Electronic Supplementary Material (ESI) for RSC Advances.

Supplementary Information

Mutagenesis of Precursor Peptide for the Generation of Nosiheptide Analogues

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Table S1 H^1 and C^{13} NMR chemical shifts of analogue 6 in DMSO-d6 (δ in ppm)

Assigment	δ_C, mult	δ_H(J in Hz)	Assigment	δ_C, mult	δ_H(J in Hz)
Ind CO	181.415		But 3	129.109	6.53 (q, 1H)
Glu CO	172.647		Pyr 4	126.894	6.43 (s, 1H)
Thz(3) 2	170.594		Thz(5) 5	126.114	8.77 (s, 1H)
Thz(4) 2	170.222		Thz(1) 5	125.893	8.51 (s, 1H)
Ala CO	169.100		Thz(3) 5	125.080	8.28 (s, 1H)
Thz(5) 2	166.483		Ind 6	124.908	7.25 (d, 1H)
Thz(2) 2	166.097		Ind 3a	124.848	
Dha CO	165.099		Ind 5	123.597	7.45 (d, 1H)
Thz(1) 2	164.650		Thz(2) 5	118.658	8.48 (s, 1H)
Thz(3) CO	163.471		Thz(4) 5	118.261	8.26 (s, 1H)
Thz(2) CO	159.825		Ind 3	114.929	
Thz(1) CO	159.568		Ind 7	103.524	7.41 (d, 1H)
Thz(5) CO	159.480		Dha 3	66.326	6.50E(s) 5.73Z(s)
Thz(4) 4	159.233		Glu 4	63.050	5.24 (s, 1H)
Pyr 3	155.383		Glu4’	49.302	5.44 (s, 1H)
Thz(1) 4	149.867		Ind 4’	47.587	5.70 (t, 1H)
Thz(5) 4	148.940		Cys 2	44.634	5.99 (m, 1H)
Thz(3) 4	148.940		Glu 3	38.944	4.06 (d, 2H)
Thz(2) 4	147.306		Cys 3	38.666	3.93 (d, 2H)
Pyr 6	138.776		Ala 2	29.630	5.79 (m, 1H)
Ind 7a	137.827		But 4	19.481	1.73 (d, 3H)
Pyr 2	136.522		Ind 3’	13.533	3.42 (m, 3H)
Dha 2	134.433		Ala 3	11.913	3.63 (d, 3H)
Ind 2	130.223				
Pyr 5	129.975				
But 2	129.763				
Ind 4	129.447				
Ind NH 10.81 (s, 1H); Dha NH 10.13 (s, 1H); But NH 9.57 (s, 1H); Cys NH 8.09 (s, 1H)					

Table S2 H^1 and C^{13} NMR chemical shifts of analogue 7 in DMSO-d6 (δ in ppm)					
Assigment	**δ_C, mult**	**δ_H(J in Hz)**	**Assigment**	**δ_C, mult**	**δ_H(J in Hz)**
Ind CO	181.871		But 3	129.160	6.48 (q, 1H)
Glu CO	172.810		Pyr 4	127.626	6.37 (s, 1H)
Thz(3) 2	169.793		Thz(5) 5	127.362	8.86 (s, 1H)
Thz(4) 2	169.221		Thz(1) 5	126.220	8.64 (s, 1H)
Ser CO	168.690		Thz(3) 5	125.112	8.20 (s, 1H)
Thz(5) 2	166.582		Ind 6	124.958	7.25 (d, 1H)
Thz(2) 2	165.959		Ind 3a	124.852	
Dha CO	165.054		Ind 5	124.410	7.56 (d, 1H)
Thz(1) 2	160.400		Thz(2) 5	123.325	8.60 (s, 1H)
Thz(3) CO	159.999		Thz(4) 5	120.181	8.11 (s, 1H)
Thz(2) CO	159.758		Ind 3	118.680	
Thz(1) CO	158.451		Ind 7	107.039	7.80 (d, 1H)
Thz(5) CO	157.958		Dha 3	103.608	6.46E(s) 5.76Z(s)
Thz(4) 4	157.713		Glu 4	66.565	5.52 (t, 1H)
Pyr 3	150.518		Ser 3	65.935	5.67 (s, 1H)
Thz(1) 4	149.839		Ind 4’	63.227	5.48 (m, 1H)
Thz(5) 4	149.713		Ser 2	54.007	5.50 (m, 1H)
Thz(3) 4	149.369		Cys 2	48.929	4.05 (d, 1H)
Thz(2) 4	148.036		Glu 2	44.791	4.02 (d, 1H)
Pyr 6	140.978		Glu 3	28.903	3.49 (m, 2H)
Ind 7a	137.585		Cys 3	28.932	3.41 (m, 2H)
Pyr 2	135.609		But 4I	13.336	1.78 (d, 3H)
Dha 2	134.358		Ind 3’	12.637	2.73 (s, 3H)
Ind 2	130.683				
Pyr 5	129.963				
But 2	129.798				
Ind 4	129.607				
Ind NH 11.29 (s, 1H); Dha NH 10.12 (s, 1H); But NH 9.70 (s, 1H); Cys NH 8.89 (s, 1H)					

Table S3 H^1 and C^{13} NMR chemical shifts of analogue 8 in DMSO-d6 (δ in ppm)					
Assigment	**δ_C, mult**	**δ_H(J in Hz)**	**Assigment**	**δ_C, mult**	**δ_H(J in Hz)**
Ind CO	181.895		But 3	127.101	6.44 (q, 1H)
Glu CO	172.994		Pyr 4	126.506	6.35 (s, 1H)
Thz(3) 2	170.014		Thz(5) 5	126.329	8.78 (s, 1H)
Thz(4) 2	169.594		Thz(1) 5	125.077	8.57 (s, 1H)
Thz(5) 2	167.321		Thz(3) 5	124.841	8.46 (s, 1H)
Val CO	166.928		Ind 6	123.442	7.19 (s, 1H)
Thz(2) 2	166.686		Ind 3a	120.036	
Dha CO	165.060		Ind 5	117.587	7.57 (d, 1H)
Thz(1) 2	164.004		Thz(2) 5	115.114	8.46 (s, 1H)
Thz(3) CO	163.183		Thz(4) 5	103.824	8.20 (s, 1H)
Thz(2) CO	159.674		Ind 3	99.766	
Thz(1) CO	159.129		Ind 7	66.359	7.68 (d, 1H)
Thz(5) CO	158.513		Dha 3	65.810	6.35E(s) 5.78Z(s)
Thz(4) 4	153.810		Glu 4	56.599	5.60 (t, 1H)
Pyr 3	153.037		Ind 4’	49.910	5.78 (s, 1H)
Thz(1) 4	150.368		Cys 2	45.117	6.04 (m, 1H)
Thz(5) 4	149.605		Glu 2	40.344	5.86 (m, 1H)
Thz(3) 4	148.779		Glu 3	40.066	4.17 (m, 2H)
Thz(2) 4	147.621		Cys 3	29.730	3.70 (d, 2H)
Pyr 6	138.112		Val 2	28.943	5.08 (d, 1H)
Ind 7a	135.266		But 4	18.477	1.70 (d, 3H)
Pyr 2	134.419		Ind 3’	12.644	2.30 (m, 3H)
Dha 2	130.447		Val 4	13.361	1.23 (m, 3H)
Ind 2	130.054		Val 4’	11.657	1.13 (m, 3H)
Pyr 5	129.927				
But 2	129.648				
Ind 4	129.020				
Ind NH 11.10 (s, 1H); Dha NH 10.05 (s, 1H); But NH 9.07 (s, 1H)					

Table S4 Primers used in this study	
Primer Name	**Primer Sequence (5'-3')**
nosM-hF	GGATCCACCAGGCTCACCAGCTCGGCGGAGA
nosM-hR	AAGCTTTCCTCGCGGGGGATGCCGTCGAACA
1001AF	CACCCAGCCCTGAACCACCTCCACG
1001AR	GGATGGCCTGGACCCAGTCGCAGAACG
primer C2S-A	GCACTCGCAGGTGGTCGACGAGGCCGACA
primer C2S-B	TGTCGGCCTCGTCGACCACCTGCGAGTGC
primer C5S-A	AGCACTCCGAGGTGGTGCACGAGGCCGACA
primer C5S-B	TGTCGGCCTCGTGCACCACCTCGGAGTGCT
primer C7S-A	CAGGAGGAGCAGGAACAGCACGACTCGCAG
primer C7S-B	CTGCGAGTCGTGCTGTTCCTGCTCCTCCTG
primer C9S-A	CAGGAGGAGCAGGAGGAGCAGCACTCGCAG
primer C9S-B	CTGCGAGTGCTGCTCCTCCTGCTCCTCCTG
primer C11S-A	TCCATCAGGAGGACGAGGAACAGCAGCACT
primer C11S-B	AGTGCTGCTGTTCCTCGTCCTCCTGATGGA
T3A-A	ACTCGCAGGTGGCGCACGAGGCCGACATGAC
T3A-B	GTCATGTCGGCCTCGTGCGCCACCTGCGAGT
T3S-A	CAGCACTCGCAGGTGGAGCACGAGGCCGACA
T3S-B	TGTCGGCCTCGTGCTCCACCTGCGAGTGCTG
T3V-A	CAGCACTCGCAGGTGACGCACGAGGCCGACA
T3V-B	TGTCGGCCTCGTGCGTCACCTTGCGAGTGCTG
T3D-A	CGCAGGTGTCGCACGAGGCCGACATGACCTT
T3D-B	TGTCGGCCTCGTGCGACACCTGCGAGTGCTG
T3K-A	CAGCACTCGCAGGTCTTGCACGAGGCCGACA
T3K-B	TGTCGGCCTCGTGCAAGACCTGCGAGTGCTG
Underlined letters were restriction sites for Hind III (AAGCTT) and BamH I (GGATCC).	

Fig. S1 LC-TOF/MS analysis of analogue 6

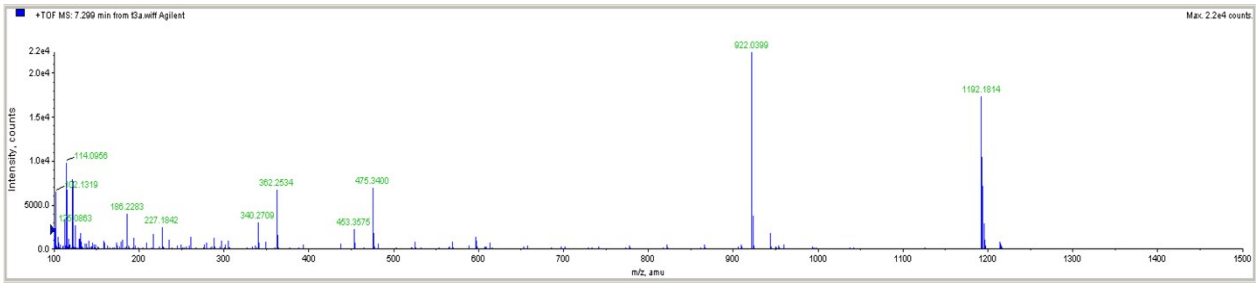


Fig. S2 LC-TOF/MS analysis of analogue 7.

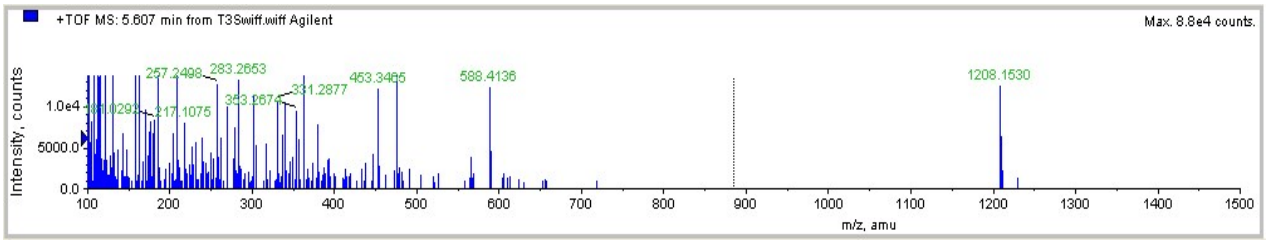


Fig. S3 LC-TOF/MS analysis of analogue 8.

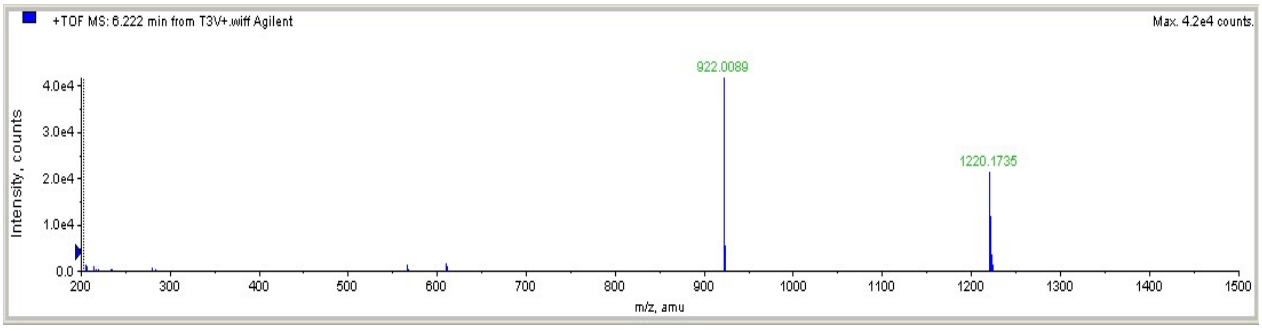


Fig. S4 Structure and numbering system used for analogue 6

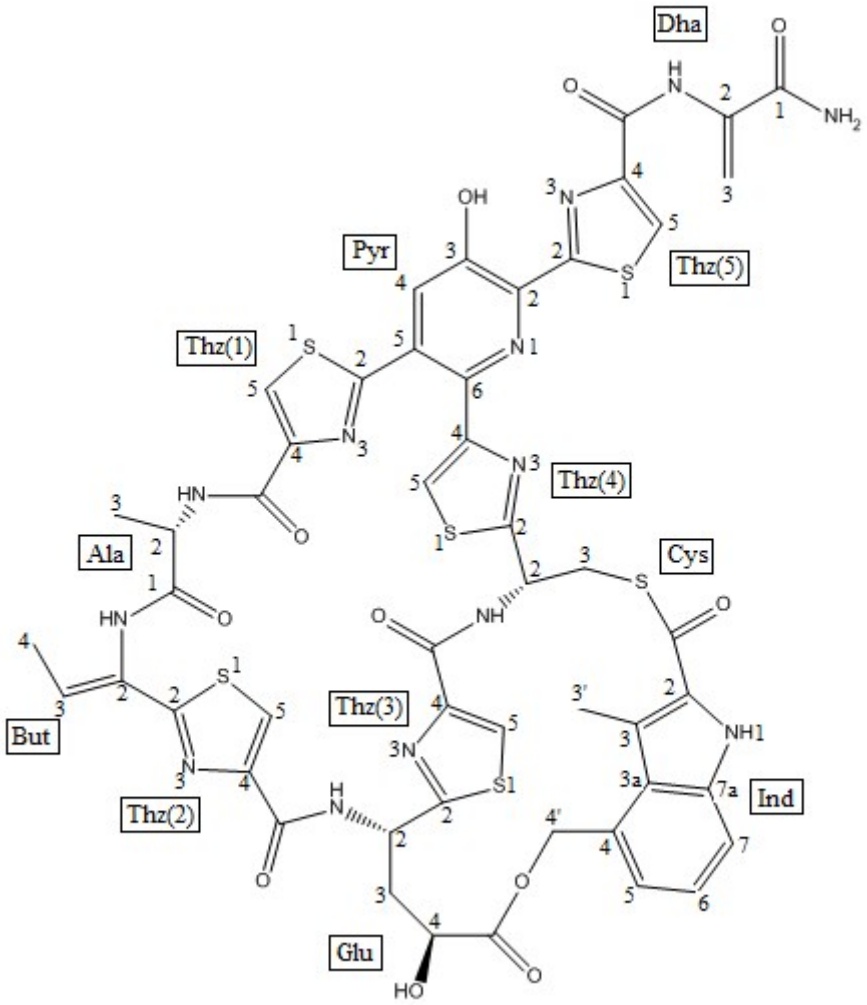
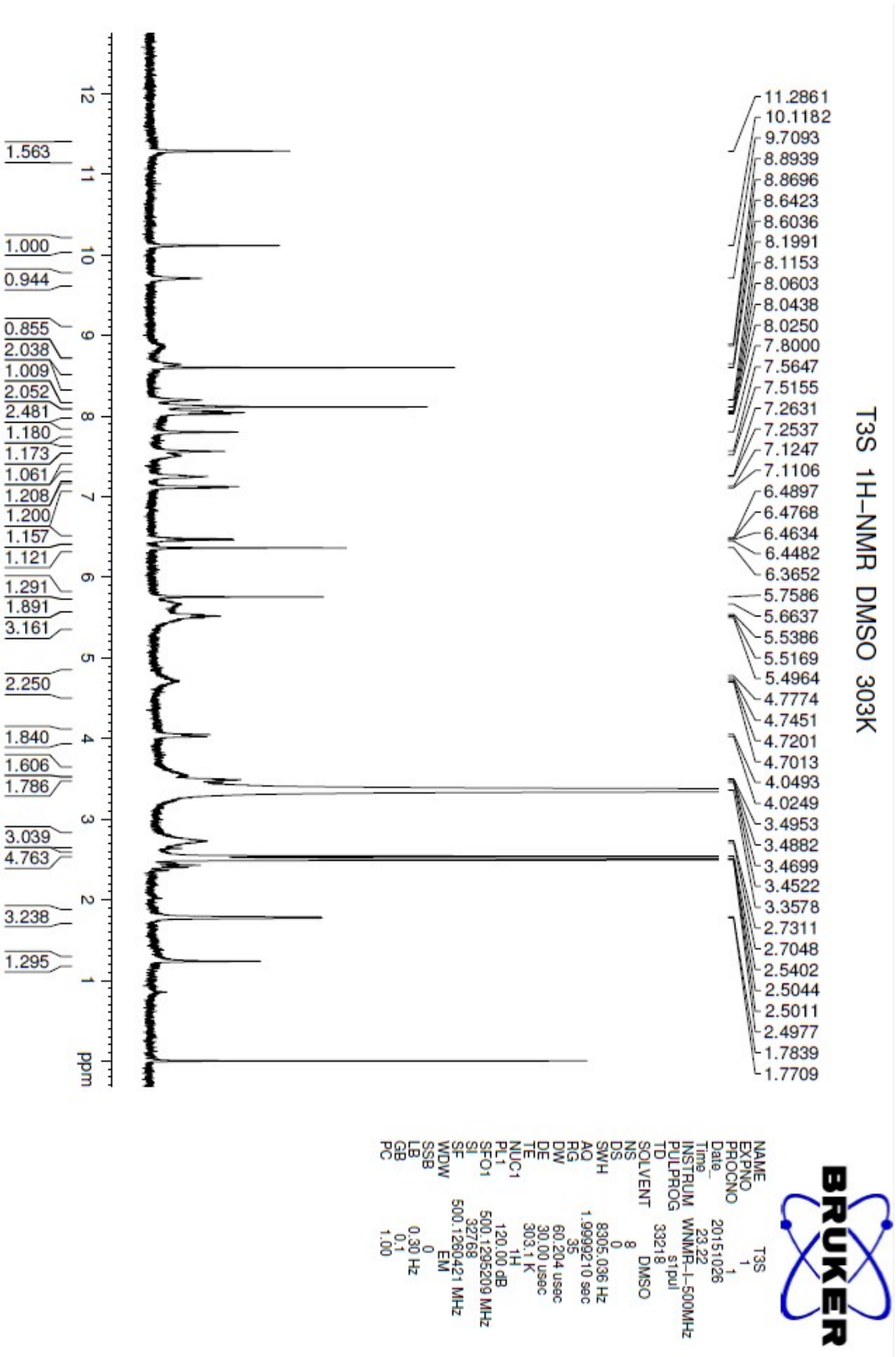


Fig. S5 NMR spectra of analogue 6. (A) H^{1}NMR (303 K, DMSO-d6). (B) C^{13}NMR (303 K, DMSO-d6).

A



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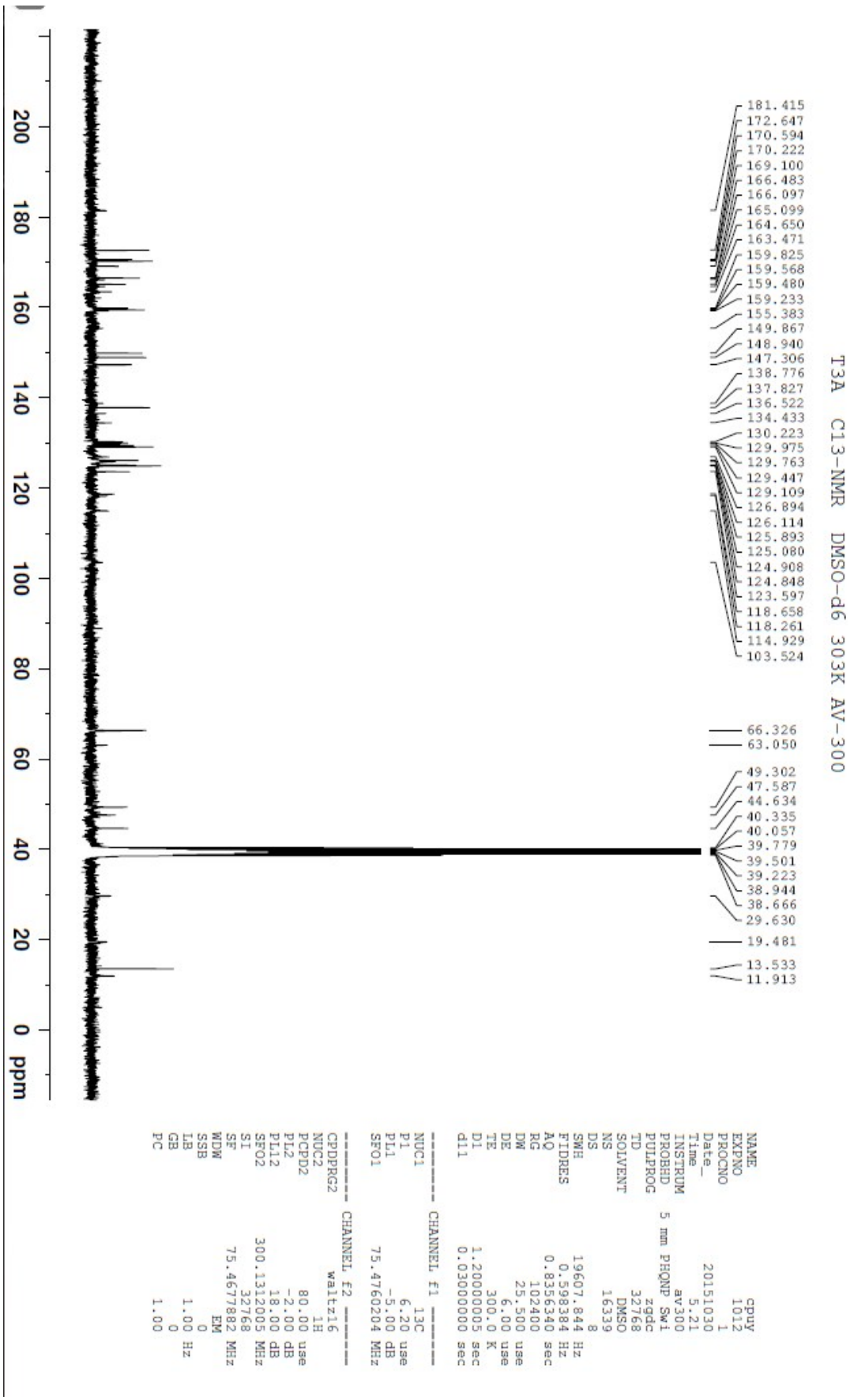
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170.222
169.100
166.483
166.097
165.099
164.650
163.471
159.825
159.568
159.480
159.233
155.383
149.867
148.940
147.306
138.776
137.827
136.522
134.433
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129.763
129.447
129.109
126.894
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125.893
125.080
124.908
124.848
123.597
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Date_ 20151030
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RG 102400
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DE 6.00 use
TE 300.0 K
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P1 6.20 use
PL1 -5.00 dB
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CHANNEL f2
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NUC2 1H
PCPD2 80.00 use
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Fig. S6 Structure and numbering system used for analogue 7.

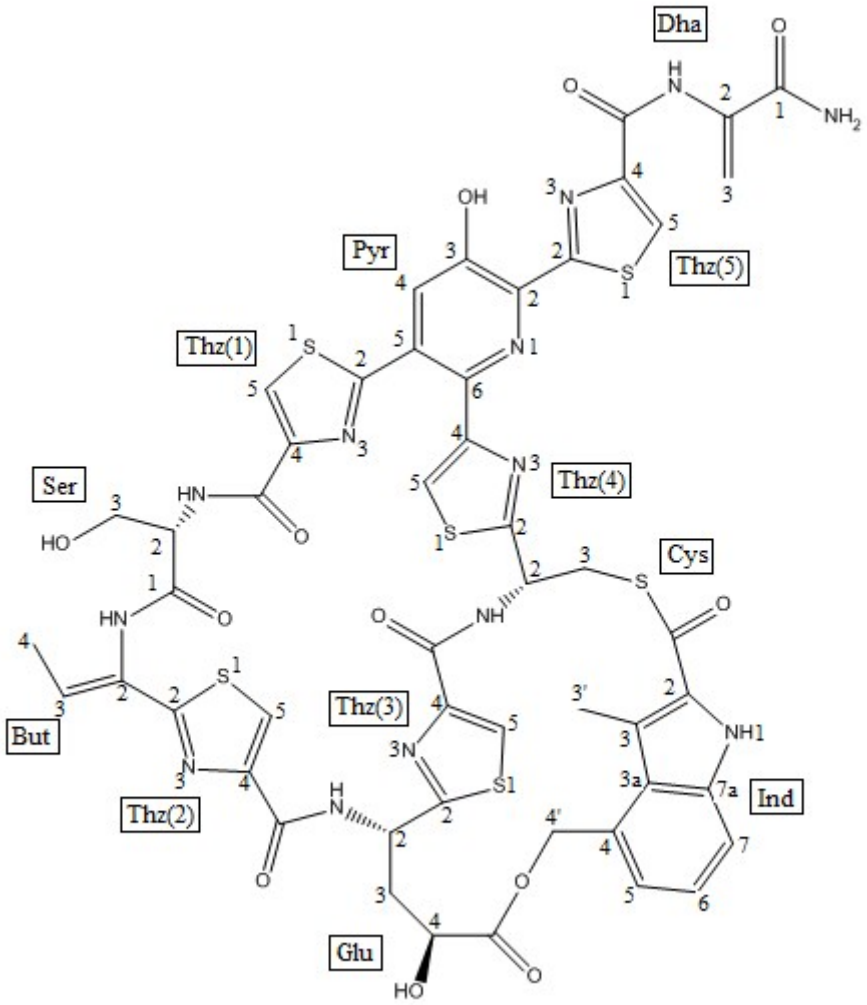
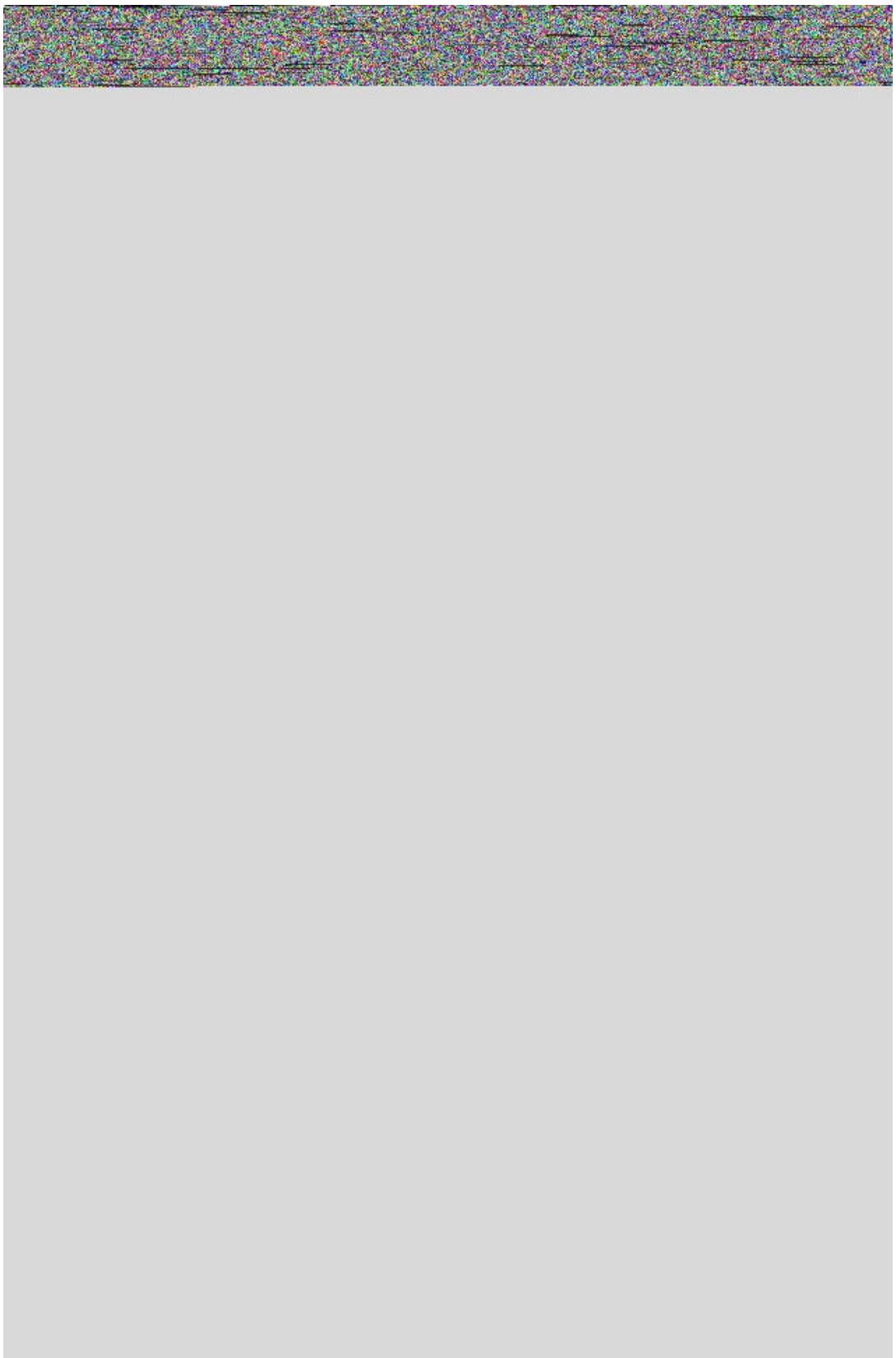


Fig. S7 NMR spectra of analogue 7. (A) H^{1}NMR (303 K, DMSO-d6). (B) C^{13}NMR (303 K, DMSO-d6**).**

A



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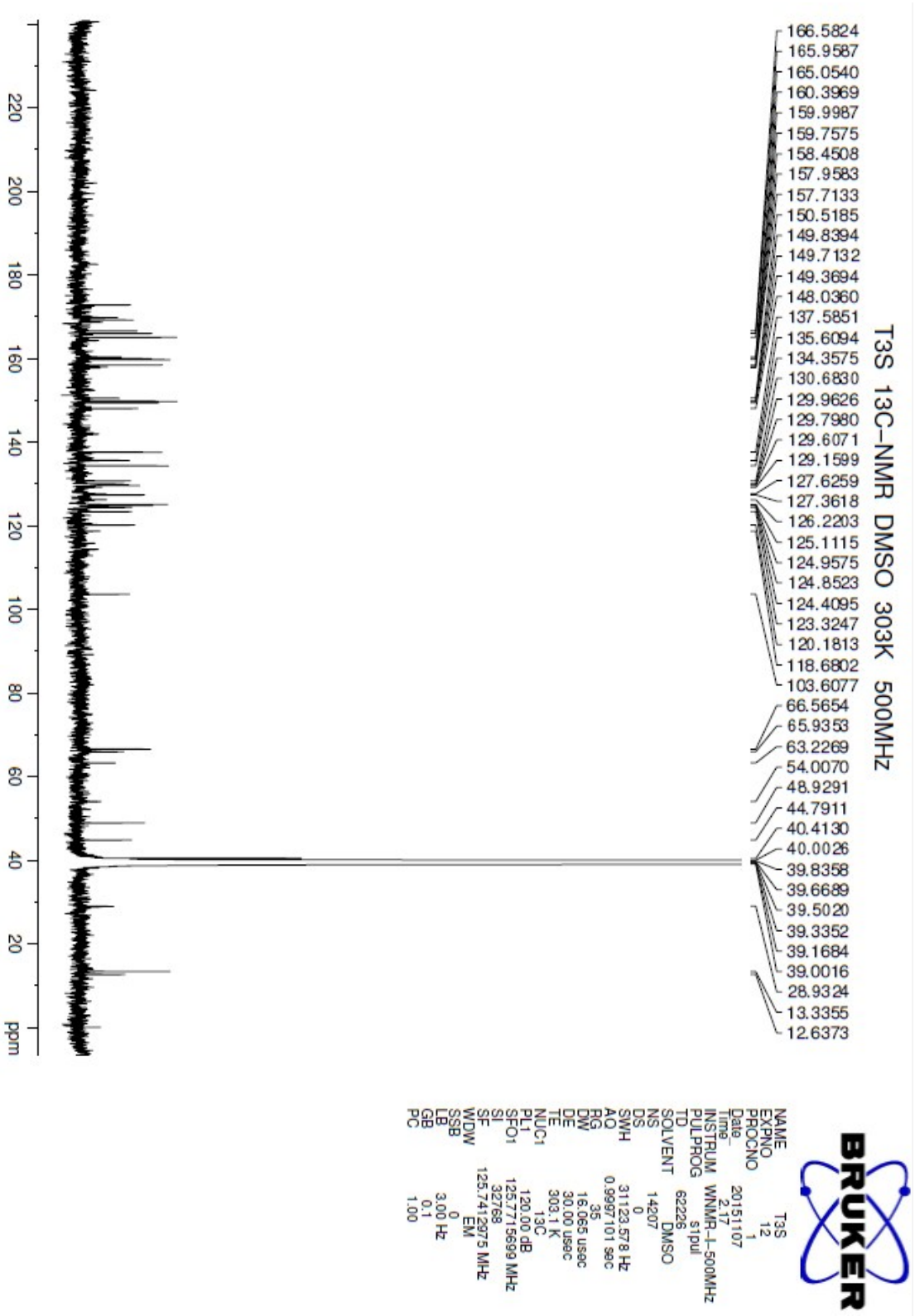
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60
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BRUKER

Fig. S8 Structure and numbering system used for analogue 8.

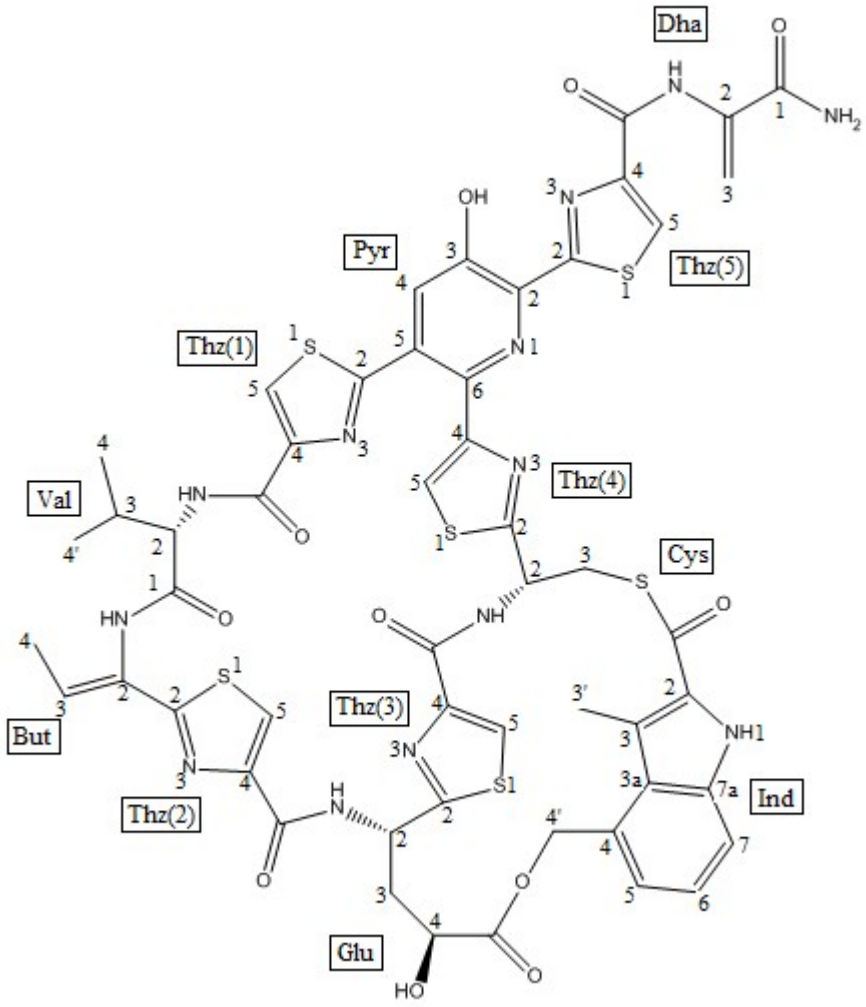
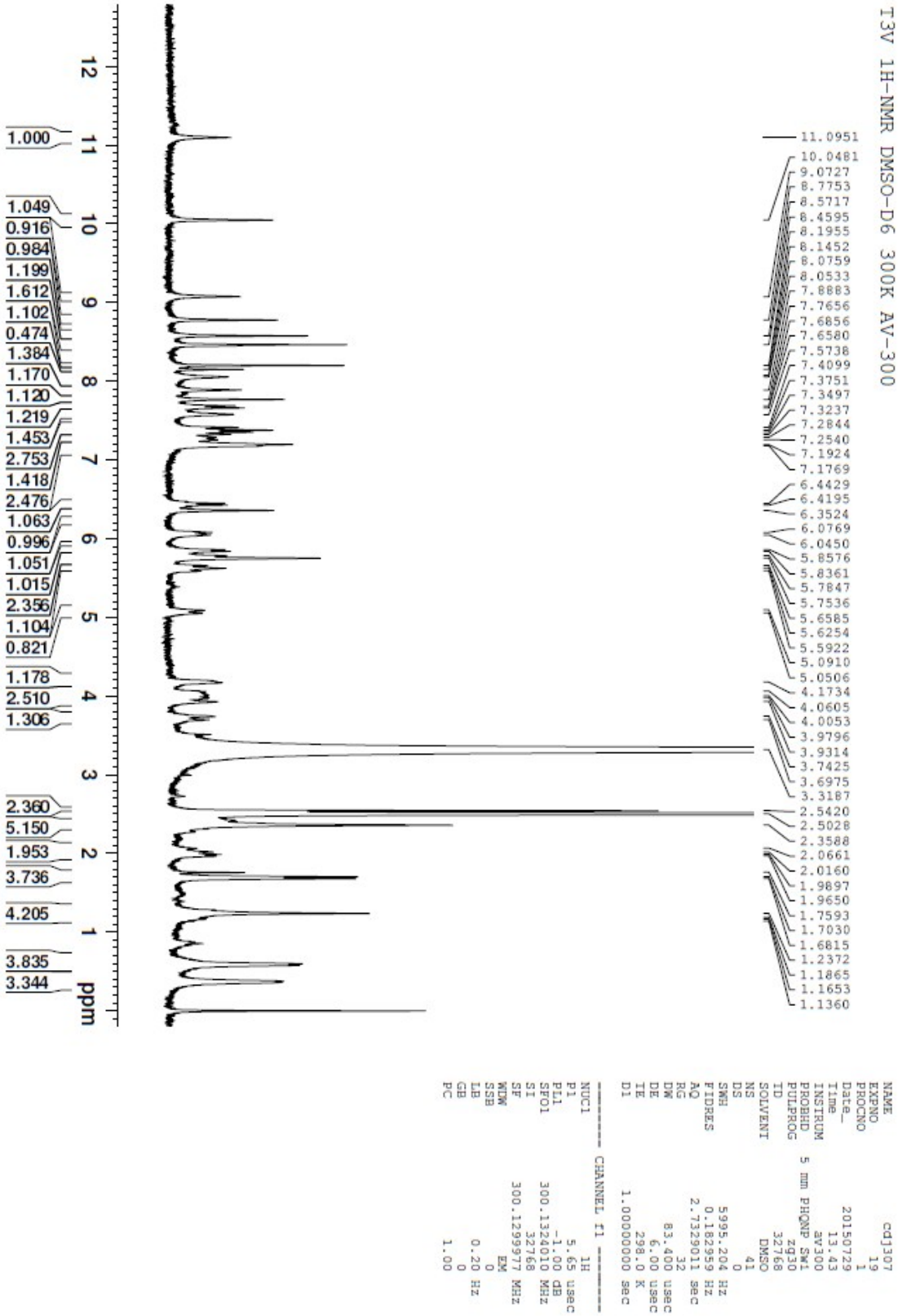


Fig. S9 NMR spectra of analogue 8. (A) H^{1}NMR (300 K, DMSO-d6). (B) C^{13}NMR (300 K, DMSO-d6).

A



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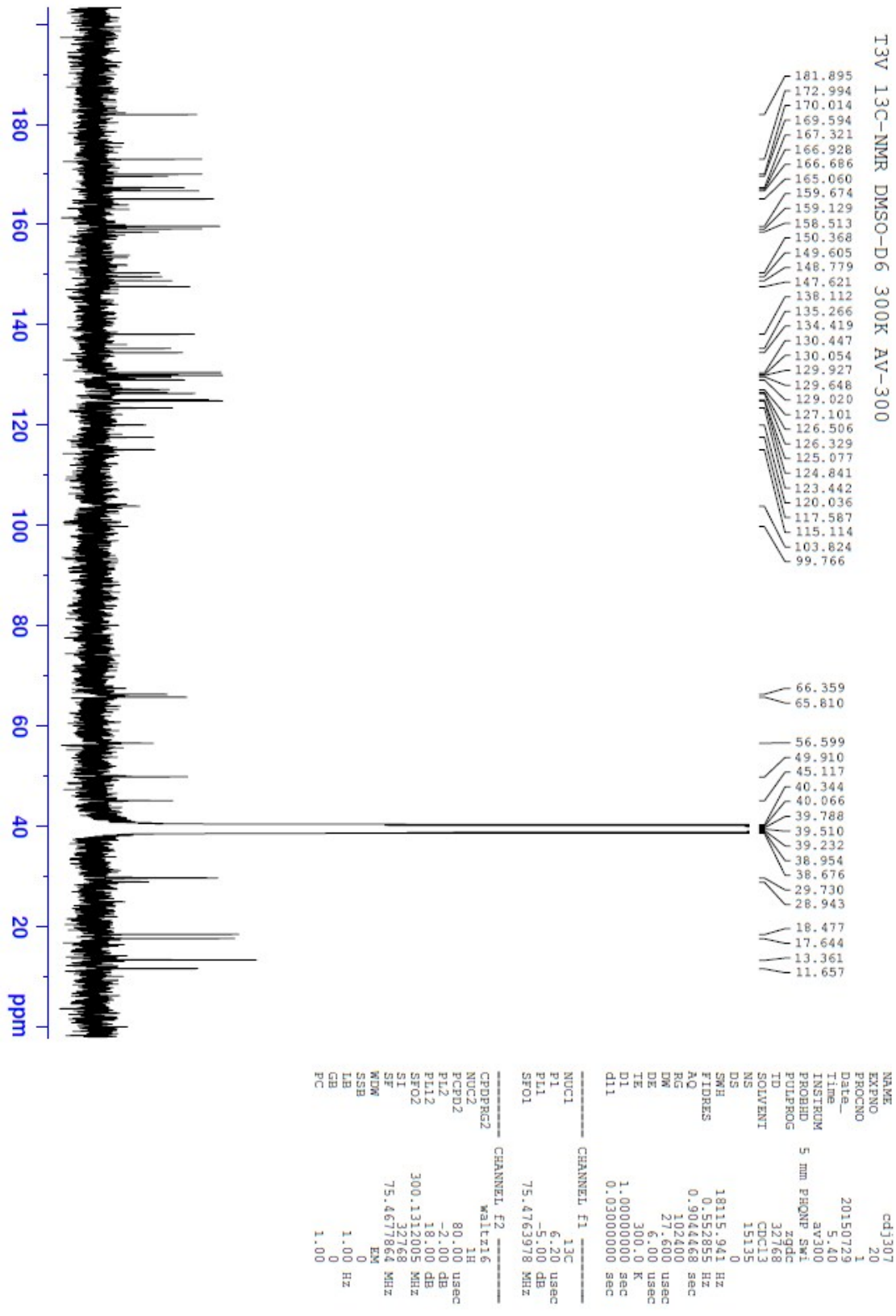
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169.594
167.321
166.928
166.686
165.060
159.674
159.129
158.513
150.368
149.605
148.779
147.621
138.112
135.266
134.419
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129.020
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125.077
124.841
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