Solid-phase synthesis of a putative heptapeptide intermediate in vancomycin biosynthesis

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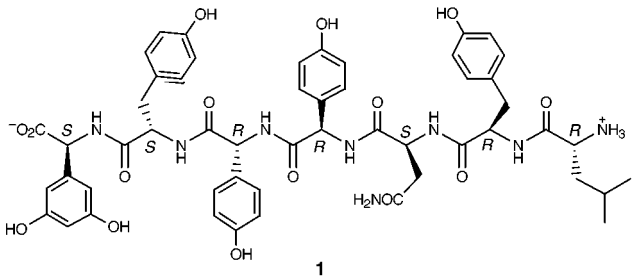
The solid-phase synthesis was completed of the heptapeptide D-Leu-D-Tyr-L-Asn-Hpg-Hpg-L-Tyr-Dhpg, a putative intermediate in vancomycin biosynthesis, where Dhpg = (*S*)-3,5-dihydroxyphenylglycine and Hpg = (*R*)-4-hydroxyphenylglycine, using 2-chlorotrityl resin, benzyl side-chain protecting groups for the aromatic residues, and Alloc chemistry for chain elongation; the heptapeptide was obtained in 16% yield with the correct relative and absolute configuration.

Important progress has been made recently in elucidating the biosynthetic pathway to glycopeptide antibiotics of the vancomycin family. The nucleotide sequence of a gene cluster from the chloroeremomycin-producing microorganism revealed a large number of open reading frames encoding putative peptide synthetases, halogenases, hydroxylases and P450-like cytochromes, amongst many others.[1,2] Similar genes have also been cloned and sequenced from the balhimycin producer,[3] and disruption of a P450-like gene in this organism has provided the first confirmation of its role in the biosynthesis of the glycopeptide antibiotic, as well as indicating a likely role in a phenol coupling reaction.[4] Presently, however, the sequence of steps in glycopeptide antibiotic biosynthesis is largely unknown. Since most, if not all, the biosynthetic genes are now available for study, progress in this area should be facilitated by access to putative biosynthetic intermediates. We report here a convenient solid-phase synthesis of the heptapeptide **1**, which



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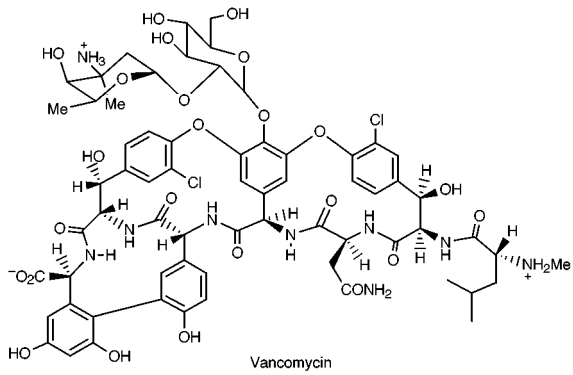
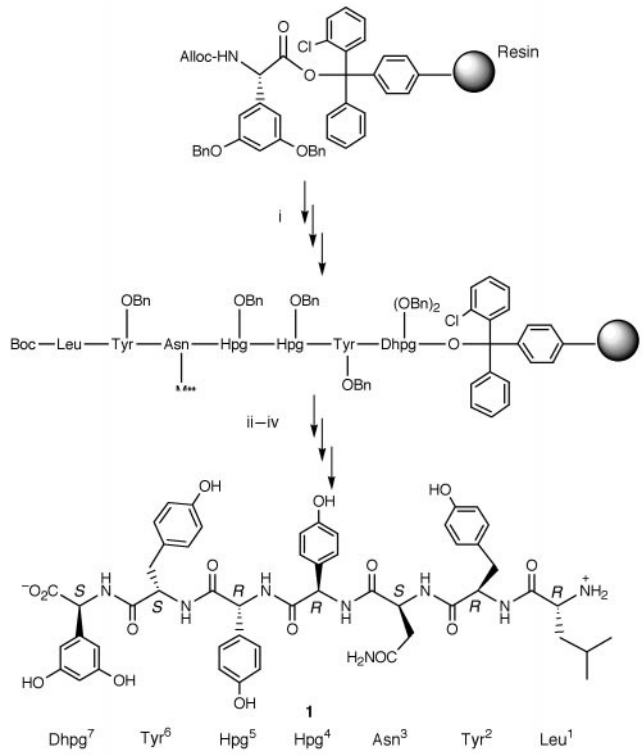


Vancomycin

may be considered[5] a possible product of the vancomycin peptide synthetase, and hence a potential substrate of downstream enzymes such as the halogenases, hydroxylases, or those catalyzing phenol coupling reactions.

A major concern in planning the solid-phase synthesis of **1** was the sensitivity of several amino acids to epimerization during chain assembly and/or subsequent deprotection steps, when using standard Fmoc or tBoc chemistry. We chose, therefore, to investigate Alloc chemistry for chain elongation, since deprotection of the N-terminus at each step of chain assembly can be performed under almost neutral conditions.[6] The amino acids (*R*)-4-hydroxyphenylglycine (Hpg) and (*S*)-3,5-dihydroxyphenylglycine (Dhpg) were synthesized by standard methods,[5] and protected on N^{α} with the Alloc group, and in the aromatic side chains with benzyl ethers. For the synthesis of **1**, (*S*)-Alloc-Dhpg$(OBn)_2$-OH was first coupled to 2-chlorotrityl chloride resin[7] in CH_2Cl_2 with NMM† as base. The peptide chain was then elaborated using for each coupling HATU/HOAt (4 equiv.) and the required amino acid derivative (4 equiv.) in DMF. For Alloc removal,[8] $Pd(PPh_3)_4$ and $PhSiH_3$ were used, as outlined in Scheme 1.

Upon completion of the solid-phase synthesis, the protected heptapeptide was first cleaved from the resin, and the N-terminal Boc and side-chain protecting groups were then



Scheme 1 *Reagents and conditions*: i, for coupling (*R*)-Alloc-Hpg(OBn)-OH, (*R*)- and (*S*)-Alloc-Tyr(OBn)-OH, L-Alloc-Asn(Mtt)-OH, or D-Boc-Leu-OH (4 equiv.), HATU and HOAt (each 4 equiv.), NMM, DMF, and for Alloc deprotection $Pd(PPh_3)_4$ (0.5 equiv.) and $PhSiH_3$ (30 equiv.) in CH_2Cl_2; ii, CF_3CH_2OH–AcOH–CH_2Cl_2 (1 : 1 : 3); iii, TFA–CH_2Cl_2 (1 : 1) + 5% Pr^i_3SiH, 0 °C, 2 h; iv, TFA, thioanisole (3 : 1), 20 °C, 3 h.

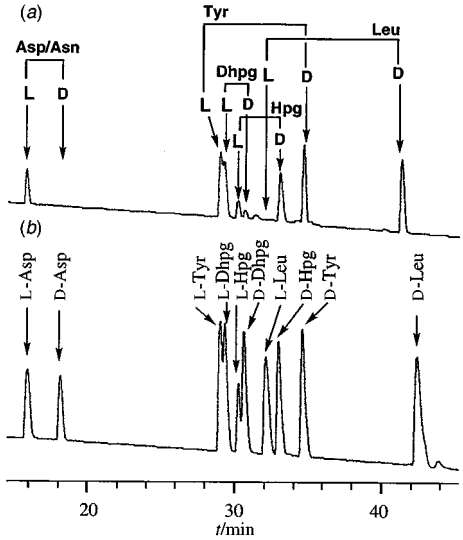


Fig. 1 HPLC chromatograms (Waters C_{18} column, 5 μm, 100 Å, 3.9 × 100 mm, flow rate: 1 ml min^{-1}, gradient, 5–100% MeCN in 0.1 M ammonium acetate buffer pH 7.4, over 130 min) showing (*a*) derivatives formed by reaction of *N*α-(2,4-dinitro-5-fluorophenyl)-L-valinamide with the amino acid hydrolysate of peptide **1** (the identities of the peaks are indicated) and (*b*) the standards prepared by reacting each optically pure amino acid with the same reagent (the identity of each peak was confirmed by MS and ^{1}H NMR spectroscopy).

removed in two steps (Scheme 1). Analysis of the crude product by HPLC revealed three main products in a ratio of *ca.* 6:2:1. The predominant product was identified by spectroscopic data as the required heptapeptide,‡ which was isolated by preparative HPLC (Vydac C_{18} 218TP1010, 10 μm, 300 Å, 10 × 250 mm, flow rate: 5 ml min^{-1}, gradient 10–40% MeCN in water + 0.1% TFA over 25 min) in *ca.* 16% overall yield. Due to difficulty in their purification, the minor products were not investigated in detail, although their mass spectra indicated the same mass as for **1**. Model studies (not described here) indicated that in particular the Hpg residues are susceptible to partial epimerization during the solid-phase synthesis.

In order to prove that the main product of the synthesis has the desired configuration, it was hydrolysed with 6 M HCl at 115 °C, under Ar, in the presence of phenol. The amino acids were immediately reacted with the modified Marfey's reagent *N*α-(2,4-dinitro-5-fluorophenyl)-L-valinamide[9] and analyzed by HPLC. Conditions were used that allowed the resolution on a C_{18} reversed phase column of a mixture of D- and L-Asp, D- and L-Tyr, D- and L-Leu, D- and L-Hpg and D- and L-Dhpg (see Fig. 1), each as a derivative with the modified chiral Marfey's reagent. In this way, the amino acid hydrolysate was seen to consist of L-Asp and D-Leu, of D- and L-Tyr, and predominantly of L- (rather than D-) Dhpg and D- (rather than L-) Hpg. This corresponds to the expected constitution of the heptapeptide **1**.

The solid-phase synthesis reported here allows the convenient preparation of **1** in multi-milligram amounts, as required for enzymatic studies of vancomycin biosynthesis. The method also appears amenable to parallel synthesis methods, that might allow the production of small combinatorial libraries of related peptides as tools to study the substrate specificities of these enzymes.

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Notes and references

† Abbreviations: HATU, *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate; HOAt, 1-hydroxy-7-azabenzotriazole; Mtt, 4-methyltrityl; NMM, *N*-methylmorpholine.

‡ *Spectral data* for **1**: *m/z* (ESI) 1035.5 $[M + H]^+$, 538 $[M + H + K]^{2+}$, 518.5 $[M + 2H]^{2+}$; $\delta_H CD_3OD–H_2O$ 1:1, *ca.* pH 5; 600 MHz; internal TSP δ = 0; with presaturation of the water resonance; 300 K) 8.82 (weak br s, HN Tyr^2), 8.29 (d, HN Hpg^4), 8.21 (d, HN Hpg^5), 8.16 (d, HN Asn^3), 8.15 (d, HN Tyr^6), 8.10 (d, HN Dhpg^7), 7.36 (s, HD^E Asn^3), 7.24 (d, H2,6 Hpg^4), 7.00 (d, H2,6 Tyr^2), 6.97 (d, H2,6 Hpg^5), 6.83 (d, H3,5 Hpg^4), 6.78 (d, H3,5 Tyr^2), 6.76 (d, H2,6 Tyr^6), 6.74 (d, H3,5 Hpg^5), 6.64 (s, HD^Z Asn^3), 6.55 (d, H3,5 Tyr^6), 6.48 (d, H2,6 Dhpg^7), 6.27 (t, H4 Dhpg^7), 5.50 (d, HA Hpg^4), 5.28 (d, HA Hpg^5), 5.02 (d, HA Dhpg^7), 4.67 (m, HA Asn^3), 4.61 (m, HA Tyr^6), 4.29 (m, HA Tyr^2), 3.87 (t, HA Leu^1), 2.97 (dd, HB2 Tyr^6), 2.94 (dd, HB2 Tyr^2), 2.88 (dd, HB1 Tyr^2), 2.70 (dd, HB1 Tyr^6), 2.51 (dd, HB2 Asn^3), 2.47 (dd, HB1 Asn^3), 1.50 (m, HG Leu^1), 1.45 (m, HB Leu^1), 0.85 (d, HD2 Leu^1), 0.81 (d, HD1 Leu^1). The assignments were made by 2D TOCSY, DQF-COSY and NOESY experiments. The connectivity within each spin system was confirmed by intra-residue *J* couplings and NOEs, and the amino acid sequence by inter-residue $d_{\alpha N}$ (*i*, *i* + 1) NOEs.

1 A. M. A. vanWageningen, P. N. Kirkpatrick, D. H. Williams, B. R. Harris, J. K. Kershaw, N. J. Lennard, M. Jones, S. J. M. Jones and P. J. Solenberg, *Chem. Biol.*, 1998, **5**, 155.
2 P. J. Solenberg, P. Matsushima, D. R. Stack, S. C. Wilkie, R. C. Thompson and R. H. Baltz, *Chem. Biol.*, 1997, **4**, 195.
3 S. Pelzer, R. Sussmuth, D. Heckmann, J. Recktenwald, P. Huber, G. Jung and W. Wohlleben, *Antimicrob. Agents Chemother.*, 1999, **43**, 1565.
4 R. D. Sussmuth, S. Pelzer, G. Nicholson, T. Walk, W. Wohlleben and G. Jung, *Angew. Chem., Int. Ed.*, 1999, **38**, 1976.
5 K. C. Nicolaou, C. N. C. Boddy, S. Bräse and N. Winssinger, *Angew. Chem., Int. Ed.*, 1999, **38**, 2096.
6 For a review, see F. Guibé, *Tetrahedron*, 1998, **54**, 2967.
7 K. Barlos, O. Chatzi, D. Gatos and G. Stavropoulos, *Int. J. Pept. Protein Res.*, 1991, **37**, 513.
8 N. Thieriet, J. Alsina, E. Giralt, F. Guibé and F. Albericio, *Tetrahedron Lett.*, 1997, **38**, 7275.
9 H. Brückner and C. Keller-Hoehl, *Chromatographia*, 1990, **30**, 621.

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