Electronic Supplementary Material (ESI) for Nanoscale.
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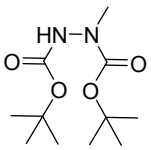
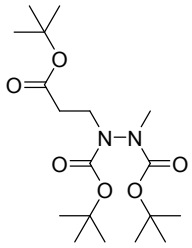
Synthesis of compounds

Di-*tert*-butyl 1-methylhydrazine-1,2-dicarboxylate[1]



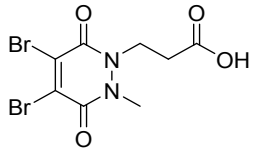
To a stirring solution of *N*-methylhydrazine **4** (157 mg, 3.42 mmol) in *i*-PrOH (4.3 mL) was added dropwise di-*tert*-butyl dicarbonate (1.6 g, 7.5 mmol, pre-dissolved in CH_2Cl_2 (3.4 mL)) over 20 min. The mixture was then stirred for 16 hr at 21°C. After this time, solvent was removed *in vacuo* and purification by flash column chromatography (20% Et_2O/petrol) yielded di-*tert*-butyl 1-methylhydrazine-1,2-dicarboxylate (407 mg, 2.05 mmol, 60%) as a white solid: m.p. 58–62°C (*lit m.p.* 54–56°C). ^{1}H NMR (600 MHz, $CDCl_3$, rotamers) δ 6.55-6.10 (br s, 1H), 3.11 (s, 3H), 1.47 (s, 18H); ^{13}C NMR (150 MHz, $CDCl_3$, rotamers) *δ* 171.2 (C), 155.9 (C), 81.3 (C), 60.4 (CH_3), 28.3 (CH_3); IR (solid) 3316, 2978, 2932, 1701 cm^{-1}. Spectra data agrees with that described in Bahou *et al.* [1]

Di-*tert*-butyl 1-(3-(tert-butoxy)-3-oxopropyl)-2-methylhydrazine-1,2-dicarboxylate[1] **5**



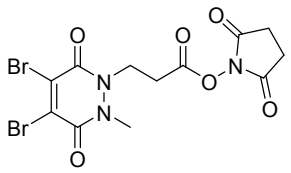
To a solution of di-*tert*-butyl 1-methylhydrazine-1,2-dicarboxylate (3.00 g, 12.2 mmol) in *t*-BuOH (5 mL), was added 10% NaOH (0.5 mL) and the reaction mixture stirred at 21°C for 10 min. After this, *tert*-butyl acrylate (5.31 mL, 36.6 mmol) was added to the solution and the reaction mixture was heated at 60°C for 24 hr. Following this, the solvent was removed *in vacuo* and the crude residue was dissolved in EtOAc (150 mL) and washed with water (3 × 50 mL). The organic layer was then dried ($MgSO_4$) and concentrated *in vacuo*. Purification of the residue by flash column chromatography (0% to 20% EtOAc/petrol) afforded di-*tert*-butyl-1-(3-(*tert*-butoxy)-3-oxopropyl)-2-methylhydrazine-1,2-dicarboxylate **5** (2.24 g, 5.98 mmol, 49%) as a clear oil. ^{1}H NMR (600 MHz, $CDCl_3$, rotamers) δ 3.85–3.52 (m, 2H), 3.06–2.99 (m, 3H), 2.51 (t, *J* = 7.2 Hz, 2H), 1.48–1.43 (m, 27H). ^{13}C NMR (150 MHz, $CDCl_3$, rotamers) δ 171.0 (C), 155.4 (C), 154.4 (C), 81.0 (C), 44.6 (CH_3), 36.6 (CH_2), 34.1 (CH_2), 28.3 (CH_3). IR (thin film) 2976, 2933, 1709 cm^{-1}. LRMS (ESI) 375 (100, $[M+H]^+$), 319 (30, $[M-C_4H_9+2H]^+$). HRMS (ESI) calcd for $C_{18}H_{35}N_2O_6$ $[M+H]^+$ 376.2524; observed 376.2516. Spectra data agrees with that described in Bahou *et al.* [1]

3-(4,5-Dibromo-2-methyl-3,6-dioxo-3,6-dihydropyridazin-1(2*H*)-yl) propanoic acid[1] **6**



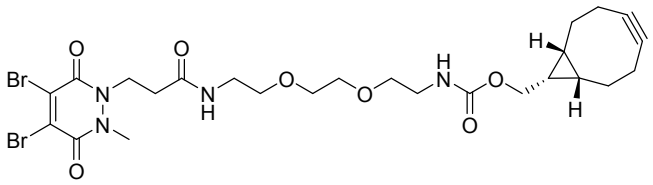
Dibromomaleic acid (4.93 g, 17.9 mmol) was dissolved in AcOH (25 mL) and heated under reflux for 30 min. To this solution, was added di-*tert*-butyl-1-(3-(*tert*-butoxy)-3-oxopropyl)-2-methylhydrazine-1,2-dicarboxylate **5** (5.60 g, 14.9 mmol) and the reaction heated under reflux for a further 4 hr. After this time, the reaction mixture was then concentrated *in vacuo* with toluene co-evaporation (3 × 30 mL, as an azeotrope) and the crude residue purified by flash column chromatography (50% to 100% EtOAc/petrol (1% AcOH)) to afford 3-(4,5-dibromo-2-methyl-3,6-dioxo-3,6-dihydropyridazin-1(2*H*)-yl) propanoic acid **6** (3.41 g, 9.57 mmol, 64%) as a yellow solid. m.p. 140–144°C ^{1}H NMR (600 MHz, DMSO-d_6) δ 4.28 (t, *J* = 7.3 Hz, 2H), 3.56 (s, 3H), 2.63 (t, *J* = 7.3 Hz, 2H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 171.9 (C), 152.7 (C), 152.4 (C), 135.3 (C), 135.0 (C), 43.1 (CH_3), 34.7 (CH_2), 31.7 (CH_2). IR (solid) 3044, 1725, 1606, 1570 cm^{-1} LRMS (ESI). 359 (50, $[M^{81}Br^{81}Br+H]^+$) 357 (100, $[M^{79}Br^{81}Br+H]^+$), 355 (50, $[M^{79}Br^{79}Br+H]^+$). HRMS (ESI) calcd for $C_8H_9Br_2N_2O_4$ $[M^{79}Br^{81}Br+H]^+$ 358.8883; observed 358.8882. Spectra data agrees with that described in Bahou *et al.* [1]

2,5-Dioxopyrrolidin-1-yl 3-(4,5-dibromo-2-methyl-3,6-dioxo-3,6-dihydropyridazin-1(2*H*)-yl) propanoate[1] **7**



A solution of 3-(4,5-dibromo-2-methyl-3,6-dioxo-3,6-dihydropyridazin-1(2*H*)-yl) propanoic acid **6** (750 mg, 2.13 mmol) in THF (10 mL) was cooled to 0°C and was added N,N'-dicyclohexylcarbodiimide (480 mg, 2.34 mmol). The homogenous solution was then stirred at 0°C for 30 min. After this time, was added *N*-hydroxysuccinimide (89 mg, 0.78 mmol) and the reaction stirred at 21°C for a further 16 hr. The newly formed heterogeneous mixture was then filtered and the filtrate concentrated in *vacuo*. Purification of the crude residue by flash column chromatography (20% to neat EtOAc/petrol) afforded 2,5-dioxopyrrolidin-1-yl 3-(4,5-dibromo-2-methyl-3,6-dioxo-3,6-dihydropyridazin-1(2*H*)-yl) propanoate **7** (511 mg, 1.13 mmol, 53%) as a white solid. m.p. 100–104°C. ^{1}H NMR (600 MHz, $CDCl_3$) δ 4.48 (t, J = 6.9 Hz, 2H), 3.68 (s, 3H), 3.11 (t, J = 6.9 Hz, 2H), 2.85 (s, 4H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 168.7 (C), 166.0 (C), 153.3 (C), 153.1 (C), 136.9 (C), 135.3 (C), 43.0 (CH_2), 35.3 (CH_3), 29.1 (CH_2), 25.7 (CH_2). IR (solid) 2992, 1814, 1782, 1735, 1634, 1576 cm^{-1}. Spectra data agrees with that described in Bahou *et al.* [1]

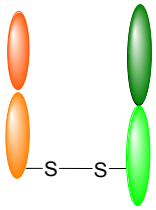
((1*R*,8*S*,9*S*)-Bicyclo[6.1.0]non-4-yn-9-yl)methyl (2-(2-(2-(3-(4,5-dibromo-2-methyl-3,6-dioxo-3,6-dihydropyridazin-1(2*H*)-yl)propanamido)ethoxy)ethoxy)ethyl) carbamate[1] **8**



To a solution of 2,5-dioxopyrrolidin-1-yl 3-(2-bromo-2-methyl-3,6-dioxo-3,6-dihydropyridazin-1(2*H*)yl) propanoate **7** (132 mg, 0.200 mmol, pre-dissolved in MeCN (10 mL)), was added *N*-[(1*R*,8*S*,9*S*)-bicyclo[6.1.0]non-4-yn-9-ylmethyloxycarbonyl]-1,8-diamino-3,6-dioxaoctane (71 mg, 0.220 mmol) and the reaction mixture was stirred at 21°C for 16 hr. After this time, the reaction mixture was concentrated *in vacuo* and the crude residue dissolved in $CHCl_3$ (10 mL) and washed with water (2 × 5 mL) and saturated aq. K_2CO_3 (10 mL). The organic layer was then dried ($MgSO_4$) and concentrated *in vacuo*. Purification of the crude residue by flash column chromatography (0% to 10% MeOH/EtOAc) afforded ((1*R*,8*S*,9*S*)-Bicyclo[6.1.0]non-4-yn-9-yl)methyl (2-(2-(2-(3-(4,5-dibromo-2-methyl-3,6-dioxo-3,6-dihydropyridazin-1(2*H*)-yl)propanamido)ethoxy)ethoxy)ethyl) carbamate **8** (105 mg, 0.160 mmol, 72%) as a yellow oil: ^{1}H NMR (600 MHz, $CDCl_3$, rotamers) δ 7.84 (s, 0.5H), 6.34 (s, 0.5H), 5.82 (s, 0.5H), 5.29 (s, 0.5H), 4.44 (t, *J* = 6.6 Hz, 2H), 4.14–4.12 (m, 2H), 3.73–3.71 (m, 3H), 3.60–3.38 (m, 12H), 2.62 (t, *J* = 6.6 Hz, 2H), 2.29-2.22 (m, 6H), 1.61–1.57 (m, 2H), 1.39–1.24 (m, 1H), 0.96–0.94 (m, 2H); ^{13}C NMR (150 MHz, $CDCl_3$, rotamers) δ 169.1 (C), 156.9 (C), 153.1 (C), 153.0 (C), 136.4 (C), 135.5 (C), 98.9 (C), 70.4 (CH_2), 70.3 (CH_2), 69.7 (CH_2), 63.0 (CH_2), 44.6 (CH_2), 40.8 (CH_2), 39.5 (CH_2), 35.1 (CH_3), 34.1 (CH_2), 29.3 (CH_2), 29.2 (CH_2), 21.6 (CH_2), 20.2 (CH_2), 17.9 (CH), 14.3 (CH); IR (thin film) 3329, 2920, 2858, 1708, 1630, 1572, 1534 cm^{-1}; LRMS (ESI), 687 (50, $[M^{81}Br^{81}Br+Na]^+$) 685 (100, $[M^{79}Br^{81}Br+Na]^+$), 683 (50, $[M^{79}Br^{79}Br+Na]^+$), 663 (60, $[M^{79}Br^{81}Br+H]^+$); HRMS (ESI) calcd for $C_{25}H_{35}Br_2N_4O_7$ $[M^{79}Br^{81}Br+H]^+$ 663.0847; observed 663.0846. Spectra data agrees with that described in Bahou *et al.* [1]

Chemical biology

CTX F(ab) **2**



CTX **1** (10 mg, 10 mg/mL) was digested with 1 mL of immobilized papain beads (CTX **1**/papain ratio 40:1) in a digest buffer (20 mM sodium phosphate monobasic, 10 mM EDTA, and 80 mM cysteine·HCl, pH = 7) for 5 hr at 37°C. Subsequently, the reaction mixture was centrifuged at 10,000 rpm for 1 min to remove the immobilised papain. The reaction mixture containing **2** was subsequently purified by passing through a Protein A column. The concentration and purity of CTX F(ab) **2** was evaluated by UV-Vis spectroscopy (ε_{280} = 68,590 $M^{-1}{\cdot}cm^{-1}$) and SDS-PAGE. The molecular weight of CTX F(ab) **2** was determined by LC-MS. Observed mass: 49788 Da. Glycosylation results in heterogeneous populations of F(ab), visible by LC-MS.

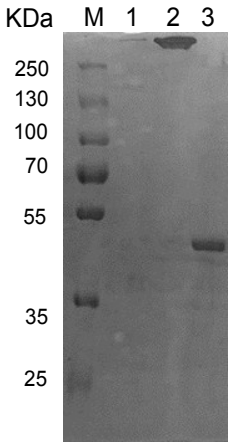


Figure S1. SDS-PAGE gel for CTX **1** digestion**: M)** Molecular weight marker. **1)** Empty. **2)** CTX **1**. **3)** CTX F(ab) **2**.

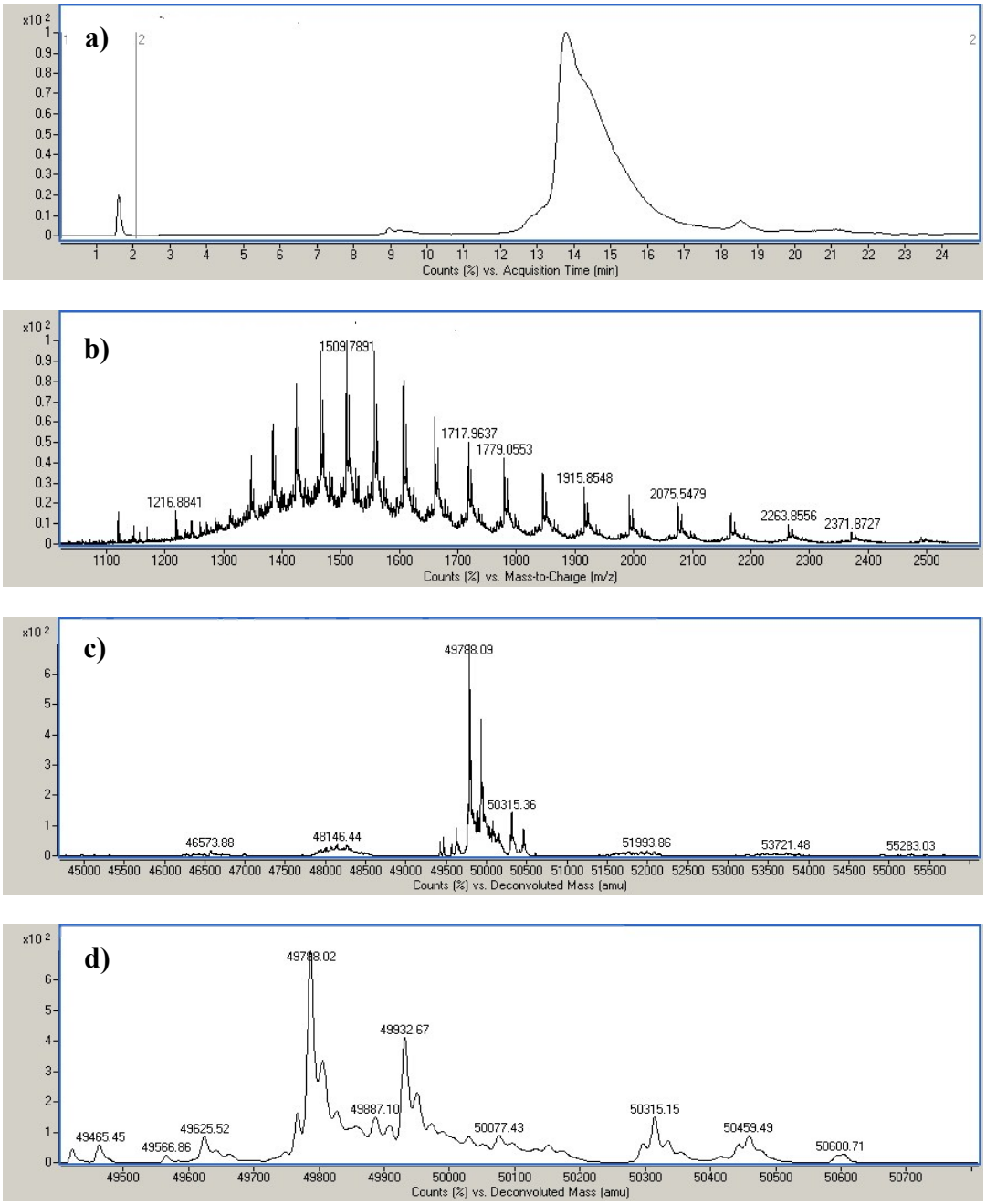


Figure S2. (a) TIC, **(b)** non-deconvoluted LC-MS trace, **(c)** deconvoluted MS data for CTX F(ab) **2**, **(d)** zoom in of deconvoluted MS data for **2**, highlighting the N-Glycan residues present in the F(ab) fragment (M+145 Da, M+528 Da, M+671 Da)

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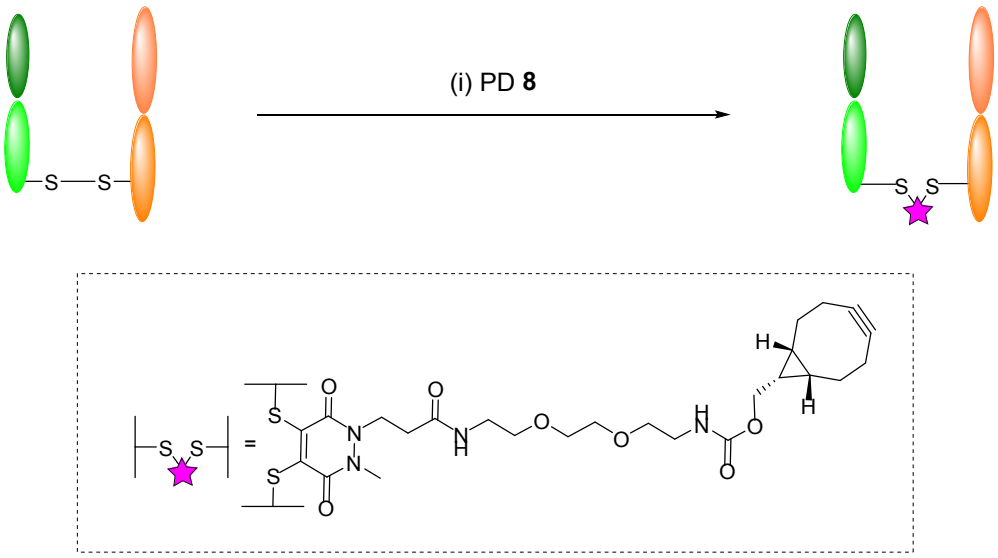
CTX F(ab) **9**



To a solution of CTX F(ab) **2** (50 µL, 20 µM, 1 eq.) in BBS (25 mM sodium borate, 25 mM NaCl, 5 mM EDTA, pH 8 + 3% DMSO) was added PD **8** (1 µL, 20 mM in DMSO, 20 eq.), followed by TCEP.HCl (1 µL, 20 mM in H_2O, 20 eq.) and the reaction mixture incubated at 21°C for 16 hr. The excess reagents were then removed by repeated diafiltration into fresh buffer using VivaSpin sample concentrators (GE Healthcare, 10,000 MWCO). Following this, analysis by SDS-PAGE, LC-MS and UV-Vis revealed > 95% conversion to CTX F(ab) **9**. Expected mass: 50,290 Da. Observed mass: 50,288 Da. Glycosylation results in heterogeneous populations of **9**.

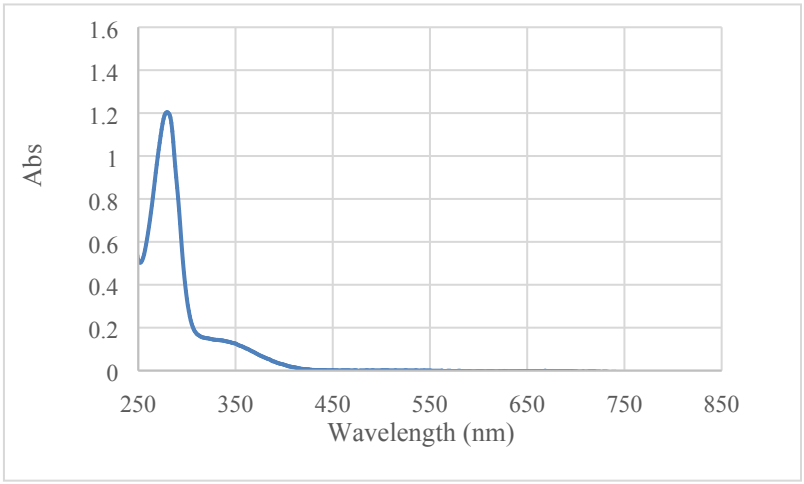


Figure S3. UV-Vis data for CTX F(ab) **9**, Pyridazinedione to antibody ratio ≈ 0.95.

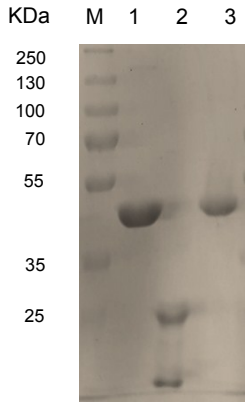


Figure S4. SDS-PAGE gel for successful formation of conjugate **9: M)** Molecular weight marker. **1)** CTX F(ab) **2**. **2)** CTX F(ab) + 20 eq. TCEP.HCl. **3)** CTX F(ab) **9**.

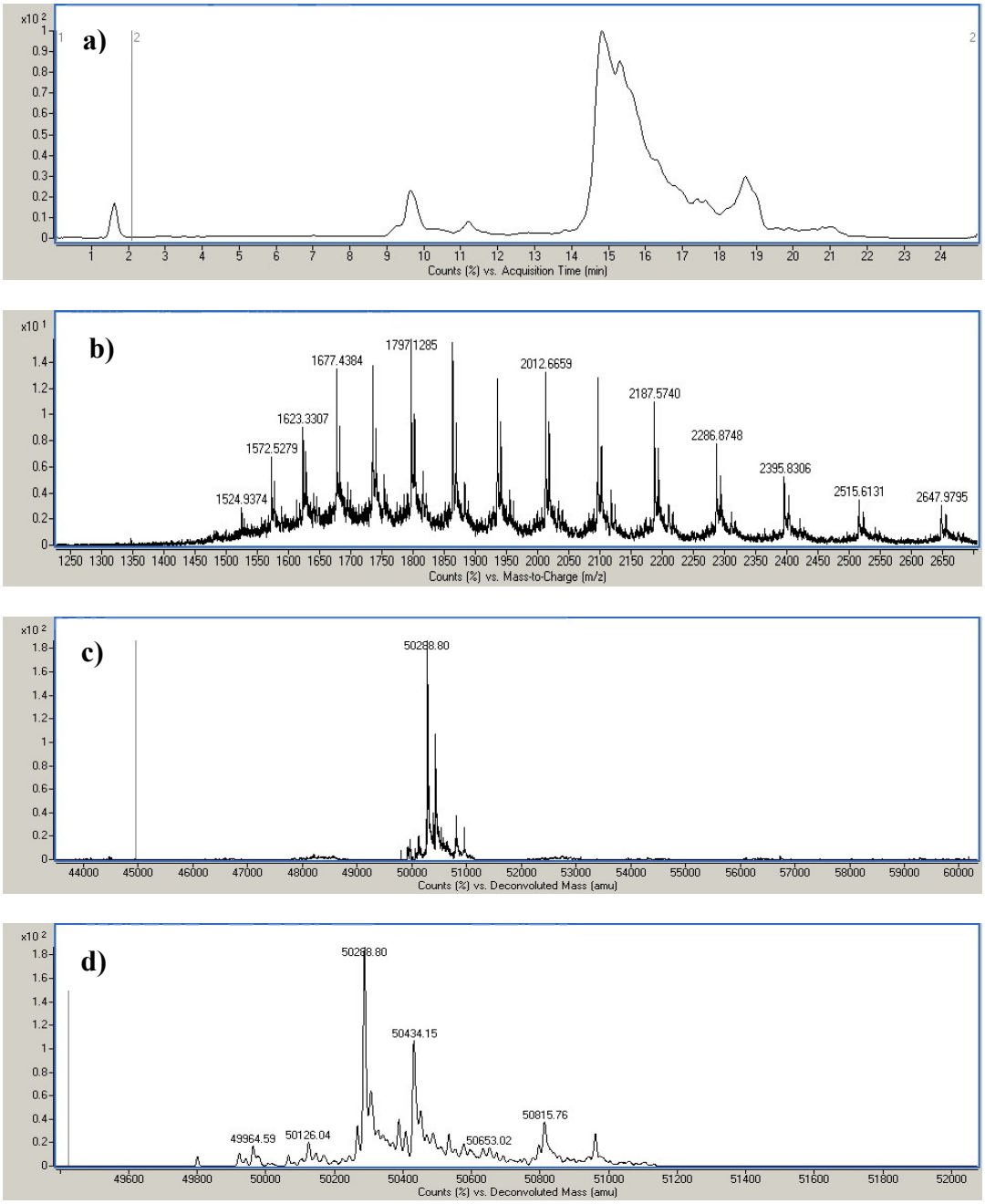
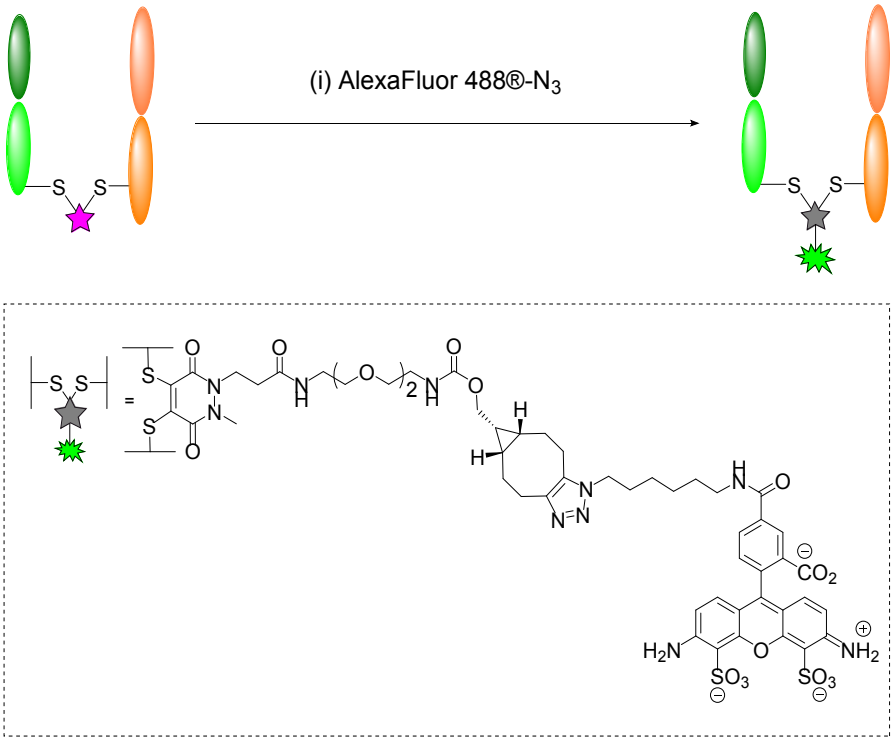


Figure S5. (a) TIC, **(b)** non-deconvoluted LC-MS trace, **(c)** deconvoluted MS data for conjugate **9** and **(d)** zoom in of deconvoluted MS data for conjugate **9**, confirming the presence of N-Glycan residues (M+145 Da, M+528 Da, M+671 Da).

CTX F(ab) **10**



To a solution of conjugate **9** (50 µL, 20 µM, 1 eq.) in PBS (pH = 7.4) was added Alexafluor®-488-N_3 (0.2 µL, 20 mM in DMSO, 4 eq.) and the reaction mixture incubated at 21°C for 2 hr. The excess reagents were then removed by repeated diafiltration into fresh buffer using VivaSpin sample concentrators (GE Healthcare, 5,000 MWCO). Successful conjugation was confirmed by SDS-PAGE, UV-Vis analysis and LC-MS. Expected mass: 50,946 Da. Observed mass: 50,946 Da. Glycosylation results in heterogeneous populations of conjugate **10**.

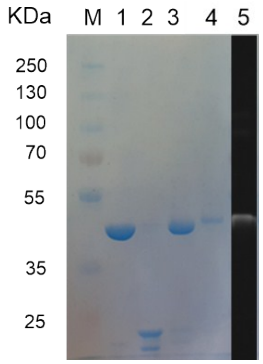


Figure S6. SDS-PAGE gel for successful formation of CTX F(ab) fluorescent conjugate **10: M)** Molecular weight marker. **1)** CTX F(ab) **2**. **2)** CTX F(ab) + 20 eq. TCEP.HCl. **3)** CTX F(ab) **9**. **4)** CTX F(ab) fluorescent conjugate **10. 5)** CTX F(ab) fluorescent conjugate under UV-Vis light.

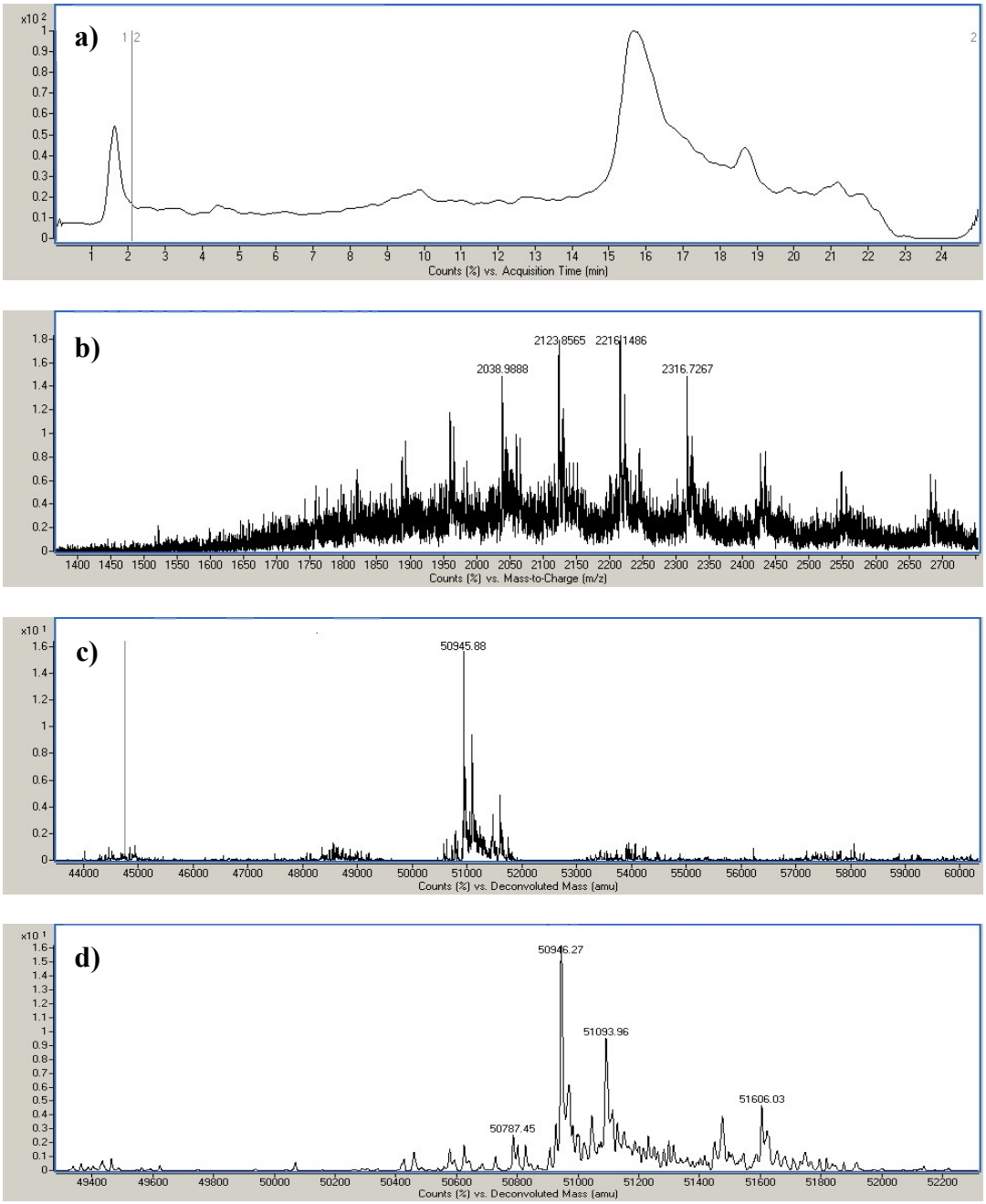


Figure S7. (a) TIC, **(b)** non-deconvoluted LC-MS trace, **(c)** deconvoluted MS data for **10 (d)** zoom in of deconvoluted MS data for **10**, confirming the presence of N-Glycan residues in the 'clicked' F(ab) fragment (M+145 Da, M+528 Da, M+671 Da).

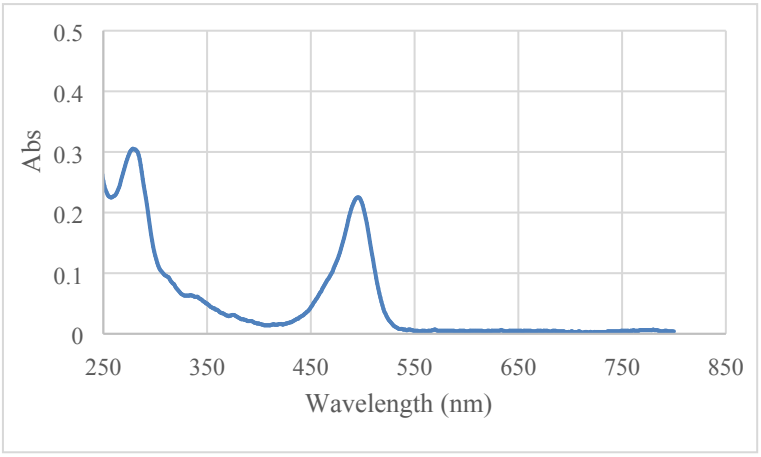


Figure S8. UV-Vis data for CTX F(ab) fluorescent conjugate **10**, Fluorophore to Antibody ratio ≈ 0.8. CTX F(ab) concentration corrected using a correction factor at 280 nm of 0.11 × A_{495} for Alexafluor®-488.

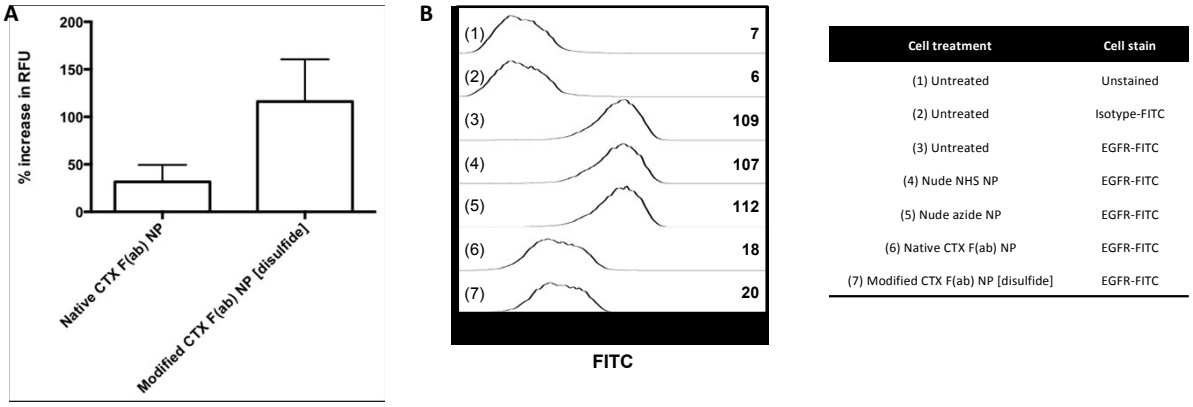


Figure S9. Binding of nanoformulations to EGFR-expressing cells. (A) MIA PaCa-2 cells were treated with fluorescent nanoformulations (600 µg polymer/mL) for 45 min at 4 °C. Cells were then washed and binding of the nanoformulations was assessed through fluorescence measurement. Results for native CTX F(ab) NP and modified CTX F(ab) NP [disulfide] are presented as % increase in RFU versus the corresponding nude NHS NP and nude azide NP controls, respectively. (B) BxPC-3 cells were treated with non-fluorescent nanoformulations (500 µg polymer/mL) for 1 hr at 4 °C. Cells were then washed, stained with FITC-labelled EGFR or isotype control antibodies and analysed by flow cytometry. Representative histograms are shown for each of the numbered treatments 1 – 7, with inset values denoting the geometric mean fluorescence intensity.

References

1) Bahou, C., Richards, D. A., Maruani, A., Love, E. A., Javaid, F., Caddick, S., Baker, J. R. & Chudasama, V. Highly homogeneous antibody modification through optimisation of the synthesis and conjugation of functionalised dibromopyridazinediones. *Org Biomol Chem* 16(8), 1359-1366 (2018).