Selective Cleavage of Vancosamine, Glucose, and *N*-Methyl-leucine from Vancomycin and Related Antibiotics

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Reaction of vancomycin and related antibiotics with trifluoroacetic acid at −15 °C for 40 h selectively cleaved the amino sugar vancosamine; the second sugar, glucose, was removed by reaction with trifluoroacetic acid at 50 °C for 3 h, and the *N*-terminal leucine moiety was cleaved using the Edman degradation procedure.

Vancomycin is an important antibiotic and is the drug of choice for treatment of serious Gram-positive infections, especially those caused by methicillin resistant *Staphylococcus aureus*. As part of a continuing study to elucidate the contributions of the various structural groups of the vancomycin molecule to its antibacterial activity[1,2] and to understand better its mechanism of action, we examined the selective and sequential cleavage of the two sugars, vancosamine and glucose, which constitute the disaccharide moiety of vancomycin and related antibiotics (Scheme 1). Finally, the *N*-methyl-leucine was removed by Edman degradation.[3]†

A compound thought to be aglucovancomycin was prepared by treating vancomycin with hot hydrochloric acid.[4] H.p.l.c. showed this product to be a mixture. Pure aglucovancomycin, isolated by preparative h.p.l.c., has *in vitro* antibacterial activity comparable to that of vancomycin.

The method for selective cleavage of vancosamine or vancosaminyl-*O*-glucose from A51568,[5] vancomycin, and M43A[6] consisted of treating the above antibiotics with trifluoroacetic acid (TFA) at controlled temperature. Reaction of antibiotics (**1**), (**2**), and (**3**) with TFA at about −10 to −20 °C removed the amino sugar vancosamine, and the desvancosamine derivatives (**4**), (**5**), and (**6**), respectively, were obtained as the major products.‡ Only minor amounts of aglucones were formed. At higher temperatures (20—70 °C), reaction of antibiotics (**1**), (**2**), and (**3**) or their corresponding desvancosamine derivatives (**4**), (**5**), and (**6**) with TFA afforded the aglucones (**7**), (**8**), and (**9**), respectively.

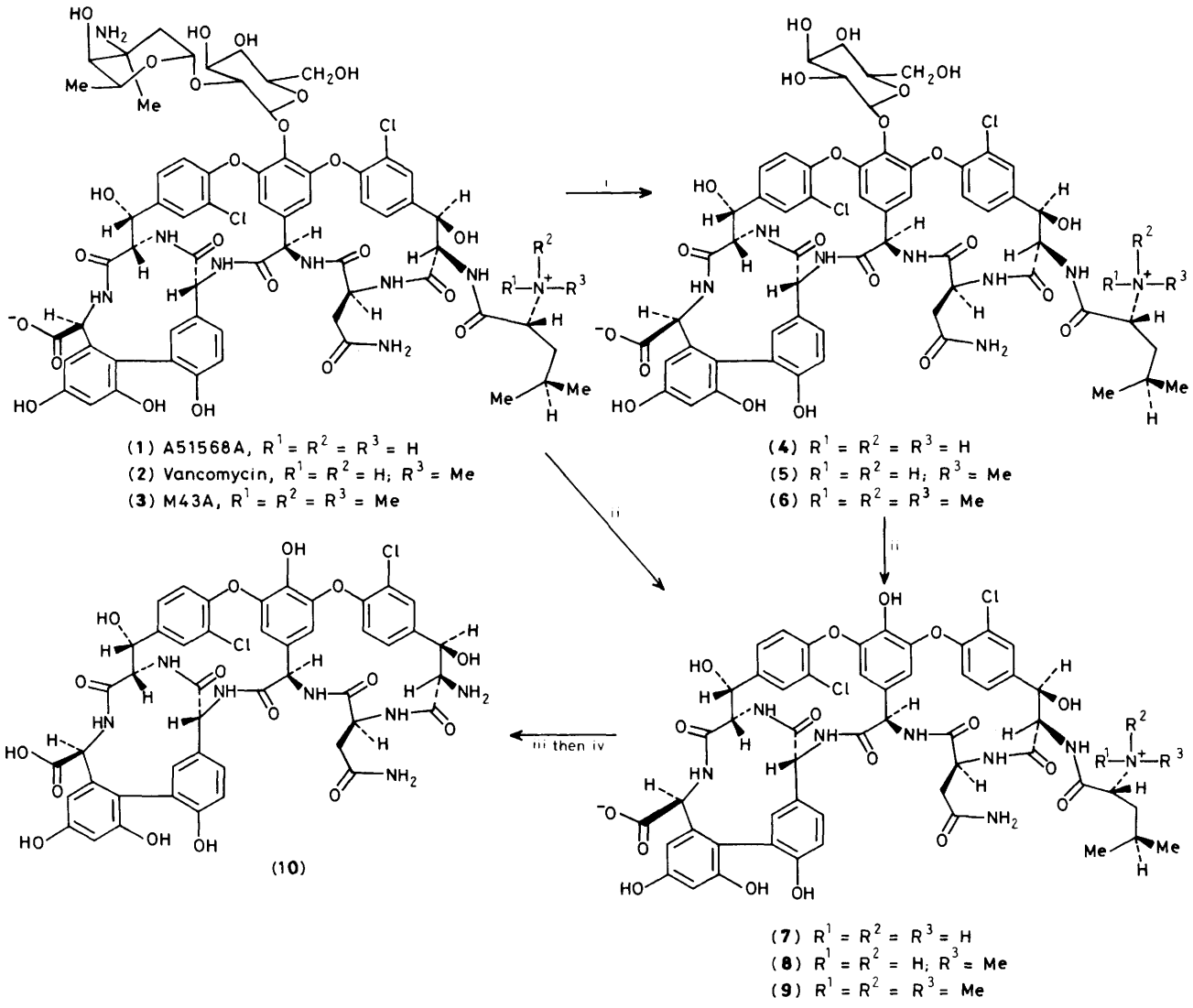
Both the desvancosamine analogues (**4**), (**5**), and (**6**) and the aglucones (**7**), (**8**), and (**9**) retain a substantial amount of the antibacterial activity. The desvancosamine compounds (**4**), (**5**), and (**6**) are 2—4 times less active than the parent antibiotics. The aglucones (**7**), (**8**), and (**9**) have similar activity to that of (**1**), (**2**), and (**3**), respectively.

Finally, reaction of aglucovancomycin (**8**) in 50% aqueous pyridine with phenyl isothiocyanate at pH 8.6 and at 50 °C for 1 h afforded the hexapeptide (**10**) in 61% yield. Compound (**10**) was devoid of antibacterial activity.

Even though the exact conformation of vancomycin and the stereochemical details of the binding of the D-Ala-D-Ala carboxy terminus of UDP-*N*-acetylmuramylpentapeptide to the *N*-terminal *N*-methyl-leucine of vancomycin are not yet known,[7] it is not surprising that the removal of the crucial *N*-methyl-leucine leads to complete destruction of the antibacterial activity of vancomycin. Details of the structure–activity

† After the completion of our work on the Edman degradation of aglucovancomycin, Williams and co-workers reported the Edman degradation of vancomycin.[3]

‡ All new compounds described in this communication gave satisfactory fast-atom-bombardment mass and ^{1}H n.m.r. spectra.



Scheme 1 *Reagents and conditions:* i, TFA at −15 °C for 40 h; ii, TFA at 50 °C for 3 h; iii, excess of PhNCS at pH 8.6 adjusted with 0.2 M NaOH at 50 °C for 2 h.

Table 1. Fast-atom-bombardment mass spectral data and h.p.l.c. retention times of vancomycin and related antibiotics and their degradation products.

Compound	*m/z* (M^+ + H)	R_t/min[a]
(**1**) A51568A	1434	6.48
(**4**) Desvancosamine A51568A	1291	10.14
(**7**) Agluco A51568A	1129	20.62
(**2**) Vancomycin	1448	7.58
(**5**) Desvancosamine vancomycin	1305	11.52
(**8**) Aglucovancomycin	1143	21.19
(**3**) M43A	1476	13.24
(**6**) Desvancosamine M43A	1333	15.42
(**9**) Agluco M43A	1171	23.03
(**10**) Hexapeptide of aglucovancomycin	1016	17.37

[a] The analytical h.p.l.c. system was as follows: column: Beckman Ultrasphere (5μ particle size) ODS, 25 cm; mobile phase: solvent A: MeCN/TEAP (5:95); solvent B: MeCN/TEAP (2:3); (TEAP = 0.5% aqueous triethylamine adjusted to pH 3 with conc. phosphoric acid); Gradient: 9% B to 70% B during 40 min; then hold for 5 min at 70% B; flow rate: 1.0 ml per min; detection: u.v. at 254 nm.

relationship of the various structural groups of the vancomycin molecule with antibacterial activity will be published elsewhere.[8]

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References

1 R. Nagarajan, A. A. Schabel, J. L. Occolowitz, F. T. Counter, and J. L. Ott, *J. Antibiotics*, submitted for publication.
2 R. Nagarajan, A. A. Schabel, J. L. Occolowitz, F. T. Counter, J. L. Ott, and A. M. Felty-Duckworth, *J. Antibiotics*, submitted for publication.
3 P. M. Booth, D. J. M. Stone, and D. H. Williams, *J. Chem. Soc., Chem. Commun.*, 1987, 1694.
4 F. J. Marshall, *J. Med. Chem.*, 1965, **8**, 18.
5 A. H. Hunt, G. G. Marconi, T. K. Elzey, and M. M. Hoehn, *J. Antibiotics*, 1984, **37**, 917.
6 R. Nagarajan, H. M. Higgens, M. M. Hoehn, A. H. Hunt, N. D. Jones, K. E. Merkel, K. H. Michel, and J. L. Occolowitz, *J. Am. Chem. Soc.*, submitted for publication.
7 J. C. T. Barna and D. H. Williams, *Ann. Rev. Microbiol.*, 1984, **38**, 339.
8 R. Nagarajan, manuscript in preparation.