Supplementary

Targeted Genome Mining – From Compound Discovery to Biosynthetic Pathway Elucidation – Supplementary Materials

Nils Gummerlich [1], Yuriy Rebets [1], Constanze Paulus[1], Josef Zapp[2] and Andriy Luzhetskyy[1,3,*]

[1] Department of Pharmaceutical Biotechnology, Saarland University, Campus C2.3, 66123, Saarbrücken, Germany; nils.gummerlich@gmx.de (N.G.), yurko.rebets@gmail.com (Y.R.), constanzepaulus@gmail.com (C.P.)
[2] Department of Pharmaceutical Biology, Saarland University, C2.3, 66123 Saarbrücken, Germany; j.zapp@mx.uni-saarland.de (J.Z.)
[3] Helmholtz-Institute for Pharmaceutical Research Saarland (HIPS), Campus E8.1, 66123, Saarbrücken, Germany.
* Correspondence: a.luzhetskyy@mx.uni-saarland.de Tel.: +49-681-302-70200 (A.L.)
* Correspondence: andriy.luzhetskyy@helmholtz-hips.de; Tel.: +49-681-302-70200 (A.L.)

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Table S 1 Oligonucleotides used in this work; All Oligonucleotides were produced by Eurofins (Eurofins Genomics Germany GmbH, Ebersberg, Germany)

Name	Sequence of the Oligonucleotide
f3E7test	GTCGTTGACCCTCAGCAAGT
r3E7test	GCCTACCACTTCTACGCGTT
f1G5test	GTAGGCGAACAACTGCAACG
r1G5test	CTTCATGCGCAAGGAGATGC
f1C15test	ACCCGATCGGCCTCAAGATC
r1C15test	AGATCACGATCATCGCGGTC
f3K5test	GCGAAGTACGACACGACGTA
r3K5test	GGGGTGTCCGGTTCTACAAG
f1F6tes	ATGCTCAGGATCGAATCGCC
r1F6test	ACAGCTACGCCTTCGACTTC
f1C7test	GAATCATCGCCGACAGCAAC
r1C7test	GTTCTCGTCGATCTTGCCGA
f3E19test	AGCCTGGTCAACCACTGTTC
r3E19test	AGCCCAACAGTTGATAGCCC
f1G11test	CCTTCGAGATCTCCGGGTTG
r1G11test	GACTCGGGAACTCCTTACGC
f3C18test	GAGTACCACATCGCCACCAA
r3C18test	AGAGGATCTCCAGGTCGTCG
f1L8test	CCACTGACGTGACGCATACT
r1L8test	GTGAGCAGTGAGGTGTGGTT
f3M21test	AGACCGTCGAGCTGAACAAG
r3M21test	CACACCGTCTGGAGGATCTG
f1E5test	AAGCTGTGGTTGTACTCCGG
r1E5test	GCTTTCGGGGTCAATTGTCG
f3A24test	AGTTCGTGGAAGCGCTGGAC
r3A24test	AAGTCCTCCAGGACCTTCACC
f3K17test	GAAGACGTCGATGCTCCACA
r3K17test	ATCTTCGCCGGCTACAACAT
f-del-54860	CACCACGCCCCGGCACGACGACCCGGCCACCGCGTCACGGC TTCCGGGGATCCGTCGACCC
r-del-54860	GGGCCGCGCCAGCACCATGGCGCTCTGGAAGCCGCCGAAAC TGTAGGCTGGAGCTGCTTCG
f-del-55110	ATGGAGCCCATCGCGATCATCGGTGTGGGGTGCCGGTTCCC TTCCGGGGATCCGTCGACCC
r-del-55110	CGCGACCTCGCGCGGTGGTTCGTCACGACGCAGCGACGGCA TGTAGGCTGGAGCTGCTTCG
f-chk-54860	GCCTGCTTCGACGCGATCAA
r-chk-54860	GATCACGCCCATGTCGAACG
f-chk-55110	GATCACGCTCTCCCAGACG
r-chk-55110	GACGAGTCCTTCATCGCCAA
f-del-54730	GTGACCACCACAAGCCCCGCCTCCACGATCGACCTCTATTCCGACGAATTT CCGGGGATCCGTCGACCC
r-del-54730	TCAGCGGTGCAGGGTGGTCGGGAGCGCGGCGATGCCGCGGATGGTGTTGTG TAGGCTGGAGCTGCTTCG
f-del-54740	ATGAGGCTGATCGTCGATCGCGACCGCTGCGAAGGGCACGGCGTGTGCGTT CCGGGGATCCGTCGACCC
r-del-54740	TCAGCCTTCCAGCTTGAGGGCGGCGACCGGGCAGATGAGCACCGAGTCGT GTAGGCTGGAGCTGCTTCG

f-del-54750	ATGGAGAGCACGGCGCGAACGGTGTTCCGCCCGGCCTCGGCCCGCACCATT CCGGGGATCCGTCGACCC
r-del-54750	CTAGGACACCCTGGTCCGGGCCGCCCGGCCCTTGAGCAGCCGGCCCAGCTG TAGGCTGGAGCTGCTTCG
f-del-54760	ATGTCAGAGGAGAGCACCGAGAACACGACCGCCATGCGGTTGCTGCGGCT TCCGGGGATCCGTCGACCC
r-del-54760	CTAGGCGCGCACTGCTTCTACGACGCTGCACAGCCCGGCGGGGACGATCTG TAGGCTGGAGCTGCTTCG
f-del-54770+80	ATGCCGGTAATCGCGGCGAACGACGGTTGTCTGACCGTCTTCAACATGTTTC CGGGGATCCGTCGACCC
r-del-54770+80	TCAGTGCGGCGGGTCGACCAGCCCCAGTTGGACGAGGATGCCCATGTCGTG TAGGCTGGAGCTGCTTCG
f-del-54790	ATGAGCACACCCCCGTCACTGGTCGTCGTCGGCGCGTCCTTGGCCGGTCTTC CGGGGATCCGTCGACCC
r-del-54790	TCAGTCGCGGTGCACCGACGTGACCGCGTCGGACCAGGTCACGCCCCGGTG TAGGCTGGAGCTGCTTCG
f-del-54800	ATGATGGAAGGCGAACTGGGGGCCTTCCTGCGCAGTCGCCGTGAAGCCGTT CCGGGGATCCGTCGACCC
r-del-54800	TCAGCCGGAGCCGACCGCGCGCAACCCCACCTGGTTCACGTTCAGCCGGTG TAGGCTGGAGCTGCTTCG
f-del-54820	TCAGGCCGAGGCGCTGTCCAATCGGGACAGTTCCGCCGCAGTCAGCTCGTT CCGGGGATCCGTCGACCC
r-del-54820	ATGCGTCCGACTCCGCTGGGCACCGCCGCGCTGGGCACCACCGGGTTGATG TAGGCTGGAGCTGCTTCG
f-del-54830	TCAGGCGGGGTGCGGCGACCCGGTCGACCAGATCAGGTGGAACGACAGCT TCCGGGGATCCGTCGACCC
r-del-54830	ATGACAGCCGAGTCCGACCTGCCGTCCCGTGCCGACGTGGGCGCGCTGCTG TAGGCTGGAGCTGCTTCG
f-del-54930	ATCGAGGACGGCCCCGACCCGCAGGCCACCGAGCGCCAGCACGCCAAGGT TCCGGGGATCCGTCGACCC
r-del-54930	TCATTGCGGCGGGTTGCCGTGCTTGCGGCTGGGCAGGTCGGCGTGCTTGTGT AGGCTGGAGCTGCTTCG
f-del-54940	GTGCAAGGTGCATTCGTGGAGTTGCGATGCGTTGCCGACCGAATACCGGTT CCGGGGATCCGTCGACCC
r-del-54940	CTATTGCCAACTCGACGGGCTGCGGTAGGCCGTAACGCGTTCCGCGCGGTG TAGGCTGGAGCTGCTTCG
f-del-54950	GTGGTAGGGCAACAAGCCGGTGAGTTAGTCAGCACATGGGAGCCCGCGCT TCCGGGGATCCGTCGACCC
f-del-54950	TCAGCCCTCGCCGAGGCGGAAACCGACACCGCGAACGGTGACGATCCACT GTAGGCTGGAGCTGCTTCG
f-del-1E5-left	GTGGTGCCGCTCTCCCTCATAGGTTTGCCAACGACGGCGTCAGTGCGTCTTC CGGGGATCCGTCGACCC
r-del-1E5-left	CTGGCAGTGCCGGTCTCAGCTCGGCCTCCCTCACCGGCGACGGCTGCCATG TAGGCTGGAGCTGCTTCG
f-del-1E5-right	TCACCACGCTCCAAGGCGGAGCCGGGACGTGCCCGCCGCCCACGGCCGCT TCCGGGGATCCGTCGACCC
r-del-1E5-right	TCACGTCACGGTGAGGCGGATGGGTATGCCGAGGCTGGGGTTGCCCGGATG TAGGCTGGAGCTGCTTCG
f-del-54730-chk	TGAACTTCGGGACCTTGACG
r-del-54730-chk	CGATCGACGATCAGCCTCAT
f-del-54740-chk	GGTACAGCCGACCGACAC
r-del-54740-chk	ATCAGCTCGATGGTGCGG

f-del-54750-chk	ATGAGGCTGATCGTCGATCG
r-del-54750-chk	GTTCTCGGTGCTCTCCTCTG
f-del-54760-chk	CGCTGACGGTGTACAAGCA
r-del-54760-chk	TCCCGCATCTCTTTGAGCAG
f-del-54770+80-chk	TGGACCTCGACATGTTGGTC
r-del-54770+80-chk	TCCTCTCCTGGGTCCTGTTC
f-del-54790-chk	GAACAGGACCCAGGAGAGGA
r-del-54790-chk	GTTCGAGACGATGCAACTGG
f-del-54800-chk	TCCCCTACTTCTGGTCGGAC
r-del-54800-chk	GTGCTCGGTCATGTCTTCCC
f-del-54820-chk	CATCGGCGTTGTCGGAAAC
r-del-54820-chk	GTCGTTCCACCTGATCTGGT
f-del-54830-chk	GGTCGTGCAGGTAGTACAGG
r-del-54830-chk	GAGTGGGAGTTCTGCGACAA
f-del-54930-chk	GAAGGGAGCAGTGCGTGAA
r-del-54930-chk	CTTCTCCACCAGAACCTCCG
f-del-54940-chk	TGGTCCGGCAGTACAAAGAC
r-del-54940-chk	TTTTCCGGTGTCATGACGGT
f-del-54950-chk	TGCGGTTCACACTCTTGAGC
r-del-54950-chk	ATGTGGATGACTGCCCGTG
f-chk-1E5-le-int	GATCGCCATTGTGTCCGTTG
r-chk-1E5-le-int	CATCTCGATCACTCCCACGG
f-chk-1E5-ri-int	TCACAAGGCACCTTACGGAC
r-chk-1E5-ri-int	CATCAGCGAAATCACTGCCG
f-chk-1E5_ri	CTCCGGGAAGTGATACTCGC
r-chk-1E5_ri	GACGTTCTCCTCAGGTGACG
f-chk-1E5_le	CACTCGACGTCCAACTGTCA
r-chk-1E5_le	GAGTGCCGCGGATATCTGAA
F-54800A3OE	AAAAAAAGGTACCTAGCAGGGCTCCAAAACTAACGCCTGATGTAGGATCAGATGAAAAAAAAAAAAGGAGGAAAATACATATGATGGAAGGCGAACTGGG
R-54800A3OE	AAAAAAAAAGCTTTCAGCCGGAGCCGACCGCGCGCAACCCCACCTGGTTCACGTT
F-54950A3OE	AAAAAAAGGTACCTAGCAGGGCTCCAAAACTAACGCCTGATGTAGGATCAGATGAAAAAAAAAAAAGGAGGAAAATACATGTGGTAGGGCAACAAGCC
R-54950A3OE	AAAAAAAAAGCTTTGTGGGTGTTCCACTCCGGA

Table S 2 Strains, Plasmids and BACs used in this work

Streptomyces	Characteristics	Reference
S. albus J1074	*S. albus G1 (DSM 41398)* derivative with the defective *SalG* I restriction modification system heterologous host	(Chater and Wilde, 1980 [1])
Streptomyces lividans Δ6	*S. lividans* TK24 derivative, deletion of 6 BGCs	(Paper in Revision)
Saccharothrix espanaensis DSM 44229 (T)	Producer of Saccharomicins	Labeda et al.[2]
E. coli	**Characteristics**	**Reference**
GB2005	General cloning host	Wang et al., 2006[3]
ET12567 pUZ8002	Strain used for intergeneric conjugation,	Kieser 2000[4]
GB2005-red-rham	*GB2005*, RhamC-BAD-γbaA used for Red/ET	Strochlic et al., 2010[5]
Plasmids	**Characteristics**	**Reference**
psmart-BAC-S	Apr^R; BAC Vector	Lucigen
pTOS	Apr^R; VWB-based *Streptomyces* integrative vector	Herrmann et al. (2012)[6]
pTOS_A3_R1	pTOS derivative; Integrated Gene 54800, influenced by A3 promotor cloned into the Kpn I and Hind III site	This work
pTOS_A3_R2	pTOS derivative; Integrated Gene 54950, influenced by A3 promotor cloned into the Kpn I and Hind III site	This work
patt-shyg	*Swa* I/*EcoR* V fragment of phyg-OK containing hyg cloned into the *EcoR* V site of patt	Myronovskyi et al. [7]
BACs	**Characteristics**	**Reference**
pSMART-BAC-S-1C15	pSMART-BAC-S derivative containing a fragment of the *S. espanaensis* chromosome (Locus in bp: 1,670,099-1,769,322)	Intact genomics
pSMART-BAC-S-3A24	pSMART-BAC-S derivative containing a fragment of the *S. espanaensis* chromosome (Locus in bp: 2,190,390-2,280,022)	Intact genomics
pSMART-BAC-S-3E7	pSMART-BAC-S derivative containing a fragment of the *S. espanaensis* chromosome (Locus in bp: 2,332,949-2,404,154)	Intact genomics
pSMART-BAC-S-1G5	pSMART-BAC-S derivative containing a fragment of the *S. espanaensis* chromosome (Locus in bp: 2,825,657-2,924,398)	Intact genomics
pSMART-BAC-S-1F6	pSMART-BAC-S derivative containing a fragment of the S. espanaensis chromosome (Locus in bp: 3,429,623-3,516,631)	Intact genomics
pSMART-BAC-S-1I20	pSMART-BAC-S derivative containing a fragment of the *S. espanaensis* chromosome (Locus in bp: 3,577,211-3,660,078)	Intact genomics
pSMART-BAC-S-3E19	pSMART-BAC-S derivative containing a fragment of the S. espanaensis chromosome (Locus in bp: 3,526,632-3,686,841)	Intact genomics
pSMART-BAC-S-3K5	pSMART-BAC-S derivative containing a fragment of the *S. espanaensis* chromosome (Locus in bp: 3,786,071-3,893,910)	Intact genomics

pSMART-BAC-S-1G11	pSMART-BAC-S derivative containing a fragment of the *S. espanaensis* chromosome (Locus in bp: 4,412,386-4,533,983)	Intact genomics
pSMART-BAC-S-3C18	pSMART-BAC-S derivative containing a fragment of the *S. espanaensis* chromosome (Locus in bp: 4,412,386-4,533,983)	Intact genomics
pSMART-BAC-S-1L8	pSMART-BAC-S derivative containing a fragment of the *S. espanaensis* chromosome (Locus in bp: 5,681,922-5,790,602)	Intact genomics
pSMART-BAC-S-3M21	pSMART-BAC-S derivative containing a fragment of the *S. espanaensis* chromosome (Locus in bp: 5,865,637-5,963,104)	Intact genomics
pSMART-BAC-S-1E5	pSMART-BAC-S derivative containing a fragment of the *S. espanaensis* chromosome (Locus in bp: 6,074,530-6,190,776)	Intact genomics
pSMART-BAC-S-1C7	pSMART-BAC-S derivative containing a fragment of the *S. espanaensis* chromosome (Locus in bp: 6,252,700-6,375,967)	Intact genomics
pSMART-BAC-S-3K17	pSMART-BAC-S derivative containing a fragment of the *S. espanaensis* chromosome (Locus in bp: 6,511,399-6,617,883)	Intact genomics
1E5Δ55110	pSMART-BAC-S-1E5 derivative; Knockout of gene BN6_55110	This work
1E5Δ54860	pSMART-BAC-S-1E5 derivative; Knockout of gene BN6_54860	This work
1E5ΔpenA	pSMART-BAC-S-1E5 derivative; Knockout of gene BN6_54730	This work
1E5ΔpenC	pSMART-BAC-S-1E5 derivative; Knockout of gene BN6_54750	This work
1E5ΔpenD	pSMART-BAC-S-1E5 derivative; Knockout of gene BN6_54760	This work
1E5ΔpenE/F	pSMART-BAC-S-1E5 derivative; Knockout of gene BN6_54770 & BN6_54780	This work
1E5ΔpenG	pSMART-BAC-S-1E5 derivative; Knockout of gene BN6_54790	This work
1E5ΔpenR1	pSMART-BAC-S-1E5 derivative; Knockout of gene BN6_54800	This work
1E5ΔpenI	pSMART-BAC-S-1E5 derivative; Knockout of gene BN6_54820	This work
1E5ΔpenJ	pSMART-BAC-S-1E5 derivative; Knockout of gene BN6_54830	This work
1E5ΔpenV	pSMART-BAC-S-1E5 derivative; Knockout of gene BN6_54940	This work
1E5ΔpenR2	pSMART-BAC-S-1E5 derivative; Knockout of gene BN6_54950	This work
1E5Δleft	pSMART-BAC-S-1E5 derivative; Deletion of left flanking region (Deletion locus in bp: 6,079,768 - 6,123,914; Deletion of 44,147 bp)	This work
1E5Δright	pSMART-BAC-S-1E5 derivative; Deletion of right flanking region (Deletion locus in bp: 6,148,356 6,184,748; Deletion of 36,393 bp)	This work

Table S 3 Preparative HPLC method for pentangumycin (CMP468) & SEK90 (CMP791); 20 mL/min flow rate

Method for 1			Method for 2			Waters AutoPurification System
Min.	%A	%B	Min.	%A	%B	Waters 2545 Binary Gradient module
0	95	5	0	95	5	Waters SFO (System Fluidics organizer)
2	95	5	2	95	5	Waters 2998 PAD (Photodiode array Detector)
16	3	97	16	3	97	Waters SQ-Detector-2
17	3	97	20	3	97	Waters 2767 Sample Manager
18	95	5	21	95	5	Nucleodur C18 Htec 250/4,6 C18 5 μM (analytical)
19	95	5	22	95	5	Nucleodur C18 Htec 250/21 C18 5 μM (preparative)

Biosynthetic pathway of pentangumycin; Roman numerals: proposed intermediates; Arabic numerals: intermediates with identified fitting masses after single gene knockouts and overexpression of the regulatory genes; Black pathway: methylation as first biosynthetic step after aromatization; Grey pathway: methylation as last biosynthetic step to complete the formation of pentangumycin (1).

Table S 4 Preparative HPLC method and yield for 8 and 9; Flow Rate: 20mL/min

Method for 1E5_CMP1 and 1E5_CMP2			Waters AutoPurification System	Purified compounds
Min.	%A	%B	Waters 2545 Binary Gradient module	8: 6 mg
0	95	5	Waters SFO (System Fluidics organizer)	9: 7 mg
1	95	5	Waters 2998 PAD (Photodiode array Detector)	
5	50	50	Waters 2767 Sample Manager	
15	5	95	Nucleodur C18 Htec 250/4,6 C18 5 μM (analytical)	
19	5	95	Nucleodur C18 Htec 250/21 C18 5 μM (preparative)	
20	95	5		
21	95	5		

Table S 5 AntiSMASH predicted biosynthetic gene clusters in *S. espanaensis*

#	Prediction:[a]	Predicted Cluster:[b]	Homology:[c]	BAC:[d]	Expressed:[e]
1	Terpene	Geosmin	100%	known	No
2	Lanthipeptide	Erythreapeptin	75%	homology	No
3	Terpene	Isorenieratene	42%	ex: 1C15	No
4	Bacteriocine			known	No
5	Furan	Asukamycin	30%	ex: 3A24	No
6	NRPS			ex: 3E7	No
7	Other	A54145	3%	not covered	No
8	NRPS	Myxochelin	50%	ex: 1G5	No
9	Indole	Frankiamicin	14%	ex: 1F6	No
10	Ladderane, NRPS	Skyllamycin	22%	ex: 1F6	No
11	Linear azole containing Peptides	A201A	6%	ex: 1I20	No
12	Type I PKS	Tylactone	6%	ex: 3E19	No
13	NRPS	Tyrobetaine	53%	ex: 3K5	No
14	NRPS			not covered	No
15	NRPS, Type I PKS	Kedarcidin	18%	not covered	No
16	Lanthipeptide	Kinamycin	5%	ex: 1G11	No
17	Oligosaccharide	Teicoplanin	4%	not covered	No
18	Melanin			known	No
19	NRPS, Type I PKS	Leinamycin	15%	not covered	No
20	NRPS, Type I PKS	Lavendiol	35%	ex: 3C18	Yes
21	Terpene	Isorenieratene	85%	homology	No
22	NRPS	Cyclomarin	13%	ex: 1L8	No
23	Aminoglycoside			ex: 3M21	No
24	NRPS	Ficellomycin	3%	ex: 3M21	No
25	RIPP	Anantin C	75%	homology	No
26	Terpene, Type II PKS, Type I PKS, NRPS	Fluostatin	23%	ex: 1E5	Yes
27	terpene,NRPS,T1PKS	Ficellomycin	27%	ex: 1C7	No
28	Oligosaccharide	Desosamine	22%	ex: 3K17	No
29	Terpene			excluded	No
30	Terpene	SF2575	6%	excluded	No
31	Lanthipeptide	Olimycin A	8%	excluded	No

a: Prediction: Predicted class of BGC;

b: Predicted cluster: Cluster with the highest homology;

c: Homology: Homology between the predicted cluster and the BGC identified in the genome of S. espanaensis;

d: Work: known: Cluster was known and excluded; homology: due to high homology of the whole cluster, the cluster was excluded; not covered: Cluster was not covered by our library; ex: "XXX" Cluster was covered by the BAC "XXX" and chosen for expression in our host;

e: Expressed: Indication of successful expression in our heterologous hosts

Table S 6 NMR spectroscopic data of 1E5_CMP1 (*$CDCl_3$*)

1E5_CMP1			
Pos.	δ_C	δ_H, m (*J* in Hz)	HMBC
1 & 1'	129,6	7,22 m	-
2 & 2'	128,8	7,33 m	-
3 & 3'	127,6	7,28 m	-
4 & 4'	120,8	5,90 s	-
5 & 5'	39,7	3,71 s	-
6	18,75	3,48 s	-
OH1 & OH1'	-	10,69 s	-

Table S 7 NMR spectroscopic data of 1E5_CMP2 (*$CDCl_3$*)

1E5_CMP2			
Pos.	δ_C	δ_H, m (*J* in Hz)	HMBC
1 & 1'	170,29	-	-
2 & 2'	168,58	-	-
3 & 3'	163,33	-	-
4 & 4'	134,5	-	-
5 & 5'	129,23	7,22 m	C-10
6 & 6'	128,86	7,33 m	C-4
7 & 7'	127,44	7,28 m	C-5
8 & 8'	104,95	-	-
9 & 9'	102,83	5,86 m	C-10, C-8, C-3, C-2
10 & 10'	39,8	3,76 d (7,1 Hz)	C-9, C-5, C-4, C-3
11	31,42	4,13 t (7,6 Hz)	C-13, C-12, C-8, C2, C-1
12	30,13	2,16 m	C-14, C-13, C-11, C-8
13	21,83	1,25 m	C-14, C-12, C-11
14	13,74	0,90 t (7,4 Hz)	C-13, C-12
OH1	-	11,37 s	-
OH1'	-	10,55 s (br)	-

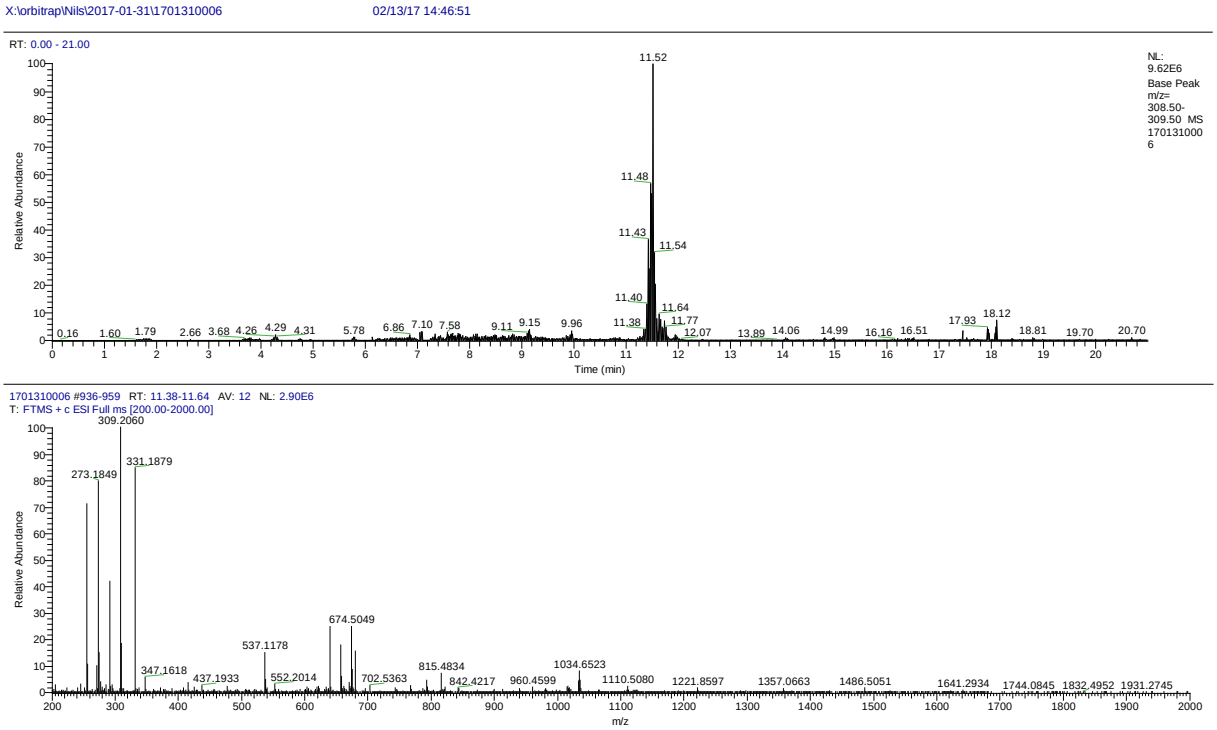


Figure S 1 HPLC-MS Extracted ion chromatogram (Extracted mass 309±0.5) of *S. lividans* ΔYA6_3C18 and the corresponding mass chromatogram

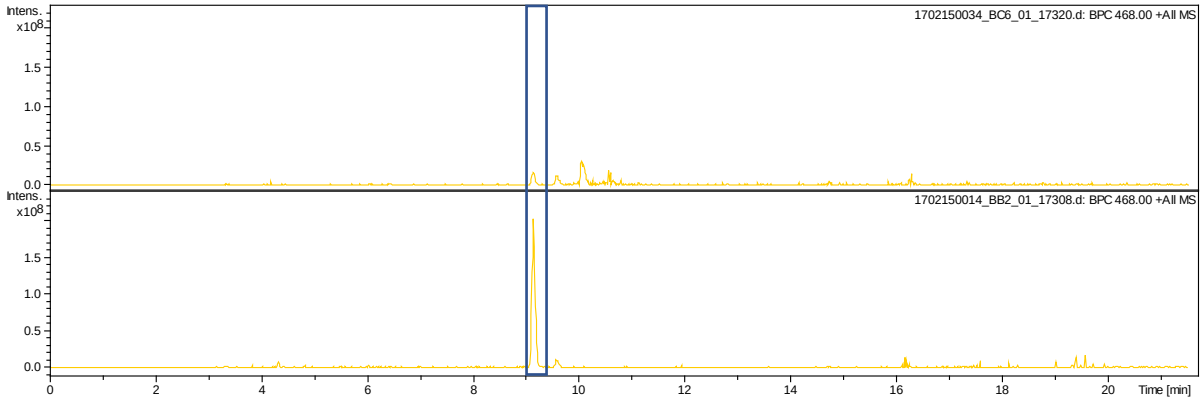


Figure S2 HPLC-MS Extracted ion chromatogram (Extracted mass 468±0.5) of *S. albus* J1074_1E5 (up) and *S. lividans Δ6*_1E5 (down)

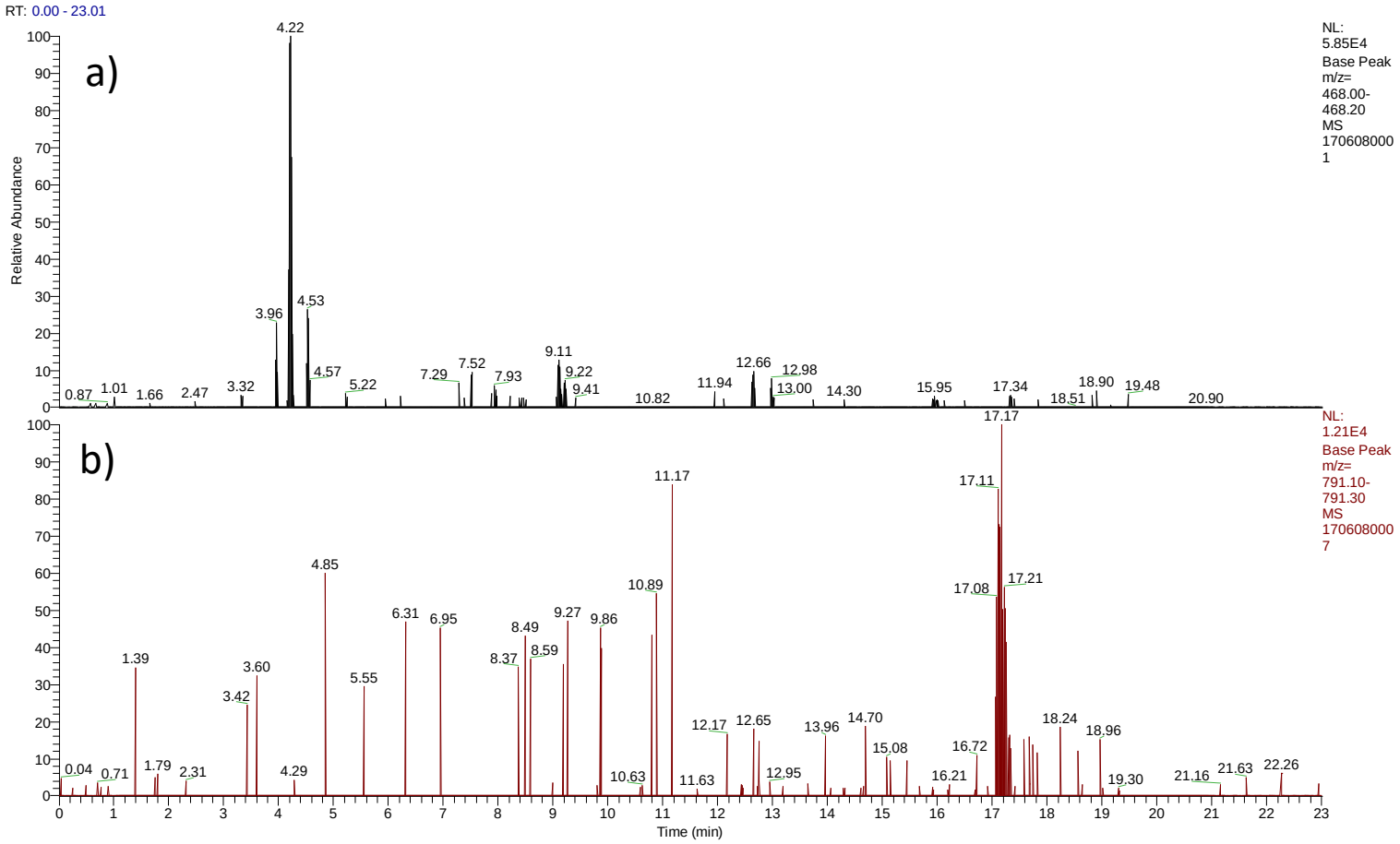


Figure S 3 HPLC-MS Extracted ion chromatogram; a) Extracted mass 468.00 – 468.20, Ethyl acetate extract of *S. espanaensis* grown in SG Medium; b) Extracted mass 791.10 – 791.30; Butanol extract of *S. espanaensis* grown in SG Medium

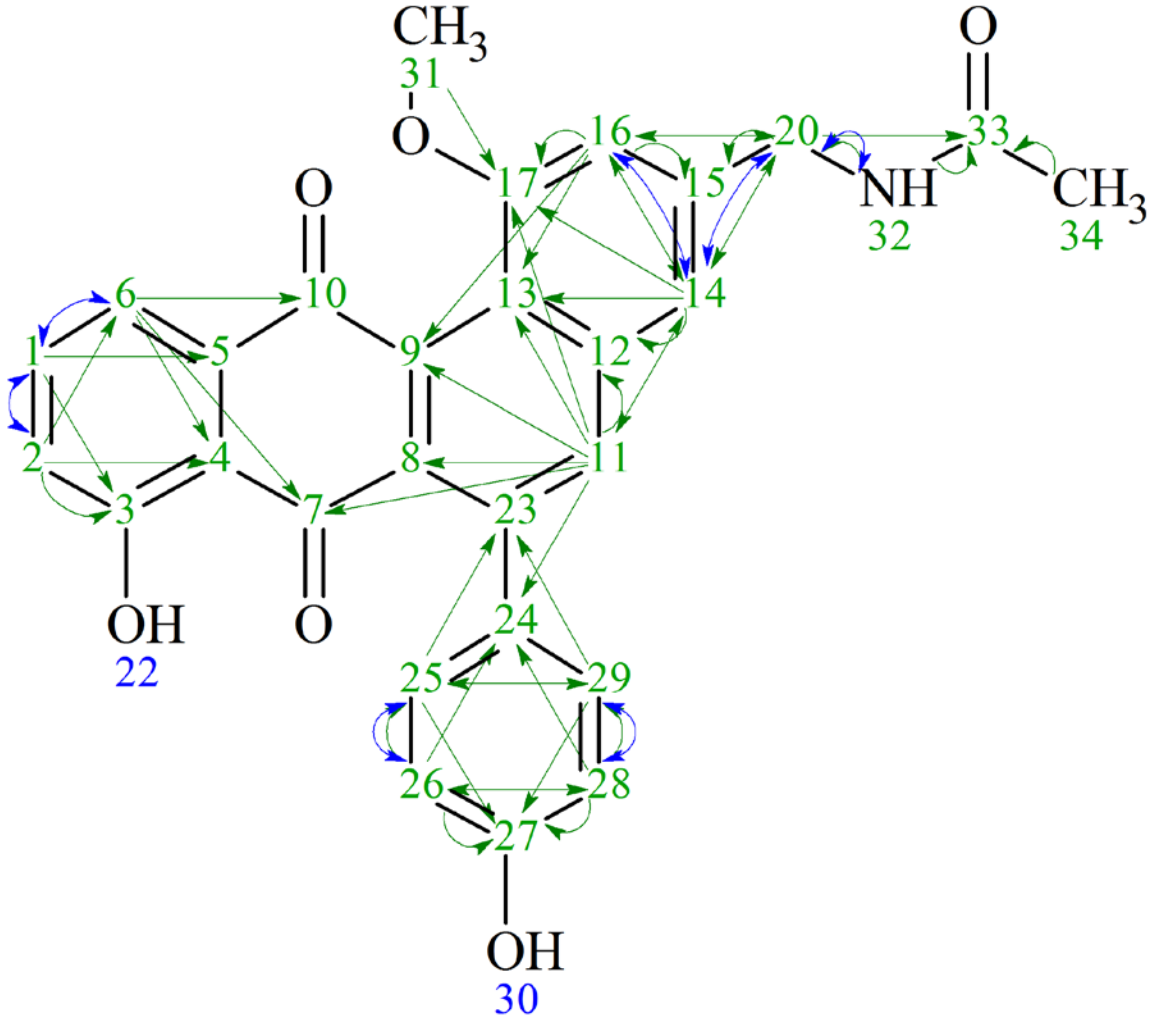


Figure S 4 Structure of Pentangumycin with all correlations (green: HMBC correlations H → C; blue: ^{1}H-^{1}H-Cosy correlation)

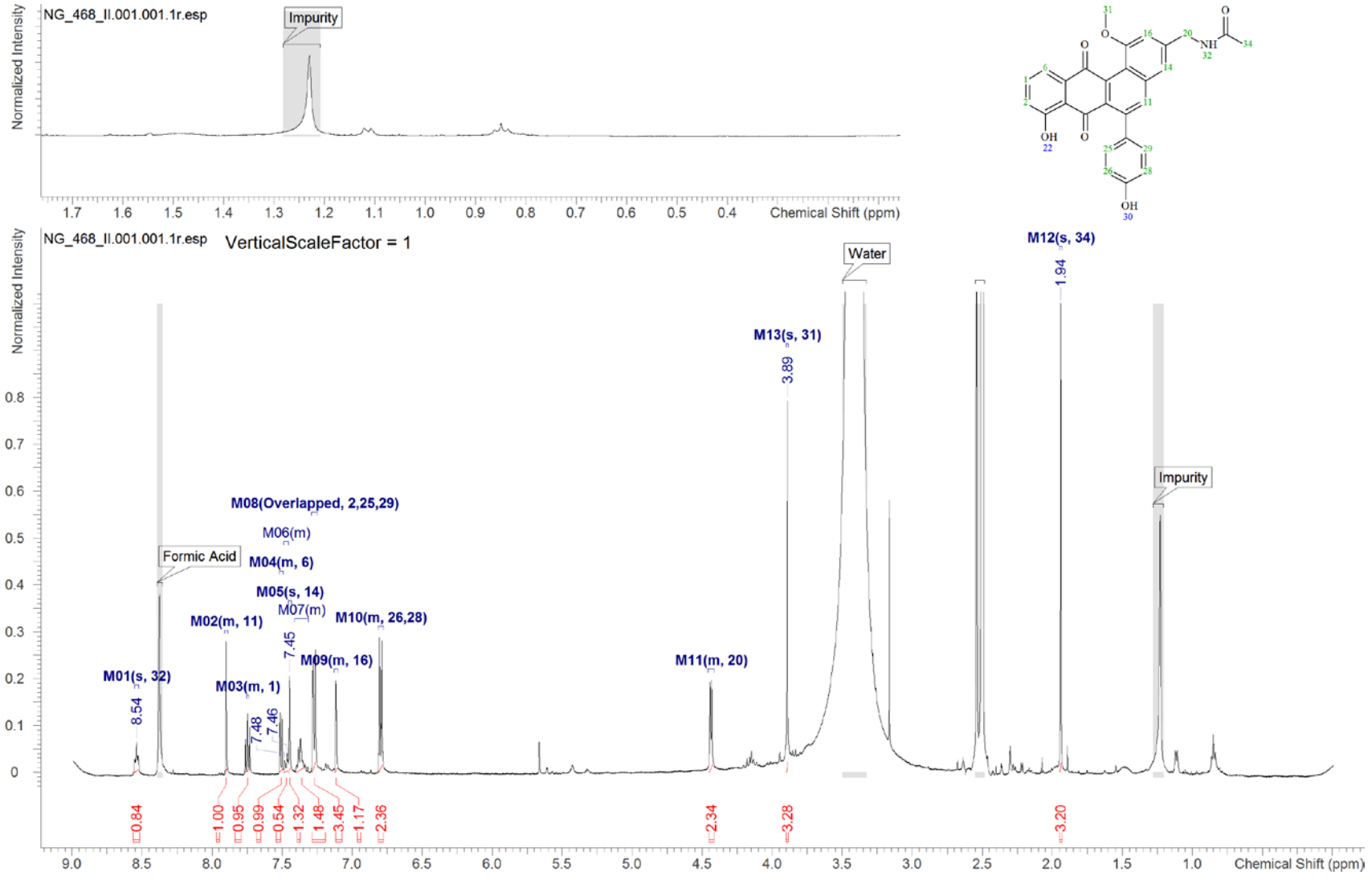


Figure S 5 ^{1}H-NMR spectrum (500 MHz, DMSO-d$_6$) of Pentangumycin; complete Spectrum and zoom from 1.7 to 0.1 ppm

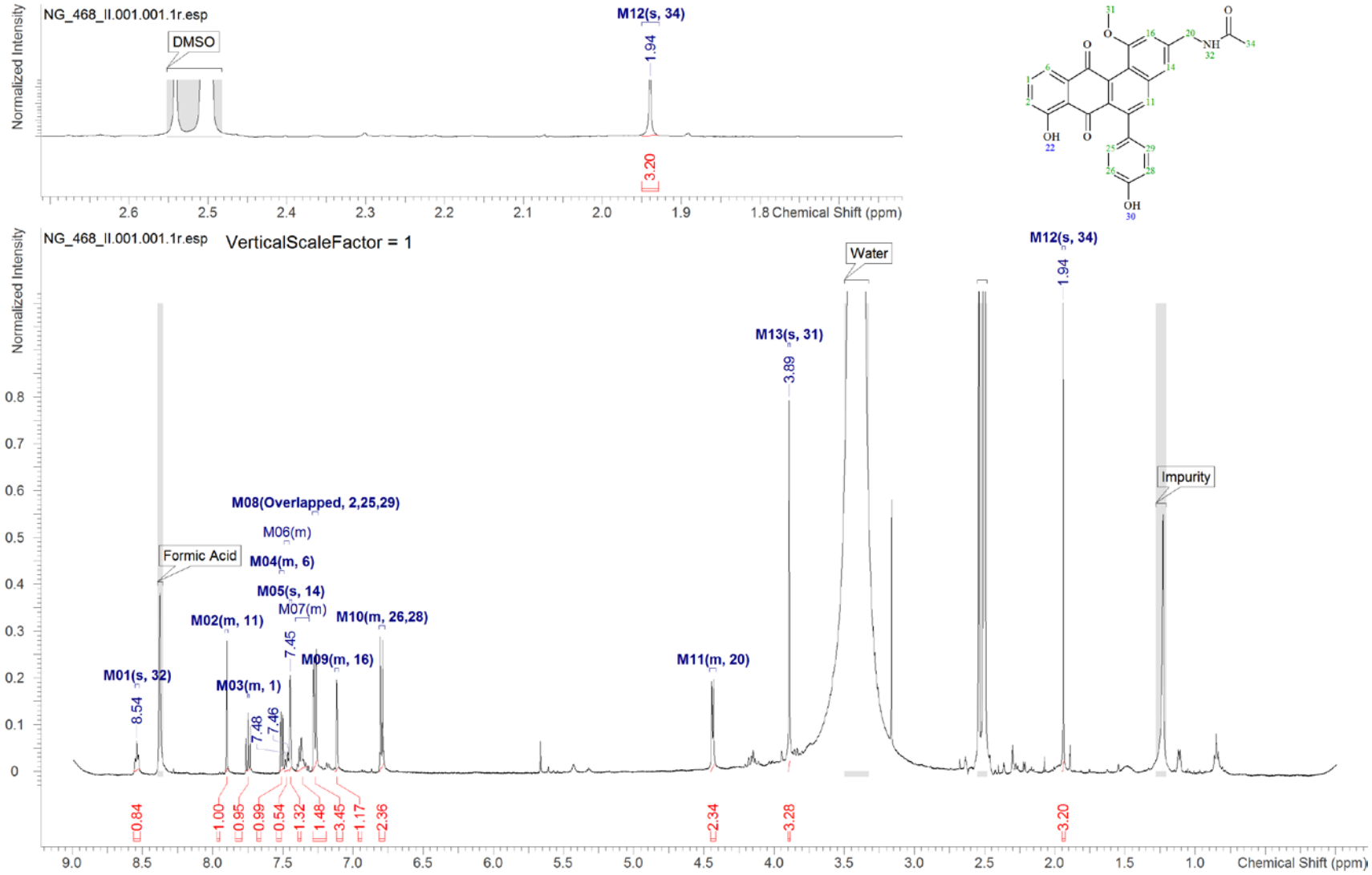


Figure S 6 ^{1}H-NMR spectrum (500 MHz, DMSO-d$_6$) of Pentangumycin; complete Spectrum and zoom from 2.7 to 1.7 ppm

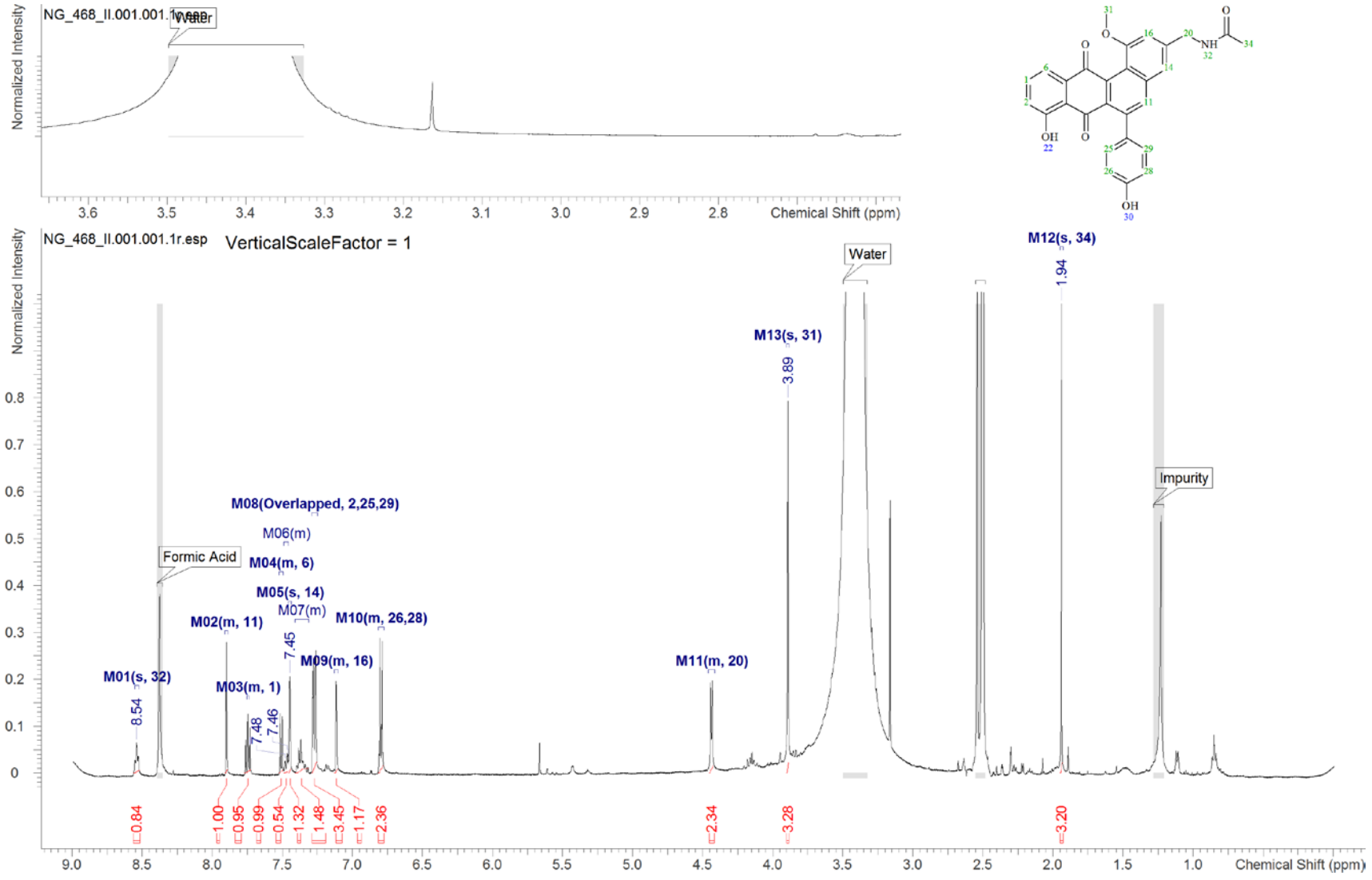


Figure S 7 ^{1}H-NMR spectrum (500 MHz, DMSO-d$_6$) of Pentangumycin; complete Spectrum and zoom from 3.65 to 2.65 ppm

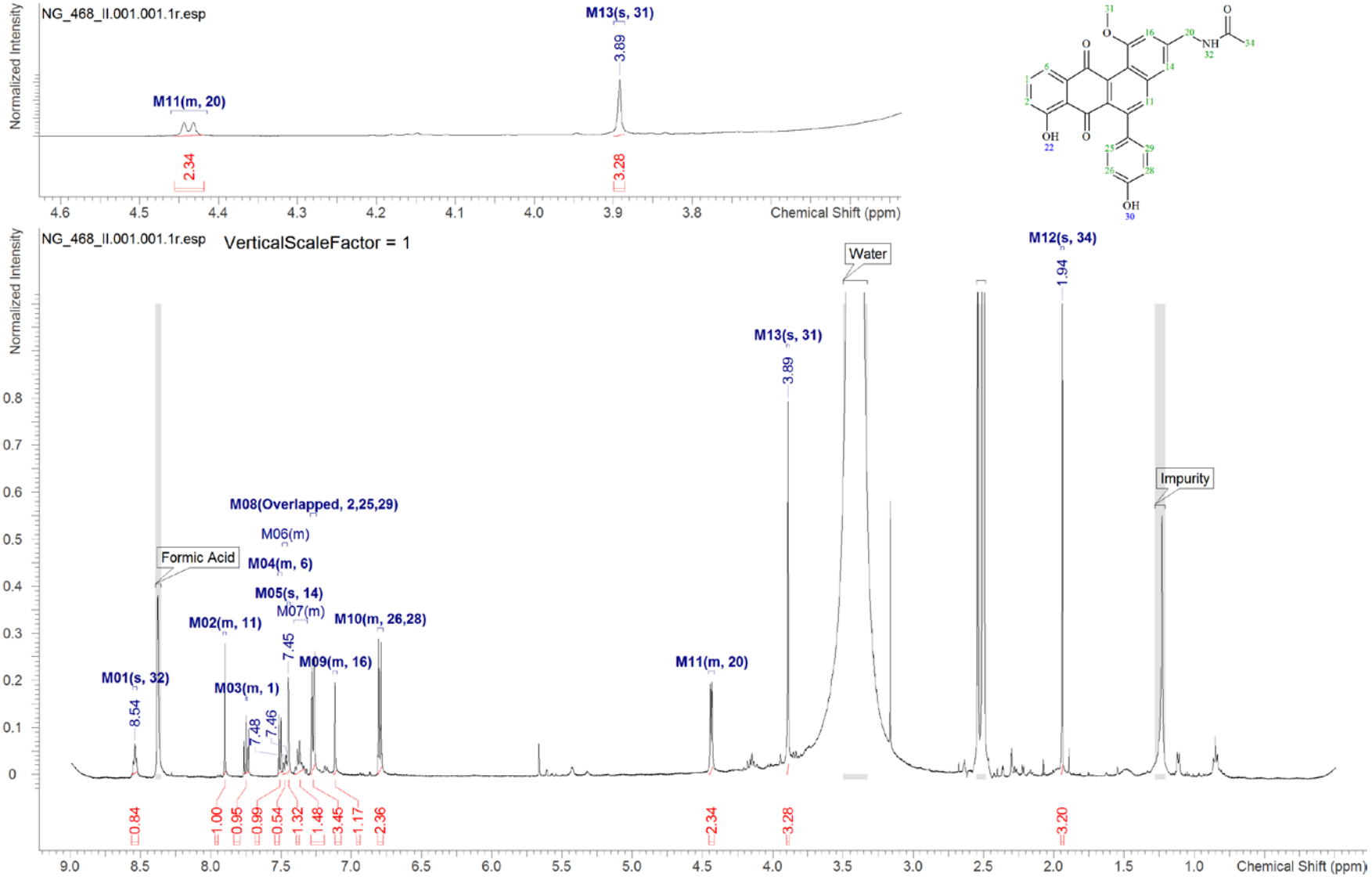


Figure S 8 ^{1}H-NMR spectrum (500 MHz, DMSO-d$_6$) of Pentangumycin; complete Spectrum and zoom from 4.6 to 3.6 ppm

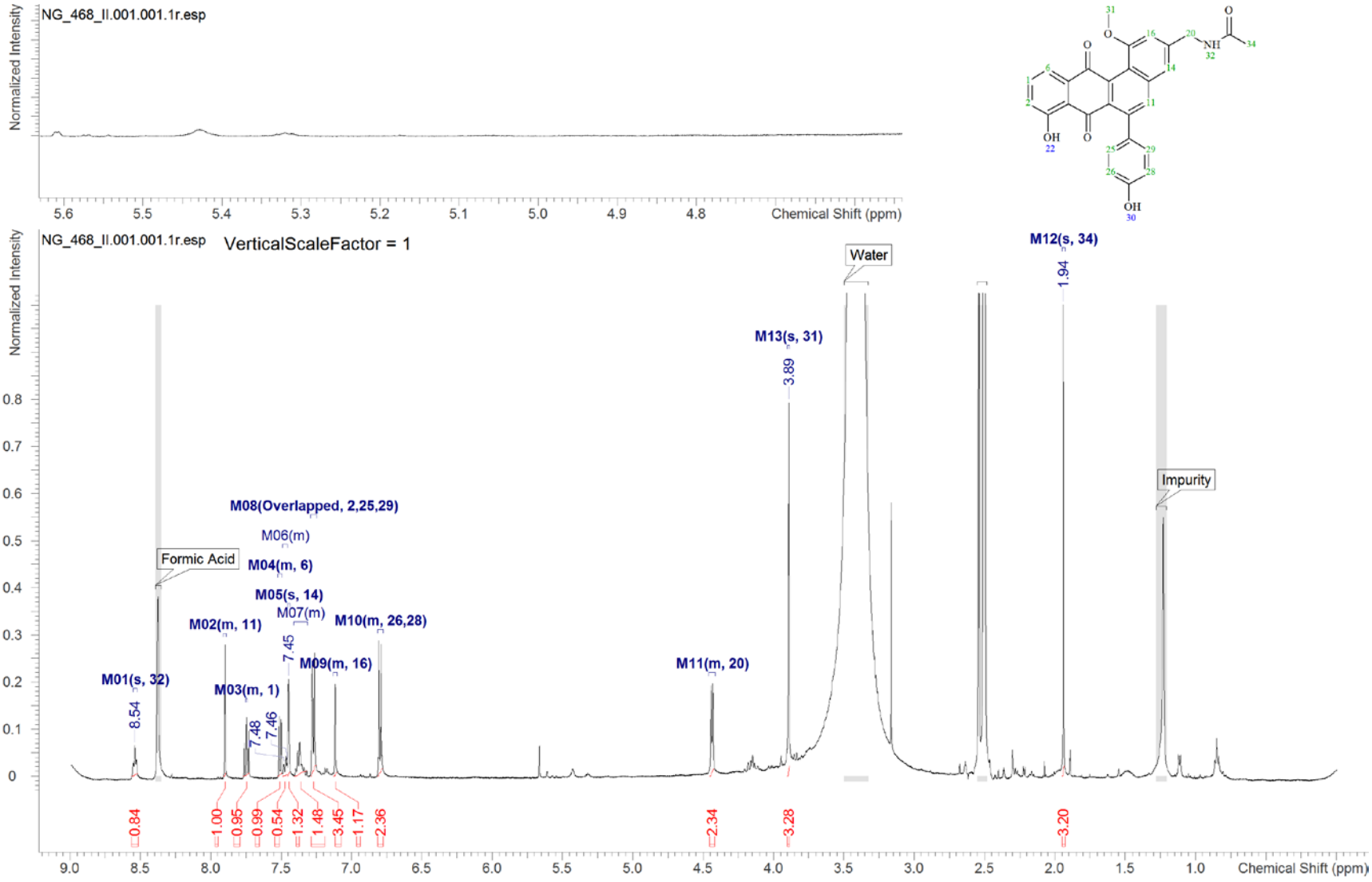


Figure S 9 ^{1}H-NMR spectrum (500 MHz, DMSO-d$_6$) of Pentangumycin; complete Spectrum and zoom from 5.6 to 4.6 ppm

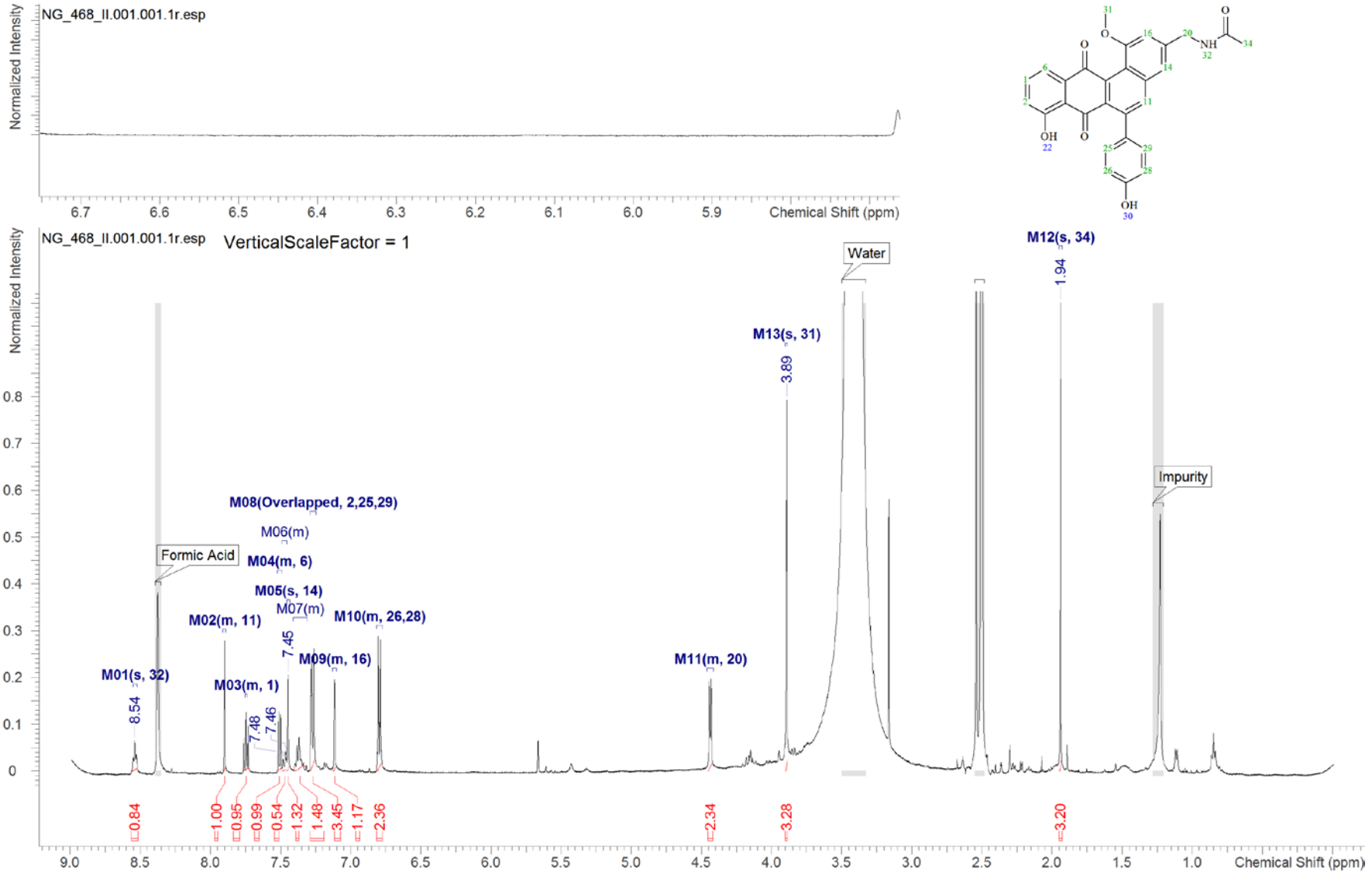


Figure S 10 ^{1}H-NMR spectrum (500 MHz, DMSO-d$_6$) of Pentangumycin; complete Spectrum and zoom from 6.7 to 5.7 ppm

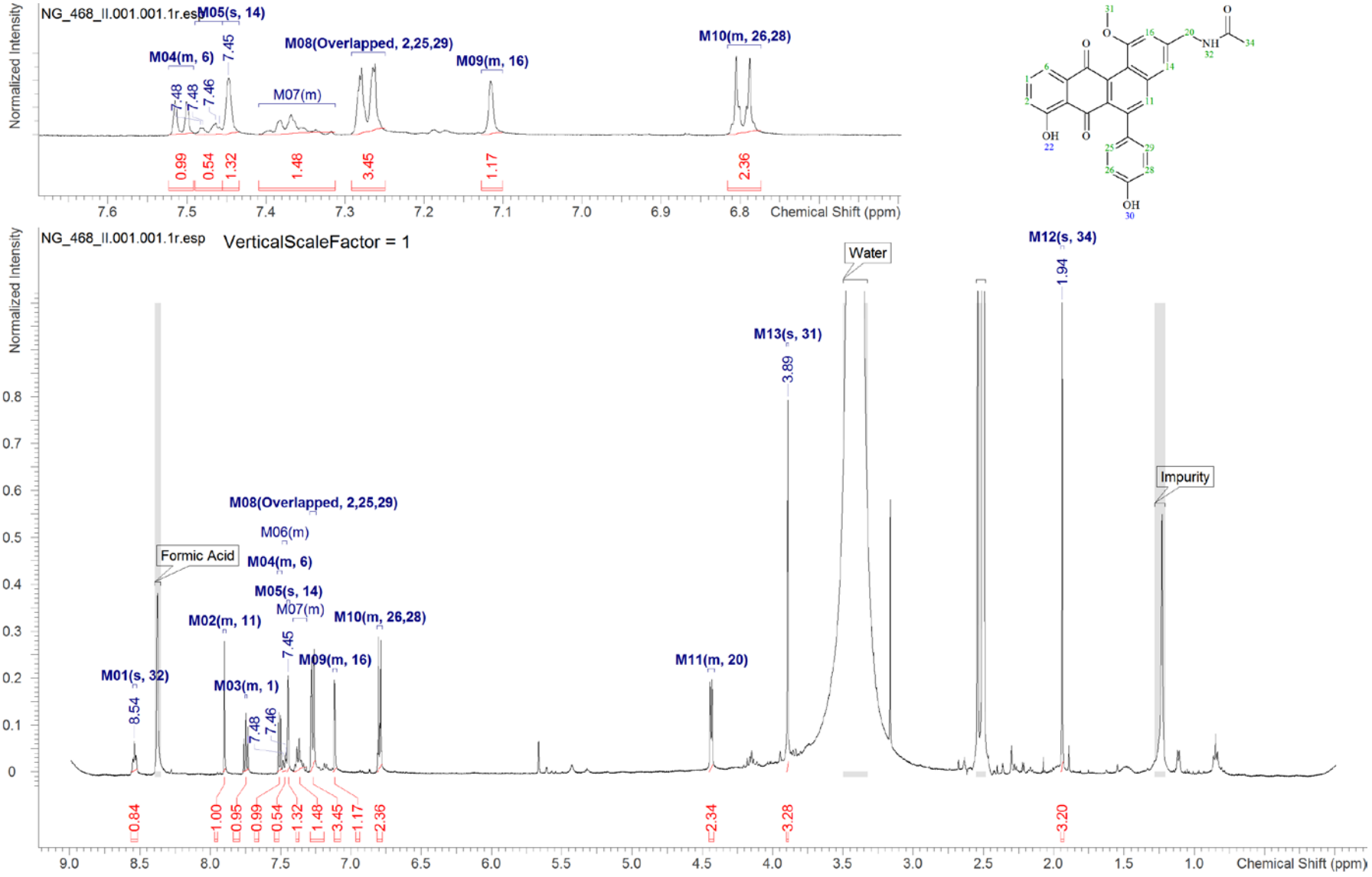


Figure S 11 ^{1}H-NMR spectrum (500 MHz, DMSO-d$_6$) of Pentangumycin; complete Spectrum and zoom from 7.6 to 6.7 ppm

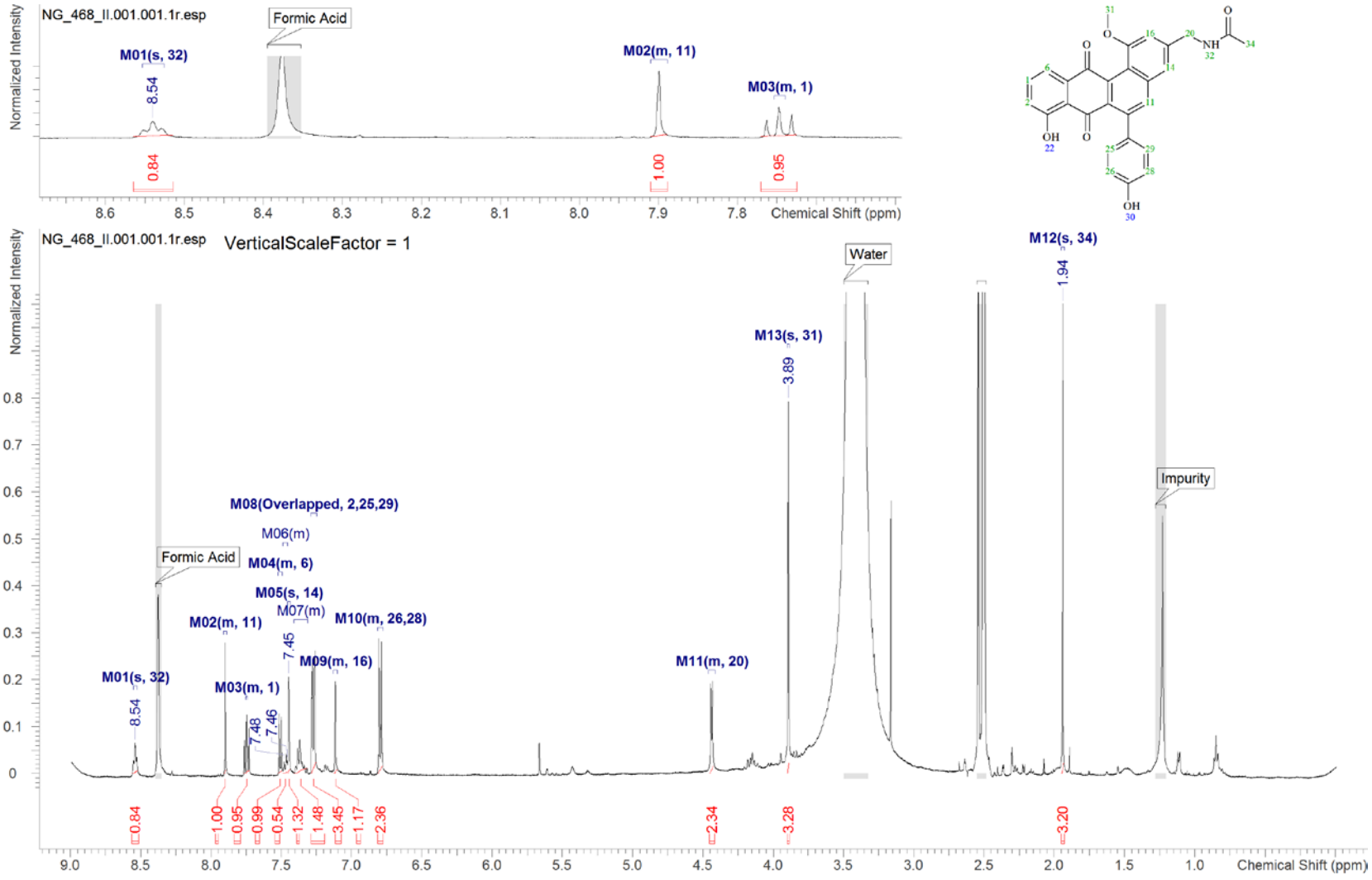


Figure S 12 ^{1}H-NMR spectrum (500 MHz, DMSO-d$_6$) of Pentangumycin; complete Spectrum and zoom from 7.6 to 6.7 ppm

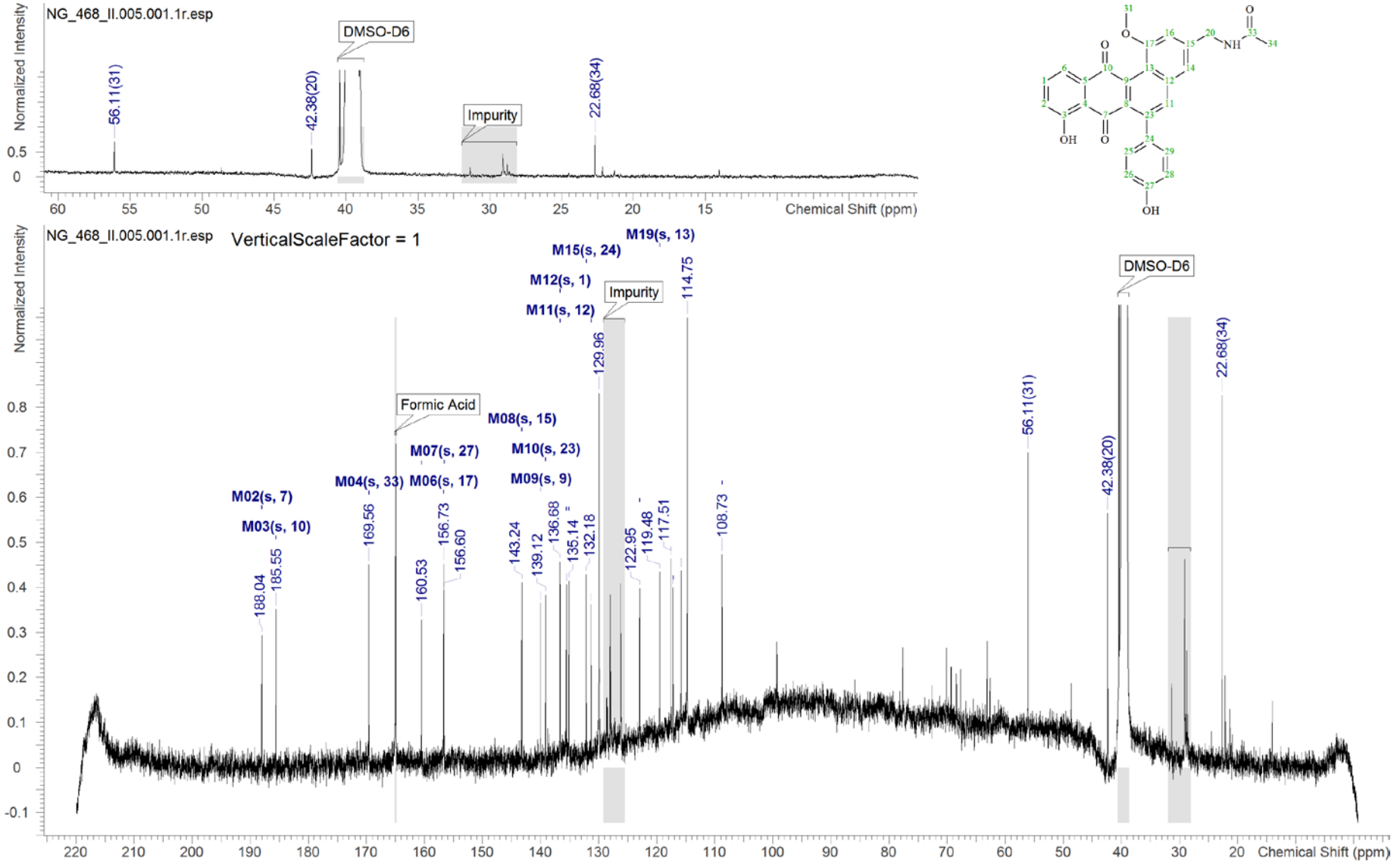


Figure S 13 ^{13}C-NMR (125 MHz, DMSO- d_6) of Pentangumycin; complete spectrum and zoom from 60 to 0 ppm.

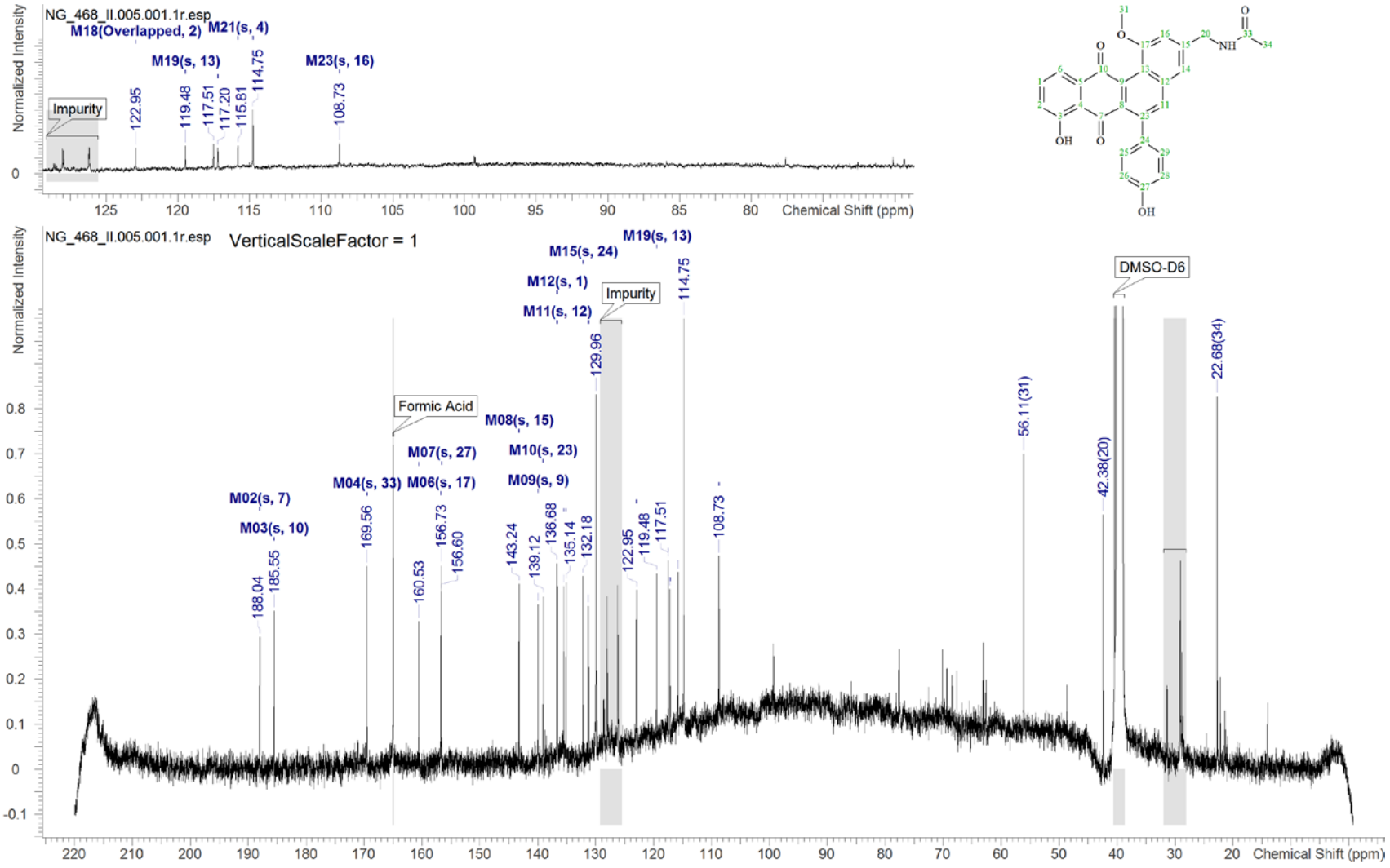


Figure S 14 ^{13}C-NMR (125 MHz, DMSO- d_6) of Pentangumycin; complete spectrum and zoom from 130 to 70 ppm.

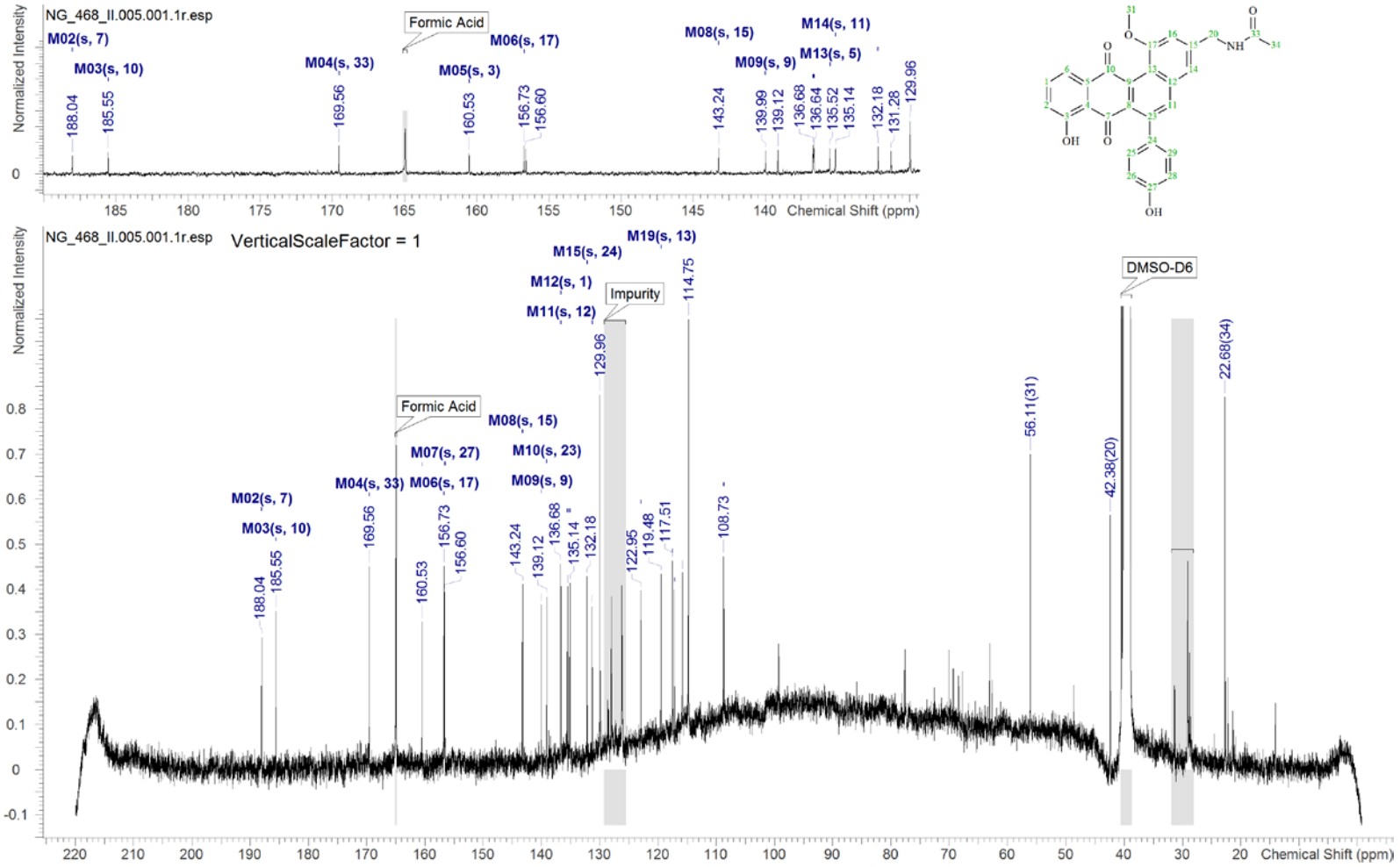


Figure S 15 ^{13}C-NMR (125 MHz, DMSO- d_6) of Pentangumycin; complete spectrum and zoom from 190 to 130 ppm.

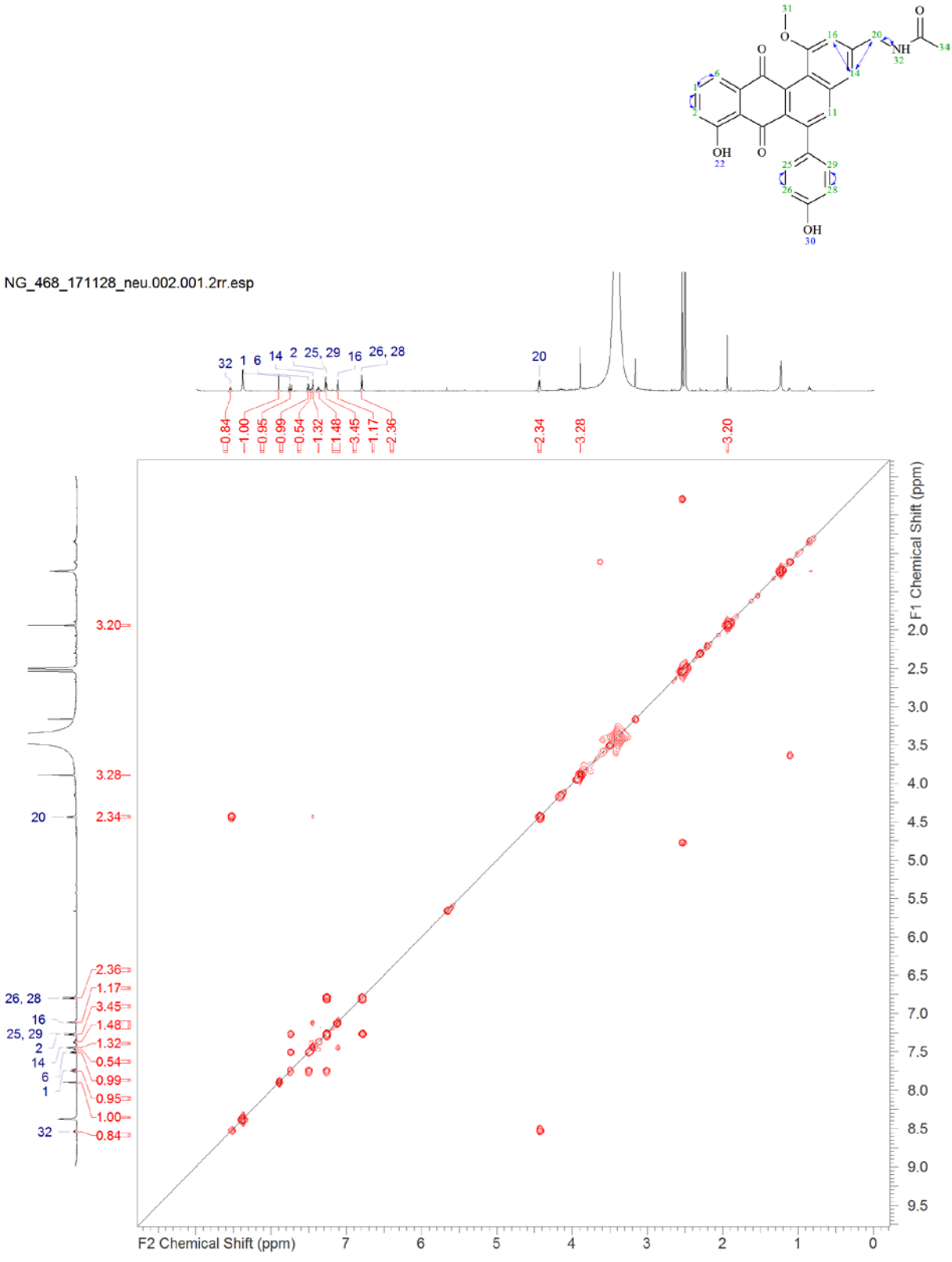


Figure S 16 ^{1}H-^{1}H- COSY spectrum (500 MHz, DMSO-d$_6$) of Pentangumycin

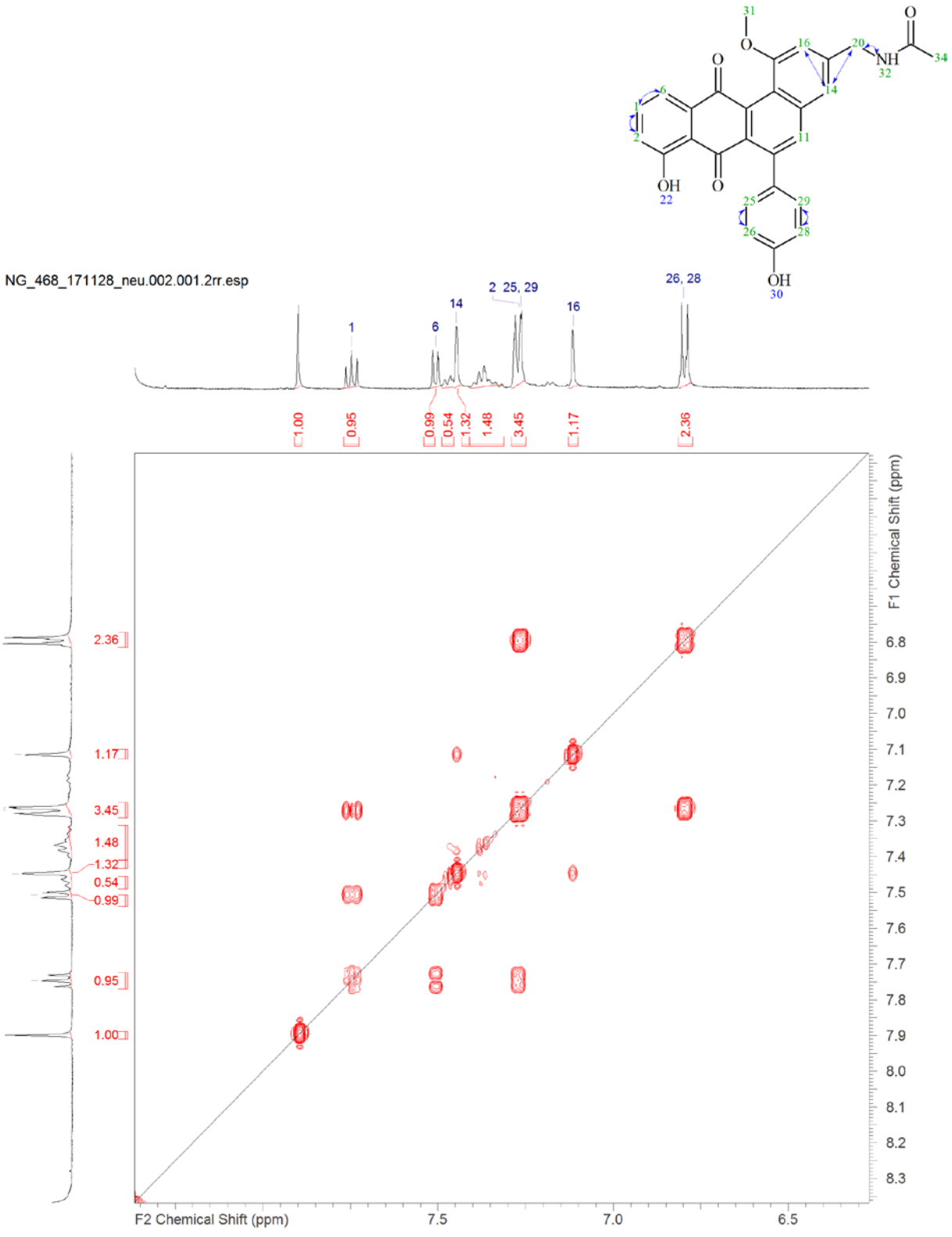


Figure S 17 ^{1}H-^{1}H- COSY spectrum (500 MHz, DMSO-d$_6$) of Pentangumycin; Zoom to aromatic region

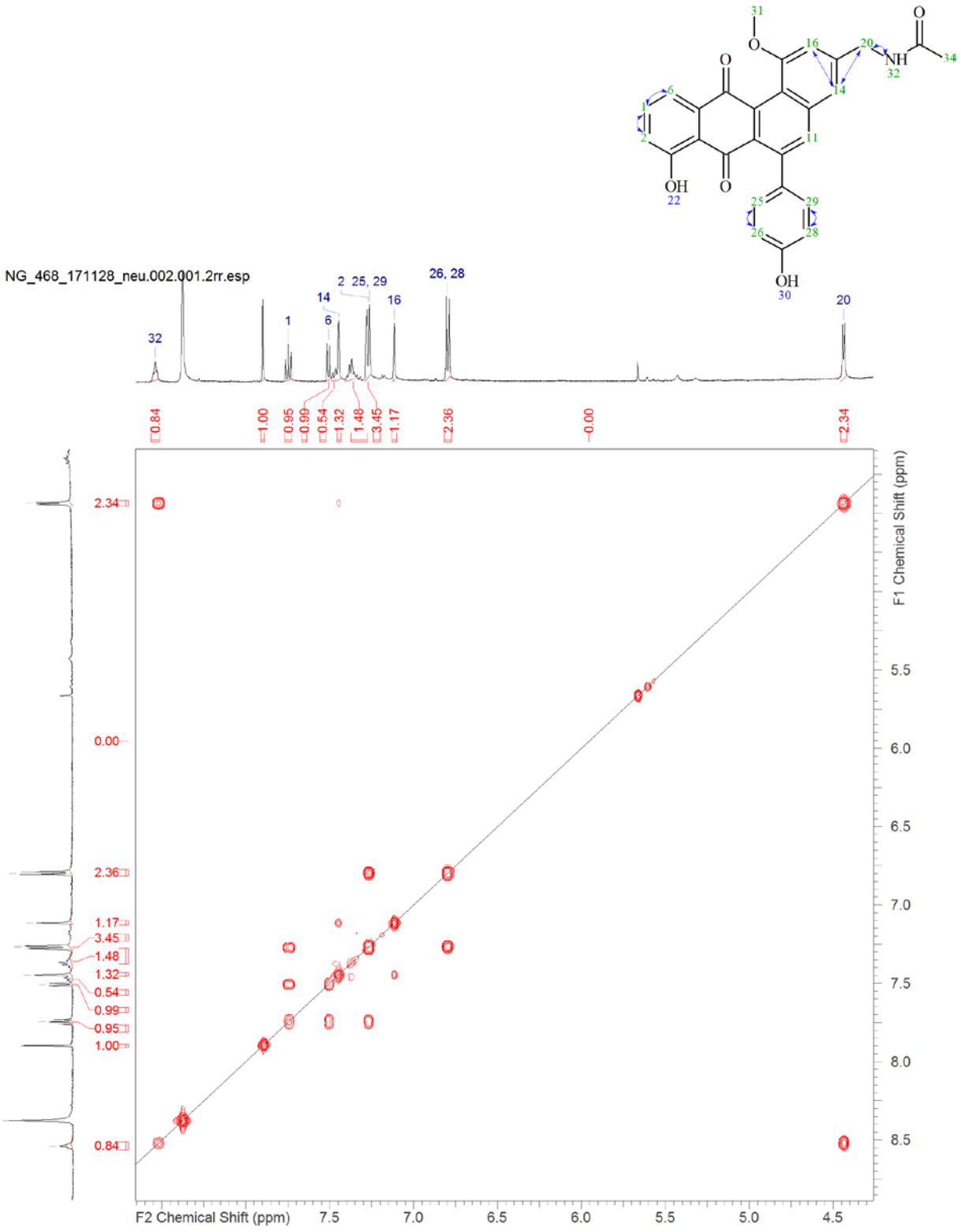


Figure S 18 ^{1}H-^{1}H- COSY spectrum (500 MHz, DMSO-d$_6$) of Pentangumycin; Zoom from 8.5 to 4.5 ppm

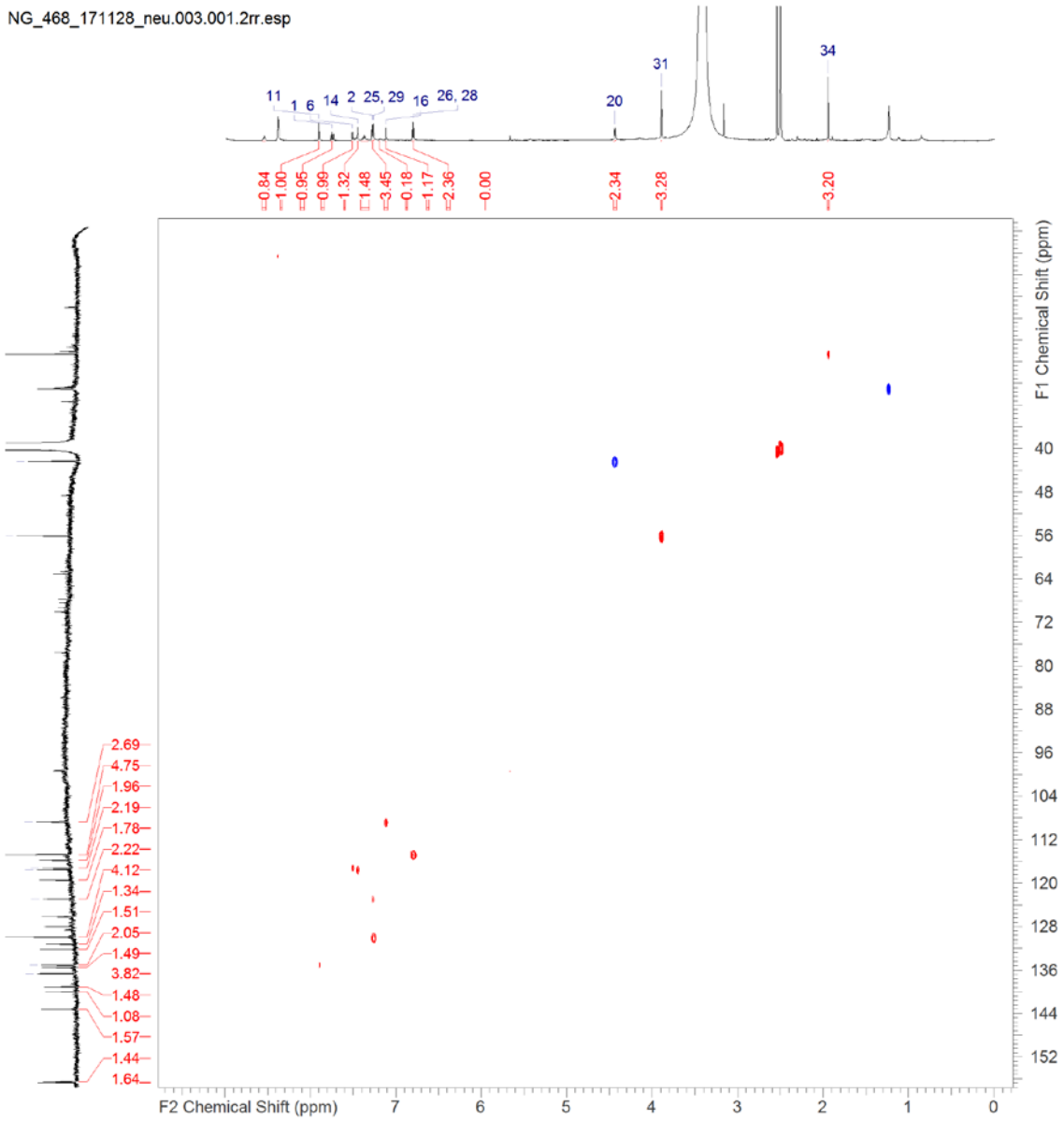


Figure S 19 HSQC-spectrum (500 MHz; 125 MHz, DMSO-d_6) of Pentangumycin; complete spectrum

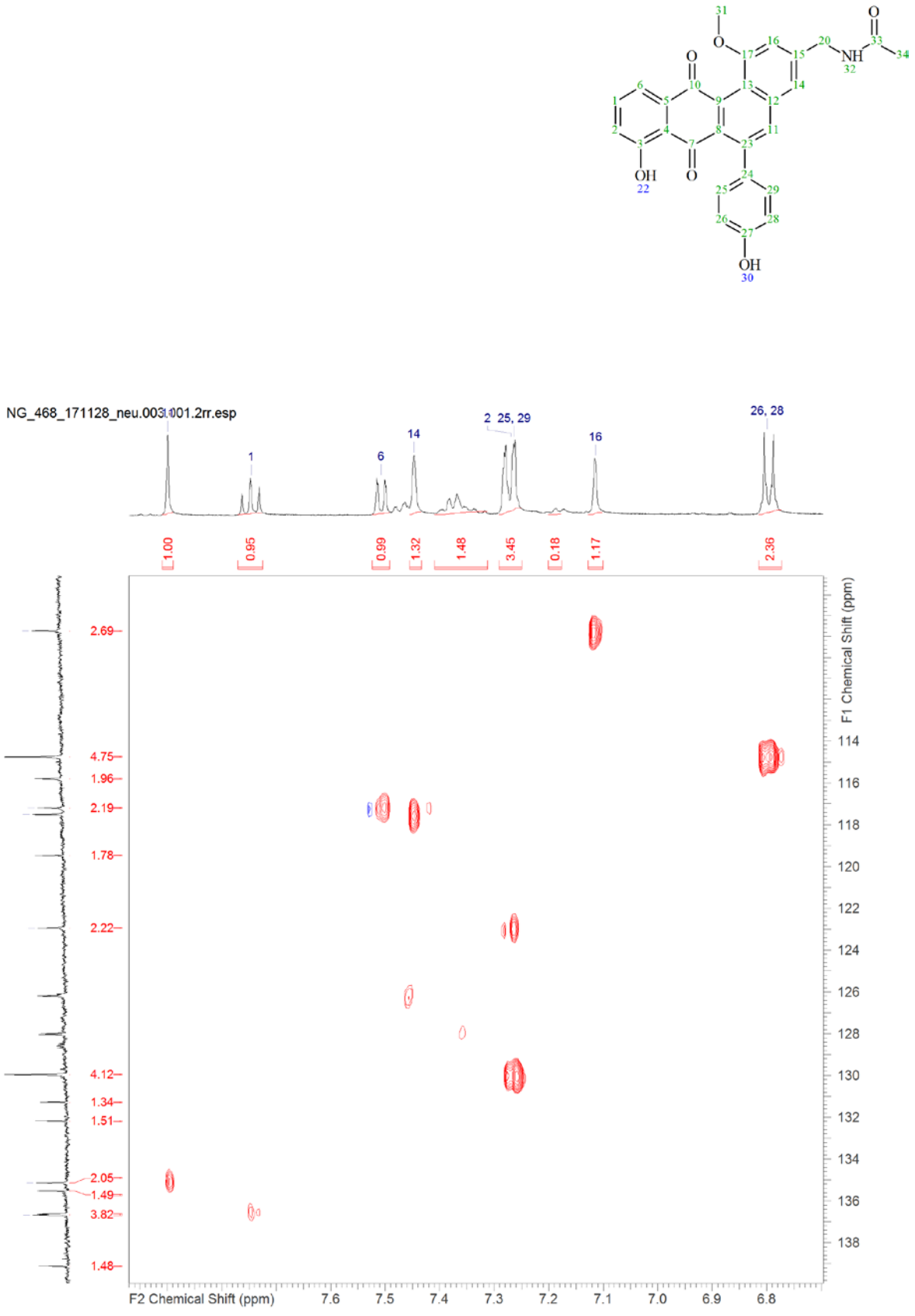
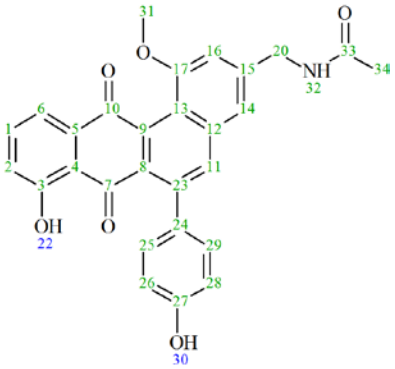


Figure S 20 HSQC-spectrum (500 MHz; 125 MHz, DMSO-d_6) of Pentangumycin; zoom from 7.9 to 6.8 ppm and 139 to 107 ppm



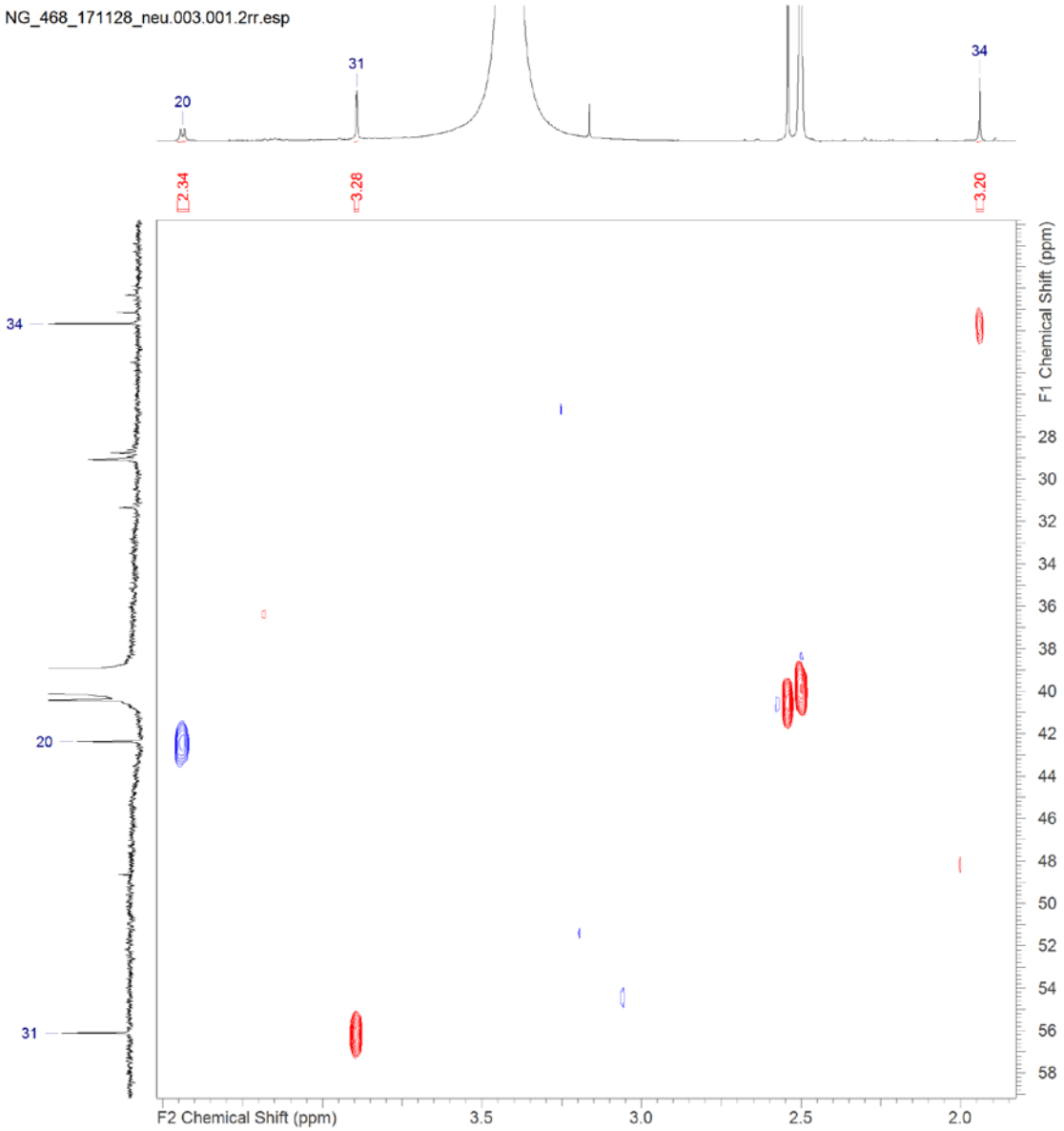


Figure S 21 HSQC-spectrum (500 MHz; 125 MHz, DMSO-d_6) of Pentangumycin; zoom from 4 to 2 ppm and 59 to 19 ppm

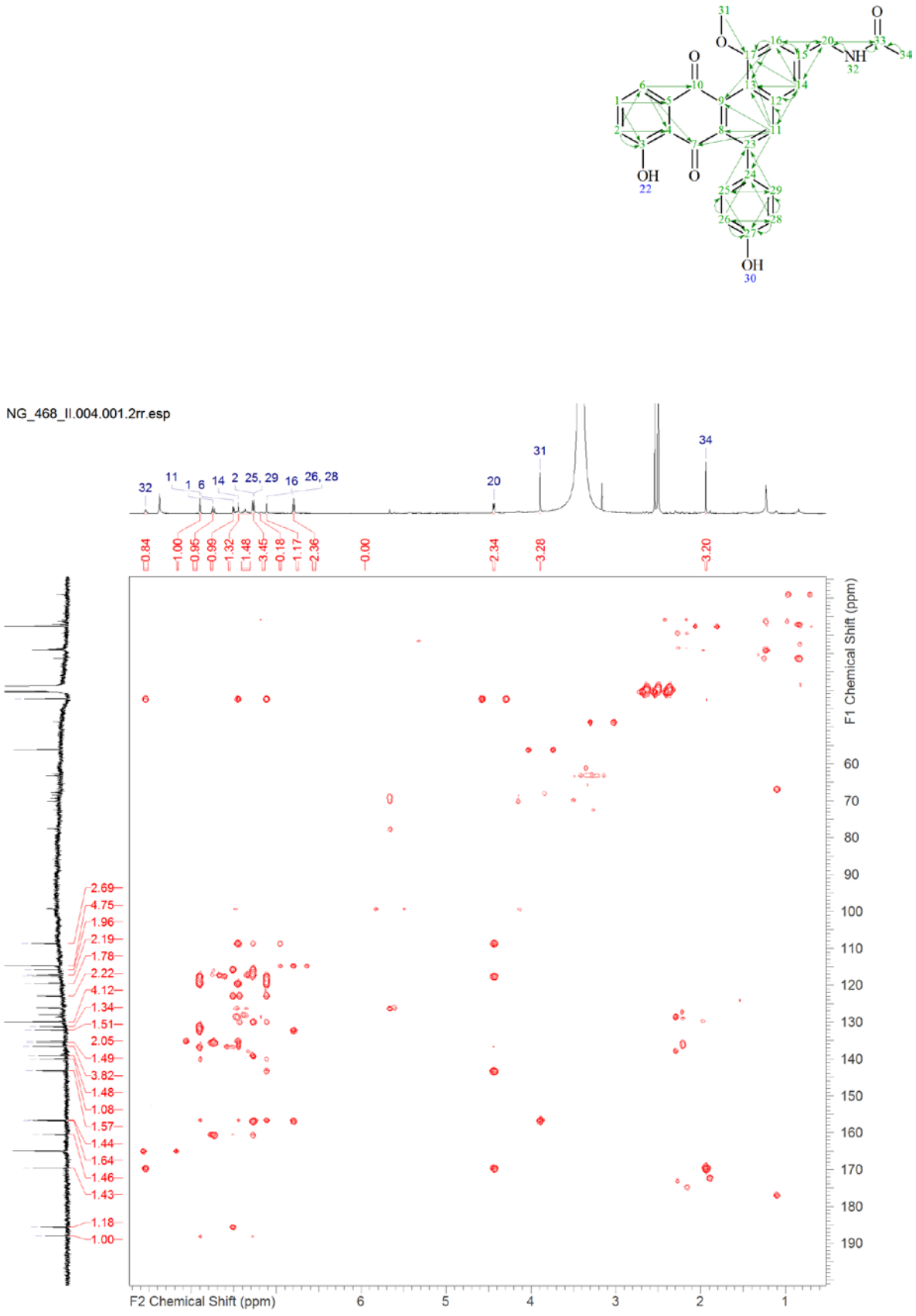


Figure S 22 HMBC-spectrum (500 MHz; 125 MHz, DMSO-d_6) of Pentangumycin; complete spectrum

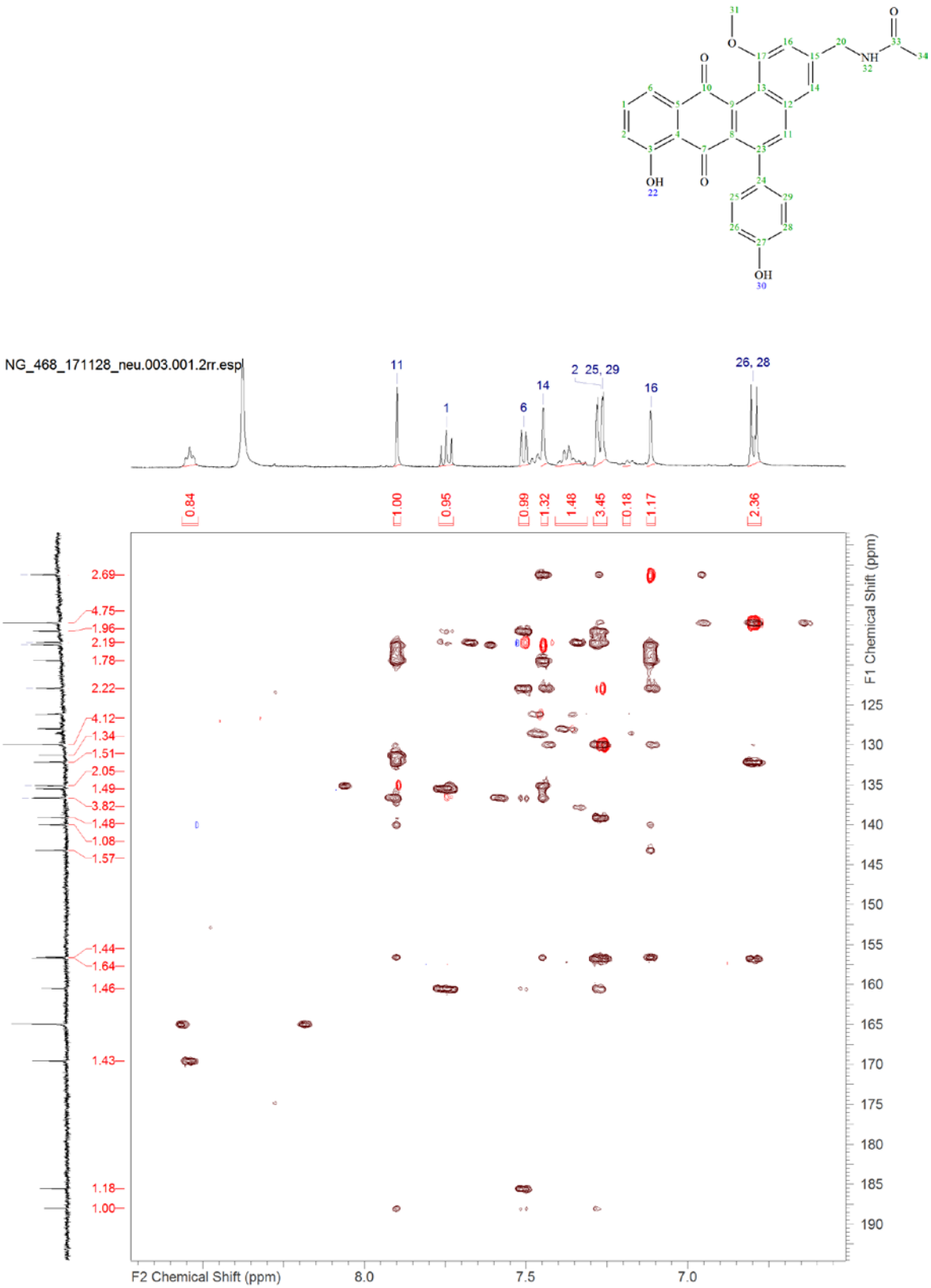


Figure S 23 HMBC - HSQC-Overlay spectrum (500 MHz; 125 MHz, DMSO-d_6) of Pentangumycin (black: HMBC; red/blue: HSQC); zoom from 9.2 to 6.5 ppm and 195 to 110 ppm

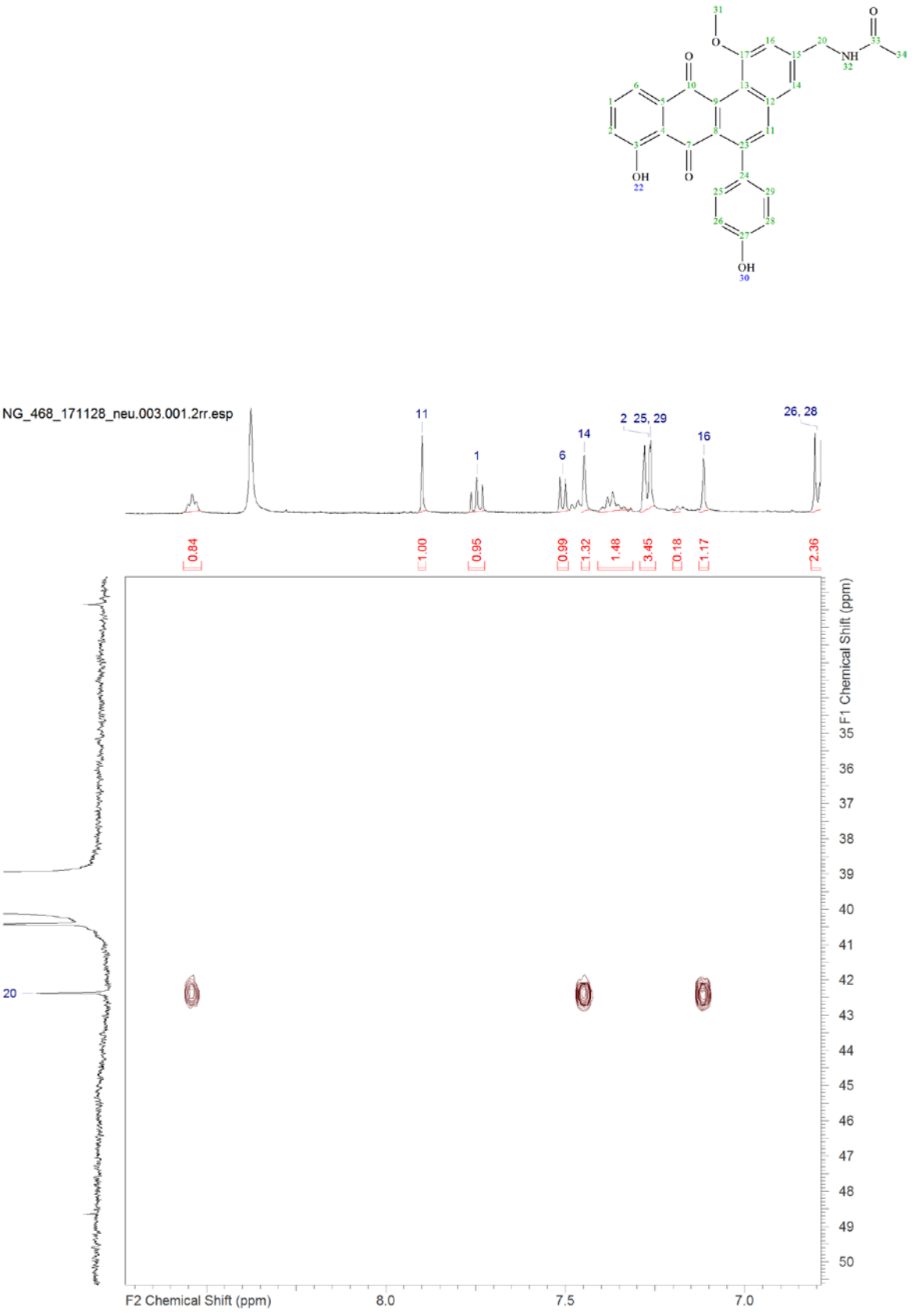
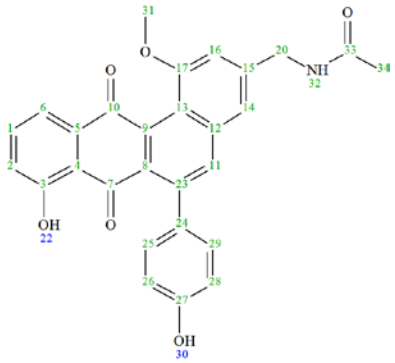


Figure S 24 HMBC - HSQC-Overlay spectrum (500 MHz; 125 MHz, DMSO-d_6) of Pentangumycin (black: HMBC; red/blue: HSQC); zoom from 9.2 to 6.5 ppm and 50 to 31 ppm



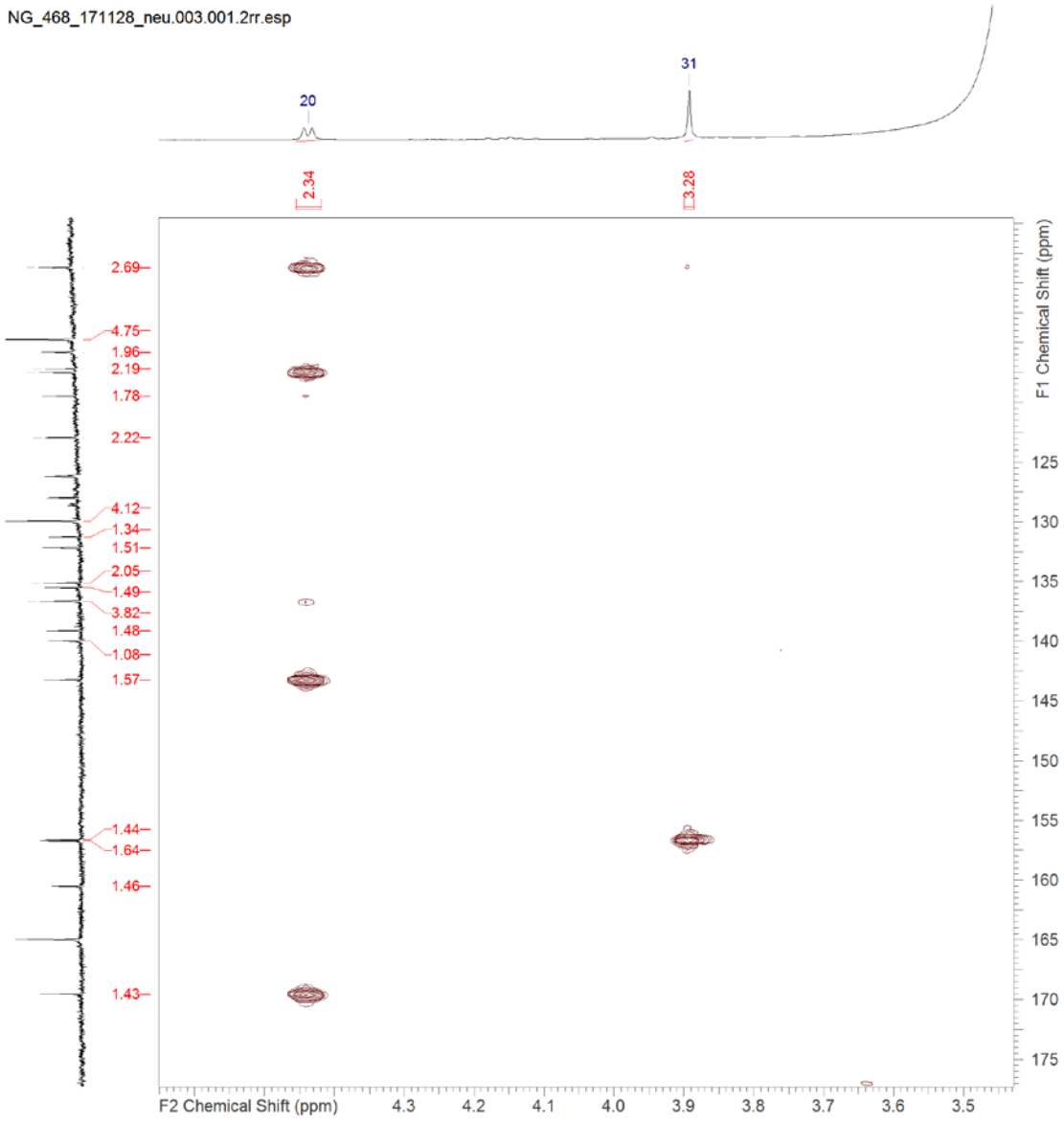
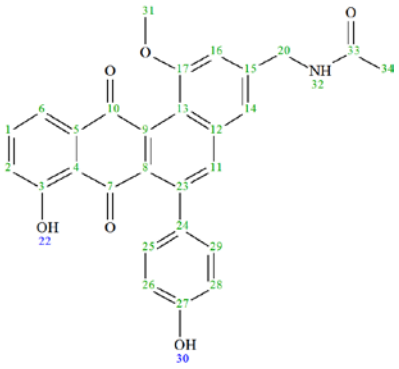


Figure S 25 HMBC - HSQC-Overlay spectrum (500 MHz; 125 MHz, DMSO-d_6) of Pentangumycin (black: HMBC; red/blue: HSQC); zoom from 4.6 to 3.5 ppm and 110 to 175 ppm



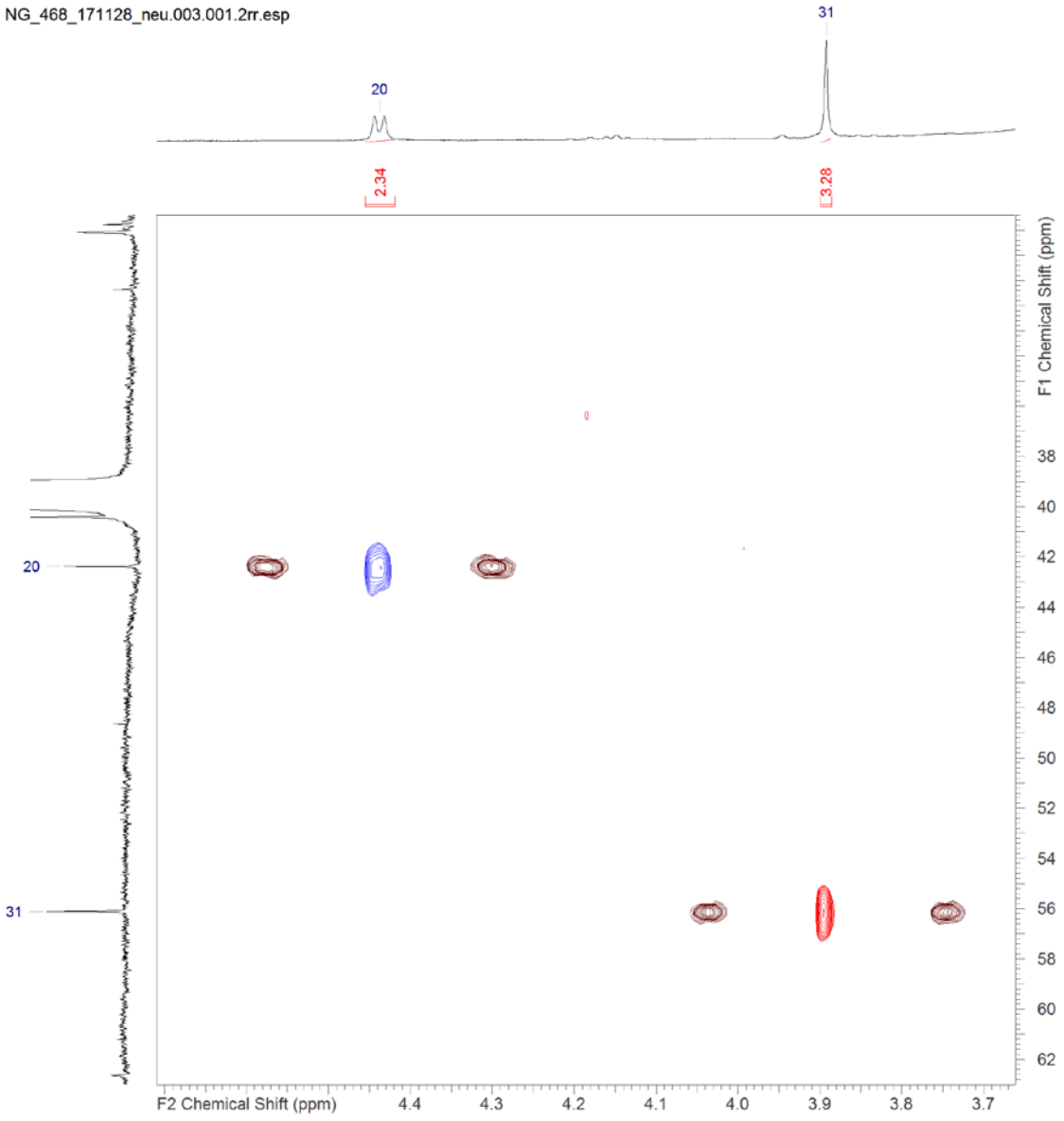


Figure S 26 HMBC - HSQC-Overlay spectrum (500 MHz; 125 MHz, DMSO-d_6) of Pentangumycin (black: HMBC; red/blue: HSQC); zoom from 4.6 to 3.7 ppm and 62 to 34 ppm

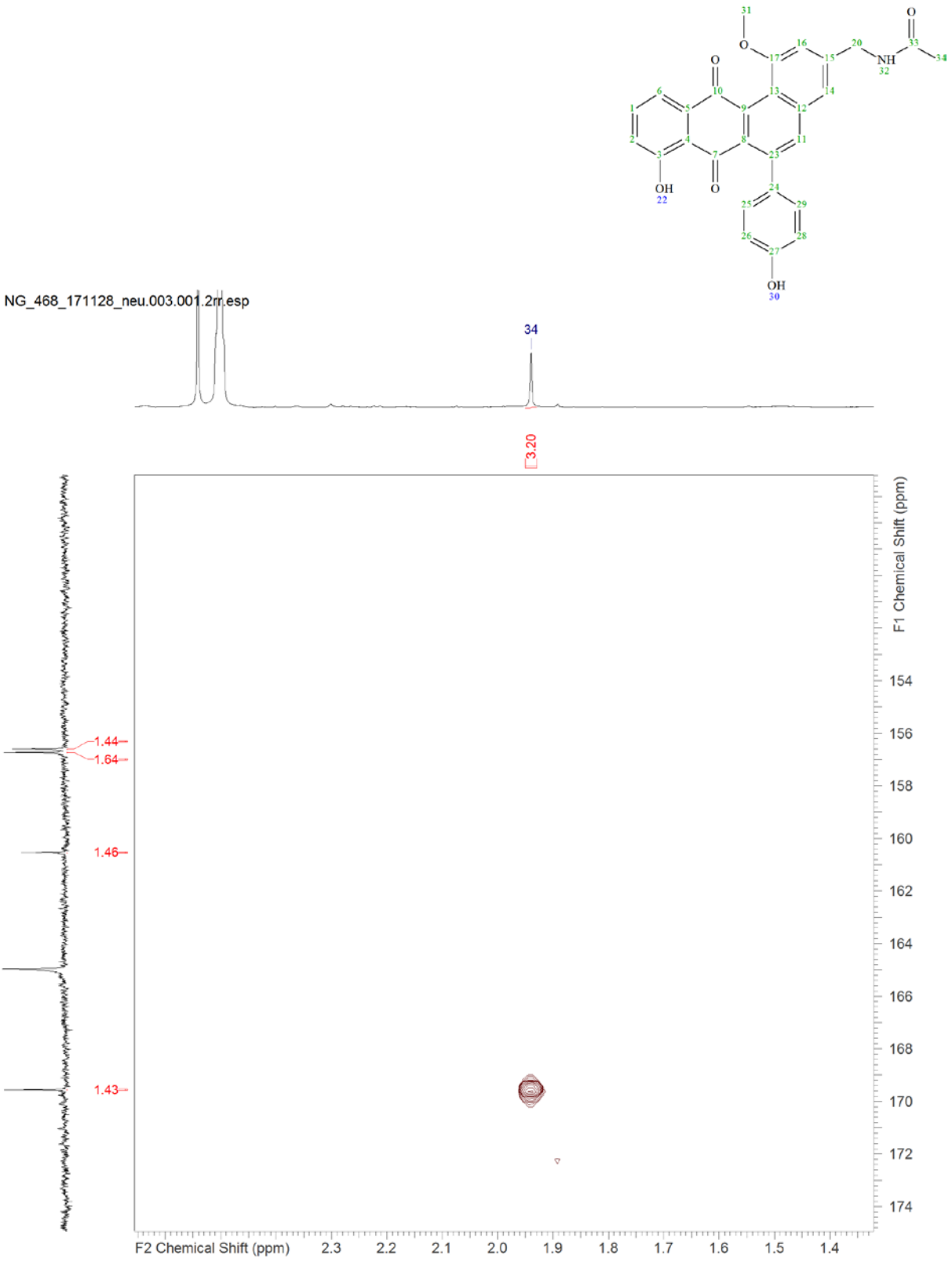


Figure S 27 HMBC - HSQC-Overlay spectrum (500 MHz; 125 MHz, DMSO-d_6) of Pentangumycin (black: HMBC; red/blue: HSQC); zoom from 2.6 to 1.4 ppm and 174 to 151 ppm

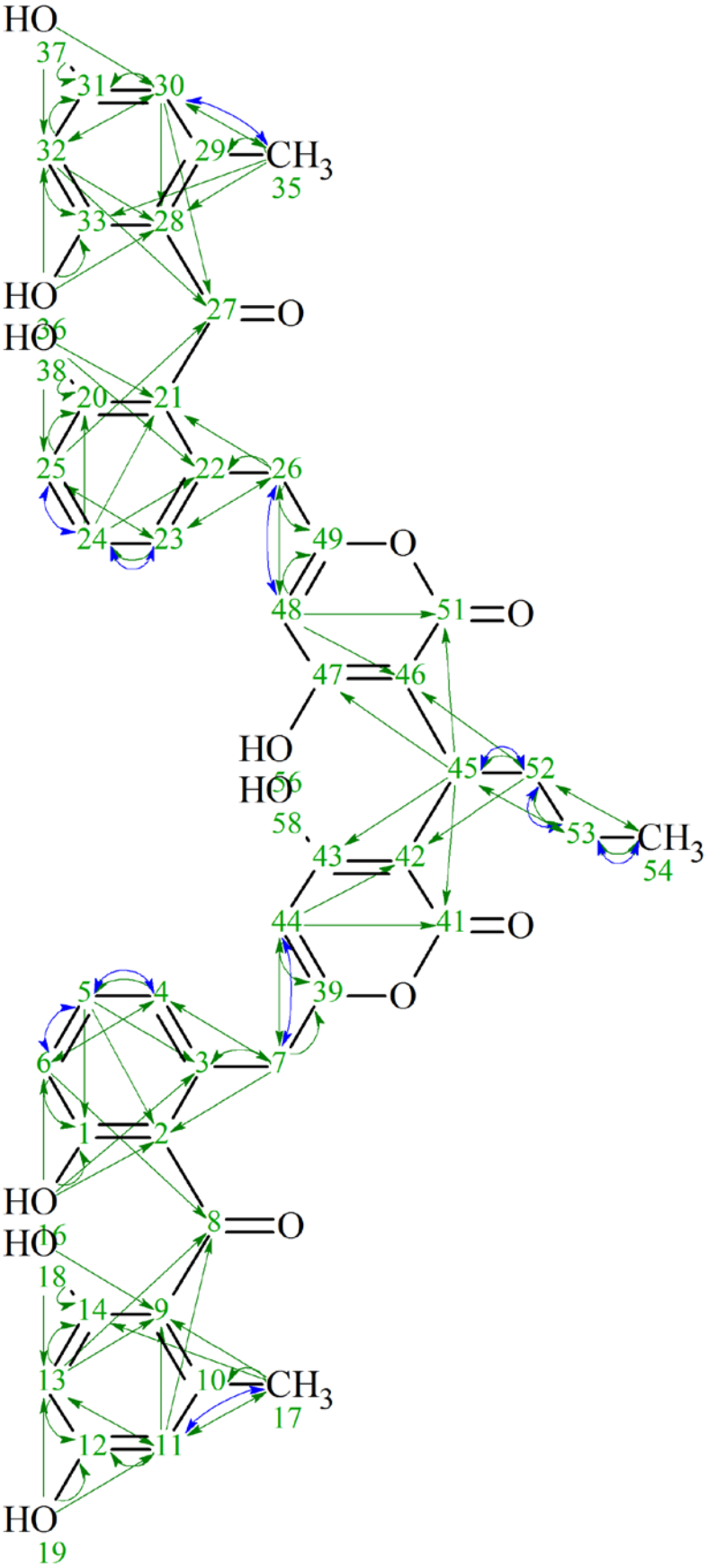


Figure S 28 Structure of SEK90 with all correlations (green: HMBC correlations H → C; blue: ^{1}H-^{1}H-Cosy correlation)

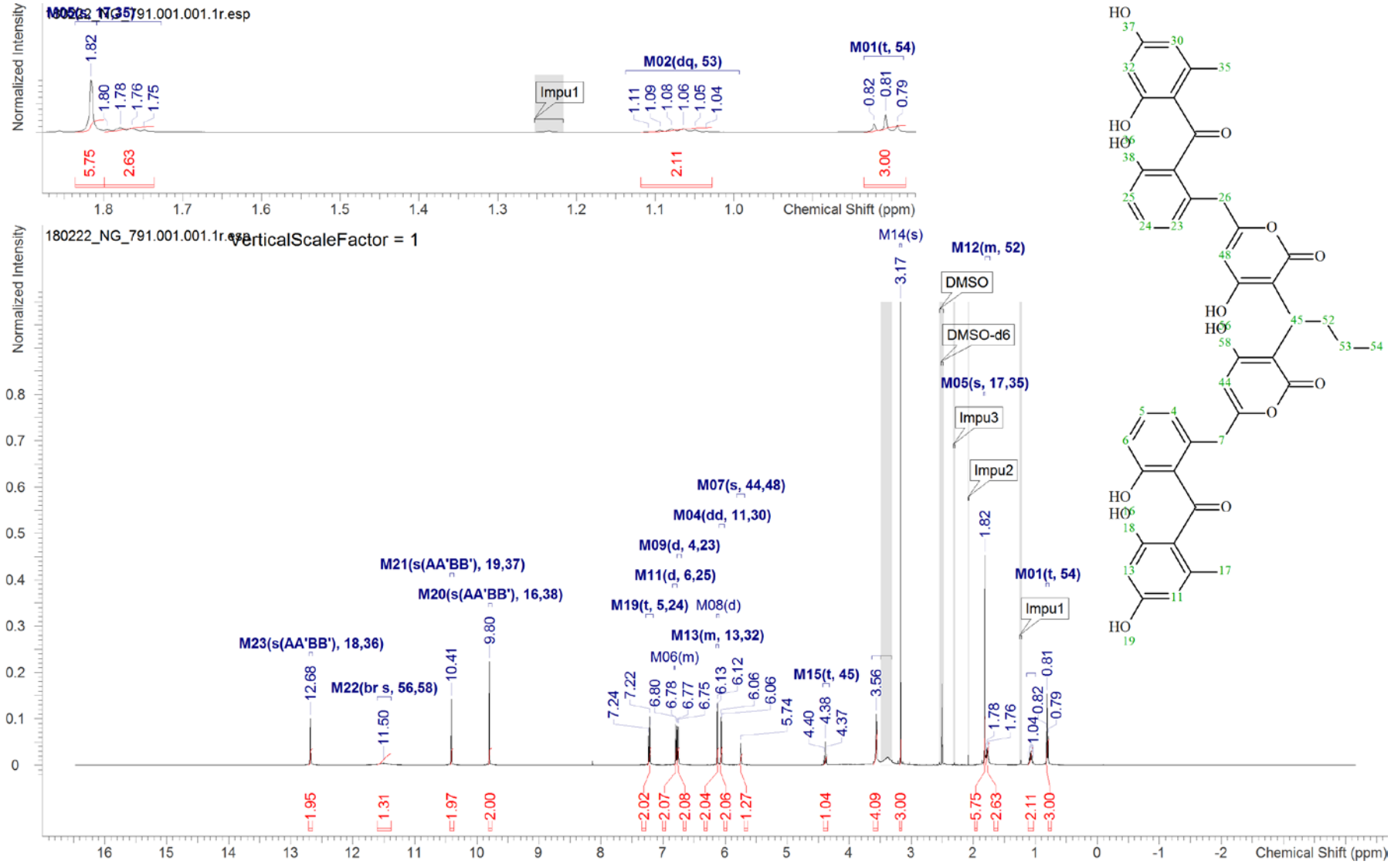


Figure S 29 ^{1}H-NMR spectrum (500 MHz, DMSO-d$_6$) of SEK90; complete Spectrum and zoom from 1.8 to 0.7 ppm

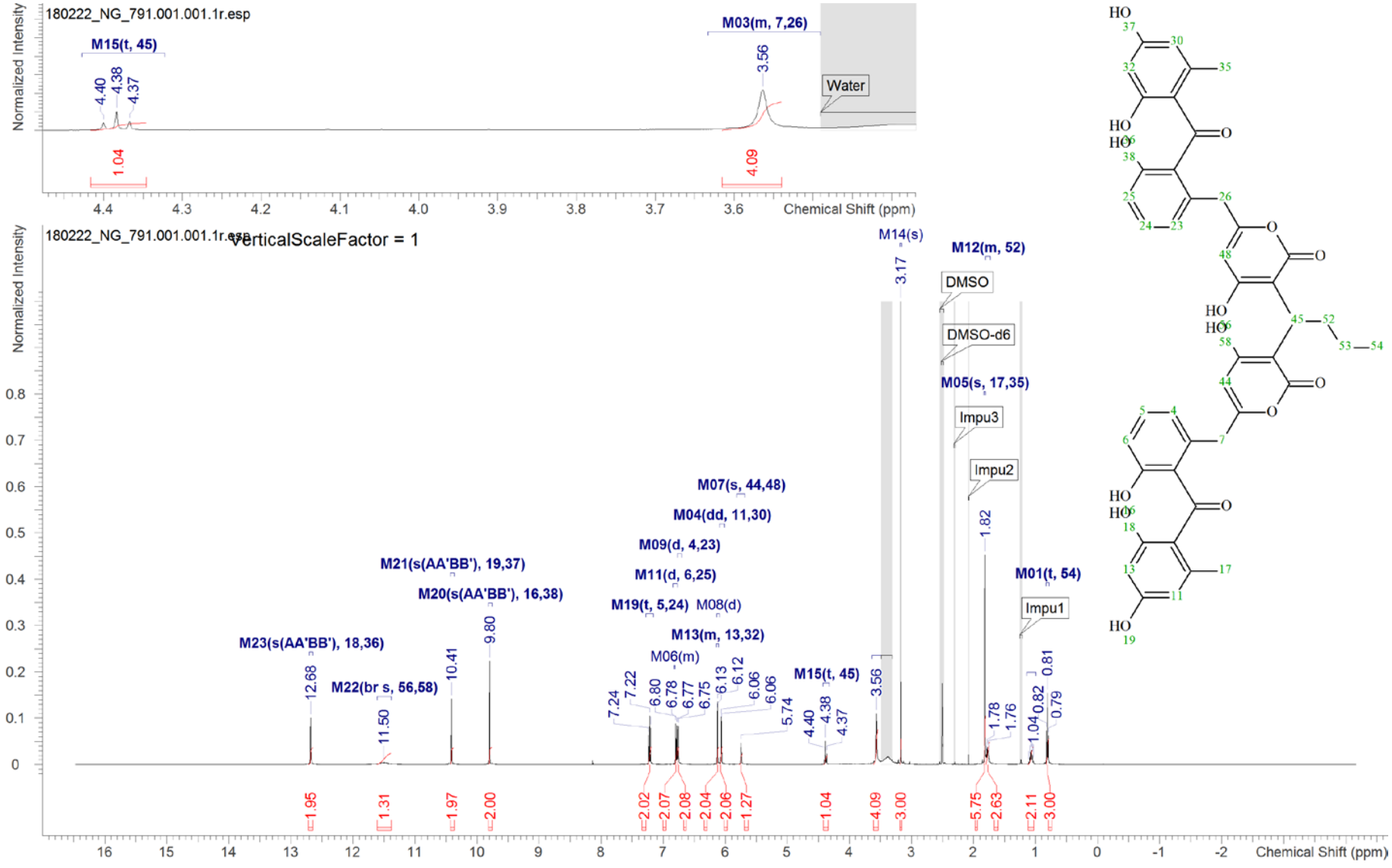


Figure S 30 ^{1}H-NMR spectrum (500 MHz, DMSO-d$_6$) of SEK90; complete Spectrum and zoom from 4.4 to 3.4 ppm

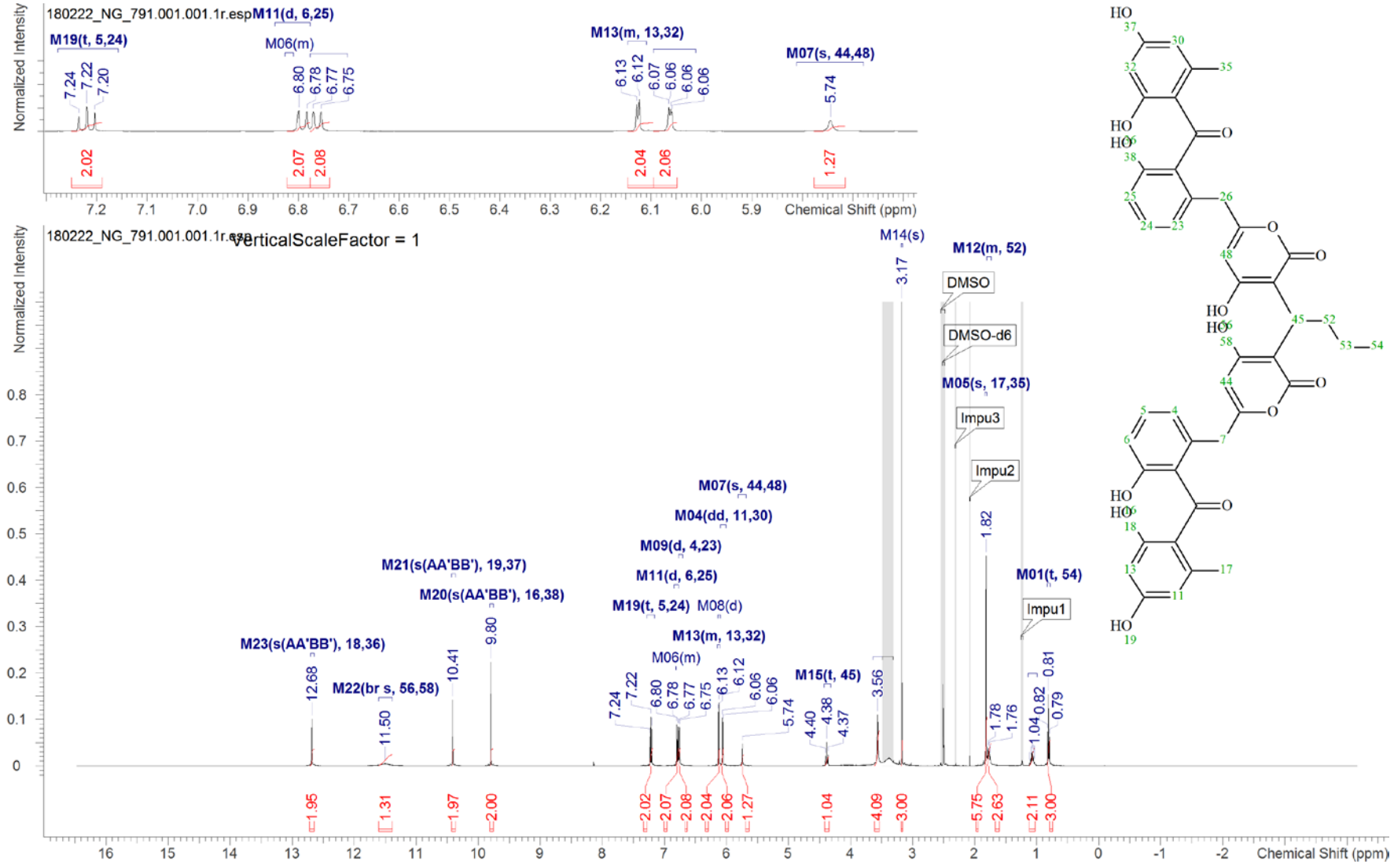


Figure S 31 ^{1}H-NMR spectrum (500 MHz, DMSO-d$_6$) of SEK90; complete Spectrum and zoom from 7.2 to 5.7 ppm

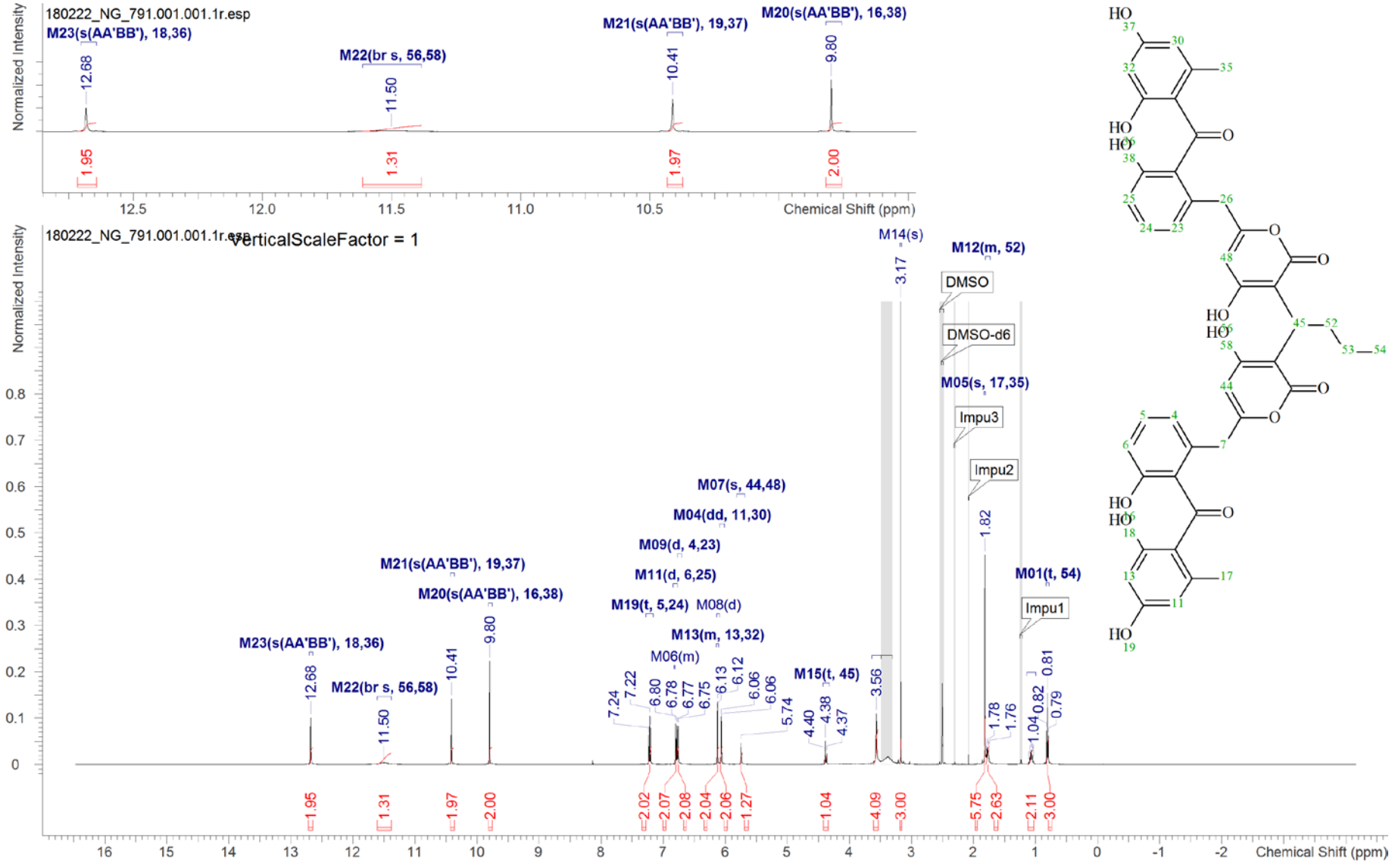


Figure S 32 ^{1}H-NMR spectrum (500 MHz, DMSO-d$_6$) of SEK90; complete Spectrum and zoom from 13 to 9.5 ppm

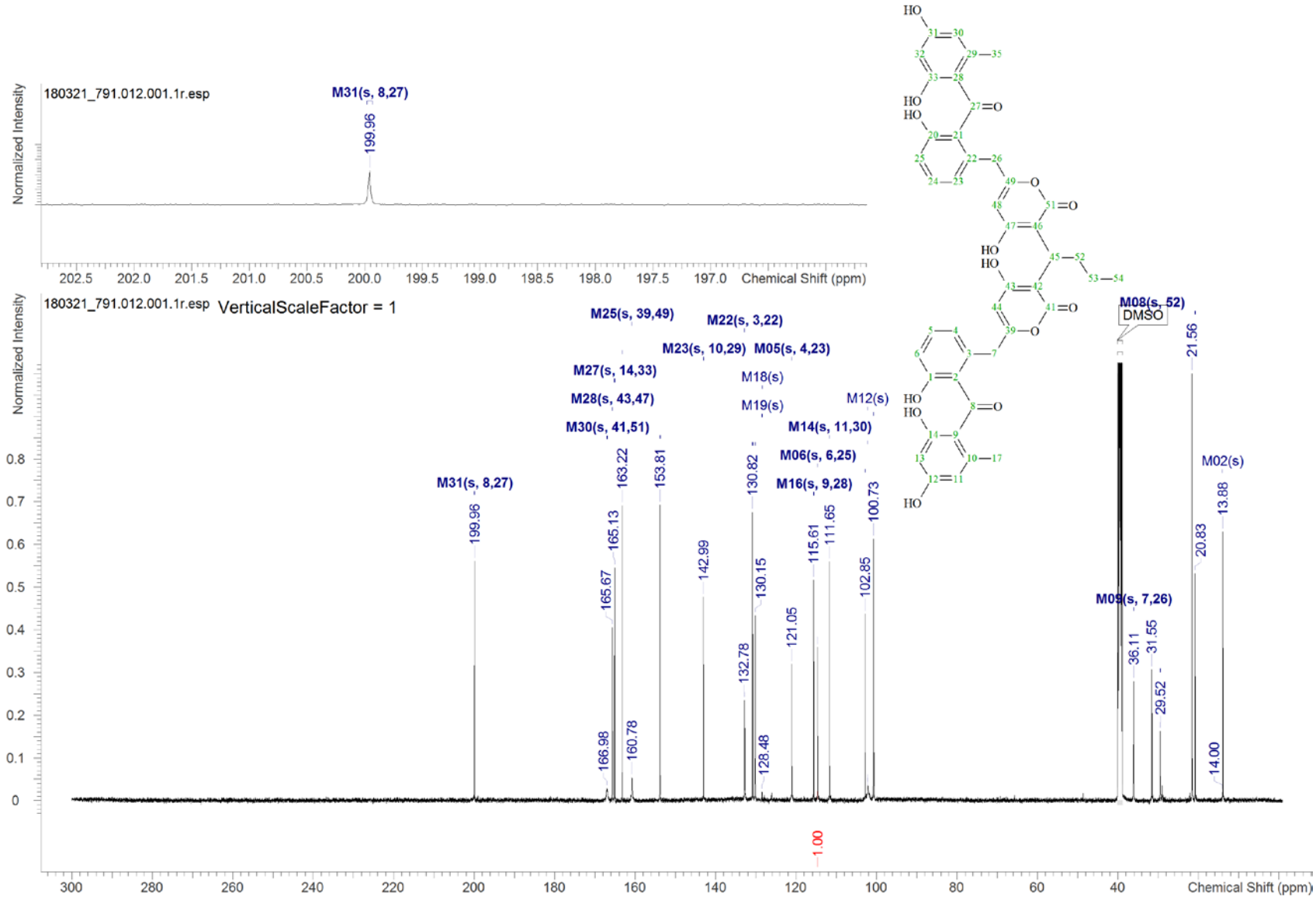


Figure S 33 ^{13}C-NMR spectrum (125 MHz, DMSO-d$_6$) of SEK90; complete Spectrum and zoom from 202 to 196 ppm

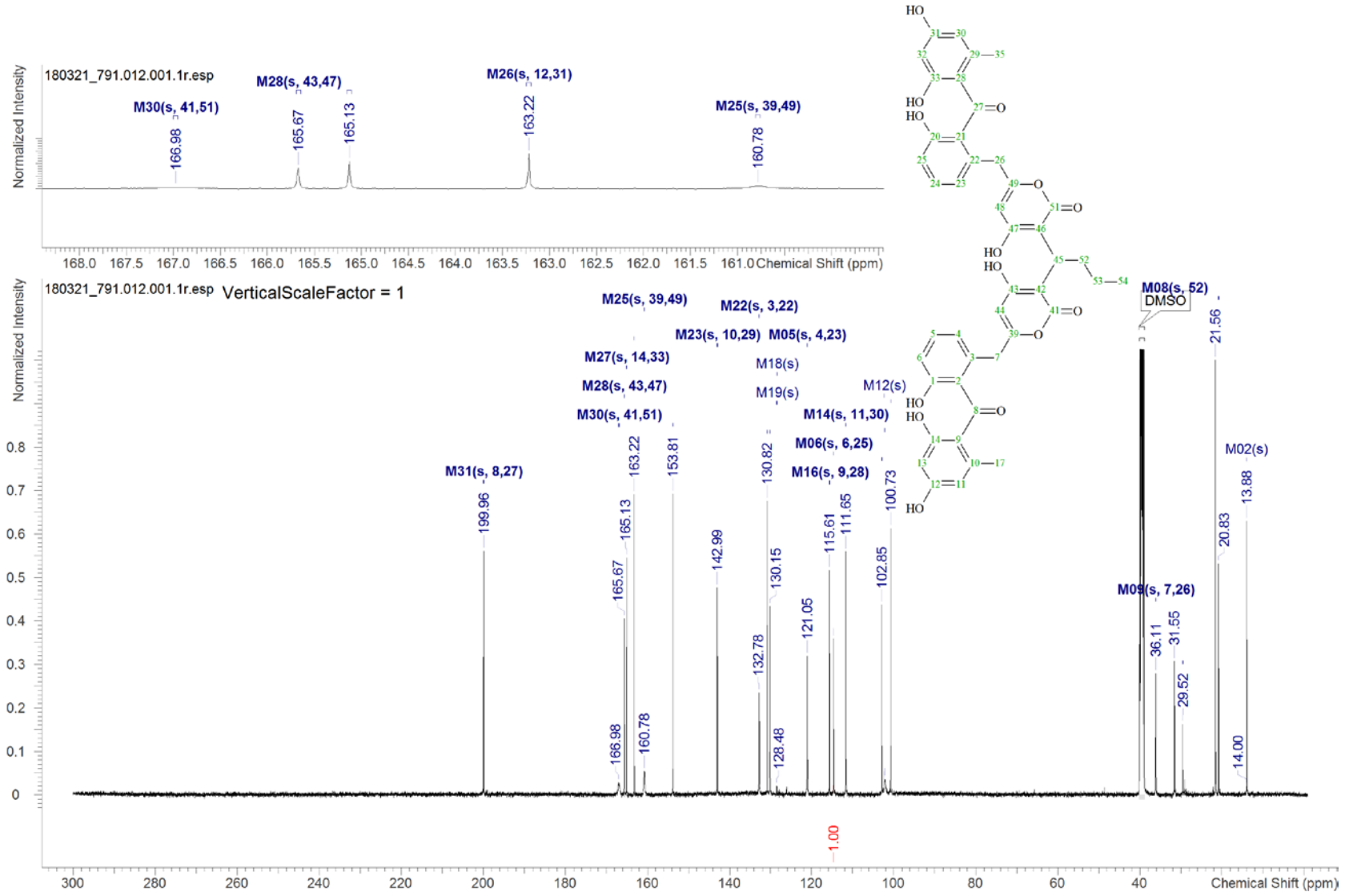


Figure S 34 ^{13}C-NMR spectrum (125 MHz, DMSO-d_6) of SEK90; complete Spectrum and zoom from 168 to 60 ppm

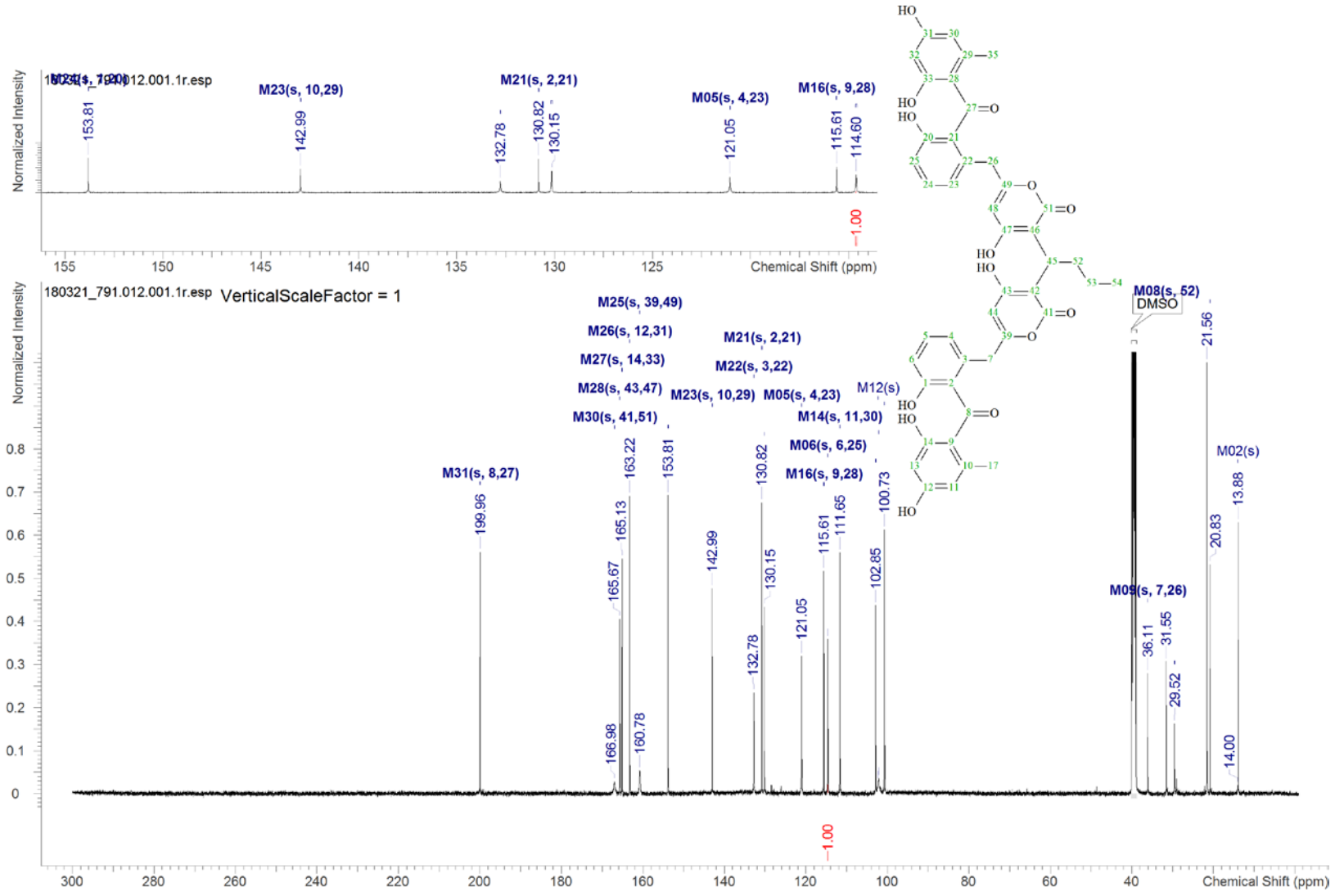


Figure S 35 13C-NMR spectrum (125 MHz, DMSO-d6) of SEK90; complete Spectrum and zoom from 155 to 113 ppm

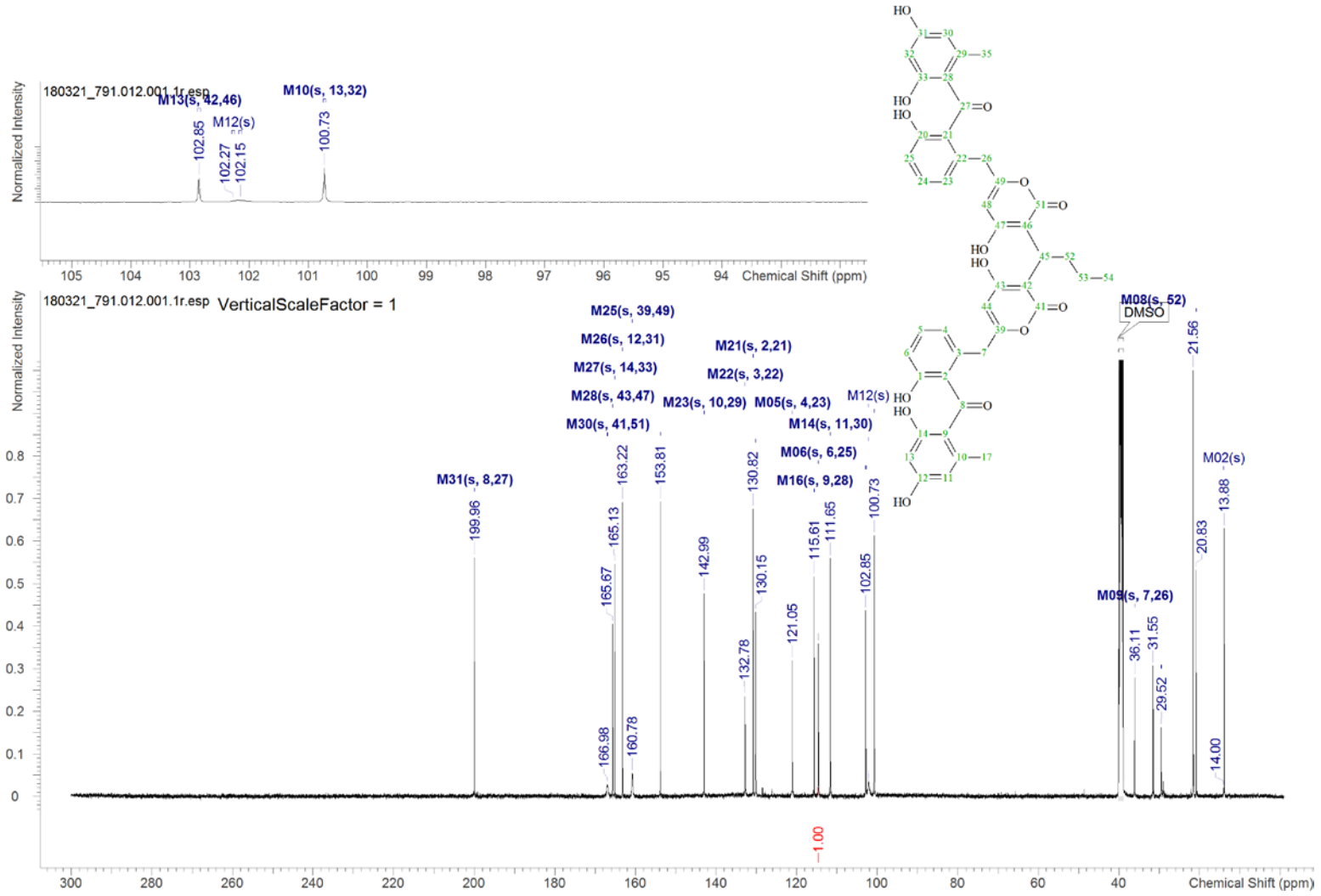


Figure S 36 ^{13}C-NMR spectrum (125 MHz, DMSO-d_6) of SEK90; complete Spectrum and zoom from 105 to 92 ppm

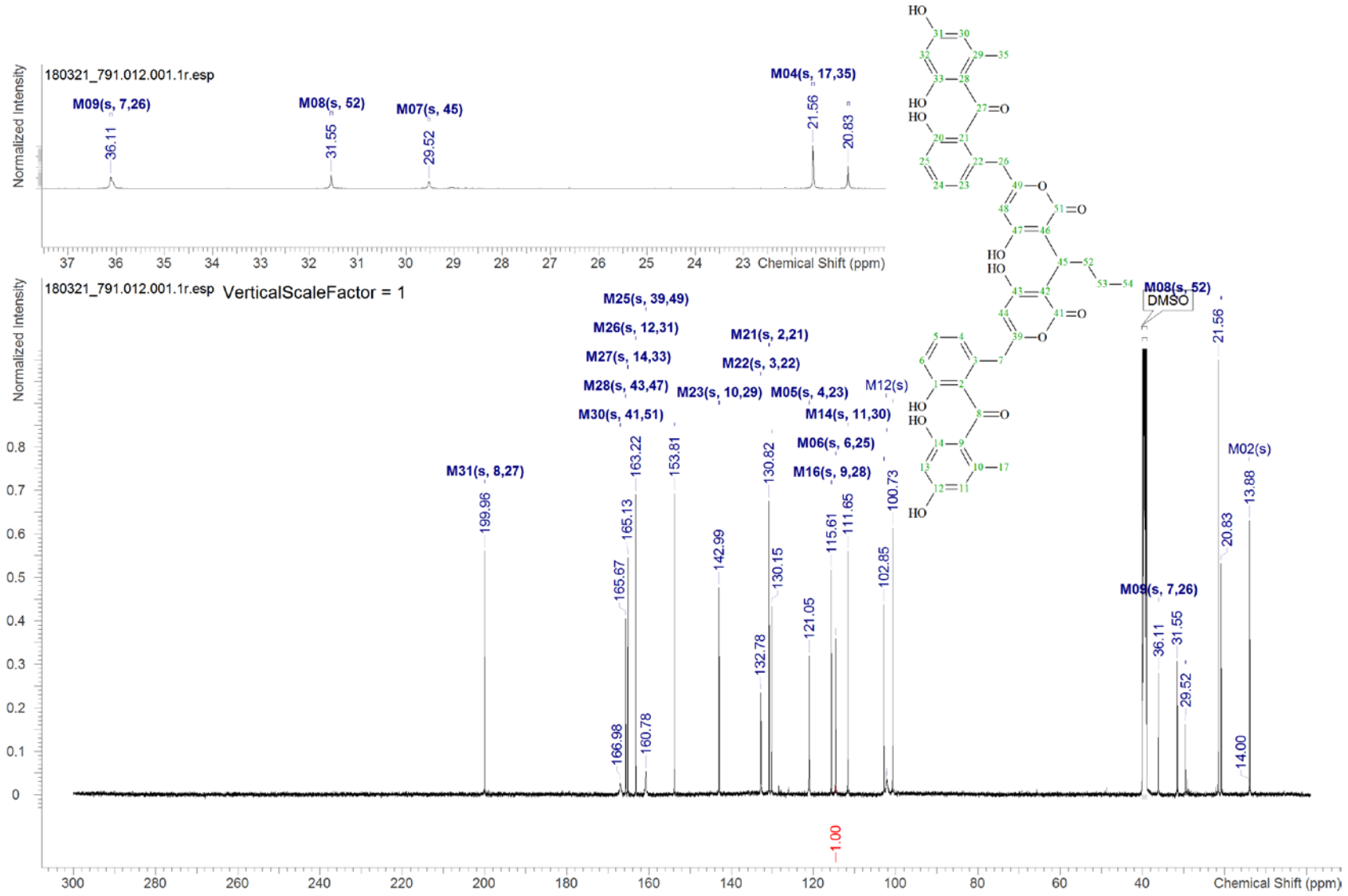


Figure S 37 ^{13}C-NMR spectrum (125 MHz, DMSO-d_6) of SEK90; complete Spectrum and zoom from 37 to 20 ppm

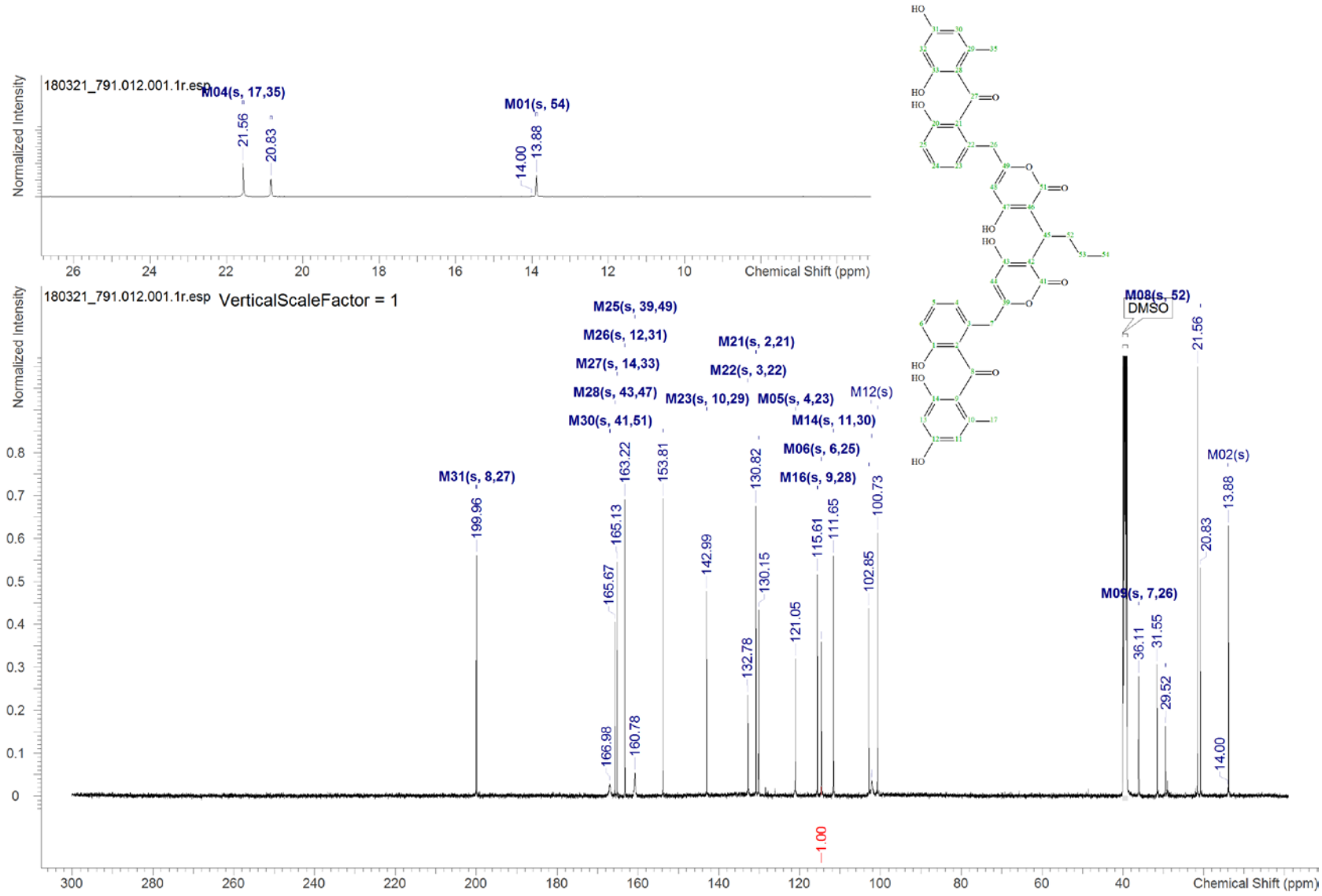


Figure S 38 ^{13}C-NMR spectrum (125 MHz, DMSO-d_6) of SEK90; complete Spectrum and zoom from 26 to 6 ppm

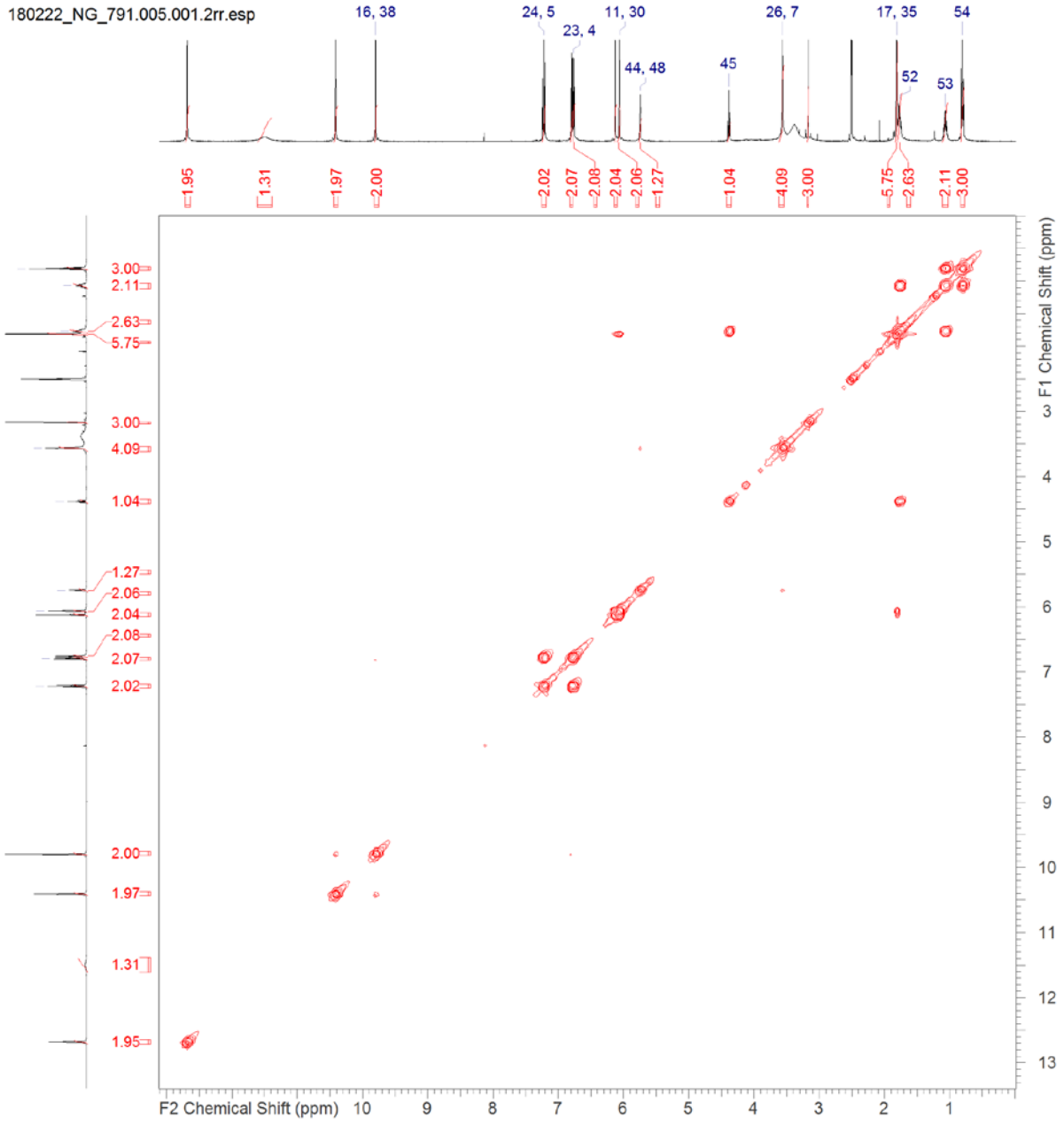


Figure S 39 ^{1}H-^{1}H- COSY spectrum (500 MHz, DMSO-d$_6$) of SEK90

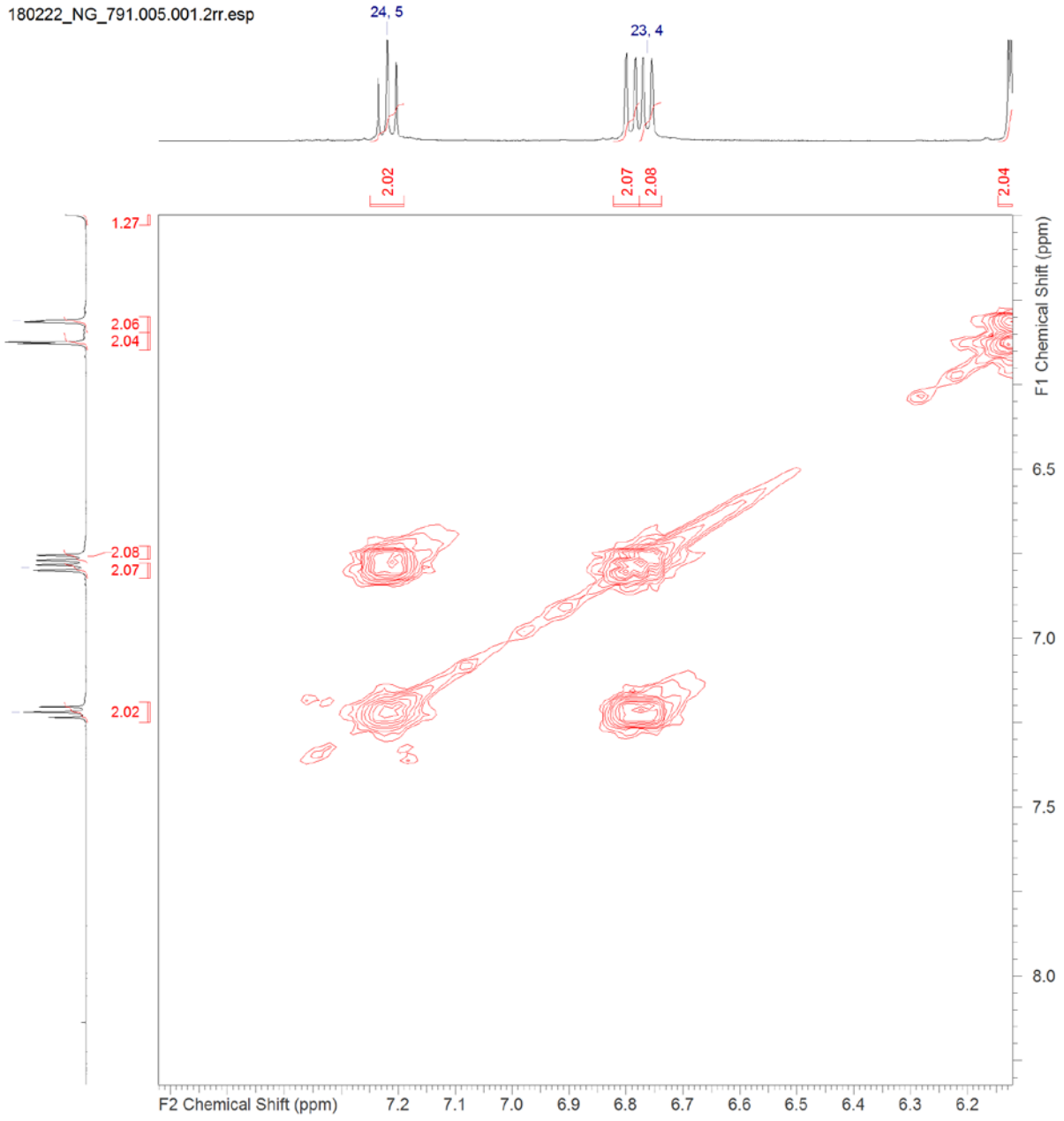


Figure S 40 ^{1}H-^{1}H- COSY spectrum (500 MHz, DMSO-d$_6$) of SEK90; zoom from 8 to 6.2 ppm

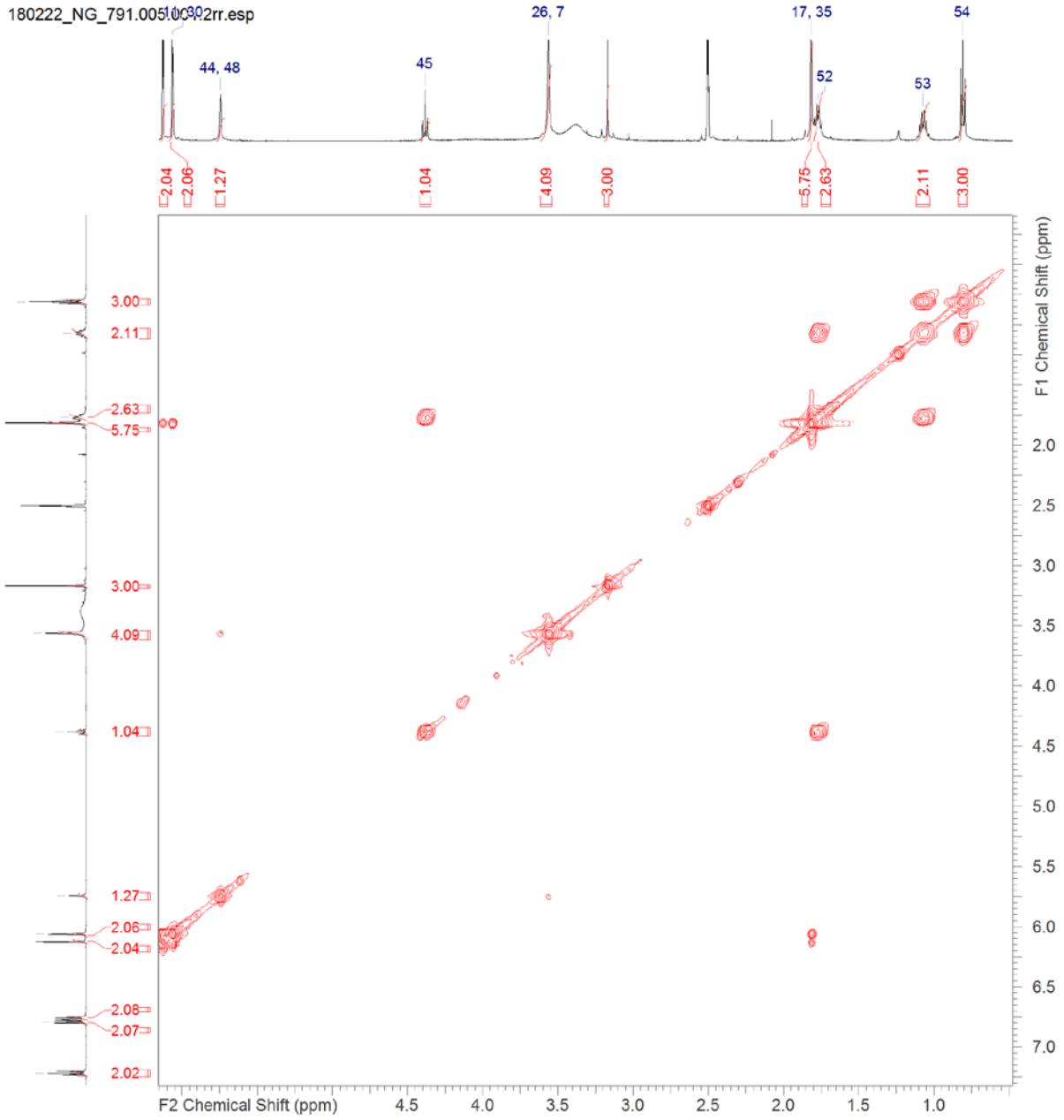


Figure S 41 ^{1}H-^{1}H- COSY spectrum (500 MHz, DMSO-d$_6$) of SEK90; zoom from 7 to 0.5 ppm

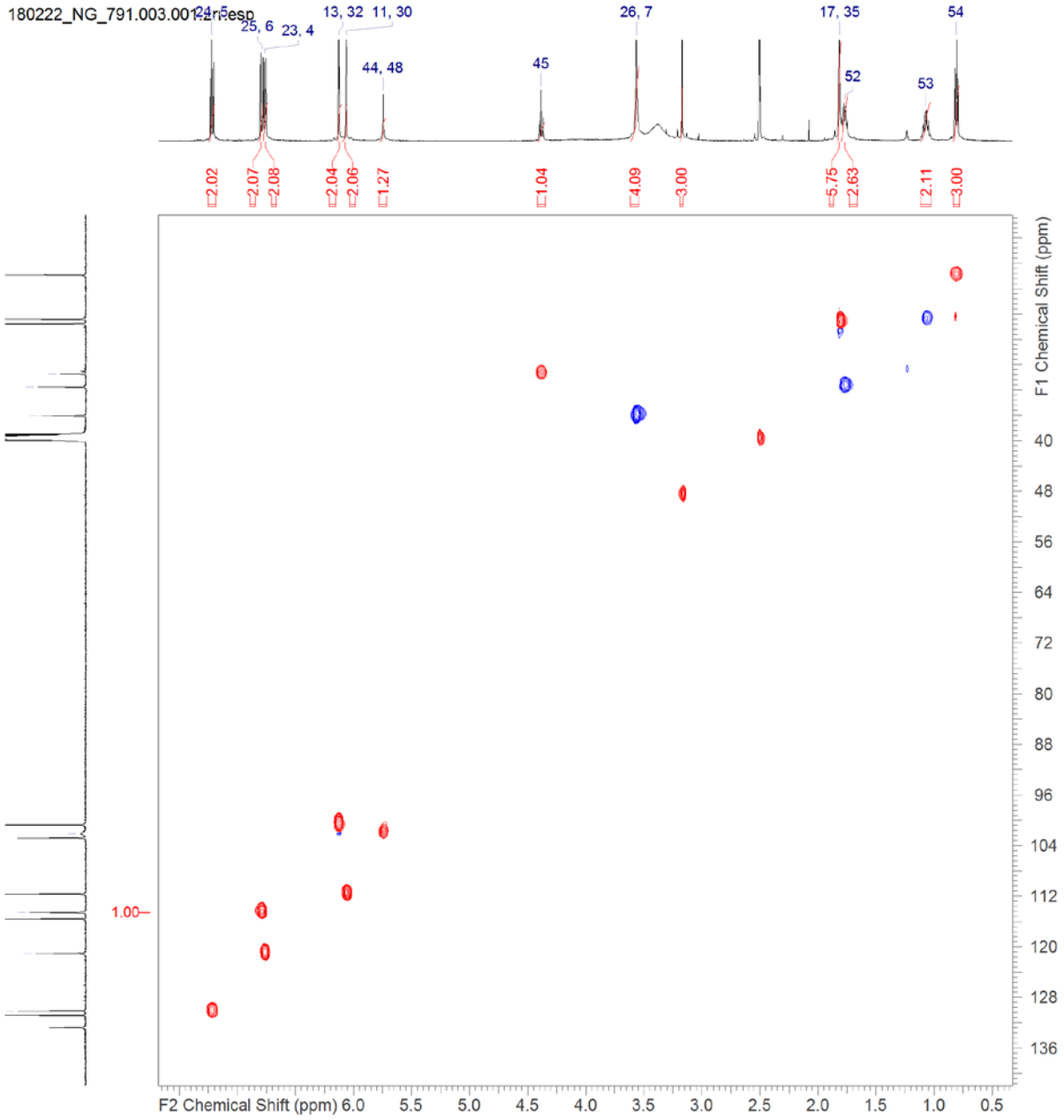


Figure S 42 HSQC-spectrum (500 MHz; 125 MHz, DMSO-d_6) of SEK90

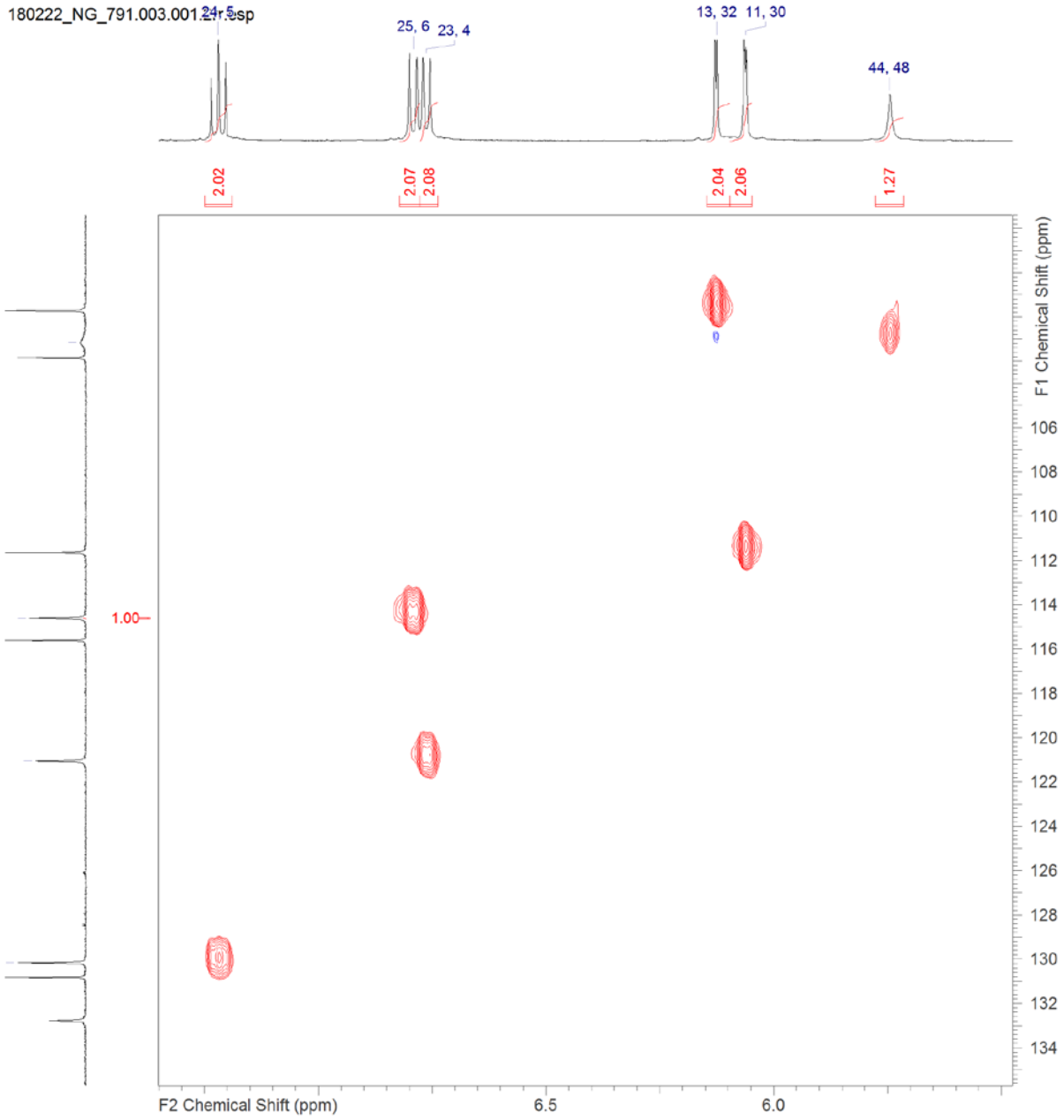


Figure S 43 HSQC-spectrum (500 MHz; 125 MHz, DMSO-d_6) of SEK90; zoom from 7.5 to 5.5 ppm and 135 to 99 ppm

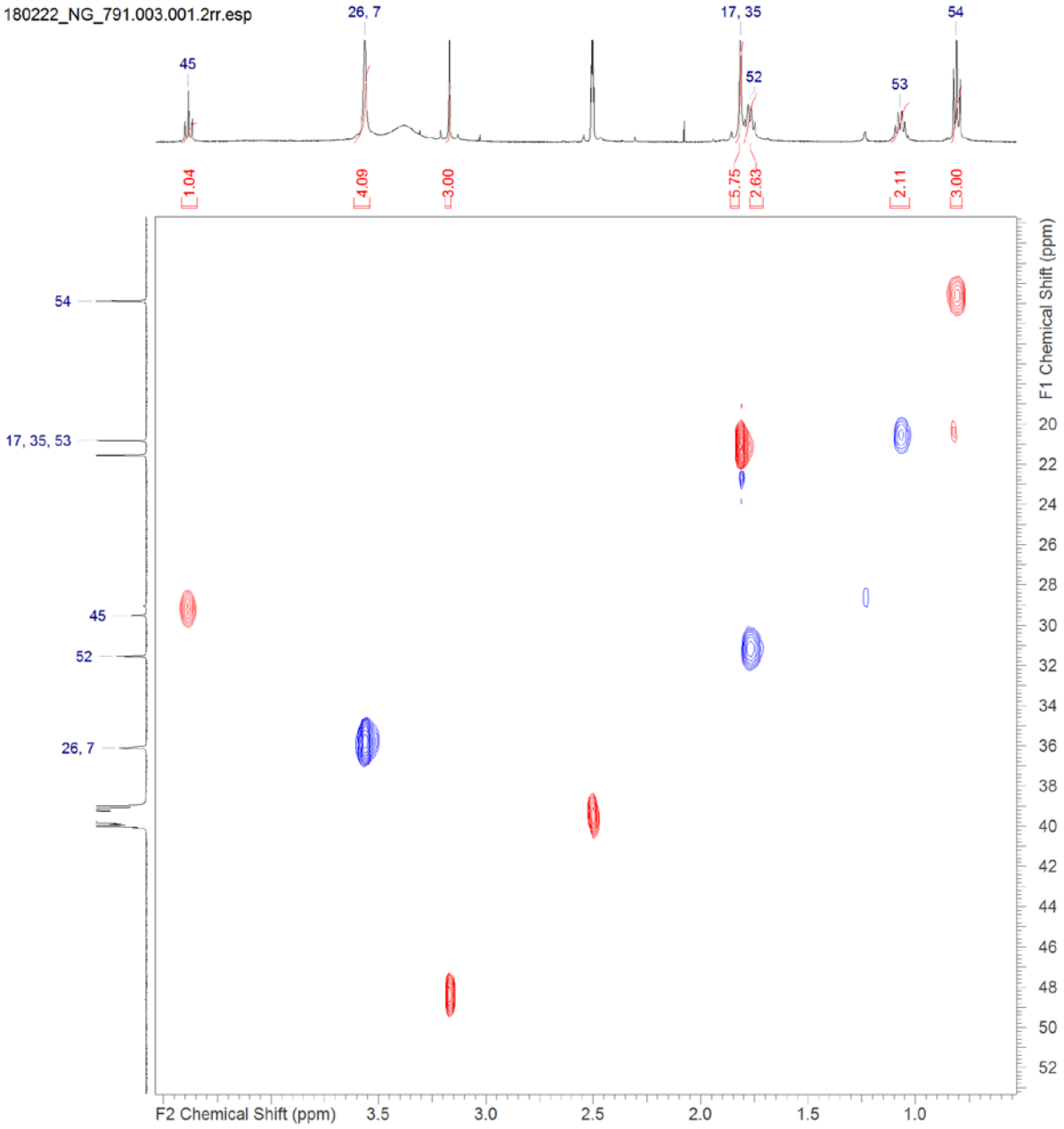


Figure S 44 HSQC-spectrum (500 MHz; 125 MHz, DMSO-d_6) of SEK90; zoom from 4 to 0,5 ppm and 53 to 16 ppm

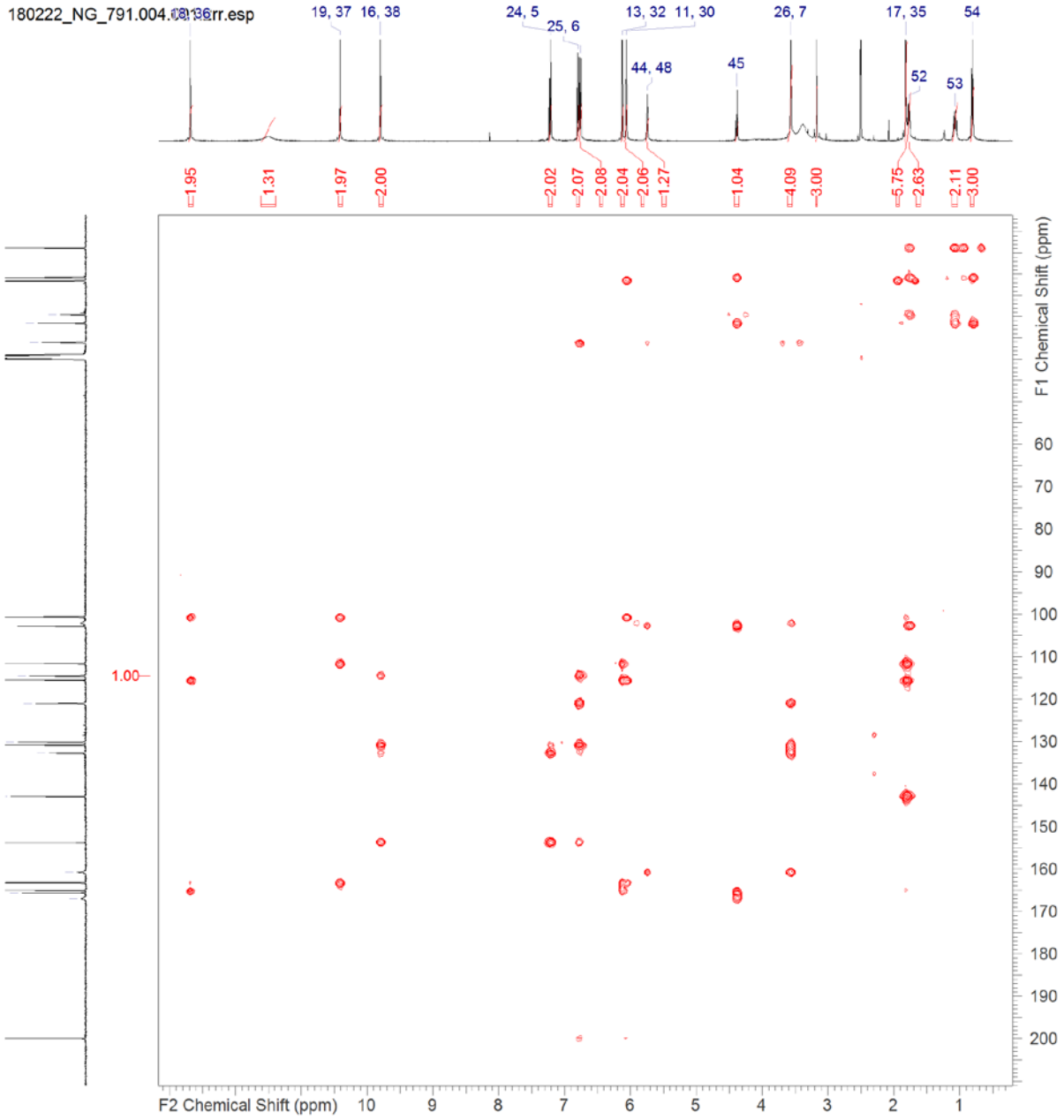


Figure S 45 HSQC-spectrum (500 MHz; 125 MHz, DMSO-d_6) of SEK90; zoom from 4 to 0.5 ppm and 53 to 16 ppm

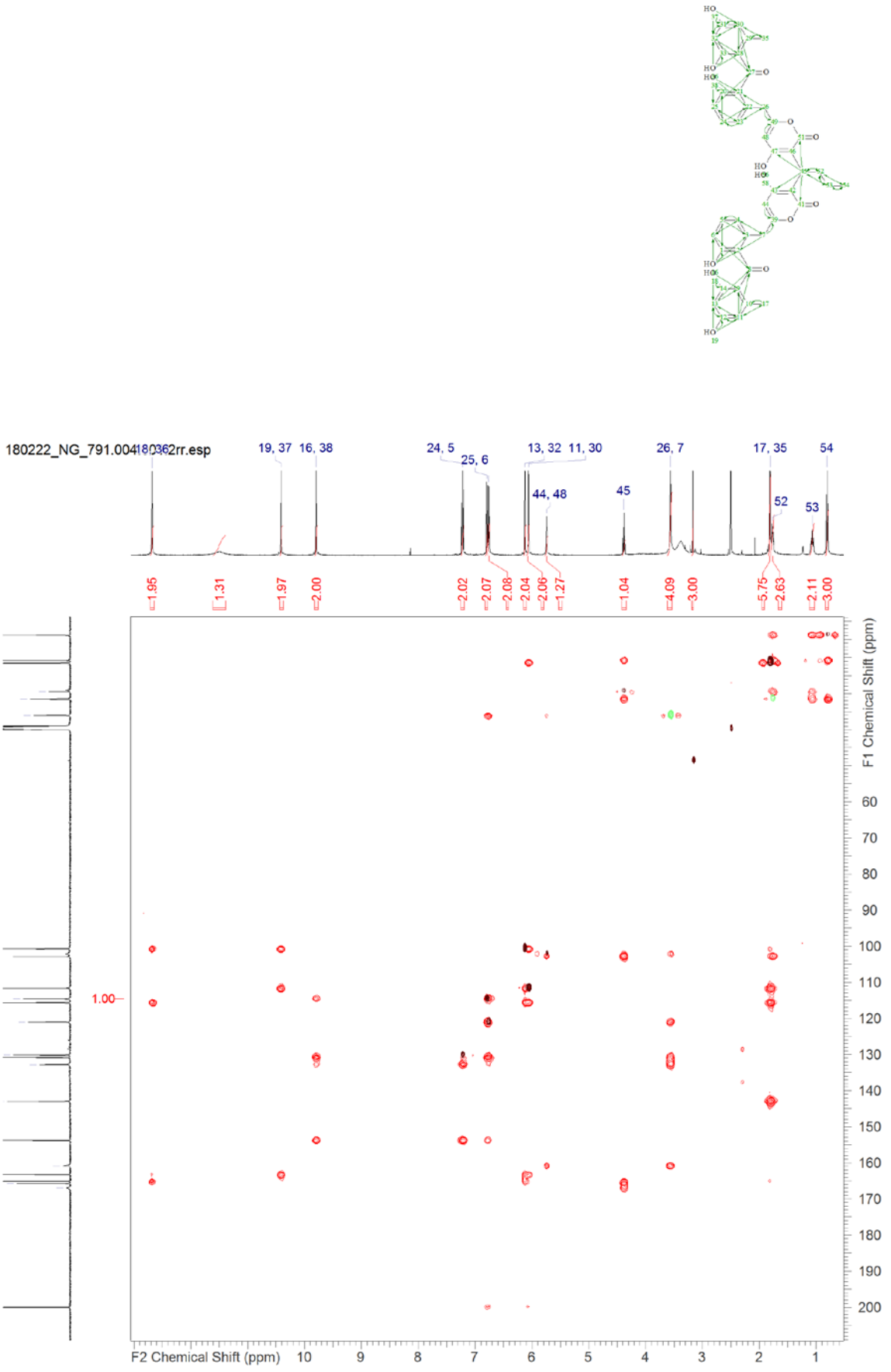


Figure S 46 HMBC - HSQC-Overlay spectrum (500 MHz; 125 MHz, DMSO-d_6) of SEK90 (red: HMBC; black/green: HSQC)

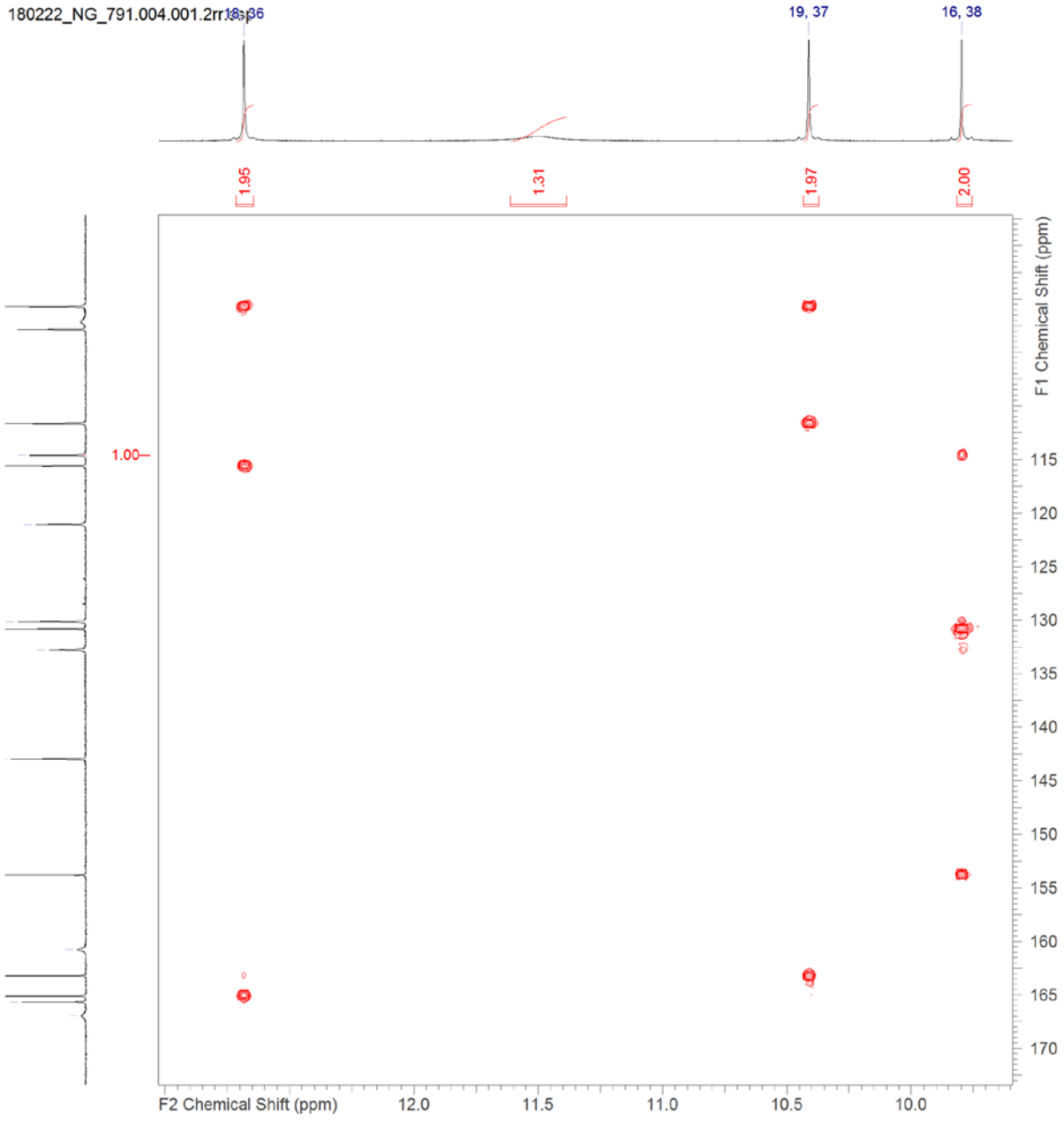


Figure S 47 HMBC - HSQC-Overlay spectrum (500 MHz; 125 MHz, DMSO-d_6) of SEK90 (black: HMBC; red/blue: HSQC); zoom from 13 to 10 ppm and 170 to 105 ppm

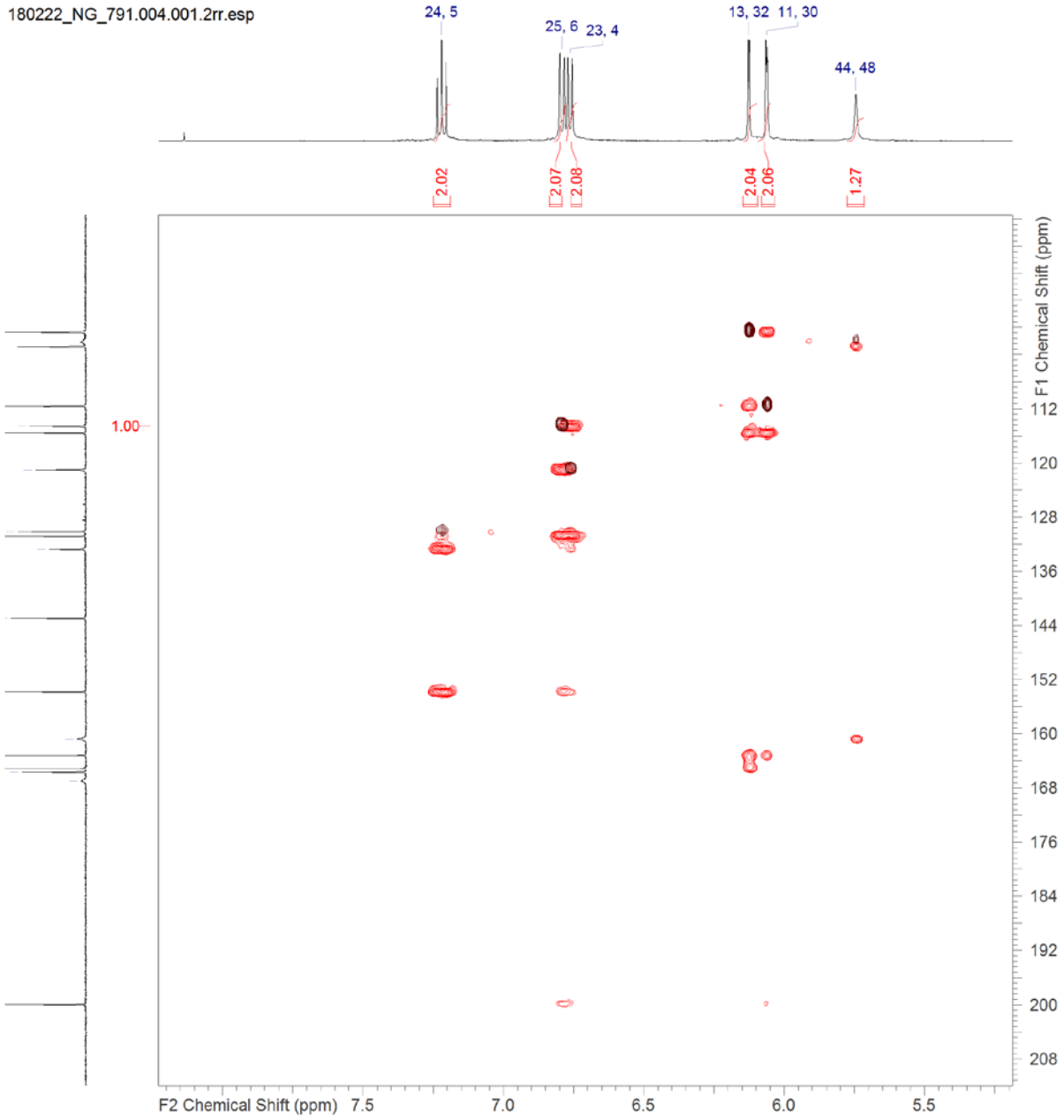


Figure S 48 HMBC - HSQC-Overlay spectrum (500 MHz; 125 MHz, DMSO-d_6) of SEK90 (black: HMBC; red/blue: HSQC); zoom from 13 to 10 ppm and 170 to 105 ppm

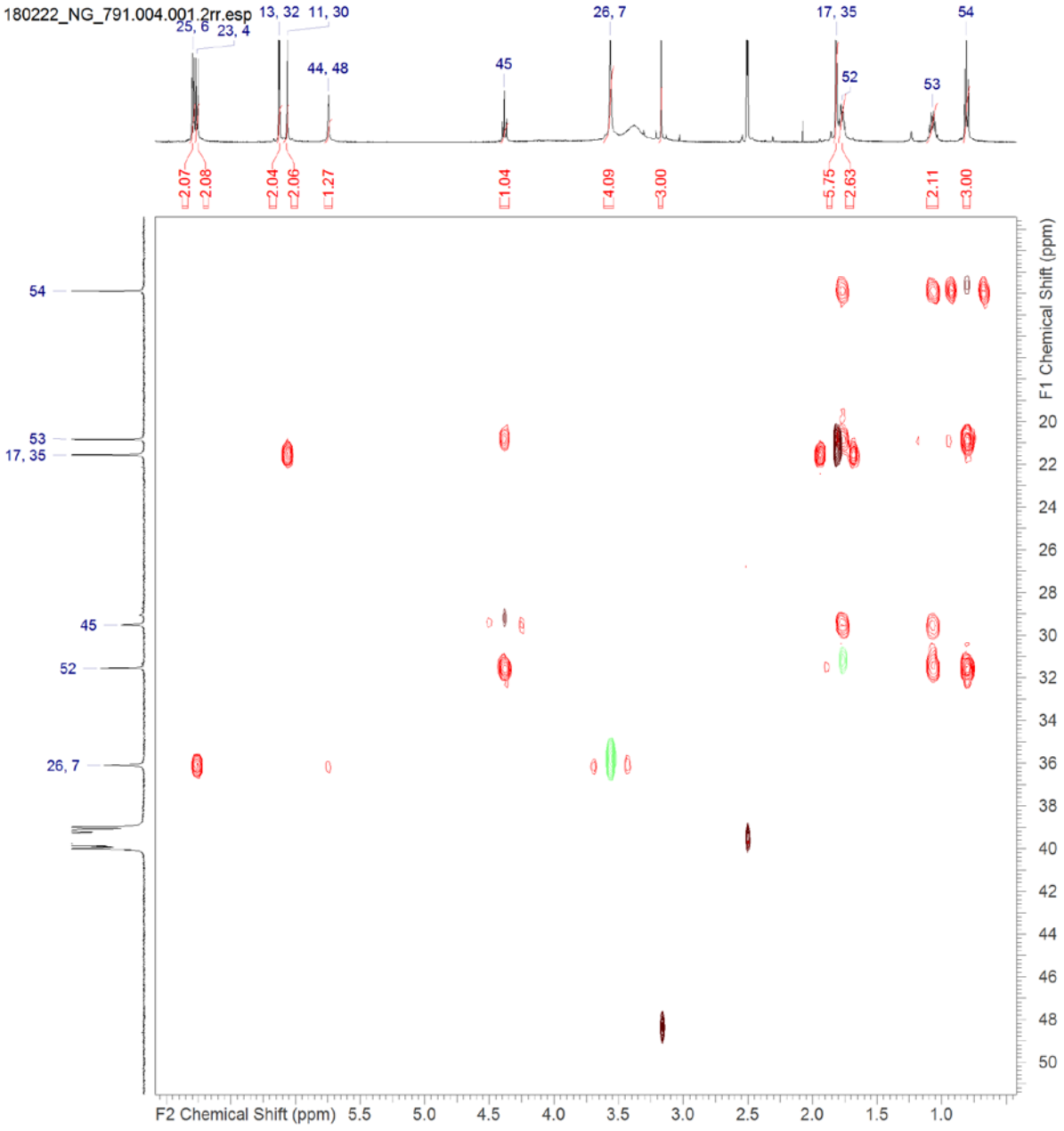


Figure S 49 HMBC - HSQC-Overlay spectrum (500 MHz; 125 MHz, DMSO-d_6) of SEK90 (black: HMBC; red/blue: HSQC); zoom from 6.5 to 0.5 ppm and 51 to 10 ppm

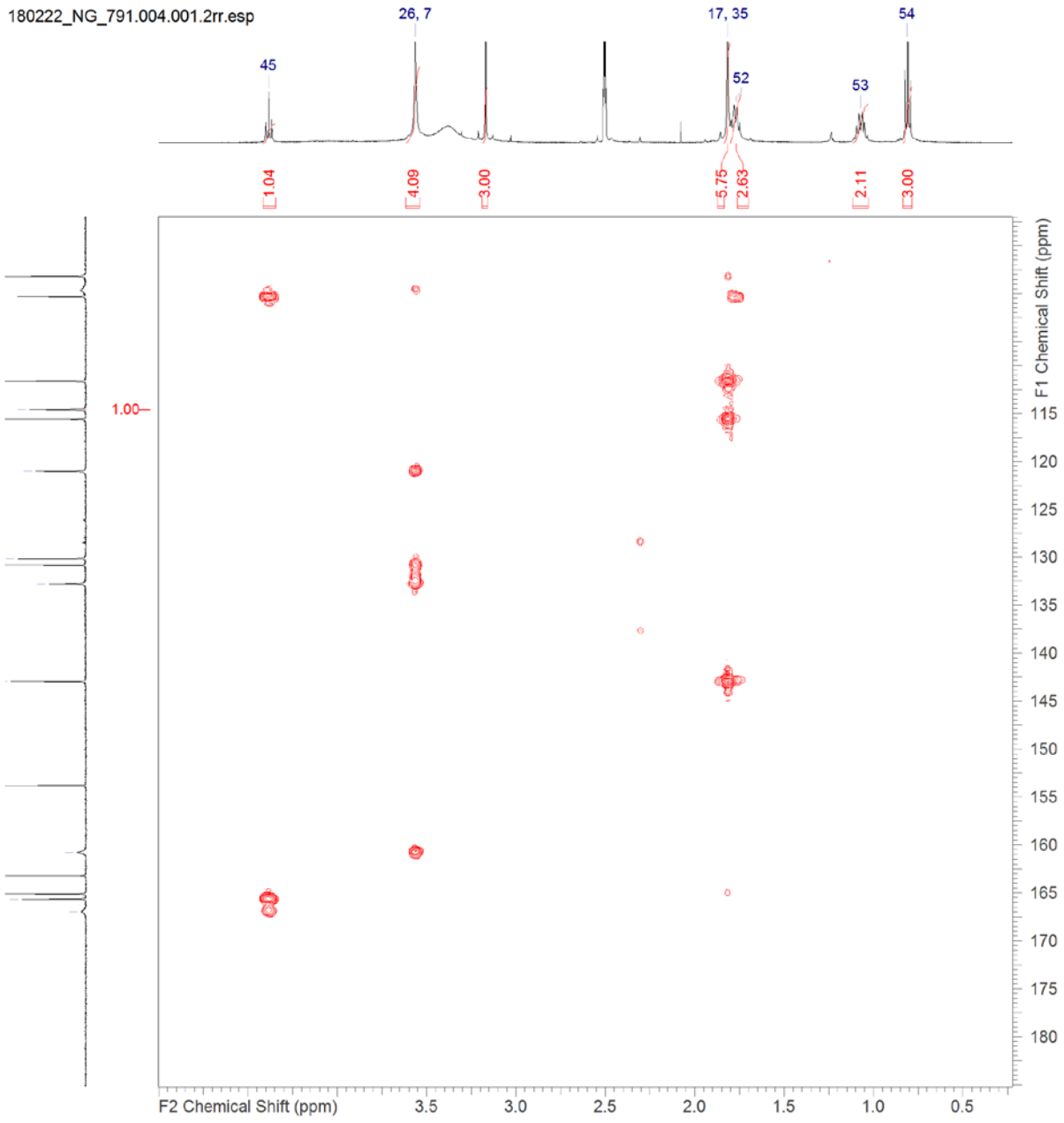
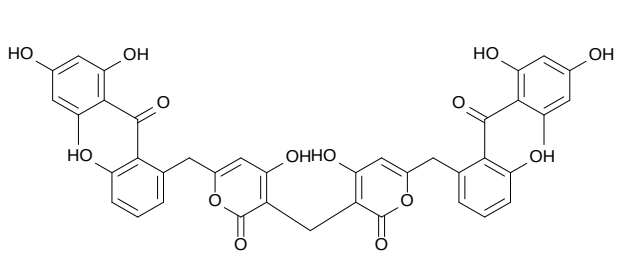
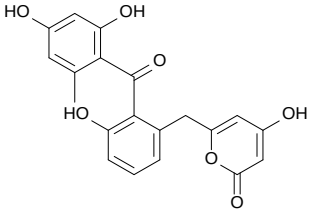


Figure S 50 HMBC - HSQC-Overlay spectrum (500 MHz; 125 MHz, DMSO-d_6) of SEK90 (black: HMBC; red/blue: HSQC); zoom from 4.5 to 0.5 ppm and 185 to 100 ppm



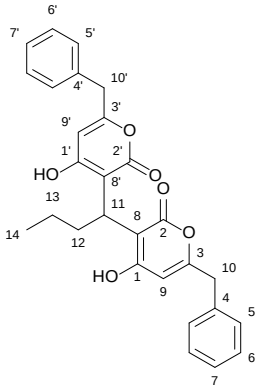
Exact Mass: 748,17921

SEK87



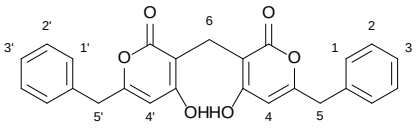
Exact Mass: 368,08960

SEK43



Exact Mass: 458,17294

1E5_CMP2



Exact Mass: 416,12599

1E5_CMP1

Figure S 51 Structures of SEK87, SEK43 and 1E5_CMP1 & 1E5_CMP2

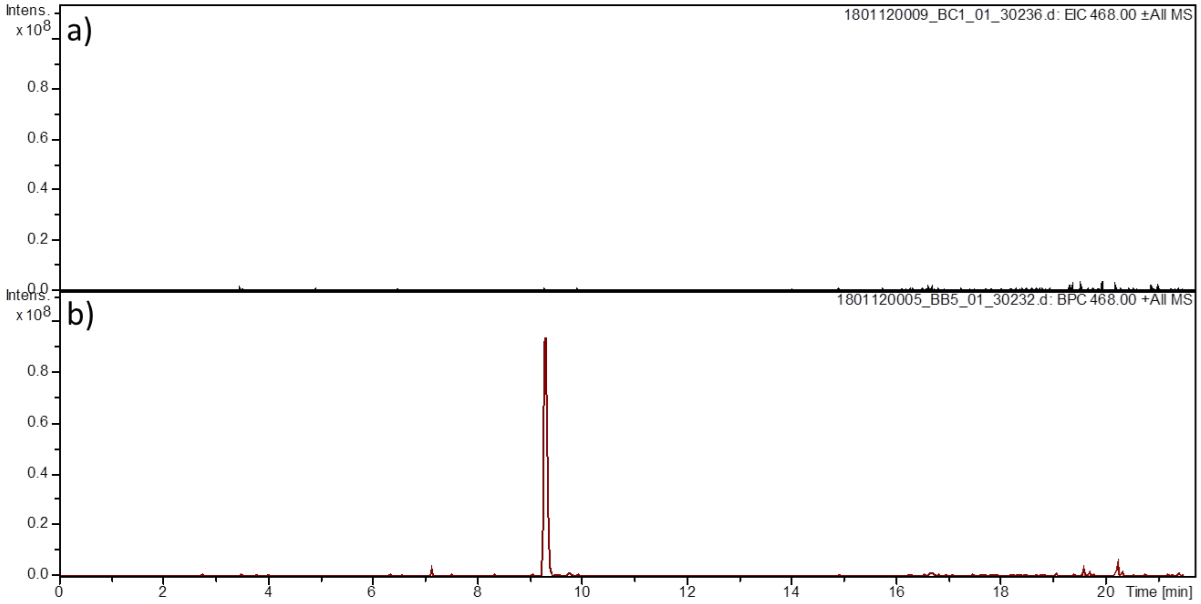


Figure S 52 HPLC-MS Extracted ion chromatogram (Extracted mass 468±0.5) of a) S. lividans ΔYA6_1E5Δ54860 and b) S. lividans ΔYA6_1E5Δ55110; The production is unchanged in the deletion of the NRPS/PKSI hybrid core gene, while the production is abolished in the created mutant lacking the type II PKS gene

Figure S 53 Genes (WP_124773686 & WP_124773691-WP124773694) of Micromonospora sp. LB39 mapped to Pentangumycins biosynthetic gene cluster

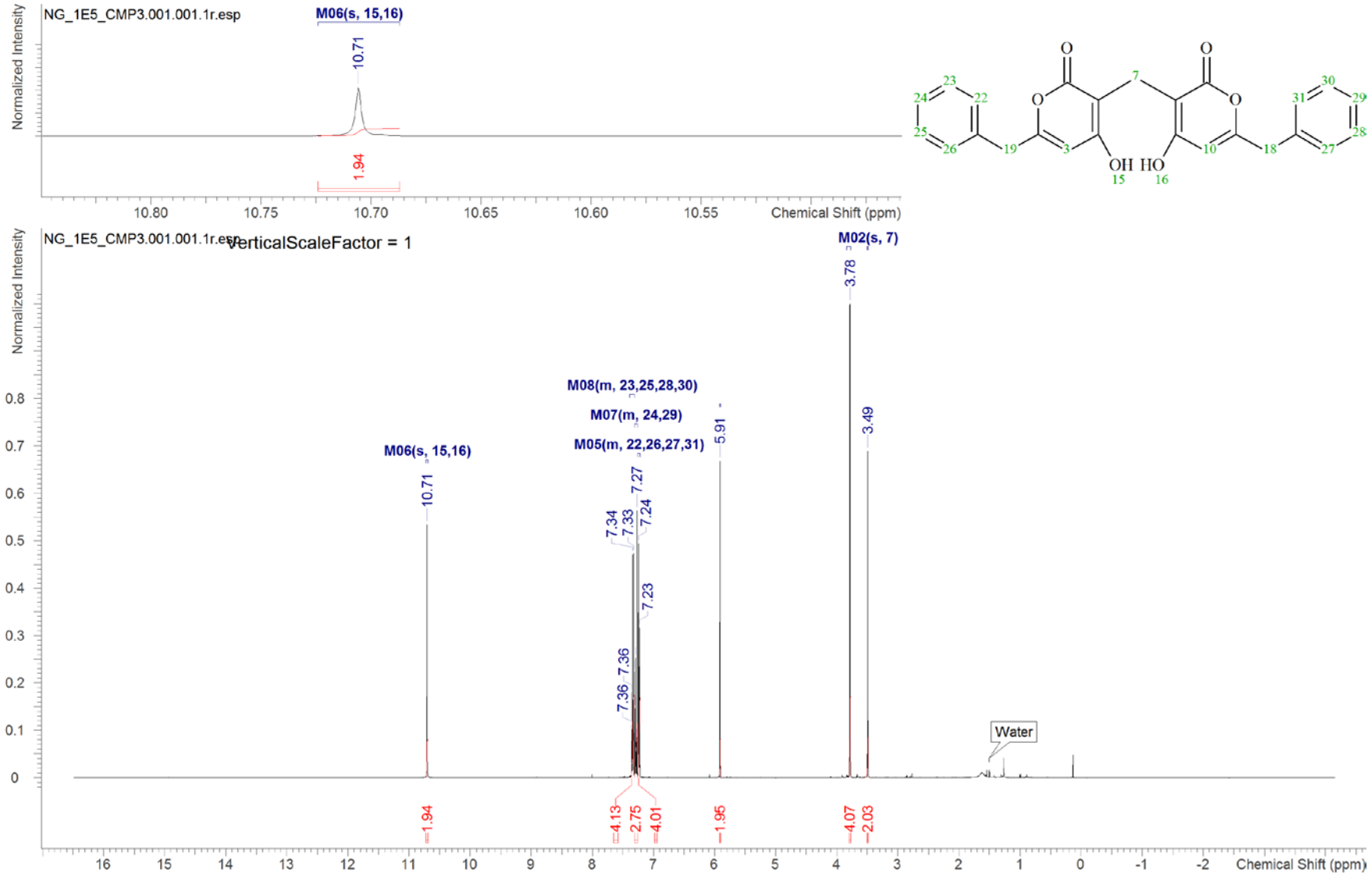


Figure S 54 ^{1}H-NMR spectrum (500 MHz, $CDCL_3$) of 1E5_CMP1; zoom from 10.8 ppm to 10.5 ppm

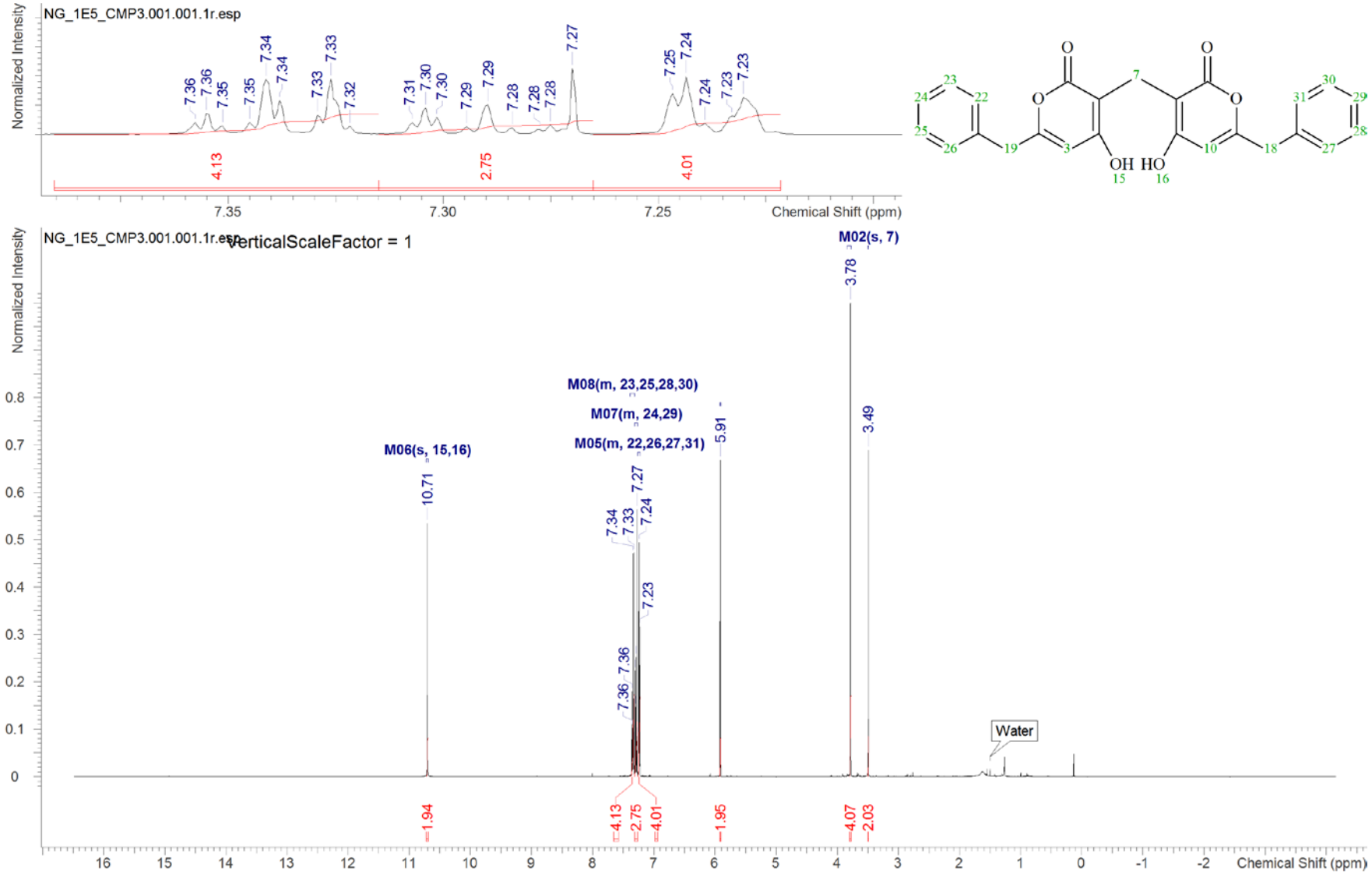


Figure S 55 ^{1}H-NMR spectrum (500 MHz, $CDCL_3$) of 1E5_CMP1; zoom from 7.4 ppm to 7.2 ppm

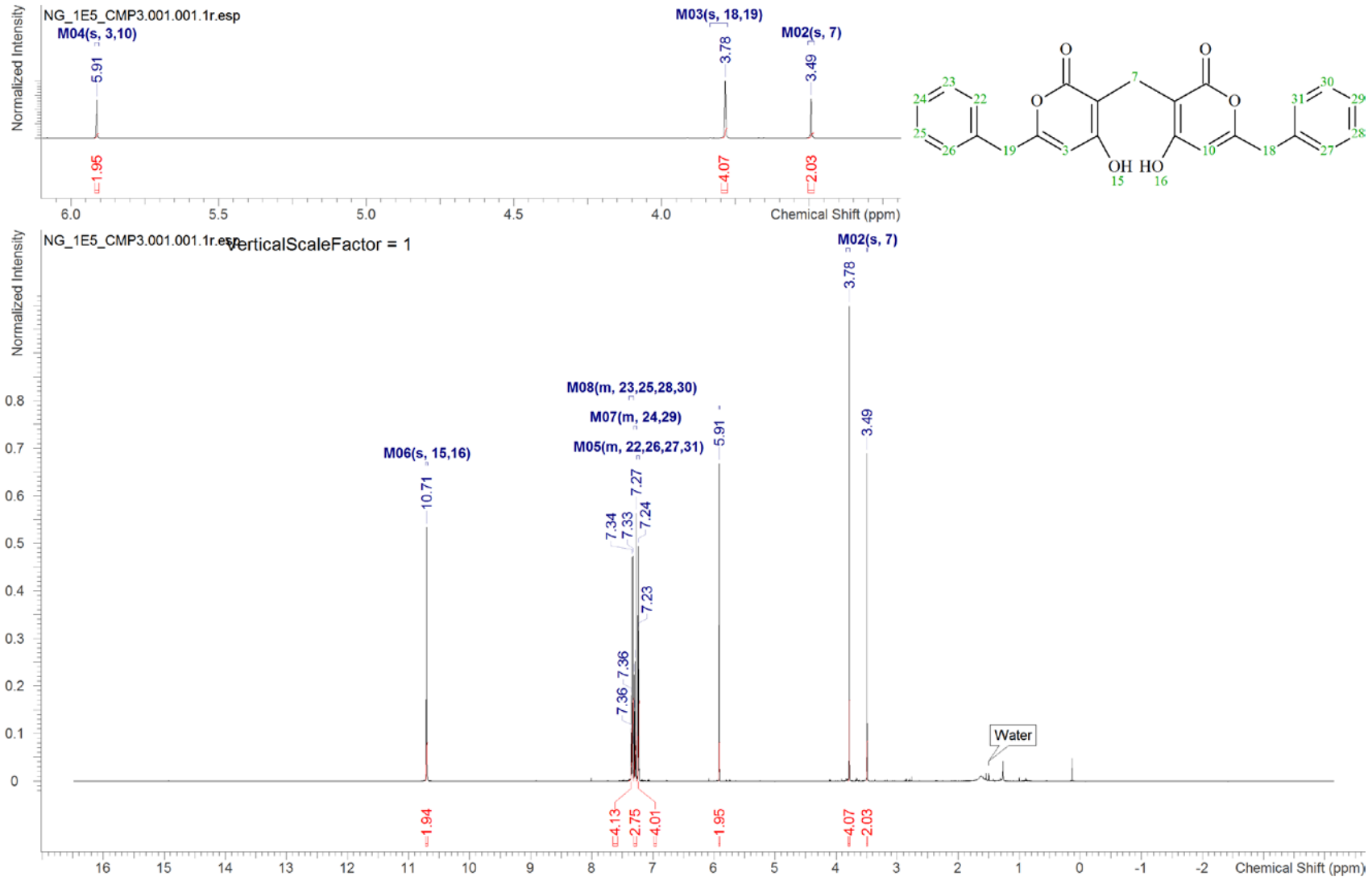


Figure S 56 ^{1}H-NMR spectrum (500 MHz, $CDCL_3$) of 1E5_CMP1; zoom from 6 ppm to 3.5 ppm

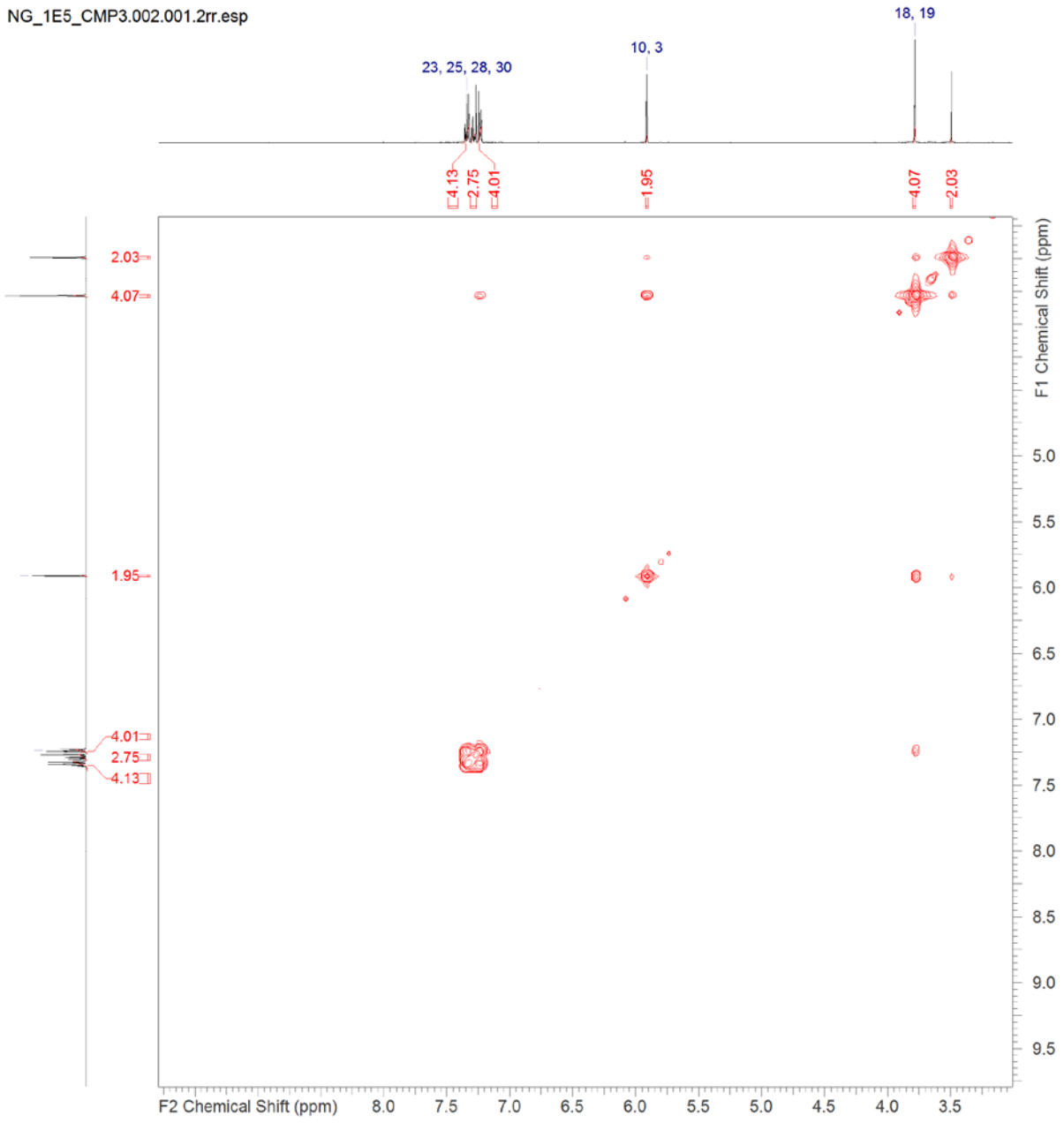


Figure S 57 ^{1}H-^{1}H- COSY spectrum (500 MHz, $CDCL_3$) of 1E5_CMP1

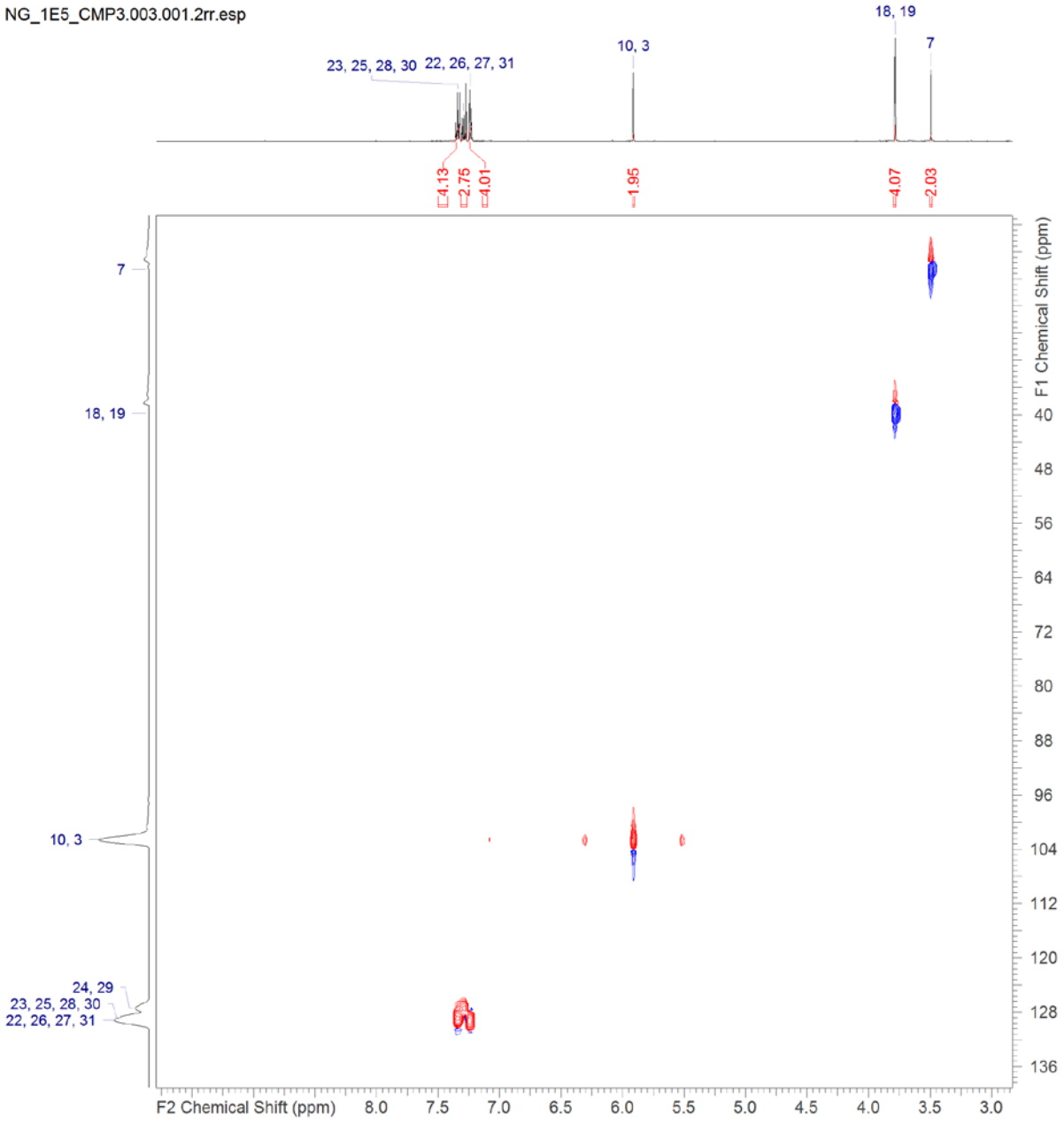


Figure S 58 HSQC-spectrum (500 MHz; 125 MHz, $CDCL_3$) of 1E5_CMP1

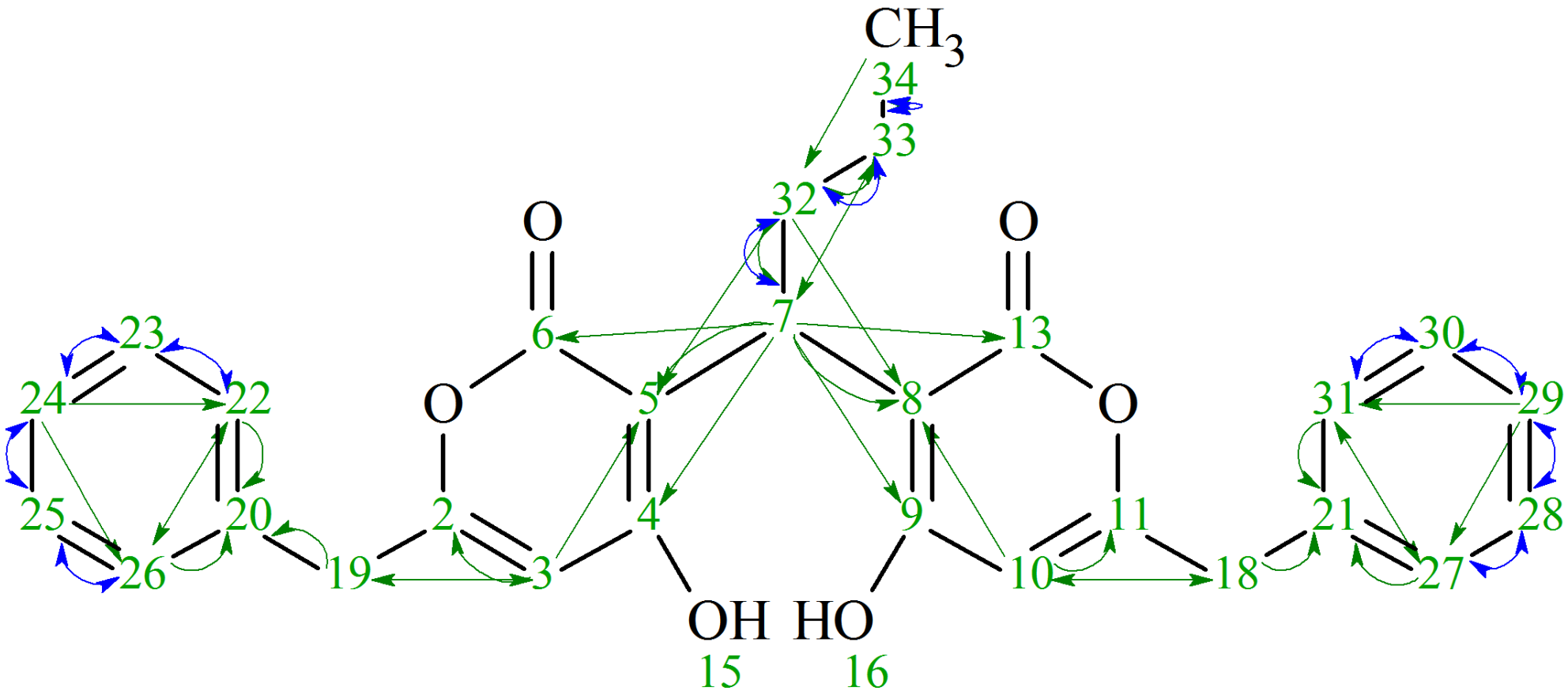


Figure S 59 Structure of 1E5_CMP2 with all obsevered correlations (HMBC: green; ^{1}H-^{1}H-Cosy: blue)

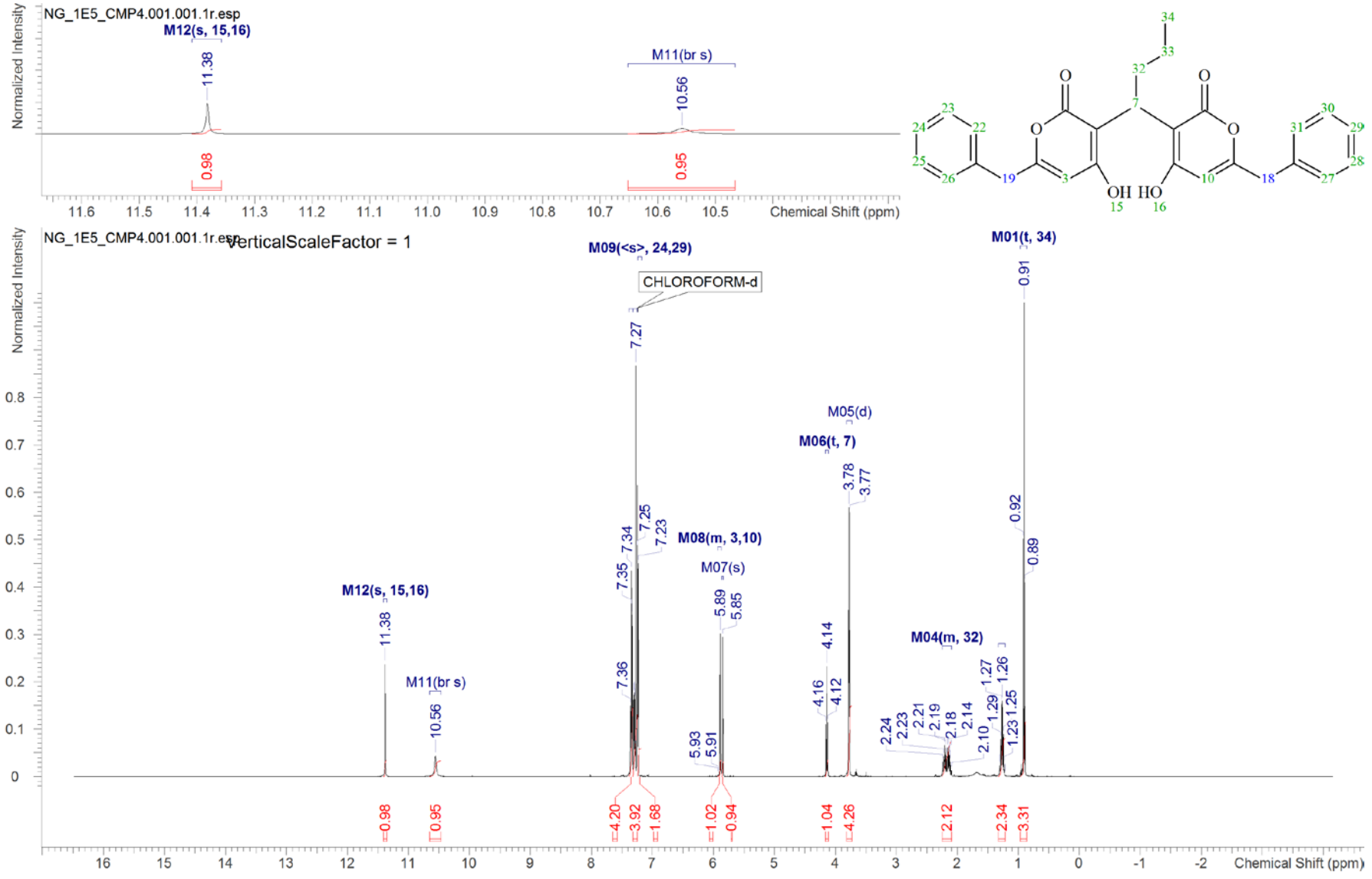


Figure S 60 ^{1}H-NMR spectrum (500 MHz, $CDCL_3$) of 1E5_CMP2; zoom from 11.6 to 9.5 ppm

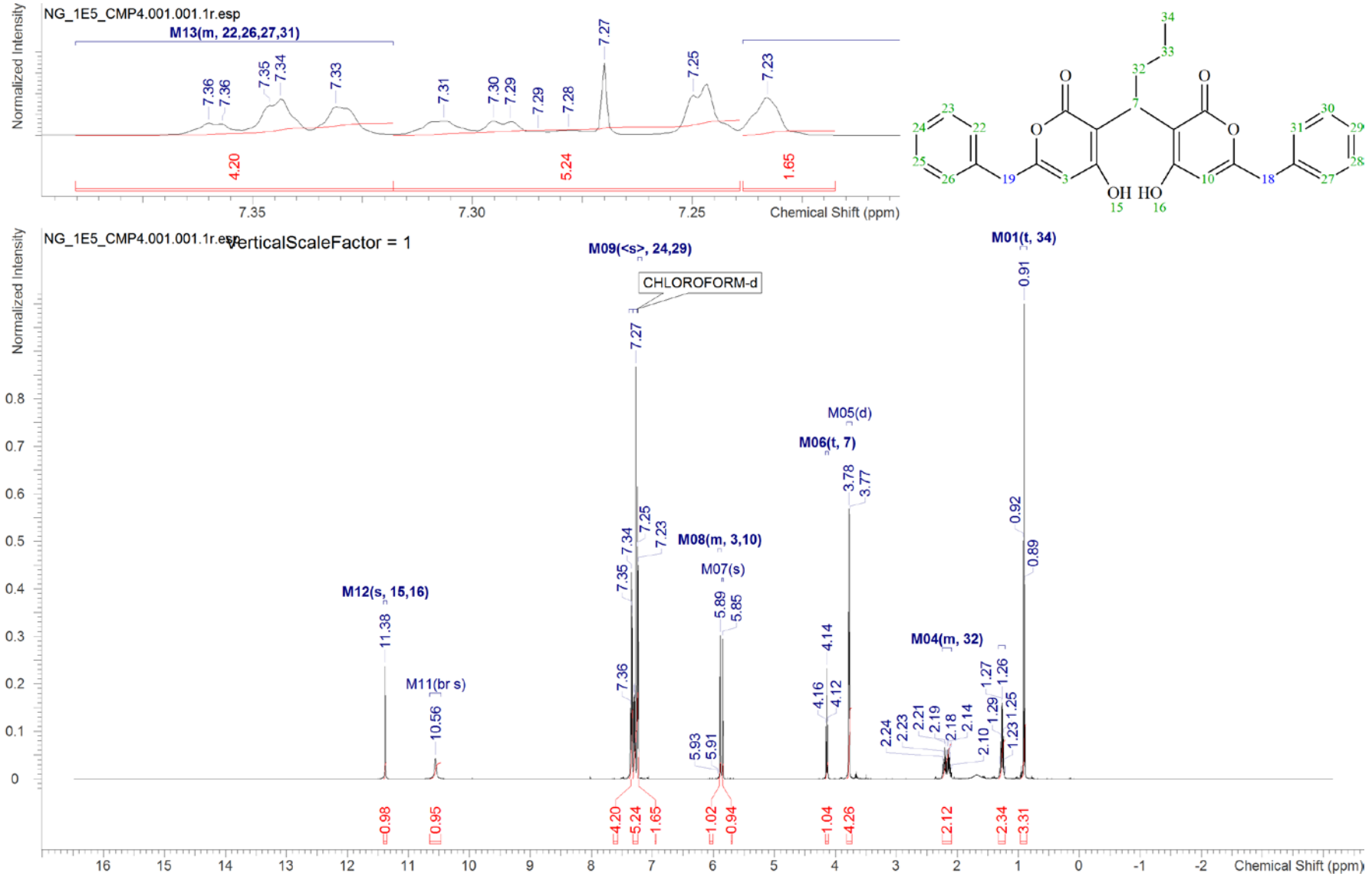


Figure S 61 ^{1}H-NMR spectrum (500 MHz, $CDCL_3$) of 1E5_CMP2; zoom from 7.4 to 7.2 ppm

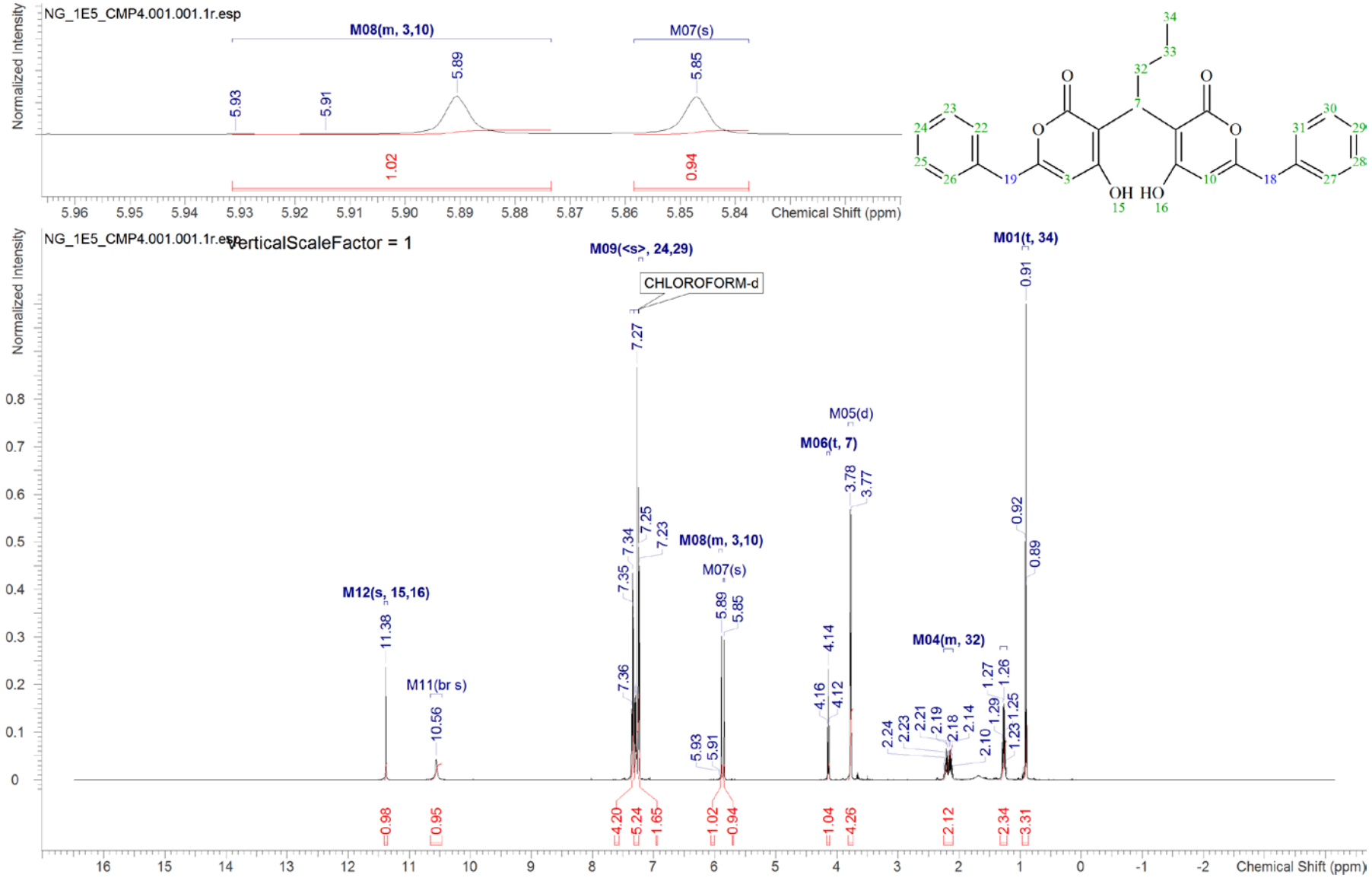


Figure S 62 ^{1}H-NMR spectrum (500 MHz, $CDCL_3$) of 1E5_CMP2; zoom from 5.96 to 5.8 ppm

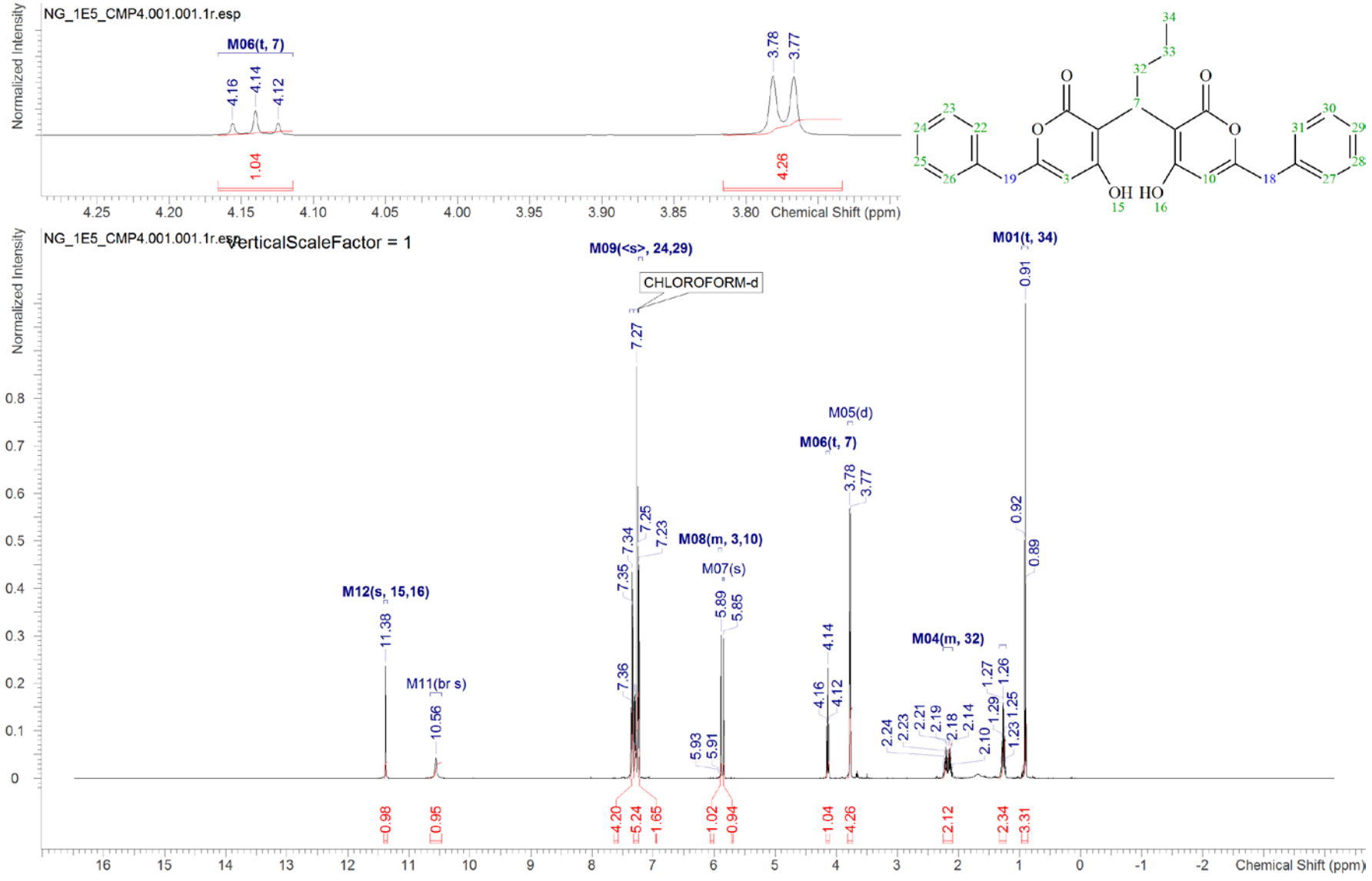


Figure S 63 ^{1}H-NMR spectrum (500 MHz, $CDCL_3$) of 1E5_CMP2; zoom from 4.25 to 3.75 ppm

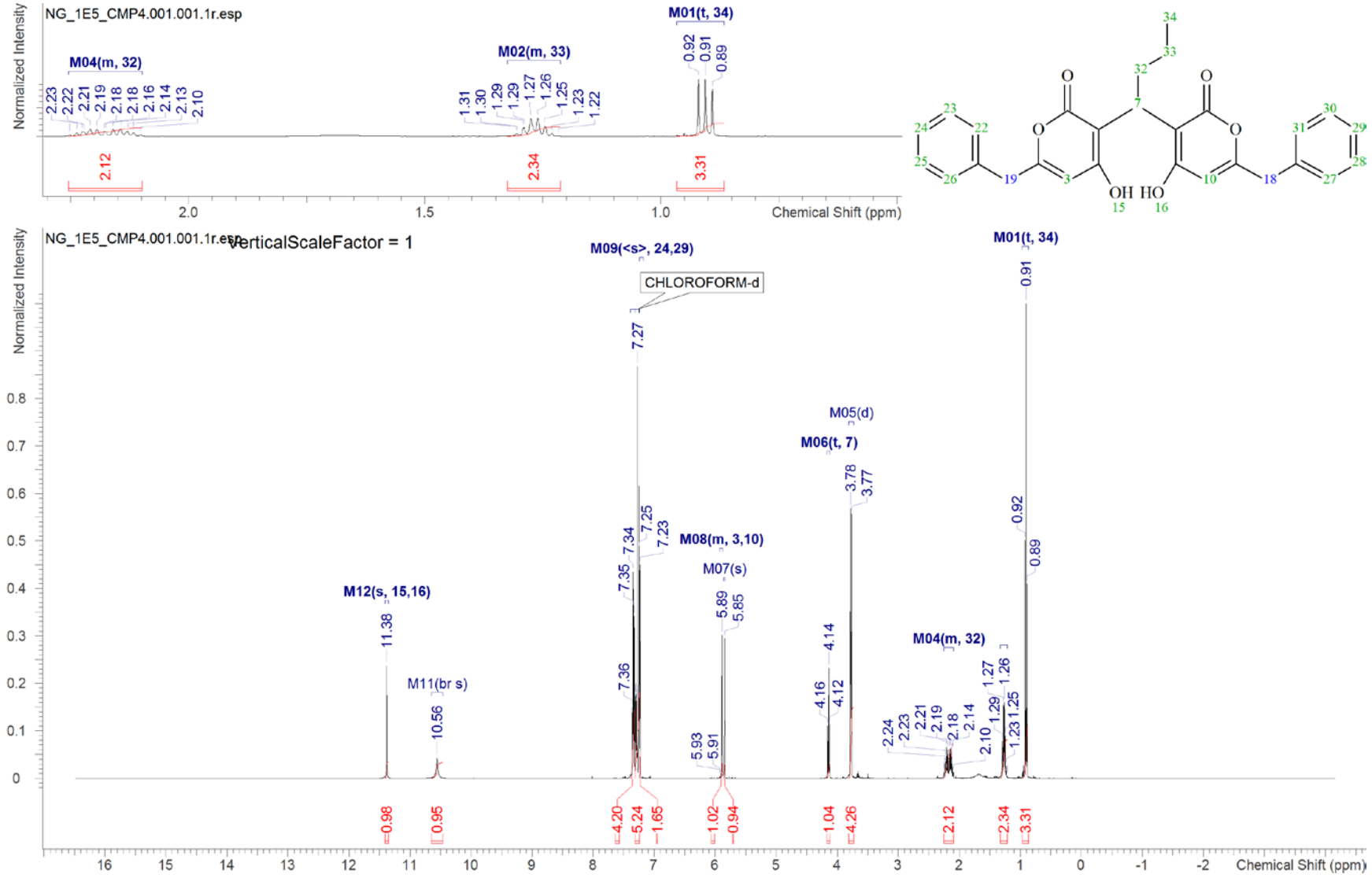


Figure S 64 ^{1}H-NMR spectrum (500 MHz, $CDCL_3$) of 1E5_CMP2; zoom from 2.5 to 0.5 ppm

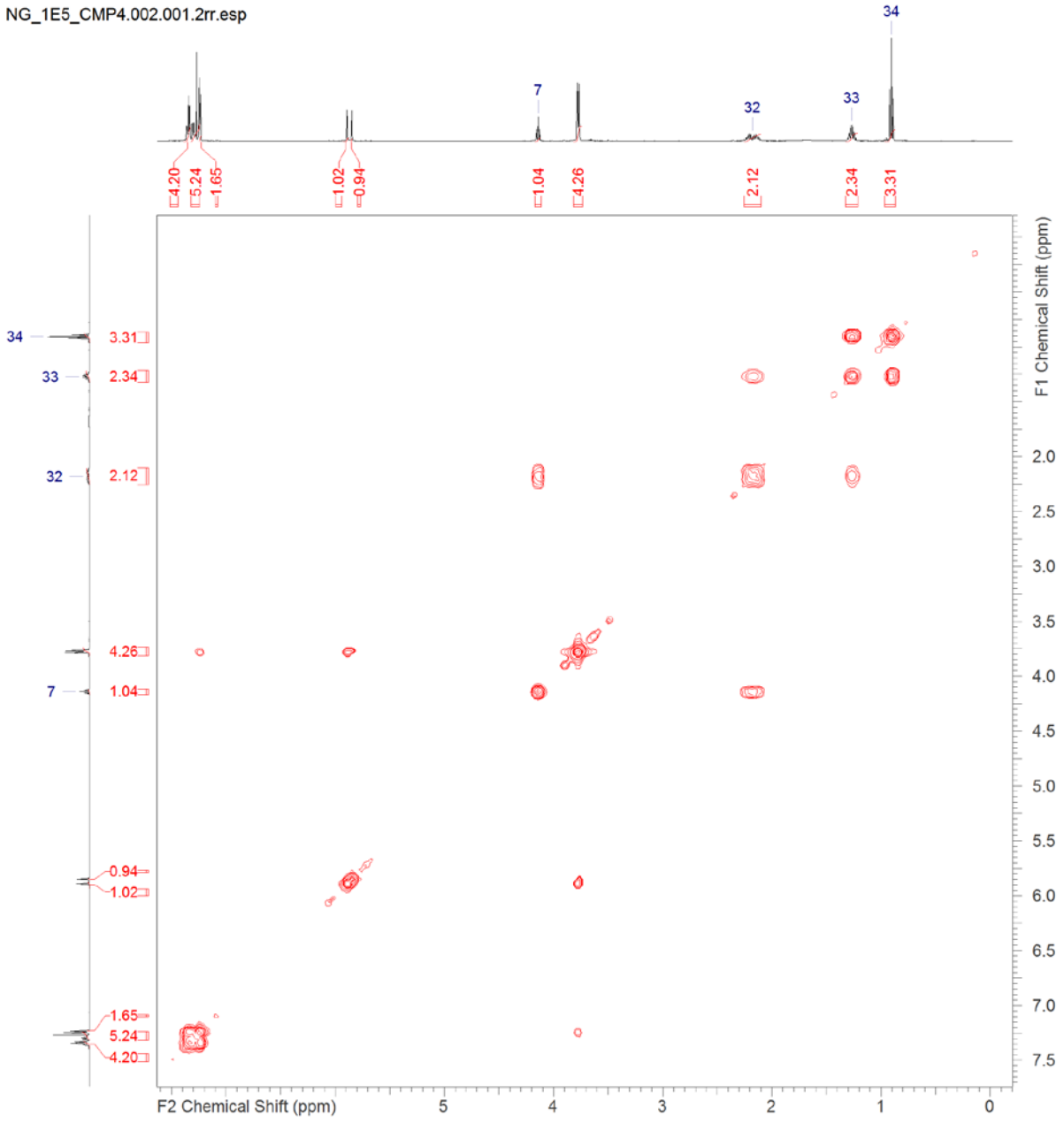


Figure S 65 ^{1}H-^{1}H- COSY spectrum (500 MHz, $CDCL_3$) of 1E5_CMP2

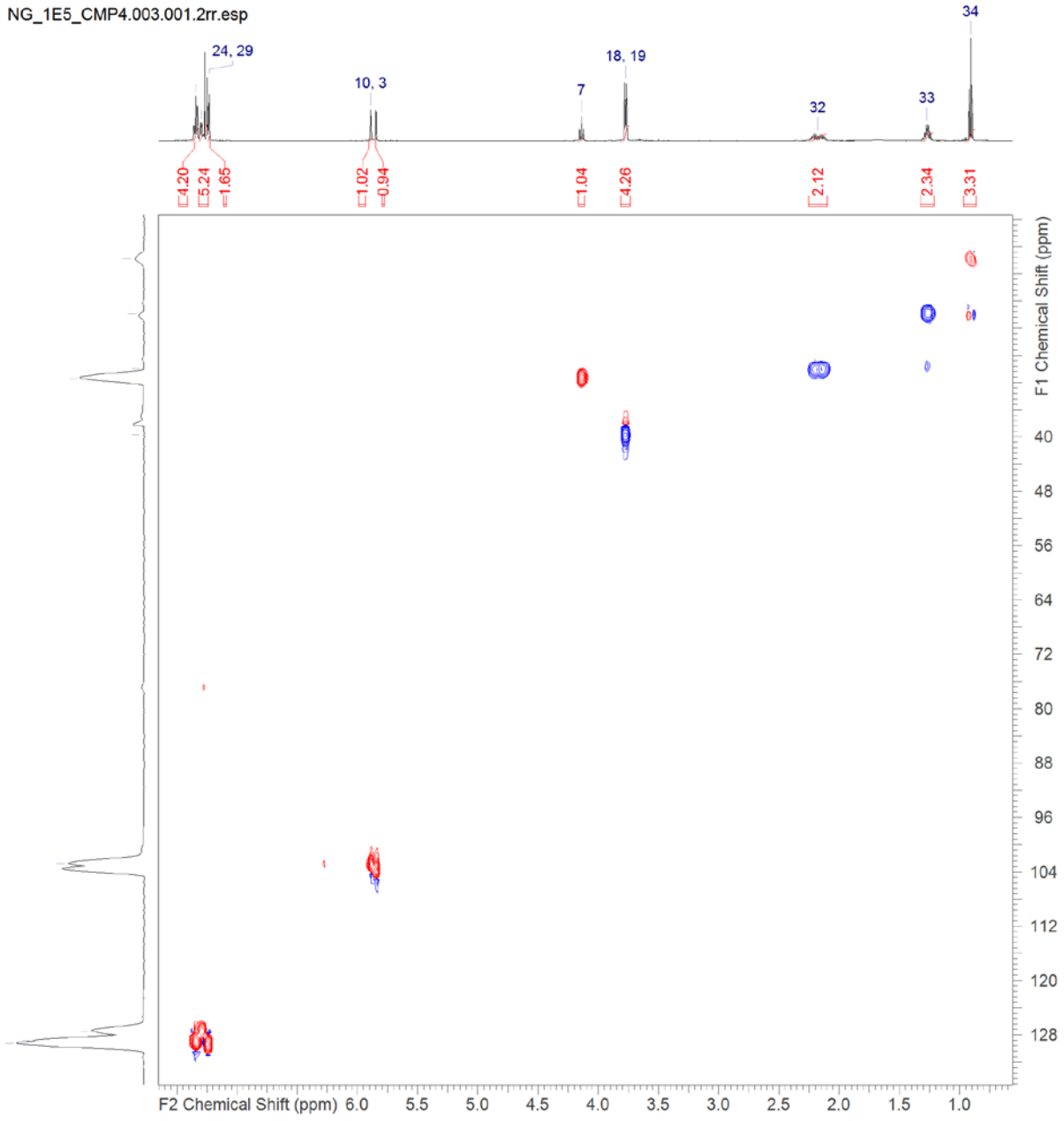


Figure S 66 HSQC-spectrum (500 MHz; 125 MHz, CDCL3) of 1E5_CMP2

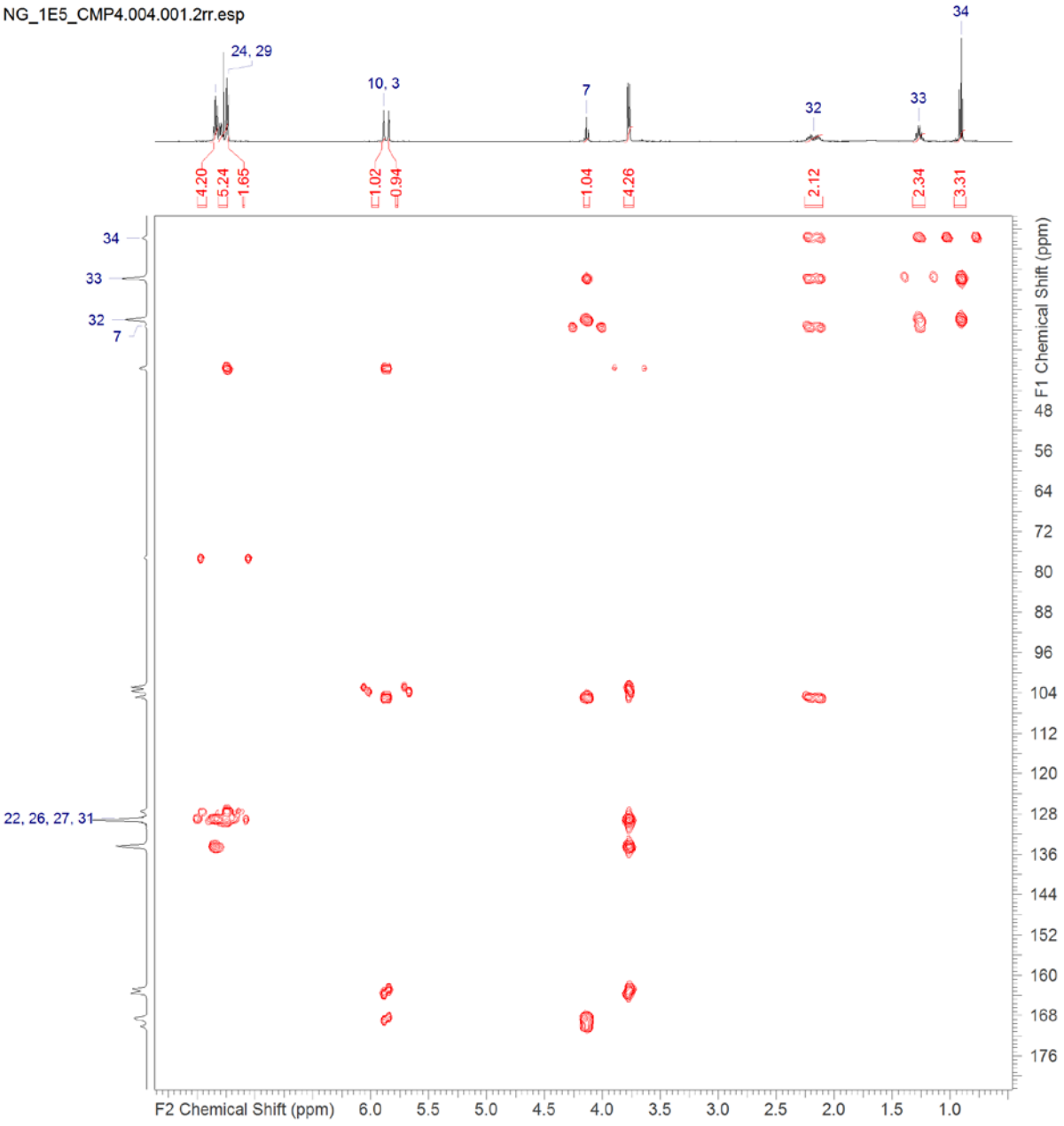


Figure S 67 HMBC-spectrum (500 MHz; 125 MHz, CDCL3) of 1E5_CMP2

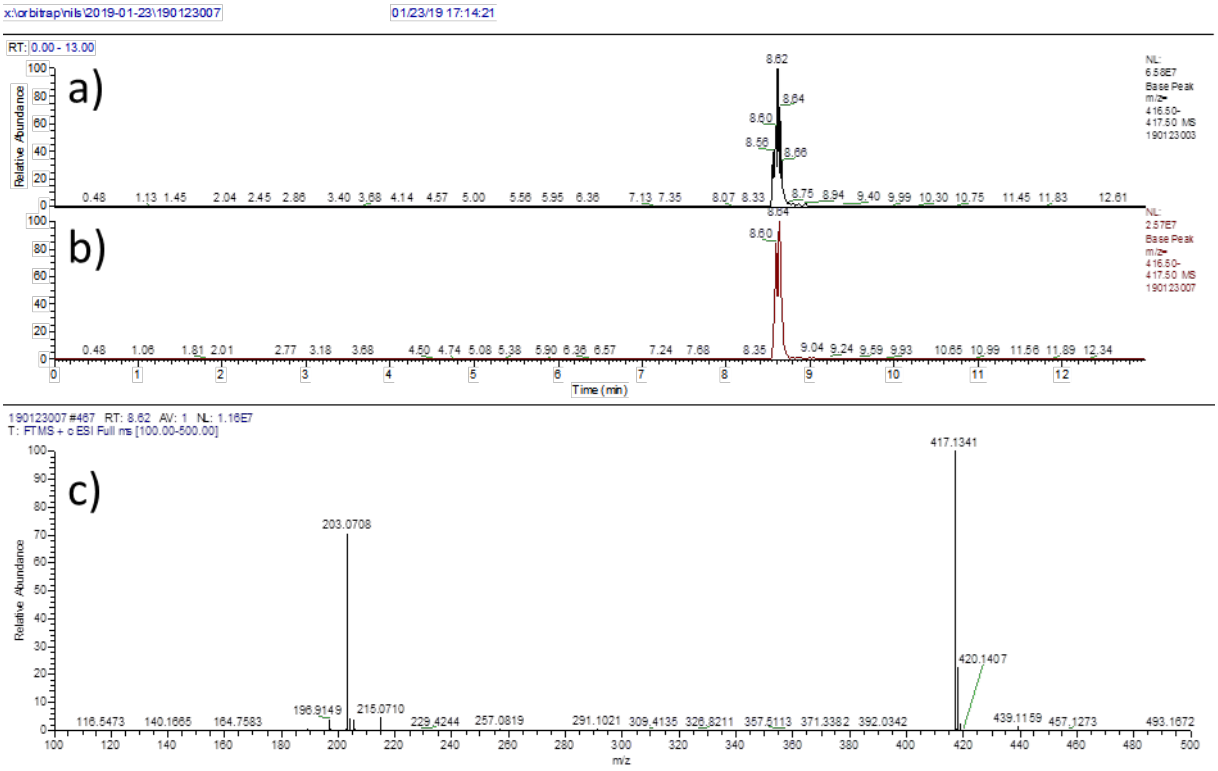


Figure S 68 HPLC-MS Extracted ion chromatogram (Extracted mass 417±0.5) of a) Spontaneously formed 8; b) synthesized 8; c) mass chromatogram of a)

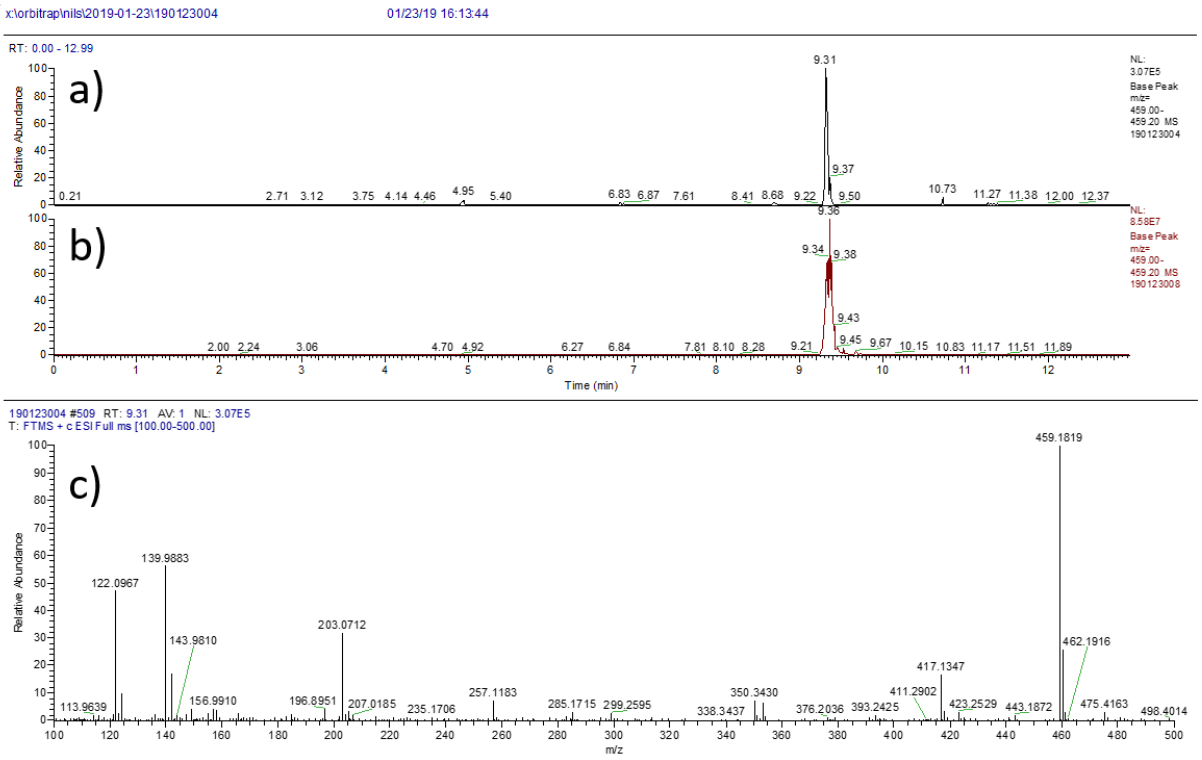


Figure S 69 HPLC-MS Extracted ion chromatogram (Extracted mass 459.1±0.1) of a) Spontaneously formed 9; b) synthesized 9; c) mass chromatogram of a)

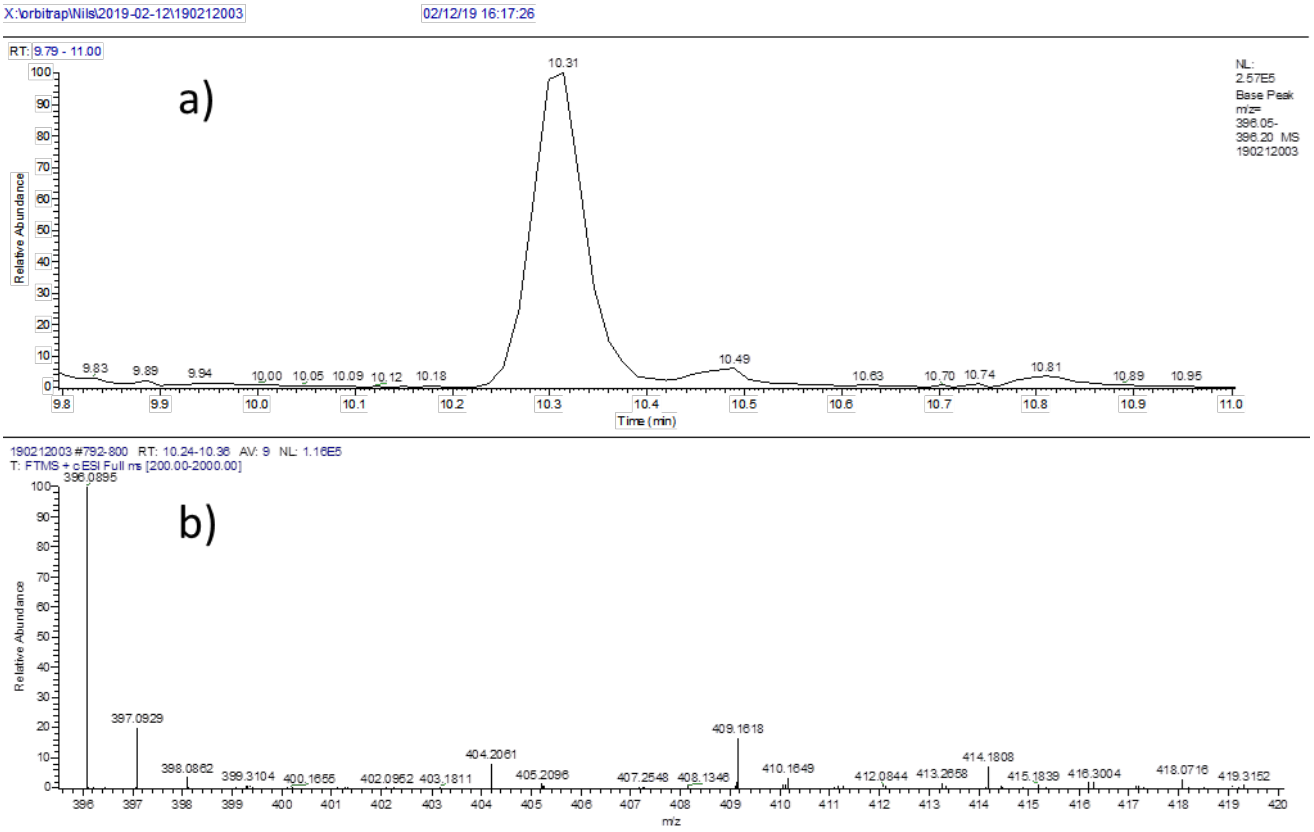


Figure S 70 HPLC Extracted ion chromatogram (Extracted mass 396.05-696.20) of *S. lividans* ΔYA6 1E5 b) mass of SEK43

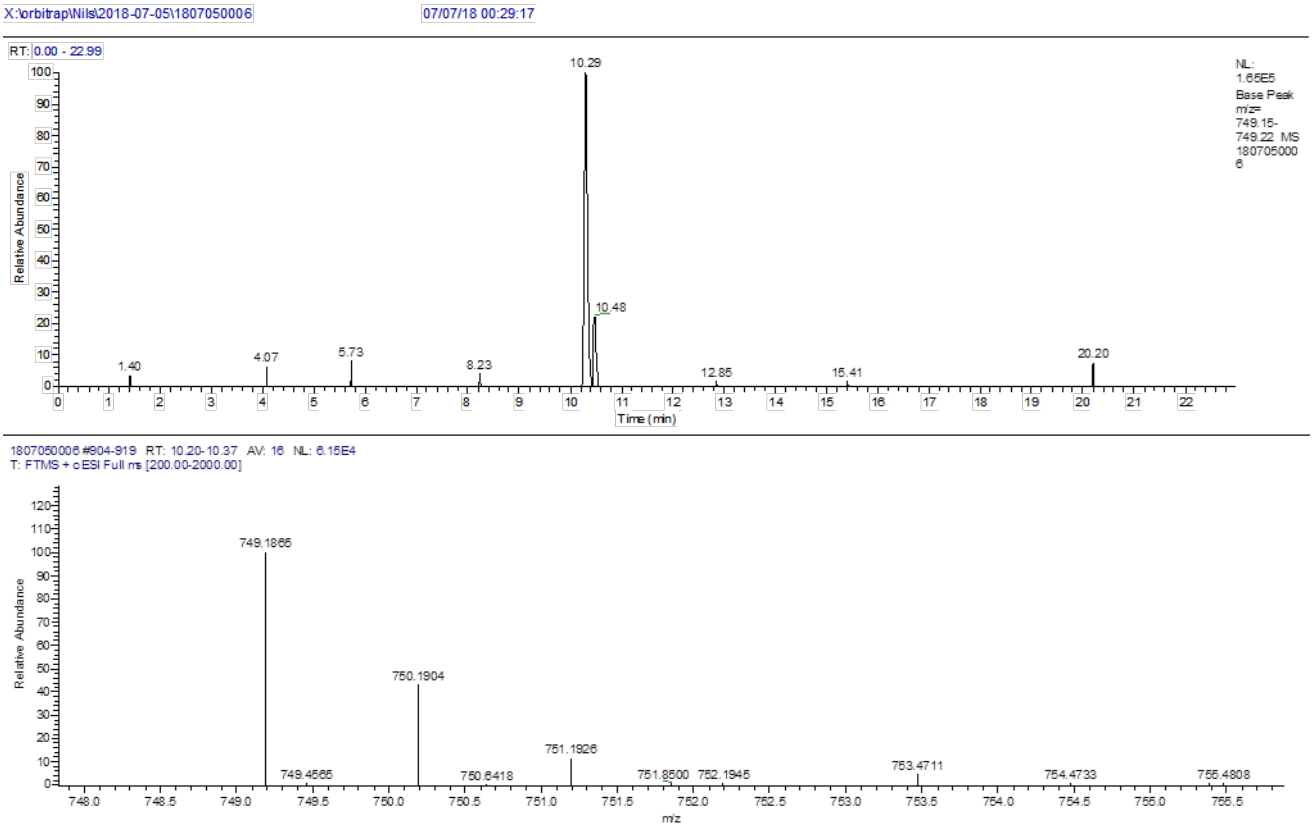


Figure S 71 HPLC Extracted ion chromatogram (Extracted mass 749.15-749.22) of *S. lividans* ΔYA6 1E5 b) mass of SEK87

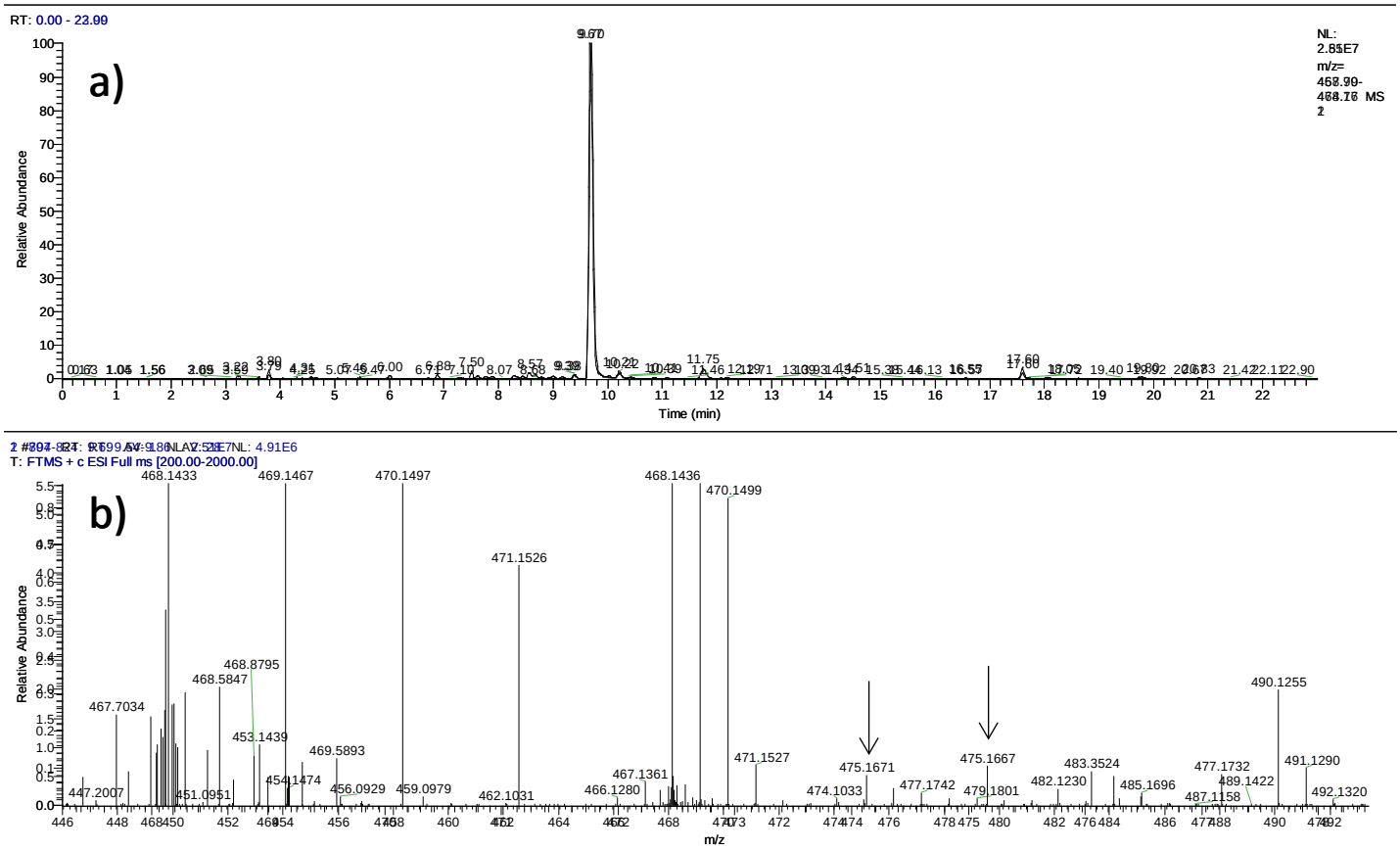


Figure S 72 HPLC-MS Extracted ion chromatogram (Extracted mass 468±0.5) of a) *S. lividans* ΔYA6_1E5 with 13C-9-15N-1-labelled L-tyrosine added to the medium b) MS-Chromatogram of a), incorporation of 13C-9-15N-1-labelled L-tyrosine visible; First repeat of a triplicate

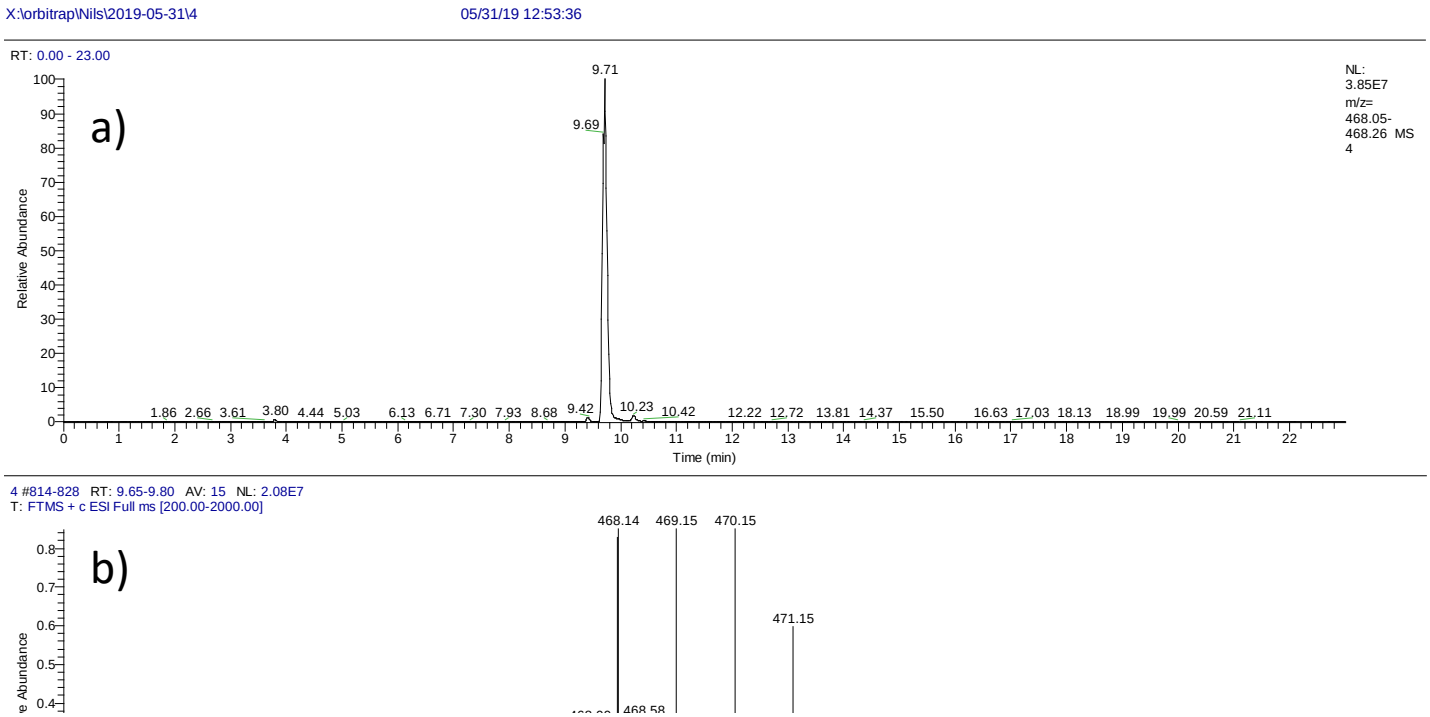


Figure S 73 HPLC-MS Extracted ion chromatogram (Extracted mass 468±0.5) of a) *S. lividans* ΔYA6_1E5 with 13C-9-15N-1-labelled L-tyrosine added to the medium b) MS-Chromatogram of a), incorporation of 13C-9-15N-1-labelled L-tyrosine visible; second repeat of a triplicate

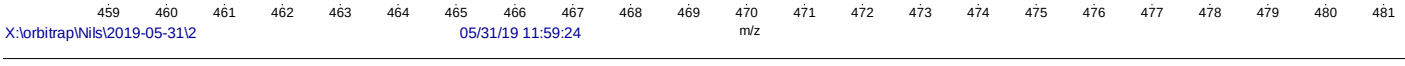


Figure S 75 HPLC-MS Extracted ion chromatogram (Extracted mass 468±0.5) of a) *S. lividans* ΔYA6_1E5 without 13C-9-15N-1-labelled L-tyrosine added to the medium b) MS-Chromatogram of a) no incorporation of 13C-9-15N-1-labelled L-tyrosine visible; first repeat of a triplicate

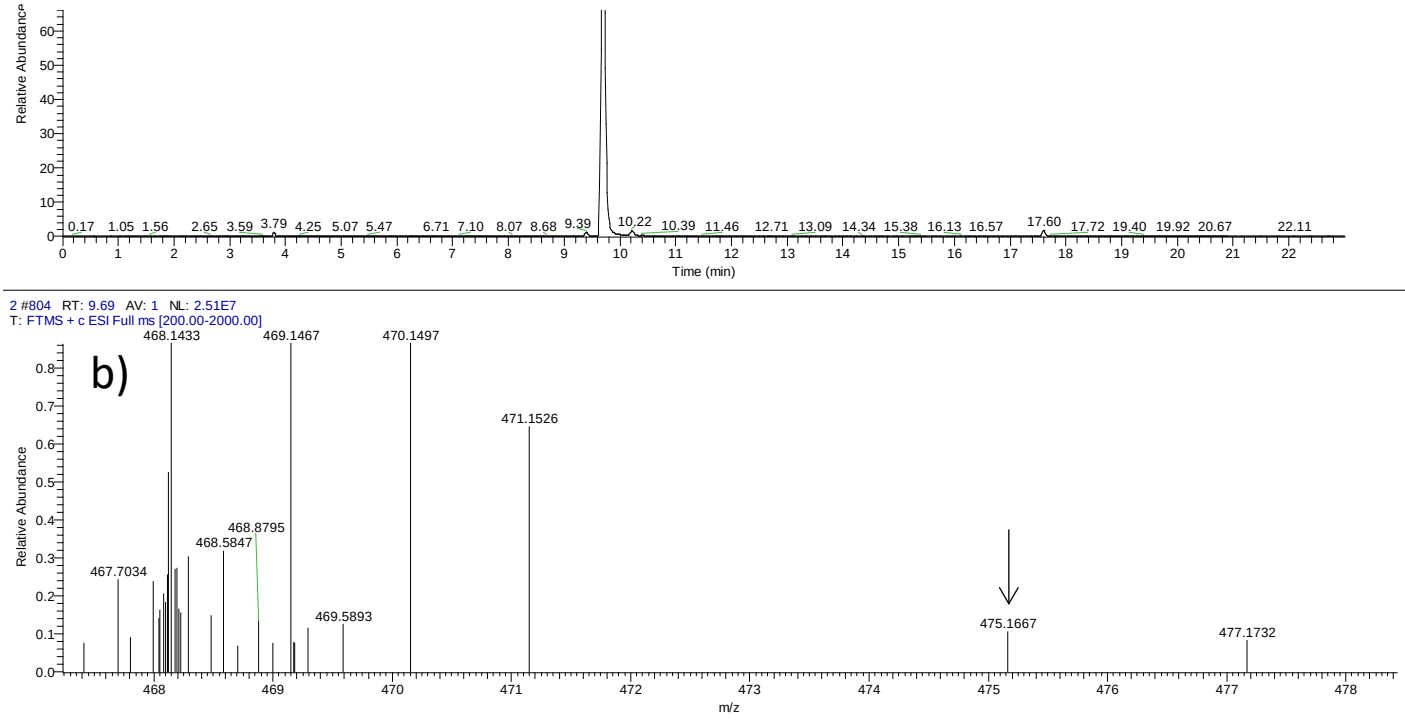


Figure S 74 HPLC-MS Extracted ion chromatogram (Extracted mass 468±0.5) of a) *S. lividans* ΔYA6_1E5 with 13C-9-15N-1-labelled L-tyrosine added to the medium b) MS-Chromatogram of a), incorporation of 13C-9-15N-1-labelled L-tyrosine visible; Third repeat of a triplicate

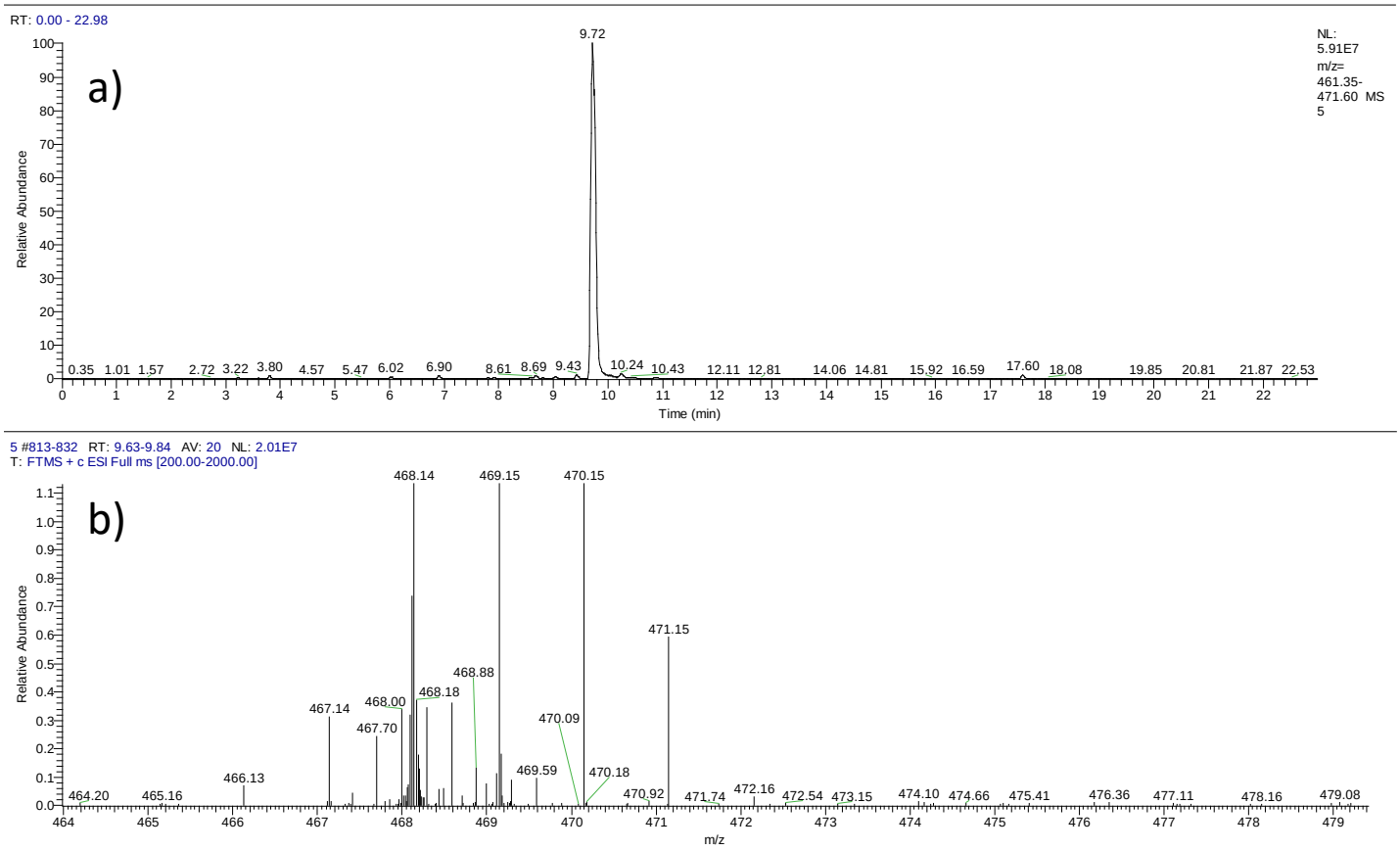


Figure S 76 HPLC-MS Extracted ion chromatogram (Extracted mass 468±0.5) of a) *S. lividans* ΔYA6_1E5 without 13C-9-15N-1-labelled L-tyrosine added to the medium b) MS-Chromatogram of a) no incorporation of 13C-9-15N-1-labelled L-tyrosine visible; second repeat of a triplicate

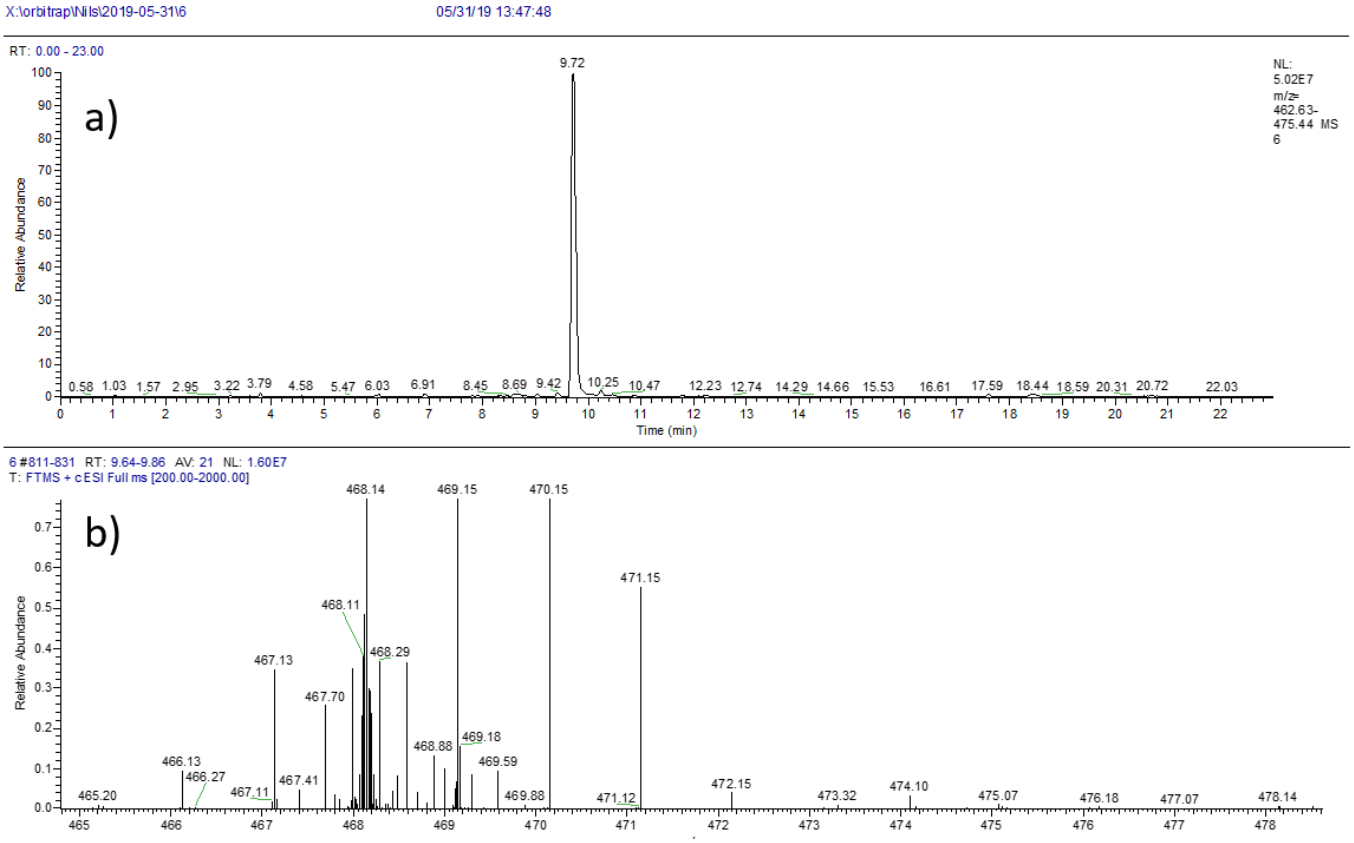


Figure S 77 HPLC-MS Extracted ion chromatogram (Extracted mass 468±0.5) of a) *S. lividans* ΔYA6_1E5 without 13C-9-15N-1-labelled L-tyrosine added to the medium b) MS-Chromatogram of a) no incorporation of 13C-9-15N-1-labelled L-tyrosine visible; third repeat of a triplicate

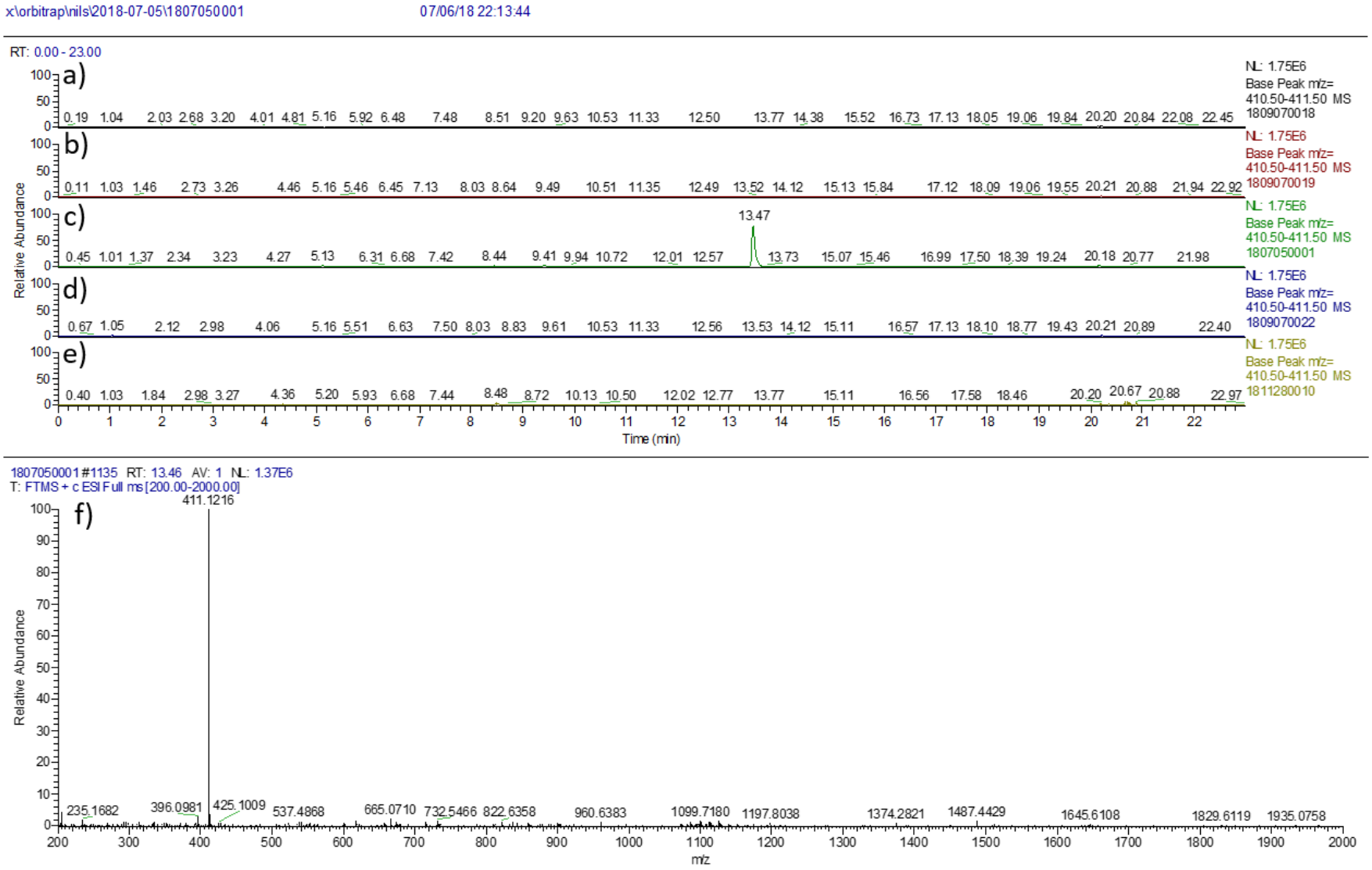


Figure S 78 HPLC-MS Extracted ion chromatogram (Extracted mass 411±0.5; corresponding mass to (3)) of a) *S. lividans* ΔYA6; b) *S. lividans* ΔYA6_1E5; c) *S. lividans* ΔYA6Δ*penA*; d) *S. lividans* ΔYA6Δ*penC; e) S. lividans* ΔYA6Δ*penD;* peak visible in c); f) ESI Full MS chromatogram of the peak visible in c) R_t=13.47

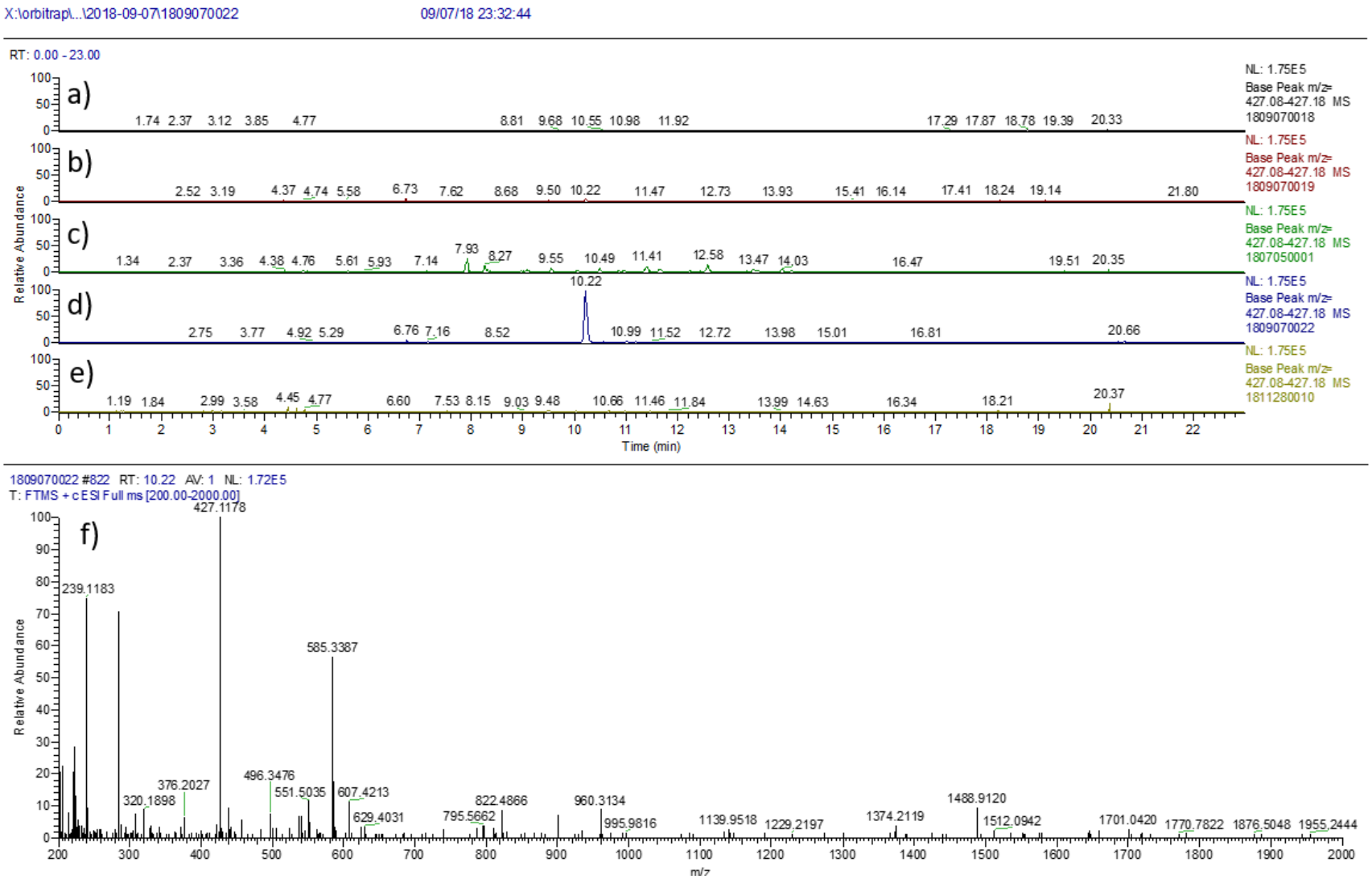


Figure S 79 HPLC-MS Extracted ion chromatogram (Extracted mass 427.09-427.18; corresponding mass to (4)) of a) *S. lividans* ΔYA6; b) *S. lividans* ΔYA6_1E5; c) *S. lividans* ΔYA6Δ*penA*; d) *S. lividans* ΔYA6Δ*penC; e) S. lividans* ΔYA6Δ*penD;* peak visible in d); f) ESI full MS of the peak visible in d) (R_t=10.22)

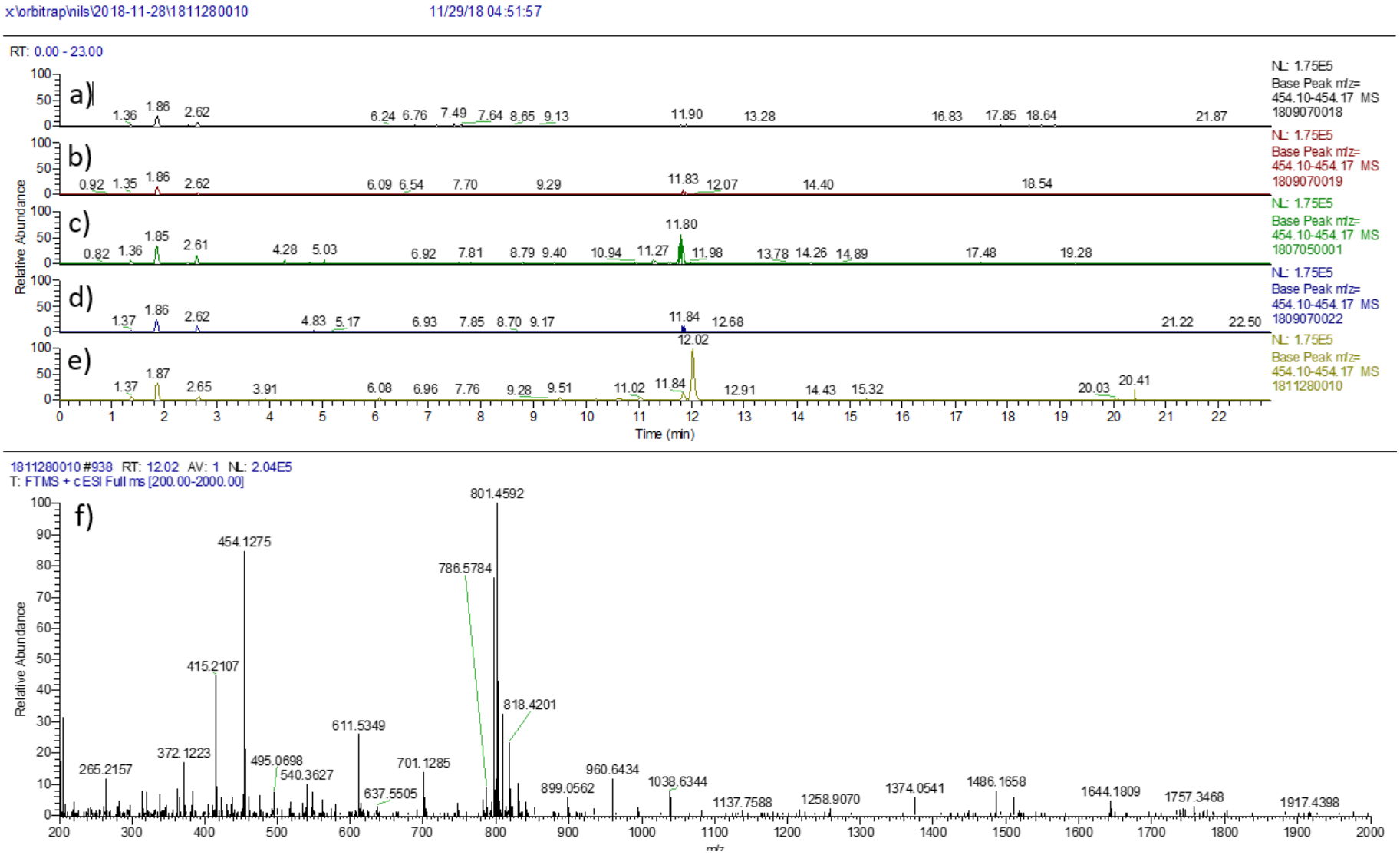


Figure S 80 HPLC-MS Extracted ion chromatogram (Extracted mass 454.10-454.17; corresponding mass to (5)) of a) *S. lividans* ΔYA6; b) *S. lividans* ΔYA6_1E5; c) *S. lividans* ΔYA6Δ*penA*; d) *S. lividans* ΔYA6Δ*penC; e) S. lividans* ΔYA6Δ*penD;* peak visible in e); f) f) ESI full MS of the peak visible in e) (R_t=12.02)

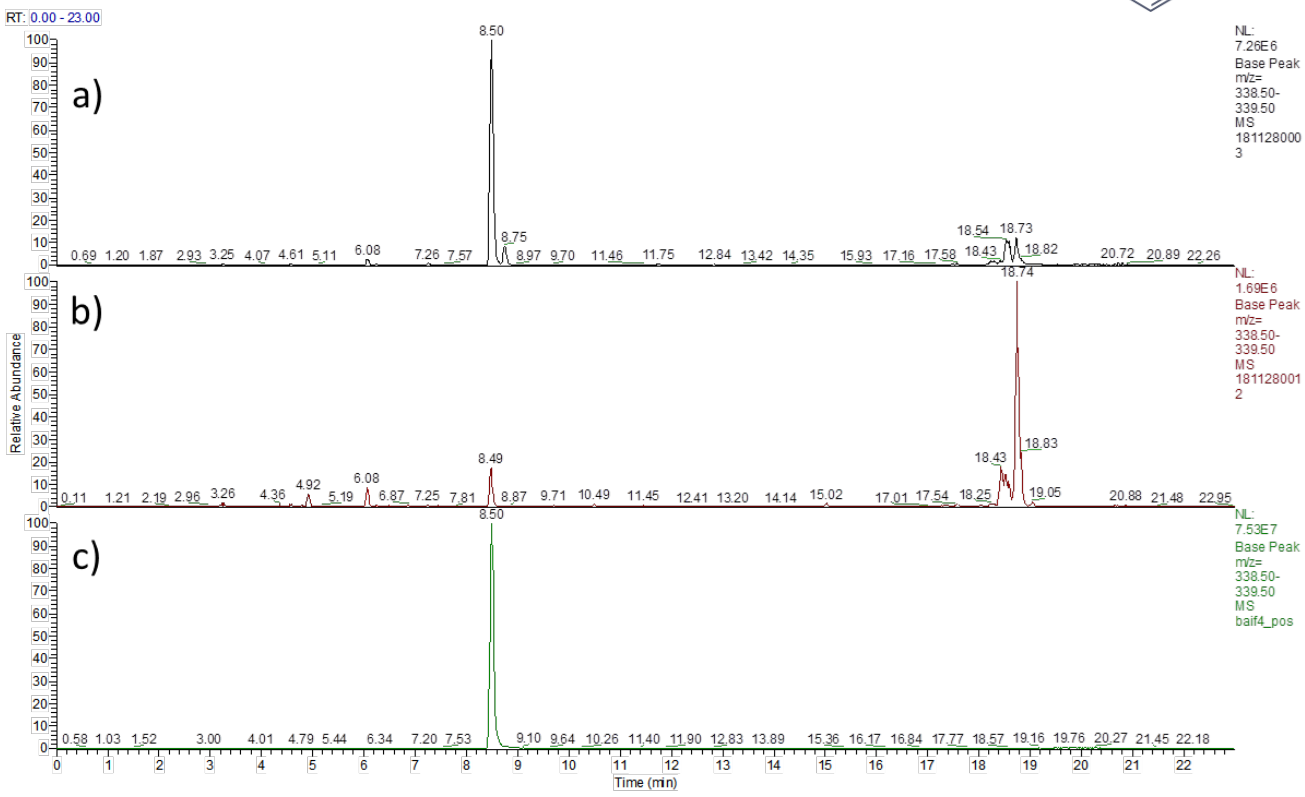


Figure S 81 HPLC-MS Extracted ion chromatogram (Extracted mass 339±0.5, Rabelomycin) of a) *S. lividans* ΔYA6 1E5, b) *S. lividans* ΔYA6 1E5ΔpenE c) pure rabelomycin as external standard

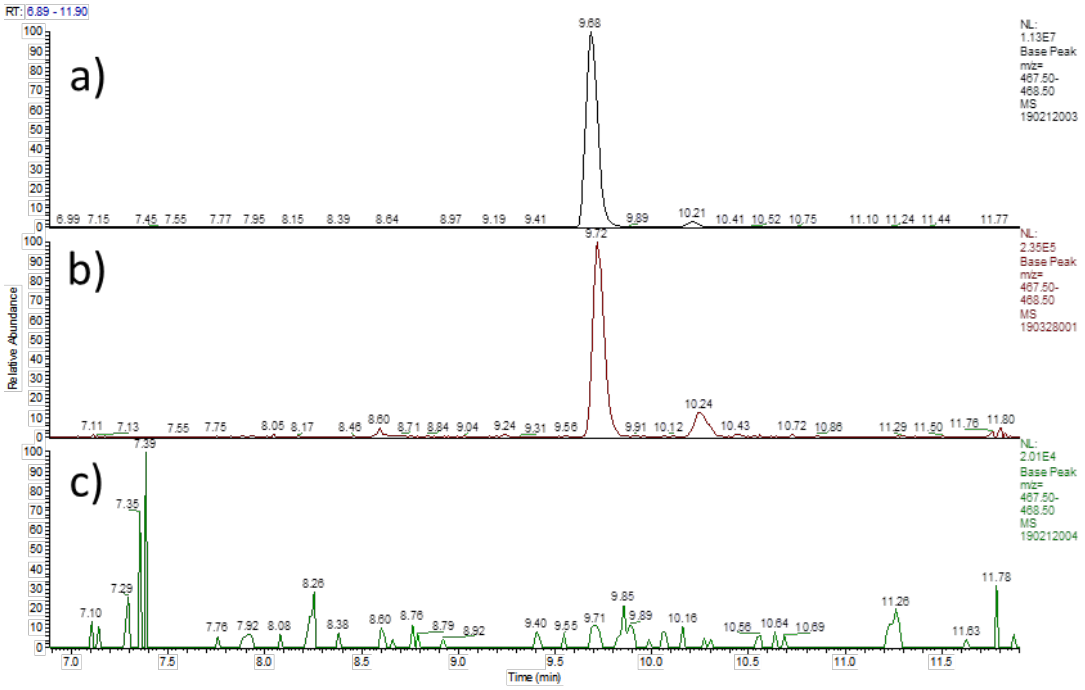


Figure S 82 HPLC-MS Extracted ion chromatogram (Extracted mass 468±0.5, (1)) of a) *S. lividans* ΔYA6 1E5, b) *S. lividans* ΔYA6 1E5ΔpenR1 and c) *S. lividans* ΔYA6 1E5ΔpenR2

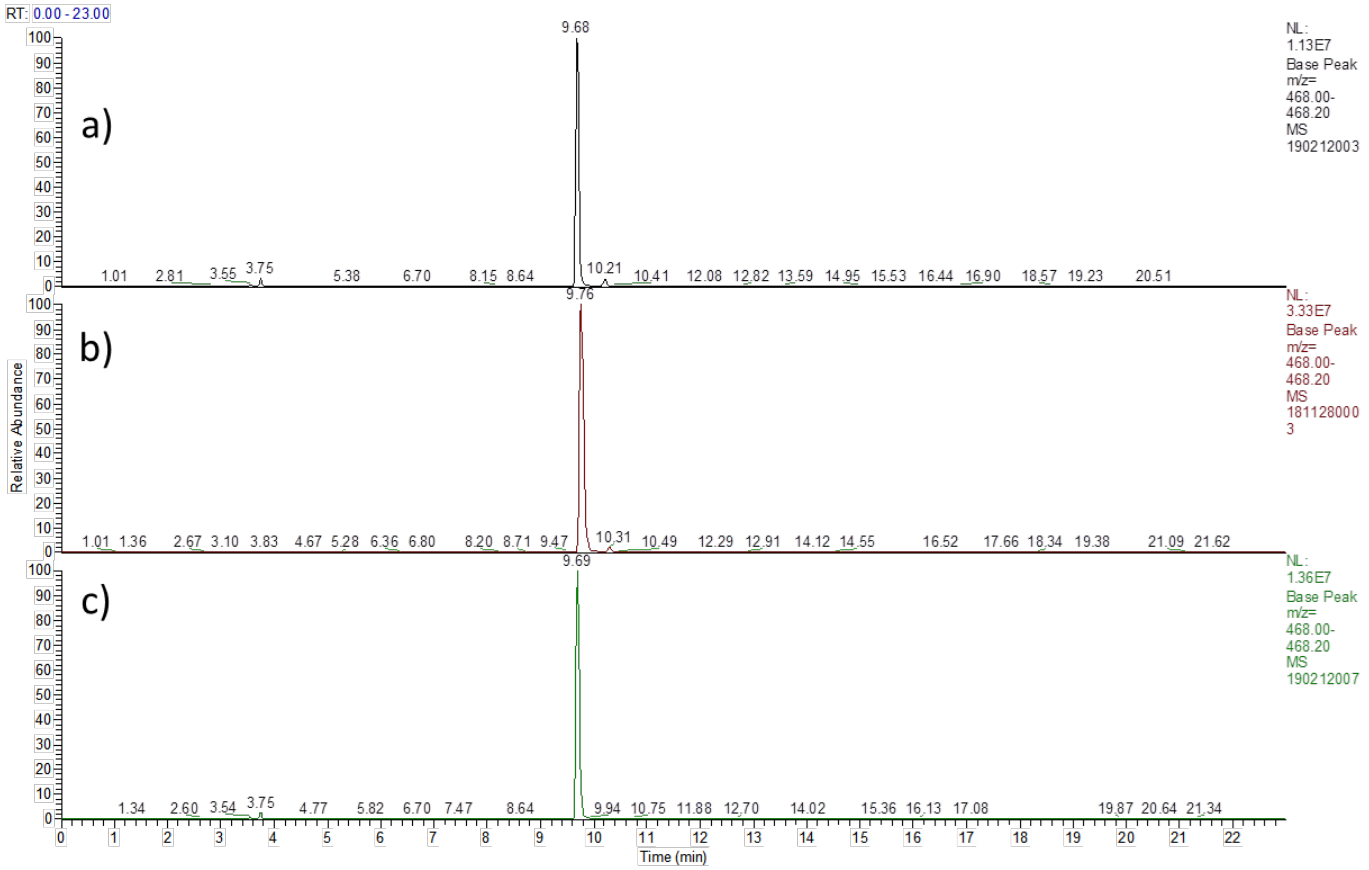


Figure S 83 HPLC-MS Extracted ion chromatogram (Extracted mass 468±0.5 (1)) of a) *S. lividans* ΔYA6 1E5, b) *S. lividans* ΔYA6 A3_penR1 1E5 and c) *S. lividans* ΔYA6 A3_penR2 1E5

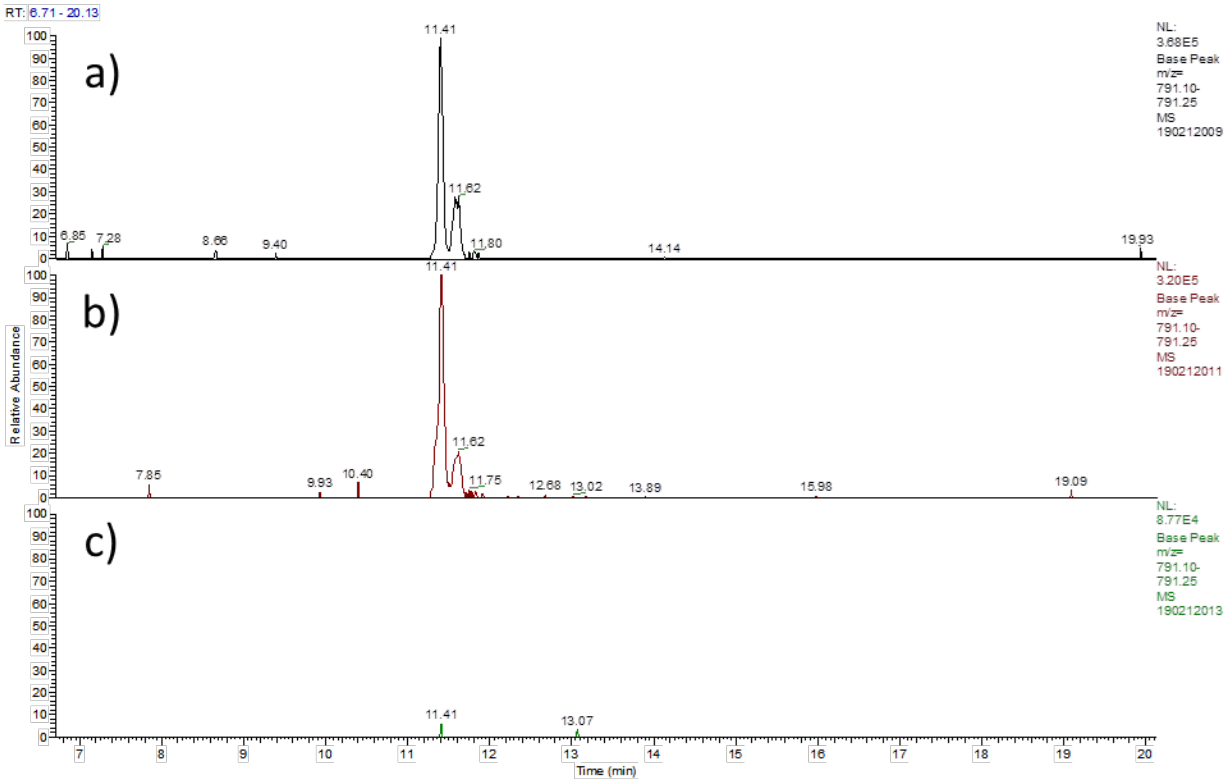


Figure S 84 HPLC-MS Extracted ion chromatogram (Extracted mass 791.10-791.25 (2)) of a) *S. lividans* ΔYA6 1E5, b) *S. lividans* ΔYA6 A3_penR1 1E5 and c) *S. lividans* ΔYA6 A3_penR2 1E5

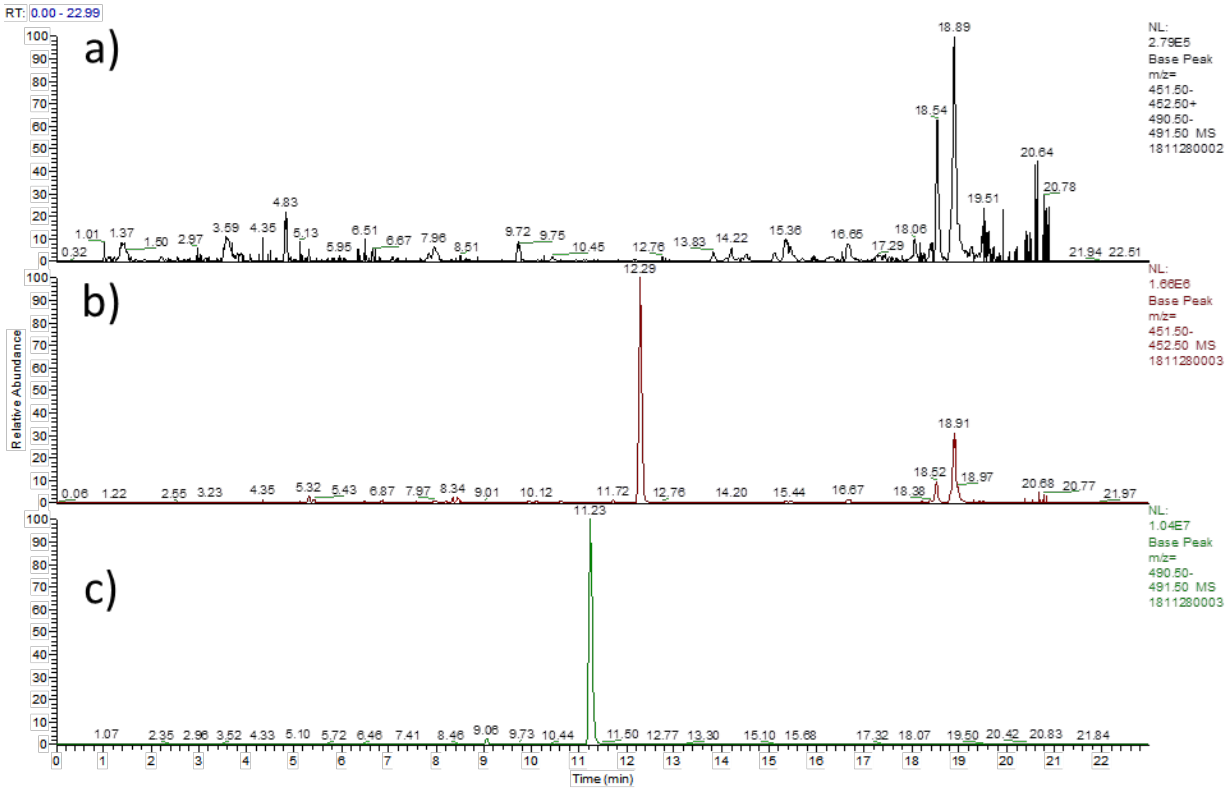


Figure S 85 HPLC-MS Extracted ion chromatogram a) *S. lividans* ΔYA6 A3_penR1 (Extracted mass 491±0.5 (6) and 452±0.5 (7)), b) *S. lividans* ΔYA6 A3_penR1 1E5 (Extracted mass 452±0.5 (7)) c) *S. lividans* ΔYA6 A3_penR1 1E5 (Extracted mass 491±0.5 (6))

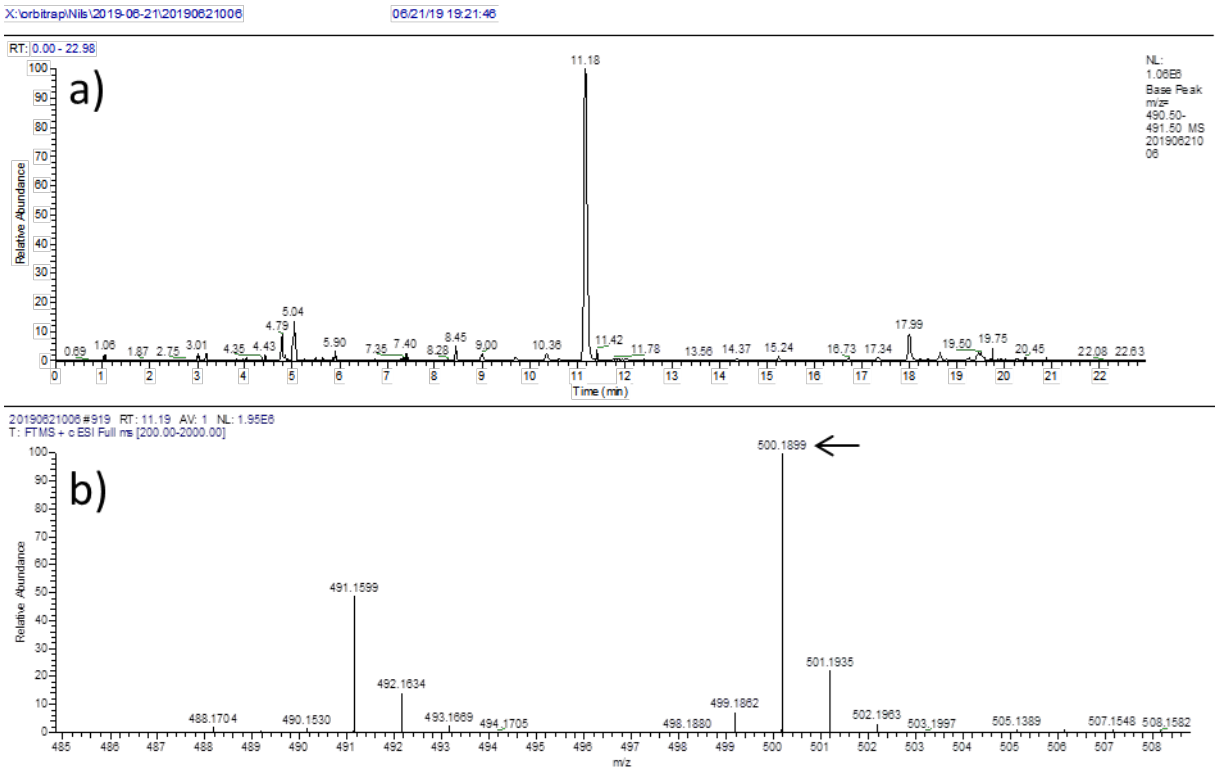


Figure S 86 HPLC-MS Extracted ion chromatogram (Extracted mass 491±0.5) of a) *S. lividans* ΔYA6_A3_penR1 1E5 with L-tryptophan 13C-11 added to the medium b) MS-Chromatogram of a), incorporation of L-tryptophan 13C-11 visible

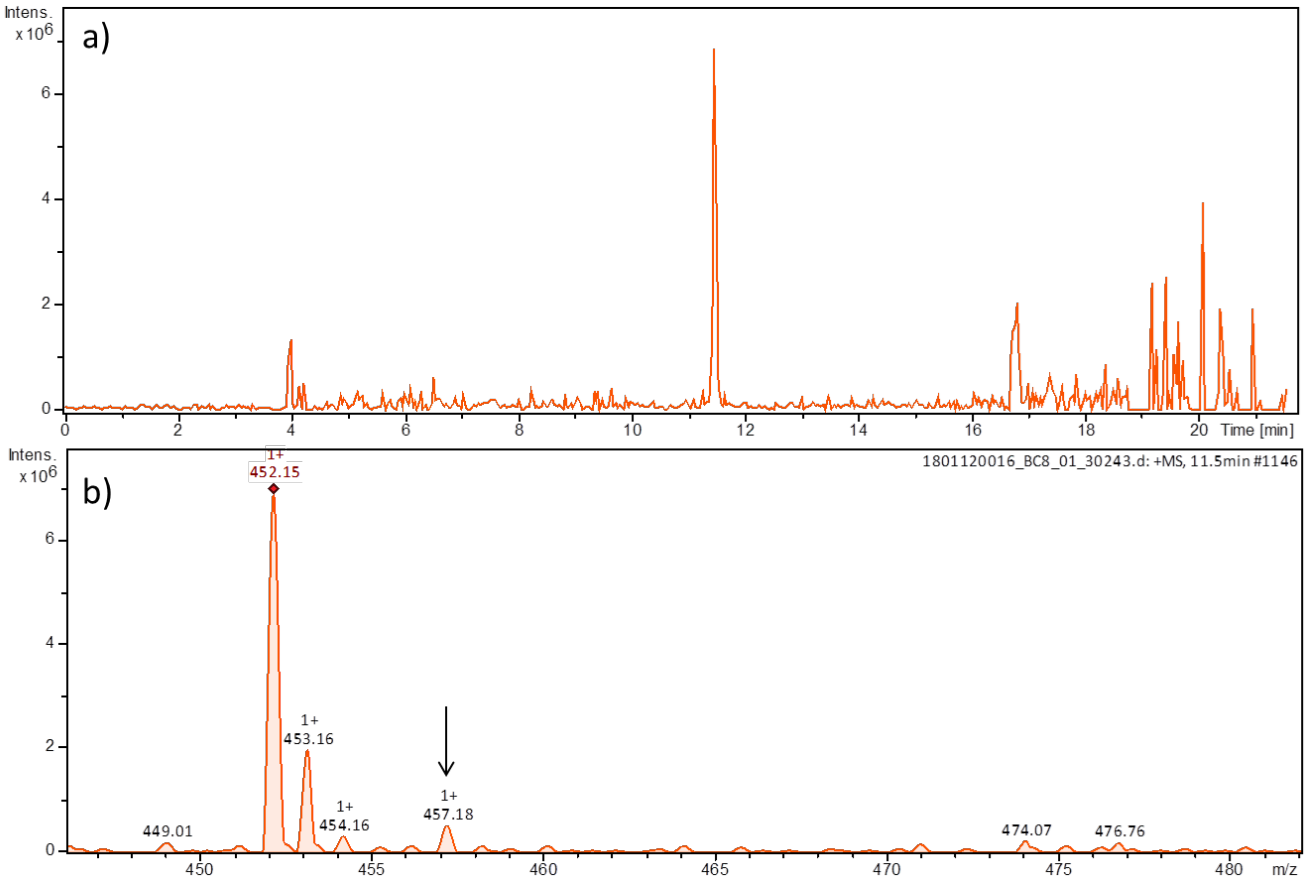


Figure S 87 HPLC-MS Extracted ion chromatogram (Extracted mass 452±0.5) of a) *S. lividans* ΔYA6_A3_penR1 1E5 with 2D-5-labelled L-Phenylalanine added to the medium b) MS-Chromatogram of a), incorporation of 2D-5-labelled L-Phenylalanine visible

1. (1) Chater, K. F.; Wilde, L. C. *J Gen Microbiol* 1980, *116*, 323-334.
2. (2) Labeda, D. P.; Lechevalier, M. P. *International Journal of Systematic and Evolutionary Microbiology* 1989, *39*, 420-423.
3. (3) Wang, J.; Sarov, M.; Rientjes, J.; Fu, J.; Hollak, H.; Kranz, H.; Xie, W.; Stewart, A. F.; Zhang, Y. *Mol Biotechnol* 2006, *32*, 43-53.
4. (4) Kieser, T.; Bibb, M. J.; Buttner, M. J.; Chater, K. F.; Hopwood, D. A. 2010.
5. (5) Strochlic, T. I.; Viaud, J.; Rennefahrt, U. E.; Anastassiadis, T.; Peterson, J. R. *Mol Cell* 2010, *40*, 493-500.
6. (6) Herrmann, S.; Siegl, T.; Luzhetska, M.; Petzke, L.; Jilg, C.; Welle, E.; Erb, A.; Leadlay, P. F.; Bechthold, A.; Luzhetskyy, A. *Appl Environ Microbiol* 2012, *78*, 1804-1812.
7. (7) Myronovskyi, M.; Rosenkranzer, B.; Luzhetskyy, A. *Appl Microbiol Biotechnol* 2014, *98*, 4557-4570.
8.