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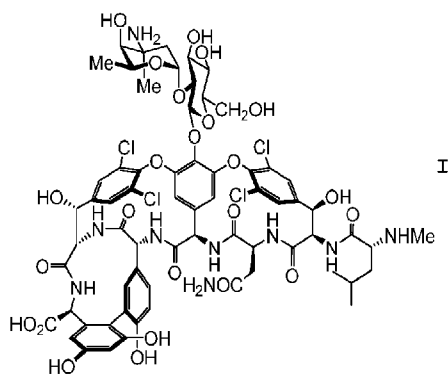
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(57) Abstract: A total synthesis of a new class of vancomycin analogues of reduced synthetic complexity was developed. The synthesis, achieved by the addition of two aryl chloride substituents to provide tetrachlorovancomycin aglycon (Compound II), tetrachlorovancomycin (Compound I), and their derivatives, permitted a streamlined total synthesis of the new class of glycopeptide antibiotics by removing atropisomer stereochemical control and enabled the simultaneous and further activated S_NAr macrocyclizations that establish the tricyclic skeleton of Compound I. In addition to the antimicrobial evaluation of tetrachlorovancomycin (Compound I), the preparation of key binding pocket and peripherally-modified derivatives, which overcome vancomycin resistance and introduce independent and synergistic mechanisms of action, revealed their exceptional antimicrobial potency and provide the foundation for use of this new class of synthetic glycopeptide analogues. Also disclosed are a pharmaceutical composition containing bactericidal amount of tetrachlorovancomycin, a derivative thereof or a salt of either dissolved or dispersed in a pharmaceutically acceptable diluent.

TETRACHLOROVANCOMYCIN AND DERIVATIVES

Description

CROSS-REFERENCE TO RELATED APPLICATION

This application claims priority to US application Serial No. 63/521,400, filed on June 16, 2023, whose disclosures are incorporated herein by reference.

GOVERNMENTAL SUPPORT

This invention was made with governmental support under CA041101 awarded by the National Institutes of Health. The government has certain rights in the invention.

BACKGROUND ART

In a series of studies, it has been 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 target D-Ala-D-Lac while maintaining binding for the unaltered target D-Ala-D-Ala found in sensitive bacteria.³ This redesign, along with peripheral modifications to the glycopeptides that introduce independent synergistic mechanisms of action, have provided an exciting advance in the development of durable antibiotics that are less susceptible to raising resistance than vancomycin itself.⁴

A remaining challenge enroute to their translation to the clinic is their accessibility, presently requiring total syntheses to obtain the targeted glycopeptide analogues.⁵ A new class of structurally simplified synthetic glycopeptide antibiotics is disclosed that is now easily accessible by total synthesis and directly addresses this challenge. The class retains all the intricate vancomycin structural features that contribute to its target binding affinity and selectivity, maintains the potent antimicrobial activity of vancomycin, and achieves

this simplification by an unusual 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 the central challenge to its synthesis (Fig. 1).⁵ In the most recent next generation total synthesis of vancomycin,⁶ the AB biaryl axis of chirality was set through a diastereoselective (>20:1 dr), 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.

Substantial improvements in the syntheses of the unnatural amino acid subunits were also introduced such that five subunits are now derived from inexpensive chiral pool starting materials of which one is commercially available, only two require asymmetric synthesis, all require ≤5 steps to access, and all but one are obtained in >50% overall yield.⁶ The extension of the work to [Y[C(=S)NH]Tpg⁴]-vancomycin for accessing pocket-modified vancomycin analogues further improved on the approach and established a scalable synthesis.⁷

The modification 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 chlorines are placed opposite those naturally present on the C and E rings (Fig. 1). This modification renders the CD and DE diaryl ethers symmetrical and eliminates the two atropisomer elements that are most challenging to control. As detailed herein, this simplification allows full control of all stereochemical features, results in a technically straightforward total synthesis with reduction in the step count [15 steps in longest linear sequence (LLS), 15% overall yield], 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.

The class of compounds (derivatives) retains all the intricate vancomycin structural features that contribute to its target

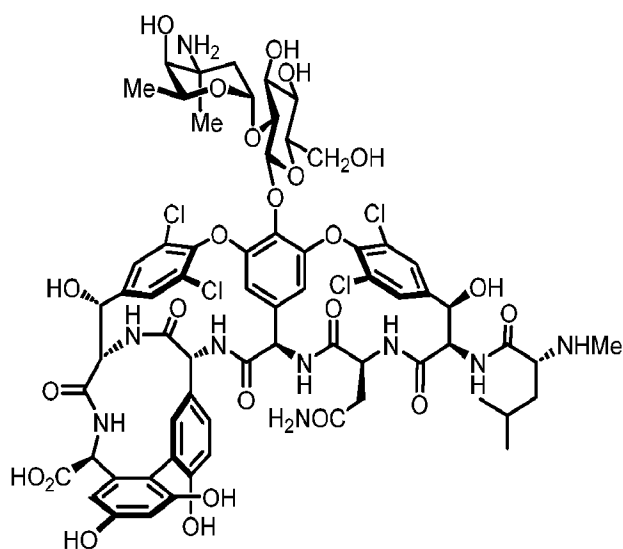
binding affinity and selectivity, maintains the potent antimicrobial activity of vancomycin, and achieves this simplification by an unusual addition, not removal, of benign substituents to the core structure.

For convenience, these 2_e,6_e-dichlorovanco-mycin analogues as tetrachlorovancomycins (e.g., 2_e,6_e-dichlorovancomycin (Compound **1**) = tetrachlorovancomycin), highlighting the four aryl chlorides now present on the core structure.

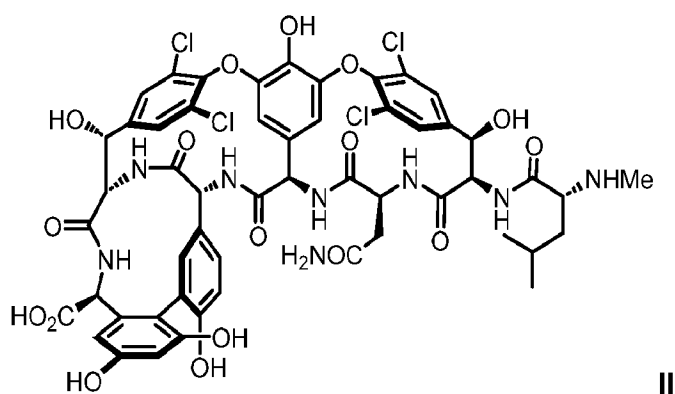
SUMMARY

The present invention contemplates a new, biologically active vancomycin analogue called tetrachlorovancomycin and its derivatives that are produced, except for glycosylation, solely by synthetic organic chemistry. Key elements of the synthetic approach include a catalyst-controlled diastereoselective formation of the AB biaryl axis of chirality (>30:1 dr), an 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 dual CD/DE ring system S_NAr cyclizations, a highly refined 4-step conversion of the product to the aglycon, and a protecting group free one-pot enzymatic glycosylation for disaccharide introduction.

The structural formula for tetrachlorovancomycin itself is shown below as Formula I.

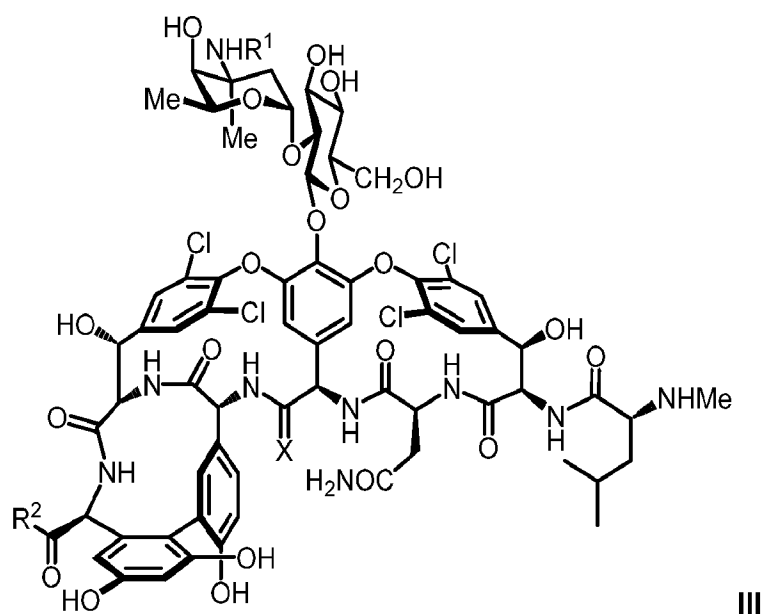


The structural formula for tetrachlorovancomycin aglycon that is produced solely using synthetic organic chemistry is shown below as Formula II.

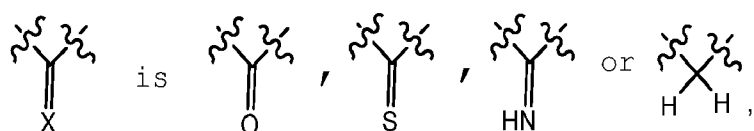


The above two compounds surprisingly exhibit activity against methicillin-resistant *S. aureus* at about a factor of 10 or less than the activity of vancomycin against vancomycin-sensitive and vancomycin-resistant bacteria. However, their derivatives substituted similarly to some of the most active vancomycin derivatives show almost the same activities. Being chemically prepared in relatively high yield provides a route to less expensive very active antibiotics.

A generic formula that can encompass tetrachlorovancomycin, contemplated derivatives and a pharmaceutically acceptable salt is shown below as Formula III.



wherein



R¹ is selected from the group consisting of hydrido (hydrogen), (C₁-C₁₆)hydrocarbyl, aryl(C₁-C₆)-hydrocarbyldiyl, heteroaryl-(C₁-C₆)hydrocarbyldiyl, (C₁-C₆)hydrocarbyldiylheteroaryl, halo(C₂-C₁₂)-hydrocarbyldiyl, and (C₁-C₁₆)amido substituents, wherein an aryl or heteroaryl group is itself optionally substituted with up to three substituents independently selected from the group consisting of:

- (i) hydroxy,
- (ii) halo,
- (iii) nitro,
- (iv) (C₁-C₆)hydrocarbyl,
- (v) halo(C₁-C₁₆)hydrocarbyl,
- (vi) (C₁-C₆)hydrocarbyloxy,
- (vii) halo(C₁-C₆)hydrocarbyloxy,
- (viii) aryl, and

(ix) aryloxy, wherein an aryl or aryloxy substituent can itself be substituted with up to three substituents independently selected from the group consisting of:

- (i) hydroxy,
- (ii) halo,
- (iii) nitro,
- (iv) (C₁-C₆)hydrocarbyl,
- (v) halo(C₁-C₁₆)hydrocarbyl,
- (vi) (C₁-C₆)hydrocarbyloxy, and
- (vii) halo(C₁-C₆)hydrocarbyloxy; and

R² is OH or $\text{---}\overset{\text{Z}}{\text{N}}\text{---}\text{HN}\text{---}\text{A}\text{---}\text{R}^3$, where Circle A is a linking

moiety having the length of a saturated chain of 2 carbon atoms and less than a saturated chain of about 12 carbon atoms, and R³ is guanidiny [H₂N(C=NH)NH-], N,N-(di-C₁-C₆-hydrocarbyl)amino, or N,N,N-(tri-C₁-C₆-hydrocarbyl)ammonium, and an optional pharmaceutically acceptable anion, Y⁻, to balance charge as needed.

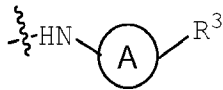
Thus, when $\text{---}\overset{\text{R}^1}{\text{C}}\text{---}\overset{\text{R}^2}{\text{N}}\text{---}$, R¹ is H (hydrido), and R² is OH,

the compound above is tetrachloro-vancomycin. When one or both of R¹ and R² are other than H and OH, respectively, a derivative of tetrachlorovancomycin is being contemplated.

In one aspect, the "X" moiety above can be H,H making the carbon to which the two hydrogens are bonded a methylene group. In another aspect, "X" is O (oxygen) double bonded to the depicted carbon atom as the carbonyl group of an amide. X can also be S (sulfur) double-bonded to the depicted carbon, making that carbon a thiocarbonyl moiety and thereby, the thiocarbonyl bonded to the -NH-group form a thioamide linkage. A compound where "X" is "S" is usually used as an intermediate to the preparation of a compound of Formula I, II and III in which "X" is "H,H" forming a methylene group as above, or is "NH", forming an amidine linkage.

Turning to the R¹ substituents other than H, those hydrophobic materials are present and discussed in one of the inventors' U.S. Patents No.9,879,049, No. 10,577,395, No. 10,934,326, well as to U.S. Patent Publication 2023/0146239, and the papers cited therein. Many of these substituents are present in commercially available in semisynthetic derivatives of vancomycin and similar glycopeptide antibiotics such as teicoplanin A2, oritavancin, dalbavancin and telavancin.

Hydrophobic R¹ substituents that are presently preferred are the benzyl, 4-chlorobenzyl, (biphenyl)methyl, (4-chlorobiphenyl)methyl [CBP], 4-fluorobenzyl, and (4-fluorobiphenyl)methyl substituent groups. Each of these six substituents can be added to the tetrachlorovancosaminy amino group by NaCNBH₄ reduction of the corresponding aldehyde as is shown in Scheme 5 hereinafter.

The R² substituents, other than OH, contain at least two nitrogen atoms separated by a linker group referred to as Circle A and depicted as , wherein the remaining valence of the nitrogen in the depicted "-HN-" group bonds to carboxyl group of the tetrachlorovancomycinyl portion of the molecule to form an amido group. The R³ contains at least a second nitrogen atom bonded directly to the Circle A linker. In one contemplated aspect, the second nitrogen atom is part of a guanidiny group [H₂N(C=NH)NH-]. In another contemplated aspect, the second nitrogen of Circle A is the nitrogen of a tertiary amine or a quaternary ammonium group, as noted above.

When R³ is a quaternary ammonium group, an optional anion, Y⁻, that is preferably pharmaceutically acceptable is also present to balance the charge. R³ is a tertiary amine or guanidiny group, both of which are typically basic, a compound containing such a group can also be present as a salt with an acid. Preferably, the acid of such an acid salt is a pharmaceutically acceptable acid, that provides the

optional anion, Y⁻. A pharmaceutical composition containing an anti-bacterially effective amount a before-described tetrachlorovancomycin or derivative, or a pharmaceutically acceptable salt dissolved or dispersed in a pharmaceutically (physiologically) diluent acceptable diluent is also contemplated. Such a composition can be in solid, liquid, gel or other appropriate form.

A method of treating a bacterial infection, particularly from Gram positive bacteria, is also contemplated.

BRIEF DESCRIPTION OF THE DRAWINGS

In the drawings forming a portion of this disclosure:

Fig. 1 shows a comparison of vancomycin and tetrachlorovancomycin that highlights the structural and synthetic simplification and atropisomerism elimination achieved by adding two benign chlorine substituents;

Fig. 2 is a schematic representation of key elements of a retrosynthetic analysis for tetrachlorovancomycin;

Fig. 3 illustrates reaction Scheme 4 that illustrates a direct synthetic route from Compound **27** to Compound **29** in 56% yield and five steps followed by the one-pot two-step enzymatic glycosylation of tetrachlorovancomycin aglycon (**29**) to form tetrachlorovancomycin (Compound **1**) that proceeded in high yield (82%) for installation of both sugar residues despite the added 2_e and 6_e aryl chlorides;

Fig. 4 outlines a synthetic pathway by which a tetrachlorovancomycin derivative of Formula III where

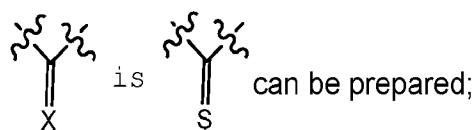


Fig. 5 shows a reaction scheme whereby the 4-thioamide derivative, Compound **41**, can be prepared from Compound **38**;

Fig. 6 shows two reaction schemes by which Compounds **40** and **39** can be prepared from Compound **38**;

Fig. 7, in two panels, as Fig.7A that illustrates two reaction schemes by which Compounds **42**, **43**, and **44** can be prepared from

Compound **41**, and in which Compounds **46** and **45** can also be prepared from Compound **44**, and Fig. 7B in which Compound **44** is used to prepare Compound **47**, that in turn is used to prepare Compounds **48** and **49**;

Fig. 8, in two panels, as Fig. 8A that illustrates tetrachlorovancomycin, Compound **1**, and its three 4-position differently substituted analogues, Compounds **41**, **42** and **43**, titration binding study results of with model ligands **A** and **B**, and similar studies with vancomycin itself and its three similarly substituted analogues in Fig. 8B;

Fig. 9, in two panels as Figs. 9A and 9B, are tables showing minimum inhibitory concentrations (MIC values) for tetrachlorovancomycin analogue Compounds **41**, **44**, **47**, **48** and **49** against bacteria that are vancomycin-sensitive (Fig. 9A) and vancomycin-resistant (Fig. 9B); data for G3,CBP-vancomycin⁴⁴;

Figs. 10 through 15 provide illustrative tetrachlorovancomycin derivative compounds with varying 4-position substituents, as well as various substituents bonded to the vancosaminylnitrogen, and also still further substituents bonded to the vancomycin usually unsubstituted (C-14) carboxyl group.

Each of the patents, patent applications and articles cited herein is incorporated by reference.

Definitions

In the context of the present invention and the associated claims, the following terms have the following meanings:

The articles "a" and "an" are used herein to refer to one or to more than one (i.e., to at least one) of the grammatical object of the article. By way of example, "an element" means one element or more than one element.

The word "hydrocarbyl" is used herein as a short-hand term for a non-aromatic group that includes straight and branched chain aliphatic as well as alicyclic groups or radicals that contain only carbon

and hydrogen. Thus, alkyl, alkenyl and alkynyl groups are contemplated, whereas aromatic hydrocarbons such as phenyl are grouped as an "aryl" group.

Where a specific aliphatic hydrocarbyl substituent group is intended, that group is recited; i.e., C₁-C₄ alkyl, methyl or tert-butyl. Exemplary hydrocarbyl groups contain a chain of 2 to about 77 carbon atoms, and preferably 2 to about 6 carbon atoms.

A particularly preferred hydrocarbyl group is an alkyl group. As a consequence, a generalized, but more preferred substituent can be recited by replacing the descriptor "hydrocarbyl" with "alkyl" in any of the substituent groups enumerated herein.

Examples of alkyl radicals include methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl, sec-butyl, tert-butyl. Examples of suitable alkenyl radicals include ethenyl (vinyl), 2-propenyl, 3-propenyl, 1,4-butadienyl, 1-butenyl, 2-butenyl, and 3-butenyl. Examples of alkynyl radicals include ethynyl, 2-propynyl, 1-propynyl, 1-butylnyl, 2-butylnyl, 3-butylnyl, and 1-methyl-2-propynyl.

As a skilled worker will understand, a substituent that cannot exist such as a C₁ alkenyl group is not intended to be encompassed by the word "hydrocarbyl", although such substituents with two or more carbon atoms are intended.

Usual chemical suffix nomenclature is followed when using the word "hydrocarbyl" except that the usual practice of removing the terminal "yl" and adding an appropriate suffix is not always followed because of the possible similarity of a resulting name to one or more substituents. Thus, a hydrocarbyl ether is referred to as a "hydrocarbyloxy" group rather than a "hydrocarboxy" group as may possibly be more proper when following the usual rules of chemical nomenclature. Illustrative hydrocarbyloxy groups include methoxy, ethoxy, n-propoxy, isopropoxy, allyloxy, n-butoxy, iso-butoxy, sec-butoxy, and tert-butoxy groups.

In the structural formulas shown throughout this application, a wavy line as shown for example in the following

representation “” is used to indicate that only a portion of a molecule is being shown, and two bonds of the carbon atom doubly bonded to X are severed from the remainder of the molecule. Similar representations are used for the same purpose with other chemical entities to save space in the text and possible confusion.

“h” = hour(s); “min” minute(s); TFA = trifluoroacetic acid; NMM = N-methylmorpholine; DMSO = dimethylsulfoxide; AcOH = acetic acid; EtOAc = ethyl acetate; MeOH = methanol; THF = tetrahydrofuran; DMF = dimethyl formamide; MeCN = acetonitrile; HBTU = 2-(1h-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate and/or o-benzotriazol-1-yl-N,N,N',N'-tetramethyluronium hexafluorophosphate; AgOAc = silver acetate; TCEP•HCl = tris(carboxyethyl)phosphine hydrochloride; T3P® = propanephosphonic acid anhydride; Bu₄NF = tetrabutylammonium fluoride; THF = tetrahydrofuran; equiv = equivalent(s); mmol = millimole(s); μmol = micromole(s); calcd = calculated; HRMS = high resolution mass spectroscopy; ESI-TOF = electrospray ionization time-of-flight mass spectroscopy; *m/z* = mass-to-charge ratio; brsm = based on recovered starting material; MHz = megaHertz; PES = polyethersulfone; PTLC = preparative thin layer chromatography; mL = microliter(s); μmol = micromole(s); ca. = circa = about.

The present invention has several benefits and advantages.

One salient benefit of the invention is the relative ease and enhanced yield of synthetically-prepared tetrachlorovancomycin and derivatives as compared to vancomycin itself when synthetically prepared, and also when compared to vancomycin preparation by fermentation using bacteria whose 4-position derivatives are very difficult to prepare.

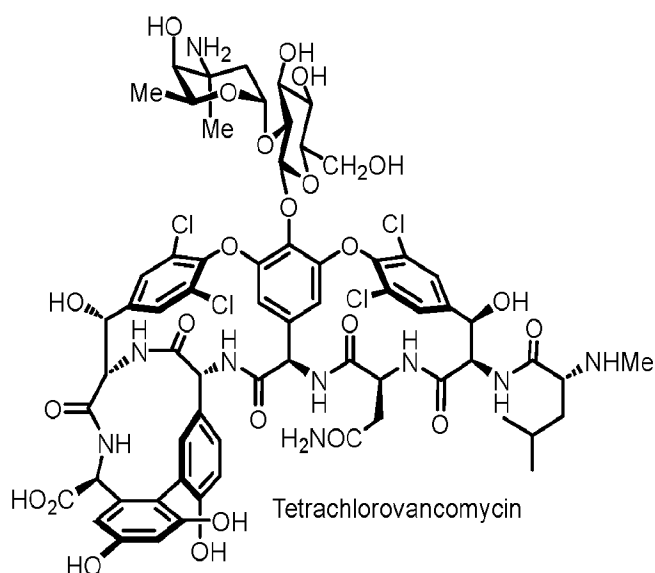
A salient advantage of the invention is that the antibacterial activity of tetrachloro-vancomycin compared to that of vancomycin itself is almost identical.

Another benefit of the invention is that the activity of the herein discussed derivatized tetrachloro-vancomycins against both vancomycin-resistant bacteria (VRE) and those bacteria that are not vancomycin-resistant compared to the activities of identically derivatized vancomycin are also almost identical.

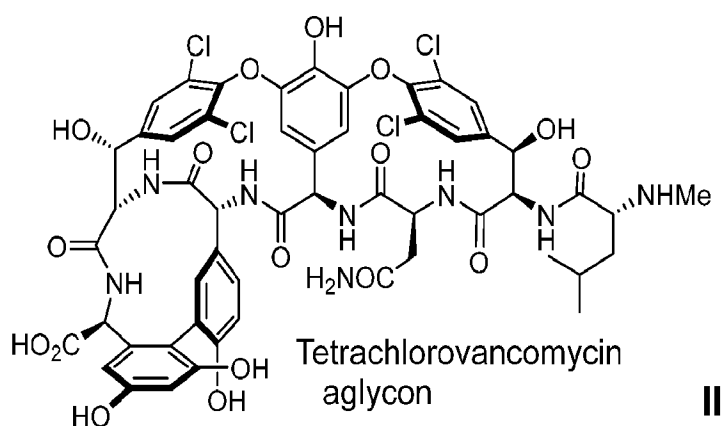
Still further benefits and advantages will be apparent to the skilled worker from the detailed description that follows.

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

The present invention contemplates a new, biologically active vancomycin derivative called tetrachlorovancomycin that is produced, except for glycosylation, solely by synthetic organic chemistry. The structural formula for tetrachlorovancomycin itself is shown below as Formula I.

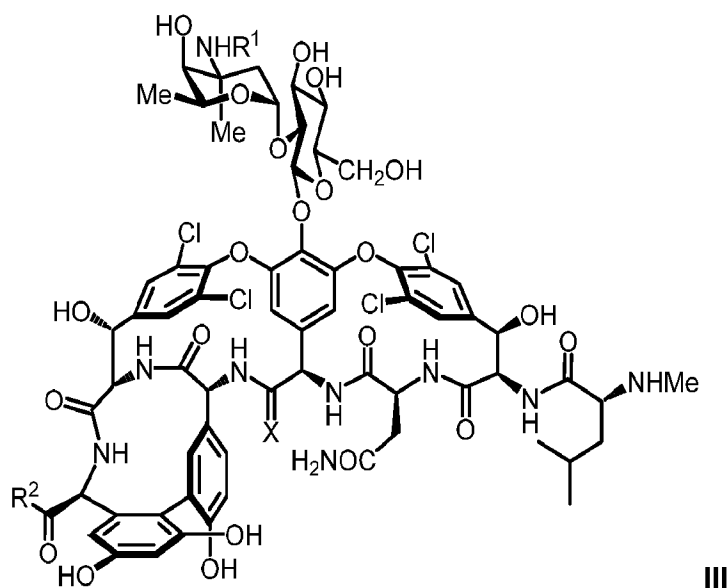


The structural formula for tetrachlorovancomycin aglycon that is produced solely using synthetic organic chemistry is shown below as Formula II.

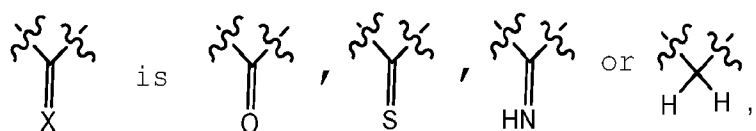


The above two compounds surprisingly exhibit activity against methicillin-resistant *S. aureus* and about a factor of 10 or less the activity of vancomycin against vancomycin-sensitive and vancomycin-resistant bacteria. However, their derivatives substituted similarly to the most active vancomycin derivatives show almost the same activities as those similarly substituted vancomycins. Being chemically prepared in relatively high yield provide a route to less expensive very active antibiotics.

A generic formula that can encompass tetrachlorovancomycin, contemplated derivatives and a pharmaceutically acceptable salt is shown below as Formula III.



wherein

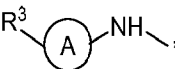


R¹ is selected from the group consisting of hydrido (hydrogen), (C₁-C₁₆)hydrocarbyl, aryl(C₁-C₆)-hydrocarbyldiyl, heteroaryl-(C₁-C₆)hydrocarbyldiyl, (C₁-C₆)hydrocarbyldiylheteroaryl, halo(C₂-C₁₂)-hydrocarbyldiyl, and (C₁-C₁₆)amido substituents, wherein an aryl or heteroaryl group is itself optionally substituted with up to three substituents independently selected from the group consisting of:

- (i) hydroxy,
- (ii) halo,
- (iii) nitro,
- (iv) (C₁-C₆)hydrocarbyl,
- (v) halo(C₁-C₁₆)hydrocarbyl,
- (vi) (C₁-C₆)hydrocarbyloxy,
- (vii) halo(C₁-C₆)hydrocarbyloxy,
- (viii) aryl, and

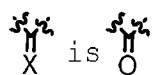
(ix) aryloxy, wherein an aryl or aryloxy substituent can itself be substituted with up to three substituents independently selected from the group consisting of:

- (i) hydroxy,
- (ii) halo,
- (iii) nitro,
- (iv) (C₁-C₆)hydrocarbyl,
- (v) halo(C₁-C₁₆)hydrocarbyl,
- (vi) (C₁-C₆)hydrocarbyloxy, and
- (vii) halo(C₁-C₆)hydrocarbyloxy; and

R² is OH or , where Circle A is a linking

moiety having the length of a saturated chain of 2 carbon atoms and less than a saturated chain of about 12 carbon atoms, and R³ is selected from the group consisting of guanidinyl [H₂N(C=NH)NH-], N,N-(di-C₁-

C₆-hydrocarbyl)amino, N,N,N-(tri-C₁-C₆-hydrocarbyl)ammonium, and N-(C₁-C₆-hydrocarbyl)-N-(C₅-C₇-cyclohydrocarbyl)ammonium, and an optional pharmaceutically acceptable anion, Y⁻, as needed to balance charge.

Thus, when  is , R¹ is H (hydrido), and R² is OH, the compound above is tetrachloro-vancomycin. When one or both of R¹ and R² are other than H and OH, respectively, a derivative of tetrachlorovancomycin is being contemplated.

The chemical syntheses of the tetrachloro-vancomycin and tetrachlorovancomycin aglycon are shown and discussed hereinafter. These syntheses require fewer steps and provide higher yields of the desired compounds in part because of the symmetry provided by the two chloro groups on each substituted phenyl ring that flanks the central substituted phenyl ring to which the vancosaminyl group is bonded. That symmetry removes two atropisomers whose presence in vancomycin itself reduces the yield of desired isomers when the compound is chemically rather than biologically prepared.

In one aspect, the “X” moiety above can be H,H making the carbon to which the two hydrogens are bonded a methylene group. In another aspect, “X” is O (oxygen) double bonded to the depicted carbon atom as the carbonyl group of an amide. X can also be S (sulfur) double-bonded to the depicted carbon, making that carbon a thiocarbonyl moiety and thereby, the thiocarbonyl bonded to the –NH- group form a thioamide linkage. A compound where “X” is S is usually used as an intermediate to the preparation of a compound of Formula I, II and III in which “X” is H,H forming a methylene group as above, or is NH, forming an amidine linkage.

Turning to the R¹ substituents other than H, those hydrophobic materials are present and discussed in the inventor’s U.S. Patents No.9,879,049, No. 10,577,395, No. 10,934,326, well as to U.S. Patent Publication 2023/0146239, and the papers cited therein. Many of these substituents are present in commercially available derivatives of

vancomycin and similar glycopeptide antibiotics such as teicoplanin A2, oritavancin, dalbavancin and telavancin.

Hydrophobic R¹ substituents that are presently preferred are the benzyl, 4-chlorobenzyl, (biphenyl)methyl, (4-chlorobiphenyl)methyl [CBP], 4-fluorobenzyl, and (4-fluorobiphenyl)methyl substituent groups. Each of these four substituents can be added to the vancosaminy amino group by NaCNBH₄ reduction of the corresponding aldehyde as is shown in Scheme 5 hereinafter.

The R² substituents, other than H, contain at least two nitrogen atoms separated by a divalent linker group referred to as Circle

A and depicted as , wherein the remaining valence of the nitrogen in the depicted "HN" group bonds to carboxyl group of the tetrachlorovancomycinyl portion of the molecule to form an amido group, and R³ contains at least a second nitrogen atom. In one contemplated aspect, the second nitrogen atom is part of a guanidiny group [H₂N(C=NH)NH-]. In another contemplated aspect, the second nitrogen of Circle A is the nitrogen of a tertiary amine or a quaternary ammonium group. The preparation of the compounds in which the second nitrogen of a Circle A group is the nitrogen of a tertiary amine or a quaternary ammonium group can be carried out as discussed in US Patent No. 10,934,326 and in Okano et al., *Proc Natl Acad Sci, USA* **114(26)**:E5052-E5061 (Pub. online 05-30-2017) for otherwise similar derivatives of vancomycin.

The chain lengths herein are measured along the longest linear atom chain in the radical between the amido nitrogen and the first nitrogen atom of a guanidiny group or the nitrogen of a tertiary amine or a quaternary ammonium group. Each atom in the chain is presumed to be carbon for ease in calculation. The lengths are thus recited in terms of carbon atoms. Such lengths can be readily determined by using published bond angles, bond lengths and atomic radii, as needed, to draw and measure a staggered chain, or by building models using

commercially available kits whose bond angles, lengths and atomic radii are in accord with accepted, published values.

For example, a 1,4-bonded 6-membered aromatic ring group (phenyl) not part of a fused ring system has a length of about a butyl group. A 1,2- or 1,3-bonded 6-ring has a length of a 2- or 3-carbon chain, respectively, as the shortest path around the ring between the two bonding position regardless of formal naming criteria. Where a 5-membered ring is present, length is calculated as the length of a 2-carbon chain. Thus, for single ring systems, length is calculated as the shortest path around the rings between the two bonding positions to the amido and guanidiny, quaternary ammonium or tertiary amine nitrogen atoms of a compound of Formula III regardless of formal naming criteria.

Radical lengths can also be determined somewhat less exactly by assuming that all atoms have bond lengths of saturated C-C bonds, that unsaturated bonds have the same lengths as saturated bonds, and that bond angles for unsaturated bonds are the same as those for saturated C-C bonds (108°), although the above-mentioned modes of measurement are preferred. Both methods produce similar results within one or two carbon atoms, and thus the use of "about".

A contemplated linker moiety Circle A can also be a hydrocarbonyl chain of two to about 12 saturated carbon atoms, or preferably two to about ten saturated carbon atoms. A more preferred linking Circle A group contains a chain of atoms that is equal to or greater than the length of two saturated carbons and is shorter than about a saturated ten carbon (decyl) chain.

More preferably still, the hydrocarbonyl chain has a chain length of two saturated carbon atoms to about eight saturated carbon (octyl) atoms. In one illustrative instance, when there is a chain of Circle A atoms linking the amido and guanidiny nitrogen atoms together, the length is simply the length of the longest chain of atoms linking those two nitrogens.

In further examining Circle A hydrocarbyl linker groups, it is noted that such groups can contain a substituent that is pendant from the chain of atoms that link the amido and second nitrogens (e.g., guanidiny) shown in Formula III. Such a substituent are selected from amino acid side chain substituents other than those containing a carboxyl group, a sulfhydryl group (-SH) or a substituent that provides a negative charge in an aqueous solution at physiological pH values, e.g., pH 7.2-7.4.

Additional pendant substituents include 2-hydroxyethyl and 2-hydroxypropyl, C₁-C₃-hydrocarbyl C₀-C₂-carboxylate, and C₀-C₂-carboxamide whose amido nitrogen is unsubstituted (-NH₂), monosubstituted (-NHR⁴) or disubstituted (-NR⁴R⁵) in which the substituent (R₄ and/or R⁵) is one or two same or different C₁-C₄-hydrocarbyl group, or whose amido nitrogen along with two substituents together form a 5- or 6-membered hydrocarbyl ring, or a heterocyclic ring containing one additional oxygen (O) atom or a N-methyl group in the ring. In the previous sentence, "C₀" is intended to indicate that the carbonyl carbon is bonded directly to an atom of the Circle A linking chain.

A contemplated linker moiety Circle A atom chain need not be entirely hydrocarbyl, but can also be contain 1, 2, or 3 oxygens in place of carbon atoms as when a -CH₂-CH₂-O-CH₂-CH₂-, -CH₂-CH₂-O-CH₂-CH₂-O-CH₂-CH₂-, or -CH₂-CH₂-O-CH₂-CH₂-O-CH₂-CH₂-O-CH₂-CH₂- Circle A linker moiety is utilized.

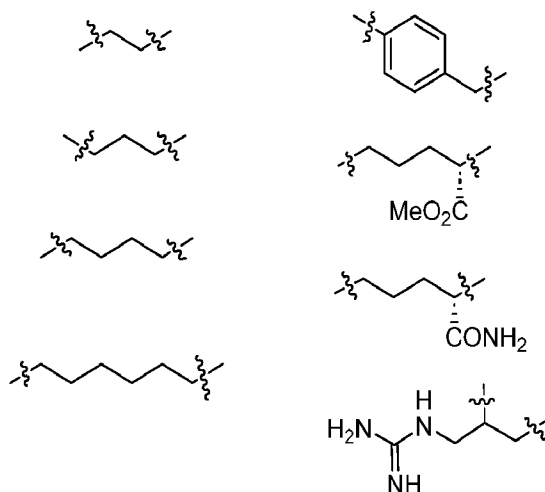
A contemplated divalent Circle A linker moiety also can comprise a ring system that can be carbocyclic or heterocyclic as discussed below. Thus, a single 5- or 6-membered ring optionally contains one or two ring hetero atoms that can independently be nitrogen, oxygen or sulfur. Individual rings can be aliphatic or aromatic, including heteroaromatic, and also be aralkyl as in a benzyl group.

Using monovalent substituent names for convenience, exemplary divalent aromatic carbocyclic ring moieties include phenyl and naphthyl groups. Again, using monovalent names for convenience, exemplary divalent heteroaryl groups include 6-membered ring substituents such as pyridyl, pyrazyl, pyrimidinyl, and pyridazinyl; 5-membered ring substituents such as 1,3,5-, 1,2,4- or 1,2,3-triazinyl, imidazyl, furanyl, thiophenyl, pyrazolyl, oxazolyl, isoxazolyl, thiazolyl, 1,2,3-, 1,2,4-, 1,2,5-, or 1,3,4-oxadiazolyl and isothiazolyl groups.

Aliphatic 5- and 6-membered carbocyclic rings are contemplated such as cyclohexyl and cyclopentyl, as well as their mono- and diethylenically unsaturated derivatives, using monovalent names for convenience. Again, using monovalent radical names for convenience, divalent aliphatic 5- and 6-membered heterocyclic rings include, piperidinyl, piperazinyl, imidazolinyl, imidazolidinyl, pyrrolinyl, pyrrolidinyl, pyrazolidinyl, pyrazolinyl, pyranyl, morpholinyl, oxazinyl, isooxazinyl, and oxathiolyl.

The present invention is exemplified in part by the illustrative listing of Circle A linker moieties shown below:

Illustrative Circle A Linker Moieties



COMPOSITION AND TREATMENT METHOD

A further aspect of the invention is a method of treating a mammal infected with a microbial infection such as a bacterial infection, typically either a Gram-positive infection or a Gram-negative bacterium; i.e., an infection caused by Gram-positive or Gram-negative bacteria, and the infected mammal is in need of antimicrobial (antibacterial) treatment. Treatment of Gram-positive bacteria are typically more successful than treatment of Gram-negative bacteria. In accordance with a contemplated method, an antibacterial-effective amount of one or more compounds of Formula III or a pharmaceutically acceptable salt of such a compound is administered to an infected mammal in need.

The compound can be administered as a solid, as a liquid formulation, as a thickened preparation e.g., as a gel, as for topical use, and is preferably administered via a pharmaceutical composition discussed hereinafter. That administration can also be oral or parenteral, as are also discussed further hereinafter.

It is to be understood that mammals are infected with bacteria and other microbes. The present invention's method of treatment is intended for use against infections of pathogenic bacteria that cause illness in the mammal to be treated. Illustrative pathogenic microbes include *S. aureus*, methicillin-resistant *S. aureus* (MRSA), VanA strains of *E. faecalis* and *E. faecium*, as well as VanB strains of *E. faecalis*. Evidence of the presence of infection by pathogenic microbes is typically understood by physicians and other skilled medical workers.

A mammal in need of treatment (a subject) and to which a pharmaceutical composition containing a Compound of Formula III or its pharmaceutically acceptable salt to be administered can be a primate such as a human, an ape such as a chimpanzee or gorilla, a monkey such as a cynomolgus monkey or a macaque, a laboratory animal such as a rat, mouse or rabbit, a companion animal such as a dog, cat, horse, or a food animal such as a cow or steer, sheep, lamb, pig, goat, llama or the like.

As is seen from the data that follow, a contemplated compound is active in *in vitro* assay studies at less than 1 µg/mL

amounts, which corresponds to a molar concentration of about 1 to about 100 nanomolar (nM), using the molecular weight of G3-CBP-tetrachlorovancomycin (Compound **31**). When used in an assay such as an *in vitro* assay, a contemplated compound is typically present in the composition in an amount that is sufficient to provide a concentration of about 0.1 nM to about 1 μ M to contact microbes to be assayed.

The amount of a compound of Formula **III** or a pharmaceutically acceptable salt of such a compound that is administered to a mammal in a before-discussed method or that is present in a pharmaceutical composition discussed below, which can be used for that administration, is an antibiotic (or antibacterial or antimicrobial) effective amount. It is to be understood that that amount is not an amount that is effective to kill all of the pathogenic bacteria or other microbes present in an infected mammal in one administration. Rather, that amount is effective to kill some of the pathogenic organisms present without also killing the mammal to which it is administered, or otherwise harming the recipient mammal as is well known in the art. As a consequence, the compound usually has to be administered a plurality of times, as is discussed in more detail hereinafter.

A contemplated pharmaceutical composition contains an effective antibiotic (or antimicrobial) amount of a Compound of Formula **III** or a pharmaceutically acceptable salt thereof dissolved or dispersed in a physiologically (pharmaceutically) acceptable diluent or carrier. An effective antibiotic amount depends on several factors as is well known in the art. However, based upon the relative potency of a contemplated compound relative to that of vancomycin itself for a susceptible strain of *S. aureus* shown hereinafter, and the relative potencies of vancomycin and a contemplated compound against the VanA *E. faecalis* and *E. faecium* strains, a skilled worker can readily determine an appropriate dosage amount.

Exemplary salts useful for a contemplated compound include but are not limited to the following: sulfate, hydrochloride, hydro bromides, acetate, adipate, alginate, citrate, aspartate, benzoate,

benzenesulfonate, bisulfate, butyrate, camphorate, camphorsulfonate, digluconate, cyclopentanepropionate, dodecylsulfate, ethanesulfonate, glucoheptanoate, glycerophosphate, hemisulfate, heptanoate, hexanoate, fumarate, hydrochloride, hydrobromide, hydroiodide, 2-hydroxy-ethanesulfonate, lactate, maleate, methanesulfonate, nicotinate, 2-naphthalenesulfonate, oxalate, palmoate, pectinate, persulfate, 3-phenyl-propionate, picrate, pivalate, propionate, succinate, tartrate, thiocyanate, tosylate, mesylate and undecanoate.

The reader is directed to Berge, *J. Pharm. Sci.* 1977

68(1):1-19 for lists of commonly used pharmaceutically acceptable acids and bases that form pharmaceutically acceptable salts with pharmaceutical compounds.

In some cases, the salts can also be used as an aid in the isolation, purification or resolution of the compounds of this invention. In such uses, the salt prepared need not be pharmaceutically acceptable.

A contemplated composition is typically administered repeatedly *in vivo* to a mammal in need thereof until the infection is diminished to a desired extent, such as cannot be detected. Thus, the administration to a mammal in need can occur a plurality of times within one day, daily, weekly, monthly or over a period of several months to several years as directed by the treating physician. More usually, a contemplated composition is administered a plurality of times over a course of treatment until a desired effect is achieved, typically until the bacterial infection to be treated has ceased to be evident.

A contemplated pharmaceutical composition can be administered orally (perorally) or parenterally, in a formulation containing conventional nontoxic physiologically acceptable carrier or diluent, adjuvant, and vehicle as desired. The term parenteral as used herein includes subcutaneous injections, intravenous, intramuscular, intrasternal injection, or infusion techniques. Formulation of drugs is discussed in, for example, Hoover, John E., *Remington's Pharmaceutical Sciences*, Mack Publishing Co., Easton, Pennsylvania; 1975 and Liberman, H.A. and Lachman, L., Eds., *Pharmaceutical Dosage Forms*, Marcel Decker, New York, N.Y., 1980.

In some embodiments, a contemplated pharmaceutical composition is preferably adapted for parenteral administration. Thus, a pharmaceutical composition is preferably in liquid form when administered, and most preferably, the liquid is an aqueous liquid, although other liquids are contemplated as discussed below, and a presently most preferred composition is an injectable preparation.

Thus, injectable preparations, for example, sterile injectable aqueous or oleaginous solutions or suspensions can be formulated according to the known art using suitable dispersing or wetting agents and suspending agents. The sterile injectable preparation can also be a sterile injectable solution or suspension in a physiologically acceptable diluent or solvent, for example, as a solution in 1,3-butanediol. Among the acceptable vehicles and solvents that can be employed are water, Ringer's solution, isotonic sodium chloride solution, and phosphate-buffered saline.

Other liquid pharmaceutical compositions include, for example, solutions suitable for parenteral administration. Sterile water solutions of a Compound of Formula III or its salt or sterile solution of a Compound of Formula III in a solvent comprising water, ethanol, or propylene glycol are examples of liquid compositions suitable for parenteral administration. In some aspects, a contemplated Compound of Formula III is provided as a dry powder that is to be dissolved in an appropriate liquid medium such as sodium chloride for injection prior to use.

In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose any bland fixed oil can be employed including synthetic mono- or diglycerides. In addition, fatty acids such as oleic acid find use in the preparation of an injectable composition. Dimethyl acetamide, surfactants including ionic and non-ionic detergents, polyethylene glycols can be used. Mixtures of solvents and wetting agents such as those discussed above are also useful.

A sterile solution can be prepared by dissolving the active component in the desired solvent system, and then passing the resulting solution through a membrane filter to sterilize it or, alternatively, by

dissolving the sterile compound in a previously sterilized solvent under sterile conditions.

Solid dosage forms for oral administration can include capsules, tablets, pills, powders, and granules. The amount of a contemplated Compound or salt of Formula III such as Compounds 48 or 49 in a solid dosage form is as discussed previously, an amount sufficient to provide an effective antibiotic (or antimicrobial) amount. A solid dosage form can also be administered a plurality of times during a one-week time period.

In such solid dosage forms, a compound of this invention is ordinarily admixed as a solution or suspension in one or more diluents appropriate to the indicated route of administration. If administered *per os*, the compounds can be admixed with lactose, sucrose, starch powder, cellulose esters of alkanolic acids, cellulose alkyl esters, talc, stearic acid, magnesium stearate, magnesium oxide, sodium and calcium salts of phosphoric and sulfuric acids, gelatin, acacia gum, sodium alginate, polyvinylpyrrolidone, and/or polyvinyl alcohol, and then tableted or encapsulated for convenient administration. Such capsules or tablets can contain a controlled-release formulation as can be provided in a dispersion of active compound in hydroxypropylmethyl cellulose. In the case of capsules, tablets, and pills, the dosage forms can also comprise buffering agents such as sodium citrate, magnesium or calcium carbonate or bicarbonate. Tablets and pills can additionally be prepared with enteric coatings.

Where an *in vitro* assay is contemplated, a sample to be assayed such as cells and tissue can be used. These *in vitro* compositions typically contain water, sodium or potassium chloride, and one or more buffer salts such as acetate and phosphate salts, Hepes or the like, a metal ion chelator such as EDTA that are buffered to a desired pH value such as pH 4.0-8.5, preferably about pH 7.2-7.4, depending on the assay to be performed, as is well known.

Preferably, the pharmaceutical composition is in unit dosage form. In such form, the composition is divided into unit doses containing appropriate quantities of the active compound. The unit

dosage form can be a packaged preparation, the package containing discrete quantities of the preparation, for example, in vials or ampules.

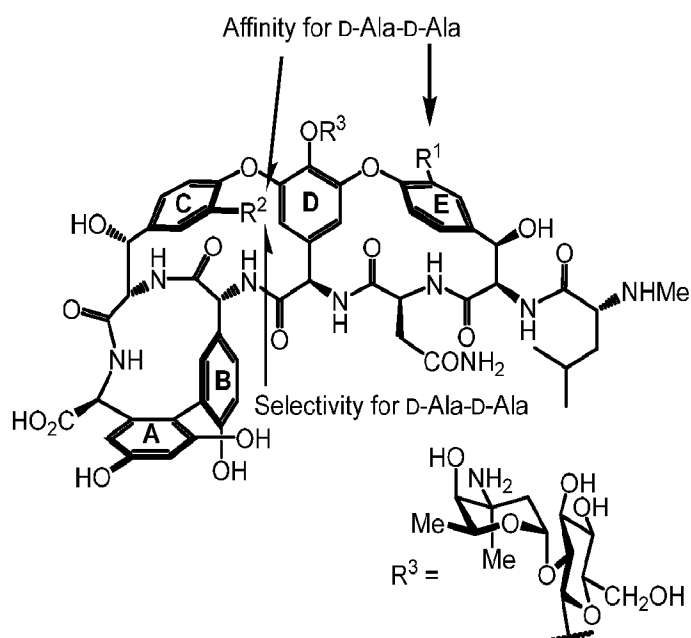
RESULTS AND DISCUSSION

Two key features were required for the present synthetic simplification to be beneficial. First, the two added chlorine substituents need to be benign and not have a significant effect on 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. 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 might be accommodated on the proximal D-ring phenol by GtfE in the initial glycosylation reaction.

Role of the Vancomycin C and E Ring Aryl Chlorides

In contrast to the unknown stringency of the glycopeptide substrate requirements for the native glycosyltransferases, a great deal is known about the impact of the aryl chlorides. The presence of both the vancomycin C and E ring aryl chlorides play important roles in ligand binding and antimicrobial activity as shown below in **Table A** that shows antimicrobial activity and D-Ala-D-Ala binding affinity of dechlorovancomycins and aglycons. Both chlorine substituents 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.⁸⁻¹⁰

Table A



Compound	R ¹	R ²	R ³	MIC (μg/mL) ^a			K _a (M ⁻¹) ^e
<i>glycopeptides</i>				<i>MSSA</i> ^b	<i>MRSA</i> ^c	<i>VSE</i> ^d	<i>Ac₂KAA</i>
4.2	Cl	Cl	sugar	1	1	2	1.5 x 10 ⁶
4.3	H	Cl	sugar	4	4	8	5.9 x 10 ⁵
4.4	H	H	sugar	8	8	32	1.6 x 10 ⁵
<i>aglycons</i> ^f				<i>MSSA</i> ^g			
4.5	Cl	Cl	H		1.25		1.4 x 10 ⁵
4.6	H	Cl	H		5		6.8 x 10 ⁴
4.7	Cl	H	H		10		3.8 x 10 ⁴
4.8	H	H	H		20		1.8 x 10 ⁴

^aRef 9. ^bMethicillin-sensitive *S. aureus* (ATCC 29213). ^cMethicillin-resistant *S. aureus* (MMX 2002). ^dVancomycin-sensitive *E. faecalis* (MMX 101). ^eRef 8. ^fRef 10. ^gATCC 25923.

In addition to its stabilizing hydrophobic interaction with the ligand terminal D-Ala methyl group, the 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 substituent that it can accommodate (Me > H >> all others).¹¹ Thus, a vancomycin structural

simplification achieved through removal of both aryl chlorides may be too detrimental to be useful.

Complementary to these studies, placement of an isomeric E-ring chloride over the binding pocket has only a small effect.¹² This observation, combined with expectations that incorporation of an isomeric C-ring chloride distal from the binding pocket was unlikely to have a significant impact,¹³ suggested that the addition of two chlorides would be more effective than removal of the two key 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 2_c,2_e,6_c,6_e-tetrachlorination pattern. Therefore, determining 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 was begun.

Synthetic Strategy

A concise route to tetrachlorovancomycin (Compound **1**, and Formula I) was designed that takes advantage of the increased symmetry (Fig. 2). Both the CD and DE macrocyclizations, with each chlorinated *o*-fluoronitrophenyl group now more activated toward S_NAr substitution, would be accomplished in a single operation as their stereochemical outcome is inconsequential following Sandmeyer chlorination. The only remaining element of atropisomerism, the AB 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 glycosyltransferases GtfE and GtfD would recognize it as a substrate for the disaccharide introduction. These native glycosyltransferases¹⁴⁻¹⁷ 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 The success of the enzymatic glycosylations of tetrachlorovancomycin, as established herein, is key to direct synthetic access to not only Compound **1**, but also future pocket-modified analogues.

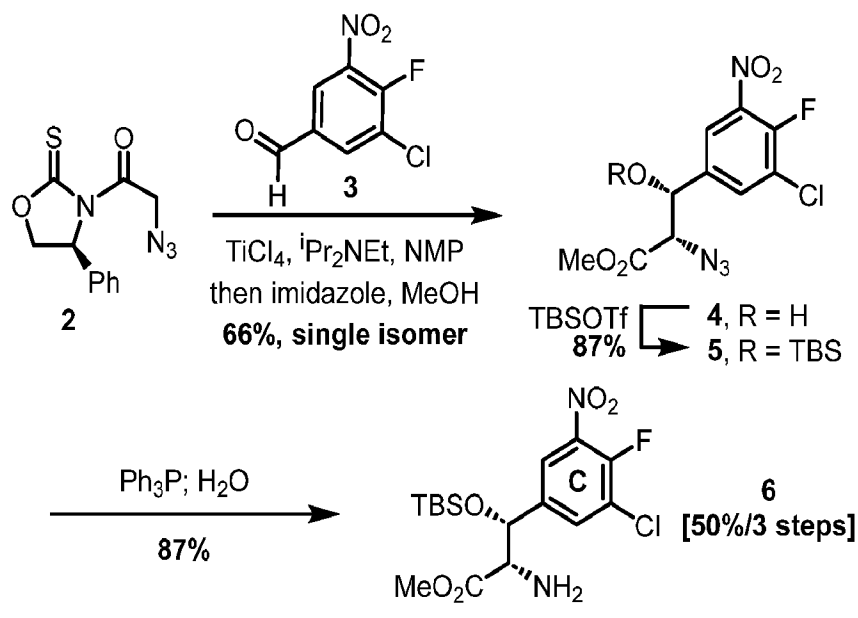
Preparation of the Modified C and E Ring Subunits

In addition to residues 1, 3, 4, 5, and 7 already in hand,⁶ preparation of the chlorinated C and E ring β -hydroxyphenylalanine subunits were required (Scheme 1, below). Diastereoselective Crimmins aldol²² addition of Compound **2** to 3-chloro-4-fluoro-5-nitro-benzaldehyde (Compound **3**)²³ followed by in situ methanolysis of the imide provided the syn aldol product Compound **4** as a single diastereomer (66%, anti-diastereomer not detected). TBS protection of the secondary alcohol to provide Compound **5** was followed by Staudinger reduction to afford the C ring (residue 6) free amine Compound **6** (50% overall yield/3 steps).

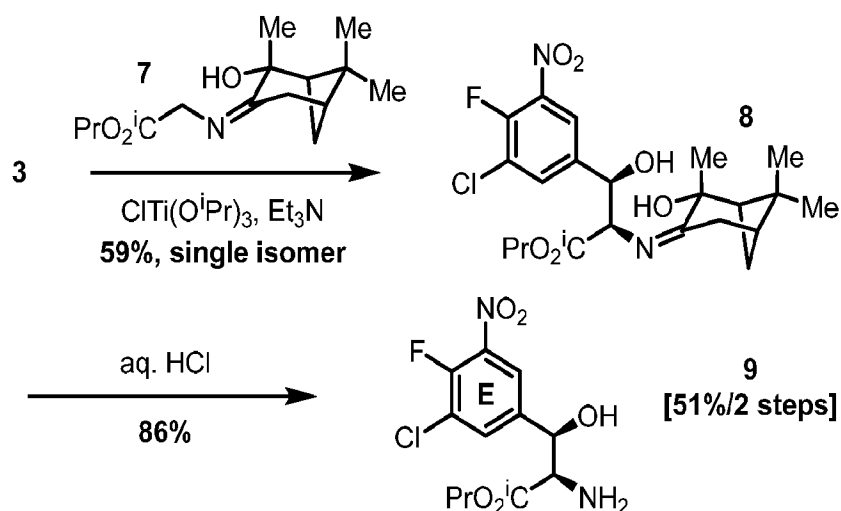
Preparation of the E ring anti β -hydroxyphenyl-alanine Compound **9** was accomplished by modification of the method of Solladié–Cavallo²⁴ that allowed use of commercially available CITi(OiPr)₃. Accordingly, diastereoselective Ti-promoted aldol

Scheme 1

A. Preparation of the chlorinated C ring



B. Preparation of the chlorinated E ring



addition of imine Compound **7** to the same aldehyde Compound **3**²³ provided the anti-product Compound **8** in good yield (59%, syn diastereomer not detected).

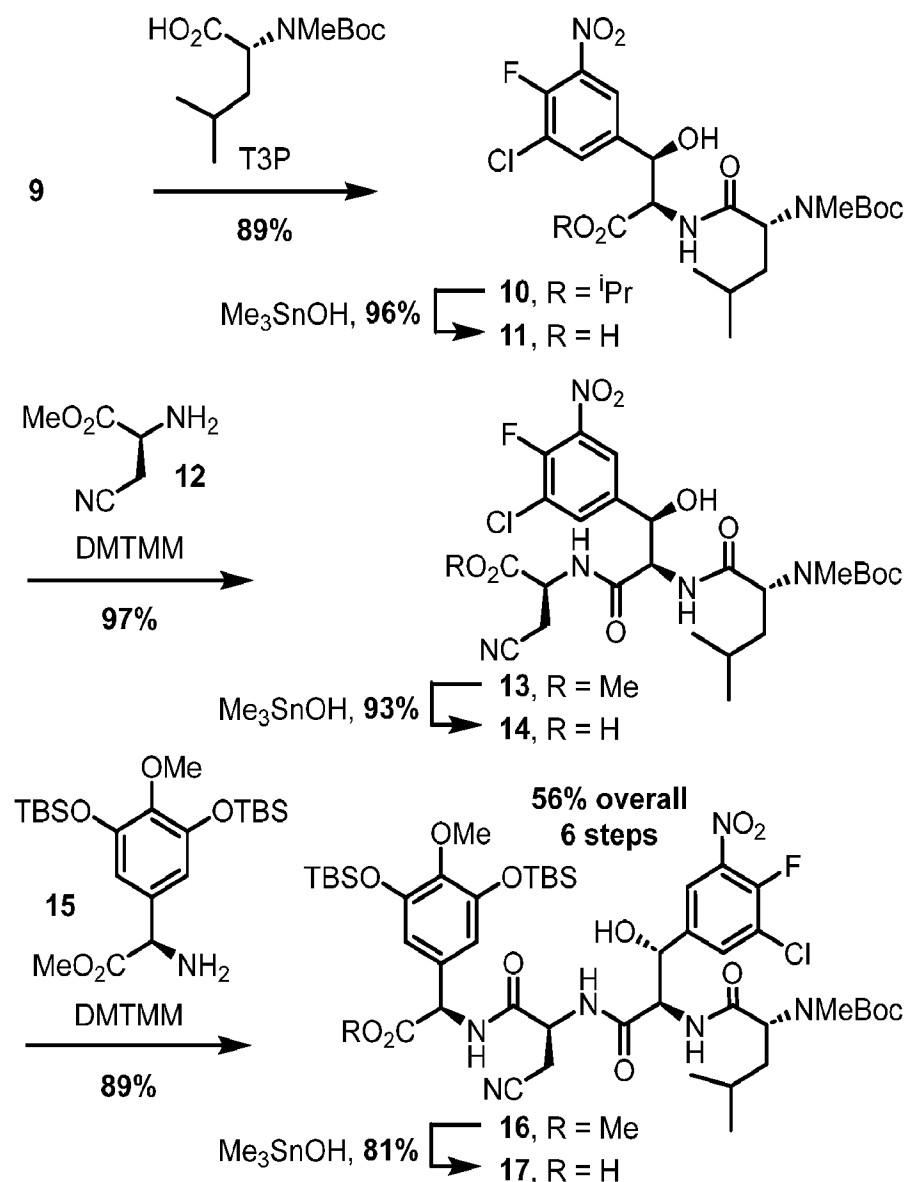
Hydrolytic removal of the chiral auxiliary with dilute aqueous HCl provided the E ring (residue 2) amine Compound **9** (86%, 51% overall yield/2 steps).

Preparation of the Linear DE Tetrapeptide

As shown in Scheme 2, below, Compound **9** was incorporated into the DE tetrapeptide Compound **17** through a series of straightforward stepwise peptide coupling reactions, starting with its coupling 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 Compound **10** (89%). Saponification of the isopropyl ester Compound **10** was surprisingly clean (Me₃SnOH,²⁶ ClCH₂CH₂Cl, 96%), providing carboxylic acid Compound **11** without detectable epimerization of the α-stereocenter. By contrast, use of even carefully controlled aqueous saponification conditions (3 equiv LiOH, 2:1 *t*-BuOH–H₂O, 0 °C, 1 h) led to significant C_α epimerization (4:1 dr).

Coupling of Compound **11** with β-cyanoalanine methyl ester Compound **12**²⁷ promoted by 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride²⁸ (DMTMM, EtOAc, 97%) provided tripeptide Compound **13**. Saponification (Me₃SnOH, ClCH₂CH₂Cl, 93%) provided carboxylic acid Compound **14**, which was coupled with the D ring free amine **15**⁶ (DMTMM, THF, 89%) to afford Compound **16**.²⁹ Methyl ester hydrolysis (Me₃SnOH, ClCH₂CH₂Cl, 81%) provided the tetrapeptide Compound **17** without C_α epimerization or competitive desilylation of the base-sensitive phenol TBS ethers.

Scheme 2

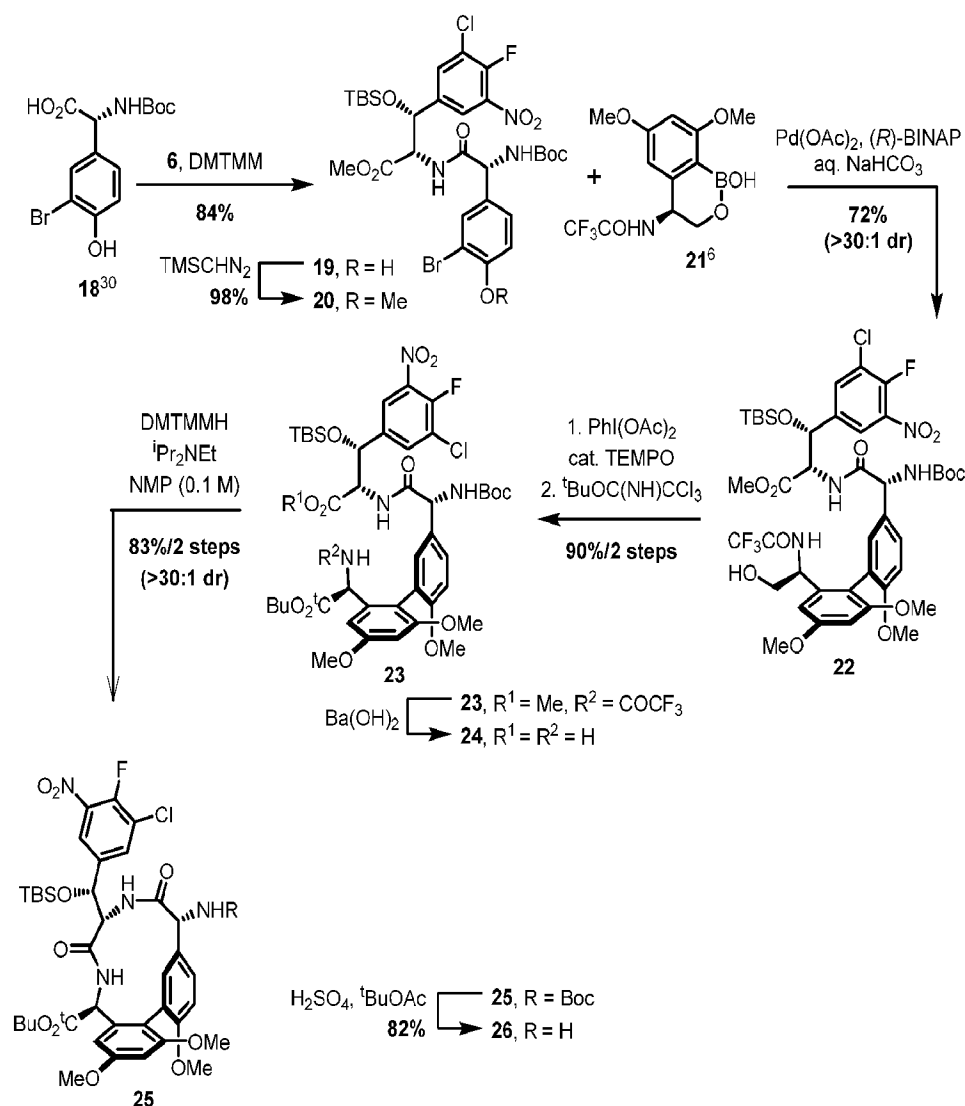


Preparation of the AB Macrocycle and Total Synthesis of Tetrachlorovancomycin

Coupling of Compound **6** with Compound **18**³⁰ (DMTMM, MeCN, 0 °C, 1 h) followed by phenol methylation (TMSCHN₂, 20% MeOH–CH₂Cl₂, 5 °C, 36 h) provided dipeptide Compound **20** (82%/2 steps) (Scheme 3, below). A one-pot Miyaura borylation–Suzuki coupling sequence conducted with an *in situ* generated (*R*)-BINAP(O)-Pd⁰ catalyst system^{6,31} provided Compound **22** exclusively as a single diastereomer (72%, >30:1 dr), setting the AB biaryl atropisomer stereochemistry. This telescoped reaction sequence, with *in situ*

generation of Compound **21** from the corresponding bromide, was conducted under mild reaction conditions nearly identical to those recently disclosed in the total synthesis of vancomycin⁶ ($\text{Pd}(\text{OAc})_2$, (*R*)-BINAP, aq NaHCO_3 , MeTHF) and did not require tailoring to accommodate the modified substrate Compound **20**, which is intrinsically more reactive toward $\text{S}_{\text{N}}\text{Ar}$ substitution and contains a potentially reactive aryl chloride.

Scheme 3



Oxidation of the C-terminus primary alcohol to the corresponding carboxylic acid ($\text{PhI}(\text{OAc})_2$, 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) provided Compound **23** cleanly (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–H₂O, 23 °C, 95%). This reagent proved to be milder³³ than LiOH and more easily removed by precipitation as BaCO₃, thus avoiding minor losses of Compound **24** due to aqueous extraction and chromatography.

Macrolactamization of Compound **24** under simulated high-dilution conditions promoted by 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methyl-morpholinium hexafluorophosphate³⁴ (DMTMMH, 1.5 equiv, 3 equiv *i*-Pr₂NEt, NMP, 0.1 M) provided the AB macrocycle Compound **25** in superb yield (83%/2 steps). Importantly, the cyclization reaction proceeds essentially instantaneously upon dropwise addition of Compound **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 Compound **25**, including the AB biaryl atropisomer stereochemistry, were confirmed in single crystal X-ray structure determination. 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 Compound **25** was accomplished under conditions that may allow reversible deprotection of the slightly acid-labile *t*-butyl ester³⁶ (8 equiv H₂SO₄, *t*-BuOAc, 0 to 23 °C, 2 h), providing Compound **26** (82%) that serves as the common precursor to tetrachlorovancomycin (Compound **1**) as well as the subsequent binding pocket-modified analogues. Strikingly, NOESY studies of the AB macrocycle Compound **26**, bearing the free amine, revealed exclusive adoption of the 5,6-*cis* amide conformation.

The final steps to the full tricyclic skeleton of tetrachlorovancomycin proved exceptionally smooth (Scheme 4, Fig. 3). Although the D ring phenylglycine is prone to epimerization,^{1,37} coupling of Compound **26** with the linear DE tetrapeptide Compound **17** mediated by 3-(diethoxy-phosphoryloxy)1,2,3-benzotriazin-4 (3*H*)-one³⁸ (DEPBT,

2 equiv, 4.5 equiv NaHCO₃, 23 °C, 17 h) proceeded in excellent yield (93%), providing heptapeptide Compound **27** without detectable epimerization (>30:1 dr). A room temperature *in situ* double S_NAr cyclization of Compound **27** was observed under the conditions of desilylation with Bu₄NF (5 equiv, MeCN, 23 °C, 4 h), establishing both the CD and DE macrocycles in a single step and providing Compound **28** in superb yield (95%) as an inconsequential mixture of atropisomers.

The ease of the two-fold intramolecular S_NAr macrocyclizations of Compound **27** is noteworthy, being conducted at room temperature with a mild desilylating agent (Bu₄NF) in a relatively nonpolar solvent that is conveniently removed by evaporation upon reaction completion (MeCN). The effortless cyclization of Compound **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 Compound **28**, as a mixture of isomers, was directly subjected to dual nitro group reduction (Fe, AcOH), two-fold Sandmeyer chlorination (BF₃•Et₂O, *t*-BuONO; CuCl, CuCl₂, CD₃CN),^{6,7} and a final global deprotection by neat TFA cleavage of the Boc group and *t*-butyl ester concurrent with nitrile hydration,⁴⁰ and subsequent removal of four methyl ethers (5:1 AlBr₃/EtSH) to afford tetrachlorovancomycin aglycon (Compound **29**, 56%/5 steps from Compound **27**) as a single diastereomer. Remarkably, the conversion of AB macrocycle Compound **26** to the fully functionalized, deprotected aglycon Compound **29** now requires only 6 steps, proceeds in 52% overall yield, and avoids the generation of undesired atropisomers or C_α diastereomers. See, Scheme 4, Fig. 3.

Highlights in this sequence include not only the mild room temperature double S_NAr cyclization of Compound **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 formation⁷ and deuterated solvent suppression⁶ of competitive reduction, a

remarkably effective TFA-mediated nitrile hydration,⁴⁰ and a scalable $\text{AlBr}_3/\text{EtSH}$ -mediated global deprotection.⁷

Finally, to find that the one-pot two-step enzymatic glycosylation of tetrachlorovancomycin aglycon (Compound **29**) proceeded in high yield (82%) for installation of both sugar residues despite the added 2_e and 6_e aryl chlorides (Scheme 4, Fig. 3) was delighting. The disaccharide introduction makes use of the two overexpressed recombinant glycosyl-transferases, GftE and GftD, involved in the native 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 phosphatase⁴² to each glycosylation reaction.

This latter feature, which results in hydrolysis of the byproduct uridine-5'-diphosphate (UDP), also prevents product inhibition and permits the sequential reactions to be conducted in a one-pot procedure without intermediate isolation of the pseudoaglycon. In addition to providing Compound **1**, the bonus of this latter work is that it also helps define the glycopeptide substrate tolerance of the native glycosyltransferases.

Combined, this allowed the total synthesis of tetrachlorovancomycin (Compound **1**) to be completed in a straightforward manner (LLS = 15 steps, 15% overall yield from the amino acid subunits), avoiding generation of minor undesired atropisomers and past problematic C α epimerizations.

Even within the constraints of an academic lab, this permits 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 approach proceeds in 15% overall yield to provide Compound **1** and nearly 20% overall yield to provide the aglycon Compound **29** with complete control

of all stereochemistry and was unimaginable at the time these studies were initiated (**Table B**, below).

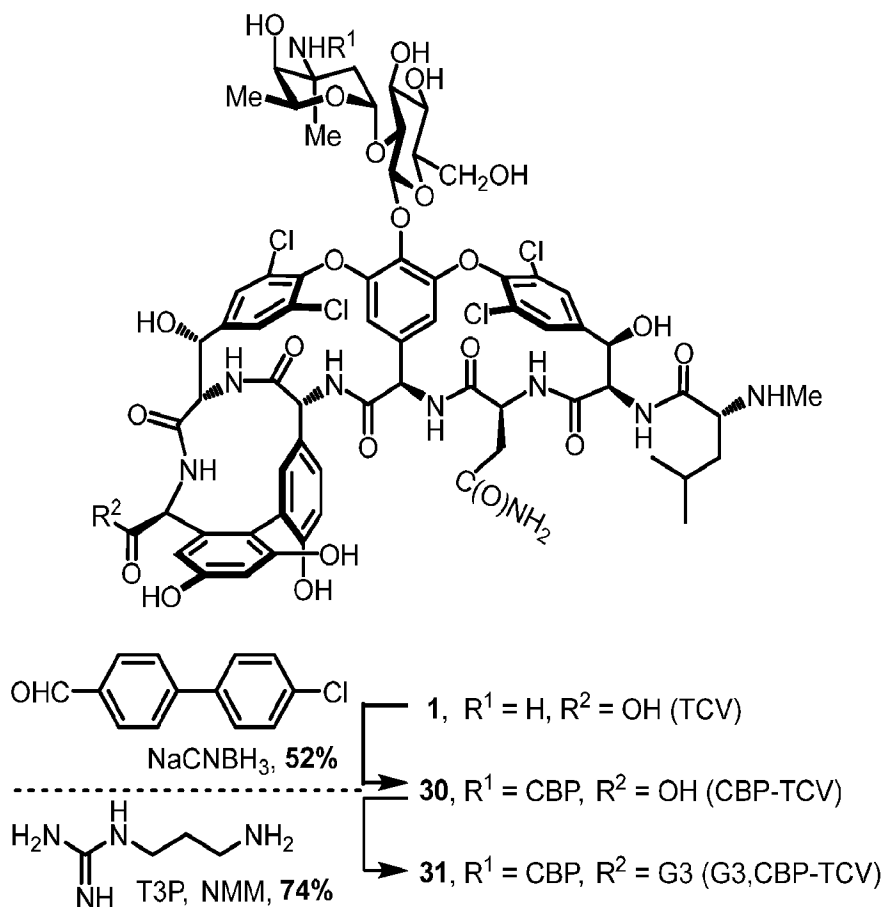
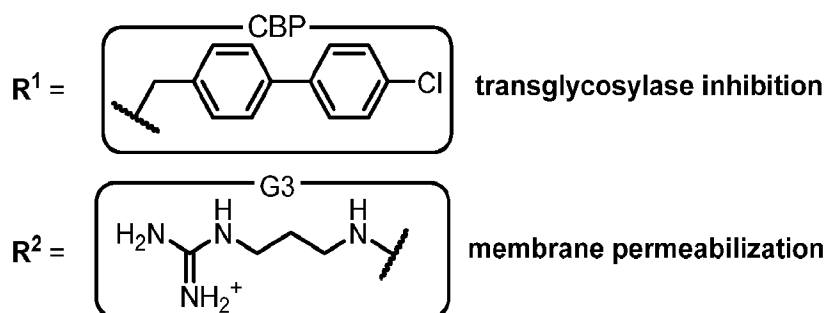
Table B
Comparison of Total Vancomycin Syntheses

Vancomycin			<u>Atroposelectivity</u>			<u>Glycosylation</u>		
Total Syntheses	LLS	Yield	AB	CD	DE	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			<u>Atroposelectivity</u>			<u>Glycosylation</u>		
Total Synthesis	LLS	Yield	AB	CD	DE	method	steps	yield
This work	15	15%	>30:1	–	–	enzymatic	1	82%

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 Compound **1** (TCV, tetrachlorovancomycin) without need for protecting groups under established conditions (Scheme 5, below).

Scheme 5



Divergent total synthesis of pocket-modified tetrachlorovancomycins

The exciting biological activity of the tetrachlorovancomycins provided the basis to pursue binding pocket-modified analogues through total synthesis of the corresponding residue 4 thioamide. The route developed to access the divergent precursor to these analogues, $[\Psi[\text{C}(=\text{S})\text{NH}]\text{Tpg}^4]$ tetrachloro-vancomycin aglycon (Compound **38**), required only slight modification for incorporation of the

residue 4 thioamide (Fig. 4). Towards this end, direct thioacylation³⁵ of AB macrocycle Compound **25** with Compound **35**⁵⁷ provided **36** in superb yield (89%). Subsequent Boc deprotection (TFA) and coupling with E ring tripeptide Compound **14** (T3P®, NMM, 0 °C) provided Compound **37** without detectable epimerization (85%, 69%/2 steps from Compound **14**) as is also shown in Fig. 4.

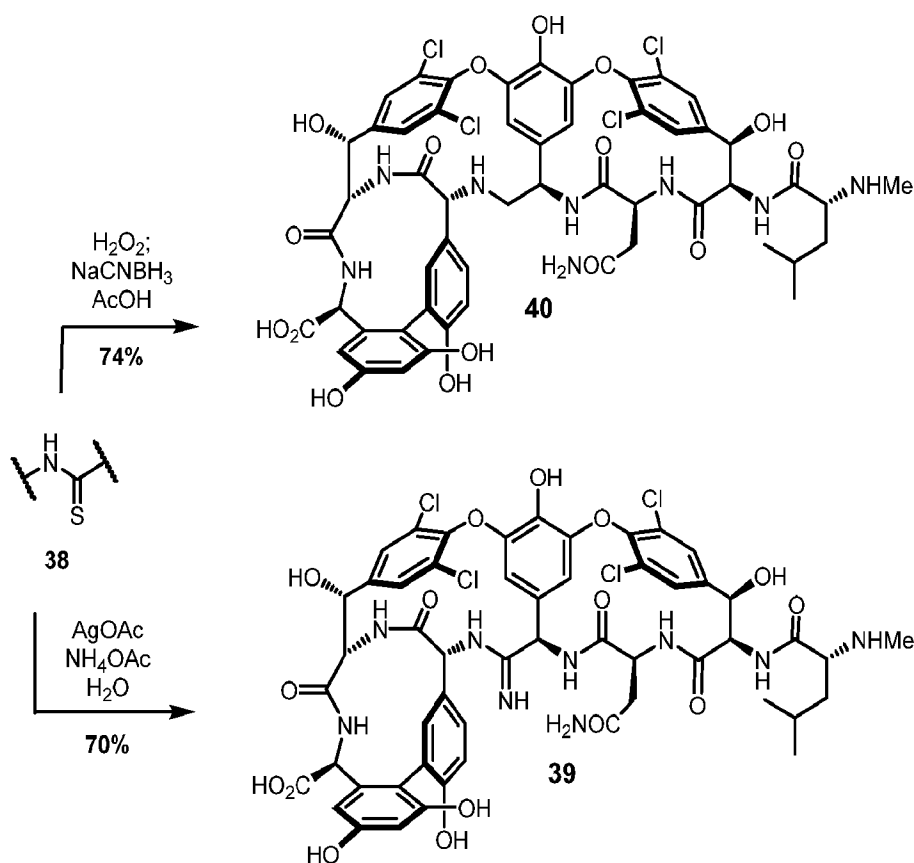
Desilylation of heptapeptide Compound **37** (Bu₄NF, MeCN, 23 °C) triggered a spontaneous double S_NAr cyclization that established the full tricyclic framework of [Ψ[C(=S)NH]Tpg⁴]tetrachlorovancomycin aglycon (Compound **38**) in a single step (Fig. 4). Dual nitro reduction (Fe, AcOH), Sandmeyer substitution (BF₃•Et₂O, *t*-BuONO; CuCl, CuCl₂), nitrile hydration with concomitant Boc and *t*-butyl ester deprotection (TFA, 23 °C), and global demethylation (5:1 AlBr₃:EtSH) provided [Ψ[C(=S)NH]Tpg⁴]tetrachloro-vancomycin aglycon (Compound **38**) in good overall yield (43%/5 steps from Compound **37**, with an average yield of 84%/step), setting the stage for the introduction of the residue 4 binding pocket modifications.

Several improvements in the present route (to Compound **38**) are worth highlighting in comparison with the total synthesis of [Ψ[C(=S)NH]Tpg⁴]vancomycin disclosed previously. First, both the CD and DE ring closures are now performed in a single operation at room temperature and with no special precautions to exclude moisture (e.g., molecular sieves, flame-dried glassware and reagents). Second, the double S_NAr cyclization of Compound **36** does not require the carefully controlled reaction conditions (5 °C, NMP) that were necessary to obtain good atroposelectivity in the CD ring closure enroute to [Ψ[C(=S)NH]Tpg⁴]vancomycin. Finally, coupling of Compound **36** to the E ring tripeptide Compound **14** prior to cyclization of the CD ring system does not result in epimerization of the sensitive^{1, 32} β-cyanoalanine residue.

Moreover, the late-stage divergent strategy developed to access pocket-modified tetrachlorovancomycin analogues directly from the fully deprotected thioamide aglycon (Compound **38**) was maintained (Scheme 6). The residue 4 amidine modification was introduced under

mild, pH-neutral conditions (100 mM AgOAc, saturated aqueous NH_4OAc , 23 °C) to provide $[\Psi[\text{C}(=\text{NH})\text{NH}]\text{Tpg}^4]\text{tetrachlorovancomycin aglycon}$ (Compound **39**, 70% yield). The unique method for reductive desulfurization developed in this work (H_2O_2 ; NaCNBH_3 , AcOH) performed similarly well for aglycon Compound **38**, delivering $[\Psi[\text{CH}_2\text{NH}]\text{Tpg}^4]\text{tetrachloro-vancomycin aglycon}$ (Compound **40**) in high yield (74%).

Scheme 6. Divergent synthesis of $[\Psi[\text{C}(=\text{NH})\text{NH}]\text{Tpg}^4]\text{tetrachlorovancomycin aglycon}$ (**39**) and $[\Psi[\text{CH}_2\text{NH}]\text{Tpg}^4]\text{tetrachlorovancomycin aglycon}$ (**40**).



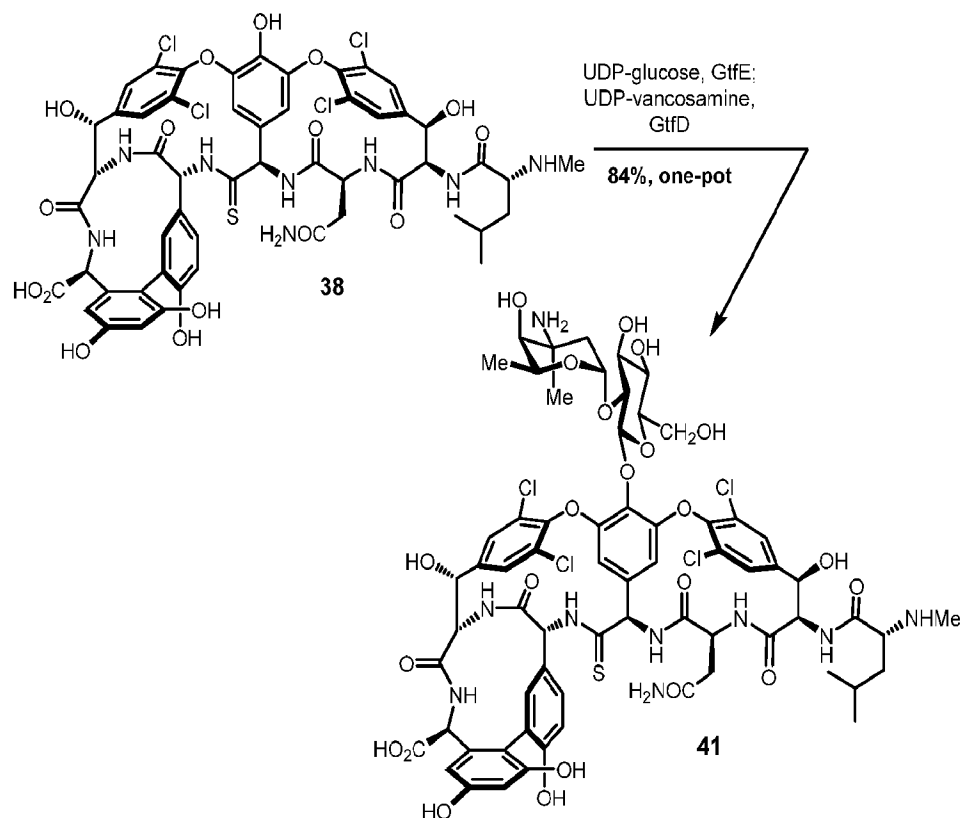
Similar, alternative syntheses of Compound **39** and **40** from Compound **38** are illustrated in Fig. 5.

The aglycon substrate Compound **38**, which additionally bears a residue 4 thioamide, was well-tolerated in the one-pot enzymatic glycosylation (UDP-glucose, GtfE; UDP-vancosamine, GtfD) to provide $[\Psi[\text{C}(=\text{S})\text{NH}]\text{Tpg}^4]\text{tetrachlorovancomycin}$ (Compound **41**) in excellent

yield (84%). Sequential conversions of Compound **41** to CBP- $[\Psi[C(=S)NH]Tpg^4]$ tetrachloro-vancomycin (Compound **44**) and G3,CBP- $[\Psi[C(=S)NH]Tpg^4]$ -tetrachlorovancomycin (Compound **47**) following our established protocols and their subsequent divergent conversions to either the amidine or aminomethylene pocket modifications following our newly devised methods smoothly provided a key series of pocket modified tetrachlorovancomycin analogues bearing one or two additional peripheral modifications that introduce one or two additional synergistic mechanisms of antibacterial activity is shown in Figs. 6A and 6B.

Although our experience in the preparative-scale enzymatic glycosylation of Compound **38** is still limited, the conversion of Compound **38** to Compound **41** appears to be nearly quantitative (84%). This conversion is shown in **Scheme 7**, below.

Scheme 7. Enzymatic glycosylation of $[\Psi[C(=S)NH]Tpg^4]$ tetrachlorovancomycin aglycon



Model Ligand Binding Studies

The binding of tetrachlorovancomycin (Compound **1**) and its aglycon Compound **29** to the model cell wall ligand Ac₂-L-Lys-D-Ala-D-Ala(**A**) and Ac₂-L-Lys-D-Ala-D-Lac (**B**)^{45,46} 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} as shown in Figs. 8A and 8B alongside those of tetrachlorovancomycin⁵⁷ as well as the corresponding vancomycin pocket modified analogues reported earlier as well as 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.

Studies established that tetrachloro-vancomycin maintains a high affinity for the model ligand **A**, displaying a binding constant approximately 5-fold lower than vancomycin. This small difference in ligand binding affinity correspondingly reduced the antimicrobial activity of relative to vancomycin, but also proved inconsequential to the activity of the more potent peripherally-modified tetrachlorovancomycin analogues where differences in potency were not distinguishable. Tetrachlorovancomycin, like vancomycin, failed to bind to an appreciable extent the model ligand of the peptidoglycan precursor found in vancomycin-resistant organisms, Ac₂-L-Lys-D-Ala-D-Lac (**B**).

As expected, the thioamide Compound **41** of tetrachlorovancomycin, like that of vancomycin, failed to bind either ligand effectively and correspondingly exhibits no appreciable antimicrobial activity. Consistent with expectations, both the amidine and aminomethylene analogues of tetrachlorovancomycin displayed dual ligand binding with near equal affinities in which the amidine was roughly 10-fold (8–12-fold) more effective.

Notably, the amidine pocket modified tetrachlorovancomycin binds both ligands with affinities roughly only two-fold lower than the affinity of tetrachlorovancomycin for Ac₂-L-Lys-D-Ala-D-Ala (**A**). In addition, and further consistent with expectations, these affinities proved to be only 3–5-fold lower than those of the corresponding vancomycin pocket modified analogues.⁵⁸ The lower antimicrobial potency of these latter two pocket modified

tetrachlorovancomycin analogues, like that of tetrachlorovancomycin itself, that smoothly follow the ligand binding affinity differences proved to incrementally diminish and ultimately disappear with each subsequent peripheral modification (CBP, G3).

Thus, the peripheral modifications, which sequentially introduce two additional independent mechanisms of action that do not rely on ligand binding,⁵⁹ not only synergistically improve potency but also largely eliminate potential small distinguishing activity differences due to relative ligand binding affinities. The key feature of which is the equipotent antimicrobial activity of the peripherally- and pocket-modified [C(=NH)NH and CH₂NH vs CONH] tetrachlorovancomycins against both vancomycin-sensitive and vancomycin-resistant Gram positive bacteria that is observed at superb potencies.

The results establish that Compound **1** maintains a high affinity for the model ligand Compound **32** ($K_a = 1.1 \times 10^5 \text{ M}^{-1}$), displaying a binding constant only 5-fold lower than vancomycin ($K_a = 5.4 \times 10^5 \text{ M}^{-1}$) and the difference was even smaller (3-fold) for the aglycons.⁴⁸ This minor difference in ligand binding affinity correspondingly reduced the antimicrobial activity of Compound **1** relative to vancomycin, but proved inconsequential for the antimicrobial activity of the more potent peripherally-modified tetrachlorovancomycin analogues as discussed hereinafter.

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), vancomycin-sensitive *E. faecium* and *E. faecalis*, as well as vancomycin-resistant *E. faecium* and *E. faecalis* (VanA and VanB VRE) strains is summarized in **Table C**, below.

Table C

Antimicrobial activity

Compound	MIC ($\mu\text{g/mL}$) ^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	10	0.08	0.08
G3-CBP-vancomycin	0.3	0.04	0.04
31 , G3-CBP-tetrachlorovancomycin	0.3	0.04	0.08

^aMinimum inhibitory concentration. ^bVancomycin-resistant *E. faecalis* (ATCC BAA-2317). ^cMethicillin-sensitive *S. aureus* (ATCC 25923).

^dMethicillin-resistant *S. aureus* (ATCC 43300).

Tetrachlorovancomycin (Compound **1**) and its aglycon Compound **29** were found to be slightly less potent than vancomycin and its aglycon (about 4-fold) consistent with their relative ligand binding affinities toward Ac₂-L-Lys-D-Ala-D-Ala. However, with incorporation of the CBP peripheral modification, the activity of CBP-tetrachlorovancomycin (Compound **30**) and CBP-vancomycin were indistinguishable in sensitive strains.

Moreover, Compound **30** displays activity against the VRE strain comparable to that of CBP-vancomycin, which is derived from direct competitive inhibition of transglycosylase (a second independent mechanism of action) that does not require Ac₂-L-Lys-D-Ala-D-Ala binding.^{49,50} This activity of CBP-tetrachloro-vancomycin (Compound **30**) is improved about 100-fold against the sensitive strains due to the expression of two independent and synergistic mechanisms of action.

Combined, these studies highlight both that the vancomycin core mechanism of action (D-Ala-D-Ala binding) is operative and highly effective in 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-tetrachloro-vancomycin (Compound **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 (2 effective mechanisms of action now including G3 induced cell permeability),⁴⁴ the antimicrobial activity of Compound **31** is superb (MIC = 0.3 µg/mL), and it is even more potent by an order of magnitude against the vancomycin sensitive strains (3 effective mechanisms of action). These results suggest that even greater VanA antimicrobial activity may be achieved with pocket-modified^{7,51} analogues of G3-CBP-tetrachloro-vancomycin (Compound **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 antimicrobial activity observed with the added peripheral modifications to tetrachlorovancomycin likely requires their incorporation in a single molecule as has been demonstrated with vancomycin⁴⁴ and its pocket-modified analogues.⁷ Second, the peripherally-modified analogues of Compound **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}

Added *In Vitro* Antimicrobial Activity of Peripherally and Pocket Modified Tetrachlorovancomycins

The antimicrobial activity of a key series of pocket modified tetrachlorovancomycin analogues against additional representative vancomycin-sensitive methicillin-susceptible and methicillin-resistant *S. aureus* (MSSA and MRSA), vancomycin-sensitive *E. faecium* and *E. faecalis*, as well as vancomycin-resistant *E. faecium* and *E. faecalis* (VanA and VanB VRE) strains is summarized in the tables of Figs. 9A and 9B.

To highlight both the synergistic activity that results from the combined peripheral and productive pocket modifications and to define the distinguishing activity derived from each modification and their

independent mechanisms of action, further tetrachlorovancomycin thioamide derivatives were examined.

[$\square$][C(=S)NH]Tpg⁴]Tetrachlorovancomycin (Compound **41**) was found to be inactive (MICs >80 μ g/mL, highest concentration tested) against all strains as expected as it is incapable of effectively binding either D-Ala-D-Ala to D-Ala-D-Lac required for inhibition of the penultimate transpeptidase-catalyzed cell wall cross-linking reaction and maturation. Sequential additions of the peripheral modifications CBP and G3 impart and incrementally improve antimicrobial activity. The CBP modification on the disaccharide provides CBP-[$\square$][C(=S)NH]Tpg⁴]-tetrachlorovancomycin (Compound **44**) that displays effective and equipotent activity (MICs 5–10 μ g/mL) against both the vancomycin-sensitive and vancomycin-resistant pathogens despite being incapable of binding either D-Ala-D-Ala or D-Ala-D-Lac.

Earlier studies have shown this activity is derived from the direct competitive inhibition of transglycosylase and does not rely on D-Ala-D-Ala or D-Ala-D-Lac binding or transpeptidase inhibition.⁵⁸ Further addition of the C-terminus G3 modification provides G3,CBP-[$\square$][C(=S)NH]Tpg⁴]tetrachlorovancomycin (Compound **47**) that now exhibits further improved antimicrobial activity (MICs 0.3–1.2 μ g/mL), displaying essentially equipotent activity against either vancomycin-sensitive or vancomycin-resistant bacteria. The increase in potency attributable to G3 is derived from a newly added mechanism of action, permeabilization of the cell envelop without membrane disruption or lysis,⁵⁸ that is independent of both the CBP-mediated transglycosylase competitive inhibition and pocket-derived ligand binding and transpeptidase inhibition.⁵⁸ Notably, Compound **47** expresses this activity now through two synergistic and independent mechanisms of action, neither of which require D-Ala-D-Ala or D-Ala-D-Lac binding.

Incorporation of the two productive pocket modifications at residue 4 that maintain binding to D-Ala-D-Ala and impart binding to D-Ala-D-Lac, the amidine and aminomethylene, into the peripherally modified tetrachlorovancomycin analogues with Compounds **48** and **49**, respectively, provided potent antimicrobial agents equally active against

vancomycin-sensitive and vancomycin-resistant bacteria. The most potent of these displayed superb activity (MICs 0.02–0.08 µg/mL), representing a 15-fold improvement in activity relative to the corresponding thioamide derivative.

This increased activity can be ascribed to the pocket dual ligand binding where that to D-Ala-D-Ala is operative and that to D-Ala-D-Lac is newly installed, inhibiting transpeptidase-catalyzed cell wall cross-linking and maturation in both vancomycin-sensitive and vancomycin-resistant bacteria. Such analogues, which we have come to refer to as maxamycins, display their activity through three independent and synergistic mechanisms of action of which only one requires D-Ala-D-Ala/D-Lac binding.

Moreover, and like the preceding studies with the original vancomycin versus tetrachloro-vancomycin series (residue 4 amide),⁵⁸ the small potency differences observed between vancomycin and tetrachlorovancomycin diminish and ultimately disappear against even sensitive bacteria with the progressive introduction of the two peripheral and subsequent pocket modifications. That is, a tetrachlorovancomycin analogue that contains two peripheral modifications and a pocket modification displays antimicrobial potency that is not distinguishable from that the corresponding vancomycin analogue, indicating the additional two chloride substitutions in the former no longer result in a measurable difference in the expression of activity.

Important general trends observed in prior studies continue to be seen with tetrachloro-vancomycin. First, the two peripheral modifications (2 > 1 > 0; CBP and G3) incrementally improve potency regardless of whether the organism is resistant or sensitive to vancomycin. This synergistic behavior is unusual and need not have been the case. It arises presumably because the peripheral modifications express their activity by mechanisms of action independent of one another as well as D-Ala-D-Ala/D-Lac binding, and all, including the pocket modifications, impact bacterial cell wall synthesis or its integrity.

In addition, the synergistic antimicrobial activity observed with the combined peripheral and pocket modifications within tetrachlorovancomycin analogues likely requires their incorporation in a single molecule as we have demonstrated with vancomycin and its pocket-modified analogues. This atypical behavior further suggests that the spatial and temporal localization of the individual effects is needed to provide the observed synergistic activity.

Finally, the peripherally-modified pocket analogues of tetrachlorovancomycin that act by three independent mechanisms of action against both vancomycin-sensitive and vancomycin-resistant bacteria are unlikely to raise resistance and represent the newest members of what can be expected to be an unusually durable antibiotic class we refer to as maxamycins.

CONCLUSION

A concise and easily scalable synthesis of a new class of structurally simplified synthetic vancomycin analogues was developed (Compound **1**, LLS = 15 steps, 15% overall yield; precursor aglycon Compound **29** in 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 potent antimicrobial activity of vancomycin, and achieves this simplification by an unusual addition of benign substituents to the core structure.

This added two chlorine substituent modification permitted a streamlined total synthesis of the new glycopeptide antibiotic analogue 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 Compound **1**.

Additional key elements of the approach include a catalyst-controlled diastereoselective formation of the AB biaryl axis of chirality (>30:1 dr), an 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 dual CD/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 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 antibiotics⁵³ and hopefully help dispel the perception that they are beyond practical synthetic access. 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 includes the preparation of binding pocket-modified analogues⁴ 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}

General Experimental

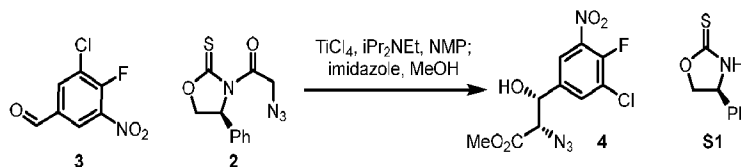
All reagents and solvents were used as supplied without further purification unless otherwise noted. $CHCl_3$ was pre-treated with alumina for at least 24 h (hours) prior to use. Preparative TLC (PTLC) and column chromatography were conducted using Millipore SiO_2 60 F254 PTLC (0.5 mm) and Zeochem ZEOprep® 60 ECO SiO_2 (40–63 μm), respectively. Analytical TLC was conducted using Millipore SiO_2 60 F254 TLC (0.250 mm) plates.

^1H and $^{13}\text{C}\{^1\text{H}\}$ NMR spectra were obtained on a Bruker Avance IIITM HD 600 MHz spectrometer equipped with either a 5 mm QCI or 5 mm CPDCH probe. Chemical shifts (δ) are reported in parts per million (ppm). Abbreviations used to designate multiplicities are: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet. Coupling constants (J) are reported in Hertz (Hz).

IR spectra were obtained on a Thermo Nicolet 380 FT-IR with a SmartOrbit Diamond ATR accessory. Specific rotations were determined at the sodium D line ($\lambda = 589$ nm) at specific temperatures and are reported as follows: $[\alpha]_D^{\text{temp}}$, concentration ($c = \text{g}/100$ mL), and solvent. Mass spectrometry analysis was performed by direct sample injection on an Agilent G1969A ESI-TOF mass spectrometer.

Melting points are uncorrected. The single crystal X-ray diffraction studies were carried out on a Bruker Platinum Pt135 CCD diffractometer equipped with Cu K α radiation ($\lambda = 1.54178$).

Preparations



Compound 4

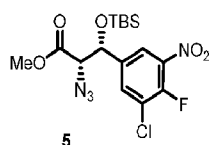
A solution of **2**⁶ (20.5 g, 76.4 mmol, 1.0 equiv) in CH_2Cl_2 (760 mL) was cooled to -78 °C and treated with TiCl_4 (8.8 mL, 80.3 mmol, 1.05 equiv). The resulting yellow solution was stirred for 15 minutes (min) at -78 °C, then treated dropwise with $i\text{-Pr}_2\text{NEt}$ (14.7 mL, 84.1 mmol, 1.1 equiv). The resulting dark purple solution was stirred at -78 °C for 1 h, then treated with NMP (14.7 mL, 153 mmol, 2.0 equiv) and stirred for an additional 15 min at -78 °C. Aldehyde **3** (20.23 g, 99.4 mmol, 1.3 equiv) in CH_2Cl_2 (99 mL) was added dropwise to this solution at -78 °C, and the reaction mixture was subsequently warmed to -20 °C and stirred for 2 h. The resulting brown solution was treated with imidazole (26.02 g, 382 mmol, 5.0 equiv) in MeOH (76 mL) and stirred at 23 °C overnight (about 16-18 h). The dark orange suspension was

warmed to 23 °C, quenched with the addition of saturated aqueous NH₄Cl (200 mL), and filtered through Celite.

The filtrate was concentrated under reduced pressure to remove MeOH, and the aqueous layer was extracted with CH₂Cl₂ (3 x 200 mL). The combined organic layers were dried with Na₂SO₄, concentrated under reduced pressure, and chromatography (SiO₂, 33% EtOAc–hexanes, rapid elution) provided an inseparable mixture of **4** (16.18 g, 66%) and (S)-4-phenyloxa-zolidine-2-thione (auxiliary **S1**) (10.39 g, 76%). The crude mixture was used in the next step without further purification.

For characterization, the material from a 4.2 mmol scale reaction was subjected directly to the following conditions to regenerate **2**, which is conveniently separated from **4**. The mixture of **4** and **S1** was combined with 2-azidoacetic acid⁵⁴ (579 mg, 5.7 mmol, 1.4 equiv) in CH₂Cl₂ (50 mL) and cooled to 0 °C. *i*-Pr₂NEt (2.16 mL, 12.4 mmol, 3 equiv) was added dropwise to this solution at 0 °C, followed by T3P (50 wt % in CH₂Cl₂, 3.95 g, 6.2 mmol, 1.5 equiv), and the reaction mixture was warmed to 23 °C and stirred for 15 min. The reaction mixture was poured into aqueous 1 M HCl (12 mL) and stirred for 5 min, then extracted with CH₂Cl₂ (3 x 50 mL). The combined organic layers were dried with MgSO₄, concentrated under reduced pressure, and the residue was purified by chromatography (SiO₂, wet load 50% CH₂Cl₂–hexanes, 50–100% CH₂Cl₂–hexanes, rapid elution) to provide **4** (872 mg, 65%) as a yellow foam and recycled **2**.

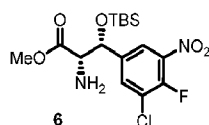
For **4**: $[\alpha]_D^{25}$ –106 (c 0.72, MeOH); ¹H NMR (600 MHz, CDCl₃) δ 7.99 (dd, *J* = 6.1, 2.2 Hz, 1H), 7.81–7.78 (m, 1H), 5.26 (d, *J* = 3.7 Hz, 1H), 4.09 (dd, *J* = 3.7, 1.3 Hz, 1H), 3.88 (d, *J* = 1.3 Hz, 3H), 3.08 (m, 1H); ¹³C{¹H} NMR (151 MHz, CDCl₃) δ 168.6, 151.4 (d, *J* = 269 Hz), 138.3 (d, *J* = 7.9 Hz), 136.7 (d, *J* = 5.3 Hz), 133.7, 124.7 (d, *J* = 17.6 Hz), 124.6, 122.3, 122.2, 72.3, 66.7, 53.5; IR (film) $\nu_{\max}$ 3506, 1740, 1613, 1542, 1486, 1438, 1349, 1271, 1211, 1101, 1013 cm⁻¹; HRMS (ESI-TOF) *m/z* [M+Cl]⁻ calcd for C₁₀H₈ClFN₄O₅, 352.9856; found, 352.9862.



Compound 5

The above crude mixture of alcohol **4** (16.18 g, 55.8 mmol, 1.0 equiv) and auxiliary **S1** (10.39 g, 58.0 mmol) was dissolved in CH₂Cl₂ (169 mL) and treated with 2,6-lutidine (23.7 mL, 203 mmol, 4.0 equiv) followed by TBSOTf (58.3 mL, 254 mmol, 5.00 equiv). The reaction mixture was warmed at reflux overnight (about 16-18 h), quenched with the addition of saturated aqueous NH₄Cl (80 mL, exothermic) at 0 °C and extracted with CH₂Cl₂ (3 × 80 mL). Column chromatography (SiO₂, 0–100% EtOAc–hexanes gradient elution) afforded **5** (19.04 g, 87%; typically 85–100%, 1–20 g scale) as a white solid.

For **5**: $[\alpha]_D^{25}$ –137 (c 3.9, MeOH); ¹H NMR (600 MHz, CDCl₃) δ 7.98 (ddd, *J* = 6.2, 2.3, 0.7 Hz, 1H), 7.75 (ddd, *J* = 5.9, 2.3, 0.6 Hz, 1H), 5.36 (d, *J* = 2.4 Hz, 1H), 3.85 (s, 3H), 3.61 (d, *J* = 2.8 Hz, 1H), 0.91 (s, 9H), 0.06 (s, 3H), –0.11 (s, 3H); ¹³C{¹H} NMR (151 MHz, CDCl₃) δ 168.3, 151.3 (d, *J* = 267 Hz), 138.2 (d, *J* = 7.7 Hz), 138.1 (d, *J* = 5.5 Hz), 133.5, 124.5 (d, *J* = 17.6 Hz), 122.1 (d, *J* = 3.1 Hz), 75.0, 67.2, 53.1, 25.5, 18.0, –4.5, –5.4; IR (film) $\nu_{\max}$ 2955, 2932, 2859, 2360, 2113, 1749, 1613, 1543, 1472, 1437, 1345, 1296, 1267, 1203, 1103, 1018 cm^{–1}; HRMS (ESI-TOF) *m/z* [M–H][–] calcd for C₁₆H₂₂ClFN₄O₅Si, 431.0954; found, 431.0947.

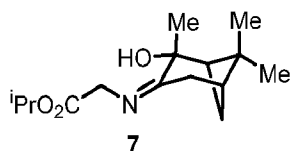


Compound 6

A solution of **5** (19.04 g, 44.0 mmol, 1 equiv) in dioxane (63 mL) was treated portion-wise with Ph₃P (11.54 g, 44.0 mmol, 1.0 equiv) at 0 °C. The resulting solution was stirred for 2 h at 23 °C before additional Ph₃P (2.31 g, 8.80 mmol, 0.20 equiv) was added. After 1 h,

the solution was treated with 75% dioxane–H₂O (63 mL) and stirred at 23 °C overnight (about 16-18 h). The reaction mixture was then concentrated under reduced pressure and the residue was purified by column chromatography (SiO₂, 10–20% EtOAc–hexanes gradient elution) to afford **6** (15.61 g, 87%) as a yellow oil.

For **6**: $[\alpha]_D^{23} +9.1$ (c 0.67, MeOH); ¹H NMR (500 MHz, CDCl₃) δ 7.99 (dd, *J* = 6.4, 2.1 Hz, 1H), 7.74 (dd, *J* = 5.7, 2.2 Hz, 1H), 5.22 (d, *J* = 2.0 Hz, 1H), 3.78 (s, 3H), 3.47 (d, *J* = 2.6 Hz, 1H), 0.90 (s, 8H), 0.02 (s, 3H), –0.12 (s, 3H); ¹³C{¹H} NMR (151 MHz, CDCl₃) δ 173.5, 151.0 (d, *J* = 267 Hz), 139.4, 138.0, 133.7, 124.0 (d, *J* = 17.4 Hz), 122.3, 74.0, 61.4, 52.5, 25.7, 18.1, –4.5, –5.3; IR (film) $\nu_{\max}$ 2954, 2931, 2857, 1744, 1542, 1347, 1257, 1096 cm^{–1}; HRMS (ESI-TOF) *m/z* [M+H]⁺ calcd for C₁₆H₂₄ClFN₂O₅Si, 407.1205; found, 407.1211.



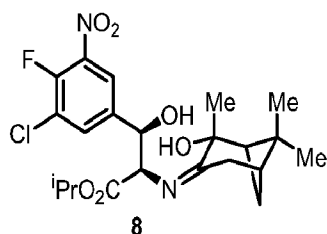
Compound 7

A suspension of isopropyl glycinate hydrochloride (5 g, 33 mmol, 2 equiv) and K₂CO₃ (8.8 g, 66 mmol, 4 equiv) in 2:1 CH₂Cl₂/H₂O (75 mL total) was stirred at 23 °C for 15 min, transferred to separatory funnel, and the organic layer was collected. The aqueous layer was extracted with additional CH₂Cl₂ (3 × 50 mL). The combined organic layers were dried over MgSO₄ and concentrated under reduced pressure to provide isopropyl glycinate (free base) as a volatile yellow oil (assumed quantitative), used directly in the following step.

The residue containing crude isopropyl glycinate (free base, 2 equiv) was combined with (1*R*,2*R*,5*R*)-(+)-2-hydroxy-3-pinane (2.7 g, 16 mmol, 1 equiv) and dissolved in PhMe (25 mL). The resulting solution was treated dropwise with BF₃•OEt₂ (0.39 mL, 3 mmol, 20 mol %) at 23 °C, and then warmed at reflux (Dean–Stark apparatus) for 3 h. The reaction mixture was cooled to 23 °C, concentrated under reduced pressure and the residue was purified by chromatography (SiO₂, 20–

60% EtOAc–hexanes + 1% Et₃N, rapid elution) to provide **7** (3.84 g, 89%) as a moisture-sensitive yellow oil.

For **7**: $[\alpha]_D^{25} +16$ (c 1.0, MeOH); ¹H NMR (600 MHz, CDCl₃) δ 5.12–5.04 (m, 1H), 4.20–4.10 (m, 2H), 2.55–2.43 (m, 2H), 2.35 (dtd, *J* = 10.7, 6.0, 2.2 Hz, 1H), 2.08 (t, *J* = 5.9 Hz, 1H), 2.04 (tt, *J* = 6.0, 3.0 Hz, 1H), 1.56 (d, *J* = 10.7 Hz, 1H), 1.52 (s, 3H), 1.38 (d, *J* = 9.8 Hz, 1H), 1.33 (s, 3H), 1.27 (d, *J* = 6.3 Hz, 6H), 0.88 (s, 3H); ¹³C{¹H} NMR (126 MHz, CDCl₃) δ 180.1, 169.8, 68.6, 53.0, 50.4, 38.7, 38.4, 33.8, 28.4, 28.2, 27.5, 23.0, 22.0; IR (film) ν_{max} 3427, 2980, 2918, 1735, 1654, 1469, 1371, 1190, 1106 cm⁻¹; HRMS (ESI-TOF) *m/z* [M+H]⁺ calcd for C₁₅H₂₅NO₃, 268.1913; found, 268.1918.

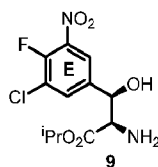


Compound 8

A solution of **7** (2.47 g, 9.24 mmol, 1 equiv) in CH₂Cl₂ (25 mL) at 0 °C was treated sequentially with TiCl(OiPr)₃ (50 wt % in CH₂Cl₂, 5.30 g, 10.2 mmol, 1.1 equiv), **3** (2.26 g in 7 mL CH₂Cl₂, 10.9 mmol, 1.2 equiv), and Et₃N (2.57 mL, 18.5 mmol, 2 equiv). The resulting white suspension was stirred at 0 °C overnight (about 16–18 h), poured into cold (0 °C) saturated aqueous NaCl (30 mL), and stirred vigorously for 10 min. The resulting suspension was filtered and the filtrate was transferred to separatory funnel. The aqueous layer was extracted with CH₂Cl₂ (3 × 30 mL). The combined organic layers were dried over MgSO₄, concentrated under reduced pressure, and the residue was purified by chromatography (SiO₂, 10–50% EtOAc–hexanes + 1% Et₃N, rapid elution) to provide **8** (2.56 g, 59%) as a moisture-sensitive yellow oil.

For **8**: ¹H NMR (400 MHz, CDCl₃) δ 8.03 (dd, *J* = 6.3, 2.2 Hz, 1H), 7.80 (dd, *J* = 6.1, 2.2 Hz, 1H), 5.23 (d, *J* = 7.1 Hz, 1H), 5.05 (p, *J* = 6.2 Hz, 1H), 4.22 (d, *J* = 7.1 Hz, 1H), 3.97 (s, 1H), 2.50–2.43 (m,

1H), 2.33–2.22 (m, 1H), 2.14 (dd, $J = 18.1, 3.0$ Hz, 1H), 2.02 (d, $J = 5.9$ Hz, 1H), 1.99 (s, 1H), 1.97 (dt, $J = 5.8, 3.1$ Hz, 1H), 1.50 (s, 3H), 1.30 (s, 3H), 1.19 (dd, $J = 6.3, 1.6$ Hz, 6H), 0.84 (s, 3H); $^{13}\text{C}\{^1\text{H}\}$ NMR (151 MHz, CDCl_3) δ 169.1, 168.4, 150.9 (d, $J = 266$ Hz), 137.9, 134.6, 134.2, 123.5 (d, $J = 17.6$ Hz), 123.3, 83.9, 76.6, 73.0, 69.8, 50.6, 38.8, 38.4, 34.6, 28.2, 28.0, 27.3, 23.0, 21.8; IR (film) ν_{max} 3388, 2982, 2924, 1726, 1655, 1609, 1543, 1480, 1372, 1347, 1271, 1208, 1104 cm^{-1} ; HRMS (ESI-TOF) m/z $[\text{M}+\text{H}]^+$ calcd for $\text{C}_{15}\text{H}_{25}\text{NO}_3$, 268.1913; found, 268.1918.



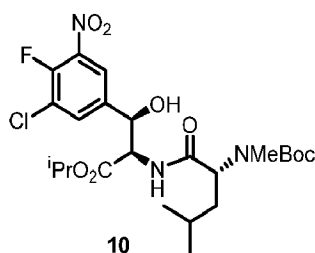
Compound 9

A solution of **8** (10.05 g, 21.34 mmol, 1 equiv) in THF (200 mL) was treated with aqueous 1 M HCl (200 mL, 200 mmol, 9 equiv) and stirred overnight (about 16–18 h) at 23 °C. The reaction mixture was concentrated under reduced pressure to remove THF, basified with the addition of concentrated aqueous NH_4OH (10 mL), and extracted with EtOAc (3 $\times$ 200 mL). The combined organic layers were dried over MgSO_4 , concentrated under reduced pressure, and the residue was purified by column chromatography (SiO_2 , 40–100% EtOAc–hexanes gradient elution) to provide **9** (5.86 g, 86%) as a yellow solid.

For **9**: ^1H NMR (600 MHz, CDCl_3) δ 7.93 (dd, $J = 6.2, 2.3$ Hz, 1H), 7.72 (dd, $J = 6.0, 2.2$ Hz, 1H), 5.06 (t, $J = 4.4$ Hz, 1H), 4.97 (p, $J = 6.3$ Hz, 1H), 3.86 (d, $J = 4.7$ Hz, 1H), 2.00 (s, 1H), 1.17 (dd, $J = 6.4, 2.4$ Hz, 6H); $^{13}\text{C}\{^1\text{H}\}$ NMR (151 MHz, CDCl_3) δ 171.8, 151.1 (d, $J = 267$ Hz), 138.0, 137.7 (d, $J = 5.4$ Hz), 133.9, 124.0 (d, $J = 17.6$ Hz), 122.5 (d, $J = 3.3$ Hz), 72.3, 70.1, 59.4, 21.9, 21.8; IR (film) ν_{max} 2985, 1732, 1613, 1542, 1485, 1348, 1269, 1102 cm^{-1} .

The workup is ideally performed immediately upon completion of the hydrolysis. Degradation of **9** is observed after prolonged exposure to the reaction conditions. Free base **9** is unstable

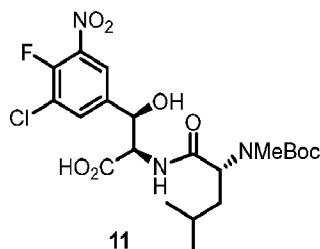
at 23 °C and should either be stored cold (≤ -20 °C), or preferably used immediately in the following step.



Compound 10

A solution of **9** (1.62 g, 5.0 mmol, 1 equiv) and BocNMe-D-Leu-OH (1.61 g, 6.5 mmol, 1.3 equiv) in THF (18 mL) was cooled to 0 °C and treated with *i*-Pr₂NEt (2.6 mL, 15 mmol, 3 equiv) followed by T3P® (50 wt % in CH₂Cl₂, 4.82 g, 7.5 mmol, 1.5 equiv). The reaction mixture was stirred for 1 h at 0 °C, quenched with the addition of H₂O (20 mL), and warmed to 23 °C. The THF was removed by concentration of the reaction mixture under reduced pressure, and the residue was extracted with CH₂Cl₂ (3 × 20 mL). The combined organic layers were dried over MgSO₄, concentrated under reduced pressure, and the residue was purified by chromatography (SiO₂, wet-load CH₂Cl₂, 5–10% EtOAc–hexanes gradient) to afford **10** (2.46 g, 89%) as a yellow foam.

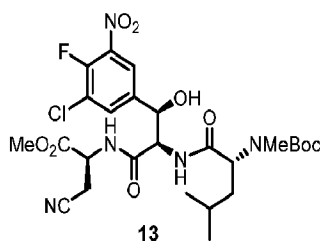
For **10**: $[\alpha]_D^{25} +26$ (c 5.9, MeOH); ¹H NMR (600 MHz, CDCl₃) δ 7.84 (dt, *J* = 6.2, 2.4 Hz, 1H), 7.67–7.63 (m, 1H), 7.13 (s, 1H), 6.86 (s, 1H), 5.06 (s, 1H), 4.79 (s, 1H), 4.59 (s, 1H), 2.79–2.58 (m, 3H), 1.68 (s, 2H), 1.52–1.44 (m, 2H), 1.41 (s, 9H), 1.31–1.14 (m, 6H), 1.00–0.80 (m, 6H); ¹³C{¹H} NMR (151 MHz, CDCl₃) δ 174.0, 167.7, 156.8, 155.2, 151.0 (d, *J* = 267 Hz), 138.0 (d, *J* = 7.6 Hz), 137.4, 133.6, 123.9 (d, *J* = 18.9 Hz), 122.1, 81.2, 73.1, 71.1, 59.6, 57.2, 36.8, 36.0, 31.0, 28.3, 25.0, 23.2, 21.8, 21.7; IR (film) $\nu_{\max}$ 3392, 2961, 1737, 1670, 1543, 1483, 1368, 1347, 1322, 1269, 1152, 1104 cm⁻¹; HRMS (ESI-TOF) *m/z* [M-H]⁻ calcd for C₂₄H₃₅ClFN₃O₈, 546.2018; found, 546.2022.



Compound 11

A solution **10** (2.67 g, 4.87 mmol, 1 equiv) in 1,2-dichloroethane (25 mL) was treated with Me_3SnOH (8.81 g, 48.7 mmol, 10 equiv) and warmed at 78 °C overnight (about 16-18 h). The reaction mixture was then cooled to 23 °C, treated with aqueous 1 M HCl (50 mL), and stirred at 23 °C until all solids dissolved. The organic layer was collected and washed with additional aqueous 1 M HCl (3 × 50 mL) and saturated aqueous NaCl (50 mL), dried over MgSO_4 , and concentrated under reduced pressure. Purification of the residue by chromatography (SiO_2 , 5% $\text{MeOH}-\text{CH}_2\text{Cl}_2$ + 2% AcOH) was followed by coevaporation with PhMe to ensure removal of residual AcOH, providing **11** (2.36 g, 96%) as a yellow oil.

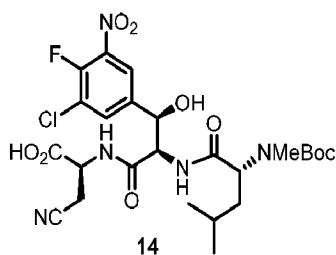
For **11**: $[\alpha]_D^{25} +28$ (c 4.32, MeOH); ^1H NMR (600 MHz, CD_3OD) δ 8.06 (dd, $J = 6.2, 2.2$ Hz, 1H), 7.88 (dd, $J = 6.1, 2.3$ Hz, 1H), 5.09 (s, 1H), 4.72–4.66 (m, 1H), 4.64 (s, 1H), 2.73 (s, 3H), 1.57 (ddd, $J = 14.1, 9.1, 5.3$ Hz, 1H), 1.52–1.46 (m, 2H), 1.45 (s, 9H), 0.99–0.88 (m, 6H); $^{13}\text{C}\{^1\text{H}\}$ NMR (151 MHz, CD_3OD) δ 173.6, 172.0, 165.4, 158.0, 157.2, 152.8, 151.0, 140.5, 139.3, 139.2, 134.9, 124.3, 124.2, 123.8, 82.1, 72.9, 59.3, 58.6, 57.4, 38.2, 30.7, 28.5, 25.9, 23.4, 21.8; IR (film) ν_{max} 3311, 2956, 1654, 1543, 1481, 1392, 1367, 1346, 1269, 1152, 1101 cm^{-1} ; HRMS (ESI-TOF) m/z $[\text{M}-\text{H}]^-$ calcd for $\text{C}_{21}\text{H}_{29}\text{ClFNO}_8$, 504.1549; found, 504.1541.



Compound 13

A solution of β -cyanoalanine methyl ester **12**,²⁷ (824 mg, 6.4 mmol, 1.5 equiv) and **11** (2.16 g, 4.3 mmol, 1 equiv) in EtOAc (20 mL) was treated with DMTMM (1.77 g, 6.4 mmol, 1.5 equiv). The reaction mixture was stirred at 23 °C overnight (about 16-18 h), quenched with the addition of aqueous 1 M HCl (20 mL) and transferred to a separatory funnel. The aqueous acid layer was discarded, and the organic layer was washed with additional aqueous 1 M HCl (3 $\times$ 20 mL). The organic layer was dried over MgSO₄, concentrated under reduced pressure, and the residue was purified by chromatography (SiO₂, 40–60% EtOAc–hexanes gradient) to provide **13** (2.18 g, 83%; typically 83–97%, 0.5–3 g scale) as a white foam.

For **13**: $[\alpha]_D^{25} +18$ (c 3.55, MeOH); ¹H NMR (500 MHz, CD₃OD) δ 8.08 (dd, J = 6.4, 2.1 Hz, 1H), 7.91 (dd, J = 6.1, 2.2 Hz, 1H), 4.96 (d, J = 7.8 Hz, 1H), 4.78 (t, J = 6.3 Hz, 1H), 4.70 (d, J = 7.7 Hz, 1H), 4.57 (br s, 1H), 3.79 (s, 3H), 3.31 (br s, 1H), 3.08–2.95 (m, 2H), 2.63 (s, 3H), 1.60 (ddd, J = 14.9, 10.6, 4.5 Hz, 1H), 1.55–1.46 (m, 2H), 1.44 (s, 9H), 0.91 (overlapping doublets, 6H); ¹³C{¹H} NMR (151 MHz, CD₃OD) δ 173.3, 171.4, 170.6, 153.0, 151.3, 140.5, 139.3, 135.4, 124.4, 124.4, 124.3, 117.9, 73.1, 73.1, 58.9, 57.4, 53.5, 50.4, 50.3, 38.3, 30.6, 28.6, 26.0, 23.5, 21.8, 20.8; IR (film) $\nu_{\max}$ 3630, 3025, 2040, 1739, 1372, 1215 cm⁻¹; HRMS (ESI-TOF) m/z [M-H]⁻ calcd for C₂₆H₃₄ClFN₅O₉, 614.2029; found, 614.2030.

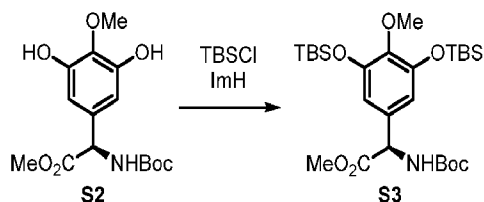


Compound 14

A suspension of **13** (3.90 g, 6.33 mmol, 1 equiv) and Me₃SnOH (5.76 g, 31.7 mmol, 5 equiv) in 1,2-dichloroethane (32 mL) was stirred at 70 °C for 1.5 h. The reaction mixture was cooled to 23 °C, quenched with the addition of aqueous 1 M HCl (30 mL), stirred

vigorously for 10 min, and the aqueous layer was discarded. The organic layer was washed with additional aqueous 1 M HCl (3 × 30 mL), dried over MgSO₄, and the residue was purified by chromatography (SiO₂, wet-load 1–2% MeOH–CH₂Cl₂, eluted with 2% MeOH–CH₂Cl₂, then 90:6:4 CH₂Cl₂/MeOH/AcOH) to provide **14** (3.53 g, 93%) as an off-white solid.

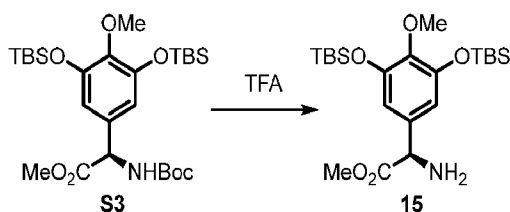
For **14**: $[\alpha]_D^{25} +27$ (c 3.8, MeOH); ¹H NMR (600 MHz, CD₃OD) δ 8.08 (d, *J* = 6.1 Hz, 1H), 7.91 (d, *J* = 5.7 Hz, 1H), 4.97 (d, *J* = 7.6 Hz, 1H), 4.74 (q, *J* = 6.7, 5.4 Hz, 2H), 4.58 (br s, 1H), 3.31 (s, 1H), 3.02 (qd, *J* = 17.1, 6.1 Hz, 2H), 2.63 (s, 3H), 1.64–1.56 (m, 1H), 1.54–1.48 (m, 2H), 1.44 (s, 9H), 0.94–0.88 (m, 6H); ¹³C{¹H} NMR (151 MHz, CD₃OD) δ 173.4, 171.6, 151.9 (d, *J* = 264 Hz), 140.4, 139.2 (d, *J* = 7.4 Hz), 135.5, 124.2 (d, *J* = 17.1 Hz), 118.1, 73.1, 73.0, 58.8, 50.4, 38.2, 28.6, 25.9, 23.5, 21.8, 21.1; IR (film) $\nu_{\max}$ 3274, 2961, 1731, 1653, 1543, 1482, 1425, 1393, 1347, 1269, 1221, 1153, 1069 cm⁻¹; HRMS (ESI-TOF) *m/z* [M+H]⁺ calcd for C₂₅H₃₃ClFN₅O₉, 602.2029; found, 602.2023.



Compound **S3**

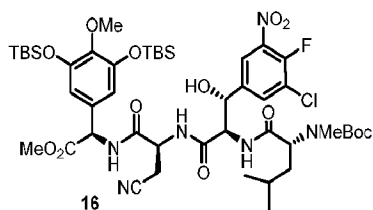
A solution of **S2**⁶ (1.03 g, 2.77 mmol, 1 equiv) and imidazole (0.565 g, 8.30 mmol, 3 equiv) in CH₂Cl₂ (10 mL) was cooled to 0 °C and treated with TBSCl (1.25 g, 8.30 mmol, 3 equiv). The reaction mixture was stirred for 1 h at 0 °C, quenched with the dropwise addition of aqueous 1 M HCl (10 mL), and warmed to 23 °C. The organic layer was collected, and the aqueous layer was extracted with additional CH₂Cl₂ (3 × 10 mL). The combined organic layers were dried over MgSO₄, concentrated under reduced pressure, and the residue was purified by chromatography (wet-load 20% CH₂Cl₂–hexanes, eluted with 5% EtOAc–hexanes) to provide **S3** (1.51 g, 98%) as a white foam.

For **S3**: $[\alpha]_D^{25} -56$ (c 6.8, MeOH); ^1H NMR (600 MHz, CDCl_3) δ 6.47 (s, 2H), 5.40 (d, $J = 7.8$ Hz, 1H), 5.14 (d, $J = 7.7$ Hz, 1H), 3.69 (s, 6H), 1.42 (s, 6H), 0.98 (s, 18H), 0.15 (s, 12H); $^{13}\text{C}\{^1\text{H}\}$ NMR (151 MHz, CDCl_3) δ 171.6, 154.8, 150.0, 143.0, 131.7, 113.4, 80.1, 60.0, 57.0, 52.6, 28.4, 25.8, 18.4, -4.5 ; IR (film) ν_{max} 2955, 2931, 2889, 2858, 1718, 1580, 1491, 1433, 1342, 1253, 1223, 1162, 1089, 1055, 1009 cm^{-1} ; HRMS (ESI-TOF) m/z $[\text{M}+\text{Na}]^+$ calcd for $\text{C}_{27}\text{H}_{49}\text{NO}_7\text{Si}_2$, 578.2945; found, 578.2946.



Compound 15

A stirred solution of **S3** (1.15 g, 2.0 mmol, 1 equiv) in CH_2Cl_2 (4 mL) at 23 °C was treated dropwise with TFA (1.2 mL). The reaction mixture was stirred at 23 °C for 1 h, diluted with PhMe (20 mL), and concentrated under a stream of N_2 . The residue was dissolved in Et_2O (20 mL) and washed with saturated aqueous NaHCO_3 (3×10 mL). The organic layer was dried over MgSO_4 and concentrated to provide **15** (assumed quantitative), which was used immediately in the following step.

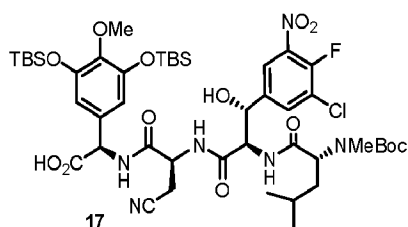


Compound 16

A solution of carboxylic acid **14** (103 mg, 0.173 mmol, 1 equiv) and amine **15** (102 mg, 0.224 mmol, 1.3 equiv) in THF (1 mL) was treated with DMTMM (71.7 mg, 0.259 mmol, 1.5 equiv) and stirred overnight (about 16-18 h) at 23 °C. The reaction mixture was diluted with H_2O (1 mL) and concentrated under reduced pressure to remove

THF. The residue was treated with saturated aqueous NH_4Cl (1 mL) and extracted with EtOAc (3×3 mL). The combined organic layers were dried over MgSO_4 , concentrated under reduced pressure, and purified by chromatography (SiO_2 , dry-load SiO_2 , washed with 6% acetone– CH_2Cl_2 , eluted with 10% acetone– CH_2Cl_2) to afford **16** (159 mg, 89%; typically 63–89%, 100–800 mg scale) as a tan solid.

For **16**: $[\alpha]_D^{25} -24$ (c 1.63, 70% *i*-PrOH– CHCl_3); ^1H NMR (600 MHz, CD_3OD) δ 8.09 (d, $J = 6.0$ Hz, 1H), 7.92 (d, $J = 5.8$ Hz, 1H), 6.61 (s, 2H), 5.30 (s, 1H), 4.96 (d, $J = 8.0$ Hz, 1H), 4.80 (t, $J = 6.5$ Hz, 1H), 4.69 (s, 1H), 4.56 (s, 1H), 3.70 (s, 3H), 3.05–2.88 (m, 2H), 2.61 (s, 3H), 1.56 (s, 1H), 1.43 (s, 11H), 1.01 (s, 18H), 0.93–0.86 (m, 6H), 0.18 (s, 12H); $^{13}\text{C}\{^1\text{H}\}$ NMR (151 MHz, CD_3OD) δ 177.7, 173.8, 173.4, 171.7, 169.6, 157.9, 157.2, 153.0, 152.2 (d, $J = 264$ Hz), 144.1, 140.6, 139.3, 135.5, 133.3, 125.9, 125.2, 124.4, 118.0, 115.3, 81.7, 73.1, 60.6, 59.3, 58.1, 57.3, 50.9, 38.2, 35.9, 35.5, 35.0, 33.1, 31.8, 30.8, 30.7, 30.6, 30.5, 30.4, 30.2, 28.6, 26.2, 25.9, 23.6, 21.9, 21.1, 19.2, 14.5, –4.37, –4.38; IR (film) ν_{max} 3312, 2928, 2357, 2219, 1748, 1644, 1580, 1539, 1492, 1433, 1391, 1345, 1255, 1227, 1154, 1092, 1010 cm^{-1} ; HRMS (ESI-TOF) m/z $[\text{M}-\text{H}]^-$ calcd for $\text{C}_{47}\text{H}_{72}\text{ClFN}_6\text{O}_{13}\text{Si}_2$, 1037.4290; found, 1037.4298.

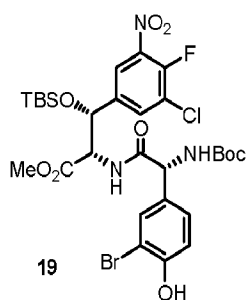


Compound 17

A suspension of **16** (747 mg, 0.718 mmol, 1 equiv) and Me_3SnOH (653 mg, 3.59 mmol, 5 equiv) in 1,2-dichloroethane (15 mL) was stirred at 70 °C overnight (about 16–18 h), cooled to 23 °C, and quenched with the addition of aqueous 1 M HCl (20 mL). The aqueous layer was discarded, and the organic layer was washed with additional aqueous 1 M HCl (3×20 mL), dried over MgSO_4 , and concentrated

under reduced pressure. Chromatography (SiO₂, 45% EtOAc–hexanes + 2% AcOH) provided **17** (593 mg, 81%) as a light tan solid.

For **17**: $[\alpha]_D^{25}$ –25 (c 0.46, MeOH); ¹H NMR (400 MHz, CDCl₃) δ 7.94–7.88 (m, 1H), 7.72 (m, 2H), 6.61 (m, 2H), 5.40 (m, 1H), 5.12 (m, 1H), 4.68 (m, 1H), 4.65–4.58 (m, 1H), 3.71 (s, 3H), 2.81 (m, 2H), 2.29 (br s, 2H), 1.58–1.43 (m, 4H), 1.38 (s, 6H), 0.96 (s, 18H), 0.92–0.81 (m, 6H), 0.15 (m, 12H); ¹³C{¹H} NMR (151 MHz, CD₃OD) δ 173.3, 171.5, 171.3, 152.9, 151.2, 140.4, 139.2, 135.4, 124.3, 124.2, 118.1, 73.12, 73.10, 58.8, 50.3, 38.2, 28.6, 28.5, 25.9, 23.5, 21.8, 21.1; IR (film) $\nu_{\max}$ 2955, 2932, 2857, 2343, 1642, 1579, 1542, 1493, 1436, 1392, 1346, 1254, 1224, 1155, 1091, 1008 cm^{–1}; HRMS (ESI-TOF) *m/z* [M–H][–] calcd for C₄₆H₇₀ClFN₆O₁₃Si₂, 1023.4134; found, 1023.4139.

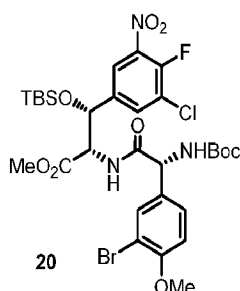


Compound 19

A stirred solution of amine **6** (11.28 g, 27.7 mmol, 1 equiv) and carboxylic acid **18**³⁰ (12.5 g, 36.1 mmol, 1.3 equiv) in MeCN (70 mL) was cooled to 0 °C and treated with DMTMM (9.98 g, 36.1 mmol, 1.3 equiv). The reaction mixture was stirred at 0 °C for 1 h, diluted with H₂O (200 mL), and extracted with PhMe (3 × 300 mL). The combined organic layers were dried over Na₂SO₄ and concentrated under reduced pressure. Chromatography (SiO₂, 20–40% EtOAc–hexane gradient) provided **19** (17.1 g, 84%) as a yellow solid.

For **19**: ¹H NMR (500 MHz, CDCl₃) δ 7.70 (d, *J* = 5.0 Hz, 1H), 7.41 (d, *J* = 5.0 Hz, 1H), 7.36 (d, *J* = 2.2 Hz, 1H), 6.96 (dd, *J* = 2.2, 8.4 Hz, 1H), 6.77 (d, *J* = 8.5 Hz, 1H), 6.58 (bs, 1H), 5.58 (d, *J* = 6.0 Hz, 1H), 5.29 (s, 1H), 4.98 (bs, 1H), 4.78 (dd, *J* = 1.5, 9.5 Hz, 1H), 3.82 (s, 3H), 1.39 (s, 9H), 0.84 (s, 9H), –0.02 (s, 3H), –0.19 (s, 3H); ¹³C{¹H} NMR (126 MHz, CDCl₃) δ 170.0, 169.4, 155.2, 153.2, 151.1 (d, *J* = 268.6 Hz),

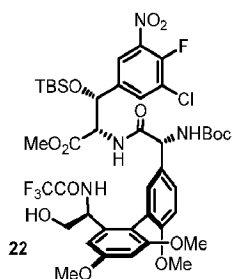
137.9 (d, $J = 7.6$ Hz), 137.5 (d, $J = 3.8$ Hz), 132.8, 131.1, 127.6, 124.1 (d, $J = 17.6$ Hz), 121.7, 116.9, 110.9, 80.7, 72.6, 58.4, 57.9, 53.2, 28.4, 25.5, 17.9, -4.7 , -5.5 ; IR (film) $\nu_{\max}$ 3414, 2954, 2931, 1678, 1543, 1493, 1346, 1257, 1161, 1103, 837, 733 cm^{-1} ; HRMS (ESI-TOF) m/z $[M+H]^+$ calcd for $\text{C}_{29}\text{H}_{38}\text{BrClFN}_3\text{O}_9\text{Si}$, 734.1312; found, 734.1321.



Compound 20

A solution of **19** (30 g, 40.8 mmol, 1 equiv) in MeOH (16 mL) and CH_2Cl_2 (65 mL) was cooled to 0 °C. The mixture was treated with TMSCHN_2 (2 M in hexane, 81.6 mL, 163 mmol, 4 equiv) and stirred at 5 °C for 36 h (TLC indicated complete reaction). The reaction mixture was cooled to 0 °C and quenched by dropwise addition of AcOH (until no further gas evolution occurred) and concentrated under reduced pressure. Column chromatography (SiO_2 , loaded with CH_2Cl_2 , 20–35% EtOAc–hexanes gradient elution) gave **20** (29.96 g, 98%) as a yellow solid.

For **20**: $[\alpha]_D^{25} -41$ (c 0.67, MeOH); ^1H NMR (600 MHz, CDCl_3) δ 7.64 (s, 1H), 7.47 (d, $J = 2.3$ Hz, 1H), 7.42 (d, $J = 4.7$ Hz, 1H), 7.07 (d, $J = 8.4$ Hz, 1H), 6.85 (d, $J = 8.5$ Hz, 1H), 6.41 (s, 1H), 5.54 (d, $J = 5.7$ Hz, 1H), 5.28 (s, 1H), 4.99 (s, 1H), 4.77 (dd, $J = 9.4, 1.8$ Hz, 1H), 3.92 (s, 3H), 3.82 (s, 3H), 1.39 (s, 9H), 0.85 (s, 9H), -0.02 (s, 3H), -0.18 (s, 3H); $^{13}\text{C}\{^1\text{H}\}$ NMR (151 MHz, CD_3OD) δ 171.3, 169.3, 155.8, 155.6, 150.9 (d, $J = 223$ Hz), 138.0, 137.3, 133.2, 131.9, 131.4, 127.2, 123.9 (d, $J = 13.9$ Hz), 122.1, 111.3 (d, $J = 22.2$ Hz), 79.5, 72.5, 57.9, 56.6, 55.1, 51.9, 27.3, 24.8, 17.6, -5.9 , -6.6 ; IR (film) $\nu_{\max}$ 2931, 1716, 1542, 1346, 1257, 1203, 1157, 837 cm^{-1} ; HRMS (ESI-TOF) m/z $[M+\text{Na}]^+$ calcd for $\text{C}_{30}\text{H}_{40}\text{BrClFN}_3\text{O}_9\text{Si}$, 770.1287; found, 770.1260.

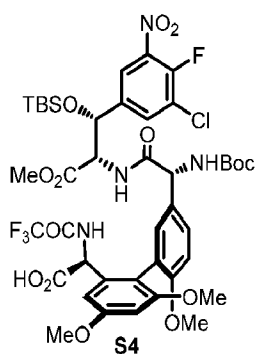


Compound 22

The following reaction has been performed on scales between 100 mg – 10 g (53–72%), and a representative procedure follows. A solution of **20** (10 g, 13.3 mmol, 1 equiv), Pd(OAc)₂ (900 mg, 4.01 mmol, 0.3 equiv), and (*R*)-BINAP (2.75 g, 4.42 mmol, 0.33 equiv) in MeTHF (134 mL) was stirred for 15 min at room temperature while sparged with Ar and then treated with solid NaHCO₃ (22.4 g, 267 mmol, 20 equiv) and H₂O (27 mL). The reaction mixture was immediately warmed to 70 °C and stirred for 45 min. A solution of boronate **21**⁶ (0.20 M in MeTHF, 200 mL, 40 mmol, 3 equiv) was added dropwise to the reaction mixture over 4 h by an addition funnel, and the resulting solution was stirred for an additional 1 h at 70 °C. The reaction mixture was then cooled to 23 °C, H₂O added (200 mL) to dissolve solid NaHCO₃, transferred to separatory funnel, and the aqueous layer was discarded. The organic layer was washed with H₂O (200 mL), dried over Na₂SO₄, and concentrated under reduced pressure. Column chromatography (SiO₂, wet-load CH₂Cl₂, 15–35% EtOAc–hexanes to recover A ring (ca. 50%) and 45–55% EtOAc–hexanes to collect product) provided **22** (9.36 g, 72%) as a light-yellow solid.

For **22**: [α]_D²⁵ +4.7 (c 0.9, MeOH); ¹H NMR (600 MHz, CDCl₃) δ 7.91 (dd, *J* = 6.3, 2.3 Hz, 1H), 7.66 (dd, *J* = 5.7, 2.2 Hz, 1H), 7.41 (m, 1H), 7.12 (m, 1H), 7.00–6.87 (m, 1H), 6.80 (d, *J* = 20.3 Hz, 1H), 6.58–6.53 (m, 1H), 6.51 (d, *J* = 2.3 Hz, 1H), 5.75 (s, 1H), 5.05 (s, 1H), 4.93 (s, 1H), 4.88–4.80 (m, 1H), 3.87 (s, 3H), 3.77 (s, 3H), 3.74 (s, 3H), 3.66 (s, 3H), 2.62 (s, 4H), 1.41 (d, *J* = 17.9 Hz, 9H), 0.87 (s, 9H), 0.03 (d, *J* = 2.4 Hz, 3H), –0.08 to –0.17 (m, 3H); ¹³C{¹H} NMR (151 MHz, CDCl₃) δ 171.2, 169.4, 160.4, 158.6, 157.7, 156.5 (q, *J* = 36.2 Hz), 155.3, 150.8 (d, *J* = 266 Hz), 138.1, 137.8, 133.3, 132.3, 129.1, 127.7, 125.1, 123.7

(d, $J = 16.7$ Hz), 122.4, 119.7, 115.8 (q, $J = 288$ Hz), 110.5, 103.3, 97.9, 79.8, 72.8, 64.0, 58.2, 55.7, 55.4, 55.3, 53.0, 52.5, 28.1, 25.4, 17.8, – 4.7, –5.5; IR (film) $\nu_{\max}$ 2931, 1713, 1543, 1504, 1203, 1161, 1092, 891 cm^{-1} ; HRMS (ESI-TOF) m/z $[M+Na]^+$ calcd for $\text{C}_{42}\text{H}_{53}\text{ClF}_4\text{N}_4\text{O}_{13}\text{Si}$, 961.3081; found, 961.3073.

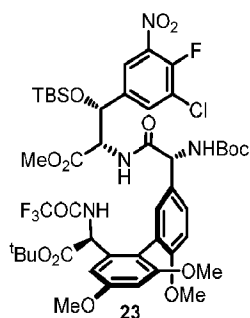


Compound S4

A stirred solution of **22** (2.00 g, 2.08 mmol, 1 equiv) in MeCN (30 mL) was diluted with H_2O (15 mL) and treated with TEMPO (195 mg, 1.25 mmol, 0.6 equiv), followed by $\text{PhI}(\text{OAc})_2$ (1.67 g, 5.20 mmol, 2.5 equiv). The reaction mixture was stirred at 23 °C for 1 h, then chromatographed directly (60 g C18, wet-load minimal MeCN– H_2O , 20–100% MeCN– H_2O + 0.1% formic acid over 300 mL) to afford semi-pure **S4** an orange solid, which was carried forward without further purification. An aliquot of this material was purified by HPLC (Phenomenex Luna® C18(2), 100 × 30 mm, 20 mL/min, 60–100% MeCN– H_2O + 0.07% TFA over 20 min, $t_R = 14.0$ min) to provide analytically pure **S4** (65 mg) as a white solid.

For **S4**: $[\alpha]_D^{25} +39$ (c 4.63, MeOH); ^1H NMR (600 MHz, CDCl_3) δ 7.88 (d, $J = 5.7$ Hz, 1H), 7.60 (s, 1H), 7.53 (s, 1H), 7.17 (d, $J = 8.5$ Hz, 1H), 7.04–6.96 (m, 2H), 6.62 (s, 1H), 6.56 (d, $J = 2.3$ Hz, 1H), 5.55 (s, 1H), 5.33 (d, $J = 8.6$ Hz, 2H), 4.98 (s, 1H), 4.89 (d, $J = 9.2$ Hz, 1H), 3.88 (s, 3H), 3.81 (s, 3H), 3.80 (s, 3H), 3.65 (s, 3H), 1.45 (s, 9H), 0.88 (s, 9H), 0.02 (s, 3H), –0.12 (s, 3H); $^{13}\text{C}\{^1\text{H}\}$ NMR (151 MHz, CD_3OD) δ 169.3, 160.6, 158.7, 158.1, 149.7, 136.2, 120.5, 98.6, 79.1, 72.7, 58.2, 54.9, 54.5, 53.8, 51.7, 27.3, 24.8, 17.5, –6.0, –6.6; IR (film) $\nu_{\max}$ 3363, 2934, 1713, 1608, 1543, 1488, 1346, 1259, 1160, 1100 837,

779 cm⁻¹; HRMS (ESI-TOF) m/z [M+Na]⁺ calcd for C₄₂H₅₁ClF₄N₄O₁₄Si, 997.2693; found, 997.2717.

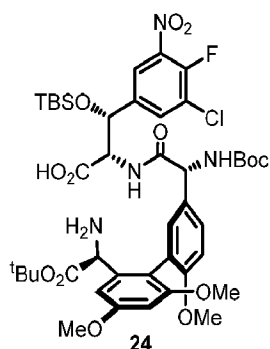


Compound 23

The semi-pure carboxylic acid **S4** from the previous step (2.0 mmol) was coevaporated with PhMe (2 × 50 mL), dissolved in CH₂Cl₂ (2 mL), and diluted with cyclohexane (6 mL). A solution of *t*-butyl trichloroacetimidate (3.6 mL, 20 mmol, 10 equiv) in cyclohexane (3.6 mL) was added to the reaction mixture over 7 h by syringe pump. The reaction mixture was stirred at 23 °C for an additional 9 h, filtered, and the filter cake was washed with minimal 30% CH₂Cl₂–hexanes (3 × 5 mL). The combined filtrate was concentrated under reduced pressure and purified by column chromatography (50 g SiO₂, wet-load CH₂Cl₂, washed with 100% CH₂Cl₂ (1.5 L) to remove trichloroacetamide, then eluted with 0–10% acetone–CH₂Cl₂ over 500 mL) to provide **23** (1.87 g, 90%) as a tan solid.

For **23**: ¹H NMR (600 MHz, CD₃OD, 2:1 rotamers, major given) δ 8.06–7.93 (m, 1H), 7.89–7.78 (m, 1H), 6.86 (s, 1H), 6.81–6.70 (m, 2H), 6.66 (s, 1H), 6.58 (s, 1H), 5.55 (s, 1H), 5.36 (s, 1H), 5.01 (s, 1H), 4.93 (d, *J* = 6.5 Hz, 1H), 3.84 (s, 3H), 3.77 (d, *J* = 11.8 Hz, 3H), 3.70 (s, 3H), 3.63 (d, *J* = 11.9 Hz, 3H), 1.54–1.21 (m, 18H), 0.84 (s, 9H), 0.03 (s, 3H), –0.16 (s, 3H); ¹³C{¹H} NMR (151 MHz, CD₃OD, rotamers, major given) δ 173.2, 170.7, 170.4, 162.0, 160.2, 159.6, 157.8, 157.1, 156.6, 152.0 (d, *J* = 217 Hz), 139.7, 137.6, 136.2, 135.2, 133.9, 130.0, 129.6, 128.7, 126.8, 126.4, 125.82, 124.4, 123.6, 122.1, 115.68 (q, *J* = 238 Hz), 111.75, 104.9, 100.0, 97.8, 59.6, 59.3, 56.5, 56.3, 56.0, 55.6, 51.1, 28.5, 28.1, 26.2, 18.9, –4.6, –5.4; IR (film) $\nu_{\max}$ 2955, 2838, 1747,

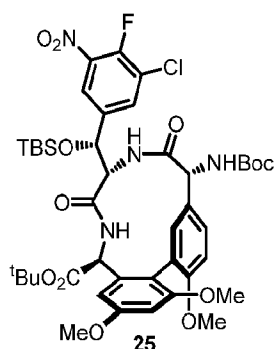
1686, 1543, 1497, 1364, 1261, 1164, 1103, 837 cm^{-1} ; HRMS (ESI-TOF) m/z $[M+H]^+$ calcd for $\text{C}_{46}\text{H}_{59}\text{ClF}_4\text{N}_4\text{O}_{14}\text{Si}$, 1053.3319; found, 1053.3284.



Compound 24

A slightly warm (25 °C) solution of **23** (450 mg, 0.44 mmol, 1 equiv) in *t*-BuOH (18 mL) was treated with saturated aqueous $\text{Ba}(\text{OH})_2$ (ca. 0.25 M, 9 mL, 2.25 mmol, 5 equiv). The reaction mixture was stirred at 23 °C for 6 h, at which point TLC (5:10:85 AcOH/MeOH/EtOAc) indicated complete consumption of **23** with <5% of the monodeprotected intermediate remaining. The reaction mixture was sparged with CO_2 (g) for 30 min and filtered through a 0.22 μm PES membrane, rinsing with MeOH (20 mL). The filtrate was concentrated under reduced pressure and lyophilized to remove residual H_2O , providing 533 mg of crude **24**, carried forward without further purification.

For **24**: HRMS (ESI-TOF) m/z $[M+H]^+$ calcd for $\text{C}_{43}\text{H}_{58}\text{ClFN}_4\text{O}_{13}\text{Si}$, 921.3520; found, 921.3503.



Compound 25

A portion of crude **24** from the above reaction (355 mg, 0.29 mmol, 1 equiv) was dissolved in NMP (1.45 mL, [substrate] = 0.2

M) and added over 30 min by syringe pump to a stirred suspension of Phenomenex Luna® (168 mg, 0.44 mmol, 1.5 equiv) and *i*-Pr₂NEt (0.15 mL, 0.87 mmol, 3 equiv) in NMP (1.45 mL, final [S] = 0.1 M). The vial and syringe used to transfer **24** were rinsed with additional NMP (2 × 0.5 mL) and added to the reaction mixture. The reaction mixture was stirred at 23 °C for an additional 15 min and purified directly by preparative HPLC (Phenomenex Luna® C18(2), 100 × 30 mm, 60–100% MeCN–H₂O + 0.07% TFA over 20 min, 20 mL/min, *t_R* = 14–19 min) to afford **25** (230 mg) as a light green solid containing minor impurities. Trituration of this sample of **25** with Et₂O (4 mL) afforded analytically pure **25** (217 mg, 83%/2 steps) as a light tan solid.

For **25**: $[\alpha]_D^{25} +16$ (c 0.57, MeOH); ¹H NMR (600 MHz, CD₃CN, 3.7:1 rotamers, major given) δ 8.12–8.08 (m, 1H), 7.93–7.91 (m, 1H), 7.07 (dd, *J* = 8.7, 2.3 Hz, 1H), 7.01 (d, *J* = 8.7 Hz, 1H), 6.97 (br s, 1H), 6.66 (br s, 1H), 6.41 (d, *J* = 2.3 Hz, 1H), 5.12 (br s, 1H), 4.81 (d, *J* = 6.2 Hz, 1H), 4.68 (s, 1H), 4.14 (d, *J* = 11.0 Hz, 1H), 3.82 (s, 3H), 3.71 (s, 3H), 3.70 (s, 3H), 1.45 (s, 9H), 1.29 (s, 9H), 0.81 (s, 9H), –0.02 (s, 3H), –0.17 (s, 3H); ¹³C{¹H} NMR (151 MHz, CD₃CN) δ 172.1, 171.9, 170.8, 170.1, 167.8, 161.3, 159.6, 158.0, 155.3, 152.0 (d, *J* = 264 Hz), 139.3, 138.92, 138.87, 136.2, 135.0, 132.0, 129.4, 128.2, 123.8, 122.3, 114.2, 112.3, 104.8, 99.7, 82.9, 79.8, 73.5, 61.7, 56.5, 56.4, 56.2, 56.1, 55.9, 28.5, 28.1, 26.0, 18.6, –4.3, –5.2; IR (film) $\nu_{\max}$ 2932, 1696, 1660, 1609, 1508, 1485, 1367, 1254, 1160 cm^{–1}; HRMS (ESI-TOF) *m/z* [M+H]⁺ calcd for C₄₃H₅₆ClFN₄O₁₂Si, 903.3415; found, 903.3391.

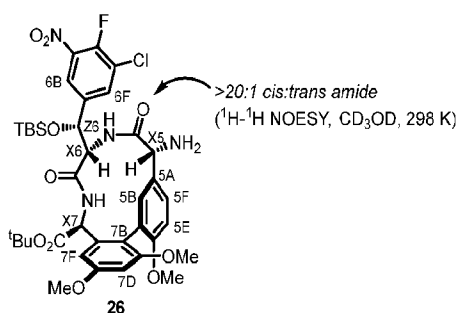
The structure, relative and absolute stereochemistry, and 5,6-cis amide conformation of **25** were confirmed with a single-crystal X-ray structure determination conducted on crystals grown from MeOH. The structure of **25** has been deposited with the Cambridge Crystallographic Data Center (CCDC 2150607).

Crystal data and structure refinement for **25**.

Report date 2022-02-04

Identification code	boger128		
Empirical formula	C43 H56 Cl F N4 O12 Si		
Molecular formula	C43 H56 Cl F N4 O12 Si		
Formula weight	903.45		
Temperature	100.15 K		
Wavelength	1.54178 Å		
Crystal system	Monoclinic		
Space group	C 1 2 1		
Unit cell dimensions:			
	a=27.4790(11) Å	a=90°	
	b=13.7793(5) Å	b=99.636(2)°	
	c=14.8814(6) Å	g=90°	
Volume	5555.2(4) Å ³		
Z	4		
Density (calculated)	1.080 Mg/m ³		
Absorption coefficient	1.293 mm ⁻¹		
F(000)	1912		
Crystal size	0.2 x 0.2 x 0.14 mm ³		
Crystal color, habit	colorless plank		
Theta range for data collection	3.012 to 68.732°.		
Index ranges	-33<=h<=32, -16<=k<=16, -17<=l<=17		
Reflections collected	46418		
Independent reflections	10131 [R(int) = 0.0482]		
Completeness to theta=67.679°	99.7 %		
Absorption correction	Semi-empirical from equivalents		
Max. and min. transmission	0.7531 and 0.6174		
Refinement method	Full-matrix least-squares on F ²		
Data / restraints / parameters	10131 / 310 / 697		
Goodness-of-fit on F ²	1.042		
Final R indices [I>2sigma(I)]	R1=0.0583, wR2=0.1643		
R indices (all data)	R1=0.0633, wR2=0.1727		
Absolute structure parameter	0.034(8)		
Largest diff. peak and hole	0.418 and -0.304 e.Å ⁻³ .		

On 3.0 gram-scale, the macrolactamization reaction was quenched with the addition of water (100 mL) and diluted with EtOAc (300 mL). The mixture was transferred to separatory funnel, and the organic layer was washed with H₂O (3 x 200 mL), saturated aqueous NaCl (200 mL), dried over Na₂SO₄, and concentrated under reduced pressure. Column chromatography (SiO₂, wet load CH₂Cl₂, 0–10% EtOAc–CH₂Cl₂) provided **25** as a white powder (1.91 g, 72%/2 steps).



Compound 26

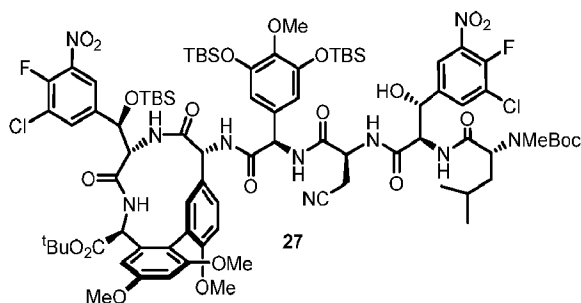
A stirred suspension of **25** (450 mg, 0.50 mmol, 1 equiv) in *t*-BuOAc (9 mL, 20 vol) was treated with H₂SO₄ (214 μ L, 4 mmol, 8 equiv) and stirred at 23 °C for 2 h. The reaction mixture was carefully quenched with the addition of Et₃N (1.4 mL, 10 mmol, 20 equiv) and concentrated under a stream of N₂. Preparative HPLC (Phenomenex Luna® C18(2) 100 × 30 mm, wet-load MeOH–H₂O, 30–90% MeCN–H₂O + 0.07% TFA, *t*_R = 14.2 min) provided **26** (TFA salt, 378 mg, 82%) as a white solid.

For **26** (TFA salt): ¹H NMR (600 MHz, CD₃OD) δ 8.82 (d, *J* = 8.3 Hz, 1H, W7), 8.26 (dd, *J* = 6.2, 2.2 Hz, 1H, 6B), 8.06 (dd, *J* = 6.0, 2.2 Hz, 1H, 6F), 7.39 (dd, *J* = 8.8, 2.5 Hz, 1H, 5F), 7.17 (d, *J* = 8.8 Hz, 1H, 5D), 7.13 (d, *J* = 2.5 Hz, 1H, 5B), 6.71 (d, *J* = 2.3 Hz, 1H, 7D), 6.52 (d, *J* = 2.3 Hz, 1H, 7F), 5.31 (d, *J* = 1.8 Hz, 1H, Z6), 5.05 (s, 1H, X5), 4.84 (d, *J* = 8.3 Hz, 1H, X7), 4.34 (d, *J* = 1.8 Hz, 1H, X6), 3.88 (s, 3H, 7E-OCH₃), 3.83 (s, 3H, 5D-OCH₃), 3.70 (s, 3H, 7C-OCH₃), 1.53 (s, 9H, C7-OC(CH₃)₃), 0.89 (s, 9H, Z6-OSi(CH₃)₃), 0.04 (s, 3H, Z6-OSiCH₃), –0.06 (s, 3H, Z6-OSiCH₃); ¹³C{¹H} NMR (151 MHz, CD₃OD) δ 170.0, 169.2, 167.4, 160.6, 158.6, 158.5, 151.7, 149.9, 138.37, 138.34, 137.99,

137.93, 136.4, 135.89, 135.84, 133.6, 127.1, 124.1, 123.3, 123.1, 122.6, 121.4, 120.6, 113.2, 104.3, 98.1, 81.9, 72.6, 60.7, 55.4, 55.3, 54.97, 54.93, 54.6, 54.2, 26.9, 24.8, 17.5, -6.0, -6.2; IR (film) $\nu_{\max}$ 3400, 2935, 1672, 1608, 1543, 1464, 1349, 1201, 1151, 836 cm^{-1} ; HRMS (ESI-TOF) m/z $[M+H]^+$ calcd for $\text{C}_{38}\text{H}_{48}\text{ClFN}_4\text{O}_{10}\text{Si}$, 803.2891; found, 803.2879.

The ^1H - ^1H NOESY spectrum of **26** (600 MHz, CD_3OD) displayed the following diagnostic nOe cross-peaks: 8.26/5.31 (6B/Z6), 8.26/4.34 (6B/X6), 8.06/5.31 (6F/Z6), 8.06/4.34 (6F/X6), 7.14/5.05 (5B/X5), 7.14/4.34 (5B/X6), 5.05/4.34 (X5/X6). The latter correlation is indicative of a 5,6-cis amide conformation.³⁵

Compound **26** was most conveniently stored as its stable TFA salt and free based immediately prior to the coupling with **17**. Minor decomposition of **26** as its free base was observed upon prolonged storage. The quantitative conversion of **26** (TFA salt) to its free base form is detailed in the preparation of **27** below.



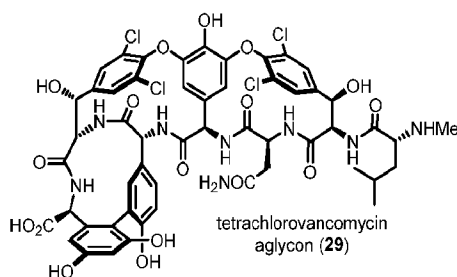
Compound 27

A solution of **26** (TFA salt, 85 mg, 93 μmol , 1 equiv) in CH_2Cl_2 (2 mL) was treated with saturated aqueous NaHCO_3 (2 mL) and stirred at 23 $^\circ\text{C}$ for 10 min. The organic layer was removed, and the aqueous layer was extracted with additional CH_2Cl_2 (3×2 mL). The combined organic layers were dried over Na_2SO_4 and concentrated to provide a light-yellow solid (free base, 75 mg, quant), used directly in the coupling reaction.

Compound **26** was combined with **17** (115 mg, 112 μmol , 1.2 equiv) and solid NaHCO_3 (34 mg, 415 μmol , 4.5 equiv) in THF (750 μL) and the resulting suspension was treated with DEPBT (60 mg, 186 μmol , 2 equiv). The reaction mixture was stirred at 23 $^\circ\text{C}$ for 17 h,

concentrated under a stream of N₂, and the residue was purified by chromatography (10 g SiO₂, wet-load 50% CH₂Cl₂–hexanes, 30–60% EtOAc–hexanes over 100 mL) to provide **27** (158 mg, 93%) as a light tan solid.

For **27**: $[\alpha]_D^{22}$ –14 (c 1.0, CH₂Cl₂); ¹H NMR (600 MHz, CD₃OD, rotameric, integration relative to major conformer) δ 8.24 (d, *J* = 6.3 Hz, 1H), 8.15 (br s, 1H), 8.11 (br s, 1H), 8.03 (br s, 1H), 8.01 (d, *J* = 6.0 Hz, 1H), 7.95 (br s, 1H), 7.93 (dd, *J* = 6.1, 2.2 Hz, 1H), 7.87 (d, *J* = 6.2 Hz, 1H), 7.51 (d, *J* = 8.6 Hz, 1H), 7.30 (d, *J* = 8.0 Hz, 1H), 7.08–6.99 (m, 3H), 6.79 (s, 1H), 6.70 (d, *J* = 2.3 Hz, 1H), 6.61 (d, *J* = 2.3 Hz, 1H), 6.60–6.55 (m, 2H), 6.49 (d, *J* = 2.3 Hz, 1H), 6.34 (br s, 1H), 5.48 (d, *J* = 2.1 Hz, 1H), 5.22 (s, 1H), 5.15 (s, 1H), 5.07–4.92 (m, 2H), 4.80 (s, 1H), 4.70–4.61 (m, 5H), 4.29–4.18 (m, 1H), 3.87 (s, 3H), 3.78 (s, 3H), 3.77 (s, 2H), 3.75 (s, 4H), 3.72 (s, 2H), 3.70 (s, 2H), 3.68 (s, 3H), 3.17–3.05 (m, 1H), 3.00 (dd, *J* = 16.9, 7.6 Hz, 1H), 2.93 (dd, *J* = 16.9, 5.5 Hz, 1H), 2.88 (dd, *J* = 17.0, 7.0 Hz, 1H), 2.74–2.61 (m, 3H), 2.59 (s, 3H), 1.52 (s, 9H), 1.49–1.37 (m, 20H), 1.31 (s, 8H), 1.08–1.03 (m, 14H), 1.03–0.98 (m, 16H), 0.96–0.84 (m, 22H), 0.79 (s, 7H), 0.23 (s, 8H), 0.15 (s, 6H), 0.13 (s, 6H), 0.02 (s, 3H), –0.09 (s, 3H), –0.12 (s, 2H); ¹³C{¹H} NMR (151 MHz, CD₃OD, rotameric) δ 173.0, 172.3, 171.2, 170.7, 170.3, 169.8, 169.5, 168.2, 167.5, 160.4, 158.8, 158.7, 157.4, 156.5, 155.7, 151.7, 149.9, 149.8, 143.0, 142.7, 139.1, 138.4, 137.9, 135.9, 135.8, 134.0, 133.4, 131.8, 130.7, 127.4, 125.9, 123.4, 123.2, 122.9, 122.6, 121.4, 121.2, 116.9, 116.6, 114.6, 113.7, 113.1, 111.1, 104.1, 103.5, 98.3, 98.0, 81.8, 81.5, 80.6, 80.2, 72.7, 71.5, 61.0, 59.3, 59.2, 58.1, 57.0, 56.7, 56.2, 56.0, 55.9, 55.4, 55.0, 54.9, 54.6, 54.5, 49.4, 36.7, 29.3, 29.0, 27.2, 26.9, 26.6, 24.9, 24.5, 22.3, 20.6, 19.6, 17.8, 17.5, –5.5, –5.7, –6.0, –6.1; IR (film) $\nu_{\max}$ 3389, 3306, 2955, 2932, 2858, 1740, 1681, 1651, 1609, 1579, 1543, 1489, 1429, 1392, 1348, 1254, 1201, 1156, 1099, 1008 cm^{–1}; HRMS (ESI-TOF) *m/z* [M–H][–] calcd for C₈₄H₁₁₆Cl₂F₂N₁₀O₂₂Si₃, 1807.6840; found, 1807.6808.



Tetrachlorovancomycin Aglycon (**29**)

S_NAr reaction: A solution of **27** (35 mg, 19 μ mol, 1 equiv) in MeCN (6.4 mL, [**27**] = 3 mM) was treated with Bu₄NF (1 M solution in THF, 100 μ L, 100 μ mol, 5 equiv), stirred at 23 °C for 4 h, and quenched with the addition of AcOH (23 μ L, 400 μ mol, 20 equiv) before concentrating under a stream of N₂ to provide crude **28** as tan oil (inconsequential mixture of atropisomers, >95% crude purity as determined by LC/MS integration at 254 nm) that was carried forward without purification.

Nitro group reduction: Crude **28** was dissolved in AcOH (2 mL) and treated with Fe powder (325 mesh, 110 mg, 1900 μ mol, 100 equiv). The reaction mixture was stirred vigorously at 35 °C for 16 h, concentrated under a stream of N₂, and resuspended in EtOAc (4 mL). The organic layer was washed sequentially with H₂O (3 mL) and saturated aqueous NaCl (3 mL), dried over Na₂SO₄, and concentrated under a stream of N₂. Rapid PTLC (SiO₂, 100% acetone) provided the bis-aniline (30 mg, inconsequential mixture of atropisomers) as an air-sensitive white solid, used immediately in the following step.

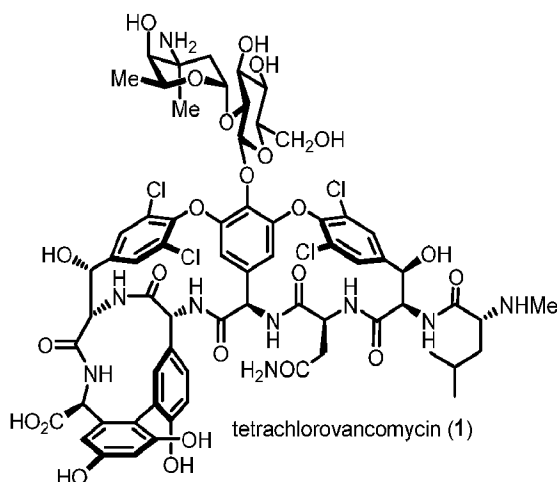
Sandmeyer substitution: A portion of the bis-aniline (20 mg, $\leq$ 13 μ mol) from the previous step was dissolved in CD₃CN (0.8 mL) and cooled to 0 °C. The reaction mixture was treated with BF₃•Et₂O (6.4 μ L, 52 μ mol, 4 equiv), followed immediately by *t*-BuONO (6.4 μ L, 52 μ mol, 4 equiv). The light yellow-green reaction mixture was stirred at 0 °C for 30 min, cooled to –35 °C, and stirred vigorously as a chilled (0 °C) suspension of CuCl (320 mg, 3.2 mmol, 250 equiv) and CuCl₂ (520 mg, 3.9 mmol, 300 equiv) in 50% CD₃CN–H₂O (1.6 mL) was added by syringe. The reaction mixture was slowly warmed to 5 °C over 2 h, added to saturated aqueous NH₄Cl (100 mL), adjusted to pH 9 with the

addition of concentrated NH_4OH , and extracted with EtOAc (100 mL). The organic layer was washed sequentially with H_2O (100 mL) and saturated aqueous NaCl (100 mL), dried over Na_2SO_4 , and concentrated under reduced pressure to provide the product as a tan solid that was carried forward without further purification.

Global deprotections: The crude Sandmeyer product was dissolved in TFA (1 mL) and stirred at 23 °C for 36 h. The reaction mixture was concentrated under a stream of N_2 , coevaporated with MeOH (1 mL), and further dried under high vacuum. The resulting solid was treated with a solution of AlBr_3 (1.5 g, 5.6 mmol, 430 equiv) in EtSH (300 μL , 4.2 mmol, 320 equiv), sonicated until homogeneous (3 min), and stirred at 23 °C for 3 h. The reaction mixture was then added dropwise to cold (0 °C) MeOH (15 mL) with vigorous stirring, rinsing the vial and pipette used to transfer the product with additional MeOH (3 $\times$ 1 mL). The MeOH was removed under a stream of N_2 at 23 °C, and the residue was purified by HPLC (Phenomenex Luna® C18(2), 250 $\times$ 4.6 mm, 5 mL/min, 20–30% MeCN– H_2O + 0.07% TFA over 20 min, t_R = 16.5 min) to provide **29** (TFA salt, 8.1 mg, 56%/5 steps) as a white solid.

For **29**: $[\alpha]_D^{25} +81$ (c 0.1, MeOH); ^1H NMR (600 MHz, CD_3OD) δ 9.44 (br s, 1H, NH), 8.88 (d, J = 6.3 Hz, 1H, NH), 8.72 (d, J = 5.9 Hz, 1H, NH), 7.81 (s, 1H), 7.69 (s, 1H), 7.65 (s, 1H), 7.64 (s, 1H), 7.08 (s, 1H), 6.98 (br s, 1H), 6.79–6.67 (m, 1H), 6.46 (d, J = 2.3 Hz, 1H), 6.42 (d, J = 2.2 Hz, 1H), 6.21 (br s, 1H), 6.13 (br s, 1H), 5.40 (d, J = 2.1 Hz, 1H), 5.34 (s, 1H), 5.27 (d, J = 3.7 Hz, 1H), 4.80 (d, J = 6.1 Hz, 1H), 4.74 (d, J = 5.9 Hz, 1H), 4.24 (d, J = 9.2 Hz, 1H), 4.20–4.14 (m, 1H), 4.05 (t, J = 7.0 Hz, 1H), 2.91 (d, J = 15.5 Hz, 1H), 2.79 (s, 3H), 1.94–1.79 (m, 2H), 1.77–1.66 (m, 2H), 0.99–0.93 (m, 6H); $^{13}\text{C}\{^1\text{H}\}$ NMR (151 MHz, CD_3OD) δ 175.8, 174.8, 172.7, 171.8, 170.0, 169.8, 169.4, 168.5, 159.2, 158.0, 156.6, 149.6, 147.7, 146.6, 142.9, 141.9, 137.4, 137.1, 136.8, 132.9, 132.6, 131.4, 130.4, 129.8, 127.9, 127.6, 127.5, 127.2, 126.5, 122.4, 118.8, 118.4, 110.2, 107.7, 106.5, 104.0, 73.4, 72.9, 63.6, 61.9, 59.0, 58.4, 56.6, 55.2, 54.0, 52.2, 40.2, 36.6, 33.1, 25.4, 23.0, 22.6; IR (film) ν_{max} 3300, 3252, 1673, 1538, 1516, 1508, 1204, 1190,

1140, 1057, 1033 cm^{-1} ; HRMS (ESI-TOF) m/z $[\text{M}+\text{H}]^+$ calcd for $\text{C}_{53}\text{H}_{50}\text{Cl}_4\text{N}_8\text{O}_{17}$, 1211.2126; found, 1211.2107.



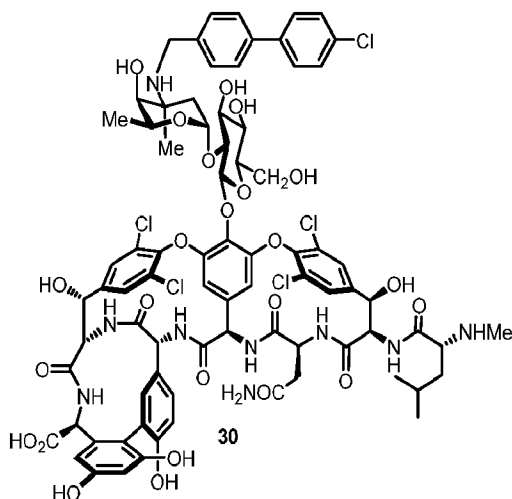
Tetrachlorovancomycin (1)

A solution of **29** (7.5 mg, 5.7 μmol , 1 equiv) in DMSO (250 μL) was treated sequentially with TCEP $\cdot\text{HCl}$ (3.5 mg, 11.4 μmol , 2 equiv), commercially available UDP-glucose $\cdot\text{2Na}$ (7 mg, 11.4 μmol , 2 equiv), aqueous 750 mM tricine- NaOH (pH 9, 0.6 mL), H_2O (2 mL), glycerol (300 μL), GtfE (50 μM , 1.2 mL, 0.06 μmol , 1 mol %)⁴¹ and commercially available calf intestinal alkaline phosphatase (CIAP, Promega, 1 U/ μL , 5 μL , 5 U).

The reaction mixture was warmed to 37 $^{\circ}\text{C}$ for 17 h, cooled to 23 $^{\circ}\text{C}$, and treated with additional TCEP $\cdot\text{HCl}$ (10.5 mg, 35 μmol , 6 equiv), 750 mM tricine- NaOH (pH 9, 1 mL), the azide precursor to UDP-vancosamine⁴¹ (45 μmol , 8 equiv), and GtfD⁴¹ (65 μM , 0.92 mL, 1 mol %). The reaction mixture was warmed to 37 $^{\circ}\text{C}$ for 16 h, cooled to 23 $^{\circ}\text{C}$, diluted with 50% MeOH–MeCN (32 mL), and filtered through a 0.22 μm PES membrane, rinsing with MeOH. The filtrate was concentrated under reduced pressure and purified by HPLC (Phenomenex Luna $^{\circ}$ C18(2), 250 $\times$ 4.6 mm, 5 mL/min, 5–30% MeCN– H_2O + 0.07% TFA, t_{R} = 16.9 min) to provide **1** (bis-TFA salt, 8.3 mg, 82%) as a white solid.

For **1**: ^1H NMR (600 MHz, CD_3OD) δ 8.91 (br s, 1H, NH), 8.70 (d, J = 5.9 Hz, 1H, NH), 8.66 (br s, 1H, NH), 7.82 (s, 1H), 7.72 (s, 1H), 7.69 (s, 1H), 7.67 (s, 1H), 7.07 (s, 1H), 6.82–6.65 (m, 2H), 6.46 (d,

$J = 2.3$ Hz, 1H), 6.40 (d, $J = 2.3$ Hz, 1H), 6.14–6.06 (m, 2H), 6.04 (s, 1H), 5.76 (d, $J = 7.8$ Hz, 1H), 5.45 (d, $J = 4.2$ Hz, 1H), 5.39 (d, $J = 2.0$ Hz, 1H), 5.35 (s, 1H), 5.27 (d, $J = 3.7$ Hz, 1H), 4.83 (q, $J = 6.7$ Hz, 1H), 4.79 (s, 1H), 4.73 (s, 1H), 4.72 (s, 1H), 4.24 (d, $J = 9.3$ Hz, 1H), 4.18 (s, 1H), 4.06 (t, $J = 6.8$ Hz, 1H), 3.93 (d, $J = 11.5$ Hz, 1H), 3.83 (t, $J = 8.2$ Hz, 1H), 3.78 (dd, $J = 12.0, 4.0$ Hz, 1H), 3.69–3.54 (m, 2H), 3.35 (s, 1H), 2.95 (d, $J = 15.2$ Hz, 1H), 2.76 (s, 3H), 2.06 (dd, $J = 13.6, 4.6$ Hz, 1H), 1.95 (d, $J = 13.3$ Hz, 1H), 1.93–1.77 (m, 2H), 1.68 (td, $J = 12.3, 11.4, 6.9$ Hz, 2H), 1.51 (s, 3H), 1.19 (d, $J = 6.4$ Hz, 3H), 0.95 (d, $J = 6.0$ Hz, 3H), 0.93 (d, $J = 6.1$ Hz, 3H); $^{13}\text{C}\{^1\text{H}\}$ NMR (151 MHz, CD_3OD) δ 174.2, 173.4, 171.6, 170.4, 168.4, 168.3, 168.0, 166.8, 157.8, 156.6, 155.3, 151.3, 149.9, 146.6, 145.1, 142.4, 141.3, 136.5, 136.1, 135.7, 132.5, 131.0, 129.5, 128.9, 128.8, 128.4, 127.0, 126.5, 126.0, 125.9, 125.6, 121.2, 117.3, 117.1, 107.6, 106.4, 104.8, 102.6, 101.3, 97.1, 78.1, 77.9, 76.8, 71.7, 71.3, 71.1, 69.7, 63.5, 62.4, 60.9, 60.3, 58.1, 57.0, 55.2, 54.4, 53.9, 51.1, 39.1, 35.2, 33.0, 31.5, 24.0, 22.0, 21.48, 21.40, 15.8; IR (film) ν_{max} 3484, 3434, 3420, 3406, 3387, 3372, 3352, 3328, 3316, 3302, 3279, 3245, 3225, 3199, 1666, 1592, 1489, 1186, 1139, 1058, 1018 cm^{-1} ; HRMS (ESI-TOF) m/z $[\text{M}+\text{H}]^+$ calcd for $\text{C}_{66}\text{H}_{73}\text{Cl}_4\text{N}_9\text{O}_{24}$, 1516.3601; found, 1516.3567.

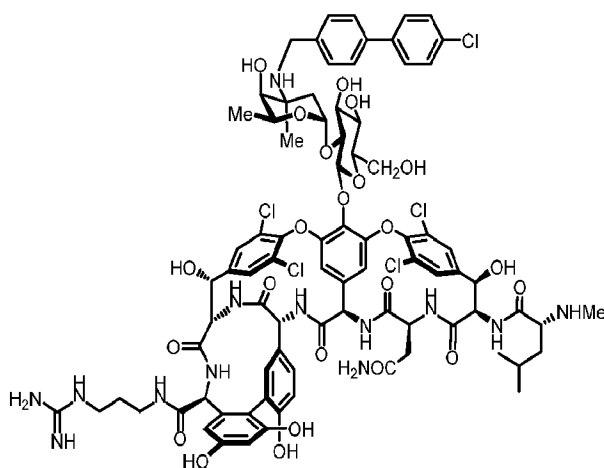


CBP-Tetrachlorovancomycin (30)

A solution of tetrachlorovancomycin (**1**, 6.9 mg, 4.0 μmol , 1 equiv), $i\text{-Pr}_2\text{NEt}$ (3.5 μL , 20 μmol , 5 equiv), and 4-(4-

chlorophenyl)benzaldehyde (1.2 mg, 5.2 μmol , 1.3 equiv) in DMF (0.69 mL, 100 vol) was warmed to 70 °C for 2 h, cooled to 50 °C, and treated with NaCNBH_3 (1 M in THF, 400 μL , 400 μmol , 100 equiv). The reaction mixture was stirred at 50 °C for 18 h, diluted with H_2O (2 mL), and purified by HPLC (Phenomenex Luna® C18(2), 250 $\times$ 4.6 mm, 5 mL/min, 10–40% $\text{MeCN-H}_2\text{O}$ + 0.07% TFA over 20 min, t_R = 19.4 min) to provide **30** (bis-TFA salt, 1.8 mg, 23%, 52% brsm) as a white solid and recovered starting material **1** (t_R = 10.0 min, 3.9 mg, 57% brsm).

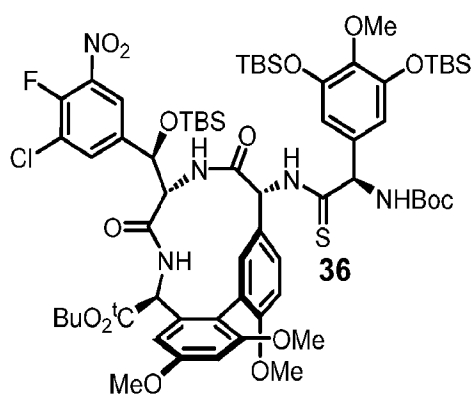
For **30**: ^1H NMR (600 MHz, CD_3OD) δ 7.78–7.68 (m, 7H), 7.66–7.62 (m, 2H), 7.59–7.55 (m, 2H), 7.51–7.46 (m, 2H), 7.07 (d, J = 2.4 Hz, 1H), 7.03–6.96 (m, 1H), 6.83 (d, J = 8.6 Hz, 1H), 6.47 (d, J = 2.3 Hz, 1H), 6.43 (d, J = 2.3 Hz, 1H), 5.97–5.90 (m, 2H), 5.68 (d, J = 7.9 Hz, 1H), 5.47–5.42 (m, 2H), 5.37 (s, 1H), 5.35 (s, 1H), 5.31 (d, J = 3.6 Hz, 1H), 4.79 (d, J = 6.2 Hz, 1H), 4.74 (d, J = 6.0 Hz, 1H), 4.24 (d, J = 9.6 Hz, 1H), 4.22–4.19 (m, 1H), 4.18 (s, 1H), 4.12 (s, 1H), 4.11–4.07 (m, 2H), 3.91–3.83 (m, 2H), 3.77 (dd, J = 11.6, 4.6 Hz, 1H), 3.64 (s, 1H), 3.60 (t, J = 9.0 Hz, 1H), 3.56 (t, J = 9.2 Hz, 1H), 3.03 (d, J = 15.5 Hz, 1H), 2.77 (s, 3H), 2.20 (dd, J = 13.6, 4.7 Hz, 1H), 2.12–1.99 (m, 2H), 1.91–1.81 (m, 1H), 1.78 (q, J = 6.6 Hz, 1H), 1.73–1.64 (m, 4H), 1.28 (d, J = 6.6 Hz, 3H), 1.04 (d, J = 6.4 Hz, 3H), 1.01 (d, J = 6.5 Hz, 3H); HRMS (ESI-TOF) m/z $[\text{M}+\text{H}]^+$ calcd for $\text{C}_{79}\text{H}_{82}\text{Cl}_5\text{N}_9\text{O}_{24}$, 1716.3994; found, 1716.3938.



G3,CBP-Tetrachlorovancomycin (31)

A solution of **30** (1.1 mg, 0.57 μ mol, 1 equiv), 1-(3-aminopropyl)guanidine⁴⁴ (bis-TFA salt, 0.96 mg, 2.8 μ mol, 5 equiv) and NMM (1.9 μ L, 17 μ mol, 30 equiv) in DMF (200 μ L) was cooled to 0 °C and treated with T3P (50 wt % in EtOAc, 3.5 μ L, 5.7 μ mol, 10 equiv). The reaction mixture was stirred at 0 °C for 10 min, diluted with H₂O (1 mL), and purified directly by HPLC (Phenomenex Luna® C18(2), 250 $\times$ 4.6 mm, 5 mL/min, 25–40% MeCN–H₂O + 0.07% TFA over 20 min, t_R = 10.1 min) to afford **31** (0.90 mg, 74%) as a white solid.

For **31**: ¹H NMR (600 MHz, CD₃OD) δ 7.78–7.69 (m, 6H), 7.67–7.61 (m, 2H), 7.58 (m, 2H), 7.53–7.44 (m, 2H), 7.11 (s, 1H), 7.05 (d, J = 8.6 Hz, 1H), 6.86 (d, J = 8.5 Hz, 1H), 6.47 (d, J = 2.3 Hz, 1H), 6.39 (d, J = 2.3 Hz, 1H), 5.96–5.83 (m, 2H), 5.67 (d, J = 7.8 Hz, 1H), 5.45 (d, J = 4.7 Hz, 1H), 5.40 (s, 1H), 5.36 (s, 1H), 5.32 (d, J = 3.6 Hz, 1H), 4.97 (s, 1H), 4.74 (d, J = 6.0 Hz, 1H), 4.63 (d, J = 5.8 Hz, 1H), 4.29–4.22 (m, 2H), 4.19 (d, J = 12.7 Hz, 1H), 4.13–4.07 (m, 2H), 3.88–3.81 (m, 2H), 3.76 (dd, J = 11.6, 4.9 Hz, 1H), 3.65 (s, 1H), 3.60 (t, J = 9.1 Hz, 1H), 3.54 (t, J = 9.3 Hz, 1H), 3.51–3.45 (m, 1H), 3.25 (t, J = 6.9 Hz, 2H), 3.03 (d, J = 16.7 Hz, 1H), 3.02–2.99 (m, 2H), 2.88 (s, 1H), 2.76 (s, 3H), 2.24–2.18 (m, 1H), 2.13–2.02 (m, 2H), 1.91–1.74 (m, 4H), 1.72–1.63 (m, 4H), 1.28 (d, J = 6.7 Hz, 3H), 1.05 (d, J = 6.5 Hz, 3H), 1.01 (d, J = 6.5 Hz, 3H); HRMS (ESI-TOF) m/z [M+3H]³⁺ calcd for C₈₃H₉₂Cl₅N₁₃O₂₃, 605.5035; found, 605.5016.

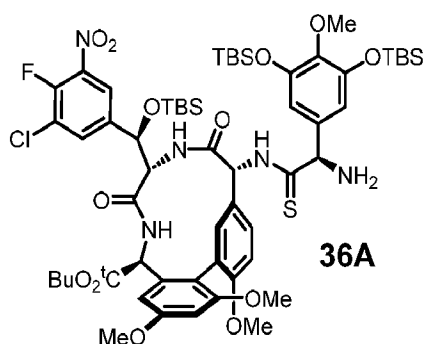


Compound **36**

A solution of **25** (TFA salt, 60 mg, 65 μ mol, 1 equiv) and **35** (82 mg, 130 μ mol, 2 equiv) in THF (0.6 mL, 10 vol) was treated with *i*-Pr₂NEt (16 μ L, 98 μ mol, 1.5 equiv) and stirred at 23 °C for 6 h. The

reaction mixture was concentrated under a stream of N₂ and purified by PTLC (SiO₂, 40% EtOAc–hexanes) to provide **36** (78.2 mg, 89%) as a white solid.

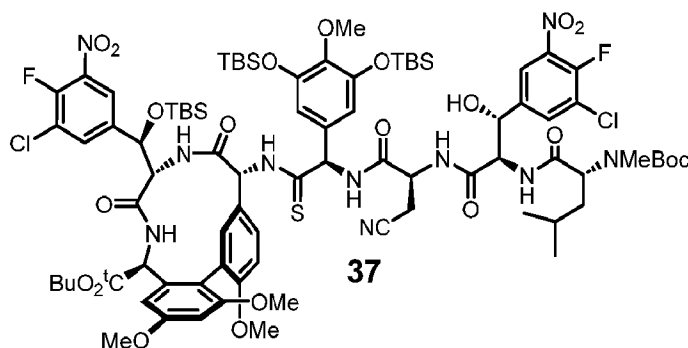
For **36**: $[\alpha]_D^{23}$ –6.0 (c 1.0, CH₂Cl₂); ¹H NMR (600 MHz, CDCl₃) δ 9.36 (s, 1H), 7.99–7.91 (m, 1H), 7.78–7.69 (m, 1H), 7.13 (d, *J* = 7.9 Hz, 1H), 7.05 (d, *J* = 2.4 Hz, 1H), 6.97 (d, *J* = 8.7 Hz, 1H), 6.55 (d, *J* = 2.3 Hz, 1H), 6.51–6.39 (m, 2H), 6.37 (d, *J* = 2.3 Hz, 1H), 6.00 (d, *J* = 10.8 Hz, 1H), 5.93–5.74 (m, 1H), 5.48 (d, *J* = 5.9 Hz, 1H), 5.28 (s, 1H), 5.05 (d, *J* = 13.8 Hz, 1H), 4.95 (d, *J* = 8.7 Hz, 1H), 4.08 (dd, *J* = 10.8, 2.1 Hz, 1H), 3.78 (s, 3H), 3.76 (s, 3H), 3.69 (s, 3H), 3.66 (s, 3H), 2.28 (s, 1H), 1.46 (s, 9H), 1.39 (s, 9H), 0.96 (d, *J* = 3.1 Hz, 18H), 0.83 (d, *J* = 4.5 Hz, 9H), 0.11 (s, 6H), 0.10–0.05 (s, 6H), 0.01 (s, 3H), –0.13 (s, 3H); ¹³C NMR (151 MHz, CDCl₃) δ 199.6, 170.3, 170.1, 165.5, 160.1, 158.8, 157.3, 154.7, 152.3, 150.6, 150.1, 149.9, 142.9, 138.0, 137.7, 137.5, 137.4, 137.2, 136.0, 134.3, 133.5, 127.4, 124.8, 124.6, 124.0, 122.6, 122.1, 121.2, 113.2, 103.8, 99.1, 82.4, 80.2, 73.1, 63.8, 61.0, 59.9, 59.5, 56.0, 55.7, 55.3, 28.3, 27.8, 25.7, 18.2, 17.9, –4.6, –4.9, –5.2; IR (film) ν_{max} 3319, 2977, 2954, 2931, 2898, 2858, 1679, 1605, 1579, 1544, 1490, 1473, 1433, 1393, 1343, 1326, 1256, 1158, 1088, 1030, 1007 cm^{–1}; HRMS (ESI-TOF) *m/z* [M+H]⁺ calcd for C₆₄H₉₃ClFN₅O₁₅SSi₃, 1342.5447; found, 1342.5472.



Compound **36A**

A solution of **36** (150 mg, 0.11 mmol, 1 equiv) in *t*-BuOAc (3 mL, 20 vol) was treated with H₂SO₄ (45 μL, 0.9 mmol, 8 equiv) and stirred at 23 °C for 1 h. The reaction mixture was quenched with the

addition of Et₃N (182 μ L, 1.32 mmol, 12 equiv) and purified directly by HPLC (Phenomenex Luna® C18(2), 100 $\times$ 30 mm, 20 mL/min, 50–100% MeCN–H₂O + 0.07% TFA over 10 min, t_R = 12.3 min) to provide semi-pure **36A** (TFA salt, 124 mg, 83%) as a light-yellow solid, used immediately in the following step. Amine **36A** is unstable, especially in its free base form, and could not be isolated without decomposition.



Compound 37

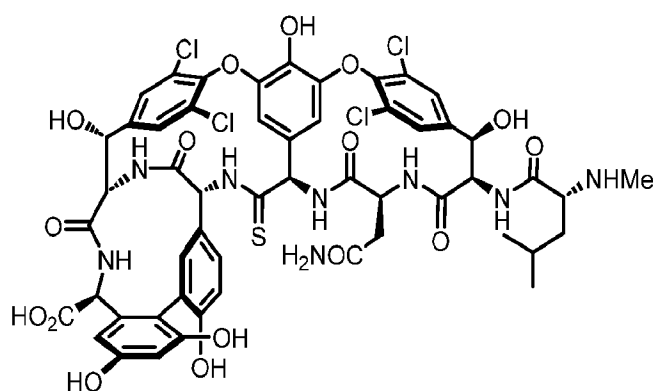
A solution of **36A** (TFA salt, 124 mg, 92 μ mol, 1 equiv) and **14** (112 mg, 150 μ mol, 1.6 equiv) in THF (2 mL) was cooled to 0 °C and treated with NMM (41 μ L, 370 μ mol, 4 equiv), followed by T3P® (50 wt % in EtOAc, 90 μ L, 150 μ mol, 1.6 equiv). The reaction mixture was stirred at 0 °C for 10 min, quenched with the addition of H₂O (5 mL), and concentrated under a stream of N₂ to remove THF. The residue was extracted with CH₂Cl₂ (5 $\times$ 3 mL) and dried over Na₂SO₄. Chromatography (10 g SiO₂, wet-load CH₂Cl₂, 0–30% Et₂O–CH₂Cl₂) provided **37** (141 mg, 85%, 69%/2 steps from **14**) as a yellow solid.

For **37**: $[\alpha]_D^{24}$ –4.0 (c 1.0, CH₂Cl₂); ¹H NMR (600 MHz, CD₃OD, rotamers, major given) δ 8.23 (dd, J = 6.2, 2.2 Hz, 1H), 8.15–8.07 (m, 1H), 7.99 (dd, J = 5.9, 2.3 Hz, 1H), 7.93 (dd, J = 6.0, 2.2 Hz, 1H), 7.33 (dd, J = 8.7, 2.3 Hz, 1H), 7.00 (d, J = 2.4 Hz, 1H), 6.68 (d, J = 2.2 Hz, 1H), 6.64–6.59 (m, 2H), 6.49 (d, J = 2.3 Hz, 1H), 5.87 (s, 1H), 5.60 (s, 1H), 5.24 (s, 1H), 5.03–4.90 (m, 2H), 4.79 (s, 1H), 4.73–4.60 (m, 1H), 4.60–4.44 (m, 1H), 4.29 (s, 1H), 3.85 (s, 3H), 3.76 (d, J = 2.9 Hz, 3H), 3.74–3.63 (m, 7H), 3.05–2.78 (m, 1H), 2.77–2.55 (m, 1H), 2.49 (s, 3H), 1.50 (s, 9H), 1.48–1.36 (m, 12H), 1.04–1.02 (m, 3H), 1.01 (m, 3H),

0.99 (s, 18H), 0.87 (s, 9H), 0.13 (s, 6H), 0.12 (s, 6H), 0.01 (s, 3H), -0.07 (s, 3H); ^{13}C NMR (151 MHz, CD_3OD , rotamers) δ 200.6, 200.5, 172.4, 170.3, 170.2, 167.8, 167.5, 160.4, 158.8, 157.7, 156.4, 155.7, 151.7, 151.6, 150.0, 149.9, 149.5, 142.7, 142.4, 139.2, 138.6, 138.5, 137.96, 137.91, 137.1, 135.8, 134.1, 133.5, 133.0, 130.7, 128.2, 123.6, 123.44, 123.42, 123.1, 123.0, 122.9, 122.4, 121.4, 121.1, 121.0, 117.0, 116.6, 114.1, 113.8, 113.7, 113.3, 112.8, 104.2, 103.4, 98.2, 98.19, 98.13, 81.8, 81.5, 80.7, 80.2, 72.6, 71.5, 66.1, 61.2, 60.3, 59.3, 59.2, 58.2, 57.0, 55.9, 55.5, 55.1, 55.0, 54.9, 54.95, 54.91, 54.6, 54.5, 53.7, 49.4, 27.3, 27.2, 26.9, 26.6, 24.99, 24.93, 24.8, 24.4, 22.3, 20.5, 19.6, 17.87, 17.82, 17.6, 17.5, -5.40, -5.44, -5.48, -5.61, -5.67, -6.0, -6.1; IR (film) ν_{max} 3274, 2931, 2857, 1738, 1654, 1607, 1580, 1543, 1489, 1431, 1391, 1346, 1252, 1201, 1155, 1089, 1007 cm^{-1} ; HRMS (ESI-TOF) m/z $[\text{M}-\text{H}]^-$ calcd for $\text{C}_{84}\text{H}_{116}\text{Cl}_2\text{F}_2\text{N}_{10}\text{O}_{21}\text{SSi}_3$, 1823.6612; found, 1823.6617.

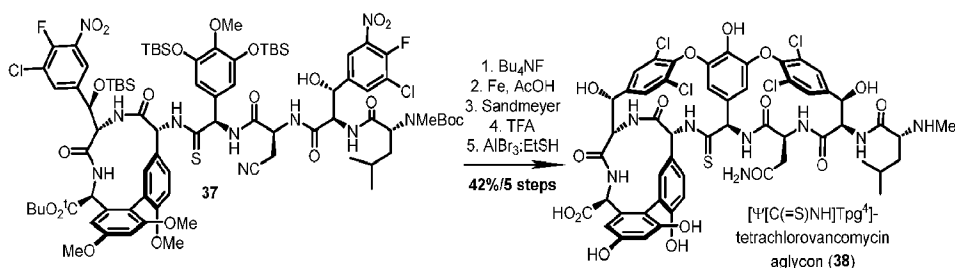
Important note:

The streamlined 5-step conversion of **37** to **38** (below) was both higher-yielding and more conveniently performed without HPLC purification of the intermediate atropisomer mixtures. Final purification of **38** by HPLC was sufficient to obtain pure samples of $[\Psi[\text{C}(=\text{S})\text{NH}]\text{Tpg}^4]\text{tetrachlorovancomycin aglycon}$.



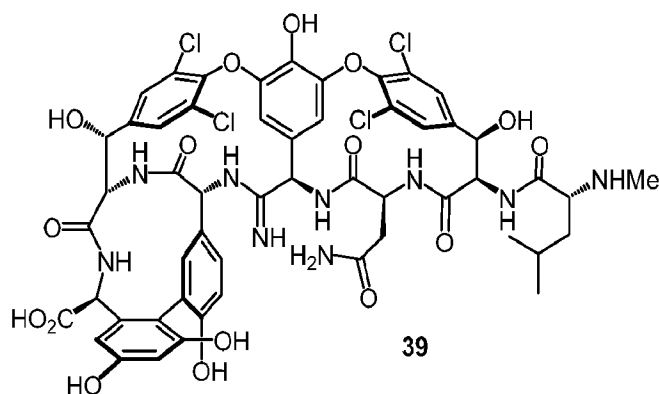
Compound 38 $[\Psi[\text{C}(=\text{S})\text{NH}]\text{Tpg}^4]\text{tetrachlorovancomycin aglycon}$

Desilylation of heptapeptide **37** (Bu₄NF, MeCN, 23 °C) triggered a spontaneous double S_NAr cyclization that established the full tricyclic framework of [Ψ[C(=S)NH]Tpg⁴]tetrachlorovancomycin aglycon (**38**) in a single step. Dual nitro reduction (Fe, AcOH), Sandmeyer substitution (BF₃•Et₂O, *t*-BuONO; CuCl, CuCl₂), nitrile hydration with concomitant Boc and *t*-butyl ester deprotection (TFA, 23 °C), and global demethylation (5:1 AlBr₃:EtSH) provided [Ψ[C(=S)NH]Tpg⁴]tetrachlorovancomycin aglycon (**38**) in good overall yield (42%/5 steps from **37**, with an average yield of 84%/step), below, setting the stage for the introduction of the residue 4 binding pocket modifications.



For **38**: [α]_D²⁴ +77 (c 1.0, MeOH); ¹H NMR (600 MHz, CD₃OD) δ 9.02 (s, 1H, NH), 8.77 (d, *J* = 5.9 Hz, 1H, NH), 7.84 (d, *J* = 1.9 Hz, 1H), 7.67 (d, *J* = 2.1 Hz, 1H), 7.65 (d, *J* = 1.9 Hz, 1H), 7.59 (d, *J* = 1.9 Hz, 1H), 7.23 (d, *J* = 2.2 Hz, 1H), 7.09 (s, 1H), 6.66 (s, 1H), 6.65 (s, 1H), 6.62 (br s, 1H), 6.61 (br s, 1H), 6.51–6.46 (m, 1H), 6.44 (d, *J* = 2.3 Hz, 1H), 6.38 (s, 1H), 5.94 (s, 1H), 5.56 (s, 1H), 5.47 (d, *J* = 2.2 Hz, 1H), 5.33 (d, *J* = 1.4 Hz, 1H), 5.22 (d, *J* = 3.9 Hz, 1H), 4.69 (d, *J* = 5.8 Hz, 1H), 4.34–4.28 (m, 1H), 4.26 (d, *J* = 2.5 Hz, 1H), 4.03 (t, *J* = 7.1 Hz, 1H), 2.90 (d, *J* = 16.5 Hz, 1H), 2.77 (s, 3H), 1.88–1.80 (m, 1H), 1.80–1.56 (m, 2H), 0.92 (d, *J* = 6.4 Hz, 3H), 0.89 (d, *J* = 6.3 Hz, 3H); ¹³C{¹H} NMR (151 MHz, CD₃OD) δ 173.5, 170.8, 170.4, 168.5, 168.2, 157.9, 156.7, 155.4, 148.4, 146.6, 146.1, 145.3, 141.5, 140.5, 137.6, 135.8, 132.6, 130.4, 129.3, 129.2, 129.0, 128.4, 128.1, 126.6, 126.1, 125.1, 125.0, 121.0, 117.3, 116.9, 106.3, 102.6, 72.0, 71.6, 62.3, 60.5, 59.5, 57.2, 52.7, 51.0, 48.5, 48.2, 38.9, 35.3, 31.9, 31.7, 29.4, 29.1, 25.5, 24.0, 22.3, 21.7,

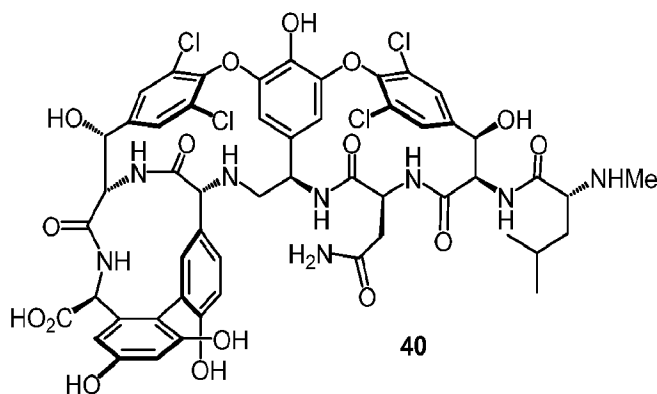
21.2, 19.5, 12.5; IR (film) $\nu_{\max}$ 3386, 3361, 3347, 3324, 3305, 3285, 3272, 3248, 3231, 3206, 1668, 1621, 1538, 1507, 1463, 1431, 1396, 1251, 1201, 1140 cm^{-1} ; HRMS (ESI-TOF) m/z $[\text{M}+\text{H}]^+$ calcd for $\text{C}_{53}\text{H}_{50}\text{Cl}_4\text{N}_8\text{O}_{16}\text{S}$, 1227.1898; found, 1227.1879.



[$\Psi[\text{C}(=\text{NH})\text{NH}]\text{Tpg}^4$]-Tetrachlorovancomycin Aglycon (39)

A solution of **38** (3.74 mg, 3.04 μmol , 1 equiv) in saturated aqueous NH_4OAc (1.2 mL) was treated with AgOAc (20.3 mg, 122 μmol , 40 equiv, $[\text{Ag}] = 100 \text{ mM}$). The mixture was stirred at 23 $^\circ\text{C}$ with protection from light for 40 h. Direct HPLC purification (Phenomenex Luna[®] C18(2), 250 $\times$ 4.6 mm, 5 mL/min, 1–15% $\text{MeCN}/\text{H}_2\text{O}$ –0.07% TFA over 3 min then 15–30% $\text{MeCN}/\text{H}_2\text{O}$ –0.07% TFA over 30 min, $t_R = 16 \text{ min}$) afforded **39** (2.81 mg, 76%) as a white solid.

For **39**: ^1H NMR (600 MHz, CD_3OD) δ 7.78 (s, 1H), 7.74 (s, 1H), 7.68 (s, 1H), 7.43 (s, 1H), 7.10 (s, 1H), 7.09 (s, 1H), 6.91 (s, 1H), 6.90 (s, 1H), 6.51 (s, 1H), 6.49 (s, 1H), 6.18 (s, 1H), 5.68 (s, 1H), 5.44 (d, $J = 5.4 \text{ Hz}$, 1H), 5.40–5.35 (m, 2H), 5.32 (s, 1H), 4.78 (s, 1H), 4.74 (s, 1H), 4.27 (s, 1H), 4.22 (s, 1H), 4.11 (s, 1H), 2.91–2.85 (m, 1H), 2.84 (d, $J = 4.0 \text{ Hz}$, 3H), 2.44 (dd, $J = 16.0, 5.5 \text{ Hz}$, 1H), 1.91–1.76 (m, 1H), 1.70–1.55 (m, 2H), 0.92 (d, $J = 6.1 \text{ Hz}$, 3H), 0.89 (d, $J = 6.2 \text{ Hz}$, 3H); HRMS (ESI-TOF) m/z $[\text{M}+2\text{H}]^{2+}$ calcd for $\text{C}_{53}\text{H}_{51}\text{Cl}_4\text{N}_9\text{O}_{16}$, 605.6182; found, 605.6172.



[Ψ[CH₂NH]Tpg⁴]Tetrachlorovancomycin Aglycon (40)

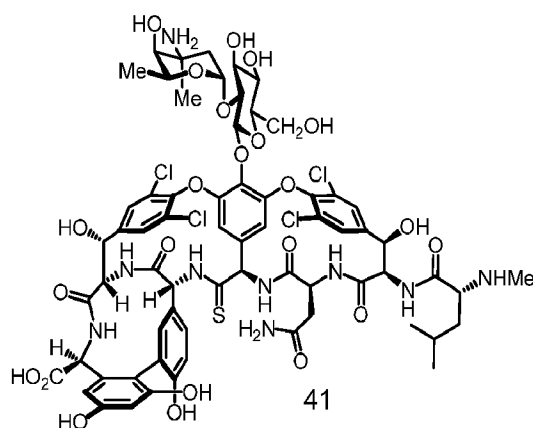
A solution of **38** (1.1 mg, 0.82 μ mol, 1 equiv) in MeOH (250 μ L) was treated with H₂O₂ (35% aqueous solution, 50 μ L, 20% v/v) and stirred at 23 °C for 1 h. The reaction mixture was cooled to 0 °C and treated with NaCNBH₃ (300 mg, 50% w/v). The resulting suspension was stirred at 0 °C for 30 min, acidified with the addition of AcOH (10 μ L), and stirred at 5 °C for 12 h. The resulting solution was warmed to 23 °C and stirred for an additional 24 h, diluted with H₂O (1 mL), and purified by HPLC (Phenomenex Luna® C18(2), 250 $\times$ 4.6 mm, 5 mL/min, 1-30% MeCN–H₂O + 0.07% TFA over 20 min, t_R = 22.7 min) to provide **40** (bis-TFA salt, 867 μ g, 74%) as a white solid.

Alternatively, a solution of **38** (3.3 mg, 2.68 μ mol, 1 equiv), anhydrous NiCl_2 (7.0 mg, 54 μ mol, 20 equiv) and 1,2-dichlorobenzene (0.3 mL) in anhydrous MeOH (3 mL) was purged with Ar and cooled to -78°C . NaBH_4 (5.1 mg, 0.135 mmol, 50 equiv) was added, and the mixture was stirred at -40°C , whereupon the solution color turned brown and then dark. After 40 min, the reaction was quenched by transferring the mixture into a saturated solution of EDTA in H_2O –MeOH (1:1, 10 mL), and the resulting mixture was stirred at 23°C for 1 h with the color changing from dark to light blue.

After filtration through a 0.22 μm PES membrane, rinsing with MeOH, the filtrate was concentrated under a nitrogen flow to remove MeOH, diluted with H₂O (10 mL), and purified by semi-preparative reverse-phase HPLC (Luna®-5 μm -C18, 100 Å, 100 $\times$ 30

mm, 15–25% MeCN/H₂O–0.07% TFA gradient over 20 min, 20 mL/min, t_R = 20.5 min) to afford **40** (2.20 mg, 68%) as a white solid.

For **40**: ¹H NMR (600 MHz, CD₃OD) δ 7.94 (d, J = 2.0 Hz, 1H), 7.89 (d, J = 2.0 Hz, 1H), 7.50 (d, J = 2.0 Hz, 1H), 7.32 (d, J = 2.1 Hz, 1H), 7.17 (dd, J = 8.6, 2.5 Hz, 1H), 7.05 (d, J = 2.5 Hz, 1H), 6.96 (d, J = 8.6 Hz, 1H), 6.47 (d, J = 2.2 Hz, 1H), 6.43 (d, J = 2.3 Hz, 1H), 5.49 (d, J = 2.0 Hz, 1H), 5.38 (d, J = 2.4 Hz, 1H), 5.29 (d, J = 5.6 Hz, 1H), 5.03 (s, 1H), 4.98 (d, J = 2.2 Hz, 1H), 4.93 (d, J = 5.6 Hz, 1H), 4.91 (s, 1H, obscured by solvent), 4.89–4.80 (m, 2H), 4.53 (dd, J = 15.2, 9.8 Hz, 1H), 4.40 (t, J = 5.2 Hz, 1H), 4.26 (dd, J = 8.9, 5.8 Hz, 1H), 4.11 (d, J = 2.7 Hz, 1H), 2.75 (s, 3H), 2.68 (d, J = 5.2 Hz, 2H), 2.24 (d, J = 15.1 Hz, 1H), 1.82 (ddd, J = 13.8, 9.0, 5.5 Hz, 1H), 1.75–1.58 (m, 2H), 1.04 (d, J = 6.4 Hz, 3H), 0.95 (d, J = 6.4 Hz, 3H); HRMS (ESI-TOF) m/z [M+H]⁺ calcd for C₅₃H₅₂Cl₄N₈O₁₆, 1197.2334; found, 1197.2291.



[Ψ[C(=S)NH]Tpg⁴]Tetrachlorovancomycin (**41**)

A solution of **38** (200 mg, 0.163 mmol, 1 equiv) and UDP-glucose•2Na (199 mg, 0.326 mmol, 2 equiv) in 750 mM tricine-NaOH (pH 9, 16 mL) was treated with GtfE (50 μ M in protein storage buffer*, 8.1 mL, 0.405 μ mol, 0.25 mol%), TCEP•HCl (93 mg, 0.324 mmol, 2 equiv) and calf intestinal alkaline phosphatase (CIAP, Promega, 1 U/ μ L in storage buffer**, 57.2 μ L, 57 U). The reaction mixture was purged with Ar and warmed at 37 °C. Compound **38** was initially not completely dissolved in the solution, but slowly goes into the solution as the reaction

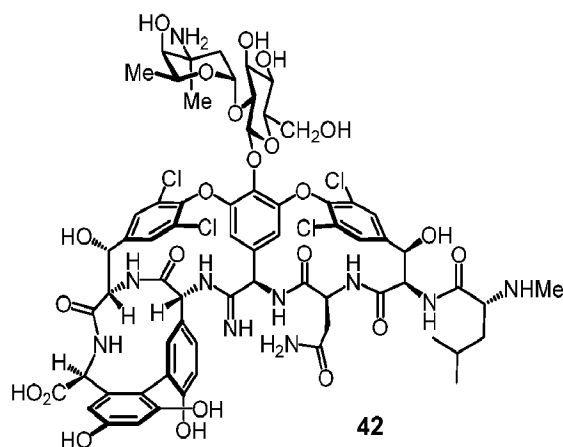
proceeds (occasional swirling the flask is needed to prevent the suspended solids from sticking to the side wall of flask).

After 60 h, LCMS indicates <2% of **38** left, and the reaction was cooled to 23 °C, and treated with additional 750 mM tricine-NaOH (pH 9, 16 mL), GtfD (65 µM in protein storage buffer*, 5.0 mL, 0.325 µmol, 0.2 mol%), TCEP•HCl (606 mg, 2.11 mmol, 13 equiv) and calf intestinal alkaline phosphatase (CIAP, Promega, 1 U/µL in storage buffer**, 57.2 µL, 57 U). The reaction mixture was warmed to 37 °C, and freshly OBz-deprotected UDP-vancosamine precursor (ca. 179 mg as a bis-ammonium salt, 0.293 mmol, 1.8 equiv, assumed 60% yield from the deprotection step) were added at a rate of 0.6 equiv/1.5 h. After the addition was complete (4 h), the reaction mixture was allowed to stand for an additional 2 h at 37 °C. LCMS indicated >97% conversion of the pseudoaglycone intermediate. The mixture was cooled to 23 °C, diluted with 500 mL of MeOH, stirred at 23 °C for 30 min, filtered through a 0.22 µm PES membrane, and rinsed with MeOH. The filtrate was concentrated under reduced pressure, redissolved in 100 mL of H₂O and purified by semi-preparative reverse-phase HPLC (Luna®-5 µm-C18, 100 Å, 100 × 30 mm, 10–28% MeCN/H₂O–0.07% TFA gradient over 25 min, 20 mL/min, *t_R* = 12.5 min) to afford **41** (209 mg, 84%/2 steps in one pot) as a white solid.

For **41**: $[\alpha]_D^{25} +9.8$ (c 0.2, MeOH); ¹H NMR (600 MHz, CD₃OD) δ 9.20 (s, 1H, NH), 8.79 (s, 1H, NH), 7.77 (s, 1H), 7.75 (s, 1H), 7.73 (s, 1H), 7.69 (s, 1H), 7.23 (s, 1H), 6.93 (d, *J* = 8.2 Hz, 1H), 6.80 (dd, *J* = 8.5, 2.5 Hz, 1H), 6.76 (s, 1H), 6.48 (t, *J* = 2.5 Hz, 1H), 6.42 (d, *J* = 2.6 Hz, 1H), 6.29 (d, *J* = 7.8 Hz, 1H), 5.94 (s, 1H), 5.71 (d, *J* = 7.7 Hz, 1H), 5.47–5.38 (m, 2H), 5.36 (s, 1H), 5.34 (s, 1H), 5.33–5.28 (m, 1H), 4.77 (dd, *J* = 6.1, 2.4 Hz, 1H), 4.36–4.30 (m, 1H), 4.30 (s, 1H), 4.10 (td, *J* = 7.3, 2.5 Hz, 1H), 3.90 (d, *J* = 11.7 Hz, 1H), 3.83 (td, *J* = 8.4, 2.5 Hz, 1H), 3.78 (dt, *J* = 11.9, 3.4 Hz, 1H), 3.64–3.51 (m, 2H), 3.36–3.34 (m, 3H), 3.03 (d, *J* = 15.9 Hz, 1H), 2.77 (d, *J* = 2.5 Hz, 3H), 2.15–2.02 (m, 2H), 1.97 (d, *J* = 13.3 Hz, 1H), 1.91–1.81 (m, 1H), 1.80–1.62 (m, 2H), 1.51 (s, 3H), 1.21 (d, *J* = 6.5 Hz, 3H), 1.01 (d, *J* = 6.5 Hz, 3H), 0.99 (d, *J*

= 6.6 Hz, 3H); $^{13}\text{C}\{^1\text{H}\}$ NMR (151 MHz, CD_3OD) δ 200.6, 175.1, 172.9, 172.2, 171.5, 170.5, 169.8, 168.2, 162.6, 162.3, 159.3, 159.1, 158.6, 157.5, 156.4, 152.1, 151.4, 147.3, 146.2, 143.3, 142.1, 138.7, 136.8, 134.4, 134.2, 133.4, 133.2, 131.8, 131.2, 130.9, 130.6, 130.5, 130.0, 129.6, 129.5, 128.5, 128.3, 128.2, 128.0, 127.1, 125.8, 122.4, 120.4, 118.8, 118.4, 116.7, 116.5, 114.8, 114.6, 107.9, 106.4, 104.1, 102.8, 98.8, 80.1, 78.6, 77.9, 72.6, 72.4, 72.2, 70.6, 65.0, 63.9, 62.1, 61.5, 60.9, 59.8, 58.3, 55.7, 52.5, 49.8, 40.3, 36.5, 34.3, 32.7, 25.3, 23.1, 22.9, 22.6, 17.1; IR (film) ν_{max} 3484, 3434, 3420, 3405, 3387, 3372, 3352, 3328, 3316, 3302, 3279, 3245, 3225, 3199, 1666, 1592, 1489, 1186, 1139, 1058, 1018 cm^{-1} ; HRMS (ESI-TOF) m/z $[\text{M}+\text{H}]^+$ calcd for $\text{C}_{66}\text{H}_{73}\text{Cl}_4\text{N}_9\text{O}_{23}\text{S}$, 1532.3372; found, 1532.3391.

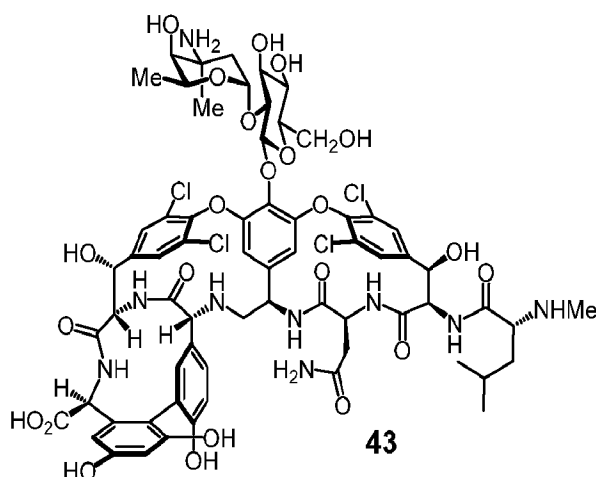
*Protein storage buffer contains 50% v/v glycerol, 30 mM Tris-HCl and 1 mM DTT (pH = 8). **Storage buffer of CIAP contains 10 mM Tris HCl (pH 8), 1 mM MgCl_2 , 0.1 mM ZnCl_2 , 50 mM KCl and 50% glycerol.



[Ψ[C(=NH)NH]Tpg⁴]Tetrachlorovancomycin (42)

A solution of **41** (2.02 mg, 1.32 μmol , 1 equiv) in saturated aqueous NH_4OAc (0.53 mL) was treated with AgOAc (8.80 mg, 52.7 μmol , 40 equiv, $[\text{Ag}] = 100 \text{ mM}$). After the resulting mixture was stirred at ambient temperature for 24 h, HPLC purification (Phenomenex Luna[®] C18(2), 250 $\times$ 4.6 mm, 1–20% MeCN/ H_2O –0.07% TFA gradient over 25 min, 5 mL/min, $t_R = 24 \text{ min}$) afforded **42** (1.54 mg, 77%) as a white solid.

For **42**: ^1H NMR (600 MHz, CD_3OD) δ 7.75 (d, J = 2.0 Hz, 3H), 7.72 (s, 1H), 7.42 (s, 1H), 7.08 (d, J = 8.6 Hz, 1H), 6.88 (d, J = 8.6 Hz, 1H), 6.47 (d, J = 23.9 Hz, 2H), 6.21 (s, 1H), 5.68 (s, 1H), 5.66 (d, J = 9.1 Hz, 1H), 5.57 (t, J = 7.4 Hz, 1H), 5.50 (s, 1H), 5.48 (d, J = 5.6 Hz, 1H), 5.43 (d, J = 4.5 Hz, 1H), 5.38 (s, 2H), 5.33 (s, 2H), 4.30 (d, J = 7.3 Hz, 1H), 3.85 (t, J = 8.1 Hz, 1H), 3.80 (d, J = 11.4 Hz, 1H), 3.76 (d, J = 11.9 Hz, 1H), 3.58 (d, J = 7.8 Hz, 2H), 3.42 (s, 1H), 2.88 (s, 1H), 2.86 (s, 3H), 2.83 (s, 1H), 2.39 (dd, J = 16.1, 4.8 Hz, 1H), 2.09–1.91 (m, 1H), 1.85–1.78 (m, 1H), 1.62 (s, 1H), 1.59 (d, J = 6.7 Hz, 2H), 1.47 (s, 3H), 1.19 (d, J = 7.2 Hz, 3H), 0.87 (d, J = 6.0 Hz, 3H), 0.85 (d, J = 6.0 Hz, 3H); HRMS (ESI-TOF) m/z $[\text{M}+2\text{H}]^+$ calcd for $\text{C}_{66}\text{H}_{74}\text{Cl}_4\text{N}_{10}\text{O}_{23}$, 758.1919; found, 758.1898.

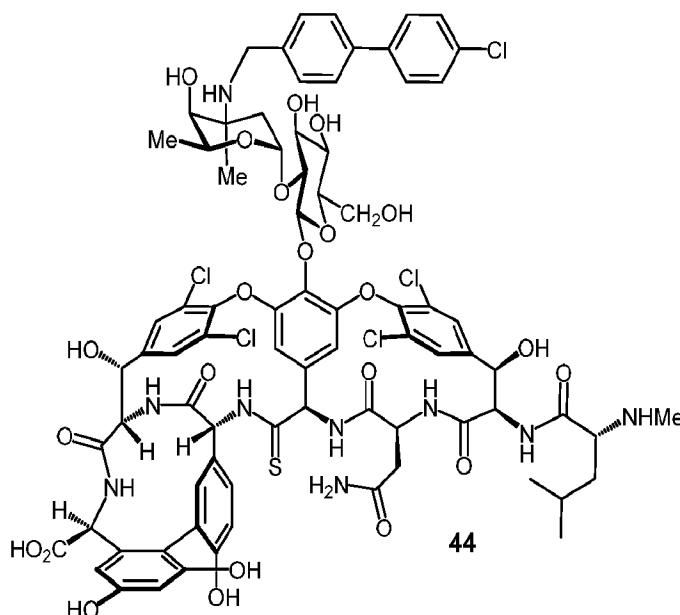


[Ψ[CH₂NH]Tpg⁴]Tetrachlorovancomycin (43**)**

A solution of **41** (2.01 mg, 1.31 μmol , 1 equiv) in anhydrous MeOH (1.5 mL) was treated with anhydrous NiCl_2 (3.38 mg, 26 μmol , 20 equiv) and 1,2-dichlorobenzene (0.15 mL). After purging with Ar and being cooled to -78°C , NaBH_4 (3.45 mg, 91 μmol , 70 equiv) was added, and the mixture was stirred at -40°C for 40 min. The reaction was quenched by addition to an EDTA suspension (saturated, H_2O –MeOH, 1:1, 10 mL), and the resulting mixture was stirred at 23°C for 1 h with the color changing from dark to light blue. After filtration through a 0.22 μm PES membrane, MeOH was removed under a nitrogen flow, and the residue was dissolved in H_2O (10 mL). Semi-preparative reverse-phase

HPLC purification (Luna®-5 μm -C18, 100 Å, 100 × 30 mm, 7–21% MeCN/H₂O–0.07% TFA gradient over 20 min, 20 mL/min, t_R = 9.5 min) provided **43** (820 μg , 42%) as a white solid.

For **43**: ¹H NMR (600 MHz, CD₃OD) δ 7.97 (s, 1H), 7.90–7.87 (m, 1H), 7.56 (s, 1H), 7.31 (d, J = 2.0 Hz, 1H), 7.14 (d, J = 8.9 Hz, 1H), 7.07 (d, J = 2.5 Hz, 1H), 6.94 (d, J = 8.7 Hz, 1H), 6.46 (d, J = 2.2 Hz, 1H), 6.41 (d, J = 2.1 Hz, 1H), 5.60 (d, J = 7.8 Hz, 1H), 5.50 (s, 1H), 5.42–5.37 (m, 2H), 5.31 (d, J = 5.7 Hz, 1H), 5.02 (d, J = 9.1 Hz, 2H), 4.98 (d, J = 5.6 Hz, 1H), 4.52–4.42 (m, 3H), 4.31–4.24 (m, 2H), 4.16 (s, 1H), 3.86–3.80 (m, 2H), 3.73 (dd, J = 11.7, 4.7 Hz, 2H), 3.58 (t, J = 9.2 Hz, 3H), 3.53 (q, J = 8.2 Hz, 2H), 2.75 (s, 2H), 2.65 (qd, J = 15.0, 5.1 Hz, 3H), 2.37 (d, J = 15.2 Hz, 2H), 2.11–2.04 (m, 2H), 1.96 (d, J = 13.4 Hz, 2H), 1.80 (s, 2H), 1.63 (td, J = 13.3, 5.9 Hz, 4H), 1.29 (s, 1H), 1.20 (d, J = 6.6 Hz, 3H), 1.00 (d, J = 6.2 Hz, 3H), 0.92 (d, J = 6.2 Hz, 3H); HRMS (ESI-TOF) m/z [M+2H]⁺ calcd for C₆₆H₇₅Cl₄N₉O₂₃, 751.6943; found, 751.6945.

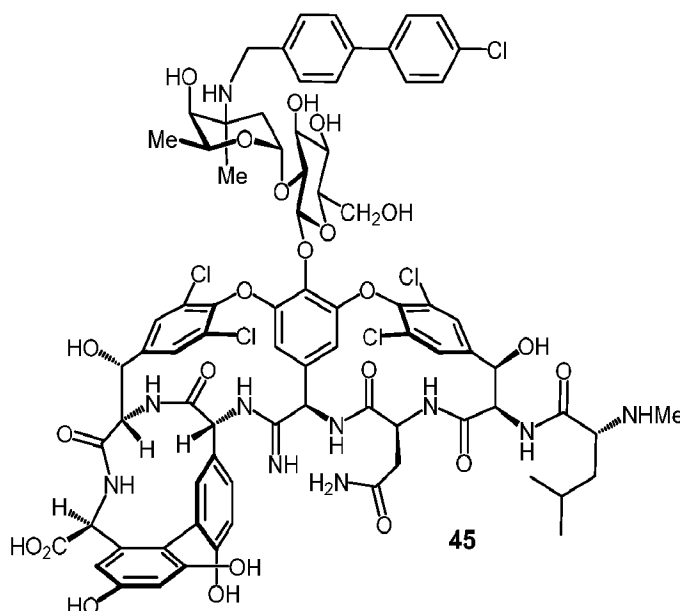


CBP-[Ψ[C(=S)NH]Tpg⁴]Tetrachlorovancomycin (**44**)

A solution of **41** (220 mg, 0.143 mmol, 1 equiv), 4-(4-chlorophenyl)benzaldehyde (40.4 mg, 0.186 mmol, 1.3 equiv), and *i*-Pr₂NEt (0.125 mL, 0.718 mmol, 5 equiv) in anhydrous DMF (5.5 mL)

was stirred at 70 °C for 2.5 h. The solution was subsequently treated with NaCNBH₃ (901 mg, 14.34 mmol, 100 equiv) and allow to stir at 70 °C for an additional 6 h. After cooling to ambient temperature, the mixture was diluted with H₂O (40 mL), and reverse-phase HPLC purification (Luna®-5 µm-C18, 100 Å, 100 × 30 mm, 1-40% MeCN/H₂O–0.07% TFA gradient over 20 min, 20 mL/min, *t_R* = 19 min) provided **44** (170 mg, 68%, 82% brsm) as a white solid and recovered starting material **41** (*t_R* = 12 min, 36.2 mg).

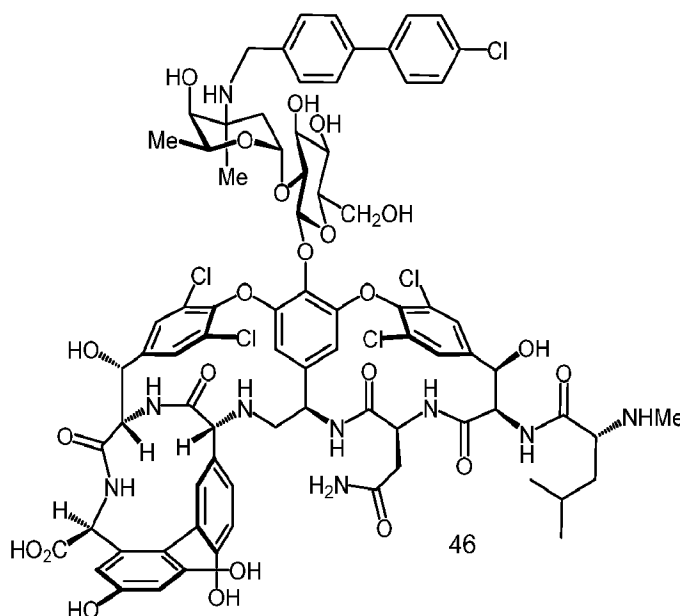
For **44**: ¹H NMR (600 MHz, CD₃OD) δ 7.77–7.68 (m, 5H), 7.62 (d, *J* = 8.6 Hz, 2H), 7.55 (d, *J* = 8.0 Hz, 2H), 7.49–7.44 (m, 2H), 7.22 (s, 1H), 6.94 (s, 2H), 6.79 (d, *J* = 8.8 Hz, 1H), 6.46 (d, *J* = 2.3 Hz, 1H), 6.40 (d, *J* = 2.3 Hz, 1H), 6.26 (s, 1H), 5.92 (s, 1H), 5.72 (s, 1H), 5.43 (s, 1H), 5.39 (s, 1H), 5.36–5.27 (m, 3H), 4.76 (d, *J* = 5.8 Hz, 2H), 4.30 (d, *J* = 12.4 Hz, 2H), 4.16 (d, *J* = 12.6 Hz, 1H), 4.11–4.05 (m, 2H), 3.93–3.79 (m, 3H), 3.77–3.74 (m, 1H), 3.64–3.54 (m, 3H), 3.02 (d, *J* = 15.9 Hz, 1H), 2.75 (s, 3H), 2.18 (d, *J* = 9.1 Hz, 2H), 2.04 (d, *J* = 13.3 Hz, 1H), 1.84 (dt, *J* = 13.8, 7.2 Hz, 2H), 1.80–1.71 (m, 2H), 1.67 (s, 3H), 1.29 (s, 2H), 1.26 (d, *J* = 6.5 Hz, 3H), 1.00 (d, *J* = 6.5 Hz, 3H), 0.97 (d, *J* = 6.0 Hz, 3H); ¹³C{¹H} NMR (151 MHz, CD₃OD) δ 200.5, 175.3, 175.1, 172.3, 171.6, 170.3, 169.8, 169.7, 168.2, 162.2, 161.9, 159.2, 158.9, 157.8, 156.6, 152.2, 151.5, 147.5, 146.4, 143.5, 142.4, 142.0, 139.9, 138.9, 137.0, 134.8, 134.2, 133.6, 131.9, 131.8, 131.5, 131.1, 130.6, 130.0, 129.7, 129.5, 129.4, 128.5, 128.1, 127.2, 125.9, 122.6, 120.3, 118.7, 118.4, 116.8, 116.5, 115.0, 114.6, 107.9, 106.7, 104.1, 102.8, 98.8, 80.0, 78.9, 78.1, 72.8, 72.5, 71.0, 70.1, 65.0, 64.0, 62.3, 61.6, 61.4, 61.0, 59.8, 58.4, 52.6, 49.8, 44.2, 40.4, 36.6, 34.4, 32.8, 25.4, 22.9, 22.7, 20.3, 17.3; HRMS (ESI-TOF) *m/z* [M+H]⁺ calcd for C₇₉H₈₂Cl₅N₉O₂₃S, 1732.3765; found, 1732.3741.



CBP-[Ψ[C(=NH)NH]Tpg⁴]Tetrachlorovancomycin (45**)**

A solution of **44** (1.51 mg, 0.87 μmol, 1 equiv) in saturated aqueous NH₄OAc (0.5 mL) was treated with AgOAc (6.0 mg, 36 μmol, 41 equiv). After the resulting mixture was stirred at ambient temperature for 24 h, HPLC purification (Phenomenex Luna® C18(2), 250 × 4.6 mm, 5 mL/min, 1–20% MeCN/H₂O–0.07% TFA over 3 min then 20–40% MeCN/H₂O–0.07% TFA over 30 min, *t_R* = 21 min) afforded **45** (1.12 mg, 75%) as a white solid.

For **45**: ¹H NMR (600 MHz, CD₃OD) δ 7.76 (s, 1H), 7.71 (d, *J* = 7.8 Hz, 2H), 7.63 (d, *J* = 8.3 Hz, 2H), 7.55 (d, *J* = 8.0 Hz, 1H), 7.47 (d, *J* = 8.4 Hz, 2H), 7.43 (s, 1H), 7.13 (s, 1H), 7.09 (d, *J* = 8.8 Hz, 1H), 6.89 (d, *J* = 8.6 Hz, 1H), 6.48 (s, 2H), 6.22 (s, 1H), 5.71 (s, 1H), 5.62 (s, 1H), 5.54–5.46 (m, 3H), 5.40 (s, 1H), 5.35 (s, 1H), 4.29 (s, 1H), 4.16 (d, *J* = 12.5 Hz, 2H), 4.06 (d, *J* = 12.6 Hz, 2H), 3.87 (d, *J* = 8.1 Hz, 2H), 3.83–3.75 (m, 3H), 3.60 (d, *J* = 10.0 Hz, 3H), 2.89 (s, 1H), 2.86 (s, 2H), 2.67 (s, 3H), 2.42 (d, *J* = 13.3 Hz, 2H), 2.19 (d, *J* = 14.3 Hz, 2H), 2.07 (dd, *J* = 19.5, 12.1 Hz, 3H), 1.87 (s, 1H), 1.76 (s, 1H), 1.65 (s, 3H), 1.35 (s, 1H), 1.27 (d, *J* = 6.6 Hz, 3H), 0.93 (m, 6H); HRMS (ESI-TOF) *m/z* [M+2H]⁺ calcd for C₇₉H₈₃Cl₅N₁₀O₂₃, 858.2116; found, 858.2078.

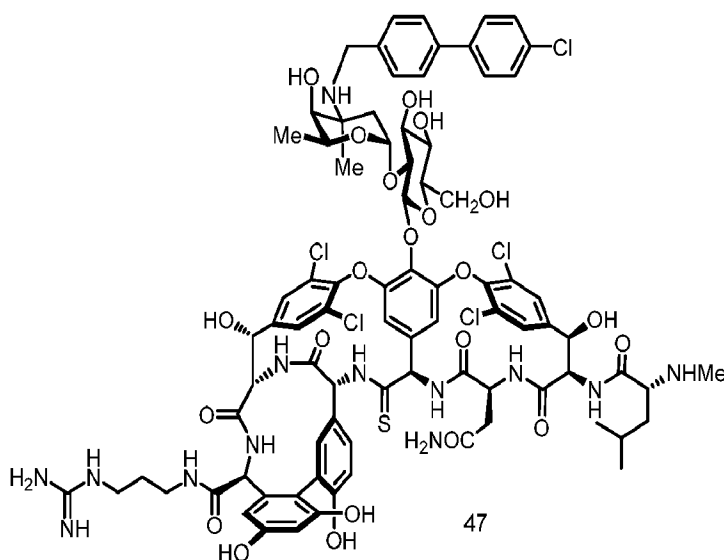


CBP- $[\Psi[\text{CH}_2\text{NH}]\text{Tpg}^4]$ Tetrachlorovancomycin (46**)**

A 4 mL glass vessel was charged with **44** (1.98 mg, 1.14 μmol , 1 equiv), anhydrous NiCl_2 (3.4 mg, 26.2 μmol , 23 equiv), anhydrous MeOH (2 mL), and 1,2-dichlorobenzene (0.2 mL). The reaction mixture was purged with Ar and cooled to -78°C . NaBH_4 (2.5 mg, 66 μmol , 60 equiv) was added and the mixture was stirred at -40°C , whereupon the solution color turned brown and then dark. After 40 min, the reaction was quenched by addition of cold H_2O (0°C , 0.07% TFA, 5 mL), and filtered through a 0.22 μm PES membrane, rinsing with MeOH. The filtrate was concentrated under a nitrogen flow to remove MeOH, diluted with H_2O (5 mL), and purified by semi-preparative reverse-phase HPLC (Luna $^{\text{®}}$ -5 μm -C18, 100 $\text{\AA}$, 100 $\times$ 30 mm, 18–28% MeCN/ H_2O –0.07% TFA gradient over 30 min, 20 mL/min, t_{R} = 24 min) to provide **46** (930 μg , 48%) as a white solid.

For **46**: ^1H NMR (600 MHz, CD_3OD) δ 7.98 (d, J = 2.0 Hz, 1H), 7.90 (d, J = 2.0 Hz, 1H), 7.70 (d, J = 8.2 Hz, 2H), 7.64–7.61 (m, 2H), 7.58–7.53 (m, 3H), 7.47 (d, J = 8.5 Hz, 2H), 7.33 (d, J = 2.0 Hz, 1H), 7.15 (dd, J = 8.6, 2.4 Hz, 2H), 7.06 (d, J = 2.4 Hz, 1H), 6.95 (d, J = 8.6 Hz, 1H), 6.45 (d, J = 2.3 Hz, 2H), 6.41 (d, J = 2.2 Hz, 2H), 5.64 (d, J = 7.8 Hz, 2H), 5.52 (s, 1H), 5.45 (d, J = 4.5 Hz, 2H), 5.39 (s, 1H), 5.31 (d, J = 5.7 Hz, 1H), 5.01 (d, J = 12.9 Hz, 3H), 4.96 (d, J = 5.0 Hz, 2H),

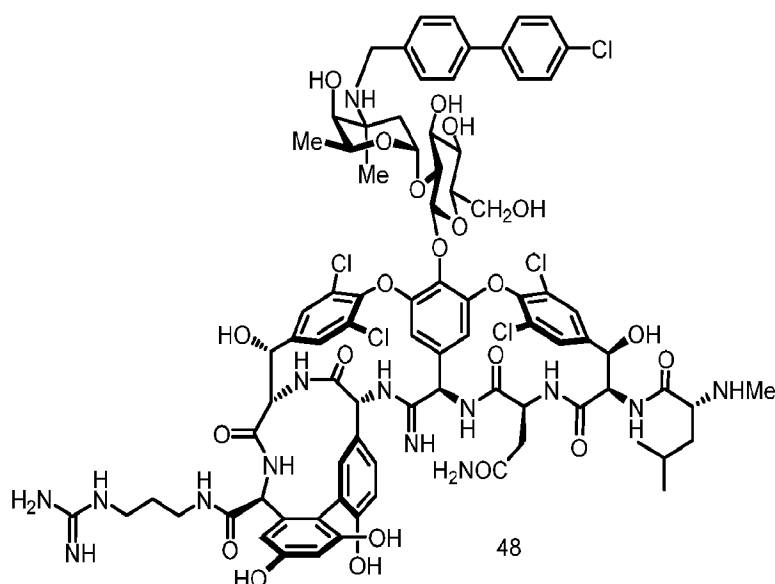
4.54–4.50 (m, 2H), 4.48 (t, $J = 5.1$ Hz, 2H), 4.28 (dd, $J = 8.9, 5.7$ Hz, 3H), 4.17 (d, $J = 12.6$ Hz, 2H), 4.14 (d, $J = 2.7$ Hz, 2H), 4.09 (d, $J = 12.6$ Hz, 2H), 3.88–3.81 (m, 4H), 3.73 (dd, $J = 11.6, 4.8$ Hz, 3H), 3.63 (s, 1H), 3.60 (t, $J = 9.2$ Hz, 4H), 3.53 (t, $J = 9.3$ Hz, 3H), 2.80 (s, 1H), 2.75 (s, 3H), 2.65 (d, $J = 5.7$ Hz, 2H), 2.37 (d, $J = 15.3$ Hz, 2H), 2.20 (d, $J = 9.3$ Hz, 2H), 2.05 (d, $J = 13.3$ Hz, 2H), 1.81 (s, 3H), 1.71 (s, 3H), 1.64–1.57 (m, 4H), 1.28 (d, $J = 6.6$ Hz, 3H), 1.01 (d, $J = 6.4$ Hz, 3H), 0.93 (d, $J = 6.3$ Hz, 3H); HRMS (ESI-TOF) m/z $[M+2H]^+$ calcd for $C_{79}H_{84}Cl_5N_9O_{23}$, 851.7140; found, 851.7128.



G3,CBP-[Ψ[C(=S)NH]Tpg⁴]Tetrachlorovancomycin (**47**)

A 4 mL glass vial was charged with **44** (10.0 mg, 5.76 μ mol, 1 equiv), 1-(3-aminopropyl)guanidine (bis-TFA salt, 20 mg, 58 μ mol, 10 equiv), *N*-methylmorpholine (NMM, 13 μ L, 0.115 mmol, 20 equiv), and anhydrous DMSO–DMF (1:1, 1.16 mL). After the resulting solution was treated with HBTU (22 mg, 58 μ mol, 10 equiv) and stirred at 23 °C under Ar for 20 min, 10 mL of H₂O was added to quench the reaction. Semi-preparative reverse-phase HPLC (Luna®-5 μ m-C18, 100 Å, 100 $\times$ 30 mm, 1–22% MeCN/H₂O–0.07% TFA gradient over 2 min then 22–35% MeCN/H₂O–0.07% TFA over 20 min, 20 mL/min, $t_R = 11.5$ min) provided **47** (9.3 mg, 88%) as a white solid.

For **47**: ^1H NMR (600 MHz, CD_3OD) δ 7.75 (s, 1H), 7.74–7.70 (m, 3H), 7.69 (s, 1H), 7.62 (d, $J = 8.2$ Hz, 2H), 7.55 (d, $J = 7.9$ Hz, 2H), 7.46 (d, $J = 8.3$ Hz, 2H), 7.24 (s, 1H), 7.02 (d, $J = 8.6$ Hz, 1H), 6.83 (d, $J = 8.5$ Hz, 1H), 6.46 (d, $J = 2.3$ Hz, 1H), 6.36 (d, $J = 2.3$ Hz, 1H), 6.22 (s, 1H), 5.85 (s, 1H), 5.67 (d, $J = 7.9$ Hz, 1H), 5.42 (d, $J = 4.6$ Hz, 1H), 5.38 (s, 1H), 5.35 (s, 1H), 5.32 (s, 2H), 4.62 (d, $J = 5.4$ Hz, 1H), 4.35 (s, 1H), 4.30 (d, $J = 9.2$ Hz, 1H), 4.17 (d, $J = 12.5$ Hz, 1H), 4.08 (d, $J = 12.9$ Hz, 2H), 3.86–3.81 (m, 2H), 3.75 (dd, $J = 11.7, 4.6$ Hz, 1H), 3.62 (s, 1H), 3.59 (d, $J = 9.3$ Hz, 1H), 3.53 (t, $J = 9.3$ Hz, 1H), 3.44 (d, $J = 13.6$ Hz, 2H), 3.35 (s, 2H), 3.23 (t, $J = 6.9$ Hz, 2H), 3.03 (d, $J = 15.8$ Hz, 1H), 2.75 (s, 3H), 2.21–2.09 (m, 3H), 2.05 (d, $J = 13.3$ Hz, 2H), 1.84 (dq, $J = 12.8, 6.7$ Hz, 3H), 1.81–1.73 (m, 2H), 1.66 (s, 3H), 1.26 (d, $J = 6.4$ Hz, 3H), 1.03 (d, $J = 6.1$ Hz, 3H), 0.98 (d, $J = 6.0$ Hz, 3H); $^{13}\text{C}\{^1\text{H}\}$ NMR (151 MHz, CD_3OD) δ 200.9, 175.1, 173.4, 172.2, 171.4, 170.5, 169.9, 168.2, 165.4, 163.0, 162.8, 162.6, 162.3, 158.5, 158.1, 157.3, 156.0, 151.9, 151.6, 147.0, 146.2, 143.2, 141.8, 139.6, 138.4, 137.8, 134.7, 133.1, 131.7, 131.6, 130.8, 130.6, 130.0, 129.3, 128.4, 127.3, 126.1, 122.6, 120.4, 118.8, 118.5, 116.6, 114.6, 107.9, 107.4, 106.1, 104.0, 102.8, 98.8, 80.2, 78.4, 77.9, 72.4, 72.3, 70.6, 70.0, 65.1, 63.9, 62.0, 61.6, 61.4, 61.2, 61.0, 60.0, 59.4, 52.5, 44.2, 40.3, 40.1, 39.9, 37.9, 37.5, 36.5, 34.3, 32.5, 29.2, 25.3, 22.9, 22.5, 20.1, 17.1; HRMS (ESI-TOF) m/z $[\text{M}+3\text{H}]^+$ calcd for $\text{C}_{83}\text{H}_{92}\text{Cl}_5\text{N}_{13}\text{O}_{22}\text{S}$, 610.8269; found, 610.8256.

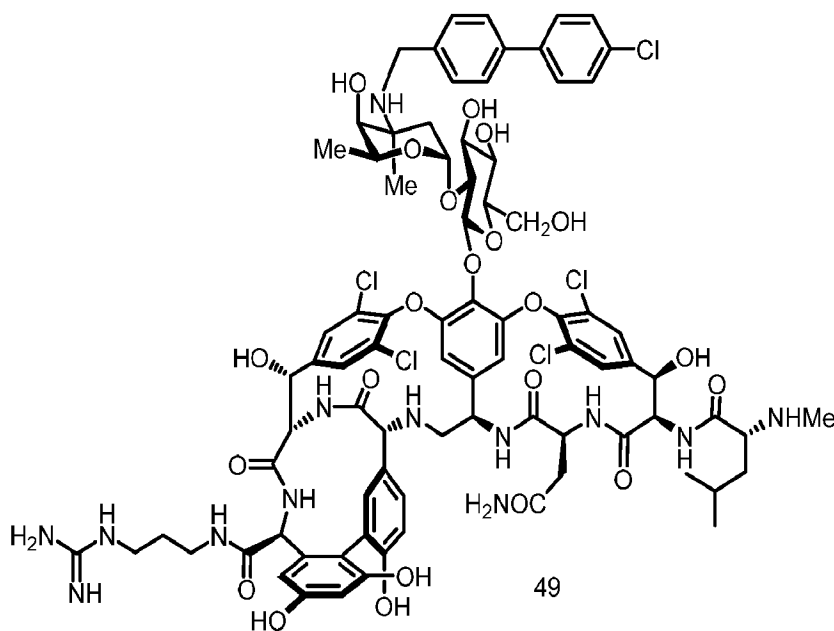


G3,CBP-[Ψ[C(=NH)NH]Tpg⁴]Tetrachlorovancomycin (**48**)

A solution of **47** (10.0 mg, 5.46 μmol, 1 equiv) in saturated aqueous NH₄OAc (2.2 mL) was treated with AgOAc (36.4 mg, 218 μmol, 40 equiv). After the resulting mixture was stirred at ambient temperature under Ar in the dark for 24 h, LCMS indicated >97% conversion of starting material. The mixture was diluted with H₂O (5 mL), and semi-preparative reverse-phase HPLC (Luna®-5 μm-C18, 100 Å, 100 × 30 mm, 1–22% MeCN/H₂O–0.07% TFA gradient over 2 min then 22–26% MeCN/H₂O–0.07% TFA over 20 min, 20 mL/min, *t_R* = 11 min) provided **48** (8.12 mg, 82%) as a white solid.

For **48**: ¹H NMR (600 MHz, CD₃OD) δ 8.33 (s, 1H), 7.83 (s, 1H), 7.76 (s, 1H), 7.75 (s, 2H), 7.71–7.68 (m, 2H), 7.62 (dd, *J* = 8.4, 3.8 Hz, 2H), 7.55 (t, *J* = 7.6 Hz, 2H), 7.46 (d, *J* = 8.3 Hz, 2H), 7.44 (d, *J* = 4.0 Hz, 1H), 7.28 (s, 2H), 7.10 (d, *J* = 8.6 Hz, 2H), 6.89 (dd, *J* = 8.6, 2.1 Hz, 1H), 6.48 (d, *J* = 1.7 Hz, 1H), 6.42 (s, 1H), 6.39 (d, *J* = 4.2 Hz, 1H), 6.20 (s, 1H), 5.69 (s, 1H), 5.62 (s, 3H), 5.52–5.45 (m, 4H), 5.35 (d, *J* = 2.0 Hz, 1H), 4.63 (s, 2H), 4.42 (s, 1H), 4.34 (s, 1H), 4.30 (s, 2H), 4.20 (d, *J* = 12.5 Hz, 1H), 4.15 (d, *J* = 12.5 Hz, 2H), 4.11 (d, *J* = 12.5 Hz, 1H), 4.05 (d, *J* = 12.6 Hz, 2H), 3.87 (t, *J* = 8.4 Hz, 2H), 3.79 (q, *J* = 12.3 Hz, 3H), 3.66 (s, 1H), 3.59 (d, *J* = 9.2 Hz, 4H), 3.44–3.41 (m, 1H), 3.23 (d, *J* = 6.9 Hz, 2H), 2.89 (s, 1H), 2.87 (d, *J* = 1.7 Hz, 3H), 2.83 (s, 1H), 2.40 (dd, *J* = 16.1, 4.9 Hz, 2H), 2.22–2.16 (m, 2H), 2.04 (d, *J* = 13.4 Hz, 2H),

1.84 (dq, $J = 15.9, 7.1$ Hz, 5H), 1.75 (s, 1H), 1.64 (d, $J = 8.7$ Hz, 3H), 1.60 (d, $J = 7.1$ Hz, 2H), 1.26 (d, $J = 6.5$ Hz, 3H), 0.86 (dd, $J = 10.4, 5.9$ Hz, 6H); $^{13}\text{C}\{^1\text{H}\}$ NMR (151 MHz, CD_3OD) δ 173.8, 173.4, 171.9, 170.8, 170.5, 169.4, 168.5, 161.9, 161.6, 159.5, 158.5, 157.5, 153.5, 152.8, 148.6, 146.7, 143.8, 142.2, 140.8, 140.0, 138.0, 137.1, 134.93, 134.88, 132.8, 132.1, 131.9, 130.9, 130.8, 130.5, 130.1, 129.9, 129.7, 129.6, 129.5, 129.2, 128.5, 127.8, 127.6, 123.5, 123.3, 120.6, 118.9, 118.7, 118.2, 116.9, 116.8, 114.8, 108.3, 106.9, 104.5, 103.9, 103.6, 98.6, 79.7, 78.8, 77.9, 72.3, 72.0, 71.2, 70.2, 65.1, 64.1, 62.5, 61.5, 61.2, 60.2, 60.1, 58.3, 55.1, 54.5, 52.4, 44.2, 40.8, 40.1, 38.0, 34.4, 32.5, 29.8, 25.3, 23.0, 22.3, 20.2, 17.3; HRMS (ESI-TOF) m/z $[\text{M}+3\text{H}]^+$ calcd for $\text{C}_{83}\text{H}_{93}\text{Cl}_5\text{N}_{14}\text{O}_{22}$, 605.1755; found, 605.1736.



G3,CBP-[Ψ[CH₂NH]Tpg⁴]Tetrachlorovancomycin (49)

A solution of **47** (5.10 mg, 2.78 μmol , 1 equiv), anhydrous NiCl_2 (7.0 mg, 54 μmol , 20 equiv) and 1,2-dichlorobenzene (0.3 mL) in anhydrous MeOH (3 mL) was purged with Ar and then cooled to -78°C . NaBH_4 (5.2 mg, 0.137 mmol, 50 equiv) was added and the mixture was stirred at -40°C for 40 min. The reaction was quenched by addition of cold H_2O (0°C , 0.07% TFA, 5 mL) and transferred into a saturated EDTA H_2O -MeOH (1:1, 5 mL), and the resulting mixture was stirred at

23 °C for 1 h with the color changing from dark to light blue. After filtration through a 0.22 µm PES membrane, rinsing with MeOH, and concentrated under reduced pressure, diluted with H₂O (5 mL), purification by semi-preparative reverse-phase HPLC (Luna®-5 µm-C18, 100 Å, 100 × 30 mm, 1–18% MeCN/H₂O–0.07% TFA gradient over 2 min then 18–28% MeCN/H₂O–0.07% TFA gradient over 20 min, 20 mL/min, *t_R* = 15 min) provided **49** (2.31 mg, 46%) as a white solid.

Alternatively, a solution of **46** (1.21 mg, 0.709 µmol, 1 equiv), 1-(3-aminopropyl)guanidine (bis-TFA salt, 1.20 mg, 3.49 µmol, 5 equiv) and *N*-methylmorpholine (NMM, 1.6 µL, 14 µmol, 20 equiv) in anhydrous DMSO and DMF (1:1, 0.3 mL) was treated with solid HBTU (2.7 mg, 7.1 µmol, 10 equiv). The mixture was stirred at ambient temperature for 30 min, and then the reaction was quenched by addition of H₂O (5 mL). Semi-preparative reverse-phase HPLC (Luna®-5 µm-C18, 100 Å, 100 × 30 mm, 1–40% MeCN/H₂O–0.07% TFA gradient over 20 min, 20 mL/min, *t_R* = 11 min) afforded **49** (994 µg, 78%) as a white solid.

For **49**: ¹H NMR (600 MHz, CD₃OD) δ 7.95 (d, *J* = 1.9 Hz, 1H), 7.89 (d, *J* = 1.9 Hz, 1H), 7.72–7.68 (m, 2H), 7.64–7.61 (m, 2H), 7.60 (d, *J* = 2.0 Hz, 1H), 7.57–7.54 (m, 2H), 7.48–7.45 (m, 2H), 7.33 (d, *J* = 2.0 Hz, 1H), 7.18 (d, *J* = 2.4 Hz, 1H), 7.17 (d, *J* = 2.4 Hz, 1H), 7.15 (d, *J* = 2.4 Hz, 1H), 6.95 (d, *J* = 8.6 Hz, 1H), 6.45 (d, *J* = 2.3 Hz, 1H), 6.35 (d, *J* = 2.3 Hz, 1H), 5.63 (d, *J* = 7.8 Hz, 1H), 5.51–5.47 (m, 2H), 5.45 (d, *J* = 4.6 Hz, 1H), 5.33 (d, *J* = 5.7 Hz, 1H), 5.08 (d, *J* = 9.6 Hz, 2H), 5.06–5.02 (m, 2H), 4.97 (d, *J* = 2.1 Hz, 1H), 4.54 (t, *J* = 5.3 Hz, 1H), 4.40 (dd, *J* = 15.2, 9.6 Hz, 2H), 4.34–4.27 (m, 3H), 4.17 (d, *J* = 12.6 Hz, 2H), 4.09 (d, *J* = 12.6 Hz, 2H), 3.87–3.80 (m, 3H), 3.74 (dd, *J* = 11.6, 4.7 Hz, 2H), 3.63 (s, 1H), 3.60 (t, *J* = 9.2 Hz, 2H), 3.54 (t, *J* = 9.3 Hz, 2H), 3.42 (d, *J* = 1.4 Hz, 1H), 3.39 (q, *J* = 6.6 Hz, 2H), 3.23 (t, *J* = 7.0 Hz, 2H), 2.77 (s, 3H), 2.70 (dd, *J* = 15.1, 4.5 Hz, 2H), 2.62 (dd, *J* = 15.1, 6.1 Hz, 2H), 2.39 (d, *J* = 15.2 Hz, 2H), 2.20 (dd, *J* = 13.6, 4.7 Hz, 2H), 2.06 (d, *J* = 13.3 Hz, 2H), 1.84 (p, *J* = 7.0 Hz, 3H), 1.81–1.76 (m, 2H), 1.71 (s, 3H), 1.63 (ddt, *J* = 21.3, 14.2, 7.7 Hz, 4H), 1.27 (d, *J* = 6.5 Hz, 3H), 0.99 (d, *J* = 6.2 Hz, 3H), 0.92 (d, *J* = 6.2 Hz, 3H); ¹³C{¹H} NMR (151 MHz, CD₃OD)

δ 175.5, 174.1, 173.0, 171.6, 170.6, 170.2, 169.5, 168.1, 165.0, 161.9, 159.2, 158.8, 157.9, 156.4, 152.7, 151.8, 147.5, 146.5, 143.7, 142.6, 142.1, 139.9, 138.0, 137.5, 137.2, 134.9, 133.7, 132.0, 131.9, 130.7, 130.5, 130.3, 130.1, 129.7, 129.4, 128.6, 128.5, 128.2, 127.8, 127.6, 127.5, 122.9, 120.5, 118.8, 116.9, 116.6, 115.0, 114.7, 108.2, 108.0, 105.7, 104.1, 102.7, 98.7, 79.8, 79.0, 78.2, 72.9, 72.6, 71.2, 70.0, 65.0, 64.0, 62.5, 61.54, 61.48, 59.6, 56.6, 55.6, 52.6, 44.2, 41.6, 40.6, 40.3, 39.6, 37.1, 36.8, 34.5, 32.5, 25.5, 23.2, 22.5, 20.3, 17.2; HRMS (ESI-TOF) m/z $[M+2H]^+$ calcd for $C_{83}H_{94}Cl_5N_{13}O_{22}$, 900.7618; found, 900.7598.

Model Ligand Binding Studies

The binding of tetrachlorovancomycin (Compound **1**) and its aglycon Compound **29** 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 Compound **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.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.

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 (Compound **33**).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 Ac₂-L-Lys-D-Ala-D-Ala (ITC $K_a = 2.9 \times 10^5 \text{ M}^{-1}$, for G3-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

Titration Binding Studies with Model D-Ala-D-Ala and D-Ala-D-Lac Ligands

The binding constants for association with the model ligands *N,N'*-Ac₂-Lys-D-Ala-D-Ala (**A**) and *N,N'*-Ac₂-Lys-D-Ala-D-Lac (**B**) were determined according to literature protocol.^{45,11a} UV difference experiments were carried out on a CARY 3E UV-Vis spectrometer. UV scans were run with a baseline correction that consisted of 20 mM sodium citrate buffer (pH = 5.1) and covered a range from 200 to 345 nm. A solution of the tetrachlorovancomycin derivative ($8 \times 10^{-5} \text{ M}$ in 20 mM sodium citrate buffer) was placed in a quartz UV cuvette (0.1 cm path length) and the UV spectrum recorded versus a reference cell containing 20 mM sodium citrate buffer. UV spectra were recorded after each addition of a solution of *N,N'*-Ac₂-Lys-D-Ala-D-Ala (**A**) or *N,N'*-Ac₂-Lys-D-Ala-D-Lac (**B**) in 20 mM sodium citrate buffer to each cell from 0.1 to up to 60.0 equiv for the weaker binding partners. The absorbance value at the λ_{max} was recorded, measuring the running change in absorbance. The binding constants were calculated from the well-defined binding curves that plot the absorbance readings versus equiv ligand added

$([\text{ligand}]/[\text{tetrachlorovancomycin analogue}])$.^{45,11a}

The accuracy of the measured binding constants, especially for the weak binding partners, was improved by titration with sufficient ligand to characterize the binding event at the dilute concentrations employed and with use of direct curve fitting methods^{45,11a} (vs Scatchard analysis) and single site binding to

quantitate the results of the well-behaved binding curves. The results are summarized in Figs. 8A and 8B alongside those of vancomycin and its tetrachloro analogues.

ITC measurements were carried out in a MicroCal™ Auto-iTC₂₀₀ system with 400 mL of antibiotic solution as the cell sample and 120 mL of ligand solution as syringe sample (2.5 mL of each injection volume). Control titration runs were conducted by using blank buffer solution against blank buffer solution, each antibiotic, and the two ligands, respectively to show no heat contribution from the individual binding components. The titration data were processed by using OriginLab software (for ITC) and “one set of sites” fitting model for curve fitting.

UV difference experiments were carried out on a CARY 3E UV-Vis spectrometer. UV scans were run with a baseline correction that consisted of 20 mM sodium citrate buffer (pH = 5.1) and covered a range from 200 to 345 nm. A solution of the tetrachloro-vancomycin derivative (8×10^{-5} M in 20 mM sodium citrate buffer) was placed in a quartz UV cuvette (0.1 cm path length) and the UV spectrum recorded versus a reference cell containing 20 mM sodium citrate buffer. UV spectra were recorded after each addition of a solution of *N,N'*-Ac₂-Lys-D-Ala-D-Ala (**A**) or *N,N'*-Ac₂-Lys-D-Ala-D-Lac (**B**) in 20 mM sodium citrate buffer to each cell from 0.1 to up to 60.0 equiv for the weaker binding partners. The absorbance value at the λ_{max} was recorded, measuring the running change in absorbance.

***In Vitro* Antimicrobial Activity Assay of Pocket and Peripherally Modified Tetrachlorovancomycins⁵⁵**

One day before studies were run, fresh cultures of vancomycin-sensitive *Staphylococcus aureus* (VSSA strain ATCC 25923), methicillin and oxacillin-resistant *Staphylococcus aureus* (MRSA strain ATCC 43300), vancomycin-resistant *Enterococcus faecium* (VanA VRE, ATCC BAA-2317), vancomycin-resistant *Enterococcus faecalis* (VanA VRE, ATCC BAA-2573) and (VanB VRE, TX-2516), vancomycin-

sensitive *Enterococcus faecium* (unknown origin) and vancomycin-sensitive *Enterococcus faecalis* (unknown origin) were inoculated and grown in an orbital shaker at 37 °C in 100% Mueller-Hinton (MH, for VSSA and MRSA) or brain-heart infusion (BHI, for VRE and VSE) broth.

After 24 h, the bacterial stock solutions were serially diluted with 5% MH (for VSSA and MRSA) or BHI (for VRE) broth supplemented by 0.002% P-80 to achieve a turbidity equivalent to a 1:100 dilution of a 0.5 M McFarland solution (bacteria concentration = 1.5×10^6 CFU/mL). This diluted bacterial stock solution was then inoculated in a 96-well U-shaped glass coated microtiter plate, supplemented with serially diluted aliquots of the antibiotic solution in DMSO (4 µL), to achieve a total assay volume of 0.1 mL. The plate was then incubated at 37 °C for 18 h, after which minimal inhibitory concentrations (MICs) were determined by monitoring the cell growth (observed as a pellet) in the wells.

The lowest concentration of antibiotic (in µg/mL) capable of eliminating cell growth in the wells is the reported MIC value. The reported MIC values for the vancomycin analogues were determined against vancomycin as a standard in the first well. For the thioamide series, the initial fresh cultures were grown in the presence of vancomycin (1 µg/mL) for resistant strains and chloramphenicol for sensitive strains (1 µg/mL).

In the instances of protein removal, filtration of the broth through an Amicon MWCO 3000 membrane was used for protein removal. Notably, assessing the antimicrobial activity of [Y(CH₂NH)]Tpg⁴]tetrachlorovancomycin (Compound **49**) with protein removal had no effect on potency against any of the strains examined, whereas small improvements in the MICs of [□[C(=NH)NH]Tpg⁴]-tetrachlorovancomycin (Compound **48**) were observed against a few strains as indicated in the tabulated data in Fig. 9.

CITATIONS

1. (a) McCormick, M. H.; McGuire, J. M.; Pittenger, G. E.; Pittenger, R. C.; Stark, W. M. Vancomycin, a new antibiotic. I. Chemical and biologic properties *Antibiot. Ann.* **1955**, 3, 606–611. (b)

- McGuire, J.; Wolfe, R.; Ziegler, D. Vancomycin, a new antibiotic. II. In vitro antibacterial studies. *Antibiot. Ann.* **1955**, 3, 612–618.
2. (a) Kahne, D.; Leimkuhler, C.; Lu, W.; Walsh, C. Glycopeptide and lipoglycopeptide antibiotics. *Chem. Rev.* **2005**, 105, 425–448. (b) Levine, D. P. Vancomycin: A history. *Clin. Infect. Dis.* **2006**, 42, S5–S12.
3. James, R. C.; Pierce, J. G.; Okano, A.; Xie, J.; Boger, D. L. Redesign of glycopeptide antibiotics: Back to the future. *ACS Chem. Biol.* **2012**, 7, 797–804.
4. Wu, Z.-C.; Boger, D. L. Maxamycins: Durable antibiotics derived by rational redesign of vancomycin. *Acc. Chem. Res.* **2020**, 53, 2587–2599.
5. Okano, A.; Isley, N. A.; Boger, D. L. Total synthesis of vancomycin related glycopeptide antibiotics and key analogues. *Chem. Rev.* **2017**, 117, 11952–11993.
6. Moore, M. J.; Qu, S.; Tan, C.; Cai, Y.; Mogi, Y.; Keith, D. J.; Boger, D. L. Next-generation total synthesis of vancomycin. *J. Am. Chem. Soc.* **2020**, 142, 16039–16050.
7. Moore, M. J.; Qin, P.; Keith, D. J.; Wu, Z.-C.; Jung, S.; Chatterjee, S.; Tan, C.; Qu, S.; Cai, Y.; Stanfield, R. L.; Boger, D. L. Divergent total synthesis and characterization of maxamycins, submitted.
8. Harris, C. M.; Kannan, R.; Kopecka, H.; Harris, T. M. The role of the chlorine substituents in the antibiotic vancomycin: preparation and characterization of mono- and didechlorovancomycin. *J. Am. Chem. Soc.* **1985**, 107, 6652–6658.
9. Wadzinski, T. J.; Gea, K. D.; Miller, S. J. A stepwise dechlorination/cross-coupling strategy to diversify the vancomycin ‘in-chloride’. *Bioorg. Med. Chem. Lett.* **2016**, 26, 1025–1028.
10. Pinchman, J. R.; Boger, D. L. Investigation into the functional impact of the vancomycin C-ring aryl chloride. *Bioorg. Med. Chem. Lett.* **2013**, 23, 4817–4819.
11. (a) Nieto, M.; Perkins, H. R. Modifications of the acyl-D-alanyl-D-alanine terminus affecting complex-formation with

- vancomycin. *Biochem. J.* **1971**, 123, 789–803. (b) Perkins, H. R. Vancomycin and related antibiotics. *Pharmacol. Ther.* **1982**, 16, 181–197.
12. Boger, D. L.; Miyazaki, S.; Loiseleur, O.; Beresis, R. T.; Castle, S. L.; Wu, J. H.; Jin, Q. Thermal atropisomerism of aglucovancomycin derivatives: Preparation of (*M,M,M*)- and (*P,M,M*)-aglucovancomycins. *J. Am. Chem. Soc.* **1998**, 120, 8920–8926.
13. Jeffs, P. W.; Muller, L.; DeBrosse, C.; Heald, S. L.; Fisher, R. The structure of aridicin A. An integrated approach employing 2D NMR, energy minimization and distance constraints. *J. Am. Chem. Soc.* **1986**, 108, 3063–3075.
14. Zmijewski, M. J.; Briggs, B. Biosynthesis of vancomycin: Identification of TDP-glucose:aglycosyl-vancomycin glucosyltransferase from *Amycolatopsis orientalis*. *FEMS Microbiol. Lett.* **1989**, 59, 129–134.
15. Losey, H. C.; Peczuh, M. W.; Chen, Z.; Eggert, U. S.; Dong, S. D.; Pelczer, I.; Kahne, D.; Walsh, C. T. Tandem action of glycosyltransferases in the maturation of vancomycin and teicoplanin aglycones: Novel glycopeptides. *Biochemistry* **2001**, 40, 4745–4755.
16. Thayer, D. A.; Wong C-H. Vancomycin analogues containing monosaccharides exhibit improved antibiotic activity: A combined one-pot enzymatic glycosylation and chemical diversification strategy. *Chem.-Asian J.* **2006**, 1, 445–452.
17. Hubbard, B. K.; Walsh, C. T. Vancomycin assembly: Nature's way. *Angew. Chem. Int. Ed.* **2003**, 42, 730–765.
18. Nakayama, A.; Okano, A.; Feng, Y.; Collins, J. C.; Collins, K. C.; Walsh, C. T.; Boger, D. L. Enzymatic glycosylation of vancomycin aglycon: Completion of a total synthesis of vancomycin and N- and C-terminus substituent effects of the aglycon substrate. *Org. Lett.* **2014**, 16, 3572–3575.
19. Thompson, C.; Ge, M.; Kahne, D. Synthesis of vancomycin from the aglycon. *J. Am. Chem. Soc.* **1999**, 121, 1237–1244.

20. (a) Nicolaou, K. C.; Mitchell, H. J.; Jain, N. F.; Winssinger, N.; Hughes, R.; Bando, T. Total synthesis of vancomycin. *Angew. Chem. Int. Ed.* **1999**, 38, 240–244. (b) Nicolaou, K. C.; Mitchell, H. J.; Jain, N. F.; Bando, T.; Hughes, R.; Winssinger, N.; Natarajan, S.; Koumbis, A. E. Total synthesis of vancomycin—part 4: Attachment of the sugar moieties and completion of the synthesis. *Chem. Eur. J.* **1999**, 5, 2648–2667.
21. Ritter, T. K.; Mong, K.-K. T.; Liu, T.; Nakatani, T.; Wong, C.-H. A programmable one-pot oligosaccharide synthesis for diversifying the sugar domains of natural products: A case study of vancomycin. *Angew. Chem. Int. Ed.* **2003**, 42, 4657–4660.
22. (a) Crimmins, M. T.; She, J. An improved procedure for asymmetric aldol additions with N-acyl oxazolidinones, oxazolidinethiones and thiazolidinethiones. *Synlett* **2004**, 1371–1374. (b) Patel, J.; Clavé, G.; Renard, P.-Y.; Franck, X. Straightforward access to protected syn α -amino- β -hydroxy acid derivatives. *Angew. Chem. Int. Ed.* **2008**, 47, 4224–4227.
23. Evans, D. A.; Wood, M. R.; Trotter, B. W.; Richardson, T. I.; Barrow, J. C.; Katz, J. L. Total syntheses of vancomycin and eremomycin aglycons. *Angew. Chem. Int. Ed.* **1998**, 37, 2700–2704.
24. (a) Solladié-Cavallo, A.; Nsenda, T. A four-step synthesis of erythro-*m*-chloro-3-hydroxytyrosine ethyl ester enantiomerically pure. *Tetrahedron Lett.* **1998**, 39, 2191–2194. (b) Crowley, B. M.; Mori, Y.; McComas, C. C.; Tang, D.; Boger, D. L. Total synthesis of the ristocetin aglycon. *J. Am. Chem. Soc.* **2004**, 126, 4310–4317.
25. Dunetz, J. R.; Xiang, Y.; Baldwin, A.; Ringling, J. General and scalable amide bond formation with epimerization prone substrates using T3P® and pyridine. *Org. Lett.* **2011**, 13, 5048–5051.
26. Nicolaou, K. C.; Estrada, A. A.; Zak, M.; Lee, S. H.; Safina, B. S. A mild and selective method for the hydrolysis of esters with trimethyltin hydroxide. *Angew. Chem. Int. Ed.* **2005**, 44, 1378–1382.

27. Boger, D. L.; Borzilleri, R. M.; Nukui, S.; Beresis, R. T. Synthesis of the vancomycin CD and DE ring systems. *J. Org. Chem.* **1997**, 62, 4721–4736.
28. Kunishima, M.; Kawachi, C.; Iwasaki, F.; Terao, K.; Tani, S. Synthesis and characterization of 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride. *Tetrahedron Lett.* **1999**, 40, 5327–5330.
29. In addition to improving the convergency of the approach, the incorporation of the D ring amino acid into tetrapeptide **17** also avoids the challenging and epimerization prone coupling of the corresponding E ring tripeptide with the D ring amine when embedded in the ABCD ring system (see cites 6 and 7).
30. Prieto, M.; Mayor, S.; Rodríguez, K.; Lloyd-Williams, P.; Giralt, E. Racemization in Suzuki couplings: A quantitative study using 4-hydroxyphenylglycine and tyrosine derivatives as probe molecules. *J. Org. Chem.* **2007**, 72, 1047–1050.
31. (a) Ji, Y.; Plata, R. E.; Regens, C. S.; Hay, M.; Schmidt, M.; Razler, T.; Qiu, Y.; Geng, P.; Hsiao, Y.; Rosner, T.; Eastgate, M. D.; Blackmond, D. G. Mono-oxidation of bidentate bis-phosphines in catalyst activation: Kinetic and mechanistic studies of a Pd/Xantphos-catalyzed C–H functionalization. *J. Am. Chem. Soc.* **2015**, 137, 13272–13281. (b) Beutner, G.; Carrasquillo, R.; Geng, P.; Hsiao, Y.; Huang, E. C.; Janey, J.; Katipally, K.; Kolotuchin, S.; La Porte, T.; Lee, A.; Lobben, P.; Lora-Gonzalez, F.; Mack, B.; Mudryk, B.; Qiu, Y.; Qian, X.; Ramirez, A.; Razler, T. M.; Rosner, T.; Shi, Z.; Simmons, E.; Stevens, J.; Wang, J.; Wei, C.; Wisniewski, S. R.; Zhu, Y. Adventures in atropisomerism: Total synthesis of a complex active pharmaceutical ingredient with two chirality axes. *Org. Lett.* **2018**, 20, 3736–3740.
32. De Mico, A.; Margarita, R.; Parlanti, L.; Vescovi, A.; Piancatelli, G. A versatile and highly selective hypervalent iodine (III)/2,2,6,6-tetramethyl-1-piperidinyloxy-mediated oxidation of alcohols to carbonyl compounds. *J. Org. Chem.* **1997**, 62, 6974–6977.

33. He, C.; Chu, H.; Stratton, T. P.; Kossler, D.; Eberle, K. J.; Flood, D. T.; Baran, P. S. Total synthesis of tagetitoxin. *J. Am. Chem. Soc.* **2020**, *142*, 13683–13688.
34. Raw, S. A. An improved process for the synthesis of DMTMM-based coupling reagents. *Tetrahedron Lett.* **2009**, *50*, 946–948.
35. Boger, D. L.; Miyazaki, S.; Kim, S. H.; Wu, J. H.; Castle, S. L.; Loiseleur, O.; Jin, Q. Total synthesis of the vancomycin aglycon. *J. Am. Chem. Soc.* **1999**, *121*, 10004–10011.
36. Lin, L. S.; Lanza, T.; de Laszlo, S. E.; Truong, Q.; Kamenecka, T.; Hagmann, W. K. Deprotection of N-tert-butoxycarbonyl (Boc) groups in the presence of tert-butyl esters. *Tetrahedron Lett.* **2000**, *41*, 7013–7016.
37. Smith, G. G.; Sivakua, T. Mechanism of the racemization of amino acids. Kinetics of racemization of arylglycines. *J. Org. Chem.* **1983**, *48*, 627–634.
38. Li, H.; Jiang, X.; Ye, Y.-h.; Fan, C.; Romoff, T.; Goodman, M. 3-(Diethoxy-phosphoryloxy)-1,2,3-benzotriazin-4 (3H)-one (DEPBT): A new coupling reagent with remarkable resistance to racemization. *Org. Lett.* **1999**, *1*, 91–94.
39. Nicolaou, K.; Boddy, C. N.; Bräse, S.; Winssinger, N., Chemistry, biology, and medicine of the glycopeptide antibiotics. *Angew. Chem. Int. Ed.* **1999**, *38*, 2096–2152.
40. Moorthy, J. N.; Singhal, N. Facile and highly selective conversion of nitriles to amides via indirect acid-catalyzed hydration using TFA or AcOH–H₂SO₄. *J. Org. Chem.* **2005**, *70*, 1926–1929.
41. Moore, M. J.; Qin, P.; Keith, D. J.; Boger, D. L. Improved preparative enzymatic glycosylation of vancomycin aglycon and analogues. *Tetrahedron* **2023**, *131*, Article 133211.
42. Unverzagt, C.; Kunz, H.; Paulson, J. C. High-efficiency synthesis of sialyloligosaccharides and sialoglycopeptides. *J. Am. Chem. Soc.* **1990**, *112*, 9308–9309.

43. Nagarajan, R.; Schabel, A.; Occolowitz, J.; Counter, F.; Ott, J.; Felty–Duckworth, A. Synthesis and antibacterial evaluation of N-alkyl vancomycins. *J. Antibiot.* **1989**, *42*, 63–72.
44. Wu, Z.–C.; Cameron, M. D.; Boger, D. L. Vancomycin C-terminus guanidine modifications and further insights into an added mechanism of action imparted by a peripheral structural modification. *ACS Infect. Dis.* **2020**, *6*, 2169–2180.
45. Perkins, H. R. Specificity of combination between mucopeptide precursors and vancomycin or ristocetin. *Biochem. J.* **1969**, *111*, 195–205.
46. Thordarson, P. Determining association constants from titration experiments in supramolecular chemistry. *Chem. Soc. Rev.* **2011**, *40*, 1305–1323.
47. McPhail, D.; Cooper, A. Microcalorimetric studies of ligand-induced vancomycin dimerisation and molecular recognition; in Coleman, A.W. ed, *Molecular Recognition and Inclusion*, Springer, 1998, pp 415–418.
48. Tetrachlorovancomycin (**1**), like vancomycin, fails to bind Ac₂-L-Lys-D-Ala-D-Lac (**33**) to an appreciable extent ($K_a < 470 \text{ M}^{-1}$) being 1000-fold less effective than its binding with Ac₂-L-Lys-D-Ala-D-Ala.
49. (a) Allen, N. E.; Hobbs, J. N., Jr.; Nicas, T. I. Inhibition of peptidoglycan biosynthesis in vancomycin-susceptible and -resistant bacteria by a semisynthetic glycopeptide antibiotic. *Antimicrob. Agents Chemother.* **1996**, *40*, 2356–2362. (b) Goldman, R. C.; Baizman, E. R.; Longley, C. B.; Branstrom, A. Chlorobiphenyl-desleucyl-vancomycin inhibits the transglycosylation process required for peptidoglycan synthesis in bacteria in the absence of dipeptide binding. *FEMS Microbiol. Lett.* **2000**, *183*, 209–214.
50. (a) Ge, M.; Chen, Z.; Russell, H.; Onishi; Kohler, J.; Silver, L. L.; Kerns, R.; Fukuzawa, S.; Thompson, C.; Kahne, D. Vancomycin derivatives that inhibit peptidoglycan biosynthesis without binding D-Ala-D-Ala. *Science* **1999**, *284*, 507–511. (b) Chen, L.; Walker, D.; Sun, B.; Hu, Y.; Walker, S.; Kahne, D. Vancomycin analogues active

against VanA-resistant strains inhibit bacterial transglycosylase without binding substrate. *Proc. Natl. Acad. Sci. USA* **2003**, *100*, 5658–5663.

51. Okano, A.; Isley, N. A.; Boger, D. L. Peripheral modifications of [Y(CH₂NH)Tpg⁴]vancomycin with added synergistic mechanisms of action provide durable and potent antibiotics. *Proc. Natl. Acad. Sci. U.S.A.* **2017**, *114*, E5052–E5061.

52. Wu, Z.-C.; Isley, N. A.; Okano, A.; Weiss, W. J.; Boger, D. L. C1-CBP-Vancomycin: Impact of a vancomycin C-terminus trimethylammonium cation on pharmacological properties and insights into its newly introduced mechanism of action, *J. Org. Chem.* **2020**, *85*, 1365–1375.

53. Wright, P. M.; Seiple, I. B.; Myers, A. G. The evolving role of chemical synthesis in antibacterial drug discovery. *Angew. Chem. Int. Ed.* **2014**, *53*, 8840–8869.

54. Dyke, J. M.; Groves, A. P.; Morris, A.; Ogden, J. S.; Dias, A. A.; Oliveira, A. M. S.; Costa, M. L.; Barros, M. T.; Cabral, M. H.; Moutinho, A. M. C. Study of the thermal decomposition of 2-azidoacetic acid by photoelectron and matrix isolation infrared spectroscopy. *J. Am. Chem. Soc.* **1997**, *119*, 6883–6887.

55. Clinical and Laboratory Standards Institute, *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically*, Approved Standard, 7th ed, CLSI document M07-A8, Clinical and Laboratory Standards Institute: Wayne, PA; 2009.

56. Moore, M. J.; Qu, S.; Tan, C.; Cai, Y.; Mogi, Y.; Keith, D. J.; Boger, D. L. Next-generation total synthesis of vancomycin. *J. Am. Chem. Soc.* **2020**, *142*, 16039–16050.

57. Moore, M. J.; Qin, P.; Yamasaki, N.; Zeng, X.; Keith, D. J.; Jung, S.; Fukazawa, T.; Graham-O'Regan, K.; Wu, Z.-C.; Chatterjee, S.; Boger, D. L. Tetrachlorovancomycin: total synthesis of a designed glycopeptide antibiotic of reduced synthetic complexity, *J. Am. Chem. Soc.* **2023**, *145*, 21132–21141.

58. Moore, M. J.; Qin, P.; Keith, D. J.; Wu, Z.-C.; Jung, S.; Chatterjee, S.; Tan, C.; Qu, S.; Cai, Y.; Stanfield, R. L.; Boger, D. L. Divergent total synthesis and characterization of maxamycins. *J. Am. Chem. Soc.* **2023**, *145*, 12837–12852.

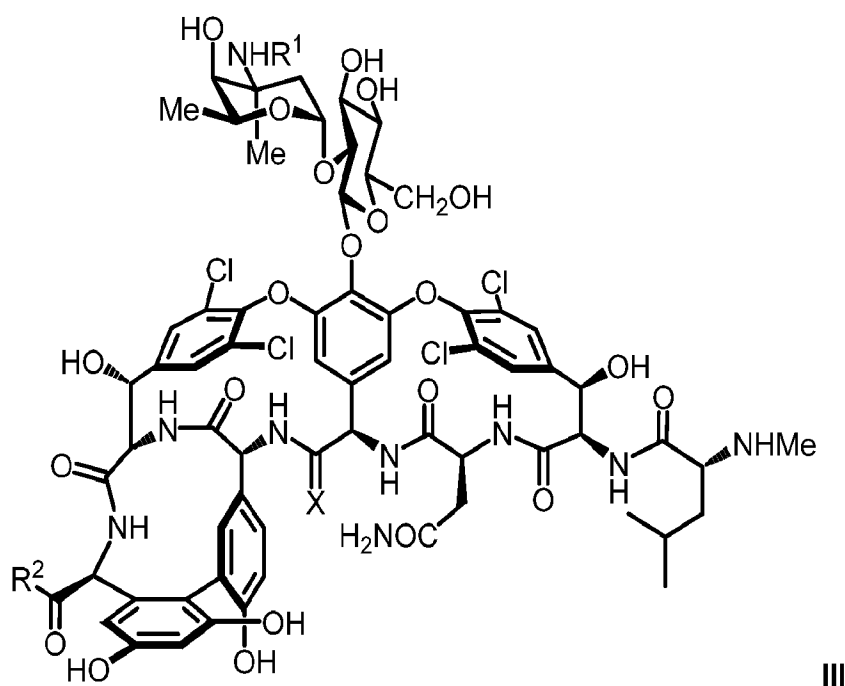
59. It has been shown elsewhere that addition of the peripheral 4-chlorobiphenylmethyl (CBP) group to vancomycin and related structures does not impact (increase) the solution phase binding affinity for model ligands: Allen, N. E.; Nicas, T. I. Mechanism of action of oritavancin and related glycopeptide antibiotics, *FEMS Microbiol. Rev.* **2003**, *26*, 511–532. Similarly, we have found that a vancomycin G3 C-terminus modification does not impact (increase) the binding to Ac₂-L-Lys-D-Ala-D-Ala.⁵⁸

60. (a) Perkins, H. R. Specificity of combination between mucopeptide precursors and vancomycin or ristocetin. *Biochem. J.* **1969**, *111*, 195–205. (b) Nieto, M.; Perkins, H. R. Modifications of the acyl-D-alanyl-D-alanine terminus affecting complex-formation with vancomycin. *Biochem. J.* **1971**, *123*, 789–803.

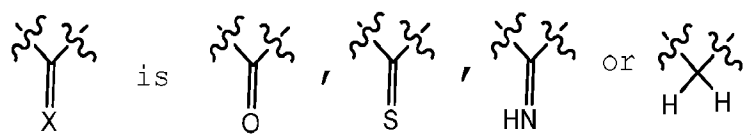
61. (a) Thordarson, P. Determining association constants from titration experiments in supramolecular chemistry. *Chem. Soc. Rev.* **2011**, *40*, 1305–1323. (b) Hibbert, D. B.; Thordarson, P. The death of the Job plot, transparency, open science and online tools, uncertainty estimation methods and other developments in supramolecular chemistry data analysis. *Chem. Commun.* **2016**, *52*, 12792–12805. The program used may be accessed free at supramolecular.org or directly at app.supramolecular.org/bindfit.

CLAIMS

1. A compound that corresponds in structure to that shown in Formula III or its pharmaceutically acceptable salt,



wherein

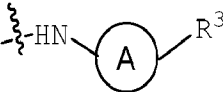


R¹ is selected from the group consisting of hydrido, (C₁-C₁₆)hydrocarbyl, aryl(C₁-C₆)-hydrocarbyldiyl, heteroaryl(C₁-C₆)hydrocarbyldiyl, (C₁-C₆)hydrocarbyldiylheteroaryl, halo(C₁-C₁₂)-hydrocarbyldiyl, and (C₁-C₁₆)amido substituents, wherein an aryl or heteroaryl group is itself optionally substituted with up to three substituents independently selected from the group consisting of:

- (i) hydroxy,
- (ii) halo,
- (iii) nitro,

(iv) (C₁-C₆)hydrocarbyl,
 (v) halo(C₁-C₁₆)hydrocarbyl,
 (vi) (C₁-C₆)hydrocarbyloxy,
 (vii) halo(C₁-C₆)hydrocarbyloxy,
 (viii) aryl, and
 (ix) aryloxy, wherein an aryl or aryloxy substituent can itself be substituted with up to three substituents independently selected from the group consisting of:

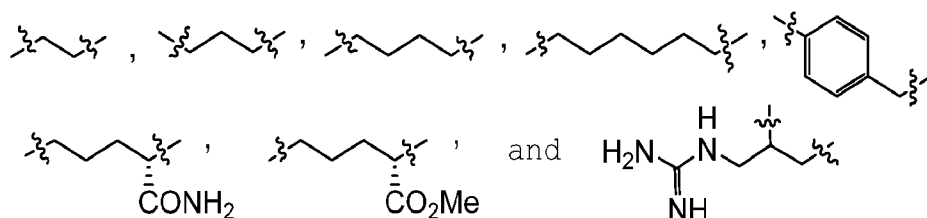
- (i) hydroxy,
- (ii) halo,
- (iii) nitro,
- (iv) (C¹-C⁶)hydrocarbyl,
- (v) halo(C¹-C¹⁶)hydrocarbyl,
- (vi) (C¹-C⁶)hydrocarbyloxy, and
- (vii) halo(C₁-C₆)hydrocarbyloxy; and

R² is OH or , where Circle A is a linking

moiety having the length of a saturated chain of 2 carbon atoms and less than a saturated chain of about 12 carbon atoms, and R³ is guanidiny [H₂N(C=NH)NH-], N,N-(di-C₁-C₆-hydrocarbyl)amino, or N,N,N-(tri-C₁-C₆-hydrocarbyl)ammonium, and an optional pharmaceutically acceptable anion, Y⁻, to balance charges needed.

2. The compound or its pharmaceutically acceptable salt according to claim 1, wherein R¹ is (C₁-C₁₆)hydrocarbyl, aryl(C₁-C₆)-hydrocarbyldiyl, or halo(C₁-C₁₂)-hydrocarbyldiyl.

3. The compound or its pharmaceutically acceptable salt according to claim 2, wherein said Circle A linker moiety is selected from the group consisting of



4. The compound or its pharmaceutically acceptable salt

according to claim 3, wherein R³ is guanidinyI $\left[\begin{array}{c} \text{HN} \\ \text{C} \\ \text{NH} \end{array} \begin{array}{c} \text{NH}_2 \\ \text{NH}_2 \end{array} \right]^+$.

5. The compound or its pharmaceutically acceptable salt

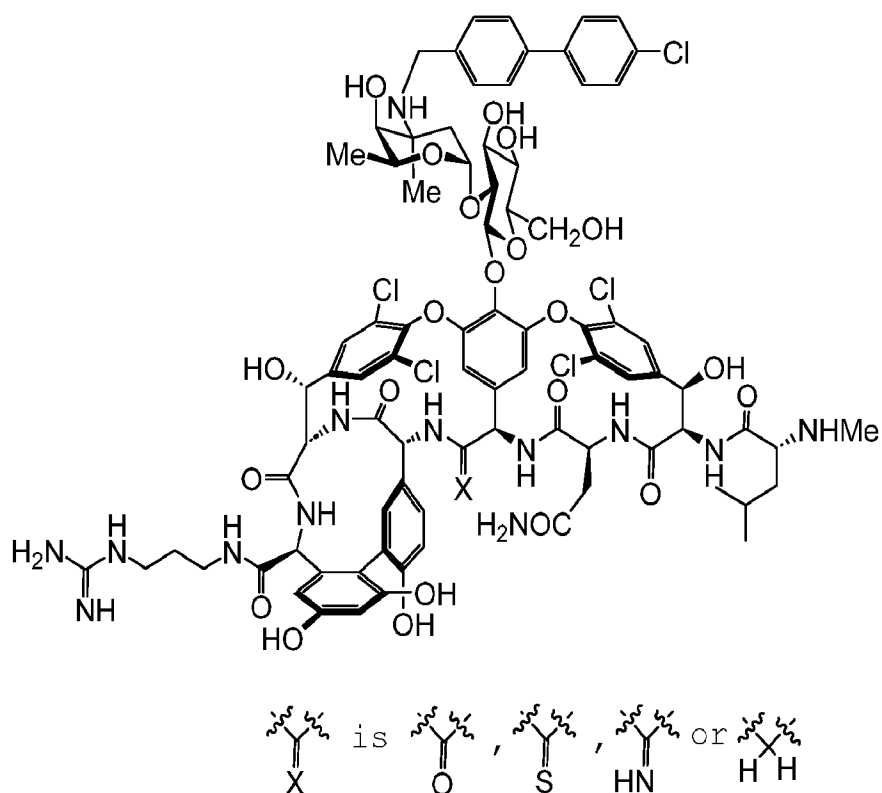
according to claim 3, wherein R³ is N,N,N-(tri-C₁-C₆-hydrocarbyl)ammonium.

6. The compound or its pharmaceutically acceptable salt

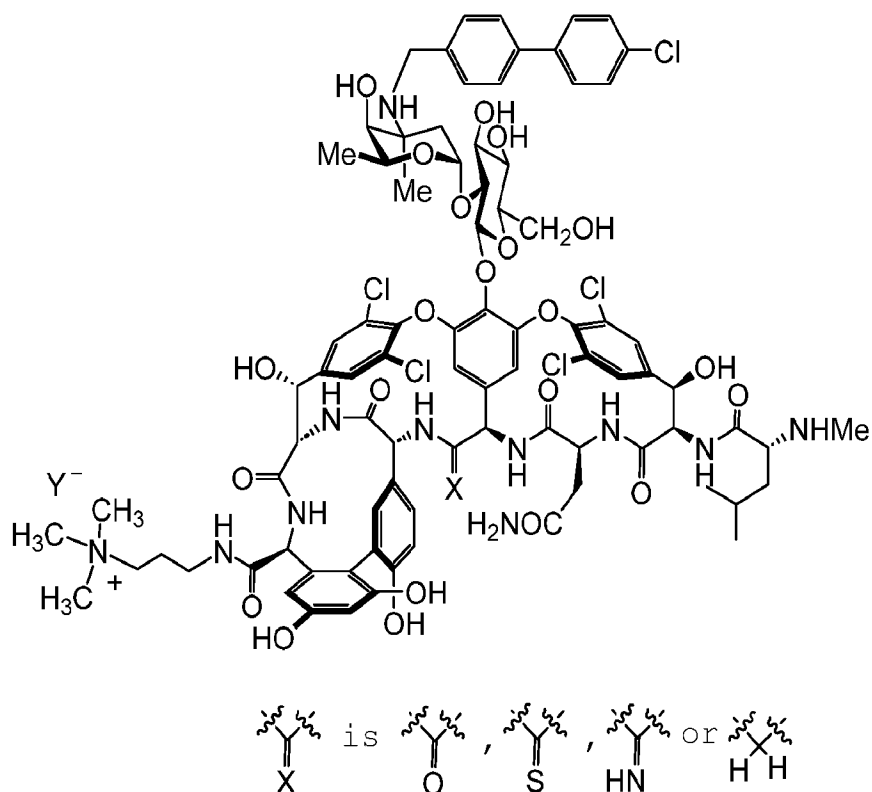
according to claim 1 that corresponds in structure to that shown in the structural formula below,

7. A compound that corresponds in structure to that shown

in the structural formula below or its pharmaceutically acceptable salt,



8. A compound that corresponds in structure to that shown in the structural formula below or its pharmaceutically acceptable salt,



9. A pharmaceutical composition that comprises an antimicrobial amount of a compound of claim 1 or a pharmaceutically acceptable salt thereof dissolved or dispersed in a physiologically acceptable diluent.

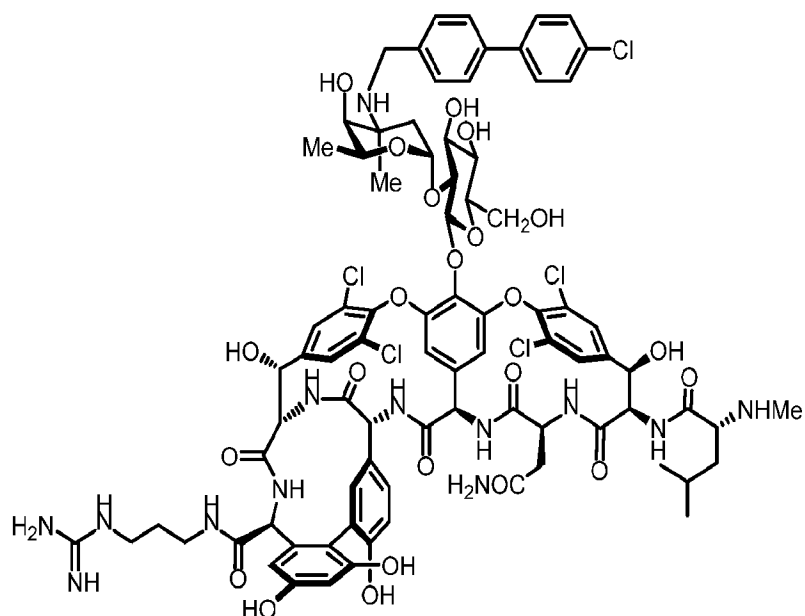
10. A method of treating a bacterially-infected mammal in need of antibacterial treatment that comprises administering an antibacterial-effective amount of a compound according to claim 1 or a pharmaceutically acceptable salt of such a compound to said infected mammal in need.

11. The method according to claim 10, wherein the bacteria that infect said bacterially-infected mammal are Gram-positive bacteria.

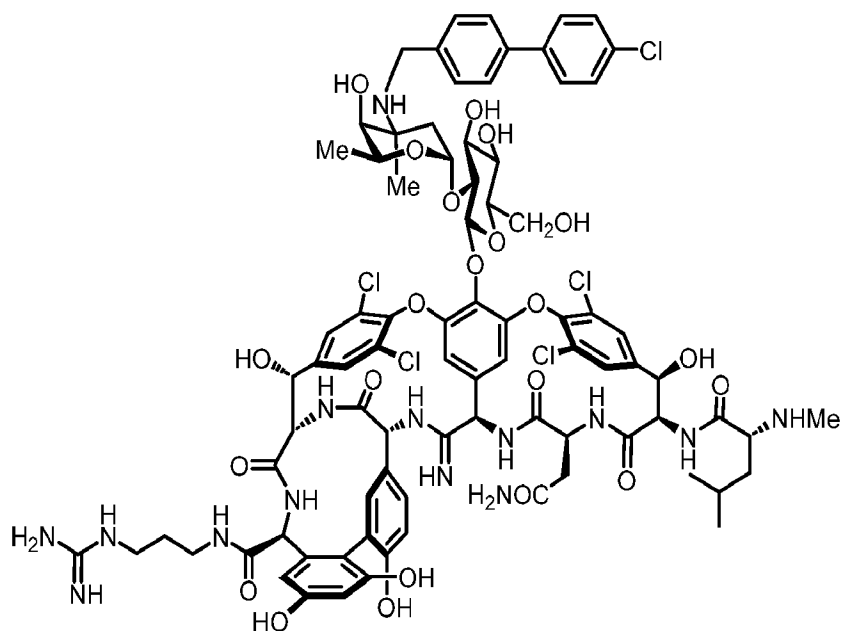
12. The method according to claim 11, wherein said Gram-positive bacteria are selected from the group consisting of one or more of *S. aureus*, methicillin-resistant *S. aureus* (MRSA), VanA *E. faecalis*, VanA *E. faecium*, and VanB *E. faecalis*.

13. The method according to claim 12, wherein said administration is repeated a plurality of times.

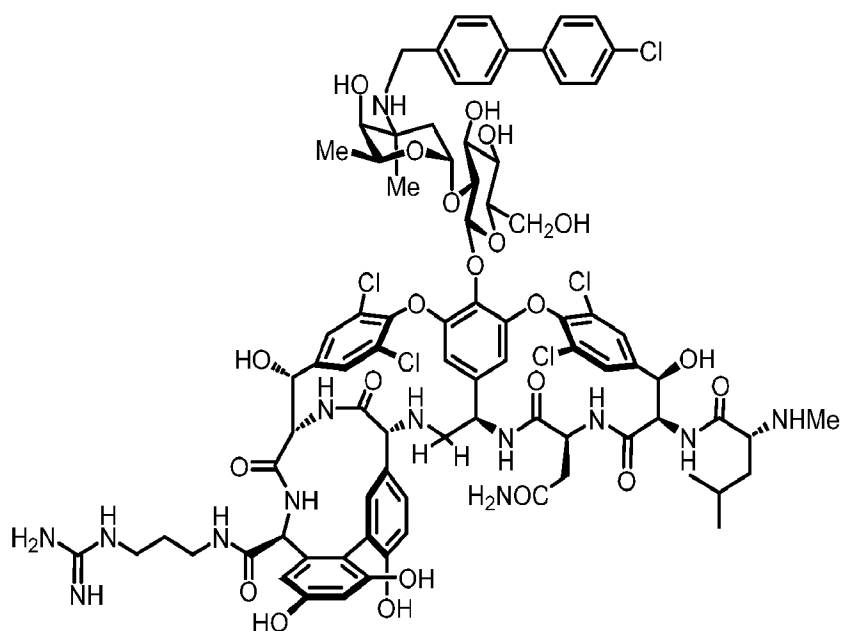
14. The method according to claim 10, wherein said administered compound corresponds in structure to the formula shown below or a pharmaceutically acceptable salt thereof



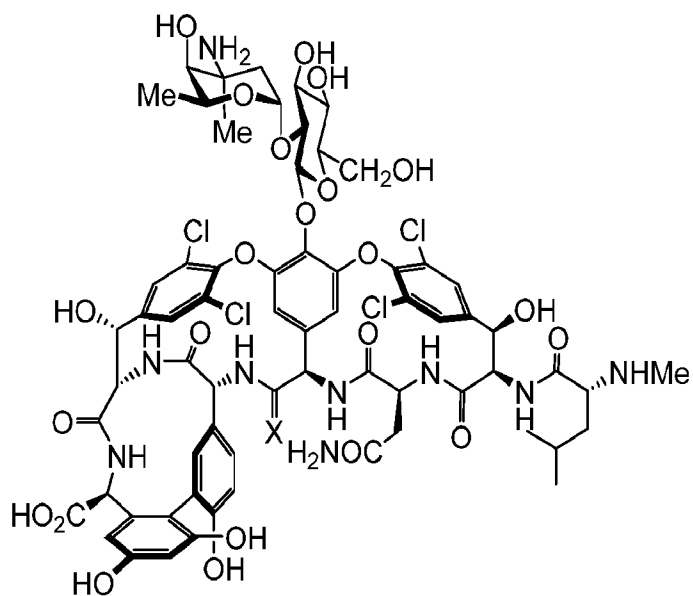
15. The method according to claim 10, wherein said administered compound corresponds in structure to the formula shown below or a pharmaceutically acceptable salt thereof



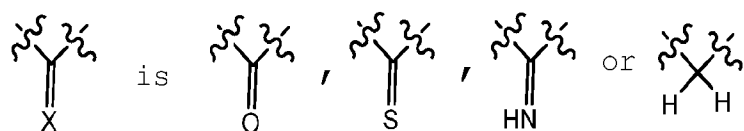
16. The method according to claim 10, wherein said administered compound corresponds in structure to the formula shown below or a pharmaceutically acceptable salt thereof



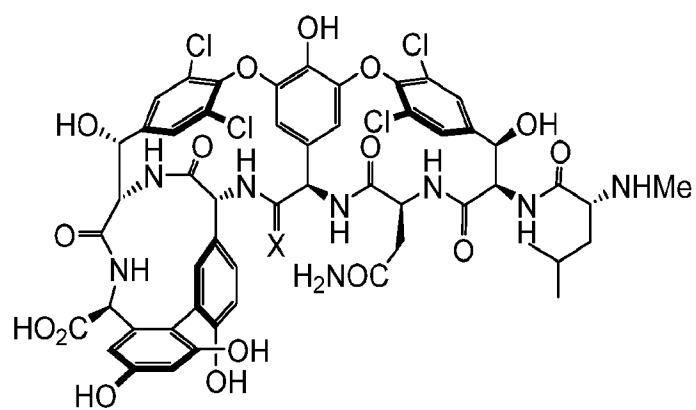
17. A compound of the structural formula shown below



wherein



18. A compound of the structural formula shown below



wherein

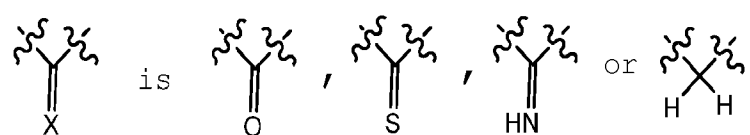
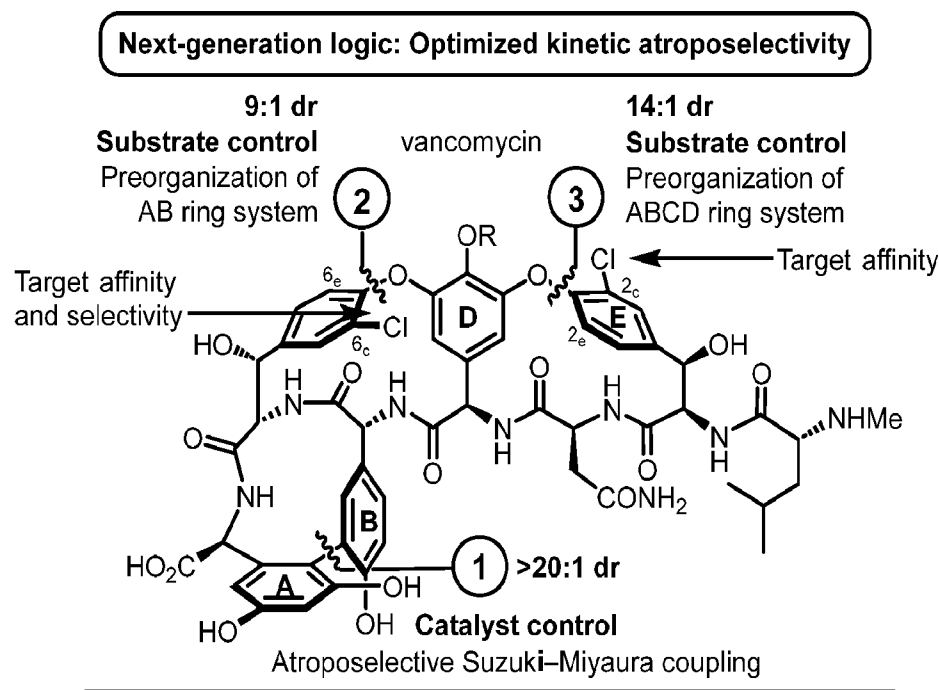
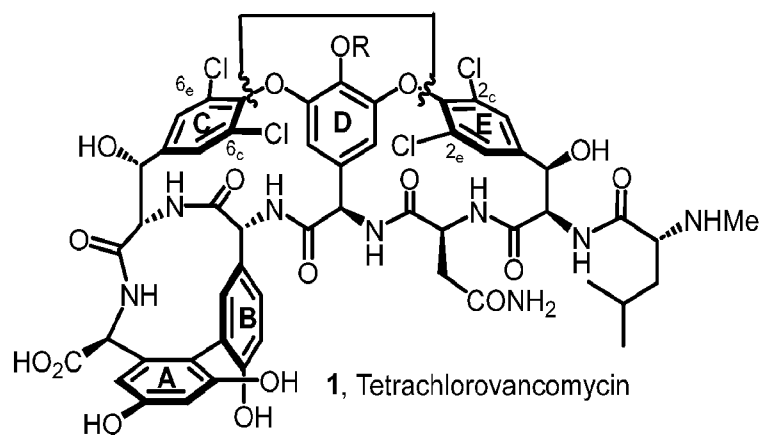


Fig. 1

**This work: Stereochemical simplification****Two elements of atropisomerism removed**Two-fold intramolecular S_NAr cyclization

- Removes synthetic challenge of atropisomer stereochemistry
- Further streamlines now technically simple synthesis
- Maintains potent antimicrobial activity

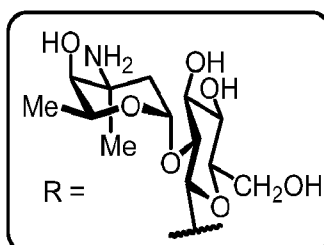


Fig. 2

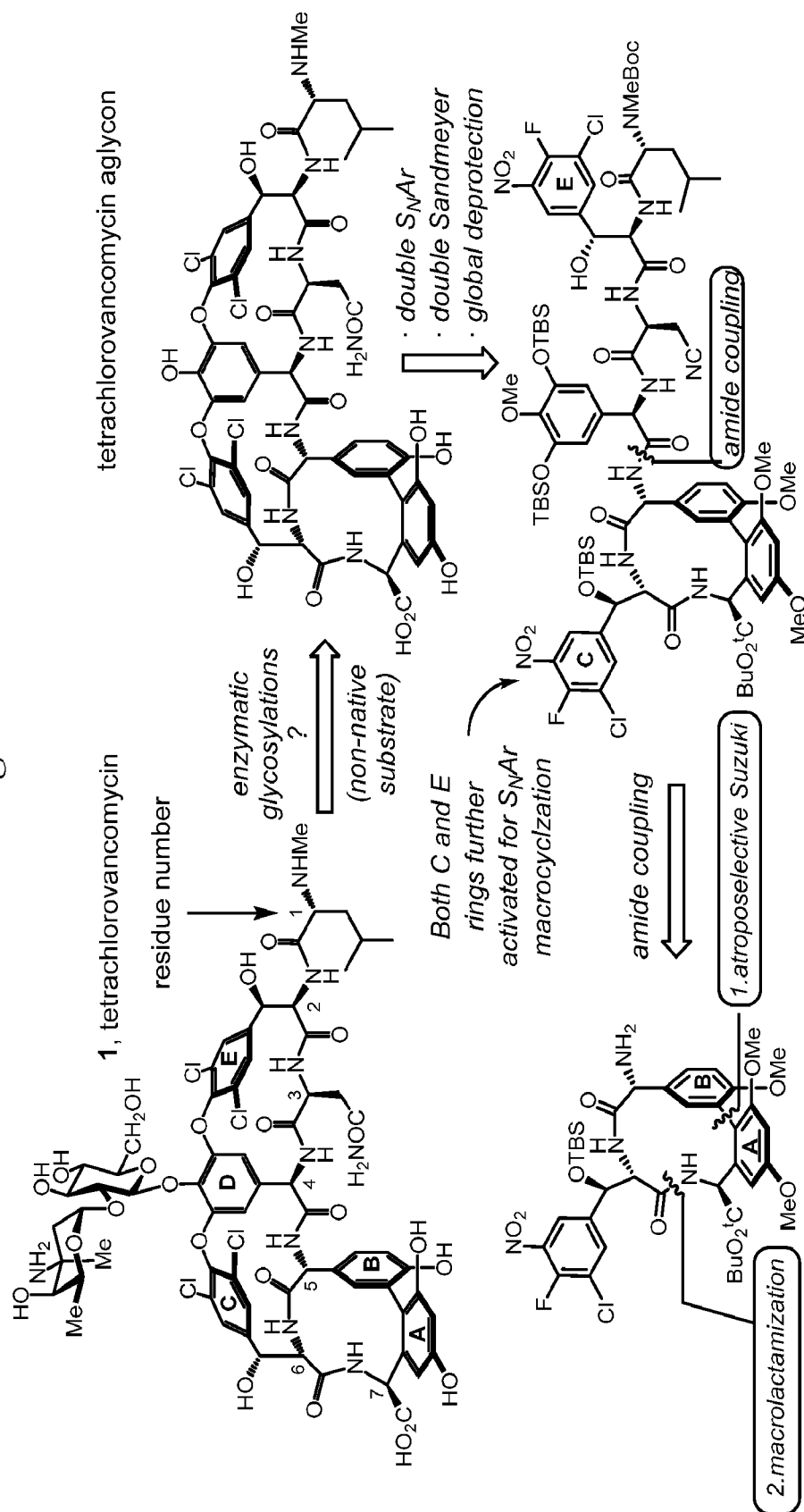


Fig. 3

Scheme 4

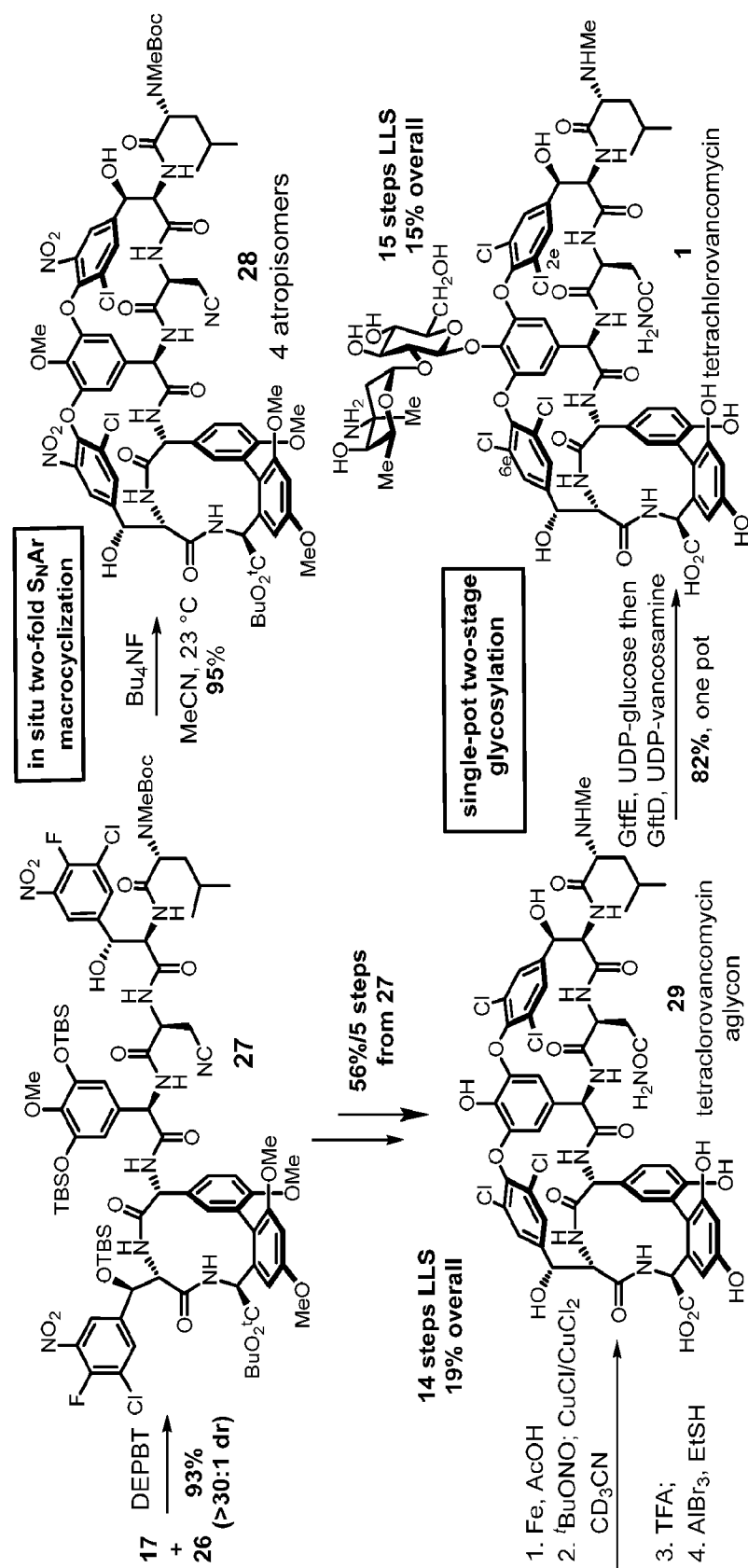


Fig. 4

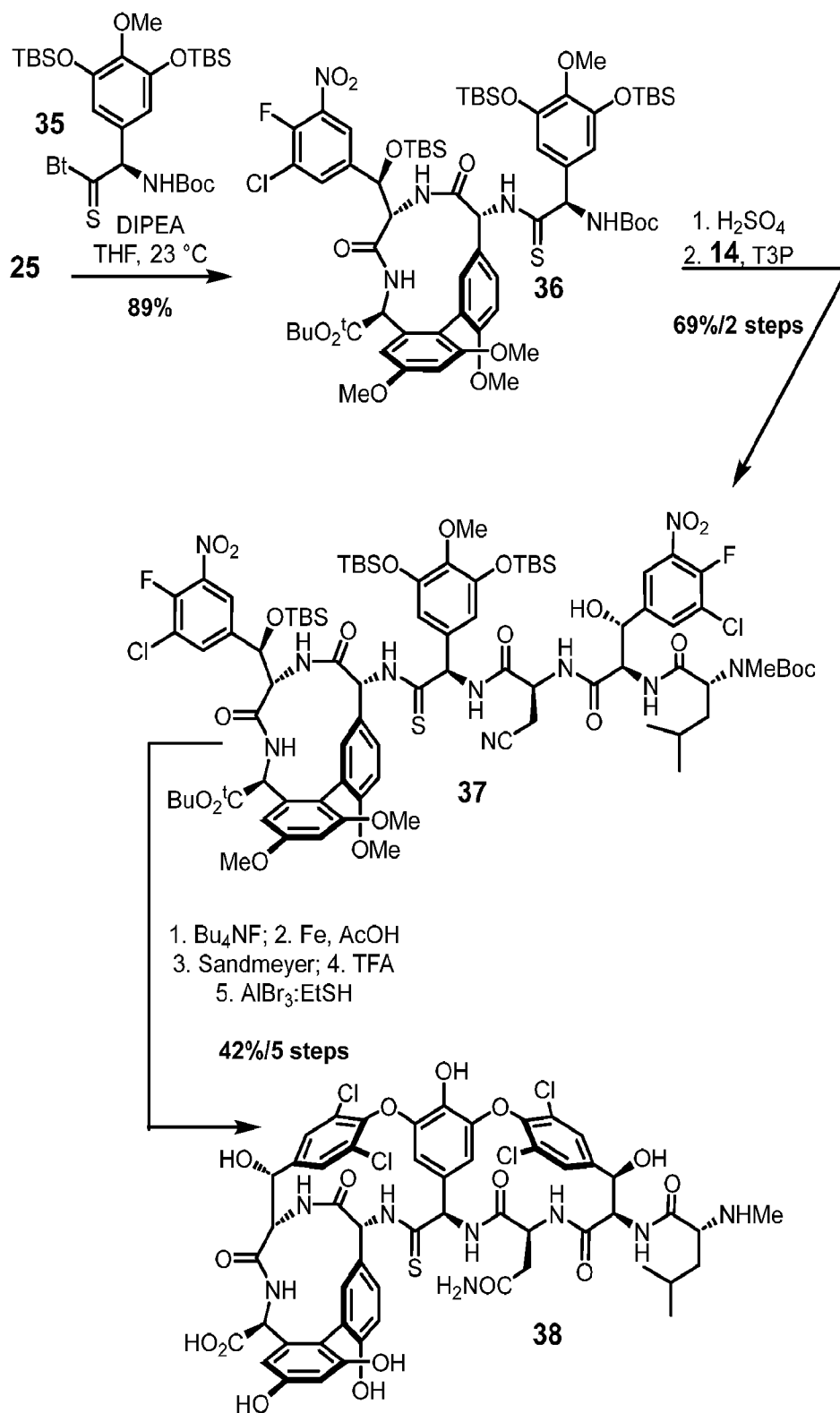


Fig. 5

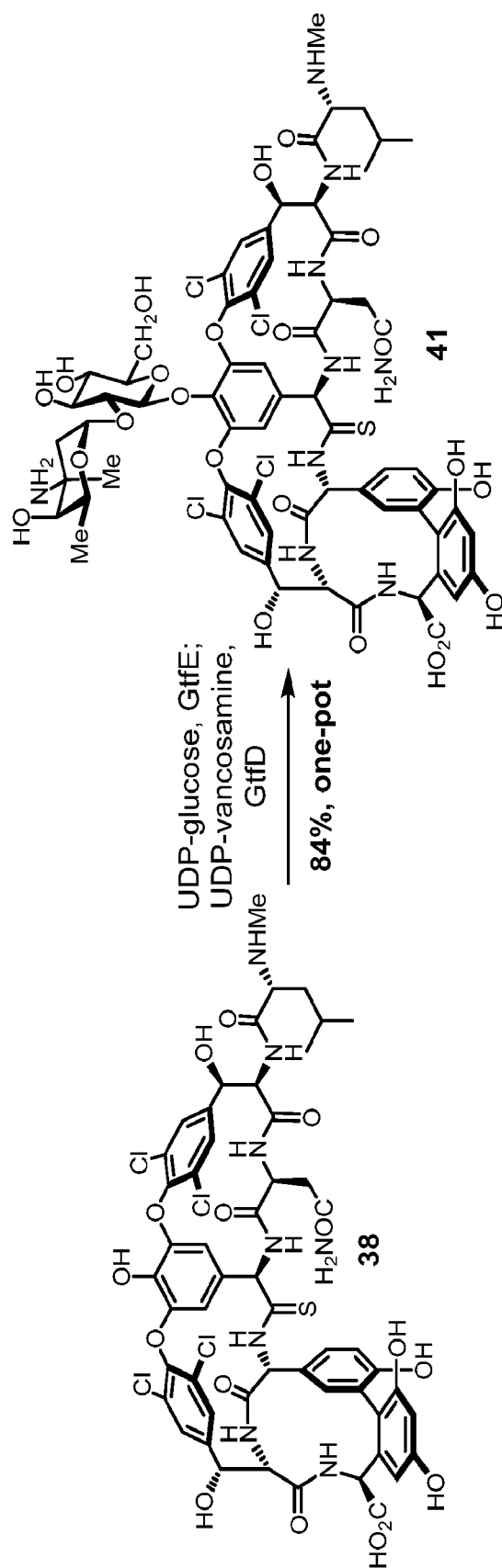


Fig. 6

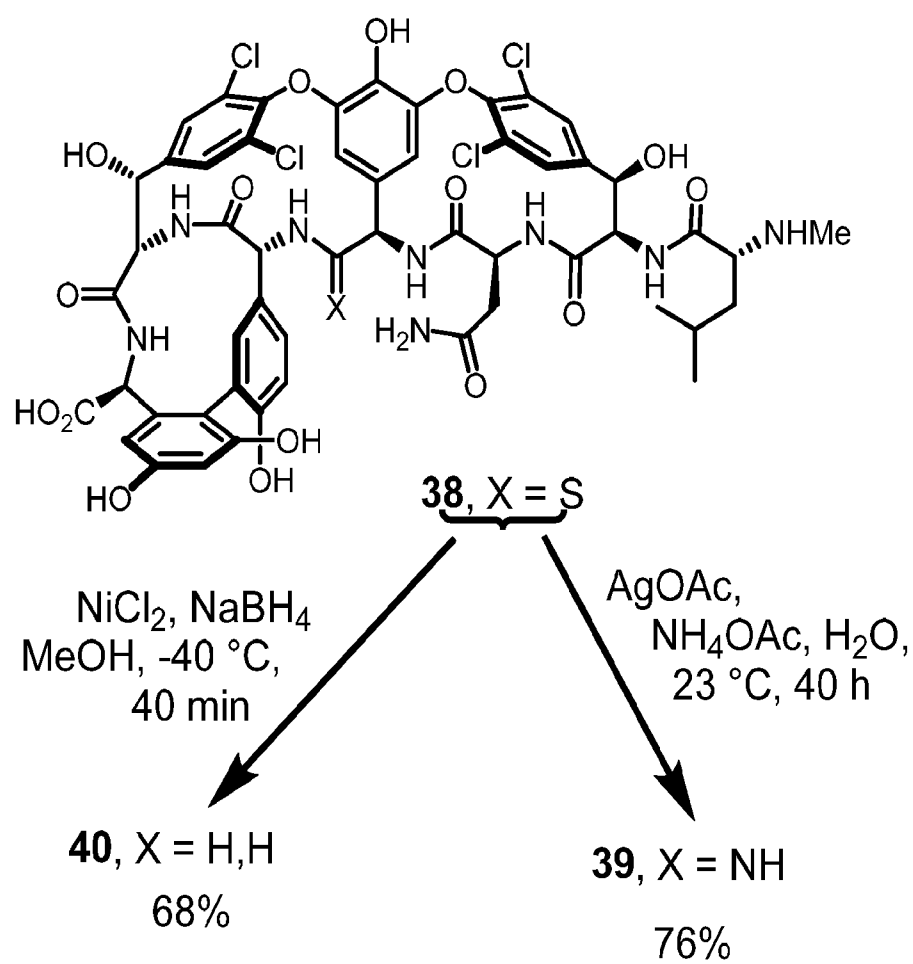


Fig. 7

Fig. 7A

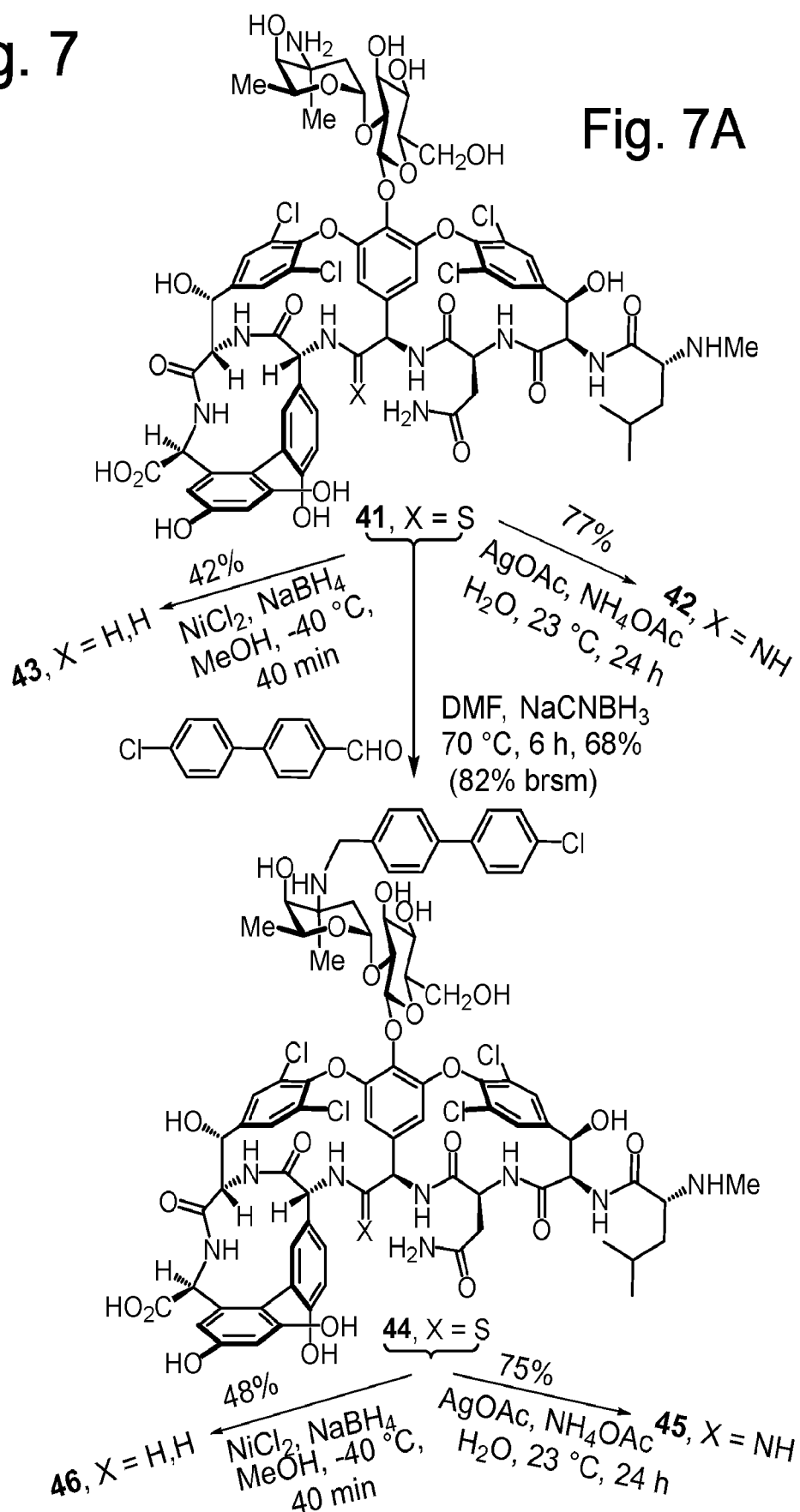


Fig. 7B

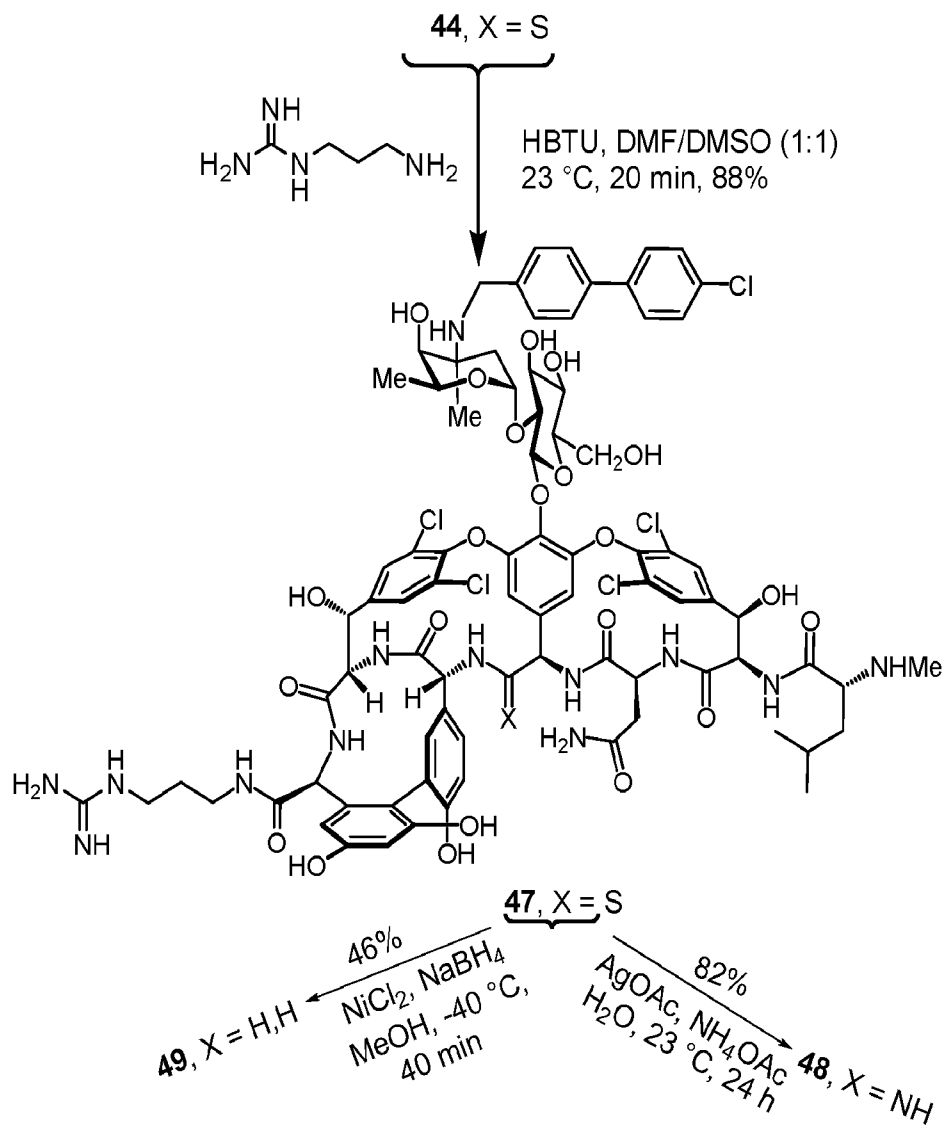
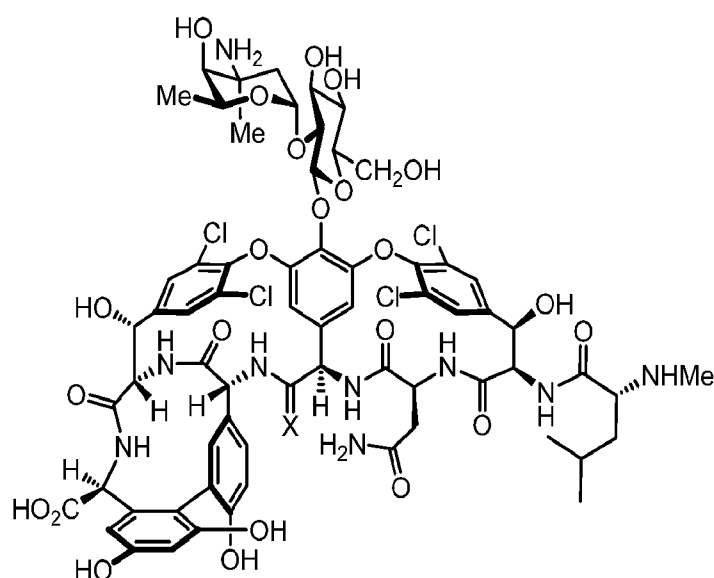


Fig. 8

Fig. 8A

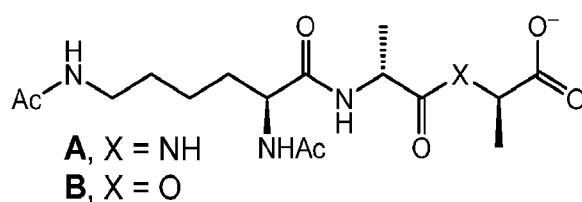


1, X = O tetrachlorovancomycin

41, X = S [Ψ [C(=S)NH]Tpg⁴]tetrachlorovancomycin

42, X = NH [Ψ [C(=NH)NH]Tpg⁴]tetrachlorovancomycin

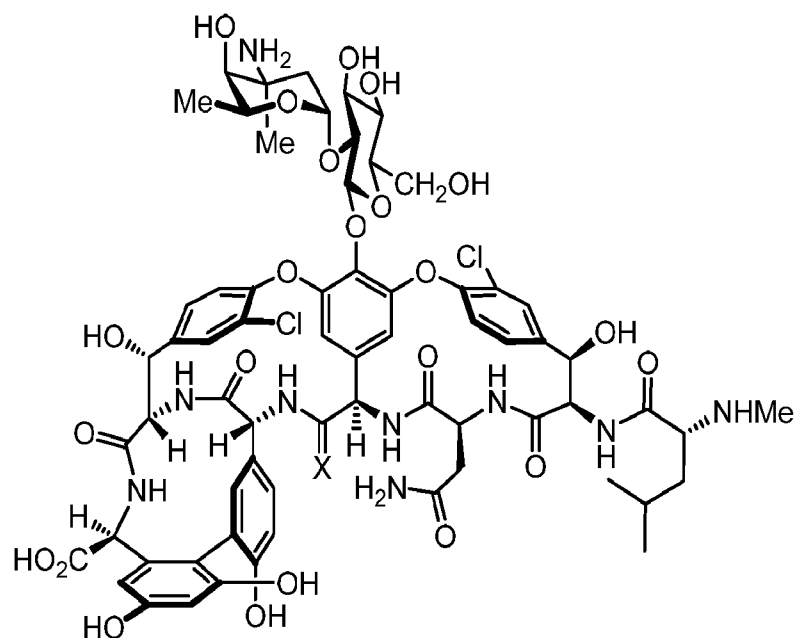
43, X = H,H [Ψ (CH₂NH)]Tpg⁴]tetrachlorovancomycin



compound	ligand, K_a (M ⁻¹) ^a		K_a (A/B)
	A, X = NH	B, X = O	
1 , X = O	3.3×10^4	nd ^b	nd
41 , X = S	2.2×10^2	nd	nd
42 , X = NH	2.1×10^4	1.6×10^4	1.3
43 , X = H,H	1.7×10^3	2.0×10^3	0.85

^a K_a = association constant. ^bNot determined (nd), binding $<1 \times 10^2$ M⁻¹.

Fig. 8B



X = O vancomycin

X = S $[\Psi[C(=S)NH]Tpg^4]$ vancomycin

X = NH $[\Psi[C(=NH)NH]Tpg^4]$ vancomycin

X = H,H $[\Psi(CH_2NH)]Tpg^4]$ vancomycin

compound	ligand, K_a (M^{-1}) ^a		$K_a(A/B)$
	A, X = NH	B, X = O	
X = O	2.0×10^5	1.8×10^2	1100
X = S	3.2×10^2	5.8×10^1	—
X = NH	7.4×10^4	7.2×10^4	1.03
X = H,H	5.5×10^3	5.7×10^3	0.96

^a K_a = association constant. ^bNot determined (nd), binding $<1 \times 10^2 M^{-1}$.

Fig. 9

Fig. 9A

Compound	MIC, µg/mL			
	vancomycin-sensitive			
	<i>S. aureus</i> ^a	MRSA <i>S. aureus</i> ^b	<i>E. faecium</i> ^c	<i>E. faecalis</i> ^c
[Ψ[C(=S)NH]Tpg ⁴]tetrachlorovancomycin (41)	>80	>80	≥80	≥80
CBP-[Ψ[C(=S)NH]Tpg ⁴]tetrachlorovancomycin (44)	5	5	10	10
G3,CBP-[Ψ[C(=S)NH]Tpg ⁴]tetrachlorovancomycin (47)	0.3	0.3	0.6	0.6
G3,CBP-[Ψ[C(=NH)NH]Tpg ⁴]tetrachlorovancomycin (48)	0.08	0.08	0.15	0.15 (0.08) ^g
G3,CBP-[Ψ(CH ₂ NH)Tpg ⁴]tetrachlorovancomycin (49)	0.02	0.02	0.04	0.04
G3,CBP-vancomycin ⁴⁴	0.04	0.02	nd	nd

^aATCC 25923. ^bATCC 43300. ^cUnknown source. ^dATCC BAA-2317. ^eATCC-2573. ^fTX-2516. ^gProteins removed.

Fig. 9B

Compound	MIC, µg/mL			
	vancomycin-resistant			
	VanA <i>E. faecium</i> ^d	VanA <i>E. faecalis</i> ^e	VanB <i>E. faecalis</i> ^f	
[Ψ[C(=S)NH]Tpg ⁴]tetrachlorovancomycin (41)	>80	>80	>80	>80
CBP-[Ψ[C(=S)NH]Tpg ⁴]tetrachlorovancomycin (44)	10	10	5	5
G3,CBP-[Ψ[C(=S)NH]Tpg ⁴]tetrachlorovancomycin (47)	0.6	1.2	1.2	1.2

G3,CBP-[Ψ[C(=NH)NH]Tpg ⁴]tetrachlorovancomycin (48)	0.08	0.6 (0.15) ^g	0.6 (0.15) ^g	0.6 (0.15) ^g
G3,CBP-[Ψ(CH ₂ NH)Tpg ⁴]tetrachlorovancomycin (49)	0.08	0.08	0.08	0.08

G3,CBP-vancomycin ⁴⁴	0.3	0.3	0.6	0.6

^aATCC 25923, ^bATCC 43300. ^cUnknown source. ^dATCC BAA-2317.
^eATCC-2573. ^fTX-2516. ^gProteins removed.

Fig. 13

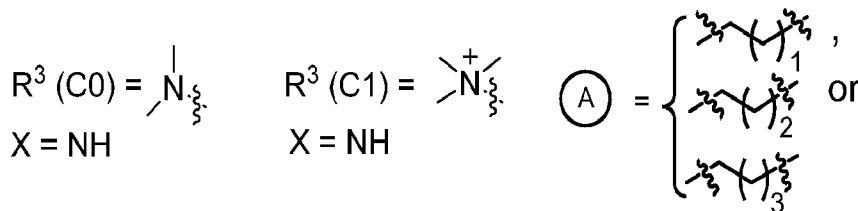
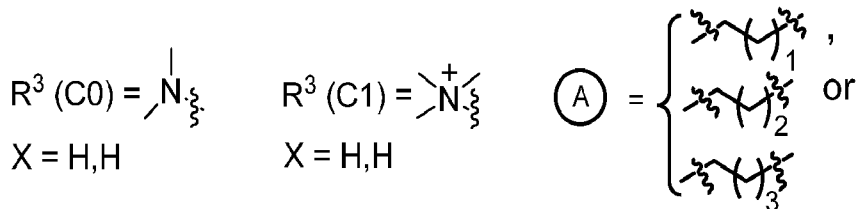
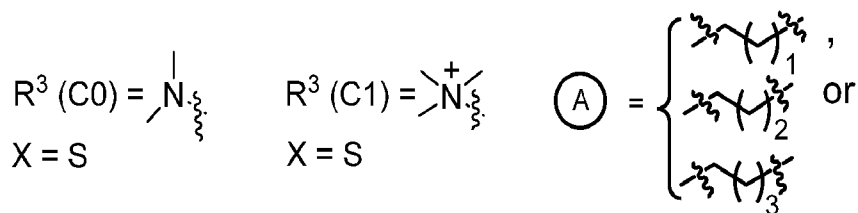
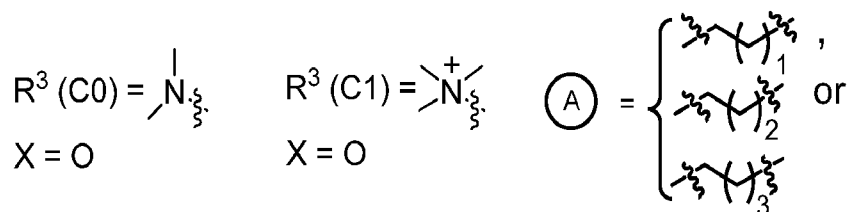
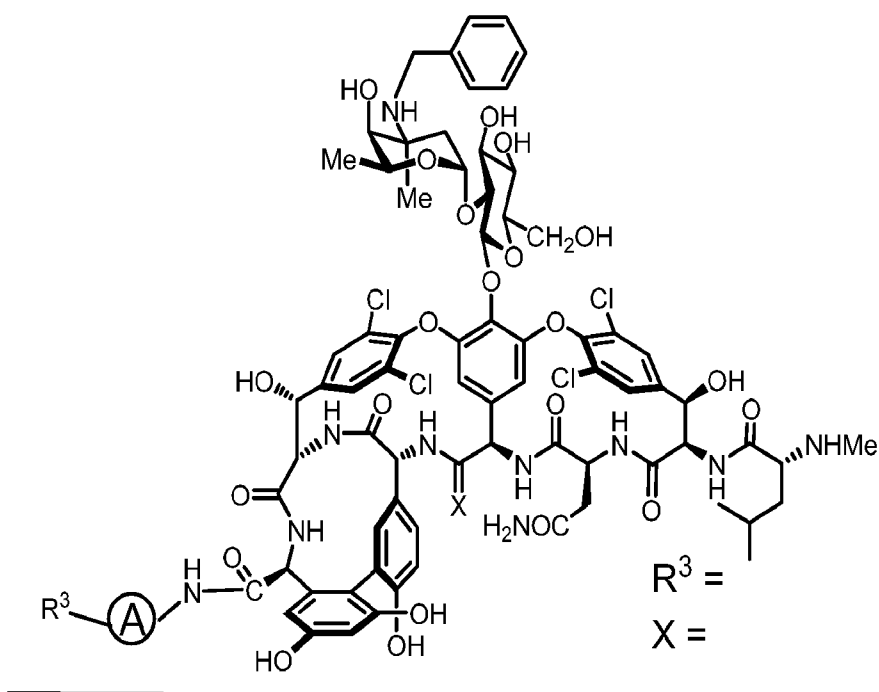


Fig. 14

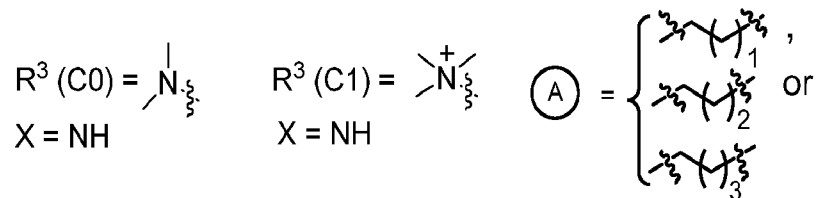
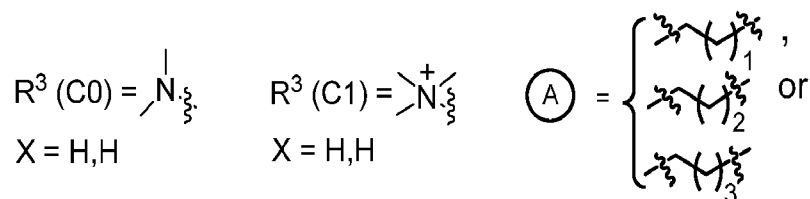
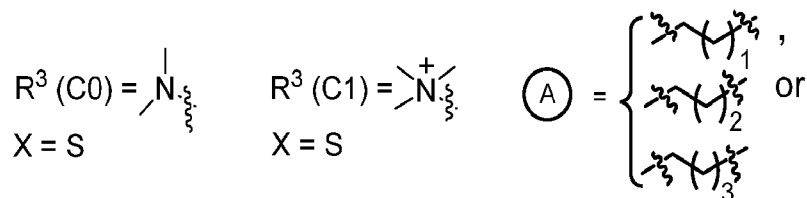
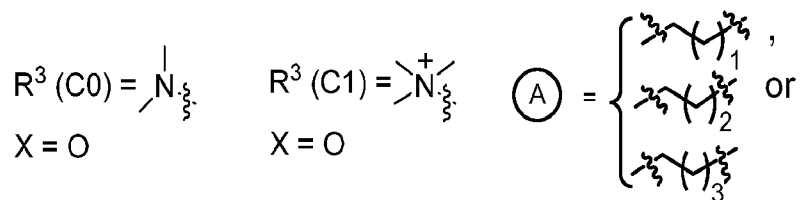
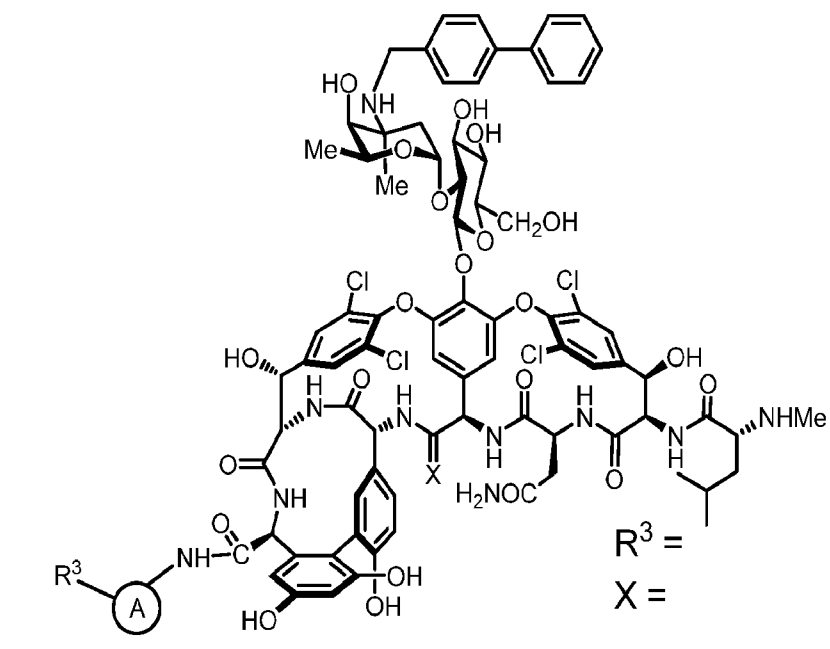
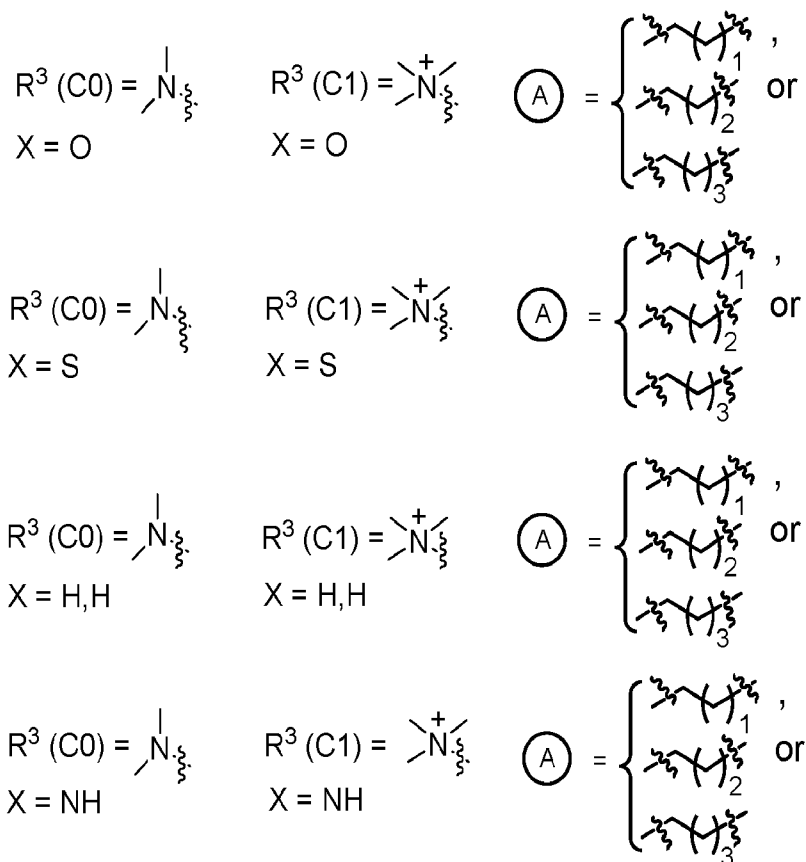
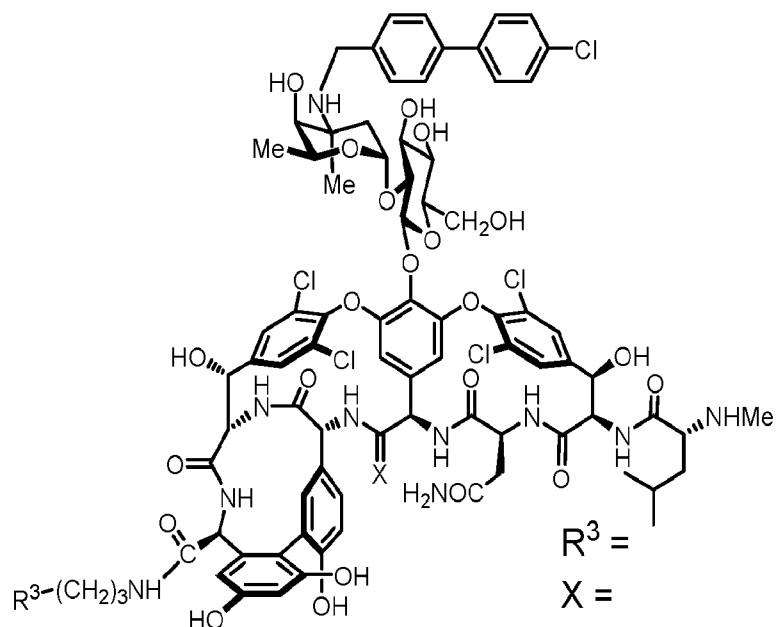


Fig. 15



INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 24/31453

A. CLASSIFICATION OF SUBJECT MATTER

IPC - INV. A61K 38/14, A61K 38/04, A61K 38/14 (2024.01)

ADD. A61K 38/12 (2024.01)

CPC - INV. A61K 38/14, A61K 38/04, A61K 38/14

ADD. A61K 38/12

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

See Search History document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

See Search History document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	OKANO et al. "Total Syntheses of Vancomycin Related Glycopeptide Antibiotics and Key Analogues", Chem Rev. 2017 September 27; 117(18): pp 11952-11993, especially: pg 4, para 4; pg 6, para 2; pg 9, para 2; pg 46, Figure 1, formula 1; pg 71, Scheme 1; pg 72 Scheme 2; pg 77, Scheme 7.	1
Y	KRAUS et al. "Second Generation Analogs of the Cancer Drug Clinical Candidate Tipifarnib for Anti-Chagas Disease Drug Discovery", J. Med. Chem. 2010, 53, pp 3887-3898, especially: pg 3888, col 2 to pg 3889, col 1, para 1; pg 3888, Figure 1; pg 3890, Table 1.	1
A	CROWLEY et al. "Total Synthesis and Evaluation of [psi[CH ₂ NH]Tpg4]Vancomycin Aglycon: Reengineering Vancomycin for Dual D-Ala-D-Ala and Dala- D-Lac Binding", J Am Chem Soc. 2006 March 8; 128(9): pp 2885-2892, especially: pg 2, para 2 to pg 3, para 1; pg 11, Figure 1, formula 1; pg 12, Figure 2, formula 5; pg 15, Scheme 2, formula 14 to formula 15.	1
A	RAMAKRISHNAN "Antibacterial Activities and Modes of Action of Vancomycin and Related Glycopeptides", ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Apr. 1991, p. 605-609.	1



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"D" document cited by the applicant in the international application

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

23 September 2024

Date of mailing of the international search report

OCT 11 2024

Name and mailing address of the ISA/US

Mail Stop PCT, Attn: ISA/US, Commissioner for Patents
P.O. Box 1450, Alexandria, Virginia 22313-1450

Facsimile No. 571-273-8300

Authorized officer

Kari Rodriguez

Telephone No. PCT Helpdesk: 571-272-4300

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 24/31453

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
See extra sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

--Box III - Lack of Unity--

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I+: Claims 1-5 and 7-18, directed to a compound that corresponds in structure to that shown in Formula III, the compound listed in claim 7, claim 8, claim 17, or claim 18, or a pharmaceutically acceptable salt of said compounds. The compound of Formula III will be searched to the extent that it encompasses the first species of claim 1, wherein -C(=X)- is the first substructure: -C(=O)-; R1 is consisting of hydrido; R2 is OH. It is believed that claims 1 encompasses this first named invention, and thus this claim will be searched without fee to the extent that it encompasses the first species of claim 1. This first named invention has been selected based on the guidance set forth in section 10.54 of the PCT International Search and Preliminary Examination Guidelines. Applicant is invited to elect additional compounds of Formula III, wherein each additional compound elected will require one additional invention fee. Applicants must specify the claims that encompass any additionally elected compound. Applicants must further indicate, if applicable, the claims which encompass the first named invention, if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched. Additionally, an exemplary election wherein different actual variables are selected is suggested. An exemplary election would be a compound of claim 1, wherein -C(=X)- is the second substructure: -C(=S)-; R1 is consisting of hydrido; R2 is OH (i.e. claim 1).

The groups of inventions listed above do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Special Technical Features:

Group I+ includes the technical feature of a unique compound of Formula III, which is not required by any other invention of Group I+.

Common Technical Features:

The inventions of Groups I+ share the technical feature of a compound that corresponds in structure to that shown in Formula III, the compound listed in claim 7, claim 8, claim 17, or claim 18, or a pharmaceutically acceptable salt of said compounds.

These shared technical features, however, do not provide a contribution over the prior art, because the shared technical features are obvious over the article entitled "Total Synthesis and Evaluation of [psi[CH₂NH]]Tpg4]Vancomycin Aglycon: Reengineering Vancomycin for Dual D-Ala-D-Ala and Dala- D-Lac Binding" by Crowley et al. (hereinafter 'Crowley') in view of the article entitled "Second Generation Analogs of the Cancer Drug Clinical Candidate Tipifarnib for Anti-Chagas Disease Drug Discovery" by Kraus et al. (hereinafter 'Kraus').

Crowley teaches a compound that corresponds in structure to that shown in Formula III wherein -C(=X)- is the first substructure; R1 is consisting of hydrido; R2 is OH (pg 11, Figure 1, formula 1), but Crowley doesn't teach the second chlorine atom on each of the phenyl rings in the structure. However, Crowley further teaches that the synthesis of the mono-chloro substituted compound is problematic because the two rings form atropisomers (pg 2, para 2 to pg 3, para 1, 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... 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; see also pg 12, Figure 2, formula 5; see also pg 15, Scheme 2, formula 14 to formula 15). Furthermore, Kraus teaches that atropisomerism (axial chirality) of a single ortho-chlorine atom was eliminated using a symmetrical disubstitution of a phenyl ring (pg 3888, col 2 to pg 3889, col 1, para 1, Although 2 is one of the most potent anti-T. cruzi compounds reported to date, subsequent studies showed that there is hindered rotation about the bond connecting the 3-chlorophenyl group and the quinolone ring presumably because of a clash between the ortho methyl group and the vinylic proton of the quinolone ring. Thus 2 is a mixture of rotamers with the ortho methyl group either above or below the plane of the quinolone ring... We thus went on to design new analogs that either lack a 2-substituent on the 3-chlorophenyl ring or have a C2-symmetric ring in order to avoid rotamer formation; see also pg 3888, Figure 1; see also pg 3888, Figure 1; see also pg 3890, Table 1, formula 2 and formulas 14-16, especially formula 15 for dichloro substitution). Therefore, it would have been obvious to a person having ordinary skill in the art to prepare the derivative of the compound of Crowley with dichloro substitution because this compound is suggested by the teachings of Crowley in view of Kraus. It would have been further obvious to combine Kraus with Crowley because Crowley teaches that axial chirality of an ortho-chloro substituted phenyl ring is synthetically problematic (pg 2, para 2 to pg 3, para 1, 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... 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; see also pg 12, Figure 2, formula 5; see also pg 15, Scheme 2, formula 14 to formula 15) and Kraus teaches that a ortho,ortho-dichloro substituted phenyl moiety eliminates axial chirality for an ortho-chloro substituted phenyl group (pg 3888, col 2 to pg 3889, col 1, para 1, Although 2 is one of the most potent anti-T. cruzi compounds reported to date, subsequent studies showed that there is hindered rotation about the bond connecting the 3-chlorophenyl group and the quinolone ring presumably because of a clash between the ortho methyl group and the vinylic proton of the quinolone ring. Thus 2 is a mixture of rotamers with the ortho methyl group either above or below the plane of the quinolone ring... We thus went on to design new analogs that either lack a 2-substituent on the 3-chlorophenyl ring or have a C2-symmetric ring in order to avoid rotamer formation; see also pg 3888, Figure 1; see also pg 3888, Figure 1; see also pg 3890, Table 1, formula 2 and formulas 14-16, especially formula 15 for dichloro substitution) and the ortho,ortho-dichloro substituted derivative of the compound of Crowley would not have this issue.

--continued on next page--

--from previous page--

As said compound was known in the art, this cannot be considered a special technical feature that would otherwise unify the inventions of Group I+.

The inventions of Group I+ thus lack unity under PCT Rule 13.

Note: Claim 6 lacks clarity. Claim 6 references "the structural formula below", but no formula is listed in claim 6, and no meaningful search could be performed for claim 6. Therefore, for the purpose of completing this ISR, claim 6 was not included in the opinion.