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Total Synthesis and Evaluation of [Ψ[CH_2NH]Tpg^4]Vancomycin Aglycon: Reengineering Vancomycin for Dual D-Ala-D-Ala and D-Ala-D-Lac Binding

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Abstract: An effective synthesis of [Ψ[CH_2NH]Tpg^4]vancomycin aglycon (**5**) is detailed in which the residue 4 amide carbonyl of vancomycin aglycon has been replaced with a methylene. This removal of a single atom was conducted to enhance binding to D-Ala-D-Lac, countering resistance endowed to bacteria that remodel their D-Ala-D-Ala peptidoglycan cell wall precursor by a similar single atom change (ester O for amide NH). Key elements of the approach include a synthesis of the modified vancomycin ABCD ring system featuring a reductive amination coupling of residues 4 and 5 for installation of the deep-seated amide modification, the first of two diaryl ether closures for formation of the modified CD ring system (76%, 2.5−3:1 kinetic atropodiastereoselectivity), a Suzuki coupling for installation of the hindered AB biaryl bond (90%) on which the atropisomer stereochemistry could be thermally adjusted, and a macrolactamization closure of the AB ring system (70%). Subsequent DE ring system introduction enlisted a room-temperature aromatic nucleophilic substitution reaction for formation of the remaining diaryl ether (86%, 6−7:1 kinetic atropodiastereoselectivity), completing the carbon skeleton of **5**. Consistent with expectations and relative to the vancomycin aglycon, **5** exhibited a 40-fold increase in affinity for D-Ala-D-Lac ($K_a = 5.2 \times 10^3$ M^{-1}) and a 35-fold reduction in affinity for D-Ala-D-Ala ($K_a = 4.8 \times 10^3$ M^{-1}), providing a glycopeptide analogue with balanced, dual binding characteristics. Beautifully, **5** exhibited antimicrobial activity (MIC = 31 μg/mL) against a VanA-resistant organism that remodels its D-Ala-D-Ala cell wall precursor to D-Ala-D-Lac upon glycopeptide antibiotic challenge, displaying a potency that reflects these binding characteristics.

The most common strains of enterococci resistant to vancomycin (**1**), VanA and VanB, possess an inducible resistance pathway in which the terminal dipeptide of the cell wall peptidoglycan precursor is modified from D-Ala-D-Ala to D-Ala-D-Lac.[1] Binding of the antibiotic to this modified ligand is reduced 1000-fold, leading to a 1000-fold drop in antimicrobial activity.[1d,k] In a recent disclosure,[2] we provided the first experimental study on the origin of this loss in binding affinity, partitioning the effect into lost H-bond and repulsive lone-pair contributions (Figure 1). Thus, the binding affinity of vancomycin for **3**, which incorporates a methylene (CH_2) in place of the linking amide NH of Ac_2-L-Lys-D-Ala-D-Ala, was compared with that of Ac_2-L-Lys-D-Ala-D-Ala (**2**) and Ac_2-L-Lys-D-Ala-D-Lac (**4**). The vancomycin affinity for **3** was approximately

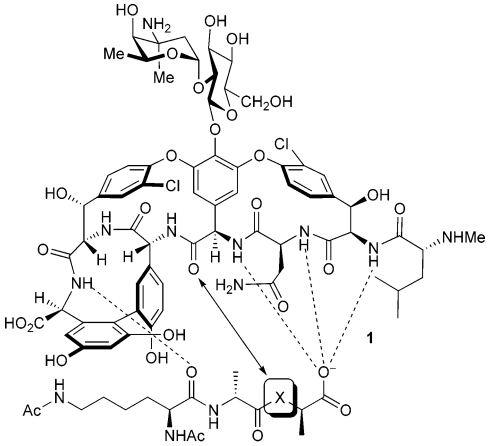

Figure 1. Binding constants of D-Ala-D-Ala, D-Ala-D-Lac, and a peptide analogue with vancomycin.

(1) Reviews: (a) Kahne, D.; Leimkuhler, C.; Lu, W.; Walsh, C. *Chem. Rev.* **2004**, *105*, 425. (b) Hubbard, B. K.; Walsh, C. T. *Angew. Chem., Int. Ed.* **2003**, *42*, 730. (c) Nicolaou, K. C.; Boddy, C. N. C.; Bräse, S.; Winssinger, N. *Angew. Chem., Int. Ed.* **1999**, *38*, 2096. (d) Williams, D. H.; Bardsley, B. *Angew. Chem., Int. Ed.* **1999**, *38*, 1172. (e) Malabarba, A.; Nicas, T. I.; Thompson, R. C. *Med. Res. Rev.* **1997**, *17*, 69. Glycopeptide resistance and analogues: (f) Malabarba, A.; Ciabatti, R. *Curr. Med. Chem.* **2001**, *8*, 1759. (g) Pootoolal, J.; Neu, J.; Wright, G. D. *Annu. Rev. Pharmacol. Toxicol.* **2002**, *42*, 381. (h) Van Bambeke, F. V.; Laethem, Y. V.; Courvalin, P.; Tulkens, P. M. *Drugs* **2004**, *64*, 913. (i) Sussmuth, R. D. *ChemBioChem* **2002**, *3*, 295. (j) Gao, Y. *Nat. Prod. Rep.* **2002**, *19*, 100. (k) Healy, V. L.; Lessard, I. A. D.; Roper, D. I.; Knox, J. R.; Walsh, C. T. *Chem. Biol.* **2000**, *7*, R109.
(2) McComas, C. C.; Crowley, B. M.; Boger, D. L. *J. Am. Chem. Soc.* **2003**, *125*, 9314.

10-fold less than that of **2**, but 100-fold greater than that of **4**. This indicated that the reduced binding affinity of **4** (4.1 kcal/mol) may be attributed to both the loss of a key H-bond and a destabilizing lone pair/lone pair interaction introduced with the ester oxygen of **4** (2.6 kcal/mol), with the latter, not the H-bond, being responsible for the greater share (100-fold) of the 1000-fold binding reduction. These observations have significant ramifications on the reengineering of vancomycin to bind D-Ala-D-Lac, suggesting that the design could focus principally on removing the destabilizing lone-pair interaction rather than reintroducing a H-bond and that this may be sufficient to compensate for two of the three orders of magnitude in binding affinity lost with D-Ala-D-Lac. Thus, synthesis of a vancomycin analogue with removal of the residue 4 carbonyl and its destabilizing lone-pair interaction could potentially restore much of the binding affinity of the antibiotic for the modified ligand. At present, such a deep-seated change in the molecule can only be achieved by total synthesis.[3] Having developed a convergent and efficient synthesis of the vancomycin aglycon,[4] and having extended its effectiveness in the synthesis of the teicoplanin[5] and ristocetin aglycons,[6] we embarked on the total synthesis of [Ψ[CH_2NH]Tpg^4]vancomycin aglycon (**5**), in which the residue 4 carbonyl has been replaced with a methylene. Through such efforts, we were able to establish the effect this structural modification has on the binding affinity for both D-Ala-D-Ala and D-Ala-D-Lac and probe its impact on the antimicrobial activity of the analogue.

Challenges and Synthetic Plan for [Ψ[CH_2NH]Tpg^4]-Vancomycin Aglycon. The desired analogue **5** was anticipated to be prepared by a route analogous to that developed for vancomycin,[4] with notable modifications. Thus, two aromatic nucleophilic substitution reactions with formation of the biaryl ethers would be enlisted for CD and DE macrocyclization, a key macrolactamization reaction would be employed for cyclization of the AB ring system, and the defined order of CD, AB, and DE ring closures was expected to permit sequential control of the atropisomer stereochemistry of each of the newly formed ring systems or their immediate precursors (Figure 2). Thus, in addition to any kinetic diastereoselection that may be achieved in the ring closures, this order was expected to permit the recycling of any undesired atropisomer for each newly introduced ring system by thermal equilibration, providing a predictable control of the stereochemistry and dependably funneling all synthetic material into one of eight possible atropodiastereomers. Key to recognition of this preferential order of ring closures was our establishment of the thermodynamic parameters of atropisomerism for the individual vancomycin

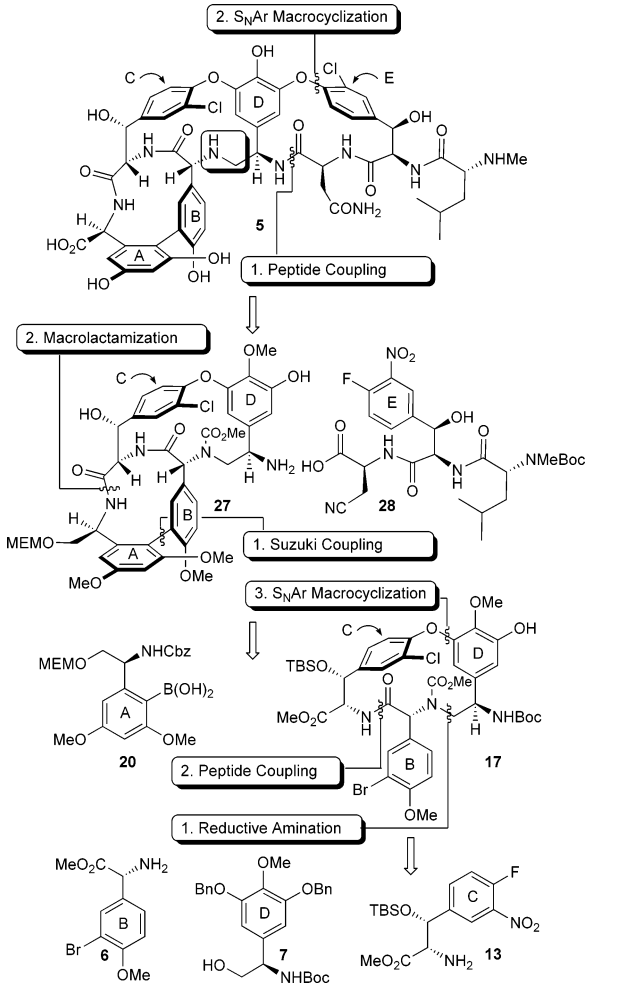

Figure 2. Plan for [Ψ[CH_2NH]Tpg^4]vancomycin aglycon.

ring systems: DE ring system[7] (E_a = 18.7 kcal/mol) < AB biaryl precursor[4] (E_a = 25.1 kcal/mol) < CD ring system[7] (E_a = 30.4 kcal/mol). Thus, the molecule was to be assembled by coupling the modified and fully functionalized ABCD ring system **27** with the E ring tripeptide **28**, followed by a diastereoselective aromatic nucleophilic substitution reaction for closure of the 16-membered DE ring system with formation of the biaryl ether linkage. Notably, the activating nitro substituent additionally serves as the precursor functionality for aryl chloride introduction, and the analogous vancomycin ring closures[4,8−10] were effected with preferential formation of the natural (*P*)-atropisomer. The E ring tripeptide **28** would be derived in the manner described for vancomycin,[4] except that the E ring subunit would be prepared enlisting an improved route developed during our more recent total synthesis of the ristocetin

(3) Efforts to modify the residue 4 carbonyl by selective reaction of the amide linking residues 4 and 5 within vancomycin aglycon derivatives have not yet been successful in our efforts, and we are unaware of any reports of such modifications. Synthetic reviews: (a) Boger, D. L. *Med. Res. Rev.* **2001**, *21*, 356. (b) Lloyd-Williams, P.; Giralt, E. *Chem. Soc. Rev.* **2001**, *30*, 145. (c) Zhang, A. J.; Burgess, K. *Angew. Chem., Int. Ed.* **1999**, *38*, 634. (d) Rao, A. V. R.; Gurjar, M. K.; Reddy, K. L.; Rao, A. S. *Chem. Rev*. **1995**, *95*, 2135. (e) Evans, D. A.; DeVries, K. M. *Drugs Pharm. Sci.* **1994**, *63*, 63.

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(5) (a) 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. (b) 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.

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(8) Boger, D. L.; Borzilleri, R. M.; Nukui, S. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 3091.

(9) Evans, D. A.; Watson, P. S. *Tetrahedron Lett.* **1996**, *37*, 3251.

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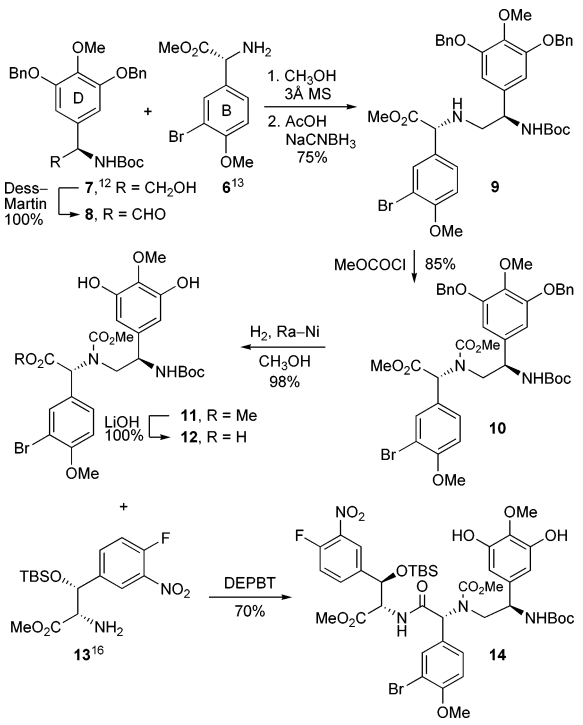
aglycon,[6] employing an α-hydroxypinanone[11] as the chiral auxiliary for a diastereoselective aldol addition. The most significant deviations rest with the required modifications in the preparation of the ABCD subunit, which houses the modified amide, and include the use of a reductive amination coupling of residues 4 and 5 (D and B rings) with protection of the newly generated amine as a methyl carbamate and an experimentally derived altered order to the assembly of the BCD tripeptide. A relatively small and robust amine protecting group was chosen to avoid the introduction of unfavorable steric interactions that may affect the CD macrocyclic ring closure and that would be stable throughout the synthesis, yet still be compatible with a final stage global deprotection. CD macrocyclization, enlisting a key aromatic nucleophilic substitution reaction for formation of 16-membered biaryl ether, followed by Suzuki coupling of the A ring subunit and AB macrolactamization, was anticipated to complete the preparation of the modified ABCD ring system **27**, enlisting a ring closure order that would permit the sequential and selective thermal adjustment of the CD and AB ring system atropisomer stereochemistry. Key unknown features of the approach include the feasibility of conducting the critical CD ring closure enlisting the residue 4 protected amine versus amide, the resulting unknown atropisomer stereochemical issues (kinetic and thermodynamic diastereoselectivity), and the impact the deep-seated structural change would have on the conformational features of the CD or ABCD ring systems and those of the final molecule. Finally, the subtle choice of a nitrile as a precursor to the residue 3 side-chain carboxamide permits a final stage amide deprotection yet conveys stability to any projected thermal atropisomer equilibrations in its presence,[7f] and the use of a protected hydroxymethyl precursor (vs a methyl ester) to the C-terminus carboxylic acid enhances the rate of the projected AB macrolactamization[4] and precludes inadvertent epimerization throughout the synthesis.

Synthesis of the BCD Tripeptide. The B and D subunits **6** and **7** were prepared following previously optimized procedures.[6,7] Oxidation of alcohol **7**[12] (2.0 equiv of Dess−Martin periodinane, CH_2Cl_2, 0−25 °C, 1 h, 100%) was followed by immediate reductive amination coupling of the sensitive aldehyde **8** with **6**[13] (1.1 equiv, CH_3OH, 3-Å molecular sieves, 0 °C, 45 min; 3.0 equiv of AcOH, 3.0 equiv of $NaBH_3CN$, −20 °C, 2 d) to afford amine **9** in good yield (75%) and excellent diastereoselectivity (12:1) (Scheme 1). Shorter reaction times (14−20 h) at higher temperatures (−15 to −5 °C) led to substandard selectivities (4:1 to 9:1), and the use of less $NaBH_3CN$ (1.5−2.0 equiv) or lower temperatures (−30 °C) led to incomplete reactions. Longer reaction times (3−8 d) led to only marginal increases in yield (82% after 8 d) and roughly equal diastereoselectivities. Initial efforts to prepare **9** directly by displacement of the mesylate derived from alcohol **7** were ineffective, as were attempts to conduct the reductive amination with the BC dipeptide and **8**. Amine protection of **9** as the methyl carbamate (10 equiv of MeOCOCl, 10 equiv of K_2CO_3, THF, 0−25 °C, 18 h, 85%), followed by benzyl ether deprotection[14] (Raney nickel, CH_3OH, 0 °C, 5 h, 98%) and saponification (3.0 equiv of LiOH, THF−H_2O, 0 °C, 6 h, 100%), provided **12**. Unexpectedly, the order of these latter two deprotections proved important. Saponification of **10**[15] under a variety of conditions (LiOH, THF−H_2O or *t*-BuOH−H_2O, −10 to 0 °C; LiOOH, THF−H_2O; Me_3SnOH, 1,2-dichloroethane, 70 °C) led to variable amounts of an epimer (5−20%) that was difficult to separate from the product. In contrast, benzyl ether deprotection of **10**, followed by saponification of **11**, reduced the amount of epimer (0−3%), presumably due to preferential deprotonation of the phenols such that subsequent C_α deprotonation at residue 5 was less facile.[15] Coupling of **12** with **13**[16] (3.0 equiv of DEPBT,[17] 3.0 equiv of $NaHCO_3$, DMF, 0−25 °C, 8 h) gave tripeptide **14** in good yield (70%) and excellent diastereoselectivity (14:1). A range of other more conventional coupling reagents (EDCI−HOAt, HATU, FDPP) also provided good conversions (65−80%) but suffered from considerable competitive racemization.

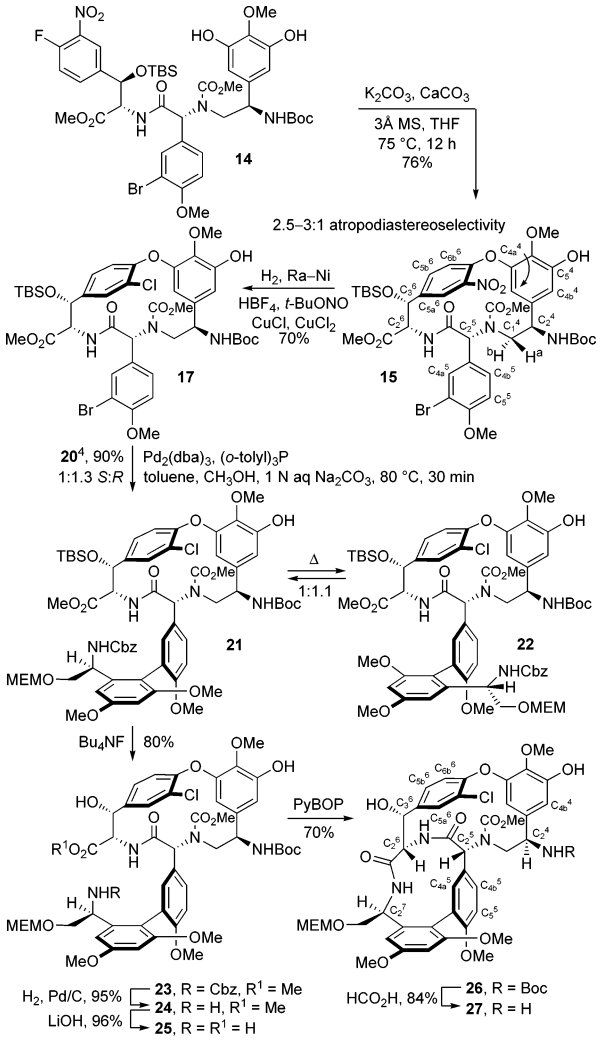
Scheme 1



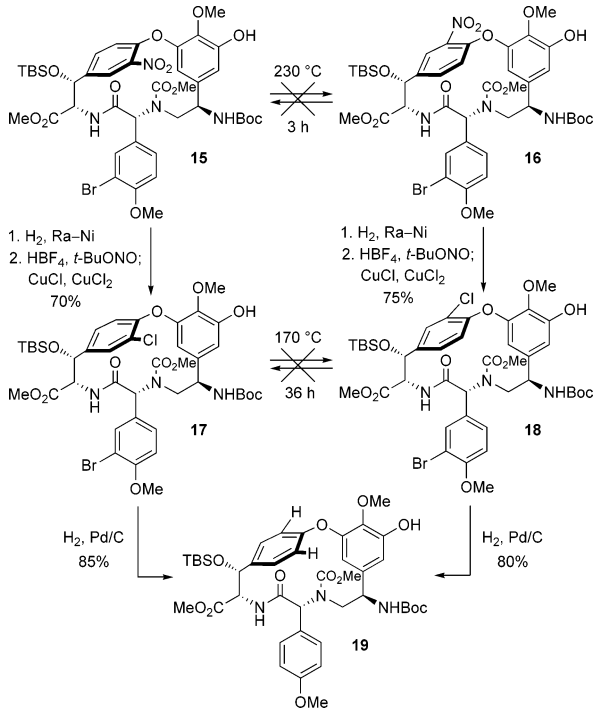
Synthesis of the ABCD Ring System. The stage was now set for a detailed examination of one of the critical reactions in the approach to **5**, entailing the cyclization of **14**. After considerable optimization (Supporting Information Tables S1 and S2), cyclization of **14** (20 equiv of K_2CO_3, 20 equiv of $CaCO_3$, 3 wt equiv of 3-Å molecular sieves, 12 mM THF, 75 °C bath temperature, 12 h) afforded **15** in good yield (54%) and good atropodiastereoselectivity (2.5:1, **15** (54%) and **16** (22%)), even when conducted on a large scale (2.7 g) (Scheme

(11) Solladié-Cavallo, A.; Nsenda, T. *Tetrahedron Lett.* **1998**, *39*, 2191.
(12) Compound **7** is available in six steps (37% overall) from methyl gallate using three recrystallizations and was scaled to 300 g, ref 6.
(13) Compound **6** is available in five steps (55% overall) from (*R*)-4-hydroxyphenyl-glycine using two recrystallizations and was scaled to 60 g, ref 7a.
(14) Benzyl ether deprotection at higher temperatures (25 °C) may lead to competitive aryl bromide reduction, although this was observed in appreciable amounts only when excess Raney nickel was employed.
(15) Saponification of **11** was considerably slower than that of **10** and occasionally required additional LiOH for complete conversion to **12**, with little effect on the amount of epimer generated in the reaction.
(16) Compound **13** is available in three steps (45% overall) from 4-fluoro-3-nitrobenzaldehyde and was scaled to 30 g, ref 6.
(17) Fan, C.-X.; Hao, X.-L.; Ye, Y.-H. *Synth. Commun.* **1996**, *26*, 1455. Li, H.; Jiang, X.; Ye, Y.-H.; Fan, C.; Romoff, T.; Goodman, M. *Org. Lett.* **1999**, *1*, 91.

Scheme 2



Scheme 3



2). The inclusion of $CaCO_3$ in the reaction mixture is critical and serves to trap the liberated fluoride arising from the aromatic nucleophilic substitution as an insoluble byproduct (CaF_2), preventing TBS ether deprotection and a subsequent competitive base-catalyzed retro aldol reaction of the free alcohol. Nearly comparable results were obtained by promoting the ring closure of **15** with the stronger base *t*-BuOK (1.0 equiv, THF, −20 °C, 18 h), providing **15** and its atropisomer **16** in 57% and 19% yields (3:1 atropodiastereoselectivity), respectively, under remarkably mild reaction conditions (−20 °C, THF). However, the use of *t*-BuOK proved more sensitive to the reaction parameters, suffered competitive racemization if excess base was employed, and proved more difficult to implement on a large scale than the reaction enlisting $K_2CO_3/CaCO_3$. The cyclization of **14** represents a considerable improvement over the analogous ring closure reaction enlisted in our original synthesis of vancomycin (50−65%, 1:1 atropisomers vs 76−87%, 2.5−3:1 atropisomers), where both the overall conversion and the atropodiastereoselectivity were lower, illustrating that the closure of **14** may benefit from the increased conformational flexibility of the cyclization substrate.[18] Unlike the vancomycin CD ring system, in which the atropisomers could be thermally equilibrated at 120−140 °C, permitting the recycling and productive use of the unnatural atropisomer, the atropisomers **15** and **16** could not be thermally interconverted, even at temperatures as high as 210−230 °C (Scheme 3).

Reduction of the nitro group (Raney nickel, 0 °C, CH_3OH, 1 h), followed by diazotization (1.3 equiv of HBF_4, 1.3 equiv of *t*-BuONO, CH_3CN, 0 °C, 30 min) and Sandmeyer substitution (50 equiv of CuCl, 60 equiv of $CuCl_2$, H_2O, 0−25 °C, 1 h, 70% from **15**), cleanly provided **17** without loss of the atropisomer stereochemistry inherent in starting **15**. The unnatural atropisomer **16** was also subjected to these conditions to cleanly give **18** (75%) (Scheme 3). The stereochemical assignments of these two compounds and their relationship as atropisomers (vs epimers) were established by 2D ROESY $^1H-^1H$ NMR experiments and confirmed chemically by their reductive dechlorination (H_2, 10%, Pd/C) to afford the identical product **19** (Scheme 3). Diagnostic NOE cross-peaks for **17** were observed between $C_{5a}{}^6-H/C_3{}^6-H$ (s), $C_{5a}{}^6-H/C_2{}^6-H$ (s), $C_{5b}{}^6-H/C_{6b}{}^6-H$ (s), $C_5{}^5-H/C_{4b}{}^5-H$ (s), $C_3{}^6-H/C_2{}^6-H$ (s), $C_2{}^5-H/C_{4a}{}^5-H$ (w), $C_2{}^5-H/C_{4b}{}^5-H$ (w), $C_2{}^4-H/C_{1b}{}^4-H$ (s), $C_2{}^4-H/C_{4b}{}^4-H$ (s), $C_{1a}{}^4-H/C_{1b}{}^4-H$ (s), $C_5{}^4-OH/C_6{}^4-OMe$ (s), and $C_5{}^5-H/C_6{}^5-OMe$ (m), and no NOE cross-peaks were observed between $C_{5b}{}^6-H/C_3{}^6-H$ and $C_{5b}{}^6-H/C_2{}^6-H$. Diagnostic NOE cross-peaks for **18** were observed between $C_{5b}{}^6-H/C_{6b}{}^6-H$ (s), $C_{5b}{}^6-H/C_3{}^6-H$ (s), $C_{5b}{}^6-H/C_2{}^6-H$ (s), $C_5{}^5-H/C_{4b}{}^5-H$ (s), $C_3{}^6-H/C_2{}^6-H$ (s), $C_2{}^6/N_1{}^6-H$ (w), $C_2{}^5-H/C_{4a}{}^5-H$ (m), $C_2{}^5-H/C_{4b}{}^5-H$ (m), $C_2{}^4-H/C_{1b}{}^4-H$ (s), $C_{1b}{}^4-H/C_{4a}{}^5-H$ (w), $C_{1b}{}^4-H/C_{4b}{}^5-H$ (w), $C_{1a}{}^4-H/C_{4b}{}^4-H$ (s), $C_{1b}{}^4-H/C_{1a}{}^4-H$ (s), $C_{4b}{}^4-H/C_5{}^4-OH$ (m), $C_5{}^4-OH/C_6{}^4-OMe$ (w), $C_5{}^5-H/C_6{}^5-OMe$ (w), $C_2{}^4-H/N_1{}^4-H$ (w), and no NOE cross-peaks were observed between $C_{5a}{}^6-H/C_3{}^6-H$ and $C_{5a}{}^6-H/C_2{}^6-H$.

Suzuki coupling of **17** with the hindered A ring boronic acid **20**[4] (0.3 equiv of $Pd_2(dba)_3$, 1.5 equiv of $(o\text{-tol})_3P$, toluene−CH_3OH−1 N aqueous Na_2CO_3 10:3:1, 80 °C, 30 min) proceeded in excellent yield (90%) under remarkably effective

(18) Recent efforts have led to improvements in the vancomycin CD ring closure, and this method was utilized for the preparation of the analogous intermediates bearing a residue 4/5 thioamide. Its use in the preparation of **5** along with additional vancomycin analogues will be disclosed in due time.

conditions[4] given the steric constraints of the substrate **20**, providing a separable 1:1.3 mixture of atropisomers (**21**:**22**) slightly favoring the unnatural configuration. Thermal equilibration of isolated **22** was carried out initially employing our reported conditions for vancomycin (*o*-dichlorobenzene, 120 °C, 18 h, 81% recovery of material)[7] to afford a 1:1.1 separable mixture, permitting the recycling of this unnatural atropisomer. An examination of the parameters for this isomerization (k = 0.12 h^{-1}, $t_{1/2}$ = 5.9 h at 120 °C and k = 0.36 h^{-1}, $t_{1/2}$ = 1.8 h at 135 °C) revealed that it proceeds with an energy of activation (E_a) of 25.6 kcal/mol ($\Delta H^{\ddagger}$ = 24.8 kcal/mol, $\Delta S^{\ddagger}$ = −0.26 eu, $\Delta G^{\ddagger}$ = 24.9 kcal/mol) essentially indistinguishable from that observed with the authentic vancomycin AB biaryl system, but it does not result in the analogous 3:1 thermodynamic preference for the natural atropisomer. However, the unusual and unexpected atropisomer stability of the CD ring system allowed us to improve on the recycling conditions. Heating the mixture in a microwave reactor at an elevated temperature (210 °C, *o*-dichlorobenzene) shortened the reaction time significantly (5 min vs 18 h) and slightly improved the recovery of material (88% vs 81%). This improvement impacted the efficiency of the recycling of **22** by allowing multiple equilibrations to be run in a single day rather than over the course of a week. Silyl ether deprotection of **21** (1.2 equiv of Bu_4NF, THF, 0 °C, 10 min), followed by N-Cbz removal (H_2, 10% Pd/C, 1% Cl_3CCO_2H−CH_3OH, 15 min, 95%) and methyl ester hydrolysis (1.0 equiv of LiOH, THF−H_2O, 0 °C, 1 h, 96%), gave amino acid **25**. Notably, N-Cbz removal in the absence of Cl_3CCO_2H[5] was much slower (11 h), and these conditions led to competitive chloride reduction.[19] Macrolactamization with closure of the AB ring system was effected by treatment of **25** with PyBOP (3.0 equiv, 6.0 equiv of $NaHCO_3$, 0.001 M CH_2Cl_2−DMF 5:1, 0−25 °C, 12 h) to afford the fully functionalized bicyclic ABCD ring system **26** in good yield (70%) with only trace amounts of competitive epimerization (<3%). Alternative coupling reagents (EDCI and HOAt or HOBt, HATU) and reaction conditions (10−100% DMF−CH_2Cl_2, 3−5 equiv of Na_2CO_3, −5 to 0 °C) led to lower conversions (30−52%) or required extended reaction times (3 d). N-Boc deprotection (HCO_2H−$CHCl_3$ 1:1, 10 h, 84%) gave the free amine **27** for coupling with the E ring tripeptide. Confirmation of the atropisomer stereochemistry and amide conformational assignments for **26** were established by 2D ROESY ^1H−^1H NMR. Diagnostic NOE cross-peaks for **26** were observed between C_5^4−OH/C_{4b}^4−H (s), C_5^4−OH/C_6^4−OMe (s), N_1^7−H/C_{4a}^5−H (s), N_1^7−H/C_2^5−H (s), N_1^7−H/C_3^6−H (m), N_1^7−H/C_2^6−H (m), C_{5a}^6−H/C_3^6−H (s), C_{5a}^6−H/C_2^6−H (s), C_{5b}^6−H/N_1^6−H (m), C_3^6−OH/N_1^6−H (s), C_{5b}^6−H/C_3^6−OH (m), C_{6b}^6−H/C_{5b}^6−H (s), C_{6b}^6−H/C_{4a}^4−H (w), N_1^4−H/C_{4b}^4−H (m), N_1^4−H/C_{4a}^4−H (w), C_{4b}^5−H/C_5^5−H (s), C_2^6−H/C_{4a}^5−H, C_{4b}^5−H/C_{1b}^4−H (m), C_{4a}^5−H/C_6^7−H (w), C_{4a}^5−H/C_2^5−H (s), C_5^5−H/C_6^5−OMe (s), C_4^7−H/C_2^7−H (s), C_4^7−H/C_{1b}^7−H (s), C_4^7−H/C_{1a}^7−H (w), C_4^7−H/C_{5b}^7−OMe (s), C_4^7−H/C_6^7−H (w), C_6^7−H/C_2^5−H (w), C_6^7−H/C_{5b}^7−OMe (s), C_6^7−H/C_{5a}^7−OMe (s), C_2^5−H/C_3^6−H (m), C_2^5−H/C_2^6−H (s), C_3^6−H/C_2^6−H (m), C_1^7−(MEM-CH_2)$_1$/C_{1a}^7−H (s), C_1^7−(MEM-CH_2)$_1$/C_1^7−(MEM-CH_2)$_2$ (s), C_2^7−H/C_{1b}^7−H (s), and C_2^7−H/C_{1a}^7−H (s), and no NOE cross-peaks were observed between C_{5b}^6−H/C_3^6−H, C_{5b}^6/C_2^6−H, C_2^6−H/C_3^6−OH, N_1^6−H/N_1^7−H, N_1^6−H/C_2^5−H, and N_1^6−H/C_{4a}^5−H. Most important in this spectroscopic assessment was not only the expected confirmation of the CD and AB atropisomer stereochemistry, but also the establishment of a vancomycin-like conformation for **26** bearing a cis amide linking the residues 5 and 6 (strong diagnostic C_2^5-H/C_2^6-H NOE), maintaining the spatial relationships and orientations of the AB ring system (strong diagnostic C_2^5−H/C_{4a}^5−H and C_2^6−H/C_{4a}^5−H NOEs) and the CD ring system (diagnostic C_{6b}^6−H/C_{4a}^4−H NOE). Although this might be considered unusual on the surface, even the natural atropisomer of the isolated AB ring system of vancomycin, without the surrounding CD ring system, adopts a conformation incorporating this cis amide structure, illustrating that it is the confines of the AB ring system, not that of the CD ring system, that defines this key cis amide conformational preference.[4] The lack of discernible NOEs to the methyl carbamate protecting the amine of the modified amide established that it extends out and away from the ABCD ring system binding pocket.

Synthesis of the Full Carbon Skeleton. Coupling of **27** and **28** (2.0 equiv of DEPBT,[17] 2.2 equiv of $NaHCO_3$, THF, 0−25 °C, 14 h, 73%) afforded **29** with excellent diastereoselectivity (12:1), arising from little competitive racemization (Scheme 4). These conditions were utilized on the basis of our experience with the teicoplanin[5] and ristocetin[6] aglycons and are superior to those originally reported for vancomycin[4] (EDCI) in terms of diastereoselectivity (12:1 vs 3:1). Closure of the DE ring system with formation of the key biaryl ether was accomplished by treatment of **29** with CsF (10 equiv, 20 equiv of $CaCO_3$,[20] 3-Å molecular sieves, DMF, 25 °C, 17 h) to afford **30** in good yield (74%) and good atropodiastereoselectivity (6−7:1). Notably, the closure of **30** was conducted under milder conditions than those originally disclosed for vancomycin[4,7−10] (DMF vs DMSO at 25 °C with added 3-Å molecular sieves and $CaCO_3$) and approaches the kinetic atropisomer diastereoselectivity observed in our efforts[4] (8:1), while surpassing that detailed in the related efforts by Evans[10] (5:1) and contrasting the closure detailed by Nicolaou[21] (1:3), where the unnatural atropisomer predominated with an alternative substrate and method of ring closure. Thus, consistent with the adoption of a vancomycin-like conformation by **26**, the amide modification in the ABCD ring system of **29** had little impact on the ease or diastereoselectivity of the DE ring closure. Reduction of the nitro group[22] (H_2, 10% Pd/C, THF, 8 h, 94%), followed by diazotization of the resulting amine **32** (1.2 equiv of HBF_4, 1.2 equiv of *t*-BuONO, CH_3CN, 0 °C, 20 min) and Sandmeyer substitution

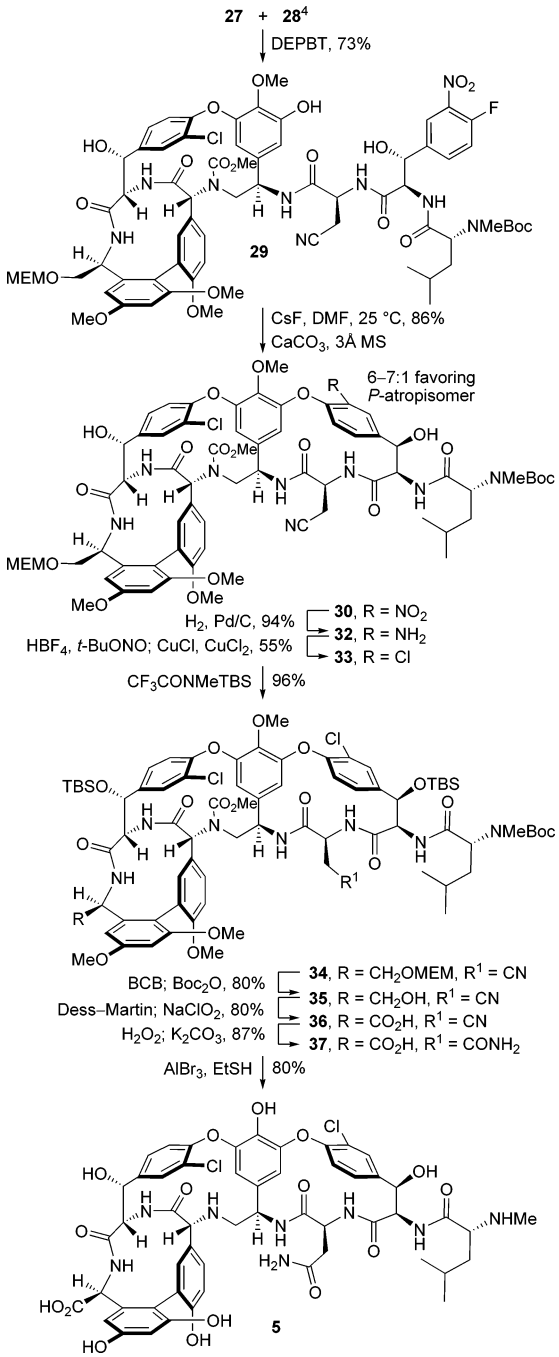
(19) Use of Raney nickel for N-Cbz removal was also successful, although lower recoveries (84%) of the product were observed.

(20) Both the added 3-Å molecular sieves and $CaCO_3$ result in cleaner conversions to product. It is not yet clear whether the soluble base under these conditions is CsF or Cs_2CO_3 with precipitation of insoluble CaF_2.

(21) (a) Nicolaou, K. C.; Takayanagi, M.; Jain, N. F.; Natarajan, S.; Koumbis, A. E.; Bando, T.; Ramanjulu, J. M. *Angew. Chem., Int. Ed.* **1998**, *37*, 2717. (b) Nicolaou, K. C.; Natarajan, S.; Li, H.; Jain, N. F.; Hughes, R.; Solomon, M. E.; Ramanjulu, J. M.; Boddy, C. N. C.; Takayanagi, M. *Angew. Chem., Int. Ed.* **1998**, *37*, 2708. (c) Nicolaou, K. C.; Jain, N. F.; Natarajan, S.; Hughes, R.; Solomon, M. E.; Li, H.; Ramanjulu, J. M.; Takayanagi, M.; Koumbis, A. E.; Bando, T. *Angew. Chem., Int. Ed.* **1998**, *37*, 2714. (d) Nicolaou, K. C.; Mitchell, H. J.; Jain, N. F.; Winssinger, N.; Hughes, R.; Bando, T. *Angew. Chem., Int. Ed.* **1999**, *38*, 240. (e) Nicolaou, K. C.; Li, H.; Boddy, C. N. C.; Ramanjulu, J. M.; Yue, T.-Y.; Natarajan, S.; Chu, X.-J.; Brase, S.; Rubsam, F. *Chem. Eur. J.* **1999**, *5*, 2584. (f) Nicolaou, K. C.; Boddy, C. N. C.; Li, H.; Koumbis, A. E.; Hughes, R.; Natarajan, S.; Jain, N. F.; Ramajulu, J. M.; Brase, S.; Solomon, M. E. *Chem. Eur. J.* **1999**, *5*, 2602. (g) Nicolaou, K. C.; Koumbis, A. E.; Takayanagi, M.; Natarajan, S.; Jain, N. F.; Bando, T.; Li, H.; Hughes, R. *Chem. Eur. J.* **1999**, *5*, 2622. (h) Nicolaou, K. C.; Mitchell, H. J.; Jain, N. F.; Bando, T.; Hughes, R.; Winssinger, N.; Natarajan, S.; Koumbis, A. E. *Chem. Eur. J.* **1999**, *5*, 2648.

(22) Reduction of the nitro group was sensitive to the choice of solvent in terms of recovery and observance of side products.

Scheme 4



(50 equiv of CuCl, 60 equiv of $CuCl_2$, H_2O, 0−25 °C, 1 h, 55%), gave **33**, which embodies the full carbon skeleton of **5**.

Completion of the Synthesis. With the full carbon skeleton in hand, we directed our attention toward completion of the synthesis (Scheme 4). TBS ether protection of the secondary alcohols (65 equiv of $CF_3CONMeTBS$, CH_3CN, 55 °C, 22 h; aqueous citric acid, 25 °C, 13 h, 96%), followed by MEM ether deprotection of **34** (12 equiv of *B*-bromocatecholborane (BCB), CH_2Cl_2, 0 °C, 2 h; 5.1 equiv of Boc_2O, 6.0 equiv of $NaHCO_3$, dioxane−H_2O 2:1, 0−25 °C, 2.5 h, 80%) and two-step oxidation of the resulting primary alcohol **35** (4.0 equiv of Dess−Martin periodinane, CH_2Cl_2, 0 °C, 15 min then 25 °C, 1 h; 9.0 equiv of 80% aqueous $NaClO_2$, 7.0 equiv of $NaH_2PO_4 \cdot H_2O$, *t*-BuOH/2-methyl-2-butene 4:1, 25 °C, 20 min, 82%), provided the carboxylic acid **36**. Hydrolysis of the residue 3 nitrile with formation of the carboxamide **37** (40 equiv of 30% aqueous H_2O_2, 8.0 equiv of 10% aqueous K_2CO_3, DMSO, 25 °C, 3.5 h, 87%)[4] set the stage for a final global deprotection.[23] In a final key reaction, **37** was treated with $AlBr_3$ (35 equiv, EtSH, 25 °C, 5 h, 80%) to afford **5,** arising from the remarkable deprotection of four aryl methyl ethers, the two TBS ethers, the N-terminus Boc group, and the critical residue 4 methyl carbamate.

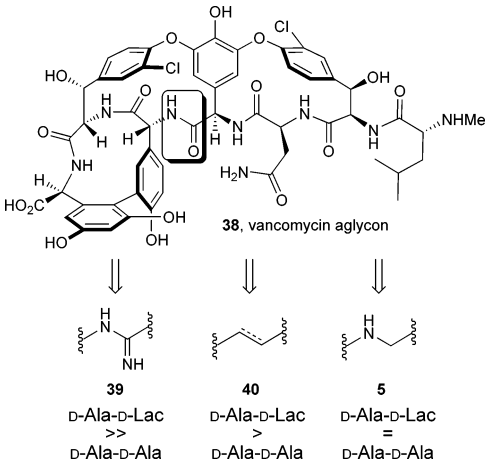


Figure 3. Potential modifications.

Assessment of [Ψ[CH_2NH]Tpg[4]]Vancomycin Aglycon. A subtle element in the design of **5** and choice of simply removing the residue 4 carbonyl rests with the projected properties of the molecule. In principle, one might consider reengineering the capabilities of a reverse H-bond into the vancomycin structure, removing the destabilizing lone-pair interaction with D-Ala-D-Lac and reinstating the lost H-bond. Such opportunities include amidine derivatives (e.g., [Ψ[C(═NH)NH]Tpg[4]]vancomycin aglycon **39**, Figure 3), and ongoing efforts have provided a thioamide intermediate that we intend to use to access such derivatives.[18] Significantly, such derivatives should enhance D-Ala-D-Lac binding, approaching the level of affinity observed with vancomycin and D-Ala-D-Ala. However, these derivatives would also accordingly reduce binding to D-Ala-D-Ala. Consequently, they would be expected to gain antimicrobial activity against constituitively resistant bacteria endowed with a D-Ala-D-Lac peptidoglycan cell wall precursor (e.g., VanD) but be inactive against sensitive and inducibly resistant bacteria (VanA and VanB) that maintain or at least start with a D-Ala-D-Ala peptidoglycan cell wall precursor. The closest modified vancomycins that would be expected to reproduce the binding results observed in Figure 1 are those that replace the amide bond linking residues 4 and 5 with a methylene (CH_2CH_2) or ethylene (CH═CH) linker. Such derivatives, by analogy with the results in Figure 1, would be expected to enhance D-Ala-D-Lac affinity 100-fold, missing only the contribution to binding derived from the H-bond. However, they might be expected to pay an additional penalty for binding D-Ala-D-Ala derived from not only the loss of the H-bond (10-fold) but also for not

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(25) McAtee, J. J.; Castle, S. L.; Jin, Q.; Boger, D. L. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 1319.

Table 1. Binding and Antimicrobial Properties

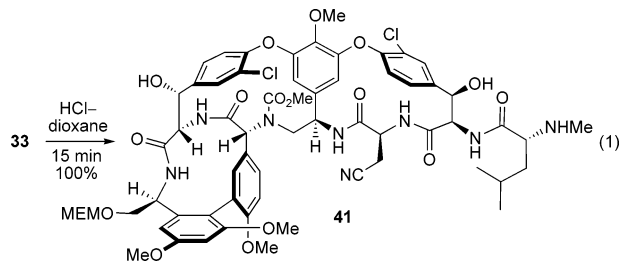
compound	K_a (M^{-1}) **2**[a]	K_a (M^{-1}) **4**[b]	K_a**2**/K_a**4**	VanA,[c] MIC (μg/mL)[d]
1, vancomycin	2.0×10^5	1.8×10^2	1100	>500 (2000)[e]
38, vancomycin aglycon	1.7×10^5	1.2×10^2	1400	>500 (640)[e]
5	4.8×10^3	5.2×10^3	0.92	31
41	1.6×10^3	4.1×10^3	0.40	31

[a] Ac$_2$-L-Lys-D-Ala-D-Ala. [b] Ac$_2$-L-Lys-D-Ala-D-Lac. [c] *Enterococcus faecalis* (VanA, BM4166). [d] Vancomycin and vancomycin aglycon exhibit MICs of 1−2.5 μg/mL against wild-type *E. faecalis*. [e] Taken from ref 25.

satisfying a ligand H-bond donor (10-fold?, for a cumulative 100-fold reduction?). As such, the derivatives might be expected to be marginally active against sensitive and inducibly resistant bacteria growing with a D-Ala-D-Ala peptidoglycan cell wall precursor (10- to 100-fold loss in activity) and more active against constituitively resistant bacteria (10-fold loss in activity) bearing the D-Ala-D-Lac precursor. Consequently, and while we intend to examine such vancomycin analogues, they were not the first to be targeted for synthesis.

The targeted analogue **5**, incorporating an amine in the linkage of residue 4 with residue 5, not only removes the offending carbonyl and the destabilizing lone-pair interaction with D-Ala-D-Lac but also maintains a local polar environment (protonated amine) that may better accommodate the binding of an electronegative group or atom (NH of D-Ala-D-Ala amide or O of D-Ala-D-Lac ester). Intuitively, we anticipated that, while this might not bind D-Ala-D-Lac quite as well as derivatives such as **40**, it would be better than **40** at binding D-Ala-D-Ala. In the best case, **5** might bind D-Ala-D-Ala and D-Ala-D-Lac with equal affinity, making it effective for the treatment of sensitive or resistant bacteria, regardless of the structure of the peptidoglycan cell wall precursor. Notably, this dual binding results from a reduction in the affinity for D-Ala-D-Ala and an enhancement in the affinity for D-Ala-D-Lac, such that the affinity for either splits that observed with vancomycin. Accordingly, while the spectrum of antimicrobial activity increases relative to vancomycin to include sensitive and resistant bacteria, the potency of the derivative would be anticipated to be reduced 30- to 40-fold.

The results of our assessment of **5** alongside vancomycin (**1**) and its aglycon **38** are compiled in Table 1.[24,25] An additional analogue **41**, derived from N-Boc deprotection of the synthetic intermediate **33** (eq 1), was also examined that bears the methoxycarbonyl protecting group on the residue 4/5 linking amine. The binding affinities of **5** for Ac$_2$-L-Lys-D-Ala-D-Ala



(1)

(**2**) and Ac$_2$-L-Lys-D-Ala-D-Lac (**4**) were essentially equivalent (4.8×10^3 vs 5.2×10^3 M^{-1}, respectively), with the D-Ala-D-Lac binding being slightly better. Impressively, this represented the anticipated results relative to the vancomycin aglycon, where the enhancement for binding D-Ala-D-Lac is 43-fold (5.2×10^3 vs 1.2×10^2 M^{-1}) and the reduction in binding affinity for D-Ala-D-Ala is 37-fold (4.8×10^3 vs 1.7×10^5 M^{-1}). In addition, the comparison of **5** with **41** seems to reflect the intuitive expectations of the impact of the polar amine (protonated) versus its carbamate derivative, where the binding affinity for D-Ala-D-Ala with **5** versus **41** increases 3-fold (4.8×10^3 vs 1.6×10^3 M^{-1}), while the impact on D-Ala-D-Lac is a more marginal 1.2-fold increase in affinity (5.2×10^3 vs 4.1×10^3 M^{-1}). Although there are additional structural features in the comparison of **5** and **41** that might impact the absolute affinities measured, in both instances the binding increases with the free amine **5**, and it is with **5** that the dual binding is balanced.

The four compounds were compared in an antimicrobial assay against VanA *Enterococcus faecalis* (BM4166) that is inducibly resistant to treatment by glycopeptide antibiotics including vancomycin and teicoplanin (Table 1). It is the most difficult of the resistant organisms to treat (vs VanB), and, characteristic of such organisms, they grow unchallenged enlisting a D-Ala-D-Ala peptidoglycan cell wall precursor, but switch to D-Ala-D-Lac upon glycopeptide treatment. As such, it represents a superb test of whether **5** and related dual D-Ala-D-Ala/D-Lac binding antibiotics might prove useful in the treatment of resistant bacteria. Beautifully, **5** as well as **41** exhibited MICs of 31 μg/mL, being roughly 40-fold more potent than vancomycin or its aglycon (MICs = 2000 and 640 μg/mL), correlating well with the ca. 40-fold increase in binding affinity for D-Ala-D-Lac. Moreover, this potency is roughly 30-fold weaker than that observed with vancomycin and its aglycon against sensitive *E. faecalis* (MICs = 1−2.5 μg/mL), correlating with the 35- to 40-fold loss in binding affinity for D-Ala-D-Ala. These results suggest that, regardless of the peptidoglycan cell wall precursor utilized by the organism, it remains equally sensitive to treatment by **5** and **41**.

Conclusions. The modification of the dipeptide terminus of peptidoglycan cell wall precursors from D-Ala-D-Ala to D-Ala-D-Lac in resistant bacteria reduces the binding affinity of vancomycin for the ligand by 1000-fold, leading to a 1000-fold loss in biological activity. We had shown that a modified peptide ligand possessing a methylene in place of the lactate oxygen restores 100-fold of this binding affinity by removal of a destabilizing lone-pair interaction. This suggested that removal of the residue 4 carbonyl in the vancomycin aglycon would produce an analogue with enhanced affinity for D-Ala-D-Lac and might restore much of the biological activity of the molecule that is lost with resistant bacteria. Moreover, and among the range of potential modifications that could be envisioned, that entailing the simple removal of the residue 4 carbonyl, providing **5**, was anticipated to bind D-Ala-D-Ala and D-Ala-D-Lac with similar affinities, providing an analogue that might be equivalently effective against sensitive (D-Ala-D-Ala) and resistant (D-Ala-D-Lac) bacteria. Consequently, we extended our efforts on the preparation of glycopeptide antibiotics to a total synthesis of the [Ψ[CH$_2$NH]Tpg^4]vancomycin aglycon (**5**), in which the residue 4 carbonyl has been replaced with a methylene. Consistent with expectations and relative to the vancomycin aglycon, **5** exhibited a 40-fold increase in affinity for D-Ala-D-Lac ($K_a = 5.2 \times 10^3$ M^{-1}) and a corresponding 35-fold reduction in affinity for D-Ala-D-Ala ($K_a = 4.8 \times 10^3$ M^{-1}),

providing a molecule with balanced, dual binding characteristics. Beautifully, **5** exhibited antimicrobial activity against a VanA-resistant organism that remodels its D-Ala-D-Ala peptidoglycan cell wall precursor to D-Ala-D-Lac upon glycopeptide challenge, displaying a potency that reflects these binding characteristics.

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Supporting Information Available: Full experimental details. This material is available free of charge via the Internet at http://pubs.acs.org.

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