Supplementary information

Preparation of recombinant strains

The first step was the construction of pKC16GNT, containing the four genes necessary for the formation of the indolocarbazole aglycon (*rebO*, *rebD*, *rebC* and *rebP*), the gene *staG* coding for a glycosyltransferase for the formation of the first N-glycosidic bond , the *staN* gene coding for a P-450 oxygenase for the second sugar-aglycon bond , and the rebeccamycin resistance gene *rebT*. For this purpose, a fragment of DNA including *staG* and *staN* was obtained by PCR using *Streptomyces longisporoflavus* DSM10189 total DNA and the oligonucleotides CS043 (5'-TATATTACTAGTCGCGGAGGCGACGTTGAC-3') and STAN2 (5'-TATCTAGAGTCAGTTCAGTACGGCGGGC-3'). This DNA fragment was cloned as a *Spe*I-*Xba*I fragment in the same sites of LITMUS 28 (New England BioLabs), generating the pLGTFstaN plasmid. This was followed by the preparation of pKC16GNT plasmid by the pKC016 tandem cloning at *Xba*I site (*Proc. Natl. Acad. Sci. USA* 2005, 102, 461-466), of three DNA fragments containing: *ermE** promoter (isolated as a *Hin*dIII-*Bam*HI fragment from pEM4 plasmid (*Mol. Microbiol.* 1998, 28, 1177-1185), pLGTFstaN insert (containing *staG* and *staN*) and *rebT* gene (obtained by PCR as described in *Proc. Natl. Acad. Sci. USA* 2005, 102, 461-466), respectively.

pKC16GNT was then introduced in *Streptomyces albus* J1074 (*J. Gen. Microbiol.* 1980, 116, 323-334), generating *Streptomyces albus* 16GNT strain. Plasmid introduction was carried out by protoplast transformation, following standard procedures (Kieser et al., *Practical Streptomyces genetics*, The John Innes Foundation, Norwich, UK, 2000). Starting with *Streptomyces albus* 16GNT strain, the following recombinant strains were obtained: *Streptomyces albus* 16GNT(pRHAM), *Streptomyces albus* 16GNT(pLNBIV), *Streptomyces albus* 16GNT(pLN2) and *Streptomyces albus* 16GNT(pLNR) through the introduction, separately, of the following plasmids: pRHAM, pLNBIV, pLN2 and pLNR, respectively. These four plasmids have been described previously (*J. Mol. Microbiol. Biotechnol.* 2000, 2, 271-276; *Chem. Biol.* 2002, 9, 721-729; *J. Nat. Prod.* 2002, 65, 1685-1689), and code for biosynthesis enzymes for the following sugars (as NDP or nucleosidyl diphosphate): L-rhamnose, L-digitoxose, L-olivose, and D-olivose, respectively.

Preparation of the compounds **3-9**

Preparation of the novel indolocarbazole analogs was carried out by preparative HPLC. For the obtention of compounds **3-9**, the strains *Streptomyces albus* 16GNT(pRHAM) *Streptomyces albus* 16GNT(pLNBIV), *Streptomyces albus* 16GNT(pLN2) and *Streptomyces albus* 16GNT(pLNR) were cultured initially in 50 ml TSB in the presence of 50 µg/ml thiostrepton and grown for 24h at 30°C and 250 rpm. After 24h, this preinoculum was used to innoculate at 2.5% 8 2L Erlenmeyer flasks, each containing 400mL medium. They were then incubated at 30°C and 250 rpm, during 4-5 days. Cultures were centrifuged at 12.000 rpm for 30 min. The majority of the compounds are found both in the culture as in the mycellium. Micelia were extracted with acetone, and supernatants were filtered using a 1µm Mini Profil cartridge (Pall). The filtered culture was submitted to a solid-phase extraction (SepPaK Vac C18, Waters). The retained compounds were eluted with a lineal gradient of MeOH and 0,1% TFA in water (0 - 100% MeOH in 60 min, at 10 ml/min), collecting fractions every 5 min.

Extracts were analyzed by HPLC, using a Waters Alliance system coupled to a 2996 diode array detector and a ZQ4000 mass spectrometer (Waters-Micromass). The column used was a C18 Symmetry (2,1 x 150 mm, Waters) using as mobile phase MeCN and 0,1% TFA in water. Elution started at 10% MeCN for 4 min, followed by a lineal gradient up to 88% MeCN at 30min, and

finishing with 100 % MeCN for 5 min, at a flow rate of 0.25ml/min. Mass spectroscopy analysis was done by electrospray ionization (ESI) in positive mode, with 3KV capillary voltage and 20, 60 and 100V cone voltage. The wavelength detection was 290nm for compounds with staurosporine-like spectrum and 316nm for compounds with rebeccamycin-like spectrum.

Following analysis, samples containing desired compounds were evaporated under vacuum, prior 0.1M pH 7.0 $NaHPO_4$ buffer addition to each sample. Extracts were dissolved in a mixture of DMSO and acetone (50:50), chromatographed in a μBondapak C18 radial compression cartridge (PrepPaK Cartridge, 25 x 100 mm, Waters), using as mobile phase mixtures of MeCN (or MeOH) and 0,1% TFA in water at 10 ml/min flow rate and collecting the compounds of interest in multiple injections. In other purifications, an XTerra column (7,8 x 300 mm, Waters) was used following the same procedure but at 3 ml/min flow rate. The fractions containing the purified product were diluted with 3 volumes of water and submitted to solid-phase extraction to eliminate the acid from the mobile phase and concentrate the compounds, which were lyophilized for final storage.

In this manner, the following compounds were obtained: from *S. albus* 16GNT(pRHAM), 1 mg N^{13}-1'-ß-L-rhamnosylarcyriaflavin (**3**) and 1.2 mg N^{12}-5'(*S*)-N^{13}-1'-(*R*)-L-rhamnosylarciriaflavin (**7**); from *S. albus* 16GNT(pLN2), 2.1 mg N^{12}-5'(*S*)-N^{13}-1'-(*R*)-L-olivosylarcyriaflavin (**8**) and 1.2 mg N^{13}-1'-ß-L-olivosylarcyriaflavin (**4**); from *S. albus* 16GNT(pLNR), 0.8 mg N^{13}-1'-ß-D-olivosylarcyriaflavin (**6**); and from *S. albus* 16GNT(pLNBIV), 1,6 mg N^{13}-1'-ß-L-digitoxosylarcyriaflavin (**5**) and 1.1 mg N^{12}-5'(*S*)-N^{13}-1'-(*R*)-L-digitoxosylarcyriaflavin (**9**).

Compounds **10-16** were prepared in a similar manner, as described in *Mol. Microbiol.* 2005, 58, 17-27.

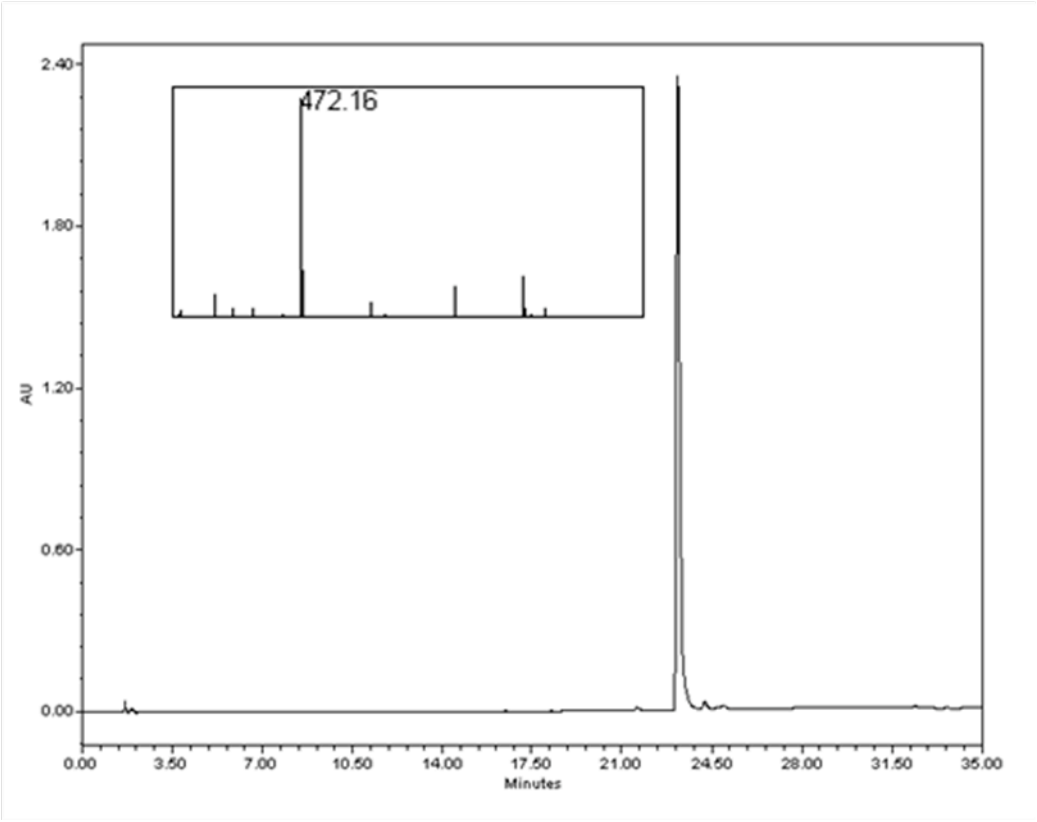
Characterization of the compounds:

Compounds were initially identified by HPLC/MS analysis by comparing UV absortion spectrum and analyzing the mass of the molecular ion. Analyses of *S. albus* 16GNT (pRHAM) strain extracts revealed two compounds, m/z 472 and 470, respectively, indicative of the presence of L-rhamnose joined by one and by both N atoms to the indolocarbazole ring (arcyriaflavin A) respectively. The chromatogram corresponding to the 16GNT(pLN2) strain extracts also showed two compounds bearing the typical indolocarbazole spectrum and m/z 454 and 456. These are the masses expected for the incorporation of L-olivose to the aglycon through one and two C-N bonds respectively. Strain 16GNT(pLNR) provided only one compound m/z 456 indicative of incorporation of D-olivose to only one of the N-atoms of the aglycon. Finally, strain 16GNT(pLNBIV) showed two compounds, m/z 456 and 454 indicating incorporation of L-digitoxose to arcyriaflavin A through one or two C-N bonds respectively.

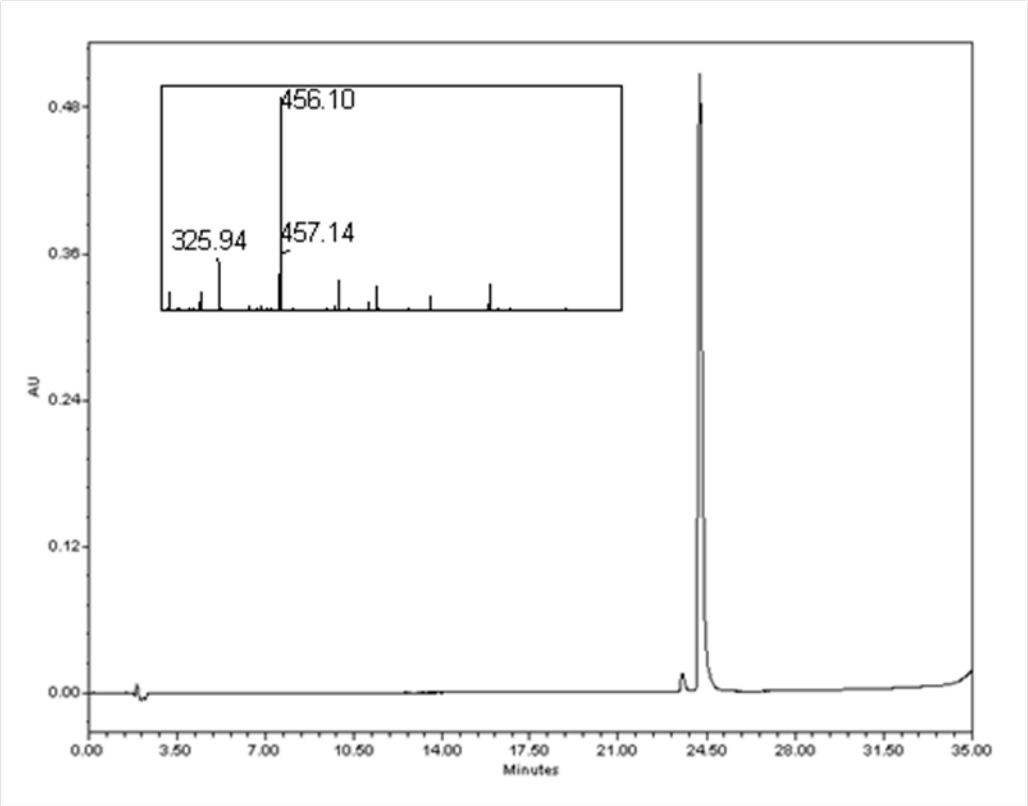
Final identification was carried out by ^{1}H- and ^{13}C-NMR. Sample preparation was done by dissolving 95-160 μg pure products in 200 μl acetone-d_6 and transferred to a 3mm NMR tube. Solvent signals were used as internal reference. NMR spectra were recorded at 298K using a Bruker Avance Ultrashield Plus 600 spectrometer equipped with a 5mm TCI cryoprobe. Homonuclear 2D ^{1}H, ^{1}H COSY/TOCSY, heteronuclear ^{1}H, ^{13}C HSQC/HMBC and selective NOE experiments were acquired. Typical parameters for 2D experiments were: COSY/TOCSY, 256 and 2048 points in F1 and F2, respectively, 16 transients each, 60 ms spin-lock in the TOCSY; HSQC, 256 and 2048 points in F1 and F2, respectively, 48 transients each; HMBC, 512 and 2048 points in F1 and F2, respectively, 64 transients each. Mixing time for 1D experiments sel-nOe was 400 ms. NMR experiments were processed using the program Topspin 1.3 (Bruker GmbH, Karlsruhe, Germany). Tables 1 to 7 of this supplementary material show data obtained for the compounds **3-9.**

Compounds **10-16** were characterized in a similar manner, as described in *Mol. Microbiol.* 2005, 58, 17-27.

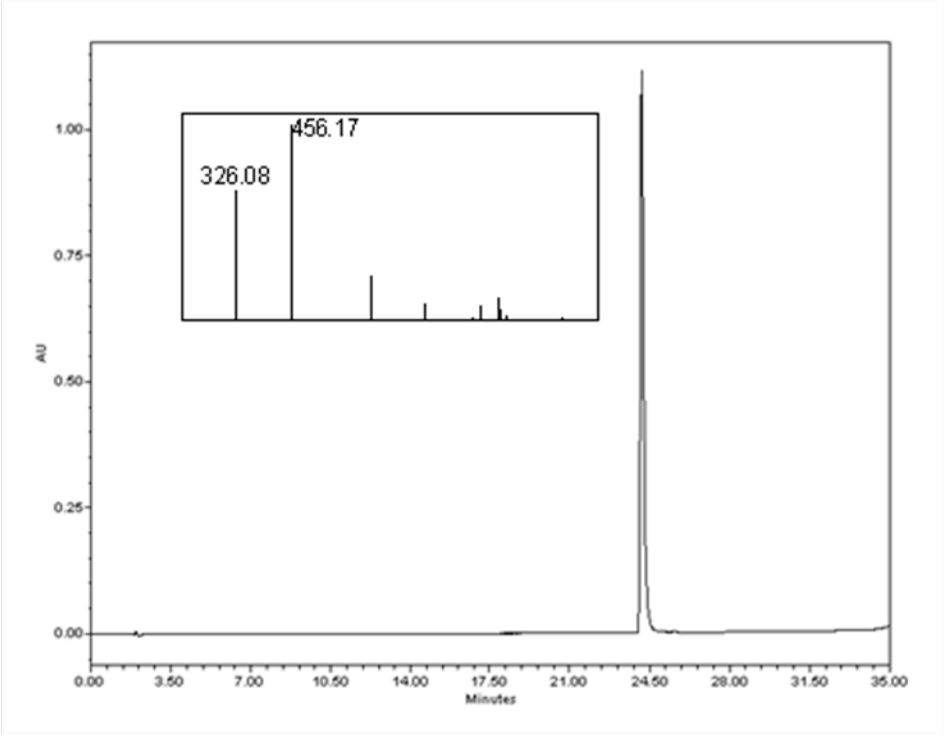
HPLC-MS for compound 3



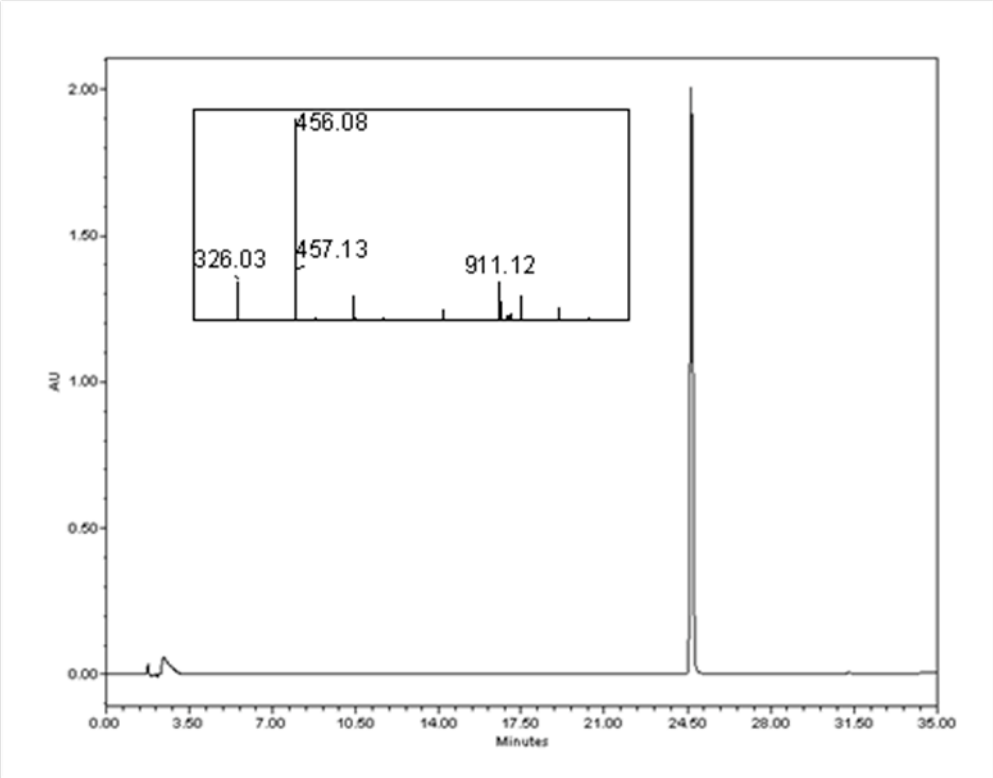
HPLC-MS for compound 4



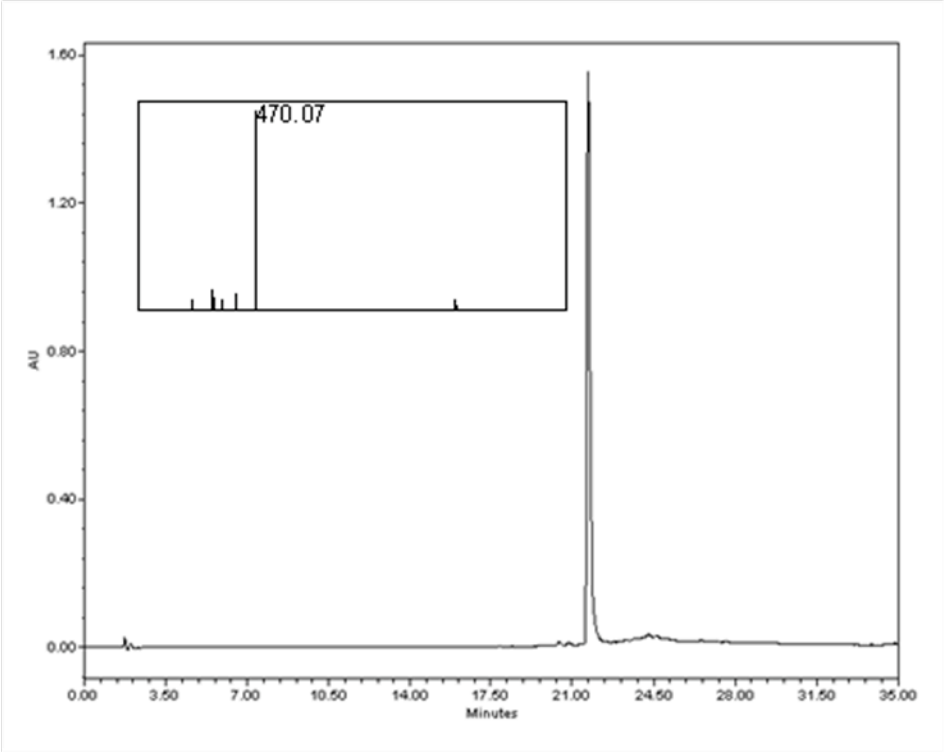
HPLC-MS for compound 5



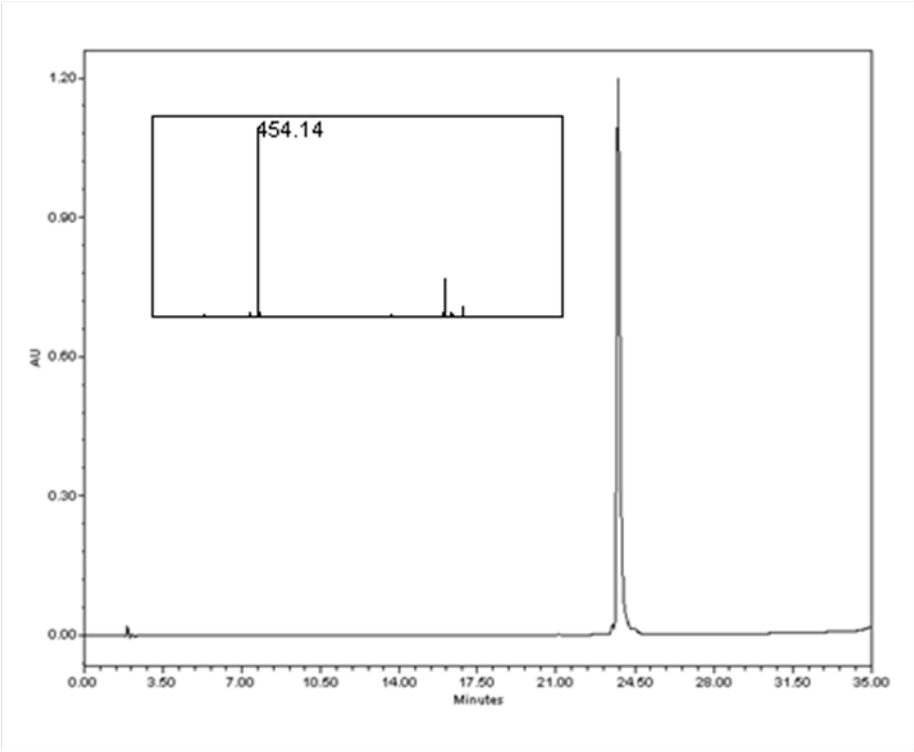
HPLC-MS for compound 6



HPLC-MS for compound 7



HPLC-MS for compound 8



HPLC-MS for compound 9

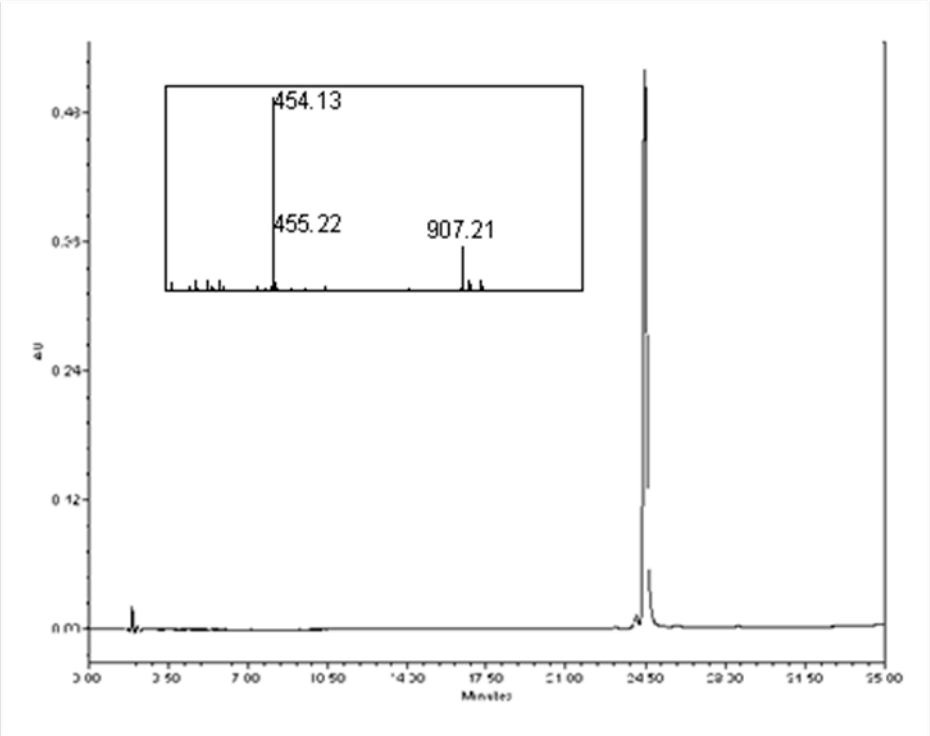


Table 1. NMR data for N^{13}-1'-ß-L-rhamnosylarcyriaflavin (compound **3**) in acetone-*d*6. (^{1}H, 600 MHz; ^{13}C, 150 MHz).

Position	$\delta^{13}C$ (ppm)	δ^{1}H (ppm)	Multipl.	*J* (H, H) (Hz)
1	110.8	7.82	d	8.3
2	127.4	7.60	td	7.5, 1.2
3	121.1	7.40	td	7.6, 0.8
4	125.7	9.33	d	7.6
4a	122.7	-		
4b	119.0	-		
4c	NO	-		
5	NO	-		
6	-	9.87	s	
7	NO	-		
7a	NO	-		
7b	117.8	-		
7c	122.4	-		
8	125.7	9.23	d	7.9
9	120.8	7.32	td	7.6, 1.1
10	127.3	7.53	td	7.5, 1.2
11	111.8	7.64	d	8.3, 8.4
12	-	11.72	s	
11a	141.6	-		
12a	NO	-		
12b	129.5	-		
13a	142.8	-		
1'	78.5	6.62	d	9.5
2'	68.7	4.77	ddd	9.9, 6.8, 3.3
2'OH	-	4.65	m	
3'	73.1	4.46	d	3.2
3'OH	-	4.65	m	
4'	72.3	4.34	d	3.6
4'OH	-	5.92	d	3.2
5'	78.1	4.68	dq	7.3, 1.3
5'CH3	15.4	1.82	d	7.3

Table 2. NMR data for N^{13}-1'-ß-L-olivosylarcyriaflavin (compound **4**) in acetone-*d*6. (^{1}H, 600 MHz; ^{13}C, 150 MHz).

Position	δ^{13}C (ppm)	δ^{1}H (ppm)	Multipl.	*J* (H, H) (Hz)
1	110.2	7.78	d	8.4
2	128.2	7.64	t	7.6
3	121.8	7.42	td	7.3, 0.8
4	126.5	9.36	d	8
4a	122.6	-		
4b	118.5	-		
4c	120.2	-		
5	NO	-		
6	-	9.87	s	
7	NO	-		
7a	120.2	-		
7b	116.2	-		
7c	122.1	-		
8	126.2	9.23	d	8.1
9	121.3	7.35	td	7.4, 1.1
10	127.8	7.54	td	7.1, 1.2
11	112.3	7.67	d	8.4
12	-	11.93	s	
11a	141.4	-		
12a	130.1	-		
12b	129.2	-		
13a	140.9	-		
1'	74.6	6.92	dd	11.6, 3.4
2'a	34.5	2.97	ddd	14.9, 11.9, 3.3
2'e	34.5	1.98	ddd	14.3, 3.1, 2.7
3'	69.9	4.46	m	
3'OH	-	4.86	m	
4'	70.3	4.07	m	
4'OH	-	5.83	m	
5'	78.4	4.73	q	7.5
5'CH3	15.6	1.84	d	7.2

Table 3: NMR data for N-1-β-D-diglucosylarcyriaflavin (compound **5**) in acetone-*d*6. (^{1}H, 600 MHz, ^{13}C, 150 MHz).

Position	δ^{13}C (ppm)	δ^{1}H (ppm)	Multipl.	*J* (H, H) (Hz)
1	110.0	7.92	d	8.7
2	127.4	7.60	td	7.4, 1.38
3	121.2	7.40	td	7.5, 0.8
4	125.8	9.36	d	7.9
4a	122.2	-		
4b	118.6	-		
4c	122.4	-		
5	NO	-		
6	-	9.86	s	
7	NO	-		
7a	122.4	-		
7b	118.0	-		
7c	122.1	-		
8	125.3	9.23	d	8
9	120.5	7.33	td	7.5, 0.8
10	127.2	7.53	dt	7.4, 1.1
11	112.1	7.74	d	7.8
11a	141.8	-		
12	-	12.18	s	
12a	130.1	-		
12b	128.9	-		
13	-			
13a	140.9	-		
1'	76.9	6.79	dd	11.7, 3.5
2'a	36.1	2.70	dd	12.5, 11.9
2'e	36.1	2.19	ddd	12.9, 5.0, 3.5
3'	65.3	4.62	ddd	11.5, 5.3, 2.0
3'OH	-	NO		
4'	71.6	4.15	t	2
4'OH	-	NO		
5'	76.5	4.73	dq	1.7, 7.2
5'CH3	14.8	1.67	d	7.2

Table 4: NMR data for N-1,3-D-olivosylarcyriaflavin (compound **6)** in acetone-*d*6. (^{1}H, 600 MHz, ^{13}C, 150 MHz).

Position	δ^{13}C (ppm)	δ^{1}H (ppm)	Multipl.	*J* (H, H) (Hz)
1	111.2	7.98	d	8.6
2	127.6	7.62	td	7.6, 1.0
3	121.7	7.43	td	7.3, 0.8
4	125.9	9.32	d	7.8
4a	122.9	-		
4b	119.5	-		
4c	NO	-		
5	NO	-		
6	-	9.95	s	
7	NO	-		
7a	NO	-		
7b	NO	-		
7c	122.9	-		
8	125.6	9.22	d	8
9	121.1	7.40	td	7.6, 0.8
10	127.7	7.60	td	7.6, 1.2
11	112.0	7.80	d	8.2
12		10.92	s	
11a	141.0	-		
12a	NO	-		
12b	NO	-		
13a	141.3	-		
1'	82.8	6.68	dd	11.2, 2.7
2'a	29.8	2.24	ddd	13.2, 11.2, 5.2
2'e	29.8	2.18	ddd	13.2, 5.2, 2.8
3'	72.0	4.10	m	
3'OH	-	NO	m	
4'	78.0	3.67	dd	9.5, 9.1
4'OH	-	NO	m	
5'	76.9	4.11	dd	9.5, 6.1
5'CH3	18.3	1.71	d	6.1

Table 5. NMR data for N^{12}-5'(S)-N^{13}-1'-(R)-L-rhamnosylarcyriaflavin (compound **7**) in acetone-*d*6. (^{1}H, 600 MHz; ^{13}C, 150 MHz).

Position	δ^{13}C (ppm)	δ^{1}H (ppm)	Multipl.	*J* (H, H) (Hz)
1	109.6	7.71	d	8.3
2	127.8	7.65	td	7.1, 1.1
3	120.9	7.46	td	6.8, 0.8
4	126.0	9.26	d	7.9
4a	122.8	-		
4b	NO	-		
4c	NO	-		
5	NO	-		
6	-	9.90	s	
7	NO	-		
7a	NO	-		
7b	NO	-		
7c	124.2	-		
8	126.0	9.38	d	8.3
9	120.8	7.36	td	7.8, 0.8
10	126.1	7.51	td	6.8, 1.5
11	117.4	8.25	d	8.6
11a	142.8	-		
12a	NO	-		
12b	129.6	-		
13a	139.0	-		
1'	88.2	6.72	d	1.8
2'	72.7	4.47	s	
2'OH	-	5.29	m	
3'	65.9	3.87	dd	11.1, 2.6
3'OH	-	4.11	m	
4'	74.4	4.66	dd	11.1, 3.7
4'OH	-	4.95	m	
5'	97.6	-		
5'CH3	29.7	2.46	s	

Table 6. NMR data for N12-5'(S), N13-1'(R)-L-olivosylarcyriaflavina (compound **8**) in acetone-*d*6. (^{1}H, 600 MHz; ^{13}C, 150 MHz).

Position	δ^{13}C (ppm)	δ^{1}H (ppm)	Multipl.	*J* (H, H) (Hz)
1	109.5	7.71	d	8.3
2	127.4	7.64	td	7.7, 1.1
3	121.2	7.44	td	7.4, 0.8
4	125.9	9.26	d	8.3
4a	123.0	-		
4b	117.9	-		
4c	NO	-		
5	NO	-		
6	-	9.89	s	
7	NO	-		
7a	NO	-		
7b	117.9	-		
7c	124.4	-		
8	125.2	8.89	d	7.9
9	120.7	7.35	t	7.1
10	126.5	7.50	dt	8.0, 1.5
11	116.7	8.22	d	8.7
11a	142.5	-		
12a	NO	-		
12b	129.6	-		
13a	138.6	-		
1'	83.3	6.95	dd	4.9, 1.5
2'a	38.9	2.58	ddd	13.8, 11.7, 5.0
2'e	38.9	2.64	ddd	13.8, 4.2, 1.5
3'	63.2	3.85	ddd	11.5, 9.8, 4.2
3'OH	NO	3.81	s	
4'	81.0	4.10	t	9.8
4'OH	-	4.10	s	
5'	97.3	-		
5'CH3	29.3	2.46	s	

Table 7. NMR data for N^{12}-5'(S)-N^{13}-13(R)-L-digitoxosylarcyriaflavin (compound **9)** in acetone-*d*6. (^{1}H, 600 MHz; ^{13}C, 150 MHz).

Posición	δ^{13}C (ppm)	δ^{1}H (ppm)	Multipl.	*J* (H, H) (Hz)
1	109.3	7.65	d	8.3
2	127.0	7.6	td	7.0, 1.3
3	120.9	7.40	td	7.3, 1.1
4	125.8	9.24	d	8
4a	123.1	-		
4b	116.1	-		
4c	NO	-		
5	NO	-		
6	-	9.78	s	
7	NO	-		
7a	NO	-		
7b	117.5	-		
7c	124.5	-		
8	125.3	9.36	d	8.2
9	120.5	7.32	dt	7.6, 0 8
10	126.3	7.48	dt	7.9, 1.6
11	116.3	8.18	d	8.7
11a	142.3	-		
12a	NO	-		
12b	131.2	-		
13a	139.1	-		
1'	80.3	6.82	dd	5.6, 1.4
2'a	34.8	2.80	ddd	15.0, 3.0, 5.6
2'e	34.8	2.70	ddd	15.0, 3.4, 1.4
3'	65.1	4.27	ddd	3.0, 3.0, 3.0
3'OH	-	NO		
4'	74.4	4.30	d	3
4'OH	-	NO		
5'		-		
5'CH3	30.3	2.40	s	

Kinase assays:

Inhibition of protein kinases was measured for compounds **3-9** and **10-16**. Analysis of kinase activity was carried out by using LabChip technology (Caliper Life Sciences), especifically by the instruments Caliper LC3000 and EZ Reader II.

The kinase assay employed measures the conversion of a fluorescent peptide (substrate) to a phophorylated product. The reaction mixture, in a microtiter plate, is introduced by means of a capillary tube into a chip where the non-phosphorylated substrate and phophorylated product are separated by electrophoresis and detected by laser-induced fluorescence. The intesity of the fluorescent signal over time is related to the progress of the reaction. The phophorylated product migrates faster than the non-phosphorylated substrate and the signals of the two peptide forms appear as two differentiated peaks. Caliper's software determines the height of the peaks and calculates the ratio of product to the sum of peaks (P/(P+S)). This value is used to compare wells with compounds and wells with controls, therefore determining the % inhibition for a given compound.

The following kinases were used for the enzymatic inhibition studies, between brackets ATP Michaelis-Menten constant, Km, for each kinase, in μM: obtained from Carna Biosciences: MAPKAPK2 (4.6), AurA (3.6), AurB (5), PKCζ (3.8), RSK1 (23.3), PRAK (5), Erk1 (33.4), PKD2 (32.1), CK1d (16.3), CHK1 (33), ABL (61.7), FYN (36), LYNa (17), CHK2 (57.8), MET (79.5), LCK (28.5), SRC (38), GSK3ß (7.3), Erk2 (62.1), PKACα (1.7), AKT2 (186.1), INSR (871.8), p38α (396.5), AKT1(48), MSK1 (21.2), PKCß2 (84.8), ROCK2 (3.3), PIM2 (4.9), AMPK (38.6), KDR (164.8), IRAK4 (196.5), SGK1 (121.8), SYK (33,5); obtained from Invitrogen: CDK2 (57.6), BTK (123), HGK (80); obtained from Upstate: MST2 (36.6), PKGα (16), PAK2 (1.9), IGF1R (320), FGFR1 (171), MARK1 (33), CAMK2δ (22.4), c-TAK1 (66), DYRK1a (18.1), CaMK4 (3.9), FLT3 (350), c-Raf (6.2), P70S6K (95).

Compounds were dissolved in 100% DMSO and were diluted 25 times the desired assay concentration. For the determination of IC_{50} values, serial dilutions were carried out along 8 concentrations to provide the inhibition curve. 1μL of each concentration is transferred, in duplicate, to a 384-well microplate. To each well, the following is added: 12μL enzymatic buffer containing the purified kinase (from the providers indicated above), 100 mM HEPES, pH 7.5, 1 mM DTT (Calbiochem), 0.002% Brij-35 (Sigma) and 10mM $MgCl_2$ as cofactor, except for INSR and IRAK4 for which 10mM $MnCl_2$ is used instead of $MgCl_2$. For kinases CAMK2δ and CAMK4, 1mM $CaCl_2$ and 6.7 μg/mL calmodulin are also added. For KDR kinase, 0,05% CHAPSO is also added. For the c-Raf kinase, 10mM $MnCl_2$ is also added. For PKCß2 kinase, 0.02 μg/mL PS/PMA is also added. For PKGα kinase, 10μM cGMP is also added. The compound and the enzyme are preincubated for 15 min, and then 12 μL peptide/ATP buffer containing 100 mM HEPES, pH 7.5, 1.5 μM fluorescein-labeled peptide (specific for the corresponding kinase), ATP (concentration equals its K_M for each kinase), and 0.002% Brij-35 are added to each well to initiate the reaction. Reactions are generally incubated for 1-1.5h at room temperature to obtain an adequate conversion (15-40%) of peptide to phosphorylated product. Reactions were terminated through the addition of 45μL buffer containing 10mM EDTA. Microplates were read by the LabChip 3000 equipment using a 12-channel LabChip. The P/(P+S) values were obtained as described above and the IC_{50} curves were generated u sing Xlfit software.

Table 8 shows the % inhibition obtained by compounds **3-6** and **10-13** when used at 100nM and 10nM concentrations. For example, Dyrk1a kinase is 87% inhibited by compound **3** and completely inhibited by compound **10** when used at 10nM concentration.

Table 8. Kinase assay of indolocarbazole compounds glycosylated only at 1N: N^{13}-1'-ß-L-rhamnosylarcyriaflavin [compound 3], N^{13}-1'-ß-L-olivosylarcyriaflavin [compound 4], N^{13}-1'-ß-L-digitoxosilarcyriaflavin [compound 5], N^{13}-1'-ß-D-olivosylarcyriaflavin [compound 6], N^{13}-1'-ß-L-rhamnosyl-k252c [compound 10], N^{13}-1'-ß-L-olivosyl-k252c [compound 11], N^{13}-1'-ß-L-digitoxosyl-k252c [compound 12], N^{13}-1'-ß-D-olivosyl-k252c [compound 13]. Numeric values refer to the remainder kinase activity (%) after treatment with the assayed compound at the concentrations of 10nM and 100nM.

	3		4		5		6		10		11		12		13	
Kinase	100nM	10nM	100nM	10nM	100nM	10nM	100nM	10nM	100nM	10nM	100nM	10nM	100nM	10nM	100nM	10nM
AmpKa1	11	1	21	3	28	8	33	5	7	1	10	-3	1	2	9	-3
AurA	5	0	19	4	22	4	13	3	3	2	24	2	5	1	20	3
CamK2a	3	-5	29	36	58	15	46	4	2	-6	27	-6	26	-7	29	-6
Chk1	15	-3	35	5	81	45	50	2	8	2	27	1	46	6	20	4
Dyrk1a	103	87	75	32	87	49	94	62	115	101	89	42	73	15	50	14
Erk2	-4	-6	-3	-2	0	-4	30	-1	-1	-3	3	-4	0	-4	-2	-4
FGFR1	4	3	21	3	34	7	25	1	5	1	7	-1	5	1	7	-2
FGFR3	-4	-10	3	-6	-1	-6	-11	-8	-1	-2	-6	-4	-11	-6	-13	-20
FLT3	25	-10	81	20	95	63	98	51	35	-7	89	34	79	20	106	76
GSK3b	72	21	69	21	81	42	69	18	8	3	9	2	7	1	0	0
HGK	89	30	62	9	90	54	83	35	91	44	76	16	78	20	89	36
Ikkb	32	-10	60	9	76	37	50	7	12	-5	33	-5	24	27	16	-5
JAK2	33	-3	74	19	65	23	88	42	39	7	79	14	25	-4	89	37
KDR	21	-7	68	21	63	28	40	2	6	-1	48	7	16	1	18	-4
MST2	10	2	15	2	54	16	65	13	19	4	33	4	34	7	78	12
p38a	-1	0	-2	-2	-2	1	-1	-1	0	-1	-3	-2	-2	-1	-3	-1
PDK1	46	-6	71	7	97	61	76	21	15	-10	81	9	94	30	81	3
RSK1	20	2	77	14	77	38	74	31	7	-4	56	7	22	-1	31	-1
SGK1	13	8	17	9	37	17	27	12	6	7	15	8	14	6	12	9
SYK	-8	2	35	11	24	13	31	5	10	8	30	12	11	-1	29	7

Cell proliferation assays

All 14 compounds (7 rebeccamycin and 7 staurosporine derivatives) were tested for cytotoxicity against three different tumor cell lines: MDA-MB-231 (breast), A549 (lung, NSCL) and HT29 (colon). Cellular growth and viability was measured using a colorimetric assay based on a reaction with sulphorhodamine B (SRB), according to the technique described by Faircloth *et al.* (*Journal of Tissue and Culture Methods* 1988, 11, 201-205).

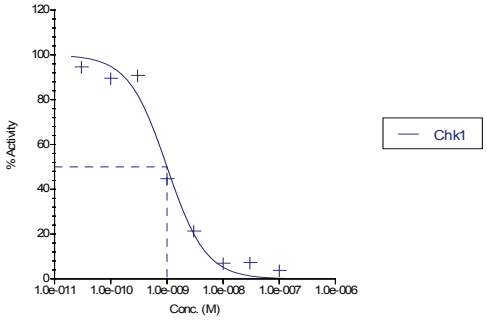
96-well microtiter wells are inoculated with cells ($5x10^3$ cells per well) in 195 μl medium aliquots, icubated for 18 h, without adding compound, to allow cells to adhere to the surface. Then, test compounds are added, as 5 μl samples, in a concentration range of 10 to 10^{-8} μg/ml, dissolved in DMSO/EtOH (0.2% in PS buffer). After 48 h exposure, the antitumoral effect is measured using the Sulphorhodamine B assay (SRB): cells are fixed by addition of 50 μl cold 50% (w/v) trichloroacetic acid and are incubated for 60 min. at 4ºC. Plates are washed with deionized water and dried. 100 μl of SRB solution (0.4% w/v in 1% acetic acid) are added to each well, and plates are incubated for 10min at room temperature. Non-bound SRB is eliminated by washing with 1% acetic acid. Plates are air-dried and the bound dye is dissolved in Tris buffer. Optical densities are read at 490nm using an automated spectrophotometer.

Table 9. Antiproliferative activity for glycosylated indolocarbazoles. GI50 in μM.

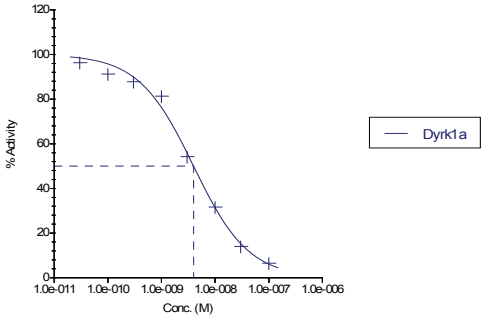
Compound	Cell line		
	MDA-MB-231 (breast)	A549 (lung, NSCL)	HT29 (colon)
1 (Reb)	-	0.76	1.55
3	4.40	4.18	2.42
4	14.1	8.79	8.79
5	4.19	4.41	6.39
6	2.43	2.21	2.16
7	1.35	2.04	2.45
8	7.50	5.73	11.2
9	8.16	5.51	8.16
2 (Sta)	0.013	0.008	0.076
10	1.97	2.84	2.10
11	1.43	1.56	1.52
12	2.49	3.40	3.40
13	3.17	4.98	3.85
14	0.59	0.61	0.87
15	1.05	1.02	1.21
16	1.84	1.96	1.52

Compound 7 IC_{50} curves

Chk1



Dyrka1



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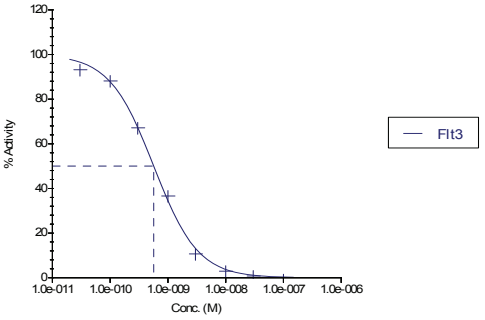
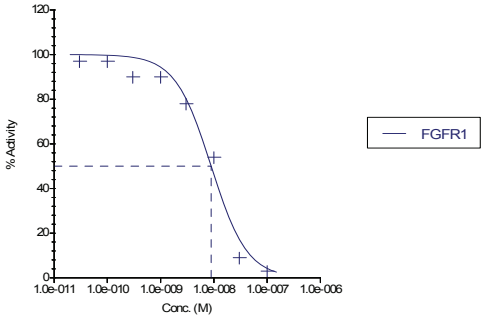
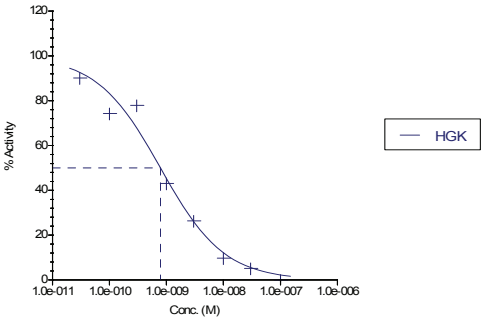


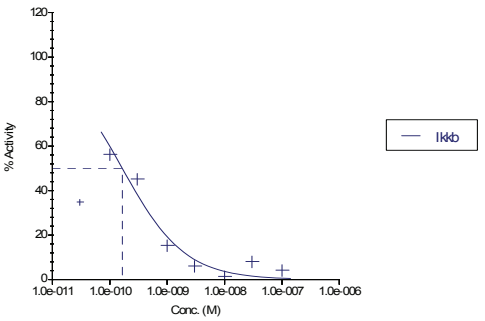
FGFR1



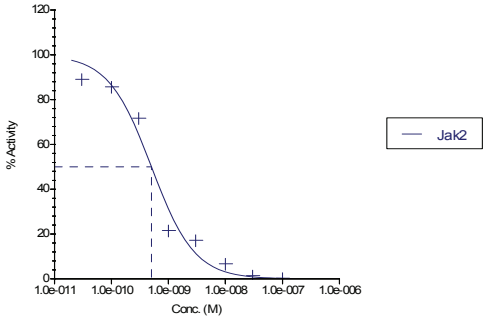
HGK



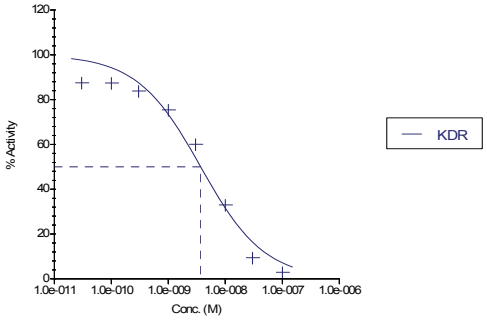
Ikkb



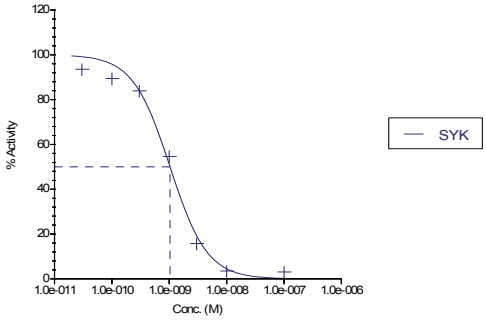
Jak2



KDR



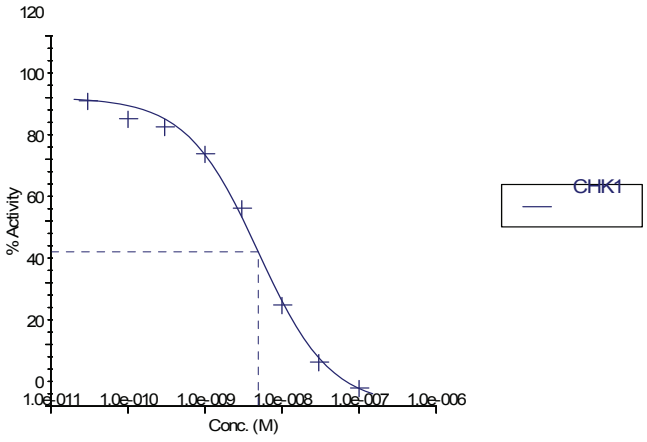
SYK



Compound 8

IC_{50} curves

Chk1



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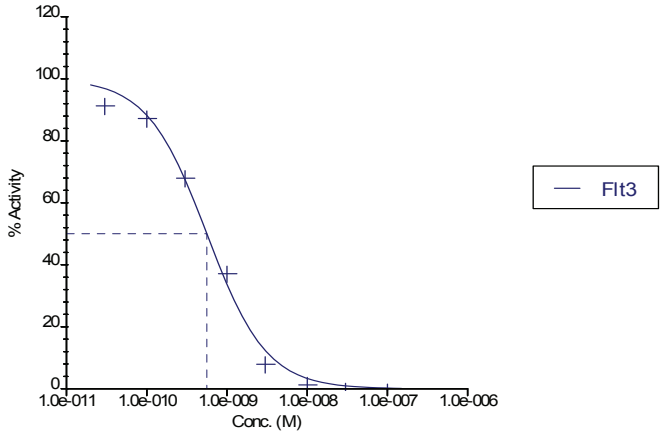

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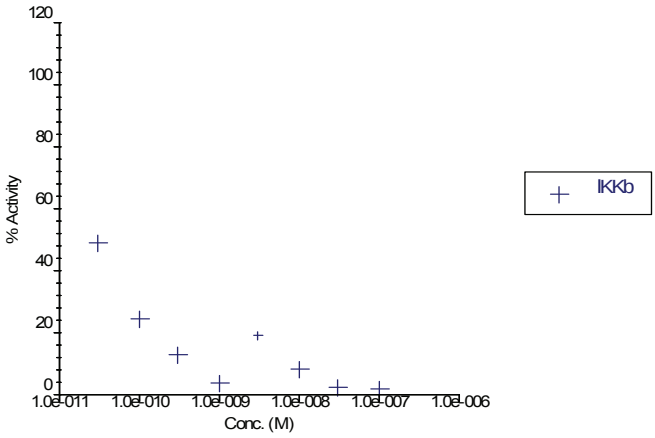

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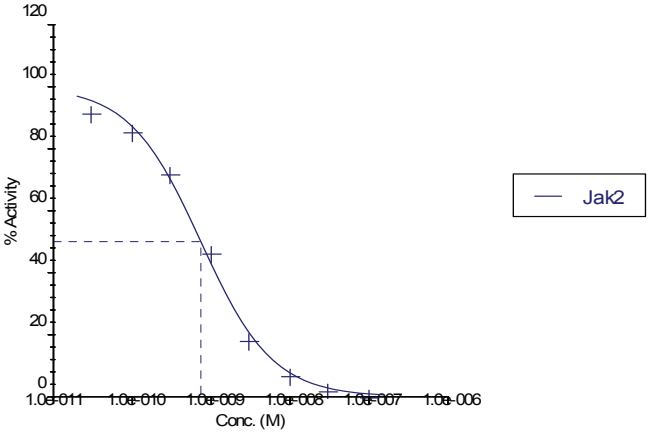

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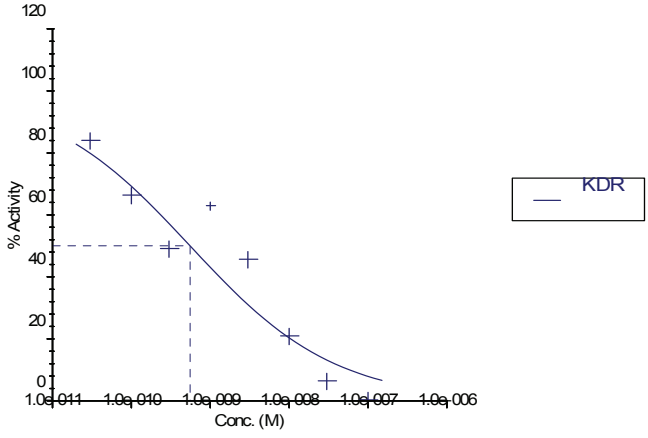
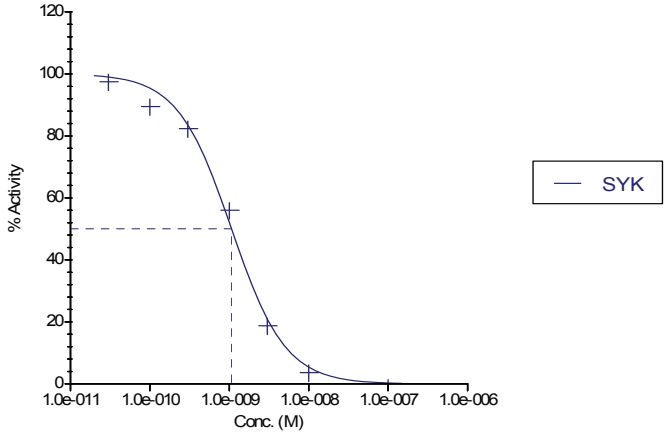
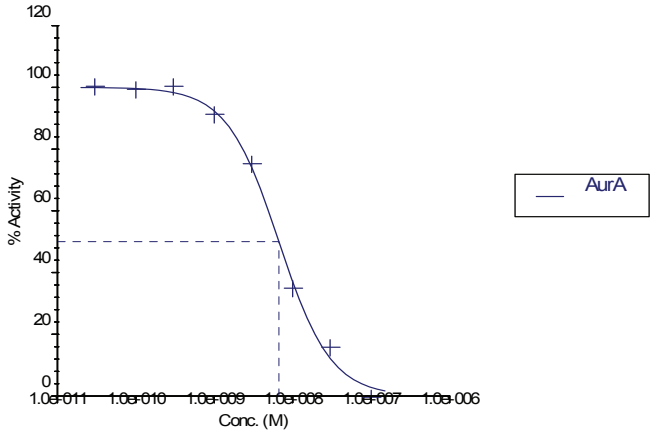


SYK

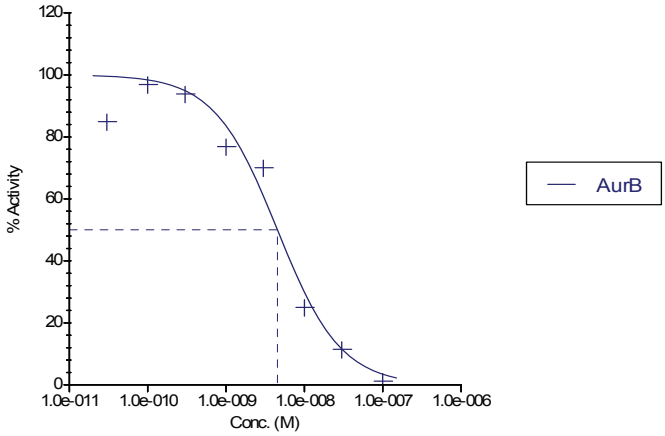


Compound 9 IC_{50} curves

AurA



AurB



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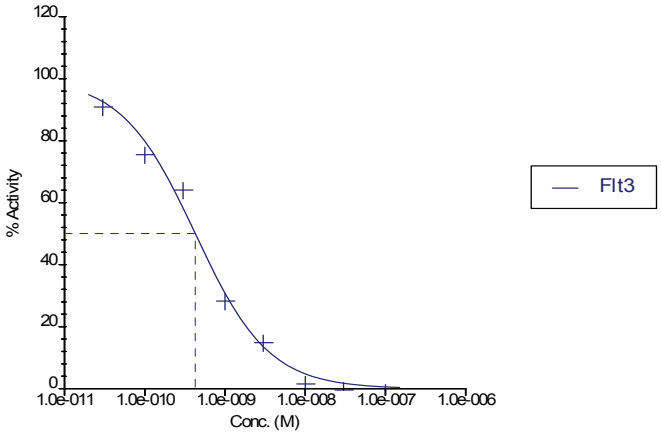
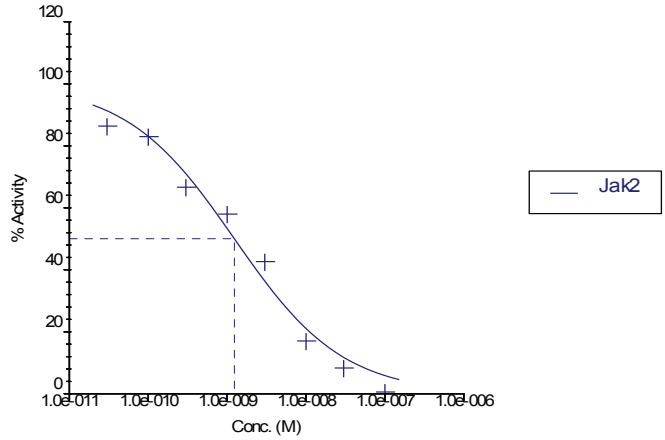


Jak2



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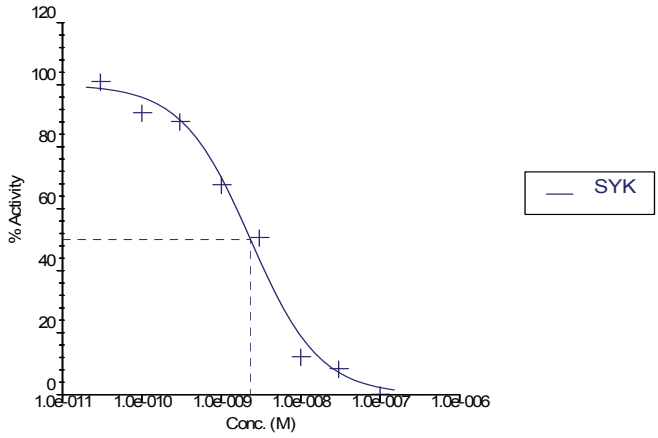
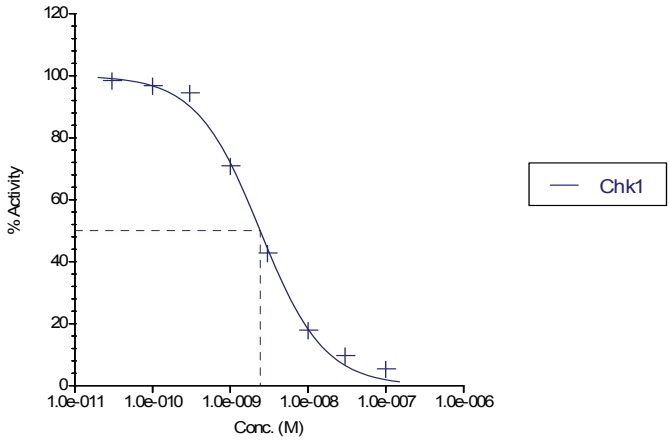


Compound 14 IC_{50} curves

Chk1



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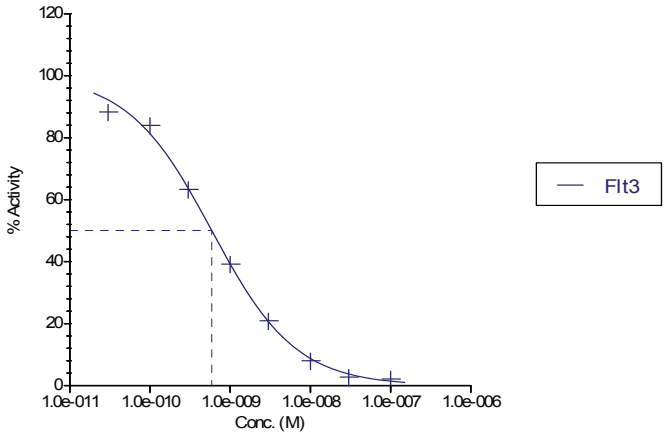

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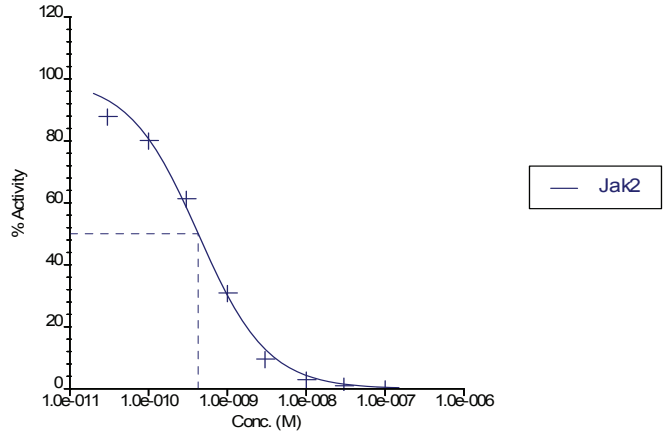
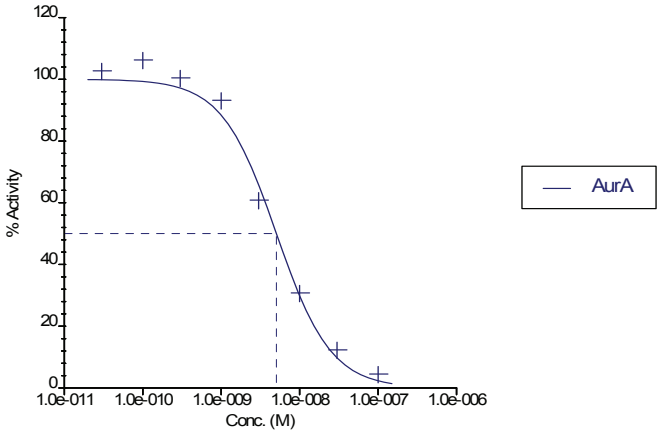


Compound 15

IC_{50} curves

AurA



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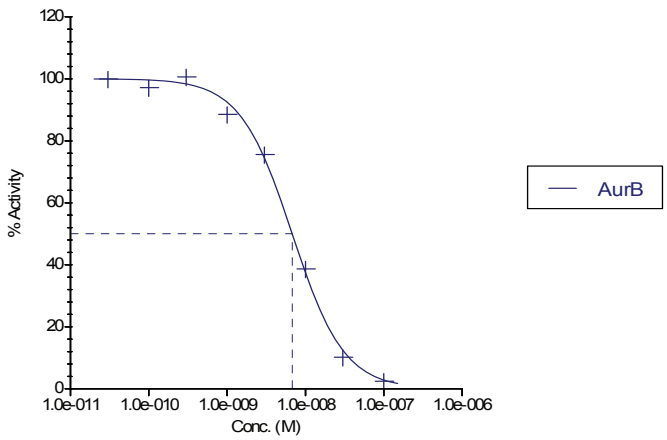

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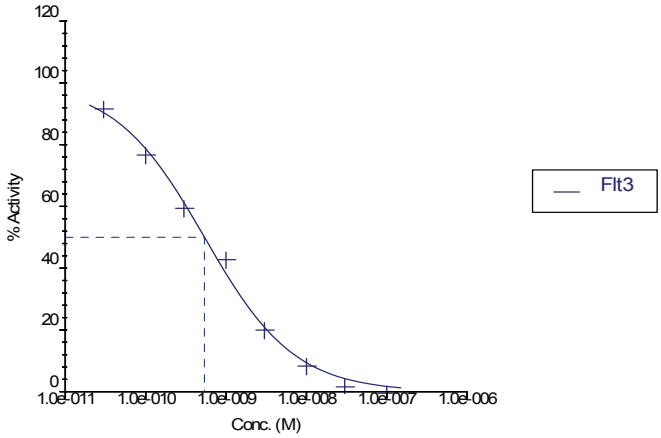

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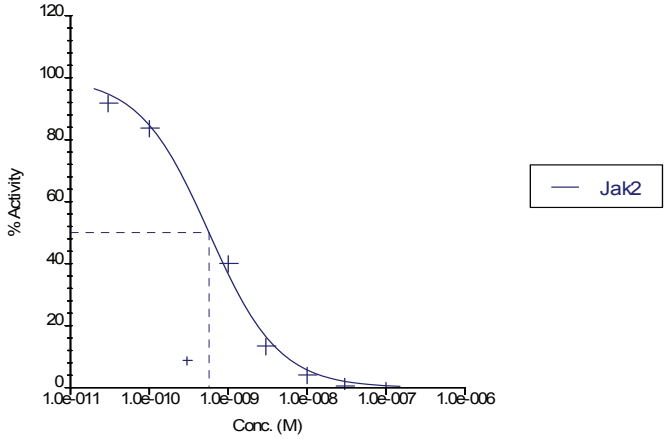
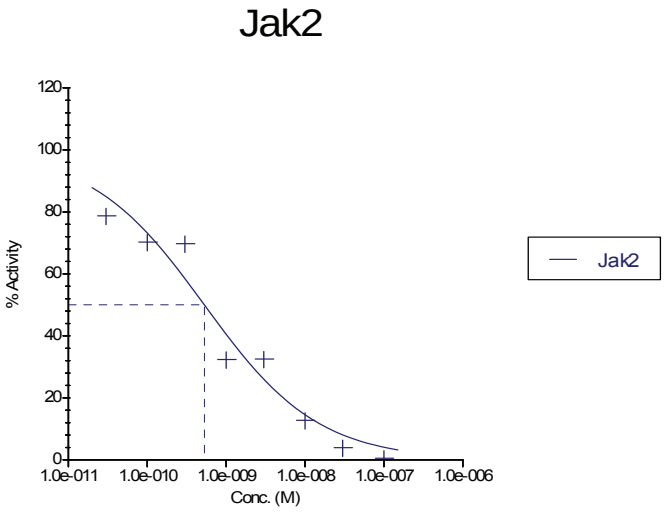
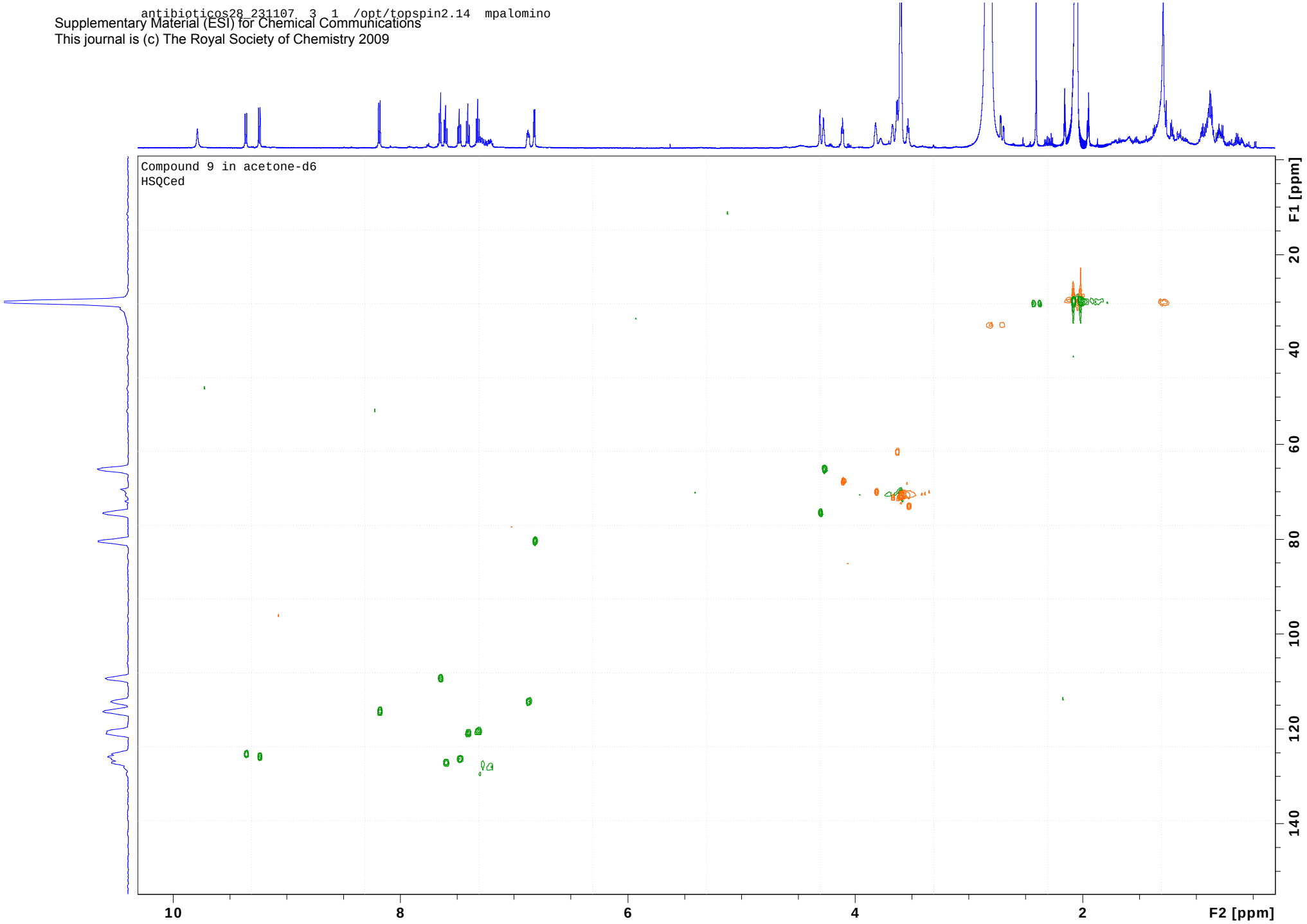
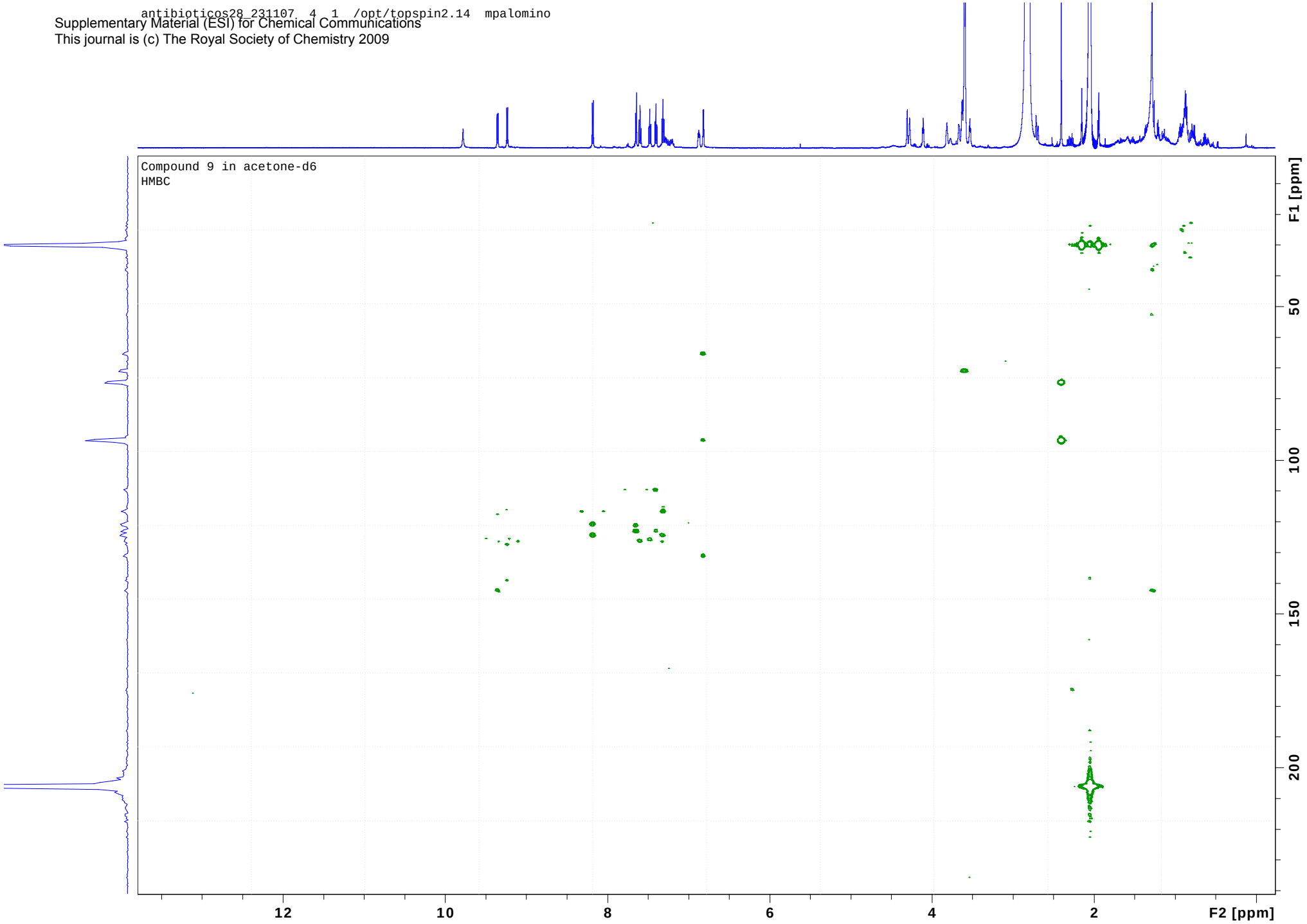


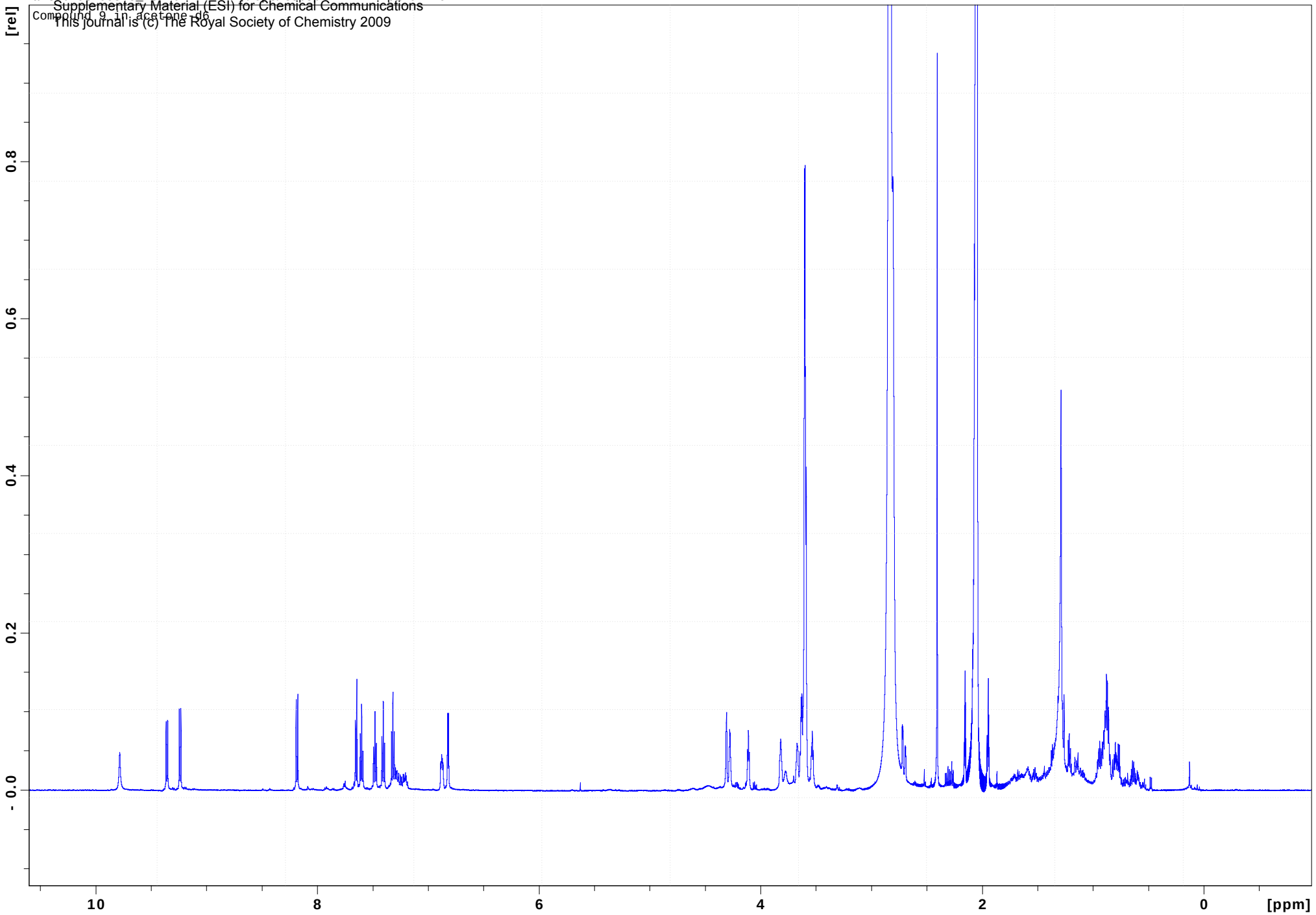
Compound 16

IC_{50} curves





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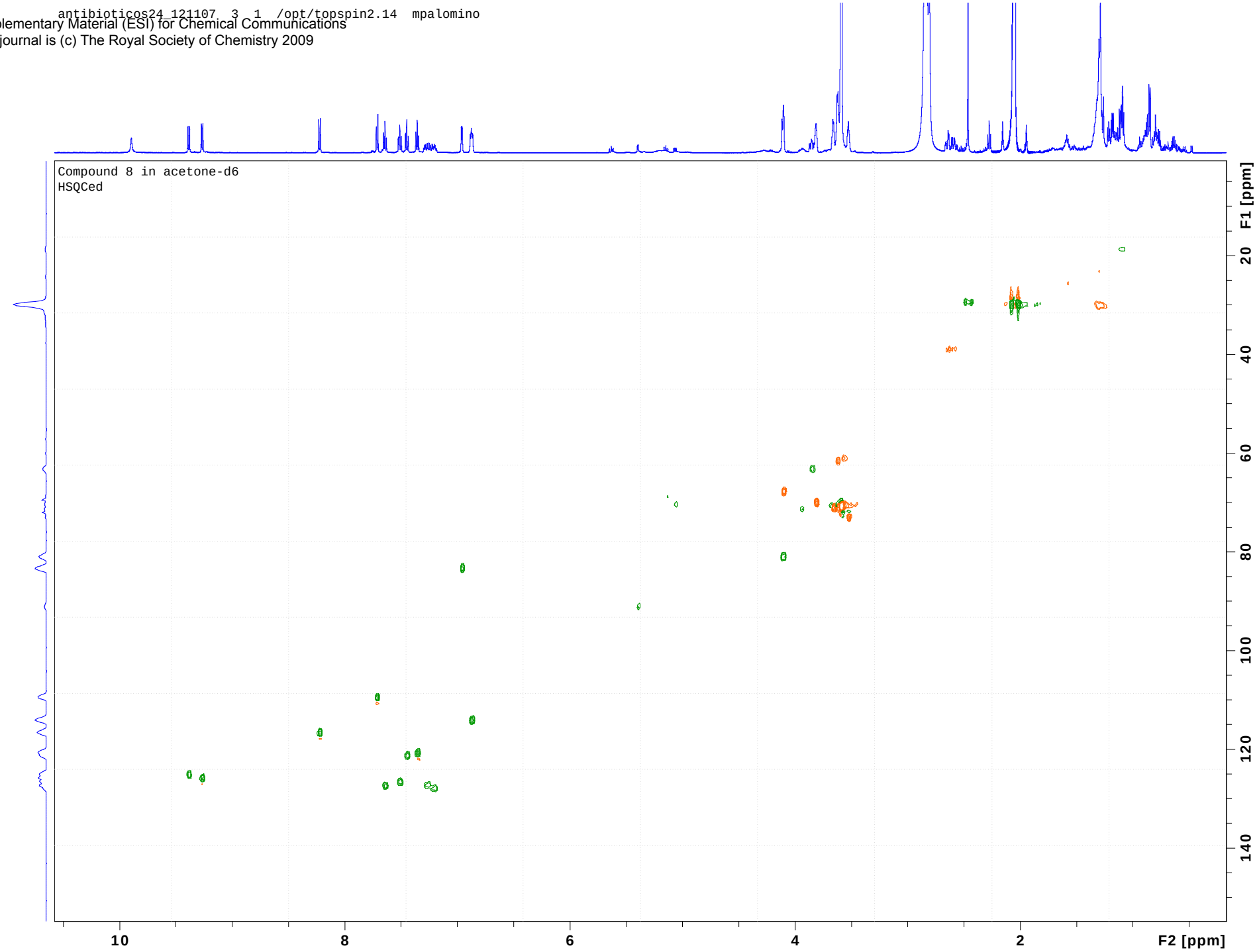

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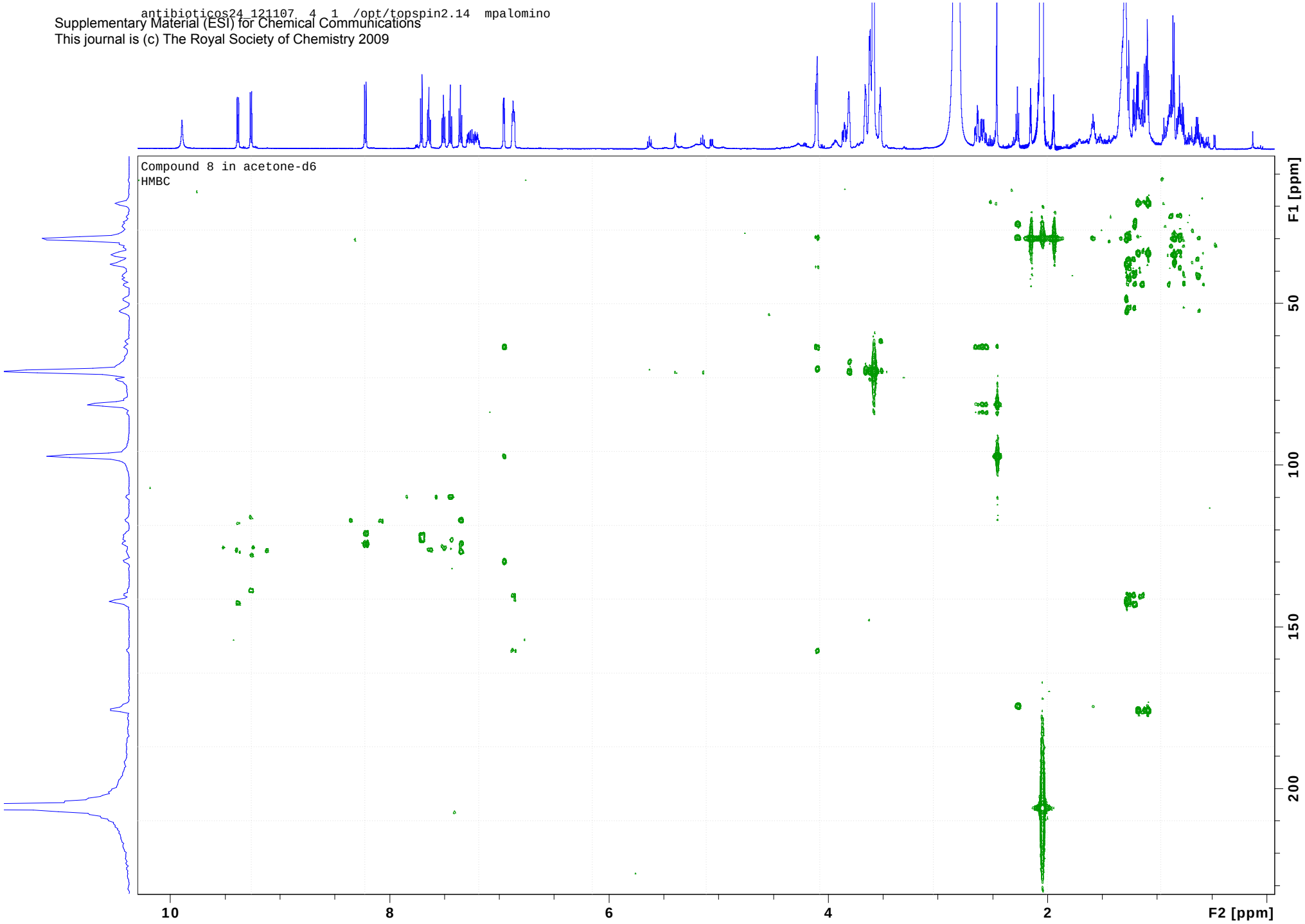

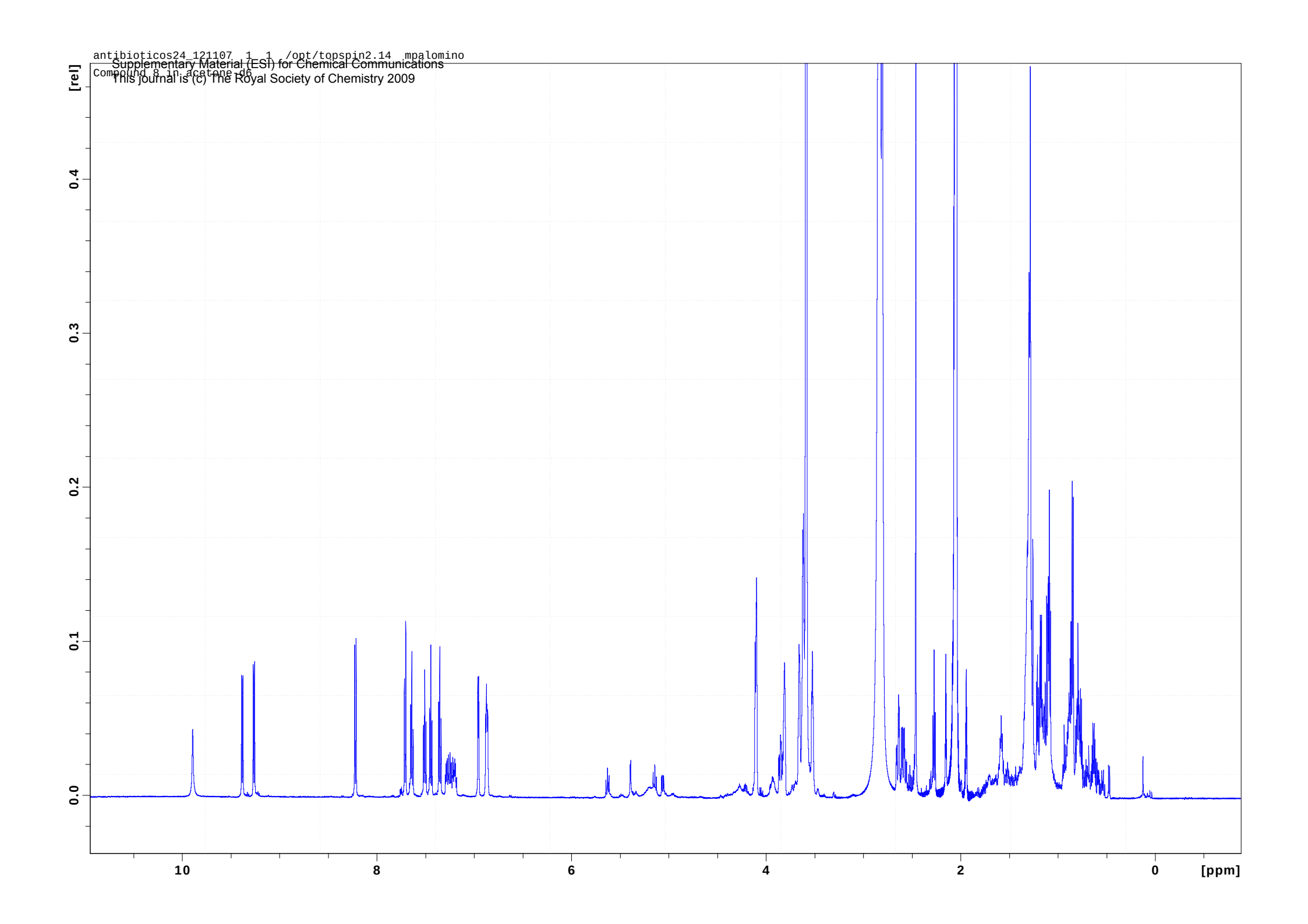
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Supplementary Material (ESI) for Chemical Communications
This journal is (c) The Royal Society of Chemistry 2009
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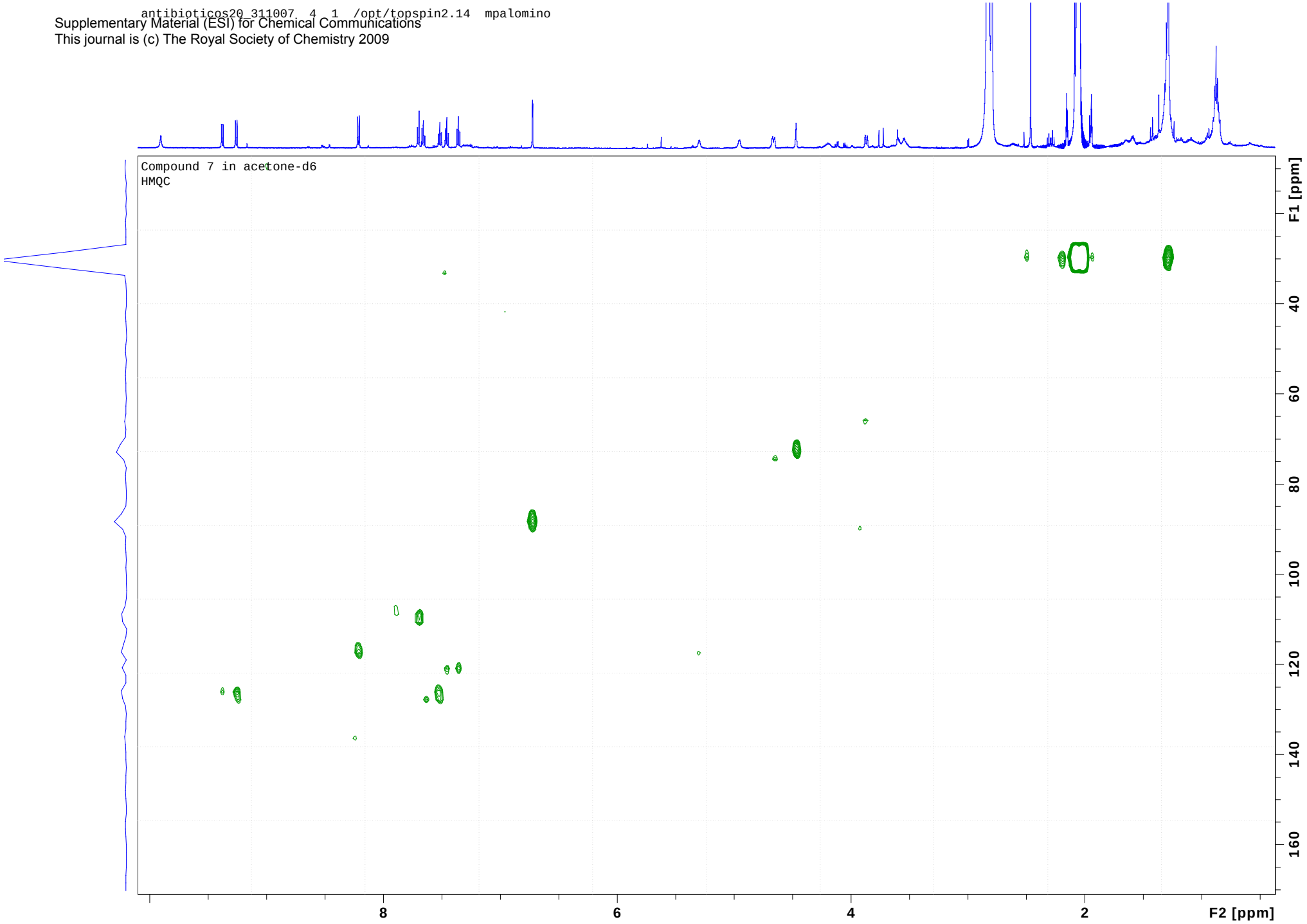

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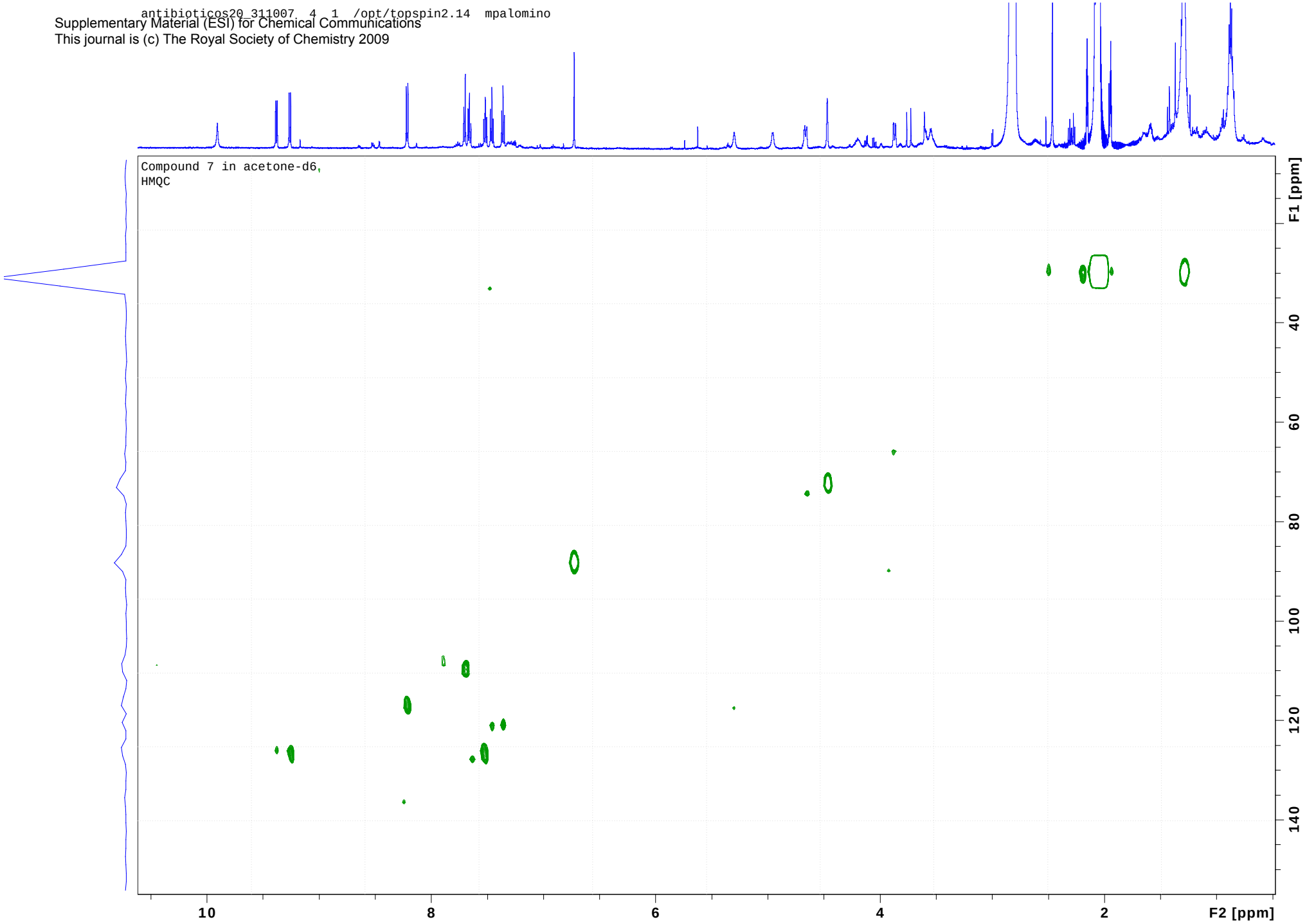

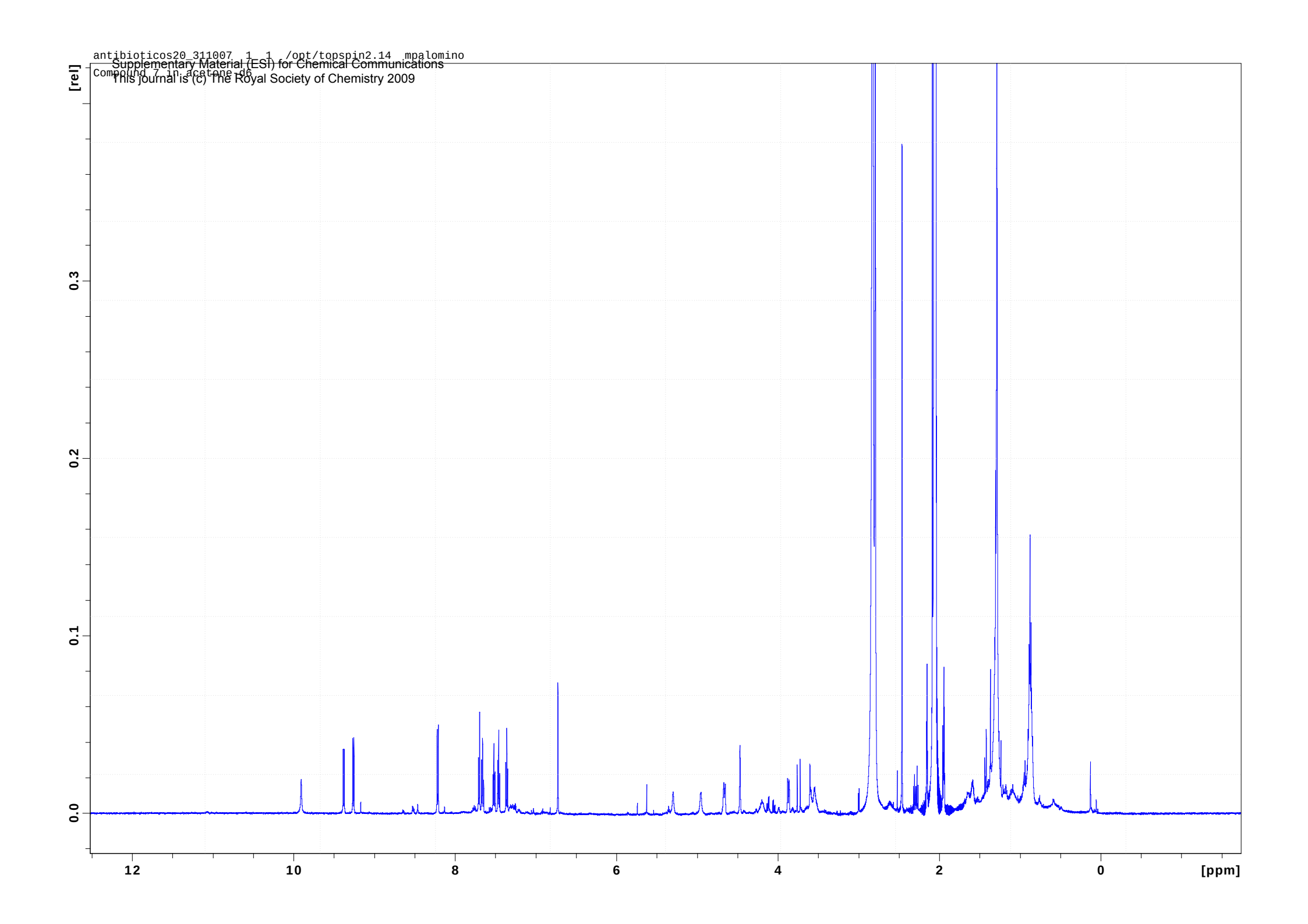
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Compound 7 in acetone-d6
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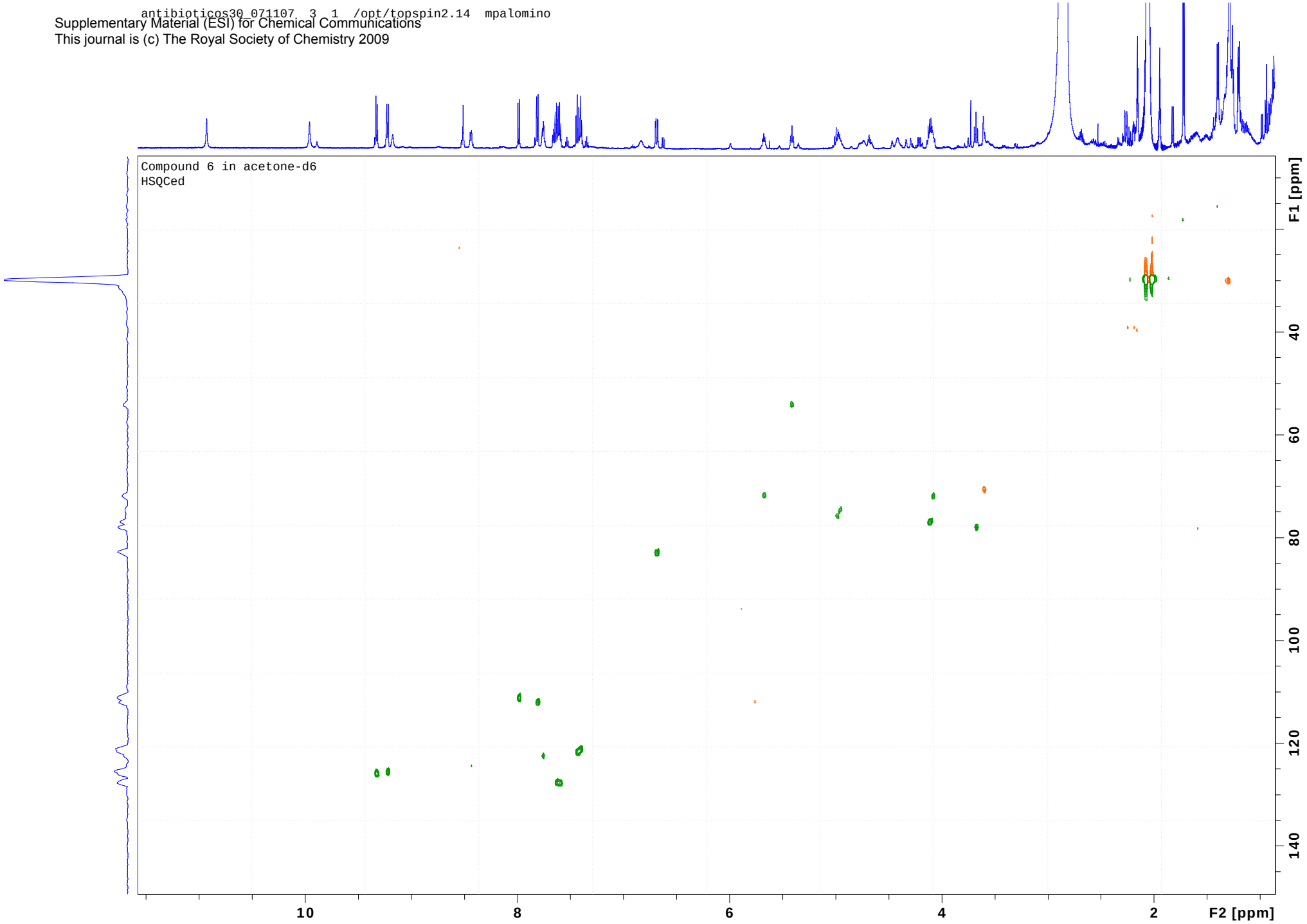
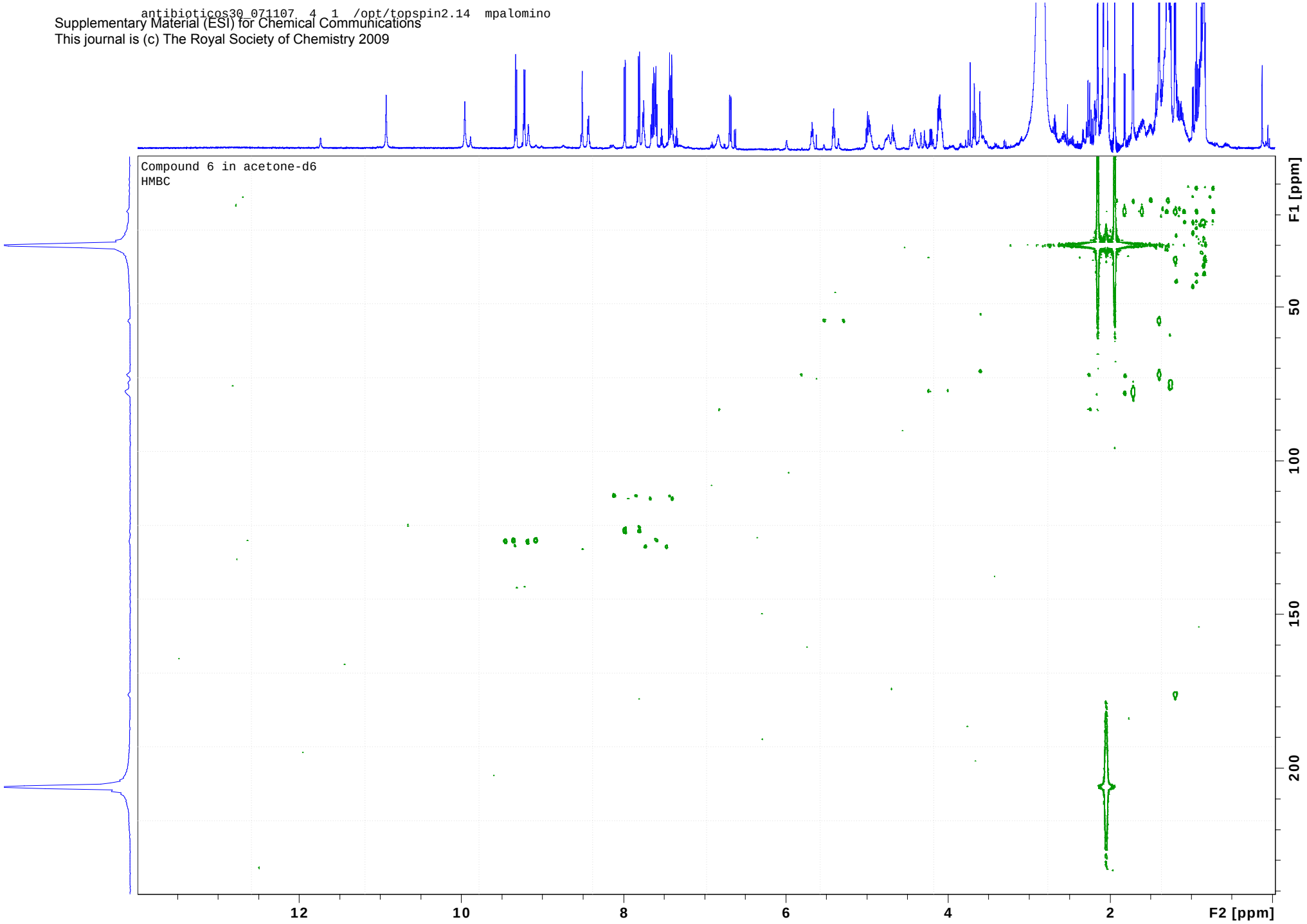
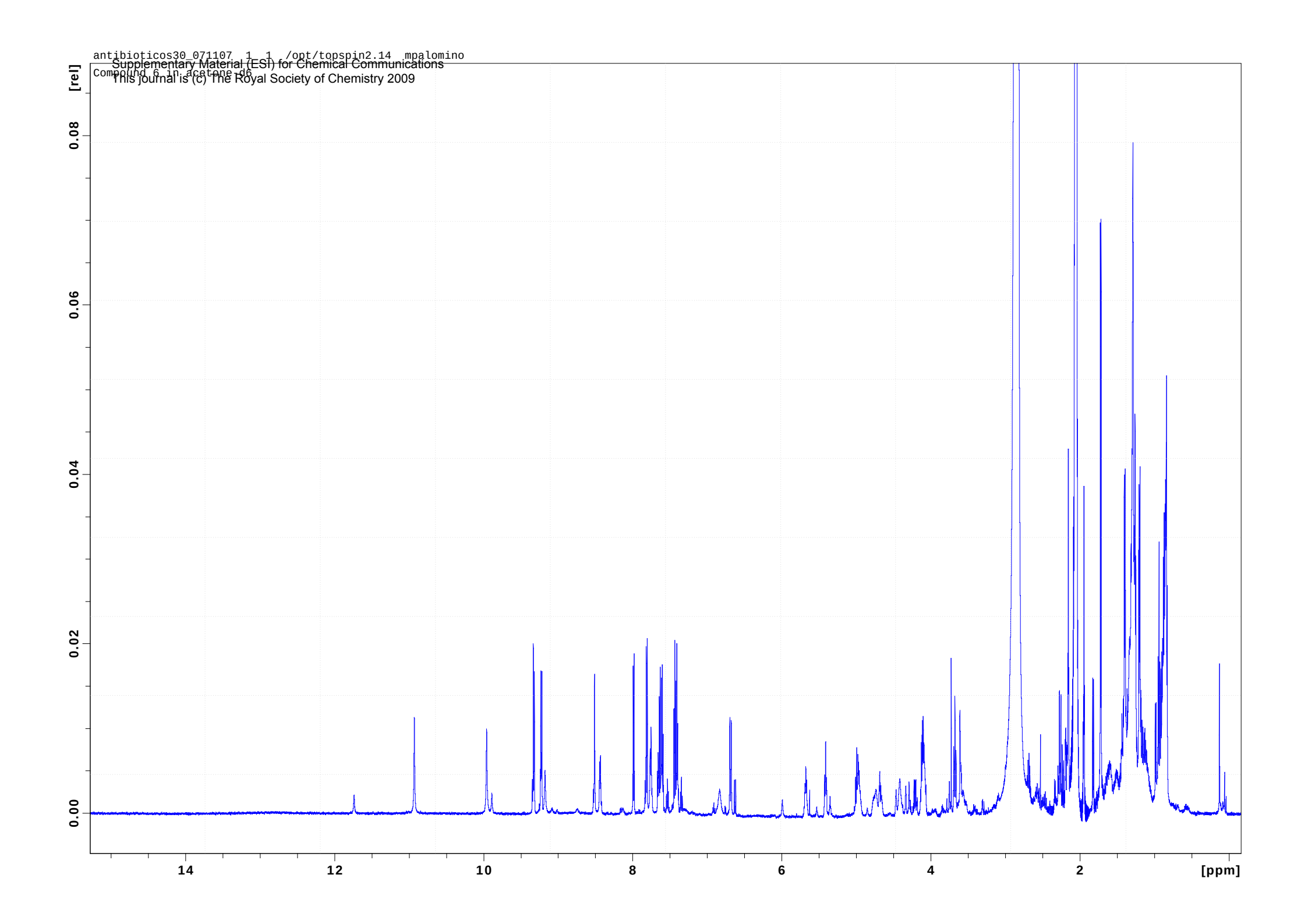
0.1
0.0
12
10
8
6
4
2
0
[ppm]

Supplementary Material (ESI) for Chemical Communications






antibioticos30_071107 1 1 /opt/topspin2.14 mpalomino
Compound 6 in acetone-d6
Supplementary Material (ESI) for Chemical Communications
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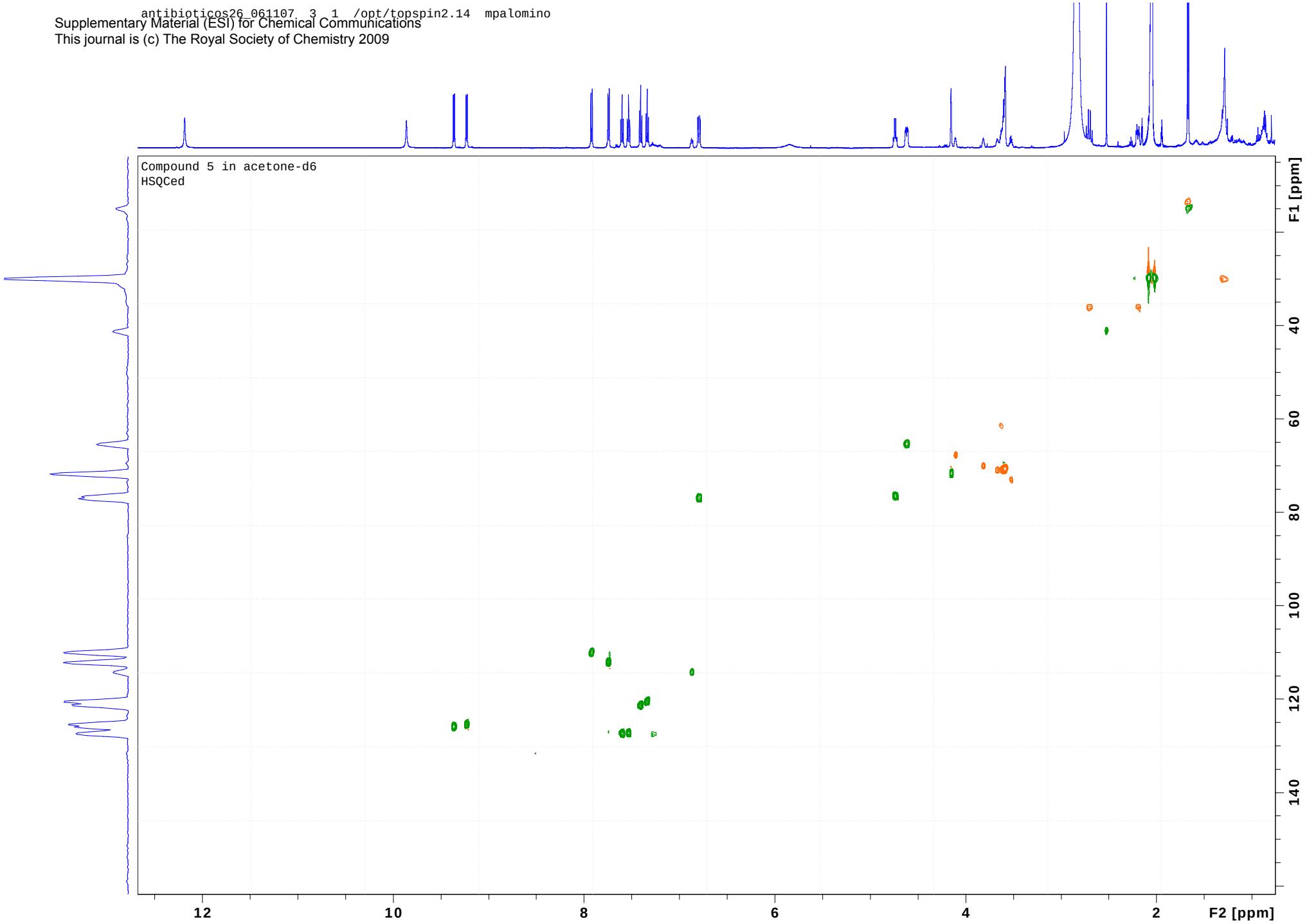
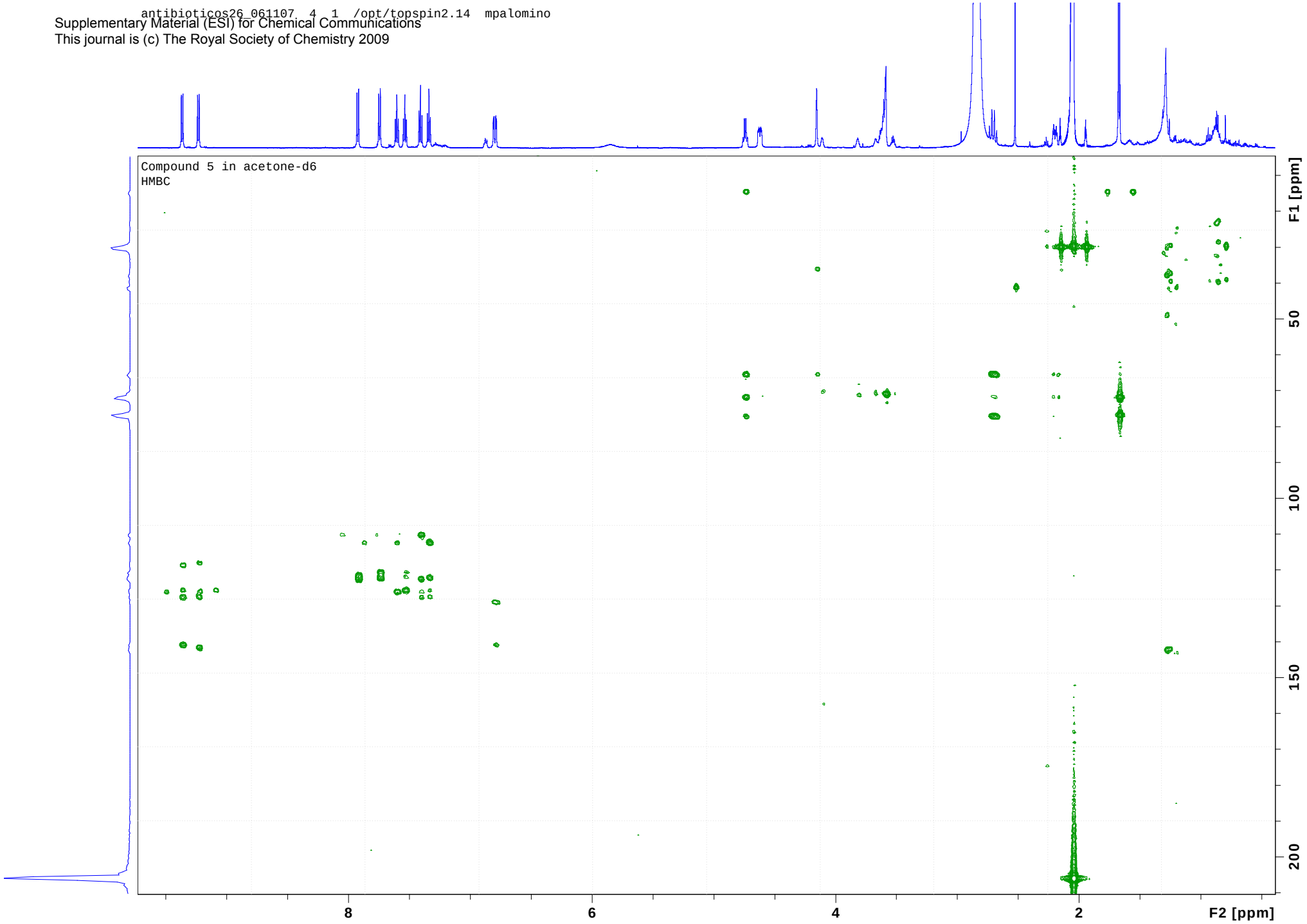
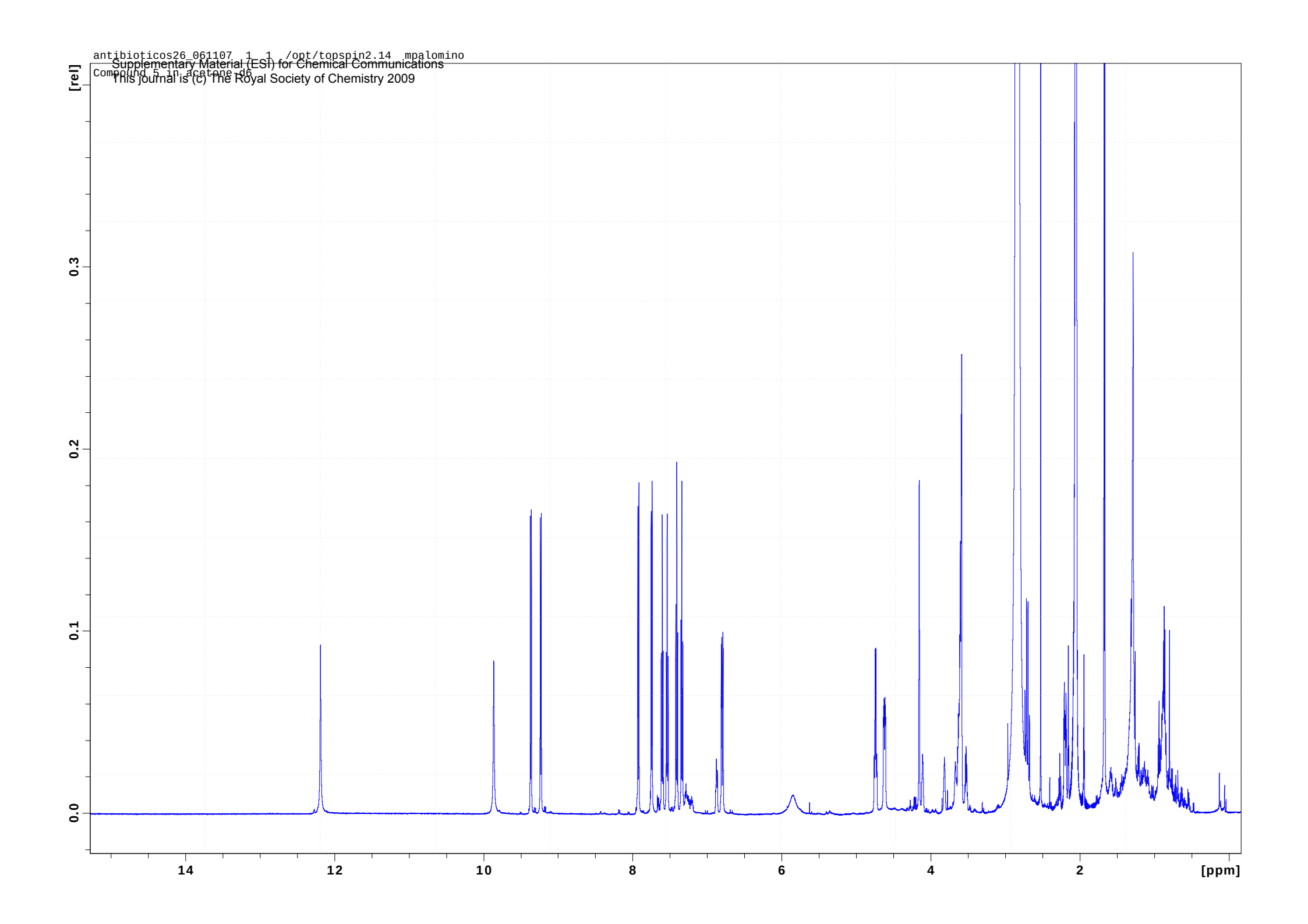
[rel]
0.08
0.06
0.04
0.02
0.00
14
12
10
8
6
4
2
[ppm]



Supplementary Material (ESI) for Chemical Communications

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Compound 5 in acetone-d6
Supplementary Material (ESI) for Chemical Communications
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[rel]
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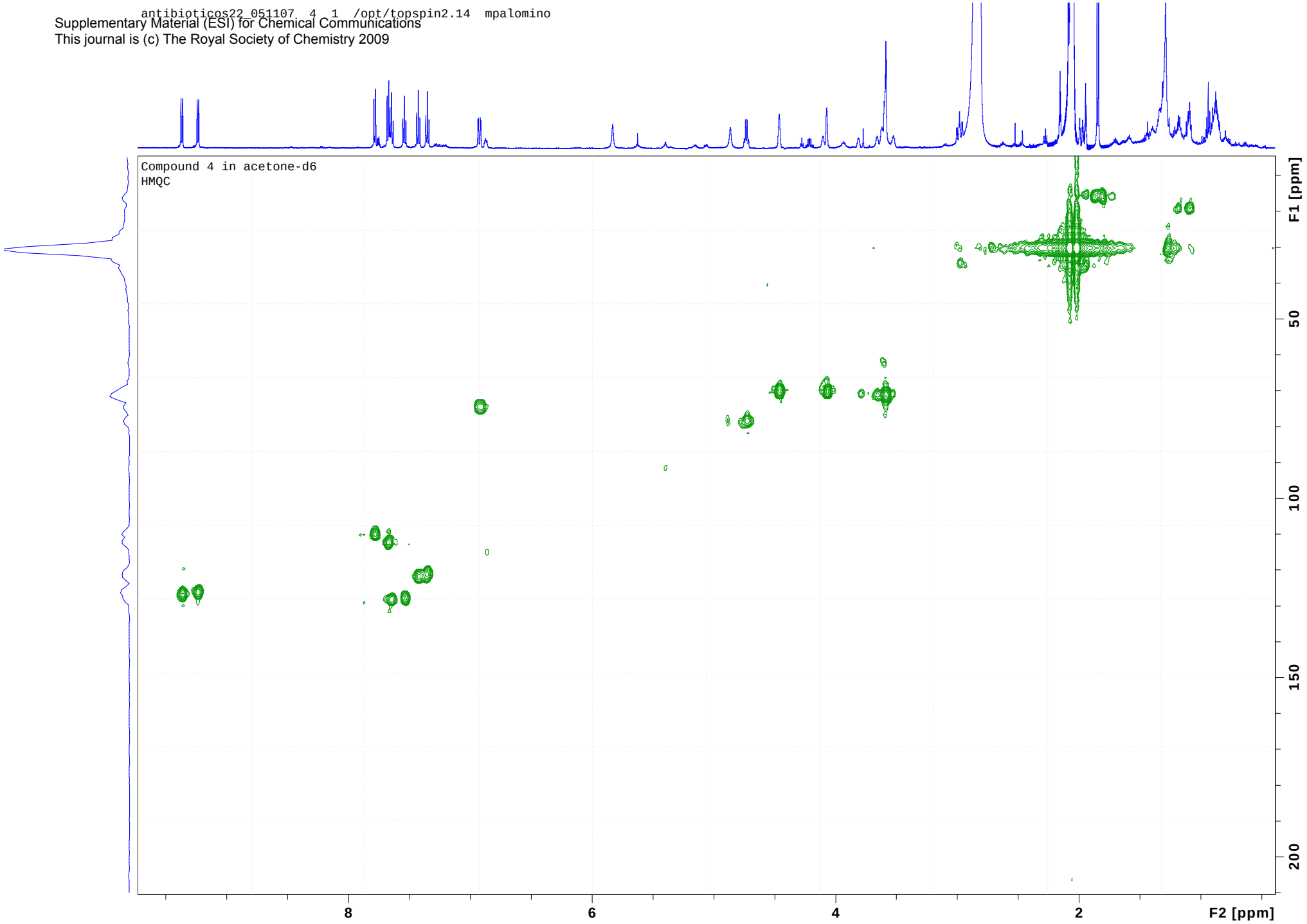
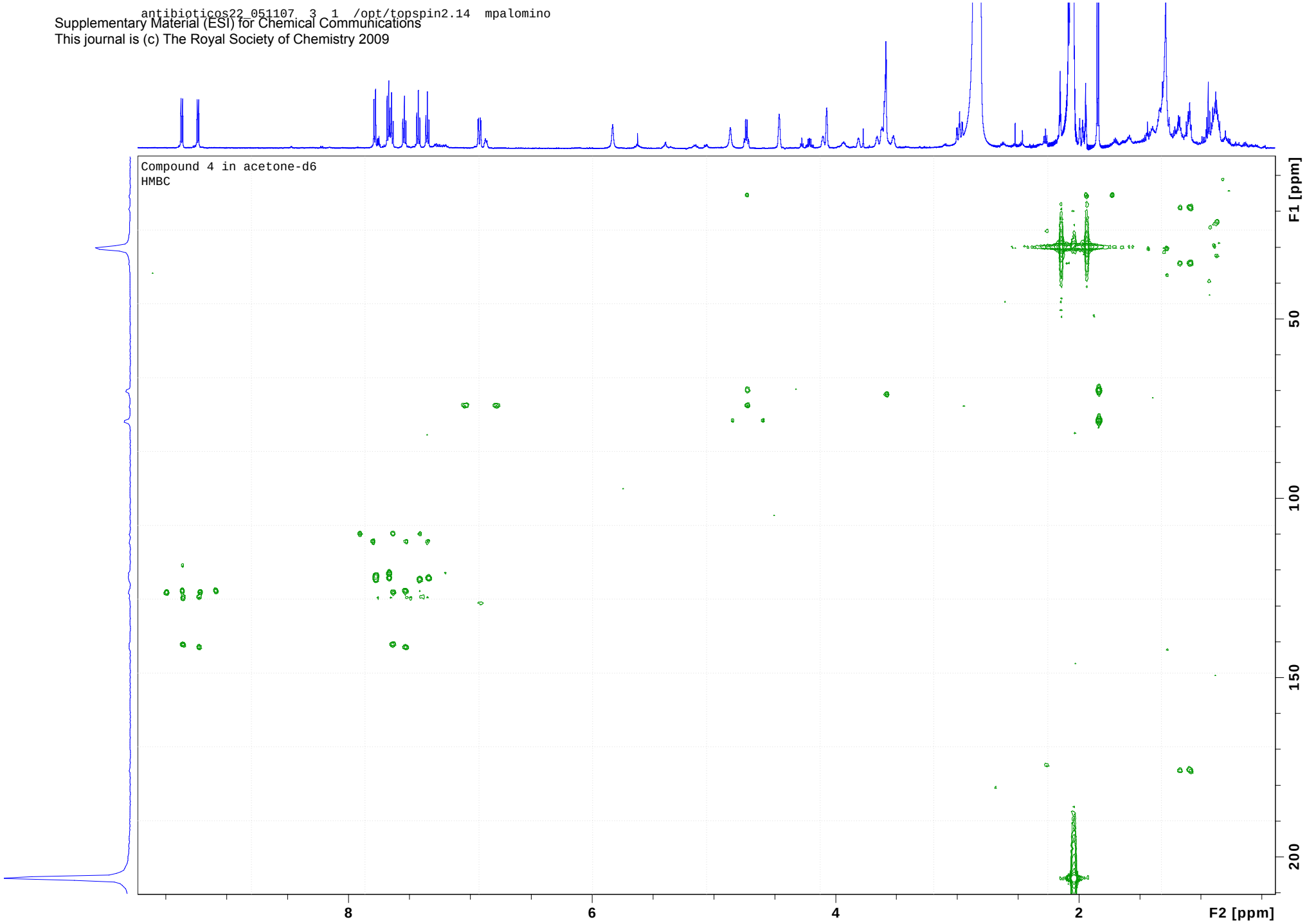
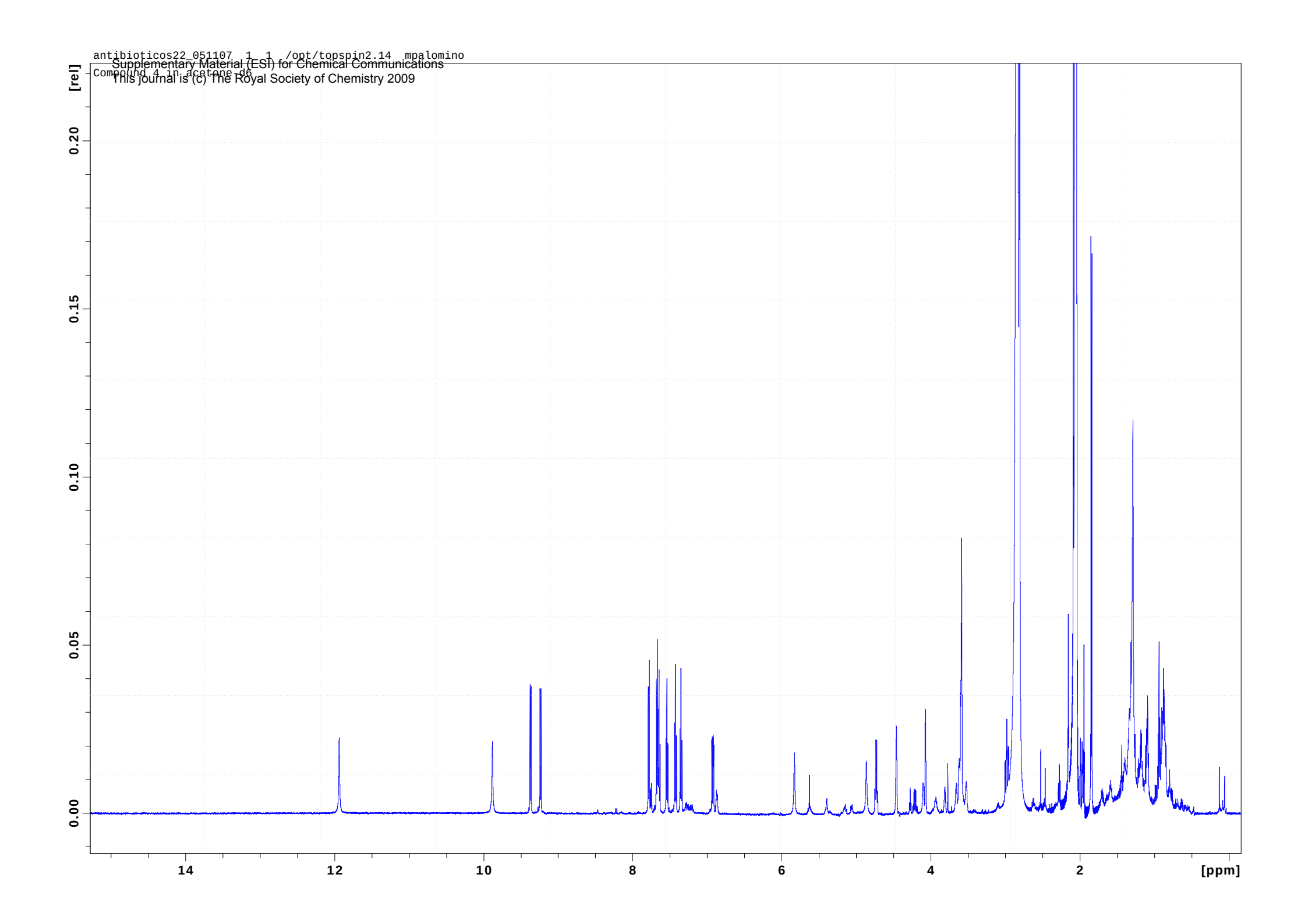
0.1
0.0
14
12
10
8
6
4
2
[ppm]

Supplementary Material (ESI) for Chemical Communications



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Compound 4 in acetone-d6
Supplementary Material (ESI) for Chemical Communications
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[rel]
0.20
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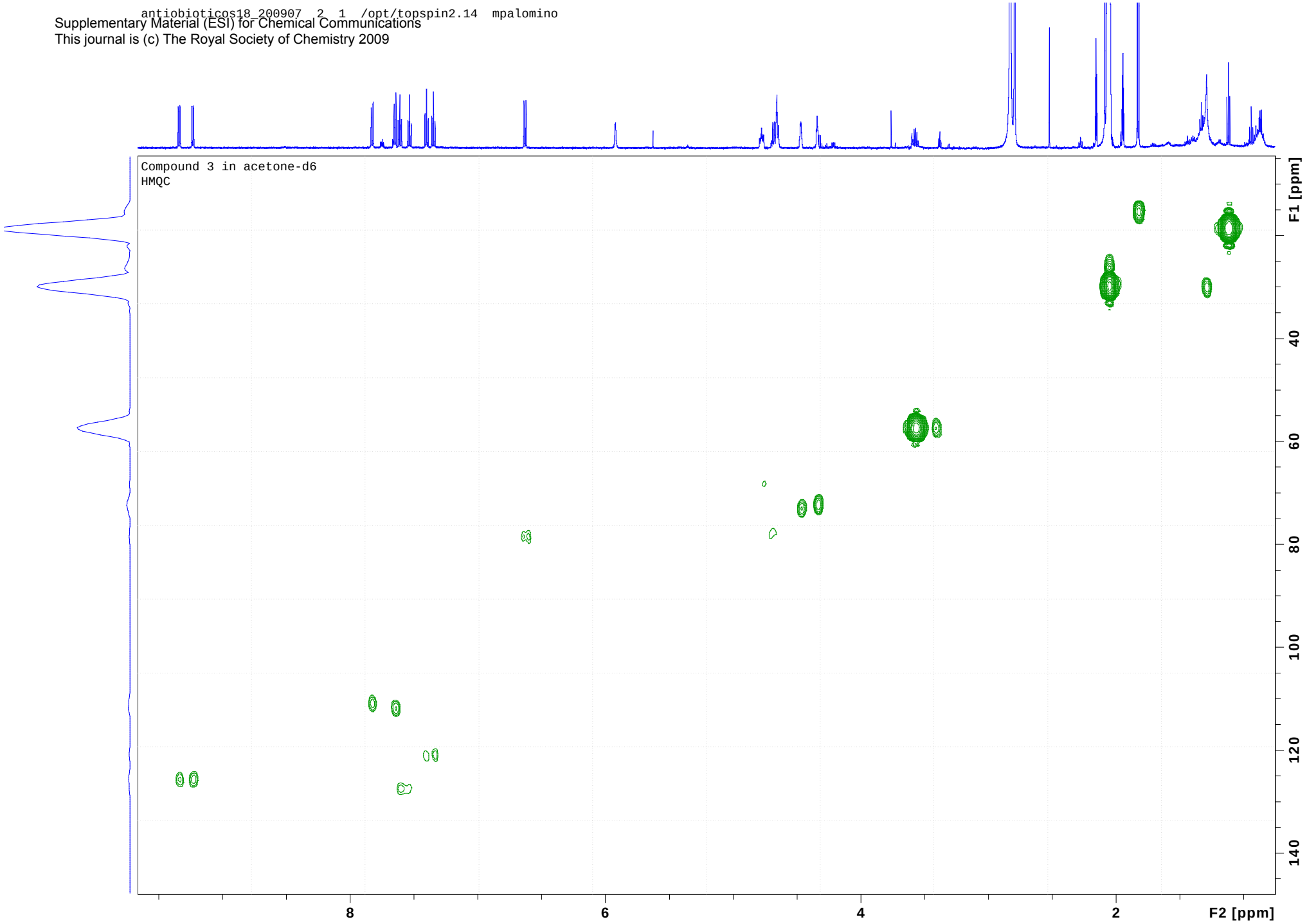
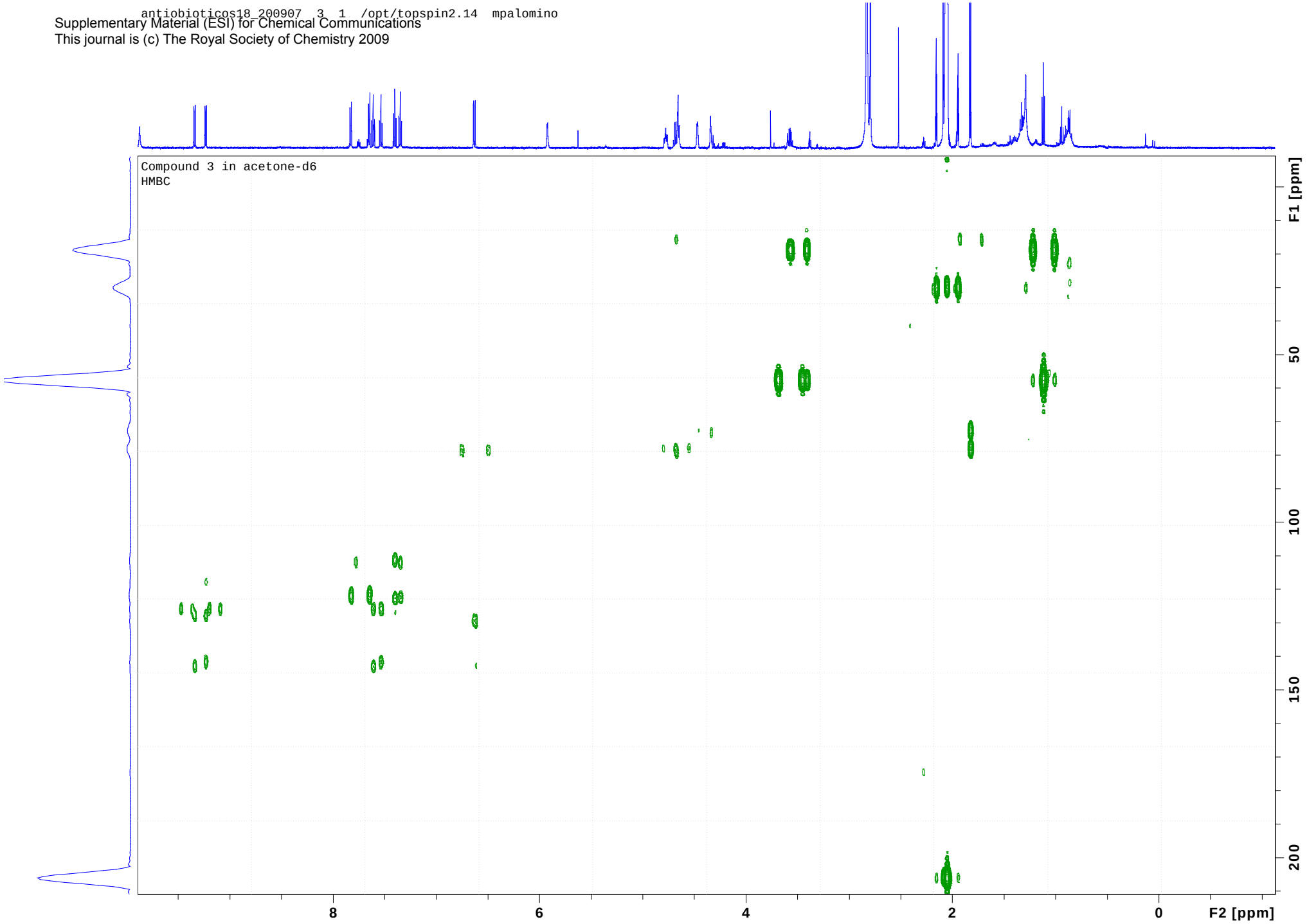
0.05
0.00
14
12
10
8
6
4
2
[ppm]

Supplementary Material (ESI) for Chemical Communications



Supplementary Material (ESI) for Chemical Communications



Compound 3 in acetone d6