

## Support Information for

# Fluorescent Derivative Probes for Subcellular Localization during Osteoclast Formation

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### Biological Procedures

Murine monocyte/macrophage cell line RAW264.7 was generously provided by the Department of Biological Sciences of Nanjing University.  $\alpha$ -Modification of Eagle's medium ( $\alpha$ -MEM) and fetal bovine serum (FBS) from HyClone (Carlsbad, CA); receptor activator for nuclear factor  $\kappa$ B ligand (RANKL) from PeproTech EC (London, UK); TRAP kit from Sigma (St. Louis, MO).

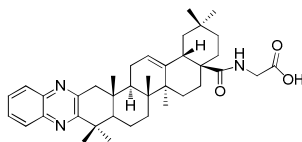
RAW264.7 cells were cultured at 37 °C in  $\alpha$ -MEM, 10% FBS, 100 U/ml penicillin and 10 mg/ml streptomycin. For proliferation the cells were cultured at 37 °C in atmosphere of 5% CO<sub>2</sub>. After reaching confluence of about 80%, the cells were trypsinated and seeded at a final density of  $1 \times 10^3$  cells/well in 96-well plates. Cells were incubated in complete medium in the presence of RANKL (40 ng/ml) or RANKL plus compounds. Cultures were fed every 36 hrs by replacing with fresh medium. After 5 days, the cells were stained with the osteoclast marker (TRAP kit), following the manufacturer's protocols. TRAP<sup>+</sup> cells with three or more nuclei were counted as osteoclasts. Data are expressed as mean  $\pm$  S.D. (n = 3). Images were acquired using a spectral confocal microscope (Leica TCS SP5, Germany).

MTT assay: RAW264.7 cells were seeded into 96-well plates at a density of  $1 \times 10^4$  cells per well. Cells were incubated with compounds (10  $\mu$ M) for 24 hrs at 37 °C. After incubation with MTT at 37 °C for 4 hrs, 150  $\mu$ L DMSO was added to each well, followed by gently shaking for 5 min to achieve complete dissolution. The absorbance was determined at 570 nm. Data are expressed as mean  $\pm$  S.D. (n = 3).

## Chemical Procedures

Commercially available reagents were used without further purification. Thin layer chromatography was carried out on precoated Kieselgel F254 plates (0.25 mm). Flash column chromatography was performed with silica gel (200-300 mesh). <sup>1</sup>H and <sup>13</sup>C NMR spectra were recorded on Bruker Avance III 400 spectrometer in CDCl<sub>3</sub> solutions using TMS ( $\delta$  = 0 ppm) as references. HR-ESI-MS spectra were obtained on a Finnigan TSQ-7000 mass spectrometer.

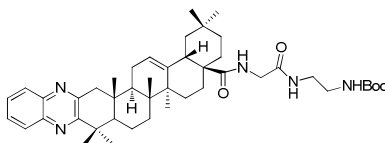
### Compound 1



To a round bottom flask (RBF) was added QOA-8a (2.0 g, 3.3 mmol, 1.0 eq), NaOH (0.3 g, 6.6 mmol, 2.0 eq) and 40 ml THF/H<sub>2</sub>O (3:1). The reaction was stirred for 4 hrs

at r.t. until TLC indicated consumption of the starting materials. 2 M Hydrochloric acid was added to adjust the pH of the mixture to pH 4. The aqueous phase was extracted with EtOAc (50 ml  $\times$  3). The combined extracts were washed with brine, dried (Na<sub>2</sub>SO<sub>4</sub>) and concentrated in vacuo to give crude products **1** as white solid (1.9 g, 95%), which was used for next reaction without further purification. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$ : 8.06–7.87 (m, 2H), 7.66–7.63 (m, 2H), 7.04 (s, 1H), 5.63 (s, 1H), 3.92 (s, 2H), 3.74 (d,  $J$  = 5.9 Hz, 1H), 2.72 (d,  $J$  = 10.6 Hz, 1H), 2.59 (d,  $J$  = 16.9 Hz, 1H), 2.38–2.12 (m, 2H), 2.02 (s, 1H), 1.91–1.49 (m, 10H), 1.49–1.17 (m, 13H), 1.11 (d,  $J$  = 12.4 Hz, 1H), 0.95 (s, 3H), 0.92 (s, 3H), 0.87 (s, 3H), 0.86 (s, 3H). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>)  $\delta$ : 178.8, 161.6, 152.1, 143.9, 142.3, 139.8, 129.3, 128.9, 126.9, 123.7, 77.3, 77.0, 76.7, 68.0, 53.5, 48.4, 46.8, 46.3, 45.5, 40.4, 39.4, 36.6, 34.2, 33.1, 32.4, 30.7, 27.3, 25.6, 25.3, 23.9, 23.6, 20.3, 16.21, 15.8.

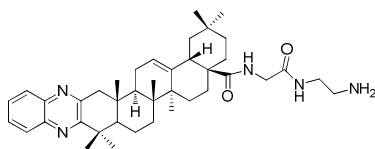
## Compound 2



To a RBF was added compound **1** (1.0 g, 1.7 mmol, 1.0 eq), tert-butyl (2-aminoethyl) carbamate (0.3 g, 2.0 mmol, 1.2 eq), HOBT (0.3 g, 2.0 mmol, 1.2 eq), EDCI (0.4 g, 2.0 mmol, 1.2 eq), TEA (0.3 g, 3.4 mmol, 2.0 eq) and DCM (30 ml). The reaction was stirred for 16 hrs at r.t.. The reaction was quenched by adding aq. NH<sub>4</sub>Cl and diluted with DCM. The aqueous phase was extracted with DCM (30 ml  $\times$  3). The combined extracts were washed with brine, dried (Na<sub>2</sub>SO<sub>4</sub>) and concentrated in vacuo. Purification by silica gel column chromatography (petroleum ether/ethyl acetate = 2/1) provided **2** (1.0 g, 82%) as white solid. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$ : 8.00–7.94 (m, 2H), 7.64–7.63 (m, 2H), 7.35 (s, 1H), 7.00 (s, 1H), 5.58 (s, 1H), 5.41–5.16 (m, 1H), 4.00 (d,  $J$  = 16.4 Hz, 1H), 3.85–3.71 (m, 1H), 3.35–3.22 (m, 5H), 2.68 (t,  $J$  = 15.4 Hz, 2H), 2.19–1.99 (m, 3H), 1.89–1.54 (m, 10H), 1.42–1.14 (m, 23H), 0.92 (s, 9H), 0.82 (s, 3H). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>)  $\delta$ : 178.9, 169.3, 160.9, 156.4, 151.8, 143.8, 142.1, 140.8, 128.8, 128.5, 128.1, 123.4, 79.4, 77.5, 77.4, 76.9, 53.4, 49.4, 46.4, 45.5,

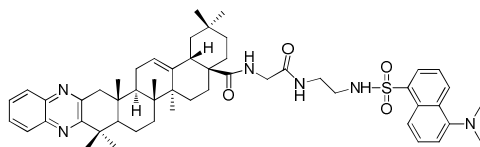
43.5, 42.1, 40.3, 39.3, 36.8, 34.1, 33.0, 32.4, 31.8, 30.7, 28.4, 27.3, 25.7, 25.3, 23.8, 23.6, 20.3, 16.1, 15.7.

### Compound 3



To a solution of **2** (1.0 g, 1.4 mmol, 1.0 eq) in DCM (20 ml) was added trifluoroacetic acid (1 ml) at r.t.. The mixture was stirred at r.t. until complete conversion of the starting material (~0.5 hr) and then directly concentrated. The crude product was purified by silica gel column flash chromatography (dichloromethane/methanol/NH<sub>3</sub>.H<sub>2</sub>O = 15/1/0.1) to give white solid (0.7 g, 81%). <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ: 8.03–7.93 (m, 2H), 7.67–7.64 (m, 2H), 6.85–6.83 (m, 2H), 5.56 (s, 1H), 4.07–3.94 (m, 1H), 3.75 (dd, *J* = 16.2, 4.3 Hz, 1H), 3.33 (m, 1H), 3.23 (d, *J* = 16.3 Hz, 1H), 2.86 (s, 1H), 2.66 (d, *J* = 16.2 Hz, 2H), 2.19–1.50 (m, 19H), 1.44 (s, 3H), 1.43 (s, 3H), 1.33–1.12 (m, 9H), 0.93 (s, 3H), 0.92 (s, 3H), 0.82 (s, 3H). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ: 179.7, 170.4, 161.0, 151.8, 143.8, 142.2, 140.8, 128.9, 128.6, 128.1, 123.4, 53.4, 49.4, 46.5, 46.4, 45.5, 43.3, 42.1, 42.0, 40.3, 39.9, 39.3, 37.5, 36.8, 34.1, 33.0, 32.3, 31.7, 30.7, 27.2, 25.7, 25.4, 23.8, 23.5, 23.4, 20.3, 16.0, 15.6.

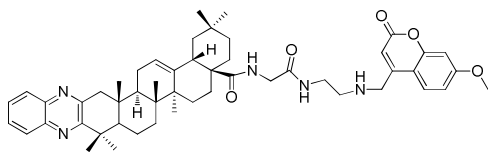
### Compound 4



To a solution of **3** (0.2 g, 0.3 mmol, 1.0 eq) in dry DCM (30 ml) was added dansyl chloride (89 mg, 0.33 mmol, 1.1 eq) and triethylamine (61 mg, 0.6 mmol, 2.0 eq) at r.t.. The mixture was stirred at r.t. until complete conversion of the starting material (~2 hrs). The reaction was quenched by adding aq. NH<sub>4</sub>Cl and diluted with DCM. The aqueous phase was extracted with DCM (20 ml × 3). The combined extracts were

washed with brine, dried (Na<sub>2</sub>SO<sub>4</sub>) and concentrated in vacuo. Purification by silica gel column chromatography (dichloromethane/methanol = 20/1) provided **4** (0.2 g, 83%) as a slightly green solid. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ: 8.52 (d, *J* = 8.5 Hz, 1H), 8.28 (d, *J* = 8.5 Hz, 1H), 8.20 (dd, *J* = 7.3, 1.1 Hz, 1H), 8.04–7.92 (m, 2H), 7.68–7.61 (m, 2H), 7.50 (dd, *J* = 16.1, 7.6 Hz, 2H), 7.15 (d, *J* = 7.5 Hz, 1H), 7.07 (t, *J* = 5.8 Hz, 1H), 6.87 (t, *J* = 4.8 Hz, 1H), 6.13 (t, *J* = 6.0 Hz, 1H), 5.54 (t, *J* = 3.2 Hz, 1H), 3.90 (dd, *J* = 16.4, 5.3 Hz, 1H), 3.69 (dd, *J* = 16.4, 4.3 Hz, 1H), 3.35–3.33 (m, 2H), 3.21 (d, *J* = 16.4 Hz, 1H), 3.06–3.02 (m, 2H), 2.86 (s, 6H), 2.70–2.62 (m, 2H), 2.15–1.98 (m, 3H), 1.86–1.49 (m, 10H), 1.46–1.33 (m, 8H), 1.30–1.11 (m, 7H), 0.92 (s, 3H), 0.92 (s, 3H), 0.87 (s, 3H), 0.79 (s, 3H). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ: 179.6, 169.9, 161.0, 151.9, 143.8, 134.7, 130.5, 129.5, 128.9, 128.6, 128.3, 128.1, 123.4, 123.2, 115.3, 77.3, 77.0, 76.7, 53.4, 49.4, 46.5, 45.5, 43.6, 42.9, 42.1, 40.3, 39.4, 36.8, 34.1, 33.0, 32.6, 30.7, 27.3, 25.7, 23.8, 20.4, 16.2, 15.6. HR-ESI-MS *m/z*: calcd for C<sub>51</sub>H<sub>65</sub>N<sub>4</sub>O<sub>6</sub>S [M + Na]<sup>+</sup> 895.4915; found 895.4918.

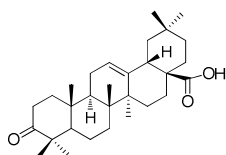
### Compound 5



To a solution of **3** (0.2 g, 0.3 mmol, 1.0 eq) and K<sub>2</sub>CO<sub>3</sub> (83.0 mg, 0.6 mmol, 2.0 eq) in dry acetone (30 ml) was added 4-bromomethyl-7-methoxycoumarin (88 mg, 0.33 mmol, 1.1 eq) at r.t.. The reaction was stirred and refluxed for 16 hrs. The reaction was quenched by adding aq. NH<sub>4</sub>Cl and diluted with DCM. The aqueous phase was extracted with DCM (20 ml × 3). The combined extracts were washed with brine, dried (Na<sub>2</sub>SO<sub>4</sub>) and concentrated in vacuo. Purification by silica gel column chromatography (dichloromethane/methanol = 20/1) provided **5** (0.15 g, 58%) as white solid. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ: 8.08–7.89 (m, 2H), 7.65 (dd, *J* = 6.3, 3.5 Hz, 2H), 7.53 (d, *J* = 9.5 Hz, 1H), 6.92 (s, 1H), 6.89–6.76 (m, 3H), 6.43 (s, 1H), 5.55 (t, *J* = 3.3 Hz, 1H), 4.05–3.93 (m, 3H), 3.86 (s, 3H), 3.84–3.75 (m, 1H), 3.44–3.43 (m, 2H), 3.22 (d, *J* = 16.4 Hz, 1H), 2.88 (t, *J* = 5.7 Hz, 2H), 2.67–2.63 (m,

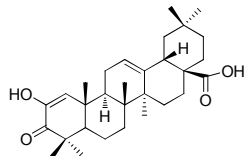
2H), 2.20–1.95 (m, 4H), 1.88–1.53 (m, 10H), 1.43 (s, 3H), 1.41 (s, 3H), 1.37–1.11 (m, 8H), 0.92 (s, 3H), 0.91 (s, 3H), 0.89 (s, 3H), 0.81 (s, 3H).  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$ : 179.2, 169.4, 162.7, 161.5, 161.0, 155.6, 153.3, 151.8, 144.0, 142.2, 140.9, 128.9, 128.6, 128.1, 124.8, 123.4, 112.5, 111.9, 110.3, 101.1, 55.8, 53.4, 49.4, 49.0, 48.6, 46.5, 45.5, 44.0, 42.1, 40.3, 39.3, 39.1, 36.9, 34.1, 33.0, 32.6, 32.3, 31.7, 30.7, 27.3, 25.7, 25.3, 23.9, 23.5, 20.3, 16.2, 15.7. HR-ESI-MS  $m/z$ : calcd for  $\text{C}_{51}\text{H}_{65}\text{N}_5\text{NaO}_5$  [ $\text{M} + \text{Na}$ ] $^+$  850.4878; found 850.4879.

### Compound 6



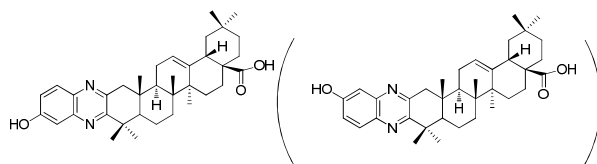
$\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$  (1.7 g, 4.8 mmol) and  $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$  (0.75 g, 4.8 mmol) were dissolved in water (95 ml).  $\text{Na}_2\text{WO}_4 \cdot 2\text{H}_2\text{O}$  (4 g, 12 mmol) was dissolved in cooled 35% aq  $\text{H}_2\text{O}_2$  (28 ml) and mixed with the previously phosphate buffer. Oleanolic acid (OA) (11 g, 24 mmol) and DMF (50 ml) were added to a RBF with heating to 90 °C. The cooled mixture of  $\text{Na}_2\text{WO}_4\text{-H}_2\text{O}_2$  with phosphete buffer was added dropwise to the DMF solution of OA for over 1 hr followed by stirring for 1 hr under the same reaction conditions. The aqueous phase was extracted with EtOAc (100 ml  $\times$  3). The combined extracts were washed with brine, dried ( $\text{Na}_2\text{SO}_4$ ) and concentrated in vacuo to give crude products **6** as white solid (9.8 g, 89%).  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$ : 5.28 (s, 1H), 2.82 (dd,  $J = 13.7, 4.0$  Hz, 1H), 2.59–2.46 (m, 1H), 2.42–2.30 (m, 1H), 2.02–1.82 (m, 4H), 1.80–1.53 (m, 6H), 1.50–1.26 (m, 7H), 1.24–1.09 (m, 6H), 1.07 (s, 3H), 1.03 (s, 3H), 1.01 (s, 3H), 0.91 (s, 3H), 0.89 (s, 3H), 0.79 (s, 3H).  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$ : 217.8, 184.4, 143.6, 122.4, 55.3, 47.4, 46.9, 46.6, 45.8, 41.7, 41.0, 39.3, 39.1, 36.8, 34.1, 33.8, 33.1, 32.4, 32.1, 30.7, 27.7, 26.4, 25.9, 23.6, 23.5, 22.9, 21.4, 19.5, 17.0, 15.0.

### Compound 7



To a solution of **6** (4.5 g, 10.0 mmol, 1.0 eq) in 100 ml t-BuOH/THF (10/1) was added t-BuOK (6.7 g, 60.0 mmol, 6.0 eq) at r.t.. The reaction was stirred and refluxed for 10 hrs at 40 °C. The reaction mixture was treated with aqueous 1M HCl (100 ml) and diluted with EtOAc. The aqueous phase was extracted with EtOAc (100 ml × 3). The combined extracts were washed with brine, dried (Na<sub>2</sub>SO<sub>4</sub>) and concentrated in vacuo to give crude products **7** as light yellow solid (3.5 g, 78%). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ: 6.35 (s, 1H), 5.97 (s, 1H), 5.34 (t, *J* = 3.4 Hz, 1H), 2.89–2.81 (m, 1H), 2.12–1.87 (m, 4H), 1.81–1.69 (m, 3H), 1.67–1.11 (m, 17H), 0.94 (s, 3H), 0.91 (s, 3H), 0.83 (s, 3H). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ: 201.0, 184.3, 143.9, 143.8, 128.2, 122.1, 53.8, 46.6, 45.6, 43.9, 43.0, 41.9, 41.1, 40.0, 38.5, 33.8, 33.1, 32.4, 30.7, 27.6, 27.2, 25.9, 23.5, 23.4, 22.8, 21.8, 19.7, 18.7, 17.5.

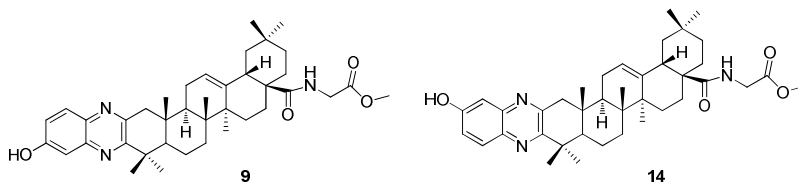
### Compound **8** and **13**



To a RBF was added **7** (3.5 g, 7.5 mmol, 1.0 eq), 3, 4-diaminophenol (0.93 g, 7.5 mmol, 1.0 eq) and EtOH (100 ml) at r.t.. The reaction was stirred and refluxed for 4 hrs at 80 °C. After being cooled down to r.t., the solvent was removed under reduced pressure. The crude product was further purified by flash column chromatography on silica gel (petroleum ether/ethyl acetate = 2/1) provided **8** and **13** without separated (3.0 g, 72%) as white solid. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ: 12.07 (s, 1H), 10.22 (d, *J* = 8.1 Hz, 1H), 7.79 (dd, *J* = 15.8, 9.1 Hz, 1H), 7.27 (d, *J* = 9.0 Hz, 1H), 7.16–7.14 (m, 1H), 5.26 (s, 1H), 2.99 (s, 1H), 2.80 (d, *J* = 10.4 Hz, 1H), 2.67 (s, 1H), 2.08–1.94 (m, 3H), 1.82–1.47 (m, 12H), 1.35 (s, 3H), 1.32 (s, 3H), 1.23–1.14 (m, 6H), 0.89 (s, 6H), 0.83 (s, 6H). <sup>13</sup>C NMR (100 MHz, DMSO-*d*<sub>6</sub>) δ: 178.6, 160.0, 157.9, 157.8,

156.6, 151.5, 148.1, 143.7, 142.8, 142.0, 136.4, 135.6, 129.5, 129.0, 121.6, 121.6, 108.9, 108.5, 52.5, 45.5, 44.7, 41.6, 41.0, 36.4, 33.3, 32.8, 32.0, 30.1, 29.0, 27.2, 25.4, 25.1, 23.3, 22.8, 22.1, 16.5, 15.3, 15.2.

#### Compound **9** and **14**

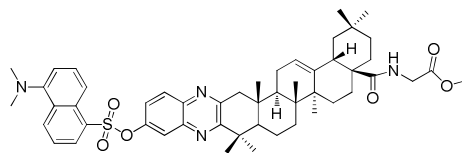


To a solution of **8** and **13** (mixture) (3.0 g, 0.5 mmol) in anhydrous CH<sub>2</sub>Cl<sub>2</sub> (50 ml) was added oxalyl chloride (2.2 ml) and stirred at room temperature for 24 hrs. The mixture was concentrated to dryness under reduced pressure. Hexane (3 × 50 ml) was added to the residue and the solution was concentrated to dryness to give acid chloride. To an anhydrous CH<sub>2</sub>Cl<sub>2</sub> (200 ml) solution of each corresponding glycine methyl ester hydrochloride (0.8 g, 0.6 mmol, 1.3 eq) and triethylamine (1 ml) was added the above acid chloride. The reaction mixture was stirred at room temperature for 2 hrs, and purified by Pre-TLC on silica gel (dichloromethane/methanol = 20/1) provided **9** (1.4 g) as white solid and **14** (1.1 g) as white solid. Compound **9**: <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ: 7.66 (d, *J* = 9.1 Hz, 1H), 7.00 (s, 1H), 6.90 (d, *J* = 8.6 Hz, 1H), 6.55 (t, *J* = 4.1 Hz, 1H), 5.46 (s, 1H), 4.12 (dd, *J* = 18.7, 5.4 Hz, 1H), 3.85 (dd, *J* = 18.6, 3.5 Hz, 1H), 3.73 (s, 3H), 3.09 (d, *J* = 16.5 Hz, 1H), 2.62 (m, 2H), 2.04 (m, 3H), 1.82–1.54 (m, 10H), 1.55–1.29 (m, 9H), 1.27–1.06 (m, 6H), 0.93 (s, 3H), 0.89 (s, 6H), 0.79 (s, 3H). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ: 178.6, 170.4, 158.2, 157.3, 150.4, 144.0, 140.7, 137.8, 130.2, 123.2, 121.5, 106.8, 53.4, 52.4, 48.4, 46.5, 46.4, 45.5, 42.2, 41.6, 40.0, 39.3, 36.7, 34.1, 33.0, 32.4, 32.3, 31.8, 30.7, 29.7, 27.3, 25.6, 25.3, 23.9, 23.5, 20.3, 16.1, 15.8. HR-ESI-MS *m/z*: calcd for C<sub>39</sub>H<sub>54</sub>N<sub>3</sub>O<sub>4</sub> [M + H]<sup>+</sup> 628.4109; found 628.4108. Compound **14**: <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ: 7.76 (d, *J* = 8.9 Hz, 1H), 7.31 (d, *J* = 2.0 Hz, 1H), 7.20 (s, 1H), 6.65 (t, *J* = 4 Hz, 1H), 5.54 (s, 1H), 4.16 (dd, *J* = 18.6, 5.5 Hz, 1H), 3.87 (dd, *J* = 18.6, 3.5 Hz, 1H), 3.76 (s, 3H), 3.20 (d, *J* = 16.3 Hz, 1H), 2.68–2.63 (m, 2H), 2.13–1.99 (m, 3H), 1.91–1.78 (m, 3H), 1.74–1.56 (m, 8H),



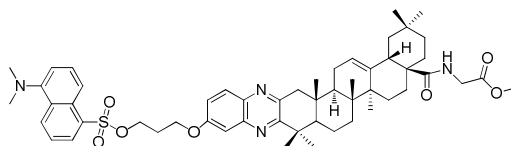


## Compound 11



To a solution of **9** (0.2 g, 0.3 mmol, 1.0 eq) in dry DCM (25 ml) was added dansyl chloride (0.9 g, 0.33 mmol, 1.1 eq) and triethylamine (0.1 ml) at r.t.. The mixture was stirred at r.t. until complete conversion of the starting material (~16 hrs). The reaction was quenched by adding aq. NH<sub>4</sub>Cl and diluted with DCM. The aqueous phase was extracted with DCM (15 ml × 3). The combined extracts were washed with brine, dried (Na<sub>2</sub>SO<sub>4</sub>) and concentrated in vacuo. Purification by silica gel column chromatography (petroleum ether/ethyl acetate = 2/1) provided **11** (0.2 g, 85%) as a slightly green solid. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 8.61 (d, *J* = 8.5 Hz, 1H), 8.53 (d, *J* = 8.7 Hz, 1H), 8.08 (dd, *J* = 7.3, 1.1 Hz, 1H), 7.87 (d, *J* = 9.1 Hz, 1H), 7.74–7.67 (m, 1H), 7.46–7.38 (m, 2H), 7.34 (dd, *J* = 9.1, 2.6 Hz, 1H), 7.28–7.26 (m, 1H), 6.53 (t, *J* = 4 Hz, 1H), 5.54 (t, *J* = 3.1 Hz, 1H), 4.14 (dd, *J* = 18.6, 5.6 Hz, 1H), 3.85 (dd, *J* = 18.6, 3.6 Hz, 1H), 3.75 (s, 3H), 3.12 (d, *J* = 16.5 Hz, 1H), 2.92 (s, 6H), 2.71–2.64 (m, 1H), 2.57 (d, *J* = 16.6 Hz, 1H), 2.14–1.97 (m, 3H), 1.83–1.54 (m, 10H), 1.44–1.10 (m, 16H), 0.94 (s, 3H), 0.93 (s, 3H), 0.86 (s, 3H), 0.81 (s, 3H). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 178.2, 170.5, 161.5, 152.9, 151.9, 149.3, 144.2, 140.7, 132.2, 131.3, 130.8, 130.3, 130.0, 129.9, 129.2, 124.0, 123.1, 123.0, 119.9, 119.5, 115.8, 53.3, 52.3, 49.3, 46.5, 46.4, 45.5, 42.3, 42.2, 41.5, 40.4, 39.3, 36.8, 34.1, 33.0, 32.4, 32.3, 31.7, 30.7, 27.3, 25.6, 25.3, 23.9, 23.6, 23.5, 20.3, 16.1, 15.7. HR-ESI-MS *m/z*: calcd for C<sub>51</sub>H<sub>65</sub>N<sub>4</sub>O<sub>6</sub>S [M + H]<sup>+</sup> 861.4619; found 861.4620.

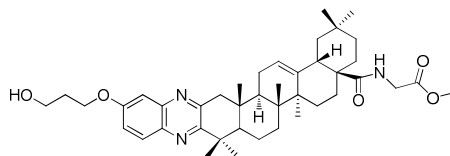
## Compound 12



To a solution of **10** (0.2 g, 0.3 mmol, 1.0 eq) in dry DCM (25 ml) was added dansyl chloride (0.9 g, 0.33 mmol, 1.1 eq) and triethylamine (0.1 ml) at r.t.. The mixture was

stirred at r.t. until complete conversion of the starting material (~16 hrs). The reaction was quenched by adding aq. NH<sub>4</sub>Cl and diluted with DCM. The aqueous phase was extracted with DCM (15 ml × 3). The combined extracts were washed with brine, dried (Na<sub>2</sub>SO<sub>4</sub>) and concentrated in vacuo. Purification by silica gel column chromatography (petroleum ether/ethyl acetate = 2/1) provided **12** (0.2 g, 81%) as a slightly green solid. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ: 8.50 (d, *J* = 8.5 Hz, 1H), 8.28 (dd, *J* = 7.3, 1.2 Hz, 1H), 8.22 (d, *J* = 8.6 Hz, 1H), 7.71 (d, *J* = 9.2 Hz, 1H), 7.55–7.41 (m, 2H), 7.04 (d, *J* = 2.6 Hz, 1H), 6.94 (m, 2H), 6.55 (t, *J* = 4 Hz, 1H), 5.57 (s, 1H), 4.25 (t, *J* = 5.9 Hz, 2H), 4.16 (dd, *J* = 18.7, 5.6 Hz, 1H), 4.02 (t, *J* = 5.8 Hz, 2H), 3.85 (dd, *J* = 18.7, 3.5 Hz, 1H), 3.76 (s, 3H), 3.18 (d, *J* = 16.3 Hz, 1H), 2.73 (s, 6H), 2.67–2.62 (m, 1H), 2.22–2.11 (m, 4H), 2.09–2.00 (m, 1H), 1.89–1.78 (m, 2H), 1.74–1.58 (m, 11H), 1.45 (s, 3H), 1.44 (s, 3H), 1.26–1.14 (m, 8H), 0.95 (s, 3H), 0.94 (s, 3H), 0.93 (s, 3H), 0.83 (s, 3H). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ: 158.4, 151.6, 149.0, 144.2, 143.3, 131.6, 131.0, 130.6, 129.8, 129.7, 128.8, 123.3, 122.9, 121.9, 119.1, 115.3, 107.0, 67.1, 63.5, 53.4, 52.4, 49.1, 46.6, 46.4, 45.6, 45.3, 42.3, 42.2, 41.5, 40.3, 39.3, 36.9, 34.2, 33.0, 32.4, 32.3, 31.8, 30.8, 29.7, 28.6, 27.3, 25.7, 25.3, 23.9, 23.6, 20.4, 16.1, 15.6. HR-ESI-MS *m/z*: calcd for C<sub>54</sub>H<sub>70</sub>N<sub>4</sub>NaO<sub>7</sub>S [M + Na]<sup>+</sup> 941.4857; found 941.4853.

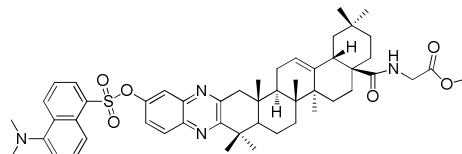
### Compound 15



The compound **15** was obtained by the same way as described for preparation of compound **10**. Yield: 85%. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ: 7.89 (d, *J* = 8.0 Hz, 1H), 7.30–7.27 (m, 2H), 6.57 (t, *J* = 4.0 Hz, 1H), 5.57 (s, 1H), 4.26 (t, *J* = 6.0 Hz, 2H), 4.15 (dd, *J* = 18.6, 5.5 Hz, 1H), 3.94–3.80 (m, 3H), 3.76 (s, 3H), 3.18 (d, *J* = 16.4 Hz, 1H), 2.68–2.63 (m, 2H), 2.17–2.04 (m, 5H), 1.87–1.57 (m, 10H), 1.47–1.34 (m, 8H), 1.30–1.13 (m, 7H), 0.94 (s, 3H), 0.93 (s, 3H), 0.91 (s, 3H), 0.82 (s, 3H). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ: 178.3, 170.6, 159.2, 158.2, 151.6, 144.2, 142.1, 138.3, 129.8,

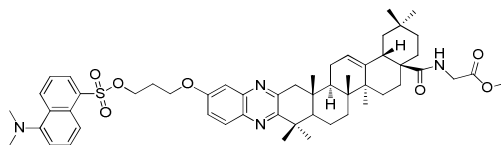
123.3, 121.9, 106.3, 76.7, 65.8, 59.9, 53.4, 52.3, 49.3, 46.6, 46.4, 45.6, 42.3, 42.2, 41.5, 40.0, 39.3, 36.8, 34.1, 33.0, 32.3, 32.3, 31.9, 31.8, 30.8, 27.3, 25.6, 25.2, 23.9, 23.6, 20.3, 16.1, 15.7. HR-ESI-MS  $m/z$ : calcd for  $C_{42}H_{60}N_3O_5$   $[M + H]^+$  686.4527; found 686.4528.

### Compound 16



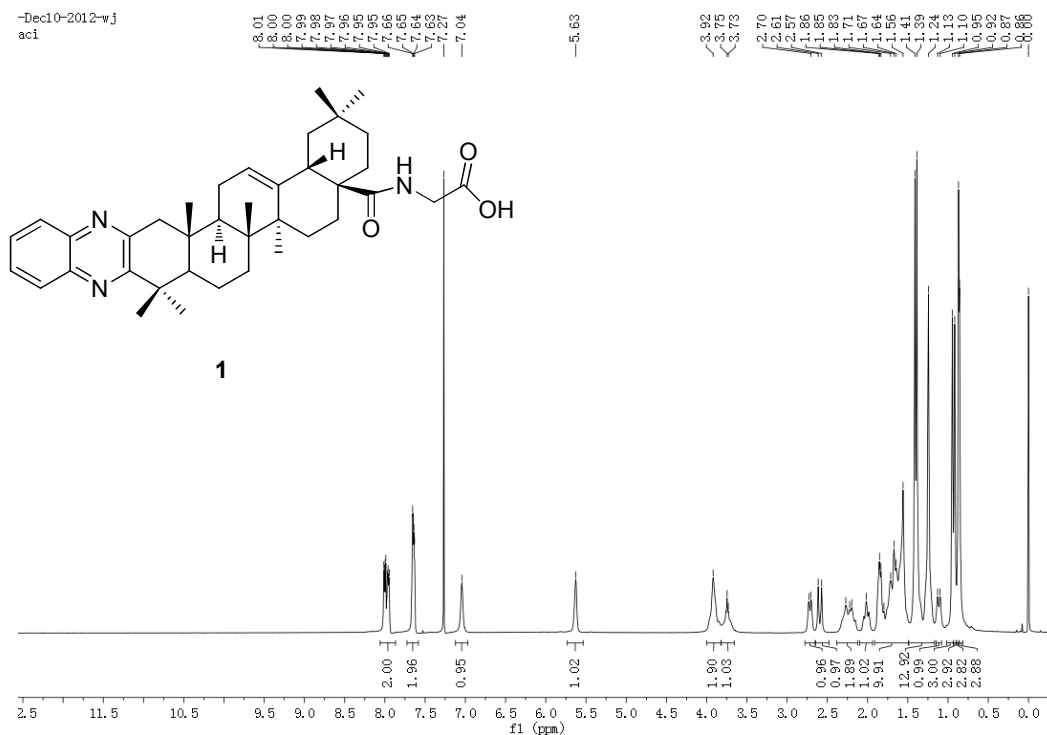
The compound **16** was obtained by the same way as described for preparation of compound **11**. Yield: 79%.  $^1H$  NMR (300 MHz,  $CDCl_3$ )  $\delta$ : 8.63 (d,  $J = 8.2$  Hz, 1H), 8.55 (d,  $J = 8.5$  Hz, 1H), 8.12 (d,  $J = 6.4$  Hz, 1H), 7.79–7.62 (m, 3H), 7.49–7.40 (m, 1H), 7.29 (d,  $J = 7.8$  Hz, 1H), 7.16 (dd,  $J = 9.1, 2.6$  Hz, 1H), 6.53 (s, 1H), 5.54 (s, 1H), 4.14 (dd,  $J = 18.6, 5.5$  Hz, 1H), 3.84 (dd,  $J = 18.6, 3.4$  Hz, 1H), 3.75 (s, 3H), 3.17 (d,  $J = 16.4$  Hz, 1H), 2.93 (s, 6H), 2.60 (m, 2H), 2.12 - 2.03 (m, 3H), 1.85–1.55 (m, 10H), 1.40–1.11 (m, 14H), 0.93 (s, 3H), 0.92 (s, 3H), 0.87 (s, 3H), 0.80 (s, 3H).  $^{13}C$  NMR (100 MHz,  $CDCl_3$ )  $\delta$ : 178.2, 170.6, 152.4, 152.0, 149.0, 144.2, 142.1, 139.3, 132.2, 131.3, 131.0, 130.1, 129.9, 129.5, 129.2, 123.9, 123.1, 123.0, 120.9, 119.4, 115.7, 53.3, 52.3, 49.3, 46.6, 46.4, 45.5, 42.3, 42.2, 41.5, 40.5, 39.3, 36.8, 34.1, 33.0, 32.4, 32.3, 31.7, 30.8, 27.3, 25.6, 25.3, 23.9, 23.6, 20.3, 18.6, 16.1, 15.7. HR-ESI-MS  $m/z$ : calcd for  $C_{51}H_{65}N_4O_6S$   $[M + H]^+$  861.4619; found 861.4625.

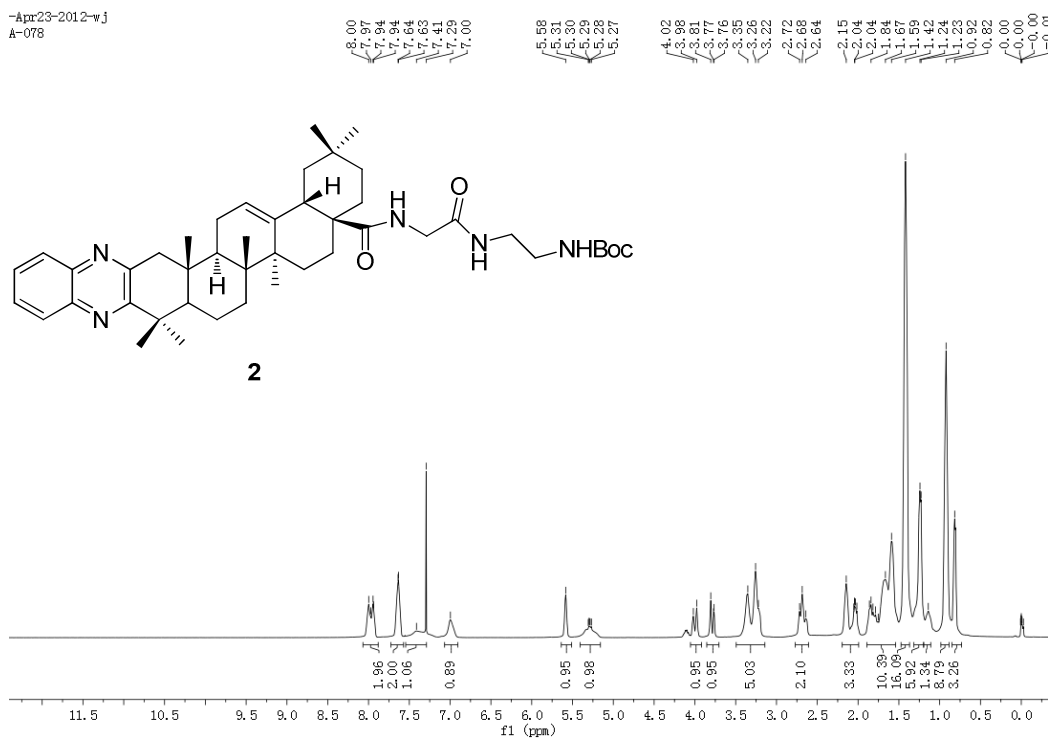
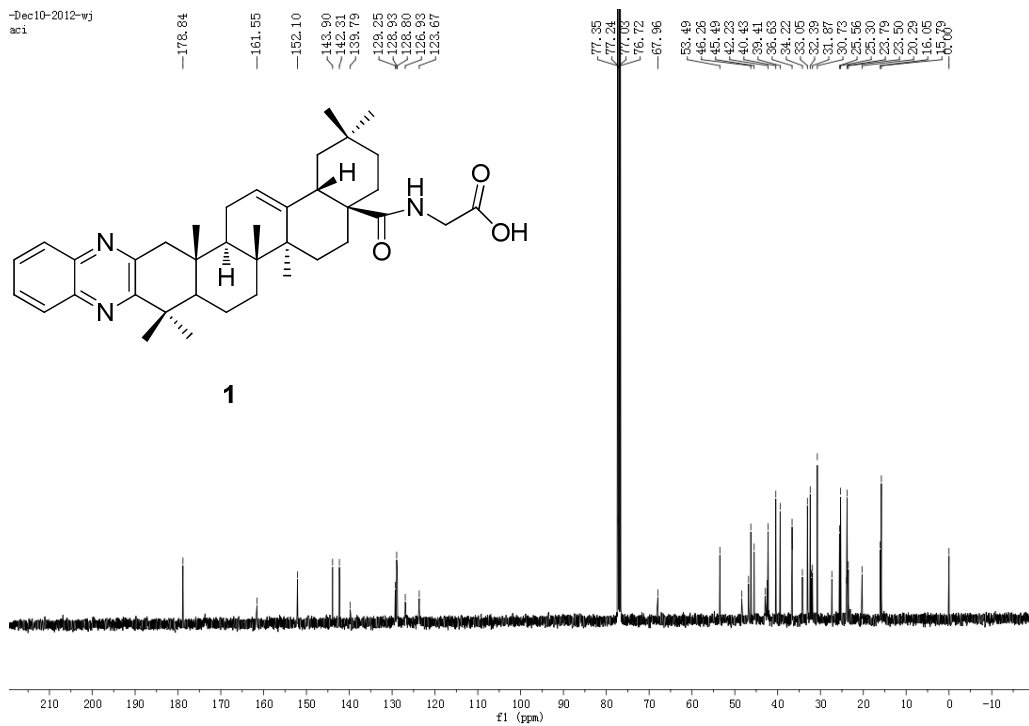
### Compound 17



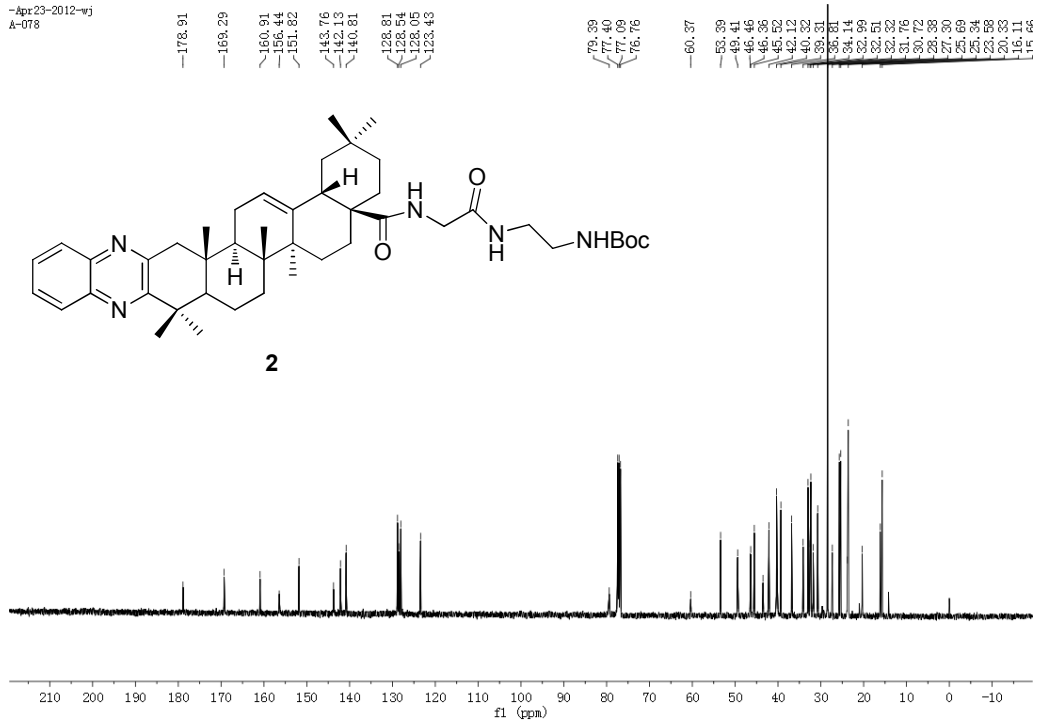
The compound **17** was obtained by the same way as described for preparation of compound **12**. Yield: 81%.  $^1H$  NMR (400 MHz,  $CDCl_3$ )  $\delta$ : 8.50 (d,  $J = 8.5$  Hz, 1H), 8.28 (dd,  $J = 7.3, 1.2$  Hz, 1H), 8.20 (d,  $J = 8.7$  Hz, 1H), 7.75 (d,  $J = 9.2$  Hz, 1H), 7.51 (dd,  $J = 8.5, 7.4$  Hz, 1H), 7.46–7.39 (m, 1H), 6.98 (s, 1H), 6.89–6.86 (m, 2H), 6.55 (s,

1H), 5.57 (s, 1H), 4.25 (t,  $J = 5.9$  Hz, 2H), 4.15 (dd,  $J = 18.7, 5.5$  Hz, 1H), 3.99 (t,  $J = 5.7$  Hz, 2H), 3.86 (dd,  $J = 18.7, 3.6$  Hz, 1H), 3.76 (s, 3H), 3.20 (d,  $J = 16.4$  Hz, 1H), 2.72–2.64 (m, 7H), 2.16–2.04 (s, 5H), 1.87–1.57 (m, 10H), 1.42 (s, 3H), 1.41 (s, 3H), 1.41–1.24 (m, 9H), 0.95 (s, 3H), 0.94 (s, 3H), 0.93 (s, 3H), 0.84 (s, 3H).  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$ : 178.3, 170.6, 158.6, 158.2, 151.6, 144.2, 138.2, 131.6, 130.9, 130.6, 129.8, 129.7, 129.6, 128.7, 123.2, 122.9, 121.6, 119.0, 115.2, 67.0, 63.3, 53.4, 52.3, 49.3, 46.6, 46.4, 45.6, 45.3, 42.3, 42.2, 41.5, 40.0, 39.3, 36.8, 34.2, 33.0, 32.4, 31.8, 30.8, 28.5, 27.3, 25.7, 25.2, 23.9, 23.6, 20.3, 16.1, 15.7. HR-ESI-MS  $m/z$ : calcd for  $\text{C}_{54}\text{H}_{70}\text{N}_4\text{NaO}_7\text{S}$   $[\text{M} + \text{Na}]^+$  941.4857; found 941.4858.

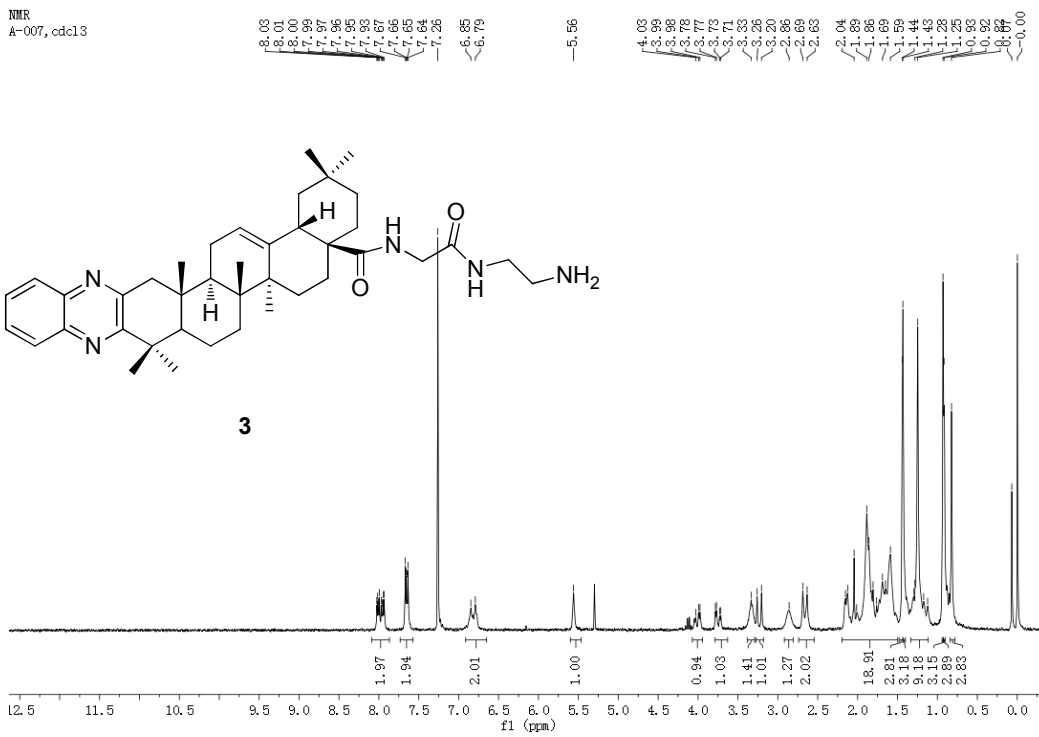




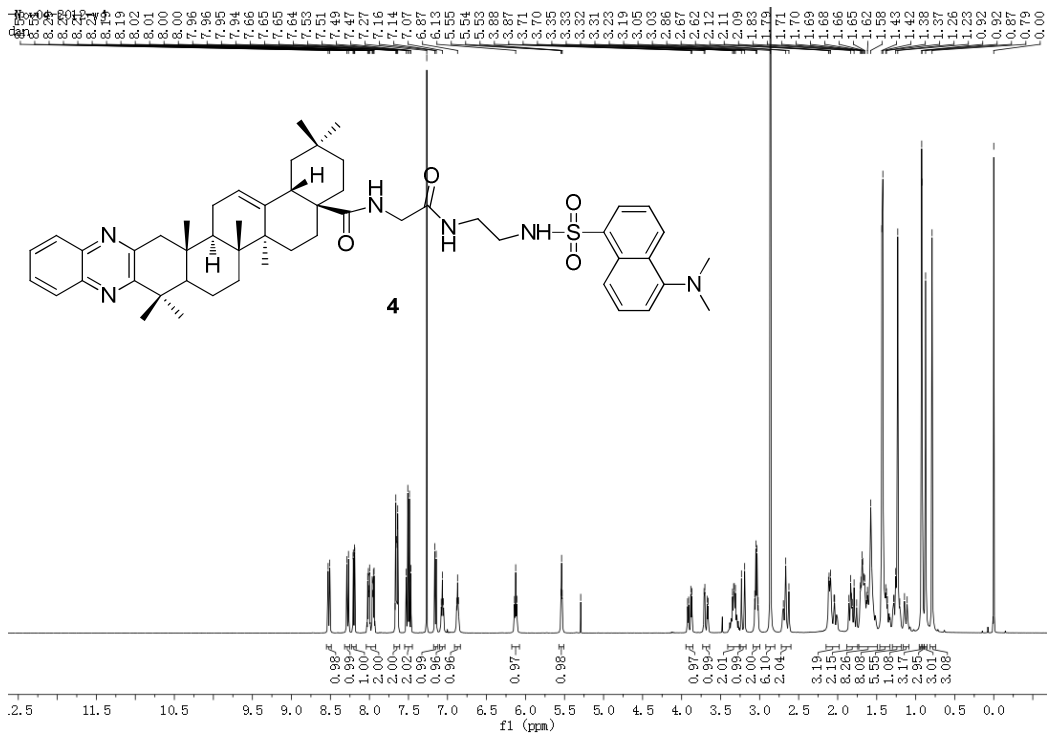
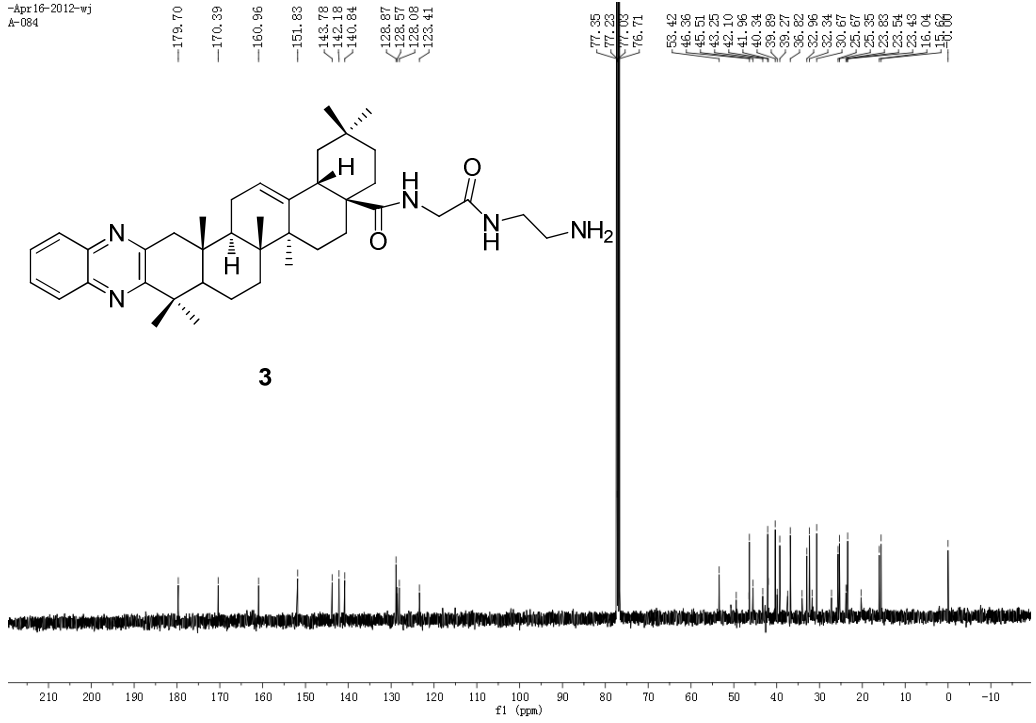
-Apr23-2012-wj  
A-078



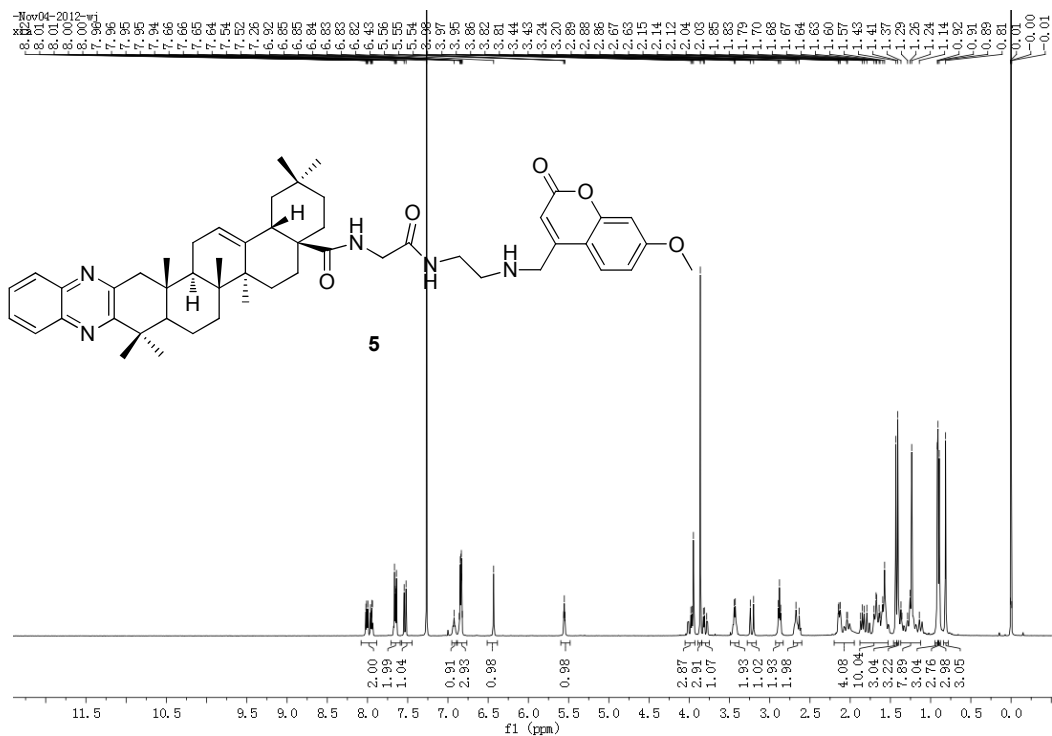
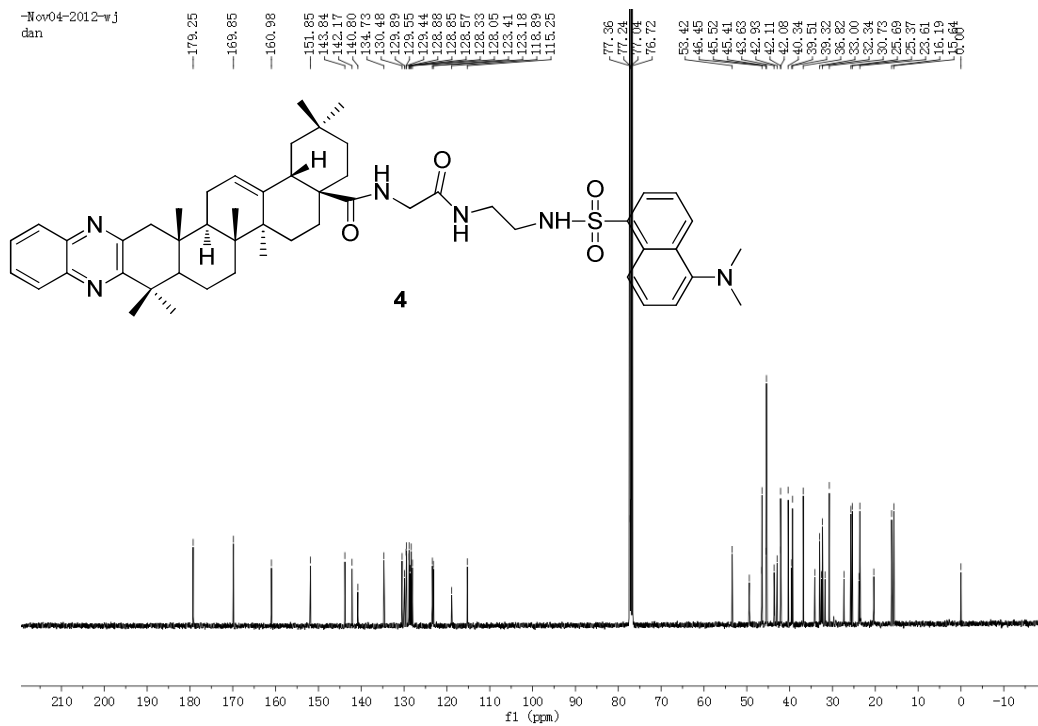
NMR  
A-007, cdcl3



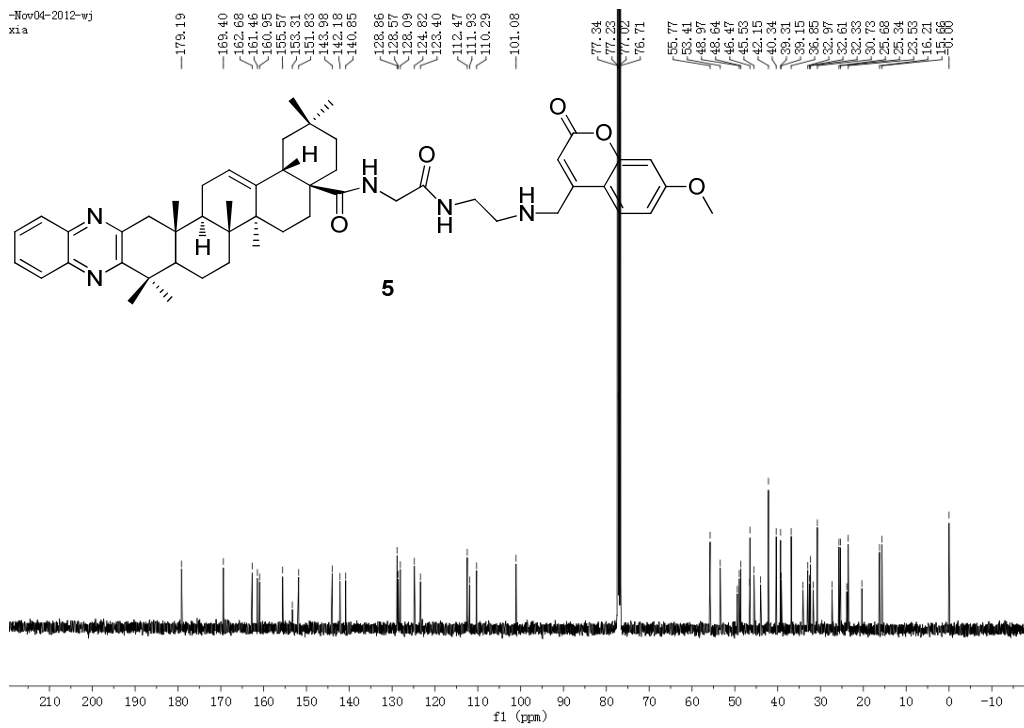
-Apr 16-2012-wj  
A-084



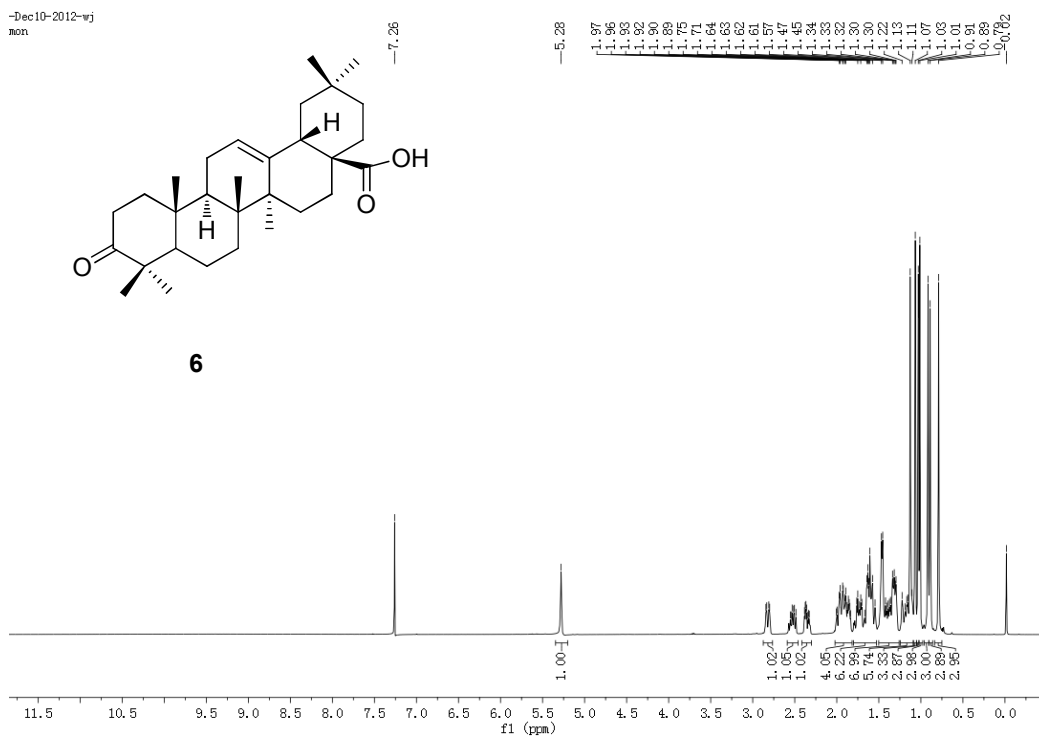


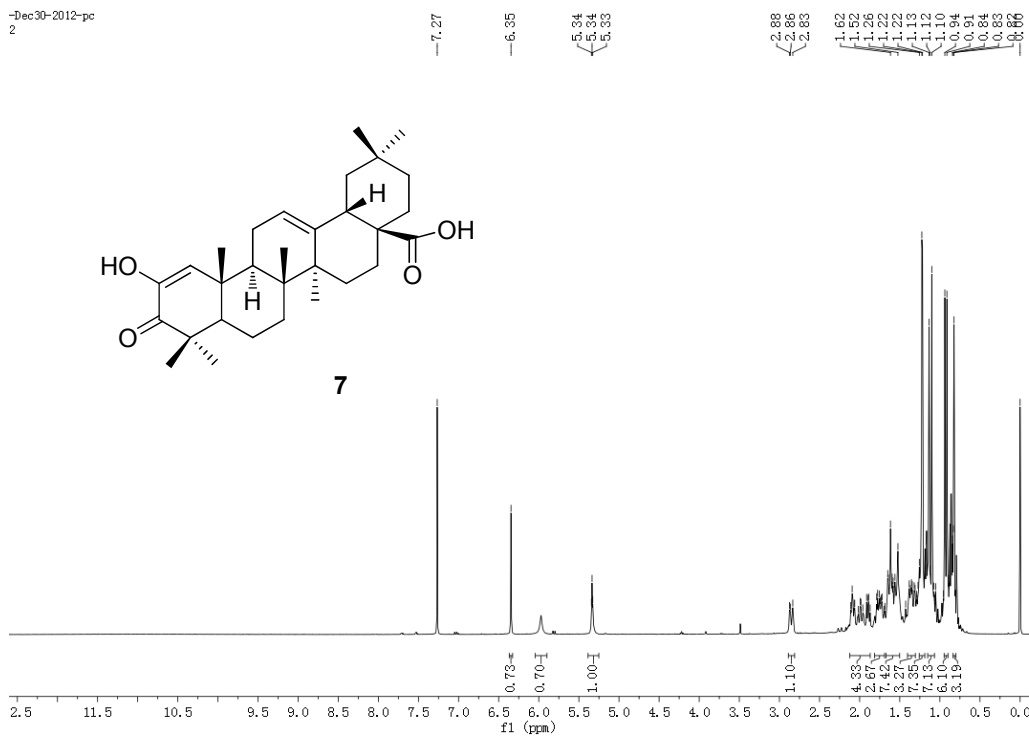
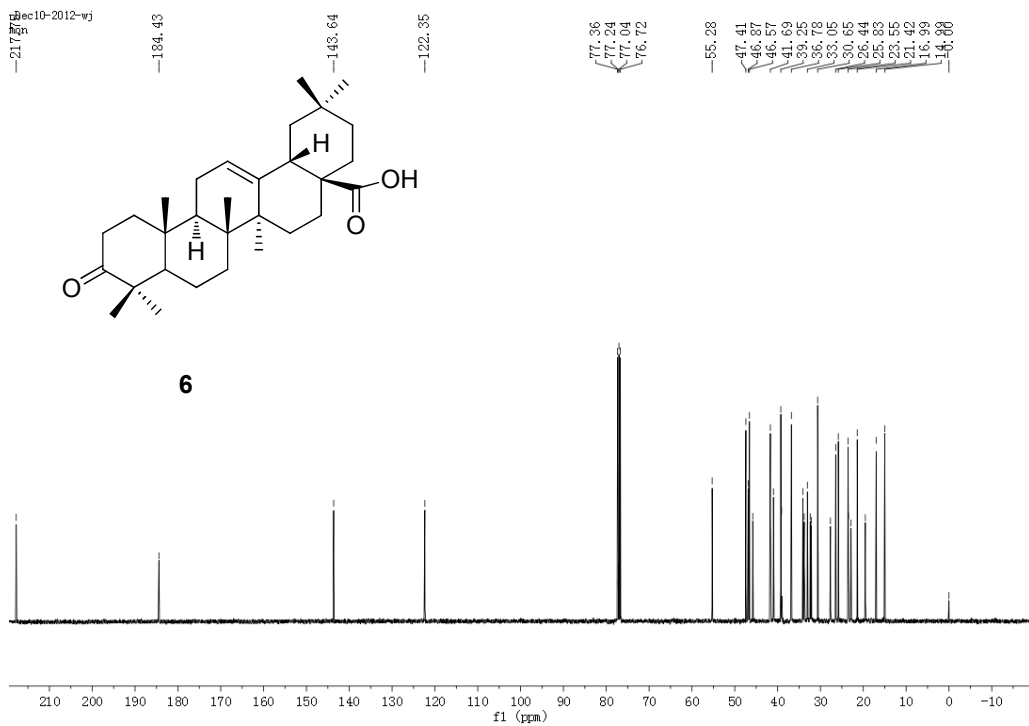


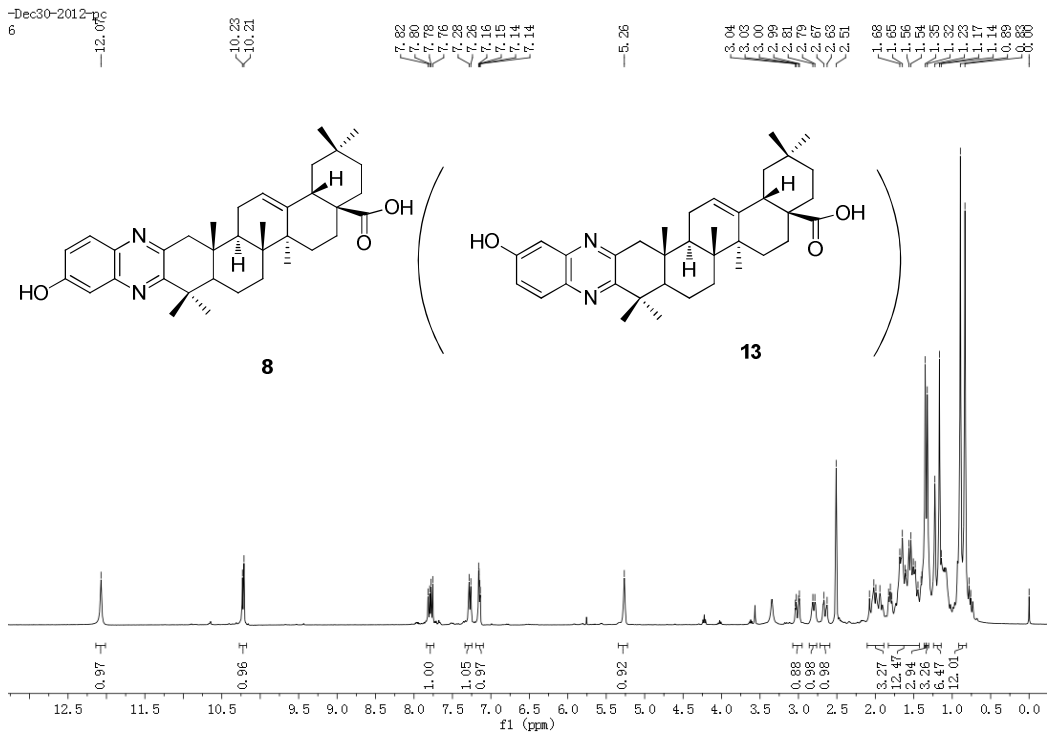
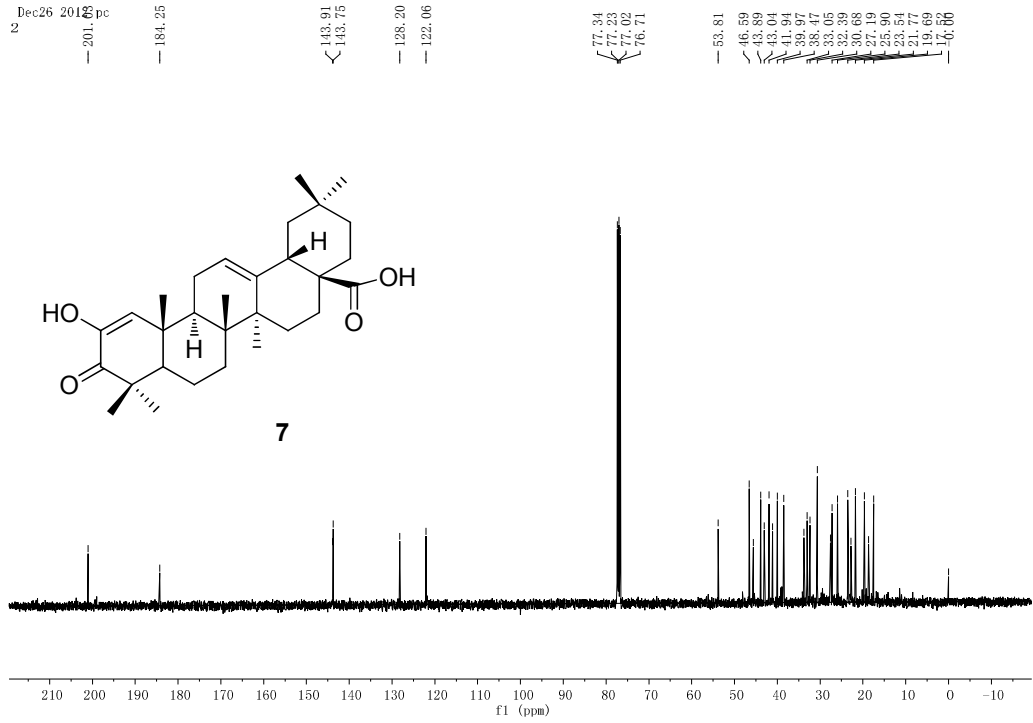
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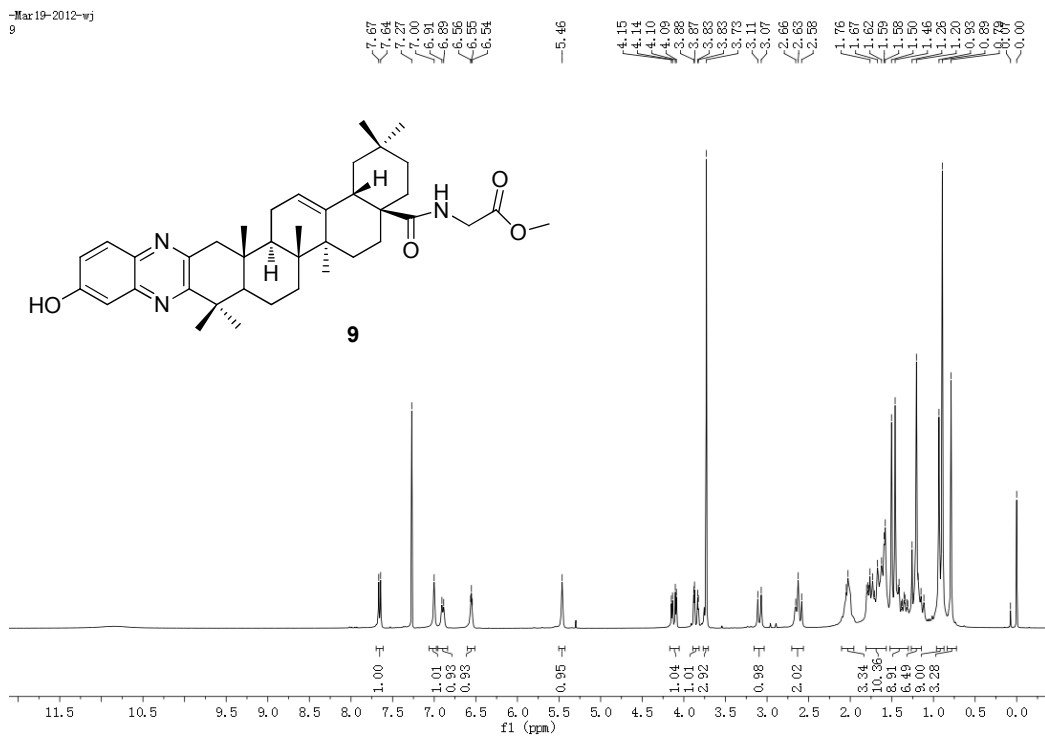
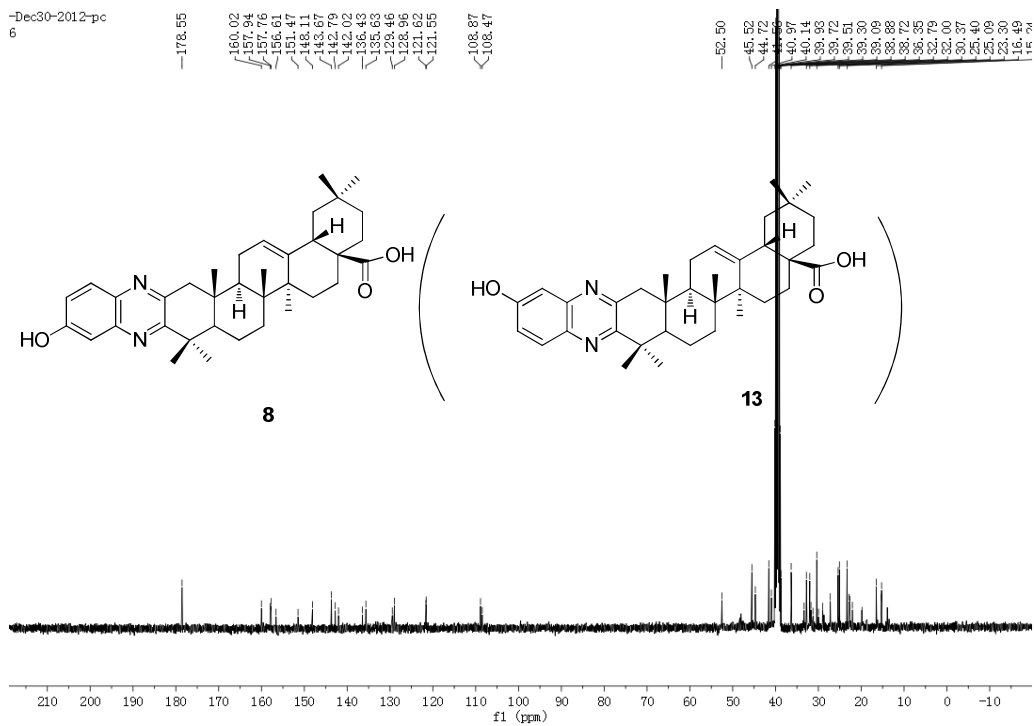


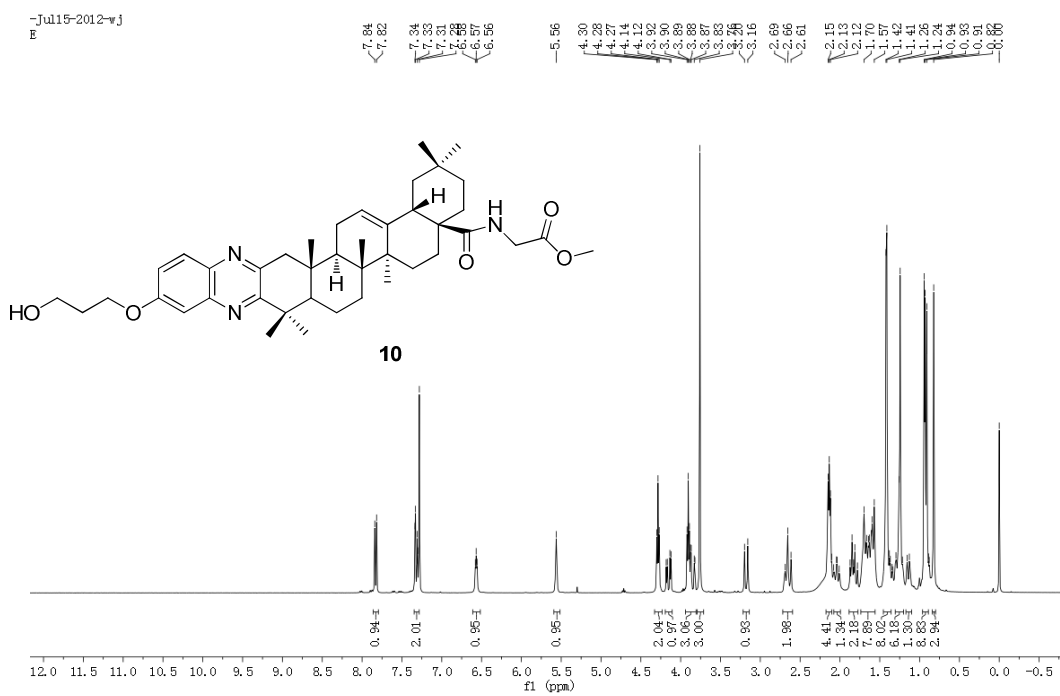
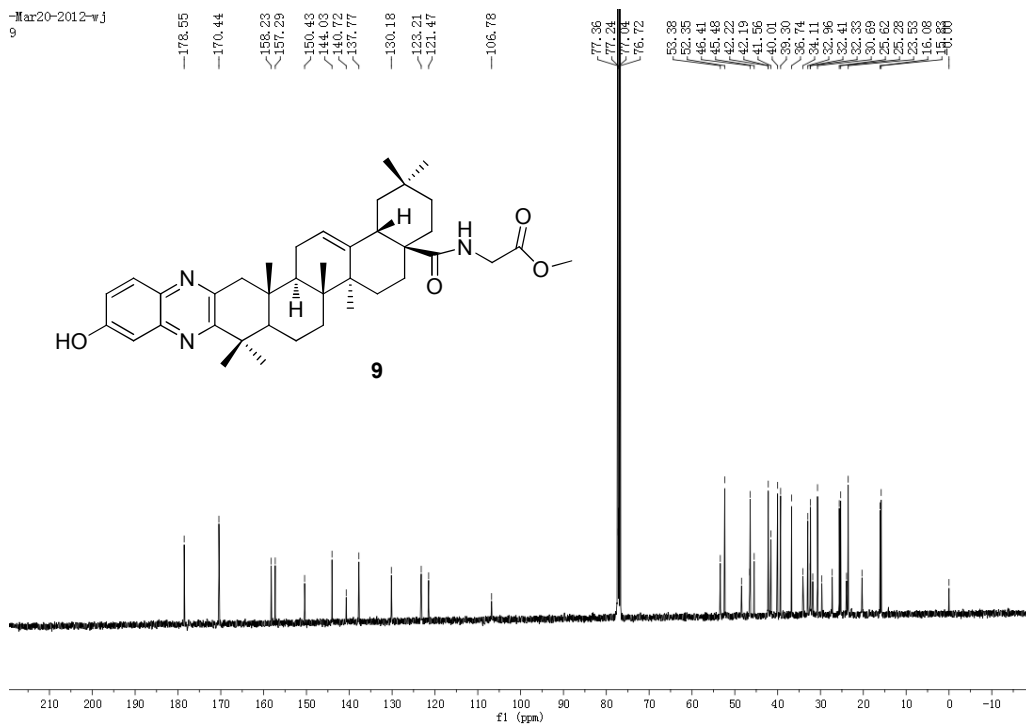
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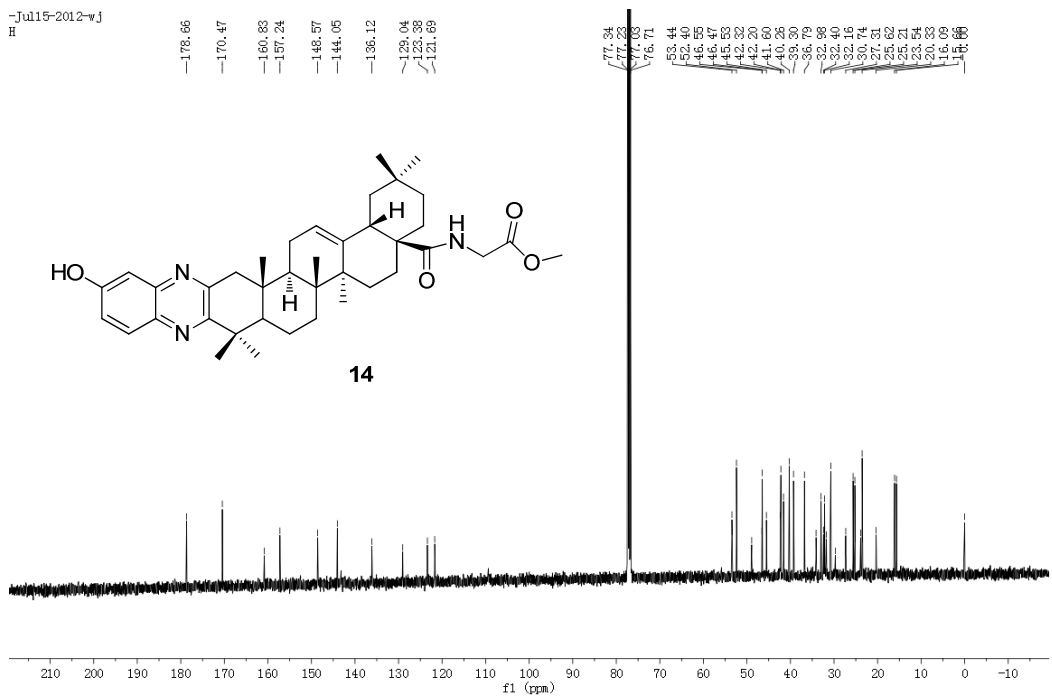
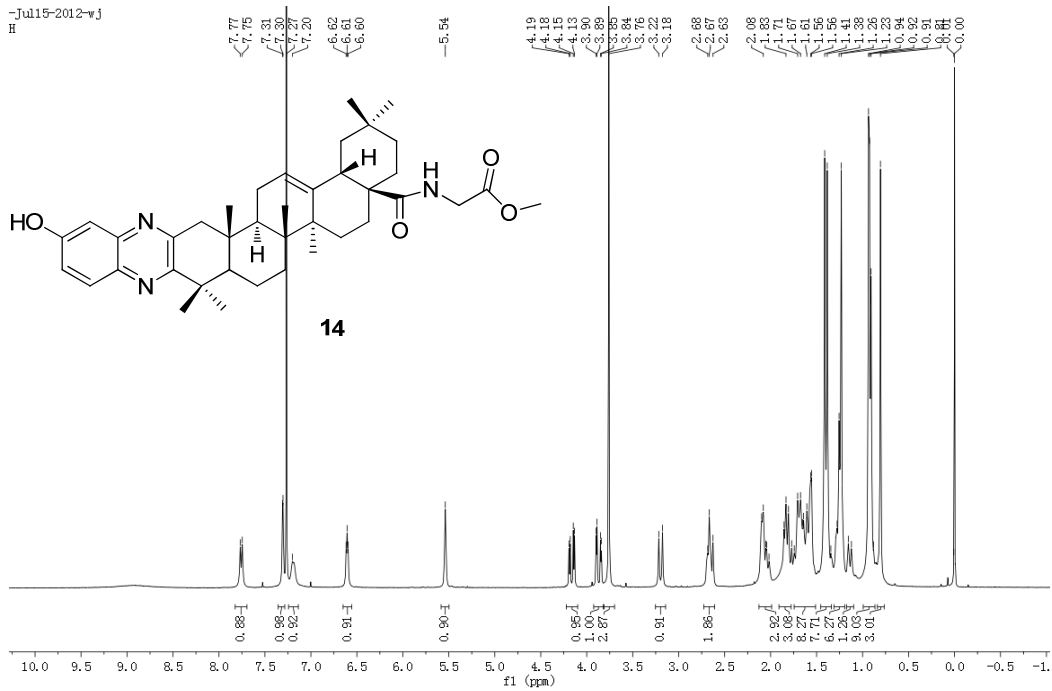




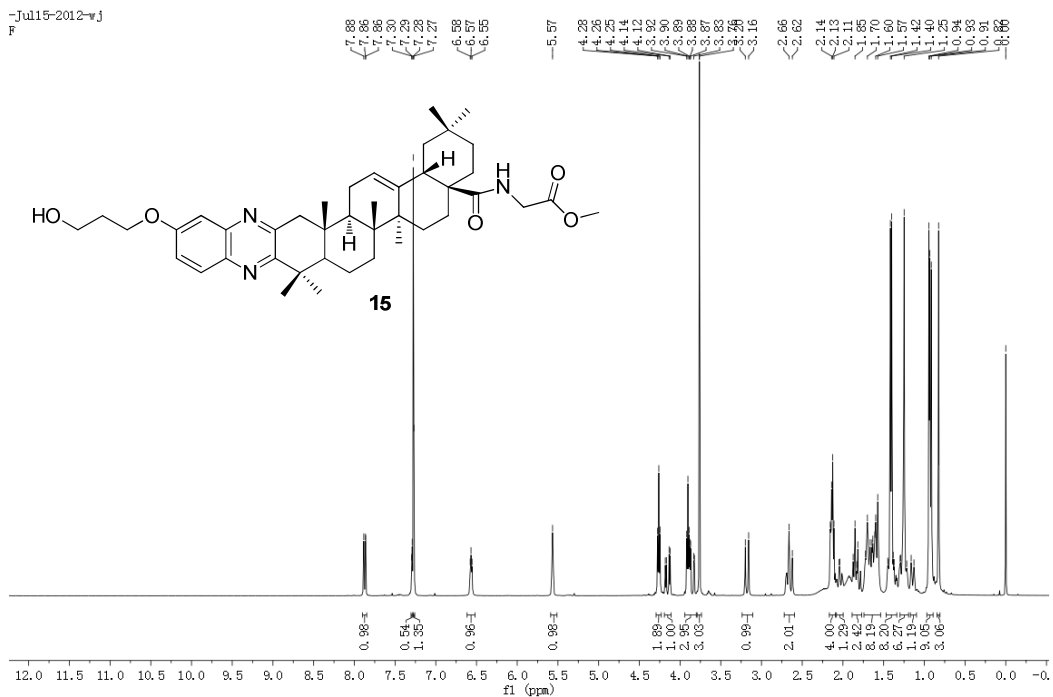




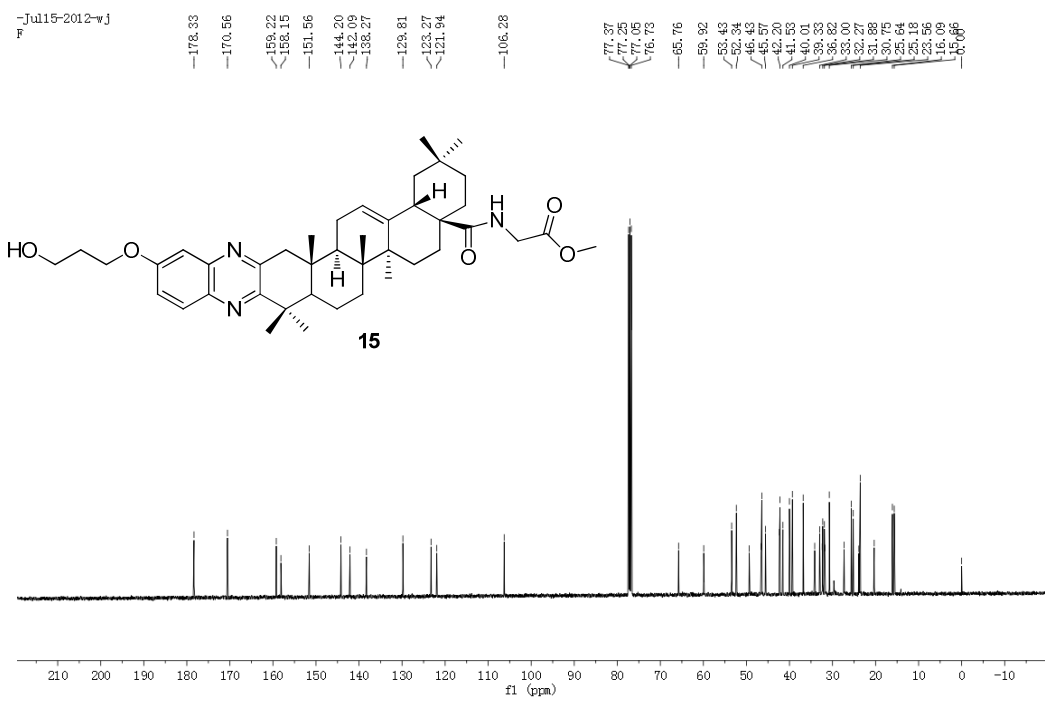




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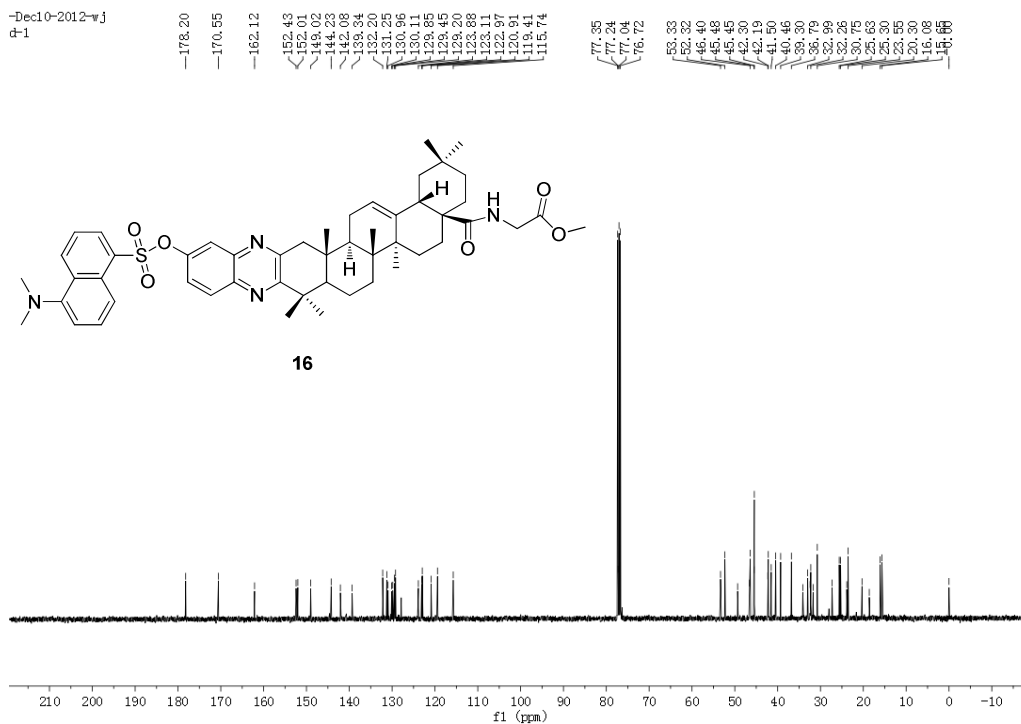


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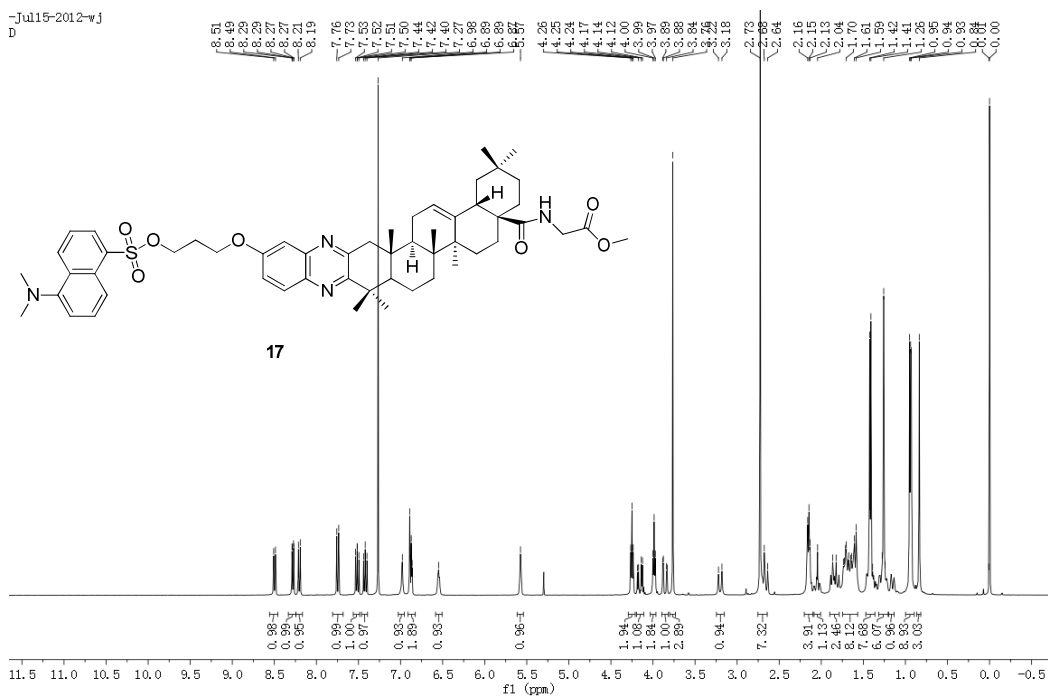




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-Jul15-2012-wj  
D



-Jul15-2012-wj  
D

