A Synthetic Analogue of the Carboxylate-binding Pocket of Vancomycin

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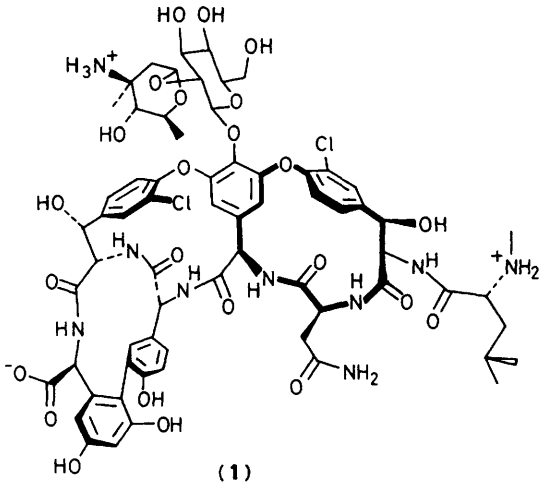
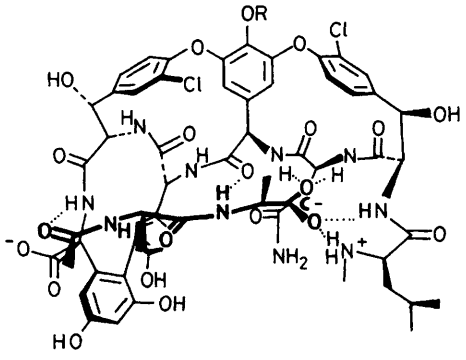
A synthetic analogue of the right-hand ring of vancomycin, the region in the antibiotic which contains the pocket for binding to the terminal carboxylate of D-Ala–D-Ala containing peptides, has been prepared and shows structural and spectroscopic similarity to the natural molecule.

Vancomycin is an antibiotic, isolated from *Streptomyces orientalis*, with bactericidal activity against gram-positive Staphylococcus and Streptococcus strains.[1] Recently there has been increasing interest in this and related antibiotics owing to the novelty of their structures and the fact that no naturally occurring strains of bacteria are known to be resistant to vancomycin.[1,2] A combination of chemical degradation, *X*-ray crystallography, and elegant n.m.r. experiments have established the structure of vancomycin as (**1**), containing a tricyclic heptapeptide with unusual biphenyl and triphenyl diether components.[3] The antibiotic functions by strongly binding to the terminal D-Ala–D-Ala residues of mucopeptides involved in bacterial cell wall biosynthesis. Williams has proposed[4] an active complex (**2**) in which vancomycin forms six hydrogen bonds to the acylated D-Ala–D-Ala unit. The primary interaction is to the terminal carboxylate of Ac–D-Ala–D-Ala where one ammonium and three amide groups act as hydrogen bonding partners within a hydrophobic pocket formed by the diphenyl ether and acetamide side chain.[1] Two further hydrogen bonds occur to the amide NH and CO groups on the dipeptide fragment. Binding is relatively substrate specific, tolerating only minor changes in the terminal dipeptide composition, and strongly stereospecific, with L-amino acid dipeptides not bound at all.[5] Thus, vancomycin represents an interesting and pharmaceutically important model for protein–substrate interactions in general.[1]

The property of substrate binding to synthetic molecular receptors is a continuing goal of host–guest chemistry.[6] However few of the reported hosts model at all closely biochemical receptor sites. Vancomycin contains a substrate-binding site of a size tractable enough to be approached by synthesis. We have initiated research aimed at the construction of synthetic hosts based on the vancomycin framework. Such systems are of interest because: (i) they should provide a



(1)

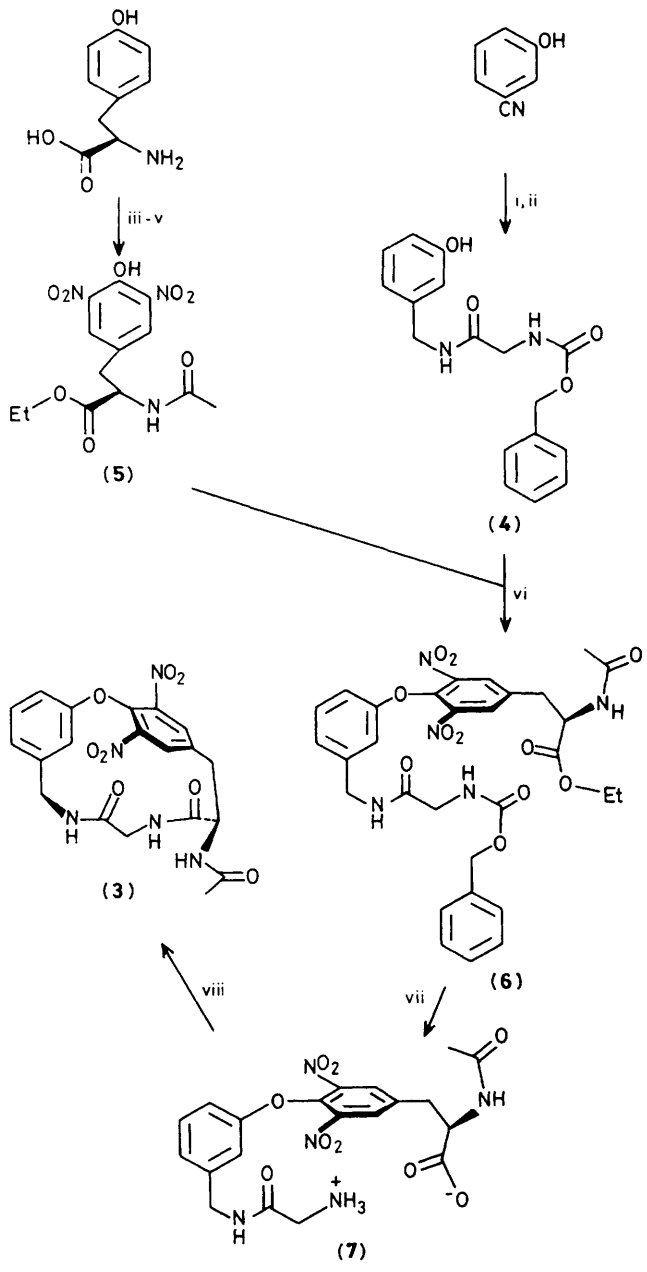


(2)

new class of receptors for peptide and other carboxylate anion derivatives and (ii) new potential antibiotics with different transport and toxicity properties to vancomycin may be developed.

Our preliminary strategy is to reduce the complexity of (**1**) while retaining key components of the binding region. For example, the carbohydrate portion and benzylic hydroxy groups in (**1**) are distant from the binding region and do not appear to interact significantly with the substrate.[1] In fact most of the key residues are found in the right-hand portion of the antibiotic. The right-hand diphenyl ether dipeptide ring [(**1**) bold lines] contains a hydrophobic pocket and three hydrogen-bonding amide NH moieties for the carboxylate group. Minor additions of an *N*-methyl-leucine residue at the *N*-terminus and carboxamide group at the central phenylglycine would provide five of the six H-bonding sites for D-Ala–D-Ala binding in a simple monocyclic molecule. This ring system will form the basis of our development of synthetic vancomycin-like receptors. We herein report the synthesis of the basic ring skeleton (**3**) and its structural and spectroscopic similarity to the equivalent portion of the natural product [(**1**) bold lines].

The synthesis of (**3**) was carried out as shown in Scheme 1.† This involves an efficient and readily modified convergent route starting from 3-cyanophenol and D- or L-tyrosine. *N*-Benzyloxycarbonylglycine was coupled to 3-aminomethylphenol with complete regioselectivity using *N*-methyl-2-chloropyridinium iodide. The second component (**5**) was obtained from tyrosine *via* nitration, acetylation, and esterification.[7] Tosylation of (**5**) at 80 °C in pyridine followed by addition of (**4**) afforded diphenyl ether (**6**) in good yield with little racemization.‡ Treatment of (**6**) with trifluoroacetic acid (TFA)–HCl (2:1) over four days cleanly removed the benzyloxycarbonyl and ethyl ester protecting groups to form amino acid (**7**) {^{1}H n.m.r. [$^2H_6$]dimethylsulphoxide (DMSO) 8.88 (b, 1H, NHCO), 8.37(s, 2H, NO_2ArH), 8.35(b, 1H, NHCO), 8.15(b, 3H, NH_3), 7.27 (t, *J* 8 Hz, 1H, NCH_2Ar5H),



Scheme 1. *Reagents:* i, BH_3–THF; ii, *N*-benzyloxycarbonylglycine, *N*-methyl-2-chloropyridinium iodide, Et_3N; iii, HNO_3, H_2SO_4; iv, Ac_2O; v, EtOH, $MeC_6H_4SO_2OH$; vi, $MeC_6H_4SO_2Cl$, pyridine; vii, HCl, TFA; viii, diphenylphosphoryl azide.

† All new compounds gave satisfactory spectroscopic and mass spectral data consistent with their structures.

‡ In addition to activating tyrosine towards nucleophilic attack, the nitro groups will, in future analogues, allow introduction of halide or other substituents to vary the steric and electronic properties of the pocket.

7.14(d, *J* 8 Hz, 1H, NCH_2Ar6H), 6.92(s, 1H, NCH_2Ar2H), 6.66(d, *J* 8 Hz, 1H, NCH_2Ar4H), 4.56(m, 1H, NCHCO), 4.33(d, *J* 6 Hz, 2H, NCH_2Ar), 3.62(m, 2H, NCH_2CO), 3.32(dd, *J* 13, 5 Hz, 1H, $CHCH_2Ar$), 3.04(dd, 1H, *J* 13, 9 Hz, $CHCH_2Ar$), and 1.80(s, 3H, MeCO)}. The final cyclization step was accomplished using diphenylphosphoryl azide[8] in dimethylformamide (DMF) (0 °C, 4 days) to provide, after silica gel preparative layer chromatography, cyclic diphenyl ether (**3**) {^{1}H n.m.r. [$^2H_6$]DMSO, 8.44(d, *J* 2 Hz, 1H, NO_2ArH), 8.10(d, *J* 2 Hz, 1H, NO_2ArH), 8.55, 8.35, 7.76(3m, 3H, NHCO), 7.32(t, *J* 8 Hz, 1H, NCH_2Ar5H), 7.09(d, 1H, NCH_2Ar4H), 6.96(d, *J* 8 Hz, 1H, NCH_2Ar6H), 5.84(s, 1H, NCH_2Ar2H), 4.78(m, 1H, NCHCO), 4.48, 3.95(2m, 2H, NCH_2Ar), 3.95, 3.42(2m, 2H, NCH_2CO), 3.25, 2.85(2m, 2H, CH_2CH), and 1.86(s, 3H, MeCO)}.

A key structural marker in the vancomycin antibiotics is the strongly upfield shifted aromatic proton signals of the central phenylglycine residue.[1,4] A comparison of the aromatic regions in the ^{1}H n.m.r. spectra of (**7**) and (**3**) (both in [$^2H_6$]-DMSO) shows a dramatic upfield shift (>1 p.p.m.) of the benzylamine 2-H signal, from δ 6.92 in (**7**) to δ 5.84 in (**3**). This is due to the peptide ring which restricts the motion of the benzylamine unit and forces its 2-proton to lie under the dinitrophenyl group. The equivalent proton in vancomycin is found at δ 5.65[9] and in ristocetin at δ 5.85.[10]

The above results confirm that cyclization has taken place and that synthetic analogue (**3**), while missing the left-hand portion of vancomycin, nonetheless shows a structural similarity to the carboxylate binding pocket of the natural antibiotic. Modification of our synthetic route to allow incorporation of the N-terminal *N*-methyl-leucine and central carboxamide residues should provide more accurate analogues capable of mimicking both the structural and functional (*i.e.* substrate binding) properties of vancomycin.

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References

1 For recent reviews see D. H. Williams, V. Rajananda, M. P. Williamson, and G. Bojesen, in 'Topics in Antibiotic Chemistry,' ed. P. Sammes, vol. 5, Part B, Ellis Horwood, Chichester, 1980, p. 123; M. P. Williamson, D. H. Williams, and S. J. Hammond, *Tetrahedron*, 1984, **40**, 569.

2 J. W. Lightbown, in 'Experimental Chemotherapy,' vol. III, eds. R. J. Schitzer and F. Hawkins, Associated Press, London, 1964, p. 271.

3 G. M. Sheldrick, P. G. Jones, O. Kennard, D. H. Williams, and G. A. Smith, *Nature* (*London*), 1978, **271**, 223; D. H. Williams and J. R. Kalman, *J. Am. Chem. Soc.*, 1977, **99**, 2768; C. M. Harris, H. Kopecka, and T. M. Harris, *ibid.*, 1983, **105**, 6915; M. P. Williamson and D. H. Williams, *ibid.*, 1981, **103**, 6580.

4 D. H. Williams and D. W. Butcher, *J. Am. Chem. Soc.*, 1981, **103**, 5697.

5 M. Nieto and H. R. Perkins, *Biochem. J.*, 1971, **123**, 789.

6 See for example R. M. Kellogg, *Top. Curr. Chem.*, 1982, **101**, 111; A. D. Hamilton in 'Comprehensive Heterocyclic Chemistry,' ed. A. R. Katritzky and C. W. Rees, Pergamon Press, London, 1984, vol. 7, p. 731.

7 J. R. Chalmers, G. T. Dickson, J. Elks, and B. A. Hems, *J. Chem. Soc.*, 1949, 3424.

8 S. F. Brady, S. L. Varga, R. M. Freidinger, D. A. Schwenk, M. Mendlowski, F. W. Holly, and D. F. Veber, *J. Org. Chem.*, 1979, **18**, 3101.

9 D. H. Williams and J. R. Kalman, *J. Am. Chem. Soc.*, 1977, **99**, 2768.

10 J. R. Kalman and D. H. Williams, *J. Am. Chem. Soc.*, 1980, **102**, 897.