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Tetrachlorovancomycin: Total Synthesis of a Designed Glycopeptide Antibiotic of Reduced Synthetic Complexity

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Abstract

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Supporting Information

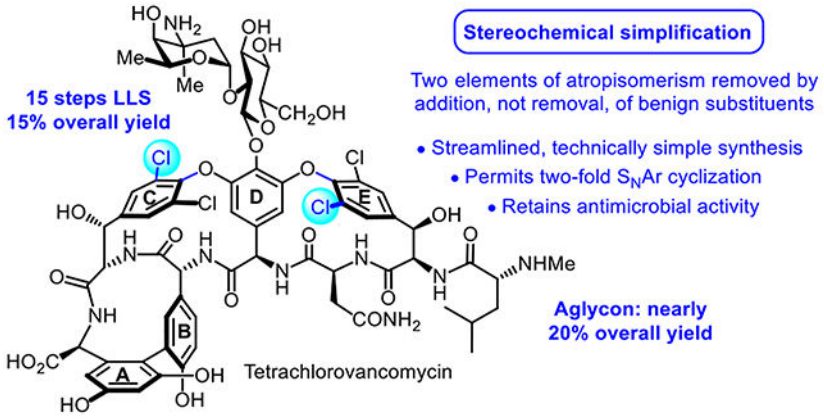
Full experimental details, summary of literature antimicrobial activity and D-Ala-D-Ala binding affinity of dechlorovancomycins and their aglycons (Figure S1), ligand binding affinity of vancomycin and tetrachlorovancomycin aglycons (Figure S2), X-ray crystal data and structure refinement for **25**, and copies of ^{1}H, ^{13}C, and 2D NMR spectra (pdf)

The Supporting Information is available free of charge on the ACS Publications website

The authors declare no competing financial interests.

A technically straightforward total synthesis of a new class of vancomycin analogues of reduced synthetic complexity was developed that provided tetrachlorovancomycin (**1**, LLS = 15 steps, 15% overall yield) and its precursor aglycon **29** (nearly 20% overall yield). The class retains all the intricate vancomycin structural features that contribute to its target binding affinity and selectivity, maintains the antimicrobial activity of vancomycin, and achieves the simplification by an unusual addition, not removal, of benign substituents to the core structure. The modification, accomplished by addition of two aryl chloride substituents to provide **1**, permitted a streamlined total synthesis of the new glycopeptide antibiotic class by removing the challenges associated with CD and DE ring system atropisomer stereochemical control. This also enabled their simultaneous and further-activated S_NAr macrocyclizations that establish the tricyclic skeleton of **1**. Key elements of the approach include catalyst-controlled diastereoselective formation of the AB biaryl axis of chirality (>30:1 dr), an essentially instantaneous macrolactamization of the AB ring system free of competitive epimerization (>30:1 dr), racemization free coupling of the E ring tetrapeptide, room temperature simultaneous CD and DE ring system cyclizations, a highly refined 4-step conversion of the cyclization product to the aglycon, and a protecting group free one-pot enzymatic glycosylation for disaccharide introduction. In addition to the antimicrobial evaluation of tetrachlorovancomycin (**1**), the preparation of key peripherally-modified derivatives, which introduce independent and synergistic mechanisms of action, revealed their exceptional antimicrobial potency and provide the foundation for future use of this new class of synthetic glycopeptide analogues.

Graphical Abstract



INTRODUCTION

In a series of studies, we have shown that deep-seated changes in the binding pocket of vancomycin[1,2] can be used to overcome vancomycin resistance by reinstating binding to the altered cell wall precursors terminating in D-Ala-D-Lac while maintaining binding for the native D-Ala-D-Ala target found in sensitive bacteria.[3] This redesign, along with peripheral modifications to the glycopeptides that introduce independent synergistic mechanisms of action, provided an exciting advance in the development of durable antibiotics that are less susceptible to raising resistance than vancomycin itself.[4] A remaining challenge for their potential translation to the clinic is their accessibility, presently requiring total syntheses to

obtain the targeted glycopeptide analogues.[5] Herein, we disclose a new class of structurally simplified synthetic glycopeptide antibiotics that is now easily accessible by total synthesis and directly addresses this challenge. The class retains all the intricate structural features of vancomycin that contribute to its target binding affinity and selectivity, maintains the antimicrobial activity of vancomycin, and achieves this simplification by addition, not removal, of benign substituents to the core structure.

The diastereoselective introduction of the three elements of atropisomerism embedded in the vancomycin structure is a central challenge to its synthesis (Figure 1).[5] In the most recent next generation total synthesis of vancomycin (LLS = 19 steps, 3.7% overall yield),[6] the AB biaryl axis of chirality was set through a diastereoselective chiral catalyst-controlled Suzuki–Miyaura coupling. Preorganization provided by the rigid AB macrocycle was used to then construct the CD and subsequently the DE macrocyclic diaryl ethers with high substrate-controlled atroposelectivity.[6] The extension of the work to [Ψ[C(=S)NH]Tpg^4]vancomycin for accessing pocket-modified vancomycin analogues further improved on the approach and established a scalable synthesis.[7]

The change in the vancomycin structure detailed herein that simplifies the total synthesis is exemplified by 2_e,6_e-dichlorovancomycin (**1**), a fully synthetic analogue of vancomycin in which two added chlorides are placed opposite those naturally present on the C and E rings (Figure 1). This modification renders the CD and DE diaryl ethers symmetrical and eliminates the two atropisomer elements that are challenging to synthetically control. As detailed herein, this simplification allows full control of all stereochemical features and results in a straightforward total synthesis of **1** (15 steps LLS, 15% overall yield) and its precursor aglycon (nearly 20% overall yield) with a further reduction in the step count and improvement in overall yield relative to vancomycin itself. It also improves the CD/DE macrocyclization rates and efficiencies that are now run concurrently and provides a synthetic glycopeptide antibiotic that maintains the ligand binding and antimicrobial activity of the natural product. For convenience, we refer to these 2_e,6_e-dichlorovancomycin analogues as tetrachlorovancomycins (e.g., 2_e,6_e-dichlorovancomycin (**1**) = tetrachlorovancomycin), highlighting the four aryl chlorides now present on the core structure.

RESULTS AND DISCUSSION

Two key features are required for the simplification to be beneficial. First, the two added chloride substituents must not significantly reduce the target D-Ala-D-Ala binding and resulting antimicrobial activity. Second, they would need to be compatible with the enzymatic glycosylations used to introduce the disaccharide.[5] The latter could only be established experimentally as little is known about the stringency of the glycopeptide substrate requirements for the native glycosyltransferases, especially what impact the added non-native chlorides proximal to the D-ring phenol might have on the initial glycosylation reaction.

Role of the Vancomycin C and E Ring Aryl Chlorides.

In contrast, much more is known about the key impact of the vancomycin native aryl chlorides. Both the vancomycin C and E ring aryl chlorides play important roles in ligand binding and antimicrobial activity. Both contribute to ligand binding affinity (C-ring > E-ring chloride) and their cumulative removal results in a >10-fold loss in ligand binding affinity and antimicrobial activity (Supporting Information Figure S1).[8–10] In addition to its stabilizing hydrophobic interaction with the ligand terminal D-Ala methyl group, the native C-ring chloride also provides a cap to the binding pocket, which provides selectivity for D-Ala-D-Ala binding by restricting the size of peptide side chain that it can accommodate (Me > H >> all others).[11] Thus, a vancomycin structural simplification achieved through removal of both native aryl chlorides is likely too detrimental to be useful. Complementary to these studies, we showed that placement of an isomeric non-native E-ring chloride over the binding pocket (2_e,6_c dichloride) has only a small effect (2_e-Cl,2_c-H vs 2_e-H,2_c-H).[12] This observation, combined with expectations that incorporation of an additional C-ring chloride distal from the binding pocket (6_e-Cl) is unlikely to have a significant impact,[13] suggested that the addition of two non-native chlorides would be more effective than removal of the two native chlorides, providing the synthetic simplification sought with modest impact on the ligand binding affinity/selectivity and antimicrobial properties.

To our knowledge, no member of the glycopeptide antibiotics has been discovered that contains the proposed symmetrical 2_c,2_e,6_c,6_e-tetrachlorination pattern. Therefore, we set out to establish the effect of the tetrachloro modification on ligand binding affinity and antimicrobial activity through the total synthesis of tetrachlorovancomycin (**1**) as well as its peripherally modified derivatives for direct comparison with vancomycin and its derivatives.

Synthetic Strategy.

The concise route to tetrachlorovancomycin (**1**) that was designed takes advantage of the increased symmetry (Figure 2). With each chlorinated *o*-fluoronitrophenyl group now more activated toward S_NAr substitution, both the CD and DE macrocyclizations would now be accomplished in a single operation as their stereochemical outcome is inconsequential following Sandmeyer chlorination. The remaining element of atropisomerism, the biaryl axis of chirality embedded in AB macrocycle, would be set by a reliable, highly diastereoselective catalyst-controlled Suzuki–Miyaura coupling.[6]

Although confident in the ability of the approach to provide tetrachlorovancomycin aglycon, it was less clear whether the native glycosyltransferases[14–17] GtfE and GtfD would recognize it as a substrate for the disaccharide introduction. Of these, the action of GtfE was of most concern as it installs the first sugar that is proximal to D-ring phenol glycosylation site and is the much slower of the two reactions. Pioneering studies of Walsh and Kahne showed that GtfE with UDP-glucose can glycosylate additional aglycons (e.g., teicoplanin vs vancomycin),[15] yet we have found that it is sensitive to peripheral as well as pocket modifications to vancomycin.[18] To our knowledge, no studies conducted to date provided assurance the added non-native 6_e and 2_e chlorides and the combined tetrachloro substitution proximal to the D-ring phenol glycosylation site would be tolerated. These native glycosyltransferases[14–17] were instrumental to our total synthesis of vancomycin,[6,18]

allowing direct aglycon glycosylation without the need for protecting groups and avoiding the less efficient chemical glycosylation methods.[19–21] Thus, the success of the enzymatic glycosylations of tetrachlorovancomycin established herein is key to accessing not only **1**, but also future pocket-modified analogues.

Preparation of the Modified C and E Ring Subunits.

Substantial improvements in the syntheses of the unnatural amino acid subunits were introduced recently such that five subunits are now derived from inexpensive chiral pool starting materials, only the C and E ring subunits require asymmetric synthesis, all require ≤5 steps to access, and all but one are obtained in >50% overall yield.[6] Because residues 1, 3, 4, 5, and 7 are already in hand from this past work,[6] only the preparation of the new chlorinated C and E ring β-hydroxyphenylalanine subunits were required to access **1** (Scheme 1). Diastereoselective Crimmins aldol[22] addition of **2** to 3-chloro-4-fluoro-5-nitrobenzaldehyde (**3**)[23] followed by in situ methanolysis of the imide provided the syn aldol product **4** as a single diastereomer (66%, anti-diastereomer not detected). TBS protection of the secondary alcohol to provide **5** followed by Staudinger reduction afforded the C ring (residue 6) free amine **6** (≥50% overall yield/3 steps).

Preparation of the E ring anti β-hydroxyphenylalanine **9** was accomplished by modification of the method of Solladié–Cavallo[24] that allowed use of commercially available $ClTi(OiPr)_3$ (Scheme 1). Accordingly, diastereoselective Ti-promoted aldol addition of imine **7** to the same aldehyde **3**[23] provided the anti-product **8** in good yield (89%, syn diastereomer not detected). Hydrolytic removal of the chiral auxiliary with dilute aqueous HCl provided the E ring (residue 2) amine **9** (92%, 82% overall yield/2 steps).

Preparation of the Linear DE Tetrapeptide.

Compound **9** was incorporated into the DE tetrapeptide **17** through a series of peptide coupling reactions (Scheme 2), starting with its reaction with commercially available BocNMe-D-Leu-OH (2,4,6-tripropyl-1,3,5,2,4,6-trioxatriphosphorinane-2,4,6-trioxide (T3P),[25] *N*-methylmorpholine (NMM), THF, 0 °C) to provide **10** (89%). Saponification of the isopropyl ester **10** was surprisingly clean (Me_3SnOH,[26] $ClCH_2CH_2Cl$, 96%), providing carboxylic acid **11** without detectable epimerization. In contrast, use of even carefully controlled aqueous saponification conditions (3 equiv LiOH, 2:1 *t*-BuOH–H_2O, 0 °C, 1 h) led to significant C_α epimerization (4:1 dr).

Coupling of **11** with β-cyanoalanine methyl ester **12**[27] promoted by 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride[28] (DMTMM, EtOAc, 97%) provided tripeptide **13**. Saponification (Me_3SnOH, $ClCH_2CH_2Cl$, 93%) provided carboxylic acid **14**, which was coupled with the D ring free amine **15**[6] (DMTMM, THF, 89%) to afford **16**.[29] Methyl ester hydrolysis (Me_3SnOH, $ClCH_2CH_2Cl$, 81%) provided the tetrapeptide **17** without epimerization or competitive desilylation of the base-sensitive phenol TBS ethers.

Preparation of the AB Macrocycle and Total Synthesis of Tetrachlorovancomycin.

Coupling of **6** with **18**[30] (DMTMM, MeCN, 0 °C, 1 h) followed by phenol methylation ($TMSCHN_2$, 20% MeOH–CH_2Cl_2, 5 °C, 36 h) provided **20** (82%/2 steps) (Scheme 3). A

one-pot Miyaura borylation–Suzuki coupling sequence conducted with an in situ generated (*R*)-BINAP(O)-Pd^0 catalyst system[6,31] provided **22** as a single detectable diastereomer (72%, >30:1 dr), setting the AB biaryl atropisomer stereochemistry. This telescoped reaction sequence, with in situ generation of **21** from the corresponding bromide, was conducted under mild reaction conditions nearly identical to those disclosed in a total synthesis of vancomycin[6] ($Pd(OAc)_2$, (*R*)-BINAP, aq $NaHCO_3$, MeTHF) and did not require tailoring to accommodate the modified substrate **20**, which is intrinsically more reactive toward S_NAr substitution.

Oxidation of the primary alcohol **21** to the corresponding carboxylic acid ($PhI(OAc)_2$, cat. TEMPO, 2:1 MeCN–H_2O, 23 °C, 1 h)[32] and esterification with *t*-butyl trichloroacetimidate (CH_2Cl_2–cyclohexane, 23 °C, 16 h) cleanly provided **23** (90%/2 steps, Scheme 3). Simultaneous hydrolysis of the methyl ester and trifluoroacetamide was best accomplished with $Ba(OH)_2$ (5 equiv, 2:1 *t*-BuOH–H_2O, 23 °C, 95%). This reagent proved to be milder[33] than LiOH and more easily removed by precipitation as $BaCO_3$, thus avoiding minor losses of **24** due to aqueous extraction and chromatography. Macrolactamization of **24** under simulated high-dilution conditions promoted by 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium hexafluorophosphate[34] (DMTMMH, 1.5 equiv, 3 equiv *i*-Pr_2NEt, NMP, 0.1 M) provided the AB macrocycle **25** in superb yield (83%/2 steps). This cyclization reaction proceeds essentially instantaneously upon dropwise addition of **24** to a solution containing DMTMMH without trace of epimerization (>30:1 dr) and benefits from the modulated nucleophilicity of the reacting amine that precludes its competitive addition to the coupling reagent.[6] The structure, relative stereochemistry, and absolute configuration of **25**, including the AB biaryl atropisomer stereochemistry, were confirmed in single crystal X-ray structure determination (Scheme 3). The cis amide between residues 5 and 6 in the crystal structure is characteristic of the strained 12-membered ring system,[35] both in related AB macrocycles and within the tricyclic core structure of vancomycin.[5] Boc deprotection of **25** was accomplished under conditions that may allow reversible deprotection of the slightly acid-labile *t*-butyl ester[36] (8 equiv H_2SO_4, *t*-BuOAc, 0 to 23 °C, 2 h), providing **26** (82%) which serves as the common precursor to tetrachlorovancomycin (**1**) as well as future binding pocket-modified analogues. Strikingly, NOESY studies of **26**, bearing the free amine, also revealed exclusive adoption of the 5,6-cis amide conformation.

The final steps to the full tricyclic skeleton of tetrachlorovancomycin proved remarkably smooth (Scheme 4). Although the D ring phenylglycine is prone to epimerization,[1,37] coupling of **26** with the linear DE tetrapeptide **17** mediated by 3-(diethoxyphosphoryloxy)-1,2,3-benzotriazin-4 (3*H*)-one[38] (DEPBT, 2 equiv, 4.5 equiv $NaHCO_3$, 23 °C, 17 h) proceeded in excellent yield (93%), providing heptapeptide **27** without detectable epimerization (>30:1 dr). A subsequent room temperature in situ double S_NAr cyclization of **27** was observed under the conditions of desilylation with Bu_4NF (5 equiv, MeCN, 23 °C, 4 h), establishing both the CD and DE macrocycles in a single step and providing **28** in superb yield (95%) as an inconsequential mixture of atropisomers. The ease of the two-fold intramolecular S_NAr macrocyclizations is noteworthy, being conducted at room temperature with a mild desilylating agent (Bu_4NF) in a relatively nonpolar solvent that is conveniently removed by evaporation upon reaction completion

(MeCN). The effortless cyclization of **27** relative to related substrates[5,35,39] can be attributed to the inductive electron-withdrawing effect of the added aryl chloride substituent on each the C and E rings, increasing the ease of S_NAr reaction.[23] The cyclized product **28**, as a mixture of isomers, was directly subjected to dual nitro group reduction (Fe, AcOH), and two-fold Sandmeyer chlorination (BF_3•Et_2O, *t*-BuONO; CuCl, $CuCl_2$, CD_3CN).[6,7] A final global deprotection that involves neat TFA cleavage of the Boc group and *t*-butyl ester concurrent with nitrile hydration[40] followed by subsequent removal of four methyl ethers (5:1 $AlBr_3$/EtSH) afforded tetrachlorovancomycin aglycon (**29**, 56%/5 steps from **27**) as a single diastereomer. Remarkably, the conversion of AB macrocycle **26** to the fully functionalized, deprotected aglycon **29** now requires only 6 steps, proceeds in 52% overall yield, and avoids the generation of undesired atropisomers or C_α diastereomers. Highlights in this sequence include not only the mild room temperature double S_NAr cyclization of **27** (<4 h, 95%), but also the clean Fe-mediated dual nitro group reduction with avoidance of hydroxylamine byproducts,[7] a highly refined two-fold Sandmeyer substitution reaction with Lewis acid-mediated diazonium salt formation[7] and deuterated solvent suppression[6] of competitive reduction, a remarkably effective TFA-mediated nitrile hydration,[40] and a scalable $AlBr_3$/EtSH-mediated global deprotection.[7]

Finally, we were delighted to find that the one-pot two-step enzymatic glycosylation of tetrachlorovancomycin aglycon (**29**) proceeded in high yield (82%) for installation of both sugar residues despite the added 2_e and 6_e aryl chlorides (Scheme 4). The disaccharide introduction makes use of the two overexpressed recombinant glycosyltransferases GftE and GftD involved in the biosynthetic glycosylation of vancomycin and the glycosyl donors UDP-glucose (commercially available) and UDP-vancosamine.[41] UDP-substrate loadings were reduced in a recent optimization of the scaled enzymatic reactions,[41] and these reversible reactions are driven to completion by addition of calf intestinal alkaline phosphatase[42] to each glycosylation reaction. This latter feature, which results in hydrolysis of the byproduct uridine-5'-diphosphate (UDP), also prevents product inhibition and allows the sequential reactions to be conducted in one-pot without pseudoaglycon isolation. In addition to providing **1**, The bonus of this latter work is that it also helps define qualitatively the glycopeptide substrate tolerance of the native glycosyltransferases. Because the initial glycosylation of tetrachlorovancomycin aglycon with GftE and UDP-glucose for installation the first sugar is proximal to the newly added non-native 6_e and 2_e chlorides, it is the most likely step to have been impacted. However, this slowest of the two glycosylation reactions could be conducted under conditions identical to those optimized for vancomycin[41] (time, temp.) even with the reduced amounts of enzyme catalyst and sugar donor.

Even within the constraints of an academic lab, this allows substantial quantities of a fully synthetic glycopeptide antibiotic to be prepared by total synthesis, and for us includes its ongoing extension to analogues bearing deep-seated binding-pocket modifications. The concise, technically straightforward total synthesis provides **1** (15 steps LLS, 15% overall yield) and its precursor aglycon **29** (nearly 20% overall yield) with complete control of all stereochemistry, avoiding the generation of minor undesired atropisomers and past problematic C_α epimerizations, and was unimaginable at the time we initiated our studies many years ago (Figure 3).

Peripherally-modified Tetrachlorovancomycins.

Two key peripheral modifications have emerged in studies with vancomycin that introduce independent mechanisms of action, further inhibit cell wall biosynthesis or its integrity, overcome vancomycin resistance, synergistically improve antimicrobial potency, reduce susceptibility toward raising resistance, and even improve *in vivo* pharmacokinetic (PK) properties.[4] For comparison purposes and representative of these modifications, the 4-chlorobiphenylmethyl (CBP)[43] derivatization of the vancosamine residue by reductive amination and the cationic 3-guanidylpropyl-1-amine amidation (G3)[44] of the C-terminus carboxylic acid were sequentially introduced in a single step each from **1** (TCV, tetrachlorovancomycin) without need for protecting groups under established conditions (Scheme 5).

Model Ligand Binding Studies.

The binding of tetrachlorovancomycin (**1**, Figure 4) and its aglycon **29** (Supporting Information Figure S2) to the model cell wall ligand Ac_2-L-Lys-D-Ala-D-Ala (**32**)[45] was examined by UV measurement of the change in absorbance upon titration of the ligand into a solution of glycopeptide (8.0×10^{-5} M, 20 mM sodium citrate buffer, pH = 5.1)[45,46] and by isothermal titration calorimetry (ITC, 8.0×10^{-5} M, 100 mM sodium citrate buffer, pH 5.1, 298 K)[47] and compared alongside vancomycin and its aglycon. The study established that **1** maintains a high affinity for the model ligand **32** ($K_a = 1.1 \times 10^5$ M^{-1}), displaying a binding constant only 5-fold lower than vancomycin ($K_a = 5.4 \times 10^5$ M^{-1}) and the difference was even smaller (3-fold) for the aglycons (Supporting Information Figure S2).[48] This small difference in ligand binding affinity correspondingly reduced the antimicrobial activity of **1** relative to vancomycin, but proved inconsequential to the activity of the more potent peripherally-modified tetrachlorovancomycin analogues (see below). Additionally, tetrachlorovancomycin (**1**), like vancomycin, fails to bind to an appreciable extent the model ligand of the peptidoglycan precursor found in vancomycin-resistant organisms, Ac_2-L-Lys-D-Ala-D-Lac (**33**), (Supporting Information Figure S3).[48] Finally, and although not examined herein, it has been shown elsewhere that addition of the peripheral 4-chlorobiphenylmethyl (CBP) group to vancomycin and related structures does not impact (increase) the solution phase binding affinity for model ligands.[43b] Similarly, we have found that a vancomycin G3 C-terminus modification does not impact (increase) the binding to Ac_2-L-Lys-D-Ala-D-Ala (ITC $K_a = 2.9 \times 10^5$ M^{-1}, for G3-vancomycin, unpublished studies).

In Vitro Antimicrobial Activity.

The antimicrobial activity of the series of tetrachlorovancomycin analogues against representative vancomycin sensitive methicillin-susceptible and methicillin-resistant *S. aureus* (MSSA and MRSA) as well as a VanA vancomycin-resistant *E. faecium* (VRE) strain is summarized in Figure 5. Resistance of VanA VRE is induced upon exposure to glycopeptide antibiotics through an intricate late-stage remodeling of the peptidoglycan precursor D-Ala-D-Ala to D-Ala-D-Lac. Tetrachlorovancomycin (**1**) and its aglycon **29** were found to be slightly less potent than vancomycin and its aglycon (ca. 4 to 8-fold) against the sensitive organisms (MSSA and MRSA) consistent with their relative ligand binding affinities toward Ac_2-L-Lys-D-Ala-D-Ala. Like vancomycin, **1** was inactive against VanA

VRE, serving to induce resistant peptidoglycan remodeling. However, after incorporation of the CBP peripheral modification, the activity of CBP-tetrachlorovancomycin (**30**) and CBP-vancomycin was indistinguishable in sensitive *S. aureus* strains. Moreover, **30** displayed activity against the VRE strain comparable to CBP-vancomycin, which is derived from direct competitive inhibition of transglycosylase (a second independent mechanism of action) that does not entail D-Ala-D-Ala binding.[49,50] The activity of CBP-tetrachlorovancomycin (**30**) is improved 50-fold relative to **1** against the sensitive strains due to the expression of two independent and synergistic mechanisms of action. Combined, these studies highlight both that **30** expresses the vancomycin core mechanism of action (D-Ala-D-Ala binding) effectively against the sensitive strains and that the added CBP peripheral modification introduces a well-established second mechanism of action independent of D-Ala-D-Ala binding (direct transglycosylase inhibition).[49,50]

Most significantly, G3,CBP-tetrachlorovancomycin (**31**) exhibited exceptional potency against all three pathogens, including the VanA vancomycin-resistant enterococci (VRE) that was indistinguishable from G3,CBP-vancomycin.[44] Even against VRE (two effective mechanisms of action now including G3 induced cell permeability),[44] the antimicrobial activity of **31** is superb (MIC = 0.3 μg/mL), and it is even an order of magnitude more potent against the vancomycin sensitive strains (three effective mechanisms of action). These results suggest that even greater VanA antimicrobial activity might be achieved with pocket-modified[7,51] analogues of G3,CBP-tetrachlorovancomycin (**31**) that reinstate binding to the modified cell wall precursor terminating in D-Ala-D-Lac. Although not examined with this tetrachlorovancomycin series, two additional features are worth noting. First, the synergistic anitimicrobial activity observed with the added peripheral modifications to tetrachlorovancomycin likely requires their incorporation in a single molecule as has been demonstrated with vancomycin[44] and its pocket-modified analogues.[7] Second, the peripherally-modified analogues of **1** that act by two or three independent mechanisms of action are unlikely to raise resistance and would be expected to be the newest members of an unusually durable antibiotic class.[4,44,51,52]

CONCLUSION

A concise and easily scalable synthesis of a new class of structurally simplified synthetic vancomycin analogues was developed that provides **1** (LLS = 15 steps, 15% overall yield)[53] and its precursor aglycon **29** (nearly 20% overall yield). The defining feature of this class is the introduction of an added chlorine substituent on the vancomycin C and E rings, which reduces synthetic complexity. The class retains the intricate vancomycin structural features that contribute to its target binding affinity and selectivity, maintains the antimicrobial activity of vancomycin, and achieves the simplification by an unusual addition of benign substituents to the core structure. This modification permitted a streamlined total synthesis of the new glycopeptide antibiotic class by removing the challenges associated with CD and DE ring system atropisomer stereochemical control and enabled their simultaneous and further activated S_NAr macrocyclizations that establish the tricyclic skeleton of **1**. Additional key elements of the approach include a catalyst-controlled diastereoselective formation of the AB biaryl axis of chirality (>30:1 dr), an essentially instantaneous macrolactamization

of the AB ring system free of competitive epimerization (>30:1 dr), an epimerization free coupling of the E ring tetrapeptide, the room temperature single-step CD and DE ring system S_NAr cyclizations, a refined 4-step conversion of the product to the aglycon, and a one-pot enzymatic glycosylation for disaccharide introduction. The results of the study not only highlight and maintain the key role of the natural product chloride substituents, improving target ligand binding affinity and selectivity, but also help define the glycopeptide substrate tolerance of the native glycosyltransferases enlisted to enzymatically introduce the disaccharide for which little is known. Finally, these studies, enabled by total synthesis, complement those detailed with other complex antibiotics[54] and hopefully help dispell the perception that they are beyond practical synthetic access. We hope to have highlighted that an otherwise challenging natural product synthetic target in which essentially every structural element and functional group is important to its properties can be reduced in complexity by adding benign substituents, rather than traditionally trying to remove structural features or truncate regions of the molecule. This redesign element provided a surprisingly simple solution even for a structure as complex as vancomycin that many might believe cannot be realistically accessed by total synthesis or is too complex for traditional medicinal chemistry to be conducted by total synthesis.

In addition to the antimicrobial evaluation of tetrachlorovancomycin, the subsequent preparation and examination of two key peripherally-modified derivatives, which introduce independent and synergistic mechanisms of action, revealed their exceptional antimicrobial potency and provide the foundation for use of this new family of synthetic glycopeptide analogues. For us, this provides the foundation for the future preparation of binding pocket-modified analogues[4] of tetrachlorovancomycin to reinstate binding to the altered target D-Ala-D-Lac of vancomycin-resistant bacteria while maintaining binding for the unaltered target D-Ala-D-Ala found in sensitive bacteria as well as extension to their even more potent and durable peripherally-modified derivatives.[4,7]

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Data Availability Statement

The data underlying this study are available in the published article and its online supplementary material.

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53. If one were to count the coupling of **25** with **17** (Boc deprotection and amide bond coupling) and entire Sandmeyer substitution reaction sequence (nitro reduction, amine diazotization, and chloride substitution) each as a single step, the LLS = 13 steps.

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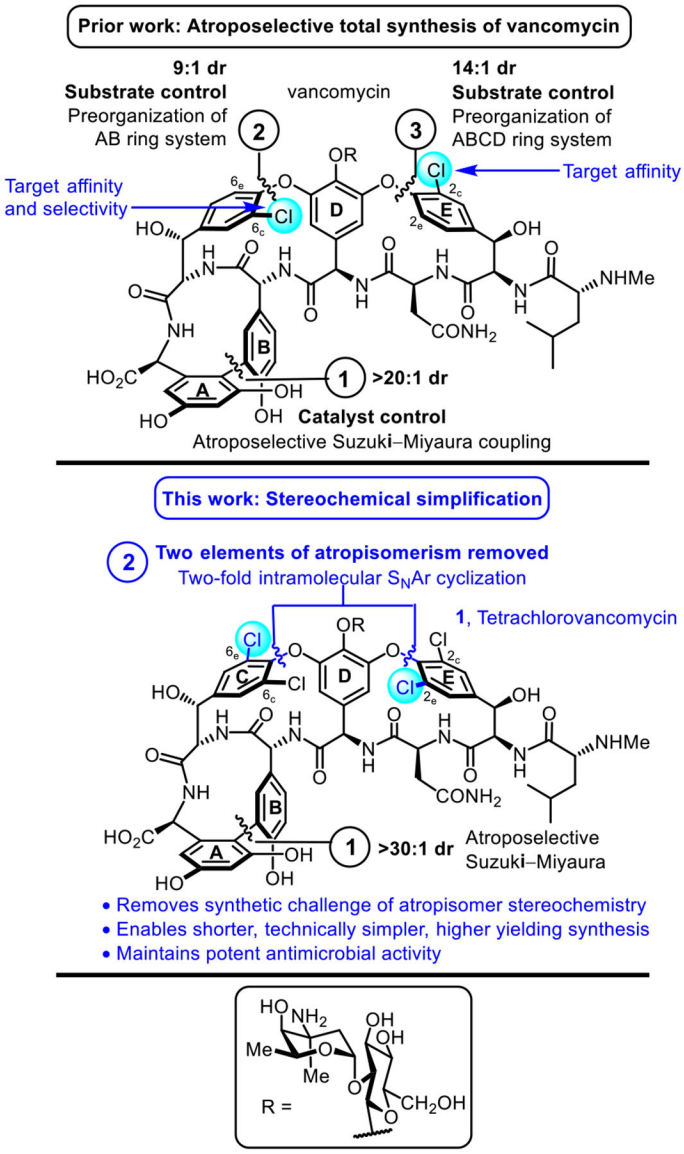


Figure 1.

Comparison of vancomycin and tetrachlorovancomycin, highlighting the synthetic simplification achieved by adding two aryl chloride substituents.

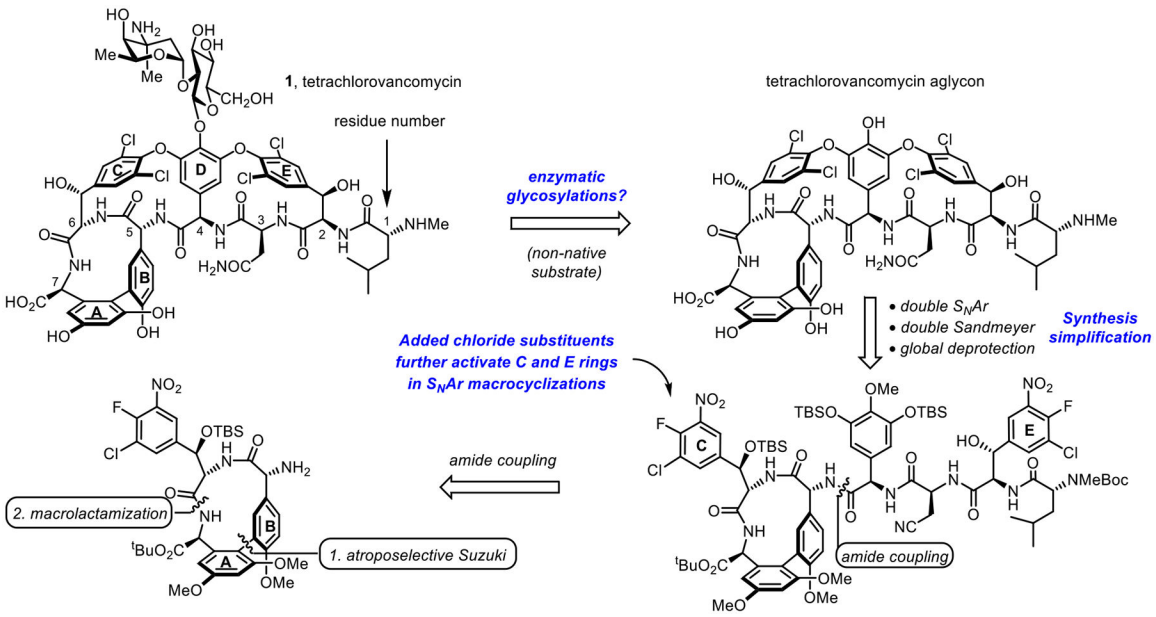


Figure 2.
Key elements of the retrosynthetic analysis for tetrachlorovancomycin.

Vancomycin Total Syntheses	LLS	Yield	Atroposelectivity AB	CD	DE	Glycosylation method	steps	yield
Nicolaou (1998)	35	0.006%	2:1	1:1	1:3	chemical	11	14%
Boger (2014)	27	0.14%	1:1	1:1	8:1	enzymatic	2	80%
Boger (2020)	19	3.7%	>20:1	8:1	14:1	enzymatic	2	80%
Tetrachlorovancomycin			**Atroposelectivity**			**Glycosylation**		
Total Synthesis	**LLS**	**Yield**	AB	CD	DE	method	steps	yield
This work	**15**	**15%**	**>30:1**	–	–	**enzymatic**	**1**	**82%**

Figure 3.
Comparison with total syntheses of vancomycin.

compound	UV, K_a[a]	ITC, K_a[a]	ΔG[b]	ΔH[b]	$-T\Delta S$[b]
vancomycin	2.0×10^5	5.5×10^5	–7.8	–10.7	+2.9
1, tetrachlorovancomycin	3.3×10^4	1.1×10^5	–6.9	–10.3	+3.4

[a]Association constant, in M^{-1}; [b]In kcal/mol

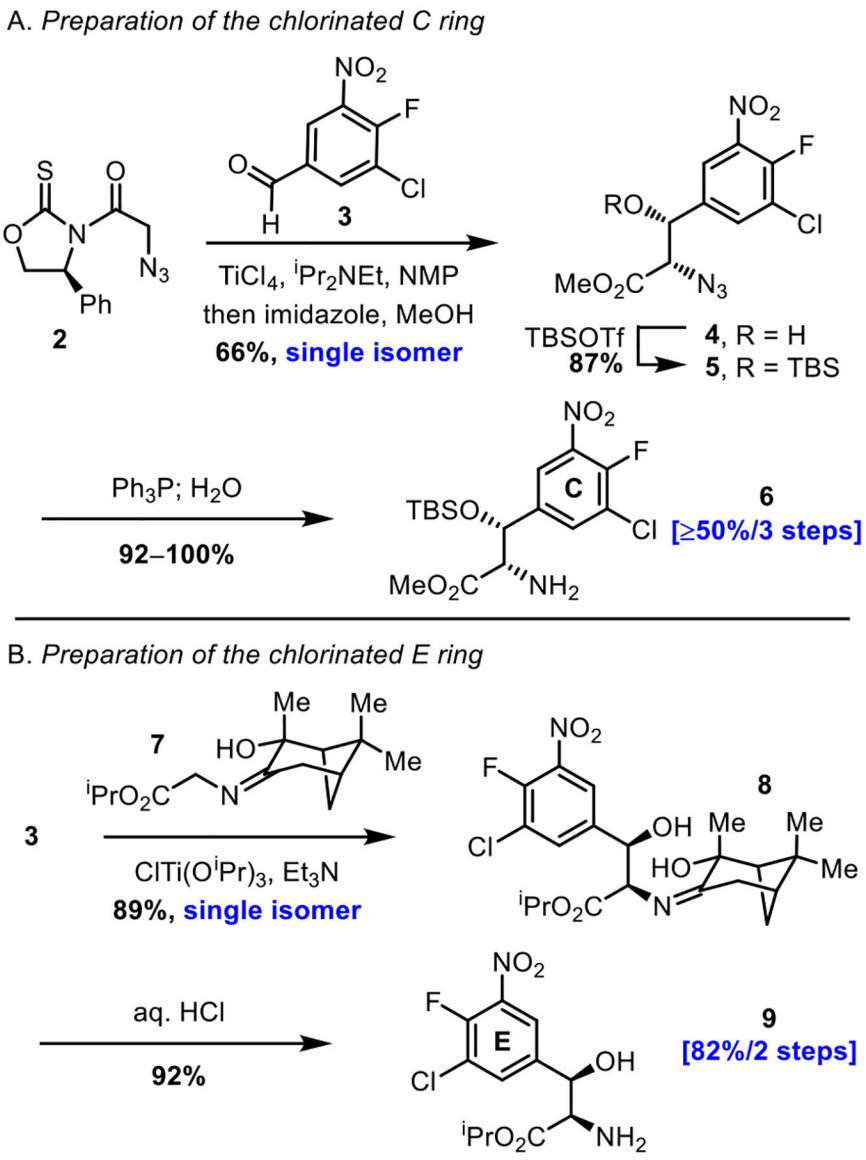
Figure 4.

Binding of **1** with Ac_2Lys-D-Ala-D-Ala (**32**) established by UV (20 mM sodium citrate buffer, pH = 5.1) and ITC (100 mM Na-citrate, pH 5.1, 298 K) titrations and thermodynamic parameters established by ITC.

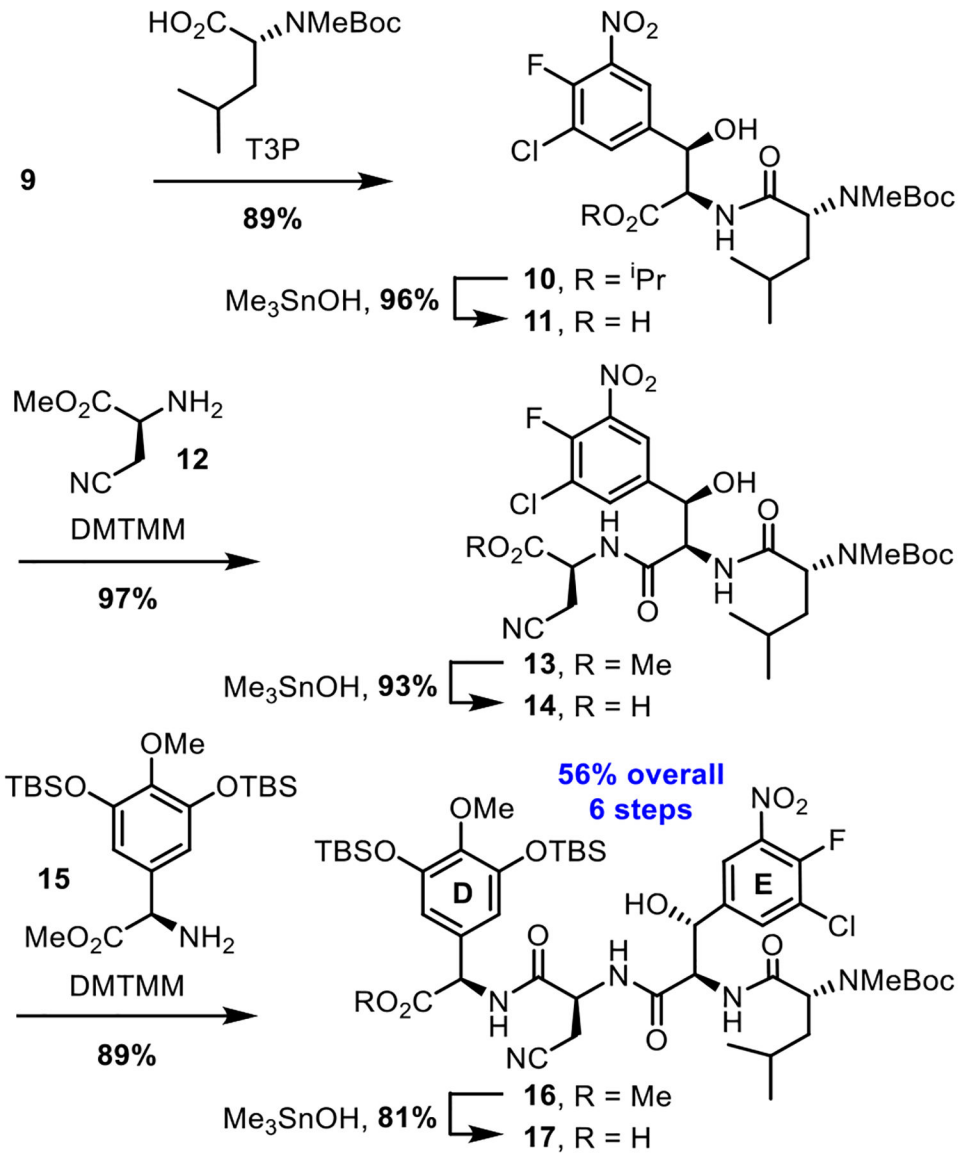
compound	MIC (μg/mL)[a] VRE[b]	MSSA[c]	MRSA[d]
vancomycin aglycon	>250	2	2
29, tetrachlorovancomycin aglycon	>250	8	8
vancomycin	250	0.5	0.5
1, tetrachlorovancomycin	>250	4	4
CBP-vancomycin	2.5	0.08	0.08
30, CBP-tetrachlorovancomycin	5	0.08	0.08
G3,CBP-vancomycin	0.3	0.04	0.04
31, G3,CBP-tetrachlorovancomycin	0.3	0.04	0.08

[a]Minimum inhibitory concentration. [b]Vancomycin-resistant *E. faecium* (ATCC BAA-2317). [c]Methicillin-sensitive *S. aureus* (ATCC 25923). [d]Methicillin-resistant *S. aureus* (ATCC 43300).

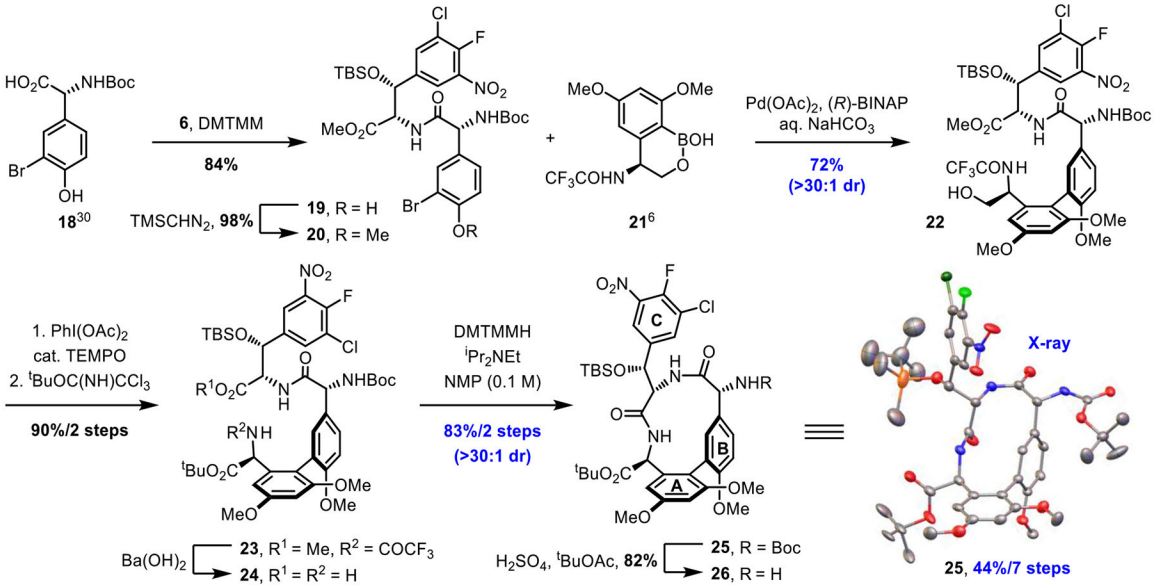
Figure 5.
Antimicrobial activity.



Scheme 1.
Preparation of the C and E ring amino acid subunits

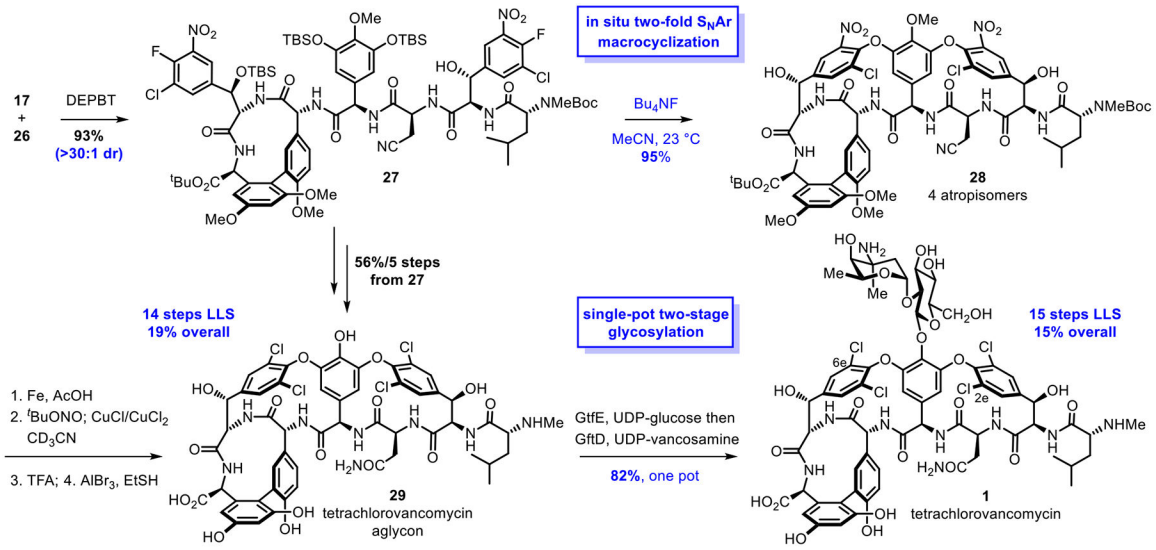


Scheme 2.
Synthesis of the linear DE tetrapeptide



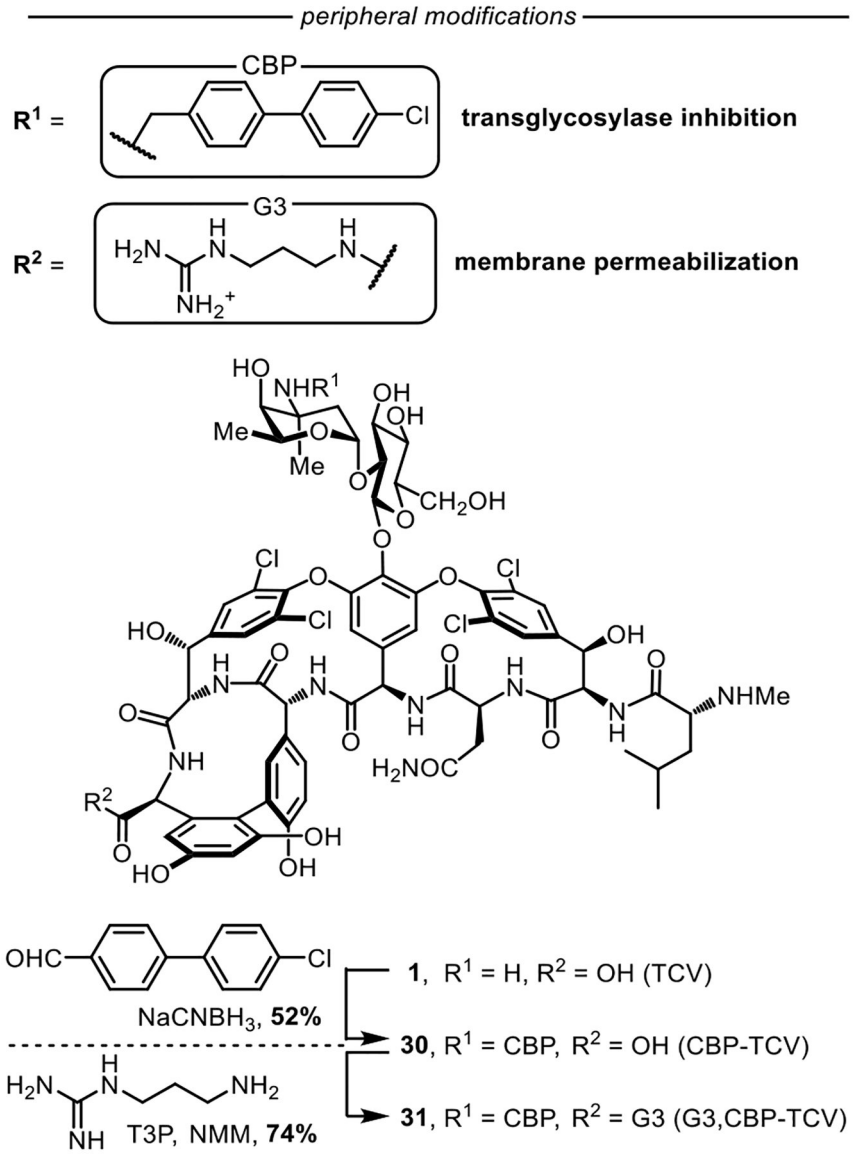
Scheme 3.

Synthesis of the macrocyclic AB ring system



Scheme 4.

Total synthesis of tetrachlorovancomycin



Scheme 5.
Peripheral modification of tetrachlorovancomycin