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Glycopeptide Carboxamides Active against Vancomycin-resistant Enterococci

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Glycopeptide antibiotics were synthesized *via* the PyBOP® mediated condensation of aliphatic, heterocyclic and aromatic amines with the *C*-terminus of vancomycin, LY264826 (A82846B) and semi-synthetic derivatives of these natural products. Amides of LY264826 and vancomycin demonstrated excellent activity against staphylococci and streptococci as compared to the parent natural product. However, the amides of *N*-alkylated LY264826 and *N*-alkylated vancomycin were active against vancomycin-resistant enterococci as well as other Gram-positive pathogens such as *Staphylococcus aureus*, *S. haemolyticus*, *S. epidermidis* and *Streptococcus pneumoniae*.

Vancomycin (Fig. 1) is currently the drug of choice for serious enterococcal and staphylococcal infections. However, the emergence and spread of vancomycin-resistant enterococci (VRE) have resulted in an intensified search for new clinical agents[1,2]. Alkylation of the disaccharide of vancomycin has previously been reported by NAGARAJAN[3,4] to improve activity against VRE, however, the improved level of activity was not sufficient to predict clinical utility[5]. On the other hand, alkylation of the LY264826 disaccharide (Fig. 1) led to compounds with enhanced potency against VRE that could be clinically relevant[6~10]. Thus, this work made evident the relationships of antibacterial activity with the additional *epi*-vancosamine and alkylation of the disaccharide on LY264826.

In an attempt to expand the SAR of the glycopeptide antibiotics and improve activity against VRE while maintaining activity against staphylococci and streptococci, amines were coupled to the carboxy-terminus of vancomycin, LY264826 and alkylated analogs of these glycopeptides. MALABARBA and CIABATTI[11] have reported success using this approach by the synthesis of teicoplanin (Fig. 1) amides with improved antibacterial activity. Herein we report our findings on the modification of the *C*-terminus of glycopeptides. We found that carboxamides of vancomycin were not active against VRE, but did show improved activity against other Gram-positive pathogens. The carboxamides of *N*-alkylated vancomycin and two carboxamides of LY264826 showed activity against VRE along with good activity against other Gram-positive isolates. Several carboxamides of *N*-alkylated LY264826 showed excellent activity against VRE and other Gram-positive pathogens.

Chemistry

The carboxamides were prepared utilizing a PyBOP® (Benzotriazol-1-yloxytripyrrolidinophosphonium hexafluorophosphate) mediated condensation reaction where the glycopeptide, the desired amine, and PyBOP® were dissolved in DMSO or a mixture of DMSO and DMF (Fig. 2)[11~14]. The mixture was stirred at room temperature under inert atmosphere, typically, for 2~24 hours. Upon completion, as determined by reverse phase HPLC, the reaction mixture was diluted with water and lyophilized or was precipitated with ether to give a solid. Preparative HPLC readily provided the desired product in yields ranging from 8~58%. In some instances, an excess of amine and/or PyBOP® was added to aid in product formation. Fast atom bombardment mass spectroscopy (FAB-MS) was utilized to determine the structures of the glycopeptide carboxamides. For example, a molecular ion

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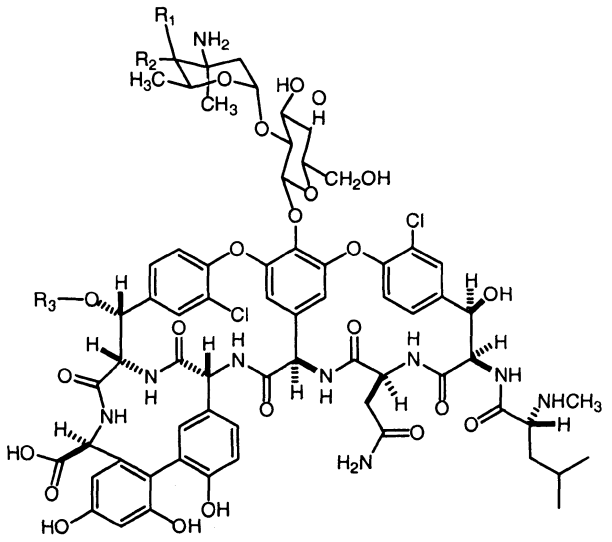
Fig. 1. Structures of vancomycin, LY264826, and teicoplanin.



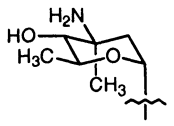
Vancomycin:

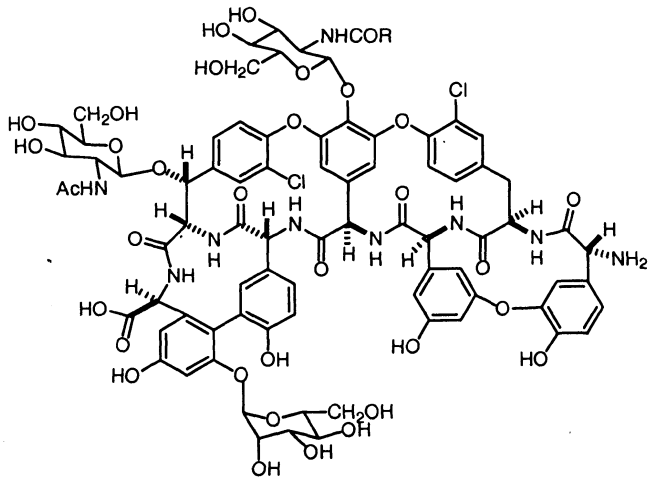
R_1 = OH, R_2 = H, R_3 = H

LY264826 (A82846B):

R_1 = H, R_2 = OH, R_3 = 4-epivancosaminyl

where 4-epivancosaminyl =





Teicoplanin: R =

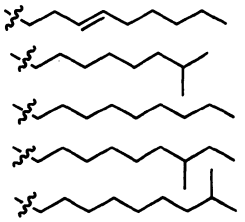


Fig. 2. Carboxamide formation of vancomycin, LY264826 and *N*-alkylated derivatives.

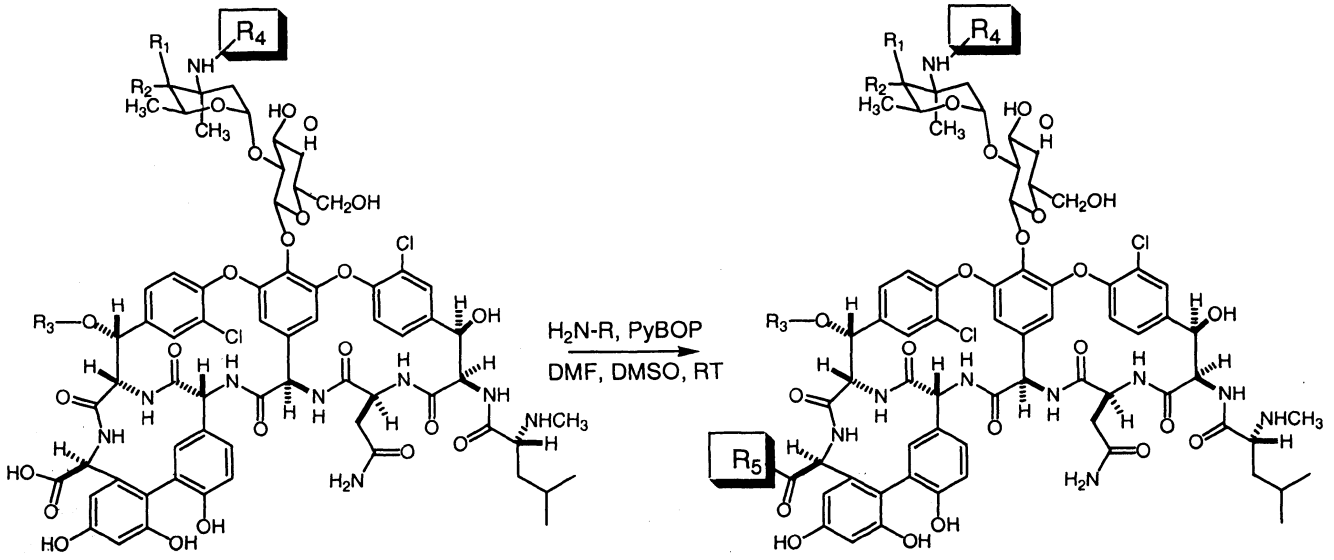
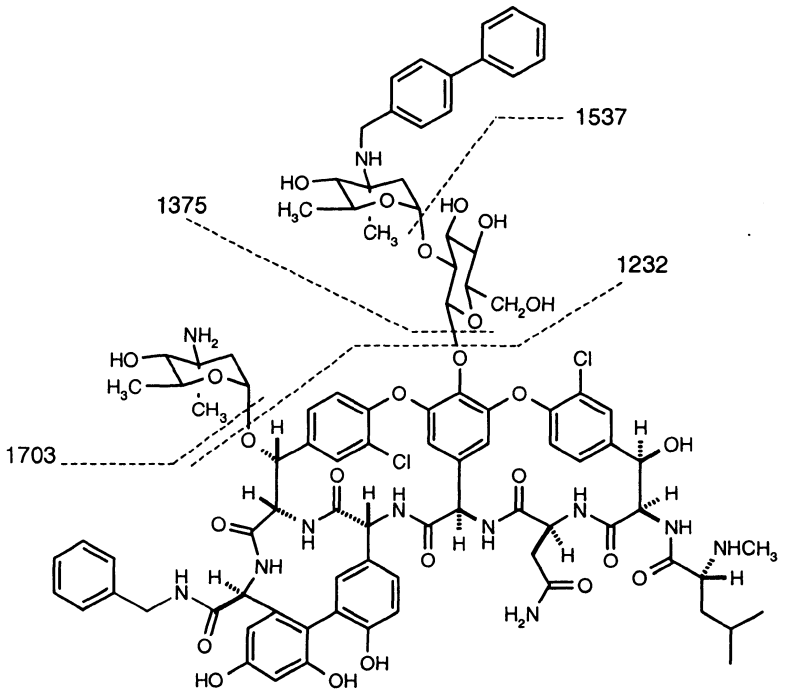


Fig. 3. FAB-MS analysis of compound **12**.



cluster for compound **12** (Fig. 3) at *m/z* 1848 was indicative of product formation. The absence of the glycopeptide aglycone peak (*m/z* 1143) and the presence of *m/z* 1232 corresponding to the carboxamide of the aglycone provided evidence of amide formation. All other ions present were supportive of product formation as previously discussed[9,13,15].

Materials and Methods

Experimental Procedure

FAB-MS spectra were generated using a ZAB-2SE mass spectrometer. Samples were dispersed in thioglycerol with added TFA and introduced into the mass spectrometer on a FAB target. All amines used in this study were commercially available. *N*-Alkylated glycopeptides were prepared as previously described[6,9].

N-(4-Phenylbenzyl) LY264826, Benzylamide, (**12**)

4-Phenylbenzyl LY264826 (500 mg, 0.28 mmol) was added to a mixture of 18.5 ml DMF (anhydrous) and 3.5 ml DMSO (anhydrous). Benzylamine (31 μl, 0.28 mmol) was added to the suspension in one portion followed by PyBOP® (148 mg, 0.28 mmol) and the reaction became homogeneous. The reaction mixture was allowed to stir at room temperature for approximately 24 hours. At that time the solution was diluted with diethyl ether to precipitate the product. The solid was collected by filtration, washed with acetone and dried at 40°C under vacuum. The solid (*ca.* 500 mg) was dissolved in 5 ml of a 1 : 1 mixture of water and acetonitrile. The solution was filtered through a 0.2 μm Anotop 25 Plus® filter disk (Watman International Ltd.) and purified by reverse phase preparative HPLC[9] to give 249 mg (47%) of a white solid; FAB-MS *m/z* 1848 (M+1).

Antimicrobial Activity (*In Vitro*)

In vitro evaluation of staphylococci and streptococci was carried out according to NCCLS recommendations for broth microdilution assay. Data for a single screening reference strain are shown. Strains shown are: *S. aureus* 489 (methicillin-resistant), *S. epidermidis* 270, *S. haemolyticus* 105 and *S. pneumoniae* Parkl[16].

In vitro evaluation of activity against enterococci was carried out by a standard broth microdilution assay using Brain Heart Infusion medium. Strains used were 4 representative vancomycin-resistant enterococci (2 *Enterococcus faecium*, 2 *E. faecalis*) with confirmed *vanA* genotypes, and 5 vancomycin-susceptible isolates, (2 *E. faecium* and 3 *E. faecalis*). Comparative data are presented

Table 1. Amide derivatives of LY264826, [MIC (μg/ml)].

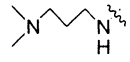
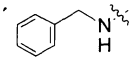
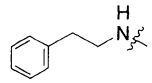
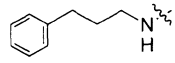
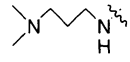
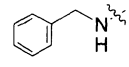
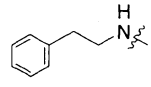
Compound	R_5	Enterococci		Staphylococci		
		VanA-Resistant	Vancomycin-Susceptible	*S. aureus 489*	*S. haemolyticus 105*	*S. epidermidis 270*
LY264826 (1)	HO	45	0.29	≤0.06	1.0	0.25
2		>128	0.50	≤0.06	≤0.06	≤0.06
3		>128	0.22	≤0.06	≤0.06	≤0.06
4		16	0.25	≤0.06	≤0.06	≤0.06
5		11.3	0.22	≤0.06	0.125	≤0.06

Table 2. Amide derivatives of vancomycin, [MIC (μg/ml)].

Compound	R_5	Enterococci		Staphylococci		
		VanA-Resistant	Vancomycin-Susceptible	*S. aureus 489*	*S. haemolyticus 105*	*S. epidermidis 270*
Vancomycin (6)	HO	362	2.6	0.50	4.0	0.50
7		>128	3.0	0.25	0.125	0.25
8		>128	1.74	≤0.06	0.125	0.125
9		>128	1.74	≤0.06	0.125	0.125

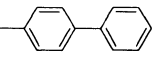
as the geometric mean MIC in μg/ml.

Results and Discussion

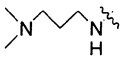
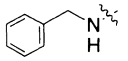
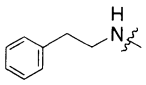
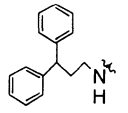
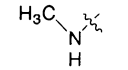
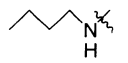
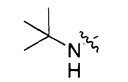
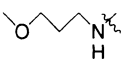
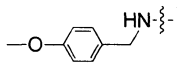
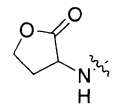
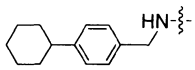
LY264826 and vancomycin are naturally occurring glycopeptides that are similar in structure. The only differences are the addition of an *epi*-vancosamine on the benzylic hydroxyl group of amino acid residue 6 and replacement of the vancosamine of vancomycin with *epi*-vancosamine in LY264826[9]. The activities of these compounds toward the reported bacteria are impressive with one noticeable exception; both compounds show little activity against VRE (Tables 1 and 2). Overall, LY264826 is eight times more active than vancomycin. In our earlier studies, *N*-alkylation of the vancosaminyl and *epi*-vancosaminyl moiety of the respective glycopeptides substantially improved activity against vancomycin sensitive enterococci (VSE) and VRE[6]. For example, N^4-(4-phenylbenzyl)LY264826, **10** (Table 3) is over 75 times more active than its parent, LY264826, and N^4-(4-phenylbenzyl)vancomycin, **28** (Table 5) is over 45 times more active than its parent, vancomycin, **6** (Table 2). We decided to take advantage of these advances in antimicrobial activity and try to further enhance activity by appending groups to the carboxy-terminus of these *N*-alkylated glycopeptides.

Parallel modifications were performed by condensing

Table 3. Amide derivatives of N^4-(4-phenylbenzyl)LY264826, **10**.

Fig. 2, R_4= 

[MIC (μg/ml)]

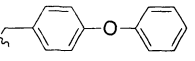
Compound	R_5	Enterococci		Staphylococci		
		VanA-Resistant	Vancomycin-Susceptible	*S. aureus* 489	*S. haemolyticus* 105	*S. epidermidis* 270
10		0.59	0.048	0.125	1.0	0.25
11		1.2	0.054	2.0	0.50	0.50
12		0.84	0.14	4.0	0.50	0.50
13		0.84	0.19	2.0	2.0	1.0
14		1.7	0.87	32	8.0	16
15		1.2	0.041	1.0	0.125	≤0.06
16		2.4	0.11	4.0	0.50	1.0
17		2.0	0.082	1.0	1.0	0.125
18		1.4	0.072	1.0	2.0	1.0
19		1.7	0.13	0.50	1.0	2.0
20		1.7	0.048	1.0	0.50	0.50
21		6.7	0.33	4.0	4.0	2.0

vancomycin (**6**), LY264826 (**1**), compounds **10**, N^4-(4-phenylbenzyl)LY264826, **22**, N^4-(4-phenoxybenzyl)-LY264826, and **28**, N^4-(4-phenylbenzyl)vancomycin, with the following amines: dimethylaminopropylamine, benzylamine and phenethylamine. All of the new derivatives elicited excellent antimicrobial activity against VSE (MIC≤3), staphylococci (MIC≤4) and *S. pneumoniae* (MIC≤0.5). Compound **14** is a noticeable exception with regard to activity against staphylococci (Table 3).

Table 1 illustrates that all amide modifications of LY264826 showed excellent activity against staphylococci and VSE: Specifically, the novel glycopeptides demonstrated activity as good as the parent natural product and improved activity against *S. epidermidis* and *S. haemolyticus*. Furthermore, compounds **2**, **3**, and **4** were active below the lower limit of the current assay against the staphylococci and *S. pneumonia*. The most active aryl amide compounds, **4** and **5**, showed at least a 2-fold improvement in activity against VRE as compared to compound **1**.

Table 2 illustrates that the amide derivatives of vancomycin had a biological profile similar to vancomycin. The amide compounds were slightly more active against staphylococci than vancomycin and were as active as

Table 4. Amide derivatives of N^4-(4-phenyloxybenzyl)LY264826, **22**.

Fig. 2, R_4= 

[MIC (μg/ml)]

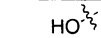
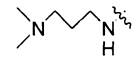
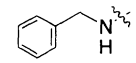
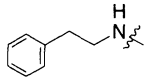
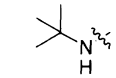
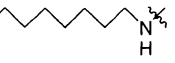
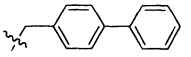
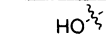
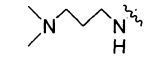
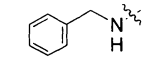
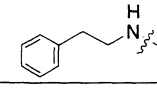
Compound	R_5	Enterococci		Staphylococci		
		VanA-Resistant	Vancomycin-Susceptible	*S. aureus* 489	*S. haemolyticus* 105	*S. epidermidis* 270
22		0.50	0.072	≤0.06	0.5	≤0.06
23		1.4	0.13	2.0	2.0	0.25
24		2.0	0.082	1.0	1.0	0.50
25		2.0	0.019	1.0	0.50	1.0
26		4.0	0.22	2.0	4.0	4.0
27		1.7	0.44	2.0	1.0	1.0

Table 5. Amide derivatives of N^4-(4-phenylbenzyl)vancomycin, **28**.

Fig. 2, R_4= 

[MIC (μg/ml)]

Compound	R_5	Enterococci		Staphylococci		
		VanA-Resistant	Vancomycin-Susceptible	*S. aureus* 489	*S. haemolyticus* 105	*S. epidermidis* 270
28		8.0	0.66	≤0.06	0.25	0.125
29		32	0.66	0.50	0.125	≤0.06
30		19	0.50	0.125	0.125	1.0
31		11	0.44	0.25	0.125	1.0

vancomycin against VSE. They were not active against VRE. Furthermore, the benefits of the additional sugar of LY264826 were seen when comparing the carboxamide series of vancomycin against the carboxamide series of LY264826.

An extensive SAR was performed centered around the phenylbenzyl analog, **10** as shown in Table 3. Compounds **11**, **12**, and **13** retained excellent activity against VRE. However, for the first time in this series, we noticed a slight drop in staphylococcal activity. As the appendage became longer and more branched, for example compound **14**, the activity against the staphylococci further deteriorated, as did the activity against VRE. Of the derivatives made, those containing non-branched, non-substituted aryl side chains possessed slightly better activity against VRE than those containing alkyl groups (compounds **12** and **13** *vs.* **15**~**18**). Overall, the Gram-positive antibacterial activity seen for all compounds in this series does not rival the activity of the

carboxamides of vancomycin and LY264826. Compounds containing hydrogen bonding acceptors (**12** and **16** as compared to **19** and **18**, respectively) offered no clear advantage over compounds without that characteristic. Finally, a combination of aryl and cyclic alkyl moieties offered no improvement in VRE or other Gram-positive activity (compound **21**).

Table 4 shows that the addition of an oxygen linker slightly improved activity of the alkylated LY264826 series against staphylococci (compound **22** *vs.* **10**) and did not affect activity against enterococci. Comparison of compounds **24**, **25** with the corresponding phenylbenzyl analogs, compounds **12**, **13**, indicated that the advantages of the oxygen containing series persisted with *S. aureus*, but decreased activity against VRE. Compounds **26** and **27** demonstrated a decrease in activity against VRE and staphylococci as the carboxamide alkyl chain became branched and longer, respectively.

As seen in the alkylated LY264826 derivatives, alkylation of vancomycin greatly improved activity against VRE as shown in Table 5. The current series displays good activity against the staphylococcal species tested. This finding is in contrast to the corresponding LY264826 molecules (Table 3) where improved activity against VRE was accompanied with a decrease in activity against staphylococci.

The most dramatic improvements in the activity of the parent glycopeptides, vancomycin, **6** and LY264826 **1** are seen by *N*-alkylation with aromatic side chains. The carboxamide-containing glycopeptides exhibited excellent activity against staphylococci, streptococci, and VSE. Furthermore, the *N*-alkylated glycopeptide carboxamides possessed activity against vancomycin-resistant enterococci. The utility of these novel glycopeptide carboxamides is under investigation.

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16) Data not shown since all MIC values are <0.06 for all derivatives except **1**, **6**, **23**, **7**, **8**, **9** which were 0.16, 0.5, 0.125, 0.5, 0.125, 0.25 μg/ml respectively.