

Synthesis of Nitric Oxide Reductase Active Site Models Bearing Key Components at both Distal and Proximal Sites

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Supporting Information

Materials and methods

All reagents were obtained from commercial suppliers and used without further purification unless otherwise indicated. All air and moisture-sensitive reactions were carried out under a nitrogen atmosphere in oven-dried glassware. Tetrahydrofuran was distilled from sodium-benzophenone. Acetonitrile and dichloromethane were distilled over calcium hydride. Flash column chromatography was performed on E. Merck silica gel 60 (230-400 mesh). NMR spectra were recorded on either a varian Mercury-400 or Inova-500 MHz spectrometers and referenced to the residual proton signals of the solvent. Mass spectra were obtained from Stanford Mass Spectrometry Laboratory. Melting points were recorded on a MEL-TEMP melting point machine from Laboratory Devices and were uncorrected. UV-vis spectra were recorded on a HP8452 diode array spectrophotometer. 3-(*o*-Methoxy-phenyl)-3*H*-imidazole-4-carboxylic acid ethyl ester (**8**), *cis*- α -(*o*-aminophenyl)- α_2 -(*o*-4-(3-methylimidazolyl)-amidophenyl)- β -(*o*-3-(1-(5-*p*-trifluoromethylphenyl)imidazolylmethyl)-benzamidophenyl)porphyrin (**5a**), and *cis*- α -(*o*-aminophenyl)- α_2 -(*o*-4-(3-methylimidazolyl)-amidophenyl)- β -(*o*-3-(1-(5-*p*-acetylenylphenyl)imidazolylmethyl)-benzamidophenyl)porphyrin (**5b**)

[3-(*o*-Methoxy-phenyl)-3*H*-imidazol-4-yl]-methanol (**11**)

A solution of **8** (2.0 g, 8.12 mmol) in dry tetrahydrofuran (20 mL) was added dropwise into a suspension of lithium aluminum hydride (95%, 648 mg, 16.24 mmol) in dry tetrahydorfuran (20 mL) under nitrogen atmosphere at 0 °C. After the addition, the mixture was stirred at 0 °C for 0.5 h, then at room temperature for 24 h. The reaction mixture was cooled to 0 °C and a 10% solution of potassium hydroxide (10 mL) was slowly added. The mixture was stirred for 15 minutes. It was condensed under vacuum to a small volume. Water was added, and the mixture was extracted with chloroform. The organic extracts were dried over Na₂SO₄. Filtration and evaporation gave **11** as a solid, mp 110-112 °C. Yield: 1.48 g (90%). ¹H NMR (CDCl₃): δ 7.45 (s, 1H), 7.41 (t, 1H, J = 8.0 Hz), 7.30 (d, 1H, J = 8.0 Hz), 7.06 (s, 1H), 7.03 (m, 2H), 4.41 (s, 2H), 3.76 (s, 3H), 3.11 (s(br), 1H). ¹³C NMR (CDCl₃) : δ 154.2, 138.7, 133.1, 130.4, 128.7, 127.7, 124.7, 121.0, 112.1, 55.8, 53.8. MS (ES): calc. for C₁₁H₁₂N₂O₂ m/z 205.1(M+H)⁺, found 205.6.

3-(*o*-Methoxy-phenyl)-3*H*-imidazole-4-carbaldehyde (12)

To a solution of imidazole alcohol **11** (2.0 g, 9.8 mmol) in dichloromethane (60 mL) was added manganese oxide activated (4.0 g, 46 mmol). The suspension was stirred at room temperature, overnight. The reaction mixture was filtered through celite. The filtrate was evaporated. The residue was subjected to chromatography on silica with ethyl acetate/hexane (3:1) as eluent to give **12** as a white solid, mp 86-88 °C. Yield: 1.82 g (92%). ¹H NMR (CDCl₃): δ 9.61 (s, 1H), 7.86 (s, 1H), 7.65 (s, 1H), 7.43 (t, 1H, J = 7.8 Hz), 7.23 (d, 1H, J = 7.8 Hz), 7.02 (m, 2H), 3.74 (s, 3H). ¹³C NMR (CDCl₃): δ 178.6, 154.1, 143.3, 140.5, 132.4, 130.8, 127.6, 124.4, 120.7, 112.0, 55.7. MS (ES): calc. for C₁₁H₁₀N₂O₂ m/z 203.1 (M+H)⁺, found 203.8.

3-(*o*-Hydroxy-phenyl)-3*H*-imidazole-4-carbaldehyde (13)

To a solution of imidazole aldehyde **12** (500 mg, 2.47 mmol) in dichloromethane (40 mL) was added a solution of boron tribromide (1.0 M in CH₂Cl₂, 20 mL) at -78 °C under nitrogen. The mixture was stirred at -78 °C for 30 min, then at room temperature for 72 h. Water (5 mL) was added to quench the reaction while the reaction mixture was cooled to 0 °C, and the mixture was stirred for 30 min. Water (50 mL) was added. The aqueous phase was neutralized to pH = 5~6. It was extracted by dichloromethane. The organic extracts were combined, dried over anhydrous MgSO₄. Filtration and evaporation gave solid residue. It was subjected to chromatography on silica eluting with 1.5% of methanol in chloroform. The starting material (260 mg) was recovered. The desired product was obtained as a white solid. Yield: 60 mg (12.9%).

3-(*o*-Hydroxy-phenyl)-3*H*-imidazole-4-carbaldehyde (13)

Imidazole aldehyde **12** (1.00 g, 4.95 mmol) was dissolved in HBr solution (48% in water, 10 mL). The mixture was heated to reflux and stirred for 24 h under N₂. The solution was cooled to room temperature. Anhydrous K₂CO₃ was added until the evolution of CO₂ ceased and the pH reached 6-7. The mixture was evaporated to dryness. The solids residue was transferred to a column built with silica and chloroform, eluting with 1.5 % -5% MeOH in CHCl₃. The major fraction was collected. Evaporation of solvent gave solids. Yield: 0.58 g (62%), mp 170-172°C. ¹H NMR (CD₃COCD₃): δ 9.68 (s, 1H), 7.90 (m, 2H), 7.35 (d, 2H, J = 6.0 Hz), 7.12 (m, 1H), 6.99 (t, 1H, J = 6.0 Hz). ¹³C NMR (CD₃COCD₃): δ 178.6, 152.3, 139.2, 130.4, 128.0, 122.5, 119.8, 119.1, 116.6, 88.5. MS (ES): calc. for C₁₀H₈N₂O₂ m/z 188.2 (M+H)⁺, found 188.9.

Methyl 5-iodopentanoate

Methyl 5-chloropentanoate (5.0 g, 33 mmol) and NaI (25.0 g, 167 mmol) were in acetone (70 mL). The suspension was stirred at room temperature for three days. Solvent was evaporated. The residue was dissolved in methylene chloride and washed with water. The organic layer was dried over MgSO₄. Filtration and evaporation gave red oil. It was purified by chromatography on silica eluting with hexanes/methylene chloride (2:1) to afford 6.0 g (80%) of the product as a light red oil. ¹H NMR (CDCl₃): δ 3.65 (s, 3H), 3.16 (t, 2H, J = 7.2

Hz), 2.31 (t, 2H, J = 7.2 Hz), 1.82 (m, 2H), 1.72 (m, 2H). ^{13}C NMR (CDCl_3): δ 173.6, 51.6, 32.8, 32.7, 25.7, 5.9.

5-[2-(5-Fornyl-imidazol-1-yl)-phenoxy]-pentanoic acid methyl ester (14)

Compound **13** (1.0 g, 5.32 mmol) and methyl 4-iodopentanoate (2.58 g, 10.7 mmol) were dissolved in dry acetonitrile (50 mL) in a round bottom flask. Anhydrous K_2CO_3 (2.94 g, 21.3 mmol) was added. The mixture was refluxed overnight. It was cooled to room temperature, evaporated to dryness. The residue was dissolved in water, extracted with ethyl acetate. The organic extracts were dried and evaporated to give an oil residue. It was subjected to chromatography on silica with eluent ethyl acetate/hexane (3:1) to give 1.20 g (75%) of solid product, mp 68-70 °C. ^1H NMR (CDCl_3): δ 9.68 (s, 1H), 7.92 (s, 1H), 7.70 (s, 1H), 7.46 (t, 1H, J = 7.5 Hz), 7.29 (d, 1H, J = 7.5 Hz), 7.06 (m, 2H), 3.98 (t, 2H, J = 6.0 Hz), 3.66 (s, 3H), 2.27 (t, 2H, J = 7.0 Hz), 1.64 (m, 4H). ^{13}C NMR (CDCl_3): δ 178.7, 173.8, 154.1, 143.4, 140.5, 132.5, 130.8, 127.5, 124.5, 120.7, 112.8, 68.0, 51.6, 33.4, 28.3, 21.3. MS (ES): calc. for $\text{C}_{16}\text{H}_{18}\text{N}_2\text{O}_4$ m/z 302.1($\text{M}+\text{H}$)⁺, found 302.9.

3-[2-(4-Methoxycarbonyl-butoxy)-phenyl]-3*H*-imidazole-4-carboxylic acid (6)

Compound **14** (1.00 g, 3.3 mmol) was dissolved in tert-butanol (30 mL) and 2-methyl-2-butene in THF (10 mL, 2.0 M). A solution of NaClO_2 (80%, 3.74 g, 33 mmol) and $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$ (3.56 g, 2.58 mmol) in water (15 mL) was added. The mixture was stirred at room temperature until TLC shows complete reaction (approximately 1.0 h). Water (100 mL) and NaCl (aqueous saturated, 100 mL) were added. It was extracted with ethyl acetate. Drying (MgSO_4) and evaporation afforded 1.05 g (99%) of solid product, mp 120-122 °C. ^1H NMR (CDCl_3): δ 11.38 (s(br), 1H), 7.83 (s, 1H), 7.68 (s, 1H), 7.36 (t, 1H, J = 7.8 Hz), 7.22 (d, 1H, J = 7.8 Hz), 6.96 (m, 2H), 3.91 (t, 2H, J = 6.0 Hz), 3.58 (s, 3H), 2.22 (t, 2H, J = 7.0 Hz), 1.60 (m, 4H). ^{13}C NMR (CDCl_3): δ 174.1, 162.2, 153.8, 142.1, 135.8, 130.5, 127.5, 125.6, 120.5, 112.6, 67.9, 51.6, 33.4, 28.3, 21.3. MS (ES): calc. for $\text{C}_{16}\text{H}_{18}\text{N}_2\text{O}_5$ m/z 318.1($\text{M}+\text{H}$)⁺, found 319.0.

2-Iodo-3-(2-methoxy-phenyl)-3*H*-imidazole-4-carbaldehyde (15)

Imidazole aldehyde **12** (1.00 g, 4.95 mmol) was dissolved in dry THF (25 mL) in a round-bottomed flask. *N*-iodosuccinimide (95%, 2.35 g, 9.92 mmol) was added in one portion. The flask was wrapped with Al foil and the mixture was refluxed under N_2 for 24 h. After cooled to room temperature, saturated $\text{Na}_2\text{S}_2\text{O}_3$ solution was added to destroy excess *N*-iodosuccinimide. Solvent was evaporated. The residue was dissolved in water, extracted with ethyl acetate. The extracts were dried over MgSO_4 . Filtration and evaporation afforded solid residue. It was purified by chromatography on silica eluting with ethyl acetate/hexanes (3:1) to give 1.46 g (90%) of solid product, mp 96-98 °C. ^1H NMR (CDCl_3): δ 9.36 (s, 1H), 7.83 (s, 1H), 7.51 (t, 1H, J = 7.8 Hz), 7.18, (d, 1H, J = 7.8 Hz), 4.09 (q, 2H, J = 7.2 Hz), 3.74 (s, 3H), 1.23 (t, 3H, J = 7.2 Hz). ^{13}C NMR (CDCl_3): δ 177.7, 154.8, 142.2, 136.3, 131.7, 129.3, 125.4, 120.8, 112.3, 104.3, 55.8. MS (ES): calc. for $\text{C}_{11}\text{H}_9\text{IN}_2\text{O}_2$ m/z 328.0 ($\text{M}+\text{H}$)⁺, found 328.9.

Methyl 4-pentyneoate

4-Pentyneoic acid (5.0 g, 51.0 mmol) and *p*-toluenesulfonic acid (97 mg, 0.51 mmol) were dissolved in MeOH (30 mL) and benzene (50 mL) in a 100 mL round-bottomed flask. The flask was fitted with a Dean-Stark trap. The mixture was refluxed for 3 days. The reaction mixture was poured into a saturated NaHCO₃ solution, then extracted with diethyl ether. The extracts were dried and evaporated. The residue was distilled at ambient pressure to provide 4.1 g (72%) of desired ester. Colorless oil, bp 141-142 °C (760 mmHg) [lit. bp 143-144 °C (760 mmHg)]. ¹H NMR (CDCl₃): δ 3.66 (s, 3H), 2.52 (m, 2H), 2.46 (m, 2H), 1.94 (t, 1H). ¹³C NMR (CDCl₃): δ 172.2, 82.5, 69.1, 51.9, 3.1, 14.3.

5-[5-Formyl-1-(2-methoxy-phenyl)-1*H*-imidazol-2-yl]-pent-4-ynoic acid methyl ester (16)

Iodoimidazole **15** (500 mg, 1.52 mmol), PdCl₂(PPh₃)₂ (53 mg, 0.076 mmol) and CuI (15 mg, 0.079 mmol) were in dry THF (30 mL) in a round-bottomed flask. The mixture was degassed by two freeze-pump-thaw cycles. Diisopropylamine (4.0 mL) was added. The mixture was degassed by two freeze-pump-thaw cycles. Methyl 4-pentyneoate (188 mg, 1.68 mmol) was added and the mixture was degassed by two freeze-pump-thaw cycles. Then the flask was placed in a 50 °C pre-heated water bath and the mixture was stirred for 20 h. A yellow solution with some white precipitates was formed. The solvent was evaporated. The residue was subjected to chromatography on silica eluting with ethyl acetate/hexanes (1:1) to afford 333 mg (70%) of the desired product, mp 65-66 °C. ¹H NMR (CDCl₃): δ 9.50 (s, 1H), 7.85 (s, 1H), 7.45 (t, 1H, J = 7.8 Hz), 7.25 (d, 1H, J = 7.8 Hz), 7.04 (m, 2H), 3.74 (s, 3H), 3.62 (s, 3H), 2.56 (t, 2H, 8.0 Hz), 2.41 (t, 2H, J = 8.0 Hz). ¹³C NMR (CDCl₃): δ 178.2, 171.6, 154.5, 140.1, 132.4, 131.1, 128.5, 124.0, 120.5, 112.0, 95.1, 70.5, 55.8, 52.0, 32.3, 15.2. MS (ES): calc. for C₁₇H₁₆N₂O₄ m/z 312.1 (M+H)⁺, found 312.9.

5-[5-Formyl-1-(2-methoxy-phenyl)-1*H*-imidazol-2-yl]-pentanoic acid methyl ester (17)

Compound **16** (1.00 g, 3.20 mmol) was dissolved in a mixture of ethyl acetate and methanol (40 mL, 1:1 vol). The solution was degassed with N₂ for 10 min. Pd/C (10% on activated carbon, 230 mg) was added. Hydrogen gas was bubbled through the stirred mixture until TLC showed complete reaction. The reaction mixture was filtered through a silica bed, evaporated to dryness. The residue was subjected to chromatography on silica eluting with ethyl acetate/hexanes (1:1 vol) to give 0.76 g (75%) of the desired compound as an oil. ¹H NMR (CDCl₃): δ 9.45 (s, 1H), 7.77 (s, 1H), 7.42 (t, 1H, J = 8.0 Hz), 7.13 (d, 1H, J = 8.0 Hz), 7.01 (m, 2H), 3.70 (s, 3H), 3.56 (s, 3H), 2.44 (t, 2H, J = 7.6 Hz), 2.19 (t, 2H, J = 7.6 Hz), 1.66 (m, 2H), 1.53 (m, 2H). ¹³C (CDCl₃): δ 178.0, 173.6, 155.5, 154.6, 140.7, 132.8, 131.0, 128.5, 124.2, 120.8, 112.0, 55.7, 51.4, 33.5, 26.6, 26.3, 24.3. MS (ES): calc. for C₁₇H₂₀N₂O₄ m/z 316.1 (M+H)⁺, found 316.9.

2-(4-Methoxycarbonyl-butyl)-3-(2-methoxy-phenyl)-3*H*-imidazole-4-carboxylic acid (7)

Compound **17** (1.00 g, 3.16 mmol) was dissolved in tert-butanol (30 mL) and 2-methyl-2-butene in THF (10 mL, 2.0 M). A solution of NaClO₂ (80%, 3.74 g, 33 mmol) and

$\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$ (3.56 g, 2.58 mmol) in water (15 mL) was added. The mixture was stirred at room temperature until TLC shows complete reaction (approximately 1.0 h). Water (100 mL) and NaCl (aqueous saturated, 100 mL) were added. It was extracted with ethyl acetate. After drying (MgSO_4) and evaporation, 1.04 g (99%) of solid product were obtained, mp 118-120 $^{\circ}\text{C}$. ^1H NMR (CDCl_3): δ 11.1 (s(br), 1H), 7.74 (s, 1H), 7.36 (t, 1H, J = 8.0 Hz), 7.08 (d, 1H, J = 8.0 Hz), 6.96 (m, 2H), 3.67 (s, 3H), 3.55 (s, 3H), 2.41 (t, 2H, J = 7.2 Hz), 2.12 (t, 2H, J = 7.2 Hz), 1.47 (m, 4H). ^{13}C NMR (CDCl_3): δ 173.7, 161.8, 154.7, 152.7, 133.1, 130.7, 128.6, 125.7, 124.8, 120.5, 111.8, 55.6, 51.4, 33.4, 26.5, 25.9, 24.1. MS (ES): calc. for $\text{C}_{17}\text{H}_{20}\text{N}_2\text{O}_5$ m/z 332.1 ($\text{M}+\text{H}$)⁺, found 332.9.

5-[2-(5-Chlorocarbonyl-imidazol-1-yl)-phenoxy]-pentanoic acid methyl ester (9)

Acid **6** (250 mg, 0.78 mmol) was suspended in dry and degassed acetonitrile (15 mL) under N_2 . Oxalyl chloride (275 μL , 3.15 mmol) was added through a syringe followed by a drop of DMF. The clear solution was stirred at room temperature for 4 h, during which a lot of white solids precipitated out. The solution was condensed under vacuum to a small volume (~3 mL). Dry diethyl ether (10 mL) was added and the mixture was stirred for 20 min. It was quickly filtered in the air. The cake was washed with 2 mL of dry diethyl ether, then left under vacuum for overnight. A white solid product was obtained, 220 mg (75%). Characterization of this white solid was achieved by reacting it with dry ethanol to give the corresponding ethyl ester. To the acyl chloride (10 mg) was added dry ethanol (0.5 ml), the solution was then evaporated under vacuum to give an oil. ^1H NMR (CDCl_3): δ 8.60 (s, 1H), 8.02 (s, 1H), 7.53 (t, 1H, J = 7.6 Hz), 7.32 (d, 1H, J = 7.6 Hz), 7.10 (t, 1H, J = 7.6 Hz), 7.04 (d, 1H, J = 7.6 Hz). ^{13}C NMR (CDCl_3): δ 173.7, 156.9, 153.4, 138.5, 132.8, 127.3, 126.0, 125.5, 123.0, 121.2, 112.8, 68.5, 62.5, 51.9, 33.5, 28.4, 21.4, 14.1.

5-[5-Chlorocarbonyl-1-(2-methoxy-phenyl)-1*H*-imidazol-2-yl]-pentanoic acid methyl ester (10)

Acid **7** (250 mg, 0.75 mmol) was dissolved in thionyl chloride (5 mL). The solution was heated to 78-80 $^{\circ}\text{C}$ and stirred for overnight (14 h) under nitrogen. Excess thionyl chloride was removed on a rotavap. The residue was washed with anhydrous ether (1 mL \times 2), then left under vacuum overnight to give a light yellow oil residue, 251 mg (86%). Characterization of this oil residue was achieved by reacting it with dry ethanol to give the corresponding ethyl ester. To the acyl chloride solution in dry acetonitrile (0.15 M, 200 μL) was added dry ethanol (0.5 ml), the solution was then evaporated under vacuum to give an oil. ^1H NMR (CDCl_3): δ 7.96 (s, 1H), 7.57 (t, 1H, J = 8.0 Hz), 7.36 (d, 1H, J = 8.0 Hz), 7.22 (m, 2H), 4.16 (q, J = 7.0 Hz), 3.76 (s, 3H), 3.57 (s, 3H), 2.82 (m, 2H), 2.22 (t, 2H, J = 7.0 Hz), 1.75 (m, 2H), 1.52 (m, 2H), 1.19 (t, 3H, J = 7.0 Hz). ^{13}C NMR: δ 173.4, 156.8, 154.2, 151.9, 132.9, 128.1, 125.1, 125.0, 121.6, 121.2, 112.3, 62.0, 56.0, 51.6, 33.1, 26.5, 24.9, 23.9, 13.9.

Synthesis of porphyrins

General procedures to make the α -trisimidazole- β -tail-porphyrins following a [2+1] method

3ab and **4ab**: Porphyrin (**5a** 30 mg, **5b** 30 mg) was dissolved in THF/CH₃CN (2:1 vol, 8 mL). Dry HCl gas was bubbled through the solution until it turned brown green. Imidazole acyl chloride **9** or **10** (2.0 equiv) was added. The solution turned green immediately. It was stirred for 10 minutes. Then a solution of Et₂NC₆H₅ (1.26 M in THF) was added dropwise until the green color disappeared. Stirring was carried on for 10 min. The above procedures were repeated until 10 equivalents of acyl chloride were added. Since the product has almost the same polarity as the starting material, the reaction cannot be monitored by TLC. After the reaction was finished, the solution was diluted with CH₂Cl₂ (100 mL). Then it was washed with saturated NaHCO₃ (100 mL) and water (100 mL \times 3). The organic phase was dried (MgSO₄) and evaporated to give purple solids.

The mixture was separated on chromatotron using 0.8% MeOH in NH₃ saturated CH₂Cl₂.

For **3ab**, only one band was observed. Therefore, the porphyrin was collected in small culture tubes (0.5 mL solution). The first 2/3 and the last 1/3 of tubes were combined separately and evaporated. ¹H NMR shows that the first 2/3 of the fraction contained the unreacted starting material and the product **1ab**, while the last 1/3 of the fraction contained the clean **1ab**. For **4ab**, the first fraction was the expected product; the second fraction was the starting material **5ab**.

Yields:

3a: 23 mg (ratio of starting material and product 1:1), 10 mg (clean product)

Yield based on the reacted starting material was 98%.

3b: 22 mg (ratio of starting material and product 2:3), 13 mg (clean product)

Yield based on the reacted starting material was 99%.

4a: 14 mg (product), 15 mg (starting material).

Yield based on the reacted starting material was 75%.

4b: 13 mg (product), 17 mg (starting material).

Yield based on the reacted starting material was 79%.

3a: ¹H NMR (CDCl₃): δ 8.88 (m, 9H), 8.46 (d, 1H, J = 8.0 Hz), 8.43 (d, 1H, J = 8.0 Hz), 8.39 (d, 1H, J = 8.0 Hz), 8.27 (s, 1H), 8.14 (d, 1H, J = 7.5 Hz), 8.06 (d, 1H, J = 7.5 Hz), 8.03 (d, 1H, J = 7.5 Hz), 7.88 (m, 5H), 7.74 (m, 2H), 7.59 (m, 4H), 7.46 (t, 1H, J = 7.5 Hz), 7.41 (s, 1H), 7.04 (m, 5H), 6.83 (t, 1H, J = 7.5 Hz), 6.80 (s, 1H), 6.65 (d, 2H, J = 8.0 Hz), 6.51 (d, 1H, J = 8.0 Hz), 6.34 (s, 2H), 6.26 (m, 2H), 6.09 (d, 1H, J = 7.5 Hz), 5.82 (s, 1H), 5.59 (s, 1H), 5.55 (s, 1H), 4.09 (s, 2H), 3.63 (s, 3H), 3.60 (s, 3H), 3.30 (m, 2H), 2.90 (s, 3H), 1.16 (m, 2H), 0.67 (m, 4H), -2.54 (2H). MS (ES): calc. for C₈₈H₆₉F₃N₁₆O₇ m/z 1519.5 (M+H)⁺, found 1519.8. $\lambda_{\text{max}}(10^{-3}\epsilon, \text{M}^{-1}\text{cm}^{-1})$ 424 (441), 518 (93)

3b: ¹H NMR (CDCl₃): δ 8.88 (m, 9H), 8.46 (d, 2H, J = 8.5 Hz), 8.42 (d, 1H, J = 8.5 Hz), 8.39 (d, 1H, J = 8.5 Hz), 8.29 (s, 1H), 8.14 (d, 1H, J = 7.5 Hz), 8.07 (d, 1H, J = 7.5 Hz), 8.04 (d, 1H, J = 7.5 Hz), 7.88 (m, 5H), 7.74 (t, 1H, J = 8.0 Hz), 7.71 (s, 1H), 7.60 (m, 4H), 7.47 (t,

1H, $J = 7.5$ Hz), 7.40 (s, 1H), 7.11 (s, 1H), 7.06 (s, 1H), 7.03 (s, 1H), 6.91 (d, 2H, $J = 8.0$ Hz), 6.89 (s, 1H), 6.82 (m, 1H), 6.74 (s, 1H), 6.64 (d, 1H, $J = 7.0$ Hz), 6.49 (d, 2H, $J = 8.0$ Hz), 6.27 (m, 4H), 6.12 (d, 1H, $J = 7.5$ Hz), 5.80 (s, 1H), 5.60 (s, 1H), 5.59 (s, 1H), 4.01 (s, 2H), 3.64 (s, 3H), 3.60 (s, 3H), 3.30 (m, 2H), 3.00 (s, 1H), 2.91 (s, 3H), 1.14 (m, 2H), 0.70 (m, 4H), -2.55 (2H). MS (ES): calc. for $C_{89}H_{70}N_{16}O_7$ m/z 1475.6 ($M+H$)⁺, found 1475.6. $\lambda_{max}(10^{-3}\epsilon, M^{-1}cm^{-1})$ 425 (454), 518 (90)

4a: ¹H NMR (CDCl₃): δ 8.88 (m, 9H), 8.45 (m, 3H), 8.00 (m, 8H), 7.70 (t, 1H, $J = 8.0$ Hz), 7.58 (m, 4H), 7.47 (s, 1H), 7.41 (t, 1H, $J = 7.6$ Hz), 7.37 (s, 1H), 7.12 (t, 1H, $J = 8.0$ Hz), 7.05 (d, 2H, $J = 8.0$ Hz), 6.98 (s, 2H), 6.94 (d, 1H, $J = 6.4$ Hz), 6.87 (s, 1H), 6.79 (s, 1H), 6.65 (d, 2H, $J = 8.0$ Hz), 6.55 (m, 2H), 6.37 (s, 1H), 6.63 (m, 2H), 6.11 (s, 1H), 5.85 5.55, 5.49, 5.40, 5.31 (3H, Im_oH4), 4.10 (s, 2H), 3.61, 3.60, 3.59, 3.57 (6H, NMe), 3.45 (s, 3H, COOMe), 3.37, 3.30 (Ph-OMe), 1.90 (m, 4H), 1.16 (m, 4H), -2.52, -2.54 (2H, porNH). MS (ES): calc. for $C_{89}H_{71}N_{16}O_7$ m/z 1533.5 ($M+H$)⁺, found 1534.0. $\lambda_{max}(10^{-3}\epsilon, M^{-1}cm^{-1})$ 424 (478), 518 (60)

4b: ¹H NMR (CDCl₃): δ 8.86 (m, 9H), 8.45 (m, 3H), 7.98 (m, 8H), 7.70 (t, 1H, $J = 8.0$ Hz), 7.58 (m, 4H), 7.41 (m, 3H), 7.09 (t, 1H, $J = 7.2$ Hz), 6.97 (s, 1H), 6.93 (d, 1H, $J = 8.0$ Hz), 6.88 (d, 2H, $J = 8.0$ Hz), 6.79 (d, 1H, $J = 4.4$ Hz), 6.72 (s, 2H), 6.55 (m, 2H), 6.47 (d, 2H, $J = 8.0$ Hz), 6.26 (m, 3H), 6.16 (d, 1H, $J = 7.2$ Hz), 5.88, 5.57, 5.50, 5.43, 5.32 (3H, Im_oH4), 4.00 (2H, benzoylCH₂), 3.61, 3.60, 3.58, 3.55 (6H, NMe), 3.454, 3.449 (3H, COOMe), 3.34, 3.27 (3H, Ph-OMe), 2.984, 2.982 (1H, Alkynyl), 1.90 (m, 4H), 1.16 (m, 4H), -2.52, -2.54 (2H, porNH). MS (ES): calc. for $C_{90}H_{72}N_{16}O_7$ m/z 1489.6 ($M+H$)⁺, found 1489.7. $\lambda_{max}(10^{-3}\epsilon, M^{-1}cm^{-1})$ 424 (475), 518 (41)

General procedures for saponification reaction of **3-4ab** to **1-2ab**

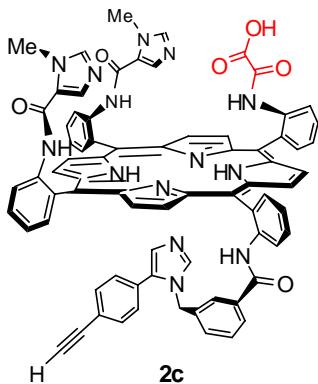
Porphyrin (**3-4ab**, 10 mg) was dissolved in MeOH/THF (10 mL, 1:1 vol). A solution of potassium hydroxide in water (1.0 g/mL, 5mL) was added. The mixture was stirred at room temperature for 2.0 h. The organic solvent was evaporated. The aqueous phase containing porphyrin was extracted with CH₂Cl₂ and washed with saturated NaHCO₃ solution. The organic extracts were dried (Na₂SO₄) and evaporated to give a purple solid. It was purified on chromatotron using 15% MeOH in NH₃ saturated CH₂Cl₂ solution. Yield: **1a** 9.3 mg (94%); **1b** 9.5 mg (96%), **2a** 8.9 mg (90%), **2b** 9.0 (91%)

1a: ¹H NMR (CDCl₃): the carboxylic acid proton is not assigned, δ 9.08 (d, 1H, $J = 4.8$ Hz), 8.96 (d, 1H, $J = 4.8$ Hz), 8.81 (m, 9H), 8.58 (d, 1H, $J = 8.0$ Hz), 8.22 (d, 1H, $J = 7.2$ Hz), 8.18 (d, 2H, $J = 8.0$ Hz), 8.02 (d, 1H, $J = 7.2$ Hz), 7.85 (m, 4H), 7.75 (t, 1H, $J = 8.0$ Hz), 7.54 (m, 5H), 7.35 (s, 1H), 7.20 (m, 2H), 7.09 (d, 1H, $J = 8.0$ Hz), 7.04 (d, 2H, $J = 8.0$ Hz), 6.98 (s, 1H), 6.94 (s, 1H), 6.83 (m, 2H), 6.78 (s, 1H), 6.69 (d, 2H, $J = 8.0$ Hz), 6.63 (d, 2H, $J = 8.0$ Hz), 6.27 (m, 4H), 6.11 (d, 1H, $J = 7.6$ Hz), 5.13 (s, 1H), 4.07 (s, 1H), 4.02 (m 2H), 3.64 (s, 3H), 3.48 (s, 3H), 1.22 (m, 8H), -2.50 (s, 2H). MS (ES): calc. for $C_{87}H_{67}F_3N_{16}O_7$ m/z 1505.5 ($M+H$)⁺, found 1505.7. HRMS (ES): calc. for $C_{87}H_{67}F_3N_{16}O_7$ m/z 1505.5409 ($M+H$)⁺, found 1505.5419. UV/Vis (CH₂Cl₂): $\lambda_{max}(10^{-3}\epsilon, M^{-1}cm^{-1})$ 424 (460), 518(65)

1b: ^1H NMR (CDCl_3): the carboxylic acid proton is not assigned, δ 9.08 (d, 1H, $J = 4.8$ Hz), 8.95 (d, 1H, $J = 4.8$ Hz), 8.81 (m, 9H), 8.58 (d, 1H, $J = 8.4$ Hz), 8.22 (d, 1H, $J = 7.6$ Hz), 8.18 (d, 2H, $J = 8.0$ Hz), 8.02 (d, 1H, $J = 7.6$ Hz), 7.85 (m, 4H), 7.75 (t, 1H, $J = 8.0$ Hz), 7.54 (m, 5H), 7.35 (s, 1H), 7.18 (m, 2H), 7.08 (d, 2H, $J = 7.6$ Hz), 6.98 (m, 1H), 6.94 (s, 1H), 6.89 (d, 2H, $J = 8.0$ Hz), 6.82 (t, 1H, $J = 8.0$ Hz), 6.78 (s, 1H), 6.70 (m, 2H), 6.46 (d, 2H, $J = 8.0$ Hz), 6.26 (m, 3H), 6.20 (s, 1H), 6.15 (d, 1H, $J = 7.6$ Hz), 5.14 (s, 1H), 4.09 (s, 1H), 3.94 (m, 2H), 3.64 (s, 3H), 3.48 (s, 3H), 2.99 (s, 1H), 1.10 (m, 8H), -2.51 (s, 2H). MS (ES): calc. for $\text{C}_{88}\text{H}_{68}\text{N}_{16}\text{O}_7$ m/z 1461.5 ($\text{M}+\text{H}$) $^+$, found 1461.9. HRMS (ES): calc. for $\text{C}_{88}\text{H}_{68}\text{N}_{16}\text{O}_7$ m/z 1461.5535 ($\text{M}+\text{H}$) $^+$, found 1461.5552. $\lambda_{\text{max}}(10^{-3}\varepsilon, \text{M}^{-1}\text{cm}^{-1})$ 425 (459), 518 (85)

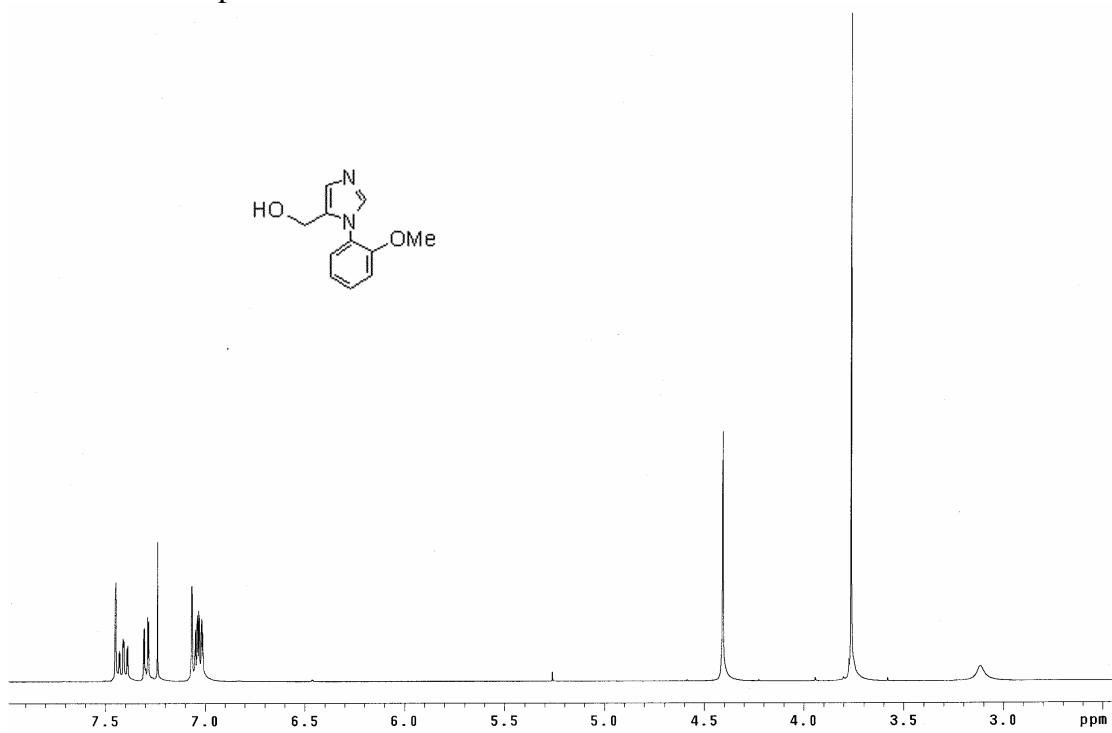
2a: ^1H NMR (CDCl_3): the carboxylic acid proton is not assigned, δ 8.90 (m, 11H), 8.57 (m, 2H), 8.34 (m, 2H), 8.14 (m, 2H), 7.98 (m, 1H), 7.85 (m, 4H), 7.70 (t, 1H, $J = 8.0$ Hz), 7.60 (m, 4H), 7.38 (m, 2H), 7.03 (m, 4H), 6.85 (s, 1H), 6.81 (s, 1H), 6.63 (m, 4H), 6.25 (m, 4H), 6.10 (m, 2H), 5.88 (s, 1H), 4.90, 4.83 (1H, Im_α 4H), 4.06 (2H, benzoyl CH_2), 3.73 (s, 3H, Im_α NMe), 3.67, 3.62 (3H, Im_α NMe), 3.23, 3.17 (3H, PhOMe), 2.01 (m, 2H), 1.67 (m, 2H), 0.95 (m, 4H), -2.55 (s, 2H). MS (ES): calc. for $\text{C}_{88}\text{H}_{69}\text{N}_{16}\text{O}_7$ m/z 1519.6 ($\text{M}+\text{H}$) $^+$, found 1520.1. HRMS (ES): calc. for $\text{C}_{88}\text{H}_{69}\text{N}_{16}\text{O}_7$ m/z 1519.5565 ($\text{M}+\text{H}$) $^+$, found 1519.5543. $\lambda_{\text{max}}(10^{-3}\varepsilon, \text{M}^{-1}\text{cm}^{-1})$ 424 (480), 518 (74)

2b: ^1H NMR (CDCl_3): the carboxylic acid proton is not assigned, δ 8.89 (m, 11H), 8.57 (m, 2H), 8.35 (m, 2H), 8.15 (m, 2H), 7.98 (m, 1H), 7.86 (m, 4H), 7.63 (m, 5H), 7.36 (m, 2H), 7.04 (m, 2H), 6.88 (m, 2H), 6.77 (d, 1H, $J = 4.0$ Hz), 6.74 (s, 1H), 6.63 (m, 3H), 6.47 (d, 2H, $J = 8.0$ Hz), 6.21 (m, 5H), 6.10 (m, 2H), 5.88 (s, 1H), 4.95, 4.87 (1H, Im_α 4H), 3.95 (2H, benzoyl CH_2), 3.72 (s, 3H, Im_α NMe), 3.66, 3.61 (3H, Im_α NMe), 3.23, 3.17 (3H, PhOMe), 2.98 (s, 1H), 2.01 (m, 2H), 1.67 (m, 2H), 0.94 (m, 4H), -2.56 (s, 2H). MS (ES): calc. for $\text{C}_{89}\text{H}_{70}\text{N}_{16}\text{O}_7$ m/z 1475.6 ($\text{M}+\text{H}$) $^+$, found 1476.1. HRMS (ES): calc. for $\text{C}_{89}\text{H}_{70}\text{N}_{16}\text{O}_7$ m/z 1475.5692 ($\text{M}+\text{H}$) $^+$, found 1475.5702. $\lambda_{\text{max}}(10^{-3}\varepsilon, \text{M}^{-1}\text{cm}^{-1})$ 424 (498), 518 (50)

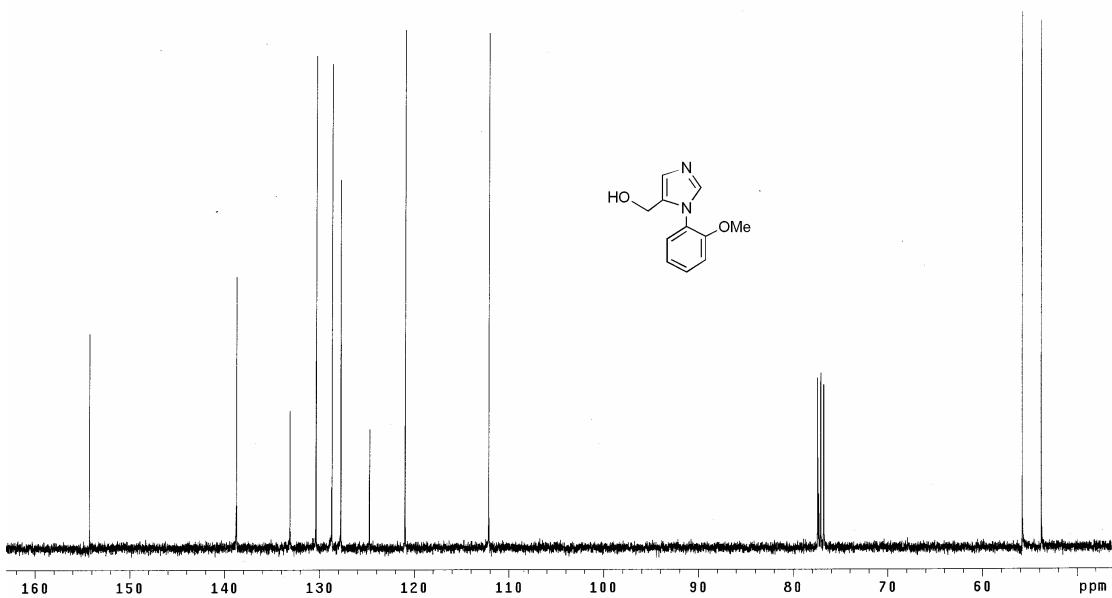


2c: ^1H NMR (CDCl_3): δ 8.77 (m, 11H), 8.45 (d, 1H, $J = 7.0$ Hz), 8.38 (s, 1H), 8.16 (d, 1H, $J = 6.0$ Hz), 8.04 (d, 1H, $J = 6.0$ Hz), 7.81 (m, 7H), 7.57 (q, 2H, $J = 6.0$ Hz), 7.50 (m, 3H), 6.86 (d, 2H, $J = 6.4$ Hz), 6.84 (s, 2H), 6.41 (d, 2H, $J = 6.4$ Hz), 6.28 (t, 1H, $J = 6.4$ Hz), 6.26 (d, 1H, $J = 6.4$ Hz), 6.14 (d, 1H, $J = 6.4$ Hz), 6.08 (s, 1H), 5.85 (s, 1H), 5.67 (s, 1H), 3.82 (s, 2H), 3.52 (s, 6H), 3.45 (s, 3H), 2.99 (s, 1H), -2.63 (s, 2H). MS (ES): calc. for $\text{C}_{75}\text{H}_{54}\text{N}_{14}\text{O}_6$ m/z 1247.5 ($\text{M}+\text{H}$) $^+$, found 1247.7. $\lambda_{\text{max}}(10^{-3}\varepsilon, \text{M}^{-1}\text{cm}^{-1})$ 424 (457), 518 (46)

¹H NMR of compound **11**



¹³C NMR of compound **11**

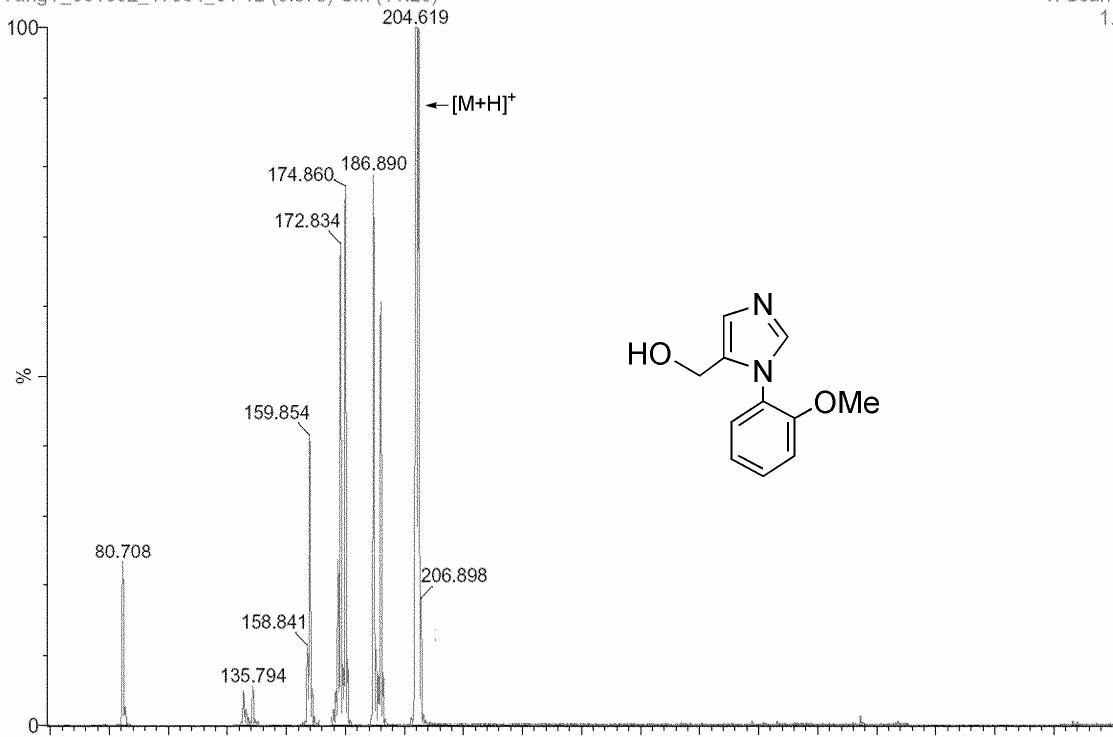


LRMS of compound **11**

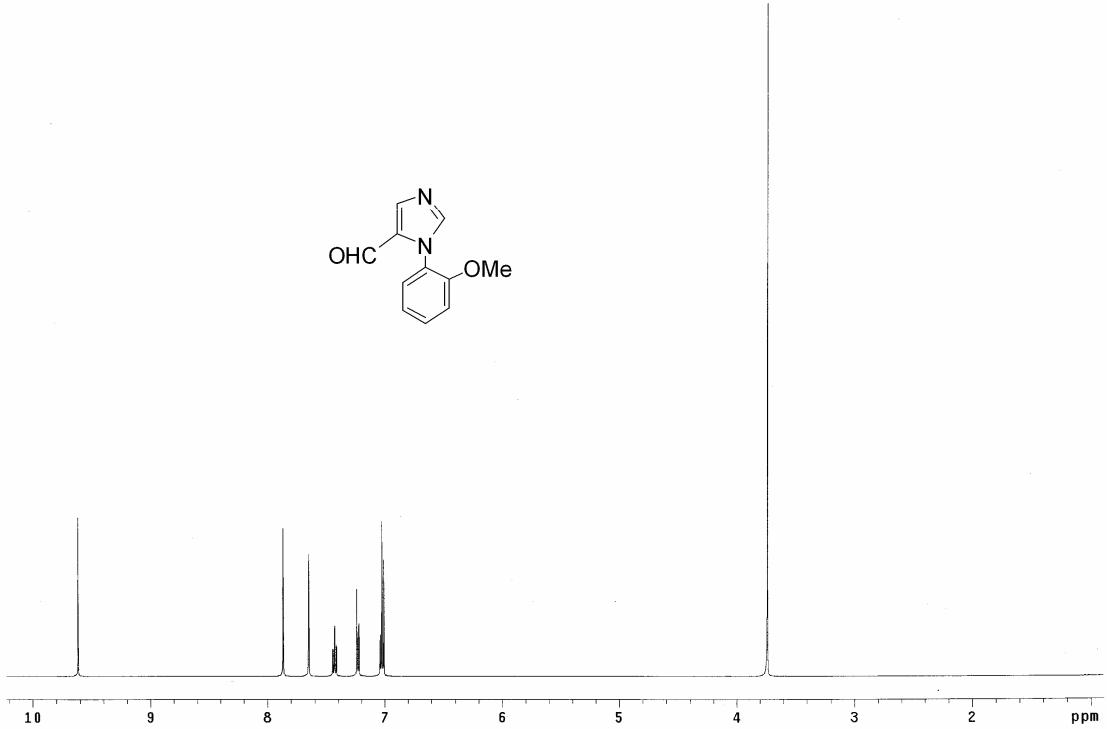
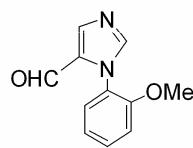
YY-III-81

YangY_061002_17564_01 12 (0.878) Cm (11:29)

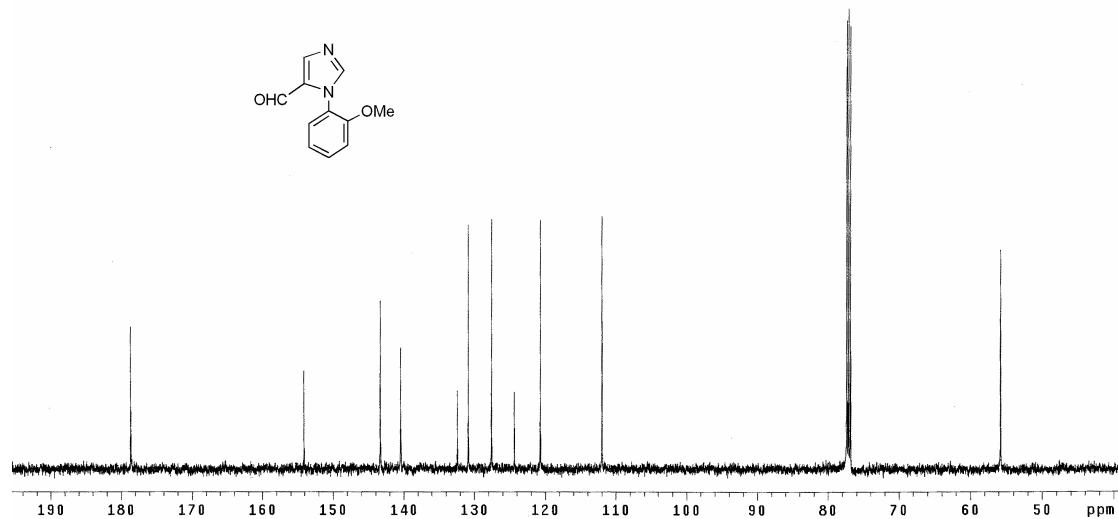
1: Scan ES+
1.34e{



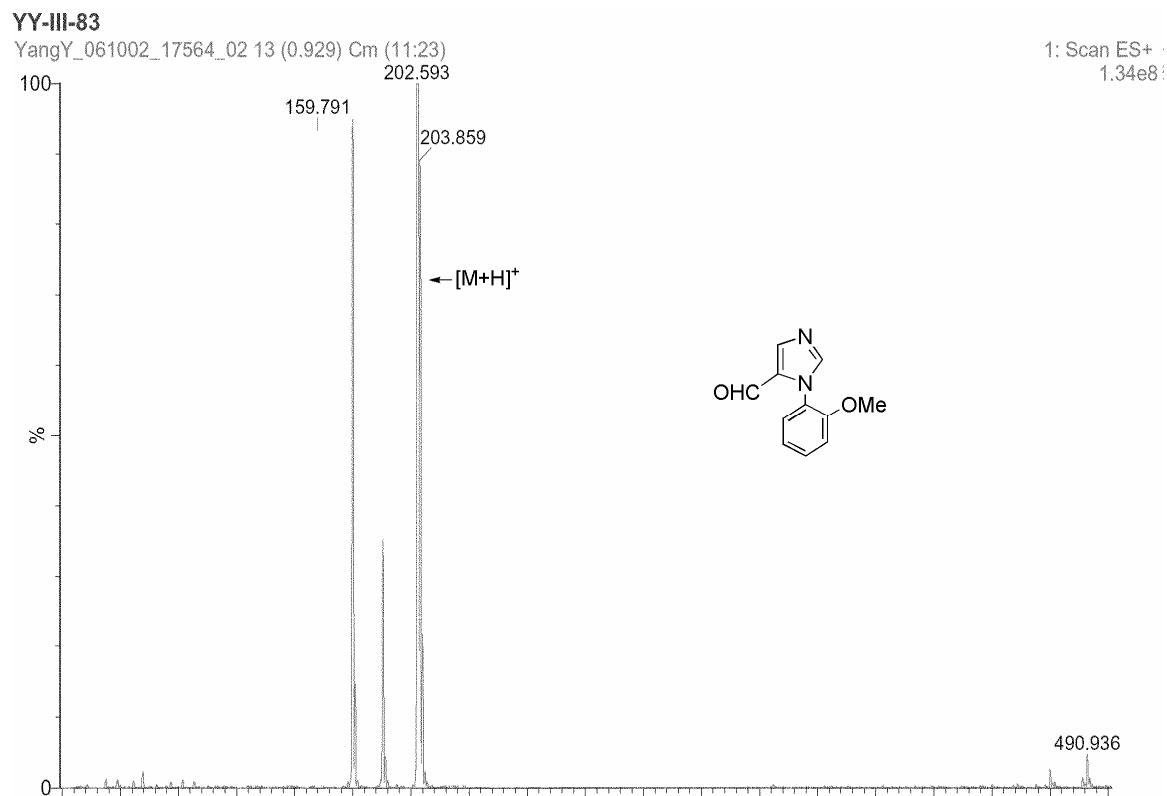
^1H NMR of compound **12**



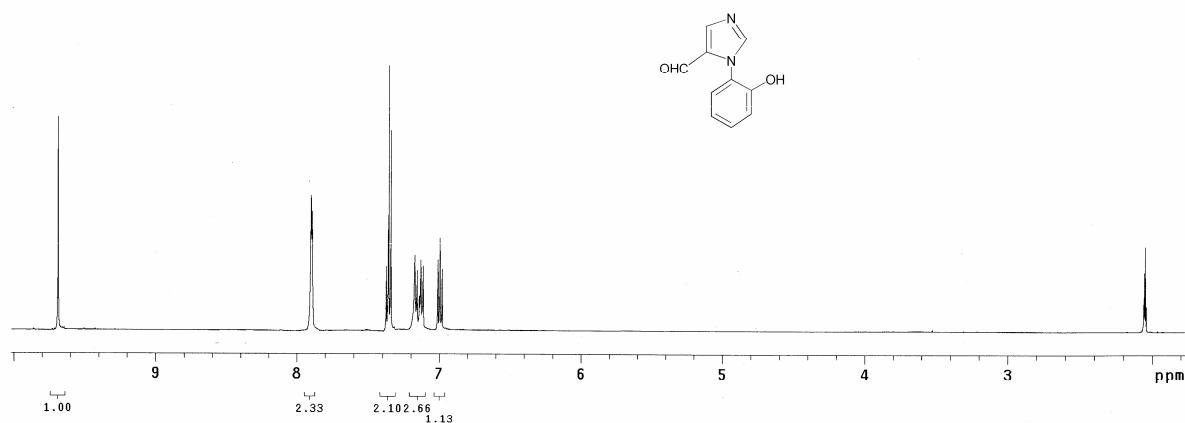
¹³C NMR of compound **12**



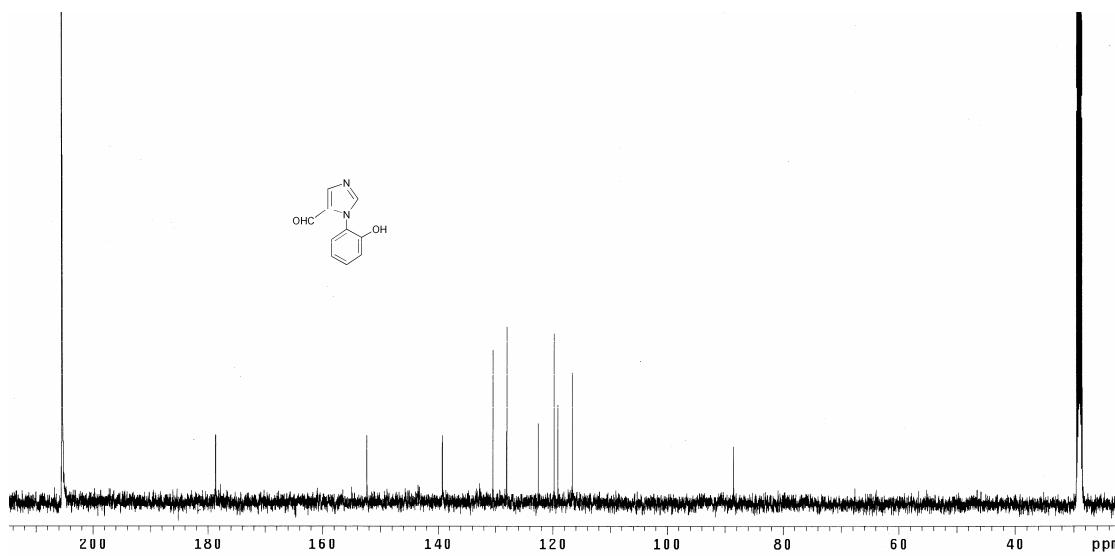
LRMS of compound **12**



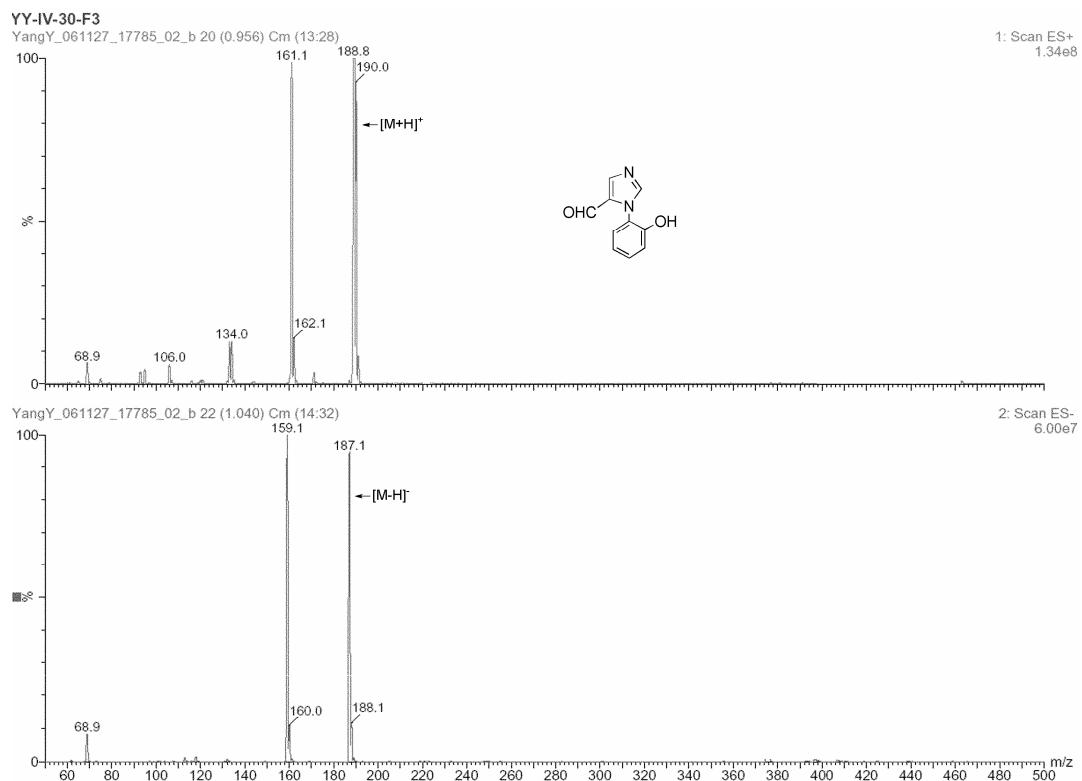
¹H NMR of compound **13**



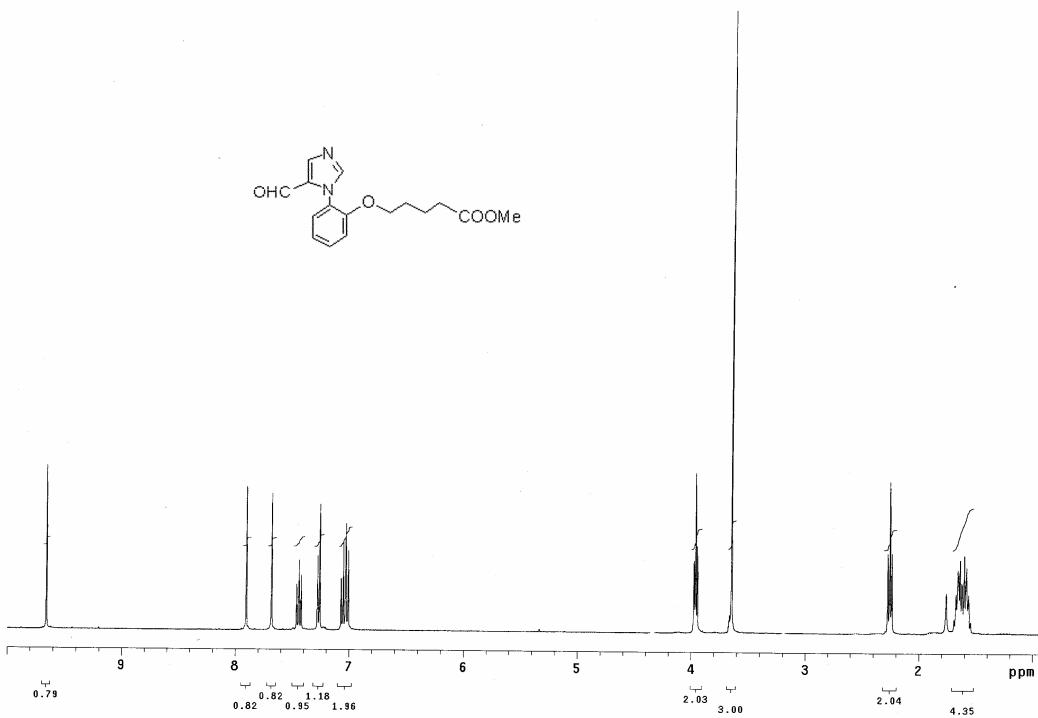
¹³C NMR of compound **13**



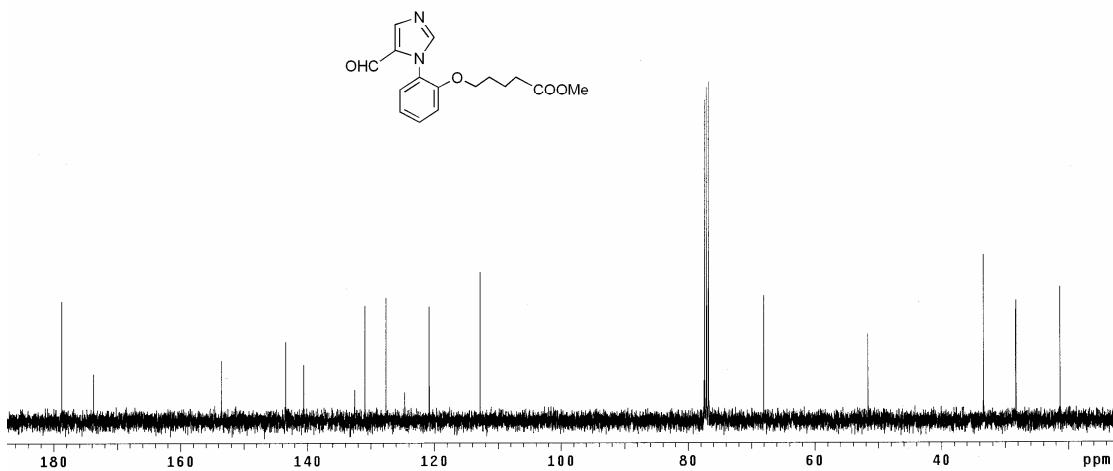
LRMS of compound 13



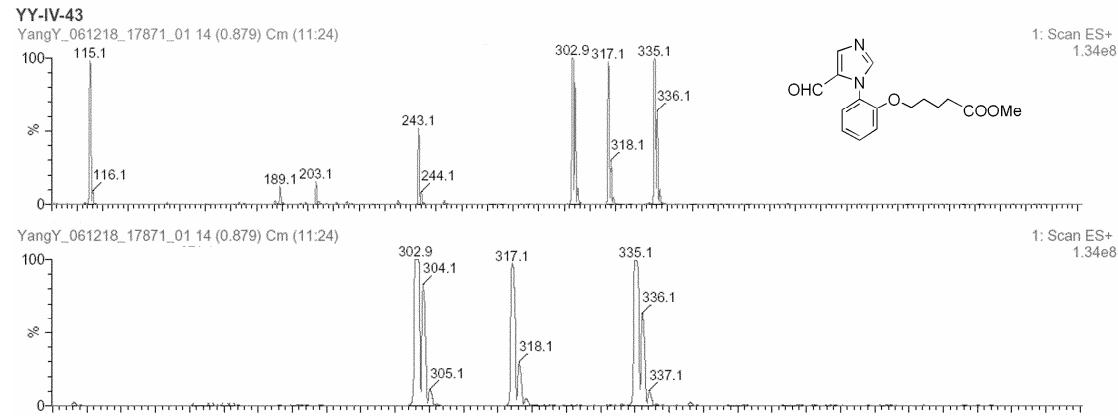
¹H NMR of compound 14



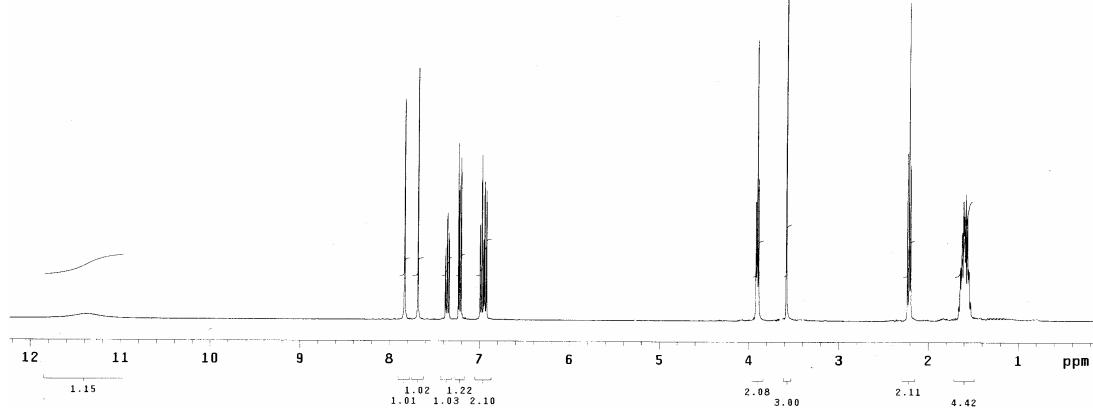
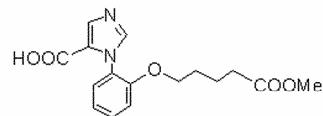
¹³C NMR of compound **14**



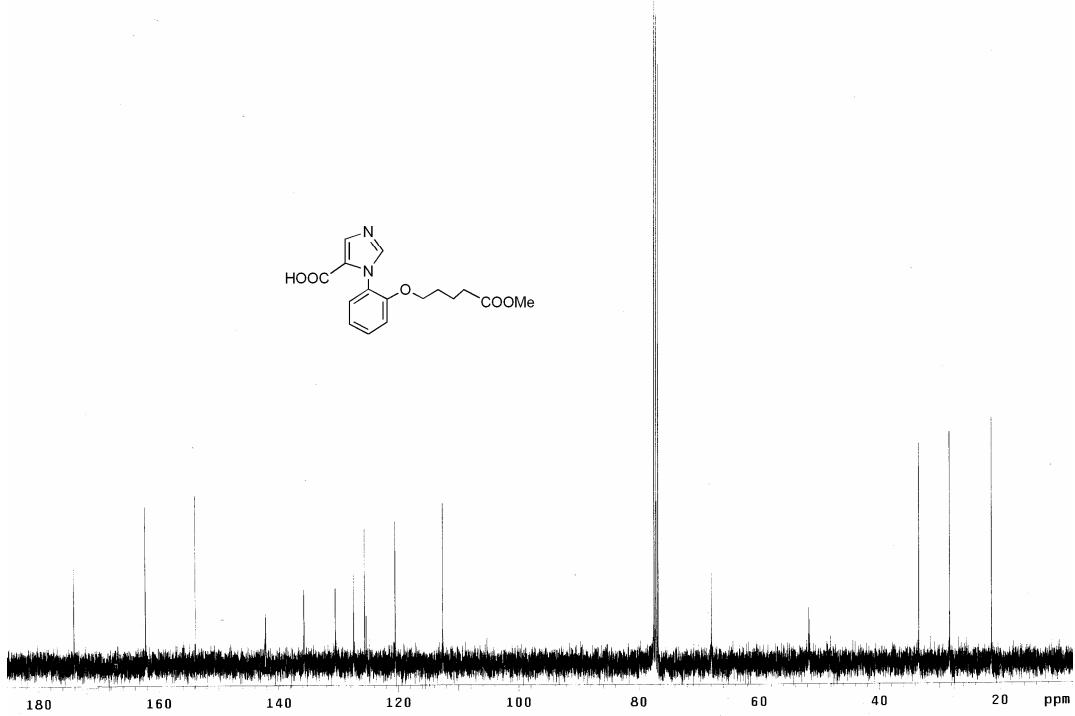
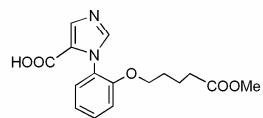
LRMS of compound **14**



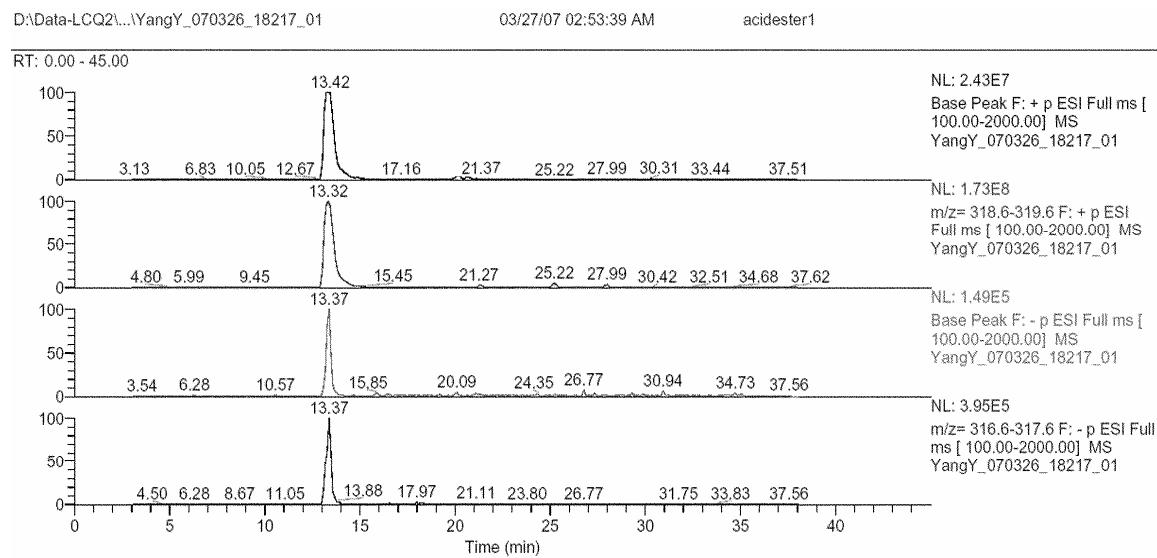
¹H NMR of imidazole acid **6**



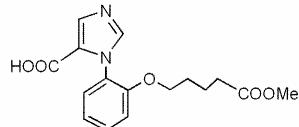
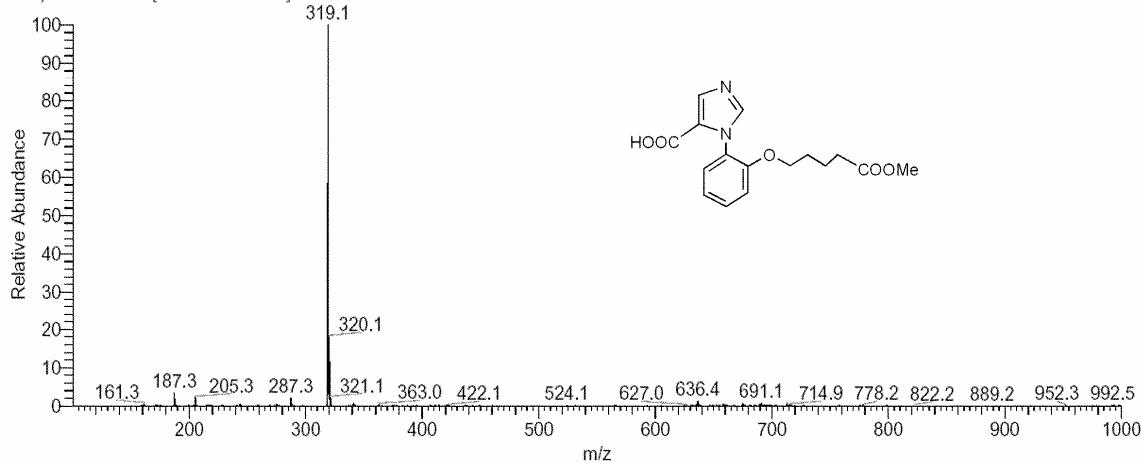
¹³C NMR of imidazole acid **6**



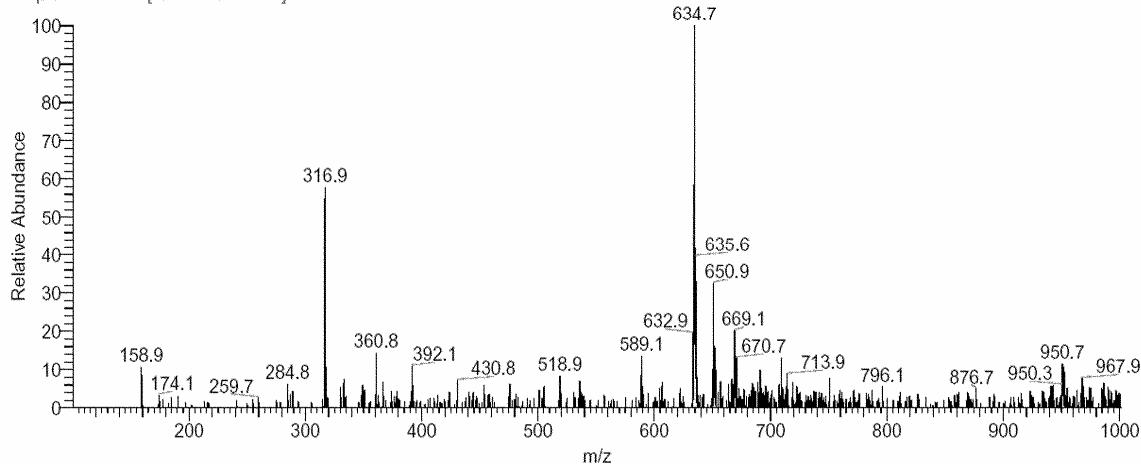
HPLC-MS of imidazole acid **6** (wavelength for HPLC detection is 254 nm)



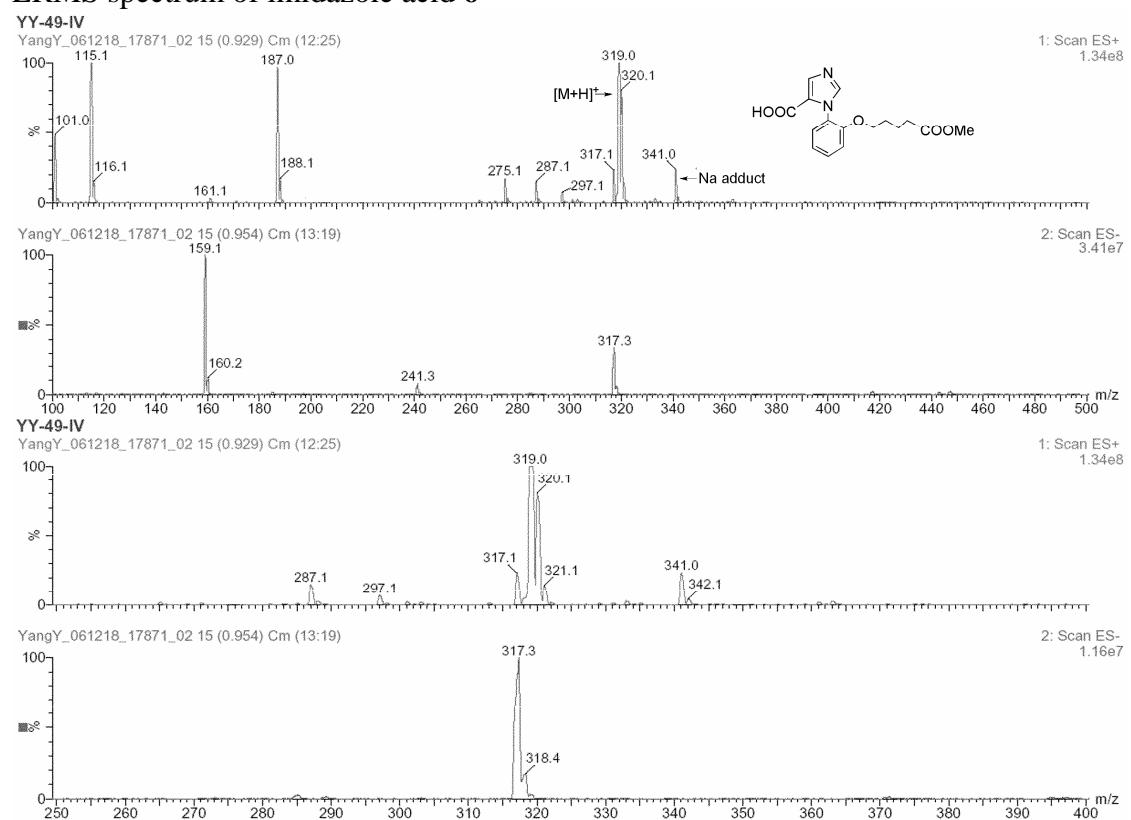
YangY_070326_18217_01 #306-322 RT: 13.03-13.83 AV: 9 NL: 1.56E7
F: + p ESI Full ms [100.00-2000.00]



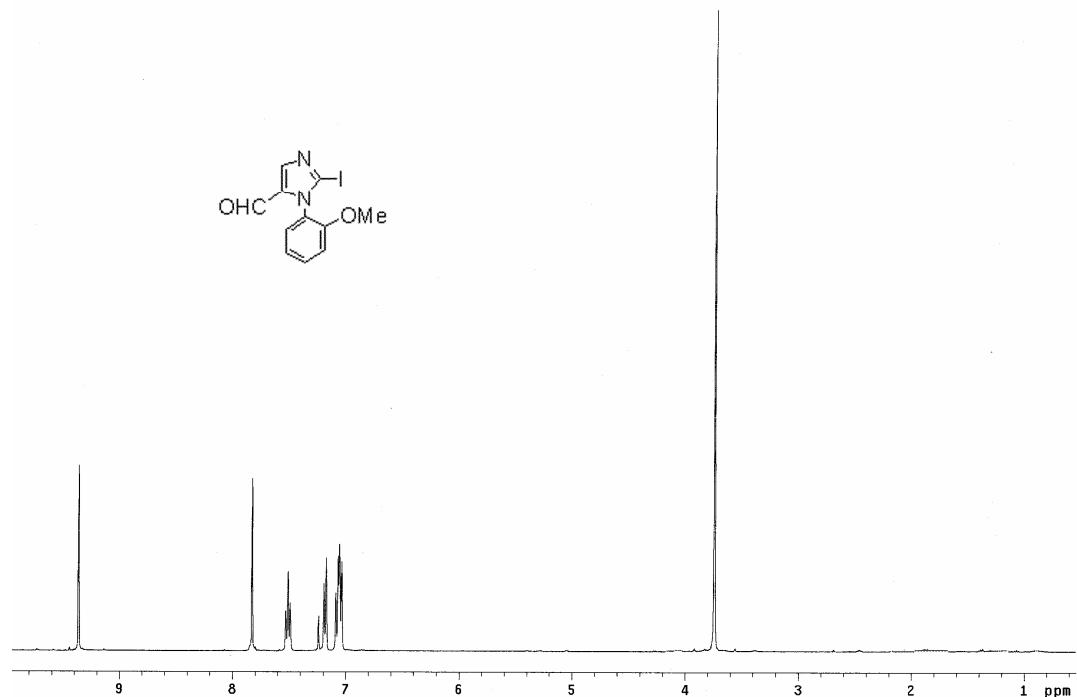
YangY_070326_18217_01 #309-316 RT: 13.18-13.47 AV: 4 NL: 8.50E4
F: - p ESI Full ms [100.00-2000.00]



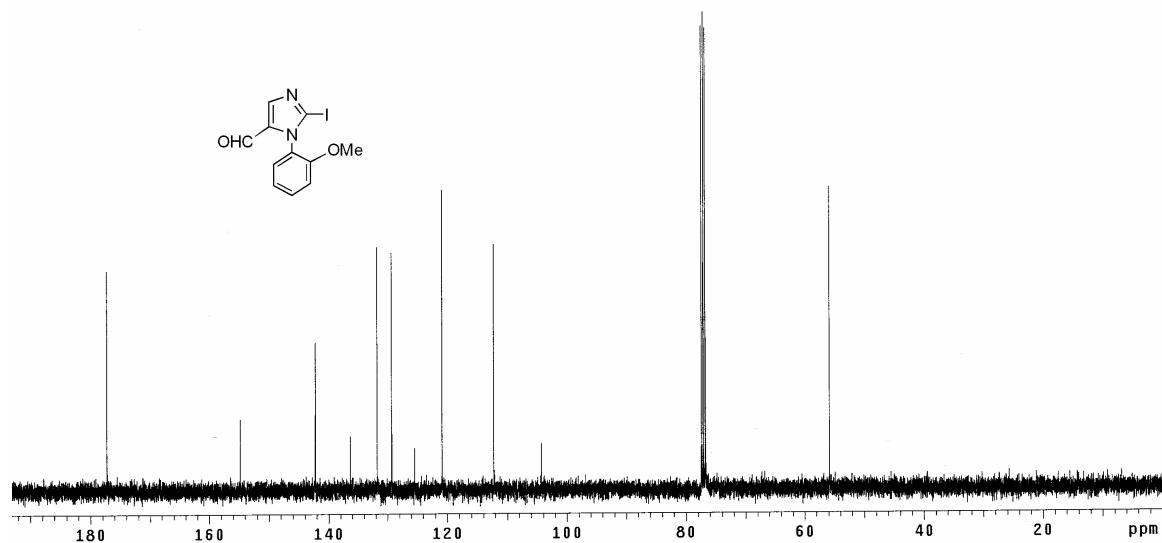
LRMS spectrum of imidazole acid **6**



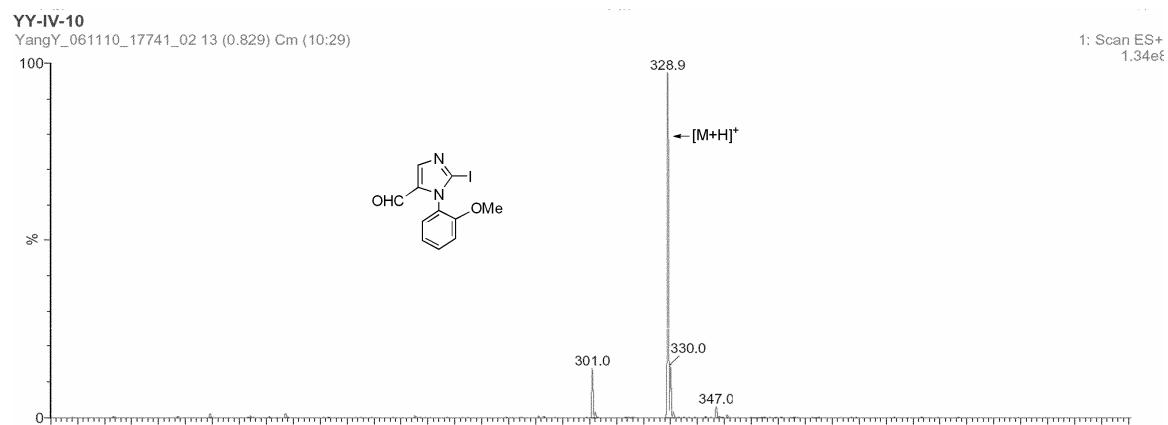
¹H NMR of compound **15**



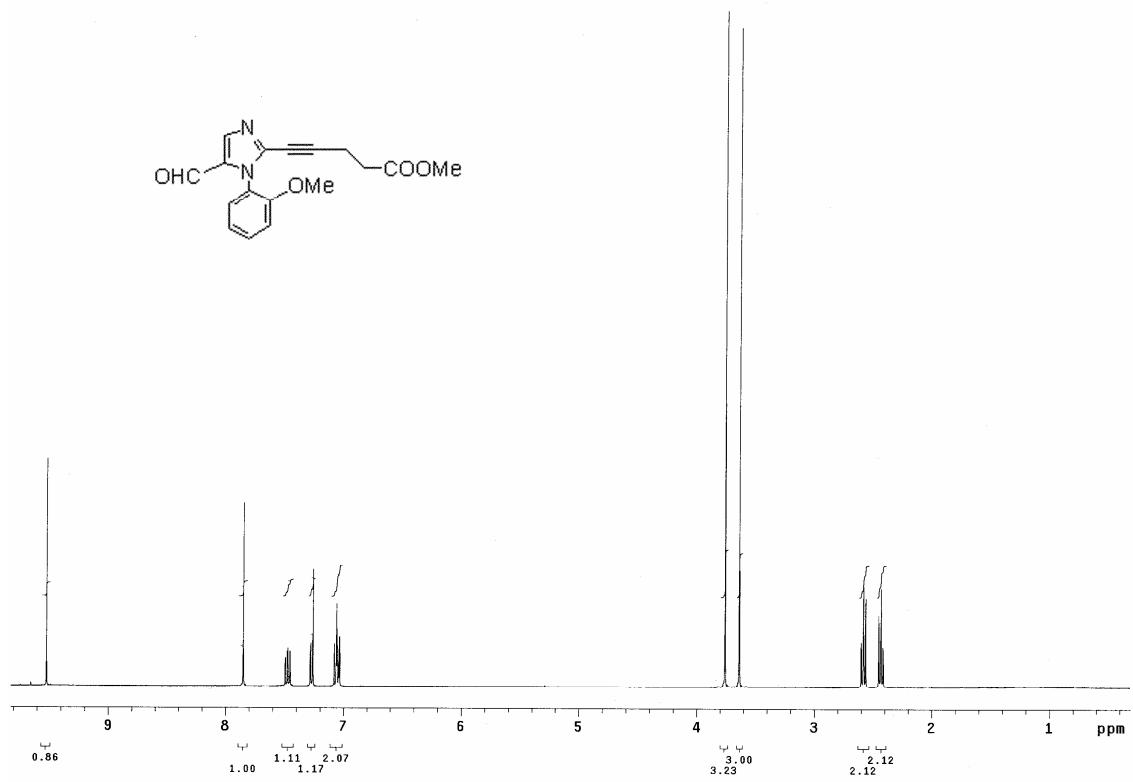
¹³C NMR of compound **15**



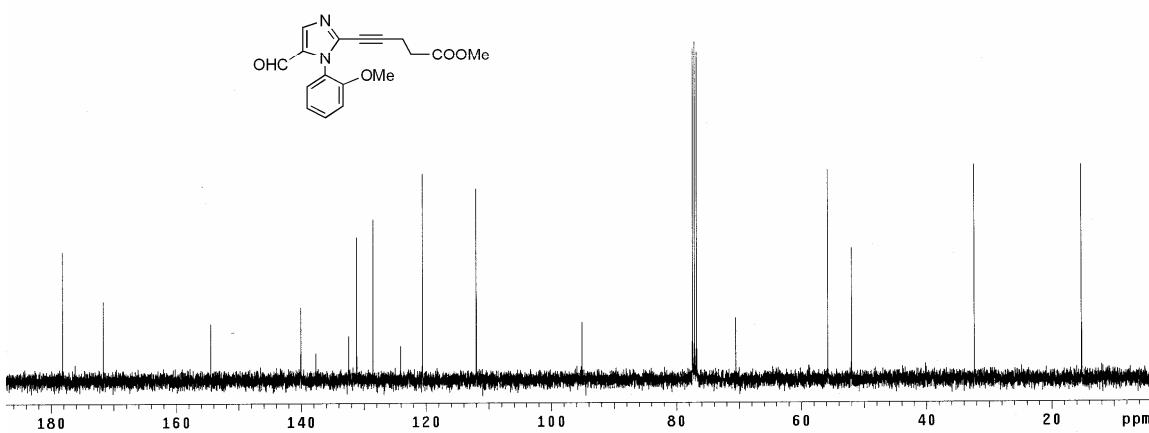
LRMS of compound **15**



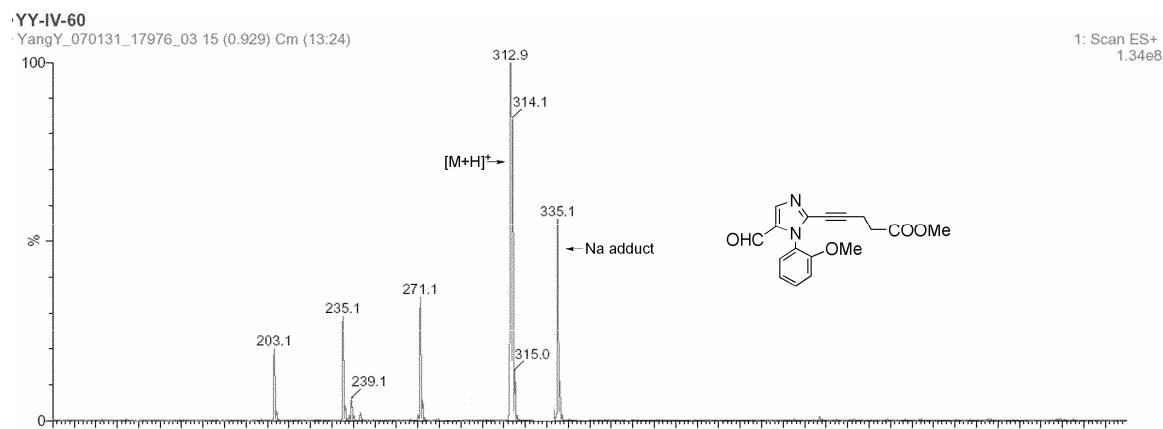
¹H NMR of compound **16**



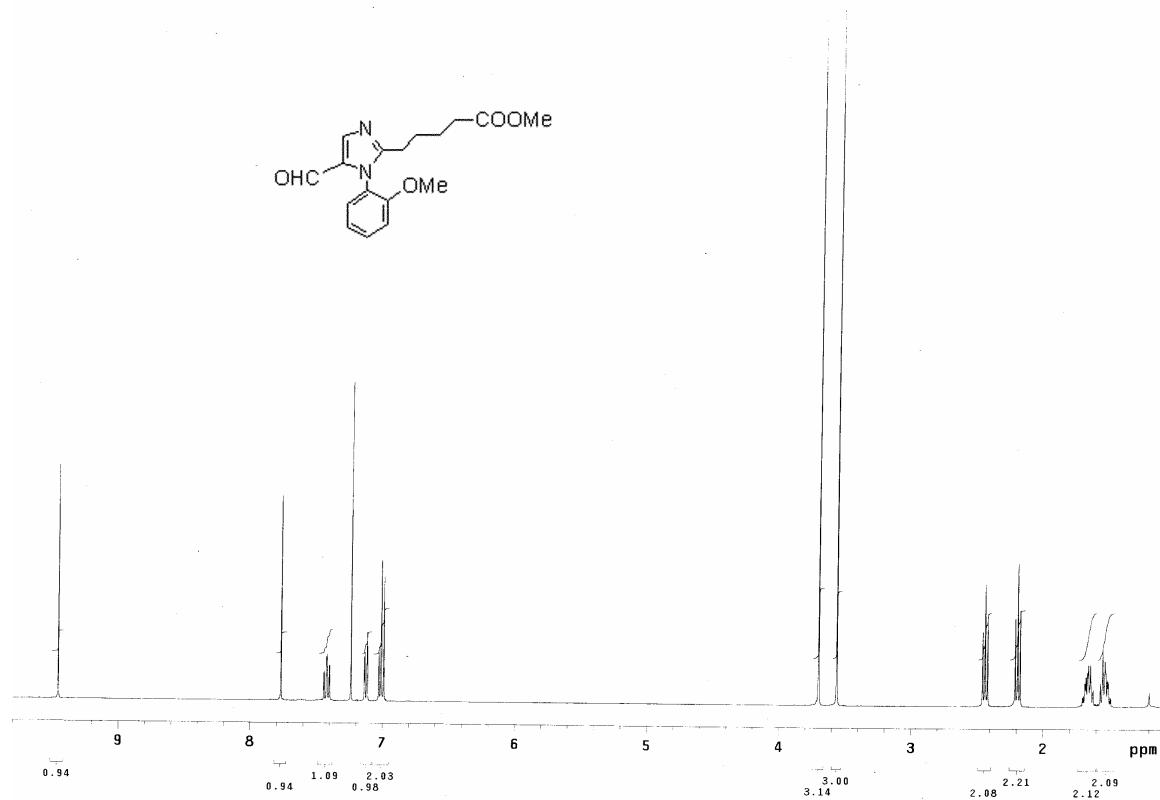
¹³C NMR of compound **16**



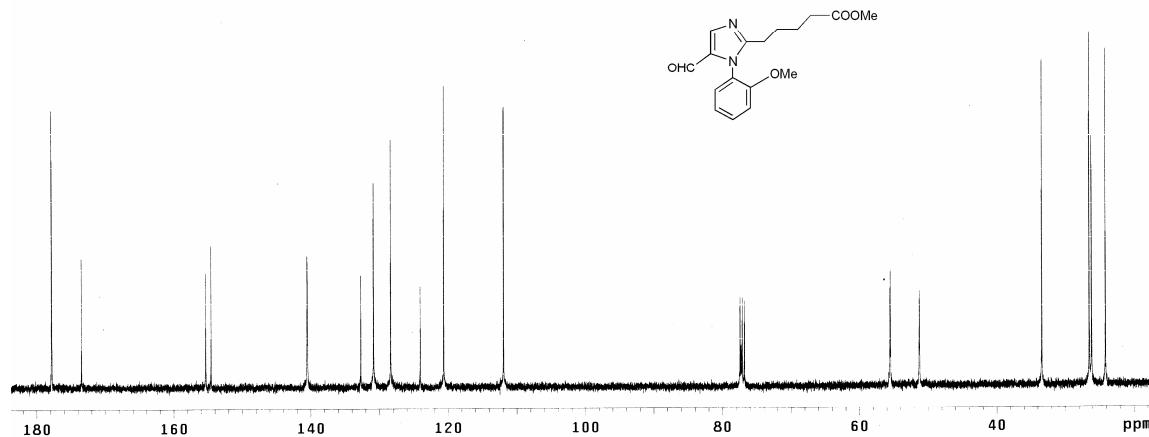
LRMS of compound **16**



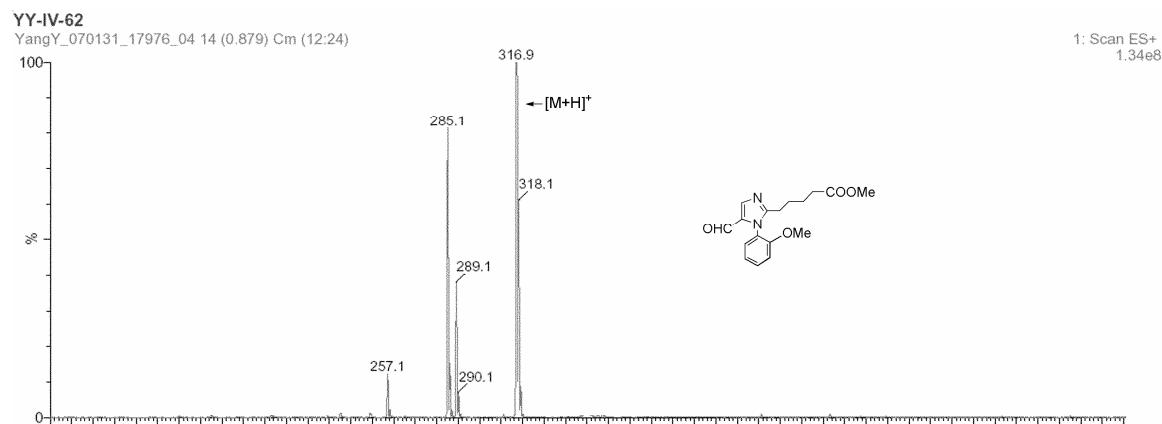
¹H NMR of compound **17**



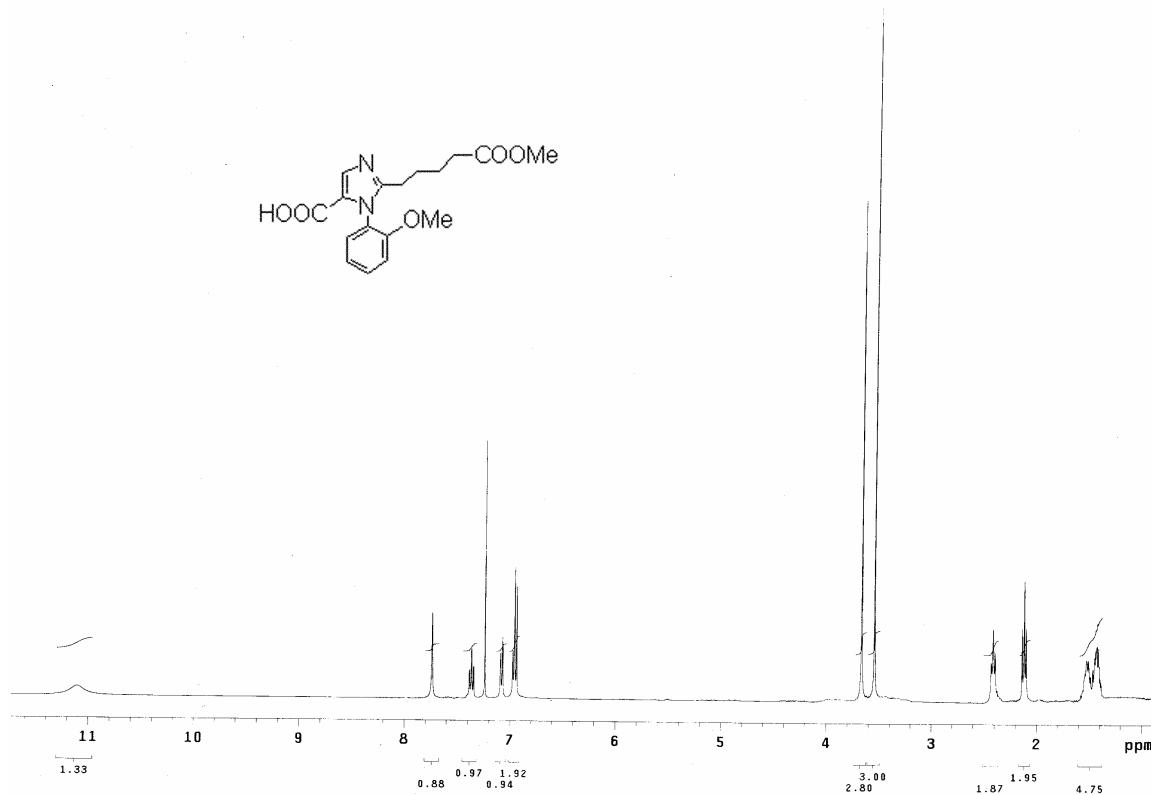
¹³C NMR of compound **17**



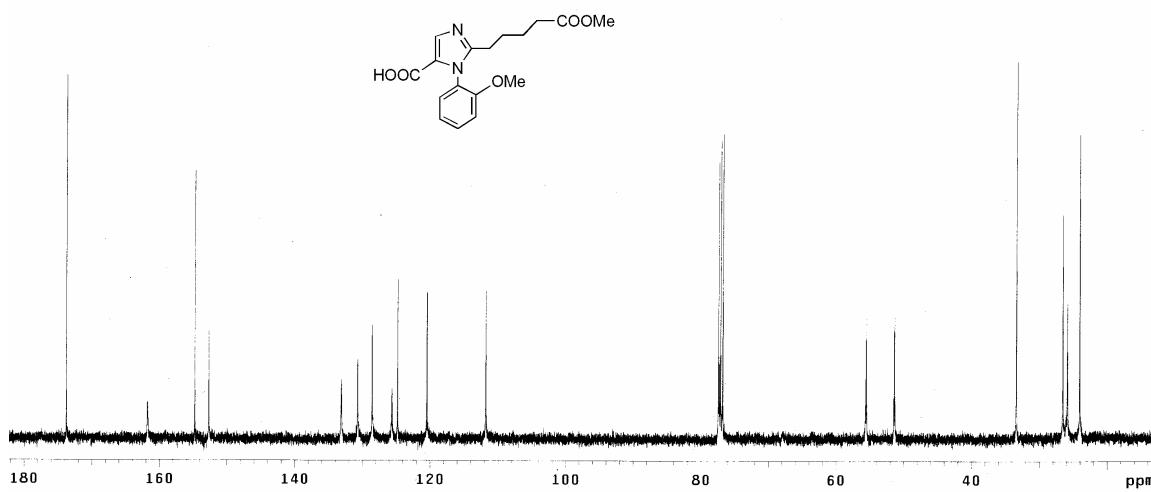
LRMS of compound **17**



¹H NMR of compound 7



¹³C NMR of compound 7

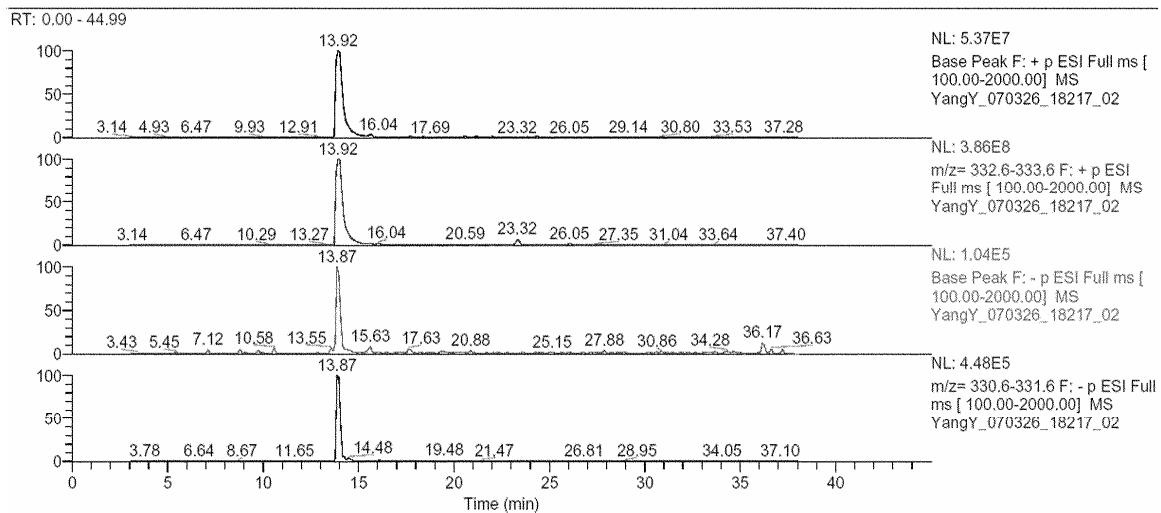


HPLC-MS of compound 7 (wavelength for HPLC detection is 254 nm)

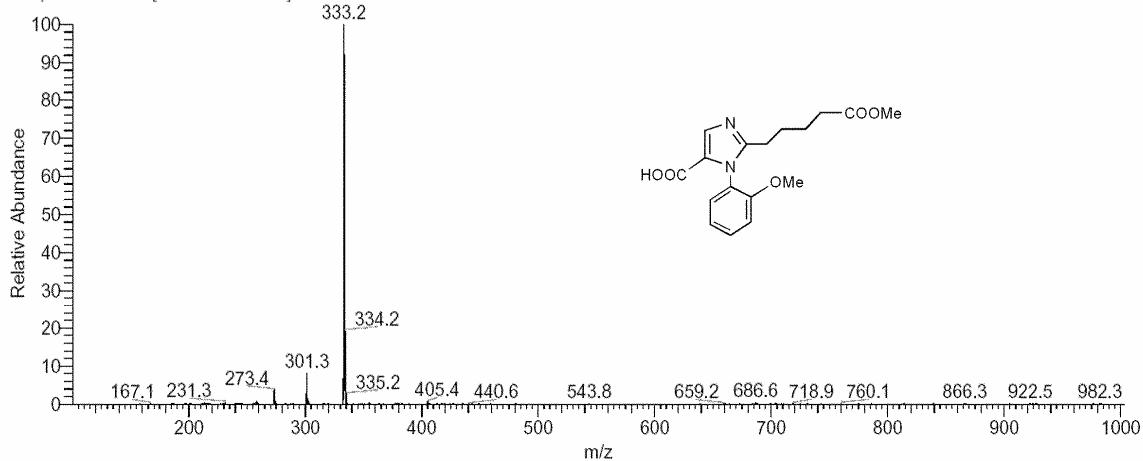
D:\Data-LCQ2\...\YangY_070326_18217_02

03/27/07 04:25:30 AM

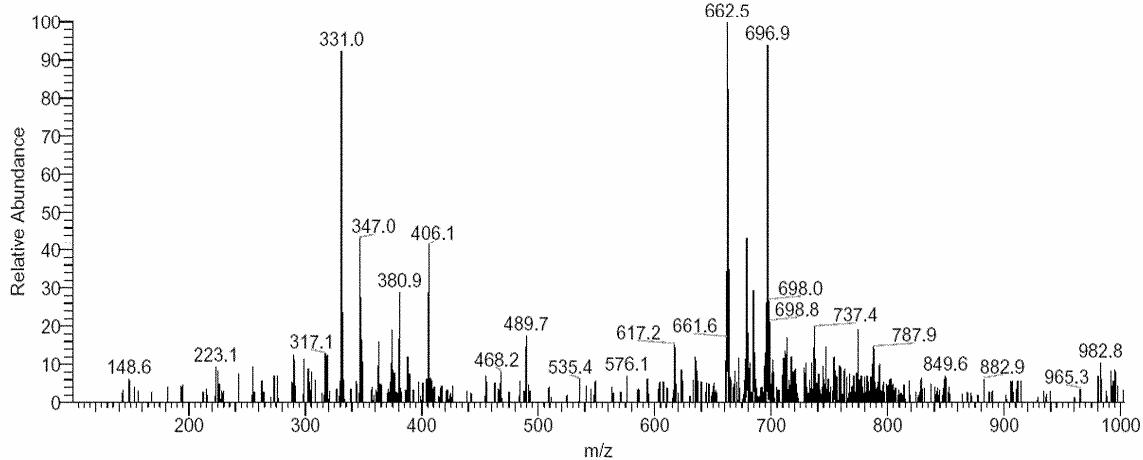
acidester2



YangY_070326_18217_02 #320-331 RT: 13.83-14.33 AV: 6 NL: 3.63E7
F: + p ESI Full ms [100.00-2000.00]



YangY_070326_18217_02 #320-326 RT: 13.87-14.07 AV: 3 NL: 5.78E4
F: - p ESI Full ms [100.00-2000.00]

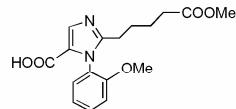
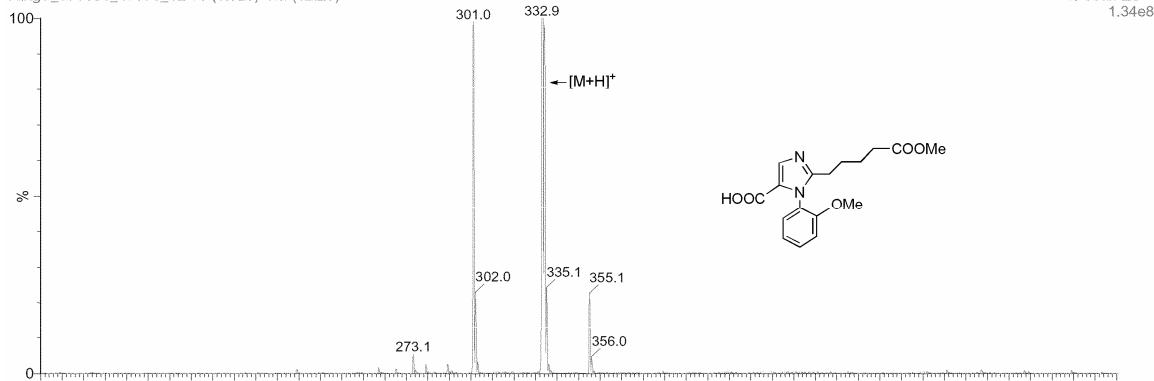


LRMS of compound 7

YY-IV-70

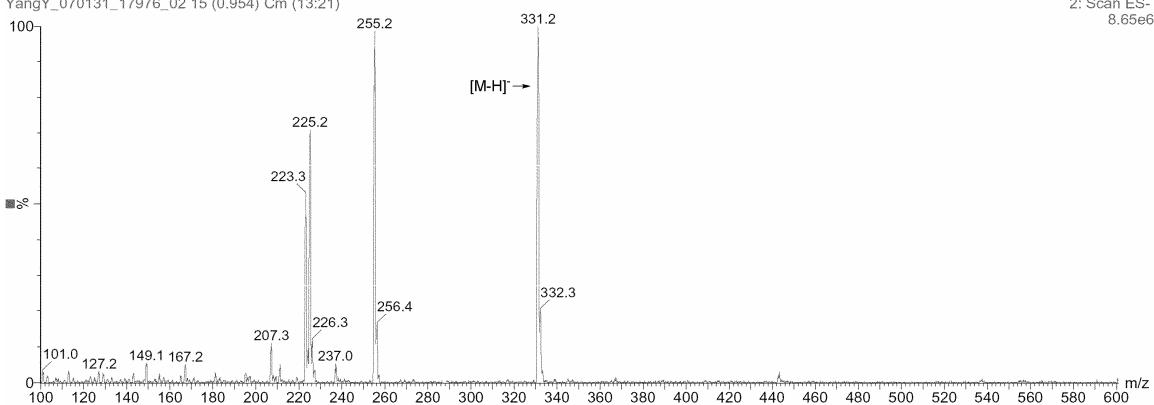
YangY_070131_17976_02 15 (0.929) Cm (12:25)

1: Scan ES+
1.34e8

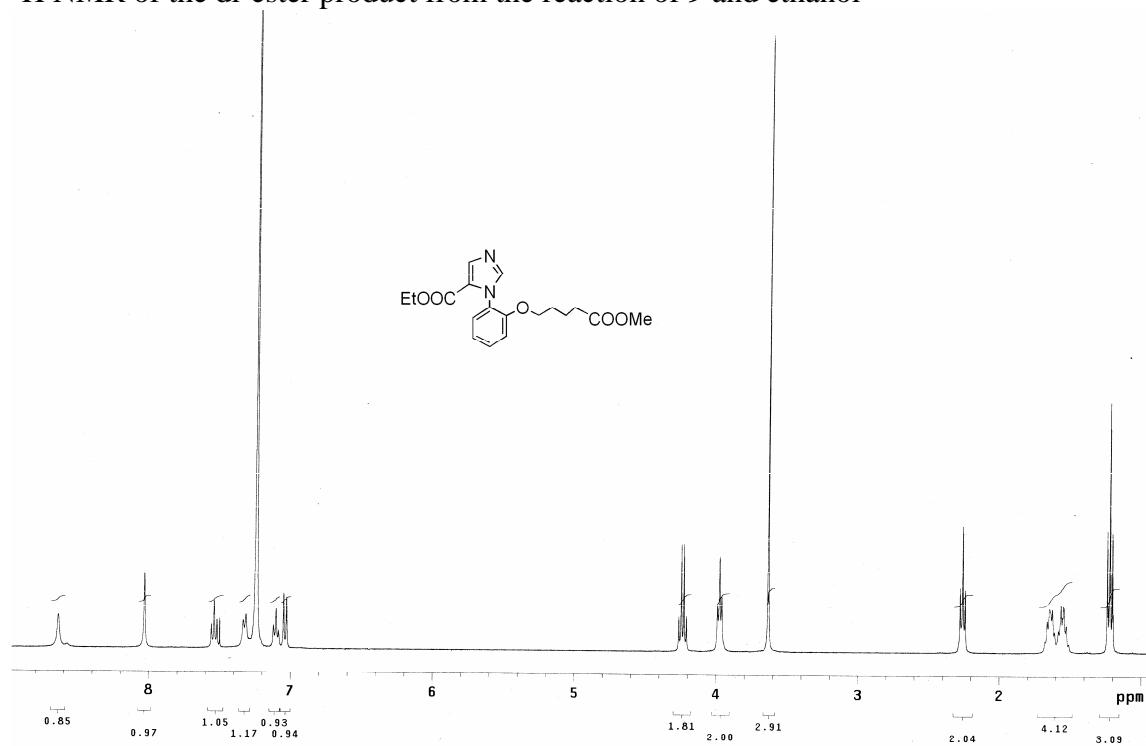


YangY_070131_17976_02 15 (0.954) Cm (13:21)

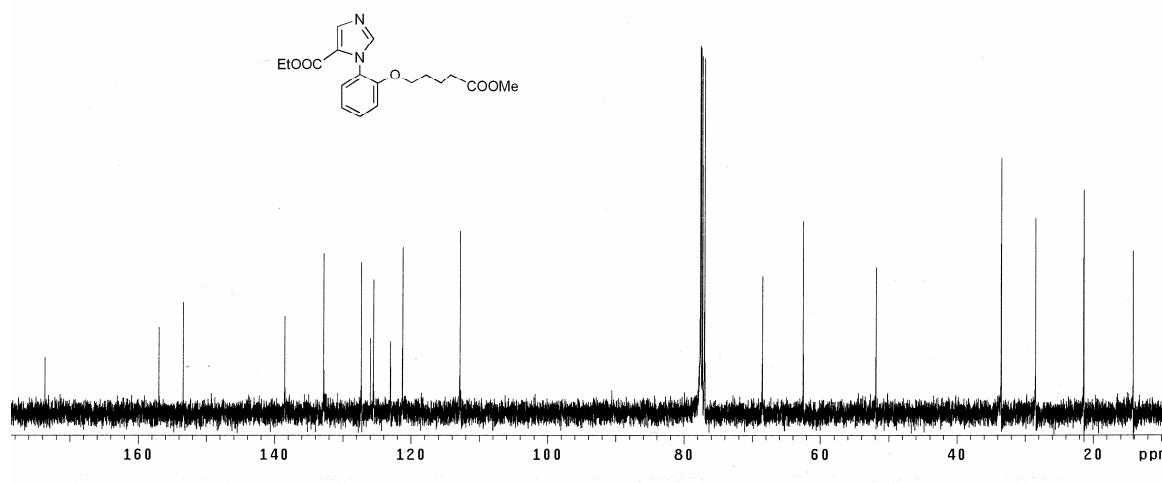
2: Scan ES-
8.65e6



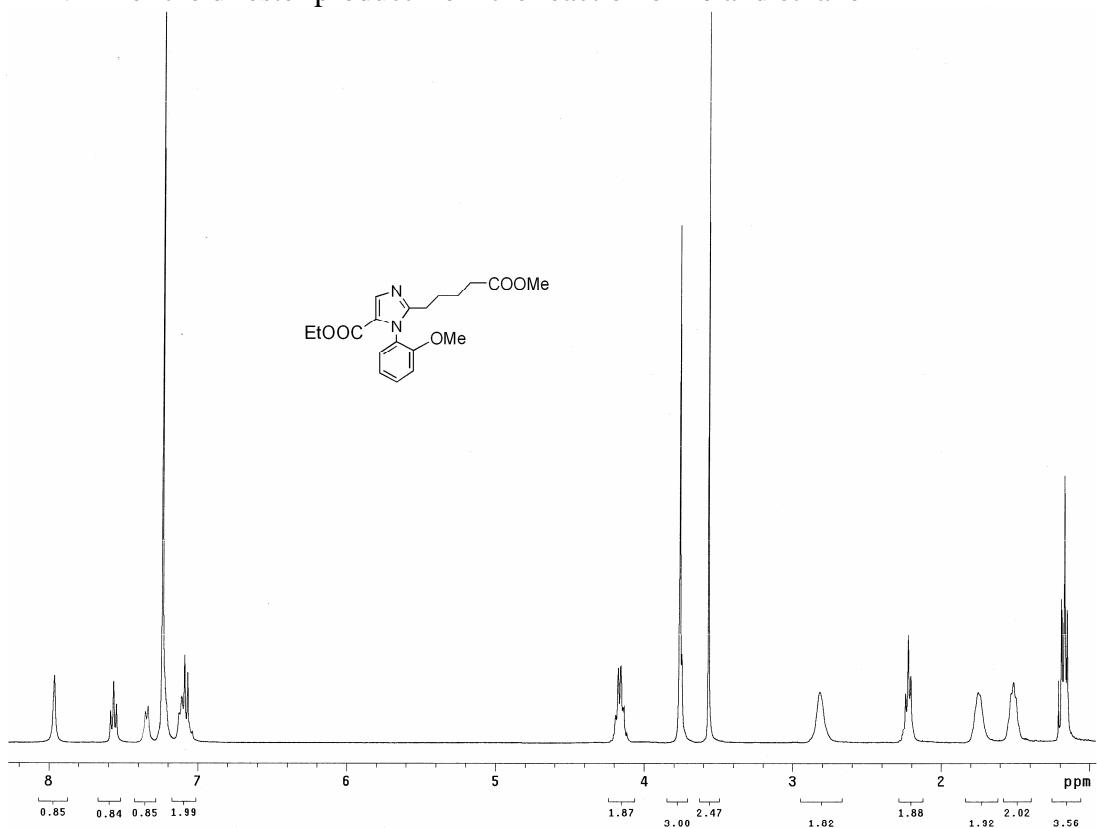
¹H NMR of the di-ester product from the reaction of **9** and ethanol



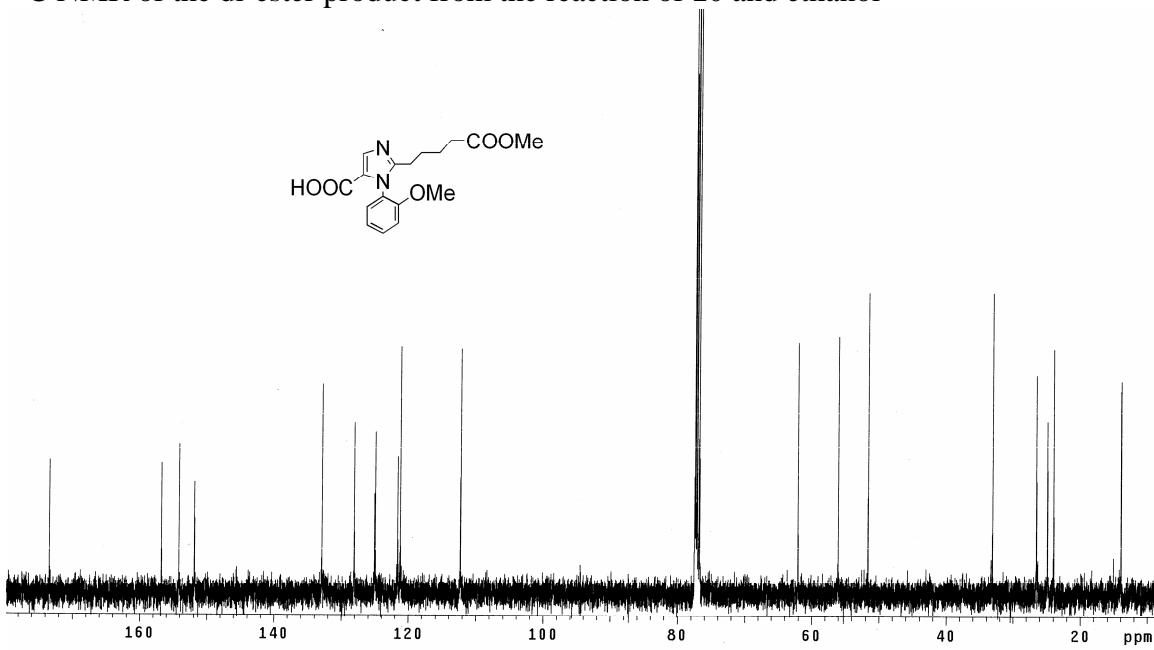
¹³C NMR of the di-ester product from the reaction of **9** and ethanol



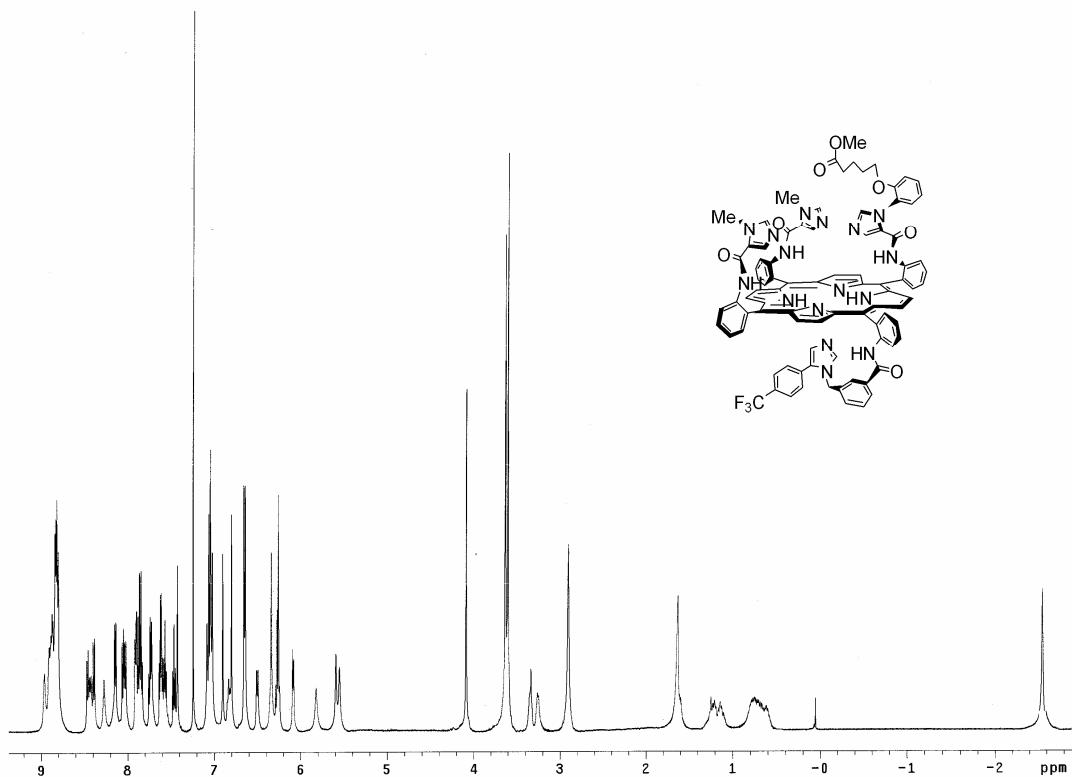
¹H NMR of the di-ester product from the reaction of **10** and ethanol



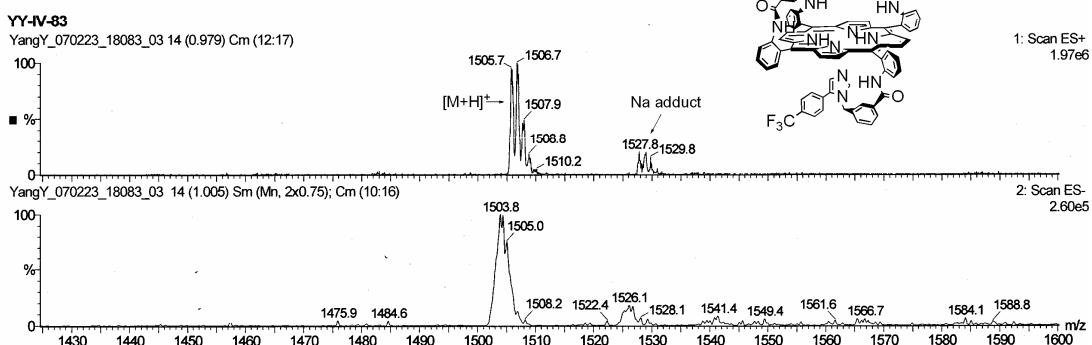
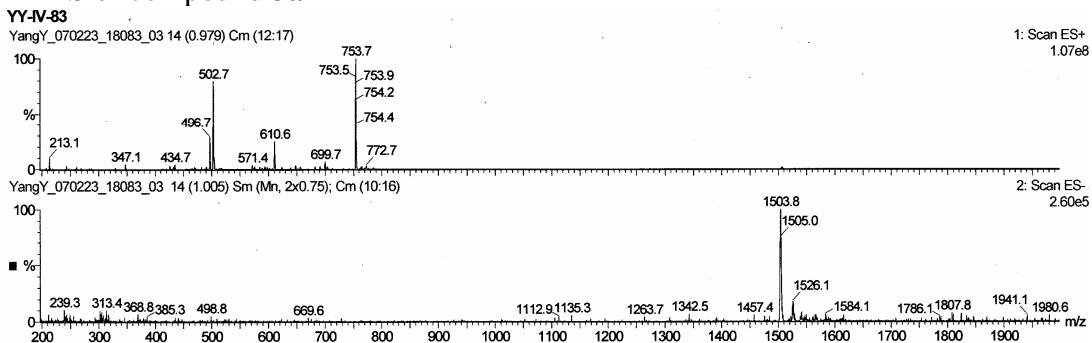
¹³C NMR of the di-ester product from the reaction of **10** and ethanol



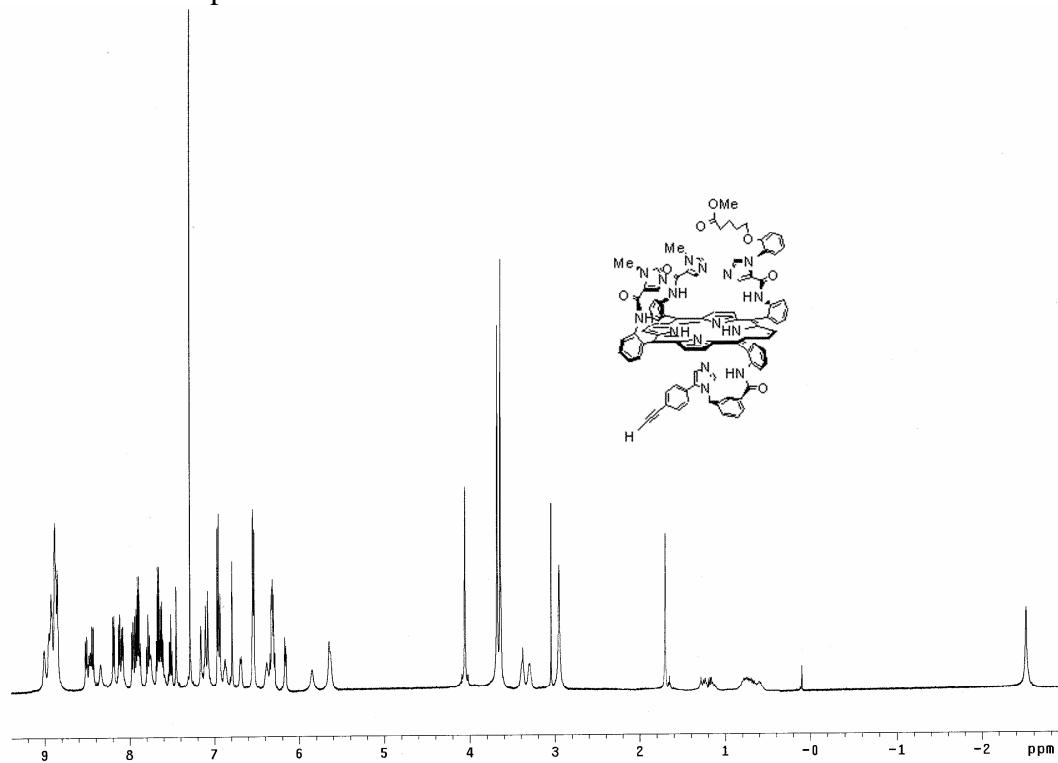
¹H NMR of compound 3a



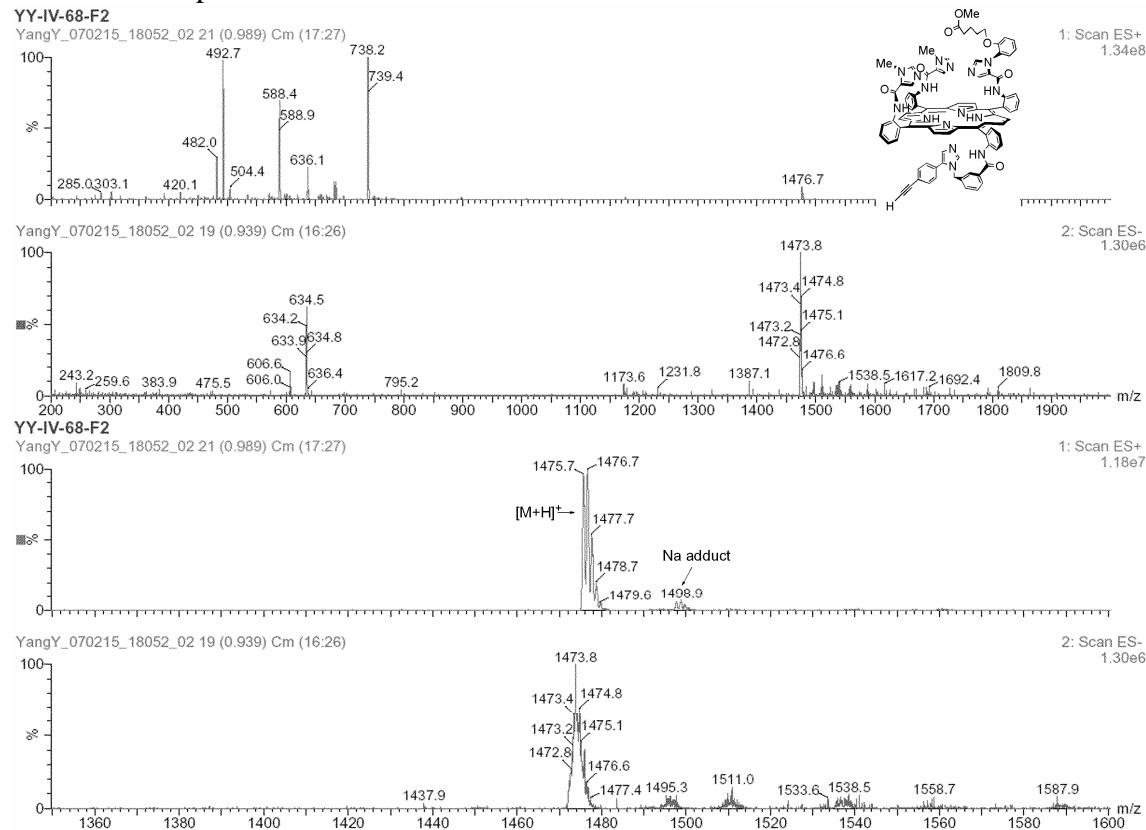
LRMS of compound 3a



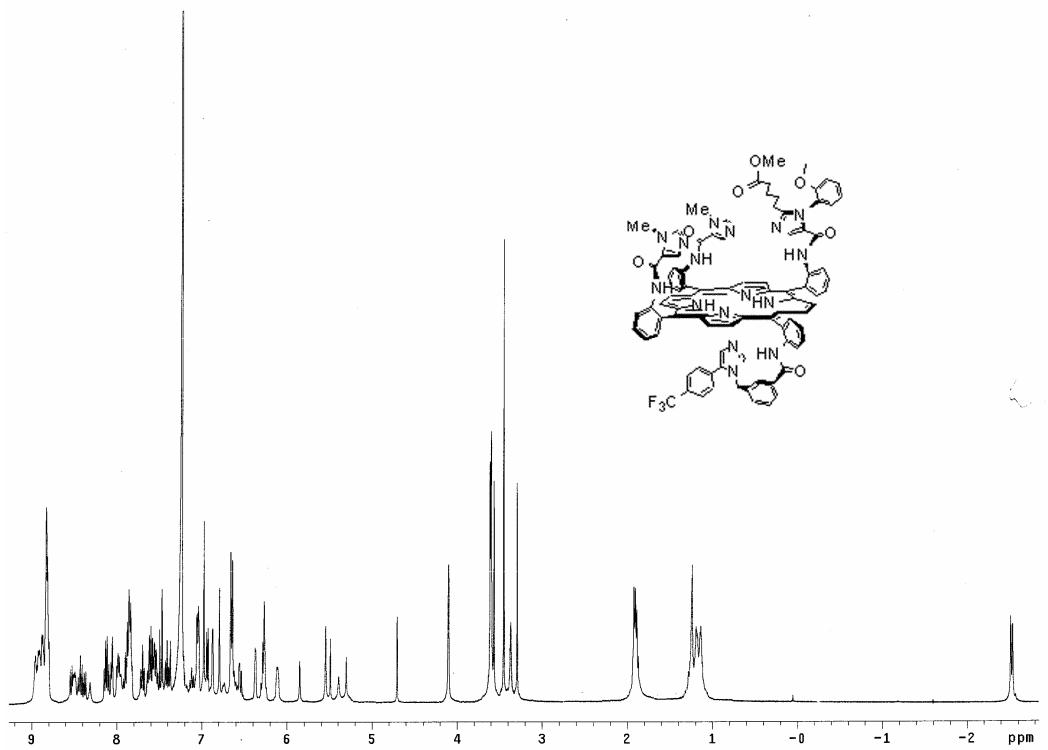
¹H NMR of compound 3b



LRMS of compound 3b



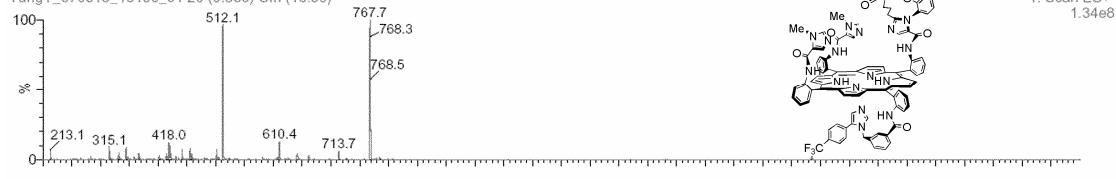
¹H NMR of compound 4a



LRMS of compound 4a

YY-IV-96

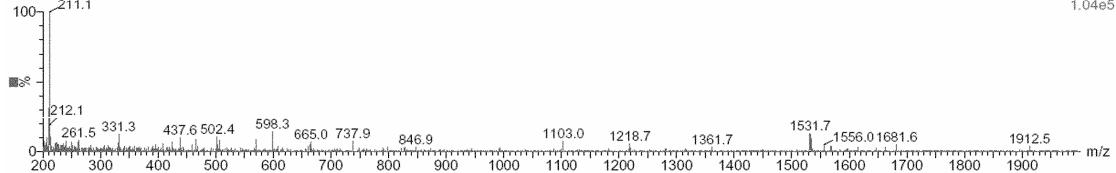
YangY_070319_18190_01 20 (0.956) Cm (16:30)



1: Scan ES+
1.34e8

YangY_070319_18190_01 20 (0.973) Sm (SG, 2x0.20); Cm (15:32)

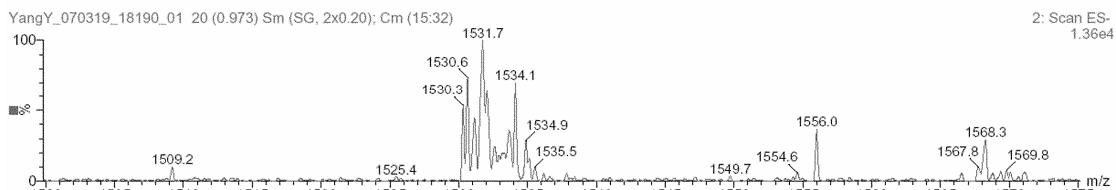
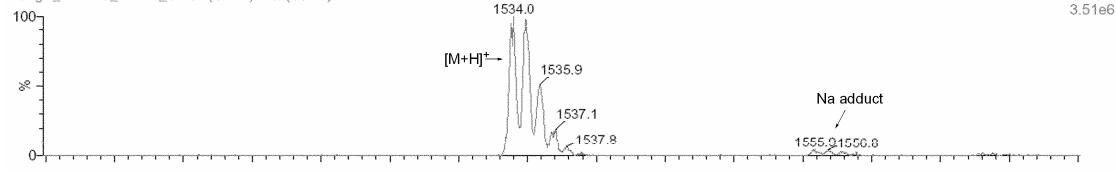
2: Scan ES-
1.04e5



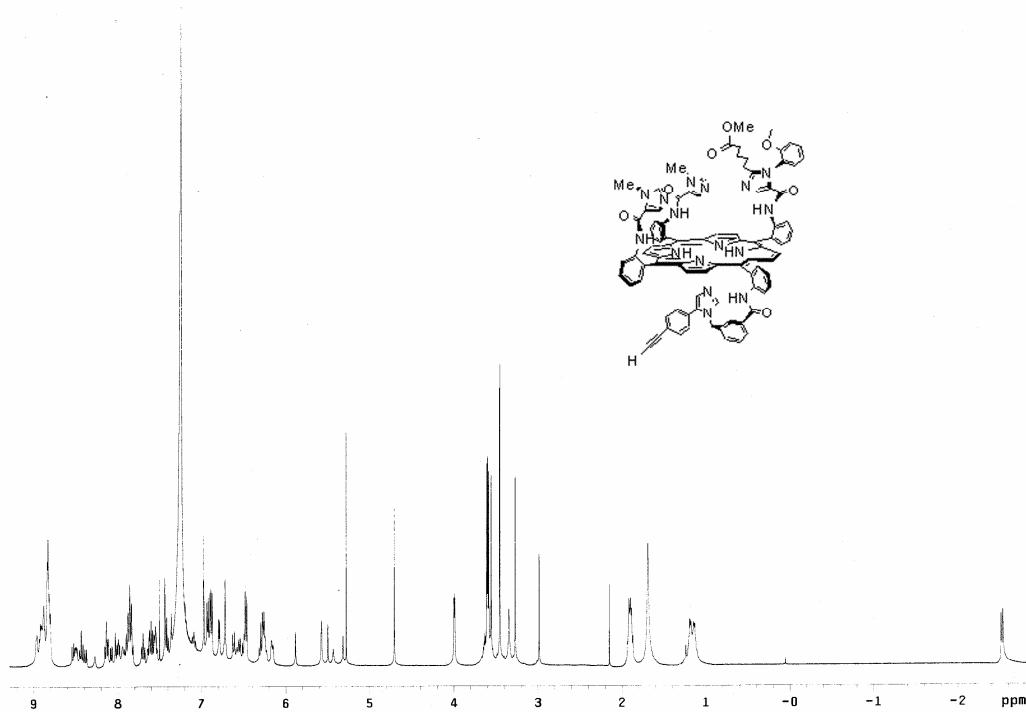
YY-IV-96

YangY_070319_18190_01 20 (0.956) Cm (16:30)

1: Scan ES+
3.51e6



¹H NMR of compound 4b

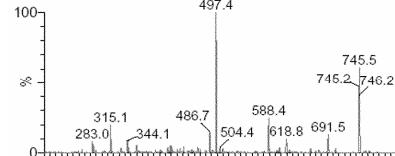


LRMS of compound 4b

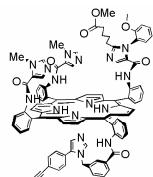
YY-IV-88-F1

YangY_070307_18157 21 (0.989) Cm (17:28)

497.4

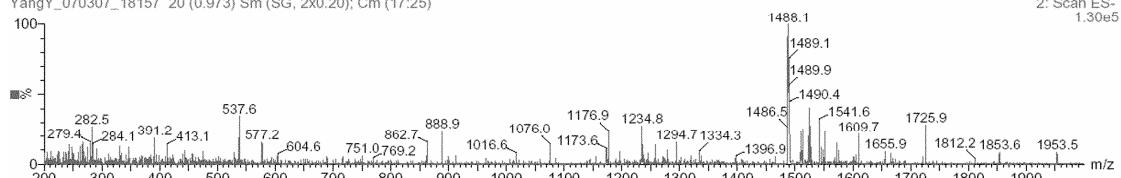


1: Scan ES+ 1.34e8



YangY_070307_18157 20 (0.973) Sm (SG, 2x0.20); Cm (17:25)

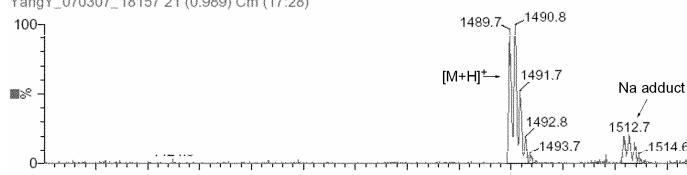
2: Scan ES- 1.30e5



YY-IV-88-F1

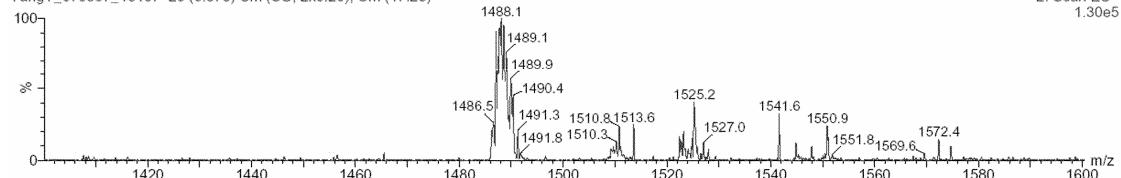
YangY_070307_18157 21 (0.989) Cm (17:28)

1: Scan ES+ 1.68e6

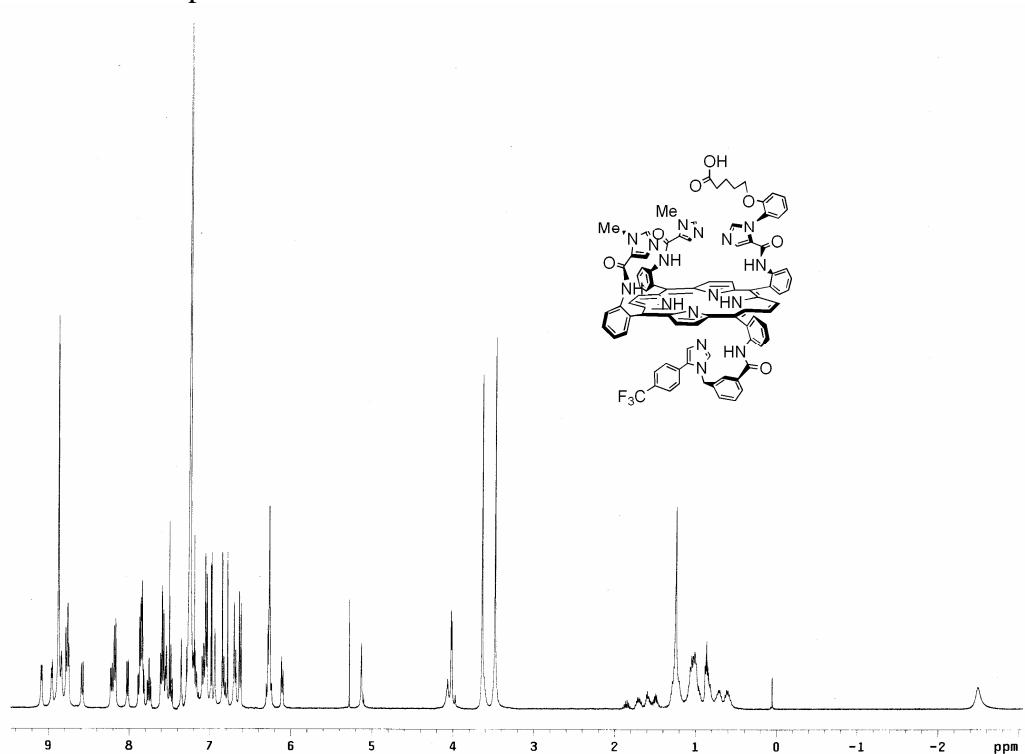


YangY_070307_18157 20 (0.973) Sm (SG, 2x0.20); Cm (17:25)

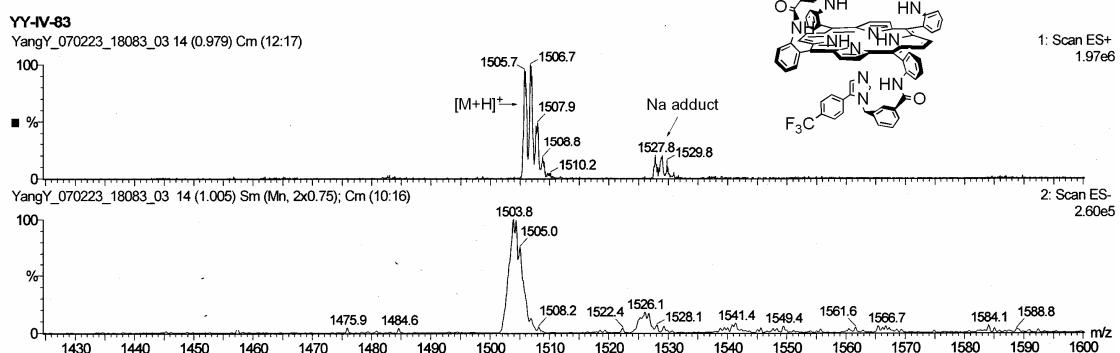
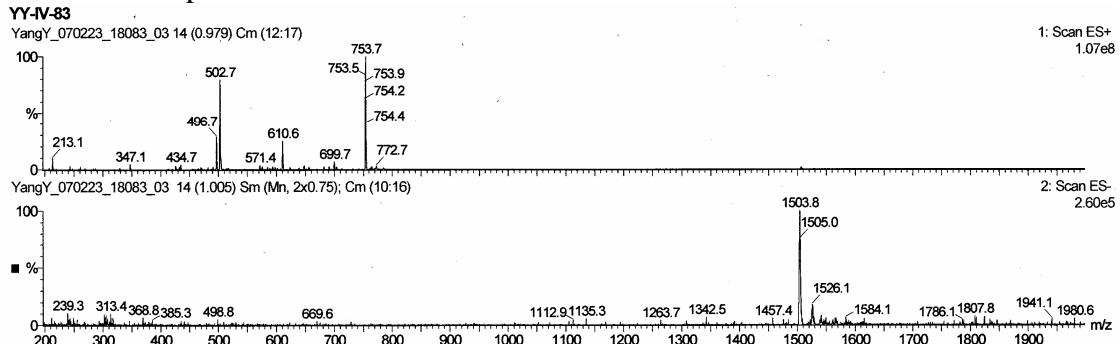
2: Scan ES- 1.30e5



¹H NMR of compound 1a



LRMS of compound 1a



HRMS of compound 1a

Elemental Composition Report

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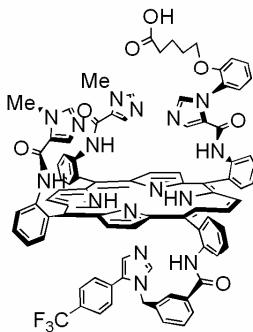
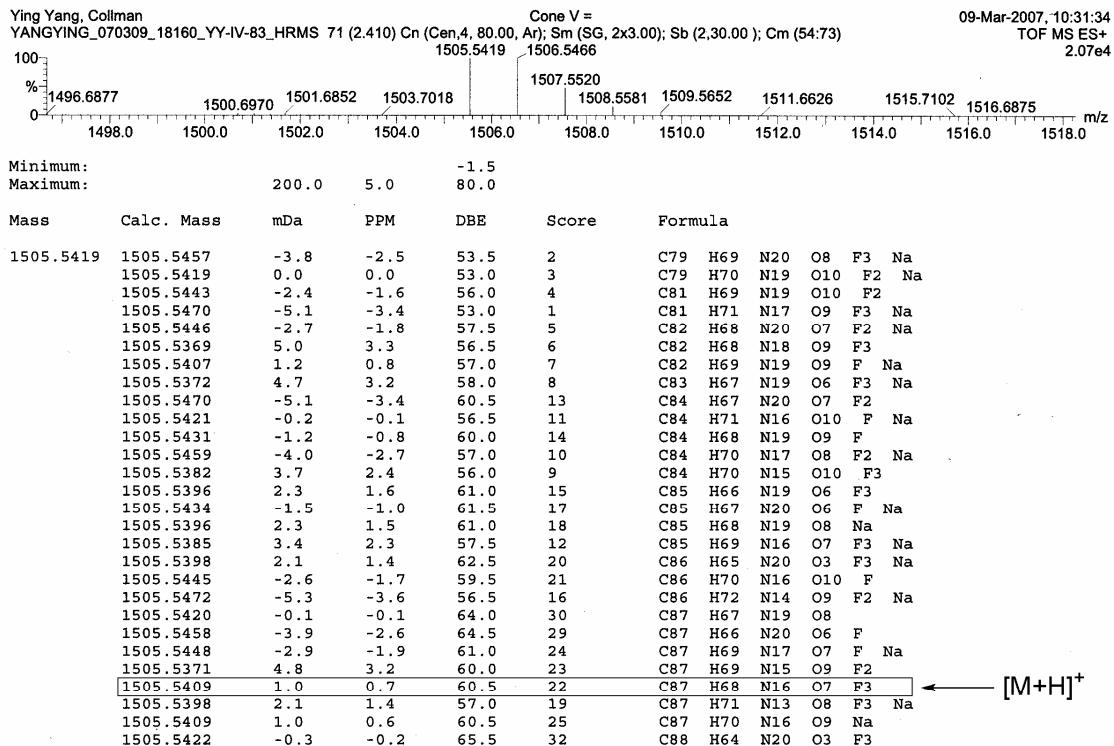
Single Mass Analysis

Tolerance = 5.0 PPM / DBE: min = -1.5, max = 80.0

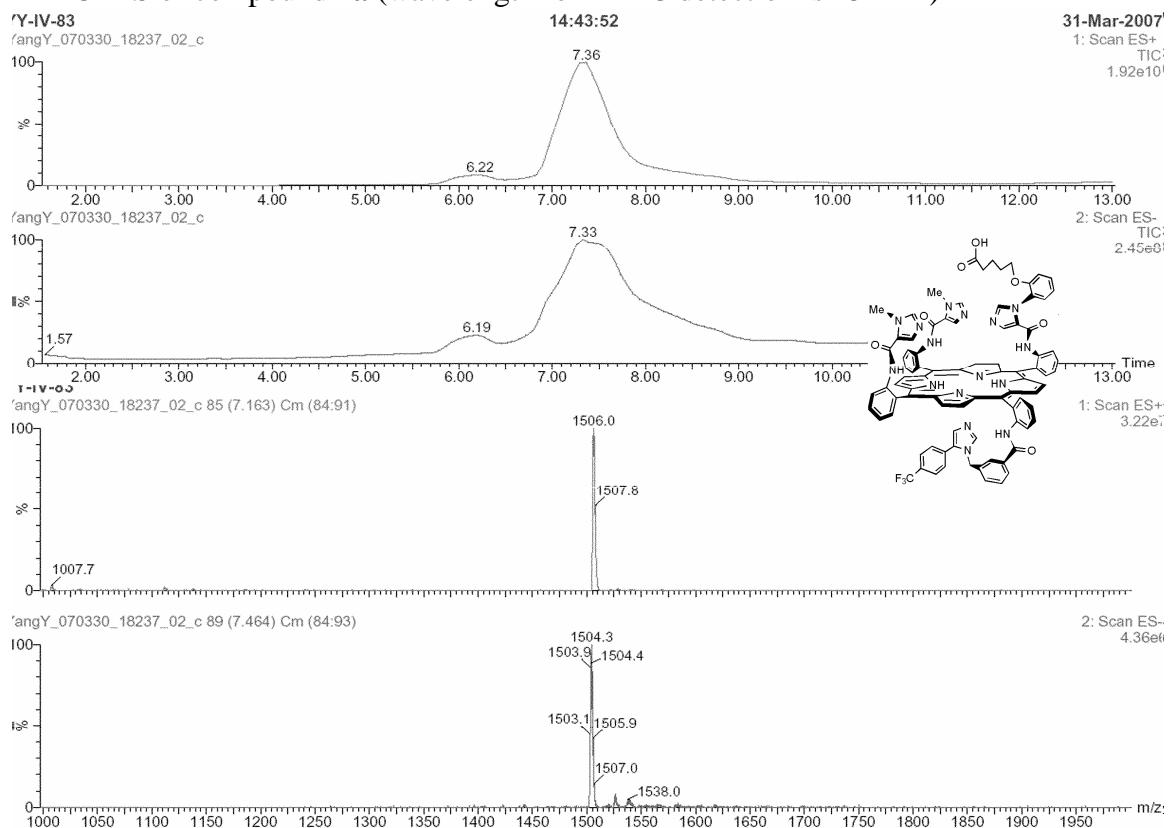
Isotope cluster parameters: Separation = 1.0 Abundance = 1.0%

Monoisotopic Mass, Odd and Even Electron Ions

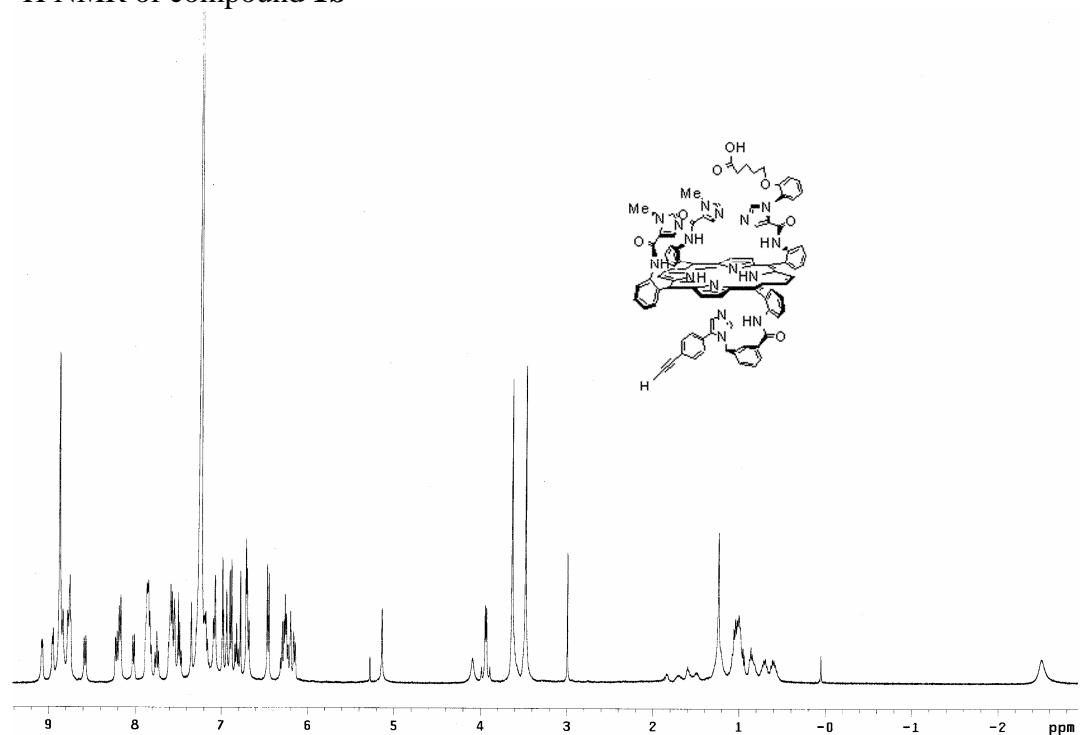
16018 formula(e) evaluated with 183 results within limits (up to 140 closest results for each mass)



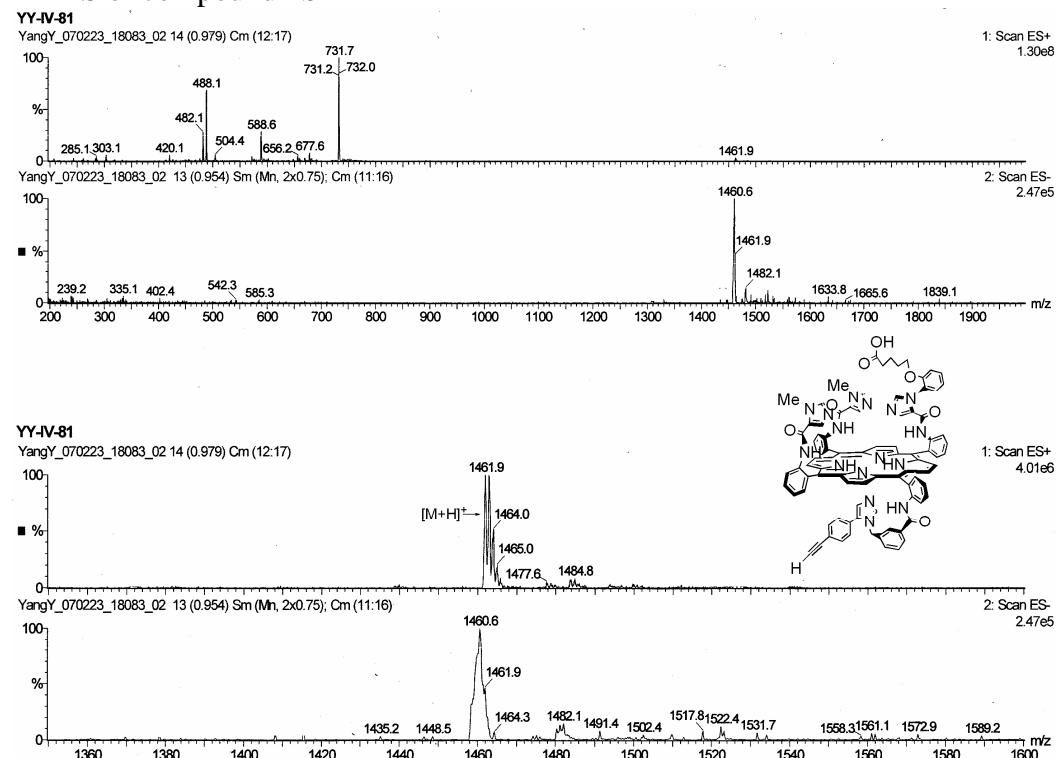
HPLC-MS of compound **1a** (wavelength for HPLC detection is 254 nm)



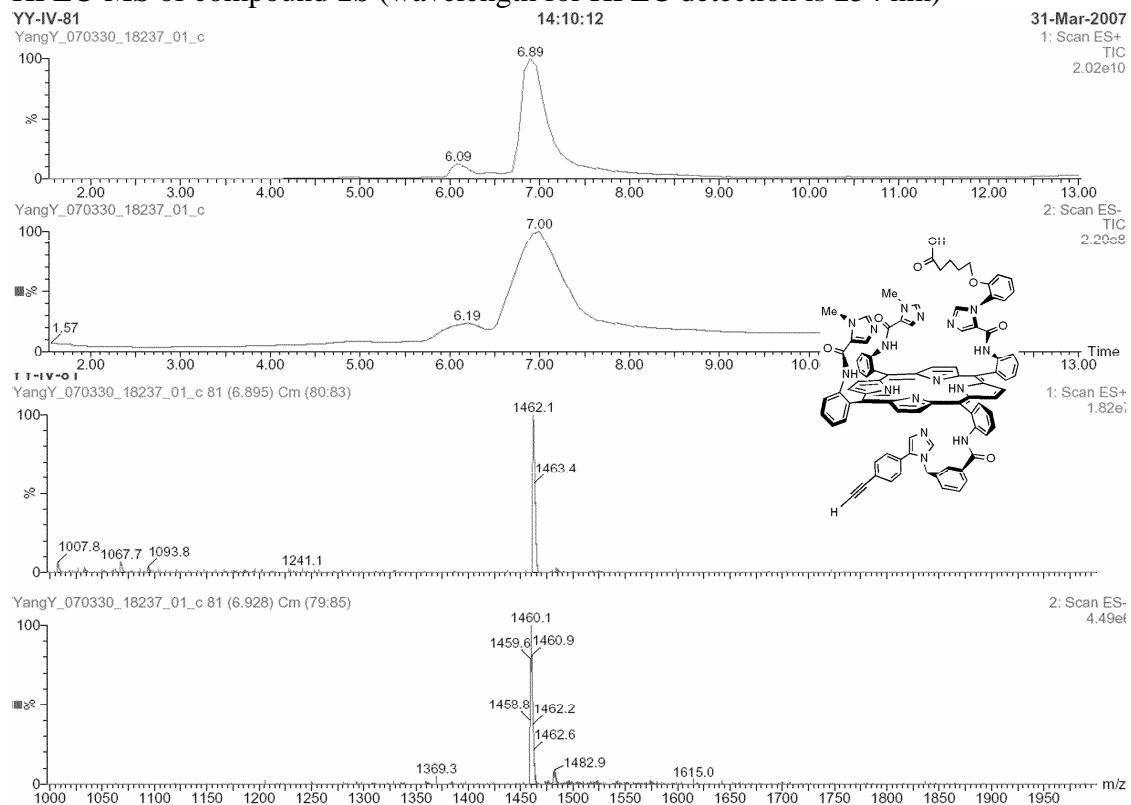
¹H NMR of compound **1b**



LRMS of compound **1b**



HPLC-MS of compound **1b** (wavelength for HPLC detection is 254 nm)



HRMS of compound **1b**

Elemental Composition Report

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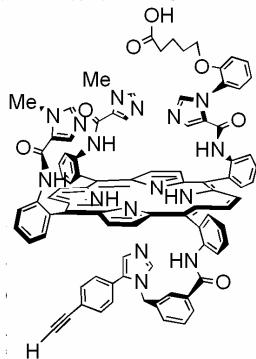
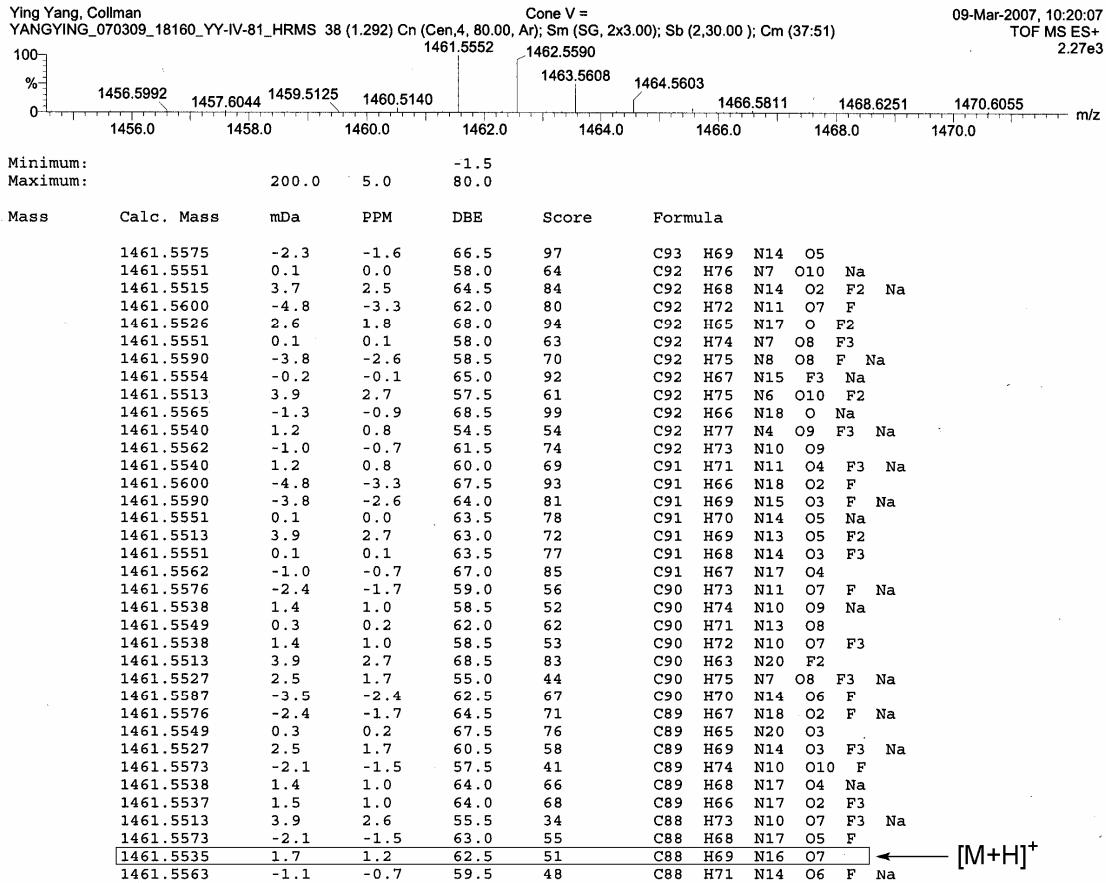
Single Mass Analysis

Tolerance = 5.0 PPM / DBE: min = -1.5, max = 80.0

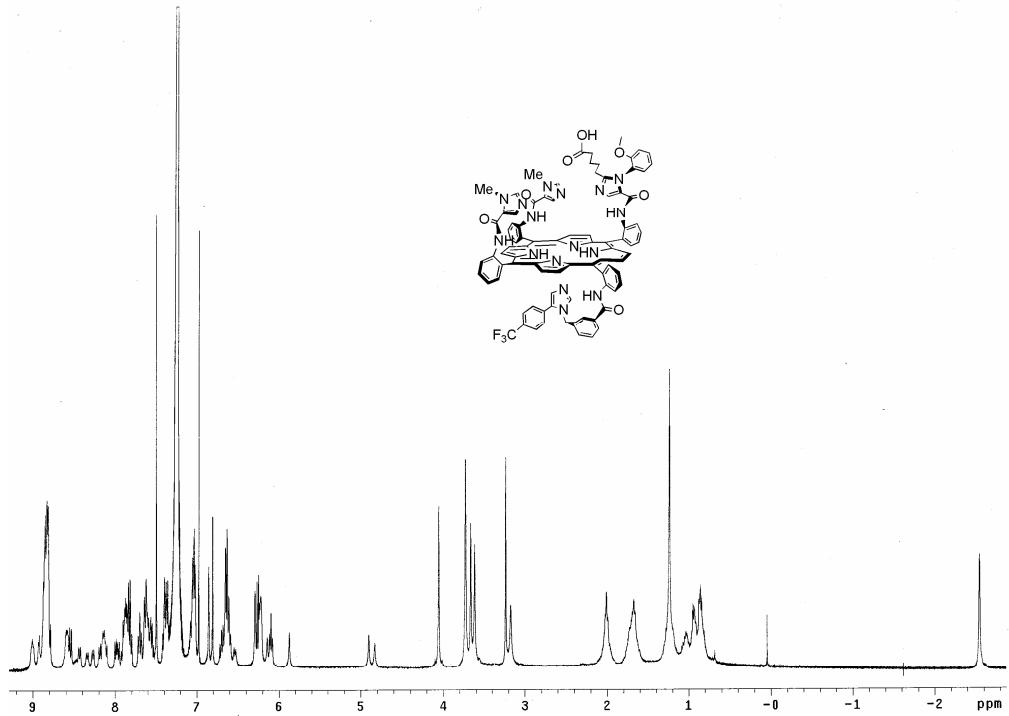
Isotope cluster parameters: Separation = 1.0 Abundance = 1.0%

Monoisotopic Mass, Odd and Even Electron Ions

19202 formula(e) evaluated with 219 results within limits (up to 140 closest results for each mass)



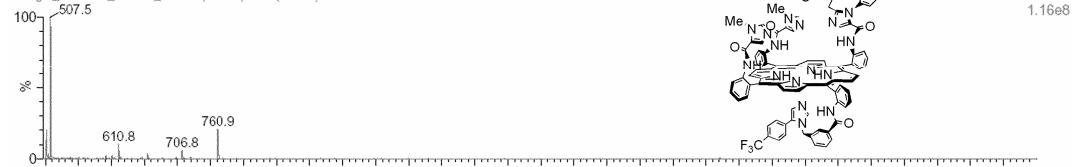
¹H NMR of compound 2a



LRMS of compound **2a**

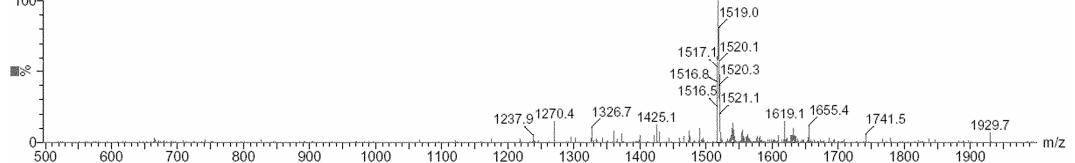
YY-IV-96-acid

YangY_070319_18190_02 86 (7.231) Cm (84:89)



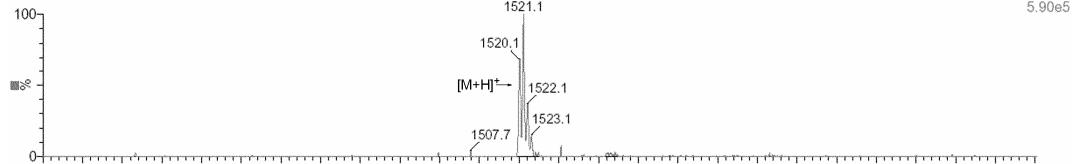
YangY_070319

Yang et al. / 3000-3020 (1,250) 3011 (3,100)

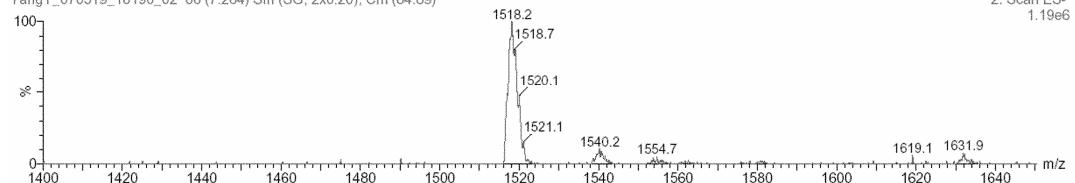


YY-IV-96-acid

YangY_070319_18190_02 86 (7,231) Sm (SG, 2x0.20); Cm (84:89)



YangY_070319_18190_02 86 (7.264) Sm (SG, 2x0.20); Cm (84:89)



HRMS of compound 2a

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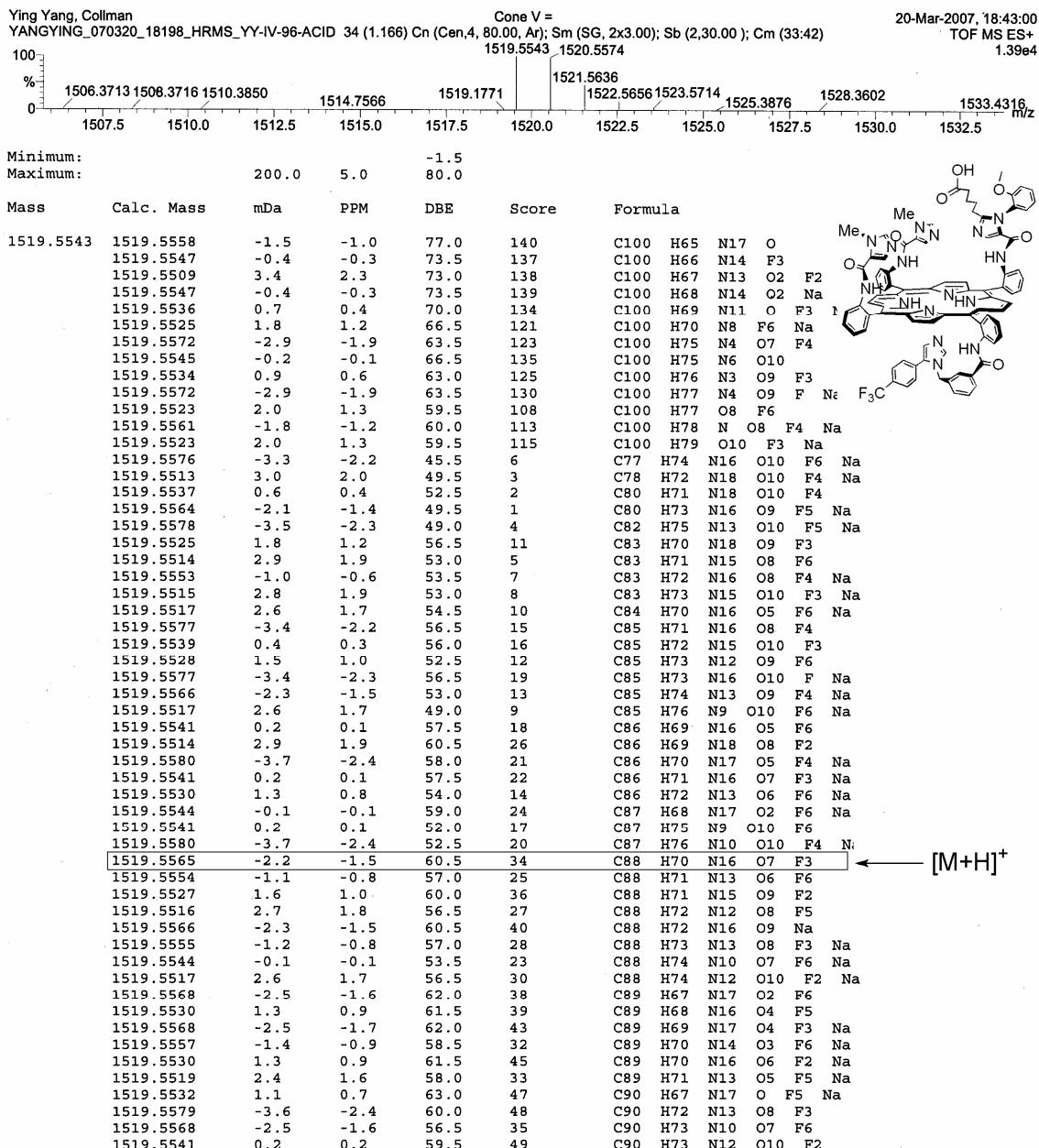
Single Mass Analysis

Tolerance = 5.0 PPM / DBE: min = -1.5, max = 80.0

Isotope cluster parameters: Separation = 1.0 Abundance = 1.0%

Monoisotopic Mass, Odd and Even Electron Ions

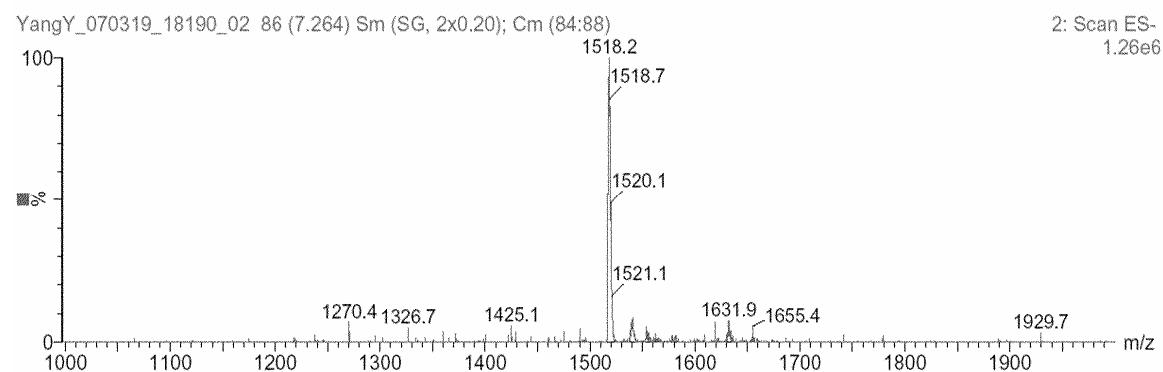
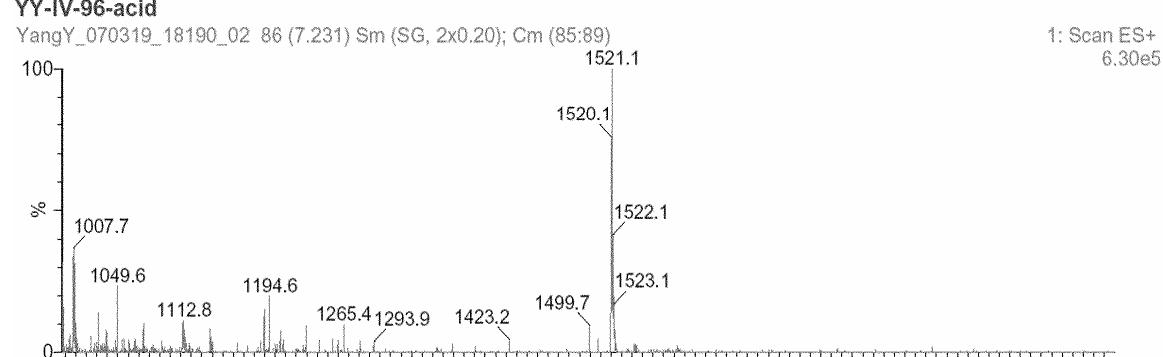
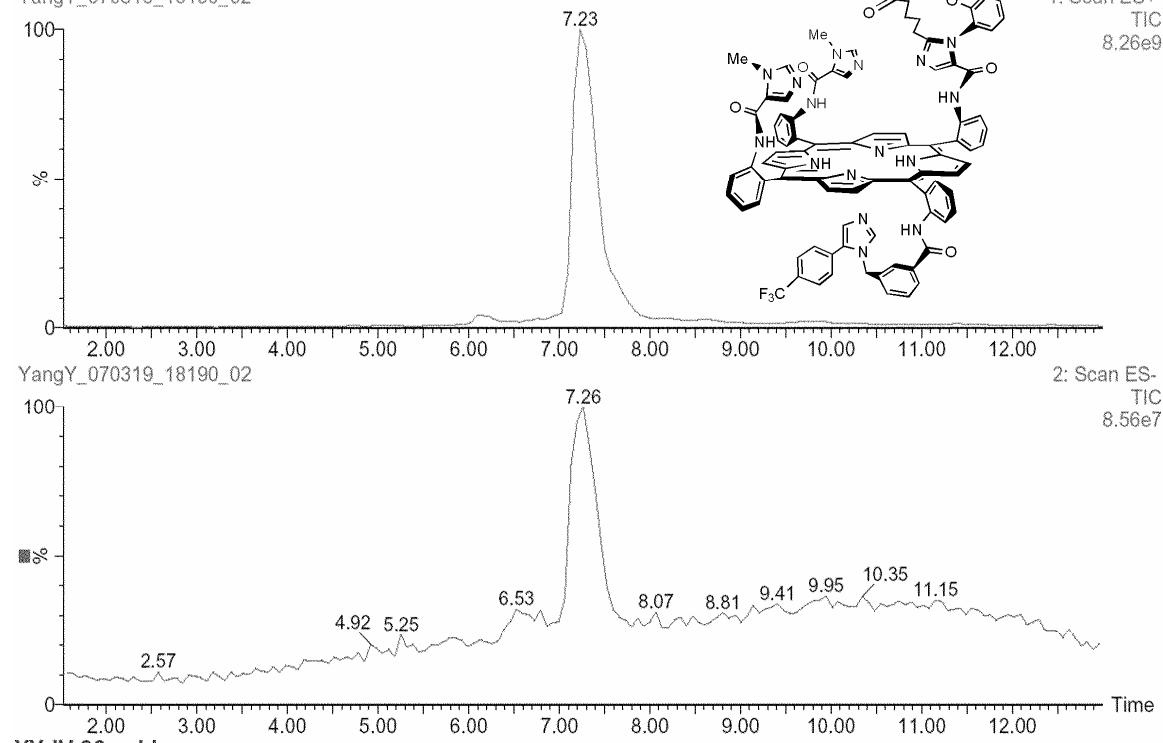
13624 formula(e) evaluated with 291 results within limits (up to 140 closest results for each mass)



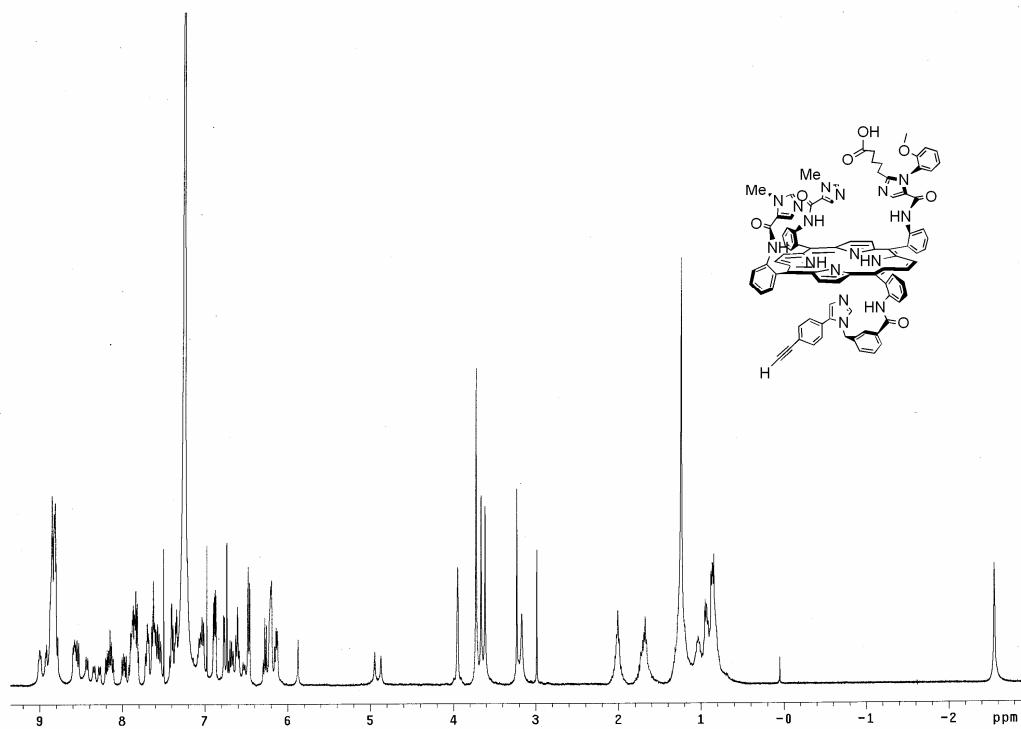
HPLC-MS of compound **2a** (wavelength for HPLC detection is 254 nm)

YY-IV-96-acid

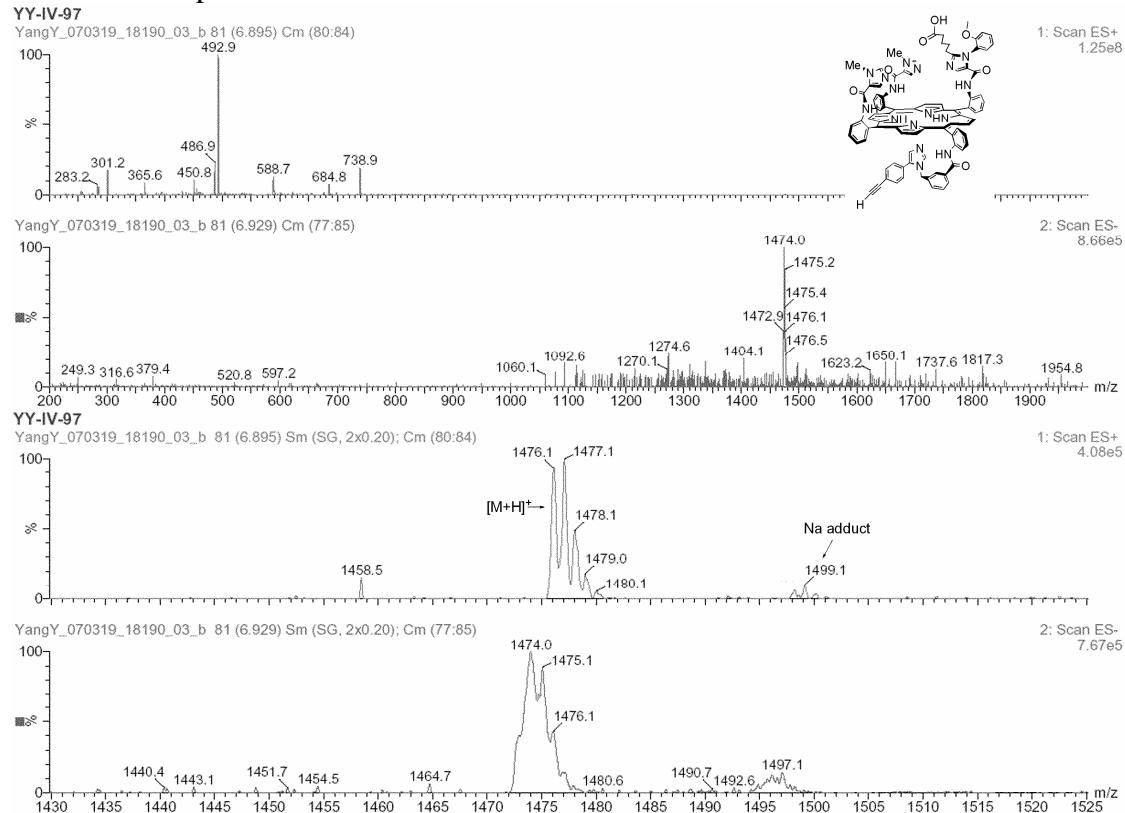
YangY_070319_18190_02



¹H NMR of compound **2b**



LRMS of compound **2b**



HRMS of compound 2b

Elemental Composition Report

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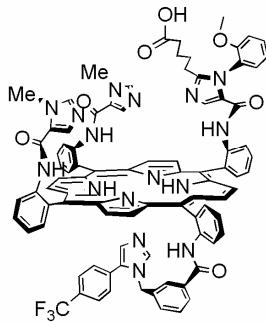
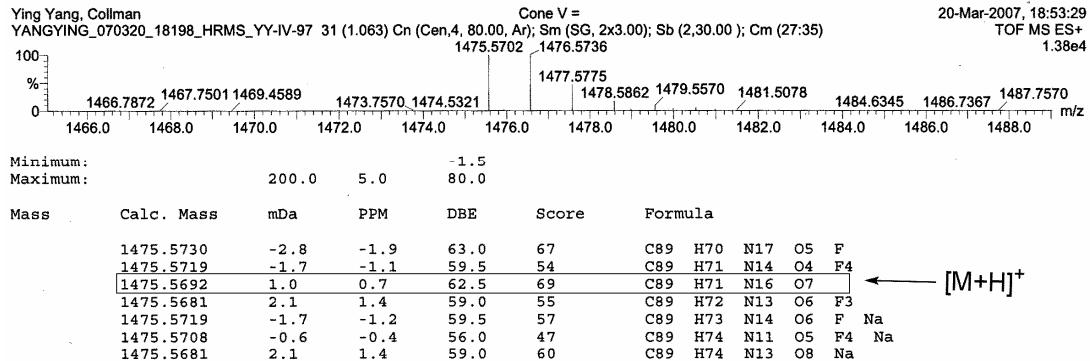
Single Mass Analysis

Tolerance = 5.0 PPM / DBE: min = -1.5, max = 80.0

Isotope cluster parameters: Separation = 1.0 Abundance = 1.0%

Monoisotopic Mass, Odd and Even Electron Ions

17296 formula(e) evaluated with 363 results within limits (up to 140 closest results for each mass)



HPLC-MS of compound **2b** (wavelength for HPLC detection is 254 nm)

