Electronic Supplementary Information

Non-symmetrically substituted phenoxazinones from laccase-mediated oxidative cross-coupling of aminophenols: an experimental and theoretical insight.

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TABLE OF CONTENT FOR THE SUPPORTING INFORMATION

Theoretical computations

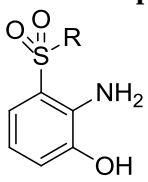
Bibliographical references for the synthesis of aminophenols and phenoxazinones

[12a] - F. Bruyneel, E. Enaud, L. Billottet, S. Vanhulle and J. Marchand-Brynaert, *Eur. J. Org. Chem.*, 2008, **1**, 71-79;

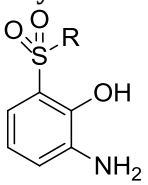
[12b] - F. Bruyneel, O. Payen, A. Rescigno, B. Tinant and J. Marchand-Brynaert, *Chem Eur. J.*, 2009, **15**, 8283-8295;

[12d] - F. Bruyneel, L. D'Auria, O. Payen, P. J. Courtoy and J. Marchand-Brynaert, *ChemBioChem*, 2010, **11**, 1451-1457.

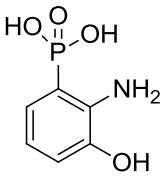
S2-*o*-Aminophenols studied by CV in this work



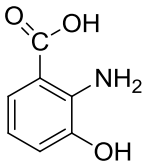
R = OH **3c**
R = NH-cyclohexyl **3f**
R = $NH(CH_2)_3NMe_2$ **3j**
R = NH_2 **3d**



R = OH **4c**
R = NH-cyclohexyl **4f**
R = $NH(CH_2)_3NMe_2$ **4j**



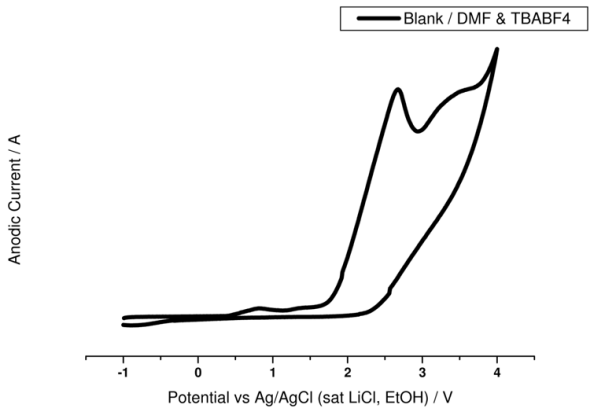
3b



3a

R = NH-phenyl **3e**
R = $NH(CH_2)_2NH_2$ **3i**
R = $NHCH_2CO_2Me$ **3g**
R = $NH(CH_2)_3OH$ **3h**

Cyclic voltammograms (CVs) were recorded on a potentiostat EG&G model 283. Data were exported in excel files and graph were generated using the software Origin 6.0 Professional (Microcal Origin).



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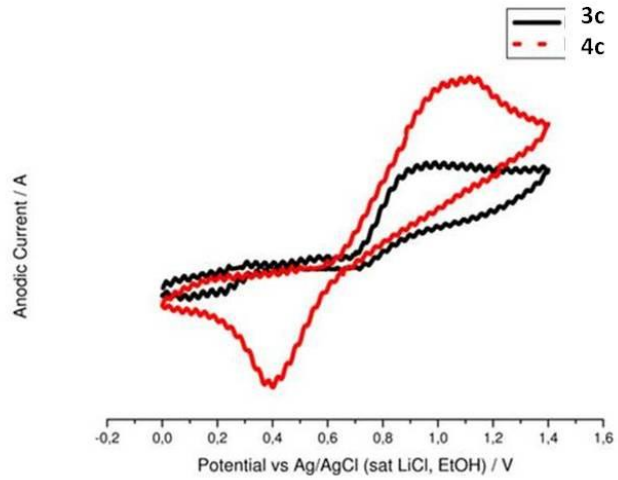

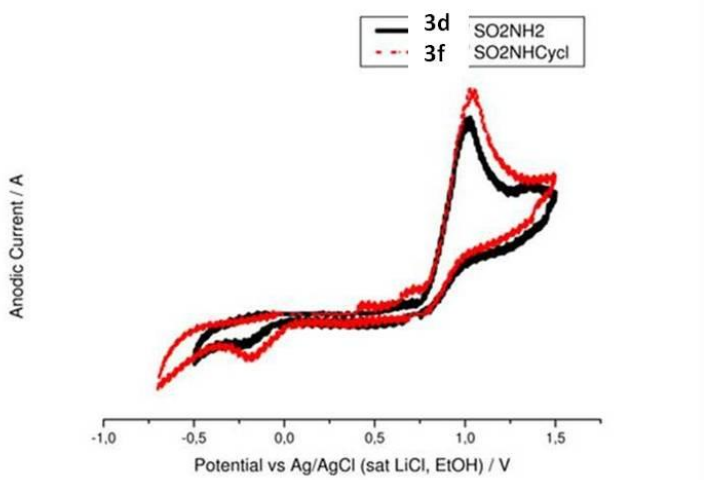

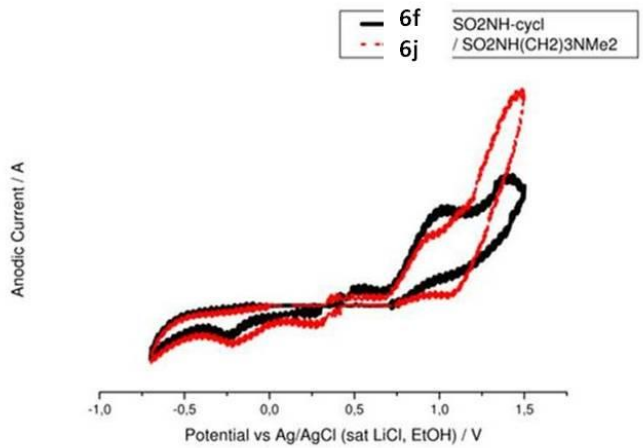

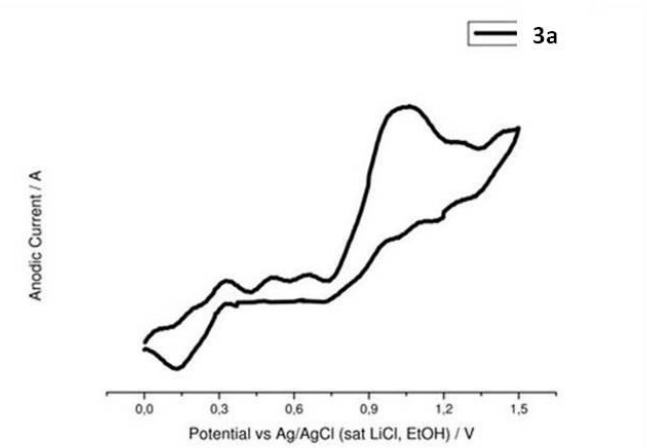

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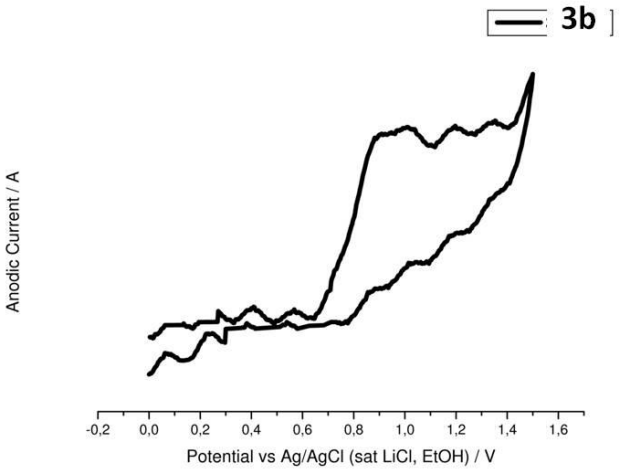
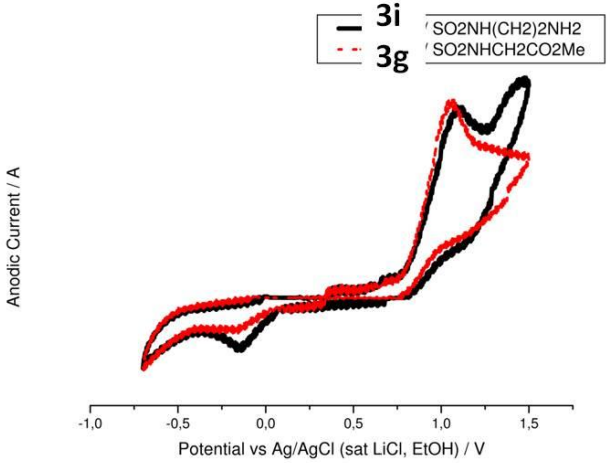
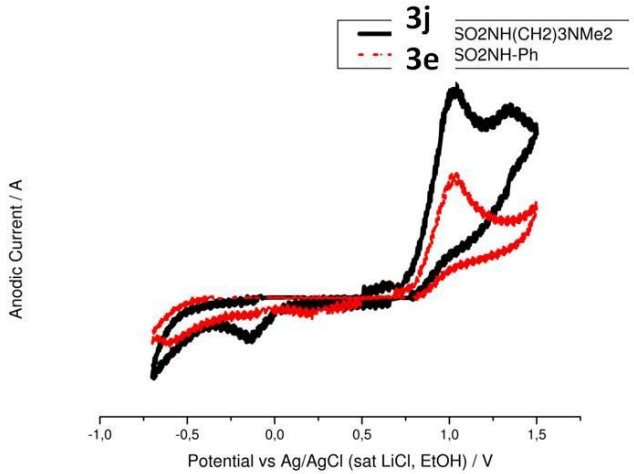
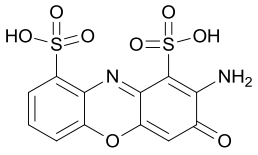




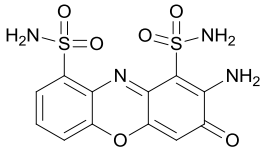


Symmetrically substituted phenoxazinones cited and used in the research



5c

$C_{12}H_8N_2O_8S_2$; 371.97 m/z



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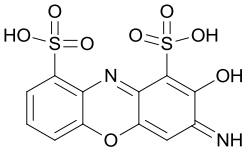
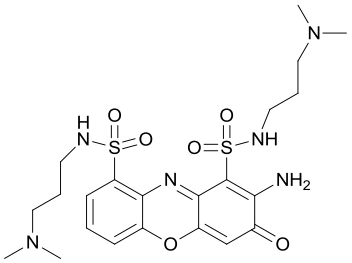


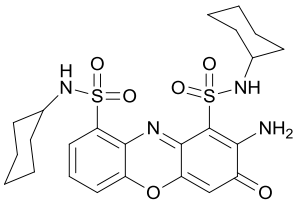
6c

$C_{12}H_8N_2O_8S_2$; 371.97 m/z



5j

$C_{22}H_{32}N_6O_6S_2$; 540.18 m/z



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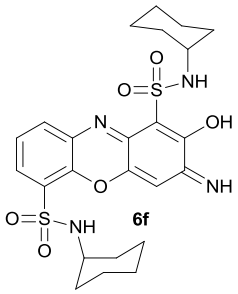
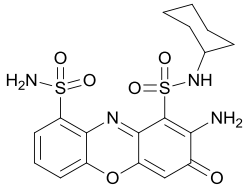
$C_{24}H_{30}N_4O_6S_2$; 534.16 m/z



6f

$C_{24}H_{30}N_4O_6S_2$; 534.16 m/z

Non-symmetrically substituted phenoxazinones cited and identified



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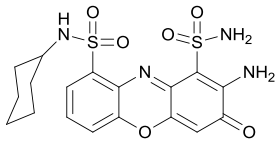

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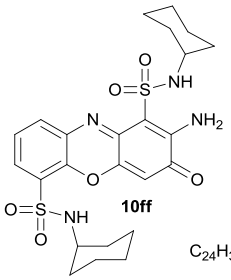
$C_{18}H_{20}N_4O_6S_2$; 452.08 m/z



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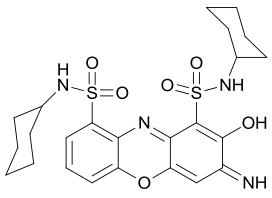

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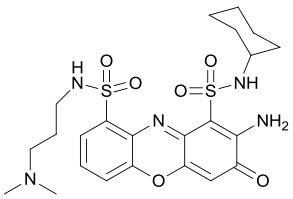
$C_{24}H_{30}N_4O_6S_2$; 534.16 m/z



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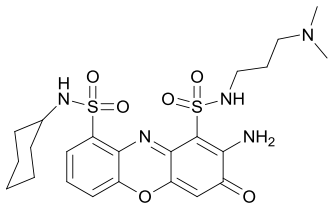

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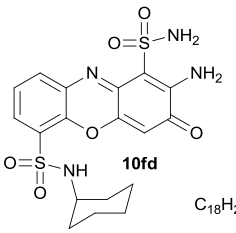
$C_{23}H_{31}N_5O_6S_2$; 537.17 m/z



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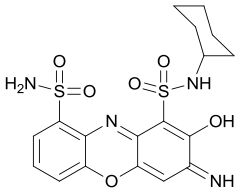
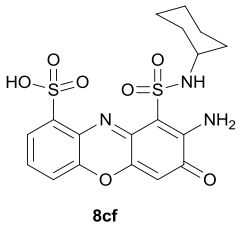


9df

$C_{18}H_{20}N_4O_6S_2$;452.08 m/z



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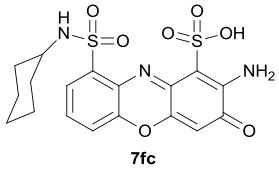

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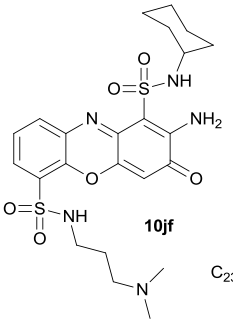
$C_{18}H_{19}N_3O_7S_2$; 453.07 m/z



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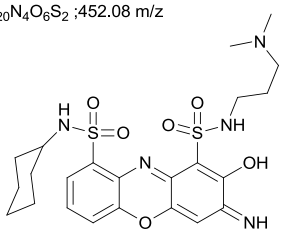

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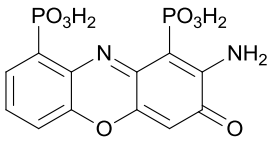
$C_{23}H_{31}N_5O_6S_2$; 537.17 m/z

S6 Symmetrical phenoxazinones from "proof concept" reactions



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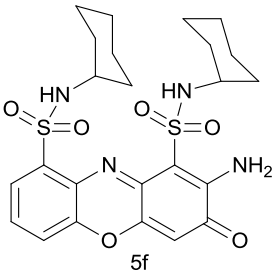

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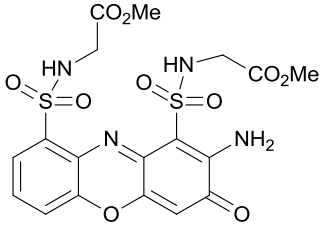

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isolated non-symmetrical phenoxazinones from "proof concept" reactions

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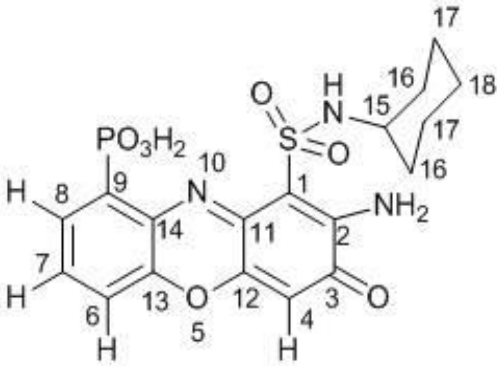

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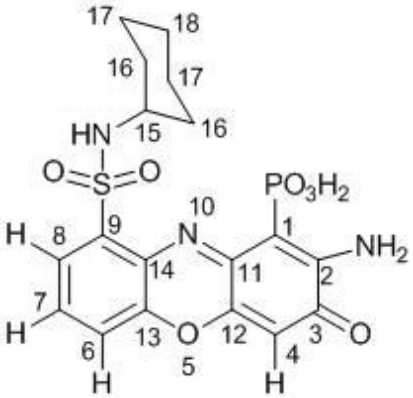

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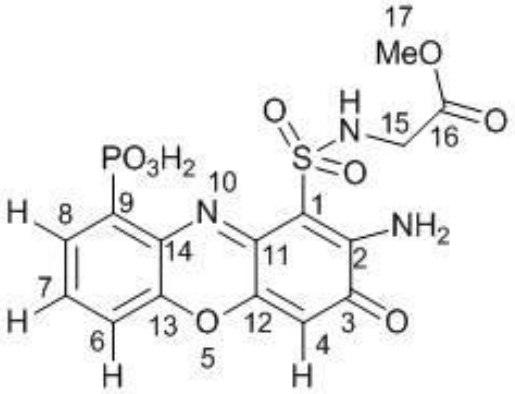

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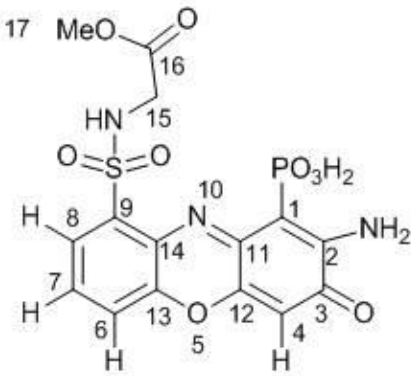


S7

HRMS Results-5d [12b]

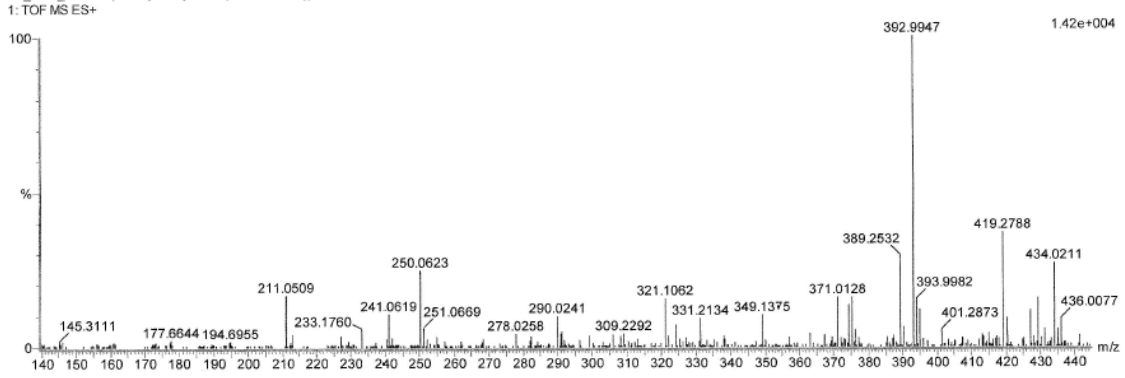
Elemental Composition Report **FB_PHX_414**

Single Mass Analysis
Tolerance = 15.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

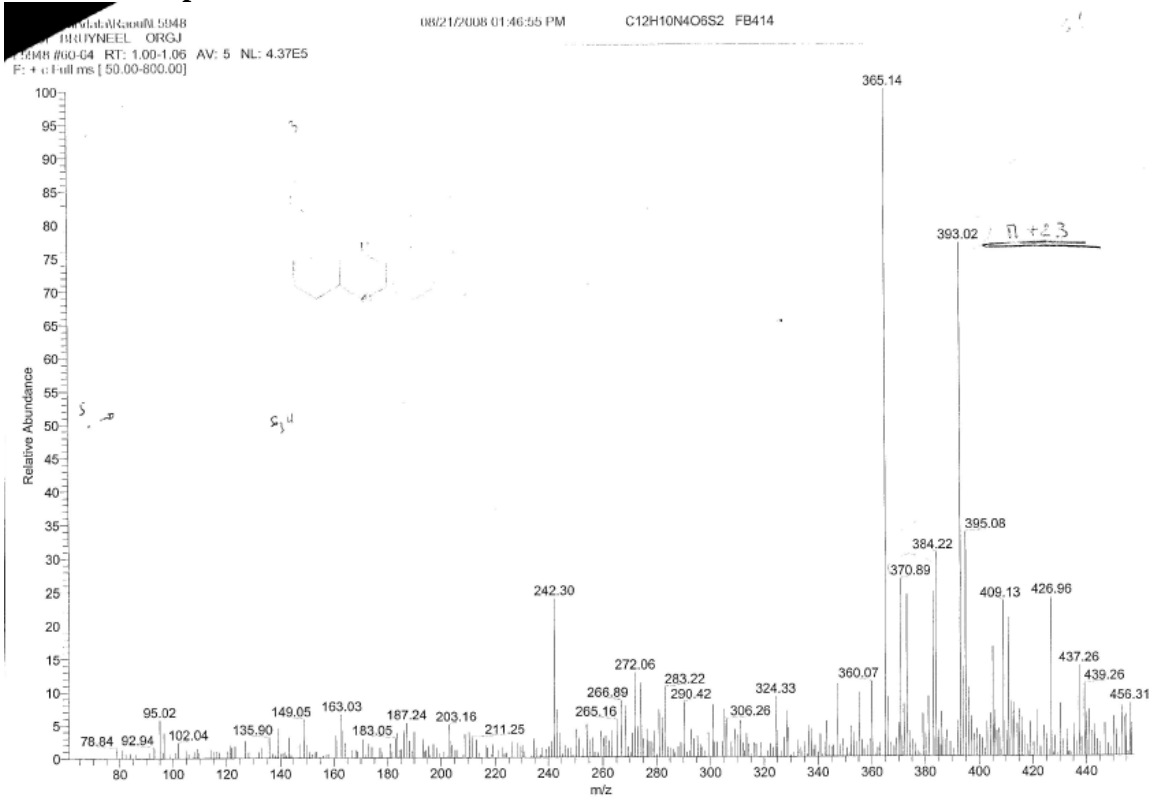
Monoisotopic Mass, Even Electron Ions
424 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 12-12 H: 0-60 N: 0-5 O: 0-11 Na: 0-1 S: 0-2

Minimum:				-1.5		
Maximum:		20.0		15.0		50.0
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
392.9947	392.9939	0.8	2.0	9.5	15.4	C12 H10 N4 O6 Na S2

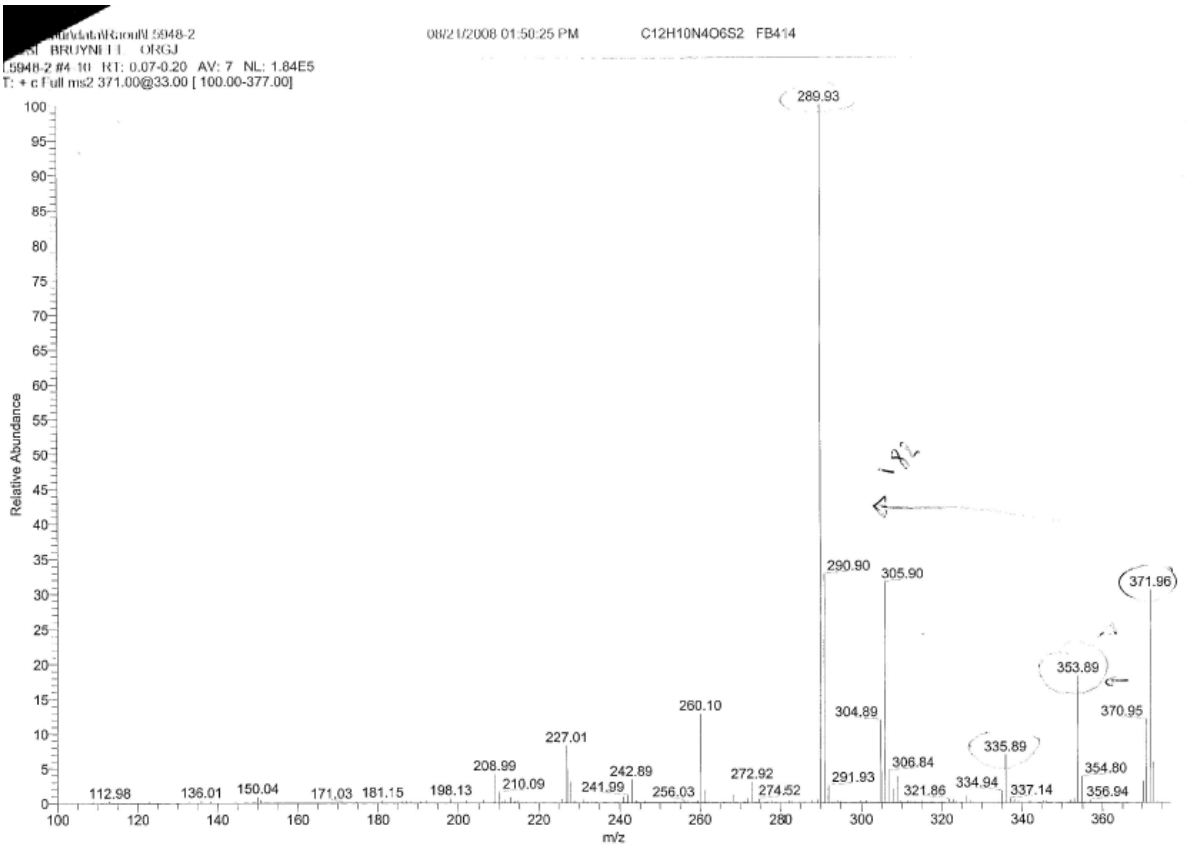
FB_PHX_414 20 (0.240) Cm (17:27-(5:11+77:104))
1: TOF MS ES+



Full MS ESI-positive of 5d



Tandem –MS/MS of 5d



HRMS of compound 5f

MODE: ES+

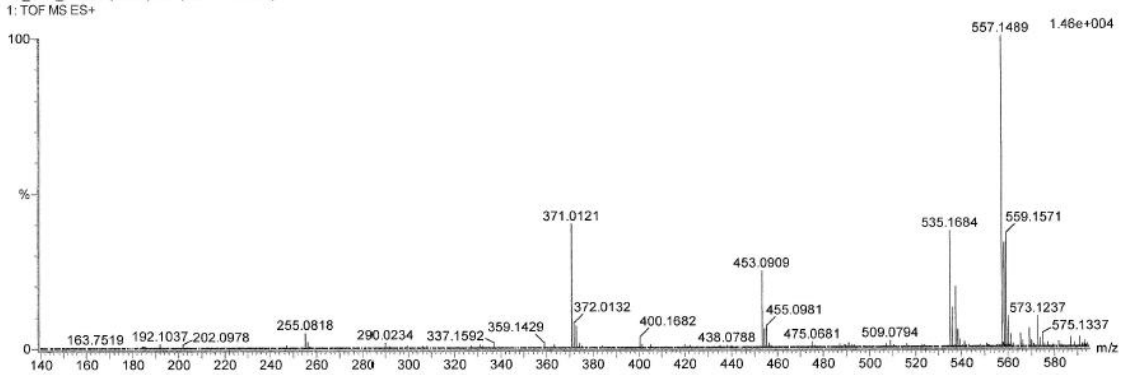
Elemental Composition Report **FB_PHX_415**

Single Mass Analysis
Tolerance = 10.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

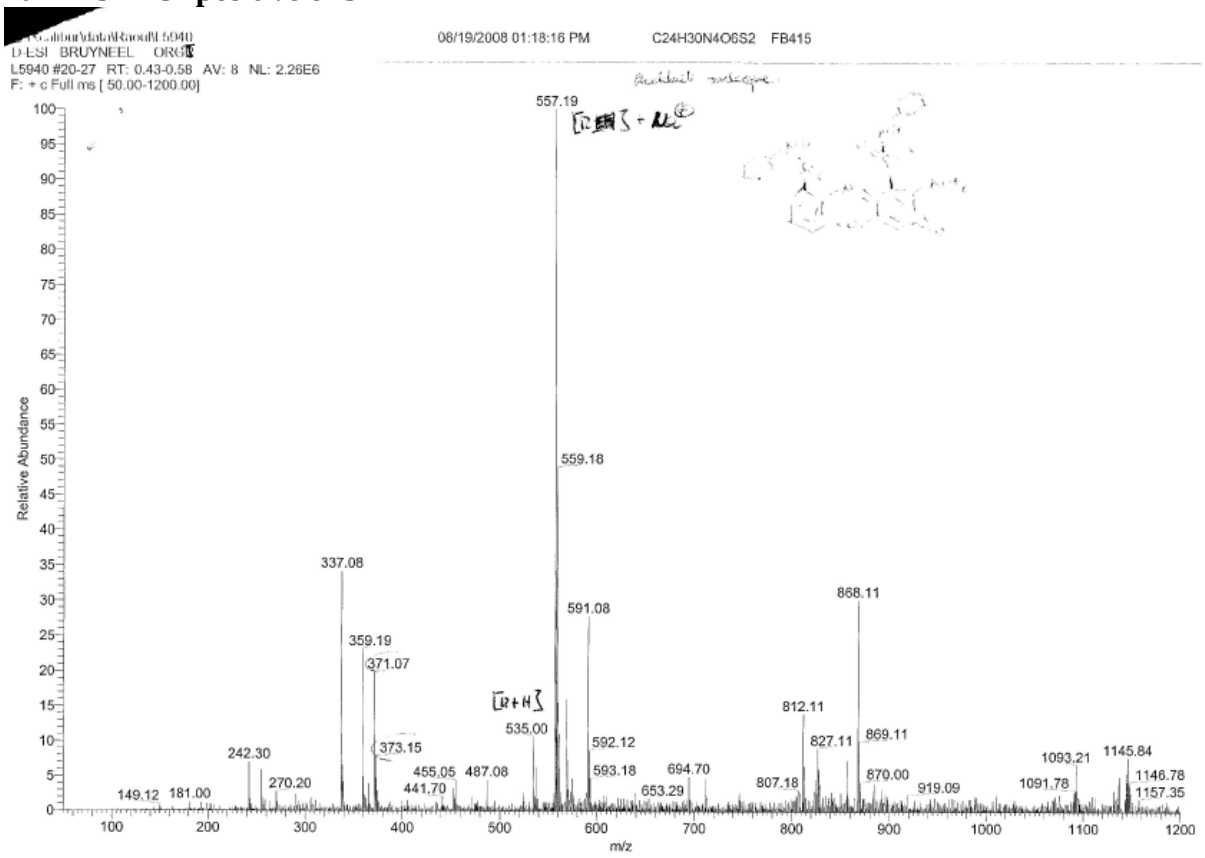
Monoisotopic Mass, Even Electron Ions
210 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 24-24 H: 0-90 N: 0-4 O: 0-6 Na: 0-1 S: 0-2

Minimum:				-1.5		
Maximum:		20.0		10.0	50.0	
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
557.1489	557.1504	-1.5	-2.7	11.5	898.2	C24 H30 N4 O6 Na S2
535.1684	535.1685	-0.1	-0.2	11.5	697.6	C24 H31 N4 O6 S2

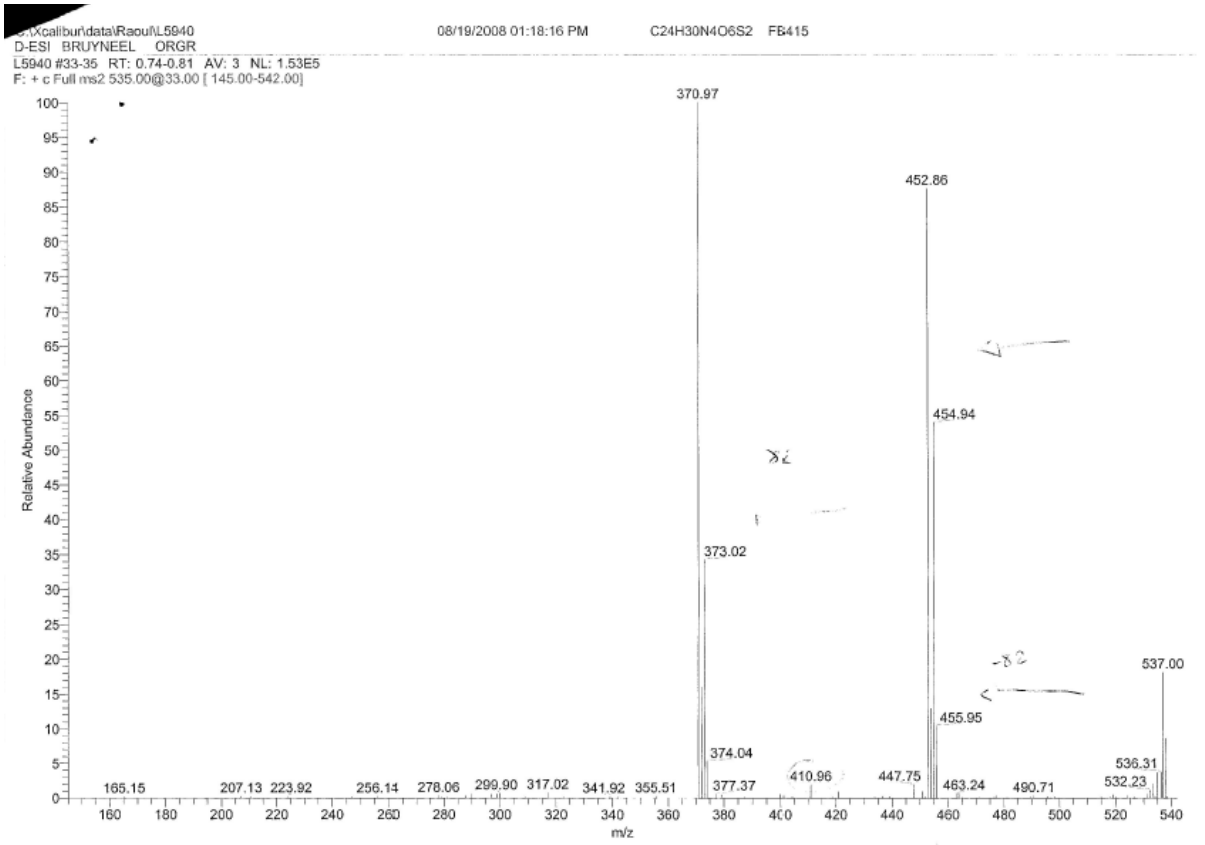
FB_PHX_415 35 (0.426) Cm (35:45-140:158)
1: TOF MS ES+



Full MS –ESI positive of 5f



Tandem MS/MS of 5f



HRMS-5j

ACCURATE MASS CALCULATION PERFORMED ON A WATERS LCT PREMIER XE

MODE: ES+

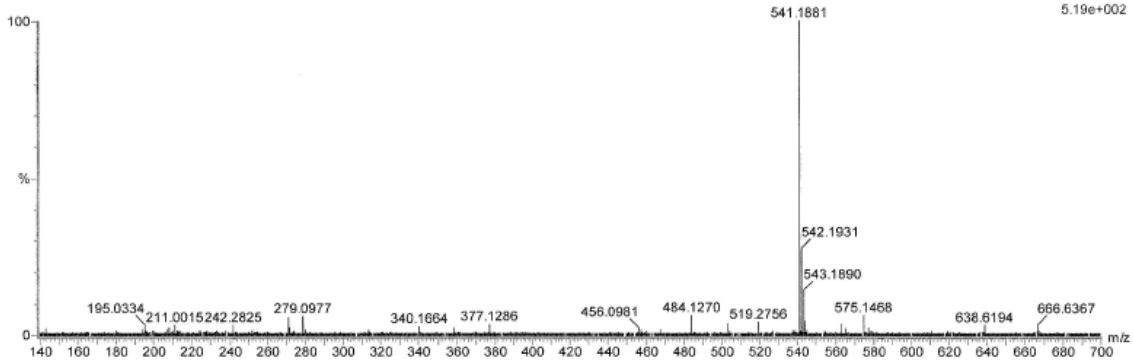
Elemental Composition Report **FB_PHX_417**

Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

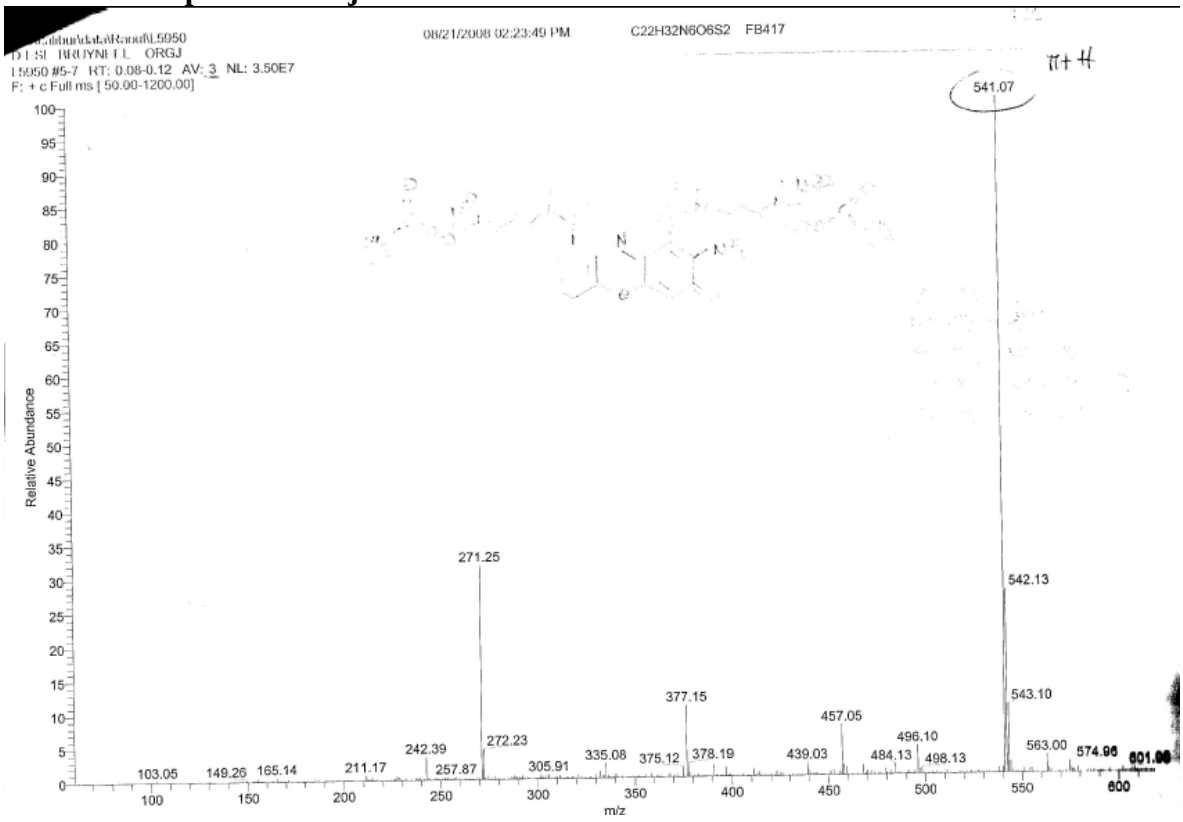
Monoisotopic Mass, Even Electron Ions
499 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 22-22 H: 0-60 N: 0-6 O: 0-11 Na: 0-1 S: 0-2

Minimum:				-1.5		
Maximum:		20.0		5.0	50.0	
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
541.1881	541.1903	-2.2	-4.1	9.5	0.2	C22 H33 N6 O6 S2

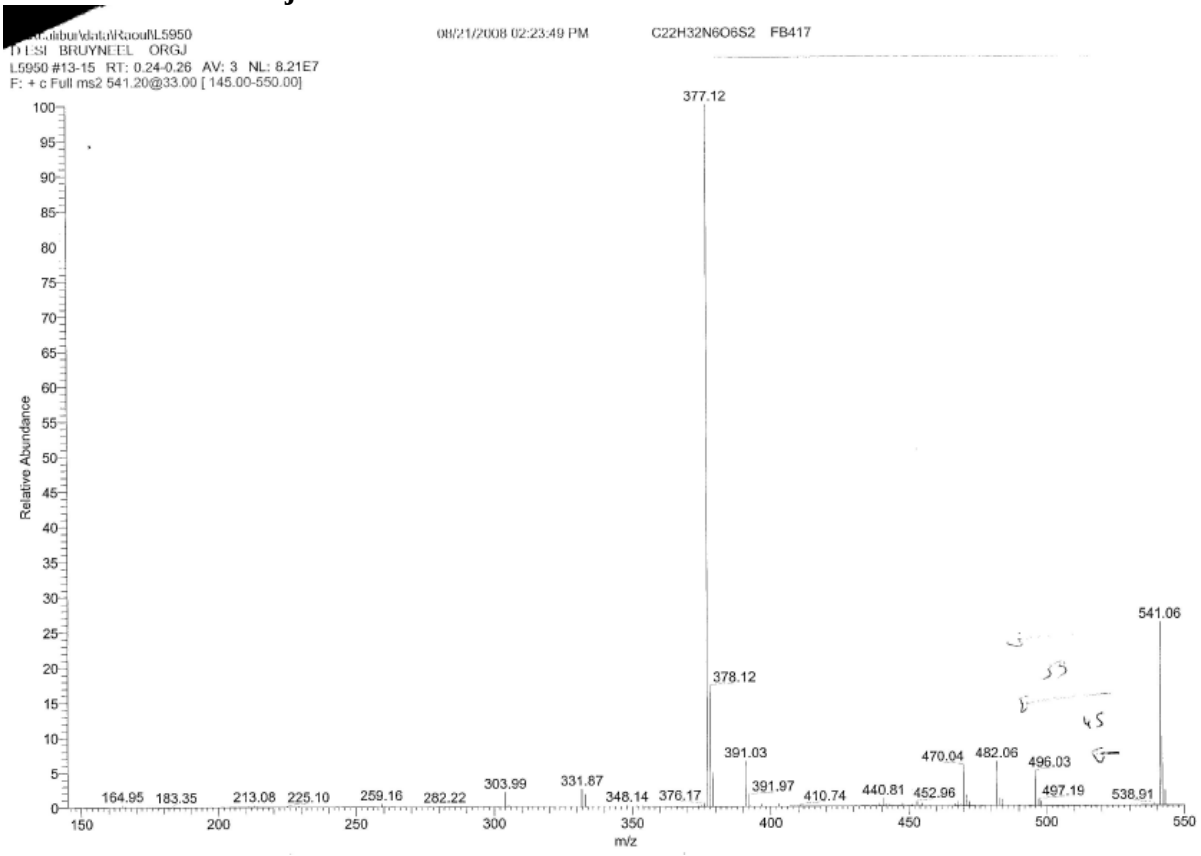
FB_PHX_417 18 (0.220) Cm (16:18)
1: TOF MS ES+



Full-MS-ESI positive of 5j



Tandem MS/MS of 5j



HMRS of 6f

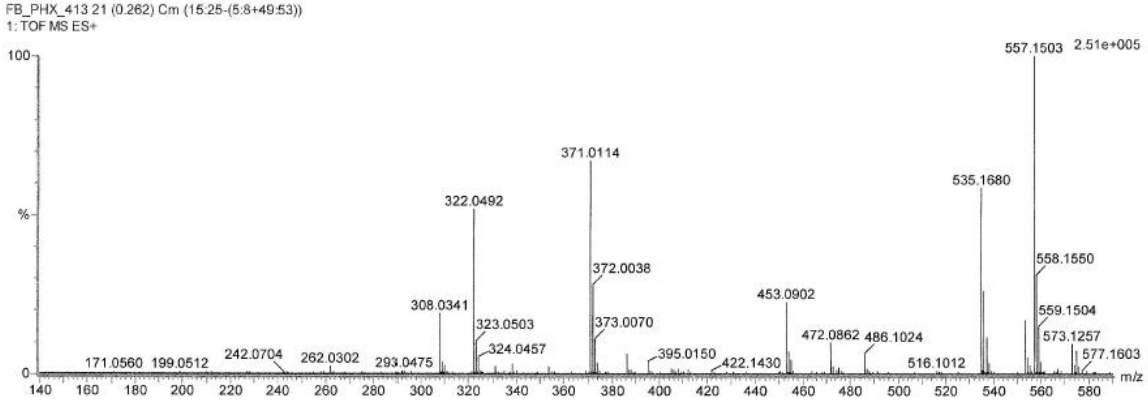
MODE: ES+

Elemental Composition Report **FB_PHX_413**

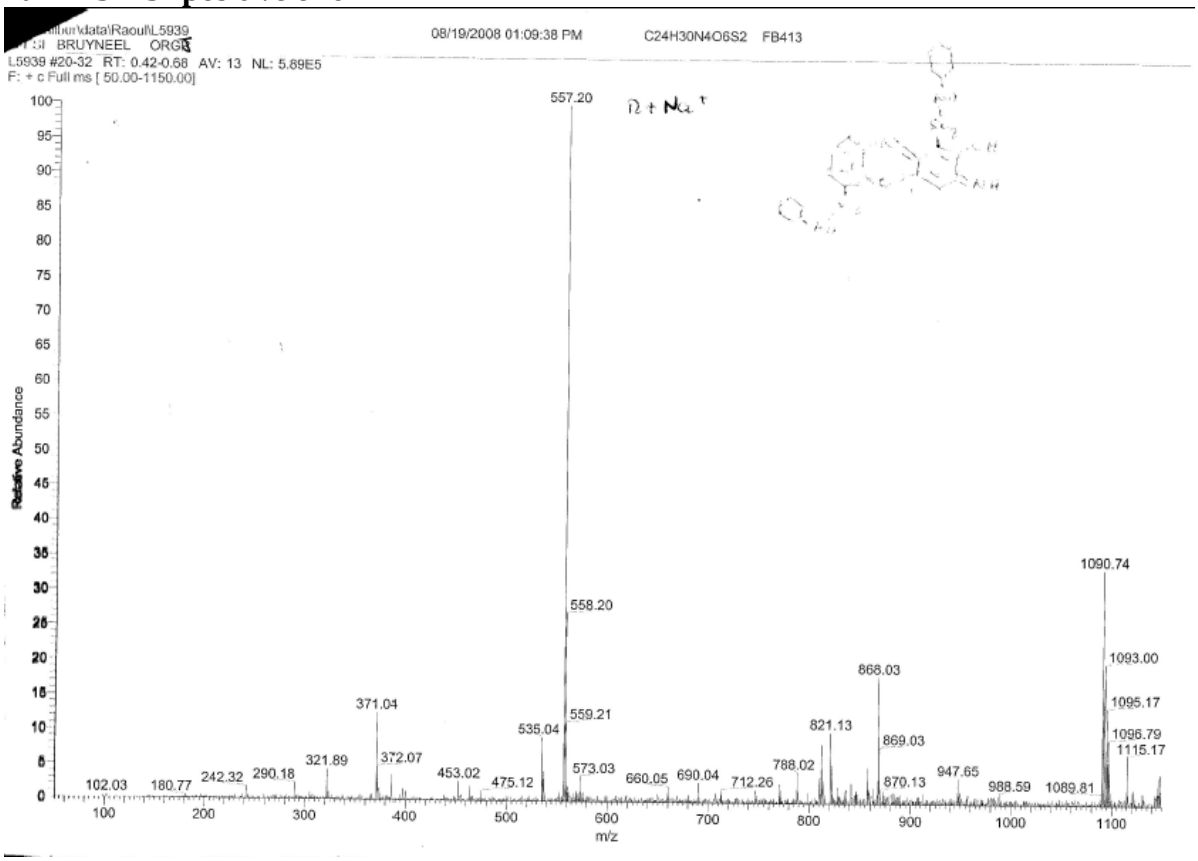
Single Mass Analysis
Tolerance = 10.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
417 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 24-24 H: 0-60 N: 0-5 O: 0-11 Na: 0-1 S: 0-2

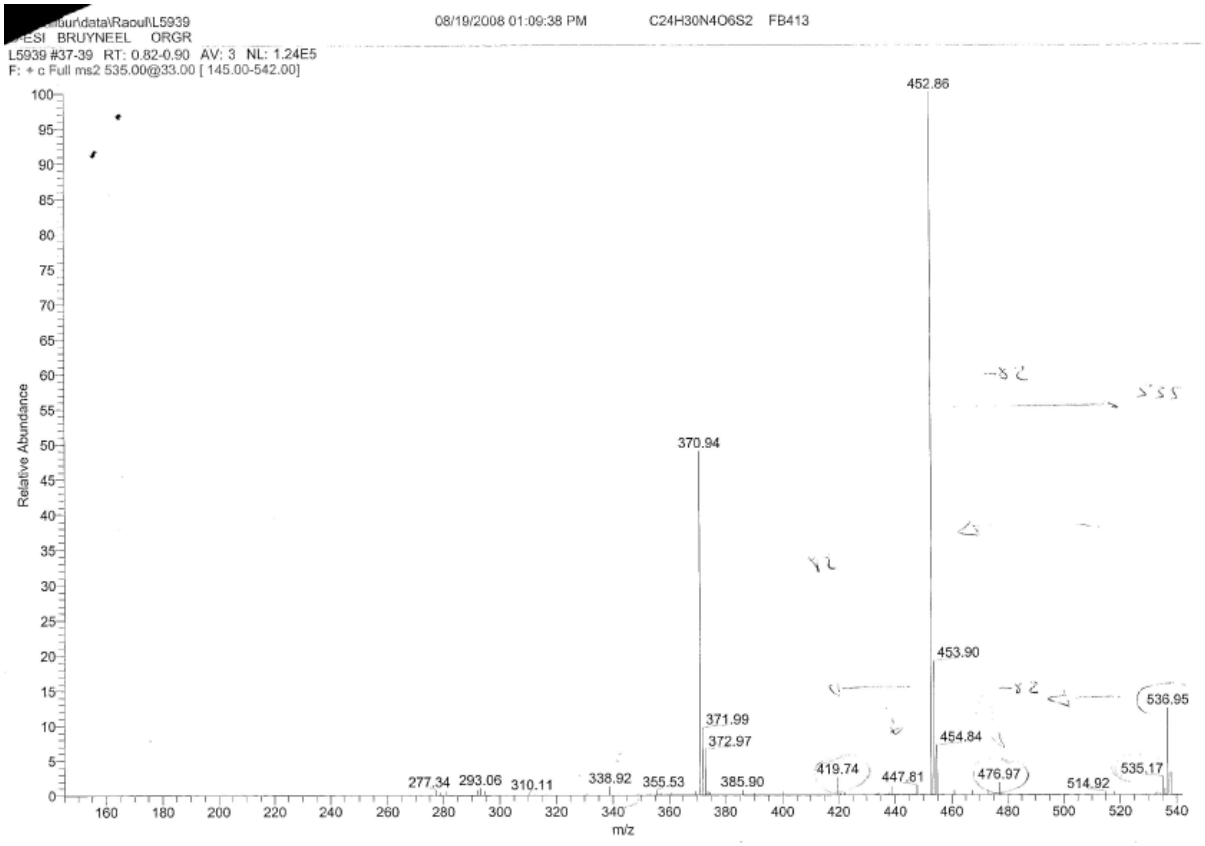
Minimum:				-1.5		
Maximum:		20.0		10.0	50.0	
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
535.1680	535.1685	-0.5	-0.9	11.5	3051.5	C24 H31 N4 O6 S2
557.1503	557.1504	-0.1	-0.2	11.5	6.1	C24 H30 N4 O6 Na S2



Full MS-ESI positive of 6f



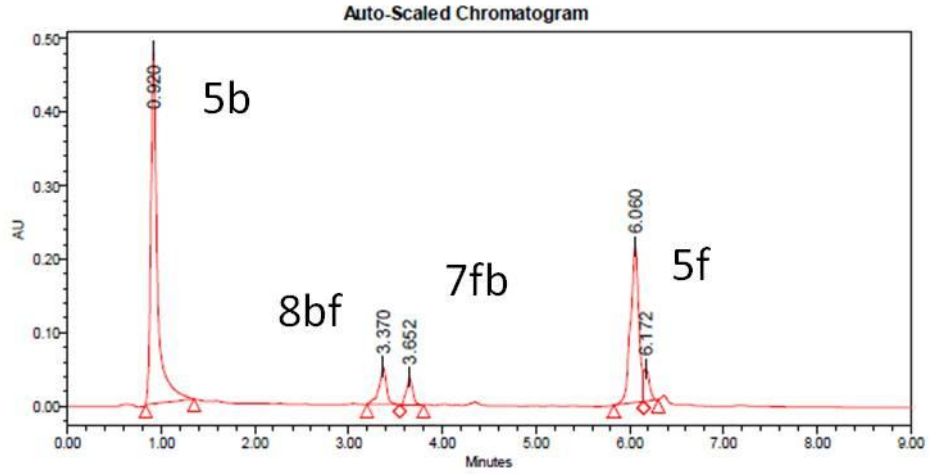
Tandem MS/MS of 6f



S13- analytic HPLC-UV elution profiles with Xbridge™ of proof reactions

For 3b & 3f

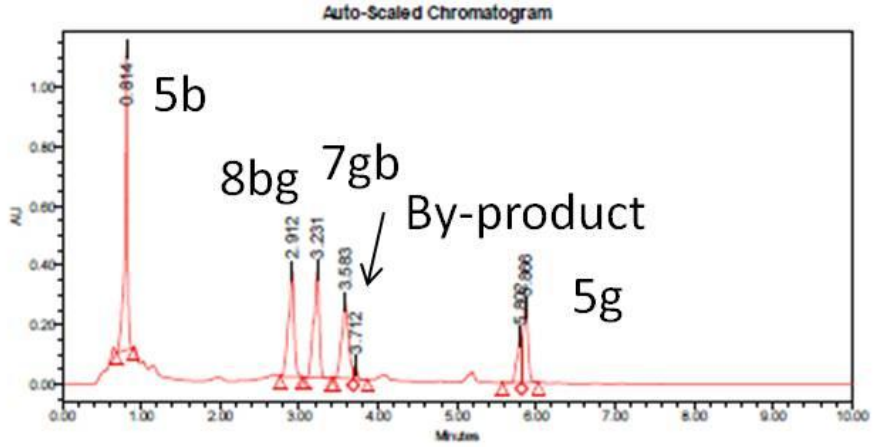
At 16h



Peak Name:

	Injection	RT	Area	% Area	Height
1	1	0.920	2337085	54.67	481081
2	1	3.370	280525	6.56	51768
3	1	3.652	170988	4.00	37662
4	1	6.060	1309420	30.63	212102
5	1	6.172	176924	4.14	44054
Mean		4.035			
Std. Dev.		2.177			

For 3b & 3g

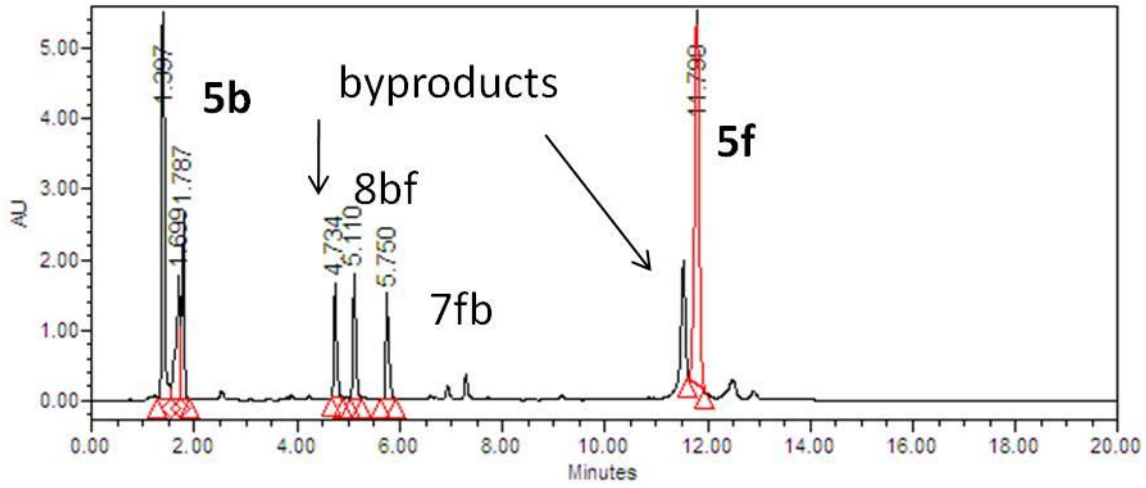


Peak Name:

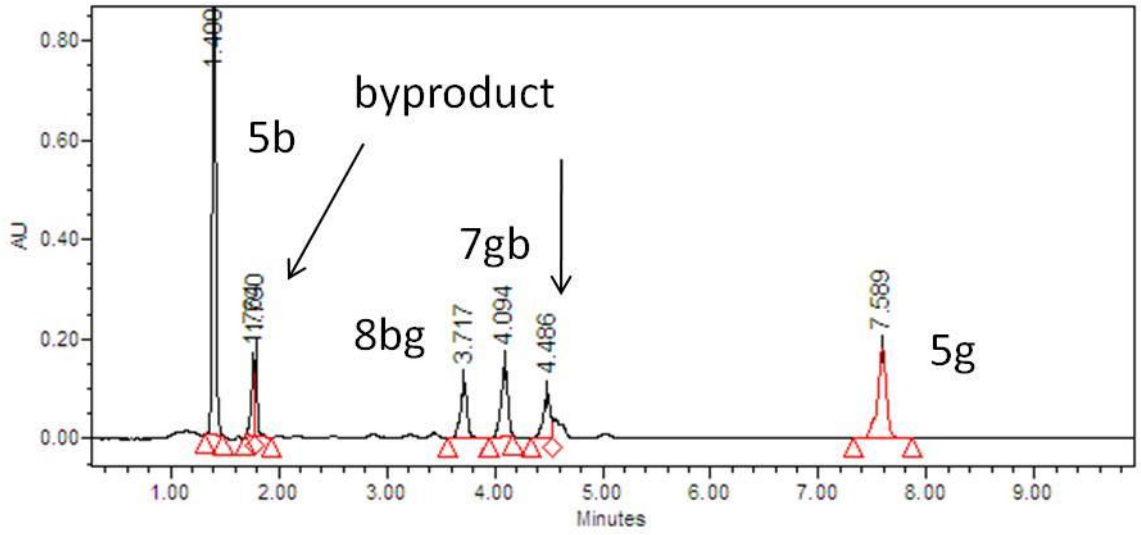
	Injection	RT	Area	% Area	Height
1	1	0.814	2406162	25.70	1015234
2	1	2.912	1753041	18.72	357653
3	1	3.583	1541940	16.47	257398
4	1	3.712	151505	1.62	45737
5	1	5.802	556580	5.94	155657
6	1	5.866	1195229	12.76	252275
7	1	3.231	1758898	18.78	363027

S14- Preparative HPLC-UV elution profiles with Xbridge™-Prep of proof reactions

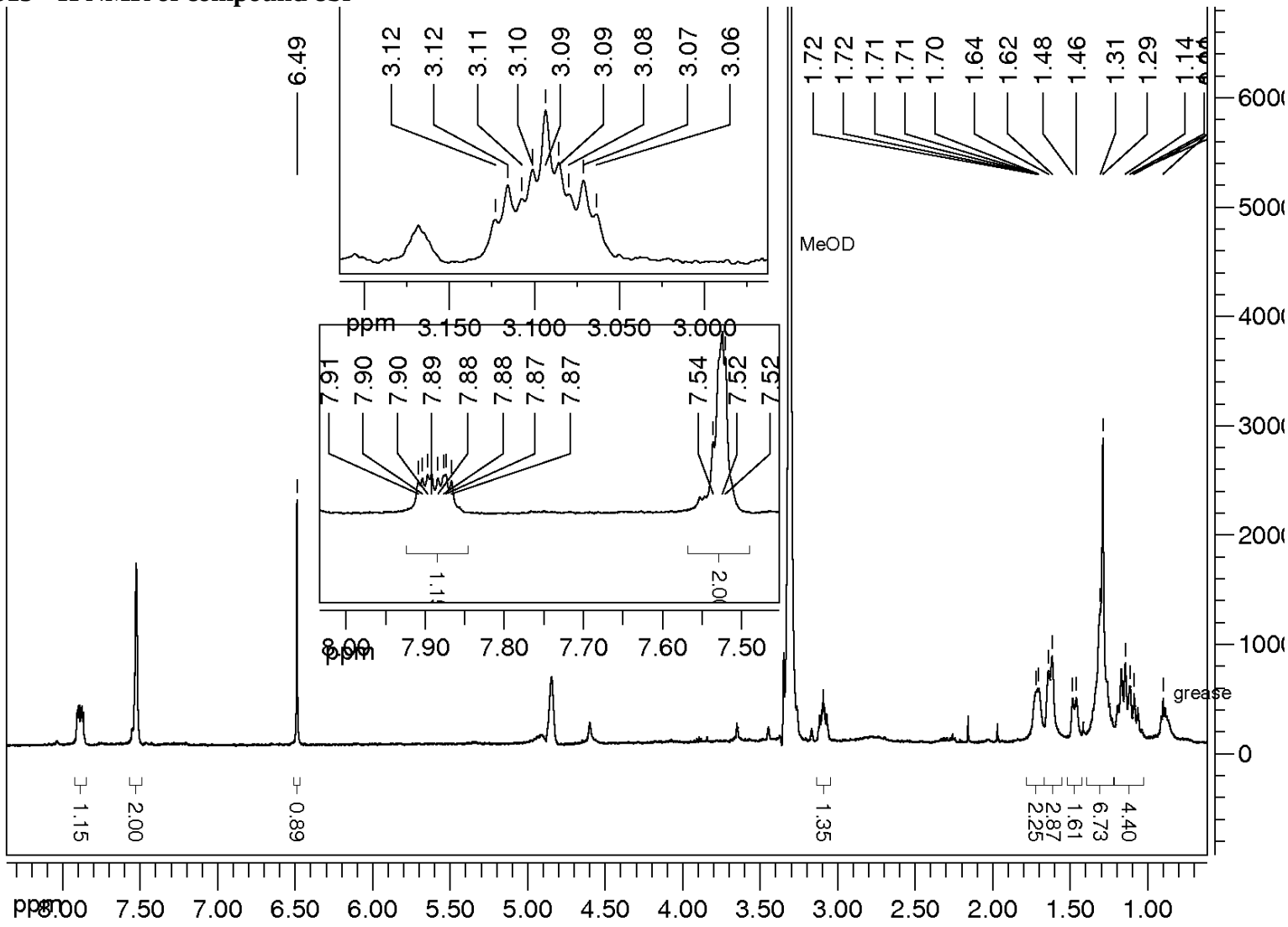
For 3b & 3f



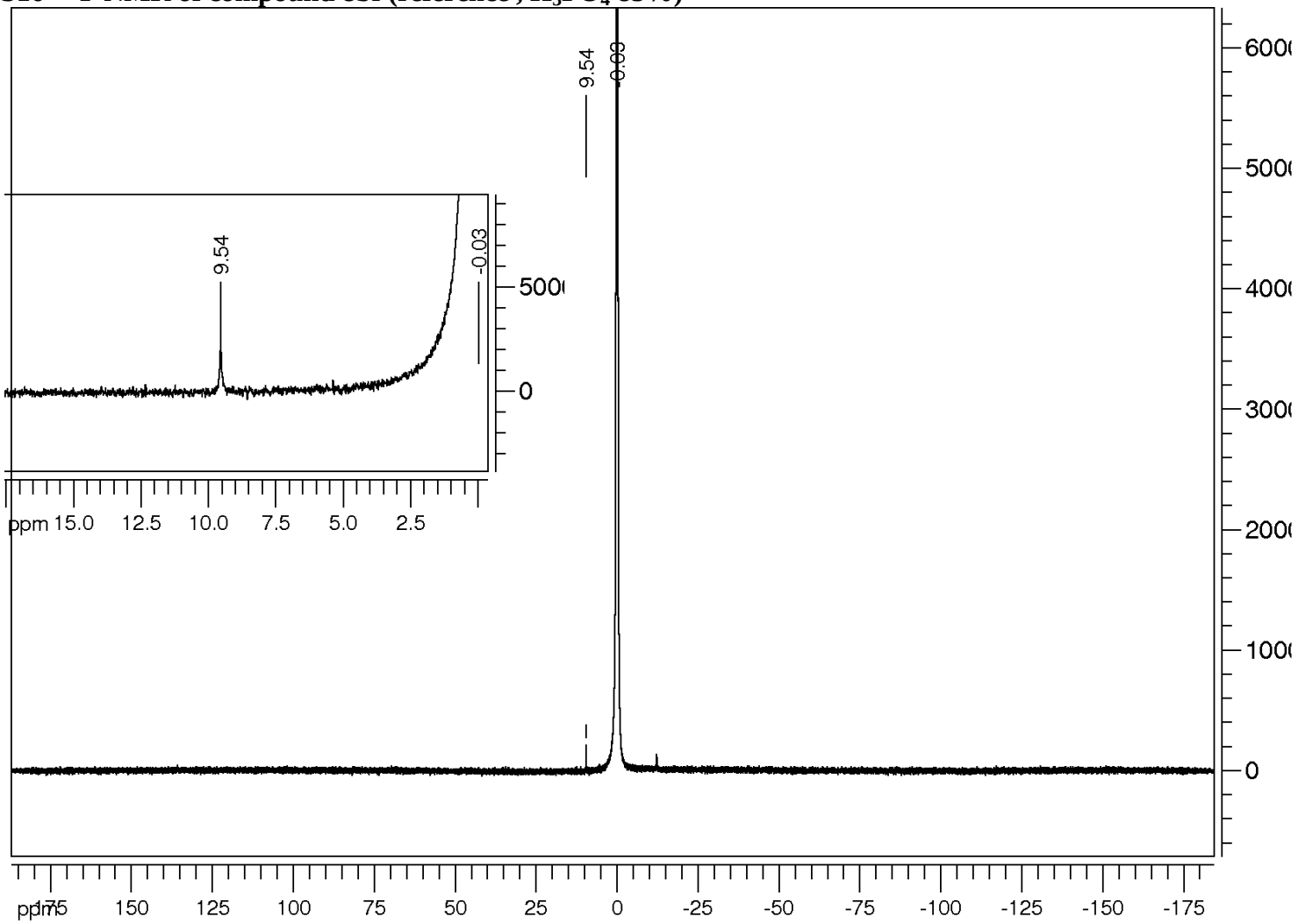
For 3b & 3g



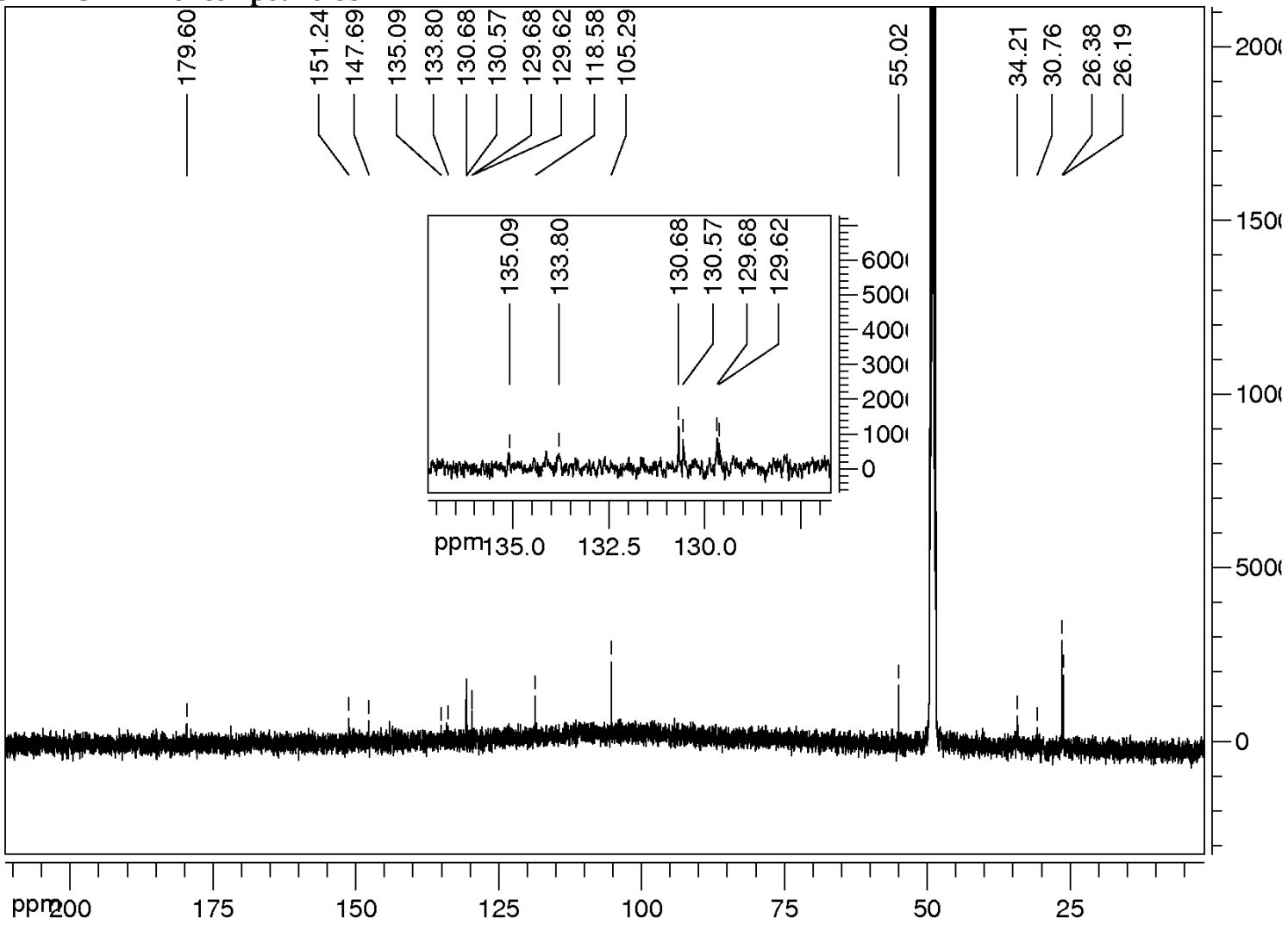
S15- ^{1}H NMR of compound 8bf



S16- ^{31}P NMR of compound 8bf (réference , H_3PO_4 85%)



S17- ^{13}C NMR of compound 8bf



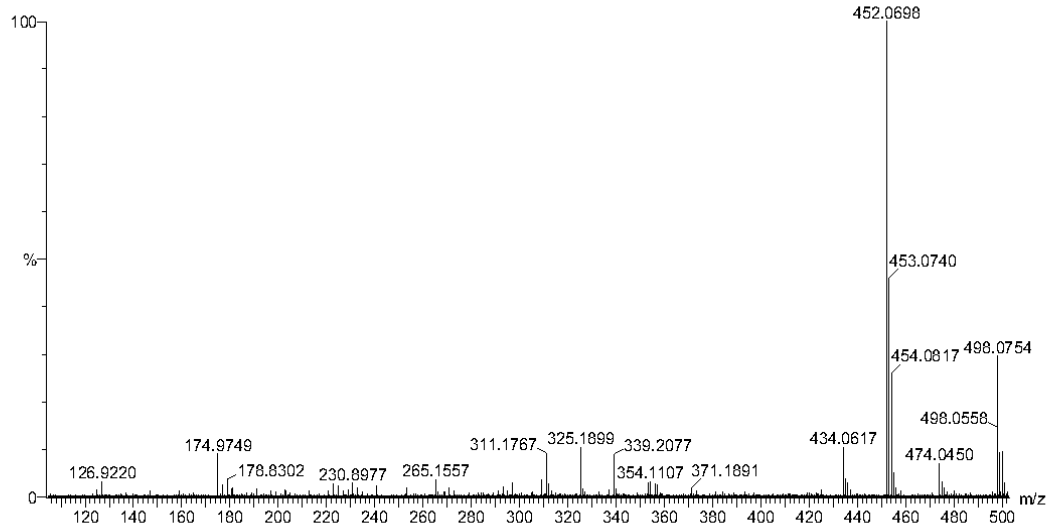
S18- HRMS of compound 8bf

Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 10.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 2

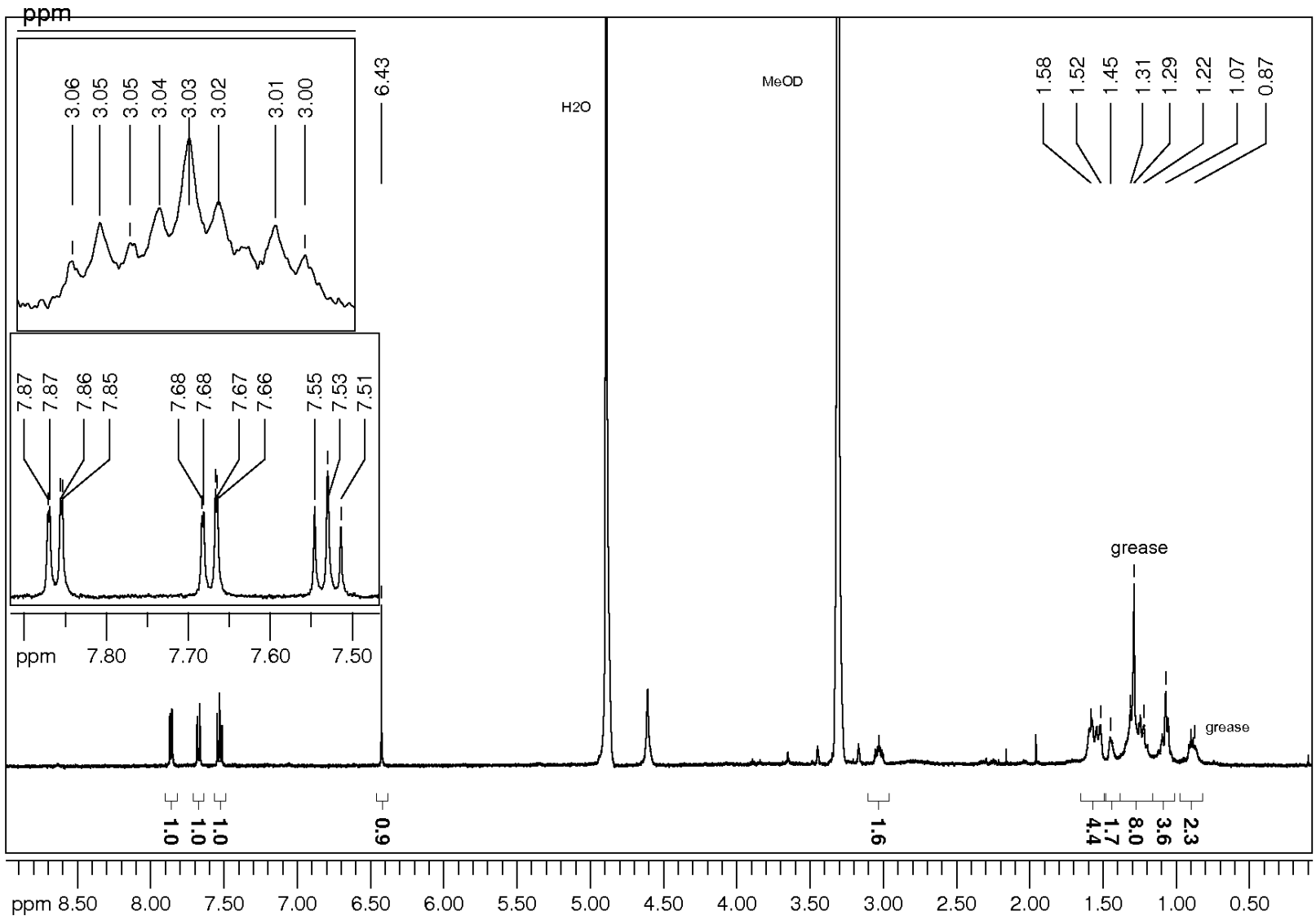
Monoisotopic Mass, Even Electron Ions
30 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 18-18 H: 4-200 N: 3-3 O: 7-10 Na: 0-1 P: 1-2 S: 1-2

LCT Premier
18-Aug-2011
FB_2011_R104_SP_P2A 15 (0.350)
2: TOF MS ES-
9.31e+002

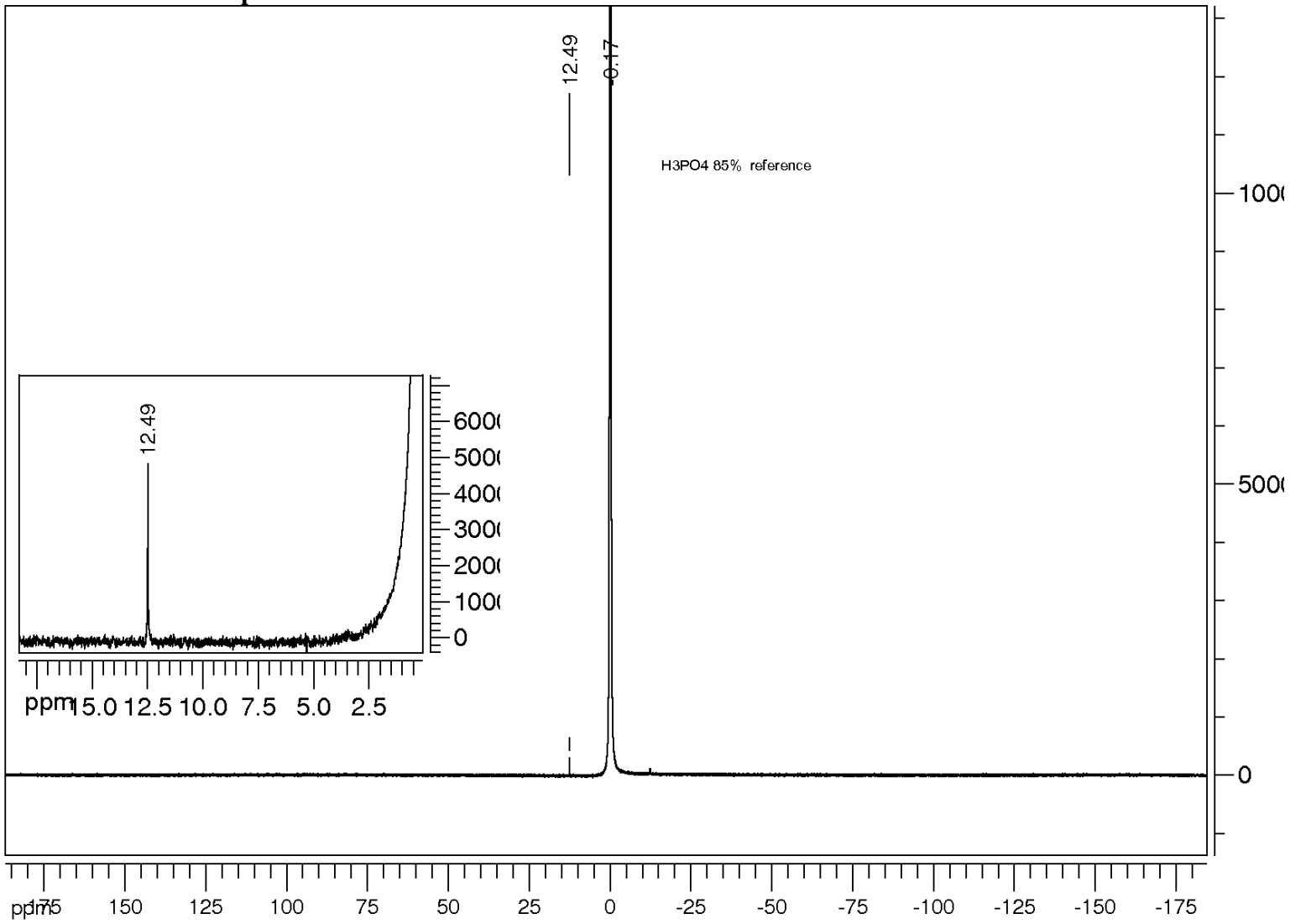


Minimum:				-1.5		
Maximum:		20.0	10.0	50.0		
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
452.0698	452.0681	1.7	3.8	11.5	50.3	C18 H19 N3 O7 P S

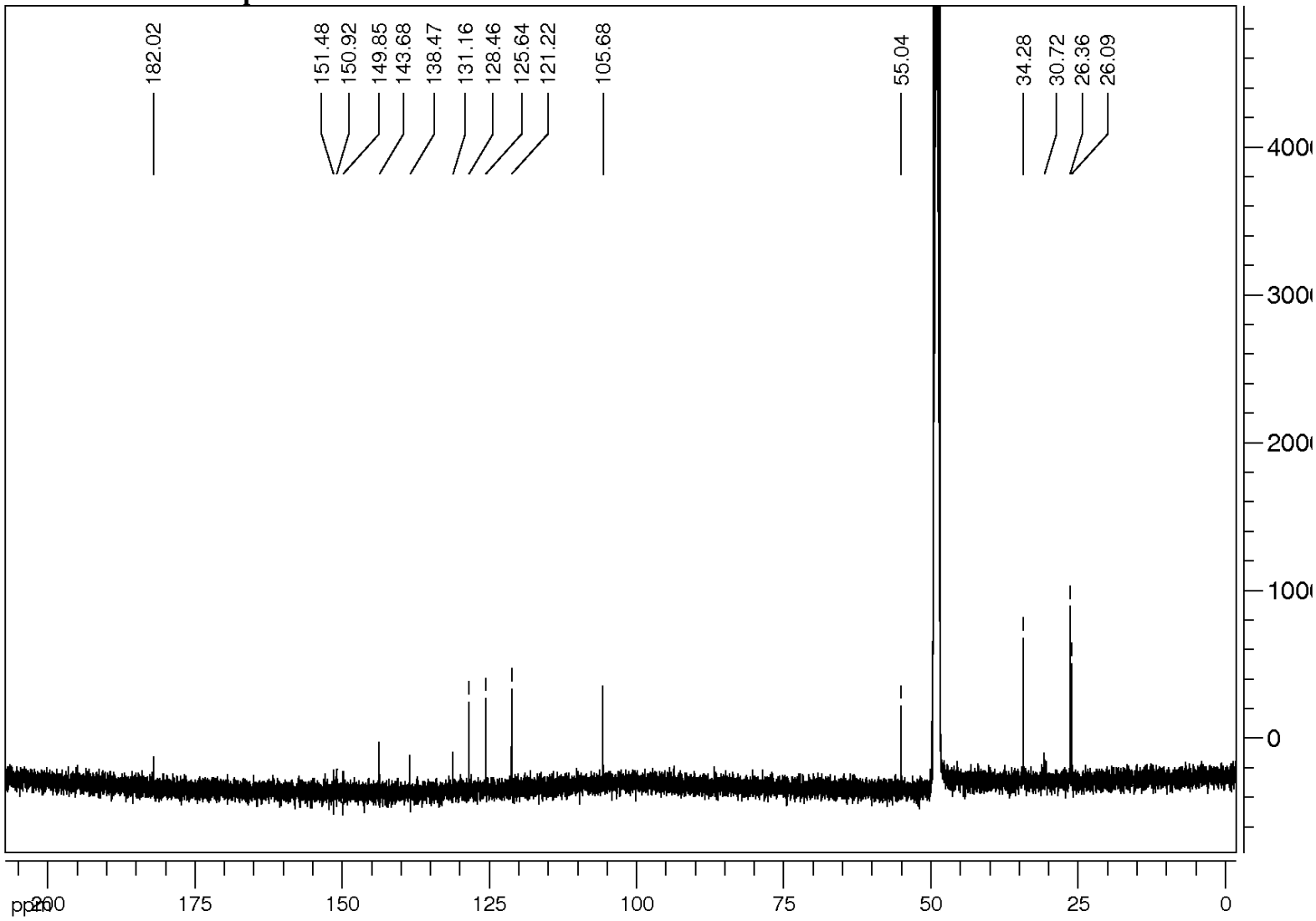
S19- ^{1}H NMR of compound 7fb



S20- ^{31}P NMR of compound 7fb



S21- ^{13}C NMR of compound 7fb



S22-HRMS data of compound 7fb

Elemental Composition Report **Page 1**

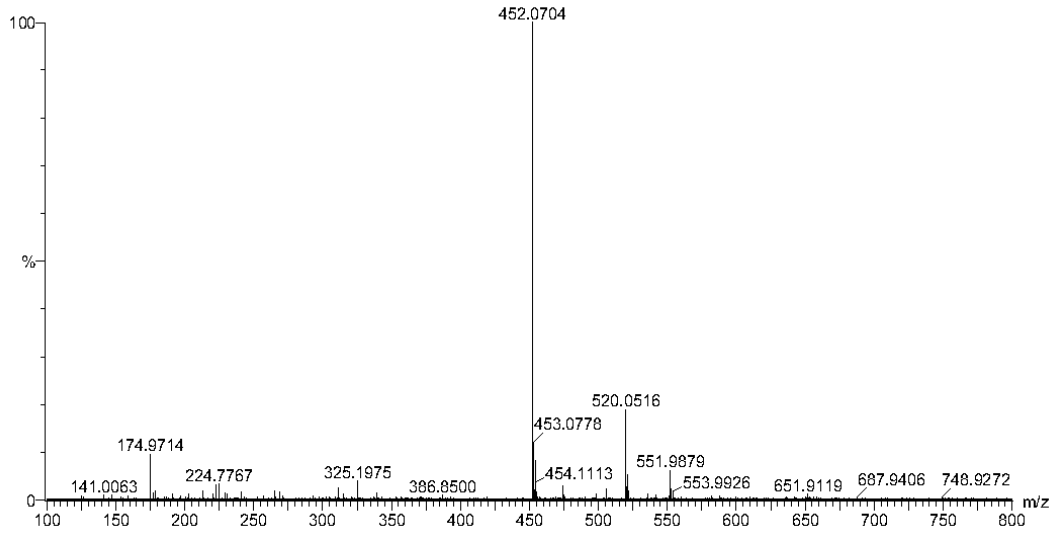
Single Mass Analysis
Tolerance = 10.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 2

Monoisotopic Mass, Even Electron Ions
180 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 18-18 H: 4-200 N: 0-5 O: 7-10 Na: 0-1 P: 1-2 S: 1-2

LCT Premier

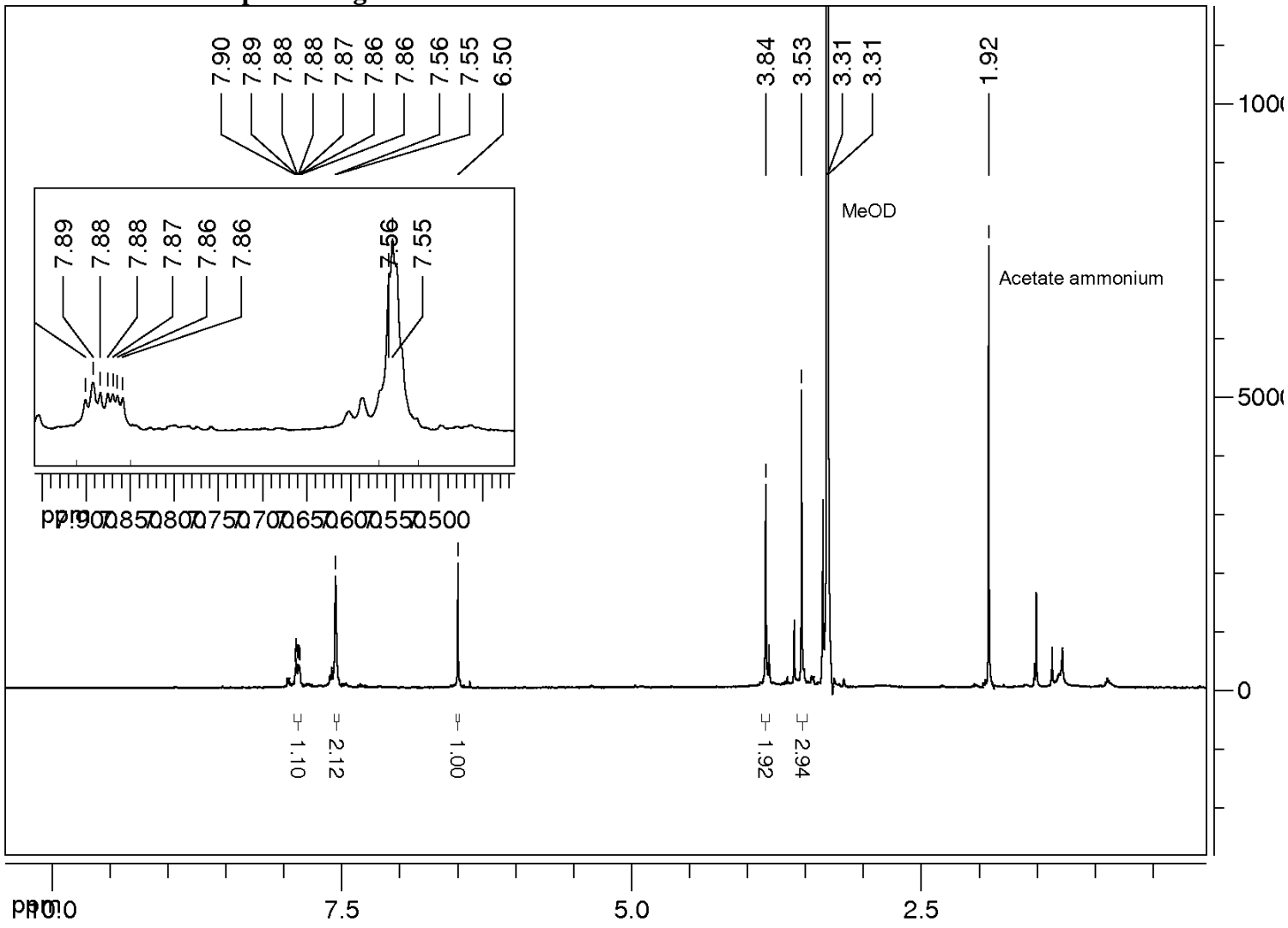
18-Aug-2011
FB_2011_R104_SP_P3A 16 (0.369)

2: TOF MS ES-
1.42e+003

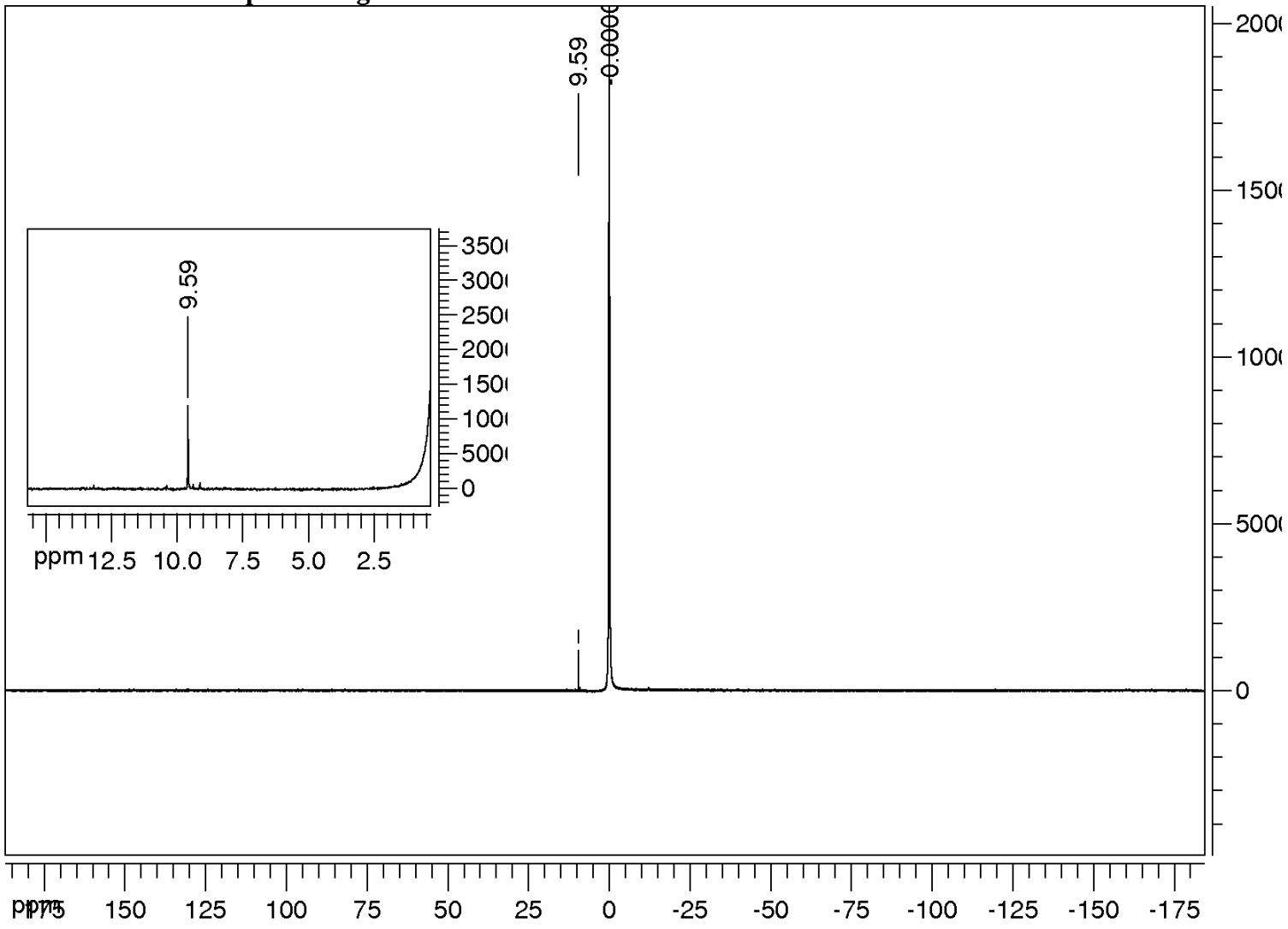


Minimum:				-1.5		
Maximum:		20.0	10.0	50.0		
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
452.0704	452.0681	2.3	5.1	11.5	44.9	C18 H19 N3 O7 P S

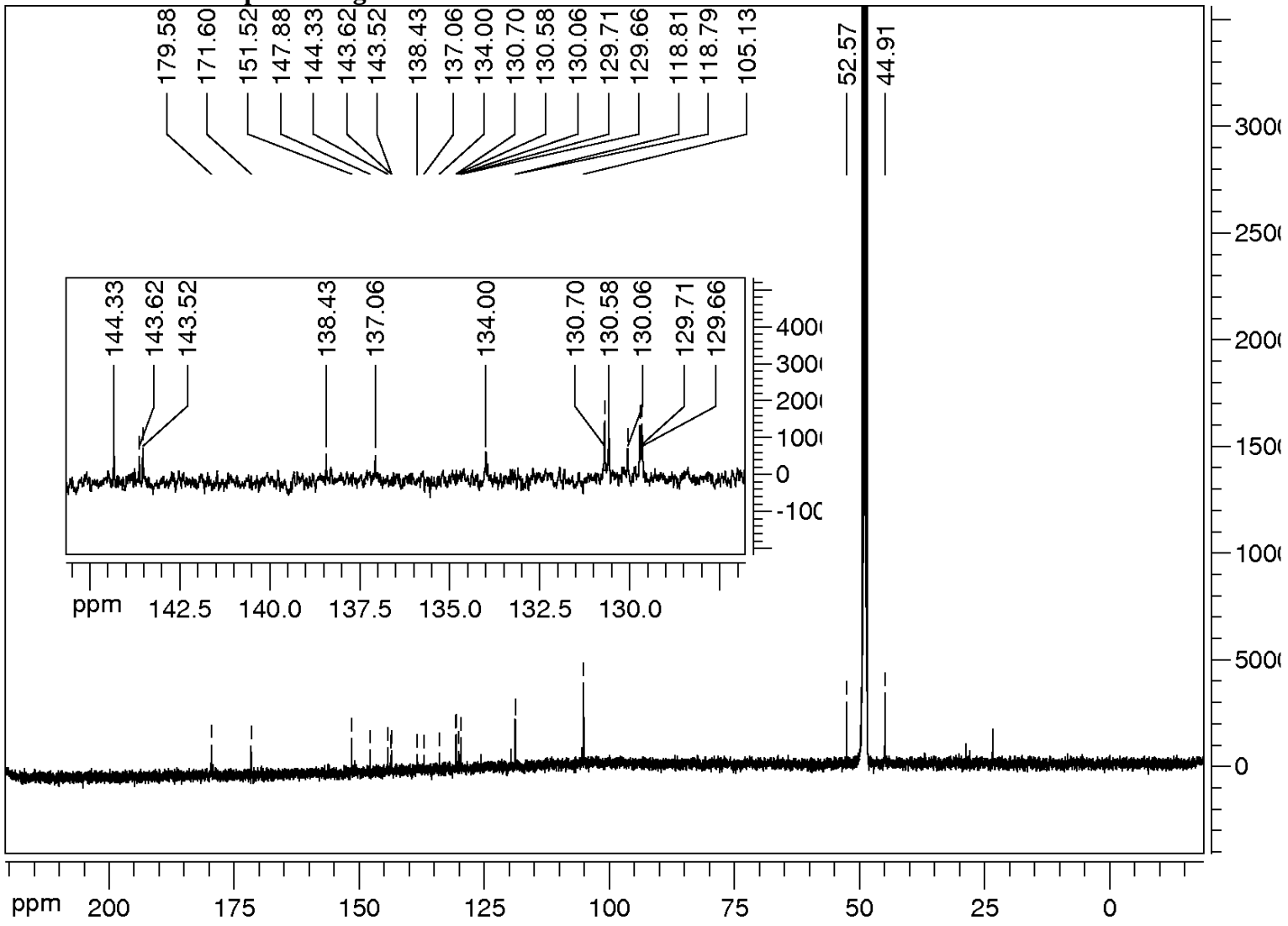
S23- ^{1}H NMR of compound 8bg



S24- ^{31}P NMR of compound 8bg



S25- ^{13}C NMR of compound 8bg



S26- HRMS of compound 8bg

Elemental Composition Report **Page 1**

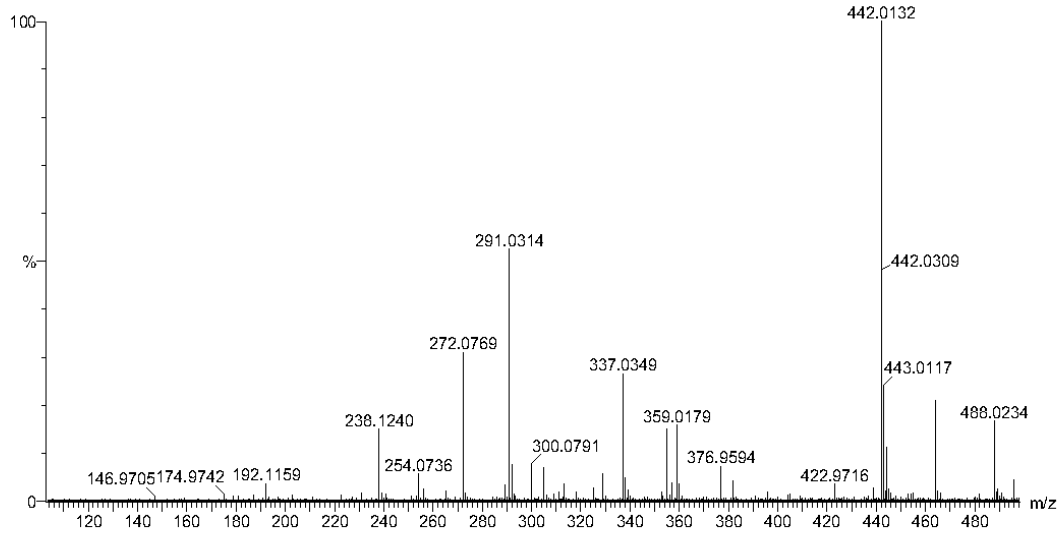
Single Mass Analysis
Tolerance = 10.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 2

Monoisotopic Mass, Even Electron Ions
15 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 15-15 H: 4-200 N: 3-3 O: 9-10 Na: 0-1 P: 1-2 S: 1-2

LCT Premier

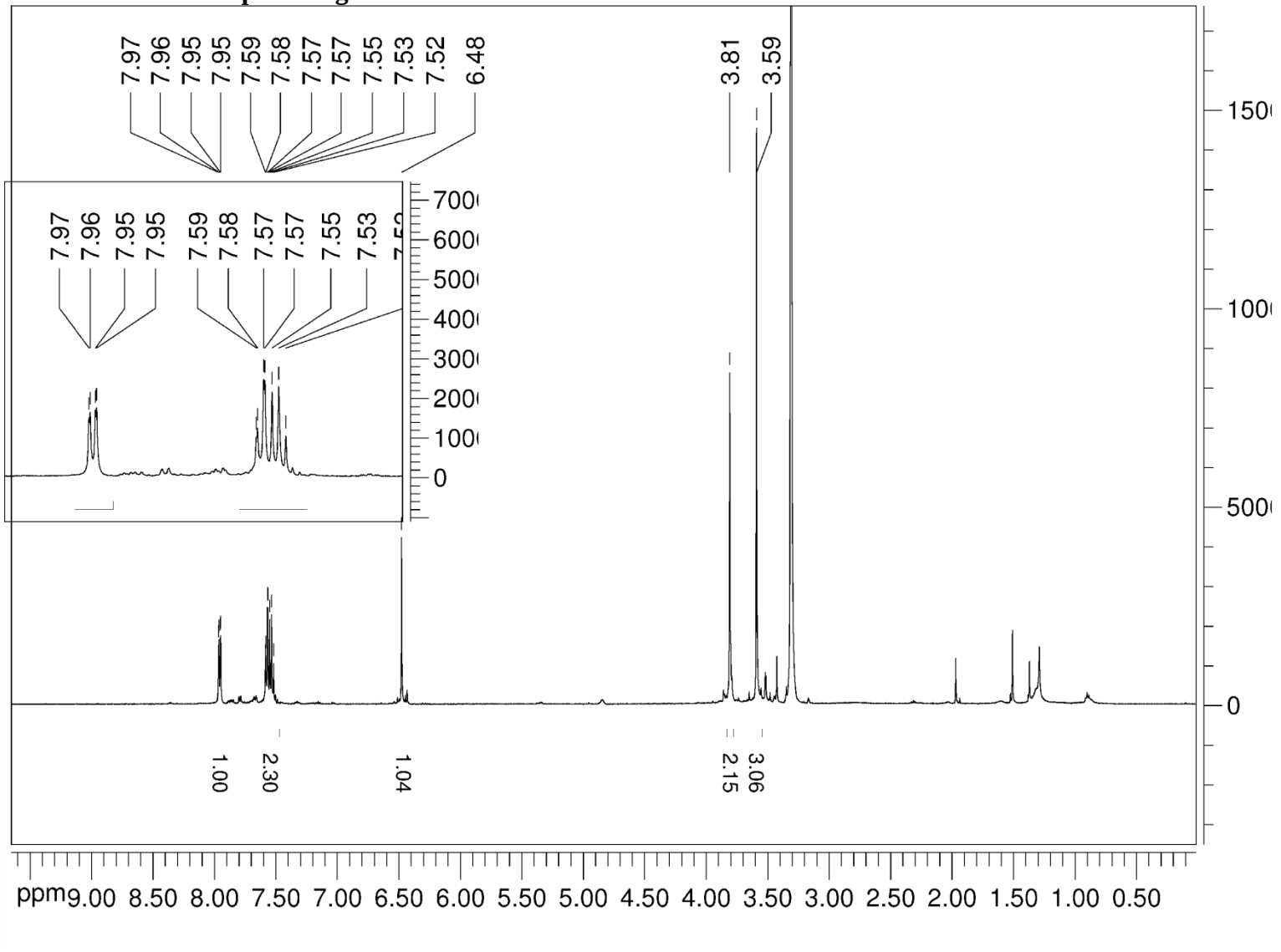
18-Aug-2011
FB_2011_R1-5_SP_P2A 15 (0.350)

2: TOF MS ES-
9.89e+002

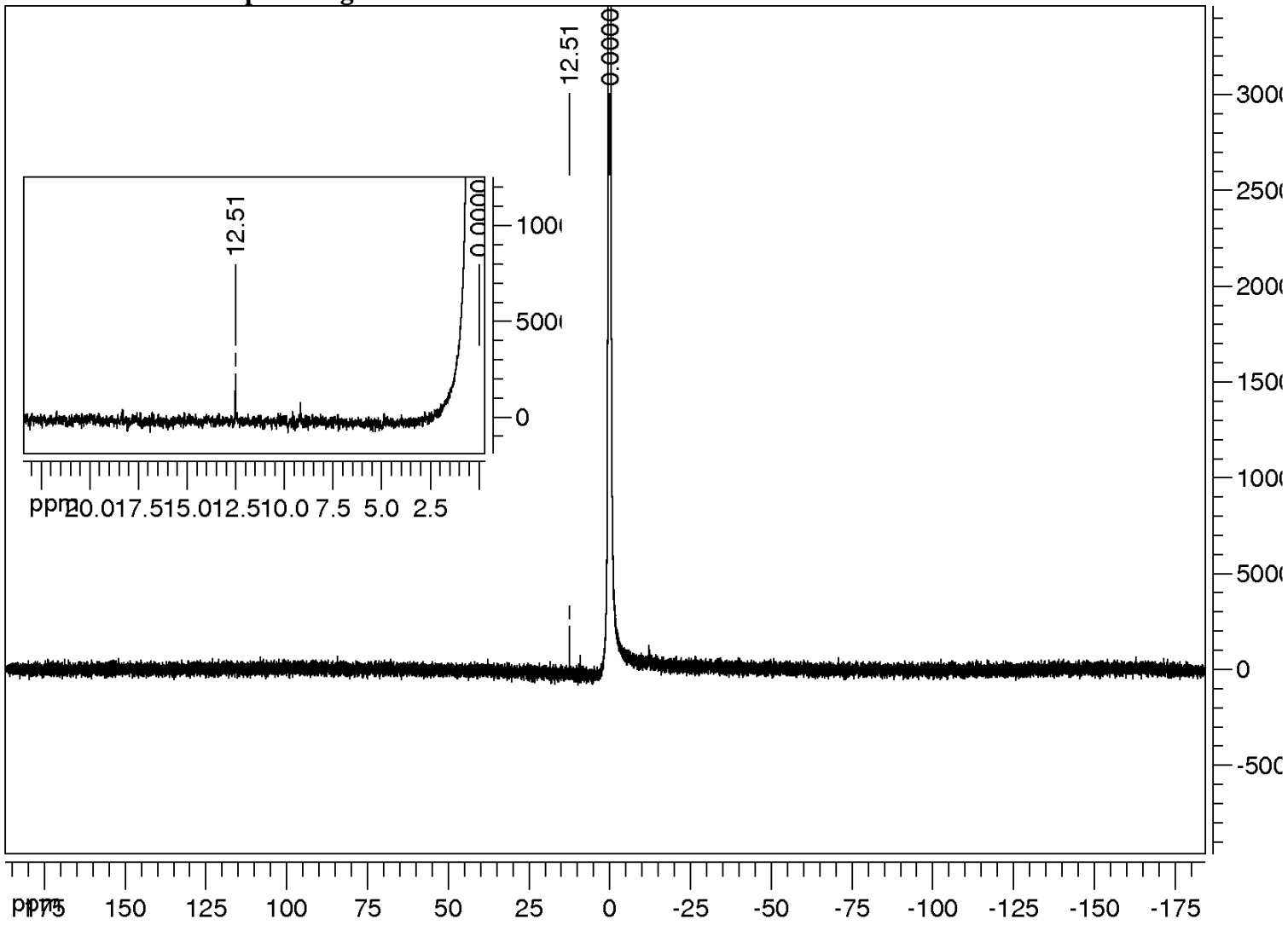


Minimum:				-1.5		
Maximum:		20.0	10.0	50.0		
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
442.0132	442.0110	2.2	5.0	11.5	4.3	C15 H13 N3 O9 P S

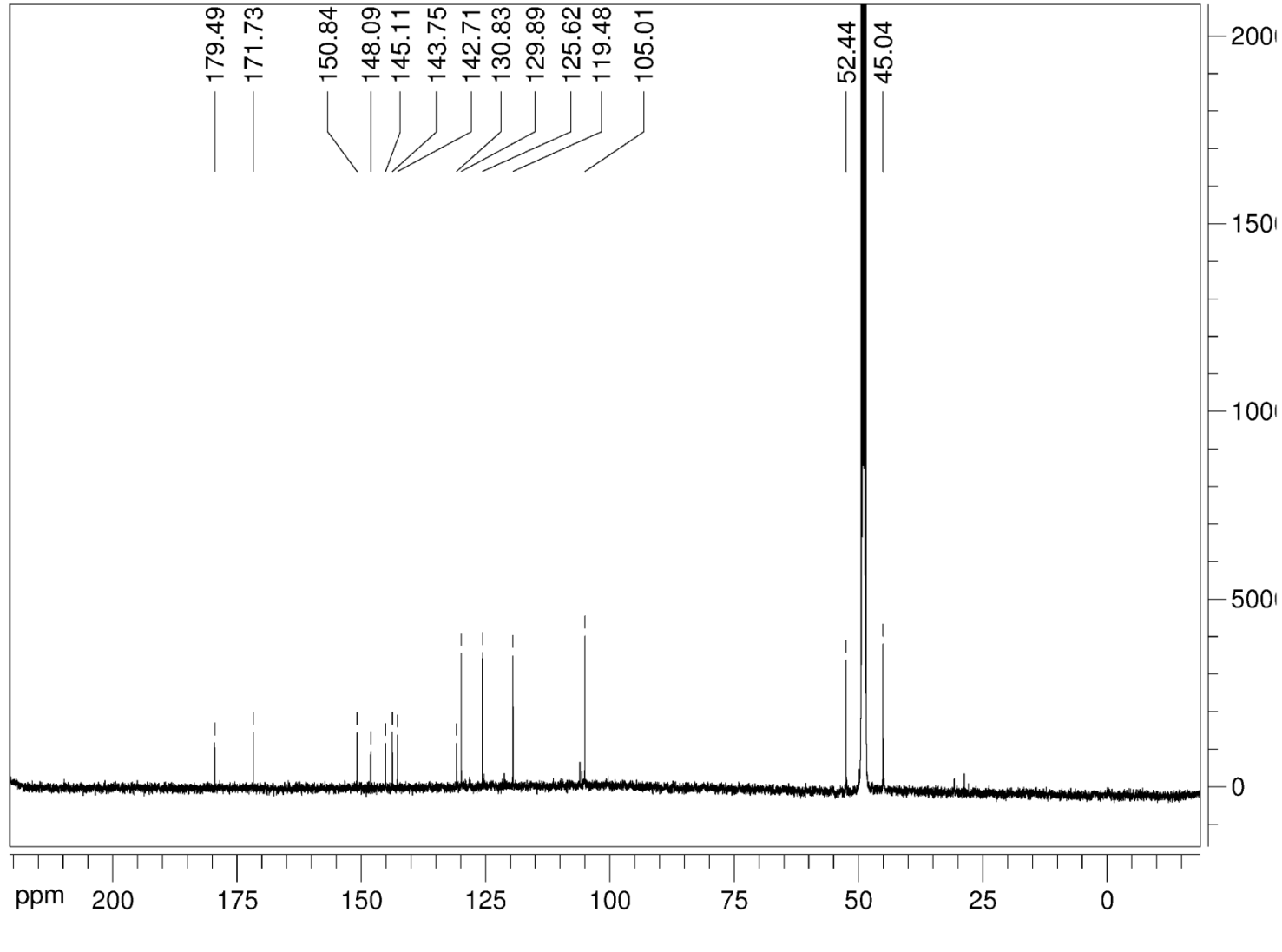
S27- ^{1}H NMR of compound 7gb



S28- ^{31}P NMR of compound 7gb



S29- ^{13}C NMR of compound 7gb



S30- HRMS of compound 7gb

Elemental Composition Report **Page 1**

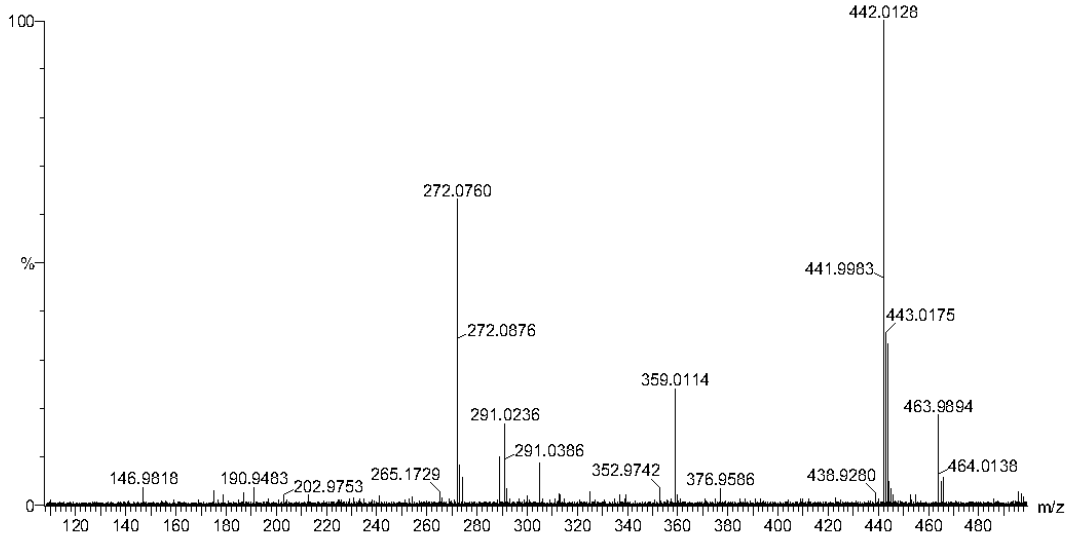
Single Mass Analysis
Tolerance = 10.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 2

Monoisotopic Mass, Even Electron Ions
15 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 15-15 H: 4-200 N: 3-3 O: 9-10 Na: 0-1 P: 1-2 S: 1-2

LCT Premier

18-Aug-2011
FB_2011_R105_SP_P3A 16 (0.369)

2: TOF MS ES-
6.19e+002



Minimum:				-1.5		
Maximum:		20.0	10.0	50.0		
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
442.0128	442.0110	1.8	4.1	11.5	21.3	C15 H13 N3 O9 P S

S31-Identification of novel phenoxazinones by HPLC-MS/MS

Pairs of selected precursors were oxidized in presence of laccase **Tv2** using identical experimental conditions. Only the compounds featuring primary/secondary sulfonamides were chosen to ease the HPLC-MS separation and ionization. Compound **4j** was included in the set to determine if it is oxidized or acts only as a nucleophilic partner. After 24 hours of reaction, samples were taken off and analyzed by HPLC-PDA at a flow rate of 0.2 $mLmin^{-1}$ and subsequently analyzed in HPLC-MS/MS.[‡-1] Reaction mixtures containing a suspension of partially water-soluble phenoxazinones (typically **5f** and **6f**) were filtered and the residual solid dissolved in methanol before both solutions were analyzed by HPLC-PDA (see ESI). Selected results are summarized in Table 3. The identification process for the pair **3f-3d** (entry 1, Table 3) is exemplified in the Figure 5. First the elution profile, analyzed by HPLC-PDA, showed the corresponding known phenoxazinones **5d** and **5f** at a retention time of respectively 2.59 and 14.54 minutes (Figure 5, top on left). Two new heterocycles featuring the phenoxazinone typical absorption spectrum (λ_{max}=230, 440 nm) were detected at respectively 10.62 and 11.03 minutes. The sample was then analyzed by HPLC-MS (full mass spectra) which allowed the identification of the peaks at 9.49 and 10.04 minutes (about 1 min shift due to different HPLC systems). Full mass spectra of both peaks showed a prominent m/z 453 ion corresponding to $[M + H]^+$ of compounds with the molecular formula $C_{18}H_{20}N_4O_6S_2$ (spectra in ESI), as expected for the heterocycles **8df** and **7fd** (see Scheme 2). Tandem mass spectrometry (CID fragmentation) was used to identify the specific fragmentation patterns which indicate phenoxazinones featuring a cyclohexyl moiety. Compared to the known dyes **5d** and **5f**, the ions at m/z 289.7 - 272.7 - 208.7 (Fg = SO_2NH_2) and m/z 370.7 - 353.7 - 305.7 (Fg = SO_2NH-cyclohexyl) were respectively identified as indicative of the core structure. MS/MS spectra (Figure 5 on the right) are detailed in the entry 1 of the Table 3. Specific loss of neutral fragments corresponding to cyclohexene (- m/z 82), sulfur dioxide (- m/z 64) and ammonia (- m/z 17) were observed. Such typical fragmentations were similarly presents in all the new compounds detected (structural information in the ESI). By mixing **3f** and the previously inert **4j** (Table 3, entry 2), a major new peak was detected in both HPLC-PDA and HPLC-MS. The corresponding ion with m/z of 538 $[M + H]^+$ ($C_{23}H_{31}N_5O_6S_2$) gave in tandem mass spectrometry the typical fragmentation along with a loss of neutral fragment of m/z 45 ($HNMe_2$) indicative of the tertiary amino group. A second compound with identical mass m/z 538 was detected in tiny amount. The same fragmentation pattern was observed. This suggests that the major compound arises from laccase-mediated oxidation of **3f** and subsequent 1,4-addition of native **4j** (Scheme 2, phenoxazinone **10jf**). The very minor compound would arise from auto-oxidation of **4j** and subsequent 1,4-addition of native **3f** (Scheme 2, phenoxazinone **9fj**). In comparison, the reaction of **3f** and **3j** (i.e the regioisomer of **4j**) gave two novel heterocyclic compounds (**8jf** and **7fj**, Table 4, entry 3) corresponding to m/z 538. They feature the expected fragmentation pattern in tandem mass spectrometry which validates the observations made for the reaction of **3f** and **4j**. Finally mixing compound **4f** with **3d** gave again two novel compounds with a prominent ion of m/z 453 (**9df** and **10fd**, see entry 4, Table 3). These experimental results indicate that non-symmetrically substituted phenoxazinones are indeed formed in various proportions most probably depending on the redox-properties of the precursors and their relative affinity for the enzyme.

S32- Fragmentation patterns observed in the identification of non-symmetrically substituted phenoxazinones

Non-symmetrically substituted phenoxazinones identified by HPLC-MS after 24 hours of reaction [a]

Entry	Cmpd	HPLC-PDA		HPLC-MS			Fragments analysis
		Dye-(min)	Dye-(min)	Dye-(min)	Dye-(min)	$[M+H]^+$ *m/z*	dye-(min) = ion (rel ab %) → daughter [loss]
1	**3f-3d**	**8fd-**(10.62)		**8fd-**(9.49)		453.20	**8fd**-(9.49) = 453.20 → 370.79 (75) [-82, C_6H_{10}]; 370.79 → 353.76 (20) [-17, NH_3]; 353.76 → 289.74 (100) [-64, SO_2]; 289.74 → 272.71 (20) [-17, NH_3], 272.71 → 208.72 (65) [-64, SO_2].
			7fd-(11.03)		**7fd-**(10.04)	453.20	**7fd**-(10.04) = 453.20 → 370.79 (70) [-82, C_6H_{10}]; 370.79 → 353.75 (10) [-17, NH_3]; 353.76 → 289.74 (100) [-64, SO_2]; 289.74 → 272.71 (15) [-17, NH_3], 272.71 → 208.73 (60) [-64, SO_2].
2	**3f-4j**	**10jf-**(6.79)	/	**10jf-**(5.68)	/	538.20	**10jf**-(5.68) = 538.11 → 455.97 (25) [-82, C_6H_{10}]; 455.97 → 438.92 (20) [-17, NH_3]; 455.97 → 410.90 (5) [-45, $HNMe_2$]; 438.92 → 374.82 (50) [-64, SO_2]; 455.97 → 374.86 (20) [-82, C_6H_{10}].
			/		**9fj-**(7.11)	538.20	**9fj**-(7.11) = 538.11 → 455.97 (25) [-82, C_6H_{10}]; 455.97 → 438.92 (20) [-17, NH_3]; 455.97 → 438.92 (15) [-45, $HNMe_2$], 438.92 → 374.82 (50) [-64, SO_2]; 455.97 → 374.86 (20) [-82, C_6H_{10}], 164.70, 85.60.
3	**3f-3j**	**8jf-**(7.77)		**8jf-**(6.46)		538.20	**8jf**-(6.46) = 538.20 → 455.97 (30) [-82, C_6H_{10}]; 455.97 → 410.86 (10) [-45, $HNMe_2$]; 382.80 (7); 376.88 ;85.71
			7fj-(8.09)		**7fj-**(6.75)	538.20	**7fj-** (6.75) = 538.20 → 455.97 (15) [-82, C_6H_{10}]; 455.97 → 410.85 (10) [-45, $HNMe_2$]; 382.80 (7); 164.66 ; 85.70
4	**4f-3d**	**9df-**(8.74)		**9df-**(7.3)		453.20	**9df**-(7.3) = 453.20 → 417.86 (50) [-17, -18, NH_3 & H_2O]; 453.20 → 387.90 (15) ; 387.90 → 371.85 (100); 371.85 → 289.70 (35) [-82, C_6H_{10}],
			10fd-(10.19)		**10fd-**(9.10)	453.20	**10fd**-(9.1) = 453.20 → 417.86 (50) [-17, -18, NH_3 & H_2O]; 453.20 → 387.90 (15) ; 387.90 → 371.85 (100); 371.85 → 289.70 (35) [-82, C_6H_{10}],

[a] Representative chromatograms recorded for the entry 1 are shown in the Figure 4. See experimental part for details about the chromatographic systems. Chromatogram 1 (top on the left) recorded with HPLC-PDA (λ=440 nm), chromatogram 2 (left on the bottom) recorded with HPLC-MS. MS-MS Spectra 1 (right, top) & 2 (right, bottom) respectively correspond to peak 1 and peak 2. Due to transposition from waters Alliance pumps to Agilent Spectra6000 pumps system and a slight modification of the eluent, a difference of about 1 minute is observed between the peaks of the chromatograms.

S33- Retention times of precursors and phenoxazinones listed in Table 2

Relative rates of conversion of selected substrates with surrogate laccase [a]

Entry	Cmpd1[b]	Cmpd2 [b]	RT-1 (min)	RT-2 (min)	Ratio at T_0 [c]	Ratio at T_3 [c]	Rel Conv[d]
1	**3f**	**3d**	3.88	1.51	1.15	0.96	**3d>3f**
2	**3f**	**3c**	3.88	1.21	0.13	0	**3c>3f** [e]
3	**3f**	**3j**	3.88	1.1-1.24	2.46	2.67	**3f>3j**
4	**4f**	**3f**	4.02	3.89	0.78	1.2	**4f>>3f**
5	**4f**	**3d**	4-02	1.51	0.87	1	**4f>3d**

[a] Representative chromatogram recorded at λ= 310nm for the entry 1 is shown in the Figure 2 (bottom). The uv-vis spectra of compounds **3f** and **3d** are also shown (top). [b] See Table 1 for the side-chains and Scheme 1 for the outline of the reaction in presence of laccase. [c] Area recorded at λ= 310nm, T_1 = 2 min after starting the reaction. Reaction mixture was spiked every 25 min, T_3 = 75 min [d] Relative rates were estimated by following the evolution of the ΔAreaCp1/ ΔAreaCp2 with ΔArea = ([Area T_1 –Area T_3]/Area T_1) recorded at 310nm over 75 min by laccase **Tv2** (100 $U.L^{-1}$) at pH 6 (0.2 M of $AcONH_4$). [e] Relative rate of consumption could not be estimated by comparison, the starting material **3c** disappeared in the first 25 min.

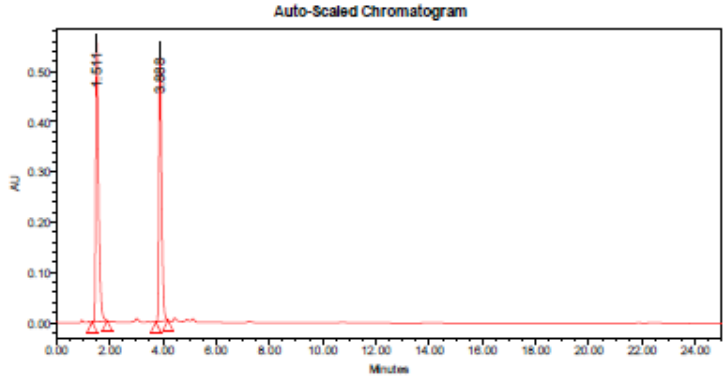
Retention times (RT in min) of laccase-mediated synthesised phenoxazinones from selected substrates in the first 2 hours of reaction.[a]

Entry	Cp1	Cp2	RT-P1	RT-P2	RT-P3	RT-P4	UV-Vis
1	**3f**	**3d**	2.92	4.97	5.17	7.62	240-260; 420-445
2	**3f**	**3c**	1.42	4.47	5.20	7.61	
3	**3f**	**3j**	1.45	4.54	5.2	7.62	
4	**4f**	**3f**	4.85	6.07	6.23	7.6 trace	
5	**4f**	**3d**	-	4.13	4.8	6.26	

[a] Representative chromatogram recorded for the entry 1 is shown in the Figure 3 (left). The peculiar uv-visible patterns are shown for the phenoxazinone compounds (right). See Table 1 for side-chains and see Schemes 1, 2 for general structures of the heterocyclic cores. Area recorded at λ= 440nm after 75 min of reaction. An intermediate is detected at retention time of 7.2 min and could arise from the first 1,4-addition step between oxidised and reduced **3f**. (see Scheme 4 for general structure).

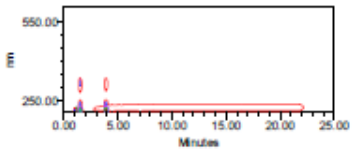
S34-HPLC-PDA analysis of the 3d-3f conversions by laccase at T1, T2, T3 at 310 nm

T1

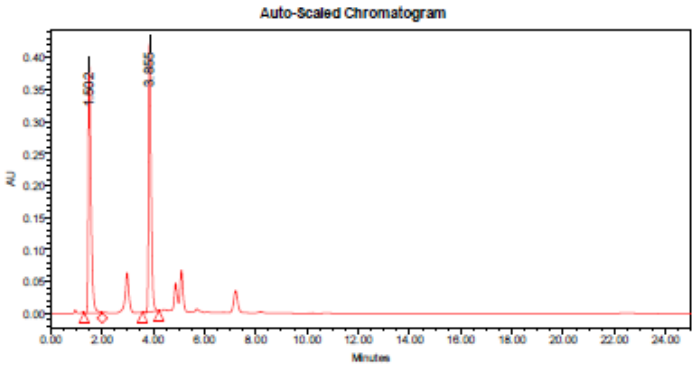


Peak Name:

	Injection	RT	Area	% Area	Height
1	1	1.511	4109396	52.72	555178
2	1	3.888	3684955	47.28	542289
Mean		2.699			
Std. Dev.		1.680			
% RSD		62.25			

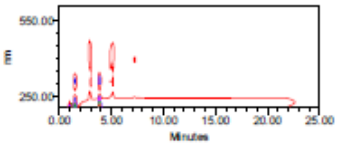


T2

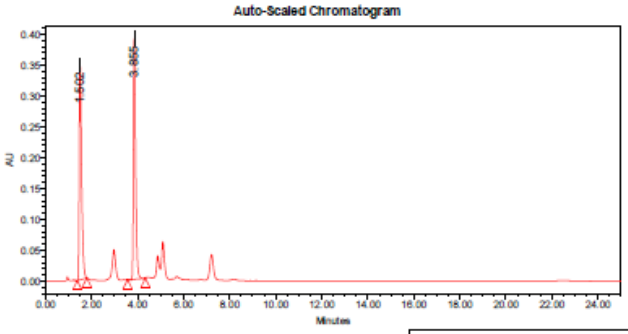


Peak Name:

	Injection	RT	Area	% Area	Height
1	1	1.502	2861604	50.38	388685
2	1	3.855	2818697	49.62	419042
Mean		2.678			
Std. Dev.		1.664			
% RSD		62.13			

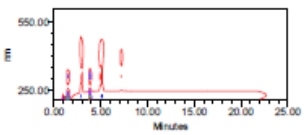


T3



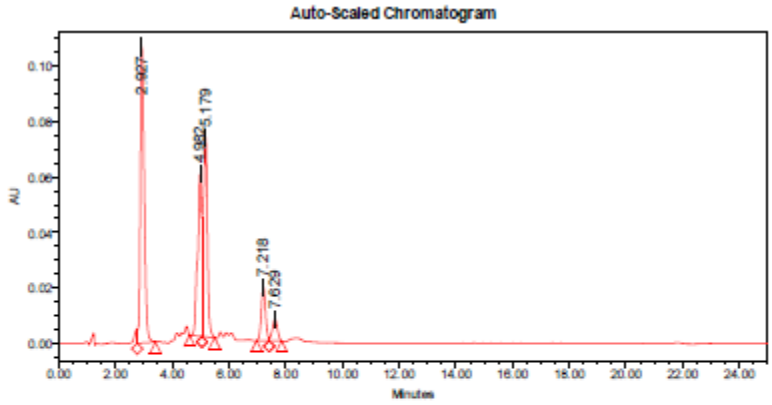
Peak Name:

	Injection	RT	Area	% Area	Height
1	1	1.502	2494594	49.04	348668
2	1	3.855	2592010	50.96	390605
Mean		2.679			
Std. Dev.		1.664			
% RSD		62.13			



S35-HPLC-PDA analysis of the 3d-3f conversions by laccase at T2, T3 (440 nm)

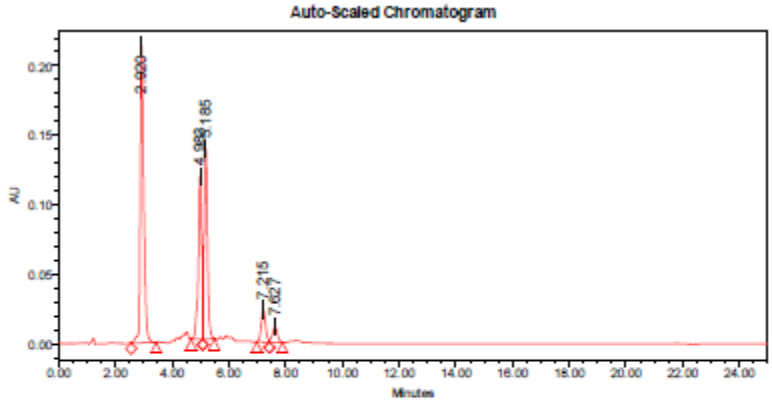
T2



Peak Name:

	Injection	RT	Area	% Area	Height
1	1	2.927	1013725	38.72	106901
2	1	4.982	559253	21.36	58451
3	1	5.179	749650	28.64	71890
4	1	7.218	199067	7.60	19308
5	1	7.629	96250	3.68	7769
Mean		5.587			
Std. Dev.		1.900			

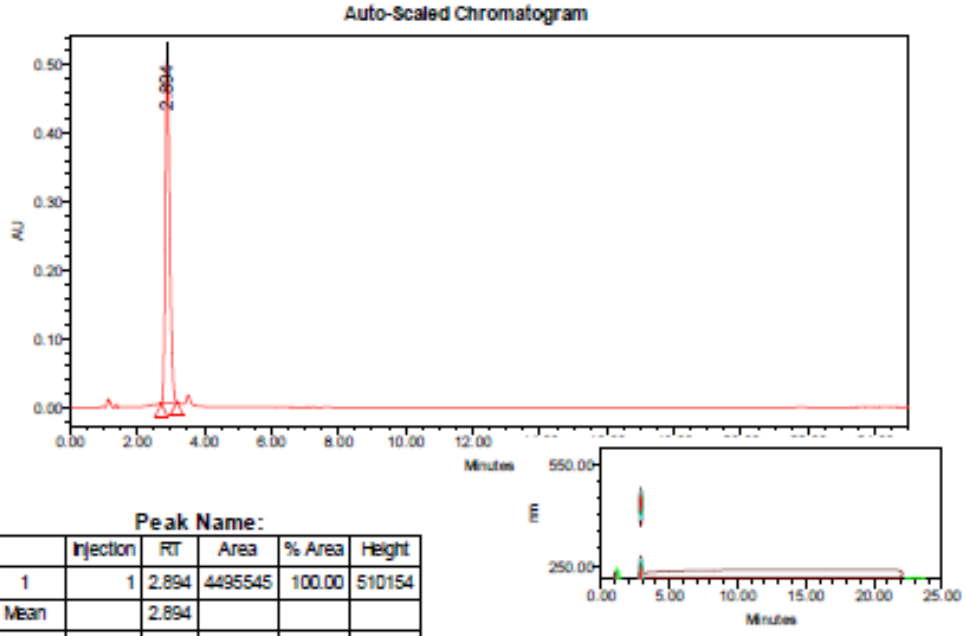
T3



Peak Name:

	Injection	RT	Area	% Area	Height
1	1	2.920	1808090	41.34	213866
2	1	4.983	1008305	23.05	117305
3	1	5.185	1180117	26.98	137251
4	1	7.215	240262	5.49	23181
5	1	7.627	136958	3.13	10966
Mean		5.586			
Std. Dev.		1.901			

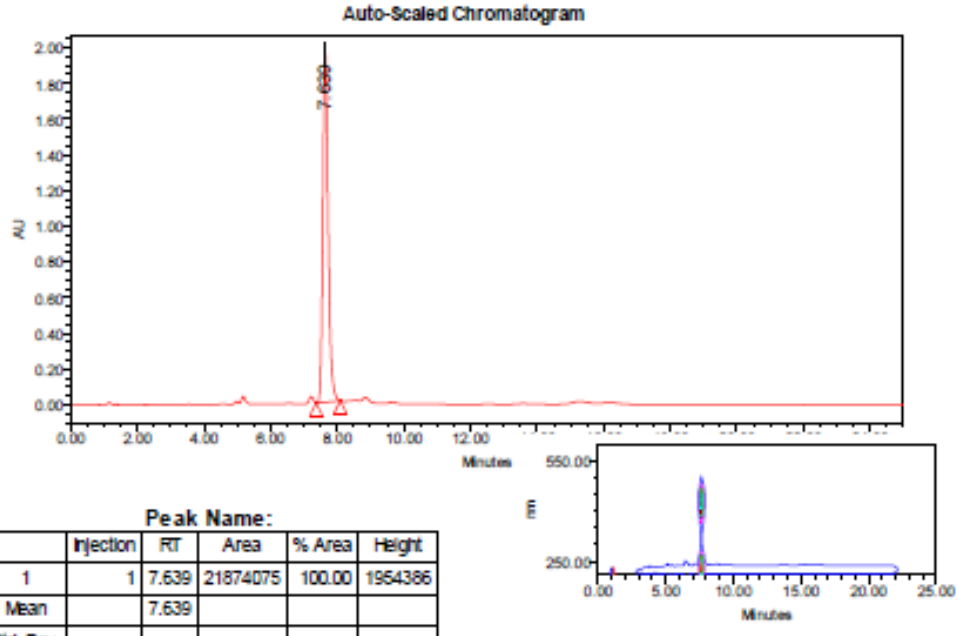
S36-Reference Phenoxazinone 5d –corresponding to analysis with system 1



Peak Name:

	Injection	RT	Area	% Area	Height
1	1	2.894	4496545	100.00	510154
Mean		2.894			
Std. Dev.					
% RSD					

Reference Phenoxazinone 5f –corresponding to analysis with system 1

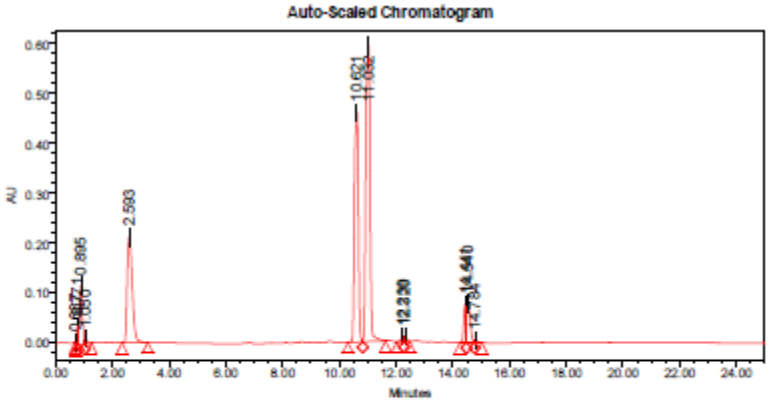


Peak Name:

	Injection	RT	Area	% Area	Height
1	1	7.639	21874075	100.00	1954386
Mean		7.639			
Std. Dev.					
% RSD					

S37-HPLC-PDA and MS of analysis of the 3d-3f conversion by laccase

HPLC-PDA with system 2

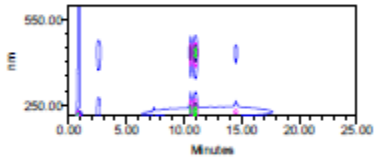


Peak Name:

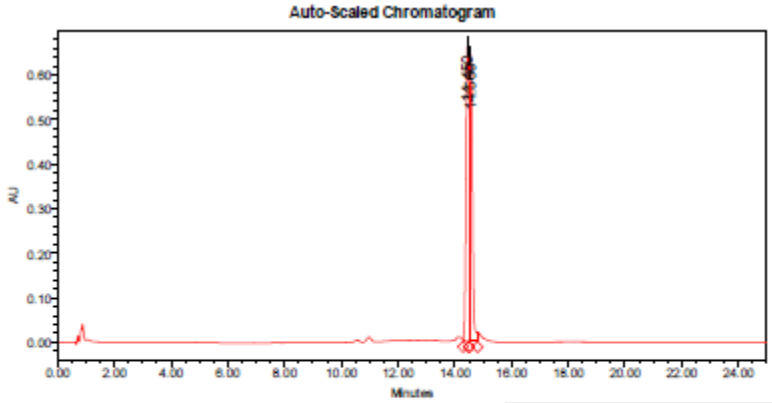
	Injection	RT	Area	% Area	Height
1	1	0.687	2319	0.02	1765
2	1	0.771	118227	0.77	35543
3	1	0.895	793783	5.15	117340
4	1	1.050	53909	0.35	9894
5	1	2.593	2640594	17.12	211038
6	1	10.621	4412624	28.60	461041
7	1	11.032	6110289	39.61	592933

Peak Name:

	Injection	RT	Area	% Area	Height
8	1	12.239	65157	0.42	10120
9	1	12.320	57093	0.37	9547
10	1	14.441	471223	3.05	76498
11	1	14.540	673823	4.37	78129
12	1	14.784	28615	0.19	3274
Mean		7.998			
Std. Dev.		6.155			
% RSD		76.96			

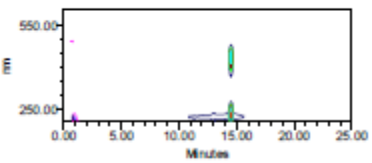


Reference phenoxazinone 5f

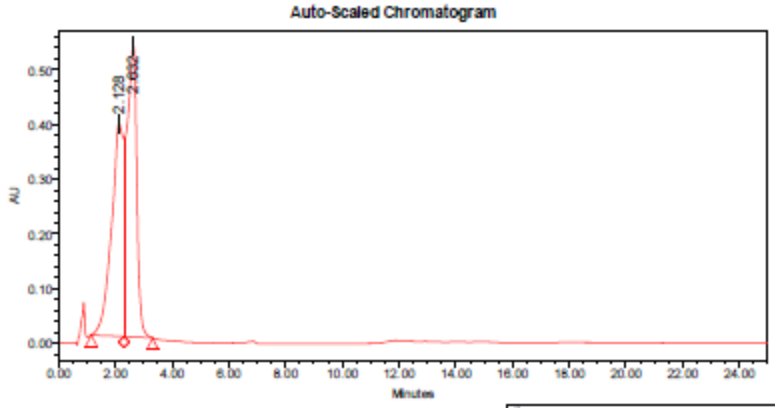


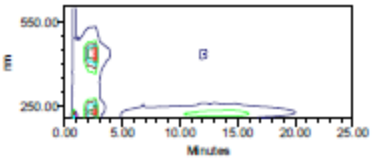
Peak Name:

	Injection	RT	Area	% Area	Height
1	1	14.450	4502963	48.56	662914
2	1	14.560	4770746	51.44	643168
Mean		14.505			
Std. Dev.		0.078			
% RSD		0.54			



Reference phenoxazinone 5d



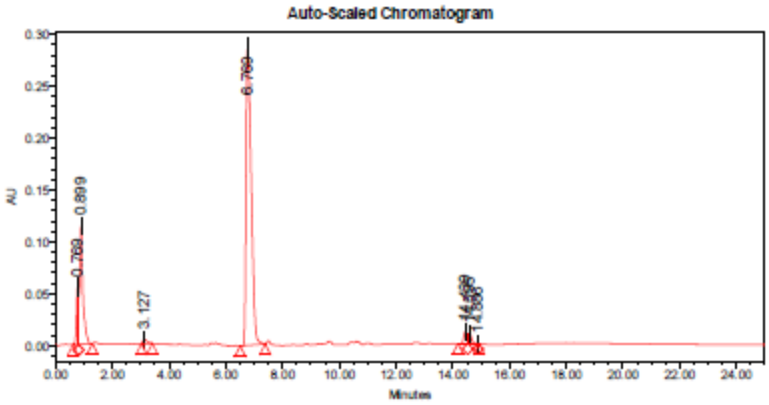


Peak Name:

	Injection	RT	Area	% Area	Height
1	1	2.128	11245883	46.14	388604
2	1	2.632	13125539	53.86	532465
Mean		2.380			
Std. Dev.		0.356			
% RSD		14.96			

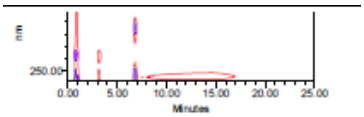
S39- HPLC-PDA of reaction 4j-3f (entry 2, table 3)

Solution after filtration

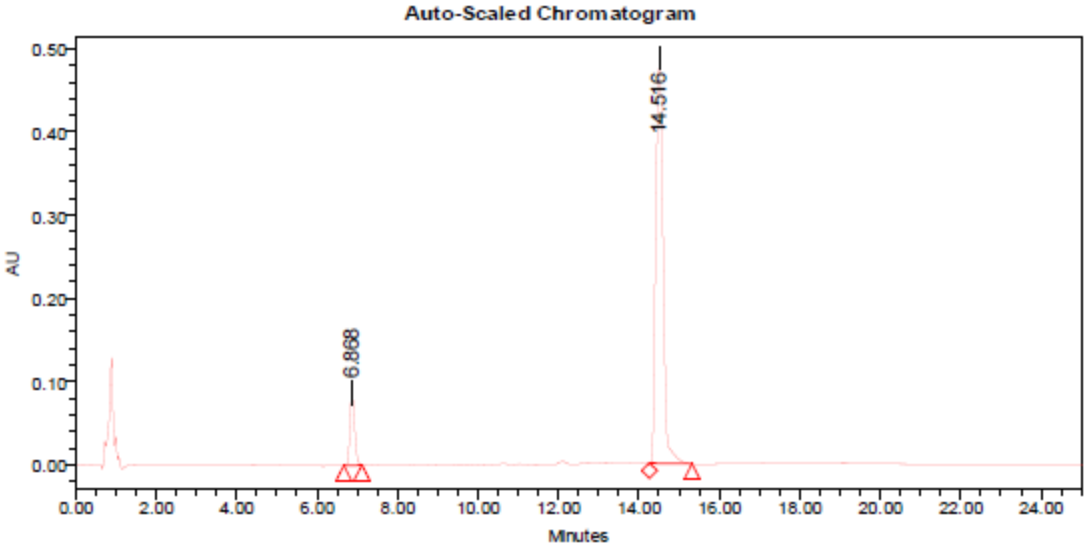


Peak Name:

	Injection	RT	Area	% Area	Height
1	1	0.769	236727	4.69	55245
2	1	0.899	944449	18.73	113512
3	1	3.127	43656	0.87	3985
4	1	6.769	3645226	72.29	287883
5	1	14.439	92384	1.83	11297
6	1	14.595	79615	1.58	9000
7	1	14.886	318	0.01	175

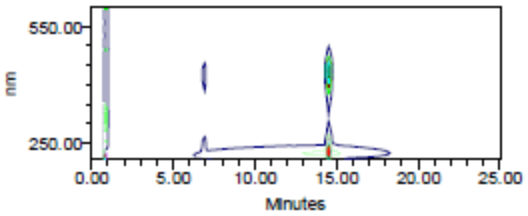


Precipitated dissolved in methanol



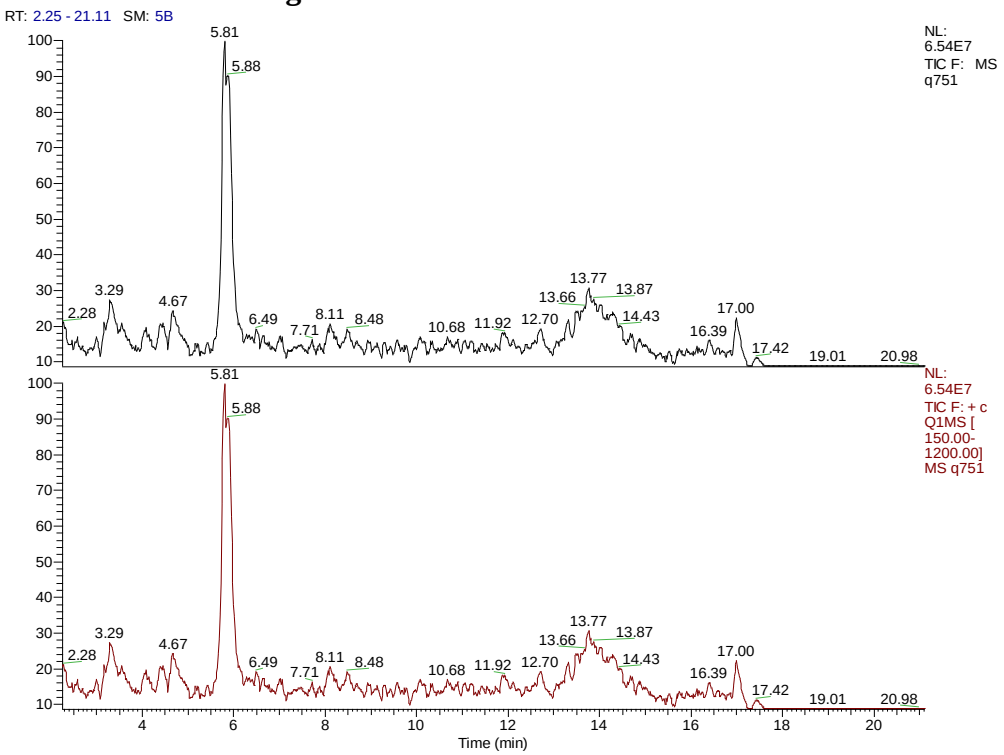
Peak Name:

	Injection	RT	Area	% Area	Height
1	1	6.868	698079	10.16	86660
2	1	14.516	6175564	89.84	486853
Mean		10.692			
Std. Dev.		5.408			
% RSD		50.58			

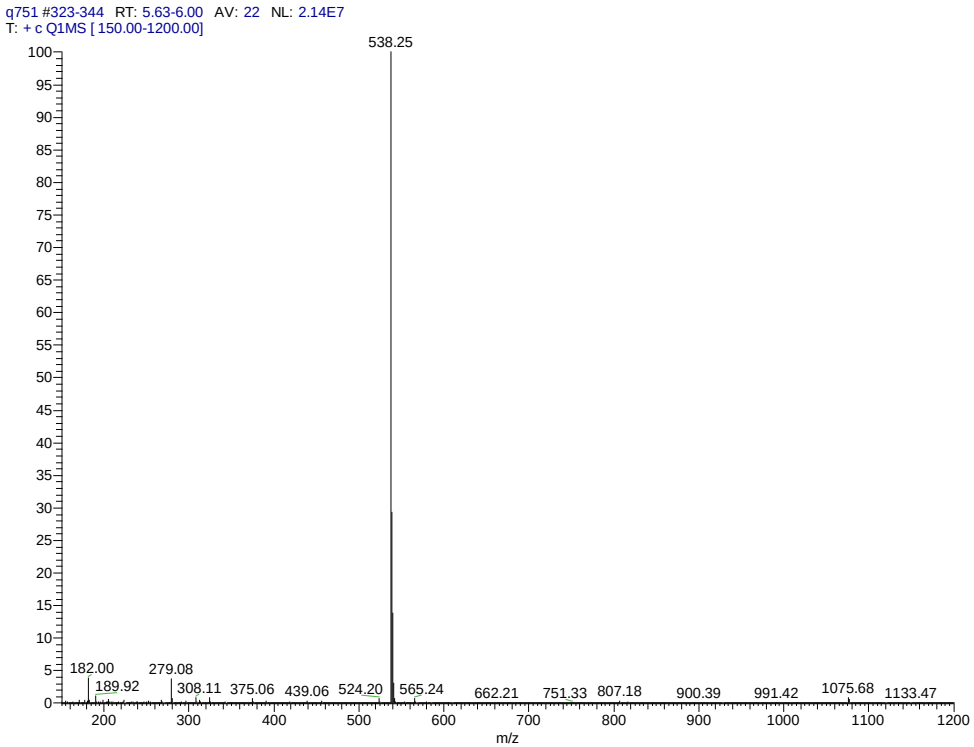


HPLC-Full MS of reaction 4j-3f (entry 2, table 3)

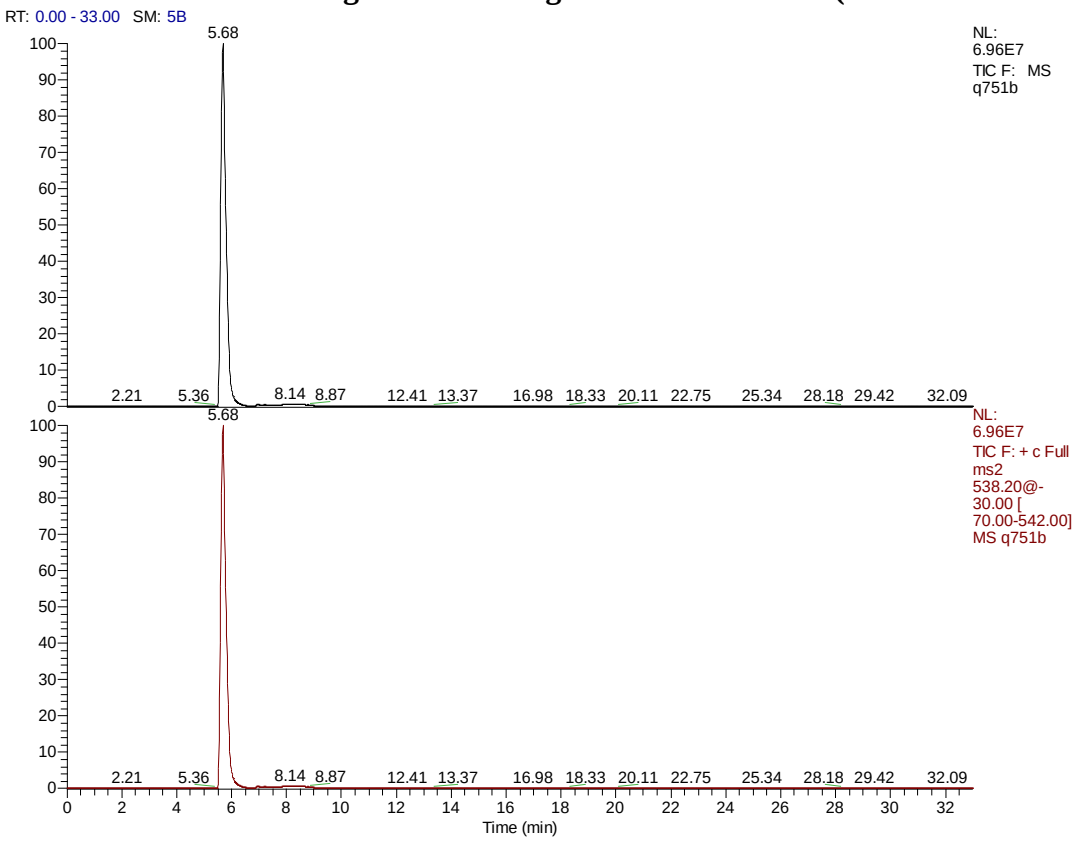
HPLC-MS chromatogram



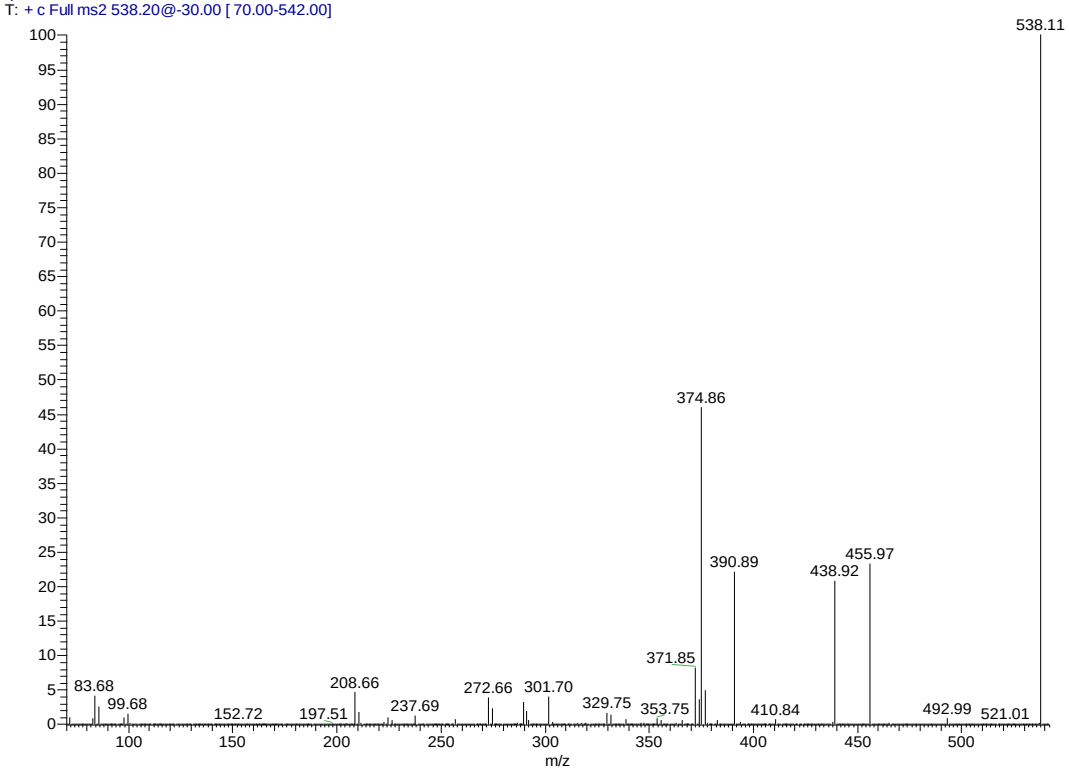
Peak 1 with 538.2 m/z



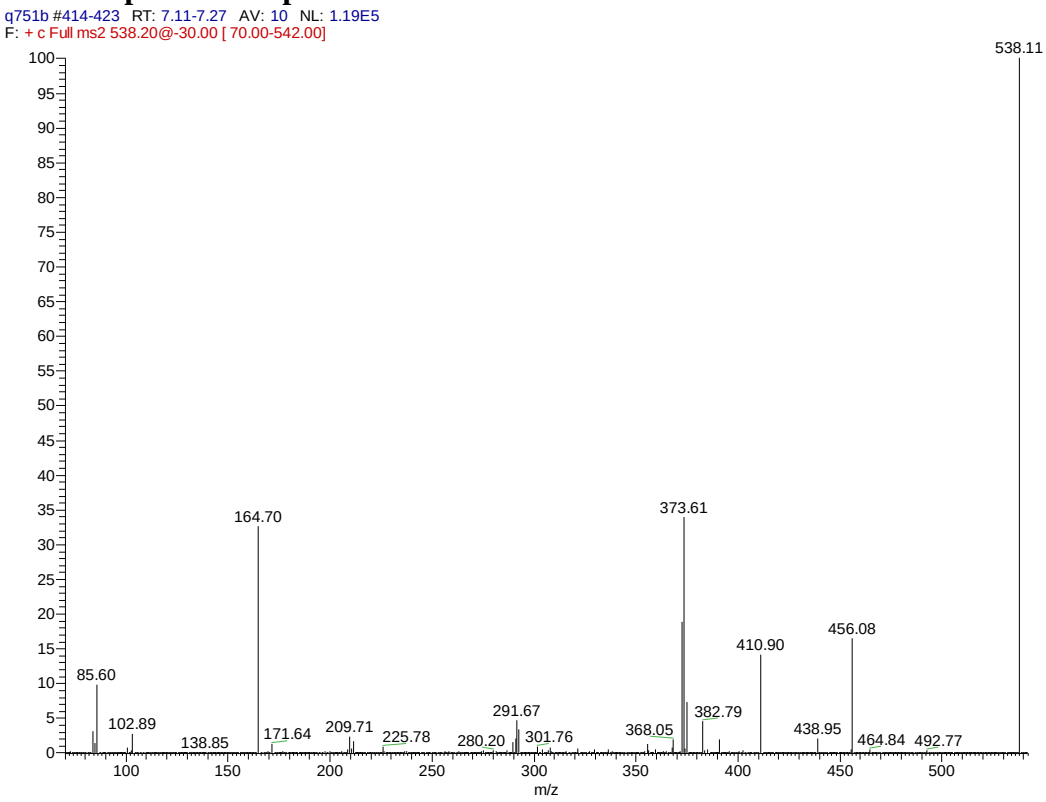
HPLC-MS/MS chromatograms with target mass = 538.2 m/z (divert valve 2-17 min)



MS/MS spectrum of peak 1

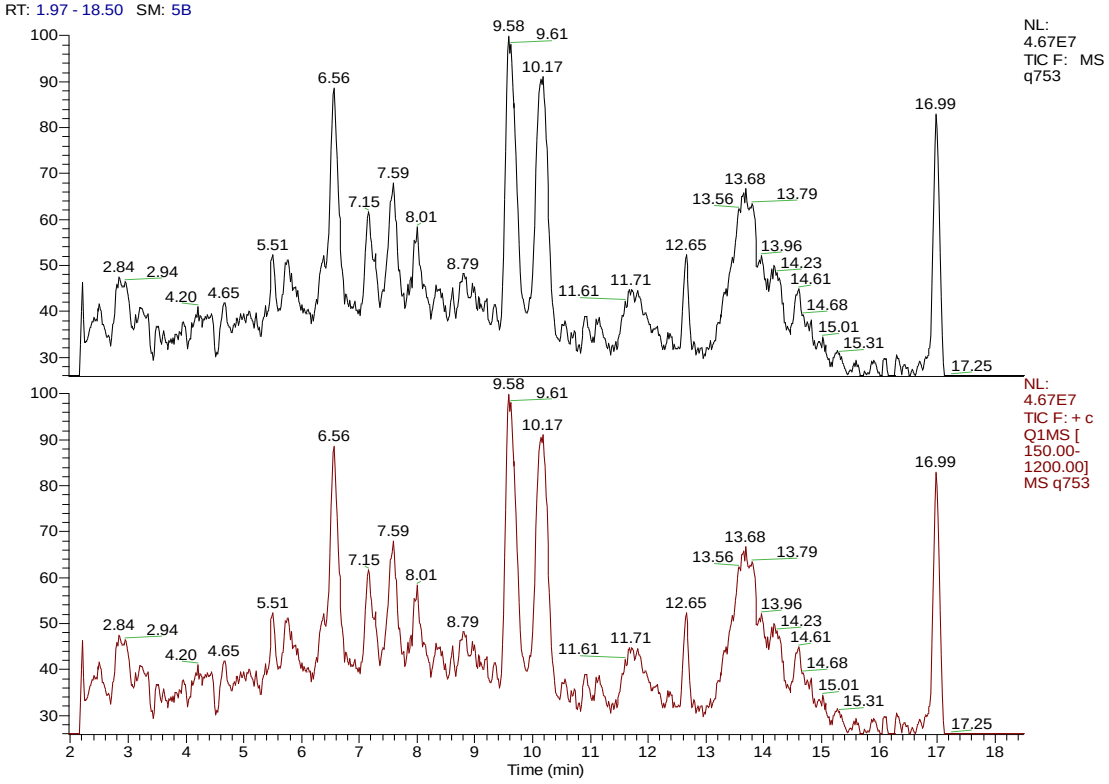


MS/MS spectrum of peak 2 detected in trace

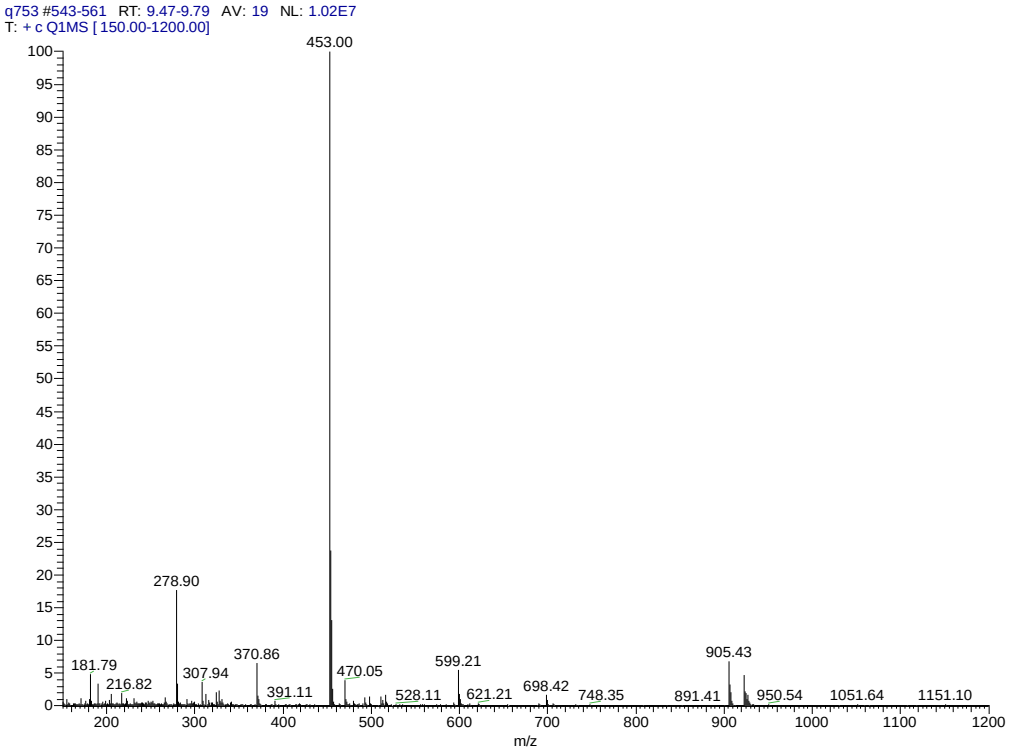


HPLC-Full MS of reaction Table 3, entry 1 (3f-3d)

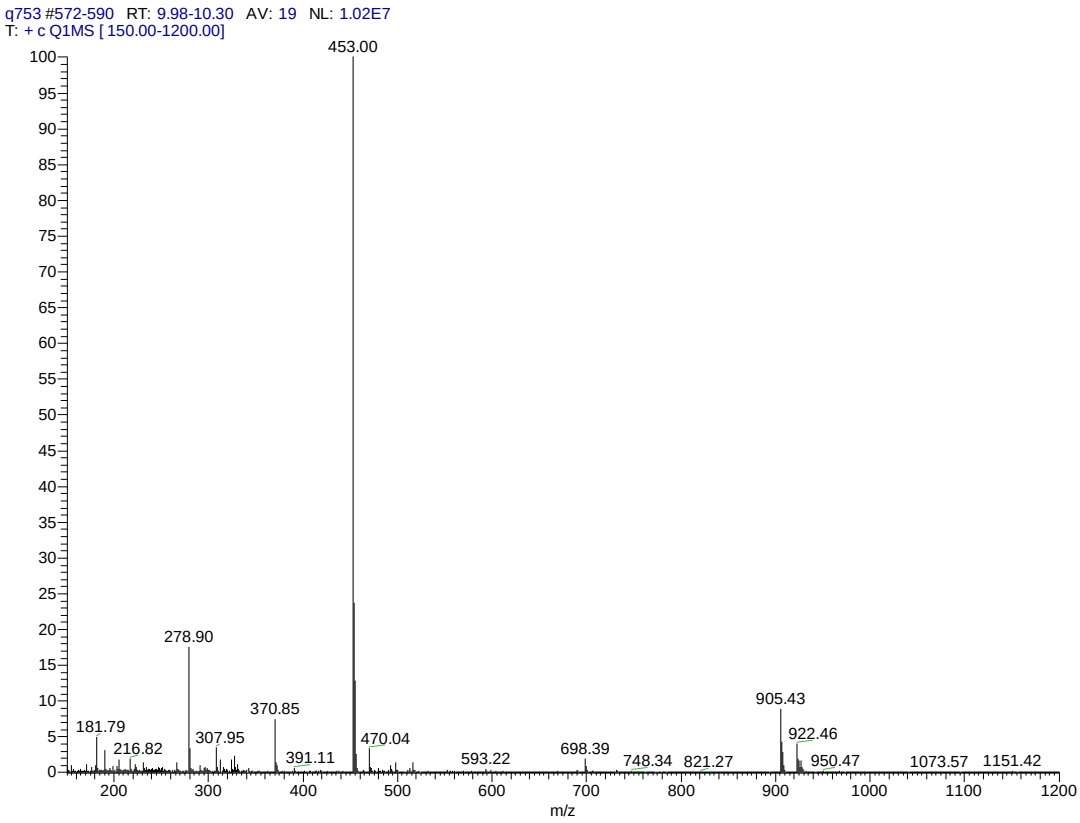
HPLC-MS chromatogram



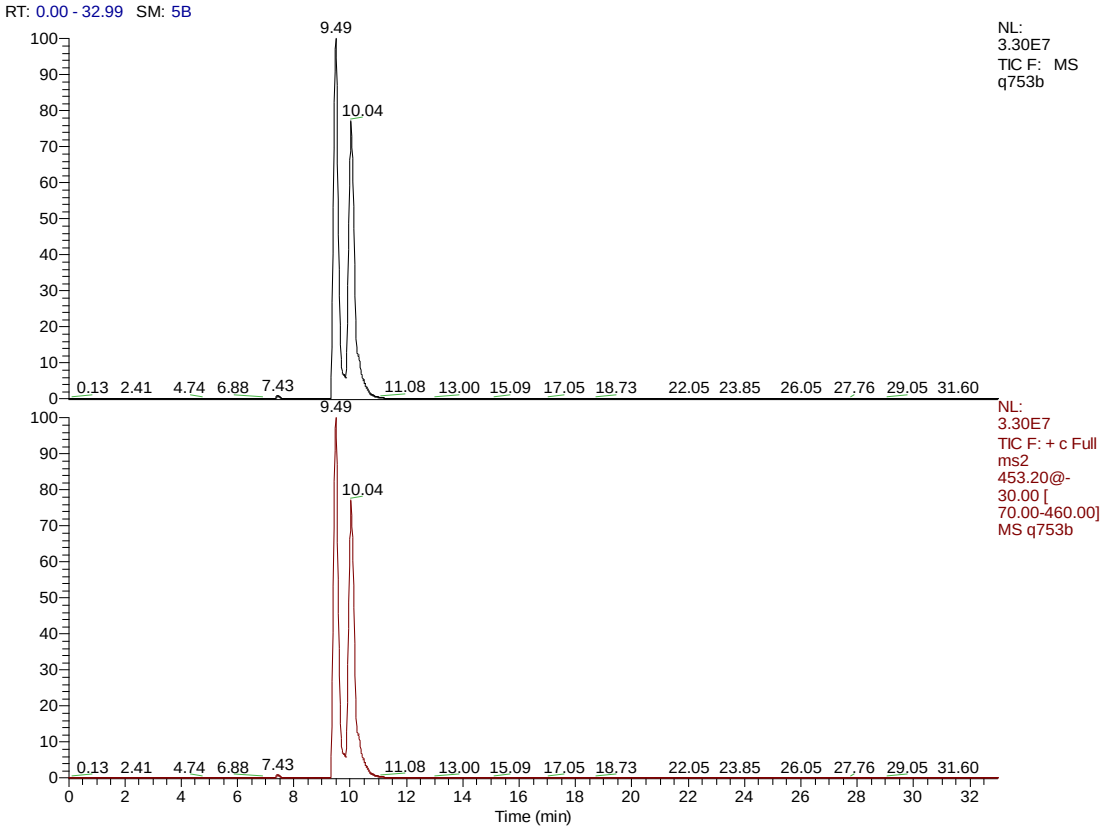
Peak 1 with 453 m/z



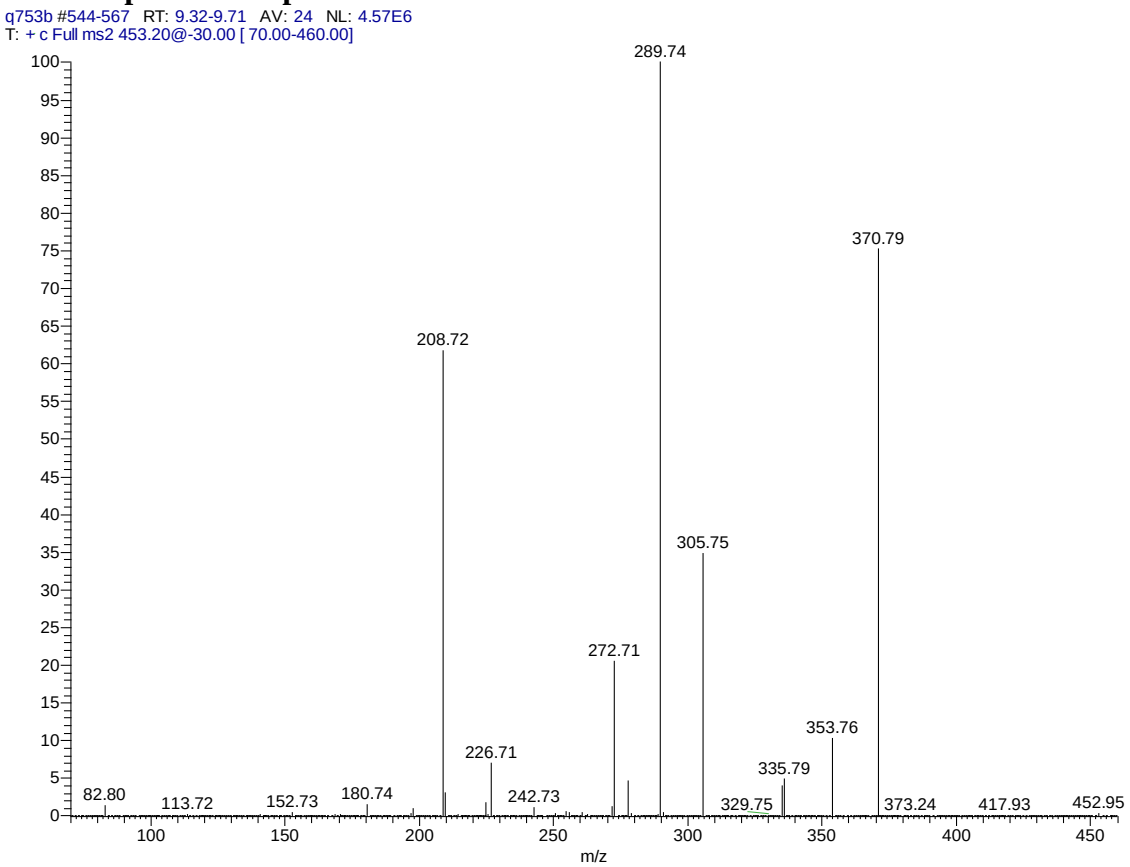
Peak 2 with 453 m/z



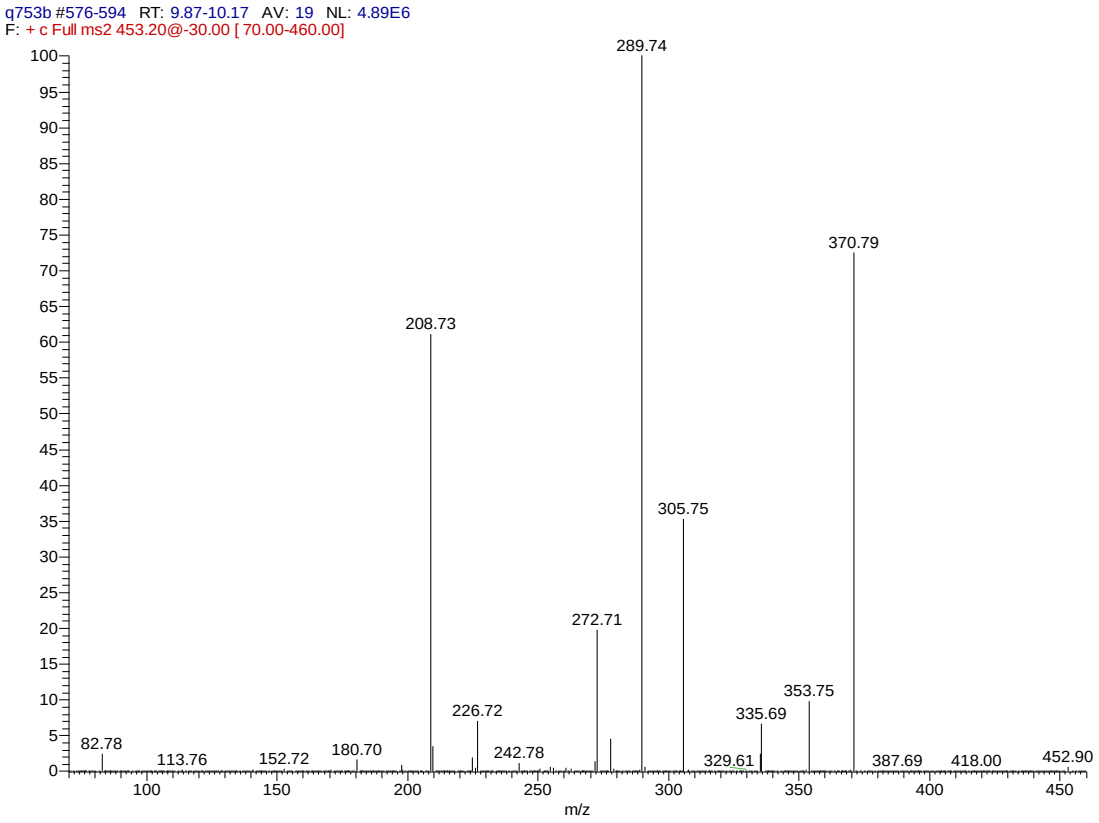
HPLC-MS/MS chromatograms with target mass = 453 m/z (divert valve 2-17 min)



MS/MS spectrum of peak 1

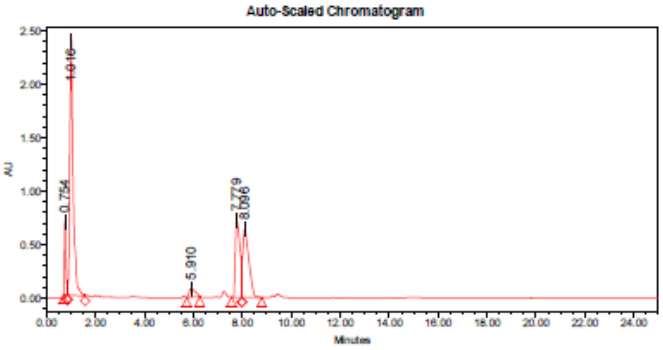


MS/MS spectrum of peak 2



HPLC-PDA & MS analysis for entry 3 of table 3 (3j-3f)

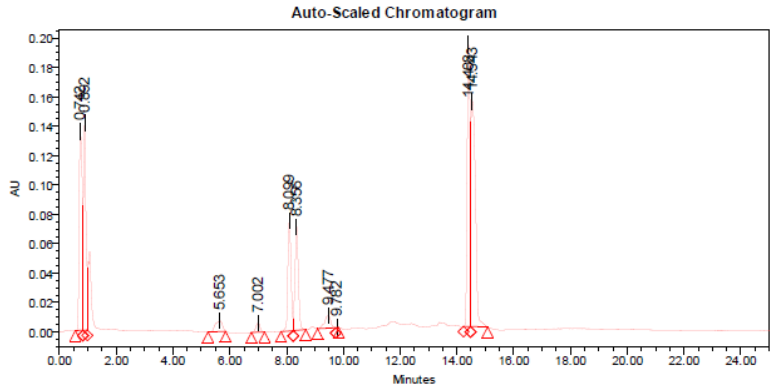
Solution after filtration



Peak Name:

	Injection	RT	Area	% Area	Height
1	1	0.754	2847907	5.98	681497
2	1	1.016	22996186	48.32	2381105
3	1	5.910	1364187	2.87	78111
4	1	7.779	9427952	19.81	712194
5	1	8.096	10958458	23.02	633688
Mean		4.711			
Std. Dev.		3.593			

Precipitated dissolved in methanol

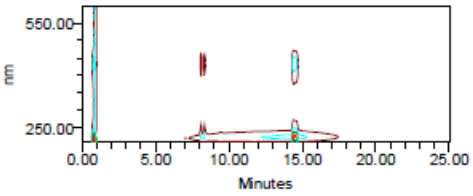


Peak Name:

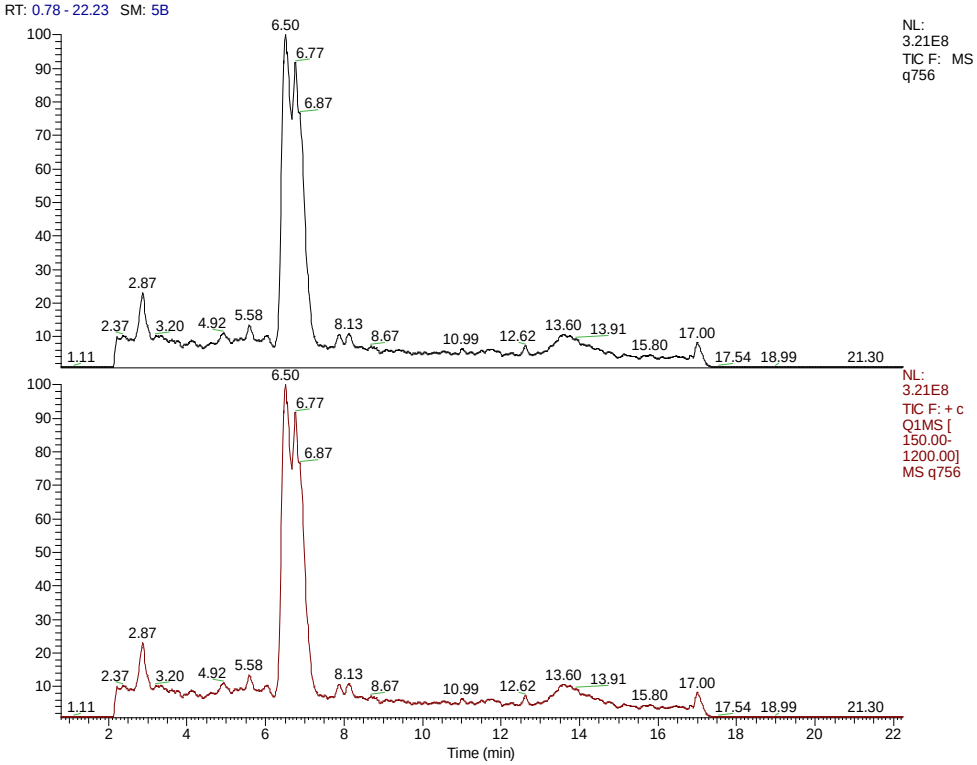
	Injection	RT	Area	% Area	Height
1	1	0.742	1030859	15.91	135621
2	1	0.892	883644	13.64	141172
3	1	5.653	127473	1.97	7478
4	1	7.002	62490	0.96	6227
5	1	8.099	589523	9.10	75068
6	1	8.356	583645	9.01	70456
7	1	9.477	126689	1.95	8383

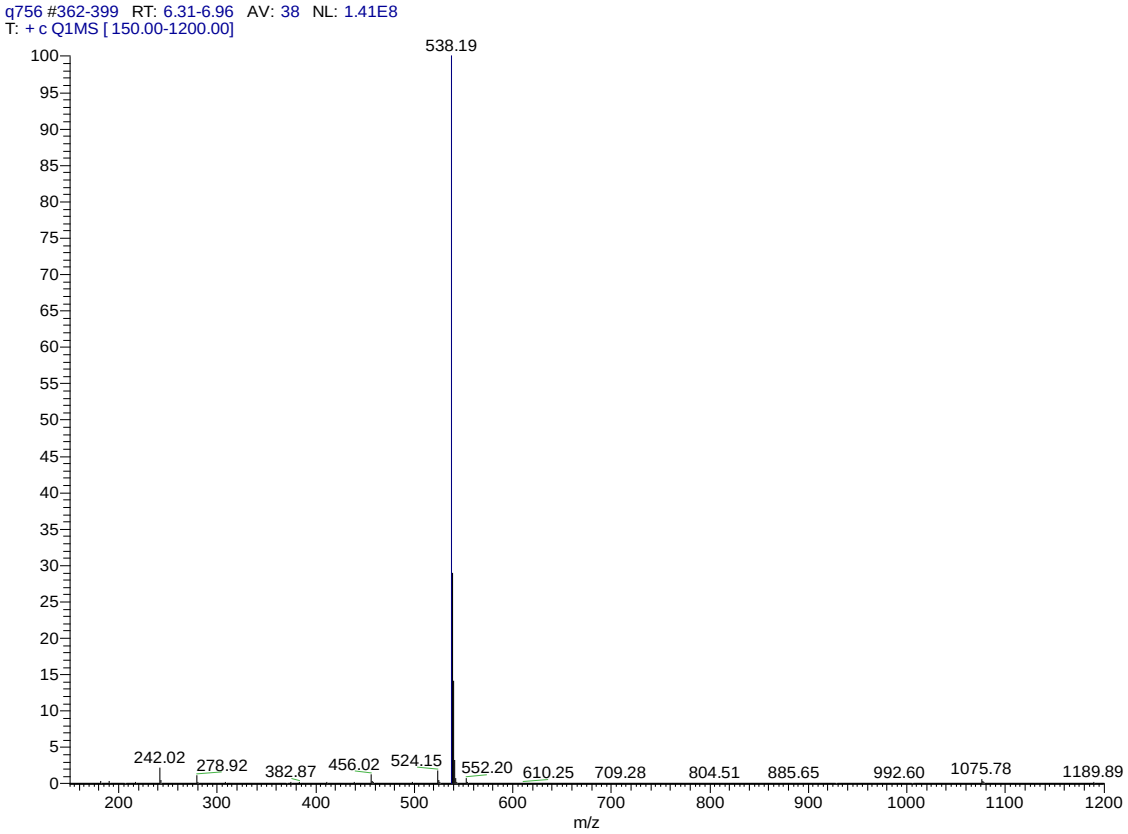
Peak Name:

	Injection	RT	Area	% Area	Height
8	1	9.782	1345	0.02	336
9	1	14.408	1357582	20.95	192418
10	1	14.543	1717104	26.50	153553
Mean		7.895			
Std. Dev.		4.702			
% RSD		59.55			

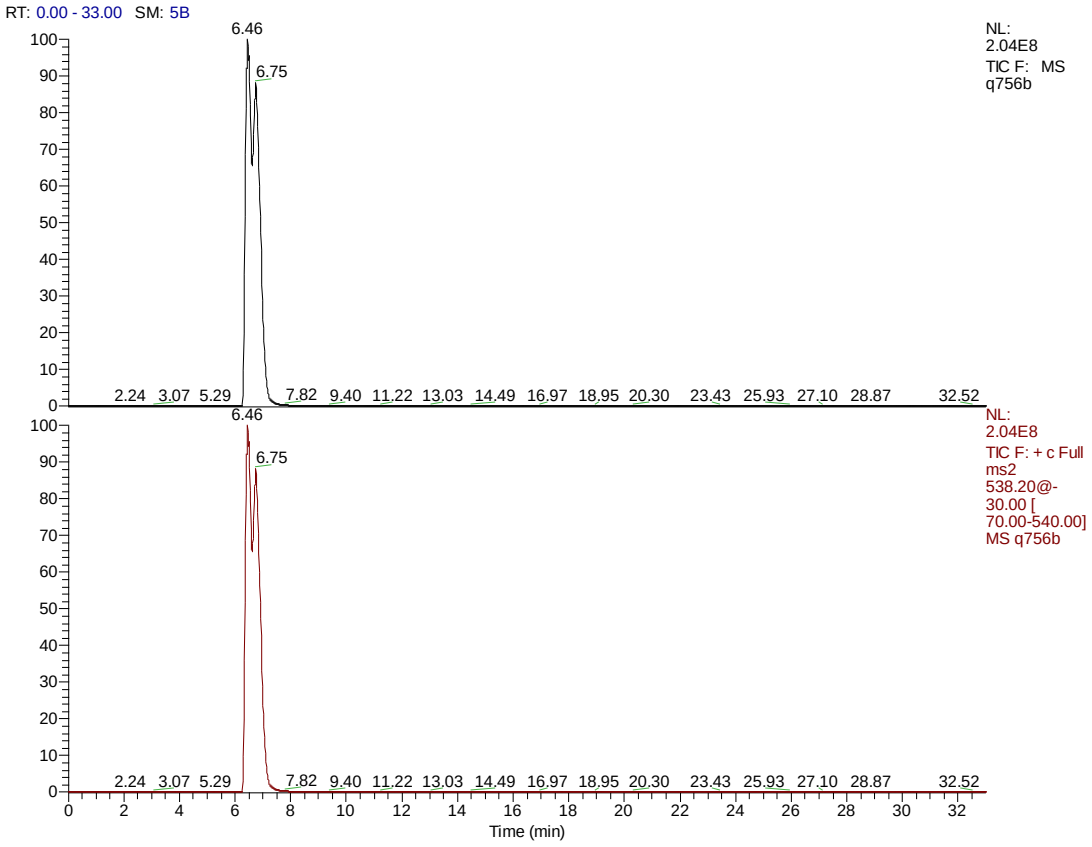


HPLC-MS chromatogram

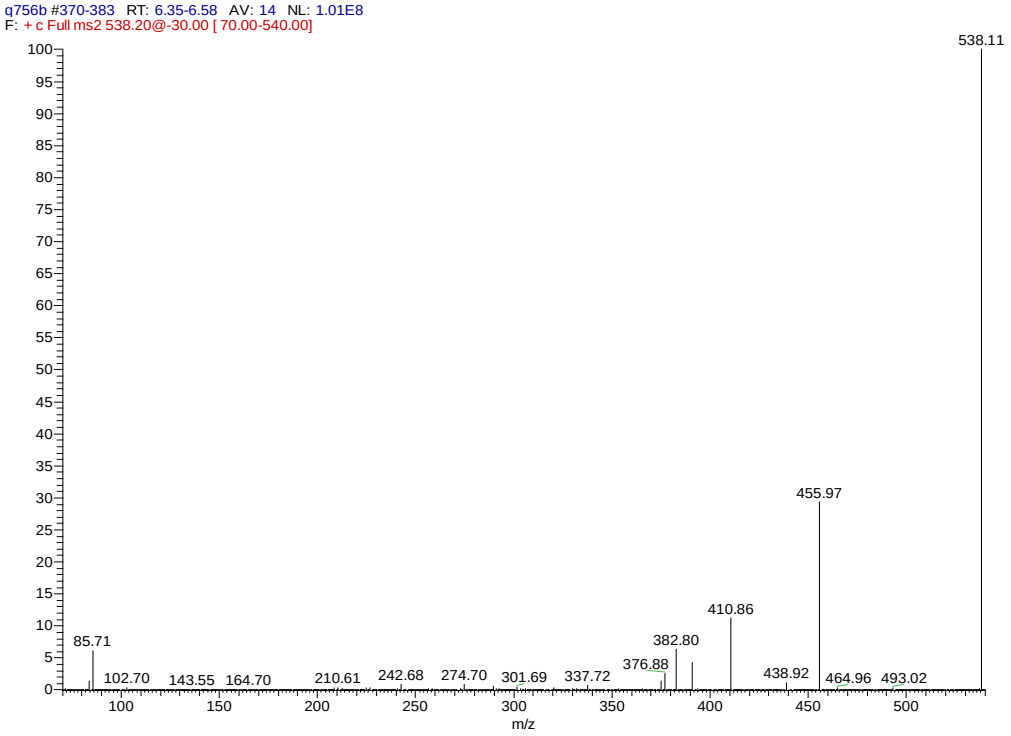




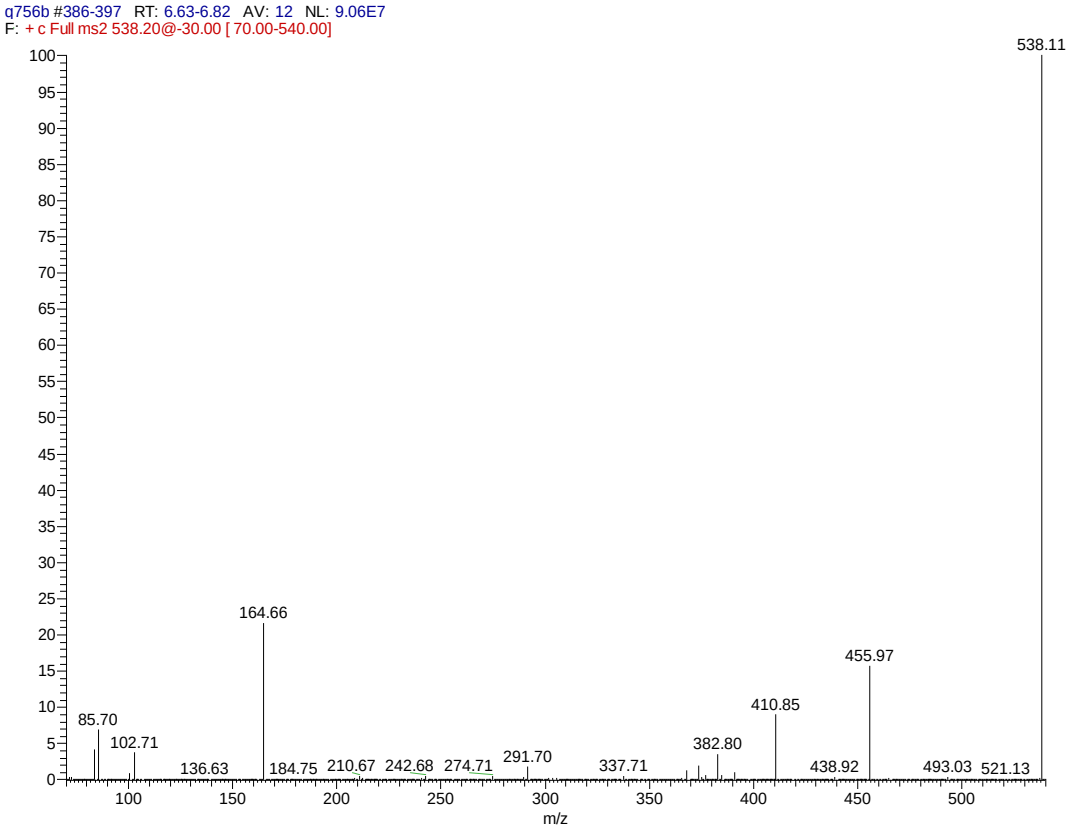
HPLC-MS/MS chromatograms with target mass = 538.2 m/z (divert valve 2-17 min)



Peak 1 with 538.2 m/z

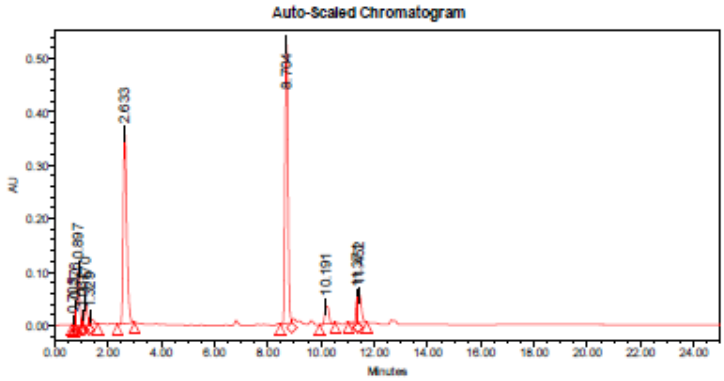


Peak 2 with 538.2 m/z



S49 HPLC-PDA of reaction 4f-3d (entry 4, table 3)

Solution after filtration

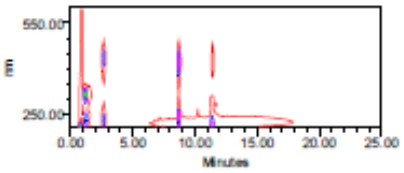


Peak Name:

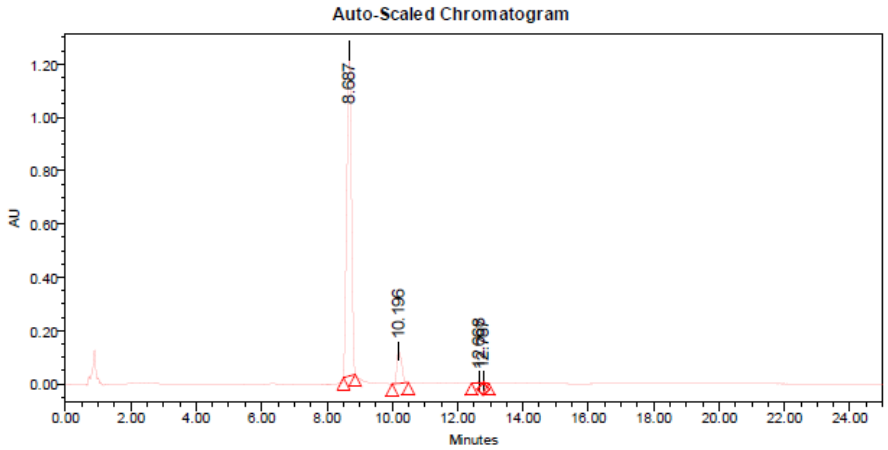
	Injection	RT	Area	% Area	Height
1	1	0.705	8215	0.08	2703
2	1	0.776	88249	0.91	32730
3	1	0.897	681756	7.05	104346
4	1	1.032	51561	0.53	12985
5	1	1.170	294467	3.05	40429
6	1	1.329	117007	1.21	11712
7	1	2.633	3288124	34.01	355594

Peak Name:

	Injection	RT	Area	% Area	Height
8	1	8.704	4085841	42.26	522886
9	1	10.191	352999	3.65	33162
10	1	11.371	238341	2.47	45698
11	1	11.452	460764	4.77	51080
Mean		4.569			
Std. Dev.		4.727			
% RSD		103.45			

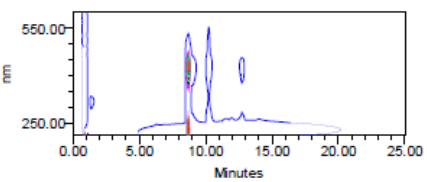


Precipitated dissolved in methanol

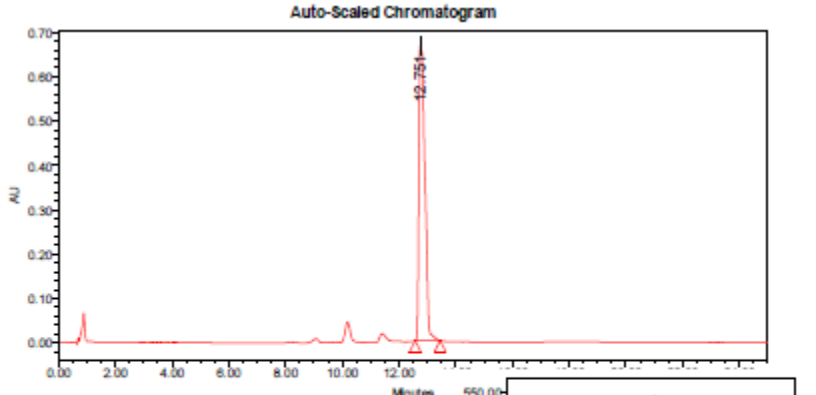


Peak Name:

	Injection	RT	Area	% Area	Height
1	1	8.687	11927385	90.00	1220857
2	1	10.196	1218878	9.20	119846
3	1	12.668	74500	0.56	7926
4	1	12.787	31329	0.24	6186
Mean		11.085			
Std. Dev.		1.996			
% RSD		18.00			



Reference phenoxazinone 6f

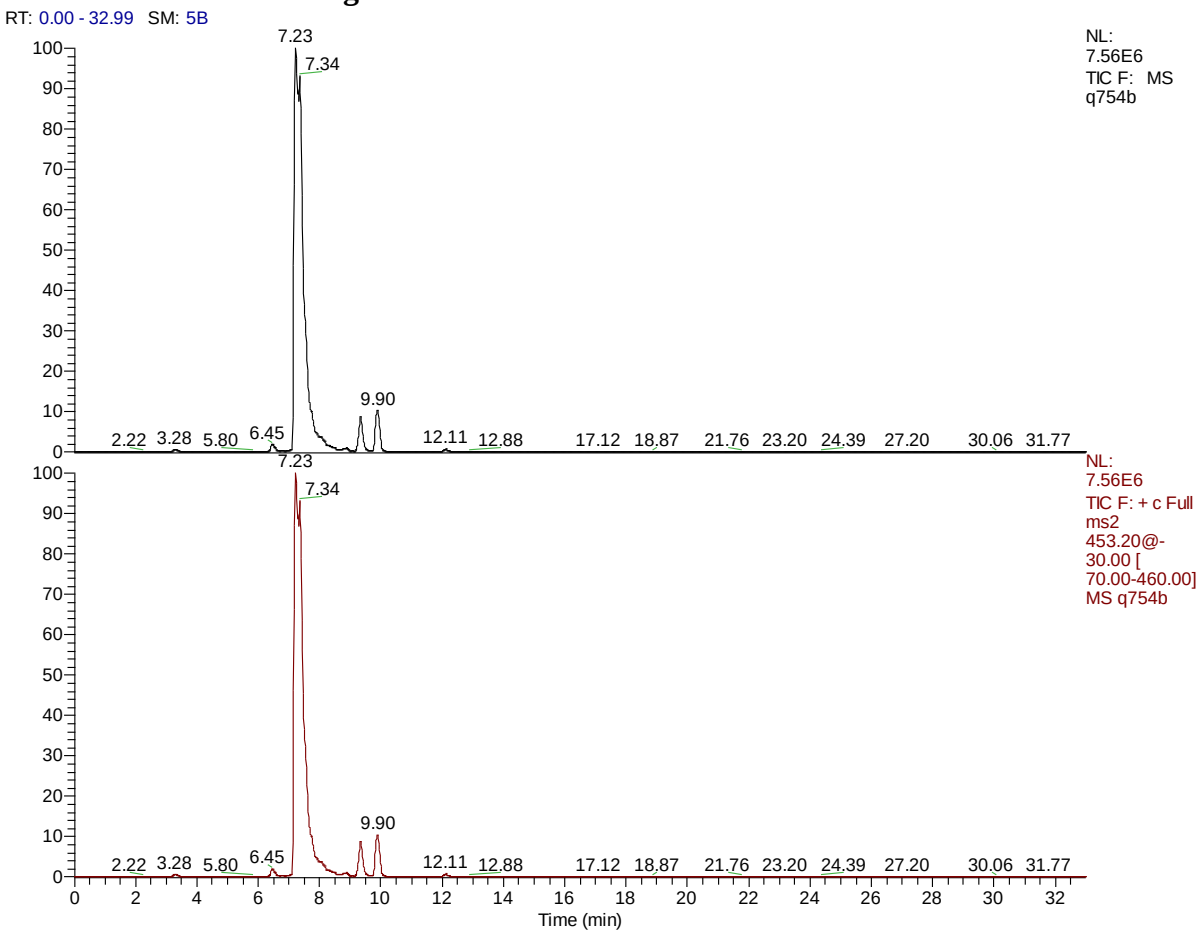


Peak Name:

	Injection	RT	Area	% Area	Height
1	1	12.751	9792417	100.00	665072
Mean		12.751			
Std. Dev.					
% RSD					

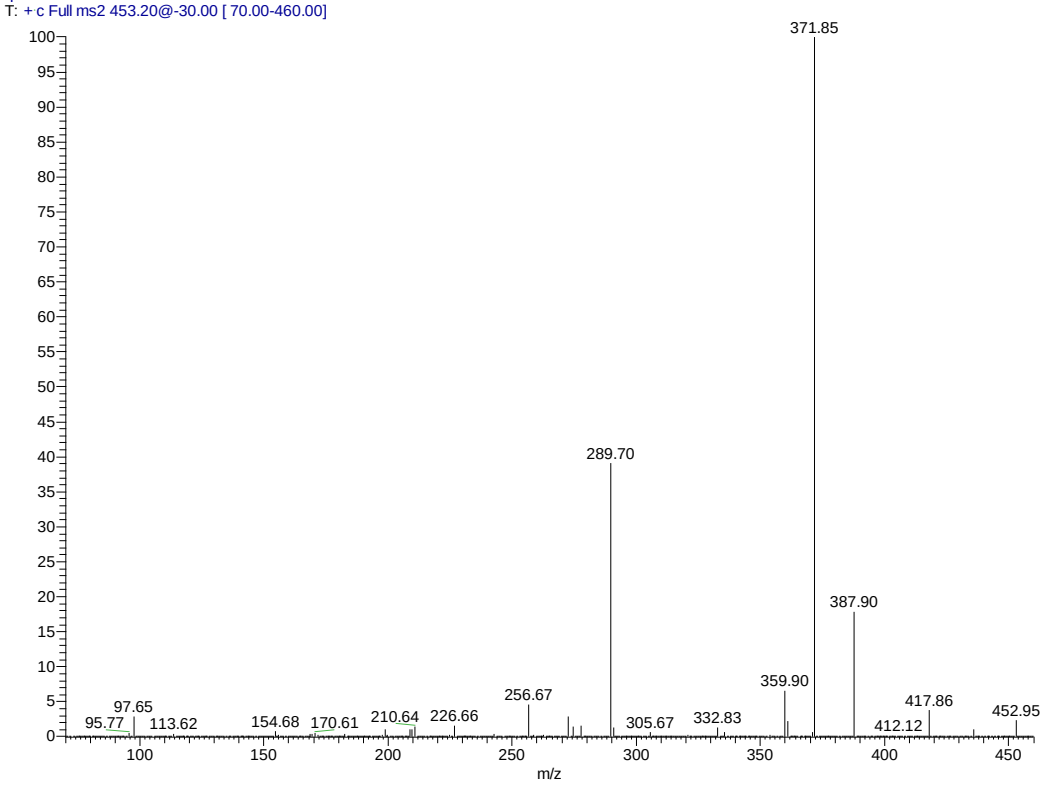
HPLC-Full MS of reaction 4f-3d (entry 4, table 3)

HPLC-MS/MS chromatogram



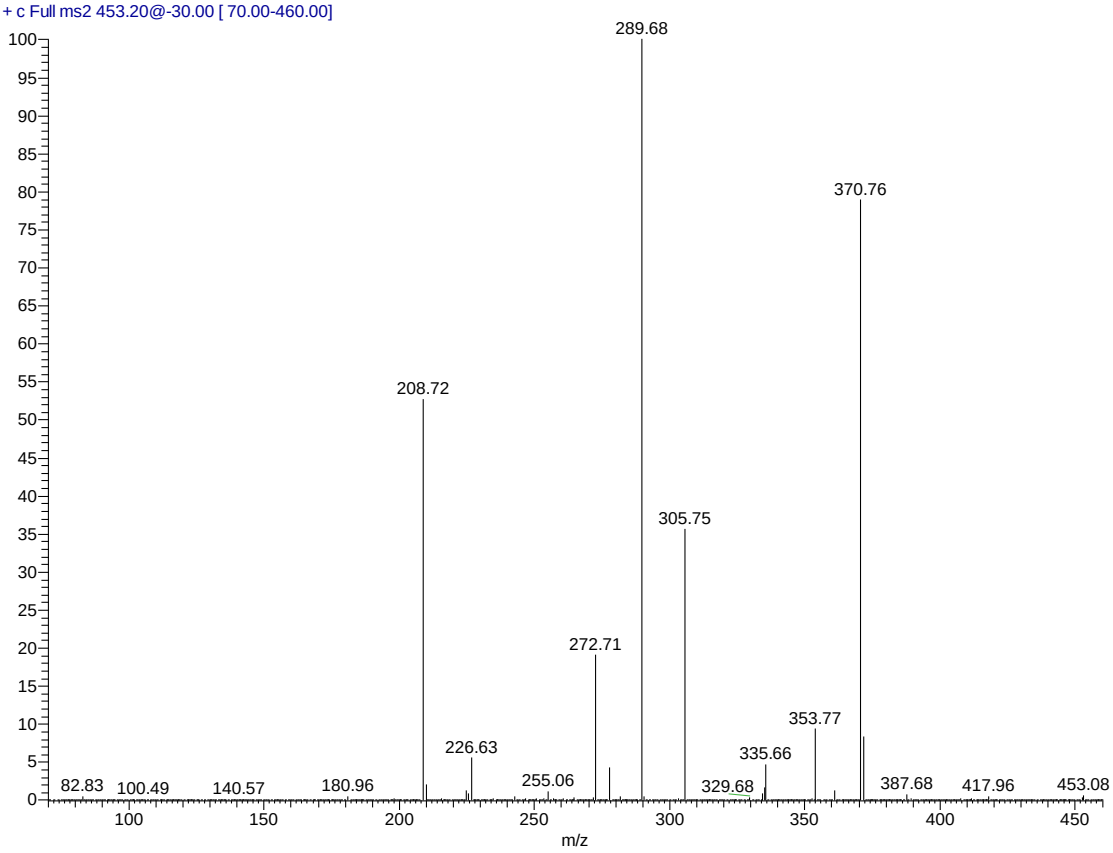
Peak 1 with 453.2 m/z (9df)

q754b #413-456 RT: 7.07-7.81 AV: 44 NL: 1.65E6
T: + c Full ms2 453.20@-30.00 [70.00-460.00]



Peak 2 with 453.2 m/z (10fd)

q754b #532-550 RT: 9.11-9.42 AV: 19 NL: 8.93E4
T: + c Full ms2 453.20@-30.00 [70.00-460.00]



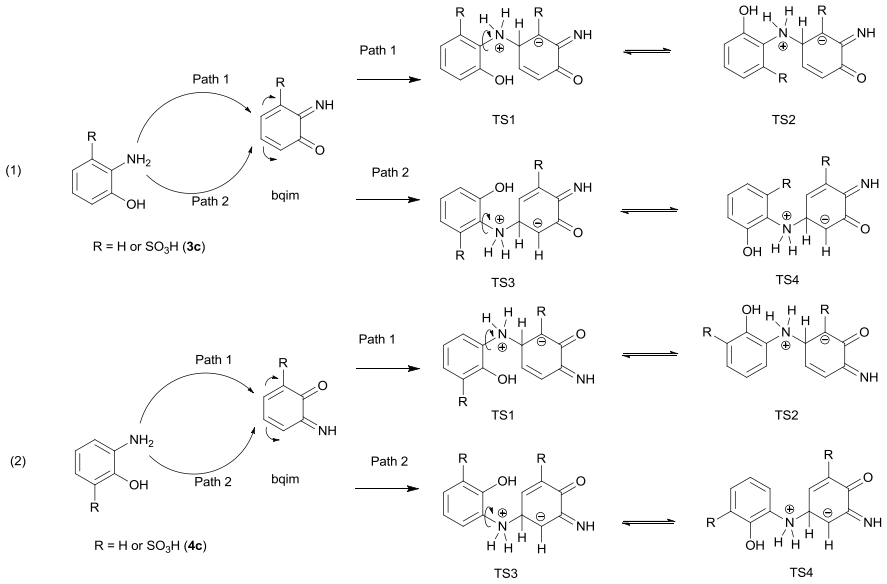
S52- RHF and B3LYP absolute and relative energies

RHF/6-31+G (see Scheme 3 and Table 4)

Entry	Substrate	Substrate, absolute energy (a.u.)	Bqim, absolute energy (a.u.)	Transition state (TS)	Absolute energy (a.u.)	ΔRel energy (Kcal mol^{-1})
1	*o*-aminophenol	-360.468505246	-359.245465197	TS1	-719.590796294	77.293
				TS2	-719.597814260	72.889
				TS3	-719.616359831	61.251
				TS4	-719.610778195	64.754
2	3c	-982.207067520	-980.969696013	TS1	-1963.08351389	58.515
				TS2	-1963.09715803	49.953
				TS3	-1963.06670886	69.060
				TS4	-1963.07997662	60.735
3	4c	-982.207374417	-980.970384217	TS1	-1963.09745014	50.394
				TS2	-1963.09433061	52.352
				TS3	-1963.05006906	80.126
				TS4	-1963.04139050	85.572

B3LYP/6-31+G(d) (see Scheme 3 and Table 4)

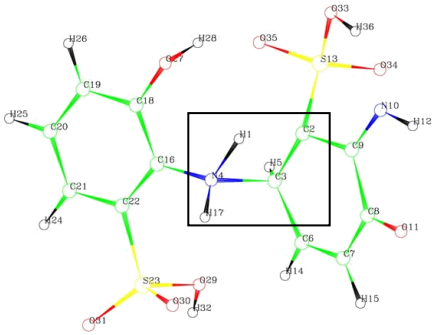
Entry	Substrate	Substrate, (a.u.)	Bqim, (a.u.)		Transition state (TS)	Absolute energy (a.u.)	ΔRel energy (Kcal mol^{-1})
1	*o*-aminophenol	-362.831183224	-361.585079052		TS1	-724.328066503	55.344
					TS2	-724.324173007	57.787
					TS3	-724.344072449	45.300
					TS4	-724.340447754	47.574
2	3c	-986.633276414	-985.381118818		TS1	-1971.95756939	35.659
					TS2	-1971.94929497	40.851
					TS3	-1971.93252745	51.373
					TS4	-1971.94523151	43.401
3	4c	-986.635710264	-985.379671675		TS1	-1971.95277334	39.285
					TS2	-1971.94960705	41.274
					TS3	-1971.91827385	60.936
					TS4	Na	Na

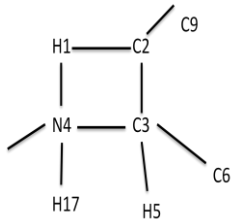


Bqim = benzoquinone-imine internediate

S53 – Representative equilibrium structure and atoms labeling,

C2-H1 : r1
C2-C3 : r2
N4-C3 : r3
C3-C2-H1 : t1
N4-C3-C2 : t2





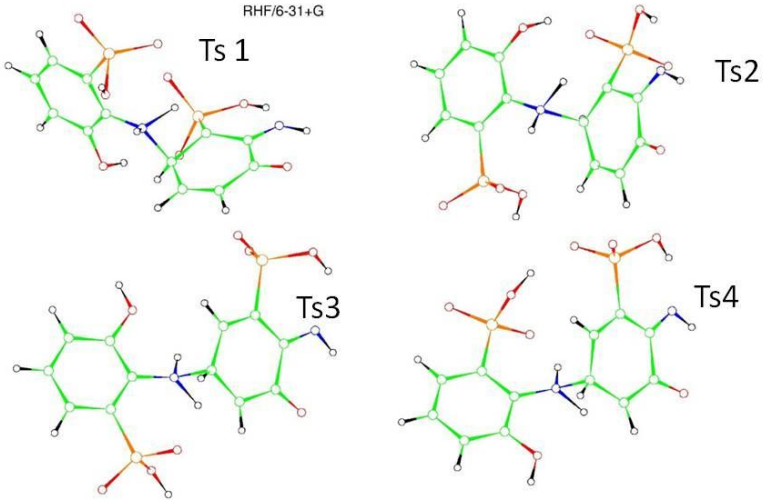
Variables used to define the Z-Matrix.

For each TS structure, the main components of the reaction coordinate at the RHF/6-31+G level are given by the eigenvector associated to the negative eigenvalue of the Hessien matrix corresponding to the imaginary frequency. The geometric parameters show the charge separation occuring at that equilibirum structure.

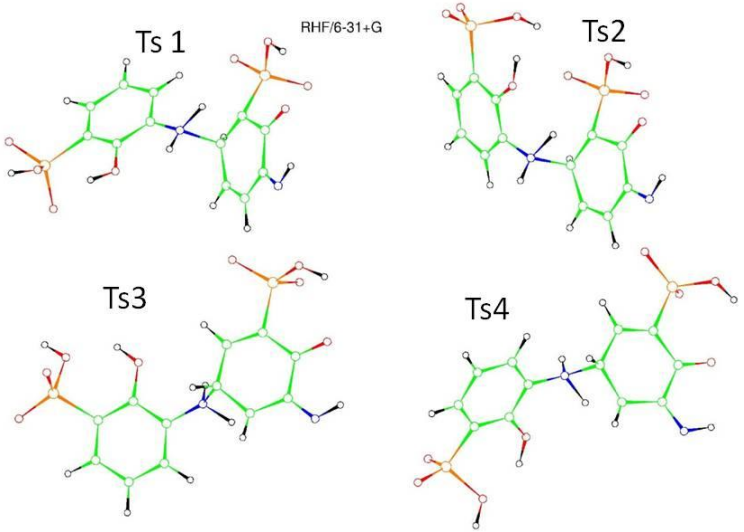
3c	TS1		TS2		TS3		TS4	
Distances in A Angles in degrees		eigenvector components						
r1	1.4957	0.76174	1.4829	0.80099	1.5372	0.76685	1.5289	0.75269
r2	1.5245	-0.15192	1.5272	-0.14364	1.5132	-0.14279	1.5151	-0.13858
r3	1.5445	0.11957	1.5667	0.11909	1.5866	0.10597	1.5600	0.07825
t1	73.169	-0.48874	73.586	-0.46581	73.335	-0.41927	72.602	-0.44636
t2	96.321	-0.25954	95.798	-0.26139	95.638	-0.28821	96.132	-0.29074

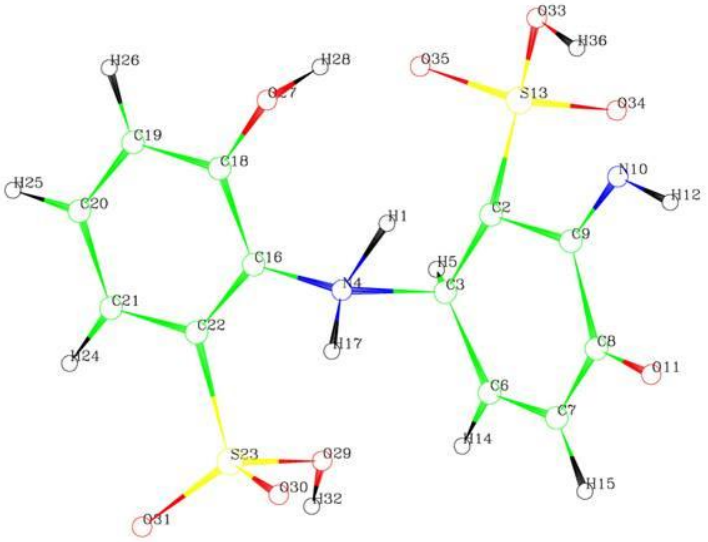
4c								
r1	1.4414	0.75759	1.4398	0.72690	1.5890	0.86990	1.5908	0.85782
r2	1.5270	-0.13982	1.5278	-0.15798	1.5147	-0.14711	1.5158	-0.14843
r3	1.5521	0.06991	1.5465	0.10804	1.5782	0.15109	1.5621	0.15040
t1	74.526	-0.48685	74.607	-0.52211	71.002	-0.32956	71.087	-0.33962
t2	95.459	-0.32205	96.053	-0.32536	97.032	-0.16064	97.103	-0.14952

S54- Equilibrium structures of the TS 1- 4 (RHF/6-31+G) For compound 3c



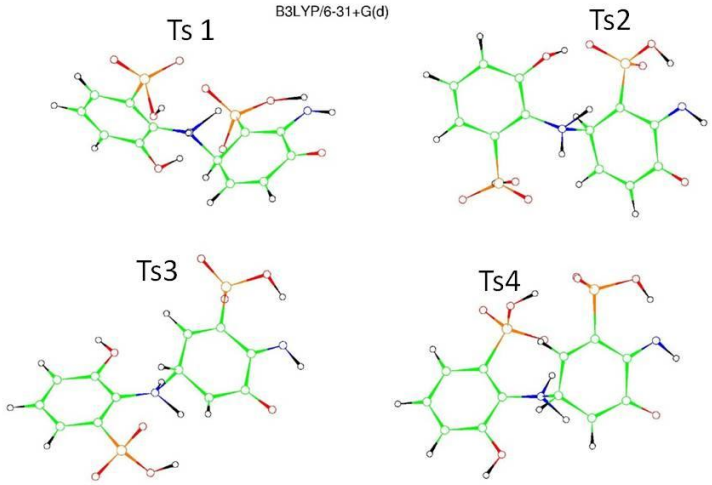
For compound 4c



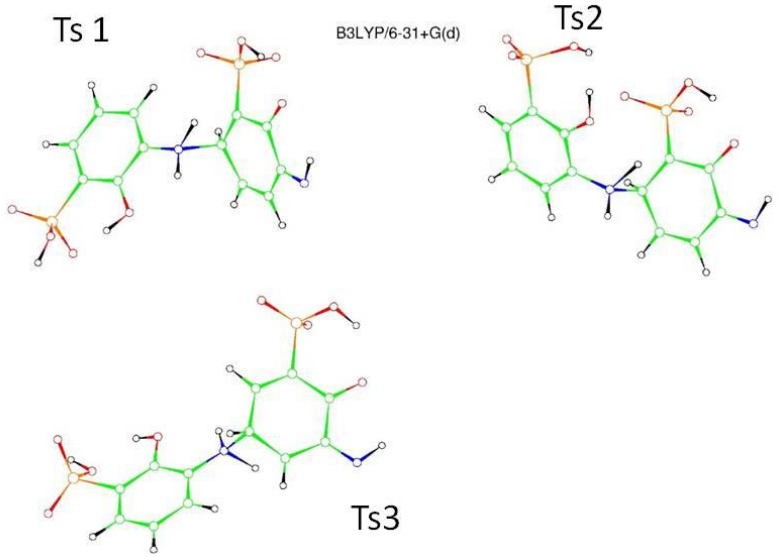


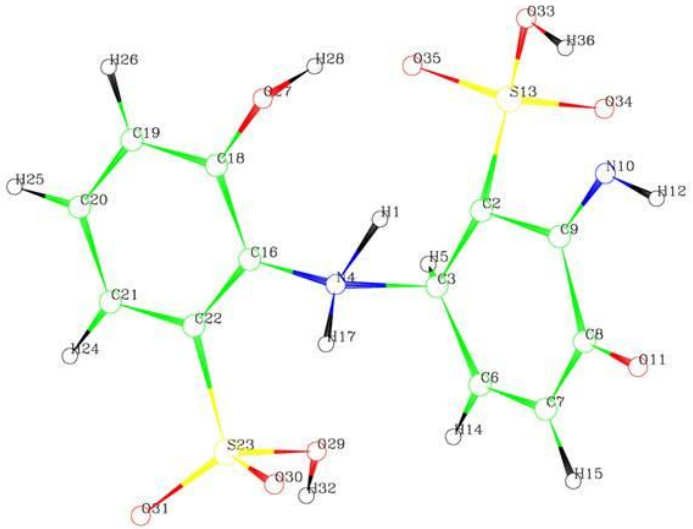
Atoms labeling (TS2 for 3c)

S55-Equilibrium structures of the TS 1- 4 (B3LYP/6-31+G(d)) For compound 3c



For compound 4c





Atoms labelling (TS2 for 3c)

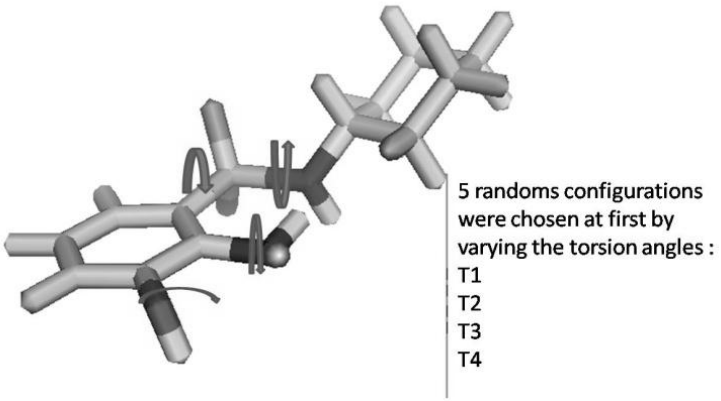
S56 Energy minimization of selected substrates for docking purpose

Optimization

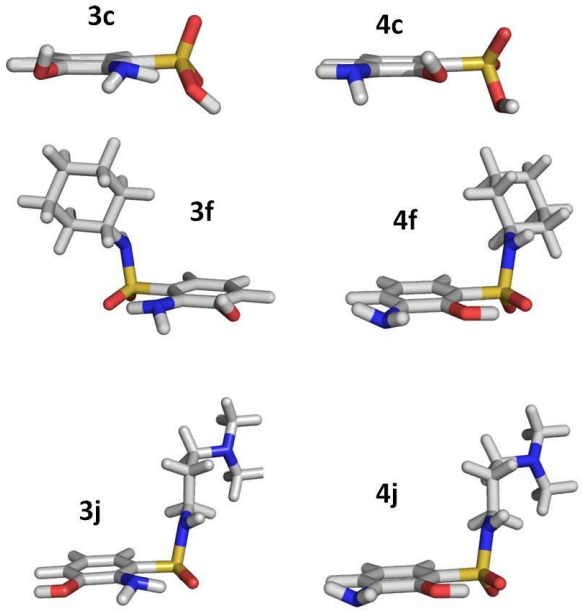
Three dimensional structures were generated using CS Chem3D from Cambridgesoft. Molecular geometries were calculated for the parent compounds. Calculations were carried out using the semi-empirical quantum-mechanical PM3 methods of the MOPAC2009™ program.[J. J. P. Stewart; J. Mol. Mod. 13 (2007) 1173; Version 7.295]

Five starting conformations were arbitrary chosen to evaluate whether or not the resulting conformations were representative enough. To do so, the dihedral angle between the aromatic plane and the proton of the phenol and aniline were modified along with the dihedral angle of the sulfonamide group. Finally, at least two initial conformations were chosen with respectively a hydrogen bong between an oxygen of the sulfonated group and a proton of the nearby aniline (or phenol), or between the proton of the sulfonamide and the nearby nitrogen (or oxygen) of the aniline (or phenol).

Example of dihedral angle varied for compound 3f

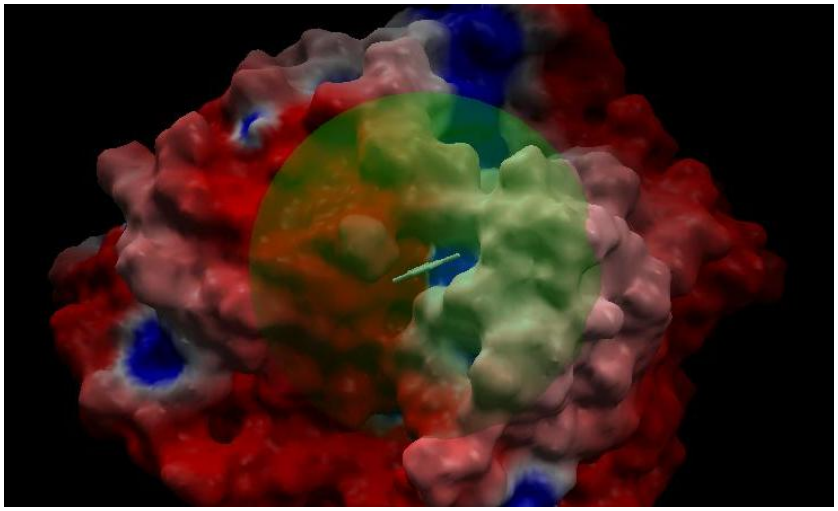


Optimized conformations

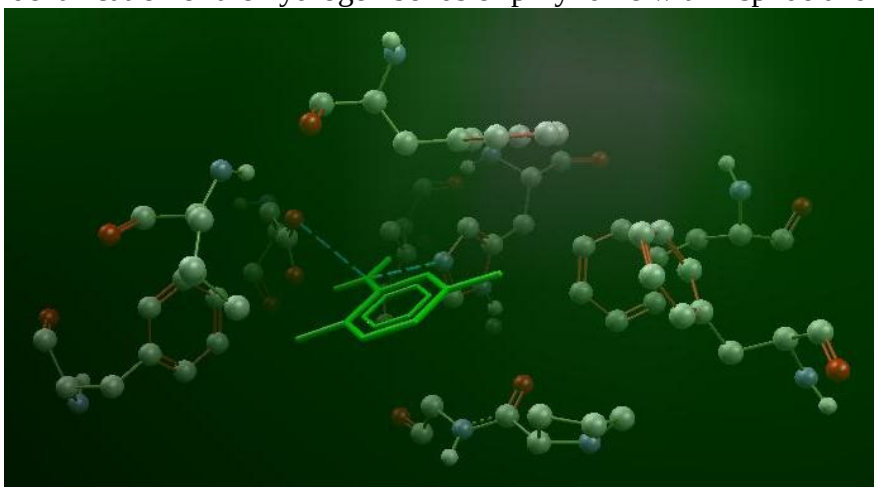


S-57 Docking of the selected substrates using Molegro Virtual Docker program

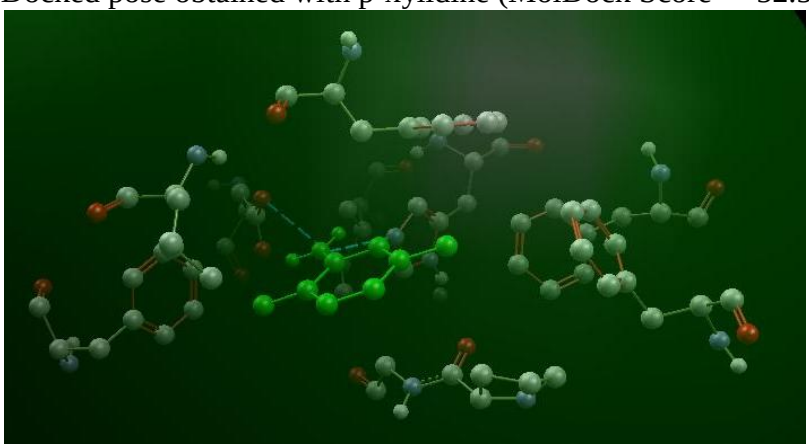
Preparation of receptor from *Trametes versicolor* co-crystallized with *p*-xylidine (PDB 1Kya), Local constraint for docking , 15 Å radius sphere



Identification of the hydrogen bonds of p-xylidine with Asp206 and His508

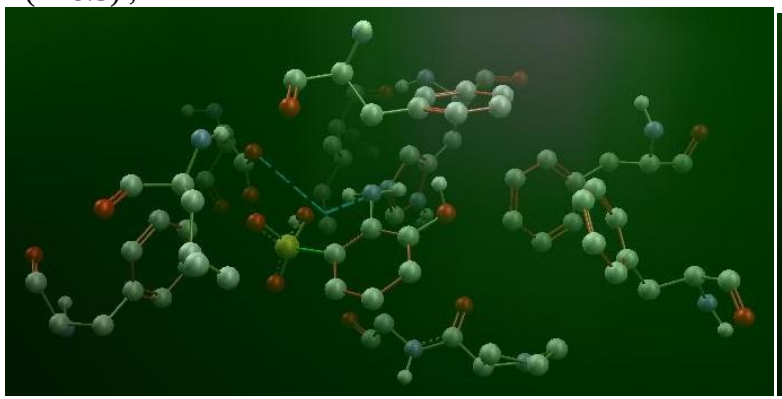


Docked pose obtained with p-xylidine (MolDock Score = -52.5 arbitrary units)

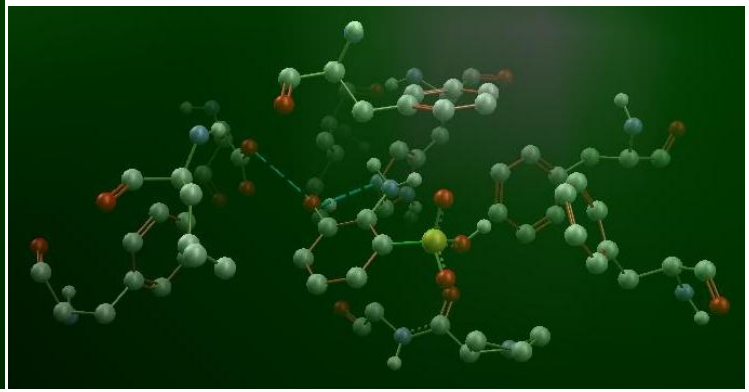


Docked pose of **3c**

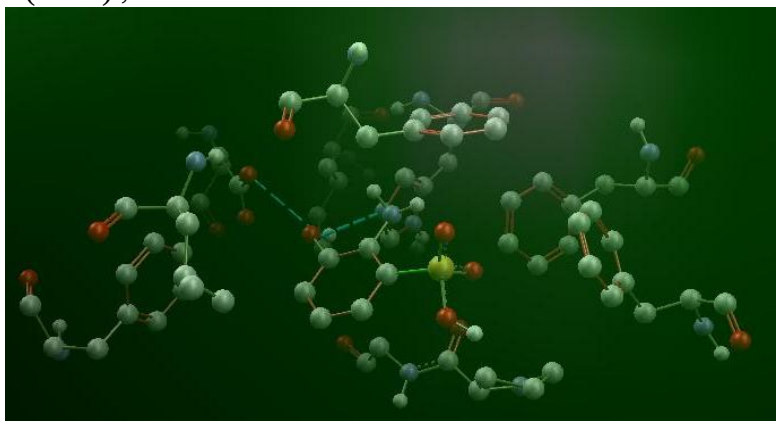
1 (-48.3) ;



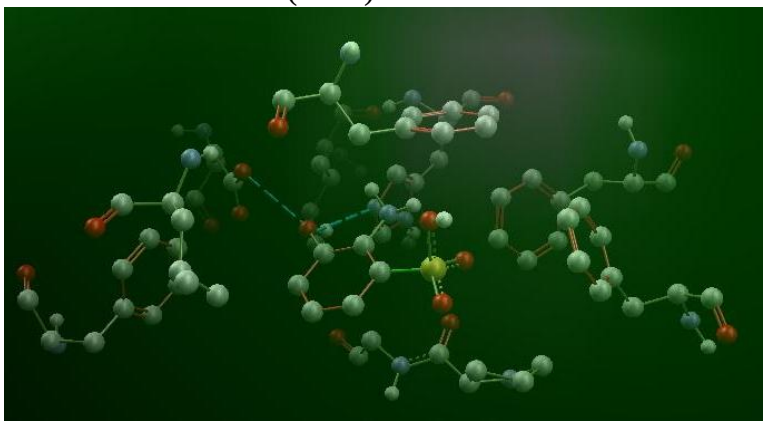
2 (-52.2) ;



3 (-54.1) ;

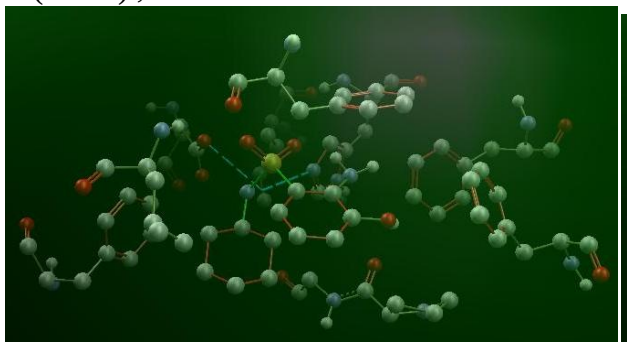


4 (-53.6)

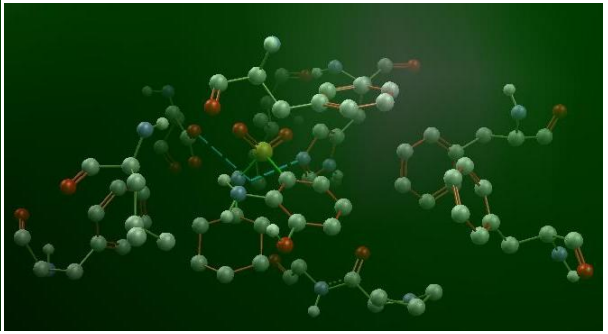


Docked pose of **3f**

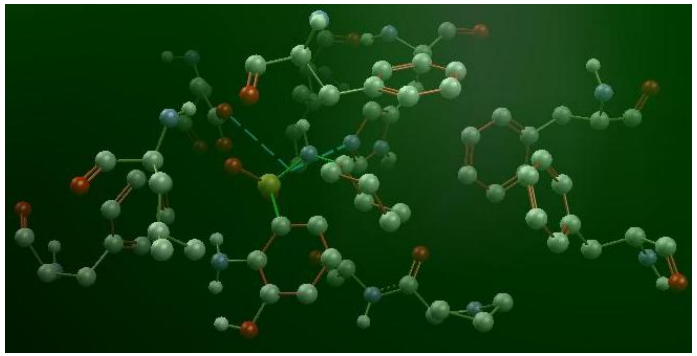
1 (-48.3) ;



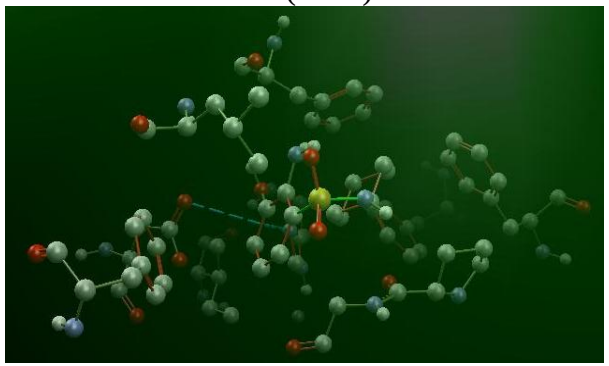
2 (-31.3) ;



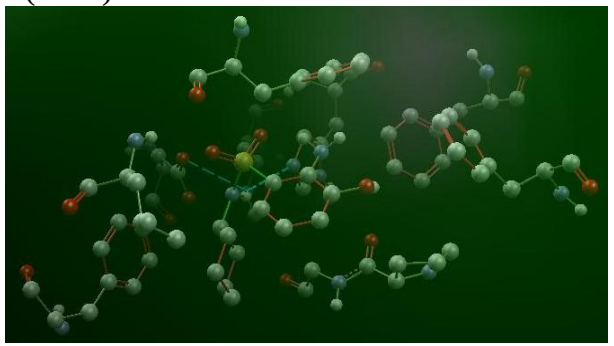
3 (-11.6) ;



4 (-36.9)

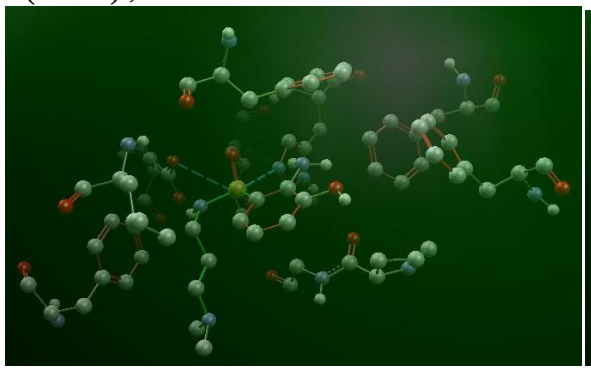


5 (-71.3)

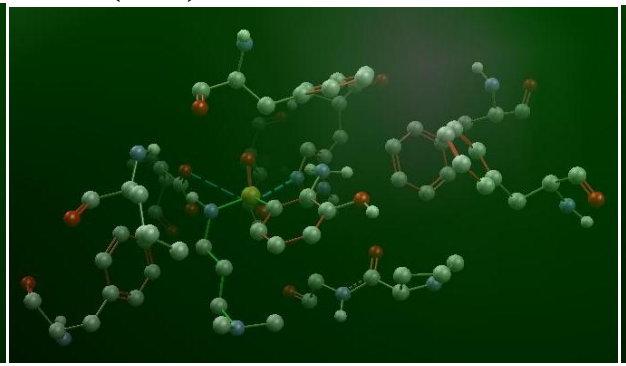


Docked pose of **3j** ;

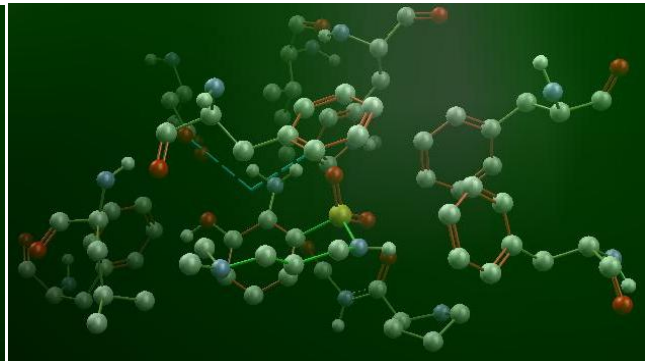
1 (-47.2) ;



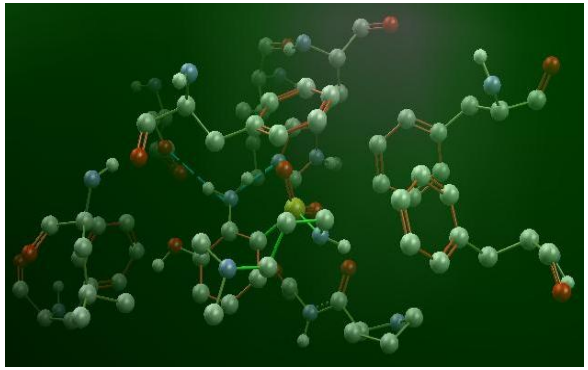
2 (-53.1) ;



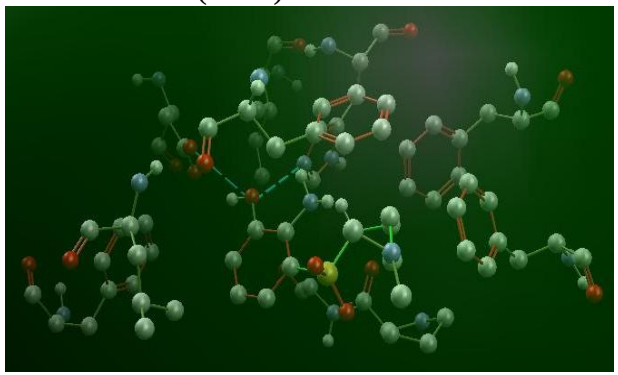
3 (-50.8)



4 (-41.5) ;

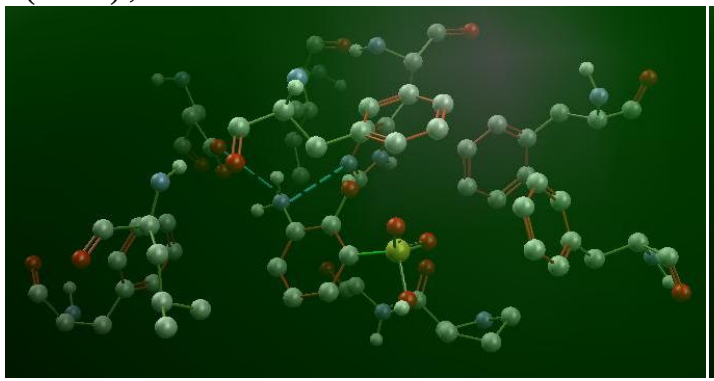


5 (-47.5)

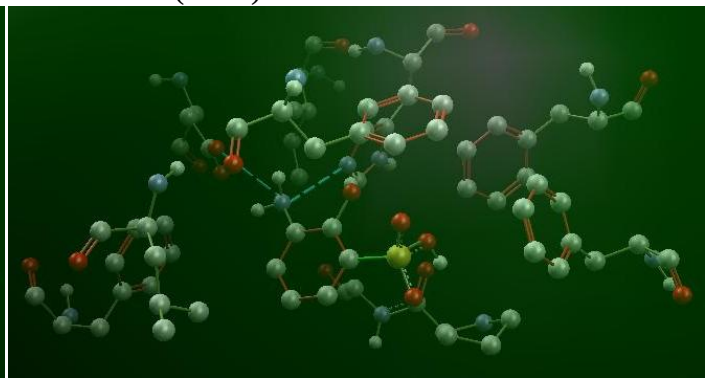


Docked pose of **4c**

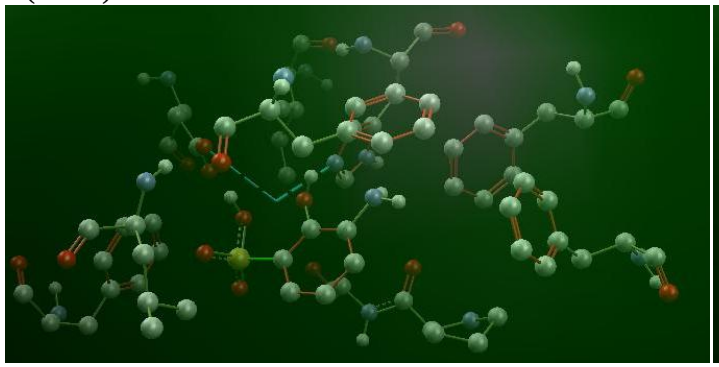
1 (-54.8) ;



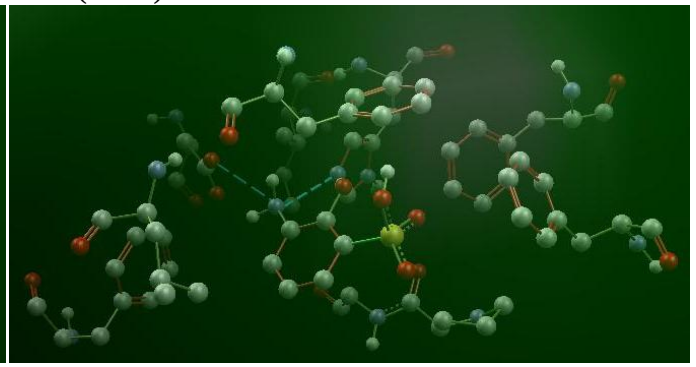
2 (-51.5)



3 (-43.1)

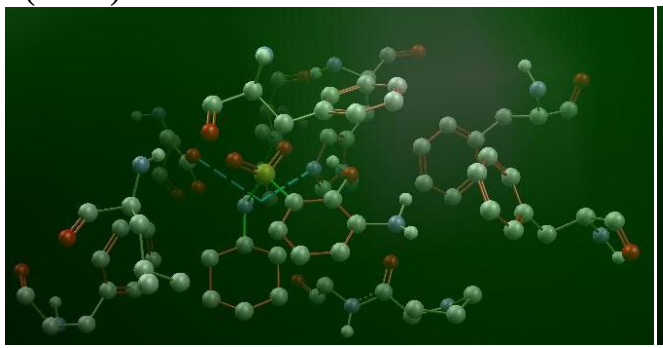


4 (-52.8)

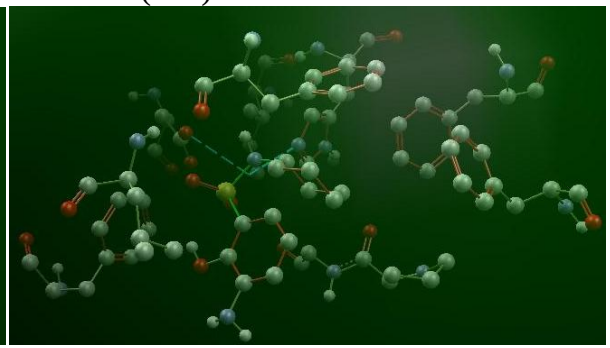


Docked pose of **4f**

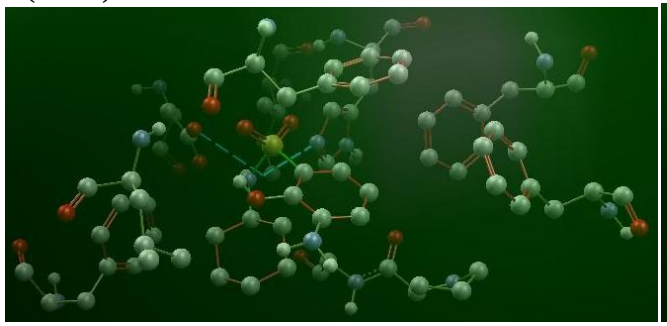
1 (-40.1)



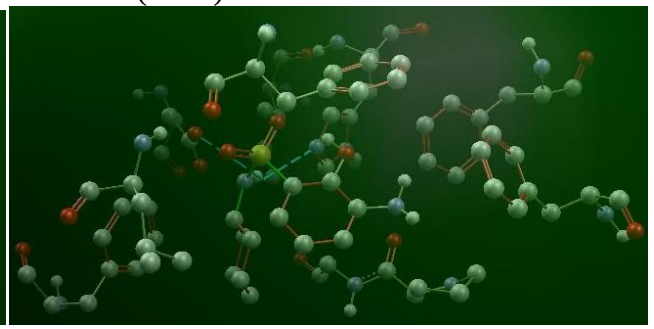
2 (-8.2)



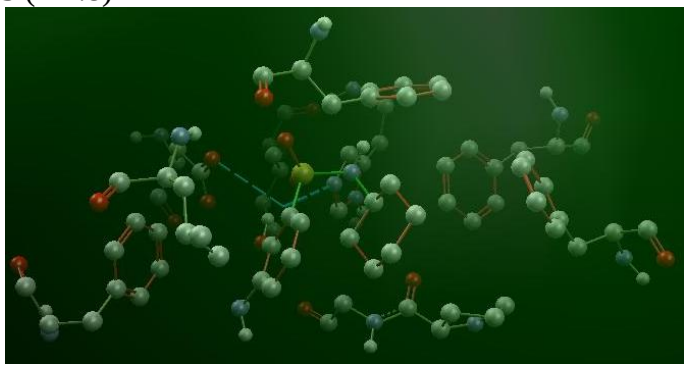
3 (-27.5)



4 (-72.4)

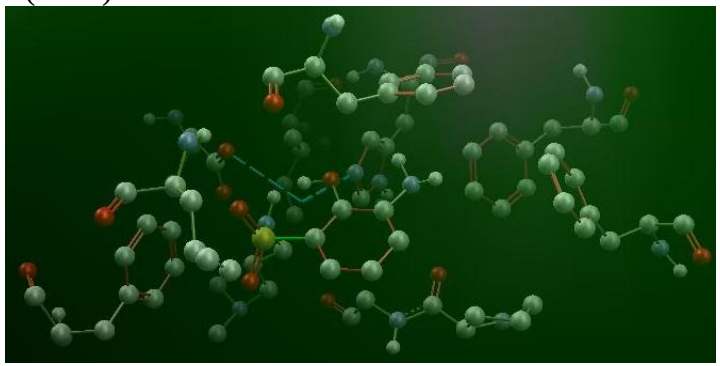


5 (-22.8)

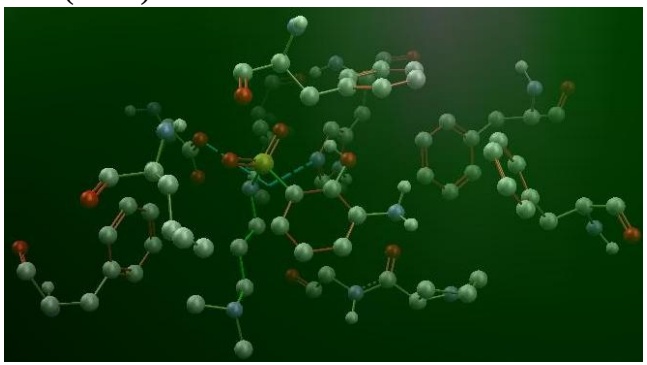


Docked pose of **4j**

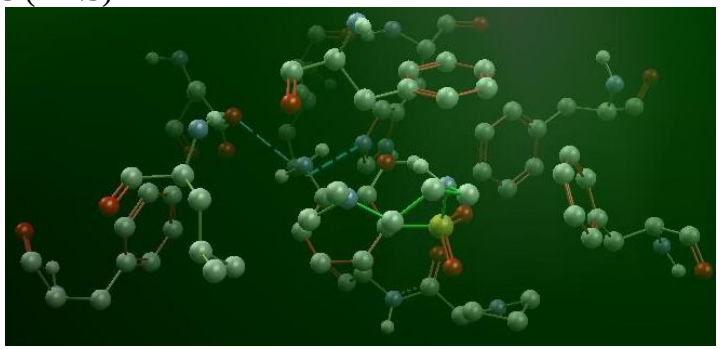
1 (-79.3)



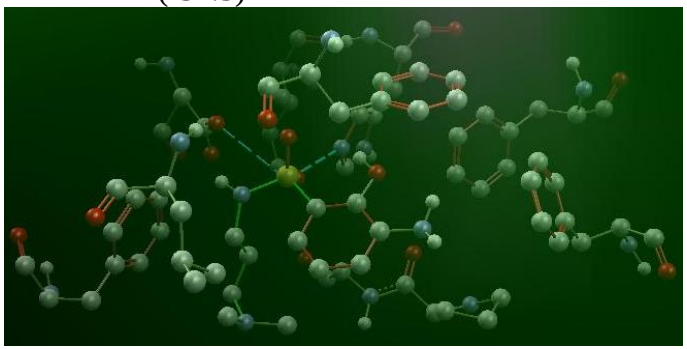
2 (-78.2)



3 (-72.3)



4 (-57.3)



5 (-67.0)