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Synthesis and antibacterial activity of O-substituted nocathiacin I derivatives

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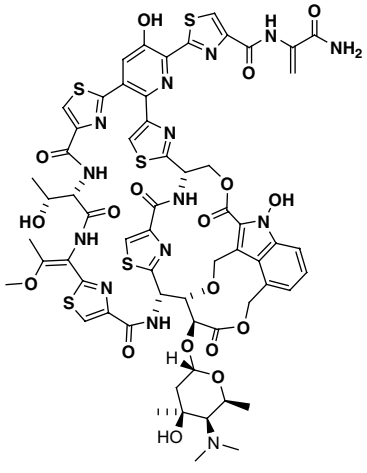
Abstract—The synthesis and antibacterial activity of a series of new nocathiacin I derivatives (**1**–**12**) containing polar water solubilizing groups is described. Most of these compounds exhibited potent antibacterial activity and have improved water solubility. In addition, compounds **5**, **7**–**9** also exhibited potent in vivo activity.

1. Introduction

Rapid development of resistance to the existing antibacterial drugs poses a major public health problem through out the world. The incidence of multidrug-resistant Gram-positive bacteria is increasing, and infections caused by these bacteria are particularly problematic.[1] In addition, due to the unprecedented movement of both goods and people across the globe, the resistant strains are transported around the world at a much faster rate now than ever before. Furthermore, recent reports suggest that bacteria are developing resistance to vancomycin, often used to treat tough bacterial infections and considered as the last option, at an alarming rate.[2] Consequently, there is a pressing need to discover and develop new antibacterial agents, which have a broad spectrum of activity against the resistant bacteria and preferably work by a different mechanism of action than the existing drugs to avoid cross-resistance development.

Nocathiacin I, a cyclic thiazolyl peptide antibiotic, was isolated from the fermentation broth of *Nocardia* sp.[3a,b] and fungus *Amicolaptosis* sp.[3c] It displays a potent antibacterial activity against a variety of Gram-positive bacteria, including a number of multiple drug resistant strains such as methicillin-resistant *Staphylococcus aureus* (MRSA), methicillin-resistant *Enterococcus faecium* (MREF), vancomycin-resistant *Enterococci* (VRE) and penicillin-resistant *Streptococcus pneumoniae* (PRSP). It disrupts the bacterial protein biosynthesis by interacting directly with the L11 protein and 23S rRNA region of the ribosome.[4a,b,c] Unlike other members of thiazolyl peptide antibiotics, such as nosiheptide and thiostrepton, nocathiacin I has bactericidal activity against *S. aureus*[4c] and shows in vivo efficacy in a mouse systemic *S. aureus* infection model.[4d]



Nocathiacin I

Keywords: Antibacterial activity; Nocathiacin; Natural product.
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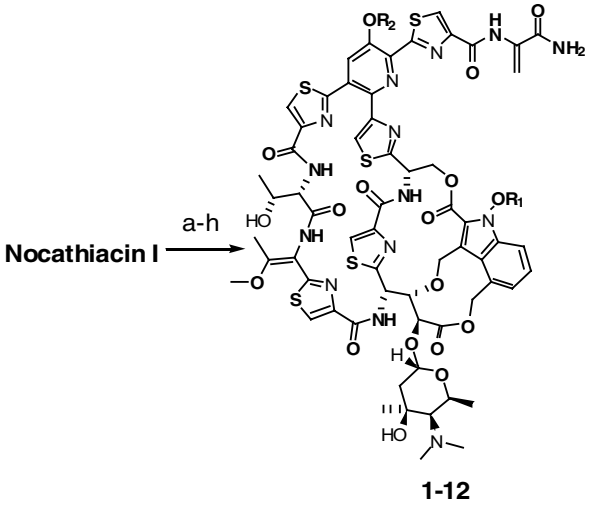
Although nocathiacin I has better solubility at low pH than other members of thiazolyl peptide antibiotics, its solubility is inadequate for development as an intravenous (iv) drug. As a result, we have undertaken an investigation to modify nocathiacin I to improve its solubility both in water and saline while maintaining its intrinsic biological activity. One of our approaches is to introduce polar water solubilizing groups into the molecule through chemical transformation.[5] Herein, we report the results of synthesis and antibacterial activities of O-substituted nocathiacin I analogues.

2. Chemistry and biology results

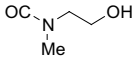
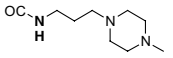
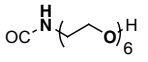
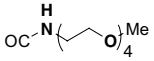
The synthesis of nocathiacin I derivatives bearing polar O-substituents[6] is outlined in Scheme 1 and results are listed in Table 1. The carbamate derivatives **1**–**6** were prepared by two different approaches. Treatment of nocathiacin I with butyl isocyanate provided the carbamate **1** whereas reaction with methyl isocyanate furnished carbamate **2**. Prolonged reaction times and poor yields coupled with limited availability of isocyanates with polar groups warranted development of an alternative route. We found that in situ conversion of nocathiacin I to the corresponding carbonate by treating with 4-nitrophenyl chloroformate followed by reacting with amine gave the best results. Thus, carbamates **3**–**6** were prepared by trapping the in situ generated carbonate with an appropriate amine.[7]

Reacting nocathiacin I with methylphosphonic dichloride [$MeP(O)Cl_2$] provided phosphonate derivatives **7**–**9**.



Scheme 1. For **1**–**2** (a) pyridine/RNCO/rt/5–7 days; For **3**–**6** (b) DMF/BTPP/rt to 0 °C, then add 4-NO_2–PhOCOCl/10 min/R′R″NH/15 min; For **7** (c) DMF/Cs_2CO_3/rt/30 min, cool to 0 °C/$MeP(O)Cl_2$/40 min/aq $NaHCO_3$; For **8** (d) DMF/BTPP/0 °C, then $MeP(O)Cl_2$/30 min/aq $NaHCO_3$; For **9** (e) CH_2Cl_2/DIEA/0 °C/$MeP(O)Cl_2$/30 min/aq $NaHCO_3$; For **10** (f) pyridine/2-chloro-1,3,2-dioxaphospholane-2-oxide/0 °C–rt/5 h; For **11** (g) CH_2Cl_2/Et_3N/2-chloro-1,3,2-dioxaphospholane-2-oxide/rt/4 h; For **12** (h) CH_2Cl_2/DMAP/2-chloro-1,3,2-dioxaphospholane-2-oxide/0 °C–rt/18 h.

Table 1. O-substituted nocathiacin I derivatives

Compd	R_1	R_2	Yield (%)[a]
1	$CONH^{n}Bu$	H	11
2	CONHMe	H	4
3		H	25
4		H	41
5		H	15
6		H	13
7	H	P(O)(Me)ONa	13
8	P(O)(Me)ONa	H	8
9	P(O)(Me)ONa	P(O)(Me)ONa	21
10	H	P(O)(OR)OH[b]	39
11	P(O)(OR)OH	H	15
12	P(O)(OR)OH[b]	P(O)(OR)OH[b]	17

[a] Isolated yields.
[b] $R = CH_2CH_2Cl$.

Thus, treatment of nocathiacin I with $MeP(O)Cl_2$ in the presence of caesium carbonate exclusively afforded the phenol hydroxyl-phosphonate derivative **7**. Alternatively, use of phosphazene base, *tert*-butyliminotri(pyrrolindino)phosphorane (BTPP), furnished the indole hydroxyl-phosphonate derivative **8**.[8] Treatment of nocathiacin I with excess $MeP(O)Cl_2$ in the presence of Hunig base produced the bis-phosphonate **9**. The phosphate derivatives **10**–**12** were prepared by reacting nocathiacin I with 2-chloro-1,3,2-dioxaphospholane-2-oxide under slightly different conditions. The monophosphate **10** was obtained by treating nocathiacin I with 2-chloro-1,3,2-dioxaphospholane-2-oxide in pyridine.[9] On the other hand, exposure of nocathiacin I to 2-chloro-1,3,2-dioxaphospholane-2-oxide in the presence of triethylamine provided the regioisomeric mono-phosphate **11**. The DMAP mediated reaction of nocathiacin I with 2-chloro-1,3,2-dioxaphospholane-2-oxide furnished the bis-phosphate **12**.

Interestingly, despite the presence of four hydroxyl groups only two hydroxyl groups, viz. pyridyl–OH and indole–N–OH, were found to be reactive towards a variety of electrophiles. Furthermore, it is gratifying that these two hydroxyl groups can also be regioselectively functionalized using slightly different conditions. The other two hydroxyl groups viz. the tertiary hydroxyl from the amino-sugar moiety and the secondary hydroxyl from the threonine moiety are less reactive. This may be because both these hydroxyls are sterically hindered consequently less accessible to electrophiles.

The semi-synthetic O-derivatives of nocathiacin I were evaluated for their in vitro and in vivo antibacterial activity and results are summarized in Table 2. The

Table 2. In vitro and in vivo antibacterial activity of O-substituted nocathiacin I derivatives

Compound	MICs (μg/mL)[a]			PD_{50}[b] (mg/kg)	Solubility,[c] mg/mL (pH)
	S. aureus A15090 (MSSA)[d]	*S. pneumo* A28272 (PRSP)	*E. faecalis* A20688 (MSEF)[d]		
Nocathiacin I	0.007	0.002	0.03	0.2 (iv)	0.34 (4.0)
1	0.001	0.007	0.03	ND	ND
2	0.125	0.001	0.06	ND	ND
3	0.03	0.0005	0.03	ND	0.003 (7.1)
4	0.003	0.0005	0.003	ND	>2.3 (3.0)
5	0.06	0.003	0.06	0.78 (sc)	>2.1 (3.5)
6	0.125	0.015	0.125	ND	ND
7	0.125	0.06	0.25	0.26 (iv)	>2.2 (8.4)
8	0.125	0.007	0.25	1.6 (iv)	5.6 (8.2)
9	0.25	0.003	0.25	5.9 (iv)	>10.0 (8.5)
10	1	0.03	1		ND
11	0.25	0.03	0.5	>10 (sc)	0.53 (6.9)
12	4	0.5	4	ND	ND

[a] Minimum inhibitory concentration (MIC): lowest concentration of drug that inhibits visible growth of the organism.[10]
[b] PD_{50} determined by mice systemic *S. aureus* infection model.[11]
[c] Equilibrium water solubility was determined with amorphous powders.
[d] MSSA: methicillin-sensitive *S. aureus*; MSEF: methicillin-sensitive *E. faecalis*. ND means not determined.

lypophilic butyl carbamate **1** is as potent as the parent, whereas the methyl carbamate **2** has reduced antibacterial activity when compared with nocathiacin I. The carbamates **3** and **4**, which contain hydroxyl and amine polar groups, respectively, have retained the in vitro potency of the parent compound. Similarly, PEG-amine derived carbamates **5** and **6** also have very good in vitro antibacterial activity. In addition carbamate analogue **5** also showed an excellent in vivo potency.

Although the mono-methylphosphonate analogue **7** has reduced in vitro antibacterial activity it has retained the in vivo potency of nocathiacin I. In contrast, the indole hydroxyl mono-methylphosphonate derivative **8** has reduced in vitro and in vivo potency when compared with the parent. This difference in the in vivo activity of the phosphonates **7** and **8** may presumably be due to more rapid conversion of **7** to the parent under physiological conditions. The bis-methylphosphonate derivative **9** also suffered from significantly reduced in vitro and in vivo activity. Furthermore, the phosphate analogues **10**–**11** are significantly less potent than nocathiacin I. Most of these semi-synthetic nocathiacin derivatives have very good aqueous solubility. In general, the nocathiacin derivatives with improved aqueous solubility have reduced in vitro antibacterial activity.

In summary, we have synthesized several mono- and bis-substituted nocathiacin I O-derivatives with polar groups such as carbamates, phosphonates and phosphates, and evaluated them for antibacterial activity. During the course of this work we have regioselectively introduced substituents either on the 3-hydroxypyridyl moiety or on the indole-N-hydroxyl moiety. Most of these synthetic analogues have retained the potent antibacterial activity of the parent. In addition, some of these analogues exhibit an excellent in vivo efficacy in the mouse systemic *S. aureus* infection model and have improved aqueous solubility.

Acknowledgements

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6. The regiochemistry of the O-substitution is assigned using the UV absorption data. The nocathiacins with free pyridyl hydroxyl group showed bathochromic shift when the pH of solution is changed from an ambient pH to basic. Whereas nocathiacins with pyridyl hydroxyl group blocked with a substituent had no significant change in the UV absorption when the pH of the solution is changed from an ambient pH to basic. Also, see Ref. 5a for regiochemical determination of O-alkylated nocathiacins using NMR data.
7. Preparation of **4**: To a solution of Nocathiacin I (0.2874 g, 0.2 mmol) in DMF (2 mL) was added phosphazene base (BTPP, 0.2 mL, 0.6 mmol) and the mixture was stirred for 10 min. Then the reaction was cooled to 0 °C, 4-nitrophenyl chloroformate (0.14 g, 0.7 mmol) was added and the mixture was stirred at 0 °C for 10 min. To this was added 1-(3-aminopropyl)-4-methylpiperazine (0.12 g, 0.6 mmol), stirred at 0 °C for 15 min. and then quenched with 1 N HCl (3 mL). The reaction mixture dissolved in DMF and purified by chromatography (preparative C18, ODS-A, S-75μm, 20–30% acetonitrile/water/0.5 mL 1 N HCl per litre) to yield the product as a yellow powder (0.1141 g, 41% yield). ^{1}H NMR (DMSO, 500 MHz): δ 11.82 (b s, 1H), 11.36 (s, 1H), 10.06 (s, 1H), 9.21 (s, 1H), 8.92 (b s, 1H), 8.76 (s, 2H), 8.60 (s, 1H), 8.54 (s, 1H), 8.22 (s, 1H), 8.11 (s, 2H), 8.02 (m, 1H), 7.88 (m, 1H), 7.75 (d, $J = 10.0$ Hz, 1H), 7.63 (s, 1H), 7.45 (s, 1H), 7.28 (d, $J = 5.0$ Hz, 1H), 7.21 (m, 1H), 6.38 (s, 1H), 6.06 (d, $J = 10.0$ Hz, 1H), 5.77 (s, 1H), 5.71 (m, 2H), 5.10 (s, 1H), 5.07 (s, 2H), 4.87 (b s, 1H), 4.35 (m, 1H), 4.26 (m, 1H), 4.11 (m, 2H), 3.92 (s, 2H), 3.86 (s, 1H), 3.59–3.30 (m, 15H), 3.10 (s, 3H), 2.88 (s, 6H), 2.77 (s, 3H), 2.13 (d, $J = 10.0$ Hz, 1H), 2.04 (s, 3H), 1.92 (d, $J = 15.0$ Hz, 1H), 1.84 (m, 1H), 1.59 (s, 3H), 1.13 (s, 3H), 0.77 (s, 3H). HRMS calcd for $C_{70}H_{78}N_{17}O_{19}S_5$ (M+H): 1620.426.; found 1620.428.
8. Preparation of **8**: To a stirred solution of Nocathiacin I (1.0 g, 0.7 mmol) in N,N-dimethylformamide (25 mL) was added phosphazene base (BTPP, 0.65 mL, 2.1 mmol) and the mixture was stirred for 5 min. The mixture was cooled to 0 °C and methylphosphonic dichloride (0.09 g, 0.7 mmol) was added and stirred at 0 °C for 30 min. The reaction mixture was quenched with saturated aq sodium bicarbonate (6 mL), and dissolved in water and purified by chromatography (preparative C18, ODS-A, S-75 μm, 15% acetonitrile/water–30% acetonitrile/water) to yield the product as a yellow powder (0.083 g, 8% yield). ^{1}H NMR (DMSO, 500 MHz): δ 11.42 (b s, 1H), 10.08 (s, 1H), 9.01 (s, 1H), 8.71 (s, 1H), 8.56 (s, 1H), 8.53 (s, 1H), 8.20 (s, 1H), 8.07 (b s, 1H), 7.91 (s, 1H), 7.88 (m, 1H), 7.83 (m, 2H), 7.61 (b s, 1H), 7.24 (t, $J = 7.5$ Hz, 1H), 7.12 (d, $J = 10.0$ Hz, 1H), 7.09 (d, $J = 10.0$ Hz, 1H), 6.35 (s, 1H), 6.02 (d, $J = 15.0$ Hz, 1H), 5.74 (s, 1H), 5.70 (d, $J = 10.0$ Hz, 1H), 5.13 (d, $J = 5.0$ Hz, 1H), 5.02 (m, 2H), 4.90 (d, $J = 10.0$ Hz, 1H), 4.55 (d, $J = 10.0$ Hz, 1H), 4.28 (d, $J = 10.0$ Hz, 1H), 4.13 (m, 2H), 3.99 (d, $J = 10.0$ Hz, 1H), 3.90 (s, 3H), 3.02 (m, 5H), 2.98 (m, 1H), 2.78 (m, 4H), 2.26 (m, 1H), 2.05 (m, 1H), 2.00 (s, 2H), 1.89 (d, $J = 15.0$ Hz, 1H), 1.73 (m, 6H), 1.55 (m, 2H), 1.19 (s, 1H), 1.15 (m, 3H), 0.90 (d, $J = 15.0$ Hz, 2H), 0.76 (m, 2H). HRMS calcd for $C_{62}H_{64}N_{14}O_{20}PS_5$ (M+H): 1515.276; found 1515.272.
9. Preparation of **10**: To suspension of Nocathiacin I (200 mg, 0.14 mmol) in pyridine (3 mL) at 0 °C was added 2-chloro-1,3,2-dioxaphospholane-2-oxide (64 μL, 0.7 mmol). Then the reaction mixture was stirred 15 min at 0 °C and slowly warmed to room temperature. The resulting clear reaction mixture was stirred at room temperature for 5 h and then purified using preparative reverse phase HPLC with methanol/water (contains 0.1% TFA). The fractions containing the product were combined, concentrated and freeze dried to afford the TFA salt of **10** (70.0 mg, 39% yield) as a bright yellow solid. ^{1}H NMR (DMSO, 500 MHz): δ 10.81 (s, 1H), 10.08 (s, 1H), 9.10 (s, 1H), 8.63 (s, 1H), 8.61 (s, 1H), 8.58 (s, 1H), 8.22 (s, 1H), 8.13 (b s, 1H), 7.93 (s, 1H), 7.85 (m, 1H), 7.74 (m, 1H), 7.62 (b s, 1H), 7.34 (m, 2H), 7.20 (m, 1H), 6.42 (b s, 1H), 6.01 (m, 1H), 5.73 (m, 3H), 5.25 (m, 1H), 5.06 (m, 2H), 4.80 (m, 1H), 4.60 (m, 1H), 4.31 (m, 1H), 4.25 (m, 1H), 4.14 (m, 4H), 4.05 (m, 1H), 3.91 (b s, 3H), 3.74 (m, 2H), 2.87 (b s, 6H), 2.47 (m, 1H), 2.12 (m, 1H), 2.02–1.92 (m, 4H), 1.61 (b s, 3H), 1.28 (m, 2H), 1.16 (m, 3H), 0.95 (m, 3H), 0.84 (m, 3H). HRMS calcd for $C_{63}H_{64}ClN_{14}O_{21}PS_5$ (M + H): 1579.248; found 1579.245.
10. The minimum inhibitory concentration (MIC) of a compound was obtained against a panel of bacteria using a conventional broth dilution assay in accordance with standards recommended by the National Committee for Clinical Laboratory Standards (NCCLS, Document M7-A6). The serial microbroth dilution method used Muller–Hinton medium except for the *Streptococcus pneumoniae*, which was tested in 50% Muller–Hinton medium and 50% Todd–Hewitt medium. The final bacterial inoculum contained approximately 5×10^5 CFU/well and was run on microtiter plates. The volume of each well was 100 μL and the plates were inoculated at 35 °C for 18 h in ambient air. The MIC was defined as the lowest drug concentration that prevented visible growth of the bacteria.
11. PD_{50} is the amount of drug required (mg/kg) to cure 50% of infected mice subjected to a lethal systemic infection of *S. aureus*. Adult female ICR mice were inoculated intraperitoneally with 5–6$\times10^6$ CFU overnight culture of *S. aureus* A15090 strain suspended in 7% sterile hog gastric mucin. Drug was prepared in a 10% DMSO/5% Tween 80/85% water vehicle and administrated subcutaneously, twice daily at 1 and 4 h after pathogen inoculation. The number of mice that survived in each experimental group was monitored up to 8 days after pathogen inoculation, and the 50% protective doses (PD_{50}s) of the drug-treated animals were determined by the Spearman–Karber nonparametric estimator method. Each experimental group consisted of 10 animals and a minimum of three different concentrations of drug was evaluated per compound.