

***N*-Acyl-*N*-carboxymethyl-2-nitroaniline and its analogues: a new class of photolabile precursor of carboxylic acids**

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Supplementary Information

Experimental

Materials and General Instrumentation. All reagents and solvents were of the highest commercial quality and were used without purification. Hexane, chloroform, dichloromethane, acetonitrile, pyridine, methanol, acetic anhydride, nitric acid and phosphate standard pH solution (pH = 7.41) were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Ethyl acetate was purchased from Kanto Chemical Co. Inc. (Tokyo, Japan). All other reagents were purchased from Tokyo Chemical Industry Co. Ltd. (Tokyo, Japan), Wako Pure Chemical Industries, Ltd (Osaka, Japan), or Aldrich Chemical Co., Inc. (Milwaukee, WI). NMR spectra were recorded on a JNM-EX270 (¹H NMR at 270 MHz; ¹³C at 67.8 MHz) or AV-400FT NMR (¹H NMR at 400 MHz; ¹³C at 100 MHz). δ values are given in parts per million relative to the peak for tetramethylsilane. Silica gel column chromatography was performed using silica gel 60N (Kanto Chemical Co. Inc., Tokyo, Japan). Absorption spectra were measured on a Shimadzu UV-1600.

***tert*-Butyl 2-(*N*-phenylacetamido)acetate (**1**).**

A mixture of *tert*-butyl 2-(phenylamino)acetate (1.98 g, 9.55 mmol), acetic anhydride (2.8 mL, 29.7 mmol), and pyridine (3.9 mL, 48.5 mmol) in dichloromethane (9 mL) was stirred at room temperature for 16 h. After the reaction was finished, the mixture was washed with 1 N HCl aqueous solution and water. The organic layer was dried over Na₂SO₄, filtered, and evaporated. The residue was purified by flash column chromatography on silica gel (ethyl acetate/hexane = 1:2 as eluent) to give **1** as a colorless oil (1.96 g, 82% yield).

¹H NMR (CDCl₃, 270 MHz) δ 1.46 (s, 9H), 1.92 (s, 3H), 4.26 (s, 2H), 7.31-7.43 (m, 5H). ¹³C NMR (CDCl₃, 100 MHz) δ 22.2, 28.1, 51.2, 81.7, 127.9, 128.1, 129.7, 143.6, 168.3, 170.7. Anal. Calcd. for C₁₄H₁₉NO₃: C, 67.45; H, 7.68; N, 5.62, Found: C, 67.07; H, 7.74; N, 5.63.

***tert*-Butyl 2-(*N*-(2-nitrophenyl)acetamido)acetate (**2**).**

To a mixture of 97% Nitric acid (0.5 mL) and acetic anhydride (1 mL) was slowly added compound **1** (511 mg, 2.05 mmol) dissolved in acetic anhydride (0.5 mL) under ice-cold (0-4 °C) conditions. After warming up to room temperature, the mixture was stirred for 6 h. The mixture was poured into ethyl acetate (50 mL) and was washed with saturated NaHCO₃ and brine, dried over Na₂SO₄, filtered and evaporated. The residue was purified by flash column chromatography on silica gel (dichloromethane/diethyl ether = 10:0 to 9:1 as eluent) to give **2** as a colorless oil (127 mg, 22% yield).

¹H NMR (CDCl₃, 270 MHz) δ 1.47 (s, 9H), 1.85 (s, 3H), 3.60 (d, *J* = 17.3, 1H), 4.84 (d, *J* = 17.3, 1H), 7.57 (dd, *J* = 7.5 Hz, *J* = 7.8 Hz, 1H), 7.69 (dd, *J* = 7.5 Hz, *J* = 7.8 Hz, 1H), 7.84 (dd, *J* = 1.5 Hz, *J* = 8.1 Hz, 1H), 7.96 (dd, *J* = 1.5 Hz, *J* = 8.1 Hz, 1H). ¹³C NMR (CDCl₃, 100 MHz) δ 21.9, 28.0, 51.6, 82.1, 125.2, 129.9, 132.4, 134.2, 136.2, 147.1, 168.1, 170.0. Anal. Calcd. for C₁₄H₁₈N₂O₅: C, 57.13; H, 6.16; N, 9.52, Found: C, 57.01; H, 6.23; N, 9.56.

2-(*N*-(2-Nitrophenyl)acetamido)acetic acid (CNA1-Ac**).**

Compound **2** (51.2 mg, 0.174 mmol) was dissolved in trifluoroacetic acid (1 mL) and was stirred at room temperature for 1 h. The solution was concentrated and the residue was washed with hexane to give **CNA1-Ac** as a white solid (36.5 mg, 88%).

¹H NMR (CDCl₃, 270 MHz) δ 1.78 (s, 3H), 3.86 (d, *J* = 17.5, 1H), 4.58 (d, *J* = 17.5, 1H), 7.59-7.80 (m, 3H), 8.02-8.06 (m, 1H), 9.44 (s, 1H). ¹³C NMR (CDCl₃, 100 MHz) δ 21.8, 50.9, 125.5, 130.2, 132.2, 134.5, 135.7, 146.8, 170.89, 172.82. Anal. Calcd. for C₁₀H₁₀N₂O₅: C, 50.42; H, 4.23; N, 11.76, Found: C, 50.37; H, 4.15; N, 11.62.

***tert*-Butyl 2-(*N*-(3-acetoxyphenyl)acetamido)acetate (**4**).**

A mixture of 3-aminophenol (3.60 g, 33.0 mmol), *tert*-butyl bromoacetate (4.4 mL, 30.0 mmol), *N,N*-diisopropylethylamine (5.4 mL, 31.3 mmol), NaI (4.54 g, 30.3 mmol) in acetonitrile (30 mL) was refluxed under N₂ atmosphere for 1 h. After evaporation of acetonitrile, ethyl acetate (100 mL) was added to the residue and was washed with 1N HCl and water. The organic layer was dried over Na₂SO₄, filtered and evaporated. The residue was purified by flash column chromatography on silica gel (ethyl acetate/hexane = 1:2 as eluent) to give *tert*-butyl 2-((3-hydroxyphenyl)amino)acetate (**3**, Scheme 1) as a colorless oil, which was used to the next reaction without further purification due to its instability.

NMR Data of **3**: ^1H NMR(CDCl_3 , 270 MHz) δ 1.48 (s, 9H), 3.77 (s, 2H), 4.27 (s, 1H), 4.87 (s, 1H), 6.06-6.21 (m, 3H), 7.00 (t, $J = 7.3$ Hz, 1H).

A mixture of compound **3** (3.20 g, 14.3 mmol), acetic anhydride (3.3 mL, 35.2 mmol), and pyridine (5.5 mL, 68.3 mmol) in dichloromethane (14 mL) was stirred at room temperature for 2 h. After the reaction was finished, ethyl acetate (100 mL) was added to the mixture and was washed with 1 N HCl aqueous solution and water. The organic layer was dried over Na_2SO_4 , filtered, and evaporated. The residue was purified by flash column chromatography on silica gel (ethyl acetate/hexane = 1:1 as eluent) to give **4** as a colorless oil (3.54 g, 80% yield).

^1H NMR(CDCl_3 , 270 MHz) δ 1.46 (s, 9H), 1.94 (s, 3H), 2.30 (s, 3H), 4.24 (s, 2H), 7.09-7.26 (m, 3H), 7.41 (t, $J = 7.8$ Hz, 1H). ^{13}C NMR (CDCl_3 , 100 MHz) δ 21.1, 22.2, 28.1, 51.9, 81.9, 121.5, 121.6, 125.3, 130.3, 144.4, 151.2, 168.1, 169.1, 170.1. Anal. Calcd. for $\text{C}_{16}\text{H}_{21}\text{NO}_5$: C, 62.43; H, 6.85; N, 4.53, Found: C, 62.53; H, 6.89; N, 4.56.

***tert*-Butyl 2-(*N*-(3-hydroxyphenyl)acetamido)acetate (**5**).**

2 N NaOH (6 mL) was added to a solution of **4** (3.54 g, 11.5 mmol) in methanol (18 mL) and was stirred at room temperature for 1 h. After the reaction was completed, the mixture was neutralized to pH = 7 by addition of 2 N HCl. After methanol was removed by evaporation, the mixture was extracted with ethyl acetate (50 mL \times 2) and the organic layer was dried over Na_2SO_4 , filtered, and evaporated. The residue was purified by flash column chromatography on silica gel (ethyl acetate/hexane = 1:1 as eluent) to give **5** as a colorless oil (2.86 g, 94% yield). ^1H NMR (CDCl_3 , 270 MHz) δ 1.46 (s, 9H), 1.95 (s, 3H), 4.25 (s, 2H), 6.85 (d, $J = 6.6$ Hz, 3H), 7.25 (t, $J = 8.1$ Hz, 1H). ^{13}C NMR (CDCl_3 , 100 MHz) δ 22.0, 28.0, 52.2, 82.0, 114.8, 115.8, 118.9, 130.6, 144.2, 157.8, 168.2, 172.0. Anal. Calcd. for $\text{C}_{14}\text{H}_{19}\text{NO}_4$: C, 63.38; H, 7.22; N, 5.28, Found: C, 63.31; H, 7.32; N, 5.12.

***tert*-Butyl 2-(*N*-(3-(2-(*tert*-butoxy)-2-oxoethoxy)phenyl)acetamido)acetate (**6**).**

A mixture of compound **5** (2.86 g, 10.0 mmol), *tert*-butyl bromoacetate (2.4 mL, 16.2 mmol), *N,N*-diisopropylethylamine (3.8 mL, 22.1 mmol), NaI (2.54 g, 16.9 mmol) in acetonitrile (8 mL) was refluxed under N_2 atmosphere for 4 h. After evaporation of acetonitrile, ethyl acetate (80 mL) was added to the residue and the mixture was washed with 1N HCl and water. The organic layer was dried over Na_2SO_4 , filtered and evaporated. The residue was purified by flash column chromatography on silica gel

(ethyl acetate/hexane = 1:1 as eluent) to give **6** as a colorless oil (3.37 g, 82% yield).

¹H NMR (CDCl₃, 270 MHz) δ 1.46 (s, 9H), 1.49 (s, 9H), 1.92 (s, 3H), 4.22 (s, 2H), 4.52 (s, 2H), 6.85-6.97 (m, 3H), 7.26-7.34 (m, 1H). ¹³C NMR (CDCl₃, 100 MHz) δ 22.1, 28.02, 28.06, 51.9, 65.6, 81.8, 82.6, 114.28, 114.29, 120.9, 130.4, 144.7, 158.7, 167.6, 168.2, 170.7. Anal. Calcd. for C₂₀H₂₉NO₆: C, 63.26; H, 7.84; N, 3.68, Found: C, 63.31; H, 7.70; N, 3.69.

***tert*-Butyl 2-(*N*-(5-(2-(*tert*-butoxy)-2-oxoethoxy)-2-nitrophenyl)acetamido)acetate (**7**).**

To a mixture of 97% Nitric acid (1.2 mL) and acetic anhydride (3.5 mL) was slowly added compound **6** (3.37 g, 8.89 mmol) dissolved in acetic anhydride (0.5 mL) under ice-cold (0-4 °C) conditions. After warming up to room temperature, the mixture was stirred for 2 h. The mixture was poured into ethyl acetate (50 mL) and was washed with saturated NaHCO₃ and brine, dried over Na₂SO₄, filtered and evaporated. The residue was purified by flash column chromatography on silica gel (ethyl acetate/hexane = 1:2 as eluent) to give **7** as a colorless oil (702 mg, 19% yield).

¹H NMR(CDCl₃, 270 MHz) δ 1.46 (s, 9H), 1.51 (s, 9H), 1.85 (s, 3H), 3.54 (d, *J* = 17.2 Hz, 1H), 4.62 (s, 2H), 4.84 (d, *J* = 17.2 Hz, 1H), 7.01 (dd, *J* = 3.0 Hz, *J* = 9.1 Hz, 1H), 7.29 (d, *J* = 3.0 Hz, 1H), 8.08 (d, *J* = 9.1 Hz, 1H). ¹³C NMR (CDCl₃, 100 MHz) δ 21.7, 28.00, 28.04, 51.6, 65.8, 82.0, 83.4, 115.6, 117.6, 127.8, 138.7, 139.9, 162.1, 166.4, 168.1, 169.7. Anal. Calcd. for C₂₀H₂₈N₂O₈: C, 56.59; H, 6.65; N, 6.60, Found: C, 56.58; H, 6.77; N, 6.75.

2-(*N*-(5-(Carboxymethoxy)-2-nitrophenyl)acetamido)acetic acid (CNA2-Ac)

Compound **2** (51.2 mg, 0.174 mmol) was dissolved in trifluoroacetic acid (1 mL) and was stirred at room temperature for 1 h. The solution was concentrated and the residue was washed with hexane to give **CNA2-Ac** as a white solid (36.5 mg, 88%).

¹H NMR(CD₃CN, 270 MHz) δ 1.80 (s, 3H), 3.84 (d, *J* = 17.5 Hz, 1H), 4.60 (d, *J* = 17.5 Hz, 1H), 4.78 (s, 2H), 7.09 (dd, *J* = 3.1 Hz, *J* = 9.4 Hz, 1H), 7.23 (d, *J* = 3.0 Hz, 1H), 8.12 (d, *J* = 9.4 Hz, 1H). ¹³C NMR (DMSO, 100 MHz) δ 22.1, 50.5, 65.6, 115.4, 118.2, 128.6, 138.6, 139.6, 162.5, 169.63, 169.67, 170.8. Anal. Calcd. for C₁₂H₁₂N₂O₈: C, 46.16; H, 3.87; N, 8.97, Found: C, 45.94; H, 3.81; N, 8.87.

***tert*-Butyl 2-(*N*-(3-methoxyphenyl)acetamido)acetate (**8**).**

A mixture of compound *tert*-butyl 2-((3-methoxyphenyl)amino)acetate (1.57 g, 6.60 mmol), acetic anhydride (1.3 mL, 13.8 mmol), and pyridine (2.7 mL, 33.5 mmol) in dichloromethane (6 mL) was stirred at room temperature for 47 h. After the reaction was finished, ethyl acetate (100 mL) was added to the mixture and was washed with 1 N HCl aqueous solution and water. The organic layer was dried over Na₂SO₄, filtered, and evaporated. The residue was purified by flash column chromatography on silica gel (ethyl acetate/hexane = 1:2 as eluent) to give **8** as a colorless oil (1.56 g, 85% yield).

¹H NMR(CDCl₃, 270 MHz) δ 1.46 (s, 9H), 1.94 (s, 3H), 3.81 (s, 3H), 4.23 (s, 2H), 6.86-6.92 (m, 3H), 7.26-7.33 (m, 1H). ¹³C NMR (CDCl₃, 100 MHz) δ 22.3, 28.2, 52.1, 55.6, 81.9, 113.7, 113.9, 120.2, 130.5, 144.9, 160.6, 168.4, 170.8. Anal. Calcd. for C₁₅H₂₁NO₄: C, 64.50; H, 7.58; N, 5.01, Found: C, 64.62; H, 7.57; N, 4.96.

***tert*-Butyl 2-(*N*-(5-methoxy-4-nitrophenyl)acetamido)acetate (**9**)**

To a mixture of 97% Nitric acid (0.25 mL) and acetic anhydride (0.75 mL) was slowly added compound **8** (508 mg, 1.82 mmol) dissolved in acetic anhydride (0.3 mL) under ice-cold (0-4 °C) conditions. The mixture was stirred under the ice-cold condition for 1 h and at room temperature for 20 min. The mixture was poured into ethyl acetate (5 mL) and was washed with saturated NaHCO₃ and brine, dried over Na₂SO₄, filtered and evaporated. The residue was purified by flash column chromatography on silica gel (dichloromethane/diethyl ether = 10:0 to 9:1 as eluent) to give *para*-nitro product **9** as a red solid (180 mg, 30% yield)

¹H NMR(CDCl₃, 270 MHz) δ 1.47 (s, 9H), 2.00 (s, 3H), 3.97 (s, 3H), 4.25 (s, 2H), 6.98 (dd, *J* = 1.8 and 8.6 Hz, 1H), 7.16 (d, *J* = 1.8 Hz, 1H), 7.89 (d, *J* = 8.6 Hz, 1H). ¹³C NMR (CDCl₃, 100 MHz) δ 22.2, 28.0, 51.7, 56.8, 82.4, 113.3, 119.6, 126.9, 138.8, 148.6, 153.8, 168.0, 169.9. Anal. Calcd. for C₁₅H₂₀N₂O₆: C, 55.55; H, 6.22; N, 8.64, Found: C, 55.24; H, 6.06; N, 8.82.

***tert*-Butyl 2-(*N*-(5-methoxy-2,4-dinitrophenyl)acetamido)acetate (**10**).**

To a mixture of 97% Nitric acid (0.075 mL) and acetic anhydride (0.25 mL) and Hβ^{ref} (35 mg) was slowly added compound **8** (508 mg, 1.82 mmol) dissolved in acetic anhydride (0.3 mL) under ice-cold (0-4 °C) conditions. The mixture was stirred under the ice-cold condition for 4 h and at room temperature for 20 h. The mixture was poured into ethyl acetate (5 mL) and was washed with saturated NaHCO₃ and brine, dried over Na₂SO₄, filtered and evaporated. The residue was purified by flash column

chromatography on silica gel (ethyl acetate/hexane = 1:1 as eluent) to give dinitro product **10** as a brown oil (43 mg, 21% yield).

¹H NMR(CDCl₃, 270 MHz) δ 1.47 (s, 9H), 1.91 (s, 3H), 3.54 (d, *J* = 17.5 Hz, 1H), 4.09 (s, 3H), 4.95 (d, *J* = 17.5 Hz, 1H), 7.70 (s, 1H), 8.67 (s, 1H). ¹³C NMR (CDCl₃, 100 MHz) δ 21.9, 28.0, 51.2, 57.9, 82.7, 116.4, 117.7, 124.1, 138.1, 142.0, 156.5, 168.3, 168.8. Anal. Calcd. for C₁₅H₁₉N₃O₈: C, 48.78; H, 5.19; N, 11.38 Found: C, 48.76; H, 5.15; N, 11.27.

2-(*N*-(5-Methoxy-2,4-dinitrophenyl)acetamido)acetic acid (CNA3-Ac).

Compound **10** was dissolved in trifluoroacetic acid (1 mL) and was stirred at room temperature for 1 h. The solution was concentrated and the residue was washed with hexane to give **CNA3-Ac** as a white solid (21 mg, 58%).

¹H NMR(CDCl₃, 270 MHz) δ 1.93 (s, 3H), 3.75 (d, *J* = 18.3 Hz, 1H), 4.10 (s, 3H), 5.09 (d, *J* = 18.3 Hz, 1H), 7.59 (s, 1H), 8.70 (s, 1H). ¹³C NMR (CDCl₃, 100 MHz) δ 21.35, 49.6, 57.8, 114.6, 117.9, 124.0, 138.1, 141.6, 156.6, 169.59, 169.64. Anal. Calcd. for C₁₁H₁₁N₃O₈: C, 42.18; H, 3.54; N, 13.42, Found: C, 42.39; H, 3.56; N, 13.26.

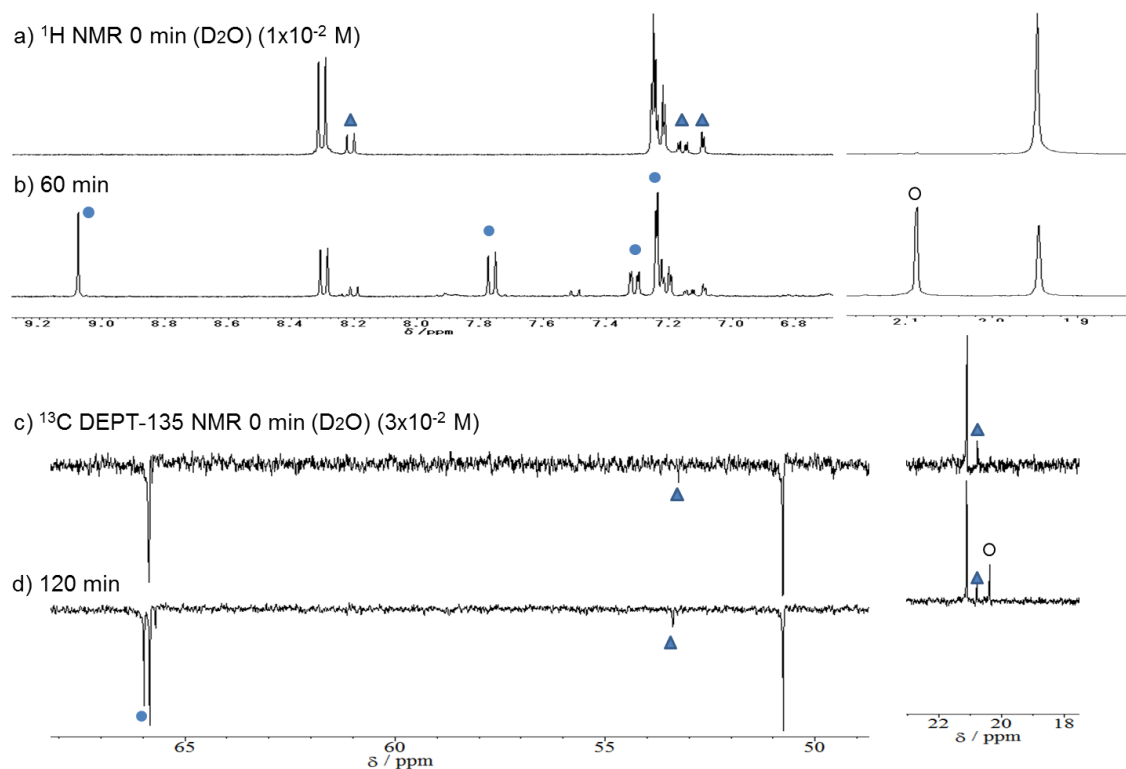


Fig. S1 Comparison of ¹H NMR and ¹³C DEPT-135 NMR spectra of CNA2-Ac before (a and c) and after (b and d) 365 nm light irradiation in D₂O. Small peaks (▲) represent the rotational isomer of CNA2-Ac. New peaks appearing after photoirradiation are due to 2-((5-(carboxymethoxy)-2-nitrosophenyl)imino)acetic acid (●) and acetic acid (○), respectively. A new peak appeared at 9.08 ppm in (b) is ascribed to the CH=N proton.