Solid-Phase Synthesis Using (Allyloxy)carbonyl(Alloc) Chemistry of a Putative Heptapeptide Intermediate in Vancomycin Biosynthesis Containing *m*-Chloro-3-hydroxytyrosine

Preliminary Communication

by **Ernst Freund**, **Francesca Vitali**, **Anthony Linden** and **John A. Robinson***

Institute of Organic Chemistry, University of Zurich, Winterthurerstrasse 190, CH-8057 Zurich

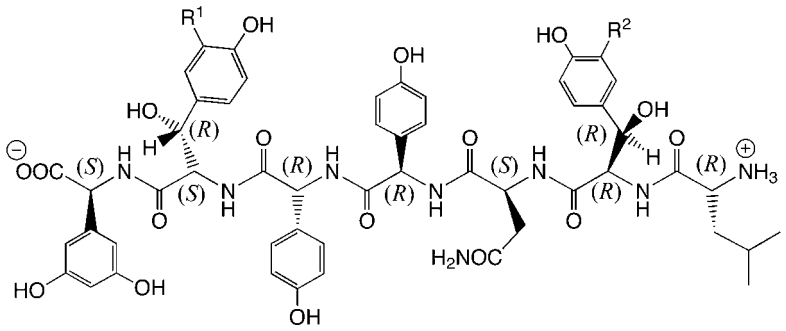
Dedicated to Professor *Albert Eschenmoser* on the occasion of his 75th birthday

A convenient method for the solid-phase synthesis of putative linear heptapeptide intermediates in vancomycin biosynthesis is described, in particular, the heptapeptide D-Leu-Cyt-L-Asn-Hpg-Hpg-Cyt′-Dhpg (Cyt = (2*R*,3*R*)-*m*-chloro-3-hydroxytyrosine, Hpg = (*R*)-2-(*p*-hydroxyphenyl)glycine, Cyt′ = (2*S*,3*R*)-*m*-chloro-3-hydroxytyrosine and Dhpg = (*S*)-2-(3,5-dihydroxyphenyl)glycine). The synthesis was performed on chlorotrityl resin and employed the (allyloxy)carbonyl protecting group for temporary $N(\alpha)$ protection during peptide-chain assembly.

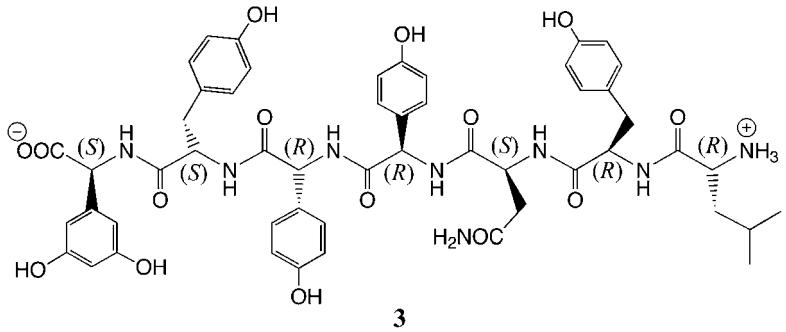
1. Introduction. – Recent studies of glycopeptide antibiotic biosynthesis have led to the cloning of glycosyl transferase genes responsible for the addition of monosaccharides to the vancomycin heptapeptide aglycon [1], from both vancomycin- and chloroeremomycin-producing strains of *Amycolatopsis orientalis.* Also, 72 kb of chromosomal DNA surrounding the glycosyl transferase genes from the chloroeremomycin producer has been sequenced [2]. This revealed 39 putative open reading frames (orfs), including many encoding enzymes likely to be involved in the biosynthesis of the antibiotic. Putative functions were assigned to these biosynthetic genes based on sequence comparisons to enzymes of known function. The genes encoding the peptide synthetase, required for assembly of the heptapeptide core, were easily identified, and more recently, biochemical studies of the recombinant peptide synthetase produced in *E. coli* were reported [3]. Another gene was shown to encode an *N*-methyltransferase, responsible for methylating the N-terminal leucine residue in vancomycin [4].

Of special interest here are the enzymes that catalyze the oxidative cross-linking of the phenolic rings during vancomycin biosynthesis. Three closely linked P450-like orfs were identified in the chloroeremomycin cluster [2], and a similar arrangement of three P450 genes (*oxyA*, *oxyB* and *oxyC*) has been found in the balhimycin producer *Amycolatopsis mediterranei* [5]. Insertional inactivation of these oxygenase gene(s) resulted in a balhimycin mutant which was unable to produce the antibiotic, but, instead, produced the linear heptapeptide **1**, which is possibly the direct product of the balhimycin peptide synthetase [5][6]. Hence, the P450-like orfs appear to be responsible for the oxidative phenol-coupling reactions.

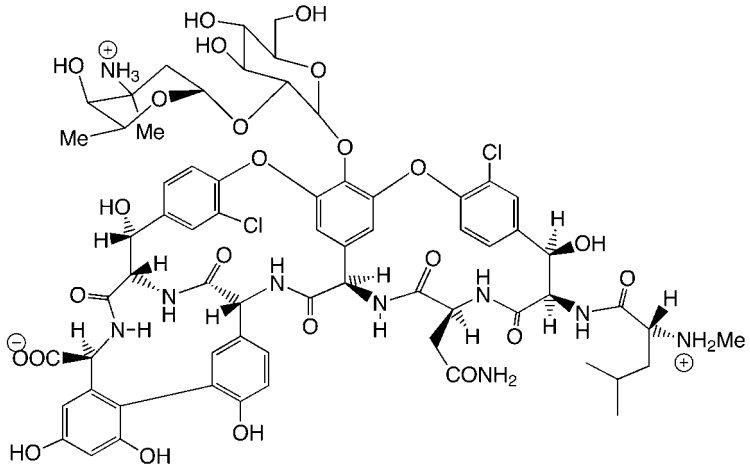
Presently, the biosynthetic route to glycopeptides of the vancomycin family is still largely unknown. In particular, the timing of the phenol-coupling reactions, and the



1 $R^1 = R^2 = Cl$
2 $R^1 = R^2 = H$



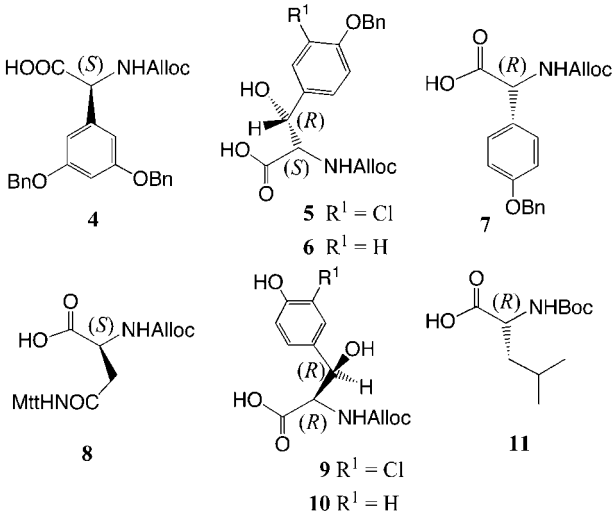
3



Vancomycin

roles and preferred substrates of the P450-like enzymes implicated in catalyzing these steps remain to be defined. To address these questions, access is required to putative heptapeptide substrates such as **1**. In earlier work [7], we reported a convenient solid-phase synthesis of the related heptapeptide **3**. We now show that the solid-phase methods developed earlier may also be used to allow convenient access to heptapeptides such as **1** and **2**.

2. Results and Discussion. – The main concern in planning a solid-phase synthesis of **1** was the sensitivity of several of the constituent amino acids to epimerization. The widely used solid-phase Fmoc- or Boc-chemistries, which require basic or acidic conditions at each step of chain elongation, appeared to be unsuitable for the synthesis. For this reason, we favoured the use of Alloc[1]) chemistry [8], and could show in earlier work that **3** can be prepared in good yield by this methodology [7]. To apply the same approach here, we first set out to synthesize the suitably protected building blocks **4** – **10** (**11** is available commercially).

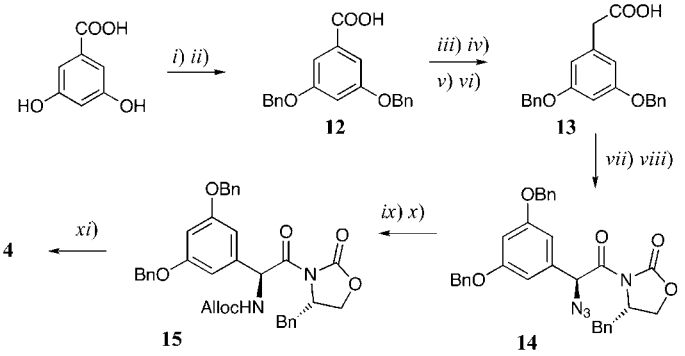


2.1. *Synthesis of Amino-Acid Building Blocks* **4** – **10**. Compound **4** was prepared from (*S*)-3,5-dihydroxybenzoic acid following a procedure of *Evans et al.* [9], as shown in *Scheme 1*. Homologation of acid **12** to **13** was achieved by the *Arndt-Eistert* reaction according to a published procedure [10]. After coupling to the chiral auxiliary (*S*)-4-benzyloxazolidin-2-one, the asymmetric aziridination was performed by the method of *Evans et al.* [9]. After reduction of the azide group to an amine, protection of the amino group, and removal of the chiral auxiliary, the product **4** was obtained in good yield, in crystalline form, with $>98\%$ optical purity (determined by ^{1}H-NMR after coupling **4** to (*S*)- and (*R*)-1-phenylethylamine).

---

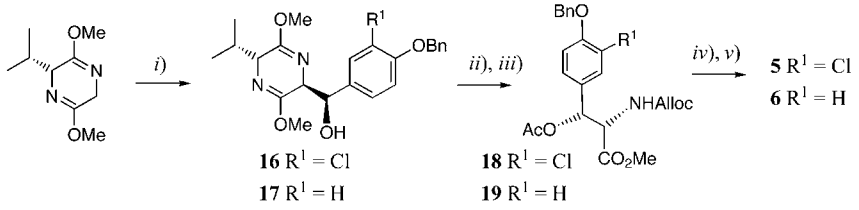
[1]) *Abbreviations:* Alloc: allyloxycarbonyl, Cyt: *m*-chloro-3-hydroxytyrosine, Dhpg: 2-(3,5-dihydroxyphenyl)glycine, HATU: 2-(1*H*-7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate, HOAt: 1-hydroxy-7-azabenzotriazole, Hpg: 2-(4-hydroxyphenyl)glycine, Mtt: 4-methyltrityl, NMM: *N*-methylmorpholine.

Scheme 1



i) BnCl, K_2CO_3, DMF, r.t. *ii*) KOH, EtOH/H_2O, r.t.; 97% over 2 steps. *iii*) $SOCl_2$. *iv*) CH_2N_2, Et_3N, THF 0°; 81% over 2 steps. *v*) MeOH, silver benzoate, Et_3N. *vi*) 1M KOH, MeOH/H_2O 9:1; 79% over 2 steps. *vii*) Et_3N, pivaloyl chloride, THF, then (*S*)-4-benzyloxazolidin-2-one, BuLi; 89%. *viii*) K salt of 1,1,1,3,3,3-hexamethyldisilazane (KHMDS), trisyl azide (trisyl = 2,4,6-triisopropylbenzenesulfonyl), THF, −78°, then AcOH; 81%. *ix*) $SnCl_2$, dioxane/H_2O. *x*) Allyl chloroformate, dioxane/aq. $NaHCO_3$; 77% over 2 steps. *xi*) LiOH, THF/H_2O 3:1; 88%.

Scheme 2



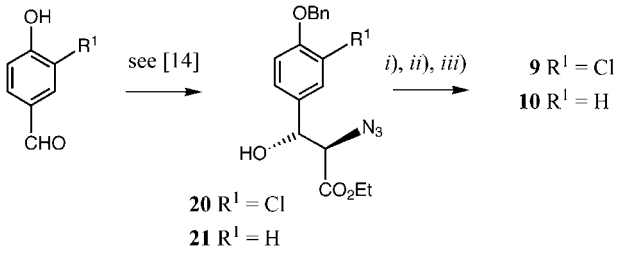
i) 1.1 equiv. BuLi, 1.1 equiv. $Ti(NEt_2)_3Cl$, 1.2 equiv. aldehyde, −78°; 76%. *ii*) Ac_2O, pyridine. *iii*) 3 equiv. TFA, H_2O/MeCN. *iv*) Allyl chloroformate, $NaHCO_3$; 60% over three steps. *v*) K_2CO_3, MeOH/water; 91%.

The synthesis of **5** and **6** was achieved using the method of *Schöllkopf* and coworkers [11][12]. The titanium derivative of the *Schöllkopf* bis-lactim ether reacted smoothly with both required aldehydes (*Scheme 2*), to afford almost exclusively the required stereoisomers **16** and **17**. These adducts were then converted to **5** and **6** by a slight adaptation of published procedures [11][12].

The amino-acid derivative **7** was prepared from D-(2-(4-hydroxyphenyl)glycine, by first alkylating the phenol group with BnBr, following the method of *Wünsch et al.* [13]. Then, the Alloc group was introduced by standard methods. The enantiomeric purity of the product (> 97%) was analyzed by ^{1}H-NMR after coupling the product to optically pure 1-phenylethylamine. Compound **8** was prepared from commercially available Fmoc-Asn(Mtt)-OH, by exchanging the Fmoc group for Alloc.

The synthesis of **9** and **10** was performed by the route described by *Rao et al.* [14]. The azide intermediates **20** and **21** were crystalline (*Scheme 3*). This allowed recrystallization to improve the optical purity to close to 100% (determined by ^{1}H-NMR with a chiral solvating agent). Also, both the relative and absolute configuration of **20** was confirmed by single-crystal X-ray diffraction, as was (only) the relative configuration of **21** (see *Fig.*). The azides **20** and **21** were then transformed in straightforward steps into **9** and **10**, respectively (*Scheme 3*).

Scheme 3



i) $SnCl_2$, dioxane/H_2O. *ii*) Allyl chloroformate, $NaHCO_3$; 83% over the last two steps. *iii*) $LiOH \cdot H_2O$, THF/H_2O; 91%.

2.2. *Peptide Synthesis.* The solid-phase synthesis of peptide **1** was performed as described in [7] on 2-chlorotrityl-chloride resin. The first amino acid, **4**, was coupled to the resin in CH_2Cl_2 with NMM as base, without significant epimerisation. The peptide chain was then extended using for each coupling HATU/HOAt (4 equiv.) and the required amino-acid derivative, **5**–**11**. For Alloc removal, $Pd(PPh_3)_4$ and $PhSiH_3$ were used, as outlined in *Scheme 4*. The assembly of the heptapeptide was monitored after each amino-acid coupling and Alloc-removal step, by cleaving a small sample of intermediate from the resin and analysis by reversed-phase HPLC. This revealed a single component at the dipeptide stage, but, after coupling of **7**, two components were detected in a 4:1 ratio, presumably due to partial epimerization during coupling of this amino acid. The same ratio of two heptapeptide epimers was also observed at the end of the synthesis. Upon completion of the synthesis, the side-chain-protected peptide was cleaved from the solid support using a mixture of CH_2Cl_2/CF_3CH_2OH/AcOH (see *Scheme 4*). The major component of the protected heptapeptide product was then treated with CF_3COOH/thioanisole/$(i\text{-}Pr)_3SiH$ to remove all protecting groups, and the product **1** was purified by reversed-phase HPLC in an overall yield (not optimized) of *ca.* 13%.

The heptapeptide **1** was characterized by MS and NMR. The electrospray MS showed a main molecular ion at *m*/*z* 1135, as well as peaks at higher mass (*m*/*z* 1136, 1137, 1138, 1139 and 1140), as expected for the isotopes of Cl. The ^{1}H-NMR spectra were recorded in H_2O ($H_2O:D_2O$ 9:1)/CD_3OD 5:2 (at 300 K), which gave sharp peaks also for the amide resonances. Analysis of 2D-NOESY, TOCSY and DQF-COSY spectra allowed a complete assignment of all non-exchangeable resonances[2]),

---

[2]) ^{1}H-NMR Assignment for the heptapeptide **1** in (H_2O/D_2O 9:1)/CD_3OD 5:2 at 300 K (pH* *ca.* 5): 8.49 (weak *d*, NH, Asn^3); 8.40 (*d*, NH, Hpg^4); 8.30 (*d*, NH, Dhpg^7); 8.29 (*d*, NH, Hpg^5); 8.24 (*d*, NH, Cyt^6); 7.45 (*s*, NH(δ) (*trans*), Asn^3); 7.23 (*d*, H–C(2), H–C(6), Hpg^4); 7.23 (*d*, H–C(2), Cyt^2); 7.15 (*d*, H–C(2), Cyt^6); 6.99 (*dd*, H–C(6), Cyt^2); 6.98 (*d*, H–C(2), H–C(6), Hpg^5); 6.92 (*d*, H–C(5), Cyt^2); 6.90 (*dd*, H–C(6), Cyt^6); 6.83 (*d*, H–C(3), H–C(5), Hpg^4); 6.79 (*s*, NH(δ) (*cis*), Asn^3); 6.76 (*d*, H–C(3), H–C(5), Hpg^5); 6.70 (*d*, H–C(5), Cyt^6); 6.51 (*d*, H–C(2), H–C(6), Dhpg^7); 6.30 (*t*, H–C(4), Dhpg^7); 5.51 (*d*, H–C(α), Hpg^4); 5.38 (*d*, H–C(α), Hpg^5); 5.18 (*d*, H–C(β), Cyt^6); 5.04 (*d*, H–C(α), Dhpg^7); 4.90 (*d*, H–C(β), Cyt^2); 4.73 (*m*, H–C(α), Asn^3); 4.72 (*m*, H–C(α), Cyt^6); 4.45 (*d*, H–C(α), Cyt^2); 3.86 (*t*, H–C(α), Leu^1); 2.77 (*dd*, 1 H–C(β), Asn^3); 2.71 (*dd*, 1 H–C(β), Asn^3); 1.38 (*m*, H–C(γ), H–C(β), Leu^1); 0.78 (*d*, 1 H–C(δ), Leu^1); 0.75 (*d*, 1 H–C(δ), Leu^1). The assignments were made by 2D-TOCSY, DQF-COSY and NOESY experiments at 600 MHz. 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. The NH resonances for Leu^1 and Cyt^2 were not observed due to exchange.

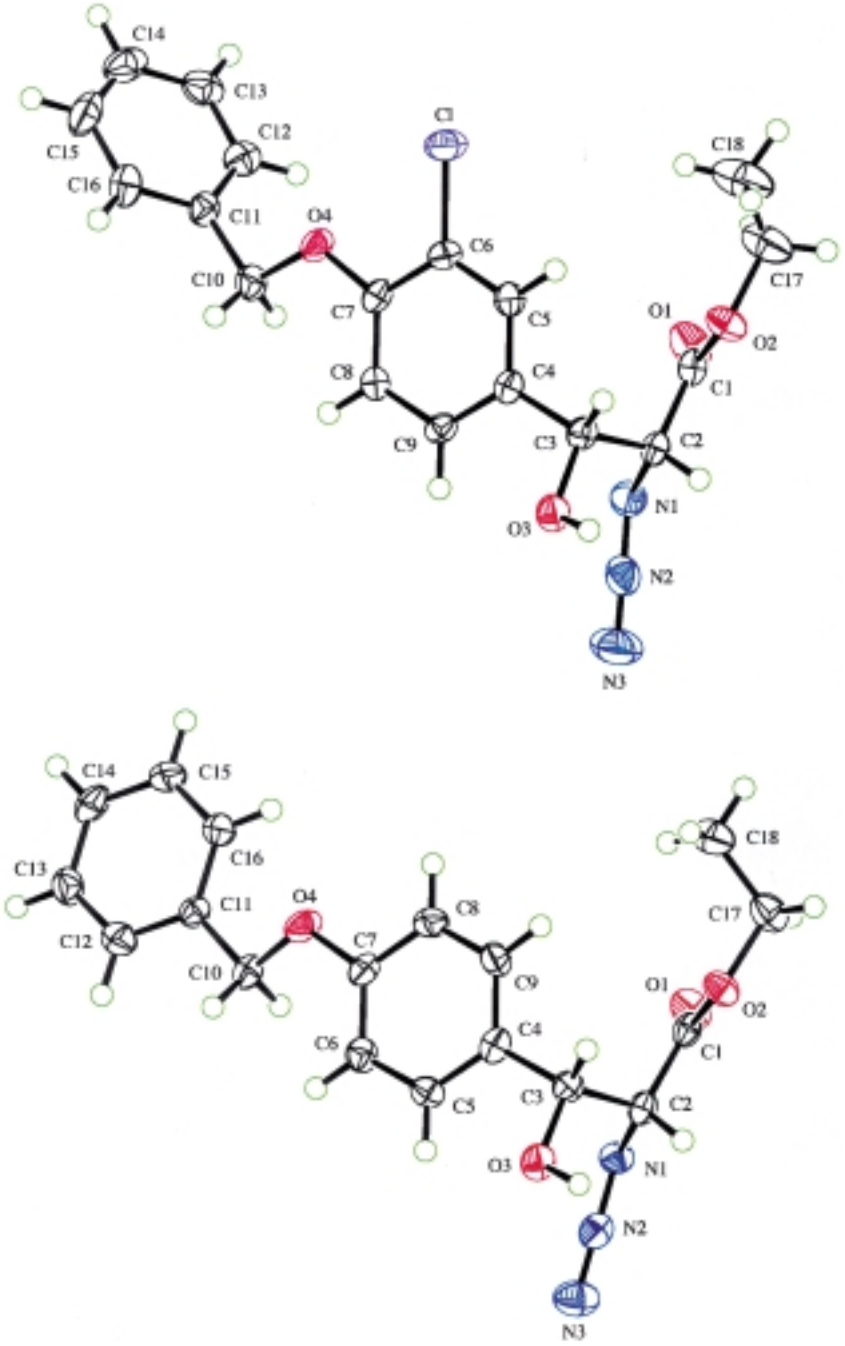
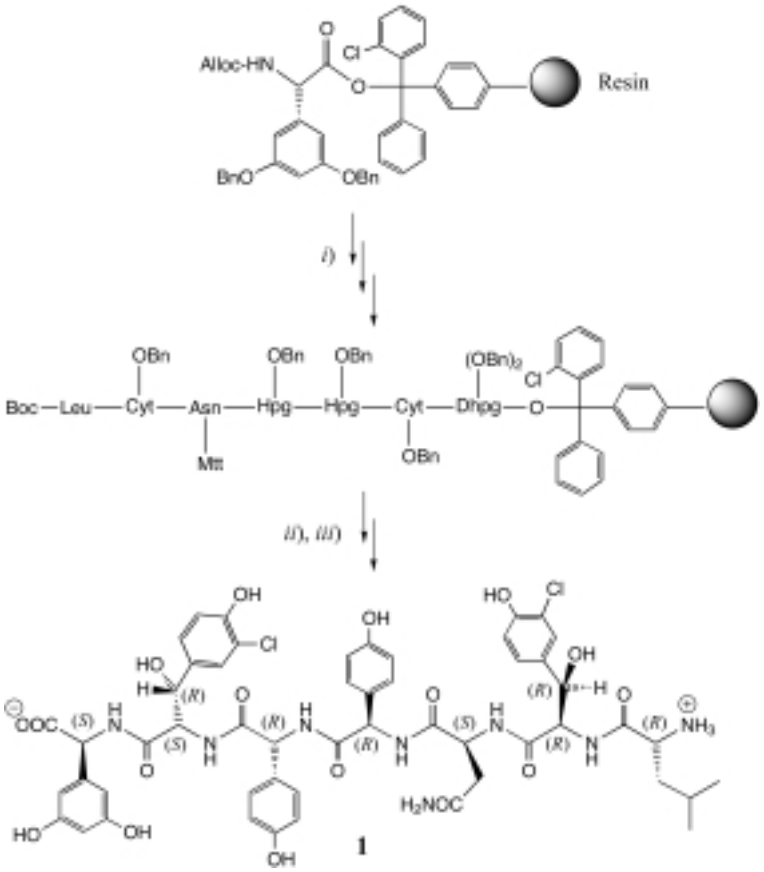


Figure. *X-Ray crystal structures of the intermediates* **20** *and* **21** (*Scheme 3*)

Scheme 4



i) For coupling **4** (Dhpg), **5** (Cyt), **7** (Hpg), **8** (Asn), **9** (Cyt) and **11** (each 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/thioanisole 3:1 + 5% $(i\text{-}Pr)_3SiH$, r.t., 2 h., 13% yield over whole synthesis.

and also provided confirmation of the constitution of the molecule. No long-range NOEs were observed in NOESY spectra, indicating that the peptide is not folded under these conditions. Finally, the assignments made from 1D-^{1}H and 2D-NOESY, TOCSY and DQF-COSY-NMR spectra recorded in (D_6)DMSO were essentially identical to those reported earlier [6] for **1** isolated from a mutant of the balhimycin producer.

In summary, a convenient method is decribed for the solid-phase synthesis, using Alloc chemistry, of putative intermediates in the biosynthesis of vancomycin, in particular, the heptapeptide **1** containing, amongst other amino acids, 2-(3,5-dihydroxyphenyl)glycine, 2-(4-hydroxyphenyl)glycine and *m*-chloro-3-hydroxytyrosine. The method should allow rapid access also to a variety of related heptapeptides that may prove useful in studies of the later steps in glycopeptide antibiotic biosynthesis.

Crystallographic data (excluding structure factors) for the structures of **20** and **21** reported in this paper have been deposited with the *Cambridge Crystallographic Data Centre* as supplementary publication Nos. CCDC-148683 and CCDC-148684, respectively. Copies of the data can be obtained, free of charge, on application to the CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44-(0)1223-336033 or e-mail: deposit@ccdc.cam.ac.uk).

The authors thank the *Swiss National Science Foundation* for financial support.

REFERENCES

[1] P. J. Solenberg, P. Matsushima, D. R. Stack, S. C. Wilkie, R. C. Thompson, R. H. Baltz, *Chem. Biol.* **1997**, *4*, 195.
[2] A. M. A. van Wageningen, P. N. Kirkpatrick, D. H. Williams, B. R. Harris, J. K. Kershaw, N. J. Lennard, M. Jones, S. J. M. Jones, P. J. Solenberg, *Chem. Biol.* **1997**, *5*, 155.
[3] J. W. Trauger, C. T. Walsh, *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 3112.
[4] D. P. O'Brien, P. N. Kirkpatrick, S. W. O'Brien, T. Staroske, T. I. Richardson, D. A. Evans, A. Hopkinson, J. B. Spenser, D. H. Williams, *Chem. Commun.* **2000**, 103.
[5] S. Pelzer, R. Sussmuth, D. Heckmann, J. Recktenwald, P. Huber, G. Jung, W. Wohlleben, *Antimicrob. Agents Chemother.* **1999**, *43*, 1565.
[6] R. D. Sussmuth, S. Pelzer, G. Nicholson, T. Walk, W. Wohlleben, G. Jung, *Angew. Chem., Int. Ed.* **1999**, *38*, 1976.
[7] E. Freund, J. A. Robinson, *Chem. Commun.* **1999**, 2509.
[8] F. Guibé, *Tetrahedron* **1998**, *54*, 2967–3042.
[9] D. A. Evans, T. C. Britton, J. A. Ellman, R. L. Dorow, *J. Am. Chem. Soc.* **1990**, *112*, 4011.
[10] N. Katagiri, J. Nakano, K. Tetsuzo, *J. Chem. Soc., Perkin Trans I* **1981**, 2710.
[11] T. Beulshausen, U. Groth, U. Schöllkopf, *Liebigs Ann. Chem.* **1991**, 1207.
[12] U. Schöllkopf, T. Beulshausen, *Liebigs Ann. Chem.* **1989**, 223.
[13] E. Wünsch, G. Fries, A. Zwick, *Chem. Ber.* **1958**, *91*, 542.
[14] A. V. Rama Rao, T. K. Chakraborty, K. L. Reddy, A. S. Rao, *Tetrahedron Lett.* **1994**, *35*, 5043–5046.

Received August 21, 2000