Bioorganic & Medicinal Chemistry Letters 18 (2008) 4051–4053

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

N-Demethylation of nocathiacin I via photo-oxidation

Wenying Li *, Stella Huang, Xiaohong Liu, John E. Leet, Joseph L. Cantone, Kin S. Lam †

Bristol-Myers Squibb Research and Development, 5 Research Parkway, Wallingford, CT 06492, USA

ARTICLE INFO

Article history:
Received 25 April 2008
Revised 28 May 2008
Accepted 29 May 2008
Available online 4 June 2008

Keywords:
Antibacterial
Nocathiacin
Photo reaction
Oxidation
Demethylation

ABSTRACT

In order to improve aqueous solubility of nocathiacin I (**1**), a potent antibacterial agent, N-demethylation of the amino-sugar moiety was sought. Irradiation of **1** in DMF/CH_2Cl_2 with UV light of 380 nm led to a cyclic product **2**, which was hydrolyzed to yield the desired nocathiacin VI (**3**). Treatment of **1** with shorter UV light caused trans–cis isomerization of a c–c double bond.

Nocathiacin I (**1**, Fig. 1) is a new antibacterial agent produced by *Nocardia* sp. ATCC 202099, and belongs to the family of cyclic thiazolyl peptide antibiotics.[1] In addition to its in vitro nanomolar potency against a broad spectrum of Gram-positive bacteria, including MRSA, MREF, and PRSP, nocathiacin I (**1**) demonstrated excellent in vivo efficacy in a systemic *Staphylococcus aureus* infection mouse model. Due to low aqueous solubility of **1**, a critical goal for development of this chemotype lies in the preparation of aqueous soluble analogs that maintain potent antibacterial activities. One of the approaches was to introduce a water solubilizing group into the molecule through enzymatic and chemical transformation. Nocathiacin III (**4**), the aglycone of **1**, had the same level of the antibiotic activity as **1**, suggesting that modification on the amino-sugar moiety might be tolerated for the activity. Therefore we aimed to generate nocathiacin IV (**3**), which would offer a secondary amine as a new modification site on the sugar for introducing water solubilizing groups.

From a culture *Amycolatopsis* sp., Sasaki and coworkers isolated **1** and **3**, as MJ347-81F4-A and MJ347-81F4-B, respectively, but no spectral data were reported.[2] The biological activity of MJ347-81F4-B was not reported. Although we were able to isolate an ample amount of **3** in a culture of *Nocardia* sp. ATCC 202099, the production of **3** was not reproducible. Therefore, we investigated semi-synthesis of **3** from **1**.

Enzymatic N-demethylation of **1** was attempted using whole cell biotransformation techniques. About seventy bacteria and fungi strains were screened for their ability to catalyze N-demeth-

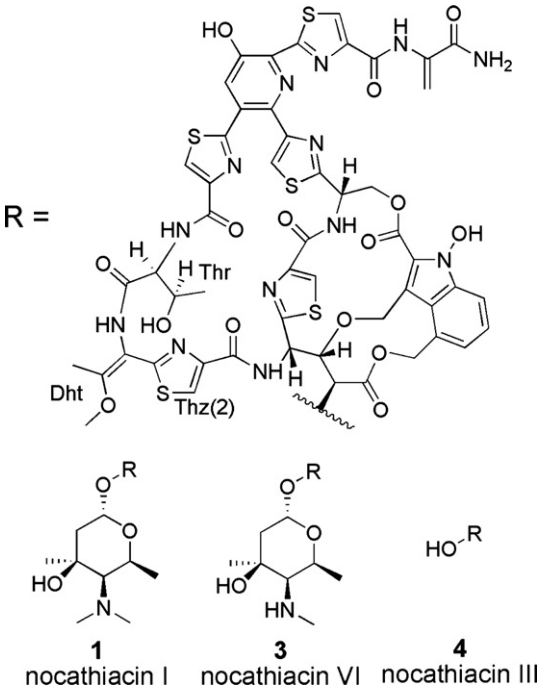


Figure 1. Nocathiacins I, III, and V.

* Corresponding author at present address: Bristol-Myers Squibb Research and Development, Route 206 & Province Line Road, Princeton, NJ 08540, USA. Tel.: +1 609 252 5792.
E-mail address: wenying.li@bms.com (W. Li).
† Present address: Nereus Pharmaceuticals, Inc., 10480 Wateridge Circle, San Diego, CA 92121, USA.

0960-894X/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2008.05.112

ylation of **1**. Although many of the strains had known oxidation (including demethylation) activities,[3] none of them was able to convert **1** to **3**.

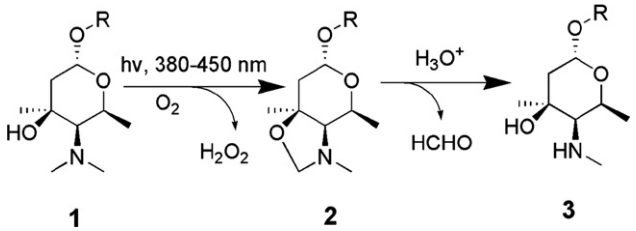
Nocathiacin I (**1**) contains multiple nucleophilic groups, hindering the use of common N-demethylation agents such as chloroformates.[4] In addition, **1** is liable to acidic and basic hydrolysis (resulting in loss of the hydroxyl indole moiety), which precludes methods requiring strong acidic or basic conditions. On the other hand, a photo N-demethylation method that has been previously described for several complex molecules[5] presented an alternative route to **3**.

Compound **1** has strong UV absorption between 200 and 400 nm and the longest UV maximum is at 360 nm. We expected that the UV chromophore in **1** could act as photosensitizer for the reaction, therefore no external photosensitizing catalyst was added to the system. A high pressure Hg lamp (450 W), equipped with a 350–450 nm dichroic mirror was used as light source. The photo reaction of **1** was run in $CHCl_3$/MeOH (3/2, v/v) at a concentration of 2 mg/ml for 13 min at room temperature and in open air. HPLC analysis of the reaction mixture revealed four major peaks: peak A (compound **5**,[6] MW 1436), peak B (MW 1434), peak C (starting material, 1, MW 1436), and peak D (compound **2**,[7] MW 1434), at ratios of 20:19:32:28. The mixture was worked up and subjected to semi-preparative HPLC, using a 1 mM aq HCl/CH_3CN mobile phase with a C-18 stationary phase.

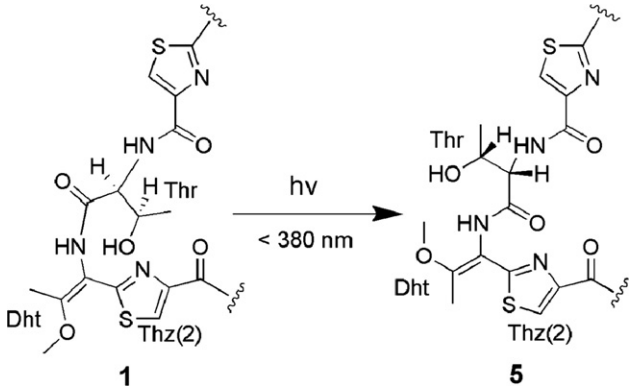
HRMS analysis of peak D (**2**) showed that it lost two hydrogen atoms (compared to **1**). The LC/MS/MS fragmentation pattern indicated that the change occurred at the sugar moiety. However, a new product with a molecular weight of 1422 was detected in the purified fraction of **2**. The NMR spectra of this new product clearly showed loss of one *N*-methyl group, indicating the desired N-demethylation product **3**[8] (Scheme 1). Compound **3** exhibited excellent in vitro and in vivo activity, comparable to **1**.

Peak A (**5**) had the same molecular formula and same MS/MS fragmentation pattern as **1**. While carbon–carbon and carbon–proton connectivities remained the same as in **1** determined by HMQC and HMBC data, some changes in proton chemical shifts were observed. A proton–proton NOE between Thr-OH and Dht-OMe in **5** was observed, while not present in **1**,[9] suggesting isomerization of the Dht double bond (Scheme 2). The trans–cis isomerization led to substantial loss of potency (compound **5** was 10- to 30-fold less potent than **1** in in vitro antimicrobial assay), possibly due to a 3D-configuration change.[10] No further characterization of peak B was carried out, as we speculated that it was formed from **5**, by the same transformation mechanism as observed from **1** to **2**.

To minimize the photoisomerization of the Dht double bond, a long pass glass filter (cut-on: 385 nm) was added to the light source to remove short UV light. In CH_2Cl_2/DMF (4/1), the photo reaction gave **2** as only major product (64% yield). NMR analysis on the crude reaction mixture indicated the cyclized amino-sugar moiety, as evidenced by a new methylene pair (δ 3.98, 4.36, J = 3.1 Hz) showing HMQC correlations to methylene carbon δ 87.4, in addition to one *N*-methyl resonance, δ 2.23.



Scheme 1. N-Demethylation of nocathiacin I (**1**) via photo-oxidation.

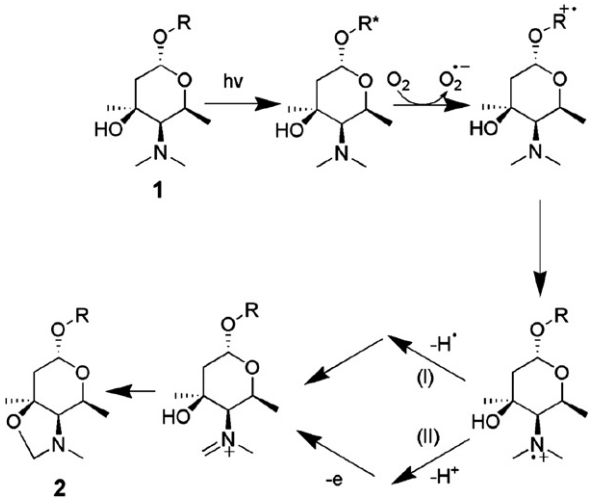


Scheme 2. Photoisomerization of nocathiacin I (**1**).

After purification by preparative HPLC, **2** was cleanly converted to **3** by maintaining the pooled fractions (in 1 mM aq HCl/CH_3CN, ~20/80) at 70–80 °C for 3–4 h (70% conversion in first hour). Under these hydrolytic conditions, other parts of molecule were not affected. Compound **3** was obtained as the HCl salt upon removal of solvents.[11]

The low solubility of 1 in many organic solvents limited the choice of solvents for the reaction. We found CH_2Cl_2/DMF (4/1) gave the best result. Generally, less polar solvents (benzene, CH_2Cl_2, $CHCl_3$) gave a cleaner photo-oxidation. UV irradiation at 350–450 nm in polar solvents such as CH_3CN, MeOH, EtOAc, and DMF favored isomerization and other side reactions.

When the reaction mixture was purged with argon, the photo-oxidation rate was reduced significantly, indicating requirement of molecular oxygen for the reaction. At the end of reaction, peroxides were detected in the mixture using a peroxide test strip. To account for these findings, a plausible photo-oxidation pathway was proposed (Scheme 3). Acting as a photosensitizer, the pyridine-thiazolyl hyper-conjugating ring system in **1** was activated by UV and quenched by molecular oxygen, resulting in a radical cation and a superoxide radical anion,[12] which could be further transformed into H_2O_2 or $HO_2\cdot$.[13] An electron was then transferred from the amine to the radical cation ring system inter- or intramolecularly, yielding an amine radical cation,[14] which could lead to an iminium ion via pathways I or II. The neighboring hydroxyl group, which is cis to the nitrogen, facilitated the nucleophilic adduction to the iminium ion.



Scheme 3. Proposed pathway for photo-oxidation of nocathiacin I (**1**).

It is interesting to note that a cyclic intermediate similar to **2** was observed in photodemethylation of a *N*,*N*-dimethylamino steroid.[5d] However, no such intermediate was reported in the case of anthracyclines,[5b,c] even though the β-hydroxyl group in the amino-sugar is cis to the nitrogen.

Recently, **2** was isolated from a fermentation broth of *Amycolatopsis fastidosa*, ATCC 202099 as a minor product.[15] Given the instability of **2** under acidic conditions, compound **2** in culture might lead to formation of **3**. It is unlikely that photo-oxidation of **1** contributed to formation of **2** in aqueous solvent. Therefore, it is speculated that formation of **3** in the nocathiacin producing culture was due to enzymatic oxidation of the *N*,*N*-dimethylamino group in **1**, via **2** as an intermediate. As production of **3** in the *Amycolatopsis* cultures was not reproducible, the photodemethylation remains the preferred route to **3**.

In summary, the photo-oxidation reaction was developed as a practical route for accessing large amount of compound **3**, allowing us to explore SAR around the amino-sugar moiety.

Acknowledgment

We thank Dr. Yasutsugu Ueda for the constructive comments on the manuscript.

References and notes

1. (a) Leet, J. E.; Ax, H. A.; Gustavson, D. R.; Brown, D. M.; Turner, L.; Brown, K.; Li, W.; Lam, K. S. U.S. Patent 6,218,398, **2001**.; (b) 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; (c) 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; (d) The strain was recently reclassified as *Amycolatopsis fastidosa*, see Ref. 15.
2. Sasaki, T.; Otani, T.; Matsumoto, H.; Hamada, M.; Takeuchi, T.; Hori, M. *J. Antibiot.* **1998**, *51*, 715.
3. (a) Chen, T. S.; Arison, B. H.; Wicker, L. S.; Inamine, E. S.; Monaghan, R. L. *J. Antibiot.* **1992**, *45*, 118; (b) Davis, P. J.; Rosazza, J. P. *J. Org. Chem.* **1976**, *41*, 2548.
4. (a) Hengeveld, J. E.; Gupta, A. K.; Kemp, A. H.; Thomas, A. V. *Tetrahedron Lett.* **1999**, *40*, 2497; (b) Malpass, J. R.; Hemmings, D. A.; Wallis, A. *Tetrahedron Lett.* **1996**, *37*, 3911; (c) Olofson, R. A.; Martz, J. T.; Senet, J.-P.; Piteau, M.; Malfroot, T. *J. Org. Chem.* **1984**, *49*, 2081.
5. (a) Ripper, J. A.; Tiekink, E. R. T.; Scammells, P. J. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 443; (b) Johdo, O.; Tone, H.; Okamoto, R.; Yoshimoto, A.; Naganawa, H.; Sawa, T.; Takeuchi, T. *J. Antibiot.* **1992**, *45*, 1653; (c) Hermentin, P.; Raab, E.; Paal, M.; Gerken, M.; Kolar, C.; Boettger, D.; Berscheid, H. G. *J. Carbohydr. Chem.* **1990**, *9*, 235; (d) Khuong-Huu-Laine, F.; Herlem-Gaulier, D. *Tetrahedron Lett.* **1970**, *42*, 3649.
6. *Characterization of compound* ***5*** *(HCl salt):* ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.08 (1H, s), 9.19 (1H, s), 8.63 (1H, s), 8.59 (1H, s), 8.52 (1H, m), 8.48 (1H, s), 8.18 (1H, m), 8.10 (1H, br s), 7.91 (1H, s), 7.78 (1H, s), 7.59 (1H, br s), 7.52 (1H, d, *J* = 9.1 Hz), 7.31 (1H, m), 7.18 (1H, d, *J* = 8.1 Hz), 6.37 (1H, s), 5.99 (1H, d, *J* = 11.8 Hz), 5.83 (1H, br d), 5.77 (1H, s), 5.53 (1H, d, *J* = 10.1 Hz), 5.43 (1H, m), 5.10 (1H, m), 5.04 (1H, d, *J* = 11.8 Hz), 4.70 (1H, d, *J* = 10.1 Hz), 4.37 (1H, m), 4.24 (1H, d, *J* = 10.1 Hz), 4.14 (1H, d, *J* = 11.8 Hz), 4.09 (1H, m), 3.96 (1H, d, *J* = 10.1 Hz) 3.48 (3H, s), 3.24 (1H, s), 2.91 (6H, br s), 2.13 (1H, m), 1.97 (3H, s), 1.93 (1H, m), 1.63 (3H, s), 1.11 (6H, br d). HRMS (ESI) calcd for $C_{61}H_{61}N_{14}O_{18}S_5$ (M+H): 1437.289; found: 1437.285. LC/MS (+ESI): *m/z* 1437.
7. *Characterization of compound* ***2*** *(as in reaction mixture):* ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.79 (1H, br), 10.08 (1H, s), 9.05 (1H, s), 8.67 (1H, s), 8.57 (1H, s), 8.54 (1H, s), 8.48 (1H, m), 8.22 (1H, s), 8.09 (1H, m), 7.96 (2H, s), 7.88 (1H, s), 7.72 (1H, d, *J* = 9.2 Hz), 7.63 (1H, br s), 7.37 (2H, m), 7.17 (1H, d, *J* = 6.9 Hz), 6.39 (1H, s), 5.96 (1H, d, *J* = 12.2 Hz), 5.86 (1H, d, *J* = 10.2 Hz), 5.78 (2H, br s), 5.26 (1H, m), 5.07 (2H, m), 4.84 (1H, m), 4.78 (1H, m), 4.53 (1H, d, *J* = 10.2 Hz), 4.40 (1H, d, *J* = 10.2 Hz), 4.36 (1H, d, *J* = 3.1 Hz), 4.23 (1H, m), 4.16 (1H, d, *J* = 8.1 Hz), 4.00 (1H, m), 3.98 (1H, d, *J* = 3.1 Hz), 3.90 (3H, s), 3.61 (1H, m), 2.65 (1H, m), 2.27 (1H, br s), 2.23 (3H, s), 2.12 (1H, m), 2.00 (3H, s), 1.73 (1H, m), 1.30 (3H, s), 1.18 (3H, br), 0.51 (3H, d, *J* = 6.9 Hz). HRMS (ESI) calcd for $C_{61}H_{59}N_{14}O_{18}S_5$ (M+H): 1435.274; found: 1435.278. LC/MS (+ESI): *m/z* 1435.
8. *Characterization of compound* ***3*** *(HCl salt):* ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.40 (1H, s), 10.83 (1H, s), 10.07 (1H, s), 9.11 (1H, s), 8.64 (1H, s), 8.61 (1H, m), 8.58 (1H, s), 8.53 (1H, s), 8.24 (1H, s), 8.17 (1H, m), 8.12 (1H, br s), 8.03 (1H, s), 7.88 (1H, s), 7.85 (1H, d, *J* = 11.1 Hz), 7.72 (1H, d, *J* = 8.4 Hz), 7.63 (1H, s), 7.34 (2H, m), 7.18 (1H, d, *J* = 7.0 Hz), 6.37 (1H, s), 6.00 (1H, d, *J* = 11.9 Hz), 5.78 (1H, s), 5.75 (1H, m), 5.69 (1H, d, *J* = 9.1 Hz), 5.22 (1H, m), 5.07 (2H, m), 4.91 (1H, d, *J* = 4.2 Hz), 4.78 (1H, d, *J* = 10.5 Hz), 4.55 (1H, d, *J* = 10.9 Hz), 4.33 (1H, d, *J* = 9.6 Hz), 4.24 (1H, m), 4.14 (1H, d, *J* = 10.8 Hz), 4.05 (1H, d, *J* = 9.6 Hz), 3.91 (3H, s), 3.78 (1H, d, *J* = 6.8 Hz), 2.82 (1H, m), 2.63 (3H, br s), 2.43 (1H, m), 2.00 (3H, s), 1.98 (1H, m), 1.87 (1H, d, *J* = 14.2 Hz), 1.57 (3H, s), 1.15 (3H, br s), 0.68 (3H, d, *J* = 6.5 Hz). HRMS (ESI) calcd for $C_{60}H_{59}N_{14}O_{18}S_5$ (M+H): 1423.274; found: 1423.277. LC/MS (+ESI): *m/z*, 1423, 1266. MS^2 spectrum of *m/z* 1423: *m/z* 1266.
9. (a) Constantine, K. L.; Mueller, L.; Lam, K. S.; Huang, X.; Li, W.; Abid, S.; Leet, J. E. *J. Am. Chem. Soc.* **2002**, *124*, 7284; (b) Huang, Xi. S.; Liu, X.; Constantine, K. L.; Leet, J. E.; Roongta, V. *Magn. Reson. Chem.* **2007**, *45*, 447.
10. Interaction between the Thr-OH and Thz(2) moiety was observed with **1**,[9] suggesting the Thr moiety pointed into the cyclic system. For **5**, interaction between the Thr-OH and Thz(2) moiety was not observed, instead, proton–proton NOE between the Thr-OH and Dht-OMe was observed, suggesting the Thr moiety now point outward, possibly due to hydrogen bonding effect.
11. *Representative experimental procedure:* **1** (200 mg) was dissolved in a mixture of 20 ml DMF and 80 ml CH_2Cl_2 in a 250 ml Pyrex beaker. The solution was irradiated under a UV light from a high pressure Hg lamp (450 W, equipped with a 350–450 nm dichroic mirror and a 385 nm cut-on long pass glass filter) for 50 min with stirring in the open air. Solvent was removed and the residue was taken in 12 ml DMSO. After purification using semi-prep HPLC on an YMS ODS-AQ column (20 × 150 mm, S-5) using 15–36% acetonitrile/1 mM aq HCl as eluent, the fraction containing **2** (about 400 ml) was incubated in a 70 °C water bath for 4 h. After removal of acetonitrile, followed by freeze drying, 92.3 mg of **3** was obtained as the HCl salt (yield, 45.5%).
12. Mori, T.; Takamoto, M.; Tate, Y.; Shinkuma, J.; Wada, T.; Inoue, Y. *Tetrahedron Lett.* **2001**, *42*, 2505.
13. Andrieux, C. P.; Hapiot, P.; Saveant, J.-M. *J. Am. Chem. Soc.* **1987**, *109*, 3768.
14. (a) Ledwith, A. *Acc. Chem. Res.* **1972**, *5*, 133; (b) Dopp, D.; Heufer, J. *Tetrahedron Lett.* **1982**, *23*, 1553.
15. Jayasuriya, H.; Herath, K.; Ondeyka, J. G.; Zhang, C.; Zink, D. L.; Brower, M.; Gailliot, F. P.; Greene, J.; Birdsall, G.; Venugopal, J.; Ushio, M.; Burgess, B.; Russotti, G.; Walker, A.; Hesse, M.; Seeley, A.; Junker, B.; Connors, N.; Salazar, O.; Genilloud, O.; Liu, K.; Masurekar, P.; Barrett, J. F.; Singh, S. B. *J. Antibiot.* **2007**, *60*, 554.