Supporting Information

Sensitive Detection of Acrolein in Serum Using Time-resolved Luminescence

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Instrumental: ^{1}H NMR and ^{13}C NMR spectra were recorded on a JEOL JNM-LA300. Mass spectra were recorded on a JEOL JMSSX102A or a JEOL JMS-T100LC (ESI+, ESI-) mass spectrometer. Preparative HPLC purification was performed on a reversed-phase ODS column (GL Sciences (Tokyo, Japan), Inertsil Prep-ODS 30 mm x 250 mm) fitted on a JASCO PU-1587 HPLC system. UV-visible spectra were obtained on a Shimadzu UV-1600 (Tokyo, Japan). Fluorescence and luminescence spectroscopic studies were performed with a Hitachi F4500 (Tokyo, Japan). Time-resolved luminescence spectra were obtained using a Perkin-Elmer LS-55 (Beaconsfield, Buckinghamshire, England). Luminescence assays on 96-well plates were performed using a plate reader instrument coupled with a Perkin-Elmer Envision 2103 Multilabel Reader.

Luminescence Spectral Measurements: Measurements were performed in 1 cm quartz cells at 25 °C, following excitation at 320 nm. The excitation slit width was 2.5 nm. The emission slit width was 2.5 nm. The photomultiplier voltage was 950 V. Time-resolved luminescence spectra were performed in 1 cm quartz cells at 25 °C, following excitation at 320 nm. The excitation slit width was 5.0 nm. The emission slit width was 2.5 nm.

Quantum Yield Measurements: Quantum yields of the complexes were estimated by a relative method with reference to a luminescence standard. For europium complexes, $[Ru(bpy)_3]Cl_2$ (F = 0.028 in air-equilibrated water) was used. The luminescence spectra were measured at 25 °C in 50 mM sodium phosphate buffer (pH 3.5) containing less than 0.1% DMSO as a cosolvent. All spectra were obtained with a Hitachi F4500 spectrofluorometer.

Analytical HPLC: To confirm that Skraup reaction of the probe had taken place, analytical RP-HPLC was performed using an Inertsil 3 ODS column (4.6 mm x 250 mm, GL Sciences, Japan), fitted on a JASCO PU-980 HPLC system. Conditions: a 20 min gradient, from 1% to 30% solvent B, and a 10 min gradient, from 30% to 100% solvent B (solvent A, 0.1 M triethylammonium acetate (pH 7.4); solvent B, 80% acetonitrile/20% 0.1 M triethylammonium acetate (pH 7.4)).

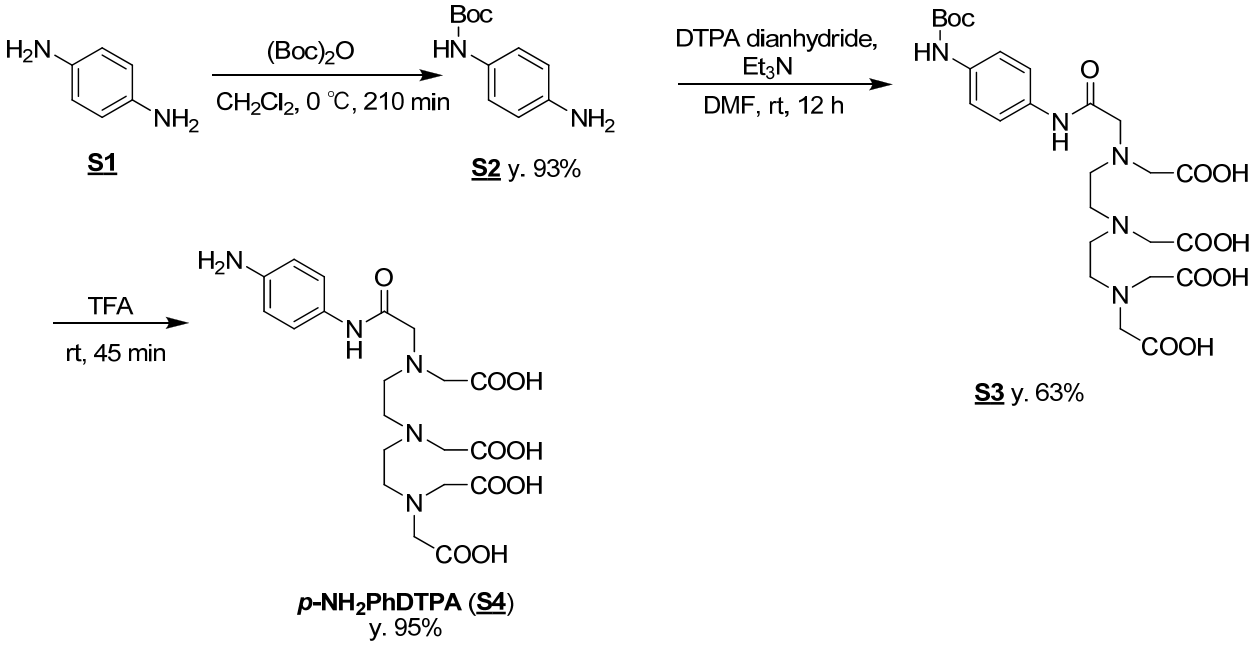
Acrolein Assay on 96-Well Plates: First, acrolein was added to 50 mM sodium phosphate buffer (pH 7.4). To 100 μl of 50 mM sodium phosphate buffer containing acrolein (1, 2, 3 μM) were added 15 μl of 100 mM *p*-NH_2PhDTPA (DMSO solution) and 35 μl of 5.5 N HCl in micro test tubes, which were sealed with parafilm to prevent evaporation of the solvent. Blanks were similarly prepared. The microtubes were heated in an oil bath for 30 min at 100 °C, then cooled in tap water. One equivalent of europium aqueous solution was added to the mixtures after dilution (100x) with 200 mM sodium phosphate buffer (finally, pH 3.5). The luminescence intensity (ex. 340 nm, em. 615 nm) was measured with delay and gate times of 100 ms and 1 ms, respectively. The excitation and emission slit widths were 60 nm and 8.5 nm, respectively. Measurements were performed on white plates (IWAKI, Microlite^{2+}) at room temperature.

Assay of Human Serum: Human sera from 3 healthy volunteers (normal) were centrifuged before use (12 krpm, 4 °C, 10 min). Informed consent was given by each participant. Acrolein diluted with 50 mM sodium phosphate buffer (pH 7.4) was

added to the supernatants to give final concentrations of 0, 5, 10, and 15 μM. The procedure followed the method as described above. After the reaction, 600 μl of 50 mM sodium phosphate buffer (pH 7.4) was added, and the mixtures were centrifuged (12 krpm, 4 °C, 10 min). TRF measurement was performed after dilution (100x) with 200 mM sodium phosphate buffer (finally, pH 3.5) as described above.

Assay of Human Serum (Using a Conventional Method): Assays were performed according to the method of Alarcon. Acrolein solutions were prepared as described above. After the reaction, centrifugation was performed and the fluorescence spectra of the supernatants were obtained with a Hitachi F4500 spectrofluorometer.

Synthesis of *p*-NH_2PhDTPA



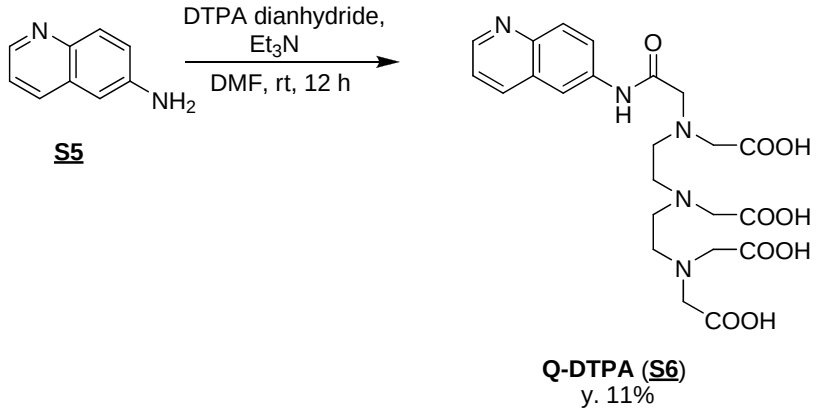
Scheme S1. Synthesis of *p*-NH_2PhDTPA

Synthesis of S2: To a solution of *p*-phenylenediamine (1.15 g, 11 mmol) in DCM (50 ml) was added dropwise a solution of di-*t*-butyl dicarbonate (463 mg, 2.1 mmol) in DCM (10 ml) under an Ar atmosphere at 0 °C. The reaction mixture was stirred at 0 °C for 3.5 hours, and evaporated to dryness. The residue was purified by silica gel column chromatography (AcOEt/n-hexane = 1/2) to afford 408 mg (2 mmol) of S2 as a white solid, 93% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.50 (s, 9H), 3.53 (s, 2H), 6.31 (s, 1H), 6.62 (d, 2H, *J* = 8.6 Hz), 7.12 (d, 2H, *J* = 8.4 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ 28.3, 79.9, 115.5, 120.9, 129.6, 142.3, 153.3. HRMS (ESI^+): m/z calcd for $(M+Na)^+$, 231.1110; Found, 231.1149.

Synthesis of S3: To a solution of diethylenetriaminepentaacetic dianhydride (72.9 mg, 0.20 mmol) in N,N-dimethylformamide (3.5 ml) was added dropwise a solution of triethylamine (55.4 μl, 0.40 mmol) at 0 °C. Then, a solution of S2 (42.5 mg, 0.20 mmol) in N,N-dimethylformamide (3.0 ml) was added dropwise to the mixture at 0 °C. The reaction mixture was stirred at room temperature for 12 hours, and evaporated to dryness. The residue was purified by preparative reverse-phase HPLC using a 20 min linear gradient from 10% to 80% solvent B (solvent A, H_2O (0.1% trifluoroacetic acid)); solvent B, acetonitrile/H_2O=80/20 (0.1% trifluoroacetic acid)). 73.0 mg of S3 (0.13 mmol) was obtained as a white solid, 63% yield. ^{1}H NMR (300 MHz, CD_3OD) δ 1.50 (s, 9H), 3.19 – 3.23 (m, 4H), 3.39 – 3.48 (m, 4H), 3.63 (m, 8H), 4.43 (s, 2H), 7.33 (d, 2H, *J* = 8.8 Hz), 7.52 (d, 2H, *J* = 8.8 Hz). ^{13}C NMR (75 MHz, CD_3OD) δ 28.7, 50.9, 51.4, 54.4, 54.5, 55.8, 56.3, 56.4, 59.1, 80.8, 120.2, 122.1, 134.2, 137.1, 155.4, 170.3, 171.0, 174.6, 174.6. HRMS (ESI^+): m/z calcd for $(M+Na)^+$, 606.2387; Found, 606.2387.

Synthesis of S4: To S3 (73.0 mg, 0.13 mmol) was added trifluoroacetic acid (2 ml) on an ice bath. The reaction mixture was stirred at 0 °C for 3.5 hours, and evaporated to dryness. The residue was purified by preparative reverse-phase HPLC using a 20 min linear gradient from 1% to 30% solvent B (solvent A, H_2O (0.1% trifluoroacetic acid)); solvent B, acetonitrile/H_2O=80/20 (0.1% trifluoroacetic acid)). 57.6 mg of S3 (0.12 mmol) was obtained as a white solid, 95% yield. M. p. 110.1 – 111.6 °C. ^{1}H NMR (300 MHz, CD_3OD) δ 3.22 – 3.25 (m, 4H), 3.50 – 3.53 (m, 4H), 3.63 (m, 6H), 3.63 (s, 2H), 4.43 (s, 2H), 7.33 (d, 2H, *J* = 8.4 Hz), 7.78 (d, 2H, *J* = 8.2 Hz). ^{13}C NMR (75 MHz, CD_3OD) δ 50.8, 51.3, 54.3, 54.6, 54.9, 55.9, 56.2, 58.8, 122.5, 124.5, 127.8, 139.9, 169.7, 171.4, 174.3, 174.5. HRMS (ESI^+): m/z calcd for $(M+H)^+$, 484.2044; Found, 484.2052.

Synthesis of Q-DTPA



Scheme S2. Synthesis of Q-DTPA

Synthesis of S6: To a solution of diethylenetriaminepentaacetic dianhydride (152 mg, 0.43 mmol) in N,N-dimethylformamide (7.0 ml) was added dropwise triethylamine (118 µl, 0.85 mmol) at 0 °C. To the mixture was added S5 (61.3 mg, 0.43 mmol) in N,N-dimethylformamide (4.5 ml) at 0 °C. The reaction mixture was stirred at room temperature for 12 hours, and evaporated to dryness. The residue was purified by preparative reverse-phase HPLC using a 20 min linear gradient from 10% to 80% solvent B (solvent A, H_2O (0.1% trifluoroacetic acid)); solvent B, acetonitrile/H_2O=80/20 (0.1% trifluoroacetic acid)). 25.6 mg of S6 (0.049 mmol) was obtained as a white solid, 95% yield. M. p. 196.2 – 198.2 °C. ^{1}H NMR (300 MHz, D_2O) δ 3.29 – 3.36 (m, 4H), 3.42 – 3.52 (m, 4H), 3.78 (s, 2H), 3.90 – 3.91 (m, 6H), 4.14 (s, 2H), 7.98 (dd, 1H, *J* = 5.5, 8.4 Hz), 8.08 (dd, 1H, *J* = 2.0, 9.3 Hz), 8.16 (d, 1H, *J* = 9.4 Hz), 8.51 (d, 1H, *J* = 2.0 Hz), 8.96 (d, 1H, *J* = 5.5 Hz), 9.01 (d, 1H, *J* = 8.4 Hz). ^{13}C NMR (75 MHz, D_2O) δ 51.4, 52.2, 52.4, 52.5, 55.1, 56.7, 57.0, 58.1, 118.4, 122.0, 122.7, 129.8, 130.3, 135.4, 138.3, 143.6, 147.6, 168.7, 171.3, 172.6, 172.9. HRMS (ESI^+): m/z calcd for $(M+ Na)^+$, 542.1863; Found, 542.1864.

Analytical HPLC: To confirm that Skraup reaction of the probe had taken place, analytical RP-HPLC was performed using an Inertsil 3 ODS column (4.6 mm x 250 mm, GL Sciences, Japan), fitted on a JASCO PU-980 HPLC system. Conditions: a 20 min gradient, from 1% to 30% solvent B, and a 10 min gradient, from 30% to 100% solvent B (solvent A, 0.1 M triethylammonium acetate (pH 7.4); solvent B, 80% acetonitrile/20% 0.1 M triethylammonium acetate (pH 7.4)).

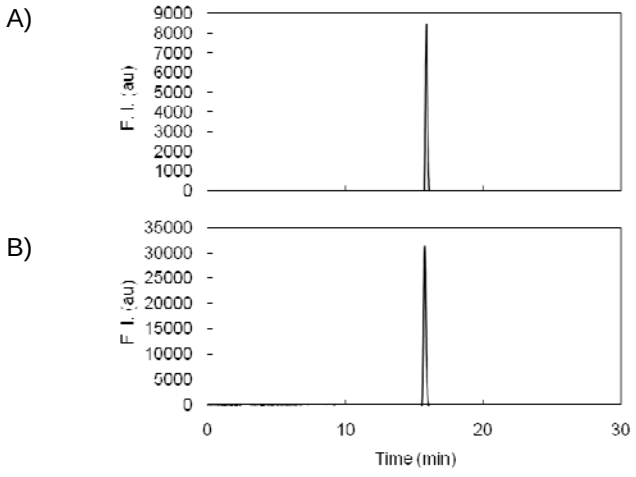
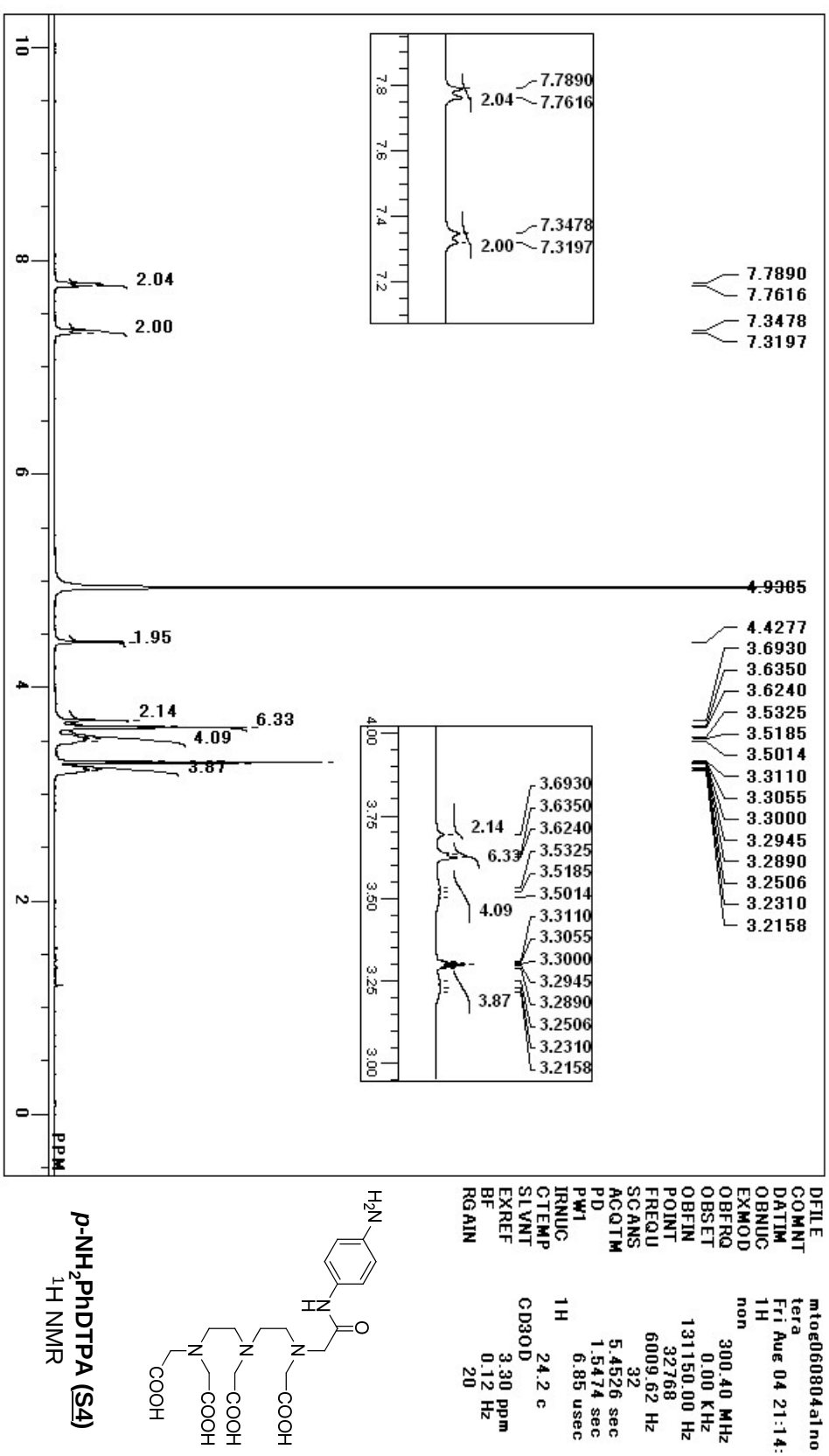


Figure S1. A) HPLC elution chart of the reaction solution recorded with fluorescence detection (Ex. / Em. = 325 / 615 nm).
B) HPLC elution chart of a mixture of the reaction solution and standard Q-DTPA-Eu.

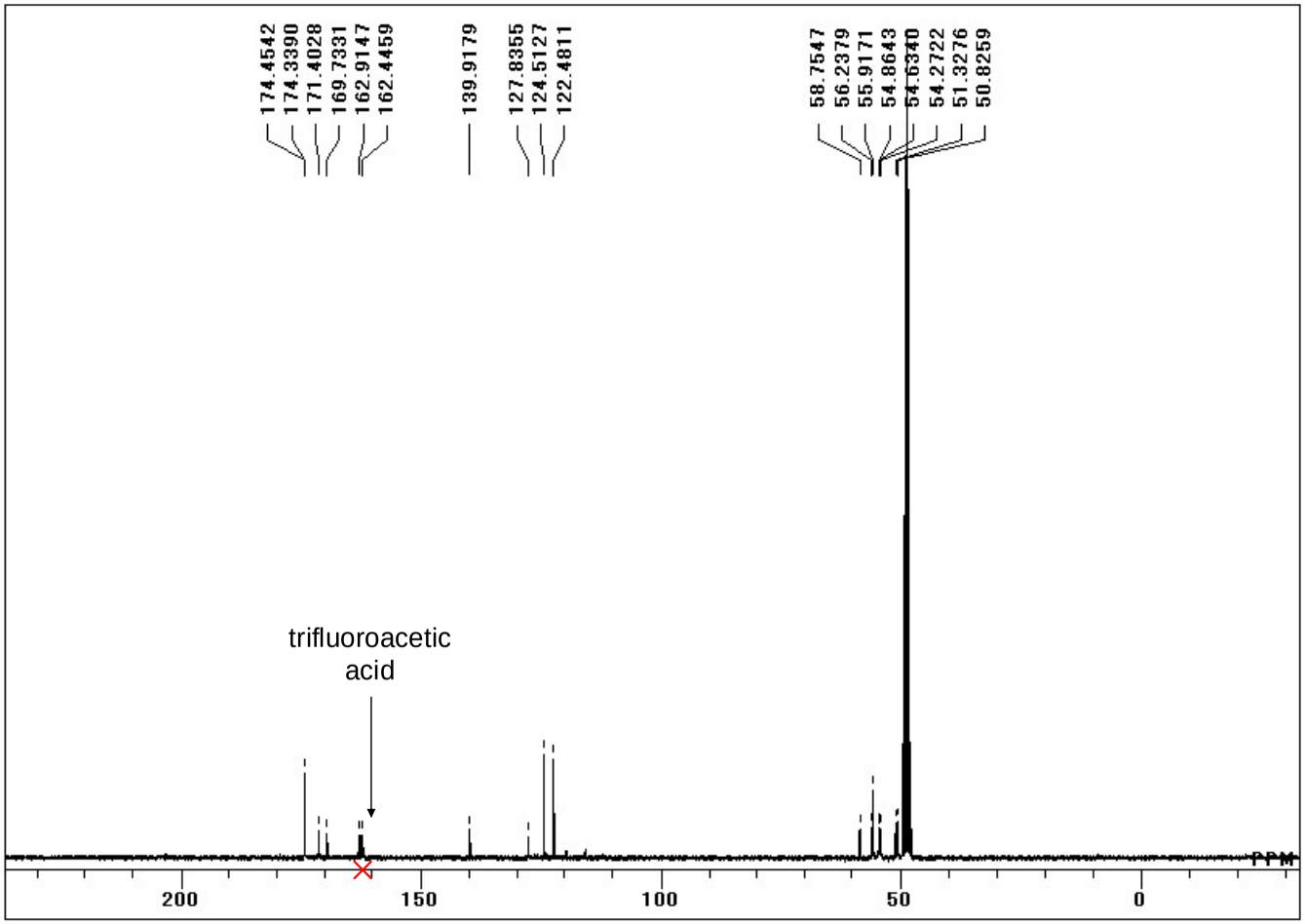
NMR spectra of key compounds



***p*-NH$_2$PhDTPA (S4)**

^{1}H NMR

p-nh2phdtpa C



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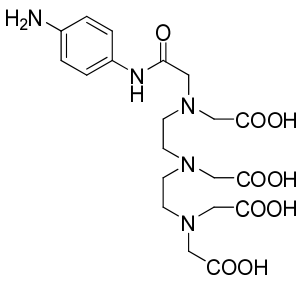
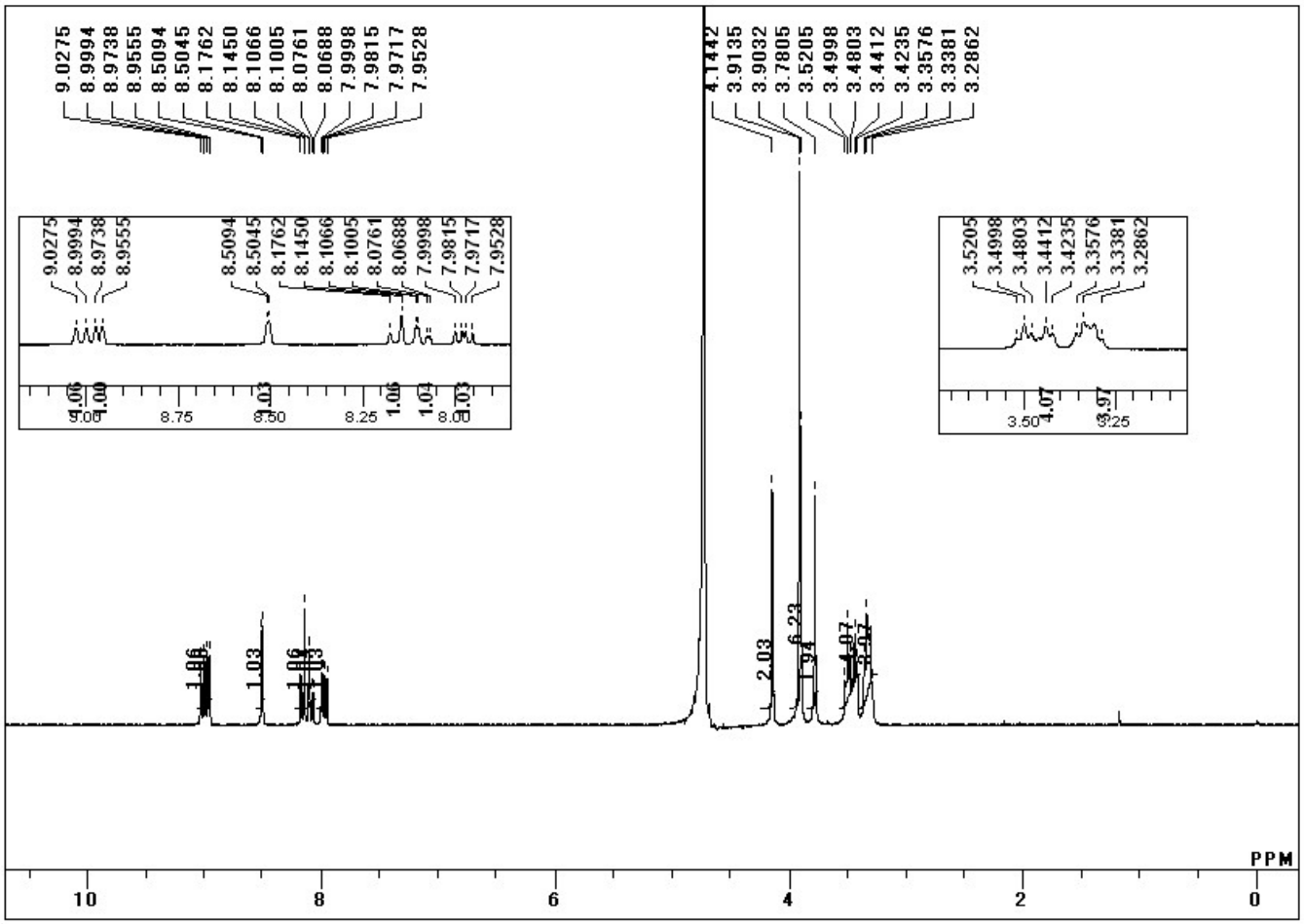


***p*-NH_2PhDTPA (S4)**
^{13}C NMR

Q-Q-DTPA



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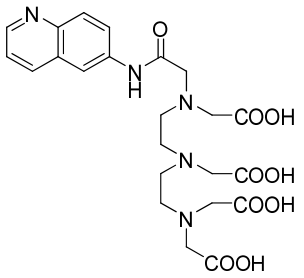
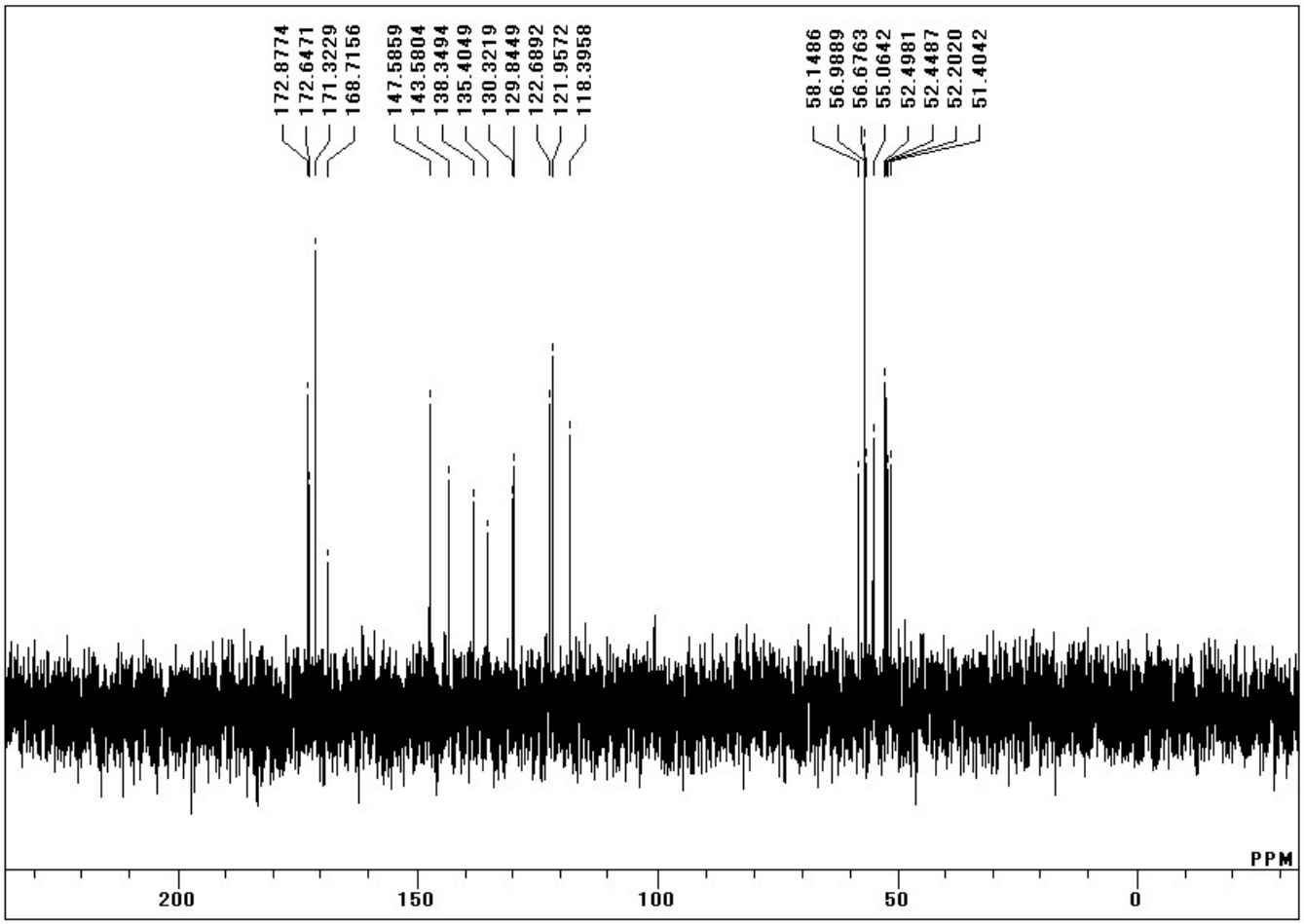


Q-DTPA (S6)

^{1}H NMR

Q-DTPA



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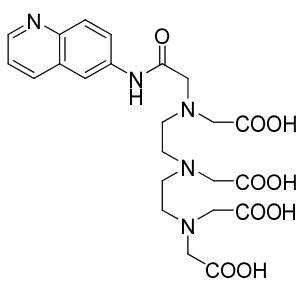

Q-DTPA (S6)
^{13}C NMR