Pergamon

Bioorganic & Medicinal Chemistry Letters 12 (2002) 849–852

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Antibacterial Activity of G6-Quaternary Ammonium Derivatives of a Lipophilic Vancomycin Analogue†

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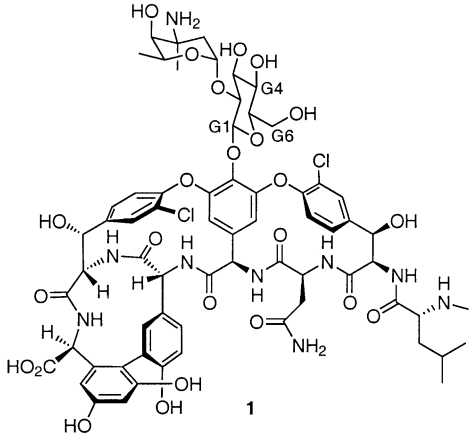
Received 2 November 2001; accepted 26 December 2001

Abstract—A series of G6-amino derivatives of a lipophilic vancomycin analogue was prepared. Antibacterial activity of the analogues was inversely proportional to the degree of substitution of the G6-nitrogen. The fully substituted (quaternary) analogues were essentially inactive against vanA phenotype VREF strains but retained substantial activity against other bacteria, a profile reminiscent of teicoplanin.

Vancomycin (**1**) was discovered more than 40 years ago.[2] Despite the development of resistance in some strains of enterococci (VREF strains),[3] vancomycin is still widely used for the treatment of serious bacterial infections and it is the antibiotic of last resort against many antibiotic-resistant staphylococcus strains, especially MRSA. To date, only intermediate-level vancomycin-resistant *Staphylococcus aureus* (VISA) strains have been detected.[4] However, the likely development of high-level vancomycin-resistant *S. aureus* (VRSA) necessitates the discovery of novel antibiotics with useful activity against such strains.



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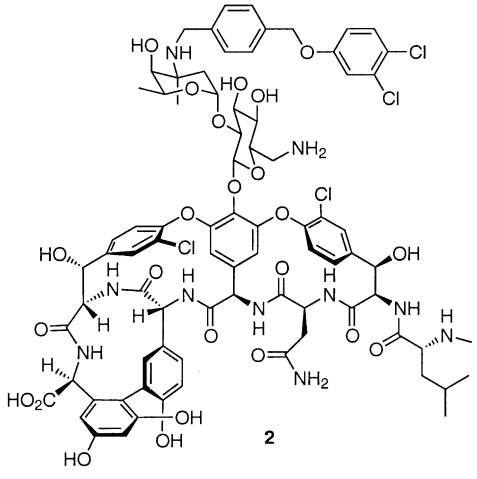
The recent discovery by Kahne et al.[5] that vancomycin could be selectively functionalized at the 6-position (G6) of the glucose residue has enabled the synthesis of a wide variety of analogues that were not previously accessible. Coupled with the observation by Nagarajan et al.[6] that incorporation of a lipophilic side chain into vancomycin resulted in a dramatic increase in activity against vancomycin-resistant bacteria, this suggested the possibility of preparing novel glycopeptide antibiotics with an improved spectrum of activity and/or improved pharmacological properties over vancomycin.[7] Indeed, Kim et al.[8] have reported the synthesis of numerous lipophilic vancomycin analogues that appear to have enhanced activity relative to vancomycin.



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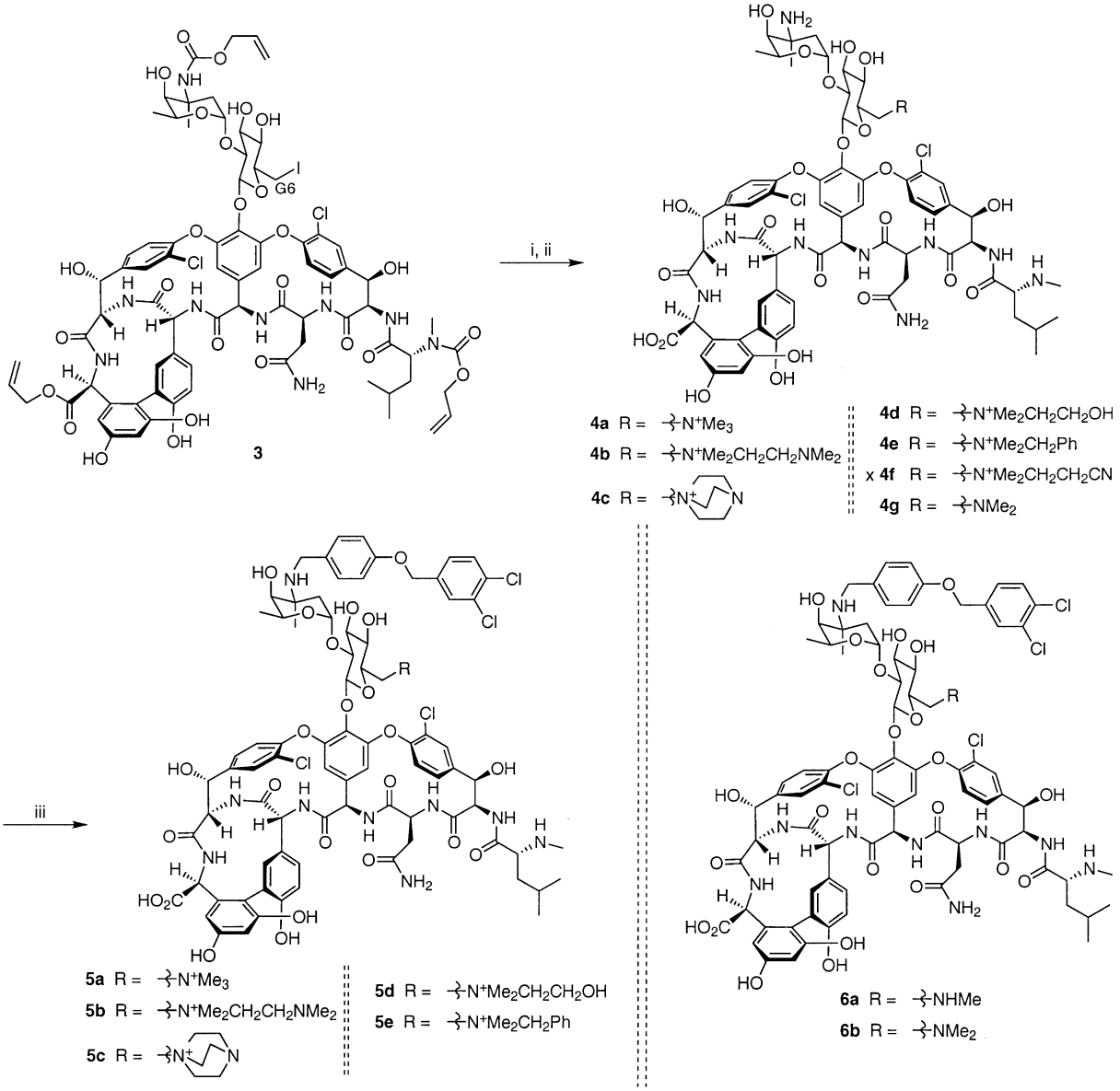
0960-894X/02/$ - see front matter
PII: S0960-894X(02)00049-5

One of the most active analogues reported to date is the 6-amino-6-deoxy derivative **2**, which has a primary amine at G6 of the glucose residue in addition to a lipophilic side chain in the vancosamine residue.[8a] The presence of the primary amino group, which would be positively charged at physiological pH, at G6 appeared to result in improved antibacterial activity for **2**. We were interested in probing the effect of alkyl substitution on the G6 nitrogen atom of **2** to see if increased steric bulk at this position would negate the advantage derived from the presence of a charged substituent. We especially wanted to determine if the presence of a tetrasubstituted nitrogen, a bulky 'hard quat' that would be charged at any pH, at G6 would result in a further improvement in antibiotic activity.

The quaternary ammonium analogues **5a**–**5e**[9] were synthesized from the protected iodide **3**[8a] via the route outlined in Scheme 1. Silver ion assisted displacement of the iodide by a tertiary amine in DMF followed by removal of the protecting groups afforded the quaternary ammonium adducts **4a**–**4e**. However, the reaction failed when the amine was 3-dimethylaminopropionitrile. In this case, the G6-dimethylamino analogue **4g** (formed by beta elimination from the initial adduct **4f**) was isolated instead. Introduction of the aralkyl side chain by reductive amination of the amine with 4-(3,4-dichloro)benzyloxybenzaldehyde afforded the final products **5a**–**5e** and **6b**.

In an in vitro antibacterial assay (Table 1), the quaternary ammonium analogues **5a**–**5e** demonstrated activity that was comparable to or slightly better than vancomycin against *Staphyloccus* and vancomycin-sensitive *Enterococcus* strains. However, they were generally less active than the unsubstituted amine **2** against these strains. Unfortunately, **5a**–**5e** were considerably less active than **2** against VREF strains. Interestingly,



Scheme 1. Reagents and conditions: (i) tertiary amine, AgOTf, DMF; (ii) $Pd_2(dba)_3$, $Ph_2P(CH_2)_3PPh_2$, piperidine, DMF; (iii) ArCHO, $NaBH_3CN$, DMF.

Table 1. In vitro antibacterial activity of vancomycin analogues (MIC μg/mL)[a]

Compound	MSSA[b] (MB2985)	MRSA[b] (COL)	MRSA[b] (MH76)	VISA[b] (CL5705)	VISA[b] C L(5706)	VSEFs[b] (MB2864)	VREFs[b] (MB4877)	VREFs[b] (MB5244)	VSEFm[b] (RLA1)	VREFm[b] (CL4931)
1	2	1	1	4	8	4	2048	16	2	2048
2	0.06	1	1	0.5	0.25	0.25	4	0.5	≤0.03	2–4
5a	0.5	0.5	0.5	2	2	1	16	1	0.5	>64
5b	2	2	2	2	4	4	>64	8	1	>64
5c	1	1	1	2	2	2	8	1	0.5	>32
5d	2	1	1	2	2	2	8	4	0.5	>64
5e	0.25	0.5	0.25	1	1	2	16	1	0.25	>64
6a	≤0.03	0.12	0.12	1	0.25	0.25	4	1	0.03	32
6b	≤0.03	0.5	0.25	1	0.5	1	16	1	≤0.03	32

[a]MIC values determined by a standard broth microdilution assay.
[b]MSSA = methicillin-sensitive *S. aureus*; MRSA = methicillin-resistant *S. aureus*; VISA = vancomycin intermediate-resistant *S. aureus*; VSEFs = vancomycin-sensitive *E. faecalis*; VREFs = vancomycin-resistant *E. faecalis* (vanB resistance phenotype); VSEFm = vancomycin-sensitive *E. faecium*; VREFm = vancomycin-resistant *E. faecium* (vanA resistance phenotype).

5a–**5e** retain significant activity against the resistant *Enterococcus faecalis* strains (vanB phenotype)[3d] but not against the resistant *Enterococcus faecium* strains (vanA phenotype).[3d] In this regard, the quaternary analogues resemble teicoplanin. As with teicoplanin, it appears that the quaternary analogues **5a**–**5e** lack the ability to induce resistance[10] in the vanB strains and thus retain activity against those strains. It has been proposed that the improved activity of lipophilic vancomycin analogues against vanA strains is due to dimerization and membrane anchoring[11] or, alternatively, to a second mechanism of action[4,12] which only becomes apparent when the usual glycopeptide mechanism of binding to D-ala-D-ala is not possible. The activity of the unsubstituted amine **2** may be explained by one of these hypotheses. However, the introduction of a quaternary ammonium group into **5a**–**5e** clearly interferes with the mechanism that confers VREF activity on **2**.

We have briefly explored the relation between size of the G6 substituent and antibacterial activity. This study was enabled by the serendipitous synthesis of the G6 dimethylamino analogue **6b**, which allowed us to examine the SAR of increasing the number of methyl groups on the G6 nitrogen. There is a direct relationship between the number of methyl groups on the G6 nitrogen and the MIC against vancomycin-resistant bacteria. This effect is most pronounced against the vancomycin-resistant *E. faecium* (VREFm) strain. Thus, the unsubstituted compound **2** (no methyl groups) is the most active against VREFm (MIC 2–4 μg/mL) while analogues **6a**[8] (one methyl) and **6b** (two methyls) are somewhat less active (MIC 32 μg/mL) and the quaternary analogue **5a** (three methyls) is essentially inactive against this strain. The exact reason for the decrease in antibacterial activity with increasing substitution at G6 is unclear. It seems likely that the greater steric demands of the quaternary ammonium group play a significant role but the presence of a permanent positive charge at G6 in analogues **5a**–**5e** might also be important.[13] Although the highly active unsubstituted analogue **2** would also have a positive charge at this position at physiological pH, that positive charge results from reversible protonation of the G6 amine. This would allow analogues such as **2**, **6a**, and **6b** to become neutral under circumstances (e.g., in a lipid environment or in proximity to another positive charge) where a positive charge is unfavorable. The presence of a permanent positive charge on G6 of analogues **5a**–**5e** thus imposes greater limitations on these analogues and this may explain their reduced activity against resistant bacteria. However, the intermediate level of activity observed for **6a** and **6b** suggests that size is also important. Whatever the reason for the loss of activity with increasing substitution, it is clear that large substituents should be avoided at G6.

Although the G6 quaternary ammonium analogues **5a**–**5e** retained some antibacterial activity, their diminished activity relative to the unsubstituted analogue **2** against the VREF strains was disappointing. It is unclear whether this loss of activity is due to steric or electronic effects. Further results in this area will be described in future reports from this laboratory.

Acknowledgements

We thank Dr. Gerard Kieczykowski and Ms. Amanda Makarewicz for the preparation of the protected vancomycin iodide **3**. We also thank Dr. Daniel Kahne and Mr. Robert Wilkening for helpful discussions.

References and Notes

1. For clarity and brevity, we utilize herein a simplified nomenclature for vancomycin derivatives wherein the glucose positions 1 to 6 are designated G1 to G6 (see structure **1**).
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13. One of the referees for this manuscript suggested that the presence of a positive charge at G6 might interfere with dimerization and membrane anchoring.