Chemical Conversion of Nocathiacin I to Nocathiacin II and a Lactone Analogue of Glycothiohexide α

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Nocathiacin I (**1**) was converted to its deoxy indole analogue, nocathiacin II (**2**), another natural product, by a unique and facile chemical process. This process was applied to nocathiacin IV (**4**), generating the lactone analogue of glycothiohexide α (**5**), which was also prepared from nocathiacin II by a mild hydrolytic process. In contrast to glycothiohexide α (**3**), this lactone analogue (**5**) was found to exhibit in vivo antibacterial efficacy in an animal (mouse) infection model.

Multidrug-resistant strains of clinically significant pathogenic Gram-positive bacteria, such as methicillin-resistant *Staphylococcus aureus* (MRSA), penicillin-resistant *Streptococcus pneumonia* (PRSP), and vancomycin-resistant enterococci (VRE), are becoming increasingly more prevalent in the United States and throughout the world, creating a serious worldwide health problem.[1] Current treatment options are very limited, and there is an urgent need for discovery and development of a new class of antibacterial agents effective against multidrug-resistant Gram-positive bacteria.[2]

Recently, we reported the discovery of nocathiacin I (**1**) and nocathiacin II (**2**), new thiazolyl peptide antibiotics from *Nocardia* sp. fermentations.[3] Concurrently, nocathiacin I (MJ347-81F4 A) (**1**) was also isolated by Otani et al. from *Amycolatopsis* sp. fermentations.[4] The nocathiacins belong to an indole-containing tricyclic group of the thiazolyl peptide class of antibiotics. Other indole-containing tricyclic thiazolyl peptide antibiotics reported are glycothiohexide α (**3**)[5] and S-54832/A-I (**6**).[6] These are structurally related to the bicyclic nosiheptide (**7**),[7] a well-known animal growth promoter. Nocathiacin II (**2**) and glycothiohexide α (**3**) do not contain a hydroxy group at the indolenitrogen, and glycothiohexide α (**3**) lacks also the dehydroalanine side-chain moiety. A major structural difference in the core ring system between the nocathiacins and glycothiohexide α is that nocathiacins have a lactone linkage, whereas glycothiohexide α has a thio-lactone linkage. All of these thiazolyl peptide antibiotics possess potent in vitro antibacterial activity against Gram-positive bacteria, including multidrug-resistant strains.

We also reported[8] recently a mild chemical conversion of nocathiacin I (**1**) to nocathiacin IV (**4**), an analogue lacking the dehydroalanine side-chain moiety. Nocathiacin IV (**4**) proved to be a useful intermediate for the synthesis of nocathiacin analogues having a water-solubilizing moiety.[9] Nocathiacin II (**2**), the deoxy indole analogue, is another potentially useful intermediate for the analogue synthesis. Herein, we report an efficient chemical transformation of **1** to **2**, as well as the chemical conversion of **1** to **5**,[10] the lactone analogue of glycothiohexide α (**3**) via **2** or **4**.

Results and Discussion

The deoxy indole analogue, nocathicin II (**2**), was isolated from the fermentation broth as a minor component, and the yield was too low to be useful as a key intermediate for analogue synthesis, although the free indole-NH of **2** offered a potential site for derivatization. An efficient chemical conversion of nocathiacin I (**1**) to **2** was deemed necessary to generate deoxy indole analogues of **2**. Several methods were attempted to convert **1** to **2** by a conventional reductive process without much success. For example, application of common reducing agents, such as alkyl phosphites or DMSO, resulted in substantial decomposition of the molecule with little or no formation of **2**. Catalytic hydrogenation (H₂/Pd−C in THF−EtOH) reduced the dehydroalanine moiety, but not the hydroxyindole moiety. However, we discovered a facile and highly effective process from **1** to **2**, while we were investigating the introduction of an ionizable pyruvate at the *N*-hydroxyindole in an effort to improve its aqueous solubility.[11] When **1** in DMF was treated with ethyl bromopyruvate in the presence of Hunig's base (diisopropylethylamine), phosphazene base, or cesium carbonate in DMF at room temperature, the reaction provided nocathiacin II (**2**) in good yield (54%), and no expected pyruvate derivative was isolated. The product isolated from this reaction showed a loss of 16 Da in the MS spectrum and was identical to the natural **2** by HPLC, LC/MS, MS/MS, and ¹H/¹³C NMR analyses. Under these conditions, **1** was deoxygenated cleanly to give **2**. Although the formation of deoxy indoles was reported by treatment of *N*-hydroxyindoles with haloacetate in the presence of base,[12] this dehydroxylation process by the use of α-halopyruvate was much more facile and complete than the reaction with haloacetate. When the above deoxygenation conditions were applied to **4**,[8] the reaction produced, as expected, deoxygenated nocathiacin V (**5**),[10] which is the lactone analogue of glycothiohexide α (**3**). Compound **5** was also prepared from **2** by cleavage of the dehydroalanine moiety (HI−MeI in THF at 45 °C).[8] The structure of **5** was fully supported by spectroscopic analysis (¹H NMR, ¹³C NMR, HRMS, and CD). A close examination of the ¹³C NMR spectrum obtained from **5** and the ¹³C NMR chemical shifts reported for glycothiohexide-α (**3**) revealed the presence of a serine residue in **5** in place of the cysteine residue in **3**. Accordingly, a downfield methylene carbon signal at δ 62.5 (Ser-C3) in **5** was observed, compared to a methylene

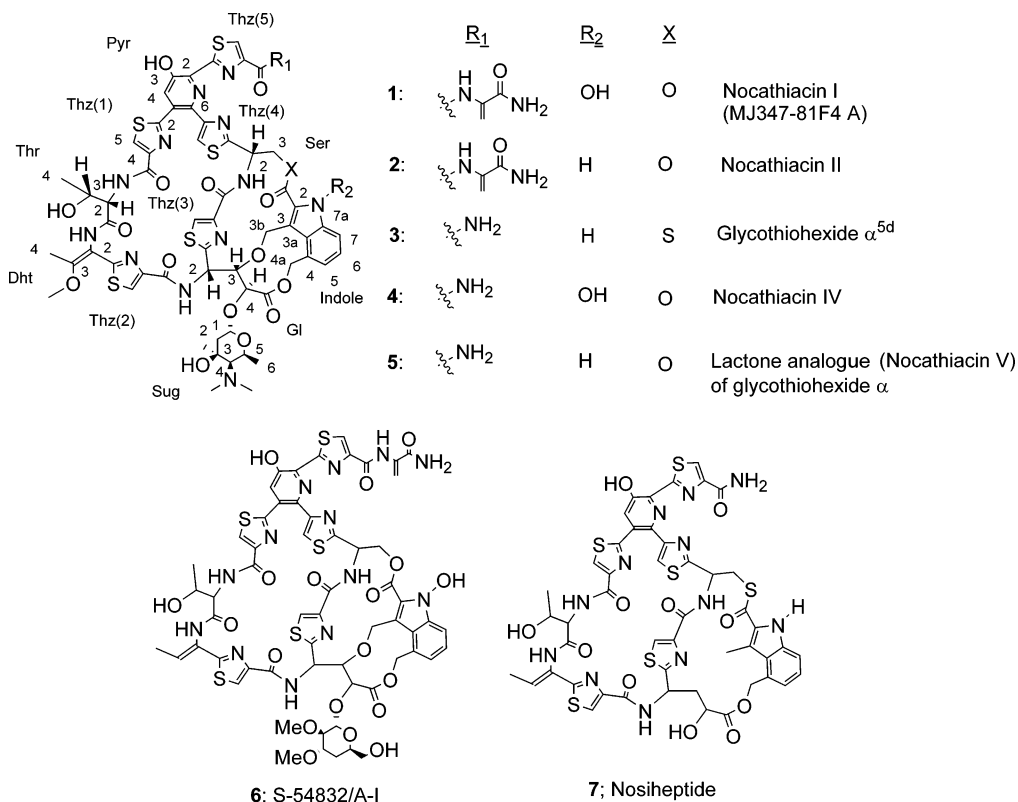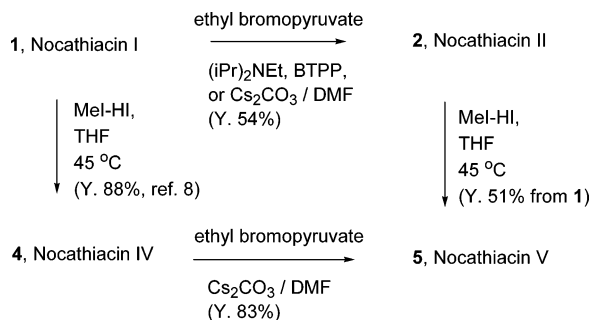* To whom correspondence should be addressed. Tel: 203-677-6308. Fax: 203-677-7702. E-mail: yasutsugu.ueda@bms.com.

Figure 1.

Scheme 1

carbon resonance at δ 30.1 (Cys-C3) in **3**. In addition, a lactone carbonyl resonance at δ 161.7 (indole-COO−) was observed in **5**, in contrast to the thiolactone carbonyl resonance at δ 183.7 (indole-COS−) reported for **3**. The chemical conversion to nocathiacin V (**5**) from **1** via **2** and **4** is outlined in Scheme 1.

Like nocathiacin IV (**4**), nocathiacin II (**2**) and nocathiacin V (**5**), prepared from nocathiacin I (**1**), are potentially useful intermediates for the synthesis of other nocathiacin analogues.

The in vitro and in vivo potency of these nocathiacin derivatives was evaluated. The results are shown in Table 1. The in vitro antibacterial activity against selected bacteria and in vivo efficacy in a *Staphylococcus aureus* infection model in mice for these antibiotics are expressed as MIC (minimum inhibitory concentration) and PD$_{50}$ (50% protective dose),[13] respectively. *Staphylococcus aureus* (A15090), *Streptococcus pneumoniae* (A28272), and *Enterococcus faecalis* (A20688) were selected as representative Gram-positive organisms. As reported previously,[3,4] **1**, **2**, and **4** exhibited highly potent in vitro antibacterial activity against Gram-positive bacteria, MICs being 0.001−0.15 μg/mL. They also showed potent in vivo efficacy in a systemic infection model in mice, having a PD$_{50}$ of ca. 1 mg/kg.

Nocathiacin V (**5**) was equally potent, displaying an MIC of 0.03−0.125 μg/mL and a PD$_{50}$ of 4 mg/kg. In contrast to other thiazolyl peptides, such as micrococcin and nosiheptide, these compounds not only exhibited potent in vitro antibacterial potency against Gram-positive bacteria but also displayed potent in vivo efficacy in a *S. aureus* infection model in mice. A further interesting and important finding is that **5**, the lactone analogue of **3**, exhibits in vivo efficacy in the animal model, whereas **3** is reported to be devoid of in vivo efficacy.[5] Nosiheptide, another thiazolyl peptide antibiotic which contains a thiolactone moiety, is also reported to be devoid of in vivo efficacy,[7b] implying that the thiolactone moiety might be responsible for the lack of in vivo efficacy. Consistent with this view, the other indole-containing tricyclic thiazolyl peptide, S-54832/A-I, which has the lactone linkage just like the nocathiacins, is reported to exhibit in vivo efficacy in an animal model.[6]

In summary, we developed a unique and facile chemical method to convert *N*-hydroxylated nocathiacin I (**1**) to *N*-deoxy nocathiacin II (**2**). Application of this chemistry to nocathiacin IV (**4**) produced nocathiacin V (**5**), the lactone analogue of glycothiohexide α (**3**), which was also prepared from **2** by a mild cleavage process for dehydroalanine units. In contrast to **3**, this lactone analogue, nocathiacin V (**5**), exhibited potent in vivo efficacy in an animal infection model.

Experimental Section

General Experimental Procedures. Solvents and other reagents were used as purchased without further purification. Reactions were monitored by LC/MS. Analytical HPLC was run with a reversed-phase column (YMC Pro-C18, 4.6 × 50 mm, 5 μm) using a 10−90% gradient elution of MeOH−H$_2$O containing 0.2% H$_3$PO$_4$, or CH$_3$CN−H$_2$O containing 0.2% TFA, as a mobile phase, unless otherwise specified. Detection by UV was at 220 nm. Compounds were purified by preparative

Table 1. In Vitro and in Vivo Antibacterial Potency of Nocathiacins I, II, IV, and V[a]

	MIC (μg/mL)			
compound	*Staph. aureus* A15090 (μg/mL)	*Strep. pneumo* A28272 (μg/mL)	*E. faecalis* A20688 (μg/mL)	PD_{50} (mg/kg) (*S. aureus*, mice, sc)
nocathiacin I	0.007−0.03	0.002	0.03	0.31−2.8
nocathiacin II	0.007	0.0005	0.15	<0.62
nocathiacin IV	0.003−0.03	0.001−0.003	0.007−0.03	1.07−1.47
nocathiacin V	0.06	0.03	0.125	4.35

[a] In vitro and in vivo potency were expressed in MICs and PD_{50}s. MIC: minimum inhibitory concentration, *Staph. aureus* or *S. aureus*: *Staphylococcus aureus*, *Strep. pneumo*: *Streptococcus pneumoniae*, PD_{50}: 50% protective dose,[13] sc: subcutaneous.

reversed-phase HPLC using a 30−100% gradient elution of MeOH−H_2O containing 0.1% TFA as mobile phase, unless otherwise specified.

Preparation of Nocathiacin II (2). To a solution of nocathiacin I (**1**,[3] 5.00 g, 3.5 mmol) in DMF (35 mL) was added a solution of phosphazene base, BTPP (*tert*-butyliminotri-(pyrrolidino)phosphorane; 2.19 g, 7.0 mmol) in DMF (15 mL), and the mixture was stirred at room temperature for 5 min. To this mixture was added ethyl bromopyruvate (1.32 mL, 10.5 mmol), and the mixture stirred at room temperature for 30 min, by which time HPLC indicated the reaction was nearly complete. The volatile materials were removed in vacuo to give a deep orange syrup, which was triturated with anhydrous diethyl ether. The resultant precipitates were collected to obtain crude **2** (7.02 g) as a yellow powder, which was purified by column chromatography (silica gel, 5−30% A in CH_2Cl_2; A = 10% H_2O in MeOH) to obtain 3.02 g (2.13 mmol, yield 61%; HPLC purity 89%) of **2** as a yellow powder. The purer fractions were used for collecting spectroscopic data. These data (^{1}H NMR, ^{13}C NMR, HPLC, LC/UV/MS) were identical within experimental error to those of natural nocathiacin II (**2**): HPLC (retention time) 9.52 min (X-Terra C-18 column, 5 μm, 4.6 × 150 mm, 0.01% TFA-CH_3CN−H_2O gradient, at 254 nm; natural nocathiacin II showed a peak at 9.49 min).

Nocathiacin II (**2**) was also prepared by use of Hunig's base or Cs_2CO_3 instead of phosphazene base (BTPP).

Nocathiacin I (**1**, 0.5 g, 0.35 mmol) in DMF (5 mL) was treated with Hunig's base (0.12 mL, 0.7 mmol) or Cs_2CO_3 (225 mg, 0.7 mmol) and ethyl bromopyruvate (0.15 mL, 1.27 mmol) at room temperature for 3 h. LC/MS indicated the reaction was complete. Ethyl ether was added, and the yellow solid precipitate formed was collected by filtration. The crude material was taken to the next step without further purification.

Preparation of Nocathiacin V (5). (A) From Nocathiacin II (2). The crude material containing **2**, obtained from **1** (0.5 g, 0.35 mmol), was suspended in THF (5 mL) and heated at 45 °C in a sealed tube in the presence of HI (57% in H_2O, 0.10 mL, 0.7 mmol) and iodomethane (0.22 mL, 3.5 mmol). After 2 h, LC/MS indicated the reaction was complete. The mixture was cooled to room temperature, and ethyl ether was added. The precipitate formed was collected by filtration and subjected to Sephadex LH-20 (100 g) column chromatography using 1:1 $CHCl_3$−MeOH as eluent. Fractions measuring 8−10 mL each were collected at a flow rate of 2−3 mL/min. Fractions were consolidated on the basis of silica gel TLC profiles ($CHCl_3$−MeOH−H_2O, 85:15:1.5 v/v/v; long-wavelength UV and/or ceric sulfate spray for detection) to afford **5** (375 mg, yield 79% from **1**, HPLC purity 64%) as a pale yellow solid. Further purification was achieved by preparative HPLC using a YMC ODS-AQ C18 column (5 μm particle size, 120 Å pore size, 20 mm × 150 mm). Elution was begun with 0.1 M ammonium acetate−CH_3CN, 75:25 v/v, with a 20 min linear gradient to 0.1 M ammonium acetate−CH_3CN, 50:50 v/v. Elution flow rate was 20 mL/min. Detection (UV) was at 254 nm. A typical sample injection size was 20−40 mg/200−400 μL DMSO. In this manner, HPLC purification of 105 mg of enriched product yielded 23 mg of highly purified product suitable for spectroscopic analyses. Compound **5**: CD (MeOH) λ nm ($\Delta\epsilon$) 210 (+32.3), 237 (−48.0), 265 (+17.2), 306 (−6.2), 327 (+3.4), 370 (+5.5); UV (MeOH) λ_{max} (log ϵ) 216 (5.07), 297 (4.70), 366 (4.41) nm; IR (KBr) ν_{max} 3423, 1726, 1656, 1534,

1473, 1249, 1192, 1087, 1012, 752 cm^{-1}; ^{1}H NMR (DMSO-d_6, 500 MHz) δ 11.34 (1H, s br, indole-NH), 9.10 (1H, s, Dht-NH), 8.64 (1H, s, Thz(1)-H5), 8.59 (1H, d, J = 8.5 Hz, Glu-NH), 8.49 (1H, s, Thz(3)-H5), 8.41 (1H, s, Thz(5)-H5), 8.22 (1H, s, Thz-(2)-H5), 7.96 (1H, s br, Pyr-C4), 7.79 (1H, s br, Thz(4)-H5), 7.69 (1H, m, Ser-NH), 7.67 (2H, s br, Thz(5)-CONH$_2$), 7.57 (1H, d, J = 8.3 Hz, indole-H7), 7.25 (1H, t, J = 7.6 Hz, indole-H6), 7.11 (1H, d, J = 7.0 Hz, indole-H5), 7.05 (1H, d, J = 8.0 Hz, Thr-NH), 5.75 (1H, dd, J = 11.0, 3.3 Hz, Ser-H2), 6.01 (1H, d, J = 12.2 Hz, indole-H4a), 5.66 (1H, d, J = 8.6 Hz, Glu-H2), 5.22 (1H, m, Ser-H3), 4.99 (1H, d, J = 12.5 Hz, indole-H4a), 4.92 (1H, m, Sug-H1), 4.92 (1H, m, indole-H3b), 4.88 (1H, m, Thr-3-OH), 4.65 (1H, d, J = 11.1 Hz, Ser-H3), 4.34 (1H, d, J = 9.6 Hz, Glu-H4), 4.21 (1H, m, Thr-H2), 4.09 (1H, d, J = 10 Hz, indole-H3b), 4.01 (1H, dd, J = 9.6, 1.5 Hz, Glu-H3), 3.88 (3H, s, Dht-3-OMe), 3.72 (1H, m, Sug-H5), 2.47 (6H, s, Sug-4-NMe$_2$), 2.10 (1H, m, Thr-H3), 1.99 (1H, m, Sug-H4), 1.98 (3H, s, Dht-H4), 1.94 (1H, m, Sug-H2), 1.76 (1H, d, J = 14.1 Hz, Sug-H2), 1.38 (3H, s, Sug-3-Me), 1.05 (3H, s br, Thr-H4), 0.51 (3H, d, J = 6.5 Hz, Sug-H6); ^{13}C NMR (DMSO-d_6, 127 MHz) δ 172.0 (C, Glu-CO), 168.1 (C, Thr-CO), 167.7 (C, Thz(4)-C2), 167.4 (C, Thz(3)-C2), 167.2 (C, Thz(5)-C2), 164.0 (C, Thz(1)-C2), 163.0 (C, Thz(2)-C2), 161.7 (C, indole-CO), 161.4 (C, Dht-C3), 161.0 (C, Thz(3)-CO), 160.6 (C, Thz(2)-CO), 158.6 (C, Thz(5)-CO), 158.2 (C, Thz(1)-CO), 154.2 (C, Thz(4)-C4), 151.3 (C, Pyr-C3), 150.9 (C, Thz(5)-C4), 150.0 (C, Thz(1)-C4), 148.9 (C, Thz(3)-C4), 145.9 (C, Thz(2)-C4), 142.4 (C, Pyr-C6), 136.8 (C, indole-C7a), 134.7 (C, Pyr-C2), 130.0 (C, Pyr-C5), 127.9 (C, indole-C4), 127.3 (C, Pyr-C4), 126.7 (CH, Thz(5)-C5), 126.6 (C, indole-C2), 126.3 (CH, Thz(1)-C5), 125.6 (CH, Thz(3)-C5), 125.3 (CH, Thz(2)-C5), 124.2 (C, indole-C3a), 123.7 (CH, indole-C6), 122.8 (CH, indole-C5), 119.9 (CH, Thz(4)-C5), 116.2 (C, indole-C3), 115.9 (CH, indole-C7), 109.8 (C, Dht-C2), 95.1 (CH, Sug-C1), 79.0 (C, Glu-C3), 70.7 (CH, Glu-C4), 68.4 (CH, Sug-C4), 67.6 (CH$_2$, indole-C4a), 67.6 (CH, Sug-C3), 66.3 (CH, Sug-C5), 65.8 (CH, Thr-C3), 64.0 (CH$_2$, indole-C3b), 62.5 (CH$_2$, Ser-C3), 56.2 (CH$_3$, Dht-OMe), 55.1 (CH, Thr-C2), 51.1 (CH, Ser-C2), 50.6 (CH, Glu-C2), 44.4 (CH$_3$, Sug-C4−NMe$_2$), 40.3 (CH$_2$, Sug-C2), 30.6 (CH$_3$, Sug-C3-Me), 17.9 (CH$_3$, Thr-C4), 17.9 (CH$_3$, Sug-C6), 13.0 (CH$_3$, Dht-C4); HRESIMS [M + H]$^+$ m/z 1352.2714 (calcd for $C_{58}H_{58}N_{13}O_{16}S_5$, 1352.2728).

(B) From Nocathiacin IV (4). To a solution of **4**[8] (1.55 g, 1.13 mmol) and Cs_2CO_3 (0.525 g, 1.58 mmol) in DMF (7.7 mL) was added ethyl bromopyruvate (0.48 mL, 3.84 mmol), and the mixture was stirred at room temperature for 1 h, by which time HPLC indicated the presence of the starting material. An additional 0.22 mL (1.7 mmol) of ethyl bromopyruvate was added and the reaction stirred for an additional hour, by which time LC/MS indicated the reaction was complete. The DMF was removed under reduced pressure, and the residue was dissolved in a minimal amount of MeOH−CH_2Cl_2. Diethyl ether was added, and the resulting precipitates were collected by vacuum filtration and air-dried to yield 0.100 g (0.074 mmol, yield 6.5%; HPLC purity 91%) of nocathiacin V (**5**) as an off-white powder. A second trituration of the filtrate gave 1.374 g (1.02 mmol, yield 90%; HPLC purity 86%) of nocathiacin V (**5**) as an off-white powder.

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