

Electronic Supporting Information for

Highly efficient synthesis of novel methyl 13²-methylene mesopyropheophorbide *a* by pH-dependent regionselective condensation with formaldehyde and its stereoselective Michael addition reaction

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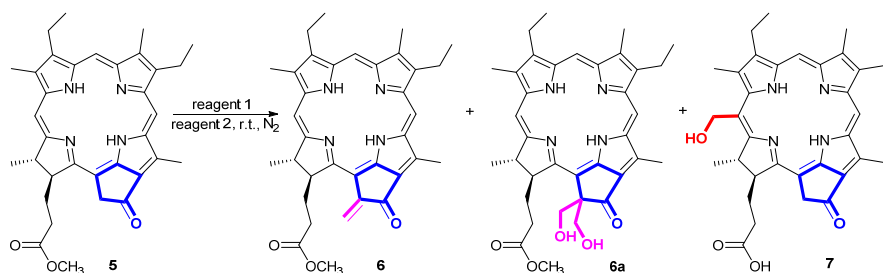
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1. Supporting Tables and Figures

Table S1. The reaction of methyl mesoporphorphorbide **5** with formaldehyde sources (40% formalin or paraformaldehyde) in various conditions.



entry	reagent 1 (eq.)	reagent 2 (eq.)	time (h)	yield (6) ^a	yield (6a) ^a	yield (7) ^a
1	40% formalin (1.2)	NaOMe (5.0)	6	15%	10%	/
2	40% formalin (5.0)	NaOMe (10.0)	6	47%	24%	/
3	40% formalin (5.0)	NaOMe (10.0)	12	45%	24%	/
4	40% formalin (10.0)	NaOMe (10.0)	6	49%	30%	/
5	40% formalin (10.0)	NaH (2.5)	6	30%	13%	/
6	40% formalin (10.0)	NaH (5.0)	6	48%	25%	/
7	40% formalin (10.0)	NaH (10.0)	6	48%	25%	/
8	paraformaldehyde ^b	NaOMe (5.0)	6	71%	12%	/
9	paraformaldehyde ^b	NaOMe (10.0)	6	85%	6%	/
10	paraformaldehyde ^b	NaOMe (20.0)	6	80%	5%	/
11	40% formalin (10.0)	TsOH (10.0)	96	/ ^c	/	/
12	40% formalin (10.0)	TFA	96	/	/	/
13	40% formalin (10.0)	conc. HCl	96	/	/	5%
14	40% formalin (10.0)	combined acid	72	/	/	65%
15	40% formalin (10.0)	combined acid	>96	/	/	64%

^a After standard treatment with 5% H₂SO₄/MeOH. ^b Equal-weight was used as methyl mesoporphorphorbide **5**. ^c No product was observed.

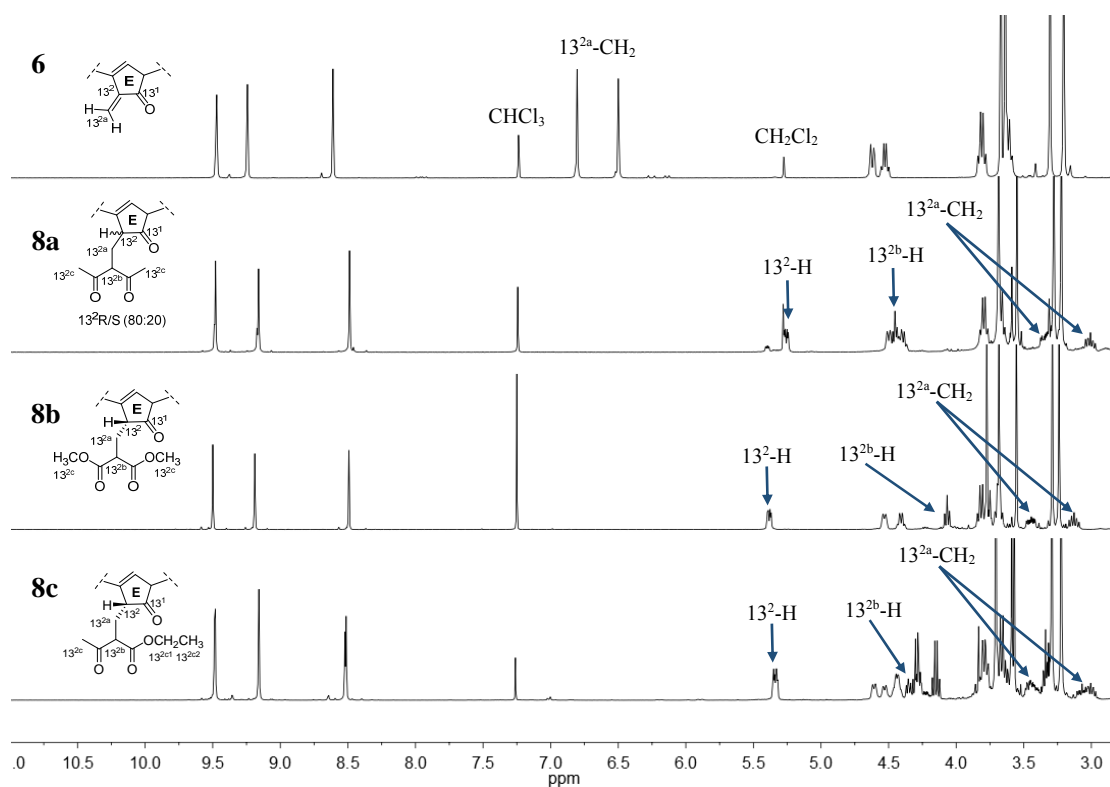


Fig. S1. The comparative ^1H NMR spectra (CDCl_3 , 400 MHz) in the region δ 2.8–11.0 ppm of methyl 13^2 -methylene mesopyropheophorbide **6** and its Michael reaction adducts **8a** - **8c**.

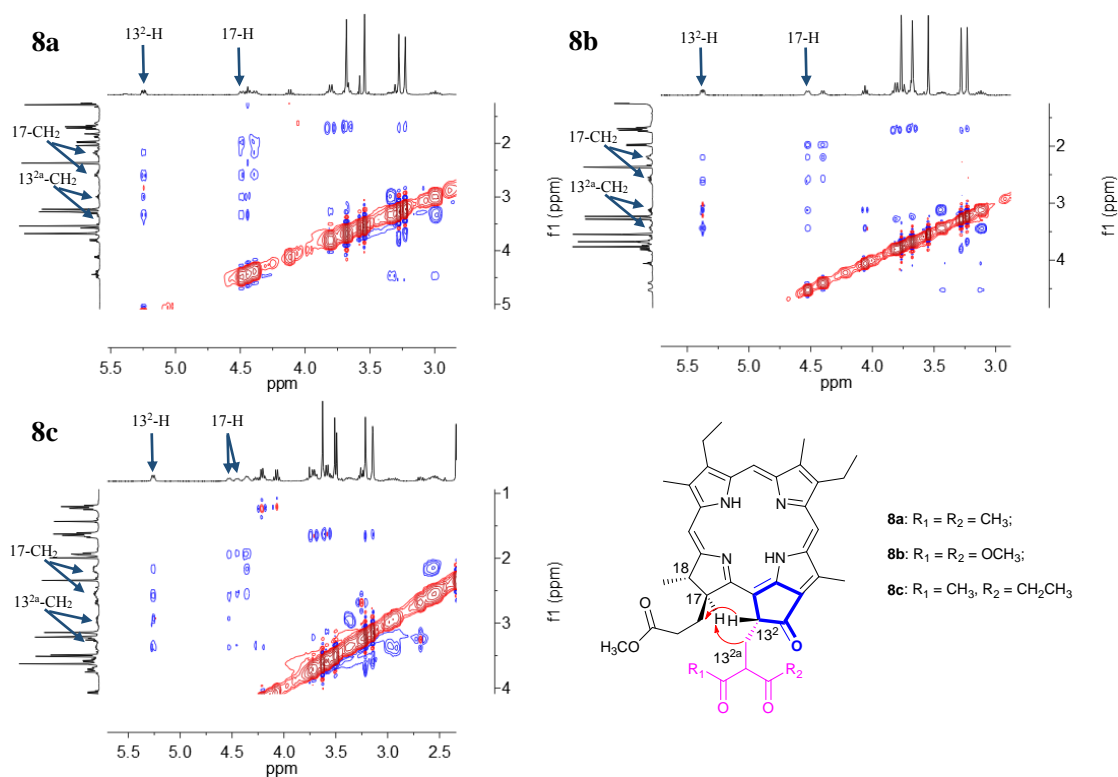


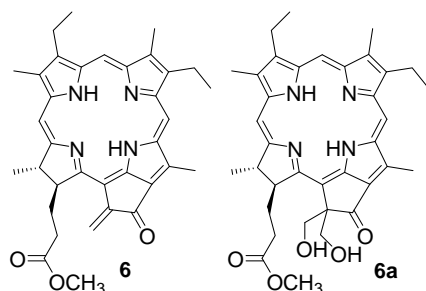
Fig. S2. NOE interactions (selected) observed in 2D/NOESY ^1H NMR spectra of Michael reaction adducts **8a** - **8c**.

2. General Information

All reactions were monitored by TLC using 0.20 mm silica gel plates with or without UV indicator (60F–254). Silica gel 60 (230–400 mesh, Merck) was used for flash column chromatography. ^1H and ^{13}C NMR spectra were measured at 400 and 101 MHz, respectively, on a Bruker Advance II spectrometer. COSY and NOESY techniques were used to assign the NMR peak. Chemical shifts (δ) are given in ppm relative to tetramethylsilane (TMS, 0 ppm) unless otherwise indicated. Electronic absorption spectra were measured on a AOE A560 UV–Vis spectrophotometer. The absorption maxima λ_{max} are given in nm and molar absorbance coefficient (ϵ) or relative intensity. MS(ESI) were obtained on a AB SCIEX API 4000 LC/MS System at Yantai University. Materials obtained from commercial suppliers were used without further purification. Methyl mesopyropheophorbide **5** was prepared according to the procedure described in the literature^[1].

3. Experimental Procedure and characterization of products

Synthesis of methyl 13²-methylene mesopyropheophorbide a 6 and methyl 13²-dihydromethyl mesopyropheophorbide a 6a

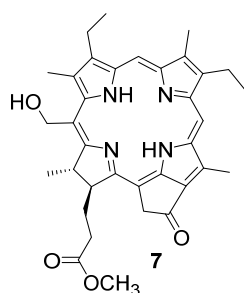


Methyl mesopyropheophorbide **5** (300 mg, 0.545 mmol) and paraformaldehyde (300 mg) was dissolved in THF (50 mL), and a solution of sodium methoxide in methanol (1mol/L, 5.45mL) was added under N_2 atmosphere, then the reaction mixture was stirring at room temperature under

N_2 atmosphere for 6 h, monitoring the progress spectroscopically. pH value of the reaction mixture was adjusted to 4-6 by adding diluted hydrochloric acid and extracted with dichloromethane. The combined organic layers were washed with water, dried over sodium sulfate, and evaporated in vacuum. The residue was dissolved in 100 mL methanol containing 5% H_2SO_4 and allowed to stir at room temperature under N_2 atmosphere overnight to recover the 17³-methyl ester structure. The mixture was diluted with water and extracted with dichloromethane. The combined organic layers were washed with water, dried over sodium sulfate, and evaporated in vacuum. The residue was separated on silica gel (eluent: dichloromethane-acetone, gradient 2-4% acetone) to give 261 mg (0.463 mmol, 85%) of the title compound **6** as dark green solid and 20 mg (0.033mmol, 6%) of **6a** as a byproduct. **6**: UV-VIS (CH_2Cl_2) λ_{max} 409 (relative intensity, 0.90), 427 (1.00), 520 (0.10), 556 (0.08), 609 (0.07), 664 (0.41) nm. ^1H NMR (400 MHz, CDCl_3) δ 9.46 (s, 1H, 10-H), 9.24 (s, 1H, 5-

H), 8.61 (s, 1H, 20-H), 6.81 (s, 1H, 13^{2a}-H), 6.50 (s, 1H, 13^{2a}-H), 4.62 (d, $J = 9.3$ Hz, 1H, 17-H), 4.53 (q, $J = 7.2$ Hz, 1H, 18-H), 3.81 (q, $J = 7.5$ Hz, 2H, 3¹-CH₂), 3.66, 3.64, 3.30, 3.20 (each s, each 3H, OCH₃ + CH₃), 3.64 – 3.56 (m, 2H, 8¹-CH₂), 2.72 – 2.60, 2.36 – 2.27, 2.07 – 1.96 (each m, total 4H, 17¹ + 17²-CH₂), 1.83 (d, $J = 7.3$ Hz, 3H, 18-CH₃), 1.71 (t, $J = 7.6$ Hz, 3H, 3²-CH₃), 1.65 (t, $J = 7.5$ Hz, 3H, 8²-CH₃), 0.08, -1.96 (each brs, each 1H, 2 × NH). ¹³C NMR (101 MHz, CDCl₃) δ 187.13, 173.53, 171.43, 161.88, 154.78, 150.44, 149.65, 149.10, 145.00, 142.01, 141.90, 136.91, 136.51, 135.97, 131.15, 128.63, 128.46, 112.82, 106.64, 104.52, 97.04, 93.31, 51.69, 51.43, 49.79, 31.24, 29.31, 23.54, 19.47, 19.42, 17.50, 17.01, 12.20, 11.29, 10.99. MS (ESI): calcd for C₃₅H₃₈N₄O₃ 562.3; found m/z 563.7 (MH⁺). Anal. calcd for C₃₅H₃₈N₄O₃ C 74.71, H 6.81, N 9.96. Found C 74.75, H 6.84, N 10.01. **6a**: UV-VIS (CH₂Cl₂) λ_{max} 408 (relative intensity, 1.0), 504 (0.09), 536 (0.09), 603 (0.08), 658 (0.35) nm. ¹H NMR (400 MHz, CDCl₃) δ 9.35 (s, 1H, 10-H), 9.06 (s, 1H, 5-H), 8.41 (s, 1H, 20-H), 5.06 (d, $J = 11.5$ Hz, 1H, 13^{2a}-CH₂), 4.84 (d, $J = 11.5$ Hz, 1H, 13^{2a}-CH₂), 4.73 (d, $J = 11.4$ Hz, 1H, 13^{2a}-CH₂), 4.55 (d, $J = 11.3$ Hz, 1H, 13^{2a}-CH₂), 4.42 – 4.32 (m, 2H, 17 + 18-H), 3.76 (q, $J = 7.5$ Hz, 2H, 3¹-CH₂), 3.64 – 3.53 (m, 2H, 8¹-CH₂), 3.58, 3.26, 3.18, 2.85 (each s, each 3H, OCH₃ + CH₃), 2.51 – 2.35, 2.28 – 2.20, 2.13 – 2.01 (each m, total 4H, 17¹ + 17²-CH₂), 1.72 (t, $J = 7.6$ Hz, 3H, 3²-CH₃), 1.67 – 1.61 (m, 6H, 8² + 18-CH₃), 0.03, -1.19 (each brs, each 1H, 2 × NH). MS (ESI): calcd for C₃₆H₄₂N₄O₅ 610.3; found m/z 611.7 (MH⁺). Anal. calcd for C₃₆H₄₂N₄O₅ C 70.80, H 6.93, N 9.17. Found C 70.83, H 6.91, N 9.20.

Synthesis of methyl 20-hydroxymethyl mesopyropheorbide a 7



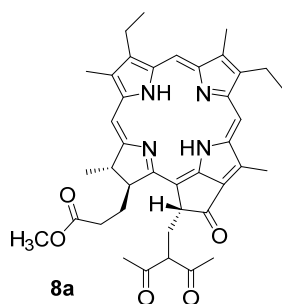
Methyl mesopyropheorbide **5** (200 mg, 0.363 mmol) and aqueous formalin (10 mL, 40%) was dissolved in a mixture of glacial acetic acid (10 mL), phosphoric acid (8 mL) and concentrated hydrochloric acid and allowed to stir at room temperature for 3 days. The reaction mixture was diluted with water and extracted with dichloromethane. The combined

organic layers were washed with water, dried over sodium sulfate, and evaporated in vacuum. The residue was dissolved in 100 mL methanol containing 5% H₂SO₄ and allowed to stir at room temperature under N₂ atmosphere overnight to recover the 17³-methyl ester structure. The mixture was diluted with water and extracted with dichloromethane. The combined organic layers were washed with water, dried over sodium sulfate, and evaporated in vacuum. The residue was separated on silica gel (eluent: dichloromethane-methanol, gradient 2-4% methanol) to give the unreacted

starting material **5** (52 mg, 0.094 mmol, 26%) as the fast-moving band and the title compound **7** as dark green solid (137 mg, 0.236 mmol, 65%). UV-VIS (CH₂Cl₂) λ_{\max} 412 (relative intensity, 1.00), 511 (0.11), 544 (0.12), 609 (0.08), 666 (0.35) nm. ¹H NMR (400 MHz, CDCl₃) δ 9.43 (s, 1H, 10-H), 9.32 (s, 1H, 5-H), 6.36 (d, J = 12.8 Hz, 1H, 20¹-H), 6.01 (d, J = 12.8 Hz, 1H, 20¹-H), 5.19 (d, J = 19.8 Hz, 1H, 13²-H), 5.13 (d, J = 19.8 Hz, 1H, 13²-H), 4.80 (q, J = 7.1 Hz, 1H, 18-H), 4.20 (dd, J = 8.2, 3.3 Hz, 1H, 17-H), 3.90 – 3.82 (m, 2H, 3¹-CH₂), 3.63, 3.54, 3.45, 3.27 (each s, each 3H, OCH₃ + CH₃), 3.66 (q, J = 7.6 Hz, 2H, 8¹-CH₂), 2.52 – 2.32, 2.23 – 2.11 (each m, total 4H, 17¹ + 17²-CH₂), 1.71 (t, J = 7.6 Hz, 3H, 3²-CH₃), 1.68 (t, J = 7.6 Hz, 3H, 8²-CH₃), 1.50 (d, J = 7.0 Hz, 3H, 18-CH₃), 0.70, -1.34 (each brs, each 1H, 2 × NH). MS (ESI): calcd for C₃₅H₄₀N₄O₄ 580.3; found m/z 581.3 (MH⁺). Anal. calcd for C₃₅H₄₀N₄O₄ C 72.39, H 6.94, N 9.65. Found C 72.28, H 6.84, N 9.69.

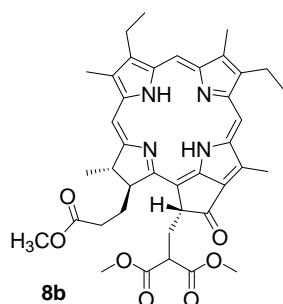
General procedure for the Michael reaction of compound 6 and the synthesis of compounds 8a-8c:

Methyl 13²-methylene mesopyropheophorbide **6** (200 mg, 0.355 mmol) and corresponding reactive methylene compound (1 mL, acetylacetone for compound **8a**; dimethyl malonate for compound **8b** and ethyl acetoacetate for compound **8c**) was dissolved in THF (20 mL), and a solution of sodium methoxide in methanol (1mol/L, 3.55 mL) was added under N₂ atmosphere, then the reaction mixture was stirring at room temperature under N₂ atmosphere for 3 h, monitoring the progress spectroscopically. pH value of the reaction mixture was adjusted to 4-6 by adding diluted hydrochloric acid and extracted with dichloromethane. The combined organic layers were washed with water, dried over sodium sulfate, and evaporated in vacuum. The residue was separated on silica gel (eluent: dichloromethane-acetone, gradient 0.5-2.0% acetone) to give the desired compound **8a-8c** as dark green solids (yield: 90-94%).

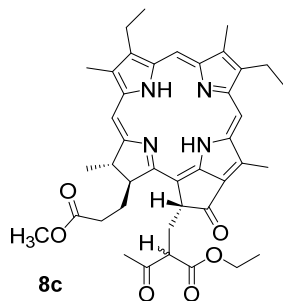


8a: Yield: 94%. UV-VIS (CH₂Cl₂) λ_{\max} 410 (relative intensity, 1.00), 504 (0.10), 535 (0.09), 603 (0.08), 656 (0.35) nm. ¹H NMR (400 MHz, CDCl₃) δ 9.47(9.48) (s, 1H, 10-H), 9.15(9.17) (s, 1H, 5-H), 8.48 (s, 1H, 20-H), 5.25(5.38) (dd, J = 8.6, 3.4 Hz, 1H, 13²-H), 4.49 (d, J = 8.8 Hz, 1H, 17-H), 4.45 (t, J = 6.3 Hz, 1H, 13^{2b}-H), 4.39 (q, J = 7.9 Hz, 1H, 18-H), 3.79 (q, J = 7.5 Hz, 2H, 3¹-CH₂), 3.66 (q, J = 7.6 Hz, 2H, 8¹-CH₂), 3.68, 3.54(3.58), 3.27, 3.21 (each s, each 3H, OCH₃ + CH₃), 3.37 – 3.31 (m, 1H, 13^{2a}-H), 3.04 – 2.96 (m, 1H, 13^{2a}-H), 2.65 – 2.51, 2.26 – 2.11 (each m, total 4H, 17¹ + 17²-CH₂), 2.37 (s, 3H, COCH₃), 1.98 (d, J = 7.3 Hz, 3H,

18-CH₃), 1.71 (t, *J* = 7.7 Hz, 3H, 3²-CH₃), 1.68 (t, *J* = 7.7 Hz, 3H, 8²-CH₃), 1.28 (s, 3H, COCH₃), 0.53, -1.60(-1.64) (each brs, each 1H, 2 × NH). ¹³C NMR (101 MHz, CDCl₃) δ 204.0, 203.6, 198.8, 180.0, 173.6, 172.4(172.3), 161.6, 155.9, 150.5, 149.0, 145.3, 142.6, 141.8, 137.5(137.3), 135.9, 131.6(131.5), 128.7, 127.7, 108.7, 104.3(104.2), 96.0, 92.7(92.5), 64.5, 55.6, 51.6(51.7), 50.6(50.7), 50.5(50.1), 31.0, 30.9, 30.1(30.0), 30.0(29.9), 29.6(29.7), 23.5(23.3), 19.5, 19.4, 17.5, 16.9, 12.1, 11.3, 11.0. MS (ESI): calcd for C₄₀H₄₆N₄O₅ 662.4; found *m/z* 663.9 (MH⁺). Anal. calcd for C₄₀H₄₆N₄O₅ C 72.48, H 7.00, N 8.45. Found C 72.46, H 6.98, N 8.49.



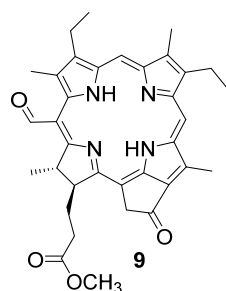
8b: Yield: 90%. UV-VIS (CH₂Cl₂) λ_{max} 410 (relative intensity, 1.00), 504 (0.10), 535 (0.08), 603 (0.07), 657 (0.36) nm. ¹H NMR (400 MHz, CDCl₃) δ 9.48 (s, 1H, 10-H), 9.17 (s, 1H, 5-H), 8.49 (s, 1H, 20-H), 5.38 (dd, *J* = 8.1, 3.2 Hz, 1H, 13²-H), 4.52 (d, *J* = 8.5 Hz, 1H, 17-H), 4.40 (q, *J* = 7.4 Hz, 1H, 18-H), 4.06 (t, *J* = 7.1 Hz, 1H, 13^{2b}-H), 3.79 (q, *J* = 7.4 Hz, 2H, 3¹-CH₂), 3.77, 3.67, 3.55, 3.28, 3.22, 2.36 (each s, each 3H, OCH₃ + CH₃), 3.67 (q, *J* = 7.3 Hz, 2H, 8¹-CH₂), 3.47 – 3.41 (m, 1H, 13^{2a}-H), 3.19 – 3.08 (m, 1H, 13^{2a}-H), 2.66 – 2.52, 2.26 – 2.13 (each m, total 4H, 17¹ + 17²-CH₂), 1.98 (d, *J* = 7.3 Hz, 3H, 18-CH₃), 1.72 (t, *J* = 7.7 Hz, 3H, 3²-CH₃), 1.69 (t, *J* = 7.7 Hz, 3H, 8²-CH₃), 0.46, -1.64 (each brs, each 1H, 2 × NH). ¹³C NMR (101 MHz, CDCl₃) δ 198.36, 173.60, 172.13, 170.02, 169.99, 161.46, 155.60, 150.45, 149.10, 145.22, 142.41, 141.77, 137.48, 137.26, 135.77, 131.48, 128.91, 127.86, 108.24, 104.34, 96.01, 92.51, 55.34, 52.67, 51.64, 51.63, 51.61, 50.64, 50.39, 48.64, 31.25, 30.97, 30.06, 23.28, 19.49, 19.37, 17.47, 16.91, 12.11, 11.28, 10.96. MS (ESI): calcd for C₄₀H₄₆N₄O₇ 694.3; found *m/z* 695.9 (MH⁺). Anal. calcd for C₄₀H₄₆N₄O₇ C 69.15, H 6.67, N 8.06. Found C 69.21, H 6.65, N 8.09.



8c: Yield: 93%. UV-VIS (CH₂Cl₂) λ_{max} 410 (relative intensity, 1.00), 504 (0.09), 534 (0.09), 601 (0.08), 657 (0.40) nm. ¹H NMR (400 MHz, CDCl₃) δ 9.50(9.49) (s, 1H, 10-H), 9.18 (s, 1H, 5-H), 8.50(8.49) (s, 1H, 20-H), 5.30 (d, *J* = 7.8 Hz, 1H, 13²-H), 4.57(4.49) (d, *J* = 8.3 Hz, 1H, 17-H), 4.40 (q, *J* = 7.2 Hz, 1H, 18-H), 4.33 – 4.19 (m, 3H, 13^{2b}-H+ 13^{2b}-CO₂CH₂CH₃), 3.81 (q, *J* = 7.6 Hz, 2H, 3¹-CH₂), 3.68 (q, *J* = 7.3 Hz, 2H, 8¹-CH₂), 3.69, 3.56(3.54), 3.29, 3.24, 2.38(1.47) (each s, each 3H, OCH₃ + CH₃), 3.48 – 3.34 (m, 1H, 13^{2a}-H), 3.06 – 2.92 (m, 1H, 13^{2a}-H), 2.78 – 2.51, 2.25 – 2.16 (each m, total 4H, 17¹ + 17²-CH₂), 1.97(1.96) (d, *J* = 7.0 Hz, 3H, 18-CH₃), 1.77 – 1.67 (m, 6H, 3² + 8²-CH₃), 1.29 (t, *J* = 7.1 Hz, 3H, 13^{2b}-CO₂CH₂CH₃), 0.49, -

1.61(-1.63) (each brs, each 1H, 2 × NH). ¹³C NMR (101 MHz, CDCl₃) δ 203.3(203.1), 198.9, 173.6, 172.2, 169.8, 169.5, 161.5(161.4), 155.7(155.6), 150.5, 149.0(148.9), 145.2(145.1), 142.4, 141.8(141.7), 137.4, 137.3, 135.8(135.7), 131.6(131.5), 128.8(128.7), 127.7, 104.24, 95.95, 92.66(92.54), 61.59, 60.64, 56.29(56.04), 55.39(55.32), 51.62(51.58), 50.65(50.44), 31.03(30.99), 30.39(30.15), 30.04(29.54), 23.44(23.27), 19.48, 19.36, 17.47, 16.91, 14.11, 12.89, 12.11, 11.28, 10.96. MS (ESI): calcd for C₄₁H₄₈N₄O₆ 692.4; found *m/z* 693.6 (MH⁺). Anal. calcd for C₄₁H₄₈N₄O₆ C 71.08, H 6.98, N 8.09. Found C 71.12, H 7.00, N 8.05.

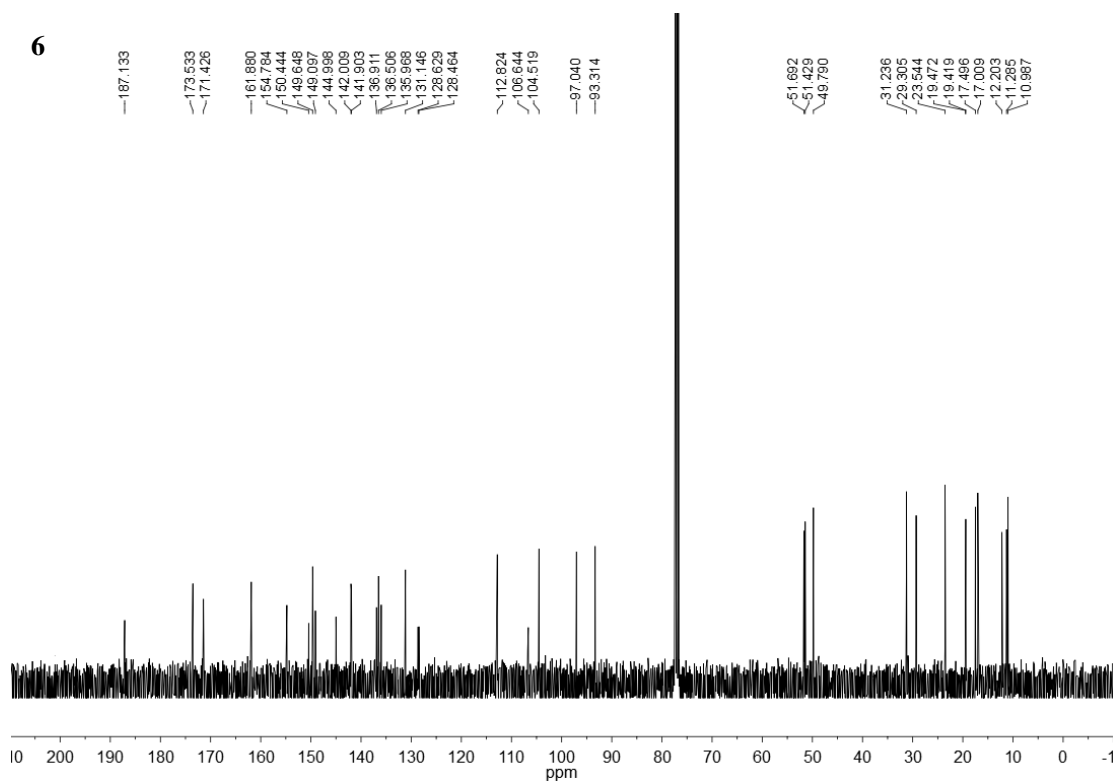
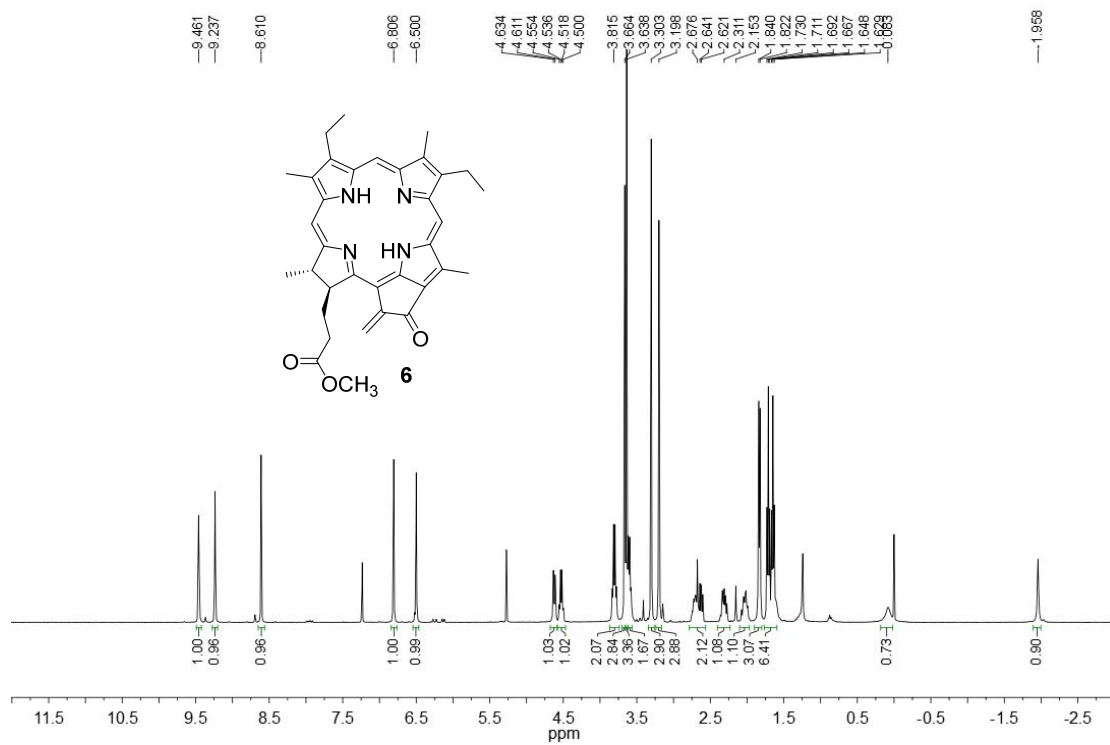
Synthesis of 20-formyl meso pyropheophorbide **9**



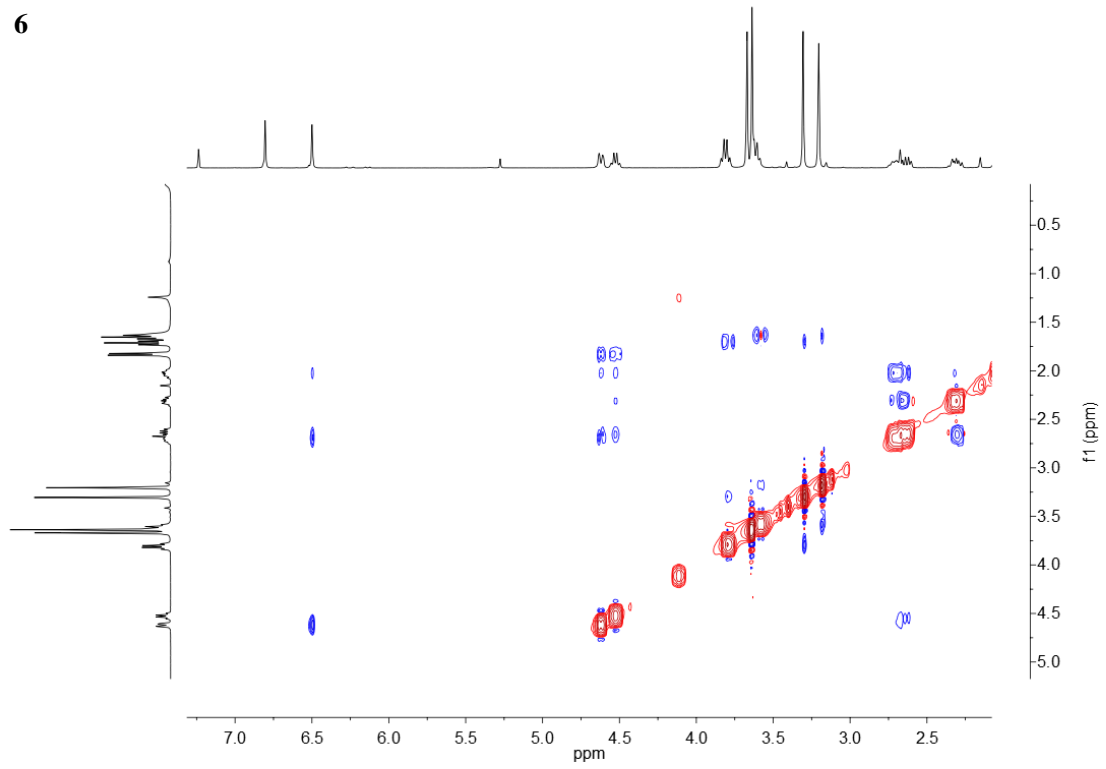
Compound **7** (100 mg, 0.172 mmol) was dissolved in 10 mL dry dichloromethane containing NMO (26 mg, 0.258 mmol). After stirring the mixture for 10 min, TPAP (10 mg, 0.028 mmol) was added and allowed to stir at room temperature for 1 h. The reaction mixture was diluted with dichloromethane and then washed with sodium sulphite solution and brine.

The organic layer was dried over sodium sulfate, and evaporated in vacuum. The residue was separated on silica gel (eluent: dichloromethane-acetone, gradient 0.5-2.0% acetone) to give the title compound **9** as dark green solid (68 mg, 0.117 mmol, 68%). UV-VIS (CH₂Cl₂) λ_{max} 412 (relative intensity, 1.00), 513 (0.07), 549 (0.13), 631 (0.08), 689 (0.29) nm. ¹H NMR (400 MHz, CDCl₃) δ 11.80 (s, 1H, 20-CHO), 9.27 (s, 1H, 10-H), 9.21 (s, 1H, 5-H), 5.18 (q, *J* = 7.0 Hz, 1H, 18-H), 5.05 (d, *J* = 20.0 Hz, 1H, 13²-H), 4.98 (d, *J* = 20.0 Hz, 1H, 13²-H), 4.03 (dd, *J* = 9.1, 3.1 Hz, 1H, 17-H), 3.74 (q, *J* = 7.6 Hz, 2H, 3¹-CH₂), 3.55 (q, *J* = 7.6 Hz, 2H, 8¹-CH₂), 3.61, 3.52, 3.24, 3.14 (each s, each 3H, OCH₃ + CH₃), 2.65 – 2.57, 2.50 – 2.42, 2.34 – 2.27, 2.17 – 2.06 (each m, total 4H, 17¹ + 17²-CH₂), 1.66 (t, *J* = 7.6 Hz, 3H, 3²-CH₃), 1.63 (t, *J* = 7.6 Hz, 3H, 8²-CH₃), 1.42 (d, *J* = 7.0 Hz, 3H, 18-CH₃), 0.88, 0.04 (each brs, each 1H, 2 × NH). MS (ESI): calcd for C₃₅H₃₈N₄O₄ 578.3; found *m/z* 579.3 (MH⁺). Anal. calcd for C₃₅H₃₈N₄O₄ C 72.64, H 6.62, N 9.68. Found C 72.68, H 6.67, N 9.70.

4. NMR (1D, 2D, ¹H, ¹³C) and MS (ESI) spectrum of products



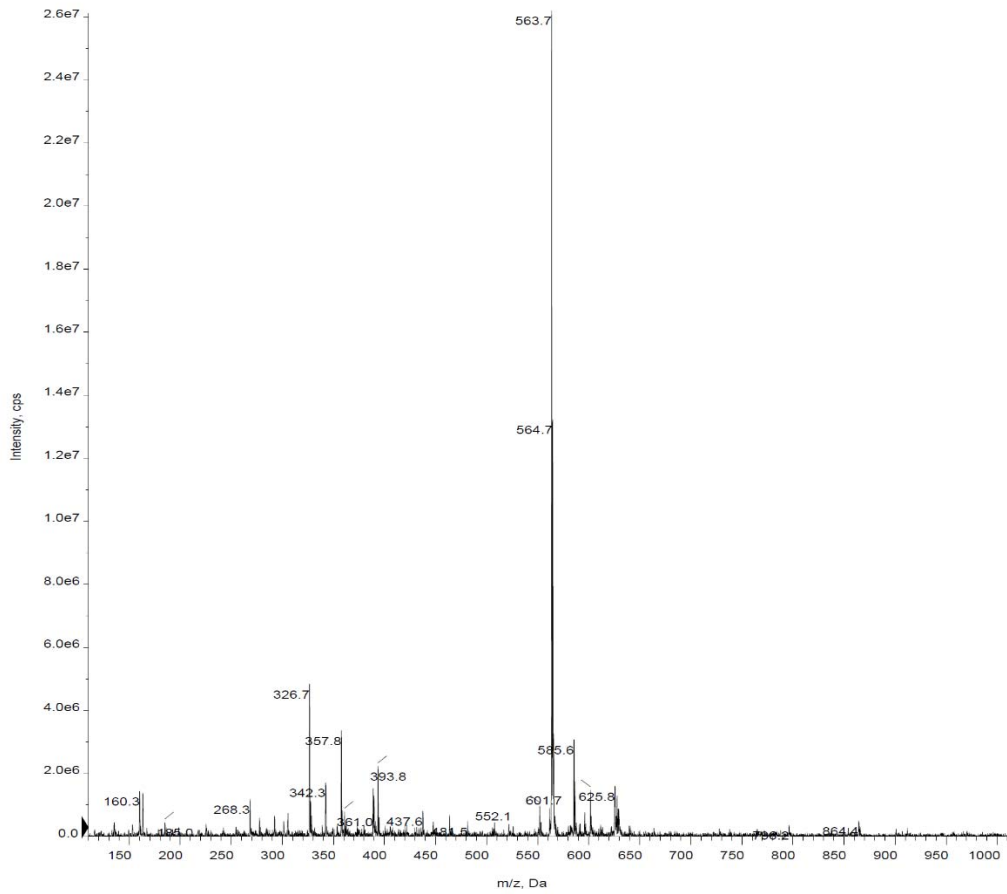
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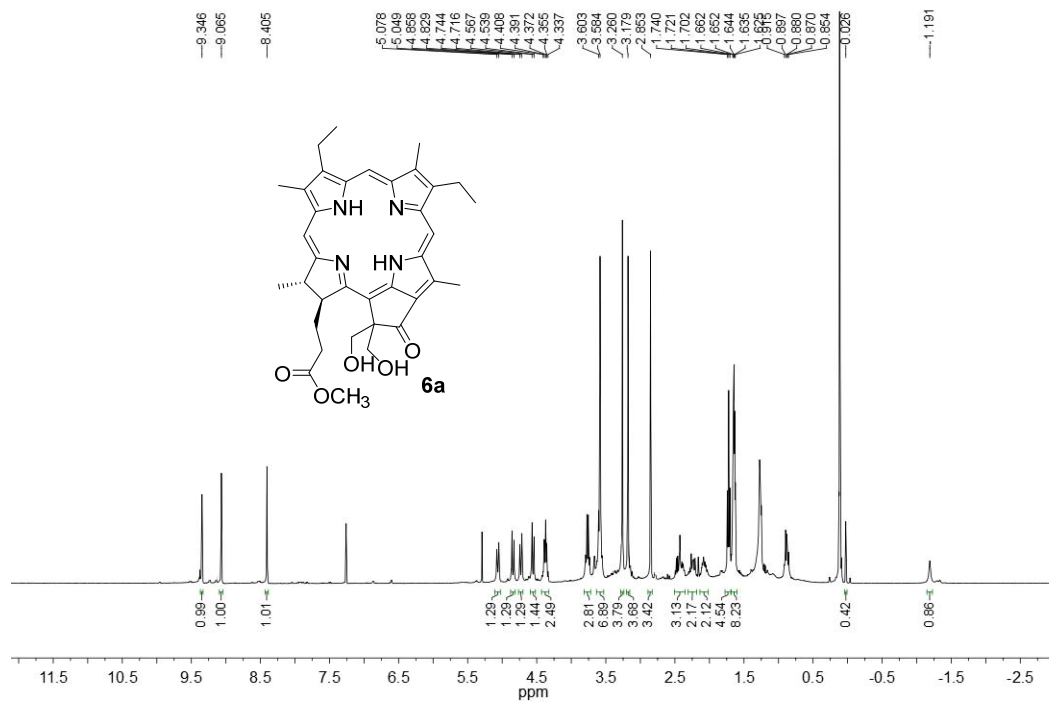


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+Q1: 238 MCA scans from Sample 3 (TuneSampleID002) of lijiazhu10.10 .wiff (Turbo Spray)

Max. 2.6e7 cps.

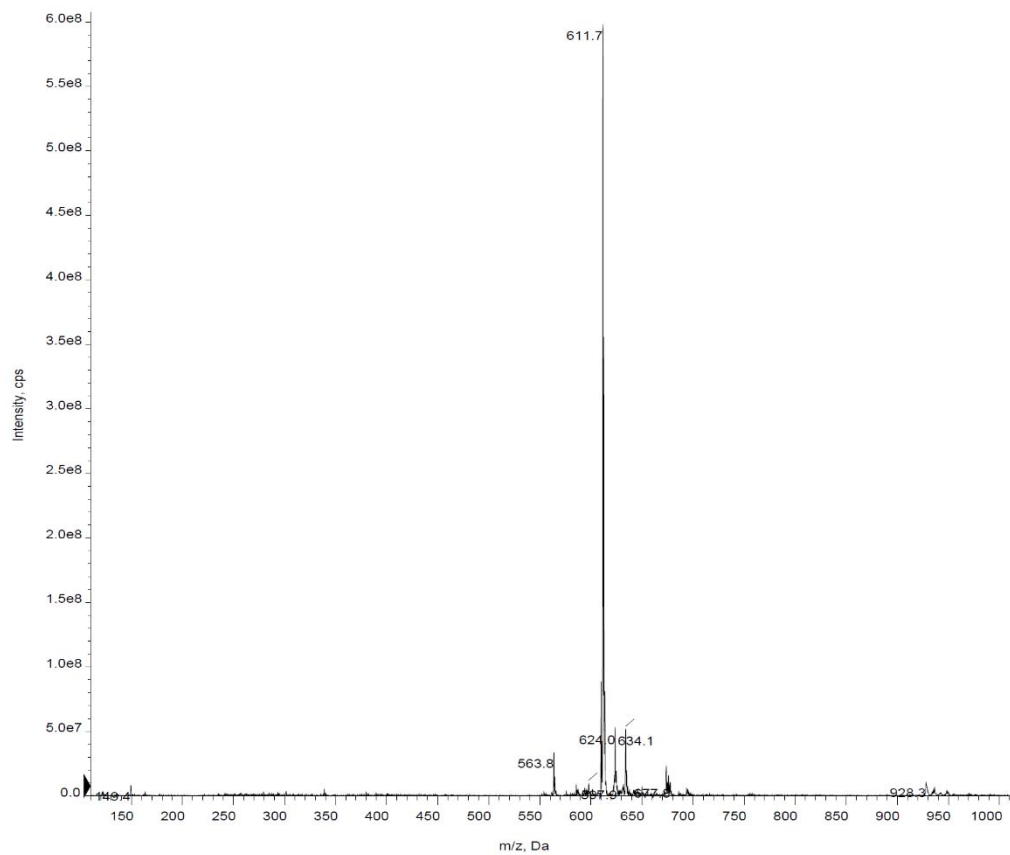


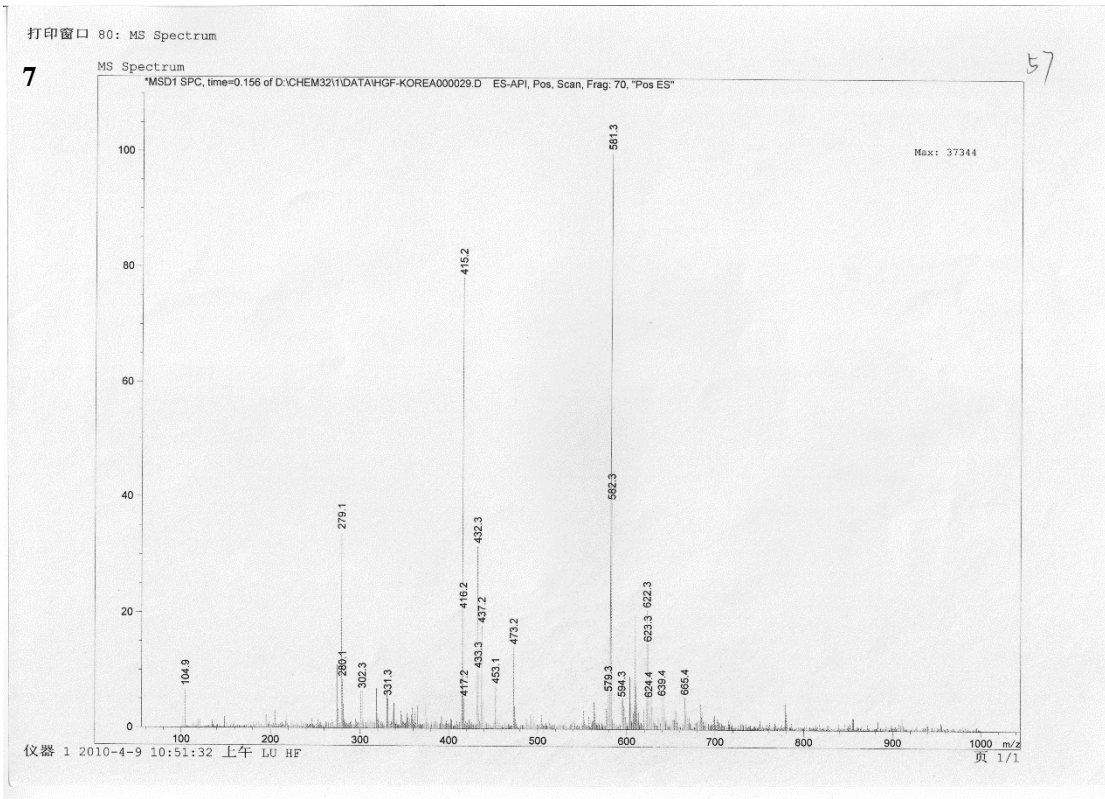
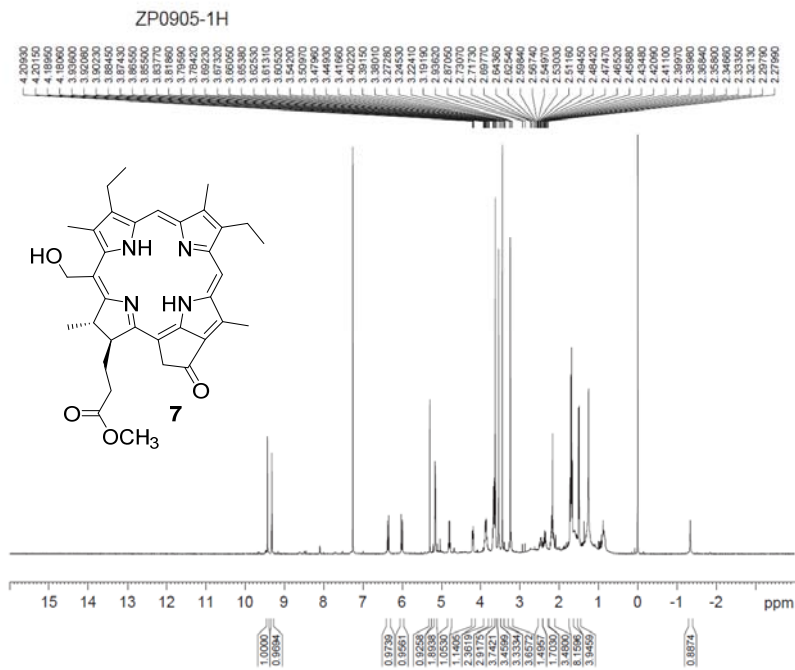


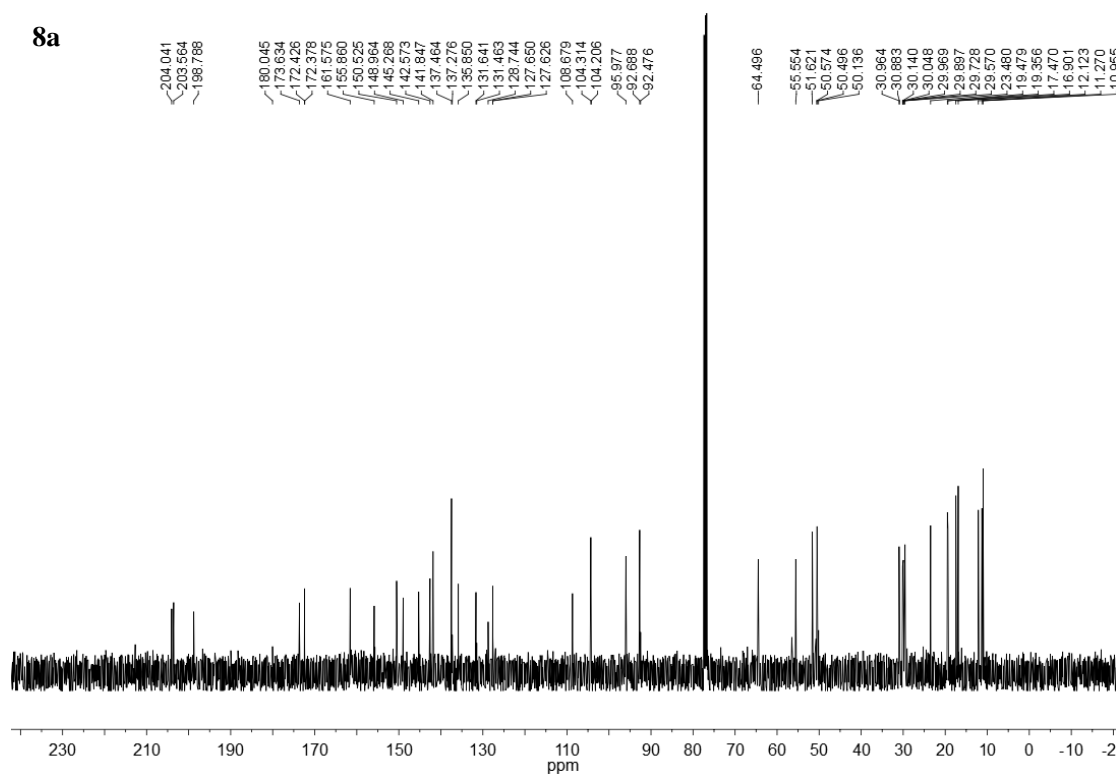
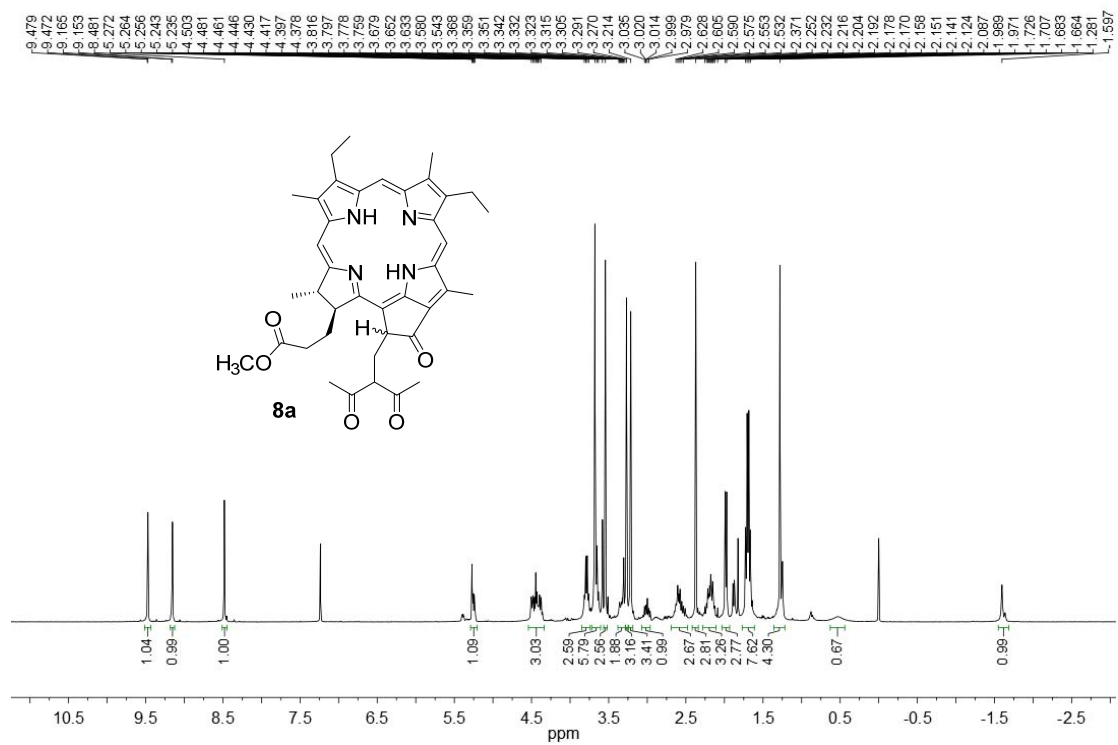
+Q1: 238 MCA scans from Sample 7 (132) of Iijiazhu10.10 .wiff (Turbo Spray)

Max. 6.0e8 cps.

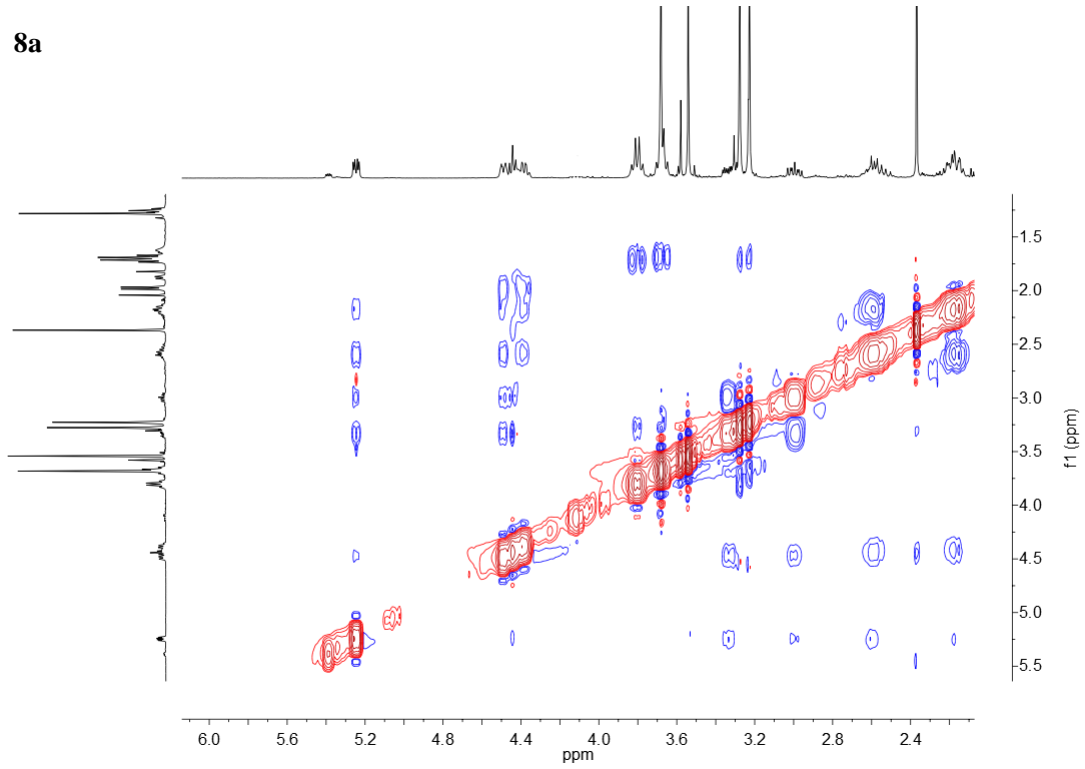
6a







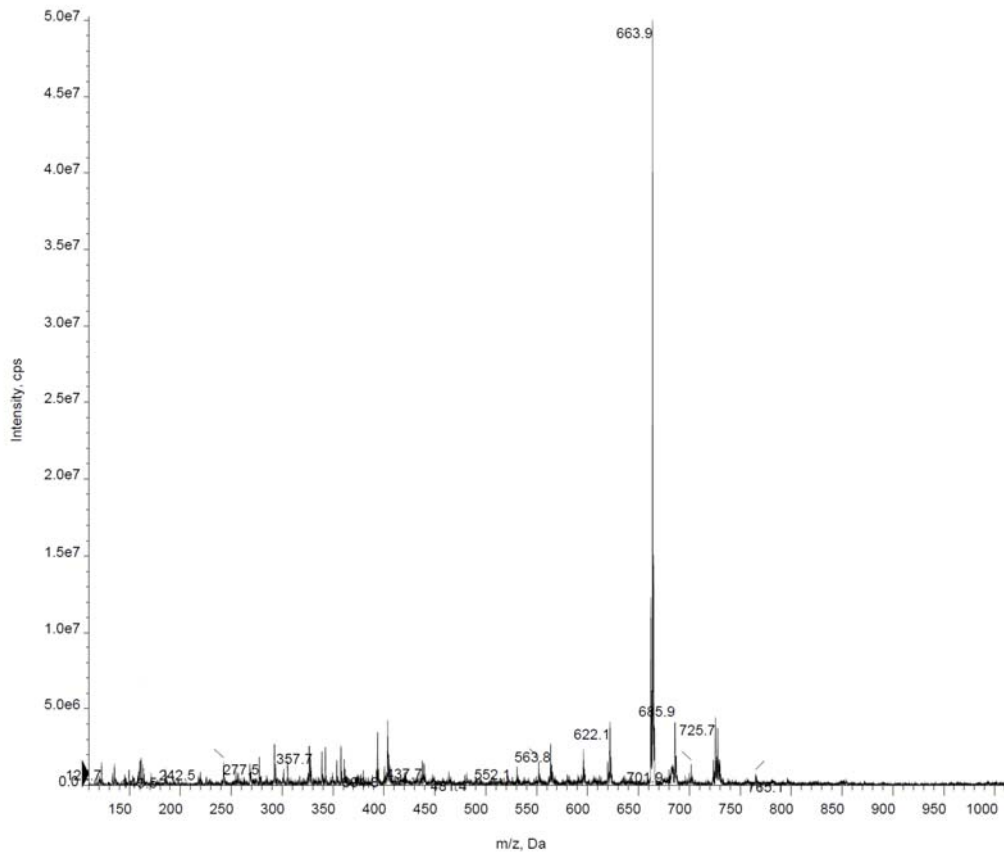
8a

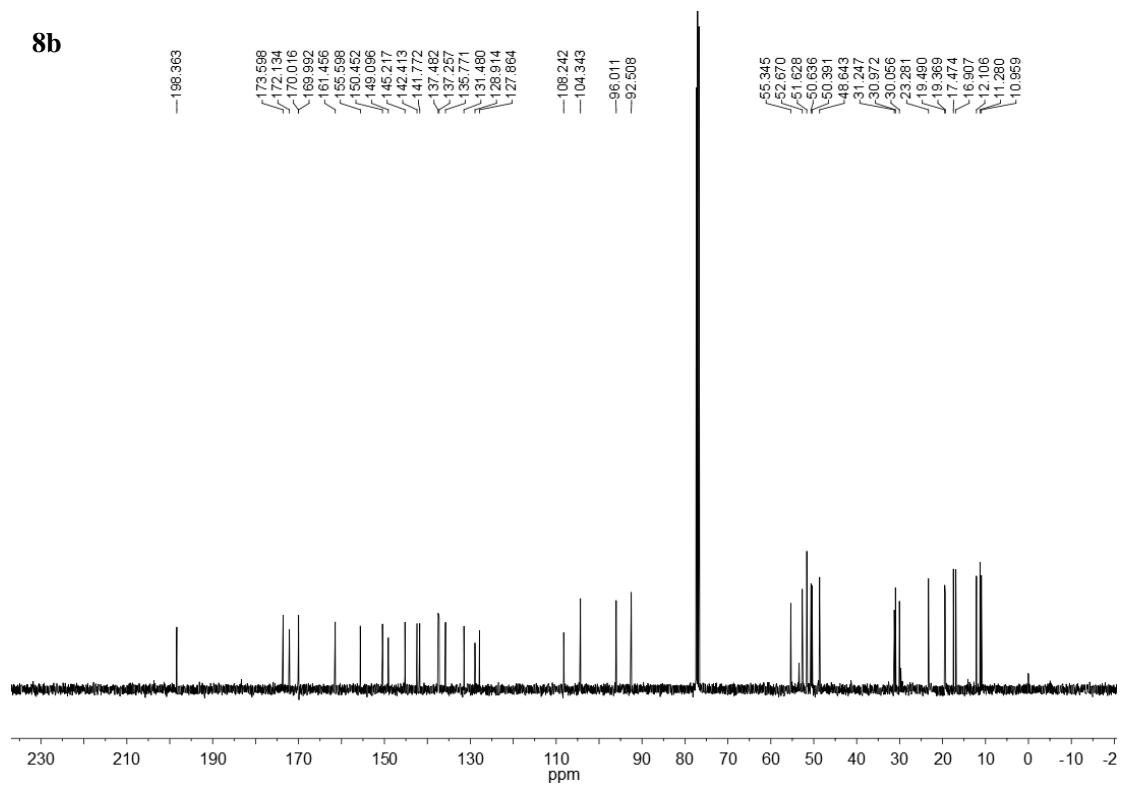
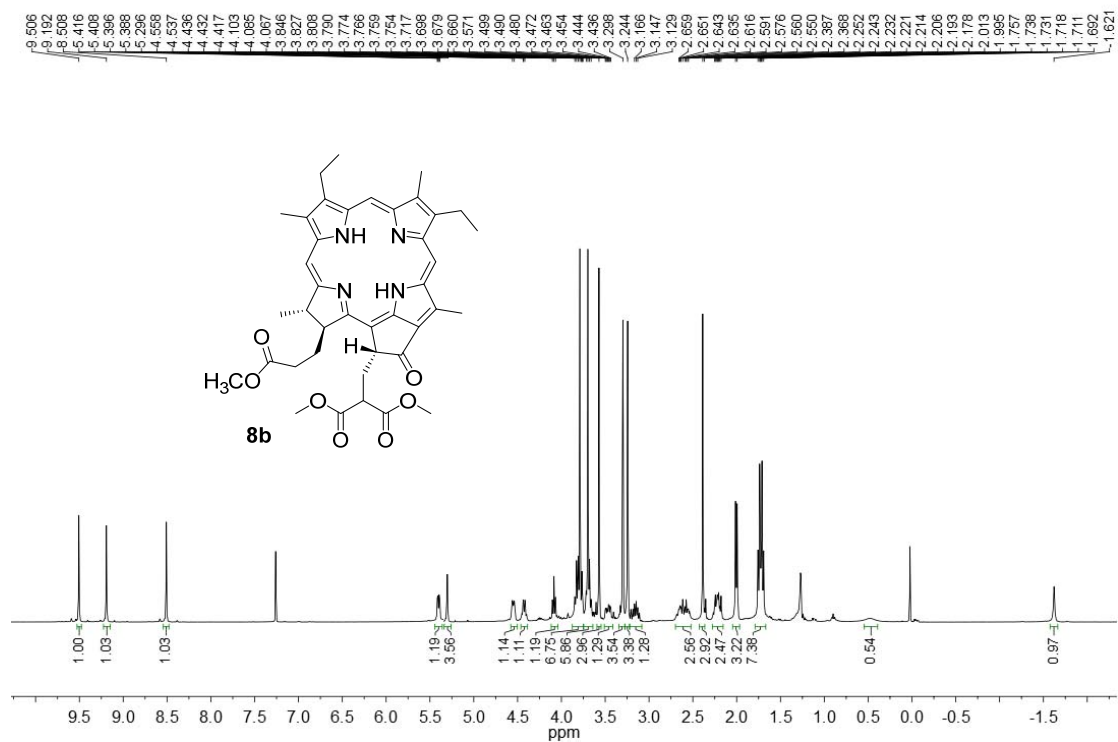


+Q1: 238 MCA scans from Sample 4 (TuneSampleID1416) of Iijiazhu10.10 .wiff (Turbo Spray)

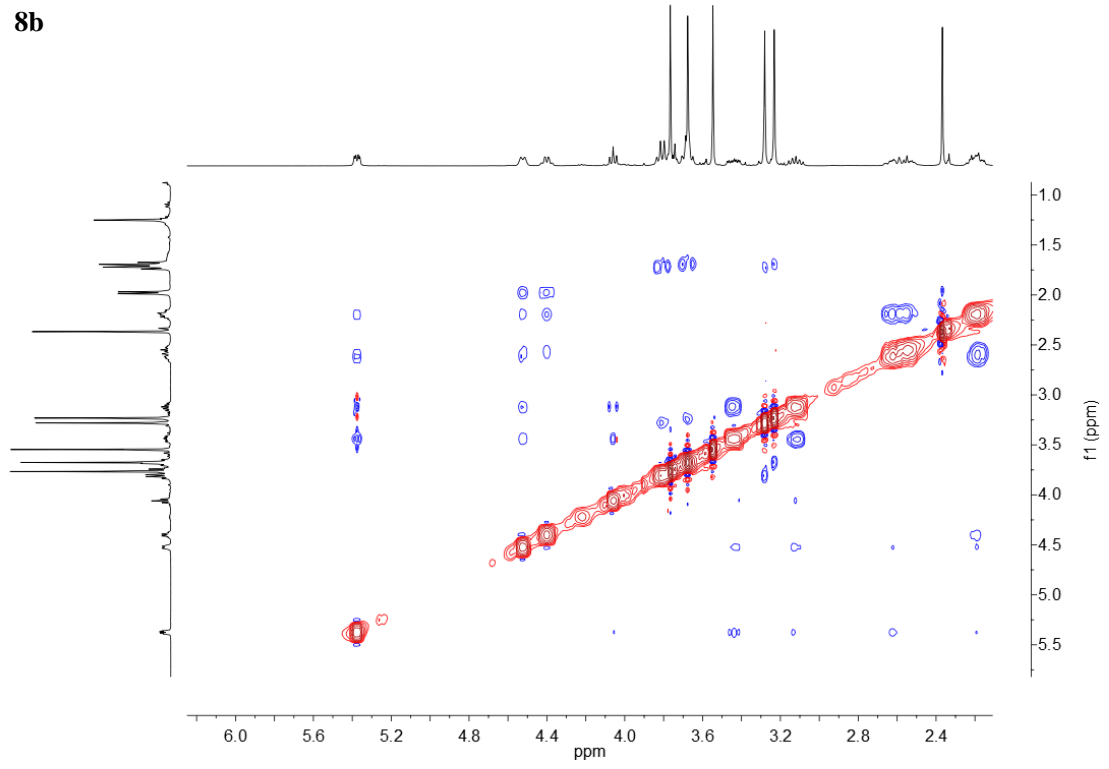
Max. 5.0e7 cps.

8a





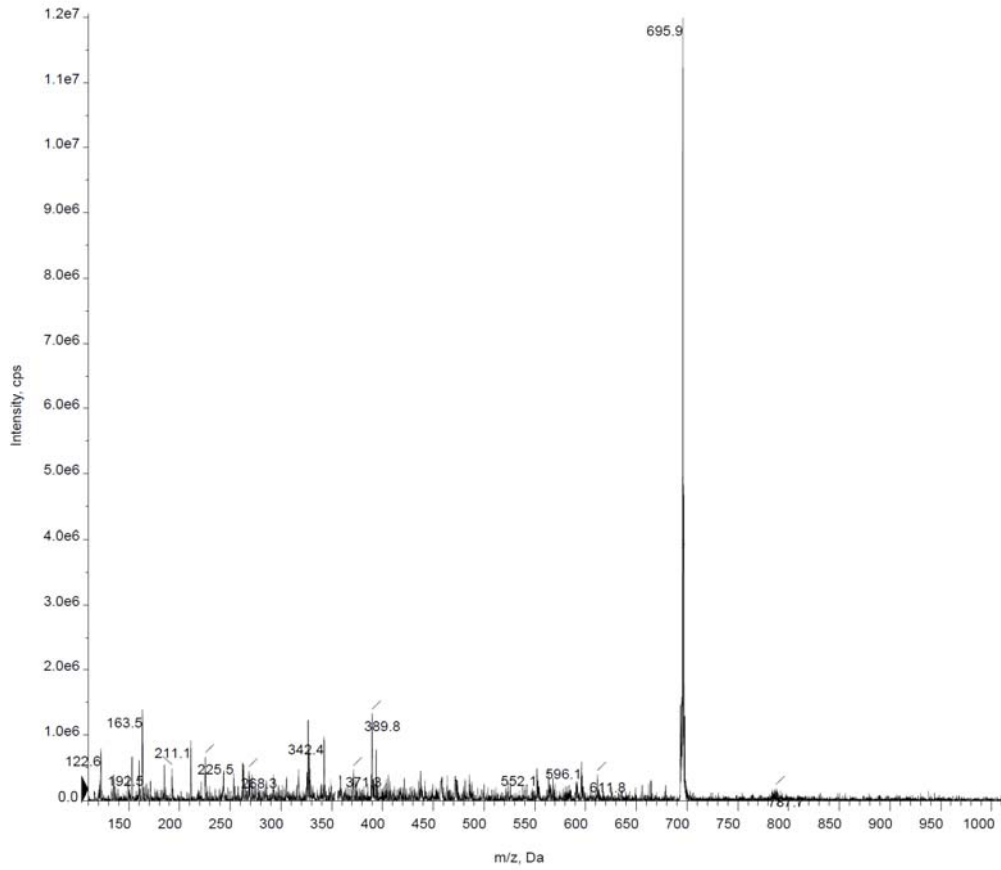
8b

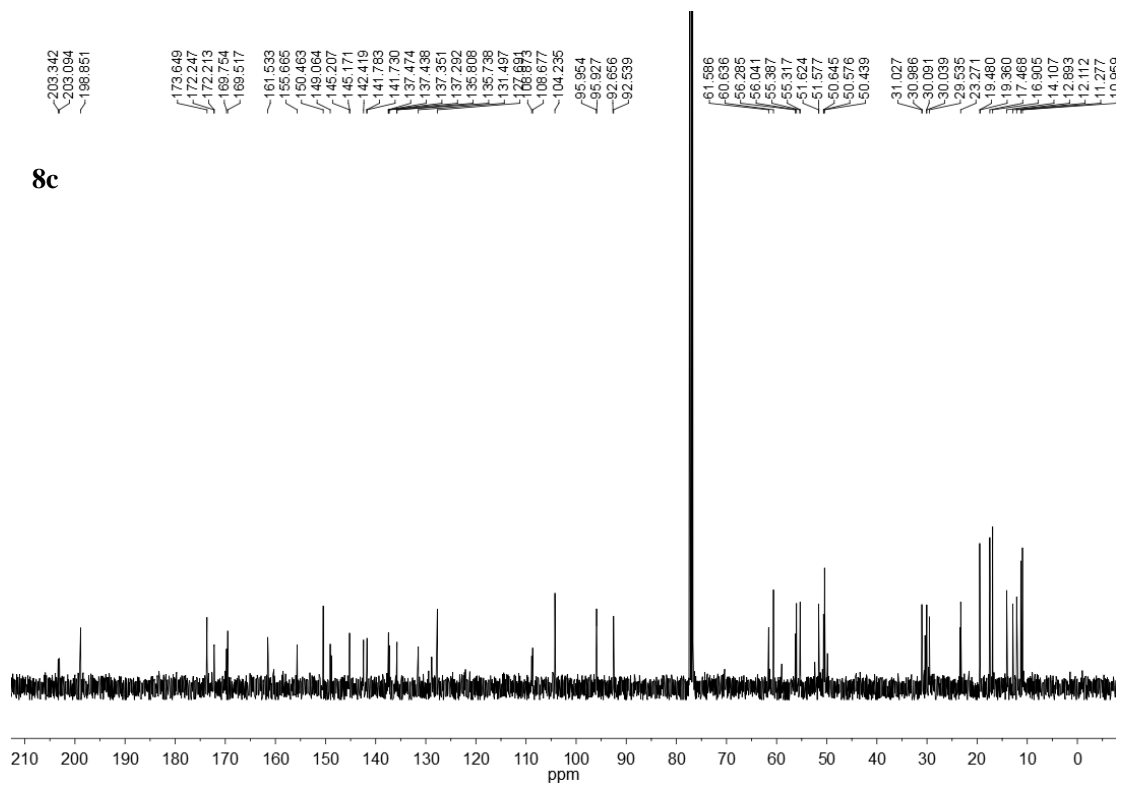
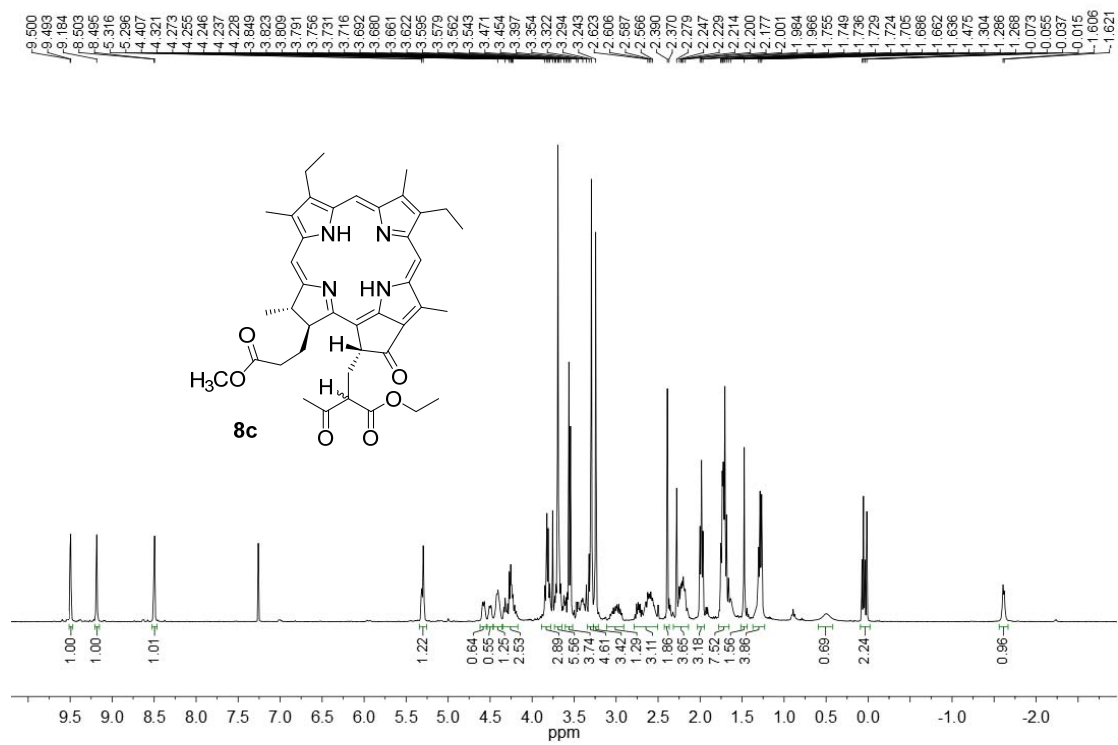


+Q1: 238 MCA scans from Sample 5 (TuneSampleID17) of Iijiazhu10.10 .wiff (Turbo Spray)

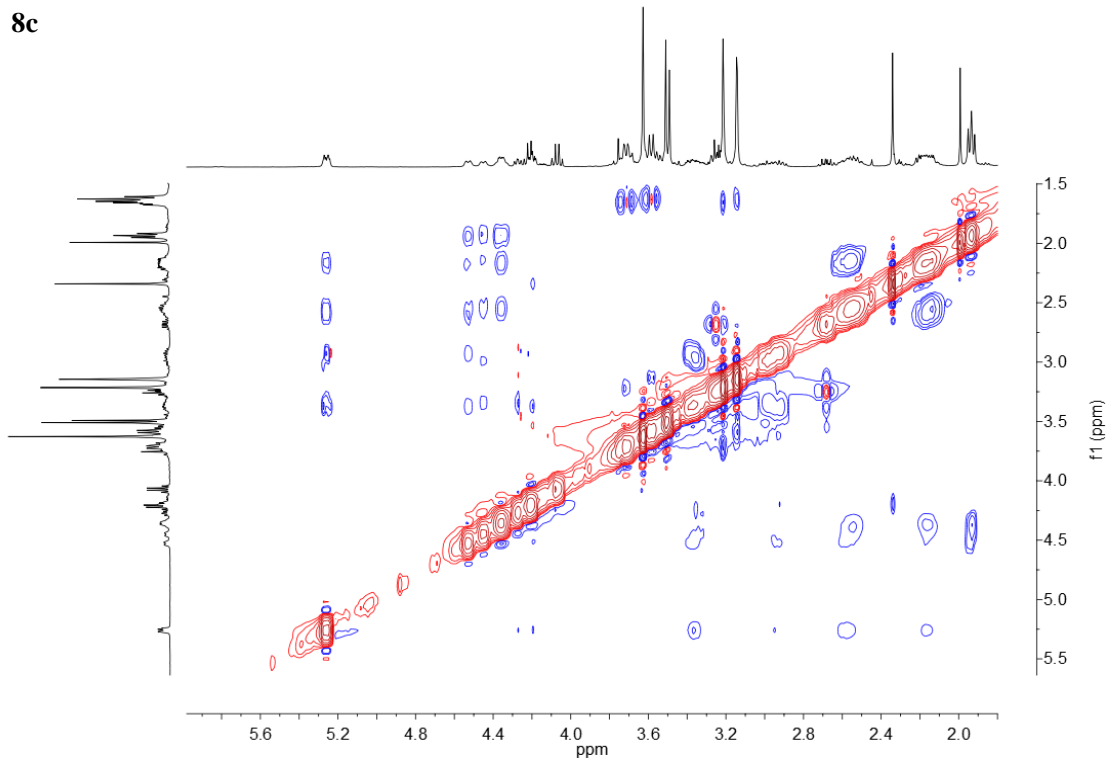
Max. 1.2e7 cps.

8b





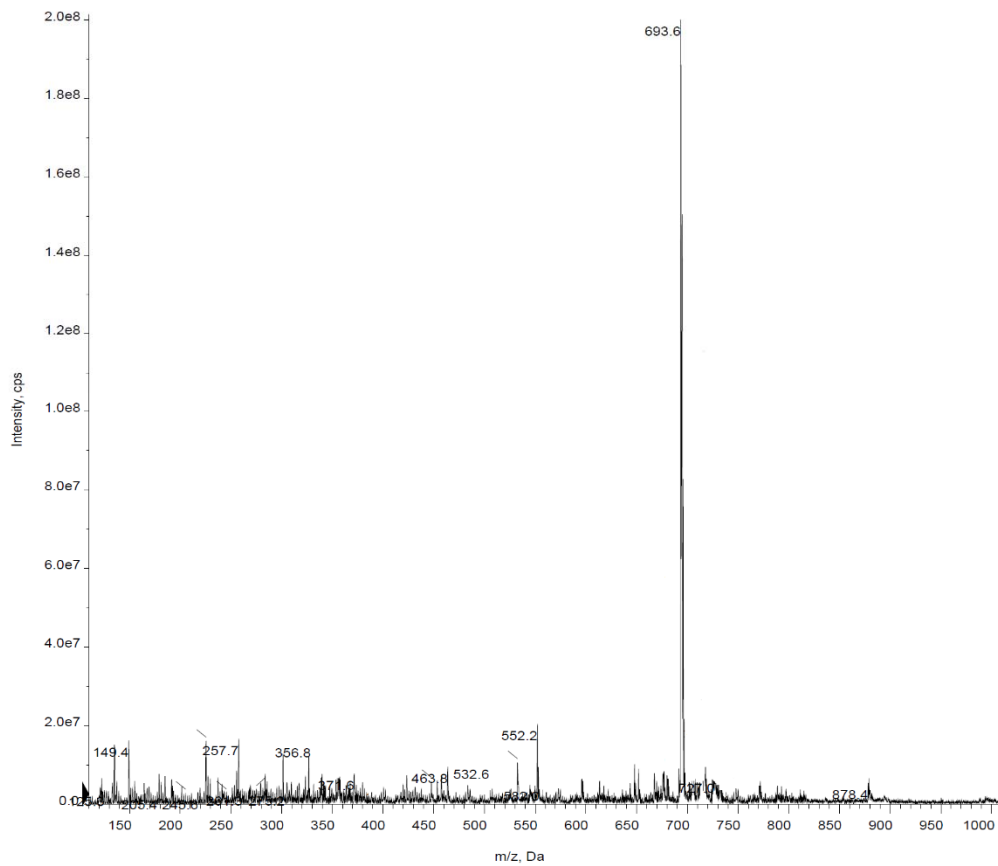
8c

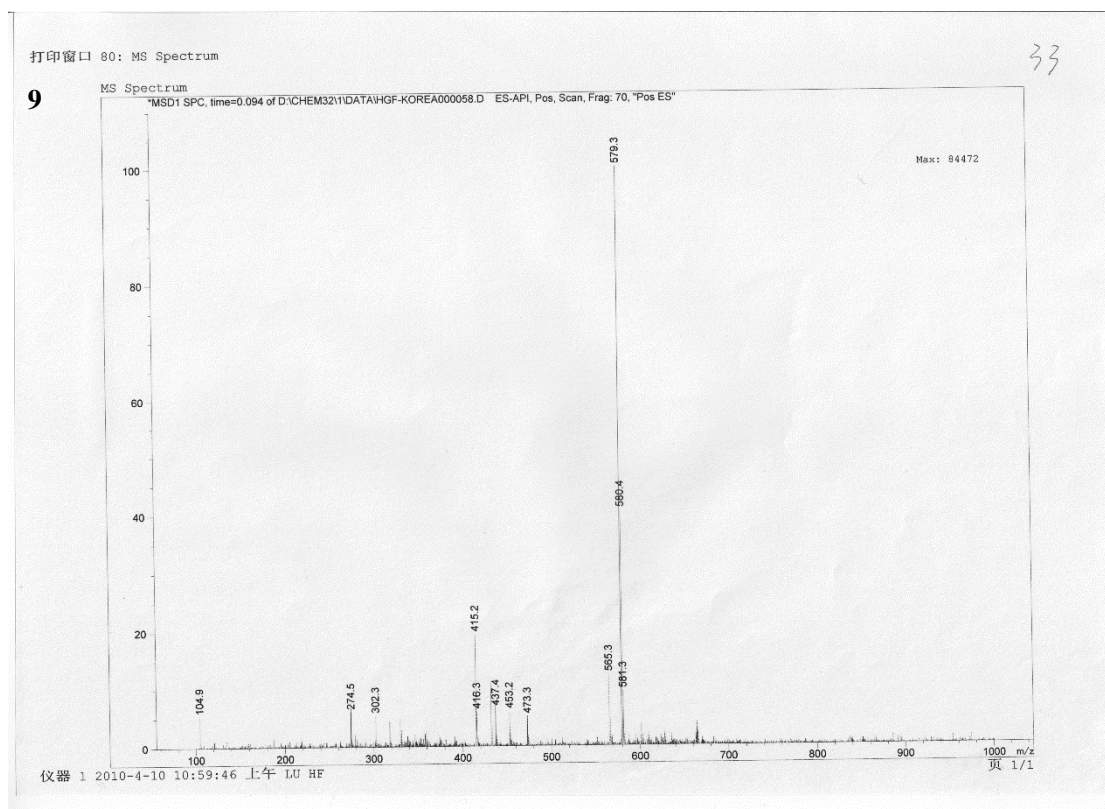
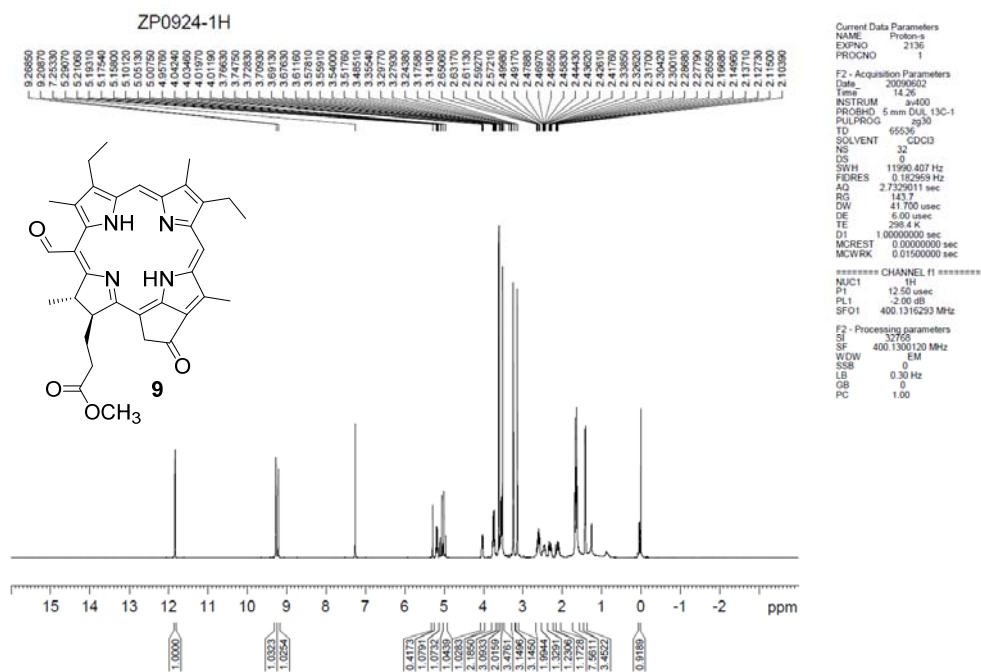


+Q1: 238 MCA scans from Sample 6 (TuneSampleID19) of Ilijazhu10.10 .wiff (Turbo Spray)

Max. 2.0e8 cps.

8c





5. References

- [1] K. M. Smith, D. A. Goff and D. J. Simpson, *J. Am. Chem. Soc.*, 1985, **107**, 4946-4954.