Supplementary Information

Reaction Engineering Enables Selective Chemoenzymatic Transformation of Alkynes into α-Bromoketones and 1,2-Dibromostyrenes

Jiangtao Sha [a, b], Huanhuan Li [b], Kun Guo *[a], Jie Zhang [b], Jianqun Peng [b], Junyi Cao [b], Bishuang Chen *[c], and Wuyuan Zhang *[b]

[a] School of Chemical Engineering and Technology, Xi'an Jiaotong University, Xi'an 710049, Shanxi, China.

[b] Key Laboratory of Engineering Biology for Low-carbon Manufacturing, Tianjin Institute of Industrial Biotechnology, Chinese Academy of Sciences, Tianjin 300308, China.

[c] School of Marine Sciences, Sun Yat-Sen University, Zhuhai 519082, Guangdong, China.

*Corresponding author: kun.guo@xjtu.edu.cn; zhangwy@tib.cas.cn; chenbsh23@mail.sysu.edu.cn

Table of Contents

Experimental Procedures

Expression information of *Ci*VCPO

Host: *Escherichia coli* BL21(DE3)

Plasmid: pET-28a (+)-*Ci*VCPO

The DNA sequence of recombinant plasmid (the blue text denotes the plasmid sequence):

ATGGGCAGCAGCCATCATCATCATCATCACAGCAGCGGCCTGGTGCCGCGCGGCAGCCATATGGCTAG
CATGACTGGTGGACAGCAAATGGGTCGCGGATCCATGGGCAGCGTTACGCCGATTCCGCTGCCGAAA
ATTGATGAACCGGAAGAATATAATACCAACTATATTCTGTTTTGGAACCATGTTGGTCTGGAACTGAATC
GTGTTACCCATACCGTGGGTGGTCCGCTGACGGGCCCTCCTCTGAGCGCACGTGCACTGGGTATGCTG
CATCTGGCAATTCATGATGCATATTTTAGCATTTGTCCTCCGACCGATTTTACCACCTTTCTGAGTCCGGA
TACCGAAAATGCGGCCTATCGTCTGCCGTCACCGAATGGTGCCAATGATGCTCGTCAGGCCGTTGCAGG
CGCAGCCCTGAAAATGCTGAGCAGTCTGTATATGAAACCTGTTGAACAGCCGAATCCGAATCCGGGCG
CAAACATTAGCGATAATGCGTATGCACAGCTGGGTCTGGTTCTGGATCGTAGTGTTCTGGAAGCCCCTG
GTGGTGTCGATCGTGAATCTGCTAGCTTTATGTTTGGTGAAGATGTTGCAGATGTTTTTTTTGCGCTGCT
GAATGATCCTCGTGGCGCATCTCAGGAAGGTTATCATCCGACCCCTGGTCGTTATAAATTTGATGATGAA
CCGACCCATCCGGTTGTGCTGATTCCTGTTGATCCTAATAATCCGAATGGTCCGAAAATGCCGTTTCGCC
AGTATCATGCGCCTTTTTATGGTAAAACCACCAAACGTTTTGCAACACAGAGCGAACATTTTCTGGCAG
ATCCTCCTGGTCTGCGTAGCAATGCAGATGAAACCGCCGAATATGATGATGCCGTCCGTGTTGCAATTG
CAATGGGCGGCGCCCAGGCCCTGAATAGCACCAAACGTAGTCCTTGGCAGACCGCACAGGGTCTGTAT
TGGGCATACGATGGCAGCAATCTGATTGGTACACCGCCGCGTTTTTATAATCAGATTGTTCGTCGTATTG
CCGTTACCTATAAAAAGGAAGAAGATTTAGCAAACAGCGAAGTTAATAATGCAGATTTTGCACGCCTGT
TTGCCCTGGTCGATGTTGCATGTACTGATGCAGGCATTTTTAGCTGGAAAGAAAAATGGGAATTTGAAT
TTTGGCGTCCTCTGAGCGGTGTTCGTGATGATGGTCGTCCGGATCATGGTGATCCTTTTTGGCTGACCC
TGGGCGCCCCGGCAACGAATACAAATGATATTCCGTTTAAACCGCCGTTTCCGGCATATCCTAGCGGTC
ATGCAACCTTTGGTGGTGCAGTTTTTCAGATGGTTCGTCGTTATTATAATGGTCGTGTTGGTACGTGGAA
AGATGATGAACCTGATAATATTGCCATTGATATGATGATTAGCGAAGAACTGAATGGTGTTAATCGTGAT
CTGCGTCAGCCGTATGATCCTACGGCGCCGATTGAAGATCAGCCGGGTATTGTTCGTACCCGTATTGTTC
GTCATTTTGATAGCGCCTGGGAACTGATGTTTGAAAATGCAATTAGTCGTATTTTTCTGGGTGTTCATTG
GCGTTTTGATGCAGCAGCAGCACGTGATATTCTGATTCCTACCACCACTAAAGATGTTTATGCAGTTGAT
AATAACGGTGCAACCGTTTTTCAGAATGTTGAAGATATTCGTTATACCACCCGTGGTACACGCGAAGAT
CCTGAAGGTCTGTTTCCGATCGGCGGCGTTCCGCTGGGTATTGAAATTGCAGATGAAATTTTTAACAAC
GGTCTGAAACCGACCCCACCTGAAATTCAGCCTATGCCACAGGAAACCCCTGTTCAGAAACCGGTGGG
TCAGCAGCCGGTTAAAGGTATGTGGGAAGAAGAACAGGCCCCGGTGGTGAAAGAAGCACCGCTCGA
GCACCACCACCACCACCACTGAGATCCGGCT

The sequence of recombinant *Ci*VCPO (the blue text denotes the sequence of tags):

HHHHHHSSGLVPRGSHMASMTGGQQMGRGSMGSVTPIPLPKIDEPEEYNTNYILFWNHVGLELNRVT
HTVGGPLTGPPLSARALGMLHLAIHDAYFSICPPTDFTTFLSPDTENAAYRLPSPNGANDARQAVAGAALK
MLSSLYMKPVEQPNPNPGANISDNAYAQLGLVLDRSVLEAPGGVDRESASFMFGEDVADVFFALLNDPR
GASQEGYHPTPGRYKFDDEPTHPVVLIPVDPNNPNGPKMPFRQYHAPFYGKTTKRFATQSEHFLADPPG
LRSNADETAEYDDAVRVAIAMGGAQALNSTKRSPWQTAQGLYWAYDGSNLIGTPPRFYNQIVRRIAVTYK
KEEDLANSEVNNADFARLFALVDVACTDAGIFSWKEKWEFEFWRPLSGVRDDGRPDHGDPFWLTLGAP
ATNTNDIPFKPPFPAYPSGHATFGGAVFQMVRRYYNGRVGTWKDDEPDNIAIDMMISEELNGVNRDLR
QPYDPTAPIEDQPGIVRTRIVRHFDSAWELMFENAISRIFLGVHWRFDAAAARDILIPTTTKDVYAVDNNG
ATVFQNVEDIRYTTRGTREDPEGLFPIGGVPLGIEIADEIFNNGLKPTPPEIQPMPQETPVQKPVGQQPVK
GMWEEEQAPVVKEAPLEHHHHHH*

Preparation of cell free extract containing *Ci*VCPO

Preparation of *Ci*VCPO was conducted as reported previously[1]. The recombinant plasmids were transformed into *Escherichia coli* BL21 (DE3) and pre-cultivated in 30 mL Luria-Bertani (LB) medium (5 g/L yeast extract, 10 g/L tryptone, and 10 g/L NaCl) containing 50 mg/L kanamycin at 37 °C and 220 rpm overnight. Then, 2 L LB medium containing 50 mg/L kanamycin was inoculated with 1 mL of an overnight culture. Cells were grown at 37 °C and 220 rpm. When the optical density at 600 nm (OD_{600}) reached 0.6-1.0, IPTG was added for inducing protein expression at the final concentration of 0.5 mM, followed by incubation at 20 °C and 180 rpm for 20 h. The cells were harvested by centrifugation (8000 rpm, 5 min, 4 °C) and washed twice with 0.85% NaCl solution. The harvested cells were resuspended in Tris-H_2SO_4 buffer (50 mM, pH 8.0) and disrupted by ultrasonication for 30 min (30% power, 3 s on and 5 s off at 4 °C). The cell lysate was centrifugated at 4 °C (10000 rpm) for 30 min, and the supernatant was filtered through a 0.45 μm filter. Then, the filtrate was packed in a dialysis bag with a molecular cutoff of 10 kDa, and the target enzyme solution was salt-exchanged with Tris-H_2SO_4 buffer (50 mM, pH 8.0, 1 mM sodium orthovanadate). Enzyme activity was monitored by using phenol red method. Stop changing the salt until the enzyme activity stops changing, collect the enzyme solution, and concentrate it using a Millipore Amicon® Ultra centrifugal filters (10 kDa, 15 mL).

Phenol red analytical method: Incubating the enzyme in sodium citrate (100 mM, pH 5.0), 100 mM H_2O_2, 50 mM KBr, 100 μM phenol red at 25 °C.

Purification of *Ci*VCPO by Ni-NTA

The harvested cells were resuspended with binding buffer (pH 8.0, 0.05 M Tris-H_2SO_4 buffer, 0.3 M NaCl and 0.05 M imidazole) and disrupted by ultrasonication for 30 min (30% power, 3 s on and 5 s off at 4 °C). The cell lysate was centrifugated at 4 °C (10000 rpm) for 30 min, and

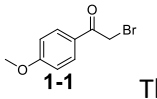
the supernatant was loaded onto a HisTrap™ FF crude column (GE, USA) equilibrated with binding buffer. Then, the samples were eluted with binding buffer at a flow rate of 5 mL/min to remove the impure proteins. Next, the elution of the target enzymes was conducted by gradually increasing the contents of an elution buffer (pH 8.0, 0.05 M Tris-H_2SO_4 buffer, 0.5 M NaCl and 0.3 M imidazole) with a flow rate of 3 mL/min. Then, the filtrate was packed in a dialysis bag with a molecular cutoff of 10 kDa, and the target enzyme solution was salt-exchanged with Tris-H_2SO_4 buffer (50 mM, pH 8.0, 1 mM sodium orthovanadate). The fractions containing target enzymes were concentrated by Millipore Amicon® Ultra centrifugal filters (10 kDa, 15 mL) with desalting buffer (pH 8.0, 0.05 M Tris-H_2SO_4 buffer). The purified protein fractions were subjected to SDS-PAGE analysis to determine its purity. Additionally, the protein concentrations were measured by the Bradford protocol. Enzyme activity was monitored by using chlorodimedon (MCD) method as previously reported.

MCD analytical method: Incubating the enzyme in sodium citrate (100 mM, pH 5.0), 5 mM H_2O_2, 5 mM KBr, 100 μM MCD (methanol solution) at 25°C.

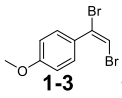
Procedure for the chemical synthesis of 1,2-dibromostyrene

The method for synthesizing 1,2-dibromostyrene was carried out according to the reported procedure with a slight modification[2]. Typically, the reaction was performed in a 1.5 mL glass vial, NBS (N-Bromosuccinimide, 0.1 mmol) was added into a mixture of the alkynes (0.05 mmol) and PPh_3 (Triphenylphosphine, 0.05 mmol) in CH_2Cl_2 (200 μL), and the mixture was placed on a Thermo Shaker Incubator at 30 °C, 500 rpm for 3 h. After the reaction was complete, a 10 μL aliquot was taken, diluted 50-fold with ethyl acetate (EA), and subjected to GC and GC-MS analysis. The results were then compared with those obtained from the enzymatic catalytic reaction.

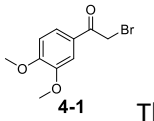
Isolation and purification of the preparative-scale reaction products



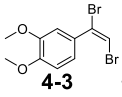
1-1 The crude product was purified by silica gel column chromatography with n-hexane: ethyl acetate = 50:1 as the eluent to give a light yellow solid (90 mg, 82% purity, Rf = 0.13).



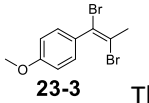
1-3 The crude product was purified by silica gel column chromatography with n-hexane as the eluent to give a colourless liquid (212 mg, 95% purity, Rf = 0.55).



4-1 The crude product was purified by silica gel column chromatography with n-hexane: ethyl acetate = 20:1 as the eluent to give a light yellow solid (133.4 mg, 79% purity, Rf = 0.07).



4-3 The crude product was purified by silica gel column chromatography with n-hexane: ethyl acetate = 20:1 as the eluent to give a white solid (275.2 mg, 97% purity, Rf = 0.39).



23-3 The crude product was purified by silica gel column chromatography with n-hexane as the eluent to give a colorless liquid (199.8 mg, 89% purity, Rf = 0.33).

Analytical procedures

Gas chromatography (GC)

GC2010 Pro or GC2030 from SHIMADZU equipped with SH-1 column (crossed 100% dimethyl polysiloxane, 30 m × 0.25 mm × 0.25 μm) was employed in this research. Gas chromatography equipped with a flame ionization detector (FID) was used to quantify the conversion of each substrate and determine the selectivity of the products. Nitrogen was used as the carrier gas, and a column flow at 3 mL min^{-1} and split ratio of 80:1 was used in all analyses. Data was analyzed using the software LabSolutions^{TM} post-run analysis from SHIMADZU. Typically, for the determination of substrates **1**-**2**, **4**-**31**, and **S1**-**S3** catalysed by *Ci*VCPO, the GC was programmed from 120 °C (held for 0.5 min) to 180 °C at 10 °C min^{-1} (held for 4 min), 30 °C min^{-1} to 320 °C (held for 1 min). For the determination of **2** catalysed by *Ci*VCPO, the GC was programmed from 120 °C (held for 0.5 min) to 180 °C at 9 °C min^{-1} (held for 6 min), 30 °C min^{-1} to 320 °C (held for 1 min).

GC-MS analysis

Electron ionisation (EI) GC-MS data were collected on GCMS-QP2010 equipped with SH-1 column (crossed 100% dimethyl polysiloxane, 30 m × 0.25 mm × 0.25 µm) was employed in this reasearch. The program for GC-MS analysis was identical to that employed in the GC protocol, as described above.

NMR analysis

^{1}H NMR (400 MHz) and ^{13}C NMR (101 MHz) spectra were recorded on a Quantum-I 400MHz spectrometer (Q.One Instruments Ltd., China) using $CDCl_3$ or DMSO-d6 as solvents. Chemical shifts are reported in ppm relative to tetramethylsilane (TMS) as an internal standard. Signal multiplicities are abbreviated as: singlet (s), doublet (d), triplet (t), quartet (q), multiplet (m). Coupling constants (J) are given in Hz and were directly extracted from spectra without correction.

Crystallography of **1-1**

Crystallization was achieved via the slow solvent evaporation method. Specifically, the product was dissolved in a mixture of ethyl acetate and petroleum ether (5:95, v/v) until just dissolved. The solution was placed in a glass vial and allowed to stand undisturbed at room temperature. Crystals formed after approximately 24 h through controlled solvent evaporation.

Further experimental results

SDS-PAGE analysis of purified *CiVCPO*

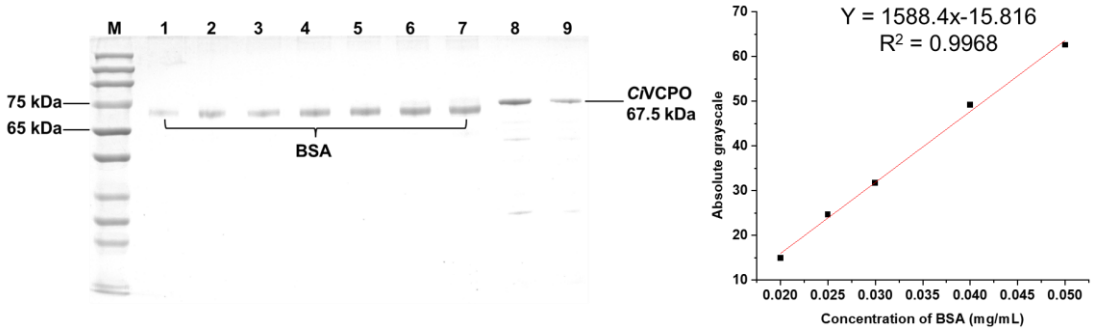


Figure S1. SDS-PAGE analysis and quantification of purified *CiVCPO*. Lane M, Marker. Lane 1-7 are different concentrations of bovine serum albumin (BSA). Lane 1, 0.01 mg/mL. Lane 2, 0.015 mg/mL. Lane 3, 0.02 mg/mL. Lane 4, 0.025 mg/mL. Lane 5, 0.03 mg/mL. Lane 6, 0.04 mg/mL. Lane 7, 0.05 mg/mL. Lane 8, *Ci*VCPO. Lane 9, *Ci*VCPO. The standard curve for gray scale analysis is Y = 1588.4*x – 15.816, R^2 = 0.9968.

Control experiments for the oxybromination of alkynes

Table S1. Control experiments for the oxybromination of alkynes

Entry	Conditions	Conversion (%)
1	10 mM **1**, 100 mM H_2O_2, 20 mM KBr with VO_4^-	R.L. N.P.
2	10 mM **1**, 100 mM H_2O_2, 20 mM KBr without *Ci*VCPO	R.L. N.P.
3	10 mM **1**, 100 mM H_2O_2, 20 mM KBr with *Ci*VCPO	99
4	10 mM **1**, 100 mM H_2O_2, 1 M KBr without *Ci*VCPO	R.L. N.P.
5	10 mM **1**, 100 mM H_2O_2, 1 M KBr with *Ci*VCPO	>99

Reaction condition: 10% DMSO in citrate buffer (100 mM, pH 5.0), [**1**] = 10 mM, [*Ci*VCPO] = 150 nM, [H_2O_2] = 100 mM, [KBr] = 20 or 1000 mM, 200 μL scale, 30 °C, 800 rpm, 0.5 h. The conversion and product selectivity were determined by GC and GC-MS. R.L. = Relatively low. N.P. = No product.

Time course of **1** (10-40 mM) catalysed by *Ci*VCPO

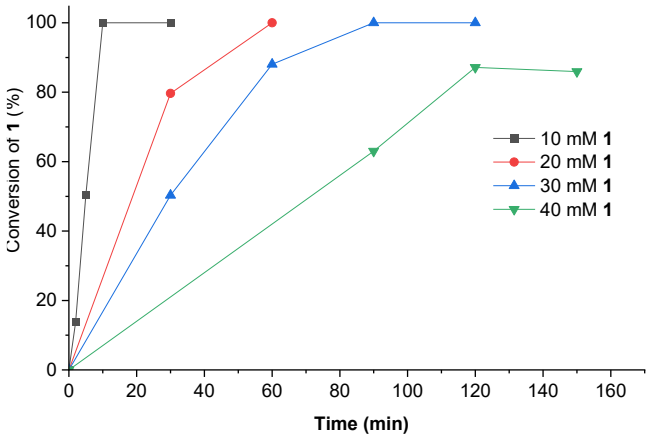


Figure S2. Time course of **1** (10-40 mM) catalysed by *Ci*VCPO. General reaction condition: 10% DMSO in citrate buffer (100 mM, pH 5.0), [**1**] = 10-40 mM, [*Ci*VCPO] = 150 nM, [KBr] = 20-80 mM, [H_2O_2] = 100-400 mM, 150 min, 200 µL scale, 30 °C, 800 rpm, 150 min. The conversion was determined by GC.

Table S2. Product distribution with different concentration of **1**. Reaction condition: 10% DMSO in citrate buffer (100 mM, pH 5.0), [**1**] = 10-40 mM, [*Ci*VCPO] = 150 nM, [KBr] = 20-80 mM, [H_2O_2] = 100-400 mM, 200 µL scale, 30 °C, 800 rpm, 150 min. The conversion and product selectivity were determined by GC and GC-MS.

Entry	c (**1**) (mM)	c (*Ci*VCPO) (nM)	c (H_2O_2) (mM)	c (KBr) (mM)	reaction time (min)	Conv. (%)	Product selectivity (%)			
							1-1	1-2	1-3	1-4
1	10	150	100	20	30	99	60	14	25	1
2	20		200	40	60	99	55	3	41	2
3	30		300	60	90	99	53	1	44	2
4	40		400	80	120	87	49	1	48	23

Organic solvents optimization experiments

TableS3. Organic solvents optimization experiments

Entry	c (**1**) (mM)	10% organic solvent	c (KBr) (mM)	c (H_2O_2) (mM)	c (*Ci*VCPO) (nM)	Conv. (%)	Product selectivity (%)			
							1-1	1-2	1-3	1-4
1	30	acetone	60	300	150	99	35	8	55	2
2		acetone	1000			67	4	1	93	3
3		methanol	60			94	45	1	52	2
4		methanol	1000			73	4	1	93	3

Reaction condition: 10% acetone or methanol in citrate buffer (100 mM, pH 5.0), [**1**] = 30 mM, [*Ci*VCPO] = 150 nM, [H_2O_2] = 300 mM, [KBr] = 60 or 1000 mM, 200 µL scale, 30 °C, 800 rpm,

120 min. The conversion and product selectivity were determined by GC.

Non-model reaction of other substrates catalysed by *Ci*VCPO

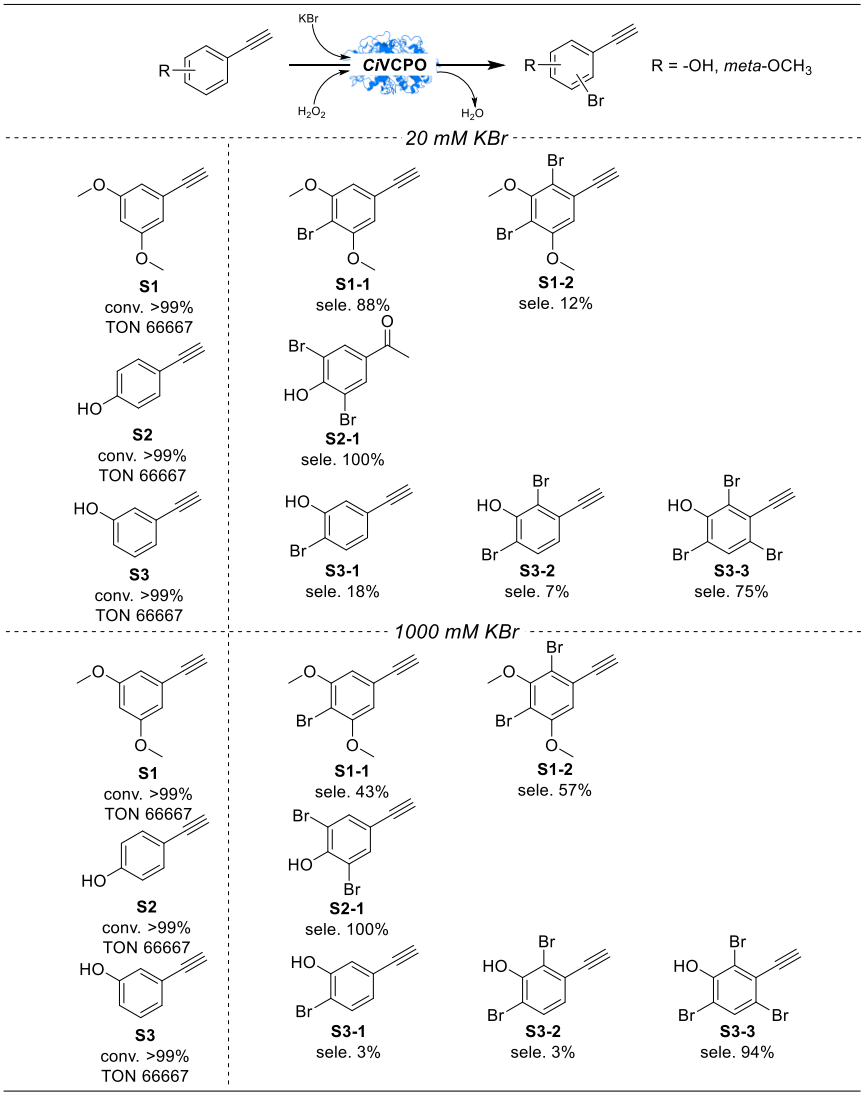
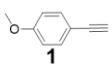
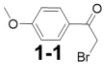
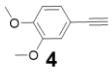
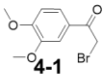
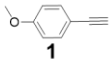
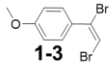
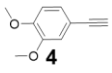
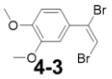
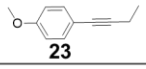
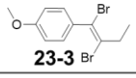


Figure S3. Non-model reaction of other substrates catalysed by *Ci*VCPO. General reaction condition: 10% DMSO in citrate buffer (100 mM, pH 5.0), [substrate] = 10 mM, [*Ci*VCPO] = 150 nM, [KBr] = 20 or 1000 mM, [H_2O_2] = 100 mM, 30 °C, 800 rpm, 0.5 h, 200 µL scale.

Isolated yield of products from preparative synthesis

Table S4. Isolated yield of products from preparative synthesis

Substrate	Condition	Product isolated yield (%)	
 1	A	 1-1	51%
 4	B	 4-1	65%
 1	C	 1-3	79%
 4	D	 4-3	97%
 23	E	 23-3	70%

Reaction condition: [substrate] = 30 mM (1 mmol), 10% DMSO, citrate buffer (100 mM, pH 5.0), 30 °C, 500 rpm. A. [*Ci*VCPO] = 150 nM, [H_2O_2] = 150 mM, [KBr] = 13.33 mM/h, 4 h; B. [*Ci*VCPO] = 150 nM, [H_2O_2] = 200 mM, [KBr] = 13.33 mM/h, 4 h; C. [*Ci*VCPO] = 150 nM, [H_2O_2] = 150 mM, [KBr] = 1 M, 3 h; D. [*Ci*VCPO] = 150 nM, [H_2O_2] = 200 mM, [KBr] = 2 M, 3 h; E. [*Ci*VCPO] = 150 nM, [H_2O_2] = 250 mM, [KBr] = 2 M, 3 h.

GC and GC-MS analysis of products from substrates **1**-**31** catalysed by *Ci*VCPO

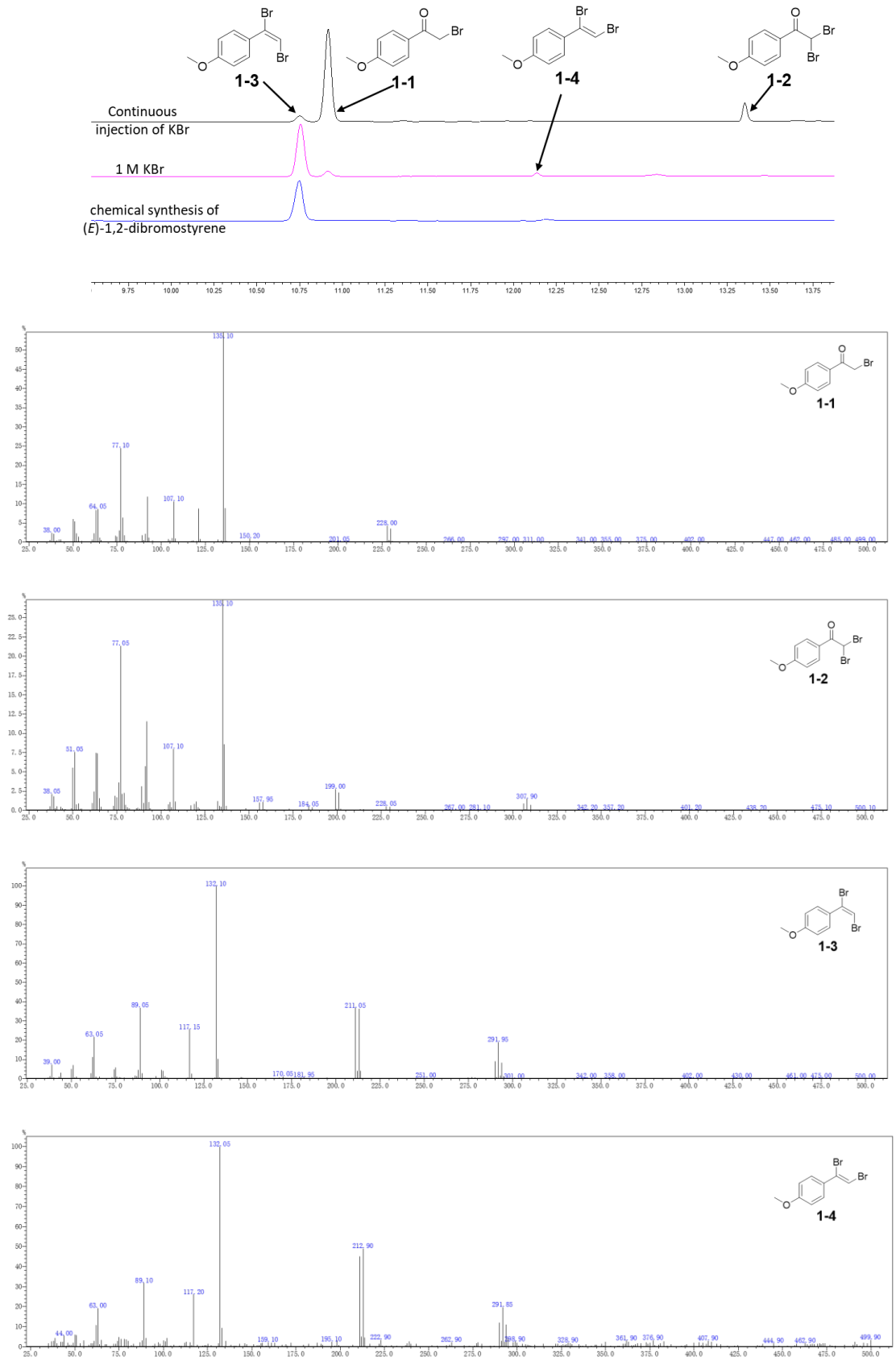


Figure S4. Representative GC and Mass spectrum of **1-1**, **1-2**, **1-3**, **1-4**

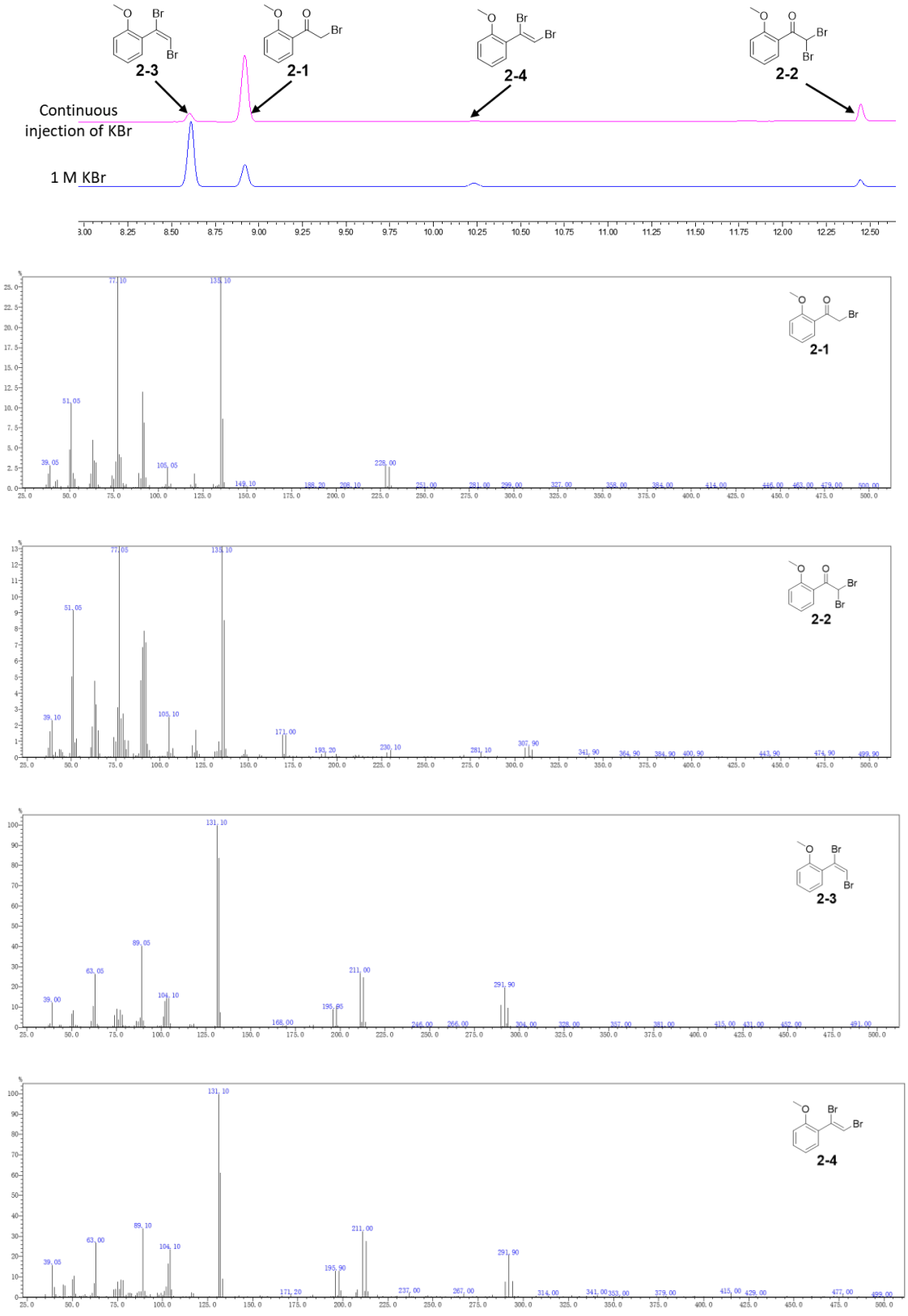


Figure S5. Representative GC and Mass spectrum of **2-1**, **2-2**, **2-3**, **2-4**

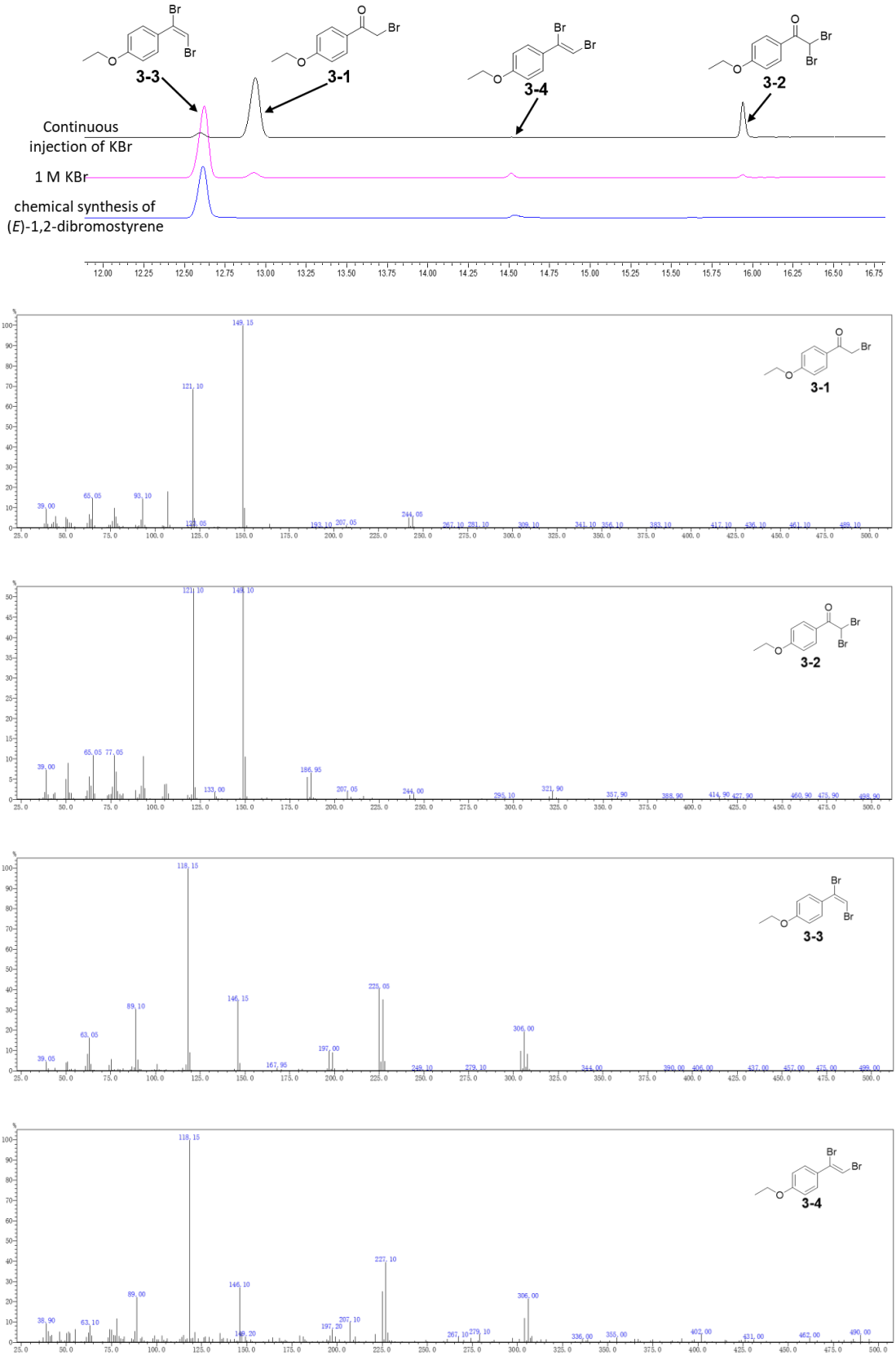


Figure S6. Representative GC and Mass spectrum of **3-1**, **3-2**, **3-3**, **3-4**

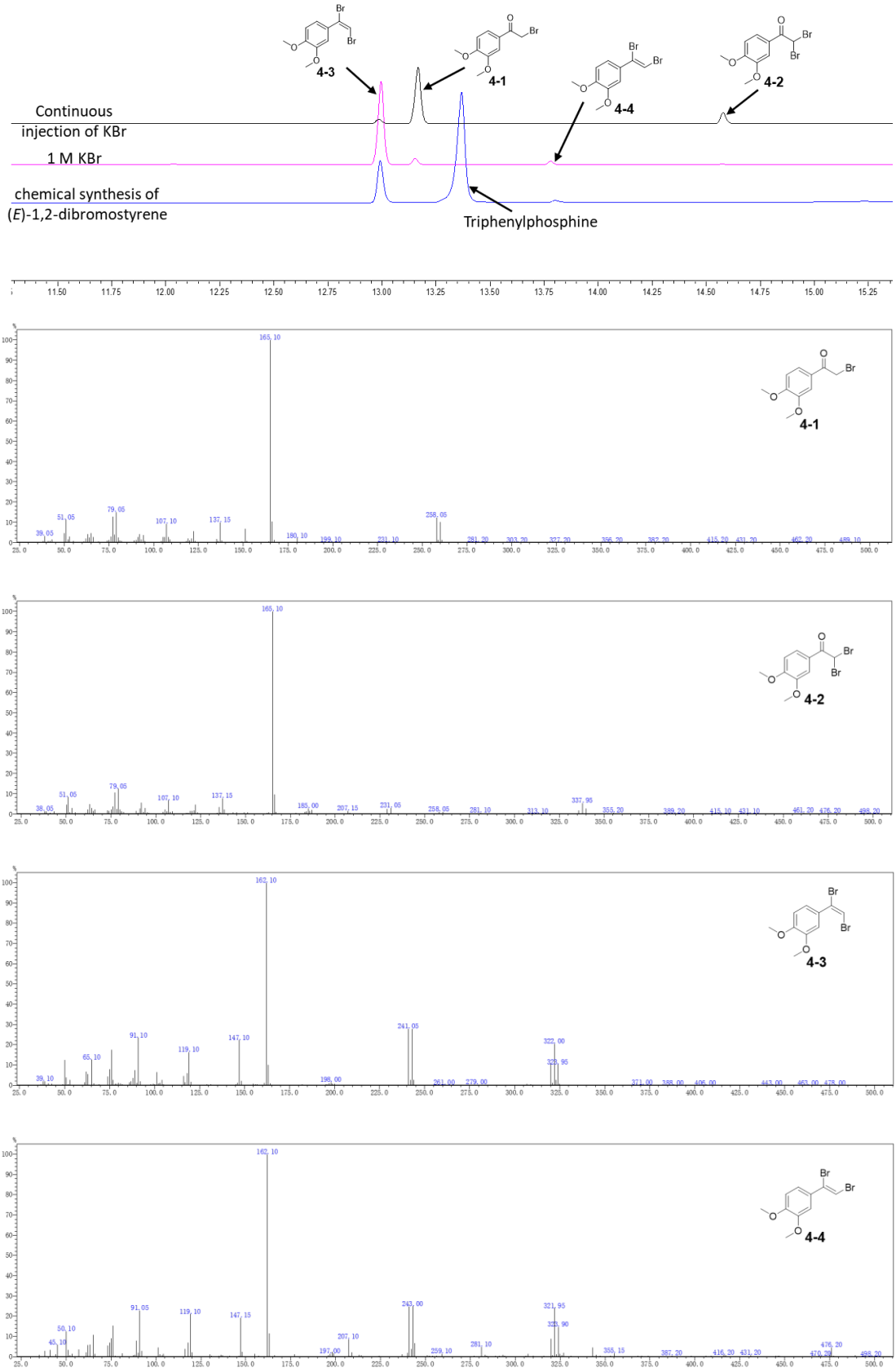


Figure S7. Representative GC and Mass spectrum of **4-1**, **4-2**, **4-3**, **4-4**

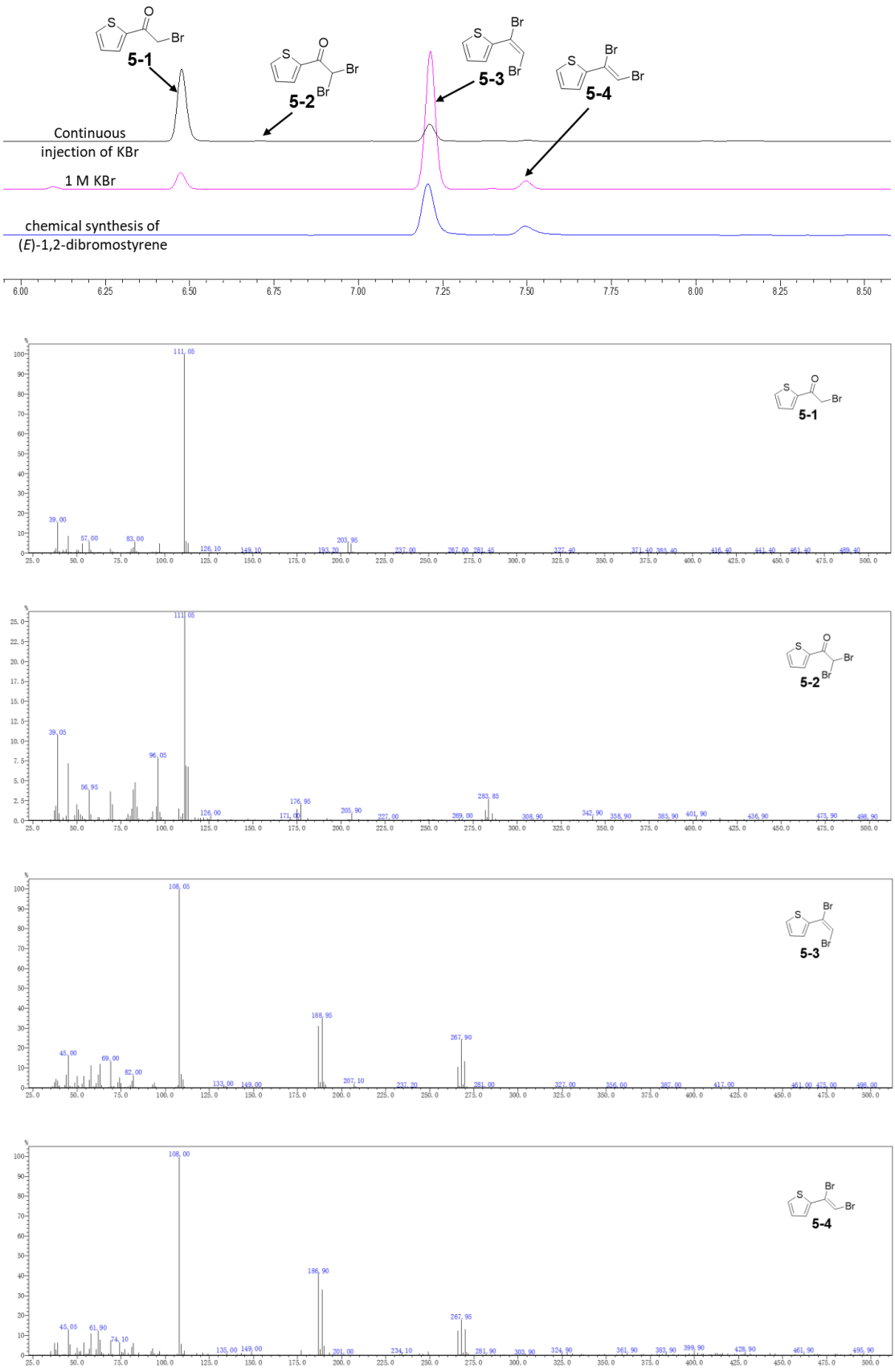


Figure S8. Representative GC and Mass spectrum of **5-1**, **5-2**, **5-3**, **5-4**

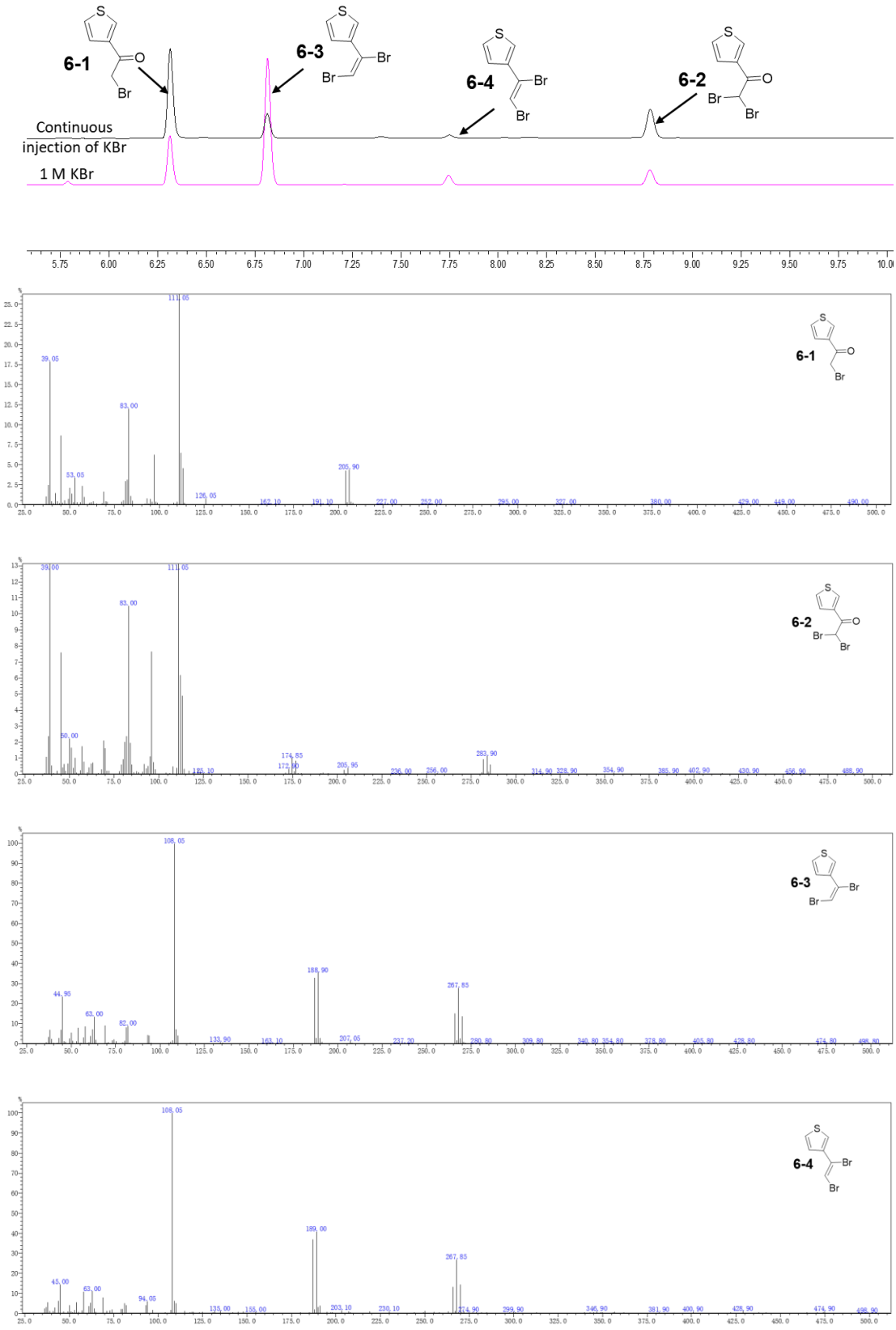


Figure S9. Representative GC and Mass spectrum of **6-1**, **6-2**, **6-3**, **6-4**

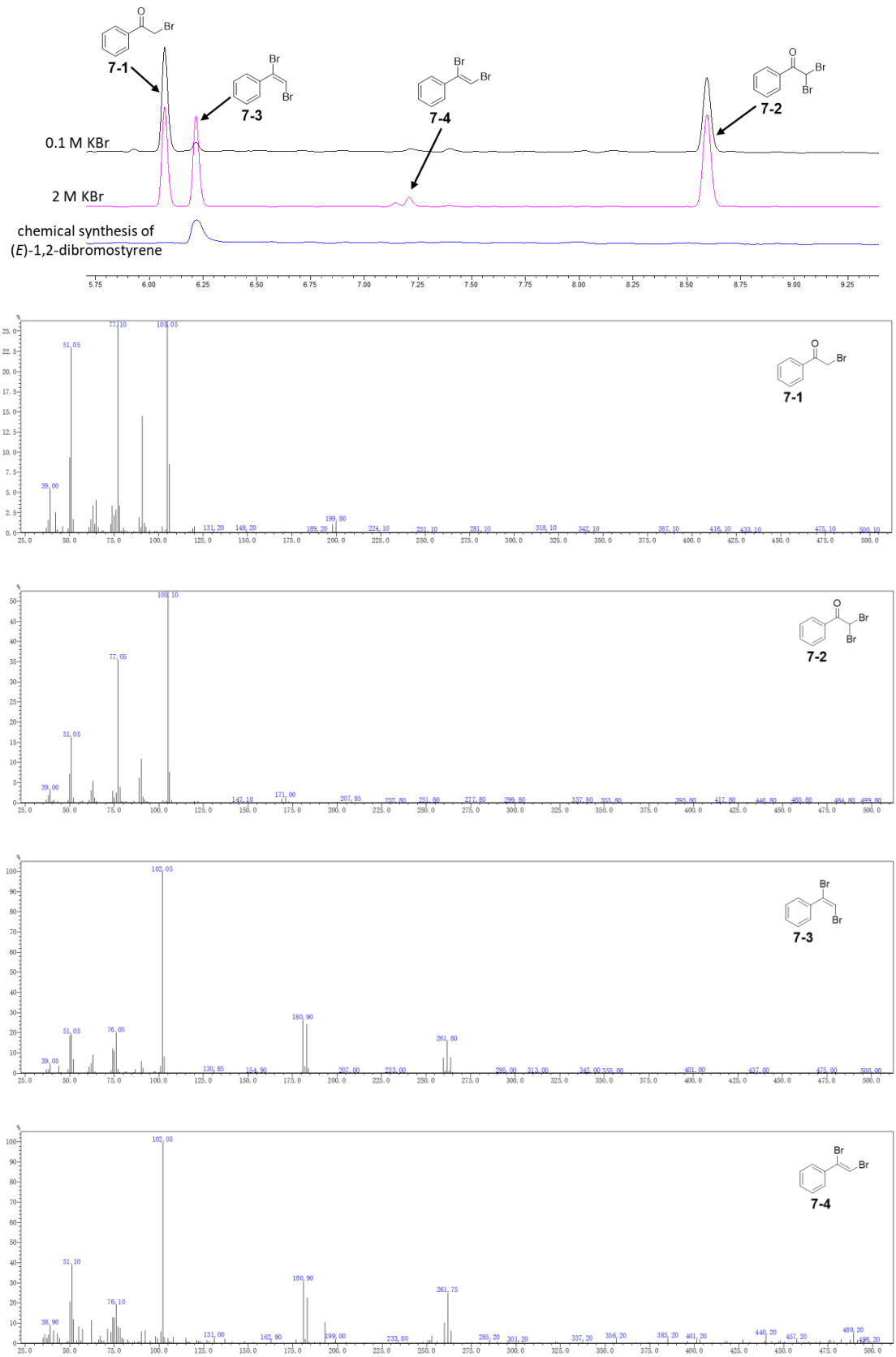


Figure S10. Representative GC and Mass spectrum of **7-1**, **7-2**, **7-3**, **7-4**

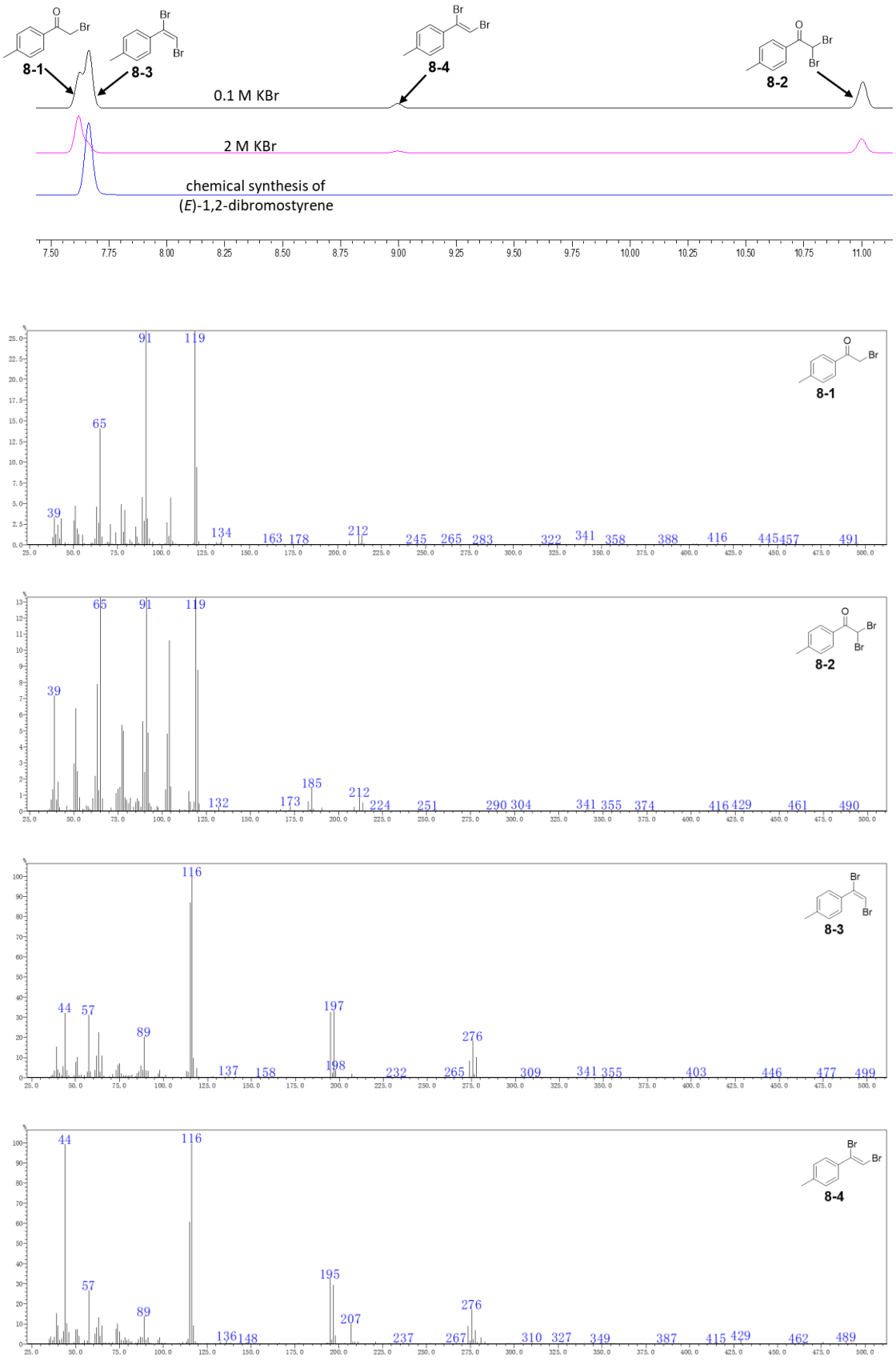


Figure S11. Representative GC and Mass spectrum of **8-1**, **8-2**, **8-3**, **8-4**

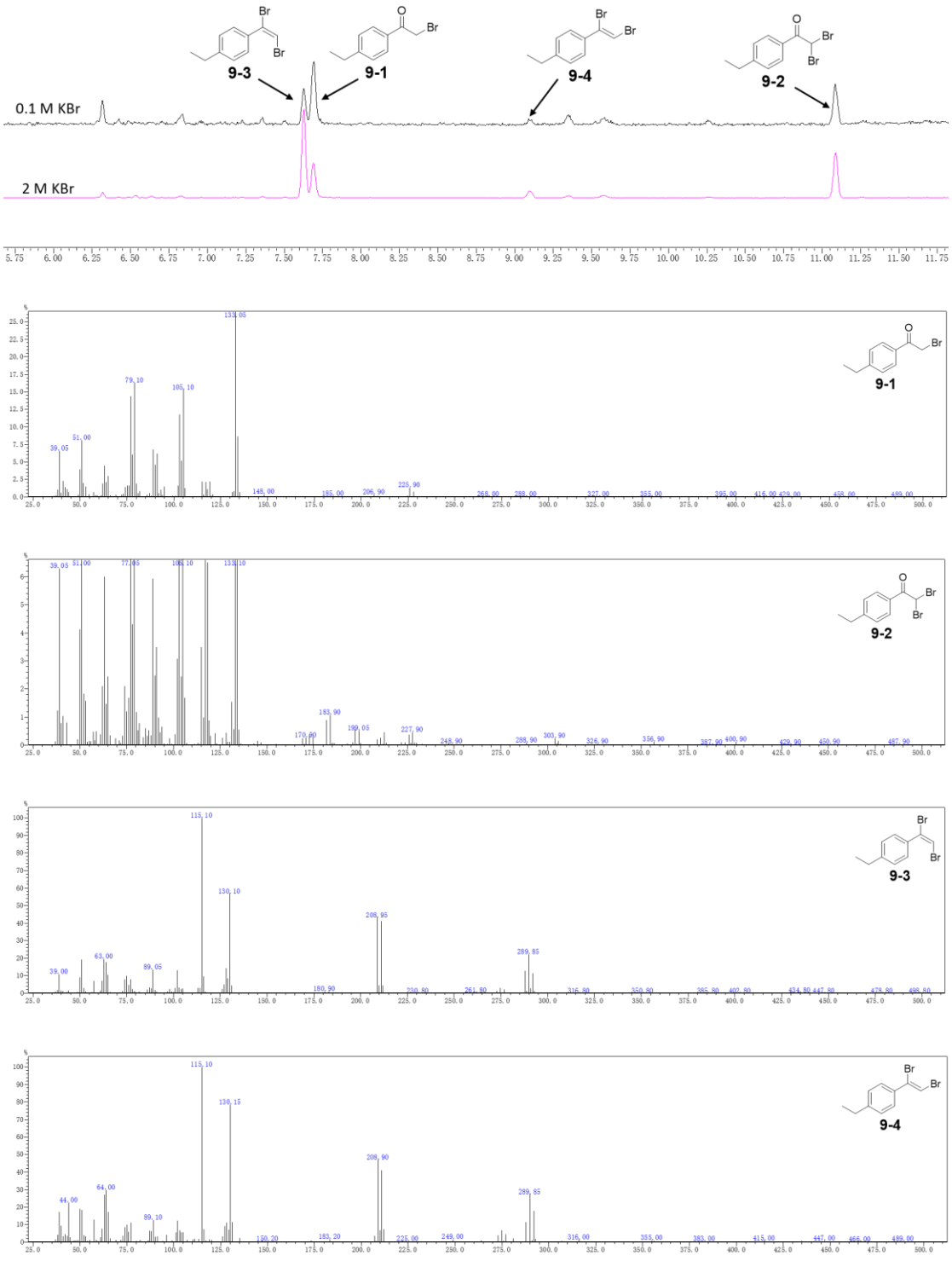


Figure S12. Representative GC and Mass spectrum of **9-1**, **9-2**, **9-3**, **9-4**

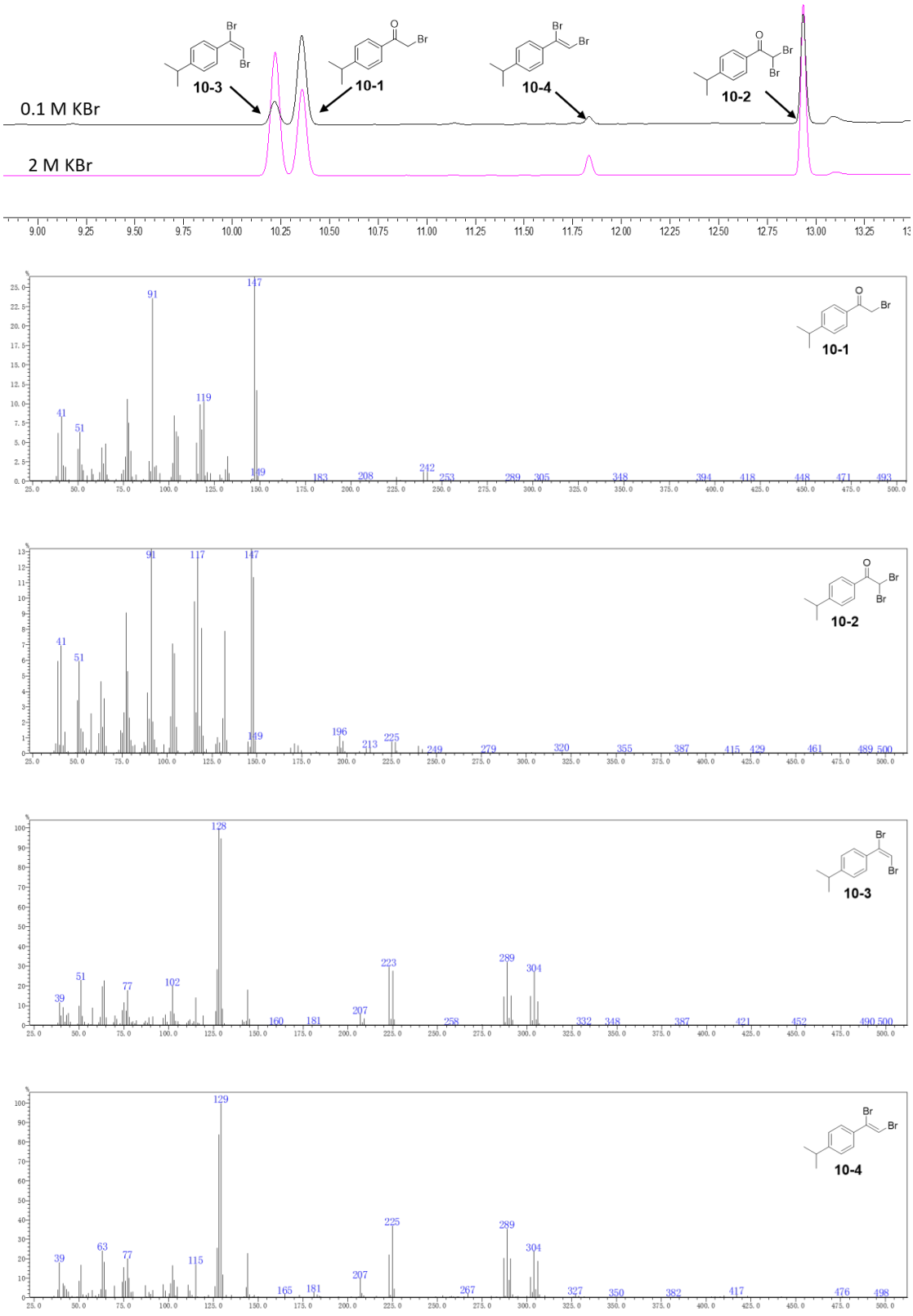


Figure S13. Representative GC and Mass spectrum of **10-1**, **10-2**, **10-3**, **10-4**

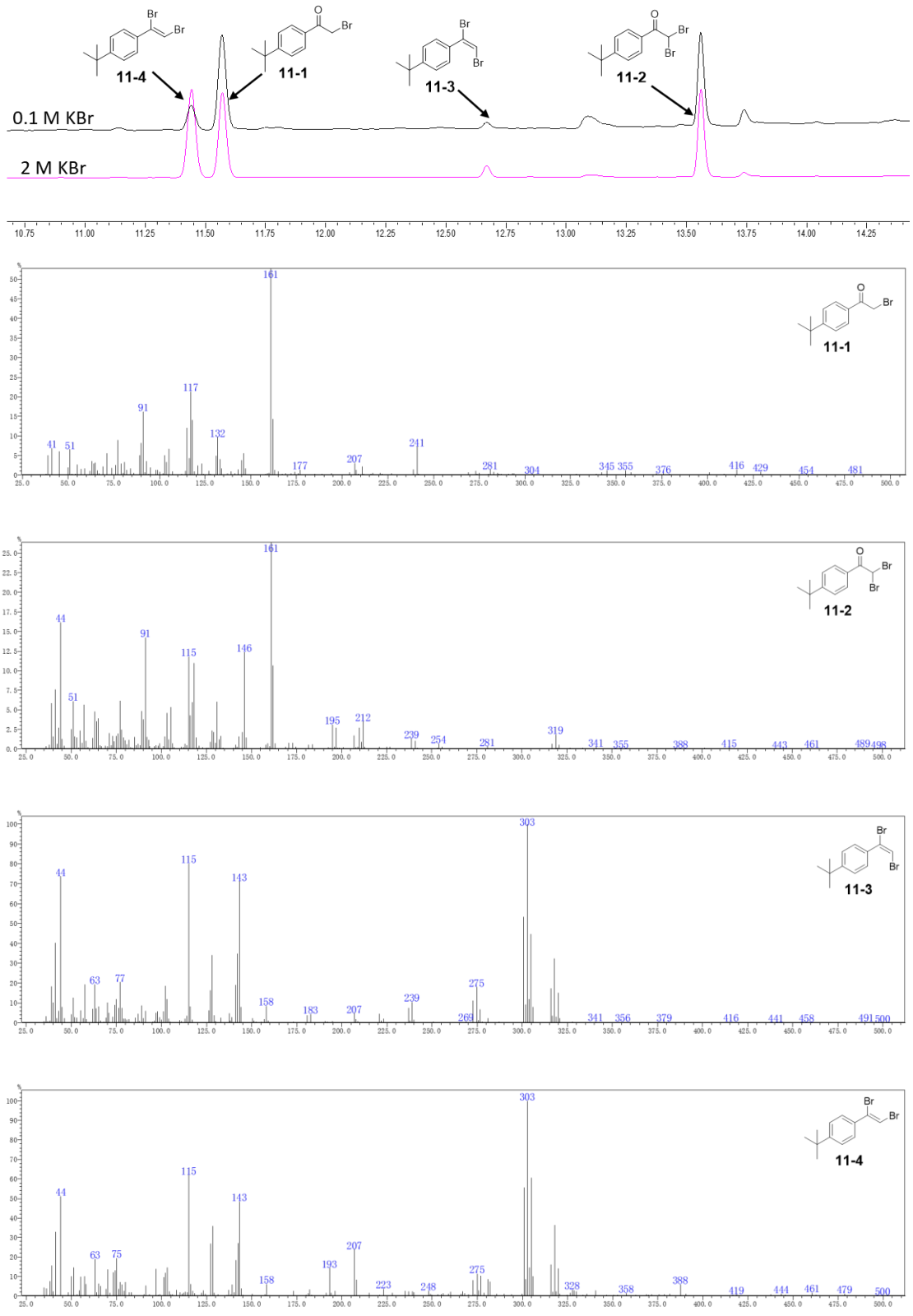


Figure S14. Representative GC and Mass spectrum of **11-1**, **11-2**, **11-3**, **11-4**

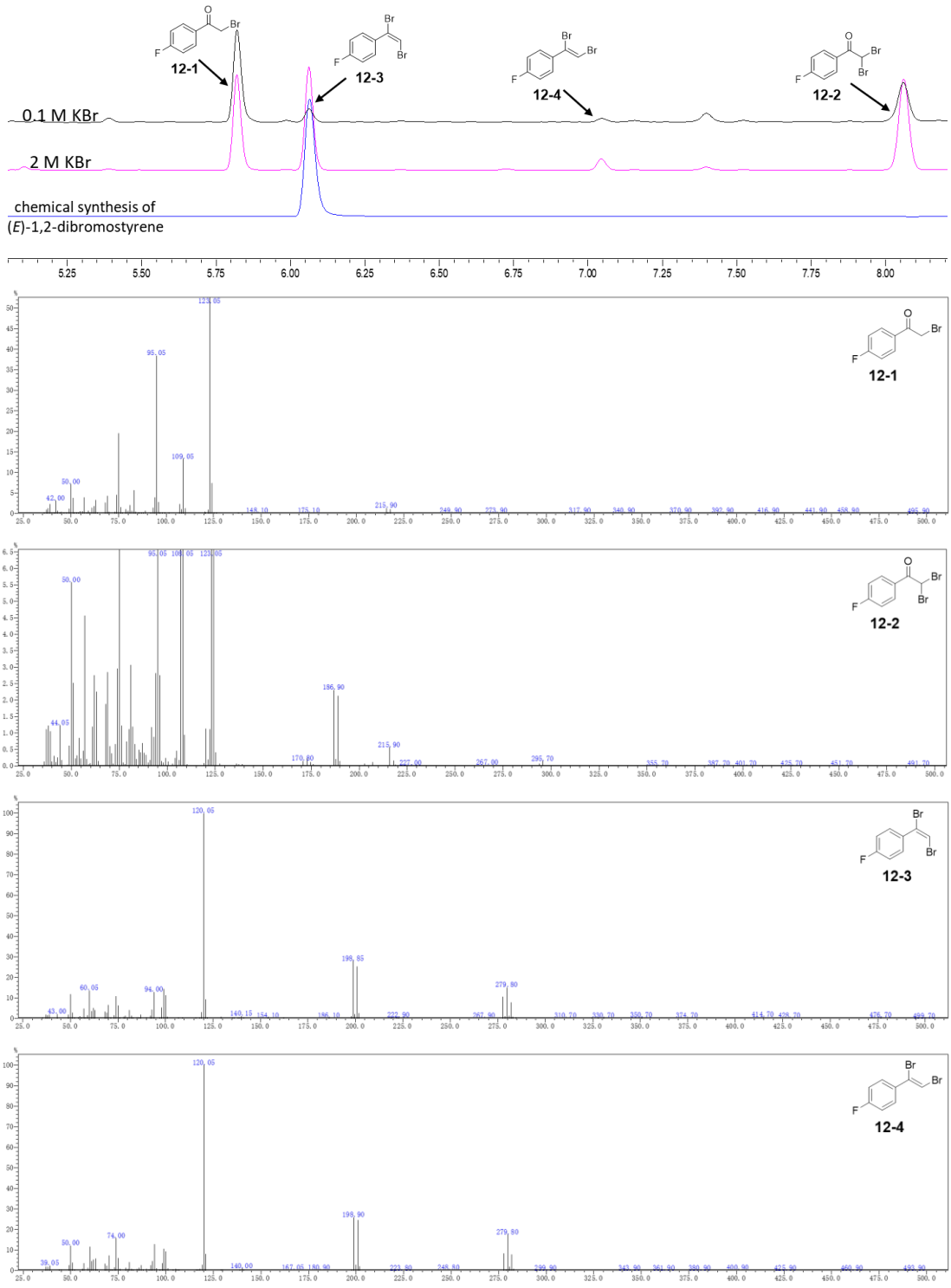


Figure S15. Representative GC and Mass spectrum of **12-1**, **12-2**, **12-3**, **12-4**

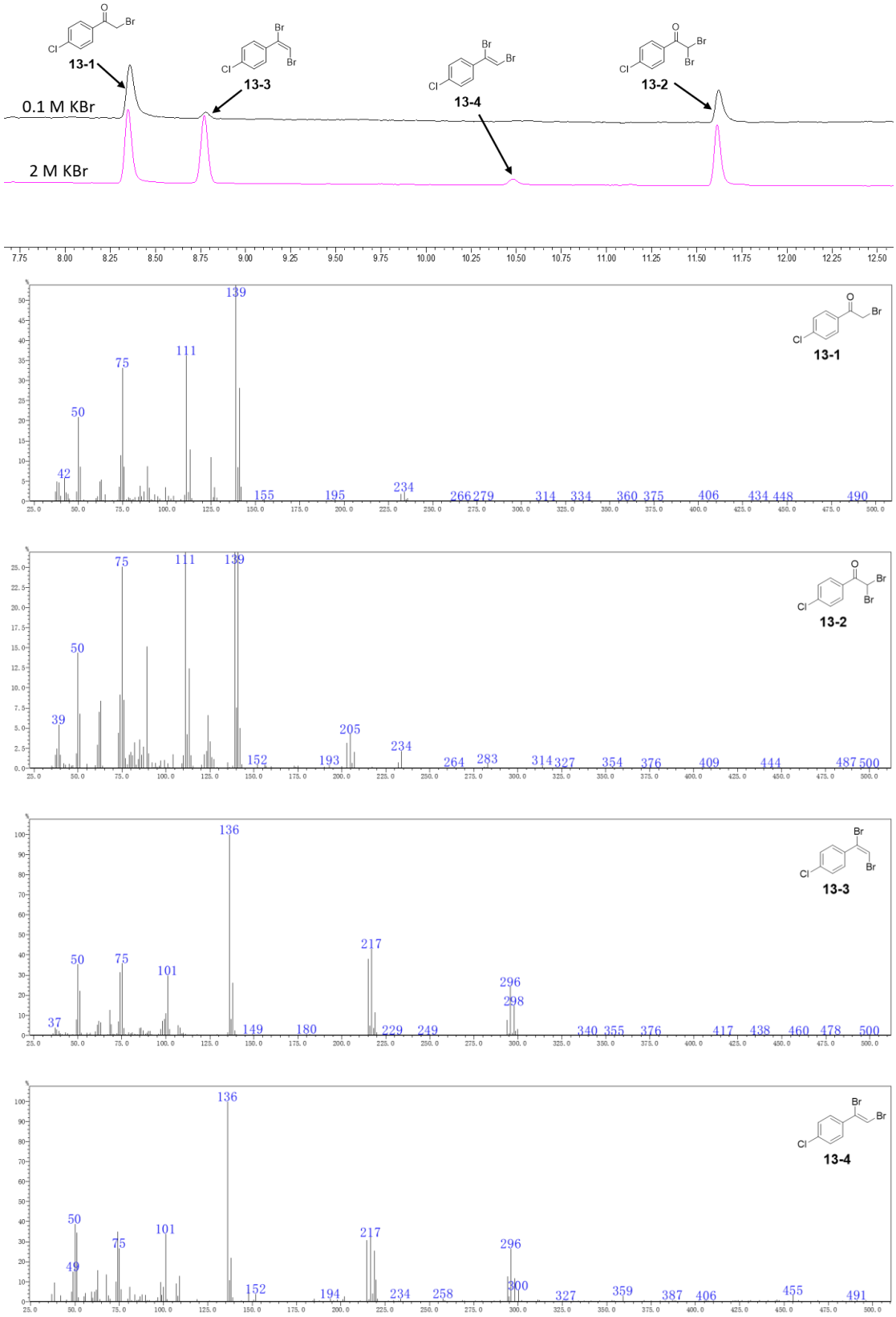


Figure S16. Representative GC and Mass spectrum of **13-1**, **13-2**, **13-3**, **13-4**

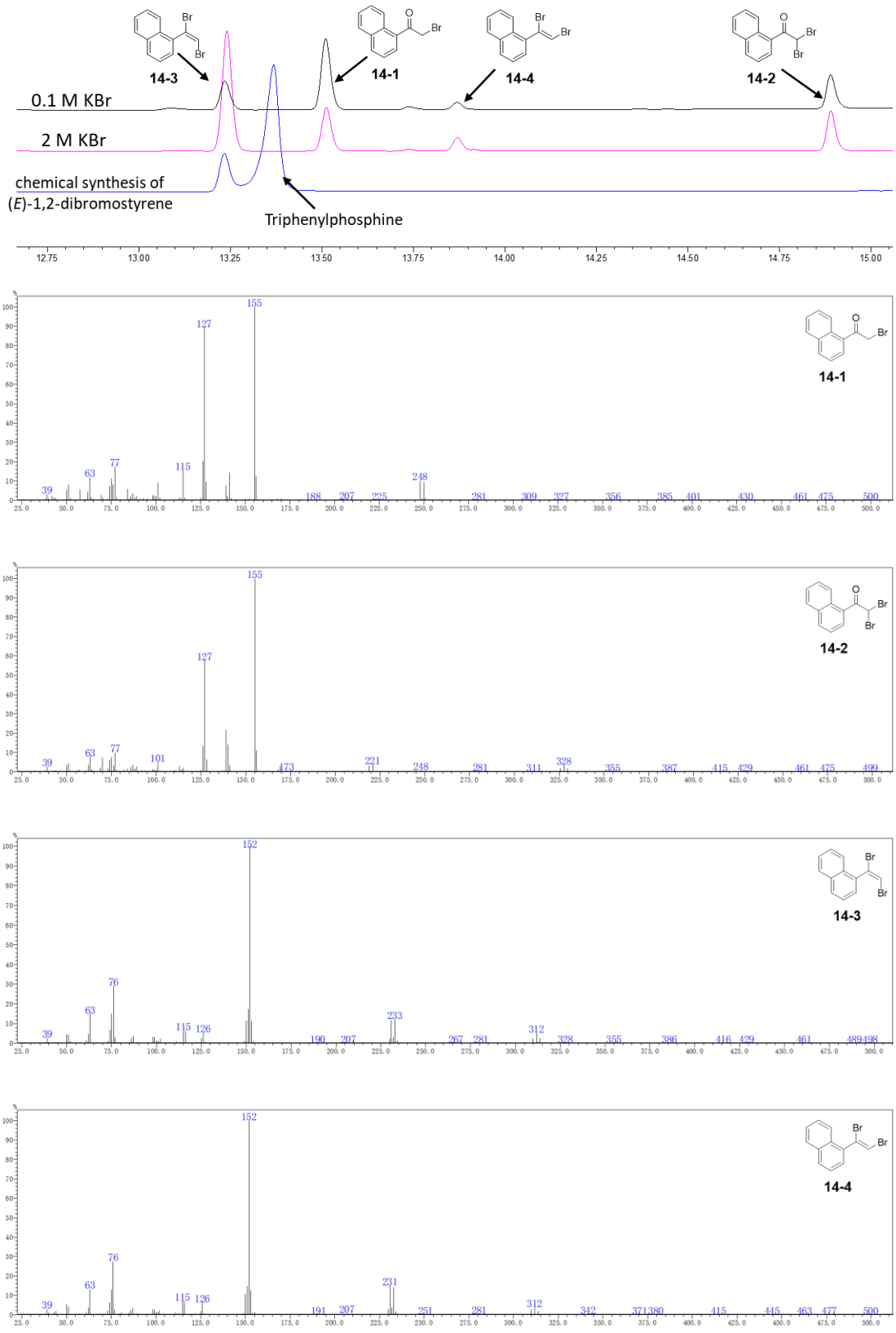


Figure S17. Representative GC and Mass spectrum of **14-1**, **14-2**, **14-3**, **14-4**

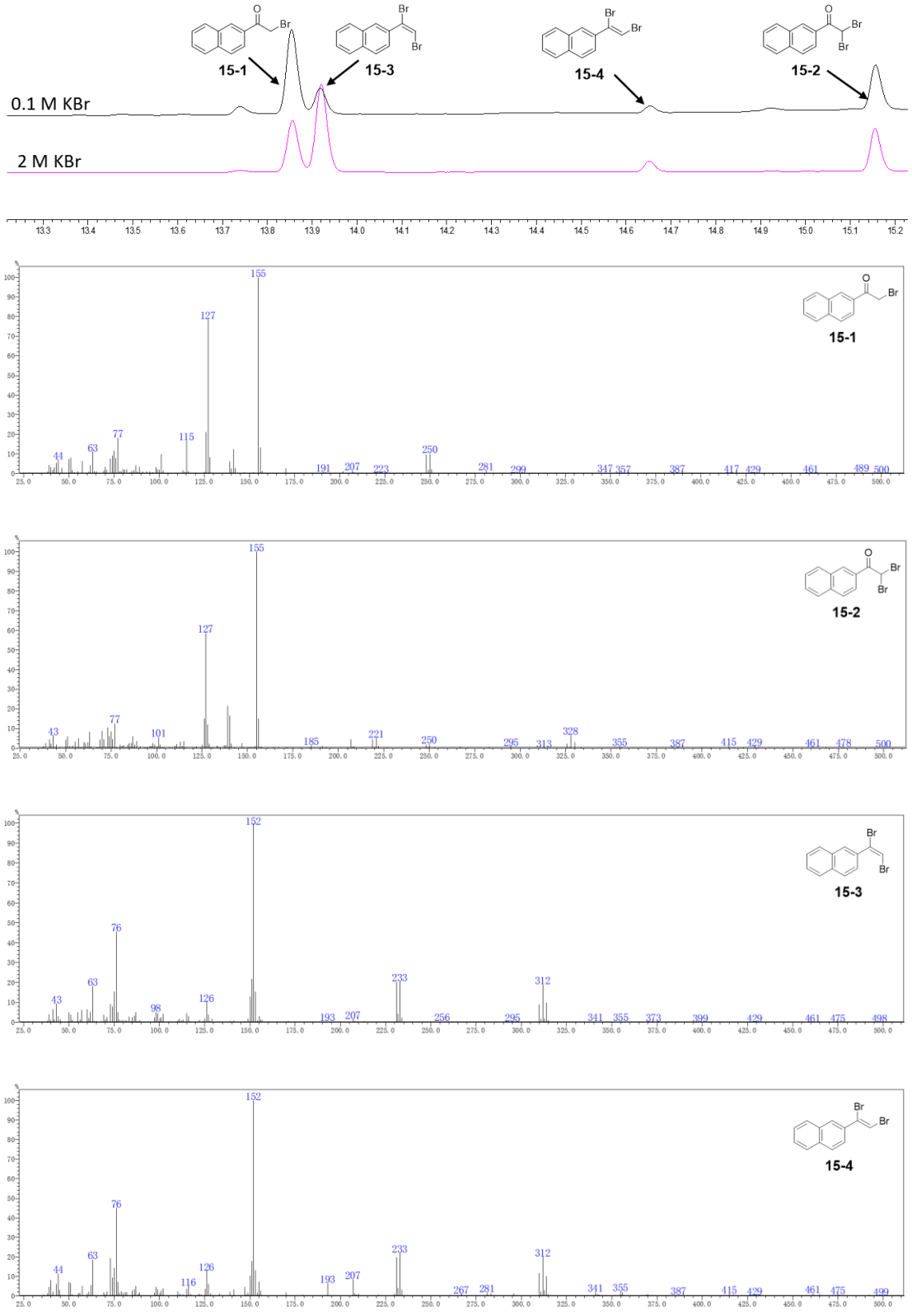


Figure S18. Representative GC and Mass spectrum of **15-1**, **15-2**, **15-3**, **15-4**

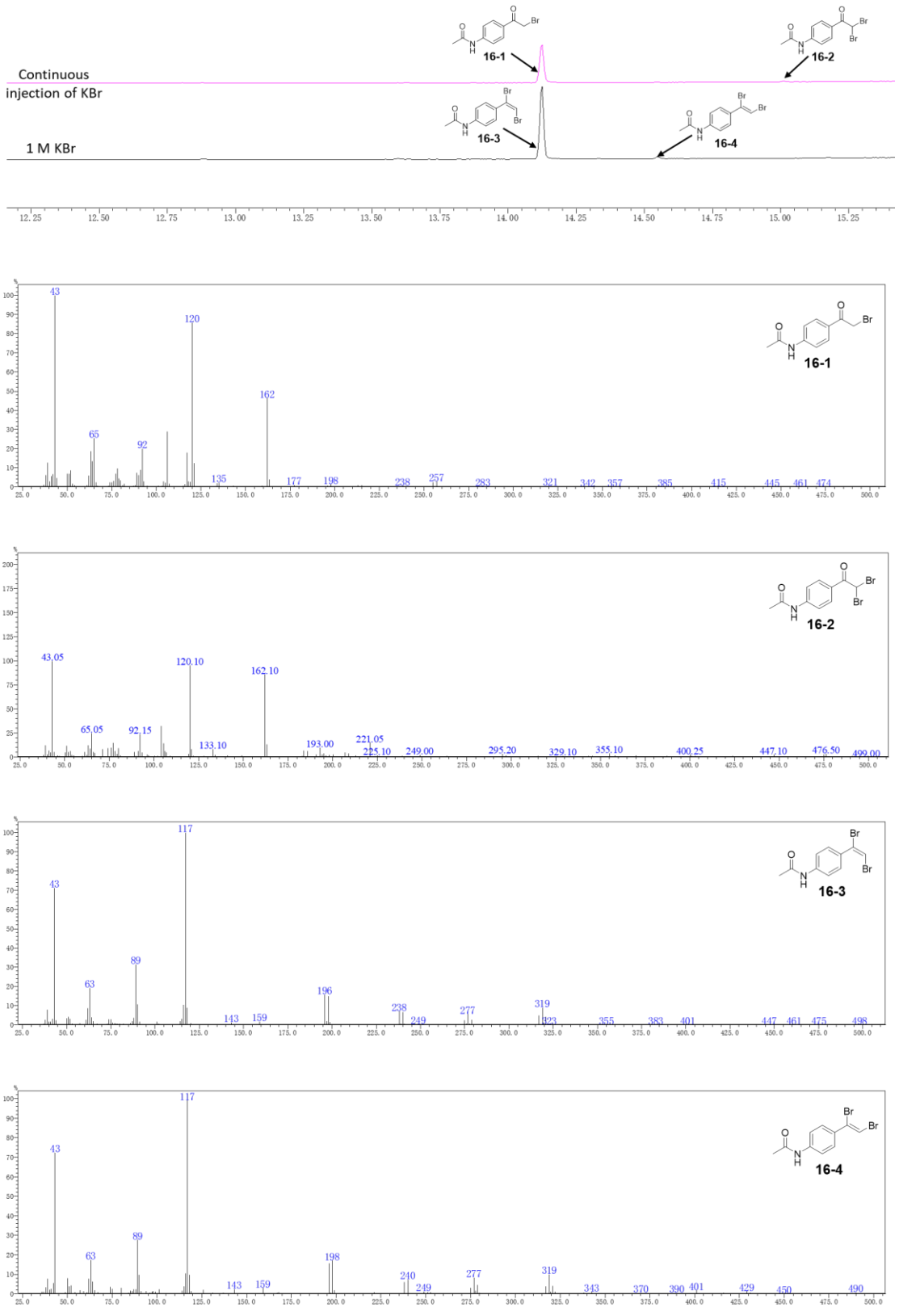


Figure S19. Representative GC and Mass spectrum of **16-1**, **16-2**, **16-3**, **16-4**

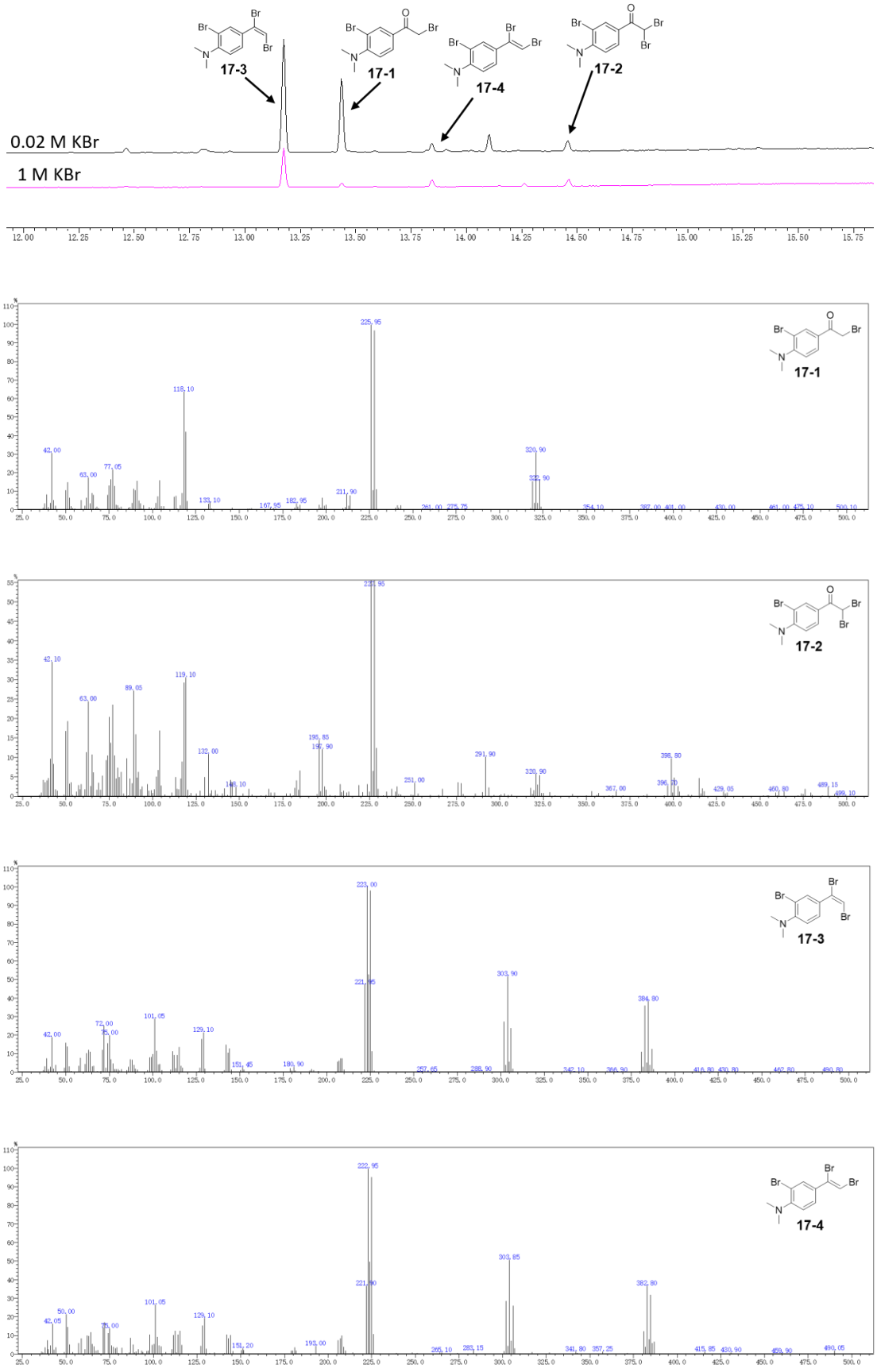


Figure S20. Representative GC and Mass spectrum of **17-1**, **17-2**, **17-3**, **17-4**.

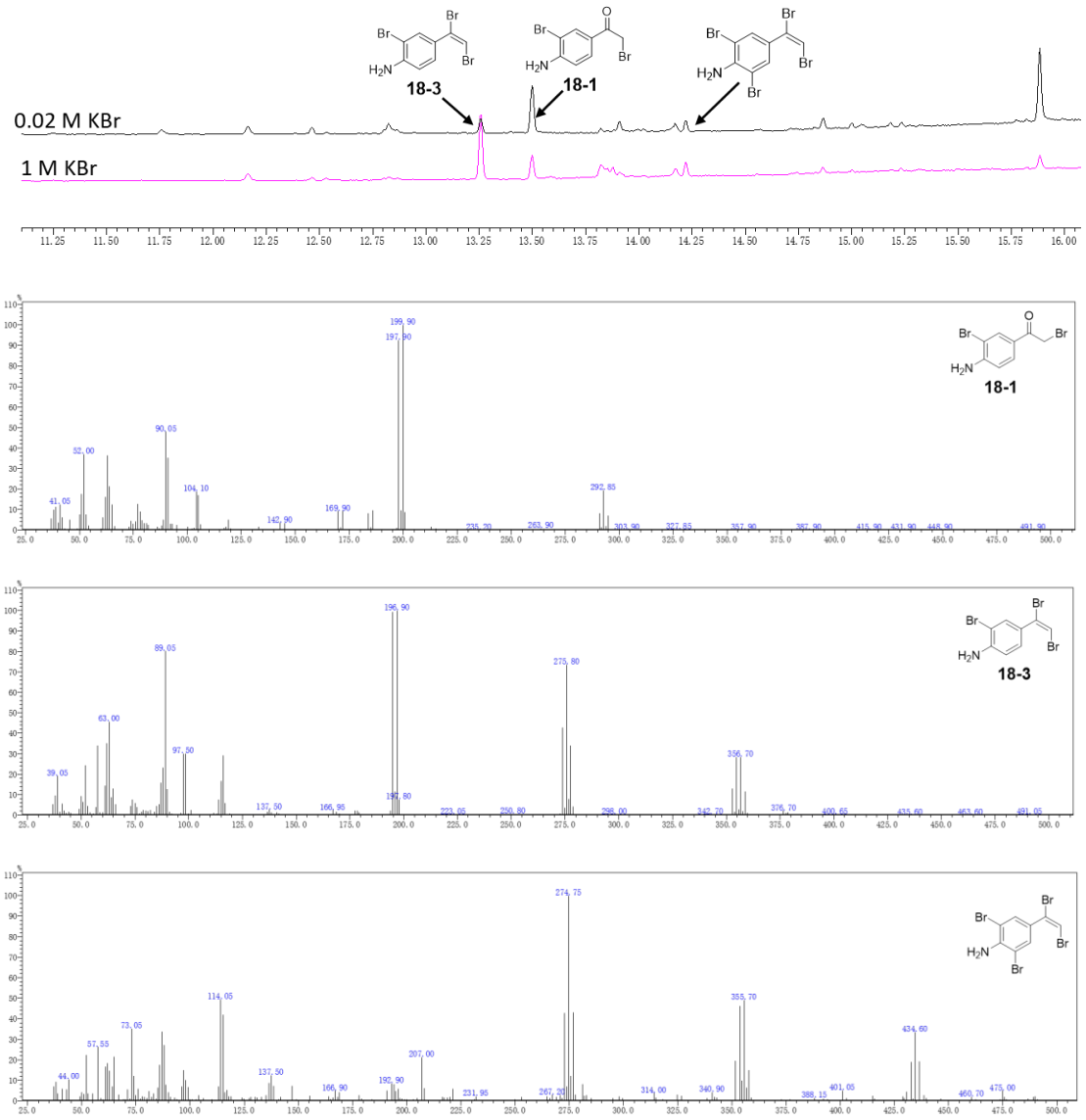
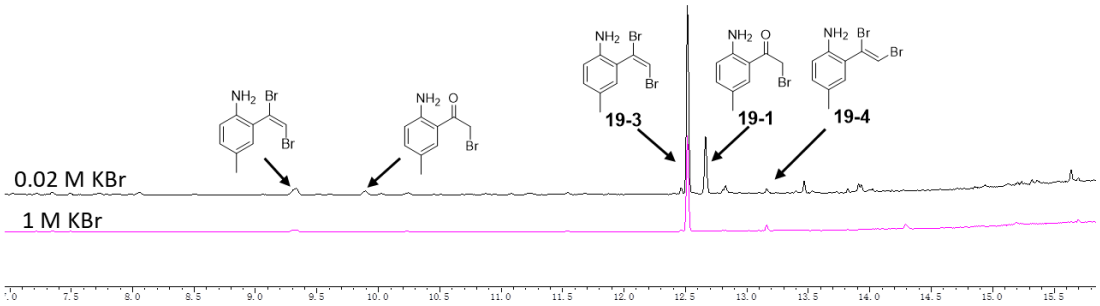
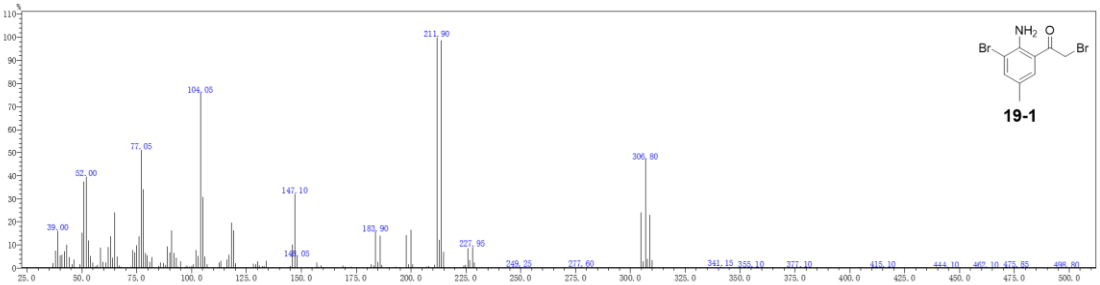
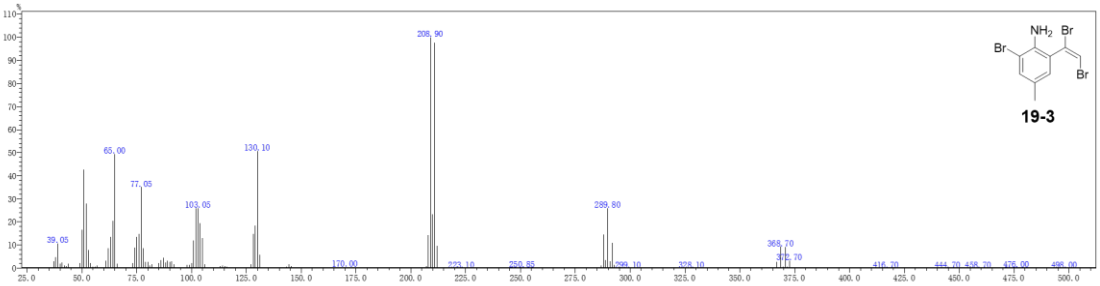
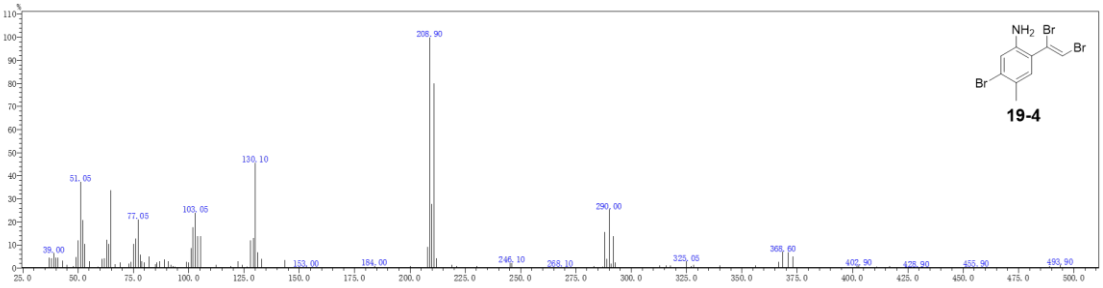


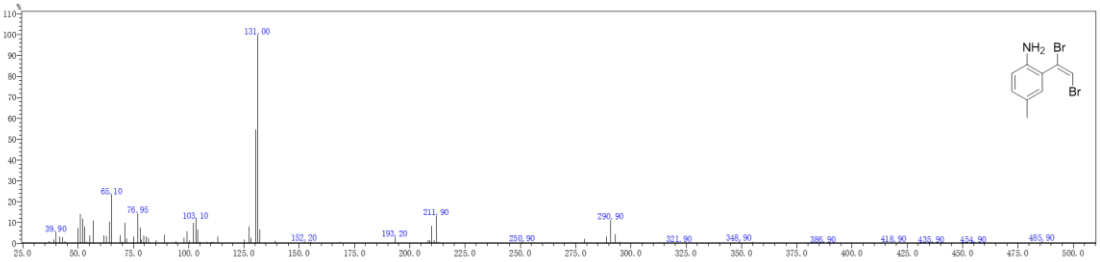
Figure S21. Representative GC and Mass spectrum of **18-1**, **18-3** and byproduct.


0.02 M KBr
1 M KBr
19-3
19-1
19-4


19-1


19-3


19-4



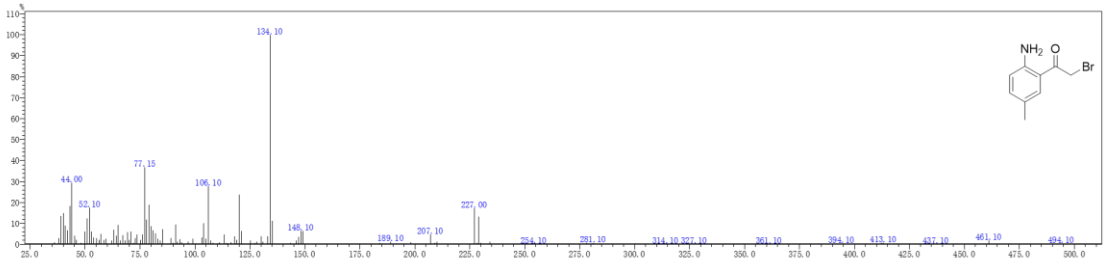


Figure S22. Representative GC and Mass spectrum of **19-1**, **19-3**, **19-4** and byproduct.

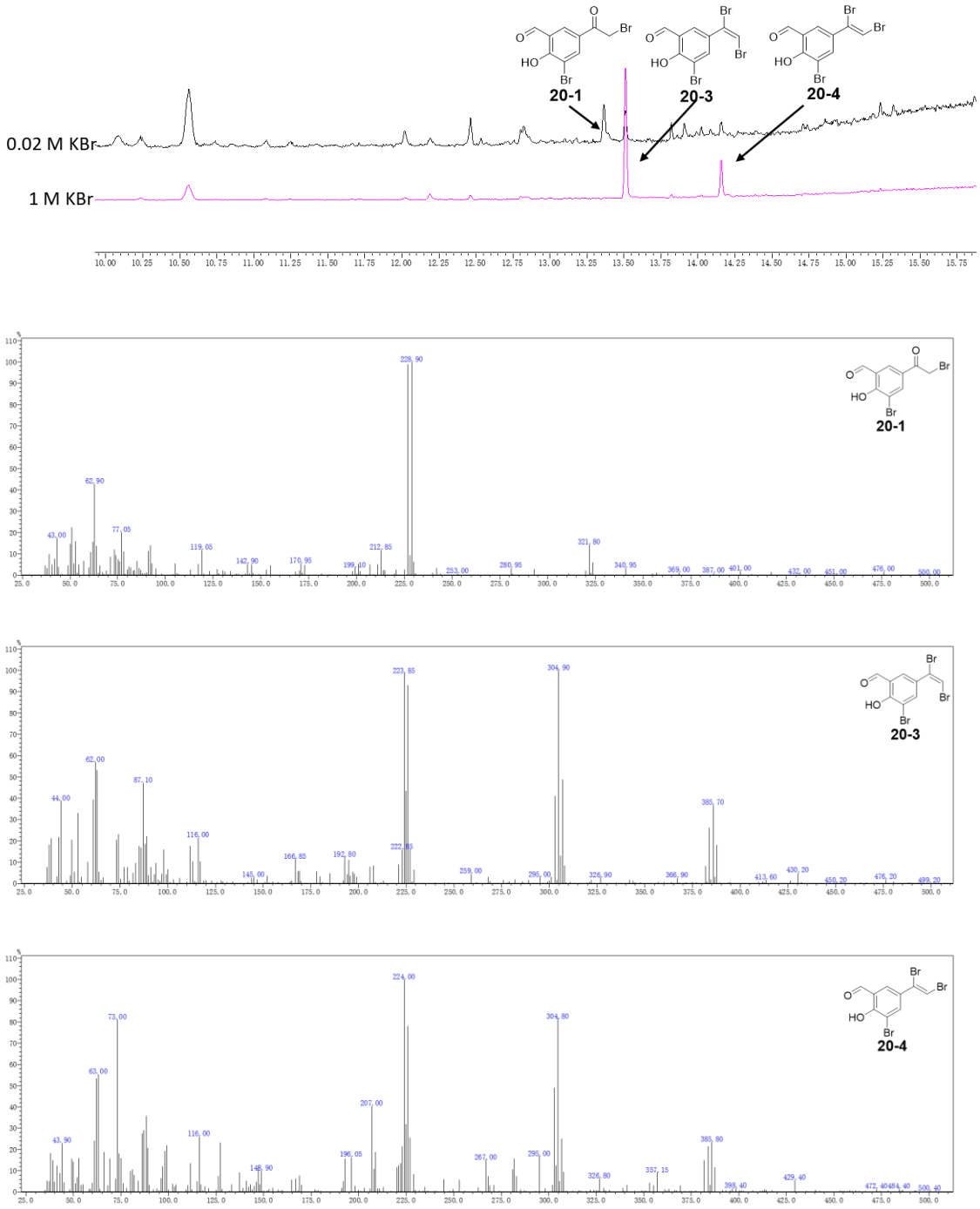


Figure S23. Representative GC and Mass spectrum of **20-1, 20-3, 20-4**.

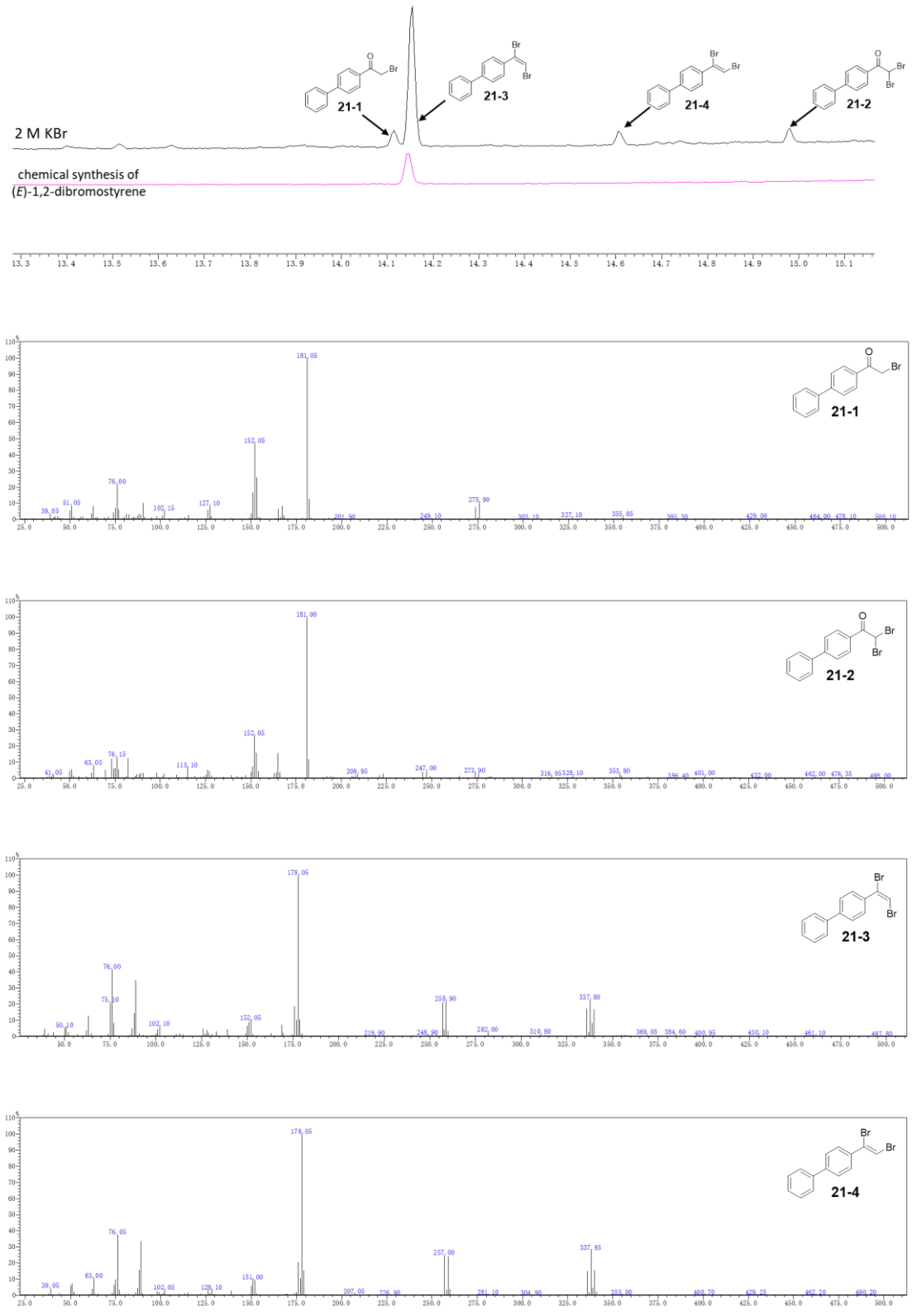


Figure S24. Representative GC and Mass spectrum of **21-1**, **21-2**, **21-3**, **21-4**

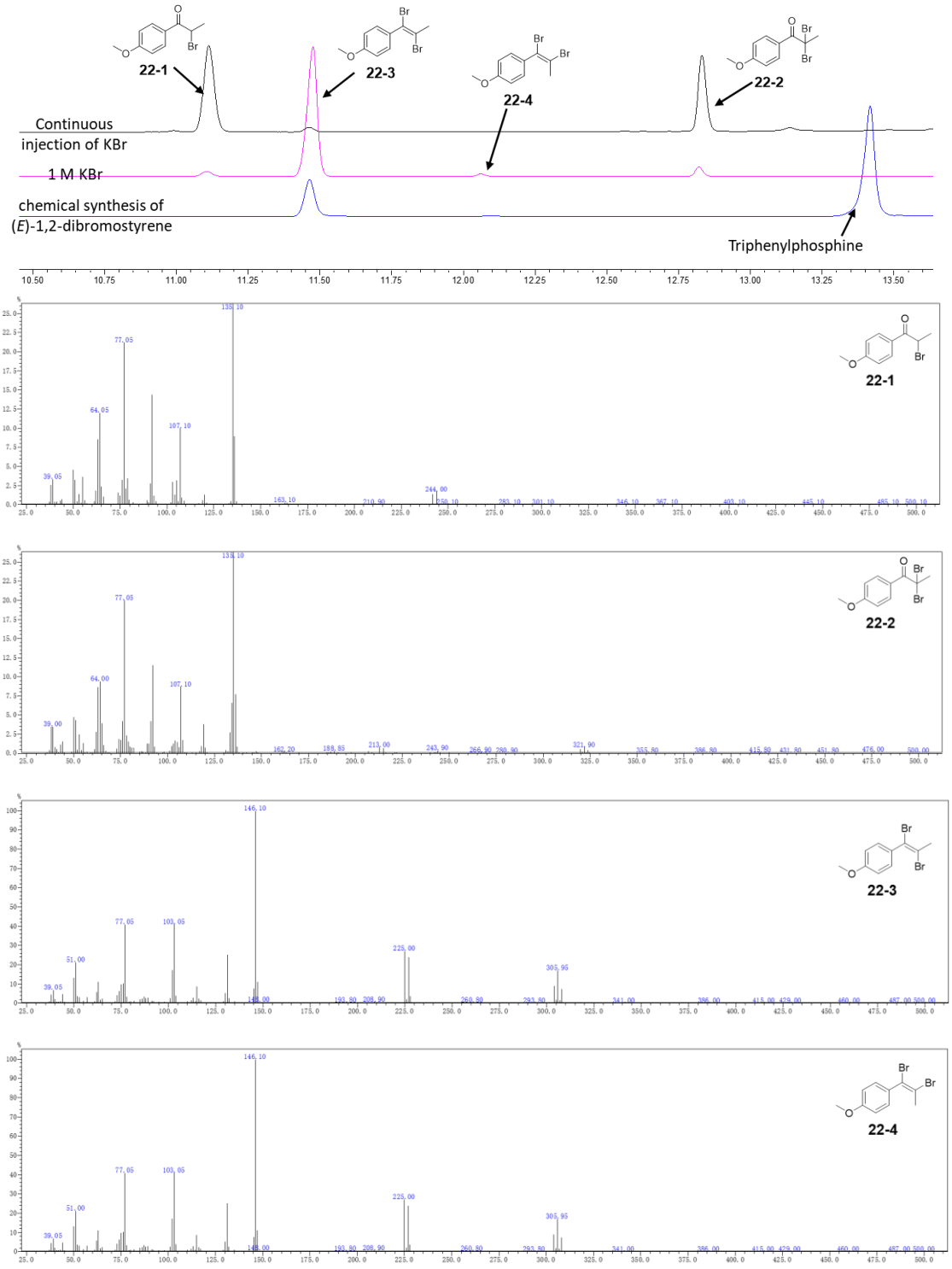


Figure S25. Representative GC and Mass spectrum of **22-1**, **22-2**, **22-3**, **22-4**

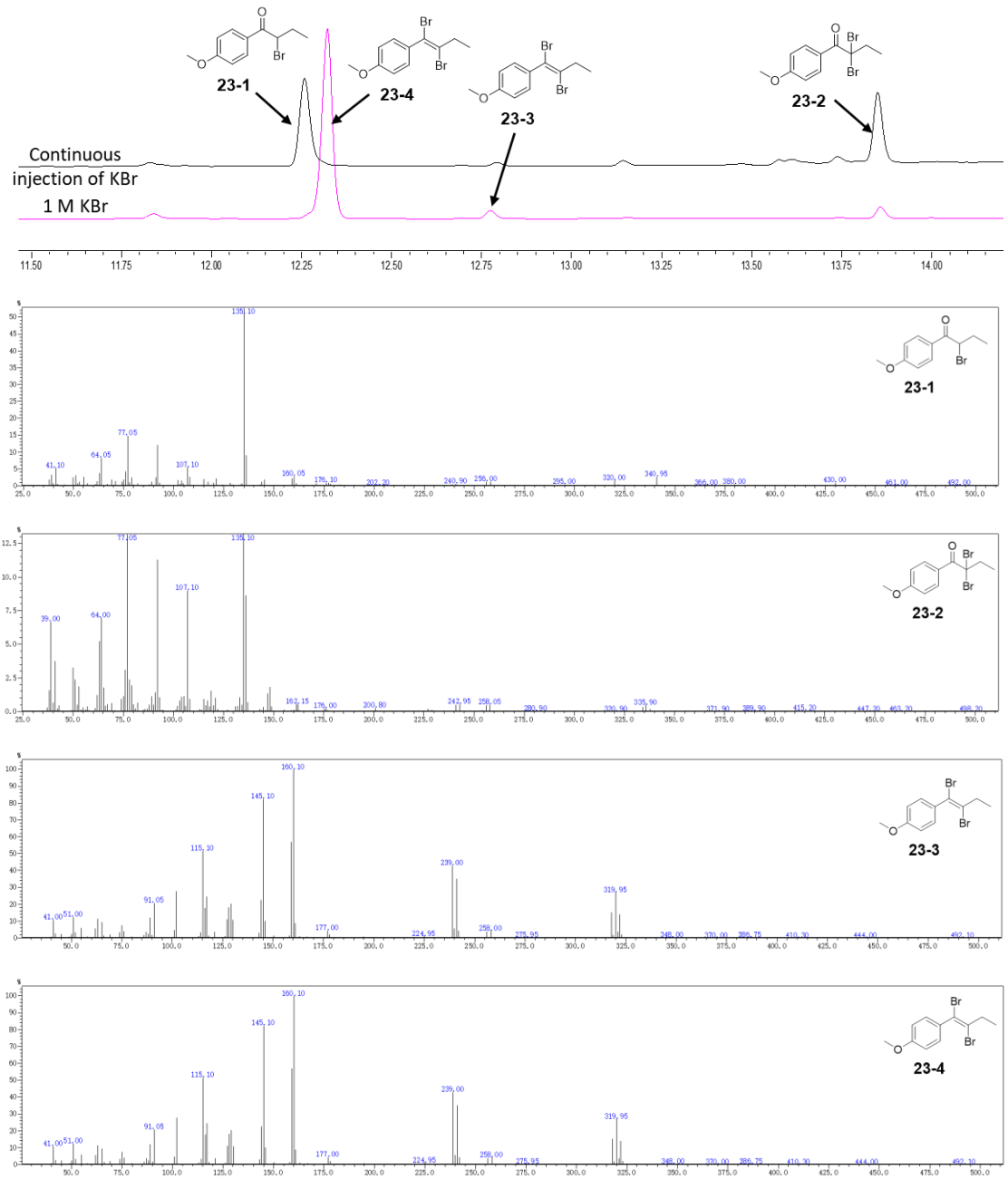


Figure S26. Representative GC and Mass spectrum of **23-1**, **23-2**, **23-3**, **23-4**

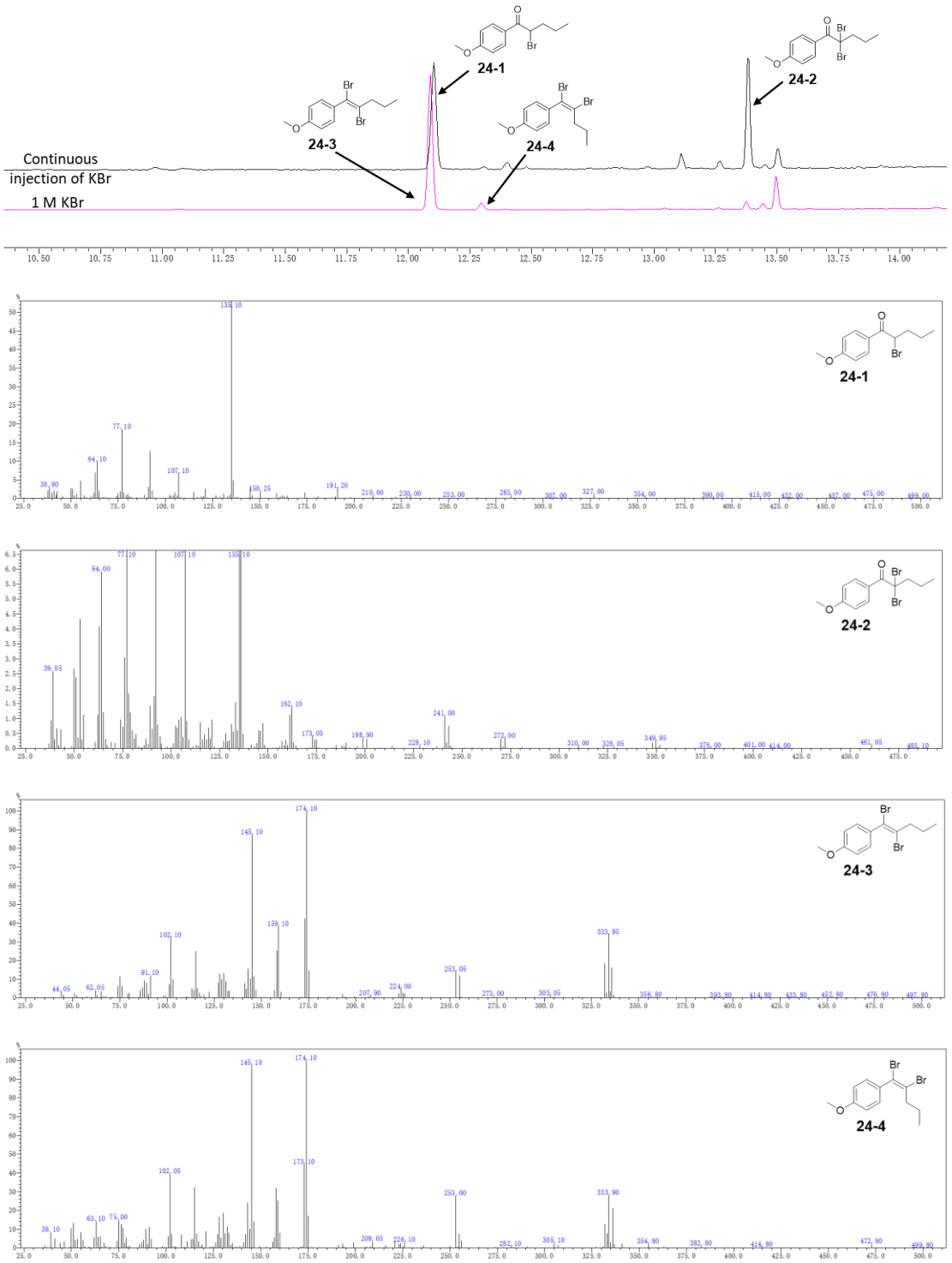


Figure S27. Representative GC and Mass spectrum of **24-1**, **24-2**, **24-3**, **24-4**

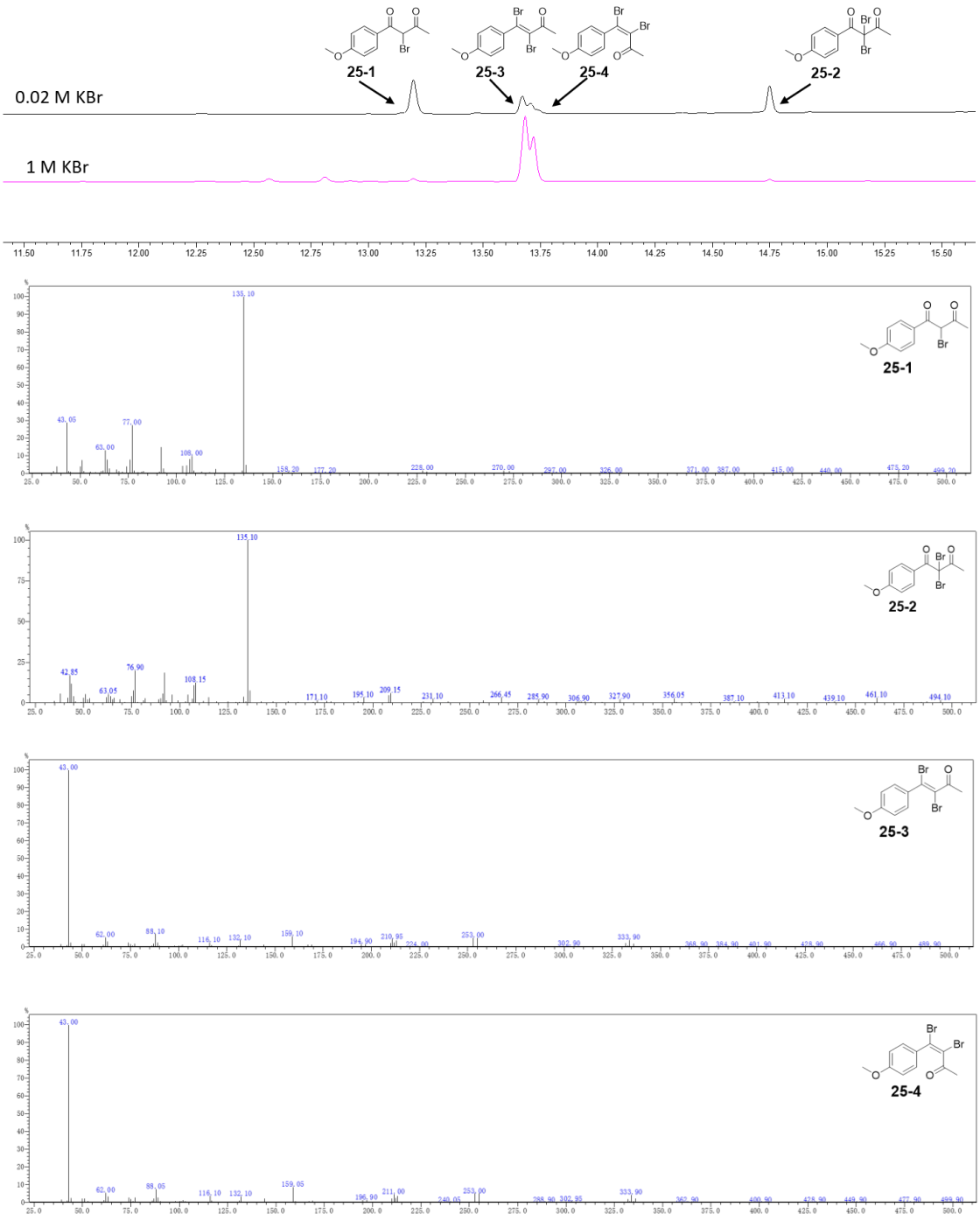


Figure S28. Representative GC and Mass spectrum of **25-1**, **25-2**, **25-3**, **25-4**

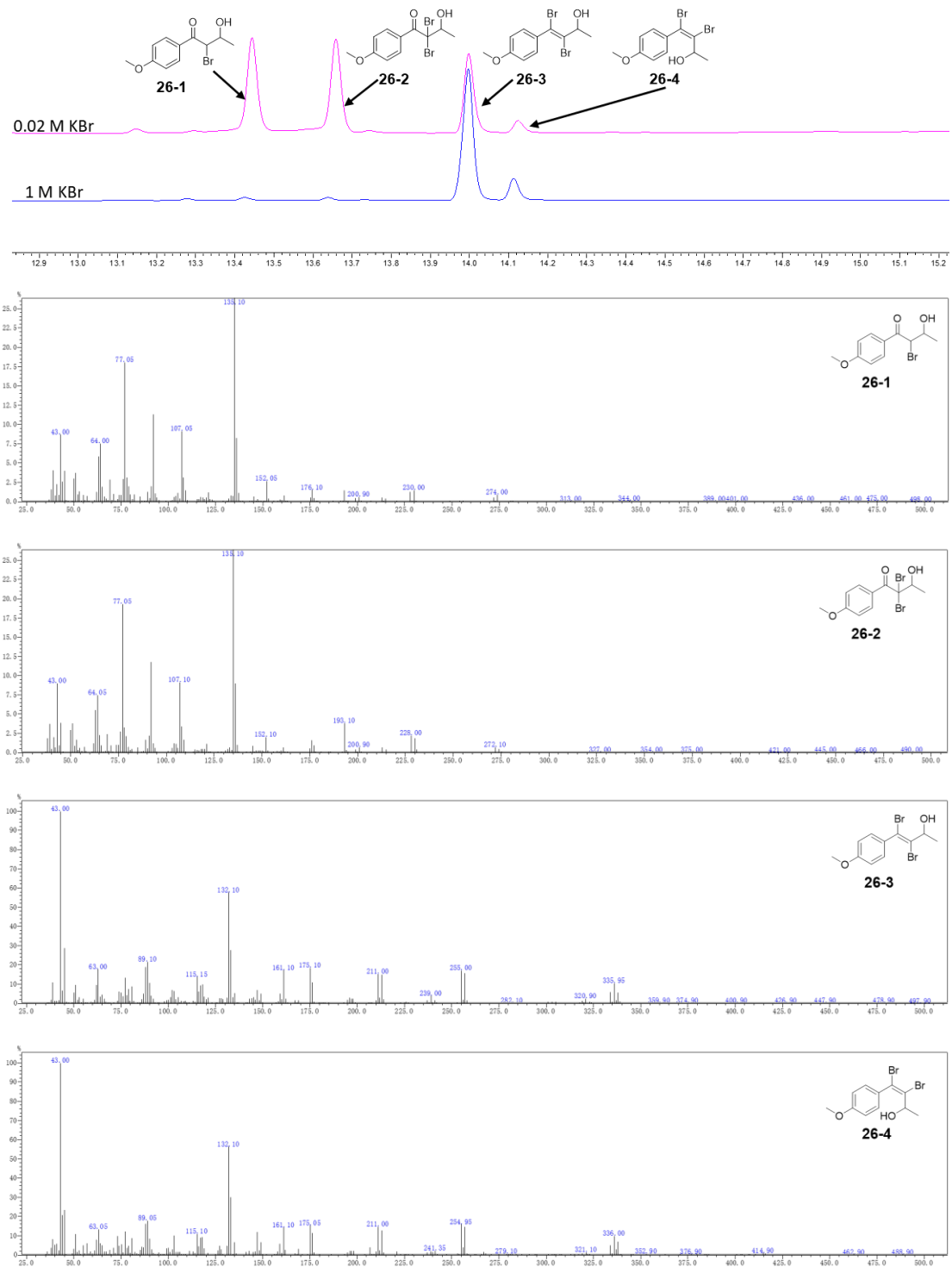


Figure S29. Representative GC and Mass spectrum of **26-1**, **26-2**, **26-3**, **26-4**

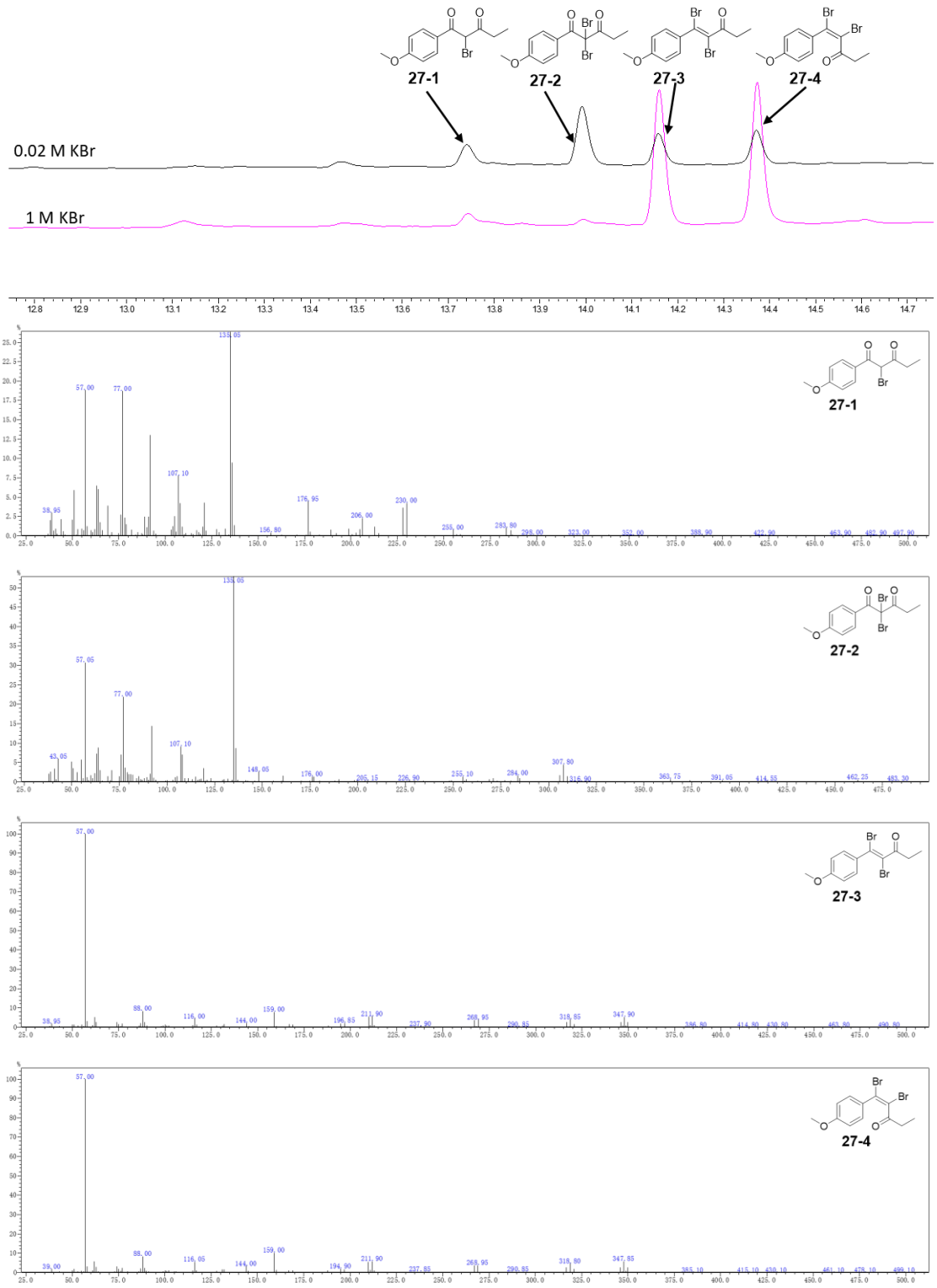


Figure S30. Representative GC and Mass spectrum of **27-1**, **27-2**, **27-3**, **27-4**

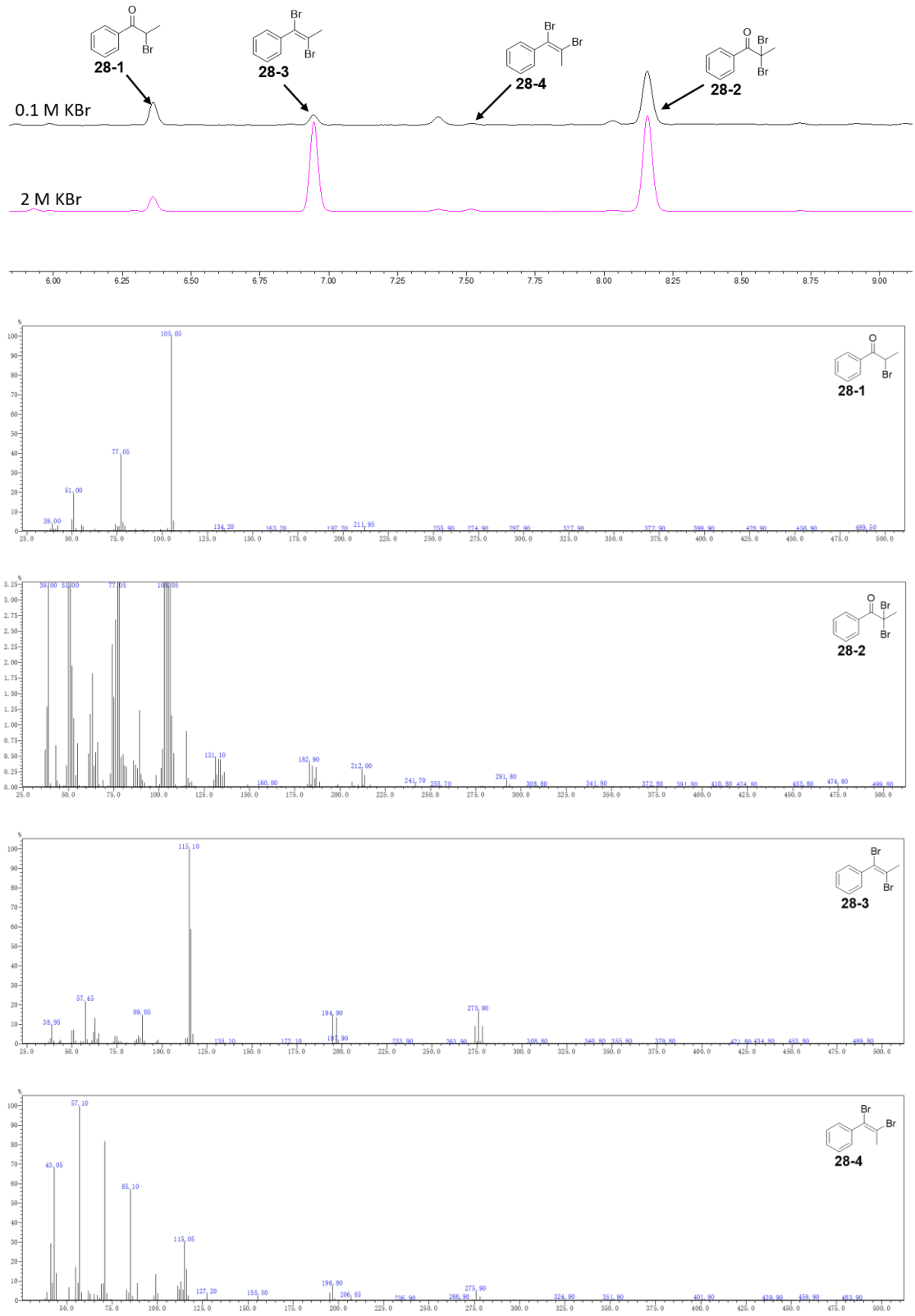


Figure S31. Representative GC and Mass spectrum of **28-1**, **28-2**, **28-3**, **28-4**

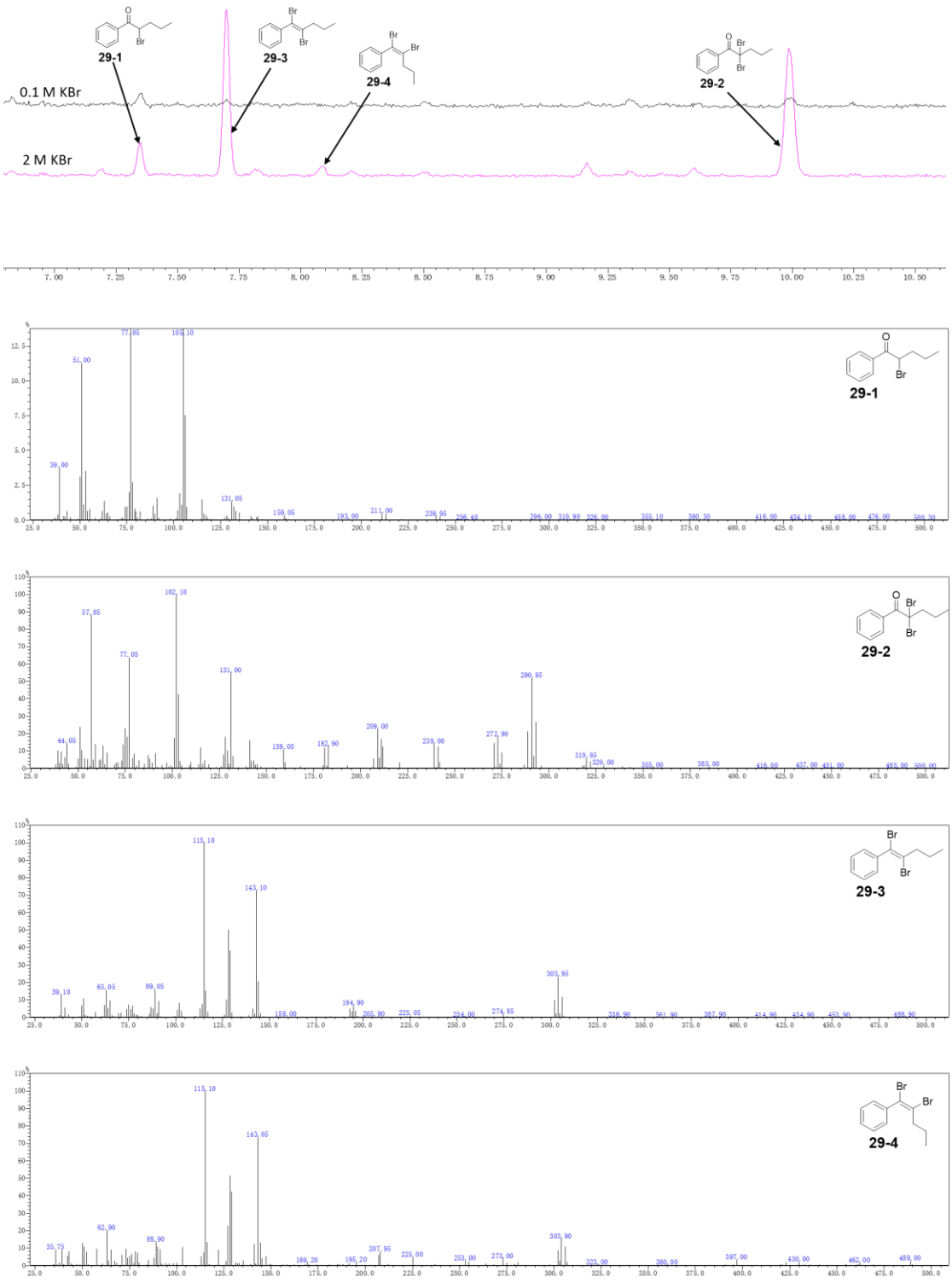


Figure S32. Representative GC and Mass spectrum of **29-1**, **29-2**, **29-3**, **29-4**

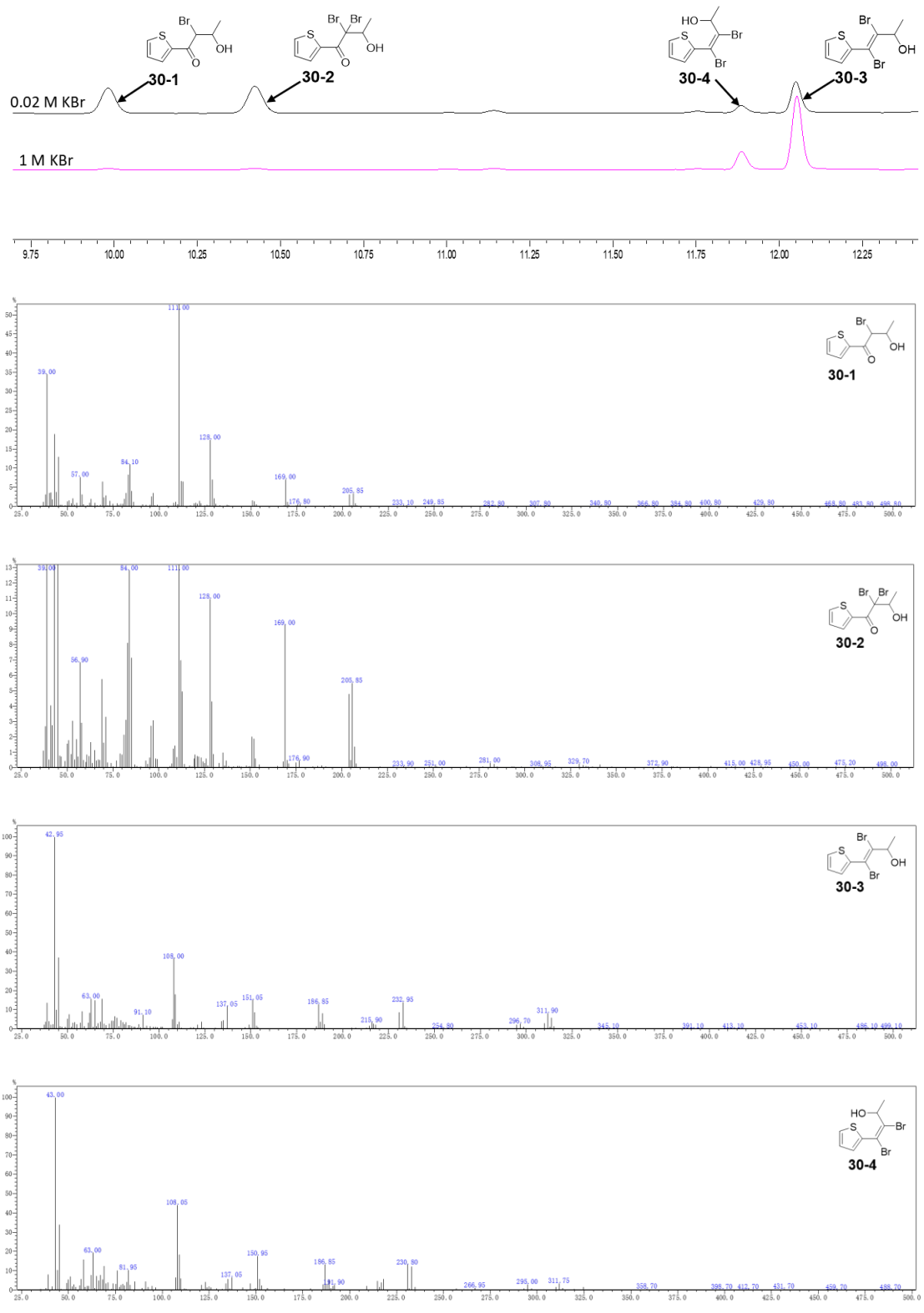


Figure S33. Representative GC and Mass spectrum of **30-1**, **30-2**, **30-3**, **30-4**

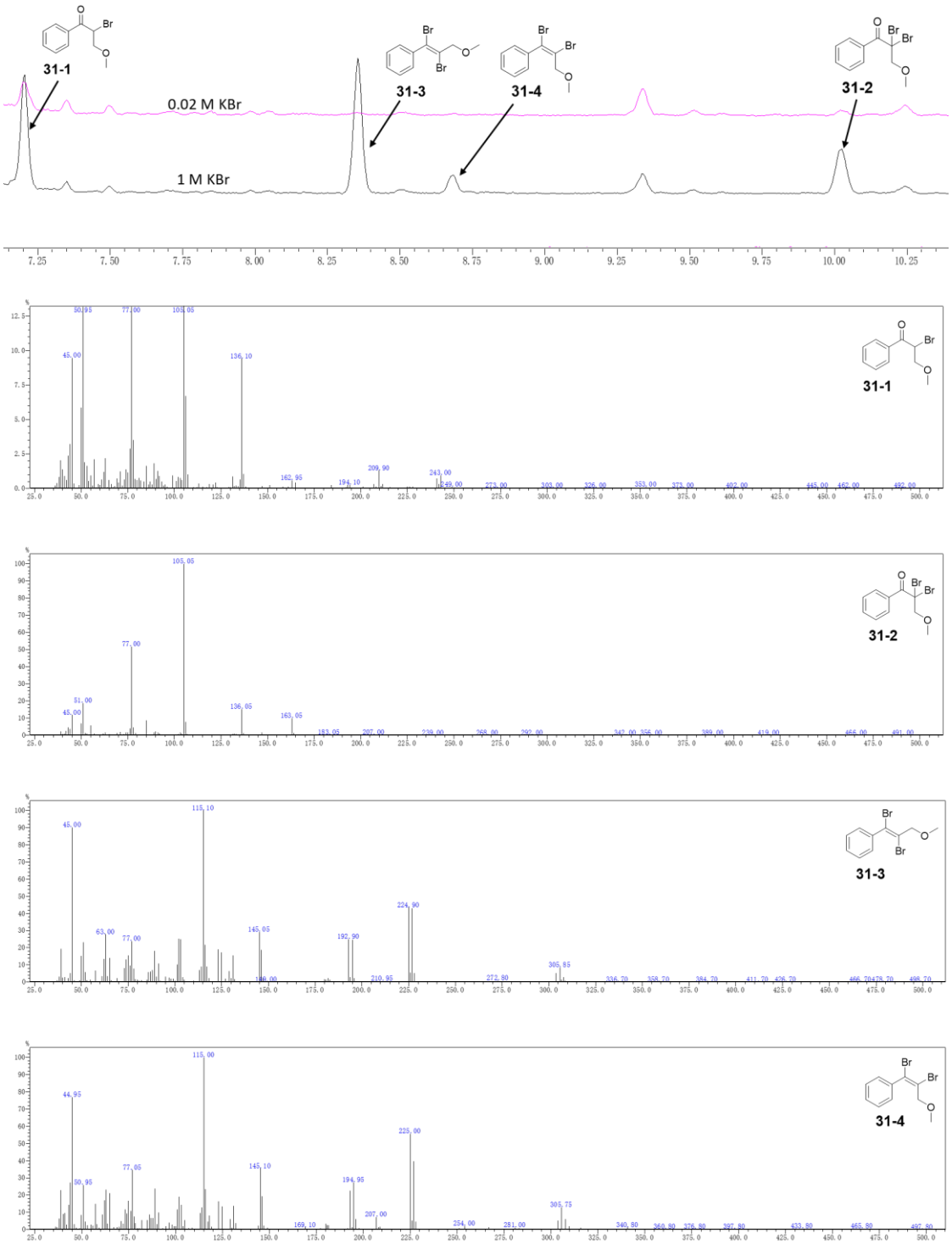


Figure S34. Representative GC and Mass spectrum of **31-1**, **31-2**, **31-3**, **31-4**

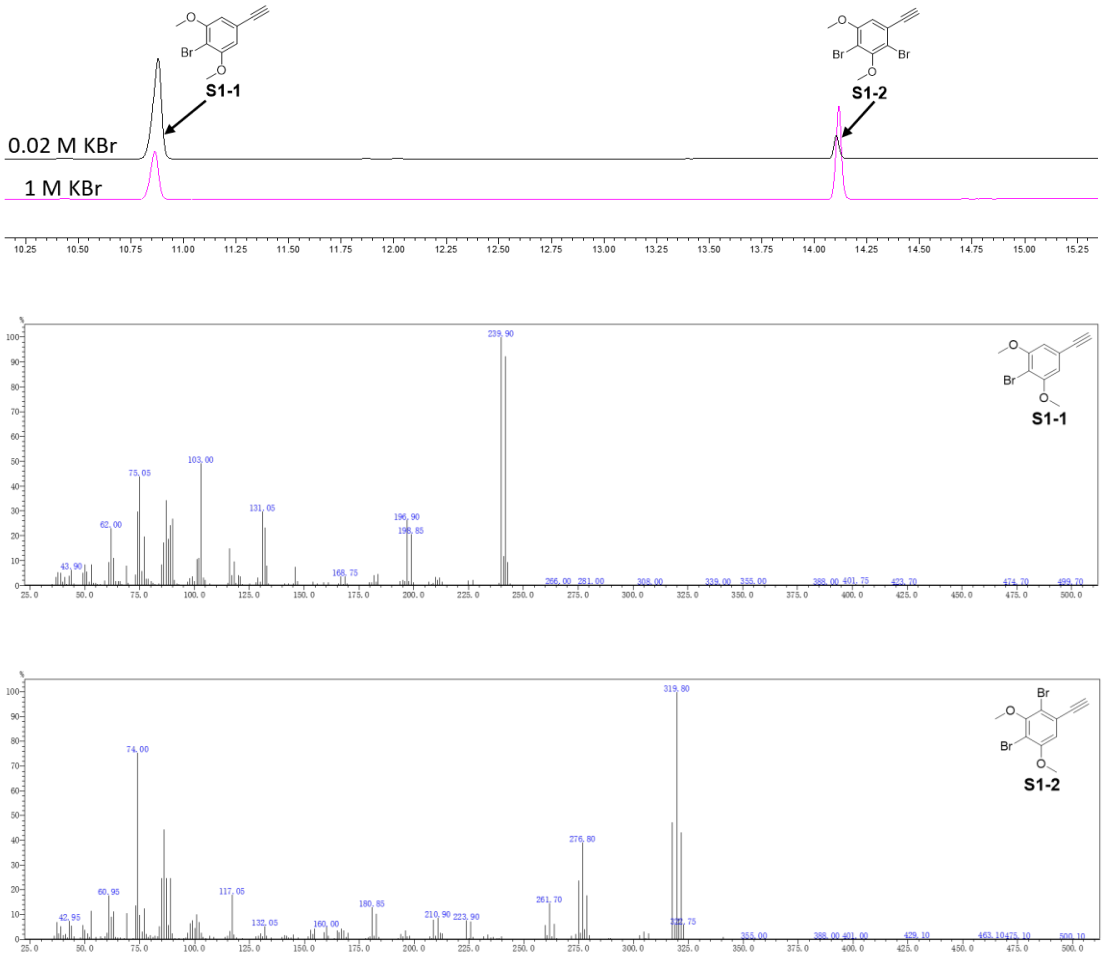


Figure S35. Representative GC and Mass spectrum of **S1-1**, **S1-2**

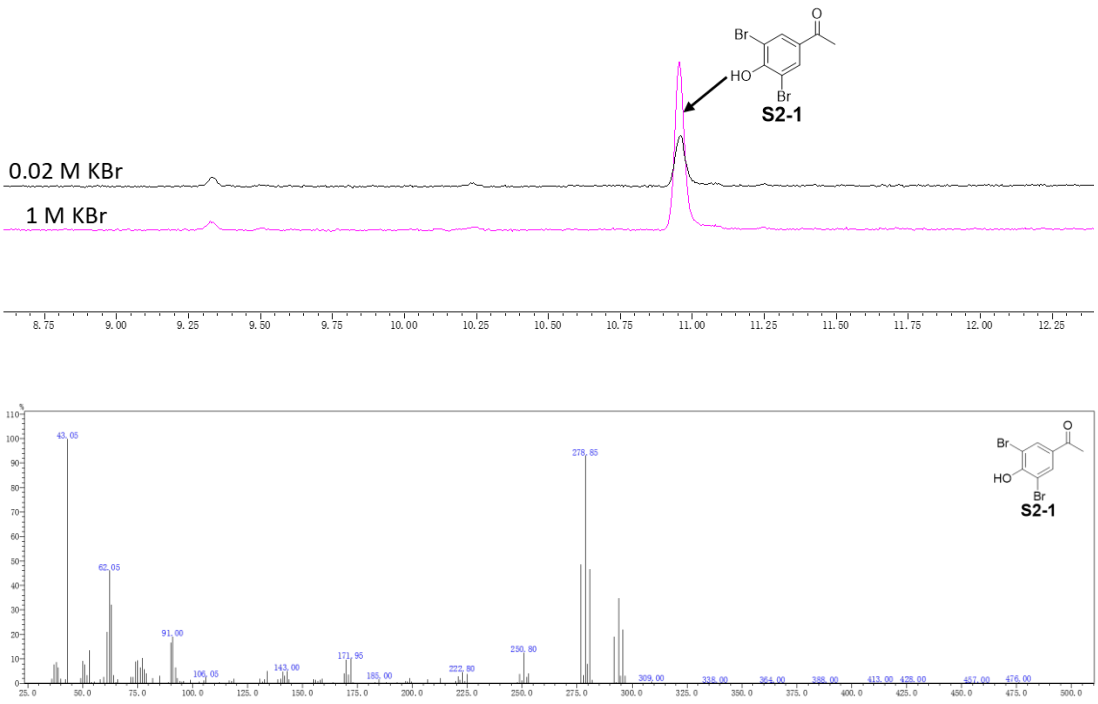


Figure S36. Representative GC and Mass spectrum of **S2-1**

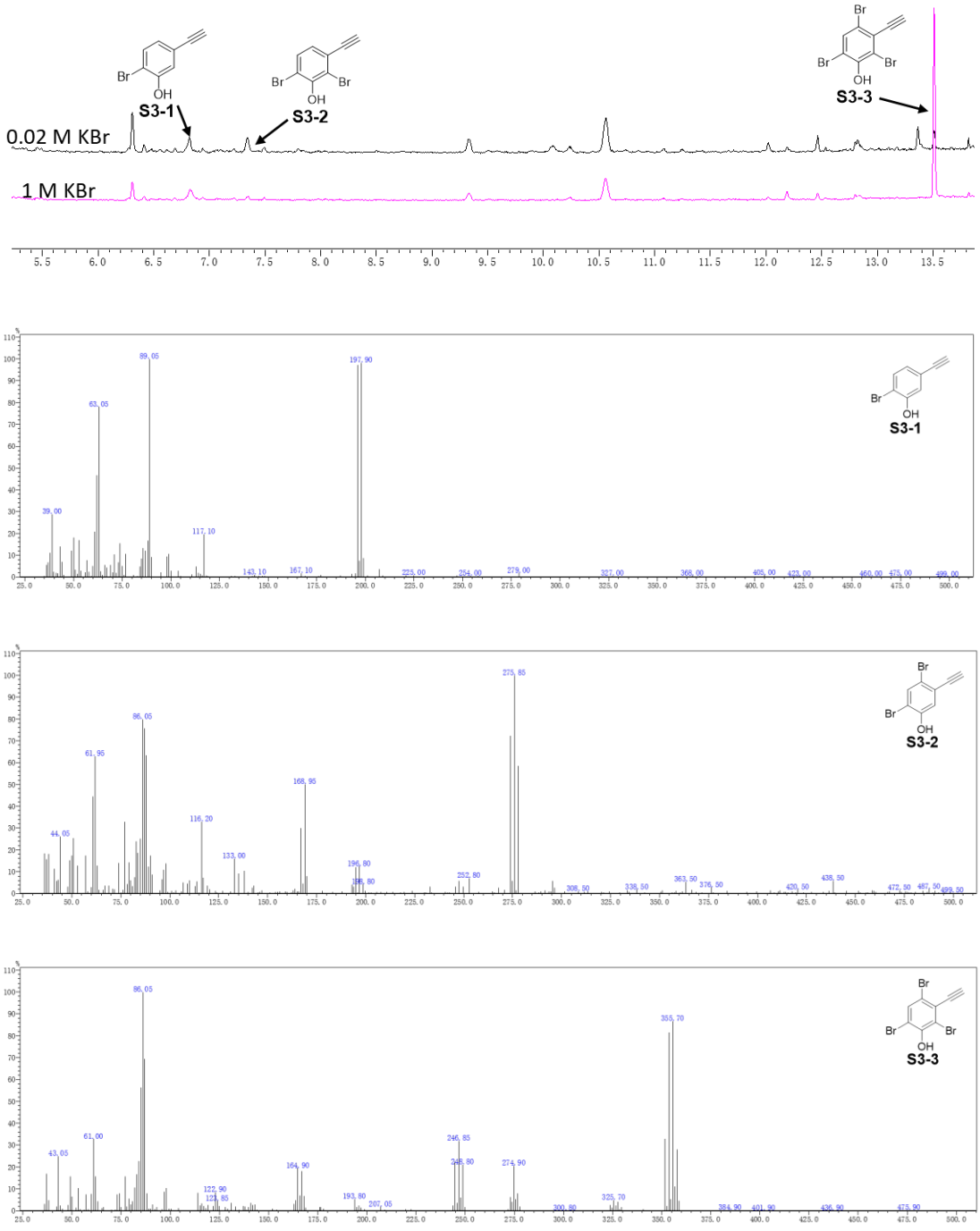
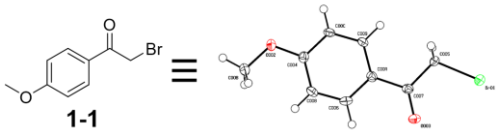


Figure S37. Representative GC and Mass spectrum of **S3-1, S3-2, S3-3**

Crystal structure of **1-1**

Table S5. Crystal structure of **1-1**



Empirical formula	**$C_9H_9BrO_2$**
Formula weight	229.07
Temperature/K	103(3)
Crystal system	monoclinic
Space group	$P2_1/c$
a/Å	7.52210(10)
b/Å	12.39280(10)
c/Å	9.91070(10)
α/°	90
β/°	110.8220(10)
γ/°	90
Volume/Å^3	863.535(17)
Z	4
ρ_{calc} mg/mm^3	1.762
μ/mm^{-1}	6.125
F(000)	456.0
Crystal size/mm^3	0.1 × 0.1 × 0.1
Radiation	Cu Kα (λ=1.54184Å)
2Θ range for data collection	11.928 to 148.648°
Index ranges	-7 ⩽ h ⩽ 9, -14 ⩽ k ⩽ 15, -12 ⩽ l ⩽ 12
Reflections collected	16475
Independent reflections	1703[R(int) = 0.0256]
Data/restraints/parameters	1703/0/110
Goodness-of-fit on F^2	1.233
Final R indexes [I>=2σ (I)]	R_1 = 0.0268, wR_2 = 0.1257
Final R indexes [all data]	R_1 = 0.0270, wR_2 = 0.1262
Largest diff. peak/hole / e Å^{-3}	0.40/-1.11

NMR analysis of products from preparative-scale synthesis

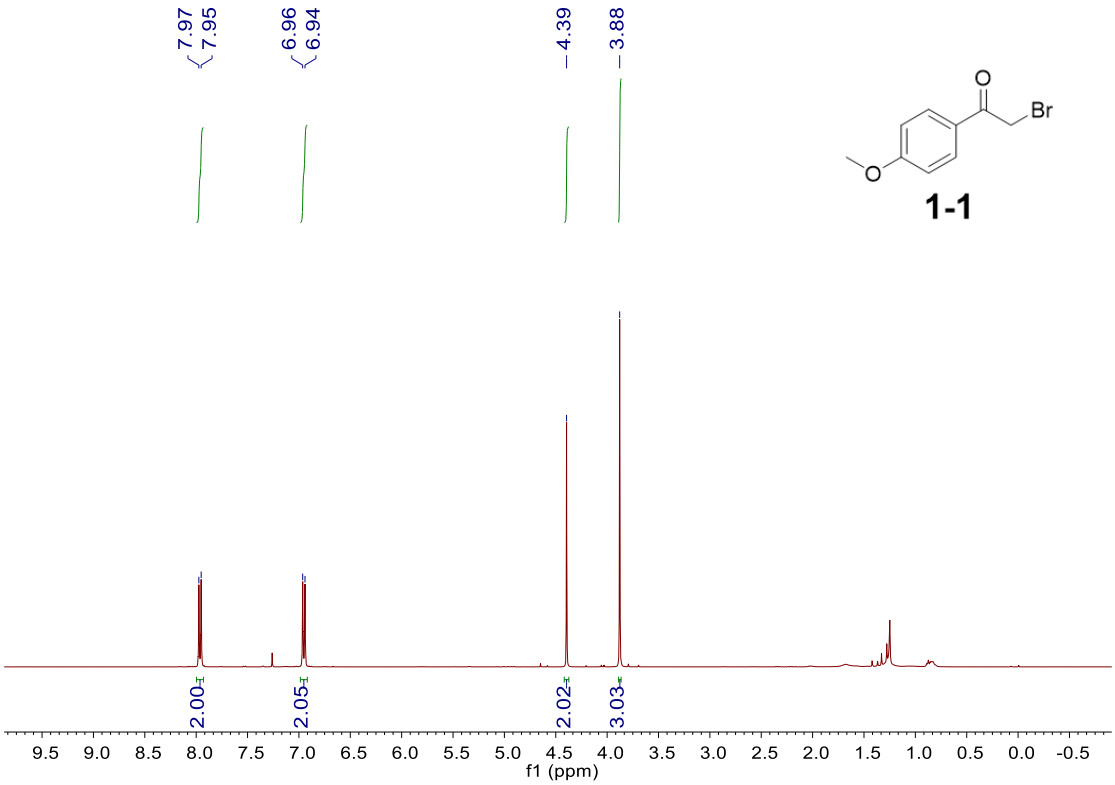


Figure S38. ^{1}H NMR of **1-1**. ^{1}H NMR (400 MHz, Chloroform-*d*): δ 7.96 (d, *J* = 9.0 Hz, 2H), 6.95 (d, *J* = 9.0 Hz, 2H), 4.39 (s, 2H), 3.88 (s, 3H).

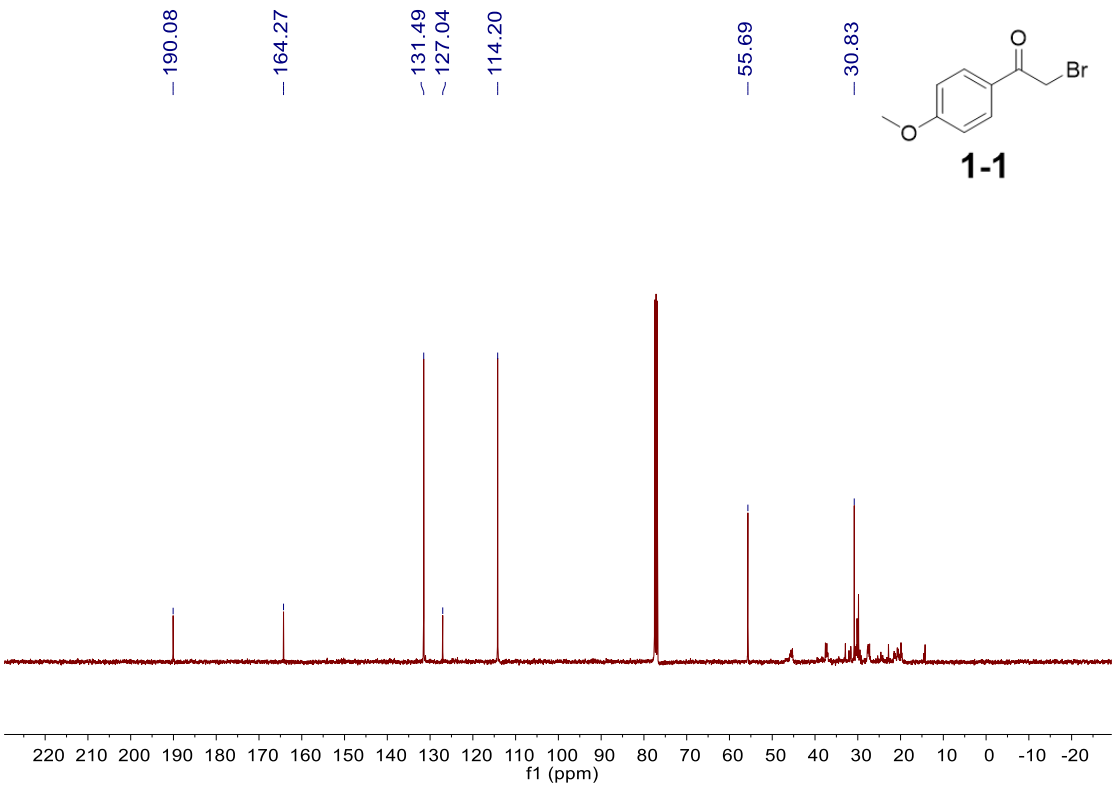


Figure S39. ^{13}C NMR of **1-1**. ^{13}C NMR (101 MHz, Chloroform-*d*): δ 190.08, 164.27, 131.49, 127.04, 114.20, 55.69, 30.83.

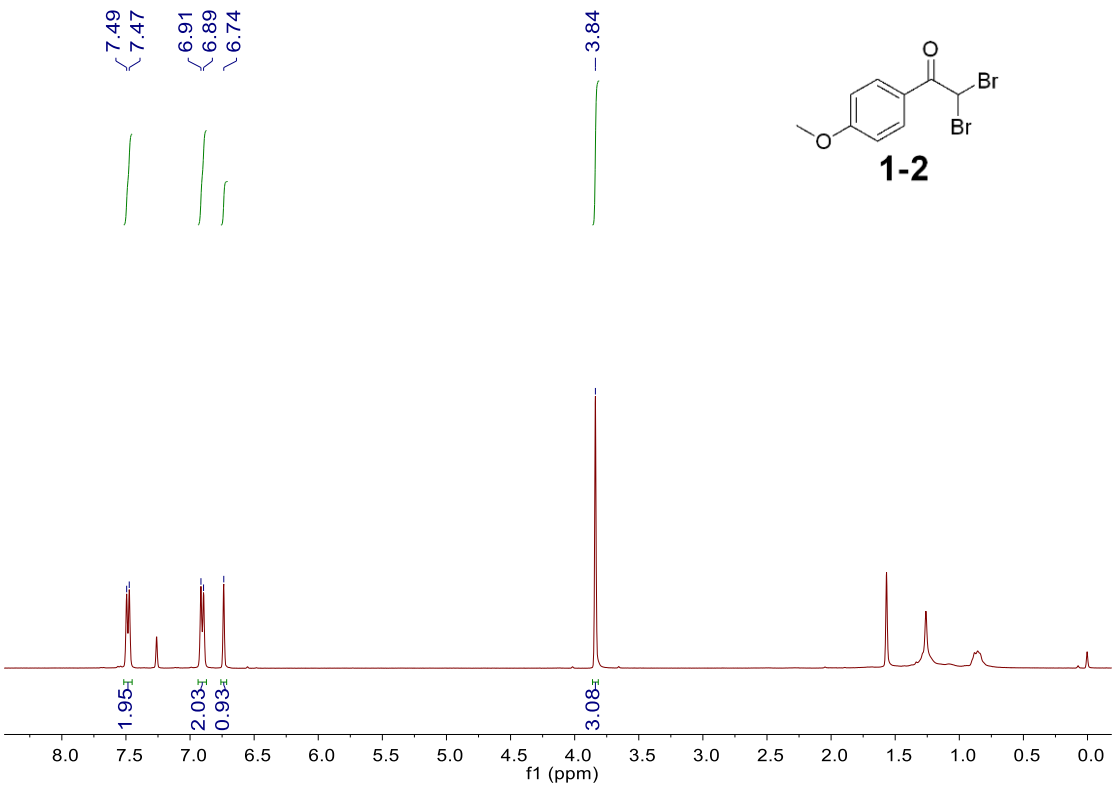


Figure S40. ^{1}H NMR of **1-2**. ^{1}H NMR (400 MHz, Chloroform-*d*): δ 7.48 (d, *J* = 8.1 Hz, 2H), 6.90 (d, *J* = 8.1 Hz, 2H), 6.74 (s, 1H), 3.84 (s, 3H).

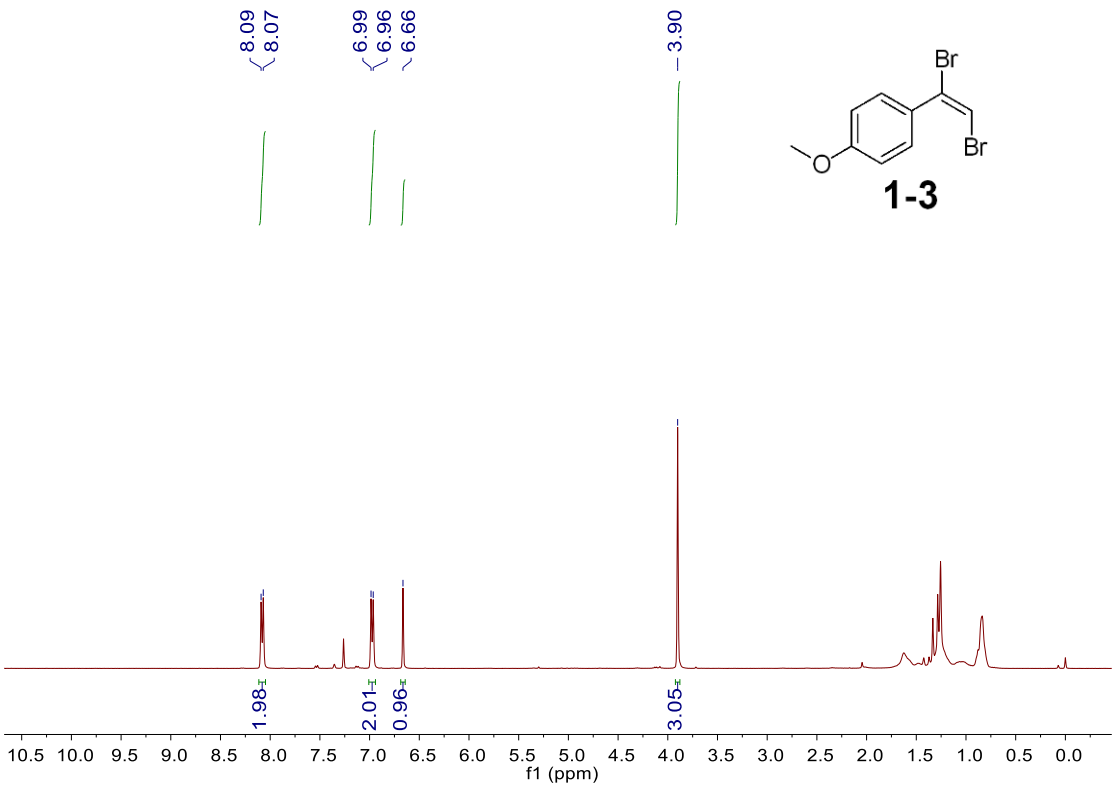


Figure S41. ^{1}H NMR of **1-3**. ^{1}H NMR (400 MHz, Chloroform-*d*): δ 8.11 – 8.05 (m, 2H), 6.97 (d, *J* = 8.8 Hz, 2H), 6.66 (s, 1H), 3.90 (s, 3H).

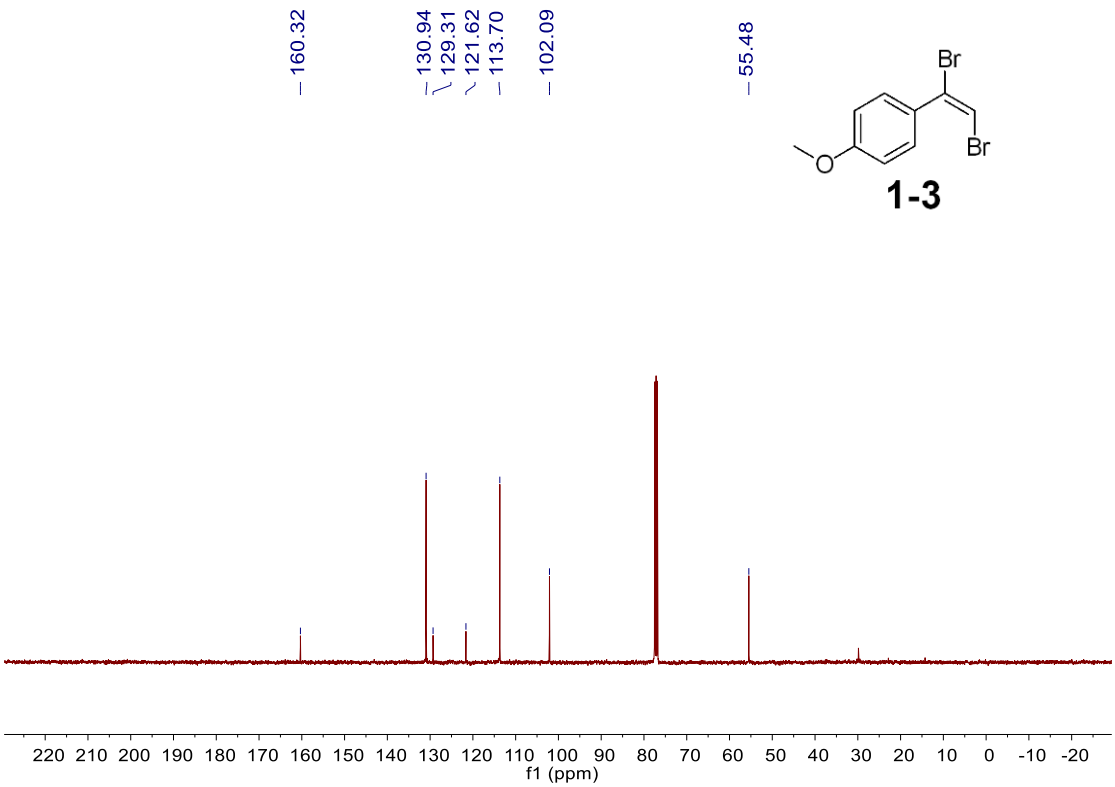


Figure S42. ^{13}C NMR of **1-3**. ^{13}C NMR (101 MHz, Chloroform-*d*): δ 160.32, 130.94, 129.31, 121.62, 113.70, 102.09, 55.48.

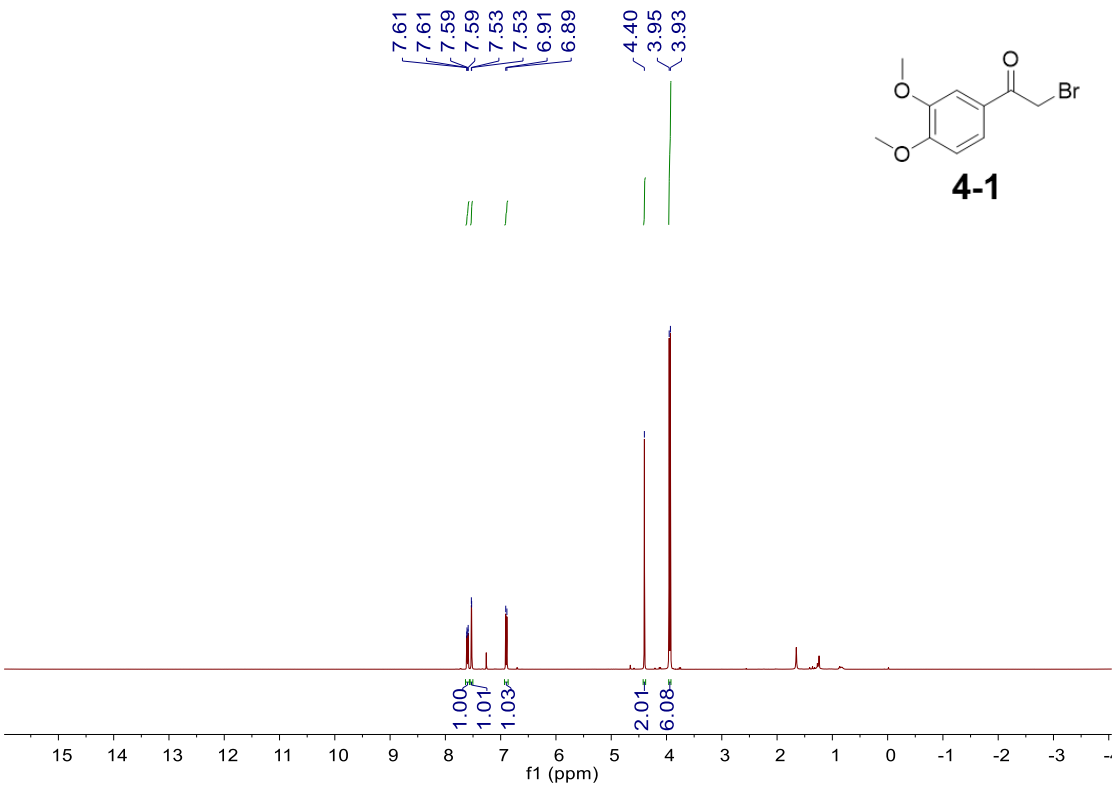


Figure S43. ^{1}H NMR of **4-1**. ^{1}H NMR (400 MHz, Chloroform-*d*): δ 7.60 (dd, *J* = 8.4, 1.9 Hz, 1H), 7.53 (d, *J* = 1.9 Hz, 1H), 6.90 (d, *J* = 8.4 Hz, 1H), 4.40 (s, 2H), 3.94 (d, *J* = 8.9 Hz, 6H).

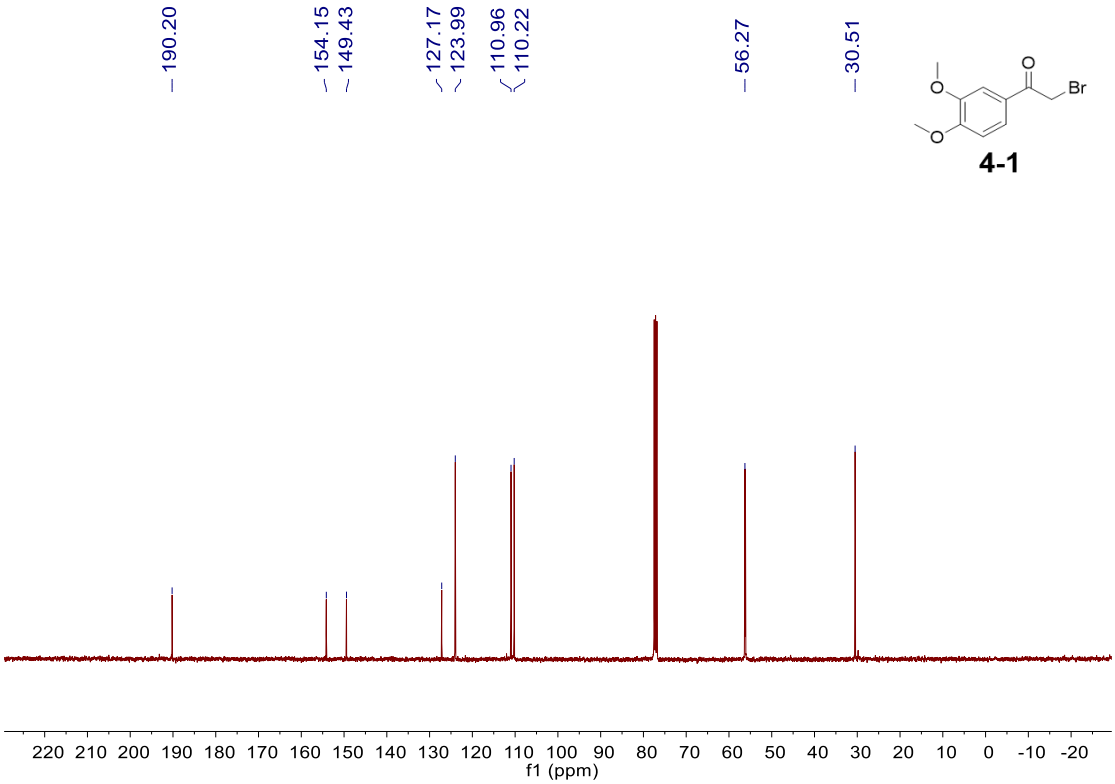


Figure S44. ^{13}C NMR of **4-1**. ^{13}C NMR (101 MHz, Chloroform-*d*): δ 190.20, 154.15, 149.43, 127.17, 123.99, 110.96, 110.22, 56.27, 30.51.

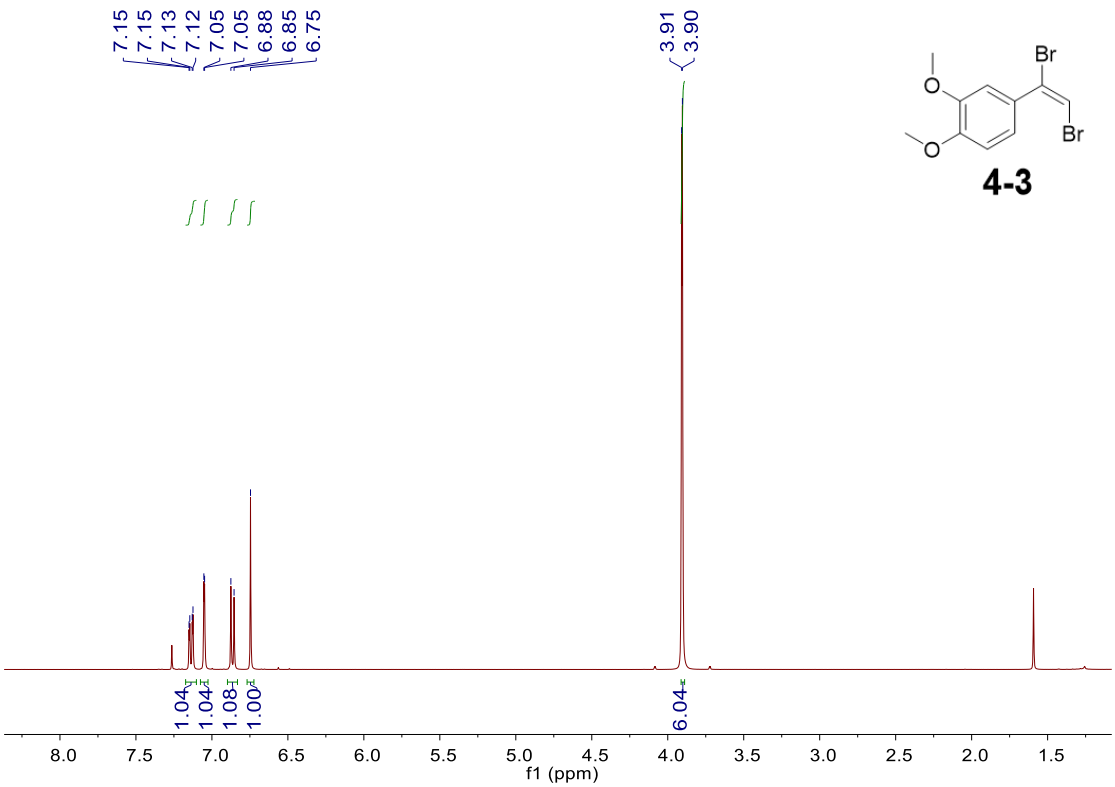


Figure S45. ^{1}H NMR of **4-3**. ^{1}H NMR (400 MHz, Chloroform-*d*): δ 7.14 (dd, *J* = 8.4, 2.0 Hz, 1H), 7.05 (d, *J* = 1.9 Hz, 1H), 6.86 (d, *J* = 8.4 Hz, 1H), 6.75 (s, 1H), 3.91 (d, *J* = 2.1 Hz, 6H).

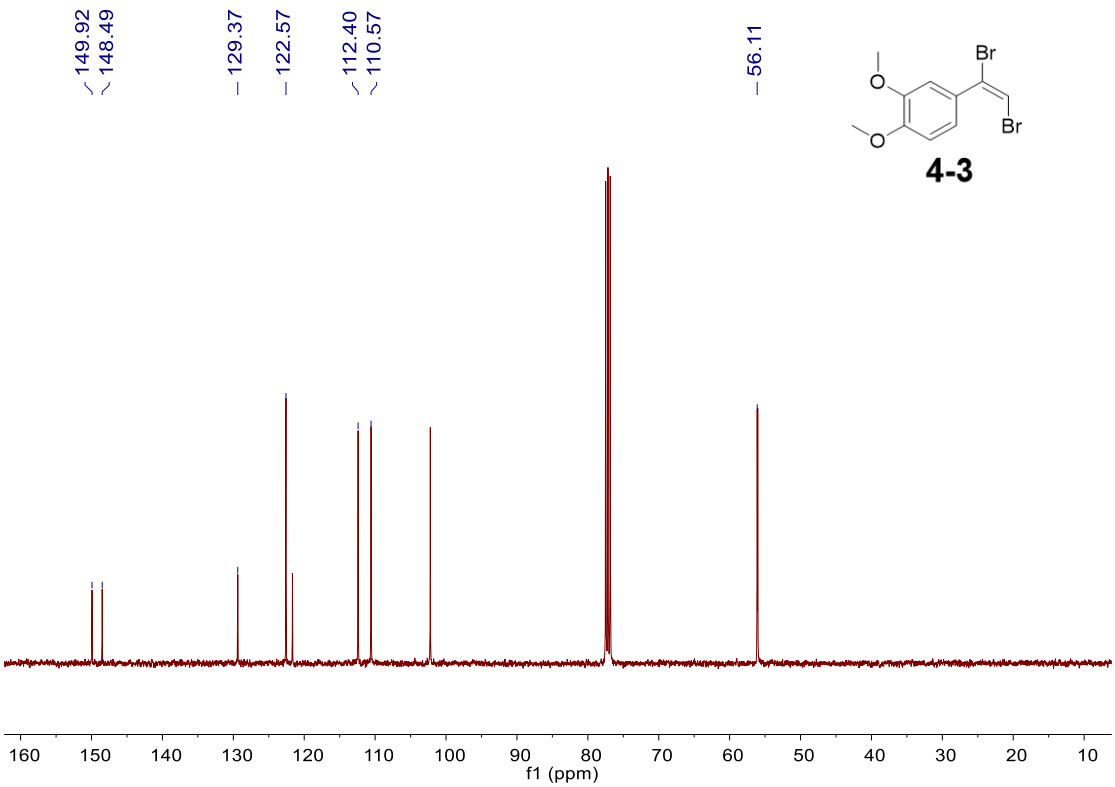


Figure S46. ^{13}C NMR of **4-3**. ^{13}C NMR (101 MHz, Chloroform-*d*): δ 149.92, 148.49, 129.37, 122.57, 112.40, 110.57, 56.11.

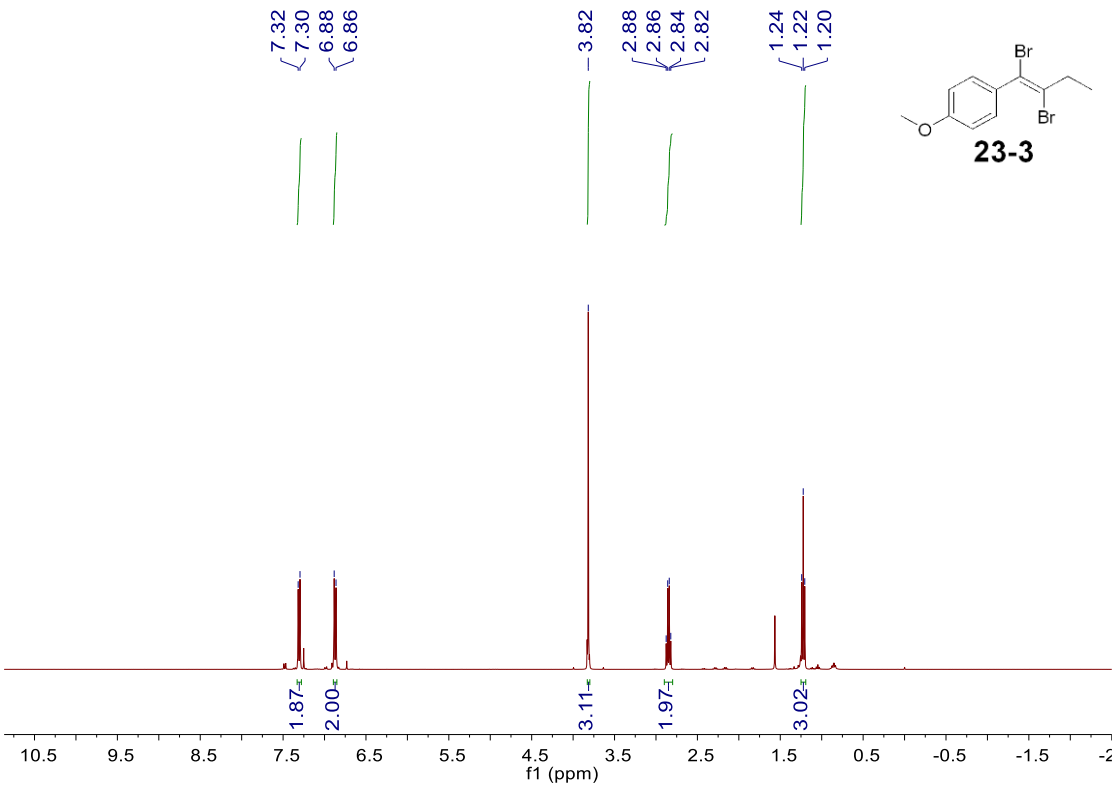


Figure S47. ^{1}H NMR of **22-3**. ^{1}H NMR (400 MHz, Chloroform-*d*): δ 7.31 (d, *J* = 8.8 Hz, 2H), 6.87 (d, *J* = 8.8 Hz, 2H), 3.82 (s, 3H), 2.85 (q, *J* = 7.4 Hz, 2H), 1.22 (t, *J* = 7.4 Hz, 3H).

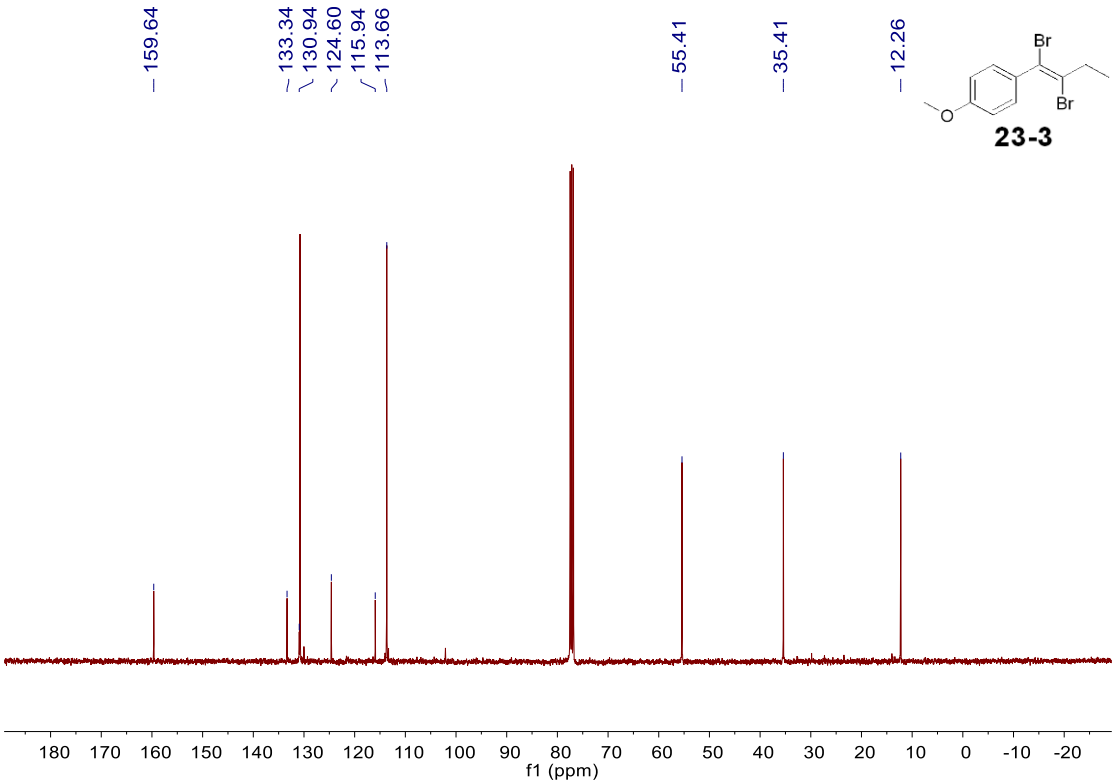


Figure S48. ^{13}C NMR of **22-3**. ^{13}C NMR (101 MHz, Chloroform-*d*): δ 159.64, 133.34, 124.60, 115.94, 113.66, 55.41, 35.41, 12.26.

Mechanism investigation

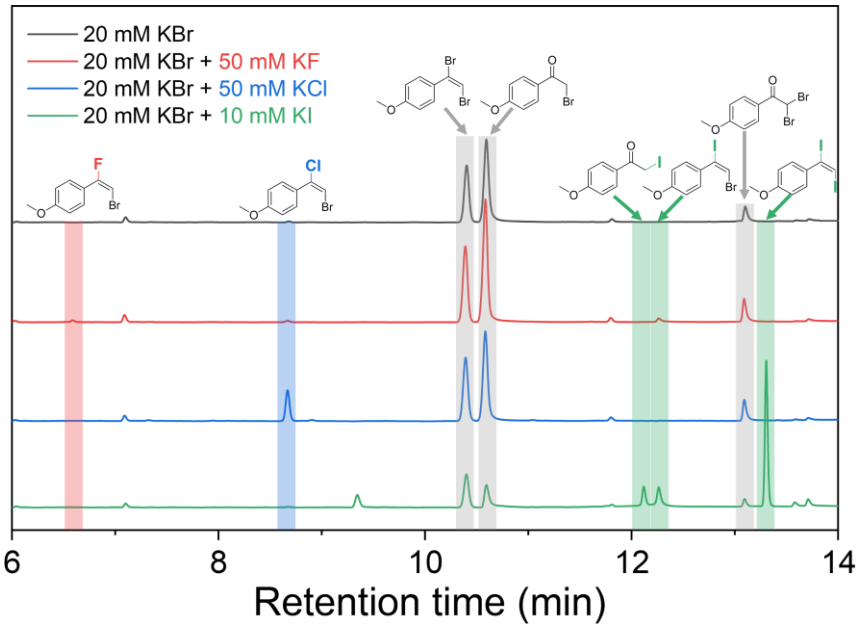


Figure S49. Trapping the carbenium ion by using exogenous nucleophiles (KF, KCl and KI). Condition: 10% DMSO in citrate buffer (100 mM, pH 5.0), [**1**] = 10 mM, [*Ci*VCPO] = 150 nM, [H_2O_2] = 100 mM, [KBr] = 20 mM, 30 °C, 800 rpm, 0.5 h. Exogenous KX: [KF] = 50 mM, [KCl] = 50 mM, [KI] = 10 mM. The products were determined by GC and GC-MS.

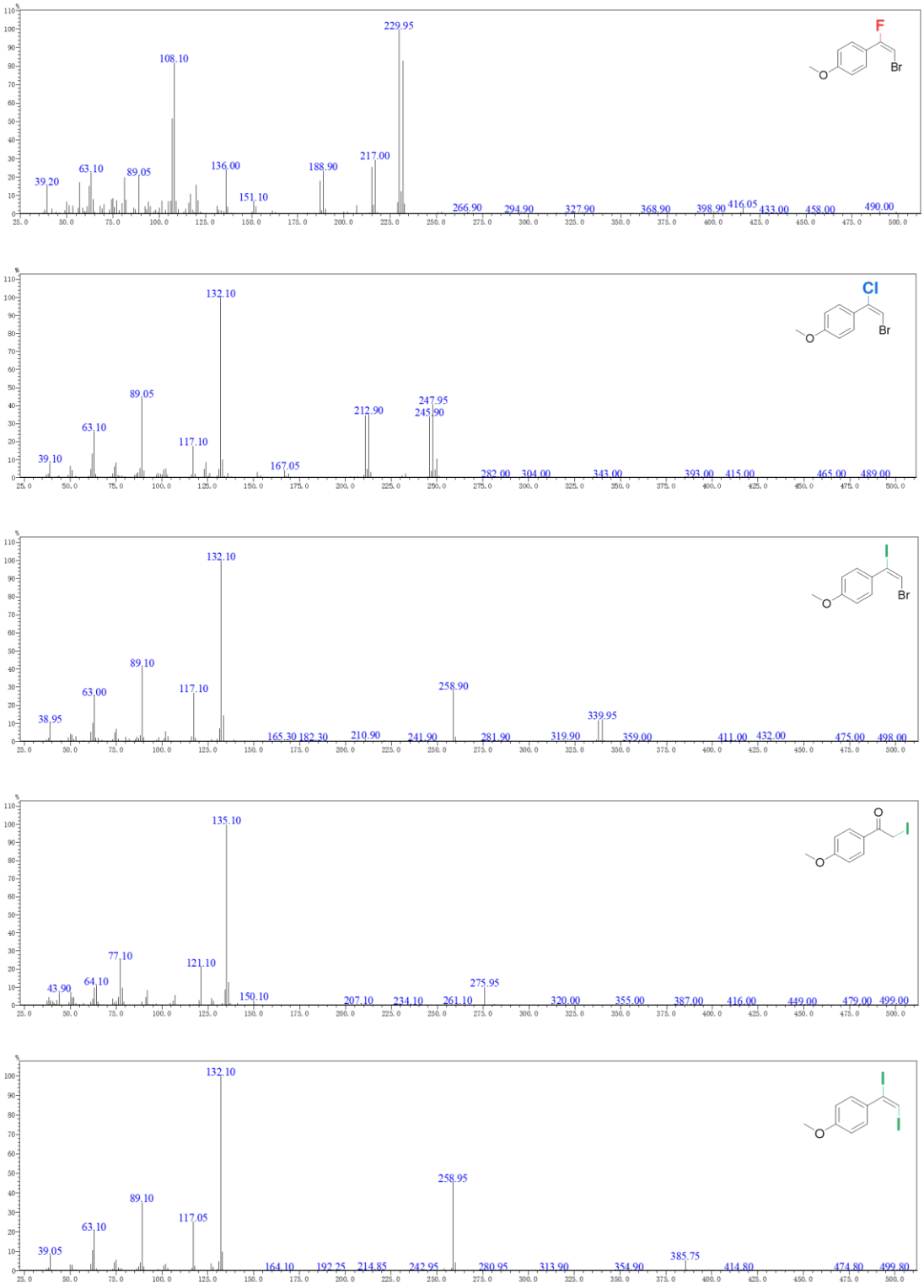


Figure S50. Mass spectrum of multi-halogenated products in figure S49.

E-factor analysis

Table S6. Comparison of E-factor for the synthesis of α-bromoketones.

Item	Mass (g)			
Substrate	0.12	0.03	0.03	0.13
Cosubstrate	0.49 (HBr)	0.04 (NBS)	0.06 (oxidants)	2.38 (KBr)
Cosolvent	1.57	1.03	3.98	3.63
Catalyst	-	0.00	0.01	0.00
Solvent for isolation	67.29	22.41	22.41	67.29
Silica gel	11.00	3.66	3.66	11.00
Sum of wastes	80.48	27.17	30.15	84.44
Product	0.16	0.03	0.03	0.09
E factor	508.09	790.93	1180.57	938
System	H_2O_2/KBr[3]	$AuCl_3$/NBS[4]	$Ph_3PAuNTf_2$/Oxidant[5]	This work

Table S7. Comparison of E-factor for the synthesis of 1,2-Dibromostyrenes

Item	Mass (g)		
Substrate	0.04	0.03	0.13
Cosubstrate	0.22 (PIDA/NaBr)	0.07 (NBS)	(Recyclable KBr)
Cosolvent	2.36	0.53	3.63
Catalyst	-	0.05 (PPh_3)	0.00
Solvent for isolation	21.83	13.10	65.50
Silica gel	3.67	2.20	11.00
Sum of wastes	28.11	15.98	80.26
Product	0.06	0.03	0.22
E factor	499	591.28	363
System	Hypervalent Iodine/NaBr[6]	NBS/PPh_3[2]	This work

References

1 H. Li, S. H. H. Younes, S. Chen, P. Duan, C. Cui, R. Wever, W. Zhang, F. Hollmann, *ACS Catal*., 2022, **12**, 4554–4559.

2 W. Wang, M. Wang, W. Xu, B. Zhao, H. Zhang, Y. Gao, S. Guo and Y. Wang, *Org. Chem. Front.*, 2023, **10**, 3903–3908.

3 G. I. Nikishin, N. I. Kapustina, L. L. Sokova, O. V. Bityukov and A. O. Terent'ev, *RSC Adv.*, 2018, **8**, 28632–28636.

4 Y. Xing, M. Zhang, S. Ciccarelli, J. Lee and B. Catano, *Eur J Org Chem*, 2017, **2017**, 781–785.

5 W. He, L. Xie, Y. Xu, J. Xiang and L. Zhang, *Org. Biomol. Chem.*, 2012, **10**, 3168.

6 Y. Li, X. Chen, D. Huang, Z. Xie and Y. Liu, *Front. Chem.*, 2022, **10**, 879789.