Supporting Information

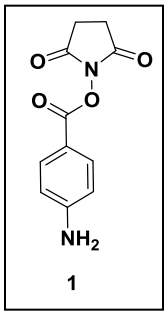
General Procedures and Materials

Unless otherwise noted, all chemicals and solvents were of analytical grade and used as received from commercial sources. Water (dd-H_2O) used in biological procedures or as a reaction solvent was deionized using Milli-Q Advantage A-10 water purification system (MilliPore, USA). UV-Vis spectra were acquired on a DU 730 spectrophotometer (Beckman Coulter, USA) with quartz cuvettes. Centrifugations were carried out in an X-22R benchtop centrifuge (Beckman Coulter, USA). Integrated DNA Technologies (IDT, Iowa, USA) supplied all DNA. MALDI-TOF analysis was performed on a Perseptive Biosystems Voyager-DE STR with a 2',4',6'-trihydroxyacetophenone matrix. Electrospray ionization mass spectrometry of the DNA-catalysts was achieved by directly infusing the sample into a Waters G2 Synapt mass spectrometer.

DNA Sequences Used

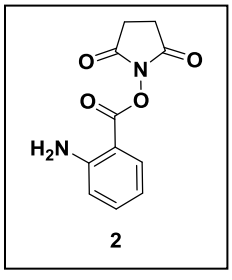
A15-aniline**:**	5'– AAAAAAAAAAAAAAA-aniline – 3'
T15**:**	5' – I linker – TTTTTTTTTTTTTTT – 3'
Fig. 2 template:	5'-CCA CAC TGG TAC TGA GAC ACG GTC CAG ACT-3'
Figure 2 catalyst sequence:	5'-AGT CTG GAC CGT GTC – 3-NH_2
Fig. 2 Acyl hydrazine:	5'-I-linker-TCA GTA CCA GTG-3'

Synthetic Procedures



1

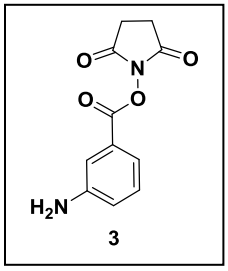
2,5-dioxopyrrolidin-1-yl 4-aminobenzoate (1): A 20 mL scintillation vial was charged with 4-amino benzoic acid (137 mg, 1.0 mmol), EDC (310 mg, 2.0 mmol), N-hydroxysuccinimide (175 mg, 1.5 mmol), and a stir bar. CH_2Cl_2 (10 mL) was added, and the suspension was stirred at room temperature. After 1 h, the solution turned clear, and after 3 h, the reaction was diluted with H_2O (5 mL), transferred to a separatory funnel, and the layers were separated. The organic phase was washed with H_2O (2 × 5 mL), brine (1 × 5 mL), and dried over $MgSO_4$. The solvent was removed by rotary evaporation to yield a white foam (72 mg, 0.31 mmol, 31%). ^{1}H NMR (DMSO-d_6, 400 MHz): δ 7.74 (2H, t, J = 7.72 Hz), δ 6.64 (2H, t, J = 6.65 Hz), δ 2.85 (4H, s). ^{13}C NMR (101 MHz, DMSO-d_6) δ 171.25, 161.88, 155.86, 132.74, 113.48, 109.64, 25.92. IR (neat): 3470, 3360, 3010, 2920, 2850, 1730, 1600, 1260, 1210, 1070, 752 cm^{-1}. ESI-MS, calculated for [M+H]: 235.07; found: 235.07.



2

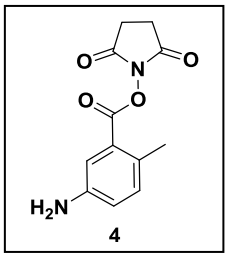
2,5-dioxopyrrolidin-1-yl 2-aminobenzoate (2): A 20 mL scintillation vial was charged with 2-amino benzoic acid (137 mg, 1.0 mmol), EDC (310 mg, 2.0 mmol), N-hydroxysuccinimide (175 mg, 1.5 mmol), and a stir bar. CH_2Cl_2 (10 mL) was added, and the suspension was stirred at room temperature. After 1 h, the solution turned clear, and after 3 h, the reaction was diluted with H_2O (5 mL), transferred to a separatory funnel, and the layers were separated. The organic phase was washed with H_2O (2 × 5 mL), brine (1 × 5 mL), and dried over $MgSO_4$. The solvent was removed by rotary evaporation to yield a light yellow foam (19 mg, 0.08 mmol, 8%). ^{1}H NMR (400 MHz, DMSO-

d_6) δ 7.79 (dd, *J* = 8.1, 1.7 Hz, 1H), 7.41 (ddd, *J* = 8.6, 7.0, 1.5 Hz, 1H), 6.89 (d, *J* = 8.6 Hz, 1H), 6.80 (s, 2H), 6.62 (td, *J* = 7.4, 6.9, 1.2 Hz, 1H), 2.88 (s, 4H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 171.14, 162.66, 153.26, 136.65, 130.48, 117.45, 115.76, 103.22, 25.97. IR (neat): 3340, 3130, 2660, 1480, 1370, 1100, 873, 821, 722 cm^{-1}. ESI-MS, calculated for [M+H]: 235.1; found: 235.1.



3

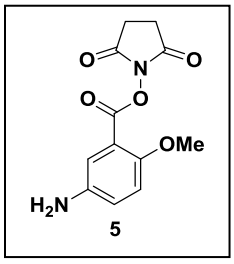
2,5-dioxopyrrolidin-1-yl 3-aminobenzoate (3): A 20 mL scintillation vial was charged with 3-amino benzoic acid (137 mg, 1.0 mmol), EDC (310 mg, 2.0 mmol), N-hydroxysuccinimide (175 mg, 1.5 mmol), and a stir bar. CH_2Cl_2 (10 mL) was added, and the suspension was stirred at room temperature. After 1 h, the solution turned clear, and after 3 h, the reaction was diluted with H_2O (5 mL), transferred to a separatory funnel, and the layers were separated. The organic phase was washed with H_2O (2 × 5 mL), brine (1 × 5 mL), and dried over $MgSO_4$. The solvent was removed by rotary evaporation to yield a white foam (144 mg, 0.62 mmol, 62%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.32 – 7.23 (m, 2H), 7.23 – 7.16 (m, 1H), 7.00 – 6.92 (m, 1H), 2.88 (s, 4H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 169.31, 162.05, 146.85, 129.73, 125.89, 121.20, 120.55, 116.15, 25.69. IR (neat): 3360, 2960, 2920, 1730, 1460, 1130, 903, 737 cm^{-1}.ESI-MS, calculated for [M+H]: 235.07; found: 235.07.



4

2,5-dioxopyrrolidin-1-yl 5-amino-2-methylbenzoate (4): A 20 mL scintillation vial was charged with 5-amino-2-methylbenzoic acid (15 mg, 0.10 mmol), EDC (31 mg, 0.20 mmol), N-hydroxysuccinimide (17 mg, 0.15 mmol), and a stir bar. A mixture of THF and CH_2Cl_2 was added (1:1, 3 mL), and the suspension was stirred at room temperature. After 3 h, the reaction was diluted with CH_2Cl_2 (10 mL) and H_2O (5 mL), transferred to a separatory funnel, and the layers were separated. The organic phase was washed with H_2O (2 × 5 mL), brine (1 × 5 mL), and dried over $MgSO_4$. The solvent was removed by rotary evaporation to yield a white foam. Purification by prepratory TLC (CH_2Cl_2:EtOH, 95:5) furnished **4** as a white solid (9.2 mg, 0.04 mmol, 32%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.42 (d, *J* = 2.6 Hz, 1H), 7.09 (d, *J* = 8.2 Hz, 1H), 6.87 (dd, *J* = 8.0, 2.4 Hz, 1H), 3.78 – 3.71 (m, 2H), 2.92 (s, 4H), 2.48 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 169.45, 162.15, 144.38, 132.76, 131.63, 124.53, 120.85, 117.10, 25.71, 20.54. IR (neat): 3430, 3380, 2960, 2920, 2850, 1730, 1500, 1200, 1190, 1070, 910 cm^{-1}. ESI-MS, calculated for [M+H]: 249.09; found: 249.09.

2,5-dioxopyrrolidin-1-yl 5-amino-2-methoxybenzoate (5): A 20 mL scintillation vial protected from light was charged with 5-amino-2-methoxybenzoic acid (42 mg, 0.25 mmol), EDC (39 mg, 0.25 mmol), N-hydroxysuccinimide (29 mg, 0.25 mmol), and a stir bar. DMF (2 mL) was added, and the mixture was stirred at room temperature. After 3 h, the reaction was diluted with EtOAc (15 mL) and transferred to a separatory funnel. The organic phase was washed with H_2O (5 × 5 mL), brine (1 × 5 mL), and dried over $MgSO_4$. The solvent was removed by rotary evaporation to yield a yellow oil, which was subjected to prepratory TLC (CH_2Cl_2:EtOH, 95:5) in the dark to provide **5** as a light yellow solid (17 mg, 0.06 mmol, 26%). Note: This compound is unstable and should be used immediately after preparation. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.39 (dd, *J* = 3.0, 0.4 Hz, 1H), 6.97 (dd, *J* = 8.9, 2.9 Hz, 1H), 6.90 (d, *J* = 8.8 Hz, 1H), 3.88 (s, 3H), 2.91 (s, 4H). ^{13}C NMR (101



5

MHz, $CDCl_3$) δ 169.44, 160.36, 153.99, 139.63, 122.94, 118.34, 114.06, 56.72, 29.71, 25.70. IR (neat): 3350, 3960, 2920, 2850, 1730, 1510, 1210, 1060, 823 cm^{-1}. ESI-MS, calculated for [M+H]: 265.08; found: 265.08.

General Synthetic Procedure for DNA-organocatalysts: A 1.4 mL Eppendorf tube was charged with DNA-3'-NH_2 (10 μL, 1.3 mM stock in ddH_2O), $NaHCO_3$ (10 μL, 300 mM in ddH_2O), and the appropriate NHS-ester (10 μL, 126 mM in DMSO). The vial was incubated overnight at 4 ˚C, after which the reaction was diluted with ddH_2O and purified with a NAP-5 column to provide an aqueous solution of the DNA-catalyst. IDT was contracted to purify the A15-aniline catalyst derivative; the remainder were purified in house as described below.

HPLC Purification of DNA-catalysts: HPLC purification of the DNA-catalysts was carried out on an HP Series 1100 HPLC fitted with an Agilent Zorbax SB-C18 column. Elution conditions were as follows: Triethylammonium bicarbonate buffer (TEAB): MeCN (100:0 → 50:50 over 40 minutes) at a rate of 1.5 mL per minute. 1.5 mL fractions were collected with an Isco Foxy Jr. fraction collector, and fractions containing products were analyzed by MALDI-MS to identify the desired product fractions. ESI-MS was carried out to provide accurate mass determinations of the DNA-catalysts.

SI Table 1. Retention times and summary of ESI-MS analysis.

Compound	Elution Time	Expected Mass	Found Mass
DNA-**1:**	20.4 min.	4914.25	4914.04
DNA-**2**:	20.7 min.	4914.25	4914.30
DNA-**3**:	20.5 min.	4914.25	4914.30
DNA-**4**:	20.5 min.	4928.25	4928.33
DNA-**5**:	20.8 min.	4944.25	4944.32

The mass spectra for these compounds are included as SI Figs. S17-22.

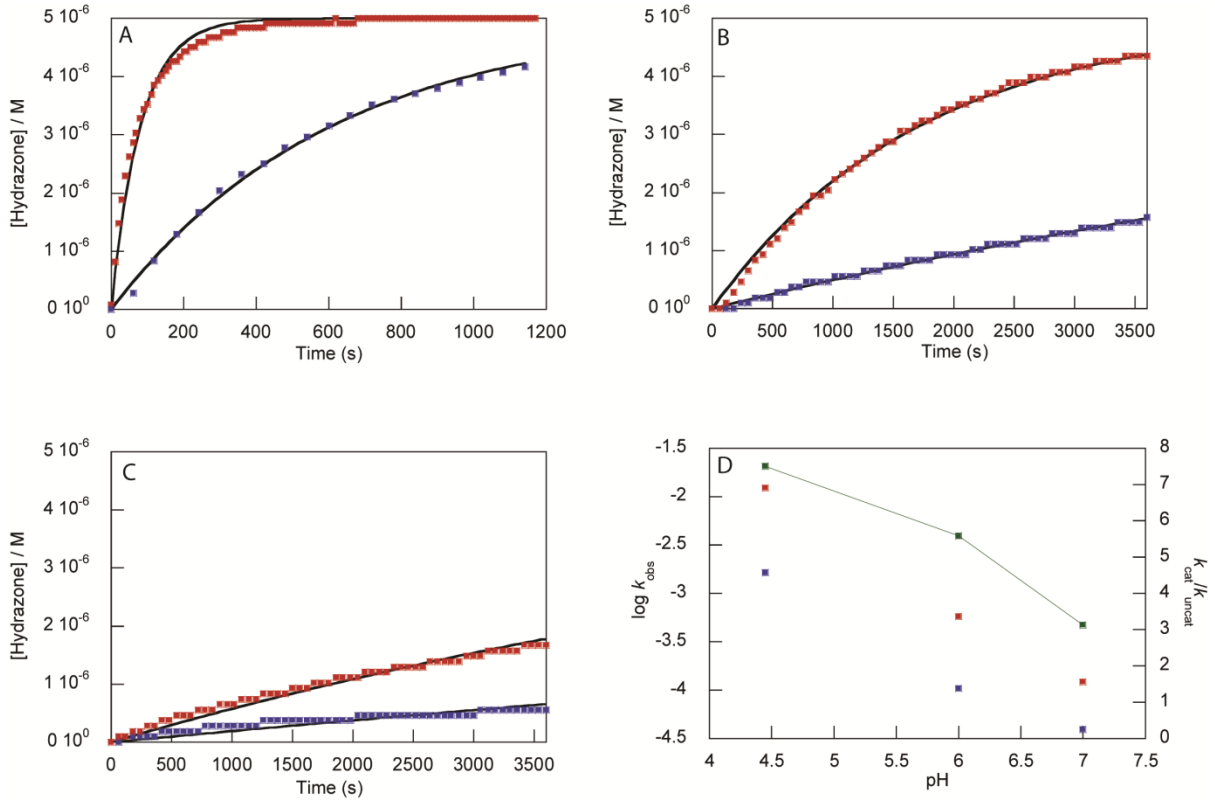


Figure S1. Plots showing the pH dependence of the hydrazone formation between acyl hydrazine-derivatized T15 DNA (5.0 μM) and 4-nitrobenzaldehyde (500 μM) in 50 mM PB with 150 mM NaCl at (A) pH 4.45, (B) pH 6.0, and (C) pH 7.0 in the absence (blue) and presence (red) of complementary A15-aniline (5.0 μM). The signal at 340 nm was measured every 60 seconds, except for the red trace in (A), which was measured every 10 seconds. (D) Plot showing the effect of pH on the rate of hydrazone formation for uncatalyzed (blue) and complementary A15-aniline-catalyzed reactions (red) and the corresponding k_{cat}/k_{uncat} values.

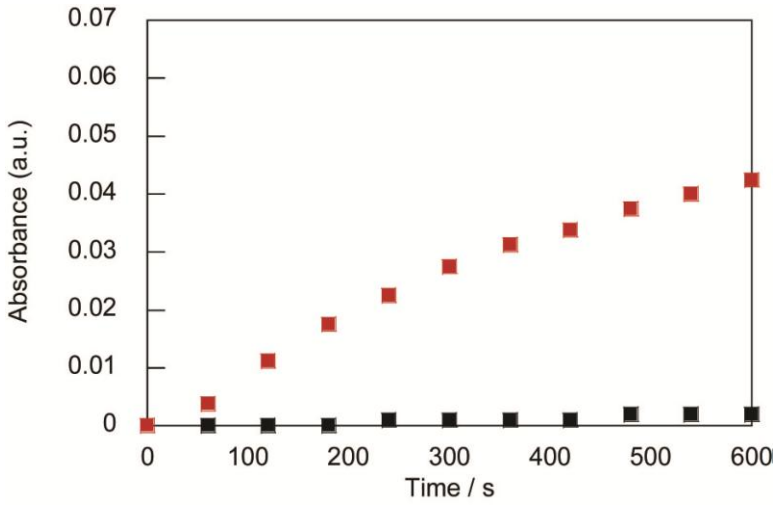


Figure S2. Contribution of the aniline Schiff base intermediate to the absorbance at 340 nm is less than 5%. (Black squares) Time course absorbance change that result from the reaction of aniline (5 μM) and 4-nitrobenzaldehyde (500 μM) in pH 4.5 buffer. (Red squares) Time course absorbance change that result from the reaction of A15-aniline (5 μM), 4-nitrobenzaldehyde (500 μM), and DNA-acyl hydrazine (5 μM) in pH 4.5 buffer.

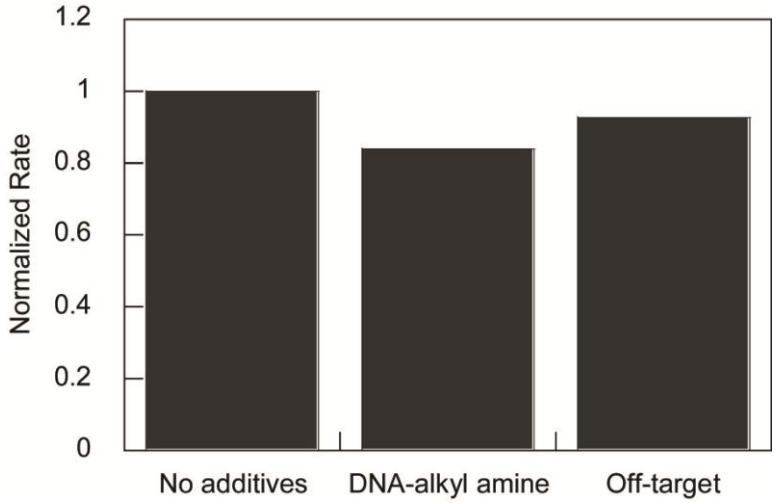


Figure S3. Control experiments that show correct basepairing and the aniline are both necessary for the observed rate enhancement to occur. Experiments were carried out with T15 acyl hydrazine DNA (1.5 μM) with 4-nitrobenzaldehyde (500 μM) in pH 5.2 50 mM HEPES, 150 mM NaCl buffer ("no additives") or with A15 DNA-3'-alkyl amine (1.5 μM), or a non-complementary acyl-hydrazine (1.5 μM) ("off target").

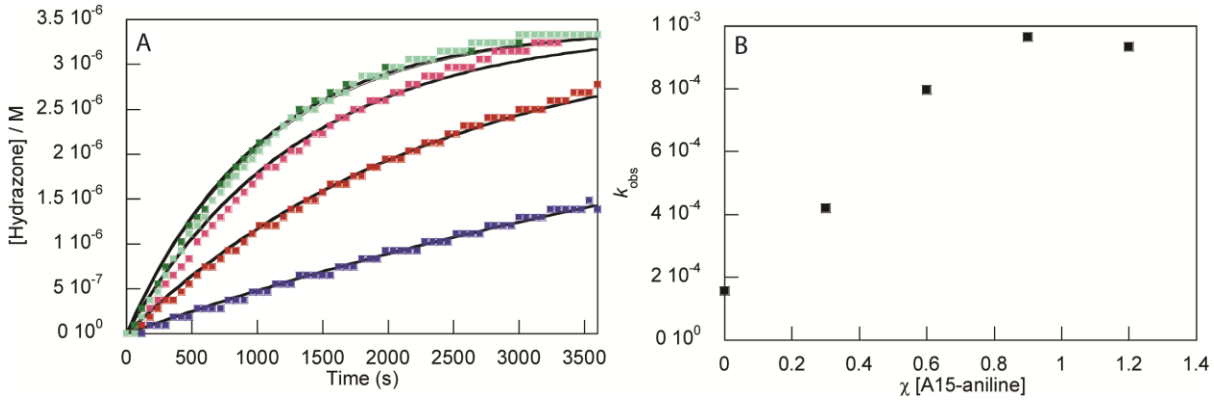


Figure S4. (A) Plots showing the rate of hydrazone formation in the absence (blue) and the presence of A15-aniline. Data points correspond to the concentration of product hydrazone as a function of time, monitored by UV-vis at 340 nm in 50 mM PB with 150 mM NaCl, pH 6.0. Traces show the rate of hydrazone formation between 3.3 μM T-15 hydrazide and 500 μM 4-nitrobenzaldehyde in the presence of 0, 1.0, 2.0, 3.0, and 4.0 μM A15-aniline. (B) Plot showing the corresponding pseudo-first order rate constants as a function of mol fraction of A15-aniline.

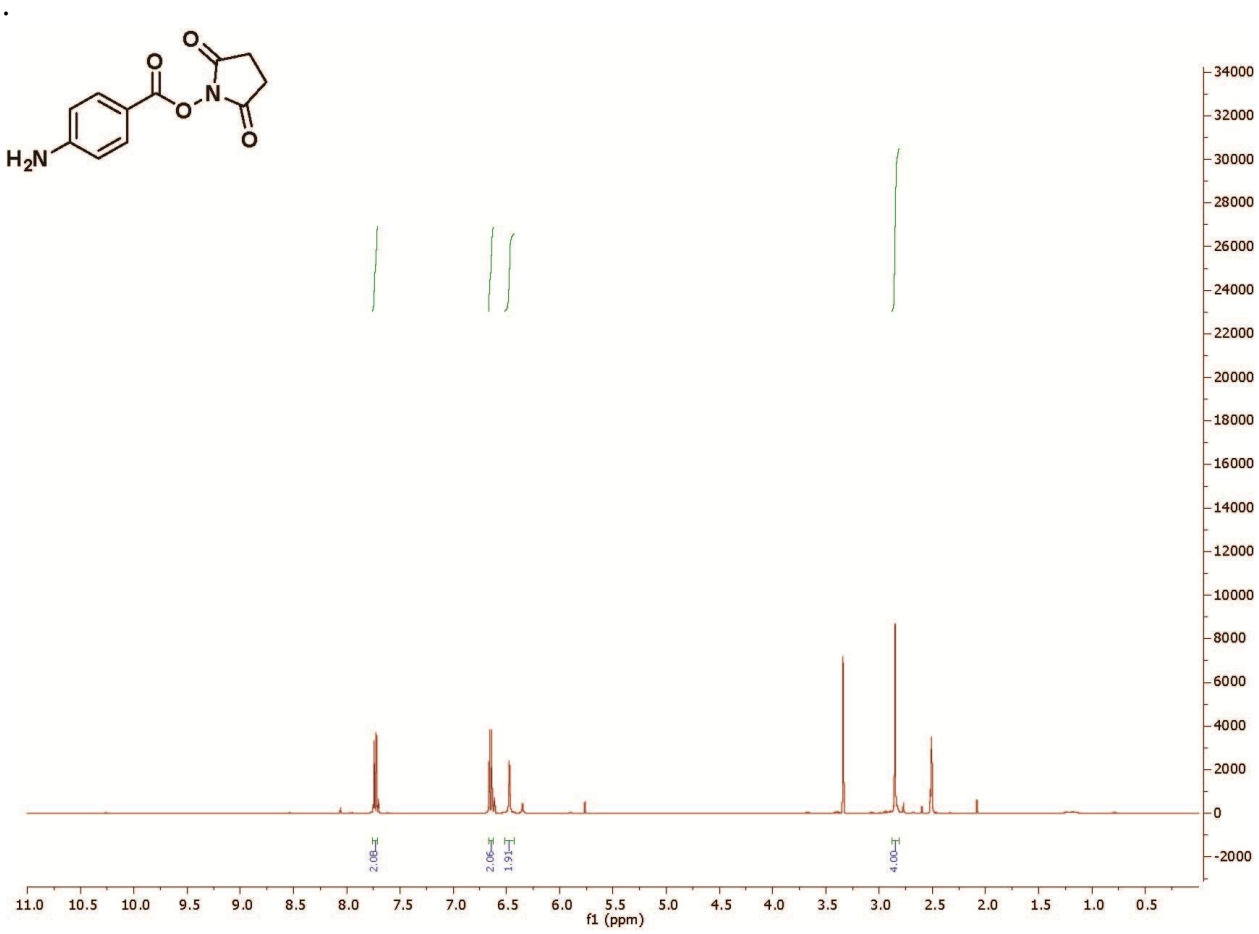


Figure S5. ^{1}H NMR spectrum of compound **1** in DMSO-d_6.

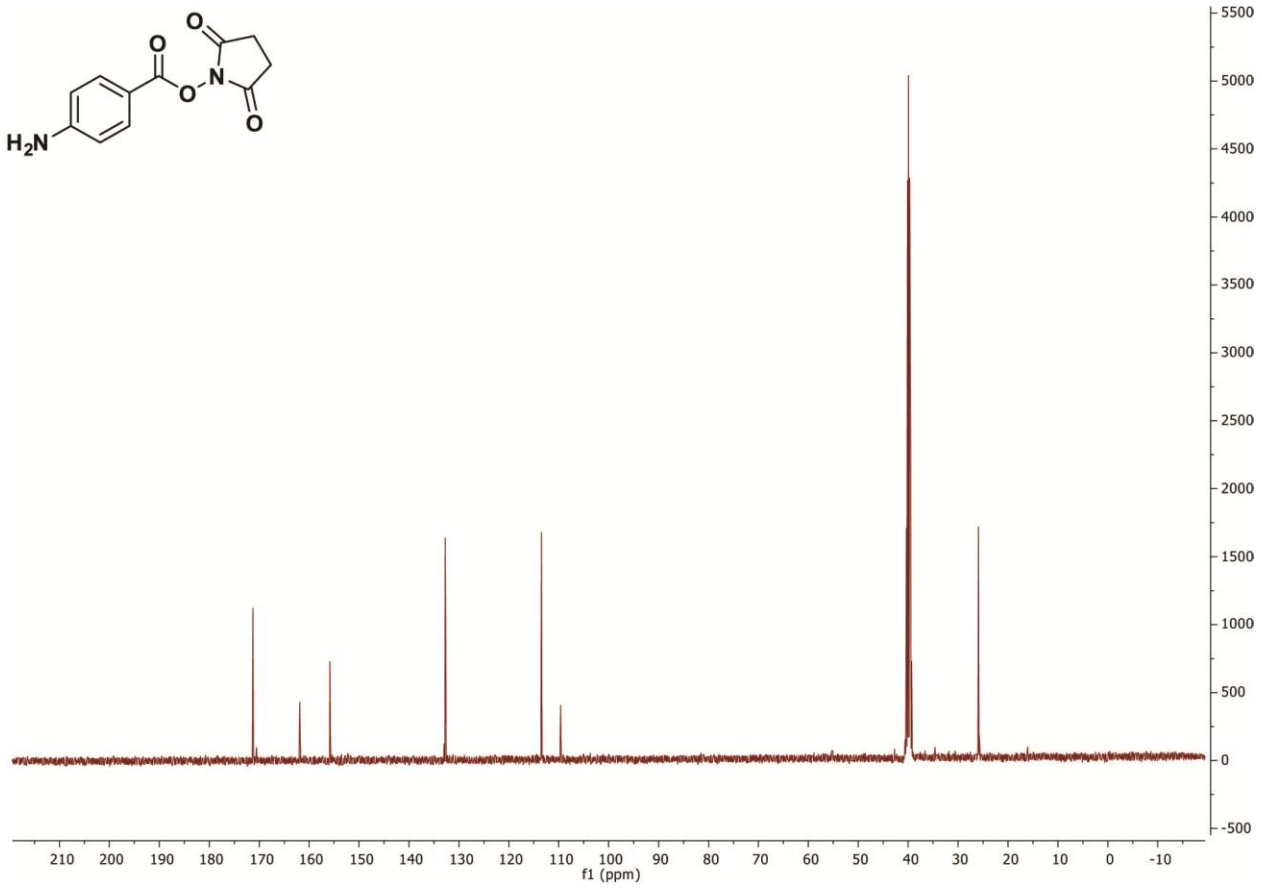


Figure S6. ^{13}C NMR spectrum of compound **1** in DMSO-d_6.

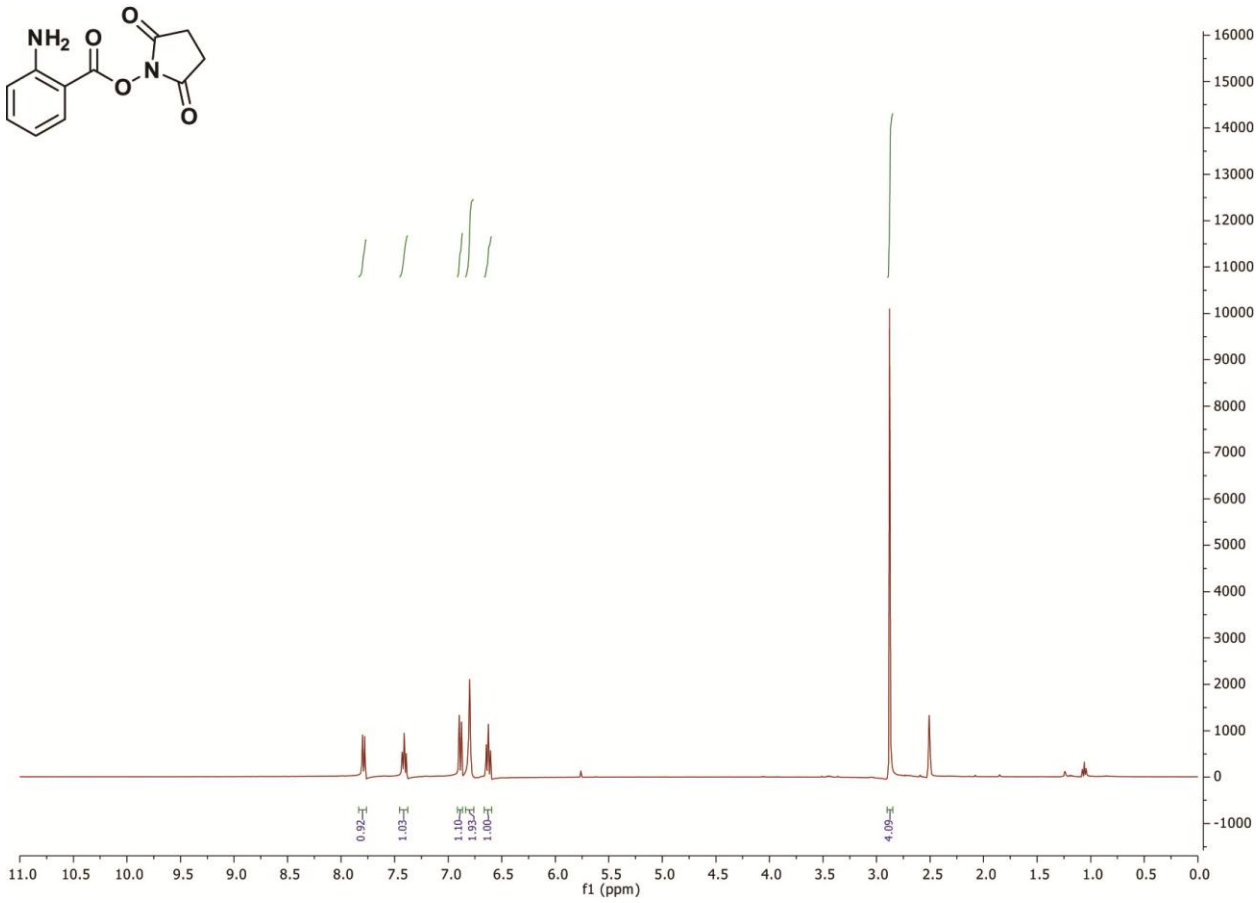


Figure S7. ^{1}H NMR spectrum of compound **2** in DMSO-d_6.

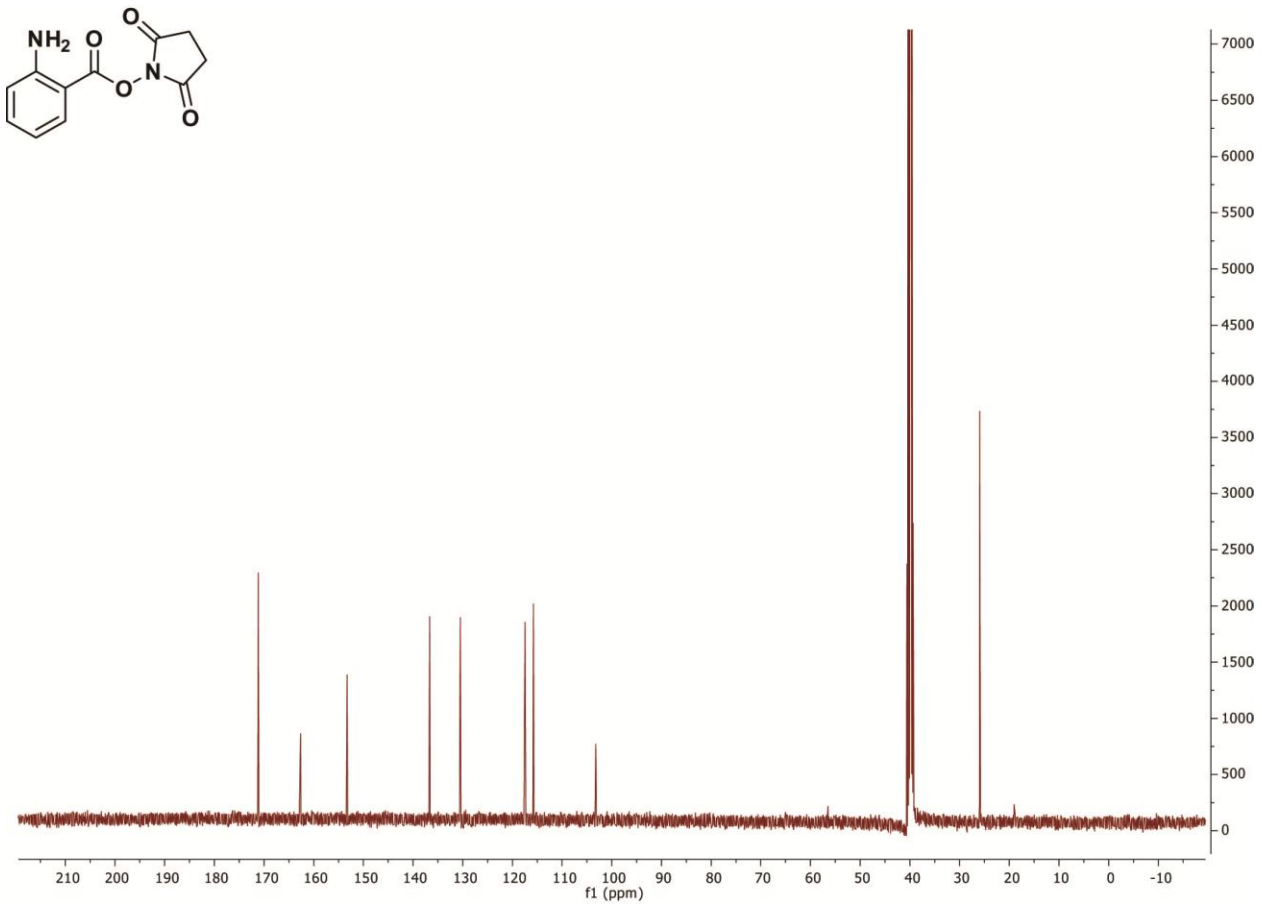


Figure S8. ^{13}C NMR spectrum of compound **2** in DMSO-d_6.

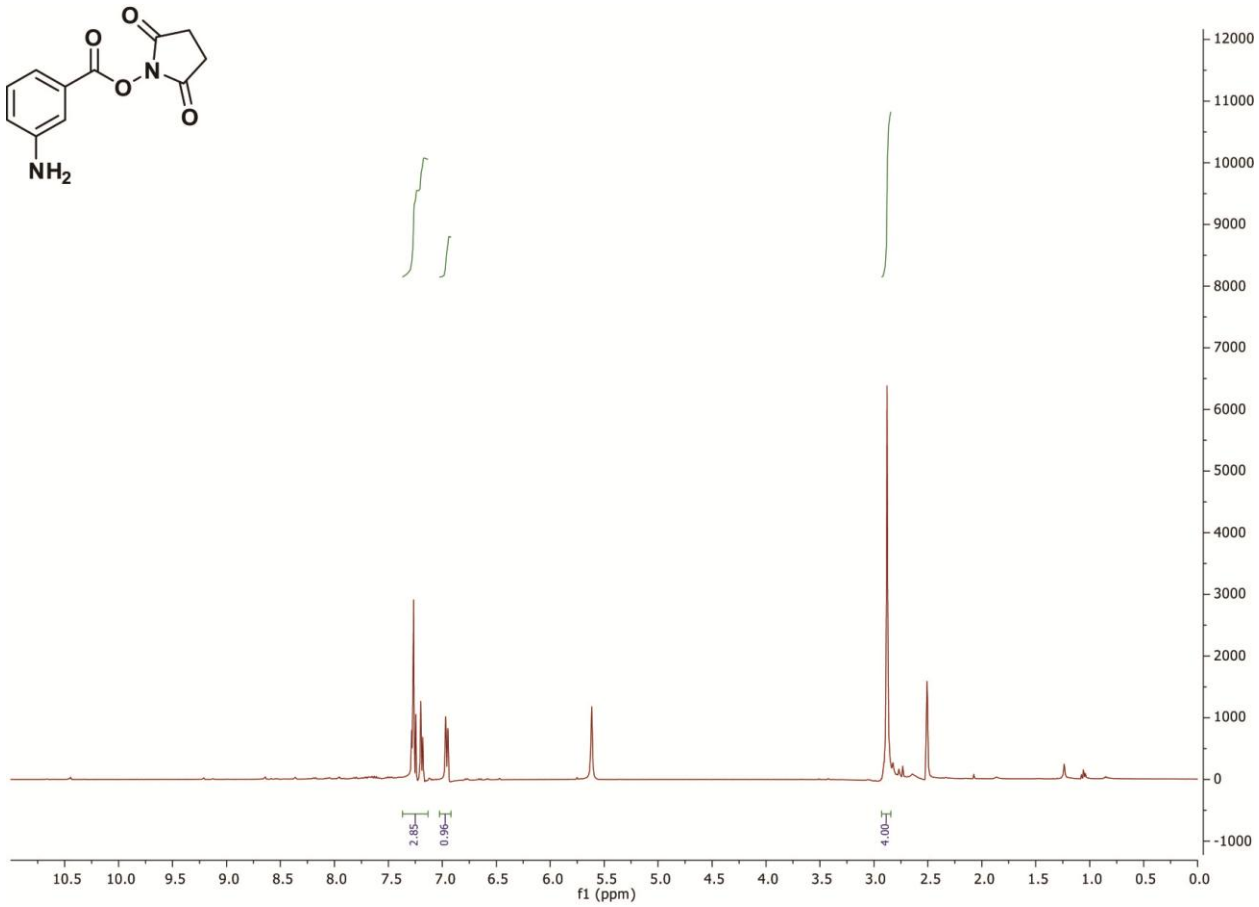


Figure S9. ^{1}H NMR spectrum of compound **3.** The spectrum was acquired in DMSO-d_6 with H_2O suppression.

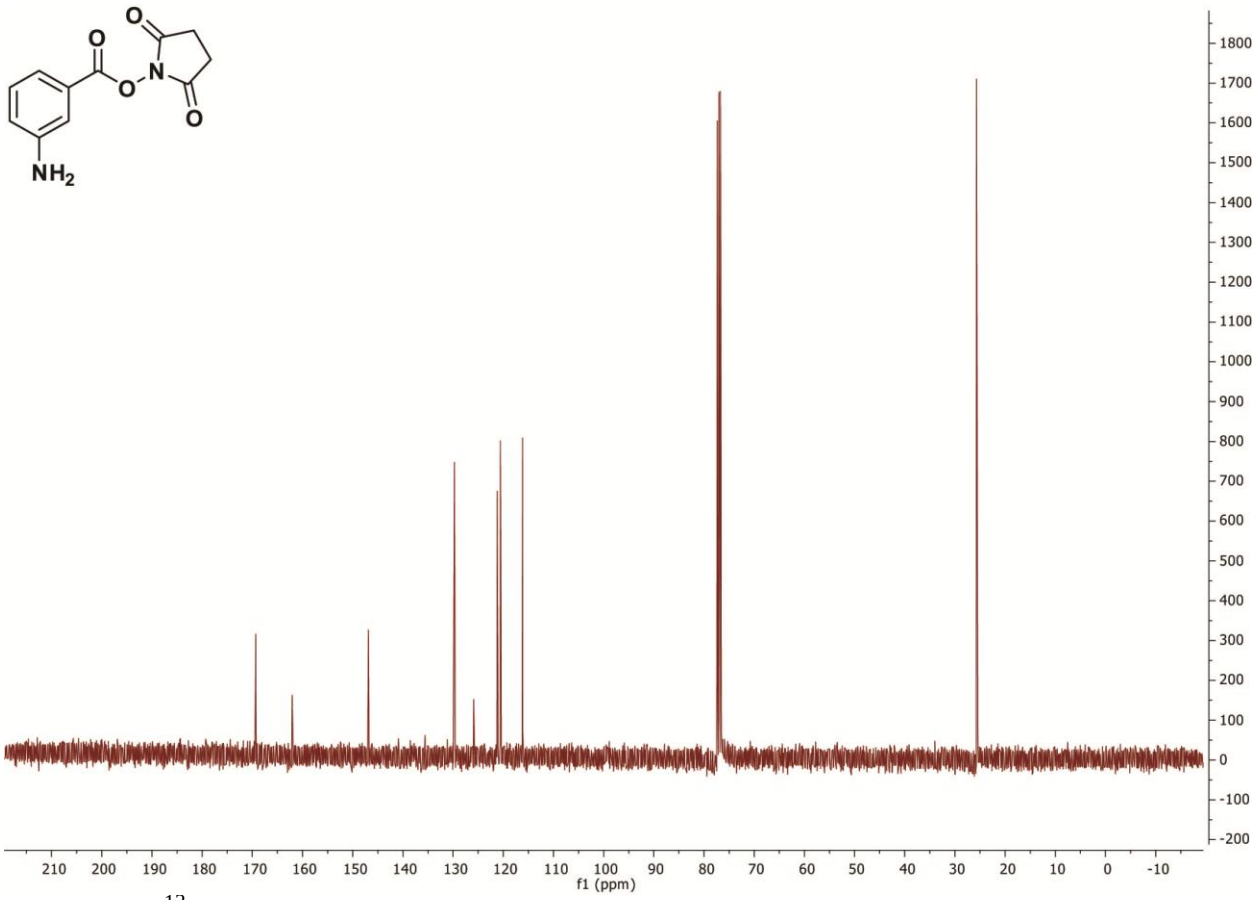


Figure S10. ^{13}C NMR spectrum of compound **3** in $CDCl_3$.

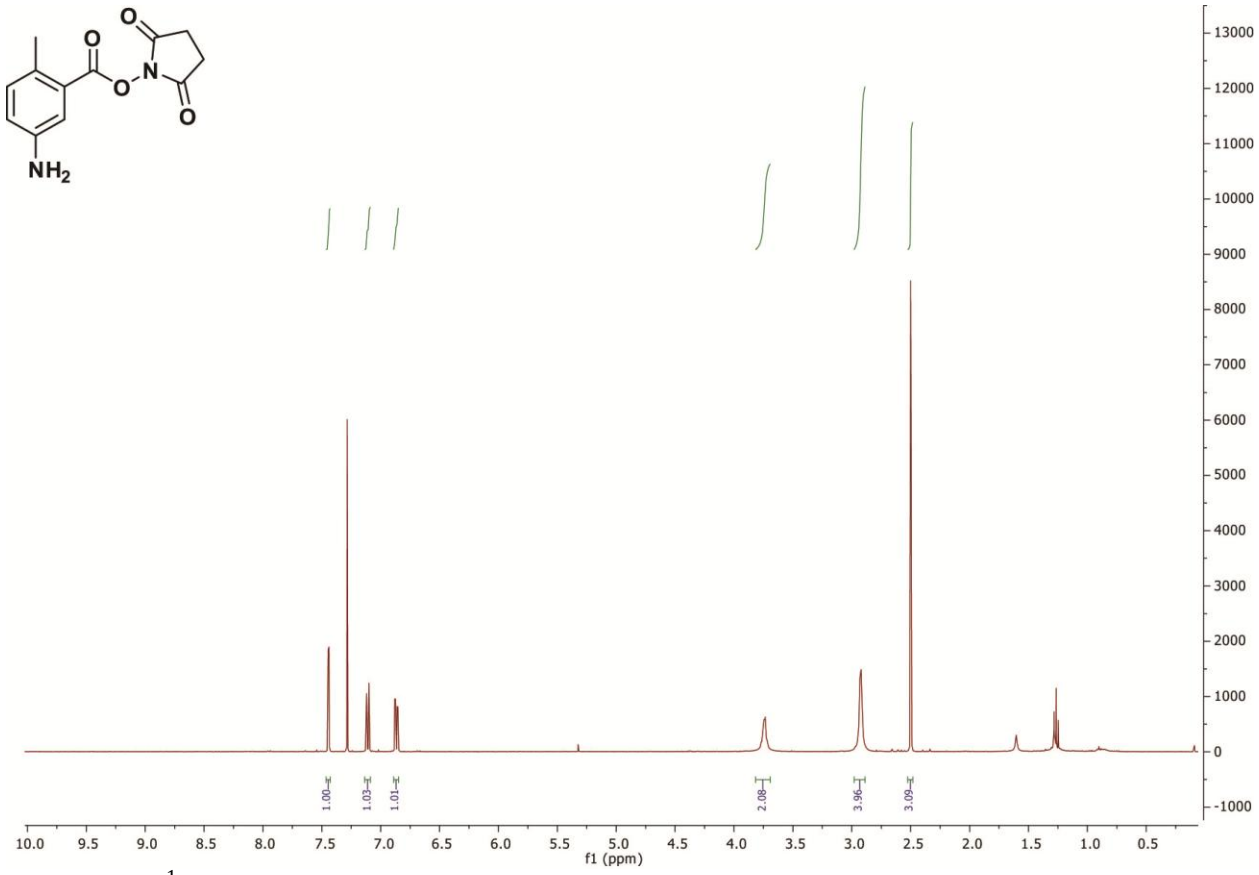


Figure S11. ^{1}H NMR spectrum of compound **4** in $CDCl_3$.

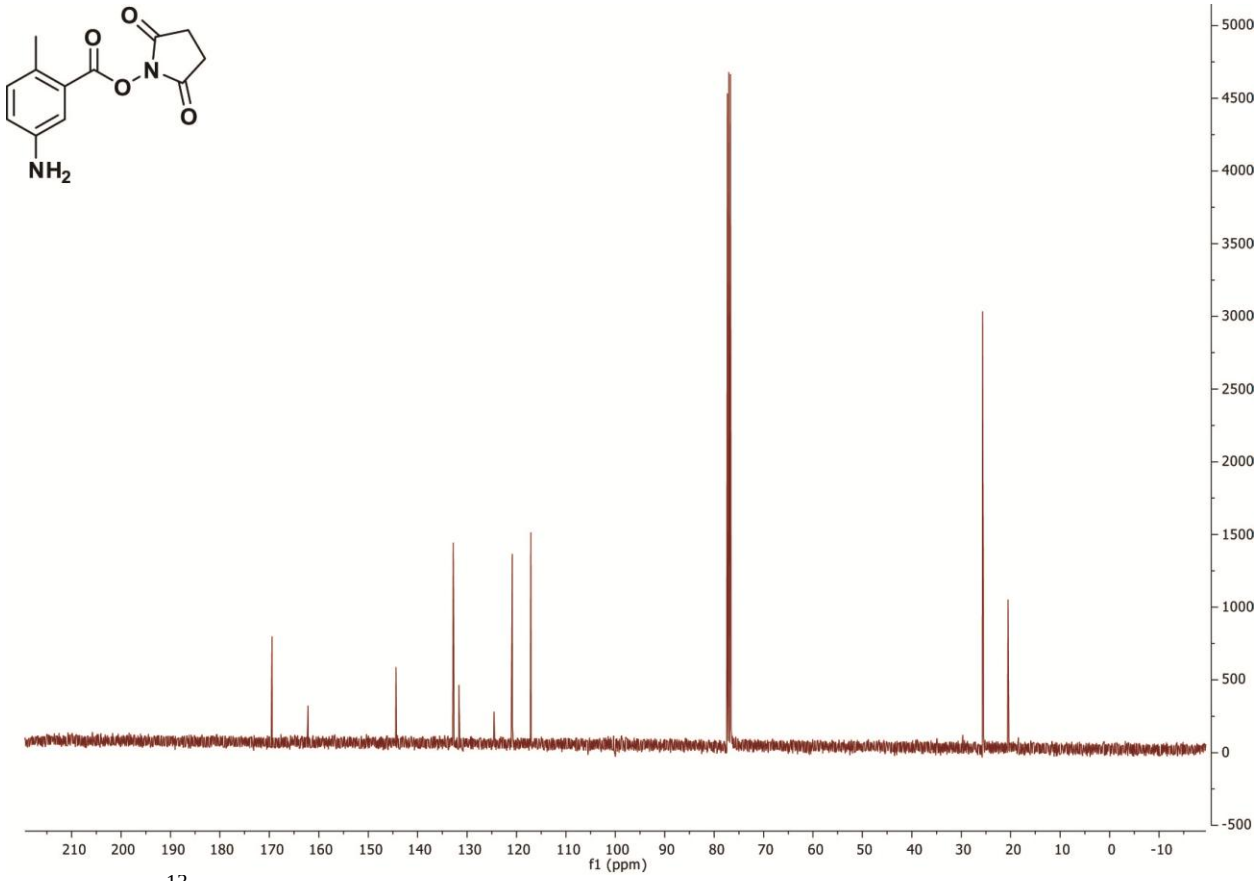


Figure S12. ^{13}C NMR spectrum of compound **4** in $CDCl_3$.

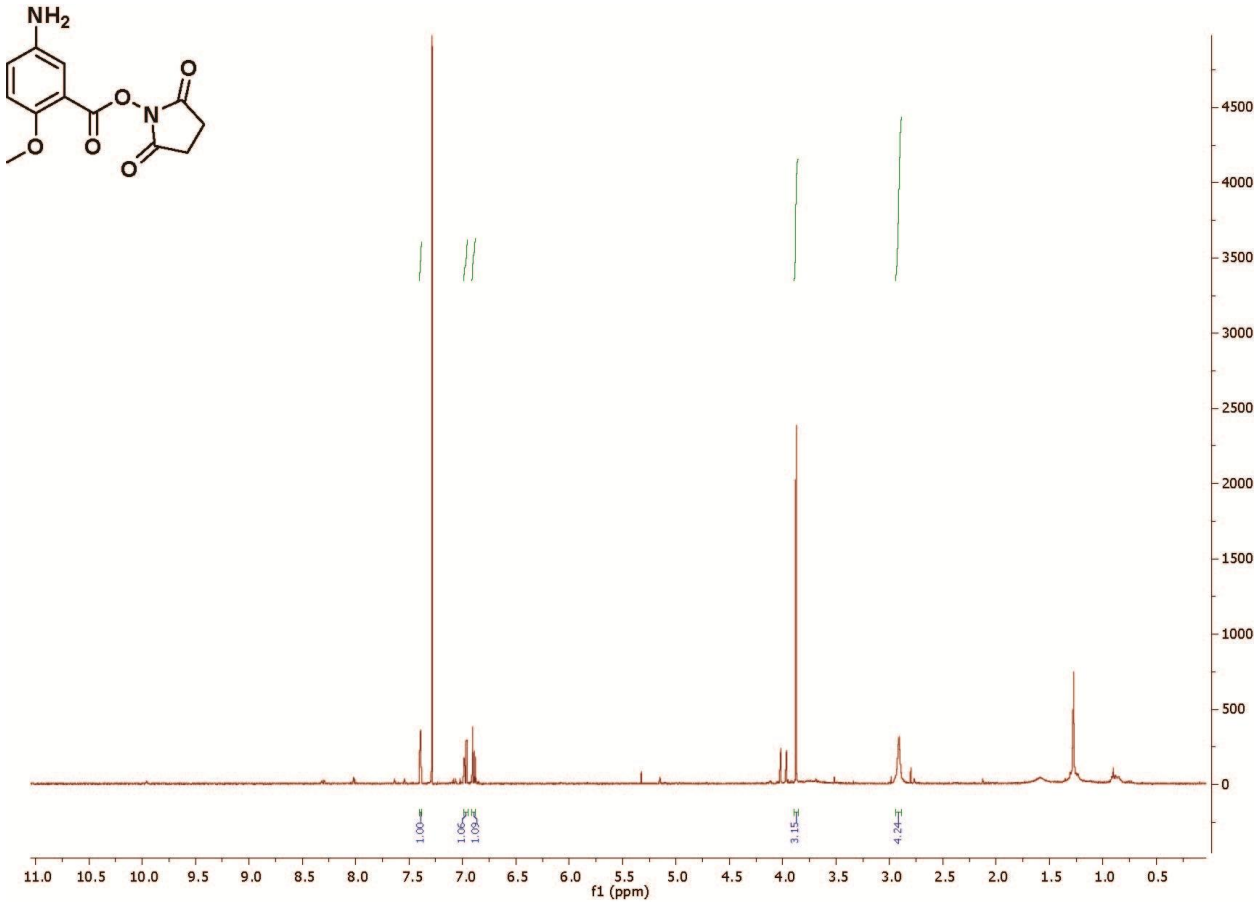


Figure S13. ^{1}H NMR spectrum of compound **5** in $CDCl_3$.

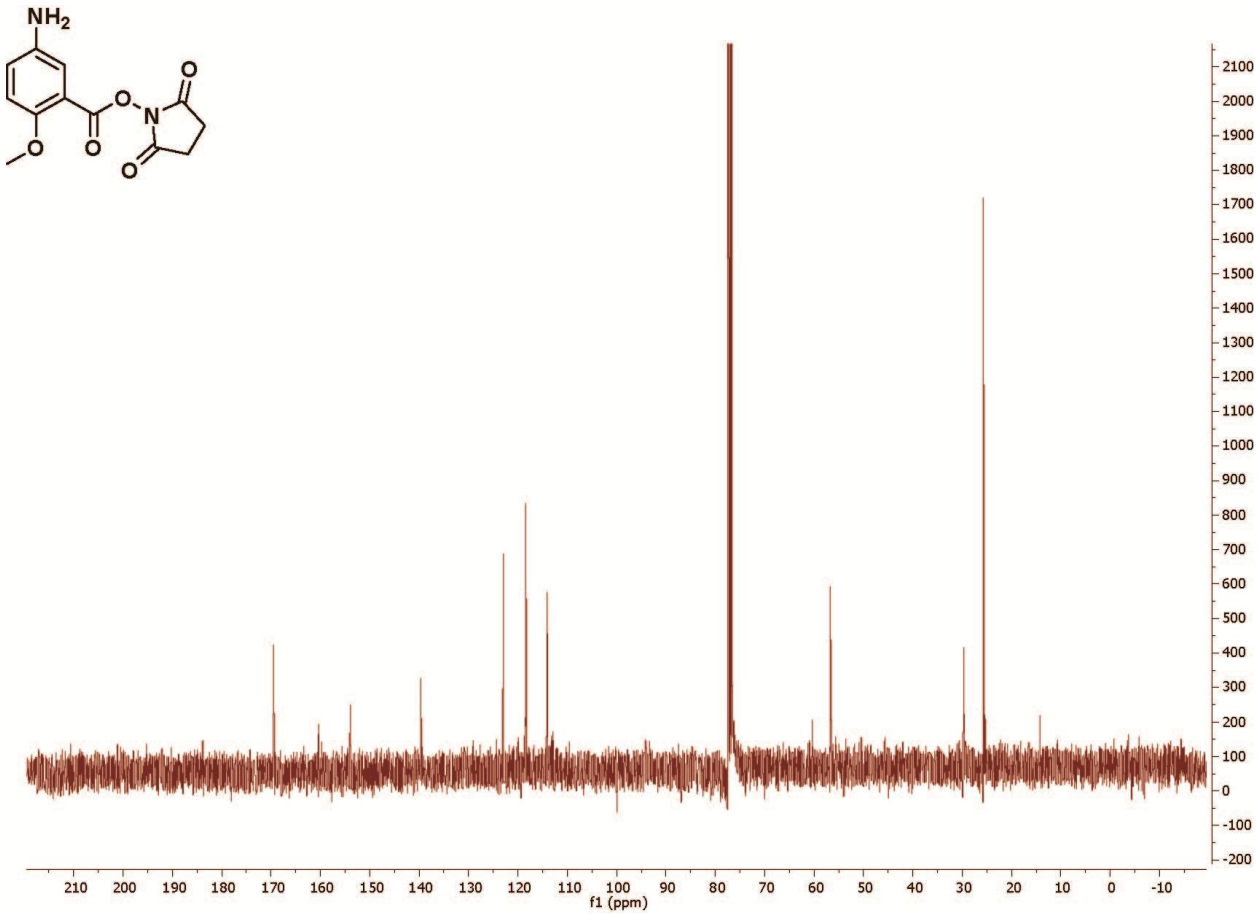


Figure S14. ^{13}C NMR spectrum of compound **6** in $CDCl_3$.

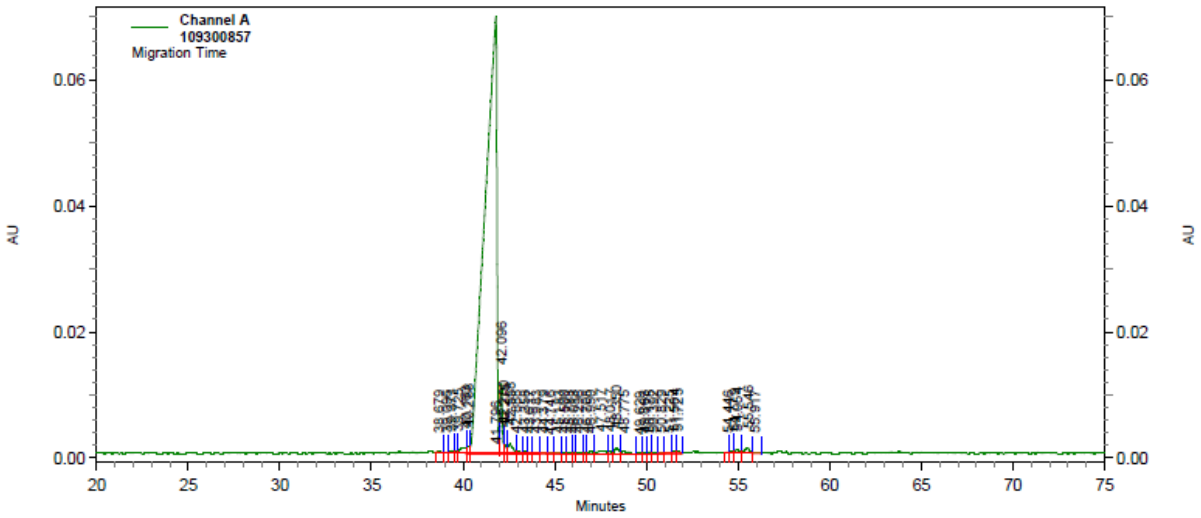


Figure S15. Analytical HPLC trace of the purified A15-aniline oligonucleotide. Analysis provided by IDT.

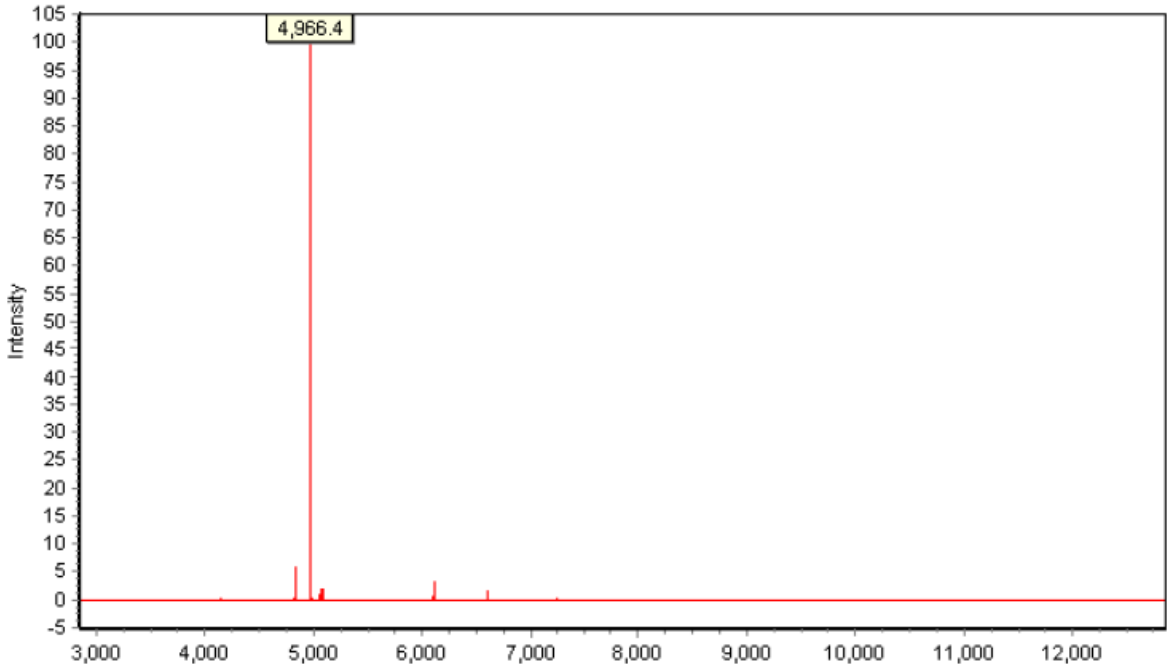


Figure S16. Oligo ESI-MS analysis of HPLC-purified A15-aniline. Expected mass: 4966.3; Found: 4966.4. Analyis provided by IDT.

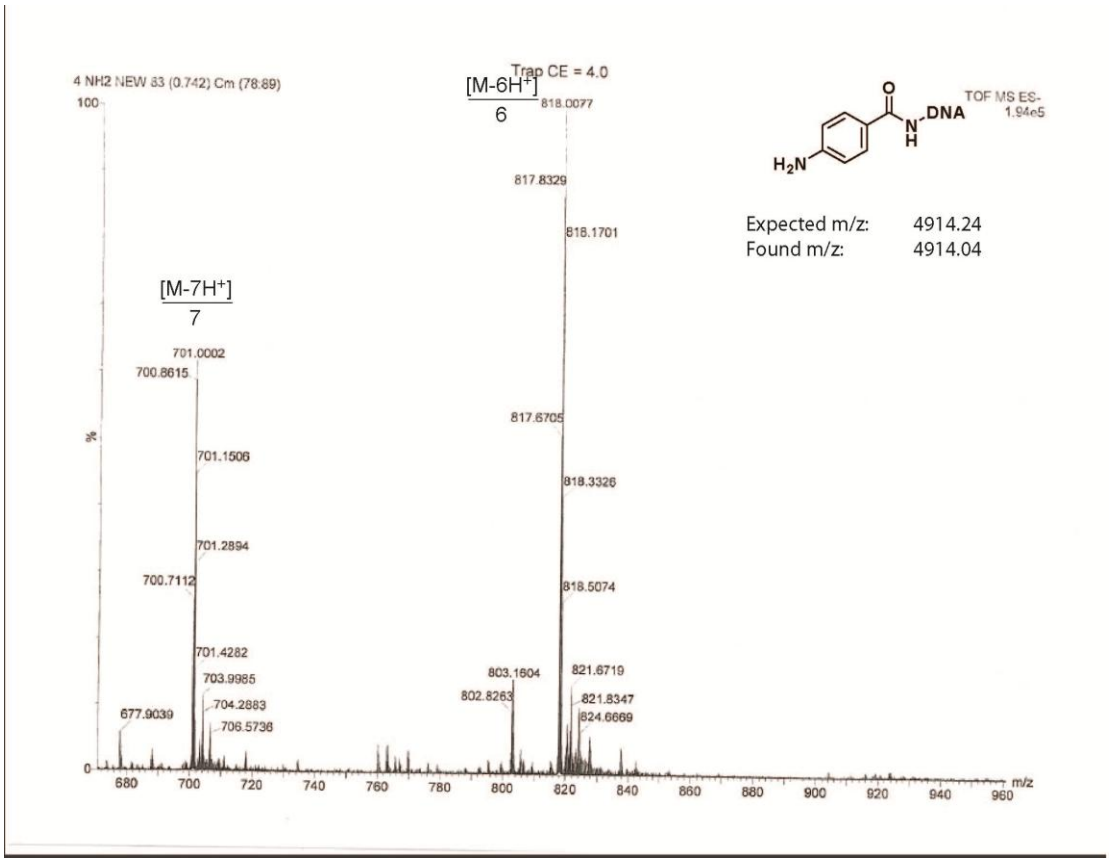


Figure S17. Oligo ESI-MS analysis of HPLC-purified **1'**. Expected mass: 4914.24; Found: 4914.04.

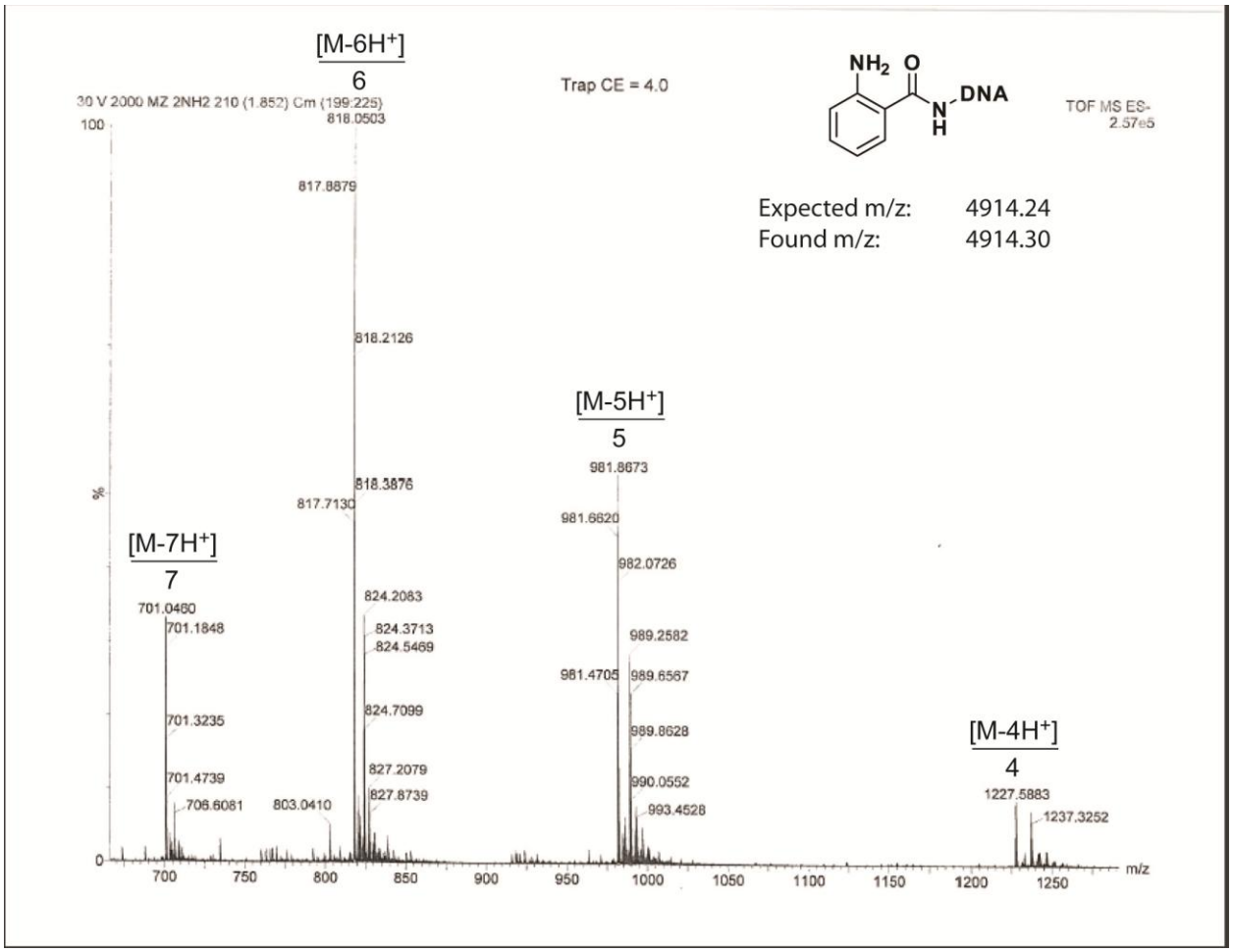


Figure S18. Oligo ESI-MS analysis of HPLC-purified **2'**. Expected mass: 4914.24; Found: 4914.30.

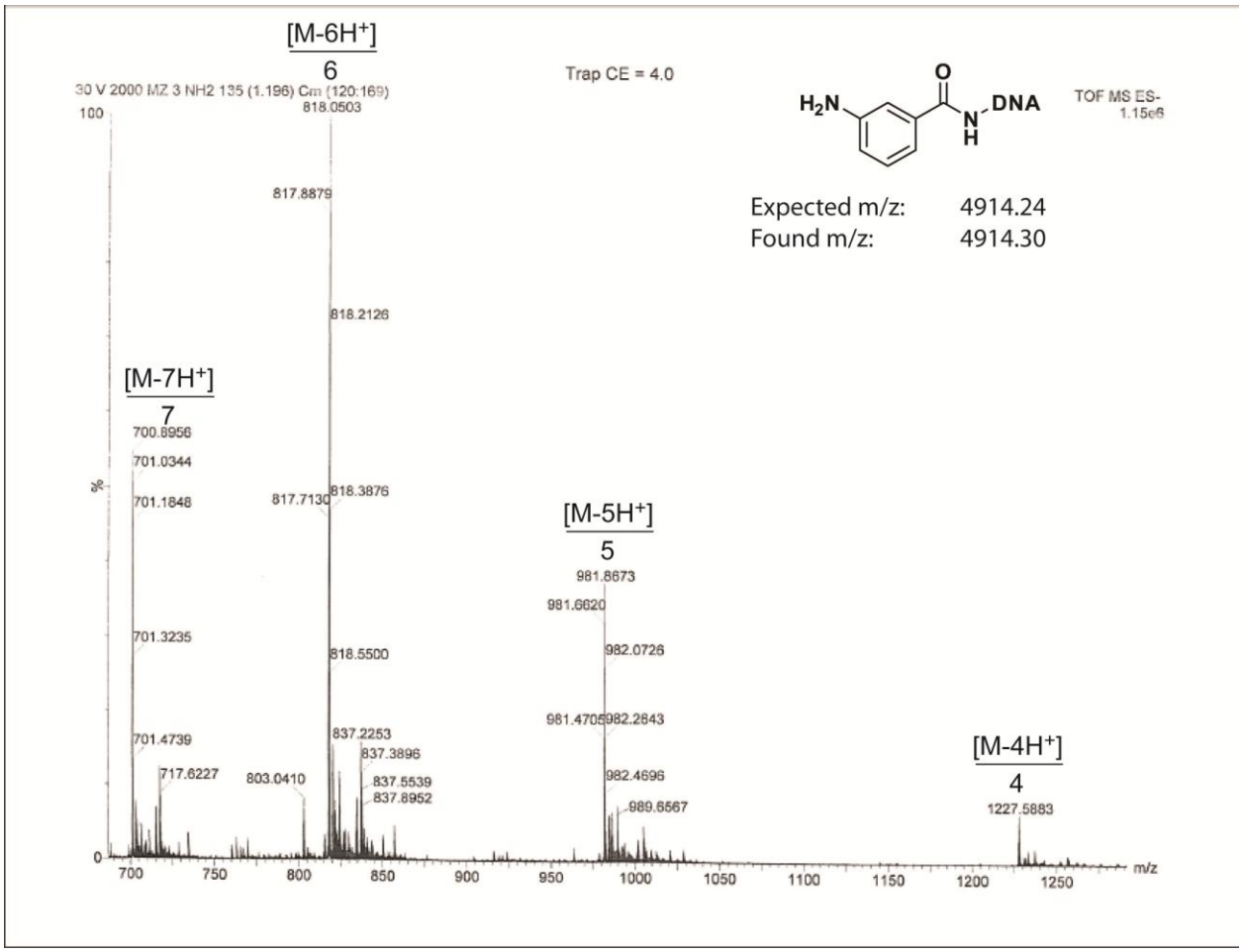


Figure S19. Oligo ESI-MS analysis of HPLC-purified **3'**. Expected mass: 4914.24; Found: 4914.30.

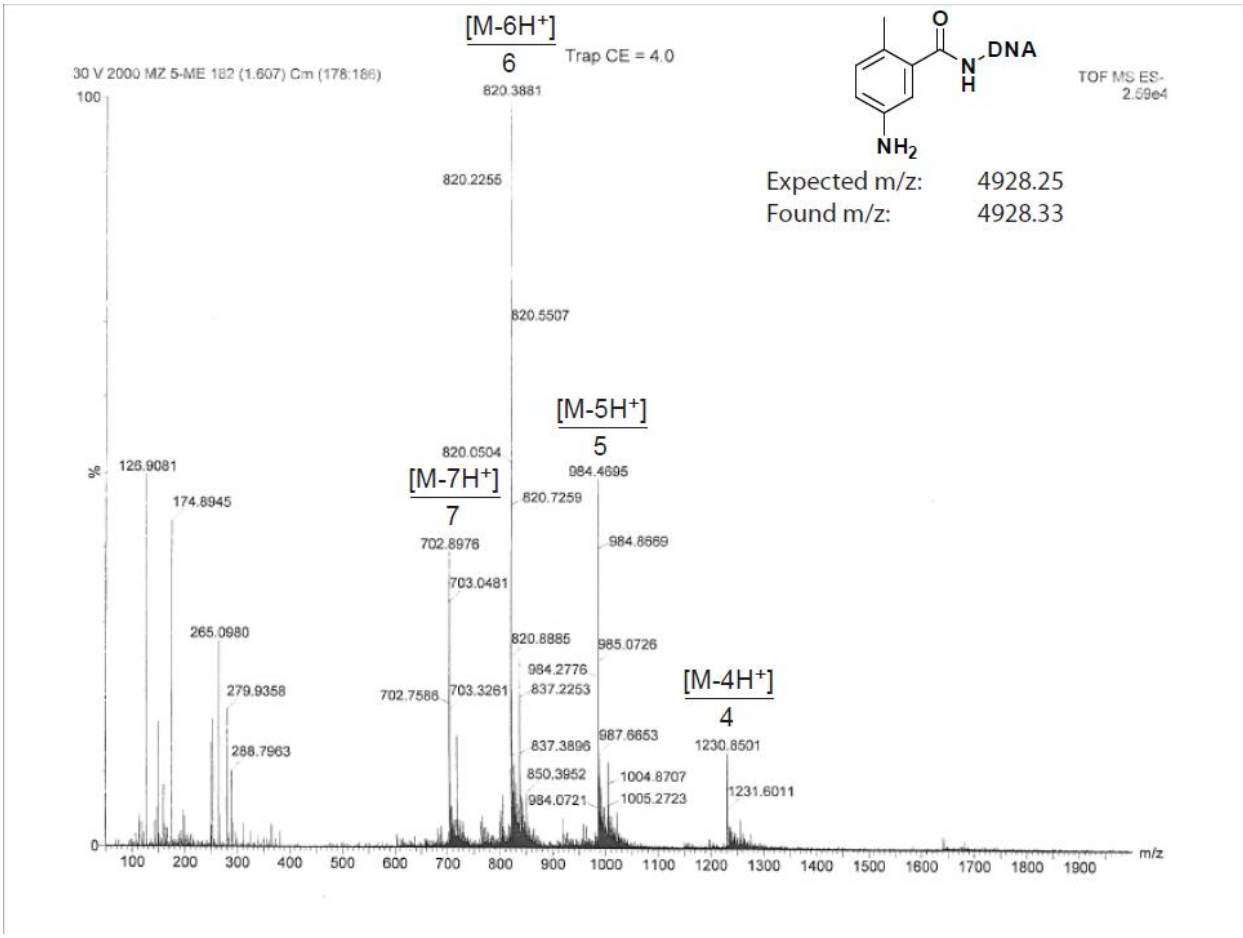


Figure S20. Oligo ESI-MS analysis of HPLC-purified **4'**. Expected mass: 4928.25; Found: 4928.33.

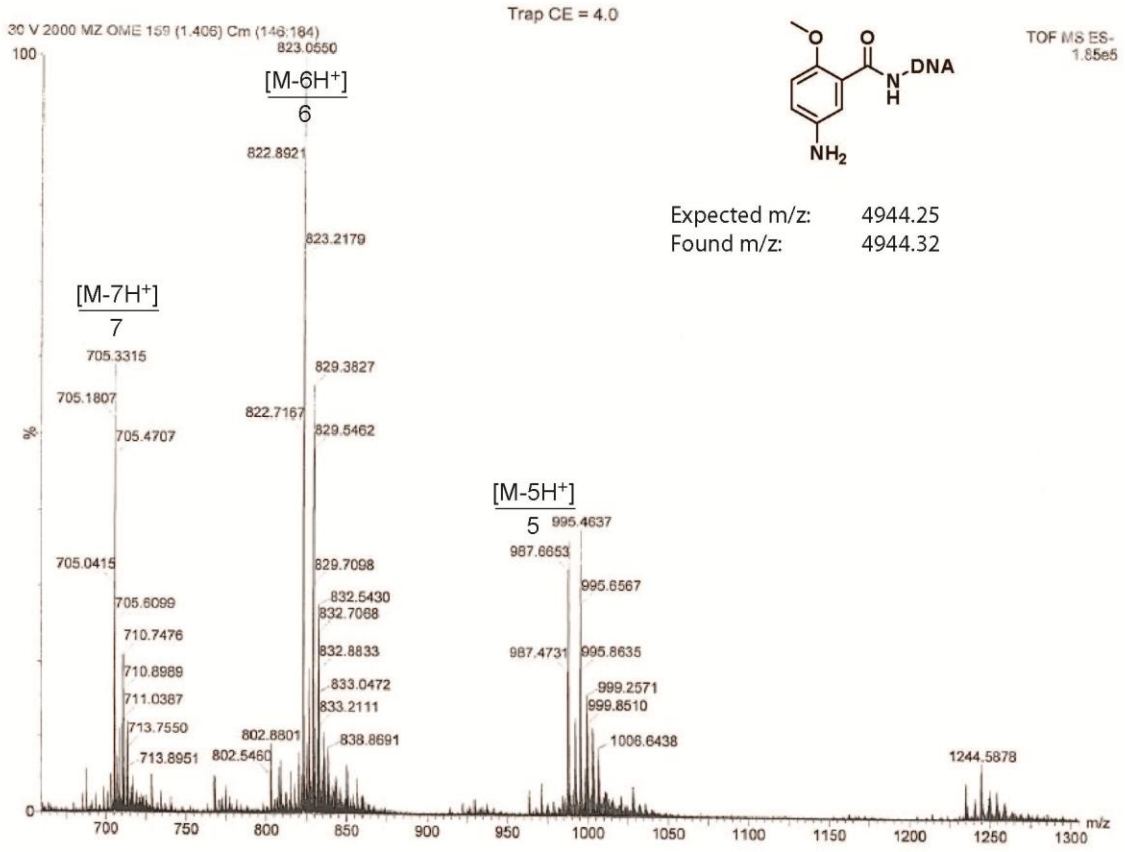


Figure S21. Oligo ESI-MS analysis of HPLC-purified **5'**. Expected mass: 4944.25; Found: 4944.32.

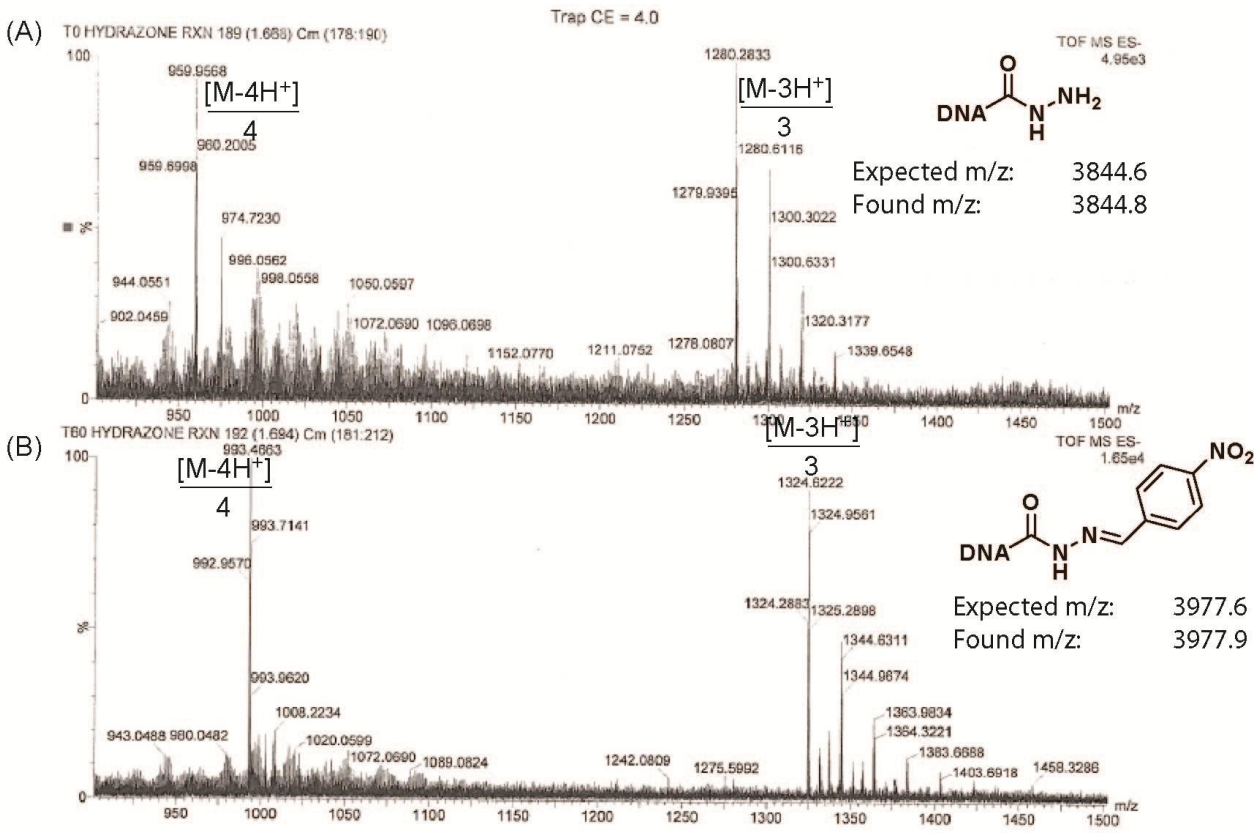


Figure S22. (A) Oligo ESI-MS analysis of acyl hydrazine used in Figure 2. Expected mass: 3844.6; Found: 3844.8. (B) Oligo ESI-MS analysis of the hydrazone formed between (A) and 4-nitrobenzaldehyde. Expected mass: 3977.6; Found: 3977.9