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SUPPORTING INFORMATION

Rhodamine-Based Cyclic Hydrazide Derivatives as Fluorescent Probes for Selective and Rapid Detection of Formaldehyde

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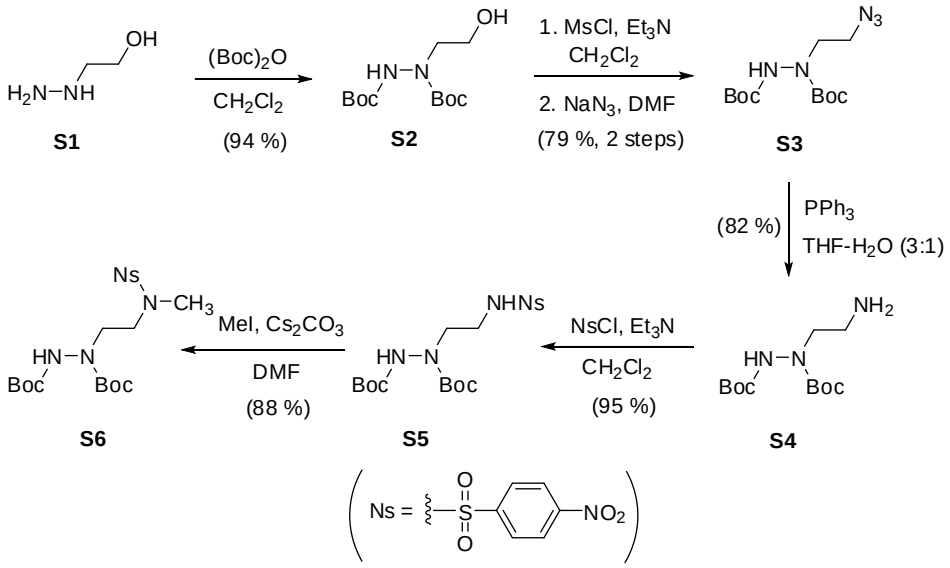
CONTENTS

General methods

General synthetic materials and methods: Silica gel 60 (230-400 mesh, Merck) was used for column chromatography. Analytical thin layer chromatography was performed using Merck 60 F254 silica gel (pre-coated sheets, 0.25 mm thick). All reagents and solvents were purchased from Sigma-Aldrich, TCI, Alfa and Acros, and used as received with the following exceptions. Dichloromethane (CH_2Cl_2) was distilled from calcium hydride (CaH_2) and tetrahydrofuran (THF) from sodium (Na) and benzophenone.

Spectroscopic materials and methods: Nuclear magnetic resonance (NMR) spectra were recorded in $CDCl_3$ unless stated otherwise with internal references ($CDCl_3$, δ = 7.26 ppm and TMS, δ = 0.00 ppm) at ambient temperature mainly on Bruker II-400 Fourier Transform Spectrometers operating at 400 MHz for ^1H and at 100 MHz for ^{13}C. Mass spectra were recorded on an Ultimate 3000 RS-Q-Exactive Orbitrap Plus mass spectrometer for both low resolution and high resolution mass spectra. The pH was recorded by HI-8014 instrument (HANNA). Infrared absorption spectra were recorded as a KBr pellet on a Vertex 70 FT-IR spectrophotometer. All UV–Vis spectra were measured with UV–visible spectrophotometer (V-650). Fluorescence spectrophotometer (LS 55) was used to obtain fluorescence emission spectrum in liquid state. The slit width was 10 nm for both excitation and emission. Samples were included in a 10.0 mm long quartz cuvette (3.5 ml volume). The photon multiplier voltage was 400 V and a circulating PBS buffer/DMSO bath was used during all experiments to regulate the temperature at 37 ^oC. The excitation-time emission spectrum at 520 nm was integrated over the range of 540-720 nm. All measurements were performed at least three times.

Synthesis



Scheme S1. Synthesis of compound **S6**.

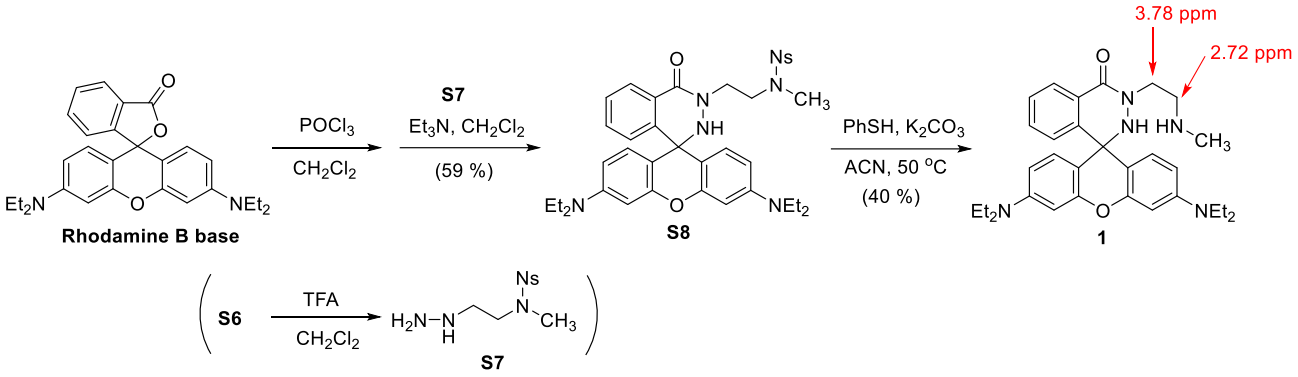
Compound S3: To a solution of di-tert-butyl 1-(2-hydroxyethyl)hydrazine-1,2-dicarboxylate (**S2,** ref 1) (1.94 g, 7 mmol) in CH_2Cl_2 (50 mL) were added Et_3N (1.5 mL, 14 mmol) and MsCl (1.26 g, 11 mmol) at -78 ºC. After stirring for 10 min at the same temperature, the reaction was quenched by addition of water (50 mL). The organic layer was extracted with CH_2Cl_2 (50 mL x3), washed with brine, dried over $MgSO_4$, filtered, and concentrated in vacuo. The crude product was treated with sodium azide (670 mg, 10 mmol). After stirring for 2 h at 90 ºC, the mixture was cooled to room temperature. The organic layer was extracted with ethyl acetate (100 mL x3), washed with brine, dried over $MgSO_4$, filtered, and concentrated in vacuo. The crude product was purified by flash column chromatography (hexane : ethyl acetate = 4:1) to give **S3** as a white solid (1.66 g, 79%). **^{1}H NMR** (400 MHz, $CDCl_3$): δ 6.43 (s, 1H), 3.64 (bs, 2H), 3.45 (bs, 2H), 1.46 (s, 18H); **^{13}C NMR** (100 MHz, $CDCl_3$): δ 155.4, 155.1, 81.8, 49.8, 49.0, 28.3; **IR** (film, cm^{-1}) 3317, 2982, 2937, 2161, 2083, 1733, 1706, 1492, 1397, 1366, 1318, 1302, 1258, 1146; **HRMS** (FAB) *m/z* calcd for $C_{12}H_{23}N_5O_4$ $[(M+H)^+]$ 302.1822; found 302.1816.

Compound S4: To a solution of **S3** (733 mg, 2.43 mmol) in THF (25 mL) at 0 ºC was slowly added triphenylphosphine (957 mg, 1.5 mmol), After stirring for 4 h at room temperature, water (8.3 mL) was added to the mixture slowly. After stirring for additional 16 h, the volatile materials were removed under reduced pressure. The residues were purified by flash column chromatography (CH_2Cl_2 : methanol = 8:1) to give **S4** as a white solid (549 mg, 82%). **^{1}H NMR** (400 MHz, $CDCl_3$): δ 6.77 (bs, 1H), 3.51 (bs, 2H), 2.87 (t, *J* = 5.6 Hz, 2H), 2.64 (bs, 2H), 1.45 (s, 18H); **^{13}C NMR** (100 MHz, $CDCl_3$): δ 155.8, 81.5, 51.9, 39.7, 28.3; **IR** (film, cm^{-1}) 3482, 3360, 3305, 3006, 2977, 2931, 1728, 1701, 1410, 1394, 1256, 1154, 1127; **HRMS** (FAB) *m/z* calcd for $C_{12}H_{25}N_3O_4$ $[(M+H)^+]$ 276.1917; found 276.1912.

Compound S5: To a solution of **S4** (380 mg, 1.3 mmol) in CH_2Cl_2 (12.2 mL) were added TEA (0.3 mL, 1.97 mmol) and 4-nitrobenzenesulfonyl chloride (353 mg, 1.59 mmol) at room temperature. After stirring for 30 min at the same temperature, the solvent was removed under reduced pressure. The crude product was purified by flash column chromatography (hexane : ethyl acetate = 3:1) to give **S5** as a yellow solid (562 mg, 95%). **^{1}H NMR** (400 MHz, $CDCl_3$): δ 8.32 (d, *J* = 8.8 Hz, 2H), 8.04 (d, *J* = 8.8 Hz, 2H), 3.55 (bs, 2H), 3.17 (bs, 2H), 1.43 (d, *J* = 10.8 Hz, 18H); **^{13}C NMR** (100 MHz, $CDCl_3$): δ 154.9, 150.0, 146.6, 128.4, 124.4, 82.7, 50.3, 41.2, 28.2; **IR** (film, cm^{-1}) 3325, 3108, 3069, 2981, 2935, 2872, 1713, 1533, 1479, 1457, 1369, 1350, 1287, 1254, 1164, 1093; **HRMS** (FAB) *m/z* calcd for $C_{18}H_{28}N_4O_8S$ $[(M+H)^+]$ 461.1700; found 461.1691.

Compound S6: To a solution of **S5** (547 mg, 1.09 mmol) in DMF (46 mL) were added a cesium carbonate (767 mg, 2.41 mmol) and methyl iodide (0.09 mL, 1.31 mmol) at room temperature. After

stirring overnight at the same temperature, the mixture was brought to pH 7 by addition of saturated aqueous ammonium chloride. The aqueous layer was extracted with ethyl acetate several times. The combined organic layers were washed with brine, dried over $MgSO_4$, filtered, and concentrated in vacuo to give **S6** as a yellow solid (460 mg, 88 %). **^{1}H NMR** (400 MHz, $CDCl_3$): δ 8.36 (d, *J* = 8.8 Hz, 2H), 7.97 (d, *J* = 8.8 Hz, 2H), 3.66 (bs, 2H), 3.25 (bs, 2H), 2.86 (s, 3H), 1.47 (s, 18H); **^{13}C NMR** (100 MHz, $CDCl_3$): δ 155.3, 150.2, 144.3, 128.4, 124.6, 81.7, 81.4, 47.6, 46.1, 34.9, 28.4; **IR** (film, cm^{-1}) 3304, 3110, 3039, 2981, 2934, 2870, 2821, 1737, 1709, 1668, 1606, 1532, 1478, 1312, 1278, 1247, 1169, 1148, 1089; **HRMS** (FAB) *m/z* calcd for $C_{19}H_{30}N_4O_8S$ [$(M+H)^+$] 475.1857; found 475.1849.



Scheme S2. Synthesis of probe **1**.

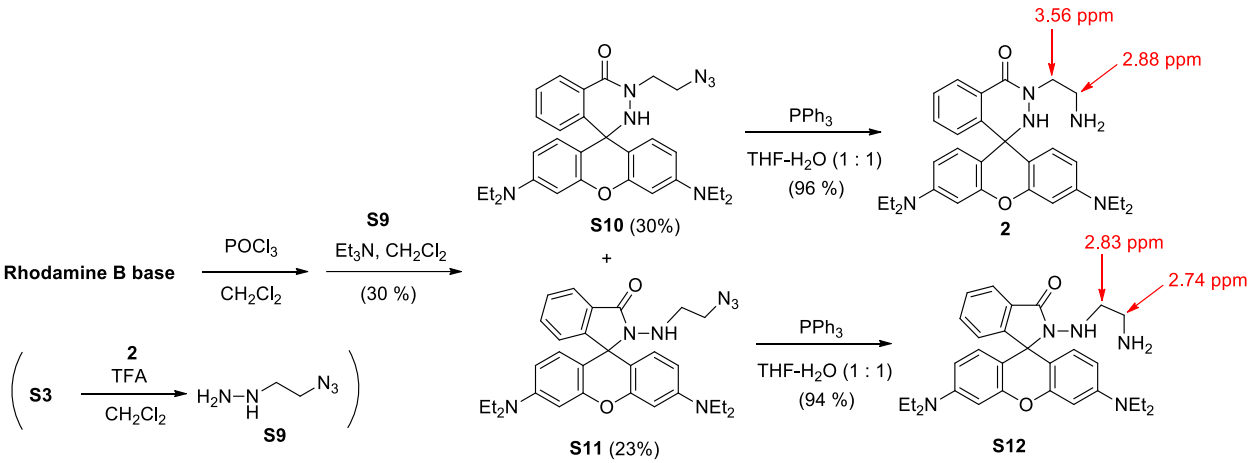
Compound S7: To a solution of **S6** (448 mg, 0.93 mmol) in CH_2Cl_2 (5 mL) was slowly added a solution of trifluoroacetic acid (10 ml) in CH_2Cl_2 (5 mL). After stirring for 30 min, the volatile materials were removed under reduced pressure to give **S7**, which was used for the next step without purification.

Compound S8: To a solution of rhodamine B base (274 mg, 0.62 mmol) in dichloromethane (5 mL) was added phosphorus oxychloride (0.15 mL, 1.7 mmol) dropwise over 2 min. The mixture was heated under reflux for 3 h. The reaction mixture was cooled to room temperature. The volatile materials were removed under reduced pressure to give rhodamine B acid chloride, which was used for the next step without purification.[2]

To a solution of the above rhodamine B acid chloride in CH_2Cl_2 (2 mL) was added slowly a solution of the crude **S7** and TEA (0.60 mL, 4.3 mmol) in CH_2Cl_2 (2.3 mL) at 0 ºC. After stirring overnight at room temperature, the mixture was brought to pH 7 by addition of saturated aqueous ammonium chloride. The aqueous layers were extracted with ethyl acetate several times. The combined organic layers were dried over $MgSO_4$, filtered, and concentrated in vacuo. The crude product was purified by flash column chromatography (hexane : ethyl acetate = 2:1) to give **S8** as a pink solid (256 mg, 59%). **^{1}H NMR** (400 MHz, $CDCl_3$): δ 8.27-8.29 (m, 2H), 8.20-8.21 (m, 1H), 7.86-7.89 (m, 2H), 7.43-7.45

(m, 2H), 7.03-7.06 (m, 1H), 6.68 (d, J = 8.8 Hz, 2H), 6.42-6.69 (m, 2H), 6.31-6.41 (m, 1H), 6.28-6.31 (m, 2H), 3.53 (t, J = 6.4 Hz, 2H), 3.34-3.31 (m, 8H), 2.96 (t, J = 6.4 Hz, 2H), 2.62 (s, 3H), 1.14-1.18 (m, 12H); **^{13}C NMR** (100 MHz, $CDCl_3$): δ 165.0, 154.0, 153.3, 150.0, 148.7, 144.2, 144.1, 132.5, 129.5, 129.2, 128.6, 128.4, 127.8, 127.8, 127.6, 124.4, 124.3, 109.6, 108.1, 107.2, 98.9.

Probe 1: To a solution of **S8** (41 mg, 0.06 mmol) in acetonitrile (3 mL) were added potassium carbonate (12 mg, 0.087 mmol) and thiophenol (0.008 mL, 0.087 mmol). The mixture was heated under reflux for 4 h. After cooling to room temperature, the solvent was removed under reduced pressure. The residues were purified by flash column chromatography (dichloromethane : methanol = 13:1) to give **1** as a pink solid (12 mg, 40%). **^{1}H NMR** (400 MHz, $CDCl_3$): δ 8.19-8.21 (m, 1H), 7.44-7.46 (m, 2H), 7.06-7.08 (m, 1H), 6.71 (d, J = 7.2 Hz, 2H), 6.44 (d, J = 4.4 Hz, 2H), 6.28-6.31 (m, 2H), 3.78 (t, J = 4.4 Hz, 2H), 3.35 (q, J = 7.2 Hz, 8H), 2.72 (t, J = 4.4 Hz, 2H), 2.36 (s, 3H), 1.16 (t, J = 6.8 Hz, 12H); **^{13}C NMR** (100 MHz, $CDCl_3$): δ 165.1, 153.4, 149.0, 143.7, 132.3, 129.5, 129.5, 128.0, 127.8, 127.5, 109.5, 106.9, 98.7, 58.5, 49.0, 46.4, 44.5, 35.5, 12.7; **IR** (film, cm^{-1}) 3589, 3434, 3060, 2970, 2930, 2871, 1736, 1686, 1633, 1614, 1544, 1514, 1459, 1306, 1274, 1233, 1222; **HRMS** (FAB) *m/z* calcd for $C_{31}H_{39}N_5O_2$ [(M+H)$^+$] 514.3176; found 514.3173.



Scheme S3. Synthesis of probes **2** and **S12**.

Compound S9 : To a solution of **S3** (100 mg, 0.33 mmol) in CH_2Cl_2 (4.5 mL) was slowly added a solution of trifluoroacetic acid (4.5 ml) in CH_2Cl_2 (4.5 mL). After stirring for 30 min, the volatile materials were removed under reduced pressure to give **S9**, which was used for the next step without purification.

Compound S10 and S11: To a solution of rhodamine B base (71 mg, 0.16 mmol) in dichloromethane (2 mL) was added phosphorus oxychloride (0.039 mL, 0.44 mmol) dropwise over 2 min. The solution was heated under reflux for 3 h. The reaction mixture was cooled to room temperature and the volatile

materials were removed under reduced pressure to give rhodamine B acid chloride.

To a solution of the above rhodamine B acyl chloride in CH_2Cl_2 (1 mL) at 0 ºC was slowly added a solution of **S9** (33 mg, 0.32 mmol) and TEA (0.22 mL, 1.6 mmol) in CH_2Cl_2 (1.5 mL) at room temperature. After stirring overnight at the same temperature, the mixture was brought to pH 7 by addition of saturated aqueous ammonium chloride. Aqueous layers were extracted with ethyl acetate several times. The combined organic layers were dried over $MgSO_4$, filtered, and concentrated in vacuo. The crude product was purified by flash column chromatography (hexane : ethyl acetate = 6:1) to give **S10** (25 mg, 30%) and the 5-membered **S11** (19 mg, 23%) as pink solids.

6-Membered azide compound S10: **^{1}H NMR** (400 MHz, $CDCl_3$): δ 8.25-8.27 (m, 1H), 7.45-7.47 (m, 2H), 7.09-7.11 (m, 1H), 6.68 (d, *J* = 8.8 Hz, 2H), 6.48 (d, *J* = 2.4 Hz, 2H), 6.28-6.31 (m, 2H), 4.53(bs, 1H), 3.54 (t, *J* = 6.8 Hz, 2H), 3.35 (q, *J* = 6.8 Hz, 8H), 3.05 (t, *J* = 6.8 Hz, 2H), 1.16 (t, *J* = 6.8 Hz, 12H); **^{13}C NMR** (100 MHz, $CDCl_3$): δ 164.8, 153.4, 149.0, 143.9, 132.4, 129.4, 129.4, 128.0, 127.8, 127.6, 109.4, 106.8, 98.7, 58.6, 47.8, 47.0, 44.5, 12.7; **IR** (film, cm^{-1}) 3185, 2967, 2931, 2897, 2870, 2099, 1686, 1649, 1633, 1613, 1574, 1544, 1509,1480, 1230, 1219, 1203, 1121, 1095, 1077; **HRMS** (FAB) *m/z* calcd for $C_{30}H_{35}N_7O_2$ [$(M+H)^+$] 526.2925; found 526.2916.

5-Membered azide compound S11: **^{1}H NMR** (400 MHz, $CDCl_3$): δ 7.91-7.93 (m, 1H), 7.45-7.51 (m, 2H), 7.13-7.45 (m, 1H), 6.40-6.42 (m, 2H), 6.26-6.29 (m, 2H), 4.32 (bs, 1H), 3.34 (q, *J* = 7.2 Hz, 8H), 2.97 (t, *J* = 6.4 Hz, 2H), 2.77-279 (m, 2H), 1.16 (t, *J* = 6.8 Hz, 12H).

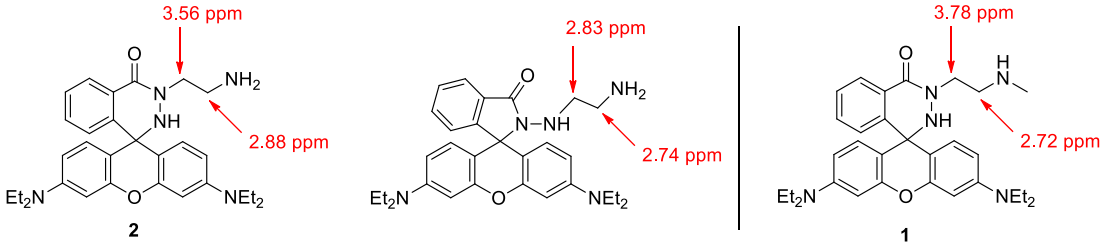
Probe 2: To a solution of **S10** (25 mg, 0.05 mmol) in THF (0.9 mL) at 0 ºC was slowly added a triphenylphosphine (18.7 mg, 0.07 mmol) at room temperature. After stirring for 4 h, water (0.9 mL) was added to the mixture. After stirring for additional 16 h, the solvent was removed under reduced pressure. The residues were purified by flash column chromatography (dichloromethane : methanol = 10:1) to give **2** as a light pink solid (23 mg, 96%). **^{1}H NMR** (400 MHz, $CDCl_3$): δ 8.13-8.15 (m, 1H), 7.28-7.37 (m, 2H), 6.98 (d, *J* = 8.8 Hz, 1H), 6.67 (d, *J* = 8.8 Hz, 2H), 6.41 (bd, 2H), 6.20-6.22 (m, 2H), 3.56 (bs, 2H), 3.20 (bq, 8H), 2.88 (bs, 2H), 1.12 (t, *J* = 6.8 Hz, 12H); **^{13}C NMR** (100 MHz, $CDCl_3$): δ 165.5, 153.5, 149.0, 143.8, 132.3, 129.5, 129.4, 128.0, 127.8, 127.5, 109.6, 106.9, 98.8, 58.5, 50.5, 44.6, 39.5, 12.7; **IR** (film, cm^{-1}) 3436, 2969, 2929, 2895, 2869, 1737, 1633, 1614, 1574, 1545, 1513, 1321, 1305, 1273, 1222, 1152; **HRMS** (FAB) *m/z* calcd for $C_{30}H_{37}N_5O_2$ [$(M+H)^+$] 500.3020; found 500.3033.

5-Membered amine compound S12: To a solution of **S11** (19 mg, 0.04 mmol) in THF (0.6 mL) at 0 ºC was slowly added a triphenylphosphine (14.9 mg, 0.06 mmol) at room temperature. After stirring for 4 h, water (0.6 mL) was added to the mixture. After stirring for additional 16 h, the solvent was removed under reduced pressure. The residues were purified by flash column chromatography (dichloromethane : methanol = 10:1) to give **S12** as a light pink solid (18 mg, 94%). **^{1}H NMR** (400 MHz, $CDCl_3$): δ 7.87-7.89 (m, 1H), 7.46-7.54 (m, 2H), 7.12 (d, *J* = 8.0 Hz, 1H), 6.37-6.39 (m, 4H),

6.24-6.27 (m, 2H), 3.96 (s, 1H), 3.34 (q, J = 6.8 Hz, 8H), 2.84 (bt, 2H), 2.75 (bt, 2H), 1.16 (t, J = 6.8 Hz, 12H); **^{13}C NMR** (100 MHz, $CDCl_3$): δ 168.7, 153.9, 151.6, 149.4, 133.6, 129.5, 128.8, 128.4, 124.1, 123.5, 108.6, 103.9, 98.0, 66.48, 45.9, 44.6, 37.5, 12.7; **IR** (film, cm^{-1}) 3434, 2967, 2926, 2870, 1689, 1634, 1615, 1515, 1466, 1526, 1329, 1264, 1232, 1119, 1020; **HRMS** (FAB) *m/z* calcd for $C_{30}H_{37}N_5O_2$ [$(M+H)^+$] 500.3020; found 499.2941.

6-Membered ring structures of probes 1 and 2

A chemical shift of C*H*$_2$ attached to amide N in the 6-membered probe **2** in the ^{1}H NMR spectrum is assigned to 3.56 ppm, but that of the corresponding C*H*$_2$ in 5-membered compound S12 to 2.83 ppm. In addition, a chemical shift of C*H*$_2$ attached to amide N in the 6-membered probe **1** in the ^{1}H NMR spectrum is assigned to 3.78 ppm that is similar to that of probe **2**. On these bases, probes **1** and **2** have the 6-membered ring structures.



Fluorescence response of 1 to various analytes. RCS, ROS, and metal cations were prepared according to the following methods. 100 μM analytes (otherwise specified) were added to 10 μM **1** in 20 mM PBS buffer (DMSO 1%) at 37 ºC throughout the experiment.[3,4]

(1) **Acetaldehyde**: 1 μL of 200 mM stock solution of acetaldehyde in distilled H_2O was added to 1999 μL of a 10 μM probe solution in PBS

(2) **Benzaldehyde**: 1 μL of 200 mM stock solution of benzaldehyde in DMSO was added to 1999 μL of a 10 μM probe solution in PBS

(3) **4-Hydorxybenzaldehyde**: 1 μL of 200 mM stock solution of 4-hydroxybenzaldehyde in DMSO was added to 1999 μL of a 10 μM probe solution in PBS

(4) **Ethyl pyruvate**: 1 μL of 200 mM stock solution of ethyl pyruvate in distilled H_2O was added to 1999 μL of a 10 μM probe solution in PBS

(5) **Ethyl glyoxalate**: 1 μL of 200 mM stock solution of ethyl glyoxalate in DMSO was added to 1999 μL of a 10 μM probe solution in PBS

(6) **Propionaldehyde**: 1 μL of 200 mM stock solution of propionaldehyde in DMSO was added to 1999 μL of a 10 μM probe solution in PBS

(7) **D-glucose (1mM)**: : 1 μL of 2 M stock solution of D-glucose in distilled H_2O was added to 1999 μL of a 10 μM probe solution in PBS

(8) **H_2O_2**: 1 μL of 200 mM stock solution of H_2O_2 in distilled H_2O was added to 1999 μL of a 10 μM probe solution in PBS

(9) **HOCl**: 1 μL of 200 mM stock solution of sodium hypochlorite in 0.1M NaOH (aq) was added to 1999 μL of a 10 μM probe solution in PBS

(10) **$NO^{\bullet}$**: 1 μL of 200 mM stock solution of SNP (sodium nitroferricyanide(III) dihydrate)in distilled H_2O was added to 1999 μL of a 10 μM probe solution in PBS

(11) **$^1O_2$**: 1 μL of 200 mM stock solution of sodium hypochlorite in 1 mM H_2O_2 was added to 1999 μL of a 10 μM probe solution in PBS

(12) **$O_2^{\bullet-}$**: 1 μL of 200 mM stock solution of potassium superoxide in DMSO was added to 1999 μL of a 10 μM probe solution in PBS

(13) **$^{\bullet}OH$**: $CuCl_2$ (copper(II) chloride) was dissolved in H_2O and H_2O_2 was diluted in H_2O respectively. 1 μL of 200 mM stock solution ($CuCl_2$ solution:H_2O_2 solution =1:10) was added to 1999 μL of a 10 μM probe solution in PBS

(14) **Cu^{2+}**: 1 μL of 200 mM stock solution of $CuCl_2 \cdot 2H_2O$ in distilled H_2O was added to 1999 μL of a 10 μM probe solution in PBS

(15) **Fe^{2+}**: 1 μL of 200 mM stock solution of $FeSO_4 \cdot 7H_2O$ in distilled H_2O was added to 1999 μL of a 10 μM probe solution in PBS

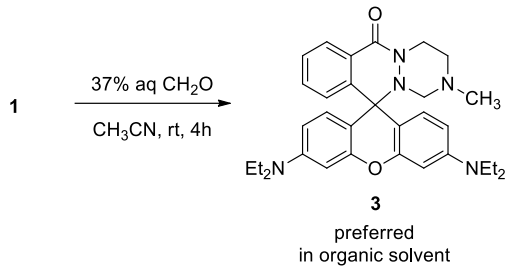
(16) **Fe^{3+}**: 1 μL of 200 mM stock solution of $Fe_2(SO_4)_3$ in distilled H_2O was added to 1999 μL of a 10 μM probe solution in PBS

(17) **K^+**: 1 μL of 200 mM stock solution of $KCOOCH_3$ in distilled H_2O was added to 1999 μL of a 10 μM probe solution in PBS

(18) **Zn^{2+}**: 1 μL of 200 mM stock solution of $Zn(NO_3)_2 \cdot 6H_2O$ in distilled H_2O was added to 1999 μL of a 10 μM probe solution in PBS

(19) **FA**: 1 μL of 200 mM stock solution of formaldehyde in distilled H_2O was added to 1999 μL of a 10 μM probe solution in PBS

Isolation of the reaction product of 1 with FA



Compound 3 : To a solution of **1** (30 mg, 0.040 mmol) in acetonitrile (3 mL) was slowly added formaldehyde (0.30 mL, 10.7 mmol). The mixture was stirred for 4 h at room temperature. The solvent was evaporated under vacuum and purified by flash column chromatography (dichloromethane : methanol = 20:1) to give **3** as a pink solid. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 8.26-8.29 (m, 1H), 7.44-7.49 (m, 2H), 7.12-7.14 (m, 1H), 6.71 (d, *J* = 8.8 Hz, 2H), 6.46 (d, *J* = 2.4 Hz, 2H), 6.26-6.29 (m, 2H), 3.70-3.71 (bt, 2H), 3.31-3.37 (m, 8H), **3.24 (s, 2H, CH_2)**, 2.56 (bt, 2H), 2.14 (s, 3H), 1.16 (t, *J* = 7.2 Hz, 12H); **^{13}C NMR** (100 MHz, $CDCl_3$): δ 165.1, 153.9, 148.6, 139.6, 132.7, 130.5, 128.6, 128.3, 127.6, 127.2, 109.7, 106.4, 98.8, **72.5 (CH_2)**, **61.3 (spiro ring carbon)**, 52.1, 44.4, 43.0, 41.3, 12.7; **IR** (film, cm^{-1}) 3435, 2968, 2929, 2894, 2792, 1735, 1653, 1633, 1613, 1511, 1397, 1356, 1273, 1252,1200, 1153, 1115; **HRMS** (FAB) *m/z* calcd for $C_{32}H_{39}N_5O_2$ [$(M+H)^+$] 526.3177; found 526.3175.

Estimation of the ratio of the close to open form of probe 1

$$[\text{probe } \mathbf{1}_{close} \rightleftharpoons \text{probe } \mathbf{1}_{open}] + FA \rightarrow [\text{adduct } \mathbf{3}_{close} \rightleftharpoons \text{adduct } \mathbf{3}_{open}]$$

We assumed that the molar extinction coefficients of the close forms ($\mathbf{1}_{close}$ and $\mathbf{3}_{close}$) are similar, and those of the open forms ($\mathbf{1}_{open}$ and $\mathbf{3}_{open}$) are also similar, because of their same core structures.

$\varepsilon_{\mathbf{1}c}$ (probe $\mathbf{1}_{close}$) ~ $\varepsilon_{\mathbf{3}c}$ (adduct $\mathbf{3}_{close}$)

$\varepsilon_{\mathbf{1}o}$ (probe $\mathbf{1}_{open}$) ~ $\varepsilon_{\mathbf{3}o}$ (adduct $\mathbf{3}_{open}$)

We also assumed that the molar extinction coefficients for probe **1** (ε = 10,540 M^{-1} cm^{-1}, see Table S1) and adduct **3** (ε = 42,500 M^{-1} cm^{-1}, see Table S1) are originated from the corresponding single major form. In this case, the probe **1** is mainly present in the close form and the adduct **3** in the open form. Thus,

$\varepsilon_{\mathbf{1}c}$ (probe $\mathbf{1}_{close}$) ~ $\varepsilon_{\mathbf{3}c}$ (adduct $\mathbf{3}_{close}$) ~ $\varepsilon_{\mathbf{1}}$ = 10,540 M^{-1} cm^{-1}

$\varepsilon_{\mathbf{1}o}$ (probe $\mathbf{1}_{open}$) ~ $\varepsilon_{\mathbf{3}o}$ (adduct $\mathbf{3}_{open}$) ~ $\varepsilon_{\mathbf{3}}$ = 42,500 M^{-1} cm^{-1}

The maximum absorbance of probe **1** and adduct **3** was determined to be 0.158 and 0.371, respectively (see Figure S1).

Absorption, A = εbc, b = 1 cm, c= 1.0 x 10^{-5} M (10 µM)

The close-open ratio coefficients:

$c_{\mathbf{1}c} + c_{\mathbf{1}o} = 1$, $c_{\mathbf{3}c} + c_{\mathbf{3}o} = 1$

$A_{\mathbf{1}}$ (for probe **1**) = $c_{\mathbf{1}c}$ x $A_{\mathbf{1}c}$ + $c_{\mathbf{1}o}$ x $A_{\mathbf{1}o}$

$A_{\mathbf{3}}$ (for adduct **3**) = $c_{\mathbf{1}c}$ x $A_{\mathbf{3}c}$ + $c_{\mathbf{1}o}$ x $A_{\mathbf{3}o}$

For the close-open ratio of probe **1**:

$A_{\mathbf{1}}$ = 0.158 = $c_{\mathbf{1}c}$ x ($\varepsilon_{\mathbf{1}}$ x 1 cm x 1.0 x 10^{-5} M) + $c_{\mathbf{1}o}$ x ($\varepsilon_{\mathbf{3}}$ x 1 cm x 1.0 x 10^{-5} M)

0.158 = $c_{\mathbf{1}c}$ x (10,540 M^{-1} x 10^{-5} M) + $c_{\mathbf{1}o}$ x (42,500 M^{-1} x 10^{-5} M)

0.158 = $c_{\mathbf{1}c}$ x (0.10540) + $c_{\mathbf{1}o}$ x (0.42500) ------ (1)

$c_{\mathbf{1}c} + c_{\mathbf{1}o} = 1$, $c_{\mathbf{1}o} = 1 - c_{\mathbf{1}c}$ ------ (2)

From eq (1) and (2), $c_{\mathbf{1}c}$ = 0.835, $c_{\mathbf{1}o}$ = 0.164

Therefore, probe **1** (close form) ~ 84%, probe **1** (open form) ~ 16%

Table S1. Extinction coefficients, quantum yields and fluorescence outputs of compounds.

	φ_{fl} (%)	ε_{514} (M^{-1} cm^{-1})	Fluorescence Output (φ_{fl} x ε_{514})
Probe **1**	23	10,540	2,424
Probe **2**	28	815	228
Adduct **3**	14	42,500	5,950

Quantum yield was determined using rhodamine B as a standard (ϕ = 0.31) according to the known procedure.[5]

The quantum yield was calculated according to the following equation: $\phi_{sample} = \phi_{standard} (Grad_{sample}/Grad_{standard})(\eta_{sample}^2/\eta_{standard}^2)$

(ϕ = quantum yield, $\phi_{standard}$ = 0.31 in water, Grad = gradient from the plot of integrated fluorescence intensity versus absorbance, η = refractive index of the solvent).

Fluorescence output of each probe ("brightness") is proportional to the product of the extinction coefficient (at the relevant excitation wavelength) and the fluorescence quantum yield.

Cell Study

Cell culture. HeLa (human cervical cancer cells), MRC-5 (human fibroblast cell line derived from normal lung tissue), MCF-7 cells (human breast cancer cells) were purchased from Korean Cell Line Bank. HaCaT cells (human keratinocytes) were kindly provided from Dr. Sung-Hyun Ko's group (Chemical Biology Research Center, Korea Research Institute of Bioscience and Biotechnology (KRIBB)) and HEK293T cells (human embryonic kidney cells) from Professor Kiwon Song's group (College of Life Science and Biotechnology, Yonsei University). These cells were cultured in DMEM (Invitrogen) or RPMI (Invitrogen), supplemented with 10% fetal bovine serum (FBS), 50 units/mL penicillin and 50 units/mL streptomycin, were cultured at 37 ºC with 5% CO_2 atmosphere.

Cell imaging. Confocal fluorescence microscopy images were obtained using a Zeiss laser scanning microscope (LSM 800 Carl Zeiss, Germany). Probes were excited at 561 nm and emission was collected between 570 and 720 nm. Hoechst 33342 was excited at 405 nm and emission was collected from 450 to 510 nm.

Quantitation of fluorescence intensity in cells. Fluorescence intensities in cells were quantified for each image using the mean region of interest (ROI) tool and ImageJ software. Specifically, a constant circular ROI was chosen to encompass cells of interest and the same ROI area size was used for background subtraction. All fluorescence intensities derived from confocal microscope images were averages of at least three independent experiments.

Cell viability assay. Cell viability was assessed by using a MTT ((3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. HeLa cells (5×10^3 cells/100 μL) were plated in triplicate in 96-well plates for 24 h and were incubated with various concentrations of **1** in culture media. MTT (20 μL of a 5 mg/mL solution) was added to culture media in each well and the resulting mixtures were incubated for 2 h. After removing culture media containing MTT, 100 μL of DMSO was added and the cells were incubated for 30 min for color development. Absorbance at 590 nm was measured using an Infinite® 200 PRO multimode microplate reader (Tecan, Switzerland).

Supplementary References

1. Brown, Michael John, PhD Thesis, "Synthesis, structure and functionalisation of 1,2-diazetidines", University of Warwick, 2011.
2. B. Biswal, D. Mallick and B. Bag, *Org. Biomol. Chem.*, 2016, **14**, 2241.
3. T. F. Brewer and C. J. Chang, *J. Am. Chem. Soc.*, 2015, **137**, 10886.
4. M. Kim, S. –K. Ko, H. Kim, I. Shin and J. Tae, *Chem. Commun.*, 2013, **49**, 7959.
5. D. Magde, G.E. Rojas and P.G. Seybold, *Photochem. Photobiol*. 1999, **70**, 737.
6. K. J. Bruemmer, R. R. Walvoord, T. F. Brewer, G. Burgos-Barragan, N. Wit, L. B. Pontel, K. J. Patel and C. J. Chang, *J. Am. Chem. Soc.*, 2017, **193**, 5338.

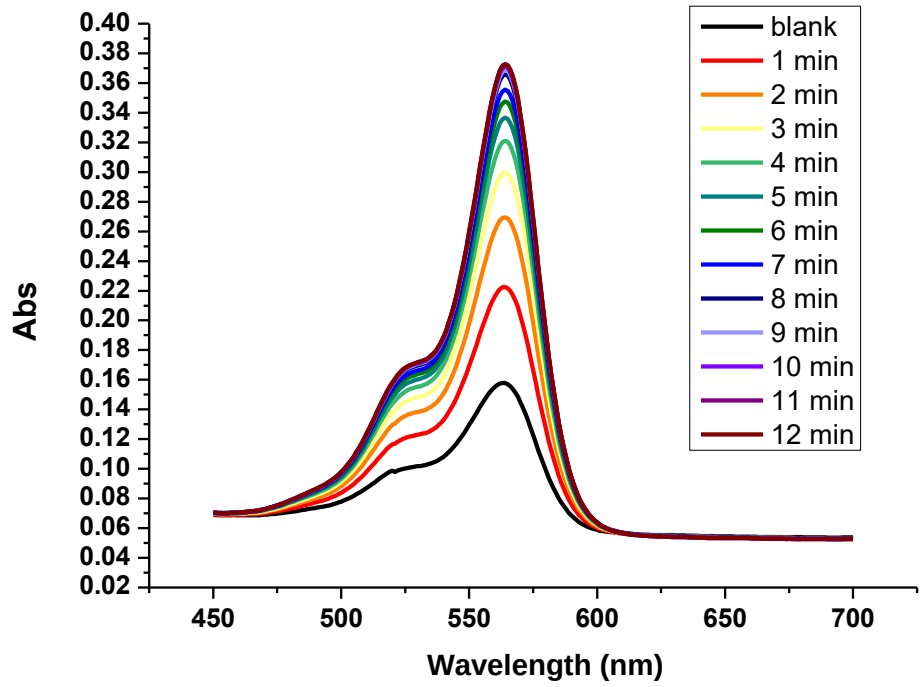


Figure S1. Time-dependent changes of the UV-VIS absorption spectra of **1** (10 μM) promoted by addition of 10 equiv FA in PBS buffer (1% DMSO, pH 7.4) at 37 ^{o}C.

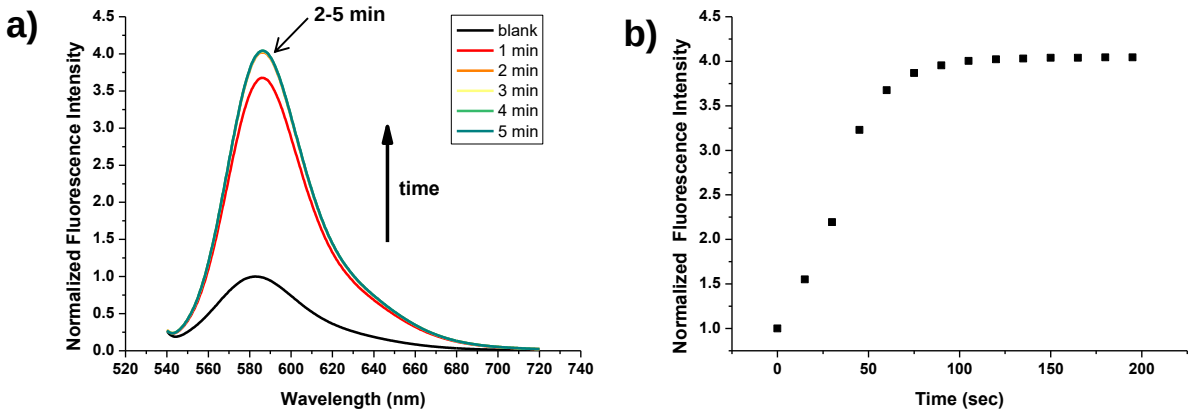


Figure S2. (a) Time-dependent change of the fluorescence spectra of **2** (10 μM) promoted by addition of 10 equiv FA in PBS buffer (1% DMSO, pH 7.4) at 37 ºC (λ_{ex} = 520 nm). (b) A plot corresponding to the time-dependent increase in fluorescence intensity of **2** following addition of FA ($\lambda_{ex}/\lambda_{em}$ = 520/583 nm).

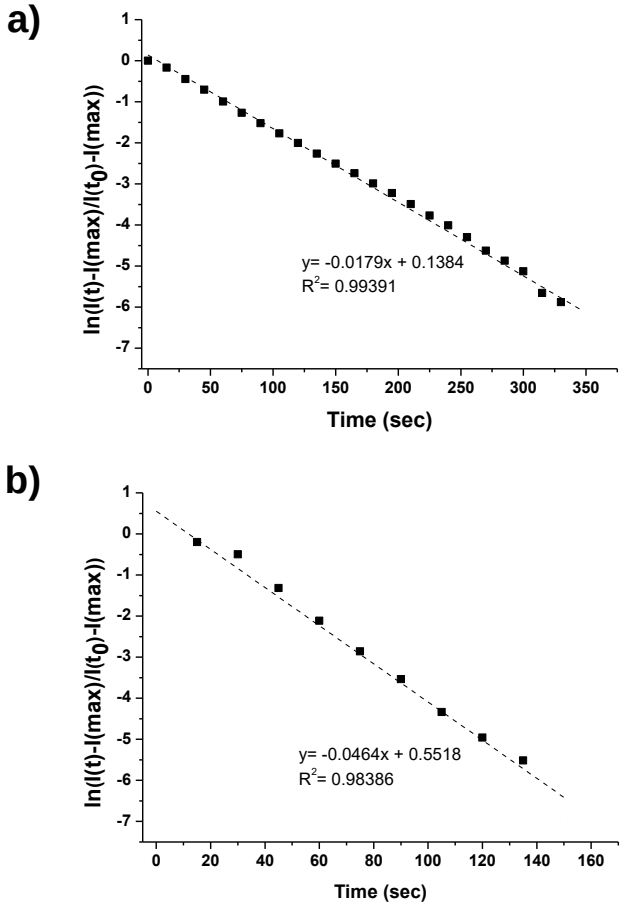


Figure S3. The plots of pseudo-first-order kinetics and linearized integrated rate law of (a) **1** (10 μM) and (2) **2** (10 μM) upon additions of FA (10 equiv) in PBS buffer (1% DMSO, pH 7.4) at 37 °C ($\lambda_{ex}/\lambda_{em}$ = 520/583 nm) (see Supplementary ref. 6).

The calculated rate constants are

$k = 1.8 \times 10^{-2}$ $M^{-1}s^{-1}$ for **probe 1**

$k = 4.6 \times 10^{-2}$ $M^{-1}s^{-1}$ for **probe 2**

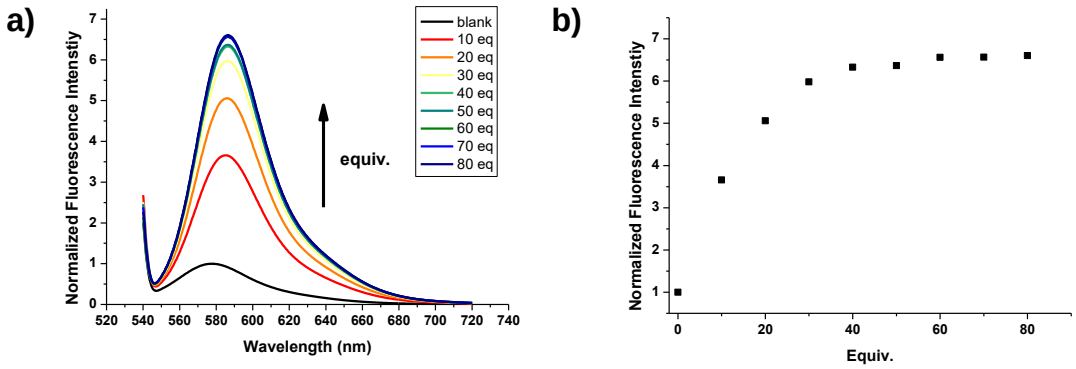


Figure S4. (a) FA concentration-dependent changes of the fluorescence spectra of **2** (10 μM), 10 min after each addition of FA (λ_{ex} = 520 nm). (b) FA concentration-dependent increase in fluorescence intensity of **2** (10 μM) ($\lambda_{ex}/\lambda_{em}$ = 520/583 nm).

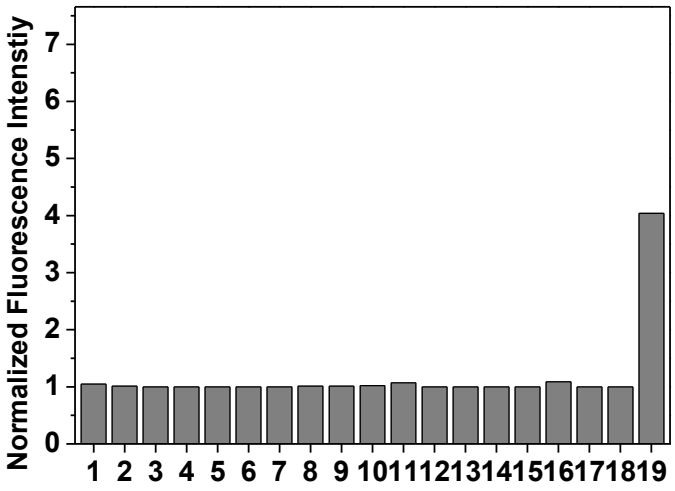


Figure S5. Change of fluorescence intensity of **2 (**10 μM) at 583 nm (λ_{ex} = 520 nm) promoted by addition of each of the biologically relevant analytes (10 equiv) in PBS buffer (1% DMSO, pH 7.4) at 37 ^{o}C. Numbering of the analytes in the graph is as follows: 1, acetaldehyde; 2, benzaldehyde; 3, 4-hydroxybenzaldehyde; 4, ethyl pyruvate; 5, ethyl glyoxalate; 6, propionaldehyde; 7, D-glucose (1 mM); 8, H_2O_2; 9, HOCl; 10, NO$^{\bullet}$; 11, $^1O_2$; 12, $O_2^{\bullet-}$; 13, $^{\bullet}$OH; 14, Cu^{2+}; 15, Fe^{2+}; 16, Fe^{3+}; 17, K^+; 18, Zn^{2+}; 19, FA.

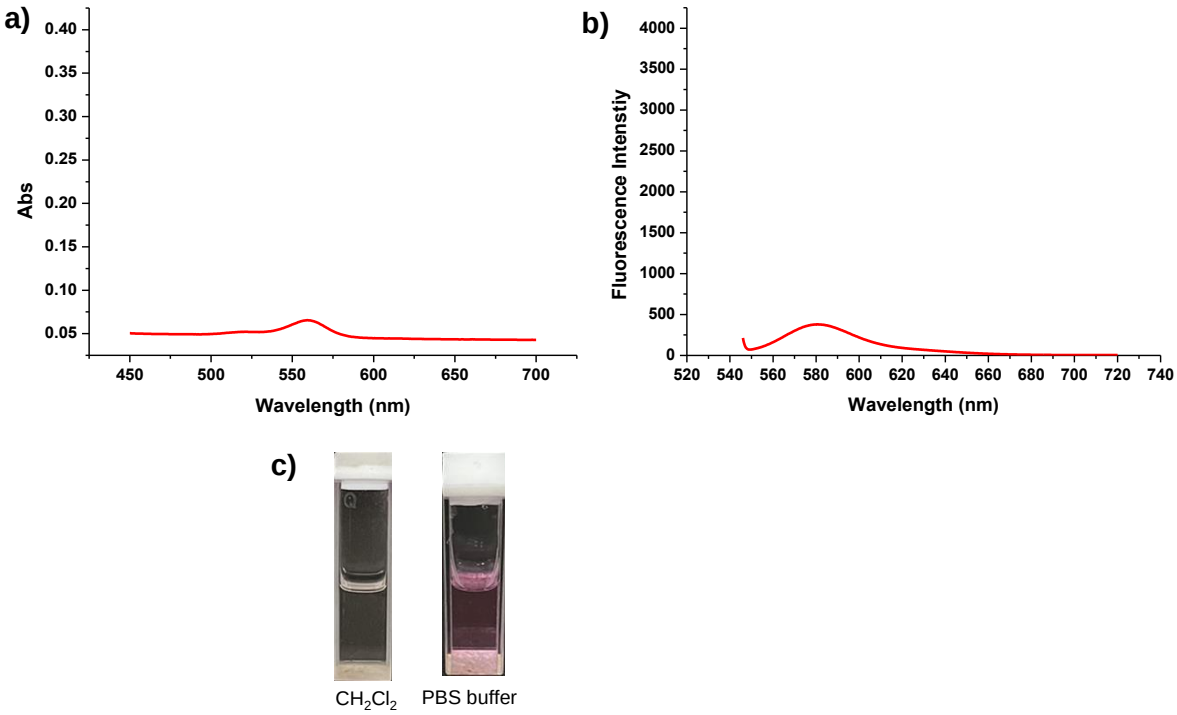


Figure S6. (a) UV-Vis absorption and (b) fluorescence spectra (λ_{ex} = 520 nm) of the isolated product (10 µM) in CH_2Cl_2. (c) Colors of the isolated adduct (10 µM) in CH_2Cl_2 and PBS buffer.

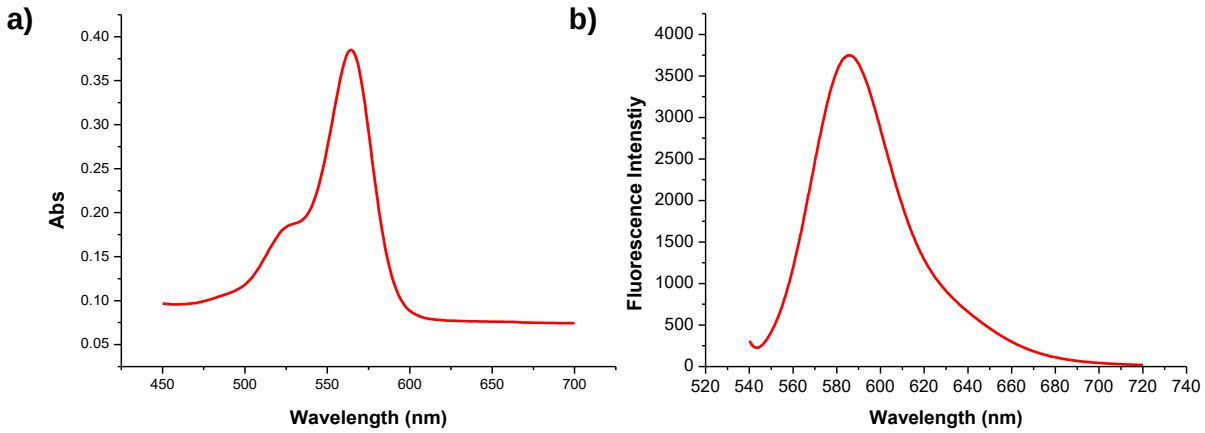
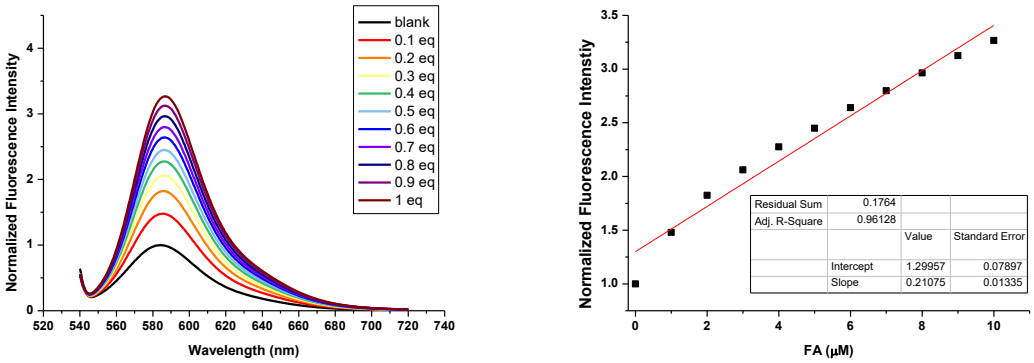


Figure S7. (a) UV-Vis absorption and (b) fluorescence spectra (λ_{ex} = 520 nm) of the isolated adduct (10 μM) in PBS buffer (1% DMSO, pH 7.4).

Probe **1**



Probe **2**

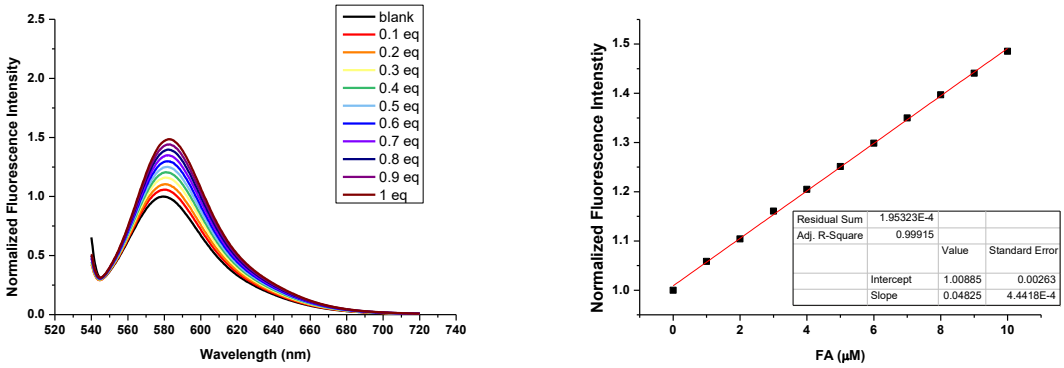


Figure S8. The limits of detection of **1** (10 μM) and **2** (10 μM) were measured by addition of 0.1 equiv FA in PBS buffer (1% DMSO, pH 7.4) at 37 ^{o}C ($\lambda_{ex}/\lambda_{em}$ = 520/583 nm). The limit of detection (LOD) was calculated using the equation, LOD = 3.3(Sy/S), where Sy is the standard deviation of the response of the curve, and S is a slope of the calibration curve. (The limits of detection of **1** and **2** for FA have been calculated to be 1.24 μM and 0.59 μM, respectively.)

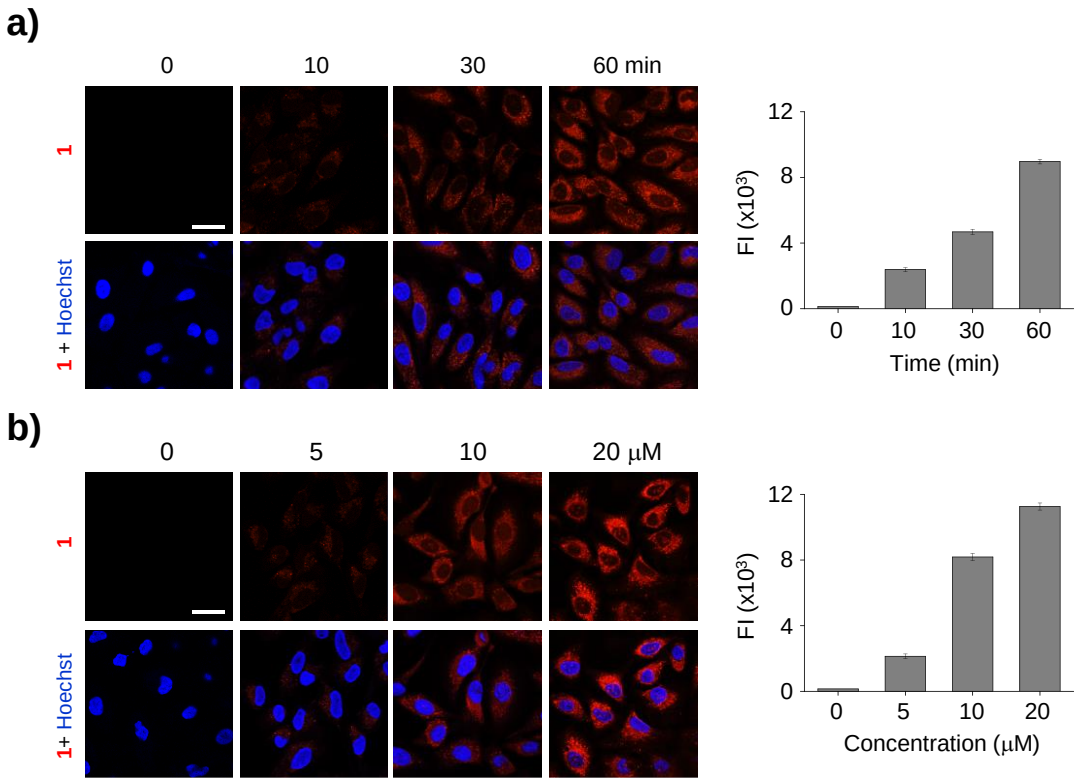


Figure S9. Time and concentration-dependent responses of **1** to FA in cells. HeLa cells were incubated with (a) 10 μM **1** for the indicated times and (b) the indicated concentrations of **1** for 1 h. Cell images were obtained using confocal fluorescence microscopy (scale bar = 10 μm). The nucleus was stained with Hoechst 33342. Graphs show fluorescence intensities (FI) in cells treated with **1** (mean ± s.d., n = 3).

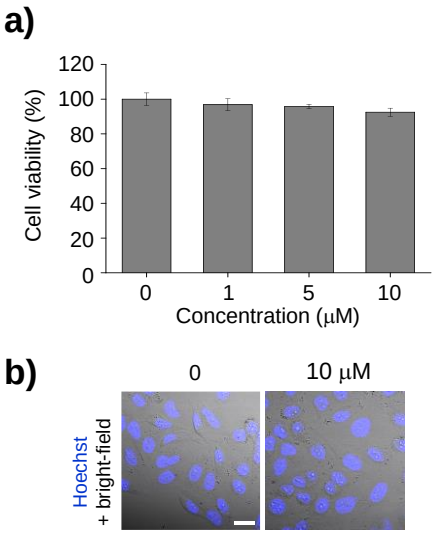


Figure S10. (a) HeLa cells were incubated with indicated concentrations of **1**. Cell death was measured by means of a MTT assay (mean ± s.d., n = 3). (b) HeLa cells were incubated with 10 μM **1** followed by treatment with 1 μM Hoechst 33342. Cell images were obtained using confocal fluorescence microscopy (scale bar = 10 μm).

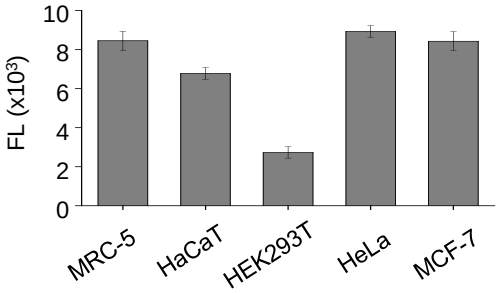


Figure S11. Measurement of endogenous FA in cell lysates using probe **1**. Lysates of each cells were incubated with **1** (10 μM) for 1 h (mean ± s.d., n = 3).

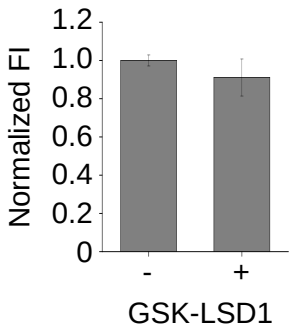
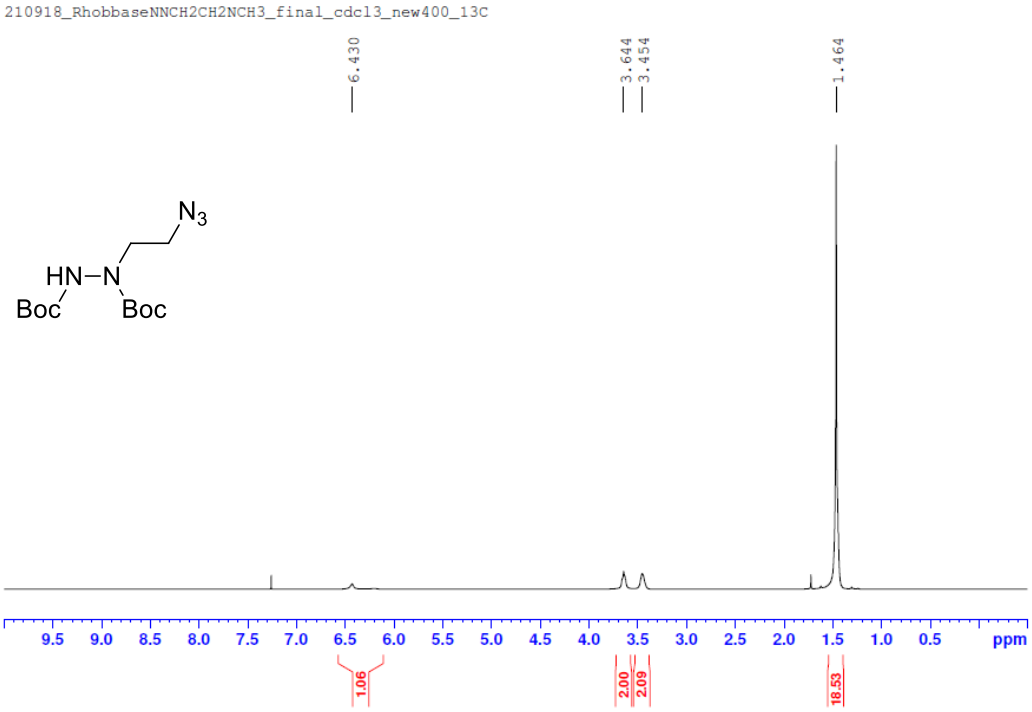
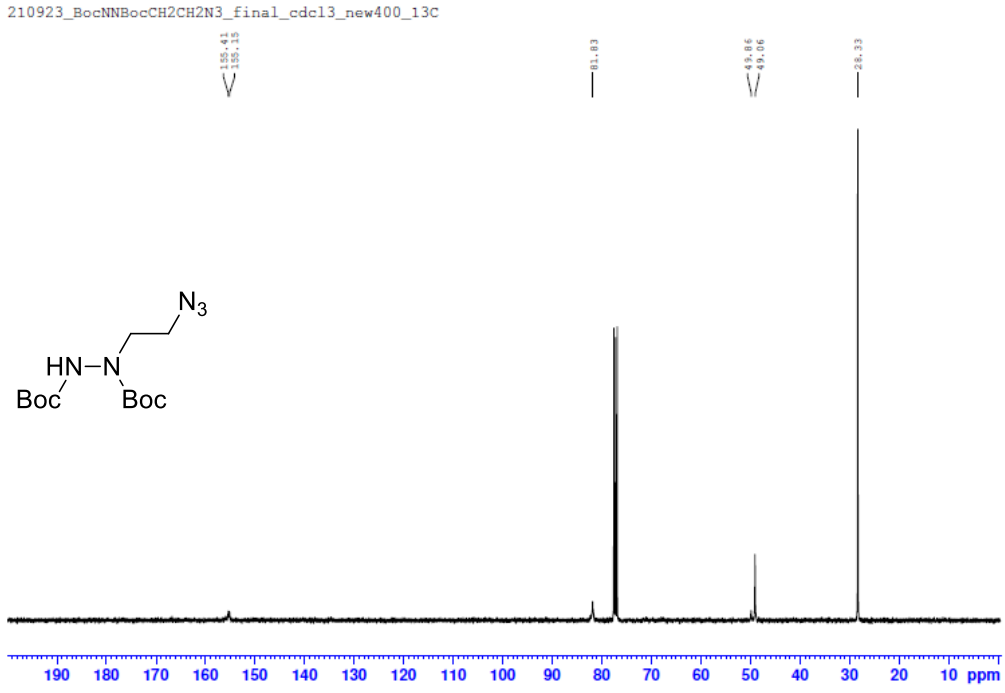


Figure S12. The effect of an inhibitor on LSD1-promoted generation of FA in cells. MCF-7 cells were incubated with or without 1 μM GSK-LSD1 for 20 h. Cell lysates were incubated with **1** (10 μM) for 1 h. A graph shows the normalized fluorescence intensity (mean ± s.d., n = 3).

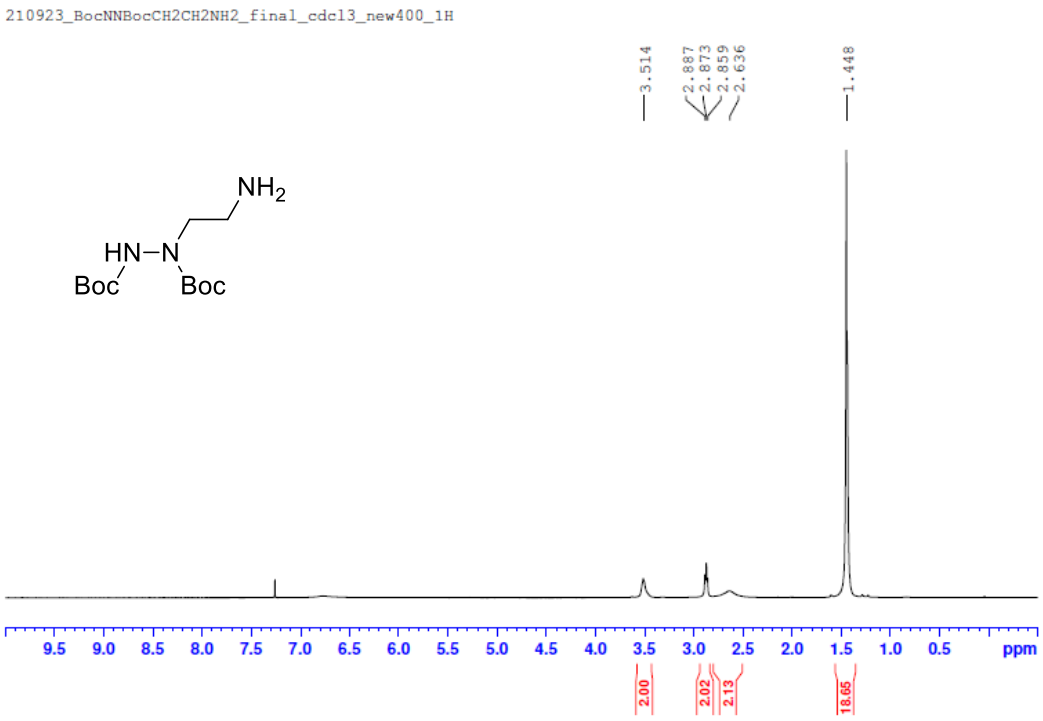
<NMR spectra>

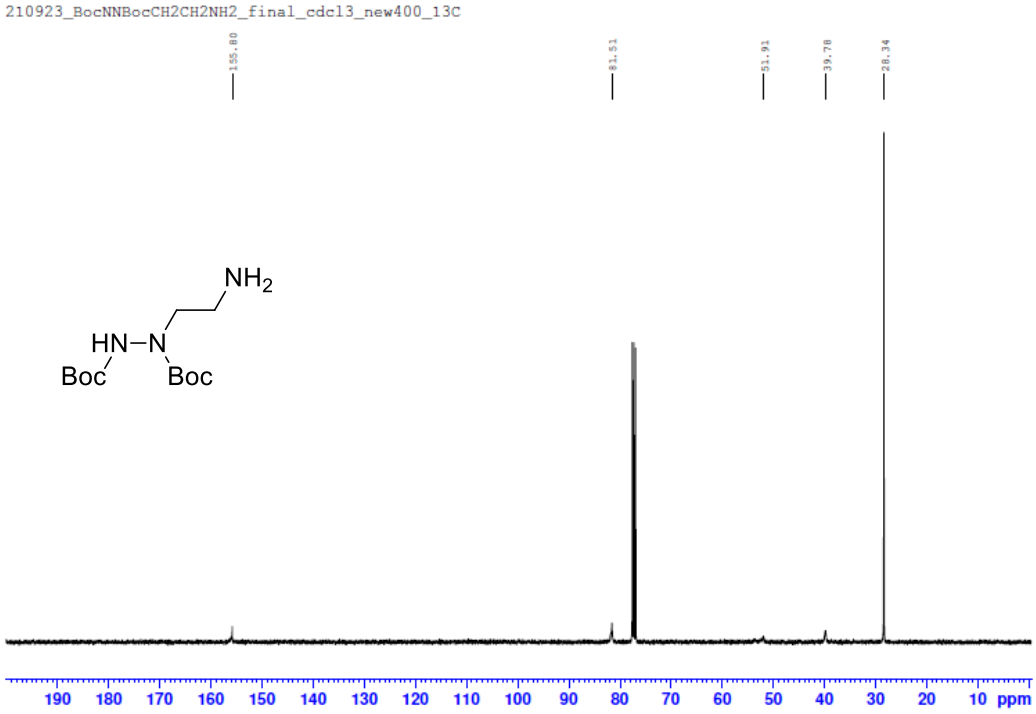
Compound S3





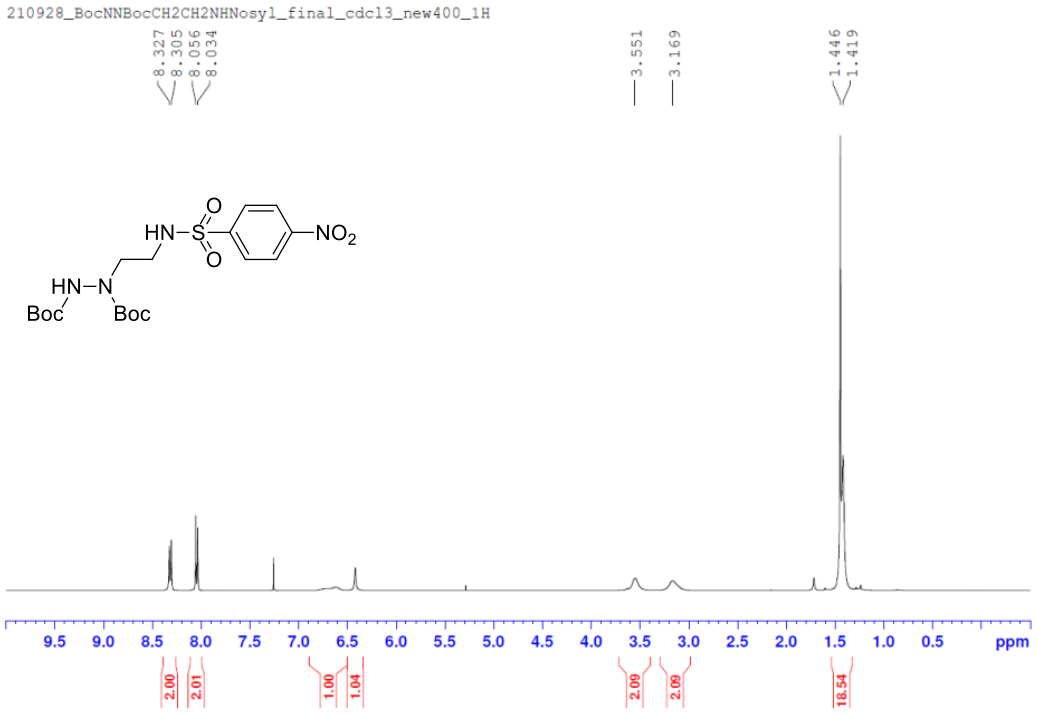
Compound S4





1

Compound S5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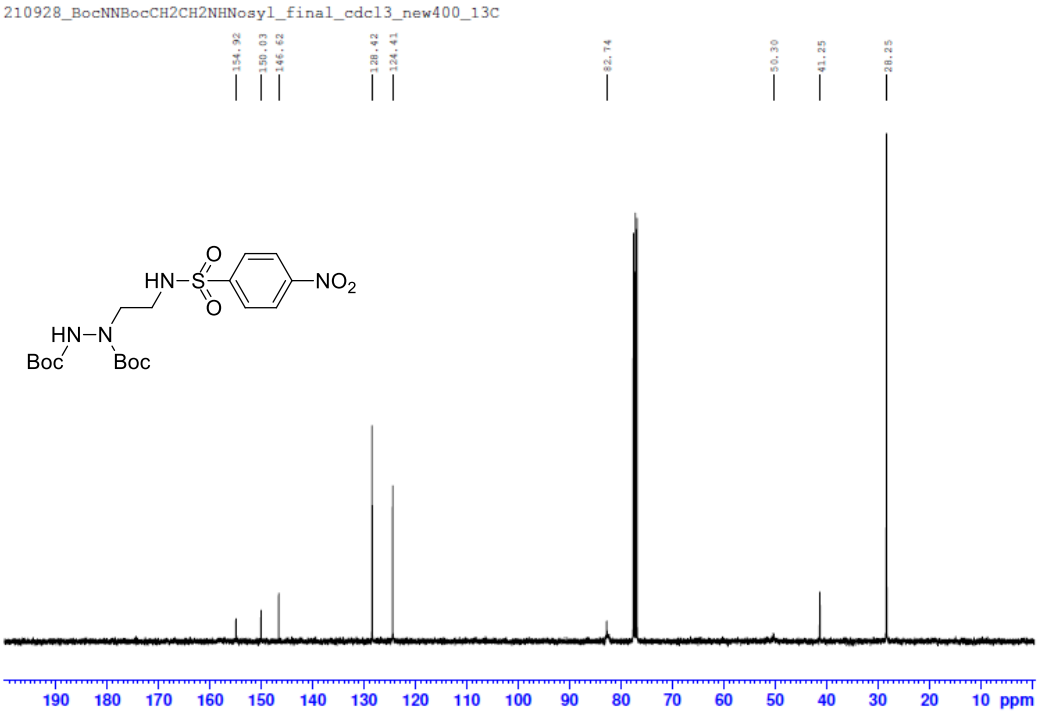
8.327
8.305
8.056
8.034
3.551
3.169
1.446
1.419
HN
S
O
O
NO2
HN
N
Boc
Boc
9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 ppm
2.00
2.01
1.00
1.04
2.09
2.09
18.54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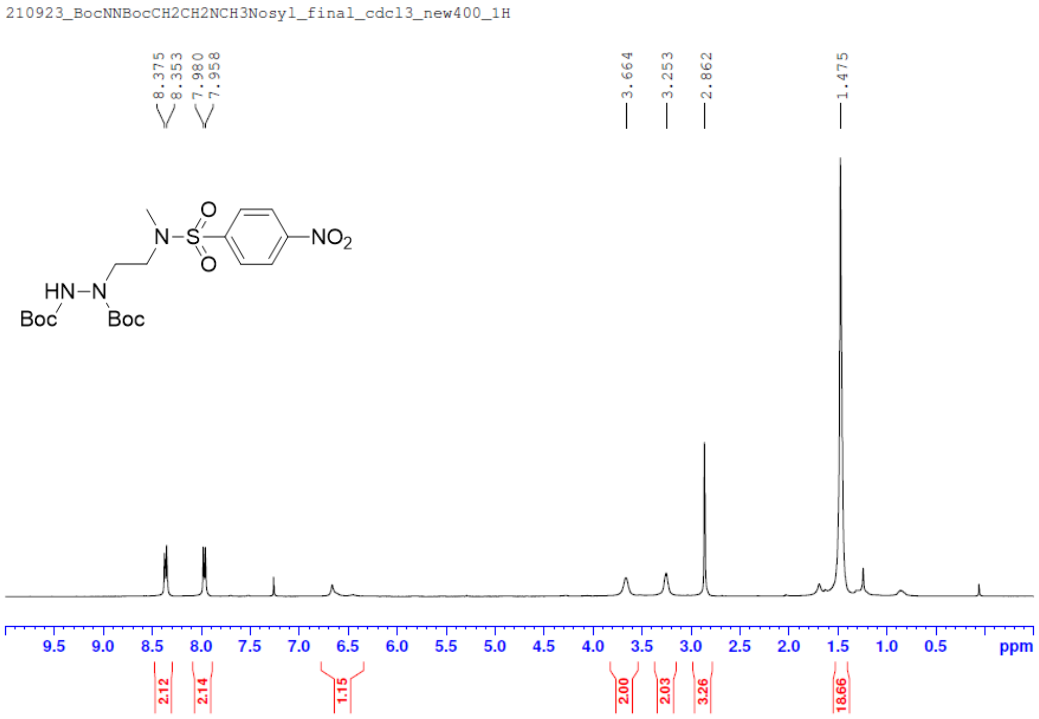
150.03
146.62
128.42
124.41
82.74
50.30
41.25
28.25
HN
S
O
O
NO2
HN
N
Boc
Boc
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 ppm

Compound S6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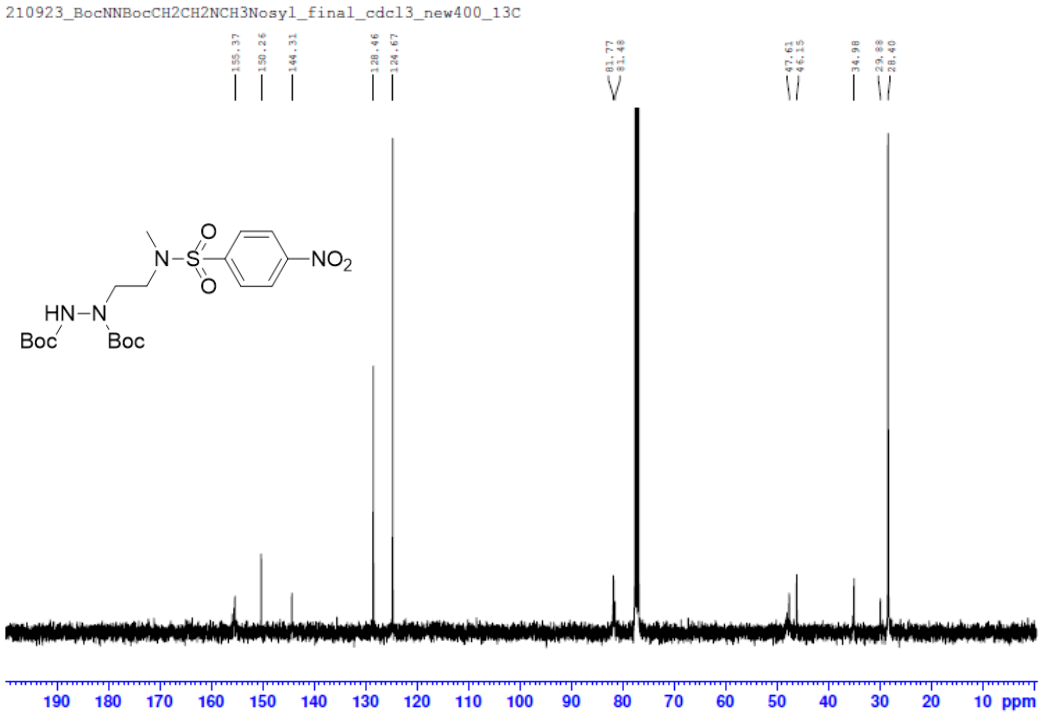
8.353
7.980
7.958
3.664
3.253
2.862
1.475
NO2
HN–N
Boc
Boc
9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 ppm
2.12
2.14
1.15
2.00
2.03
3.26
18.66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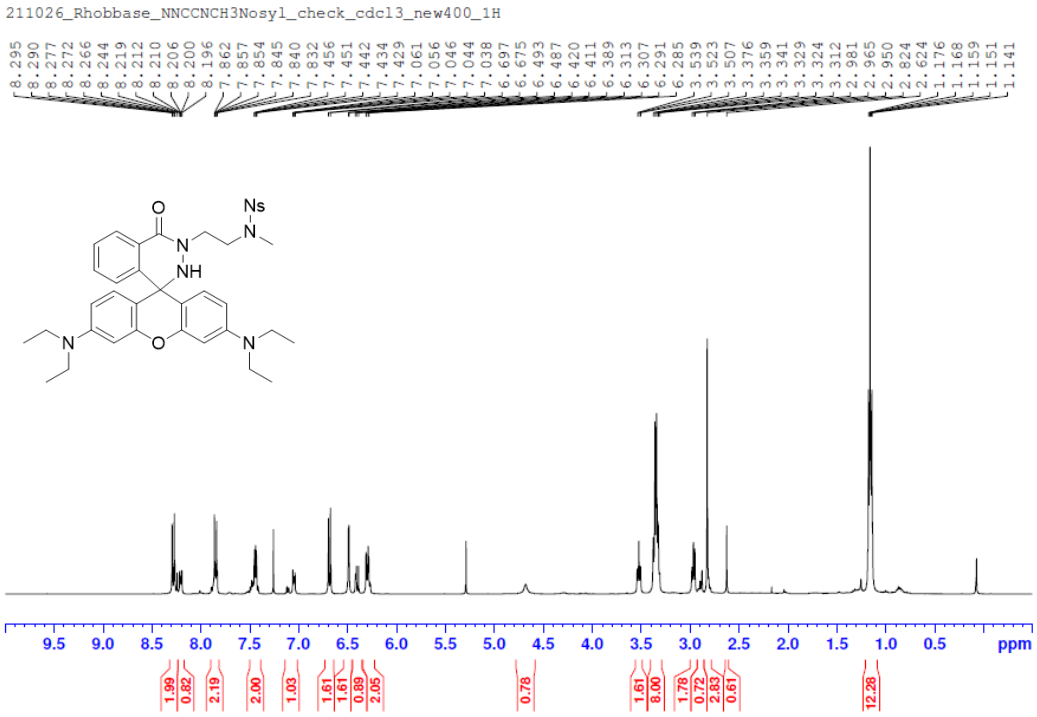
150.26
144.31
128.46
124.67
81.77
81.48
47.61
46.15
34.98
29.88
28.40
NO2
HN–N
Boc
Boc
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 ppm

Compound S8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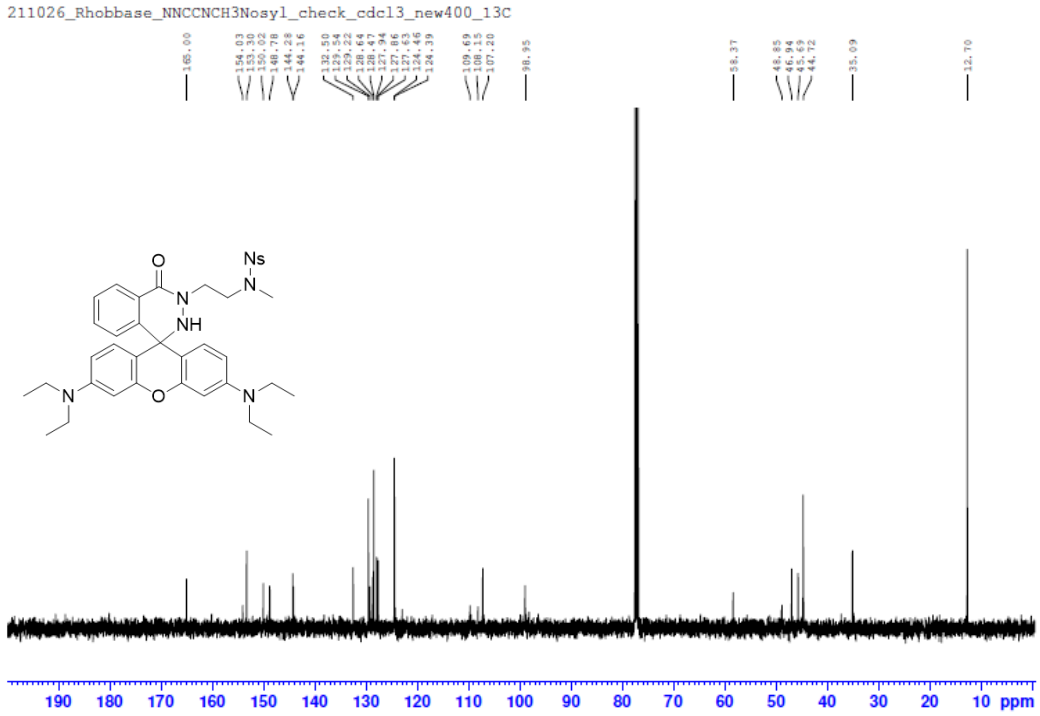
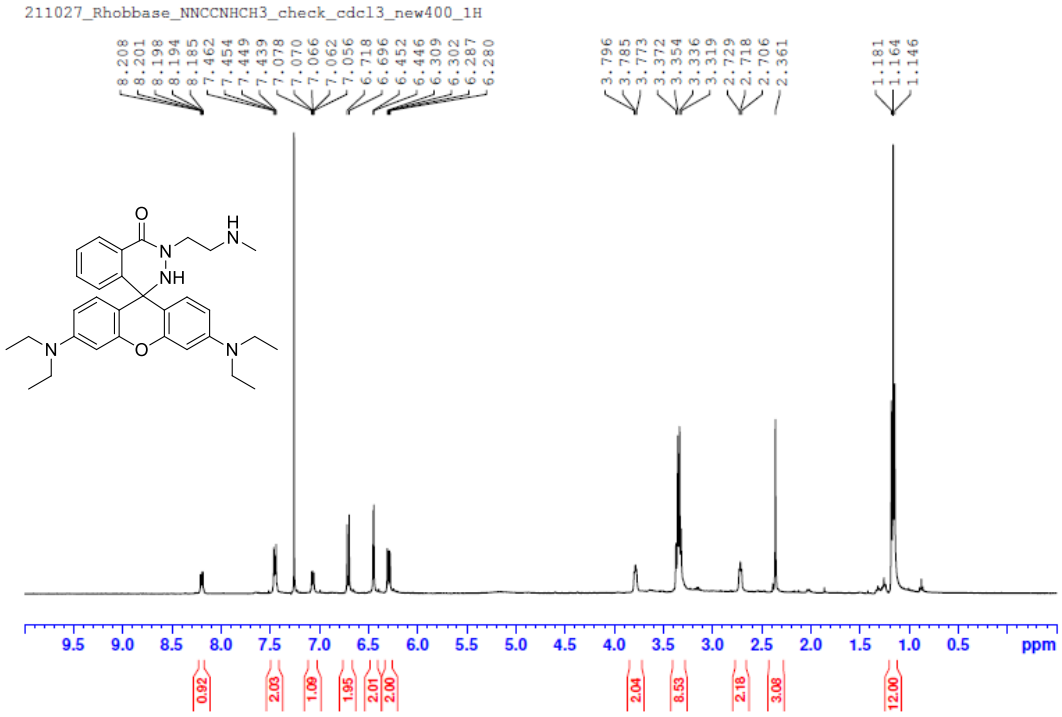
8.290
8.277
8.272
8.266
8.244
8.219
8.212
8.210
8.206
8.200
8.196
7.862
7.857
7.854
7.845
7.840
7.832
7.456
7.451
7.442
7.434
7.429
7.061
7.056
7.046
7.044
7.038
6.697
6.675
6.493
6.487
6.420
6.411
6.389
6.313
6.307
6.291
6.285
3.539
3.523
3.507
3.376
3.359
3.341
3.329
3.324
3.312
2.981
2.965
2.950
2.824
2.624
1.176
1.168
1.159
1.151
1.141
O
Ns
N
N
NH
N
O
N
9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 ppm
1.99
0.82
2.19
2.00
1.03
1.61
1.61
0.89
2.05
0.78
1.61
8.00
1.78
0.72
2.83
0.61
12.28


211026_Rhobbase_NNCCNCH3Nosyl_check_cdcl3_new400_13C
165.00
154.03
153.30
150.02
148.78
144.28
144.16
132.50
129.54
129.22
128.64
128.47
127.86
127.63
124.46
124.39
109.69
108.15
107.20
98.95
58.37
46.85
46.44
45.69
44.72
35.09
12.70
O
Ns
N
N
NH
N
O
N
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 ppm

Probe 1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8.201
8.198
8.194
8.185
7.462
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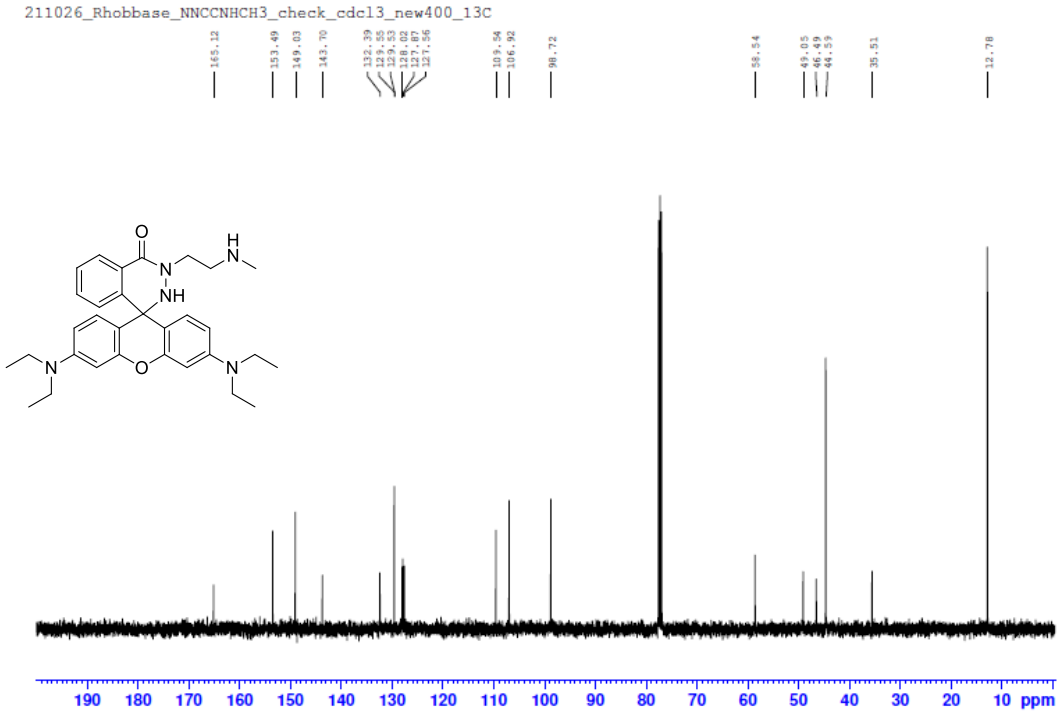
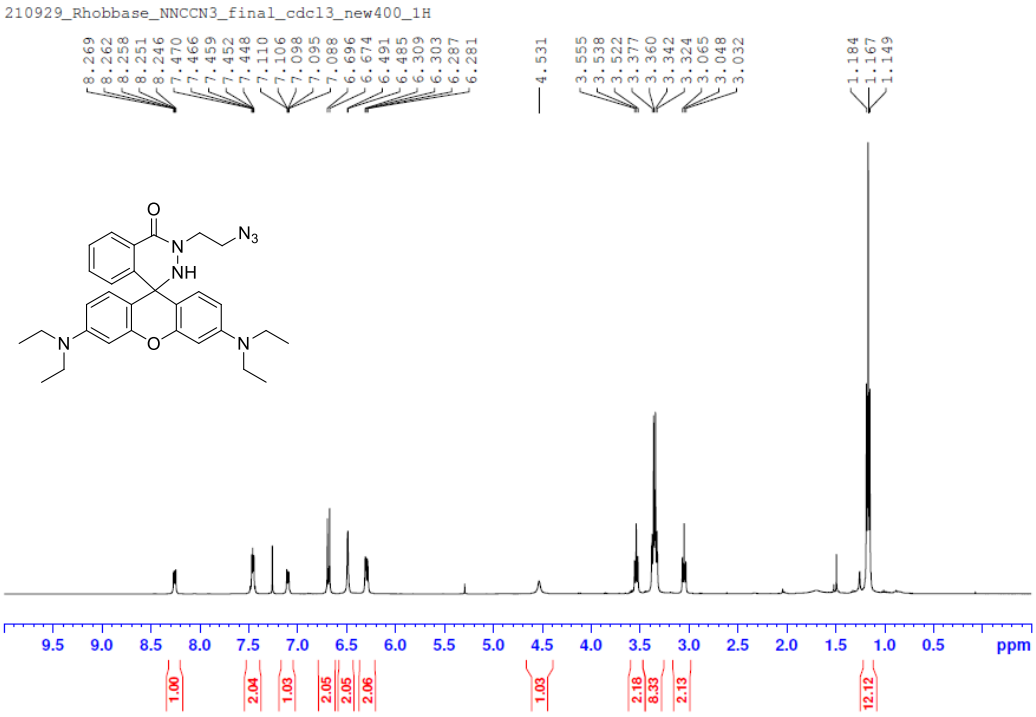
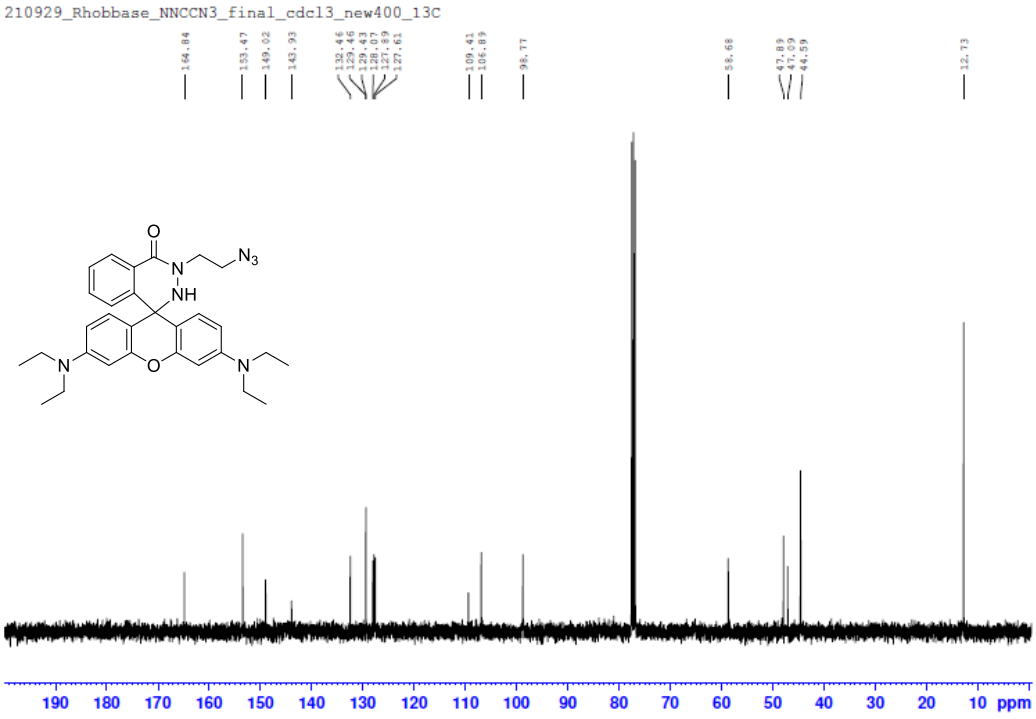
7.439
7.078
7.070
7.066
7.062
7.056
6.718
6.696
6.452
6.446
6.309
6.302
6.287
6.280
3.796
3.785
3.773
3.372
3.354
3.336
3.319
2.729
2.718
2.706
2.361
1.181
1.164
1.146
9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 ppm
0.92
2.03
1.09
1.95
2.01
2.00
2.04
8.53
2.18
3.08
12.00

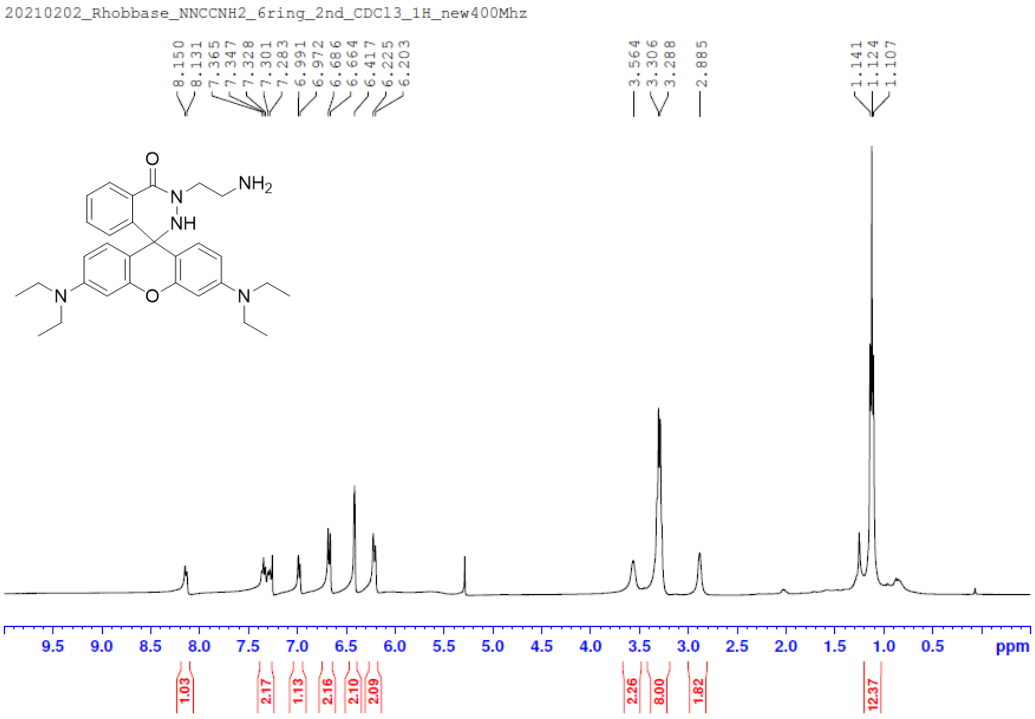

211026_Rhobbase_NNCCNHCH3_check_cdcl3_new400_13C
165.12
153.49
149.03
143.70
132.39
129.55
129.53
128.02
127.87
127.56
109.54
106.92
98.72
58.54
49.05
46.49
44.59
35.51
12.78
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 ppm

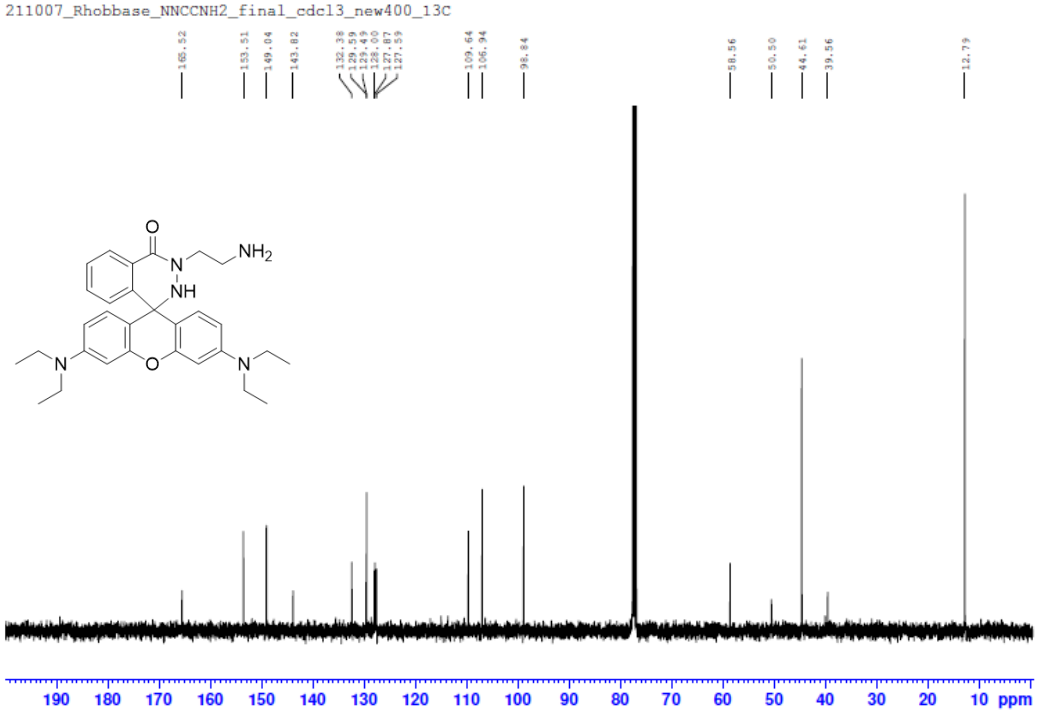
Compound S10



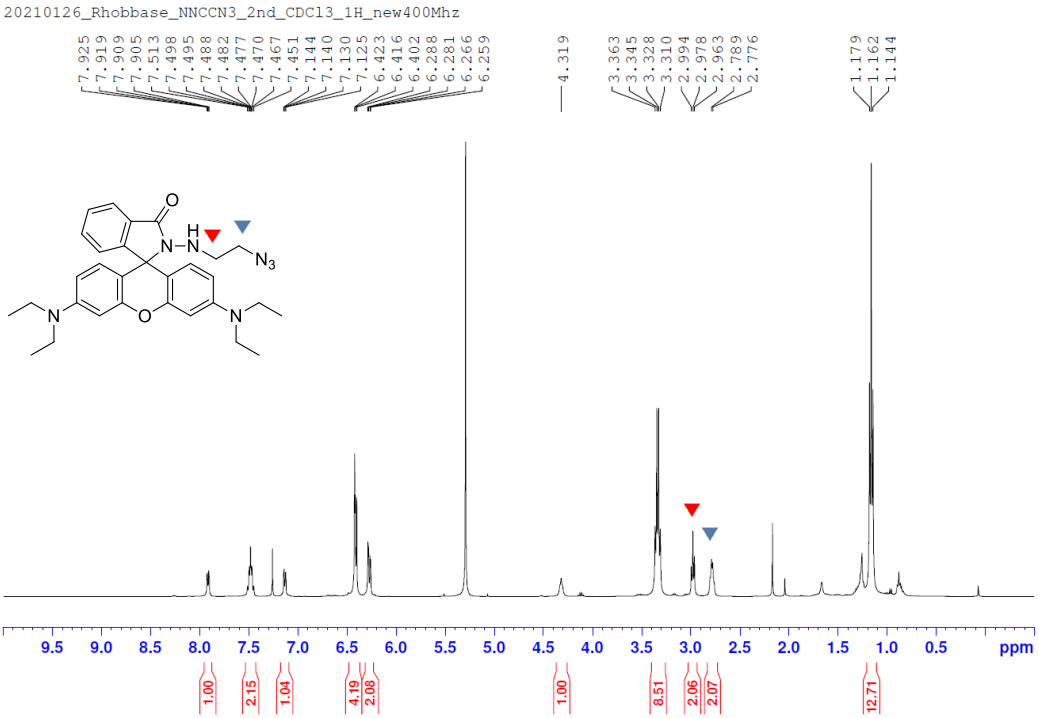


Probe 2

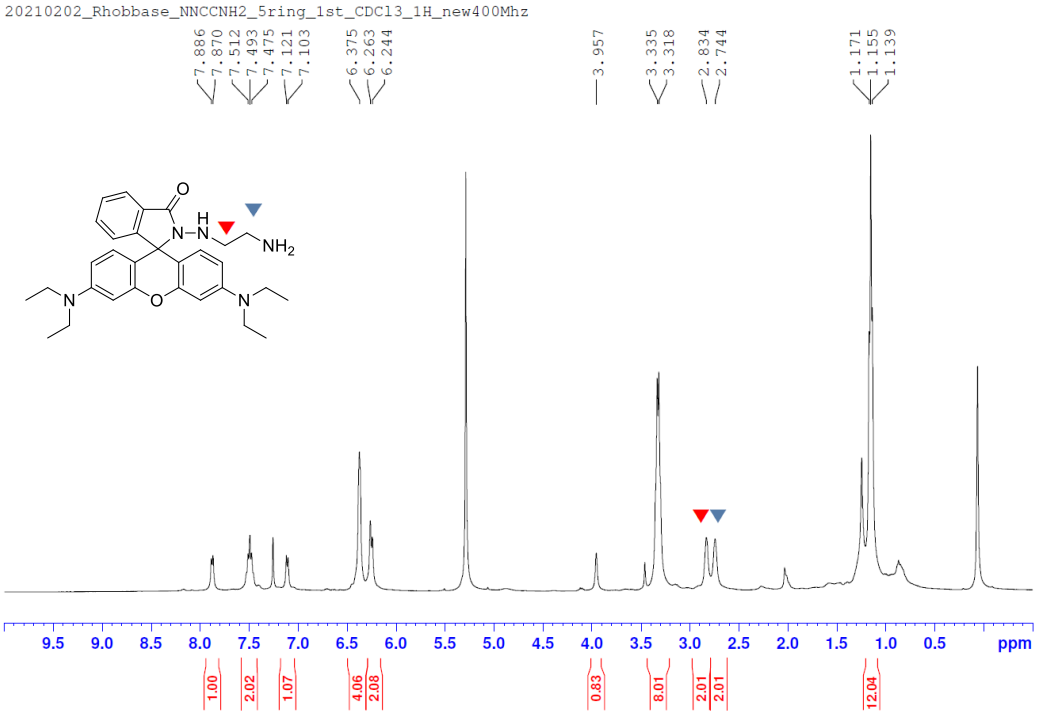

20210202_Rhobbase_NNCCNH2_6ring_2nd_CDCl3_1H_new400Mhz
8.150
8.131
7.365
7.347
7.328
7.301
7.283
6.991
6.972
6.686
6.664
6.417
6.225
6.203
3.564
3.306
3.288
2.885
1.141
1.124
1.107
NH2
NH
N
O
9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 ppm
1.03
2.17
1.13
2.16
2.10
2.09
2.26
8.00
1.82
12.37

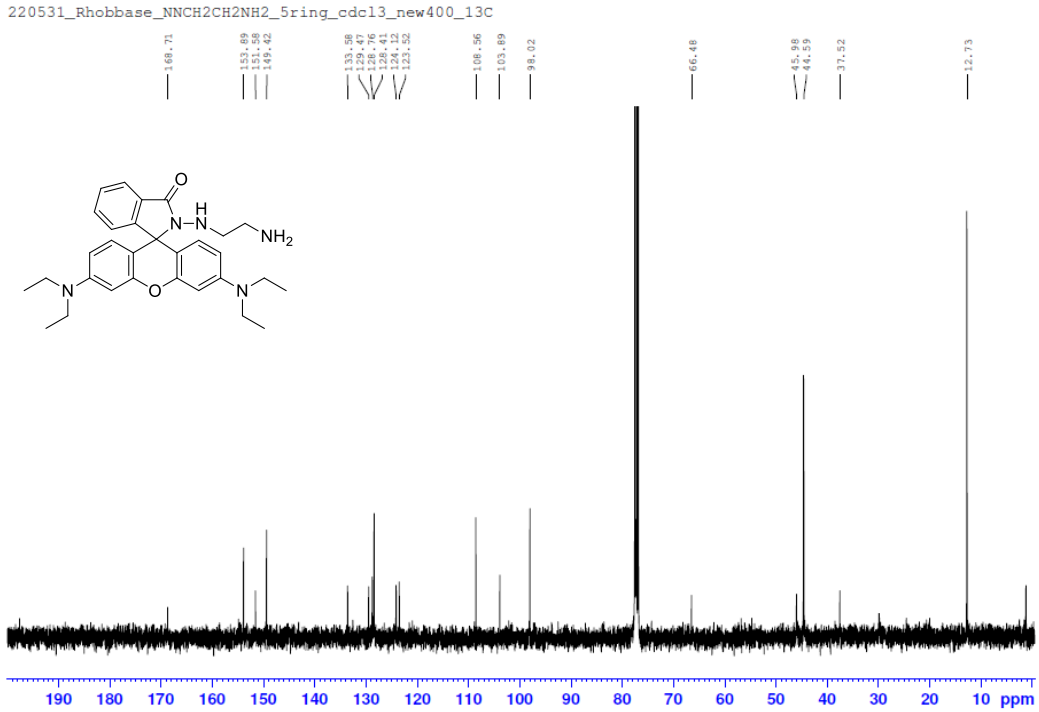

211007_Rhobbase_NNCCNH2_final_cdcl3_new400_13C
165.52
153.51
149.04
143.82
130.38
129.53
129.49
128.00
127.87
127.59
109.64
106.94
98.84
58.56
50.50
44.61
39.56
12.79
NH2
NH
N
O
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 ppm

5-Membered Azide: Compound S11



5-Membered Amine: Compound S12





Adduct 3 in $CDCl_3$