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Synthesis of a D-Ala-D-Ala peptide isostere via olefin cross-metathesis and evaluation of vancomycin binding

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ABSTRACT

The alkene peptide isostere for the D-Ala-D-Ala dipeptide was synthesized via a convergent approach utilizing olefin cross-metathesis. The new isostere was then evaluated for binding to the last resort antibiotic, vancomycin. The alkene isostere exhibited a $K_D = 90\ \mu M$ in comparison to the native peptide ($K_D = 2.3\ \mu M$) and Lac mutant ($K_D = 2300\ \mu M$). This study demonstrates that loss of binding in vancomycin resistant strains as a result of a D-Ala to D-Lac mutation is from both the loss of a crucial hydrogen bond and introduction of a repulsive lone pair interaction.

The dipeptide D-Ala-D-Ala has received a great deal of attention due to its involvement in bacterial cell wall biosynthesis. This short peptide sequence is the target for vancomycin, commonly referred to as the antibiotic of last resort. Vancomycin inhibits cell wall biosynthesis by binding to a peptidoglycan precursor (terminating with D-Ala-D-Ala) and blocking the action of transpeptidase, which completes the cross-linking of the cell wall.[1] In 1978, Williams and co-workers established that a series of hydrogen bonds between the short peptide sequence Ac_2-Lys-D-Ala-D-Ala and vancomycin is responsible for binding and recognition.[2] Even with vancomycin's effectiveness in combating resistant strains of bacteria, Vancomycin Resistant Enterococci (VRE) were first reported in 1988.[3] Walsh and co-workers discovered that a single mutation in the peptide resulting in replacement of the D-Ala-D-Ala amide with an ester (called D-Ala-D-Lac) is responsible for vancomycin resistance (see Fig. 1).[4] This single atom mutation leads to a 1000-fold decrease in binding of vancomycin and hence a pathway of resistance for bacteria. The weak binding of D-Ala-D-Lac is hypothesized to result from the loss of a crucial hydrogen bond (loss of NH) and the emergence of a repulsive lone pair interaction (incorporation of oxygen). We sought to understand the importance of each effect on binding by replacing the backbone amide of D-Ala-D-Ala with a functional group that would mimic the three-dimensional structure of the amide bond while removing the potential for this amide to participate in hydrogen bonding. These types of biomimics are known as peptide isosteres.

Peptide isosteres are useful probes to study binding interactions with biological targets. Often these probes are designed to modify the peptides susceptibility to protease cleavage, or to study the presence or absence of molecular interactions with the backbone amide. Numerous functional groups have been successfully employed as peptide isosteres. The use of esters, known as depsipeptides, has been extensively employed. A recent example employed depsipeptides to study the kinetics and thermodynamics of β-sheet folding.[5] Hydroxymethylenes and hydroxyethylamines have been successfully used as HIV protease inhibitors in the treatment of AIDS.[6] A ketone isosteric replacement has been elegantly employed by Boger and co-workers to study the D-Ala-D-Ala interaction with vancomycin.[7] They found that the repulsive lone pair interaction from the ester mutation contributed slightly more to the binding loss, than removal of the H-bond. However, the study noted that the free-rotation of their alkane-mimic could have an impact on the finding, so we sought a complementary exercise that would use a conformationally rigid isostere for D-Ala-D-Ala. Due to the planar structure of the amide-backbone, *trans*-alkenes have also been widely used to study the peptide backbone. Alkene isosteres lack the ability to form hydrogen bonds and do not introduce negative electrostatic interactions by incorporation of a heteroatom. Alkene isosteres have been successfully employed in the study of Alzheimer's amyloidogenesis,[8] gramicidin S,[9] integrin receptors,[10] collagen stability,[11] and asymmetric catalysis.[12]

While numerous syntheses of alkene isosteres have been developed, they are often lengthy and specific to a particular target.[13] We sought a synthesis of the D-Ala-D-Ala alkene isostere that was convergent and modeled the method in which peptides are chemically synthesized. Peptides generated either in the laboratory or at

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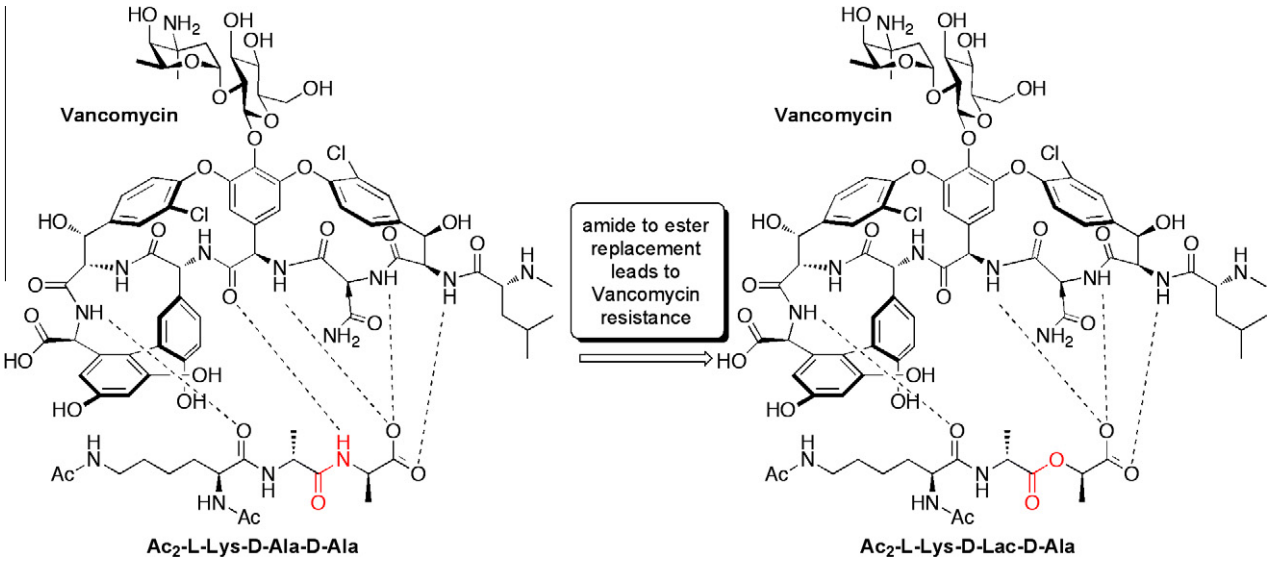
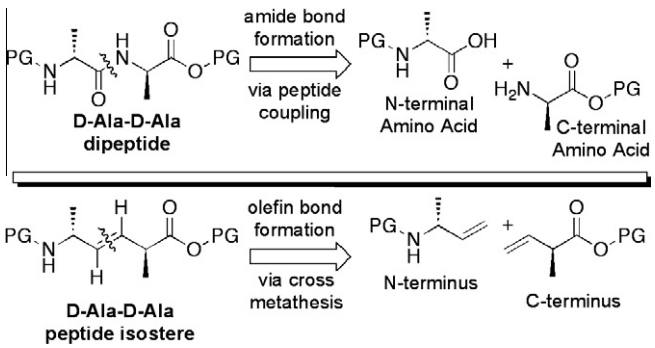


Figure 1. Hydrogen bonding interactions of vancomycin with bacterial cell wall peptides.

the ribosome are assembled by forming the amide bond between two corresponding amino acids. In mirroring this approach to alkene-isostere synthesis, the central alkene would be generated in a coupling reaction (see Scheme 1). The power of olefin cross-metathesis for generating coupled alkenes presented itself as a potential retrosynthetic disconnection.[14] The continual discovery of new and improved metathesis catalysts and the propensity for these reactions to favor the *trans*-alkene emboldened our endeavor. Olefin cross-metathesis has been previously explored in the synthesis of alkene isosteres where the C-terminal amino acid is glycine (side chain = hydrogen).[15] We sought to investigate whether two α-disubstituted alkenes would be competent partners in a cross-metathesis reaction.

Our synthetic approach began with the synthesis of the N- and C-terminus necessary to test the key cross-metathesis step. D-Ala N-terminus (**1**) is available in high optical purity in three steps from commercially available Boc-D-Ala using modified literature procedures.[16] To obtain a suitable C-terminus optically pure for the cross-metathesis reaction, we investigated diastereoselective alkylation of crotonates. α-Substituted-β-γ-eneoates have been successfully synthesized by deconjugative alkylation of chiral crotonates using Evans' chiral auxiliary.[17] The desired α-center is generated with high levels of diastereoselectivity. Since the Evans' auxiliary is readily removed under oxidative hydrolysis to afford the corresponding carboxylic acid,[18] we hoped it would serve as a suitable protecting group during the key cross-metathesis step. This would provide convergent access to our desired alkene isostere

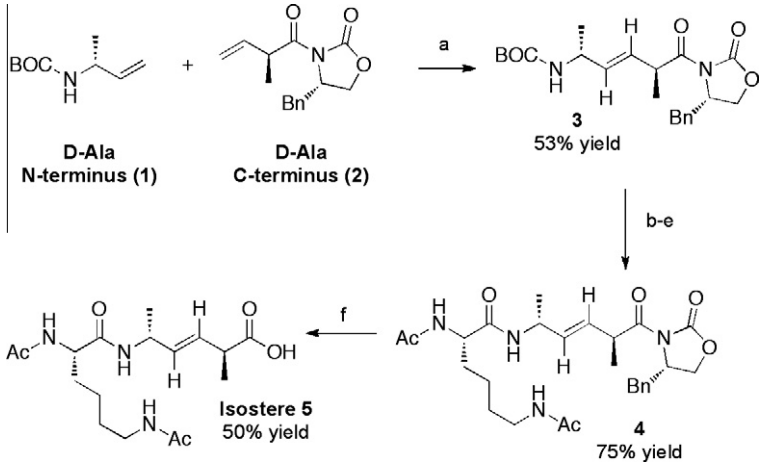


Scheme 1. Retrosynthesis of peptide isostere mirrors peptide synthesis.

target, avoiding unnecessary protection and deprotection steps. To this end, alkylation of the crotonate oxazolidinone derived from (*S*)-phenylalaninol with methyl iodide provided desired D-Ala-C-terminus (**2**).[19] With both the N- and C-terminus in hand, the merits of the key cross-metathesis step were examined. Reaction with Grubbs first and second generation catalysts resulted in only recovered starting material. However, the more active Hoveyda–Grubbs second generation catalyst generated both the desired heterodimer (**3**) and two homodimers corresponding to the termini coupling with themselves. In the course of optimizing the cross-metathesis to favor the desired heterodimer, it was discovered that the N-terminus was more reactive. By using a 1:3 ratio of N-terminus to C-terminus, the desired protected dipeptide isostere (**3**) was obtained in 53% isolated yield (the C-terminal homodimer was formed in 16% yield, while the N-terminal homodimer was formed in 7% yield). We are currently investigating the scope and rational behind this finding and will disclose the results in due course.[20]

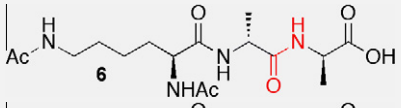
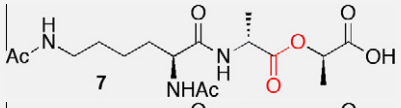
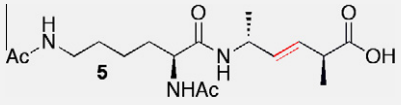
In order to compare our isostere mimic with data that had previously been collected for vancomycin binding, the isostere needed to be coupled with di-Ac-Lys and deprotected at the C-terminus. This was accomplished by removing the BOC-protecting group under acidic conditions, followed by standard peptide coupling with Boc-Lys(Ac)-OH. The terminal BOC-carbamate on the new tripeptide was then converted to an acetate to generate protected tripeptide (**4**) in 75% yield for the four-step sequence.[21] The chiral oxazolidinone that was used for installation of the C-terminus side chain and as the carboxylic acid protecting group, was then removed to form isostere (**5**) in 50% yield. All new compounds were fully characterized (Scheme 2).[22]

With a completed synthesis of our target, attention then focused on testing the binding of our isostere to vancomycin. For comparison, the native bacterial cell wall tripeptide, Ac-Lys(Ac)-D-Ala-D-Ala-OH (**6**) was synthesized via standard solution phase peptide synthesis. We conducted a ultra-violet absorbance-binding assay developed in 1969 by Perkins and used in 2003 by Boger and co-workers.[1,7] The assay monitors the differential absorbance of vancomycin at 279 nm with increasing concentration of ligand (see Table 1). Our results for the native peptide (**6**) were consistent with those reported by Boger and Perkins and provided a K_D = 2.3 μM. The ester to amide mutation that results from replacement of D-Ala-D-Ala to D-Ala-D-Lac (compound **7**) has previously been determined to have a binding constant three orders of magnitude weaker then the native peptide (K_D = 2.3 mM).[7] When our



Scheme 2. Synthesis of isostere (**5**). Reagents: (a) 10 mol % Hoveyda–Grubbs second generation catalyst, benzene, CH_2Cl_2, reflux; (b) TFA, CH_2Cl_2; (c) BOC-Lys(Ac)-OH, EDC·HCl, HOBt, NEt_3, CH_2Cl_2; (d) TFA, CH_2Cl_2; (e) Ac_2O, pyridine, CH_2Cl_2; (f) LiOH, H_2O_2, H_2O, THF.

Table 1
Dissociation constants (K_D) and binding free energy (ΔG) for peptide ligands binding to vancomycin[a]

Compound	K_D (μM)	ΔG (kcal/mol)
	2.3 ± 0.8	−7.7
	2300[b]	−3.6
	90 ± 20	−5.5

[a] Experiments performed at 25 °C, 100 mM vancomycin in 0.02 M sodium citrate, pH 5.1. UV measured at 279 nm, data is the average of five runs.
[b] Data obtained from Ref. 7.

isostere probe (**5**) was tested for vancomycin binding, a K_D = 90 μM was obtained. Since removal of the key hydrogen bond in the isostere–vancomycin interaction does not lead to the same dissociation constant as the native peptide or complete loss of binding, the Lac-mutation must work via a dual mechanism. This result is consistent with an explanation that loss of activity in VRE results from both the loss of a key hydrogen bond (2.2 kcal/mol) and generation of a negative electrostatic interaction (1.9 kcal/mol). It should be noted that the rigid alkene isostere **5** is a weaker binder then Boger's keto-methylene isostere, which exhibited a K_D = 30 μM.[7] This difference in affinity for vancomycin could be the result of some flexibility being necessary for binding. The next generation of antibiotics for VRE can use the need for flexibility and the dual action mechanism for the loss of binding in the design of better pharmaceuticals.[23] Isostere **5** could also serve as an antibiotic, if it demonstrated significant affinity for the bacterial enzyme transpeptidase. Peptide isosteres have been successfully employed as pharmaceuticals for the treatment of HIV,[6] and the D-Ala-D-Ala peptide sequence has already been established as a target for inhibitors of transpeptidase.[24]

In conclusion, we have demonstrated that alkene peptide isosteres can be synthesized in a convergent fashion utilizing olefin cross-metathesis. The two-alkene termini for the metathesis can be synthesized in optically pure form from the corresponding amino acid and diastereoselective alkylation. This method was used to generate the novel D-Ala-D-Ala alkene isostere via a convergent approach. The alkene isostere was then evaluated for binding to vancomycin. The study demonstrated that removal of the hydrogen bond is not solely responsible for the loss of binding in the Lac-mutation but is a combination of this loss as well as introduction of a repulsive lone pair interaction, from incorporation of an ester oxygen. Current work in our lab is focused on studying the key cross-metathesis reaction for the synthesis of other alkene peptide isosteres as well as elucidating the factors that govern selectivity for the formation of the desired heterodimer.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.06.065.

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21. Attempts to directly couple Ac-Lys(Ac)-OH to the dipeptide isostere lead to significant racemization of the Lys chiral center as detected by NMR. The four-step procedure with the BOC-carbamate protecting group lead to no detectable racemization.
22. *Protected dipeptide isostere* (**3**): ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.36–7.20 (m, 5H), 5.72 (dt, J = 1.3, 7.9, 15.5 Hz, 1H), 5.61 (dd, J = 3.2, 15.2 Hz, 1H), 4.65 (m, 1H), 4.46 (m, 2H), 4.20 (m, 3H), 3.27 (dd, J = 3.2, 13.3 Hz, 1H), 2.78 (dd, J = 9.7, 13.3 Hz, 1H), 1.44 (s, 9H), 1.33 (d, J = 6.9 Hz, 3H), 1.20 (d, J = 6.7 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 174.7, 154.9, 152.7, 135.0, 134.0, 129.2, 128.7, 127.9, 127.2, 79.0, 65.8, 55.2, 47.0, 40.3, 37.6, 28.2, 20.8, 17.7; IR (film, cm^{-1}) 3367, 2977, 2926, 2870, 1776, 1693, 1498, 1454; TLC R_f 0.23 (50% ether/petroleum ether); Exact mass calcd for [$C_{22}H_{30}N_2O_5$ + H] + requires m/z 403.2233. Found 403.2248 (FAB). *Protected tripeptide isostere* (**4**): ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.35–7.20 (m, 5H), 6.35 (d, J = 7.9 Hz, 1H), 6.22 (d, J = 8.4 Hz, 1H), 5.76 (bs, 1H), 5.71 (dt, J = 1.3, 7.6, 15.7, 1H), 5.61 (dd, J = 4.8, 15.8 Hz, 1H), 4.66 (m, 1H), 4.52 (m, 1H), 4.44 (m, 1H), 4.31 (m, 1H), 4.24 (d, J = 7.9 Hz, 1H), 4.17 (dd, J = 2.5, 9.1 Hz, 1H), 3.26 (m, 3H), 2.78 (dd, J = 9.5, 13.3 Hz, 1H), 2.05 (s, 3H), 1.98 (s, 3H), 1.82 (m, 1H), 1.68 (m, 1H), 1.53 (m, 2H), 1.35 (m, 2H), 1.32 (d, J = 6.9 Hz, 3H), 1.23 (d, J = 6.9 Hz, 3H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 174.7, 171.4, 169.7, 169.6, 153.6, 136.2, 133.7, 130.2, 129.2, 128.6, 127.6, 79.6, 66.8, 55.2, 53.1, 46.0, 40.3, 37.3, 32.7, 29.5, 23.5, 23.3, 23.1, 21.3, 18.1; IR (film, cm^{-1}) 3271, 3068, 2927, 2861, 1772, 1699, 1628, 1542; TLC R_f 0.25 (10% methanol/dichloromethane); Exact mass calcd for [$C_{27}H_{38}N_4O_6$ + H] + requires m/z 515.2870. Found 515.2888 (FAB). Isostere **5**: ^{1}H NMR (D_2O w/0.75% 3-(trimethylsilyl)-propionic-2,2,3,3-d_4 acid, sodium salt, 400 MHz) δ 8.15 (d, J = 8.2 Hz, 1H), 5.67 (dd, J = 7.0, 15.7 Hz, 1H), 5.60 (dd, J = 4.4, 15.7 Hz, 1H), 4.40 (m, 1H), 4.17 (m, 1H), 3.16 (m, 3H), 2.02 (s, 3H), 1.97 (s, 3H), 1.73 (m, 2H), 1.52 (m, 2H), 1.37 (m, 2H), 1.21 (d, J = 6.7 Hz, 3H), 1.20 (d, J = 6.9 Hz, 3H); ^{13}C NMR (D_2O w/0.75% 3-(trimethylsilyl)-propionic-2,2,3,3-d_4 acid, sodium salt, 400 MHz) δ 184.1, 176.9, 176.8, 176.0, 134.5, 132.4, 57.1, 49.4, 46.1, 41.9, 33.6, 30.7, 25.2, 24.7, 24.5, 22.2, 19.5; IR (film, cm^{-1}) 3283.1, 3068, 2967, 2926, 2860, 1709, 1629, 1544; Exact mass calcd for [$C_{17}H_{29}N_3O_5$ + H] + requires m/z 356.2185. Found 356.2189 (FAB).
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