Supporting Information

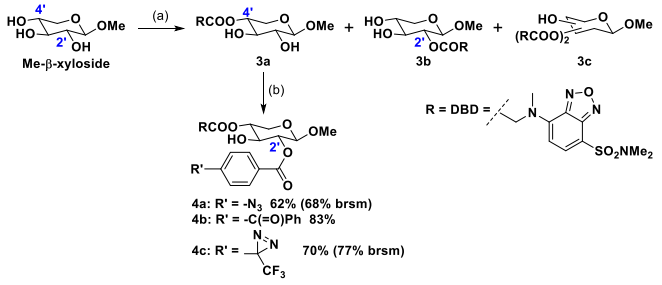
Synthesis of a Fluorescent Photoaffinity Probe of OSW-1 by Site-Selective Acylation of an Inactive Congener and Biological Evaluation

Kaori Sakurai,* Masato Hiraizumi, Naho Isogai, Rina Komatsu, Takahiro Shibata, Yoshihiro Ohta

Department of Biotechnology and Life Science, Tokyo University of Agriculture and Technology, Tokyo, 184-8588, Japan

1. General Experimental Methods

NMR experiments were performed at 293 K on JEOL ECX 300, 400 or ECA 500 using 5 mm z-gradient probes and data were processed with Delta software. The spectra are referenced internally according to residual solvent signals of $CDCl_3$ (^{1}H NMR; δ = 7.26 ppm, ^{13}C NMR; δ = 77.0 ppm) and pyridine-d_5 (^{1}H NMR; δ = 8.74 ppm, ^{13}C NMR; δ = 149.9 ppm). Positive ion ESI-TOF-MS data were obtained by JEOL AccuTOF mass spectrometer. Unless noted otherwise, all chemical reagents were purchased from Wako Chemicals, TCI and Sigma-Aldrich. Preparative thin-layer chromatography (TLC) was performed using PLC Silica gel 60 F254 pre-coated plates (Merck Millipore). Flash column chromatography was performed using Silica gel 60 (spherical, particle size 40-100 μm; Kanto Chemical). Liquid chromatography was performed using Biotage® SNAP cartridge KP-C18-HS 12 g column (particle size 37-70; Biotage Japan) on Biotage Isolera One system (Biotage Japan, Tokyo). Analytical scale HPLC experiments were performed using a 4.6 × 25.0 mm (5 μm) Waters Xbridge ODS column on JASCO LC-2000 Plus system (Tokyo) equipped with a photodiode array detection unit MD-2018. UV-visible spectra were recorded on Varian Cary 50 UV-visible spectrophotometer. Fluorescence spectra were recorded on JASCO ETC 273 spectrophotometer.



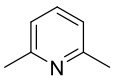
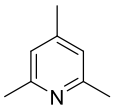
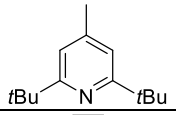
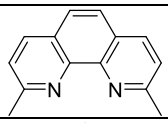
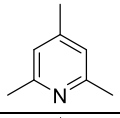
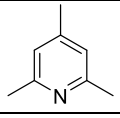
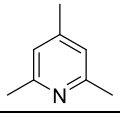
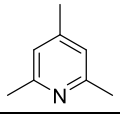
Reagents and conditions: (a) RCOCl (1.5 equiv.), Me_2SnCl_2 (1.5 equiv.), base, THF, rt, 2h.; (b) R'COCl (1.5 equiv.), Me_2SnCl_2 (1.5 equiv.), DIPEA (4.0 equiv.), THF (0.25 M), rt, 2h.

2. General procedure for Me_2SnCl_2-mediated acylation of β-methyl xyloside

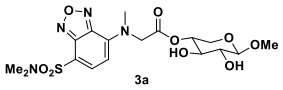
To a solution of β-methyl-xylose (2.39 mg, 15.0 μmol) in THF (60 μL) were added Me_2SnCl_2 (4.94 mg, 22.5μmol), base and 4-(*N*,*N*-dimethylaminosulfonyl)-7-(*N*-chloroformylmethyl-*N*-methylamino)-2,1,3-benzoxadiazole (DBD-COCl, 7.48 mg, 22.5 μmol) stirred at room temperature. After 2 h, the mixture was diluted with EtOAc (5 mL) and was

washed with 3% aqueous HCl, water, saturated $NaHCO_3$, then brine. The organic layer was dried with Na_2SO_4, which was filtered and concentrated in vacuo. The residue was purified by flash column chromatography ($CHCl_3$/MeOH = 9/1) to give **3a**−**3c** as yellow solid.

Table S1. Selective acylation at C4'-OH of β-methyl-xylose.

Entry	Me_2SnCl_2 (equiv.)	DBD-COCl (equiv.)	Base	THF (M)	Yield (%)		
					3a	**3b**	**3c**
1	-	1.0	DIPEA	0.25	2	11	13
2	1.0	1.0	DIPEA	0.25	46	12	11
3	1.0	1.0	TEA	0.25	25	4	7
4	1.0	1.0	K_2CO_3	0.25	37	3	3
5	1.0	1.0		0.25	18	5	5
6	1.0	1.0		0.25	51	7	2
7	1.0	1.0		0.25	55	8	4
8	1.0	1.0		0.25	N. D.	N. D.	N. D.
9	1.0	1.0		0.25	41	9	2
10	1.5	1.5		0.25	63	7	9
11	2.0	2.0		0.25	47	3	46
12	1.0	1.0		0.1	51	2	N. D.
13	1.5	1.5		0.1	53	3	2

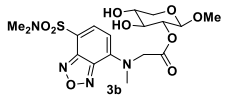
Compound 3a



3a

Compound **3a** was obtained as yellow solid: ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.91 (1H, d, *J* = 8.3 Hz), 6.21 (1H, d, *J* = 8.3 Hz), 5.01(1H, m), 4.97 (1H, d, *J* = 3.0 Hz), 4.89 (1H, m), 4.78 (1H, d, *J* = 4.5 Hz), 4.31 (1H, d, *J* = 6.2 Hz), 4.07 (1H, dd, *J* = 4.5, 12.0 Hz), 3.76 (1H, t, *J* = 7.6 Hz), 3.52 (3H, s), 3.52-3.34 (2H, m), 3.34 (3H, s), 2.88 (7H, s), 2.61 (1H, br); ^{13}C NMR (125 MHz, $CDCl_3$): δ168.8, 146.9, 144.8, 142.1, 138.5, 110.4, 103.2, 103.0, 77.2, 72.4, 72.2, 61.3, 56.9, 56.5, 41.4, 37.8 (×2); HRMS (ESI-TOF) calcd for $C_{17}H_{24}N_4NaO_9S$ ($M+Na^+$): 483.11617; found: 483.11615.

Compound 3b



3b

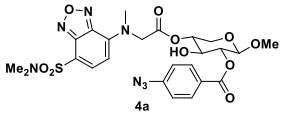
Compound **3b** was obtained as yellow solid: ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.91 (1H, d, *J* = 7.9 Hz), 6.21 (1H, d, *J* = 8.3 Hz), 5.06-4.75 (3H, m), 4.23 (1H, d, *J* = 6.9 Hz), 4.07 (1H, dd, *J* = 4.5, 5.2 Hz), 3.83 (1H, m), 3.64 (1H, br), 3.53 (3H, s), 3.49 (1H, m), 3.37 (3H, s), 2.88 (6H, s), 2.61 (1H, br), 2.39 (1H, m); LRMS (ESI-TOF) calcd for $C_{17}H_{24}N_4NaO_9S$ ($M+Na^+$): 483.11617; found: 483.10522.

3. General procedure for preparation of acylating reagent[1]

To a solution of carboxylic acid (1.0 equiv.) in dry CH_2Cl_2 (1.5 M) were added oxalyl chloride (1.3 equiv.) and the reaction mixture was stirred for 2 h at room temperature. The mixture was concentrated *in vacuo* and was used in the subsequent reaction without further purification.

4. Me_2SnCl_2-catalyzed 2'-OH selective acylation of 4'-DBD-xylose derivative

Compound 4a

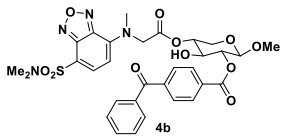


4a

To a solution of compound **3a** (4.66 mg, 10.0 μmol) in THF (40 μL) were added Me_2SnCl_2 (2.20 mg, 10.0 μmol), DIPEA (5.17 mg, 40.0 μmol) and 4-azidobenzoyl chloride (2.72 mg, 15.0 μmol) stirred at room temperature. After 2 h, the mixture was diluted with EtOAc (5 mL) and was washed with 3% aqueous HCl, water, saturated $NaHCO_3$, then brine. The organic layer was dried with Na_2SO_4, which was filtered and concentrated in vacuo. The residue was purified by flash column chromatography ($CHCl_3$/MeOH = 93/7) then by preparative TLC ($CHCl_3$/MeOH = 93/7) to give compound **4a** (3.75 mg, 6.19 μmol, 62%) as yellow solid: ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.11 (2H, d, *J* = 8.6 Hz), 7.88 (1H, d, *J* = 7.9 Hz), 7.08 (2H, d, *J* = 9.0 Hz), 6.16 (1H, d, *J* = 8.3 Hz), 5.03 (1H, dd, *J* = 5.9, 4.8 Hz), 4.97-4.95 (1H, m), 4.90 (1H, d, J = 11.0 Hz), 4.72 (1H, d, J = 4.5 Hz), 4.68 (1H, d, J = 9.3 Hz), 4.20 (1H, dd, *J* = 3.8, 12.7 Hz), 3.97 (1H, m), 3.61 (1H,

dd, *J* = 5.5, 12.7 Hz), 3.50 (3H, s), 3.27 (3H, s), 3.26 (1H, br), 2.86 (6H, s); ^{13}C NMR (125 MHz, $CDCl_3$): δ 168.9, 165.1, 147.0, 145.6, 145.4, 142.2, 138.6, 131.9, 125.9, 119.0, 110.7, 103.2, 100.4, 71.8, 71.3, 69.2, 59.7, 56.6, 56.4, 41.4, 37.9 (×2); HRMS (ESI-TOF) calcd for $C_{24}H_{27}N_7NaO_{10}S$ ($M+Na^+$): 628.1437; found: 628.1436.

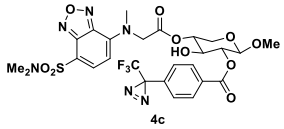
Compound 4b



4b

To a solution of compound **3a** (3.33 mg, 7.20 μmol) in THF (29 μL) were added Me_2SnCl_2 (1.58 mg, 7.20 μmol), DIPEA (3.72 mg, 28.8 μmol) and 4-benzoylbenzoyl chloride (2.64 mg, 10.8 μmol) stirred at room temperature. After 2 h, the mixture was diluted with EtOAc (5 mL) and was washed with 3% aqueous HCl, water, saturated $NaHCO_3$, then brine. The organic layer was dried with Na_2SO_4, which was filtered and concentrated in vacuo. The residue was purified by flash column chromatography ($CHCl_3$/MeOH = 93/7) then by preparative TLC ($CHCl_3$/MeOH = 93/7) to give compound **4b** (3.98 mg, 5.95 μmol, 83%) as yellow solid: ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.25 (2H, d, *J* = 8.6 Hz), 7.89 (1H, d, *J* = 8.0 Hz), 7.86 (2H, d, *J* = 8.6 Hz), 7.78 (2H, d, *J* = 8.0 Hz), 7.62 (1H, t, *J* = 7.5 Hz), 7.49 (2H, t, *J* = 8.0 Hz), 6.18 (1H, d, *J* = 8.6 Hz), 5.10 (1H, dd, *J* = 4.6, 6.3 Hz), 4.99-4.96 (1H, m), 4.91 (1H, d, *J* = 18.3 Hz), 4.76 (1H, d, J = 18.3 Hz), 4.75 (1H, d, J = 4.0 Hz), 4.20 (1H, dd, *J* = 3.4, 12.6 Hz), 4.01 (1H, dd, *J* = 6.3, 12.6 Hz), 3.63 (1H, dd, *J* = 5.2, 12.6 Hz), 3.52 (3H, s), 3.29 (3H, s), 3.10 (1H, d), 2.87 (6H, s); ^{13}C NMR (125 MHz, $CDCl_3$): δ 195.9, 179.5, 168.8, 146.9, 144.8, 142.1, 141.9, 138.5, 136.7, 133.1, 132.4, 130.1, 129.9 (×2), 128.5, 110.8, 103.1, 100.1, 71.6, 71.2, 68.9, 59.5, 56.5, 56.4, 41.3, 37.8(×2); HRMS (ESI-TOF) calcd for $C_{31}H_{32}N_4NaO_{11}S$ ($M+Na^+$):691.1680; found: 691.1681.

Compound 4c

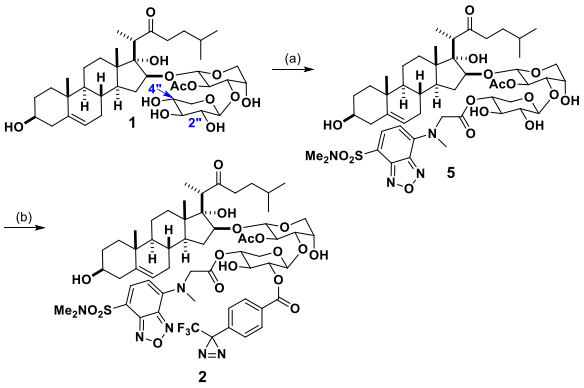


4c

To a solution of compound **3a** (5.50 mg, 12.0 μmol) in THF (48 μL) were added Me_2SnCl_2 (2.63 mg, 12.0 μmol), DIPEA (6.20 mg, 48.0 μmol) and 4-[3-(trifluoromethyl)-3*H*-diazirin-3-yl]benzoic chloride[2] (4.47 mg, 18.0 μmol) stirred at room temperature. After 2 h, the mixture was diluted with EtOAc (5 mL) and was washed with 3% aqueous HCl, water, saturated $NaHCO_3$, then brine. The organic layer was dried with Na_2SO_4, which was filtered and concentrated in vacuo. The residue was purified by flash column chromatography ($CHCl_3$/MeOH = 93/7) then by preparative TLC ($CHCl_3$/MeOH = 93/7) to give compound **4c** (5.63 mg, 8.37 μmol, 70%) as yellow solid: ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.15 (2H, d, *J* = 8.6 Hz), 7.90 (1H, d, *J* = 8.6 Hz), 7.27 (2H, d, *J* = 8.0 Hz), 6.19 (1H, d, *J* = 8.0 Hz), 5.05 (1H, dd, *J* = 4.6, 6.3 Hz), 4.97-4.94 (1H, m), 4.91 (1H, d, *J* = 18.3 Hz), 4.73 (1H, d, *J* = 18.3 Hz), 4.69 (1H, d, *J* = 4.6 Hz), 4.17 (1H, dd, *J* = 4.0, 12.6 Hz), 3.97 (1H, m), 3.58 (1H, dd, *J* = 5.7, 12.6 Hz), 3.50 (3H, s), 3.28 (3H, s), 3.03 (1H, br), 2.88 (6H, s); ^{13}C NMR (125 MHz, $CDCl_3$): δ 168.8, 164.7, 146.9, 144.8, 142.1, 138.5, 134.4, 130.5 (×2), 130.2 (×2), 126.4, 120.8, 117.6, 110.8, 103.2, 100.2, 71.7, 71.5, 69.3, 59.8, 56.5, 56.3, 41.3, 37.8 (×2); HRMS

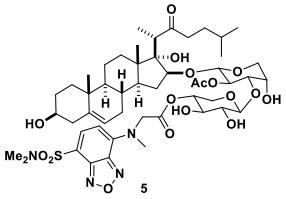
(ESI-TOF) calcd for $C_{26}H_{27}F_3N_6NaO_{10}S$ ($M+Na^+$): 695.1359; found: 693.1358.

5. Synthesis of fluorescent photoaffinity probe of OSW-1 (2)



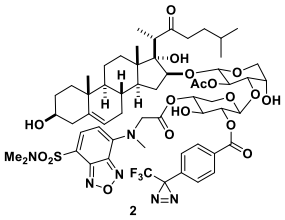
Reagents and conditions: (a) DBD-COCl (1.5 equiv.), Me_2SnCl_2 (1.5 equiv.), collidine (8.0 equiv.), THF (0.1 M), rt, 2h, 48%; (b) 4-[3-(trifluoromethyl)-3*H*-diazirin-3-yl]benzoic chloride (2.0 equiv.), Me_2SnCl_2 (2.0 equiv.), DIPEA (8.0 equiv.), THF (0.1 M), rt, 2h, 50 %.

4”-DBD-deacylated OSW-1 (5)



To a solution of compound **1** (5.87 mg, 7.90 μmol) in THF (79 μL) were added Me_2SnCl_2 (3.47 mg, 15.8 μmol), collidine (7.09 mg, 63.2 μmol) and DBD-COCl (5.26 mg, 15.8 μmol) at room temperature, which was stirred for 2 h. The reaction mixture was diluted in EtOAc (5 mL) and washed with 3% HCl, water, saturated $NaHCO_3$ aqueous solution and brine. The organic layer was dried over Na_2SO_4, filtered and concentrated in vacuo. The residue was purified by flash column chromatography ($CHCl_3$/MeOH = 19/1) to give compound **5** (4.09 mg, 3.95 mol, 50%) as yellow solid: ^{1}H NMR (500 MHz, pyridine-d_5): 8.04 (1H, d, *J* = 8.0 Hz), 6.25 (1H, d, *J* = 8.6 Hz), 5.87-5.84 (1H, m), 5.38 (1H, br), 5.33-5.28 (1H, m), 5.12-4.91 (2H, m), 4.86 (1H, s), 4.63 (1H, d, *J* = 6.9 Hz), 4.40 (1H, s), 4.28-4.16 (5H, m), 3.82-3.73 (2H, m), 3.63-3.59 (2H, m), 3.33-3.28 (1H, m), 3.28 (3H, s), 2.83 (6H, s), 2.83-2.79 (2H, m), 2.61 (2H, d, *J* = 7.45 Hz), 2.39 (3H, s), 2.39-2.33 (1H, m), 2.13-2.02 (2H, m), 2.00-1.88 (3H, m), 1.86-1.76 (2H, m), 1.72-1.43 (8H, m), 1.31 (3H, d, *J* = 7.5 Hz), 1.12-1.07 (1H, br), 1.07 (3H, s), 0.96-0.94 (1H, br), 0.95 (3H, s), 0.93 (3H, s), 0.91 (3H, s) ; ^{13}C NMR (125 MHz, pyridine-d_5): δ 218.9, 167.0, 169.9, 147.7, 145.4, 142.9, 142.0, 139.4, 121.1, 109.6, 106.8, 103.2, 101.4, 88.1, 85.6, 80.7, 74.5, 74.4, 73.9, 72.2, 71.3, 69.0, 67.6, 63.0, 56.5, 50.2, 48.6, 46.6, 46.4, 43.6, 41.2, 39.5, 37.8 (×2), 37.7, 36.9, 35.0, 32.9, 32.8, 32.6, 32.4, 32.1, 27.9, 22.8, 22.6, 21.6, 20.9, 19.6, 13.5, 11.8; HRMS (ESI-TOF) calcd for $C_{50}H_{74}N_4NaO_{17}S$ ($M+Na^+$):1057.46674; found: 1057.46671.

Fluorescent photoaffinity probe of OSW-1 (2)



2

To a solution of compound **5** (5.75 mg, 5.60 μmol) in THF (56 μL) were added Me_2SnCl_2 (2.46 mg, 11.2 mol), DIPEA (5.79 mg, 44.8 μmol) and 4-[3-(trifluoromethyl)-3*H*-diazirin-3-yl]benzoic chloride[4] (2.78 mg, 11.2 μmol) at room temperature, which was stirred for 2 h at room temperature. The reaction mixture was diluted in EtOAc (5 mL) and washed with 3% aqueous HCl, water, saturated $NaHCO_3$ aqueous solution and brine. The organic layer was dried over Na_2SO_4, filtered and concentrated in vacuo. The residue was purified by flash column chromatography ($CHCl_3$/MeOH = 19/1) to give compound **2** (3.49 mg, 2.80 μmol, 50%) as yellow solid. 1: ^{1}H NMR (500 MHz, pyridine-d_5): 8.31 (2H, d, *J* = 8.02 Hz), 8.03 (1H, d, *J* = 8.02 Hz), 7.37 (2H, d, *J* = 8.0 Hz), 6.24 (1H, d, *J* = 8.0 Hz), 5.67-5.62 (1H, m), 5.56-5.51 (1H, m), 5.38-5.32 (2H, m), 5.24 (1H, d, *J* = 6.9 Hz), 5.17 (1H, d, *J* = 18.3 Hz), 4.99 (1H, d, *J* = 18.3 Hz), 4.60 (1H, d, *J* = 5.7 Hz), 4.49-4.41 (2H, m), 4.38 (1H, s), 4.25-4.16 (3H, m), 3.86-3.78 (1H, br), 3.77-3.59 (2H, m), 3.27 (3H, s), 3.23-3.16 (1H, m), 2.84 (2H, d, *J* = 5.2 Hz), 2.82 (6H, s), 2.63-2.57 (3H, m), 2.48-2.22 (2H, m), 2.09-2.04 (2H, m), 1.94 (3H, s), 1.94-1.84 (2H, br), 1.82-1.72 (2H, m), 1.65 (1H, d, *J* = 12.6 Hz), 1.61-1.41 (6H, m), 1.28 (3H, d, *J* = 7.5 Hz), 1.10-1.04 (1H, br), 1.04 (3H, s), 0.97 (3H, s), 0.97-0.92 (1H, br), 0.85 (3H, d, *J* = 6.2 Hz), 0.83 (3H, d, *J* = 5.8 Hz) ; ^{13}C NMR (125 MHz, pyridine-d_5): δ 218.7, 169.7, 169.2, 164.6, 147.7, 145.3, 142.7, 141.9, 139.4, 130.8, 129.4, 128.7, 126.8, 125.8, 121.0, 109.5, 103.3, 102.2, 100.7, 88.7, 85.6, 83.4, 80.0, 75.7, 75.1, 73.2, 71.8, 71.5, 71.4, 71.3, 71.0, 62.2, 56.5, 50.9, 48.5, 46.5, 46.4, 43.5, 41.2, 39.3, 37.8 (×3), 36.8, 34.2, 32.7, 32.6, 32.2, 32.0, 27.8, 22.9, 22.7, 22.4, 21.3, 20.9, 20.8, 19.6, 13.6, 11.8; ^{19}F NMR (282 MHz, $CDCl_3$): δ -66.06; HRMS (ESI-TOF) calcd for $C_{59}H_{77}F_3N_6NaO_{18}S$ ($M+Na^+$):1269.48649; found: 1269.48652.

6. Isolation of deacylated OSW-1 (1) from *Ornithogalum saundersiae*

Deacylated OSW-1 (**1**) was isolated from the roots of *Ornithogalum saundersiae* based on the previously published protocol.[3] The fresh roots were diced and lyophilized (204 g) which were washed with hexane then were refluxed with methanol (400 mL) for 1 h. After cooling to ambient temperature, the pulps were filtered. The filtered residues were subjected to two additional rounds of methanolic extraction. The combined methanolic extract was concentrated *in vacuo* and was diluted in 80 % MeOH/H_2O solution. The aqueous methanol layer was washed with hexane (× 2) and CH_2Cl_2 (× 3). The CH_2Cl_2 layer was collected and was reduced in volume *in vacuo*, which was washed with brine (× 2). The CH_2Cl_2 layer was dried over Na_2SO_4 and was concentrated *in vacuo*. The crude fraction (8 g) was purified by silica gel chromatography (0%→2%→3%→4%→5%→10%→20%→50% MeOH/$CHCl_3$) twice and the fraction eluted with 4−5% MeOH/$CHCl_3$ was collected at each round. The crude sample was then purified by ODS chromatography on Biotage Isolera One system (25−50% MeCN/H_2O gradient) to collect the fraction eluted 36−39%

MeCN/H_2O gradient. The collected fraction was further purified by an analytical scale reversed-phase HPLC using a 4.6 × 25.0 mm (5 μm) Waters Xbridge ODS column at a flow rate of 1 mL/ min (45−51% MeCN/H_2O gradient) with UV detection at 192 nm to give compounds **1** (9 mg): ^{1}H NMR (500 MHz, pyridine-d_5): 5.85 (1H, t, *J* = 7.0 Hz), 5.38 (1H, d, *J* = 4.0 Hz), 4.90 (1H, d, *J* = 7.5 Hz), 4.86 (1H, s), 4.63 (1H, d, *J* = 6.0 Hz), 4.45 (1H, m), 4.33 (1H, dd, *J* = 14.5 Hz, *J* = 8.0 Hz), 4.27-4.20 (3H, m), 4.14 (1H, m), 4.08 (1H, t, *J* = 8.5 Hz), 3.86-3.76 (3H, m), 3.69 (1H, t, *J* = 10.5 Hz), 3.32 (1H, dd, *J* = 7.5 Hz, *J* = 14.5 Hz), 2.82 (2H, t, *J* = 7.5 Hz), 2.61 (2H, d, *J* = 7.5 Hz), 2.38 (1H, m,), 2.36 (3H, s), 2.13-2.04 (2H, m), 2.00-1.87 (2H, m), 1.84-1.76 (2H, m), 1.73-1.40 (8H, m), 1.31 (3H, d, *J* = 7.5 Hz), 1.24 (1H, br), 1.11 (1H, m), 1.07 (3H, s), 0.98 (1H, br), 0.95 (3H, s), 0.93 (3H, d, *J* = 6.0 Hz), 0.91 (3H, d, *J* = 6.5 Hz); ^{13}C NMR (125 MHz, pyridine-d_5): δ 219.0, 170.0, 141.9, 121.1, 106.9, 101.4, 88.2, 85.7, 80.2, 78.3, 74.2, 72.2, 71.3, 70.9, 68.8, 67.2, 66.7, 50.1, 48.6, 46.5, 46.4, 43.5, 39.5, 37.7, 36.8, 35.1, 32.8, 32.7, 32.6, 32.2, 32.0, 27.9, 22.8, 22.5, 21.5, 20.9, 19.6, 13.5, 11.9; HRMS (ESI-TOF) calcd for $C_{39}H_{62}NaO_{13}$ ($M+Na^+$):761.40881; found: 761.40605.

7. Fluorescence spectroscopic analysis of fluorescent photoaffinity probe 2

Fluorescence spectra were recorded for **2** prepared as 1 μM solution in PBS. The excitation spectra or emission spectra for an appropriate spectral window were recorded on JASCO ETC 273 spectrophotometer (JASCO) using the excitation scan or emission scan mode. Fluorescence excitation was detected at 555 nm and emission spectra was excited at 445 nm.

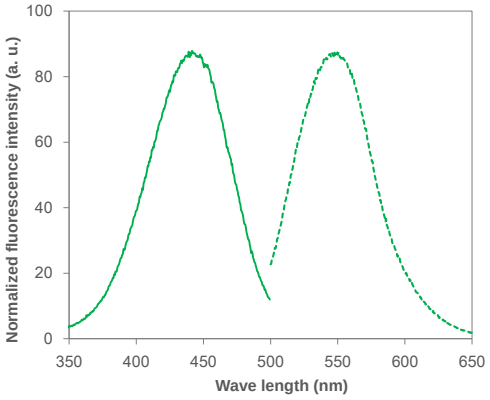


Figure S1. Fluorescence excitation (single line) and emission spectra (dashed line) of **2** at 1 μM in PBS.

8. Cell cultures

HeLa cells (RIKEN bioresource center) were cultured in DMEM (Gibco) supplemented with 10% FBS 100 U/mL penicillin and 100 μg/mL streptomycin sulfate in a humidified 5% CO_2 atmosphere at 37 °C.

9. XTT Assay

HeLa cells were trypsinized and suspended in culture media (DMEM supplemented with 10% FBS 100 U/mL penicillin and 100 μg/mL streptomycin sulfate) at a final concentration of 1×10^4 cells/mL, which were seeded in a

96-well microtiter plate. Varied concentrations of compounds (OSW-1, **1**, **2**, **5**, **7**[1]) or DMSO were added to each well in triplicate, and then were incubated for 72 h. They were incubated with 2 mg/mL XTT (Sigma-Aldrich) in culture media for 4 h. The amounts of viable cells were determined by measuring the UV absorbance at 490 nm on Micro Plate Reader Model 550 (Biorad). The IC_{50} values were obtained by non-linear regression curve-fitting to a Hill's equation using the Prism program (GraphPad).

10. Fluorescence cell imaging studies

HeLa cells were seeded in a glass-bottom dish (IWAKI) at 3×10^5 cells/mL in DMEM and were cultured for 20 h. For mitochondria staining, cells were treated with DBD-tagged probes **2** or **7** (1 μM) at 37 ℃ for 1 h and with tetramethyl rhodamine ethyl ester (TMRE, 25 nM) for further 10 min and then were imaged live. For ER staining, cells were preincubated with 0.25 μM ER-Tracker™ Red (BODIPY® TR-glibenclamide, Thermo Fisher Scientific) in HBSS buffer (0.34 mM Na_2HPO_4, 0.44 mM $KHPO_4$, pH 7.2, 137.9 mM NaCl, 1.26 mM $CaCl_2$, 0.49 mM $MgCl_2$, 0.41 mM $MgSO_4$, 5.33 mM KCl, 4.17 mM $NaHCO_3$;Gibco) at 37 ℃ for 1 h then treated with DBD-probe **2** or **7** (0.5 μM) at 37 ℃ for 1.5 h for **2** or 1 h for **7**, washed with chilled PBS, fixed with 4% paraformaldehyde and were analyzed. For Golgi staining, cells were preincubated with 3 μM BODIPY® TR-ceramide (Thermo Fisher Scientific) in HBSS buffer at 17 ℃ for 30 min, washed with HBSS buffer then were incubated with DBD-probe **2** (1 μM) or **7** (0.5 μM) at 37 ℃ for 30 min and after washing with chilled PBS were imaged live. For lysosome staining, cells were preincubated with 100 nM Lysotracker® Red (Thermo Fisher Scientific) in DMEM at 37 ℃ for 1 h, washed with DMEM, then treated with DBD-probe **2** or **7** (0.5 μM) at 37 ℃ for 1.5 h, washed with chilled PBS, fixed with 4% paraformaldehyde and were analyzed. For fluorescence microscopic imaging, the glass-bottom dish was placed on the stage of an inverted epifluorescence microscope (IX-70; Olympus) equipped with a 40×objective lens (Uapo 40×3/340, NA =0.9; Olympus). Fluorescence was elicited by illumination with a 75 W xenonlamp through a 15nm band-pass filter centered at 535nm. Fluorescence at >580nm was collected with a cooled CCD camera (Sensicam QE (6.45 μm /pixel at 1×), PCO AG, Kelheim, Germany). For DBD fluorescence, excitation between 450 and 480nm and emission between 515 and 550 nm were used. The images were acquired with binning pixels 2×2. The exposure time for each frame was 1 s. The intensity of illumination was also reduced to 25% with a neutral density filter to avoid photodynamic injury to cells. All procedures described above were performed at room temperature. The image data with the resolution of 12 bits per pixel were analyzed with Image J.

(a) (b)

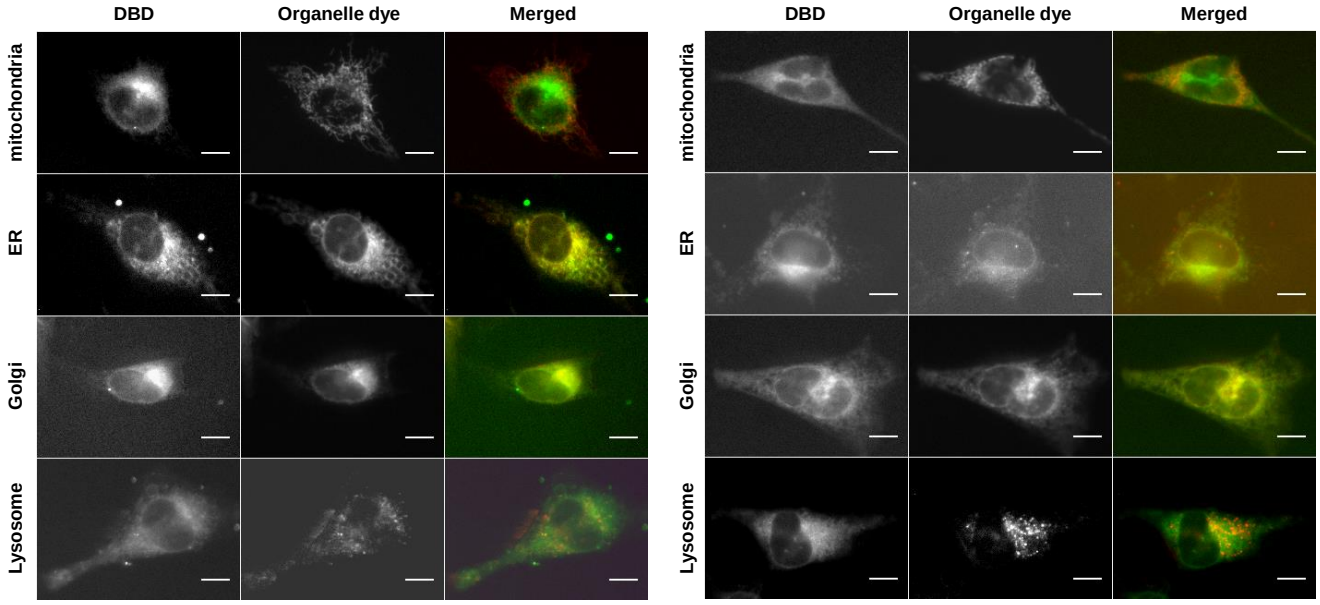


Figure S2. Intracellular localization analysis of (a) fluorescent OSW-1 photoaffinity probe **2** and (b) 4”-DBD-tagged OSW-1 **7** in HeLa cells by fluorescent microscopy. Cells were treated with the DBD-probe (1 μM) and TMRE as a mitochondria marker, ER tracker as an ER marker or BODIPY TR-ceramide as a Golgi marker or Lysotracker Red as a lysosome marker. In merged images, fluorescence of organelle specific stains are shown in red and that of DBD are shown in green. Scale bars: 10 μm.

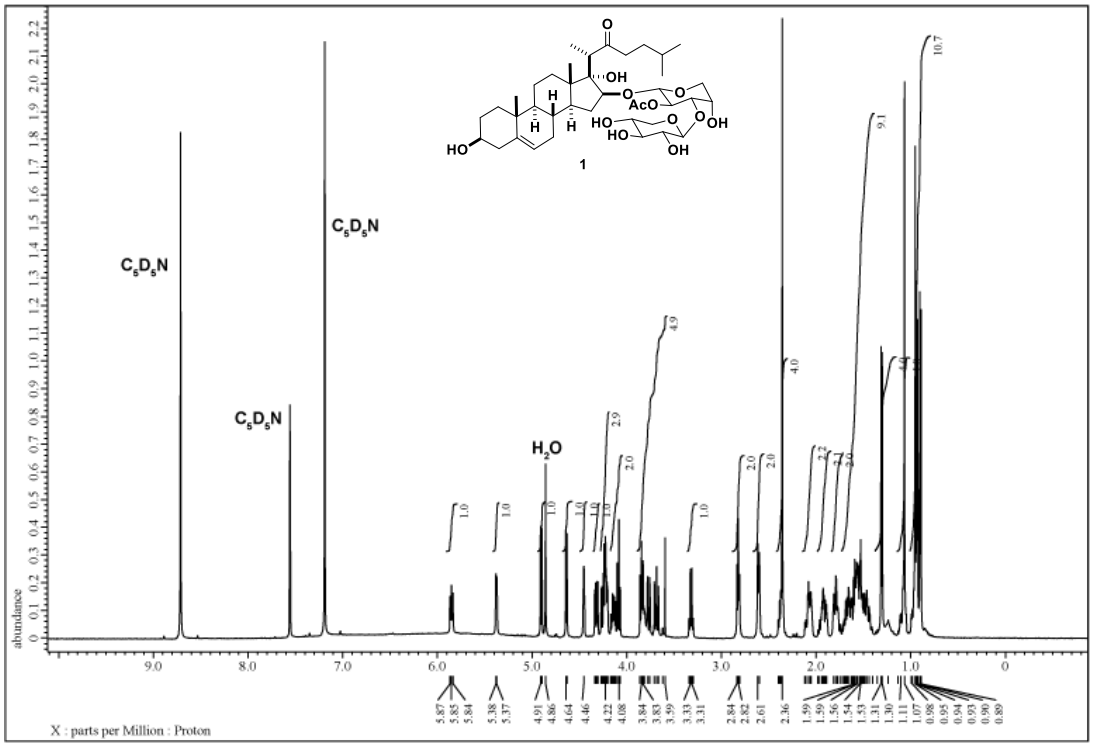
References

1. K. Sakurai, T. Takeshita, M. Hiraizumi, R. Yamada, *Org. Lett.* 2014, **16**, 6318.

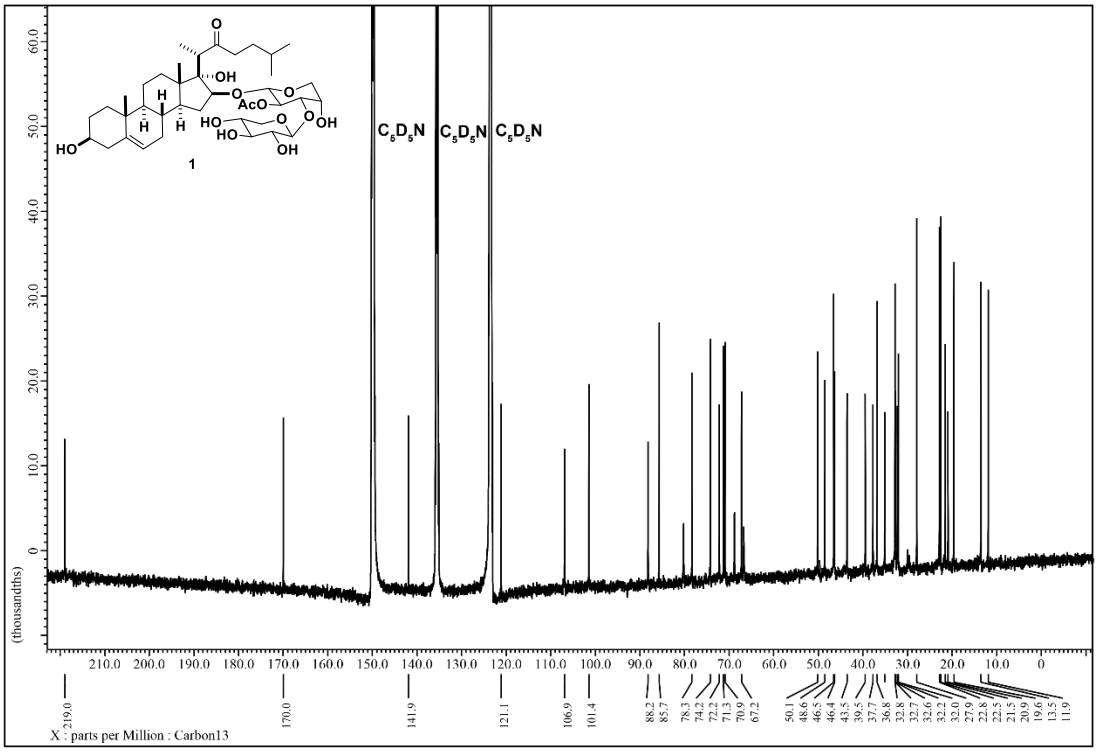
2. K. Sakurai, Y. Yasui, S. Mizuno, *Asian. J. Org. Chem.* 2015, ***4***, 724.

3. K. Sakurai, T. Fukumoto, K. Noguchi, N Sato, H. Asaka, N. Moriyama M. Yohda., *Org. Lett.* 2010, **12**, 5732.

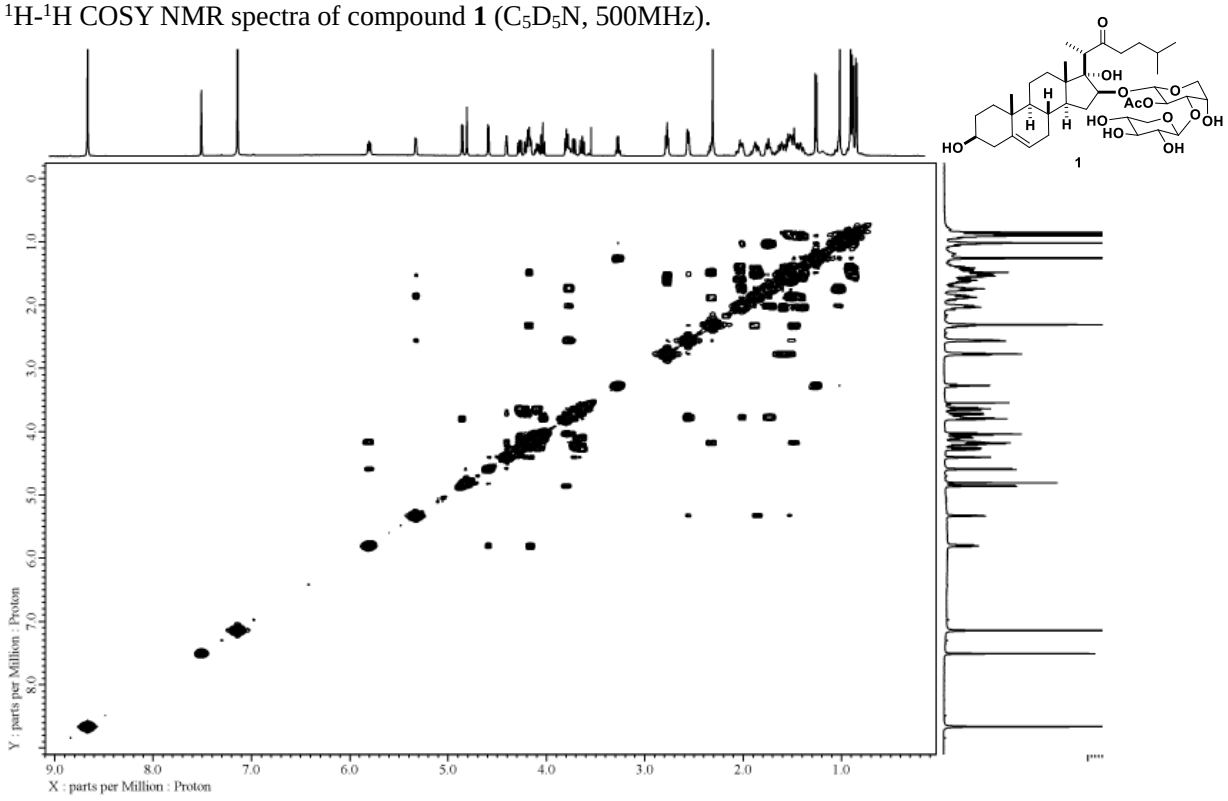
^{1}H NMR spectra of compound **1** (C_5D_5N, 300MHz).



^{13}C NMR spectra of compound **1** (C_5D_5N, 75MHz).

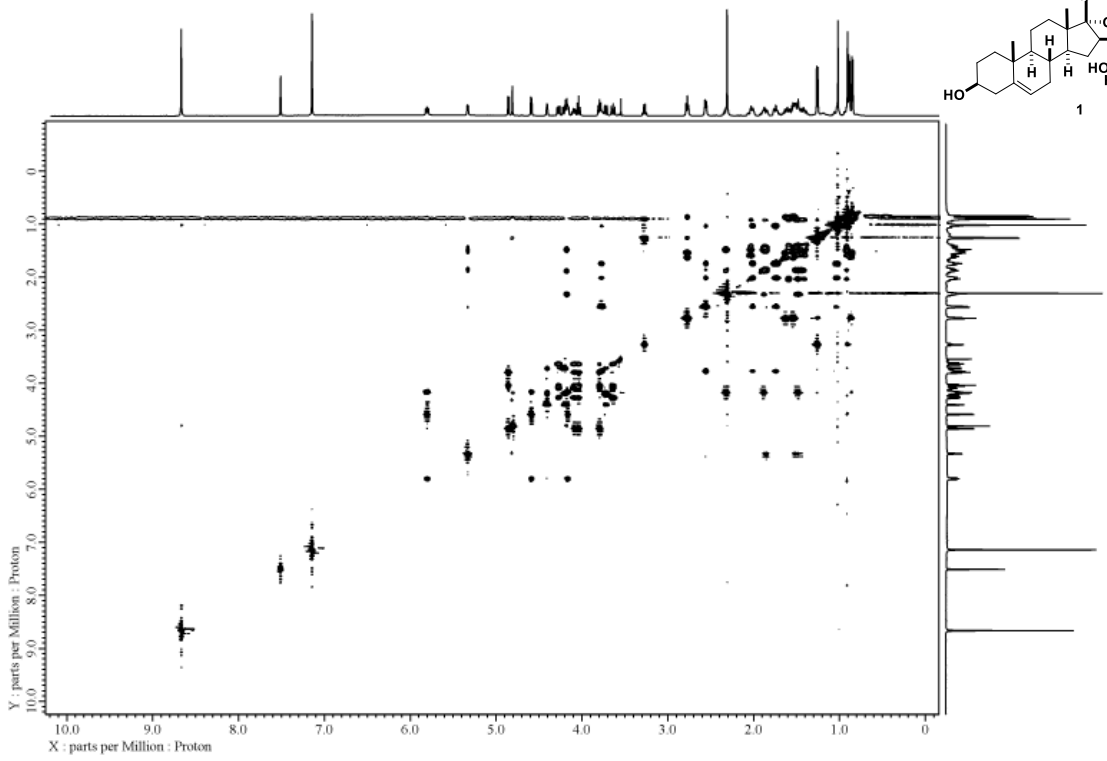


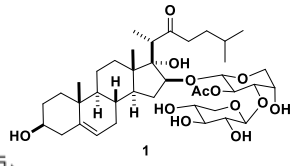
^{1}H-^{1}H COSY NMR spectra of compound **1** (C_5D_5N, 500MHz).



1

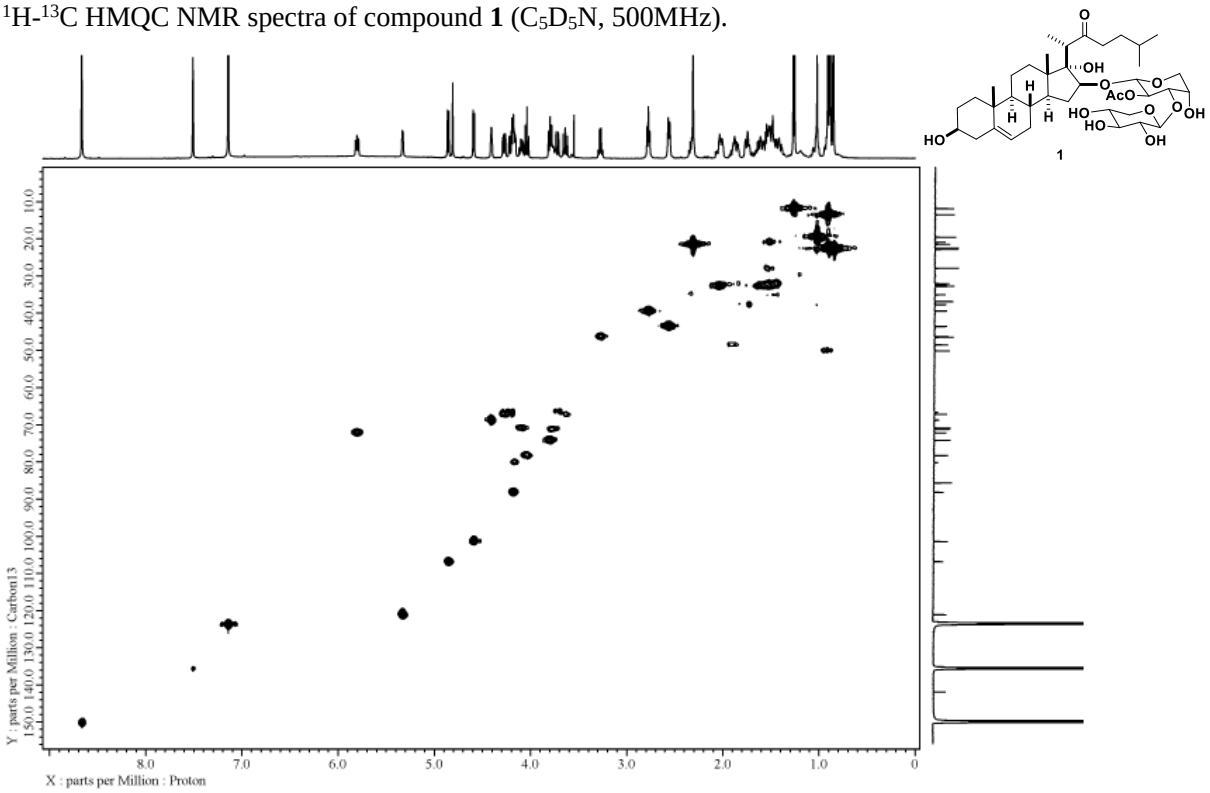
^{1}H-^{1}H TOCSY NMR spectra of compound **1** (C_5D_5N, 500MHz).



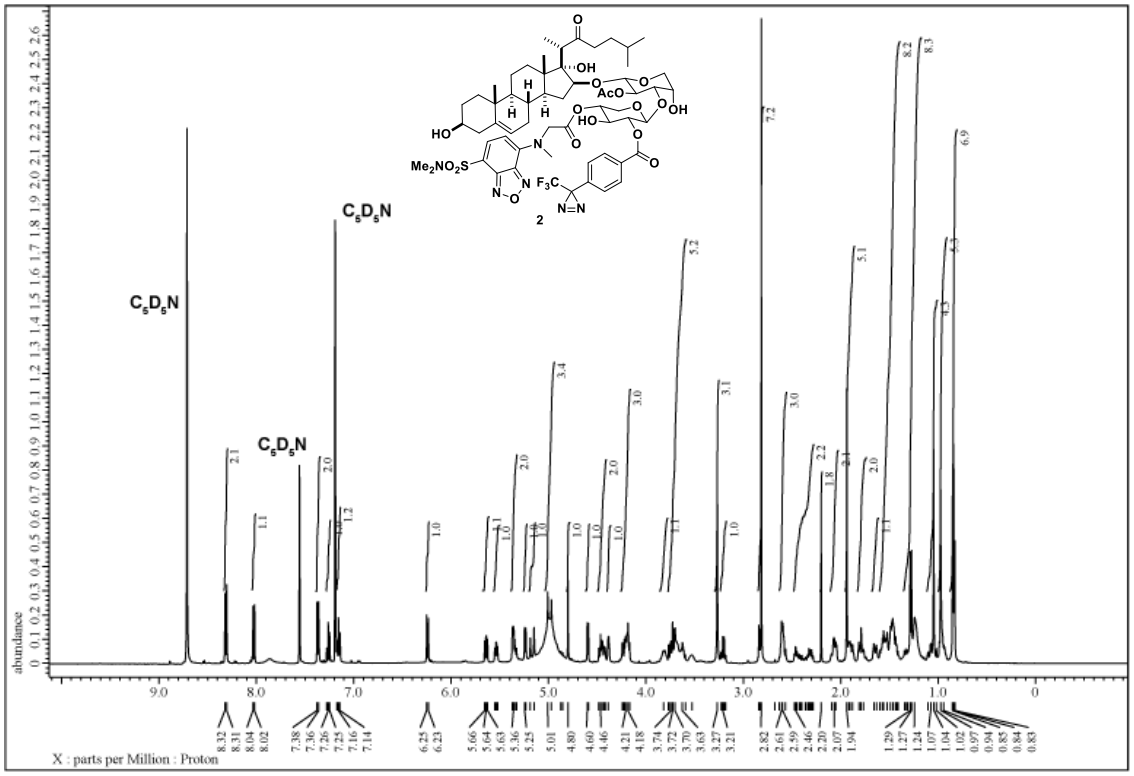


1

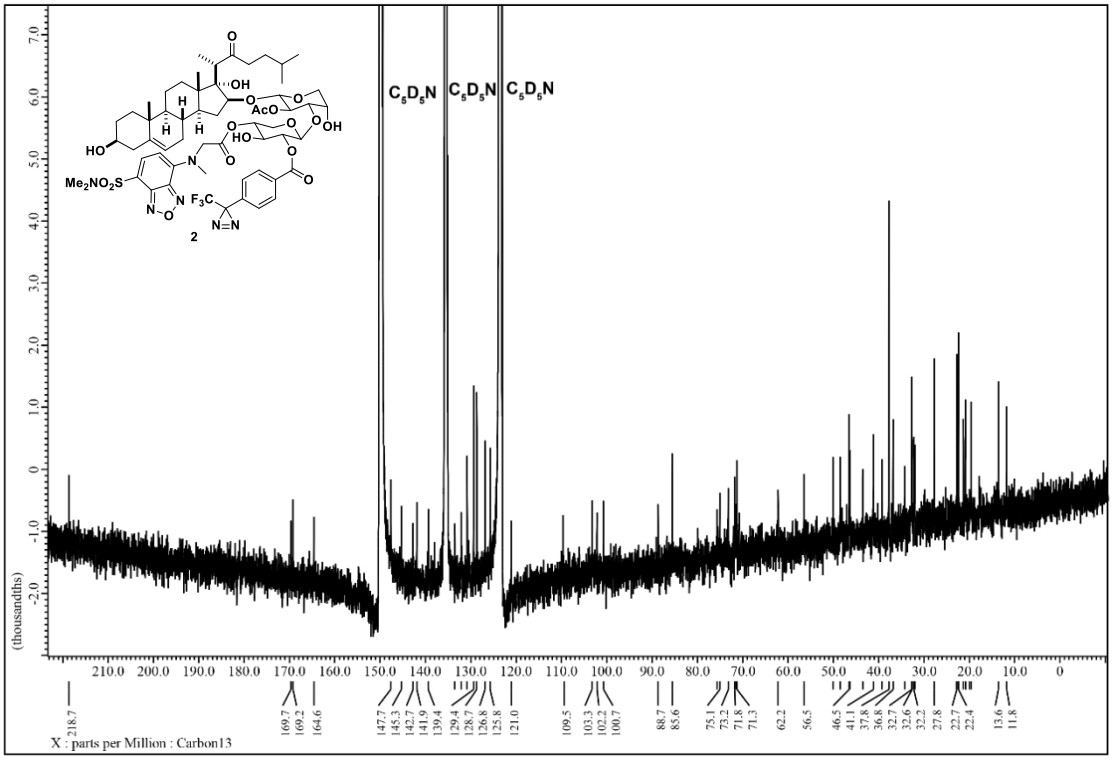
^{1}H-^{13}C HMQC NMR spectra of compound **1** (C_5D_5N, 500MHz).



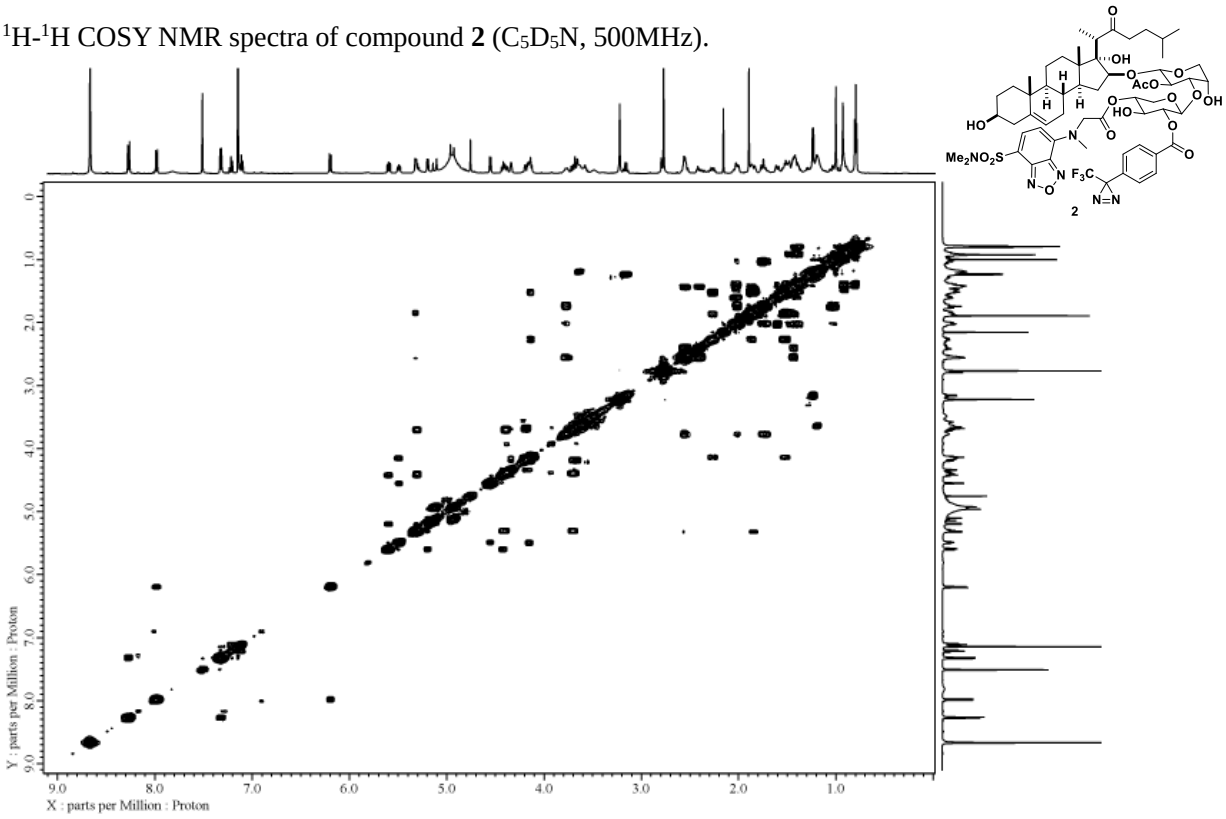
^{1}H NMR spectra of compound **2** (C_5D_5N, 500MHz).



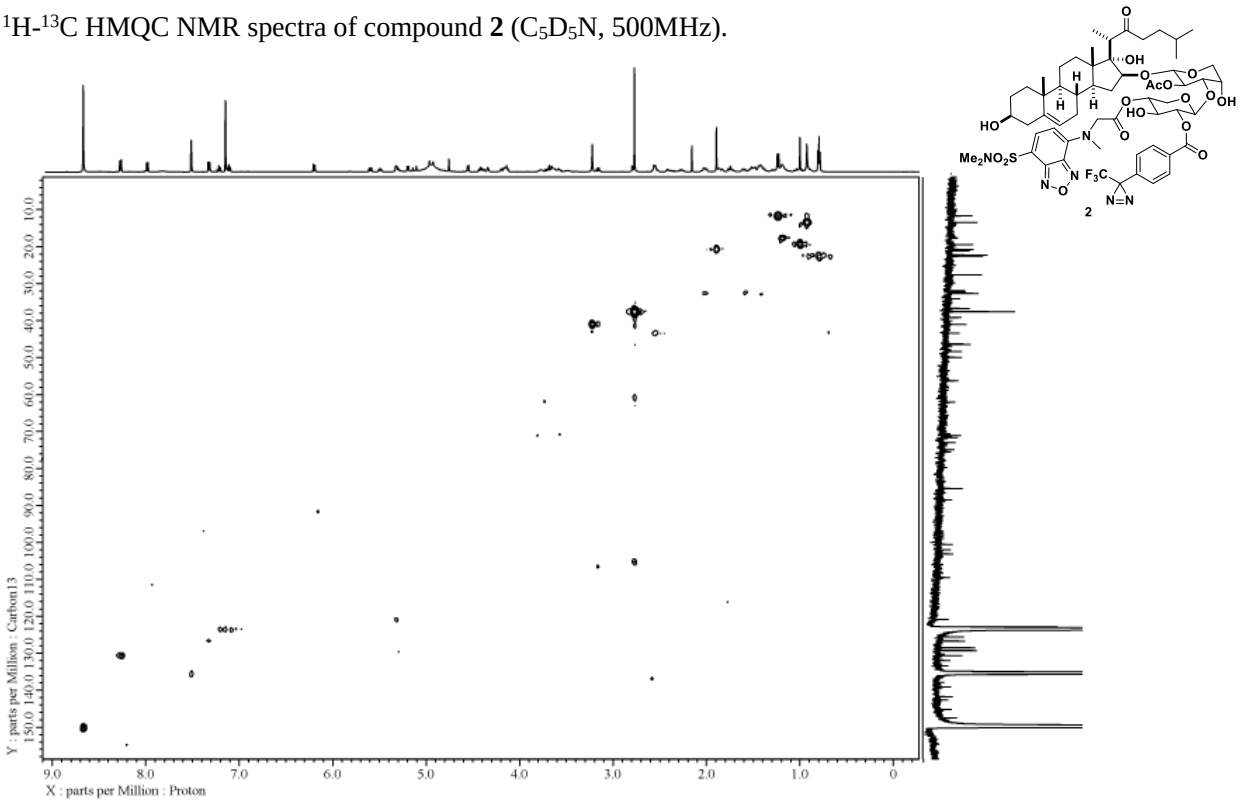
^{13}C NMR spectra of compound **2** (C_5D_5N, 125MHz).



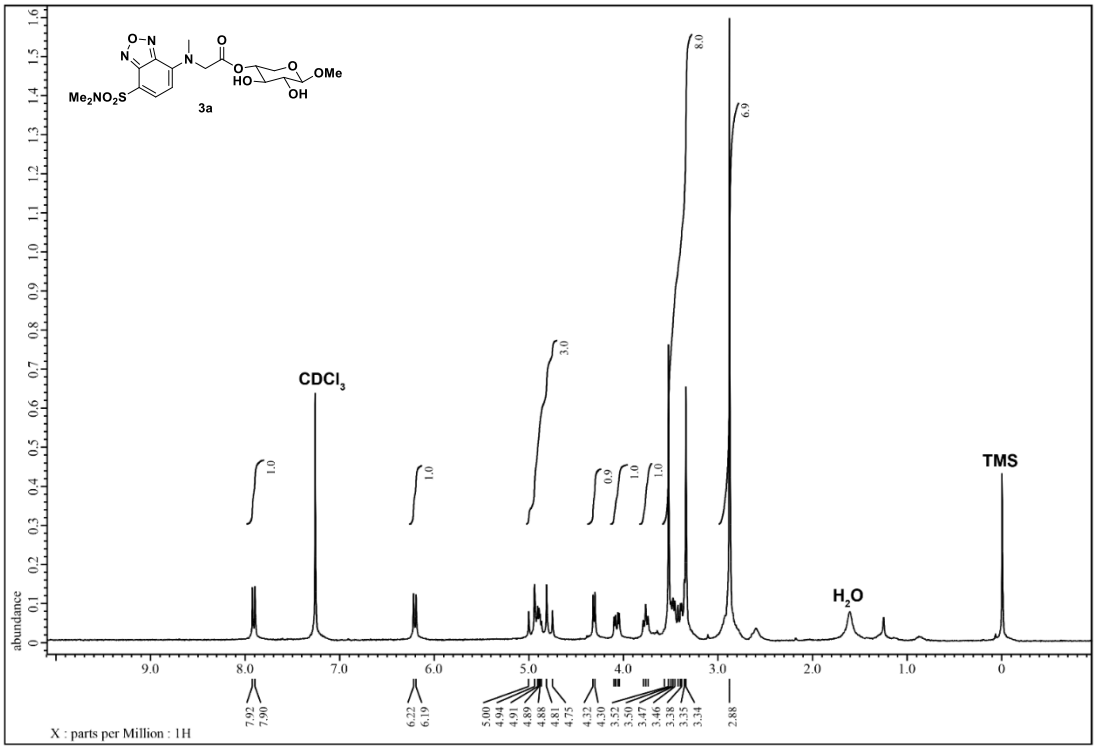
^{1}H-^{1}H COSY NMR spectra of compound **2** (C_5D_5N, 500MHz).



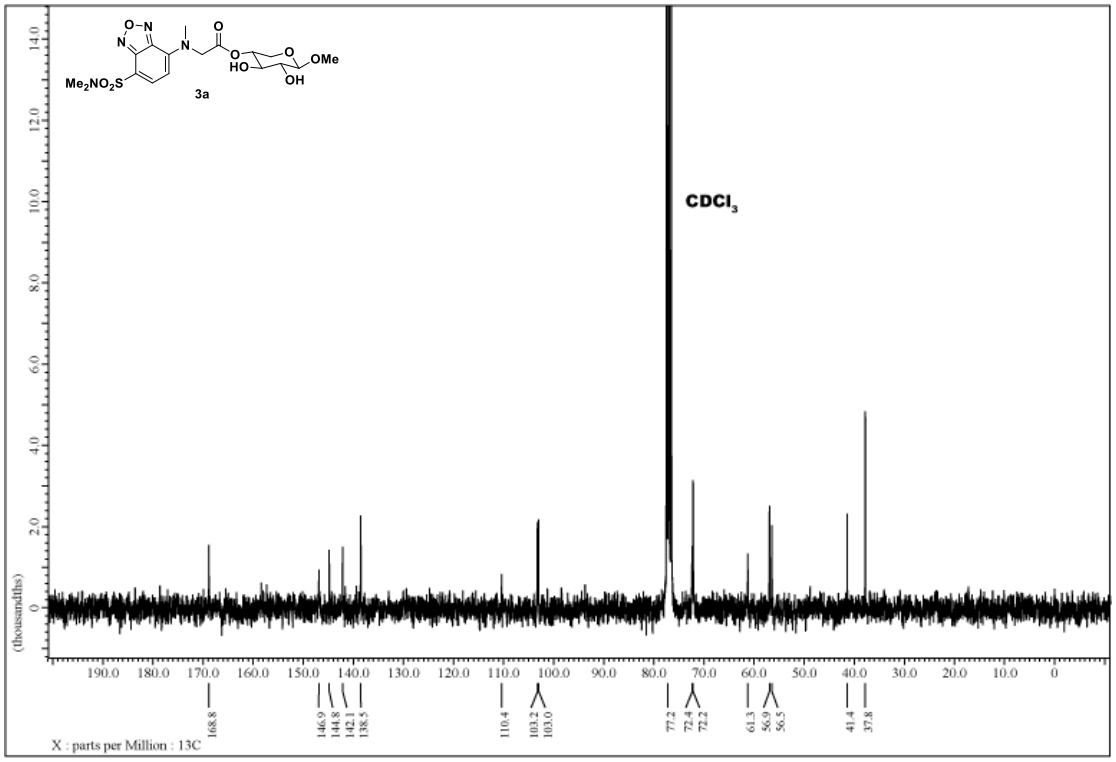
^{1}H-^{13}C HMQC NMR spectra of compound **2** (C_5D_5N, 500MHz).



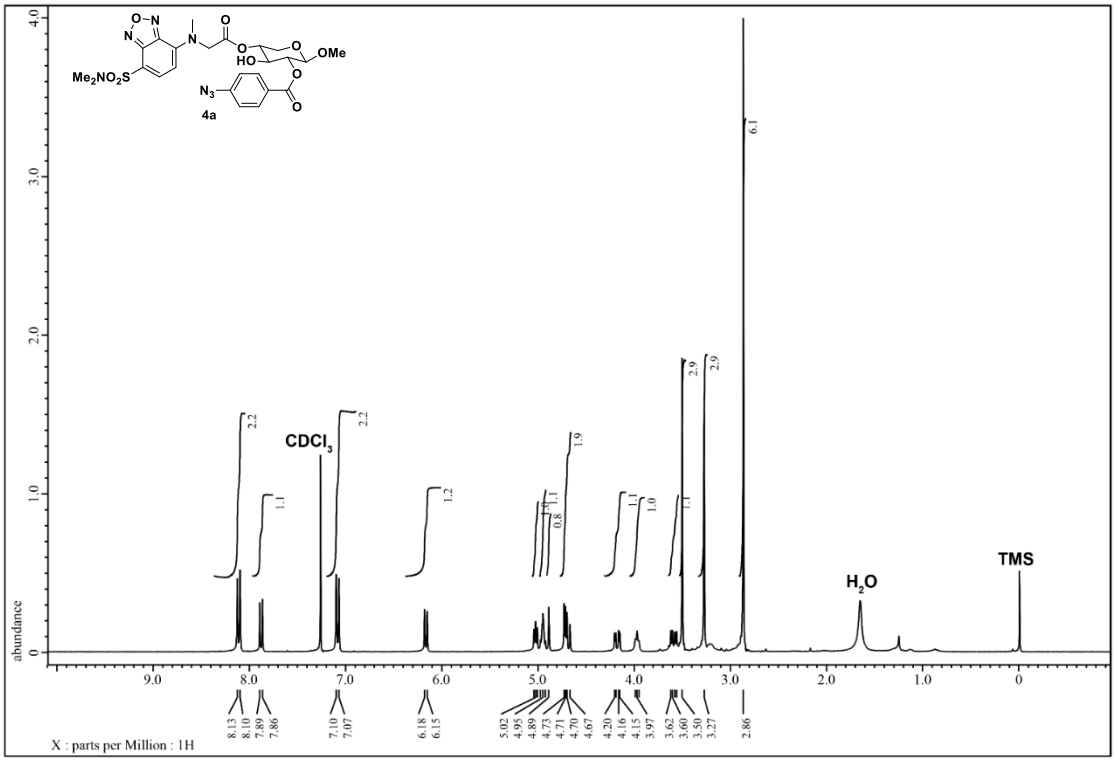
^{1}H NMR spectra of compound **3a** ($CDCl_3$, 500MHz).



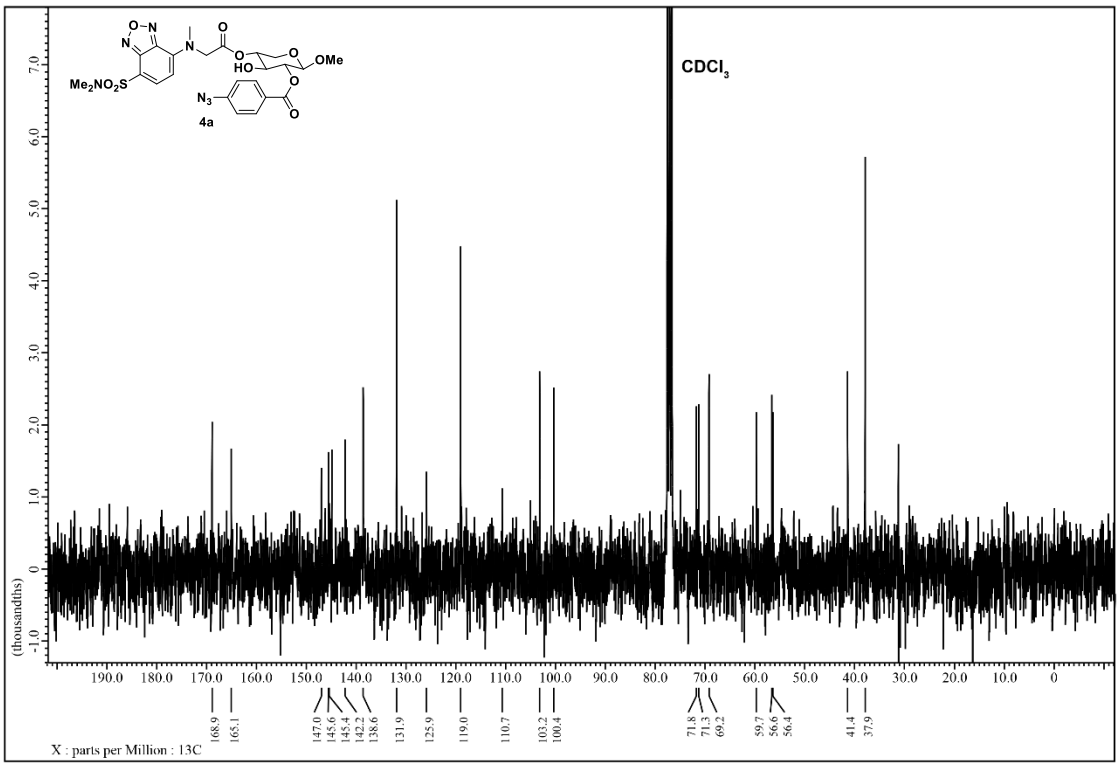
^{13}C NMR spectra of compound **3a** ($CDCl_3$, 125MHz).



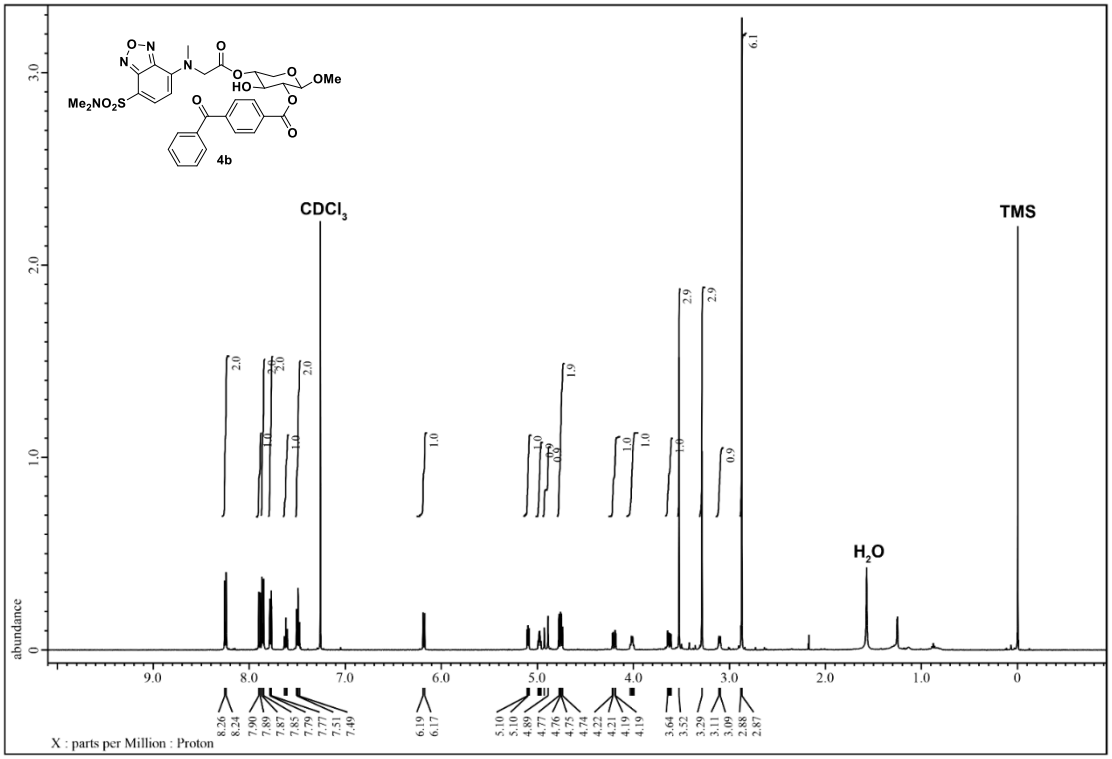
^{1}H NMR spectra of compound **4a** ($CDCl_3$, 300MHz).



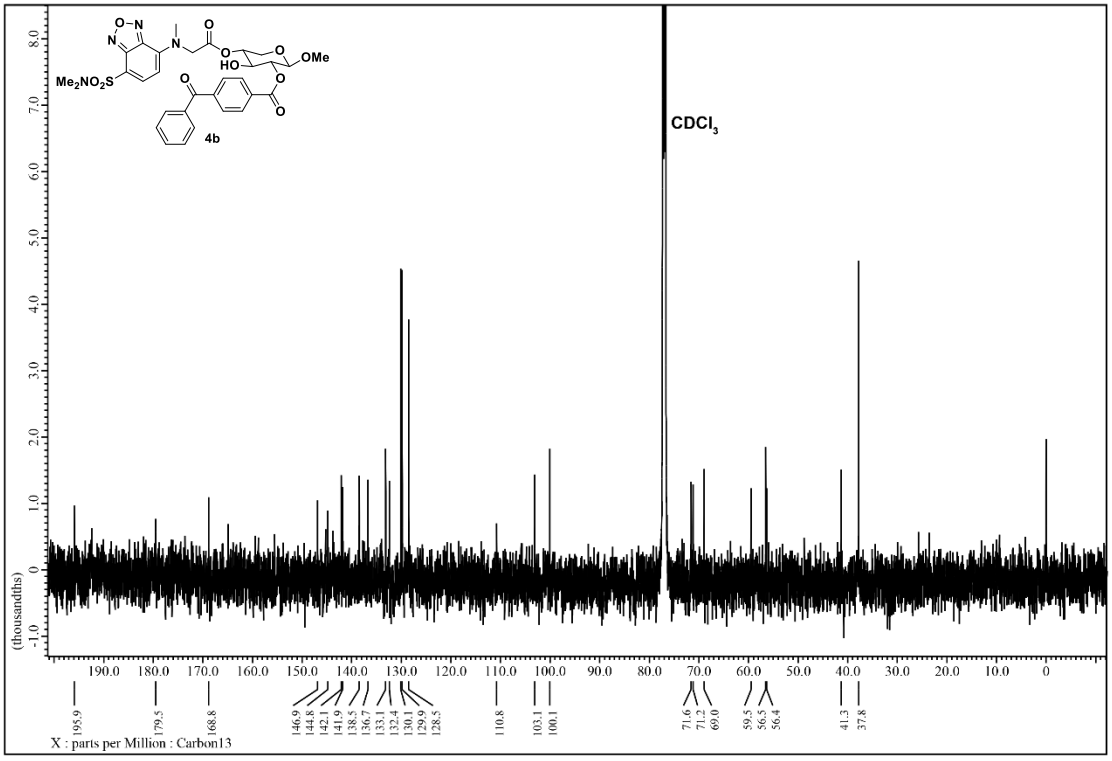
^{13}C NMR spectra of compound **4a** ($CDCl_3$, 75MHz).



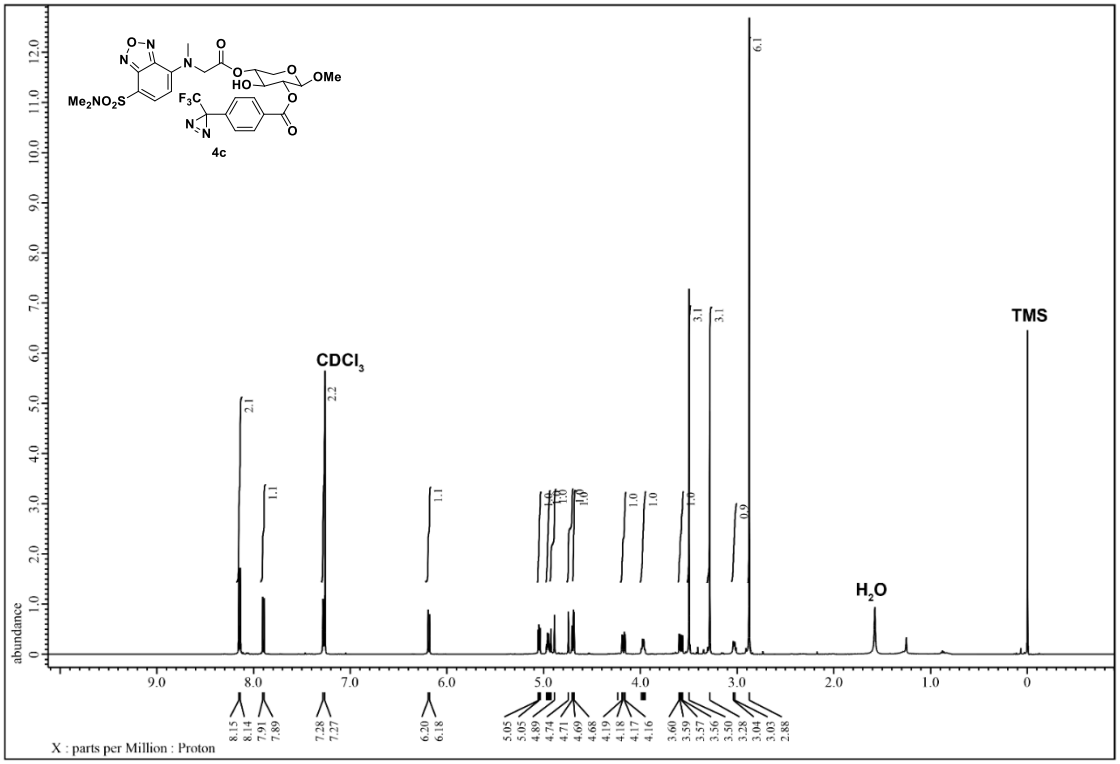
^{1}H NMR spectra of compound **4b** ($CDCl_3$, 500MHz).



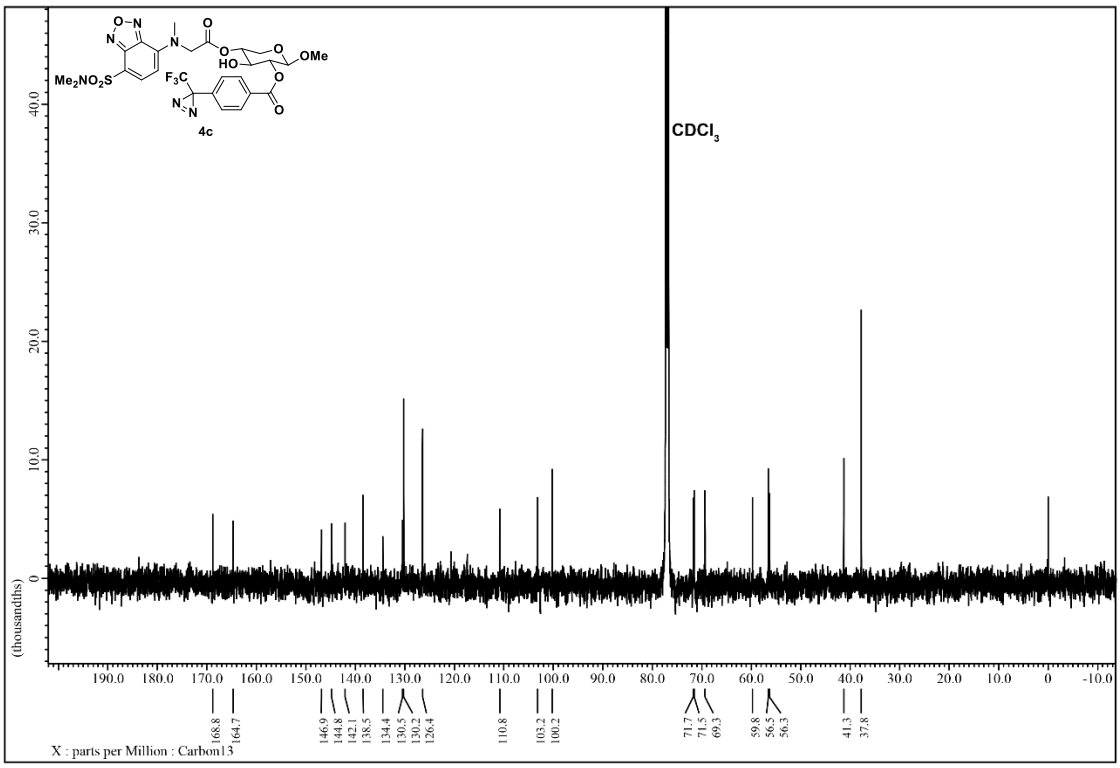
^{13}C NMR spectra of compound **4b** ($CDCl_3$, 125MHz).



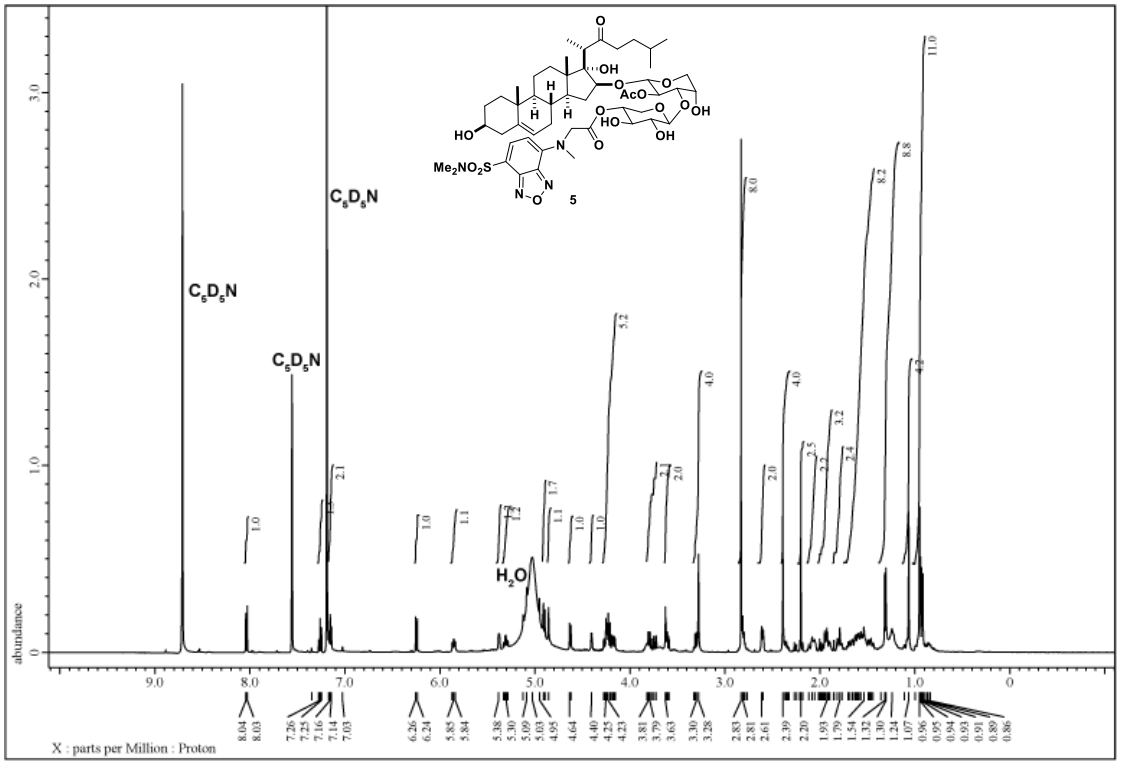
^{1}H NMR spectra of compound **4c** ($CDCl_3$, 500MHz).



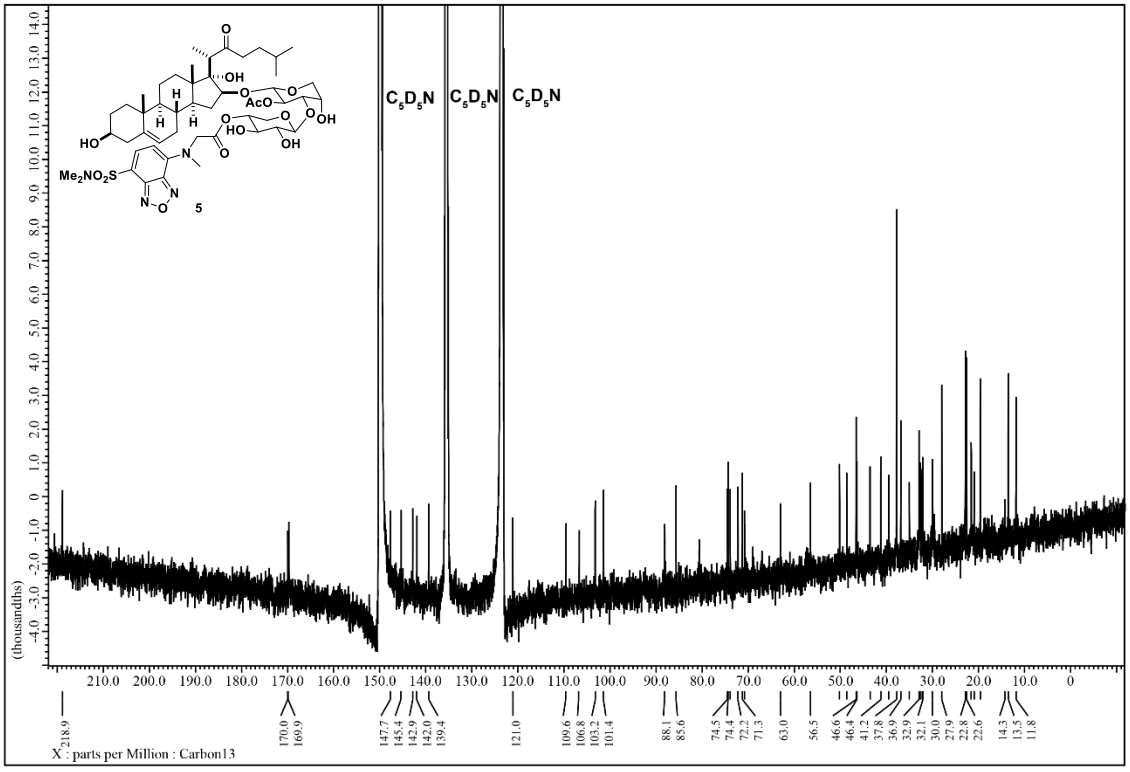
^{13}C NMR spectra of compound **4c** ($CDCl_3$, 125MHz).



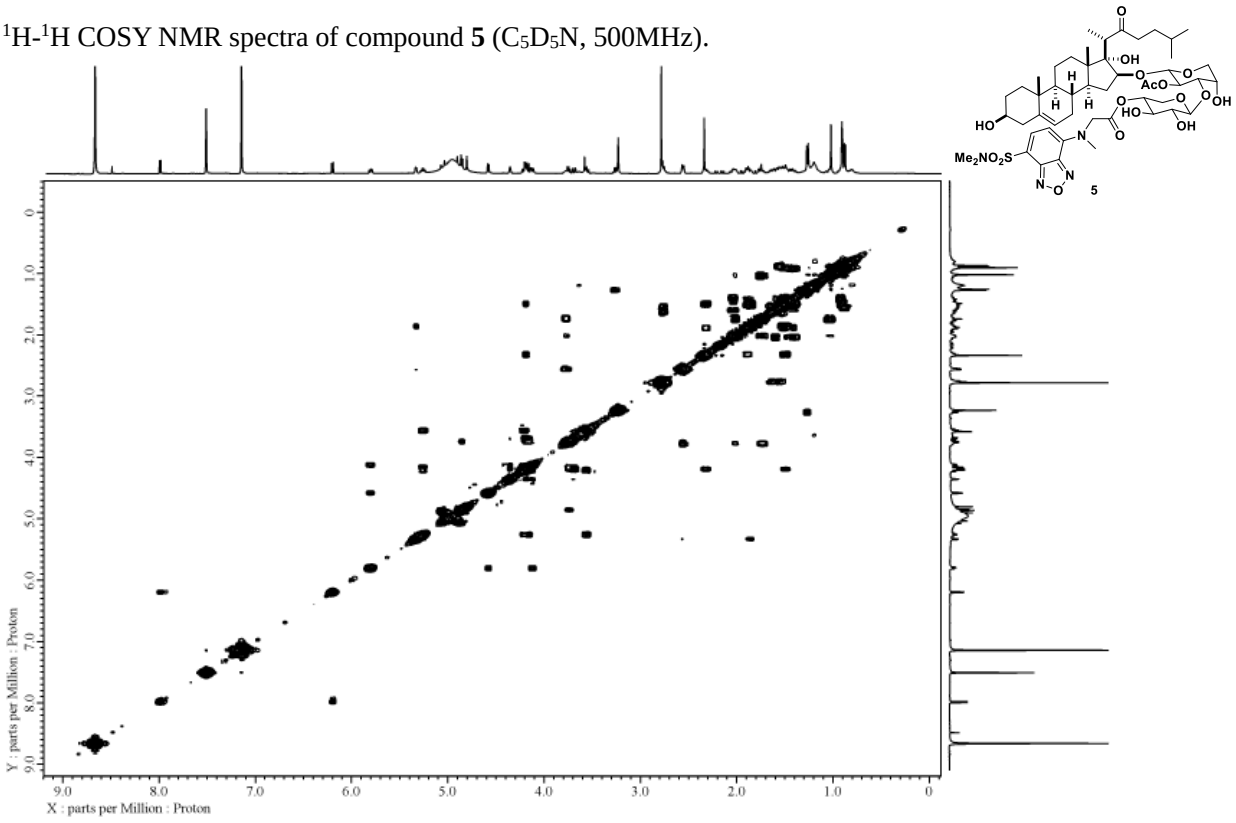
^{1}H NMR spectra of compound **5** (C_5D_5N, 500MHz).



^{13}C NMR spectra of compound **5** (C_5D_5N, 125MHz).



^{1}H-^{1}H COSY NMR spectra of compound **5** (C_5D_5N, 500MHz).



^{1}H-^{13}C HMQC NMR spectra of compound **5** (C_5D_5N, 500MHz).