Electronic Supplementary Information

Aromatic hydroxylation of substituted benzenes by an unspecific peroxygenase from *Aspergillus brasiliensis*

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1 Material & Methods

1.1 Fed-batch process

P. pastoris X-33::pPICZA_*Abr*UPO was chosen for fed-batch fermentation in a 7.5 l bioreactor (Infors, Bottmingen, Switzerland). A total of 3 l basal salt medium (per 1 l: 0.47 g $CaSO_4$ x 2 H_2O, 8 ml H_3PO_4 (85 %), 9.1 g K_2SO_4, 4.2 g KOH, 3.66 g $MgSO_4$, 43.5 g glycerol (100 %), supplemented with 0.87 mg biotin, 4.35 ml *Pichia* trace metals (per 1 l of PTM_1 solution: 6 g $CuSO_4$ x 5 H2O, 0.08 g NaI, 3 g $MnSO_4$.x H_2O, 0.5 g $CoCl_2$, 20 g $ZnCl_2$,0.02 g H_3BO_3, 0.2 g Na_2Mo_4 x 2 H_2O, 65 g $FeSO_4$x 7 H_2O, 0.2 g biotin, 5 ml H_2SO_4)) was inoculated to an OD_{600} of 0.5 from a preculture. The preculture was grown overnight at 30 °C and 200 rpm in 200 ml BMGY containing 100 µg/ml Zeocin™, and the cells were washed with sterile 0.9 % sodium chloride solution before inoculation of the fermenter. During the entire fermentation, the pH was kept at pH 5.0 by titrating 10 % phosphoric acid and 25 % ammonium hydroxide. The stirring rate was set at 800 rpm and the oxygen was supplied with 3 l/min. Until the primary carbon source glycerol was completely consumed, the temperature was set to 30 °C. Afterwards 0.5 % (*v/v*) MeOH with 12 g/l PTM_1 solution was added as carbon source and as inducer for the gene expression, and the temperature was reduced to 25 °C. Additionally, 10 µM hemin was added to allow efficient loading of *Abr*UPO. 0.5 % (*v/v*) MeOH was automatically added when MeOH in the fermentation broth was consumed as indicated by a spike in dissolved oxygen. After 9 days, the cells were harvested (11,325 x *g*, 4 °C, 20 min). The volumetric activity towards ABTS, OD_{600} and protein concentration were determined at different time points throughout the whole fermentation.

1.2 LC/MS elution profile for detection of 23

Conversion of **23** was analysed by liquid chromatography coupled to mass spectrometry (LC/MS) on a Prominence/LCMS2020 device (Shimadzu) like described before.[1] A Chromolith® Performance RP-18e column (100×4.6 mm, Merck) was used. Solvent A was ddH2O with 0.1% formic acid, while solvent B was methanol. 1 µl of each sample was injected and separated with a flow rate of 1 ml min^{-1} at 30 °C. The substances were ionized by electron spray ionization (ESI) and atmospheric pressure chemical ionization (APCI) in dual ionization mode. Mass fragments were detected in positive and negative scan mode in a range of 100-500 m/z.

23	Gradient from 10 % B to 75 % B for 10 min, hold 75 % B for 5 min, equilibration at 10 % B for 5 min

Table S1: Temperature profiles of the GC/MS programmes

Compound	Temperature profile
1-3, 5-6	Maintained at 80 °C for 5 min, ramped to 250 °C at 20 °C min^{-1}, held for 2 min
4, 7, 8-10, 14	Maintained at 100 °C for 1 min, ramped to 150 °C at 5 °C min^{-1}, held for 1 min, ramped to 250 °C at 20 °C min^{-1}, held at 250 °C for 1 min
11-13	Maintained at 70 °C for 2 min, ramped to 100 °C at 5 °C min^{-1}, ramped to 250 °C at 15 °C min^{-1}, held at 250 °C for 2 min

15	Maintained at 130 °C for 2 min, ramped to 300 °C at 20 °C min^{-1}, held at 300 °C for 8 min
16	Maintained at 120 °C for 1.5 min, ramped to 180 °C at 5 °C min^{-1}, held for 1 min, ramped to 200 °C at 10 °C min^{-1}, held for 1 min, ramped to 300 °C at 20 °C min^{-1}, held at 300 °C for 1 min
17-18	Maintained at 40 °C, ramped to 300 °C at 5 °C min^{-1}, held at 300 °C for 2 min
19-20	Maintained at 90 °C for 5 min, ramped to 320 °C at 15 °C min^{-1}, held at 320 °C for 5 min
21	Maintained at 80 °C for 3 min, ramped to 300 °C at 15 °C min^{-1}, held at 300 °C for 2 min
22	Maintained at 200 °C for 4 min, ramped to 300 °C at 10 °C min^{-1} held for 4 min, ramped to 320 °C at 5 °C min^{-1}, held at 320 °C for 2 min
24	Maintained at 120°C for 1 min, ramped to 210°C at 10°C min-1, ramped to 300°C at 40°C min-1, held at 300°C for 1 min.
25-27	Maintained at 150°C for 1 min, ramped to 260°C at 10 °C min-1, ramped to 300°C at 40 °C min-1, held at 300°C for 3 min.

2 Results

2.1 Recombinant expression and characterization of *Abr*UPO

Table S2: Purification of recombinant *Abr*UPO

	Spec. activity [U/mg]	Total Units[c] [U]	Yield[d] [%][a]	Purification factor [x-fold]	Protein concentration [mg/ml][e]	Volume [l]
Supernatant [a]	18.17 ± 1.5	93,970	(100)	1.0	1.29	4.6
Ultrafiltration [b]	20.30 ± 0.9	39,467	100	1.1	8.84	0.22
HIC	22.7 ± 3.0	191	11	1.2	7.02	0.0012
IEX	31.7 ± 1.4	170	9.7	1.7	5.36	0.001

[a] Cell-free supernatant after fed-batch fermentation
[b] Ultrafiltration retentate of supernatant using tangential flow filtration (TFF). Concentrated sample was collected in three steps (eluates) with different enzyme activities and protein concentrations.
[c] Enzyme activity was determined with ABTS
[d] Yield based on the enzyme applied to HIC
[e] Protein concentration was determined with Bradford assay

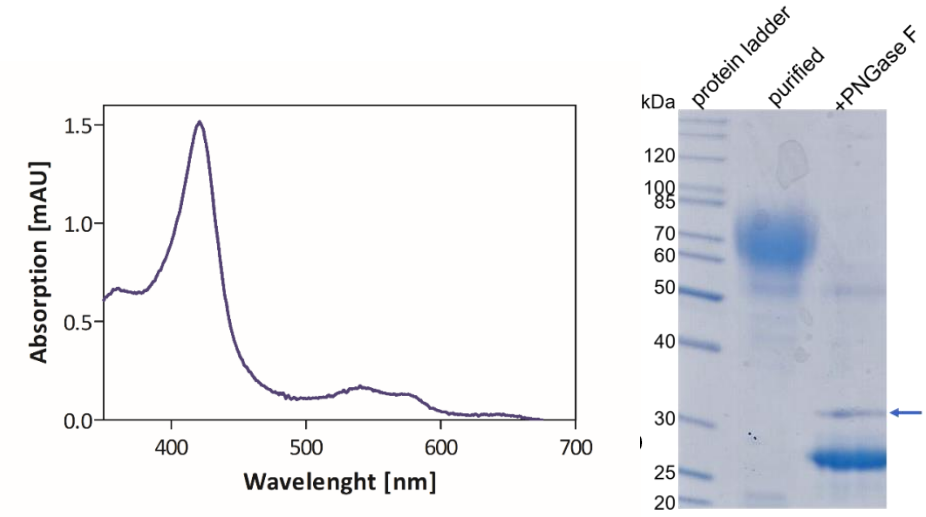


Figure S1: left - UV-VIS spectrum of 1 µM purified *Abr*UPO, right - SDS-PAGE analysis of purified and PNGase F treated *Abr*UPO using a 12.5 % resolving gel. Blue Arrow indicates PNGase F (36 kDa).

Table S3: N-glycosylation degree of different UPOs

Name	Expression host	N-glycosylation [%]	Reference
*Abr*UPO	*P. pastoris*	~55	This work
r*Aae*UPO	*P. pastoris*	30	[2]
r*Ani*UPO	*P. pastoris*	50	[3]
r*Cab*UPO1	*P. pastoris*	~14	[3]
r*Cci*UPO	*Aspergillus oryzae*	14-44	[4]
*Cgl*UPO	*Chaetomium globosum*	19	[5]
*Cra*UPO	*Coprinus radians*	37	[6]
*Hsp*UPO	*P. pastoris*	~50	[7]
*Mro*UPO	*Marasmius rotula*	16	[8]

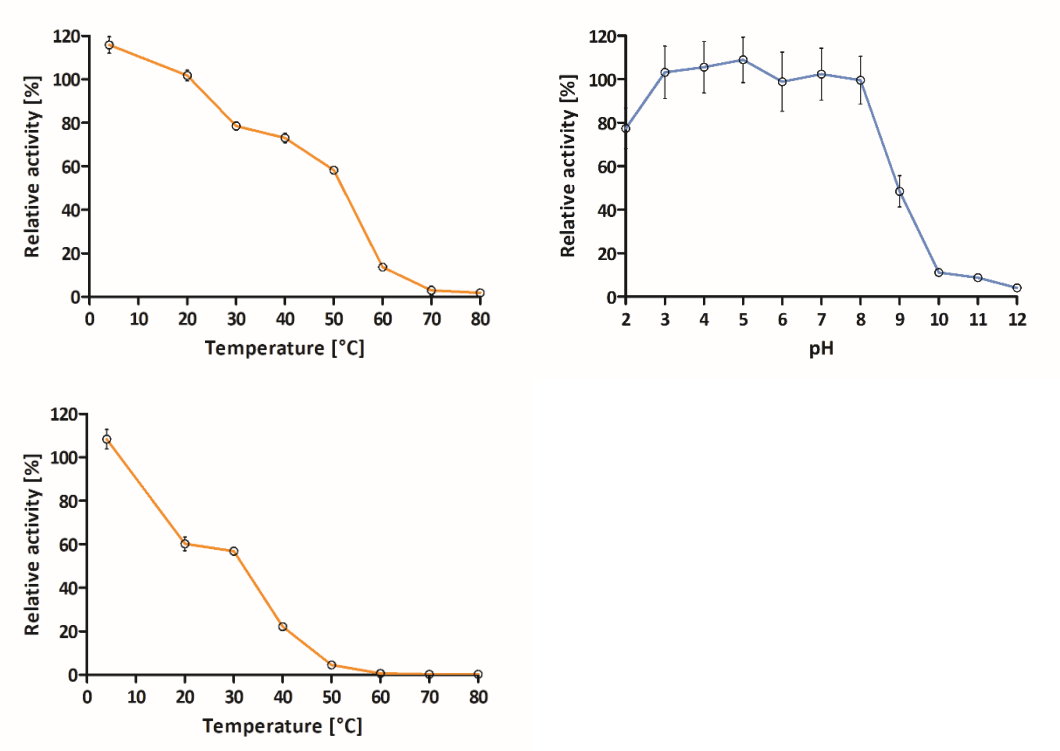
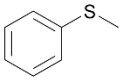
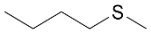
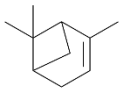
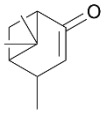
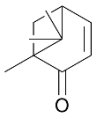
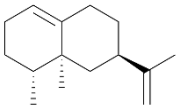
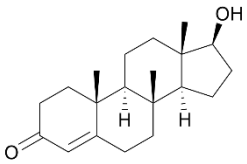


Figure S2: Determination of T_{50} (left upper), thermal stability after 240 min (left lower) and pH stability (right). Residual activity of *Abr*UPO dissolved in 50 mM sodium phosphate buffer pH 7.0, 2 mM $MgCl_2$ after 10 min (upper) or 240 min (lower) incubation at temperatures between 4 - 80 °C. For pH stability, residual activity of *Abr*UPO after 60 min incubation in 100 mM Britton-Robinson buffer in a pH range between 2 to12 was measured.

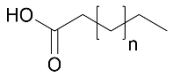
2.2 Catalytic activity of *Abr*UPO

Table S4: Further substrates of *Abr*UPO: Reactions were conducted in 50 mM sodium phosphate pH 7.0 with 2 mM $MgCl_2$, 1 mM substrate, 4 mM hydrogen peroxide, 1.3 µM *Abr*UPO, 8 mM ascorbic acid at 25 °C and 600 rpm for 180 min.

	Compound	Structure	Substrate depletion [%]
17	Thioanisole		>99
18	Butyl methyl sulfide		>99
19	α-Pinene		84
20	(1*S*)-(-)-Verbenone		14
21	(1*R*)-(+)-Camphor		n. d.
22	Valencene		n. d.
23	Testosterone		10

n. d. = not detected

Table S5: Product distribution for fatty acids: Reactions were conducted in 50 mM sodium phosphate pH 7.0 with 2 mM $MgCl_2$, 200 µM substrate, 500 µM hydrogen peroxide, 0.8 µM *Abr*UPO at 25 °C and 600 rpm for 180 min.



24 Capric acid (C10) n = 6
25 Undecanoic acid (C11) n = 7
26 Lauric acid (C12) n = 8
27 Tridecanoic acid (C13) n = 9

Product distribution [%]

	ω	ω-1	ω-2	ω-3	ω-4	ω-5	ω-6	ω-7	ω-8	γ	other
24	-	32	3	13	1	17	19	3	-	-	12
25	-	46	7	16	16	4	3	3	5	-	-
26	-	34	6	16	11	19	3	3	2	6	-
27	-	19	6	16	11	11	7	19	-	8	3

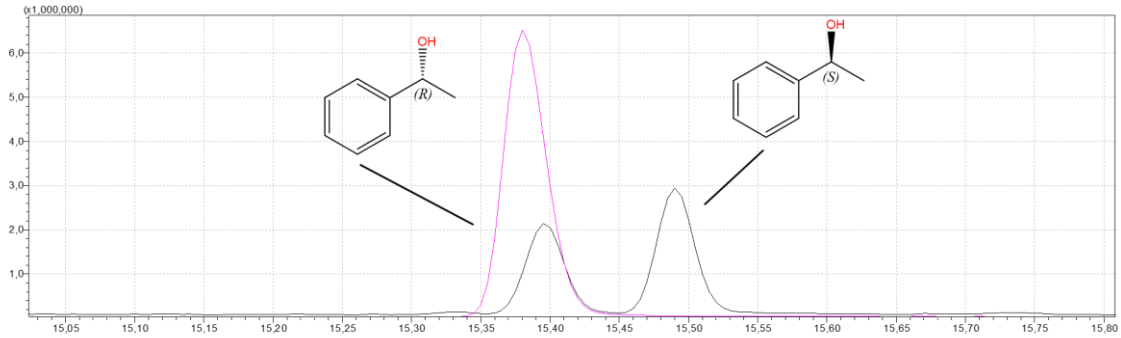


Figure S3: Determination of the enantiomeric excess of 1a. Black: after reaction, pink: authentic standard (*R*)-1-phenylethanol **1a**. Reactions were conducted in 50 mM sodium phosphate buffer pH 7.0 with 2 mM $MgCl_2$, 1 mM substrate, 4 mM hydrogen peroxide, 1.3 µM *Abr*UPO, 8 mM ascorbic acid at 25 °C and 600 rpm for 180 min.

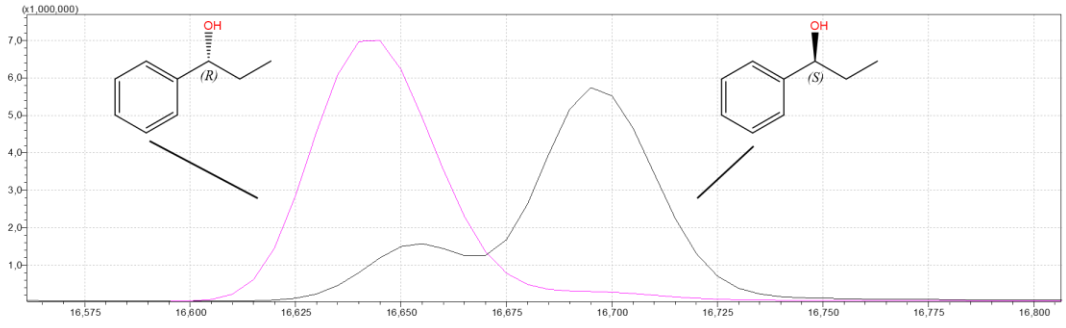


Figure S4: Determination of the enantiomeric excess of 2a. Black: after reaction, pink: authentic standard (*R*)-1-phenyl-1-propanol **2a**. Reactions were conducted in 50 mM sodium phosphate buffer pH 7.0 with 2 mM $MgCl_2$, 1 mM substrate, 4 mM hydrogen peroxide, 1.3 µM *Abr*UPO, 8 mM ascorbic acid at 25 °C and 600 rpm for 180 min.

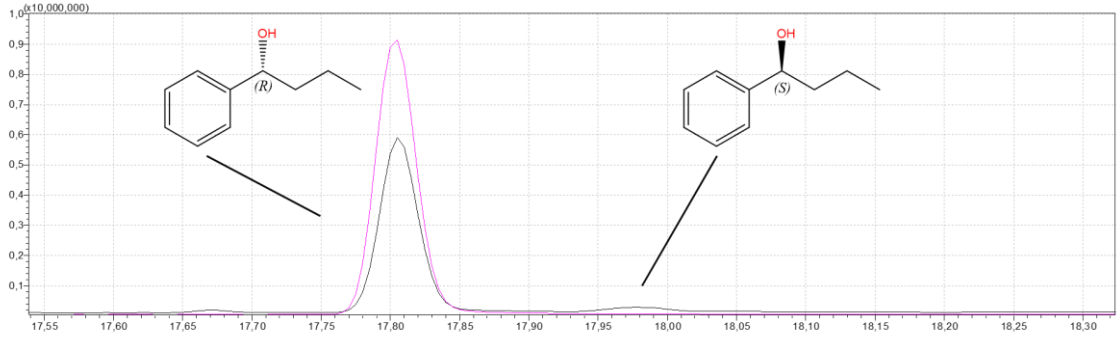


Figure S5: Determination of the enantiomeric excess of 3a. Black: after reaction, pink: authentic standard (*R*)-1-phenyl-1-butanol **3a**. Reactions were conducted in 50 mM sodium phosphate buffer pH 7.0 with 2 mM $MgCl_2$, 1 mM substrate, 4 mM hydrogen peroxide, 1.3 µM *Abr*UPO, 8 mM ascorbic acid at 25 °C and 600 rpm for 180 min.

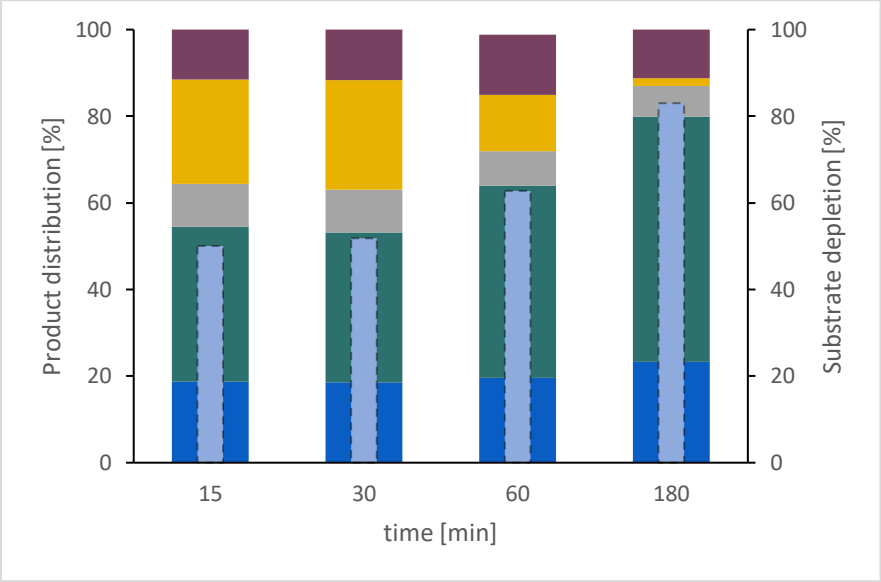


Figure S6: Time course *ρ*-cymene 5 conversion. Blue: *ρ*-cymene-8-ol **5a**; teal: thymohydroquinone **5b**; grey: thymoquinone **5c**; yellow; aromatic mono-hydroxylated **5d**; purple: other unidentified products; light blue: substrate depletion. Reactions were conducted in 50 mM sodium phosphate buffer pH 7.0 with 2 mM $MgCl_2$, 1 mM substrate, 4 mM hydrogen peroxide, 1.3 µM *Abr*UPO, 8 mM ascorbic acid at 25 °C and 600 rpm. Samples were taken after 15, 30, 60 and 180 minutes.

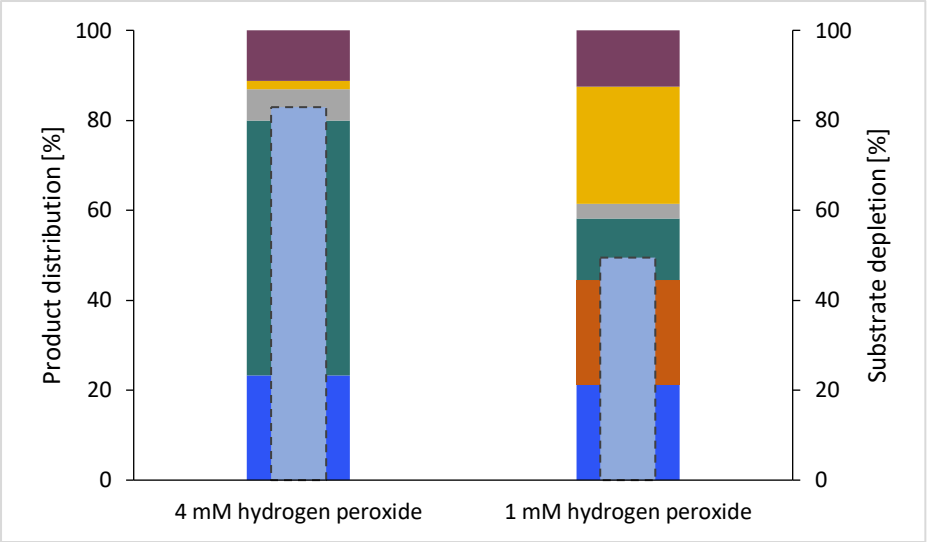


Figure S7: Influence of peroxide concentration on *ρ*-cymene 5 conversion. Blue: *ρ*-cymene-8-ol **5a**; teal: thymohydroquinone **5b**; grey: thymoquinone **5c**; yellow; aromatic mono-hydroxylated **5d**; purple: other unidentified products; orange: *ρ*-isopropyl benzaldehyde; light blue: substrate depletion. Reactions were conducted in 50 mM sodium phosphate buffer pH 7.0 with 2 mM $MgCl_2$, 1 mM substrate, either 1 mM or 4 mM hydrogen peroxide, 1.3 µM *Abr*UPO, 8 mM ascorbic acid at 25 °C and 600 rpm for 180 min.

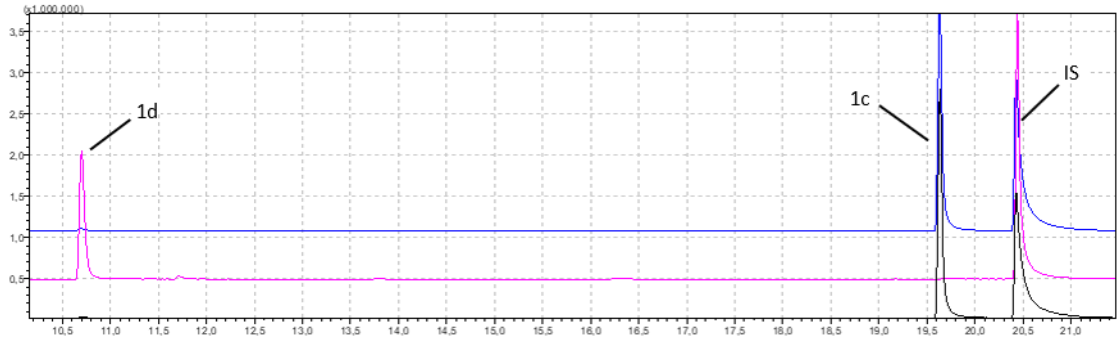


Figure S8: GC/MS chromatogram of conversions of 2-ethylbenzene-1,4-diol 1c. Black: with 8 mM ascorbic acid, pink: without ascorbic acid, blue: without enzyme, with 8 mM ascorbic acid. 10.7 min: 2-ethyl-1,4-benzoquinone **1d**, 19.6 min: 2-ethylbenzene-1,4-diol **1c**, 20.5 min: IS. Reactions were conducted in 50 mM sodium phosphate buffer pH 7.0 with 2 mM $MgCl_2$, 1 mM substrate, 4 mM hydrogen peroxide, 1.3 µM *Abr*UPO, at 25 °C and 600 rpm for 180 min.

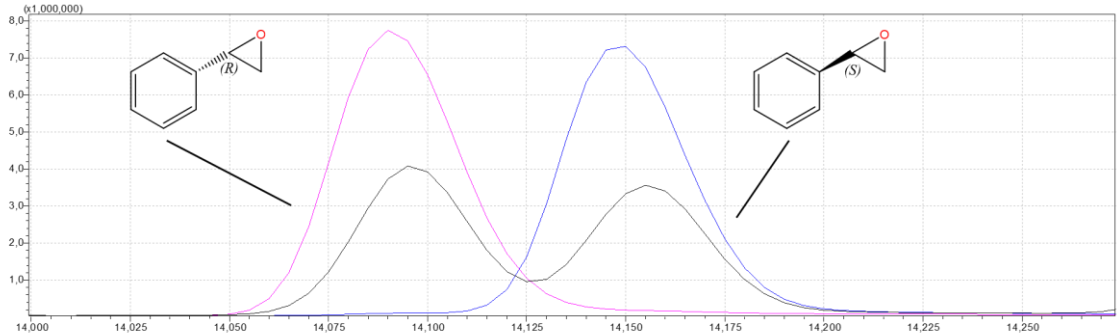
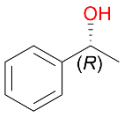
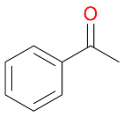
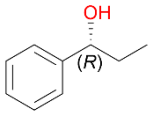
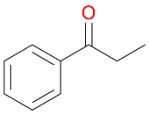
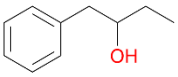
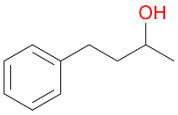
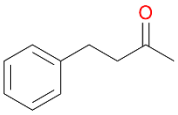
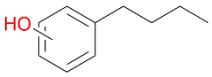
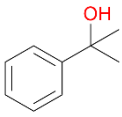
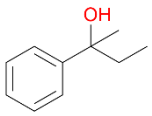
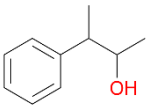
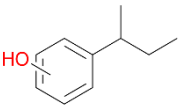
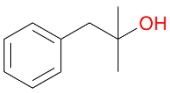
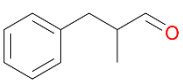
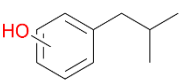
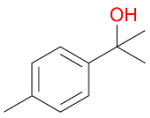
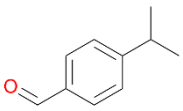


Figure S9: Determination of the enantiomeric excess of 11a. Black: after reaction, pink: authentic standard (*R*)-styrene oxide **11a**, blue: authentic standard (*S*)-styrene oxide. Reactions were conducted in 50 mM sodium phosphate buffer pH 7.0 with 2 mM $MgCl_2$, 1 mM substrate, 4 mM hydrogen peroxide, 1.3 µM *Abr*UPO, 8 mM ascorbic acid at 25 °C and 600 rpm for 180 min

Table S6: Product distribution of PaDa-I-catalysed reactions. Reactions were conducted in 50 mM sodium phosphate pH 7.0 with 2 mM $MgCl_2$, 1 mM substrate, 4 mM hydrogen peroxide, 1.3 µM PaDa-I, 8 mM ascorbic acid at 25 °C and 600 rpm for 180 min.

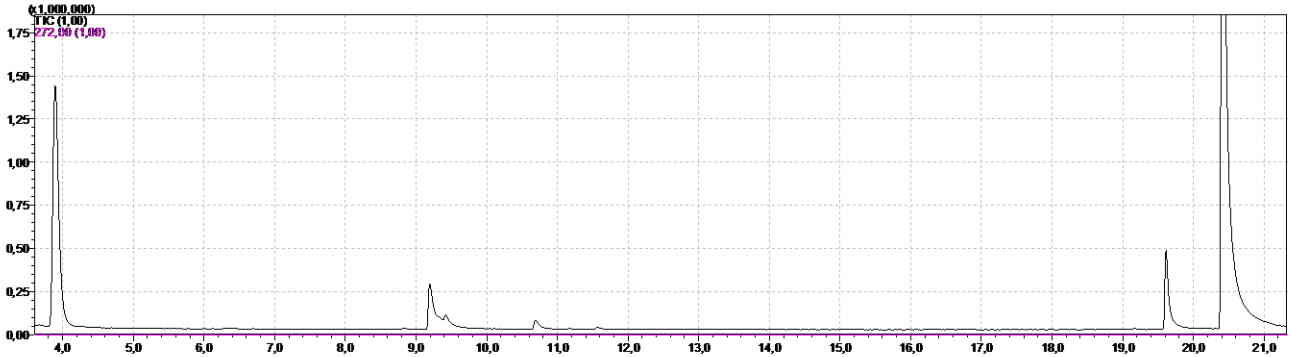
Compound	Substrate depletion [%]	Product distribution [%]			
1	96	 **1a**[a], 56, *ee* = >99 %	 **1b**[a], 44		
2	72	 **2a**[a], 95, *ee* = >99 %	 **2b**[b], 2		
3	42	 **3f**[b], **7**	 **3g**[b], 84	 **3h**[a], 6	 **3i**[b], 3**
4	84	 **4a**[a], 98			
5	24	 **5a**[a], 41	 **5b**[b], 38	 **5f**[b], 16**	
6	30	 **6e**[b], 87	 **6f**[b], 5	 **6g**[b], 6	
7	81	 **7a**[a], 13	 **7e**[b], 67		

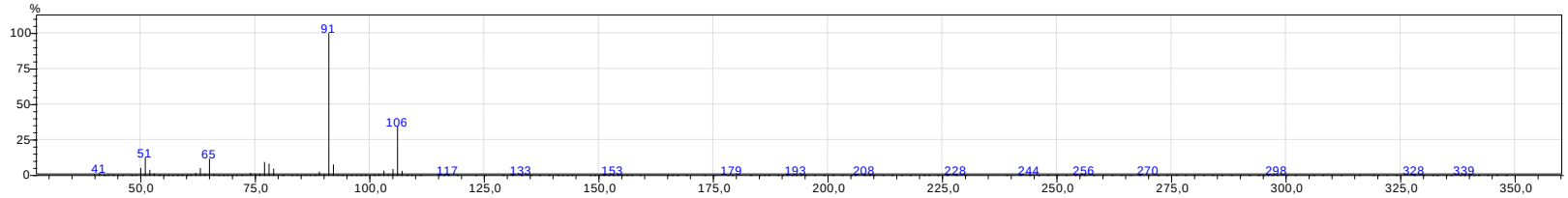
[a] verified by MS and reference substance

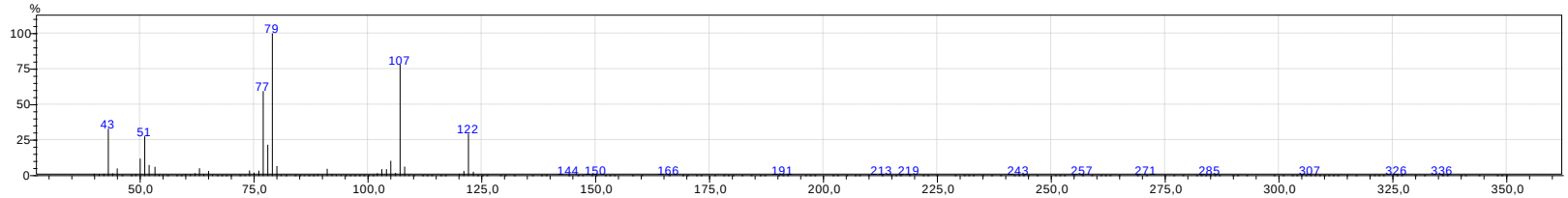
[b] verified by MS

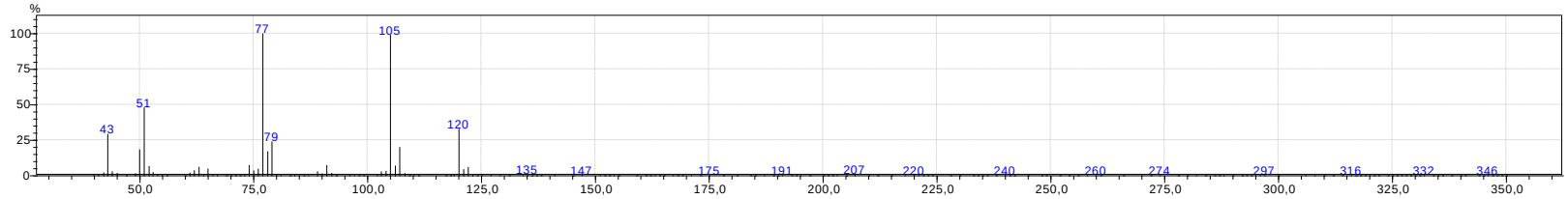
** Different retention time compared to *Abr*UPO. Hydroxylated either at *meta* or *para* position.

3 GCMS chromatograms









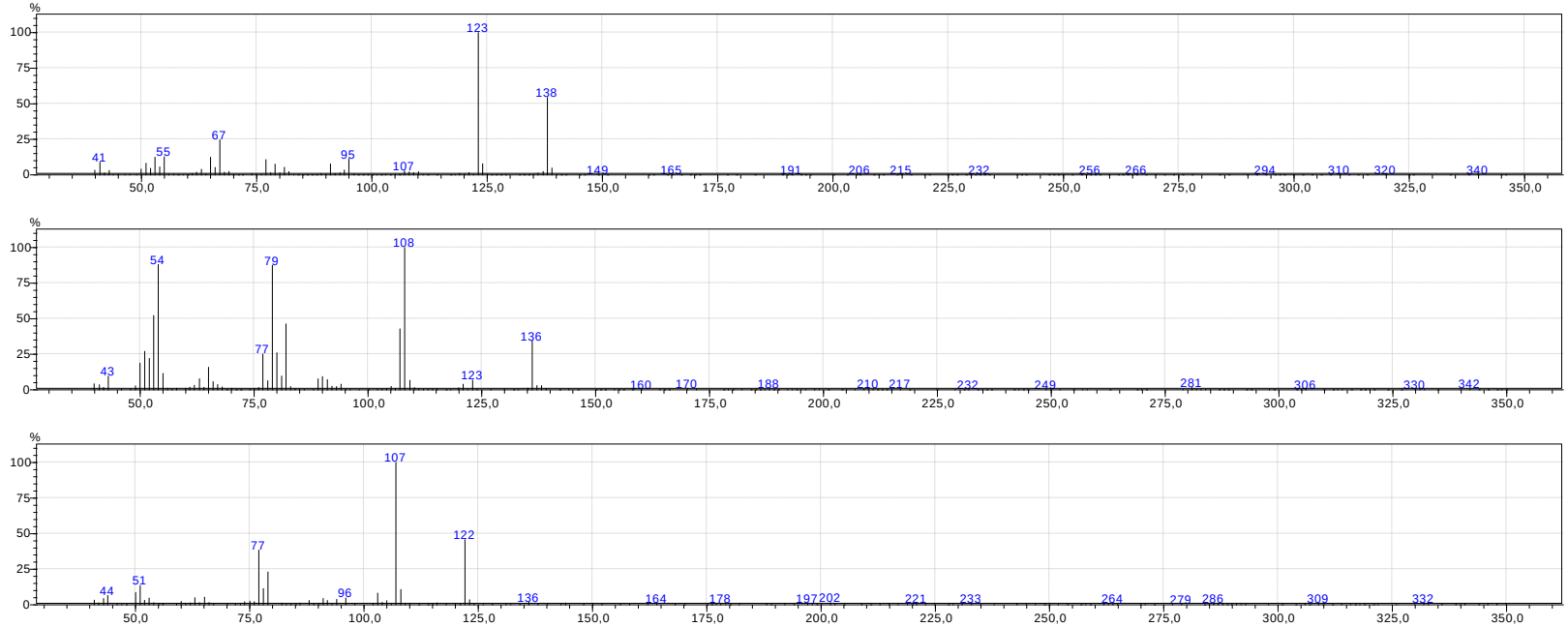
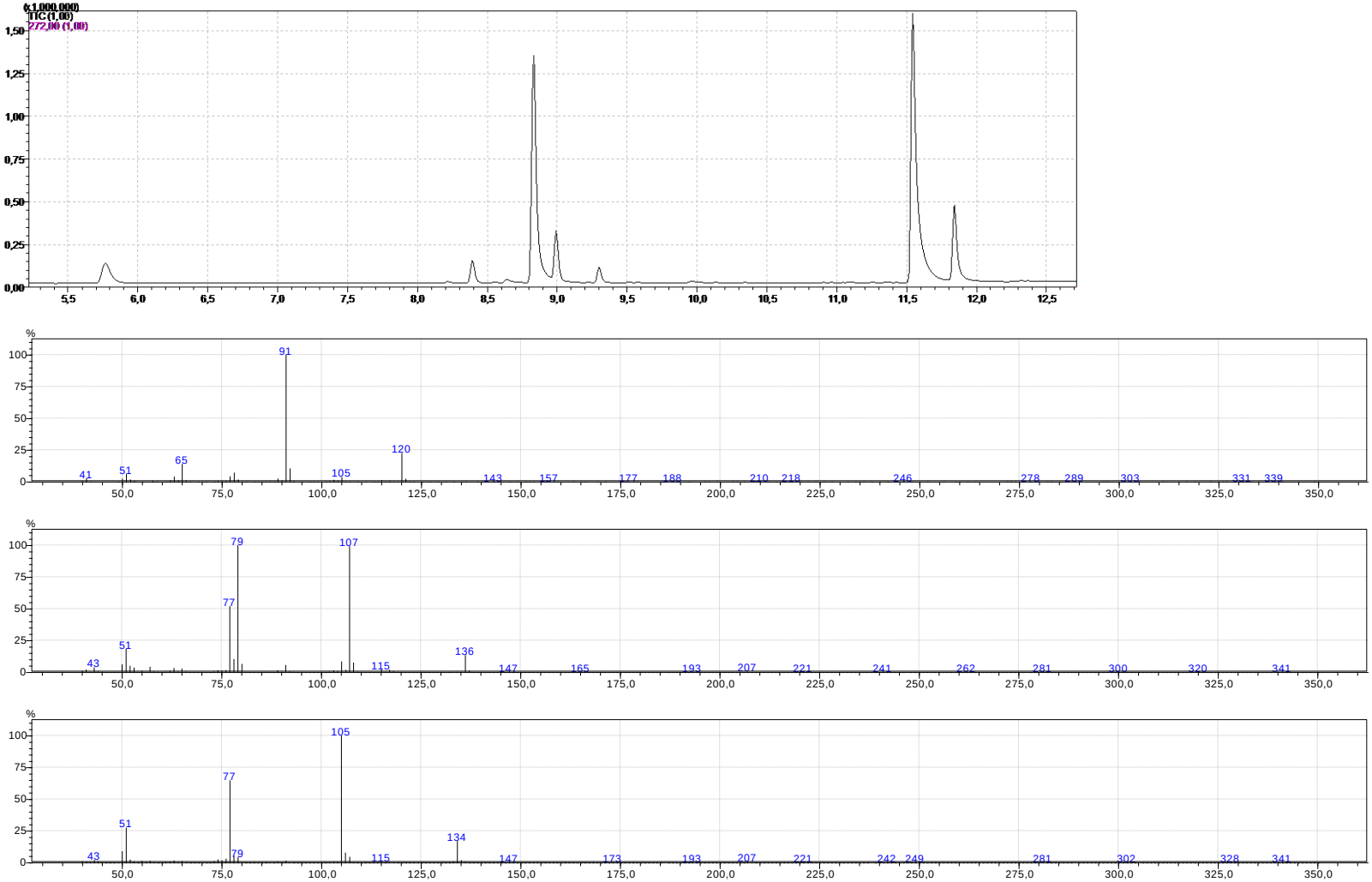


Figure S10: GC and MS chromatograms of conversion of **1**. Reactions were conducted in 50 mM sodium phosphate buffer pH 7.0 with 2 mM $MgCl_2$, 1 mM substrate, 4 mM hydrogen peroxide, 1.3 µM *Abr*UPO, 8 mM ascorbate at 25 °C and 600 rpm for 180 min. MS chromatograms of **1** (3.896 min), **1a** (9.195 min), **1b** (9.420 min), **1c** (19.550 min), **1d** (10.691 min) and **1e** (11.532 min) in sorted order.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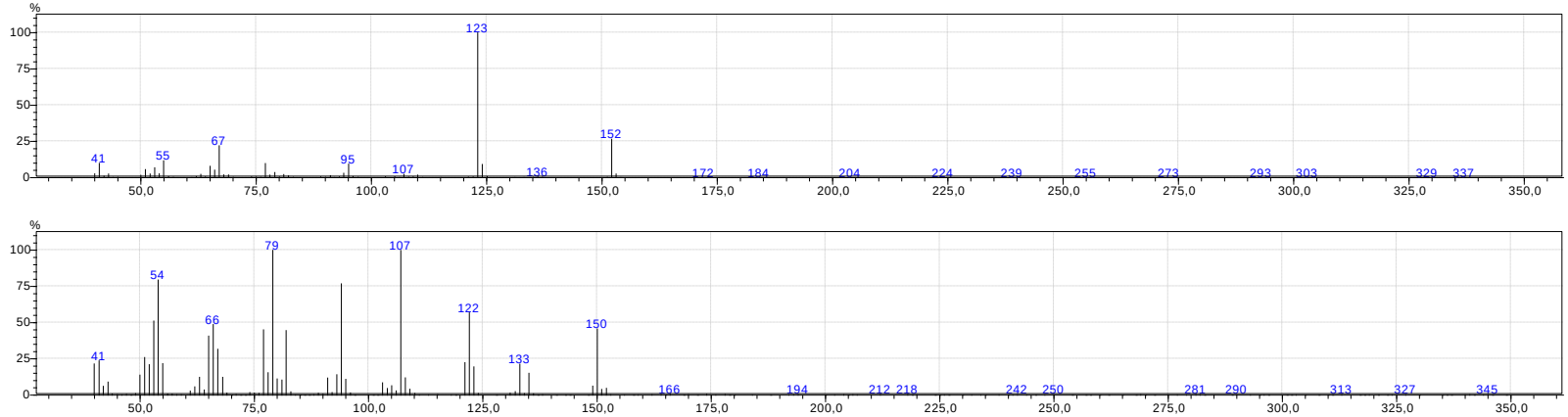
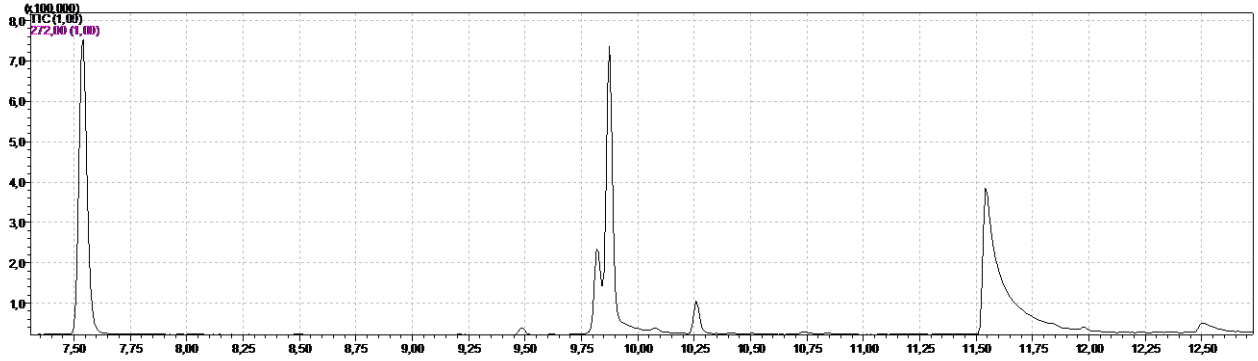


Figure S11: GC and MS chromatograms of conversion of **2**. Reactions were conducted in 50 mM sodium phosphate buffer pH 7.0 with 2 mM $MgCl_2$, 1 mM substrate, 4 mM hydrogen peroxide, 1.3 µM *Abr*UPO, 8 mM ascorbate at 25 °C and 600 rpm for 180 min. MS chromatograms of **2** (5.783 min), **2a** (8.831 min), **2b** (8.992 min), **2c** (11.842 min) and **2d** (9.300 min), in sorted order.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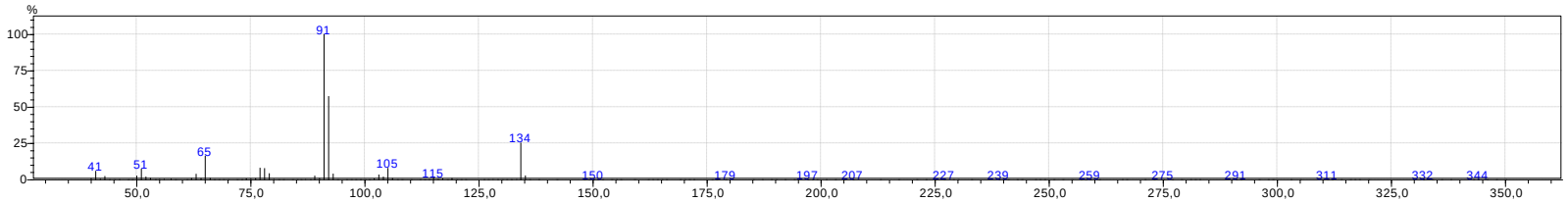
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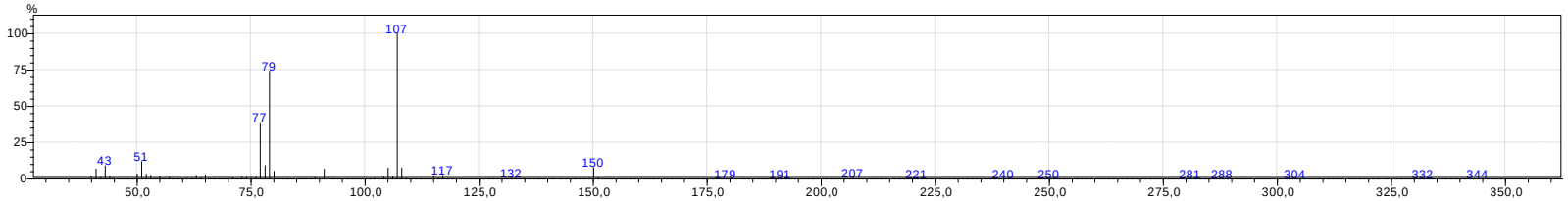
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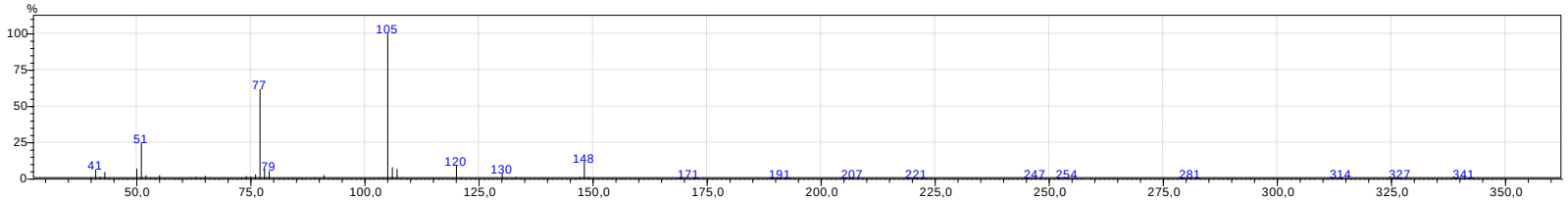
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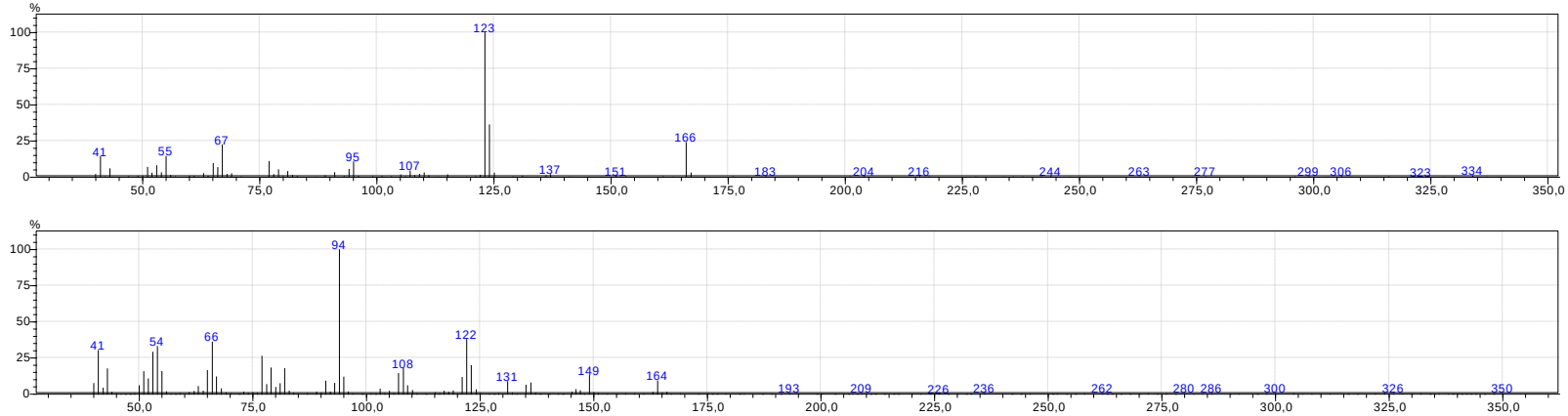
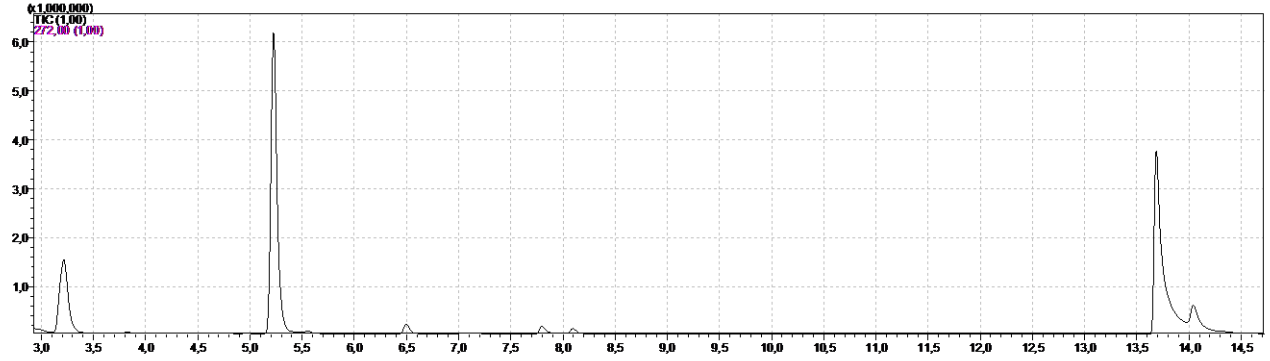


Figure S12: GC and MS chromatograms of conversion of **3**. Reactions were conducted in 50 mM sodium phosphate buffer pH 7.0 with 2 mM $MgCl_2$, 1 mM substrate, 4 mM hydrogen peroxide, 1.3 µM *Abr*UPO, 8 mM ascorbate at 25 °C and 600 rpm for 180 min. MS chromatograms of **3** (7.549 min), **3a** (9.820 min)**, 3b** (9.873 min), **3c** (12.506 min) and **3d** (10.260 min) in sorted order.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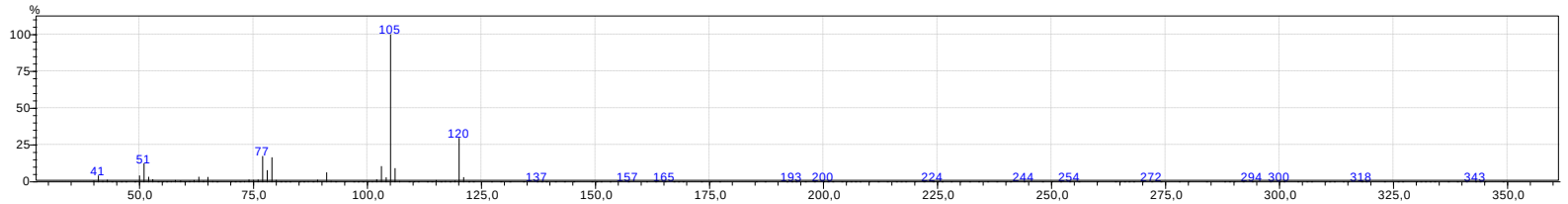
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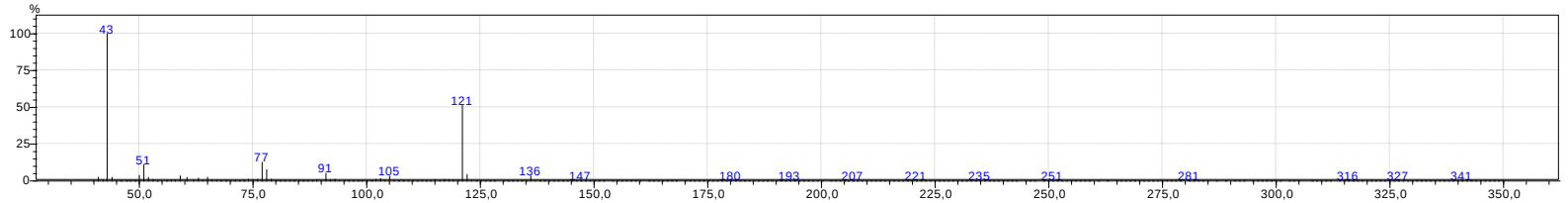
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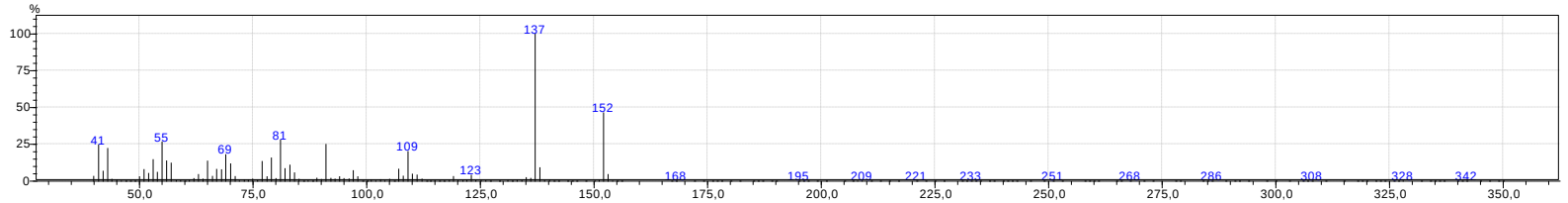
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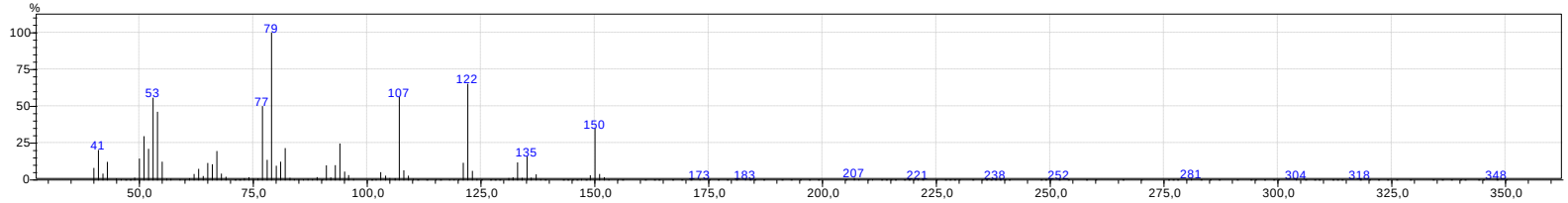
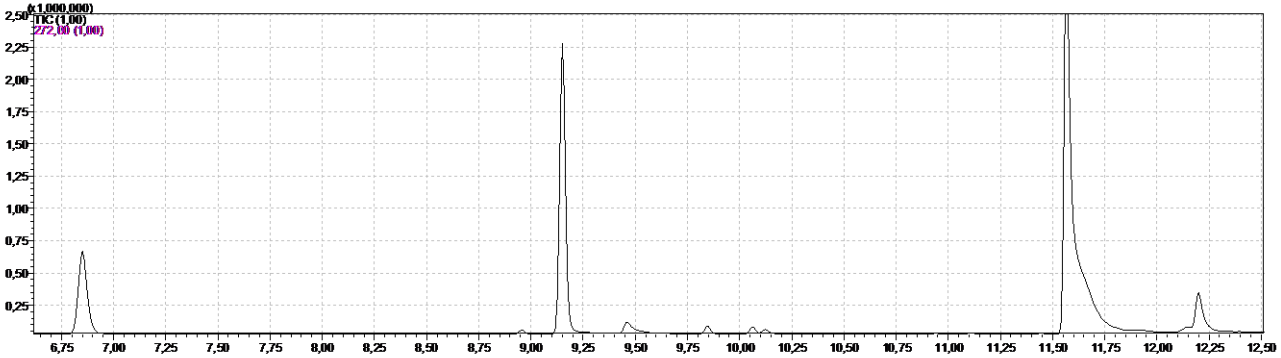


Figure S13: GC and MS chromatograms of conversion of **4**. Reactions were conducted in 50 mM sodium phosphate buffer pH 7.0 with 2 mM $MgCl_2$, 1 mM substrate, 4 mM hydrogen peroxide, 1.3 µM *Abr*UPO, 8 mM ascorbate at 25 °C and 600 rpm for 180 min. MS chromatograms of **4** (3.207 min), **4a** (5.244 min)**, 4b** (13.527 min) and **4c** (6.542min) in sorted order.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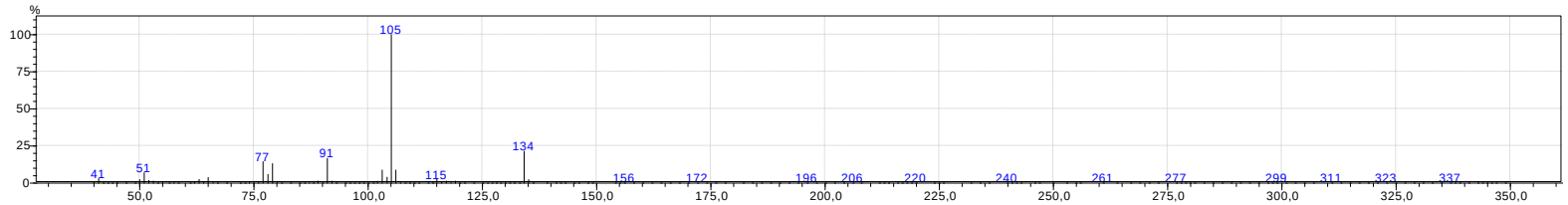
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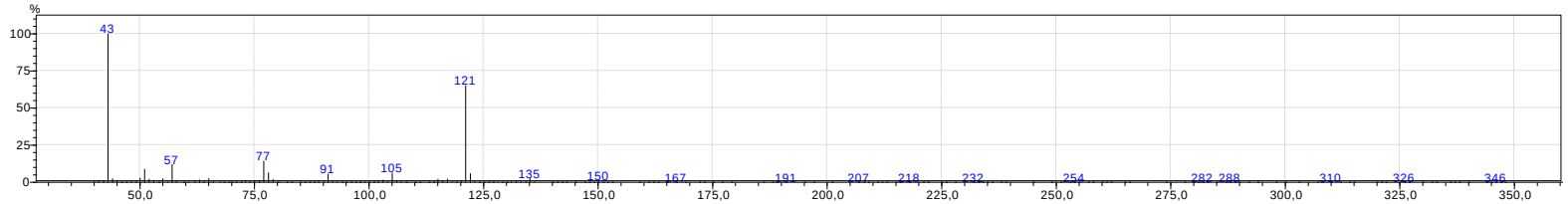
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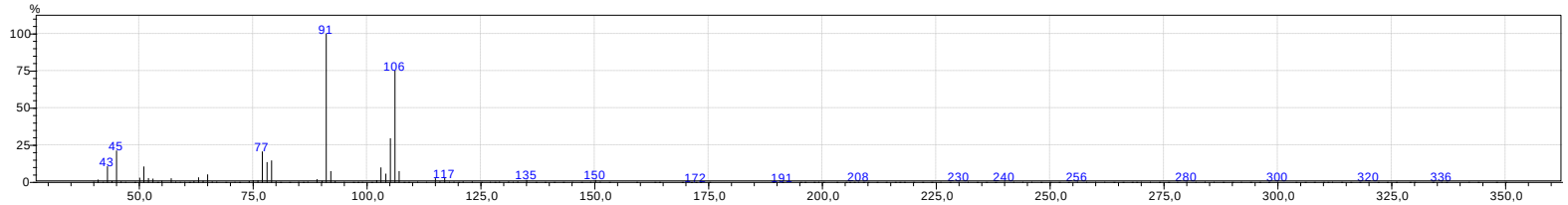
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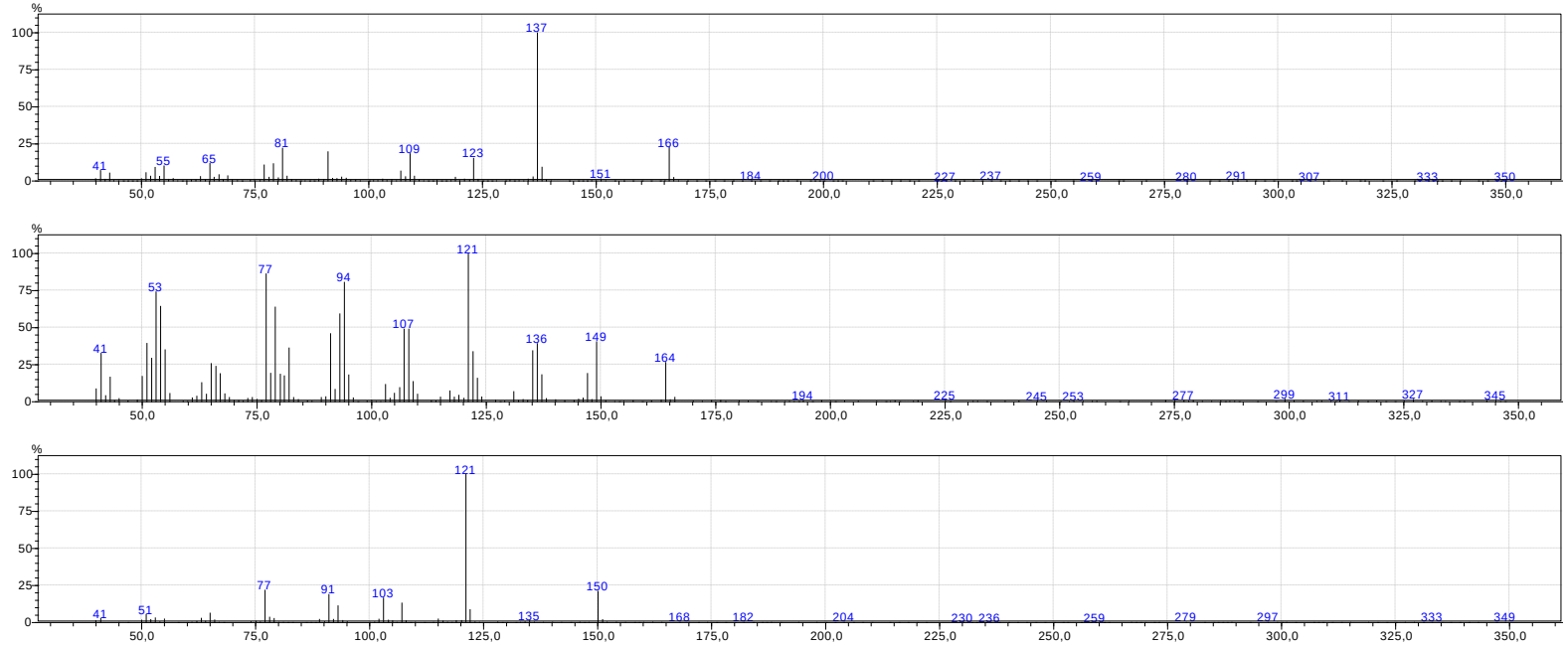
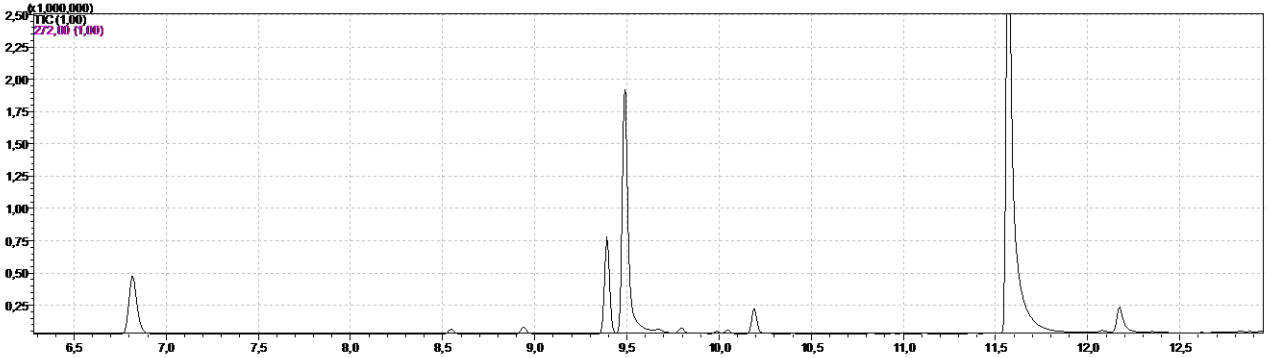


Figure S14: GC and MS chromatograms of conversion of **5**. Reactions were conducted in 50 mM sodium phosphate buffer pH 7.0 with 2 mM $MgCl_2$, 1 mM substrate, 4 mM hydrogen peroxide, 1.3 µM *Abr*UPO, 8 mM ascorbate at 25 °C and 600 rpm for 180 min. MS chromatograms of **5** (6.838 min), **5a** (9.151 min)**, 5b** (9.461 min), **5c** (12.200 min), **5d** (9.846 min) and **5e** (10.062 min) in sorted order.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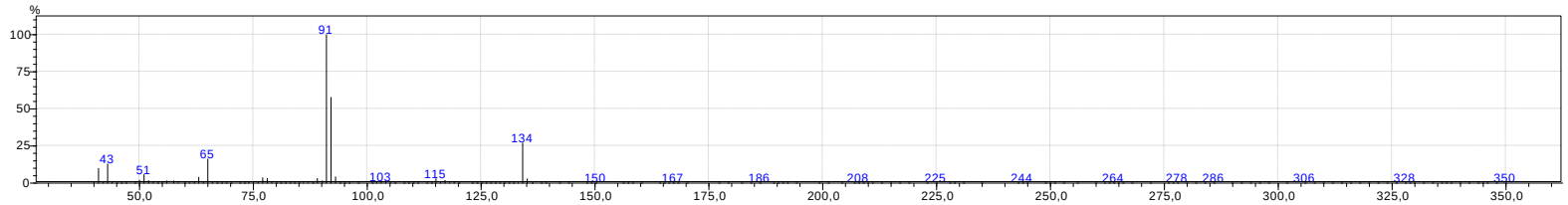
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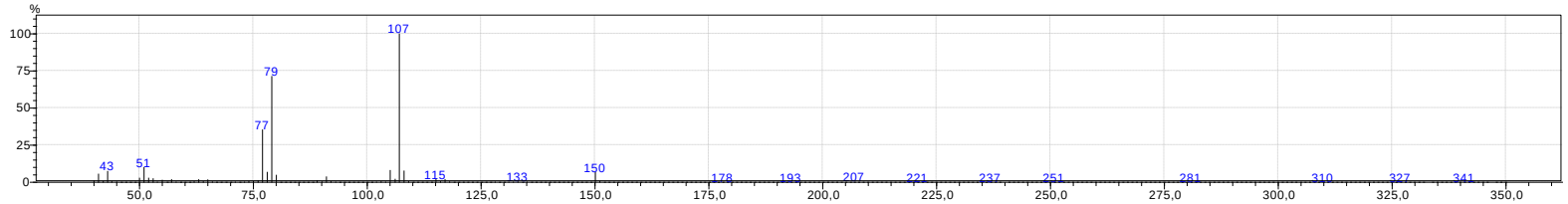
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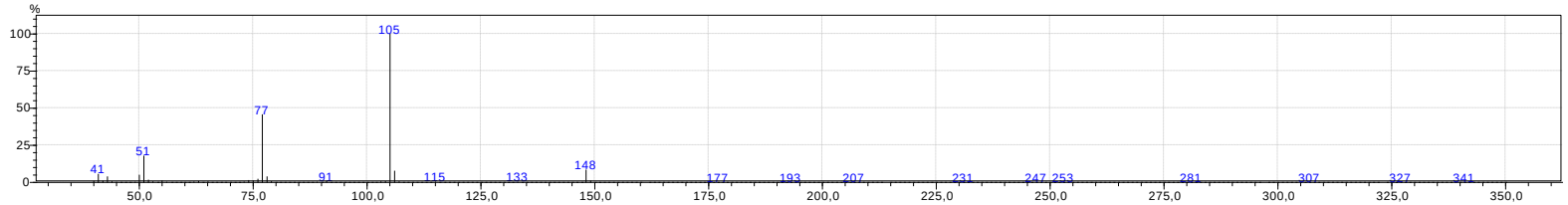
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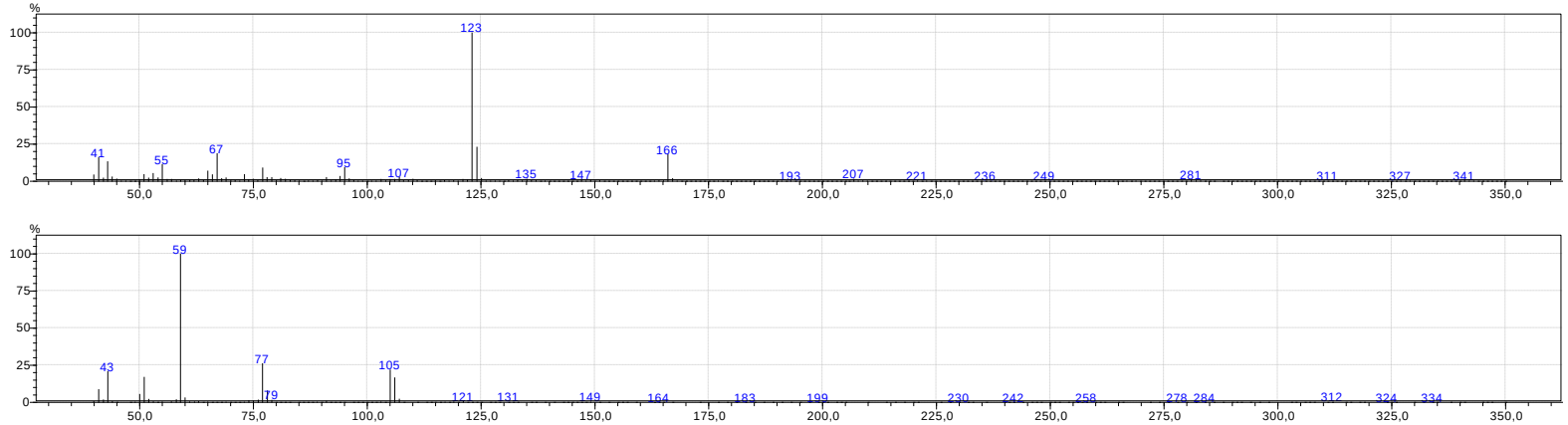
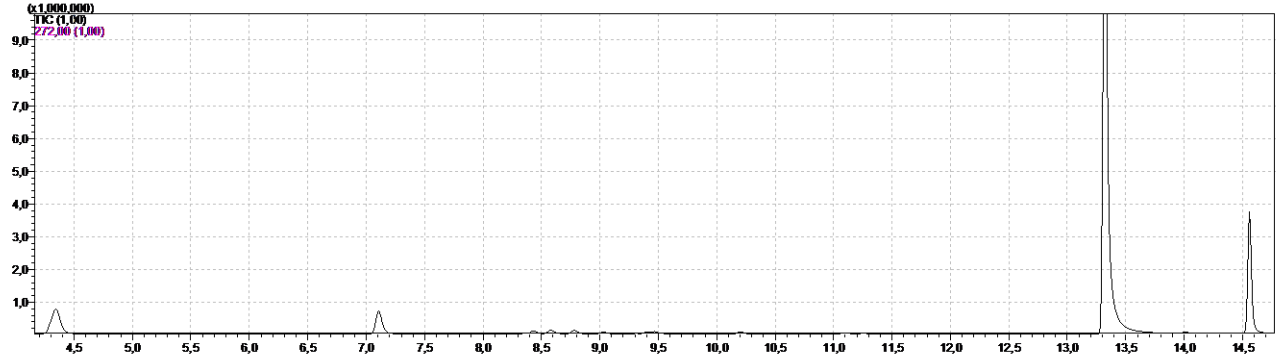


Figure S15: GC and MS chromatograms of conversion of **6**. Reactions were conducted in 50 mM sodium phosphate buffer pH 7.0 with 2 mM $MgCl_2$, 1 mM substrate, 4 mM hydrogen peroxide, 1.3 µM *Abr*UPO, 8 mM ascorbate at 25 °C and 600 rpm for 180 min. MS chromatograms of **6** (6.828 min), **6a** (9.488 min)**, 6b** (9.392 min), **6c** (12.133 min) and **6d** (10.190 min) in sorted order.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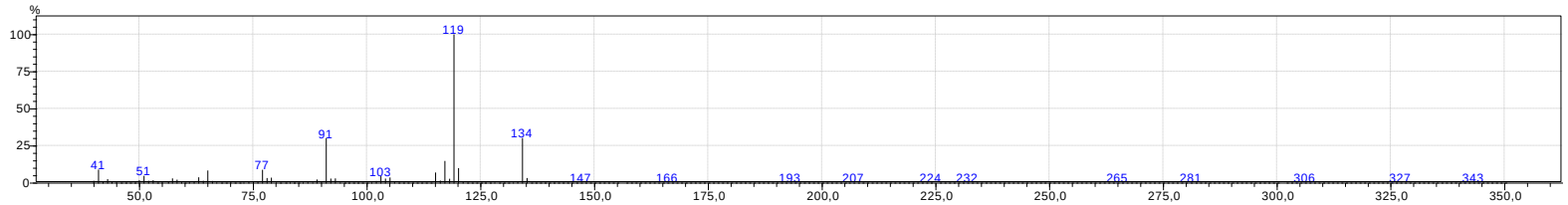
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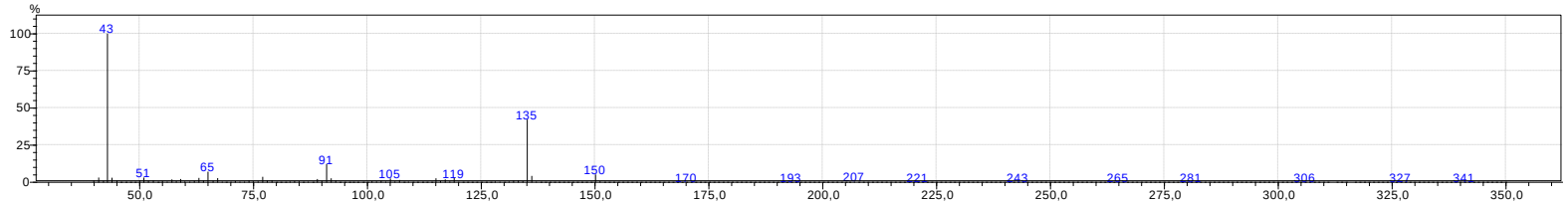
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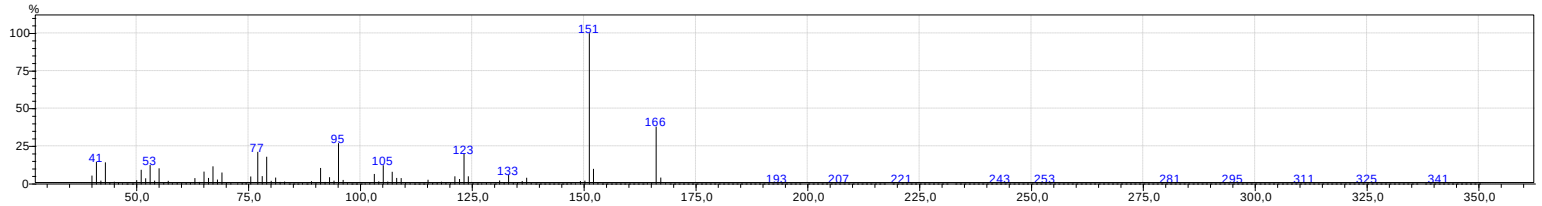
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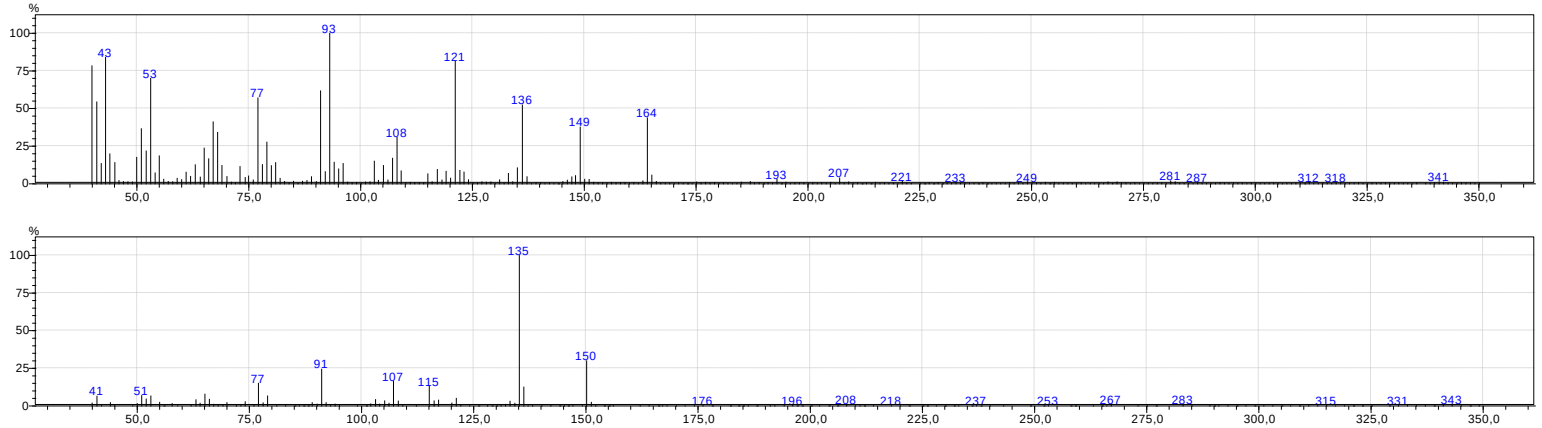


Figure S16: GC and MS chromatograms of conversion of **7**. Reactions were conducted in 50 mM sodium phosphate buffer pH 7.0 with 2 mM $MgCl_2$, 1 mM substrate, 4 mM hydrogen peroxide, 1.3 µM *Abr*UPO, 8 mM ascorbate at 25 °C and 600 rpm for 180 min. MS chromatograms of **7** (4.306 min), **7a** (7.079 min)**, 7b** (14.549 min), **7c** (8.398min) and **7d** (9.440 min) in sorted order.

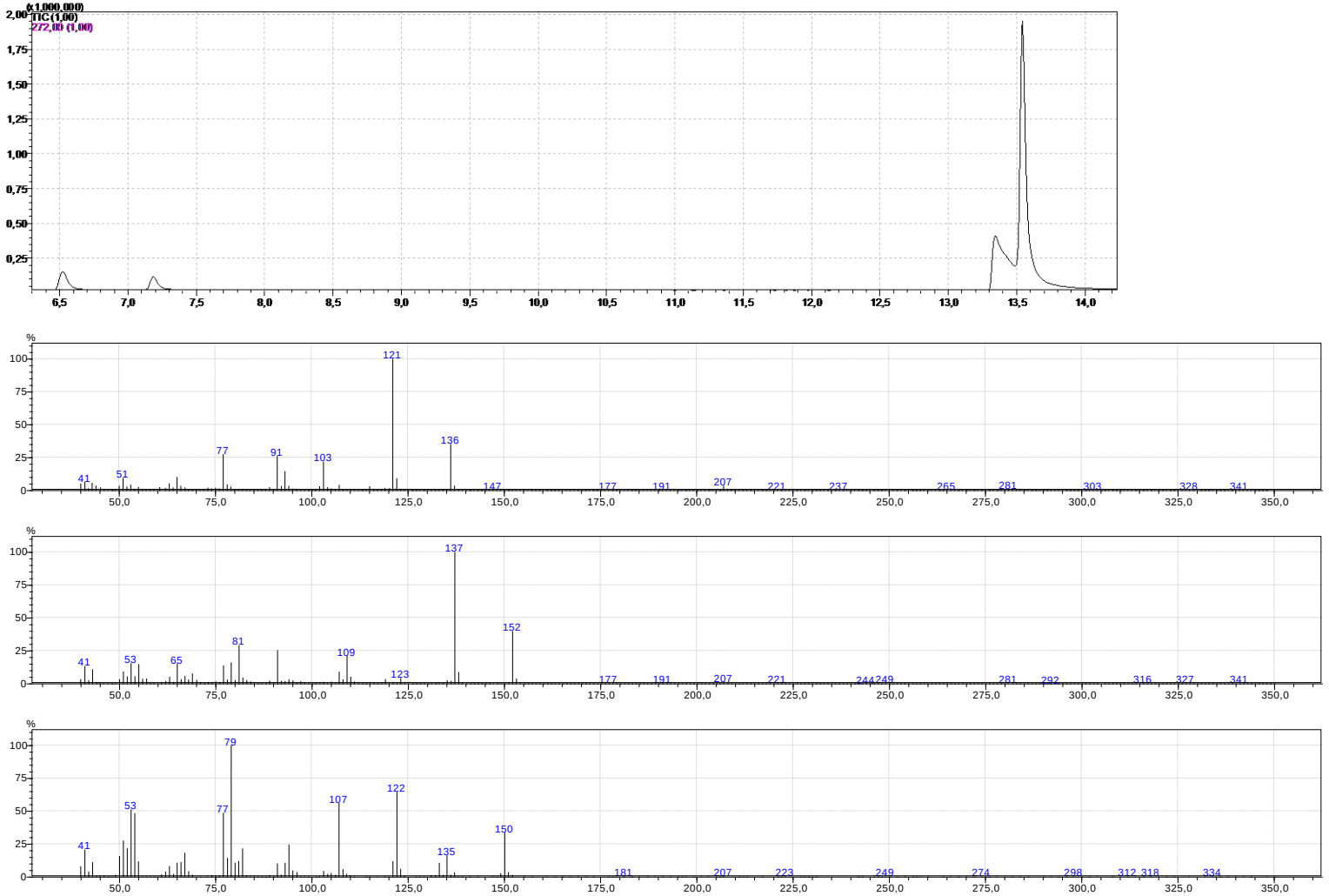


Figure S17: GC and MS chromatograms of conversion of **8**. Reactions were conducted in 50 mM sodium phosphate buffer pH 7.0 with 2 mM $MgCl_2$, 1 mM substrate, 4 mM hydrogen peroxide, 1.3 µM *Abr*UPO, 8 mM ascorbate at 25 °C and 600 rpm for 180 min. MS chromatograms of **8** (7.193 min), **8a** (6.522 min) and **8b** (13.543 min) in sorted order.

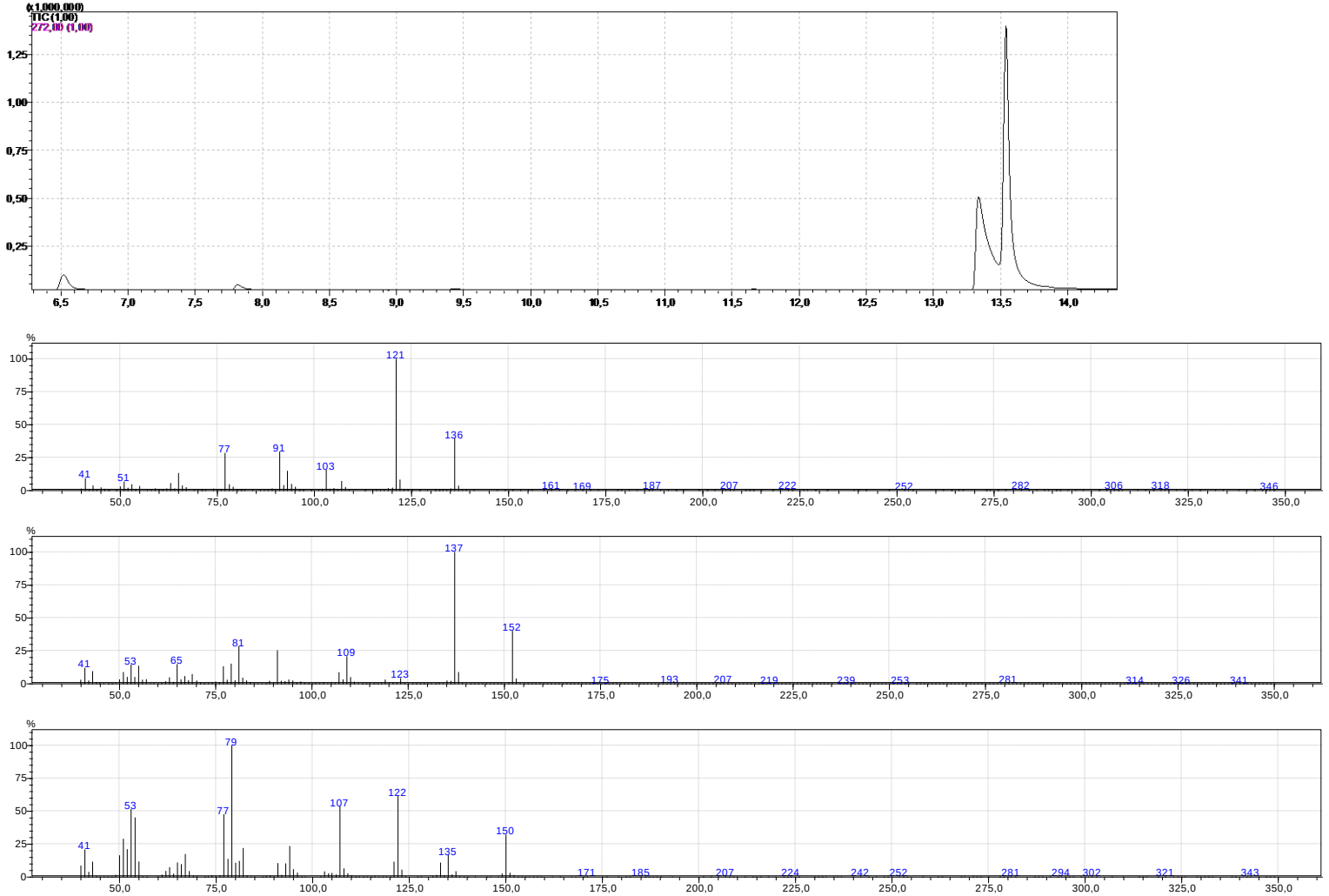


Figure S18: GC and MS chromatograms of conversion of **9**. Reactions were conducted in 50 mM sodium phosphate buffer pH 7.0 with 2 mM $MgCl_2$, 1 mM substrate, 4 mM hydrogen peroxide, 1.3 µM *Abr*UPO, 8 mM ascorbate at 25 °C and 600 rpm for 180 min. MS chromatograms of **9** (7.833 min), **9a** (6.521 min) and **9b** (13.540 min) in sorted order.

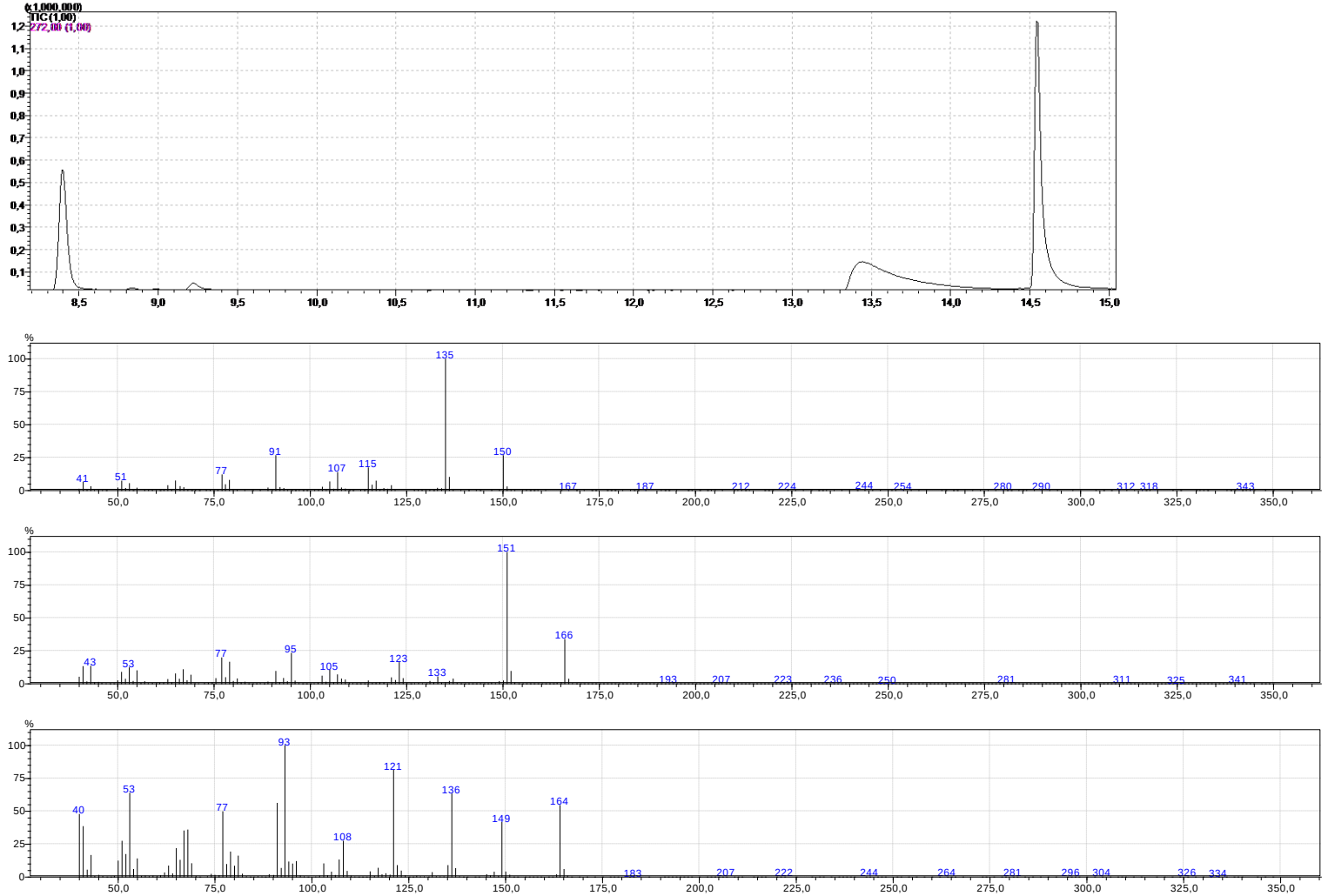


Figure S19: GC and MS chromatograms of conversion of **10**. Reactions were conducted in 50 mM sodium phosphate buffer pH 7.0 with 2 mM $MgCl_2$, 1 mM substrate, 4 mM hydrogen peroxide, 1.3 µM *Abr*UPO, 8 mM ascorbate at 25 °C and 600 rpm for 180 min. MS chromatograms of **10** (9.247 min), **10a** (8.397 min) and **10b** (14.545 min) in sorted order.

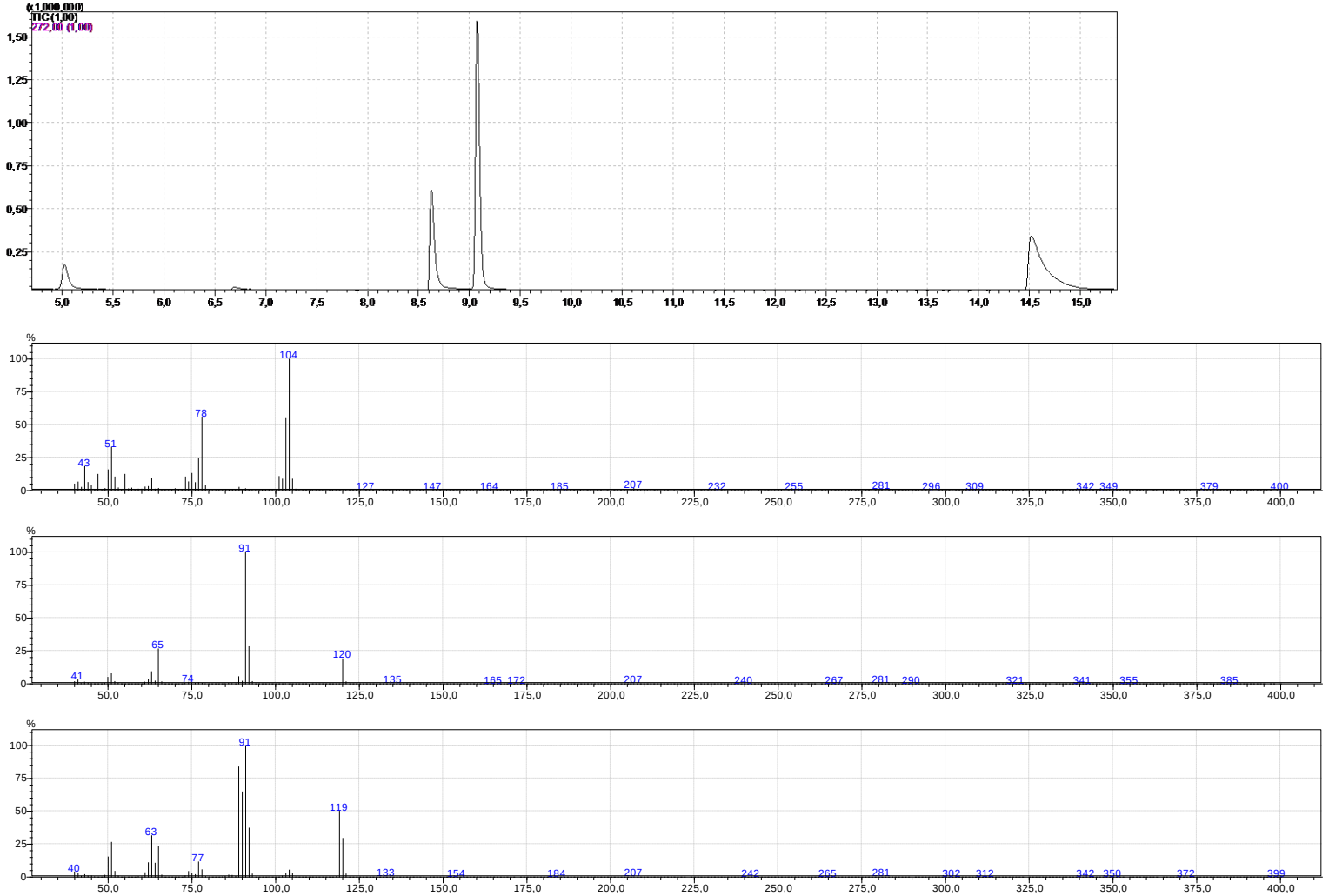


Figure S20: GC and MS chromatograms of conversion of **11**. Reactions were conducted in 50 mM sodium phosphate buffer pH 7.0 with 2 mM $MgCl_2$, 1 mM substrate, 4 mM hydrogen peroxide, 1.3 µM *Abr*UPO, 8 mM ascorbate at 25 °C and 600 rpm for 180 min. MS chromatograms of **11** (5.053 min), **11a** (8.628 min) and **11b** (9.078 min) in sorted order.

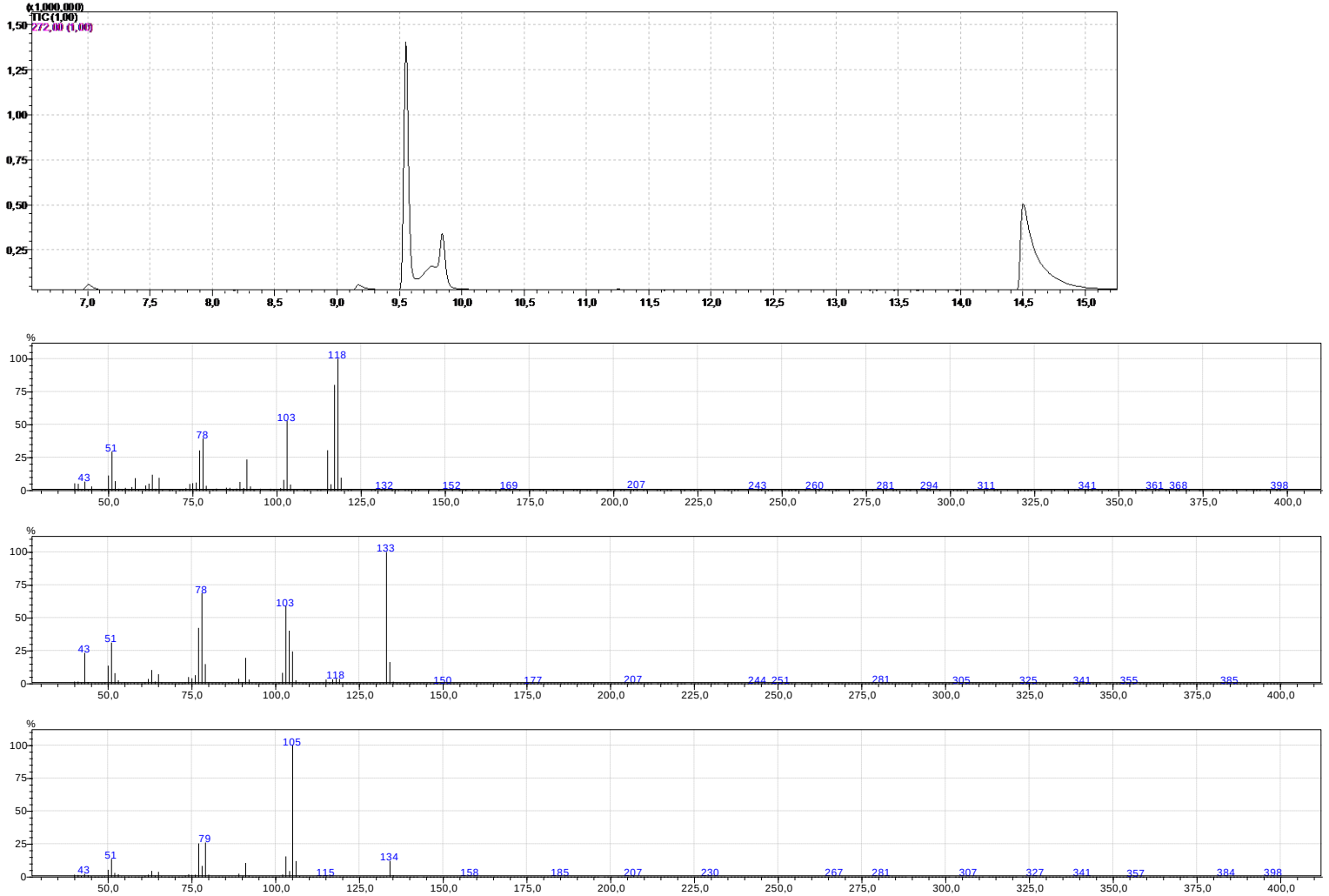


Figure S21: GC and MS chromatograms of conversion of **12**. Reactions were conducted in 50 mM sodium phosphate buffer pH 7.0 with 2 mM $MgCl_2$, 1 mM substrate, 4 mM hydrogen peroxide, 1.3 µM *Abr*UPO, 8 mM ascorbate at 25 °C and 600 rpm for 180 min. MS chromatograms of **12** (6.986 min), **12a** (9.553 min) and **12b** (9.845 min) in sorted order.

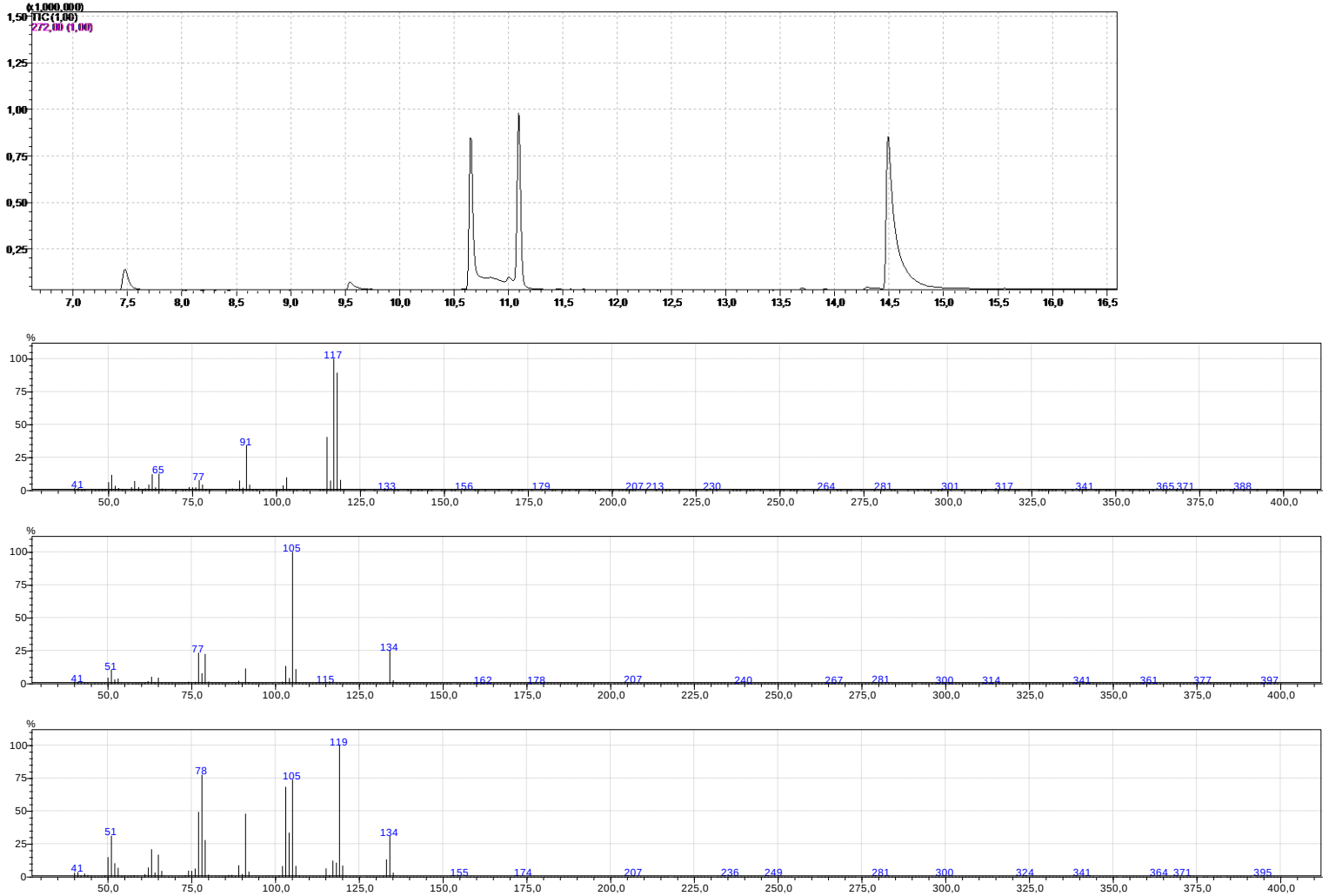
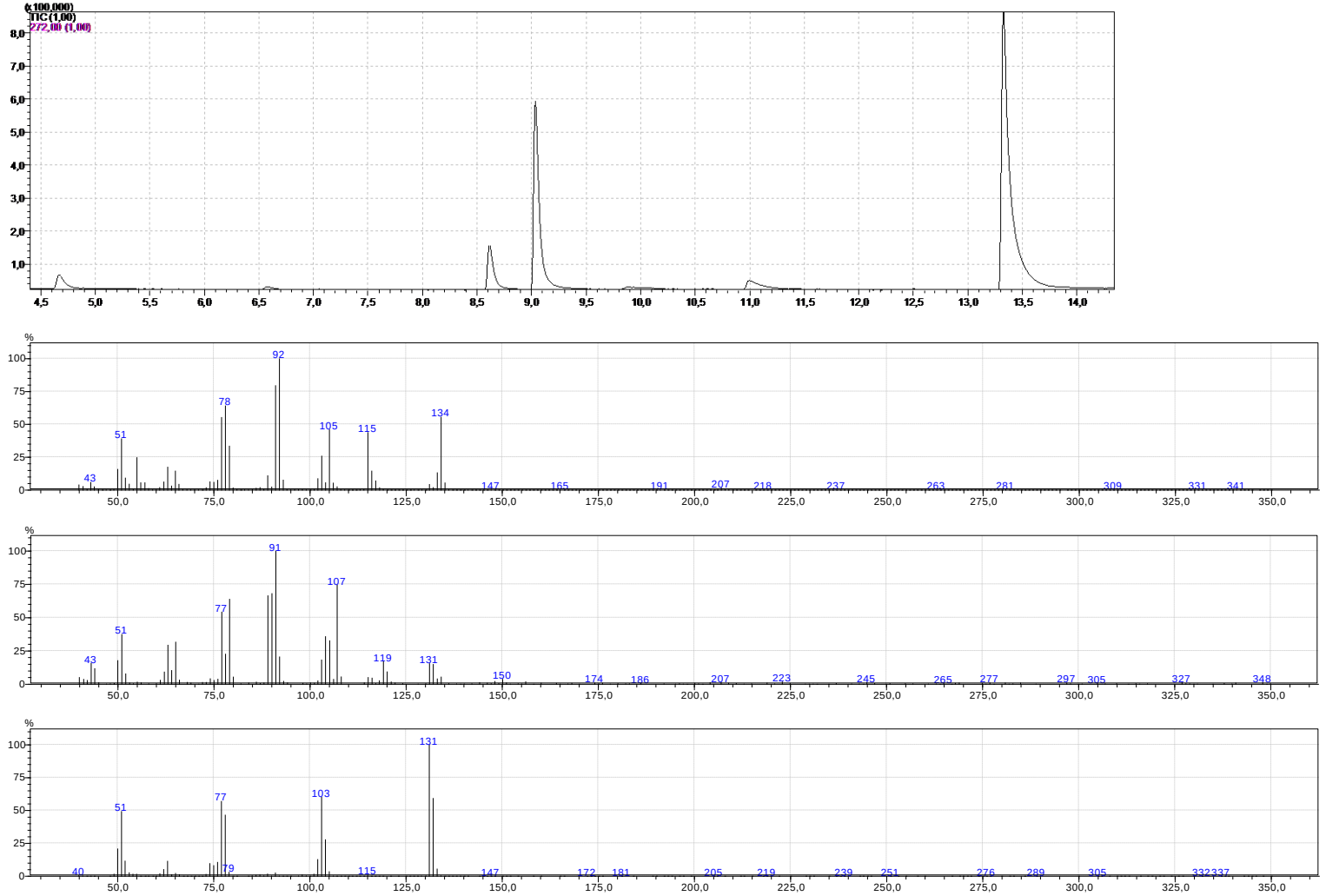


Figure S22: GC and MS chromatograms of conversion of **13**. Reactions were conducted in 50 mM sodium phosphate buffer pH 7.0 with 2 mM $MgCl_2$, 1 mM substrate, 4 mM hydrogen peroxide, 1.3 µM *Abr*UPO, 8 mM ascorbate at 25 °C and 600 rpm for 180 min. MS chromatograms of **13** (7.483 min), **13a** (10.654 min) and **13b** (11.095 min) in sorted order.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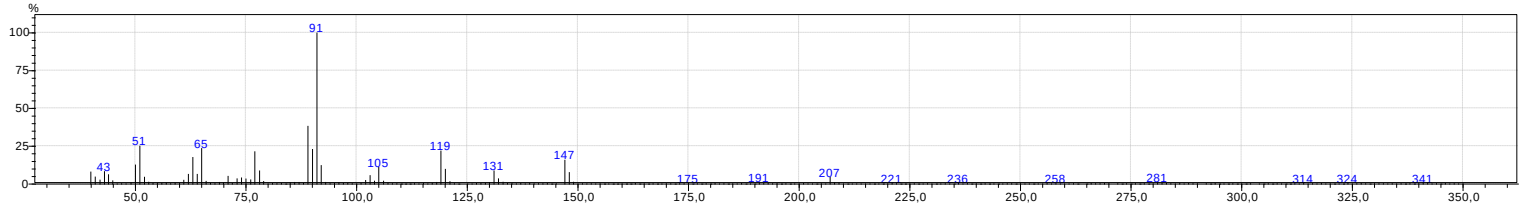


Figure S23: GC and MS chromatograms of conversion of **14**. Reactions were conducted in 50 mM sodium phosphate buffer pH 7.0 with 2 mM $MgCl_2$, 1 mM substrate, 4 mM hydrogen peroxide, 1.3 µM *Abr*UPO, 8 mM ascorbate at 25 °C and 600 rpm for 180 min. MS chromatograms of **14** (9.738 min), **14a** (10.994 min)**, 14b** (9.033 min) and **14c** (8.619 min) in sorted order.

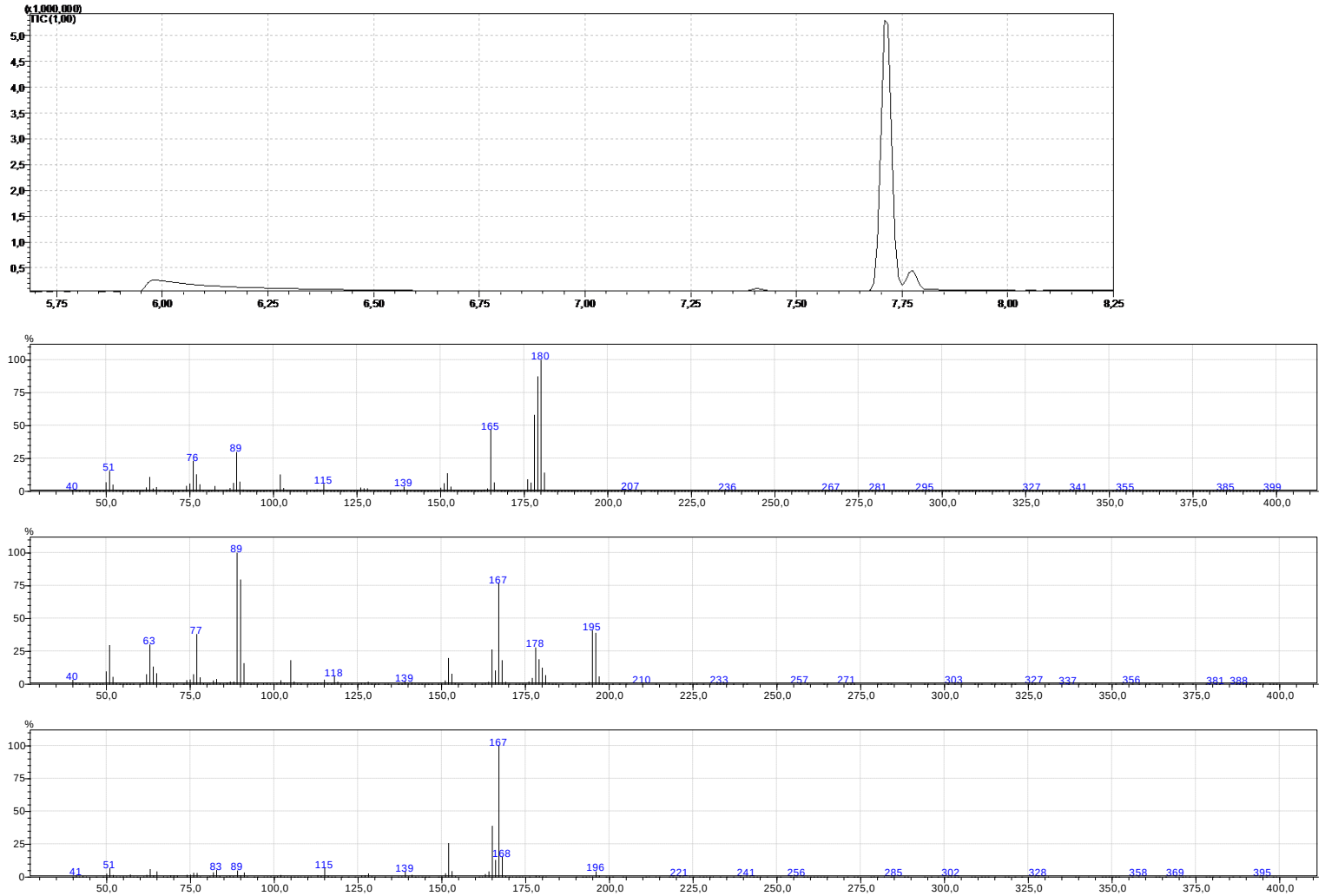
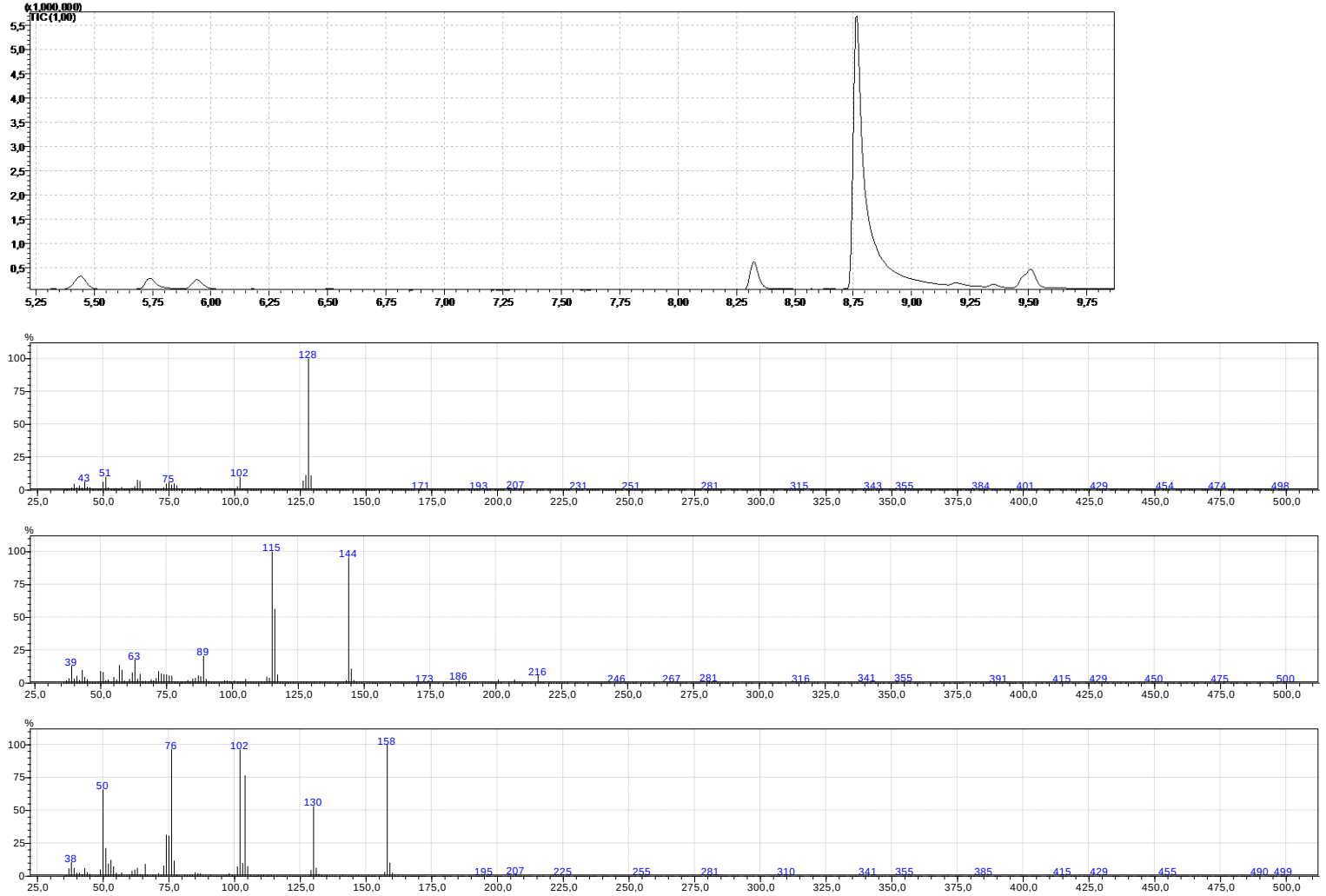


Figure S24: GC and MS chromatograms of conversion of **15**. Reactions were conducted in 50 mM sodium phosphate buffer pH 7.0 with 2 mM $MgCl_2$, 1 mM substrate, 4 mM hydrogen peroxide, 1.3 µM *Abr*UPO, 8 mM ascorbate at 25 °C and 600 rpm for 180 min. MS chromatograms of **15** (7.709 min), **15a** (7.771 min) and **15b** (7.407 min) in sorted order.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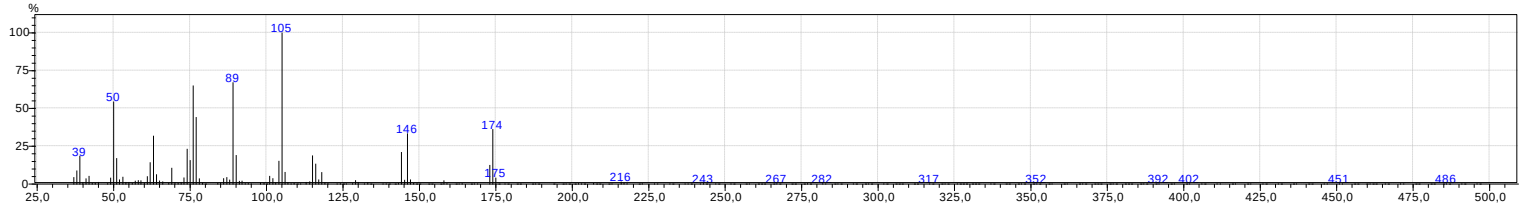


Figure S25: GC and MS chromatograms of conversion of **16**. Reactions were conducted in 50 mM sodium phosphate buffer pH 7.0 with 2 mM $MgCl_2$, 1 mM substrate, 4 mM hydrogen peroxide, 1.3 µM *Abr*UPO, 8 mM ascorbate at 25 °C and 600 rpm for 180 min. MS chromatograms of **16** (5.441 min), **16a** (9.950 min)**, 16b** (8.324 min) and **16c** (9.510min) in sorted order.

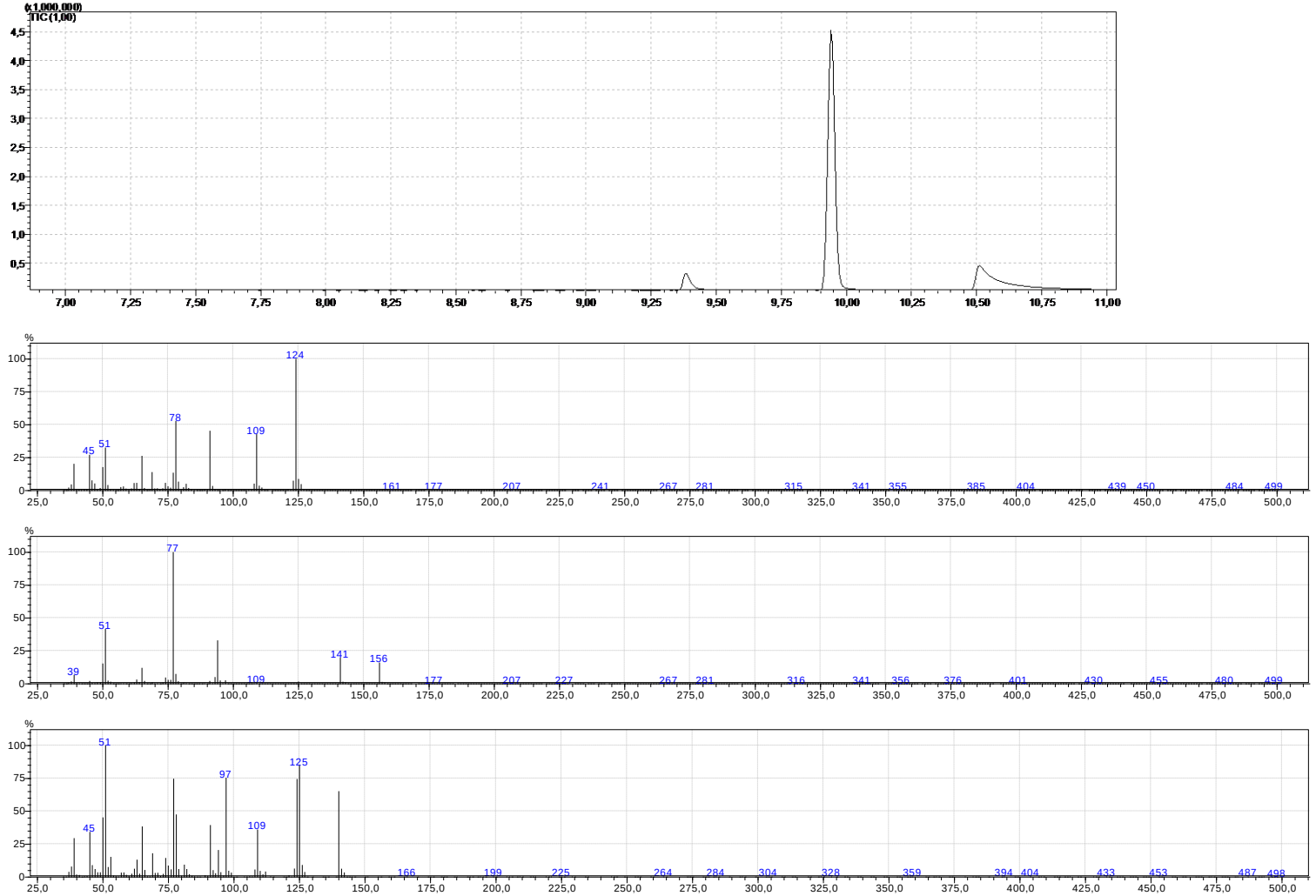


Figure S26: GC and MS chromatograms of conversion of **17**. Reactions were conducted in 50 mM sodium phosphate buffer pH 7.0 with 2 mM $MgCl_2$, 1 mM substrate, 4 mM hydrogen peroxide, 1.3 µM *Abr*UPO, 8 mM ascorbate at 25 °C and 600 rpm for 180 min. MS chromatograms of **17** (6.850 min), **17a** (9.942 min) and **17b** (9.383 min) in sorted order.

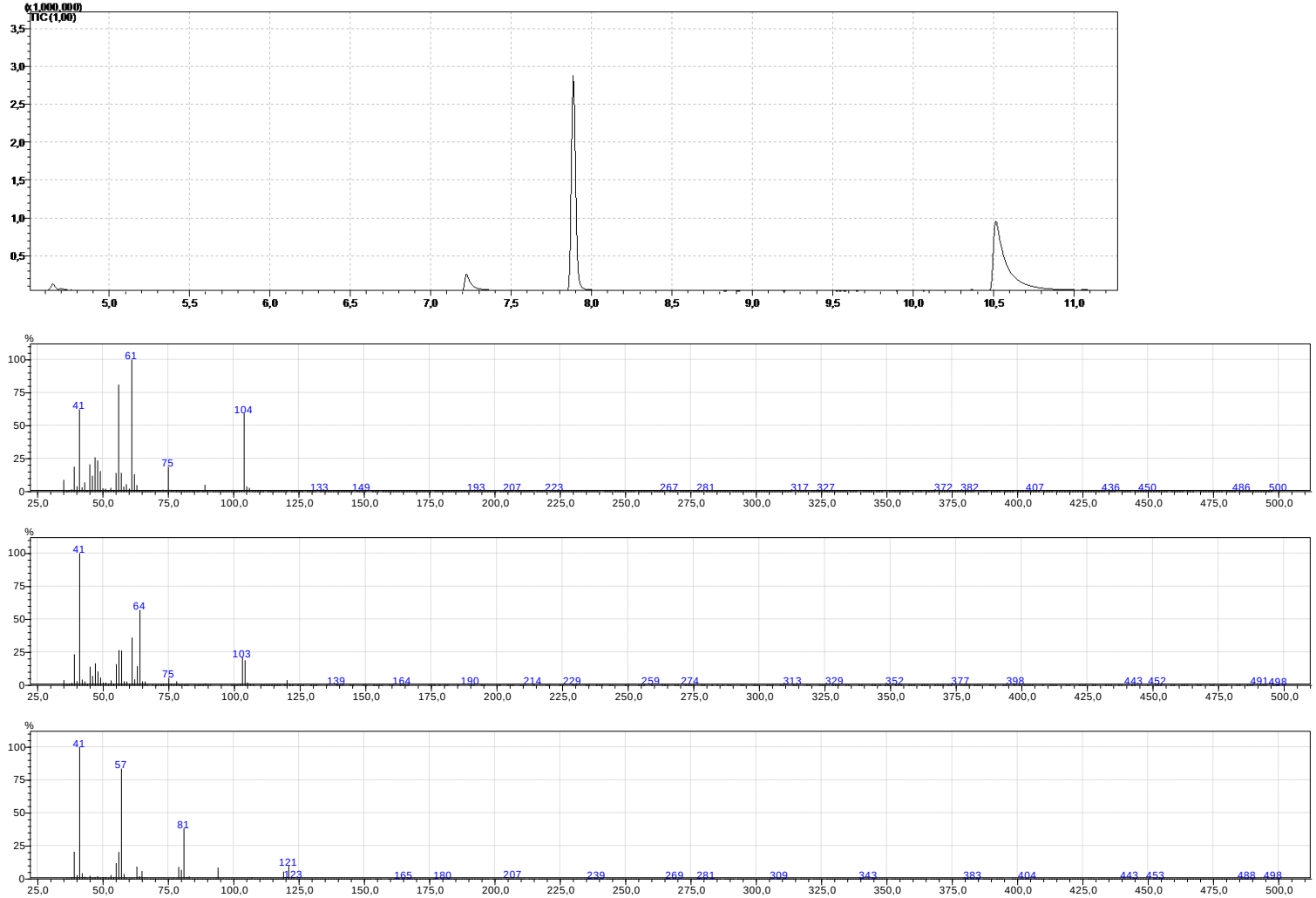
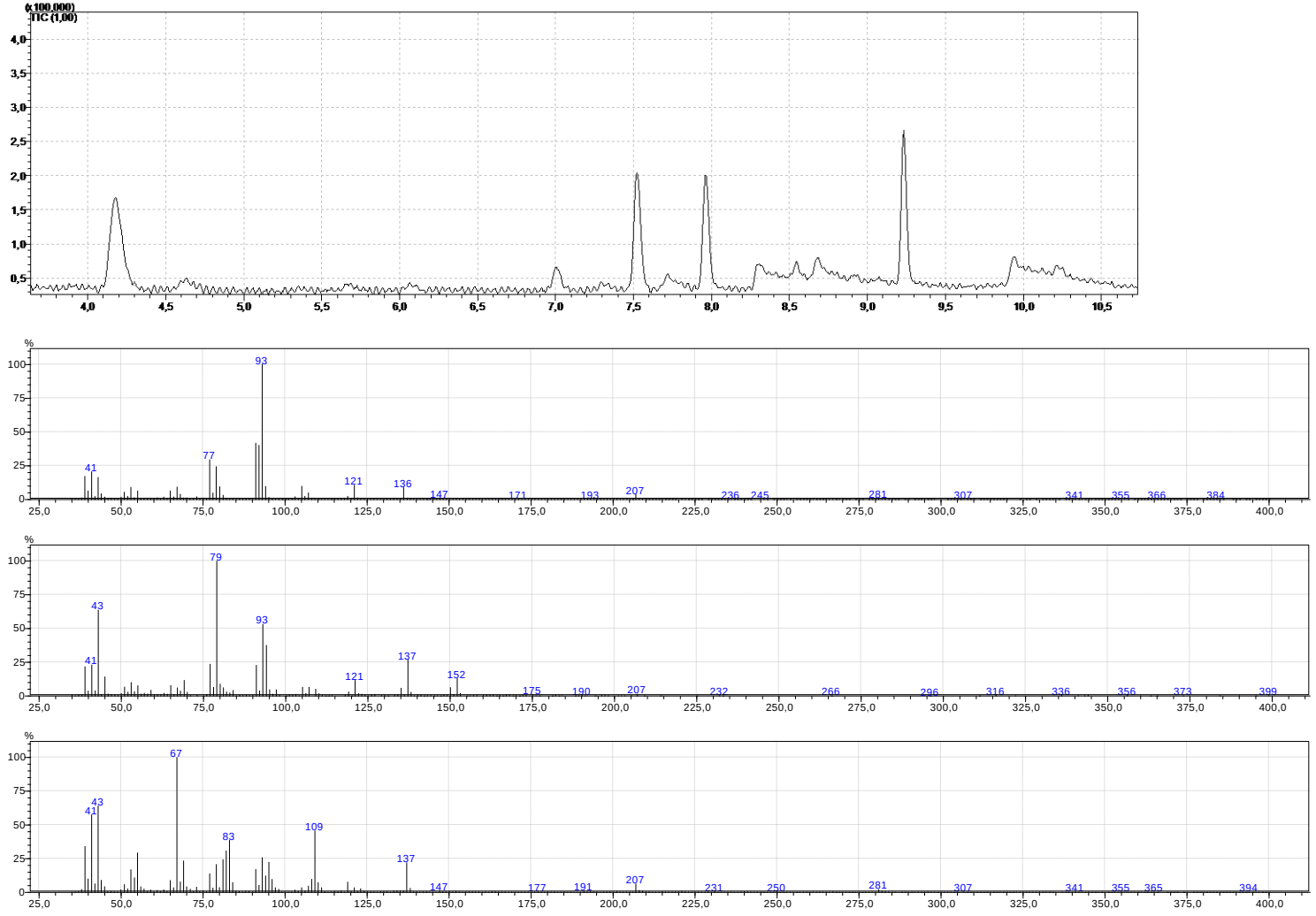


Figure S27: GC and MS chromatograms of conversion of **18**. Reactions were conducted in 50 mM sodium phosphate buffer pH 7.0 with 2 mM $MgCl_2$, 1 mM substrate, 4 mM hydrogen peroxide, 1.3 µM *Abr*UPO, 8 mM ascorbate at 25 °C and 600 rpm for 180 min. MS chromatograms of **18** (3.833 min), **18a** (7.222 min) and **18b** (7.887 min) in sorted order.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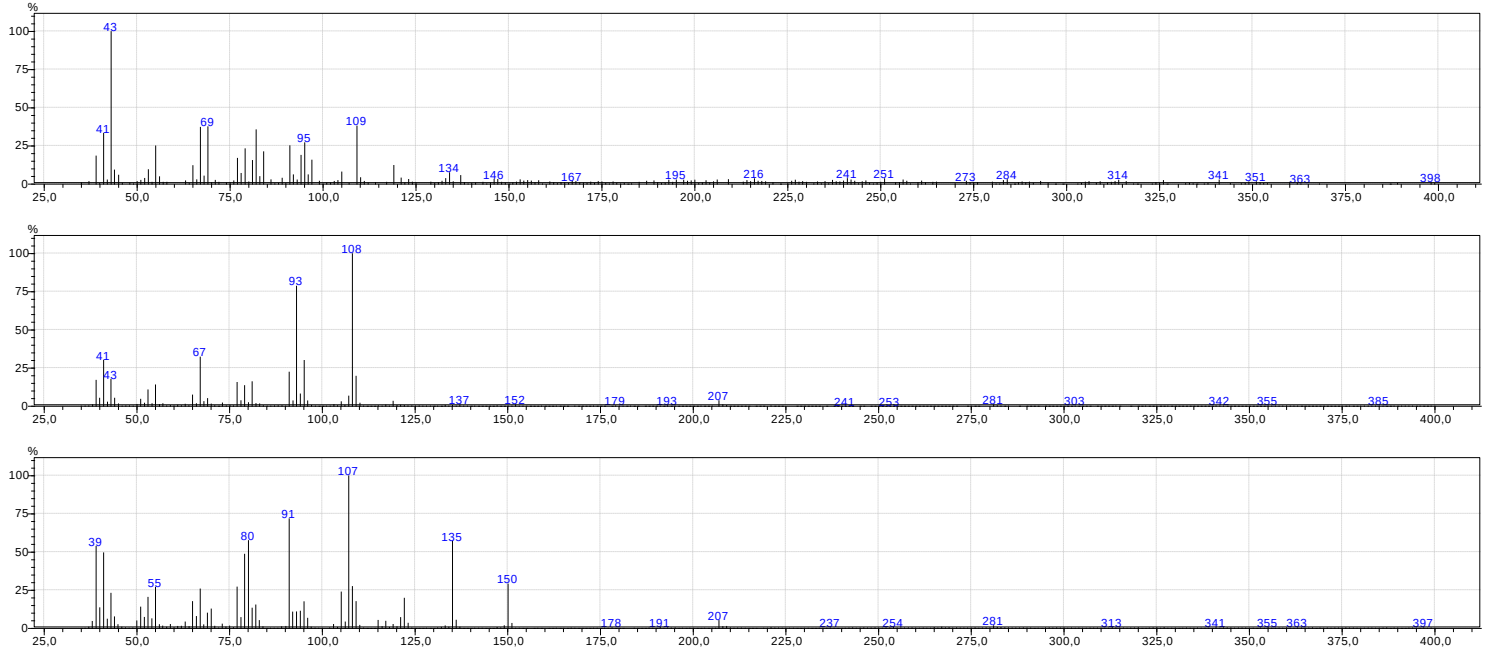


Figure S28: GC and MS chromatograms of conversion of **19**. Reactions were conducted in 50 mM sodium phosphate buffer pH 7.0 with 2 mM $MgCl_2$, 1 mM substrate, 4 mM hydrogen peroxide, 1.3 µM *Abr*UPO, 8 mM ascorbate at 25 °C and 600 rpm for 180 min. MS chromatograms of **19** (4.178 min), **19a** (7.020min), **19b** (7.522 min)**, 19c** (7.722 min), **19d** (7.964 min) and **19e** (9.234 min) in sorted order.

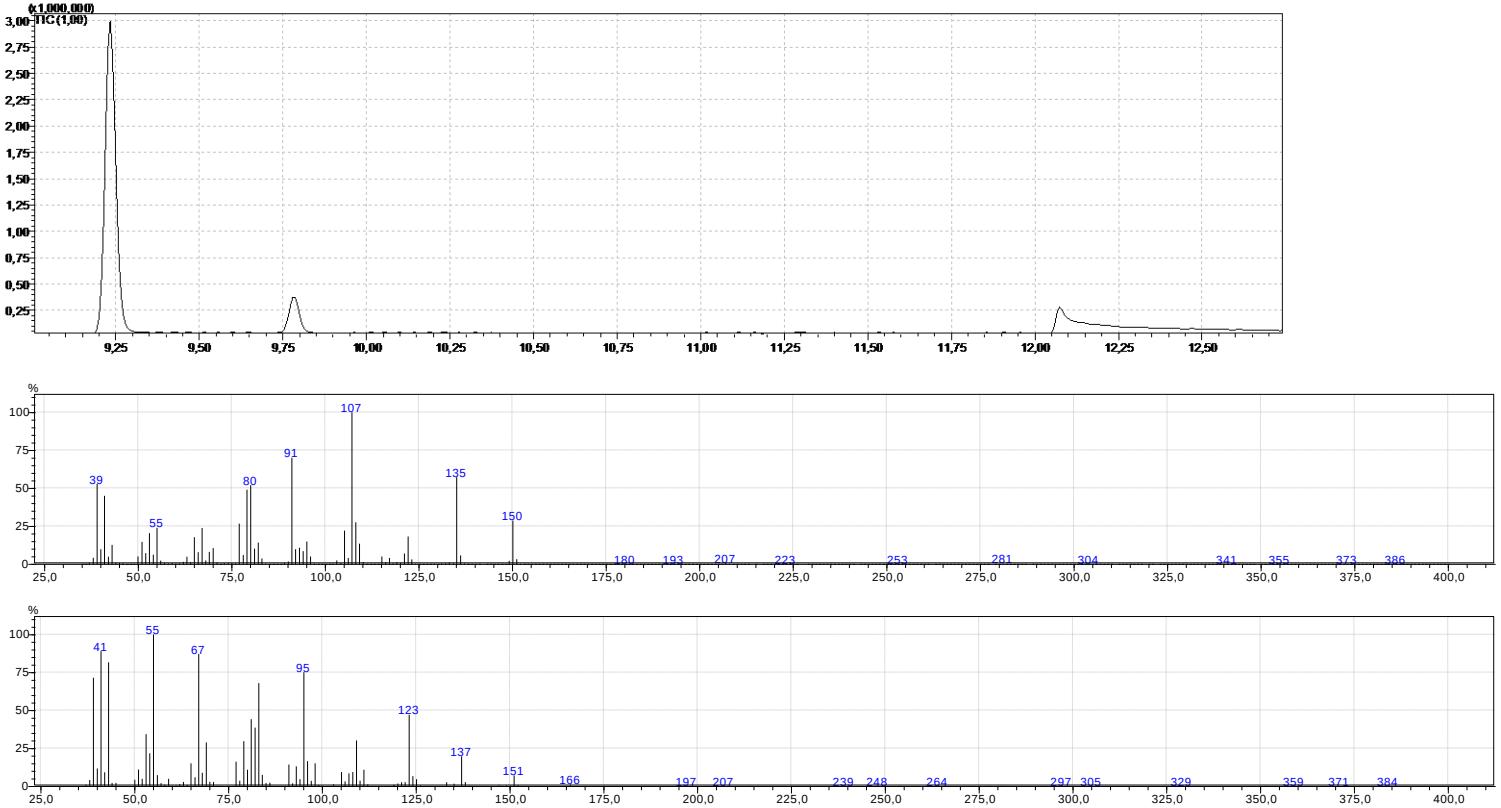


Figure S29: GC and MS chromatograms of conversion of **20**. Reactions were conducted in 50 mM sodium phosphate buffer pH 7.0 with 2 mM $MgCl_2$, 1 mM substrate, 4 mM hydrogen peroxide, 1.3 µM *Abr*UPO, 8 mM ascorbate at 25 °C and 600 rpm for 180 min. MS chromatograms of **20** (9.223 min) and **20a** (9.783 min) in sorted order.

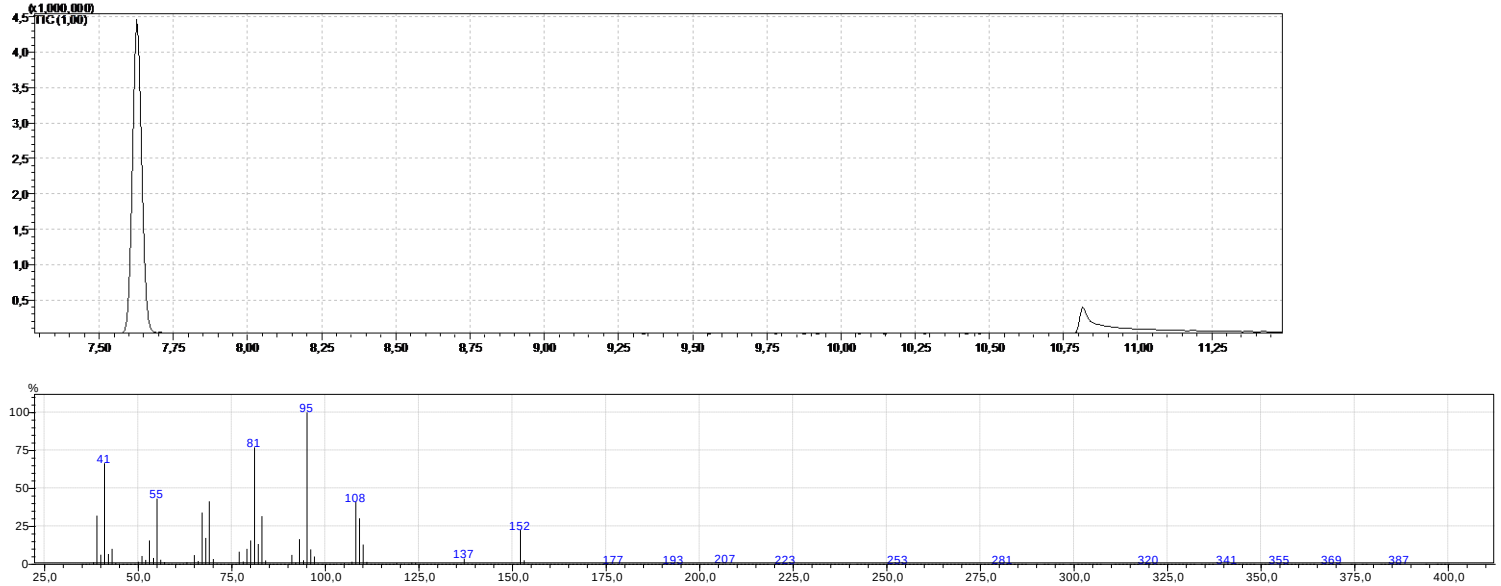


Figure S30: GC and MS chromatograms of conversion of **21** (7.632 min). Reactions were conducted in 50 mM sodium phosphate buffer pH 7.0 with 2 mM $MgCl_2$, 1 mM substrate, 4 mM hydrogen peroxide, 1.3 µM *Abr*UPO, 8 mM ascorbate at 25 °C and 600 rpm for 180 min.

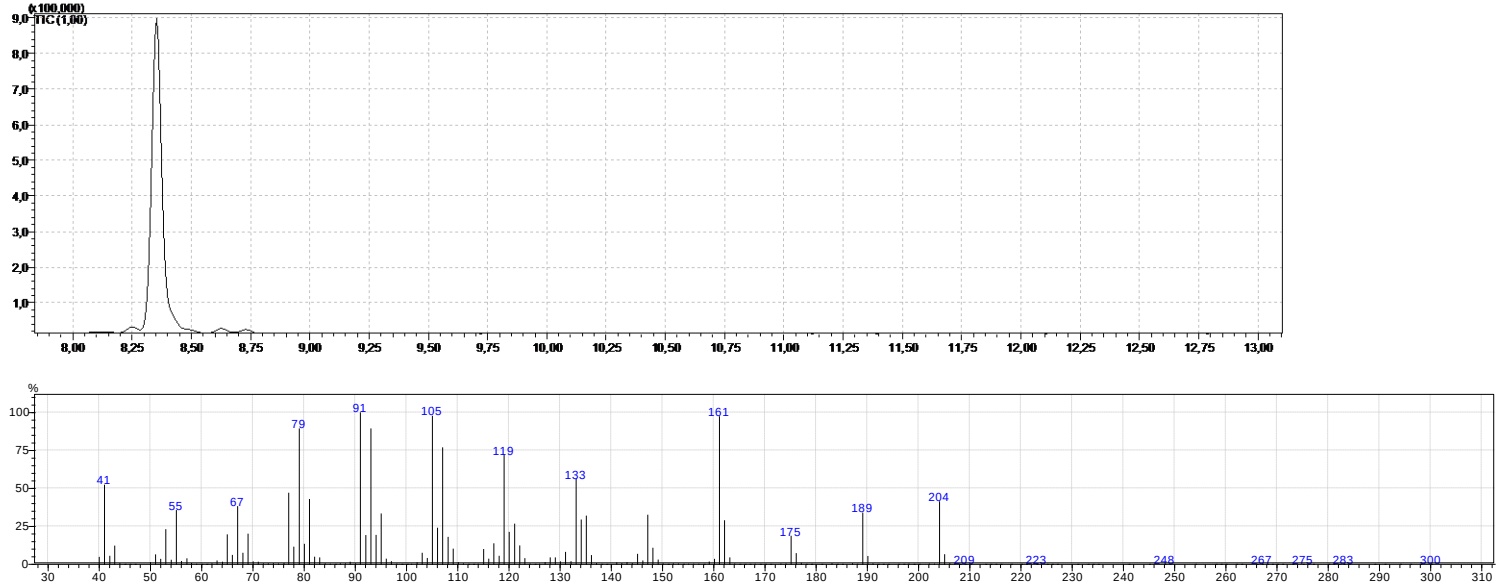


Figure S31: GC and MS chromatograms of conversion of **22** (8.365 min). Reactions were conducted in 50 mM sodium phosphate buffer pH 7.0 with 2 mM $MgCl_2$, 1 mM substrate, 4 mM hydrogen peroxide, 1.3 µM *Abr*UPO, 8 mM ascorbate at 25 °C and 600 rpm for 180 min.

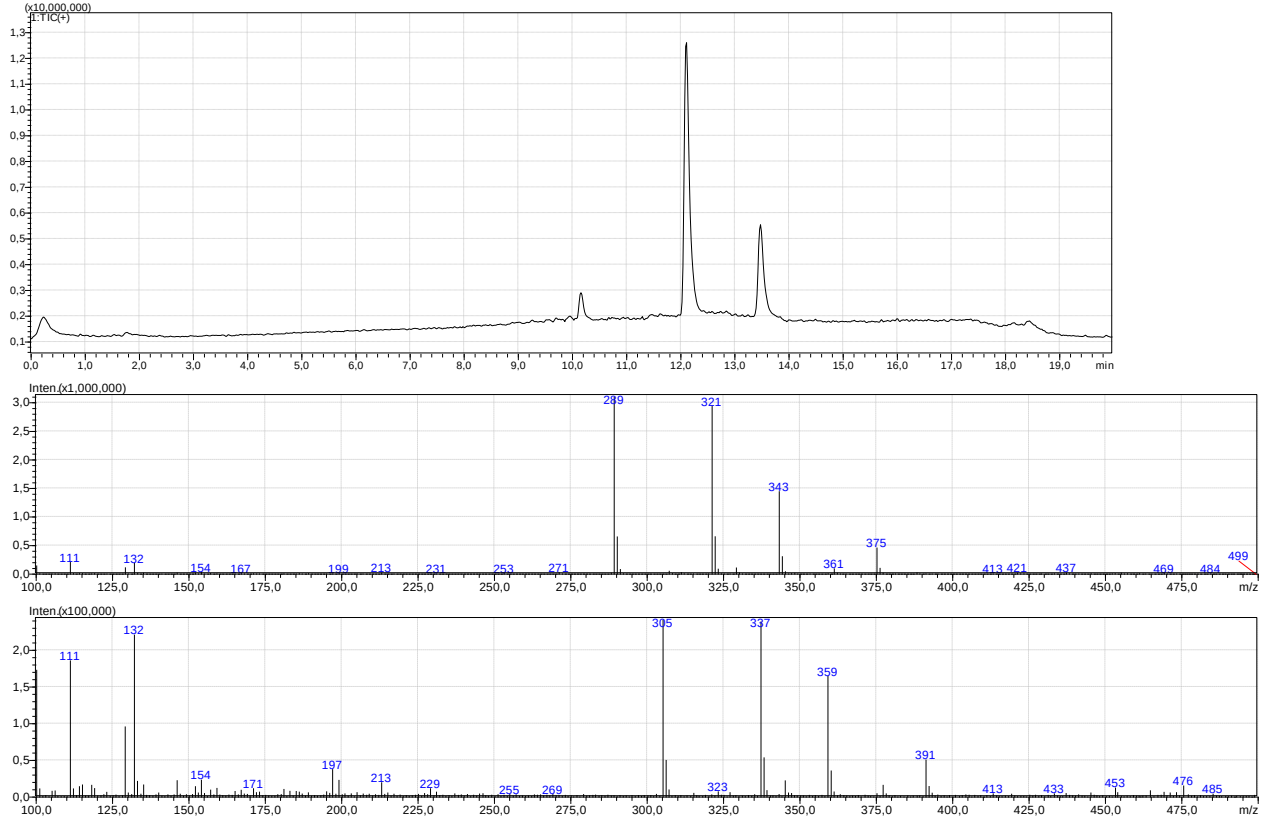
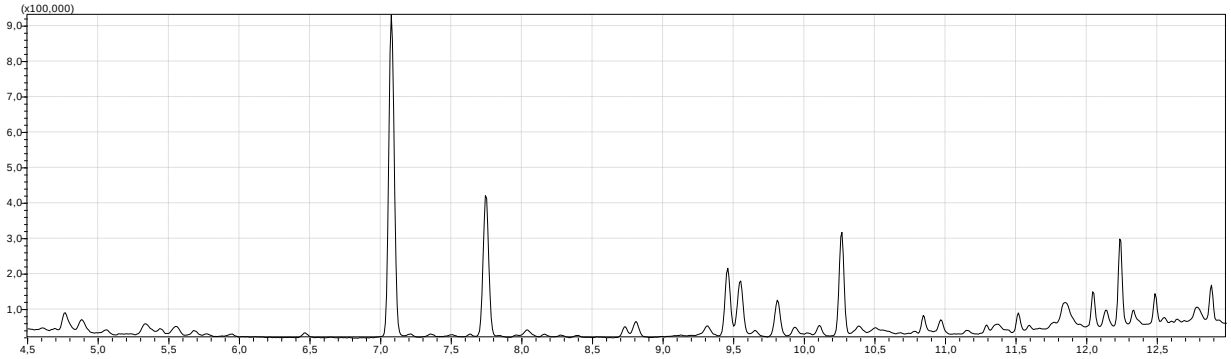


Figure S32: LC and MS chromatograms of conversion of **23**. Reactions were conducted in 50 mM sodium phosphate buffer pH 7.0 with 2 mM $MgCl_2$, 1 mM substrate, 4 mM hydrogen peroxide, 1.3 µM *Abr*UPO, 8 mM ascorbate at 25 °C and 600 rpm for 180 min. MS chromatograms of **23** (12.100 min) and **23a** (10.150 min) in sorted order.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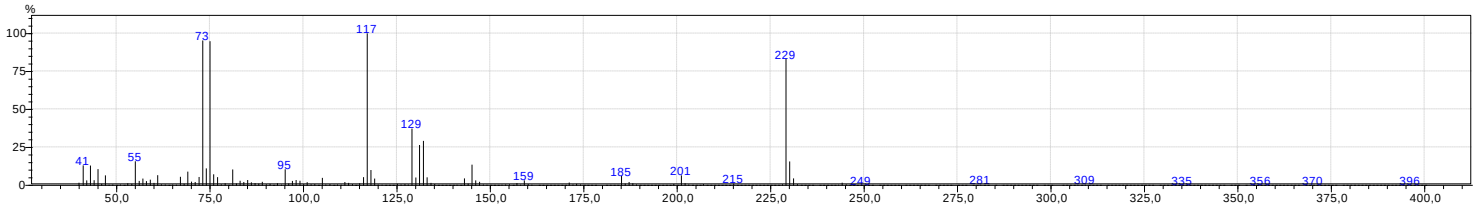
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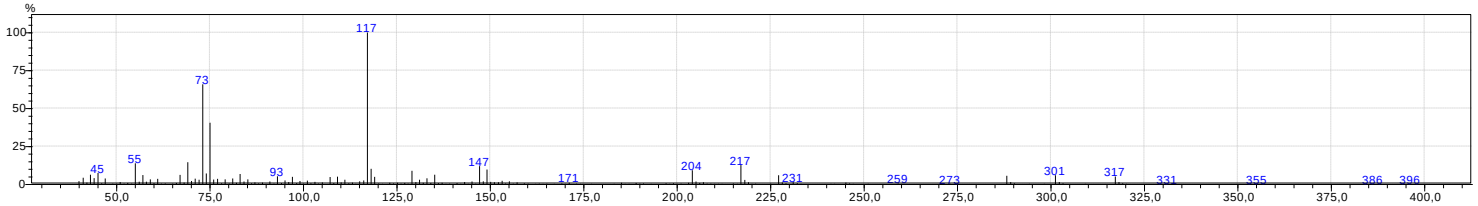
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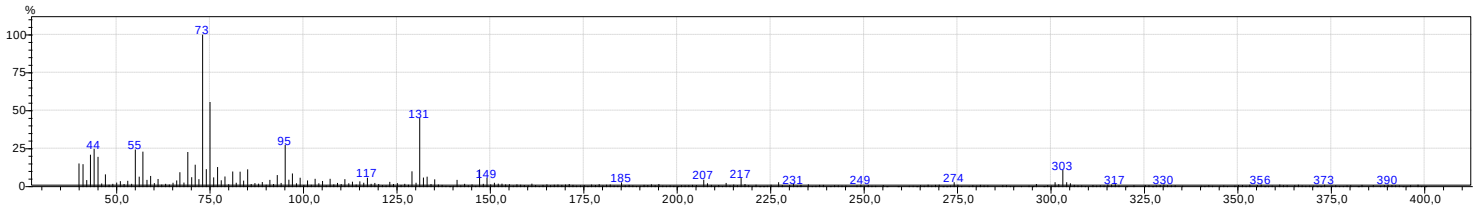
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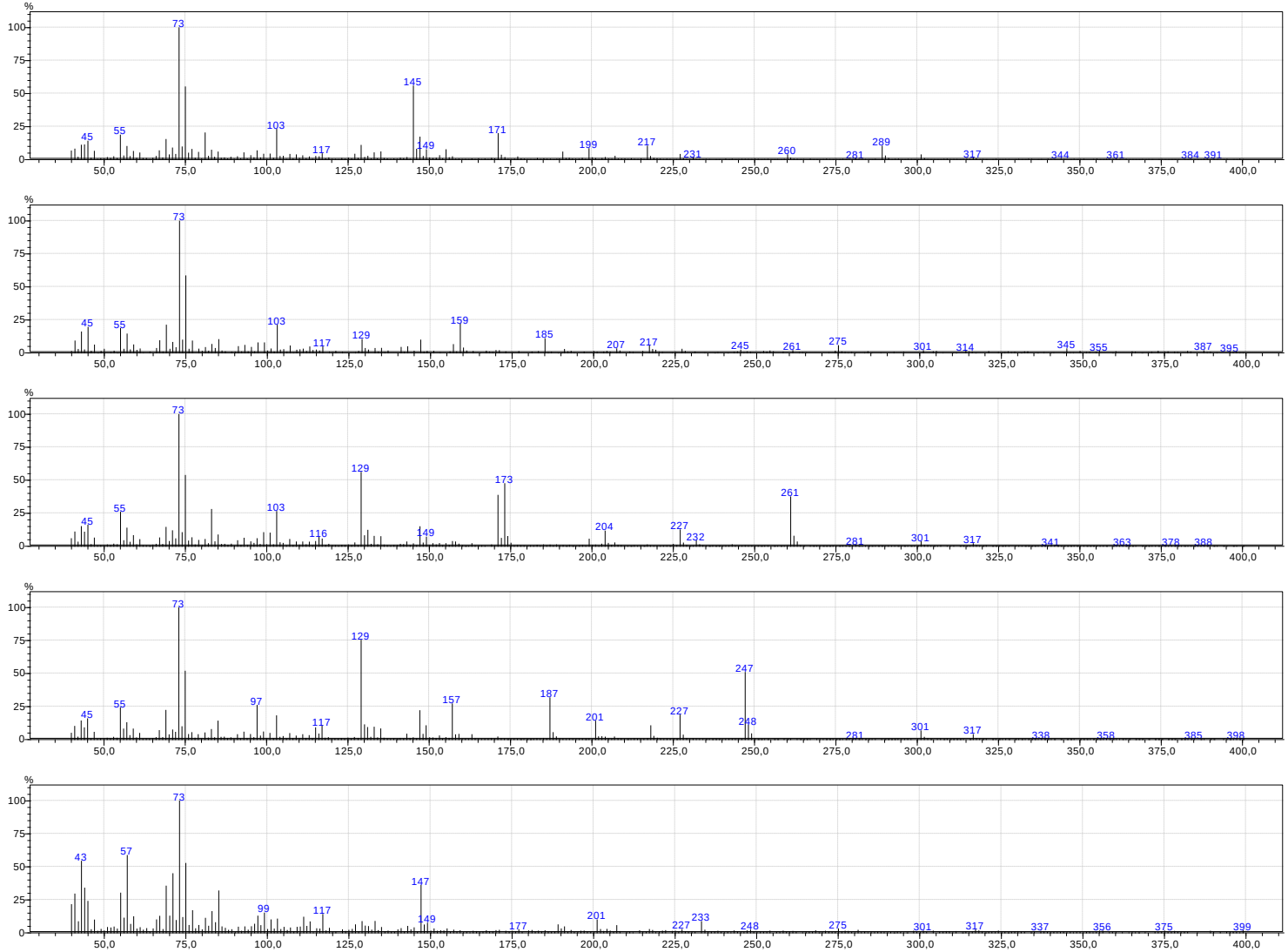
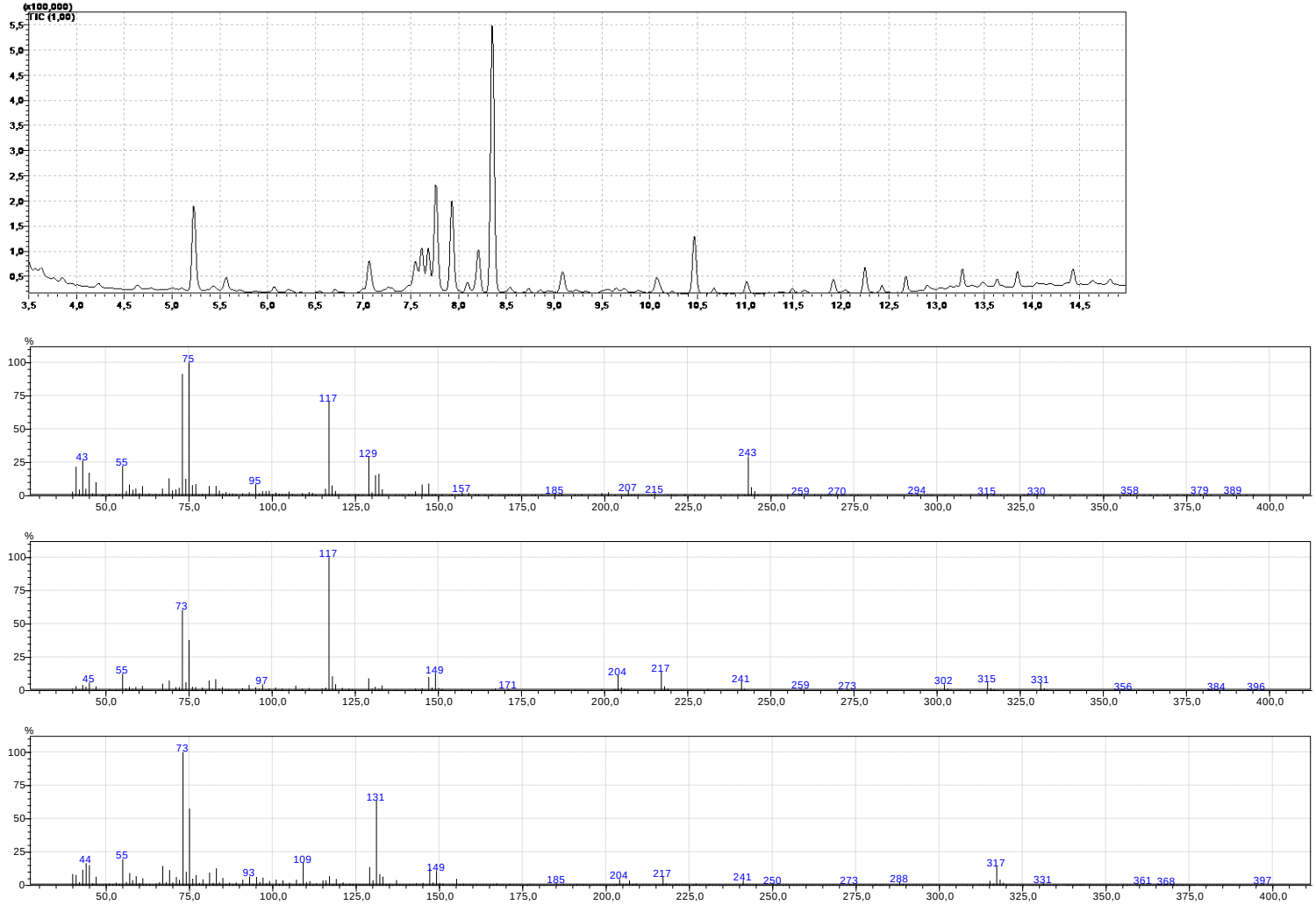


Figure S33: GC and MS chromatograms of conversion of **24**. Reactions were conducted in 50 mM sodium phosphate buffer pH 7.0 with 2 mM $MgCl_2$, 1 mM substrate, 4 mM hydrogen peroxide, 1.3 µM *Abr*UPO, 8 mM ascorbate at 25 °C and 600 rpm for 180 min. MS chromatograms of **24** (7.076 min), ω-1 (10.270 min), ω-2(10.115 min), ω-3 (9.816 min), ω-4 (9.653 min), ω-5 (9.551 min), ω-6 (9.463 min) and ω-7 (9.313 min) in sorted order.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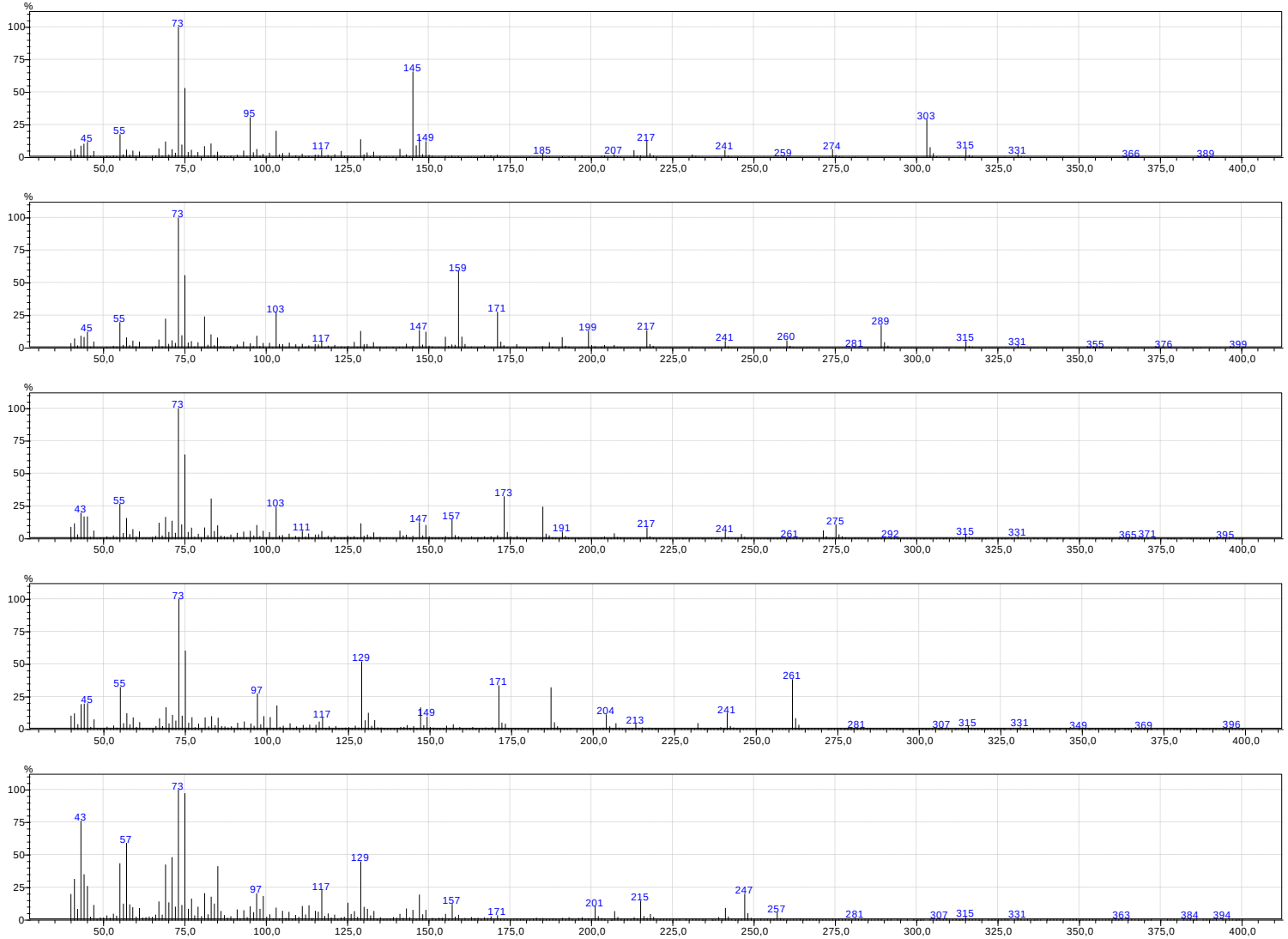
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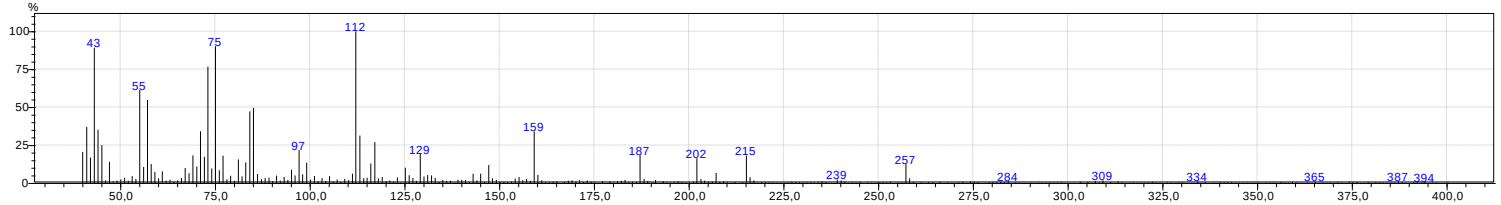
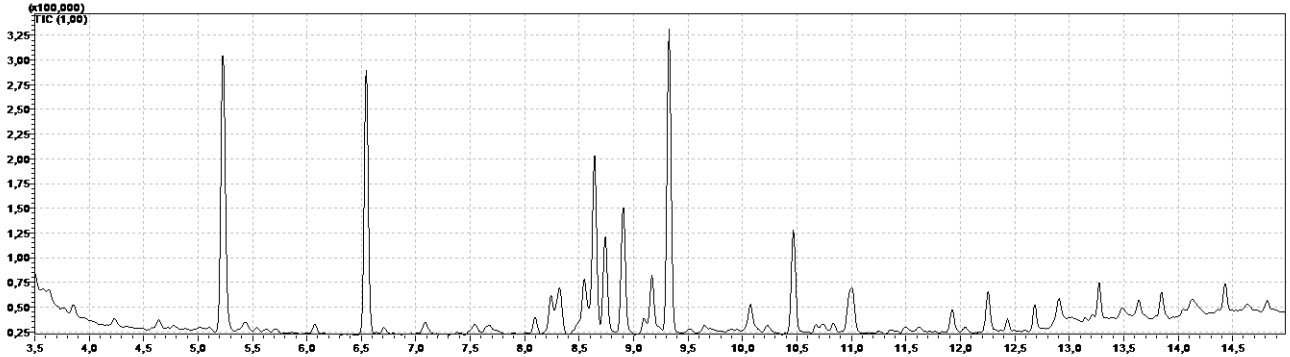


Figure S34: GC and MS chromatograms of conversion of **25**. Reactions were conducted in 50 mM sodium phosphate buffer pH 7.0 with 2 mM $MgCl_2$, 1 mM substrate, 4 mM hydrogen peroxide, 1.3 µM *Abr*UPO, 8 mM ascorbate at 25 °C and 600 rpm for 180 min. MS chromatograms of **25** (5.567 min), ω-1 (8.350 min), ω-2 (8.209 min), ω-3 (7.934min), ω-4 (7.761 min), ω-5 (7.685 min), ω-6 (7.621 min), ω-7 (7.554 min) and ω-8 (7.067 min) in sorted order.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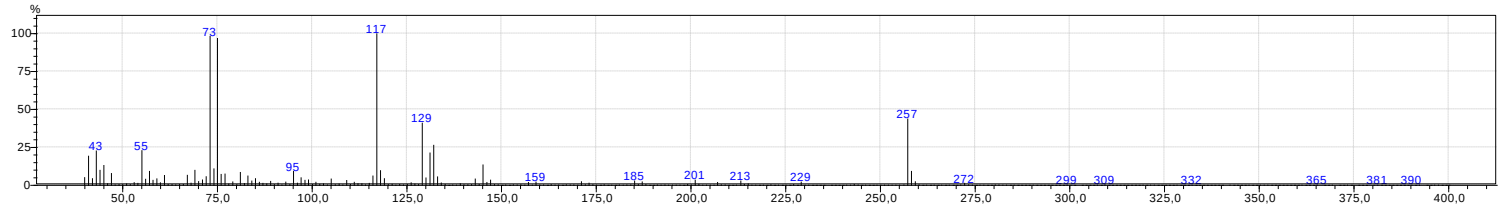
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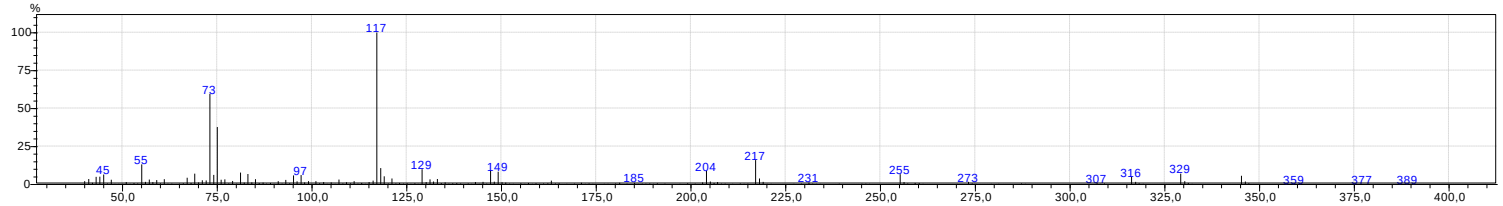
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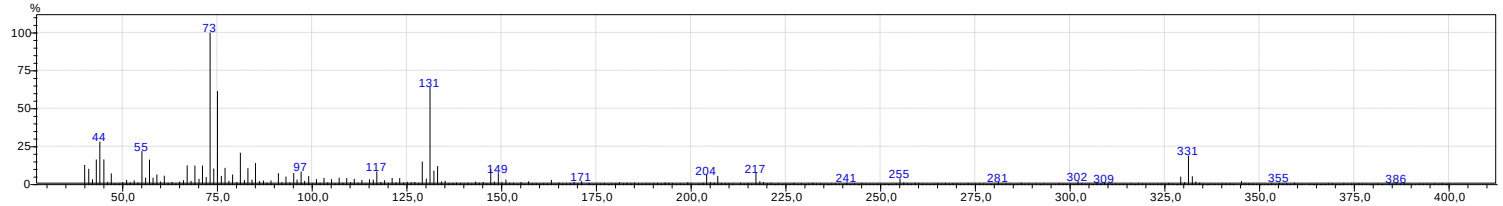
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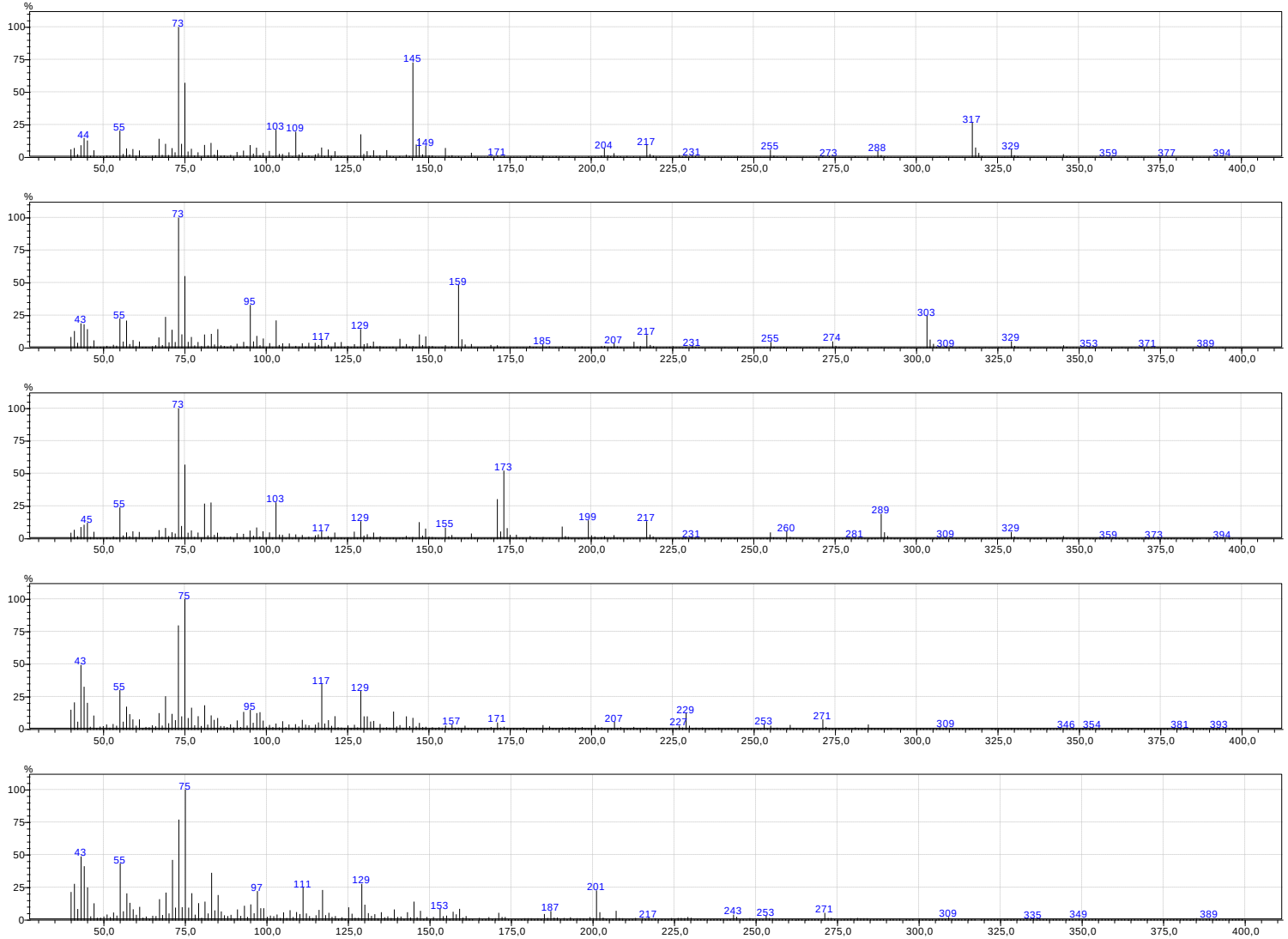
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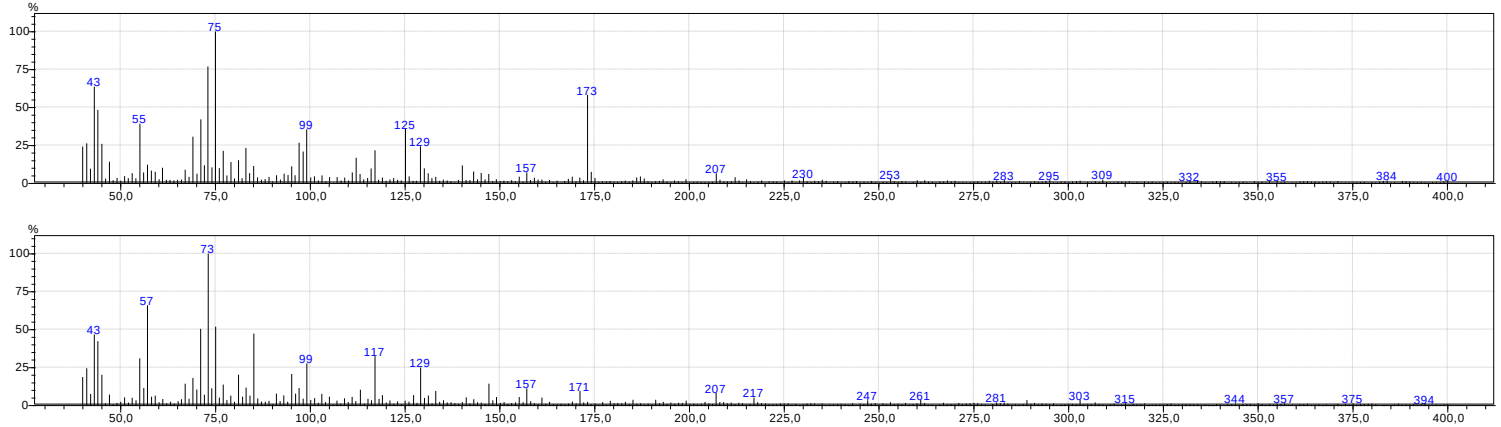
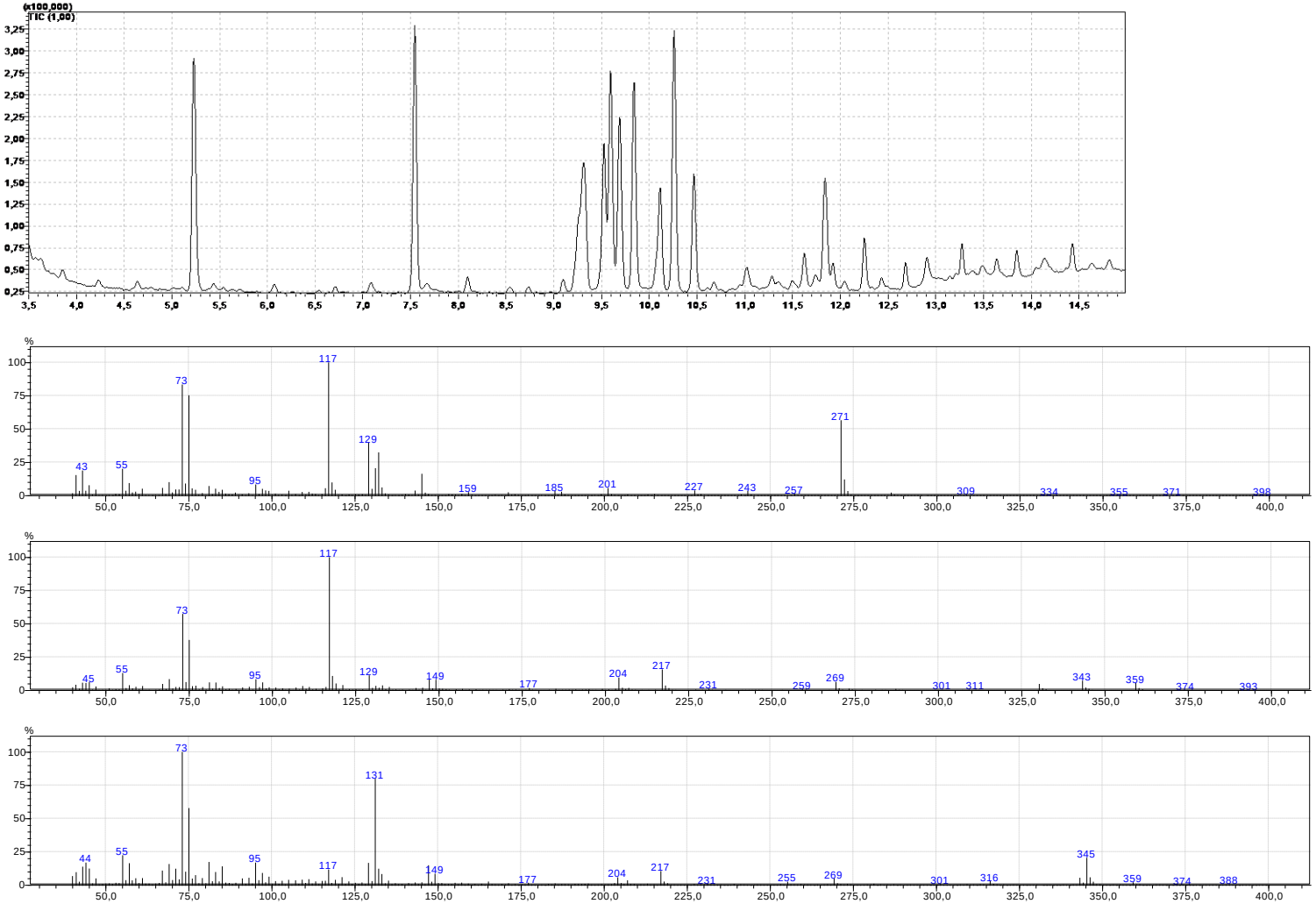


Figure S35: GC and MS chromatograms of conversion of **26**. Reactions were conducted in 50 mM sodium phosphate buffer pH 7.0 with 2 mM $MgCl_2$, 1 mM substrate, 4 mM hydrogen peroxide, 1.3 µM *Abr*UPO, 8 mM ascorbate at 25 °C and 600 rpm for 180 min. MS chromatograms of **26** (6.550 min), ω-1 (9.330 min), ω-2 (9.171 min), ω-3 (8.908 min), ω-4 (8.738 min), ω-5 (8.644 min), ω-6 (8.547min), ω-7 (8.322 min), ω-8 (8.244 min) and γ (10.997 min) in sorted order.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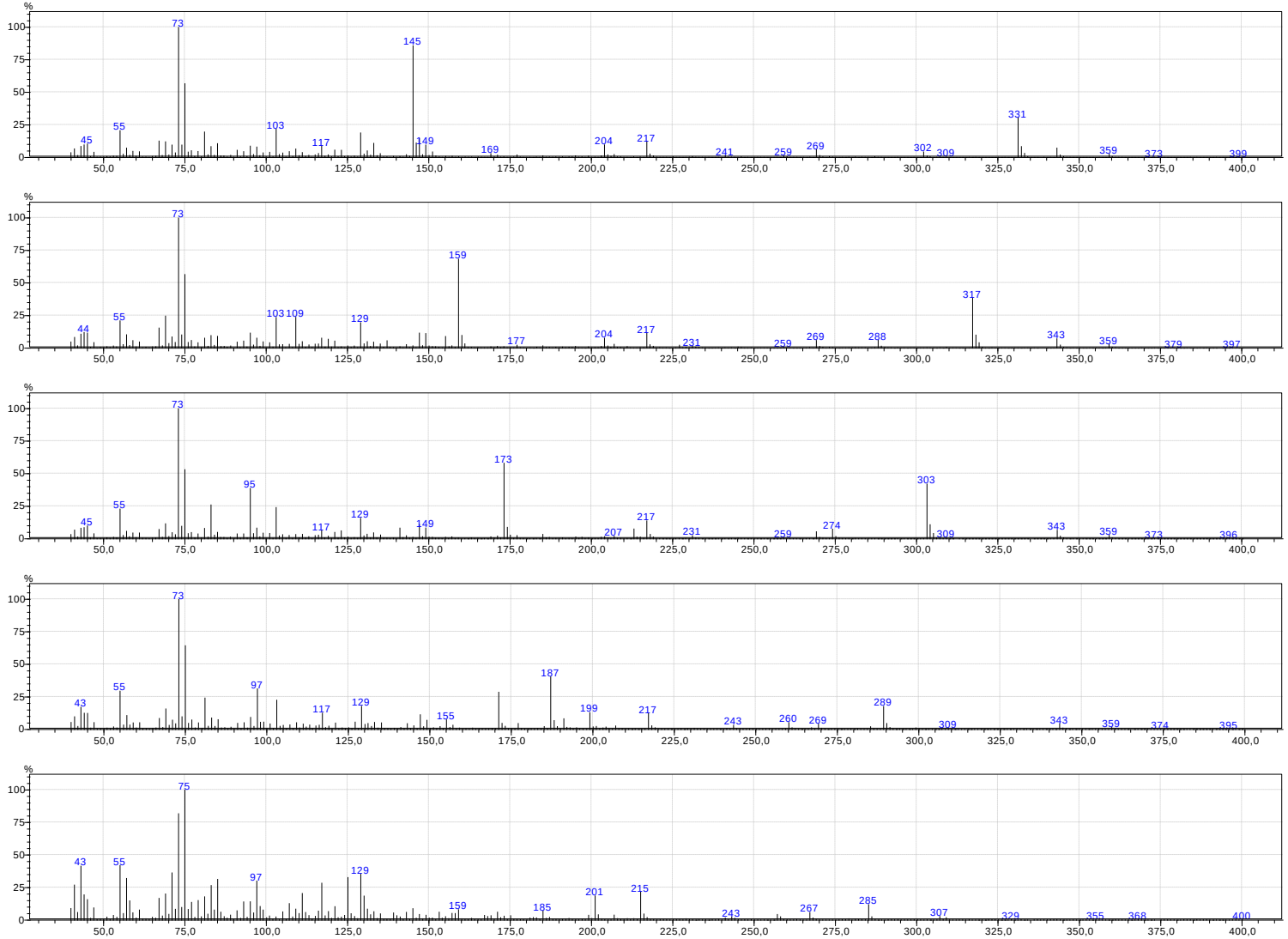
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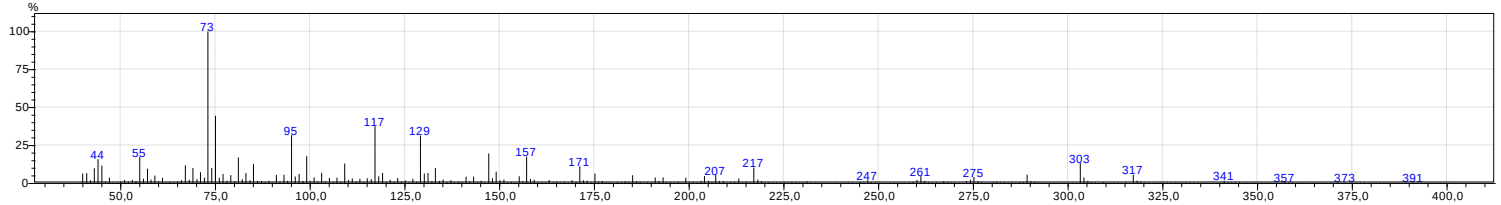


Figure S36: GC and MS chromatograms of conversion of **27**. Reactions were conducted in 50 mM sodium phosphate buffer pH 7.0 with 2 mM $MgCl_2$, 1 mM substrate, 4 mM hydrogen peroxide, 1.3 µM *Abr*UPO, 8 mM ascorbate at 25 °C and 600 rpm for 180 min. MS chromatograms of **27** (7.543 min), ω-1 (10.263 min), ω-2 (10.109 min), ω-3 (9.850 min), ω-4 (9.696 min), ω-5 (9.598 min), ω-6 (9.531 min), ω-7 (9.313 min) and γ (11.845 min) in sorted order.

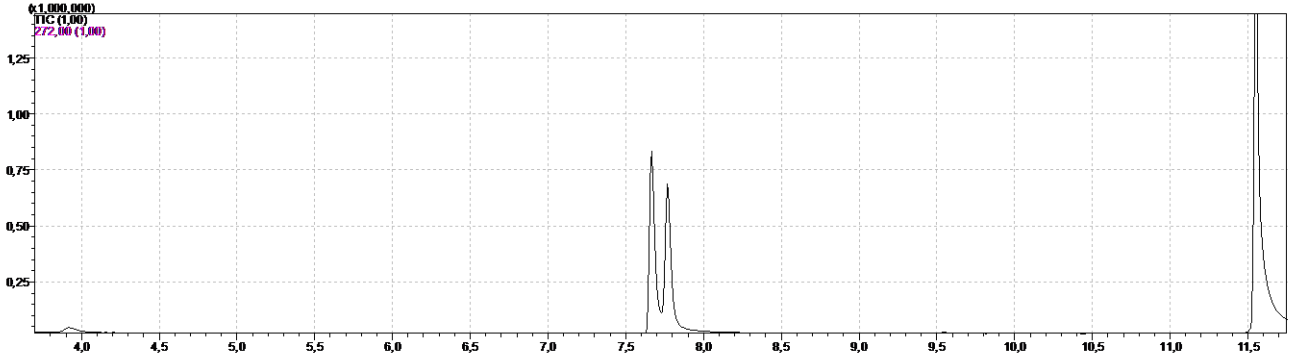
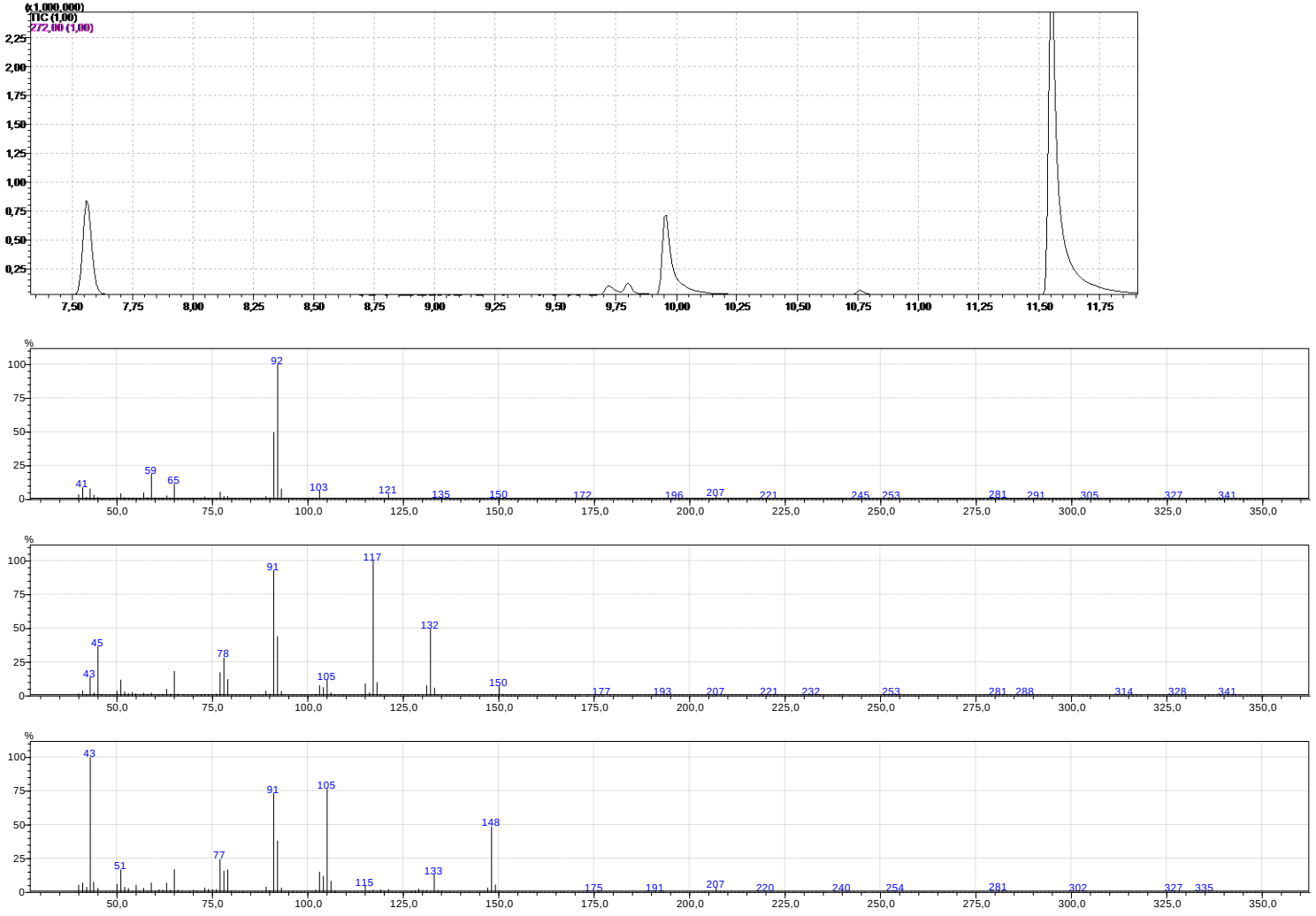


Figure S37: GC and MS chromatograms of conversion of **1**. Reactions were conducted in 50 mM sodium phosphate buffer pH 7.0 with 2 mM $MgCl_2$, 1 mM substrate, 4 mM hydrogen peroxide, 1.3 µM PaDa-I, 8 mM ascorbate at 25 °C and 600 rpm for 180 min.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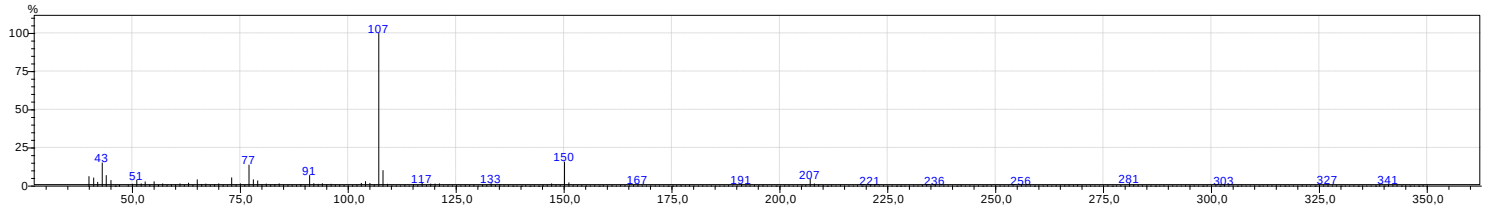

Figure S38: GC and MS chromatograms of conversion of **3**. Reactions were conducted in 50 mM sodium phosphate buffer pH 7.0 with 2 mM $MgCl_2$, 1 mM substrate, 4 mM hydrogen peroxide, 1.3 µM PaDa-I, 8 mM ascorbate at 25 °C and 600 rpm for 180 min. MS chromatograms of **3f** (9.720 min), **3g** (9.955 min), **3h** (9.800 min) and **3i** (10,761 min) in sorted order.

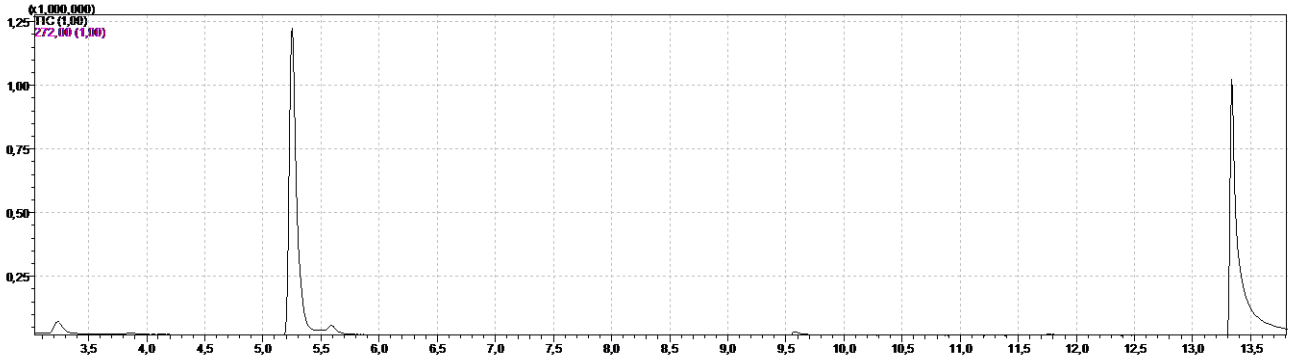

Figure S39: GC and MS chromatograms of conversion of **4**. Reactions were conducted in 50 mM sodium phosphate buffer pH 7.0 with 2 mM $MgCl_2$, 1 mM substrate, 4 mM hydrogen peroxide, 1.3 µM PaDa-I, 8 mM ascorbate at 25 °C and 600 rpm for 180 min.

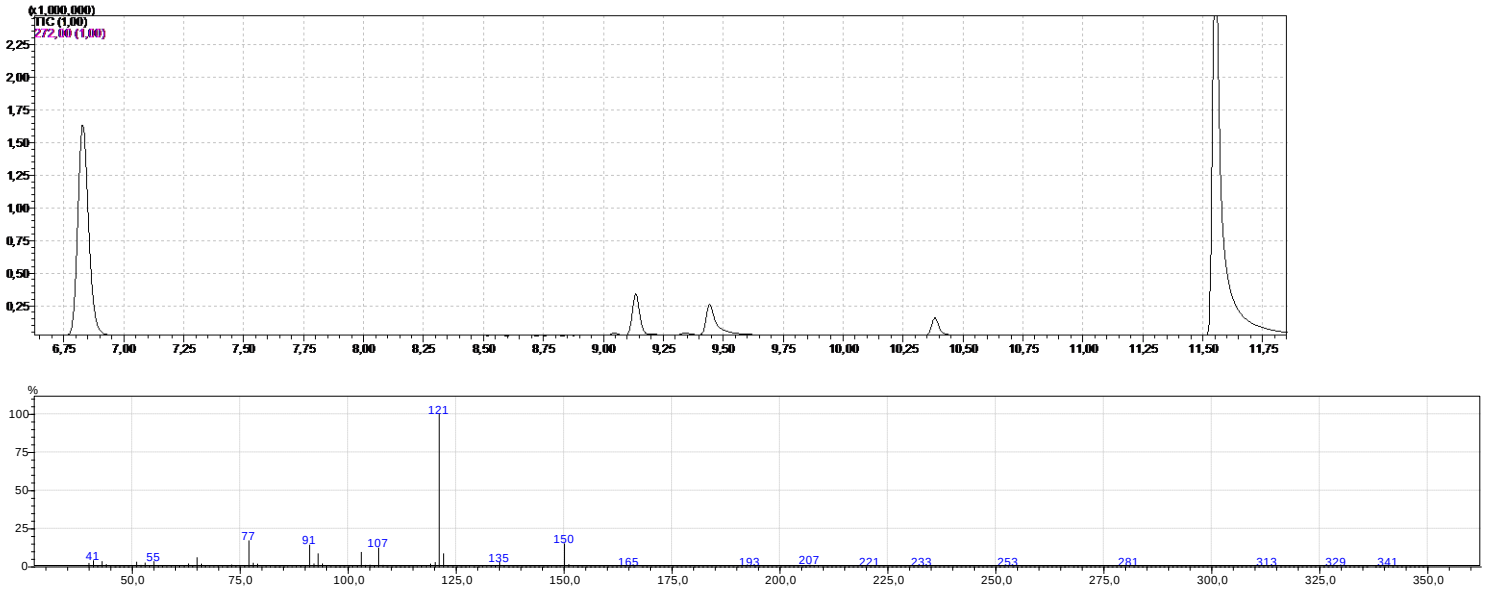

Figure S40: GC and MS chromatograms of conversion of **5**. Reactions were conducted in 50 mM sodium phosphate buffer pH 7.0 with 2 mM $MgCl_2$, 1 mM substrate, 4 mM hydrogen peroxide, 1.3 µM PaDa-I, 8 mM ascorbate at 25 °C and 600 rpm for 180 min. MS chromatogram of **5f** (10.383 min).

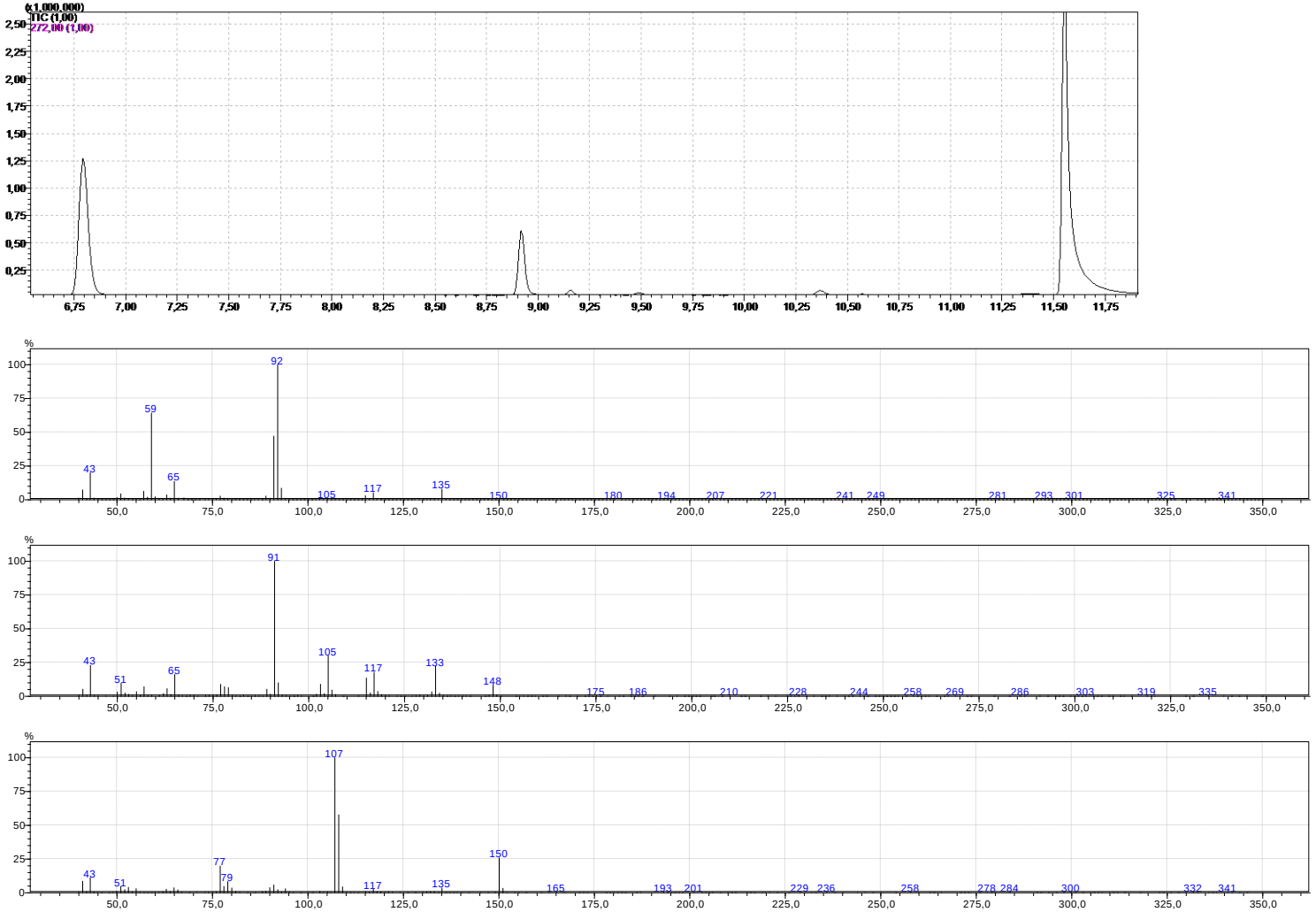

Figure S41: GC and MS chromatograms of conversion of **6**. Reactions were conducted in 50 mM sodium phosphate buffer pH 7.0 with 2 mM $MgCl_2$, 1 mM substrate, 4 mM hydrogen peroxide, 1.3 µM PaDa-I, 8 mM ascorbate at 25 °C and 600 rpm for 180 min. MS chromatograms of **6e** (8.919 min), **6f** (9.157 min) and **6g** (10.367 min).

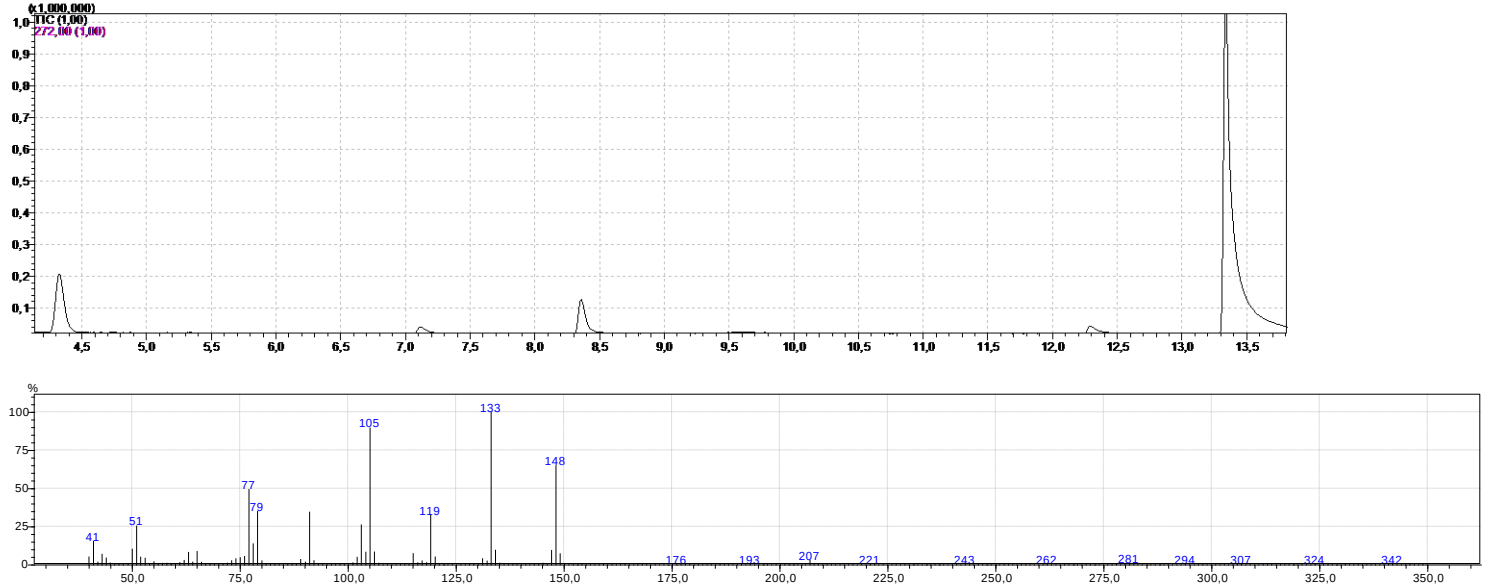

Figure S42: GC and MS chromatograms of conversion of **7**. Reactions were conducted in 50 mM sodium phosphate buffer pH 7.0 with 2 mM $MgCl_2$, 1 mM substrate, 4 mM hydrogen peroxide, 1.3 µM PaDa-I, 8 mM ascorbate at 25 °C and 600 rpm for 180 min. MS chromatogram of **7e** (8,358 min).

4 References

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