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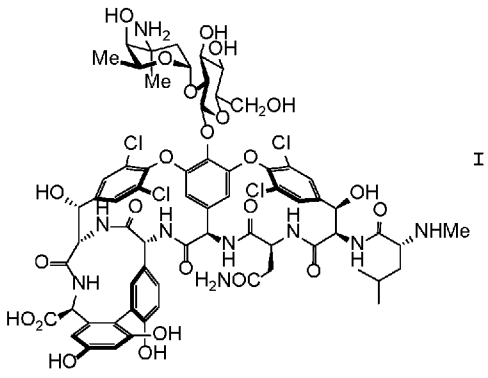

I

(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 tetrachlorovancomcyin aglycon (Compound II), tetrachlorovancomcyin (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-modi fied 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.

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TETRACHLOROVANCOMYCIN AND DERIVATIVES

Description

CROSS-REFEREENCE 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 [Ψ[C(=S)NH]Tpg^4]-vancomycin for accessing pocket-modified vancomycin analogues further improved on the approach and established a scalable synthesis.[7]

The 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.

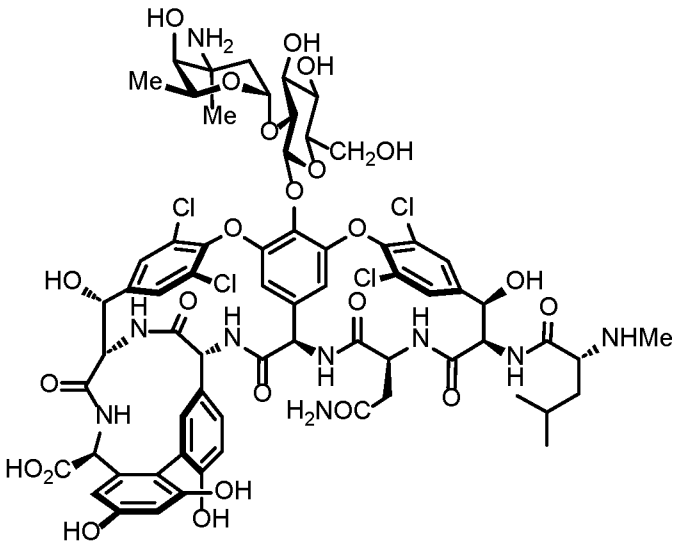
For convenience, these $2_e,6_e$-dichlorovancomycin analogues as tetrachlorovancomycins (e.g., $2_e,6_e$-dichlorovancomycin (Compound **1**) = tetrachlorovancomycin), highlighting the four aryl chlorides now present on the core structure.

SUMMARY

The present invention contemplates a new, biologically active vancomycin analogue called

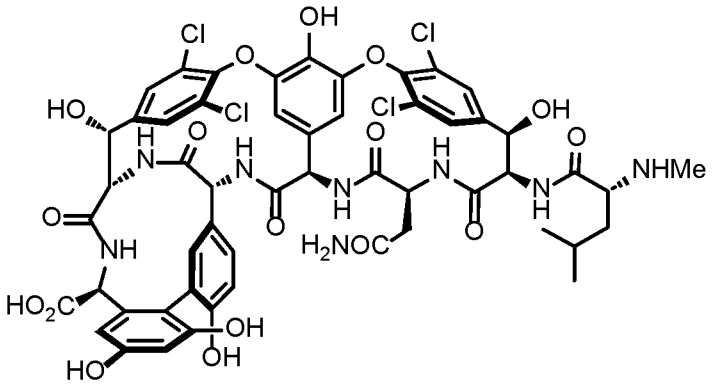
tetrachlorovancomycin and its derivatives that are produced, except for glycosylation, solely by synthetic organic chemistry. Key elements of the synthetic approach include a catalyst-controlled diastereoselective formation of the AB biaryl axis of chirality (>30:1 dr), an instantaneous macrolactamization 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 tetrachlrovancomycin itself is shown below as Formula **I**.



I

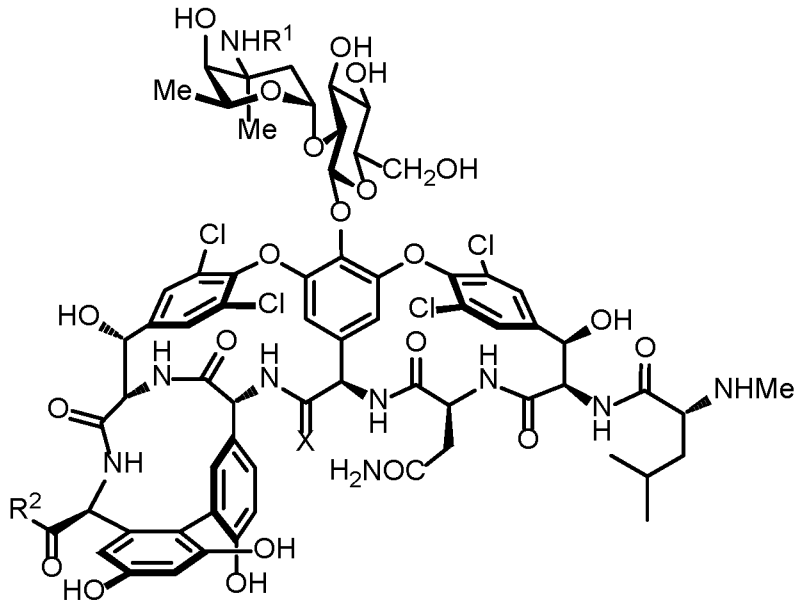
The structural formula for tetrachlrovancomycin aglycon that is produced solely using synthetic organic chemistry is shown below as Formula **II**.



II

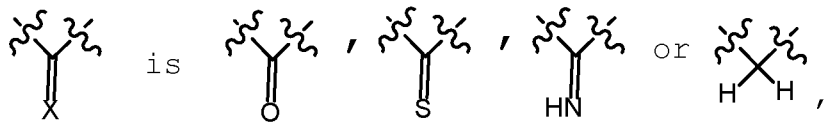
The above two compounds surprisingly exhibit activity against methicillin-resistant *S. aureus* at about a factor of 10 or less than the activity of vancomycin against vancomycin-sensitive and vancomycin-resistant bacteria. However, their derivatives substituted similarly to some of the most active vancomycin 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**.



III

wherein



R^1 is selected from the group consisting of hydrido (hydrogen), (C_1-C_{16})hydrocarbyl, aryl(C_1-C_6)-hydrocarbyldiyl, heteroaryl-(C_1-C_6)hydrocarbyldiyl, (C_1-C_6)hydrocarbyldiylheteroaryl, halo(C_2-C_{12})-hydrocarbyldiyl, and (C_1-C_{16})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) (C_1-C_6)hydrocarbyl,

(v) halo(C_1-C_{16})hydrocarbyl,

(vi) (C_1-C_6)hydrocarbyloxy,

(vii) halo(C_1-C_6)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,

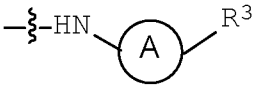
(iii) nitro,

(iv) (C_1-C_6)hydrocarbyl,

(v) halo(C_1-C_{16})hydrocarbyl,

(vi) (C_1-C_6)hydrocarbyloxy, and

(vii) halo(C_1-C_6)hydrocarbyloxy; and

R^2 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 R^3 is guanidinyl [$H_2N(C=NH)NH-$], N,N-(di-C_1-C_6-hydrocarbyl)amino, or N,N,N-(tri-C_1-C_6-hydrocarbyl)ammonium, and an optional pharmaceutically acceptable anion, Y^-, to balance charge as needed.

Thus, when is  , R^1 is H (hydrido), and R^2 is OH, the compound above is tetrachloro-vancomycin. When one or both of R^1 and R^2 are other than H and OH, respectively, a derivative of tetra-chlorovancomycin 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 R^1 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 R^1 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 tetrachlorovancosaminyl amino group by $NaCNBH_4$ reduction of the corresponding aldehyde as is shown in Scheme 5 hereinafter.

The R^2 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 R^3 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 guanidinyl group [$H_2N(C=NH)NH-$]. 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 R^3 is a quaternary ammonium group, an optional anion, Y^-, that is preferably pharmaceutically acceptable is also present to balance the charge. R^3 is a tertiary amine or guanidinyl 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;

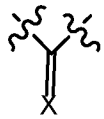
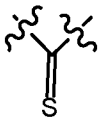
Fig. 4 outlines a synthetic pathway by which a tetrachlorovancomycin derivative of Formula **III** where  is  can be prepared;

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-vancomycin[44];

Figs. 10 through 15 provide illustrative tetrachlorovancomycin derivative compounds with

varying 4-position substituents, as well as various substituents bonded to the vancosaminyl nitrogen, 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., C_1-C_4 alkyl, methyl or tert-butyl. Exemplary hydrocarbyl groups contain a chain of 2 to about77 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-butynyl, 2-butynyl, 3-butynyl, and 1-methyl-2-propynyl.

As a skilled worker will understand, a substituent that cannot exist such as a C_1 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; Bu_4NF = 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-postiion derivatives are very difficult prepare.

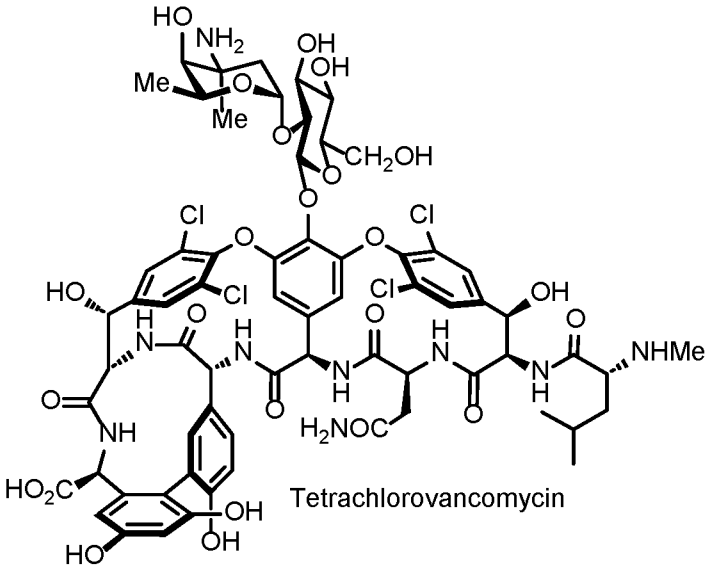
A salient advantage of the invention is that the antibacterial activity of tetrachloro-vancomycin compared to that of vancomycin itself is almost identical.

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

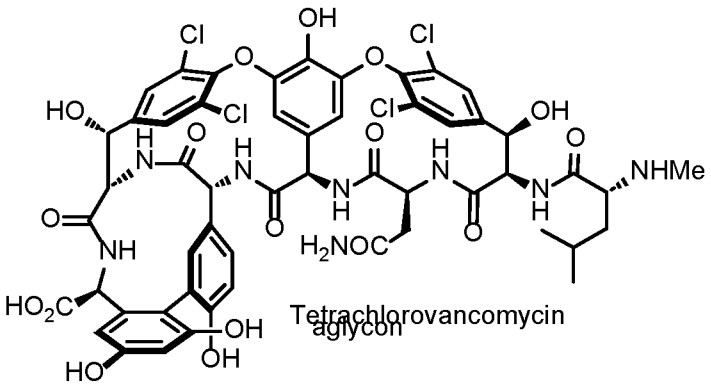
The present invention contemplates a new, biologically active vancomycin derivative called tetrachlorovancomycin that is produced, except for glycosylation, solely by synthetic organic chemistry. The structural formula for tetrachlrovancomycin itself is shown below as Formula **I**.



Tetrachlorovancomycin

I

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



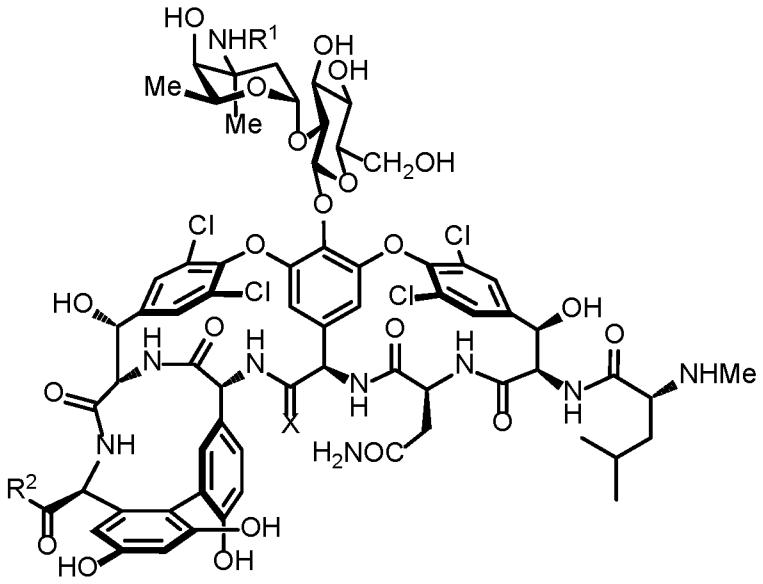
Tetrachlorovancomycin aglycon

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

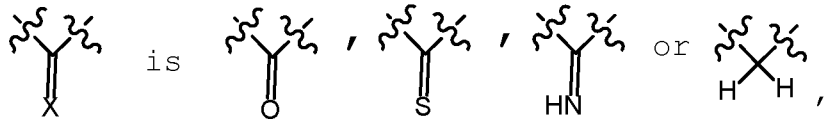
high yield provide a route to less expensive very active antibiotics.

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



III

wherein



R^1 is selected from the group consisting of hydrido (hydrogen), $(C_1\text{-}C_{16})$hydrocarbyl, aryl$(C_1\text{-}C_6)$-hydrocarbyldiyl, heteroaryl-$(C_1\text{-}C_6)$hydrocarbyldiyl, $(C_1\text{-}C_6)$hydrocarbyldiylheteroaryl, halo$(C_2\text{-}C_{12})$-hydrocarbyldiyl, and $(C_1\text{-}C_{16})$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) (C_1-C_6)hydrocarbyl,

(v) halo(C_1-C_{16})hydrocarbyl,

(vi) (C_1-C_6)hydrocarbyloxy,

(vii) halo(C_1-C_6)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,

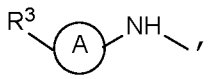
(iii) nitro,

(iv) (C_1-C_6)hydrocarbyl,

(v) halo(C_1-C_{16})hydrocarbyl,

(vi) (C_1-C_6)hydrocarbyloxy, and

(vii) halo(C_1-C_6)hydrocarbyloxy; and

R^2 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 R^3 is selected from the group consisting of guanidinyl [$H_2N(C=NH)NH$-], N,N-(di-C_1-C_6-hydrocarbyl)amino, N,N,N-(tri-C_1-C_6-hydrocarbyl)ammonium, and N-(C_1-C_6-hydrocarbyl)-N-(C_5-C_7-cyclohydrocarbyl)ammonium, and an optional pharmaceutically acceptable anion, Y^-, as needed to balance charge.

Thus, when  is  , R^1 is H (hydrido), and R^2 is OH, the compound above is tetrachloro-vancomycin. When one or both of R^1 and R^2 are other

than H and OH, respectively, a derivative of tetrachlorovancomycin is being contemplated.

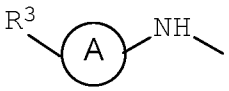
The chemical syntheses of the tetrachlorovancomycin and tetrachlorovancomycin aglycon are shown and discussed hereinafter. These syntheses require fewer steps and provide higher yields of the desired compounds in part because of the symmetry provided by the two chloro groups on each substituted phenyl 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 R^1 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 R^1 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 vancosaminyl amino group by $NaCNBH_4$ reduction of the corresponding aldehyde as is shown in Scheme 5 hereinafter.

The R^2 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 R^3 contains at least a second nitrogen atom. In one contemplated aspect, the second nitrogen atom is part of a guanidinyl group [$H_2N(C=NH)NH-$]. 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 guanidinyl 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 guanidinyl, 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^{O}), 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 hydrocarbyl 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 hydrocarbyl 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 guanidinyl 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., guanidinyl) 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, C_1-C_3-hydrocarbyl C_0-C_2-carboxylate, and C_0-C_2-

carboxamide whose amido nitrogen is unsubsubstituted ($-NH_2$), monosubstituted ($-NHR^4$) or disubstituted ($-NR^4R^5$) in which the substituent (R_4 and/or R^5) is one or two same or different C_1-C_4-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, "C_0" 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 $-CH_2-CH_2-0-CH_2-CH_2-$, $-CH_2-CH_2-0-CH_2-CH_2-0-CH_2-CH_2-$, or $-CH_2-CH_2-0-CH_2-CH_2-0-CH_2-CH_2-0-CH_2-CH_2-$ 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.

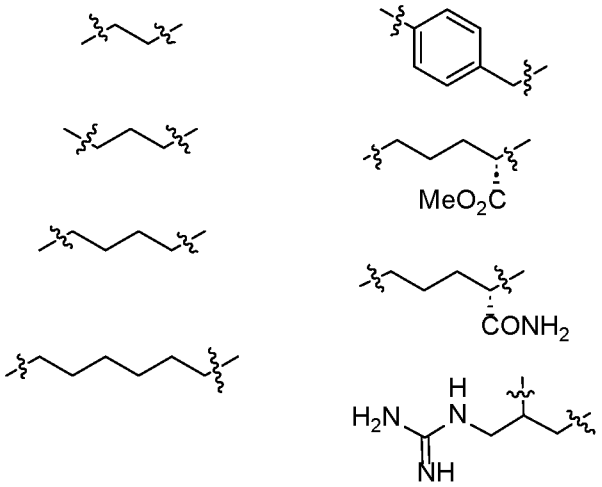
Using monovalent substituent names for convenience, exemplary divalent aromatic carbocyclic ring moieties include phenyl and naphthyl groups. Again, using monovalent names for convenience, exemplary divalent heteroaryl groups include

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 that 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*, methicilin-resistant *S. aureus* (MRSA), VanA strains of *E. faecalis* and *E. feacium*, 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 μM 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 alkanoic

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 and 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.

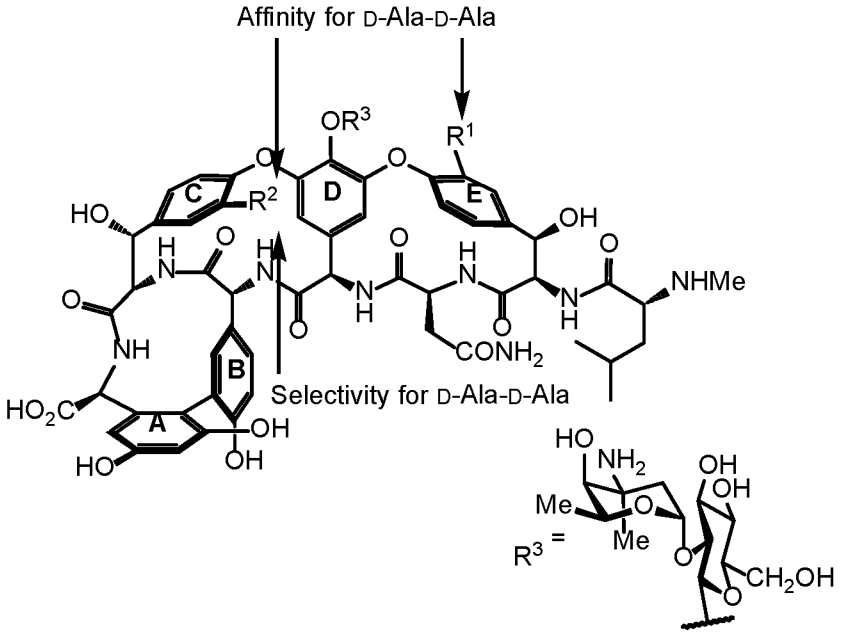
RESULTS AND DISCUSSION

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]
glycopeptides				*MSSA*[b]	*MRSA*[c]	*VSE*[d]	*Ac_2KAA*
4.2	Cl	Cl	sugar	1	1	2	1.5×10^6
4.3	H	Cl	sugar	4	4	8	5.9×10^5
4.4	H	H	sugar	8	8	32	1.6×10^5
aglycons[f]					*MSSA*[g]		
4.5	Cl	Cl	H		1.25		1.4×10^5
4.6	H	Cl	H		5		6.8×10^4
4.7	Cl	H	H		10		3.8×10^4
4.8	H	H	H		20		1.8×10^4

[a]Ref 9. [b]Methicillin-sensitive *S. aureus* (ATCC 29213). [c]Methicillin-resistant *S. aureus* (MMX 2002). [d]Vancomycin-sensitive *E. faecalis* (MMX 101). [e]Ref 8. [f]Ref 10. [g]ATCC 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 glycosyltransferases[14-17] were instrumental to our total synthesis of vancomycin,[6,18] allowing direct aglycon glycosylation without the need for protecting groups and avoiding the less efficient chemical glycosylation methods.[19-21] 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.

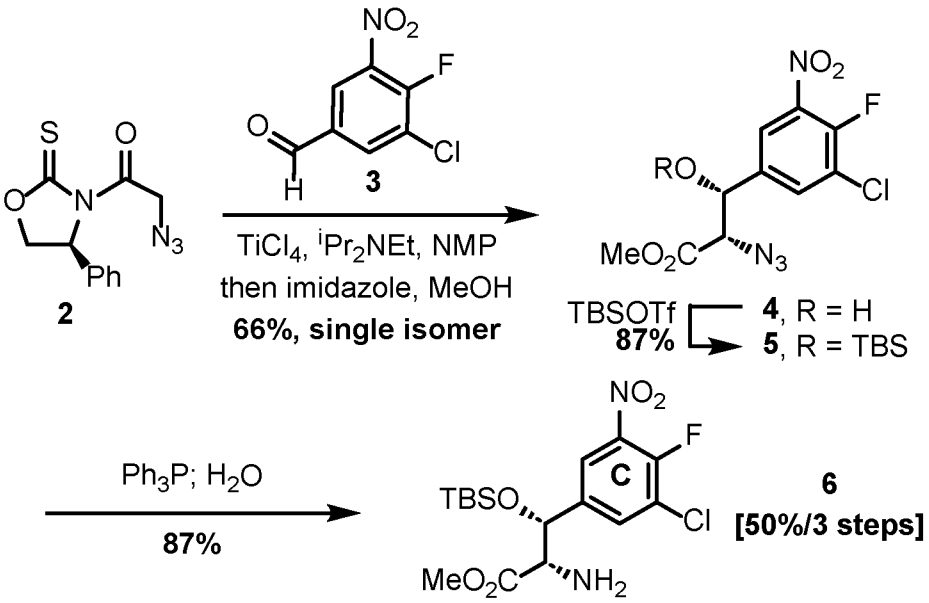
Preparation of the Modified C and E Ring Subunits

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 aldol[22] addition of Compound **2** to 3-choro-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).

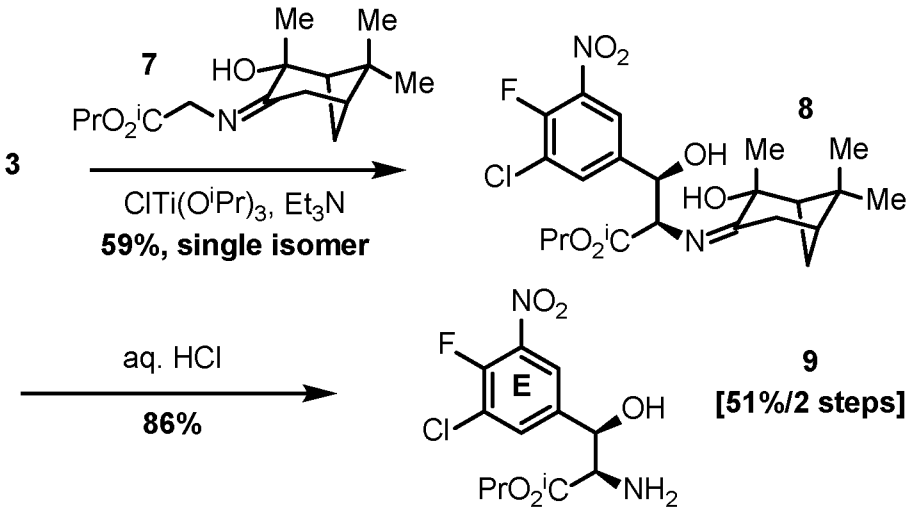
Preparation of the E ring anti β-hydroxyphenyl-alanine Compound **9** was accomplished by modification of the method of Solladié-Cavallo[24] that allowed use of commercially available $ClTi(OiPr)_3$. Accordingly, diastereoselective Ti-promoted aldol

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).

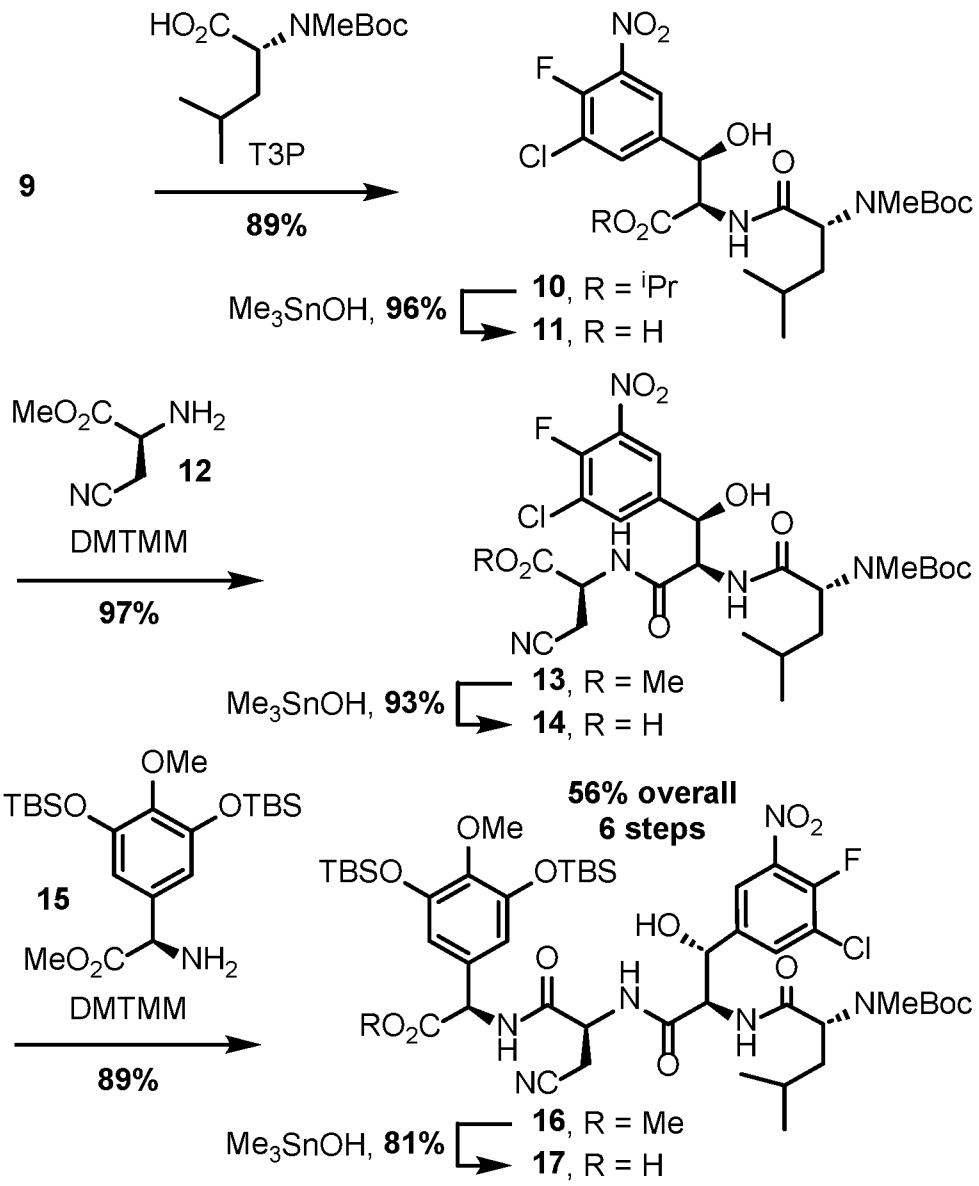
Hydrolytic removal of the chiral auxiliary with dilute aqueous HCl provided the E ring (residue 2) amine Compound **9** (86%, 51% overall yield/2 steps).

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 (Me_3SnOH,[26] $ClCH_2CH_2Cl$, 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–H_2O, 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 chloride[28] (DMTMM, EtOAc, 97%) provided tripeptide Compound **13.** Saponification (Me_3SnOH, $ClCH_2CH_2Cl$, 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 (Me_3SnOH, $ClCH_2CH_2Cl$, 81%) provided the tetrapeptide Compound **17** without C_α epimerization or competitive desilylation of the base-sensitive phenol TBS ethers.

Scheme 2

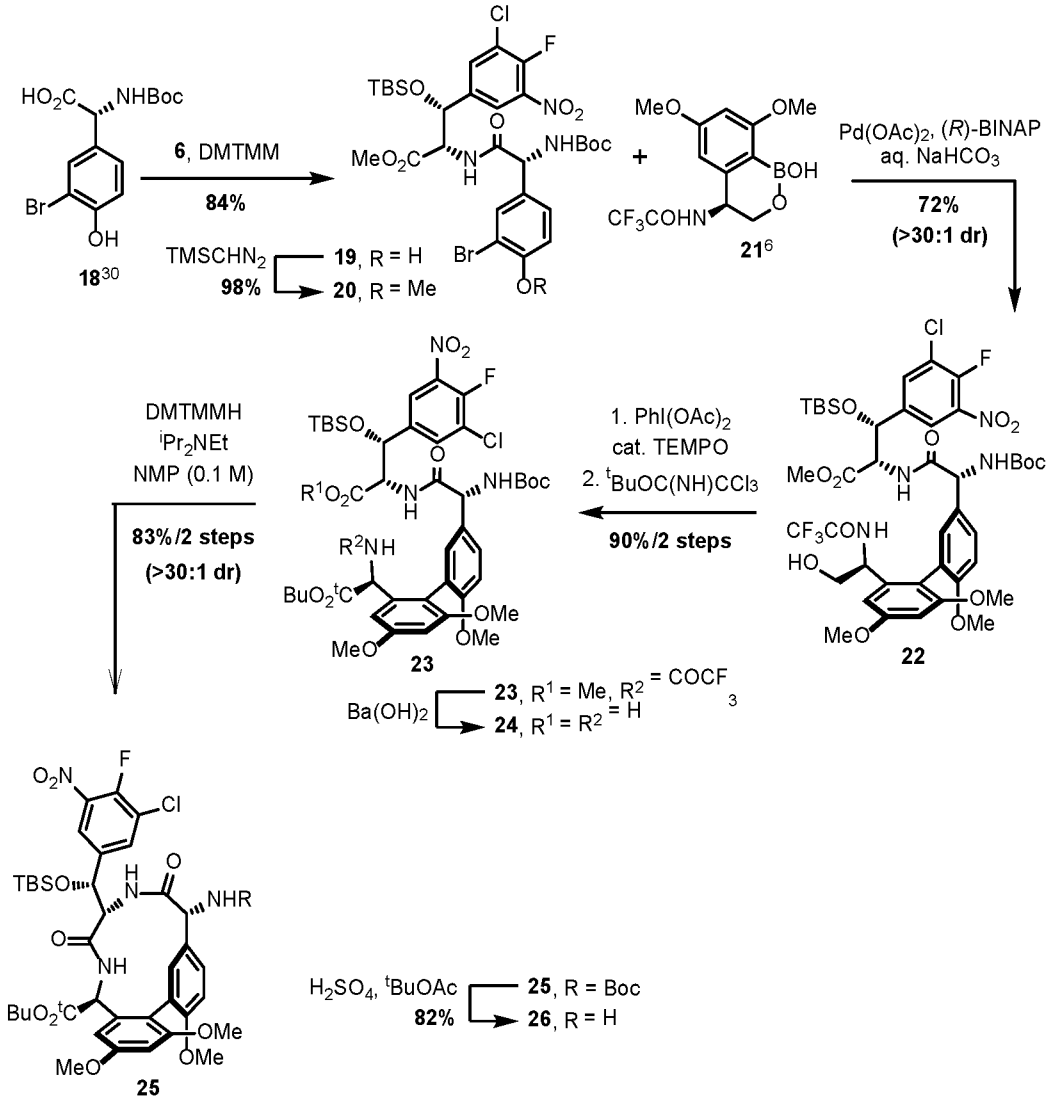


Preparation of the AB Macrocycle and Total Synthesis of Tetrachlorovancomycin

Coupling of Compound **6** with Compound **18**[30] (DMTMM, MeCN, 0 °C, 1 h) followed by phenol methylation ($TMSCHN_2$, 20% MeOH–CH_2Cl_2, 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)-Pd^0 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 vancomycin[6] ($Pd(OAc)_2$, (*R*)-BINAP, aq $NaHCO_3$, MeTHF) and did not require tailoring to accommodate the modified substrate Compound **20**, which is intrinsically more reactive toward S_NAr substitution and contains a potentially reactive aryl chloride.

Scheme 3



Oxidation of the C-terminus primary alcohol to the corresponding carboxylic acid ($PhI(OAc)_2$, cat. TEMPO, 2:1 MeCN–H_2O, 23 °C, 1 h)[32] and esterification with *t*-butyl trichloroacetimidate (CH_2Cl_2–cyclohexane, 23 °C, 16 h) 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–H_2O, 23 °C, 95%). This reagent proved to be milder[33] than LiOH and more easily removed by precipitation as $BaCO_3$, thus avoiding minor losses of 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 hexafluorophosphate[34] (DMTMMH, 1.5 equiv, 3 equiv *i*-Pr_2NEt, 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 ester[36] (8 equiv H_2SO_4, *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 ~~future~~ 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*)-one[38] (DEPBT, 2 equiv, 4.5 equiv $NaHCO_3$, 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 Bu_4NF (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 (Bu_4NF) 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 ($BF_3•Et_2O$, *t*-BuONO; CuCl, $CuCl_2$, CD_3CN),[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 $AlBr_3$/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 formation[7] and deuterated solvent suppression[6] of competitive reduction, a remarkably effective TFA-mediated nitrile hydration,[40] and a scalable $AlBr_3$/EtSH-mediated global deprotection.[7]

Finally, 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 phosphatase[42] to each glycosylation reaction.

This latter feature, which results in hydrolysis of the byproduct uridine-5′-diphosphate (UDP), also prevents product inhibition and 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

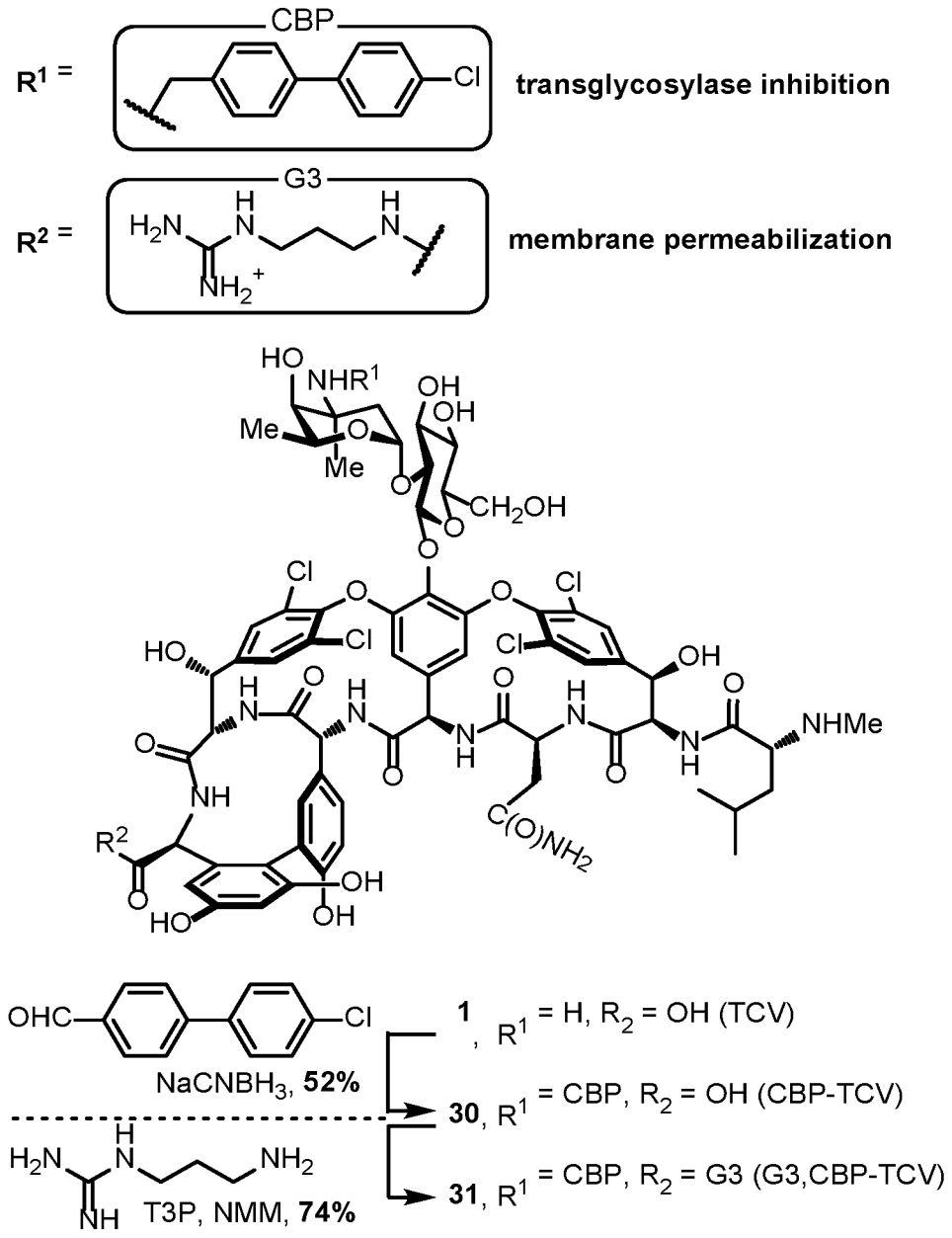
Vancomycin Total Syntheses	LLS	Yield	Atroposelectivity AB	CD	DE	Glycosylation method	steps	yield
Nicolaou (1998)	35	0.006%	2:1	1:1	1:3	chemical	11	14%
Boger (2014)	27	0.14%	1:1	1:1	8:1	enzymatic	2	80%
Boger (2020)	19	3.7%	>20:1	8:1	14:1	enzymatic	2	80%
Tetrachlorovancomycin Total Synthesis	**LLS**	**Yield**	**Atroposelectivity AB**	**CD**	**DE**	**Glycosylation 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, [Ψ[C(=S)NH]Tpg^4]tetrachloro-vancomycin aglycon (Compound **38**), required only slight modification for incorporation of the residue 4 thioamide (Fig. 4). Towards this end, direct thioacylation[35] 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.

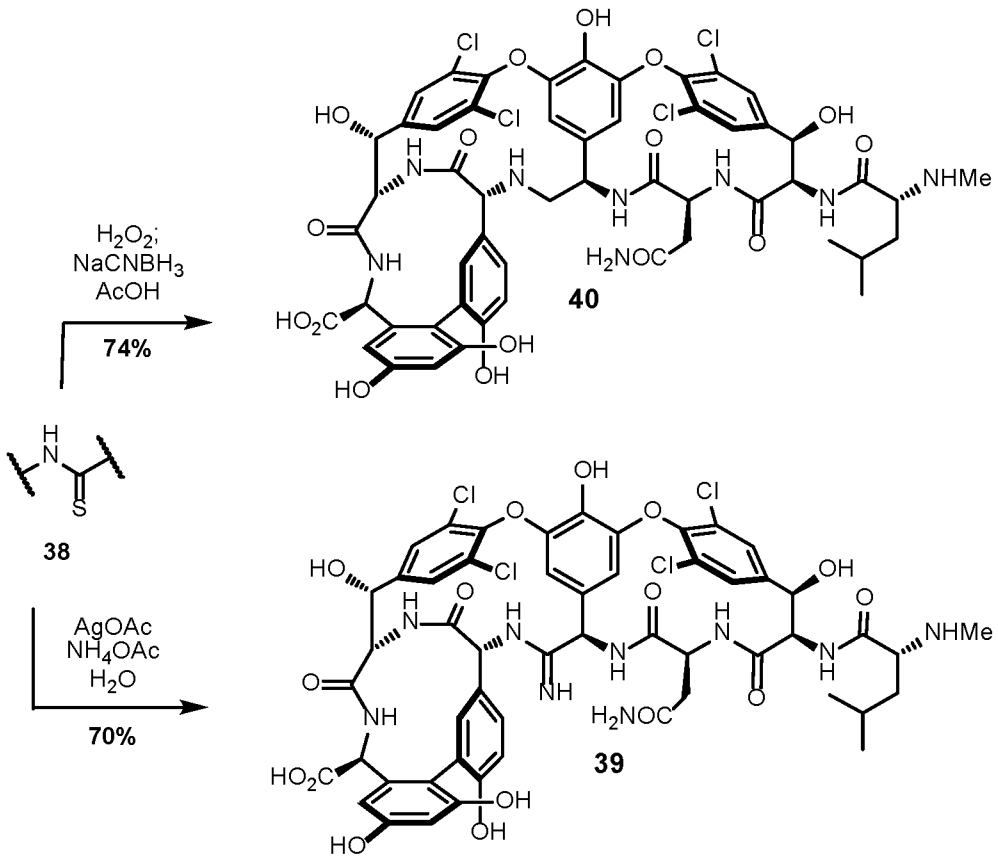
Desilylation of heptapeptide Compound **37** (Bu_4NF, MeCN, 23 °C) triggered a spontaneous double S_NAr cyclization that established the full tricyclic framework of [Ψ[C(=S)NH]Tpg^4]tetrachlorovancomycin aglycon (Compound **38**) in a single step (Fig. 4). Dual nitro reduction (Fe, AcOH), Sandmeyer substitution (BF_3•Et_2O, *t*-BuONO; CuCl, $CuCl_2$), nitrile hydration with concomitant Boc and *t*-butyl ester deprotection (TFA, 23 °C), and global demethylation (5:1 $AlBr_3$:EtSH) provided [Ψ[C(=S)NH]Tpg^4]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]Tpg^4]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]Tpg^4]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 [Ψ[C(=NH)NH]Tpg^4]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 [Ψ[CH_2NH]Tpg^4]-tetrachloro-vancomycin aglycon (Compound **40**) in high yield (74%).

Scheme 6. Divergent synthesis of [Ψ[C(=NH)NH]Tpg^4]tetrachlorovancomycin aglycon (**39**) and [Ψ[CH_2NH]Tpg^4]tetrachlorovancomycin aglycon (**40**).



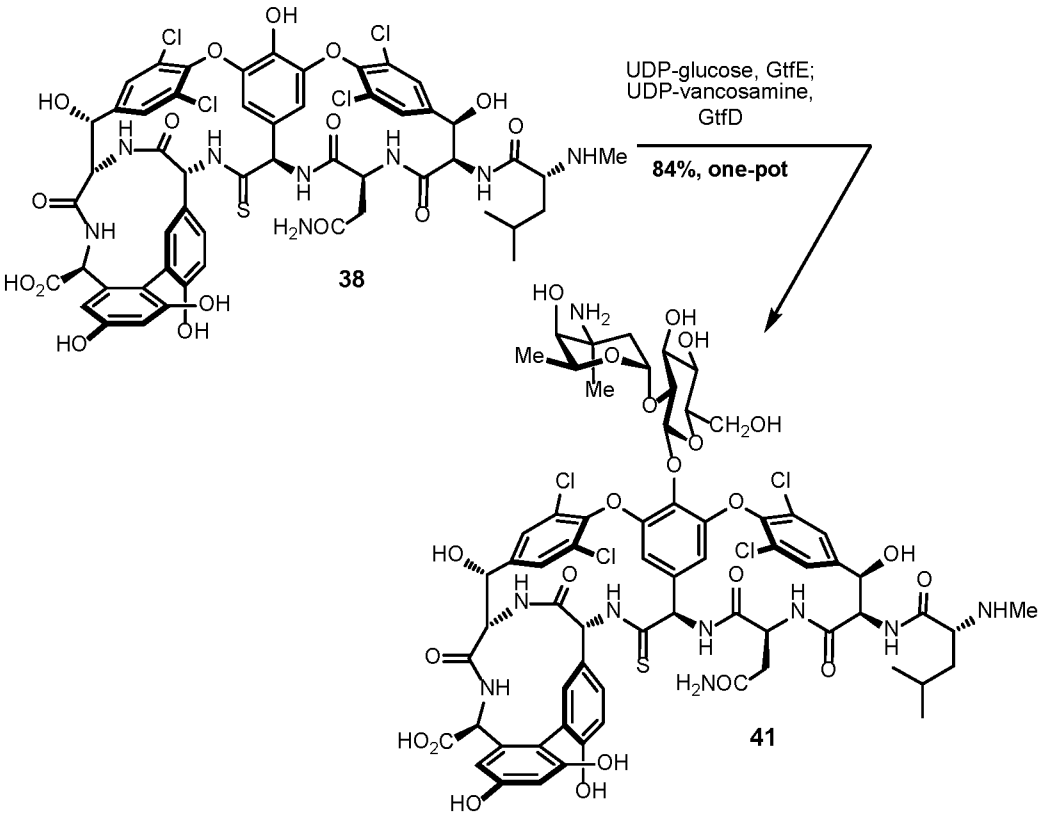
Similar, alternative syntheses of Compounds Compound **39** and **40** from Compound **38** are illustrated in Fig. 5.

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 [Ψ[C(=S)NH]Tpg^4]tetrachlorovancomycin (Compound **41**) in excellent yield (84%). Sequential conversions of Compound **41** to CBP-[Ψ[C(=S)NH]Tpg^4]tetrachloro-vancomycin (Compound **44**) and G3,CBP-[Ψ[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 tetrachlorovancomyin 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 [Ψ[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 Ac_2-L-Lys-D-Ala-D-Ala(**A**) and Ac_2-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 tetrachlorovancomycin[57] 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, Ac_2-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 Ac_2-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 CH_2NH 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 x 10^5 M^{-1}), displaying a binding constant only 5-fold lower than vancomycin (K_a = 5.4 x 10^5 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 (μg/mL)[a]		
	VRE[b]	MSSA[c]	MRSA[d]
vancomycin aglycon	>250	2	2
29, tetrachlorovancomycin aglycon	>250	8	8
vancomycin	250	0.5	0.5
1, tetrachlorovancomycin	>250	4	4
CBP-vancomycin	2.5	0.08	0.08
30, CBP-tetrachlorovancomycin	10	0.08	0.08
G3-CBP-vancomycin	0.3	0.04	0.04
31, G3-CBP-tetrachlorovancomycin	0.3	0.04	0.08

[a]Minimum inhibitory concentration. [b]Vancomycin-resistant *E. faecalis* (ATCC BAA-2317). [c]Methicillin-sensitive *S. aureus* (ATCC 25923). [d]Methicillin-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 Ac_2-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 Ac_2-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 vancomycin[44] 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.

[Ψ[C(=S)NH]Tpg^4]Tetrachlorovancomycin (Compound **41**) was found to be inactive (MICs >80 μg/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-[Ψ[C(=S)NH]Tpg^4]-tetrachlorovancomycin (Compound **44**) that displays effective and equipotent activity (MICs 5–10 μg/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-[Ψ[C(=S)NH]Tpg^4]tetrachlorovancomycin (Compound **47**) that now exhibits further improved antimicrobial activity (MICs 0.3–1.2 μg/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 antibiotics[53] 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 analogues[4] of tetrachlorovancomycin to reinstate binding to the

altered target D-Ala-D-Lac of vancomycin-resistant bacteria while maintaining binding for the unaltered target D-Ala-D-Ala found in sensitive bacteria as well as extension to their even more potent and durable peripherally-modified derivatives.[4,7]

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.

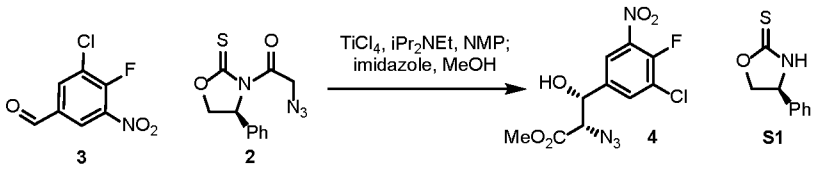
^1H and $^{13}C\{^1H\}$ NMR spectra were obtained on a Bruker Avance III™ HD 600 MHz spectrometer equipped with either a 5 mm QCI or 5 mm CPDCH probe. Chemical shifts (δ) are reported in parts per million (ppm). Abbreviations used to designate multiplicities are: s = singlet, d = doublet, t = triplet, 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 (λ = 589 nm) at specific temperatures and are reported as follows: $[\alpha]_D^{temp}$, concentration (*c* = 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 Ka radiation (l = 1.54178).

Preparations



Compound 4

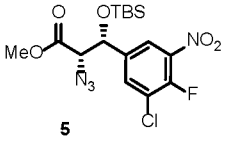
A solution of **2**[6] (20.5 g, 76.4 mmol, 1.0 equiv) in CH_2Cl_2 (760 mL) was cooled to −78 °C and treated with $TiCl_4$ (8.8 mL, 80.3 mmol, 1.05 equiv). The resulting yellow solution was stirred for 15 minutes (min) at −78 °C, then treated dropwise with *i*-Pr_2NEt (14.7 mL, 84.1 mmol, 1.1 equiv). 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 NH_4Cl (200 mL), and filtered through Celite.

The filtrate was concentrated under reduced pressure to remove MeOH, and the aqueous layer was extracted with CH_2Cl_2 (3 x 200 mL). The combined organic layers were dried with Na_2SO_4, concentrated under reduced pressure, and chromatography (SiO_2, 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 acid[54] (579 mg, 5.7 mmol, 1.4 equiv) in CH_2Cl_2 (50 mL) and cooled to 0 °C. *i*-Pr_2NEt (2.16 mL, 12.4 mmol, 3 equiv) was added dropwise to this solution at 0 °C, followed by T3P (50 wt % in CH_2Cl_2, 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 CH_2Cl_2 (3 x 50 mL). The combined organic layers were dried with $MgSO_4$, concentrated under reduced pressure, and the residue was purified by chromatography (SiO_2, wet load 50% CH_2Cl_2–hexanes, 50–100% CH_2Cl_2–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); ^{1}H NMR (600 MHz, $CDCl_3$) 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); $^{13}C\{^1H\}$ NMR (151 MHz, $CDCl_3$) 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) ν_{max} 3506, 1740, 1613, 1542, 1486, 1438, 1349, 1271, 1211, 1101, 1013 cm^{-1}; HRMS (ESI-TOF) *m/z* $[M+Cl]^-$ calcd for $C_{10}H_8ClFN_4O_5$, 352.9856; found, 352.9862.

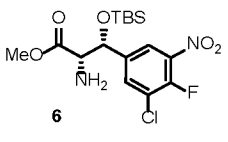


5

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 CH_2Cl_2 (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 NH_4Cl (80 mL, exothermic) at 0 °C and extracted with CH_2Cl_2 (3 × 80 mL). Column chromatography (SiO_2, 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, $CDCl_3$) 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); $^{13}C\{^1H\}$ NMR (151 MHz, $CDCl_3$) 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) ν_{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 $C_{16}H_{22}ClFN_4O_5Si$, 431.0954; found, 431.0947.

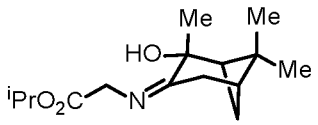


6

Compound 6

A solution of **5** (19.04 g, 44.0 mmol, 1 equiv) in dioxane (63 mL) was treated portion-wise with Ph_3P (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 Ph_3P (2.31 g, 8.80 mmol, 0.20 equiv) was added. After 1 h, the solution was treated with 75% dioxane–H_2O (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 (SiO_2, 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, $CDCl_3$) 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); $^{13}C\{^1H\}$ NMR (151 MHz, $CDCl_3$) 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) ν_{max} 2954, 2931, 2857, 1744, 1542, 1347, 1257, 1096 cm^{-1}; HRMS (ESI-TOF) *m/z* $[M+H]^+$ calcd for $C_{16}H_{24}ClFN_2O_5Si$, 407.1205; found, 407.1211.



7

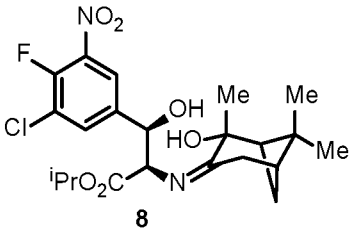
Compound 7

A suspension of isopropyl glycinate hydrochloride (5 g, 33 mmol, 2 equiv) and K_2CO_3 (8.8 g, 66 mmol, 4 equiv) in 2:1 CH_2Cl_2/H_2O (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 CH_2Cl_2 (3 × 50 mL). The combined organic layers were dried over $MgSO_4$ 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-pinanone (2.7 g, 16 mmol, 1 equiv) and dissolved in PhMe (25 mL). The resulting solution was treated dropwise with BF_3•OEt_2 (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 (SiO_2, 20–60% EtOAc–hexanes + 1% Et_3N, 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, $CDCl_3$) 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); $^{13}C\{^1H\}$ NMR (126 MHz, $CDCl_3$) 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 $C_{15}H_{25}NO_3$, 268.1913; found, 268.1918.



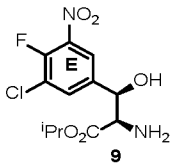
8

Compound 8

A solution of **7** (2.47 g, 9.24 mmol, 1 equiv) in CH_2Cl_2 (25 mL) at 0 °C was treated sequentially with $TiCl(OiPr)_3$ (50 wt % in CH_2Cl_2, 5.30 g, 10.2 mmol, 1.1 equiv), **3** (2.26 g in 7 mL CH_2Cl_2, 10.9 mmol, 1.2 equiv), and Et_3N (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 CH_2Cl_2 (3 × 30 mL). The combined organic layers were dried over $MgSO_4$, concentrated under reduced pressure, and the residue was purified by chromatography (SiO_2, 10–50% EtOAc–hexanes + 1% Et_3N, rapid elution) to provide **8** (2.56 g, 59%) as a moisture-sensitive yellow oil.

For **8**: ^1H NMR (400 MHz, $CDCl_3$) 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); $^{13}C\{^1H\}$ 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* $[M+H]^+$ calcd for $C_{15}H_{25}NO_3$, 268.1913; found, 268.1918.



9

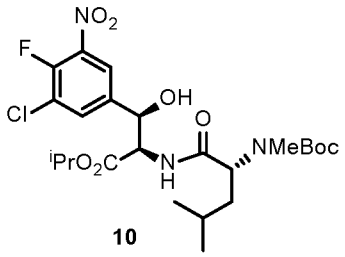
Compound 9

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 × 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); $^{13}C\{^1H\}$ 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.



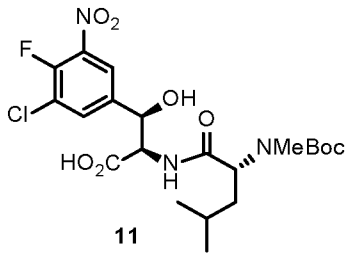
10

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*-Pr_2NEt (2.6 mL, 15 mmol, 3 equiv) followed by T3P® (50 wt % in CH_2Cl_2, 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 H_2O (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 CH_2Cl_2 (3 × 20 mL). The combined organic layers were dried over $MgSO_4$, concentrated under reduced pressure, and the residue was purified by chromatography (SiO_2, wet-load CH_2Cl_2, 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, $CDCl_3$) 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); $^{13}C\{^1H\}$ NMR (151 MHz, $CDCl_3$) 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) ν_{max} 3392, 2961, 1737, 1670, 1543, 1483, 1368, 1347, 1322, 1269, 1152, 1104 cm^{-1}; HRMS (ESI-TOF) *m/z* $[M-H]^-$ calcd for $C_{24}H_{35}ClFN_3O_8$, 546.2018; found, 546.2022.



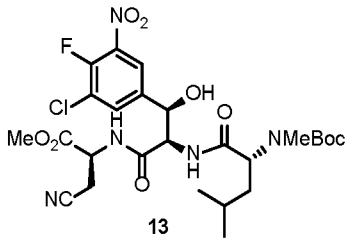
11

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% MeOH–CH_2Cl_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); $^{13}C\{^1H\}$ 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* $[M-H]^-$ calcd for $C_{21}H_{29}ClFN_3O_8$, 504.1549; found, 504.1541.



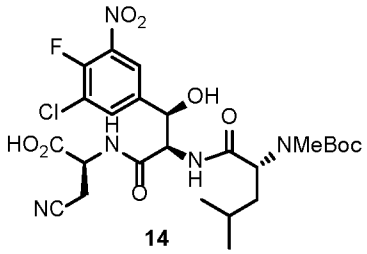
13

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 × 20 mL). The organic layer was dried over $MgSO_4$, concentrated under reduced pressure, and the residue was purified by chromatography (SiO_2, 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, CD_3OD) 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); $^{13}C\{^1H\}$ NMR (151 MHz, CD_3OD) 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) ν_{max} 3630, 3025, 2040, 1739, 1372, 1215 cm^{-1}; HRMS (ESI-TOF) *m/z* $[M-H]^-$ calcd for $C_{26}H_{34}ClFN_5O_9$, 614.2029; found, 614.2030.



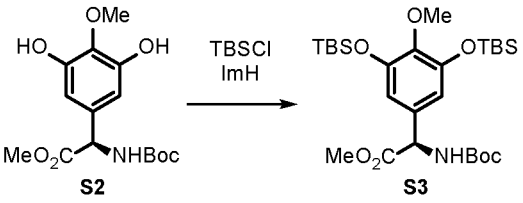
14

Compound 14

A suspension of **13** (3.90 g, 6.33 mmol, 1 equiv) and Me_3SnOH (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 $MgSO_4$, and the residue was purified by chromatography (SiO_2, wet-load 1–2% MeOH–CH_2Cl_2, eluted with 2% MeOH–CH_2Cl_2, then 90:6:4 CH_2Cl_2/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, CD_3OD) 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); $^{13}C\{^1H\}$ NMR (151 MHz, CD_3OD) 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,

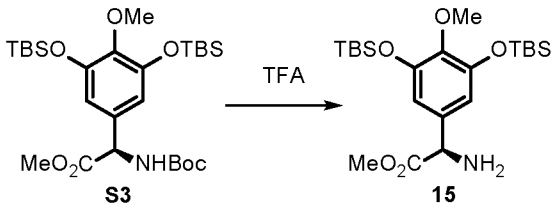
58.8, 50.4, 38.2, 28.6, 25.9, 23.5, 21.8, 21.1; IR (film) ν_{max} 3274, 2961, 1731, 1653, 1543, 1482, 1425, 1393, 1347, 1269, 1221, 1153, 1069 cm^{-1}; HRMS (ESI-TOF) *m/z* $[M+H]^+$ calcd for $C_{25}H_{33}ClFN_5O_9$, 602.2029; found, 602.2023.



Compound S3

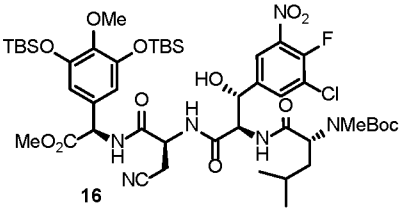
A solution of **S2**[6] (1.03 g, 2.77 mmol, 1 equiv) and imidazole (0.565 g, 8.30 mmol, 3 equiv) in CH_2Cl_2 (10 mL) was cooled to 0 °C and treated with TBSCl (1.25 g, 8.30 mmol, 3 equiv). The reaction mixture was stirred for 1 h at 0 °C, quenched with the dropwise addition of aqueous 1 M HCl (10 mL), and warmed to 23 °C. The organic layer was collected, and the aqueous layer was extracted with additional CH_2Cl_2 (3 × 10 mL). The combined organic layers were dried over $MgSO_4$, concentrated under reduced pressure, and the residue was purified by chromatography (wet-load 20% CH_2Cl_2–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); $^{13}C\{^1H\}$ 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* $[M+Na]^+$ calcd for $C_{27}H_{49}NO_7Si_2$, 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.

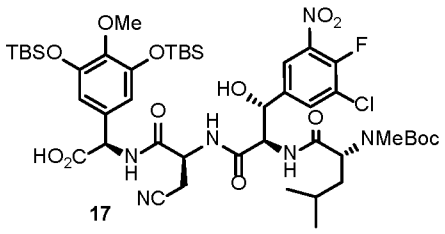


Compound 16

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–$CHCl_3$); ^{1}H 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}C{^{1}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* $[M-H]^-$ calcd for $C_{47}H_{72}ClFN_6O_{13}Si_2$, 1037.4290; found, 1037.4298.



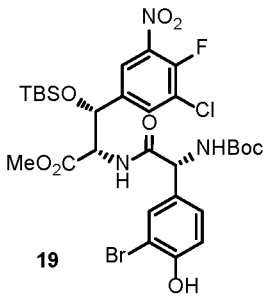
17

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 (SiO_2, 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, $CDCl_3$) 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); $^{13}C\{^1H\}$ NMR (151 MHz, CD_3OD) 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) ν_{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 $C_{46}H_{70}ClFN_6O_{13}Si_2$, 1023.4134; found, 1023.4139.



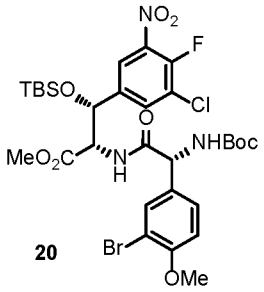
19

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 H_2O (200 mL), and extracted with PhMe (3 × 300 mL). The combined organic layers were

dried over Na_2SO_4 and concentrated under reduced pressure. Chromatography (SiO_2, 20–40% EtOAc–hexane gradient) provided **19** (17.1 g, 84%) as a yellow solid.

For **19**: ^{1}H NMR (500 MHz, $CDCl_3$) 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); ^{13}C{^{1}H} NMR (126 MHz, $CDCl_3$) 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) ν_{max} 3414, 2954, 2931, 1678, 1543, 1493, 1346, 1257, 1161, 1103, 837, 733 cm^{-1}; HRMS (ESI-TOF) m/z [M+H]$^+$ calcd for $C_{29}H_{38}BrClFN_3O_9Si$, 734.1312; found, 734.1321.



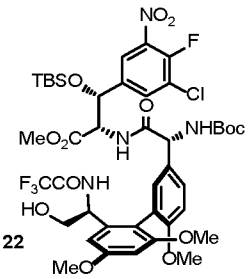
20

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); ^{1}H 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); $^{13}C\{^1H\}$ 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) ν_{max} 2931, 1716, 1542, 1346, 1257, 1203, 1157, 837 cm^{-1}; HRMS (ESI-TOF) *m/z* $[M+Na]^+$ calcd for $C_{30}H_{40}BrClFN_3O_9Si$, 770.1287; found, 770.1260.

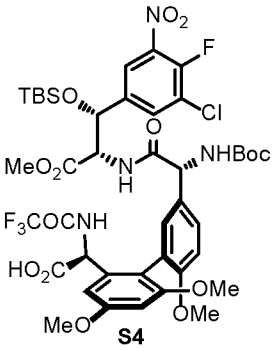


Compound 22

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 $NaHCO_3$ (22.4 g, 267 mmol, 20 equiv)

and H_2O (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, H_2O added (200 mL) to dissolve solid $NaHCO_3$, transferred to separatory funnel, and the aqueous layer was discarded. The organic layer was washed with H_2O (200 mL), dried over Na_2SO_4, and concentrated under reduced pressure. Column chromatography (SiO_2, wet-load CH_2Cl_2, 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**: $[\alpha]_D^{25}$ +4.7 (*c* 0.9, MeOH); ^1H NMR (600 MHz, $CDCl_3$) 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); $^{13}C\{^1H\}$ NMR (151 MHz, $CDCl_3$) 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) ν_{max} 2931, 1713, 1543, 1504, 1203, 1161, 1092, 891 cm^{-1}; HRMS (ESI-TOF) *m/z* $[M+Na]^+$ calcd for $C_{42}H_{53}ClF_4N_4O_{13}Si$, 961.3081; found, 961.3073.



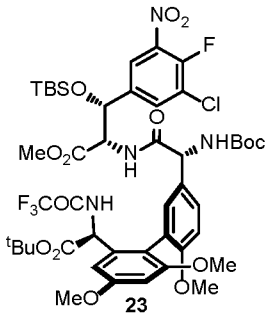
S4

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 $PhI(OAc)_2$ (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, 3Hz), 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); $^{13}C\{^1H\}$ 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) ν_{max}

3363, 2934, 1713, 1608, 1543, 1488, 1346, 1259, 1160, 1100 837, 779 cm^{-1}; HRMS (ESI-TOF) *m/z* $[M+Na]^+$ calcd for $C_{42}H_{51}ClF_4N_4O_{14}Si$, 997.2693; found, 997.2717.

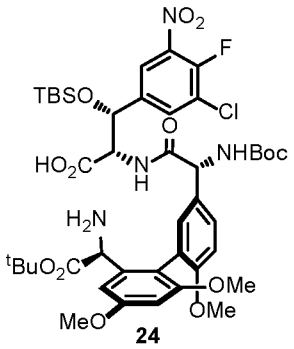


Compound 23

The semi-pure carboxylic acid **S4** from the previous step (2.0 mmol) was coevaporated with PhMe (2 × 50 mL), dissolved in CH_2Cl_2 (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% CH_2Cl_2–hexanes (3 × 5 mL). The combined filtrate was concentrated under reduced pressure and purified by column chromatography (50 g SiO_2, wet-load CH_2Cl_2, washed with 100% CH_2Cl_2 (1.5 L) to remove trichloroacetamide, then eluted with 0–10% acetone–CH_2Cl_2 over 500 mL) to provide **23** (1.87 g, 90%) as a tan solid.

For **23**: ^1H NMR (600 MHz, CD_3OD, 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); $^{13}C\{^{1}H\}$ NMR (151 MHz, CD_3OD,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) ν_{max} 2955, 2838, 1747, 1686, 1543, 1497, 1364, 1261, 1164, 1103, 837 cm^{-1}; HRMS (ESI-TOF) *m/z* $[M+H]^+$ calcd for $C_{46}H_{59}ClF_4N_4O_{14}Si$, 1053.3319; found, 1053.3284.

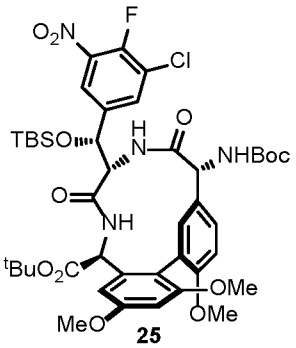


24

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 $Ba(OH)_2$ (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 $C_{43}H_{58}ClFN_4O_{13}Si$, 921.3520; found, 921.3503.



25

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*-Pr_2NEt (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–H_2O + 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 Et_2O (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, CD_3CN, 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); $^{13}C\{^{1}H\}$ NMR (151 MHz, CD_3CN) 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) ν_{max} 2932, 1696, 1660, 1609, 1508, 1485, 1367, 1254, 1160 cm^{-1}; HRMS (ESI-TOF) m/z $[M+H]^+$ calcd for $C_{43}H_{56}ClFN_4O_{12}Si$, 903.3415; found, 903.3391.

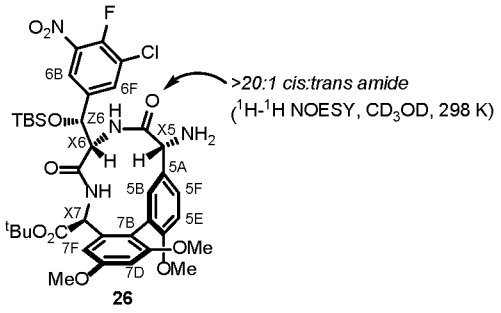
The structure, relative and absolute stereochemistry, and 5,6-cis amide conformation of **25** were confirmed with a single-crystal X-ray structure determination conducted on crystals grown from MeOH. The structure of **25** has been deposited with the Cambridge Crystallographic Data Center (CCDC 2150607

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) Å3
Z	4
Density (calculated)	1.080 Mg/m3
Absorption coefficient	1.293 mm-1
F(000)	1912
Crystal size	0.2 x 0.2 x 0.14 mm3
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 F2
Data / restraints / parameters	10131 / 310 / 697
Goodness-of-fit on F2	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 H_2O (3 x 200 mL), saturated aqueous NaCl (200 mL), dried over Na_2SO_4, and concentrated under reduced pressure. Column chromatography (SiO_2, wet load CH_2Cl_2, 0–10% EtOAc–CH_2Cl_2) provided **25** as a white powder (1.91 g, 72%/2 steps).



26

Compound 26

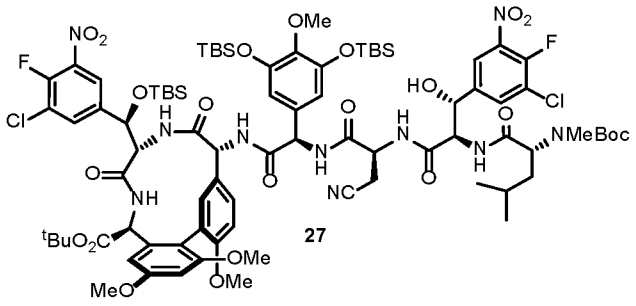
A stirred suspension of **25** (450 mg, 0.50 mmol, 1 equiv) in *t*-BuOAc (9 mL, 20 vol) was treated with H_2SO_4 (214 µL, 4 mmol, 8 equiv) and stirred at 23 °C for 2 h. The reaction mixture was carefully quenched with the addition of Et_3N (1.4 mL, 10 mmol, 20 equiv) and concentrated under a stream of N_2. Preparative HPLC (Phenomenex Luna® C18(2) 100 × 30 mm, wet-load MeOH–H_2O, 30–90% MeCN–H_2O + 0.07% TFA, t_R = 14.2 min) provided **26** (TFA salt, 378 mg, 82%) as a white solid.

For **26** (TFA salt): ^{1}H NMR (600 MHz, CD_3OD) 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-OC**H$_3$**), 3.83 (s, 3H, 5D-OC**H$_3$**), 3.70 (s, 3H, 7C-OC**H$_3$**), 1.53 (s, 9H, C7-OC(C**H$_3$**)$_3$), 0.89 (s, 9H, Z6-OSiC(C**H$_3$**)$_3$), 0.04 (s, 3H, Z6-OSiC**H$_3$**), −0.06 (s, 3H, Z6-OSiC**H$_3$**); ^{13}C{^{1}H} NMR (151 MHz, CD_3OD) 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) ν_{max} 3400, 2935, 1672, 1608, 1543, 1464, 1349, 1201, 1151, 836 cm^{-1}; HRMS (ESI-TOF) *m/z* $[M+H]^+$ calcd for $C_{38}H_{48}ClFN_4O_{10}Si$, 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.



27

Compound 27

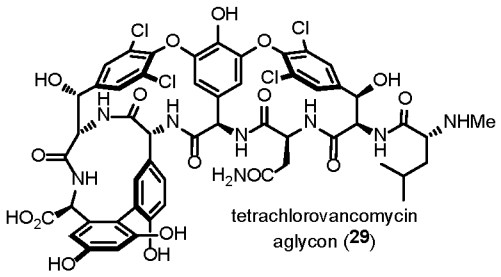
A solution of **26** (TFA salt, 85 mg, 93 µmol, 1 equiv) in CH_2Cl_2 (2 mL) was treated with saturated aqueous $NaHCO_3$ (2 mL) and stirred at 23 °C for 10 min. The organic layer was removed, and the aqueous layer was extracted with additional CH_2Cl_2 (3 × 2 mL). The combined organic layers were dried 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 °C for 17 h, concentrated under a stream of N_2, and the residue was purified by chromatography (10 g SiO_2, wet-load 50% CH_2Cl_2–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, CH_2Cl_2); ^1H NMR (600 MHz, CD_3OD, 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); $^{13}C\{^1H\}$ NMR (151 MHz, CD_3OD, 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) ν_{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 $C_{84}H_{116}Cl_2F_2N_{10}O_{22}Si_3$, 1807.6840; found, 1807.6808.



tetrachlorovancomycin aglycon (**29**)

Tetrachlorovancomycin Aglycon (29)

S_NAr reaction: A solution of **27** (35 mg, 19 µmol, 1 equiv) in MeCN (6.4 mL, [**27**] = 3 mM) was treated with Bu_4NF (1 M solution in THF, 100 µL, 100 µmol, 5 equiv), stirred at 23 °C for 4 h, and quenched with the addition of AcOH (23 µL, 400 µmol, 20 equiv) before concentrating under a stream of N_2 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 μmol, 100 equiv). The reaction mixture was stirred vigorously at 35 °C for 16 h, concentrated under a stream of N_2, and resuspended in EtOAc (4 mL). The organic layer was washed sequentially with H_2O (3 mL) and saturated aqueous NaCl (3 mL), dried over Na_2SO_4, and concentrated under a stream of N_2. Rapid PTLC (SiO_2, 100% acetone) provided the bis-aniline (30 mg, inconsequential mixture of atropisomers) as an air-sensitive white solid, used immediately in the following step.

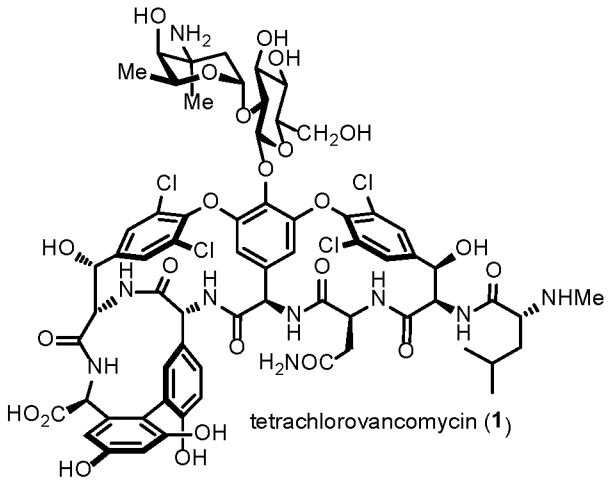
Sandmeyer substitution: A portion of the bis-aniline (20 mg, ≤13 μmol) from the previous step was dissolved in CD_3CN (0.8 mL) and cooled to 0 °C. The reaction mixture was treated with BF_3•Et_2O (6.4 μL, 52 μmol, 4 equiv), followed immediately by *t*-BuONO (6.4 μL, 52 μmol, 4 equiv). The light yellow-green 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 $CuCl_2$ (520 mg, 3.9 mmol, 300 equiv) in 50% CD_3CN–H_2O (1.6 mL) was added by syringe. The reaction mixture was slowly warmed to 5 °C over 2 h, added to saturated aqueous NH_4Cl (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 × 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 × 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); ^{1}H 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); $^{13}C\{^1H\}$ 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* $[M+H]^+$ calcd for $C_{53}H_{50}Cl_4N_8O_{17}$, 1211.2126; found, 1211.2107.



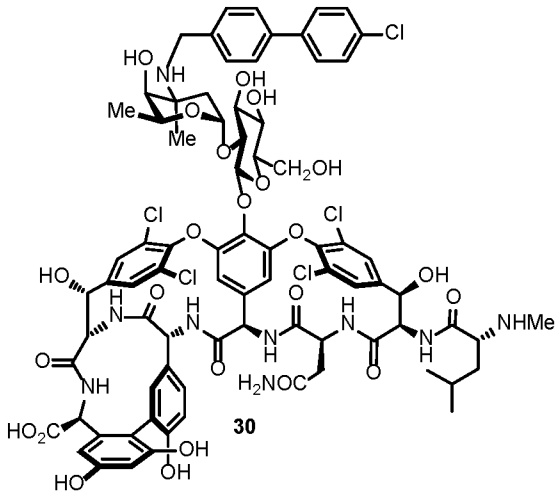
Tetrachlorovancomycin (1)

A solution of **29** (7.5 mg, 5.7 µmol, 1 equiv) in DMSO (250 µL) was treated sequentially with TCEP•HCl (3.5 mg, 11.4 µmol, 2 equiv), commercially available UDP-glucose•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 °C for 17 h, cooled to 23 °C, and treated with additional TCEP•HCl (10.5 mg, 35 µmol, 6 equiv), 750 mM tricine-NaOH (pH 9, 1 mL), the azide precursor to UDP-vancosamine[41] (45 µmol, 8 equiv), and GtfD[41] (65 µM, 0.92 mL, 1 mol %). The reaction mixture was warmed to 37 °C for 16 h, cooled to 23 °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® C18(2), 250 × 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**: ^{1}H 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}C{^{1}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* $[M+H]^+$ calcd for $C_{66}H_{73}Cl_4N_9O_{24}$, 1516.3601; found, 1516.3567.



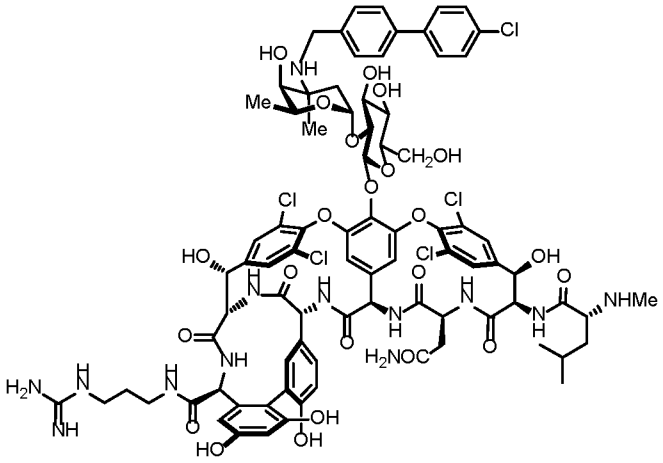
30

CBP-Tetrachlorovancomycin (30)

A solution of tetrachlorovancomycin (**1**, 6.9 mg, 4.0 µmol, 1 equiv), *i*-Pr_2NEt (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 × 4.6 mm, 5 mL/min, 10–40% MeCN–H_2O + 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 $[M+H]^+$ calcd for $C_{79}H_{82}Cl_5N_9O_{24}$, 1716.3994; found, 1716.3938.

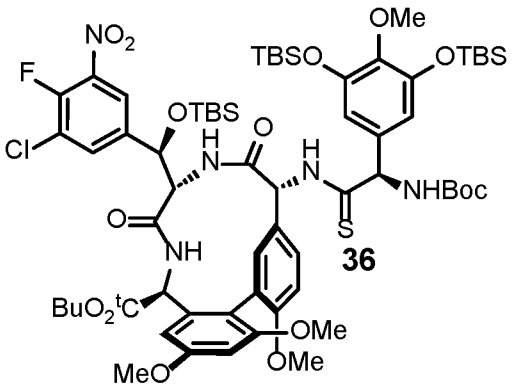


G3,CBP-Tetrachlorovancomycin (31)

A solution of **30** (1.1 mg, 0.57 µmol, 1 equiv), 1-(3-aminopropyl)guanidine[44] (bis-TFA salt, 0.96 mg, 2.8 µmol, 5 equiv) and NMM (1.9 µL, 17 µmol, 30 equiv) in DMF (200 µL) was cooled to 0 ° C and treated with T3P (50 wt % in EtOAc, 3.5 µL, 5.7 µmol, 10 equiv). The reaction mixture was stirred at 0 °C for 10 min, diluted with H_2O (1 mL), and purified directly by HPLC (Phenomenex Luna® C18(2), 250 × 4.6 mm, 5 mL/min, 25–40% MeCN–H_2O + 0.07% TFA over 20 min, t_R = 10.1 min) to afford **31** (0.90 mg, 74%) as a white solid.

For **31**: ^{1}H NMR (600 MHz, CD_3OD) 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 $C_{83}H_{92}Cl_5N_{13}O_{23}$, 605.5035; found, 605.5016.

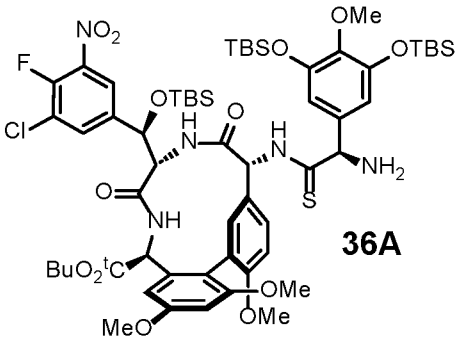


Compound **36**

A solution of **25** (TFA salt, 60 mg, 65 µmol, 1 equiv) and **35** (82 mg, 130 µmol, 2 equiv) in THF (0.6 mL, 10 vol) was treated with i-Pr_2NEt (16 µL, 98 µmol, 1.5 equiv) and stirred at 23 °C for 6 h. The reaction mixture was concentrated under a stream of N_2 and purified by PTLC (SiO_2, 40% EtOAc–hexanes) to provide **36** (78.2 mg, 89%) as a white solid.

For **36**: $[\alpha]_D^{23}$ -6.0 (c 1.0, CH_2Cl_2); ^1H NMR (600 MHz, $CDCl_3$) 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); ^{13}C NMR (151 MHz, $CDCl_3$) 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 $C_{64}H_{93}ClFN_5O_{15}SSi_3$, 1342.5447; found, 1342.5472.

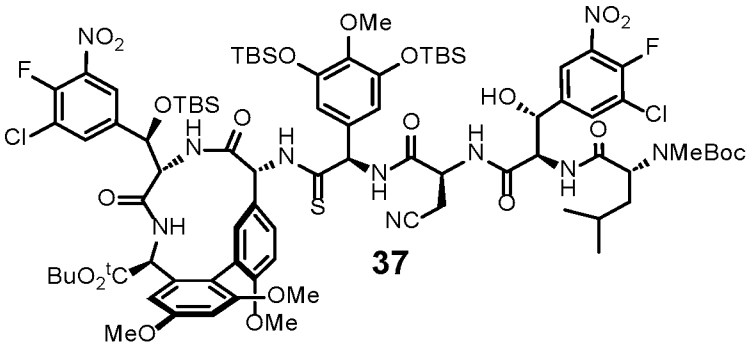


36A

Compound **36A**

A solution of **36** (150 mg, 0.11 mmol, 1 equiv) in *t*-BuOAc (3 mL, 20 vol) was treated with H_2SO_4 (45 µL, 0.9 mmol, 8 equiv) and stirred at 23 °C

for 1 h. The reaction mixture was quenched with the addition of Et_3N (182 µL, 1.32 mmol, 12 equiv) and purified directly by HPLC (Phenomenex Luna® C18(2), 100 × 30 mm, 20 mL/min, 50–100% MeCN–H_2O + 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.



37

Compound 37

A solution of **36A** (TFA salt, 124 mg, 92 µmol, 1 equiv) and **14** (112 mg, 150 µmol, 1.6 equiv) in THF (2 mL) was cooled to 0 °C and treated with NMM (41 µL, 370 µmol, 4 equiv), followed by T3P® (50 wt % in EtOAc, 90 µL, 150 µmol, 1.6 equiv). The reaction mixture was stirred at 0 °C for 10 min, quenched with the addition of H_2O (5 mL), and concentrated under a stream of N_2 to remove THF. The residue was extracted with CH_2Cl_2 (5 × 3 mL) and dried over Na_2SO_4. Chromatography (10 g SiO_2, wet-load CH_2Cl_2, 0–30% Et_2O–CH_2Cl_2) provided **37** (141 mg, 85%, 69%/2 steps from **14**) as a yellow solid.

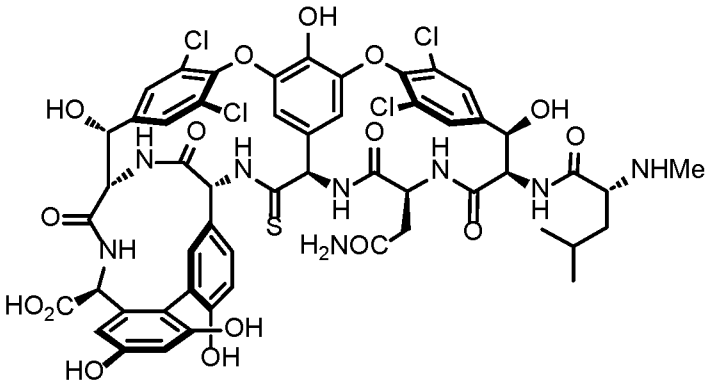
For **37**: $[\alpha]_D^{24}$ -4.0 (*c* 1.0, CH_2Cl_2); ^1H NMR (600 MHz, CD_3OD, rotamers, major given) δ 8.23 (dd, *J* = 6.2, 2.2 Hz, 1H), 8.15–8.07 (m, 1H), 7.99 (dd, *J* =

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* $[M-H]^-$ calcd for $C_{84}H_{116}Cl_2F_2N_{10}O_{21}SSi_3$, 1823.6612; found, 1823.6617.

Important note:

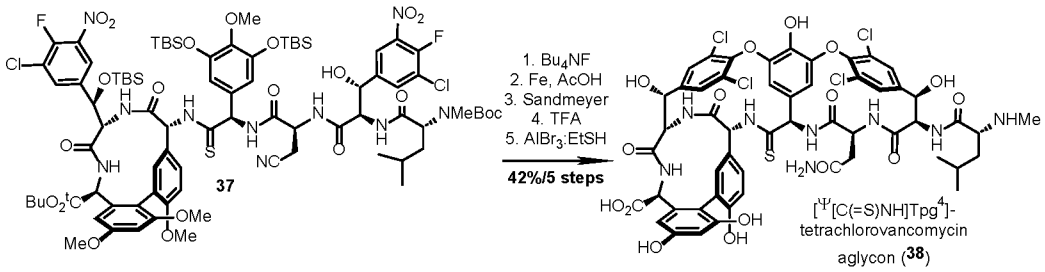
The streamlined 5-step conversion of **37** to **38** (below) was both higher-yielding and more conveniently performed without HPLC purification of

the intermediate atropisomer mixtures. Final purification of **38** by HPLC was sufficient to obtain pure samples of [Ψ[C(=S)NH]Tpg^4]tetrachlorovancomycin aglycon.

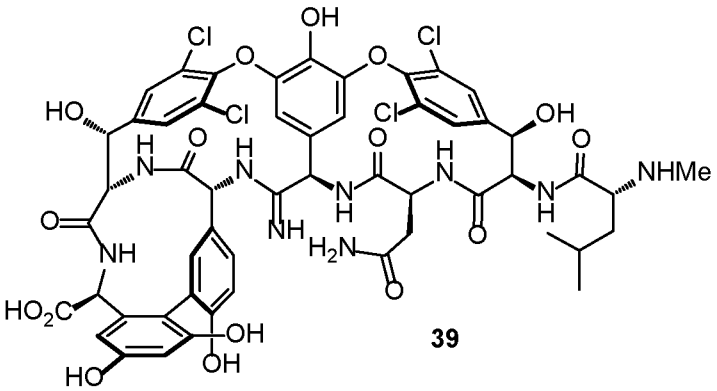


Compound 38 [Ψ[C(=S)NH]Tpg^4]tetrachlorovancomycin aglycon

Desilylation of heptapeptide **37** (Bu_4NF, MeCN, 23 °C) triggered a spontaneous double S_NAr cyclization that established the full tricyclic framework of [Ψ[C(=S)NH]Tpg^4]tetrachlorovancomycin aglycon (**38**) in a single step. Dual nitro reduction (Fe, AcOH), Sandmeyer substitution (BF_3•Et_2O, *t*-BuONO; CuCl, $CuCl_2$), nitrile hydration with concomitant Boc and *t*-butyl ester deprotection (TFA, 23 °C), and global demethylation (5:1 $AlBr_3$:EtSH) provided [Ψ[C(=S)NH]Tpg^4]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**: $[\alpha]_D^{24}$ +77 (*c* 1.0, MeOH); ^{1}H NMR (600 MHz, CD_3OD) 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); ^{13}C{^{1}H} NMR (151 MHz, CD_3OD) 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) ν_{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* $[M+H]^+$ calcd for $C_{53}H_{50}Cl_4N_8O_{16}S$, 1227.1898; found, 1227.1879.

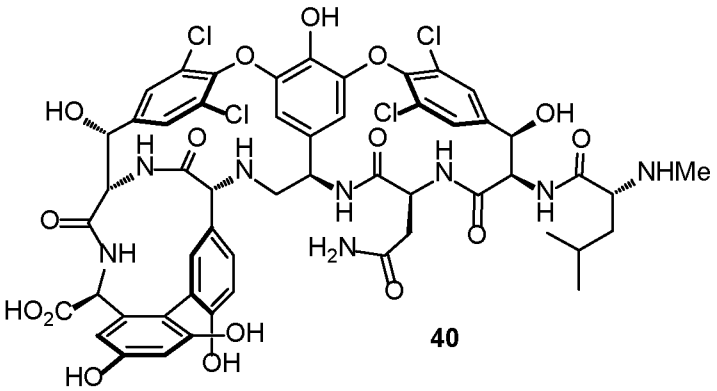


39

[Ψ[C(=NH)NH]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, [Ag] = 100 mM). The mixture was stirred at 23 °C with protection from light for 40 h. Direct HPLC purification (Phenomenex Luna® C18(2), 250 × 4.6 mm, 5 mL/min, 1–15% MeCN/H_2O–0.07% TFA over 3 min then 15–30% MeCN/H_2O–0.07% TFA over 30 min, t_R = 16 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 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 Hz, 3H), 2.44 (dd, J = 16.0, 5.5 Hz, 1H), 1.91–1.76 (m, 1H), 1.70–1.55 (m, 2H), 0.92 (d, J = 6.1 Hz, 3H), 0.89 (d, J = 6.2 Hz, 3H); HRMS (ESI-TOF) m/z $[M+2H]^{2+}$ calcd for $C_{53}H_{51}Cl_4N_9O_{16}$, 605.6182; found, 605.6172.



40

[Ψ[CH_2NH]Tpg^4]Tetrachlorovancomycin Aglycon (40)

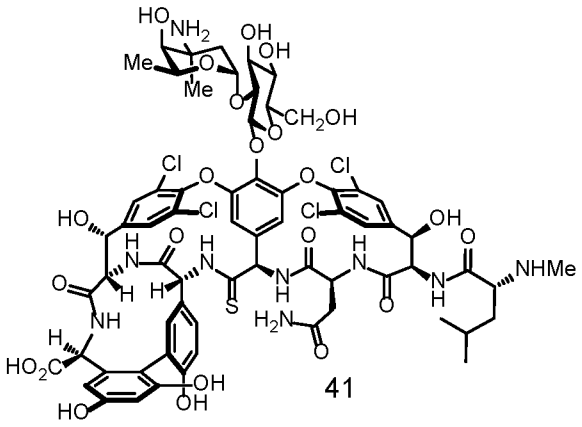
A solution of **38** (1.1 mg, 0.82 μmol, 1 equiv) in MeOH (250 μL) was treated with H_2O_2 (35% aqueous solution, 50 μL, 20% v/v) and stirred at 23 °C for 1 h. The reaction mixture was cooled to 0 °C and treated with $NaCNBH_3$ (300 mg, 50% w/v). The resulting suspension was stirred at 0 °C for 30 min, acidified with the addition of AcOH (10 μL), 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 H_2O (1 mL), and purified by HPLC (Phenomenex Luna® C18(2), 250 × 4.6 mm, 5 mL/min, 1-30% MeCN–H_2O + 0.07% TFA over 20 min, t_R = 22.7 min) to provide **40** (bis-TFA salt, 867 μg, 74%) as a white solid.

Alternatively, a solution of **38** (3.3 mg, 2.68 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 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 H_2O (10 mL), and purified by semi-preparative reverse-phase HPLC (Luna®-5 µm-C18, 100 Å, 100 × 30 mm, 15–25% MeCN/H_2O–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**: ^{1}H NMR (600 MHz, CD_3OD) 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 $C_{53}H_{52}Cl_4N_8O_{16}$, 1197.2334; found, 1197.2291.



41

[Ψ[C(=S)NH]Tpg^4]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 μM in protein storage buffer*, 8.1 mL, 0.405 μmol, 0.25 mol%), TCEP•HCl (93 mg, 0.324 mmol, 2 equiv) and calf intestinal alkaline phosphatase (CIAP, Promega, 1 U/μL in storage buffer**, 57.2 μL, 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).

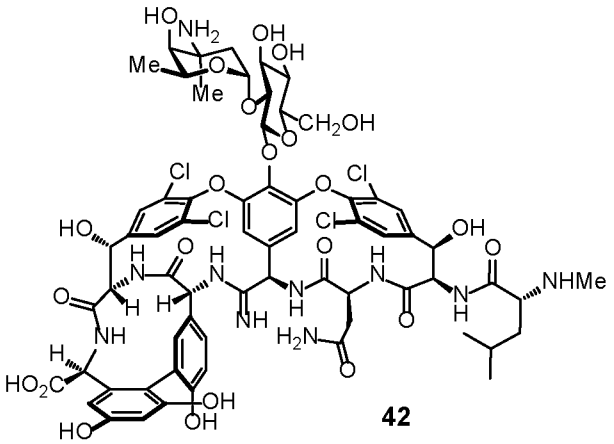
After 60 h, LCMS indicates <2% of **38** left, and the reaction was cooled to 23 °C, and treated with additional 750 mM tricine-NaOH (pH 9, 16 mL), GtfD (65 μM in protein storage buffer*, 5.0 mL, 0.325 μmol, 0.2 mol%), TCEP•HCl (606 mg, 2.11 mmol, 13 equiv) and calf intestinal alkaline phosphatase (CIAP, 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 H_2O and purified by semi-preparative reverse-phase HPLC (Luna®-5 µm-C18, 100 Å, 100 × 30 mm, 10–28% MeCN/H_2O–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); ^{1}H NMR (600 MHz, CD_3OD) 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); $^{13}C\{^1H\}$ 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* $[M+H]^+$ calcd for $C_{66}H_{73}Cl_4N_9O_{23}S$, 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.



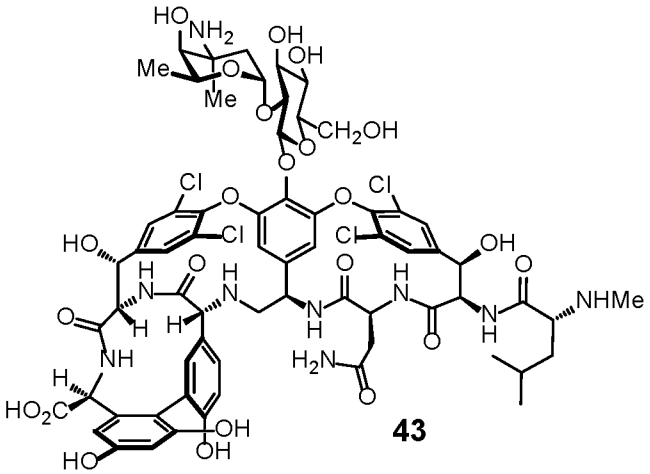
42

[Ψ[C(=NH)NH]Tpg^4]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, [Ag] = 100 mM). After the resulting mixture was

stirred at ambient temperature for 24 h, HPLC purification (Phenomenex Luna® C18(2), 250 × 4.6 mm, 1–20% MeCN/H_2O–0.07% TFA gradient over 25 min, 5 mL/min, t_R = 24 min) afforded **42** (1.54 mg, 77%) as a white solid.

For **42**: ^{1}H 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* $[M+2H]^+$ calcd for $C_{66}H_{74}Cl_4N_{10}O_{23}$, 758.1919; found, 758.1898.

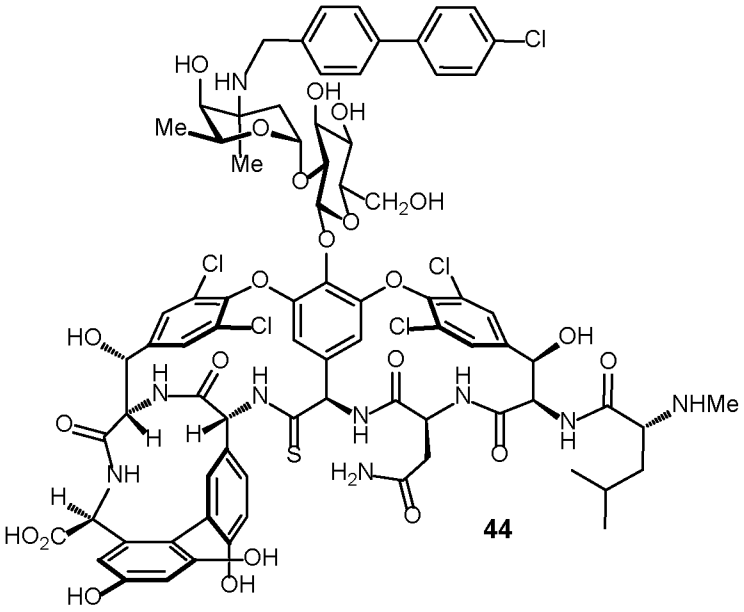


43

[Ψ[CH_2NH]Tpg^4]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 and 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/H_2O–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**: ^{1}H NMR (600 MHz, CD_3OD) 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 $C_{66}H_{75}Cl_4N_9O_{23}$, 751.6943; found, 751.6945.



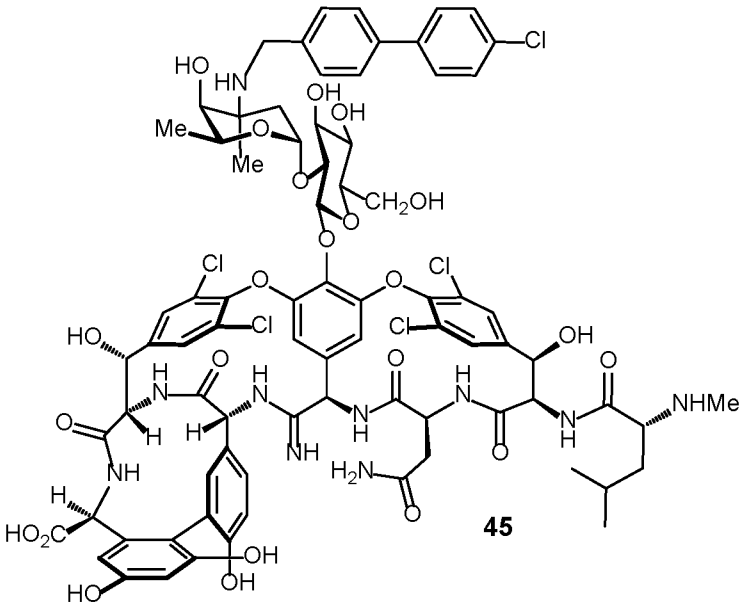
44

CBP-[Ψ[C(=S)NH]Tpg^4]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*-Pr_2NEt (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 $NaCNBH_3$ (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 H_2O (40 mL), and reverse-phase HPLC purification (Luna®-5 µm-C18, 100 Å, 100 × 30 mm, 1-40% MeCN/H_2O–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, CD_3OD) 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); $^{13}C\{1H\}$ NMR (151 MHz, CD_3OD) 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 $C_{79}H_{82}Cl_5N_9O_{23}S$, 1732.3765; found, 1732.3741.

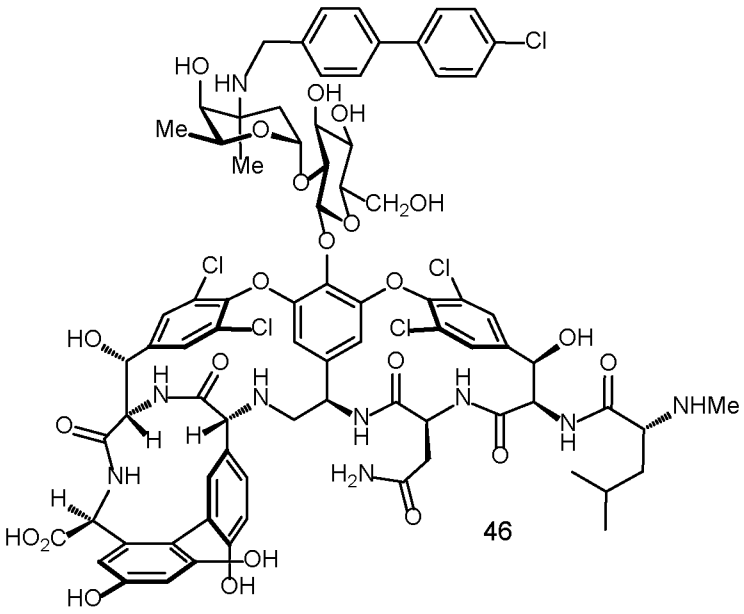


45

CBP-[Ψ[C(=NH)NH]Tpg^4]Tetrachlorovancomycin (45)

A solution of **44** (1.51 mg, 0.87 μmol, 1 equiv) in saturated aqueous NH_4OAc (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/H_2O–0.07% TFA over 3 min then 20–40% MeCN/H_2O–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, CD_3OD) 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 $C_{79}H_{83}Cl_5N_{10}O_{23}$, 858.2116; found, 858.2078.



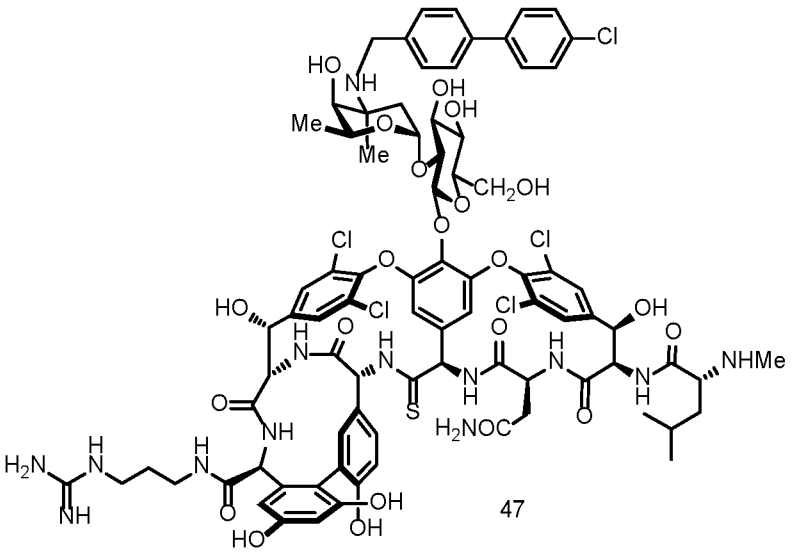
46

CBP-[Ψ[CH_2NH]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®-5 µm-C18, 100 Å, 100 × 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.



47

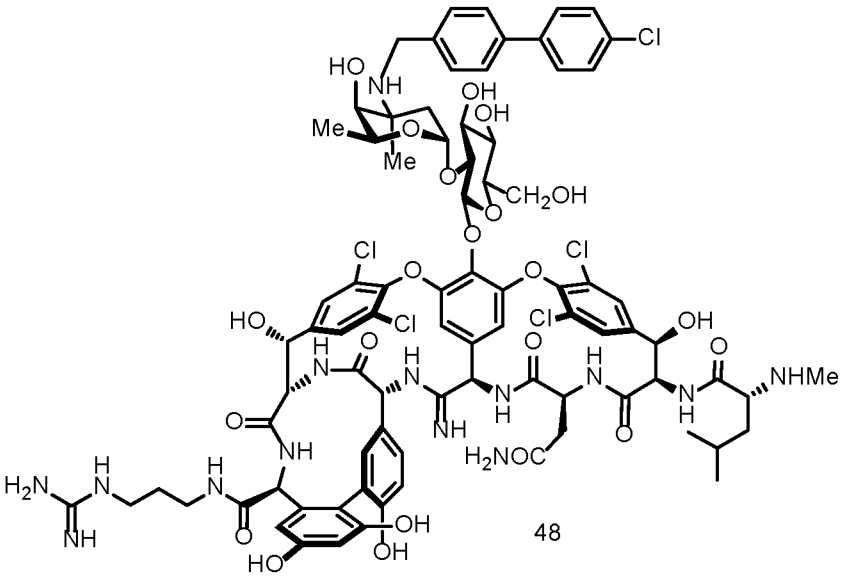
G3,CBP-[Ψ[C(=S)NH]Tpg^4]Tetrachlorovancomycin (47)

A 4 mL glass vial was charged with **44** (10.0 mg, 5.76 µmol, 1 equiv), 1-(3-aminopropyl)guanidine

(bis-TFA salt, 20 mg, 58 μmol, 10 equiv), *N*-methylmorpholine (NMM, 13 μL, 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 μmol, 10 equiv) and stirred at 23 °C under Ar for 20 min, 10 mL of H_2O was added to quench the reaction. Semi-preparative reverse-phase HPLC (Luna®-5 μm-C18, 100 Å, 100 × 30 mm, 1–22% MeCN/H_2O–0.07% TFA gradient over 2 min then 22–35% MeCN/H_2O–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**: ^{1}H 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); $^{13}C\{^1H\}$ 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* $[M+3H]^+$ calcd for $C_{83}H_{92}Cl_5N_{13}O_{22}S$, 610.8269; found, 610.8256.

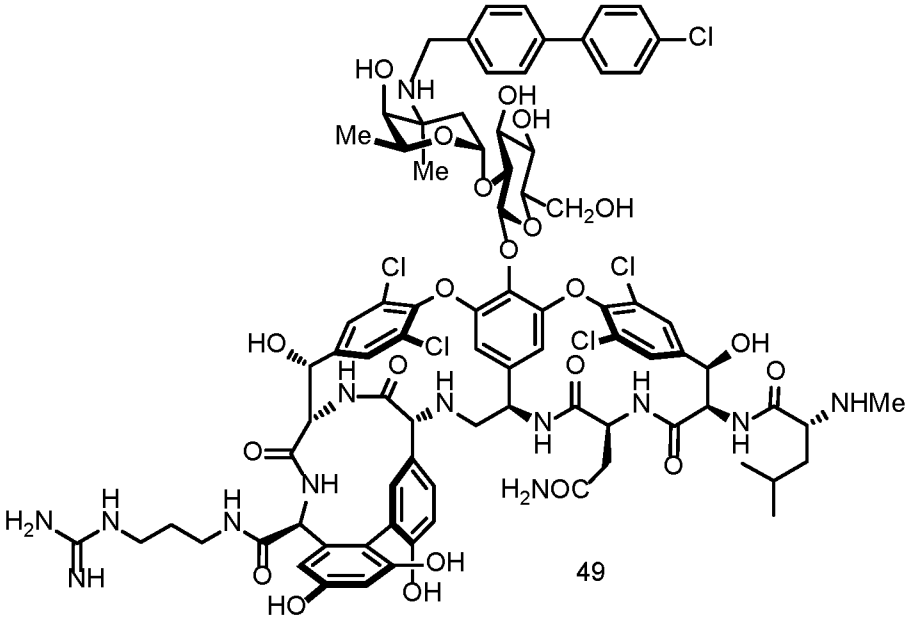


48

G3,CBP-[Ψ[C(=NH)NH]Tpg^4]Tetrachlorovancomycin (48)

A solution of **47** (10.0 mg, 5.46 µmol, 1 equiv) in saturated aqueous NH_4OAc (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 H_2O (5 mL), and semi-preparative reverse-phase HPLC (Luna®-5 µm-C18, 100 Å, 100 × 30 mm, 1–22% MeCN/H_2O–0.07% TFA gradient over 2 min then 22–26% MeCN/H_2O–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**: ^{1}H NMR (600 MHz, CD_3OD) 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}C{^{1}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* $[M+3H]^{+}$ calcd for $C_{83}H_{93}Cl_5N_{14}O_{22}$, 605.1755; found, 605.1736.



49

G3,CBP-[Ψ[CH_2NH]Tpg^4]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 H_2O (5 mL), purification by semi-preparative reverse-phase HPLC (Luna®-5 μm-C18, 100 Å, 100 × 30 mm, 1–18% MeCN/H_2O–0.07% TFA gradient over 2 min then 18–28% MeCN/H_2O–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 H_2O (5 mL). Semi-preparative reverse-phase HPLC (Luna®-5 µm-C18, 100 Å, 100 × 30 mm, 1–40% MeCN/H_2O–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**: ^{1}H NMR (600 MHz, CD_3OD) 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); $^{13}C\{^{1}H\}$ NMR (151 MHz, CD_3OD) 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 x 10^{-5} M, 20 mM sodium citrate buffer, pH = 5.1)[45,46] and by isothermal titration calorimetry (ITC, 8.0 x 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 x 10^5 M^{-1}), displaying a binding constant only 5-fold lower than vancomycin (K_a = 5.4 x 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 Ac_2-L-Lys-D-Ala-D-Ala (ITC K_a = 2.9 x 10^5 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 x 10^5	5.5 x 10^5	−7.8	−10.7	+2.9
1, tetrachlorovancomycin	3.3 x 10^4	1.1 x 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'*-Ac_2-Lys-D-Ala-D-Ala (**A**) and *N,N'*-Ac_2-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 (8×10^{-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'*-Ac_2-Lys-D-Ala-D-Ala (**A**) or *N,N'*-Ac_2-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 ([ligand]/[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-iTC_{200} 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'*-Ac_2-Lys-D-Ala-D-Ala (**A**) or *N,N'*-Ac_2-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 Tetrachlorovancomycins [55]

One day before studies were run, fresh cultures of vancomycin-sensitive Staphlococcus aureus (VSSA strain ATCC 25923), methicillin and oxacillin-resistant Staphlococcus 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 serial 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 serial 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 [Ψ(CH_2NH)]Tpg^4]tetrachlorovancomycin (Compound **49**) with protein removal had no effect on potency against any of the strains examined, whereas small improvements in the MICs of [Ψ[C(=NH)NH]Tpg^4]-tetrachlorovancomycin (Compound **48**) were observed

against a few strains as indicated in the tabulated data in Fig. 9.

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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 Ac_2-L-Lys-D-Ala-D-Ala.[58]

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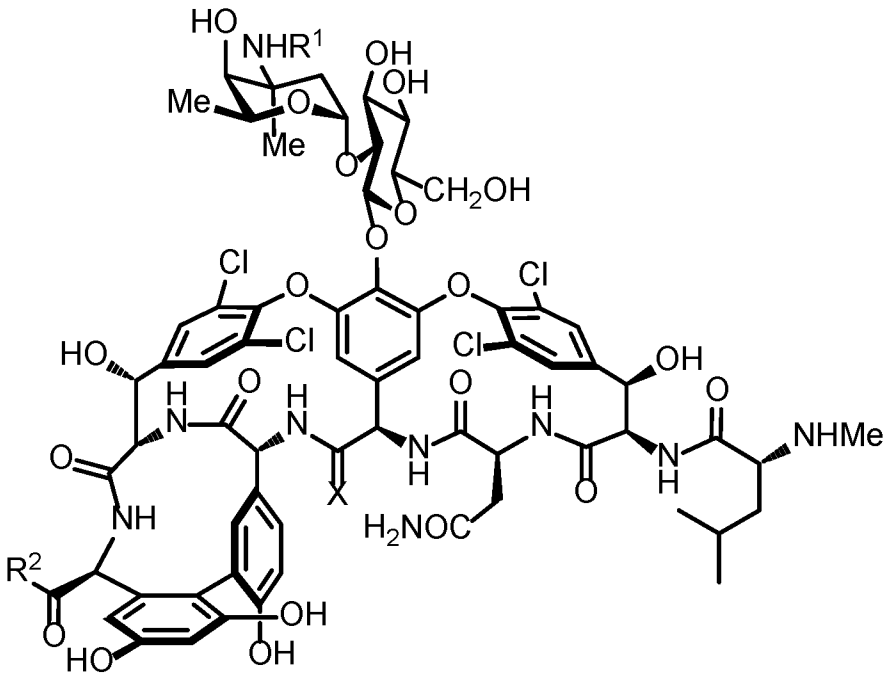
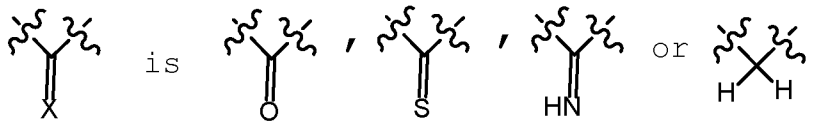
CLAIMS

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



III

wherein



R^1 is selected from the group consisting of hydrido, $(C_1\text{-}C_{16})$hydrocarbyl, aryl$(C_1\text{-}C_6)$-hydrocarbyldiyl, heteroaryl$(C_1\text{-}C_6)$hydrocarbyldiyl, $(C_1\text{-}C_6)$hydrocarbyldiylheteroaryl, halo$(C_1\text{-}C_{12})$-hydrocarbyldiyl, and $(C_1\text{-}C_{16})$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) (C_1-C_6)hydrocarbyl,

(v) halo(C_1-C_{16})hydrocarbyl,

(vi) (C_1-C_6)hydrocarbyloxy,

(vii) halo(C_1-C_6)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) (C^1-C^6)hydrocarbyl,

(v) halo(C^1-C^{16})hydrocarbyl,

(vi) (C^1-C^6)hydrocarbyloxy, and

(vii) halo(C_1-C_6)hydrocarbyloxy; and

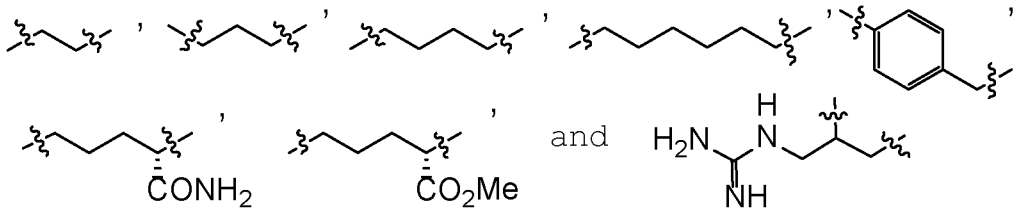
R^2 is OH or 

, 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 R^3 is guanidinyl [$H_2N(C=NH)NH$-], N,N-(di-C_1-C_6-hydrocarbyl)amino, or N,N,N-(tri-C_1-C_6-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 R^1 is

(C_1-C_{16})hydrocarbyl, aryl(C_1-C_6)-hydrocarbyldiyl, or halo(C_1-C_{12})-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



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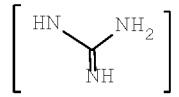
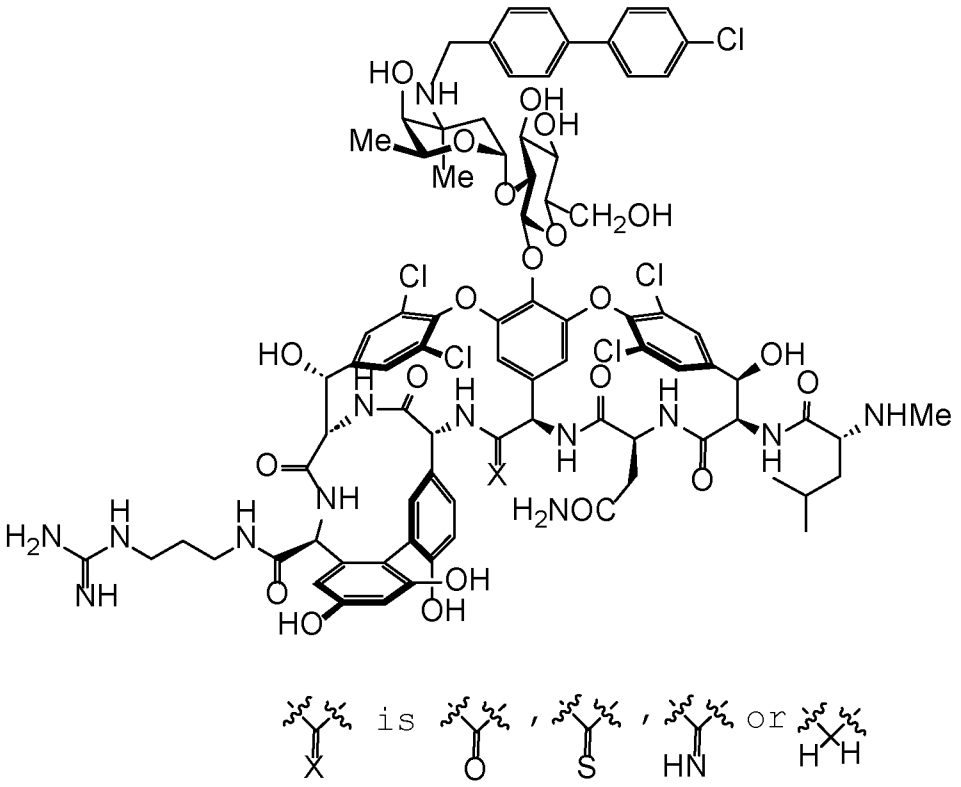
4. The compound or its pharmaceutically acceptable salt according to claim 3, wherein R^3 is guanidinyl 

.

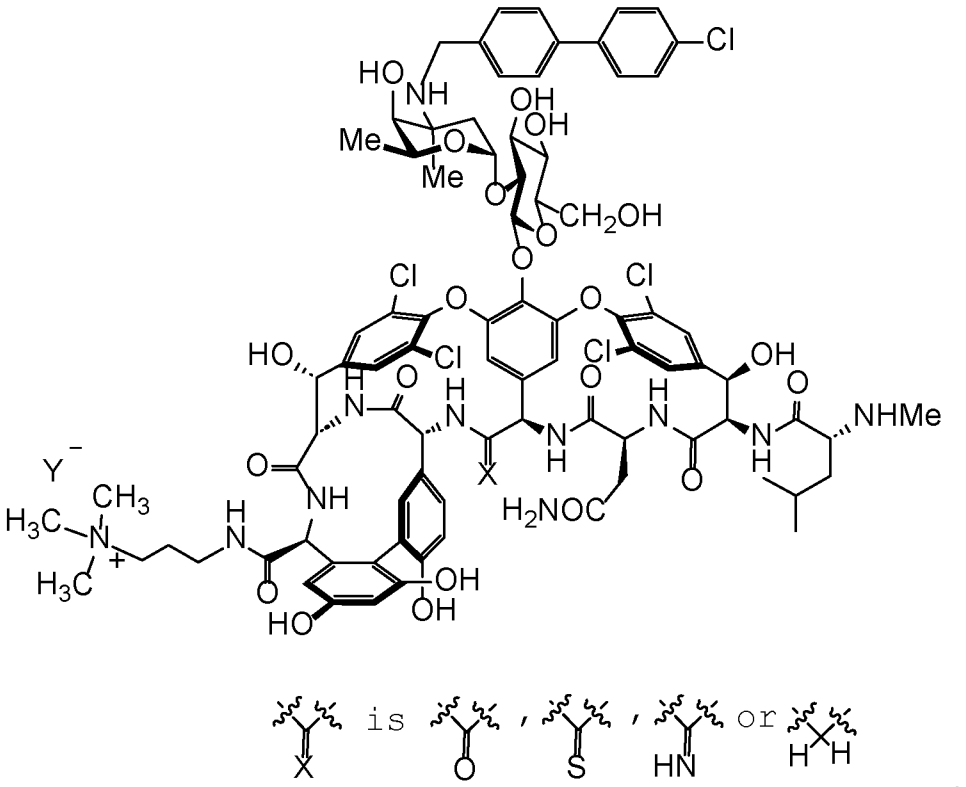
5. The compound or its pharmaceutically acceptable salt according to claim 3, wherein R^3 is N,N,N-(tri-C_1-C_6-hydrocarbyl)ammonium.

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.

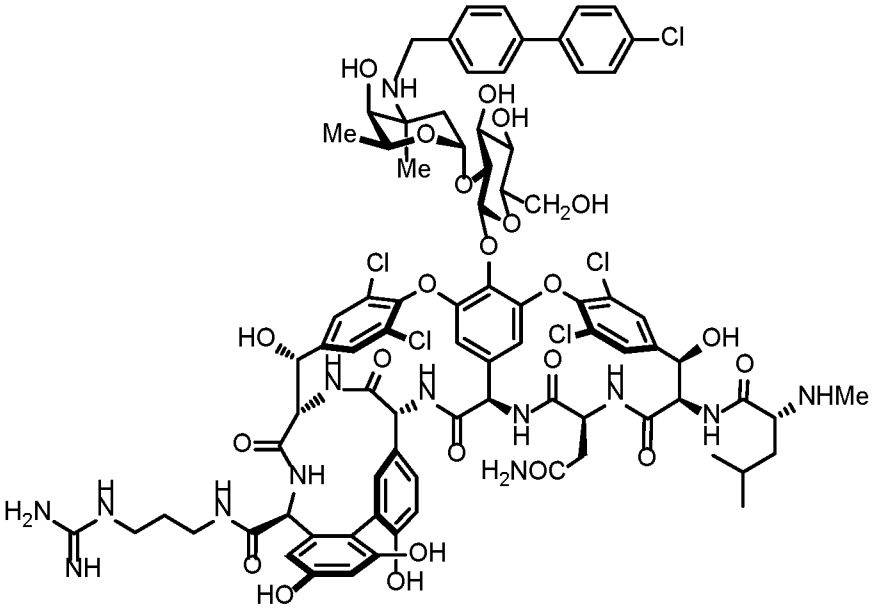
10. A method of treating a bacterially-infected mammal in need of antibacterial treatment that comprises administering an antibacterial-effective amount of a compound according to claim 1 or a pharmaceutically acceptable salt of such a compound to said infected mammal in need.

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*.

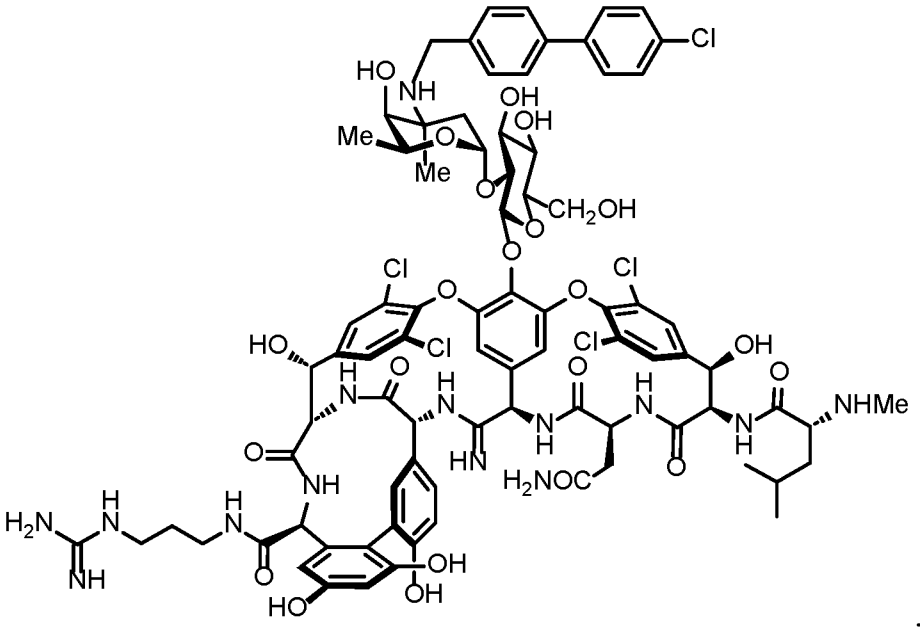
13. The method according to claim 12, wherein said administration is repeated a plurality of times.

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

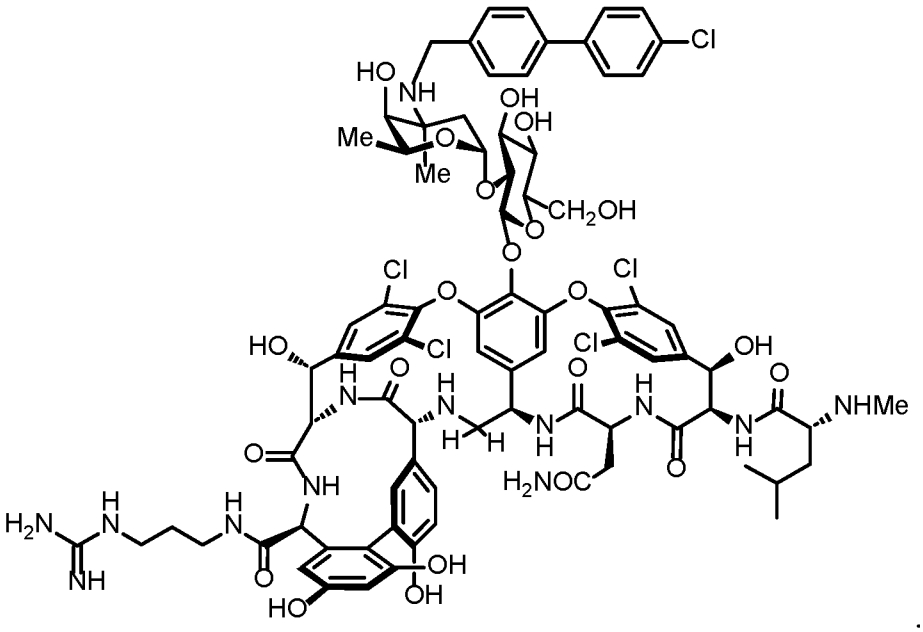


.

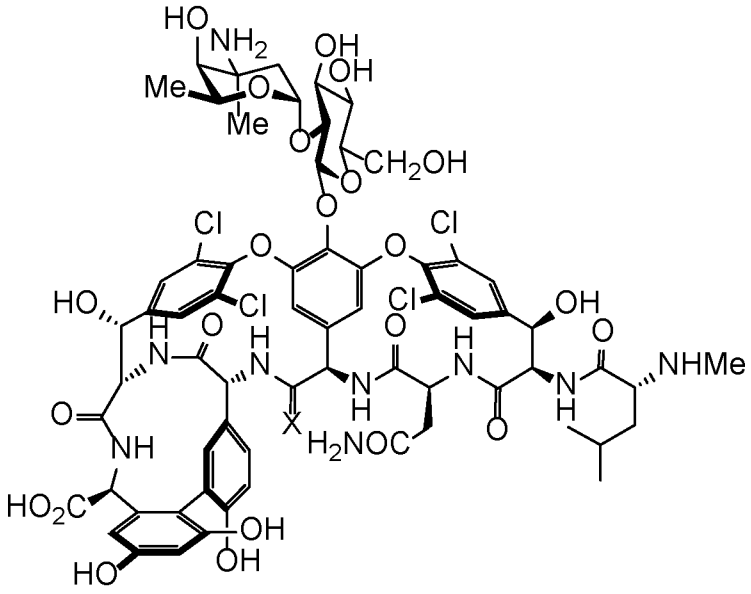
15. The method according to claim 10, wherein said administered compound corresponds in structure to the formula shown below or a pharmaceutically acceptable salt thereof



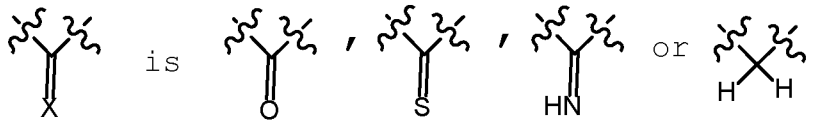
16. The method according to claim 10, wherein said administered compound corresponds in structure to the formula shown below or a pharmaceutically acceptable salt thereof



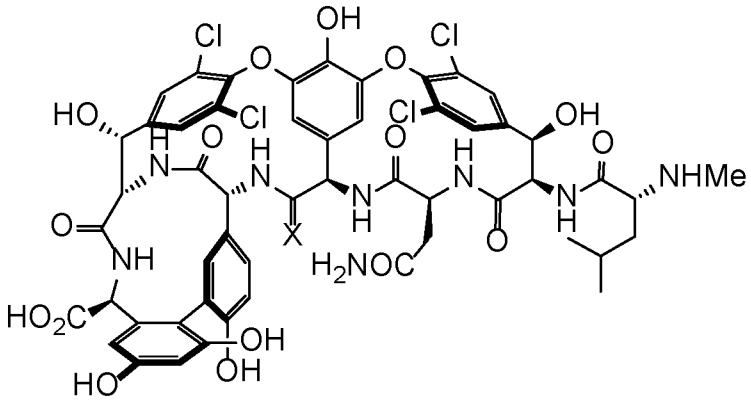
17. A compound of the structural formula shown below



wherein



18. A compound of the structural formula shown below



wherein

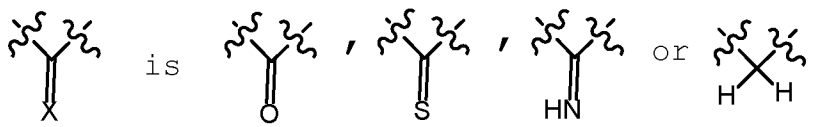


Fig. 1

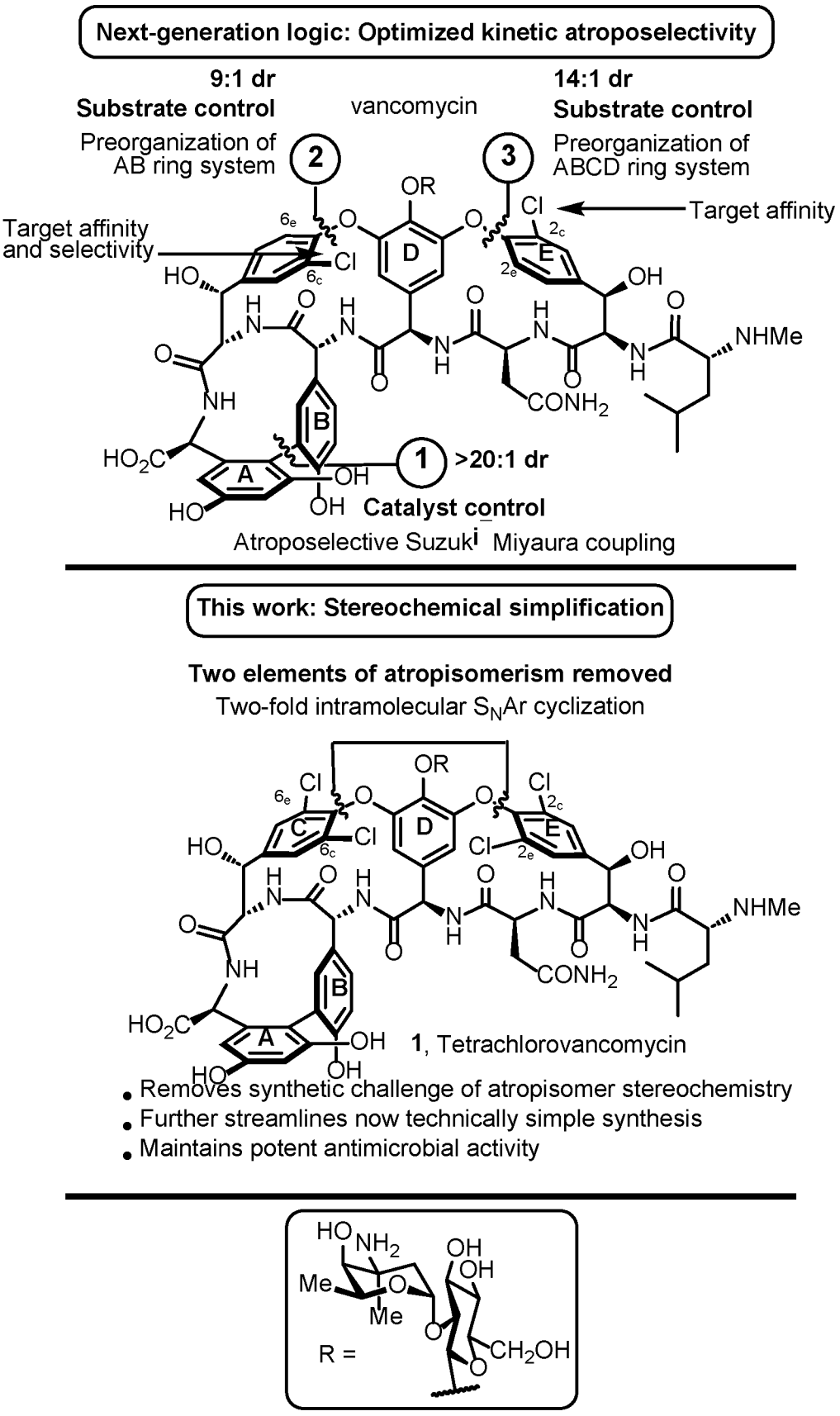

Next-generation logic: Optimized kinetic atroposelectivity
9:1 dr
Substrate control
Preorganization of AB ring system
vancomycin
14:1 dr
Substrate control
Preorganization of ABCD ring system
Target affinity
Target affinity and selectivity
>20:1 dr
Catalyst control
Atroposelective Suzuki Miyaura coupling
This work: Stereochemical simplification
Two elements of atropisomerism removed
Two-fold intramolecular SNAr cyclization
1, Tetrachlorovancomycin
Removes synthetic challenge of atropisomer stereochemistry
Further streamlines now technically simple synthesis
Maintains potent antimicrobial activity
R =

Fig. 2

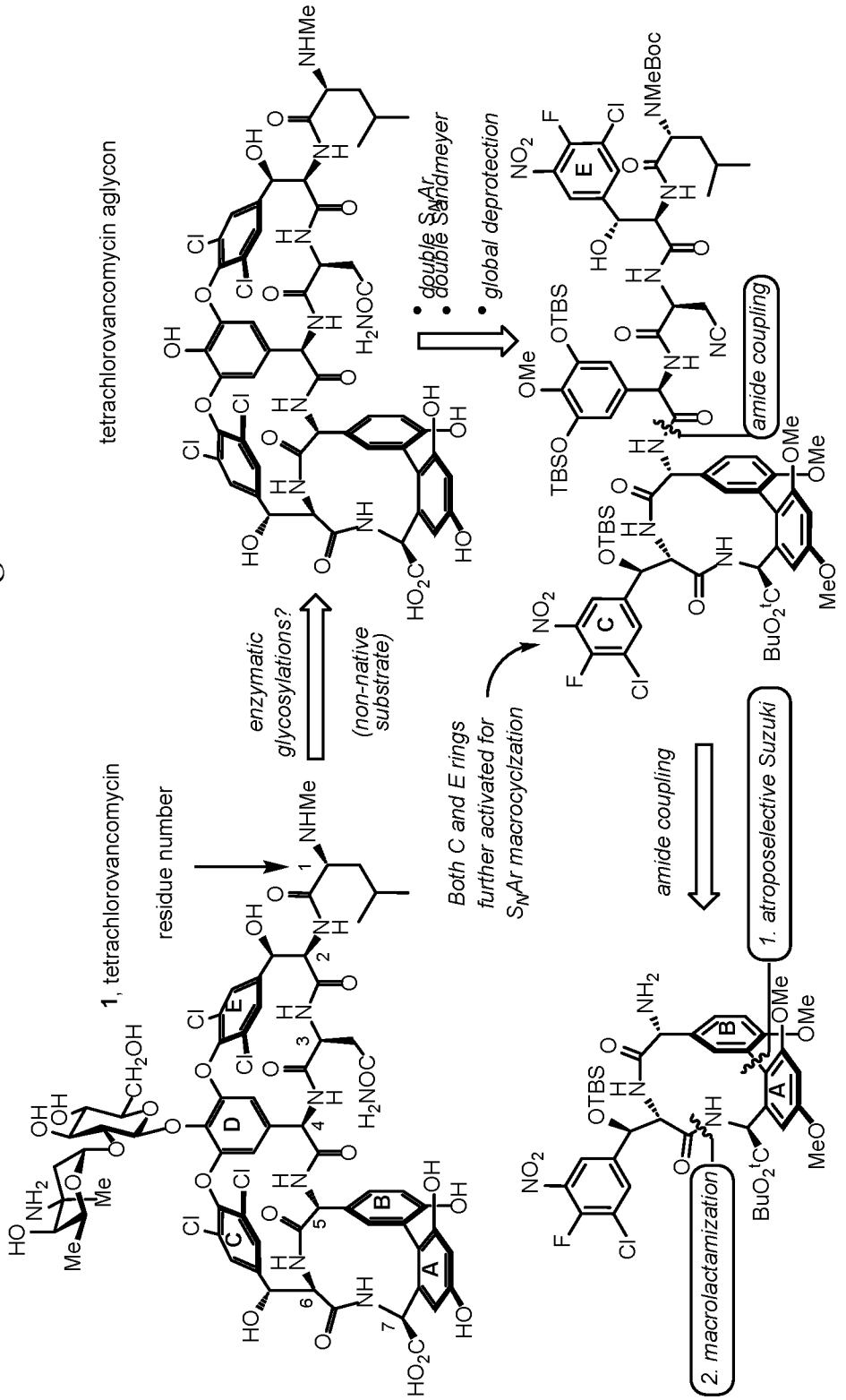

1, tetrachlorovancomycin
residue number
tetrachlorovancomycin aglycon
enzymatic glycosylations?
(non-native substrate)
double SNAr
double Sandmeyer
global deprotection
Both C and E rings further activated for SNAr macrocyclization
amide coupling
amide coupling
1. atroposelective Suzuki
2. macrolactamization

Fig. 3

Scheme 4

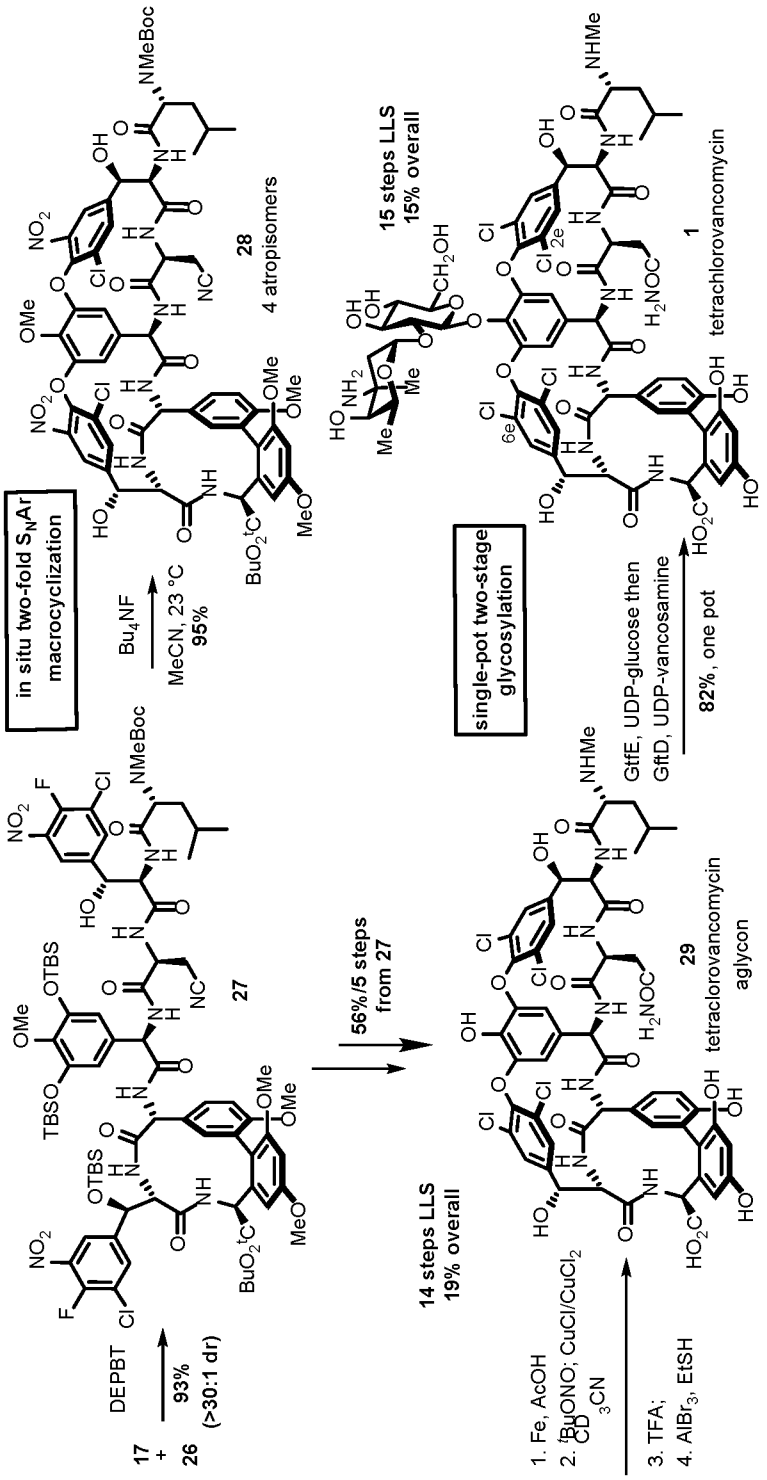


Fig. 4

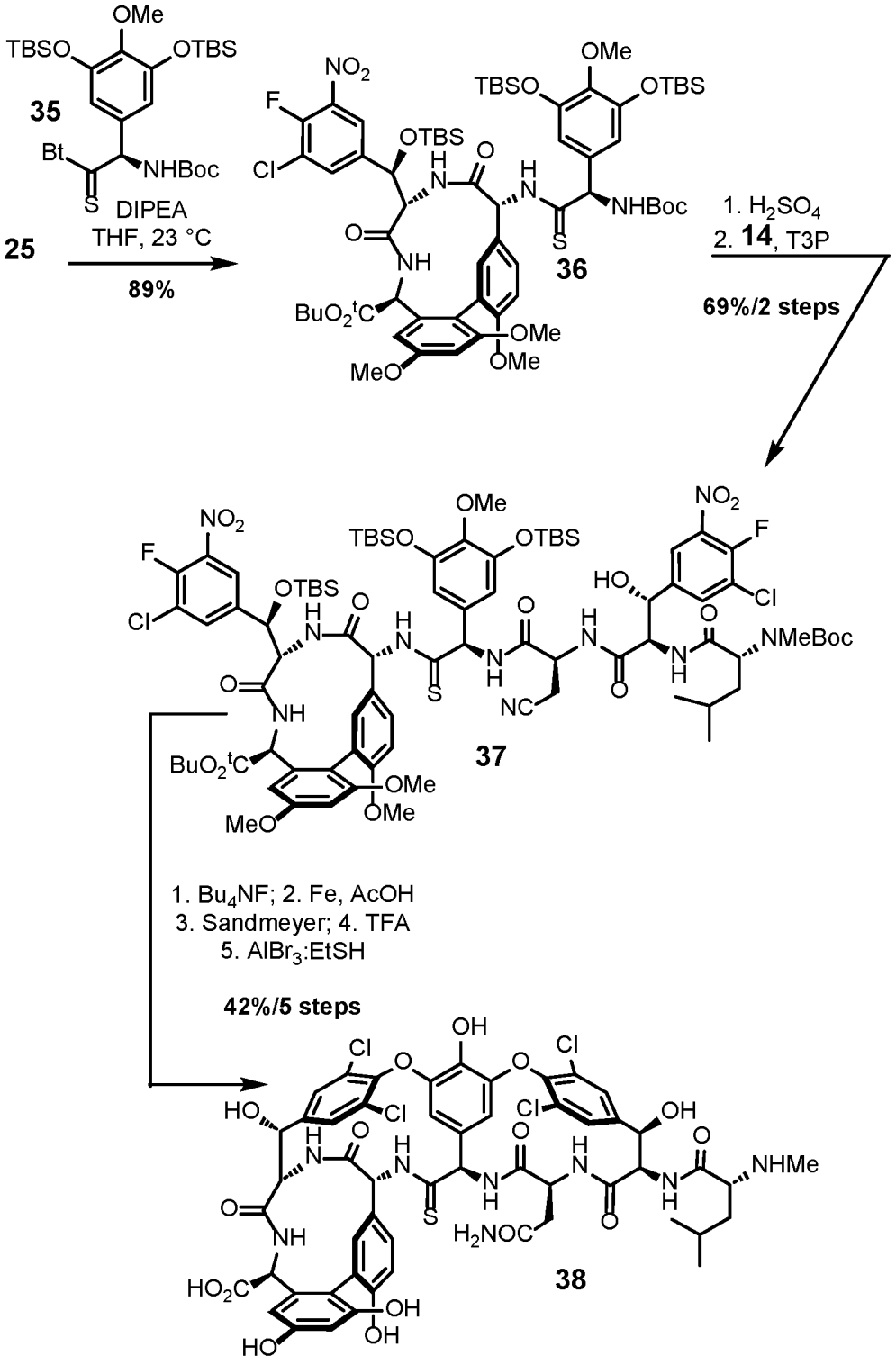

35
25
DIPEA
THF, 23 °C
89%
36
1. H_2SO_4
2. 14, T3P
69%/2 steps
37
1. Bu_4NF; 2. Fe, AcOH
3. Sandmeyer; 4. TFA
5. $AlBr_3$:EtSH
42%/5 steps
38

Fig. 5

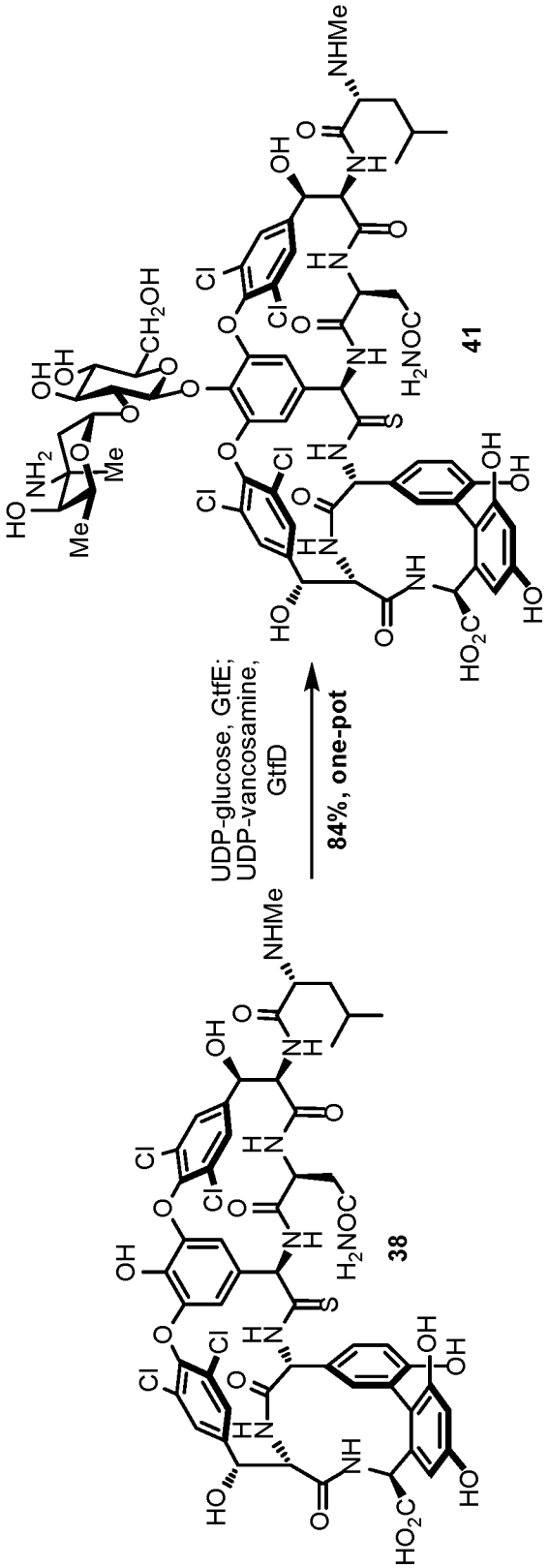


Fig. 6

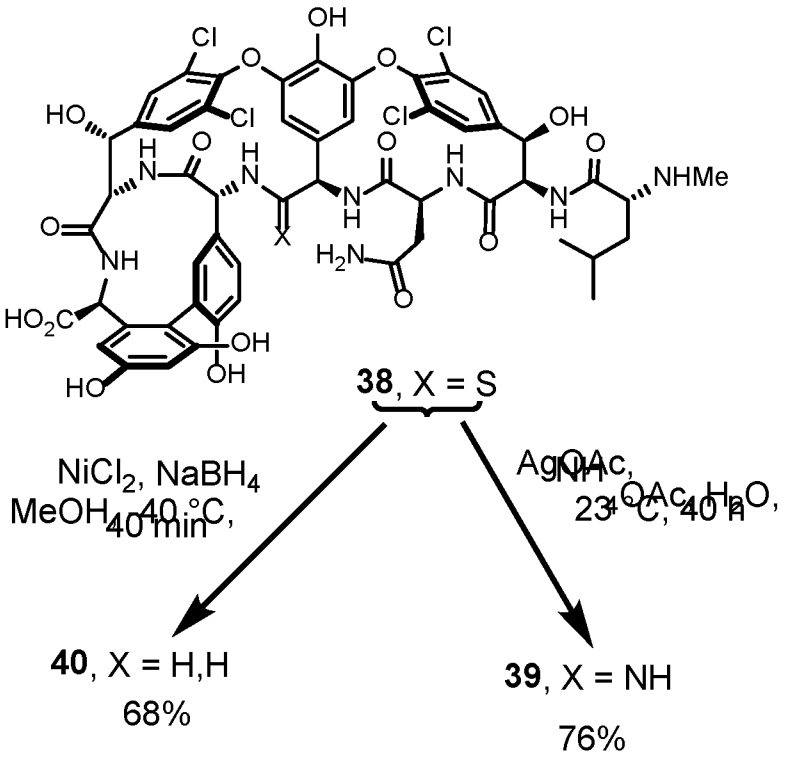

38, X = S
NiCl2, NaBH4
MeOH, 40 °C,
40 min
AgNH4OAc,
23 °C, 40 h
40, X = H,H
68%
39, X = NH
76%

Fig. 7

Fig. 7A

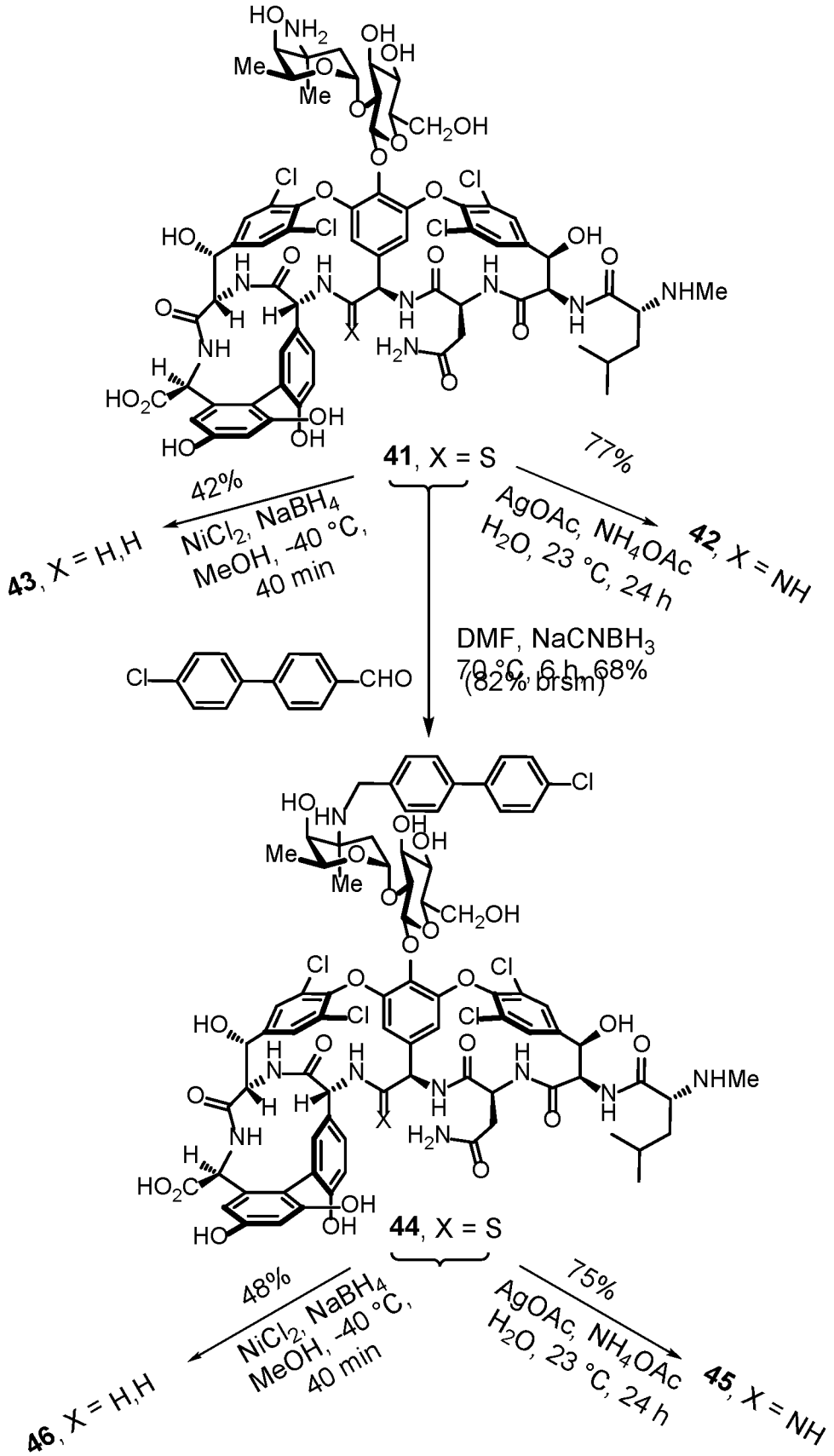

41, X = S
77%
AgOAc, NH4OAc
H2O, 23 °C, 24 h
42, X = NH
42%
NiCl2, NaBH4
MeOH, -40 °C,
40 min
43, X = H,H
DMF, NaCNBH3
70 °C, 6 h, 68%
(82% brsm)
44, X = S
75%
AgOAc, NH4OAc
H2O, 23 °C, 24 h
45, X = NH
48%
NiCl2, NaBH4
MeOH, -40 °C,
40 min
46, X = H,H

Fig. 7B

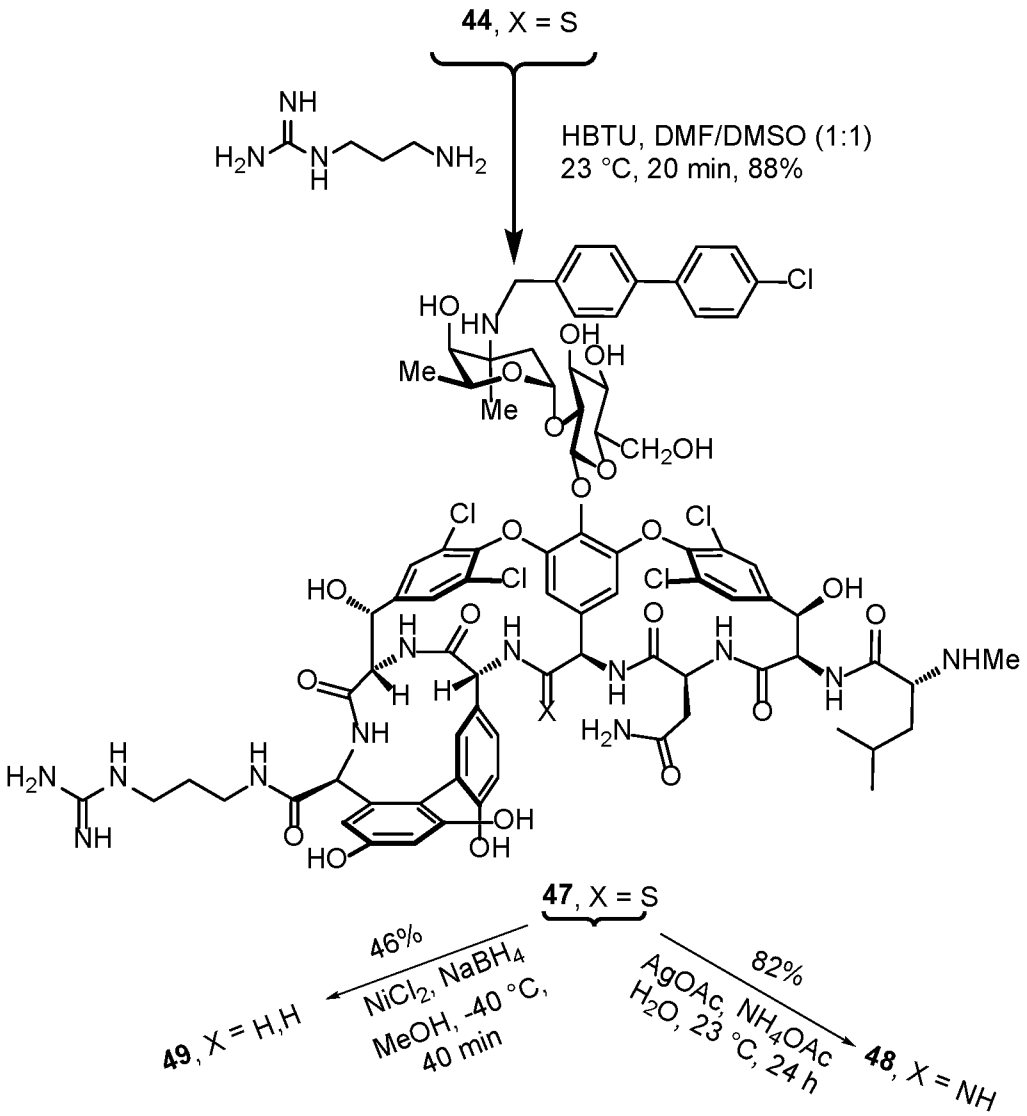
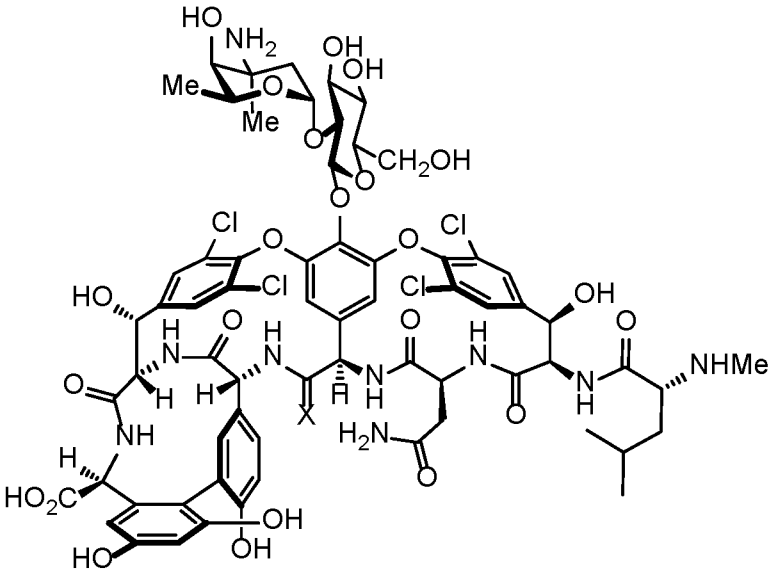
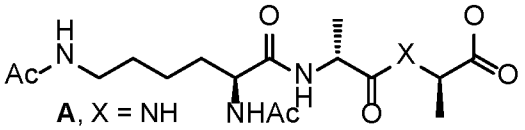

44, X = S
HBTU, DMF/DMSO (1:1)
23 °C, 20 min, 88%
47, X = S
46%
NiCl2, NaBH4
MeOH, -40 °C,
40 min
49, X = H,H
82%
AgOAc, NH4OAc
H2O, 23 °C, 24 h
48, X = NH

Fig. 8

Fig. 8A



1, X = O tertrachlorovancomycin
41, X = S [Ψ[C(=S)NH]Tpg^4]tetrachlorovancomycin
42, X = NH [Ψ[C(=NH)NH]Tpg^4]tetrachlorovancomycin
43, X = H,H [Ψ(CH$_2$NH)]Tpg^4]tetrachlorovancomycin

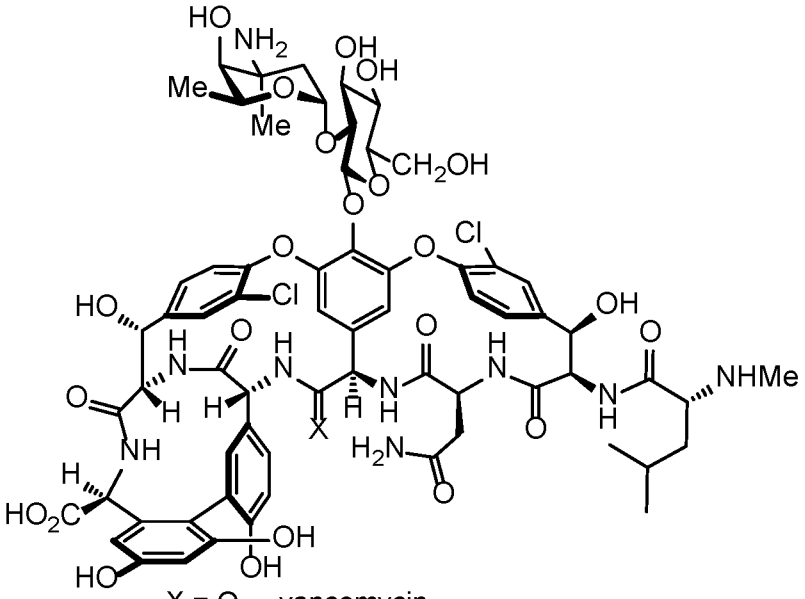


A, X = NH
B, X = O

compound	ligand, K_a (M^{-1})[a] **A**, X = NH	**B**, X = O	K_a(**A**/**B**)
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. [b]Not determined (nd), binding <1×10^2 M^{-1}.

Fig. 8B



X = O vancomycin
X = S [Ψ[C(=S)NH]Tpg^4]vancomycin
X = NH [Ψ[C(=NH)NH]Tpg^4]vancomycin
X = H,H [Ψ(CH$_2$NH)]Tpg^4]vancomycin

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

[a]K_a = association constant. [b]Not determined (nd), binding <1 x 10^2 M^{-1}.

Fig. 9

Fig. 9A

Compound	MIC, μg/mL			
	vancomycin-sensitive			
	S. aureus[a]	MRSA *S. aureus*[b]	*E. faecium*[c]	*E. faecalis*[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_2NH)Tpg^4]tetrachlorovancomycin (**49**)	0.02	0.02	0.04	0.04
G3,CBP-vancomycin[44]	0.04	0.02	nd	nd

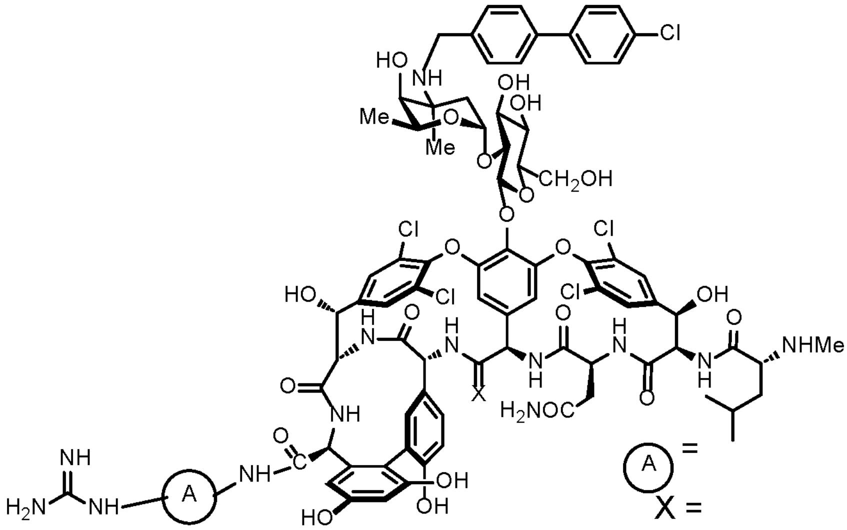
[a]ATCC 25923. [b]ATCC 43300. [c]Unknown source. [d]ATCC BAA-2317. [e]ATCC-2573.
[f]TX-2516. [g]Proteins removed.

Fig. 9B

Compound	MIC, μg/mL		
	vancomycin-resistant		
	VanA *E. faecium*[d]	VanA *E. faecalis*[e]	VanB *E. faecalis*[f]
[$^{\Psi}$[C(=S)NH]Tpg^{4}]tetrachlorovancomycin (**41**)	>80	>80	>80
CBP-[$^{\Psi}$[C(=S)NH]Tpg^{4}]tetrachlorovancomycin (**44**)	10	10	5
G3,CBP-[$^{\Psi}$[C(=S)NH]Tpg^{4}]tetrachlorovancomycin (**47**)	0.6	1.2	1.2
G3,CBP-[$^{\Psi}$[C(=NH]NH]Tpg^{4}]tetrachlorovancomycin (**48**)	0.08	0.6 (0.15)[g]	0.6 (0.15)[g]
G3,CBP-[$^{\Psi}$(CH_2NH)Tpg^{4}]tetrachlorovancomycin (**49**)	0.08	0.08	0.08
G3,CBP-vancomycin[44]	0.3	0.3	0.6

[a]ATCC 25923. [b]ATCC 43300. [c]Unknown source. [d]ATCC BAA-2317.
[e]ATCC-2573. [f]TX-2516. [g]Proteins removed.

Fig. 10



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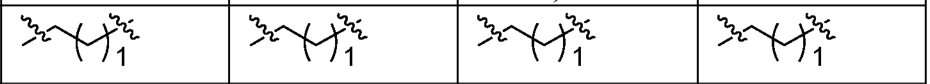
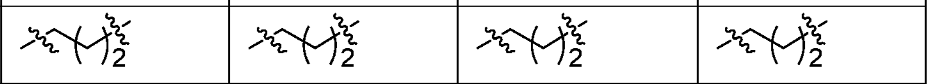
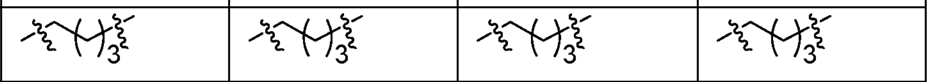
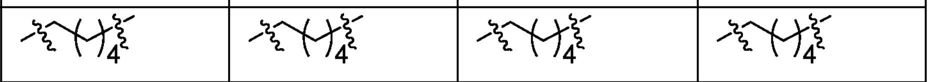
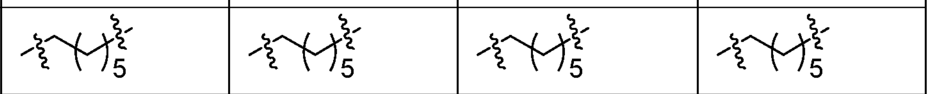
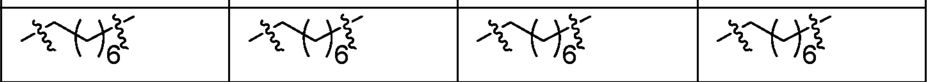
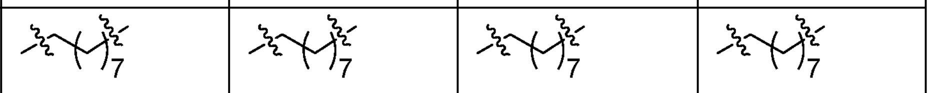
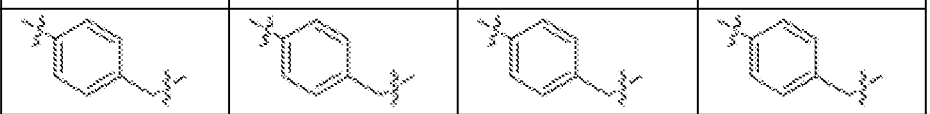
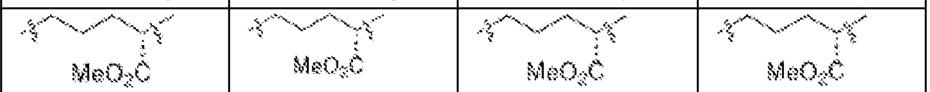
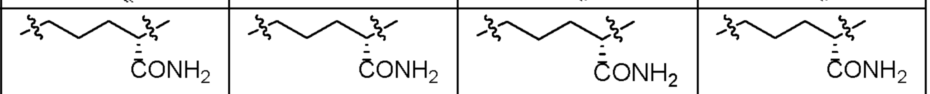
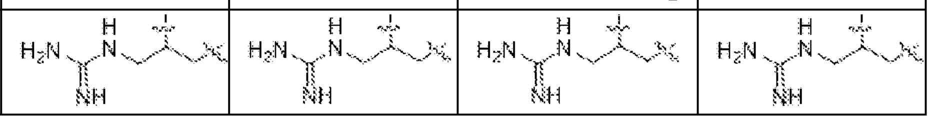
<tr><td>Circle A</td><td colspan="4">  </td></tr>
<tr><td>Circle A</td><td colspan="4">  </td></tr>
<tr><td>Circle A</td><td colspan="4">  </td></tr>
<tr><td>Circle A</td><td colspan="4">  </td></tr>
<tr><td>Circle A</td><td colspan="4">  </td></tr>
<tr><td>Circle A</td><td colspan="4">  </td></tr>
<tr><td>Circle A</td><td colspan="4">  </td></tr>
<tr><td>Circle A</td><td colspan="4">  </td></tr>
<tr><td>Circle A</td><td colspan="4">  </td></tr>
<tr><td>Circle A</td><td colspan="4">  </td></tr>
<tr><td>Circle A</td><td colspan="4">  </td></tr>
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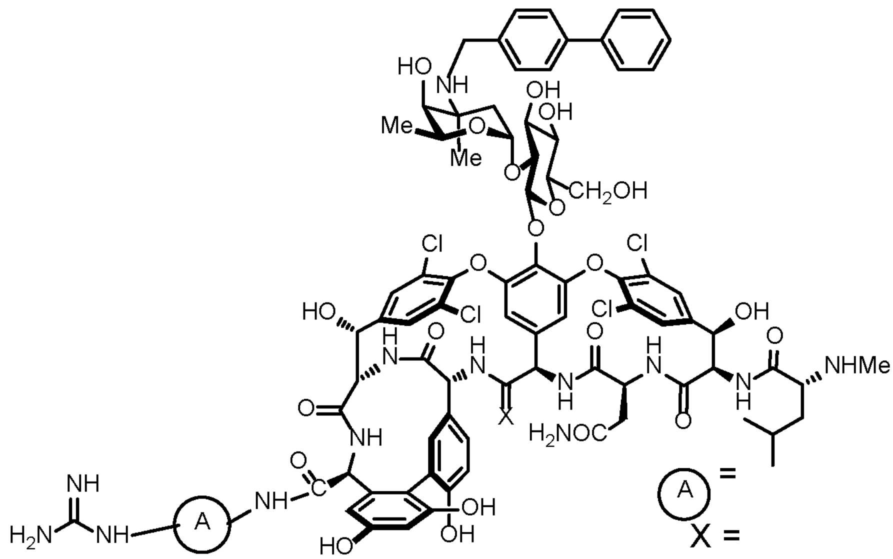
Fig. 11



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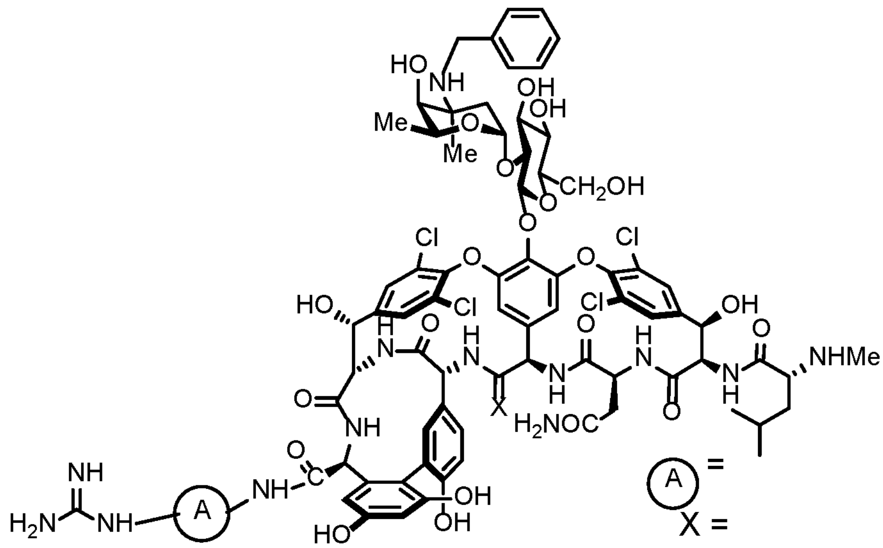
Circle A	1	1	1	1
Circle A	2	2	2	2
Circle A	3	3	3	3
Circle A	4	4	4	4
Circle A	5	5	5	5
Circle A	6	6	6	6
Circle A	7	7	7	7
Circle A				
Circle A	MeO_2C	MeO_2C	MeO_2C	MeO_2C
Circle A	$CONH_2$	$CONH_2$	$CONH_2$	$CONH_2$
Circle A	H_2N H N NH	H_2N H N NH	H_2N H N NH	H_2N H N NH

Fig. 12



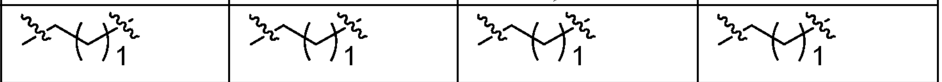
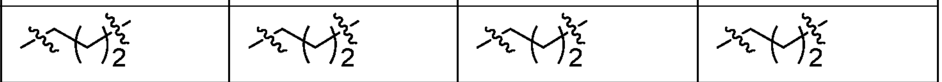
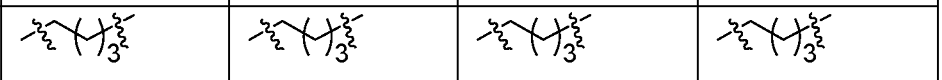
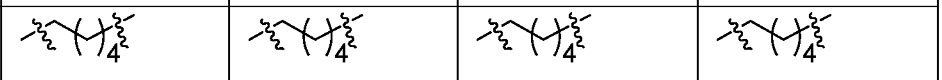
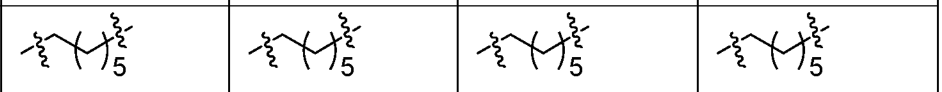
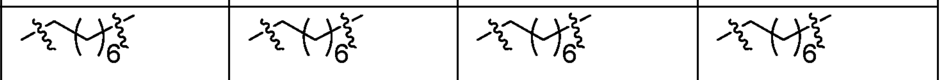
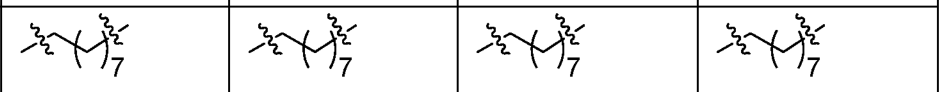
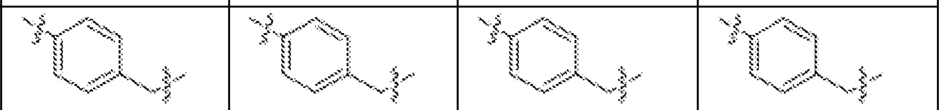
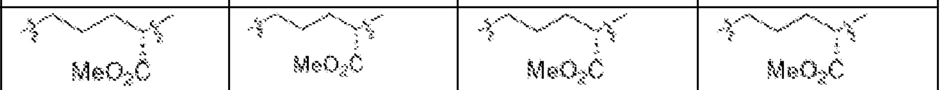
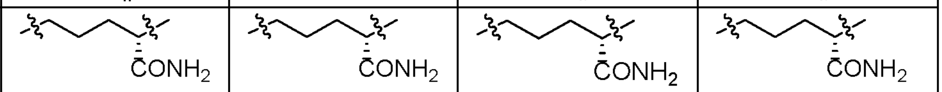
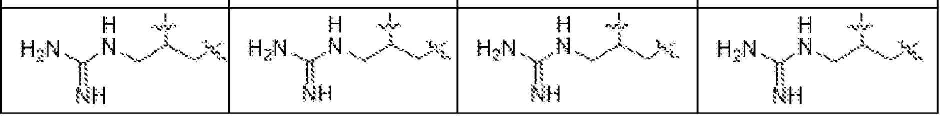
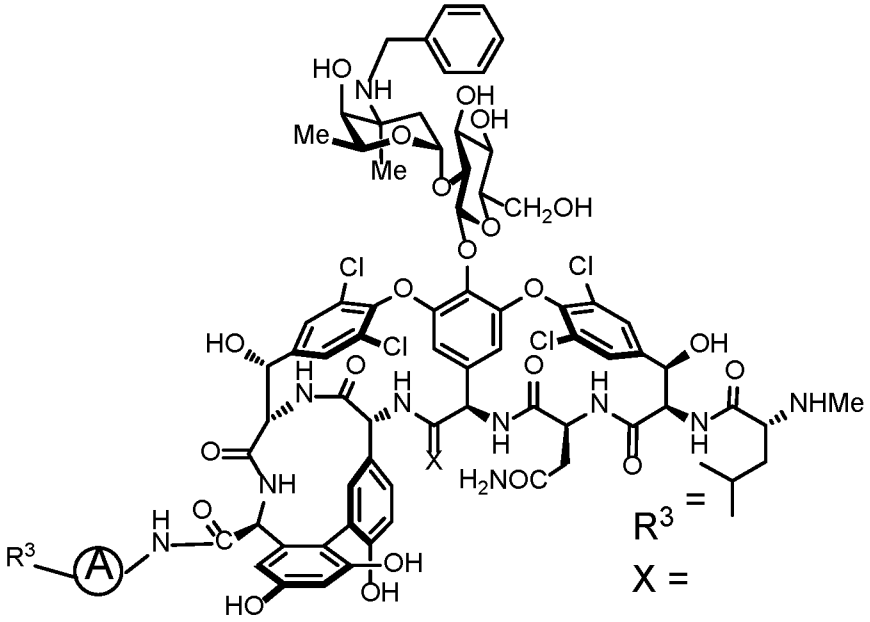
	X = O	X = S	X = H,H	X = NH
Circle A				
Circle A				
Circle A				
Circle A				
Circle A				
Circle A				
Circle A				
Circle A				
Circle A				
Circle A				
Circle A				

Fig. 13



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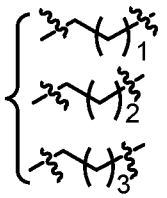
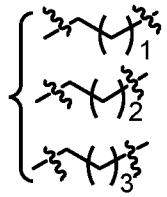
R^3 (C1) = 
X = O

A =  or

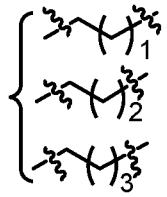
R^3 (C0) = 
X = S

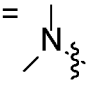
R^3 (C1) = 
X = S

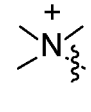
A =  or

R^3 (C0) = 
X = H,H

R^3 (C1) = 
X = H,H

A =  or

R^3 (C0) = 
X = NH

R^3 (C1) = 
X = NH

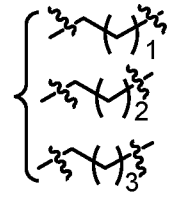
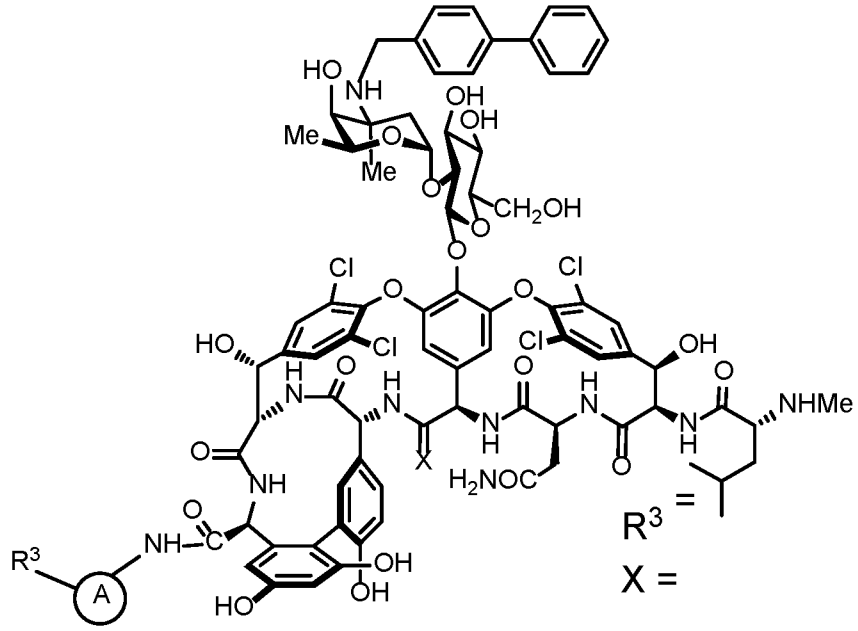
A =  or

Fig. 14


HO
NH
Me
O
Me
OH
OH
CH2OH
Cl
Cl
HO
Cl
Cl
OH
NHMe
H2NOC
NH
NH2
R3
A
OH
OH
HO
R3 =
X =

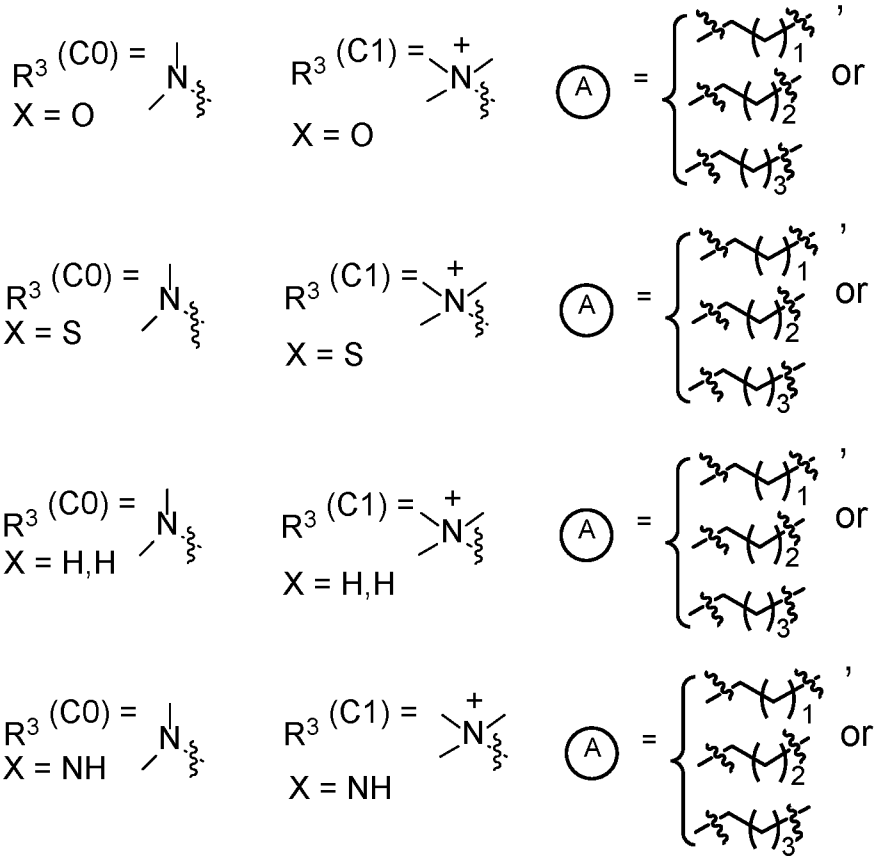
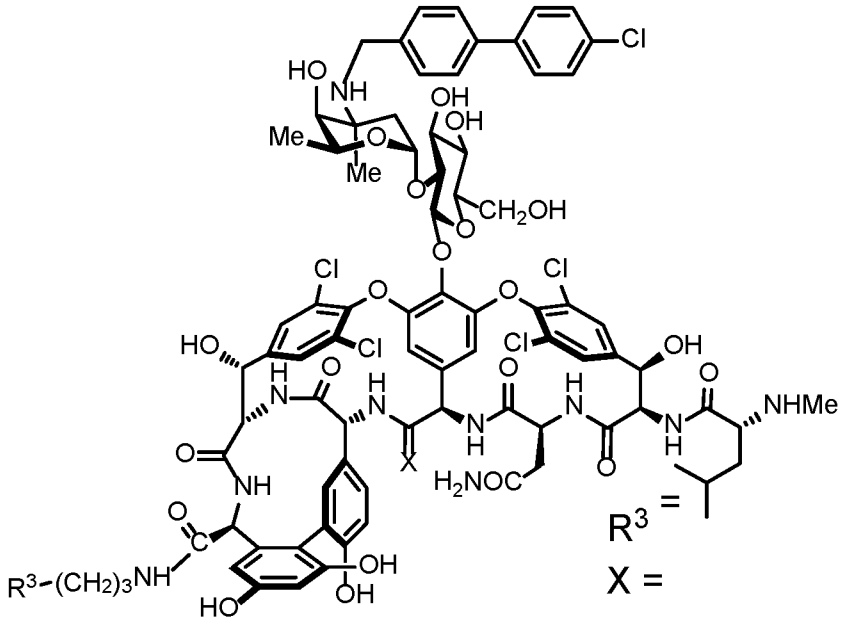
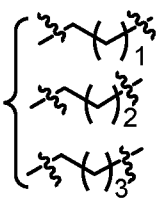

R3 (C0) =
X = O
R3 (C1) =
X = O
A =
or
R3 (C0) =
X = S
R3 (C1) =
X = S
A =
or
R3 (C0) =
X = H,H
R3 (C1) =
X = H,H
A =
or
R3 (C0) =
X = NH
R3 (C1) =
X = NH
A =
or

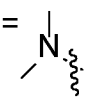
Fig. 15



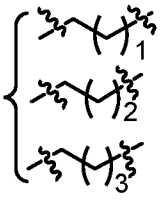
R^3 (C0) = 
X = O

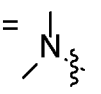
R^3 (C1) = 
X = O

A =  or

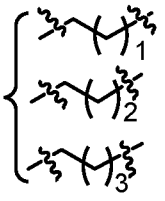
R^3 (C0) = 
X = S

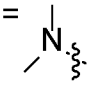
R^3 (C1) = 
X = S

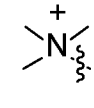
A =  or

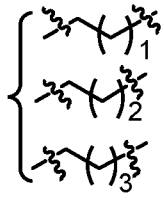
R^3 (C0) = 
X = H,H

R^3 (C1) = 
X = H,H

A =  or

R^3 (C0) = 
X = NH

R^3 (C1) = 
X = NH

A =  or