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An improved solid-phase methodology for the synthesis of putative hexa- and heptapeptide intermediates in vancomycin biosynthesis†

Dong Bo Li and John A. Robinson*

Organic Chemistry Institute, University of Zurich, Winterthurerstrasse 190, 8057-Zurich, Switzerland. E-mail: robinson@oci.unizh.ch; Fax: (+41) 1-635-6833

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The biosynthesis of the vancomycin aglycone involves three oxidative phenol coupling reactions, each catalyzed by a discrete cytochrome P450-like enzyme. Studies on the mechanism and specificity of the enzyme (called OxyB) catalyzing the first coupling, require access to suitable linear peptide precursors, each conjugated as a thioester to a peptide carrier domain of the vancomycin non-ribosomal peptide synthetase. An efficient route to representative free linear peptides is described here. The method makes use of Alloc-chemistry during solid-phase assembly of the peptide backbone, but importantly and in contrast to earlier efforts, largely avoids the use of amino acid side chain protecting groups. In this way, the target linear peptides can be released directly from the solid support under very mild conditions.

Introduction

The biosynthesis of vancomycin and related glycopeptide antibiotics is presently attracting attention.[1,2] Amongst the many interesting enzymic transformations involved in the assembly of these complex natural products (Fig. 1) are several oxidative phenol coupling reactions (OPCRs). These reactions lead to cross-linking of aromatic amino acid side chains, which together constrain the antibiotic aglycone into a conformation that is ideal for binding to its biological target, an *N*-acyl-D-Ala-D-Ala intermediate of peptidoglycan assembly in Gram-positive bacteria.[3,4] The importance of OPCRs in natural product biosynthesis, for example in alkaloid biosynthesis, is well appreciated. Recently, it has become clear that a family of cytochrome P450 proteins have evolved in various organisms to catalyze specific OPCRs. However, the P450s of plant origin studied so far, for example those acting in the biosynthesis of benzylisoquinoline alkaloids, are associated with microsomes,[5–8] which has no doubt hindered structural and mechanistic studies.

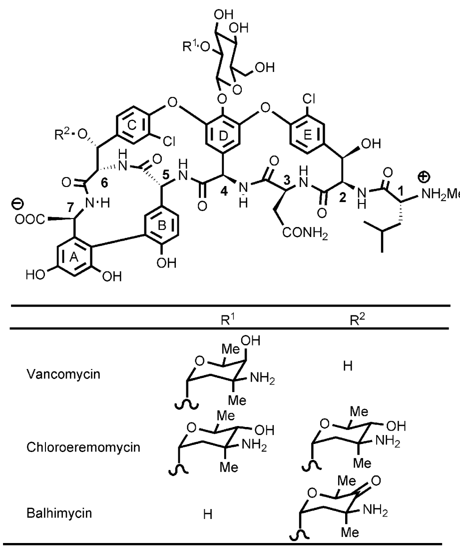

Fig. 1 Structure of vancomycin and related glycopeptides.

† Electronic supplementary information (ESI) available: experimental details of the synthesis of the amino acid building blocks. See http://www.rsc.org/suppdata/ob/b4/b418908f/

No such obstacle exists with the P450 proteins implicated in OPCRs during glycopeptide antibiotic biosynthesis, where the relevant genes have now been cloned and sequenced from several glycopeptide producers,[9–17] and two of these proteins (OxyB and OxyC from the vancomycin producer) have been crystallized and their 3D structures determined.[17,18]

Functions have been assigned by gene inactivation experiments to the three P450 proteins catalyzing OPCRs during balhimycin biosynthesis (balhimycin shares the same aglycone with vancomycin).[19–21] The first OPCR, catalyzed by OxyB (the Oxy lettering corresponds to the order of the three contiguous genes in the gene cluster, *viz. oxyA*-*oxyB*-*oxyC*), occurs between rings C and D, the second catalyzed by OxyA occurs between rings D and E, and the final coupling catalyzed by OxyC takes place between rings A and B (Fig. 1). Recently, it was shown that OxyB, cloned from the vancomycin producer, catalyzes the conversion of **1** to **2** shown in Fig. 2. The linear hexapeptide **1**, must be attached through its C-terminus to a holo-peptide carrier domain (PCD) derived from the vancomycin non-ribosomal peptide synthetase (NRPS) in order to function as a substrate for OxyB.[22] The corresponding peptide with a free C-terminus was not turned-over by OxyB. It is currently not clear, however, whether the enzyme can also catalyze an OPCR, and perhaps more efficiently, on a heptapeptide–PCD conjugate, such as **3a** or **3b** (Fig. 3). The influence on the rate of the OxyB

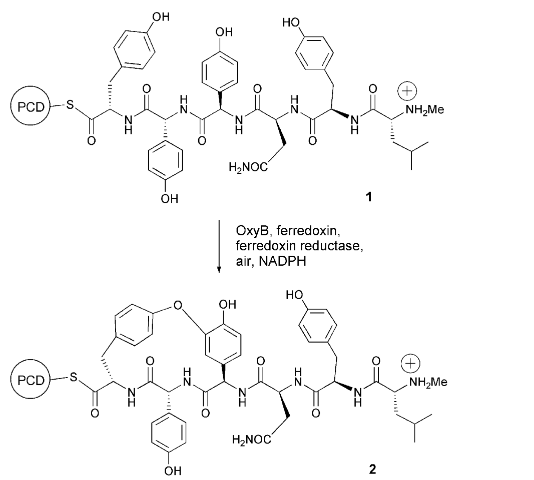

Fig. 2 OxyB transforms peptide **1** linked to a peptide carrier domain (PCD) into **2**.[22]

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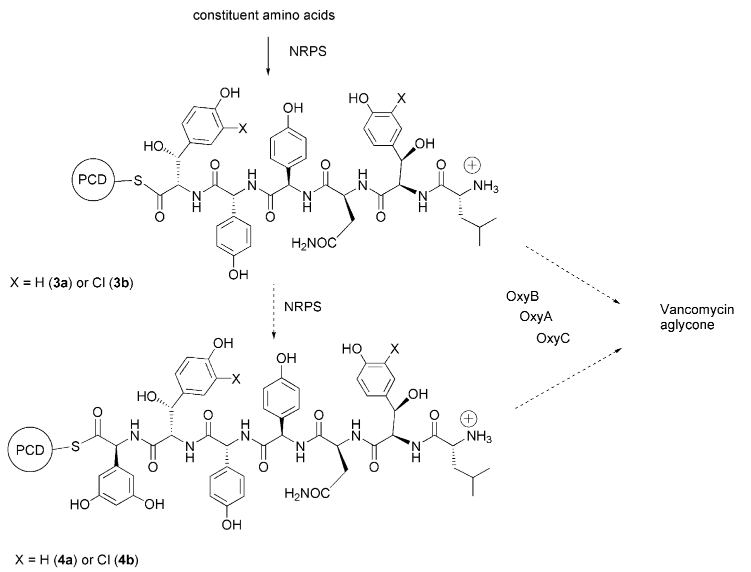


Fig. 3 The preferred biosynthetic route to vancomycin aglycone is presently not clear (*i.e.* the steps indicated by dotted arrows).[1,2] The first OPCR catalyzed by OxyB may occur preferentially on **3a/b** and/or **4a/b**.

reaction of the β-hydroxy groups and *m*-chlorine substituents in the *m*-chloro-3-hydroxytyrosine residues (compare **4a/4b** and **3a/b** with **1**) also remain to be defined.

More detailed studies of the substrate specificity of OxyB, as well as of its mechanism of action, now hinge upon the availability of suitable linear peptide–PCD conjugates. A method has been established for linking peptides *N*-methylated at the N-terminus to PCDs (*e.g.* **1**).[22,23] As long as the N-terminus is *N*-methylated, the peptide C-terminus can be efficiently activated to a thioester for coupling to the PCD; the *N*-methylamino N-terminus does not react rapidly with the thioester under the conditions used. More challenging, however, is the synthesis of the required linear peptides, because they contain amino acids, which due to their ease of epimerization and sensitivity to acids and bases, are incompatible with the two current standard methods of solid-phase peptide assembly (Boc- and Fmoc-chemistry). For this reason, we introduced earlier a method for solid-phase peptide assembly of such peptides, under neutral conditions, based on (allyloxy)carbonyl (Alloc) protection of the α-amino groups. Benzyl ether protection was still used for the phenolic groups, and methyltrityl (Mtt) for the side chain of Asn.[24,25] Although this synthetic method provided small amounts of heptapeptides **5** and **6** (Fig. 4), it was problematic, because a final side chain deprotection with strong acid is still required. This deprotection step can lead to rapid degradation, poor reproducibility, and a difficult and inefficient HPLC purification of the end product. Also, the required protected amino acids are not commercially available and demand considerable effort to produce in multi-gram amounts. In this work, we describe a new, more efficient, epimerization-free approach to the synthesis of a representative collection of hexa- and heptapeptides (**7–11**), which should facilitate studies of these interesting glycopeptide OPCRs. The key improvements are methods that largely eliminate the need for amino acid *side chain* protecting groups during peptide assembly. This in turn allows cleavage of the desired end product directly from the solid support under very mild conditions. No further functional group manipulations are then needed. Relevant methods for the stereoselective synthesis of suitably protected amino acid building blocks are also reviewed.

Results and discussion

In planning the syntheses of peptides **7–11**, key considerations were the use of the Alloc group as a temporary *N*-α-amino protecting group, and the avoidance of side chain protecting groups that require removal with acid. This required the optimization of methods for activating and coupling amino acids to the growing chain on the resin, whilst avoiding acylation of the phenolic groups and dehydration of the asparagine side chain.

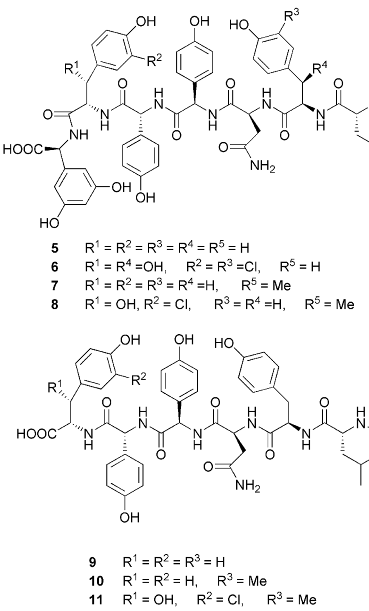
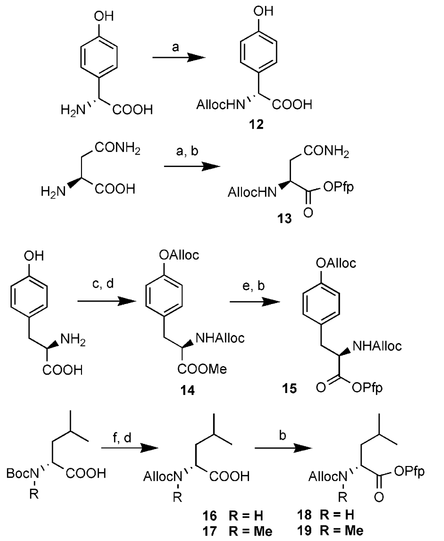


Fig. 4 Peptides **5–11**.

For the synthesis of hexapeptides **9** and **10**, the building blocks **12, 13, 15, 18** and **19** were prepared (Scheme 1). Thus, D-4-hydroxyphenylglycine was converted into **12** in high yield and without detectable racemization upon reaction with Alloc-*O*-(*N*-hydroxysuccinimide) (Alloc-OSu). This reaction did not proceed smoothly using Alloc-Cl. Alloc-OSu was also used to produce the crystalline pentafluorophenyl ester (OPfp) **13** from L-asparagine, using DCC for activation. D-Tyrosine was converted in four steps *via* **14** into the protected and activated derivative **15**. Finally, both *N*-Alloc-D-Leu-OPfp (**18**) and *N*-Alloc-*N*-methyl-D-Leu-OPfp (**19**) were prepared from the corresponding commercially available Boc-protected amino acids (Scheme 1).

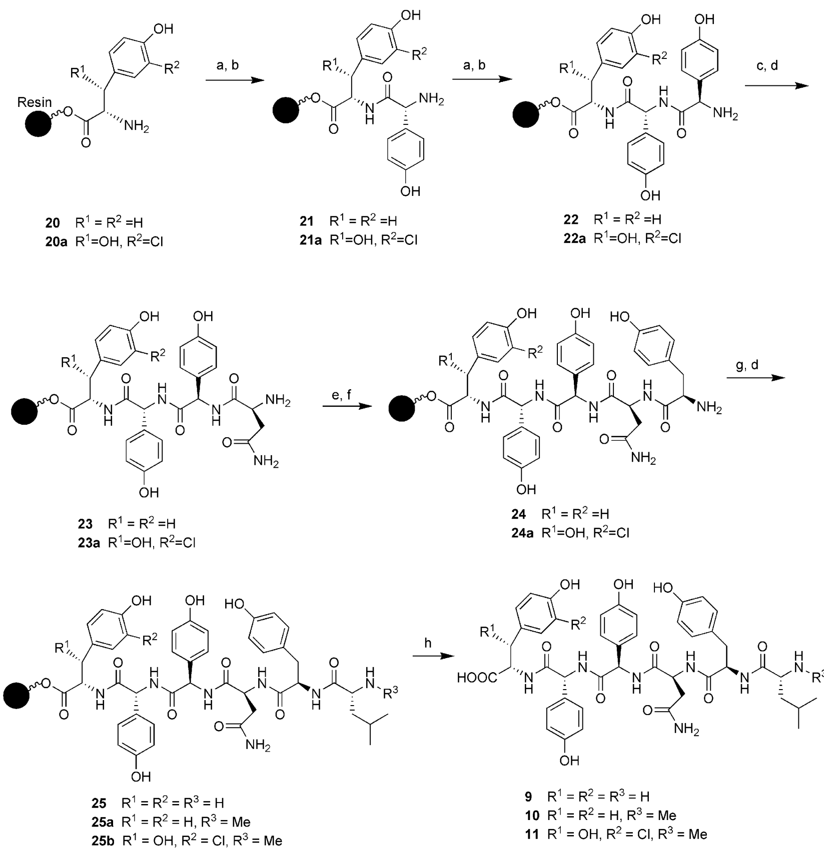
The assembly of **9** and **10** (Scheme 2) was performed on 2-chlorotrityl chloride resin, following loading of Fmoc-Tyr-OH and treatment with piperidine to remove the Fmoc-group. The first coupling between **20** and **12** was performed with



Scheme 1 *Reagents*. a) Alloc-OSu, $NaHCO_3$, acetone–water (1 : 1), rt; b) DCC, Pfp-OH, dioxane, 0 °C–rt; c) AcCl, MeOH, reflux, 99%; d) Alloc-Cl, $NaHCO_3$, dioxane–water (1 : 1), rt; e) LiOH, THF–water (1 : 1), 0 °C; f) TFA–CH_2Cl_2 (1 : 1) containing 5% TIS.

DIC–HOBt activation in DMF, and subsequent Alloc-removal was with $Pd(PPh_3)_4$–$PhSiH_3$ for 3 h. HPLC-MS analysis of material cleaved (0.6% TFA–CH_2Cl_2) from a small portion of the resin showed clean formation of the desired resin-bound dipeptide **21**. Elaboration to the tripeptide **22** in the same way also proceeded cleanly (>95%). Further extension to the tetrapeptide **23** was performed with **13** and subsequent Alloc removal with $Pd(PPh_3)_4$–Bu_3SnH. It was necessary to change the allyl scavenger, since HPLC-MS analysis at this stage showed that $PhSiH_3$ could not efficiently remove the *N*-Alloc group in the so formed tetrapeptide. Further chain elongation to pentapeptide **24** was achieved smoothly by coupling **15** and subsequent $Pd(PPh_3)_4$–Bu_3SnH treatment to remove the two Alloc groups. Finally, the last coupling of **18** or **19** onto resin bound pentapeptide **24** followed again by Alloc removal, gave **25** and **25a**, respectively. The final products (**9** and **10**) could then be released from the resin with 0.6% TFA in CH_2Cl_2, and purified by HPLC in up to 52% overall yield. A full assignment of the ^{1}H NMR spectra of **9** and **10** was possible from an analysis of 2D NMR (TOCSY, COSY and ROESY) spectra. Typical HPLC chromatograms of crude **10** from the resin, and after HPLC purification, are shown in Fig. 5. This method has afforded 100 mg quantities of **10**, which were sufficient to establish methods for coupling **10** to a PCD (Fig. 2) and for assays of the conjugate with OxyB, as reported elsewhere.[22]

Our next target was the assembly of hexapeptide **11**, which contains (2*S*,3*R*)-*m*-chloro-β-hydroxytyrosine (Cht) (Fig. 4), using the same protocol developed from the synthesis of **9** and **10**. The required building block **29** was prepared following the procedure of Evans and Weber (Scheme 3).[26] Following the $Sn(OTf)_2$-mediated aldol reaction between isothiocyanate **26** and aldehyde **27**, the aldol adduct was treated with magnesium methoxide in MeOH to afford the corresponding methyl ester **28**. This was subjected to Alloc-protection and further transformation into **29**. The final hydrolysis step was marred by an accompanying oxazolidinone (**29a**) formation, which could nevertheless be recycled to **29**, but lowered the overall yield. Alloc-Cht(Allyl)-OH (**29**) was then loaded onto 2-chlorotrityl chloride resin and the Alloc- and allyl-groups were removed to afford **20a**, which was ready for assembly of hexapeptide **11** (Scheme 2). HPLC-MS analysis of resin at each stage of the assembly revealed no evidence of epimerization or major side reactions. After cleaving **25b** from the resin, analytical



Scheme 2 *Reagents*. a) **12** (4 eq.), DIC (4 eq.) and HOBt (8 eq.), DMF, overnight; b) $Ph(PPh_3)_4$ (1 eq.), $PhSiH_3$ (60 eq.), CH_2Cl_2, 3 h; c) **13** (5 eq.), HOBt (10 eq.), DMF, overnight; d) $Ph(PPh_3)_4$ (1 eq.), Bu_3SnH (60 eq.), CH_2Cl_2, 3 h; e) **15** (5 eq.), HOBt (10 eq.), DMF, overnight; f) $Ph(PPh_3)_4$ (2 eq.), Bu_3SnH (120 eq.), CH_2Cl_2, 3 h; g) for **25, 18** (5 eq.), HOBt (10 eq.), DMF, overnight; for **25a** and **25b, 19** (5 eq.), HOBt (10 eq.), DMF, overnight; h) 0.6% TFA in CH_2Cl_2.

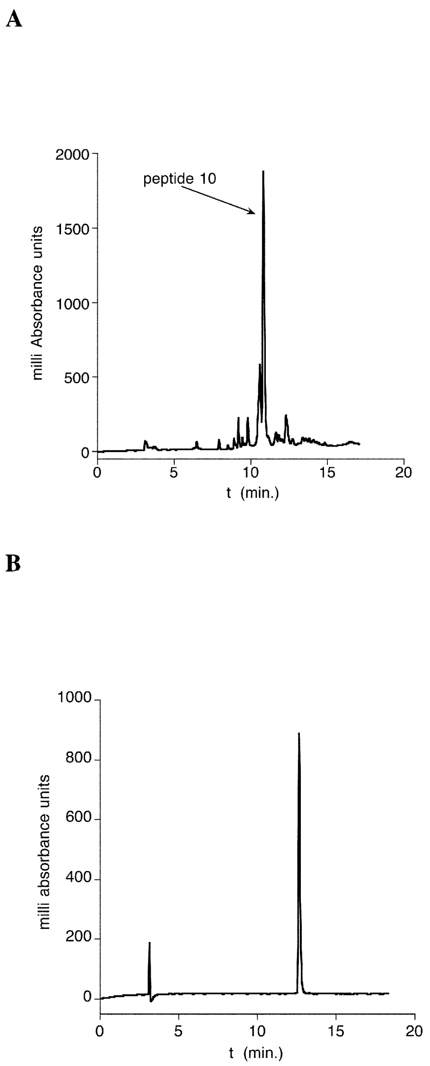
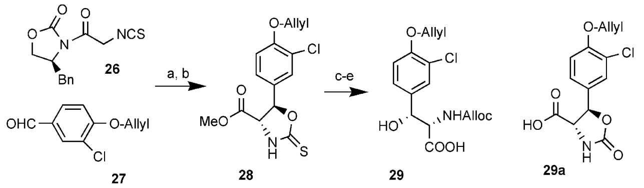


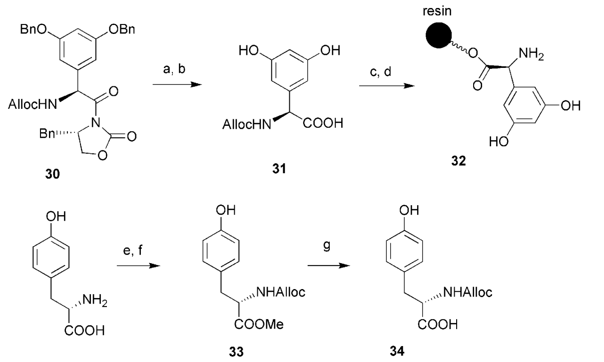
Fig. 5 HPLC chromatograms showing: **A**, the crude product (gradient from 5–40% MeCN–H_2O + 0.15% TFA over 15 min) from the synthesis of **10**; and **B**, after HPLC purification (gradient from 5–35% MeCN–H_2O + 0.15% TFA over 15 min).



Scheme 3 *Reagents*. a) $Sn(OTf)_2$, *N*-ethylpiperidine, THF, −78 °C, 81%; b) MeMgBr, MeOH, 0 °C, 87%; c) Alloc-Cl, Et_3N, DMAP, CH_2Cl_2, rt; d) HCO_2H–35% H_2O_2, 0 °C, 76% over two steps; e) LiOH, dioxane–water (10 : 1), rt (**29**, 19%; **29a**, 58%).

HPLC-MS of the crude product showed only one main peak with the correct mass. The overall yield of hexapeptide **11** after HPLC purification was *ca.* 13% and its structure was confirmed by MS and NMR spectroscopy. These results demonstrate the compatibility of a free unprotected β-hydroxy group in residue-6 with the method of peptide assembly and cleavage from the resin.

As a next step, we show that this method is also amenable to the efficient synthesis of heptapeptides **7** and **8**. The building blocks **31** and **34** required for heptapeptide **7** were synthesized as shown in Scheme 4. Preparation of the (*S*)-3,5-dihydroxyphenylglycine derivative **31** proceeded *via* oxazolidinone **30**, reported in earlier work.[24] After removal of the chiral auxiliary and deprotection with TFA–thioanisole, Alloc-Dhpg-OH **31** was obtained in 81% yield. This was loaded onto 2-chlorotrityl chloride resin and then treated with $Pd(PPh_3)_4$–$PhSiH_3$ to afford resin **32**. (*S*)-Alloc-Tyr-OH **34** was conveniently prepared by a three-step sequence starting from L-Tyr-OH (Scheme 4).



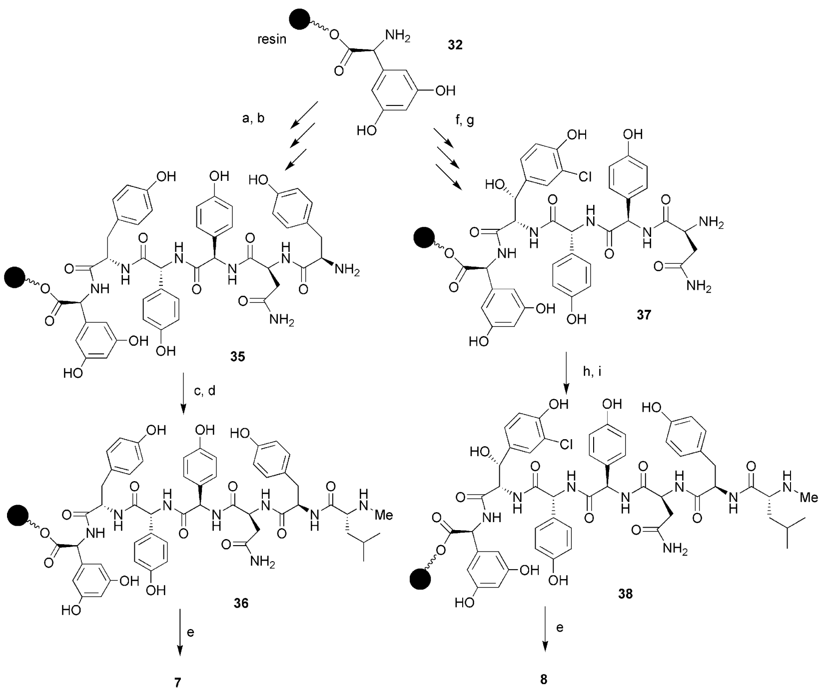
Scheme 4 *Reagents*. a) LiOH, THF–H_2O (3 : 1), 0 °C; b) TFA–thioanisole (3 : 1), rt, 81% over two steps; c) 2-chlorotrityl chloride resin, NMM, DMF–CH_2Cl_2 (10 : 1), overnight; d) $Ph(PPh_3)_4$, $PhSiH_3$, CH_2Cl_2, 3 h; e) AcCl, MeOH, reflux, 97%; f) Alloc-OSu, $NaHCO_3$, acetone–water (1 : 1), rt, 100%; g) LiOH, THF–water (1 : 1), 0 °C, 87%.

The solid-phase assembly of heptapeptide **7**, illustrated in Scheme 5, was straightforward until hexapeptide **35**. However, after the final coupling of OPfp ester **19** and subsequent Alloc-deprotection and cleavage from the resin, one main byproduct was observed by HPLC/HPLC-MS, in addition to the desired heptapeptide. MS and NMR analysis indicated that the byproduct lacked the *N*-methylleucine moiety, although the heptapeptide appeared to be stereochemically homogeneous. This indicated that no epimerization(s) had occurred during the assembly, but that a significant byproduct was formed due to inefficient coupling in the final step. In order to overcome this problem, we explored the use of a dipeptide building block in the last phase of the assembly. Thus, Alloc-*N*(Me)-D-Leu-D-Tyr(OAllyl)-OPfp **41** was prepared (Scheme 6) and used in the assembly of heptapeptide **8**. For the synthesis of **41**, the known[27] tyrosine derivative **39** was treated with TFA and then coupled to Alloc-*N*(Me)-D-(Leu)-OH. Subsequent hydrolysis and coupling to Pfp-OH provided **41**, which was used directly for peptide assembly without further purification. Starting from Dhpg-resin **32**, pentapeptide **37** (Scheme 5) was assembled using the same protocol as for **35**. Thereafter, coupling of the dipeptide OPfp ester **41** afforded resin-bound heptapeptide **38** as a single main component. HPLC-MS analysis revealed no epimerization at this dipeptide coupling step. Finally, after cleavage from the resin and HPLC purification, heptapeptide **8** was obtained in 11% overall yield and was characterized by MS and ^1H 1D and 2D NMR. The use of **41** as a viable building block for heptapeptide assembly was thus established.

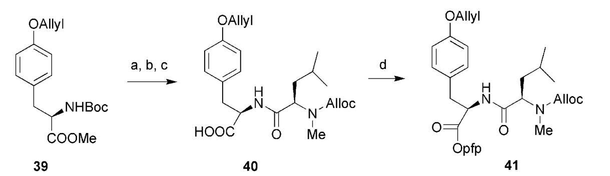
In summary, a judicious choice of protecting groups and coupling strategies has provided efficient access to a range of hexa- and heptapeptides that are of interest in ongoing studies of vancomycin biosynthesis. The coupling conditions were optimized to allow an essentially epimerization-free assembly of these peptides on a solid support. Also, the avoidance as far as possible of side-chain protecting groups allowed the cleavage of the end products from the resin under very mild conditions. The methodology presented here should be readily applicable to the synthesis of other related peptides, as potential precursors of PCD-conjugates to explore the specificity of the OPCRs catalyzed by OxyB.

Experimental

For solid-phase synthesis, DMF was redistilled under reduced pressure from ninhydrin, CH_2Cl_2 was redistilled under N_2 from CaH_2 and HPLC-grade MeOH was used. For ^1H NMR



Scheme 5 *Reagents*. a) for coupling **34** and **12** (each 4 eq.), DIC–HOBt (4 and 8 eq.), DMF, overnight; for deprotection $Ph(PPh_3)_4$ (1 eq.) and $PhSiH_3$ (60 eq.), CH_2Cl_2, 3 h; b) for coupling **13** and **15** (each 5 eq.), HOBt (10 eq.), DMF, overnight; for deprotection of **13**, $Ph(PPh_3)_4$ (1 eq.), Bu_3SnH (60 eq.), CH_2Cl_2, 3 h; of **15**, $Ph(PPh_3)_4$ (2 eq.), Bu_3SnH (120 eq.), CH_2Cl_2, 3 h; c) **19** (5 eq.), HOBt (10 eq.), DMF, overnight; d) $Ph(PPh_3)_4$(1 eq.), Bu_3SnH (60 eq.), CH_2Cl_2, 3 h; e) 0.6% TFA in CH_2Cl_2; f) for coupling: **29** and **12** (each 4 eq.), DIC–HOBt (4 and 8 eq.), DMF, overnight; for deprotection of **29**, $Ph(PPh_3)_4$ (2 eq.), $PhSiH_3$ (120 eq.), CH_2Cl_2, 3 h; of **12**, $Ph(PPh_3)_4$ (1 eq.) and $PhSiH_3$ (60 eq.), CH_2Cl_2, 3 h; g) for coupling **13** (5 eq.), HOBt (10 eq.), DMF, overnight; for deprotection $Ph(PPh_3)_4$ (1 eq.), Bu_3SnH (60 eq.), CH_2Cl_2, 3 h; h) **41** (5 eq.), HOBt (10 eq.), DMF, overnight; i) $Ph(PPh_3)_4$ (2 eq.), Bu_3SnH (120 eq.), CH_2Cl_2, 3 h.



Scheme 6 *Reagents*. a) TFA–thioanisole (3 : 1), rt; b) **17**, EDC–HOBt, DIEA, 0 °C–rt, DMF; c) LiOH, THF–H_2O (1 : 1), 80% yield over three steps; d) DCC, Pfp-OH, dioxane, 0 °C–rt.

assignments of the hexa- and heptapeptides, see Tables 1–5. A description of the synthesis of each of the amino acid building blocks is given in the electronic supplementary information†.

Table 1 ^{1}H NMR assignment of **9** (d_6 DMSO, 300 K, 600 MHz)

Residue	OH	NH	α	β	Others
Leu[1]	—	—	3.71	1.55	1.55 (γ), 0.86/0.85(δ)
D-Tyr[2]	9.18	8.59	4.53	2.89, 2.66	7.04 (2,6), 6.64 (3,5)
Asn[3]	—	8.33	4.64	2.41, 2.28	7.30 (NH), 6.92(NH)
Hpg[4]	9.34	8.01	5.52	—	7.18 (2,6), 6.67 (3,5)
Hpg[5]	9.37	8.75	5.42	—	7.08 (2,6), 6.65 (3,5)
L-Tyr[6]	9.15	8.25	4.25	2.78, 2.66	6.71 (2,6), 6.51 (3,5)

Table 2 ^{1}H NMR assignment of **10** (d_6 DMSO, 300 K, 600 MHz)

Residue	OH	NH	α	β	Others
N(Me)Leu[1]	—	—	3.59	1.50	1.40 (γ), 1.96 (NMe), 0.84/0.79(δ)
D-Tyr[2]	9.17	8.81	4.77	2.97, 2.61	7.04 (2,6), 6.63 (3,5)
Asn[3]	—	8.45	4.68	2.48, 2.33	7.30 (NH), 6.92(NH)
Hpg[4]	9.33	8.07	5.53	—	7.18 (2,6), 6.67 (3,5)
Hpg[5]	9.35	8.75	5.43	—	7.08 (2,6), 6.65 (3,5)
L-Tyr[6]	9.15	8.26	4.26	2.78, 2.61	6.72 (2,6), 6.51 (3,5)

Table 3 ^{1}H NMR assignment of **11** (d_6 DMSO, 300 K, 500 MHz)

Residue	OH	NH	α	β	Others
N(Me)Leu[1]	—	—	3.56	1.50	1.39 (γ), 1.98 (NMe), 0.85/0.80(δ)
D-Tyr[2]	9.17	8.79	4.75	2.97, 2.58	7.05 (2,6), 6.63(3,5)
Asn[3]	—	8.43	4.68	2.47, 2.33	7.30 (NH), 6.92(NH)
Hpg[4]	9.32	8.04	5.53	—	7.17 (2,6), 6.67(3,5)
Hpg[5]	9.28	8.69	5.58	—	6.98 (2,6), 6.61(3,5)
Cht[6]	9.95	8.14	4.33	4.97	7.25(2), 6.87 (6), 6.74(5)

Table 4 ^{1}H NMR assignment of **7** (d_6 DMSO, 300 K, 600 MHz)

Residue	OH	NH	α	β	Others
N(Me)Leu[1]	—	—	3.60	1.52	1.41 (γ), 1.97 (NMe), 0.85/0.80 (δ)
D-Tyr[2]	9.17	8.81	4.77	2.97, 2.59	7.05 (2,6), 6.64 (3,5)
Asn[3]	—	8.45	4.68	2.43, 2.33	7.30 (NH), 6.93 (NH)
Hpg[4]	9.33	8.05	5.52	—	7.19 (2,6), 6.68 (3,5)
Hpg[5]	9.31	8.69	5.58	—	6.98 (2,6), 6.62 (3,5)
L-Tyr[6]	9.08	8.08	4.53	2.81, 2.59	6.78 (2,6), 6.49 (3,5)
Dhpg[7]	9.33	8.69	5.06	—	6.28 (2,6), 6.18 (4)

L-Tyr-resin (20)

Fmoc-Tyr-OH (564 mg, 1.4 mmol) and NMM (400 μL, 3.6 mmol) in CH_2Cl_2 and DMF (17 ml, 10 : 1) were added to 2-chlorotrityl chloride resin (817 mg, 1.4 mmol g^{-1}) and the mixture was agitated overnight. MeOH (5 mL) was added and the mixture was agitated for 10 min. The resin was filtered and washed with DMF (4 × 25 mL), MeOH (4 × 25 mL), CH_2Cl_2 (4 × 25 mL) and DMF (4 × 25 mL). The resin was treated with a 20% solution of piperidine in DMF (15 mL) with agitation for 2 h. The resultant resin was filtered and washed with DMF (4 × 25 mL), CH_2Cl_2 (4 × 25 mL) and DMF (4 × 25 mL). The loading of L-Tyr was *ca.* 0.25 mmol g^{-1}.

Table 5 ^{1}H NMR assignment of **8** (d_6 DMSO, 300 K, 600 MHz)

Residue	OH	NH	α	β	Others
N(Me)Leu[1]	—	—	3.58	1.50	1.40 (γ), 1.96 (NMe), 0.84/0.78 (δ)
D-Tyr[2]	9.17	8.80	4.75	2.96, 2.55	7.04 (2,6), 6.62 (3,5)
Asn[3]	—	8.43	4.65	2.45, 2.31	7.29 (NH), 6.91 (NH)
Hpg[4]	9.31	8.03	5.47	—	7.16 (2,6), 6.65 (3,5)
Hpg[5]	9.34	8.67	5.46	—	7.00 (2,6), 6.65 (3,5)
Cht[6]	9.88	7.78	4.56	4.84	7.16 (2), 6.70 (6), 6.65(5), 5.57 (β-OH)
Dhpg[7]	9.32	8.50	5.11	—	6.27 (2,6), 6.16 (4)

Cht-resin (20a)

To Alloc-Cht(Allyl)-OH **29** (240 mg, 0.68 mmol) and NMM (187 µL, 1.7 mmol) in CH_2Cl_2 and DMF (17 mL, 10 : 1) was added 2-chlorotrityl chloride resin (600 mg, 0.95 mmol g^{-1}) and the mixture was agitated overnight. MeOH (5 mL) was added and the mixture was agitated for 10 min. The resin was then filtered and washed with DMF (4 × 25 mL), MeOH (4 × 25 mL) and CH_2Cl_2 (4 × 25 mL). Under Ar-atmosphere and in the dark, a solution of $Pd(PPh_3)_4$ (416 mg, 0.36 mmol) and $PhSiH_3$ (2.7 mL, 22 mmol) in CH_2Cl_2 (15 mL) was added to the resin (600 mg) and the mixture was agitated for 3 h. The resin was then filtered and washed with DMF (4 × 25 mL), CH_2Cl_2 (4 × 25 mL) and DMF (4 × 25 mL). The loading of Cht was *ca.* 0.15 mmol g^{-1} as determined after coupling Fmoc-Gly-OH, by Fmoc and HPLC analyses.

Dhpg-resin (32)

To Alloc-Dhpg-OH **31** (380 mg, 1.42 mmol) and NMM (400 µL, 3.55 mmol) in CH_2Cl_2 and DMF (17 mL, 10 : 1) was added 2-chlorotrityl chloride resin (1.20 g, 0.95 mmol g^{-1}) and the mixture was agitated overnight. MeOH (5 mL) was added and the mixture was agitated for 10 min. The resultant resin was filtered and washed with DMF (4 × 25 mL), MeOH (4 × 25 mL) and CH_2Cl_2 (4 × 25 mL). Under Ar-atmosphere and in the dark, a solution of $Pd(PPh_3)_4$ (208 mg, 0.18 mmol) and $PhSiH_3$ (1.3 mL, 11 mmol) in CH_2Cl_2 (15 mL) was added to the resin (600 mg) and the mixture was agitated for 3 h. The resulting resin was filtered and washed with DMF (4 × 25 mL), CH_2Cl_2 (4 × 25 mL) and DMF (4 × 25 mL). The substitution level of Dhpg-resin **32** was *ca.* 0.25 mmol g^{-1} as determined after coupling Fmoc-Gly-OH by Fmoc and HPLC analyses.

Hexapeptides 9 and 10

Step 1. A solution of **12** (4 eq.), DIC (4 eq.) and HOBt (8 eq.) in DMF (20 mL) was added to L-Tyr-resin **20** (660 mg, 0.25 mmol g^{-1}) and the mixture was agitated overnight. The resin was then filtered and washed with DMF (4 × 25 mL) and CH_2Cl_2 (4 × 25 mL). Under Ar-atmosphere and in the dark, a solution of $Pd(PPh_3)_4$ (1 eq.) and $PhSiH_3$ (60 eq.) in CH_2Cl_2 (15 mL) was added to the resin and the mixture was agitated for 3 h. The resultant resin **21** was filtered and washed with DMF (4 × 25 mL), CH_2Cl_2 (4 × 25 mL) and DMF (4 × 25 mL).

Step 2. The coupling of **12** was repeated as above to give **22**.

Step 3. A solution of **13** (5 eq.) and HOBt (10 eq.) in DMF (20 mL) was added to **22** and the mixture was agitated overnight. The resin was then filtered and washed with DMF (4 × 25 mL) and CH_2Cl_2 (4 × 25 mL). Under Ar-atmosphere and in the dark, a solution of $Pd(PPh_3)_4$ (1 eq.) and Bu_3SnH (60 eq.) in CH_2Cl_2 (15 mL) was added to the resin and the mixture was agitated for 3 h. The resultant resin **23** was filtered and washed with DMF (4 × 25 mL), CH_2Cl_2 (4 × 25 mL) and DMF (4 × 25 mL).

Step 4. A solution of **15** (5 eq.) and HOBt (10 eq.) in DMF (20 mL) was added to **23** and the mixture was agitated overnight. The resin was then filtered and washed with DMF (4 × 25 mL) and CH_2Cl_2 (4 × 25 mL). Under Ar-atmosphere and in the dark, a solution of $Pd(PPh_3)_4$ (2 eq.) and Bu_3SnH (120 eq.) in CH_2Cl_2 (15 mL) was added to the resin and the mixture was agitated for 3 h. The resultant resin **24** was filtered, washed with DMF (4 × 25 mL) and CH_2Cl_2 (4 × 25 mL), and divided into two equal parts.

Hexapeptide 9

Step 5. A solution of **18** (5 eq.) and HOBt (10 eq.) in DMF (20 mL) was added to **24** and the mixture was agitated overnight. The resultant resin was filtered and washed with DMF (4 × 25 mL) and CH_2Cl_2 (4 × 25 mL). Under Ar-atmosphere and in the dark, a solution of $Pd(PPh_3)_4$ (1 eq.) and Bu_3SnH (60 eq.) in CH_2Cl_2 (15 mL) was added to the resin and the mixture was agitated for 3 h. The resultant resin **25** was filtered and washed with DMF (4 × 25 mL), CH_2Cl_2 (4 × 25 mL) and DMF (4 × 25 mL). A 0.6% solution of TFA in CH_2Cl_2 (20 mL) was added to **25** and the mixture was agitated for 5 min, and then filtered. After repeating this cleavage procedure four times, the resultant resin was washed with MeOH (4 × 20 mL) and the resultant filtrate was collected, concentrated under reduced pressure to give a yellow oil, and product was purified by HPLC (preparative C_{18} column, gradient from 5–35% MeCN–H_2O + 0.15% TFA) to afford **9** (≥95% purity by HPLC) as a white powder (16 mg, *ca.* 22% overall yield). MS(ESI): 870.5 [M + H^+].

Hexapeptide 10

Step 5′. Compound **19** was coupled to **24** and cleavage from the resin gave product **10**, as above, white powder (90 mg, *ca.* 52% overall yield). MS(ESI): 884.5 [M + H^+].

Hexapeptide 11

MS(MALDI): 934.4 [M + H^+].

Heptapeptide 7

MS(ESI): 1049.4 [M + H^+].

Heptapeptide 8

MS(ESI): 1099.6 [M + H^+].

Abbreviations

Alloc, allyloxycarbonyl (= (prop-2-enyloxy)carbonyl); Boc, (*tert*-butoxy)carbonyl; Cht, β-hydroxy-*m*-chlorotyrosine; Dhpg, D-3,5-dihydroxyphenylglycine; DMAP, 4-*N*,*N*-di-methylaminopyridine; DCC, dicyclohexyl-carbodiimide; DIC, 1,3-diisopropylcarbodiimide; DIEA, *N*,*N*′-diisopropylethylamine; DMF, *N*,*N*-dimethylformamide; EDC, 1-(3-(dimethylamino)propyl)-3-ethylcarbodiimide; Fmoc, [(9*H*-fluorenyl)-methoxy]carbonyl; HOBt, 1-hydroxybenzotriazole; Hpg, D-4-hydroxyphenylglycine; NMM, *N*-methylmorpholine; Pfp-OH, pentafluorophenol; TFA, trifluoroacetic acid.

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