H_2O$ (4/1) and then an excess of 0.01 M HCl in the same solvent mixture was added and titration was carried out with 0.01 N NaOH.

Elemental analyses were obtained with a Carlo Erba 1106 analyzer. Where analyses are indicated only by symbols of the elements, results were within ±0.4% of theoretical values.

**42-$\alpha$-D-Mannosyl-56-(*N*-acyl-$\beta$-D-glucosaminyl)-35,52-didehydro-34-deoxyteicoplanin–Aglycon Complex (1) Hydrochloride.** A solution of 50 g of commercial 85% KOH in 1 L of MeOH was added at room temperature to a stirred solution of 23 g (~12 mmol) of CTA in 2.5 L of DMF/DMSO (3/2). After the mixture was stirred for 48 h, 1 L of MeOH was added followed by 1 L of $Et_2O$. A solid separated, which was collected, washed with $Et_2O$, and dried in vacuo overnight, yielding 21 g of crude **1**. This material was dissoved in 1.2 L of $CH_3CN/H_2O$ (1/2) and the resulting solution was adjusted at pH 2.8 with glacial AcOH, and then silanized silica gel (85 g) and 1-BuOH (1.5 L) were added. Solvents were evaporated, and the residue was loaded at the top of a column of 2.4 kg of the same silica gel in $H_2O$. The column was developed with a linear gradient from 10% of $CH_3CN$ in 0.001 N HCl to 70% of $CH_3CN$ in 0.01 N HCl in 30 h at the flow rate of 300 mL/h, while 25-mL fractions were collected, which were checked by HPLC. Those containing pure **1** were pooled and $CH_3CN$ and $H_2O$ were evaporated after adding enough 1-BuOH to obtain a concentrated (~120 mL) dry butanolic cloudy solution. After addition of 1.5 mL of concentrated HCl and 1 L of $Et_2O$, the precipitated solid was collected and dried in vacuo at 30 °C overnight to give 15.1 g of **1**, as the hydrochloride: IR 3280 ($\nu(NH_2$, PhOH)), 1725 ($\nu$(C=O), acid), 1645 ($\nu$(C=O), amide I), 1590 ($\delta(NH_3^+)$), 1510 ($\delta$(NH), amide II), 1230, 1060, and 1025–1010 ($\delta$(OH), $\nu$(C–O)), 970 cm$^{-1}$ (bending vibration of mannose).

**42-$\alpha$-D-Mannosyl-35,52-didehydro-34-deoxyteicoplanin Aglycon (2). (a) From TB.** Starting from 1.1 g (~0.7 mmol) of TB and following exactly the same procedure as described above for the preparation of **1** from CTA gave 0.6 g of **2**, as the hydrochloride: IR 3240 ($\nu(NH_2$, PhOH)), 1725 ($\nu$(C=O), acid), 1650 ($\nu$(C=O), amide I), 1590 ($\delta(NH_3^+)$), 1515 ($\delta$(NH), amide II), 1225, 1060, and 1005 ($\delta$(OH), $\nu$(C–O)), 970 cm$^{-1}$ (bending vibration of mannose).

**(b) From 1.** A solution of 5.0 g (~3 mmol) of **1** in 60 mL of 90% aqueous TFA was stirred at room temperature for 90 min, and then 240 mL of $Et_2O$ was added. The precipitated solid was collected (4.6 g) and redissolved in 350 mL of 1-BuOH/$MeOH/H_2O$ (1/3/3/). The resulting solution was brought to pH 6.7 with 1 N NaOH and concentrated to a small volume (~30 mL). The precipitated solid was collected by centrifugation, washed with $H_2O$ (50 mL), and dried in vacuo at 45 °C overnight, yielding 4.0 g of **2**, as the internal salt: IR 1610 cm$^{-1}$ ($\nu(COO^-)$).

**35,32-Didehydro-34-deoxyteicoplanin Aglycon (3). (a) From Compounds 1 or 2.** Dry HCl was slowly bubbled into a stirred suspension of 1 mmol of **1** (or **2**) in 100 mL of DME, while the internal temperature was maintained at 15–20 °C. A clear solution formed within a few hours; afterward the solvent was evaporated. The residue was dissolved in 200 mL of $CH_3CN/H_2O$ (25/75) and the resulting solution was loaded at the top of a column of 200 g of silanized silica gel in $H_2O$. The column was developed with a linear gradient from 25 to 60% of $CH_3CN$ in 0.001 N HCl in 20 h at a rate of 300 mL/h, while 20-mL fractions were collected. Those containing pure **3** (HPLC) were pooled and worked up as for **1** and **2**, thus obtaining 0.7 g of **3**, as the hydrochloride: IR 3300 ($\nu(NH_2$, PhOH)), 1720 ($\nu$(C=O), acid), 1655 ($\nu$(C=O), amide I), 1590 ($\delta(NH_3^+)$), 1515 ($\delta$(NH), amide II), 1230, 1060, and 1010 cm$^{-1}$ ($\delta$(OH), $\nu$(C–O)).

**(b) From 5.** A solution of **5** (5 mg) in 1 N HCl (10 mL) was stirred at room temperature for 6 h to give a 95/5 mixture (HPLC) of **3** and **5**. The corresponding peaks were identified by comparison with authentic samples.

**34,35-Didehydro-34-deoxy-(36,37-"cis",51,52-"trans")-teicoplanin Aglycon (4). (a)** A solution of 7.5 g of commercial 85% KOH in 200 mL of MeOH was added at room temperature to a stirred solution of 5 g (~3.5 mmol) of TC in 200 mL of DMF.[15] After 20 h, 10 mL of AcOH was added at 5–10 °C and the resulting solution was poured into 1.5 L of $Et_2O$. The precipitated solid was collected by centrifugation and redissolved in 3 L of 1-BuOH/$MeOH/H_2O$ (2/1/3) while the pH was adjusted to 3.5 with glacial AcOH. After addition of 2 L of 1-BuOH/$H_2O$ (1/1), the mixture was stirred for 30 min, and then the organic layer was separated, washed with $H_2O$ (1 L), and concentrated to a small volume (~200 mL). By addition of $Et_2O$ (250 mL), a solid separated, which was collected, washed with $Et_2O$ (500 mL), and dried in vacuo at room temperature overnight, yielding 1.7 g of **4**, as

(15) In DMF/DMSO (3/2), the reaction was slower and about 3 days were required to obtain complete transformation of TC into 4.

the internal salt: IR 3400–3280 ($\nu$($NH_2$, PhOH)), 1650 ($\nu$(C=O), amide I), 1615 ($\nu$($COO^-$)), 1590 ($\delta$($NH_3^+$)), 1520 ($\delta$(NH), amide II), 1230, 1200, 1160, 1060, 1020, and 1005 $cm^{-1}$ ($\delta$(OH), $\nu$(C—O)).

(b) **4** was also obtained from **3** or **5** under the same experimental conditions and with the same yields as above.

**34,35-Didehydro-34-deoxy-(36,37-"trans",51,52-"trans")-teicoplanin Aglycon (5). (a) From TC.** A solution of 6 g (0.11 mol) of freshly prepared NaOMe in 300 mL of MeOH was added at room temperature to a stirred solution of 2.8 g (2 mmol) of TC in 300 mL of DMF/DMSO (4/1).[16] After 72 h, 7 mL of glacial AcOH was added at 0–10 °C and MeOH was evaporated. The resulting suspension was filtered and the clear filtrate was poured into 1.5 L of $H_2O$. The resulting cloudy solution was adjusted at pH 6 with glacial AcOH and extracted with 2 L of 1-BuOH/EtOAc (3/1). The organic layer was concentrated to a small volume (~60 mL) and $Et_2O$ (340 mL) was added. The precipitated solid was collected, washed with $Et_2O$, and dried in vacuo at 40 °C overnight to give 0.95 g of product, as the internal salt: IR 3250 ($\nu$(NH, PhOH)), 1650 ($\nu$(C=O), amide I), 1610 ($\nu$($COO^-$)), 1590 ($\delta$($NH_3^+$)), 1510 ($\delta$(NH), amide II), 1230, 1200, 1135, 1060, and 1005 $cm^{-1}$ ($\delta$(OH), $\nu$(C—O)).

(b) **From 3.** A solution of **3** (5 mg) and 10 mg of $NaHCO_3$ in 10 mL of $CH_3CN/H_2O$ (1/1) was stirred at room temperature for 1 h to give a 9/1 mixture (HPLC) of **5** and **3**. The identity of **5** was confirmed by comparison with an authentic sample.

**Microbiological Activity Determination.** Antibacterial activity expressed as MIC (minimal inhibitory concentration in $\mu$g/mL) was determined by the 2-fold dilution method in microtiter using Difco Todd-Hewitt broth (*Strepto. pyogenes* and *Strepto. pneumoniae*) or Oxoid Iso-Sensitest broth (staphylococci, *Strepto. faecalis*, and Gram-negative organisms). Final inoculum was ~$10^4$ cfu/mL. MIC was read as the lowest concentration that showed no visible growth after overnight incubation at 37 °C. Coagulase-negative staphylococci were identified by API STAPH (Profile Index, I Ed.) according to the classification of Kloos and Schleifer.[17]

(16) Or 450 mL of DMF.

Experimental infection was carried out by using groups of five mice infected intraperitoneally with *Strepto. pyogenes* C 203. Inocula were adjusted so that untreated animals died of septicemia within 48 h. Animals were treated subcutaneously once immediately after infection. On the seventh day, $ED_{50}$ (50% effective dose) was calculated[18] on the basis of the percentage of surviving animals at each dose.

**Binding Assay.** The interaction of $Ac_2$-L-Lys-D-Ala-D-Ala with teicoplanins and the unsaturated derivatives was determined by UV differential spectroscopy.[12] Experiments were run on a Perkin-Elmer 320 double-beam UV spectrophotometer with 4-cm-pathlength nonthermostated cells. The temperature was 24 ± 2 °C. The initial volume of antibiotic solution was 10 mL at a 30 $\mu$M concentration in 10% MeOH in sodium phosphate buffer (pH 5 or 9). The difference in absorbance ($\Delta A$) developed on addition of test peptide was monitored at the wavelength (294 nm) that showed the maximum change. Association constants ($K_a$) for complex formation were obtained from the slope of the straight line resulting from a Schatchard's plot, $\Delta A/(\Delta A_{max} \times C)$ vs $\Delta A/\Delta A_{max}$, of the data. Binding constants of $10^5$–$10^6$ or greater were obtained with a standard deviation of about 10%.[19] The values of $K_a$ determined in alkaline buffer were always about 10 times lower than those obtained at pH 5.[13]

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(17) (a) Schleifer, K. H.; Kloos, W. E. *J. Syst. Bacteriol.* **1975**, *25*, 50. (b) Kloos, W. E.; Schleifer, K. H. *Int. J. Syst. Bacteriol.* **1975**, *25*, 62. (c) Kloos, W. E.; Schleifer, K. H.; Smith, R. F. *Ibid.* **1976**, *26*, 22.
(18) Finney, D. J. In *Statistical Method in Biological Assay*; Griffin, G. and Co. Ltd.: London, 1952; p 524.
(19) Harris, C. M.; Fesik, S. W.; Thomas, A. M.; Kannan, R.; Harris, T. M. *J. Org. Chem.* **1986**, *51*, 1509–1513.

# Synthesis of Congeners and Prodrugs. 3.[1] Water-Soluble Prodrugs of Taxol with Potent Antitumor Activity

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Taxol has shown good in vivo antitumor activity in a number of test systems. The formulation of taxol for antitumor testing has been difficult. Esterification at either C-2′ or C-7 resulted in loss of in vitro tubulin assembly activity but not cytotoxicity. These observations suggested that esters at C-2′ and/or C-7, which would tend to promote water solubility, might serve as useful prodrugs of taxol. The reaction of taxol with either succinic anhydride or glutaric anhydride in pyridine solution at room temperature gave the crystalline mono 2′-adducts **1b** and **1f**, respectively. Salts of these acids (**1b**, **1f**, **1i**) were formed by the addition of 1 equiv of the corresponding base, followed by evaporation and/or freeze-drying of the solvent(s). The salts had improved antitumor activity as compared to the free acids. The triethanolamine and *N*-methylglucamine salts showed greatly improved aqueous solubility and were more active than the sodium salts. The glutarate series was preferred because of the higher activity and the higher yields obtained. 2′-Glutaryltaxol (**1f**) was coupled with 3-(dimethylamino)-1-propylamine, using CDI, to form in excellent yield the amino amide **1o**. The hydrochloride salt (**1p**) showed good solubility and was extremely potent and active. At 10 mg/kg, in the B16 screen, **1p** gave a T/C of 352 with 5 out of 10 cures. In the MX-1 breast xenograft assay, this prodrug gave values of −100 at doses of 40 and 20 mg/kg, with all live animals being tumor free.

The natural product taxol (**1a**) was first isolated in 1971 from the Western Yew, *Taxus brevifolia* Nut. (Taxaceae) by Wani, Wall, and co-workers[2] who established its structure by chemical and X-ray crystallographic methods. A summary of pertinent taxol literature has been published.[3] Numerous studies have indicated that taxol and various taxane derivatives are highly cytotoxic and possess strong in vivo antitumor activity in a number of test systems.[2-10] The mechanism of action of taxol has been

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(1) For paper 2 in this series, see: Deutsch, H. M.; Gelbaum, L. T.; McLaughlin, M.; Fleischmann, T. J.; Earnhart, L. L.; Haugwitz, R. D.; Zalkow, L. H. *J. Med. Chem.* **1986**, *29*, 2164.
(2) Wani, M. C.; Taylor, H. L.; Wall, M. E.; Coggon, P.; McPhail, A. T. *J. Am. Chem. Soc.* **1971**, *93*, 2325.
(3) Zee-Cheng, R. K-Y.; Cheng, C. C. *Drugs Future* **1986**, *11*, 45.

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