Supporting information

BRUNSVICAMIDE A-C, SPONGE-RELATED CYANOBACTERIAL PEPTIDES WITH *MYCOBACTERIUM TUBERCULOSIS* PROTEIN TYROSINE PHOSPHATASE INHIBITORY ACTIVITY

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1. Purity data of brunsvicamides A-C

Purity of brunsvicamides A-C was assessed with two different HPLC systems:

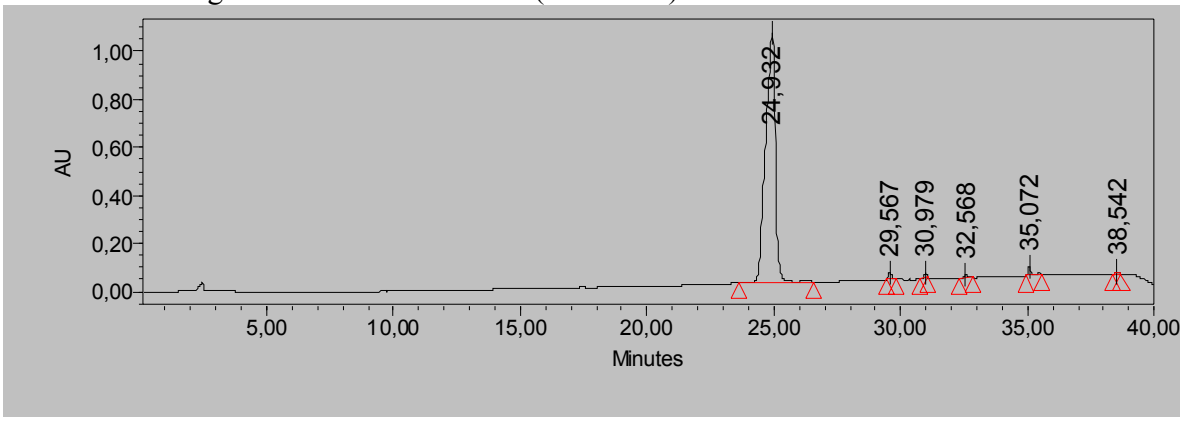
Method A: Waters HPLC system equipped with a 996 PDA detector, a 600 pump and 717plus autosampler; column: Macherey-Nagel Nucleodur C_{18} Sphinx, 5µm, 250 x 8mm; mobile phase: MeOH/H_2O; gradient elution from 50 % MeOH to 100 % MeOH in 25 min; flow: 1.0 mL.

Method B: Agilent 1100 HPLC system equipped with an API2000 Applied Biosystems/MDS Sciex; column: Macherey-Nagel Nucleodur 100 C_{18}, 5µm, 125 x 2mm; mobile phase: MeOH/H_2O (2 mM NH_4Ac); gradient elution from 10 % MeOH to 100 % MeOH in 20 min., then isocratic for 10 min; flow: 0.25 mL.

Table 1: Purity analysis of brunsvicamides A-C with HPLC

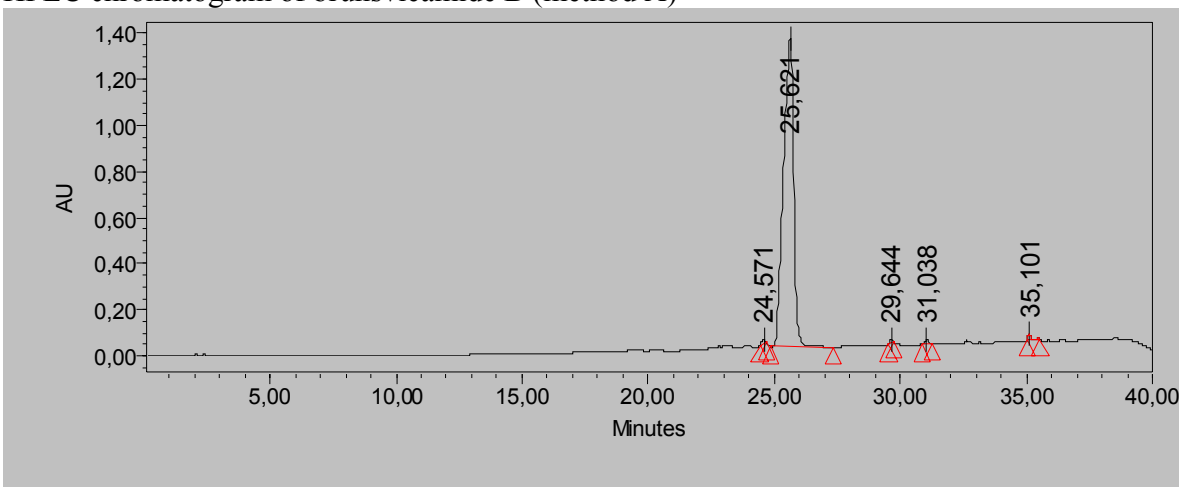
Compound	Method	Wavelength	Retention Time	Purity
Brunsvicamide A	A	220 nm	24.93 min	96 %
Brunsvicamide B	A	220 nm	25.62 min	98 %
Brunsvicamide C	A	220 nm	25.02 min	98 %
Brunsvicamide A	B	-	18.19 min	> 98 %
Brunsvicamide B	B	-	17.52 min	> 98 %
Brunsvicamide C	B	-	16.98 min	> 98 %

HPLC chromatogram of brunsvicamide A (method A)



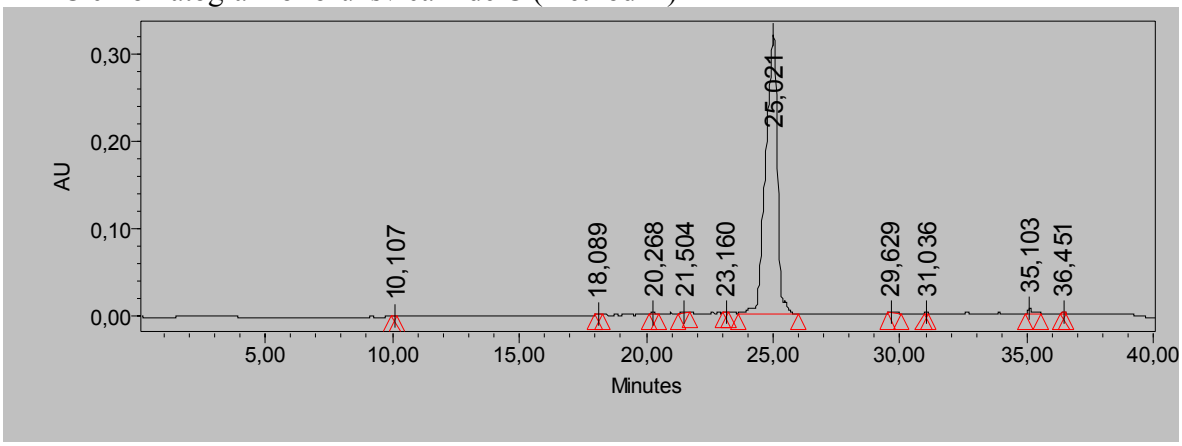
Retention Time	Area	% Area	Height
24,932	27623888	96,43	1043451
29,567	262390	0,92	25946
30,979	114520	0,40	16660
32,568	149417	0,52	13857
35,072	396999	1,39	38652
38,542	100722	0,35	9057

HPLC chromatogram of brunsvicamide B (method A)



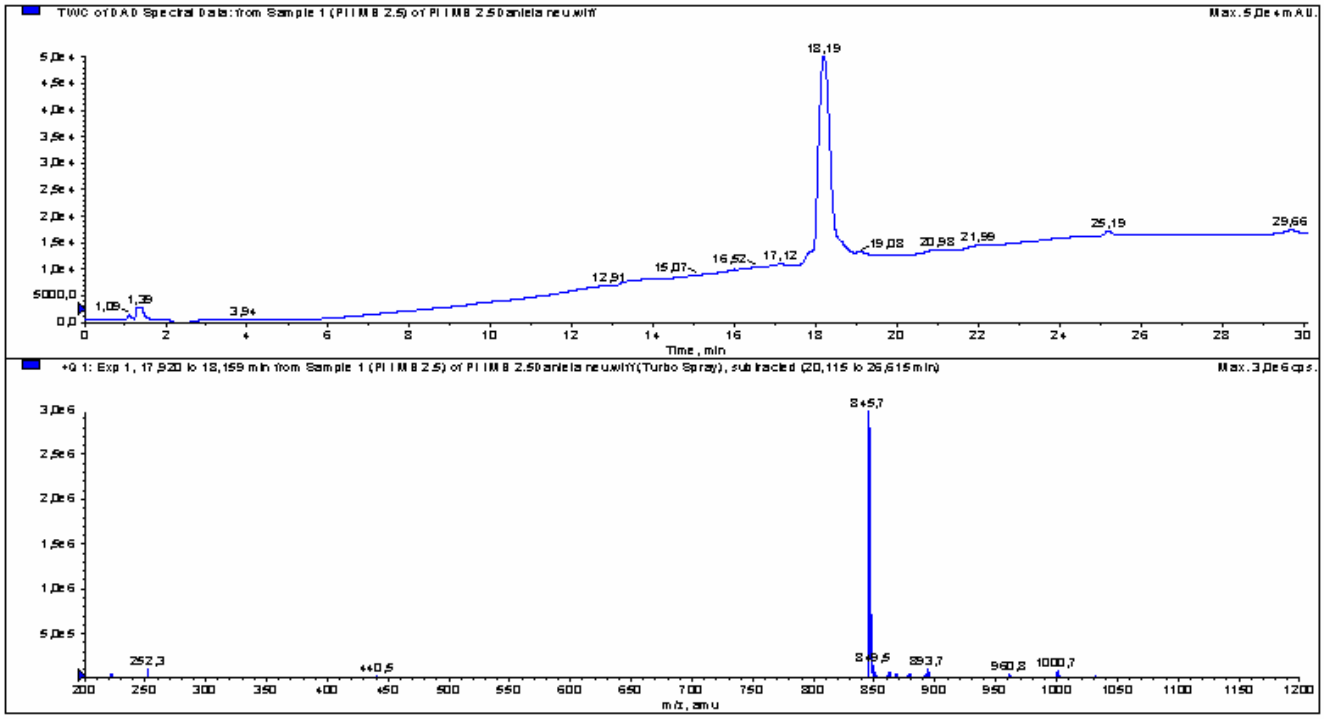
Retention Time	Area	% Area	Height
24,571	170689	0,44	19321
25,621	37861468	98,08	1337460
29,644	132448	0,34	17562
31,038	186548	0,48	20509
35,101	250170	0,65	25820

HPLC chromatogram of brunsvicamide C (method A)

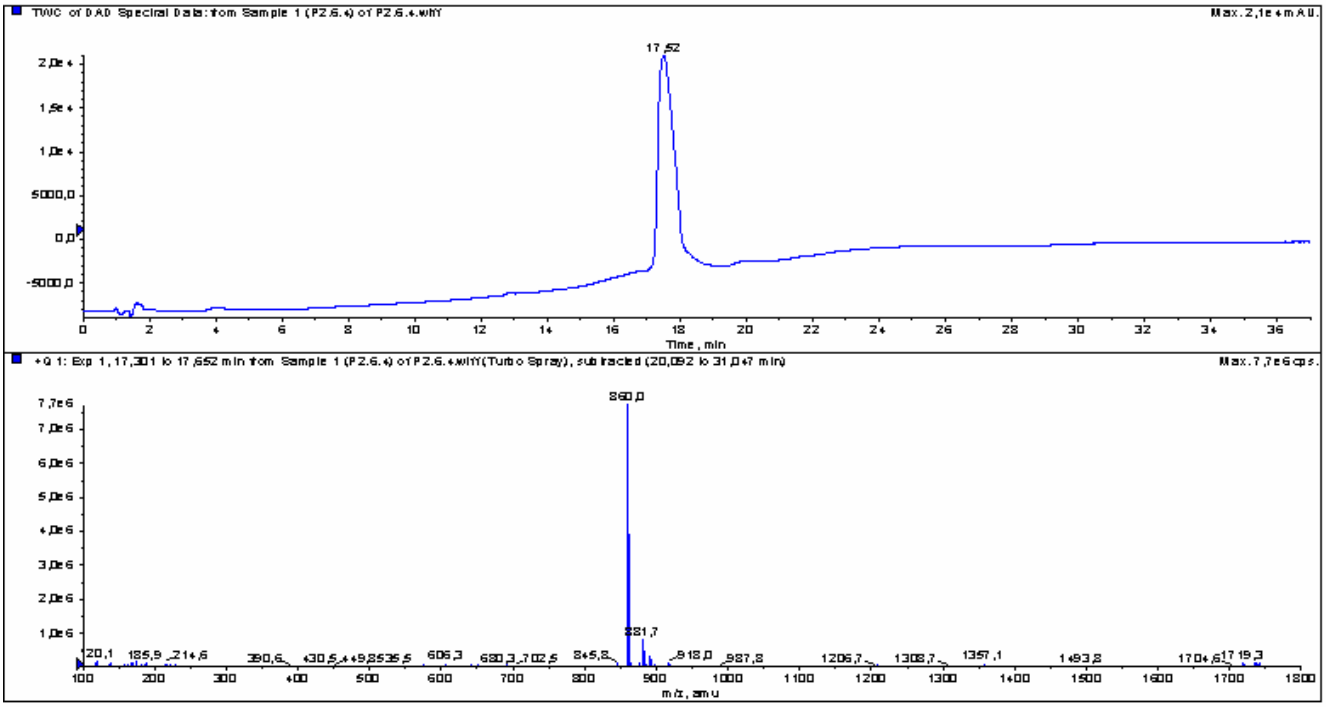


Retention Time	Area	% Area	Height
10,107	3959	0,04	519
18,089	9028	0,09	994
20,268	18951	0,18	1389
21,504	22738	0,22	1512
23,160	8367	0,08	924
25,021	10095898	98,38	316569
29,629	19542	0,19	1584
31,036	10216	0,10	1520
35,103	60728	0,59	5656
36,451	12360	0,12	1718

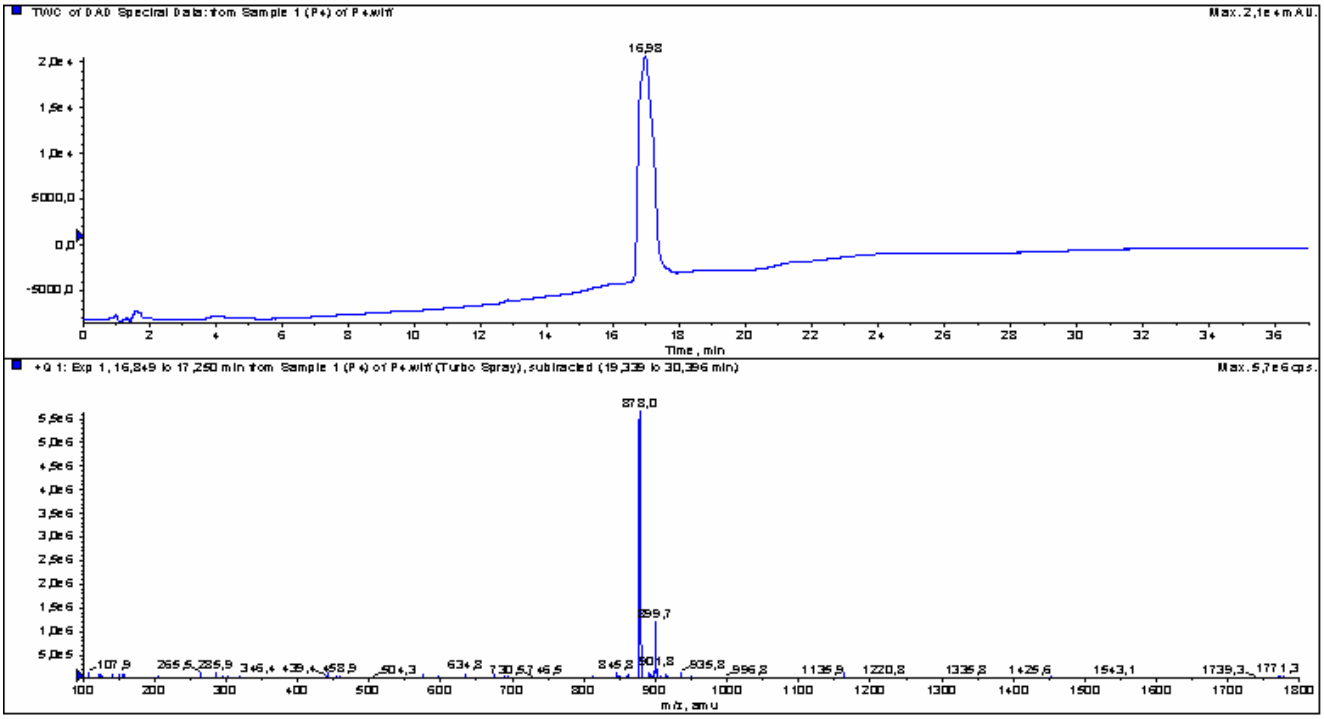
HPLC chromatogram and ESI-MS (positive mode) of brunsvicamide A (method B)



HPLC chromatogram and ESI-MS (positive mode) of brunsvicamide B (method B)



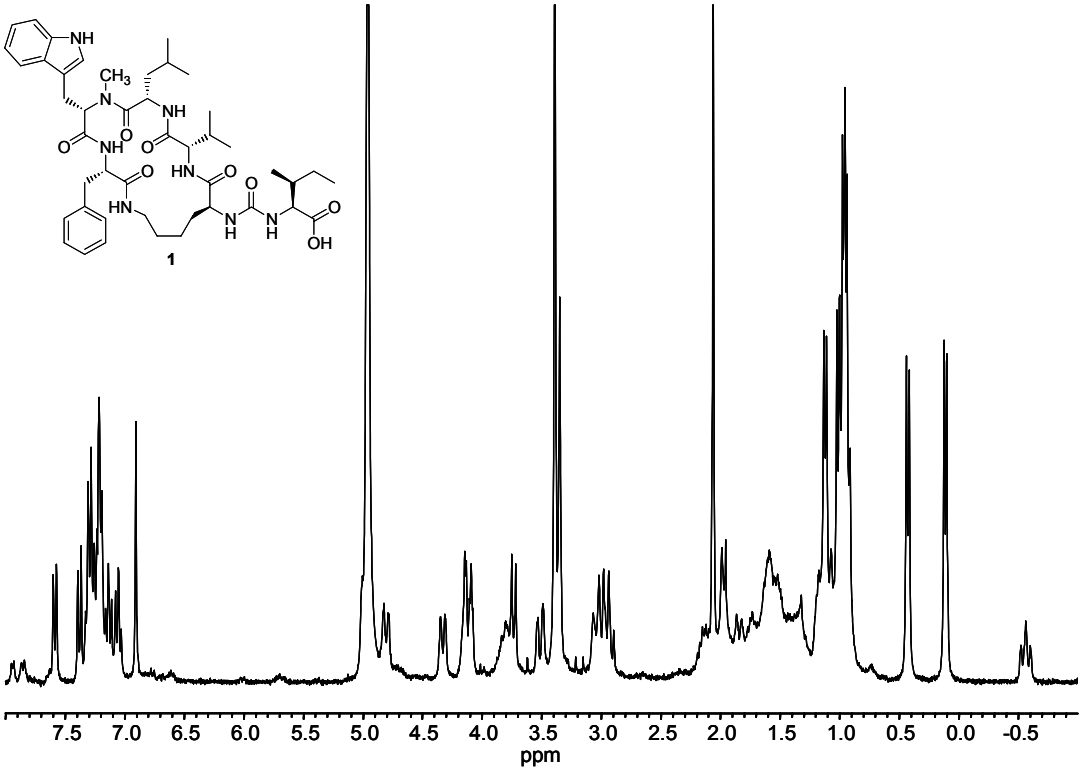
HPLC chromatogram and ESI-MS (positive mode) of brunsvicamide C (method B)



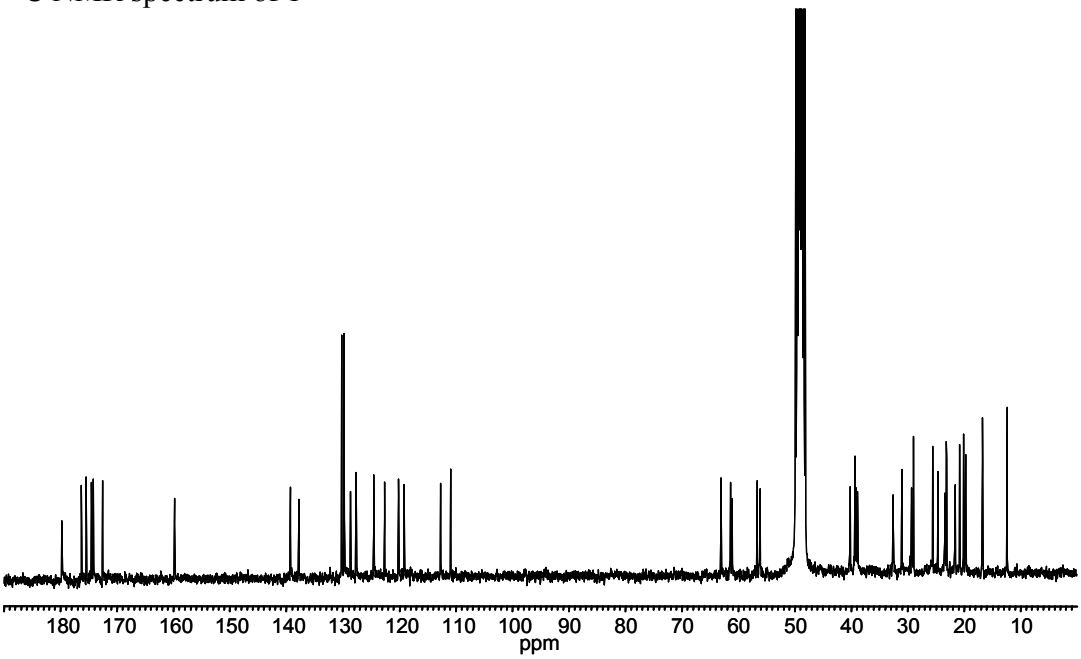
2. NMR spectra of brunsvicamide A (1)

All spectra were recorded in CD_3OD either using a Bruker Avance 300 DPX or 500 DRX spectrometer operating at 300 or 500 MHz for proton and at 75 or 125 MHz for ^{13}C, respectively. Spectra were referenced to residual solvent signals with resonances at $\delta_{H/C}$ 3.35/49.0 (CD_3OD).

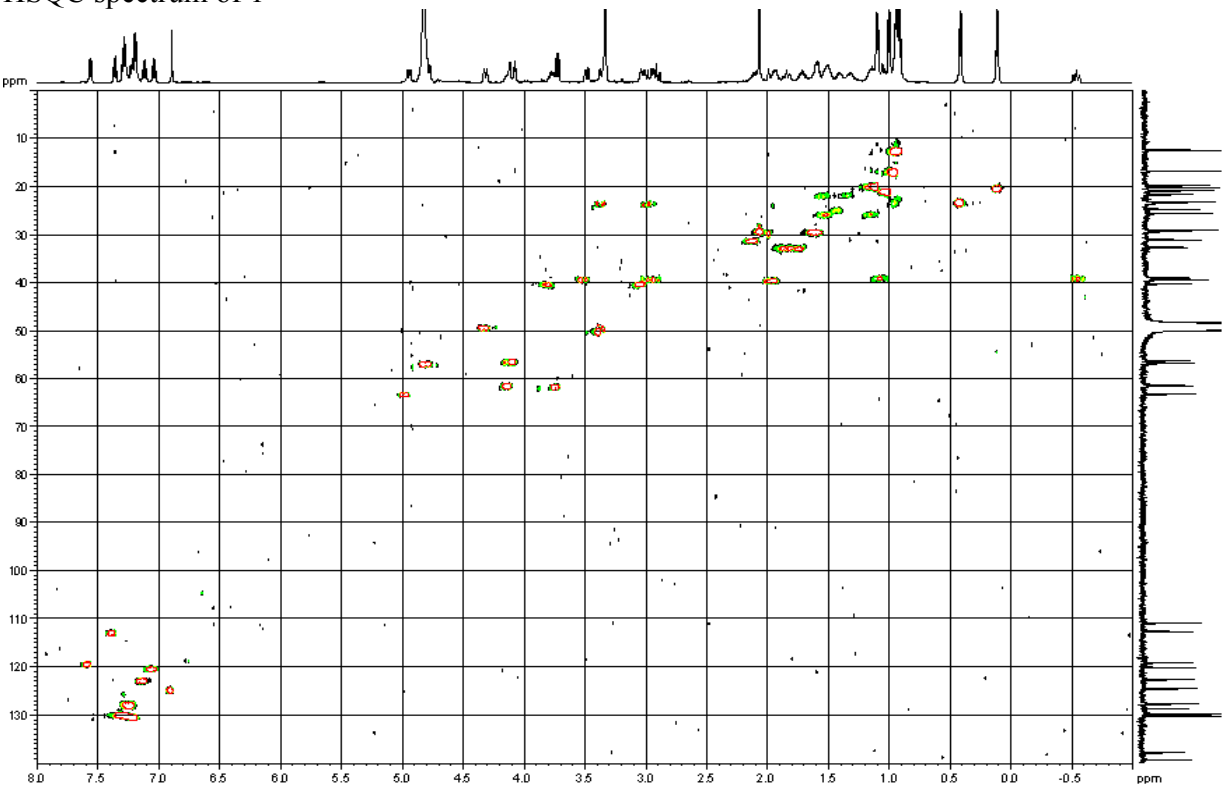
^1H NMR spectrum of 1



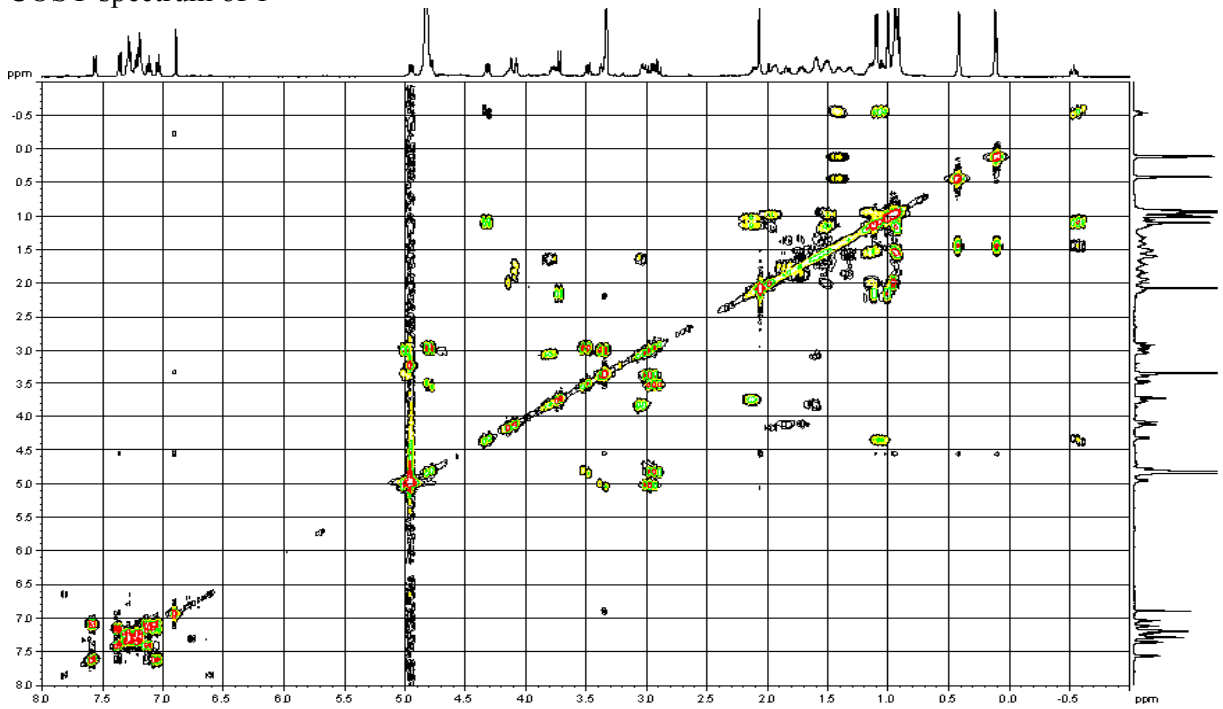
^{13}C NMR spectrum of 1



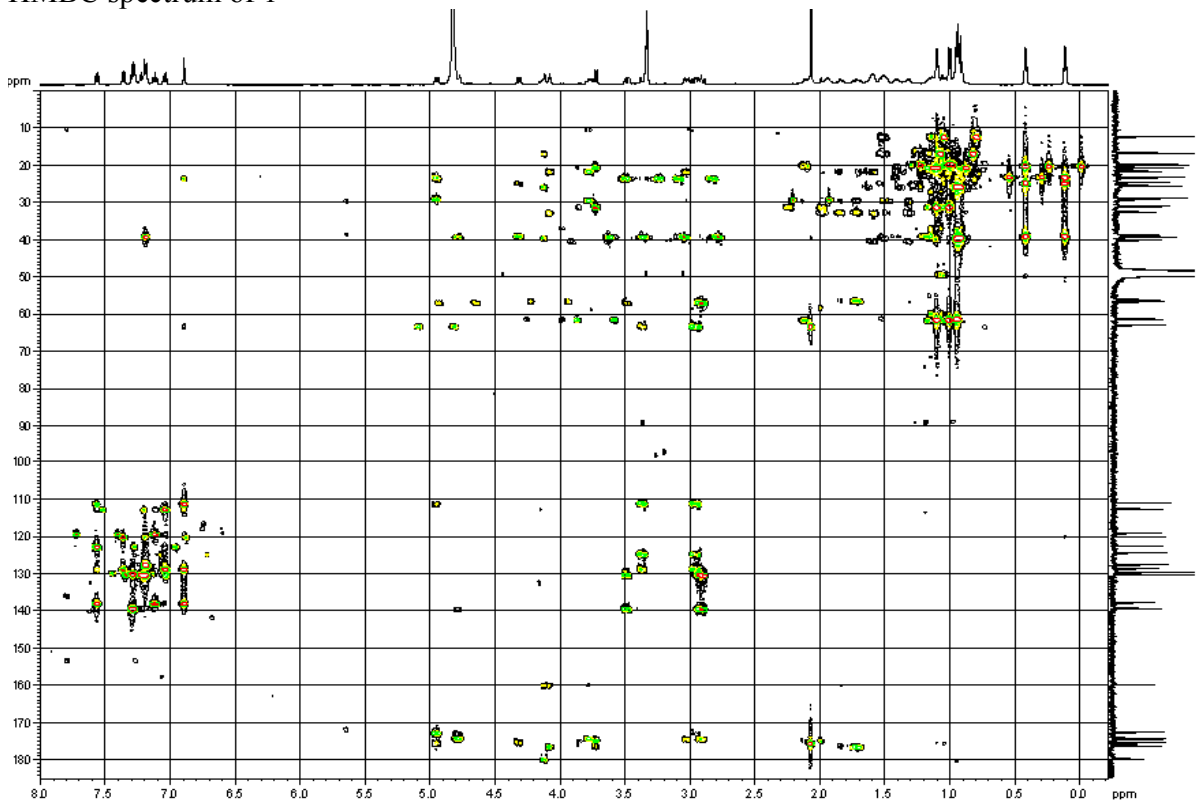
HSQC spectrum of 1



COSY spectrum of 1



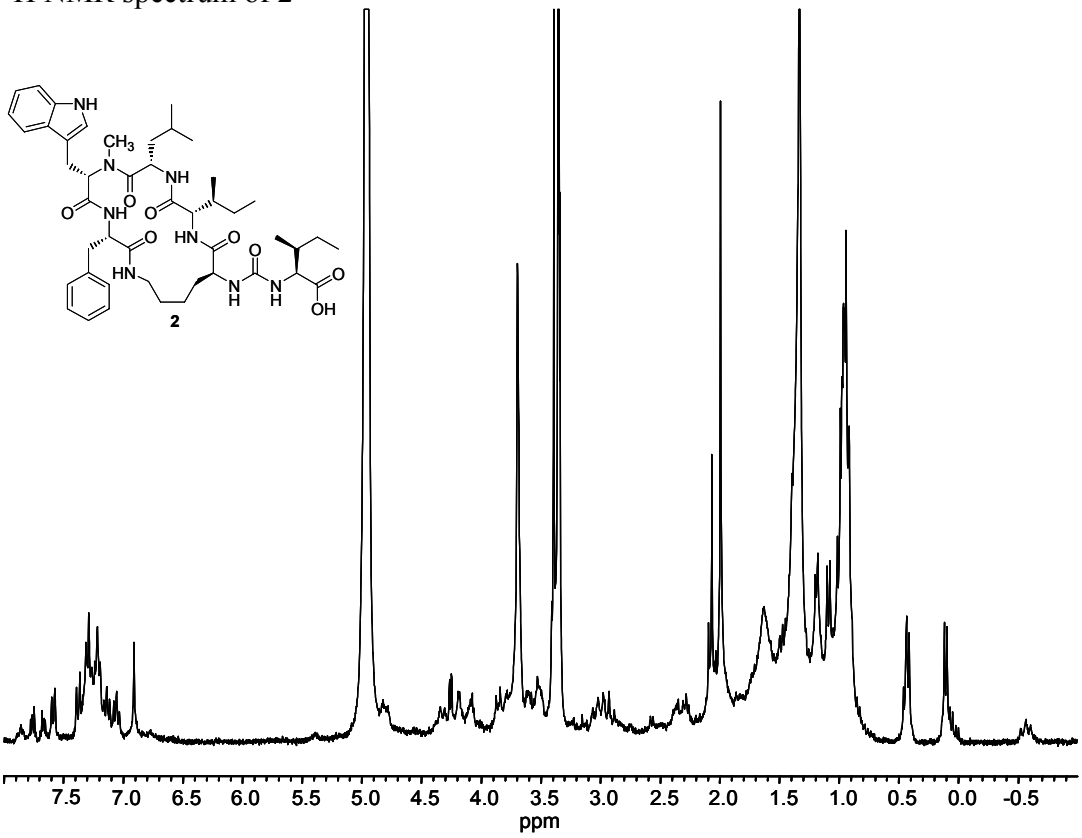
HMBC spectrum of 1



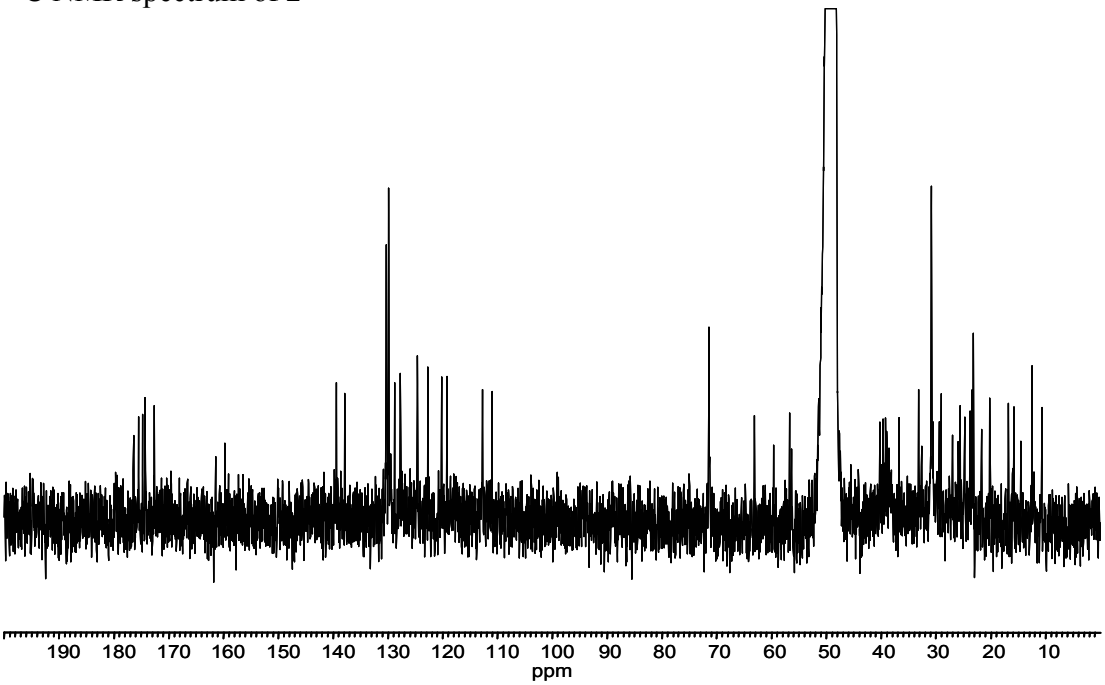
3. NMR spectra of brunsvicamide B (2)

All spectra were recorded in CD_3OD either using a Bruker Avance 300 DPX or 500 DRX spectrometer operating at 300 or 500 MHz for proton and at 75 or 125 MHz for ^{13}C, respectively. Spectra were referenced to residual solvent signals with resonances at $\delta_{H/C}$ 3.35/49.0 (CD_3OD).

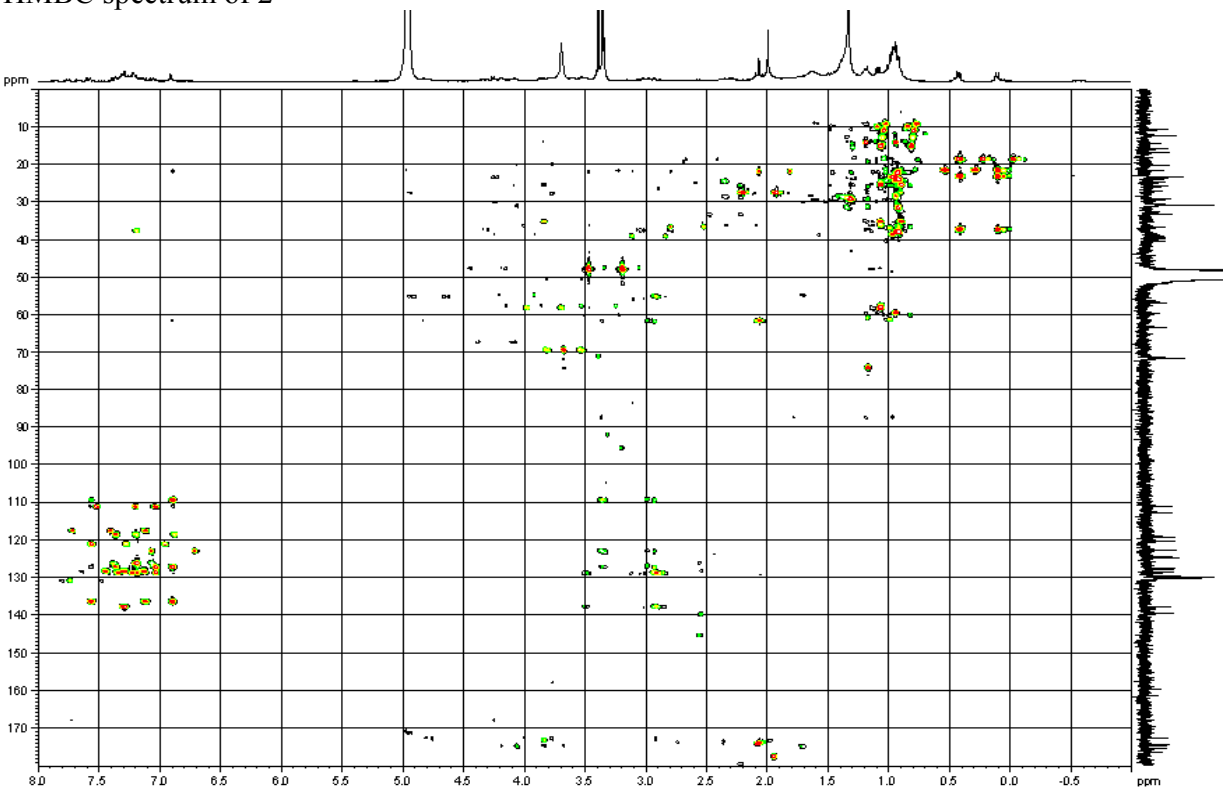
^1H NMR spectrum of 2



^{13}C NMR spectrum of 2



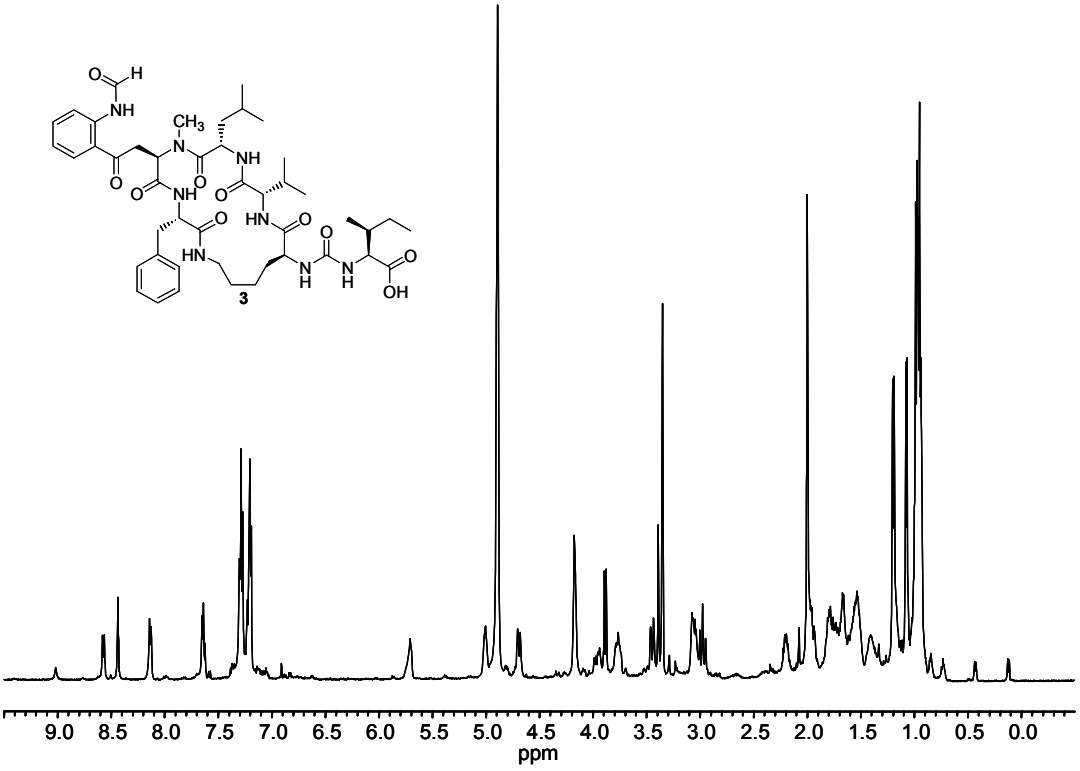
HMBC spectrum of 2



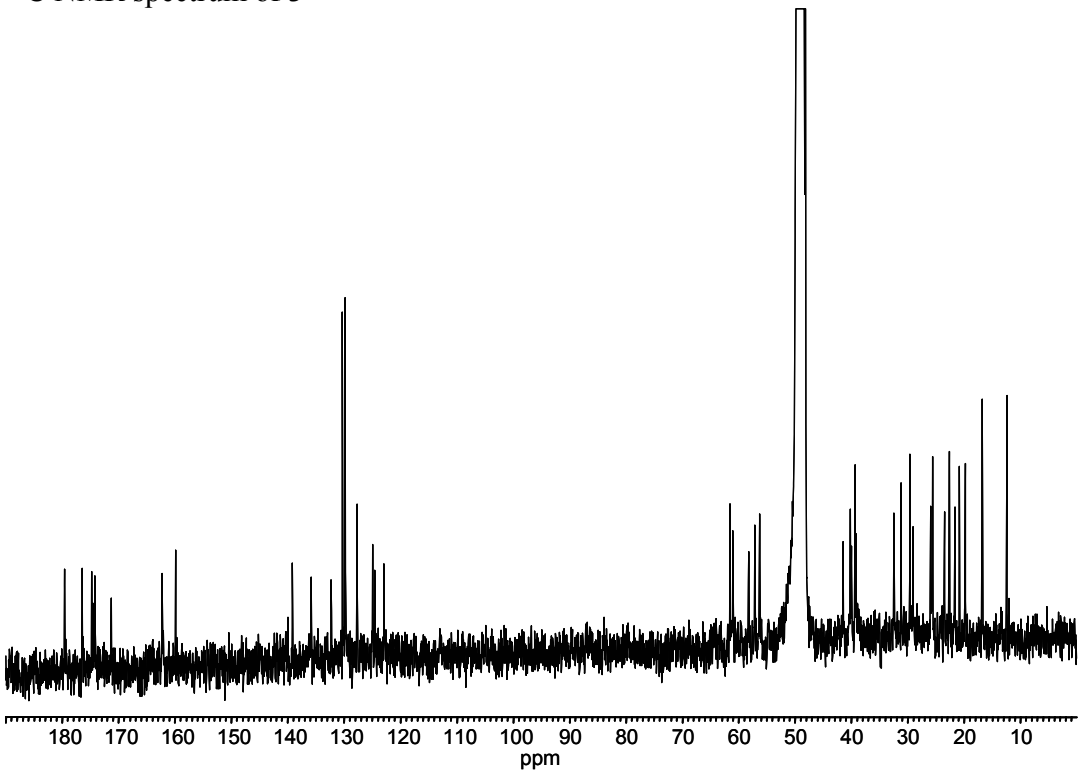
4. NMR spectra of brunsvicamide C (3)

All spectra were recorded in CD_3OD either using a Bruker Avance 300 DPX or 500 DRX spectrometer operating at 300 or 500 MHz for proton and at 75 or 125 MHz for ^{13}C, respectively. Spectra were referenced to residual solvent signals with resonances at $\delta_{H/C}$ 3.35/49.0 (CD_3OD).

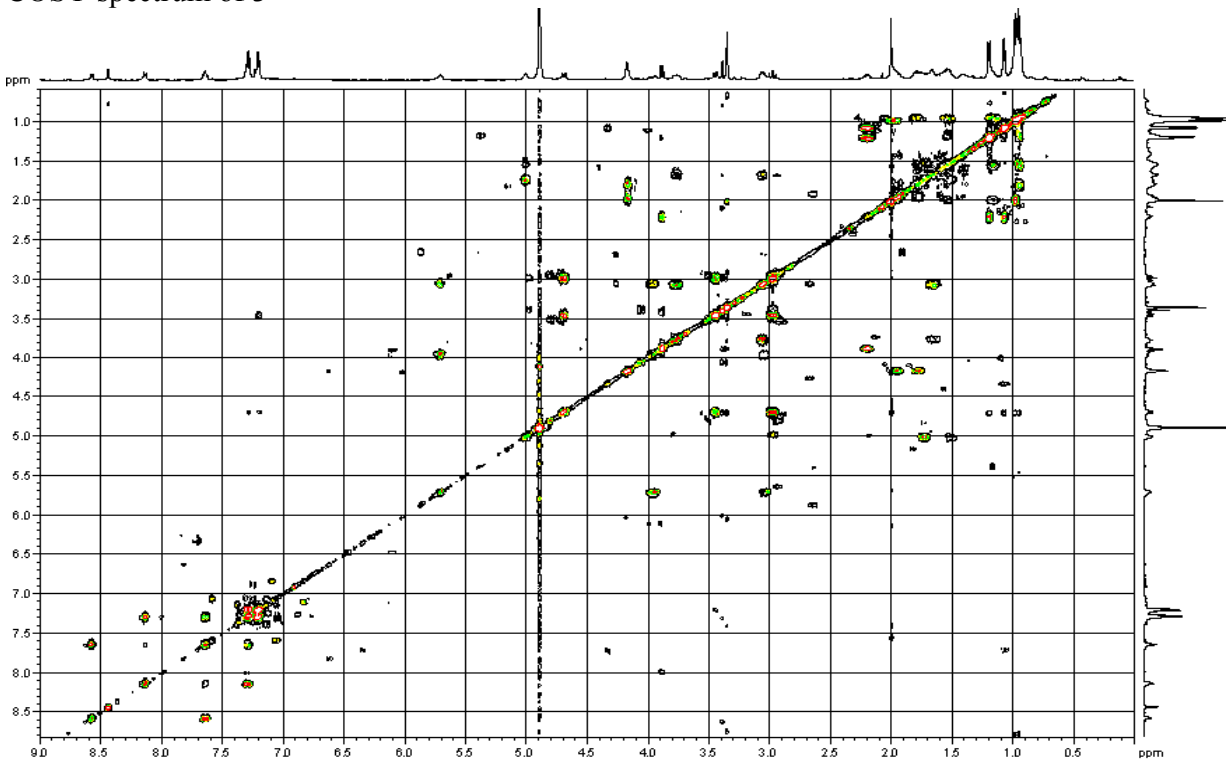
^1H NMR spectrum of 3



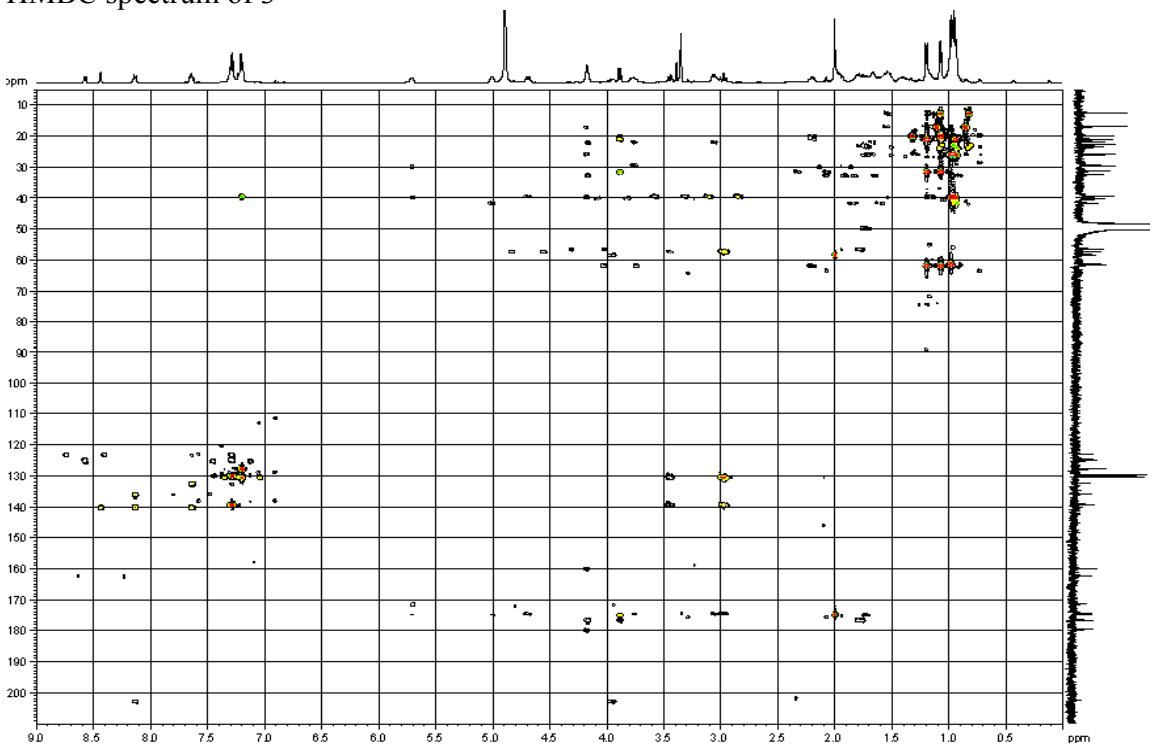
^{13}C NMR spectrum of 3



COSY spectrum of 3

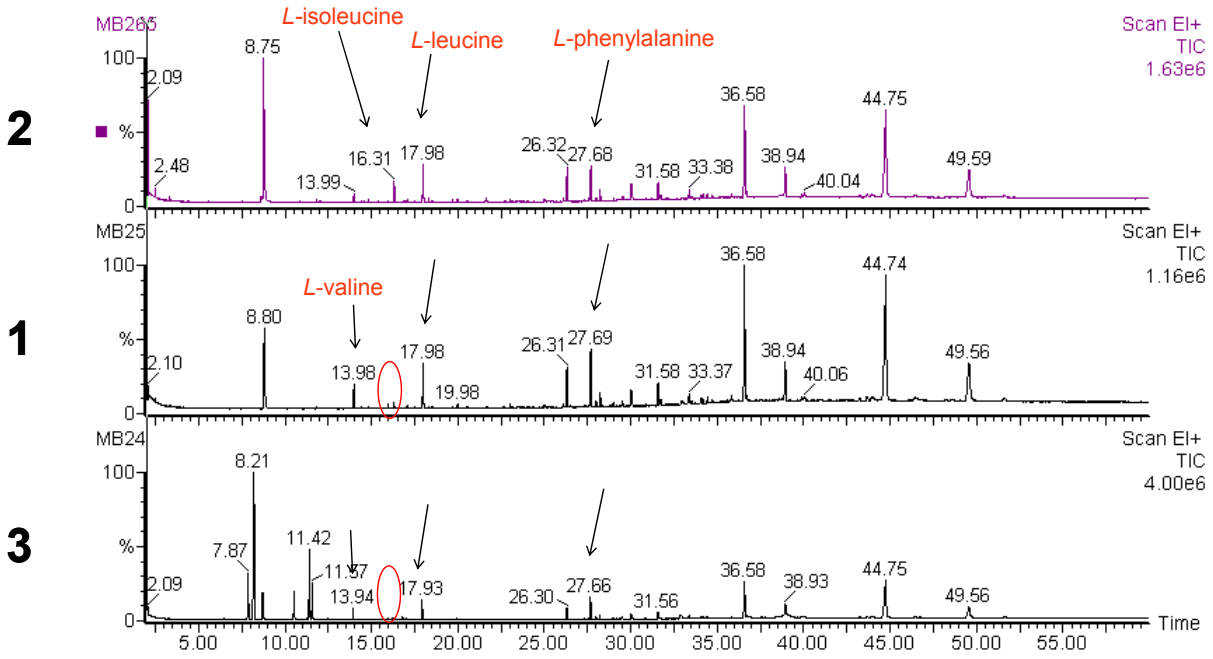


HMBC spectrum of 3



5. Chiral GC-MS of acid hydrolysate of brunsvicamides

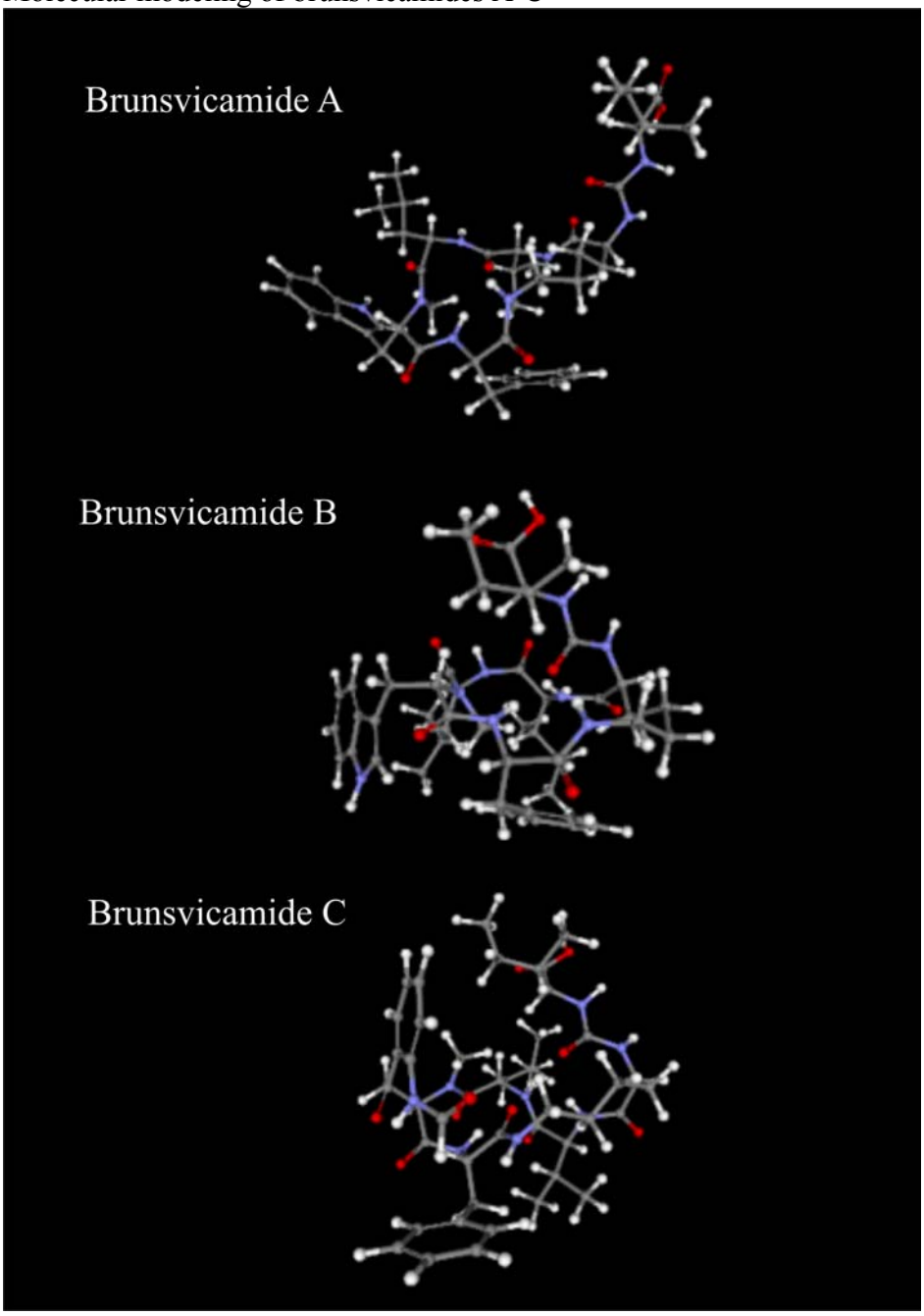
After acid hydrolysis the *N*-pentafluoropropionyl isopropyl ester derivatives of the brunsvicamide A-C (**1-3**) were analysed by GC-MS using an Alltech Capillary Chirasil-Val column (25 m x 0.25 mm; 0.16 µm; program rate: column temperature held at 50°C for 3 min; 50°C – 180°C at 4°C/min; flow: 0.6 mL/min; Inj.: 250°C, for *N*-Me-Asp analysis the program rate was as follow: initial column temperature 70°C, 70°C – 100°C at 0.5°C/min, 100°C – 180°C at 2°C/min, flow: 0.5 mL/min; Inj.: 200°C). The retention times of the *N*-pentafluoropropionyl isopropyl ester derivatives of the amino acids were compared with those of standards that had been derivatised in the same manner. Red circles indicate the absence of *L*-isoleucine in GC chromatograms of brunsvicamide A and C (**1** and **3**) after acidic hydrolysis.



6. Molecular modeling of brunsvicamides A-C

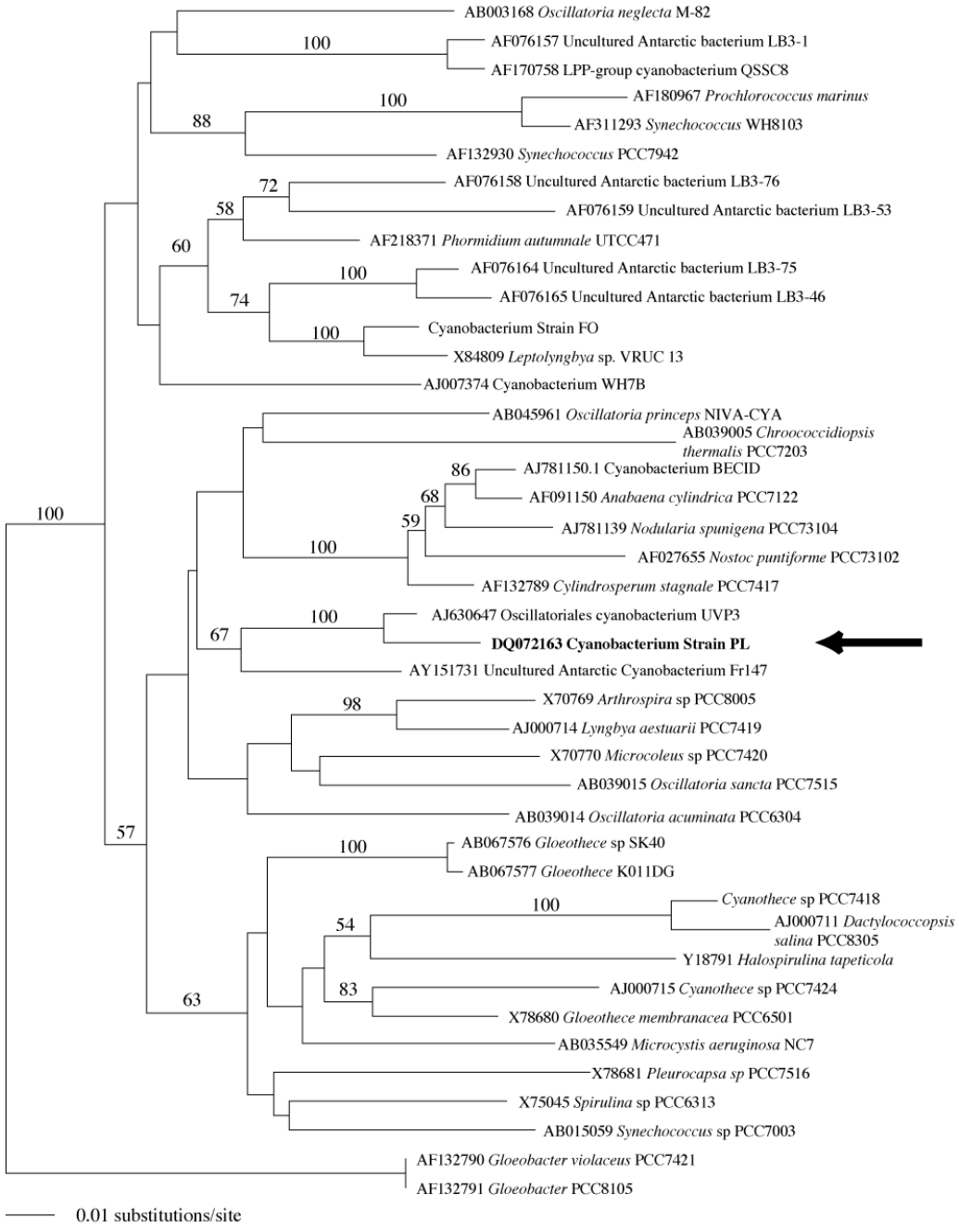
Brunsvicamides A-C were mimicked by conformation search (Boltzmann Jump) using a cff1.01 force field (Cerius^2 4.0 molecular modeling software package, MSI). Significant NOE correlations were implemented as distance restraints in the calculations. All models were refined with 500 iterations of smart minimisation, followed by conjugate gradient minimisation. Calculations were performed using a Silicon Graphics O2 workstation (Irix 6.5.6).

Molecular modeling of brunsvicamides A-C



7. Phylogenetic tree

The phylogenetic tree based on 16S rDNA sequences shows the correlation of the investigated cyanobacterial strain *Tychonema* sp. (DQ072163 cyanobacterium strain PL, see arrow) with other cyanobacteria. The scale bar indicates the evolutionary distance and represents 0.01 substitutions per site. A close relationship of the investigated strain Pl 1 to two cyanobacterial strains could be observed: Oscillatoriales cyanobacterium UVP3 (GenBank accession number AJ630647) and an uncultured Antarctic cyanobacterium Fr147 (GenBank accession number AY151731). Comparison of the 16S rDNA sequences displayed an evolutionary distance of 3% (Oscillatoriales cyanobacterium UVP3) and 8% (uncultured Antarctic cyanobacterium Fr147). This analysis was conducted in collaboration with Dr. M. Hart and Dr. F. C. Küpper (CCAP, Dunstaffnage, Scotland, UK).


AB003168 Oscillatoria neglecta M-82
100
AF076157 Uncultured Antarctic bacterium LB3-1
AF170758 LPP-group cyanobacterium QSSC8
100
AF180967 Prochlorococcus marinus
88
AF311293 Synechococcus WH8103
AF132930 Synechococcus PCC7942
72
AF076158 Uncultured Antarctic bacterium LB3-76
58
AF076159 Uncultured Antarctic bacterium LB3-53
AF218371 Phormidium autumnale UTCC471
60
100
AF076164 Uncultured Antarctic bacterium LB3-75
74
AF076165 Uncultured Antarctic bacterium LB3-46
100
Cyanobacterium Strain FO
X84809 Leptolyngbya sp. VRUC 13
AJ007374 Cyanobacterium WH7B
AB045961 Oscillatoria princeps NIVA-CYA
AB039005 Chroococcidiopsis thermalis PCC7203
86
AJ781150.1 Cyanobacterium BECID
68
AF091150 Anabaena cylindrica PCC7122
100
59
AJ781139 Nodularia spunigena PCC73104
AF027655 Nostoc puntiforme PCC73102
AF132789 Cylindrosperum stagnale PCC7417
100
AJ630647 Oscillatoriales cyanobacterium UVP3
67
DQ072163 Cyanobacterium Strain PL
AY151731 Uncultured Antarctic Cyanobacterium Fr147
98
X70769 Arthrospira sp PCC8005
AJ000714 Lyngbya aestuarii PCC7419
X70770 Microcoleus sp PCC7420
AB039015 Oscillatoria sancta PCC7515
AB039014 Oscillatoria acuminata PCC6304
57
100
AB067576 Gloeothece sp SK40
AB067577 Gloeothece K011DG
100
Cyanothece sp PCC7418
54
AJ000711 Dactylococcopsis salina PCC8305
Y18791 Halospirulina tapeticola
83
AJ000715 Cyanothece sp PCC7424
63
X78680 Gloeothece membranacea PCC6501
AB035549 Microcystis aeruginosa NC7
X78681 Pleurocapsa sp PCC7516
X75045 Spirulina sp PCC6313
AB015059 Synechococcus sp PCC7003
100
AF132790 Gloeobacter violaceus PCC7421
AF132791 Gloeobacter PCC8105
0.01 substitutions/site