Supplementary Material (ESI) for Chemical Communications

Electronic Supporting Information

Bile Acid-Based Polyaminocarboxylate Conjugates as Targeted Antitumor Agents

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Experimental Section

General. ^{1}H and ^{13}C NMR spectra were obtained using a Bruker 300MHz instrument, and chemical shifts are reported in ppm on the δ scale relative to TMS, TSP, or solvent. All reagents were purchased from Aldrich and used as received unless otherwise noted. Fast atom bombardment (FAB) high resolution mass spectra (HRMS) were obtained on JEOL double sector JMS-AX505HA mass spectrometer (University of Notre Dame, IN). The analytical HPLC was performed on Agilent 1200 equipped with a dioarray detector (λ = 254 nm), themostat set at 35 ^{o}C and a Zorbax Eclipse XDB-C18 column (4.6 x 150 mm, 80Å). An isocratic mobile phase (method 1: 100% CH_3CN/10 min, method 2: 100% CH_3OH/10 min) or a binary gradient mobile phase (method 3: 50%-20%A/30min, 50%-80%B/30 min; solvent A = H_2O; solvent B = CH_3CN) at a flow rate of 1 mL/min was used for analytical HPLC. Fluorescence Spectra were recorded on a PC1 Photon counting spectrofluorometer (ISS, Inc., Champaign, IL) with excitation at 446 nm and bandwidth of 8 nm. Flurorescence images were obtained using Olympus DSU Spinning disk confocal microscope (Olympus America Inc., Melville, NY) with a band-pass filter set at 446/20nm (Excitation) and 535/30 nm (Emission). All UV absorbance measurements

were obtained on an Agilent 8453 diode array spectrophotometer equipped with a 8-cell transport system (designed for 1-cm cell).

4-(3,12-Dihydroxy-10,13-dimethyl-hexadecahydro-cyclopenta[a]phenanthren-17-yl)-1-(2-thioxo-thiazolidin-3-yl)-pentan-1-one (2). To a vigorously stirred solution of deoxycholic acid (1 g, 2.6 mmol) in CH_2Cl_2 (60 mL) was added DCC (684 mg, 3.6 mmol), 2-mercaptothiazoline (354 mg, 3 mmol), and catalytic amount (40 mg) of *N*,*N*-dimethyl amino pyridine (DMAP). The reaction mixture was stirred for 24 h at room temperature at which time the reaction mixture was transferred to a separate-funnel and sequentially washed with 0.5N NaOH solution (100 mL × 2), and 0.1N HCl (100 mL × 2), and water (100 mL). The organic layer was dried over $MgSO_4$, filtered, and concentrated in *vacuo* to provide pure yellow solid **2** (1.07 g, 82%). The crude product was directly used for the next step without further purification. ^{1}H NMR ($CDCl_3$) δ 0.68 (s, 3H), 0.90 (s, 3H), 0.98 (d, 3H), 1.10-1.95 (m, 23H), 3.25-3.35 (m, 4H), 3.53-3.67 (m, 1H), 3.98 (s, 1H), 4.57 (t, 2H); ^{13}C NMR ($CDCl_3$) δ 12.73, 17.49, 23.16, 23.76, 26.17, 27.21, 27.63, 28.33, 28.60, 30.40, 30.74, 33.52, 34.11, 35.32, 35.48, 35.87, 36.00, 36.40, 42.08, 46.52, 47.44, 48.09, 53.59, 56.16, 71.53, 72.99, 175.35, 201.52. HRMS (Positive ion FAB) Calcd for $C_{27}H_{44}NO_3S_2$ $[M + H]^+$ *m*/*z* 494.2763 Found: $[M + H]^+$ *m*/*z* 494.2744. Analytical HPLC (t_R = 2.7 min, method 2).

4-(3,7-Dihydroxy-10,13-dimethyl-hexadecahydro-cyclopenta[a]phenanthren-17-yl)-1-(2-thioxo-thiazolidin-3-yl)-pentan-1-one (3). To a vigorously stirred solution of chenodeoxycholic acid (2 g, 5.1mmol) in CH_2Cl_2 (30 mL) was added DCC (1.26 g, 6.1 mmol), followed by the addition of 2-mercaptothiazoline (730 mg, 6.1 mmol) and catalytic amount (50 mg) of *N*,*N*-dimethyl amino pyridine (DMAP). The reaction mixture

was stirred for 24 h, the white precipitate of dicyclohexyl urea was filtered off and the filtrate volume was adjusted to 100 mL with CH_2Cl_2. The organic layer was washed with 0.5N NaOH solution (3×50 mL), and 0.1N HCl (2 × 50 mL), and water (50 mL), dried over anhydrous magnesium sulfate, filtered, and concentrated in *vacuo* to yield the crude product. Purification by column chromatography on silica gel using ethyl acetate and hexane (1:3) afforded activated chenodeoxycholic acid derivative **3** (2.24 g, 89%) as a yellow crystalline solid. The crude product was directly used for the next step without further purification. ^{1}H NMR ($CDCl_3$) δ 0.65 (s, 3H), 0.90 (s, 3H), 0.95 (d, 3H), 1.10-2.30 (m, 28H), 3.15-3.35 (m, 2H), 3.26 (t, 2H), 3.35-3.60 (m, 1H), 3.85 (s, 1H), 4.56 (t, 2H).; ^{13}C NMR (300 MHz, $CDCl_3$) δ 11.81, 18.55, 20.60, 22.82, 23.71, 28.22, 28.28, 30.65, 30. 78, 32.82, 34.61, 35.05, 35.35, 35.43, 35.62, 39.41, 39.63, 39.80, 41.50, 42.71, 50.42, 55.94, 56.11, 68.49, 71.96, 175.46, 201.52. HRMS (Positive ion FAB) Calcd for $C_{27}H_{43}NO_3S_2$ $[M + H]^+$ *m*/*z* 493.2684 Found: $[M + H]^+$ *m*/*z* 493.2678. Analytical HPLC (t_R = 3.8 min, method 1).

***tert*-butyl[4-(2-(Bis-carboxymethyl-amino)-3-{4-[4-(3,7,12-trihydroxy-10,13-dimethylhexadecahydro-cyclopenta[a]phenanthren-17-yl)-pentanoylamino]-phenyl}-propyl)-7-carboxymethyl-[1,4,7]triazonan-1-yl]-acetic acid (6).** To a solution of **5** (95 mg, 0.13 mmol) in CH_2Cl_2 (2 mL) was added Et_3N (10.2 mg, 0.1 mmol) and **1** (66 mg, 0.13 mmol). The reaction mixture was refluxed for 3 days. The resulting solution was evaporated, and the residue was purified via column chromatography with neutral alumina eluting with 2.5% CH_3OH-CH_2Cl_2 starting from CH_2Cl_2 to afford pure **6** as a creamy solid (31 mg, 21%). ^{1}H NMR ($CDCl_3$) δ 0.68 (s, 3H), 0.89 (s, 3H), 1.03–2.98 (m, 80H), 3.25–3.49 (m, 9H), 3.85 (s, 1H), 3.97 (s, 1H), 7.07 (d, 2H), 7.48 (d, 2H). ^{13}C NMR

($CDCl_3$) δ 12.50, 17.55, 22.47, 23.31, 26.46, 28.19, 34.76, 39.49, 41.55, 41.76, 46.42, 52.17, 56.12, 68.48, 71.91, 73.16, 81.12, 120.06, 129.56, 137.03, 170.77, 170.97, 171.03. HRMS (Positive ion FAB) Calcd for $C_{63}H_{106}N_5O_{12}$ $[M + H]^+$ *m*/*z* 1124.7838. Found: $[M + H]^+$ *m*/*z* 1124.7819. Analytical HPLC (t_R = 1.5 min, method 1).

[4-*tert*-butoxycarbonylmethyl-7-(2-(tert-butoxycarbonylmethyl-amino)-3-{4-[4-(3,12-dihydroxy-10,13-dimethyl-hexadecahydro-cyclopenta[a]phenanthren-17-yl)-pentanoylamino]-phenyl}-propyl)-[1,4,7]triazonan-1-yl]-acetic acid tert-butyl ester (8). To a solution of **4** (270 mg, 0.44 mmol) in CH_2Cl_2 (6 mL) was added Et_3N (44 mg, 0.44 mmol) and **2** (191 mg, 0.44 mmol). The reaction mixture was refluxed for 2 days. The resulting solution was evaporated, and the residue was purified via column chromatography with neutral alumina eluting with 1.5 % CH_3OH starting from CH_2Cl_2 to afford pure **8** as a creamy solid (223 mg, 54%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.68 (s, 3H), 0.91 (s, 3H), 0.93–1.93 (m, 54H), 2.20–2.93 (m, 20H), 3.20–3.45 (m, 6 H), 3.52–3.68 (m, 1H), 3.98 (s, 1H), 7.10 (d, 2H), 7.43 (d, 2H). ^{13}C NMR (300 MHz, $CDCl_3$) δ 12.67, 17.49, 23.16, 23.79, 26.22, 27.29, 27.61, 28.10, 28.21, 28.46, 33.56, 33.76, 34.20, 35.20, 35.38, 36.05, 36.39, 38.88, 42.15, 46.52, 47.90, 49.89, 53.43, 55.58, 55.90, 59.40, 71.63, 73.11 80.63, 80.86, 119.81, 129.56, 129.97, 136.78, 171.26, 171.64, 172.48. HRMS (Positive ion FAB) Calcd for $C_{57}H_{96}N_5O_9$ $[M + H]^+$ *m*/*z* 994.7208 Found: $[M + H]^+$ *m*/*z* 994.7182. Analytical HPLC (t_R = 1.42 min, method 1).

[4-*tert*-Butoxycarbonylmethyl-7-(2-(tert-butoxycarbonylmethyl-amino)-3-{4-[4-(3,7-dihydroxy-10,13-dimethyl-hexadecahydro-cyclopenta[a]phenanthren-17-yl)-penta-noylamino]-phenyl}-propyl)-[1,4,7]triazonan-1-yl]-acetic acid tert-butyl ester (9). To a solution of **4** (92 mg, 0.15 mmol) in CH_2Cl_2 (5 mL) was added Et_3N (15 mg, 0.15

mmol) and **3** (73 mg, 0.15 mmol). The reaction mixture was refluxed for 2 days. The resulting solution was evaporated, and the residue was purified via column chromatography with neutral alumina eluting with 2.5% methanol starting from CH_2Cl_2 to afford pure **9** as a creamy solid (36 mg, 24%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.70 (s, 3H), 0.93 – 3.50 (m, 82H) 3.80 (s, 1H), 3.80 (s, 1H), 7.16 (d, 2H), 7.50 (d, 2H). ^{13}C NMR (300 MHz, $CDCl_3$) δ 10.81, 17.59, 20.39, 22.00, 23.25, 27.08, 27.91, 29.36, 29.96, 31.82, 32.66, 33.58, 34.52, 34.82, 35.16, 35.58, 39.07, 39.36, 39.67, 41.76, 42.30, 50.16, 55.95, 67.63, 71.44, 81.01, 120.10, 129.19, 129.20, 136.80, 171.01, 173.71. HRMS (Positive ion FAB) Calcd for $C_{57}H_{96}N_5O_9$ $[M + H]^+$ *m/z* 994.7208 Found: $[M + H]^+$ *m/z* 994.7234. Analytical HPLC (t_R = 1.8 min, method 2).

[4-(2-(Bis-carboxymethyl-amino)-3-{4-[4-(3,7,12-trihydroxy-10,13-dimethyl-hexa-decahydrocyclopenta[a]phenanthren-17-yl)-pentanoylamino]-phenyl}-propyl)-7-carboxymethyl-[1,4,7]triazonan-1-yl]-acetic acid (CA-NETA). To a solution of **6** (15 mg, 0.014 mmol) was dropwise added 4M HCl in 1,4-dioxane (5 mL) at 0–5 ^{o}C. After the addition, the reaction mixture was gradually increased to room temperature and stirred for 18 h. To this solution, ether (30 mL) was added and continuously stirred for 30 min. The resulting mixture was placed in the freezer for 2 h**.** Solid residue was quickly filtered, washed with ethyl ether (5 mL), and immediately dissolved in DI H_2O and lyophilized to provide pure CA-NETA as a light brownish solid (13 mg, 90%). ^{1}H NMR (CD_3OD) δ 0.67 (s, 3H), 0.91 (s, 3H), 1.04–2.54 (m, 33H), 2.98–4.54 (m, 30H), 7.28 (d, 2H), 7.57 (d, 2H); ^{1}H NMR (CD_3OD) δ11.48, 16.23, 21.64, 22.72, 26.36, 27.24, 28.06, 29.64, 31.64, 33.46, 34.36, 34.95, 35.47, 38.92, 39.47, 41.51, 41.65, 45.96, 53.18, 54.47, 54.67, 67.54, 71.37, 72.54, 120.31, 129.45, 137.98, 166.70, 173.88, HRMS (ESI) Calcd for

$C_{47}H_{73}N_5O_{12}(NH_3)_3$ $[M + H]^+$ *m/z* 950.6052 Found: $[M + H]^+$ *m/z* 950.6069. Analytical HPLC (t_R = 1.3 min, method 2).

[4-Carboxymethyl-7-(2-(carboxymethyl-amino)-3-{4-[4-(3,12-dihydroxy-10,13-dimethylhexadecahydro-cyclopenta[a]phenanthren-17-yl)-pentanoylamino]-phenyl}-propyl)-[1,4,7]triazonan-1-yl]-acetic acid (DCA-NE3TA). To a solution of **8** (20 mg, 0.02 mmol) in 1,4 dioxane (1 mL) was dropwise added 4M HCl in 1,4-dioxane (1 mL) at 0–5 ^oC. After the addition, the reaction mixture was gradually increased to room temperature and stirred for 18 h. To this solution, ether (30 mL) was added and continuously stirred for 30 min. The resulting mixture was placed in the freezer for 2 h**.** Solid residue was quickly filtered, washed with ethyl ether (5 mL), and immediately dissolved in DI H_2O and lypophilized to provide pure **DCA-NE3TA** as a light brownish solid (19 mg, 98%). ^1H NMR (CD_3OD) 0.71 (s, 3H), 0.92– 3.90 (m, 55H), 3.97 (s, 1H), 4.09 (s, 1H), 7.28 (d, 2H), 7.57 (d, 2H); ^{13}C NMR (CD_3OD) 11.86, 16.39, 22.35, 23.50, 26.09, 27.02, 27.33, 28.53, 29.68, 31.79, 33.43, 33.63, 33.73, 33.91, 35.04, 35.55, 35.79, 36.05, 42.21, 44.44, 46.17, 71.16, 72.65, 120.41, 129.55, 130.54, 167.99, 173.96 HRMS (Positive ion FAB) Calcd for $C_{45}H_{72}N_5O_9$ $[M + H]^+$ *m/z* 826.5330 Found: $[M + H]^+$ *m/z* 826.5355. Analytical HPLC (t_R = 1.5 min, method 1).

[4-Carboxymethyl-7-(2-(carboxymethyl-amino)-3-{4-[4-(3,7-dihydroxy-10,13-dimethylhexadecahydro-cyclopenta[a]phenanthren-17-yl)-pentanoylamino]-phenyl}-propyl)-[1,4,7]triazonan-1-yl]-acetic acid (CDCA-NE3TA). To a solution of **3** (16 mg, 0.016 mmol) in 1,4-dioxane (1 mL) was dropwise added 4M HCl in 1,4-dioxane (1 mL) at 0–5 ^oC. After the addition, the reaction mixture was gradually increased to room temperature and stirred for 18 h. To this solution, ether (30mL) was

added and continuously stirred for 30 min. The resulting mixture was placed in the freezer for 2 h**.** Solid residue was quickly filtered, washed with ethyl ether (5 mL), and immediately dissolved in DI H_2O and lypophilized to provide pure **CDCA-NE3TA** as a light brownish solid (15 mg, 97 %). ^{1}H NMR (CD_3OD) δ 0.69 (s, 3H), 0.93 (s, 3H), 0.80–3.40 (m, 55H), 3.79 (s, 1H), 4.08 (s, 1H), 7.28 (d, 2H), 7.57 (d, 2H); ^{13}C NMR (CD_3OD) δ 10.76, 17.56, 20.38, 21.97, 23.23, 27.90, 29.94, 31.75, 32.66, 33.54, 34.50, 34.81, 35.13, 35.59, 39.06, 39.34, 39.66, 41.75, 42.29, 50.16, 55.92, 67.64, 71.44, 120.40, 129.28, 130.67, 138.07, 168.05, 173.87. HRMS (Positive ion FAB) Calcd for $C_{45}H_{72}N_5O_9$ $[M + H]^+$ *m/z* 826.5330 Found: $[M + H]^+$ *m/z* 826.5349. Analytical HPLC (t_R = 1.8 min, method 1).

1-[(2S,5S,9R,15R,16S)-9,16-dihydroxy-2,15-dimethyl-14-[5-oxo-5-(3-sulfanylidene-1,2-thiazolidin-2-yl)pentan-2-yl]tetracyclo[8.7.0.0^{2,7}.0^{11,15}}]heptadecan-5-yl]triaz-2-yn-2-ium-1-ide (11). To a vigorously stirred solution of azacholic acid **10**[35] (648 mg, 1.50 mmol) in CH_2Cl_2 (35 mL) was added EDC (431 mg, 2.25 mmol) followed by the addition of 2-mercaptothiazoline (215 mg, 1.80 mmol) and catalytic amount (27 mg) of *N*,*N*-dimethyl amino pyridine (DMAP). The reaction mixture was stirred for 24 h and diluted with CH_2Cl_2 (30 mL). The organic layer was washed with water (50 mL), 0.1M aq. NaOH solution (3 × 50 mL), 2M HCl (50 mL), and water (50 mL). The organic layer was dried over anhydrous magnesium sulfate, filtered and concentrated in *vacuo* to yield the crude product **11** (650 mg, 82%) as yellow oil which was directly used without further purification. ^{1}H NMR ($CDCl_3$) δ 0.7 (s, 3H), 0.9 (s, 3H), 1.0 (d, 3H), 1.1-2.45 (m, 24H), 3.1-3.4 (m, 1H), 3.3 (t, 2H), 3.85 (s, 1H), 4.0 (s, 1H), 4.56 (t, 2H).; ^{13}C NMR ($CDCl_3$) δ 12.53, 17.57, 22.61, 23.23, 26.62, 26.81, 27.58, 28.28, 30.74, 34.50, 34.77,

35.40, 35.47, 35.83, 39.46, 41.83, 41.94, 46.62, 47.62, 56.14, 61.35, 68.26, 72.98, 175.39, 201.58. HRMS (Positive ion FAB) Calcd for $C_{27}H_{43}N_4O_3S_2$ $[M + H]^+$ *m*/*z* 535.2777 Found: $[M + H]^+$ *m*/*z* 535.2764. Analytical HPLC (t_R = 2.3 min, method 2).

1-[(2S,5S,9R,15R,16S)-14-(4-{[4-(3-{4,7-bis[2-(tert-butoxy)-2-oxoethyl]-1,4,7-triazo-nan-1-yl}-2-{bis[2-(tert-butoxy)-2-oxoethyl]amino}propyl)phenyl]carbamoyl}-butan-2-yl)-9,16-dihydroxy-2,15-dimethyltetracyclo[8.7.0.0^{2,7}.0^{11,15}]heptadecan-5-yl]triaz-2-yn-2-ium-1-ide (12). To a solution of **4** (652.4 mg, 1.05 mmol) in CH_2Cl_2 (10 mL) was added Et_3N (106 mg, 1.05 mmol) and **11** (563 mg, 1.05 mmol). The reaction mixture was refluxed for 2 days. The resulting solution was evaporated, and the residue was purified via neutral alumina eluting with 1% CH_3OH/CH_2Cl_2 to afford pure **12** as a creamy solid (566 mg, 52%). ^{1}H NMR ($CDCl_3$) δ 0.65 (s, 3H), 0.87 (s, 3H), 0.92- 1.15 (m, 3H), 1.20-2.05 (m, 20H), 2.15-2.90 (m, 10H), 3.10-3.56 (m, 5H), 3.85 (s, 1H), 3.96 (s, 1H), 7.02 (d, 2H), 7.53 (d, 2H); ^{13}C NMR (CD_3OD) δ 11.83, 16.58, 21.95, 22.93, 26.51, 27.21, 27.25, 28.26, 31.85, 33.05, 33.70, 34.41, 34.60, 35.30, 35.36, 35.64, 37.39, 39.59, 41.66, 42.03, 46.58, 48.47, 61.39, 67.44, 72.56, 81.04, 120.09, 126.72, 133.25, 137.36, 171.13, 173.64. HRMS (Positive ion FAB) Calcd for $C_{57}H_{95}N_8O_9$ $[M + H]^+$ *m*/*z* 1035.7222 Found: $[M + H]^+$ *m*/*z* 1035.7218. Analytical HPLC (t_R = 2.0 min, method 3).

1-[(2S,5R,9R,15R,16S)-14-{4-[(4-{3-[4,7-bis(carboxymethyl)-1,4,7-triazonan-1-yl]-2-[bis(carboxymethyl)amino]propyl}phenyl)carbamoyl]butan-2-yl}-9,16-dihydroxy-2,15-dimethyltetracyclo[8.7.0.0^{2,7}.0^{11,15}]heptadecan-5-yl]triaz-2-yn-2-ium-1-ide (13). To a solution of **12** (111 mg, 0.11 mol) in 1,4-dioxane (4.5 mL) was dropwise added 4M HCl in 1,4-dioxane (4.5 mL) at 0–5 ^{o}C. After the addition, the reaction mixture was gradually warmed to room temperature and stirred for 22 h. To the resulting mixture was

added ether (30 mL), and the resulting mixture was continuously stirred for 30 min. The resulting mixture was placed in the freezer for 2 h**.** Solid residue was quickly filtered, immediately dissolved in MeOH, lypophilized to provide **13** as a white solid (97 mg, 96%). ^{1}H NMR (CD_3OD) δ 0.72 (s, 3H), 0.93 (s, 3H), 1.06-1.08 (m, 3H), 1.28-2.10 (m, 24H), 2.20-2.48 (m, 4H), 2.85-3.62 (m, 23H), 3.80-4.13 (m, 7H), 7.23 (d, 2H), 7,62 (d, 2H). ^{13}C NMR (CD_3OD) δ 11.71, 16.50, 21.84, 22.87, 26.52, 27.39, 28.19, 31.82, 33.69, 33.84, 34.33, 34.56, 35.23, 35.36, 35.65, 39.55, 41.68, 41.99, 44.64, 46.12, 49.57, 56.65, 61.27, 67.38, 72.49, 120.41, 129.72, 130.47, 138.14, 167.97, 174.01. HRMS (Positive ion FAB) Calcd for $C_{45}H_{71}N_8O_9$ $[M + H]^+$ *m/z* 867.5344. Found: $[M + H]^+$ *m/z* 867.5364. Analytical HPLC (t_R = 1.8 min, method 1).

2-{[1-(4-{4-[(2S,5R,9R,15R,16S)-5-amino-9,16-dihydroxy-2,15-dimethyltetracyclo-[8.7.0.0^{2,7}.0^{11,15}]heptadecan-14-yl]pentanamido}phenyl)-3-[4,7-bis(carboxymethyl)-1,4,7-triazonan-1-yl]propan-2-yl](carboxymethyl)amino}acetic acid (14). To a solution of **13** (93 mg, 0.092 mmol) in MeOH (15 mL) in a reaction bottle for hydrogenation was added dry 10% Pd/C (47 mg). The reaction mixture was subject to hydrogenation for 23 h in a Parr hydrogenator and filtered via celite bed and washed thoroughly with MeOH. The filtrate was concentrated in *vacuo* to provide **14** as a white waxy solid (90 mg, 96%). ^{1}H NMR (CD_3OD) δ 0.73 (s, 3H), 0.96 (s, 3H), 1.06-3.80 (m, 61H), 3.82 (s, 1H), 4.01 (s, 1H), 7.23 (d, 2H), 7,62 (d, 2H). ^{13}C NMR (CD_3OD) δ 11.66, 16.41, 21.67, 22.78, 25.34, 26.60, 27.40, 28.23, 31.81, 33.58, 33.72, 34.07, 34.17, 34.36, 34.65, 35.68, 39.61, 41.57, 41.69, 46.59, 51.27, 55.99, 59.31, 67.22, 72.37, 120.38, 129.23, 130.94, 137.99, 168.33, 173.92. HRMS (Positive ion FAB) Calcd for

$C_{45}H_{73}N_6O_9 \cdot 5HCl$ $[M + H]^+$ *m/z* 1021.4273. Found: $[M + H]^+$ *m/z* 1021.4321. Analytical HPLC (t_R = 1.5 min, method 1).

NBD-CA-NE3TA. To a solution of **14** (43 mg, 0.051 mmol) in MeOH (3 mL) in an ice-bath was added 7-chloro-4-nitrobenzo-2-oxa-1,3-diazole (NBD-Cl, 16 mg, 0.08 mmol). The resulting mixture was stirred for 1 day, and Et_3N (11 μL, 0.08 mmol) was added to the reaction mixture which was further stirred for 1 day. The reaction mixture was concentrated in *vacuo*. Pure **NBD-CA-NE3TA** (19 mg, 38%) was isolated using prep-TLC eluted with CH_3CN: H_2O (30:8). ^{1}H NMR (CD_3OD) 0.65 (s, 3H), 0.82-3.80 (m, 63H), 3.90 (s, 1H), 6.68 (d, 1H), 7.12 (d, 2H), 7.47 (d, 2H), 8.52 (d, 1H). ^{13}C NMR (CD_3OD) δ 11.75, 16.55, 21.76, 22.85, 25.37, 26.61, 27.45, 28.29, 29.39, 31.84, 33.78, 34.17, 34.40, 34.64, 34.74, 35.41, 39.63, 41.40, 41.73, 46.13, 49.08, 51.31, 57.48, 67.18, 72.33, 104.94, 120.19, 129.27, 131.22, 135.20, 138.00, 144.11, 145.45, 155.37, 169.46, 174.99. HRMS (Positive ion FAB) Calcd for $C_{51}H_{73}N_9O_{12}(NH_3)_3$ $[M + H]^+$ *m/z* 1054.6175. Found: $[M + H]^+$ *m/z* 1054.6177. Analytical HPLC (t_R = 1.2 min, method 3)

Cell culture. Human cervix HeLa cell line was obtained from ATCC (Rockville, MD) and cultured in minimum essential medium (MEM) with L-glutamine (2 mM), Earle's BSSand sodium bicarbonate (1.5 g/L), supplemented with 10% fetal bovine serum (FBS), non-essential amino acids (0.1 mM), sodium pyruvate (1 mM) and antibiotic/antimycotic solution in a humidified atmosphere with 5% CO_2 at 37 ^oC. Human colon cancer cell line HT29 was kindly provided by professor Raj Metha (Illinois Institute of Technology Research institute, Chicago, IL) and maitained in a humidified atmosphere with 5% CO_2 at 37 ^oC in RPMI-1640 medium, containing 10% FBS with L-glutamine and antibiotic/antimycotic.

Antiproliferative activity. Cells were seeded onto 96-well plate at density of 2,000 cells for Hela cells or 5,000 cells for HT29 cells per well in 0.1 mL complete medium and allowed to attach for 24 h. Varying concentrations of the test compounds in the final volume of 0.1 mL complete medium were then added in at least five series dilutions and incubated for 72 h. To measure cell proliferation, the Cell Titer 96 aqueous nonreactive cell proliferation assay (Promega Life Sciences, Madison, WI) was used according to the manufacturer's instructions. Briefly, MTS (2 mg/mL) and PMS (0.92 mg/ml) were mixed in a ratio of 20:1. An aliquot (20 μL) of the MTS/PMS mixture was added into each well, and the plate was incubated for 3 h at 37 °C. Optical absorbance at 490 nm was then recorded with an enzyme-linked immunosorbent assay (ELISA) microtiter plate reader (Biotek). Each experiment was done at least in triplicate. Antiproliferative activity of the test compounds was expressed as the fraction of optical densities of treated the cells relative to the untreated solvent controls.[32] The data were plotted in GraphPad Prizm 3.0. Nonlinear regression analysis was used to determine IC_{50} values. IC_{50} of the compounds was expressed as the concentration of the drugs inhibiting cell growth by 50%.

TEM measurement. Stock solutions of compounds CA-NETA and CDCA-NE3TA were prepared in 18 MΩ H_2O and diluted to the final concentrations of CA-NETA (1 mM, pH = 7) and CDCA-NE3TA (10 mM, pH = 7). A 5 μL aliquot of CDCA-NE3TA solution was added to a cavity of a Micro-Test Staining Dish (Cat# 71564, Electron Microscopy Sciences, PA), and a gold-coated grid was inverted into the solution in the plate and air-dried for 10 min. The grid was further dipped into a drop of the solution (10 μL) in a cavity of the plate and air-dried overnight. The grid was transferred into a desiccator and further dried *in vacuo* for 1.5 days. TEM images were obtained at room temperature on a

Hitachi HF-2000 High resolution TEM (Hitachi) operated at 80 kV, equipped with a charged-coupled device (CCD) camera.

Fluorescence and UV spectra of NBD-CA-NE3TA. Fluorescence Spectra were recorded on a PC1 Photon counting spectrofluorometer (ISS, Inc., Champaign, IL) with excitation at 446 nm, bandwidth of 8 nm, data collection every 1 nm at 20 ^{o}C. Stock solution (1 mM) of NBD-CA-NE3TA was prepared by dissolving sample in 0.005% DMSO-H_2O. UV-Vis measurements were carried out by adding 10 μL aliquots of the stock solution via a micropipette into 2 mL of H_2O in a quartz cuvette, while the measurement of fluorescence was carried out by adding 1 μL aliquots of the stock solutions into 1 mL H_2O in a quartz cuvette. The mixtures were stirred briefly for equilibration prior to data acquisition.

Flurorescence Imaging of live cancer cells. HT29 cancer cells were plated in glass cover slips which placed in 6-well plates, and were incubated with growth media in a humidified atmosphere with 5% CO_2 at 37 ^{o}C overnight. Control cells or cells containing NBD-CA-NE3TA (50 μM) were incubated with media for 0.5 h under 5% CO_2 at 37 ^{o}C. At the end of the incubation time, cells were rinsed with PBS three times and subsequently observed under the Olympus DSU Spinning disk confocal microscope with a band-pass filter set at 446/20nm (Excitation) and 535/30 nm (Emission).

Acknowledgement

The authors thank Dr. Nick Menhart for the flurorescence spectroscopy and Dr. Vytas Bindokas for the assistance in obtaining the flurorescence images. We thank Dr. Sankar Bhuniya for preparation of compound **3** and Mr. Haisung Lee for preparing the samples for TEM measurements.

Figure S1. Effects of bile acid-based ligands CA-NE3TA (▲), DCA-NE3TA (■), CDCA-NE3TA(♦), NE3TA (○),[22] DTPA (×),[22] and DFO (●)[22] on viability of Hela cancer cells.

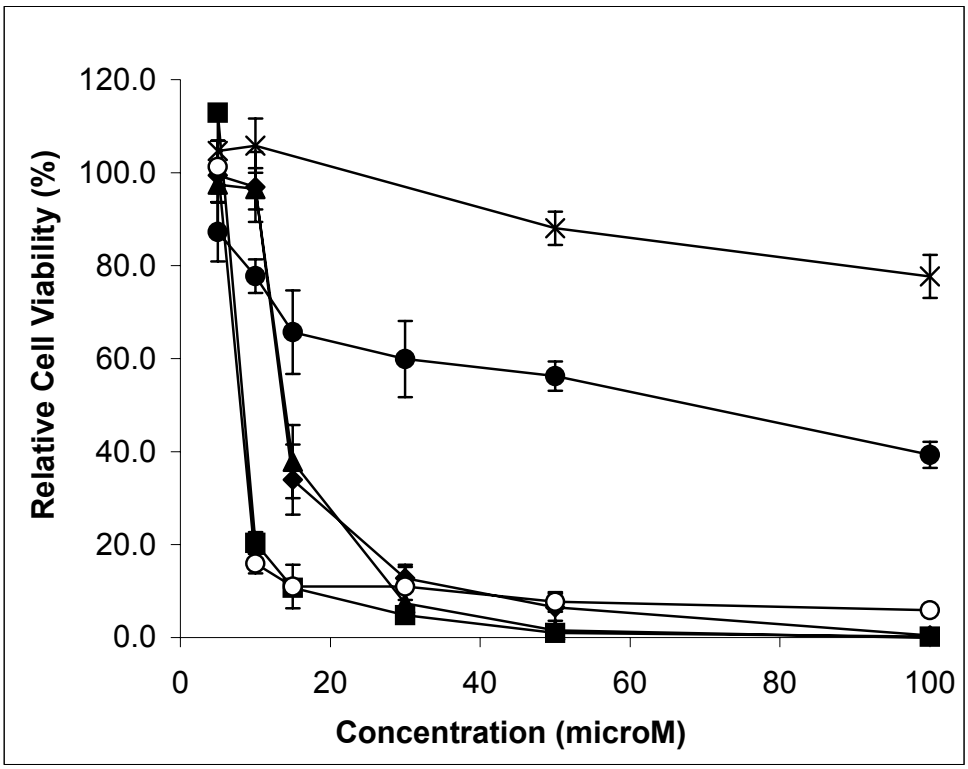


Figure S2. Effects of bile acid-based ligands CA-NE3TA (▲), DCA-NE3TA (■), CDCA-NE3TA (♦), NE3TA (○),[22] DTPA (×),[22] and DFO (●)[22] on viability of HT29 cancer cells.

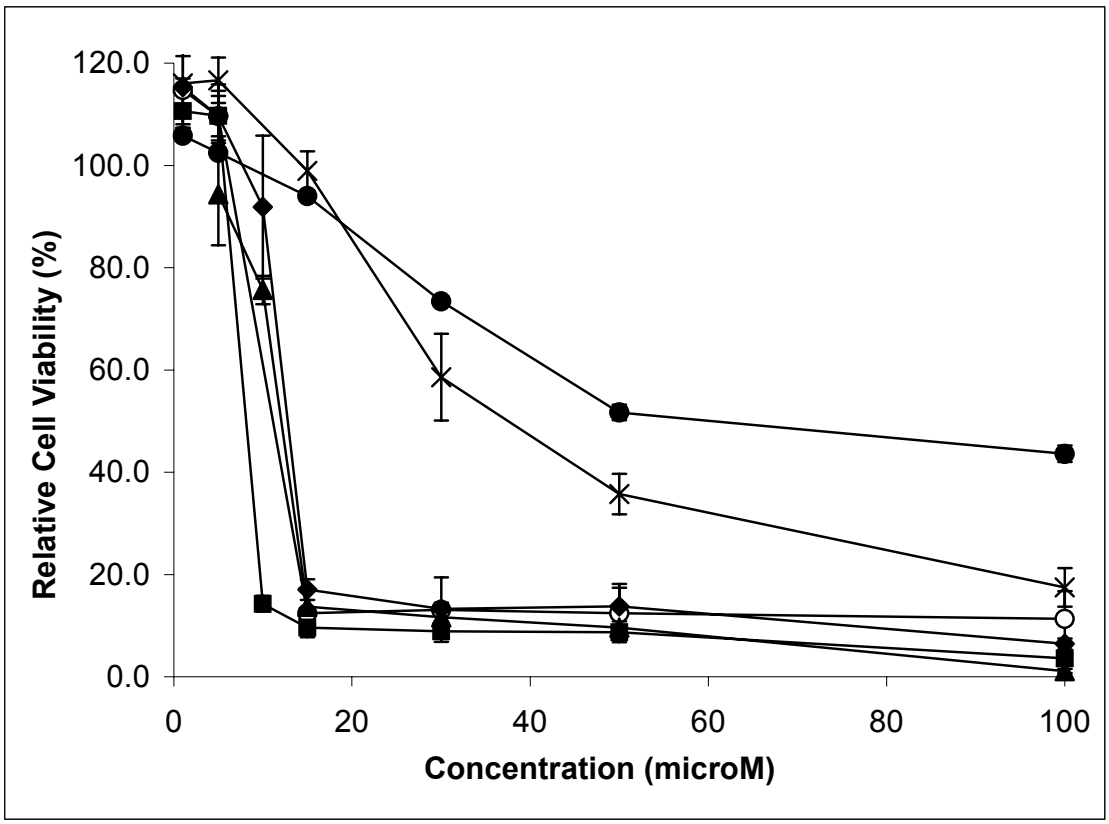
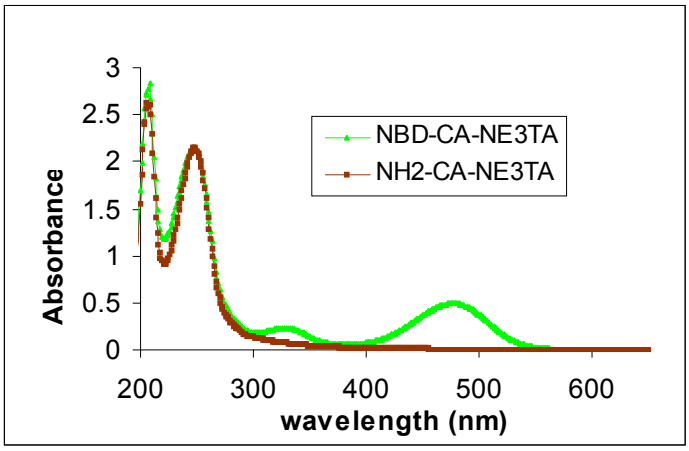


Figure S3. UV and Fluorescence spectra of NH_2-CA-NE3TA (14) and NBD-CA-NE3TA.



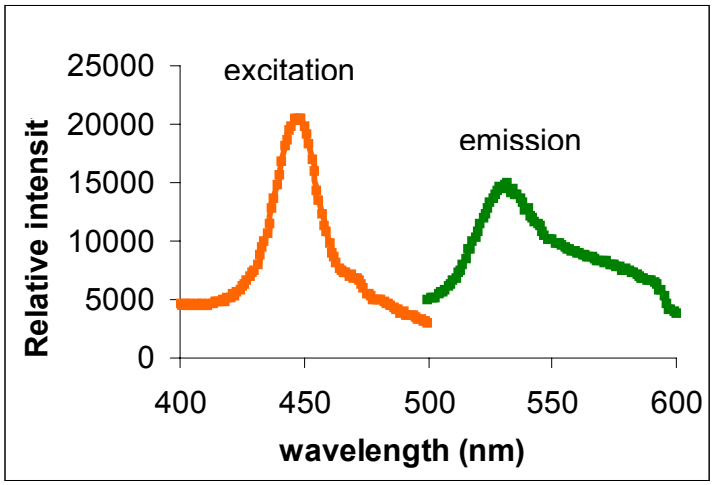
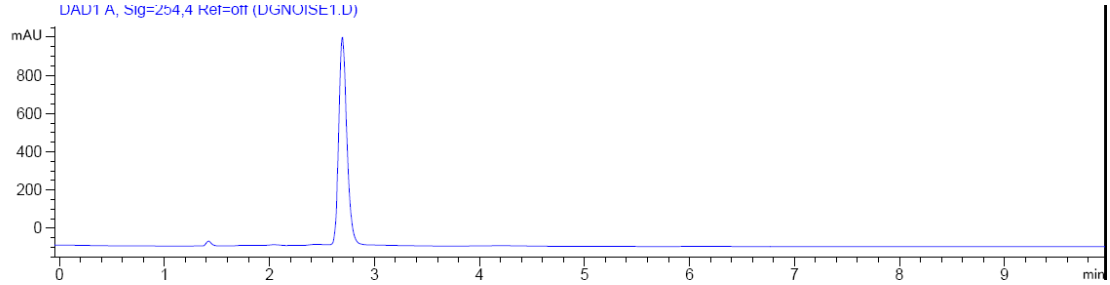
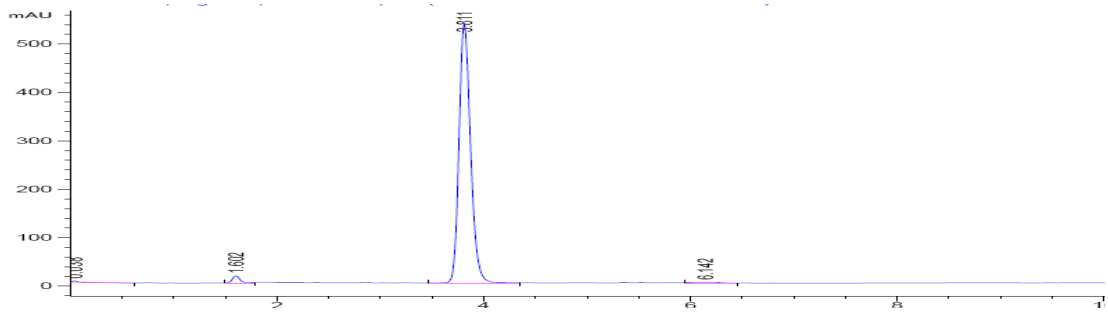


Figure S4. HPLC chromatograms of the new compounds

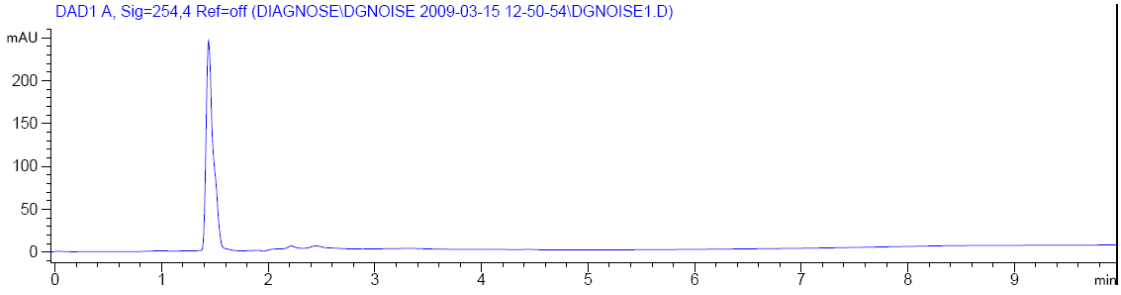
(A) Compound 2 (Method 2)



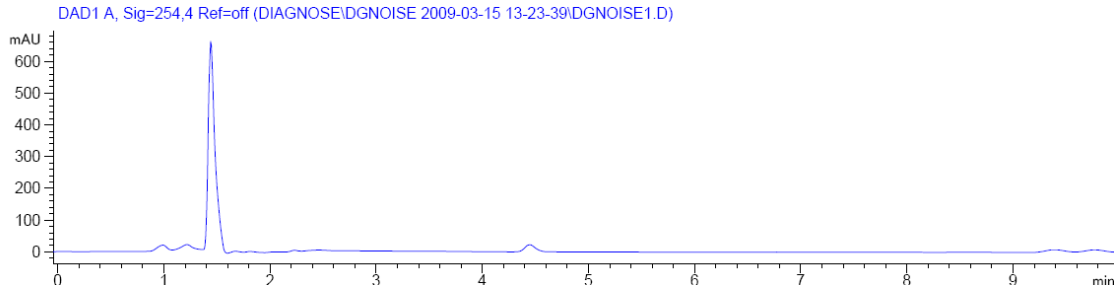
(B) Compound 3 (Method 1)



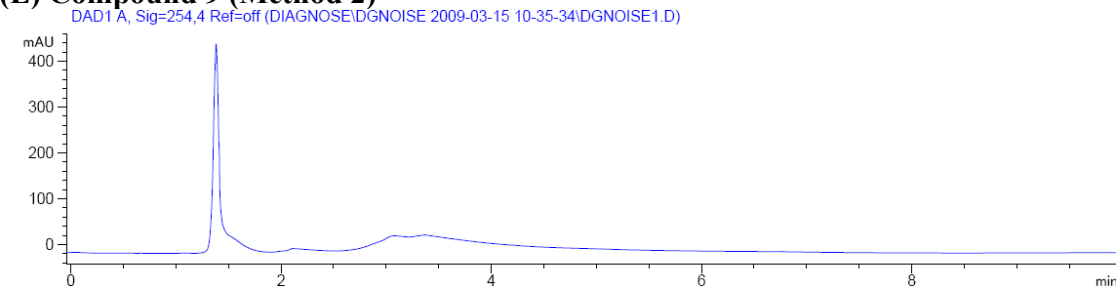
(C)Compound 6 (Method 1)



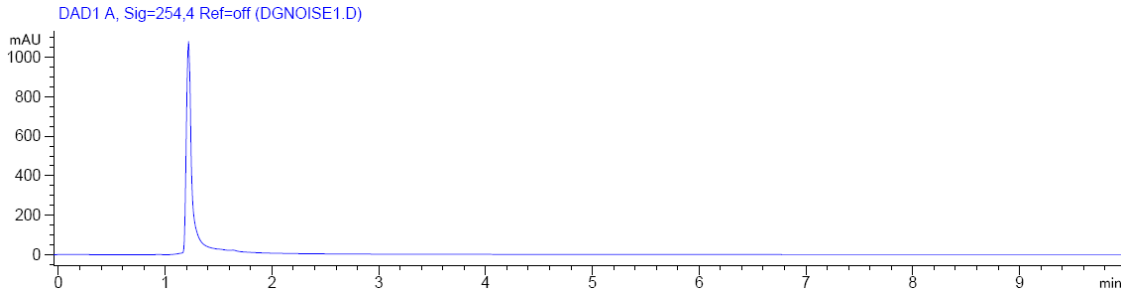
(D) Compound 8 (Method 1)



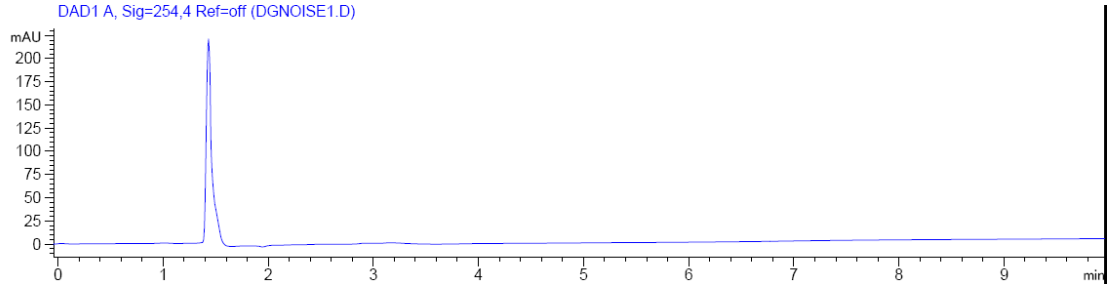
(E) Compound 9 (Method 2)



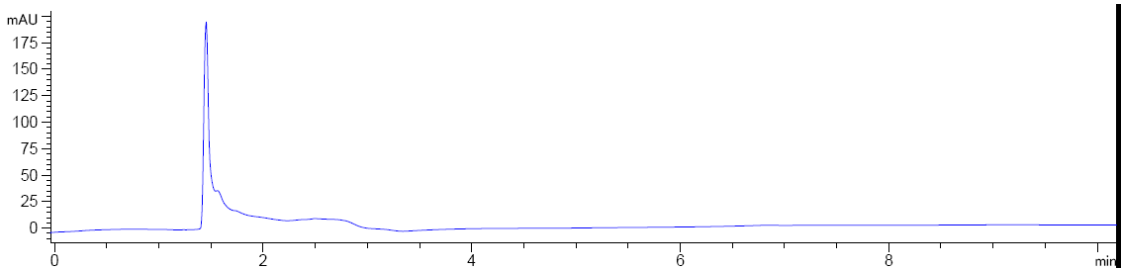
(F) CA-NETA (Method 2)



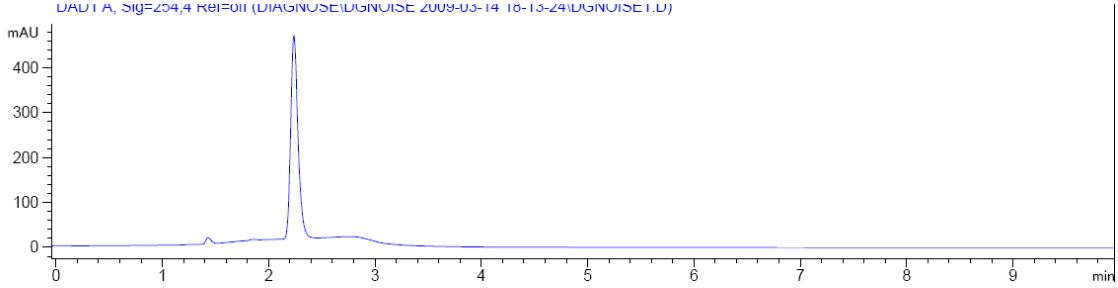
(G) DCA-NE3TA (Method 1)



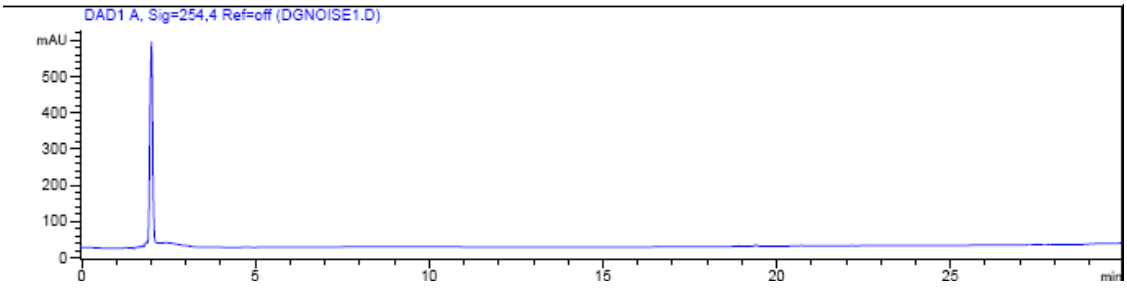
(H) CDCA-NE3TA (Method 1)



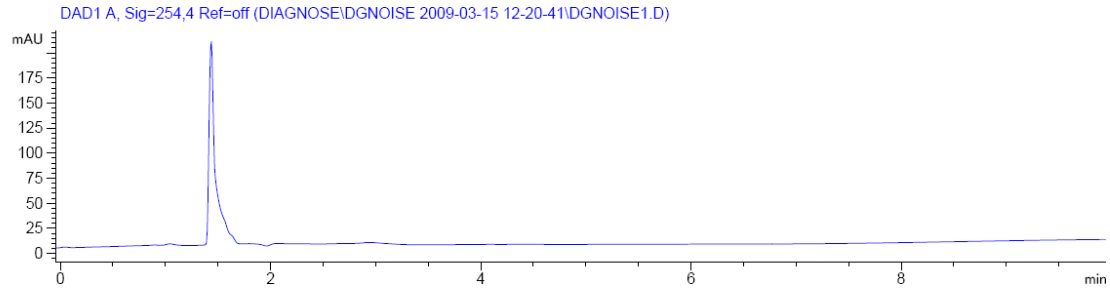
(I) Compound 11 (method 2)



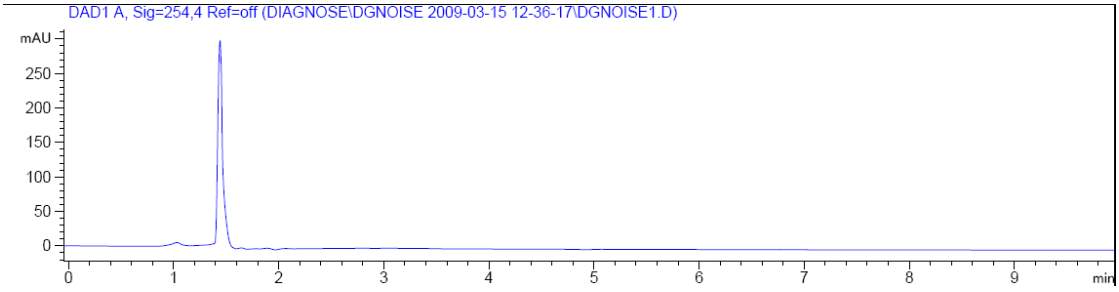
(J) Compound 12 (Method 3)



(K) Compound 13 (method 1)



(L) Compound 14 (method 1)



(M) NBD-CA-NE3TA (method 3)