

*Supplementary Material*

**Genome Sequencing of *Streptomyces atratus* SCSIOZH16  
and Activated Production of Nocardamine via Metabolic  
Engineering**

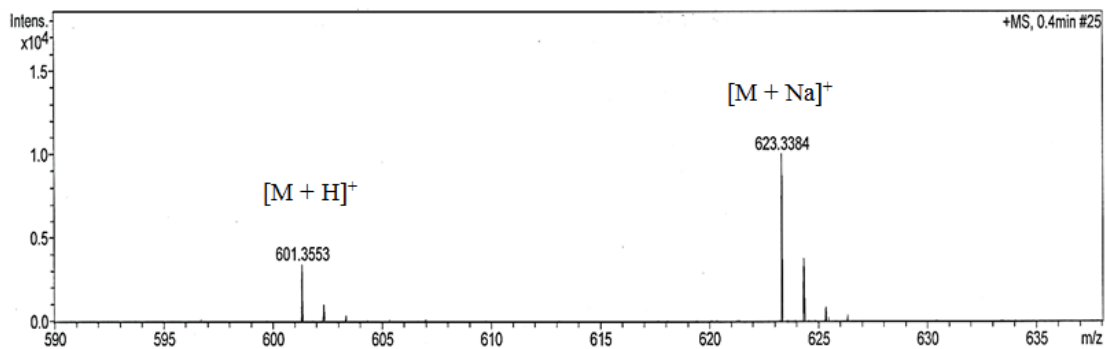
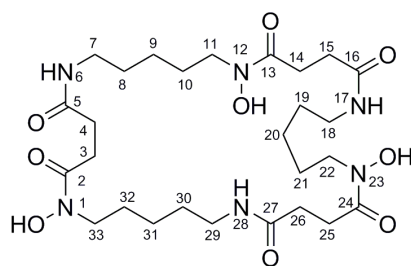
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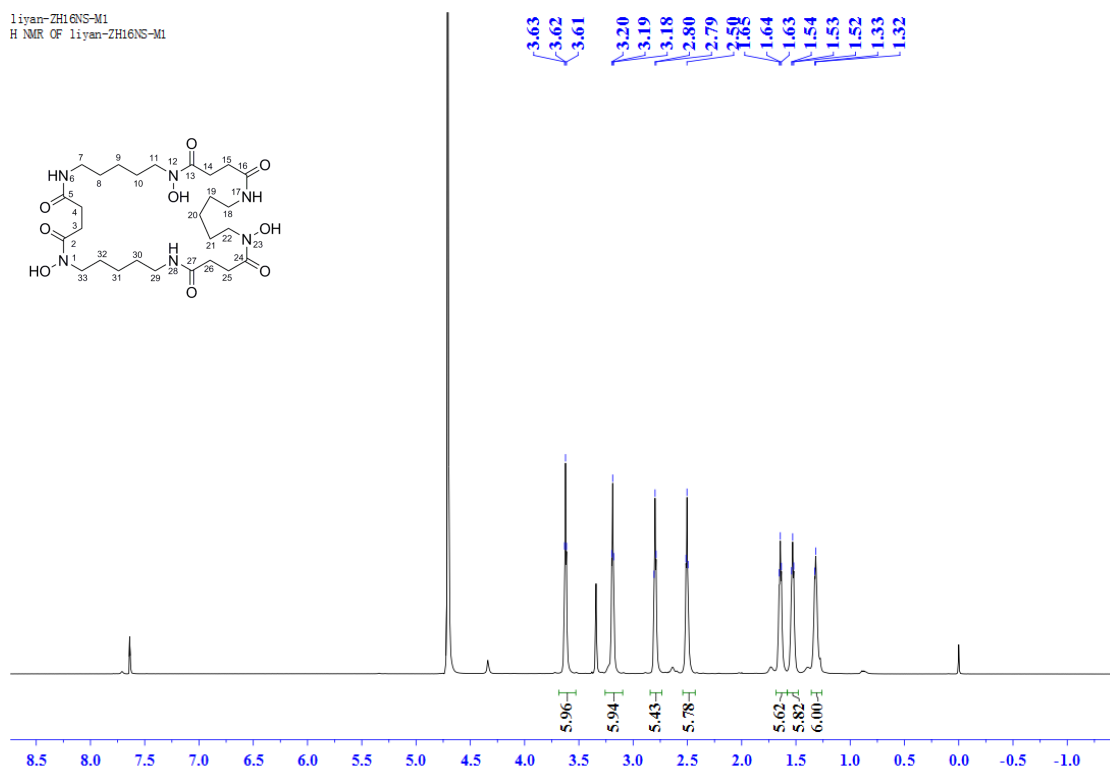
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Meas. m/z	#	Ion Formula	Score	m/z	err [ppm]	err [mDa]	mSigma	rdb	e <sup>-</sup> Conf	N-Rule
601.355333	1	C27H49N6O9	100.00	601.355554	-0.4	-0.2	12.5	6.5	even	ok
	2	C31H53O11	12.66	601.358239	-4.8	-2.9	23.8	5.5	even	ok
	3	C28H45N10O5	37.51	601.356891	2.6	1.6	24.1	11.5	even	ok
	4	C38H49O6	4.13	601.352366	-4.9	-3.0	61.4	14.5	even	ok
	5	C39H45N4O2	8.15	601.353703	-2.7	-1.6	73.1	19.5	even	ok
623.338399	1	C29H40N14NaO	52.18	623.340173	-2.8	-1.8	11.0	16.5	even	ok
	2	C31H52NaO11	46.81	623.340183	2.9	1.8	16.2	5.5	even	ok
	3	C28H44N10NaO5	100.00	623.338835	-0.7	-0.4	18.1	11.5	even	ok
	4	C27H48N6NaO9	62.64	623.337498	-1.4	-0.9	28.4	6.5	even	ok
	5	C39H44N4NaO2	14.09	623.335647	-4.4	-2.8	34.1	19.5	even	ok

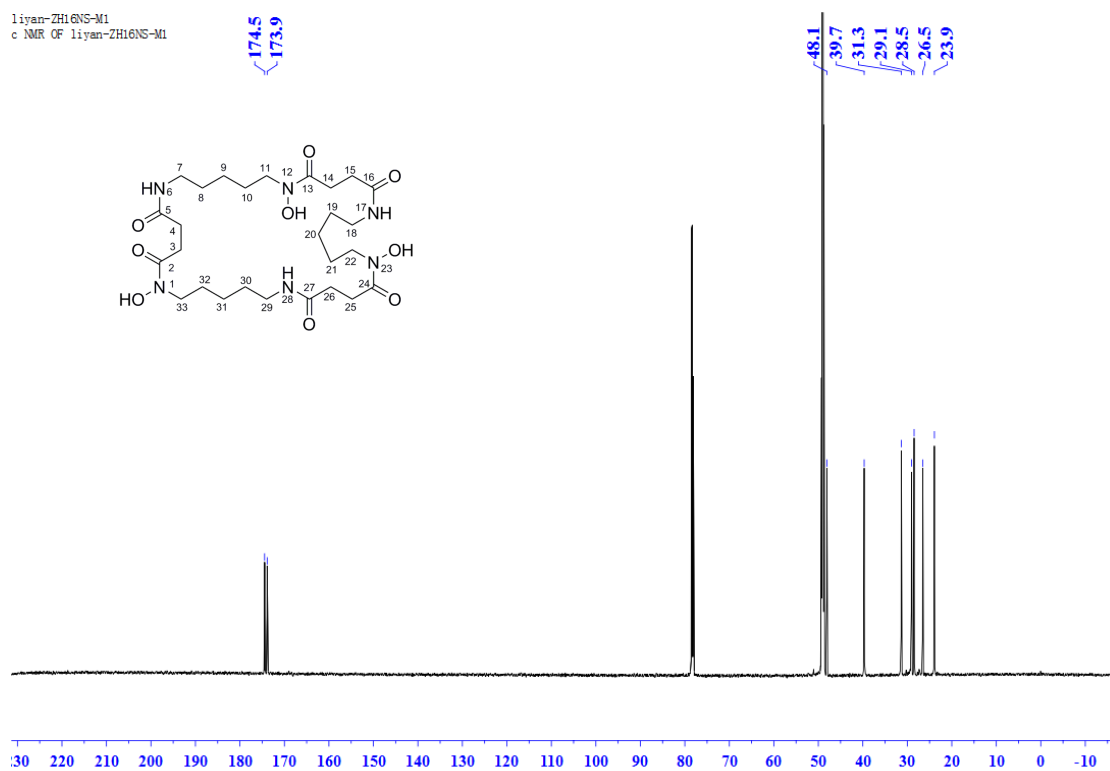
Figure S1. The HRESIMS spectrum of nocardamine.

liyan-ZH16NS-M1  
H NMR OF liyan-ZH16NS-M1



**Figure S2.** The <sup>1</sup>H NMR spectrum of nocardamine in CDCl<sub>3</sub>/MeOD (1:1) at 750 MHz.

liyan-ZH16NS-M1  
c NMR OF liyan-ZH16NS-M1



**Figure S3.** The  $^{13}\text{C}$  NMR spectrum of nocardamine in  $\text{CDCl}_3/\text{MeOD}$  (1:1) at 175 MHz.

**Table S1.** Strains and plasmids were used and constructed in this study.

Strains /Plasmids	Description	Reference
<i>E. coli</i>		
DH5 $\alpha$	Host strain for cloning	invitrogen
BW25113/pIJ790	Host strain for PCR tatgeting	1
BW25113/ cosmid 113D1	Donor strain for conjugation	This study
ET12567/pUZ8002	Donor strain for conjugation	2
ET12567/pUB307	Assistant strain for conjugation	3
Streptomyces		
<i>Streptomyces atratus</i>	Wild-type strain	4
SCSIO ZH16		
<i>Streptomyces atratus</i>	<i>Streptomyces atratus</i> SCSIO ZH16 derivative where <i>ilaS</i> gene was in-frame deleted	This study
SCSIO ZH16S		
<i>Streptomyces atratus</i>	<i>Streptomyces atratus</i> SCSIO ZH16 derivative where <i>ilaN-ilaS</i> genes were in-frame deleted	This study
SCSIO ZH16NS		
Plasmids		
pIJ773	the template for amplifying the Apr <sup>R</sup> cassette	5
Cosmid 47H	genomic library cosmid of <i>S. atratus</i> SCSIO ZH16	This study
Cosmid 47H1	Cosmid 47H derivative where 8kb fragment were disrupted by insertional mutation with <i>aac(3)IV</i>	This study
Cosmid 47H2	Cosmid 47H1 derivative with the digestion by <i>SpeI</i>	This study
Cosmid 47H3	Cosmid 47H2 derivative where the <i>neo</i> gene was replaced with <i>aac(3)IV-oriT</i> cassette	This study

**Table S2.** Primers were used in this study.

Primers	Sequence
For inframe deletion mutant	
YilaN- <i>SpeI</i> -F	CTCCTGGCTGATGACCCGGCAGCCGACTGCACGGCGAT <u>ACTAGT</u> ATTCCG GGGATCCGTCGACC
YilaS- <i>SpeI</i> -R	CGACGCACTGCCCACACCCGACCACCCAGCAACCGCCC <u>ACTAGT</u> TGTAG GCTGGAGCTGCTTC
YilaS- <i>SpeI</i> -F	GCACTTCAGCACAACGCTCTGATGTTTGAAGTGCCG <u>ACTAGT</u> ATTCCGGGG ATCCGTCGACC
YilaS- <i>speI</i> -R	CACGTCGTTGAGCGCGGCCTCCAGCGAACCGGCGTCCAG <u>ACTAGT</u> TGTAG GCTGGAGCTGCTTC
For verification of inframe deletion mutant	
YIDilaN-F	AACACCGGAGACAGCCGT
YIDilaS-R	CCAACTGCTCCAACCCAG
YIDilaS-F	GCACCCGCTGATAAGGCTGTC
YIDilaS-R	CAGGTCCGTGAGGCGAGTCT
Clu11SVF	TCTGGATCGCGGGCGAGTC
Clu11SVR	GCTGTCGAGCTCGTCGAGG
For screen genome library	
Clu11SUF	GCTCGGTTTTCTGCCGGTCCG
Clu11SUF	GTCGGTCGTCGTCTTGCGG
Clu11SDF	TGTTCTGGCGTTCGTGGTC
Clu11SDF	GTTGTGCTGGTGCGGGTTGC

Table S3. General genome features of *S. atratus* SCSIO ZH16.

Features	Chromosome
Genome topology	linear
Assembly size (bp)	9,641,288
DNA-coding regions (bp)	8,407,203
CDS	9245
G + C content (%)	69.5%
Gene density (genes per kb)	0.958
tRNA genes	69
rRNA genes	18
Secondary metabolite gene clusters	26

Table S4. The gene clusters in *S. atratus* SCSIO ZH16.

Cluster No.	NP type	Size (Kb)	most similar known cluster	Similarity
Cluster 1	Nrps	118.0	Lipopeptide	22%
Cluster 2	Otherks-Nrps	98.9	Marformycins	12%
Cluster 3	T2pks-Terpene	65.4	Spore_pigment	83%
Cluster 4	Lantipeptide	75.7	Stenothricin	13%
Cluster 5	Phosphonate	40.9	Ansatrienin	7%
Cluster 6	T1pks-Nrps	54.7	Ilamycin	—
Cluster 7	Terpene	21.1	Steffimycin	19%
Cluster 8	Ectoine	10.4	Ectoine	100%
Cluster 9	Butyrolactone	11.1	$\gamma$ -butyrolactone	100%
Cluster 10	T1pks	55.4	Tetronasin	3%
Cluster 11	Siderophore	11.8	Desferrioxamine B	100%
Cluster 12	T3pks	41.1	Naringenin	100%
Cluster 13	Nrps	88.9	Skyllamycin	48%
Cluster 14	Siderophore	14.7	Unknown	—
Cluster 15	Bacteriocin	11.3	Unknown	—
Cluster 16	Terpene	26.6	Hopene	84%
Cluster 17	Bacteriocin	12.6	Griselimycin	7%
Cluster 18	Other	44.1	Unknown	—
Cluster 19	Bacteriocin	10.8	Tautomycin	6%
Cluster 20	T1pks-Nrps	119.4	Herboxidiene	10%
Cluster 21	Terpene-Otherks- T1pks-Nrps	205.2	Vicenistatin	70%
Cluster 22	Terpene	59.0	Echosides	11%
Cluster 23	Nrps	63.8	Cyclomarin	13%
Cluster 24	Lantipeptide-Nrps	79.5	Streptolydigin	10%
Cluster 25	T1pks	46.4	Sporolide	8%
Cluster 26	Nrps	118.0	Enduracidin	16%



### Supplementary References

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4. Ma, J.; Huang, H.; Xie, Y.; Liu, Z.; Zhao, J.; Zhang, C. (2017) Biosynthesis of ilamycins featuring unusual building blocks and engineered production of enhanced anti-tuberculosis agents. *Nat. Commun.* 8, 391.
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