# SUPPLEMENTARY INFORMATION

## Discovery of Cryptic Polyketide Metabolites from Dermatophytes using Heterologous Expression in *Aspergillus nidulans*

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Running Title: Dermatophyte Polyketide Metabolite

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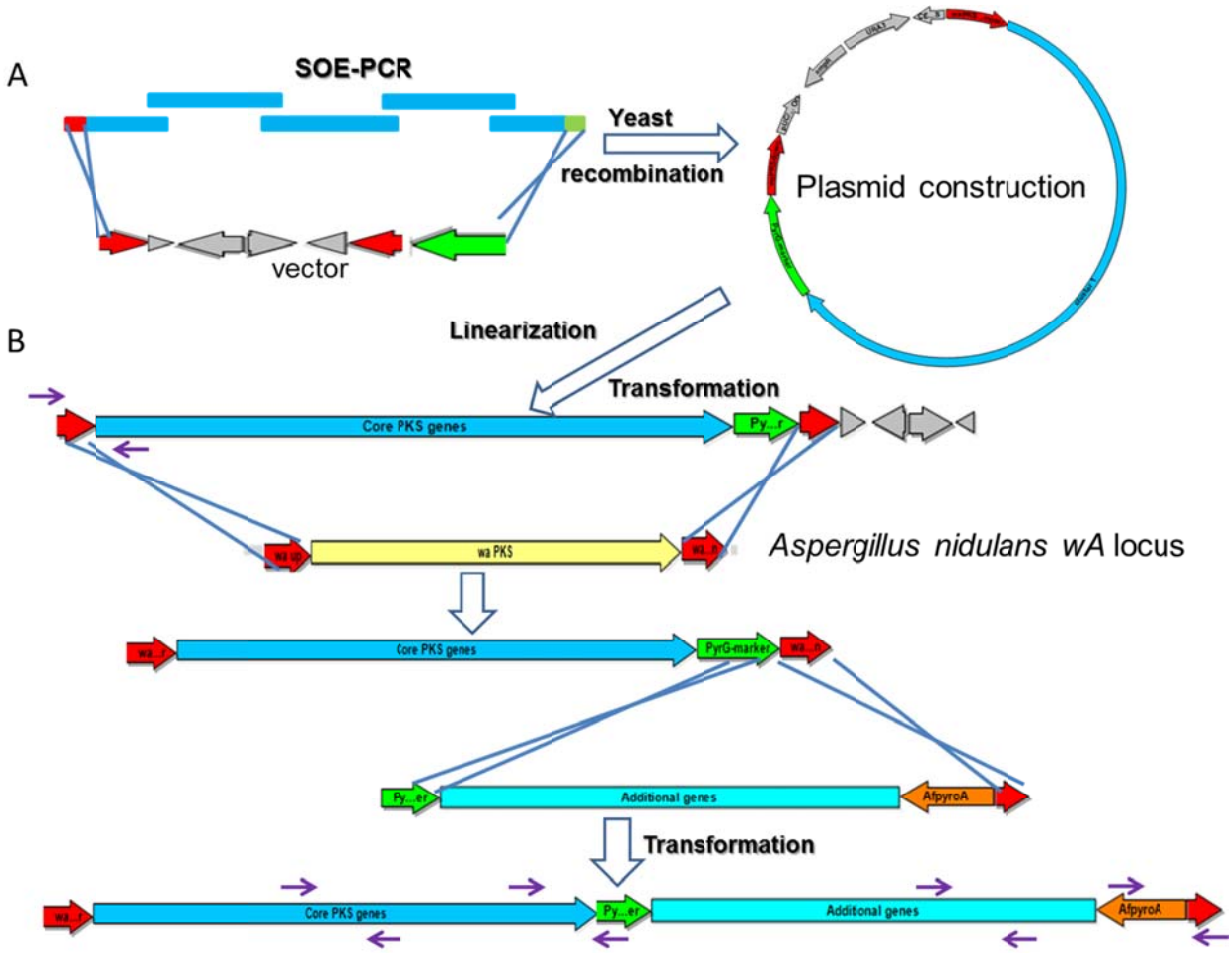



**Figure S1. Strategies for assembling of DNA fragment in yeast and transformation of *A. nidulans***

(A) SOE PCR and yeast homologous recombination were used for assembling of large DNA fragments as described previously (*1*). (B) Transformation was performed by replacing of *A. nidulans* wA PKS gene with targeted core PKS genes plus *A. fumigatus* pyrG (AfpyrG) maker for the first part of DNA fragment integration. The second integration of DNA fragment with *A. fumigatus* pyroA (AfpyroA) was inserted to the same wA locus with 1 kb flanking regions of AfpyrG and wA. The recipient strain is RJMP1.49 (*pyrG89, pyroA4, ΔnkuA::argB, veA*). The screening of transformants is performed by picking white spore strains in combination with diagnostic PCR using primers as shown in purple arrows.

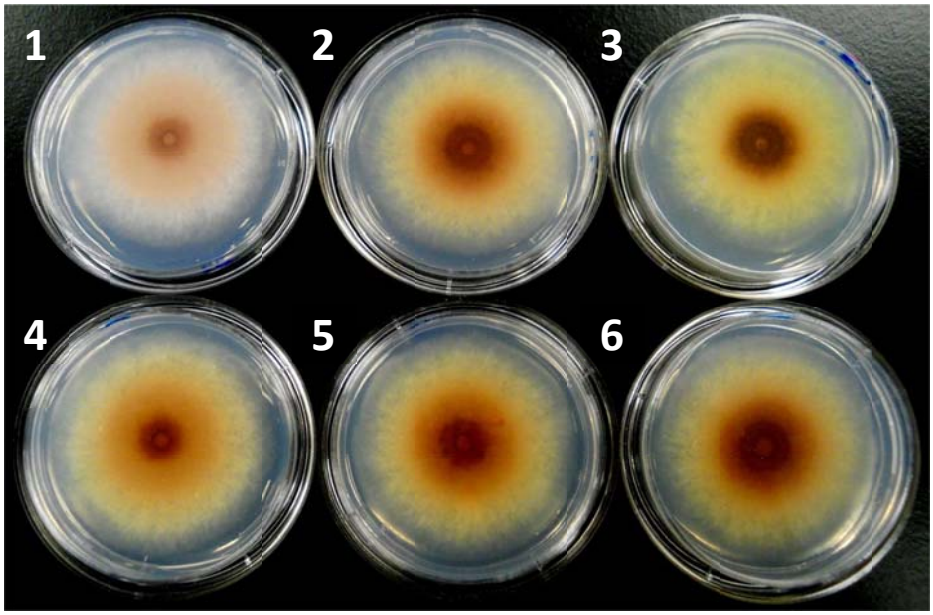



**Figure S2. Phenotypes of control strain and dermatophyte cluster transformants in *A. nidulans.***

Strains were point-inoculated and grown on minimal medium for 3 days at 37 °C. 1: CK (TWY2.2), 2: TESG_06695 to 6702 (TWY1.1), 3: MCYG_03596 to 3602 (TWY3.3), 4: TESG_06695 to 6706 (TWY7.1), 5: TESG_06695-6706 (-6696) (TWY8.1), 6: TESG_06695 to 6702 (+6701) (TWY9.1).

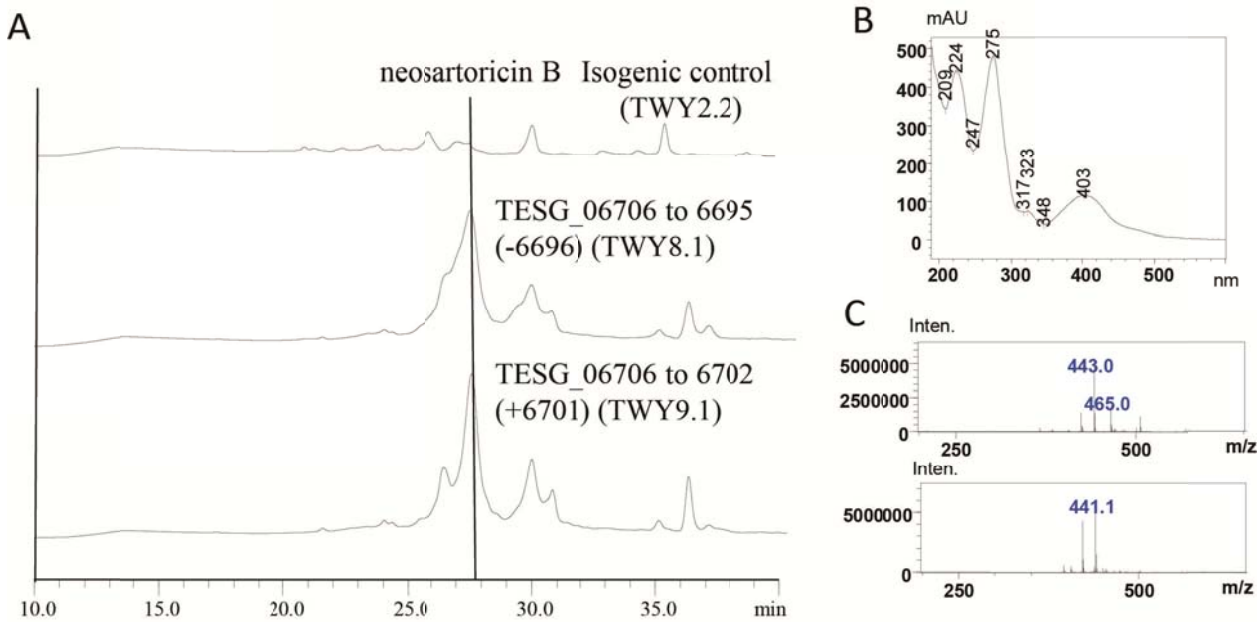



**Figure S3. LC-MS analysis of *A. nidulans* mutants.**

(A) HPLC traces (400 nm) of organic extracts from different strains. Trace 1: isogenic control (TWY2.2); Trace 2: TESG_06706 to 6695 (-6696) (TWY8.1); Trace 3: TESG_06706 to 6702 (+6701) (TWY9.1); (B) UV absorption of neosartoricin B; (C) Positive and negative masses of neosartoricin B, m/z 443.0 $[M+H]^+$, 465.0 $[M+Na]^+$, 441.1 $[M-H]^-$.

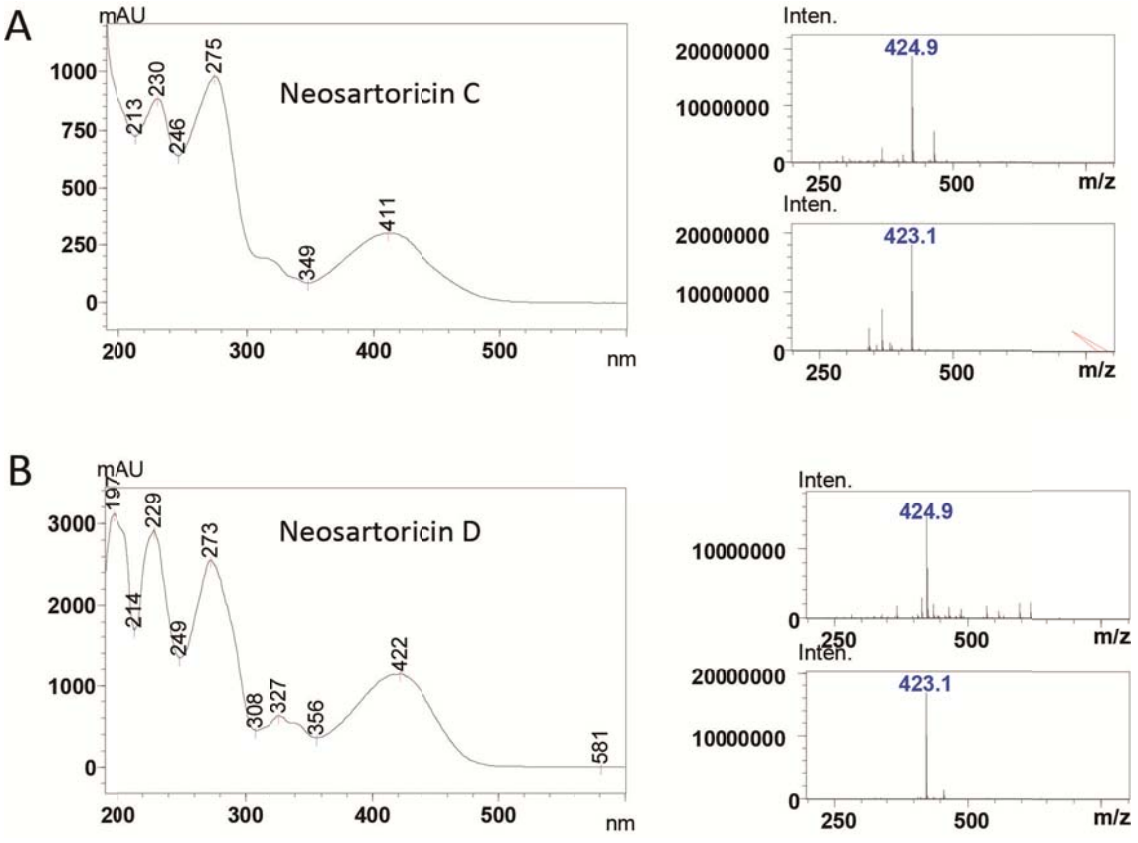



**Figure S4. UV absorption and masses *of* neosartoricins C and D.**

(A) UV absorption, positive and negative masses of neosartoricin C, m/z 424.9 $[M+H]^+$, 423.1 $[M-H]^-$; (B) UV absorption, positive and negative masses of neosartoricin D, m/z 424.9 $[M+H]^+$, 423.1 $[M-H]^-$.

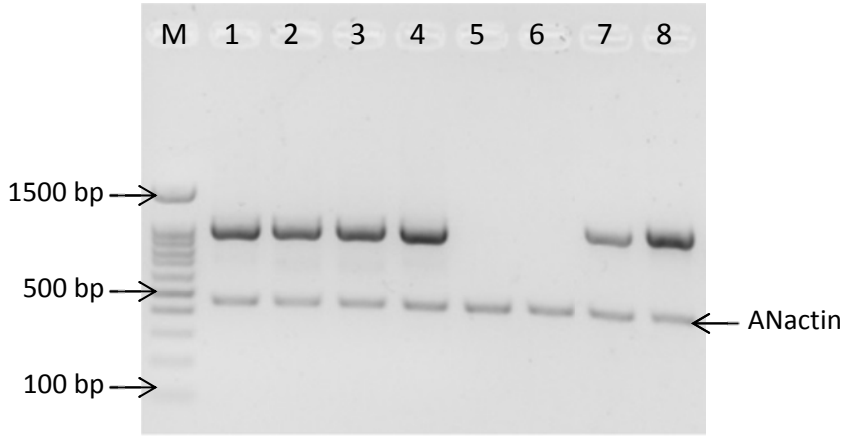



**Figure S5. RT-PCR analysis of dermatophyte gene transcription in *A. nidulans* mutants**

RT-PCR products were loaded into 1.5 % agarose gel. ANactin from *A. nidulans* was used for the relative quantification of the cDNA template. Lane M: 100 bp DNA Ladder (lamda Biotech); lane 1 and 2: TF1 (TESG_06706) and NR-PKS (TESG_06702) from TWY1.1; lane 3 to 6: TF1, NR-PKS, TESG_06698 and TESG_06701 from TWY7.1; lane 7 and 8: MCYG_03602 and MCYG_03598 from TWY3.3.

## Supplementary Tables

### Table S1. Fungal strains and plasmids used in this study

| Strain/plasmid | Descrption | Reference |
|---|---|---|
| RJMP1.49 | *pyrG89, pyroA4, ΔnkuA::argB, veA* | Yin *et al.* (*2*) |
| TWY1.1 | *pyroA4, pyrG89, ΔwApks::gpdA(p)::TESG_06706 to 6702::AfpyrG, ΔnkuA::argB, veA* | This study |
| TWY2.2 | *pyroA4, pyrG89, ΔwApks::AfpyrG, ΔnkuA::argB, veA* | This study |
| TWY3.3 | *pyroA4, pyrG89, ΔwApks::gpdA(p):: gpdA::MCYG_03602 to 3596::AfpyrG, ΔnkuA::argB, veA* | This study |
| TWY7.1 | *pyroA4, pyrG89, ΔwApks::gpdA(p)::TESG_06706 to 6702::AfpyrG::TESG_06701 to 6695::AfpyroA, ΔnkuA::argB, veA* | This study |
| TWY8.1 | *pyroA4, pyrG89, ΔwApks::gpdA(p)::TESG_06706 to 6702::AfpyrG::TESG_06701 to 6695(-6696)::AfpyroA, ΔnkuA::argB, veA* | This study |
| TWY9.1 | *pyroA4, pyrG89, ΔwApks::gpdA(p)::TESG_06706 to 6702::AfpyrG::TESG_06701::AfpyroA, ΔnkuA::argB, veA* | This study |
| pYH-WA-pyrG | *URA3, WA flanking, AfpyrG, Amp* | This study |
| pWY13.2 | *gpdA::TESG_06706 to 6702 in pYH-WA-pyrG* | This study |
| pWY14.1 | *gpdA::MCYG_03602 to 3596 in pYH-WA-pyrG* | This study |
| pWY15 | *TESG_06701 to 6695 in pYH-WA-pyrG* | This study |
| pWY16 | *TESG_06701 to 6695(-6696) in pYH-WA-pyrG* | This study |
| pWY17.1 | *TESG_06701AfpyroA in pYH-WA-pyrG* | This study |

pXX = plasmid, TXX = original transformant

126 **Table S2. PCR primer sets utilized in this study**

| Name of the primer | Oligonucleotide sequence (5'-3') | Uses |
|---|---|---|
| pYUCA-wAup-F | GATACGAGGCGCGTGTAAGTTACCTCTGGAACAGTCTCGCCGT | Wapks upstream amplification |
| pyrG-wAup-R | CATATTTCGTCAGACACAGAATAACTCTCGCTAGCGATCAGGAGAAGGAGAGTCAAGTC | |
| wAup-pyrG-R | GACTTGACTCTCCTTCTCCTGATCGCTAGCGAGAGTTATTCTGTGTCTGACGAAATATG | AfpyrG amplification |
| wAdn-pyrG-R | TGTCATAGTAAAGTGATTCGCGTCATGCGGCCGCATTCTGTCTGAGAGGAGGCACTG | |
| pyrG-wAdn-F | CAGTGCCTCCTCTCAGACAGAATGCGGCCGCATGACGCGAATCACTTTACTATGACA | wAPKS downstream amplification |
| pEamp-wAdn-R | GTTCTTTCCTGCGTTATCCCCTGTGCTGTCAGTACGCGAAGATCT | |
| wAdn-pEamp-F | AGATCTTCGCGTACTGACAGCACAGGGGATAACGCAGGAAAGAAC | pMB1 Ori + AmpR amplification |
| pYUCA-pEamp-R | TGGACCCTGATAACTTCGTATAATGTATGGCTCATGAGACAATAACCCTGA | |
| pEamp-pYUCA-F | TCAGGGTTATTGTCTCATGAGCCATACATTATACGAAGTTATCAGGGTCCA | CEN/ARS + ura3 amplification |
| wAup-pYUCA-R | ACGGCGAGACTGTTCCAGAGGTAACTTACACGCGCCTCGTATC | |
| pgpdA_F | GTCTGCTCATAGAAGATCGC | gpdA amplification |
| pgpdA_R | TTTGGTTTCGTTTCTCCATGTCCGGTTCTGTGCTGAATAGCGATTAAAGGTTCTTGGATG | |
| p1MCY_F | CTATTCAGCACAGAACCGGAC | SOE PCR for MCYG_03602 to 3596 amplification |
| p1MCY_R | CGGCTGTGTTTGAGAATAAGGC | |
| p2MCY_F | GCATCCACAGCTACAGAGCAG | |
| p2MCY_R | GTGTAGCACTTGTCGGTAGTTG | |
| p3MCY_F | GAGGATGCGGAAGCTGACAATG | |
| p3MCY_R | GCCTCATGTTCAGAGCATGAC | |
| p4MCY_F | CGCTGCAGAAACCTGTCTGAG | |
| p4MCYpyrG_R | ACACAACATATTTCGTCAGACACAGAATAACTCTCGCTAGCCTATGTCTTACCGATCTGC | |
| pScr1_F | CAACCTGAGGAAACTGCGTC | MCYG_03602 to 3596 screening |
| pScr1_R | CTATCTCGATCTTTTCCGCG | |
| pScr2_F | CTCTGGCCCGTCATTCAATC | |
| pScr2_R | GCTTTCTGAAGCGCTGGAATG | |
| pgpdAttr_R | GAGCAGCTTGCTGCCGTCGTTTTGGGCCTCGTTTCTCCATCGATTAAAGGTTCTTGGATG | SOE PCR for TESG_06706 to 6702 amplification |
| p1TTR_F | ATGGAGAAACGAGGCCCAAAAC | |
| p1TTR_R | CAAGAGGCTGTTCGACGGTC | |
| p2TTR_F | GGAAGTGTGTATGACTTGGGAG | |
| p2TTR_R | CGATGGTAGCAAGAATCCGATC | |
| p3TTR_F | CATGCTGTTCCAAATGGCGAAC | |
| p3TTR_R | GCATTCGACGAGAAGACGTAG | |
| p4TTR_F | GCACGGAAGATCGTATCACTC | |
| p4TTRpyrG_R | CACAACATATTTCGTCAGACACAGAATAACTCTCGCTAGCGATGATATCAATCACTCTGC | |
| p5TTR_F | TTGGCATCACGCATCAGTGCCTCCTCTCAGACAGA | SOE PCR for |

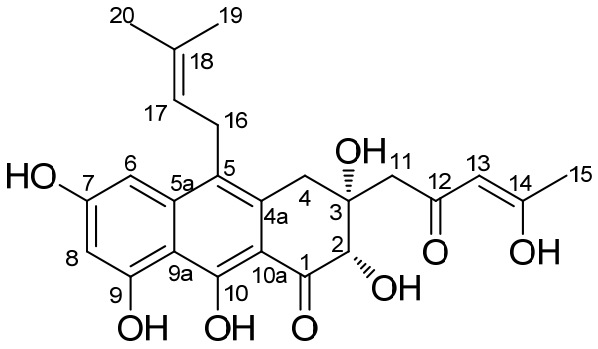
| | | |
|---|---|---|
| | ATGCATCGGGATTGATTATCAAATG | TESG_06701 to |
| p5TTR_R | GCATACTTGCCAGAATTTGCAG | 6695 amplification |
| p6TTR_F | CTTACTACAAAAGTCCCTTGC | |
| p6TTR_R | GTTTCAGTTAATTCTCCCTTTC | |
| p6AbTv_R | AGGATGCTGATAACTAGTTAACGTAACGGCATGCT GGAGTTTCAGTTAATTCTCCCTTTC | SOE PCR for TESG_06701 to |
| p7TTR_F | GATATTTATCGTCCGCTGTAAG | 6695(-6696) |
| p7AbTv_F | GCTCTTCTCGCAGCGGGAAAGGGAGAATTAACTGA AACTCCAGCATGCCGTTACGTTAAC | amplification |
| p7TTR_R | CGTTTATTTACAAACGCCGAACTTGAATACACACCC TGGTCTCGCTAACTAACTAACCAG | |
| pAfpyroA_F | CGGAGCATCTAGCTAGCTGGTTAGTTAGTTAGCGA GACCAGGGTGTGTATTCAAGTTCGG | *A fumigatus* pyroA amplification |
| pAfpyroA_R | CCTTTTCGCCCTTTGTCATAGTAAAGTGATTCGCGT CATGAGTGTTCGCGTTGGCATGCG | |
| ppyrscr_F | CCAGTCATCTAGCAAGTGAG | screening and |
| p5Ttscr_R | GGCAAGTGGACAATCTGTTC | sequencing |
| ppyroscr_F | GTAACATCCTCTTCTGAGATCG | |
| ppyroscr_R | GATGTCCCTATGAATAAACCCG | |
| ppyroseq_R | CTGAGACCACTACCCAGTTG | sequencing |
| pTtDo_F | TGGCATCACGCATCAGTGCCTCCTCTCAGACAGAA TGCCATTGCCCAGGGATTCTATTTC | TESG_06701 amplification |
| pTtDo_R | ACGTTTATTTACAAACGCCGAACTTGAATACACACC CTGGTTTCCTAGAGGTAACTATTG | |
| pAfpyroAdo_F | TACTTTACTTAACAGATCAATAGTTACCTCTAGGAA ACCAGGGTGTGTATTCAAGTTCGG | |
| pWAscr_F | GAGTTACGACCGGCCGAATG | transformant |
| pgpdAscr_R | CACACCAGCCTTTCCACTTC | screening |
| pWY15Scr_F | GATGCGACATTAGCTCGTG | transformant |
| pWY15Scr_R | CAGGATCTCGCAGAGAGC | screening |
| pWY16Scr_R | CGCATCATAATCGTGGAC | |
| AfpyrG_F | GAGAGTTATTCTGTGTCTGAC | *A fumigatus* pyroA |
| AfpyrG_R | CTGTCTGAGAGGAGGCACTG | screening |
| Tt6698_F | CCCTTTGTCTCCTTGATC | RT-PCR |
| Tt6698_R | ATGCGCGTCTCGATCATC | RT-PCR |
| Tt6701_F | CCTTGGGTCGATATTCCAG | RT-PCR |
| Tt6701_R | CAGATGATGTGAAGGAGC | RT-PCR |
| Tt6702_F | CCAGGACAGAGTGTTCTC | RT-PCR |
| Tt6702_R | GTCTGTGTTGCGTTTTCCG | RT-PCR |
| Tt6706_F | GAGGTCAGAGGATCCAAG | RT-PCR |
| Tt6706_R | GCTGTCGTCCAAGACCAG | RT-PCR |
| Mc3598_F | GTTCCATCGCGAACTGTC | RT-PCR |
| Mc3598_R | CCTGCATCAAAATAGCCTG | RT-PCR |
| Mc3602_F | CGTATGGACGTTAATGGC | RT-PCR |
| Mc3602_R | GTGAAAGCTCTGTTGCTG | RT-PCR |
| ANactin_F | GATCGGTATGGGTCAGAAGGAC | RT-PCR |
| ANactinM_R | CTTCATCAGGTAGTCCGTCAGG | RT-PCR |

**Table S3. Genes and enzymes predicted through deep bioinformatic annotation of the putative *nrc*-like cluster in fungi**

| Protein | Identity (%) to genes of | | | | | | | | putative function |
|---|---|---|---|---|---|---|---|---|---|
| *Trichophyton tonsurans CBS 112818* | *Trichophyton equinum CBS 127.97* | *Arthroderma benhamiae CBS 112371* | *Trichophyton verrucosum HKI 0517* | *Arthroderma gypseum CBS 118893* | *Trichophyton rubrum CBS 118892* | *Arthroderma otae CBS 113480* | *Neosartorya fischeri NRRL 181* | *Aspergillus fumigatus Af293* | |
| TESG_06706 | TEQG_05350 (100 %) | ARB_00534 (88%) | TRV_00390 (83%) | MGYG_06592 (75%) | TERG_08361 (90%) | MCYG_03602 (77 %) | NFIA_112200 (57%) | AFUA_7G00200 (52%) | C6 transcription factor |
| TESG_06705 | TEQG_05349 (100 %) | ARB_00535 (91%) | TRV_00389 (75%) | MGYG_06591 (79%) | TERG_08360 (91%) | MCYG_03601 (79 %) | NFIA_112210 (66%) | AFUA_7G00190 (65%) | metallo- β-lactamase-like thioesterase |
| TESG_06704 | TEQG_05348 (41 %)* | ARB_00536 (90%) | TRV_00388 (91%) | MGYG_06590 (80%) | TERG_08359 (89%) | MCYG_03600 (83 %) | NFIA_112220 (67%) | AFUA_7G00180 (67%) | flavin-dependent monooxygenase |
| TESG_06703 | TEQG_05347 (99 %) | ARB_00537 (96%) | TRV_00387 (95%) | MGYG_06589 (92%) | TERG_08358 (94%) | MCYG_03599 (90 %) | NFIA_112230 (64%) | AFUA_7G00170 (65%) | prenyltransferase |
| TESG_06702 | TEQG_05346 (98 %) | ARB_00538 (94%) | TRV_00386 (93%) | MGYG_06588 (88%) | TERG_08357 (94%) | MCYG_03598 (89 %) | NFIA_112240 (77%) | AFUA_7G00160 (76%) | non-reducing polyketide synthase |
| TESG_06701 | TEQG_05345 (71 %) | ARB_00539 (93%) | TRV_00385 (93%) | MGYG_06587 (90%) | TERG_08356 (74%) | - | - | - | dioxygenase |
| TESG_06700 | TEQG_05344 (95 %) | ARB_00540 (88%) | TRV_00384 (88%) | MGYG_08867 (90%) | - | - | - | - | hypothetical protein |
| TESG_06699 | TEQG_05343 (91 %) | ARB_00541 (88%) | TRV_00383 (88%) | MGYG_08866 (76%) | - | - | - | - | major facilitator superfamily transporter |
| TESG_06698 | Poor sequence* | ARB_00542 (97%) | TRV_00382 (96%) | MGYG_08865 (95%) | - | - | - | - | asp/glu racemase |
| TESG_06697 | TEQG_05342 (100 %) | ARB_00543 (95%) | TRV_00381 (96%) | MGYG_08864 (92%) | - | - | - | - | dioxygenase |
| TESG_06696 | TEQG_05341 (99 %) | - | - | MGYG_08861 (42%) | - | - | - | - | glycoside hydrolase |
| TESG_06695 | TEQG_05340 (99 %) | ARB_00544 (90%) | TRV_00380 (90%) | MGYG_08860 (91%) | TERG_08355 (94%) | - | | | alcohol dehydrogenase |

* Poor sequencing in the genome database to lead to TEQG_05348 shorter coding region than other FMO homologs.

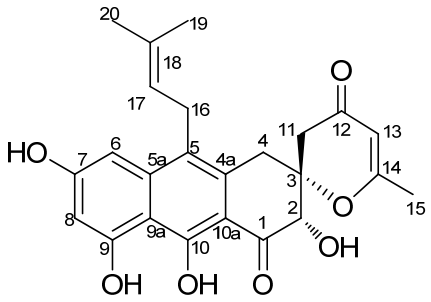
**Table S4. NMR assignment for neosartoricin B in $CDCl_3$ (500 MHz)**




| no. | $\delta_H$, mult. ($J$ in Hz) | $\delta_C$ | HMBC |
|---|---|---|---|
| 1 | | 200.4 | |
| 2 | 4.45, s | 76.6 | C-1 |
| 3 | | 75.5 | |
| 4 | 3.04, d (17.2) | 36.4 | C-3, C-4a, C-5 |
| | 3.37, d ( 17.5) | | |
| 4a | | 140.8 | |
| 5 | | 130.2 | |
| 5a | | 125.8 | |
| 6 | 6.61, d, 1.6 | 100.5 | C-5a, C-8 |
| 7 | | 160.7 | |
| 8 | 6.32, brs | 101.6 | C-6, C-7, C-9a |
| 9 | | 160.2 | |
| 9a | | 106.6 | |
| 10 | | 163.1 | |
| 10a | | 107.6 | |
| 11 | 2.78, d (15) | 46.7 | C-3, C-4, C-12 |
| | 2.93, d (15) | | C-3, C-4, C-12 |
| 12 | | 193.2 | |
| 13 | 5.68, s | 102.2 | C-14, C-15, C-11 |
| 14 | | 190.4 | |
| 15 | 2.11, s | 24.5 | C-13, C-14 |
| 16 | 3.42, d (6.3)<br>3.36, d (6.7) | 27.3 | C-4a, C-5, C-5a, C-17 |
| 17 | 4.98, s | 121.6 | C-20, C-19, C-5 |
| 18 | | 132.8 | |
| 19 | 1.81, s | 18.2 | C-17, C18, C-20 |
| 20 | 1.69, s | 25.8 | C-17, C-19, C18 |
| 2-OH | 2.31 (1H, s) | | C-1, C-2 |
| 3-OH | 6.04 (1H, brs) | | |
| 7-OH | n.a | | |
| 9-OH | 9.79 (1H, s) | | C-9, C-8, C-9a |
| 10-OH | 14.9 (1H, s) | | C-10, C-9a, C-9 |
| 14-OH(*a*) | 15.37 (1H, brs) | | |

n.a: not assigned

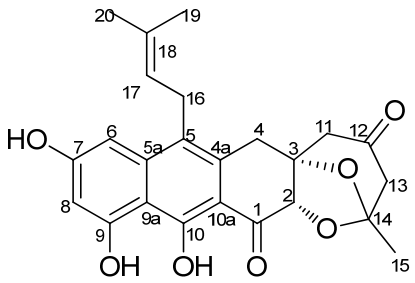
**Table S5. NMR assignment for neosartoricin C in $CDCl_3$ (500 MHz)**




| no. | $\delta_H$, mult. ($J$ in Hz) | $\delta_C$ | HMBC |
|---|---|---|---|
| 1 | | 199.2 | |
| 2 | 4.45, s | 76.2 | C-1 |
| 3 | | 84.6 | |
| 4 | 2.69, d (17.0) | 32.4 | C-5, C-4a |
| | 3.86, d (17.0) | | C-3, C-2, C-10a, C-5, C-4a |
| 4a | | 129.3 | |
| 5 | | 125.0 | |
| 5a | | 140.8 | |
| 6 | 6.75, d (2.1) | 100.5 | C-8, C-9a, C-7, C-5 |
| 7 | | 160.6 | |
| 8 | 6.52, d ( 2.1) | 102.0 | C-6, C-9a, C-9 |
| 9 | | 161.0 | |
| 9a | | 107.8 | |
| 10 | | 163.0 | |
| 10a | | 106.5 | |
| 11 | 2.43, d (16.4) | 41.8 | C-12, C-4 |
| | 3.44, d (16.4) | | C-12, C-4, C-3, C-2 |
| 12 | | 191.3 | |
| 13 | 5.34, s | 103.9 | C-11, C-14, C-15 |
| 14 | | 172.1 | |
| 15 | 1.73, s | 21.1 | C-14, C-13 |
| 16 | 3.38, m | 27.2 | C-18, C-4a, C-17, C5, C5a |
| 17 | 4.82, m | 121.1 | C-5, C-19, C-20 |
| 18 | | 133.4 | |
| 19 | 1.78, s | 18.3 | |
| 20 | 1.66, s | 25.7 | |
| 10-OH | 14.86, s | | C-10, C-10a, C-9a |
| 9-OH | 9.85, s | | C-9, C9a, C-8 |

n.a: not assigned

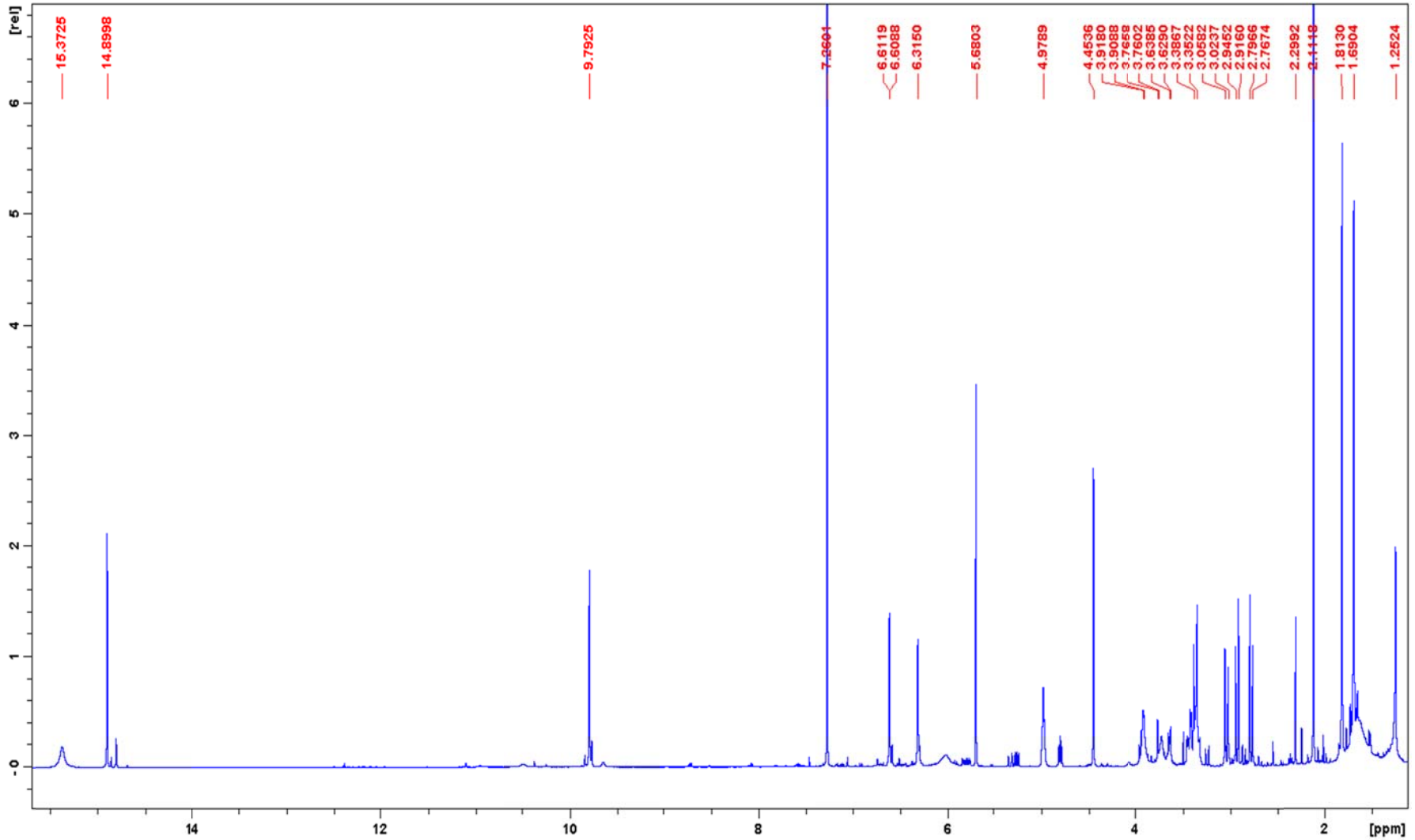
**Table S6. NMR assignment for neosartoricin D in $CDCl_3$ (500 MHz)**



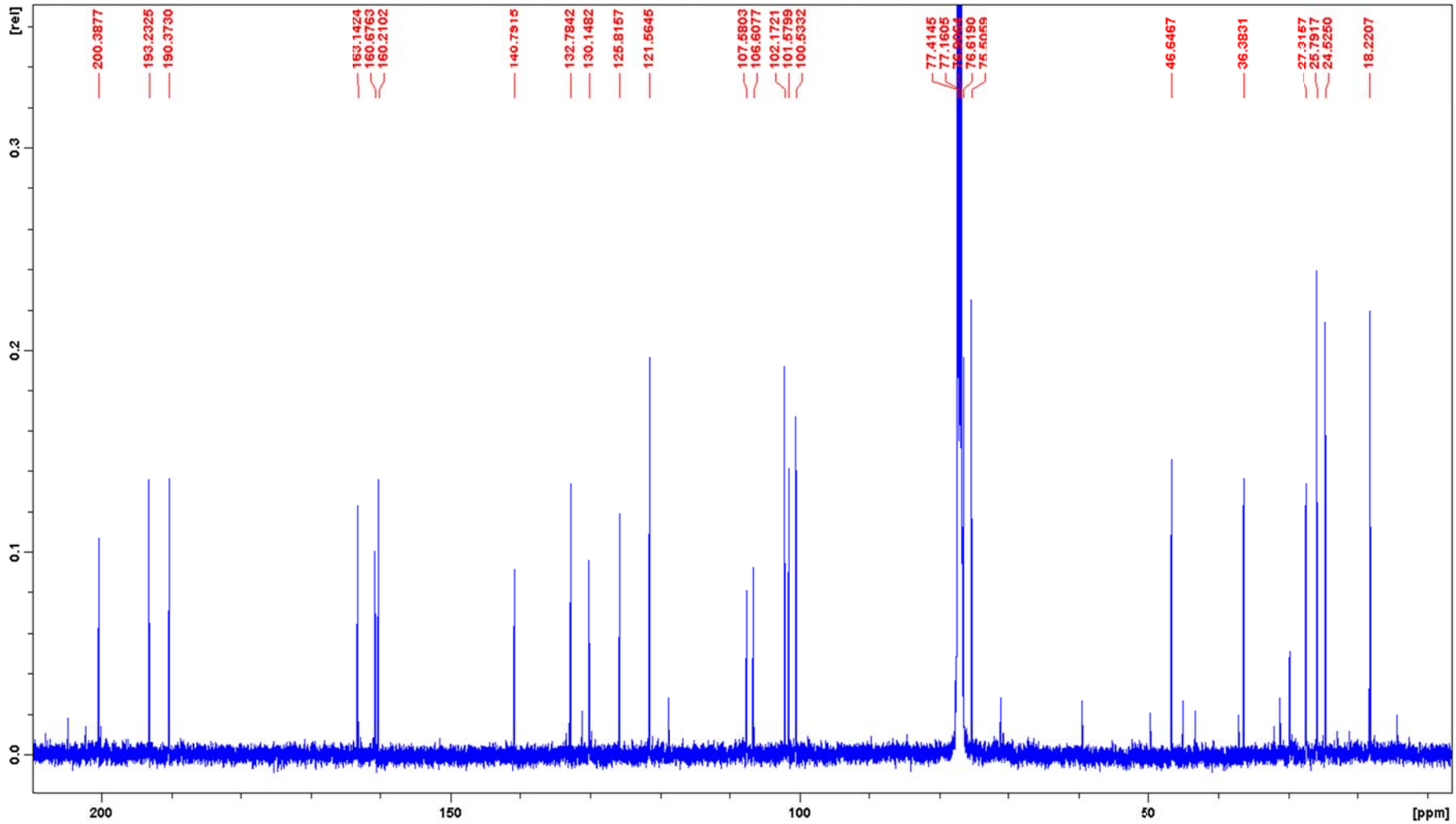

| no. | $\delta_H$, mult. ($J$ in Hz) | $\delta_C$ |
|---|---|---|
| 1 | | 203.6 |
| 2 | 4.46, s | 80.5 |
| 3 | | 78.3 |
| 4 | 2.99, d (17.6) | 32.2 |
| | 3.63, d (17.6) | |
| 4a | | 128.9 |
| 5 | | 125.2 |
| 5a | | 141.5 |
| 6 | 6.69, d (2.1) | 100.9 |
| 7 | | 160.9 |
| 8 | 6.46, d ( 2.1) | 101.7 |
| 9 | | 162.0 |
| 9a | | 108.2 |
| 10 | | 168.9 |
| 10a | | 106.2 |
| 11 | 2.59, d (16.8) | 51.1 |
| | 2.76, d (16.8) | |
| 12 | | 196.3 |
| 13 | 2.70, s | 50.1 |
| 14 | | 108.6 |
| 15 | 1.72, s | 24.6 |
| 16 | 3.48, m | 27.4 |
| 17 | 4.99, t (6.7) | 121.2 |
| 18 | | 133.4 |
| 19 | 1.85, s | 18.4 |
| 20 | 1.51, s | 25.8 |
| 10-OH | n.a | |
| 9-OH | 10.4, s | |
| 7-OH | 5.46, s | |

n.a: not assigned

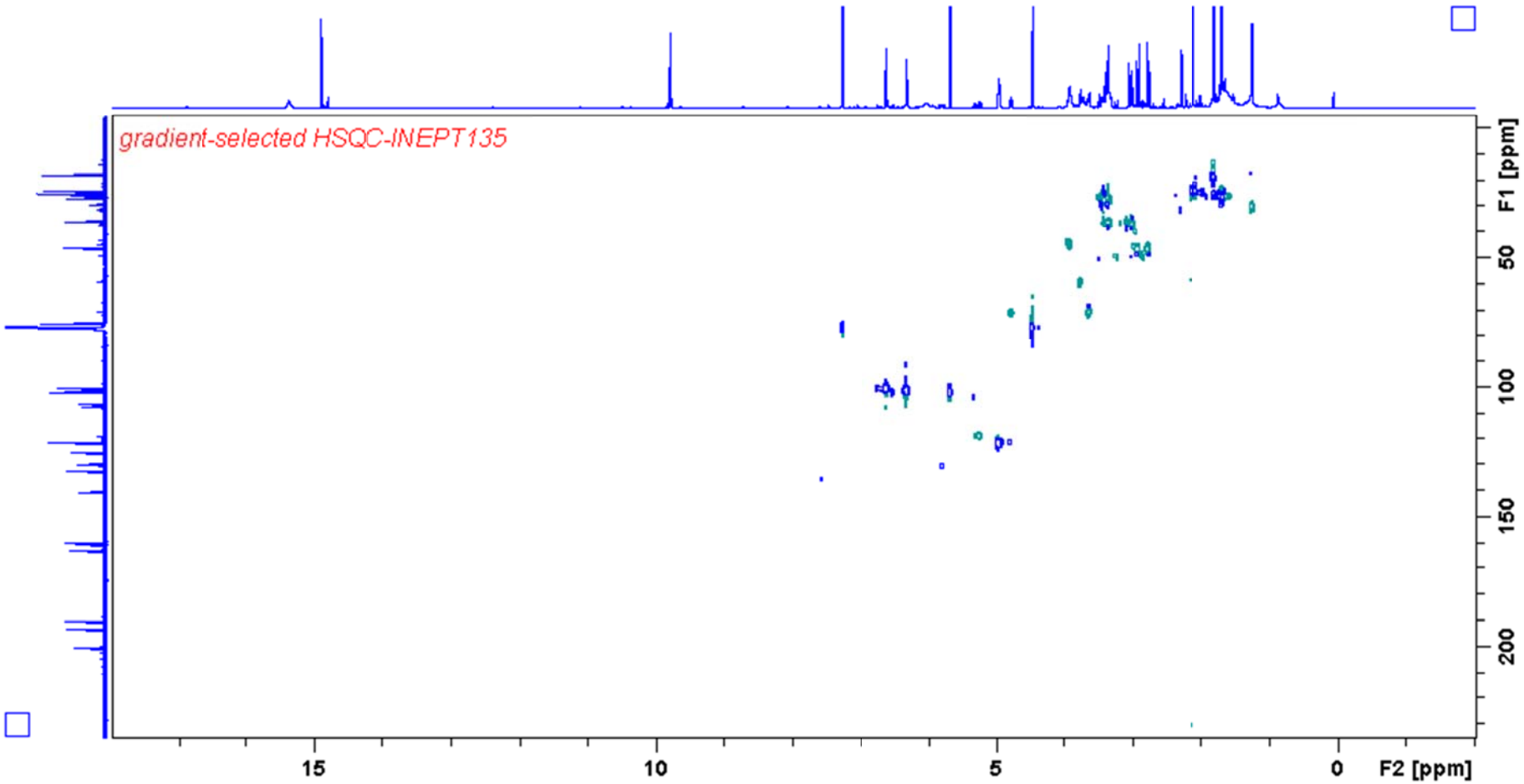
**Supplementary NMR spectra for neosartoricin B**



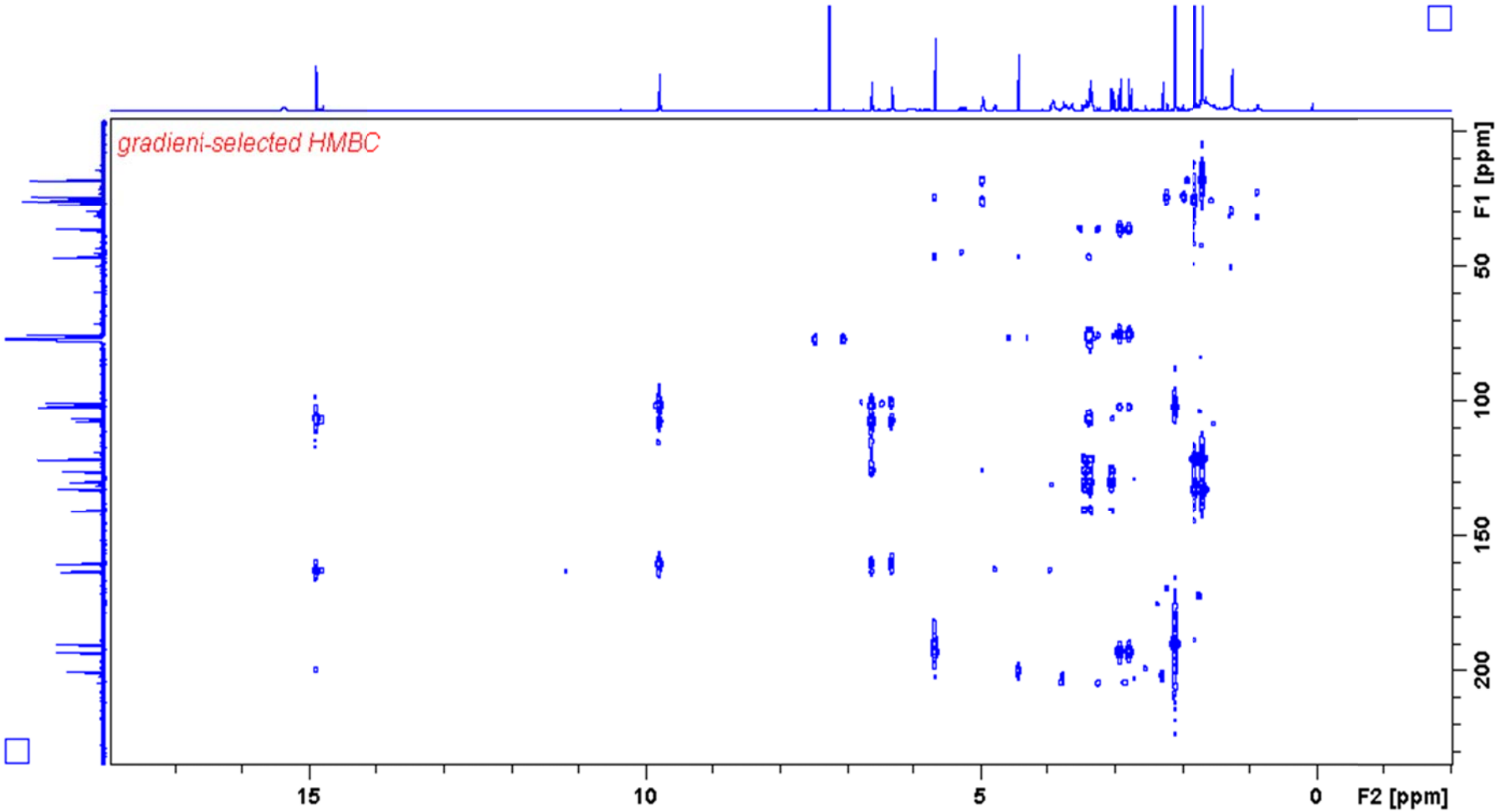

**$^{1}$H-NMR spectrum of neosartoricin B in $CDCl_3$ measured on 500 MHz Bruker NMR spectrometer (see Table S4 for peak assignments)**




**$^{13}$C-NMR spectrum of neosartoricin B in $CDCl_3$ measured on 500 MHz Bruker NMR spectrometer (see Table S4 for peak assignments)**

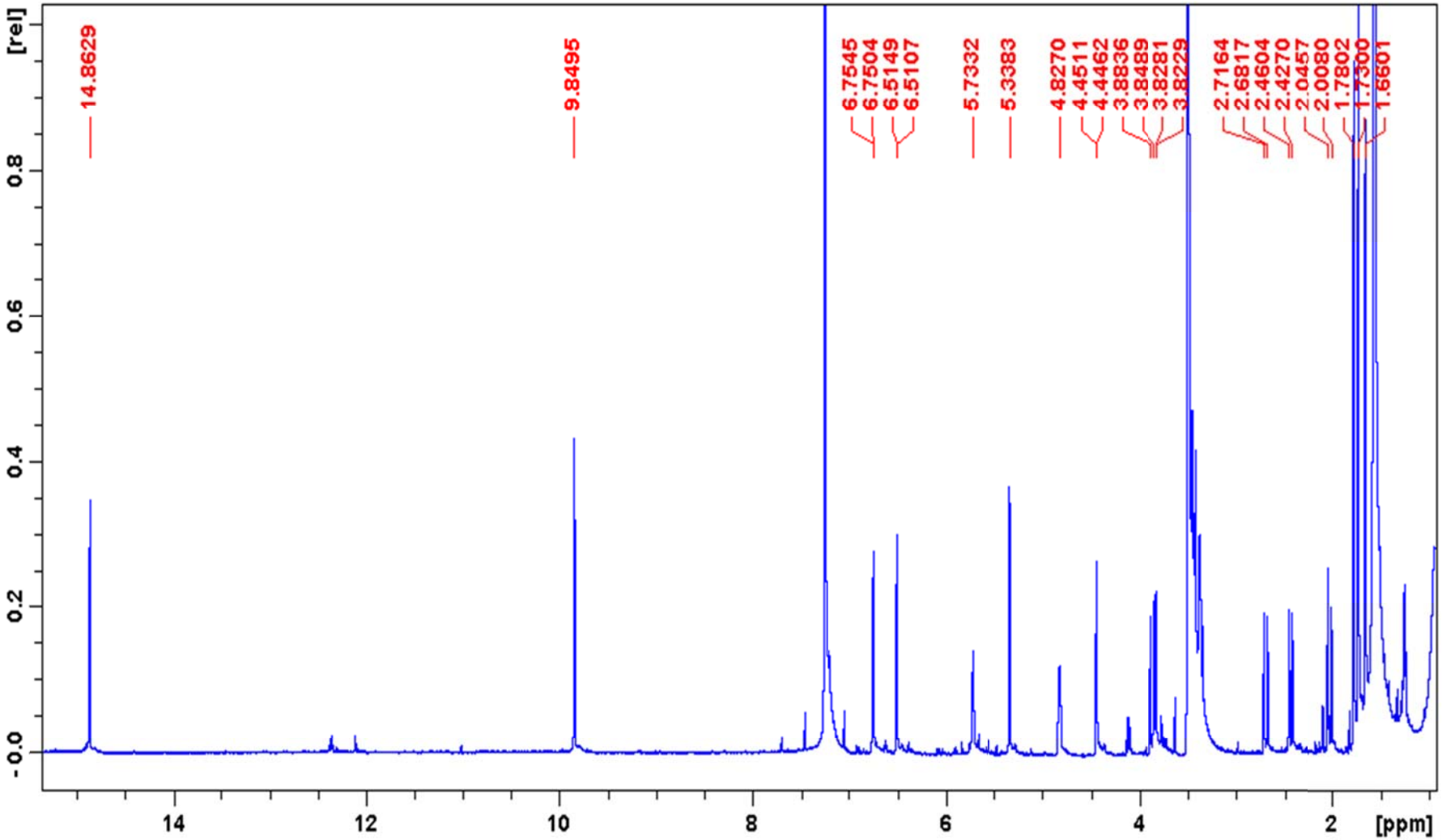



**$^{1}$H-$^{13}$C HSQC spectrum of neosartoricin B in $CDCl_3$ measured on 500 MHz Bruker NMR spectrometer (see Table S4 for peak assignments)**

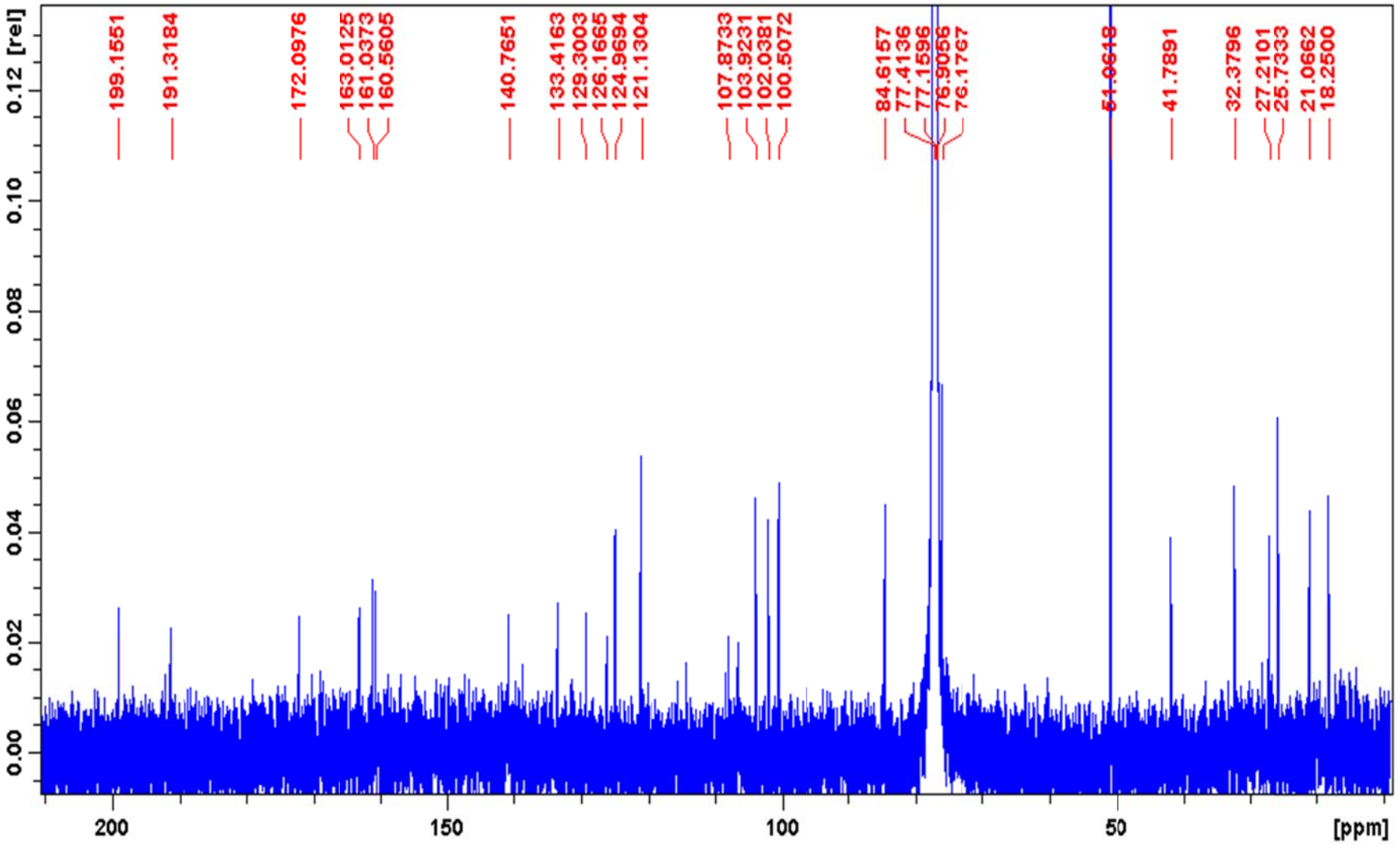



**$^{1}$H-$^{13}$C HMBC spectrum of neosartoricin B in $CDCl_3$ measured on 500 MHz Bruker NMR spectrometer (see Table S4 for peak assignments)**

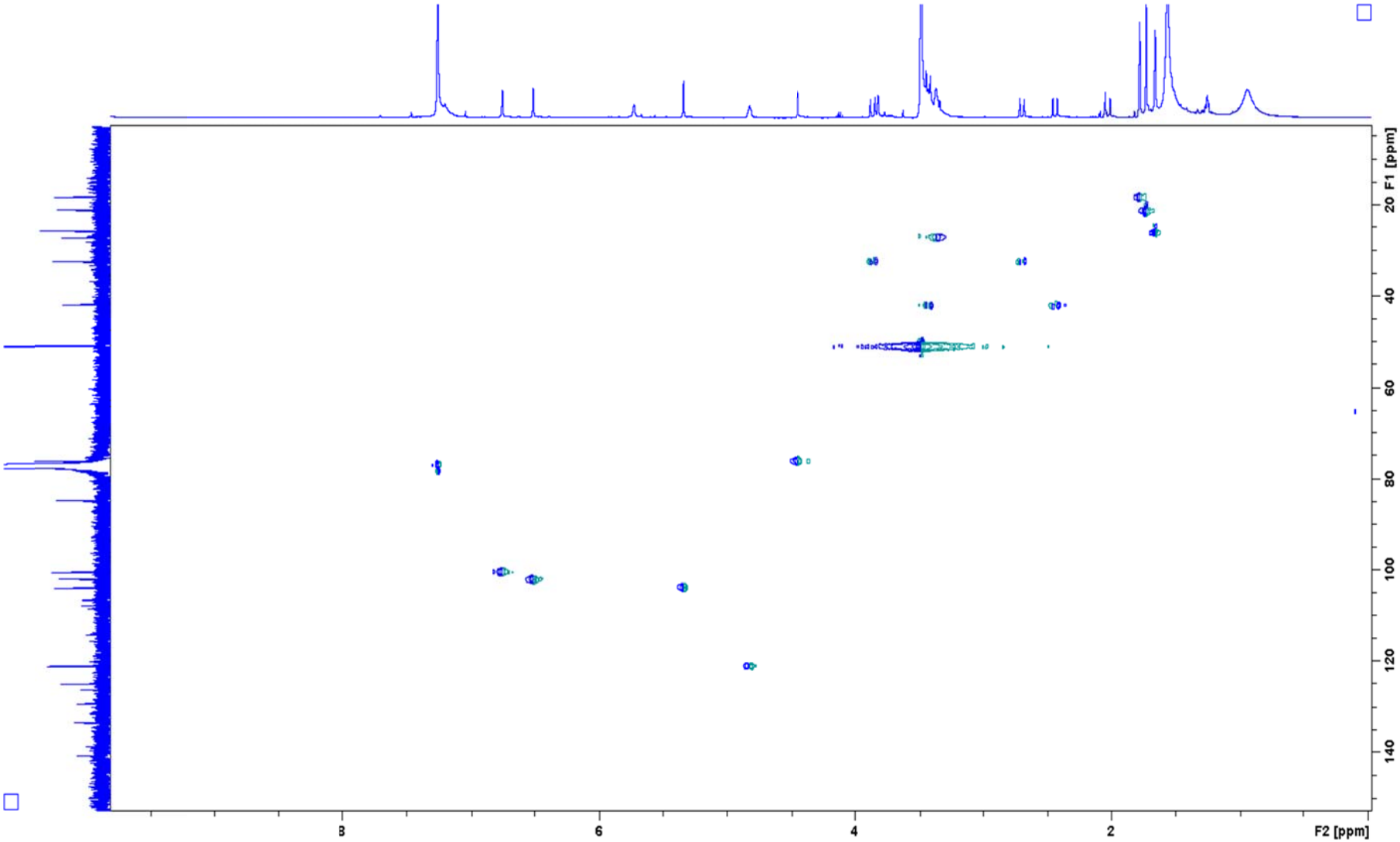
**Supplementary NMR spectra for neosartoricin C**



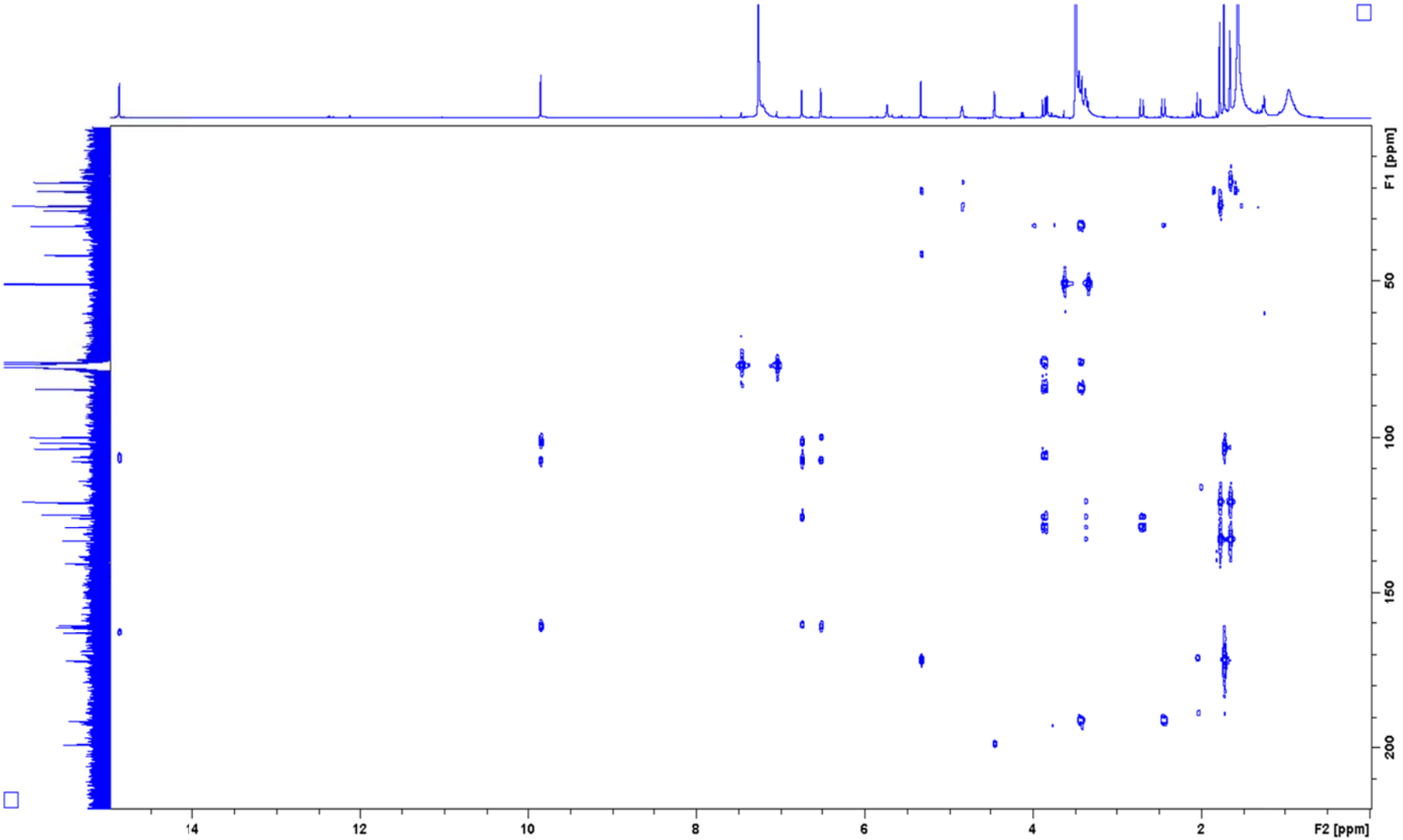

**$^{1}$H-NMR spectrum of neosartoricin C in $CDCl_3$ measured on 500 MHz Bruker NMR spectrometer (see Table S5 for peak assignments)**




**$^{13}$C-NMR spectrum of neosartoricin C in $CDCl_3$ measured on 500 MHz Bruker NMR spectrometer (see Table S5 for peak assignments)**

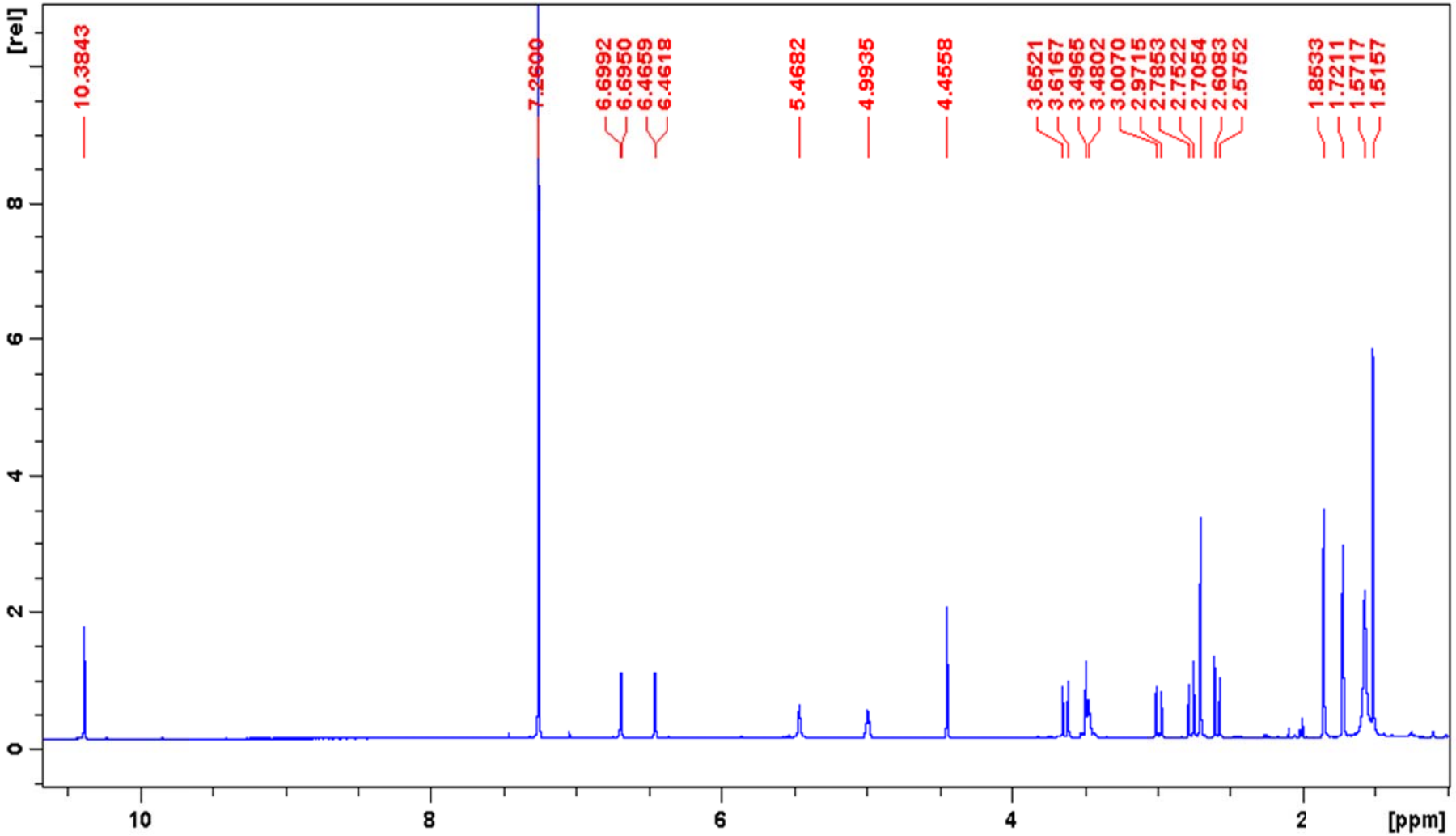



**$^{1}$H-$^{13}$C HSQC spectrum of neosartoricin C in $CDCl_3$ measured on 500 MHz Bruker NMR spectrometer (see Table S5 for peak assignments)**

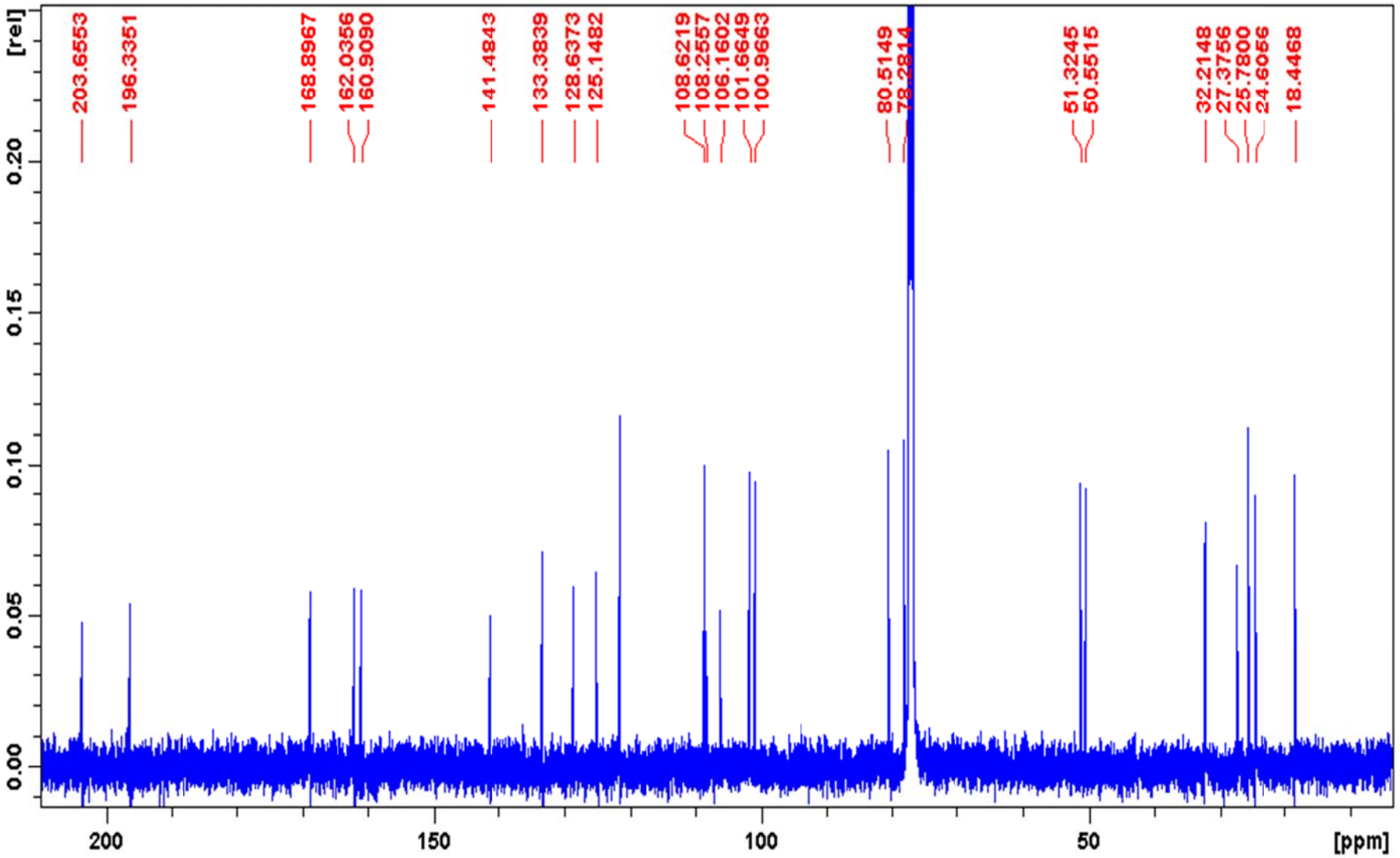



**$^{1}$H-$^{13}$C HMBC spectrum of neosartoricin C in $CDCl_3$ measured on 500 MHz Bruker NMR spectrometer (see Table S5 for peak assignments)**

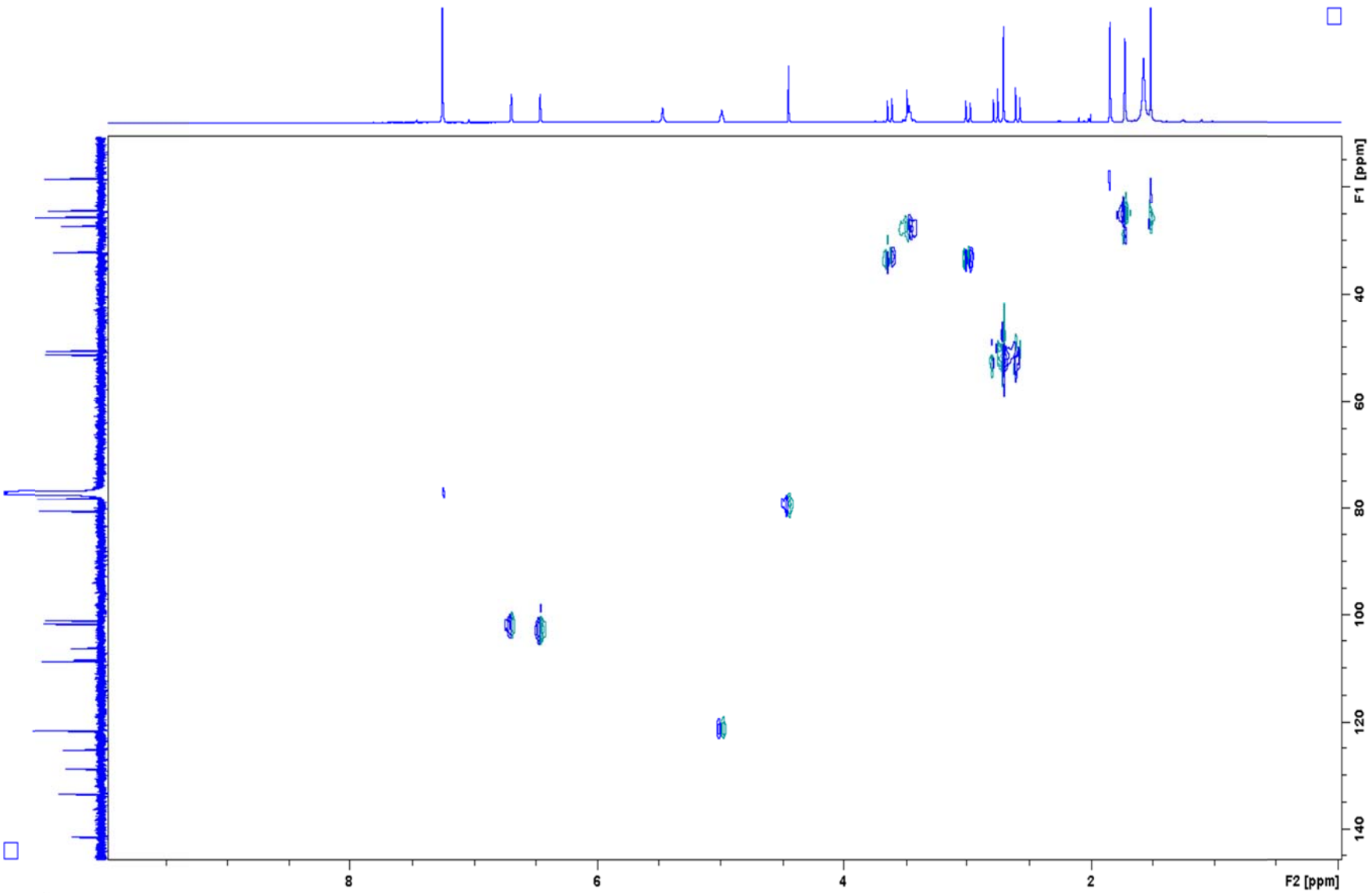
**Supplementary NMR spectra for neosartoricin D**




**$^{1}$H-NMR spectrum of neosartoricin D in $CDCl_3$ measured on 500 MHz Bruker NMR spectrometer (see Table S6 for peak assignments)**




**$^{13}$C-NMR spectrum of neosartoricin D in $CDCl_3$ measured on 500 MHz Bruker NMR spectrometer (see Table S6 for peak assignments)**




**$^{1}$H-$^{13}$C HSQC spectrum of neosartoricin D in $CDCl_3$ measured on 500 MHz Bruker NMR spectrometer (see Table S6 for peak assignments)**

## Supplementary References


1. Colot, H. V., Park, G., Turner, G. E., Ringelberg, C., Crew, C. M., Litvinkova, L., Weiss, R. L., Borkovich, K. A., and Dunlap, J. C. (2006) A high-throughput gene knockout procedure for *Neurospora* reveals functions for multiple transcription factors, *Proc Natl Acad Sci U S A 103*, 10352-10357.
2. Yin, W. B., Amaike, S., Wohlbach, D. J., Gasch, A. P., Chiang, Y. M., Wang, C. C., Bok, J. W., Rohlfs, M., and Keller, N. P. (2012) An *Aspergillus nidulans* bZIP response pathway hardwired for defensive secondary metabolism operates through *aflR*, *Mol. Microbiol. 83*, 1024-1034.