SUPPORTING INFORMATION

Nocardioazines: A Novel Bridged Diketopiperazine Scaffold from a Marine-Derived Bacterium Inhibits P-Glycoprotein

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General experimental details

Chiroptical measurements ($[\alpha]_D$) were obtained on a JASCO P-1010 polarimeter in a 100 × 2 mm cell. UV-visible spectra were obtained on a Cary 50 spectrophotometer in 1 cm quartz cells. NMR spectra were obtained on a Bruker Avance DRX600 spectrometer, in the solvents indicated and referenced to residual ^{1}H and ^{13}C signals in deuterated solvents. Electrospray ionization mass spectra (ESIMS) were acquired using an Agilent 1100 Series separations module equipped with an Agilent 1100 Series LC/MSD mass detector in both positive and negative ion modes. High-resolution ESIMS measurements were obtained on a Bruker micrOTOF mass spectrometer by direct infusion in MeCN at 3 μL/min using sodium formate clusters as an internal calibrant. HPLC was performed using an Agilent 1100 Series separations module equipped with Agilent 1100 Series diode array and/or multiple wavelength detectors and Agilent 1100 Series fraction collector, controlled using ChemStation Rev.9.03A and Purify version A.1.2 software. Energy minimization (MM2) was performed using Chem3D v12.0 (Cambridgesoft). Curve fitting was performed using Prism 5 (GraphPad Software).

Collection, cultivation and taxonomic identification of CMB-M0232

Strain CMB-M0232 was isolated from a sediment sample obtained from South Molle Island from a depth of 55 m. The fresh sediment sample was transferred to the laboratory in a sealed 50 mL Falcon tube at room temperature (16 h), where it was stored in the dark at –30 °C for one week. Approximately 1 g of the thawed sediment sample was suspended in 4 mL of Ocean Nature seawater (Aquasonic, Australia) and then subjected to heat-shock at 55 °C for 8 min after which 50 μL was transferred on to agar plates (comprising 25 mL of 1% starch, 0.4% yeast extract, 0.2% peptone, 1.8% agar, and 0.0005% rifampicin). The resulting agar plate was incubated at 27 °C for four weeks. Pure strains of individual colonies were obtained by standard microbiological techniques, and were grown to dense colonies on a single agar plate.

Genomic DNA extraction was performed using the DNeasy blood and tissue kit (Qiagen) as per the manufacturers protocol. The 16S rRNA genes were amplified from genomic DNA by PCR using the primers FC 27 (5′-AGAGTTTGATCCTGGCTCAG-3′) and RC 1492 (5′-TACGGCTACCTTGTTACGACTT-3′). The 50-μL PCR mixture contained 25 to 45 ng of DNA, 250 pmol of each primer, 2.5 U of Taq DNA polymerase and 100 μM deoxynucleoside triphosphate mixture. Amplification products were examined by agarose gel electrophoresis. The 16S rRNA gene sequence (below) showed 99% homology with other members of the genus *Nocardiopsis* by the use of the BLAST database.

GCAAGTCGAGCGGTAAGGCCCTTCGTAGGTACACGAGCGGCGAACGGGTGAGTAACACGTGAGCAACCTGCCCCTGACTC
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 Length=1498 Score = 2512 bits (1360),  Expect = 0.0
 Identities = 1383/1394 (99%), Gaps = 1/1394 (0%) Strand=Plus/Plus

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SOURCE      Nocardiopsis sp. 13651T
ORGANISM    Nocardiopsis sp. 13651T
            Bacteria; Actinobacteria; Actinobacteridae; Actinomycetales;
            Streptosporangineae; Nocardiopsaceae; Nocardiopsis.

REFERENCE   1  (bases 1 to 1498)
AUTHORS     Solano,G., Rojas-Jimenez,K., Tamayo-Castillo,G., Jaspars,M. and
            Goodfellow,M.

TITLE       Diversity of culturable actinomycetes in Pacific and Caribbean
            waters of Costa Rica (Unpublished)
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Isolation, purification and characterisation of metabolites

A frozen stock culture (1.2 mL) of *Nocardiopsis* sp. (CMB-M0232) was used to inoculate a 250 mL Schott flask containing 80 mL of M1 broth (1% starch, 0.4% yeast extract and 0.2% peptone dissolved in deionized water). The flask was shaken at 150 rpm in a rotary shaking incubator for 6 d at 27 °C. An aliquot of this seed culture (5.0 mL) was used to inoculate each of six 2 L Schott flasks containing 500 mL of M1 broth supplemented with trace elements, and fermentation was continued for a further 8 d (at 27 °C and with rotary shaking at 190 rpm). After 8 d of cultivation, the whole broth was extracted with an equal volume of EtOAc (500 mL per flask) and the combined organic phase concentrated *in vacuo* to yield a combined crude extract (197 mg). The crude extract was triturated sequentially with hexane (25 mL), CH_2Cl_2 (25 mL) and MeOH (25 mL), to afford fractions of 65.7 mg, 40.2 mg and 12.6 mg respectively. The CH_2Cl_2 fraction was subsequently subjected to HPLC fractionation (Zorbax CN 5 μm, 250 × 9.4 mm column, 4 mL/min gradient elution from 60% H_2O/MeOH to 100% MeOH over 55 min, with a hold at 100% MeCN for 5 min) to yield nocardioazine A (**1**) (t_R = 23.0 min, 0.6 mg, 0.3 %), nocardioazine B (**2**) (t_R = 24.1 min, 0.4 mg, 0.2%), *cyclo*-(L-Trp-L-Trp) (t_R = 12.4 min, 0.5 mg, 0.2%) and *cyclo*-(L-Trp-D-Trp) (t_R = 13.0 min, 0.5 mg, 0.2%) (Note: The yields of metabolites were calculated mass to mass against the total crude extract).

Nocardioazine A (**1**): pale white powder; $[\alpha]^{21}_D$ +37 (*c* 0.05, MeOH); UV (MeOH) λ_{max} (log ε) 293 (3.92), 249 (4.40), 207 (4.87) nm; NMR (600 MHz, $CDCl_3$) see Table S1; NMR (600 MHz, benzene-d_6) see Table S2; HRESI(+)MS *m/z* 483.2391 (calc for $C_{29}H_{31}N_4O_3$, 483.2396).

Nocardioazine B (**2**): pale white powder; $[\alpha]^{21}_D$ +17 (*c* 0.04, MeOH); UV (MeOH) λ_{max} (log ε) 293 (3.91), 249 (4.39), 207 (4.86) nm; NMR (600 MHz, $CDCl_3$) see Table S3; HRESI(+)MS *m/z* 491.2415 (calc for $C_{29}H_{32}N_4O_2Na$, 491.2417).

cyclo-(L-Trp-L-Trp): clear oil; $[\alpha]_D$ –52 (*c* 0.05, MeOH); UV (MeOH) λ_{max} (log ε) 218 (4.71), 273 (3.88), 281 (3.99), 288 (3.86) nm; NMR (600 MHz, methanol-d_4) see Table S4; HRESI(+)MS *m/z* 395.1481 (calc for $C_{22}H_{20}N_4O_2Na$, 395.1478).

cyclo-(L-Trp-D-Trp): clear oil; $[\alpha]_D$ –3 (*c* 0.05, MeOH); UV (MeOH) λ_{max} (log ε) 218 (4.70), 273 (3.88), 281 (3.99), 288 (3.85) nm; NMR (600 MHz, methanol-d_4) see Table S5; HRESI(+)MS *m/z* 395.1483 (calc for $C_{22}H_{20}N_4O_2Na$, 395.1478).

Cell lines and cell culture

The parental cell line SW620 (American Type Culture Collection, Manassas, VA, CCL-227), is a human colon cell line that was originated from a lymph node metastasis in the patient with primary adenocarcinoma of the colon. The multi-drug resistant (MDR) cell line, SW620 Ad300, which over-expresses Permeable-glycoprotein (P-gp), was selected from SW620 by growth in the presence of increasing concentrations of doxorubicin. SW620 and SW620 Ad300 were grown in RPMI medium 1640 as adherent mono-layer in flasks supplemented with 10% foetal bovine serum, 0.1 mM non-essential amino acids (NEAA), 2 mM L-glutamine, 100 unit/mL penicillin and 100 μg/mL streptomycin in a humidified incubator containing of 5% CO_2 at 37 °C. After SW620 Ad300 exhibited stable phenotype of P-gp, the cells were maintained in 300 ng/mL doxorubicin.

Cell cytotoxicity assay

The MTT colorimetric assay has been described previously.[1] Cells to be tested (SW620 or SW620 Ad300) were harvested with trypsin and seeded (2×10^3 cells/well) evenly into a clear 96-well plate in a final volume of 160 μL in RPMI 1640. Cells were incubated for 18 h at 37 °C, 5% carbon dioxide (CO_2) to allow cells to attach to the plate. Compounds of interest were added in duplicate in seven concentrations ranging from 0.03 μM to 30 μM. Control wells were incubated with 1% dimethylsulfoxide (DMSO) as a vehicle control. Three 'media only' wells, which did not contain cells, were also included as a background measurement. The cells were incubated for 92 h at 37 °C, 5% CO_2. Subsequently, 20 μL of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, Sigma Chemical CO., St Louis, Mo, USA) in PBS was added to each well to a final concentration of 0.5 mg/mL. The plate was then incubated for 4 h at 37 °C, 5% CO_2. After media was removed, formazan crystals were resuspended in 100 μL of DMSO followed by shaking the plate at room temperature for 10 min to dissolve the crystals. The absorbance was read at 590 nm with a Microplate Scanning Spectrophotometer from BioTek Instruments, Inc (Winooski, VT, USA). The IC_{50} were calculated as the concentration of the compound required for 50% inhibition of the cancer cells.

Reversal of resistance assay

Reversal of resistance of SW620 Ad300 cells to doxorubicin assay was performed as described for the standard cytotoxicity assay (above), but with the addition of either PBS, nocardioazine A/B (20 μM) or verapamil (10 μM) 1 h before addition of a doxorubicin dilution series.

P-gp inhibitor assay

The flow cytometry assay has been described previously.[2] Cells that overexpress P-gp (SW620 Ad300) were harvested with trypsin and resuspended in completed medium to give a final concentration of 50×10^4 cells/mL. Cells were then pre-incubated with nocardioazine A (20 μM) or verapamil (10 μM) for 30 min at 37 °C in 5% CO_2. Subsequently cells were incubated with 2.5 μM calcein AM (calcein acetoxymethyl ester) for 1 h and then washed twice with cold PBS. Finally, samples were analysed on a BD FACSCantoTM II flow cytometer (Becton Dickinson, San Jose, CA). Calcein fluorescence was detected with a 488 nm argon laser and a 530 nm band pass filter. Data were analysed by FCSexpress 4 (De Novo Software, Los Angeles, CA).

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[1] Zhou, Y.; Hopper-Borge, E.; Shen, T.; Huang, X. C.; Shi, Z.; Kuang, Y. H.; Furukawa, T.; Akiyama, S.; Peng, X. X.; Ashby, C. R., Jr.; Chen, X.; Kruh, G. D.; Chen, Z. S. *Biochem Pharmacol* **2009**, *77*, 993.

[2] Henrich, C. J.; Bokesch, H. R.; Dean, M.; Bates, S. E.; Robey, R. W.; Goncharova, E. I.; Wilson, J. A.; McMahon, J. B. *J. Biomol. Screen.* **2006**, *11*, 176.

Antimicrobial assay

One colony of the microorganism to be tested was transferred into Bacto Tryptic Soy Broth (25 mL), which was incubated with shaking at 37 °C for 48 h. An aliquot (100 μL) of this culture was used to seed Tryptic Soy Agar (50 mL) at 40 °C, which was then poured into sterile Petri plates and allowed to set at room temperature. An aliquot (10 μL) of each compound to be tested (2.5 mg/mL in DMSO) was transferred onto sterile filter paper discs (8 mm), which were air dried for 1 h. The discs were then placed onto the inoculated agar plates and incubated for a further 24 h at 37 °C. Zones of inhibition (measured as diameters, including discs) were then measured to quantify the antimicrobial activity. Rifampicin (1 mg/mL in DMSO) and cycloheximide (2.5 mg/mL) were used as positive controls.

The antimicrobial assay was performed using *Escherichia coli* (ATCC 11775), *Staphylococcus aureus* (ATCC 25923, ATCC 9144), *Bacillus subtilis* (ATCC 6051, ATCC 6633) and *Candida albicans* (ATCC 10231). No zones of inhibition were observed for nocardioazines A (**1**) or B (**2**), nor for related metabolites *cyclo*-(L-Trp-L-Trp) and *cyclo*-(L-Trp-D-Trp).

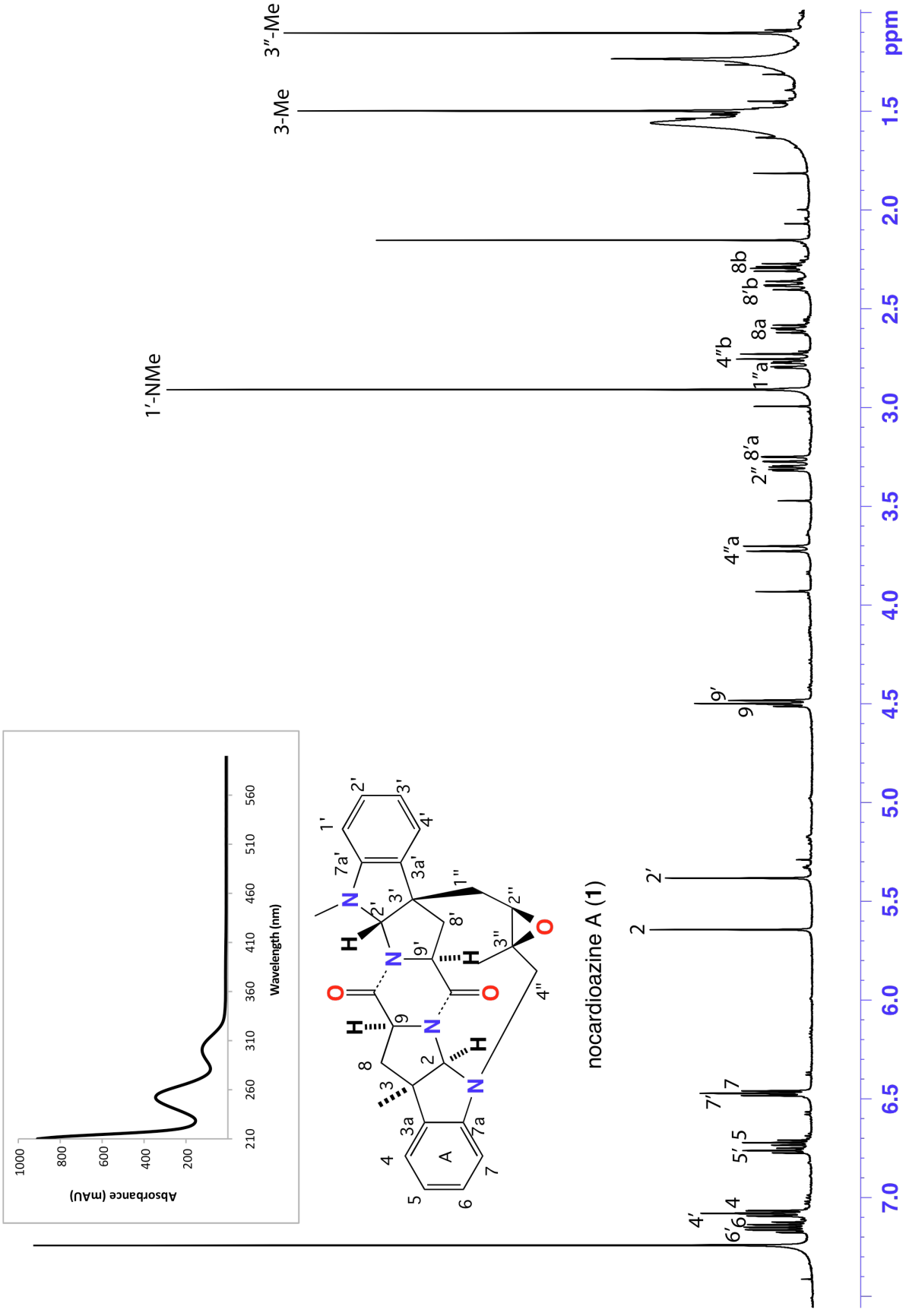

Figure S1. ^{1}H NMR (600 MHz, $CDCl_3$) and UV-vis (inset) spectra of nocardioazine A (**1**)

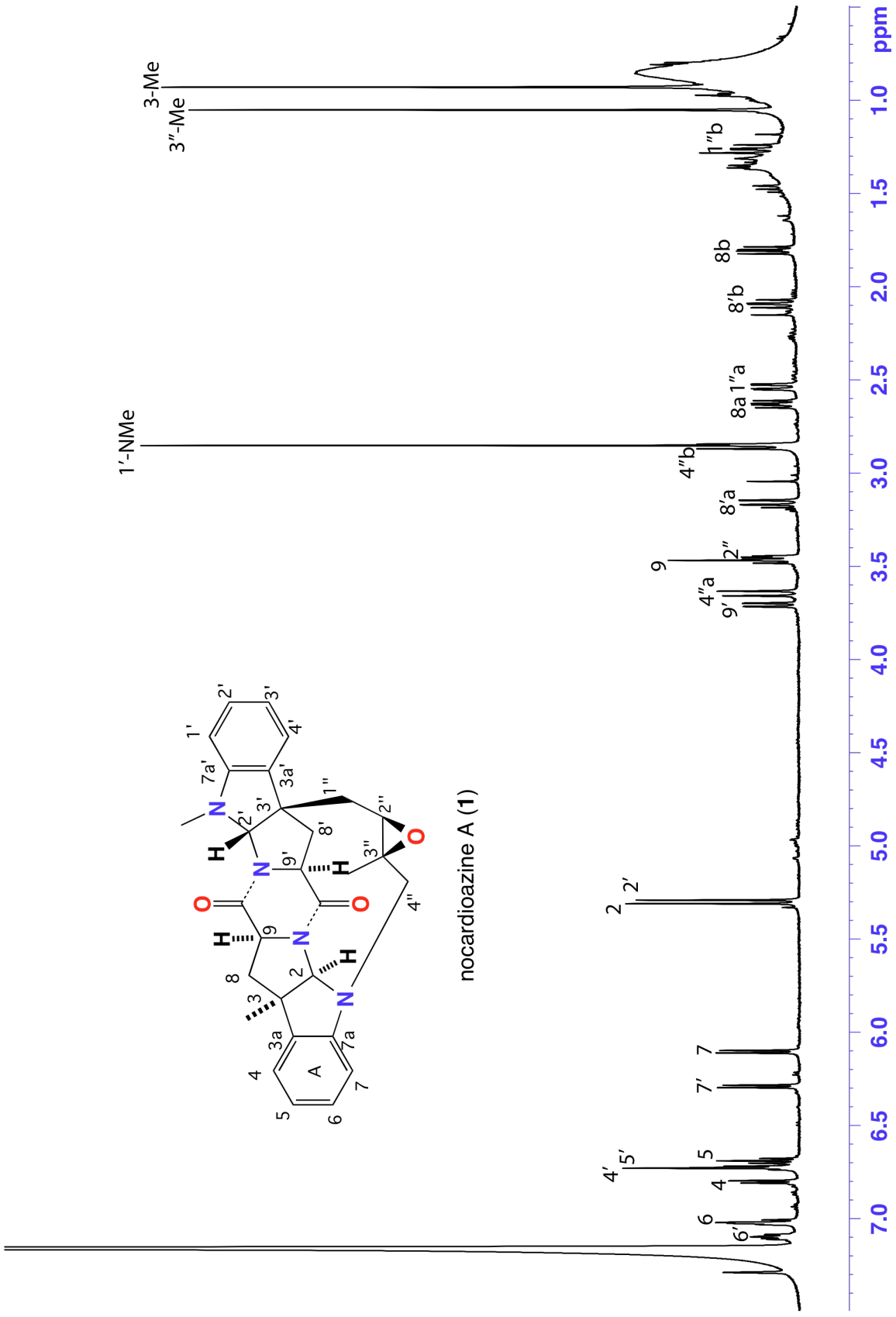

Figure S2. ^{1}H NMR (600 MHz, benzene-d_6) spectrum of nocardioazine A (**1**)

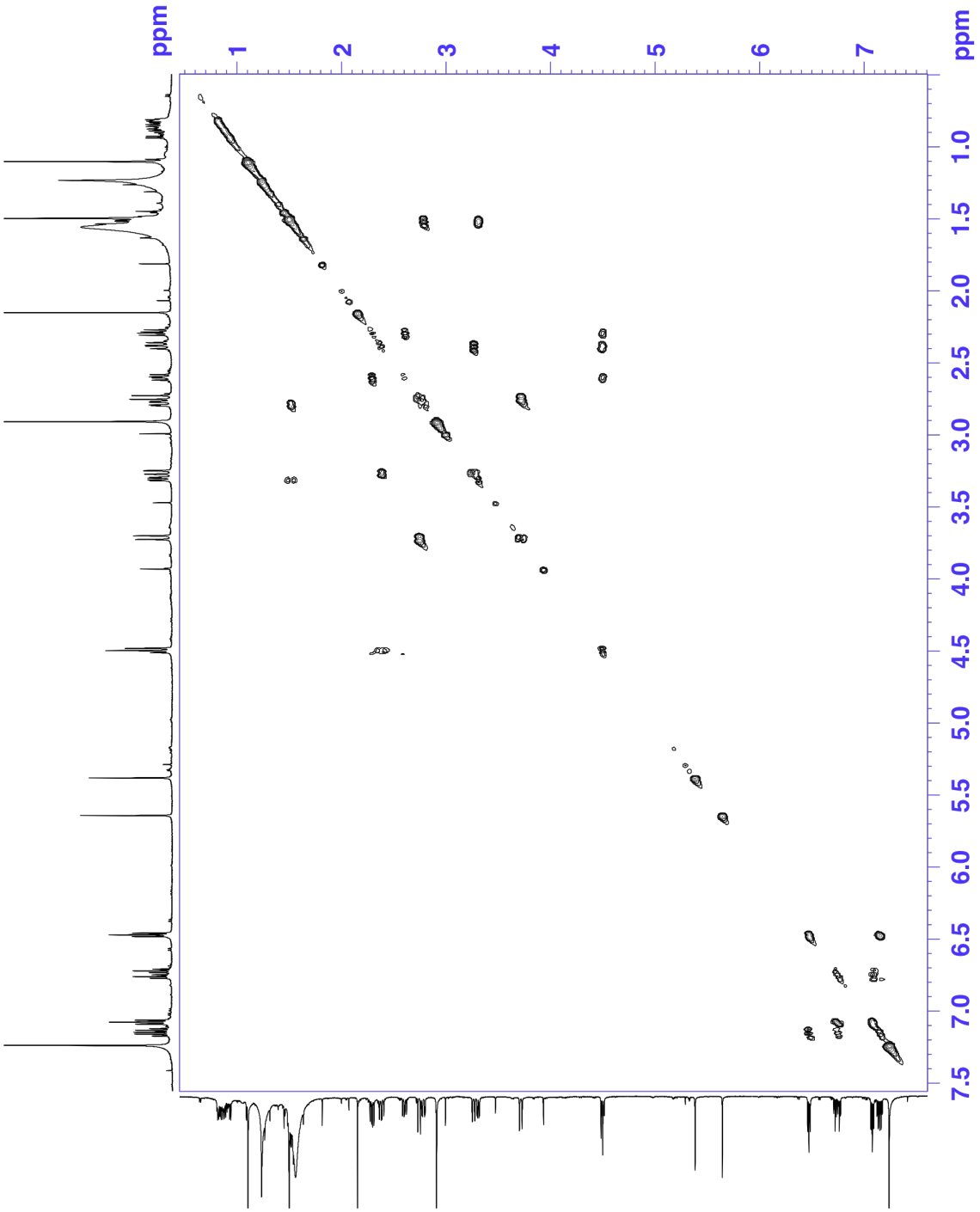

Figure S3. COSY (600 MHz, $CDCl_3$) spectrum of nocardioazine A (**1**)

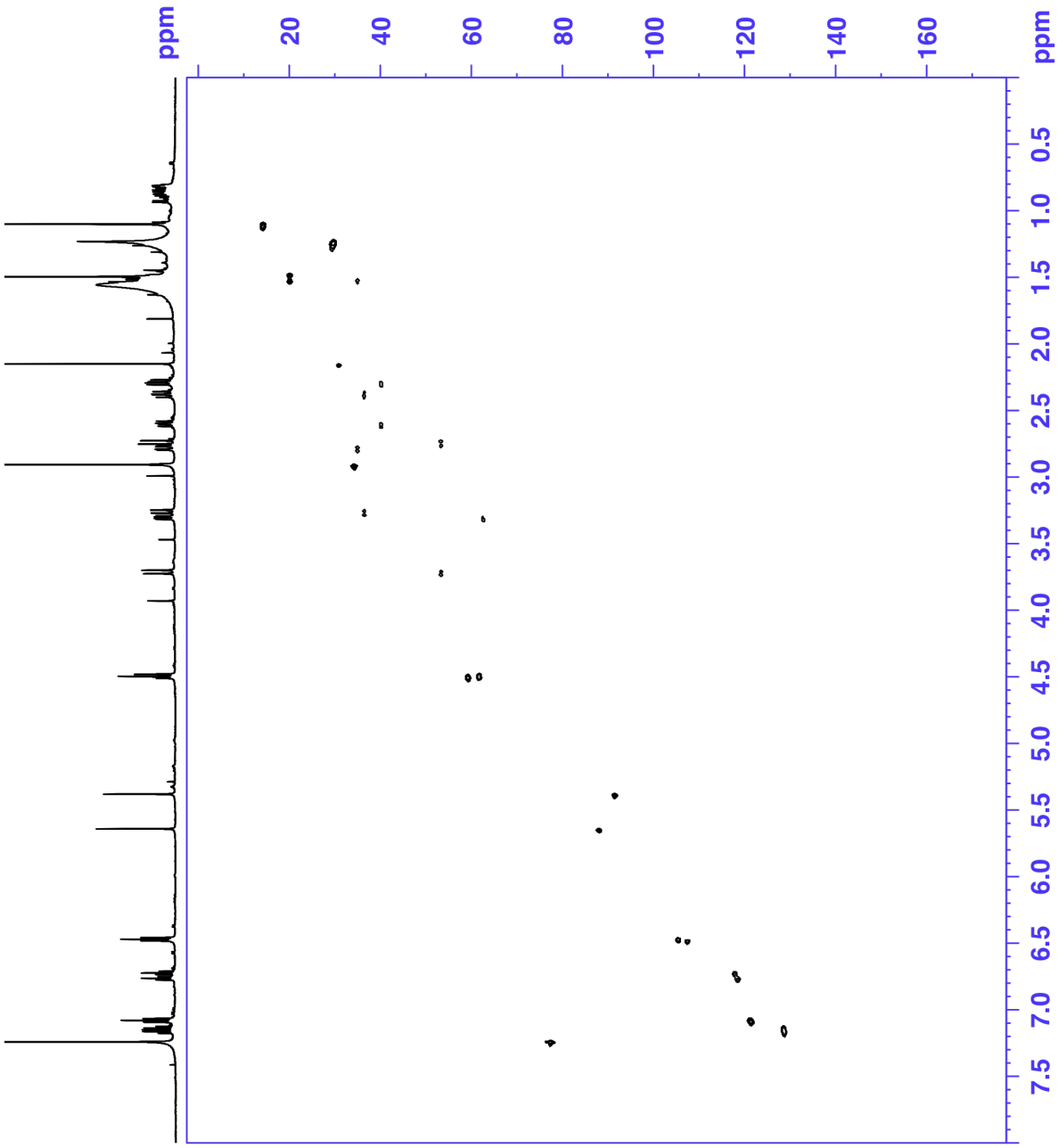

Figure S4. ^{1}H – ^{13}C HSQC (600 MHz, $CDCl_3$) spectrum of nocardioazine A (**1**)

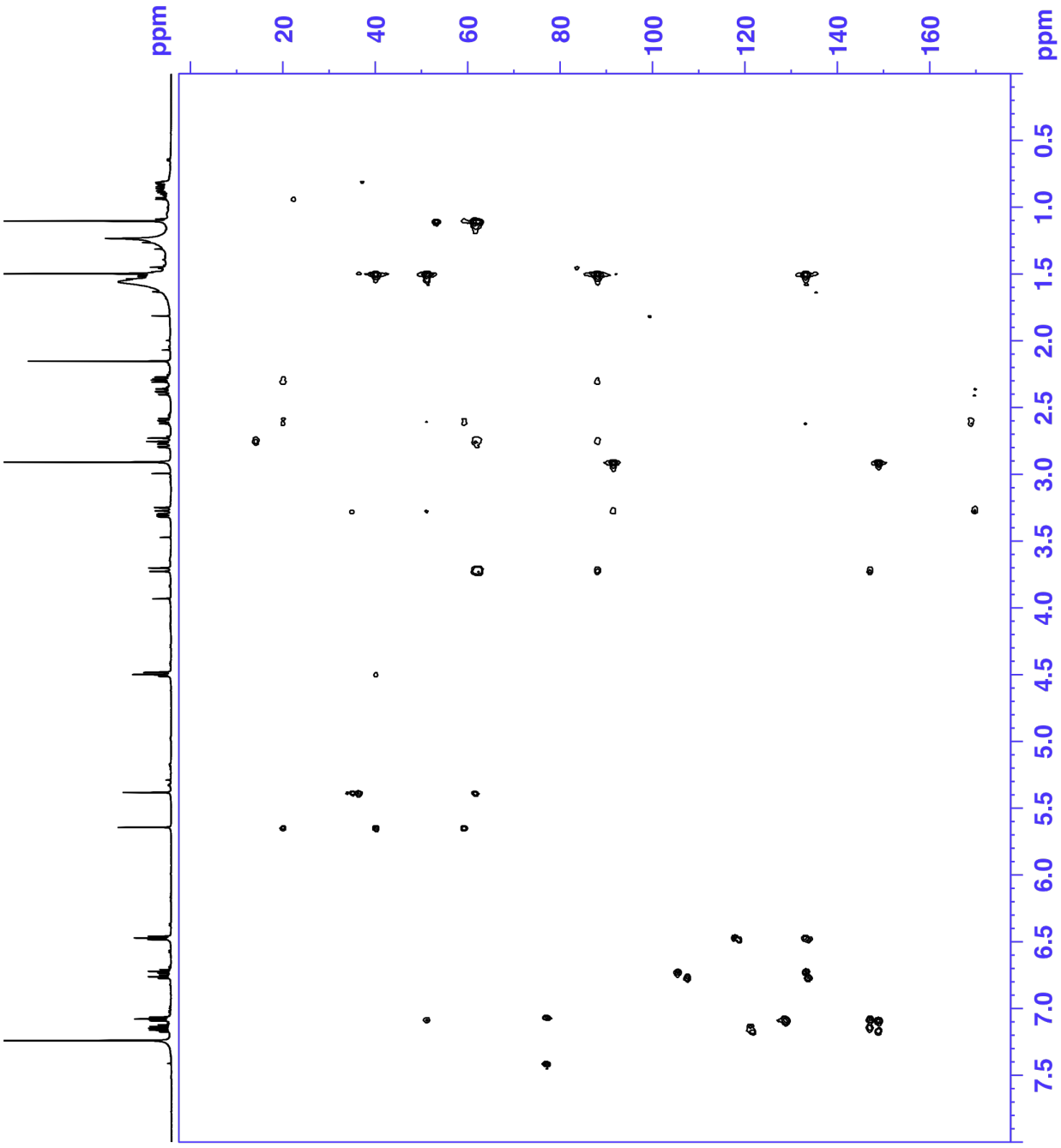

Figure S5. ^{1}H – ^{13}C HMBC (600 MHz, $CDCl_3$) spectrum of nocardioazine A (**1**)

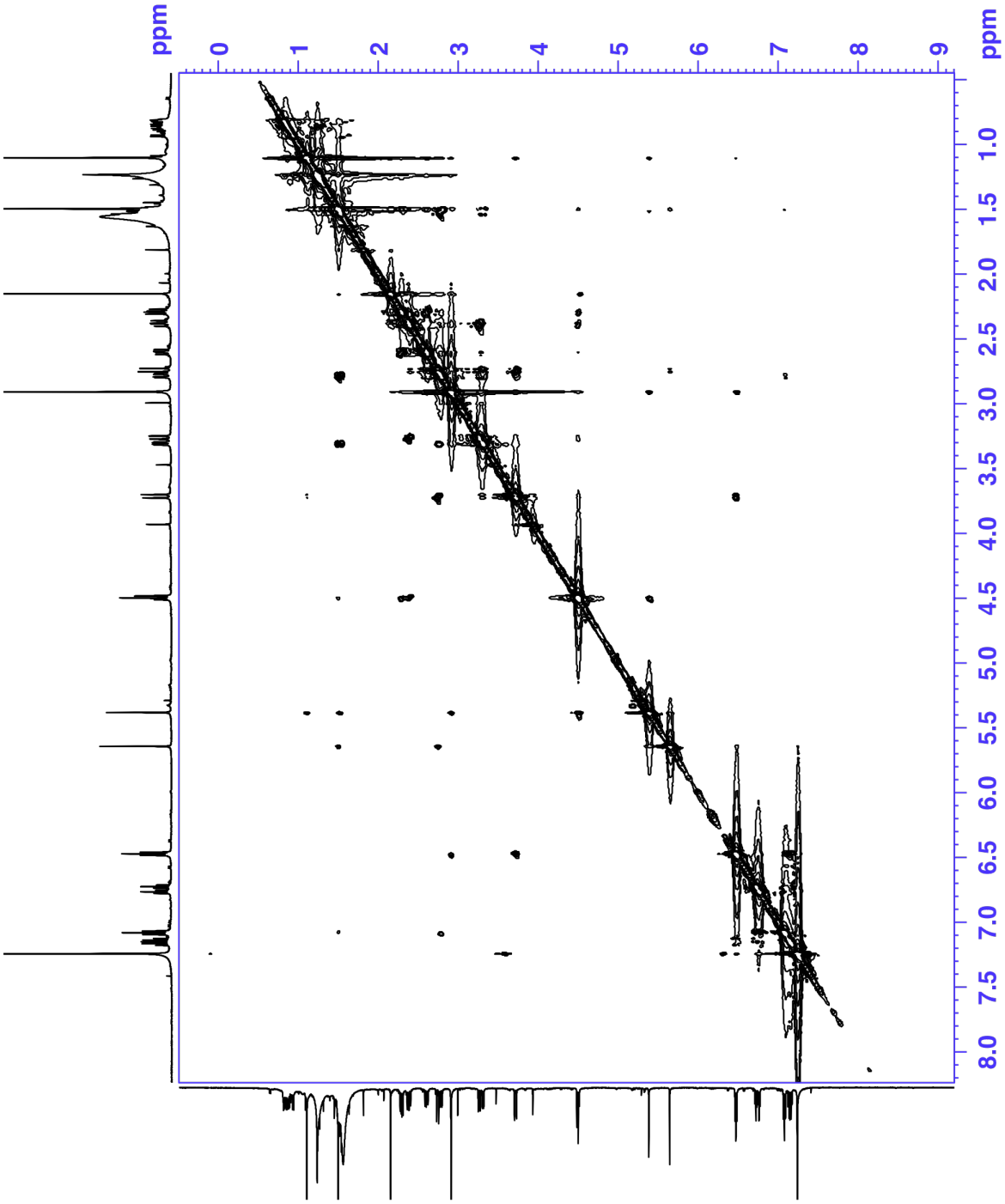

Figure S6. ROESY (600 MHz, $CDCl_3$) spectrum of nocardioazine A (**1**)

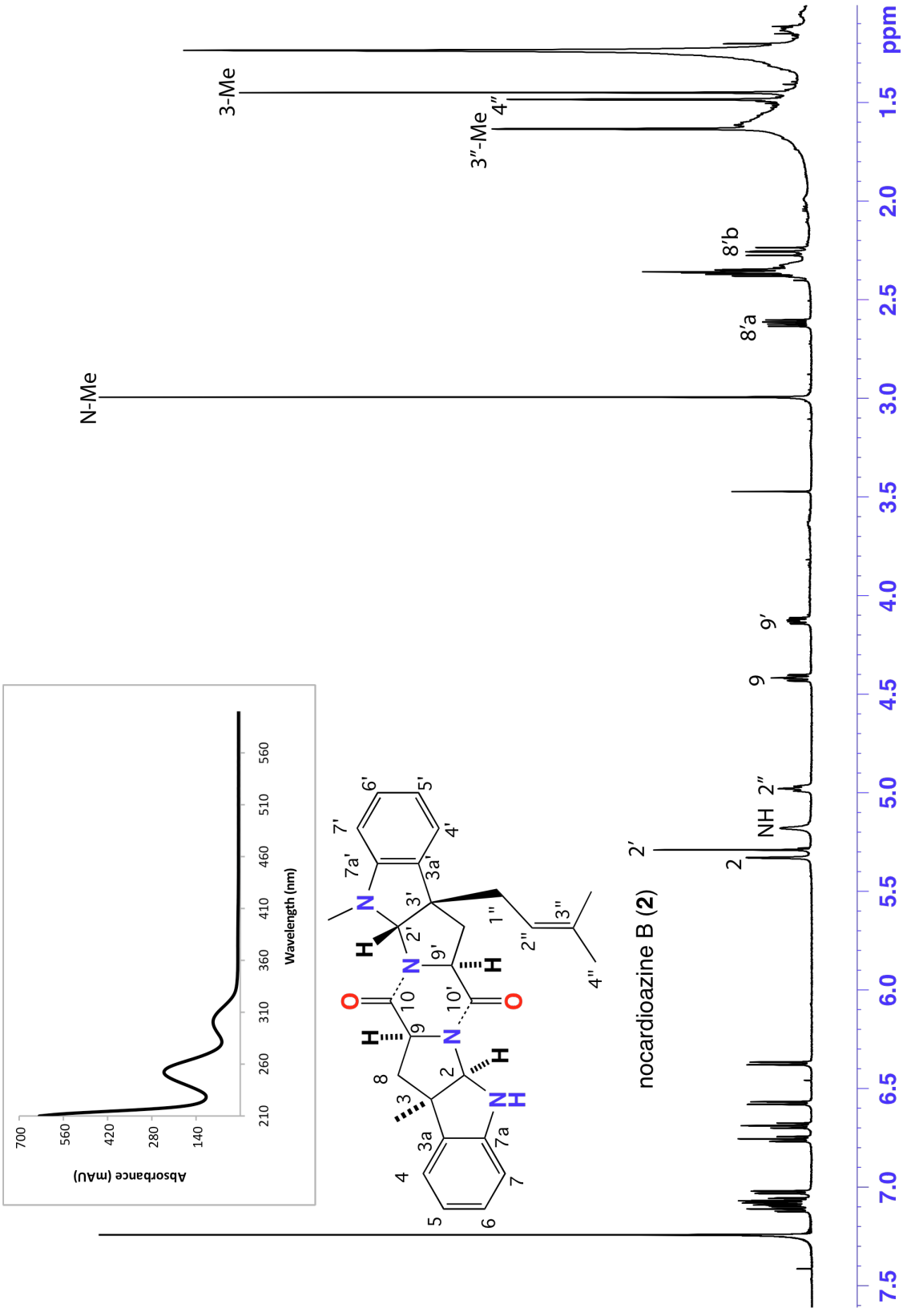

Figure S7. ^{1}H NMR (600 MHz, $CDCl_3$) and UV-vis (inset) spectra of nocardioazine B (**2**)

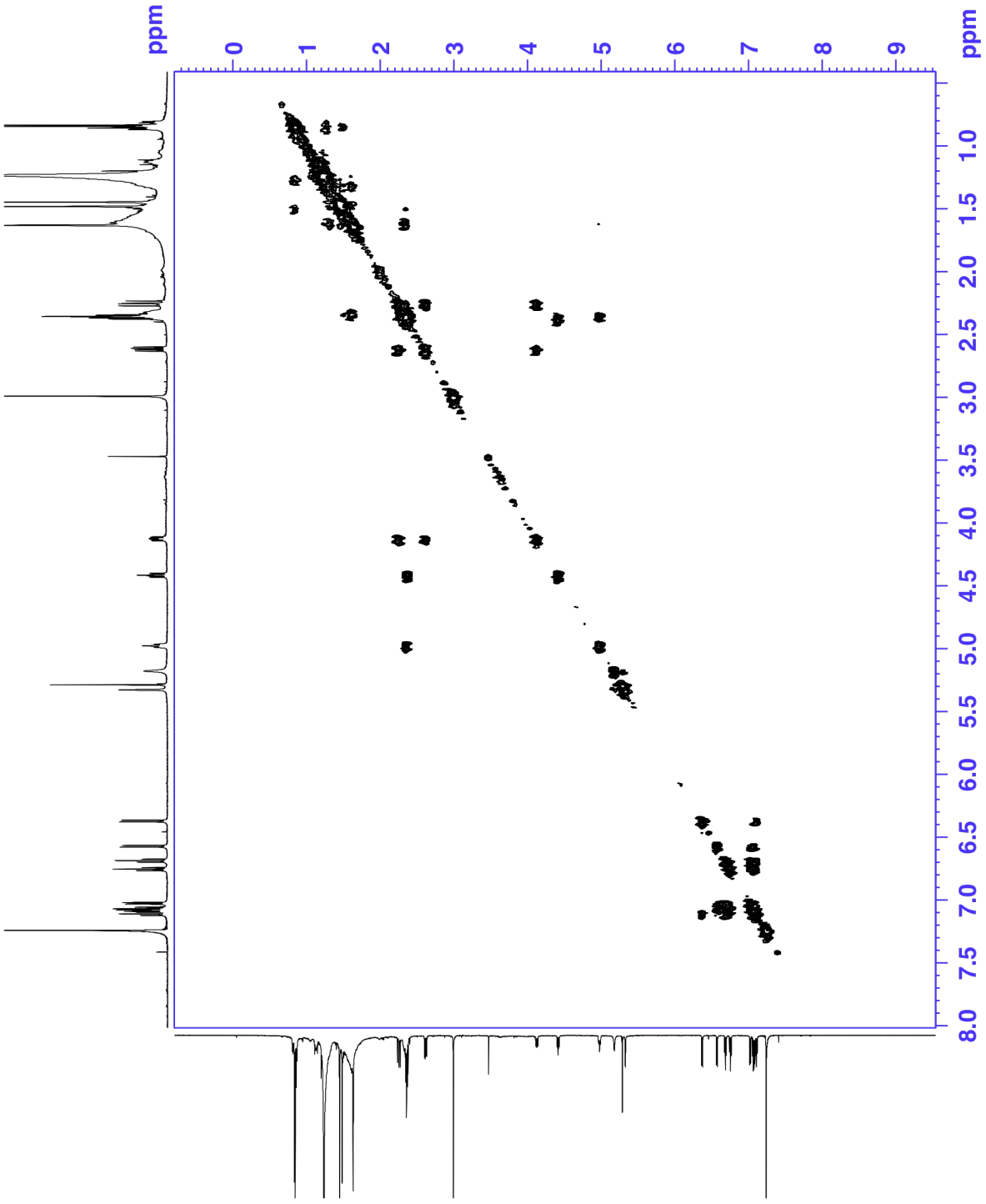

Figure S8. COSY (600 MHz, $CDCl_3$) spectrum of nocardioazine B (**2**)

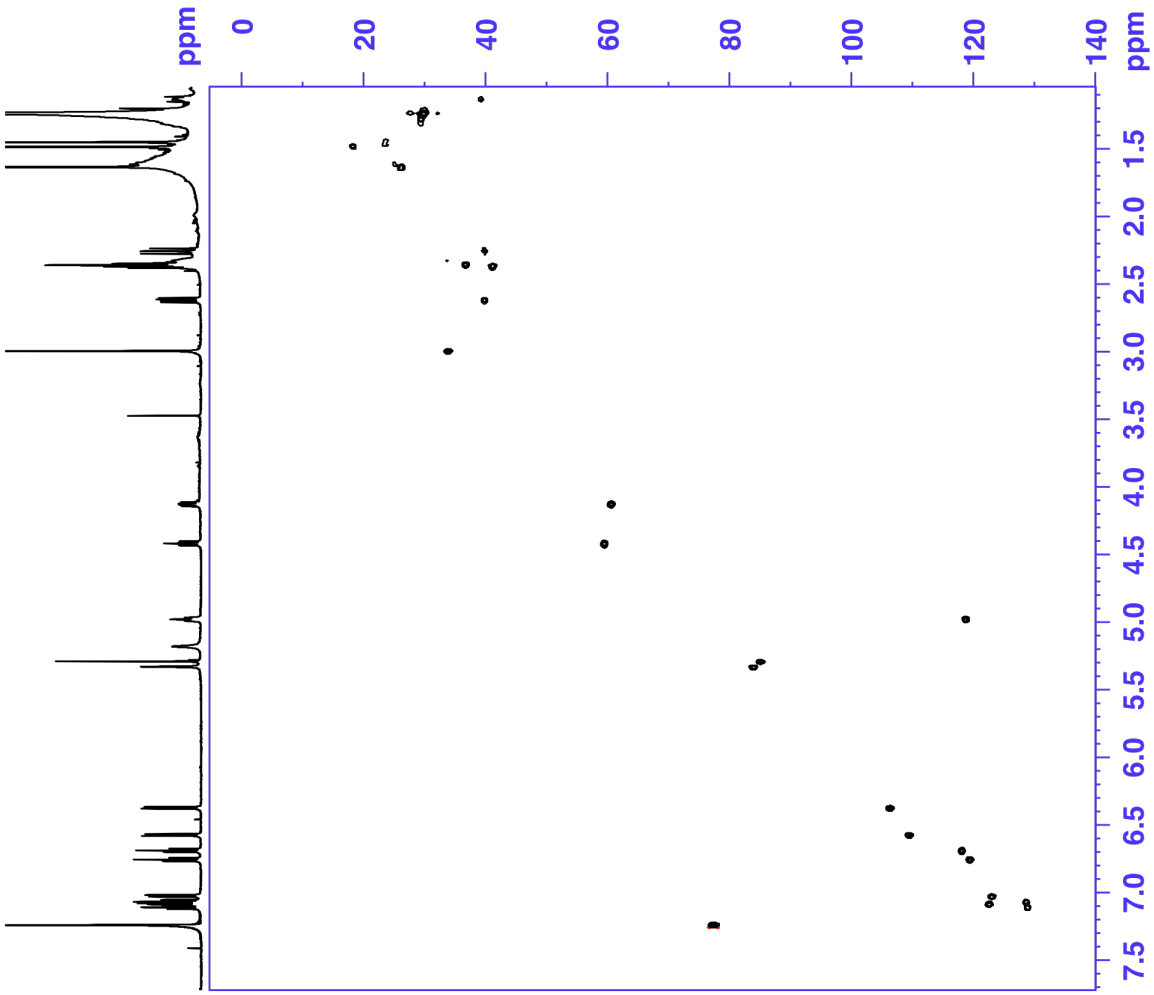

Figure S9. ^{1}H – ^{13}C HSQC (600 MHz, $CDCl_3$) spectrum of nocardioazine B (**2**)

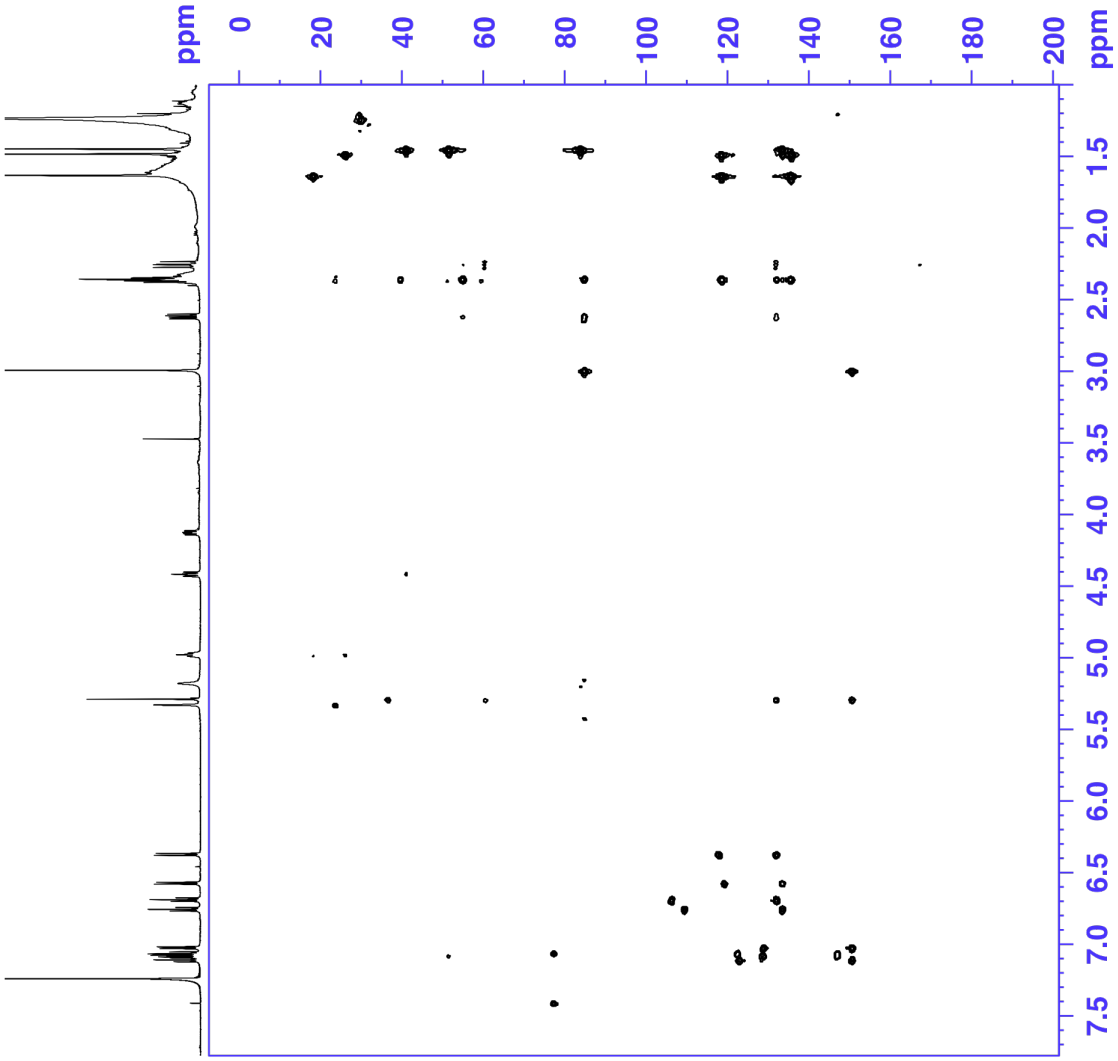

Figure S10. ^{1}H – ^{13}C HMBC (600 MHz, $CDCl_3$) spectrum of nocardioazine B (**2**)

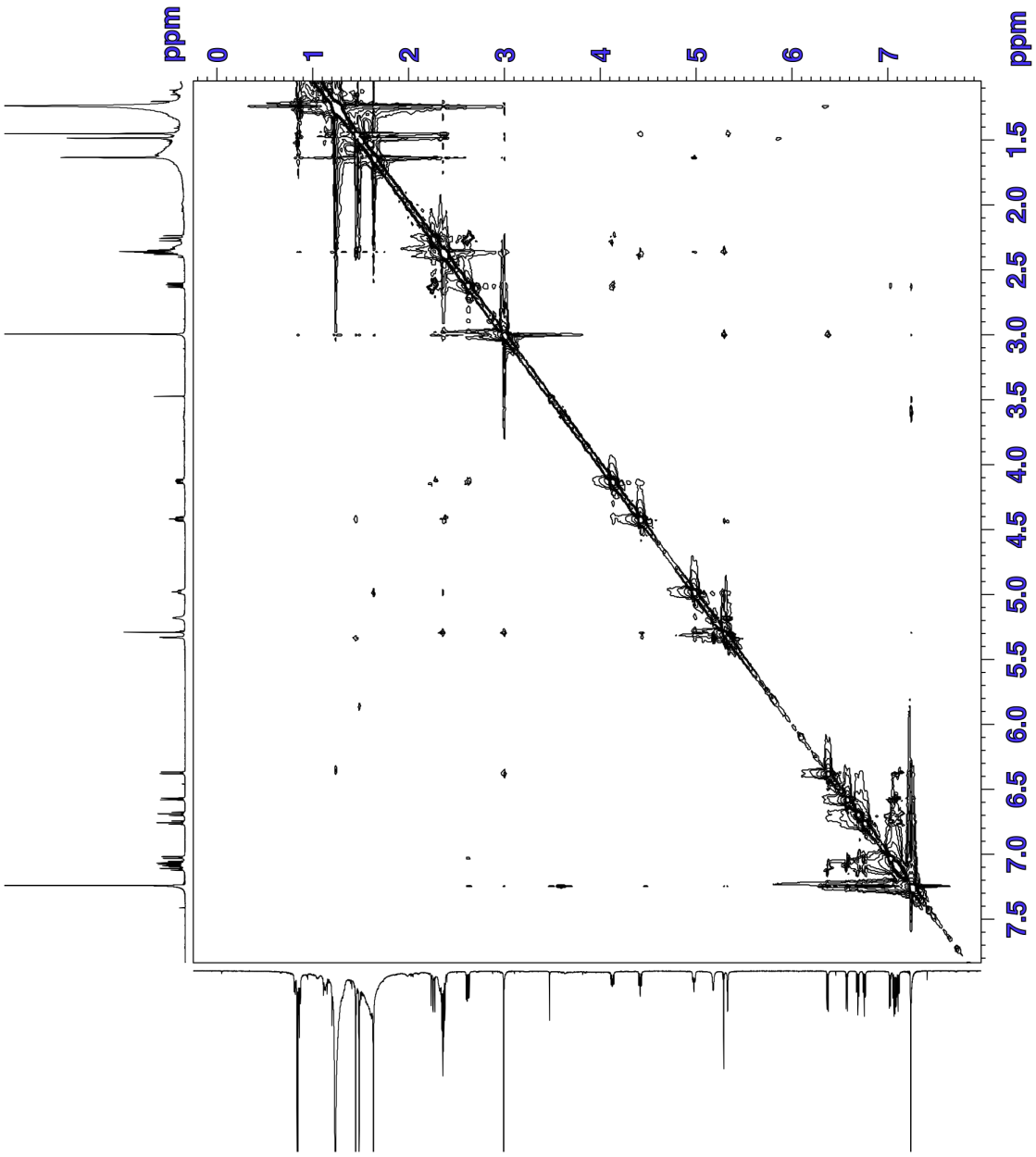

Figure S11. ROESY (600 MHz, $CDCl_3$) spectrum of nocardioazine B (**2**)

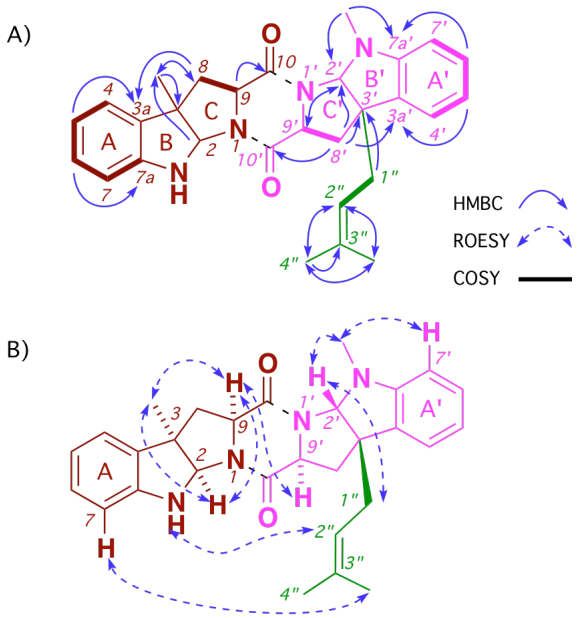

Figure S12. a) HMBC, COSY and b) ROESY 2D NMR correlations of nocardioazine B (**2**)

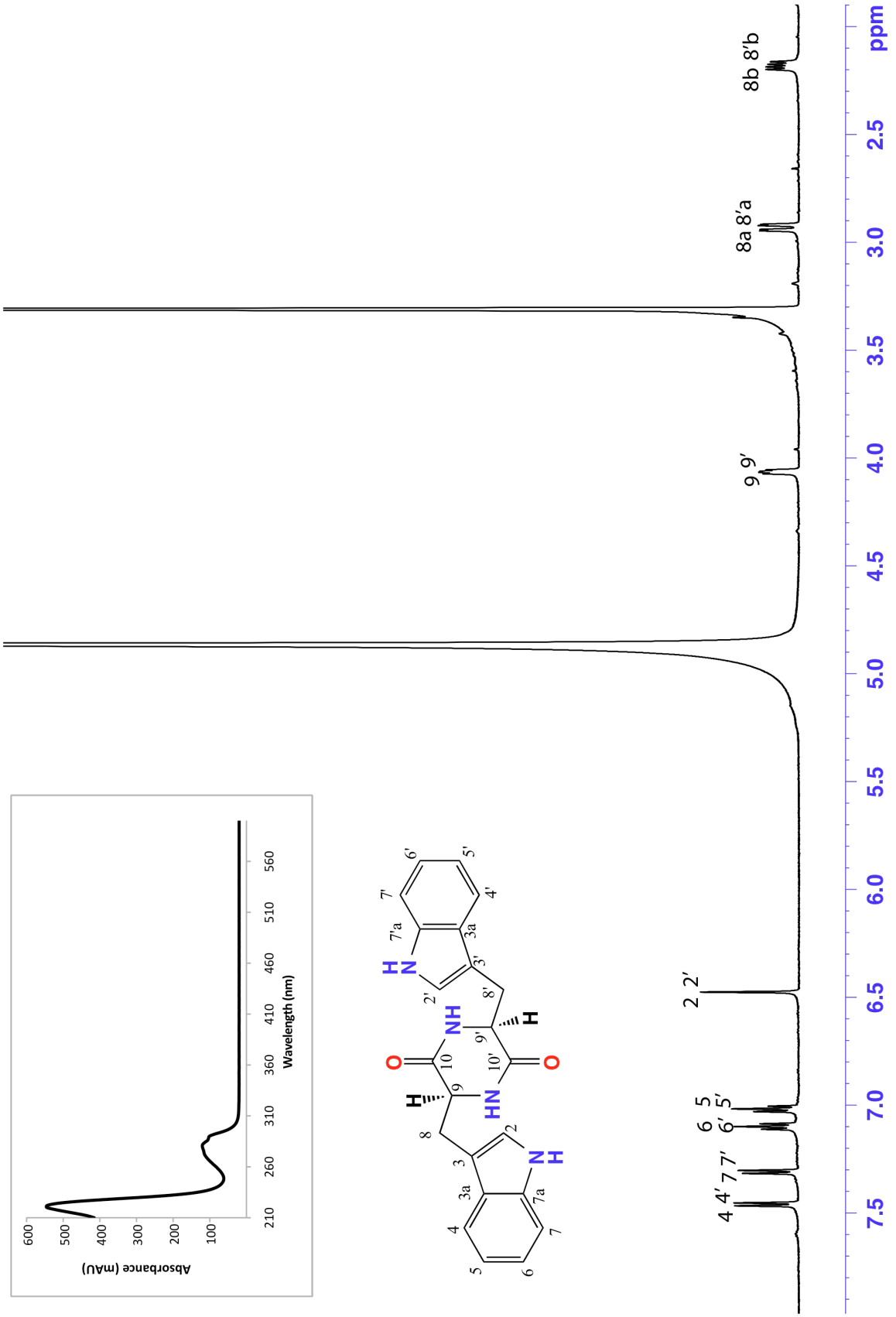

Figure S13. ^{1}H NMR (600 MHz, methanol-d_4) and UV-vis (inset) spectra of *cyclo*(L-Trp-L-Trp)

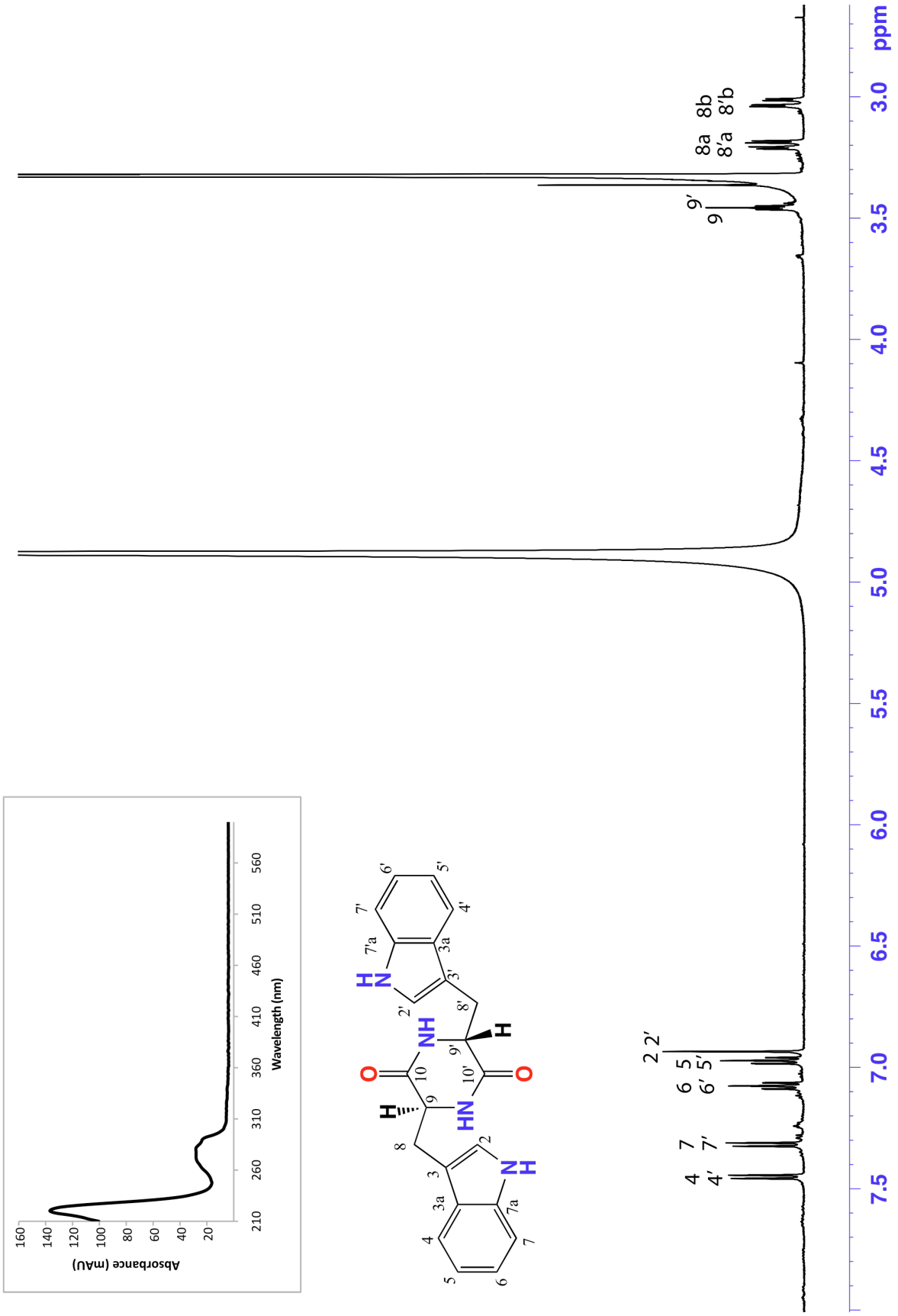

Figure S14. ^{1}H NMR (600 MHz, methanol-d_4) and UV-vis (inset) spectra of cyclo(L-Trp-D-Trp)

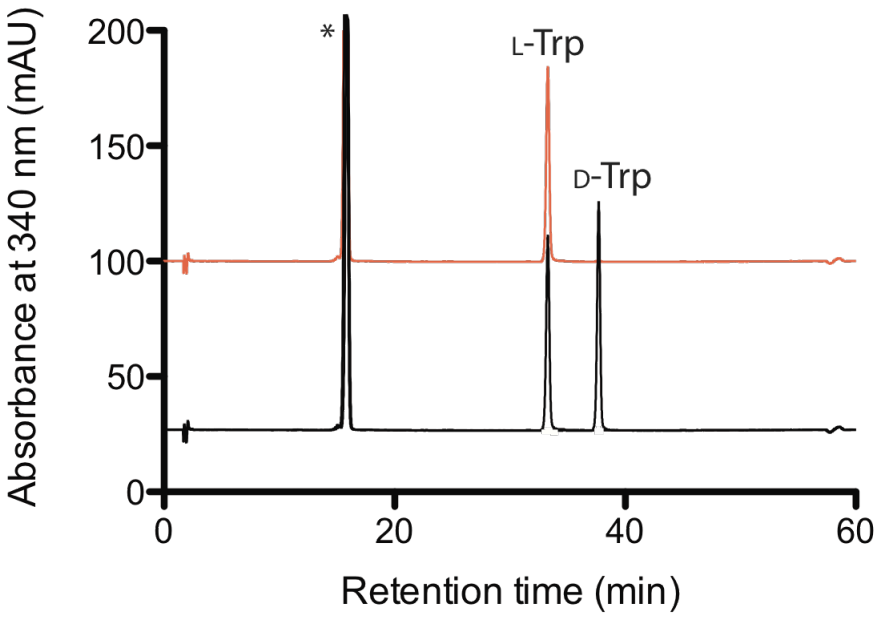

Figure S15. HPLC traces (340 nm) from LC-MS Marfey's analysis of *cyclo*-(L-Trp-L-Trp) and *cyclo*-(L-Trp-D-Trp). Identity of amino acids was confirmed by retention time and molecular weight. (In red) acid hydrolysed *cyclo*-(L-Trp-L-Trp) (50 μg) showing the presence of L-Trp (t_R=33.3 min). (In Black) acid hydrolysed *cyclo*-(L-Trp-D-Trp) (50 μg) showing the presence of L-Trp (t_R=33.3 min) and D-Trp (t_R=37.7 min). HPLC conditions, Zorbax SB-C_3 column (150 × 4.6 mm, 5 μm), 1 mL/min, gradient of 15-60% MeOH/H_2O (isocratic 5% MeCN containing 1% formic acid) over 55 min. * residual Marfey's reagent

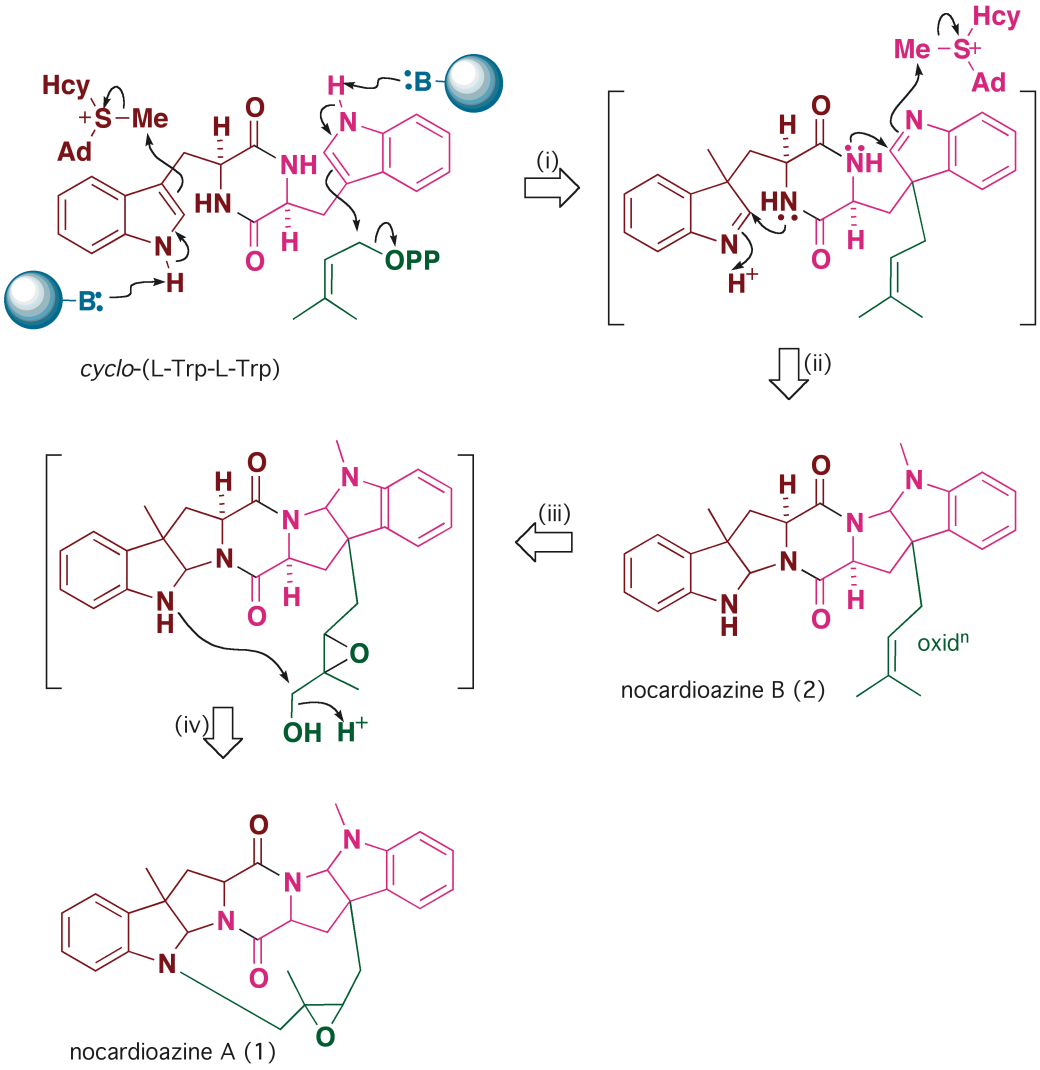

Figure S16. Proposed biosynthesis of the nocardioazines

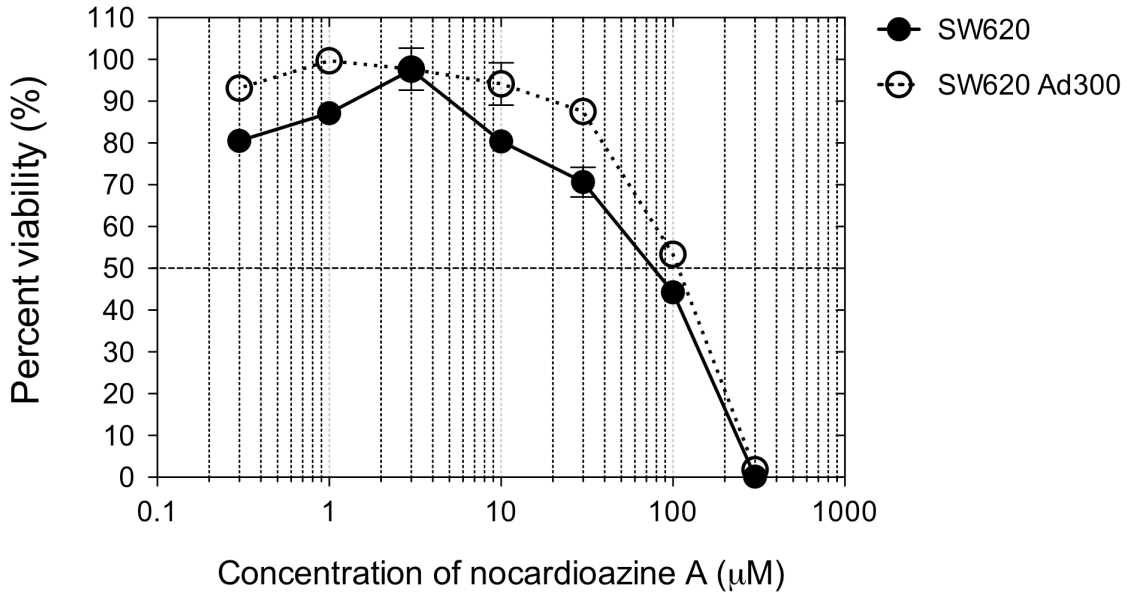

Figure S17. Cytotoxicity of nocardioazine A (**1**) to parental (SW620; IC_{50} = 59 μM) and MDR (SW620 Ad300; IC_{50} = 100 μM) colon cancer cell lines.

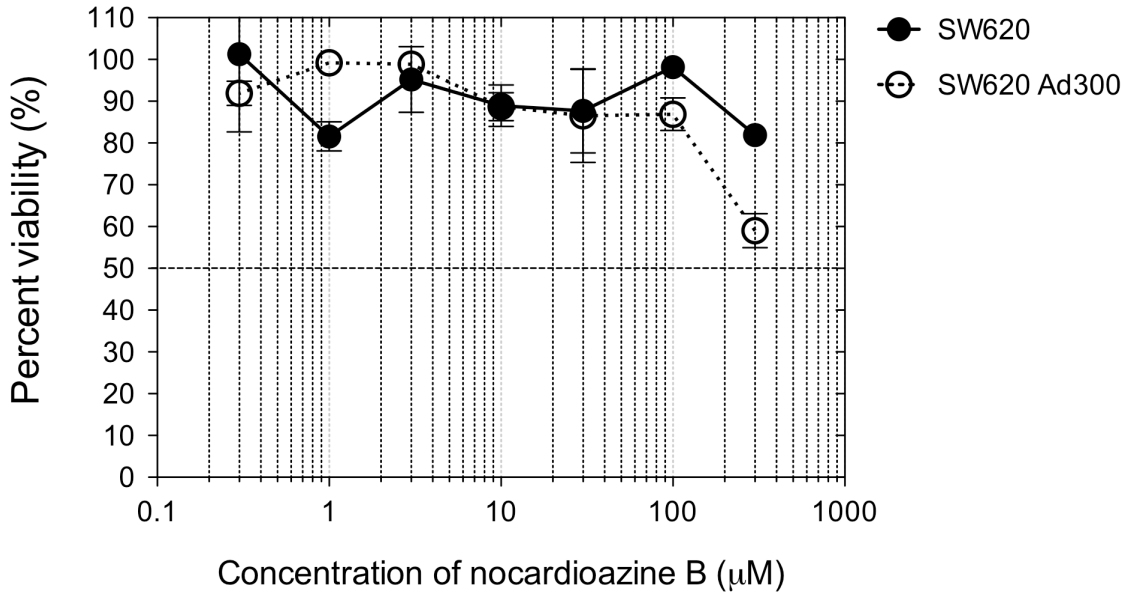

Figure S18. Cytotoxicity of nocardioazine B (**2**) to parental (SW620; IC_{50} > 300 μM) and MDR (SW620 Ad300; IC_{50} > 300 μM) colon cancer cell lines.

Table S1. NMR (600 MHz, $CDCl_3$) for nocardioazine A (**1**)

No:	^{1}H ppm (mult, *J* in Hz)	^{13}C ppm	COSY	ROESY	HMBC
2	5.64, s	88.0		4″b, 3-Me	8, 9, 3-Me
3		51.0			
3a		133.0			
4	7.07, dd, (*7.4, 1.1*)	121.1	5	3-Me	6, 7a
5	6.73, ddd, (*7.7, 7.4, 0.7*)	117.8	4, 6		3a, 7
6	7.14, ddd, (*7.7, 7.7, 1.2*)	128.4	5, 7		4, 7a
7	6.47, dd, (*7.7, 0.7*)	105.4	6	4″a, 3″-Me	3a, 5
7a		146.9			
8a	2.60, dd, (*13.7, 9.1*)	40.1	8b, 9	8b	3, 3a, 3-Me, 9, 10
8b	2.30, dd, (*13.7, 8.7*)		8a, 9	8a	2, 3, 3a, 3-Me, 10
9	4.50, m	59.2	8a/b	8b, 3-Me	8, 10
10		168.8			
2′	5.38, s	91.5		1″b, 1′-NMe, 3″-Me	1″, 8′, 9′
3′		50.9			
3′a		133.6			
4′	7.09, dd, (*7.6, 1.2*)	121.5	5′	1″a	6′, 7′a
5′	6.76, ddd, (*7.6, 7.5, 0.8*)	118.4	4′, 6′		3′a, 7′
6′	7.16, ddd, (*7.7, 7.5, 1.2*)	128.8	5′, 7′		4′, 7′a
7′	6.48, dd, (*7.7, 0.8*)	107.4	6′	1′-NMe	3′a, 5′
7′a		148.8			
8′a	3.26, dd, (*14.4, 1.6*)	36.4	8′b	8′b	1″, 2′, 3′, 10′
8′b	2.38, dd, (*14.4, 11.0*)		8′a, 9′	8′a	1″, 3′a, 10′
9′	4.49, m	61.7	8′b	8′a/b	
10′		169.6			
1″a	2.79, dd, (*11.0, 3.7*)	34.9	1″b	4′	
1″b	1.51, obscured		1″a, 2″	2′	
2″	3.30, dd, (*11.0, 3.7*)	62.4	1″b	4″b	
3″		61.4			
4″a	3.72, d, (*15.5*)	53.3	4″b	3″-Me, 7	2, 2″, 3″, 7a
4″b	2.74, d, (*15.5*)		4″a	2, 2″	2, 3″, 3″-Me, 7a
3″-Me	1.10, s	14.1		2′, 4″a	2″, 3″, 4″
3-Me	1.50, s	19.9		2, 4, 9	2, 3, 3a, 8
1′-NMe	2.91, s	34.2		2′, 7′	2′, 7′a

Table S2. NMR (600 MHz, benzene-d_6) for nocardioazine A (**1**)

pos	δ_H, mult (*J* in Hz)	δ_C	COSY	ROESY	$^1H - ^{13}C$ HMBC
2	5.31	88.3		3-Me, 4″b, 9	3-Me, 8, 9
3		51.4			
3a		134.1			
4	6.81, dd (*7.4*, *0.8*)	121.4	5		6, 7a
5	6.69, ddd (*7.4*, *7.4*, *0.7*)	118.1	4, 6		3a, 7
6	7.02, ddd, (*7.9*, *7.4*, *0.8*)	129.2	5, 7		4, 7a
7	6.11, dd (*7.9*, *0.7*)	105.9	6	3″-Me, 4″a	3a, 5
7a		148.0			
8a	2.63, dd (*13.7*, *9.0*)	40.9	8b, 9		3, 3a, 9, 10
8b	1.80, dd (*13.7*, *9.0*)		8a, 9	3-Me	2, 3-Me
9	3.47[a], m	59.3	8a/b	2, 3-Me	8, 10
10		169.3			
2′	5.29, s	92.0		1″b, 1′-NMe, 3″-Me	1″, 8′, 9′
3′		51.5			
3′a		135.1			
4′	6.73[b], m	122.3	5′		
5′	6.73[b], m	118.9	4′, 6′		
6′	7.10, m	129.2	5′, 7′		4′, 7′a
7′	6.29, d (*7.8*)	107.8	6′	1′-NMe	3′a, 5′
7′a		149.5			
8′a	3.16, dd (*14.2*, *1.7*)	36.7	8′b, 9		1″, 3′, 9′, 10′
8′b	2.09, dd (*14.2*, *11.0*)		8′a, 9		3′a, 10′
9′	3.70, dd (*11.0*, *1.7*)	61.9	8′a/b		3′, 8, 10′
10′		169.9			
1″a	2.54, dd (*14.7*, *11.1*)	35.7	1″b, 2″	2″, 8′a	2′
1″b	1.26, dd (*14.7*, *11.1*)		1″a, 2″	2′	2′, 3″, 8′
2″	3.46[a], m	62.8	1″a/b	1″a, 4″b, 8′a, 8b	
3″		62.9			
4″a	3.65, d (*15.2*)	54.0	4″b	3″-Me, 7	2, 2″, 3″, 7a
4″b	2.86, d (*15.2*)		4″a	2	2, 2″, 3″, 3″-Me
3″-Me	1.05, s	14.7		2′, 4″a, 7	3″, 4, 9′
3-Me	0.93, s	19.8		2, 8b, 9	2, 3, 3a, 8
1′-NMe	2.85, s	34.4		7′	2′, 7′a

[[a,b] overlapping signals]

Table S3. NMR (600 MHz, $CDCl_3$) for nocardioazine B (**2**)

pos	δ_H, mult (*J* in Hz)	δ_C	COSY	ROESY	$^1H - ^{13}C$ HMBC
1	5.18, s			2″	
2	5.33, s	83.7		3-Me	3-Me
3		51.2			
3a		133.3			
4	7.06, dd (*7.4*, *1.0*)	128.5	5		6, 7a
5	6.75, ddd (*7.4*, *7.*4, *0.9*)	119.2	4, 6		3a, 7
6	7.08, ddd (*7.7*, *7.4*, *1.0*)	122.4	5, 7		4, 7a
7	6.57, dd (*7.7*, *0.9*)	109.4	6		3a, 5
7a		146.9			
8	2.37[a], m	41.0	9		3-Me, 3a, 3, 9
9	4.42, ddd (*9.8*, *7.6*, *1.8*)	59.3	8	3-Me, 9′	8
10		NR			
2′	5.29, s	85.0		1″, 1′-NMe, 8′b	1″, 3′a, 7′a, 9′
3′		54.6			
3′a		132.0			
4′	7.02, dd (*7.4*, *1.1*)	122.8	5′	8′a	6′, 7′a
5′	6.69, ddd (*7.4*, *7.4*, *0.9*)	117.9	4′, 6′		3′a, 7′
6′	7.11, ddd (*7.7*, *7.4*, *1.1*)	128.8	5′, 7′		4′, 7′a
7′	6.38, dd (*7.7*, *0.9*)	106.2	6′	1′-NMe	3′a, 5′
7′a		150.6			
8′a	2.62, dd (*12.9*, *6.5*)	39.8	8′b, 9	4′, 9′	3′a, 2′, 3′
8′b	2.25, dd (*12.9*, *10.7*)		8′a, 9	2′	3′a, 9′, 10′
9′	4.13, ddd (*10.7*, *6.5*, *1.7*)	60.4	8′a/b	8′a, 9	
10′		167.1			
1″	2.35[a], m	36.7	2″	2′	2′, 2″, 3′, 3″, 8′
2″	4.98, m	118.6	1″	4″	3″-Me, 4″
3″		135.5			
3″-Me	1.48, s	17.9		3″-Me, 7	2″, 3″, 4″
4″	1.63, s	26.0		2″	2″, 3″, 3″-Me
3-Me	1.45, s	23.4		2, 9	2, 3, 8
1′-NMe	2.99, s	33.7		2′, 7′	2′, 7′a

[[a] overlapping signals], NR: not recorded

Table S4. NMR (600 MHz, methanol-d_4) data for *cyclo*-(L-Trp-L-Trp)

pos	δ_H, mult (*J* in Hz)	δ_C	COSY	HMBC
2, 2′	6.48, s	125.6		3 (3′), 3a (3′a), 7a (7′a)
3, 3′		109.3		
3a, 3′a		128.3		
4, 4′	7.46, d (*7.9*)	119.4	5 (5′)	3 (3′), 6 (6′), 7a (7′a)
5, 5′	7.02, dd (*8.1*, *7.9*)	119.8	4 (4′), 6 (6′)	3a (3′a), 7 (7′)
6, 6′	7.10, dd (*8.1*, *7.9*)	122.3	5 (5′), 7 (7′)	4 (4′), 7a (7′a)
7, 7′	7.31, d (*7.9*)	112.3	6 (6′)	3a (3′a), 5 (5′)
7a, 7′a		137.8		
8a, 8′a	2.93, dd (*14.4*, *3.6*)	31.4	8b (8′b), 9 (9′)	
8b, 8′b	2.18, dd (*14.4*, *6.9*)		8a (8′a), 9 (9′)	2 (2′), 3 (3′), 3a (3′a), 9 (9′)
9, 9′	4.06, dd (*6.9*, *3.6*)	56.7	8a/b (8′a/b)	10 (10′)
10, 10′		169.7		

Table S5. NMR (600 MHz, methanol-d_4) data for *cyclo*-(L-Trp-D-Trp)

pos	δ_H, mult (*J* in Hz)	δ_C	COSY	HMBC
2, 2′	6.92, s	125.1		3 (3′), 3a (3′a), 7a (7′a)
3, 3′		109.0		
3a, 3′a		128.4		
4, 4′	7.44, d (*8.0*)	119.4	5 (5′)	3 (3′), 6 (6′), 7a (7′a)
5, 5′	6.96, dd (*8.0*, *7.7*)	119.8	4 (4′), 6 (6′)	3a (3′a), 7 (7′)
6, 6′	7.06, dd (*8.0*, *7.7*)	122.3	5 (5′), 7 (7′)	4 (4′), 7a (7′a)
7, 7′	7.30, d (*8.0*)	118.3	6 (6′)	3a (3′a), 5 (5′)
7a, 7′a		137.7		
8a, 8′a	3.18, dd (*14.9*, *5.1*)	29.9	8b (8′b), 9 (9′)	2 (2′), 3 (3′), 9 (9′)
8b, 8′b	3.01, dd (*14.9*, *4.5*)		8a (8′a), 9 (9′)	2 (2′), 3 (3′), 9 (9′), 10 (10′)
9, 9′	3.44, dd (*5.1*, *4.5*)	56.1	8a/b (8′a/b)	3 (3′), 10 (10′)
10, 10′		170.6		