Supporting Information

Tumor-Specific Novel Taxoid-Monoclonal Antibody Conjugates

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Synthesis and characterization of new taxoids

General Methods and Materials: ¹H and ¹³C NMR spectra were measured on a Bruker AC-250 NMR spectrometer or a Varian 300 NMR spectrometer using tetramethylsilane as the internal standard. Melting points were measured on a Thomas Hoover Capillary melting point apparatus and are uncorrected. Optical rotations were measured on a Perkin-Elmer Model 241 polarimeter. TLC was performed on Merck DC-alufolien with Kieselgel 60F-254 and column chromatography was carried out on silica gel 60 (Merck; 230-400 mesh ASTM). High resolution mass spectra were obtained from the University of California, Riverside Mass Spectrometry Facility, Riverside, CA; or Mass Spectrometry Laboratory, University of Illinois at Urbana-Champaign, Urbana, IL. 3-Methyldisulfanylpropanoic acid and methyldisulfanylacetic acid were prepared by the literature method.¹ *N*-Succinimidyl-4-(2-pyridyldithio)pentanoate (SPP) was prepared using a method previously described for the related compound by Carlsson et al.² Taxoids **1**, **10a** and **10b** were prepared by the literature methods.³

10-Acetyl-3'-dephenyl-3'-(2-methylprop-1-enyl)-7-triethylsilyl-2'-triisopropylsilyldocetaxel (**5**)³:

To a solution of 7-triethylsilylbaccatin III (**3**) (70 mg, 0.099 mmol) and β -lactam **4** (59 mg, 0.148 mmol) in THF (6 mL) at -40 °C was added lithium hexamethyldisilazide (0.15 mL, 0.148 mmol). The reaction mixture was stirred for 40 minutes at -40 °C and then quenched with saturated NH₄Cl solution and extracted with EtOAc (20 mL x 3). The organic layers were combined and dried with anhydrous MgSO₄. The solvent was removed by reduced pressure vacuum and the residue was purified by column chromatography (hexanes/EtOAc = 9/1) to afford **5** as a white solid (102 mg, 93%): ¹H NMR (CDCl₃) δ 0.57 (m, 6 H), 0.93 (m, 9 H), 1.10 (m, 21 H), 1.17 (s, 3 H), 1.21 (s, 3 H), 1.33 (s, 9 H), 1.67 (s, 3 H), 1.74 (s, 3 H), 1.78 (s, 3 H), 1.67-1.78 (m, 1 H), 1.80-1.92 (m, 1 H), 1.99 (s, 3 H), 2.16 (s, 3 H), 2.35 (s, 3 H), 2.40-2.56 (m, 2 H), 3.83 (d, *J* = 6.9 Hz, 1 H), 4.18 (d, *J* = 8.4 Hz, 1 H), 4.29 (d, *J* = 8.4 Hz, 1 H), 4.42 (d, *J* = 3.0 Hz, 1 H), 4.46 (m, 1 H), 4.76 (d, *J* = 9.3 Hz, 1 H), 4.80 (t, *J* = 9.9 Hz, 1 H), 4.92 (d, *J* = 8.4 Hz, 1 H), 5.32 (d, *J* = 8.4 Hz, 1 H), 5.67 (d, *J* = 6.9 Hz, 1 H), 6.08 (t, *J* = 9.3 Hz, 1 H), 6.46 (s, 1 H), 7.44 (m, 2 H), 7.56 (m, 1 H), 8.09 (d, *J* = 7.5 Hz, 2 H). All data are consistent with the reported values.³

3'-Dephenyl-3'-(2-methylprop-1-enyl)-7-triethylsilyl-2'-triisopropylsilyldocetaxel (6):

To a solution of **5** (62 mg, 0.056 mmol) in ethanol (2 mL) was added hydrazine monohydrate (1 mL). The reaction mixture was stirred overnight and diluted with EtOAc and washed with saturated NH₄Cl solution, water and brine. The organic layer was dried with anhydrous MgSO₄. The solvent was removed by reduced pressure vacuum and the residue was purified by column chromatography (hexanes/EtOAc = 3/1) to afford **6** as a white solid (52 mg, 87%): ¹H NMR (CDCl₃) δ 0.57 (m, 6 H), 0.93 (m, 9 H), 1.04 (s, 1 H), 1.12 (m, 21 H), 1.21 (s, 3 H), 1.33 (s, 9 H), 1.69 (s, 3 H), 1.73 (s, 3 H), 1.74 (s, 3 H), 1.67-1.78 (m, 1 H), 1.79 (s, 3 H), 1.80-1.92 (m, 1 H), 1.91 (s, 3 H), 2.37 (s, 3 H), 2.24-2.50 (m, 2 H), 3.83 (d, *J* = 6.9 Hz, 1 H), 4.19 (d, *J* = 8.4 Hz, 1 H), 4.24 (d, *J* = 1.5 Hz, 1 H), 4.30 (d, *J* = 8.4 Hz, 1 H), 4.38 (m, 1 H), 4.43 (d, *J* = 2.7 Hz, 1 H), 4.76 (d, *J* = 8.1 Hz, 1 H), 4.83 (t, *J* = 9.0 Hz, 1 H), 4.92 (d, *J* = 8.1 Hz, 1 H), 5.12 (s, 1 H), 5.32 (d, *J* = 8.1 Hz, 1 H), 5.64 (d, *J* = 7.2 Hz, 1 H), 6.14 (t, *J* = 9.0 Hz, 1 H), 7.44 (m, 2 H), 7.58 (m, 1 H), 8.09 (d, *J* = 7.2 Hz, 2 H).

3'-Dephenyl-3'-(2-methylprop-1-enyl)-10-(3-methyldisulfanylpropanoyl)-7-triethylsilyl-2'-triisopropylsilyldocetaxel (8a):

To a solution of **6** (49 mg, 0.047 mmol), 3-methyldisulfanylpropanoic acid **7a** (70.6 mg, 0.470 mmol) and DMAP (catalytic amount) in dichloromethane (1 mL) was added 1,3-diisopropylcarbodiimide (DIC, 80 μL, 517 mmol). The reaction mixture was stirred overnight and diluted with EtOAc and washed with saturated NH₄Cl solution, water and brine. The organic layer was dried with anhydrous MgSO₄. The solvent was removed by reduced pressure vacuum and the residue was purified by column chromatography (hexanes/EtOAc = 3/1) to afford **8a** as a white solid (38 mg, 80%): ¹H NMR (CDCl₃) δ 0.58 (m, 6 H), 0.92 (m, 9 H), 1.11 (m, 21 H), 1.18 (s, 3 H), 1.21 (s, 3 H), 1.33 (s, 9 H), 1.68 (s, 3 H), 1.67-1.78 (m, 1 H), 1.75 (s, 3 H), 1.88 (s, 3 H), 1.80-1.92 (m, 1 H), 2.00 (s, 3 H), 2.35 (s, 3 H), 2.24-2.50 (m, 2 H), 2.42 (s, 1 H), 2.80-2.31 (m, 4 H), 3.83 (d, *J* = 6.9 Hz, 1 H), 4.19 (d, *J* = 8.1 Hz, 1 H), 4.30 (d, *J* = 8.1 Hz, 1 H), 4.43 (d, *J* = 2.7 Hz, 1 H), 4.38 (m, 1 H), 4.77 (d, *J* = 9.6 Hz, 1 H), 4.81 (t, *J* = 9.6 Hz, 1 H), 4.93 (d, *J* = 9.0 Hz, 1 H), 5.32 (d, *J* = 8.7 Hz, 1 H), 5.67 (d, *J* = 7.2 Hz, 1 H), 6.08 (t, *J* = 8.4 Hz, 1 H), 6.49 (s, 1 H), 7.45 (m, 2 H), 7.59 (m, 1 H), 8.09 (d, *J* = 7.5 Hz, 2 H).

3'-Dephenyl-3'-(2-methylprop-1-enyl)-10-(3-methyldisulfanylpropanoyl)docetaxel (9a):

To a solution of **8a** (52 mg, 0.044 mmol) in pyridine (1 mL) and acetonitrile (1 mL) was added dropwise HF/pyridine (70:30, 0.60 mL) at 0 °C, then the mixture was allowed to warm to room temperature and stirred for 17 h. The reaction mixture was diluted with EtOAc and washed with saturated NaHCO₃ (10 mL), saturated CuSO₄ solution (10 mL x 4), water (10 mL x 3) and brine (10 mL). The organic layer was dried with anhydrous MgSO₄. The solvent was removed by reduced pressure vacuum and the residue was purified by column chromatography (hexanes/EtOAc = 1/1) to afford **9a** as a white solid (32 mg, 80%): ¹H NMR (CDCl₃) δ 1.15 (s, 3 H), 1.25 (s, 3 H), 1.35 (s, 9 H), 1.68 (s, 3 H), 1.67-1.78 (m, 1 H), 1.77 (b, 6 H), 1.90 (s, 3 H), 1.80-1.92 (m, 1 H), 2.35 (s, 3 H), 2.24-2.50 (m, 2 H), 2.42 (s, 1 H), 2.80-2.31 (m, 4 H), 3.42 (d, 2 H), 3.81 (d, *J* = 6.9 Hz, 1 H), 4.18

(d, $J = 8.4$ Hz, 1 H), 4.30 (d, $J = 8.4$ Hz, 1 H), 4.42 (m, 1 H), 4.73 (dt, $J = 8.4$ Hz, 2.4 Hz, 1 H), 4.79 (t, $J = 9.0$ Hz, 1 H), 4.96 (d, $J = 8.1$ Hz, 1 H), 5.32 (d, $J = 7.8$ Hz, 1 H), 5.67 (d, $J = 6.9$ Hz, 1 H), 6.17 (t, $J = 8.7$ Hz, 1 H), 6.34 (s, 1 H) 7.47 (m, 2 H), 7.60 (m, 1 H), 8.09 (d, $J = 7.2$ Hz, 2 H); ^{13}C NMR (CDCl_3) δ 9.6, 14.2, 15.0, 18.6, 21.9, 22.4, 23.2, 23.5, 25.7, 26.7, 28.2, 29.7, 31.6, 32.1, 34.2, 35.6, 43.2, 45.7, 51.6, 58.6, 72.1, 72.3, 73.08, 75.0, 75.8, 79.1, 80.0, 81.0, 84.4, 120.7, 128.6, 129.0, 130.1, 132.7, 133.7, 137.9, 142.8, 155.5, 166.9, 170.1, 172.0, 173.0, 203.6. HRMS (FAB): m/z calcd for $\text{C}_{45}\text{H}_{61}\text{O}_{15}\text{S}_2\text{Na}^+$ 942.337800, found: 942.338034 ($\Delta = 0.2$ ppm).

3'-Dephenyl-3'-(2-methylprop-1-enyl)-10-(methyldisulfanylacetyl)docetaxel (9b):

To a solution of **6** (42 mg, 0.047 mmol), methyldisulfanylacetic acid **7b** (870 mg, 0.470 mmol) and DMAP (10 mg) in dichloromethane (1 mL) was added 1,3-diisopropylcarbodiimide (DIC, 89 mL, 0.520 mmol). The reaction mixture was stirred overnight and diluted with EtOAc and washed with saturated NH_4Cl , water and brine. The organic layer was dried over anhydrous MgSO_4 . The solvent was removed under reduced pressure and the residue was purified by column chromatography on silica gel (hexanes:EtOAc = 3:1) to afford a white solid (52 mg, 0.044 mmol). To a solution of this solid in pyridine (1 mL) and acetonitrile (1 mL) was added dropwise HF:pyridine (70:30, 0.60 mL) at 0 °C. The mixture was allowed to warm to room temperature and stirred for 17 h. The reaction mixture was diluted with EtOAc and washed with saturated NaHCO_3 (10 mL), saturated cupric sulfate (10 mL x 4), water (10 mL x 3) and brine (10 mL). The organic layer was dried over anhydrous MgSO_4 . The solvent was removed under reduced pressure and the residue was purified by column chromatography on silica gel (hexanes/EtOAc = 1/1) to afford **9b** as a white solid (32 mg, 80%): ^1H NMR (CDCl_3) δ 1.15 (s, 3 H), 1.26 (s, 3 H), 1.35 (s, 9 H), 1.69 (s, 3 H), 1.67–1.78 (m, 1 H), 1.77 (br, 6 H), 1.92 (s, 3 H), 1.80–1.92, (m, 1 H), 2.36, (s, 3 H), 2.24–2.50 (m, 2 H), 2.42 (s, 1 H), 2.49 (s, 3 H), 3.68 (s, 2 H), 3.83 (m, 2 H), 4.18 (d, $J = 8.4$ Hz, 1 H), 4.30 (d, $J = 8.4$ Hz, 1 H), 4.42 (m, 1 H), 4.77 (dt, $J = 8.4$ Hz, 2.4 Hz, 1 H), 4.79 (t, $J = 9.0$ Hz, 1 H), 4.96 (d, $J = 8.1$ Hz, 1 H), 5.32 (d, $J = 7.8$ Hz, 1 H), 5.67 (d, $J = 6.9$ Hz, 1 H), 6.17 (t, $J = 8.7$ Hz, 1 H), 6.34 (s, 1 H) 7.47 (m, 2 H), 7.60, (m, 1 H), 8.09 (d, $J = 7.2$ Hz, 2 H); ^{13}C NMR (CDCl_3) δ 9.6, 15.0, 18.6, 21.9, 22.4, 23.1, 23.4, 25.7, 26.6, 28.2, 29.7, 35.6, 35.7, 41.0, 42.6, 43.1, 45.7, 51.6, 58.6, 72.1, 72.3, 73.8, 75.0, 79.1, 80.0, 81.0, 84.4, 120.6, 128.6, 129.2, 130.1, 132.6, 133.7, 137.9, 143.0, 155.5, 166.9, 170.0, 170.1, 173.0, 203.6. HRMS (FAB): m/z calcd for $\text{C}_{44}\text{H}_{59}\text{NO}_{15}\text{S}_2\text{H}^+$ 906.3404, found 906.3401 ($\Delta = 0.4$ ppm).

3'-Dephenyl-3'-(2-methylprop-1-enyl)-7-(3-methyldisulfanylpropanoyl)-10-propanoyldocetaxel (11a):

(a) To a solution of compound **10a**¹ was added a solution of 0.1 N HCl in ethanol (5 mL) at 0 °C. The reaction mixture was allowed to warm to room temperature with stirring for 16 h. The reaction was quenched with saturated NaHCO_3 solution and extracted with EtOAc. The organic layers were washed with saturated NaHCO_3 solution and brine, dried over MgSO_4 , and concentrated in vacuo. The crude product mixture was then purified by column chromatography (hexanes/EtOAc = 2/1 to 1/1) to afford 3'-dephenyl-3'-(2-methylprop-1-enyl)-10-pro-

panoyl-2'-triisopropylsilyldocetaxel as a white solid: ^1H NMR (CDCl_3) δ 1.10 (m, 21 H), 1.30 (s, 9 H), 1.63 (s, 3 H), 1.73 (s, 2 H), 1.82 (m, 1 H), 1.88 (s, 3 H), 2.36 (s, 3 H), 2.40 (s, 2 H), 2.46 (m, 1 H), 2.49 (m, 2 H), 3.25 (br s, 1 H), 3.79 (d, $J = 7.0$ Hz, 1 H), 4.09 (d, $J = 8.3$ Hz, 1 H), 4.16 (br s, 1 H), 4.27 (d, $J = 8.3$ Hz, 1 H), 4.38 (dd, $J = 10.2, 6.7$ Hz, 1 H), 4.57 (d, $J = 9.5$ Hz, 1 H), 4.94 (d, $J = 8.0$ Hz, 1 H), 5.64 (d, $J = 7.0$ Hz, 1 H), 6.13 (m, 1 H), 6.30 (s, 1 H), 7.43 (t, 2 H), 7.56 (t, 1 H), 8.08 (d, $J = 7.4$ Hz, 2 H).

(b) To a solution of afford 3'-dephenyl-3'-(2-methylprop-1-enyl)-10-propanoyl-2'-triisopropylsilyldocetaxel (20 mg, 0.020 mmol), 3-(methyldithio)propanoic acid **7a** (15.2 mg, 0.10 mmol), and DMAP (24 mg, 0.2 mmol) in CH_2Cl_2 (2 mL), EDC hydrochloride (38 mg, 0.2 mmol) was added. The mixture was stirred for 2 h. Brine (10 mL) was added to quench the reaction. The solution was extracted with EtOAc, dried over anhydrous MgSO_4 and concentrated *in vacuo*. The residue was then purified on a silica gel column (hexanes/EtOAc = 3/1) to give 3'-dephenyl-3'-(2-methylpropenyl)-7-(3-methyldifulfanylpropanoyl)-10-propanoyl-2'-triisopropylsilyldocetaxel (20 mg, 89%) as a white solid: ^1H NMR (CDCl_3) δ 1.10 (m, 21 H), 1.30 (s, 9 H), 1.63 (s, 3 H), 1.73 (s, 2 H), 1.82 (m, 1 H), 1.88 (s, 3 H), 2.36 (s, 3 H), 2.40 (s, 2 H), 2.46 (m, 1 H), 2.49 (m, 2 H), 3.25 (br s, 1 H), 3.79 (d, $J = 7.0$ Hz, 1 H), 4.09 (d, $J = 8.3$ Hz, 1 H), 4.16 (br s, 1 H), 4.27 (d, $J = 8.3$ Hz, 1 H), 4.38 (dd, $J = 10.2, 6.7$ Hz, 1 H), 4.57 (d, $J = 9.5$ Hz, 1 H), 4.94 (d, $J = 8.0$ Hz, 1 H), 5.30 (d, $J = 7.8$ Hz, 1 H), 5.64 (d, $J = 7.0$ Hz, 1 H), 6.13 (m, 1 H), 6.30 (s, 1 H), 7.43 (t, 2 H), 7.56 (t, 1 H), 8.08 (d, $J = 7.4$ Hz, 2 H).

(c) To a solution of 3'-dephenyl-3'-(2-methylpropenyl)-7-(3-methyldifulfanylpropanoyl)-10-propanoyl-2'-triisopropylsilyldocetaxel (20 mg, 0.018 mmol) in 4 mL of pyridine/acetonitrile (1/1) was added dropwise HF/pyridine (70/30, 0.4 mL) at 0°C , and the mixture was stirred at room temperature for 12 h. The reaction was quenched with aqueous saturated NaHCO_3 solution (5.0 mL). The mixture was then diluted with EtOAc (70 mL), washed with aqueous saturated copper sulfate solution (10 mL x 3) and water (10 mL), dried over anhydrous MgSO_4 and concentrated *in vacuo*. The crude product mixture was purified on a silica gel column using hexanes/EtOAc (2/1 followed by 1/1) as the eluant to afford pure taxoid **11a** (15 mg, 86%) as a white solid: m.p. $118\text{--}120^\circ\text{C}$; ^1H NMR (CDCl_3) δ 0.96 (m, 6 H), 1.13 (s, 3 H), 1.22–1.27 (m, 6 H), 1.30 (s, 9 H), 1.63 (s, 3 H), 1.73 (s, 2 H), 1.82 (m, 1 H), 1.88 (s, 3 H), 2.36 (s, 3 H), 2.40 (s, 2 H), 2.46 (m, 1 H), 2.49 (m, 2 H), 2.55 (m, 4 H), 2.60–2.86 (m, 4 H), 3.25 (br s, 1 H), 3.79 (d, $J = 7.0$ Hz, 1 H), 4.09 (d, $J = 8.3$ Hz, 1 H), 4.16 (br s, 1 H), 4.27 (d, $J = 8.3$ Hz, 1 H), 4.38 (dd, $J = 10.2, 6.7$ Hz, 1 H), 4.57 (d, $J = 9.5$ Hz, 1 H), 4.95 (d, $J = 8.0$ Hz, 1 H), 5.30 (d, $J = 7.8$ Hz, 1 H), 5.60 (m, 1 H), 5.67 (d, $J = 7.0$ Hz, 1 H), 6.14 (m, 1 H), 6.28 (s, 1 H), 7.48 (t, 2 H), 7.62 (t, 1 H), 8.09 (d, $J = 7.4$ Hz, 2 H); ^{13}C NMR (CDCl_3) δ 10.8, 14.7, 18.6, 21.4, 22.3, 23.4, 25.7, 26.4, 28.2, 32.3, 33.4, 34.5, 35.5, 43.2, 46.9, 51.6, 56.0, 66.5, 71.7, 72.1, 73.7, 74.5, 76.2, 76.4, 76.5, 77.0, 77.5, 78.7, 79.9, 80.9, 84.0, 120.1, 120.5, 124.2, 126.7, 130.1, 131.0, 132.8, 137.9, 142.0, 142.5, 155.4, 165.9, 170.1, 174.6, 203.4. HRMS: m/e calcd for $\text{C}_{48}\text{H}_{65}\text{O}_{16}\text{NH}^+$ 976.3823, found 976.3826 ($\Delta = 0.3$ ppm).

3'-Dephenyl-3'-(2-methylprop-1-enyl)-7-(3-methyldifulfanypropanoyl)-10-(4-morpholine-1-carbonyl)-docetaxel (11b):

(a) Preparation of 3'-dephenyl-3'-(2-methylprop-1-enyl)-10-(4-morpholine-1-carbonyl)-2'-triisopropylsilyldocetaxel: Prepared using the same procedure (a) as described above for that of **11a**: White solid; ^1H NMR (CDCl_3) δ 1.10 (m, 21 H), 1.33 (s, 9 H), 1.65 (s, 3 H), 1.75 (br s, 6 H), 1.84-1.93 (m, 5 H), 2.34 (br s, 4 H), 2.46-2.58 (m, 1 H), 3.11 (d, $J = 3.5$ Hz, 1 H), 3.39-3.86 (m, 9 H), 4.11-4.18 (m, 2 H), 4.28 (d, $J = 8.4$ Hz, 1 H), 4.38-4.48 (m, 1 H), 4.72 (td, $J = 8.7, 2.5$ Hz, 1 H), 4.83 (d, $J = 8.7$ Hz, 1 H), 4.95 (d, $J = 8.2$ Hz, 1 H), 5.29 (d, $J = 8.6$ Hz, 1 H), 5.63 (d, $J = 7.0$ Hz, 1 H), 6.15 (t, $J = 8.7$ Hz, 1 H), 6.27 (s, 1 H), 7.45 (t, $J = 7.4$ Hz, 2 H), 7.59 (t, $J = 7.4$ Hz, 1 H), 8.08 (d, $J = 7.4$ Hz, 2 H).

(b) Preparation of 3'-dephenyl-3'-(2-methylprop-1-enyl)-7-(3-methyldifulfanypropanoyl)-10-(4-morpholine-1-carbonyl)-2'-triisopropylsilyldocetaxel: Prepared using the same procedure (b) as described above for that of **11a**: White solid; ^1H NMR δ 1.10 (m, 21 H), 1.12 (s, 3 H), 1.22 (s, 3 H), 1.33 (s, 9 H), 1.65 (s, 3 H), 1.75 (br s, 6 H), 1.84-1.93 (m, 5 H), 2.34 (br s, 4 H), 2.46-2.58 (m, 1 H), 3.11 (d, $J = 3.5$ Hz, 1 H), 3.39-3.86 (m, 9 H), 4.11-4.18 (m, 2 H), 4.28 (d, $J = 8.4$ Hz, 1 H), 4.32 (d, $J = 8.1$ Hz, 1 H), 4.75 (td, $J = 8.7, 2.5$ Hz, 1 H), 4.83 (d, $J = 8.7$ Hz, 1 H), 4.95 (d, $J = 8.2$ Hz, 1 H), 5.30 (d, $J = 8.6$ Hz, 1 H), 5.90 (m, 1 H), 5.67 (d, $J = 7.0$ Hz, 1 H), 6.15 (t, $J = 8.7$ Hz, 1 H), 6.26 (s, 1 H), 7.47 (t, $J = 7.4$ Hz, 2 H), 7.62 (t, $J = 7.4$ Hz, 1 H), 8.09 (d, $J = 7.4$ Hz, 2 H).

(c) Preparation of **11b** by final deprotection using the same procedure (c) as described above for that of **11a**: Obtained as a white solid: m.p. 143-145 $^\circ\text{C}$; $[\alpha]_D^{20} -62.6$ (c 0.015, CHCl_3); ^1H NMR (CDCl_3) δ 1.12 (s, 3 H), 1.22 (s, 3 H), 1.33 (s, 9 H), 1.65 (s, 3 H), 1.75 (br s, 6 H), 1.84-1.93 (m, 5 H), 2.34 (br s, 4 H), 2.46-2.58 (m, 1 H), 3.11 (d, $J = 3.5$ Hz, 1 H), 3.39-3.86 (m, 9 H), 4.11-4.18 (m, 2 H), 4.28 (d, $J = 8.4$ Hz, 1 H), 4.32 (d, $J = 8.1$ Hz, 1 H), 4.75 (td, $J = 8.7, 2.5$ Hz, 1 H), 4.83 (d, $J = 8.7$ Hz, 1 H), 4.95 (d, $J = 8.2$ Hz, 1 H), 5.30 (d, $J = 8.6$ Hz, 1 H), 5.90 (m, 1 H), 5.67 (d, $J = 7.0$ Hz, 1 H), 6.15 (t, $J = 8.7$ Hz, 1 H), 6.26 (s, 1 H), 7.47 (t, $J = 7.4$ Hz, 2 H), 7.62 (t, $J = 7.4$ Hz, 1 H), 8.09 (d, $J = 7.4$ Hz, 2 H).

1 H), 4.75 (td, $J = 8.7, 2.5$ Hz, 1 H), 4.83 (d, $J = 8.7$ Hz, 1 H), 4.95 (d, $J = 8.2$ Hz, 1 H), 5.30 (d, $J = 8.6$ Hz, 1 H), 5.90 (m, 1 H), 5.67 (d, $J = 7.0$ Hz, 1 H), 6.15 (t, $J = 8.7$ Hz, 1 H), 6.26 (s, 1 H), 7.47 (t, $J = 7.4$ Hz, 2 H), 7.62 (t, $J = 7.4$ Hz, 1 H), 8.09 (d, $J = 7.4$ Hz, 2 H); ^{13}C NMR (CDCl_3) δ 10.8, 14.7, 18.6, 21.4, 22.3, 23.4, 25.7, 26.4, 28.2, 32.3, 33.4, 34.5, 35.5, 43.2, 46.9, 51.6, 56.0, 66.5, 71.7, 72.1, 73.7, 74.5, 76.2, 76.4, 76.5, 77.0, 77.5, 78.7, 79.9, 80.9, 84.0, 120.6, 128.7, 129.1, 130.1, 132.7, 133.8, 138.0, 141.4, 153.8, 155.4, 166.9, 170.0, 170.8, 171.8, 203.4. HRMS: m/e calcd for $\text{C}_{50}\text{H}_{68}\text{O}_{17}\text{N}_2\text{S}_2\text{Na}^+$: 1055.3857, found 1055.3857 ($\Delta = 0.0$ ppm).

3'-Dephenyl-3'-(2-methylprop-1-enyl)-2'-(3-methyldisulfanylpropanoyl)-10-propanoyldocetaxel (12):

To a solution of **1**³ (20 mg, 0.024 mmol) in 1 mL of CH_2Cl_2 was added DIC (9.1 mg, 0.048 mmol), DMAP (5.8 mg, 0.048 mmol) and 3-methyldisulfanylpropanoic acid **7a** (4.0 mg, 0.026 mmol) at room temperature. Then the mixture was stirred for 12 h and the reaction quenched with saturated NH_4Cl solution (5 mL). The aqueous layer was then extracted with CH_2Cl_2 (20 mL x 3) and the combined extracts were then dried over anhydrous MgSO_4 and concentrated *in vacuo*. The residue was purified on a silica gel column using hexanes/EtOAc (3/1 followed by 2/1) as the eluant to afford **12** as a white solid (19 mg, 81%); m.p. 118-119 °C; ^1H NMR (CDCl_3) δ 1.14 (s, 3 H), 1.22 (s, 3 H), 1.25 (s, 3 H), 1.34 (s, 9 H), 1.67 (s, 3 H), 1.76 (s, 6 H), 1.85 (m, 1 H) (H_{6a}), 1.90 (s, 3 H), 2.04 (s, 1 H), 2.36 (s, 3 H) (OAc), 2.38 (MeSS) (s, 3 H) 2.39 (s, 1 H), 2.53 (m, 3 H) (H_{6b}), 2.96 (m, 3 H), 3.15 (t, 1 H), 3.82 (d, $J = 7.1$ Hz, 1 H), 4.18 (d, $J = 8.4$ Hz, 1 H) (H_{20a}), 4.31 (d, $J = 8.4$ Hz, 1 H) (H_{20b}), 4.45 (m, 1 H), 4.76 (d, $J = 9.0$ Hz, 1 H), 4.98 ($\text{H}_5, \text{H}_2, \text{H}_4$) (m, 3 H), 5.20 (d, $J = 8.4$ Hz, 1 H), 5.67 (d, $J = 7.1$ Hz, 1 H) (H_2), 6.19 (t, $J = 8.5$ Hz, 1 H), 6.31 (s, 1 H) (H_{10}), 7.48 (t, $J = 7.5$ Hz, 2 H), 7.61 (t, $J = 7.3$ Hz, 1 H), 8.11 (d, $J = 7.4$ Hz, 2 H); ^{13}C NMR (CDCl_3) δ 9.0, 9.6, 14.8, 18.6, 22.1, 22.4, 23.1, 25.8, 26.6, 27.6, 28.2, 29.7, 31.8, 33.5, 33.7, 35.5, 43.2, 45.6, 49.0, 59.5, 71.8, 72.2, 74.9, 75.2, 75.5, 77.5, 79.3, 80.0, 81.0, 84.5, 119.9, 128.7, 129.3, 130.2, 132.5, 133.6, 143.2, 167.0, 168.2, 169.7, 174.6, 204.0. HRMS (FAB, DCM/NBA): m/z calcd for $\text{C}_{48}\text{H}_{66}\text{NO}_{16}\text{S}$ 976.3823, found 976.3826 ($\Delta = -0.3$ ppm).

3'-Dephenyl-3'-(2-methylprop-1-enyl)-10-(3-methyldisulfanylbenzoyl)docetaxel (13):

To a stirred solution of **6** (27 mg, 0.03 mmol), 3-methyldisulfanylbenzoic acid (22 mg, 0.11 mmol), and DMAP (12 mg, 0.11 mmol) in CH_2Cl_2 (1 mL) was added DIC (0.02 mL, 0.11 mmol). The solution was allowed to stir for 5 h, and was diluted with EtOAc and the reaction quenched with saturated aqueous NaHCO_3 . The organic layer was washed with water and brine, dried over MgSO_4 , filtered and concentrated. The resulting solid was purified by column chromatography on silica gel (hexanes/EtOAc = 3/1). The resulting white solid was dissolved in a 3 mL of a 1:1 mixture of pyridine and CH_3CN and cooled to 0 °C. To this cooled solution was added 0.75 mL of HF-pyridine, and the solution was allowed to warm to room temperature over night. The reaction mixture was diluted with EtOAc and the reaction quenched with saturated aqueous NaHCO_3 . The organic layer was washed with saturated aqueous CuSO_4 , water and brine, dried over MgSO_4 , filtered and concentrated. The resulting oil

was purified by column chromatography on silica gel (hexanes/EtOAc = 1/1) to afford **13** (27 mg, 91% for 2 steps) as a white powder: m.p. 135-136 °C; ^1H NMR (250 MHz, CDCl_3) δ 1.28 (s, 3 H), 1.31 (s, 3 H), 1.34 (s, 9 H), 1.60 (m, 1 H), 1.71 (s, 3 H), 1.75 (s, 3 H), 1.77 (s, 3 H), 1.91 (m, 1 H), 1.96 (s, 3 H), 2.37 (s, 3 H), 2.38 (s, 3 H), 2.59 (m, 2 H), 3.35 (bs, 1 H), 4.22 (m, 2 H), 4.35 (d, J = 8.4 Hz, 1 H), 4.51 (m, 1 H), 4.76 (m, 2 H), 4.98 (d, J = 8.1 Hz, 1 H), 5.32 (m, 1 H), 5.71 (d, J = 6.9 Hz, 1 H), 6.21 (m, 1 H), 6.57 (s, 1 H), 7.46 (m, 3 H), 7.59 (m, 1 H), 7.65 (s, 1 H), 7.89 (m, 1 H), 8.04 (d, J = 7.8 Hz, 2 H), 8.22 (s, 1 H); ^{13}C NMR (63 MHz, CDCl_3) δ 9.6, 15.0, 18.6, 22.1, 22.4, 22.9, 25.7, 26.8, 28.2, 35.6, 35.7, 43.3, 45.8, 51.6, 58.7, 72.2, 72.3, 73.8, 75.1, 76.2, 79.2, 80.0, 81.1, 84.4, 120.6, 128.2, 128.2, 128.7, 129.2, 130.1, 130.2, 132.1, 132.7, 133.7, 138.0, 138.1, 143.0, 155.5, 165.8, 167.0, 170.1, 173.1, 203.5. HRMS: m/z calcd for $\text{C}_{49}\text{H}_{61}\text{NO}_{15}\text{S}_2\cdot\text{H}^+$ 968.356268, found 968.356500 (Δ = -0.2 ppm).

10-Acetyl-2-debenzoyl-2-(3-methyldisulfanylbzoyl)-3'-dephenyl-3'-(2-methylprop-1-enyl)docetaxel (14):

(a) To a stirred solution of 79 mg (0.08 mmol) of **5** in 3.75 mL of CH_2Cl_2 at -78 °C was added 75 μL of a 40% w/w solution of Triton-B in MeOH.⁴ The solution was allowed to stir for 10 min at -78 °C and was then transferred to a cold bath (-10 °C). After about 20 min at -10 °C the reaction mixture was cooled to -78 °C and the reaction quenched with 3.75 mL of 0.1 N HCl. The solution was diluted with EtOAc and washed with water. The organic layer was dried over MgSO_4 , filtered, and concentrated. The resulting solid was purified by column chromatography on silica gel (hexanes/EtOAc = 2/1) to afford 10-acetyl-2-debenzoyl-3'-dephenyl-3'-(2-methylprop-1-enyl)-2'-triisopropylsilyl-7-triethylsilyldocetaxel (55 mg, 78%) as a white solid: ^1H NMR (250 MHz, CDCl_3) δ 0.49 (m, 6 H), 0.89 (m, 9 H), 1.08 (s, 24 H), 1.17 (s, 3 H), 1.38 (s, 9 H), 1.62 (s, 3 H), 1.70 (s, 6 H), 1.91 (s, 3 H), 2.13 (s, 3 H), 2.19 (s, 3 H), 2.62 (m, 3 H), 3.41 (d, J = 6.8 Hz, 1 H), 3.90 (m, 1 H), 4.36 (m, 2 H), 4.61 (m, 3 H), 4.89 (m, 2 H), 5.29 (d, J = 8.5 Hz, 1 H), 6.04 (m, 1 H), 6.38 (s, 1 H); ^{13}C NMR (63 MHz, CDCl_3) δ 5.3, 6.7, 10.2, 12.5, 14.2, 17.5, 17.9, 18.0, 18.7, 20.8, 21.1, 22.5, 25.6, 26.0, 28.3, 29.7, 35.5, 37.3, 42.8, 46.6, 52.0, 58.3, 72.0, 72.3, 74.5, 75.1, 75.2, 78.2, 79.5, 82.3, 83.8, 121.9, 133.5, 136.1, 140.5, 155.2, 169.2, 169.4, 171.2, 171.8, 202.5.

(b) To a solution of 2-debenzoyl-taxoid thus obtained (55 mg, 0.06 mmol), 3-methyldisulfanylbzoylbenzoic acid (58 mg, 0.30 mmol), and DMAP (35 mg, 0.30 mmol) in CH_2Cl_2 (1 mL) was added DIC (0.05 mL, 0.30 mmol). The solution was allowed to stir overnight and diluted with EtOAc, and the reaction was quenched with saturated aqueous NaHCO_3 . The organic layer was washed with water and brine, dried over MgSO_4 , filtered and concentrated. The resulting white solid was purified by column chromatography on silica gel (hexanes/EtOAc = 4/1) to afford 10-acetyl-2-debenzoyl-2-(3-methyldisulfanylbzoyl)-3'-dephenyl-3'-(2-methylprop-1-enyl)-7-triethylsilyl-2'-triisopropylsilyldocetaxel (65 mg, 92%) as a white solid: ^1H NMR (250 MHz, CDCl_3) δ 0.54 (q, J = 7.8 Hz, 6 H), 0.93 (t, J = 7.8 Hz, 9 H), 1.10 (s, 21 H), 1.17 (s, 3 H), 1.21 (s, 3 H), 1.32 (s, 9 H), 1.67 (s, 3 H), 1.73 (s, 3 H), 1.77 (s, 3 H), 1.85 (m, 1 H), 1.99 (s, 3 H), 2.16 (s, 3 H), 2.37 (s, 3 H), 2.46 (s, 3 H), 2.51 (m, 1 H), 3.83 (d, J = 6.8 Hz, 1 H), 4.16 (d, J = 8.3 Hz, 1 H), 4.29 (d, J = 8.3 Hz, 1 H), 4.44 (m, 2 H), 4.80 (m, 2 H), 4.93

(d, $J = 8.3$ Hz, 1 H), 5.30 (d, $J = 8.3$ Hz, 1 H), 5.66 (d, $J = 6.5$ Hz, 1 H), 6.05 (m, 1 H), 6.46 (s, 1 H), 7.43 (t, $J = 7.8$ Hz, 1 H), 7.76 (d, $J = 8$ Hz, 1 H), 7.94 (d, $J = 7.8$ Hz, 1 H), 8.25 (s, 1 H); ^{13}C NMR (63 MHz, CDCl_3) δ 5.3, 6.7, 10.0, 12.5, 14.4, 17.5, 17.9, 18.0, 18.8, 20.8, 21.2, 22.6, 22.9, 25.7, 26.2, 28.2, 35.3, 37.2, 43.2, 46.7, 52.1, 58.4, 71.8, 72.2, 75.1, 75.2, 75.3, 78.8, 79.4, 81.0, 84.2, 122.1, 128.5, 128.9, 129.3, 130.3, 132.0, 133.2, 136.3, 138.1, 141.1, 155.1, 166.2, 169.2, 169.8, 171.9, 201.9.

(c) To a solution of 2-(3-methyldisulfanylbenzoyl)taxoid thus obtained (65 mg, 0.05 mmol) in 2 mL of a 1:1 mixture of pyridine and CH_3CN at 0°C was added 0.3 mL of HF-pyridine. The mixture was allowed to warm to room temperature overnight. The reaction mixture was then diluted with EtOAc and the reaction quenched with saturated aqueous NaHCO_3 . The organic layer was washed with saturated aqueous CuSO_4 , water, brine, dried over MgSO_4 , filtered, and concentrated. The resulting white solid was purified by column chromatography on silica gel (hexanes/EtOAc = 1/1) to afford **14** (49 mg, 98%) as a white powder: m.p. $145\text{--}150^\circ\text{C}$; $[\alpha]_D^{20}$ -87 (c 1.0 CHCl_3); ^1H NMR (300 MHz, CDCl_3) δ 1.15 (s, 3 H), 1.25 (s, 3 H), 1.35 (s, 9 H), 1.67 (s, 3 H), 1.75 (s, 3 H), 1.76 (s, 3 H), 1.90 (s, 4 H), 2.24 (s, 3 H), 2.37 (s, 3 H), 2.45 (s, 3 H), 2.55 (m, 1 H), 3.35 (bs, 1 H), 3.82 (d, $J = 6.9$ Hz, 1 H), 4.18 (m, 2 H), 4.30 (d, $J = 8.2$ Hz, 1 H), 4.44 (m, 1 H), 4.72 (m, 2 H), 4.97 (d, $J = 8.2$ Hz, 1 H), 5.31 (d, $J = 7.8$ Hz, 1 H), 5.65 (d, $J = 7.2$ Hz, 1 H), 6.14 (t, $J = 8.2$ Hz, 1 H), 6.31 (s, 1 H), 7.45 (dd, $J = 8.1$ Hz, 7.5 Hz, 1 H), 7.76 (d, $J = 8.7$ Hz, 1 H), 7.94 (d, $J = 7.5$ Hz, 1 H), 8.27 (s, 1 H); ^{13}C NMR (63 MHz, CDCl_3) δ 9.5, 14.2, 15.0, 18.7, 20.8, 21.8, 22.4, 22.9, 25.7, 26.6, 28.2, 29.7, 35.5, 43.2, 45.6, 51.7, 58.6, 72.2, 72.3, 73.7, 75.3, 75.6, 79.1, 81.0, 84.4, 120.5, 128.4, 128.8, 129.3, 130.1, 132.1, 132.7, 138.0, 138.2, 142.7, 155.4, 166.2, 170.1, 171.2, 203.7. HRMS: m/z calcd for $\text{C}_{44}\text{H}_{59}\text{NO}_{15}\text{S}_2\cdot\text{H}^+$ 906.339926, found 906.340100 ($\Delta = -0.2$ ppm).

Monoclonal Antibodies.

The monoclonal antibody N901 (a murine IgG1 that binds to the CD56 antigen found on small cell lung carcinoma cells) was prepared by the literature method.⁵ Murine anti-EGFR antibodies KS61 (IgG2a), KS77 (IgG1), and KS78 (IgG2a) were produced against human EGFR by immunizing mice using the literature methods.⁶

General Procedures of Preparation of Antibody-Taxoid Conjugates.

Taxoid **9a** was reduced with dithiothreitol to give a thiol-containing taxoid (HS-taxoid), which was purified by HPLC. The antibody was first modified with *N*-succinimidyl-4-(2-pyridyldithio)pentanoate (SPP) to introduce dithiopyridyl groups. The antibody in 50 mM potassium phosphate buffer (pH 6.5), containing NaCl (50 mM) and EDTA (2 mM), was treated with SPP (11 molar equivalents in ethanol). The final ethanol concentration was 1.4% (v/v). After 90 min at ambient temperature, lysine (50 mM) was added to help to remove any non-covalently bound SPP. The reaction was allowed to proceed for two hours, and then purified by gel filtration through a Sephadex G25 column equilibrated in the above buffer. Antibody-containing fractions were pooled and the degree of modification was determined by treating a sample with dithiothreitol and measuring the change in absorbance

at 343 nm (release of pyridine-2-thiol with $\epsilon_{343} = 8,080 \text{ M}^{-1} \text{ cm}^{-1}$). Recovery of the antibody was about 90 %, with 4-5 dithiopyridyl groups, in average, linked per antibody molecule.

The modified antibody was diluted with 50 mM potassium phosphate buffer (pH 6.5) containing NaCl (50 mM) and EDTA (2 mM). HS-Taxoid **17** (1.7 equivalent per dithiopyridyl group) in ethanol (10 % v/v in final reaction mixture) was then added to the modified antibody solution. The reaction proceeded at ambient temperature under argon for 24 hours. The progress of the reaction was monitored spectrophotometrically at 343 nm for release of pyridine-2-thiol caused by disulfide exchange between the **17** and the dithiopyridyl groups on the antibody. The increase in absorbance at 343 nm indicated that the taxoid had linked to all of the SPP sites in the antibody. The reaction mixture was then loaded on to a Sephacryl S300 gel filtration column equilibrated with phosphate-buffered saline (PBS, pH 6.5) containing 20% propylene glycol. The major peak comprised the antibody-taxoid conjugate. The recovery of the conjugate was 65-70%. The concentration of the conjugate was determined by measuring the absorbance at 280 nm. The conjugate was formulated with Tween 80 (0.05%) and human serum albumin (HSA, 1 mg/mL).

General Procedure of *in vitro* Cytotoxicity Assay.

The *In vitro* cytotoxicity was determined in a clonogenic assay.⁷ The cytotoxicity of the anti-EGFR antibody conjugate with taxoid **9a**, KS78-**9a**, towards antigen positive A-431 cells was compared with the non-binding antibody conjugate with taxoid **9a**, mN901-**9a** (Figure 1). Human epidermoid carcinoma A-431 cells were exposed to KS78-**9a** or mN901-**9a** for 24 h. The drugs were then washed off and cells were incubated for an additional period of 6 days, stained with crystal violet, and surviving fractions determined.

As Figure 1 shows, mN901-**9a** exhibits no cytotoxicity against the A431 cell line, expressing EGFR. In sharp contrast, KS78-**9a** shows high potency (IC_{50} : 1.5 nM) against the same A431 cell line.

Figure 1. Cytotoxicity of mAb-taxoid conjugates against A-431 cell line

General Procedure of *in vivo* Tumor Growth Inhibition Assay.

Anti-tumor effect of anti-EGFR-MAb-taxoid conjugates on human squamous cancer (A431) xenografts in SCID mice:

The anti-tumor effect of two different anti-human epidermal growth factor receptor antibody-taxoid conjugates (anti-EGFR-mAb-taxoid conjugates, KS61-**9a** and KS77-**9a**) was evaluated in a human tumor xenograft model in SCID mice. Five weeks old female SCID mice (25 animals) were inoculated subcutaneously into the right flank with A-431 human squamous cancer cells (1.5×10^6 cells/mouse) in 0.1 mL of serum-free medium. The tumors were allowed to grow for 11 days to an average size of 100.0 mm^3 (range of $54 - 145 \text{ mm}^3$). The mice were then randomly divided into four groups (3 to 5 animals per group) according to their tumor size. The first group received KS61-**9a** conjugate (10 mg/kg, qd x 5) administered i.v. The second group received the KS77-**9a** conjugate (10 mg/kg, qd x 5) administered i.v. A third group received free **9a** (0.24 mg/kg, qd x 5, i.v.) at the

same dose as that present in the conjugate. A control group of mice received PBS using the same treatment schedule as in groups 1-3. The tumors sizes were measured twice weekly and the tumor volumes were calculated with the formula: $1/2(\text{length} \times \text{width} \times \text{height})$. The weight of the mice was also measured twice per week.

As shown in [Figure 2](#), mice treated with conjugates showed no body weight loss therefore those conjugates were essentially non-toxic to the mice.

Figure 2. Body weight loss of mice from Experiments shown in the Figure 1 of the main text

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