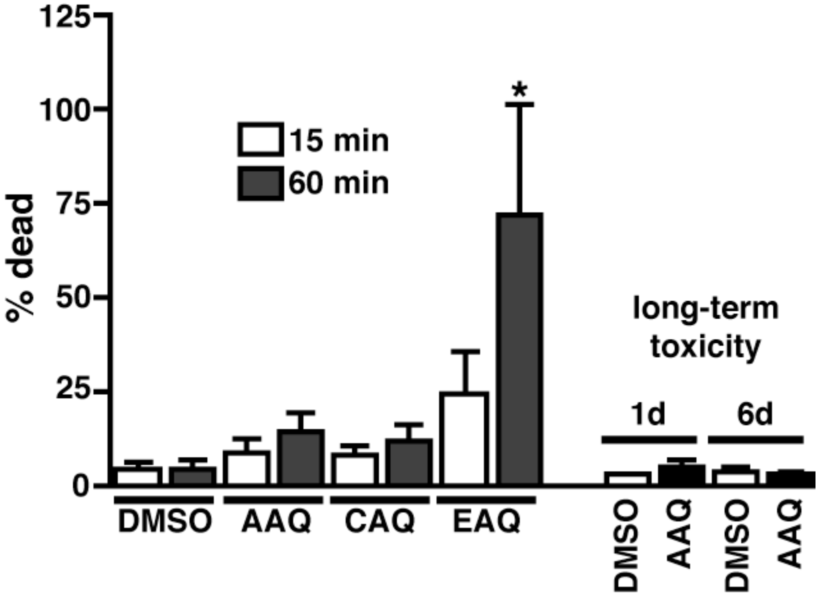
Supplementary Figure 1. Neuronal survival after PAL treatment

Cultured hippocampal neurons were incubated with PALs for the indicated time and processed immediately after treatment for a Live/Dead Assay (Molecular Probes) to quantify cell survival. EAQ, but not AAQ and CAQ, caused more toxicity than vehicle alone (* $p < 0.001$ One-way ANOVA, Tukey's post-test). To assess long-term toxicity, neurons were treated with AAQ for 15 minutes then returned to their normal growth medium for an additional 1 or 6 days. AAQ treatment had no effect on long-term survival (n = 4-8 field of cells for each condition).



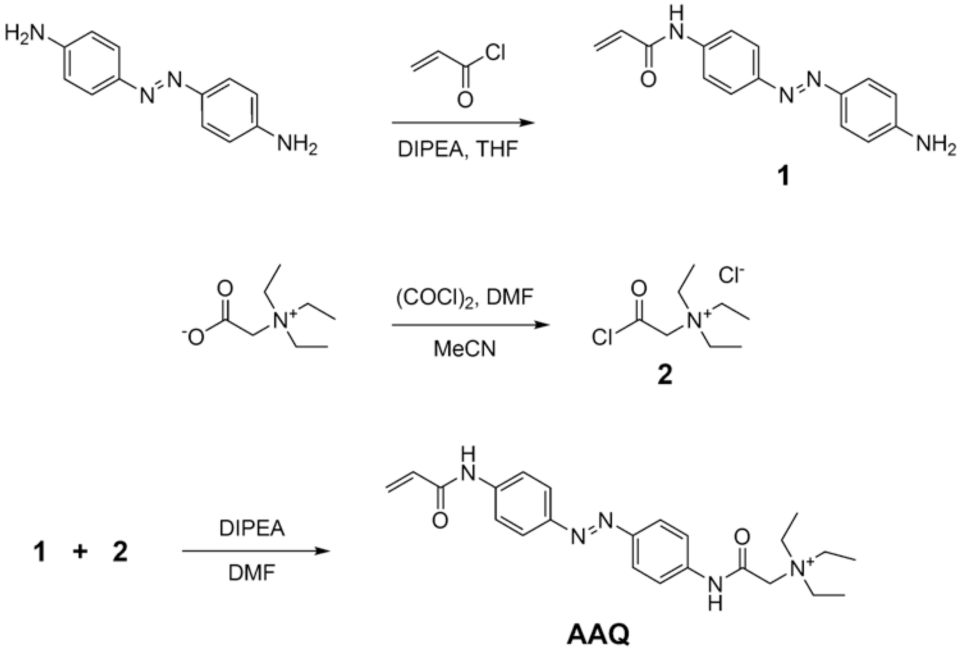
Supplementary methods

Electrophysiology. The bath solution for voltage-gated K^+ channel recordings contained in mM: 138 NaCl, 1.5 KCl, 1.2 $MgCl_2$, 2.5 $CaCl_2$, 5 HEPES, 10 glucose, pH 7.4 and when necessary, 20 μM bicuculline, 25 μM 6,7-dinitroquinoxaline-2,3-[1*H*,4*H*]-dione (DNQX) and 1 μM tetradotoxin (TTX). The intracellular solution contained in mM: 10 NaCl, 135 K-gluconate, 10 HEPES, 2 $MgCl_2$, 2 MgATP, 1 EGTA, pH 7.4. For recordings of BK-mediated currents, 1 mM $CaCl_2$ was added to the intracellular solution. For recordings of voltage-gated Na^+ channels, the bath solution contained in mM: 150 NaCl, 2 $CaCl_2$, 0.5 $CdCl_2$, 10 HEPES, 5 glucose, pH 7.4; and the intracellular solution contained in mM: 100 CsCl, 30 NaCl, 10 EGTA, 1 $CaCl_2$, 2 $MgCl_2$, 2 ATP, 0.05 GTP, 10 HEPES, 5 glucose, pH 7.4. L-type calcium channel currents were recorded in a bath solution containing (in mM): 105 Tris-HCl, 0.8 $MgCl_2$, 5.4 KCl, 20 $BaCl_2$, 0.02 TTX, 10 HEPES, 5 glucose, pH 7.4. The intracellular solution contained (in mM): 70 Cs_2-Aspartate, 20 HEPES, 11 EGTA, 1 $CaCl_2$, 5 $MgCl_2$, 5 glucose, 5 ATP, pH 7.4. Solutions were adjusted to 300-310 mOsm. Cerebellar slices recordings were performed in aCSF containing in mM: 119 NaCl, 26 $NaHCO_3$, 11 glucose, 2.5 KCl, 2.5 $CaCl_2$, 1.3 $MgCl_2$, and 1 NaH_2PO_4 and saturated with 95% O_2 and 5% CO_2. RGC recording saline solution contained (in mM): 125 NaCl, 2.5 KCl, 2.5 $CaCl_2$, 1.5 $MgCl_2$, 1.25 NaH_2PO_4, 33.7 $NaHCO_3$, and 10 glucose, pH 7.4.

General chemical methods. Reactions were carried out under N_2 atmosphere in flame-dried glassware. Tetrahydrofuran (THF) was distilled from Na/benzophenone immediately prior to use. Acetonitrile (MeCN), and diisopropylethylamine (DIPEA) were distilled from CaH_2 immediately prior to use. All other reagents and solvents were used without further purification from commercial sources. Flash column chromatography was carried out with EcoChrom ICN SiliTech 32–63 D 60 Å silica gel. Reverse-phase chromatography was carried out with Waters Preparative C18 Silica Gel WAT010001 125 Å and Waters Sep-Pak Vac 20 cc C18 Cartridges WAT036925. Reactions and chromatography fractions were monitored with either Merck silica gel 60F254 plates or Analtech C18 silica gel RPS-F 52011 plates, and visualized with 0.1N HCl. NMR spectra were measured in specified solvents and calibrated from residual solvent signal on a Bruker DRX spectrometer at 500 MHz for ^1H spectra and 125 MHz for ^{13}C spectra and either a

Bruker AVB or Bruker AVQ spectrometer at 400 MHz for ^{1}H spectra and 100 MHz for ^{13}C spectra. IR spectra were measured with a Genesis FT-IR spectrometer by thin film.

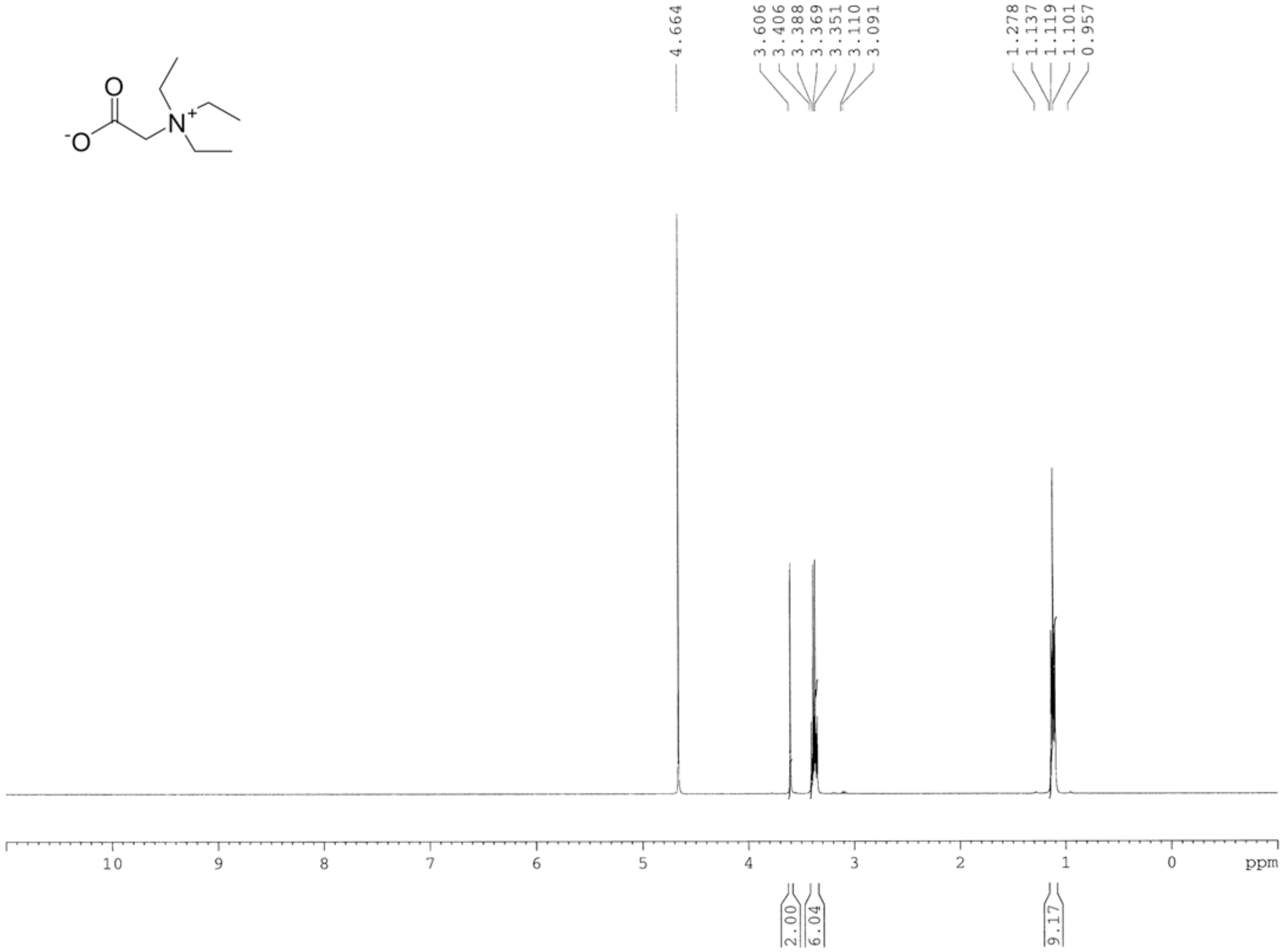
Scheme for synthesis of AAQ



4-acrylamido-4'-aminoazobenzene (1). To a solution of 4,4'-diaminoazobenzene **2** (200 mg, 0.94 mmol) and DIPEA (0.1 ml, 0.56 mmol) in THF (300 ml) at 0ºC was added acryloyl chloride (0.04 ml, 0.47 mmol) in THF (5 ml) over 1 h. The reaction was stirred for 15 min, warmed to room temperature and stirred for 1 h, at which time the crude mixture was removed of solvent *in vacuo* and immediately dry loaded onto silica gel (2 g). Silica gel chromatography through a wide column (10% EtOAc in DCM gradient to 75%) provided 4-acrylamido-4'-azobenzene as an orange solid (91 mg, 0.34 mmol, 72% yield): ^{1}H (CD_3CN, 300MHz): 4.77 (s, 2H); 5.74-5.78 (m, 1H); 6.36-6.38 (m, 2H); 6.73 (d, 2H, J=8.7); 7.70 (d, 2H, J=8.7); 7.79 (s, 4H); 8.66 (s, 1H). ^{13}C (CD_3CN, 125MHz): 114.9, 120.8, 123.7, 125.7, 127.9, 132.4, 141.3, 145.3, 149.9, 152.5, 164.5. IR (thin film): 2925, 1671, 1598, 1536, 1244. HRMS (FAB+): calculated for $C_{15}H_{15}N_4O$ – 267.1256, found – 267.1246 (M+). The remaining 4,4'-azodiaminoazobenzene was isolated for reuse.

2-triethylammonium acetic acid chloride chloride (2). Triethylammonium acetate was prepared as described previously[1]. ^{1}H (D_2O, 400MHz): 1.12 (t, 9H, J=7.2); 3.38 (q, 6H, J=7.2); 3.61 (s, 2H). ^{13}C (D_2O, 100MHz): 6.7, 53.2, 57.4, 169.5. IR (thin film): 1626, 1458, 1395. HRMS (ESI+): calculated for $C_8H_{18}NO_2$ – 160.1338, found – 160.1341 (MH+). To a solution of triethylammonium acetate (520 mg, 3.25 mmol) in MeCN (3 ml) was added a 2 M solution of oxallyl chloride in DCM (1.6 ml, 3.25 mmol) followed by several drops of DMF. The solution was stirred at ambient temperature for 15 min, removed of solvent *in vacuo* and dried under vacuum for 1 hr to remove residual HCl. The product was then taken up in DMF (10 ml) and used without further purification.

Acryl-Azo-QA (AAQ) (3). To a solution of 4-acrylamido-4'-aminoazobenzene **1** (90 mg, 0.33 mmol) and DIPEA (0.12 ml, 0.66 mmol) in DMF (5 ml) at 0°C was added 2-triethylammonium acetic acid chloride chloride **2** (0.41 mmol) in DMF and stirred for 15 min, then warmed to ambient temperature and stirred for 1 h at which time the solvent was removed *in vacuo*. Reverse phase silica gel chromatography (0.1% formic acid in H_2O to 50% MeCN: 0.1% formic acid in H_2O) provided **AAQ** as an orange solid (109 mg, 0.24 mmol, 70% yield): ^{1}H (CD_3CN, 400MHz): 1.30 (t, 9H, J=7.2); 3.52 (q, 6H, J=7.2); 4.47 (s, 2H); 5.72-5.75 (m, 1H); 6.33-6.38 (m, 1H); 6.54-6.61 (m, 1H); 7.75-7.79 (m, 4H); 7.87-7.90 (m, 4H); 8.72 (s, 1H); 10.21 (s, 1H); 12.88 (s, 1H). ^{13}C (CD_3CN, 100MHz): 8.1, 55.4, 57.7, 120.8, 121.4, 124.2, 124.3, 127.6, 132.8, 142.1, 143.0, 149.3, 149.7, 163.4, 154.9, 168.1. IR (thin film): 2986, 1684, 1594, 1541, 1250. HRMS (FAB+): calculated for $C_{23}H_{30}N_5O_2$ – 408.2400, found – 408.2395 (M+).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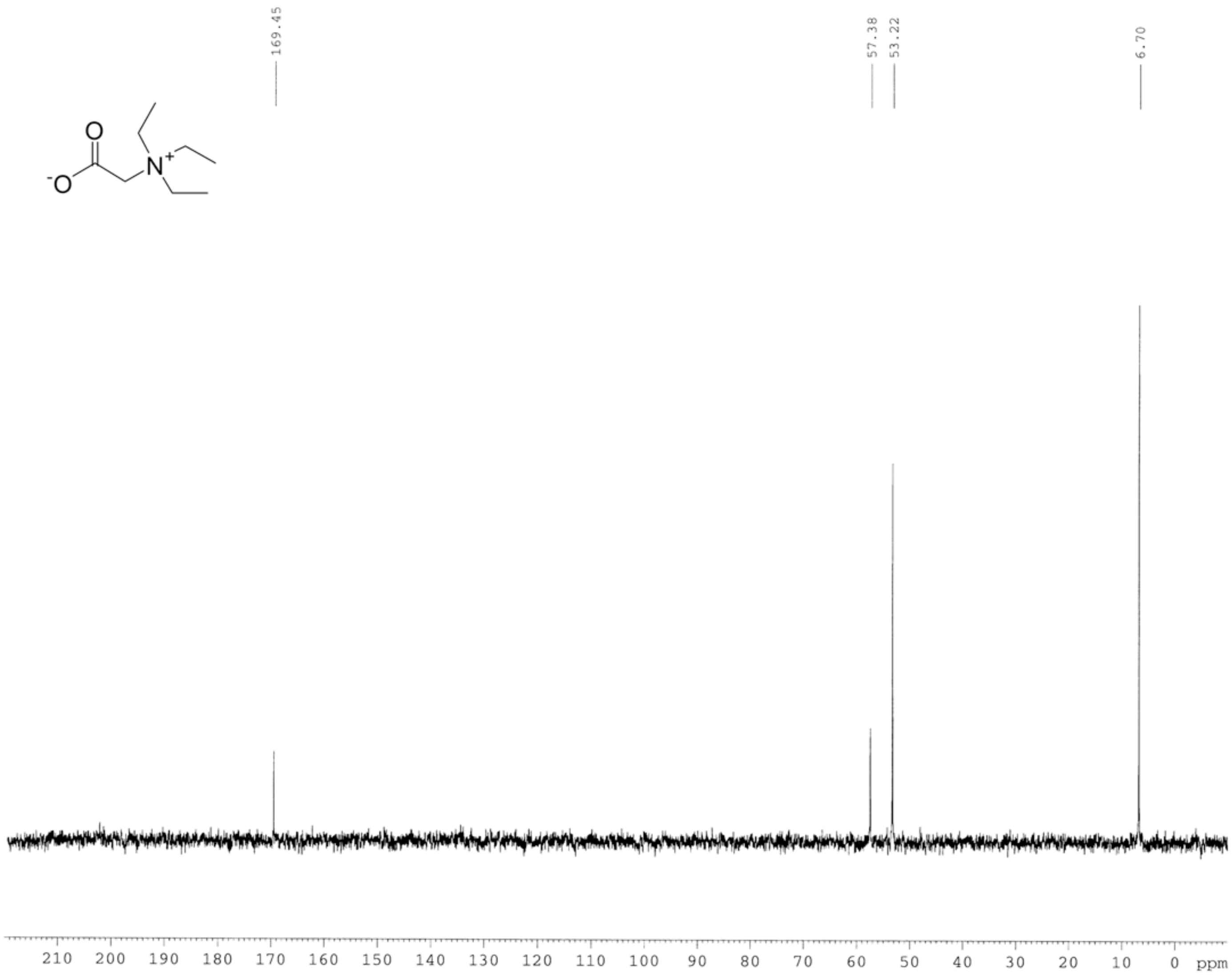
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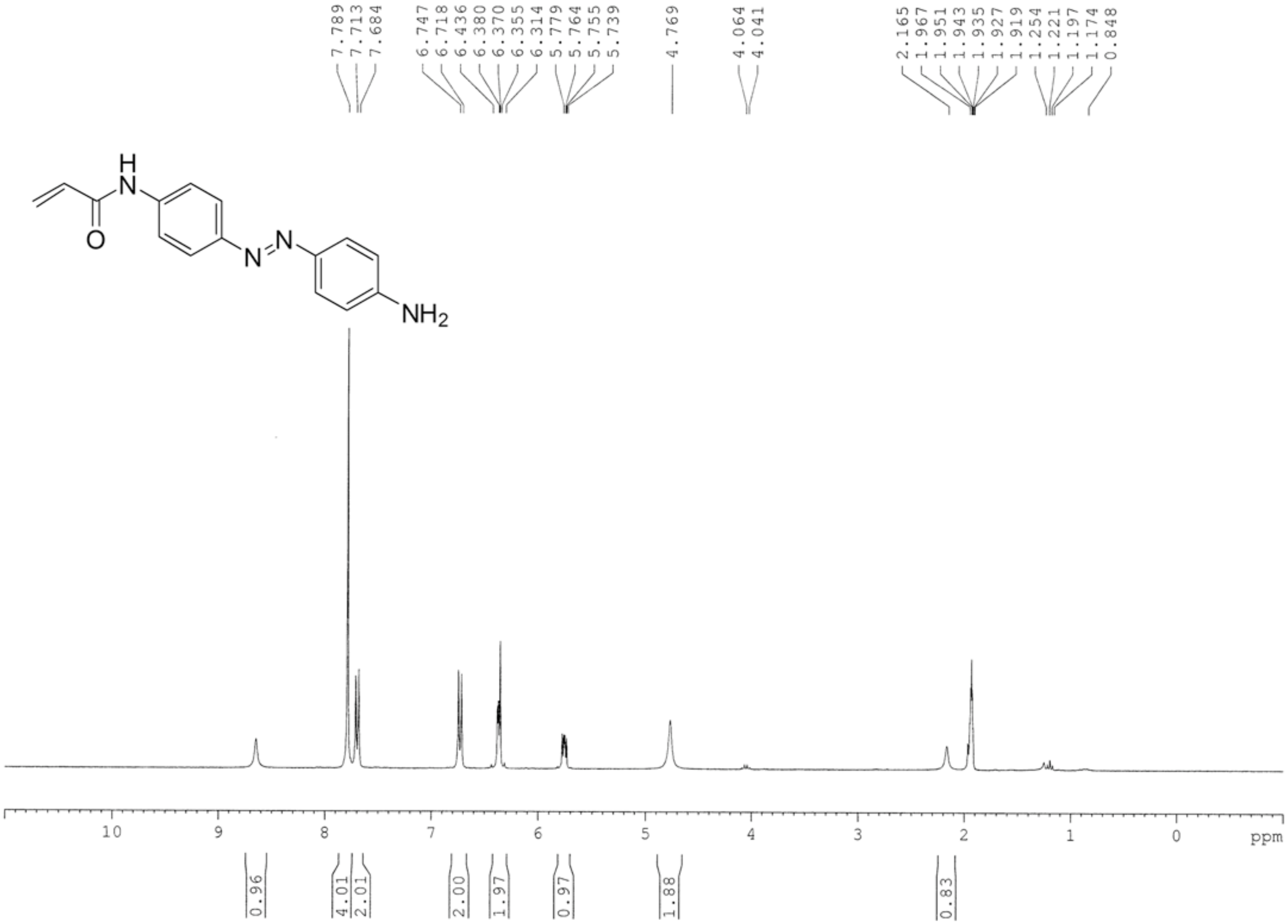
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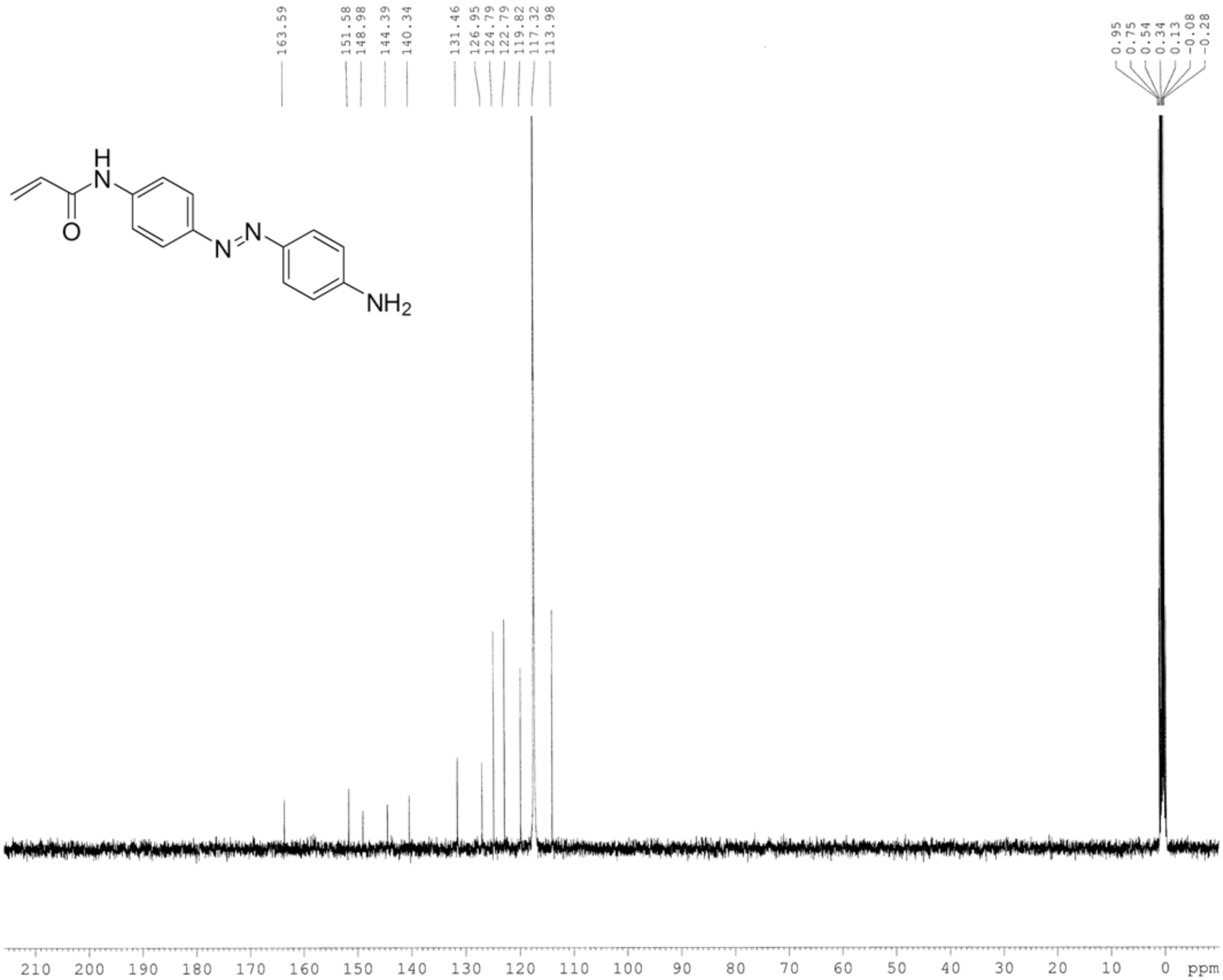
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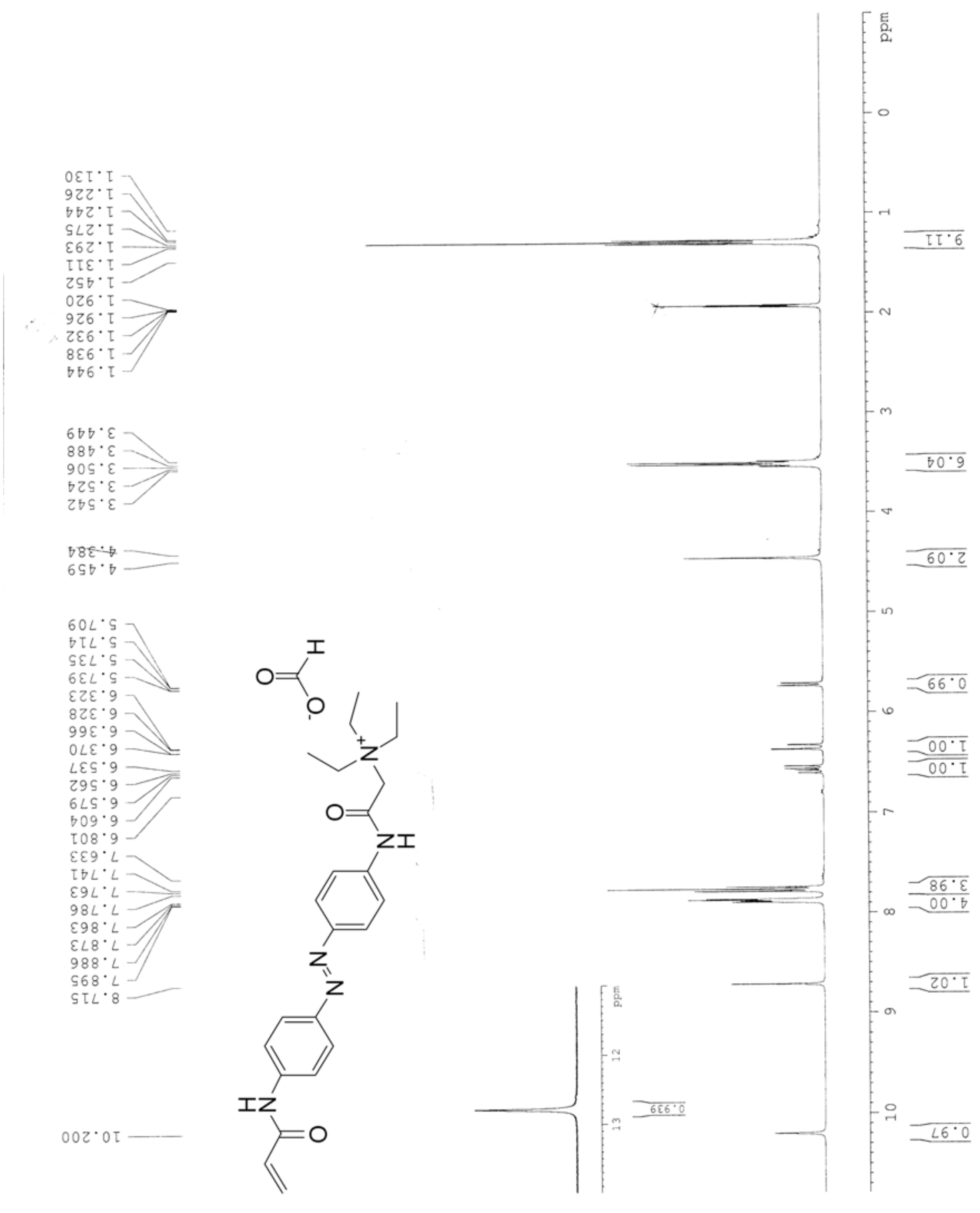
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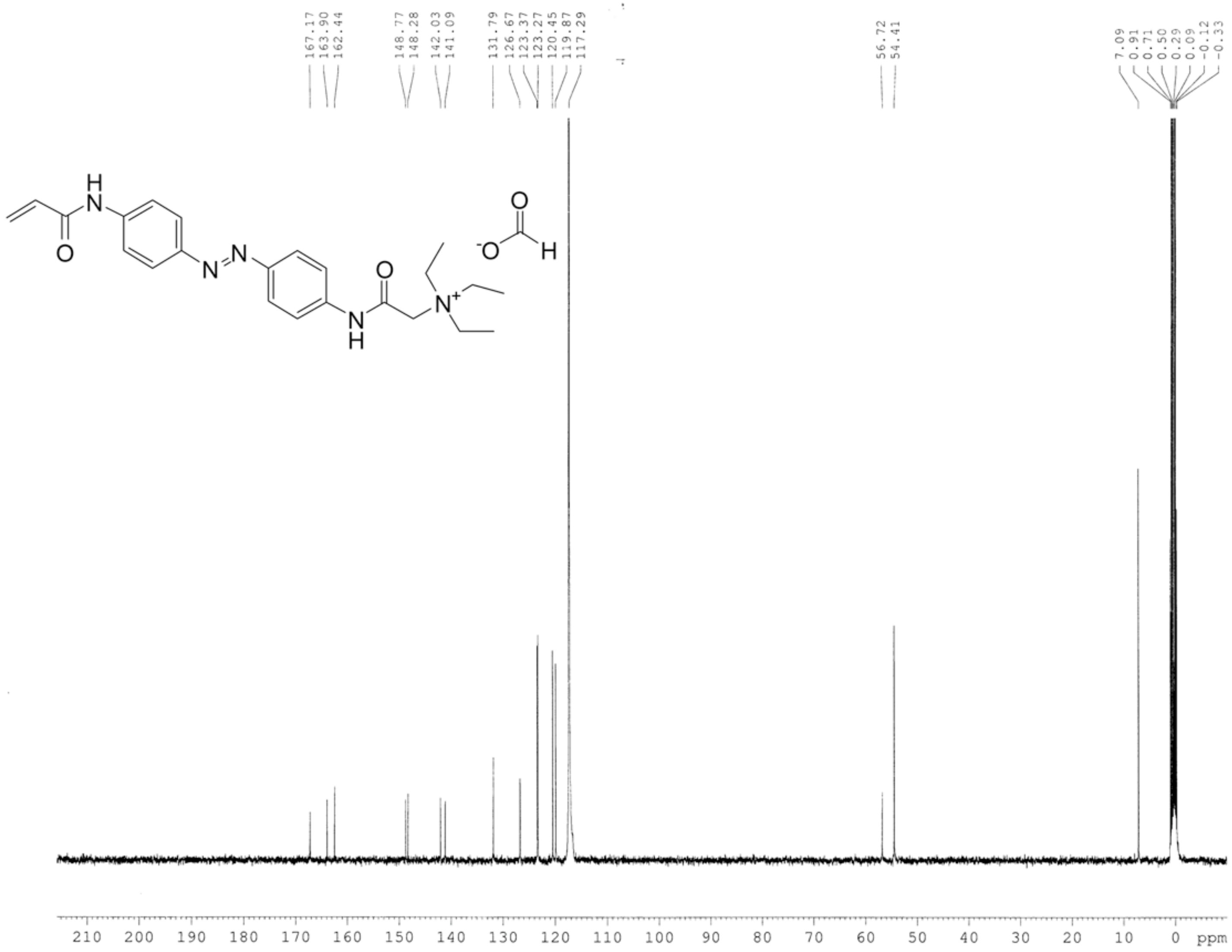
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References

1. Challenger, F.; Taylor, P.; Taylor, B. *Journal of the Chemical Society, Abstracts* **1942**, 48-55.