Pergamon

Bioorganic & Medicinal Chemistry Letters 12 (2002) 3033–3036

BIOORGANIC &
MEDICINAL
CHEMISTRY
LETTERS

An Efficient and Practical Method for Solid-Phase Synthesis of Tripeptide-Bearing Glycopeptide Antibiotics: Combinatorial Parallel Synthesis of Carboxamide Derivatives of Chloroorienticin B

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Received 28 June 2002; accepted 9 August 2002

Abstract—An efficient and practical method was established for solid-phase parallel synthesis of the peptide-bearing carboxamide derivatives of chloroorienticin B, and over 80 compounds were synthesized simultaneously. Among the derivatives prepared, compounds having both tryptophan and tyrosine residues (**1**–**3**) were found to possess potent antibacterial activity against VRE.

Introduction

In the preceding paper,[1] we described the synthesis of derivatives of chloroorienticin B,[2] a vancomycin-related glycopeptide antibiotic, by modification of amino sugar and resorcinol moieties of the parent chloroorienticin B, and presented novel derivatives having potent antibacterial activity. The C-terminus carboxyl group of the glycopeptide backbone is one of the most accessible modification sites and many examples of carboxamide derivatives of glycopeptide antibiotics have been reported.[3,4] However, little is known about the carboxamide derivatives having a peptide chain at the C-terminus. Griffin et al. have reported the introduction of the peptide chain into the C-terminus of vancomycin by solid-phase synthesis, but they prepared only a limited number of compounds (about 5) and did not describe any antibacterial activities of the derivatives prepared.[3b]

Each amino acid has a unique functionality on its own side chain, so we assumed that the introduction of a peptide chain into glycopeptide antibiotics would afford a variety of the derivatives, which could lead to novel derivatives with potent antibacterial activity.[5]

Here, we report an efficient and practical method for the solid-phase parallel synthesis of carboxamide derivatives of chloroorienticin B bearing various tripeptides at the C-terminus and also present peptide derivatives with potent antibacterial activity against methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant enterococci (VRE) (see Fig. 1).

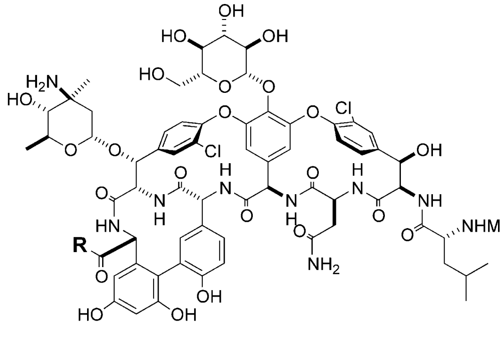

Fig. 1. Chloroorienticin B (**R** = OH) and its tripeptide-bearing carboxamide derivatives (**R** = HO-A_1-A_2-A_3-)

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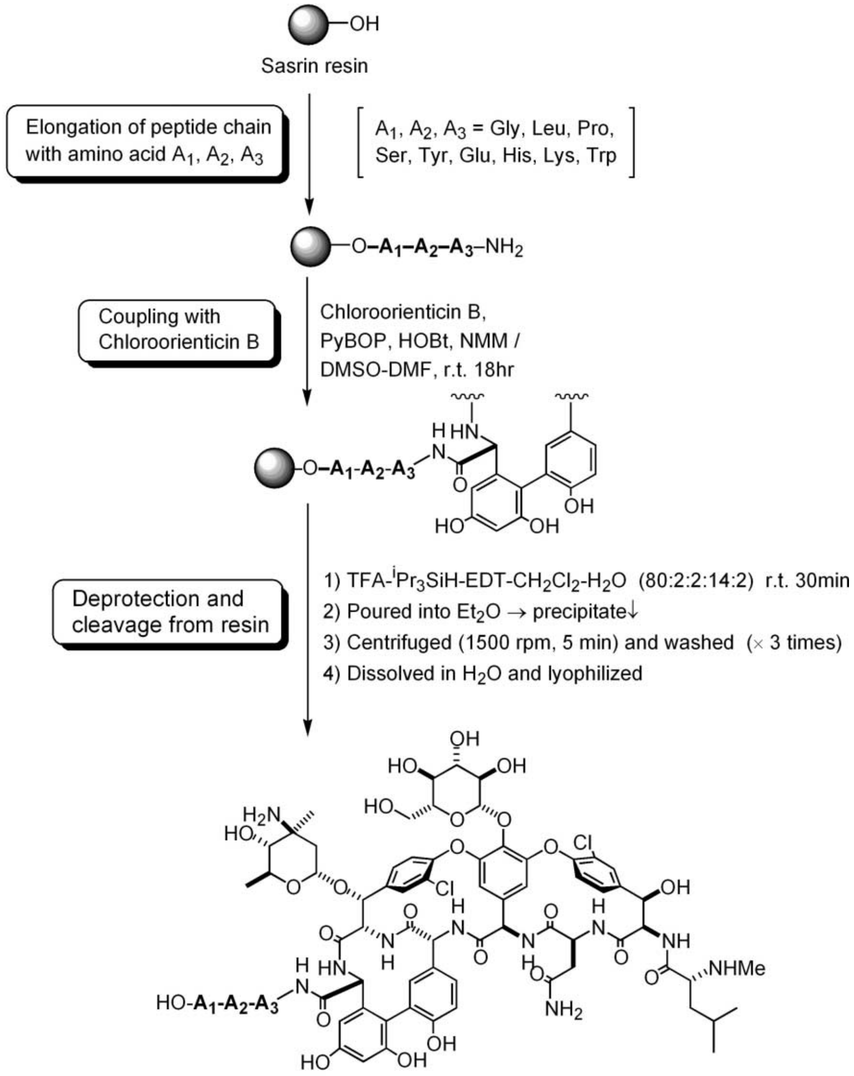
PII: S0960-894X(02)00665-0



Scheme 1. Solid-phase parallel synthesis of carboxamide derivatives of chloroorienticin B bearing various tripeptides.

Results and Discussion

In order to elucidate the effects of the amino acid side chain on antibacterial activity, we chose nine typical amino acids (Gly, Leu, Pro, Ser, Tyr, Glu, His, Lys, Trp)[6] as building blocks of the peptide chain. Chloroorienticin B derivatives bearing various tripeptides at the C-terminus were prepared as illustrated in Scheme 1.

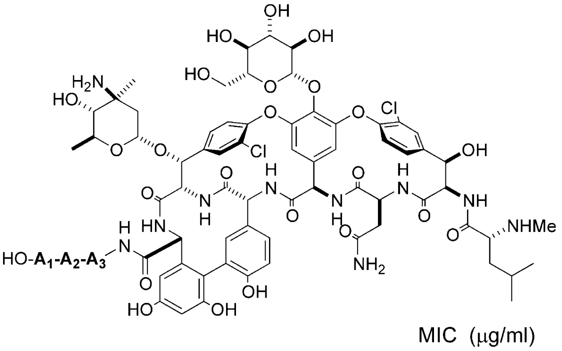
Tripeptide elongation was achieved using standard Fmoc peptide synthesis protocol[7] (30 μmol scale) on Sasrin resin[8] (super acid-sensitive resin). Each resin bearing tripeptide was coupled with chloroorienticin B by agitation with chloroorienticin B, PyBOP®,[9] HOBt (1-hydroxybenzotriazole) and NMM (*N*-methyl morpholine) in DMSO–DMF for 18 h. The completion of the coupling reaction was confirmed by the ninhydrin test.[10]

After successive washing with DMSO and CH_2Cl_2, each resin was treated with TFA(CF_3CO_2H)–$^iPr_3SiH$-EDT(ethanedithiol)–CH_2Cl_2–H_2O (80:2:2:14:2) for 30 min to cleave the product from the resin and remove the protecting groups on their amino acid side chain. Each resulting resin was filtered off, and each filtrate was poured into Et_2O, affording the precipitate of the crude products. Each precipitate was collected by centrifugation (1500 rpm, 5 min) and washed with Et_2O (repeated three times). Lyophilization of each aqueous solution of the precipitate afforded the desired tripeptide derivatives as trifluoroacetate salts. HPLC and mass spectral analysis revealed that each product obtained was almost a single compound except that each contained 10–20% of the deglucosylated by-product as an impurity.[11]

We synthesized over 80 compounds simultaneously by the method described above and examined their antibacterial activity against MRSA and VRE. Part of the results is shown in Table 1.[12]

Among the tripeptide derivatives prepared, the compounds having both tryptophan and tyrosine residue (**1**–**3**)

Table 1. In vitro antibacterial activity against MRSA and VRE



Compd	A1-A2-A3-	MIC (μg/mL)		
		Staphylococcus aureus SR3637 (MRSA)	*Enterococcus faecalis* SR7914 (VRE)	*Enterococcus faecium* SR7917 (VRE)
1	Leu-Trp-Tyr	0.5	16	8
2	Tyr-Trp-His	0.5	16	8
3	Tyr-Trp-Ser	0.5	32	16
4	Leu-Trp-Lys	0.5	32	16
5	Lys-Trp-Gly	0.25	64	32
6	Trp-Pro-His	0.5	64	16
7	Trp-His-Gly	0.25	32	16
8	Trp-Lys-His	<0.125	64	8
9	Tyr-Gly-Lys	0.5	128	64
10	Lys-His-Tyr	1	128	64
11	Leu-Tyr-Lys	0.5	128	32
12	Tyr-Gly-Glu	4	>128	>128
13	Glu-Trp-Tyr	8	>128	>128
Chloroorienticin B		0.5	>128	>128

were found to have potent activity against VRE together with their activity against MRSA comparable to the parent chloroorienticin B. Compounds having the combination of tryptophan-lysine or tryptophan-histidine (**4**–**8**) also exhibited activity against VRE. It was interesting to find that compound **8** with Trp-Lys-His tripeptide showed potent activity against MRSA. Tryptophan residue was considered to be essential for the potent activity against VRE, since compounds with a tyrosine and lysine combination (**9**–**11**) were not as active as those with the tryptophan residue. The introduction of glutamic acid (**12**–**13**) resulted in reduced activity against MRSA and no activity against VRE even though tryptophan was present.

Table 1 suggests that amino acids having basic and somewhat hydrophobic characters are essential for activity against VRE.[13] The mechanism of the antibacterial activity against VRE is still not completely understood,[14] but affinity to cell wall peptidoglycan of VRE is considered to be one of the most important factors.[14b] Basic and hydrophobic functionalities (tryptophan and tyrosine) are thought to increase the affinity to cell wall peptidoglycan of VRE, and the acidic functionality (glutamic acid), to reduce the affinity. It is also suggested that the factor responsible for increased activity against VRE is different from that for activity against MRSA, since the compounds with basic and hydrophobic functionalities (**1**–**7**) have potent activity against VRE, but their activities against MRSA were not improved when compared to the parent chloroorienticin B.[15]

The present study offers a simple and practical method for preparing a variety of peptide-bearing derivatives of glycopeptide antibiotics. This methodology also provides an efficient tool for searching for effective combinations of functional groups and is considered to be useful for finding novel derivatives of glycopeptide antibiotics with potent activity.

Acknowledgements

We are indebted to Dr. Yuji Ikenishi (Discovery Research Laboratories, Shionogi & Co., Ltd.) for the measurement of the mass spectra.

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12. The in vitro antibacterial activity is given as minimum inhibitory concentration (MIC) in µg/mL as determined by National Committee for Clinical Laboratory Standards (NCCLS)-recommended microbroth dilution methods using cation-adjusted Mueller Hinton broth (Difco Laboratories, Detroit, MI, USA).

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