

Histone Deacetylase Inhibitors as a Tool to Up-regulate New Fungal Biosynthetic Products - Isolation of EGM-556, a Cyclodepsipeptide, from *Microascus* sp.

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General Experimental Procedures.

Optical rotations were obtained on a digital polarimeter (Jasco P-2000). NMR spectra were recorded in DMSO-*d*₆ on a 500 MHz Unity Inova (125 MHz for carbon spectra) and a Unity Inova 600 MHz for all proton-detected experiments. LC-ELSD-MS analyses were carried out on a 4.6 x 150mm luna 5 μ column (Phenomenex) equipped with S.e.d.e.r.e (France) Sedex 55 ELSD detector, a Waters 996 Photodiode Array Detector and a Mariner Biospectrometry Workstation mass spectrometer. To obtain high-resolution analysis for accurate mass determination of EGM-556, two references were used for calibration purposes: Pravastatin Sodium (Mw 446.5 Da) and Etoposide (Mw 588.6 Da). To determine the accurate mass of acyclic methyl ester **2**, Pravastatin Sodium and Rifampicin (Mw 822.9 Da) were used as calibration standards.

Table S1 shows the fungal strains used for epigenetic manipulation experiments. Each strain was cultured 166 mL Dzapek-Dox/artificial seawater media in the presence of 10⁻⁴ M SAHA, which was added after 24 hrs of growth, as well as grown in a control culture treated with 200 μ L DMSO only. Cultures were allowed to grow for 17 subsequent days. Culture broths, including the mycelia were homogenized and exhaustively extracted with 200 mL ethyl acetate (3 times) and evaporated to dryness. The crude extracts were analyzed by LC-ELSD-MS at 5mg/ml.

The crude extract of fungal strain 098059A was taken to semi-preparative reversed phase C18 HPLC using 50% acetonitrile in water as eluent. Under these conditions EGM-556 eludes at ~30 min.

Table S1. Fungal strains used in epigenetic experiments

Entry #	Strain #	Strain ID	Observations ^a
1	098008A		B
2	098046C	<i>Penicillium griseofulvum</i>	D ^c
3	098049A		B
4	098059A	<i>Microascus</i> sp.	C
5	098060A		A
6	098065A		A
7	098069A		B
8	098070A		B
9	098076A	identical/related to 098065A	A
10	098078A		B
11	098079A		A
12	098079B	identical/related to 098065A	A

^a Legend to Observations: A: No significant change in metabolome. B: Significant change in metabolome, Mw < 300Da. C: New metabolites observed, Mw > 300Da. D: Known metabolites disappeared. ^b Identification based on chemistry produced: griseofulvin, dechlorogriseofulvin. ^c Known metabolites: griseofulvin, dechlorogriseofulvin.

Identification of Fungal Strain 098059A.

The fungal strain was identified by means of morphological studies and genetic studies. *Microascus* species are usually identified based upon their sexual fruiting structure (teleomorph). Strain 098059A failed to form sexual structures under laboratory conditions and was first described as a *Scopulariopsis* anamorph (asexual form). However, genetic identification using isolated genomic DNA and PCR using three rDNA primer sets (U1/U2 (28S subunit), ITS-1/ITS-4 (ITS region) and NL-1/NL-4 (D1/D2 region)) indicated that strain 098059A was most closely identified to *Microascus* sp, in particular *M. trigonosporous*. The following primers were used for PCR:

U1: 5'-GTGAAATTGTTGAAAGGGAA-3'

U2: 5'-GACTCCTTGGTCCGTGTT-3'

ITS-1: 5'-TCCGTAGGTGAACCTGCGG-3'

ITS-4: 5'-TCCTCCGCTTATTGATATGC-3'

NL-4: 5'-GGTCCGTGTTTCAAGACGG-3'

NL-1: 5'-GCATATCAATAAGCGGAGGAAAAG-3'

The following sequences were obtained by PCR:

U1/U2, forward:

ATGCGCCGGTCCGANAGCCGTCGCTTGC GGCGGTGCACTCCGGCGGGCTCGGGCCAGCATCAGTTC
NCCTCGGGGGGAGAAAGGCGGCAGGAATGTGGCTCTTCGGAGTGTTATAGCCTGCCGTATAATACCC
CCGGCGGACTGAGGACCGCGCGTATGCAAGGATGCTGGCGTAATGGTCGTCAGCGACCCGTCTTG
AAACACGGACCCAAGGAGTCAA

U1/U2, reverse:

GCCGCATCCTTGCATACGCGCGGTCTCAGTTCGCCCGGGGGTATTATACGGCAGGCTATANCACTC
CAAGAGCCACATTCCTGCCGCTTTCTCCCCCGAGGCGAACTGATGCTGGCCCCGAGCCCGCCGGA
GTGCACCGCCGCAAGCGACGGCTGATCCGACGGGCGCGAGTCTGGTCGCAAGCGCTTCCCTTTNAA
CAATTTCAAA

ITS-1/ITS-4 (ITS region):

TCCGTAGGAACCAGCGGAGGGATCATTACGGAAGTTACTCTTCATACCCTTTTGTGAACACTACCCC
ATTGCCGCGGTTGCCTCGGCGGGGAGGTCTGGGGGGTTGCGCTCCGGCGCTCCCCCGGGCCCCC
GCCGGCCGCGCCGAACTCTTCATTTGCAAAGCGGACTGTACGATCTGATTATATCTTGAAAAACAAG
TCAAACCTTTCAACAACGGATCTCTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGT
AATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCCGGCAGCAATCTG
CCGGGCATGCCTGTCCGAGCGTCATTTCTGCCCTCGAGCGCGGTTCCGGCCCCTAGCGGGCCGGCCGC
CGCCCGGTGTTGGGGCGCTGCGGGCCCTCGTGCCCGCAGGCCCTGAAATGAAGTGGCGGTCCC GCC
GCGGCGCCCCCTGCGTAGTAGTAAAGCACCTCGCATCGGGTCCC GGCGGAGGCCAGCCGTGCAACC
TCTCTTTTGATGGTTTGACCTCGGATCAGGTAGGGTTACCCGCTGAACTTAAGCATATCAATAAGCGG
AGGA

NL-1/NL-4 (D1/D2 region):

GCATATCAATAAGCGGAGGAAAAGAAACCAACAGGGATTGCCCCAGTAACGGCGAGTGAAGCGGC
AACAGCTCAAATTTGAAATCTGGTCCCCCGCGGGGCCGAGTTGTAATTTGAAGAGGATGCTTCTG
GCAAGGTGCCGTCCGAGTTCCTTGAACGGGACGCCCGAGAGGGTGAGAGCCCCGTACGGTCGGA
CGCCGAGCCTCTGTGAAGCTCCTTCGACGAGTCGAGTAGTTTGGGAATGCTGCTCAAATGGGAGG
TAAACCCCTTCTAAAGCTAAATACCGGCCAGAGACCGATAGCGCACAAGTAGAGTGATCGAAAGAT
GAAAAGCACTTTGAAAAGAGAGTTAAAAAGCACGTGAAATTGTTGAAAGGGAAGCGCTTGCGACC
AGACTCGCGCCCGTCGGATCAGCCGTCGCTCGTCGGCGGCGCACTCCGGCGGGCTCGGGCCAGCAT
CAGTTCGCCTCGGGGGGAGAAAGGCGGCGGGAATGTGGCTCTACGGAGTGTTATAGCCCCGCCGCT
AATACCCCGGGCGGACTGAGGACCGCGCGTATGCAAGGATGCTGGCGTAATGGTCGTCAGCGACC
CGTCTTGAAACACGGACC

Table S2. BLASTn results of rDNA sequences obtained after genomic DNA extraction and PCR on fungal strain 098059A using indicated primers.

Primers Used: U1 and U2, forward sequence

Accession	Description	Max score	Total score	Query coverage	E value	Max ident
AF400878.1	Scopulariopsis croci large subunit ribosomal RNA gene, partial sequence	322	322	80.00%	6.00E-85	98.00%
AF400870.1	Microascus trigonosporus large subunit ribosomal RNA gene, partial sequence	337	337	91.00%	2.00E-89	96.00%
AF400853.1	Kernia hyalina large subunit ribosomal RNA gene, partial sequence	337	337	91.00%	2.00E-89	96.00%
DQ470958.1	Microascus trigonosporus isolate AFTOL-ID 914 28S large subunit ribosomal RNA gene, partial sequence	357	357	98.00%	2.00E-95	95.00%
U26854.1	Scopulariopsis brumptii isolate 2-89 28S rRNA gene, partial sequence	315	315	89.00%	9.00E-83	95.00%
AF400864.1	Microascus griseus large subunit ribosomal RNA gene, partial sequence	326	326	93.00%	4.00E-86	94.00%
AF400881.1	Scopulariopsis hibernica large subunit ribosomal RNA gene, partial sequence	324	324	93.00%	2.00E-85	94.00%
AF400859.1	Microascus cinereus large subunit ribosomal RNA gene, partial sequence	324	324	92.00%	2.00E-85	94.00%
AF400867.1	Microascus senegalensis large subunit ribosomal RNA gene, partial sequence	320	320	92.00%	2.00E-84	94.00%
AB566305.1	Microascus sp. IFM 59326 gene for large subunit ribosomal RNA, partial sequence, strain: IFM 59326	322	322	94.00%	6.00E-85	94.00%

Primers Used: U-1 and U-2, reverse sequence

Accession	Description	Max score	Total score	Query coverage	E value	Max ident
AF400878.1	Scopulariopsis croci large subunit ribosomal RNA gene, partial sequence	363	363	99.00%	3.00E-97	97.00%
AF400870.1	Microascus trigonosporus large subunit ribosomal RNA gene, partial sequence	337	337	99.00%	2.00E-89	95.00%
AF400853.1	Kernia hyalina large subunit ribosomal RNA gene, partial sequence	337	337	99.00%	2.00E-89	95.00%
DQ470958.1	Microascus trigonosporus isolate AFTOL-ID 914 28S large subunit ribosomal RNA gene, partial sequence	333	333	99.00%	2.00E-88	94.00%
AF400859.1	Microascus cinereus large subunit ribosomal RNA gene, partial sequence	320	320	99.00%	2.00E-84	93.00%
AF400867.1	Microascus senegalensis large subunit ribosomal RNA gene, partial sequence	318	318	99.00%	7.00E-84	93.00%
U26854.1	Scopulariopsis brumptii isolate 2-89 28S rRNA gene, partial sequence	272	272	85.00%	5.00E-70	93.00%
AF400864.1	Microascus griseus large subunit ribosomal RNA gene, partial sequence	316	316	99.00%	2.00E-83	93.00%
U47835.1	Microascus trigonosporus large subunit ribosomal RNA gene, partial sequence	313	313	99.00%	3.00E-82	92.00%
AB566305.1	Microascus sp. IFM 59326 gene for large subunit ribosomal RNA, partial sequence, strain: IFM 59326	309	309	99.00%	4.00E-81	92.00%

Primers Used: ITS-1 and ITS-4

Accession	Description	Max score	Total score	Query coverage	E value	Max ident
GQ120995.1	Fungal endophyte isolate 282C 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	1077	1077	98.00%	0.00E+00	99.00%
DQ491513.1	Microascus trigonosporus isolate AFTOL-ID 914 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence	872	872	83.00%	0.00E+00	97.00%

AM774156.1	Microascus trigonosporus var. trigonosporus 18S rRNA gene (partial), ITS1, 5.8S rRNA gene, ITS2 and 28S rRNA gene (partial), strain CBS 665.71	983	983	96.00%	0.00E+00	97.00%
FJ713076.1	Microascus trigonosporus var. trigonosporus isolate R9(9) internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence	959	959	94.00%	0.00E+00	96.00%
FJ713095.1	Scopulariopsis chartarum isolate LB3A internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence	953	953	93.00%	0.00E+00	96.00%
AY625066.1	Scopulariopsis chartarum strain ATCC 16279 18S ribosomal RNA gene, partial sequence; int transcr spacer 1, 5.8S ribosomal RNA gene, and int transcr spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	904	904	95.00%	0.00E+00	94.00%
FJ914669.1	Graphium dubautiae strain HSAUP042788 18S ribosomal RNA gene, partial sequence; int transcr spacer 1, 5.8S ribosomal RNA gene, and int transcr spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	937	937	100.00%	0.00E+00	94.00%
GU056013.1	Uncultured Hypocreales isolate 31 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	915	915	98.00%	0.00E+00	94.00%
AM774160.1	Scopulariopsis chartarum ITS1, 5.8S rRNA gene, ITS2 and 28S rRNA gene (partial), strain FMR 3997	856	856	93.00%	0.00E+00	93.00%
FJ946484.1	Scopulariopsis hibernica 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	898	898	100.00%	0.00E+00	93.00%

Primers Used: NL-1 and NL-4

Accession	Description	Max score	Total score	Query coverage	E value	Max ident
AF275539.1	Microascus cirrosus large subunit ribosomal RNA gene, partial sequence	1050	1050	94.00%	0.00E+00	99.00%
U47835.1	Microascus trigonosporus large subunit ribosomal RNA gene, partial sequence	1083	1083	99.00%	0.00E+00	98.00%
AB566305.1	Microascus sp. IFM 59326 gene for large subunit ribosomal RNA, partial sequence, strain: IFM 59326	1040	1040	96.00%	0.00E+00	98.00%
AB470531.1	Microascus sp. 795_2_CY01 gene for 28S rRNA, partial sequence	953	953	91.00%	0.00E+00	97.00%
AF275541.1	Pseudallescheria africana large subunit ribosomal RNA gene, partial sequence	942	942	94.00%	0.00E+00	96.00%
AB363754.1	Scopulariopsis brevicaulis gene for large subunit rRNA, partial sequence, strain: IFM 54315	998	998	100.00%	0.00E+00	96.00%
DQ470958.1	Microascus trigonosporus isolate AFTOL-ID 914 28S large subunit ribosomal RNA gene, partial sequence	880	880	85.00%	0.00E+00	96.00%
AY494711.1	Scopulariopsis flava large subunit ribosomal RNA gene, partial sequence	867	867	87.00%	0.00E+00	96.00%
AB297478.1	Scopulariopsis brevicaulis gene for large subunit ribosomal RNA, partial sequence	992	992	100.00%	0.00E+00	96.00%

Figure S1. ^1H NMR spectrum of EGM-556 (**1**), (600 MHz, $\text{DMSO-}d_6$)

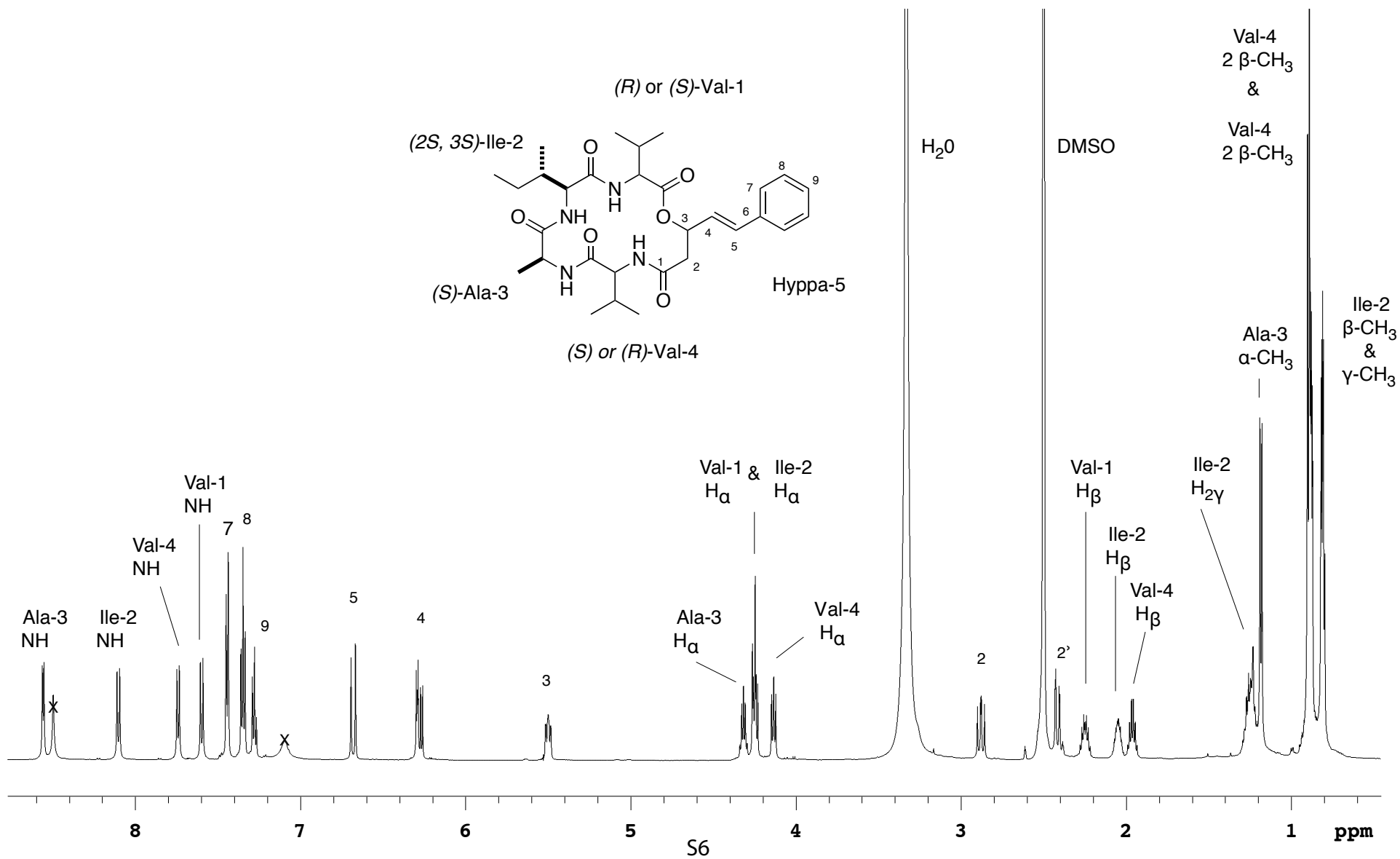


Figure S1a . Expanded regions of the ^1H NMR spectrum of EGM-556 (**1**), (600 MHz, $\text{DMSO-}d_6$)

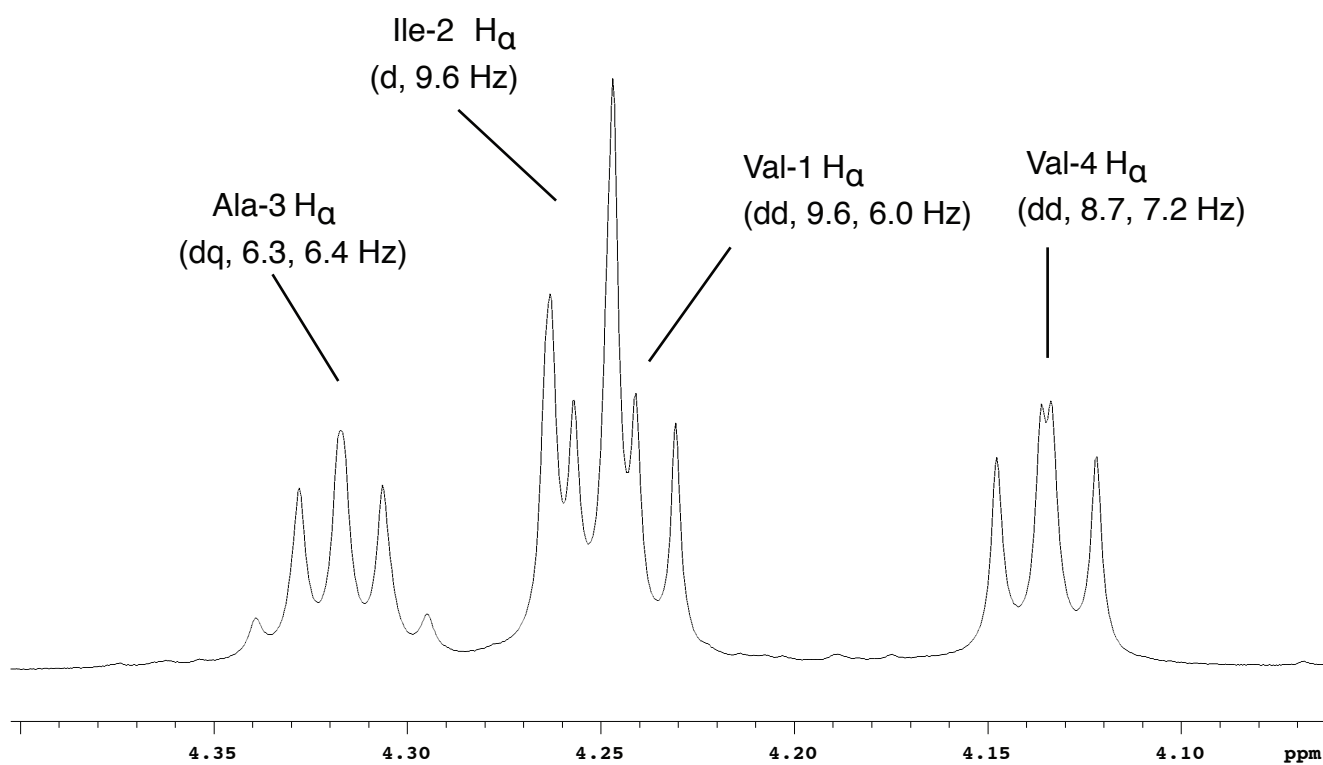
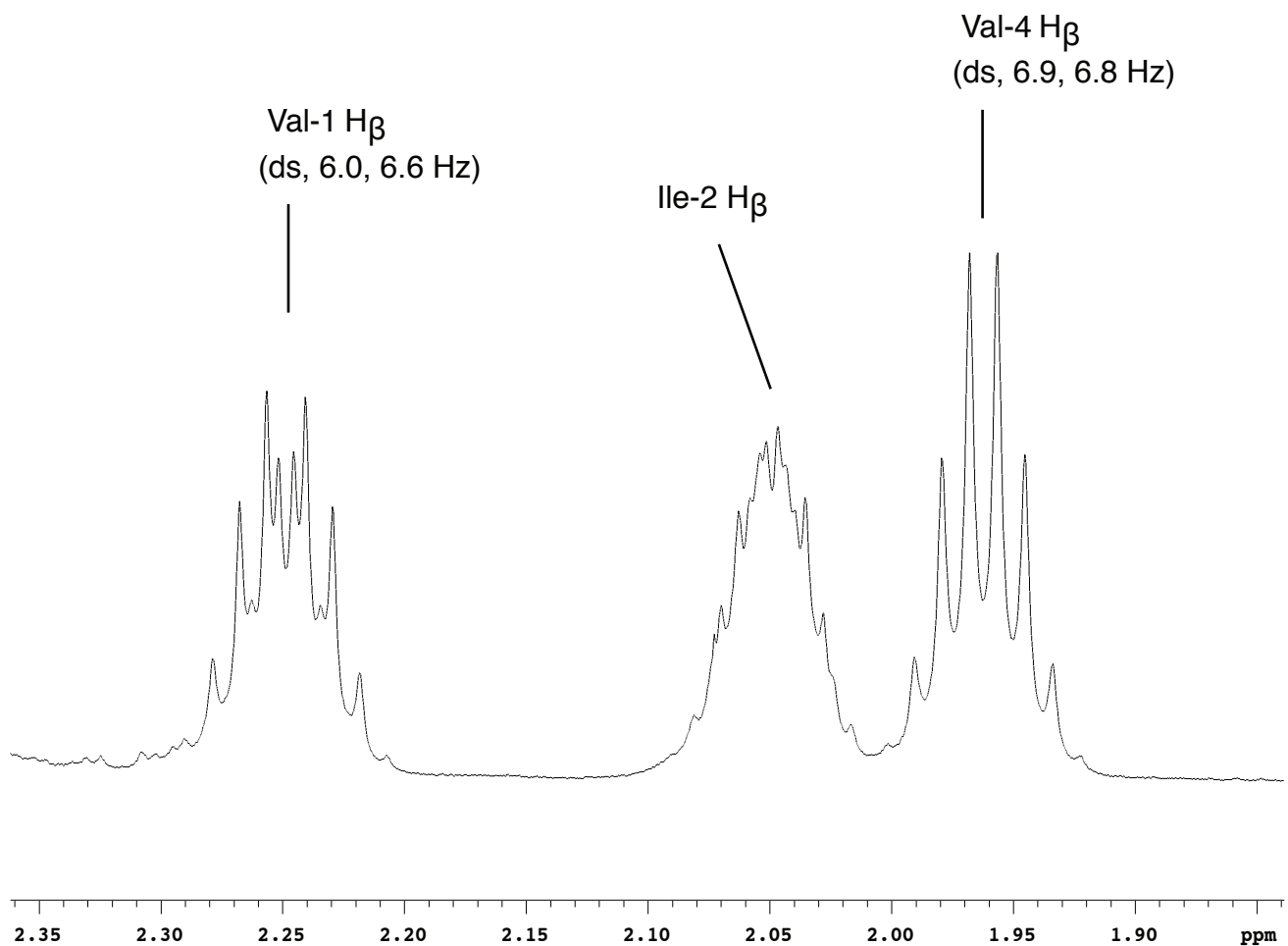


Figure S2. ^{13}C NMR spectrum of EGM-556 (**1**), (150 MHz, $\text{DMSO-}d_6$)

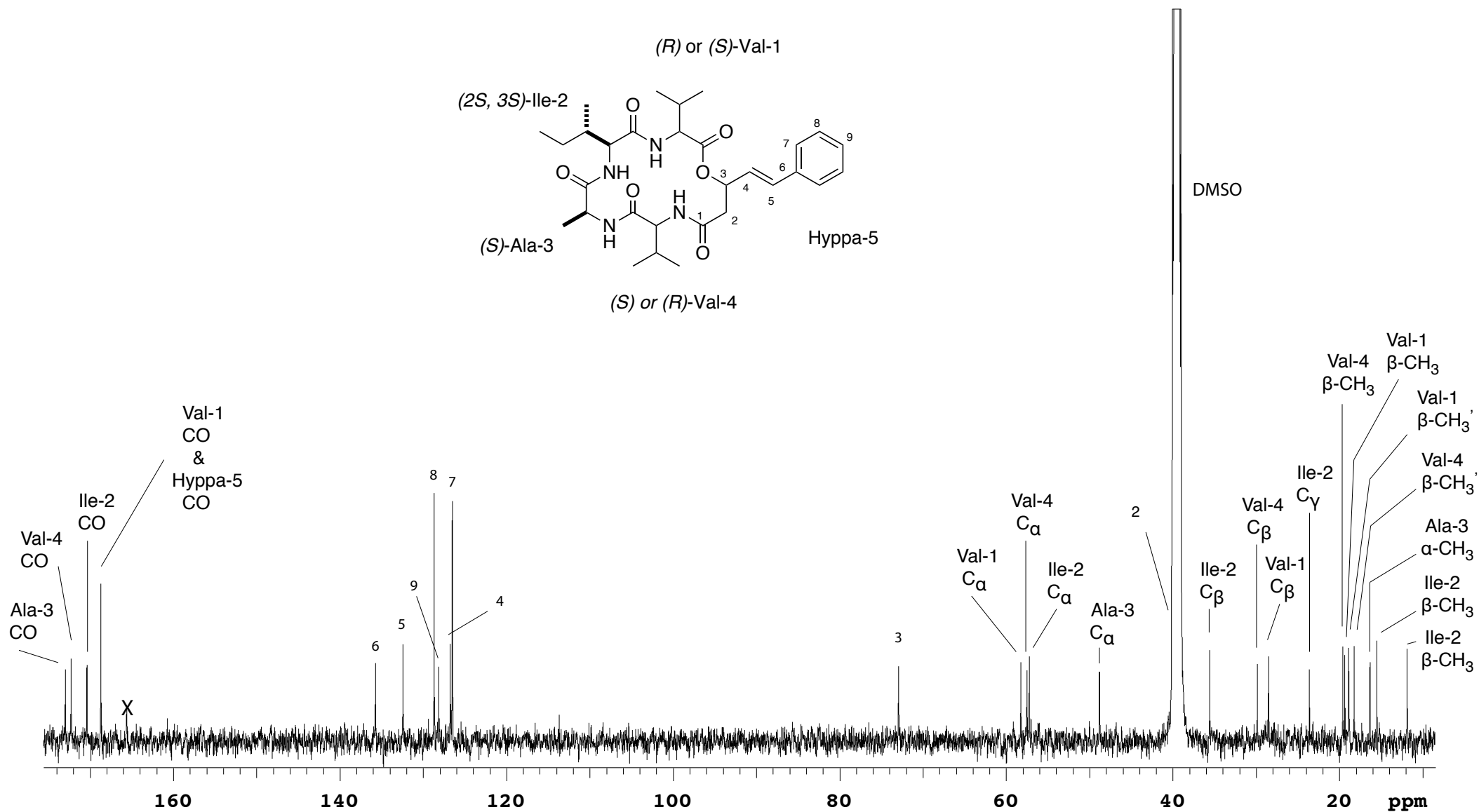


Figure S3. DEPT NMR spectrum of EGM-556 (**1**), (150 MHz, DMSO-*d*₆)

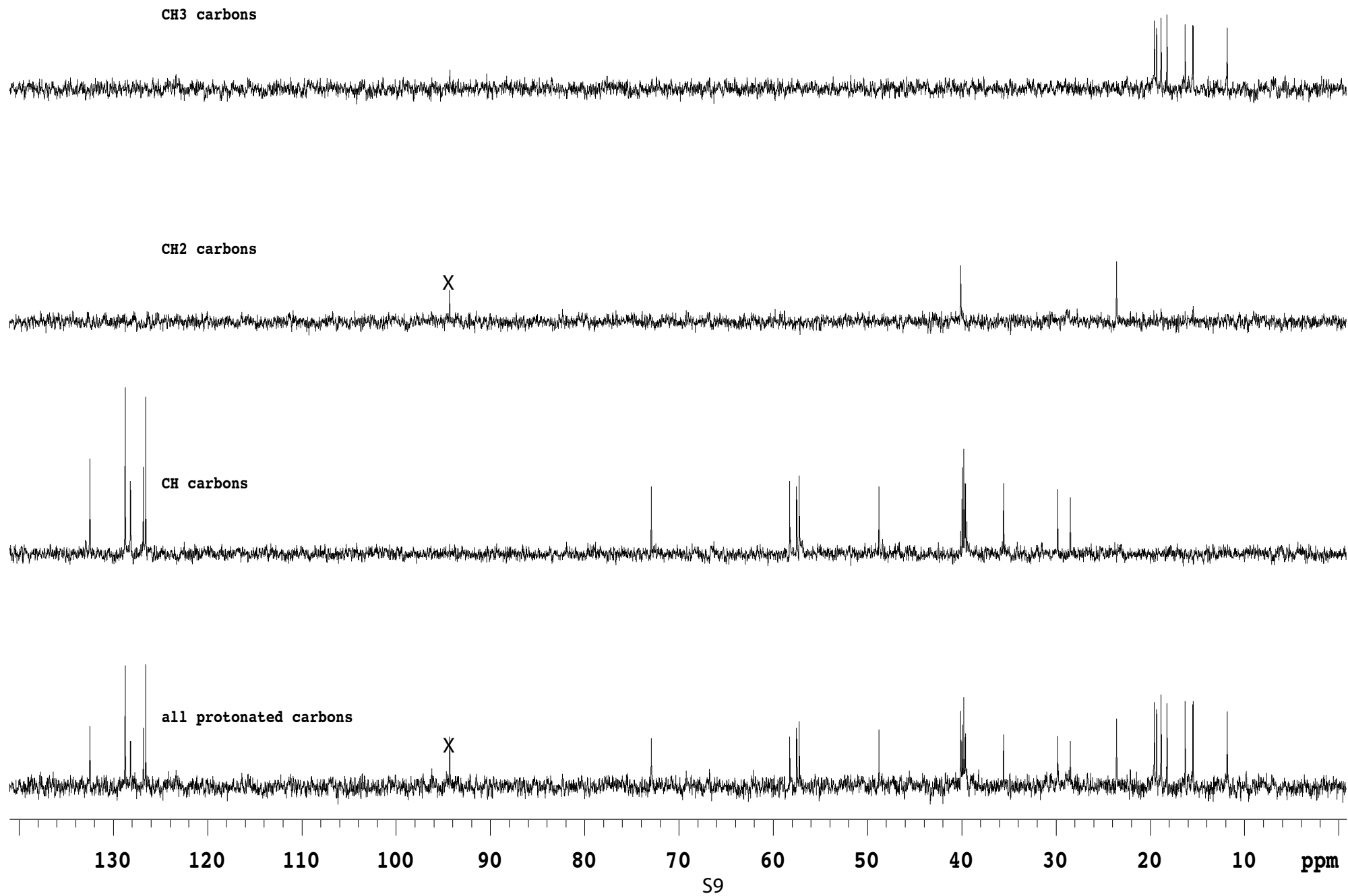


Figure S4. ^1H - ^1H COSY spectrum of EGM-556 (**1**), (600 MHz, $\text{DMSO-}d_6$)

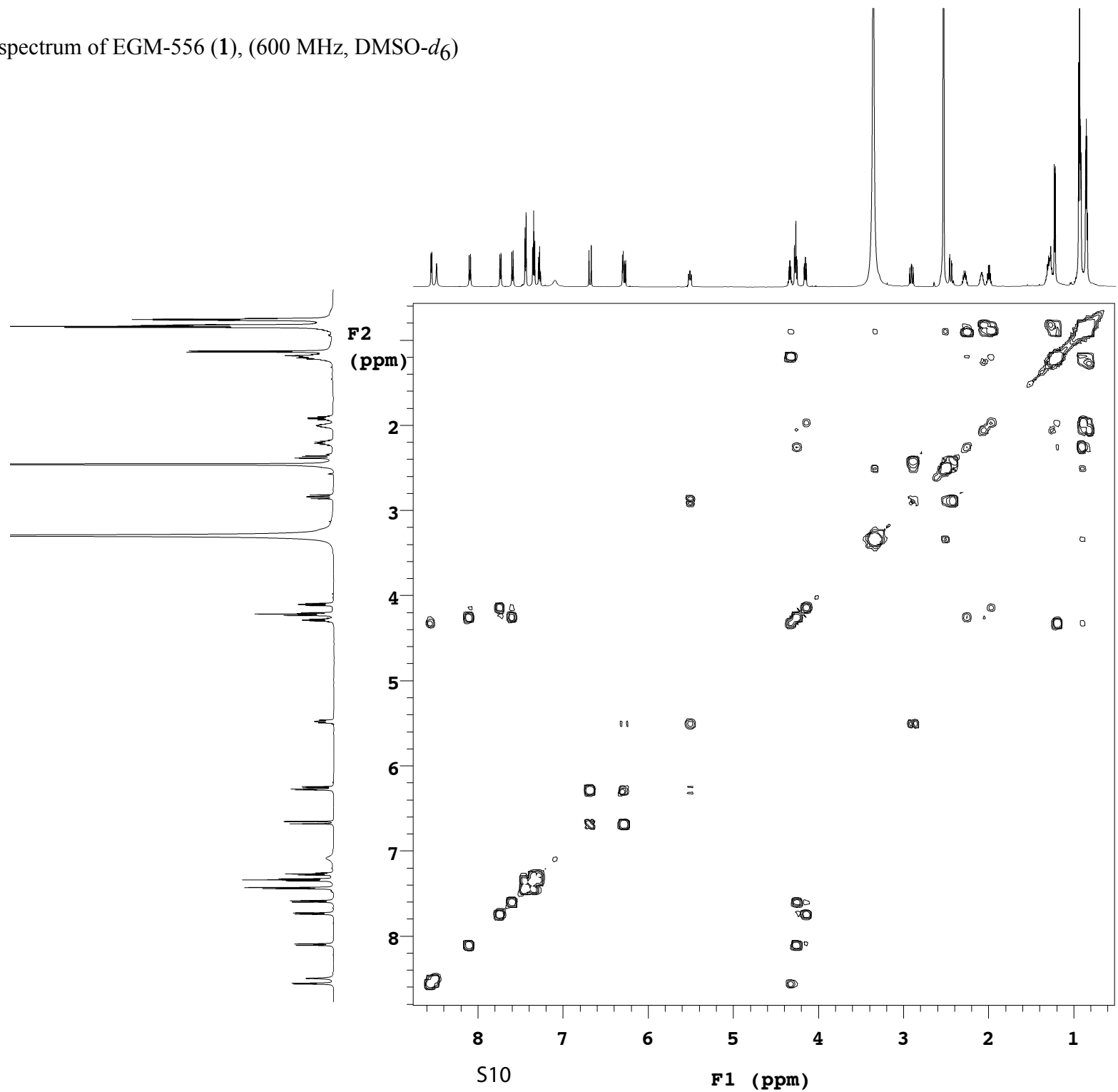


Figure S5. gHMQC spectrum of EGM-556 (**1**), (600 MHz, DMSO-*d*₆)

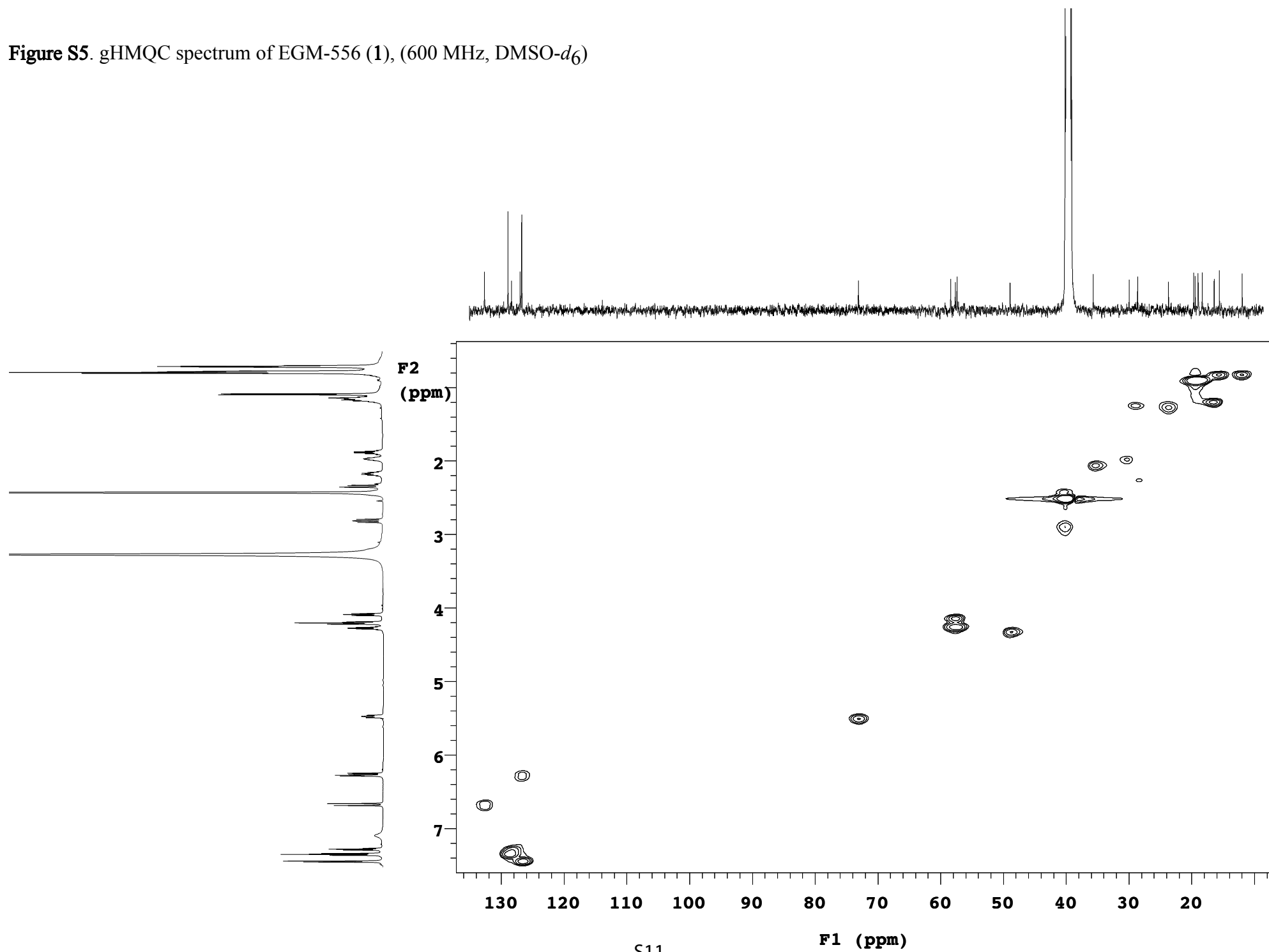


Figure S6. gHMBC spectrum of EGM-556 (**1**), (600 MHz, DMSO-*d*₆)

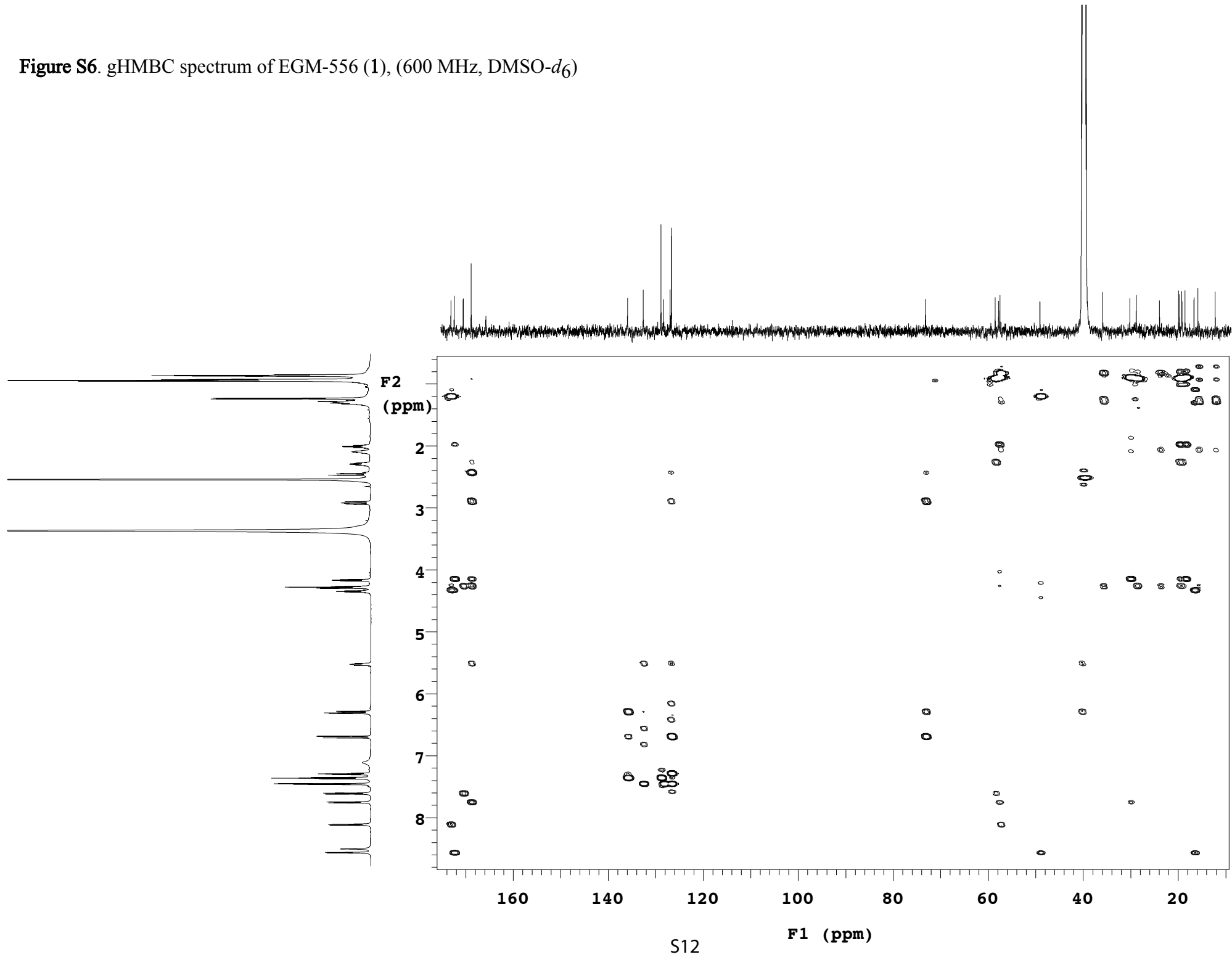


Figure S7. ^1H NMR spectrum of acyclic methyl ester (**2**), (600 MHz, $\text{DMSO-}d_6$)

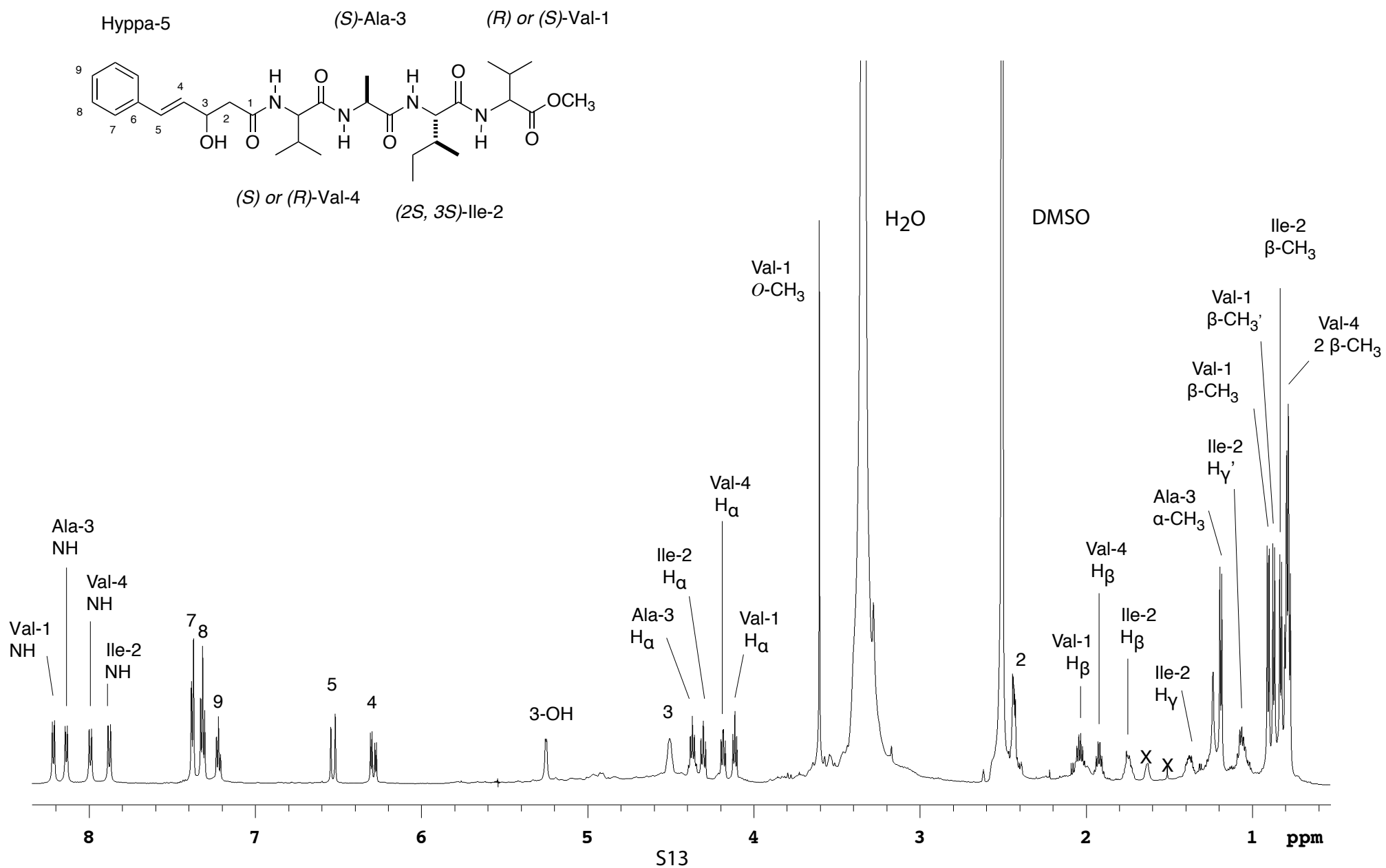


Figure S8. ^{13}C NMR spectrum of acyclic methyl ester (**2**), (150 MHz, $\text{DMSO-}d_6$)

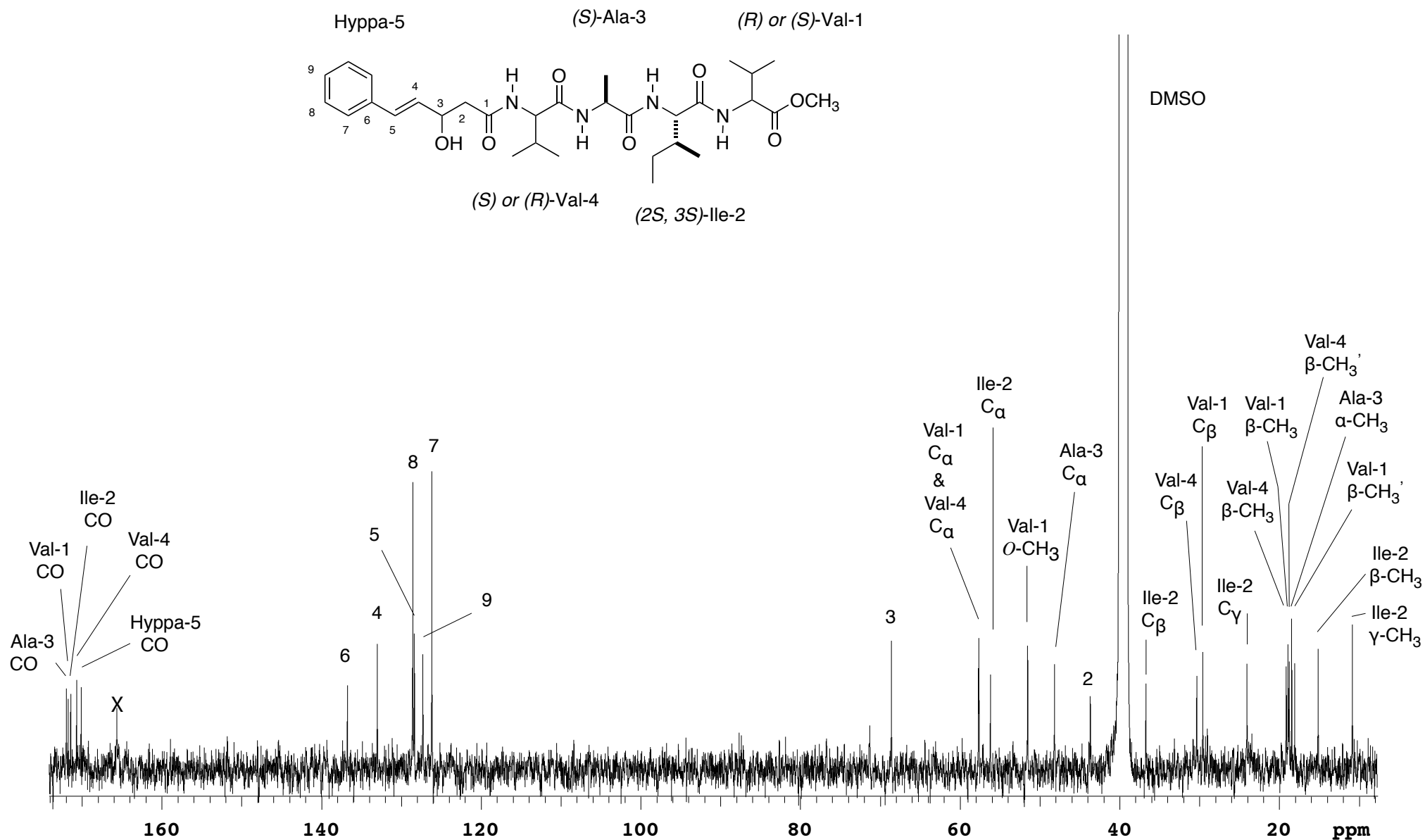


Figure S9. DEPT NMR spectrum of acyclic methyl ester (**2**), (150 MHz, DMSO- d_6)

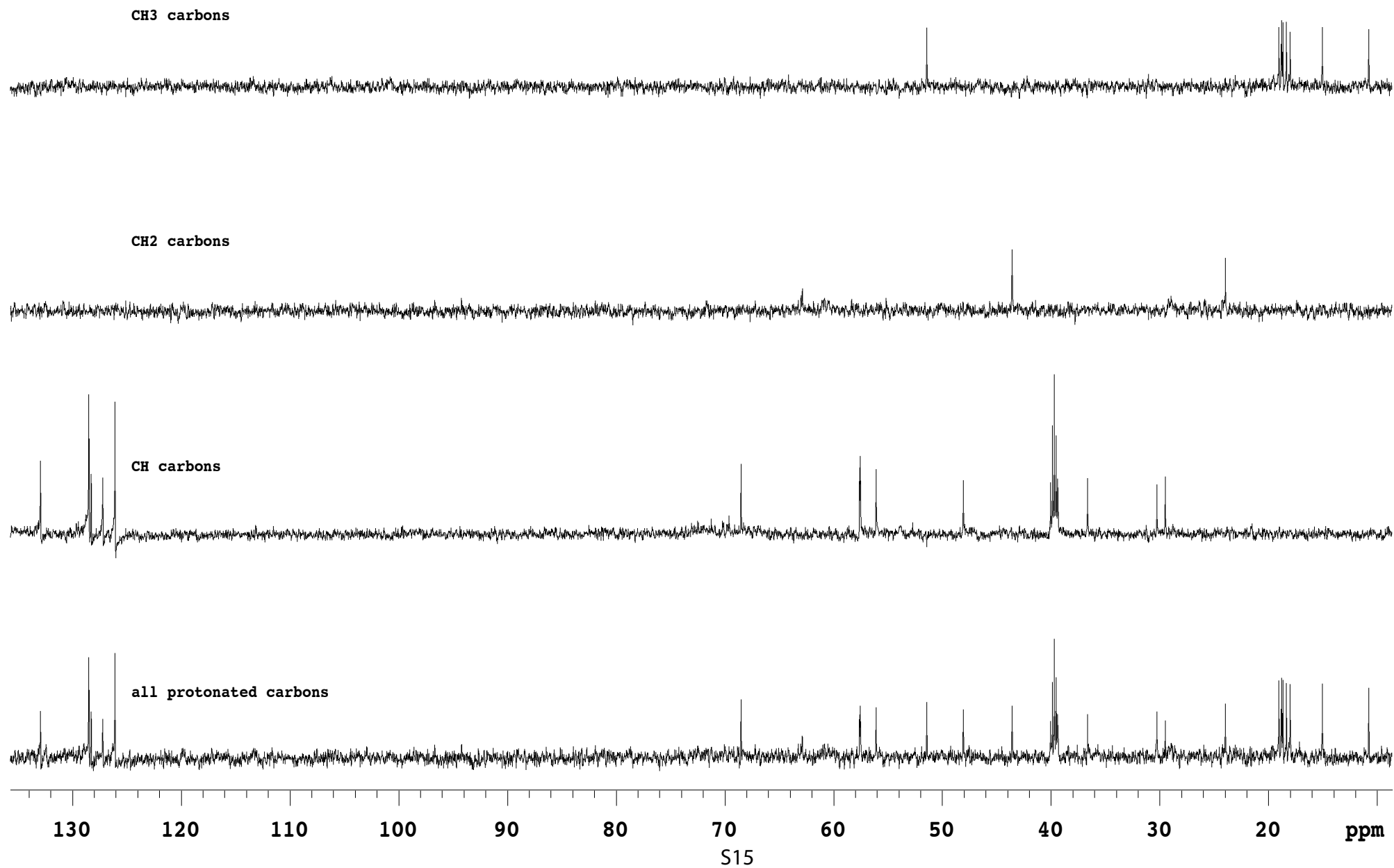


Figure S10. ^1H - ^1H COSY spectrum of acyclic methyl ester (**2**), (600 MHz, $\text{DMSO-}d_6$)

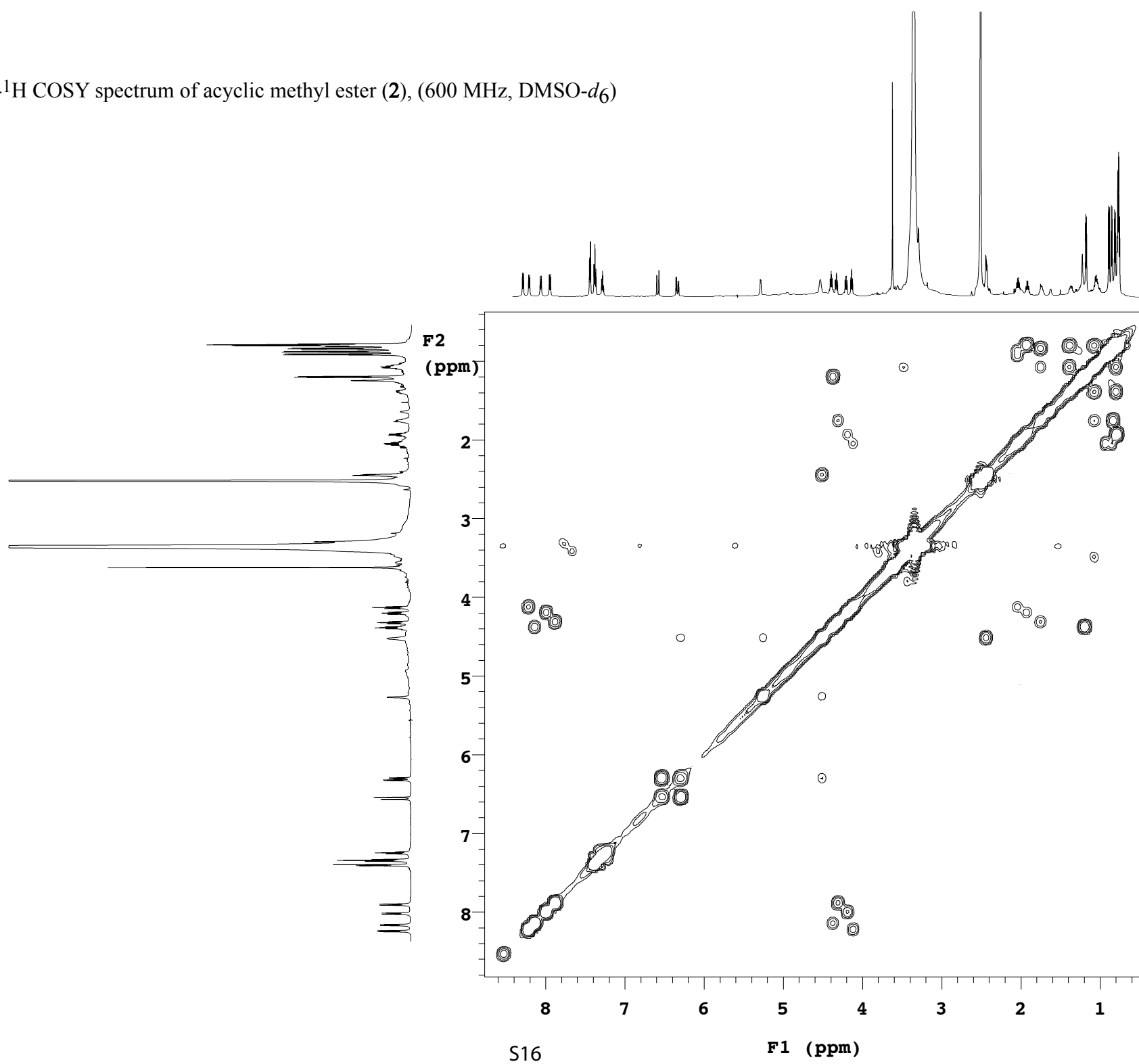


Figure S11. gHMQC spectrum of acyclic methyl ester (**2**), (600 MHz, DMSO- d_6)

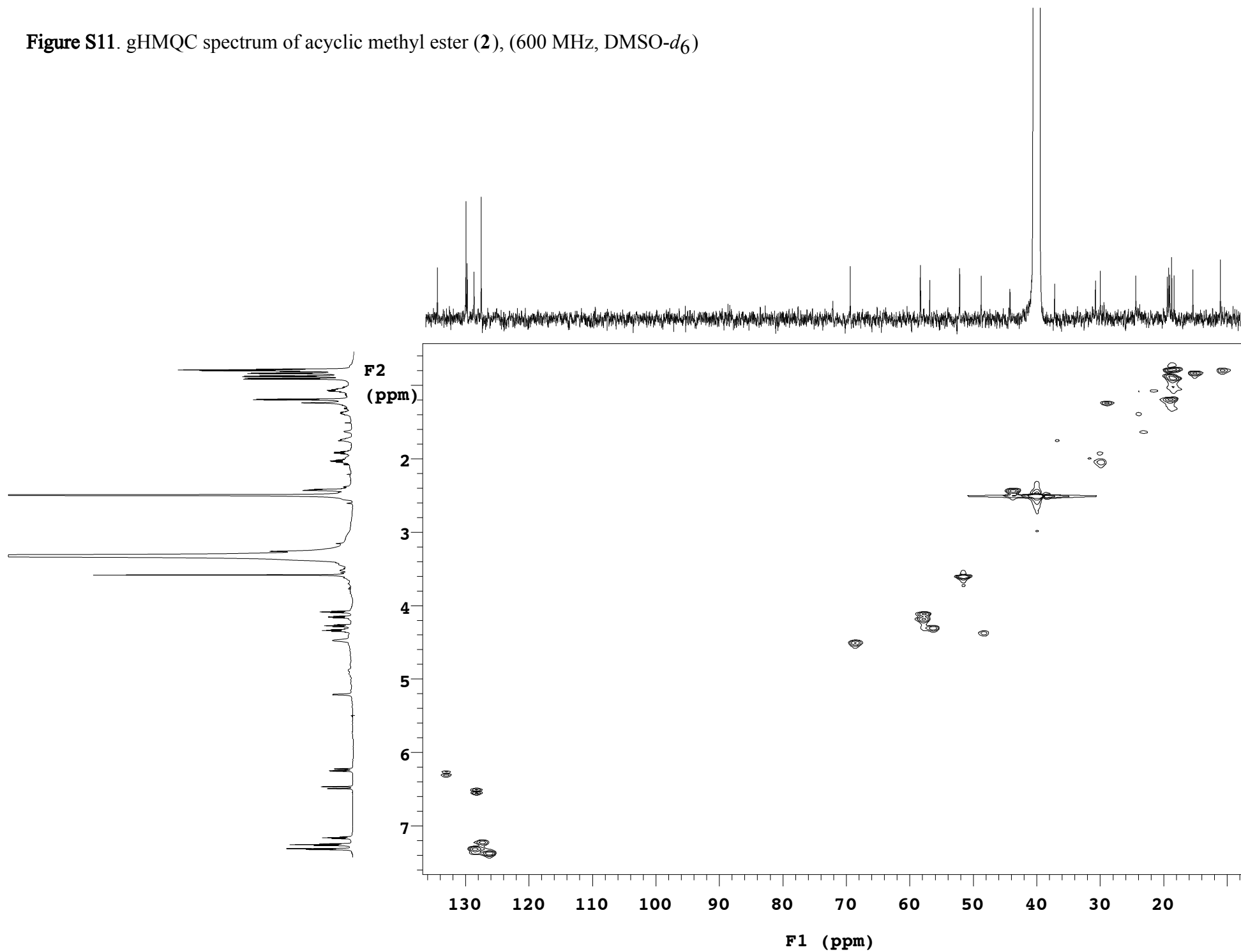


Figure S12. gHMBC spectrum of acyclic methyl ester (**2**), (600 MHz, DMSO-*d*₆)

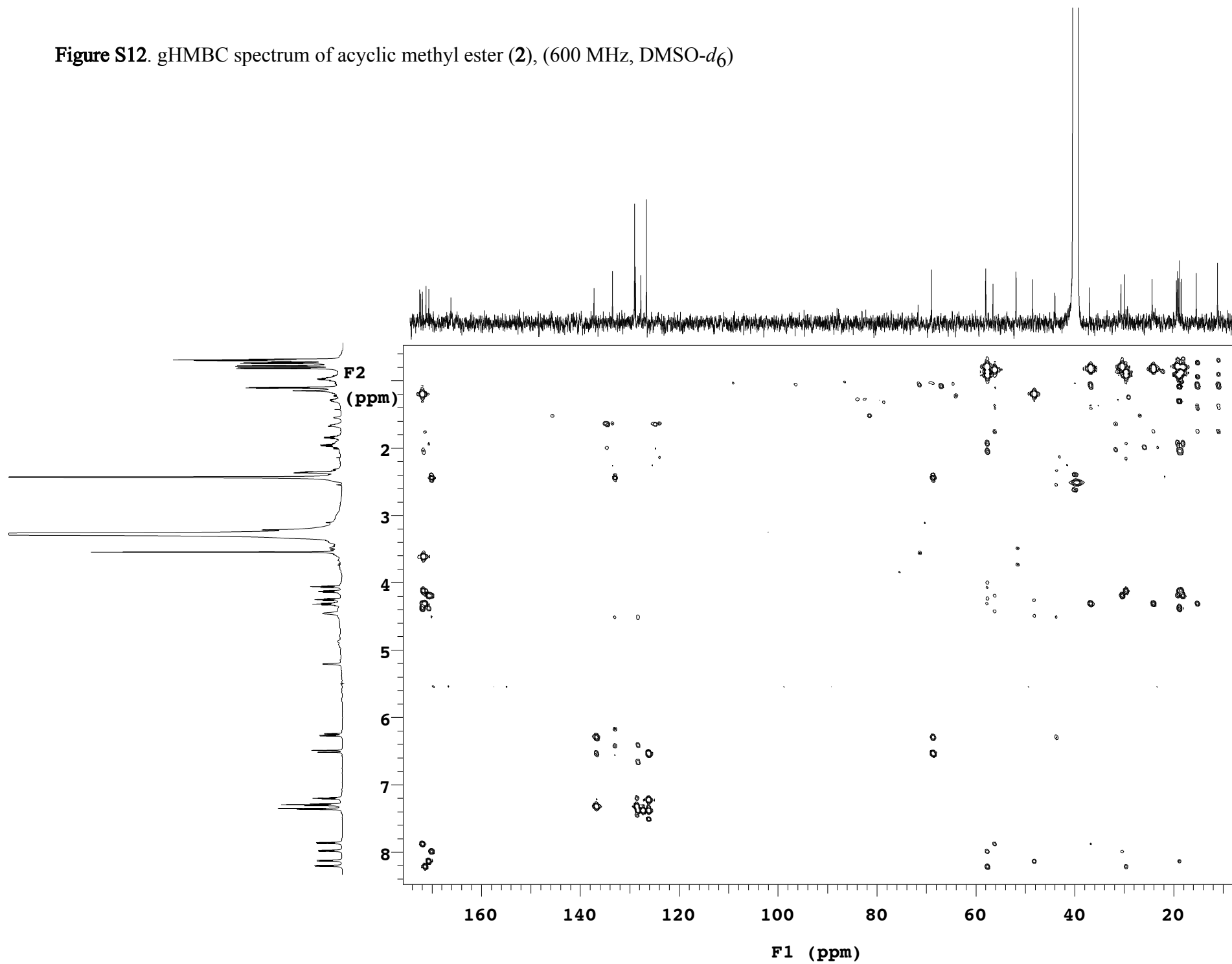


Figure S13. RP C18 LC-ELSD-MS traces of EGM-556 (**1**) hydrolysate (solid line) and comparison to free amino acids (dashed line), derivatized with Marfey's reagent (FDDA).

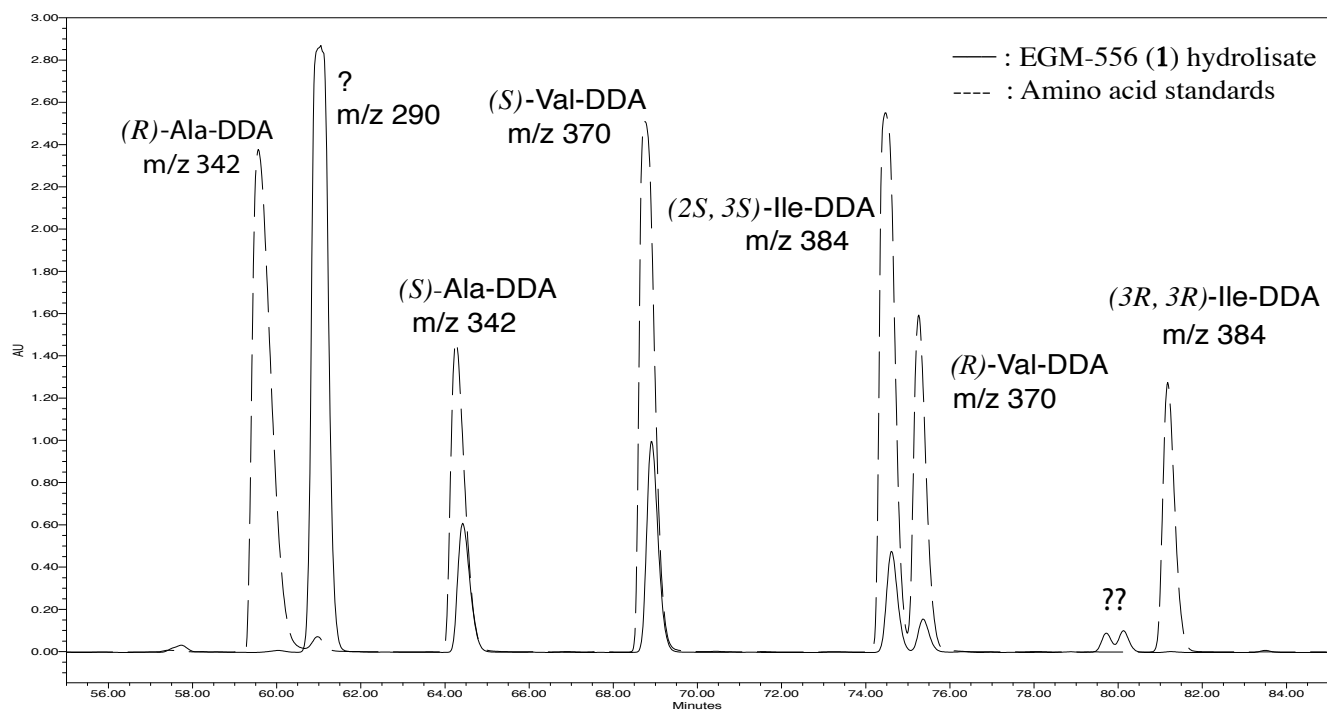


Figure S14. Chiral separation of $(2S, 3S)$ -isoleucine and $(2R, 3S)$ -isoleucine and comparison with the hydrolysate of EGM-556 (**1**), derivatised with Marfey's reagent (FDDA).

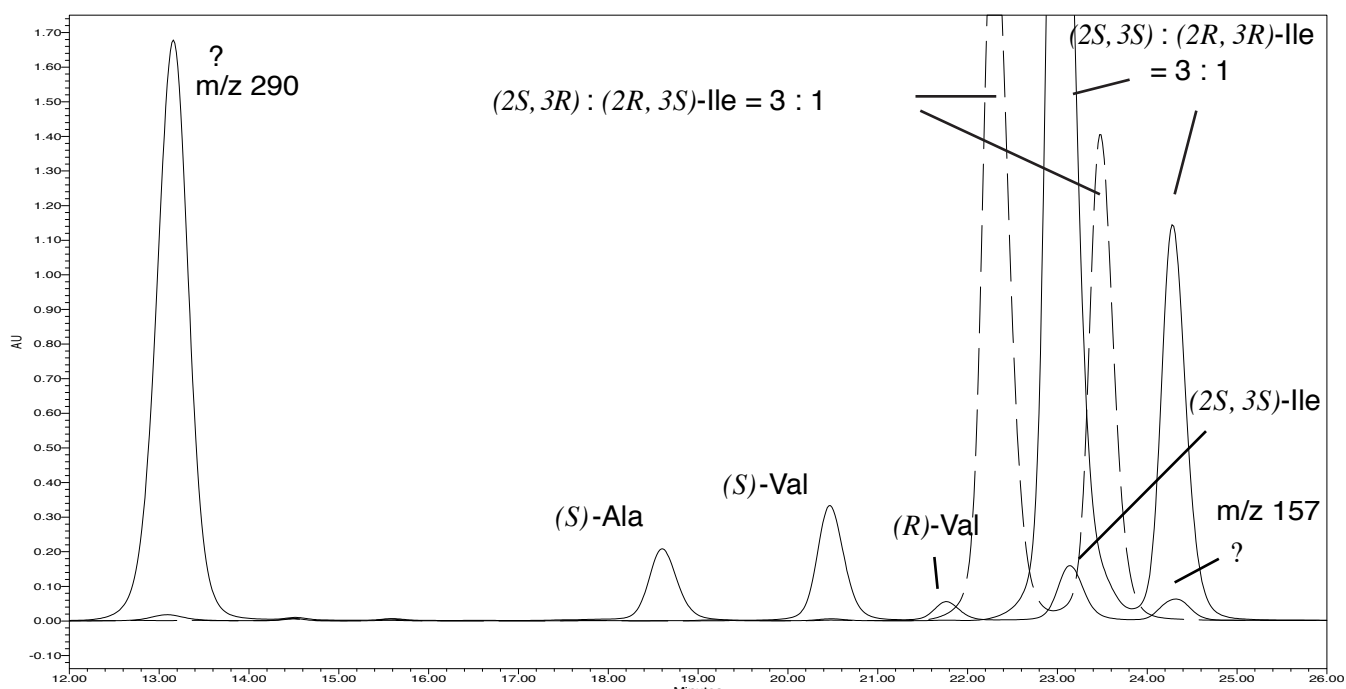


Table S3. NMR Assignments for EGM-556 (1) in DMSO-*d*₆

Structural Unit	Position	¹³ C ^a δ, type ^b	¹ H δ (m, J(Hz), int)	COSY	gHMBC
Val-1	CO	168.7, qC			4.24, 5.50, 2.25
	α	58.2, CH	4.24 (dd, 9.6, 6.0, 1H)	2.25	7.60, 2.25, 0.89
	β	28.5, CH	2.25 (ds, 6.0, 6.6, 1H)	4.24, 0.89	4.24, 0.89
	β-CH ₃	18.9, CH ₃	0.89 (d, 6.6, 3H)	2.25	4.24, 0.89
	β-CH ₃	19.3, CH ₃	0.89 (d, 6.6, 3H)	2.25	4.24, 2.25, 0.89
	NH		7.60 (d, 9.6, 1H)	4.24	
Ile-2	CO	170.4, qC			7.60, 4.26, 4.24
	α	57.2, CH	4.26 (d, 9.6, 1H)	8.10	8.10, 2.05, 1.26, 0.81
	β	35.6, CH	2.05 (m, 1H)	1.26, 0.81	4.26, 1.26, 0.81
	γ	23.6, CH ₂	1.26 (m, 2H)	2.05, 0.81	4.26, 2.05, 0.81
	β-CH ₃	15.5, CH ₃	0.81 (d, 6.6, 3H)	2.05, 1.26	4.26, 2.05, 1.26
	γ-CH ₃	11.9, CH ₃	0.81 (t, 7.5, 3H)	2.05, 1.26	2.05, 1.26
Ala-3	NH		8.10 (d, 9.6, 1H)	4.26	
	CO	173.0, qC			8.10, 4.32, 1.18
	α	48.8, CH	4.32 (dq, 6.3, 6.4, 1H)	8.56, 1.18	8.56, 1.18
	α-CH ₃	16.3, CH ₃	1.18 (d, 7.2, 3H)	4.32	8.56, 4.32
Val-4	NH		8.56 (d, 6.3, 1H)	4.32	
	CO	172.3, qC			8.56, 4.14, 1.96
	α	57.5, CH	4.14 (dd, 8.7, 7.2, 1H)	7.74, 1.96	7.74, 1.96, 0.89
	β	29.8, CH	1.96 (ds, 6.9, 6.8, 1H)	4.14, 0.89	7.74, 4.14, 0.89
	β-CH ₃	19.6, CH ₃	0.89 (d, 6.6, 3H)	1.96	4.14, 1.96, 0.89
	β-CH ₃	18.2, CH ₃	0.89 (d, 6.6, 3H)	1.96	4.14, 1.96, 0.89
Hyppa-5	NH		7.74 (d, 8.7, 1H)	4.14, 0.89	
	1	168.7, qC			7.74, 5.50, 2.88, 2.42 4.14, 1.96
	2	40.1, CH ₂ *	2.42 (dd, 14.4, 1.8, 1H)	2.88	6.28, 5.50
			2.88 (dd, 14.4, 10.5, 1H)	2.42, 5.50	
	3	72.9, CH	5.50 (ddd, 10.5, 7.1, 1.8, 1H)	2.88, 6.28	6.68, 6.28, 2.88, 2.42
	4	126.7, CH	6.28 (dd, 15.9, 7.1, 1H)	6.68, 5.50	6.68, 2.88, 2.42
	5	132.4, CH	6.68 (d, 15.9, 1H)	6.28	7.44, 5.50
	6	135.7, qC			7.36, 6.68, 6.28
	7	126.5, CH	7.44 (d, 7.2, 2H)	7.36	7.44, 7.28, 6.68
8	128.7, CH	7.36 (dd, 7.5, 7.2, 2H)	7.44, 7.28	7.36	
9	128.1, CH	7.28 (t, 7.5, 1H)	7.36	7.44	

^a Measured at 150 MHz. ^b Carbon type established from ¹³C DEPT and/or gHMQC experiments.

*Overlapping with DMSO-*d*₆

Table S4. NMR Assignments for Acyclic Methyl Ester (2) in DMSO-*d*₆

Structural Unit	Position	¹³ C ^a δ, type ^b	¹ H δ (m, J(Hz), int)	COSY	gHMBC
Val-1	O-CH ₃	51.5, CH ₃	3.61 (s, 3H)		
	CO	171.7, qC			4.11, 3.61, 2.04
	α	57.6, CH	4.11 (dd, 7.2, 6.6, 1H)	8.22, 2.04	8.22, 2.04, 0.90, 0.87
	β	29.6, CH	2.04 (ds, 7.2, 6.6, 1H)	0.90, 0.87	8.22, 4.11, 0.90, 0.87
	α-CH ₃	18.9, CH ₃	0.87 (d, 7.2, 3H)	2.04	4.11, 2.04, 0.90
	α-CH ₃	18.4, CH ₃	0.90 (d, 7.2, 3H)	2.04	4.11, 2.04, 0.87
	NH		8.22 (d, 7.2, 1H)	4.11	
Ile-2	CO	171.4, qC			8.22, 4.30
	α	56.2, CH	4.30 (dd, 9.0, 8.0, 1H)	7.88, 1.75	1.75, 1.38, 1.06, 0.83
	β	36.7, CH	1.75 (m, 1H)	1.06, 0.83	4.30, 1.38, 1.06, 0.83, 0.80
	γ	24.0, CH ₂	1.06 (m, 1H)	1.75, 1.38, 0.80	4.30, 1.75, 0.83, 0.80
			1.38 (m, 1H)	1.06, 0.80	
	β-CH ₃	15.1, CH ₃	0.83 (d, 6.6, 3H)	1.75	4.30, 1.75, 1.38, 1.06
	γ-CH ₃	10.8, CH ₃	0.80 (tr, 7.2, 3H)	1.38, 1.06	1.75, 1.38
	NH		7.88 (d, 9.0, 1H)	4.30	
Ala-3	CO	172.0, qC			7.88, 4.37, 1.19
	α	48.1, CH	4.37 (dq, 7.8, 7.2, 1H)	8.14, 1.19	8.14, 1.19
	α-CH ₃	18.7, CH ₃	1.19 (d, 7.2, 3H)	4.37	8.14, 4.37
		NH		8.14 (d, 7.8, 1H)	4.37
Val-4	CO	170.7, qC			8.14, 4.37, 4.18
	α	57.7, CH	4.18 (dd, 8.7, 6.9, 1H)	7.93, 1.92	7.93, 1.92, 0.79, 0.78
	β	30.3, CH	1.92 (ds, 7.2, 6.9, 1H)	4.18, 0.79, 0.78	7.93, 4.18, 0.79, 0.78
	β-CH ₃	19.1, CH ₃	0.79 (d, 7.2, 3H)	1.92	4.18, 1.92, 0.78
	β-CH ₃	18.1, CH ₃	0.78 (d, 7.2, 3H)	1.92	4.18, 1.92, 0.79
		NH		7.93 (d, 8.7, 1H)	4.18
Hyppa-5	1	170.1, qC			7.93, 4.51, 4.18, 2.44
	2	43.7, CH ₂	2.44 (m, 2H)	4.51	6.29, 4.51
	3	68.6, CH	4.51 (m, 1H)	6.29, 5.25, 2.44	6.53, 6.29, 2.44
	4	133.0, CH	6.29 (dd, 15.9, 6.0, 1H)	6.53, 4.51	6.53, 4.51, 2.44
	5	128.3, CH	6.53 (d, 15.9, 1H)	6.29	7.38, 4.51
	6	136.7, qC			7.32, 6.53, 6.29
	7	126.2, CH	7.38 (d, 7.8, 2H)	7.32	7.38, 7.22, 6.53
	8	128.5, CH	7.32 (dd, 7.8, 7.2, 2H)	7.38, 7.22	7.32
	9	127.3, CH	7.22 (t, 7.2, 1H)	7.32	7.38
		OH		5.25 (d, 3.6, 1H)	4.51

^a Measured at 150 MHz. ^b Carbon type established from ¹³C DEPT and/or gHMQC experiments.