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Diversifying Vancomycin via Chemoenzymatic Strategies

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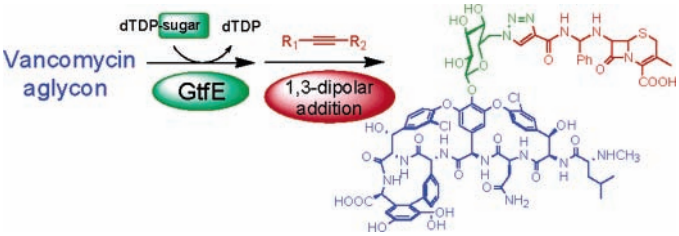
ABSTRACT



The rapid diversification of glycopeptides via glycorandomization reveals that significantly diverse substitutions are tolerated and suggests there may be a synergistic benefit to the construction of mechanistically related natural product core scaffold fusions. This work also further highlights the utility of chemoenzymatic approaches to diversify complex natural product architectures.

The emergence of vancomycin-resistant enterococci and staphylococci (VRE/VRS) clinical isolates in conjunction with the demonstrated antiviral properties of certain glycopeptides continues to promote the search for efficient routes of rapid glycopeptide diversification.[1−3] Toward this goal, recent chemical and chemoenzymatic alterations have revealed that alterations to vancomycin's L-vancosaminyl-1,2-D-glucosyl disaccharide attachment impact both the molecular target and organism specificity.[4−6] Herein, we extend the application of glycorandomization, a process utilizing chemical synthesis to provide a repertoire of unique sugar precursors to three promiscuous enzymes that activate (anomeric sugar kinases,[7] GalK; nucleotidylyltransferases,[8] Ep) and attach (glycosyltransferases,[9] GtfE) these carbohy-

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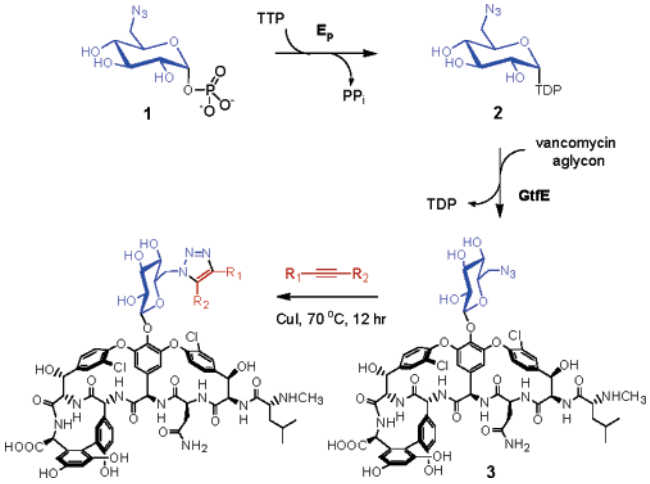
drate libraries to various complex natural product aglycons. The entire process is then followed by downstream chemoselective ligation for further library diversification.[5,10] This present extension of vancomycin glycorandomization surprisingly reveals that a variety of diverse substitutions upon the first sugar attached to vancomycin are tolerated to present analogues that rival the parent natural product.

An engineered *Salmonella* E_p mutant (L89T)[8c] was employed to convert 6-azido-6-deoxy glucose-1-phosphate (Scheme 1, **1**) to the desired nucleotide sugar **2**. Specifically,

Scheme 1. Vancomycin Glycorandomization Process



incubation of 4.2 mM **1**,[5] 4.5 mM TTP, 250 U of E_p, 40 U of inorganic pyrophosphatase in pH 8.0 Tris-HCl buffer (3.8 mL) at 37 °C for 2 h led to the production of **2** in >95% yield based upon HPLC analysis.[5,8] To a portion of this solution (200 μL) was added an equal volume of 2 mM vancomycin aglycon[11] and 20 U of purified vancomycin glucosyltransferase GtfE[12] in 150 mM tricine-NaOH buffer, pH 9, and the reaction mixture was incubated for an additional 12 h at 37 °C to provide the starting material **3** in 58 ± 5% yield for the intended studies. Compound **3** was then further diversified, via 1,3-dipolar cycloaddition,[13] as illustrated in Scheme 1. Regioselectivity of this Huisgen coupling was controlled by the addition of Cu(I) to give the preferred 1,4-disubstituted 1,2,3-triazole in the presence of excess alkyne.[5,14,15] Specifically, the dipolar cycloaddition reactions were accomplished at 70 °C in either methanol or H_2O−DMSO (4:1 or 2:1) with a 150:5:1 alkyne−CuI−azide (3.75 mM) molar ratio. After 12 h, reaction progress was assessed via LC-MS, and HRMS was subsequently utilized to confirm product formation.

For the present study, 24 different alkynes were utilized, and their structures with representative cycloaddition yields are presented in Figure 1. Of these 24, 2 (**26** and **27**) were

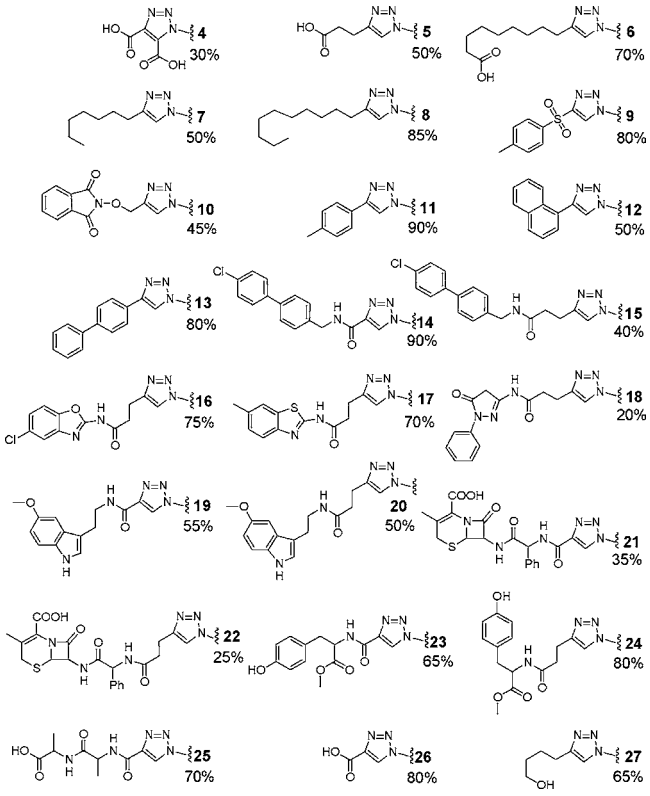


Figure 1. Products of Huisgen 1,3-dipolar cycloaddition reactions. HPLC retention times and MS characterization of **4**−**25** are presented in Table S1 (Supporting Information).

previously reported and have been included for comparison to the earlier study.[5] A number of factors were considered in selecting representative alkynes for the current study. For example, aliphatic and/or "lipidlike" alkynes (Figure 1, **6**−**8**, **26**, **27**) were selected to mimic and enhance the membrane anchor component of various bioactive glycopeptide antibiotics.[4b,6d] Two of these (Figure 1, **26** and **27**) were previously shown to have either enhanced antibiotic activity or very distinct species preference.[5] A variety of aromatic analogues were targeted (Figure 1, **9**−**20**, **23**, **24**), some of which were based upon the known ability of chlorobiphenyl substitution to alter glycopeptide mechanism of action (Figure 1, **12**−**15**).[6d,16] The remainder within this set were

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loosely based upon the reported enhancement of glycopeptide antibiotic dimerization via substitution with certain hydrogen-bonding pharmacophores (Figure 1, **16**−**25**)[17] and/or the ease of incorporating potential antiviral pharmacophores (Figure 1, **17**).[18] In addition, the simplicity of this chemistry also allows one to probe the potential advantage of covalently attaching two antibiotics with distinct mechanisms of cell wall biosynthesis inhibition (for example, a glycopeptide and β-lactam, Figure 1, **21** and **22**) or the potential of enhancing interactions with alternative targets (for example, by appending with D-ala-D-ala, Figure 1, **25**). In most cases, the reactions proceeded as expected, and even a few representative alkynes lacking adjacent electron-withdrawing substituents led to products (**8** and **24**, for example) in high yield.[19,20]

The entire set of analogues was tested for their antibacterial activity, and those analogues showing favorable activities against methicillin-resistant *Staphylococcus aureus*, vancomycin-sensitive *Enterococcus faecalis*, and/or *Enterococcus faecium* are highlighted in Table 1.[21] In comparison to the parent natural product or **3**, three analogues (**6**, **7**, and **26**) show slightly better activity against all three pathogens. While the carboxylate of **26** was previously shown to be essential for its enhanced anti-MRSA activity,[5] a comparison of **4**−**8**, **26**, and **27** may implicate side chain length as favoring the desired activities. Interestingly, triazole substitution via long alkyl chains (e.g., **7** and **8**) favors *S. aureus* activity, while a carboxylate extension (**6**) to this unit slightly favors activity toward *Enterococcus*. Covalent fusion of two cell-wall-directed agents, a glycopeptide and a β-lactam, presented chimeric natural product analogues (**21** and **22**), one of which with slightly enhanced activity.[22] However, these chimera appear to be quite sensitive to linker length, as the synergistic effects can be abolished via the simple addition of an ethyl bridge. It is also noteworthy that the chlorobiphenyl and fused aromatic variants **11**−**15** surprisingly lacked beneficial contributions.

Table 1. Minimum Inhibitory Concentrations (μg mL^{-1})

compd	*S. aureus* (ATCC 700699)	*E. faecalis* (ATCC 700802)	*E. faecium* (ATCC 700221)
Van	12 ± 3^c	23 ± 4^b	23 ± 2^b
3	21 ± 3^b	21 ± 2^b	23 ± 5^b
6	11 ± 4^a	10 ± 1^a	10 ± 3^a
7	7 ± 1^a	12 ± 1^a	11 ± 2^a
8	13 ± 3^c	$>24^b$	$>24^b$
21	$>24^b$	16 ± 1^b	12 ± 2^c
26	10 ± 1^a	20 ± 3^b	19 ± 2^b

[a] Range = 6−12 μg. [b] Range = 12−24 μg. [c] Range = 6−24 μg.

In conclusion, the rapid diversification of glycopeptides via glycorandomization reveals that significantly diverse substitutions are tolerated and can lead to analogues that rival the parent natural product. The present study also illustrates that there may be a synergistic effect of mechanistically related natural product core scaffold chimera and highlights the benefits of glycorandomization in generating such complex natural product analogues.

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Supporting Information Available: Experimental methods, table of Huisgen reaction yields, HPLC retention times, and MS characterization of **5**−**25**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(20) Under the conditions described, notable degradation was observed with most β-lactams tested, including derivatives of 6-aminopenicilanic acid and 7-aminocephalosporanic acid (= 5% yield). Under these conditions, decarboxylation en route to **4** was also observed to give **26** as the predominate product (~45%).

(21) Compounds not specifically listed in Table 1 exceeded 24 μg mL^{-1} in all three strains.

(22) For comparison, the MIC of a control mixture containing an equimolar mixture of **3** and each derivatized cephalexin exceeded 24 μg mL^{-1} (of each compound) in all three strains.