Pergamon

Tetrahedron Letters 39 (1998) 4917–4920

TETRAHEDRON LETTERS

Synthesis of Covalent Head-to-Tail Dimers of Vancomycin

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Received 8 April 1998; accepted 27 April 1998

Abstract: The synthesis of covalent dimers of vancomycin which are linked from C- to N-terminus (head-to-tail linkage) is described. Such dimers have the potential to exploit additional cooperative interactions when binding to bacterial cell-wall precursors at a surface.

Keywords: Acylation; Antibiotics; Dimers; Molecular recognition

Vancomycin (**1**) and teicoplanin are currently the drugs of choice against antibiotic-resistant bacteria such as methicillin-resistant *Staphylococcus aureus* (MRSA).[1,2] They belong to the glycopeptide family of antibiotics which act by binding to bacterial cell-wall precursors terminating in –Lys-D-Ala-D-Ala, thereby inhibiting crosslinking of the cell wall, leading to cell death.[3,4]

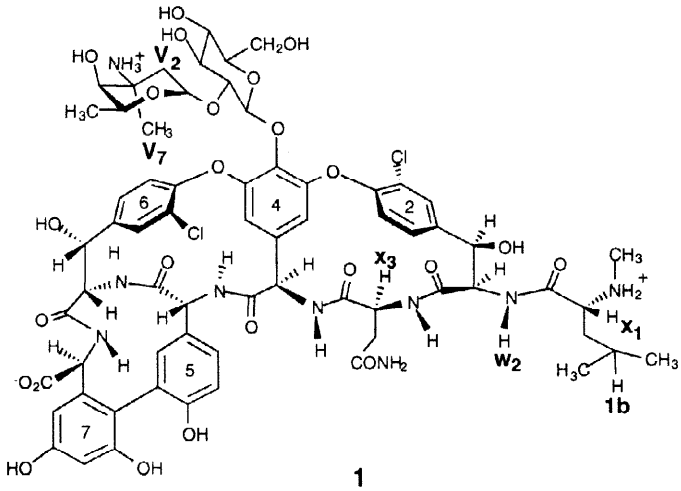

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Figure 1. The structure of vancomycin (**1**). Protons referred to in the text are labelled.

Recent studies have reported that back-to-back dimerisation of these antibiotics (except teicoplanin) is important in their antibacterial activity.[5] When an antibiotic binds to a cell-wall precursor on the surface of a bacterium as a dimer, subsequent binding of a second precursor to the second half of the dimer is effectively intramolecular, and is thus entropically favoured.[6] NMR solution studies have also revealed an enhancement to dimerisation constants in the presence of bound ligand (bacterial cell-wall precursor analogues), *i.e.*, dimerisation and ligand binding are cooperative (Figure 2).[6]

In the light of recent reports of vancomycin-resistant bacteria,[7,8] there is a strong incentive for the development of more potent antibiotics. Recently, it has been demonstrated that *N*-alkylation of glycopeptide amino sugar residues can increase antibacterial potency.[9,10] The preparation of vancomycin dimers covalently linked head-to-head through their C-termini (HH-cov-dimers) has also been reported.[11,12]

0040-4039/98/$19.00
PII: S0040-4039(98)00895-8

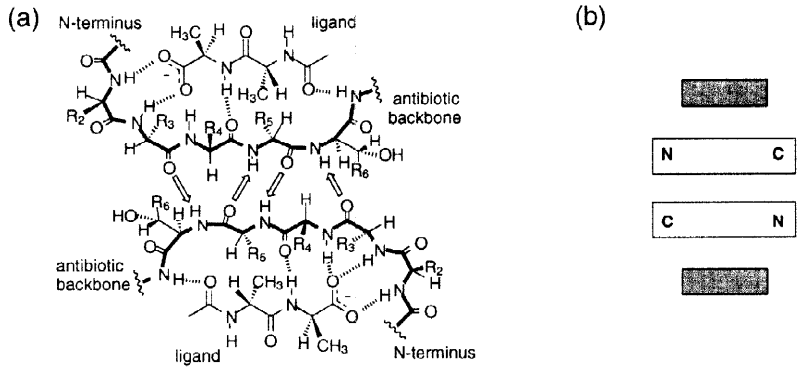


Figure 2. **(a)** Hydrogen bonding network in a vancomycin dimer complex with the cell-wall precursor analogue *N*-acetyl-D-Ala-D-Ala. The backbone of the antibiotic is shown in bold. Solid arrows represent hydrogen bonds between the two halves of the dimer, whereas dashed lines represent hydrogen bonds between cell-wall peptide analogue and antibiotic. **(b)** Schematic representation of **(a)**; white boxes represent antibiotics and grey boxes represent ligands.

These covalent dimers exhibited up to a sixty-fold improvement over vancomycin in their antibacterial activity against vancomycin-resistant enterococci (VRE), but were still insufficiently active for therapeutic use. We have prepared glycopeptide dimers that are covalently linked from the N-terminus to the C-terminus (*i.e.*, a head-to-tail linkage), with the intention of exploiting additional cooperative interactions to enhance activity. Appropriately linked head-to-tail covalent dimers (HT-cov-dimers) may form dimers of HT-cov-dimer complexes as shown in Figure 3a. Contrastingly, HH-cov-dimers are unable to form such dimers, but may form non-covalent dimers where only two of the four vancomycin moieties interact (Figure 3b). It is also possible that both kinds of cov-dimers form polymeric species.

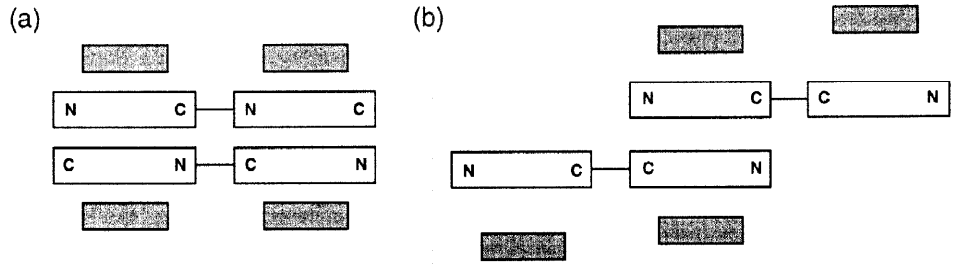
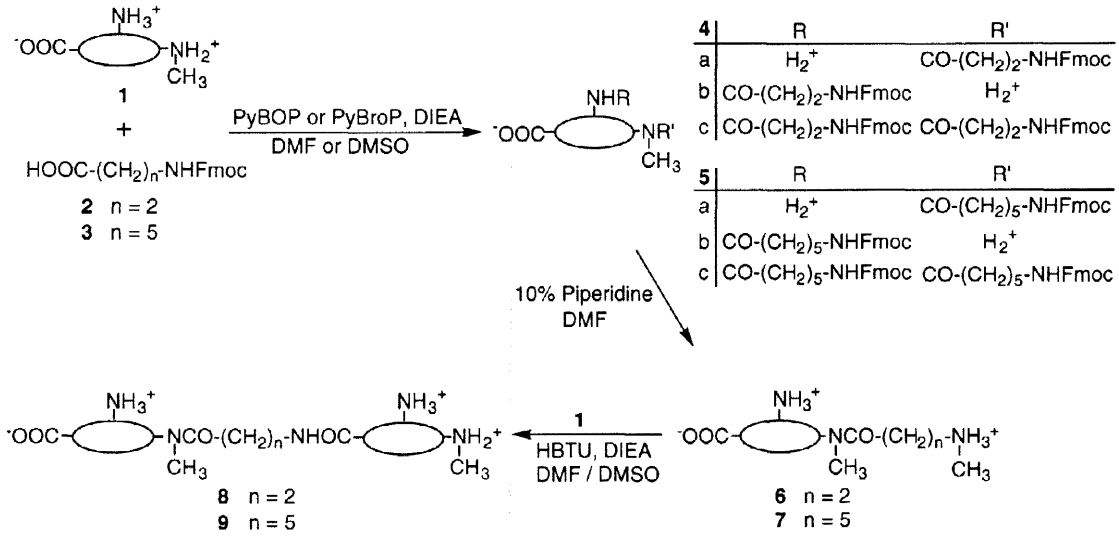


Figure 3. Schematic representation of dimers of cov-dimers bound to ligand. **(a)** Shows a dimer of HT-cov-dimers which can form double the number of hydrogen bonds relative to a normal glycopeptide dimer (compare with Fig. 2b). **(b)** Shows a dimer of HH-cov-dimers where only a partial interaction is possible.

CPK models of two vancomycin molecules linked via β-alanine and 6-aminohexanoic acid showed that these linkers might allow formation of dimers of HT-cov-dimers. The synthesis of HT-cov-dimers of vancomycin is outlined in Scheme 1.[13] The first step involves the coupling of the Fmoc-protected linker to the secondary amino group of the N-terminus of the antibiotic. Although vancomycin also bears a primary amino group at the vancosamine sugar, previous studies showed that the site of acylation depends on the reaction conditions.[14-16] HPLC analysis of test reactions using linkers **2** and **3**, and two different solvents and coupling reagents, confirmed the different reactivity towards the linkers (Table 1). The three possible products were analysed by reversed-phase (RP) HPLC, electrospray ionisation high resolution mass spectrometry (ESI HRMS) and ^{1}H NMR (500 MHz). As reported previously for other acylated glycopeptide antibiotics,[14] we found that the retention times of acylated vancomycins on a RP HPLC column were: acylated at vancosamine < acylated at N-terminus < diacylated.



Scheme 1

Table 1. Ratios of Products after Reversed-phase HPLC Analysis of Coupling Reactions of Vancomycin (**1**) with Linkers **2** and **3**.

Linker	Coupling reagent	Solvent	Recovered **1**	Substitution at N-term.	Substitution at sugar	Disubst.
2	PyBOP	DMF	15	15	40	30
2	PyBroP	DMF	20	60	< 1	20
3	PyBOP	DMF	10	15	45	30
3	PyBroP	DMF	less than 5 % product formation			
3	PyBOP	DMSO	20	30	< 5	50
3	PyBroP	DMSO	less than 5 % product formation			

The two monoacylated compounds could be differentiated in ESI MS by increasing the declustering potential in order to induce in-source fragmentation, leading to loss of the vancosamine moiety (modified or unmodified). Following the loss of this sugar, the molecular weight of the remainder of the molecule depends on the presence of a substituent at the N-terminus. ^{1}H NMR spectra reveal characteristic changes of the chemical shifts of selected protons of vancomycin on acetylation of the vancosamine or of the N-terminus.[15] Consistent with these results, acylation of the N-terminus led to an upfield shift of proton 1b and a downfield shift of protons x_1, x_3 and those of the *N*-methyl group, whereas modification of the vancosamine resulted in a downfield shift of V_{2a} and V_7 (see Figure 1, Table 2).

Table 2. Chemical Shifts of Some Protons that are Affected by Acylation of the Amino Groups of Vancomycin (500 MHz, DMSO-d_6, 300 K).

Proton	**1**	**4a**	**4b**	**4c**	**5a**	**5b**	**8** int.	**8** ext.	**9** int.	**9** ext.
x_1	3.92	5.26	3.96	5.26	5.28	3.96	5.26	3.98	5.29	3.98
1b	1.70	1.44	1.66	1.44	1.43	1.66	1.47	1.67	1.44	1.67
N-Me	2.62	2.87	2.66	2.86	2.87	2.66	2.93	2.65	2.87	2.65
x_3	4.30	4.49	4.25	4.49	4.51	4.26	4.45	4.22	4.48	4.22
V_{2a}	1.75	1.74	1.94	1.98	1.77	2.03	1.73		1.74	
V_7	1.35	1.31	1.44	1.43	1.32	1.44	~1.30		~1.30	

The coupling of the Fmoc-protected linker **2** to vancomycin (**1**) afforded **4a** in 35 % yield and 58 % recovered **1**.[17] Similarly, **5a** was obtained in 33 % yield and 24 % **1** was recovered. Removal of the Fmoc-amino goups with 10 % piperidine in DMF quantitatively afforded **6** and **7**, respectively. In the subsequent couplings, **6** and **7** were added to two equivalents of vancomycin. An excess of vancomycin was used in order to minimise self-coupling of the acylated compound bearing a relatively unhindered primary amino group at the linker moiety. Thus, the HT-cov-dimers **8** and **9** were obtained in 58 % and 36 % yield, respectively.[18]

Compounds **8** and **9** were characterised by ESI HRMS and ^{1}H NMR spectroscopy. The latter clearly showed that two different N-terminal regions were present in the cov-dimers (internal and external, respectively), whereas the vancosamine amino groups were not modified (Table 2).

Although derivatives **4b** and **5b** have potential for the synthesis of covalent vancomycin dimers linked *via* the vancosamine moiety, in this study we concentrated on the head-to-tail dimers because of their potential to form the dimer of dimers shown in Figure 3a.

Further investigations concerning the dimerisation of the novel HT-cov-dimers and their biological activity against vancomycin-susceptible and resistant bacteria are currently being carried out.

Acknowledgements:

T. S. thanks the DAAD (NATO scholarship) and the EPSRC for financial support.

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13. The following abbreviations are used: PyBOP = benzotriazol-1-yl-oxy-tris-pyrrolidino-phosphonium hexafluorophosphate; PyBroP = bromo-tris-pyrrolidino-phosphonium hexafluorophosphate; HBTU = 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate; DIEA = *N*,*N*-diisopropylethylamine.
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17. In a typical procedure, Fmoc-β-alanine (34.6 mg, 111.1 μmol) and PyBroP (51.8 mg, 111.1 μmol) were dissolved in DMF (0.3 ml), and DIEA (17.6 ml, 111.1 μmol) was added. After stirring for 15 min, the solution was added to a solution of vancomycin hydrochloride (100.0 mg, 67.3 μmol) and DIEA (12.9 μl, 74.2 μmol) in DMF (4 ml). After 12 h, the solvent was evaporated under reduced pressure. RP HPLC yielded **4a** (44.2 mg, 35 %) and recovered **1** (65.1 mg, 58 %).
18. General procedure: compound **6** (20.0 mg, 12.2 μmol) was dissolved in a mixture of DMSO (75 μl) and DMF (100 μl), and DIEA (2.5 μl, 14.3 μmol) was added. After 15 min, this solution was slowly added to a mixture of vancomycin hydrochloride (40.0 mg, 26.9 μmol) and HBTU (5.1 mg, 13.4 μmol) in DMSO (150 μl), which had been stirred for 30 min. After 45 min, the solvent was evaporated under reduced pressure. RP HPLC afforded **8** (23.3 mg, 58 %) and recovered **1** (17.1 mg, 30 %).