Supplement Information

Catalytic cleavage of disulfide bonds in small molecules and linkers of antibody-drug conjugates

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Table 1S. Concentrations of thiol species (GSH, GSSG, cysteine, cysteine) in plasma, whole blood, and blood cells of human and rat

		GSH (µM)	GSSG (µM)	Cysteine (µM)	Cystine (µM)
Human	Plasma	5.8 ± 0.6	<2.4	<2.4	26.1 ± 3.2
	Blood	284 ± 9	234 ± 25	<2.4	20.6 ± 4.0
	Blood cells	855 ± 50	315 ± 42	<2.4	11.9 ± 5.0
Rat	Plasma	7.8 ± 0.9	13.7 ± 1.1	<2.4	29.8 ± 1.3
	Blood	518 ± 26	92.0 ± 5.9	<2.4	23.7 ± 1.5
	Blood cells	1450 ± 64	160 ±8	29.4 ± 6.9	12.9 ± 3.0

The samples were treated with 5 volumes of 0.5 N perchloric acid with vortex and sonication for 5 min. The samples were analyzed by LC/MS/MS.

N=4, LLOQ = 1 µM (GSH), 2.4 µM (cysteine), 2.4 µM (GSSG), and 0.4 µM (cystine).

Table S2. Product formation of disulfide-containing prodrugs and ADC conjugates in incubation with TRX and GRX under various conditions

Reaction/ Condition	Cofactor	**Parent→**	**3**	**5**	**10**	**12**	**13**		
		Product→	**1**	**1**	**1**	**2**	**2**	**16**	**11**
+hTRX 2h	+NADPH		++	+	+	++	++	+	++
+hTRX 1h	+NADPH		ND	ND	ND	++	++	-	++
+rTRX 1h	+NADPH		ND	ND	ND	++	++	+	++
+hGRX 2h	+GSH		-	+	+	+	+	+	++
+hGRX 1h	+GSH		ND	ND	ND	+	-	+	+
+hGRX 2h	-GSH		ND	ND	ND	+	-	-	-
+hTRX+inhibitor 2h	+NADPH		ND	ND	ND	-	-	-	-
+hTRX 2h	-NADPH		-	-	-	-	-	-	-
-hTRX 2h	+NADPH		ND	ND	ND	-	-	-	-
-hGRX	+GSH		-	-	-	-	-	-	-

Note: ++, +, -, and ND shows decreasing amounts.

Figure 1S. Stability of disulfide-containing prodrugs in human and rat blood incubations

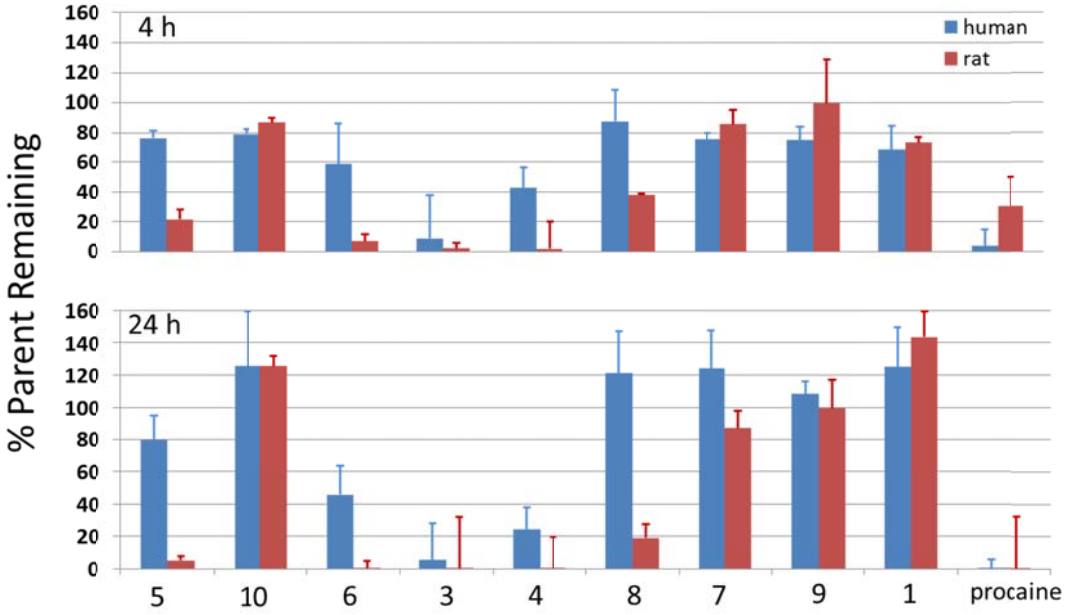
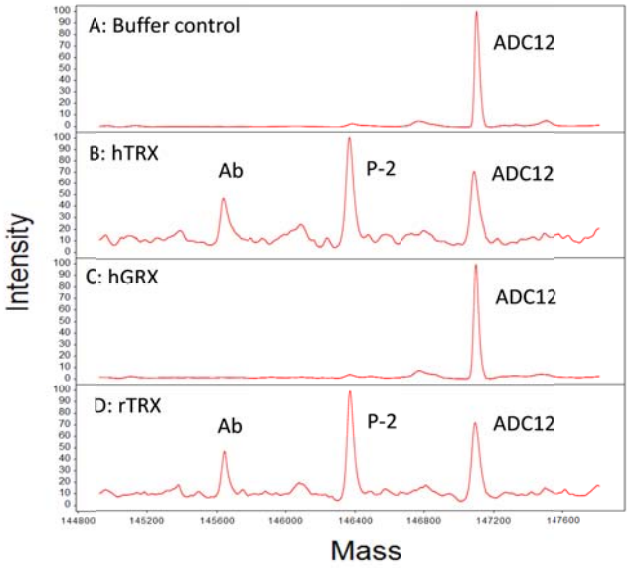


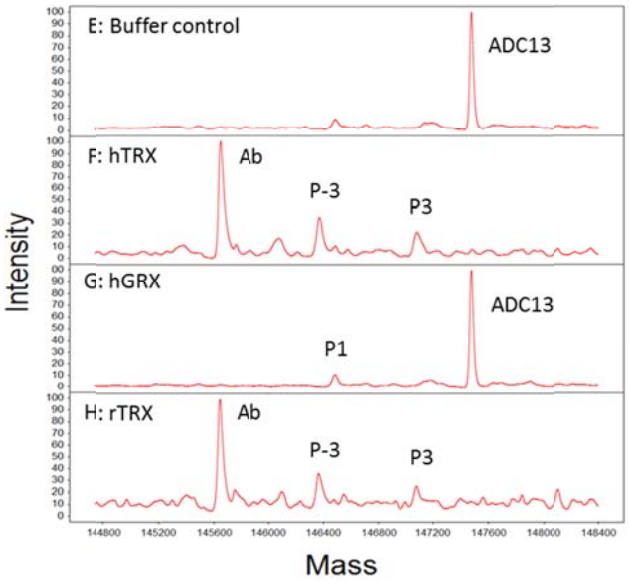
Figure 2S. Antibody-related product profiles of ADC **12** and **13** in incubations with TRX and GRX.

ADC12 = Ab + 2*LD; P-2 = Ab + LD

ADC13 = Ab + 2*LD; P-3=Ab+LD-196Da; P1=Ab+LD+CYS-196Da;

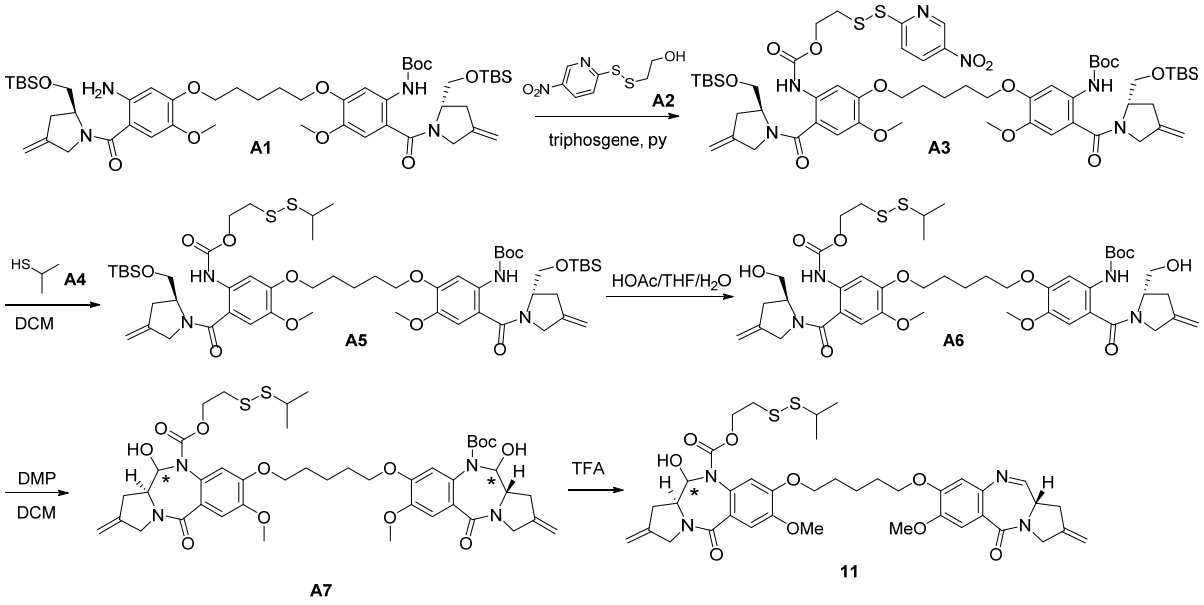
P3=Ab+2*LD-2*196Da; P4=Ab+2*LD-196Da





Synthesis of compounds

Compound 11



To a solution of triphosgene (89.43 mg, 0.300 mmol) and 4Å molecular sieves (50 mg) in DCM (5.0 mL) was added a solution of compound **A2** (165.0 mg, 0.710 mmol) and pyridine (168.58 mg, 2.13 mmol) in dichloromethane (DCM) (5.0 mL). The mixture was stirred at 0 °C for 30 min. The resulting mixture was added dropwise to a solution of compound **A1** (745 mg, 0.780 mmol), pyridine (169 mg, 2.13 mmol) and 4Å MS in DCM (5.0 mL). It was stirred at 0 °C for 30 min, and washed with water (5.0 mL). The organic phase was dried, concentrated and purified by flash column chromatography (5% MeOH in DCM) to give the product **A3** (698 mg, 81%) as a yellow oil. LC/MS (5-95, AB, 1.5 min): RT =1.187 min, m/z=606.5 [M/2+1]+.

To a solution of compound **A3** (698.0 mg, 0.580 mmol) in DCM (10.0 mL) was added 2-propanethiol (439 mg, 5.76 mmol). After the mixture was stirred at 20 °C for 1

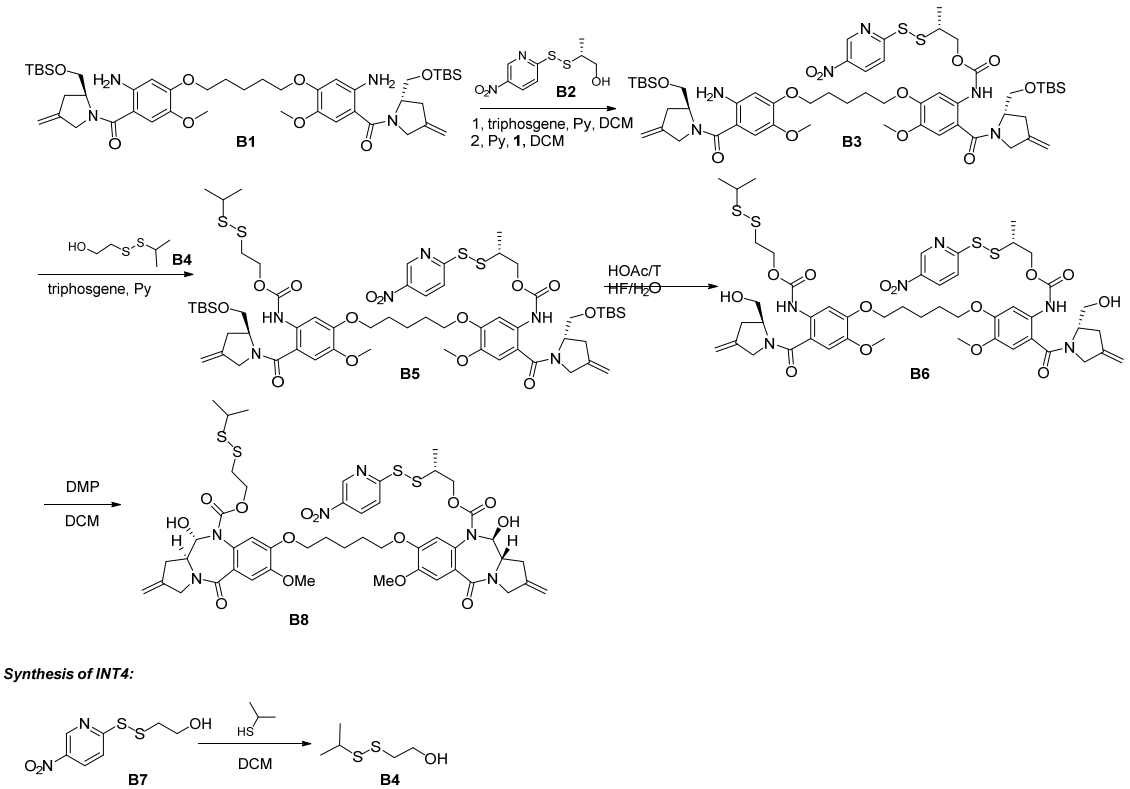
h, MnO_2 (100 mg) was added and stirred for 5 min, and filtered. The filtrate was concentrated and purified by prep-TLC (50% EtOAc in petroleum ether) to give compound **A5** (620 mg, 95%) as a yellow solid. LC/MS (5-95, AB, 1.5 min): RT =1.221 min, m/z=1131.4 [M+1]+.

To a solution of compound **A5** (620.0 mg, 0.550 mmol) in THF (6.0 mL) and water (6.0 mL) was added HOAc (3.29 g, 54.8 mmol). The mixture was stirred at 40 °C for 16 h and concentrated. It was purified by column chromatography (10% MeOH in DCM) to afford compound **A6** (208 mg, 42%) as yellow oil. LCMS (5-95, AB, 1.5 min): RT = 0.854 min, m/z=903.3 [M+1]+.

To a solution of compound **A6** (208.0 mg, 0.230 mmol) in DCM (8.0 mL) was added 4Å molecular sieves, DMP (224.7 mg, 0.530 mmol). The mixture was stirred at 20 °C for 2 h and was quenched with saturated $NaHCO_3$ and $Na_2S_2O_3$ solution (2.0 mL/2.0 mL). After it was stirred for 5 min, DCM (5.0 mL) was added and separated. The DCM phase was washed with water (2 x 5 mL). It was dried, concentrated and purified by prep-TLC (5% MeOH in DCM, Rf=0.2) to afford compound **A7** (121 mg, 58%) as a light yellow foam. LC/MS (5-95, AB, 1.5 min): RT =0.783 min, m/z=781.3 [M-100+1]+.

TFA (1.0 mL, 13.5 mmol) was added to compound **A7** (121.0 mg, 0.130 mmol) at 0 °C. After the mixture was stirred for 10 min, it was added to a cold saturated $NaHCO_3$ solution (20 mL) and extracted with DCM (3 x 10 mL). The combined organic layers were concentrated and purified by prep-TLC (10% MeOH in DCM, Rf = 0.2) followed by prep-HPLC (ACN, acetonitrile: 42~62%, 0.225%FA) to afford the title compound **11** (7.2 mg, 7.0%). LC/MS (5-95, AB, 1.5 min): RT =0.868 min, m/z=781.3 [M+1]+.

Compound B8



To a mixture of compound **B7** (200 mg, 0.86 mmol) in DCM (10 mL) was added 2-propanethiol (328 mg, 4.31 mmol). The mixture was stirred at 15 °C for 12 h. The solid was filtered and the solution was concentrated. The residue was purified by chromatography on silica (100% DCM) to give compound **B4** (90 mg, 69%) as a colorless oil. ^{1}HNMR (400 MHz, $CDCl_3$) δ 3.91-3.86 (m, 2 H), 3.05-3.00 (m, 1H), 2.86-2.84 (m, 2H), 2.04 (t, J = 6.4 Hz, 1H), 1.32 (d, J = 6.4 Hz, 1H).

To a solution of triphosgene (301 mg, 1.01 mmol) in DCM (5.0 mL) was added a mixture of compound B**2** (500 mg, 2.03 mmol) and pyridine (161 mg, 2.03 mmol) in DCM (5.0 mL) at 0 °C under N_2. The reaction mixture was stirred at 16 °C for 30 min. The mixture was concentrated in vacuo and used in the next step directly. To a solution

of the above mixture in DCM (5.0 mL) was added a mixture of compound B**1** (2.76 g, 3.24 mmol) and pyridine (128 mg, 1.62 mmol) in DCM (45.0 mL) at 0 °C. The reaction mixture was stirred at 0 °C for 1 h. TLC (50% EtOAc in petroleum ether Rf = 0.6) showed that the product B**3** was formed. The reaction mixture was concentrated in vacuo and purified by chromatography on silica (0-50% EtOAc in petroleum ether) to give compound B**3** (1.50 g, 78.2%) as a yellow solid. LC/MS (5-95, AB, 1.5 min): R_T = 1.09 min, *m/z* = 1126.7 $[M+1]^+$.

To a mixture of compound **B3** (200 mg, 0.18 mmol) and Et_3N (36 mg, 0.36 mmol) in DCM (4.0 mL) was added triphosgene (26 mg, 0.09 mmol) in DCM (2.0 mL) at 0 °C. After addition, the mixture was stirred at 15 °C for 20 min. LCMS (sample quenched with MeOH, 5-95, AB, 1.5 min): R_T = 1.164 min, *m/z* = 1183.4 $[M+32+1]^+$. The mixture was used directly in the next step. To above mixture was added a solution of compound **B4** (38 mg, 0.25 mmol) and Et_3N (34 mg, 0.33 mmol) in DCM (1.0 mL). After the reaction mixture was stirred at 15 °C for 2 h, it was concentrated and purified by prep-TLC (33% EtOAc in petroleum ether, Rf=0.4) to give compound **B5** (160 mg, 72%) as a yellow oil. LC/MS (5-95, AB, 1.5 min): R_T = 1.26 min, *m/z* = 1304.2 $[M+1]^+$.

To a mixture of compound **B5** (160 mg, 0.12 mmol) in THF (2.0 mL) and water (2.0 mL) was added HOAc (3.91 mL, 68 mmol) dropwise. After addition, the mixture was stirred at 15 °C for 12 h. TLC (5% MeOH in DCM, Rf = 0.5) showed was complete. The mixture was poured into EtOAc (30 mL), and was washed with water (10 mL x 2), saturated $NaHCO_3$ (10 mL x 2) and brine (10 mL). The organic layer was dried over Na_2SO_4 and purified by prep-TLC (5% MeOH in DCM, Rf = 0.5) to give compound **B6**

(120 mg, 93%) as a yellow solid. LC/MS (5-95, AB, 1.5 min): R_T = 0.88 min, *m/z* = 1075.5 $[M+1]^+$.

To a mixture of compound **B6** (60 mg, 0.06 mmol) in DCM (5.0 mL) was added DMP (71 mg, 0.17 mmol). The reaction mixture was stirred at 15 °C for 1 h. LCMS (5-95AB/1.5min): RT =0.80 min, $[M+H]^+$1071.2 showed 39% of desired product. The mixture was concentrated and the residue was purified by prep-TLC (7% MeOH in DCM, Rf = 0.5), followed by prep-HPLC (acetonitrile 45-75/10mM NH_4HCO_3-ACN) to give **B8** (10.1 mg, 17%) as a white solid. LC/MS (5-95, AB, 1.5 min): R_T = 0.81 min, *m/z* = 1053.1 $[M-18+1]^+$. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.22 (s, 1H), 8.30 (d, *J* = 7.6 Hz, 1H), 7.63 (d, *J* = 8.8 Hz, 1H), 7.27 (s, 1H), 7.21 (s, 1H), 6.86 (s, 1H), 6.70 (s, 1H), 5.59-5.56 (m, 2H), 5.15 (s, 4H), 4.43-4.29 (m, 4H), 4.17-3.64 (m, 19 H), 3.22-3.20 (m, 1H), 2.94-2.70 (m, 6H), 1.91 (d, *J* = 6.4 Hz, 4H), 1.64 (s, 2H), 1.26-1.20 (m, 9H).