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Vancomycin disulfide derivatives as antibacterial agents

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Abstract—A series of lipidated vancomycin analogues **1** bearing disulfide bonds within their lipid chains was designed and synthesized to optimize their ADME profiles while retaining antibacterial potency. These compounds exhibited good activity against resistant organisms and low accumulation in tissues such as kidney and liver.

Vancomycin is a glycopeptide antibiotic that is widely used in the treatment of Gram-positive bacterial infections. Over the past decades, resistance to vancomycin has become an increasing problem.[1] This has created an urgent need for new clinical agents with activity against resistant organisms.[2] Over the past 20 years, extensive research has been done on the chemical modification of vancomycin.[3–5] Through this work, it has been found that vancomycin derivatives bearing hydrophobic substituents on the disaccharide moiety have potent activity against resistant strains of bacteria including methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant enterococci (VRE).[3,6,7] However, lipophilic modifications through alkyl-linked groups on vancosamine impart unfavorable absorption, distribution, metabolism and excretion (ADME) properties such as high tissue accumulation and long elimination half-life.[3,10] We hypothesized that these unfavorable ADME properties were correlated with the increased hydrophobicity of these analogues, as the parent vancomycin is not accumulated in tissues and is cleared quite efficiently in the urine. Our program in this area sought to retain potent antibacterial properties in compounds while improving ADME profiles relative to the parent molecules in this class. One approach to this would be to introduce a metabolically labile linkage into the lipid tail, which can be degraded in vivo to a more hydrophilic product for subsequent excretion.

We designed a series of analogues (e.g., **1**) bearing disulfide bonds within their lipid appendages. We anticipated that these disulfide analogues could be metabolized to more water-soluble thiol derivatives in vivo and be excreted in urine. The latent reactive functionality could also allow self-dimerization to form vancomycin disulfide dimers linked at vancosamine, which also had the potential for the activity against resistant organisms.[8,9] Herein, we report the synthesis of vancomycin disulfide analogues as well as their antibacterial properties and rat ADME profiles (Chart 1).

Reductive *N*-alkylation on the vancosamine was carried out with aldehydes bearing an unsymmetrical disulfide linkage (see Scheme 1).[10] The unsymmetric disulfide bond was formed between a thiol-ester **2** and an alkane-thiol **3** using diethyl azodicarboxylate (DEAD) as the oxidizing agent.[11] This reaction proceeded under mild and neutral

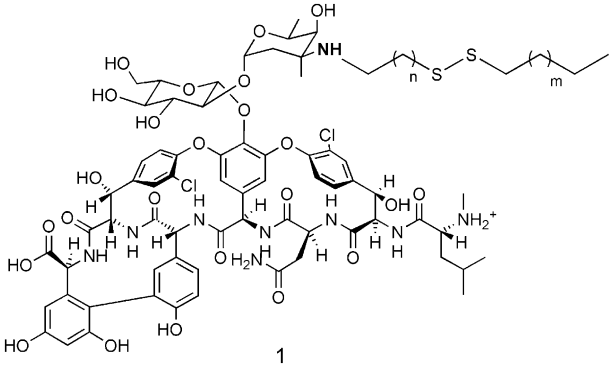

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Chart 1. Lipidated vancomycin derivatives with disufide bonds within lipid appendages.

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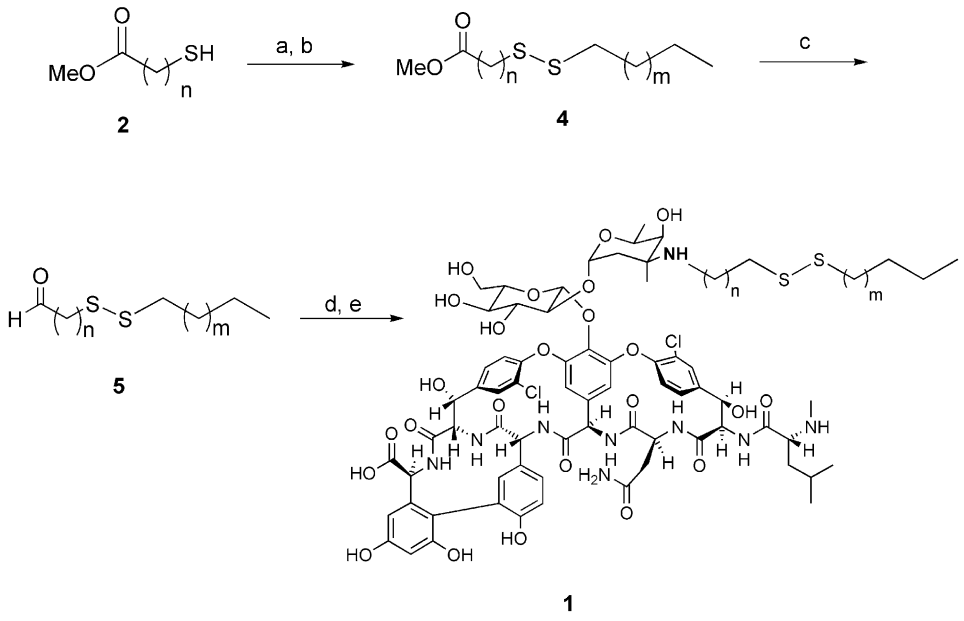
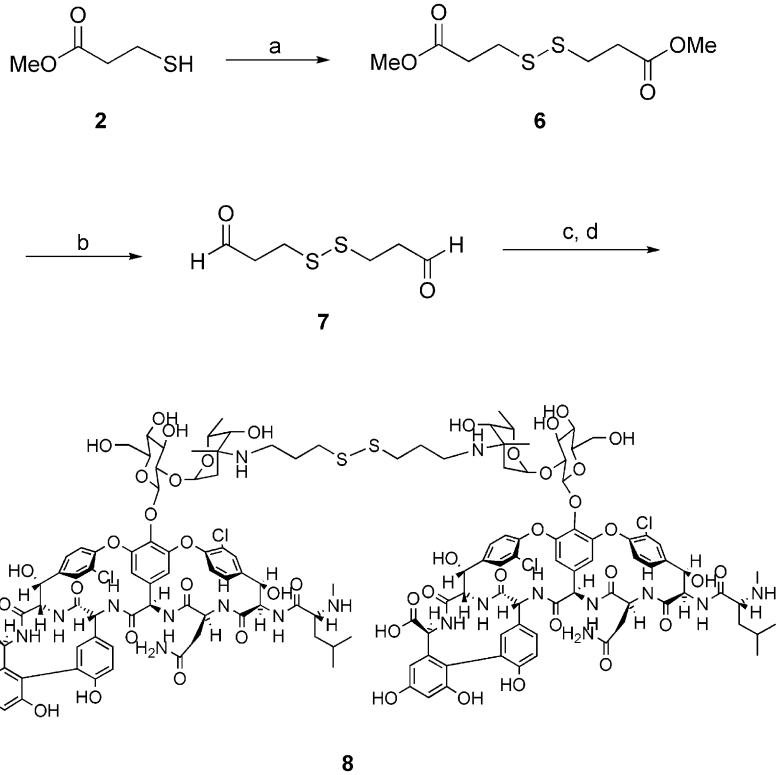
conditions giving **4** in good yield. The esters **4** were then reduced to aldehydes **5** using DIBAL-H at −78 °C without concomitant reduction of the disulfide bond. Reductive alkylation of vancomycin with aldehydes **5** could yield two mono-*N*-alkylated products, on the vancosamine and *N*-methyl leucine residue, as well as one bis-*N*-alkylated product. Under our optimized conditions, the highly selective reductive alkylation of vancomycin yields product **1** substituted at the amino group of vancosamine.[12,13] The final products were purified via reversed-phase HPLC and characterized by ion-spray mass spectrometry.

In order to study the metabolites of vancomycin disulfide-lipid analogues, we also prepared compound **8**, a vancomycin dimer linked at vancosamine via a disulfide linker (Scheme 2). The symmetric disulfide bond was



Scheme 1. General synthesis of vancomycin disulfide analogues. Reagents and conditions: (a) diethyl azodicarboxylate, CH_2Cl_2, rt, 1.5 h; (b) $C_2H_5(CH_2)_mCH_2SH$ **3**, rt, 12 h; (c) DIBAL-H, ether, −78 °C, 2 h; (d) vancomycin, diisopropylethylamine, DMF, rt, 1 h; (e) $NaCNBH_3$, TFA, methanol, rt, 1 h.

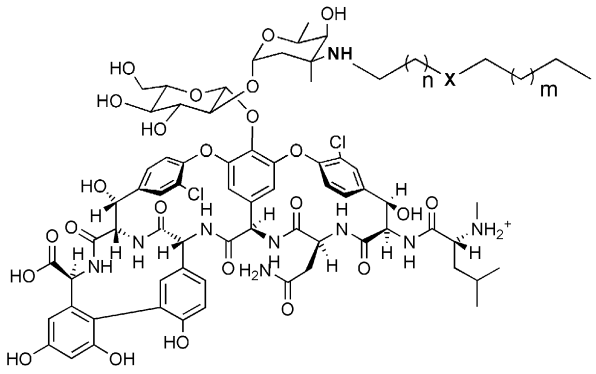


Scheme 2. Synthesis of vancomycin dimer **8**. Reagents and conditions: (a) I_2, methanol, rt, 5 h; (b) DIBAL-H, ether, −78 °C, 2 h; (c) vancomycin, diisopropylethylamine, DMF, rt, 1 h; (d) $NaCNBH_3$, TFA, methanol, rt, 1 h.

formed by iodine oxidation of thiol-ester **2**, followed by DIBAL-H reduction to give bis-aldehyde **7**. Reductive alkylation of vancomycin with **7** gave disulfide dimer **8**.

The antibacterial activities of vancomycin disulfide derivatives against a selection of pathogenic Gram-positive strains including staphylococci and enterococci were determined by standard in vitro broth microdilution susceptibility tests. The data is summarized in Table 1.[14] Hydrophobic vancomycin monomers with a disulfide bond within the lipid tail (**1a**–**e**) were as potent as their nitrogen (**1f**) and carbon (**1g**) analogues against staphylococci and against VRE strains of both the VanB and VanA phenotypes. Within the disulfide series there was a trend favoring shorter lipids for activity against MRSA. Compound **1b**, which has a 12-atom lipid chain length, was 12-fold more active against MRSA than **1e** (lipid length of 14 atoms). These compounds were rapidly bactericidal, retaining a key property of hydrophobically substituted glycopeptides. Time–kill curves against MRSA and VRE are shown in Figure 1. Again, in comparison with their close analogues bearing an amine-nitrogen **1f**, or the saturated hydrocarbon **1g**, the

Table 1. Antibacterial activity of lipidated vancomycin derivatives **1a**–**g** and metabolite **8**



Compd	X	*n*	*m*	MSSA	MRSA	VanB	VanA Fm	VanA Fs
1a	S–S	2	5	3.1	0.4	1.8	4.7	3.8
1b	S–S	1	5	0.2	0.2	2.4	6.3	4.7
1c	S–S	1	6	0.3	0.6	1.2	6.3	3.1
1d	S–S	2	6	0.6	1.2	1.8	3.1	3.1
1e	S–S	1	7	1.2	2.4	0.6	3.1	3.1
1f	N	1	7	0.4	0.4	1.8	4.1	3.6
1g	CH_2	1	7	2.8	1.5	1.6	4.1	3.8
8	S–S	2	Dimer	6.3	18.8	4.7	6.3	12.5
Vanco	na	na	na	0.7	2.0	>256	>256	>256

Results are expressed as minimal inhibitory concentration (μg/mL). na, not available.

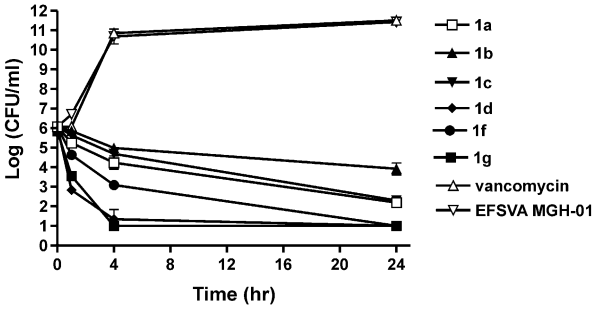


Figure 1. Bactericidal activity of vancomycin disulfides. Top: time–kill curves of MRSA 33591 in the presence of vancomycin derivatives at 4 μg/mL. Bottom: time–kill curves of VRE strain MGH-01 in the presence of vancomcycin derivatives at 10 μg/mL.

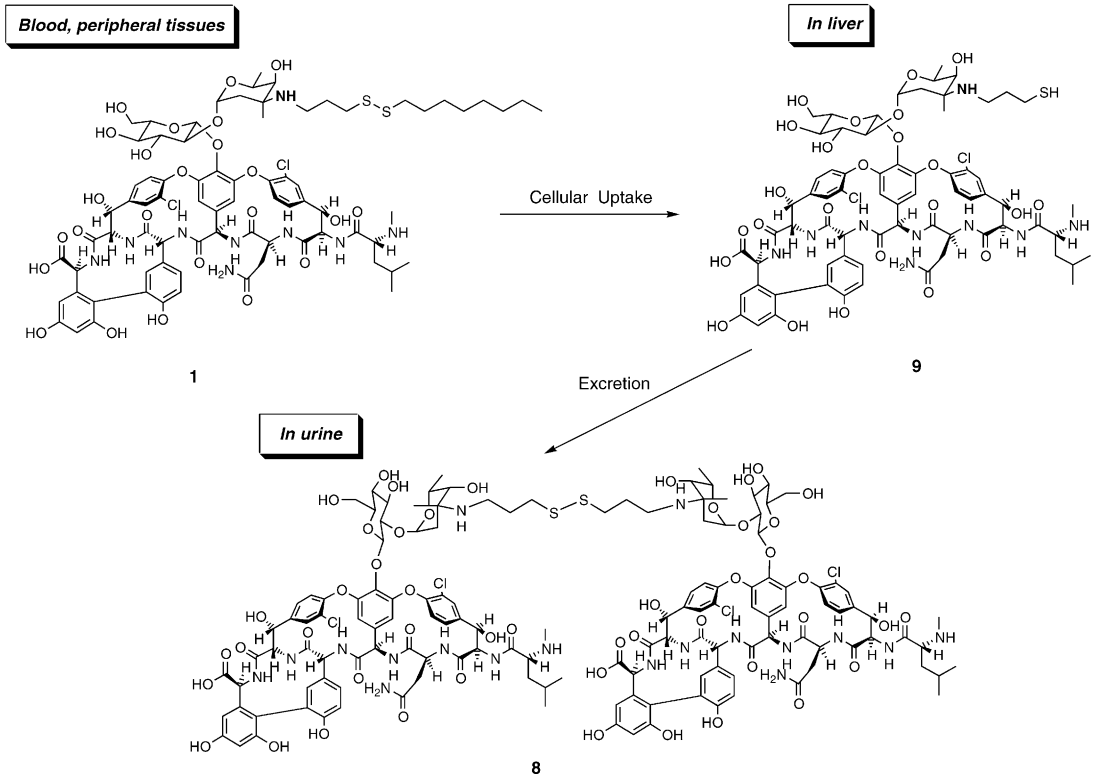


Figure 2. Possible metabolic pathway for **1a**.

Table 2. Tissue distribution and metabolites of lipidated vancomycin derivatives **1a**, **1f** and **1g**

Compd	X	% dose in liver	% dose in kidney	% dose in urine	% dimer **8** in urine[15]	% thiol **9** in urine[15]
1a	S–S	4.9	0.5	1	14	2.3
1f	N	37	22	16	na	na
1g	CH_2	70	3.4	0.4	na	na

introduction of disulfide linkage into the lipid tail retained good bactericidal activity against MRSA and VRE.

To test the hypothesis that lipidated vancomycin disulfide analogues can be cleaved in vivo, we studied the rat ADME properties of compound **1a** as well as its nitrogen and hydrocarbon analogues, **1f** and **1g** respectively. The tissue distribution data was determined 24 h after a 50 mg/kg dose was administered intravenously to male Sprague–Dawley rats. The data is summarized in Table 2.

As shown above, introducing a disulfide bond into the lipid appendage had a significant effect on the tissue distribution. The lipid chains of **1a**, **1g** and **1f** are all 13 atoms in length. However, the accumulations of **1a** in liver and kidney are much lower than that of **1g** and **1f**. Intriguingly, there was 15% of dimer **8** observed in urine along with 1% of parent compound **1a** and 2.3% of free thiol metabolite **9**. These findings support our hypothesis that lipophilic vancomycin disulfides can be metabolized into more hydrophilic compounds such as **8** and **9** for urinary excretion. The thiol **9** was concentrated during urinary production, and allowed to self-dimerize to form **8** linked via a disulfide bond (Fig. 2). Compound **8** still exhibited antibacterial activity against VRE (Table 1). The concentration of **8** in urine is well above its MIC level, which should be effective for the treatment of VRE-associated urinary tract infections.

In summary, we have successfully synthesized vancomycin derivatives bearing disulfide bonds in the lipid tail. These compounds retained good potency and rapid bactericidal activity against vancomycin-resistant strains. In vivo ADME studies showed that lipophilic vancomycin disulfide analogues exhibited an improved ADME profile in that they could be converted into more hydrophilic metabolites and excreted in urine.

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13. General procedure for the reductive alkylation on vancosamine: To the mixture of vancomycin (1 mmol, 1 equiv) and aldehyde (1.3 mmol, 1.3 equiv) in DMF (10 mL) was added diisopropylethyl amine (2 mmol, 2 equiv). The reaction was stirred at rt for 1 h. A solution of $NaCNBH_3$ (1 mmol, 1 equiv) in methanol (5 mL) was added to the reaction, followed by 3 mmol TFA, and stirring was continued for an additional h. The reaction mixture was concentrated and then precipitated in acetonitrile. Purification on RP-HPLC gave the desired product as a TFA salt.
14. MIC was determined against clinical isolates of MRSA (ATCC 33591), MSSA (ATCC 13709) and vancomycin resistant *Enterococcus faecium* (KPB-01) and *E. faecalis* (MGH-01) of Van A and Van B (ATCC 51575) phenotype utilizing the NCCLS broth microdilution method. Bactericidal activity was evaluated by time–kill analysis.
15. Calculation was based on the peak area ratio of metabolite peak versus parent peak.