Reductive Hydrolysis of the 59,60-Amide Bond of Teicoplanin Antibiotics: A Key Step from Natural to Synthetic Glycopeptides

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Received June 16, 1994

A current challenge for glycopeptides of the dalbaheptide group,[1] to which teicoplanin (Chart 1) belongs, is the emerging resistance in enterococci.[2]

In glycopeptide-susceptible bacteria, these antibiotics inhibit the cell-wall biosynthesis by specifically binding to peptidoglycan precursors terminating in D-Ala-D-Ala.[3] The primary binding interaction modelled *in vitro* using short cell-wall precursors involves peptide-NH groups of amino acid fragments 2, 3, and 4 and the carboxylate anion of D-Ala-D-Ala (Chart 2). An important role in the initial binding formation and in the antibacterial activity of glycopeptides is also played by the terminal amino group of amino acid 1.[4] Secondary hydrogen bonding systems,[5] favored by the particular configuration of the heptapeptide structure, strengthen the antibiotic–dipeptide complex. In glycopeptide-resistant strains, the replacement of target dipeptide by a D-Ala-D-hydroxy acid[6] does not allow a tight interaction between these antibiotics and the modified cell-wall mucopeptide precursors.

Changes in binding properties could be pursued by replacing amino acids 1 and 3 with new suitably selected amino acids. Although many glycopeptides have been discovered so far, the differences in the structure of the binding pocket are limited,[7] so that it is unlikely to expect significantly different naturally derived glycopeptides in the near future. Also the probability to systematically modify the structure of the active site by bioconversion of naturally occurring molecules is actually low or negligible. Hence, a strategy to replace chemically amino acids 1 and 3 was investigated.

Teicoplanin (CTA), its acidic hydrolysis pseudoaglycons (TB, TC) and aglycon (TD), in which amino acids 1 and 3 are linked together through a diphenyl ether bridge, were considered as suitable substrates for this study. Key to our strategy was a chemoselective process to cleave the peptide bond between amino acids 2 and 3 without affecting the other peptide linkages.

Selective hydrolysis of the 59,60-amide bond was effected under reductive conditions by treatment of well defined hydroalcoholic (H_2O/EtOH 65/35) solutions of CTA, TB, TC, or TD with a large excess of $NaBH_4$ at room temperature (Chart 3). The resulting open compounds (RH-CTA, 82%; RH-TB, 75%; RH-TC, 62%; and RH-TD, 47%) are pentapeptide derivatives in which the original peptide bond linking amino acid fragments 2 and 3 is hydrolyzed and the carbonyl group of amino acid 2 is reduced to primary alcohol. Epimerization at

Chart 1. Structures of Teicoplanin (CTA), Its Pseudoaglycons (TB, TC), and Aglycon (TD)

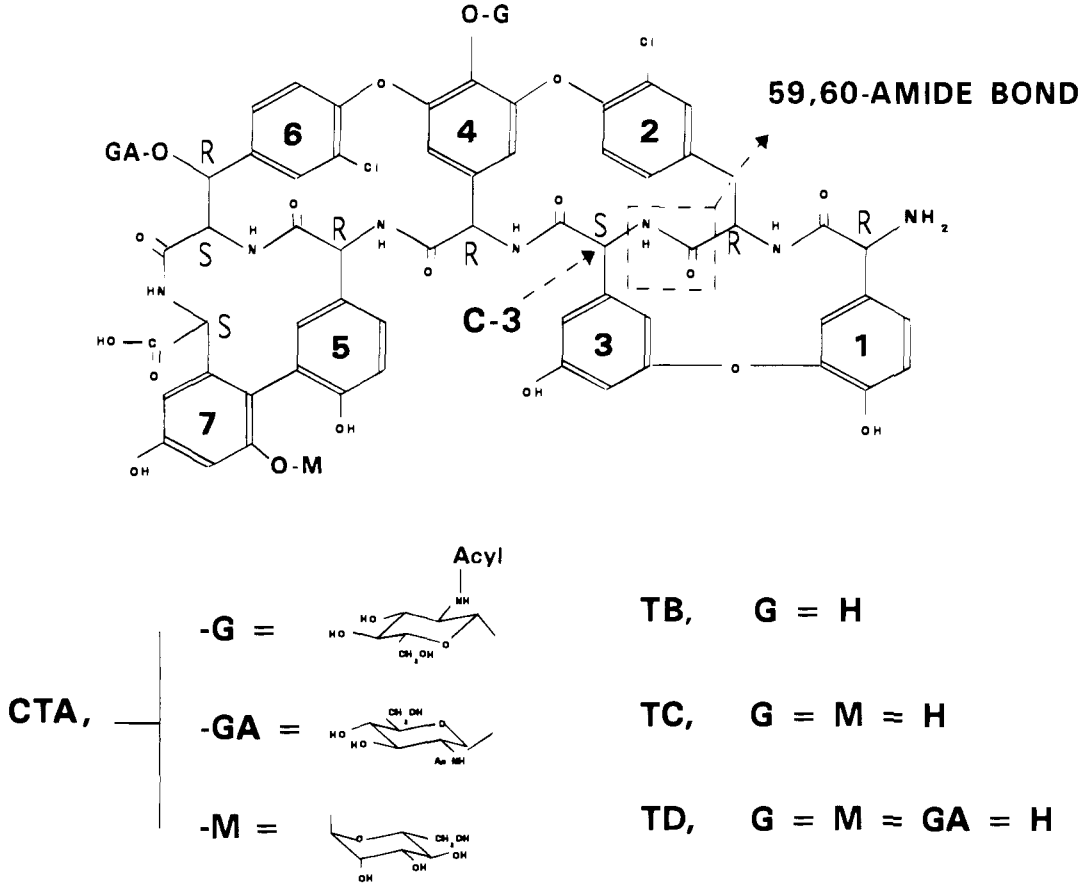

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Chart 2. Peptide Binding Interaction in Teicoplanin

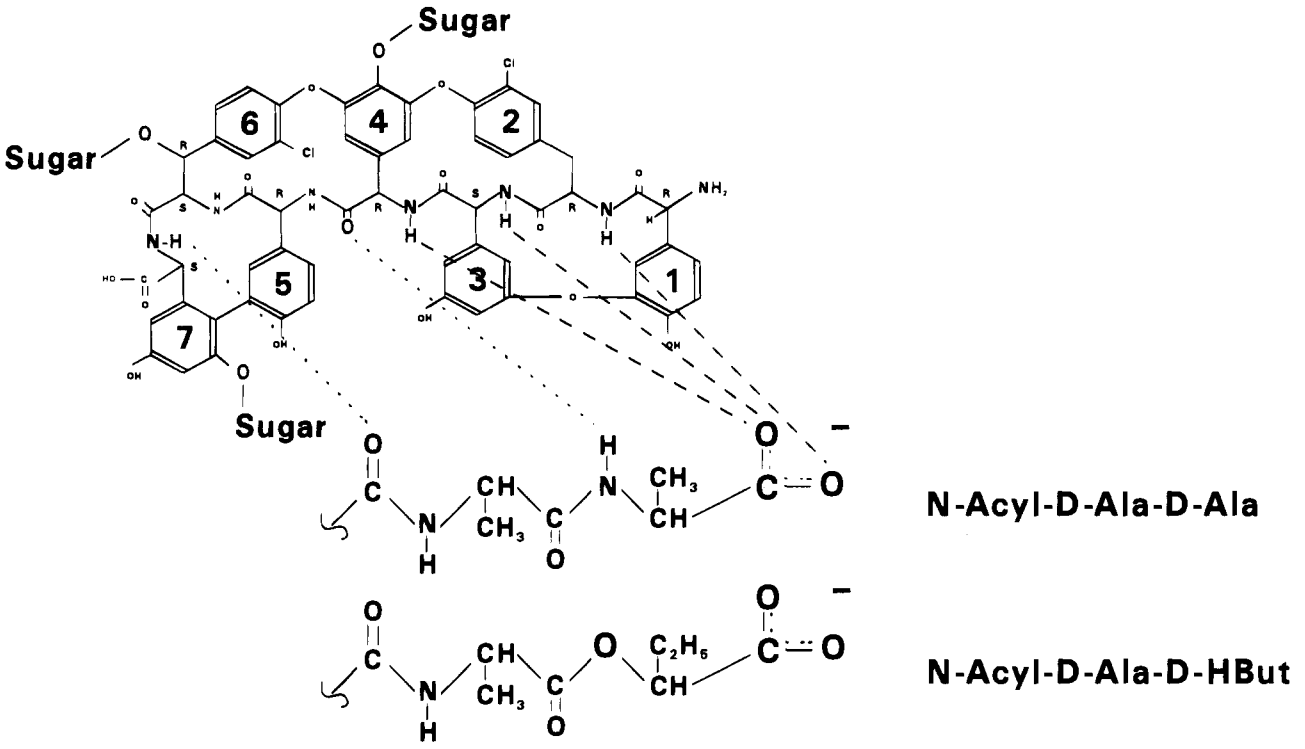
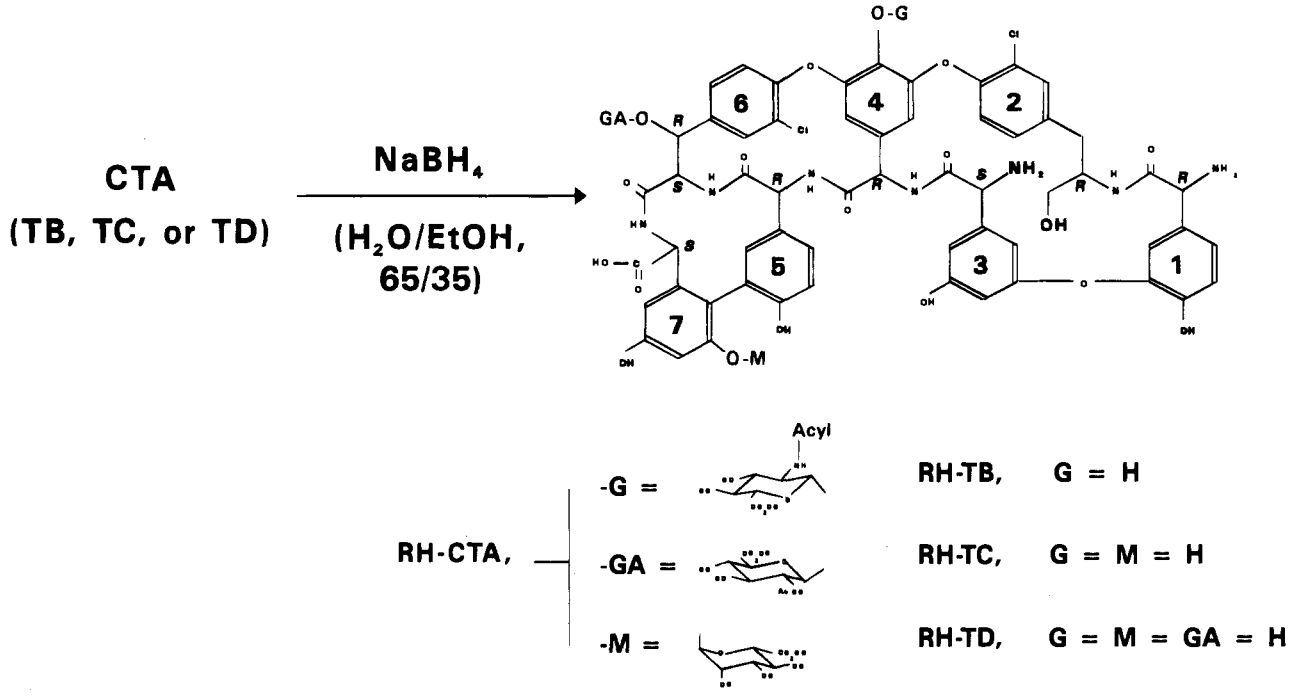


Chart 3. Synthesis of RH-Teicoplanins



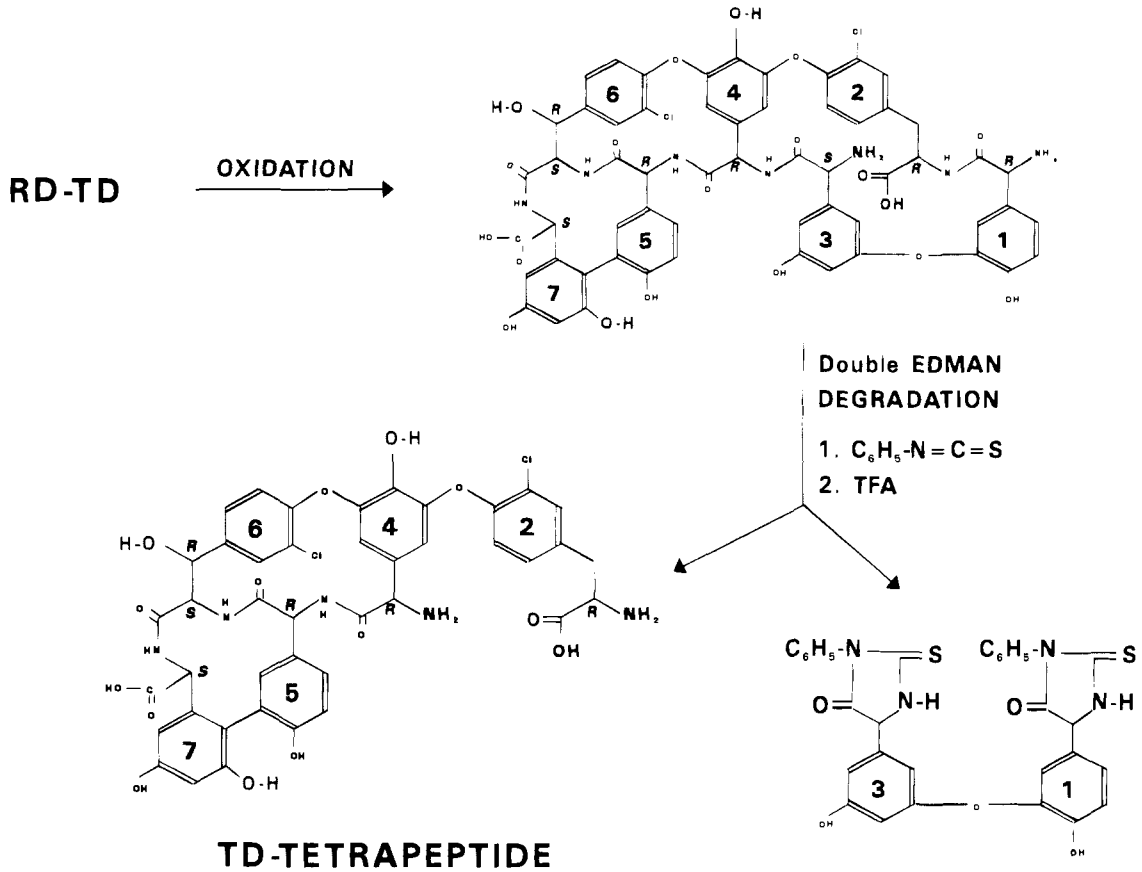
C-3,[8] which generally occurs in teicoplanin antibiotics under prolonged basic treatment, was only observed with TD under the above reaction conditions.[9] The structure of the reductive hydrolysis products was determined by 2D NMR spectroscopy and confirmed by FAB MS spectrometry.

Further, key synthons (teicoplanin derived tetrapeptides), suitable for the synthesis of modified glycopeptides differing in amino acids 1 and 3, can be prepared by simultaneous removal of amino acids 1 and 3 from RH-teicoplanins. This is accomplished by a double Edman degradation after oxidation of the resultant hydroxymethyl group in fragment 2 (Chart 4).[10]

Following the same procedure, different tetrapeptides can be obtained from other natural glycopeptides of the dalbaheptide group.[11] These intermediates will be used as substrates for a semisynthetic modification program aimed at better understanding and overcoming the emerging glycopeptide antibiotic resistance in pathogenic bacteria.

Acknowledgment. Dr. Jürgen Kettenring (NMR spectra) and Dr. Károly Vékey (FAB MS spectra) are gatefully acknowledged for the structure elucidation of RH-teicoplanins.

Chart 4. Synthetic Approach to Tetrapeptide Derived from TD



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(7) All naturally occurring glycopeptides are highly modified linear peptides made up of seven amino acids, five of which are aryl amino acids and are common to all members of the group. The remaining two amino acids in critical positions 1 and 3 are just those which mainly differentiate these molecules. Although they can be both aliphatic or aromatic, or one aliphatic and one aromatic, the structural differences among them are limited so that only four families of natural glycopeptides are currently known.

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(9) Although these reactions are all generally completed within 2 h, the different conversion rate (CTA > TB > TC > TD) seems to play a role in the epimerization of teicoplanin antibiotics. Epimers, once formed, are not susceptible to reductive hydrolysis under treatment with $NaBH_4$. This is likely the reason why RH-TD is obtained with relatively low yields with respect to the other RH-teicoplanins. Also the presence of the sugar moieties seems to favour this transformation while preventing epimerization.

(10) This CH_2OH might be oxidized to COOH after protection of sugar and phenolic hydroxyl groups of RH-CTA, RH-TB, and RH-TC. Its oxidation in RH-TD should only require protection of phenolic functions. In any case, a preliminary protection of the free amino groups would be necessary.

(11) The reductive hydrolysis of the peptide bond between amino acids 2 and 3 was also assessed with a number of these compounds.