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Novel semi-synthetic nocathiacin antibiotics: synthesis and antibacterial activity of bis- and mono-*O*-alkylated derivatives

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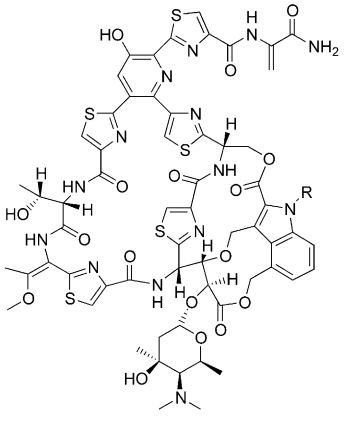
Abstract—Several semi-synthetic bis- and mono-*O*-alkyl nocathiacin derivatives were synthesized and evaluated for antibacterial activity. Mono-*O*-alkyl *N*-hydroxyindole analogues **3a**–**l** were prepared by regioselective alkylation. Bis-*O*-alkyl nocathiacins **4a**–**f** were obtained by treatment with base and excess electrophile. A one-pot protection–alkylation–deprotection strategy was developed for the preparation of mono-*O*-alkyl hydroxypyridine analogues **5a**,**b**. Most of the bis- and mono-*O*-alkyl nocathiacins maintained good in vitro activity but showed reduced in vivo efficacy when compared with the natural product. The excellent in vivo activity and improved water solubility of phosphate analogues **3m** and **4g** suggest their use as potential pro-drugs.

Resistance against antibacterial agents used in current therapy is a problem worldwide.[1] The increasing number of infections caused by methicillin-resistant *Staphylococcus aureus* (MRSA), penicillin-resistant *Streptococcus pneumoniae* (PRSP) and most recently, Vancomycin-resistant *enterococci* (VRE) emphasize the need for new and alternative antibacterial agents.[2]

Nocathiacin I and II are tricyclic thiazolyl peptide antibiotics,[3] (Fig. 1) isolated from fermentation of *Nocardia* sp.[4] or *Amycolatopsis* sp.[5] They possess excellent in vitro potency against multiple resistant strains of Gram-positive bacteria, including PRSP, MRSA and VRE. This class of natural products act as inhibitors of the elongation step in bacterial protein synthesis.[6] More importantly, in contrast to other thiazolyl peptide antibiotics such as glycothiohexide[7] and nosiheptide,[8] nocathiacins exhibit in vivo efficacy against *staphylococci* and *pneumococci* infection models in mice. Nocathiacin I (**1**) is more soluble at lower pH than thiostrepton or nosiheptide, presumably due to the presence of a dimethylamino sugar moiety. However its solubility is not sufficient for development as an intravenous agent.

As part of our efforts in the development of novel antibacterial agents, we set out to investigate chemical modifications of nocathiacins with the goal of increasing their water solubility while maintaining biological activity. We have previously reported two useful strategies for this purpose. One approach consisted of Michael addition of amines and thiols[9] to the dehydroalanine moiety. The second approach involved cleavage of the dehydroalanine moiety[10] followed by further



1: R = OH; Nocathiacin I, (MJ347-81F4-A)
2: R = H; Nocathiacin II

Figure 1. Structure of nocathiacins I and II.

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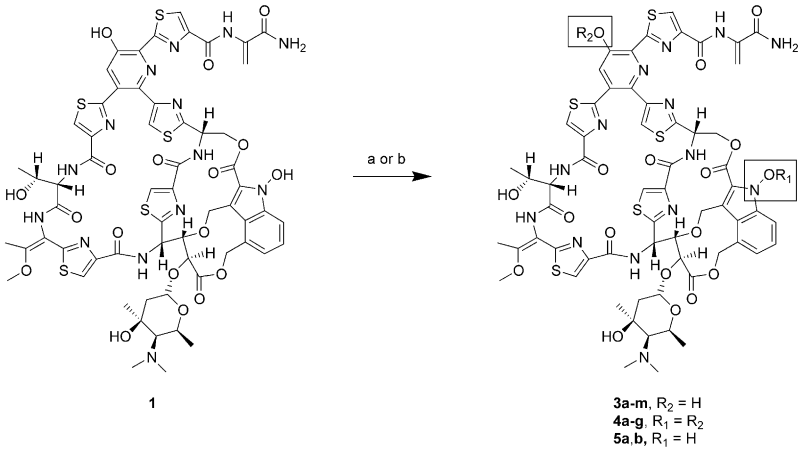
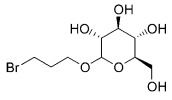
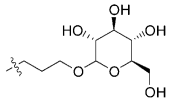
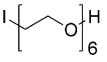
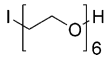
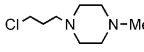
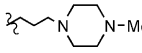
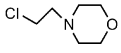
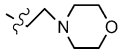
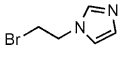
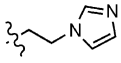
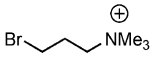
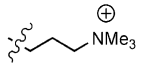
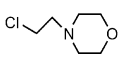
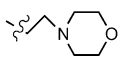
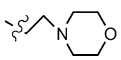


Scheme 1. Reagents and conditions: (a) base, electrophile (1 equiv for **3a**–**m**; 2.5 equiv for **4a**–**g**), DMF; (b) (i) BTPP, Boc_2O, DMF, 10 min; (ii) electrophile (1 equiv), 18 h; (iii) TFA/acetonitrile/water for **5a,b**.

Table 1. Semi-synthetic *O*-alkyl nocathiacin derivatives

Entry	Electrophile	Base	Time	Compd	R_1	R_2	Yield (%)
1	$TMSCHN_2$	none[a]	5 min	**3a**	CH_3	H	34
2		Cs_2CO_3, NaI	24 h	**3b**		H	14
3		Cs_2CO_3, TBAI	5 h	**3c**		H	30
4	$ICH_2P(O)(OEt)_2$	DIEA	24 h	**3d**	$CH_2P(O)(OEt)_2$	H	50
5	$BrCH_2CH_2CH_2NH_2$	BTPP	10 min	**3e**	$CH_2CH_2CH_2NH_2$	H	47
6		BTPP	18 h	**3f**		H	83
7		TEA[b]	17 h	**3g**		H	51
8		BTPP	30 min	**3h**		H	32
9		BTPP	4 h	**3i**		H	69
10	$BrCH_2CH_2COOH$	BTPP	24 h	**3j**	CH_2CH_2COOH	H	20
11	1,3-propanesultone	BTPP	8 h	**3k**	$CH_2CH_2CH_2SO_3H$	H	37
12	$ClCH_2OP(O)(O^tBu)_2$[16]	BTPP	5 h	**3l, m**	$CH_2OP(O)(OR)_2$[d]	H	11[e]
13	Me_2SO_4	Cs_2CO_3, TBAI	3 h	**4a**	CH_3	CH_3	93
14	$ICH_2CH=CH_2$	DIEA[c]	19 h	**4b**	$CH_2CH=CH_2$	$CH_2CH=CH_2$	89
15	$BrCH_2CONMe_2$	Cs_2CO_3	2 h	**4c**	CH_2CONMe_2	CH_2CONMe_2	38
16		TEA[b]	17 h	**4d**			10
17	1,3-propanesultone	BTPP	8 h	**4e**	$CH_2CH_2CH_2SO_3H$	$CH_2CH_2CH_2SO_3H$	50
18	$ClCH_2OP(O)(O^tBu)_2$ [16]	BTPP	5 h	**4f, g**	$CH_2OP(O)(OR)_2$[d]	$CH_2OP(O)(OR)_2$[d]	4[e]
19	MeI	BTPP	12 h	**5a**	H	CH_3	14[f]
20	1,3-propanesultone	BTPP	18 h	**5b**	H	$CH_2CH_2CH_2SO_3H$	30[f]

[a] THF/MeOH used as solvent.
[b] H_2O was used as solvent.
[c] CH_2Cl_2 was used as solvent.
[d] *t*-Butylphosphates **3l** and **4f** (R = ^{t}Bu) were treated in situ with TFA in CH_2Cl_2 to afford phosphates **3m** and **4g** (R = H), respectively.
[e] Yield for two steps.
[f] Yield for three steps.

manipulation of the resulting amide.[11] The presence of several hydroxyl functionalities in nocathiacins presents an alternate opportunity for the introduction of water soluble substituents.[12] Herein, we report the semi-synthesis of bis- and mono-*O*-alkyl nocathiacin derivatives via alkylation of the hydroxypyridine and/or hydroxyindole moieties. The structure–activity relationships for *O*-alkyl nocathiacins are also reported.

In pursuit of our goal of increasing water solubility, we primarily focused on the introduction of substituents with a polar or an ionizable group such as sugar, amine, ammonium salt, carboxylic or sulfonic acid. However, some lipophilic groups (e.g., methyl, allyl), which may help to explore the chemistry and provide useful information on the SAR of nocathiacins, were also introduced.

Semi-synthetic mono- and bis-*O*-alkyl nocathiacin I derivatives are listed in Table 1.[13] Regioselective mono-alkylation of indole *N*-hydroxyl was achieved using one equivalent of electrophile and a variety of inorganic and organic bases (entries 1–6 and 8–11).[14] In general, phosphazene base [*t*-butylimino-tri(pyrrolidino)phosphorane, BTPP] mediated-alkylations in DMF gave better results.[15] Surprisingly, several attempts to synthesize the morpholinoethyl derivative **3g** in organic solvents using a variety of bases and electrophiles were unsuccessful. However, triethylamine-mediated alkylation of nocathiacin I with 1-(2-chloroethyl)morpholine in water proceeded smoothly to afford both the mono-alkyl derivative **3g** and the bis-alkyl derivative **4d** (entries 7 and 16). Reaction of nocathiacin I with excess electrophile resulted in bis-alkylation of both the indole and pyridine hydroxyls (entries 13–15 and 17–18). *t*-Butylphosphates **3l** and **4f**[16] (entries 12 and 18) were treated with 10% TFA in CH_2Cl_2 to afford the free monophosphate **3m** and diphosphate **4m**, respectively (not shown).

In order to obtain mono-alkylated derivatives of the hydroxypyridine moiety, we developed an efficient one pot protection–alkylation–deprotection strategy (entries 19 and 20). The *N*-hydroxy indole in **1** was temporarily protected as the *N*-Boc derivative by treatment with 3 equiv of BTPP and one equiv of Boc_2O in anhydrous DMF for 10 min. In situ addition of electrophile provided alkylation of the hydroxypyridine which can be monitored by LC/MS. Upon completion, the addition of TFA in acetonitrile/water to deprotect the *N*-Boc, furnished the corresponding mono-*O*-alkyl nocathiacin derivatives **5a**,**b**.[17] Interestingly, we observed that timely addition of the electrophile is essential for the desired selectivity. Full protection of the *N*-hydroxyindole with Boc_2O took place in 10 min, as judged by LC/MS. However longer reaction times led to partial migration of the Boc-protecting group to the hydroxypyridine. This resulted, after addition of electrophile, in formation of a mixture of regioisomeric monoalkylated products. Under all the above experimental conditions, alkylation of the remaining alcohols functionalities present in nocathiacin I, threonine hydroxyl and the tertiary hydroxyl group in the sugar moiety was not observed (Scheme 1).

All new analogues were evaluated for in vitro antibacterial activity (MICs) against a panel of Gram-positive organisms (*S. aureus, S. pneumoniae* and *Enterococcus faecalis)*.[18] The in vivo efficacy (PD_{50}) against *staphylococci* was also assessed in mouse infection model.[19] The results are summarized in Table 2.

Table 2. In vitro[18] and in vivo[19] antibacterial activity of bis- and mono-*O*-alkyl nocathiacin derivatives

Compd	MIC (μg/mL)[a]			PD_{50} (mg/kg)[b]
	S. aureus	*S. pneumoniae*	*E. faecalis*	*S. aureus*
Vancomycin	0.2	ND	<0.5	1.1–3.3[c]
1	0.007–0.03	0.002	0.03	0.3–2.8
3a	0.03	0.003	0.125	<0.62
3b	16	0.06	>128	>10
3c	0.125	0.007	0.5	>10
3d	0.015	0.001	0.03	1.6
3e	0.25	0.06	1	>10
3f	1	0.03	1	8.7
3g	0.125–0.25	0.003–0.015	0.06–0.125	3.4
3h	0.5	0.015	1	>10
3i	>128	0.5	2	>10
3j	0.125	0.03	0.25	10
3k	0.125	0.007	0.125	4
3m	0.125	0.015	0.015	3
4a	0.003–0.007	0.0005–0.001	0.015	3.7
4b	0.06	0.0005	0.25	>10
4c	8	0.125	4	>10
4d	1	0.03	0.25	>10
4e	>128	0.5	128	ND
4g	0.125	0.015	0.25	0.15[c]
5a	0.015	0.003	0.015	>10
5b	2	0.25	32	>10

[a] The MIC was defined as the lowest concentration that prevented visible growth.
[b] PD_{50} is defined as the dose of the test compound given which protects 50% of mice from infections (sc).
[c] Administered by intravenous route.

Methyl ether **3a** and diethylphosphonate analogue **3d** showed excellent in vitro and in vivo antibacterial activity while analogues with polar neutral groups such as glycoside **3b** and polyethyleneglycol **3c** displayed much reduced in vivo efficacy. In general, the introduction of basic functionalities, (compounds **3e–h**) resulted in loss of in vitro and in vivo potency. One notable exception was the morpholinoethyl *N*-hydroxyindole derivative **3g**, which displayed good in vitro and in vivo activity. Ammonium derivative **3i** and carboxylic acid **3j** showed no useful activity. However, sulfonic acid **3k** and phosphoric acid **3l** exhibited excellent in vitro potency and in vivo efficacy. Bis-*O*-alkyl-derivatives bearing lipophilic groups **4a–c** maintained good in vitro potency although reduced in vivo efficacy. Other bis-*O*-alkyl analogues **4d–e** were, in general, less potent than the mono-*O*-alkyl derivatives. Mono-*O*-methyl hydroxypyridine analogue **5a** showed comparable in vitro potency to its regioisomer **3a** but lacked in vivo activity. Interestingly, propanesulfonic acid **5b** was inferior to its regioisomer **3k** in both assays.

In general, the introduction of neutral or basic polar moieties improved aqueous solubility at acidic pH (>1 mg/mL), while liphophilic substituents, not surprisingly, diminished it. Carboxylic acid derivative **3j** had a disappointingly low aqueous solubility (~0.07 mg/mL at neutral pH). However, other anionic groups such as sulfate (**3k**, **4e**) and phosphates (**3m**, **4g**) improved the solubility significantly to >2 mg/mL at neutral pH. Furthermore, phosphates **3m** and **4g** exhibited excellent in vivo efficacy, presumably due to the in vivo generation of the parent natural product, nocathiacin I. It is known that hydrolysis of the phosphonooxymethylether moiety by alkaline phosphatase present in animal tissues followed by spontaneous chemical degradation of the resulting hemi-acetal intermediate can generate the parent hydroxy compound.[20] Therefore, phosphates **3m** and **4g** are potential pro-drugs of nocathiathin I.

In summary, mono-*O*-alkyl nocathiacins exhibited in vitro activity superior to vancomycin and comparable to nocathiacin I. Some of the *N*-hydroxyindole alkyl derivatives had useful in vivo efficacy, although they were not as good as the parent natural product. Introduction of substituents to the hydroxypyridine moiety, in both mono- and bis-*O*-alkyl derivatives, resulted in diminished in vitro activity and lack of in vivo efficacy in most of cases. The excellent in vivo efficacy and improved aqueous solubility of compounds **3m** and **4g** make them suitable candidates for pro-drugs.

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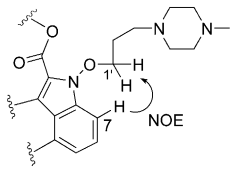
References and notes

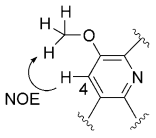
1. Sefton, A. M. *Drugs* **2002**, *62*, 557.
2. Bush, K.; Macielag, M.; Clancy, J. *Emerg. Drugs* **2000**, *5*, 347, and references therein.
3. (a) Leet, J. E.; Li, W.; Ax, H. A.; Matson, J. A.; Huang, S.; Huang, R.; Cantone, J. L.; Drexler, D.; Dalterio, R. A.; Lam, K. S. *J. Antibiot.* **2003**, *56*, 232. (b) Constantine, K. L.; Mueller, L.; Huang, S.; Abid, S.; Lam, K. S.; Li, W.; Leet, J. E. *J. Am. Chem. Soc.* **2002**, *124*, 7284.
4. Li, W.; Leet, J. E.; Ax, H. A.; Gustavson, D. R.; Brown, D. M.; Turner, L.; Brown, K.; Clark, J.; Yang, H.; Fung-Tomc, J.; Lam, K. S. *J. Antibiot.* **2003**, *56*, 226.
5. Sasaki, T.; Otani, T.; Matsumoto, H.; Hamada, M.; Takeuchi, T.; Hori, M. *J. Antibiot.* **1998**, *51*, 715.
6. (a) Porse, B. T.; Leviev, I.; Mankin, A. S.; Garrett, R. A. *J. Mol. Biol.* **1998**, *276*, 391. (b) Rodnina, M. V.; Savelsbergh, A.; Matassova, N. B.; Katunin, V. I.; Semenkov, Y. P.; Wintermeyer, W. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 9586.
7. Steinberg, D. A.; Bernan, V. S.; Montenegro, D. A.; Abbanat, D. R.; Pearce, C. J.; Korshalla, J. D.; Jacobus, N. V.; Petersen, P. J.; Mroczenski-Wildey, M. J.; Maiese, W. M.; Greenstein, M. *J. Anibiot.* **1994**, *47*, 887.
8. Benazet, F.; Cartier, M.; Florent, J.; Godard, C.; Jung, G.; Lunel, J.; Mancy, D.; Pascal, C.; Renaut, J.; Tarridec, P.; Tissier, R.; Dubost, M.; Ninet, L. *Experentia* **1980**, *36*, 414.
9. Naidu, B. N.; Li, W.; Sorenson, M. E.; Connolly, T. P.; Witchtowski, J. A.; Zhang, Y.; Kim, O. K.; Matiskella, J. D.; Lam, K. S.; Bronson, J. J.; Ueda, Y., submitted for publication.
10. Regueiro-Ren, A.; Ueda, Y. *J. Org. Chem.* **2002**, *67*, 8699.
11. Hrnciar, P.; Ueda, Y.; Huang, S.; Leet, J. E.; Bronson, J. J. *J. Org. Chem.* **2002**, *67*, 8789.
12. Nocathiacin I (**1**) contains four hydroxyl groups in its structure: *N*-hydroxyindole, hydroxypyridine, a secondary alcohol, belonging to the threonine aminoacid moiety and a tertiary alcohol in the sugar moiety.
13. The identity and purity of all new compounds were established by ^{1}H NMR and HRMS. For instance, analytical data for **3f**: ^{1}H NMR (DMSO, 500 MHz) δ 10.8 (1H, s), 10.3 (1H, s), 9.21 (1H, s), 8.60 (2H, m), 8.54 (1H s), 8.53 (1H, s), 8.22 (1H, s), 8.11 (2H, s), 7.87 (1H, s), 7.77 (1H, d, J = 10.7 Hz), 7.64 (1H, s), 7.58 (1H, d, J = 8.5 Hz), 7.45 (2H, dd, J = 8.3, 7.3 Hz), 7.30 (1H, d, J = 7.15 Hz), 7.14 (1H, d, J = 7.15 Hz), 6.38 (1H, s), 6.03 (1H, d, J = 12.5 Hz), 5.88 (1H, m), 5.76 (1H, s), 5.72 (2H, m), 5.38 (1H, m), 5.15 (m, 1H), 5.07 (2H, m), 4.83 (1H, d, J = 10.5 Hz), 4.40 (1H, d, J = 11.5 Hz), 4.12 (2H, m), 3.92 (3H, s), 3.90 (3H, m), 3.86 (2H, m), 3.42 (1H, m), 2.87 (6H, br), 2.73 (2H, br), 2.54 (2H, s), 2.51 (2H, s), 2.41 (1H, m), 2.12 (1H, m), 2.02 (3H, s), 1.95 (1H, d, J = 14.5 Hz), 1.84 (1H, m), 1.58 (3H, br), 1.25 (1H, m), 1.14 (3H, br), 0.78 (3H, d, J = 6.9 Hz); HRMS (ES) calcd for $C_{69}H_{76}N_{16}O_{18}S_5$ $[M+H]^+$: 1577.421; found 1577.421.
14. For example, NOE between proton H-7 in the hydroxyindole moiety and protons H-1′ in the side chain of compound **3f** were observed.



15. In a typical experiment, nocathiacin I (**1**) (236 mg, 0.16 mmol) in 2 mL of *N*,*N*-dimethylformamide was treated with BTPP (0.19 mL, 0.64 mmol) and 1-(3-chloropropyl)-4-methylpiperazine dichloride (40 mg, 0.16 mmol) and the mixture was stirred at room temperature for 18 h. The

reaction was quenched with 0.5 mL of 1N HCl, and chromatographed in a reverse-phase medium pressure column to afford **3f** (208 mg, 83%) as a pale yellow solid.

16. The preparation of bis-*t*-butyl chloromethyl phosphate [$ClCH_2OP(O)(OtBu)_2$] is described in: Ueda, Y.; Matiskella, J. D.; Golik, J.; Hudyma, T. W.; Chen, C.-P. U.S. Patent, US 6,362,172, March 26, 2002; *Chem. Abstr*. **2001**, *135*, 122628.
17. For instance, NOE between the newly introduced methyl group in **5a** and H-4 in the hydroxypyridine moiety was observed.



18. The minimum inhibitory concentrations (MICs) were obtained using the conventional broth microdilution assay (serial dilution method) in accordance with standards recommended by the NCCLS.
19. The animals were infected intraperitonially (IP) with 6.5×10^6 CFU of *S. aureus* A15090. The test compound was administered subcutaneously (sc) at 1 and 4 h post-infection.
20. Golik, J.; Wong, H. S. L.; Chen, S. H.; Doyle, T. W.; Wright, J. J. W.; Knipe, J.; Rose, W. C.; Casazza, A. M.; Vyas, D. M. *Bioorg. Med. Chem. Lett*. **1996**, *6*, 1837.