Supplementary information

Biocatalyzed asymmetric reduction of benzils to either benzoins or hydrobenzoins: pH dependent switch

Mohan Pal, Gautam Srivastava, Amar Nath Sharma, Suneet Kaur and Ravinder S. Jolly*

Department of Chemistry, CSIR-Institute of Microbial Technology, Sector 39, Chandigarh 160 036, India

INDEX

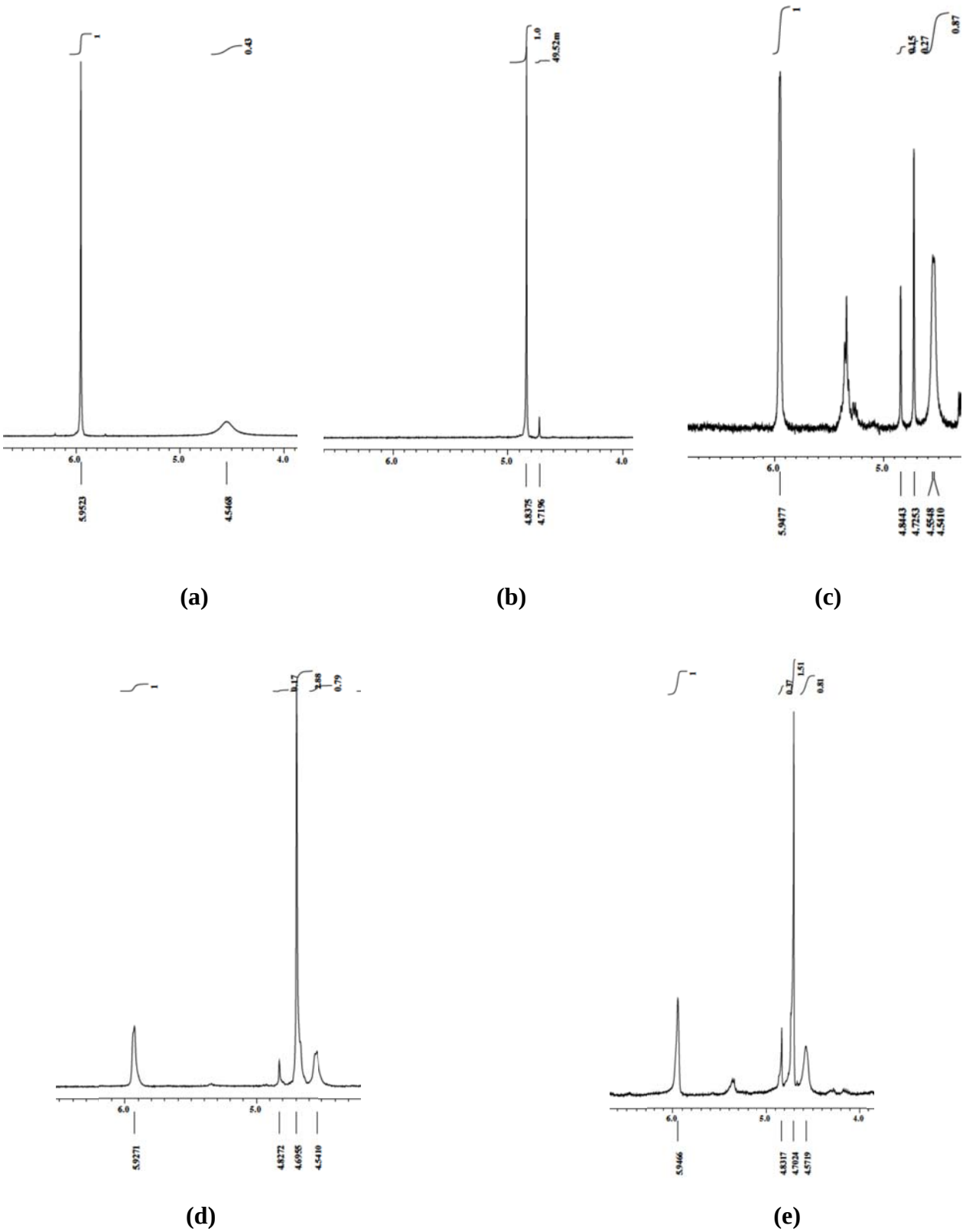


Figure 1S: ^{1}H NMR showing selected portion of a) *rac*-benzoin (**2a**) b) *dl/meso*-hydrobenzoin (**3a**) prepared by $NaBH_4$ reduction and biocatalyzed mixture of **2a**/**3a** by (c) *Penicillium sp.* (d) *Alternaria alternata* and (e) *Talaromyces flavus*

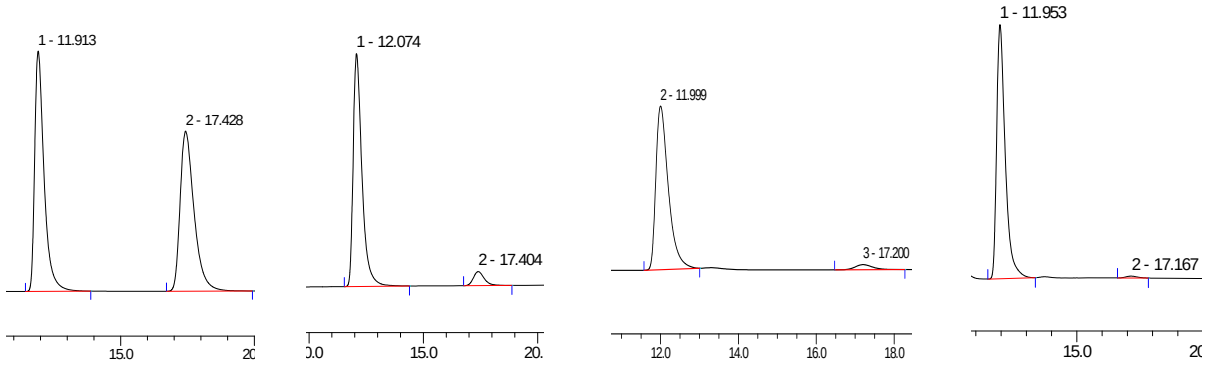


Figure 2S: HPLC profiles of (a) *rac*-benzoin **(2a)** and biocatalyzed (*S*)-benzoin **(2a)** by (b) *Penicillium sp.* (c) *Alternaria alternata* and (d) *Talaromyces flavus*

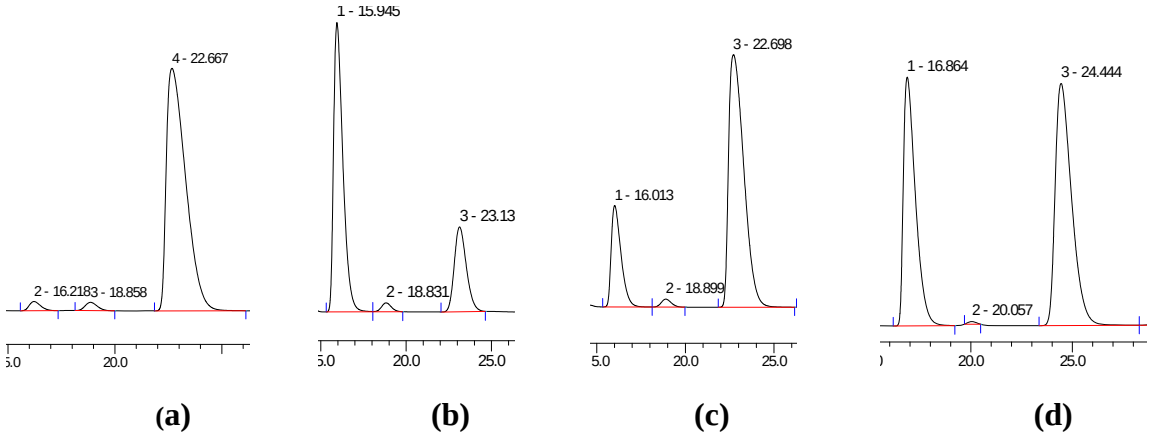


Figure 3S: HPLC profiles of **3a** obtained from (a) borohydride reduction (b) *Penicillium sp.* (c) *Alternaria alternata* and (d) *Talaromyces flavus* catalyzed reduction

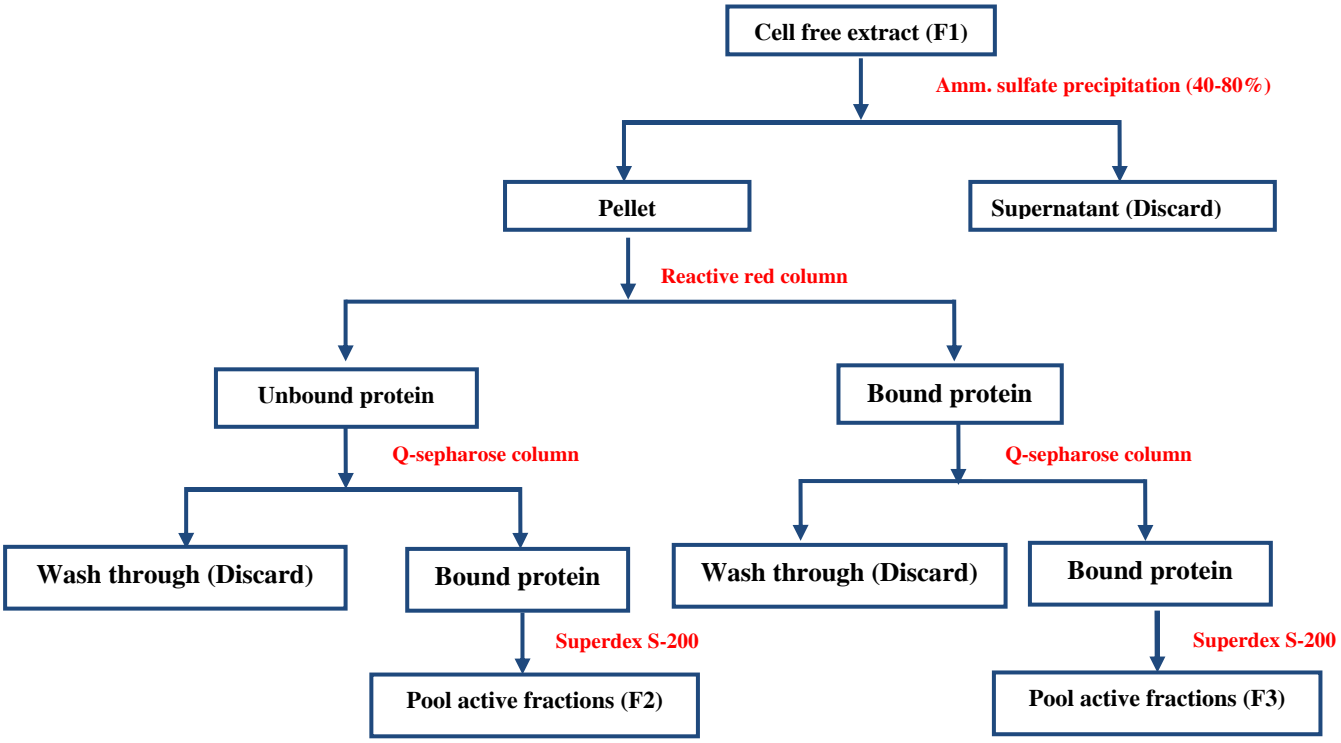


Figure 4S: Summary of attempted fractionation of proteins of *T. flavus*. Fraction 1 to 3 were used for asymmetric reduction of benzil **(1a)**, *rac*-benzoin **(2a)** and (*S*)-benzoin **(2a)**

Table 1S: Summary of the 5-step protein purification procedure

Entry	Protein purification Step	Total protein (mg)	Total activity ($\mu molmin^{-1}$)	Specific activity ($\mu molmin^{-1}mg^{-1}$ protein)	Fold purification
1	Cell free extract	487	114.5	0.235	1
2	(40-80%) amm. sulfate pellet	192	95.6	0.497	2.11
3	Reactive red	7.6	11.7	1.54	6.55
4	Q-sepharose	1.08	3.2	2.96	12.59
5	Gel filtration	0.4	1.9	4.75	20.2

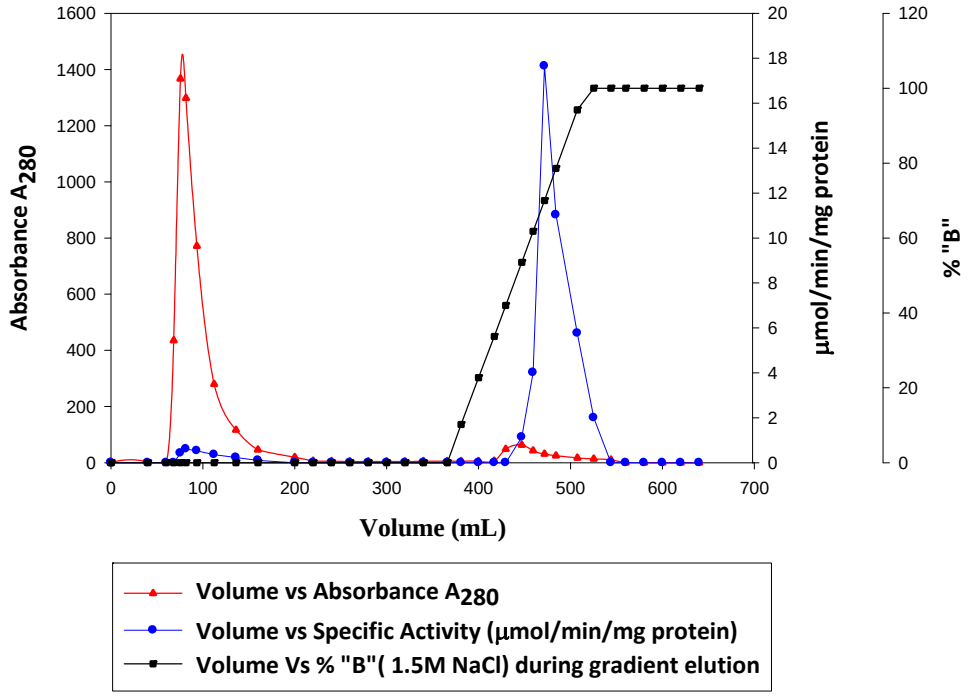


Figure 5S: Purification profile of dehydrogenase of *T. flavus* on reactive red column. The reactive red column (2×13 cm) was pre-equilibrated with phosphate buffer (10 mM, pH 8.0). The bound proteins were eluted with linear gradient of 0-1.5 M NaCl in phosphate buffer (10 mM, pH 6.5) at a flow rate of 1 mL/min. Fractions were analyzed for amount of protein by Bradford method. The enzyme activity was determined by monitoring the decrease in absorbance of NADPH at 340 nm spectrophotometrically. The active fractions were pooled and concentrated in an ultrafiltration cell using a 10 kDa membrane

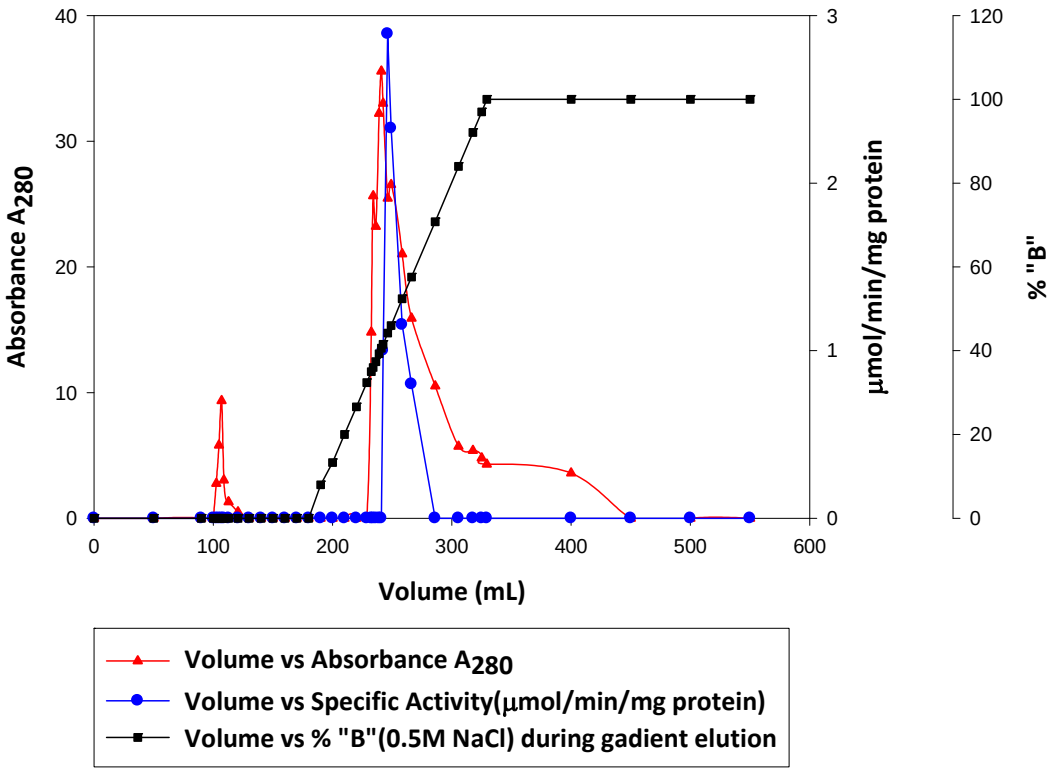


Figure 6S: Purification profile of dehydrogenase of *T. flavus* on Q-Sepharose column. The Q-sepharose column (2×5 cm)was pre-equilibrated with Tris-HCl buffer (10 mM, pH 8.0). The bound proteins were eluted with linear gradient of 0-0.5 M NaCl in with Tris-HCl buffer (10 mM, pH 8.0) at a flow rate of 1 mL/min. Fractions were analyzed for amount of protein by Bradford method. The enzyme activity was determined by monitoring the decrease in absorbance of NADPH at 340 nm spectrophotometrically. The active fractions were pooled and concentrated in an ultrafiltration cell using a 10 kDa membrane.

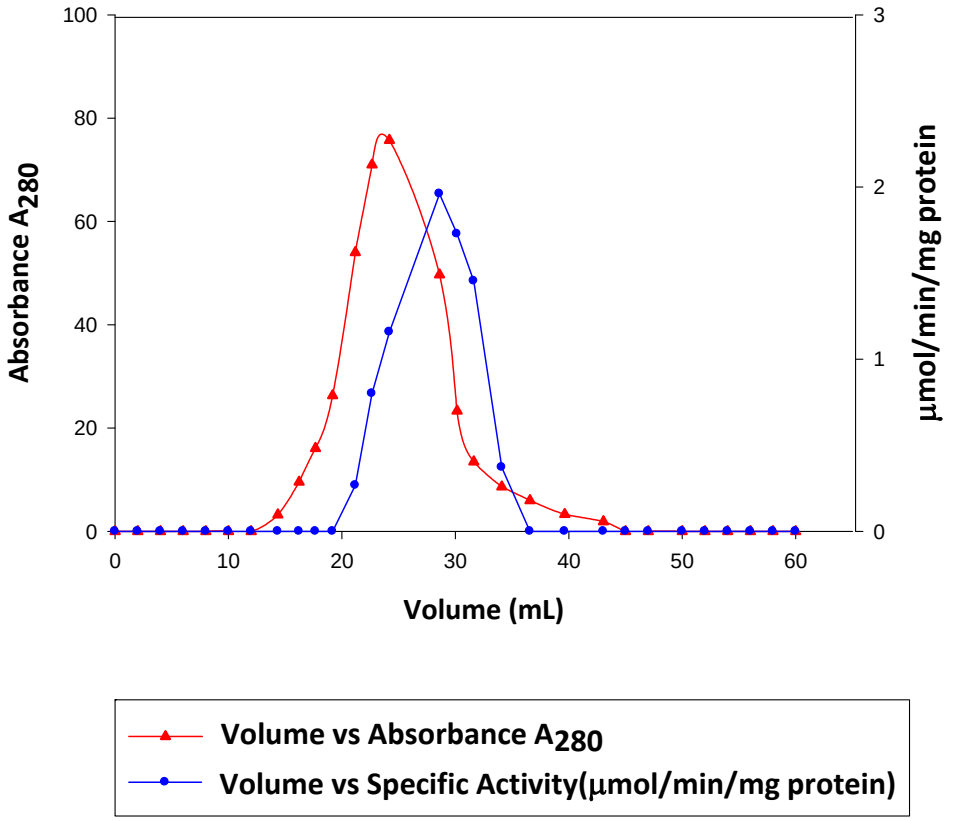
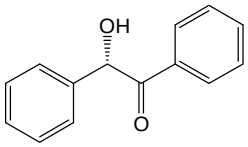
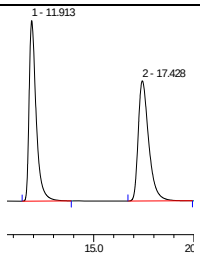
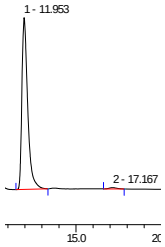
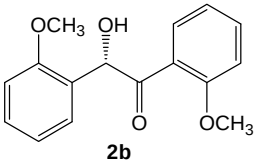
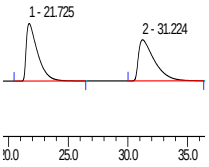
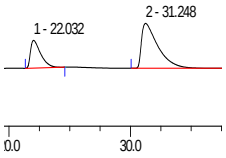
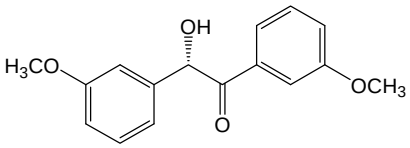
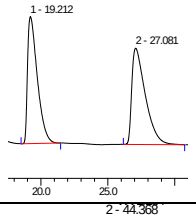
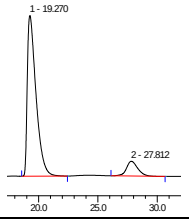
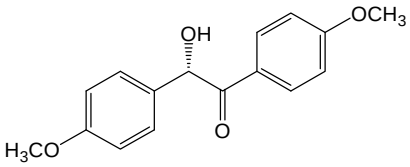
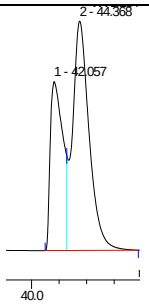
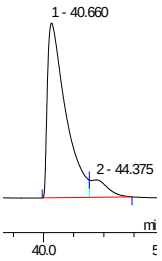
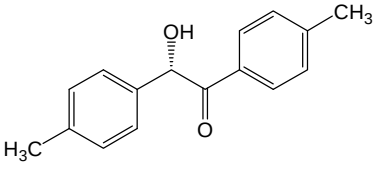
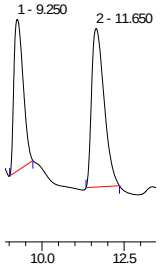
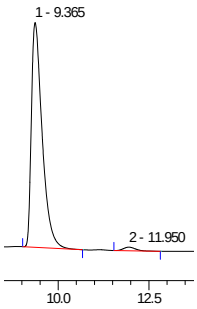
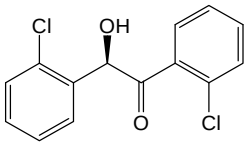
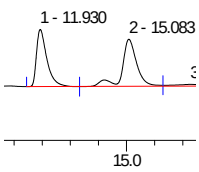
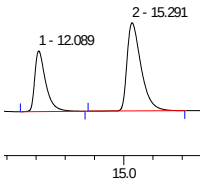


Figure 7S: Purification profile of dehydrogenase of *T. flavus* on superdex S-200 column. 0.5 mL of concentrated protein sample from Q-sepharose chromatography step was loaded on a superdex S-200 column (2×60 cm, 43 mL bed volume) previously equilibrated with Tris-HCl buffer (50 mM, pH 7.5) containing 0.15 M NaCl. The enzyme was eluted with same buffer at flow rate of 0.5 mL/min and active fractions were pooled and concentrated in an ultra filtration cell using a 10 kDa membrane. The enzyme activity was determined by monitoring the decrease in absorbance of NADPH at 340 nm spectrophotometrically.

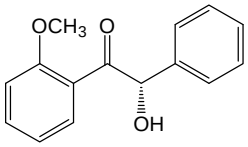
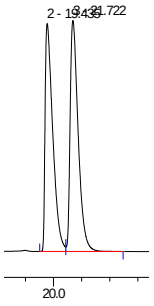
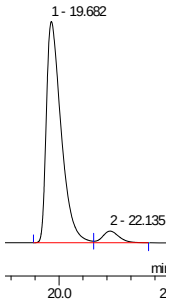
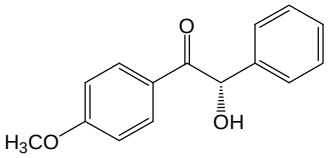
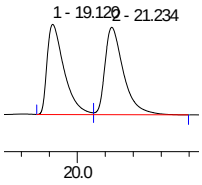
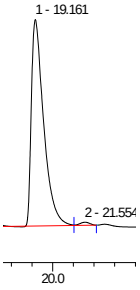
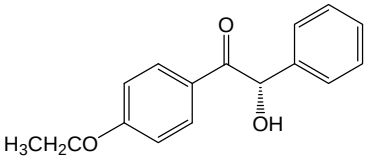
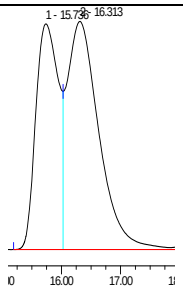
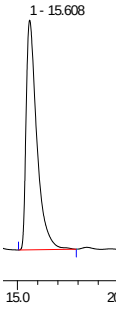
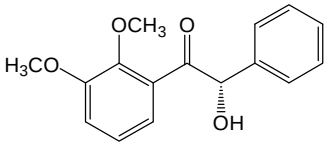
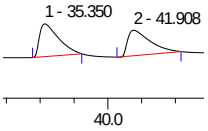
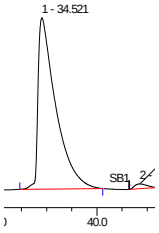
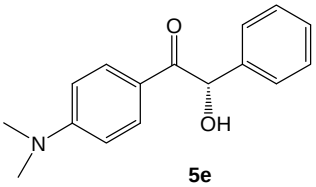
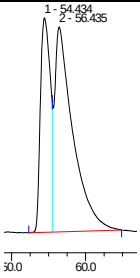
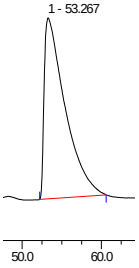
HPLC traces for biocatalyzed products

Table 2S. HPLC profiles of the products obtained from asymmetric reduction of symmetrical benzil derivatives with *T. flavus* at pH 5.0[a,b]

Entry	Biocatalyzed product	*rac.* benzoin	biocatalyzed benzoins
1	 **2a**		
2	 **2b**		
3	 **2c**		
4	 **2d**		
5	 **2e**		
6	 **2g**		

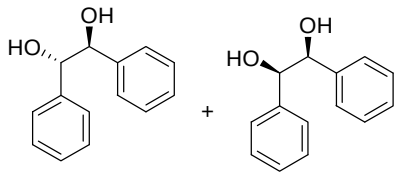
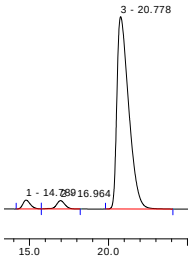
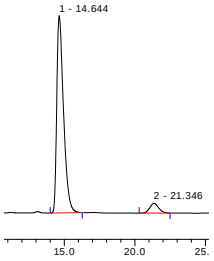
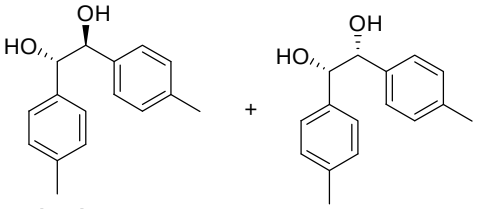
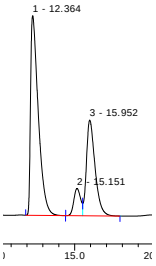
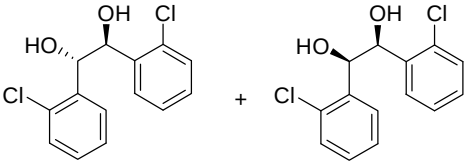
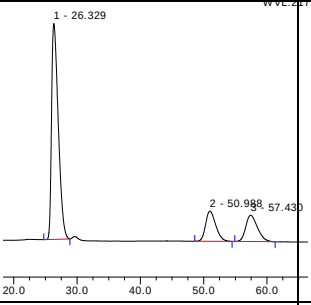
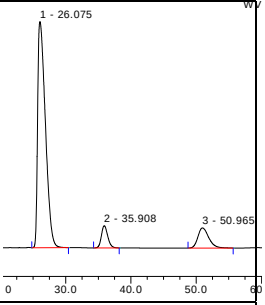
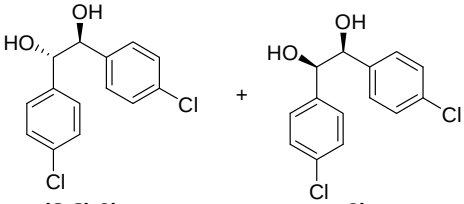
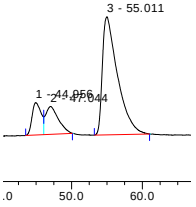
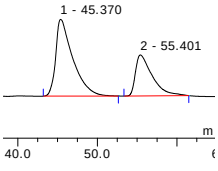
[a]e.e. was determined by chiral HPLC on Chiralcel OD-H (Diacel, Japan) column. [b]Absolute configuration was assigned based on comparison of sign of optical rotation with literature.

Table 3S. HPLC profiles of the products obtained from asymmetric reduction of un-symmetrical benzil derivatives with *T. flavus* at pH 5.0[a,b]

Entry	Biocatalyzed product	*rac.* benzoin	biocatalyzed benzoins
1	 **5a**		
2	 **5b**		
3	 **5c**		
4	 **5d**		
5	 **5e**		

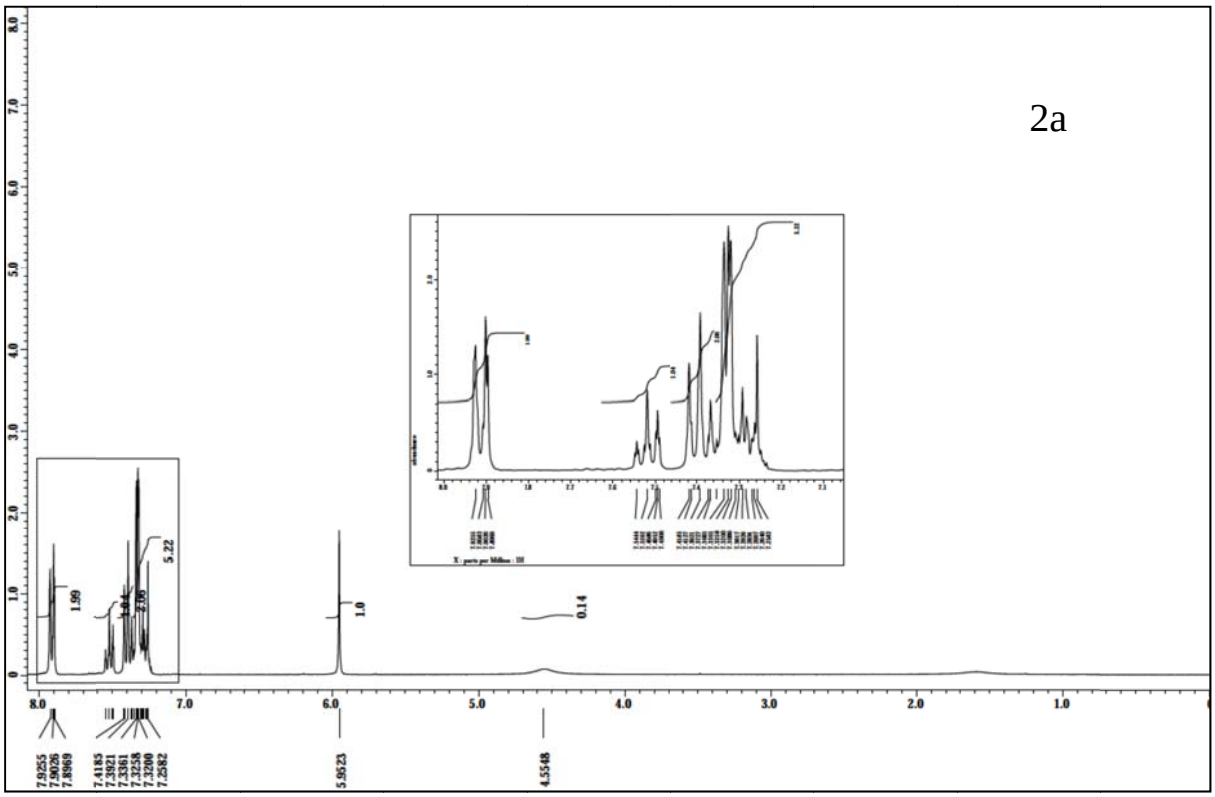
[a]e.e. was determined by chiral HPLC on Chiralcel OD-H (Diacel, Japan) column. [b] Configuration tentative

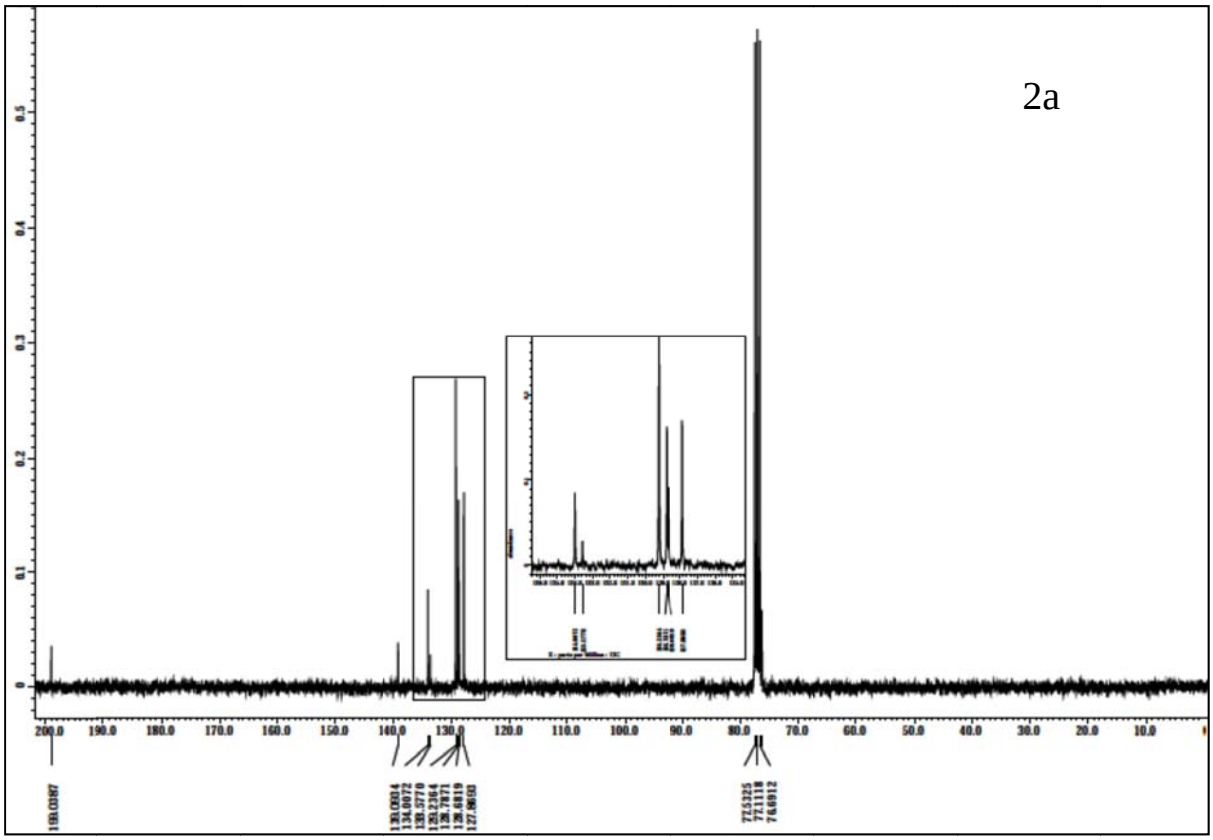
Table 4S. HPLC profiles of the products obtained from asymmetric reduction of symmetrical benzil derivatives with *T. flavus* at pH 7.0[a]

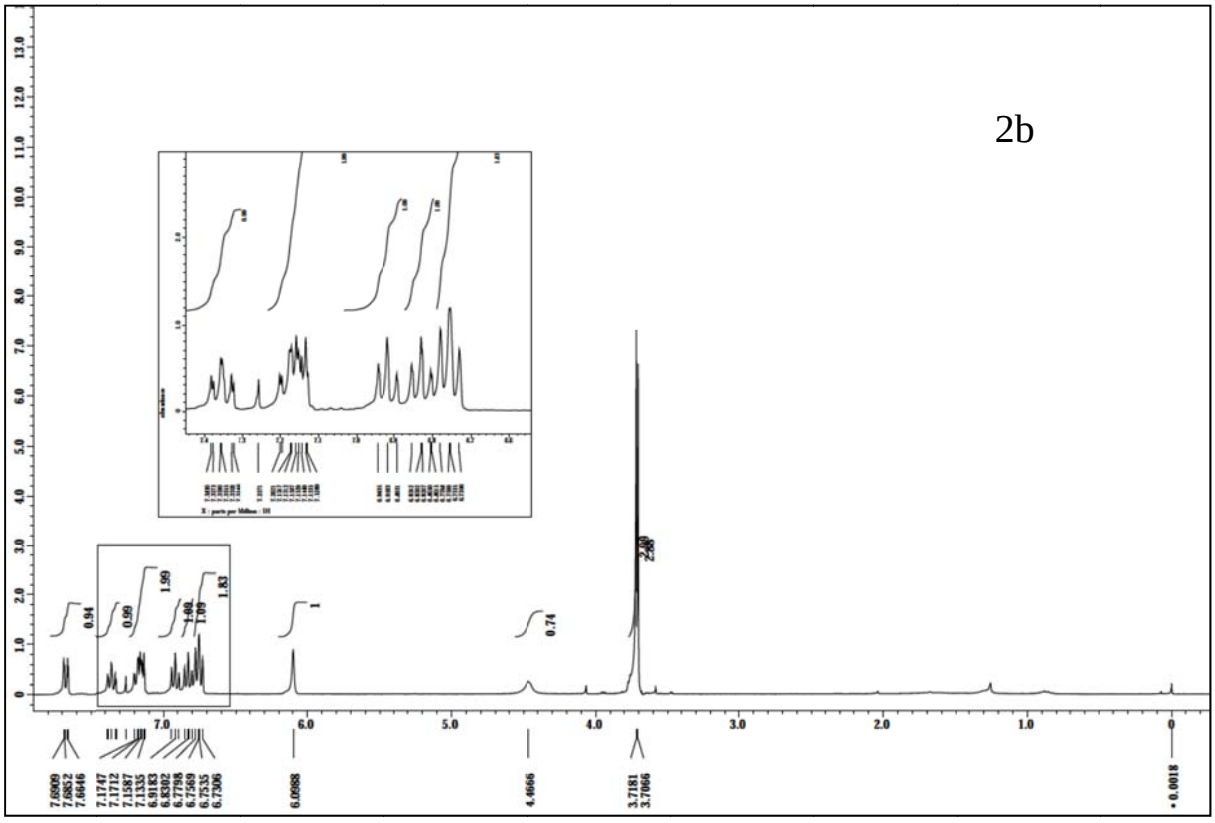
Entry	Biocatalyzed product	*rac.* benzoin	biocatalyzed benzoins
1	 **(*S,S*)-3a** **meso-3a**		
2	 **(*S,S*)-3e** **meso-3e**		
3	 **(*S,S*)-3g** **meso-3g**		
4	 **(*S,S*)-3i** **meso-3i**		

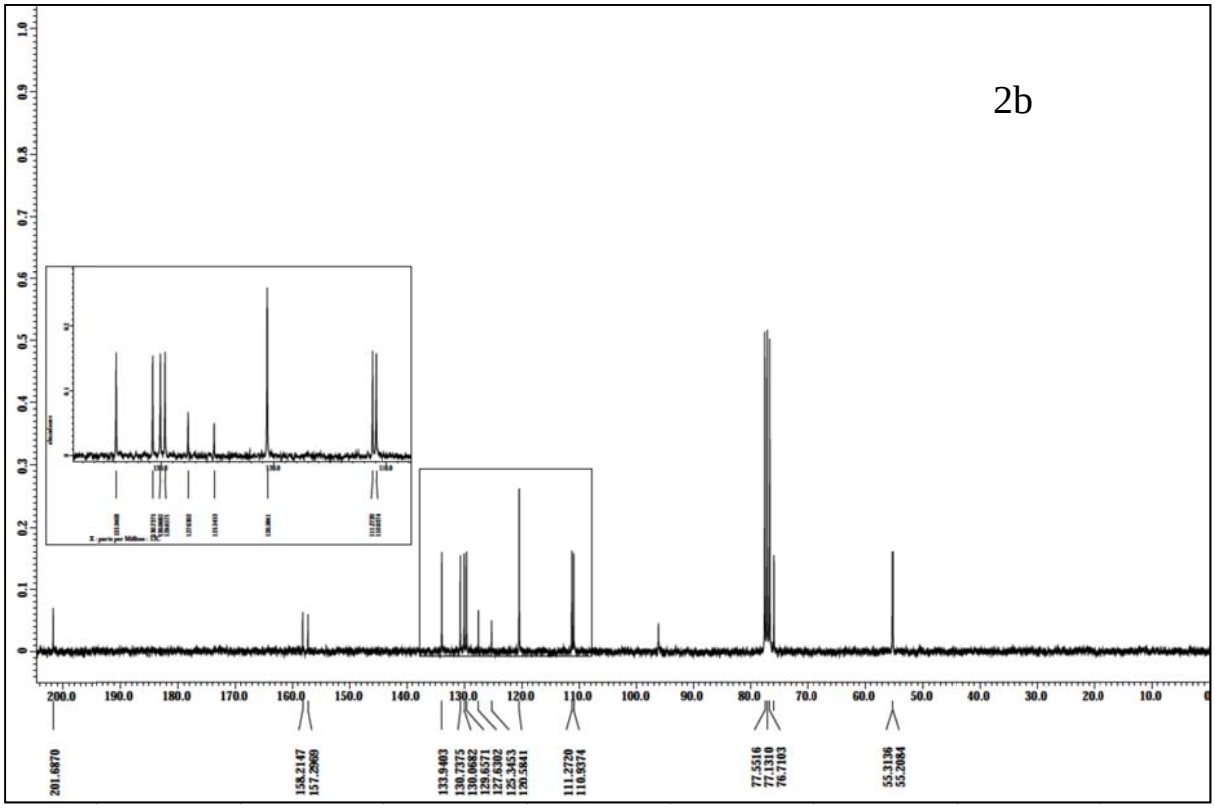
[a]e.e. was determined by chiral HPLC on Chiralcel OJ-H (Diacel, Japan) column

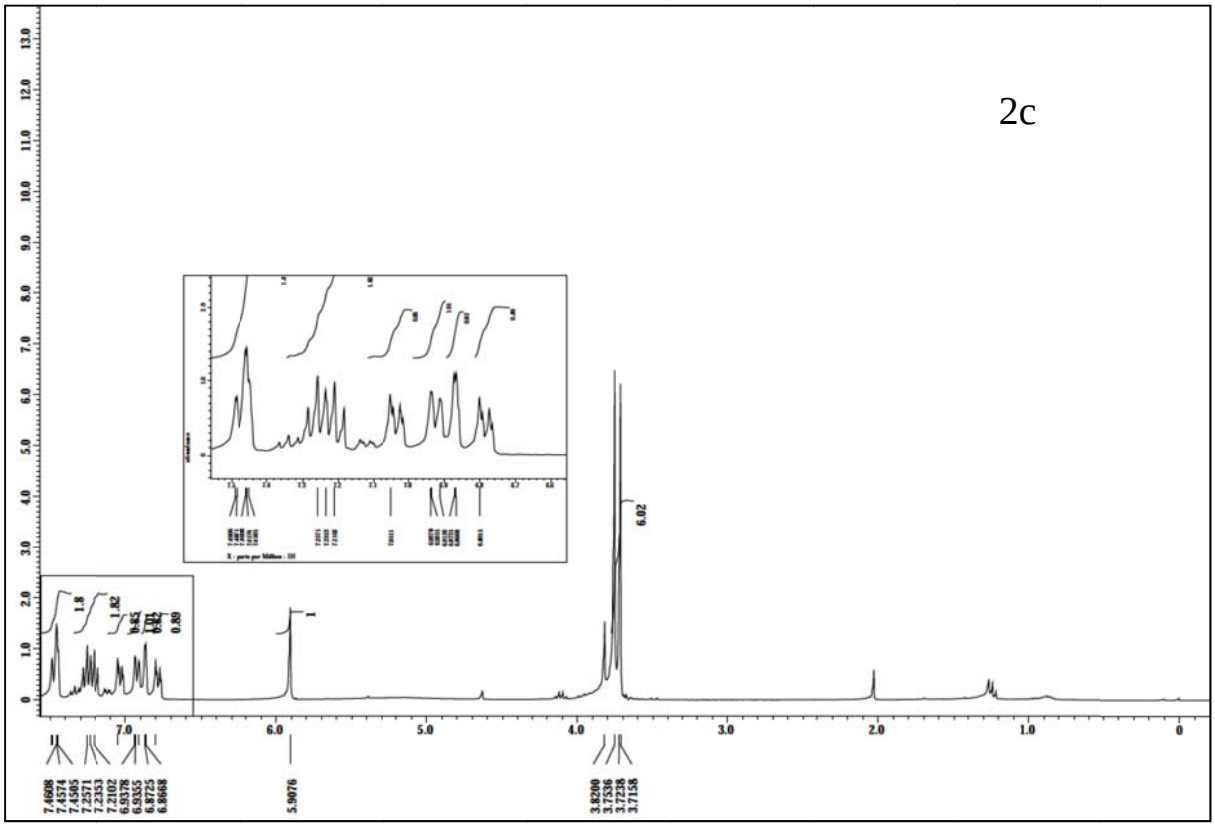
NMR images

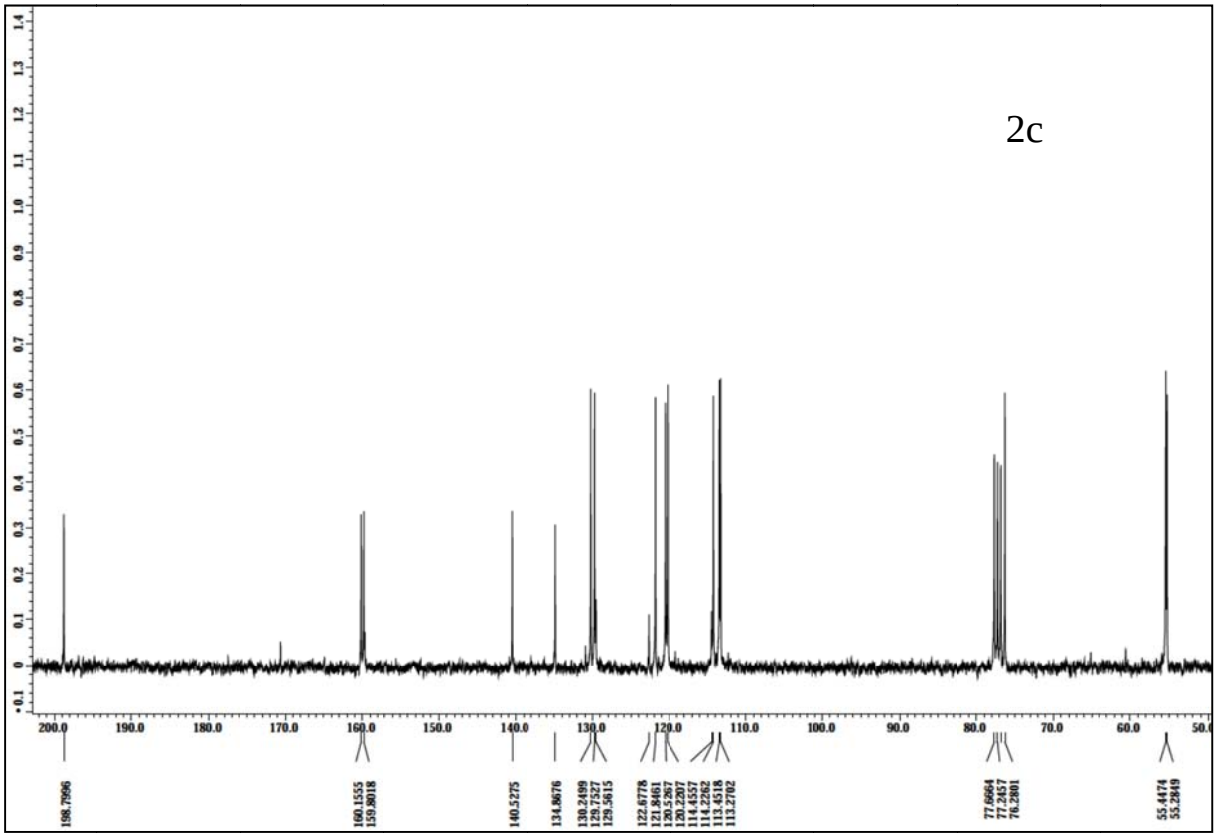

2a
1.99
5.22
1.0
0.14
7.9255
7.9026
7.8969
7.4185
7.3921
7.3361
7.3258
7.3200
7.2582
5.9523
4.5548

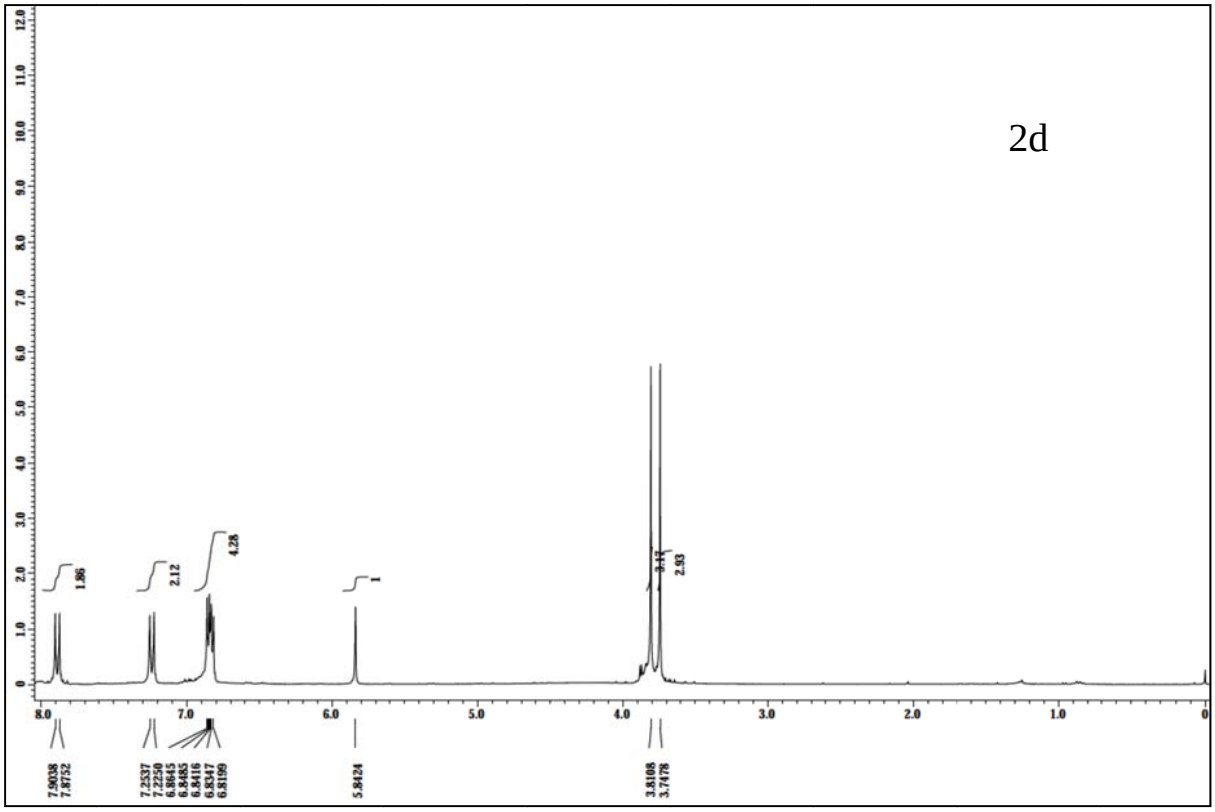

2a
193.0387
130.0034
134.0072
133.5770
129.2364
128.7871
128.6819
127.9650
77.5225
77.1118
76.6912

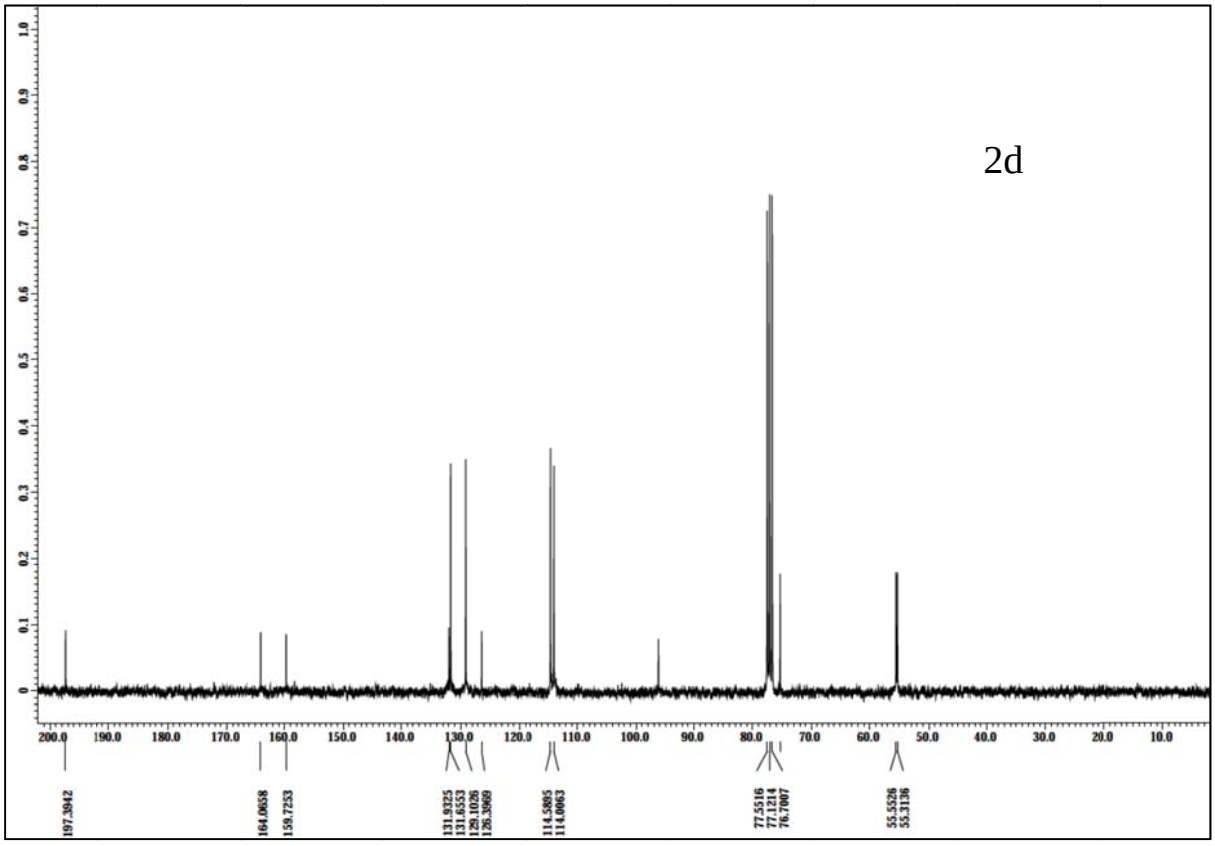

2b

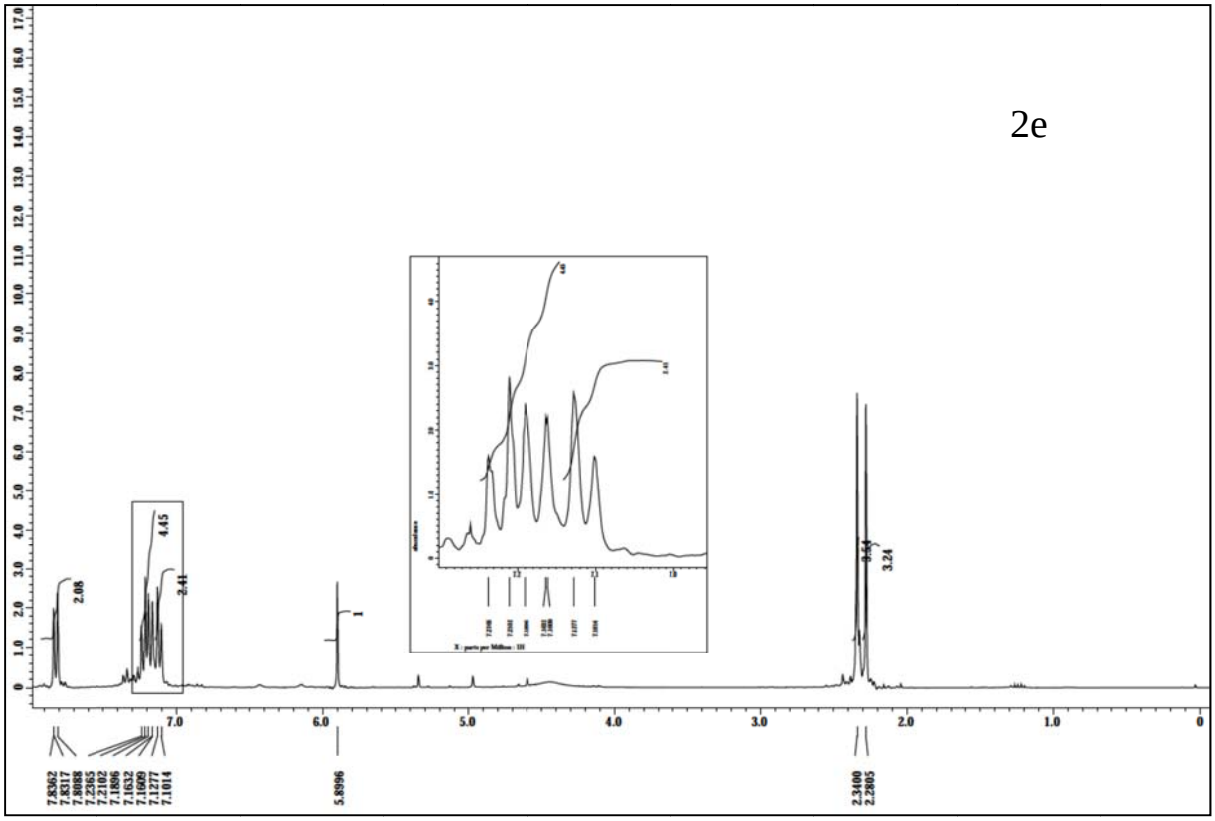

2b

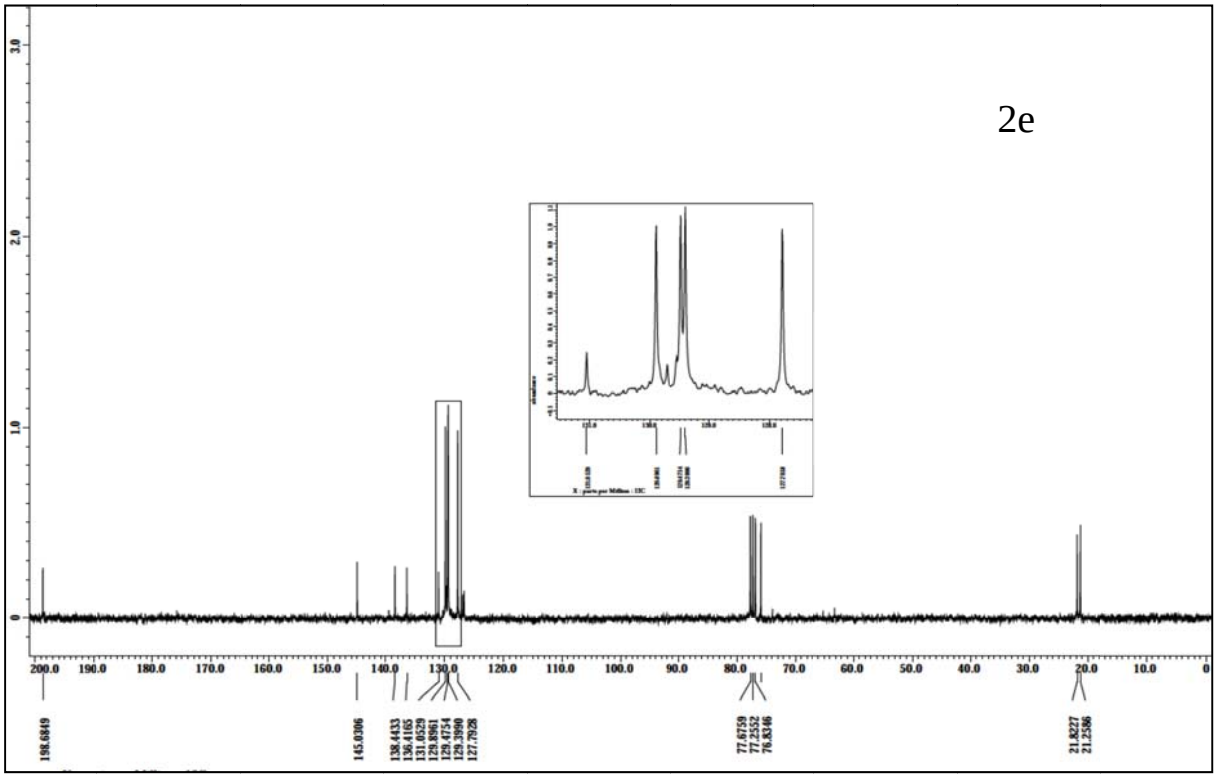

2c
1.8
1.82
0.89
1
6.02
7.4698
7.4574
7.4565
7.2571
7.2353
7.2102
6.9378
6.9355
6.8725
6.8668
5.9076
3.8260
3.8236
3.7238
3.7158

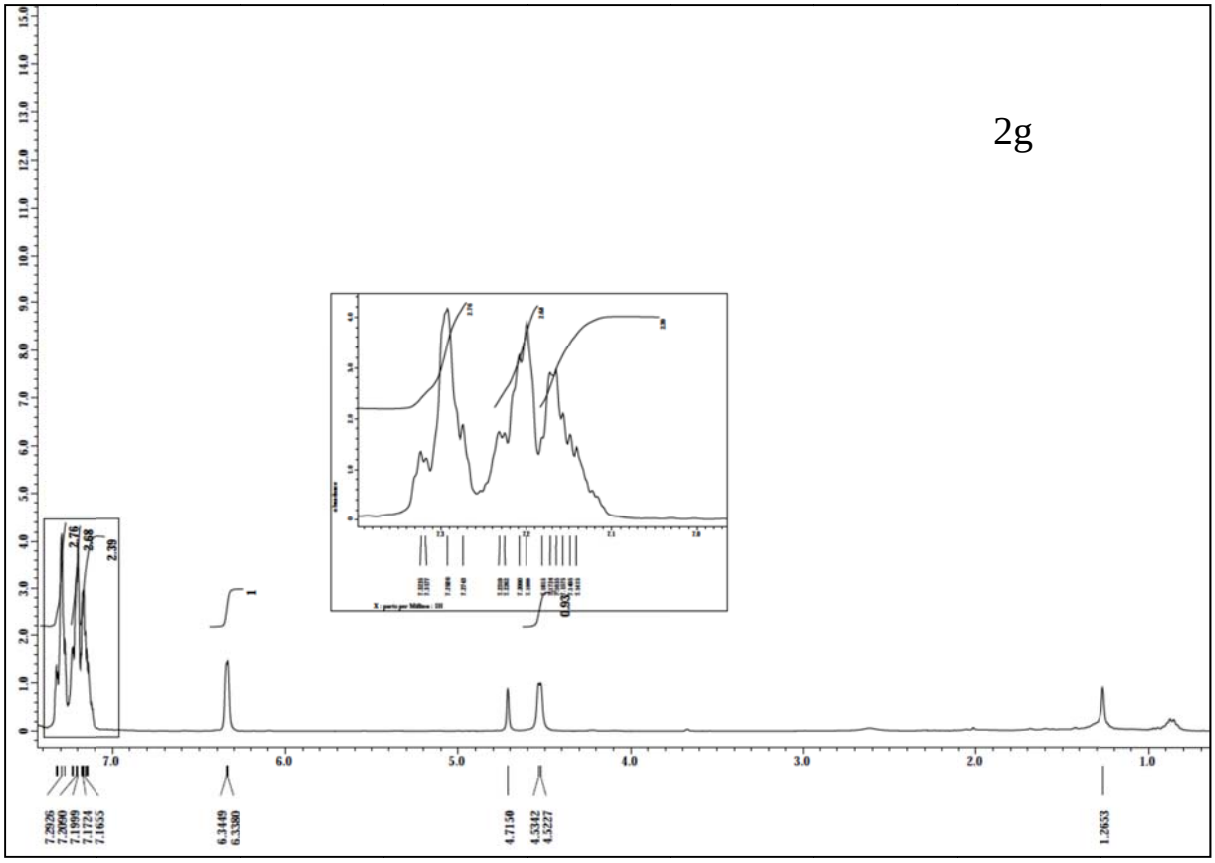

2c
198.7996
160.1555
159.8018
140.5275
134.8676
130.2499
129.7527
129.5615
122.6778
121.8461
120.5267
120.2207
114.4557
114.2782
113.4518
113.2702
77.6664
77.2457
76.2801
55.4474
55.2849

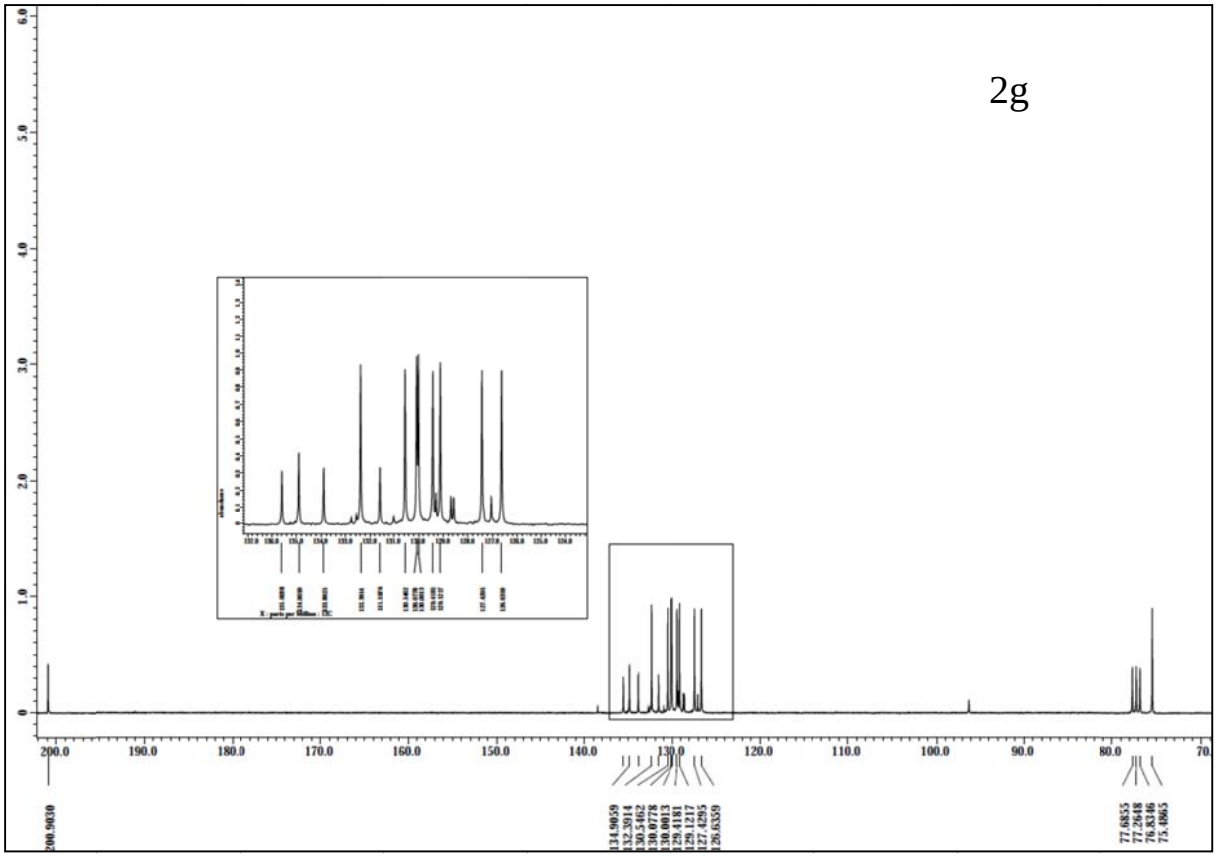

2d
1.86
2.12
4.28
1
2.93
7.9038
7.8752
7.2537
7.2250
6.8645
6.8485
6.8416
6.8347
6.8199
5.8424
3.8108
3.7478

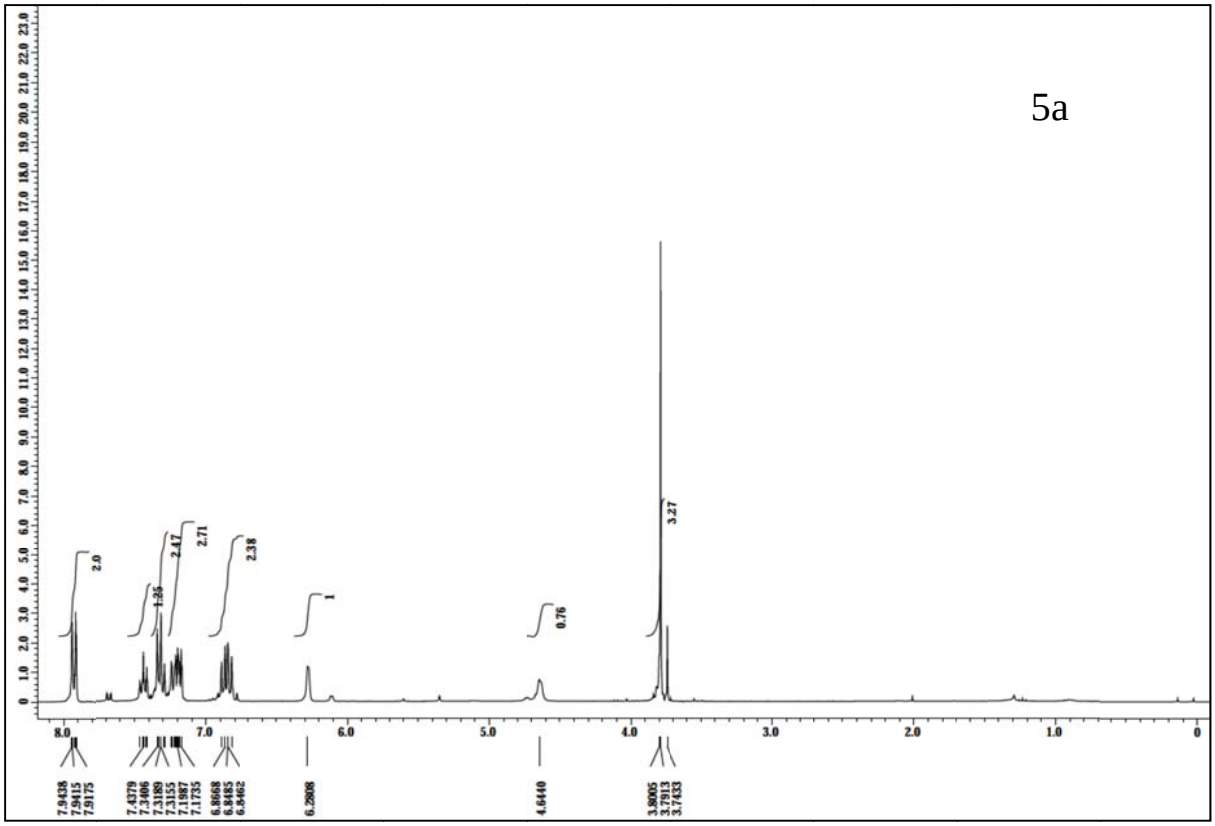

2d
197.3942
164.0658
159.7253
131.8325
131.6553
129.1026
126.3969
114.5805
114.0063
77.5516
77.1214
76.7007
55.5526
55.3136

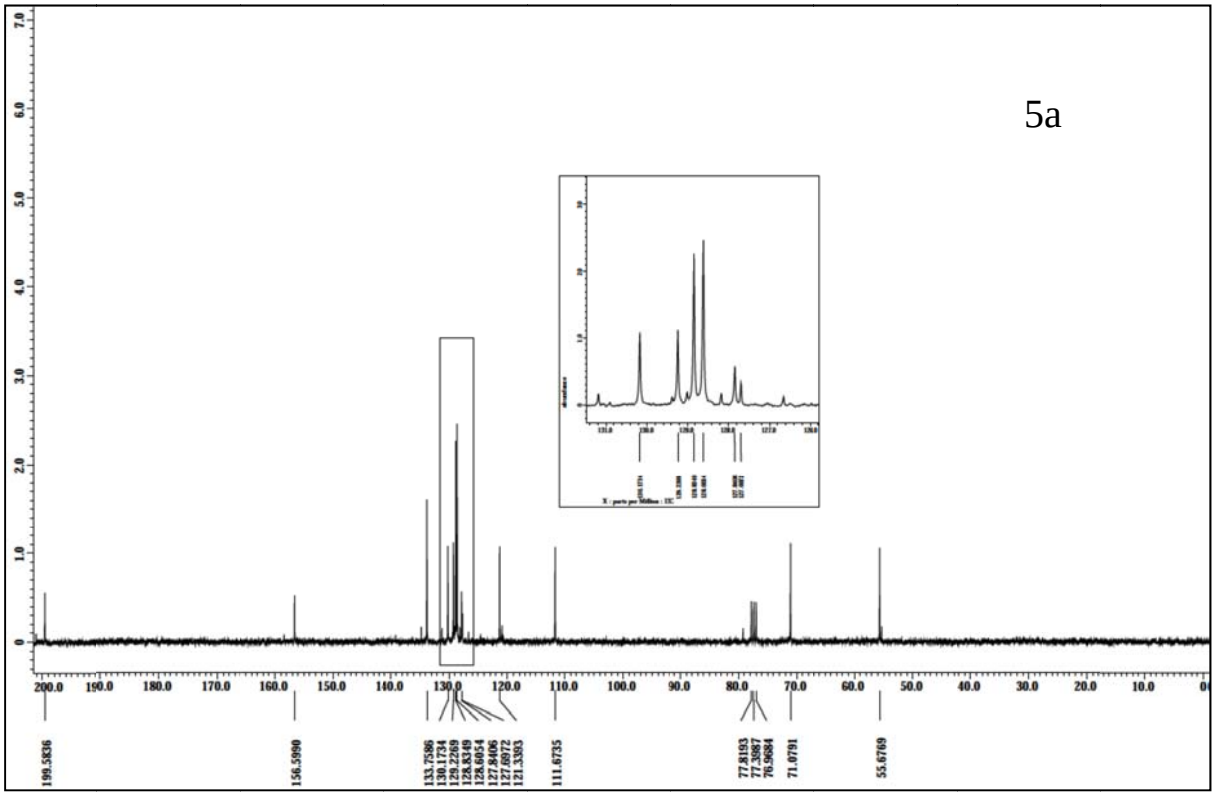

2e
2.08
4.45
2.41
1
3.24
7.8362
7.8317
7.8088
7.2765
7.2185
7.1896
7.1632
7.1609
7.1277
7.1014
5.8996
2.3400
2.2805

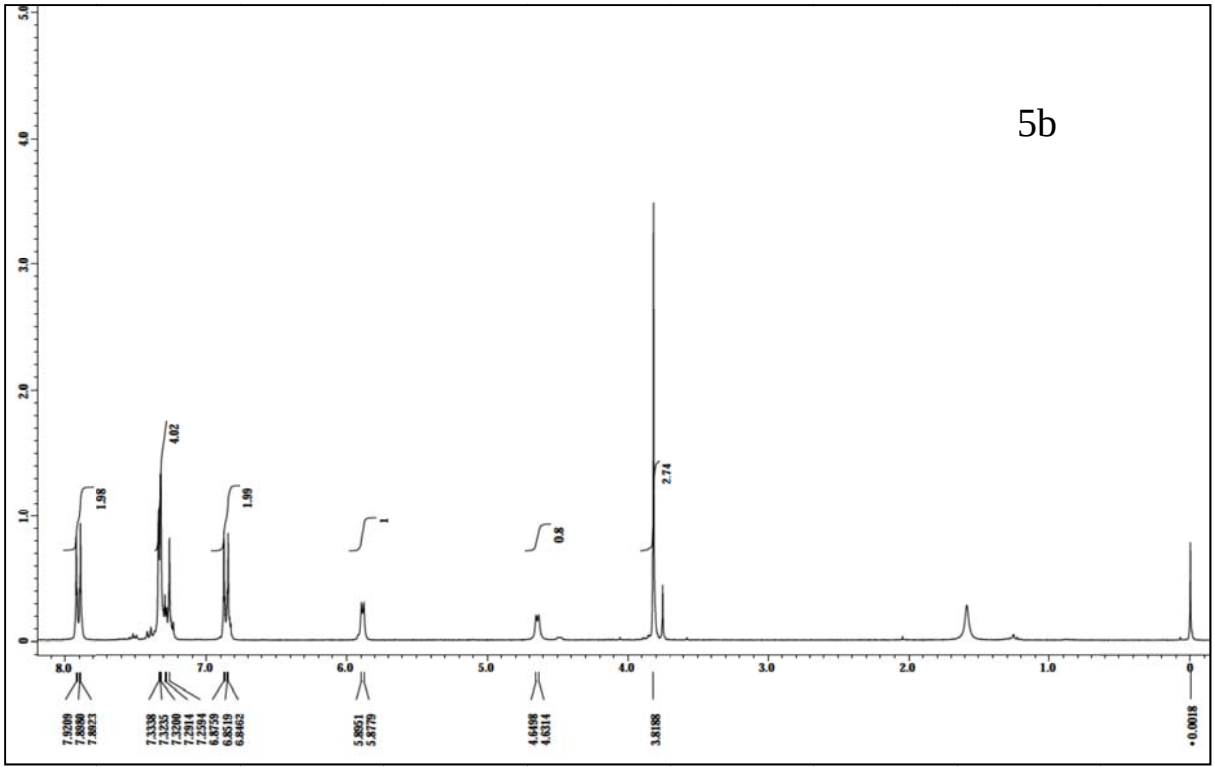

2e
198.6849
145.0306
138.4433
136.4165
131.0529
129.8961
129.4754
129.3990
127.7928
77.6759
77.2552
76.8346
21.8227
21.2586

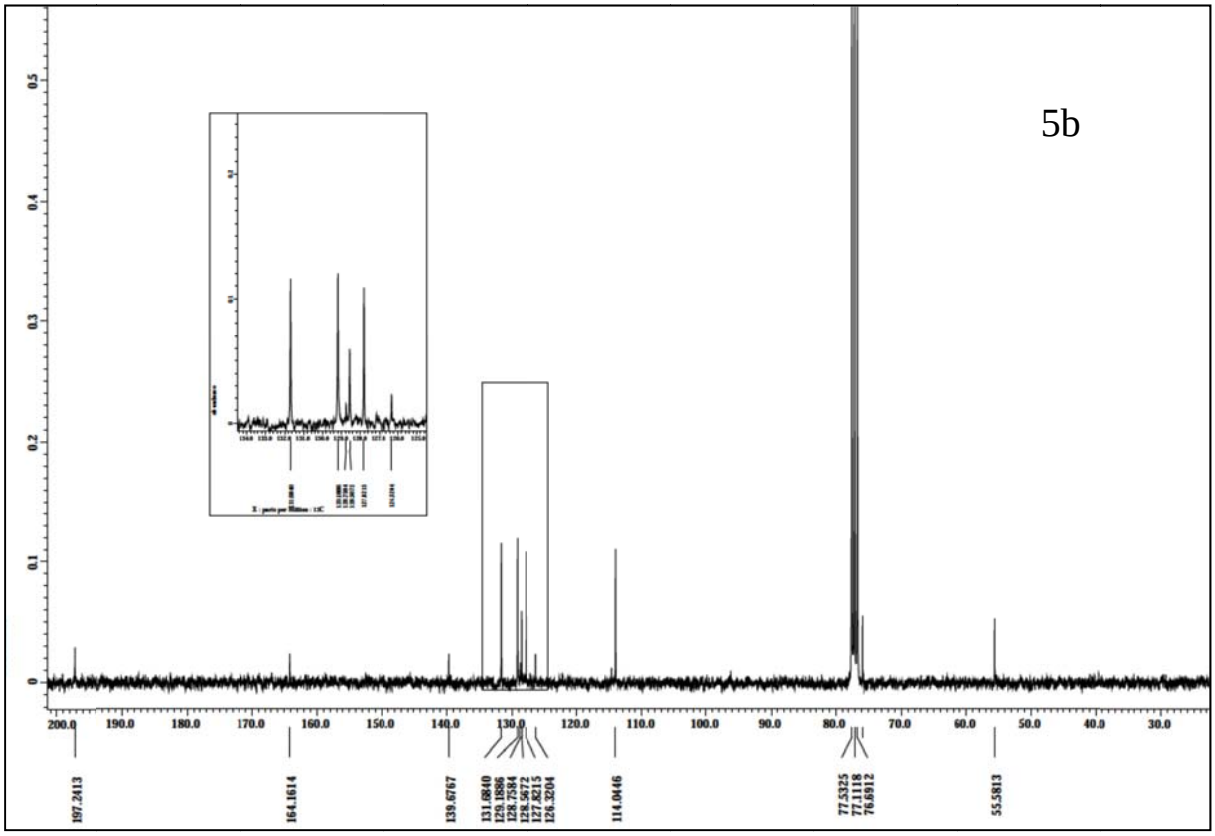

2g
2.76
2.68
2.39
1
7.2926
7.2890
7.1999
7.1724
7.1655
6.3449
6.3380
4.7150
4.5342
4.5227
1.2653

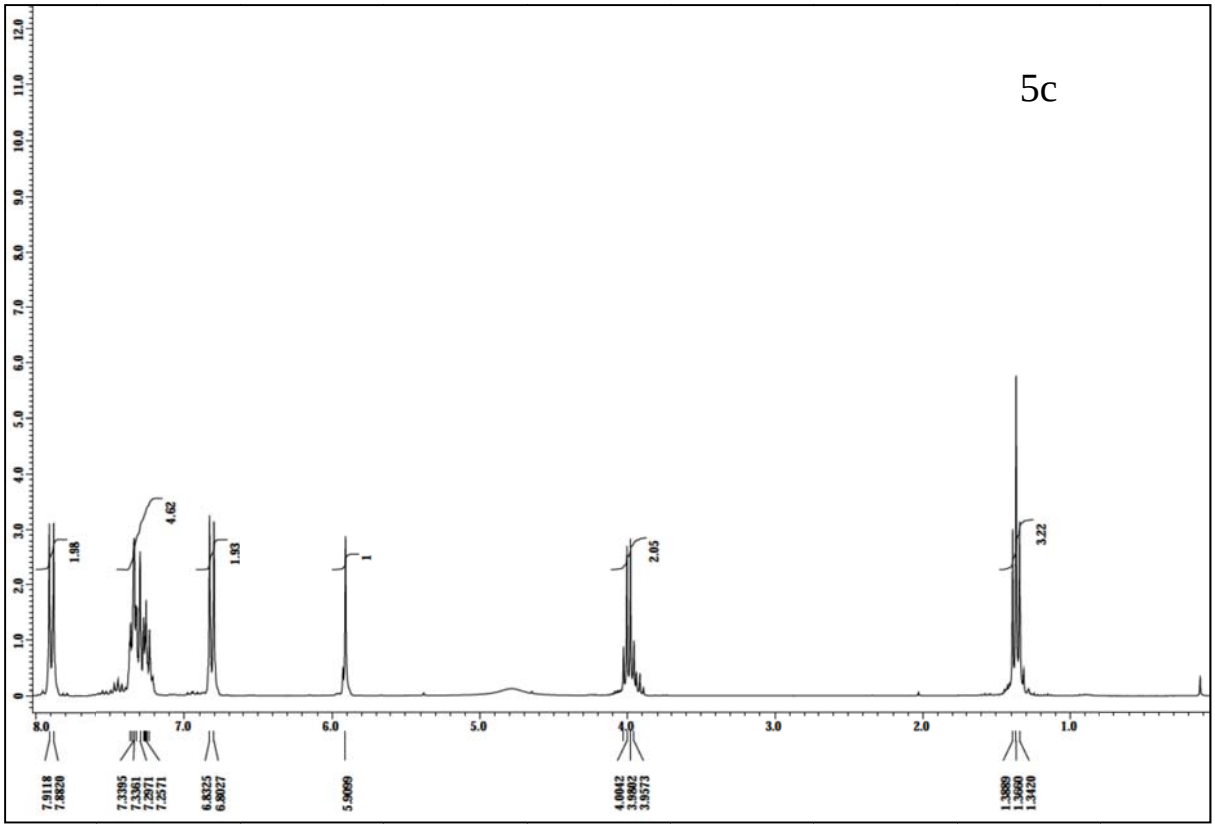

2g
200.9030
134.9059
132.3914
130.5462
130.0778
130.0013
129.1431
129.1217
127.4295
126.6359
77.6855
77.2648
76.8346
75.4865

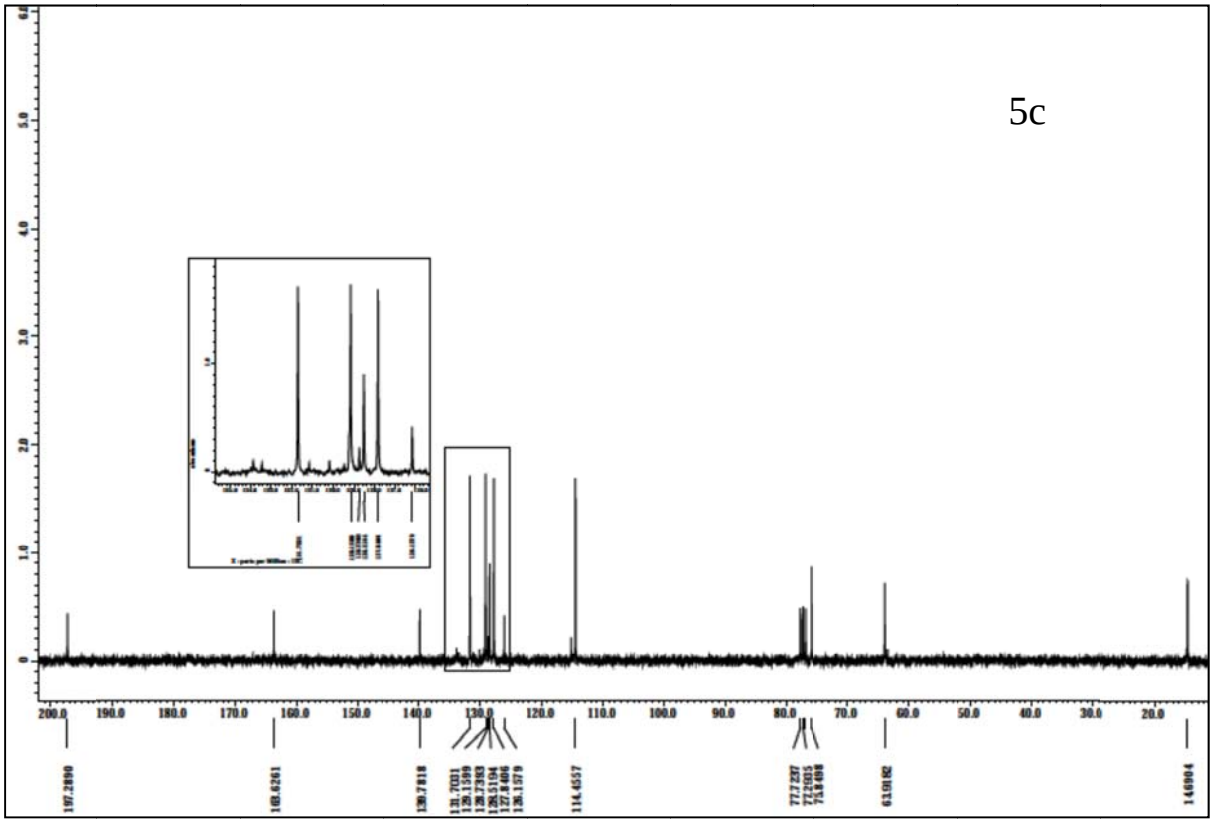

5a
2.0
1.25
2.47
2.71
2.38
1
0.76
3.27

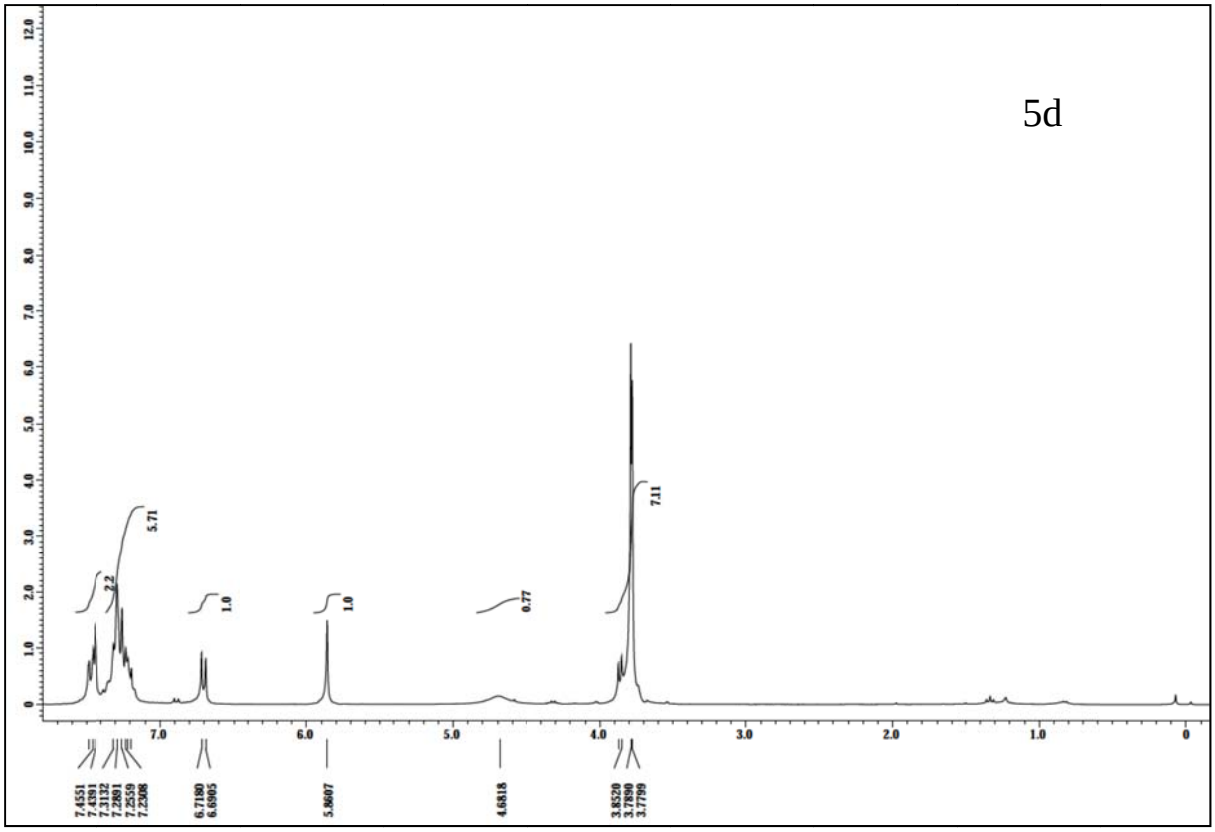

5a
199.5836
156.5990
133.7586
130.1734
129.2749
128.6854
128.6054
127.8406
127.6972
121.3393
111.6735
77.8193
77.3087
76.9684
71.0791
55.6769

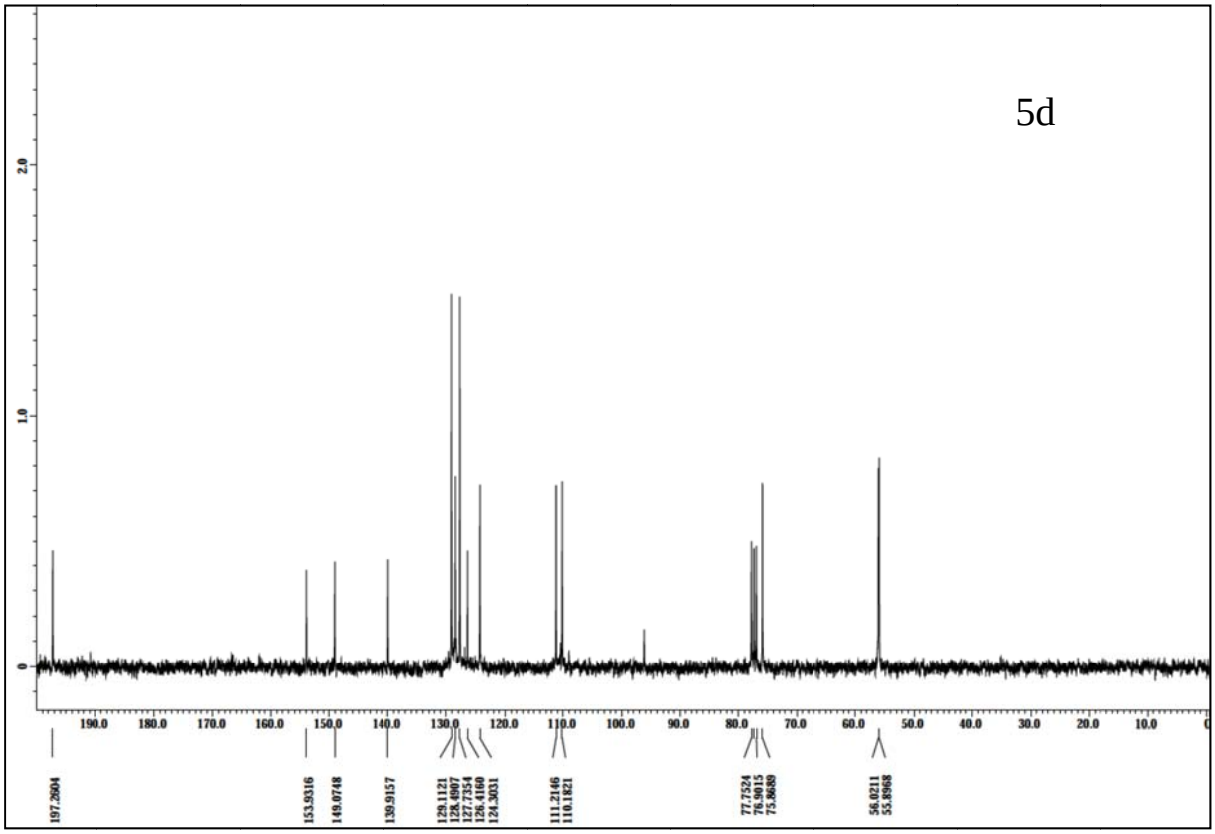

5b
1.98
4.02
1.99
1
0.8
2.74
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0
7.9209
7.8890
7.8823
7.3338
7.3235
7.2200
7.2014
7.2004
6.8759
6.8519
6.8462
5.8851
5.8779
4.6498
4.6314
3.8188
+0.0018

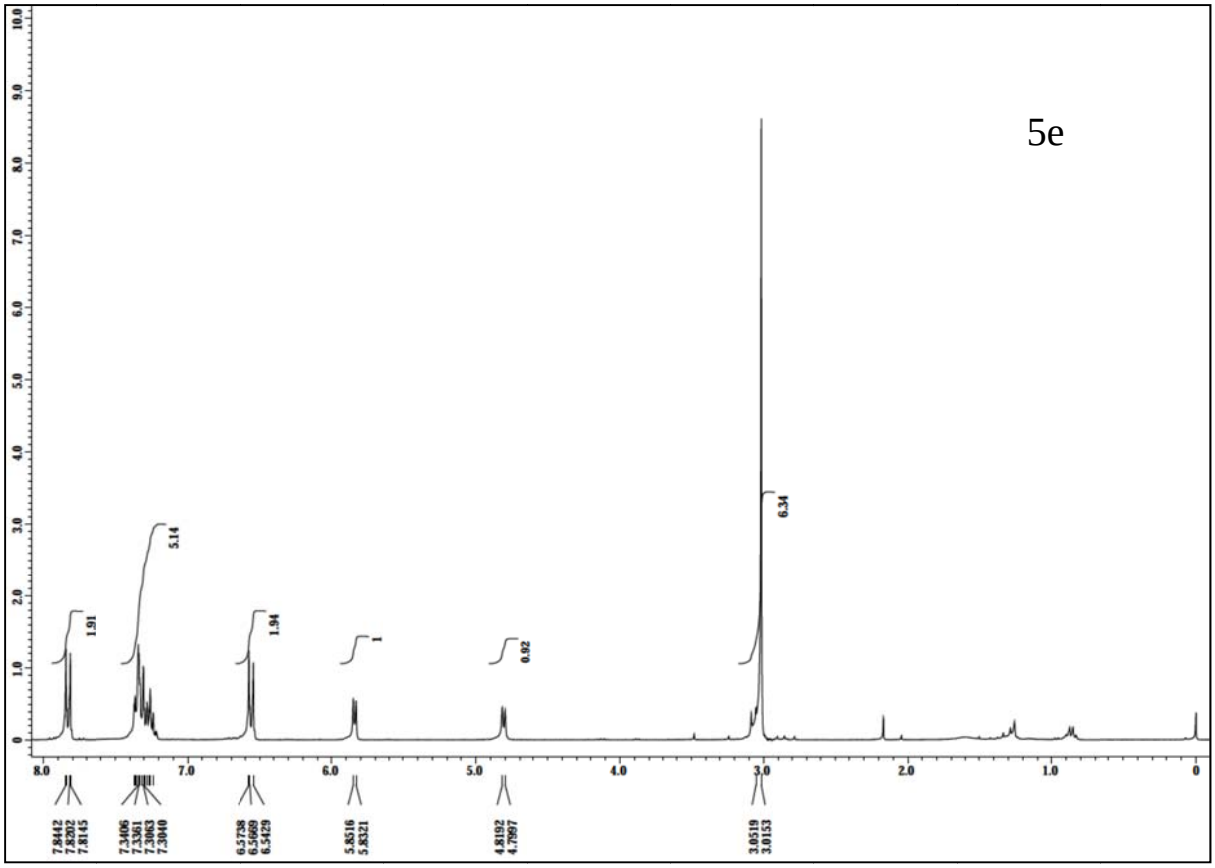

5b
200.0
190.0
180.0
170.0
160.0
150.0
140.0
130.0
120.0
110.0
100.0
90.0
80.0
70.0
60.0
50.0
40.0
30.0
197.2413
164.1614
139.6767
131.0840
129.1886
128.7384
128.3672
127.8215
126.3204
114.0446
77.5325
77.1118
76.6912
55.5813

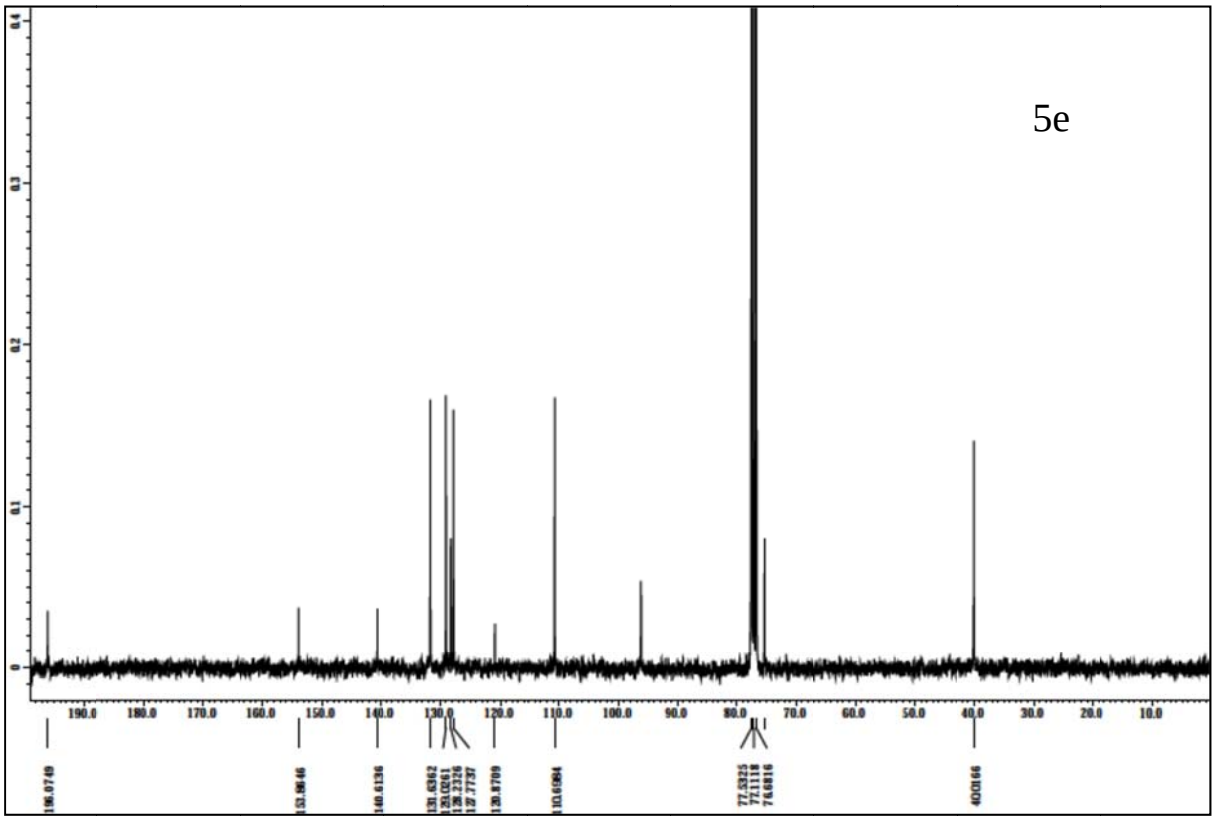

5c

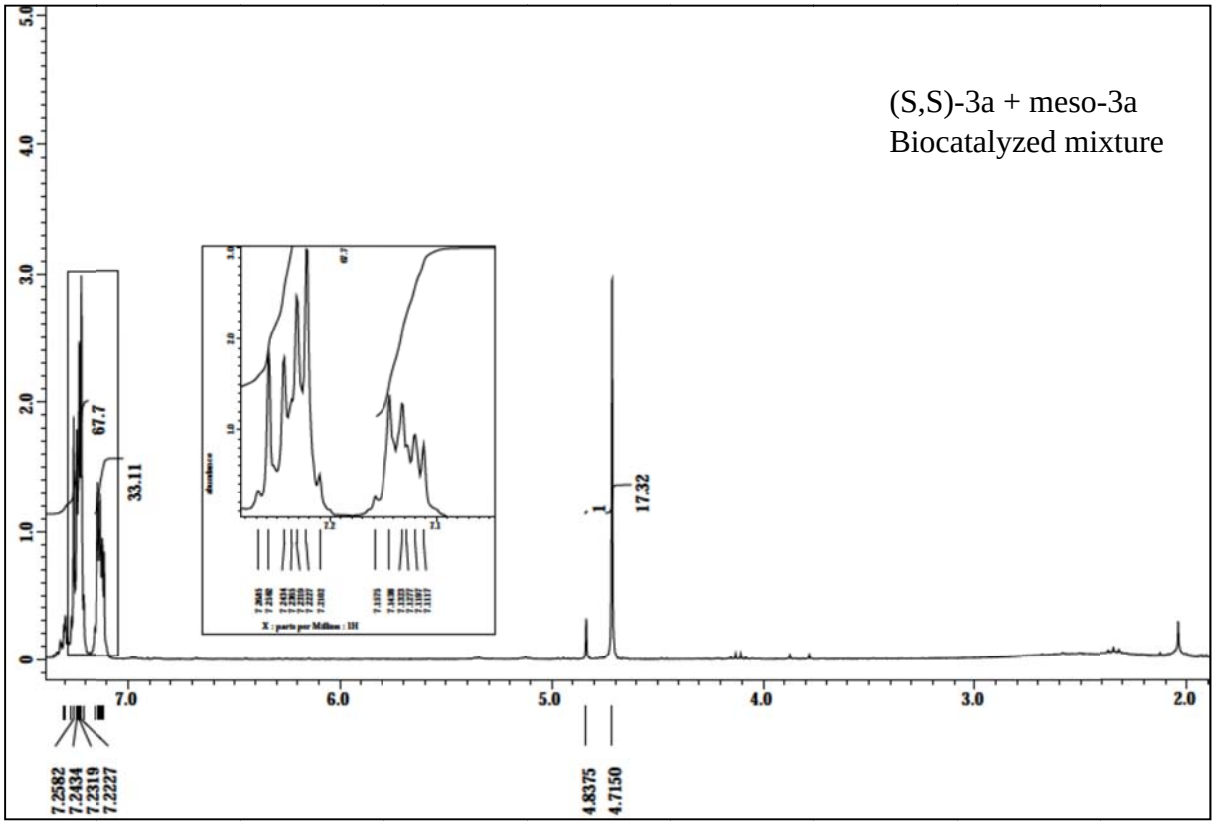

5c

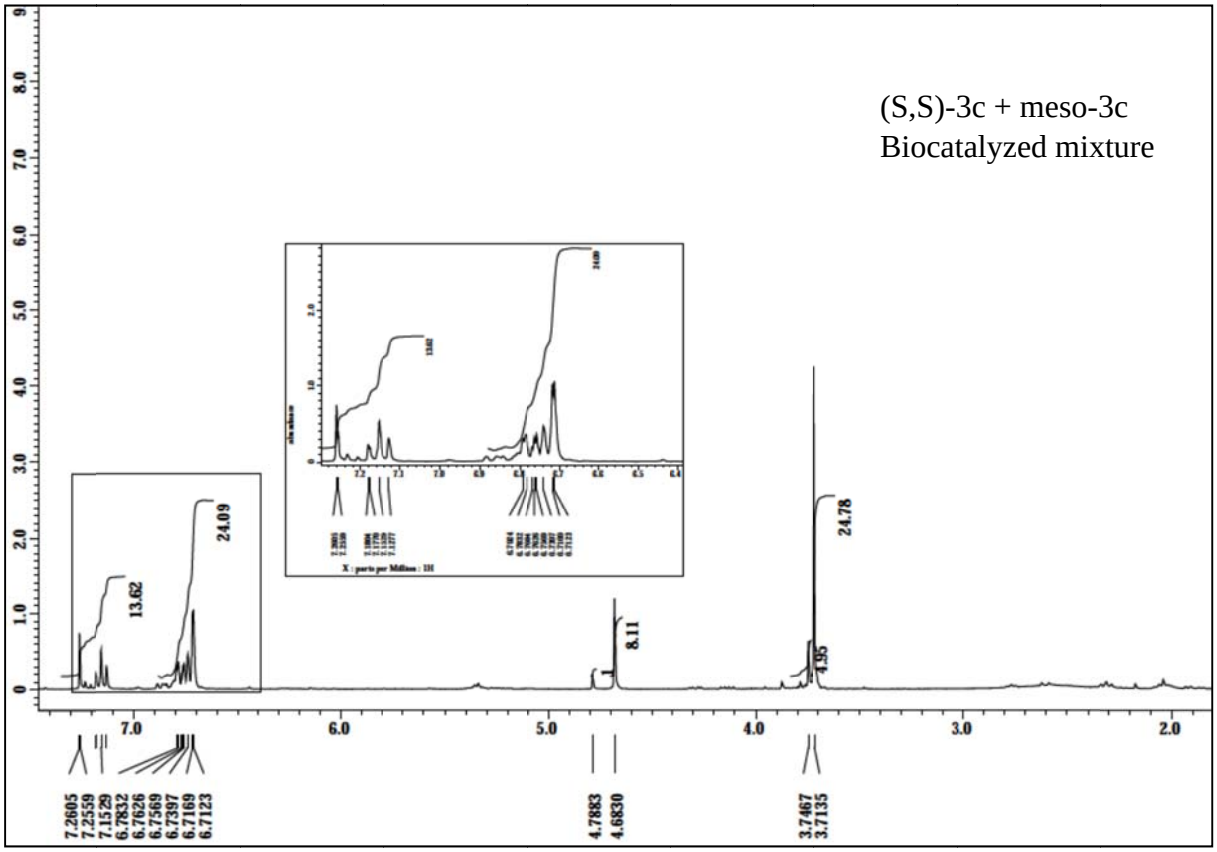

5d
5.71
2.2
1.0
1.0
0.77
7.11
7.4551
7.4391
7.3132
7.2981
7.2559
7.2308
6.7180
6.6905
5.8607
4.6818
3.8520
3.7890
3.7799

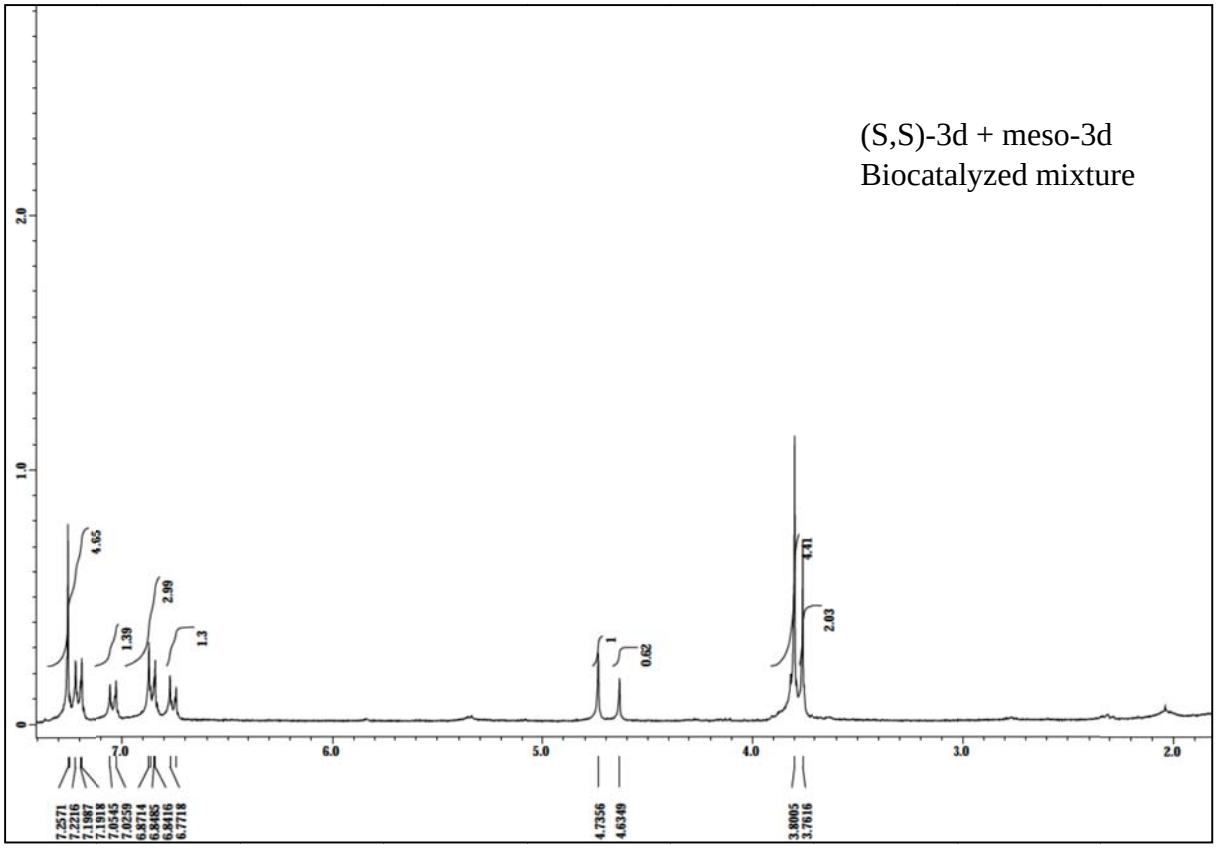

5d
197.2604
153.9316
149.0748
139.9157
128.1121
125.4997
122.7354
120.4160
124.3031
111.2146
110.1821
77.7524
76.9015
75.8689
56.0211
55.8968

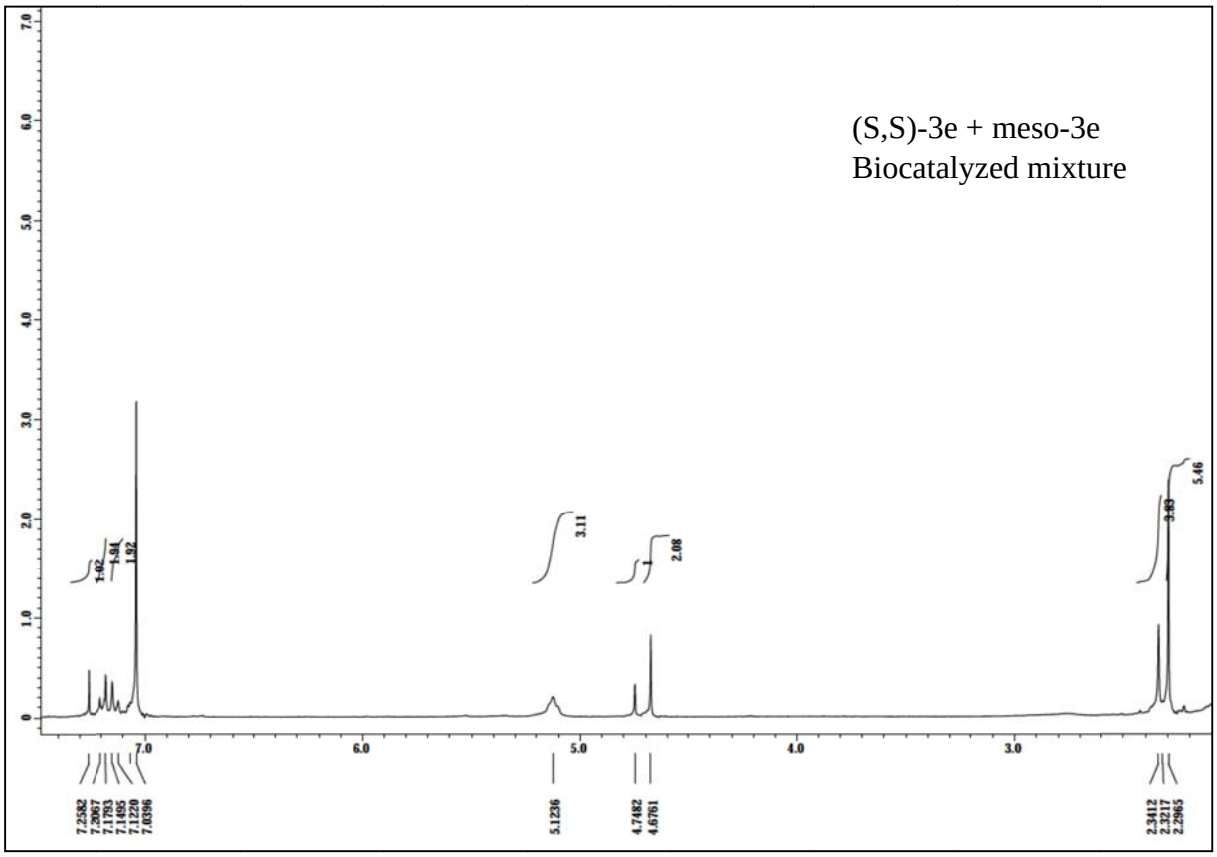

5e
1.91
5.14
1.94
1
0.82
6.34
7.8442
7.8202
7.8145
7.3406
7.3361
7.3063
7.3040
6.5738
6.5069
6.5429
5.8516
5.8321
4.8192
4.7997
3.0519
3.0153

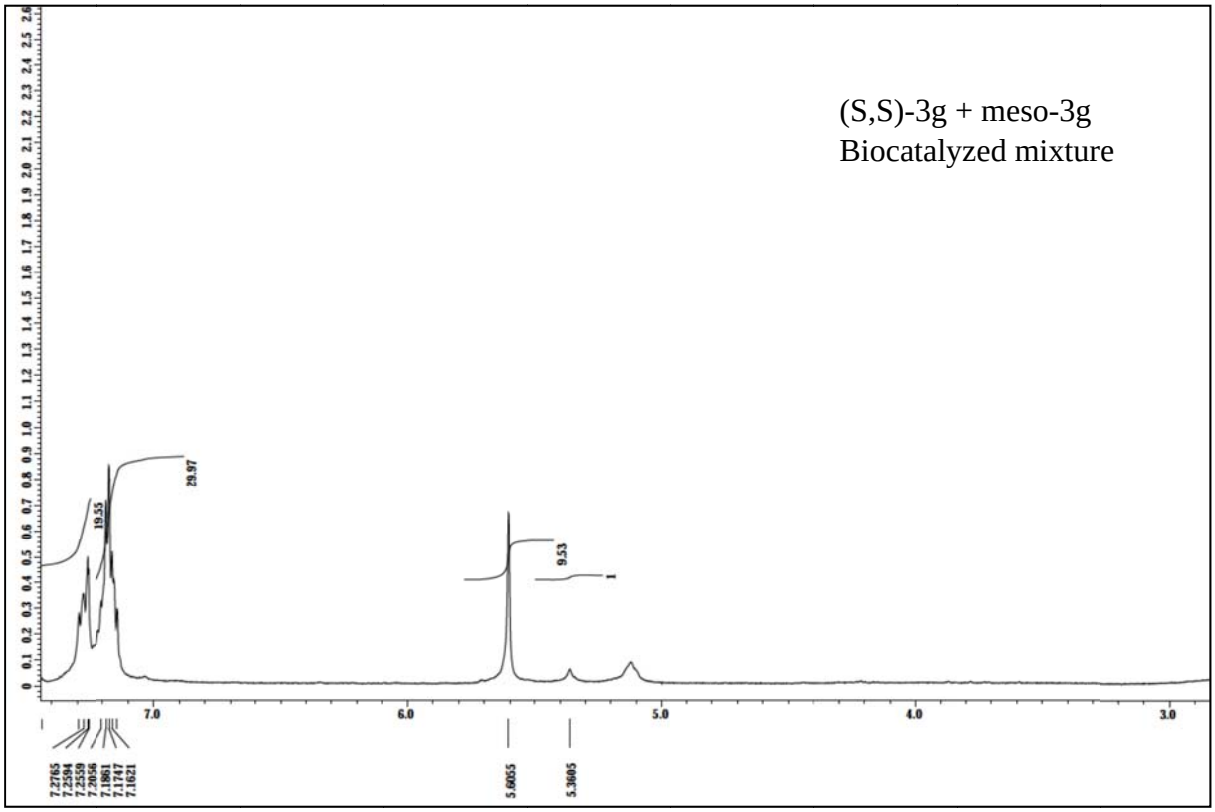

5e
77.5325
77.1118
76.6816

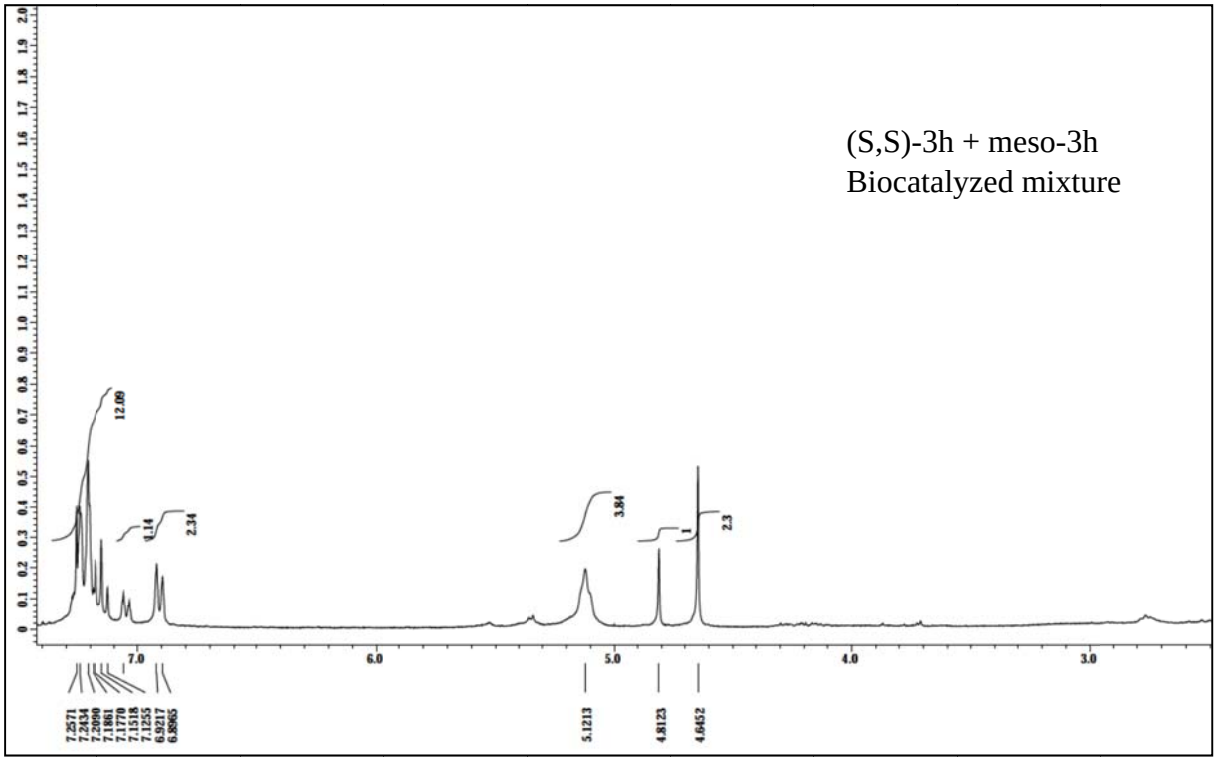

(S,S)-3a + meso-3a
Biocatalyzed mixture
67.7
33.11
17.32
7.2582
7.2434
7.2319
7.2227
4.8375
4.7150

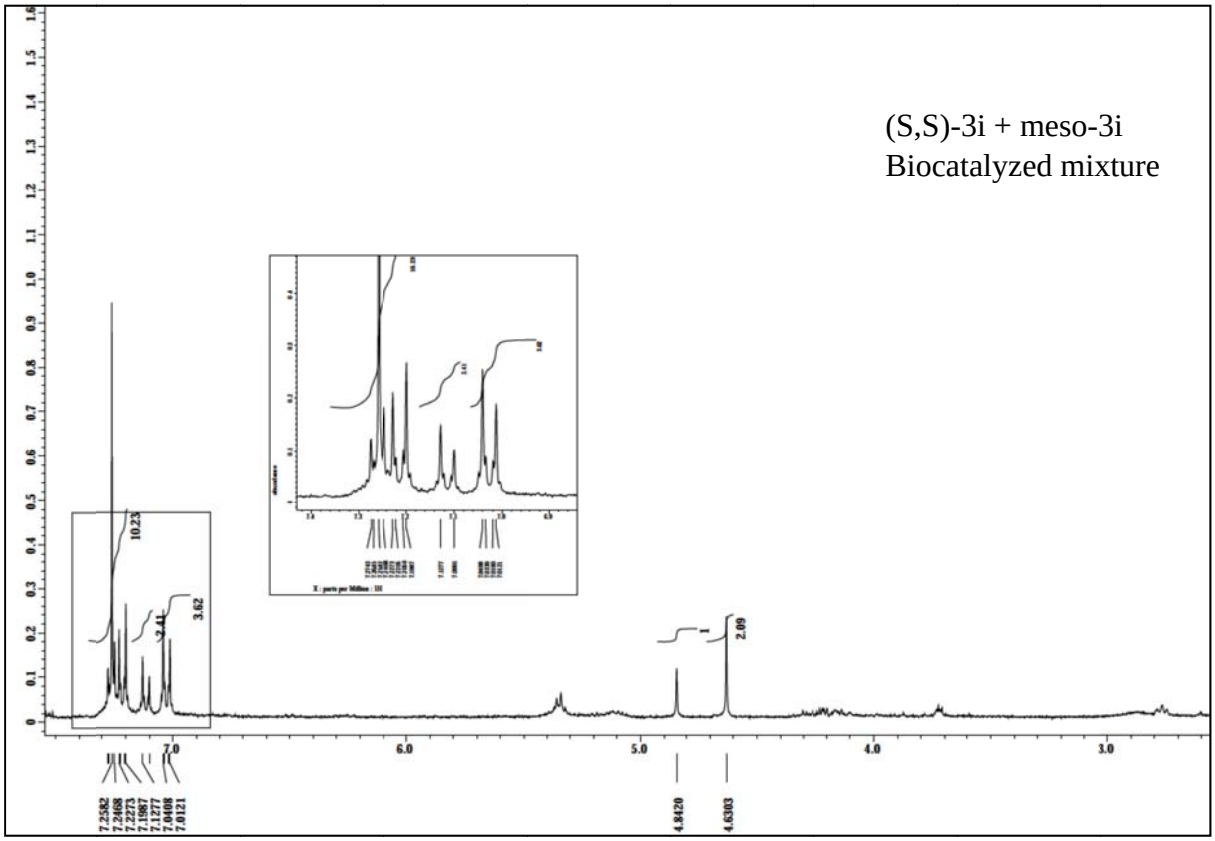

(S,S)-3c + meso-3c
Biocatalyzed mixture
24.09
13.62
8.11
24.78
4.95
7.2605
7.2559
7.1529
6.7832
6.7626
6.7569
6.7397
6.7169
6.7123
4.7883
4.6830
3.7467
3.7135


(S,S)-3d + meso-3d
Biocatalyzed mixture
4.65
1.39
2.99
1.3
0.62
4.41
2.03
7.2571
7.2216
7.1987
7.1918
7.0545
7.0259
6.8714
6.8485
6.8416
6.7718
4.7356
4.6349
3.8005
3.7616


(S,S)-3e + meso-3e
Biocatalyzed mixture
1.92
3.11
2.08
5.46
7.2582
7.2067
7.1793
7.1485
7.1220
7.0396
5.1236
4.7482
4.6761
2.3412
2.3217
2.2965


(S,S)-3g + meso-3g
Biocatalyzed mixture
29.97
19.55
9.53
1
7.0
6.0
5.0
4.0
3.0
7.2765
7.2594
7.2559
7.2056
7.1861
7.1747
7.1621
5.6055
5.3605


(S,S)-3h + meso-3h
Biocatalyzed mixture
12.09
2.34
3.84
1
2.3
7.0
6.0
5.0
4.0
3.0
7.2571
7.2434
7.2090
7.1861
7.1770
7.1518
7.1255
6.9217
6.8965
5.1213
4.8123
4.6452


(S,S)-3i + meso-3i
Biocatalyzed mixture
10.23
3.62
2.09
7.0
6.0
5.0
4.0
3.0