JACS
JOURNAL OF THE AMERICAN CHEMICAL SOCIETY
Article
pubs.acs.org/JACS

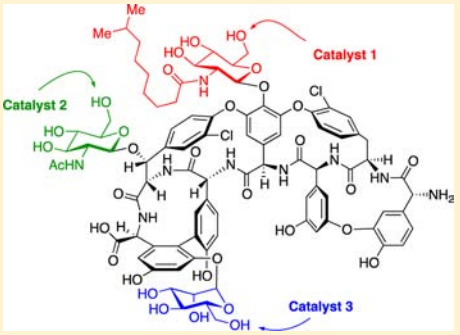
Asymmetric Catalysis at a Distance: Catalytic, Site-Selective Phosphorylation of Teicoplanin

Sunkyu Han and Scott J. Miller*

Department of Chemistry, Yale University, P.O. Box 208107, New Haven, Connecticut 06520-8107, United States

Supporting Information

ABSTRACT: We report three distinct, peptide-based catalysts that enable site-selective phosphorylation of three distinct hydroxyl groups within the complex glycopeptide antibiotic teicoplanin A_2-2. Two of the catalysts are based on a design that capitalizes on a catalyst−substrate interaction that mimics the biological mechanism of action for teicoplanin. These catalysts are based on a DXaa−DXaa peptide motif that is known to target the teicoplanin structure in a specific manner. The third was identified through evaluation of a set of catalysts that had been developed for historically distinct projects. Each catalyst contains additional functionality designed to dispose a catalytic moiety (a nucleophilic alkylimidazole) at a different region of the glycopeptide structure. A combination of mass spectrometry and 2D-NMR spectroscopy allowed structural assignment of the distinct phosphorylated teicoplanin derivatives. Mechanistic studies are also reported that support the hypotheses that led to the discovery of the catalysts. In this manner, small molecule catalysts have been achieved that allow rational, catalytic control over reactions at sites that are separated by 11.6, 16.5, and nearly 17.7 Å, based on the X-ray crystal structure of teicoplanin A_2-2. Finally, we report the biological activity of the new phosphorylated teicoplanin analogs and compare the results to the natural product itself.



INTRODUCTION

Site-selective bond formation at redundantly occurring functional groups within complex molecules is a formidable challenge that, until recently, was largely viewed as the purview of enzymatic catalysis.[1−5] As the sites become more remotely localized, and when the stereochemical relationship is not enantiotopic, particular challenges may exist. Catalysts that target large molecules may not exhibit high preferences for one sector of a structure over the other, which will increase the difficulty of functionalizing an intrinsically less reactive site in the presence of competing more reactive sites.[6] Even so, advances have been recorded. Desymmetrization reactions provide inspirational examples of equivalent functional group differentiation, even when reaction sites are somewhat remotely localized.[7−9] Site-selective reactions of increasingly complex molecules are also being developed.[6,10−13] In our own laboratory, we observed that peptide-based catalysts could effect site-selective phosphorylation of the enantiotopic 1- and 3-positions of the inositol ring, with the reacting sites separated by 2.7 Å based on the X-ray crystal structure of *myo*-inositol[14] (Figure 1a).[15,16] Later, we developed catalysts that were effective for desymmetrization of a pharmaceutically relevant diarylmethane bis(phenol) where the competing enantiotopic sites were separated by nearly a nanometer (Figure 1b).[17,18] More recently, we have endeavored to discover catalysts that enable rapid diversification of truly complex molecules, including the complex glycopeptide antibiotics vancomycin[11,12] and teicoplanin.[13] For example, we found two catalysts that can target the G6-hydroxyl group of a vancomycin derivative (**1**) with high selectivity, and another that functionalizes the Z6-position with high selectivity (Figure 1c). These findings, in the context of thiocarbonylation reactions, enabled the synthesis of the corresponding deoxyvancomycins. Distinct from vancomycin, teicoplanin A_2-2 (**2**, Figure 1d) consists of three different sugar moieties located remotely from each other. Notably, the primary hydroxyl groups of each sugar subunit are separated by 11.6, 16.5, and 17.7 Å, based on the crystal structure of teicoplanin A_2-2 (**2**, Figure 1d).[19] This increased molecular complexity of teicoplanin A_2-2 (**2**) necessitated the development of novel catalysts that can selectively functionalize the previously unexplored sugar units. In the present study, we report the identification of three distinct catalysts that exhibit high selectivity for phosphorylation of hydroxyl groups located at three different sugar moieties of teicoplanin A_2-2 derivative **3**.

Our interest in the glycopeptides stems from (a) the chemical challenge of developing effective site-selective catalysts that tolerate the dense arrays of chemical functionality, including the intrinsically varied reactivity of all the competing functional groups, and (b) the opportunity to contribute new analogs that might reveal aspects of the structure-activity relationships for these important molecules. Vancomycin (**4**, Figure 2), teicoplanin,[20] and their relatives have emerged as important compounds in the campaign against antibiotic resistance.[21−25] Studies of their chemical synthesis,[26−32] semisynthesis,[33−35] and biosynthesis[36−40] continue to contrib-

Received: June 17, 2013
Published: August 7, 2013

dx.doi.org/10.1021/ja406067v | J. Am. Chem. Soc. 2013, 135, 12414−12421

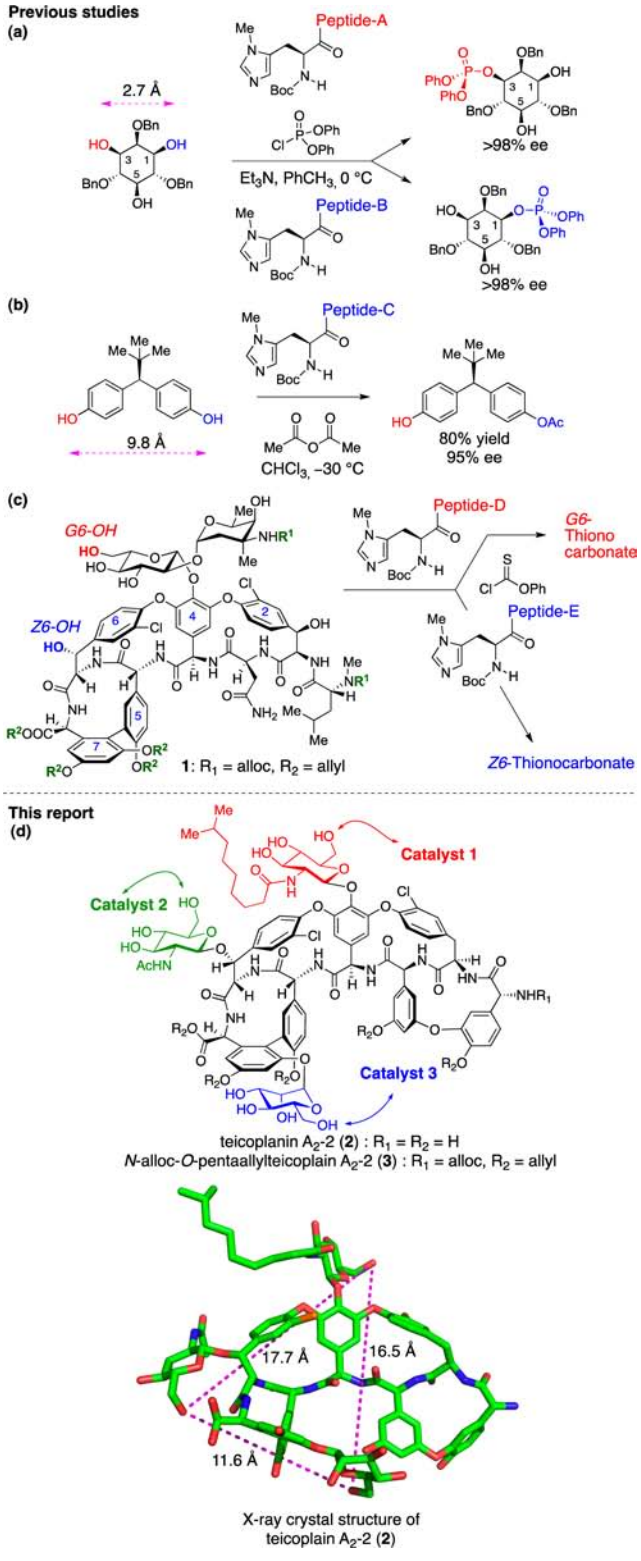


Figure 1. Representative examples of substrates for site-selective reactions with competing functional groups at a distance. (a) Enantioselective phosphorylation of inositol derivative. (b) Desymmetrizaton of bis(phenol). (c) Site-selective thiocarbonylation of vancomycin derivative **1**. (d) Site-selective functionalization of teicoplanin A_2-2 derivative **3** and X-ray crystal structure of teicoplanin A_2-2 (**2**, adapted from PDB 2XAD).[19]

ute mightily to our understanding of their modes of action, and also to promising new therapies in clinical settings. Teicoplanin,

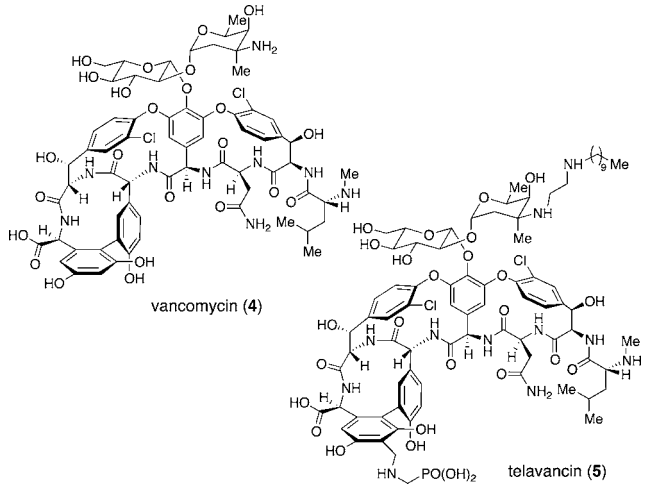


Figure 2. Structure of the representative glycopeptide drugs vancomycin (**4**) and telavancin (**5**).

in particular, is an attractive compound for our interests, not only because of its high degree of molecular complexity but also its higher degree of potency as an antibiotic.[41] Yet, the onset of resistance to vancomycin (**4**), teicoplanin, and their relatives motivates further the objective of developing new chemistry to manipulate the scaffold.[42,43] Among the interesting glycopeptides in the clinic is the compound telavancin (**5**, Figure 2), a phosphonated vancomycin derivative.[44] In part due to its phosphonated nature, we set out to learn if it might be possible to develop catalysts capable of catalytic phosphorylation of teicoplanin derivative in a site-selective manner.

RESULTS AND DISCUSSION

Initial Design of the Catalysts. The mechanism of antibacterial activity for vancomycin or teicoplanin has been studied extensively.[45] Among the conclusions is the canonical binding of the glycopeptides to the growing bacterial cell wall through a specific association between vancomycin/teicoplanin and the DAla–DAla moiety of the growing peptidoglycan. Five hydrogen bonds between the antibiotic and substrate play a key role in inhibiting the cell wall synthesis of the bacteria. We have been exploring the possibility of developing catalysts that specifically modify the glycopeptides by mimicking these biologically validated interactions. For example, we developed catalysts for selective thiocarbonylation[12] and bromination[11] of vancomycin, and bromination[13] of teicoplanin on this basis.

Loll and co-workers reported the X-ray crystal structure of the complex of DAla–DAla peptide moieties and teicoplanin using a carrier protein strategy (Figure 3a).[46] Based on this crystal structure data, we envisioned that a superposition of the terminal $DAla_1$ with a D-π-methylhistidine (DPmh) would bring the nucleophilic alkylimidazole group in close proximity to the *N*-decanoylglucosamine unit (Figure 3b, *N*-decanoylglucosamine shown in red). Alkylimidazoles, such as that localized in Pmh, have been shown to be effective nucleophilic catalysts for the delivery of a variety of functional groups to hydroxyl sites.[6,12,16,17,47,48] As noted above, these residues are quite well suited for catalytic phosphoryl group transfer, and they may also be considered, in some sense, mimics of kinases.[49] Hence, we envisioned that a catalyst with a Xaa-DAla-DPmh sequence would be optimal for the functionalization of the hydroxyl groups present in the "top/red" *N*-decanoylglucosamine moiety (Figure 3b). Indeed, this catalyst design was applied to the

dx.doi.org/10.1021/ja406067v | J. Am. Chem. Soc. 2013, 135, 12414–12421

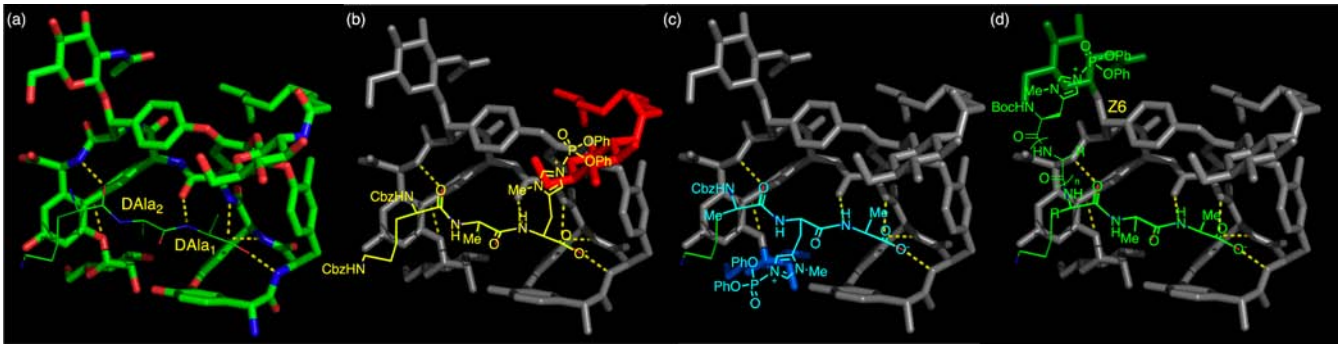


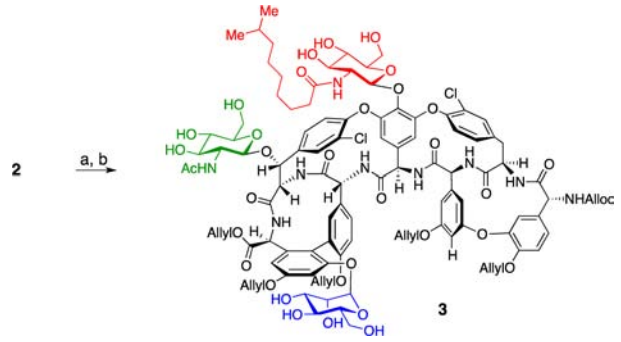
Figure 3. Design of peptide catalysts based on the X-ray crystal structure of DAla−DAla containing peptide bound to teicoplanin: (a) X-ray crystal structure of Lys-DAla-DAla-O$^-$ (**6**, lines) bound to 6c-dechloroteicoplanin (**7**, sticks, adapted from PDB 3VFG).[46] (b) Proposed model for delivery of the diphenylphosphoryl group to the *N*-decanoylglucosamine ring by Z-Lys(Z)-DAla-DPmh-O$^-$. (c) Proposed model for delivery of the diphenylphosphoryl group to the mannose ring by Z-Ala-DPmh-DAla-O$^-$. (d) Proposed model for delivery of the diphenylphosphoryl group to the *N*-acetylglucosamine ring by Pmh-(Xaa)$_n$-DAla−DAla-O$^-$.

thiocarbonyl group transfer at the G6 position of vancomycin derivative **1** (Figure 1c).[12] For the selective functionalization of the mannose ring ("bottom/blue" sugar), we envisioned a catalyst where the DAla$_2$ is substituted with DPmh (Figure 3c). The superposition of the DPmh residue in this locus suggested that the binding of Xaa-DPmh-DAla peptide to teicoplanin would position the nucleophilic alkylhistidine moiety proximal to the mannose sugar. The design of the peptide-based catalyst for selective functionalization of the *N*-acetylglucosamine ring ("left/green" sugar) was most challenging due to the long distance from the DAla−DAla binding pocket to the sugar moiety (Figure 3d). The X-ray crystal structure and molecular model of teicoplanin A$_2$-2 (**2**) bound to DAla−DAla based peptide show that the *N*-acetylglucosamine ring is positioned away from the peptide moiety due to the *R*-configuration of the Z6 carbon, which rendered the design of catalyst selective for this sugar unit uniquely challenging. We envisioned that an extended, well-defined peptide sequence, beyond a DAla−DAla initiator moiety, would be required for this goal.

Development of the Catalyst for Selective Phosphorylation of the "Top/Red" *N*-Decanoylglucosamine. Our study commenced with the proper protection of the teicoplanin A$_2$-2 (**2**). The primary amine present in teicoplanin A$_2$-2 (**2**) was efficiently protected with *N*-(allyloxycarbonyloxy)-succinimide in the presence of sodium bicarbonate. Next, the carboxylate and phenolic hydroxyl groups were allylated with allyl bromide and cesium carbonate (Scheme 1).[50−52] These functional groups had a dual role of masking the reactive amine and phenol functionalities and increasing the solubility of the substrate in organic solvents.

We then began to explore the phosphorylation of **3** (eq 1). When teicoplanin derivative **3** was treated with excess diphenyl phosphoryl chloride (DPCP, 6 equiv) and 1,2,2,6,6-pentamethylpiperidine (PEMP, 8 equiv) in the absence of nucleophilic catalyst, no reaction occurred (Table 1, entry 1). On the other hand, in the presence of *N*-methylimidazole (NMI, 1 equiv), DPCP (6 equiv), and PEMP (8 equiv), partial conversion to various mono- and bisphosphorylated products was observed (Table 1, entry 2). We could identify three different teicoplanin derivatives, each monophosphorylated at the primary hydroxyl groups of the *N*-decanoylglucosamine, *N*-acetylglucosamine, and mannose sugars (**8**/**10**/**9** = 2.4:1:2.2), and a bisphosphorylated product **12**. A description of the structural assignments of the products is presented below (*vide infra*).[52] In terms of catalyst-dependent selectivity, when the reaction

Scheme 1. Synthesis of *N*-alloc-*O*-pentaallyl-teicoplanin (3)[a]



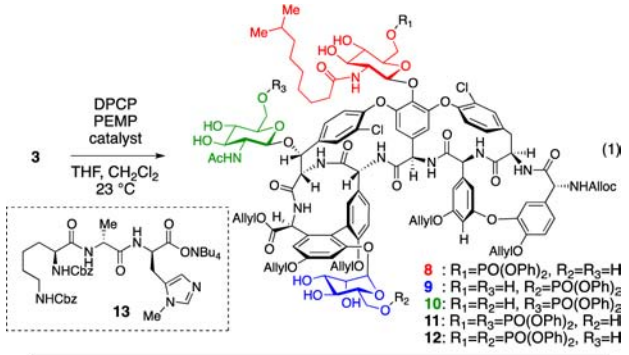
[a]Reagents and conditions: (a) *N*-(allyloxycarbonyloxy)succinimide, NaHCO$_3$, H$_2$O, 1,4-dioxane, 23 °C (89%). (b) Allylbromide, Cs$_2$CO$_3$, DMF, 50 °C (50%).

was run in the presence of designed catalyst Cbz-Lys(Cbz)-DAla-DPmh-ONBu$_4$ (**13**; Table 1, entry 3), phosphorylation occurred selectively at the primary alcohol of the "top/red" *N*-decanoylglucosamine, in accord with our hypothesis (Figure 3b). The reaction could be run with 10 mol % of the peptide catalyst **13** to give 42% isolated yield of the "top/red" *N*-decanoylglucosamine phosphorylated product **8**. This result is consistent with the selective G6 thiocarbonylation of vancomycin derivative **1** using the same catalyst **13**.[12] The portability of peptide-based catalyst from vancomycin thiocarbonylation to teicoplanin phosphorylation implies the potential application of this catalyst to broad arrays of glycopeptides for various transformations, enabling the generation of new antibiotic derivatives.[21,23,25] It is interesting to note that the HPLC traces for these catalytic runs reflect reactions that are quite clean under the conditions we have developed. The isolated yield of 42% (as opposed to a higher yield) may reflect an intrinsic loss of material during the C18 HPLC purification process, as well as formation of undetermined minor byproducts.

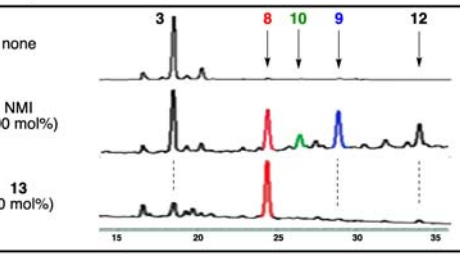
Development of the Catalyst for Selective Phosphorylation of the "Bottom/Blue" Mannose. We next sought to develop a catalyst for selective phosphorylation of the mannose ring of teicoplanin derivative **3** (eq 2). We first tested the catalyst **14**, which contained DPmh in the second amino acid and was therefore expected to functionalize the mannose unit of **3** (Figure 3c). Interestingly, when teicoplanin derivative **3**

dx.doi.org/10.1021/ja406067v | J. Am. Chem. Soc. 2013, 135, 12414−12421

Table 1. Development of a "Top/Red" *N*-Decanoylglucosamine-Selective Phosphorylation Catalyst



Entry	catalyst	HPLC trace[a]
1	none	
2	NMI (100 mol%)	
3[b]	**13** (10 mol%)	



Reaction Conditions: DPCP (6 equiv), PEMP (8 equiv), catalyst, THF, CH_2Cl_2, 23 °C.
a. HPLC traces were recorded at 280 nm. b. **8** was isolated in 42% yield.

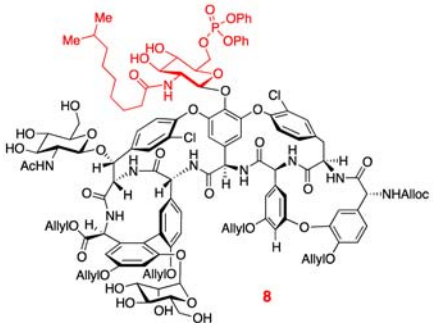
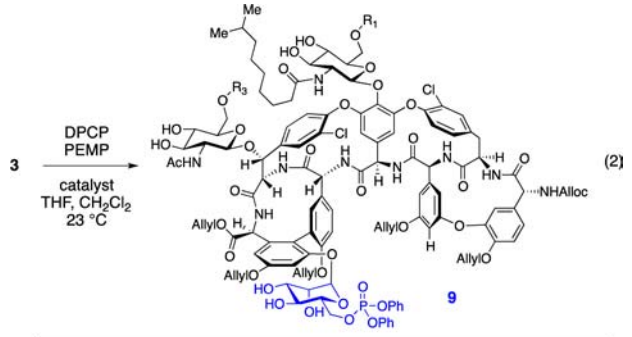
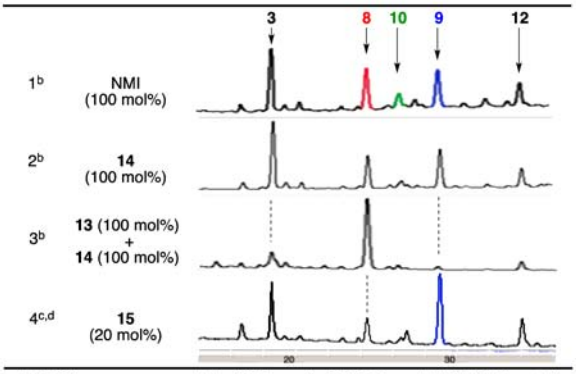


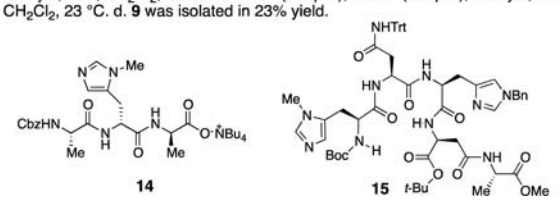
Table 2. Development of a "Bottom/Blue" Mannose-Selective Phosphorylation Catalyst



Entry	catalyst(s)	HPLC trace[a]
1[b]	NMI (100 mol%)	
2[b]	**14** (100 mol%)	
3[b]	**13** (100 mol%) + **14** (100 mol%)	
4[c,d]	**15** (20 mol%)	



a. HPLC traces were recorded at 280 nm. b. DPCP (6 equiv), PEMP (8 equiv), catalyst, THF, CH_2Cl_2, 23 °C. c. DPCP (3 equiv), PEMP (4 equiv), catalyst, THF, CH_2Cl_2, 23 °C. d. **9** was isolated in 23% yield.



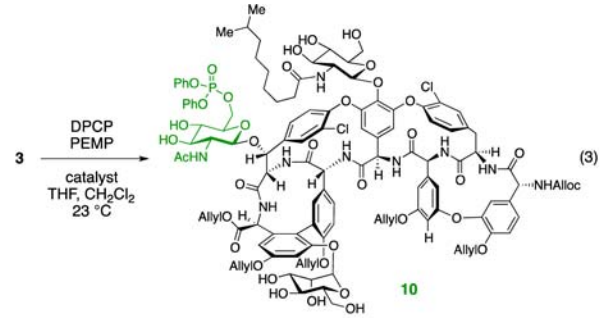
was treated with 1 equiv of peptide **14** along with excess DPCP and PEMP, mono- and bisphosphorylated products were obtained with a similar distribution ratio to that of the NMI control experiment (Table 2, entries 1 and 2). We reasoned that the DPmh caused a weaker binding between the peptide and the teicoplanin derivative **3**, resulting in nonselective reaction. In fact, in 1971, Nieto and Perkins reported that the association constant of Ac-Lys(Ac)-DAla-DAla (5.9×10^5 L/mol) to structurally relevant ristocetin B is ten times larger than that of Ac-Lys(Ac)-DLeu-DAla (5.8×10^4) while it is similar to that of Ac-Lys(Ac)-DAla-DLeu (6.1×10^5).[53] These historical results are consistent with the successful design of catalyst **13** (for the "top/red" sugar) and less successful catalyst **14** (designed for the "bottom/blue" sugar). Moreover, when teicoplanin derivative **3** was challenged with a mixture of catalysts **13** and **14** (**13**/**14** = 1:1), DPCP, and PEMP, only the "top/red" *N*-decanoylglucosamine phosphate **8** was observed (Table 2, entry 3), consistent with our rationalization.

In light of these results, we performed a screen of 15 additional catalysts available from previous projects in our group. Intriguingly, we found that peptide **15** (20 mol %) gave the desired mannose phosphorylated teicoplanin derivative **9** as a major product (23% isolated yield, Table 2, entry 4). Notably, only 3 equiv of DPCP were employed in this experiment, a function of the very high activity associated with catalyst **15**. (Six equivalents of phosphorylating agent resulted in the formation of overphosphorylated teicoplanin derivatives.) The identification of catalyst **15** also presents philosophical questions. For example, catalyst **15** was initially reported in 2001 and later exploited for the desymmetrization of *myo*-inositol derivatives.[15,16,54−58] The fact that it emerges in the context of selective derivatization of teicoplainin analog **3** highlights its portability to a very different scaffold, suggesting that it may be "privileged" in some sense.[59]

Development of the Catalyst for Selective Phosphorylation of the "Left/Green" *N*-Acetylglucosamine. We next set our goal to develop a catalyst that can selectively phosphorylate the *N*-acetylglucosamine ring of teicoplanin derivative **3** (eq 3). Notably, the primary hydroxyl group of the "left/green" *N*-acetylglucosamine is the least reactive among those of three different sugar units present in **3** upon phosphorylation in the presence of NMI (Table 3, entry 1). The intrinsic low reactivity of the *N*-acetylglucosamine, coupled with its remote location to the DAla−DAla binding pocket (*vide supra*), presented significant challenge regarding the development of a catalyst selective for this sugar unit. In fact, the localization of the "left/green" sugar is aptly described as on the opposite hemisphere of the teicoplanin core relative to the DAla−DAla binding site. Thus, a major alteration to our catalyst design strategy was necessary to target this site.

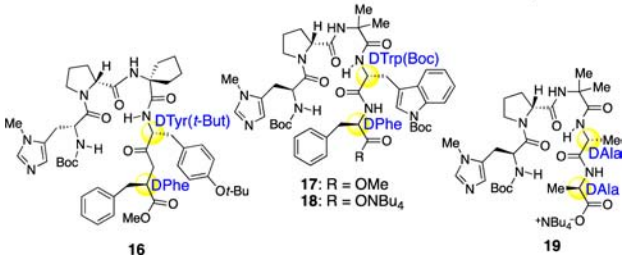
dx.doi.org/10.1021/ja406067v | J. Am. Chem. Soc. 2013, 135, 12414−12421

Table 3. Development of a "Left/Green" Acetylglucosamine-Selective Phosphorylation Catalyst



Entry	catalyst	HPLC trace[a]
1	NMI (100 mol%)	
2	**16** (100 mol%)	
3	**17** (100 mol%)	
4	**18** (100 mol%)	
5[b]	**19** (30 mol%)	

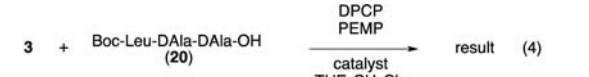
Reaction Conditions: DPCP (6 equiv), PEMP (8 equiv), catalyst, THF, CH_2Cl_2, 23 °C. a. HPLC traces were recorded at 280 nm. b. **10** was isolated in 41% yield.



A clue emerged from our evaluation of the 15 additional peptide catalysts noted above. In this screen, we identified catalysts **16** and **17**, which showed modest increases in the extent of functionalization of the "left/green" sugar (leading to phosphorylated product **10**) in comparison to NMI (Table 3, entries 1−3). In particular, peptide **16** showed moderate conversion to the top, left, and bottom phosphates **8**, **10**, and **9** (**8**/**10**/**9** = 1:1:1.2); peptide **17** afforded *N*-acetylglucosamine phosphorylated teicoplanin derivative **10** as a major product, for the first time in our studies. Interestingly, both peptides contain β-turn-promoting motifs,[60−63] possibly rendering structural rigidity to the catalysts via internal hydrogen bonding. Also striking, two D-amino acid residues were present at the C-terminal end of these hits (Table 3). We also noticed that peptide **18**, synthetized via hydrolysis of the C-terminal methyl ester of peptide **17**, showed increased selectivity and conversion to **10** (Table 3, entry 4). These observations, in conjunction with our initial peptide design plan for the left sugar (Figure 3d), stimulated us to merge these concepts in a new catalyst. These concepts culminated in the design of peptide **19**, which merges both the DAla−DAla binding moiety and a β-turn-promoting motif within a unique catalyst. In accord with our design, when protected teicoplanin **3** was treated with DPCP and PEMP in the presence of 30 mol % catalyst **19**, the desired left phosphorylated teicoplanin derivative **10** was obtained with high selectivity and good conversion (41% isolated yield, Table 3, entry 5). Notably, in subsequent studies, we found that any stereochemical changes in the Pmh or DPro residues (Pmh-Pro, DPmh-DPro, or DPmh-Pro instead of Pmh-DPro) of the catalyst resulted in significantly lower reactivity and selectivity for this reaction.[52] These observations reflect the importance of a stereochemical match of the catalyst to selectively phosphorylate the least reactive carbohydrate moiety, the "left/green" *N*-acetylglucosamine residue, within teicoplanin. In addition, they document a unique strategy for targeting hydroxyl sites that are substantially distal from the DAla−DAla binding site of teicoplainin, with the capacity to reach a number of angstroms beyond the binding site itself. The merger of the DAla−DAla concept with the β-turn-promoting motif we have studied extensively previously[64] thus constitutes a potentially new approach for accessing catalytic reactivity at quite remote sites.

In order to gain further insights about the importance of the DAla−DAla binding to the substrate for the catalysis, we envisioned experiments that might be conducted in the presence of competitive inhibitors for the catalysts. Thus, we ran the phosphorylation reaction of teicoplanin derivative **3** in the presence of 1 equiv of Boc-Leu-DAla-DAla-OH peptide (**20**, eq 4), a putative competitor for the substrate-binding site. Under these conditions, when teicoplanin derivative **3** was treated with DPCP and PEMP in the presence of peptide catalyst **13**, no reaction was observed (Table 4, entry 1).[65]

Table 4. Importance of the DAla−DAla Binding for Catalysts 13 and 19



Entry	catalyst	HPLC trace[a]
1[b]	**13** (10 mol%)	
2[b]	**19** (50 mol%)	
3[c]	**15** (20 mol%)	

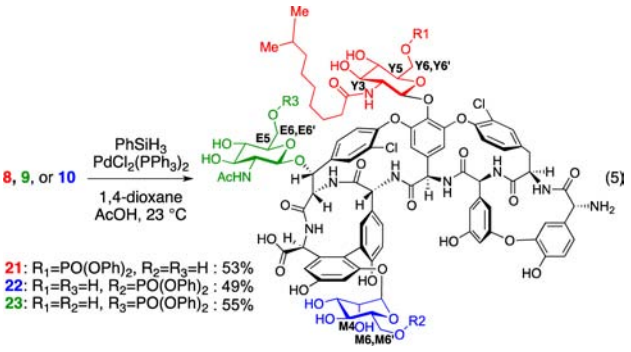
[a]HPLC traces were recorded at 280 nm. [b]DPCP (6 equiv), PEMP (8 equiv), **20** (1 equiv), catalyst, THF, CH_2Cl_2, 23 °C. [c]DPCP (4.0 equiv), PEMP (5.3 equiv), **20** (1 equiv), catalyst, THF, CH_2Cl_2, 23 °C.

Similarly, in the presence of 1 equiv of competitive Boc-Leu-DAla−DAla-OH (**20**) peptide, 50 mol % of left-sugar selective peptide **19** exhibited significantly lowered reactivity (Table 4, entry 2). On the other hand, catalyst **15**, which does not contain a DAla−DAla based binding site, gave phosphorylated products **8** and **9**, albeit in lower conversion and selectivity, perhaps due to the conformational change of the teicoplanin derivative upon binding to peptide **20** (Table 4, entry 3).[66] These experiments are consistent with the targeted notions of teicoplanin/catalyst binding at the heart of our experimental designs.

dx.doi.org/10.1021/ja406067v | J. Am. Chem. Soc. 2013, 135, 12414−12421

Deprotection of the allyl/alloc Protecting Groups and Structural and Biological Characterization. With efficient synthetic access to three monophosphorylated teicoplanin derivatives, **8**, **9**, and **10**, the deprotection conditions of the alloc and allyl groups were investigated. Palladium-mediated reduction using phenylsilane as reductant, conditions previously used in our group for the vancomycin derivatives deprotection,[12] cleanly afforded the alloc and allyl deprotected products **21**, **22**, and **23**. Straightforward reverse phase HPLC isolations resulted in yields of 53, 49, and 55%, respectively (Scheme 2). Structural assignments of phosphorylated

Scheme 2. Deprotection of the alloc and allyl Protecting Groups



teicoplanin derivatives were realized using mass spectrometry (MS) and NMR spectroscopy. ESI MS/MS data of **21**, **22**, and **23** provided distinct fragmentation patterns diagnostic of loss of each of the sugar moieties, which enabled the assessment of the site of phosphorylation (Figure 4). Furthermore, HSQC, HMBC, COSY, NOESY, and TOCSY data enabled unambiguous assignments of all proton and carbon chemical shifts of the sugar regions of **21**, **22**, and **23**. Figure 4 shows the HSQC overlay of the sugar regions of each phosphorylated teicoplanin (gray) with teicoplanin A_2-2 (**2**, colored) (Figure 4).[52] In all cases, the biggest chemical shift difference was that of the methylene (Y6,6′, M6,6′, E6,6′) protons and carbons consistent with the phosphorylation at the primary hydroxyl groups.

As a mode of biological characterization, we evaluated each of the new diphenylphosphoryl derivatives of teicoplanin A_2-2 for their antibacterial activities (Table 4). The newly synthesized analogs **21**, **22**, and **23** showed attenuated activities in four of the standard strains that were examined (methicillin-susceptible *Staphylococcus aureus* (MSSA), methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-susceptible *Enterococcus* (VanS), and vancomycin-resistant *Enterococcus* VanB (VanB)) and somewhat improved activity against vancomycin-resistant *Enterococcus* VanA (VanA) in comparison to teicoplanin itself (Table 5). It is conceivable that the site-selective functionalization capability we have established in this study may allow for design of new variants that will allow for a high precision SAR to be derived.

■ CONCLUSIONS

In summary, we identified three different peptide catalysts that selectively phosphorylate three distinct sugar moieties present in teicoplanin A_2-2 (**2**). Rational design of catalysts based on the X-ray crystal structure and modeling enabled the identification of peptide **13**, which selectively phosphorylated

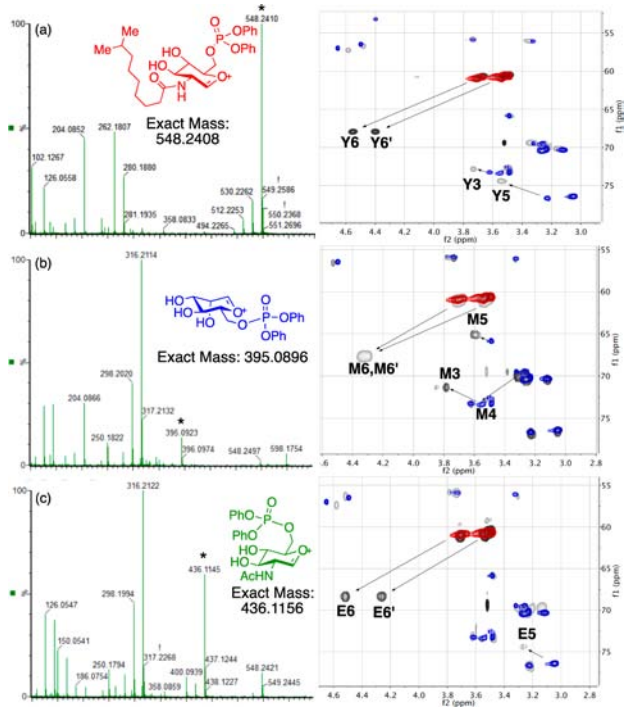


Figure 4. Structural data for **21**, **22**, and **23**. (a) ESI MS/MS data and overlay of HSQC spectrum (sugar region) of **21** (gray) with **2** (colored). (b) ESI MS/MS data and overlay of HSQC spectrum (sugar region) of **22** (gray) with **2** (colored). (c) ESI MS/MS data and overlay of HSQC spectrum (sugar region) of **23** (gray) with **2** (colored).

Table 5. MIC Data for New Phosphorylated Teicoplanin Analogues 21−23[a]

compd	*S. aureus* MSSA[b]	*S. aureus* MRSA[c]	*E. faecalis* VanS[d]	*E. faecalis* VanB[e]	*E. Faecalis* VanA[f]
21	4	4	1	4	32
22	8	8	1	8	32
23	4	4	0.5	4	62
vancomycin	1	1	2	16	>64
teicoplanin	0.5	0.5	0.25	0.25	>64
linezolid	4	4	2	2	2

[a]MIC values reported in μg/mL. [b]MSSA: methicillin-susceptible *S. aureus*. [c]MRSA: methicillin-resistant *S. aureus*. [d]VanS: vancomycin-susceptible. [e]VanB: VanB resistance genotype phenotype. [f]VanA: VanA resistance genotype/phenotype.

the "top/red" *N*-decanoylglucosamine moiety. However, our designs for the other sugar sites required editing based on experimental information and new insights acquired through evaluation of fifteen additional, diverse catalysts. From this set, we identified peptide **15**, which led to substantial selectivity for the "bottom/blue" phosphorylated teicoplanin **9**. Finally through rational optimization of the initial hits from this screen, we conceived and synthesized peptide **19**, an embodiment of both a well-ordered β-turn unit and a DAla−DAla targeting moiety. This catalyst enabled the selective phosphorylation of the least reactive "left/green" sugar of teicoplanin A_2-2 derivative **3**. Notably, the new catalysts are considerably smaller than the substrates upon which they operate, an unusual situation for many classes of catalysts, including enzymes and many small molecule catalysts based on metal complexes and organocatalysts. The chemical tools presented

dx.doi.org/10.1021/ja406067v | J. Am. Chem. Soc. 2013, 135, 12414−12421

here expand our understanding of site-selective catalysis on complex chemical scaffolds. These results illustrate a possible step forward for the development of synthetic catalysts that operate distinctively and selectively over quite long dimensions. As noted, the hydroxyl groups we have been able to target in these studies are separated by as much as 17.7 Å in a teicoplanin crystal structure,[46] which is a dimension characteristic of structural subunits in proteins.[67]

■ ASSOCIATED CONTENT

Supporting Information

Experimental details and characterization data and spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

scott.miller@yale.edu

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We are grateful to the National Institutes of General Medical Sciences of the National Institute of Health (GM-068649) for support. We thank Mr. Seth Alexander and the Schepartz laboratory for assistance with the LCMS.

■ REFERENCES

(1) Riva, S. *Curr. Opin. Chem. Biol.* **2001**, *5*, 106.
(2) Gonzalez-Sabin, J.; Moran-Ramallal, R.; Rebolledo, F. *Chem. Soc. Rev.* **2011**, *40*, 5321.
(3) Chang, S.; Lee, N. H.; Jacobsen, E. N. *J. Org. Chem.* **1993**, *58*, 6939.
(4) Lee, S. H.; Cheong, C. S. *Tetrahedron* **2001**, *57*, 4801.
(5) Bastian, A. A.; Marcozzi, A.; Herrmann, A. *Nat. Chem.* **2012**, *4*, 789.
(6) Lewis, C. A.; Miller, S. J. *Angew. Chem., Int. Ed.* **2006**, *45*, 5616.
(7) Holland, J. M.; Lewis, M.; Nelson, A. *Angew. Chem., Int. Ed.* **2001**, *40*, 4082.
(8) García-Urdiales, E.; Alfonso, I.; Gotor, V. *Chem. Rev.* **2004**, *105*, 313.
(9) Atodiresei, I.; Schiffers, I.; Bolm, C. *Chem. Rev.* **2007**, *107*, 5683.
(10) Lewis, C. A.; Longcore, K. E.; Miller, S. J.; Wender, P. A. *J. Nat. Prod.* **2009**, *72*, 1864.
(11) Pathak, T. P.; Miller, S. J. *J. Am. Chem. Soc.* **2012**, *134*, 6120.
(12) Fowler, B. S.; Laemmerhold, K. M.; Miller, S. J. *J. Am. Chem. Soc.* **2012**, *134*, 9755.
(13) Pathak, T. P.; Miller, S. J. *J. Am. Chem. Soc.* **2013**, *135*, 8415.
(14) For an X-ray crystal structure of *myo*-inositol, see: Rabinovich, I. N.; Kraut, J. *Acta Crystallogr.* **1964**, *17*, 159.
(15) Sculimbrene, B. R.; Miller, S. J. *J. Am. Chem. Soc.* **2001**, *123*, 10125.
(16) Sculimbrene, B. R.; Morgan, A. J.; Miller, S. J. *J. Am. Chem. Soc.* **2002**, *124*, 11653.
(17) Lewis, C. A.; Chiu, A.; Kubryk, M.; Balsells, J.; Pollard, D.; Esser, C. K.; Murry, J.; Reamer, R. A.; Hansen, K. B.; Miller, S. J. *J. Am. Chem. Soc.* **2006**, *128*, 16454.
(18) Lewis, C. A.; Gustafson, J. L.; Chiu, A.; Balsells, J.; Pollard, D.; Murry, J.; Reamer, R. A.; Hansen, K. B.; Miller, S. J. *J. Am. Chem. Soc.* **2008**, *130*, 16358.
(19) Chan, H.-C.; Huang, Y.-T.; Lyu, S.-Y.; Huang, C.-J.; Li, Y.-S.; Liu, Y.-C.; Chou, C.-C.; Tsai, M.-D.; Li, T.-L. *Mol. Biosyst.* **2011**, *7*, 1224.
(20) Commercially available teicoplanin exists as a mixture of natural products with varying alkyl chains of the decanoylglucosamine ring. This study was conducted with purifyed teicoplanin A_2-2 (**2**). See Supporting Information for details.
(21) Bambeke, F.; Laethem, Y.; Courvalin, P.; Tulkens, P. *Drugs* **2004**, *64*, 913.
(22) Kahne, D.; Leimkuhler, C.; Lu, W.; Walsh, C. *Chem. Rev.* **2005**, *105*, 425.
(23) Jeya, M.; Moon, H.-J.; Lee, K.-M.; Kim, I.-W.; Lee, J.-K. *Curr. Pharm. Biotechnol.* **2011**, *12*, 1194.
(24) Li, T.-L.; Liu, Y.-C.; Lyu, S.-Y. *Curr. Opin. Chem. Biol.* **2012**, *16*, 170.
(25) Ashford, P.-A.; Bew, S. P. *Chem. Soc. Rev.* **2012**, *41*, 957.
(26) Evans, D. A.; Wood, M. R.; Trotter, B. W.; Richardson, T. I.; Barrow, J. C.; Katz, J. L. *Angew. Chem., Int. Ed.* **1998**, *37*, 2700.
(27) Nicolaou, K. C.; Mitchell, H. J.; Jain, N. F.; Winssinger, N.; Hughes, R.; Bando, T. *Angew. Chem., Int. Ed.* **1999**, *38*, 240.
(28) Boger, D. L.; Miyazaki, S.; Kim, S. H.; Wu, J. H.; Castle, S. L.; Loiseleur, O.; Jin, Q. *J. Am. Chem. Soc.* **1999**, *121*, 10004.
(29) Boger, D. L.; Kim, S. H.; Miyazaki, S.; Strittmatter, H.; Weng, J.-H.; Mori, Y.; Rogel, O.; Castle, S. L.; McAtee, J. J. *J. Am. Chem. Soc.* **2000**, *122*, 7416.
(30) Boger, D. L.; Kim, S. H.; Mori, Y.; Weng, J.-H.; Rogel, O.; Castle, S. L.; McAtee, J. J. *J. Am. Chem. Soc.* **2001**, *123*, 1862.
(31) Evans, D. A.; Katz, J. L.; Peterson, G. S.; Hintermann, T. *J. Am. Chem. Soc.* **2001**, *123*, 12411.
(32) Xie, J.; Okano, A.; Pierce, J. G.; James, R. C.; Stamm, S.; Crane, C. M.; Boger, D. L. *J. Am. Chem. Soc.* **2011**, *134*, 1284.
(33) Shi, Z.; Griffin, J. H. *J. Am. Chem. Soc.* **1993**, *115*, 6482.
(34) Nicolaou, K. C.; Winssinger, N.; Hughes, R.; Smethurst, C.; Cho, S. Y. *Angew. Chem., Int. Ed.* **2000**, *39*, 1084.
(35) Pace, J. L.; Yang, G. *Biochem. Pharmacol.* **2006**, *71*, 968.
(36) Bischoff, D.; Pelzer, S.; Höltzel, A.; Nicholson, G. J.; Stockert, S.; Wohlleben, W.; Jung, G.; Süssmuth, R. D. *Angew. Chem., Int. Ed.* **2001**, *40*, 1693.
(37) Bischoff, D.; Pelzer, S.; Bister, B.; Nicholson, G. J.; Stockert, S.; Schirle, M.; Wohlleben, W.; Jung, G.; Sussmuth, R. D. *Angew. Chem., Int. Ed.* **2001**, *40*, 4688.
(38) Losey, H. C.; Peczuh, M. W.; Chen, Z.; Eggert, U. S.; Dong, S. D.; Pelczer, I.; Kahne, D.; Walsh, C. T. *Biochemistry* **2001**, *40*, 4745.
(39) Losey, H. C.; Jiang, J.; Biggins, J. B.; Oberthür, M.; Ye, X.-Y.; Dong, S. D.; Kahne, D.; Thorson, J. S.; Walsh, C. T. *Chem. Biol.* **2002**, *9*, 1305.
(40) Bister, B.; Bischoff, D.; Nicholson, G. J.; Stockert, S.; Wink, J.; Brunati, C.; Donadio, S.; Pelzer, S.; Wohlleben, W.; Sussmuth, R. D. *ChemBioChem* **2003**, *4*, 658.
(41) Schmit, J. L. *Clin. Infect. Dis.* **1992**, *15*, 302.
(42) Mainardi, J.-L.; Shlaes, D. M.; Goering, R. V.; Acar, J. F.; Goldstein, F. W. *J. Infect. Dis.* **1995**, *171*, 1646.
(43) Howden, B. P.; Davies, J. K.; Johnson, P. D. R.; Stinear, T. P.; Grayson, M. L. *Clin. Microbiol. Rev.* **2010**, *23*, 99.
(44) Higgins, D. L.; Chang, R.; Debabov, D. V.; Leung, J.; Wu, T.; Krause, K. M.; Sandvik, E.; Hubbard, J. M.; Kaniga, K.; Schmidt, D. E., Jr.; Gao, Q.; Cass, R. T.; Karr, D. E.; Benton, B. M.; Humphrey, P. P. *Antimicrob. Agents Chemother.* **2005**, *49*, 1127.
(45) Perkins, H. R. *Pharmacol. Ther.* **1982**, *16*, 181.
(46) Economou, N. J.; Zentner, I. J.; Lazo, E.; Jakoncic, J.; Stojanoff, V.; Weeks, S. D.; Grasty, K. C.; Cocklin, S.; Loll, P. J. *Acta Crystallogr.* **2013**, *D69*, 520.
(47) Fierman, M. B.; O'Leary, D. J.; Steinmetz, W. E.; Miller, S. J. *J. Am. Chem. Soc.* **2004**, *126*, 6967.
(48) Evans, J. W.; Fierman, M. B.; Miller, S. J.; Ellman, J. A. *J. Am. Chem. Soc.* **2004**, *126*, 8134.
(49) Endicott, J. A.; Noble, M. E. M.; Johnson, L. N. *Annu. Rev. Biochem.* **2012**, *81*, 587.
(50) Thompson, C.; Ge, M.; Kahne, D. *J. Am. Chem. Soc.* **1999**, *121*, 1237.
(51) Griffith, B. R.; Krepel, C.; Fu, X.; Blanchard, S.; Ahmed, A.; Edmiston, C. E.; Thorson, J. S. *J. Am. Chem. Soc.* **2007**, *129*, 8150.
(52) See Supporting Information for details.
(53) Nieto, M.; Perkins, H. R. *Biochem. J.* **1971**, *124*, 845.

(54) Sculimbrene, B. R.; Morgan, A. J.; Miller, S. J. *Chem. Commun.* **2003**, *0*, 1781.
(55) Sculimbrene, B. R.; Xu, Y.; Miller, S. J. *J. Am. Chem. Soc.* **2004**, *126*, 13182.
(56) Morgan, A. J.; Wang, Y. K.; Roberts, M. F.; Miller, S. J. *J. Am. Chem. Soc.* **2004**, *126*, 15370.
(57) Xu, Y.; Sculimbrene, B. R.; Miller, S. J. *J. Org. Chem.* **2006**, *71*, 4919.
(58) Morgan, A. J.; Komiya, S.; Xu, Y.; Miller, S. J. *J. Org. Chem.* **2006**, *71*, 6923.
(59) Yoon, T. P.; Jacobsen, E. N. *Science* **2003**, *299*, 1691.
(60) Awasthi, S. K.; Raghothama, S.; Balaram, P. *Biochem. Biophys. Res. Commun.* **1995**, *216*, 375.
(61) Haque, T. S.; Little, J. C.; Gellman, S. H. *J. Am. Chem. Soc.* **1996**, *118*, 6975.
(62) Stanger, H. E.; Gellman, S. H. *J. Am. Chem. Soc.* **1998**, *120*, 4236.
(63) Fisk, J. D.; Powell, D. R.; Gellman, S. H. *J. Am. Chem. Soc.* **2000**, *122*, 5443.
(64) Jarvo, E. R.; Copeland, G. T.; Papaioannou, N.; Bonitatebus, P. J.; Miller, S. J. *J. Am. Chem. Soc.* **1999**, *121*, 11638.
(65) For an example of the use of a competitive inhibitor to test the specific binding, see: Wang, H.; Koshi, Y.; Minato, D.; Nonaka, H.; Kiyonaka, S.; Mori, Y.; Tsukiji, S.; Hamachi, I. *J. Am. Chem. Soc.* **2011**, *133*, 12220.
(66) Note the conformational difference between DAla-DAla peptide bound teicoplanin derivative (Figure 3a) and free teicoplanin (Figure 1d).
(67) For example, these metrics are approaching those that are characteristic of at least small proteins, of which green fluorescent protein (GFP) may be emblematic, with its barrel diameter of approximately 25 Å. See: Ormö, M.; Cubitt, A. B.; Kallio, K.; Gross, L. A.; Tsien, R. Y.; Remington, S. J. *Science* **1996**, *273*, 1392.