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Biosynthetic studies of Nocathiacin-I

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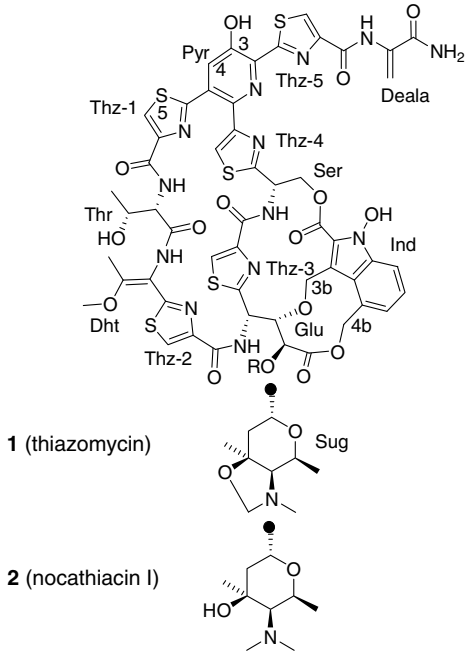
ABSTRACT

Nocathiacin-I is one of the newest members of thiazolyl peptide class of antibiotics. It is a potent inhibitor of bacterial protein synthesis and showed potent in vitro and in vivo Gram-positive antibacterial activity. Understanding of the biosynthesis of natural products is important for improvement of titer and precursor directed biosynthesis for new compounds. Biosynthesis of nocathiacin-I in *Amycolatopsis fastidiosa* using stable isotope precursor incorporation is described.

Thiazolyl peptides are rigid polycyclic natural products possessing a large number of thiazole rings and are represented by over 90 members. The oldest members, micrococcin and thiostrepton, were reported in late 1940s.[1] These compounds are produced by soil-dwelling bacteria and are highly potent Gram-positive antibiotics. They selectively inhibit bacterial protein synthesis and do not show cross-resistance to other classes of antibiotics including protein synthesis inhibitors.[1] Poor physical properties, particularly poor aqueous solubility, prevented their further development as clinically useful antibiotics. Recently, we reported the discovery of thiazomycin (**1**),[2,3] which is a congener of nocathiacin-I (**2**).[4–6] While the titer of nocathiacin-I was relatively high (400 mg/L), the titer of thiazomycin was poor (2–10 mg/L). Extensive medicinal chemistry efforts on these compounds required large quantities of material and hence, a need for improvement of titers. An understanding of the precursors involved in the biosynthesis is extremely helpful for the improvement of not only titers, but also precursor-directed biosynthesis of new compounds. Therefore, we initiated biosynthetic studies of the major compound, nocathiacin-I (**2**). Biosynthetic studies of nosiheptide (**3**)[7–9] and thiostrepton[10,11] have been reported and guided our studies. Precursor-based biosynthesis also provided material that can be used for metabolite disposition and profiling studies in animals.

There are five major structural differences between nocathiacin-I (**2**) and nosiheptide (**3**). Nocathiacin-I possesses a lactone (vs thiolactone), a methyl ether in the dehydrothreonine residue, an aminoglycoside, C-3 oxidation of the glutamic acid moiety, and cyclic ether formation involving indole methyl group and Glu-C3 to form the third macrocycle. No biosynthetic studies of thiazolyl peptides with amino sugar moieties including nocathiacins have been reported.

Nosiheptide is produced by *Streptomyces actuosus* and is known to incorporate serine, cysteine, methionine, threonine, and glutamic acid. Serine has been shown to incorporate in all but three (Thr, But, and Glu) constituent amino acid moieties. Therefore, [3-^{13}C] serine became a precursor of choice for our studies. Nocathiacin-I and thiazomycin are produced by submerged culture of *Amycolatopsis fastidiosa* MA7344. The culture was grown in a production medium consisting of in g/L: glucose 35, acid hydrolyzed casein 5.2, MOPS 20, yeast extract 6.5, and soy peptone type SL 6.5. Aqueous solution of [3-^{13}C]-L-serine (0.5 g/L, 15 mg/flask) was fed at day 1 in the shake flask culture consisting of 30 mL production medium in 250 mL Erlenmeyer flask at 32 °C. The amount of the labeled precursor feeding, time of the precursor feeding, and the harvest time were optimized for maximal incorporation. The



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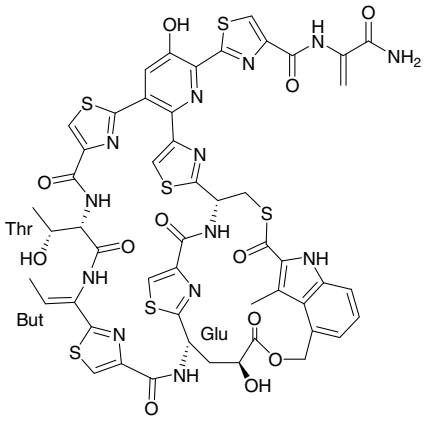

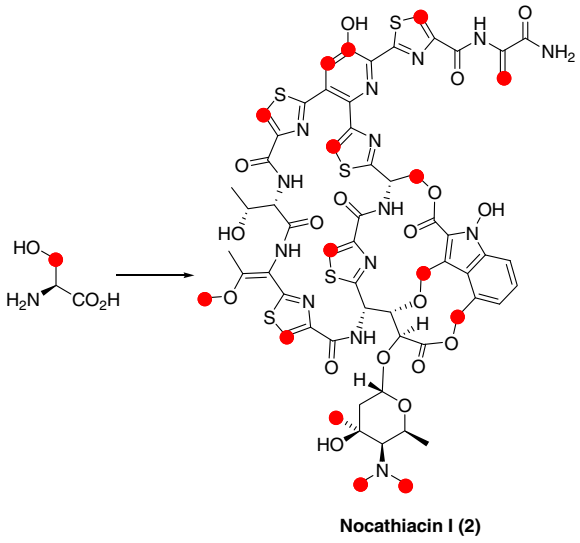

Figure 2. Labeling patterns of nocathiacin-I.

fermentation was harvested at day 7 and extracted with equal volumes of acetone (titer of nocathiacin-I was 335 mg/L). The acetone was removed under reduced pressure and product was extracted with EtOAc. EtOAc extract was concentrated and dissolved in DMF and directly chromatographed by reversed-phase HPLC (Zorbax SB-phenyl, 21.2 × 250 mm) eluting with a 15 min gradient of 40–50% aqueous CH_3CN + 0.1% TFA at a flow rate of 20 mL/min. Nocathiacin-I-containing fractions were lyophilized to give 16 mg of yellow powder. The labeled sample of nocathiacin-I was analyzed by ^{13}C NMR spectroscopy with 1 s relaxation delay. The enrichment factor (fold over natural abundance represented as % enrichment) was determined by using external calibration to a natural abundance spectrum of equal concentration recorded under identical conditions. The enrichment level was so strong that in 64 scan (Fig. 1) the ^{13}C NMR spectrum showed all enriched carbon resonances which was essentially identical to the spectrum obtained by subtraction of natural abundance spectrum from the labeled spectrum.

Analysis of the ^{13}C NMR spectrum of the labeled nocathiacin-I·TFA showed strong enrichments of each of the protonated carbons (C-5) of all five thiazoles, C-3 and C-4 of pyridine, the C-3 methylene carbon of dehydroalanine, C-3 of serine, C-3b and C-4b of indole, the methoxy carbon of dehydrothreonine, both carbons of the dimethyl amino group, and the methyl group at C-3 of the sugar moiety. The labeling pattern of nocathiacin-I is shown in Figure 2 and fold enrichment over natural abundance is presented in Table 1 measured by dividing the normalized peak area of the labeled peak with the normalized peak area of the corresponding natural abundance peak. Very high levels of incorporation were observed in the labeled nocathiacin-I produced by *A. fastidiosa* from [3-^{13}C] serine, which varied from 19 to 83-fold.

The ^{13}C labeling pattern of nocathiacin-I from labeled serine in this study was consistent with the reported labeling pattern observed for nosiheptide (**3**) from [3-^{13}C]-L-serine. However, it appears that the level of incorporation in this study is significantly (upwards of 10 times) higher than that observed in nosiheptide produced by *S. actuosus*. It is clear that all of the thiazoles are derived from the condensation of three carbon atoms of cysteine which is derived from serine. The fourth carbon of the thiazole residue originates from the carboxyl carbon of the adjacent amino acid. In general, the incorporation levels of serine in thiazole carbon (C-5) was high but not as high as incorporation levels observed for pyridine (C-3, C-4), Deala (C-3), and serine (C-3) carbons. These carbons showed incorporation levels of 66–83-fold suggesting a

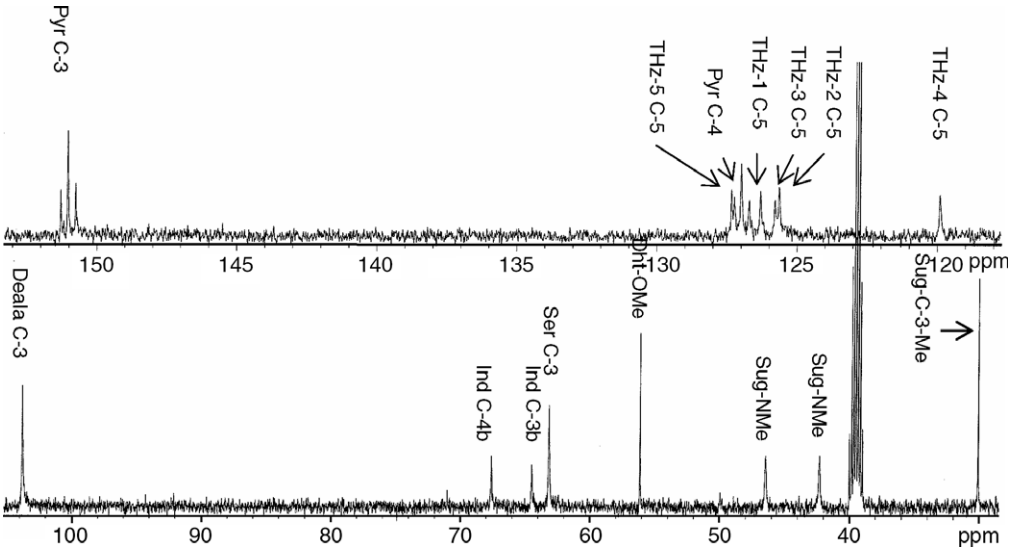

Figure 1. ^{13}C NMR spectrum (DMSO-d_6) of labeled nocathiacin-I·TFA showing only labeled carbons.

Table 1
^{13}C enrichment of nocathiacin-I carbons labeled from feeding [3-^{13}C]-L-serine

Assignment	δ_C	Fold (%) enrichment	J_{CC} in Hz
Pyr (C-3)	151.03	77.5	63.5
Pyr (C-4)	126.99	69.8	63.5
Thz-1 (C-5)	126.30	23.3	
Thz-2 (C-5)	125.63	19.0	
Thz-3 (C-5)	125.77	19.0	
Thz-4 (C-5)	119.89	22.8	
Thz-5 (C-5)	127.33	28.0	
Deala (C-3)	103.77	66.8	
Ind (C-4b)	67.59	32.4	
Ind (C-3b)	64.48	34.4	
Ser (C-3)	63.16	83.3	
Dht (OMe)	56.13	32.8	
Sug (NMe-1)	44.46	38.2	
Sug (NMe-2)	42.30	37.3	
Sug (C-3-Me)	30.11	27.9	

direct uptake of the labeled serine. The [3-^{13}C]-L-serine labeled two adjacent pyridine carbons confirming a tail-to-tail condensation of the C-3 carbons of each of the two serines in a fashion similar to that elucidated for nosiheptide and thiostrepton. These observations suggest similar biosynthetic pathways for the synthesis of the tetrasubstituted pyridine unit involving two units of serine each contributing their C-2/C-3 carbons and a unit of serine/cysteine contributing its carboxyl group for the formation of pyridine ring in all three compounds (Fig. 3).[7–9]

The labeled nocathiacin-I showed incorporations at C-3b and C-4b of the indole moiety. It has been shown that tryptophan is a precursor for the biosynthesis of the indolic acid moiety in nosiheptide. It was also shown that C-2 and C-3 of L-tryptophan are derived from C-2 and C-3 of L-serine. It has been proposed that tryptophan undergoes a rearrangement where C-2 of tryptophan is lost and C-1 of tryptophan migrates to C-2 of indolic acid. Thus the labeling by [3-^{13}C]-L-serine produced the expected labeling of the oxymethylene carbon C-3b, which is equivalent to the methyl group of nosiheptide. Like nosiheptide, C-4b was also labeled from [3-^{13}C]-L-serine. In nosiheptide, it was independently demonstrated that it originated from the methylation by *S*-adenosine methionine (SAM).[7–9] The nucleophilic attack by the C-3 hydroxy group of Glu to the putative CH_2X (X is a leaving group, e.g., Cl) of the indole C-3b would form the ether bridge and the third macrocycle of nocathiacin-I.

Serine interacts with the coenzyme, tetrahydrofolic acid (FH_4), transfers a methyl group (C-3 carbon of serine) to the coenzyme, loses a mole of water and produces glycine and thus produces the C_1 pool. This methyl group is eventually transferred to homocysteine to produce methionine (Met) which is a universal methylating agent (Fig. 4).

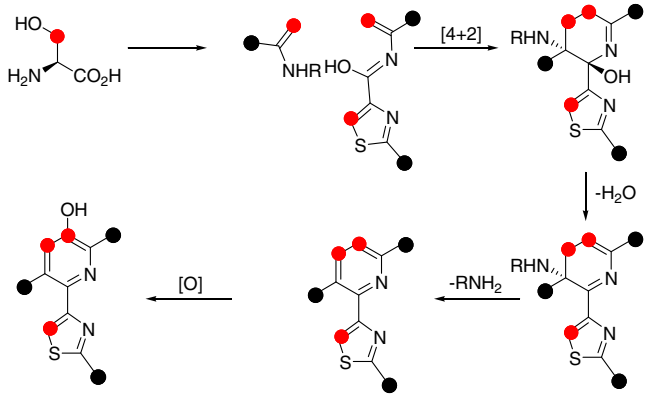

Figure 3. Biosynthesis of tetrasubstituted pyridine of nocathiacin-I.

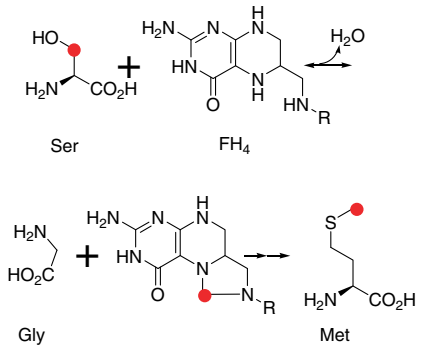

Figure 4. Conversion of C-3 of serine to methionine (C_1 pool).

Nocathiacin-I from [3-^{13}C]-L-serine showed high level of enrichments of the *O*-methyl carbon of dehydrothreonine, both *N*-methyl groups (because of partial protonation by TFA these signals appeared at δ_C 42.32 and 46.48 both in the labeled and unlabeled nocathiacin-I), and the C-3 methyl group of the sugar moiety. The extent of the incorporation was highest for the *N*-methyl groups (37–38%) and lowest to the sugar methyl at C-3 (27.9%). It is evident that this methyl group at C-3 of the sugar moiety comes from C_1 pool likely from SAM. It is also clear that the OMe and the *N*-Me groups are derived from SAM derived from serine via C_1 pool.

The producing organism of nocathiacin-I also produces[6] smaller compounds, for example, nocathiacin-III that lacks the sugar moiety, and nocathiacin-IV that lacks the terminal Deala moiety, suggesting that these moieties are added post cyclic thiazolyl peptide synthesis. It has been also suggested but not conclusively demonstrated that the indolic acid is attached post thiazole synthesis in nosiheptide.[9] In the case of nocathiacins, the likely smallest first linear peptide sequence that can produce nocathiacin-IV may involve NH_2-L-Ser-L-Cys-L-Thr-L-Thr-L-Cys-L-Glu-L-Cys-L-Ser-L-Cys-L-Ser-L-Cys-CO_2H. It remains unclear whether the acyclic peptide is formed first and if all oxidation steps take place post linear peptide synthesis or if it takes place in a step-wise fashion. This question could be answered from NPRS cluster analysis and reconstitution of enzymes. Such attempts have been made in part but no full clusters have been isolated and characterized for this class.[12]

In summary, we have reported herein the first biosynthetic study of the newest members of thiazolyl peptides by feeding the ^{13}C labeled L-serine, the most universal precursor for the biosynthesis of thiazolyl peptides. The biosynthesis of the core units mirrors the biosynthesis of nosiheptide. While it appears that the C-3 methyl group of the sugar moiety originates from SAM, further studies are needed to fully elucidate the biosynthesis of the deoxy sugar moiety and determine the sequence of events in the assembly of the peptide chains and indolic acid moiety.

References and notes

1. Bagley, M. C.; Dale, J. W.; Merritt, E. A.; Xiong, X. *Chem. Rev.* **2005**, *105*, 685–714.
2. 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. (Tokyo)* **2007**, *60*, 554–564.
3. Singh, S. B.; Occi, J.; Jayasuriya, H.; Herath, K.; Motyl, M.; Dorso, K.; Gill, C.; Hickey, E.; Overbye, K. M.; Barrett, J. F.; Masurekar, P. *J Antibiot. (Tokyo)* **2007**, *60*, 565–571.
4. Constantine, K. L.; Mueller, L.; Huang, S.; Abid, S.; Lam, K. S.; Li, W.; Leet, J. E. *J. Am. Chem. Soc.* **2002**, *124*, 7284–7285.

5. Pucci, M. J.; Bronson, J. J.; Barrett, J. F.; DenBleyker, K. L.; Discotto, L. F.; Fung-Tomc, J. C.; Ueda, Y. *Antimicrob. Agents Chemother.* **2004**, *48*, 3697–3701.
6. 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. (Tokyo)* **2003**, *56*, 226–231.
7. Houck, D. R.; Chen, L. C.; Keller, P. J.; Beale, J. M.; Floss, H. G. *J. Am. Chem. Soc.* **1987**, *109*, 1250–1252.
8. Houck, D. R.; Chen, L. C.; Keller, P. J.; Beale, J. M.; Floss, H. G. *J. Am. Chem. Soc.* **1988**, *110*, 5800–5806.
9. Mocek, U.; Knaggs, A. R.; Tsuchiya, R.; Nguyen, T.; Beale, J. M.; Floss, H. G. *J. Am. Chem. Soc.* **1993**, *115*, 7557–7568.
10. Zhou, P.; O'Hagan, D.; Mocek, U.; Zeng, Z.; Yuen, L. D.; Frenzel, T.; Unkefer, C. J.; Beale, J. M.; Floss, H. G. *J. Am. Chem. Soc.* **1989**, *111*, 7274–7276.
11. Mocek, U.; Zeng, Z.; O'Hagan, D.; Zhou, P.; Fan, L. D. G.; Beale, J. M.; Floss, H. G. *J. Am. Chem. Soc.* **1993**, *115*, 7992–8001.
12. Carnio, M. C.; Stachelhaus, T.; Francis, K. P.; Scherer, S. *Eur. J. Biochem.* **2001**, *268*, 6390–6401.