Colchicine Glycorandomization Influences Cytotoxicity and Mechanism of Action

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General Methods. Proton nuclear magnetic resonance (^{1}H and ^{13}C NMR) spectra were recorded in deuterated solvents on Varian Unity-Inova 400 MHz or 500 MHz spectrometers. Chemical shifts are reported in parts per million (ppm, δ) relative to tetramethylsilane (0.00) for *d*-chloroform, or the residual protic solvent peak for other solvents. ^{1}H NMR splitting patterns with observed first order coupling are designated as singlet (s), doublet (d), triplet (t), or quartet (q). Splitting patterns that could not be interpreted or easily visualized are designated as multiplet (m), broad (br), or apparent (a). Mass spectra (MS) were obtained with the Agilent 1100 HPLC-MSD SL Ion Trap Mass Spectrometer using electrospray ionization. High resolution mass spectrometry data for new compounds in Table 1 were obtained at the University of Wisconsin Biotechnology Center Mass Spectrometry Facility and are provided in Table S5. Commercially available reagents and solvents were used without further purification. Analytical thin layer chromatography (TLC) was carried out on TLC plates pre-coated with silica gel 60 (250 µm layer thickness). Visualization was accomplished using either a UV lamp or potassium permanganate stain (2 g $KMnO_4$, 13.3 g K_2CO_3, 2 mL 2M NaOH, 200 mL H_2O). Flash column chromatography was performed on 40-60 µm silica gel (230-400 mesh). Solvent mixtures used for TLC and flash column chromatography are reported in v/v ratios.

Benzyl (2-methoxyimino)acetate (3): To a stirred solution of glyoxylic acid monohydrate (75.1 g, 0.82 mol) in pyridine/MeOH (1:1, 1.4 L) under argon was added methoxylamine hydrochloride salt (75.0 g, 0.90 mol). The reaction mixture was stirred at room temperature for one hour and quenched with saturated aqueous NH_4Cl solution. The aqueous layer was extracted with EtOAc (3x, 700 mL), the organic layers were combined and dried over Na_2SO_4 and concentrated. Purification of the crude material by flash chromatography (8:1 EtOAc/MeOH) furnished methoxyimino-acetic acid as a colorless solid in 83.3 g, 99% yield. R_f = 0.57 (50% $CHCl_3$/MeOH); ^{1}H δ (CD_3OD): 7.45 (s, 1H), 3.98 (s, 3H); ^{13}C δ (CD_3OD): 163.8, 141.1, 62.5; Electrospray ionization-MS *m/z* [M–H]$^-$ calculated for $C_3H_4NO_3$, 102.0; observed 102.0.

To methoxyimino-acetic acid (21.0 g, 202.3 mmol) in DMF (65 mL) was added $NaHCO_3$ (68.0 g, 0.81 mol) and BnBr (96.1 mL, 0.81 mol). The reaction mixture was allowed to stir at room temperature for one hour and at 70 °C for 16 h. After the reaction was complete (based upon TLC), the mixture was cooled to 0 °C and quenched with dIH_2O. The aqueous layer was extracted with EtOAc (3x, 500 mL), and the combined organic layers were washed with brine (2x, 500 mL), dried over Na_2SO_4 and filtered The solvent was removed under reduced pressure and the crude product was purified by flash chromatography (5% EtOAc/Hexanes) to furnish benzyl (2-methoxyimino) acetate **3** (31.2 g, 80%) as colorless oil. R_f = 0.61 (20% EtOAc/Hexanes); ^{1}H δ ($CDCl_3$): 7.52 (s, 1H), 7.40 (m, 5H), 5.29 (s, 2H), 4.05 (s, 3H); ^{13}C δ161.5, 140.3, 135.0, 128.4, 128.36, 128.34, 66.9, 63.3; Electrospray ionization-MS *m/z* [M + Na]$^-$ calculated for $C_{10}H_{11}NO_3Na$, 216.0; observed 216.1.

Benzyl [*N*-(tert-Butoxycarbonyl)-*N*-methoxy-amino]acetate (4): To a solution of benzyl (2-methoxyimino) acetate (8.5 g, 43.8 mmol) in EtOH (5 mL) at 0 °C under argon atmosphere was added borane-pyridine complex (8.3 mL, 66.1 mmol, 8 M solution in pyridine). To this solution, 6 M HCl in ethanol (65 mL) was added in a dropwise fashion over a period of 3 h. The reaction mixture was stirred overnight at room temperature and neutralized (pH = 7) via drop wise addition of saturated aqueous NaOH solution at 0 °C. After removal of the solvent *in vacuo,* the crude residue was partitioned between water/CH_2Cl_2 (1:1, 400 mL). The aqueous layer was extracted with CH_2Cl_2 (3x, 150 mL), the combined organic layers were dried over Na_2SO_4, filtered, and concentrated to furnish the crude residue. Purification of the crude material by flash chromatography (20% EtOAc/petroleum ether) furnished 7.5 g (88 %) of benzyl (2-methoxyamino) acetate as colorless oil. R_f = 0.46 (20% EtOAc/petroleum ether); ^{1}H δ ($CDCl_3$): 7.36 (m, 5H), 5.19 (s, 2H), 4.64 (s, 1H), 3.64 (s, 2H), 3.53 (s, 3H); ^{13}C δ 171.2, 135.7, 128.9, 128.7, 128.6, 67.1, 61.8, 53.2; Electrospray ionization-MS *m/z* [M + Na]$^-$ calculated for $C_{10}H_{13}NO_3Na$, 218.0; observed 218.0.

To a solution of benzyl (2-methoxyamino) acetate (6.0 g, 30.7 mmol) in THF/H_2O (2:1, 50 mL) was added $NaHCO_3$ (6.5 g, 76.9 mmol) and the mixture stirred for 20 minutes. To this stirred solution, $(Boc)_2O$ (13.4 g, 61.5 mmol) was added and the reaction was allowed to continue, with stirring, for 16 h. Upon completion (as determined via TLC), the reaction mixture was diluted with ddH_2O (100 mL) and diethyl ether (100 mL). The aqueous layer was extracted with diethyl ether (3x, 150 mL), the combined organic layers were washed with 1 M aqueous HCl (2x, 100 mL), dried over Na_2SO_4, filtered and concentrated *in vacuo*. Purification of the crude material by flash chromatography furnished 8.73 g, 96% of the pure Benzyl [*N*-(tert-Butoxycarbonyl)-*N*-methoxy-amino]acetate **4**. R_f = 0.71 (20% EtOAc/petroleum ether); ^1H δ ($CDCl_3$): 7.33 (b, 5H), 5.16 (bs, 2H), 4.19 (s, 2H), 3.69 (s, 3H), 1.44 (s, 9H); ^{13}C δ 168.2, 156.3, 135.1, 128.3, 128.1, 128.1, 81.8, 66.7, 62.4, 51.3, 27.9; Electrospray ionization-MS *m/z* $[M + H]^+$ calculated for $C_{15}H_{22}NO_5$, 318.1; observed 318.1.

[*N*-(*tert*-Butoxycarbonyl)-*N*-methoxy]pentafluoro-phenyl-acetate (5): Hydrogen was bubbled through a solution of **4** (7.0 g, 23.7 mmol) containing 5 % Pd-$BaSO_4$ (10 mol %) in ethanol (10 mL). After 90 minutes, TLC analysis revealed the disappearance of the starting material. The reaction mixture was diluted with MeOH (20 mL) and filtered through celite to remove catalyst. The solvent was removed under reduced pressure to furnish the pure [*N*-(*tert*-Butoxycarbonyl)-*N*-methoxy]amino acetic acid (4.86 g, 100%). R_f = 0.12 (20% EtOAc/petroleum ether); ^1H δ ($CDCl_3$): 10.84 (broad, 1H), 4.14 (s, 2H), 3.64 (s, 3H), 1.41 (s, 9H); ^{13}C δ 173.4, 156.4, 82.3, 62.4, 60.0, 27.8; Electrospray ionization-MS *m/z* $[M - H]^-$ calculated for $C_8H_{14}NO_5$, 203.99; observed 203.99.

To a 0 ^oC solution of [*N*-(*tert*-Butoxycarbonyl)-*N*-methoxy]amino acetic acid (4.8 g, 23.2 mmol) in CH_2Cl_2/dioxane (1:1) was added pentafluorophenol (6.8 g, 37.1 mmol) and diisopropyl carbodiimide (5.8 g, 46.4 mmol). The resulting reaction mixture was stirred at room temperature for 16 h, then diluted with CH_2Cl_2 (50 mL) and filtered through celite. The filtrate was concentrated *in vacuo* and the crude residue was purified by flash chromatography (10% EtOAc/Hexanes) to furnish pure product **5** (6.75 g, 78 %). R_f = 0.62 (20% EtOAc/Hexanes); ^1H δ ($CDCl_3$): 4.49 (s, 2H), 3.74 (s, 3H), 1.46 (s, 9H); ^{13}C δ 164.9, 156.0, 142.2, 139.7, 139.6, 136.5, 82.7, 62.8, 50.9, 27.8; Electrospray ionization-MS *m/z* $[M + H]^+$ calculated for $C_{14}H_{15}F_5NO_5$, 372.0; observed 372.0.

Synthesis of 19-*N*-methoxyamino-colchicine (8): A mixture of deacetyl colchicine **6** (2.0 g, 5.6 mmol) and activated ester **5** (2.5 g, 6.7 mmol) in CH_2Cl_2 (40 mL) was stirred at room temperature for 20 hours. The reaction mixture was concentrated under reduced pressure and the crude material was purified by flash chromatography (8% MeOH/$CHCl_3$) to furnish the pure product **7** as a fluffy yellow solid (3.0 g, 98%). R_f = 0.53 (8% MeOH/$CHCl_3$) ^1H δ ($CDCl_3$): 7.40 (s, 1H), 7.35 (d, 1H, 6.8), 7.30 (d, 1H, 10.8), 6.85 (d, 1H, 10.8), 6.48 (s, 1H), 4.61 (apparent ddd, 1H, 11.6, 6.8, 6.2) 4.128 (d, 2H, 4.0), 3.90 (s, 3H), 3.87 (s, 3H), 3.84 (s, 3H), 3.64 (s, 3H), 3.56 (s, 3H), 2.50-2.45 (1H), 2.36-2.28 (1H), 2.23 (1H), 1.87-1.79 (1H), 1.42 (s, 9H); ^{13}C δ 179.1, 168.1, 163.8, 156.2, 153.4, 151.5, 150.9, 141.4, 136.6, 135.6, 133.9, 130.3, 125.2, 112.8, 107.1, 82.6, 62.1, 61.3, 61.1, 56.1, 55.8, 52.4, 52.2, 36.5, 29.5, 27.9; Electrospray ionization-MS *m/z* $[M + H]^+$ calculated for $C_{28}H_{37}N_2O_9$, 545.2; observed 545.2.

To a stirred solution of 19-[*N*-(*tert*-Butoxycarbonyl)-*N*-methoxy]amino-colchicine **7** (3.0g, 5.5 mmol) in MeOH (10 mL) was added trifluoroacetic acid (75 mL, excess) over a period of 2 days. After stirring for an additional 24 hours the TLC analysis indicated the completion of the reaction. The reaction mixture was concentrated under reduced pressure, and diluted with saturated aq. citric acid solution (50 mL) and neutralized with 1 M aq. NaOH solution at 0 ^oC to pH = 10. The aqueous layer was extracted with CH_2Cl_2 (4x, 100 mL), the combined organic layers were dried over Na_2SO_4 and concentrated *in vacuo*. The crude residue was purified by flash chromatography (8% MeOH/$CHCl_3$) to furnish a yellow solid, 19-N-methoxyamino-colchicine **8** (1.8g, 73%). R_f = 0.33 (8% MeOH/$CHCl_3$); ^1H δ ($CDCl_3$): 7.76 (broad, 1H), 7.41 (s, 1H), 7.27 (d, 1H, 10.0), 6.80 (d, 1H, 10.8), 6.50 (s, 1H), 4.67 (m, 1H), 3.95 (s, 3H), 3.90 (s, 3H), 3.86 (s, 3H), 3.62 (s, 3H), 3.60-3.43 (3H), 3.49 (s, 3H), 2.52-2.47 (1H), 2.42-2.33 (1H), 2.29-2.20 (1H), 1.89-1.82 (1H); ^{13}C δ 179.3, 169.7, 163.9, 153.3, 151.1, 151.1, 141.5, 136.2, 135.1, 134.0, 130.7, 125.5, 112.3, 107.2, 61.7, 61.4, 61.2, 56.2, 56.0, 54.3, 51.8, 36.7, 29.7; Electrospray ionization-MS *m/z* $[M + H]^+$ calculated for $C_{23}H_{29}N_2O_7$, 445.2 observed 445.2.

19-(*N*-methoxyamino-*N*-D-glucosyl)colchicine (Col0): 19-*N*-methoxyamino-colchicine **8** (33 mg, 74.2 µmol) and D-glucose (27 mg, 150 µmol) in DMF/AcOH (3:1, 820 µL) were stirred at 40 ^oC for 24 h. The reaction mixture upon concentration furnished the crude colchicine neoglucoside, which was determined by ^1H NMR to

be an isomeric mixture of β:α = 87:13. The crude material was filtered through a plug of flash silica to remove traces of unreacted starting materials to furnish the pure material (30mg, 65%). R_f = 0.53 (20% MeOH/$CHCl_3$); ^{1}H δ (DMSO-*d_6*): 8.54 (d, 0.9H, 7.2), 8.24 (d, 0.1H, 5.2H), 7.22 (s, 1H), 7.12 (d, 1H, 10.8), 7.03 (d, 1H, 11.2), 6.78 (s, 1H), 5.15 (d, 0.1H, 5.2), 5.10 (d, 0.1H, 8.4), 5.05 (d, 0.1H, 5.6), 4.98 (d, 0.9H, 4.4), 4.95 (d, 0.9H, 5.2), 4.84 (d, 0.9H, 3.6), 4.46-4.40 (2H), 3.99 (d, 1H, 8.4), 3.90 (s, 3H), 3.87 (s, 3H), 3.84 (s, 3H), 3.66 (broad dd, 1.4H, 4.4, 4.8), 3.61-3.60 (0.6H), 3.55 (s, 3H), 3.22-3.15 (2H), 3.09-3.02 (2H), 2.61 (dd, 1H, 6.4, 6.0), 2.29-2.20 (1H), 2.08-1.90 (2H); ^{1}H δ (500 MHz, 1:1 DMSO-d_6:D_2O): 7.29 (s, 1H), 7.26 (d, 1H, 6.5), 7.15 (d, 1H, 11.5), 6.74 (s, 1H), 4.35 (dd, 1H, 6.0, 11.5), 6.74 (s, 1H), 4.35 (dd, 1H, 6.0, 11.5), 4.02 (d, 1H, 8.0), 3.85 (s, 3H), 3.77 (s, 3H), 3.74 (s, 3H), 3.67 (bd, 1H, 12.0), 3.58 (bd, 1H, 16.0), 3.50-3.46 (2H), 3.48 (s, 3H), 3.43 (s, 3H), 3.31-3.25 (2H), 3.18-3.09 (2H), 2.58-2.56 (1H), 2.16-2.07 (2H), 1.96-1.92 (1H); ^{13}C δ (100 MHz, DMSO-*d6*): 177.9, 168.5, 163.52, 152.9, 150.4, 150.38, 140.7, 135.1, 134.4, 134.2, 130.7, 125.4, 112.1, 107.7, 93.0, 78.5, 77.6, 70.1, 69.9, 61.2, 61.1, 60.8, 60.7, 56.1, 56.0, 55.8, 51.3, 35.6, 29.2; ^{1}H, ^{13}C, gCOSY, gDQCOSY, and gHSQC spectra are included in this supplemental information (Figures S2-S10). For simplification of certain ^{1}H NMR experiments, D_2O was added as a co-solvent to eliminate coupling to sugar hydroxyl groups. Electrospray ionization-MS *m/z* [M + Na]$^+$ calculated for $C_{29}H_{38}N_2O_{12}Na$, 629.2; observed 629.2. HRMS: calcd for $C_{29}H_{38}N_2O_{12}Na$, 629.23170; observed 629.23224.

19-(*N*-methoxyamino-*N*-22-deoxy-D-ribosyl)colchicine (**Col21**): was prepared from 19-*N*-methoxyamino-colchicine **8** (118 mg, 0.26 mmol) and 2-deoxy-D-ribose (72 mg, 0.53 mmol) following a procedure identical to the preparation of **Col0** [116 mg, 78%, 81:19 isomeric purity by LC-MS] R_f = 0.64 (16% MeOH/$CHCl_3$); ^{1}H δ (500 MHz, DMSO-*d_6*): 9.56 (d, 0.1H, 7.5), 8.64 (bt, 0.3H, 6.2), 8.59 (t, 0.6H, 8.0), 7.20 (s, 0.3H), 7.19 (s, 0.3H), 7.189 (s, 0.4H), 7.11 (bd, 1H, 10.5), 7.02 (d, 1H, 11.0), 6.76 (s, 1H), 6.26-6.21 (0.1H), 5.34 (d, 0.1H, 4.8), 5.0 (dd, 0.2H, 9.0, 5.0), 4.90-4.83 (0.4H), 4.69 (d, 0.3H, 6.1), 4.66-4.60 (0.5H), 4.57 (t, 0.1H, 5.5), 4.42-4.35 (1.2H), 4.31-4.28 (0.1H), 4.17 (bd, 0.3H, 11.1), 4.10-4.05 (0.2H), 4.02-3.97 (0.2H), 3.95 (b, 0.3H), 3.88 (s, 3H), 3.85 (s, 3H), 3.80 (s, 3H), 3.56 (s, 3H), 3.52-3.47 (2H), 3.43-3.38 (4H), 3.37 (s, 3H), 2.60-2.57 (0.9H), 2.30-2.19 (1.1H), 2.04-1.90 (2.3H), 1.84-1.70 (1.3H), 1.65-1.62 (0.4H); ^{1}H δ (500 MHz, DMSO-d_6:D_2O): 7.20 (bs, 1H), 7.18 (d, 0.9H, 10.9), 7.07 (d, 0.9H, 10.6), 7.04 (d, 0.1H, 4.5), 7.00 (d, 0.1H, 4.0), 6.71 (s, 1H), 6.19 (dd, 0.1H, 15.8, 8.0), 6.10 (bt, 0.1H, 6.0), 4.82 (t, 0.2H, 6.5), 4.76 (t, 0.1H, 6.0), 4.38 (bd, 0.3H, 10), 4.31 (bdd, 1.2H, 13.0, 6.5), 4.04-3.94 (1H), 3.84 (s, 3H), 3.77 (s, 3H), 3.74 (s, 3H), 3.67-3.61 (0.9H), 3.58-3.52 (1.4H), 3.49 (s, 3H), 3.47-3.42 (1.4H), 3.40-3.28 (4.3H, includes OMe), 2.56-2.52 (0.9H), 2.31-2.23 (0.2H), 2.16-2.09 (1H), 2.06-1.98 (1H), 1.96-1.89 (1.3H), 1.82-1.61 (1.6H); ^{13}C δ (125MHz, DMSO-*d_6*): 194.3, 177.9, 168.3, 168.2, 168.1, 163.5, 159.8, 152.9, 150.4, 150.5, 140.7, 135.2, 134.3, 134.2, 130.7, 130.6, 130.5, 125.5, 112.1, 107.7, 94.2, 93.1, 89.7, 87.1, 86.4, 86.0, 79.2, 71.2, 70.7, 70.1, 68.3, 68.1, 66.9, 66.3, 64.9, 64.8, 62.4, 61.7, 61.6, 61.1, 61.08, 60.8, 60.7, 56.5, 56.0, 55.8, 55.3, 51.4, 51.3, 36.8, 36.1, 35.5, 34.2, 31.9, 29.2, 21.2; Electrospray ionization-MS *m/z* [M + H]$^+$ calculated for $C_{28}H_{37}N_2O_{10}$, 561.2; observed 561.2.

General procedure for neoglyoside library synthesis and purification. A mixture of 19-*N*-methoxyamino-colchicine **8** (~34 μmol) and appropriate sugar (2.0 eq.), in DMF/AcOH (3:1, 90-100 mM 19-*N*-methoxyamino-colchicine **8** final concentration) were added in 4 mL glass vials equipped with magnetic stirrer fleas. The reaction mixtures were stirred at 40 ^{o}C for 24 h utilizing a 48 well reaction block stirplate equipped with a contact thermometer for temperature control. Following removal of the solvent via Speed-Vac (55 ^{o}C, 3-4 h), 8% MeOH/$CHCl_3$ (0.5-1 mL) was added to the crude reaction mixtures and vortexed for 45-60 seconds. The crude suspended library members were purified with 1 g Alltech silica solid phase extraction disposable columns eluting first with 8% MeOH/$CHCl_3$ to first remove any remaining aglycon, followed by elution of library members with 16% MeOH/$CHCl_3$. Following removal of solvent by speed vac, the library members were dried under vaccum to furnish pure neoglycoside products. The stock solutions of neoglycosides in DMSO (20 mM) were prepared and these stock solutions were further diluted to 0.1 mM in MeOH and were characterized by LC-MS utilizing reverse phase HPLC (3 X 150 mm Phenomenex Luna C18 Column, 2 μL injection, flow rate 0.8 mL/min, linear gradient 20-80% CH_3CN/H_2O containing 0.1% formic acid, 5%/min gradient over 25 min) and electrospray ionization. Purity and isomeric ratio of the library members were determined by division of the sum of peak areas with desired neoglycoside mass that were observed at 350 nm/245 nm to the total area of all peaks [210-700 nm]. The average purity of the library was 96.2% and the average isomeric ratio was 81:19. A tabular description of calculated and observed mass, purity, isomeric ratio for all library members is provided in Table S1.

Cytotoxicity Assays

All cell lines were maintained as previously reported (reference S1). Cells were harvested by trypsinization using 0.25% trypsin and 0.1% EDTA and then counted in a ViCell XR coulter counter in duplicate, before and after dilution for assay plating. Cell plating, compound handling and assay set up were performed as previously reported (reference S1). Calcein AM (acetoxymethyl ester) reagent (30 μL, 1M) was added and the cells were incubated for 30 min at 37 °C. Plates were read for emission by using a fluorescein filter (λ_{ex} 485 nm, λ_{em} 535 nm). An equal volume (30 μL) of cell titer-glow reagent (Promega Corporation, Inc.) was added and incubated for 10 min at room temperature with gentle agitation to lyse the cells. Each plate was re-read for luminescence to confirm the inhibition observed in the fluorescent Calcein AM assay. IC_{50} calculation for library members were done by plotting percent inhibitions as a function of log[concentration] and then fit to a four parameter logistic model which allowed for a variable Hill slope utilizing XL*fit* 4.2 software as previously reported (reference S1). 57 library members were tested in a panel of 9 human cancer cell lines and 1 normal mouse control cell line. IC_{50} were determined using a threshold of 10 μM (3 orders of magnitude greater than the IC_{50} of the parent molecule colchicine) established as the "non-toxic" cut off. Dose response experiments were performed in triplicate and repeated on a separate day for all compounds below the non-toxic cutoff in each cell line. All 57 library members had an IC_{50} below the non-toxic cutoff in at least 1 cell line. 15 library members had an IC_{50} of less than 1 μM and were further tested in secondary assays to determine the mechanism of action. Library member **Col53** was excluded from further analysis due to precipitation in cell culture media. All cytotoxicity screen data is presented in Table S2.

Tubulin Polymerization Assay

In vitro tubulin polymerization assays were performed using a fluorescence-based assay (Cytoskeleton, Inc.). Test compounds and control stocks were prepared at a final concentration of 15 μM and 3 μM in room temperature sterile ddH_2O. Aliquots (5 μL) of each compound or control were added to a 96-well black half area plate (Corning Costar, Inc.) pre-warmed to 37 °C. Cold tubulin reaction mix (50 μL 1X Buffer - 80 mM piperazine-*N,N'*-bis[2-ethanesulfonic acid] sequisodium salt; 2.0 mM magnesium chloride; 0.5 mM ethylene glycol-bis[β-amino-ethyl ether) *N,N,N',N'*-tetra-acetic acid, pH 6.9, 10 μM fluorescent reporter; 20% tubulin glycerol buffer - 80 mM piperazine-*N,N'*-bis[2-ethanesulfonic acid] sequisodium salt; 2.0 mM magnesium chloride; 0.5 mM ethylene glycol-bis(β-amino-ethyl ether) *N,N,N',N'*-tetra-acetic acid, 60 % v/v glycerol, pH 6.9;, 1 mM GTP; and 2 mg mL^{-1} tubulin stock) was added to each of the compounds. The reaction was immediately read using a 350 excitation and a 435 emission filter on a Safire[2] microplate reader (Tecan Instruments), reading every 60 seconds for 61 cycles with temperature control set to 37 °C and shaking the plate for 5 seconds before the first read. Polymerization curves were generated in Excel after background correction and the effects of test compounds were compared to controls. Library members that accelerated the rate of tubulin polymerization were deemed microtubule stabilizers, whereas compounds that decelerated the rate of tubulin polymerization were deemed microtubule de-stabilizers. Fifteen library members (**6**-**8**, **Col6**, **Col16**, **Col19**, **Col21**, **Col34**, **Col38**, **Col44**, **Col45**, **Col56**, **Col53**, **Col18**, **Col65**) were tested in duplicate on at least two separate days in the *in vitro* tubulin polymerization assay. Compounds **6**-**8**, **Col6**, **Col16**, **Col34**, **Col38**, **Col44**, and **Col45** destabilized microtubules like the parent molecule colchicine. Library members **Col18**, **Col56** and **Col65** had no effect on tubulin polymerization in this assay and two library members (**Col19** and **Col21**) stabilized the microtubules like paclitaxel. Library member **Col53** precipitated and was not further tested. The library members that had no effect (**Col18**, **Col56** and **Col65**) were further tested in a wound healing assay at a sub-toxic concentration (the IC_{10}) and were shown to inhibit the migration of MB-MDA-231 cells by at least 50 % similar to both colchicine and paclitaxel tested in the same assay.

Wound Healing Assay

A highly metastatic and migratory human breast adenocarcinoma cell line, MB-MDA-231, was used to assess inhibition of cell migration by test compounds that did not stabilize or destabilize microtubules in the *in vitro* tubulin polymerization assay. Fifty thousand cells per well were plated and allowed to attach to each well of a black tissue culture treated 96 well microtiter plate (Corning Costar, Inc.). Cells are grown overnight at 37 °C to allow attachment and a monolayer to form. A uniform 1.58 mm wound was created using a 96-well floating pin tool (V & P Scientific) as a guide that forms a wound along the X-axis of each well of a 96 well plate. Wounded monolayers were treated with compounds for 96 hours to allow full wound closure. Following incubation, wounded monolayers were washed 1X in phosphate buffered saline pH 7.4, and stained with Calcein AM (acetoxymethyl ester) reagent (30 μL, 1 M) for 30 minutes at 37 °C. Plates were read at excitation 485 nm and emission 535 nm in both the area of the wound and the whole well. Amount of wound healing

was determined by dividing the fluorescence in the area of the wound by the total fluorescence per well. Percent inhibition was determined by dividing the amount of wound healing in treated wells by the amount of wound healing in cells treated with solvent only (DMSO).

Multiple Drug Effect Analysis

Ten thousand A549 cells per well were plated in triplicate in 96 well black tissue culture treated plates. Cells were incubated for 1 hr at 37 °C to allow cells to attach. Cells were treated with different test compounds in combination with paclitaxel or colchicine. The concentration of colchicine and paclitaxel was held constant at the calculated IC_{10} (concentration of compound that gives 10% growth inhibition) from the cytotoxicity assay in A549 cells. Each compound was tested in triplicate serial dilutions starting at the calculated IC_{10} in combination with paclitaxel or colchicines. The combination index (CI) method of Chou and Talalay (reference S2) was used to analyze the nature of the interaction between the test compounds and taxol or colchicines by determining a CI using Calcusyn software (Biosoft, Inc.) CI values of less than or greater than 1 indicate synergism or antagonism, respectively.

Specifically, **6**-**8**, **Col6**, **Col16**, **Col19**, **Col21**, **Col34**, and **Col45** were tested in combination with paclitaxel and colchicine and the synergistic effects were calculated using the Chou Talalay method (reference S2) by determining a combination index in Calcusyn software (Biosoft, Inc.). The results are presented in Table S3 and the guide for data interpretation presented in Table S4. Library members **6**, **8**, **Col6**, and **Col16** all displayed synergy with taxol (and antagonism with colchicines) similar to colchicine. In a similar fashion, library members **Col21**, **Col34**, and **Col45** showed strong synergism with taxol and strong antagonism with colchicine. Library members **Col19** and **Col21** showed synergism with colchicine and antagonism with taxol with **Col19** displaying reproducibly stronger effects. All synergy experiment results were replicated at least 3 times and the overall effects analyzed by averaging all combination indexes generated if the fraction affected was greater than 0.2. **Col38** and **Col44** were eliminated from the analysis due to impurities.

Figure S1. The colchicine-neoglycoside library (grey colored neoglycosides represent failed reactions).

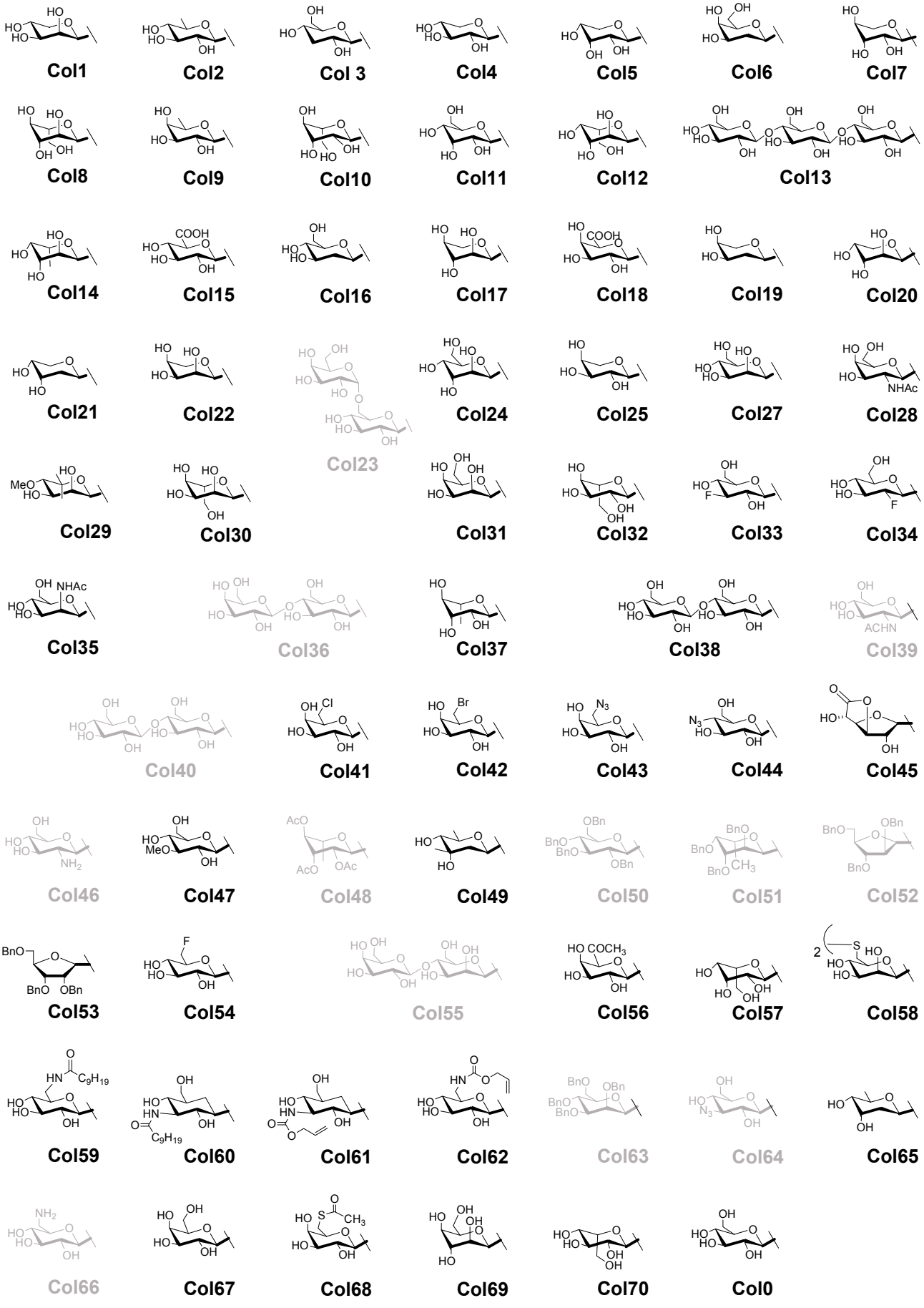

Figure S2. ^{1}H NMR of **ColI0** (500 MHz, DMSO-d_6).

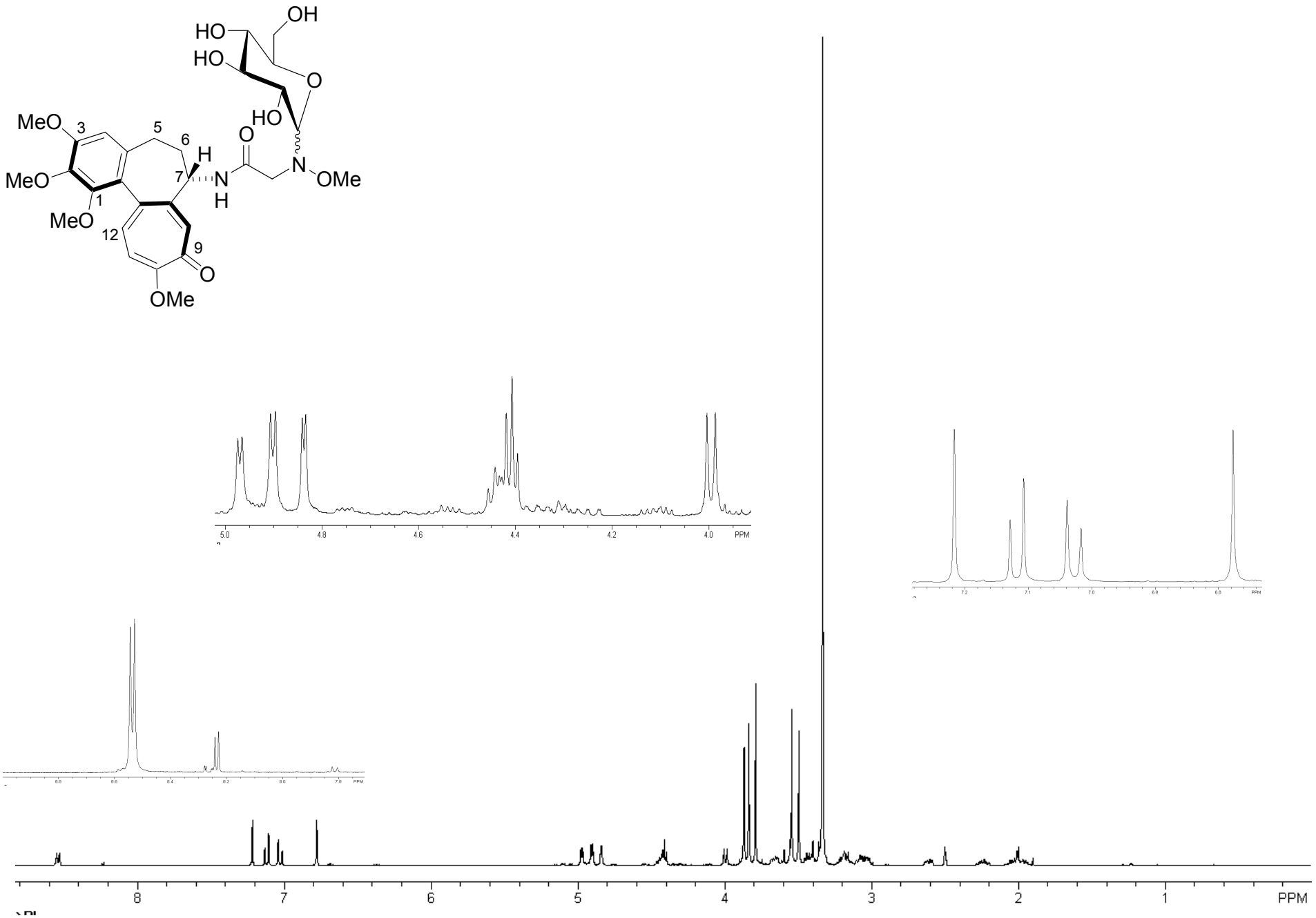

Figure S3. ^{1}H NMR of **ColI0** (500 MHz, 1:1 DMSO-d_6 : D_2O).

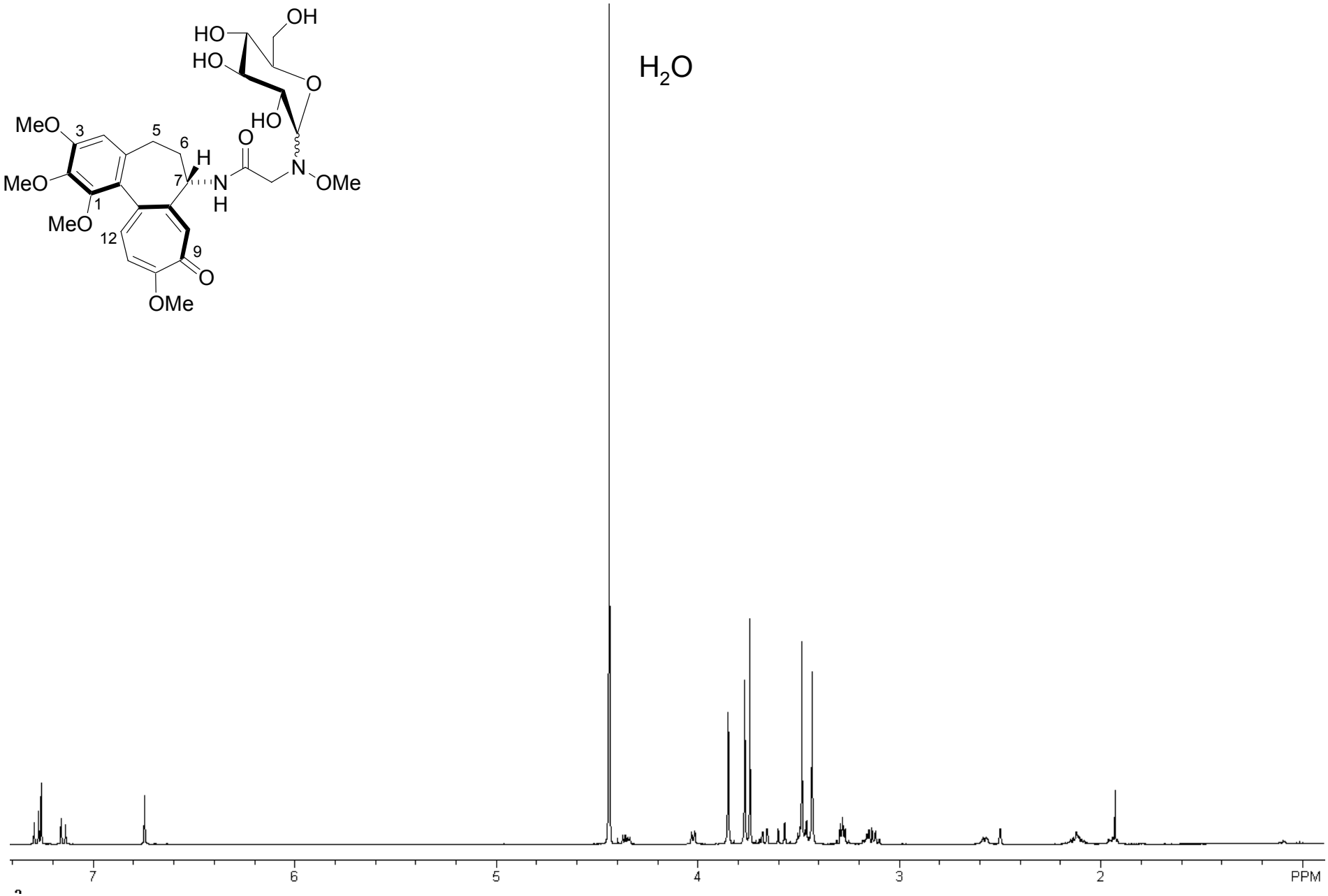

Figure S4. ^{13}C NMR of **Col0** (100 MHz, DMSO-d_6).

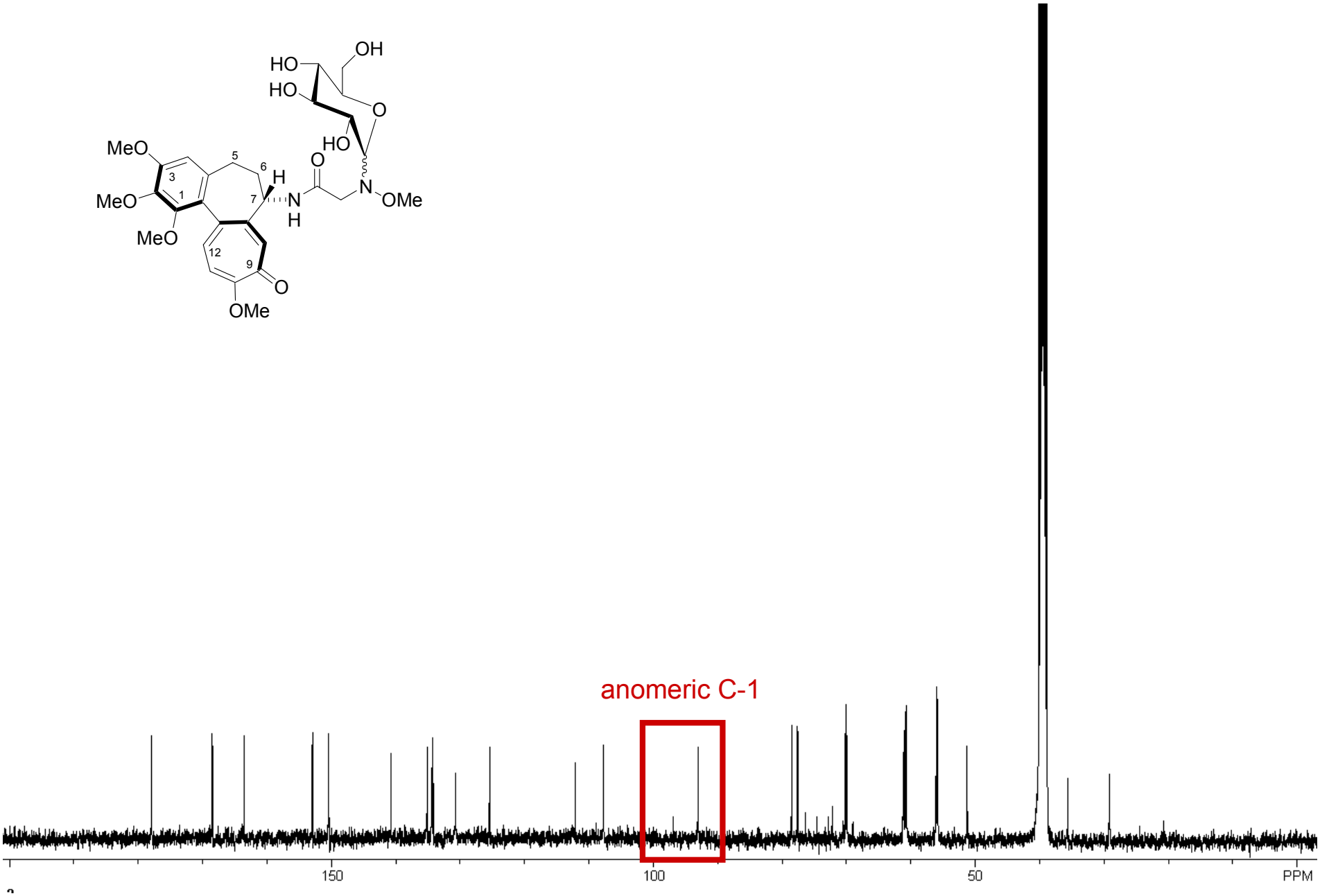

Figure S5. gCOSY of **Col0** (500 MHz, DMSO-d_6).

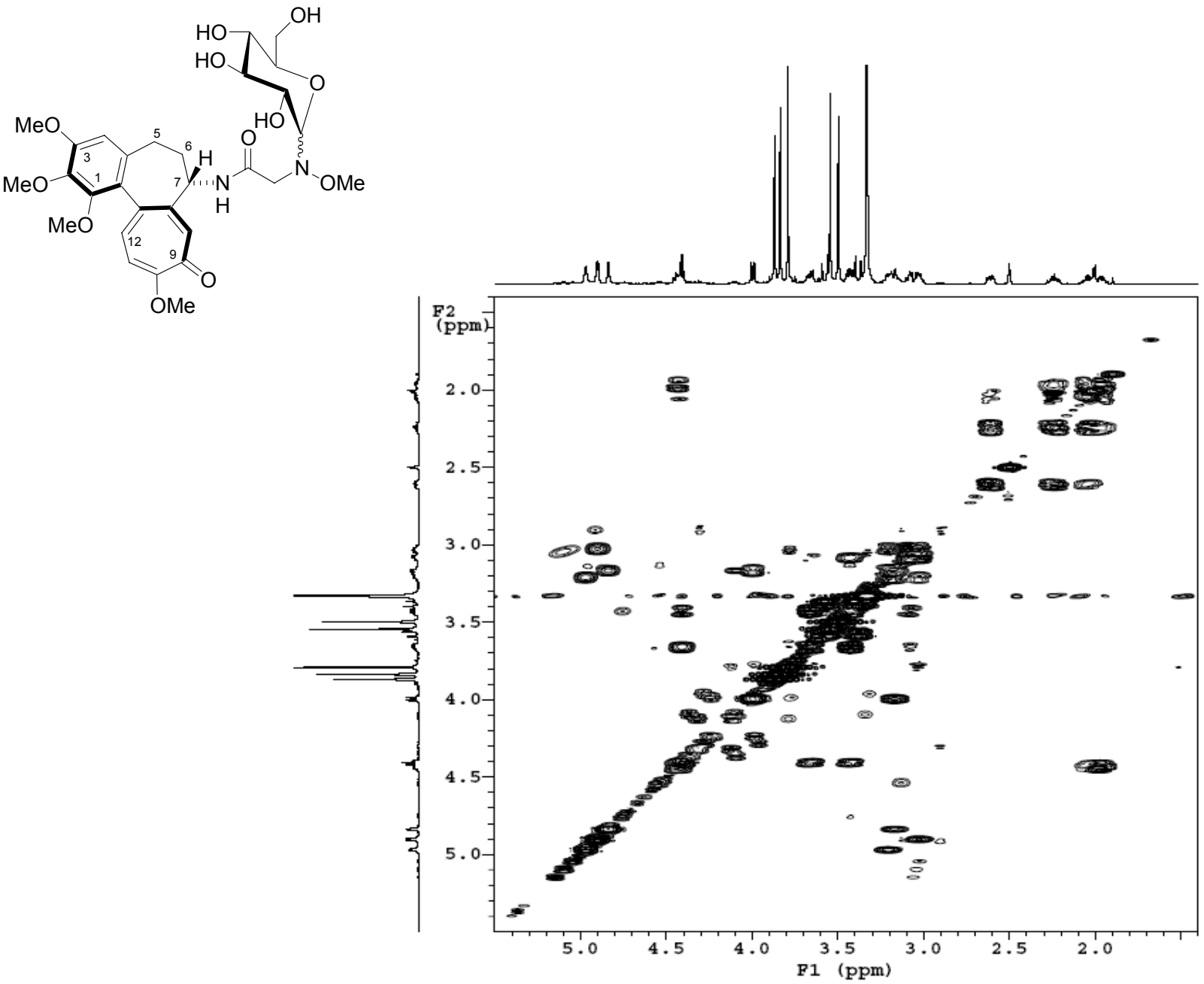

Figure S6. gCOSY of **Col0** (500 MHz, 1:1 DMSO-d_6 : D_2O).

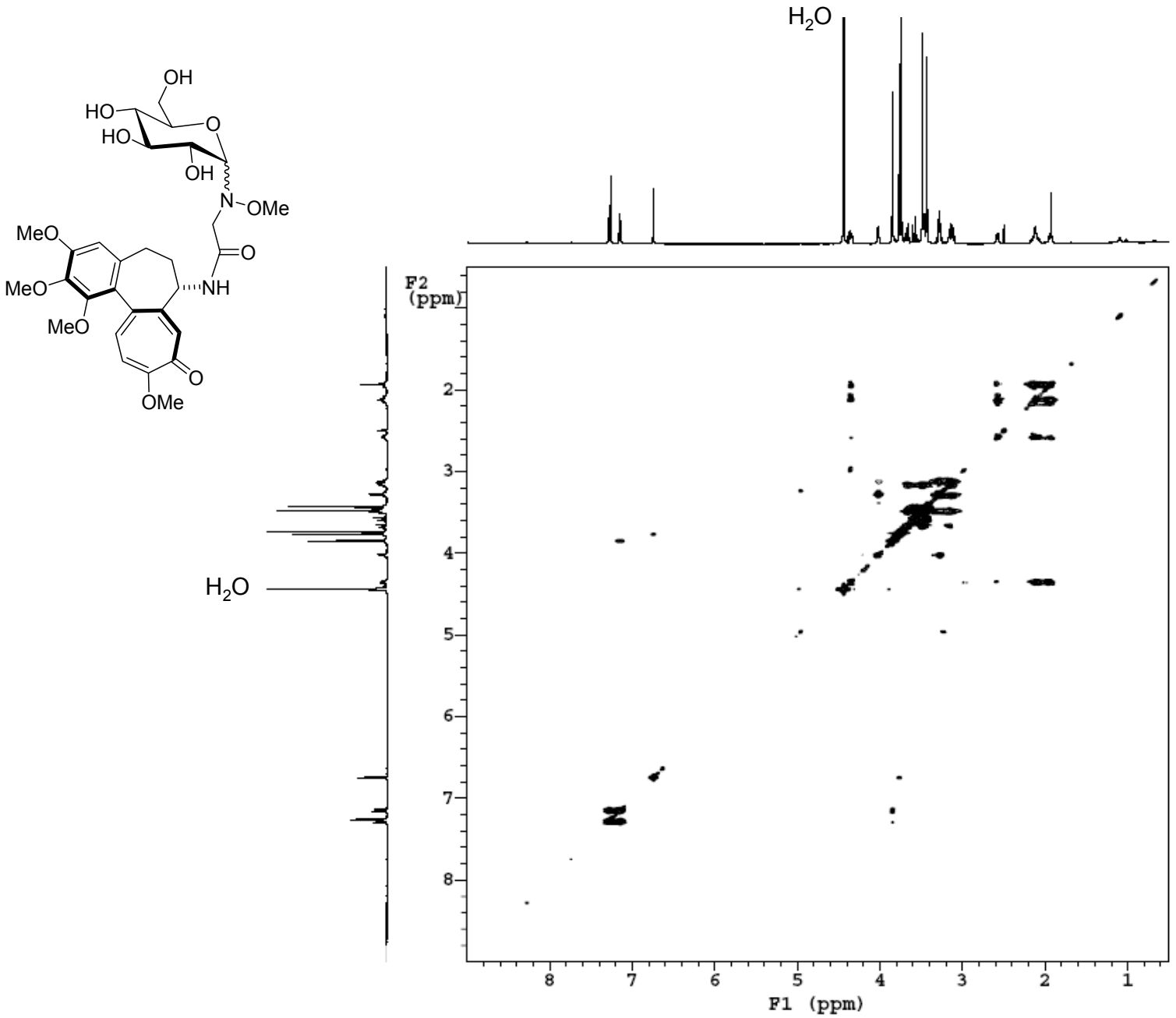

Figure S7. gCOSY of **Col0** (500 MHz, 1:1 DMSO-d_6 : D_2O).

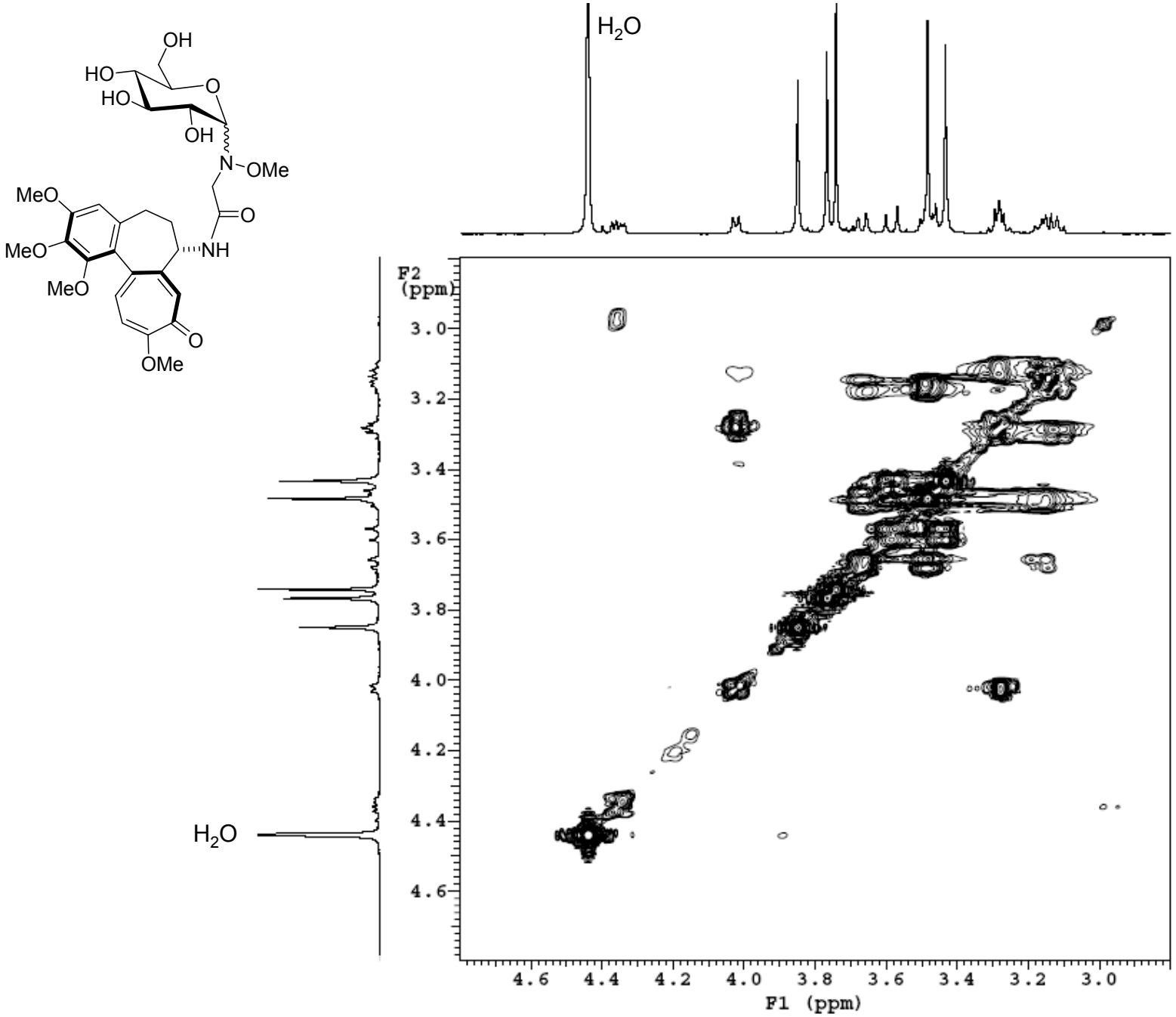

Figure S8. gDQCOSY of **Col0** (500 MHz, DMSO-d_6).

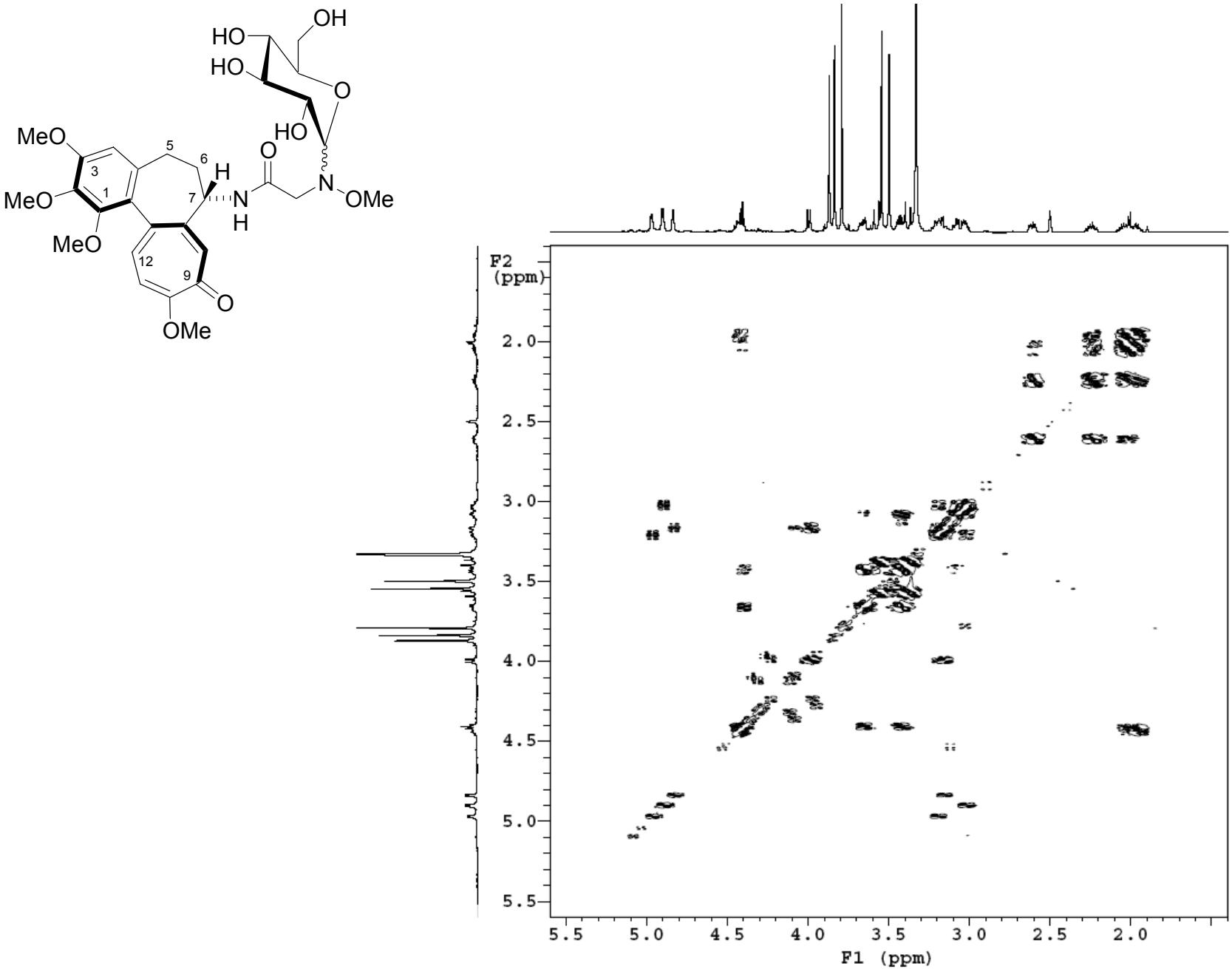

Figure S9. gHSQC of **Col0** (500 MHz, DMSO-d_6).

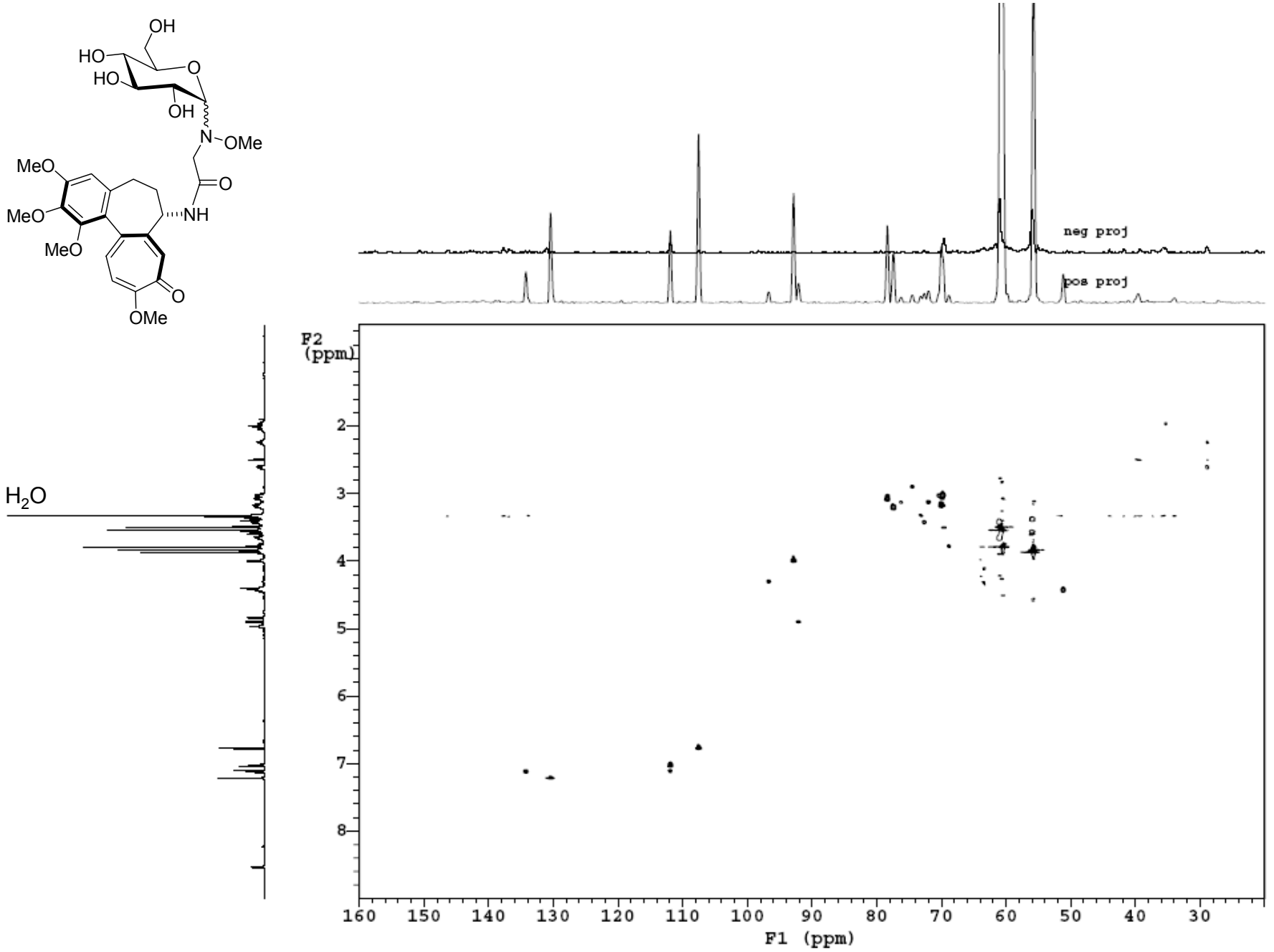

Figure S10. gHSQC of **Col0** (500 MHz, DMSO-d_6).

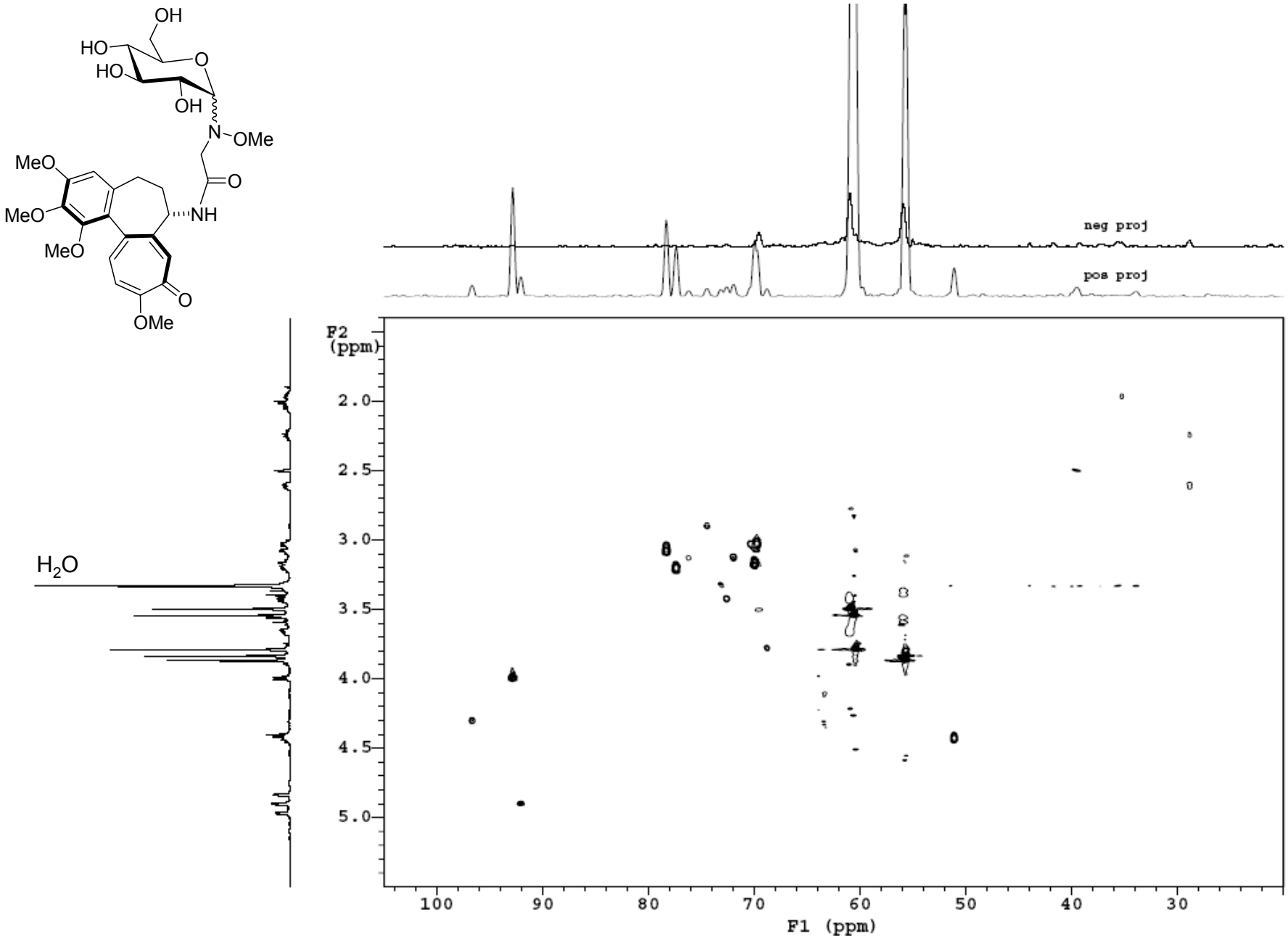

Table S1. LCMS information for colchicine-neoglycoside library.

Neoglycoside	Calculated Mass	Observed Mass	Percent purity	LC-MS-Peak ratio
D-lyxoside (**Col1**)	576.2	576.2	100	78:22
6-deoxy-D-glucoside (**Col2**)	590.2	590.2	100	80:20
3-deoxy-D-glucoside (**Col3**)	590.2	590.2	100	81:19
D-xyloside (**Col4**)	576.2	576.2	100	81:19
D-riboside (**Col5**)	576.2	576.2	100	82:18
2-deoxy-D-galactoside (**Col6**)	590.2	590.2	100	76:24
L-lyxoside (**Col7**)	576.2	576.2	100	88:12
L-glucoside (**Col8**)	606.2	606.2	100	86:14
D-fucoside (**Col 9**)	590.2	590.2	100	88:12
L-mannoside (**Col10**)	606.2	606.2	100	87:13
D-alloside (**Col11**)	606.2	606.2	100	81:19
L-galactoside (**Col12**)	606.2	606.2	100	76:24
maltotrioside (**Col13**)	930.3	930.3	100	79:21
L-fucoside (**Col14**)	590.2	590.2	100	86:14
D-glucuronoside (**Col15**)	620.2	620.2	100	88:12
2-deoxy-D-glucoside (**Col16**)	590.2	590.2	100	84:16
L-xyloside (**Col17**)	576.2	576.2	100	84:16
D-Galacturonoside (**Col18**)	620.2	620.2	76	100:0
2-deoxy-L-riboside (**Col19**)	560.2	560.2	100	71:19
D-arabinoside (**Col20**)	576.2	576.2	100	80:20
2-deoxy-D-riboside (**Col21**)	560.2	560.2	88	81:19
L-riboside (**Col22**)	576.2	576.2	100	83:17
D-melibioside (**Col23**)	768.3	XXXXXX	XXXXX	XXXXX
D-altroside (**Col24**)	606.2	606.2	100	81:19
L- arabinoside (**Col25**)	576.2	576.2	100	81:19
D-mannoside (**Col27**)	606.2	606.2	100	73:27
N-acetyl-D-galactosaminoside (**Col28**)	647.2	647.2	100	73:27
novioside (**Col29**)	618.3	618.3	100	76:24
L-alloside (**Col30**)	606.2	606.2	100	77:23
D-taloside (**Col31**)	606.2	606.2	100	78:22
L-altroside (**Col32**)	606.2	606.2	100	78:22
3-fluoro-3-deoxy-D-glucoside (**Col33**)	608.2	608.2	53	84:16
2-fluoro-2-deoxy-D-glucoside (**Col34**)	608.2	608.2	62	58:42
N-acetyl-D-mannosaminoside (**Col35**)	647.2	647.2	92	76:24
D-lactoside (**Col36**)	768.3	XXXXXX	XXXXX	XXXXXX
L-rhamnoside (**Col37**)	590.3	590.3	100	92:8
D-maltoside (**Col38**)	768.3	768.3	trace	product
N-acetyl-D-glucosaminoside (**Col39**)	647.3	XXXX	XXXX	XXXX
D-cellobioside (**Col40**)	768.8	XXXX	XXXX	XXXX
6-deoxy-6-chloro-D-galactoside (**Col41**)	624.2	624.1	100	88:12
6-deoxy-6-bromo-D-galactoside (**Col42**)	668.1	668.1	100	81:19
6-deoxy-6-azido-D-galactoside (**Col43**)	631.2	631.2	100	81:19
4-deoxy-4-azido-D-glucoside (**Col44**)	631.2	631.2	25	100:0
D-glucorono-6,3-lactonide (**Col45**)	602.2	602.1	>94	89:11
2-deoxy-2-amino-D-glucoside (**Col46**)	642.1	XXXXX	XXXX	XXXXX
3-*O*-methyl-D-glucoside (**Col47**)	620.2	620.2	100	70:30
2,3,4-tri-*O*-acetyl-L-rhamnoside (**Col48**)	716.28	XXXXX	XXXX	XXXX
mycaroside (**Col49**)	588.3	588.3	93	87:13
2,3,4,6-tetra-*O*-benzyl-D-glucopyranoside (**Col50**)	966.4	XXXXX	XXXX	XXXXX
2,3,4-tri-*O*-benzyl -L-fucopyranoside (**Col51**)	861.0	XXXXX	XXXX	XXXXX
2,3,5-tri-*O*-benzyl -D-arabinofuranoside (**Col52**)	846.9	XXXX	XXXX	XXXX
2,3,5-tri-*O*-benzyl-D-ribofuranoside (**Col53**)	846.4	846.4	100	81:19
6-deoxy-6-fluoro-D-glucoside (**Col54**)	608.2	602.2	100	84:16
4-*O*-(β-D-galacto pyranosyl-D-mannopyranoside) (**Col55**)	768.3	XXXXX	XXXXX	XXXX
6-deoxy-6-acyl-D-galactoside (**Col56**)	618.2	618.2	100	70:30
L-taloside (**Col57**)	606.2	606.2	100	83:17
6-thio-D-mannose dimer (**Col58**)	816.2	816.2	100	88:12
6-deoxy-6-*N*-decanoyl-D-glucosaminoside (**Col59**)	759.4	759.4	100	100:0
3-deoxy-3-*N*-decanoyl-D-glucosaminoside (**Col60**)	759.4	759.4	100	100:0
3-deoxy-3-carbamic acid allyl ester-D-glucoside (**Col61**)	689.3	689.3	100	87:13
6-deoxy-3-carbamic acid allyl ester-D-glucoside (**Col62**)	689.3	689.3	100	53:47
2,3,4,6-tetra-*O*-benzyl-D-mannopyranoside (**Col63**)	967.1	XXXXX	XXXX	XXXX
3-deoxy-3-azido-D-glucoside (**Col64**)	631.6	632.2	XXXX	XXXX
D-Digitoxoside (**Col65**)	574.2	574.3	100	86:14
6-deoxy-6-amino-D-glucoside (**Col66**)	605.6	606.2	XXXX	XXXX
D-galactoside (**Col67**)	606.2	606.2	100	66:34
6-deoxy-6-thio-acyl-D-galactoside (**Col68**)	664.2	664.2	100	91:9
D-idoside (**Col69**)	606.2	606.2	100	100:0
L- idoside (**Col70**)	606.2	606.2	100	100:0
D-guloside (**Col71**)	606.2	606.2	100	74:26
D-glucoside (**Col0**)	606.2	606.2	100	72:28

Table S2. Cytotoxicity data for all colchicine neoglycosides and relevant standards (in μM with % error in parentheses).

		Col1	Col2	Col3	Col4	Col5	Col6	Col7	Col8
Du145	calcein	2.00	>7.79	6.56	>10	6.78	0.958 (0.164)	4.73	>10
	CTG	1.97	>10	>10	>10	>10	0.892 (0.084)	>10	>10
HCT-116	calcein	0.71	>6.01	1.55	9.89	7.57	0.792 (0.156)	1.06	>0.30
	CTG	3.13	>9.56	5.00	>10	9.81	0.624 (0.060)	5.08	>5.0
Hep 3B	calcein	3.69	>10	4.07	>3.35	6.52	1.124 (0.181)	4.74	>10
	CTG	3.50	>10	5.16	>10	9.86	0.817 (0.072)	4.89	>10
SF-268	calcein	4.00	>10	8.42	8.93	4.59	0.691 (0.150)	4.90	>10
	CTG	3.86	8.43	5.32	8.26	5.01	0.632 (0.042)	4.97	>10
SK-OV-3	calcein	4.00	>10	4.21	>10	>10	0.381 (0.054)	5.04	>10
	CTG	4.00	9.86	4.42	>10	>10	0.388 (0.116)	>10	>10
NCI/ADR RES	calcein	1.91	>10	>10	>10	5.06	0.887 (0.173)	>10	>10
	CTG	2.63	>10	>10	>10	4.84	0.877 (0.069)	>10	>10
NCI-H460	calcein	3.93	5.42	1.96	>10	5.76	0.948 (0.128)	5.69	>9.8
	CTG	4.51	7.24	8.37	>10	5.06	0.745 (0.053)	7.08	>10
MCF7	calcein	1.33	4.77	>10	>10	2.88	2.215 (0.340)	3.99	>8.03
	CTG	1.90	4.86	3.25	>10	2.84	0.665 (0.063)	2.65	>5.81
A549	calcein	1.21	7.94	>10	>10	>10	0.942 (0.192)	9.76	>10
	CTG	2.91	8.78	9.91	>10	>10	0.782 (0.055)	>10	>10
NmuMG	calcein	6.49	>10	>10	>10	>10	4.173 (0.588)	>10	>10
	CTG	>10	>10	>10	>10	>10	3.261 (0.294)[2]	>10	>10

		Col9	Col10	Col11	Col12	Col13	Col14	Col15	Col16
Du145	calcein	>9.14	6.97	>10	5.20	>10	>10	1.64	0.910 (0.137)
	CTG	>10	5.32	>10	5.66	>10	>10	1.27	0.705 (0.019)
HCT-116	calcein	>10	1.80	>10	7.87	>10	6.22	0.70	0.947 (0.136)
	CTG	>9.44	4.53	>10	5.24	>10	6.21	1.03	0.648 (0.018)
Hep 3B	calcein	>10	>10	>10	>10	>10	>10	3.44	1.439 (0.305)
	CTG	>10	7.81	>10	>10	>10	>10	3.28	1.915 (0.164)
SF-268	calcein	>10	2.71	>10	5.14	>10	9.89	1.02	1.544 (0.199)
	CTG	>10	5.00	>10	5.25	>10	5.28	1.17	1.068 (0.091)
SK-OV-3	calcein	>7.83	>10	>10	>10	>10	>10	2.88	0.625 (0.111)
	CTG	>10	5.16	>10	>10	>10	>10	1.07	0.432 (0.036)
NCI/ADR RES	calcein	>10	9.49	>10	9.17	>10	>10	1.54	0.899 (0.149)
	CTG	>10	8.122	>10	9.42	>10	>10	1.66	1.675 (0.201)
NCI-H460	calcein	>10	5.34	>10	>10	2.58	>10	0.96	0.808 (0.138)
	CTG	>10	4.55	6.69	9.53	>10	9.03	1.08	0.946 (0.036)
MCF7	calcein	>8.75	6.90	>10	6.97	>10	>10	4.41	1.921 (0.664)
	CTG	>9.96	6.565	>10	7.8	9.86	4.95	0.99	2.653 (0.230)
A549	calcein	>10	>10	>10	>10	>10	>10	3.92	1.682 (0.303)
	CTG	>10	6.08	>10	>10	>10	>10	2.21	3.903 (0.260)
NmuMG	calcein	>10	>10	>10	>10	>10	>10	>5	4.415 (0.499)
	CTG	>10	>10	>10	>10	>10	>10	>5	1.610 (0.305)[2]

Table S2 (cont.). Cytotoxicity data for all colchicine neoglycosides and relevant standards (in μM with % error in parentheses).

		Col17	Col18	Col19	Col20	Col21	Col22	Col24	Col25
Du145	calcein	>10	1.187 (0.204)	0.262 (0.064)	3.74	0.294 (0.053)	>10	>10	>10
	CTG	8.48	1.125 (0.077)	0.369 (0.049)	2.41	0.344 (0.030)	2.38	>10	7.40
HCT-116	calcein	7.35	0.865 (0.097)	0.431 (0.070)	5.00	0.344 (0.078)	2.09	>10	2.95
	CTG	5.48	0.886 (0.064)	0.299 (0.057)	4.90	0.444 (0.138)	1.54	>10	2.09
Hep 3B	calcein	>10	2.269 (0.199)	0.437 (0.078)	>10	1.291 (0.186)	3.95	>10	>10
	CTG	>10	2.004 (0.117)	0.376 (0.036)	>10	0.633 (0.198)	2.44	>10	>10
SF-268	calcein	9.72	1.107 (0.135)	0.575 (0.097)	3.95	0.349 (0.080)	4.86	>10	4.46
	CTG	5.93	0.834 (0.044)	0.284 (0.026)	2.54	0.206 (0.017)	4.16	>10	6.80
SK-OV-3	calcein	>10	1.072 (0.148)	0.538 (0.123)	>10	0.296 (0.040)	4.84	>10	2.51
	CTG	>10	1.083 (0.063)	0.240 (0.035)	>10	0.362 (0.034)	4.84	>10	8.51
NCI/ADR RES	calcein	>10	0.828 (0.098)	0.315 (0.037)	7.74	0.209 (0.048)	3.56	>10	5.53
	CTG	>10	1.110 (0.074)	0.359 (0.033)	7.846	0.333 (0.055)	2.23	>10	5.14
NCI-H460	calcein	5.61	1.130 (0.115)	0.191 (0.029)	3.71	0.355 (0.042)	2.00	>10	5.78
	CTG	5.17	0.993 (0.061)	0.415 (0.045)	5.21	0.313 (0.013)	1.16	>10	4.41
MCF7	calcein	5.09	1.493 (0.134)	2.823 (0.328)[2]	2.35	0.609 (0.168)	2.87	>10	3.43
	CTG	6.22	1.163 (0.076)	0.916 (0.157)	4.44	0.514 (0.097)	4.27	>10	3.02
A549	calcein	>10	1.074 (0.189)	0.636 (0.117)	>10	0.248 (0.054)	9.44	>10	5.26
	CTG	9.40	1.103 (0.076)	0.376 (0.053)	9.07	0.286 (0.027)	4.22	>10	5.02
NmuMG	calcein	>10	2.615 (0.322)[2]	2.847 (0.436)	>10	0.507 (0.071)	>10	>10	>10
	CTG	>10	2.087 (0.226)[2]	2.907 (0.381)	>10	0.415 (0.048)	>10	>10	5.70

		Col27	Col28	Col29	Col30	Col31	Col32	Col33	Col34
Du145	calcein	>10	>10	0.55	>10	3.20	>10	5.44	0.328 (0.064)
	CTG	>10	>10	1.24	>10	3.07	>10	9.60	0.142 (0.018)
HCT-116	calcein	2.64	2.11	1.01	2.71	1.58	2.27	0.65	0.293 (0.050)
	CTG	2.51	5.10	1.05	2.08	3.27	2.22	1.46	0.172 (0.012)
Hep 3B	calcein	3.88	>10	2.94	>10	>10	>10	1.99	1.174 (0.249)
	CTG	>10	>0	2.47	>10	>10	>10	2.67	0.545 (0.106)
SF-268	calcein	4.00	3.56	1.28	5.24	3.62	4.41	2.33	0.452 (0.081)
	CTG	2.50	5.70	1.12	>10	7.14	2.58	0.86	0.281 (0.021)
SK-OV-3	calcein	3.07	7.32	4.35	>10	3.58	6.30	1.89	0.800 (0.609)
	CTG	7.38	5.18	1.03	>10	6.19	7.28	1.25	0.325 (0.048)
NCI/ADR RES	calcein	3.89	>10	1.18	7.17	3.63	1.04	>10	>1
	CTG	2.70	9.95	1.56	>10	4.83	8.39	>10	>1
NCI-H460	calcein	3.77	>10	3.37	>10	2.09	1.92	>10	>1
	CTG	2.55	9.76	0.98	>10	4.59	1.14	4.39	>1
MCF7	calcein	1.51	6.67	0.30	>10	0.39	6.65	6.13	0.254 (0.057)
	CTG	1.70	4.87	0.61	>10	2.00	4.37	6.64	0.242 (0.045)
A549	calcein	5.84	>10	2.18	>10	4.12	2.89	1.87	0.778 (0.086)
	CTG	4.69	>10	1.86	>10	8.04	5.33	2.02	0.545 (0.059)
NmuMG	calcein	2.90	9.57	>5	>10	>10	>10	>10	0.760 (0.241)
	CTG	3.17	>10	>5	>10	>10	>10	5.93	0.451 (0.143)

Table S2 (cont.). Cytotoxicity data for all colchicine neoglycosides and relevant standards (in μM with % error in parentheses).

		Col35	Col37	Col38	Col41	Col42	Col43	Col44	Col45
Du145	calcein	>10	4.36	0.342 (0.045)	6.74	4.26	4.19	>1	0.939 (0.135)
	CTG	5.26	5.81	0.215 (0.022)	6.69	4.47	4.87	>1	1.143 (0.144)
HCT-116	calcein	4.51	4.31	0.307 (0.062)	4.94	4.00	>10	0.389 (0.068)	0.529 (0.109)
	CTG	4.87	4.53	0.354 (0.078)	4.90	4.87	9.97	0.588 (0.103)	0.834 (0.093)
Hep 3B	calcein	>5	9.57	1.387 (0.202)	5.99	>10	3.89	0.671 (0.053)	0.962 (0.100)
	CTG	>5	8.26	0.229 (0.025)	8.80	>10	5.30	0.453 (0.019)	0.793 (0.038)
SF-268	calcein	5.02	4.68	0.319 (0.065)	7.87	3.31	8.46	0.412 (0.075)	0.462 (0.072)
	CTG	5.28	4.79	0.160 (0.014)	6.93	6.37	5.15	0.229 (0.013)	0.398 (0.036)
SK-OV-3	calcein	1.37	>10	0.316 (0.064)	7.95	>10	5.79	>1	0.887 (0.091)
	CTG	4.73	5.58	0.252 (0.043)	5.61	8.36	>10	>1	0.836 (0.052)
NCI/ADR RES	calcein	6.11	4.48	0.173 (0.024)	3.68	2.23	2.51	>1	0.403 (0.157)
	CTG	8.46	4.56	0.199 (0.016)	4.97	2.49	5.04	>1	1.138 (0.135)
NCI-H460	calcein	7.12	6.70	0.487 (0.091)	2.31	>10	2.36	>1	1.024 (0.128)
	CTG	4.83	3.72	0.112 (0.009)	5.48	4.40	5.01	>1	0.775 (0.076)
MCF7	calcein	4.38	1.56	0.313 (0.103)	1.85	3.69	3.68	0.676 (0.213)[2]	0.679 (0.104)
	CTG	2.46	3.81	0.291 (0.051)	3.36	4.90	4.91	0.596 (0.073)	0.760 (0.090)
A549	calcein	0.75	7.28	0.508 (0.136)	1.07	2.64	>10	0.649 (0.112)	1.055 (0.160)
	CTG	0.55	7.07	0.244 (0.035)	3.70	3.94	>10	0.315 (0.031)	1.100 (0.078)
NmuMG	calcein	>10	7.76	0.875 (0.188)	9.27	2.92	3.93	3.66 (0.805)[2]	>10
	CTG	>10	7.72	0.976 (0.116)	>10	2.83	3.82	1.425 (0.134)	1.461 (0.511)

		Col47	Col49	Col53	Col54	Col56	Col57	Col58	Col59
Du145	calcein	>10	8.56	0.032 (0.005)	>10	1.094 (0.116)	4.69	2.12	7.09
	CTG	5.22	5.20	0.041 (0.003)	>10	1.146 (0.100)	4.71	2.09	5.28
HCT-116	calcein	2.40	7.08	0.026 (0.006)	4.48	0.669 (0.178)	4.23	2.51	7.42
	CTG	2.49	5.31	0.039 (0.005)	4.74	0.761 (0.064)	3.86	1.90	5.17
Hep 3B	calcein	5.11	>10	0.069 (0.006)	4.66	2.228 (0.286)	>10	5.02	7.36
	CTG	9.61	9.76	0.047 (0.002)	>10	1.479 (0.141)	>10	8.62	5.70
SF-268	calcein	5.82	7.86	0.018 (0.003)	>10	>1	8.12	9.09	9.44
	CTG	4.77	9.94	0.037 (0.004)	>10	1.756 (0.146)	5.28	8.013	5.53
SK-OV-3	calcein	4.93	>10	0.036 (0.005)	>10	1.182 (0.178)	10	7.56	7.11
	CTG	5.00	>10	0.042 (0.002)	>10	0.901 (0.072)	9.61	6.07	5.08
NCI/ADR RES	calcein	6.10	>10	0.037 (0.006)	>10	0.994 (0.117)	4.26	1.57	7.97
	CTG	5.03	5.38	0.046 (0.002)	>10	1.161 (0.099)	4.76	3.60	5.16
NCI-H460	calcein	>10	9.31	0.071 (0.013)	4.90	1.094 (0.104)	>10	>10	5.21
	CTG	>10	10	0.041 (0.002)	3.82	1.550 (0.115)	>10	>10	4.95
MCF7	calcein	2.33	5.21	0.039 (0.011)	>10	0.812 (0.204)	3.29	3.86	6.19
	CTG	1.87	4.38	0.049 (0.004)	>10	3.13 (0.277)	4.49	2.50	5.51
A549	calcein	>10	>10	0.062 (0.008)	>10	2.094 (0.364)	4.85	4.62	>10
	CTG	8.17	>10	0.048 (0.003)	>10	1.909 (0.137)	4.93	3.92	8.14
NmuMG	calcein	>10	8.84	0.392 (0.083)	>10	1.493 (0.241)	9.87	>10	8.43
	CTG	>10	>10	0.120 (0.021)	>10	1.522 (0.228)	8.31	6.39	>10

Table S2 (cont.). Cytotoxicity data for all colchicine neoglycosides and relevant standards (in μM with % error in parentheses).

		Col60	Col61	Col62	Col65	Col67	Col68	Col69	Col70
Du145	calcein	6.20	7.51	9.92	0.939 (0.207)	1.64	1.73	0.78	0.80
	CTG	4.79	7.81	>10	0.850 (0.074)	1.71	4.19	1.56	2.05
HCT-116	calcein	6.56	5.95	>10	0.897 (0.081)	1.46	3.87	1.13	1.65
	CTG	4.68	5.05	9.68	0.918 (0.076)	1.65	3.37	1.51	2.18
Hep 3B	calcein	>10	>10	1.36	1.875 (0.219)	10	7.92	2.33	>10
	CTG	4.97	9.10	9.99	1.698 (0.064)	7.93	5.22	3.47	>10
SF-268	calcein	4.13	7.91	5.88	0.665 (0.205)	1.20	3.27	0.79	1.09
	CTG	5.03	5.20	>10	0.800 (0.045)	1.75	3.85	1.86	1.76
SK-OV-3	calcein	4.15	>10	>10	1.031 (0.187)	2.14	2.43	1.78	1.21
	CTG	4.38	7.99	>10	0.634 (0.035)	2.15	4.50	1.73	2.69
NCI/ADR RES	calcein	4.65	7.19	8.69	0.694 (0.167)	1.30	2.04	2.06	1.56
	CTG	4.98	8.23	>10	0.893 (0.048)	1.99	4.88	1.71	1.64
NCI-H460	calcein	5.88	5.77	>10	0.744 (0.106)	2.54	3.12	1.03	5.24
	CTG	3.98	5.00	>10	0.862 (0.046)	1.69	4.41	1.34	0.76
MCF7	calcein	3.04	3.71	9.20	0.648 (0.134)	1.84	2.54	4.02	5.67
	CTG	3.36	6.02	8.76	0.946 (0.038)	1.40	2.42	1.22	1.43
A549	calcein	>10	4.98	>10	2.301 (0.328)[2]	3.87	7.67	4.41	5.44
	CTG	>10	>10	>10	1.491 (0.162)	2.97	4.77	2.51	3.47
NmuMG	calcein	6.04	>10	>10	3.318 (0.594)[2]	8.38	9.44	>10	>10
	CTG	8.37	9.74	>10	7.747 (0.576)[2]	7.54	9.83	7.90	6.81

		Col71	Col0	6	7	8	colchine	doxorubicin	paclitaxel
Du145	calcein	4.67	>10	0.084 (0.010)	0.174 (0.026)	0.084 (0.010)	0.022 (0.003)	0.339 (0.061)	0.290 (0.132)
	CTG	>10	>10	0.064 (0.011)	0.142 (0.012)	0.064 (0.011)	0.169 (0.032)	0.842 (0.138)	0.432 (0.189)[2]
HCT-116	calcein	8.02	4.84	0.098 (0.021)	0.221 (0.039)	0.098 (0.021)	0.091 (0.033)	0.524 (0.157)	0.275 (0.200)[2]
	CTG	>10	9.64	0.116 (0.021)	0.192 (0.023)	0.116 (0.021)	0.153 (0.029)	0.812 (0.102)	0.168 (0.201)[2]
Hep 3B	calcein	9.38	5.14	0.284 (0.066)	0.506 (0.104)	0.284 (0.066)	-[1]	0.268 (0.039)[2]	0.166 (0.021)
	CTG	>10	>10	0.141 (0.021)	0.431 (0.029)	0.141 (0.021)	0.329 (0.041)[2]	0.519 (0.033)[2]	-[1]
SF-268	calcein	>10	9.70	0.191 (0.039)	0.104 (0.012)	0.191 (0.039)	0.035 (0.011)[2]	0.385 (0.041)[2]	0.315 (0.076)
	CTG	>10	6.15	0.116 (0.033)	0.156 (0.013)	0.116 (0.033)	0.067 (0.028)[2]	0.249 (0.026)[2]	-[1]
SK-OV-3	calcein	7.62	>10	0.316 (0.082)	0.097 (0.008)	0.316 (0.082)	0.024 (0.003)[2]	0.621 (0.207)[2]	0.034 (0.011)[2]
	CTG	>10	>10	0.455 (0.071)	0.184 (0.041)	0.455 (0.071)	0.035 (0.008)[2]	0.394 (0.031)[2]	0.042 (0.004)[2]
NCI/ADR RES	calcein	6.32	>10	0.065 (0.010)	0.127 (0.013)	0.065 (0.010)	0.027 (0.002)	0.174 (0.041)	0.043 (0.005)[2]
	CTG	>10	>10	0.079 (0.009)	0.181 (0.021)	0.079 (0.009)	0.018 (0.002)	0.570 (0.081)	-[1]
NCI-H460	calcein	>10	8.92	0.072 (0.012)	4.082 (0.412)	0.072 (0.012)	0.022 (0.015)	1.001 (0.164)[2]	0.105 (0.022)
	CTG	>10	4.79	0.087 (0.008)	1.495 (0.465)	0.087 (0.008)	0.027 (0.006)	0.651 (0.120)	0.053 (0.008)
MCF7	calcein	6.37	3.30	0.163 (0.036)	0.128 (0.032)	0.163 (0.036)	0.21 (0.04)[2]	0.240 (0.021)	0.195 (0.028)
	CTG	5.15	3.94	0.072 (0.009)	0.087 (0.034)	0.072 (0.009)	0.221 (0.008)[2]	0.311 (0.054)	4.558 (0.340)[2]
A549	calcein	>10	>10	0.222 /0.030)	0.203 (0.103)	0.222 (0.030)	0.118 (0.025)	0.770 (0.135)	0.075 (0.142)[2]
	CTG	>10	2.00	0.131 (0.015)	0.274 (0.028)	0.131 (0.015)	0.059 (0.013)	0.318 (0.021)	-[1]
NmuMG	calcein	>10	>10	1.645 (0.430)	0.553 (0.094)	1.645 (0.430)	0.231 (0.046)[2]	0.671 (0.098)[2]	0.893 (0.201)[2]
	CTG	>10	>10	0.630 (0.056)	0.513 (0.107)	0.630 (0.056)	-[1]	0.942 (0.071)	0.021 (0.007)

[1]not determined; [2]data from four replicates.

Table S3. Drug combination studies.

library member	plus colchicine (mean CI/median CI)		plus paclitaxel (mean CI/median CI)	
taxol	0.67	0.42	N/A	N/A
colchicine	N/A	N/A	0.77	0.18
6	2.32	1.96	0.57	0.20
7	3.16	2.20	0.21	0.09
8	2.98	1.47	0.90	0.57
Col 6	1.30	1.19	0.88	0.52
Col16	5.97	5.33	0.40	0.11
Col19	0.54	0.50	43.20	13.40
Col21	1.01	0.64	4.96	2.50
Col34	31.49	21.50	0.18	0.12
Col45	107.40	102.90	0.12	0.07

Table S4. Recommended symbols descriptors for the combination index (CI) method.

range of combination Index (CI)	symbol	description
<0.1	+++++	very strong synergism
0.1-0.3	++++	strong synergism
0.3-0.7	+++	synergism
0.7-0.85	++	moderate synergism
0.85-0.90	+	slight synergism
0.90-1.10	+ -	nearly additive
1.10-1.20	-	slight antagonism
1.20-1.45	--	moderate antagonism
1.45-3.3	---	antagonism
3.3-10	----	strong antagonism
>10	-----	very strong antagonism

Table S5. HRMS data for compounds in Table 1.

Library Member	Calculated Formula $[M+H]^+$	Calculated m/z	Observed m/z
Col 0	$C_{29}H_{38}N_2O_{12}Na$	629.23170	629.23224
Col 6	$C_{29}H_{39}N_2O_{11}$	591.25484	591.25216
Col45	$C_{29}H_{35}N_2O_{12}$	603.21845	603.21605
Col19	$C_{28}H_{37}N_2O_{10}$	561.24427	561.24143
Col21	$C_{28}H_{37}N_2O_{10}$	561.24427	561.24057
Col56	$C_{30}H_{39}N_2O_{12}$	619.24975	619.24712
Col65	$C_{29}H_{39}N_2O_{10}$	575.25992	575.25753

References.

S1. Langenhan, J. M.; Peters, N. R.; Guzei, I. A.; Hoffmann, F. M.; Thorson, J. S. *Proc. Natl. Acad. Sci.* U.S.A. **2005**, *102*, 12305.

S2. (a) Chou T.-C.; Hayball, M. P. *Dose effect analysis; software and manua*l. *Biosoft*, Cambridge, U.K., **1996**. (b) Chou, T.-C.; Talalay, P. *Adv. Enzyme Regulation* **1984**, *22*, 27.