Supplementary Material (ESI) for Analyst

SUPPORTING ON LINE SUPPLEMENTARY MATERIAL FOR

Use of Isotope Mass Probes for Metabolic Analysis of the Jasmonate Biosynthetic Pathway

Yun-Qing Huang[a], Jia-Qi Liu[a], Hanyu Gong[b], Jing Yang[b], Yangsheng Li[b]

Yu-Qi Feng[a]*

[a]*Key Laboratory of Analytical Chemistry for Biology and Medicine (Ministry of Education), Department of Chemistry, Wuhan University, Wuhan 430072, China*

[b]*College of Life Sciences, Wuhan University, Wuhan 430072, P.R. China*

* Corresponding author. Tel.:+86-27-68755595; fax: +86-27-68755595.

E-mail address: **yqfeng@whu.edu.cn**

Supporting figures

Figure S1. Procedure for pretreatment of rice seedlings and isotope mass probe labeling. MeOH, methanol; Et_2O, ethyl ether; BPB, ω-bromoacetonylpyridinium bromide; d^5-BPB, d^5-ω-bromoacetonylpyridinium bromide.

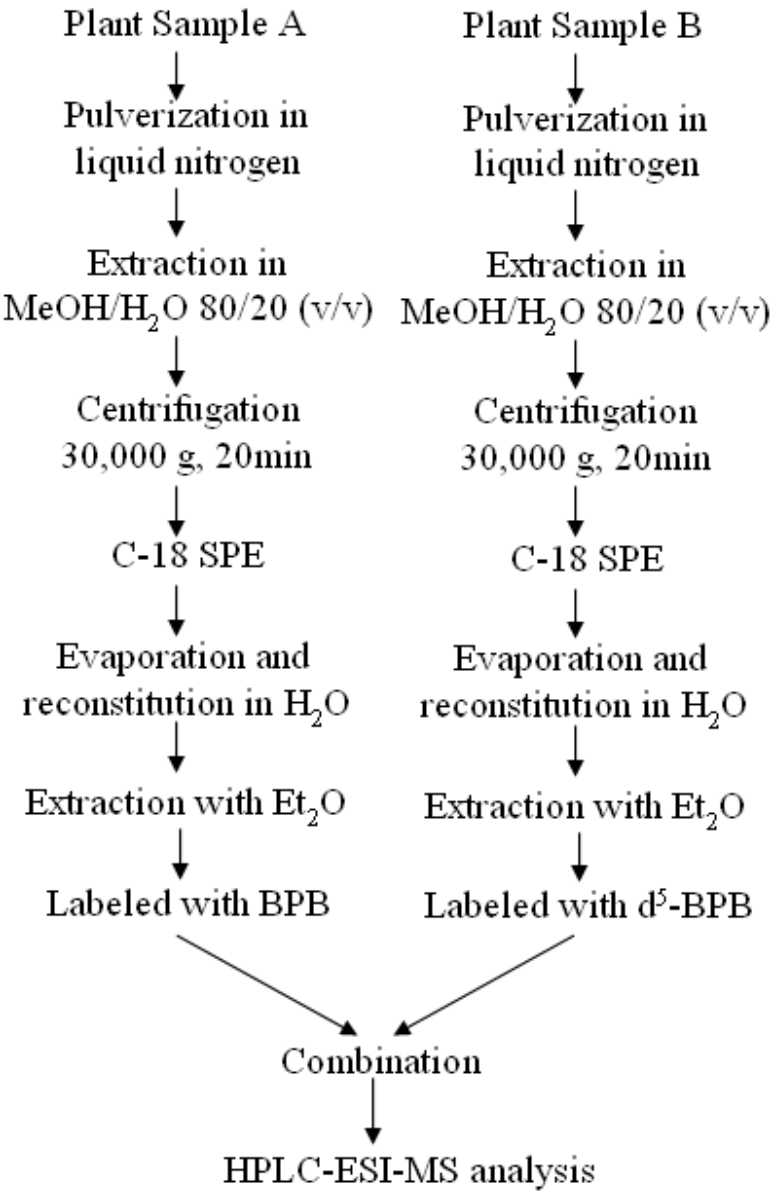


Figure S2. Optimization of labeling procedures from standard solutions. (A) Optimization of TEA concentration. The concentration of BPB was fixed at 3 nmol/mL (10 molar excess). The molar ratio of TEA over total amount of LA (0.1 nmol/mL), OPDA (0.1 nmol/mL) and JA (0.1 nmol/mL) was varied from 2 to 15. Reactions were carried out at room temperature for 2 hours. (B) Optimization of BPB molar ratios over a total amount of LA (0.1 nmol/mL), OPDA (0.1 nmol/mL) and JA (0.1 nmol/mL) for labeling. BPB was mixed with LA, OPDA and JA at molar ratios of 2 to 15 in the presence of TEA (10 molar excess) at room temperature for 2 hours. (C) Optimization of the reaction temperature. The reactions were performed with BPB at a molar ratio of 10:1 in the presence of TEA (10 molar excess) for 120 minutes. (D) Optimization of labeling time. The time was varied from 10 to 120 minutes. BPB was mixed with LA, OPDA and JA at a molar ratio of 10:1 in the presence of TEA (10 molar excess). The reactions were performed at constant room temperature at time periods of 10 min, 20 min, 30 min, 60 min and 120 min.

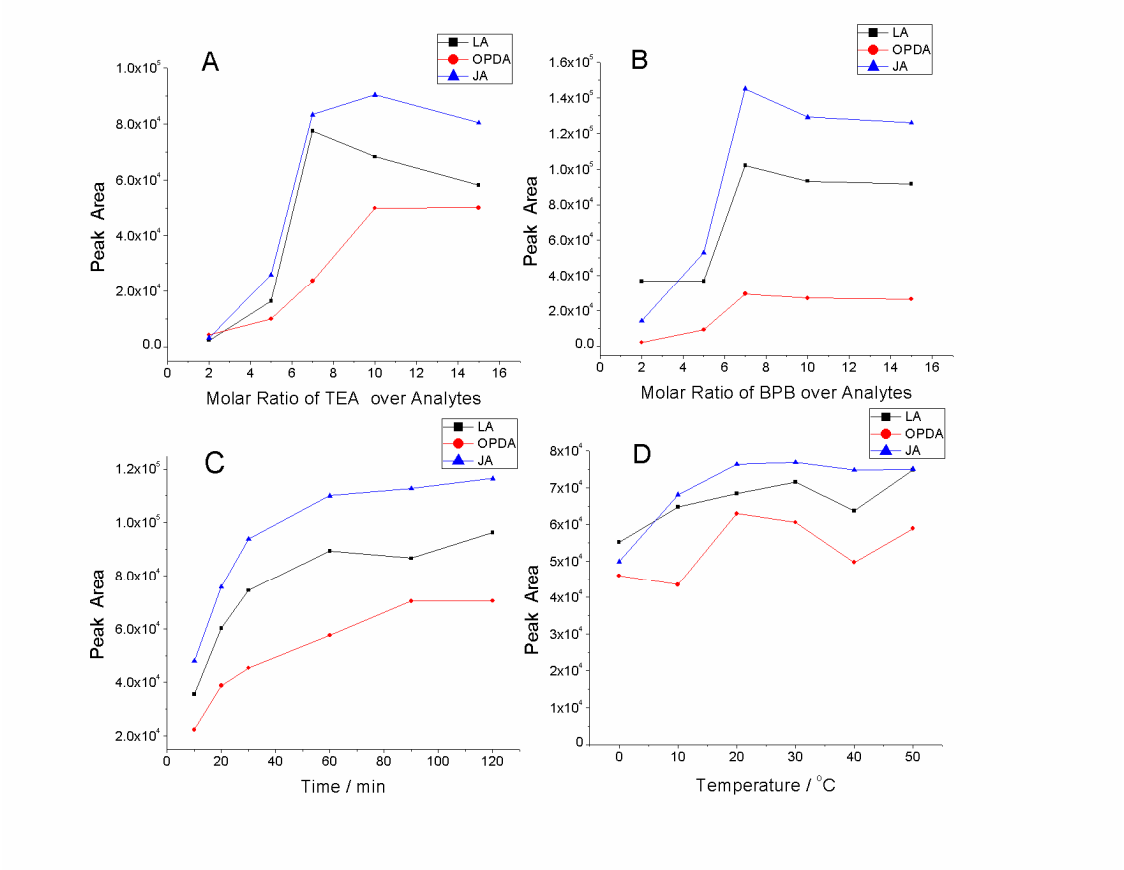


Figure S3. Examination of the stability of labeled derivatives. The change of the derivatives' intensity in solution was monitored over a period of 24 hours.

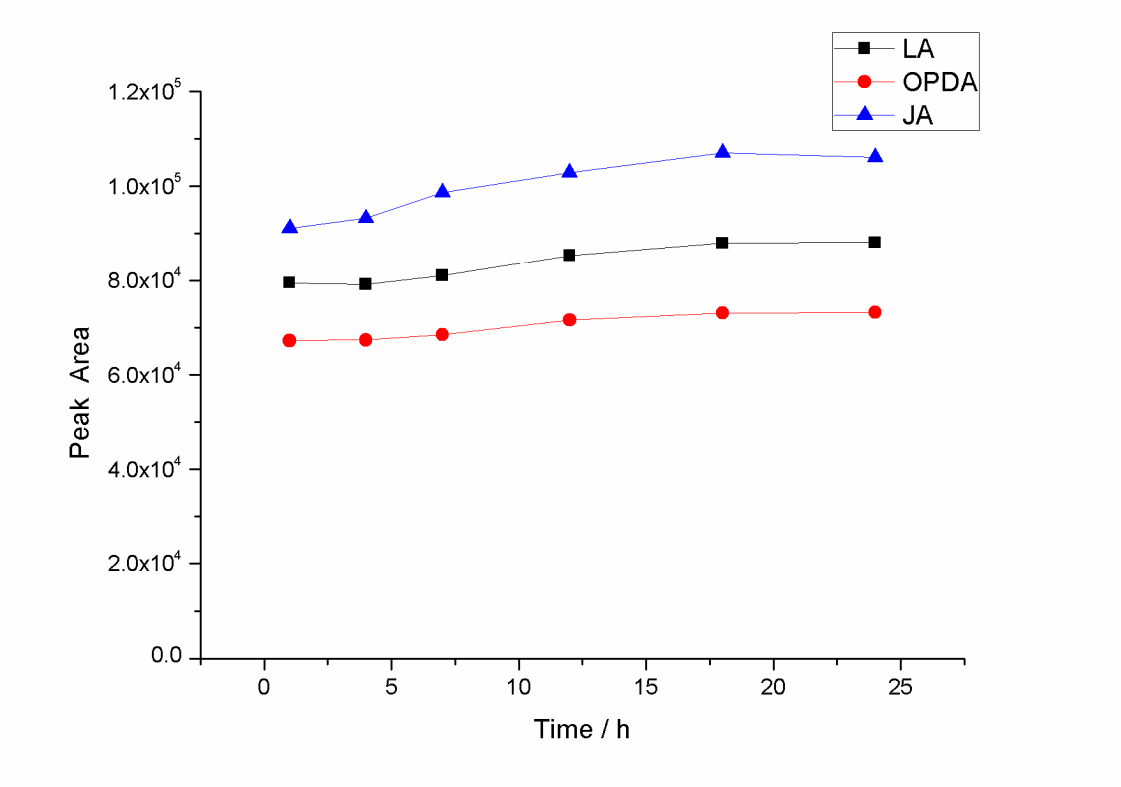


Figure S4. Optimization of labeling procedures of rice samples. (A) Optimization of TEA concentration for rice samples. The concentrations of TEA was varied from 0.5-2.5 μmol/mL. The concentration of BPB was set at 2.0 μmol/mL and the reactions were carried out at room temperature for 2 hours. (B) Optimization of BPB concentration in rice samples. The concentrations of BPB was varied from 0.25-2.0 μmol/mL. The concentration of BPB was set at 1.5 μmol/mL and performed at room temperature for 2 hours.

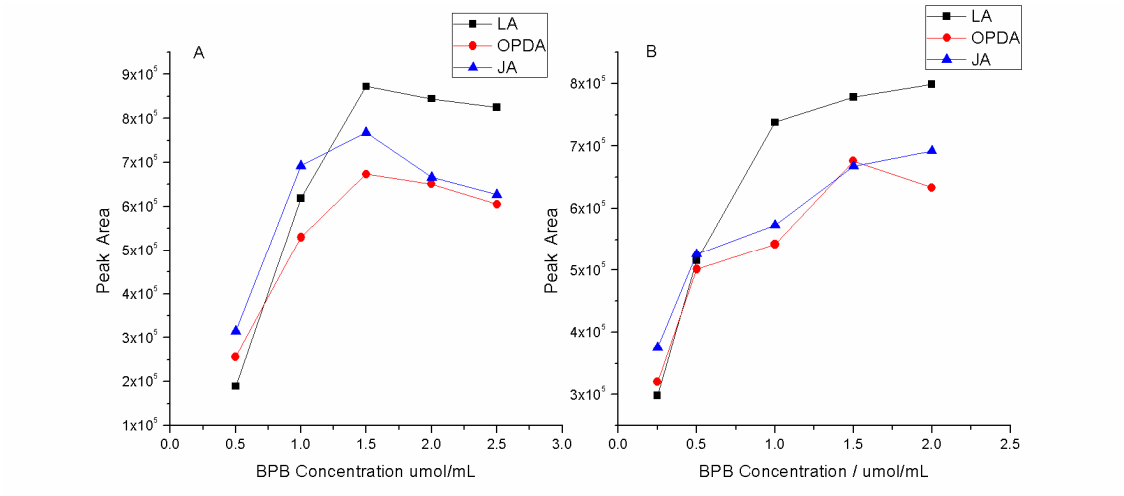


Figure S5. Comparison of ion signals between unlabeled (A) and labeled (B) analytes. The concentrations of LA, OPDA, OPC-8, OPC-6 and JA were set at 200 ng/mL each.

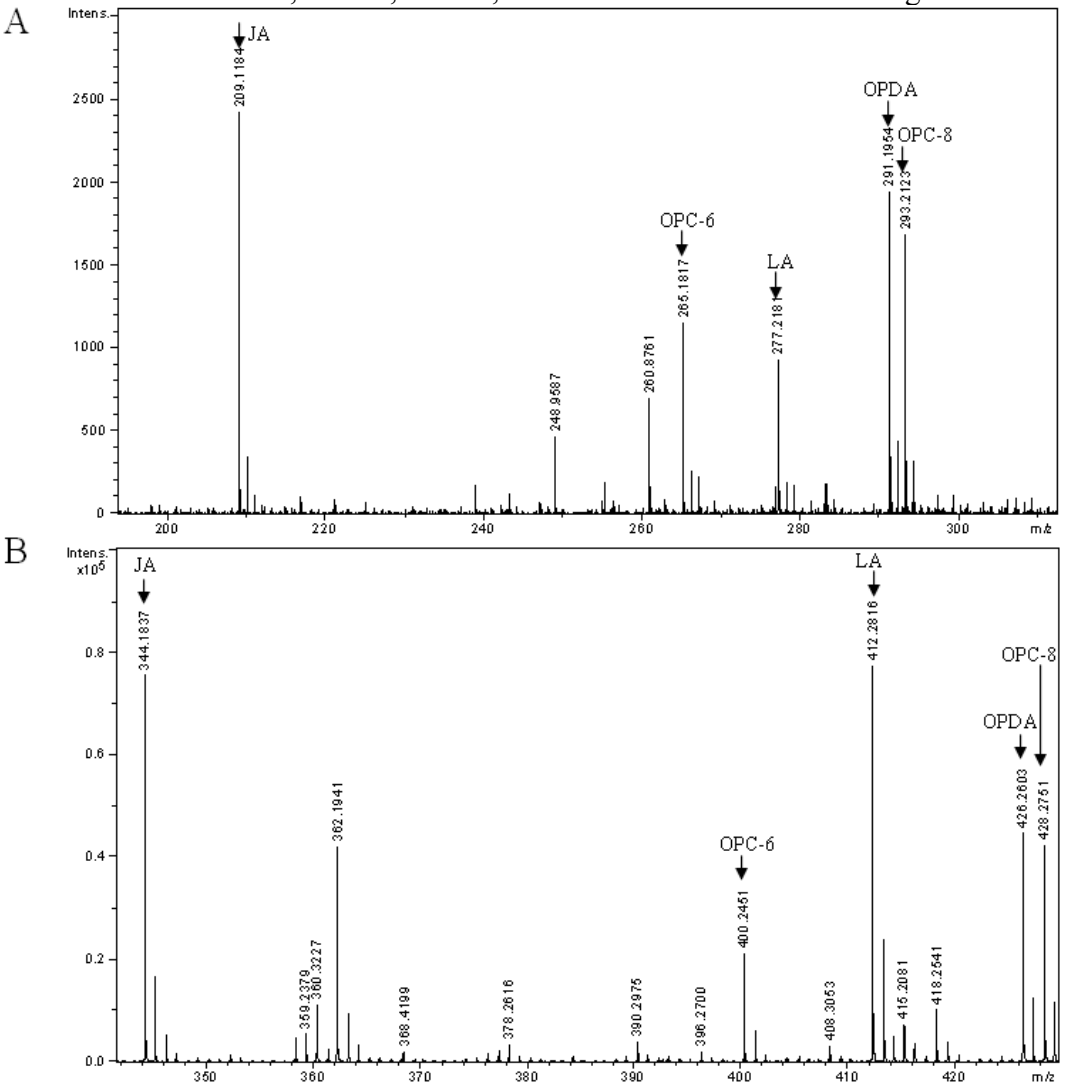


Figure S6. Linear regression plots of BPB/d^5-BPB labeled (A) LA, (B) OPDA, (C) OPC-8, (D) OPC-6 and (E) JA. The d^5-BPB labeled analytes were fixed at a concentration of 50 ng/mL, and the concentrations of BPB labeled analytes were varied from 5 ng/mL to 500 ng/mL. The relative standard deviation (RSD) of measured peak area ratios varied from 1.7-7.7%. Calibration parameters for the results were based on three replicate measurements.

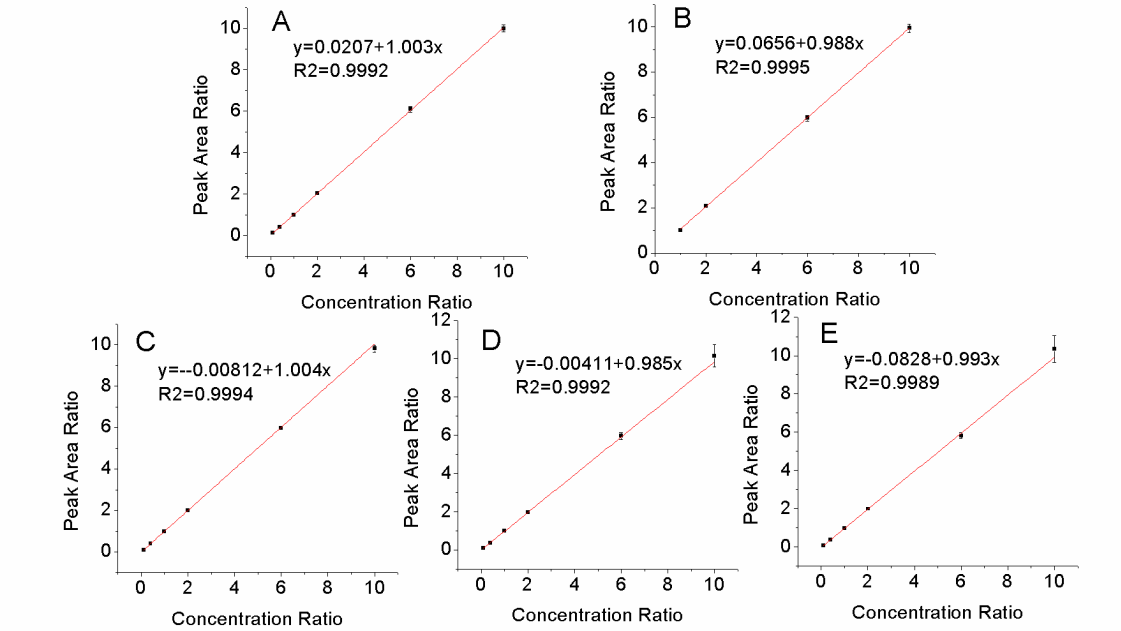


Figure S7. HPLC chromatograms of jasmonate biosynthetic pathway components from normally growing rice seedlings and salt treated rice seedlings. Normal growing rice seedlings were labeled with BPB (light) and salt treated rice seedlings were treated with d^5-BPB (heavy). Chromatographic conditions were: column, Shiseido RP/SCX (150×2.1 mm, 5 μm, Japan); sample injection volume, 10 μL; mobile phase, 80% and 20% aqueous solution (containing 30 mmol/L ammonium acetate). LA, linolenic acid; HPOT, (13S)-hydroperoxyoctadecatrienoic acid; OPDA, cis-(+)-12-oxophytodienoic acid; OPC-8, 3-oxo-2-(2'-pentenyl)-cyclopentane-1-octanoic acid; OPC-6, 3-oxo-2(2'-pentenyl)-cyclopentane-1-hexanoic acid; JA, jasmonic acid; JA-Val, jasmonic acid-valine conjugate.

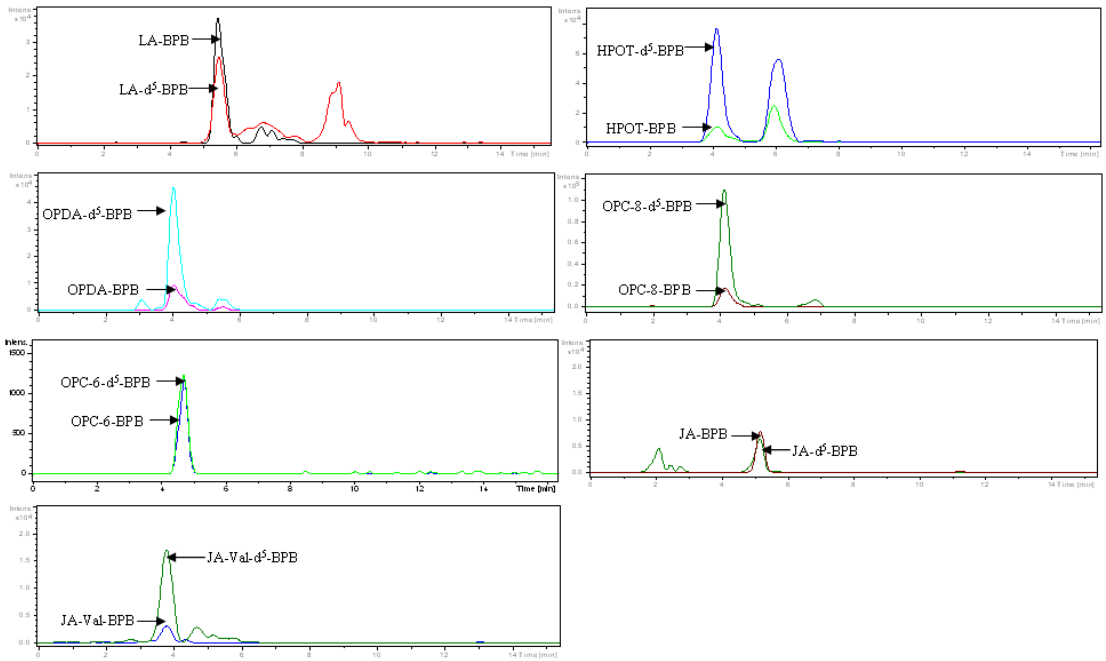
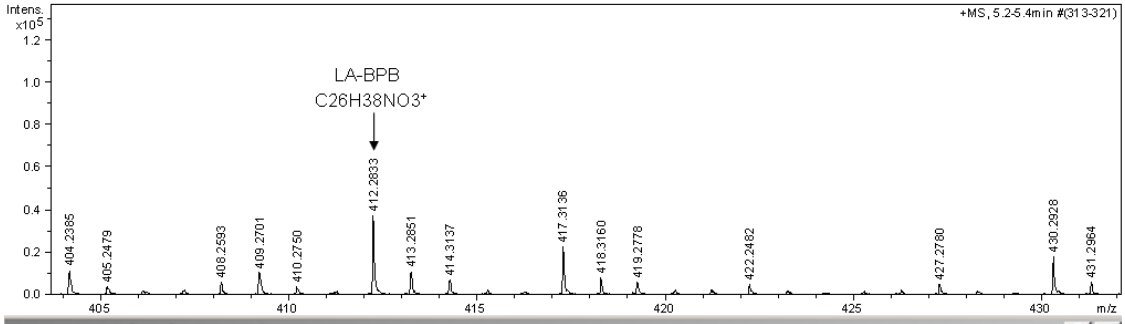


Figure S8. MS spectrums of peaks of the extracted traces and analytical results using Generate Molecular Formula (GMF) software. The theoretical values for $[LA\text{-}BPB]^+$, $[HPOT\text{-}BPB]^+$, $[OPDA\text{-}BPB]^+$, $[OPC\text{-}8\text{-}BPB]^+$, $[OPC\text{-}6\text{-}BPB]^+$, $[JA\text{-}BPB]^+$ and $[JA\text{-}Val\text{-}BPB]^+$ ions are m/z 412.2846, 444.2744, 426.2638, 428.2795, 400.2482, 344.1856 and 443.2540, respectively. The correct formulas were selected from among the many potential candidates using GMF and isotope abundance matching.



Generate Molecular Formula

Min C_{20} | Max | C 20-n

Generate | Save Results... | Help

Note: for m < 2000 the elements C, H, N, and O are considered implicitly.

Measured m/z 412.2833 | Tolerance 2 mDa | Charge 1

#	Mol. Formula	m/z	err [mDa]	|err| [ppm]	err [ppm]	mean err [ppm]	Sigma	Sigma Rank	rdb	N rule	e⁻
1	C 26 H 38 N 1 O 3	412.2846	1.29	3.1	3.1	-0.9	0.0788	1	8.5	ok	even
2	C 24 H 36 N 4 O 2	412.2833	-0.05	0.1	-0.1	-4.0	0.0819	2	9.0	ok	odd
3	C 23 H 40 O 6	412.2819	-1.39	3.4	-3.4	-9.9	0.0828	3	4.0	ok	odd
4	C 22 H 34 N 7 O 1	412.2819	-1.40	3.4	-3.4	-10.4	0.0855	4	9.5	ok	even

☐ Automatically locate monoisotopic peak | Maximum number of formulas 200

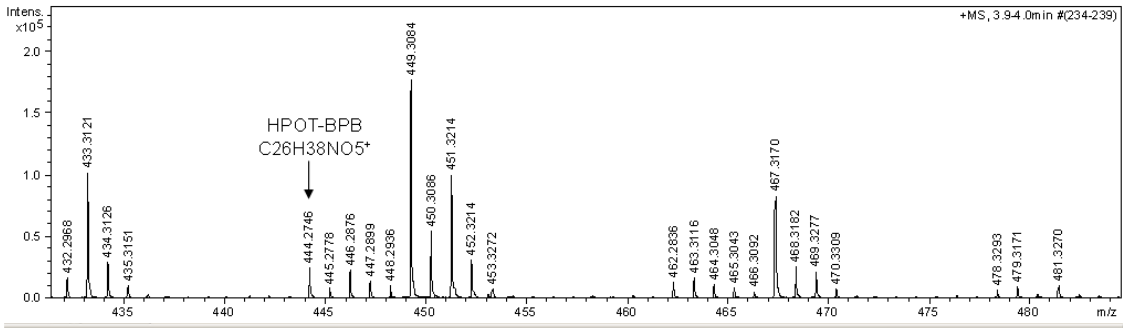
☐ Check rings plus double bonds | Minimum 0 | Maximum 0

☐ Apply nitrogen rule | Electron configuration both

☐ Filter H/C element ratio | Minimum H/C 0 | Maximum H/C 3

☑ Estimate carbon number | ☐ Generate immediately

Save for Reporting | Show Pattern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HPOT-BPB
C26H38NO5+
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449.3084
451.3214
467.3170
444.2746
450.3086
452.3214
468.3182
469.3277
432.2968
434.3126
435.3151
445.2778
446.2876
447.2899
448.2936
453.3272
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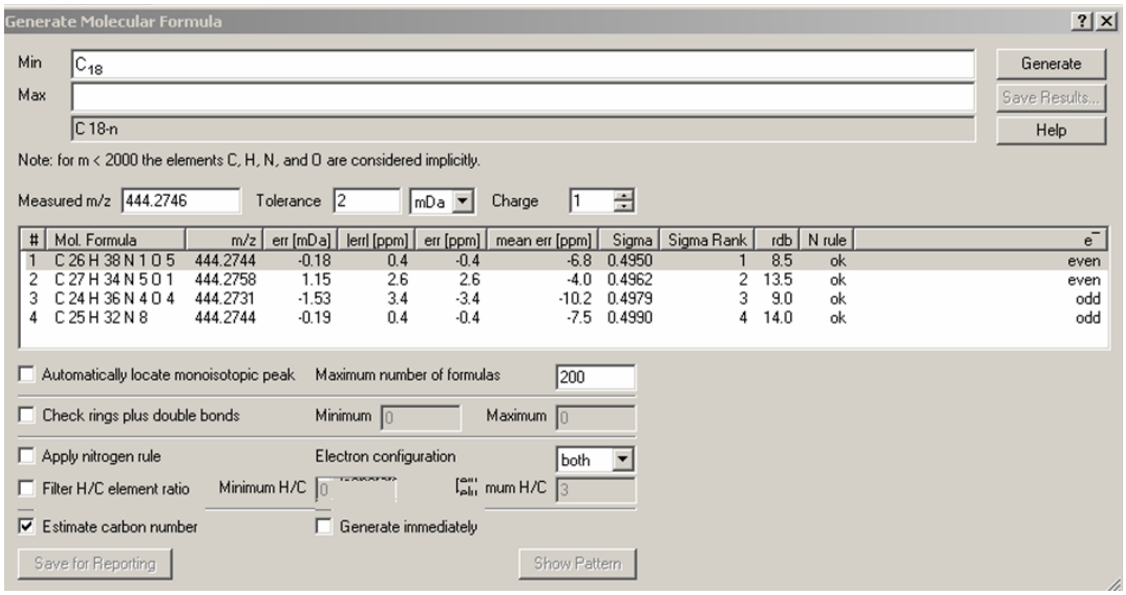
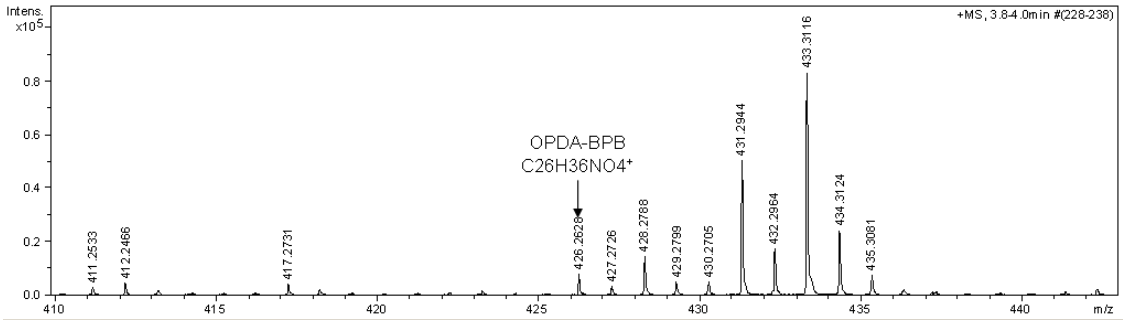
478.3293
479.3171
481.3270
m/z


Generate Molecular Formula
Min
C18
Max
C 18-n
Generate
Save Results...
Help
Note: for m < 2000 the elements C, H, N, and O are considered implicitly.
Measured m/z 444.2746
Tolerance 2
mDa
Charge 1
Mol. Formula m/z err [mDa] |err| [ppm] err [ppm] mean err [ppm] Sigma Sigma Rank rdb N rule e⁻
1 C 26 H 38 N 1 O 5 444.2744 -0.18 0.4 -0.4 -6.8 0.4950 1 8.5 ok even
2 C 27 H 34 N 5 O 1 444.2758 1.15 2.6 2.6 -4.0 0.4962 2 13.5 ok even
3 C 24 H 36 N 4 O 4 444.2731 -1.53 3.4 -3.4 -10.2 0.4979 3 9.0 ok odd
4 C 25 H 32 N 8 444.2744 -0.19 0.4 -0.4 -7.5 0.4990 4 14.0 ok odd
Automatically locate monoisotopic peak
Maximum number of formulas 200
Check rings plus double bonds
Minimum 0
Maximum 0
Apply nitrogen rule
Electron configuration both
Filter H/C element ratio
Minimum H/C 0
mum H/C 3
Estimate carbon number
Generate immediately
Save for Reporting
Show Pattern



Generate Molecular Formula

Min C_0 | Max | C 0-n

Generate | Save Results... | Help

Note: for m < 2000 the elements C, H, N, and O are considered implicitly.

Measured m/z 426.2628 | Tolerance 2 mDa | Charge 1

#	Mol. Formula	m/z	err [mDa]	|err| [ppm]	err [ppm]	mean err [ppm]	Sigma	Sigma Rank	rdb	N rule	e⁻
1	C 26 H 36 N 1 O 4	426.2639	1.08	2.5	2.5	-12.3	0.6123	1	9.5	ok	even
2	C 23 H 38 O 7	426.2612	-1.60	3.8	-3.8	-18.8	0.6127	2	5.0	ok	odd
3	C 24 H 34 N 4 O 3	426.2625	-0.27	0.6	-0.6	-16.0	0.6145	3	10.0	ok	odd
4	C 22 H 32 N 7 O 2	426.2612	-1.61	3.8	-3.8	-19.8	0.6167	4	10.5	ok	even
5	C 11 H 42 N 2 O 14	426.2631	0.25	0.6	0.6	-16.3	0.6180	5	-8.0	ok	odd
6	C 12 H 38 N 6 O 10	426.2644	1.59	3.7	3.7	-13.6	0.6197	6	-3.0	ok	odd
7	C 9 H 40 N 5 O 13	426.2617	-1.09	2.6	-2.6	-20.0	0.6203	7	-7.5	ok	even
8	C 10 H 36 N 9 O 9	426.2631	0.24	0.6	0.6	-17.4	0.6220	8	-2.5	ok	even
9	C 11 H 32 N 13 O 5	426.2644	1.58	3.7	3.7	-15.0	0.6237	9	2.5	ok	even
10	C 8 H 34 N 12 O 8	426.2617	-1.10	2.6	-2.6	-21.3	0.6242	10	-2.0	ok	odd
11	C 9 H 30 N 16 O 4	426.2630	0.24	0.6	0.6	-19.3	0.6259	11	3.0	ok	odd
12	C 10 H 26 N 20	426.2644	1.57	3.7	3.7	-18.0	0.6277	12	8.0	ok	odd
13	C 7 H 28 N 19 O 3	426.2617	-1.11	2.6	-2.6	-23.8	0.6282	13	3.5	ok	even

☐ Automatically locate monoisotopic peak | Maximum number of formulas 200

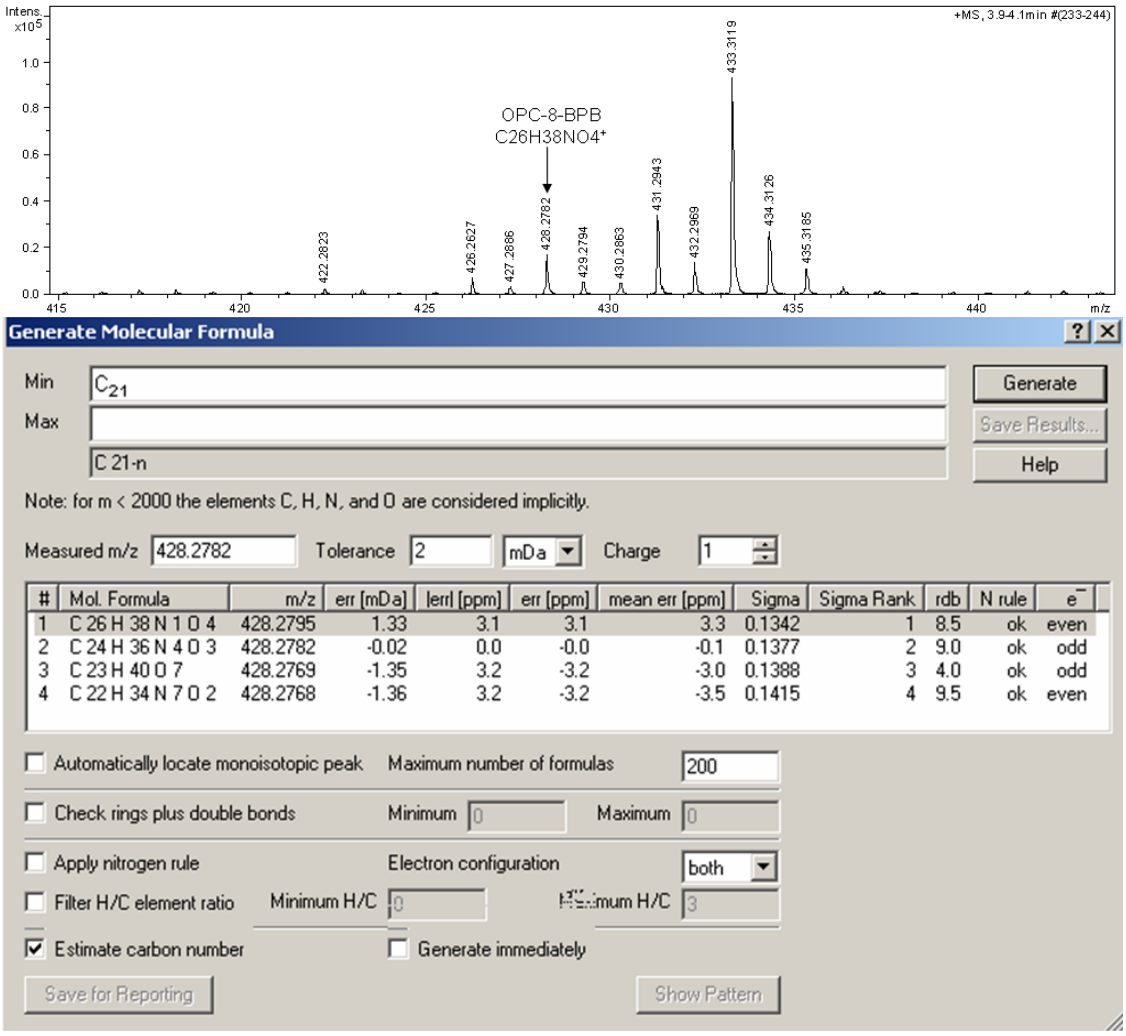
☐ Check rings plus double bonds | Minimum 0 | Maximum 0

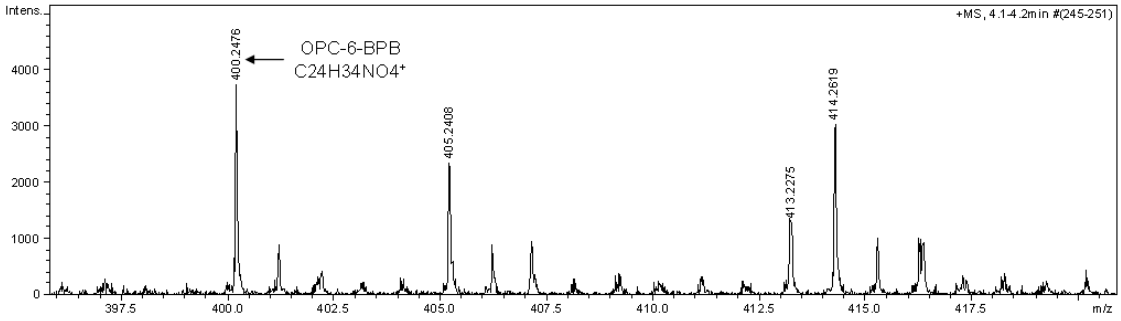
☐ Apply nitrogen rule | Electron configuration both

☐ Filter H/C element ratio | Minimum H/C 0 | Maximum H/C 3

☑ Estimate carbon number | ☐ Generate immediately

Save for Reporting | Show Pattern


Intens.
x10^5
+MS, 3.9-4.1min #(233-244)
OPC-8-BPB
C26H38NO4+
422.2823
426.2627
427.2886
428.2782
429.2794
430.2863
431.2943
432.2969
433.3119
434.3126
435.3185
m/z
Generate Molecular Formula
Min
C21
Max
C 21-n
Generate
Save Results...
Help
Note: for m < 2000 the elements C, H, N, and O are considered implicitly.
Measured m/z 428.2782
Tolerance 2
mDa
Charge 1
Mol. Formula m/z err [mDa] |err| [ppm] err [ppm] mean err [ppm] Sigma Sigma Rank rdb N rule e⁻
1 C 26 H 38 N 1 O 4 428.2795 1.33 3.1 3.1 3.3 0.1342 1 8.5 ok even
2 C 24 H 36 N 4 O 3 428.2782 -0.02 0.0 -0.0 -0.1 0.1377 2 9.0 ok odd
3 C 23 H 40 O 7 428.2769 -1.35 3.2 -3.2 -3.0 0.1388 3 4.0 ok odd
4 C 22 H 34 N 7 O 2 428.2768 -1.36 3.2 -3.2 -3.5 0.1415 4 9.5 ok even
Automatically locate monoisotopic peak
Maximum number of formulas 200
Check rings plus double bonds
Minimum 0
Maximum 0
Apply nitrogen rule
Electron configuration both
Filter H/C element ratio
Minimum H/C 0
Maximum H/C 3
Estimate carbon number
Generate immediately
Save for Reporting
Show Pattern



Generate Molecular Formula

Min C_{20} | Max | C 20-n

Generate | Save Results... | Help

Note: for m < 2000 the elements C, H, N, and O are considered implicitly.

Measured m/z 400.2476 | Tolerance 2 mDa | Charge 1

#	Mol. Formula	m/z	err [mDa]	|err| [ppm]	err [ppm]	mean err [ppm]	Sigma	Sigma Rank	rdb	N rule	e⁻
1	C 22 H 32 N 4 O 3	400.2469	-0.74	1.9	-1.9	-1.9	0.1503	1	9.0	ok	odd
2	C 24 H 34 N 1 O 4	400.2482	0.60	1.5	1.5	1.5	0.1571	2	8.5	ok	even
3	C 25 H 30 N 5	400.2496	1.94	4.8	4.8	4.8	0.1704	3	13.5	ok	even

☐ Automatically locate monoisotopic peak — Maximum number of formulas 200

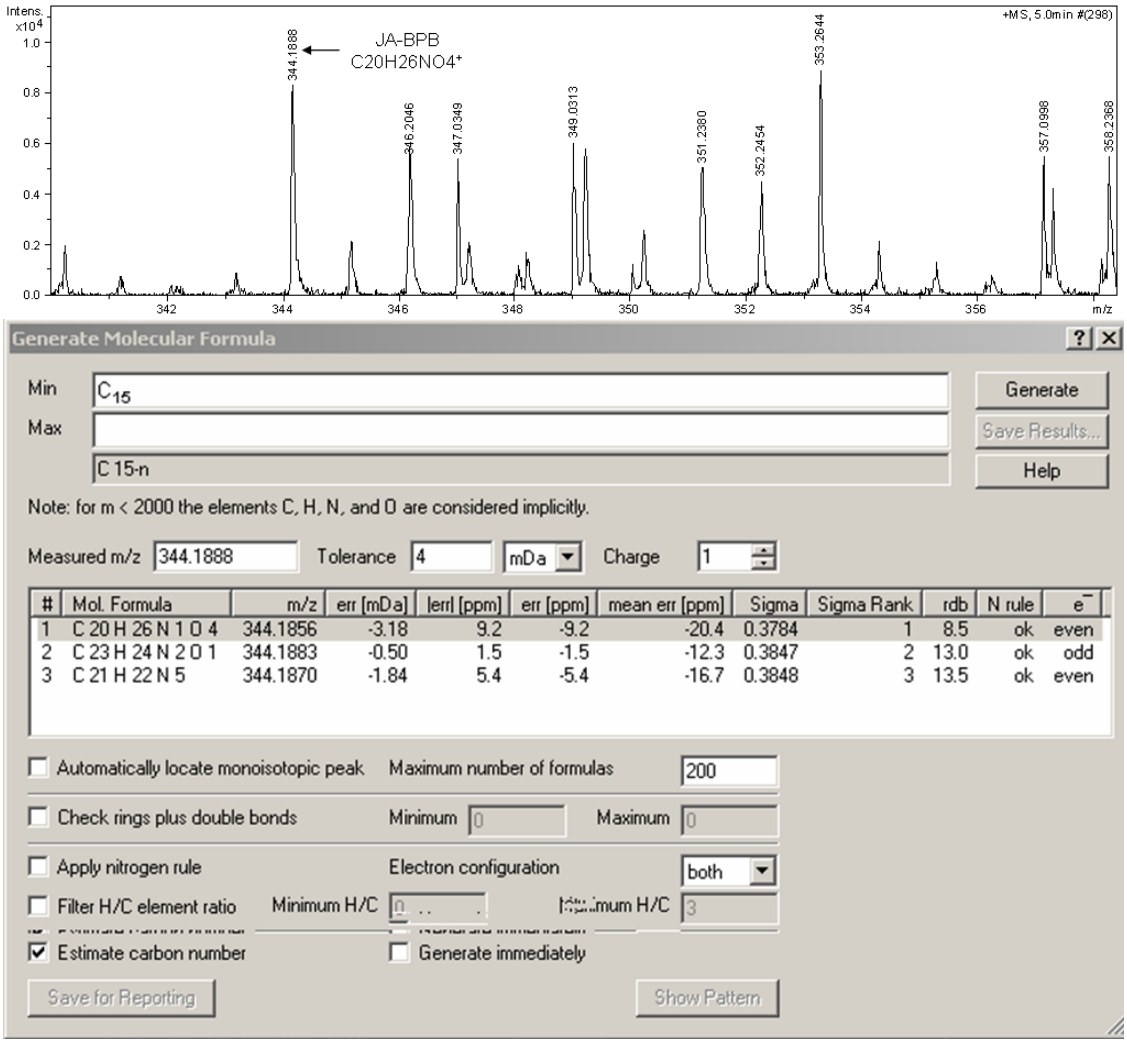
☐ Check rings plus double bonds — Minimum 0 — Maximum 0

☐ Apply nitrogen rule — Electron configuration both

☐ Filter H/C element ratio — Minimum H/C 0 — Maximum H/C 3

☑ Estimate carbon number ☐ Generate immediately

Save for Reporting | Show Pattern


Intens.
x10^4
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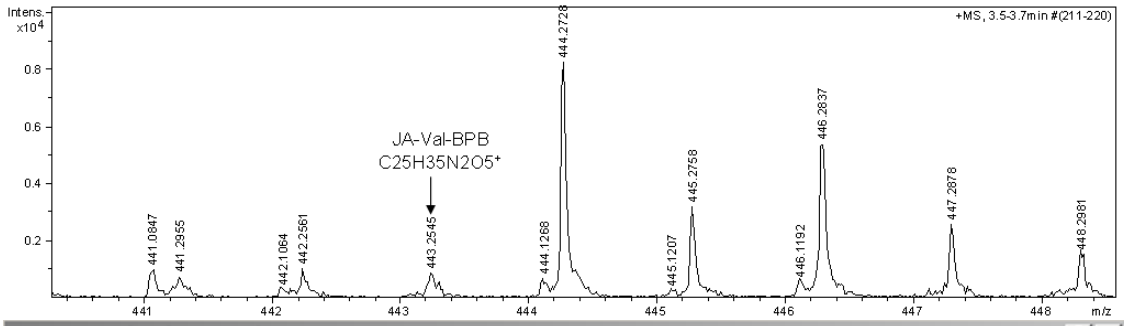
JA-BPB
C20H26NO4+
344.1888
346.2046
347.0349
349.0313
351.2380
352.2454
353.2644
357.0998
358.2368
m/z
Generate Molecular Formula
Min
C15
Max
C 15-n
Generate
Save Results...
Help
Note: for m < 2000 the elements C, H, N, and O are considered implicitly.
Measured m/z 344.1888
Tolerance 4
mDa
Charge 1
Mol. Formula m/z err [mDa] |err| [ppm] err [ppm] mean err [ppm] Sigma Sigma Rank rdb N rule e⁻
1 C 20 H 26 N 1 O 4 344.1856 -3.18 9.2 -9.2 -20.4 0.3784 1 8.5 ok even
2 C 23 H 24 N 2 O 1 344.1883 -0.50 1.5 -1.5 -12.3 0.3847 2 13.0 ok odd
3 C 21 H 22 N 5 344.1870 -1.84 5.4 -5.4 -16.7 0.3848 3 13.5 ok even
Automatically locate monoisotopic peak
Maximum number of formulas 200
Check rings plus double bonds
Minimum 0
Maximum 0
Apply nitrogen rule
Electron configuration both
Filter H/C element ratio
Minimum H/C 0
Maximum H/C 3
Estimate carbon number
Generate immediately
Save for Reporting
Show Pattern



Generate Molecular Formula

Min C_{20}

Max

C 20-n

Generate | Save Results... | Help

Note: for m < 2000 the elements C, H, N, and O are considered implicitly.

Measured m/z 443.2545 Tolerance 4 mDa Charge 1

#	Mol. Formula	m/z	err [mDa]	lerr| [ppm]	err [ppm]	mean err [ppm]	Sigma	Sigma Rank	rdb	N rule	e⁻
1	C 26 H 31 N 6 O 1	443.2554	0.85	1.9	1.9	-30.3	0.6837	1	14.5	ok	even
2	C 24 H 29 N 9	443.2540	-0.49	1.1	-1.1	-34.0	0.6883	2	15.0	ok	odd
3	C 25 H 35 N 2 O 5	443.2540	-0.49	1.1	-1.1	-32.7	0.6915	3	9.5	ok	even
4	C 23 H 33 N 5 O 4	443.2527	-1.83	4.1	-4.1	-36.4	0.6962	4	10.0	ok	odd

☐ Automatically locate monoisotopic peak Maximum number of formulas 200

☐ Check rings plus double bonds Minimum 0 Maximum 0

☐ Apply nitrogen rule Electron configuration both

☐ Filter H/C element ratio Minimum H/C 0 Maximum H/C 3

☑ Estimate carbon number ☐ Generate immediately

Save for Reporting | Show Pattern

Figure S9. ^{1}H-NMR spectrum of 1,3-Dibromo-2,2-dimethoxypropane in $CDCl_3$.

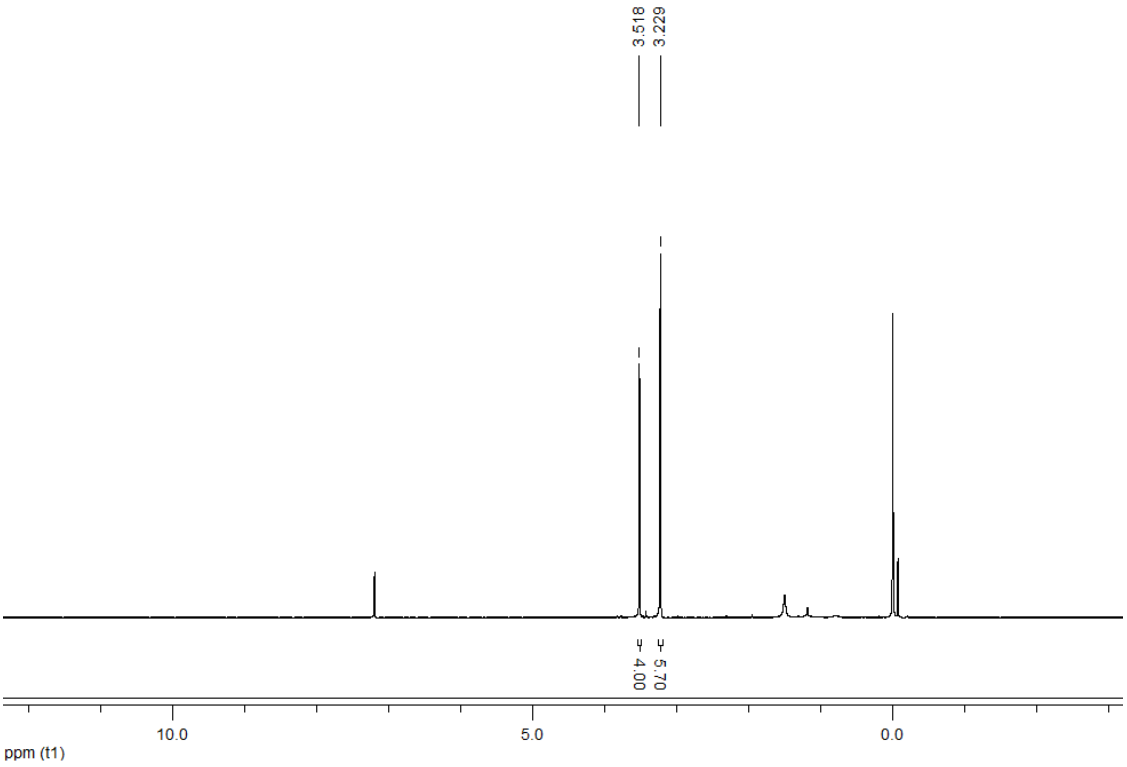


Figure S10. GC-MS chromatogram and mass spectrum of 1,3-dibromo-2,2-dimethoxypropane.

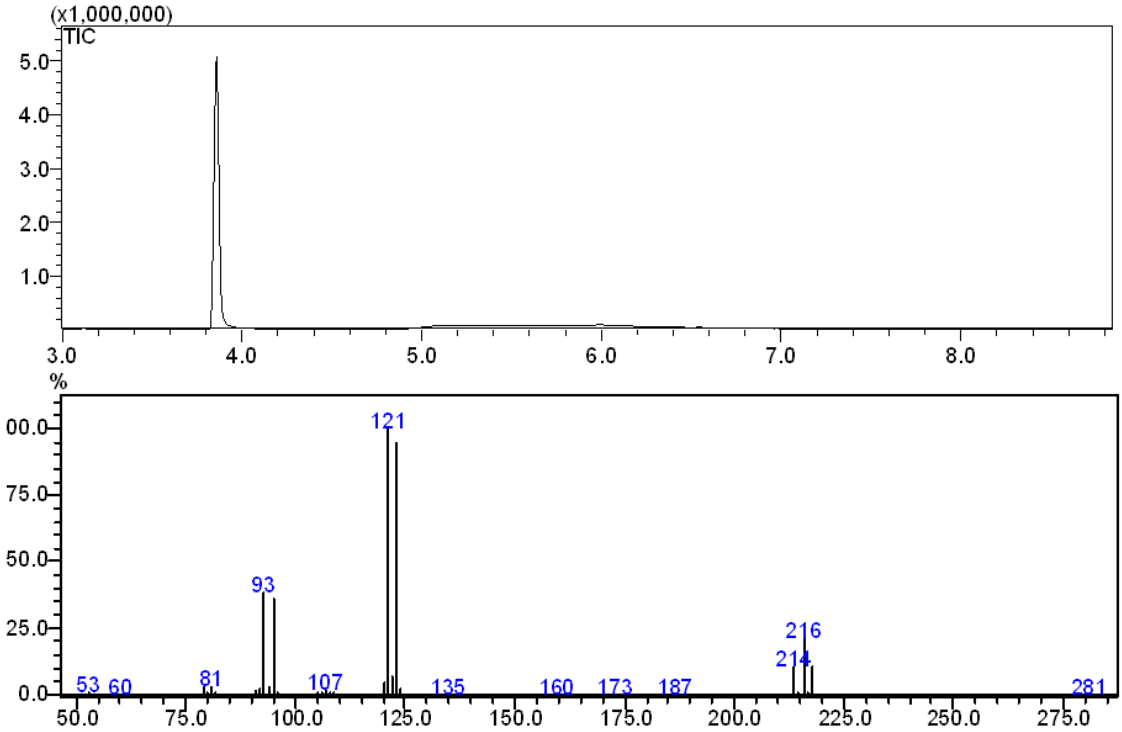


Figure S11. ^{1}H-NMR spectrum of 1,3-dibromoacetone in $CDCl_3$.

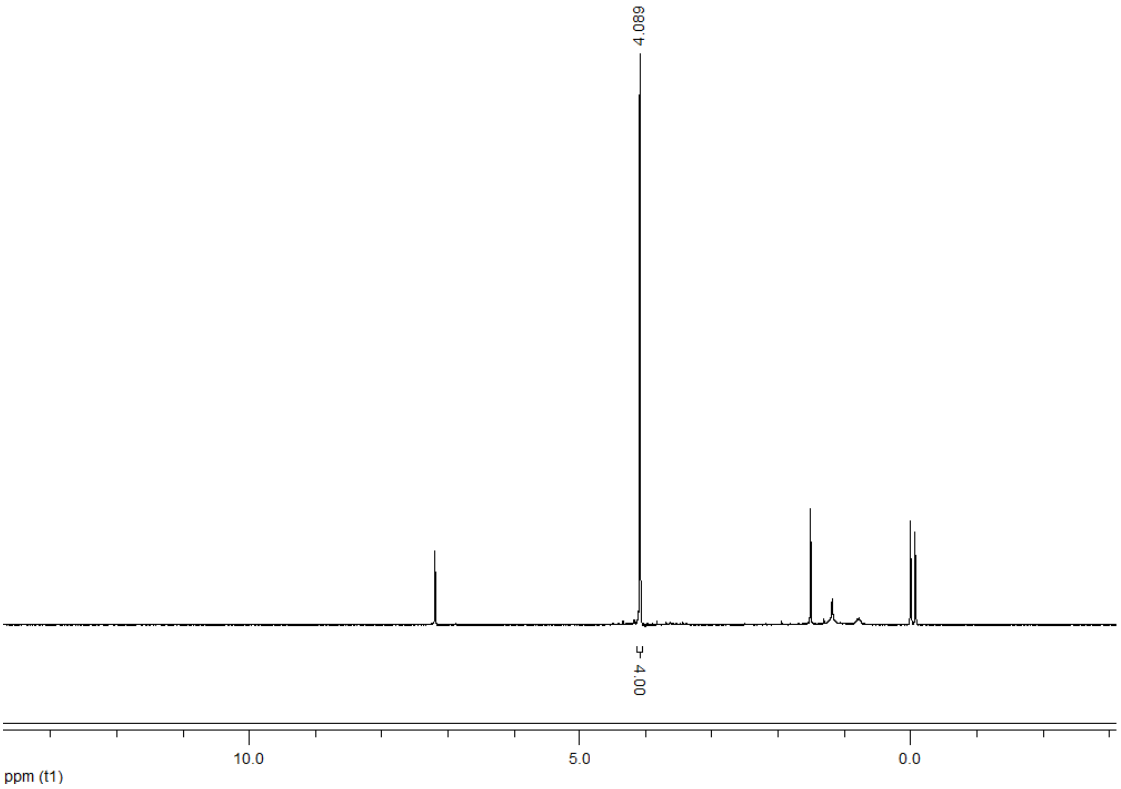


Figure S12. GC-MS chromatogram and mass spectrum of 1,3-dibromoacetone.

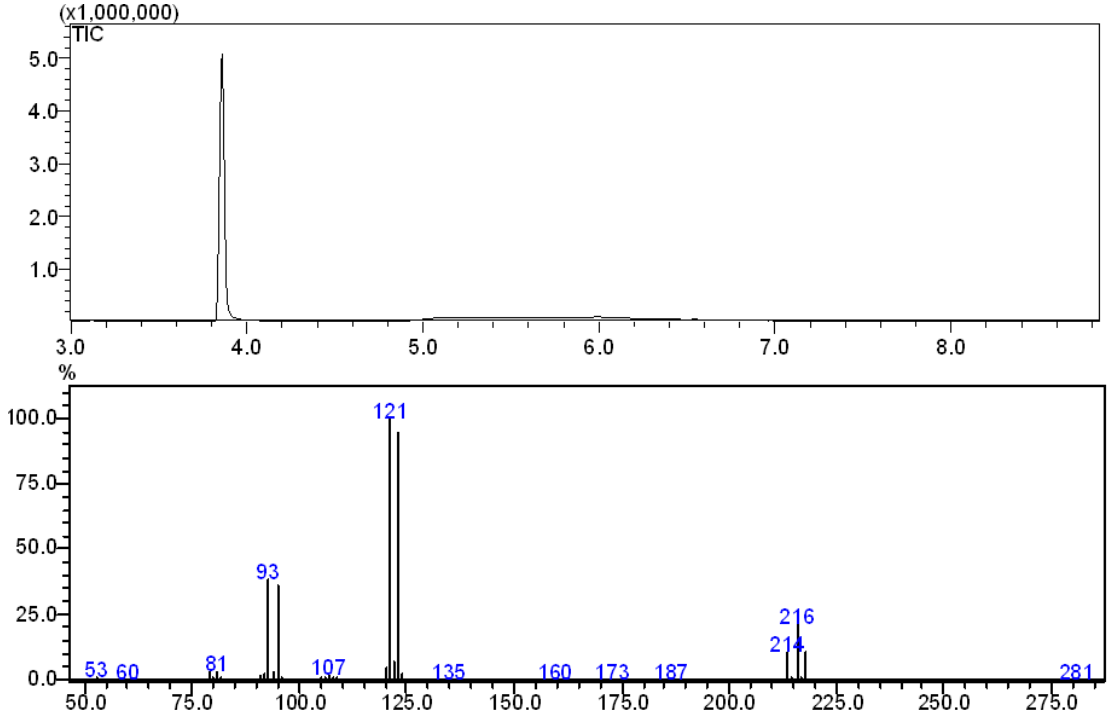


Figure S13. ^{1}H-NMR spectrum of BPB in DMSO-d6.

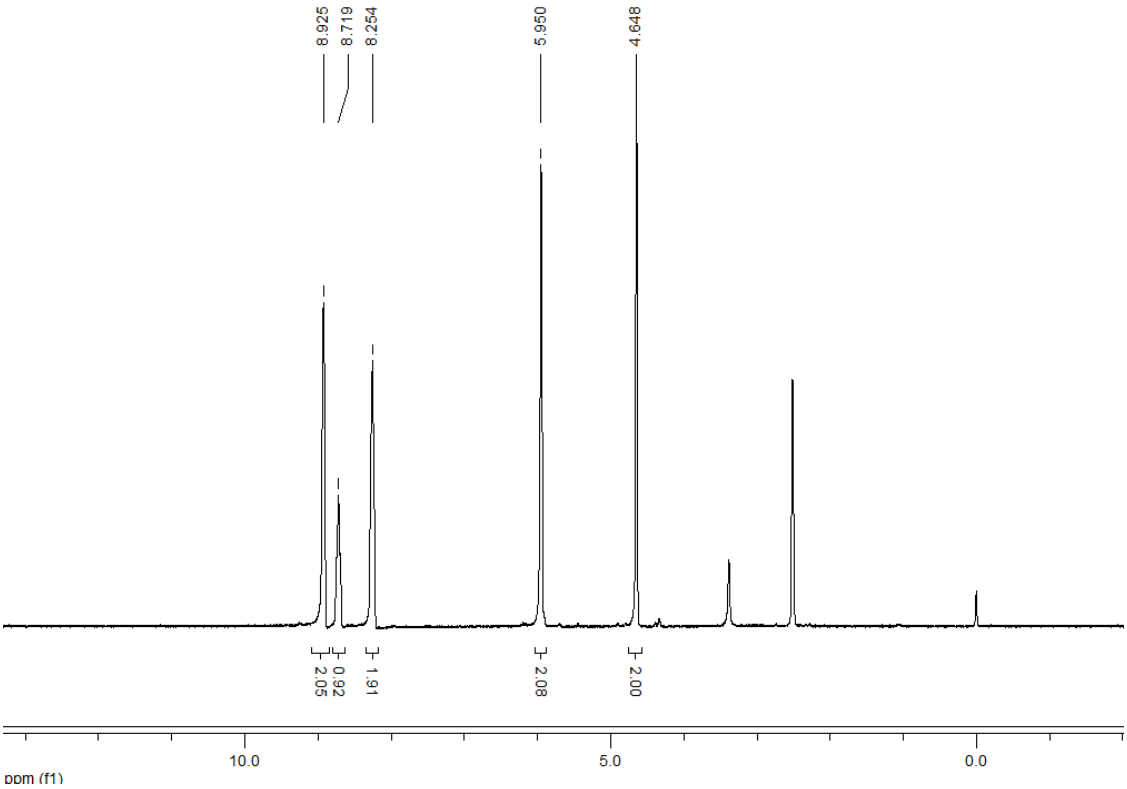


Figure S14. ^{1}H-NMR spectrum of d^{5}-BPB in DMSO-d6.

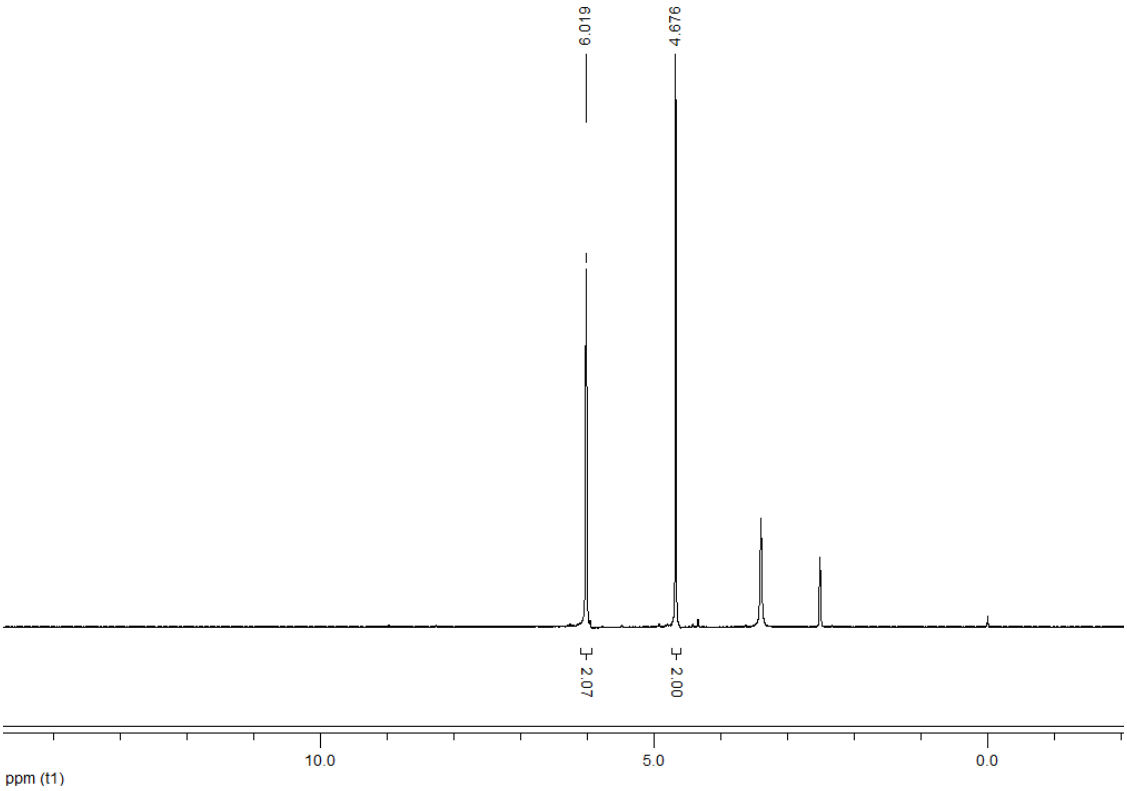


Figure S15. HR-MS spectrum of BPB.

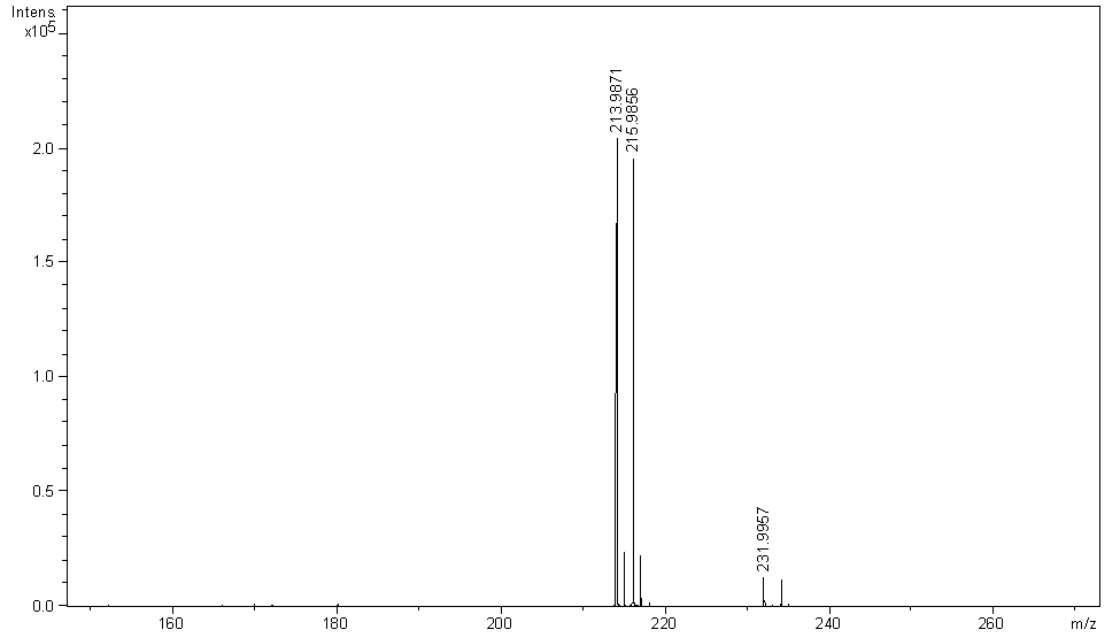


Figure S16. HR-MS spectrum of d^5-BPB.