

# Chemical Assay-guided Natural Product Isolation via Solid-supported Chemodosimetric Fluorescent Probe

Hongjun Jeon, Chaemin Lim, Ji Min Lee, and Sanghee Kim\*

College of Pharmacy, Seoul National University, Seoul 151-742, Korea

## Supplementary Information

### Table of Contents

1. General Information.....	S1
2. Synthesis of solid supported alkyne sensory bead <b>1</b> and related compounds .....	S2–S5
3. Absorption and fluorescence emission spectra of compound <b>2</b> and <b>3</b> .....	S6
4. Standard curve of triazolylcoumarin <b>3</b> .....	S7
5. Representative procedure for the chemical assay with bead <b>1</b> .....	S8
6. LC/MS spectra for validation of the sensory system.....	S9–S22
7. The list of the screened extracts of various natural plants.....	S23
8. Isolation procedure of the natural compound <b>8</b> from the extract of <i>C. morifolium</i> .....	S24
9. <sup>1</sup> H NMR and <sup>13</sup> C NMR Spectra .....	S25–S31
10. References.....	S32

\*To whom correspondence should be addressed: Tel: 82-2-880-2487. Fax: 82-2-888-0649.

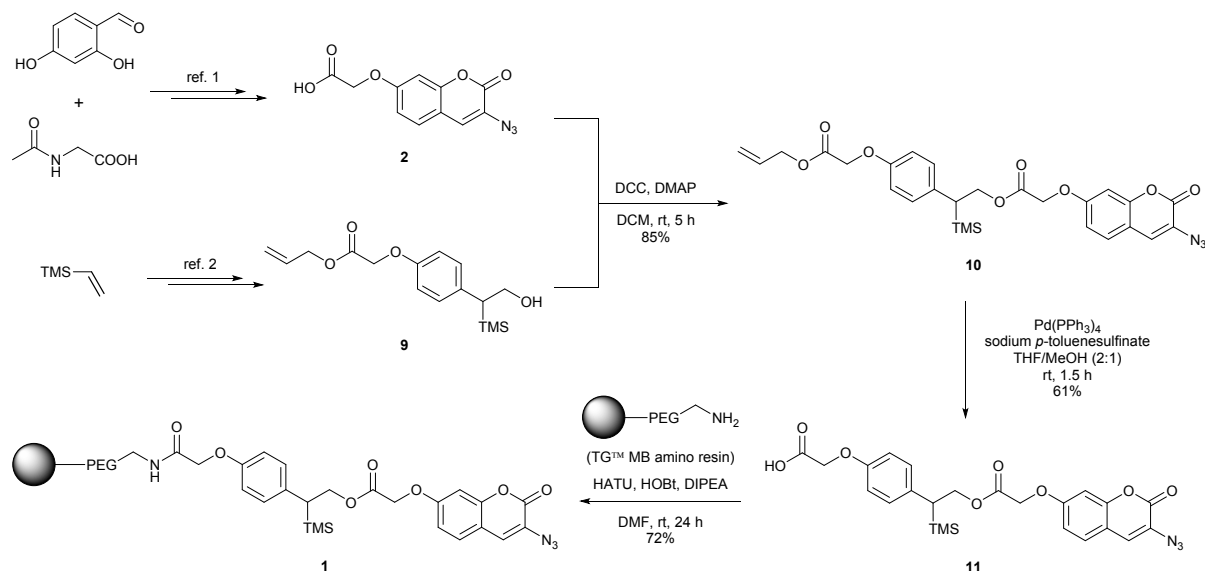
E-mail: pennkim@snu.ac.kr.

## 1. General information

All chemicals were reagent grade and were used as received. The reactions were monitored by TLC analysis using silica-gel 60 F-254 TLC plates. Flash column chromatography was performed on silica gel (230-400 mesh).  $^1\text{H}$  NMR and  $^{13}\text{C}$  NMR spectra were recorded in  $\delta$  units relative to the non-deuterated solvent as an internal reference. The IR spectra were measured on a Fourier-transform infrared spectrometer. High-resolution mass spectra (HRMS) were recorded using fast atom bombardment (FAB). UV absorption and fluorescence emission spectra were recorded using a UV-Vis (Hitachi U-3010) and a fluorescence spectrophotometer (JASCO FP-6500), respectively. Fluorescence images were acquired using a fluorescence microscope (Nikon Eclipse Ti-U, 10 $\times$  objective lens).

## 2. Synthesis of solid-supported alkyne sensory bead **1** and related compounds

### (1) Preparation of alkyne sensory bead **1**



**3-Azido-7-(carboxymethoxy)-chromen-2-one (2).** Synthesis of the 3-azido-7-(carboxymethoxy)-chromen-2-one (**2**) was performed using the procedure reported in the literature.<sup>1</sup> IR (neat)  $\nu_{\max}$  3081, 2919, 2781, 2151, 2128, 1726, 1615; <sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>/MeOD (1:1)):  $\delta$  7.40 (d,  $J$  = 8.7 Hz, 1H), 7.29 (s, 1H), 6.93 (dd,  $J$  = 8.7, 2.5 Hz, 1H), 6.85 (d,  $J$  = 2.3 Hz, 1H), 4.67 (s, 2H); <sup>13</sup>C-NMR (75 MHz, CDCl<sub>3</sub>/MeOD (1:1)):  $\delta$  171.84, 161.51, 159.42, 154.07, 129.87, 128.26, 124.88, 114.88, 114.79, 102.92, 66.58; HRMS (FAB,  $m/z$ ): [M-H]<sup>-</sup> calcd. for C<sub>11</sub>H<sub>6</sub>N<sub>3</sub>O<sub>5</sub> 260.0307, found 260.0301.

**Allyl 4-[2-hydroxy-1-(trimethylsilyl)ethyl]-phenoxyacetate (9).** Allyl 4-[2-hydroxy-1-(trimethylsilyl)ethyl]-phenoxyacetate (**9**) was performed using the procedure reported in the literature.<sup>2</sup> IR (CHCl<sub>3</sub>)  $\nu_{\max}$  3426, 2955, 1760, 1740, 1508; <sup>1</sup>H-NMR (300 MHz, CDCl<sub>3</sub>):  $\delta$  6.98–7.04 (m, 2H), 6.81–6.87 (m, 2H), 5.90 (tdd,  $J$  = 17.3, 10.4, 5.9 Hz, 1H), 5.31 (qd,  $J$  = 17.3, 1.4 Hz, 1H), 5.24 (qd,  $J$  = 10.5, 1.2 Hz, 1H), 4.69 (td,  $J$  = 5.7, 1.4 Hz, 2H), 4.61 (s, 1H), 4.06 (t,  $J$  = 11.3 Hz, 1H), 3.93 (dd,  $J$  = 11.3, 4.4 Hz, 1H), 2.37 (dd,  $J$  = 11.4, 4.5 Hz, 1H), -0.06 (s, 9H); <sup>13</sup>C-NMR (75 MHz, CDCl<sub>3</sub>):  $\delta$  186.76, 155.69, 133.55, 131.42, 128.78, 119.00, 114.99, 65.78, 65.58, 63.19, 40.81, -2.65; HRMS (FAB,  $m/z$ ): [M+H]<sup>+</sup> calcd. for 309.1522, found 309.1525.

**Allyl 2-(4-(2-(2-((3-azido-2-oxo-2H-chromen-7-yl)oxy)acetoxyl)-1-(trimethylsilyl)ethyl)phenoxy)acetate (10).** To a solution of **9** (970 mg, 3.14 mmol, 1.0 eq.) in CH<sub>2</sub>Cl<sub>2</sub> (16 mL) were added 3-azidocoumarin **2** (904 mg, 3.46 mmol, 1.1 eq.), *N,N'*-dicyclohexylcarbodiimide (973 mg, 4.72 mmol,

1.5 eq.) and 4-dimethylaminopyridine (38 mg, 0.31 mmol, 0.1 eq.). The reaction mixture was stirred at room temperature for 5 h under nitrogen, filtered through Celite 545, and concentrated *in vacuo*. The residue was purified by silica-gel column chromatography (20% EtOAc/hexane) to give **10** (1.5 g, 2.7 mmol, 85% yield) as a clear oil. IR (neat)  $\nu_{\max}$  2957, 2118, 1756, 1727, 1613, 1511;  $^1\text{H-NMR}$  (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  7.24 (d,  $J = 8.7$  Hz, 1H), 7.15 (s, 1H), 6.85–6.92 (m, 2H), 6.73–6.79 (m, 2H), 6.66 (dd,  $J = 8.7, 2.4$  Hz, 1H), 6.61 (d,  $J = 2.4$  Hz, 1H), 5.91 (tdd,  $J = 17.1, 10.2, 5.7$ , 1H), 5.31 (qd,  $J = 17.3, 1.4$  Hz, 1H), 5.24 (qd,  $J = 10.5, 1.2$  Hz, 1H), 4.65–4.74 (m, 3H), 4.61 (s, 2H), 4.47–4.55 (m, 3H), 2.51 (dd,  $J = 12.0, 4.5$  Hz, 1H),  $-0.04$  (s, 9H);  $^{13}\text{C-NMR}$  (75 MHz,  $\text{CDCl}_3$ ):  $\delta$  168.74, 168.32, 159.59, 157.50, 155.56, 152.57, 132.90, 131.44, 128.22, 126.07, 123.80, 118.98, 114.65, 113.38, 113.04, 101.56, 66.61, 65.78, 65.47, 65.27, 36.43,  $-2.72$ ; HRMS (FAB,  $m/z$ ):  $[\text{M}+\text{H}]^+$  calcd. for 552.1802, found 552.1804.

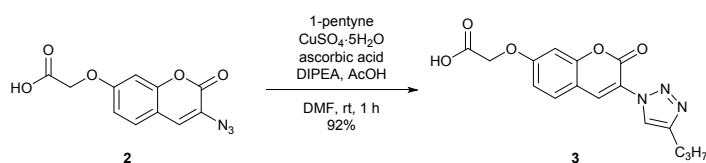
**2-(4-(2-(2-((3-Azido-2-oxo-2H-chromen-7-yl)oxy)acetoxyl)-1-(trimethylsilyl)ethyl)phenoxy)acetic acid (11).** A solution of **10** (723 mg, 1.31 mmol, 1.0 eq.) in THF (18 mL) and MeOH (9 mL) was degassed by bubbling nitrogen for 15 min. Then, sodium *p*-toluenesulfinate (382 mg, 1.97 mmol, 1.5 eq.) and tetrakis(triphenylphosphine)palladium(0) (91 mg, 0.08 mmol, 0.06 eq.) were added, and the reaction mixture was stirred at room temperature for 1.5 h in the dark. After completion of the reaction, the reaction mixture was evaporated *in vacuo* and purified by silica-gel column chromatography (10% MeOH/ $\text{CH}_2\text{Cl}_2$  + 0.2% acetic acid) to afford acid **11** (409 mg, 0.80 mmol, 61% yield) as a light-yellow oil. IR ( $\text{CHCl}_3$ )  $\nu_{\max}$  2951, 2119, 1734, 1712, 1620, 1507;  $^1\text{H-NMR}$  (300 MHz,  $\text{DMSO-d}_6$ ): 12.95 (br s, 1H), 7.62 (s, 1H), 7.52 (d,  $J = 8.4$  Hz, 1H), 6.98 (d,  $J = 8.4$  Hz, 2H), 6.93 (d,  $J = 2.4$  Hz, 1H), 6.85 (dd,  $J = 8.6, 2.6$  Hz, 1H), 6.77 (d,  $J = 8.7$  Hz, 2H), 4.79 (s, 2H), 4.59 (s, 2H), 4.48–4.65 (m, 2H), 2.50–2.58 (m, 1H),  $-0.05$  (s, 9H);  $^{13}\text{C-NMR}$  (75 MHz,  $\text{DMSO-d}_6$ ):  $\delta$  170.29, 168.39, 159.49, 157.10, 155.23, 152.25, 132.64, 128.76, 128.12, 127.02, 122.54, 114.08, 113.03, 101.33, 66.05, 64.95, 64.43, 35.42,  $-2.70$ ; HRMS (FAB,  $m/z$ ):  $[\text{M}-\text{H}]^-$  calcd. for 510.1333, found 510.1324.

**Preparation of sensory bead 1.** In a 20 mL vial, TentaGel<sup>TM</sup> MB-NH<sub>2</sub> resin (Sigma–Aldrich, 140–170  $\mu\text{m}$  beads,  $\sim 0.40$  mmol/g, 200 mg, 0.08 mmol) was pre-swollen in DMF (7 mL) for 1 h. To this solution were added acid **11** (49 mg, 0.10 mmol, 1.2 eq.), 1-[bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-*b*]pyridinium 3-oxid hexafluorophosphate (91 mg, 0.24 mmol, 3.0 eq.), 1-hydroxybenzotriazole hydrate (32 mg, 0.24 mmol, 3.0 eq.), and *N,N*-diisopropylethylamine (42  $\mu\text{L}$ , 0.24 mmol, 3.0 eq.). The vial was shaken at room temperature for 24 h in the dark and then filtered. The resin was washed two times each with DMF (10 mL), H<sub>2</sub>O (10 mL), MeOH (10 mL) and  $\text{CH}_2\text{Cl}_2$  (10 mL). Pyridine/acetic anhydride (3:1; 10 mL) was added, and the mixture was shaken for 3 h. The



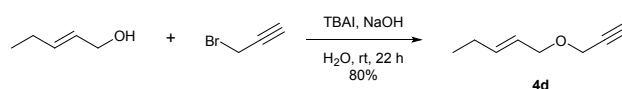
resin was subsequently washed three times with DMF (10 mL), H<sub>2</sub>O (10 mL), MeOH (10 mL), and CH<sub>2</sub>Cl<sub>2</sub> (10 mL). The resin was dried *in vacuo* to afford the loaded sensory bead **1** (230 mg). The loading was determined by UV absorption of the 3-azidocoumarin **2** obtained by treatment of the loaded resin (5 mg) in CH<sub>2</sub>Cl<sub>2</sub> (900  $\mu$ L) with TBAF·3H<sub>2</sub>O solution (100  $\mu$ L, 10 mg/mL in CH<sub>2</sub>Cl<sub>2</sub>) and subsequent shaking of the reaction mixture at room temperature for 15 min. Loading:  $c = 0.30$  mmol/g (which corresponds to a coupling yield of 72%).

## (2) Preparation of triazolylcoumarin **3**



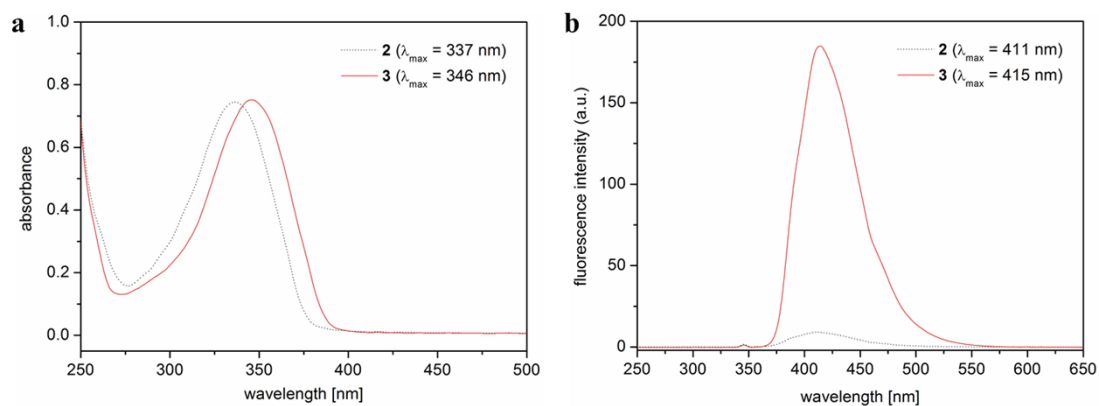
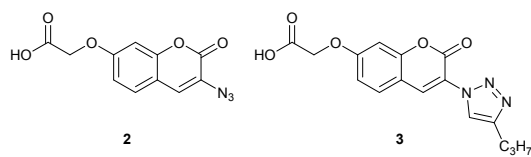
**2-((2-Oxo-3-(4-propyl-1H-1,2,3-triazol-1-yl)-2H-chromen-7-yl)oxy)acetic acid (**3**)**. To a solution of 3-azidocoumarin **2** (55 mg, 0.21 mmol, 1 eq.) in DMF (2 mL) were added 1-pentyne (104  $\mu$ L, 1.06 mmol, 5 eq.), *N,N*-diisopropylethylamine (37  $\mu$ L, 0.21 mmol, 1 eq.), acetic acid (12  $\mu$ L, 0.21 mmol, 1 eq.), copper(I) sulfate pentahydrate (10 mg, 0.04 mmol, 0.2 eq.), and ascorbic acid (15 mg, 0.08 mmol, 0.4 eq.). The reaction mixture was stirred at room temperature under nitrogen. After 1 h, the mixture was diluted with EtOAc (10 mL) and water (10 mL). The layers were separated, and the aqueous layer was extracted with EtOAc (2  $\times$  10 mL). The combined organic layers were dried with MgSO<sub>4</sub>, filtered, and concentrated under vacuum. The residue was purified by silica-gel column chromatography (10% MeOH/CH<sub>2</sub>Cl<sub>2</sub> + 0.2% acetic acid) to afford triazolylcoumarin **3** (64 mg, 0.19 mmol, 92% yield) as a white solid. IR (neat)  $\nu_{\max}$  3180, 3040, 2922, 2875, 1730, 1713, 1610, 1513; <sup>1</sup>H-NMR (400 MHz, DMSO-d<sub>6</sub>):  $\delta$  8.61 (s, 1H), 8.33 (s, 1H), 7.83 (d,  $J = 8.6$  Hz), 7.10–7.13 (m, 1H), 7.07 (dd,  $J = 8.6, 2.3$  Hz, 1H), 4.84 (s, 2H), 2.69 (t,  $J = 7.4$  Hz, 2H), 1.62–1.73 (m, 2H), 0.95 (t,  $J = 7.3$  Hz, 3H); <sup>13</sup>C-NMR (75 MHz, DMSO-d<sub>6</sub>):  $\delta$  169.40, 161.73, 156.08, 154.10, 146.92, 135.16, 130.41, 122.66, 120.50, 113.59, 111.81, 101.38, 65.18, 26.78, 22.06, 13.48; HRMS (FAB,  $m/z$ ): [M–H]<sup>–</sup> calcd. for 328.0933, found 328.0939.

## (3) Preparation of alkyne **4d**



**(E)-1-(Prop-2-yn-1-yloxy)pent-2-ene (4d).** Synthesis of (E)-1-(prop-2-yn-1-yloxy)pent-2-ene (**4d**) was achieved using almost the same procedure as that reported in the literature.<sup>3</sup> To a vigorously stirring suspension of *trans*-2-penten-1-ol (1.9 mL, 18.80 mmol, 1 eq.), tetrabutylammonium iodide (TBAI) (69 mg, 0.19 mmol, 0.01 eq.), and NaOH (2.3 g, 56.40 mmol, 3 eq.) in H<sub>2</sub>O (5 mL), propargyl bromide solution (80 wt.% in toluene, 2.1 mL, 18.80 mmol, 1 eq.) was added slowly at 0 °C. The reaction mixture was allowed to equilibrate to room temperature and stirred for 22 h. After completion of the reaction, the reaction mixture was poured into water, extracted with ether, and washed with brine and saturated aqueous Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> solution. The organic layer was dried with MgSO<sub>4</sub> and filtered. The residue was partially evaporated to remove ether under reduced pressure. The crude mixture was then purified using a Kugelrohr distillation apparatus under reduced pressure to afford the alkyne **4d** (1.9 g, 15.04 mmol, 80% yield) as a clear liquid. IR (neat)  $\nu_{\max}$  3297, 2964, 2935, 2874, 2853, 2116, 1670, 1458, 1441, 1355; <sup>1</sup>H-NMR (300 MHz, CDCl<sub>3</sub>):  $\delta$  5.72–5.82 (m, 1H), 5.44–5.57 (m, 1H), 4.09 (d, *J* = 2.4 Hz, 2H), 3.95–4.00 (m, 2H), 2.38 (t, *J* = 2.4 Hz, 1H), 1.98–2.50 (m, 2H), 0.97 (t, *J* = 7.5 Hz, 3H); <sup>13</sup>C-NMR (75 MHz, CDCl<sub>3</sub>):  $\delta$  137.39, 124.25, 79.82, 74.14, 70.35, 56.66, 25.24, 13.21; HRMS (FAB, *m/z*): [M–H]<sup>–</sup> calcd. for 123.0810, found 123.0807.

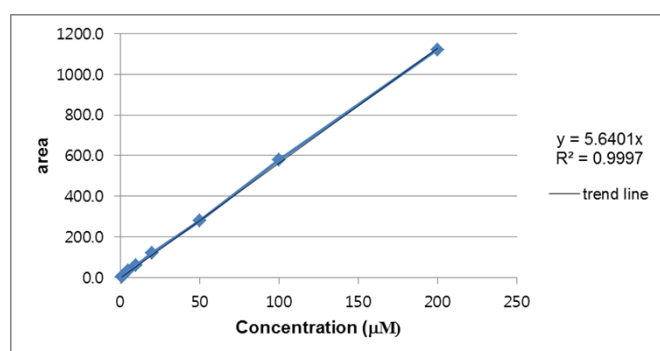
### 3. Absorption and fluorescence emission spectra of compounds **2** and **3**



**Fig. S1** (a) Comparison of the UV/Vis absorption spectra of compounds **2** (black dotted line) and **3** (red solid line) ( $30 \mu\text{M}$  in  $\text{CHCl}_3$ ); (b) Comparison of the fluorescence emission spectra ( $\lambda_{\text{exc}} = 345 \text{ nm}$ ) of compounds **2** (black dotted line) and **3** (red solid line) ( $30 \mu\text{M}$  in  $\text{CHCl}_3$ ).

#### 4. Standard curve of triazolylcoumarin **3**

The standard curve of triazolylcoumarin **3** was generated by the injection of 5  $\mu\text{L}$  of stock standard solutions of **3** to an Agilent 1260 Infinity LC (Agilent Technologies, Palo Alto, CA, USA). The stock standard solutions of **3** were prepared at concentrations of 1, 2, 5, 10, 20, 50, 100, and 200  $\mu\text{M}$  in methanol. The  $x$ -axis is the concentration of the stock solutions, and the  $y$ -axis is the area of peaks in the LC chromatograms (at 345 nm). The linearity of the standard curve was determined by linear regression analysis, which revealed a coefficient of determination ( $r^2$ ) greater than 0.999.



**Fig. S2.** Standard curve of triazolylcoumarin **3**

## 5. Representative procedure for the chemical assay with bead 1

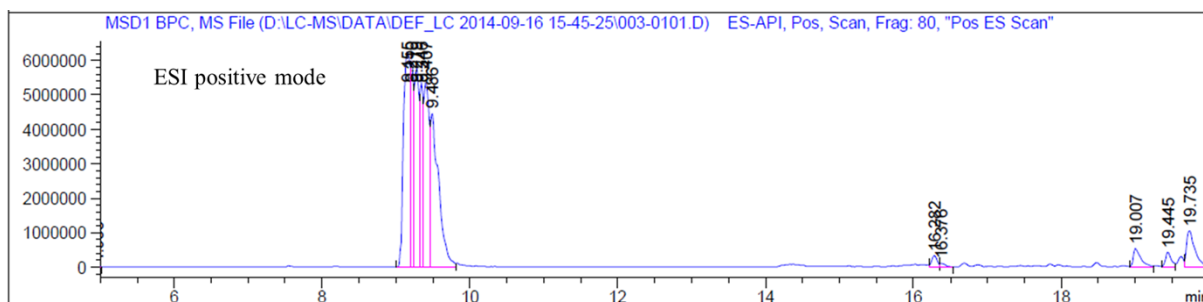
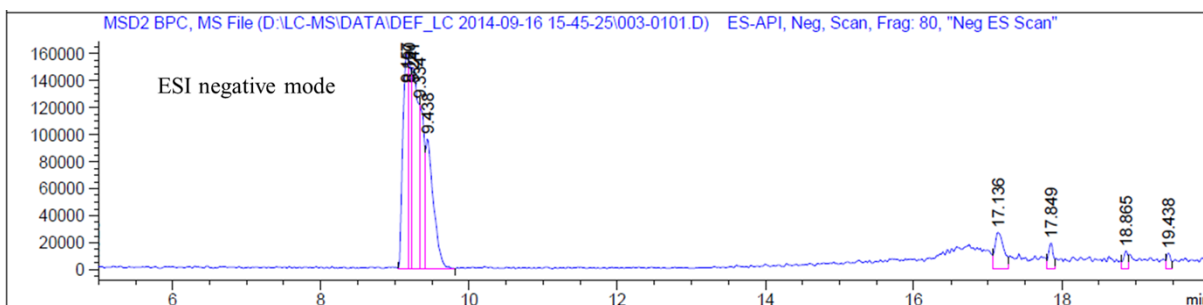
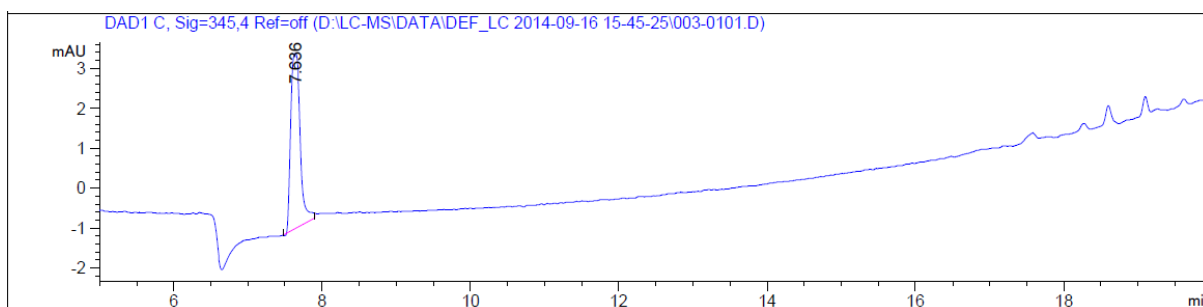
**Representative procedure for the chemical assay with the sensory bead 1:** The sensory beads 1 (0.5 or 1 mg, 0.15 or 0.30  $\mu\text{mol}$ ) were pre-swollen in 50  $\mu\text{L}$  DMF in a 96-well round-bottom, polypropylene plate at room temperature for 1 h. The DMF was removed with a multichannel pipette, and a solution of terminal alkynes or natural product extracts in DMF (ca. 30  $\mu\text{L}$ ) was added. To the suspension were added tris[(1-benzyl-1*H*-1,2,3-triazol-4-yl)methyl]amine (300 mM in DMF, 10  $\mu\text{L}$ ), acetic acid (1.6  $\mu\text{L}$ ), *N,N*-diisopropylethylamine (2.5  $\mu\text{L}$ ), copper(I) sulfate pentahydrate (300 mM in  $\text{H}_2\text{O}$ , 5  $\mu\text{L}$ ), and ascorbic acid (1500 mM in DMF, 5  $\mu\text{L}$ ). The resulting suspensions were shaken on a horizontal shaker (IKA HS 260 control, Janke & Kunkel & Co. IKA Labortechnik, Staufen, Germany) at 250 rpm at room temperature in the dark. After 20 h, the resulting beads were washed with DMF (200  $\mu\text{L}$ ),  $\text{H}_2\text{O}$  (200  $\mu\text{L}$ ), MeOH (200  $\mu\text{L}$ ), and  $\text{CH}_2\text{Cl}_2$  (200  $\mu\text{L}$ ) three times each. For the cleavage procedure, the beads were treated with a solution of tetrabutylammonium fluoride trihydrate (12  $\mu\text{L}$ , 10 mg/mL in  $\text{CH}_2\text{Cl}_2$ ) in  $\text{CH}_2\text{Cl}_2$  (288  $\mu\text{L}$ ) and shaken at room temperature for 15 min. An aliquot (5 or 10  $\mu\text{L}$ ) of the reaction solution was analyzed by LC/MS.

**LC/MS analysis:** LC/MS analysis was performed by using Agilent 6100 Series Single Quad LC/MS systems (Agilent Technologies, Palo Alto, CA, USA). Mobile phase A consisted of 0.1% formic acid in HPLC-grade water. HPLC analysis was performed using a reverse-phase Agilent Eclipse Plus C18 column (4.6  $\times$  100 mm, 3.5  $\mu\text{m}$ ) at a flow rate of 0.7 mL/min (20–100% aqueous MeOH with 0.1% formic acid over 20 min and 100% MeOH with 0.1% formic acid from 20 to 25 min). Mass spectra were acquired in both positive and negative ion modes with the capillary voltage set at 4000 eV.

## 6. LC/MS spectra for validation of the sensory system

### (a) A solution of only TBAF·3H<sub>2</sub>O in CH<sub>2</sub>Cl<sub>2</sub>

```
=====
Acq. Operator   : Y. Kwon                      Seq. Line :    1
Acq. Instrument : Instrument 1                 Location  : Vial 3
Injection Date  : 16/09/2014 3:46:35 PM      Inj       :    1
                                           Inj Volume: 10.000 µl
Different Inj Volume from Sequence ! Actual Inj Volume : 5.000 µl
Acq. Method     : D:\LC-MS\DATA\DEF_LC 2014-09-16 15-45-25\JUN (4 UV, 20PERCEN
Last changed    : 25/06/2014 4:00:39 PM by Y. Kwon
Analysis Method : C:\CHEM32\1\METHODS\JUN (4 UV, 20PERCENTMEOH).M
Last changed    : 18/09/2014 8:09:19 PM by Y. Kwon
                 (modified after loading)
Method Info     : Reserpine SIM Method for the G6130B Quadrupole LC/MS System
                 ESI Positive Ion Sensitivity Test
=====
```



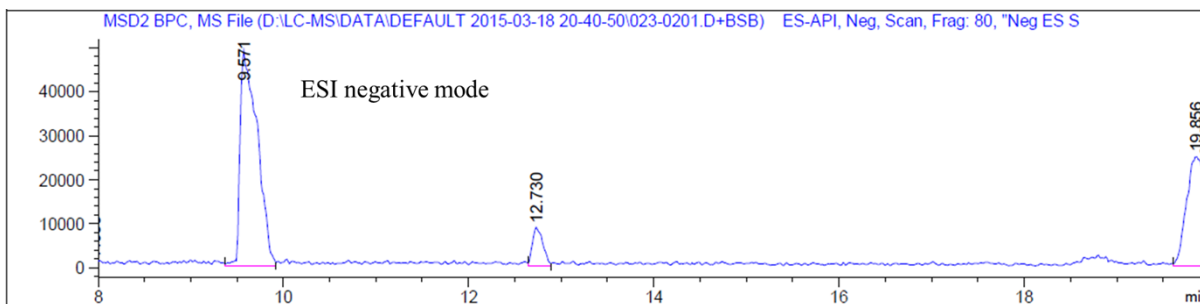
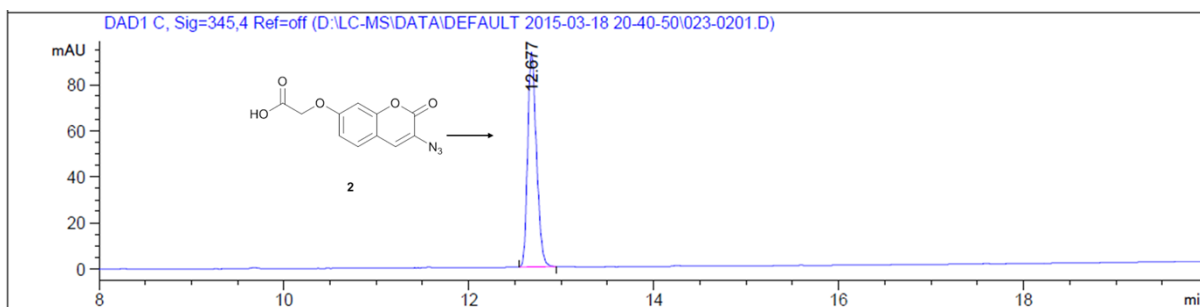
### (b) Reaction with 1-pentyne

The LC/MS data was obtained by injection of 5 µl of the bead-released sample to LC/MS after 10-fold dilution with DCM.

**- LC/MS spectrum of analyte A**

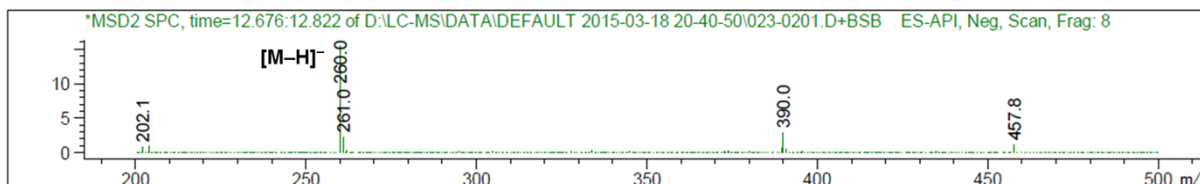
```

=====
Acq. Operator   : [BSB1]                      Seq. Line :    2
Acq. Instrument : Instrument 1                 Location  : Vial 23
Injection Date  : 18/03/2015 9:13:01 PM      Inj       :    1
                                           Inj Volume: 10.000 µl
Different Inj Volume from Sequence !      Actual Inj Volume : 5.000 µl
Acq. Method     : D:\LC-MS\DATA\DEFAULT 2015-03-18 20-40-50\JUN (4 UV, 20PERCEN
Last changed    : 18/03/2015 9:12:07 PM
                (modified after loading)
Analysis Method : C:\CHEM32\1\METHODS\DEFAULT.M
Last changed    : 19/03/2015 4:10:31 PM
                (modified after loading)
Method Info     : Reserpine SIM Method for the G6130B Quadrupole LC/MS System
                ESI Positive Ion Sensitivity Test
    
```



```

12.735      495839      390.05 I
                261.00 I
                260.05 I
    
```



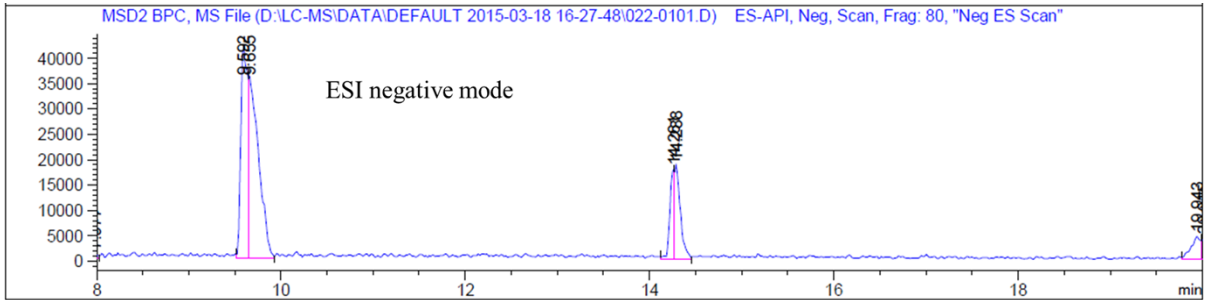
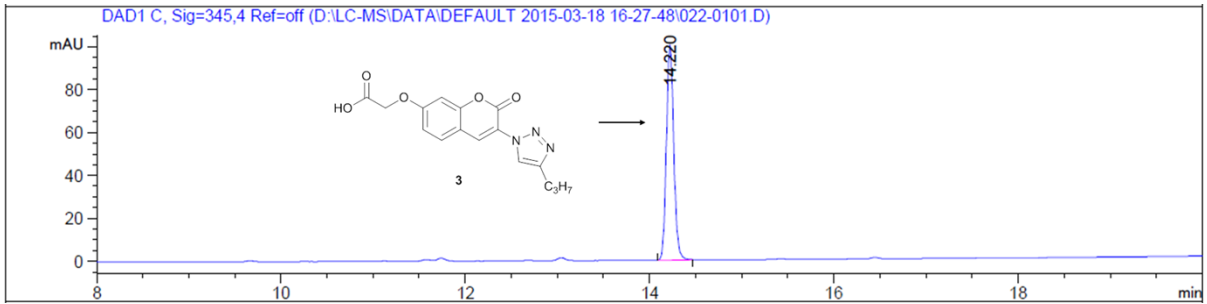
**- LC/MS spectrum of analyte B**

```

=====
Acq. Operator   :                               Seq. Line :    1
Acq. Instrument : Instrument 1                   Location  : Vial 22
Injection Date  : 18/03/2015 4:28:53 PM         Inj       :    1
                                                Inj Volume: 10.000 µl
Different Inj Volume from Sequence ! Actual Inj Volume : 5.000 µl
Acq. Method     : D:\LC-MS\DATA\DEFAULT 2015-03-18 16-27-48\JUN (4 UV, 20PERCEN
Last changed    : 25/06/2014 4:00:39 PM by Y. Kwon
Analysis Method : C:\CHEM32\1\METHODS\DEFAULT.M
Last changed    : 19/03/2015 4:29:56 PM
                  (modified after loading)
Method Info     : Reserpine SIM Method for the G6130B Quadrupole LC/MS System
                  ESI Positive Ion Sensitivity Test
  
```

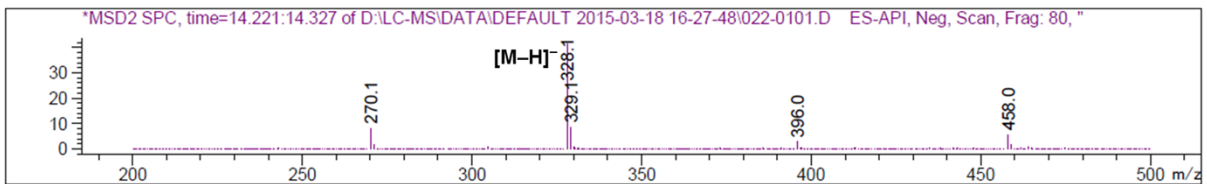
Signal 3: DAD1 C, Sig=345,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	1.534	BB	0.0557	7.68445	1.96138	1.2840
2	7.741	BB	0.0952	33.38545	4.68211	5.5784
3	14.220	BB	0.0870	557.40454	99.99574	93.1376

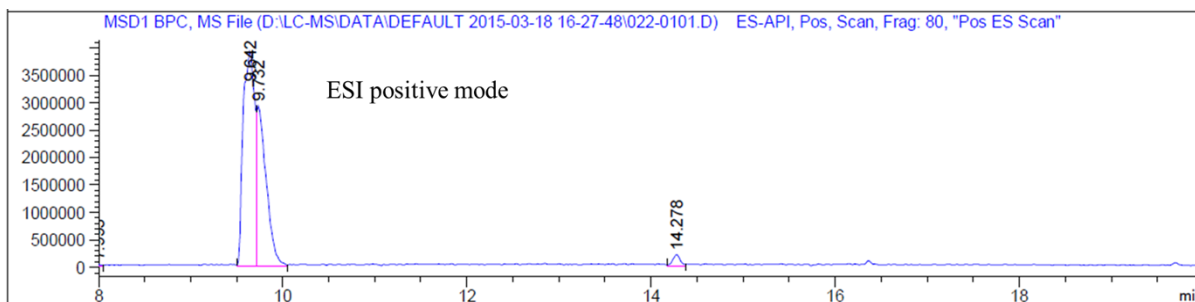


```

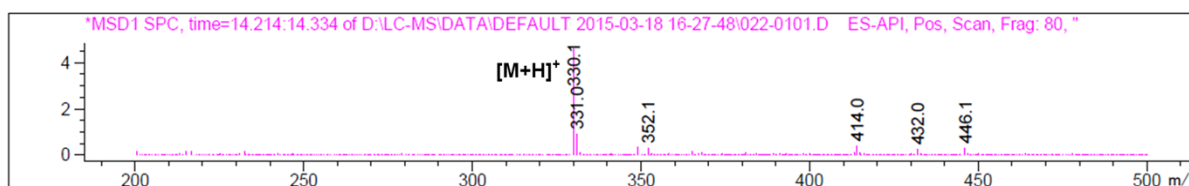
14.277      592935      458.05 I
              329.15 I
              328.15 I
              270.10 I
  
```





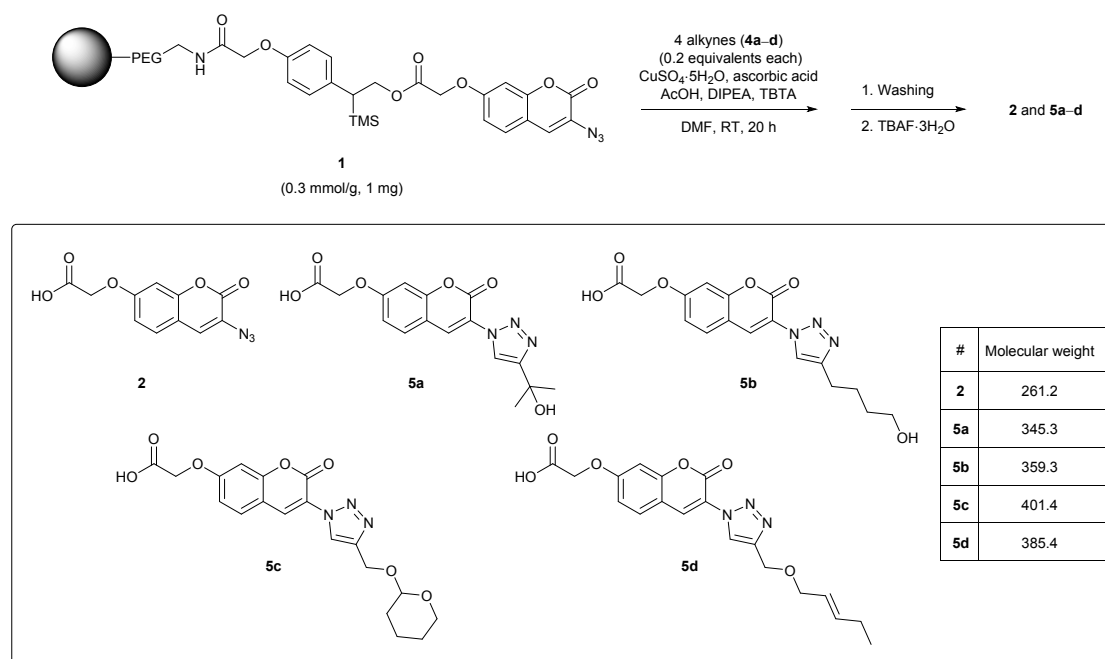


14.274      8740345      331.05 I  
330.15 I



### (c) Reaction with a set of terminal alkynes

The LC/MS data was obtained by injection of 5  $\mu$ l of the bead-released sample to LC/MS after 10-fold dilution with DCM.



**Fig. S3** The designed chemical assay with a set of alkynes (4a-d). The structures of 3-azidocoumarin 2 and triazolylcoumarins 5a-d corresponding to alkynes 4a-d are listed, along with their molecular weights.

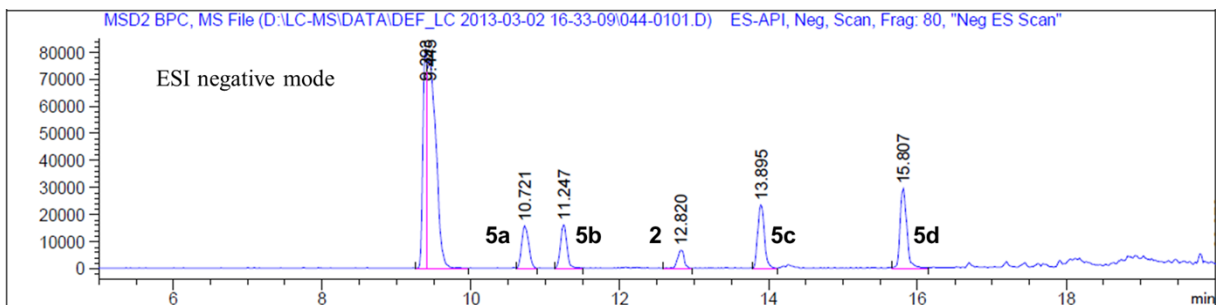
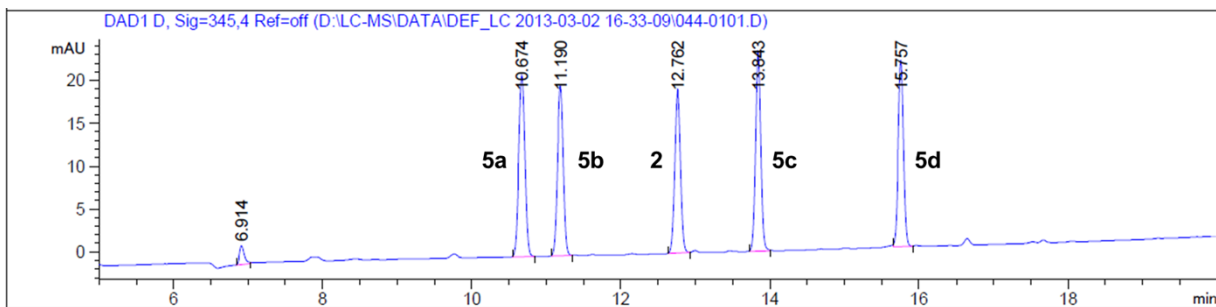
```

=====
Acq. Operator   :                               Seq. Line :    1
Acq. Instrument : Instrument 1                   Location  : Vial 44
Injection Date  : 2/03/2013 4:35:35 PM          Inj       :    1
                                                    Inj Volume: 10.000 µl

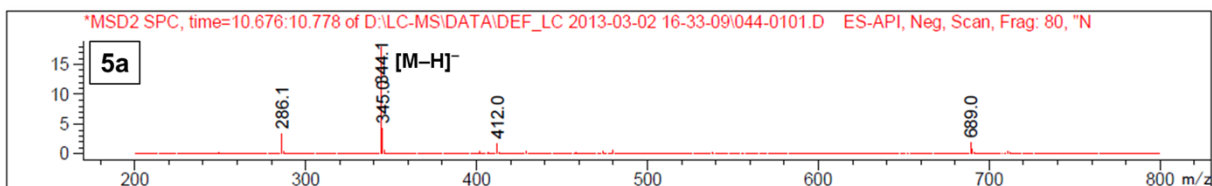
Acq. Method    : D:\LC-MS\DATA\DEF_LC 2013-03-02 16-33-09\JUN (4 UV, 20PERCEN
Last changed   : 30/01/2013 4:05:39 PM
Analysis Method : C:\CHEM32\1\METHODS\JUN (4 UV, 20PERCENTMEOH).M
Last changed   : 8/07/2014 5:12:31 PM by Y. Kwon
                (modified after loading)
Method Info    : Reserpine SIM Method for the G6130B Quadrupole LC/MS System
                ESI Positive Ion Sensitivity Test
  
```

Signal 3: DAD1 D, Sig=345,4 Ref=off

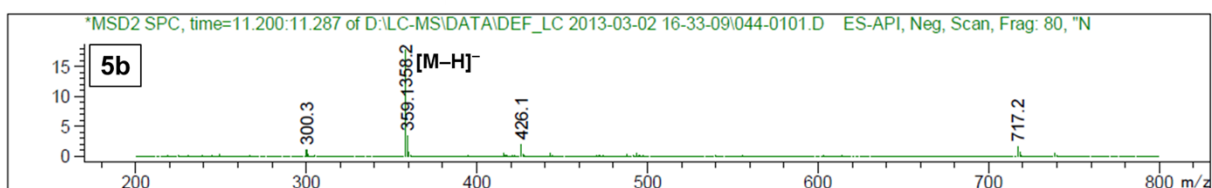
Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	1.589	BB	0.1491	153.53854	13.06573	21.0608
2	6.914	BB	0.0696	10.09527	2.19869	1.3848
3	10.674	BB	0.0919	123.51051	21.21645	16.9418
4	11.190	BB	0.0860	109.35860	19.92295	15.0006
5	12.762	BB	0.0860	104.79527	19.09686	14.3747
6	13.843	BB	0.0794	119.36368	23.45429	16.3730
7	15.757	BB	0.0779	108.36459	21.84091	14.8643



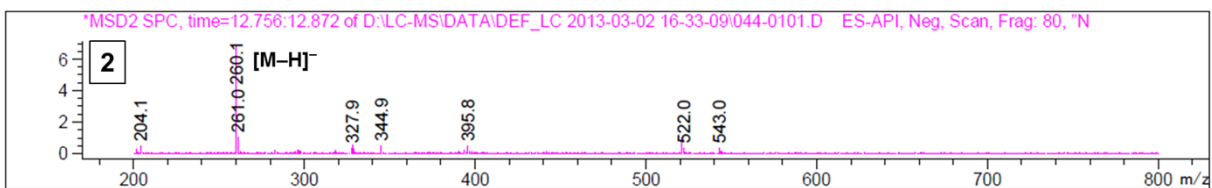
10.731      415839      689.05 I  
 345.05 I  
 344.15 I  
 286.10 I



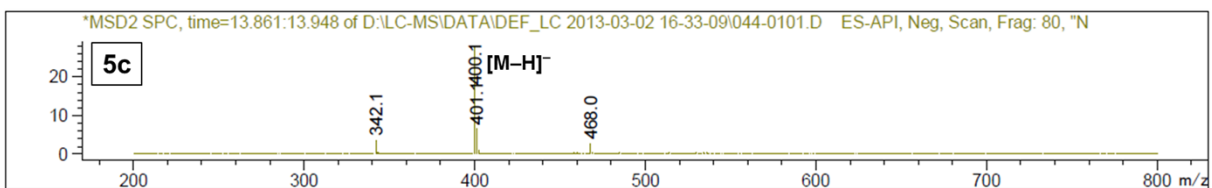
11.252      347807      426.15 I  
 359.10 I  
 358.20 I



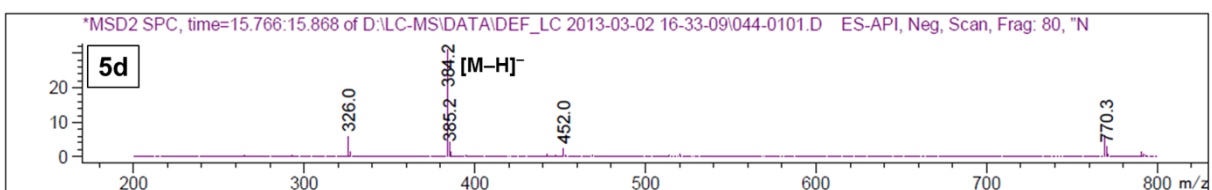
12.820      292731      521.05 I  
 261.00 I  
 260.10 I



13.897      478256      401.10 I  
 400.15 I  
 342.10 I



15.809      658559      769.25 I  
 385.05 I  
 384.20 I  
 326.05 I



(d) Validation of the sensory chemical assay system with the methanol extract of *Litsea japonica*

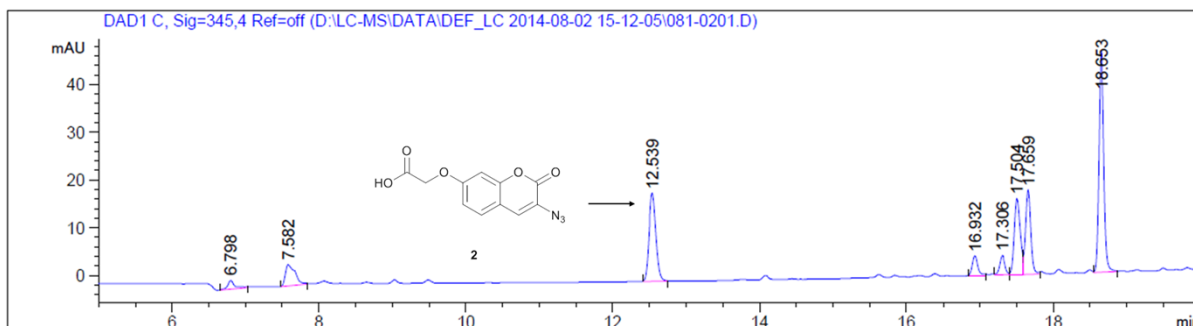
- Methanol extract of leaves of *L. japonica* (3 mg)

```

=====
Acq. Operator   : [BSB1]Y. Kwon                Seq. Line :    2
Acq. Instrument : Instrument 1                  Location  : Vial 81
Injection Date  : 2/08/2014 3:44:16 PM        Inj       :    1
                                                Inj Volume: 10.000 µl
Different Inj Volume from Sequence ! Actual Inj Volume : 5.000 µl
Acq. Method    : D:\LC-MS\DATA\DEF_LC 2014-08-02 15-12-05\JUN (4 UV, 20PERCEN
Last changed   : 2/08/2014 3:43:23 PM by Y. Kwon
                (modified after loading)
Analysis Method : C:\CHEM32\1\METHODS\JUN (4 UV, 20PERCENTMEOH).M
Last changed   : 30/09/2014 8:49:37 PM by Y. Kwon
                (modified after loading)
Method Info    : Reserpine SIM Method for the G6130B Quadrupole LC/MS System
                ESI Positive Ion Sensitivity Test
    
```

Signal 1: DAD1 C, Sig=345,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	1.533	BB	0.0507	5.76296	1.73646	0.9438
2	6.798	BB	0.0923	11.98157	1.83250	1.9622
3	7.582	BB	0.1249	40.45161	4.41418	6.6246
4	12.539	BB	0.1018	120.78407	18.57892	19.7804
5	16.932	BB	0.0769	20.97437	4.15377	3.4349
6	17.306	BV	0.0761	20.29920	4.07687	3.3243
7	17.504	VV	0.0853	89.26628	15.93954	14.6189
8	17.659	VB	0.0750	86.38739	17.66754	14.1474
9	18.653	VB	0.0720	214.71689	46.37693	35.1635





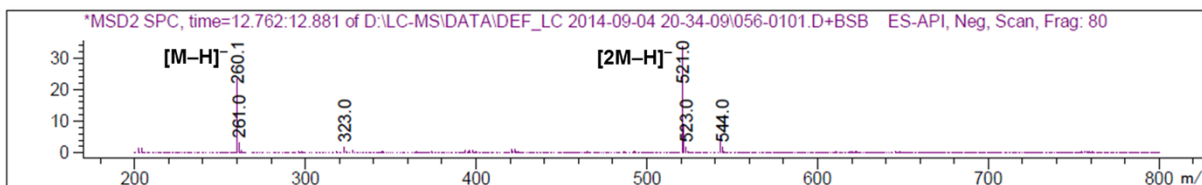




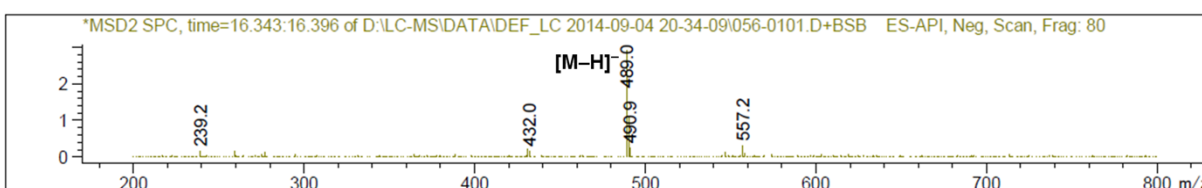




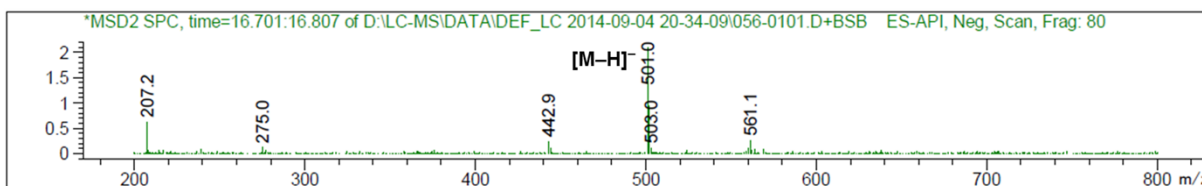
12.807      2443524      542.95 I  
 521.95 I  
 521.05 I  
 260.15 I



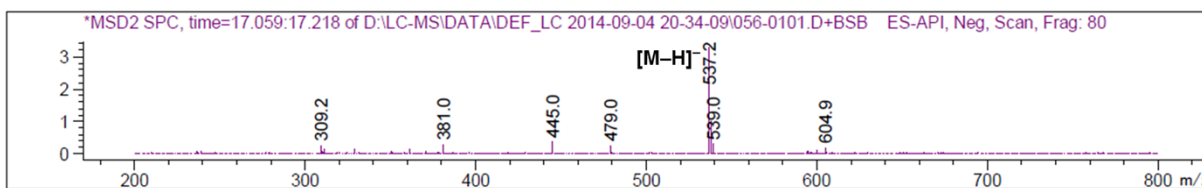
16.371      84598      557.15 I  
 490.10 I  
 489.05 I



16.757      170657      502.05 I  
 501.05 I  
 207.20 I



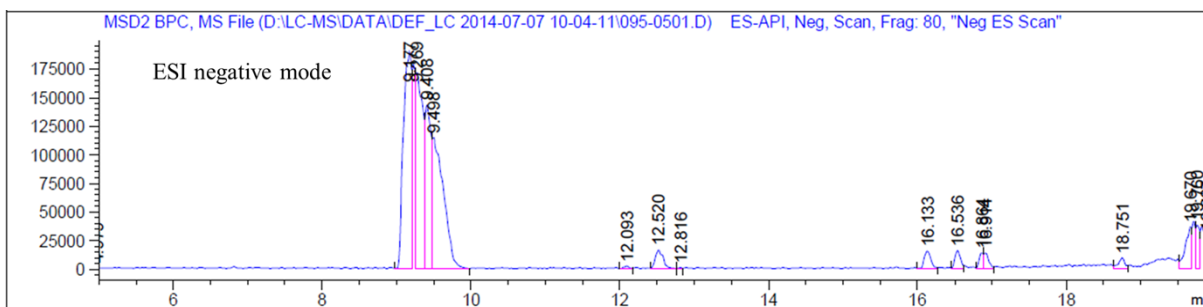
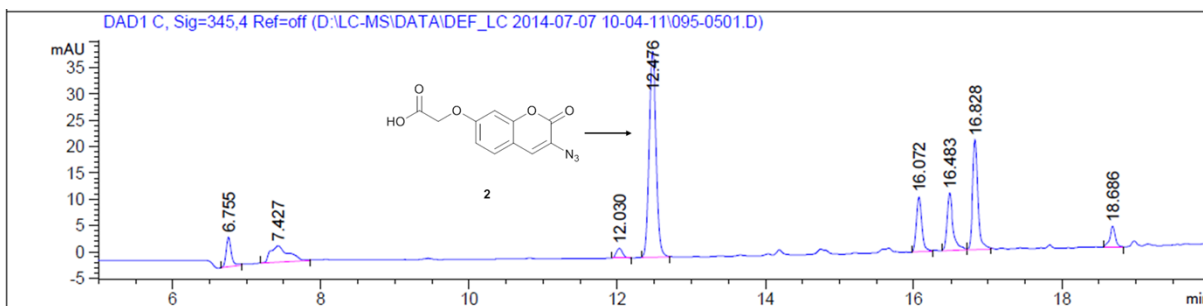
17.145      352936      538.05 I  
 537.15 I  
 445.00 I



- Hexane extract (fractionated from methanol extract) of whole plants of *C. morifolium* (10 mg)

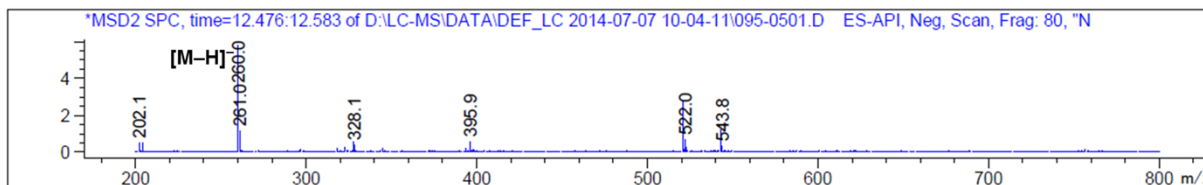
```

=====
Acq. Operator   : Y. Kwon                      Seq. Line :    5
Acq. Instrument : Instrument 1                 Location  : Vial 95
Injection Date  : 7/07/2014 12:09:23 PM      Inj       :    1
                                           Inj Volume: 10.000 µl
Different Inj Volume from Sequence ! Actual Inj Volume : 5.000 µl
Acq. Method    : D:\LC-MS\DATA\DEF_LC 2014-07-07 10-04-11\JUN (4 UV, 20PERCENT
Last changed   : 7/07/2014 12:08:29 PM by Y. Kwon
               (modified after loading)
Analysis Method: C:\CHEM32\1\METHODS\JUN (4 UV, 20PERCENTMEOH).M
Last changed   : 7/07/2014 3:19:55 PM by Y. Kwon
               (modified after loading)
Method Info    : Reserpine SIM Method for the G6130B Quadrupole LC/MS System
                 ESI Positive Ion Sensitivity Test
  
```

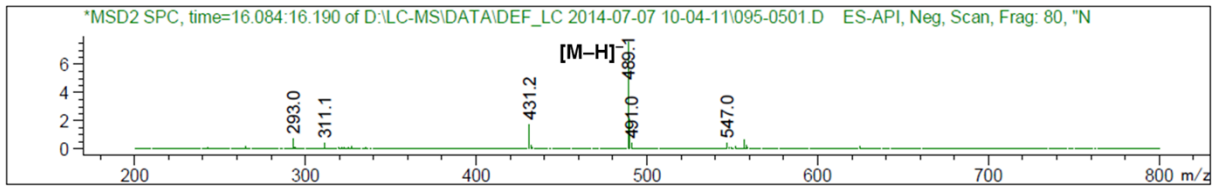


```

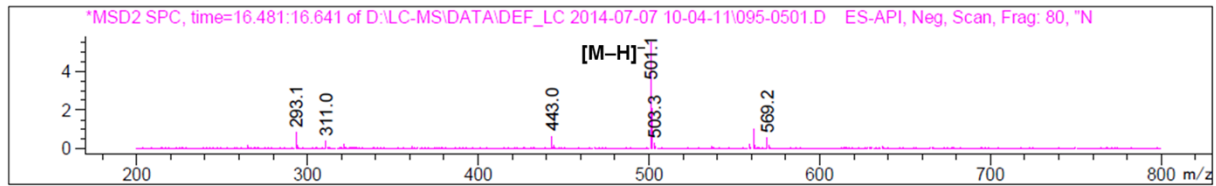
12.528      601566      542.95 I
              522.05 I
              520.90 I
              261.05 I
              260.15 I
              259.95 I
  
```



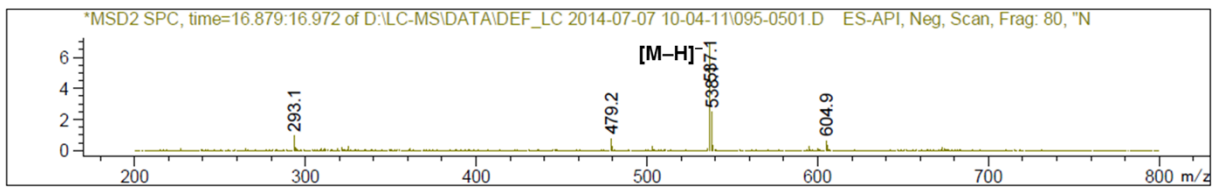
16.129      410650      490.05 I  
489.15 I  
431.20 I



16.530      501116      569.15 I  
561.10 I  
502.10 I  
501.10 I  
443.05 I  
293.10 I



16.901      238989      538.10 I  
537.10 I  
479.20 I  
293.10 I



## 7. The list of the screened extracts of various natural plants

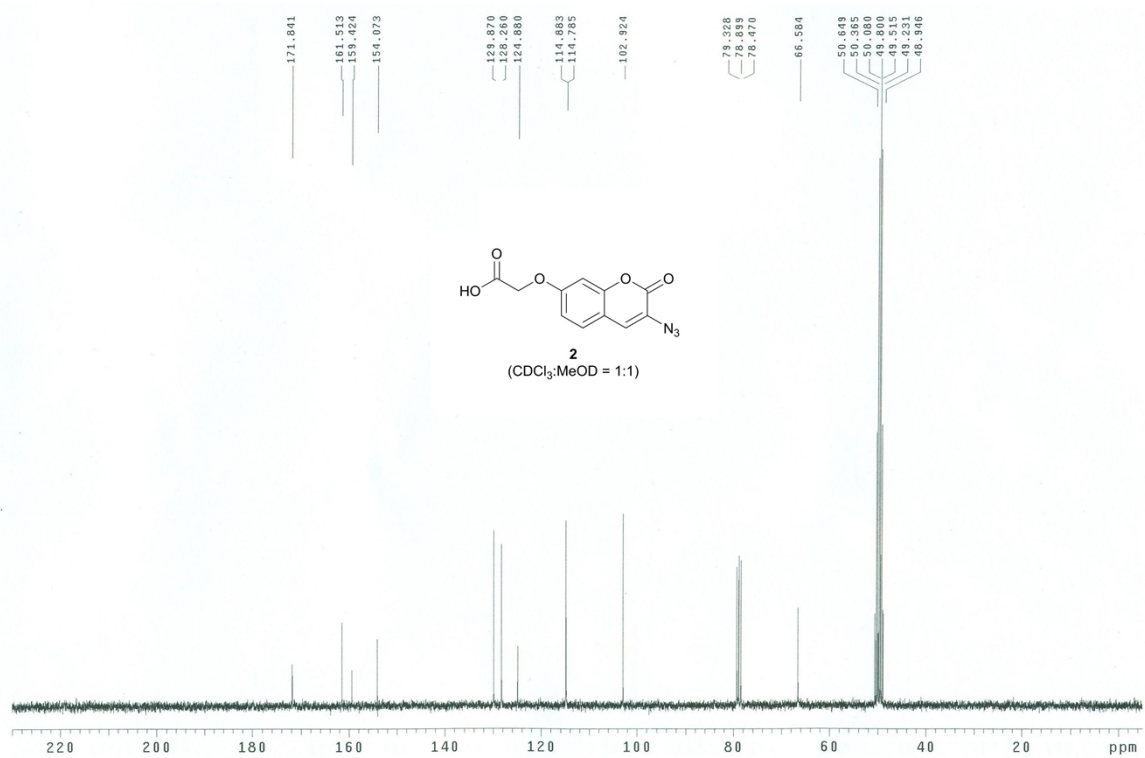
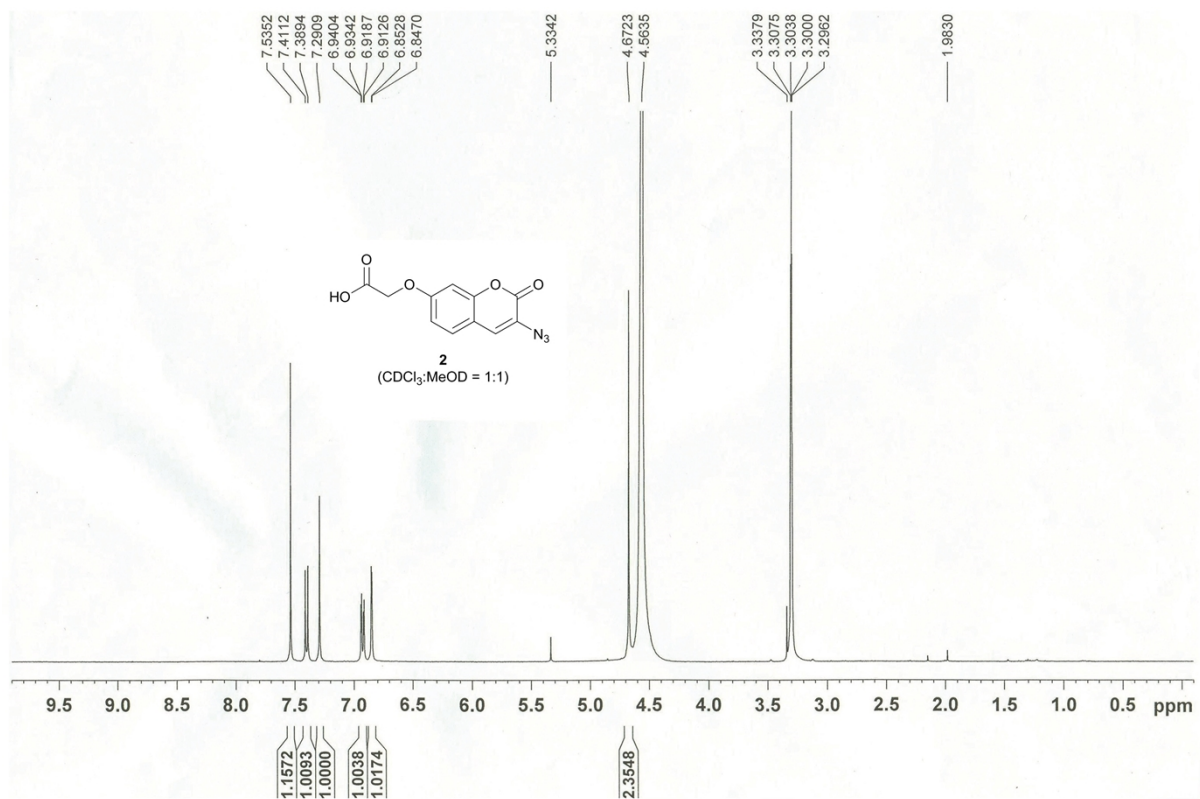
The methanol extracts of the tested plants were purchased from the Plant Extract Bank of Korea (Daejeon, Korea).

Entry	Scientific name	Part	Family	Assayed amount	Fluorescence response
1	<i>Achillea sibirica</i>	whole plant	Compositae	5 mg	–
2	<i>Actinodaphne lancifolia</i>	leaves	Lauraceae	5 mg	–
3	<i>Ainsliaea acerifolia</i>	whole plant	Compositae	5 mg	–
4	<i>Angelica dahurica</i>	whole plant	Umbelliferae	5 mg	–
5	<i>Artemisia iwayomogi</i>	whole plant	Compositae	5 mg	–
6	<i>Artemisia princeps</i> var. <i>orientalis</i>	whole plant	Compositae	10 mg	–
7	<i>Betula chinensis</i>	leaves	Betulaceae	5 mg	–
8	<i>Calendula arvensis</i>	whole plant	Compositae	5 mg	–
9	<i>Callistephus chinensis</i>	whole plant	Compositae	5 mg	–
10	<i>Camellia sinensis</i>	stems, leaves	Theaceae	5 mg	–
11	<i>Centaurea cyanus</i>	whole plant	Compositae	5 mg	–
12	<i>Cephalonoplos segetum</i>	whole plant	Compositae	5 mg	–
13	<i>Chamaecyparis obtusa</i>	leaves	Cupressaceae	10 mg	–
14	<i>Chamaecyparis pisifera</i>	leaves, stems	Cupressaceae	10 mg	–
15	<i>Chrysanthemum boreale</i>	whole plant	Compositae	5 mg	–
16	<i>Chrysanthemum indicum</i>	whole plant	Compositae	5 mg	–
17	<i>Chrysanthemum morifolium</i>	whole plant	Compositae	10 mg	<b>O</b>
18	<i>Cinnamomum camphora</i>	leaves	Lauraceae	5 mg	–
19	<i>Dahlia pinnata</i>	whole plant	Compositae	10 mg	–
20	<i>Dendropanax morbifera</i>	whole plant	Dendropanax	5 mg	–
21	<i>Eclipta prostrata</i>	whole plant	Compositae	10 mg	–
22	<i>Erigeron annuus</i>	whole plant	Compositae	5 mg	–
23	<i>Humulus japonicus</i>	whole plant	Cannabinaceae	5 mg	–
24	<i>Hypolepis punctata</i>	whole plant	Pteridaceae	5 mg	–
25	<i>Ixeris polycephala</i>	whole plant	Compositae	5 mg	–
26	<i>Ligularia taquetii</i>	whole plant	Compositae	5 mg	–
27	<i>Lindera glauca</i>	leaves	Lauraceae	10 mg	–
28	<i>Lindera sericea</i>	leaves, stems, fruits	Lauraceae	5 mg	–
29	<i>Lonicera insularis</i>	leaves	Caprifoliaceae	5 mg	–
30	<i>Lythrum salicaria</i>	seeds	Lythraceae	10 mg	–
31	<i>Machilus japonica</i>	leaves, stems	Lauraceae	5 mg	–
32	<i>Machilus thunbergii</i>	stem bark	Lauraceae	5 mg	–
33	<i>Neolitsea sericea</i>	stem bark	Lauraceae	5 mg	–
34	<i>Oenanthe javanica</i>	whole plant	Umbelliferae	5 mg	–
35	<i>Oplonanax elatus</i>	leaves	Araliaceae	5 mg	–
36	<i>Saussurea ussuriensis</i>	whole plant	Compositae	5 mg	–
37	<i>Senecio pseudo-sonchus</i>	whole plant	Compositae	5 mg	–
38	<i>Sonchus asper</i>	whole plant	Compositae	5 mg	–
39	<i>Trillium kamschaticum</i>	whole plant	Liliaceae	5 mg	–
40	<i>Vaccinium bracteatum</i>	leaves	Ericaceae	5 mg	–
41	<i>Vicia amoena</i>	whole plant	Leguminosae	5 mg	–
42	<i>Youngia denticulata</i>	whole plant	Compositae	5 mg	–

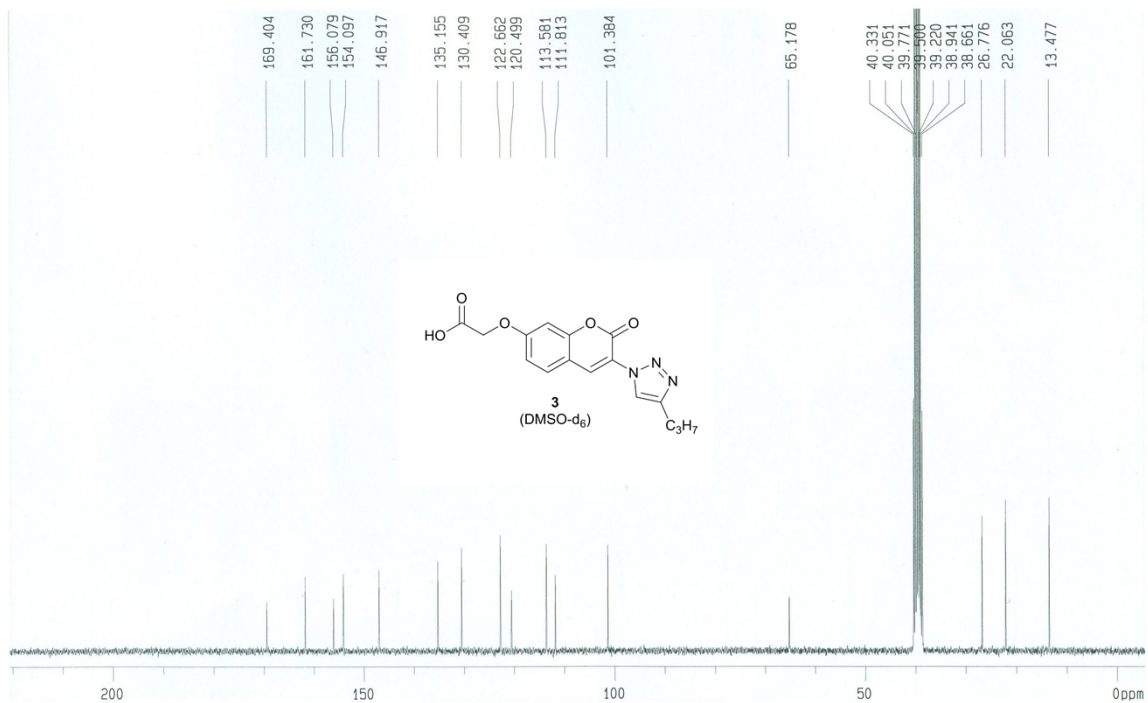
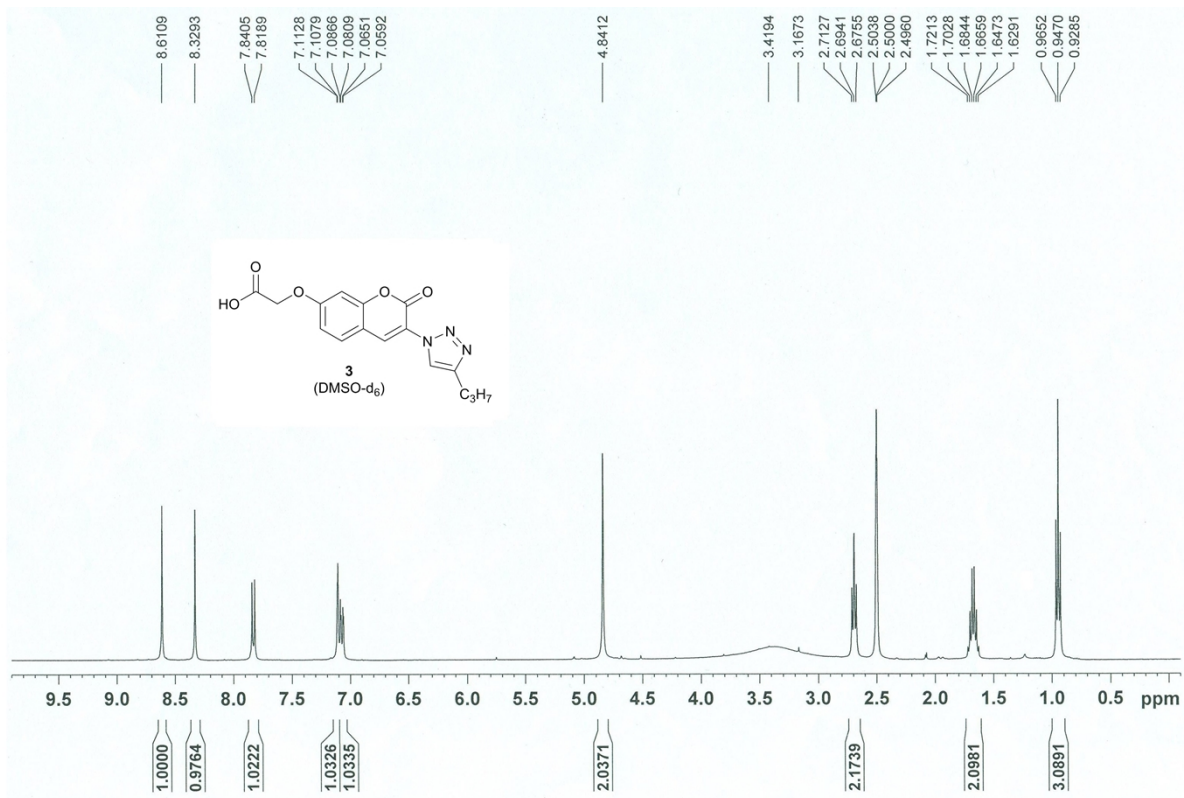
## 8. Isolation procedure of the natural compound **8** from the extract of *C. morifolium*

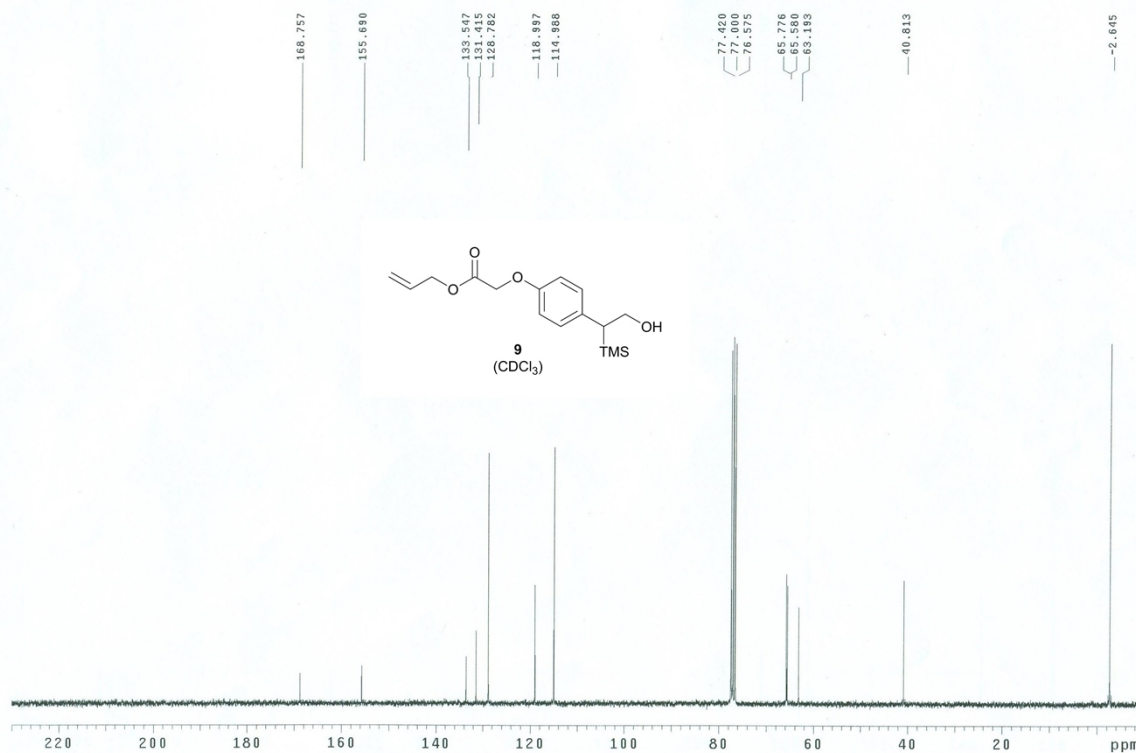
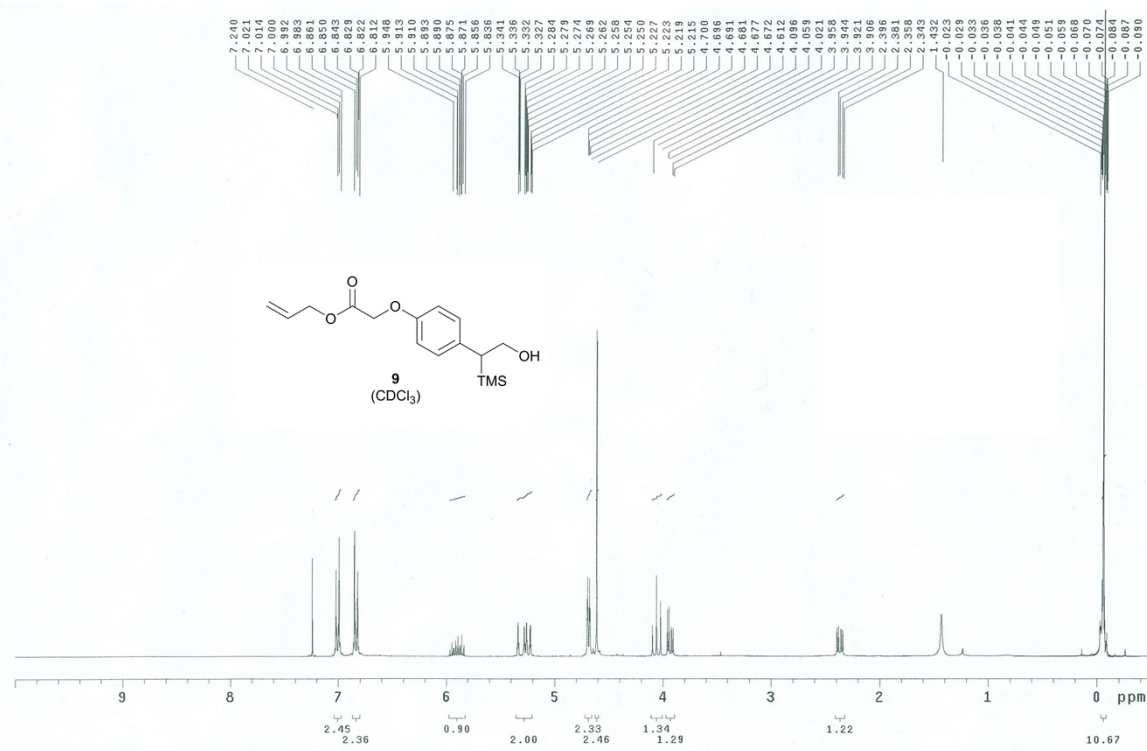
The methanol extract (30 g) of the whole plant of *Chrysanthemum morifolium* was suspended in H<sub>2</sub>O (300 mL) and successively extracted with hexane (3 × 200 mL), CHCl<sub>3</sub> (3 × 200 mL), EtOAc (3 × 200 mL), and *n*-BuOH (3 × 200 mL) to give dried hexane (4.2 g), CHCl<sub>3</sub> (1.8 g), EtOAc (2.2 g), and *n*-BuOH (4.6 g) extracts. Among the extracts, only the hexane portion exhibited fluorescence emission after the click reaction with sensory bead **1**. The hexane fraction was subjected to silica-gel column chromatography and eluted with a gradient mixture of hexane–EtOAc (12:1 to 1:1) and CH<sub>2</sub>Cl<sub>2</sub>–MeOH (10:1 to 6:1) to give fifteen fractions (A–O). After click reactions with each fraction, the fluorescence response was observed in fractions L and M. After the cleavage step, LC/MS analysis showed that the peak with a molecular ion of *m/z* 537 ([M–H]<sup>–</sup>) was predominant in fraction M. This finding indicates that fraction M contains a larger amount of the target compound (real molecular mass of 277 Daltons) compared to fraction L. Subsequently, fraction M (61 mg) was purified by semi-preparative reverse-phase HPLC conducted with a Phenomenex Luna 10 μm C18(2) column (250 × 10.00 mm) at a flow rate of 5 mL/min (30–100% aqueous MeCN with 0.1% formic acid over 20 min and 100% MeCN with 0.1% formic acid from 20 to 25 min) to give pure diyne **8** (1.2 mg) as a white solid. IR (CHCl<sub>3</sub>)  $\nu_{\max}$  3291, 3060, 3030, 2917, 2226, 1653, 1625, 1610, 1544, 1260; <sup>1</sup>H-NMR (500 MHz, CDCl<sub>3</sub>):  $\delta$  7.10–7.34 (m, 5H), 6.18 (dd, *J* = 15.0, 11.0 Hz, 1H), 6.01–6.09 (m, 1H), 5.73 (d, *J* = 14.5 Hz, 1H), 5.45 (br s, 1H), 3.61 (q, *J* = 6.5 Hz, 2H), 2.85 (t, *J* = 6.8 Hz, 2H), 2.37–2.45 (m, 4H), 1.98 (s, 1H); <sup>13</sup>C-NMR (125 MHz, CDCl<sub>3</sub>):  $\delta$  165.96, 140.57, 139.20, 138.90, 129.74, 128.79, 128.67, 126.53, 122.93, 68.22, 65.49, 65.03, 40.66, 35.67, 31.25, 18.80; HRMS (FAB, *m/z*): [M+H]<sup>+</sup> calcd. for 278.1545, found 278.1543.

# 9. <sup>1</sup>H NMR and <sup>13</sup>C NMR Spectra

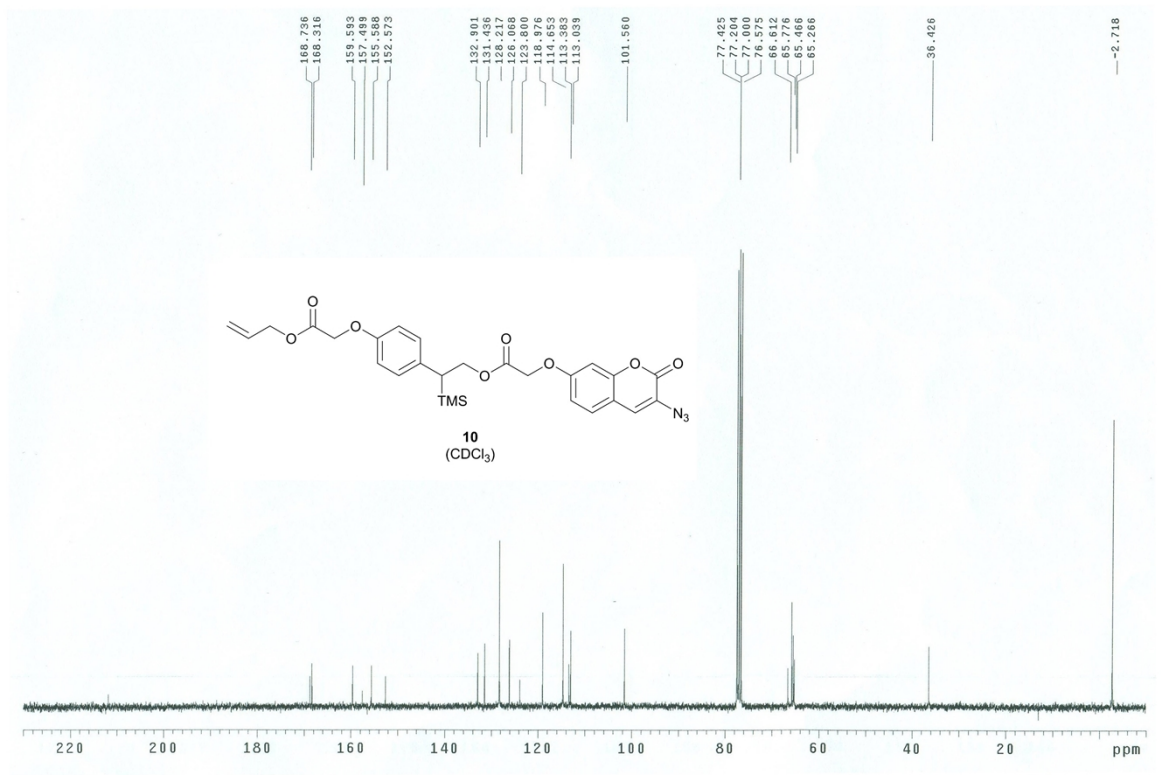
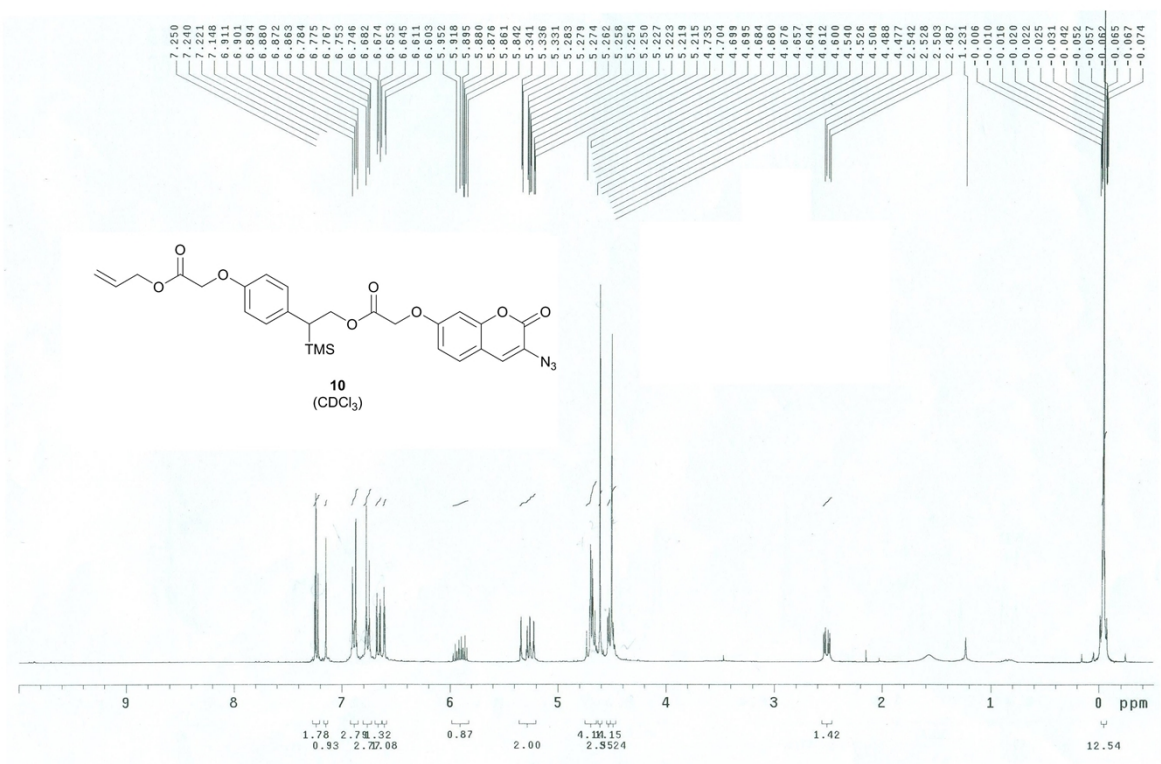


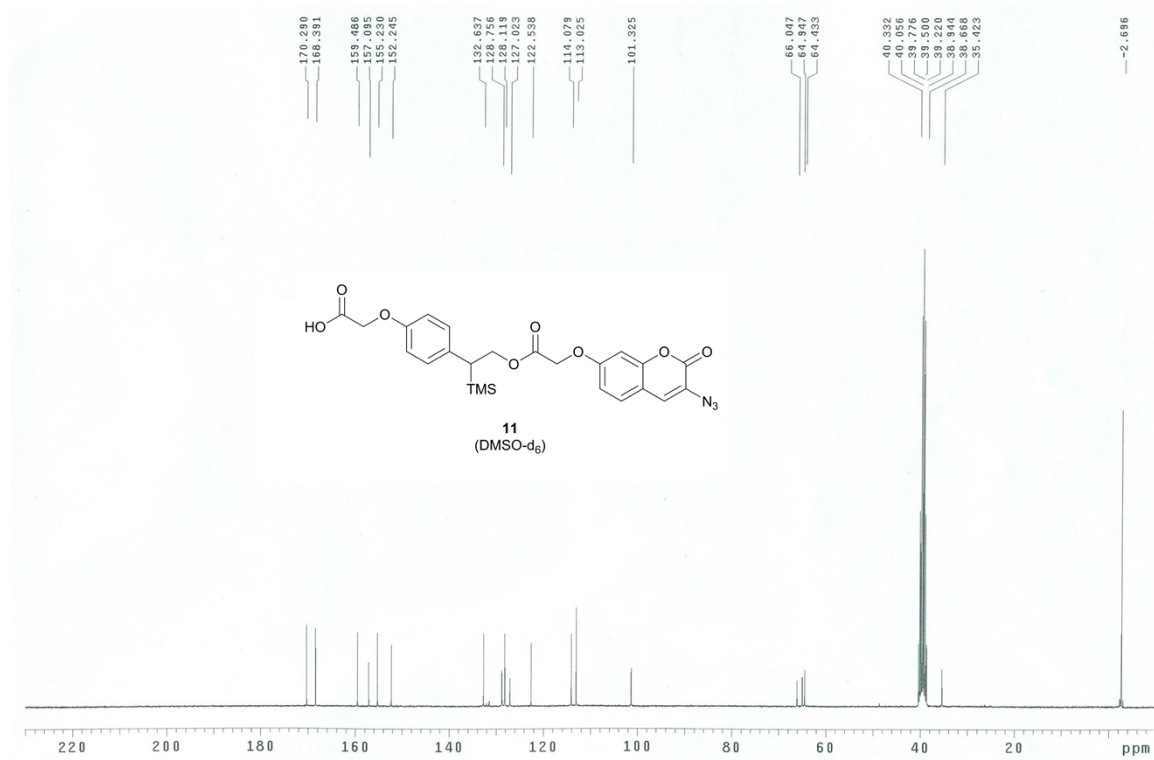
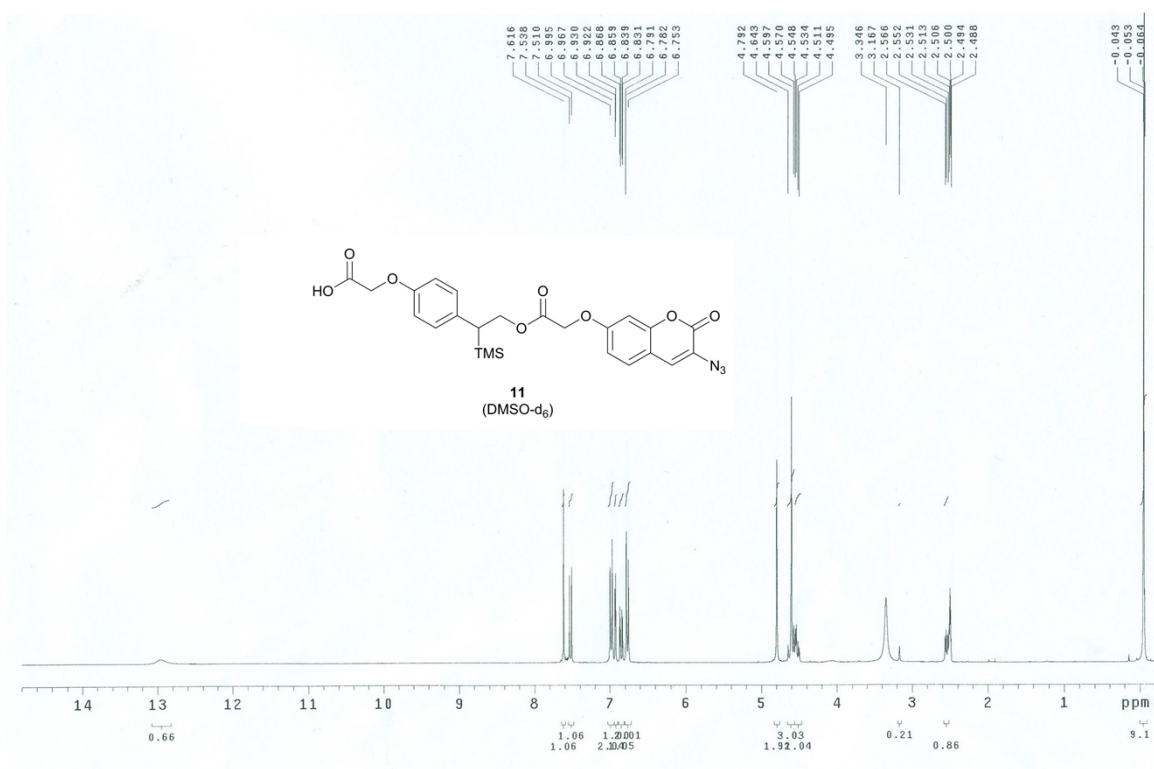












Current Data Parameters  
 NAME nov17-ph-jhj  
 EXPNO 1  
 PROCNO 1

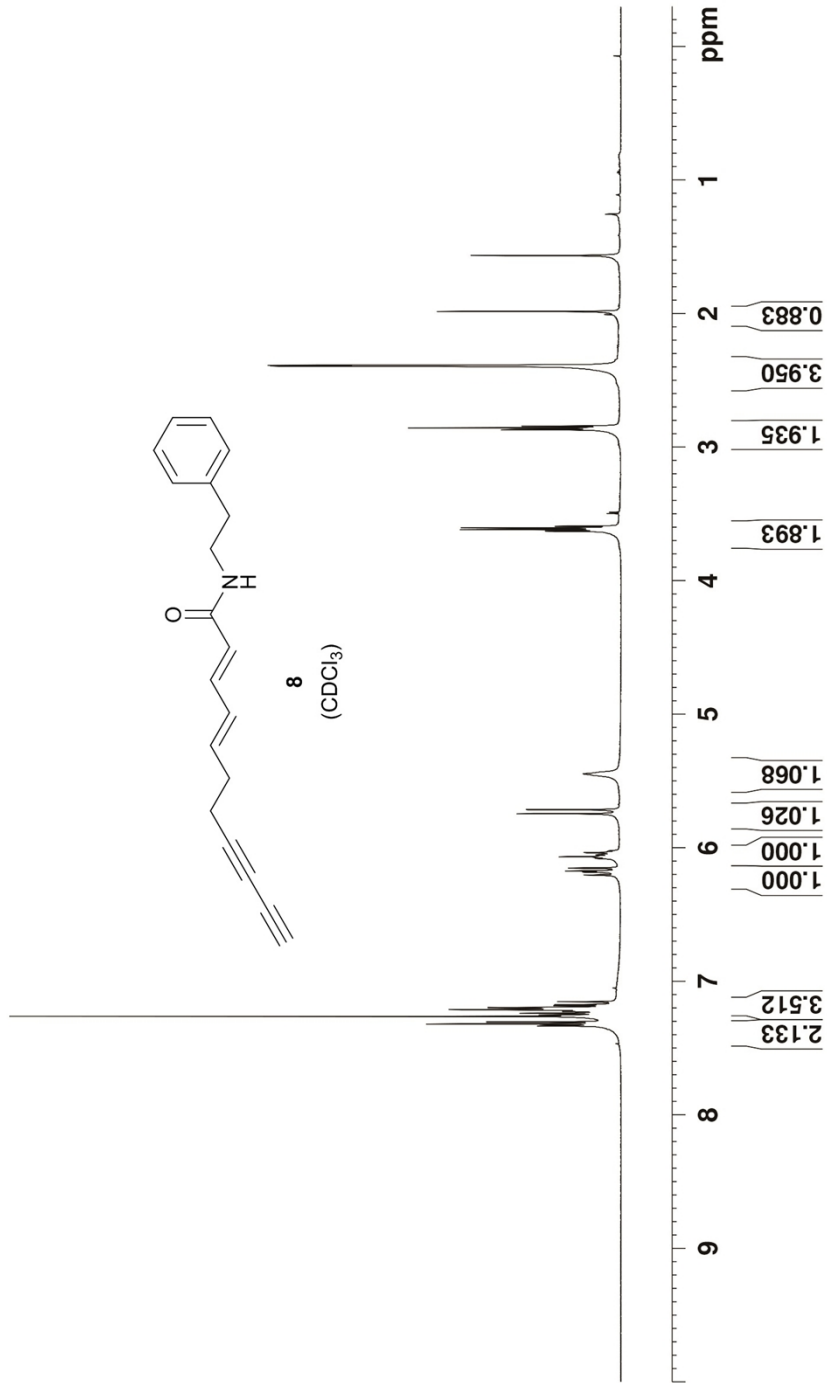
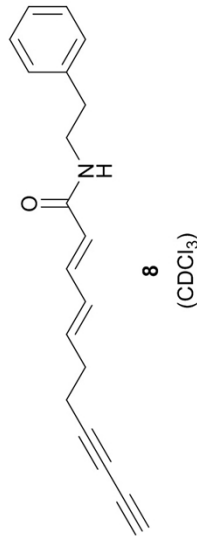
F2 - Acquisition Parameter:

Date\_ 20141117  
 Time 13.20  
 INSTRUM spect  
 PROBHD 5 mm PABBO BB/  
 PULPROG zg30  
 TD 32768  
 SOLVENT CDCl3  
 NS 64  
 DS 4  
 SWH 8012.820 Hz  
 FIDRES 0.244532 Hz  
 AQ 2.0447233 sec  
 RG 181  
 DW 62.400 usec  
 DE 6.50 usec  
 TE 298.0 K  
 D1 1.00000000 sec  
 TD0 1

===== CHANNEL f1 =====  
 SFO1 500.1332508 MH:  
 NUC1 1H  
 P1 10.20 usec  
 PLW1 19.00000000 W

F2 - Processing parameters  
 SI 16384  
 SF 500.1300133 MH:  
 EM  
 WDW 0  
 SSB 0 0.30 Hz  
 LB 0  
 GB 0  
 PC 1.00

7.331  
7.317  
7.302  
7.260  
7.254  
7.252  
7.237  
7.233  
7.222  
7.220  
7.211  
7.209  
7.202  
7.195  
7.180  
7.172  
7.150  
6.202  
6.180  
6.172  
6.150  
6.077  
6.070  
6.064  
6.058  
6.051  
6.040  
6.034  
6.028  
6.020  
5.742  
5.713  
5.445  
3.628  
3.615  
3.603  
3.589  
2.867  
2.854  
2.840  
2.391  
2.385  
1.981  
1.563



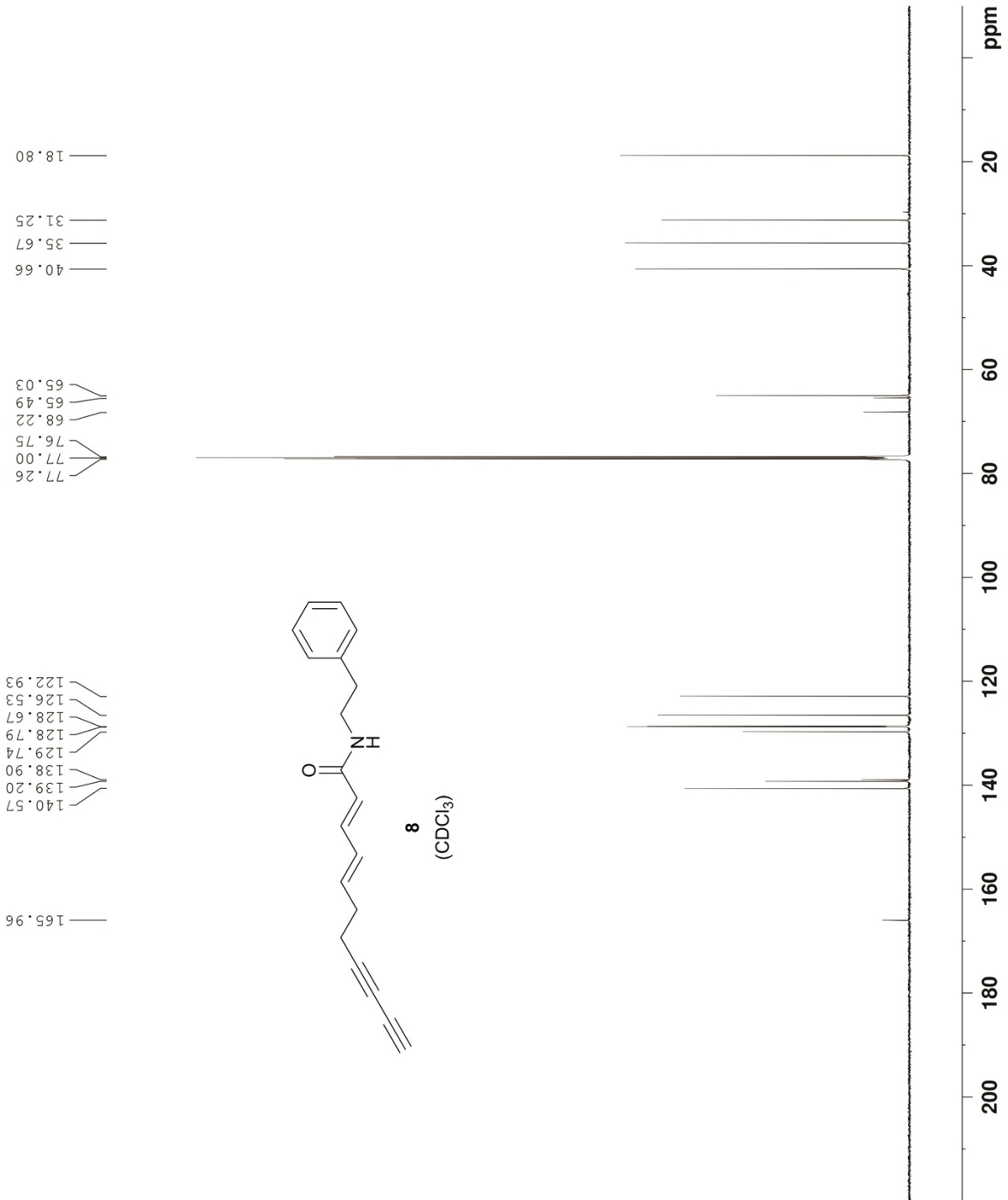
Current Data Parameters  
 NAME nov12-ph-jhj  
 EXPNO 1  
 PROCNO 1

F2 - Acquisition Parameters  
 Date\_ 20141112  
 Time 17.49  
 INSTRUM spect  
 PROBHD 5 mm PABBO BB/  
 PULPROG zgdc  
 TD 32768  
 SOLVENT CDCl3  
 NS 122880  
 DS 4  
 SWH 29761.904 Hz  
 FIDRES 0.908261 Hz  
 AQ 0.5505024 sec  
 RG 2050  
 DW 16.800 usec  
 DE 6.50 usec  
 TE 298.0 K  
 D1 2.0000000 sec  
 D11 0.0300000 sec  
 TD0 1

===== CHANNEL f1 =====  
 SFO1 125.7709936 MHz  
 NUC1 13C  
 P1 10.00 usec  
 PLW1 90.0000000 W

===== CHANNEL f2 =====  
 SFO2 500.1320005 MHz  
 NUC2 1H  
 CPDPRG[2] waltz16  
 PCPD2 80.00 usec  
 PLW2 19.0000000 W  
 PLW12 0.30886999 W

F2 - Processing parameters  
 SI 16384  
 SF 125.7577907 MHz  
 WDW EM  
 SSB 0  
 LB 1.00 Hz  
 GB 0  
 PC 1.40



## 10. References

- (1) R. I. Jølck, H. Sun, R. H. Berg and T. L. Andresen, *Chem. Eur. J.*, 2011, **17**, 3326–3331.
- (2) M. Wagner, S. Dziadek and H. Kunz, *Chem. Eur. J.*, 2003, **9**, 6018–6030.
- (3) K. Mikami, S. Kataoka, Y. Yusa and K. Aikawa, *Org. Lett.*, 2004, **6**, 3699–3701.