Synthesis and evaluation of the aldolase antibody-derived chemical-antibodies targeting alpha(5)beta(1) integrin

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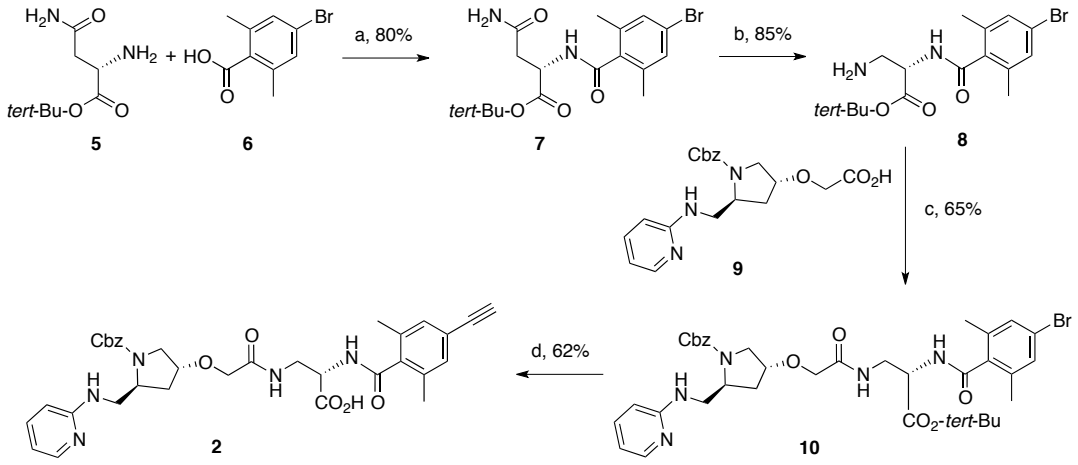
Supporting Information

1. General Methods. All reagents and solvents were obtained from commercial sources and used as received. All air-sensitive reactions were performed under argon atmosphere. In the course of aqueous work-up process, reactions were quenched with a saturated solution of ammonium chloride and extracted with ethyl acetate or chloroform, and the combined organic layers were washed with water and brine, and dried over anhydrous Na_2SO_4. ^1H and ^{13}C NMR spectra were recorded on Bruker 300 and 400 MHz, and Varian instruments 500 and 600 MHz using deuterated chloroform (99.8%D) as solvents, unless otherwise stated. ^1H Chemical shifts values (d) are reported as ppm downfield with respect to tetramethylsilane (δ at 0) as standard. Mass spectra were measured in positive mode electrospray ionization (ESI) on Agilent LC/MSD TOF instrument. TLC was performed on silica gel 60 F_{254} glass plates, and column chromatography was performed using silica gel (35-75 mesh).

2. Synthesis of targeting compound 2. Prepared as shown in Scheme S-1.



Scheme S-1. Synthesis of compound **2**. Key: Reagents and conditions: (a) 4-pyrrolidinopyridine, HBTU, DIPEA, CH_2Cl_2, 0 ˚C-RT, 5 h; (b) PIFA, Py, CH_3CN, 0 ˚C-RT, 12 h; (c) HBTU, DIPEA, DMF, RT, 12 h; (d) (i) TMS acetylene, $(Ph_3P)_2PdCl_2$, CuI, Et_3N:THF (2:1), 90 ˚C, 12 h, (ii) LiOH, THF:H_2O (2:1), RT, 24 h.

2.1. Compound 7. 4-Bromo-2,6-dimethylbenzoic acid, **6**, (352 mg, 1.54 mmol), 4-pyrrolidinopyridine (6.4 μL, 0.064 mmol), DIPEA (0.67 mL, 3.84 mmol) and HBTU (584 mg, 1.54 mmol) were dissolved in CH_2Cl_2 (5 mL) and cooled to 0 ˚C. After 10 minutes, compound **5** (288 mg, 1.28 mmol) was added and stirring was continued at RT under N_2 atmosphere for 5 h. The reaction mixture was concentrated, and worked up using EtOAc (2 x 30 mL), washed

sequentially with water and brine, and dried over Na_2SO_4. The organic layer was separated and concentrated, and the residue was purified using silica gel column chromatography to give compound **7** (408 mg, 80% yield).

2.2. Compound 8. Compound **7** (140 mg, 0.35 mmol) was dissolved in CH_3CN (2 mL) and H_2O (1 mL) and cooled using ice-water bath. PIFA (166 mg, 0.39 mmol) was added and stirred for 10 minutes. Pyridine (60 μL, 0.70 mmol) was added and stirring was continued at RT for additional 12 h. CH_3CN was removed in vaccuo and the aqueous layer was acidified with 1N HCl. The combined aqueous layer was neutralized using solid $NaHCO_3$ and then extracted using EtOAc (3 x 20 mL). The combined organic layer was dried over Na_2SO_4, concentrated in vacuum, and purified using silica gel column chromatography to give compound **8** (110 mg, 85% yield). ^{1}HNMR (300 MHz): δ 7.20 (s, 2H), 6.69 (m, 1H), 4.72 (m, 1H), 3.20 (dd, J = 12.9, 4.9 Hz, 2H), 2.30 (s, 6H), 1.51 (s, 9H).

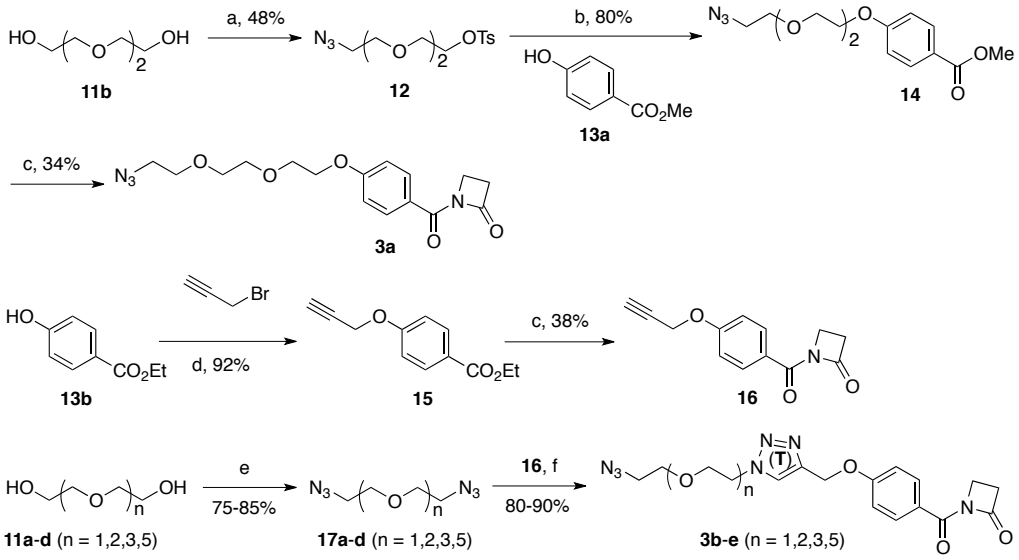
2.3. Compound 10. The known intermediate **9** (82 mg, 0.212 mmol), HBTU (82 mg, 0.215 mmol) and DIPEA (80 μL, 0.432 mmol) were dissolved in DMF (2 ml) and stirred for 10 minutes at RT under N_2 atmosphere. Compound **8** (80 mg, 0.215 mmol) in DMF (1 ml) was added to the reaction mixture and stirring was continued for additional 12 h. The reaction mixture was concentrated under vaccuo, and worked-up using water-EtOAc (3 x 15 ml). The organic layer was washed with water and brine, and dried over Na_2SO_4. The organic phase was separated and concentrated, and the residue was purified using silica gel column chromatography to get compound **10** (102 mg) in 65% yield. ^{1}HNMR (300 MHz): δ 8.01 (bs, 1H), 7.35 (s, 5H), 7.15 (s, 2H), 6.99 (t, J = 6.0 Hz, 1H), 6.81 (m, 1H), 6.53 (t, J = 6.2 Hz, 1H), 6.46 (d, J = 7.8 Hz, 1H), 6.08 (bs, 1H), 5.09 (q, J = 18.0, 12.0 Hz, 2H), 4.79 (m, 1H), 4.24 (m, 1H), 4.11 (bs, 1H), 3.88 (s, 2H), 3.75-3.65 (m, 3H), 3.56-3.40 (m, 3H), 2.27 (s, 6H), 2.18-2.12 (m, 2H), 1.47 (s, 9H).

2.4. Compound 2. TMS-acetylene (19.1 μL, 0.135 mmol), followed by CuI (2 mg, 8.5 x 10^{-3} mmol) and $(Ph_3P)_2PdCl_2$ (3 mg, 3.4 x 10^{-3} mmol) were added to a degassed solution of compound **10** (50 mg, 0.068 mmol) in Et_3N:THF (2:1, 3 mL), and the reaction mixture was flushed with argon and heated at 90 ˚C under argon atmosphere for 12 h. The solvents were removed under vaccuo, and the residue purified using silica gel column chromatography to afford the TMS-protected derivative of compound **2** (45 mg, 88% yield). ^{1}HNMR (300 MHz): δ 7.92 (bs, 1H), 7.29 (s, 5H), 7.09 (s, 2H), 6.99-6.80 (m, 2H), 6.48 (m, 2H), 5.08 (q, J_1 = 12.0 Hz, J_2 = 18.0 Hz,

2H), 4.72 (m, 1H), 4.19-4.02 (m, 2H), 3.81 (s, 2H), 3.69-3.48 (m, 6H), 2.20 (s, 6H), 2.19-2.13 (m, 2H), 1.41 (s, 9H), 0.19 (s, 6H). MS-ESI: 756.3 (M+H), 779.2 (M+Na).

The above-described compound (30 mg, 0.04 mmol) was saponified using LiOH (3 mg, 0.12 mmol) in THF: H_2O (2:1, 0.9 ml) at RT, 24h. The reaction mixture was concentrated under vaccuo, brought to pH 6 using dilute (1N) HCl, and worked up using $CHCl_3$ (3 x 15 mL). Organic layer was washed with water and brine, and dried over Na_2SO_4. Solvents were removed, and the crude residue was purified using silica gel column chromatography to give compound **2** (17.4 mg, 70% yield). ^{1}HNMR (300 MHz): δ 7.89 (bs, 1H), 7.35 (s, 5H), 7.11 (s, 2H), 6.95-6.82 (m, 2H), 6.50 (m, 2H), 5.09 (q, *J* = 18.0, 12.0 Hz, 2H), 4.71 (m, 1H), 4.15 (m, 2H), 3.80 (s, 2H), 3.69-3.45 (m, 6H), 3.10 (s, 1H), 2.20 (s, 6H), 2.19-2.13 (m, 2H). MS-ESI: 628.2 (MH^+).

3. Synthesis of linkers 3a-e. Prepared using the readily available intermediates as shown in Scheme S-3.



Scheme S-2. Synthesis of compounds **3a**-**e**. Key: (a) (i) TsCl, Et_3N, CH_3CN, 0 ˚C-RT, 5 h, (ii) NaN_3, DMF, 60 ˚C, 4 h, (iii) TsCl, Et_3N, CH_3CN, 0 ˚C-rt, 6 h; (b) K_2CO_3, DMF, RT, 12 h; (c) (i) LiOH, THF:H_2O:MeOH (3:1:1), RT, 12 h, (ii) $(COCl)_2$, cat. DMF, CH_2Cl_2, RT, 12 h, (iii) 2-Azetidinone, ^{n}BuLi, THF, -78°C- 0°C, 30 min; (d) (i) TsCl, Et_3N, CH_3CN, 0 ˚C-RT, 10 h, (ii) NaN_3, DMF, 60 ˚C, 4 h; (e) Cu (powder), $CuSO_4$ (1 M Aq. solution), CH_3CN, 50 ˚C.

3.1. Compound 12. Commercially available triethylene glycol **11b** (12 mL, 89.9 mmol) was treated with TsCl (8.56 g, 45 mmol) in the presence of Et_3N (28 mL, 198 mmol) in CH_3CN (200

mL) at 0°C under N_2 atmosphere and the resulting mixture was stirred for 5 h at RT. The reaction was finally quenched with saturated aqueous solution of NH_4Cl (60 mL). CH_3CN was removed under vaccuo and reaction mixture was worked up with EtOAc (2 x 200 mL), washed with brine and finally dried over Na_2SO_4. The organic phase was concentrated and purified by column chromatography to give mono tosyl triethylene glycol (16.5 g) in 60% yield.

The above-described monotosyl-triethylene glycol (5.0 g, 16.37 mmol) was taken in DMF (40 mL). NaN_3 (2.12 g, 32.75 mmol) was added and the reaction mixture was heated at 60 ˚C for 4 h under N_2 atmosphere. DMF was removed and diluted with water (20 mL). The residue was worked up with EtOAc (2 x 100mL), washed sequentially with water, brine and finally dried over Na_2SO_4. The organic layer was concentrate in vaccuo and purified with column chromatography to get mono azide triethylene glycol (2.58 g) in 90% yield. ^{1}HNMR (300 MHz): δ 3.72-3.61 (m, 8H), 3.57 (t, *J* = 4.8 Hz, 2H), 3.35 (t, *J* = 4.8 Hz, 2H).

The above prepared mono azide triethylene glycol (2.5 g, 14.28 mmol) was subjected to tosylation using TsCl (2.99 g, 15.71 mmol) and Et_3N (2.98 mL, 21.42 mmol) in CH_3CN (20 mL) for 6h under N_2 atmosphere using the same protocol described above to give compound **12** (4.14 g) in 88% yield. ^{1}HNMR (300 MHz): δ 7.80 (d, *J* = 7.8 Hz, 2H), 7.34 (d, *J* = 7.8 Hz, 2H), 4.16 (t, *J* = 4.8 Hz, 2H), 3.70 (t, *J* = 4.8 Hz, 2H), 3.64 (t, *J* = 4.8 Hz, 2H), 3.60 (m, 4H), 3.36 (t, *J* = 4.8 Hz, 2H), 2.45 (s, 3H).

3.2. Compound 14. A mixture of compound **12** (1.99 g, 6.06 mmol), **13a** (920 mg, 6.06 mmol) and K_2CO_3 (1.08 g, 7.88 mmol) in DMF (20 mL) was heated to 50 ˚C under N_2 atmosphere for 12 h. The reaction mixture was worked up using Et_2O (3 x 50 mL). The combined organic layer was washed using water and brine, and dried over Na_2SO_4. Solvents were removed and the residue was purified using silica gel column chromatography to give compound **14** (1.49 g, 80% yield).

3.3. Compound 3a. Compound **14** (1.4 g, 4.53 mmol) was saponified using LiOH (326 mg, 13.59 mmol) in THF:MeOH:H_2O (3:1:1) (15 ml) at RT for 12h. Reaction mixture was concentrated, acidified using 1N HCl, and extracted with $CHCl_3$. The organic layer was washed with water and brine, and dried over Na_2SO_4. Solvents were removed, and the crude acid (1.2 g, 90% yield) was taken to next reaction without further purification. ^{1}HNMR (300 MHz): δ 8.01

(d, *J* = 8.7 Hz, 2H), 6.93 (d, *J* = 8.7 Hz, 2H), 4.17 (t, *J* = 4.8 Hz, 2H), 3.87 (t, *J* = 4.8 Hz, 2H), 3.74-3.62 (m, 6H), 3.36 (t, *J* = 4.8 Hz, 2H).

$(COCl)_2$ (5 mL) and DMF (1-2 drops) were added to a mixture of the above-described crude acid (1.2 g, 4.06 mmol) was dissolved in CH_2Cl_2 (10 ml) in presence of catalytic amount of and at RT under N_2 atmosphere. After the reaction mixture was stirred for 12 h at RT, solvents were removed under vaccuo, and the crude residue was taken to next step without further purification.

Commercially available 2-azetidinone (259 mg, 3.65 mmol) was dissolved in THF (3 mL) under N_2 atmosphere and cooled to -78 ˚C. ^{n}BuLi (1.6 M in hexane, 2.16 mL, 3.47 mmol) was added to it and the reaction mixture was brought to 0 ˚C within 5 minutes. The crude acid chloride (prepared from 4.06 mmol acid) obtained from the above reaction was dissolved in THF (4 mL) and cannulated to the reaction mixture. After 30 minutes stirring at 0 ˚C, the reaction was quenched with 10% aqueous citric acid (3 mL). The resulting solution was worked up using $CHCl_3$ (2 x 20 mL) and water. The combined organic layer was washed using water and brine, dried over Na_2SO_4, and concentrated in vaccuo. The resulting residue was purified using silica gel column chromatography to afford compound **3a** (567 mg, 40% yield based on **14**). ^{1}HNMR (300 MHz): δ 8.03 (d, *J* = 8.8 Hz, 2H), 6.95 (d, *J* = 8.8 Hz, 2H), 4.18 (t, *J* = 4.8 Hz, 2H), 3.88 (t, *J* = 4.8 Hz, 2H), 3.79-3.65 (m, 8H), 3.37 (t, *J* = 4.8 Hz, 2H), 3.08 (t, *J* = 5.4 Hz, 2H).

3.4. Compound 16. Ester **15**[1] (2.0 g, 10.53 mmol) was hydrolyzed using aqueous LiOH (758 mg, 31.58 mmol) as described earlier for compound **14** affording the corresponding acid (1.67 g, Yield 90%). ^{1}HNMR ($CDCl_3$+MeOD, 300 MHz): δ 8.03 (d, *J* = 8.8 Hz, 2H), 6.98 (d, *J* = 8.8 Hz, 2H), 4.75 (d, *J* = 1.9 Hz, 2H), 2.55 (d, *J* = 1.9 Hz, 1H).

The above-described acid (1.67 g, 9.48 mmol) was converted to acid chloride as described earlier using $SOCl_2$, and reacted with N-Li-2-azetidinone (prepared using 613 mg 2-azetidinone, 8.63 mmol, and 4.9 ml n-BuLi, 7.77 mmol, in 20 ml THF) under N_2 atmosphere to afford compound **16** (651 mg, Yield 30%). ^{1}HNMR (300 MHz): δ 8.00 (d, *J* = 8.8 Hz, 2H), 7.04 (d, *J* = 8.8 Hz, 2H), 4.75 (d, *J* = 2.0 Hz, 2H), 3.76 (t, *J* = 5.4 Hz, 2H), 3.08 (t, *J* = 5.4 Hz, 2H), 2.58 (d, *J* = 2.0 Hz, 1H). MS (ESI): 230.2 $(M+H)^+$, 252.2 $(M+Na)^+$.

3.5. Compounds 17a-d. Prepared from PEG, **11a-d**, *via* the corresponding ditosylates.

Step 1. TsCl (4.4 g, 23.19 mmol) was added in portions to diethylene glycol (**11a**, 1 mL, 10.54 mmol) and Et_3N (3.67 mL, 26.35 mmol) in CH_3CN (30 ml) at 0 ˚C. After the reaction mixture was stirred for 12 hrs at RT under N_2 atmosphere, it was worked-up using saturated aqueous solution of NH_4Cl and EtOAc. The organic layer was washed with water and brine, and dried over Na_2SO_4. Solvents were removed and the residue was purified giving the ditosylate of compound **17a** (3.72 g, Yield 85%).

Similarly, ditosylates of **17b**-**d** were prepared in 80-90% yield.

11a-ditosylate. ^{1}HNMR (300 MHz): δ 7.73 (d, *J* = 8.1 Hz, 4H), 7.30 (d, *J* = 8.1 Hz, 4H), 4.04 (t, *J* = 4.8 Hz, 4H), 3.56 (t, *J* = 4.8 Hz, 4H), 2.42 (s, 6H).

11b-ditosylate. ^{1}HNMR (300 MHz): δ 7.73 (d, *J* = 8.4 Hz, 4H), 7.31 (d, *J* = 8.4 Hz, 4H), 4.12 (t, *J* = 4.8 Hz, 4H), 3.64 (t, *J* = 4.8 Hz, 4H), 3.54 (s, 4H), 2.41 (s, 6H).

11c-ditosylate. ^{1}HNMR (300 MHz): δ 7.75 (d, *J* = 8.4 Hz, 4H), 7.30 (d, *J* = 8.4 Hz, 4H), 4.11 (t, *J* = 4.8 Hz, 4H), 3.64 (t, *J* = 4.8 Hz, 4H), 3.52 (s, 8H), 2.41 (s, 6H).

11d-ditosylate. ^{1}HNMR (300 MHz): δ 7.72 (d, *J* = 8.1 Hz, 4H), 7.30 (d, *J* = 8.1 Hz, 4H), 4.18 (t, *J* = 4.8 Hz, 4H), 3.70 (t, *J* = 4.8 Hz, 4H), 3.64 (s, 8H), 3.59 (s, 8H), 2.41 (s, 6H).

Step 2. NaN_3 (516 mg, 7.5 mmol) was added to a solution of the above-described ditosylate of **11a** (1.5 g, 3.6 mmol) in DMF (40 mL), and the reaction mixture was heated at 60 ˚C for 4 h under N_2 atmosphere. DMF was removed, and the residue was diluted with water (20 mL) and extracted using EtOAc. The organic layer was washed sequentially with water and brine, and dried over Na_2SO_4. Solvents were removed, and the residue was purified giving compound **17a** (535 mg, Yield 94%).

Similarly, compounds **17b**-**d** were prepared from the ditosylates of compounds **11b**-**d**.

Compound 17a. ^{1}HNMR (300 MHz): δ 3.70 (t, *J* = 5.4 Hz, 4H), 3.42 (t, *J* = 4.8 Hz, 4H).

Compound 17b. ^{1}HNMR (300 MHz): δ 3.68-3.64 (m, 8H), 3.37 (t, *J* = 4.8 Hz, 4H).

Compound 17c. ^{1}HNMR (300 MHz): δ 3.72-3.68 (m, 12H), 3.42 (t, *J* = 4.8 Hz, 4H).

Compound 17d. ^{1}HNMR (300 MHz): δ 3.73-3.66 (m, 20H), 3.42 (t, *J* = 4.8 Hz, 4H).

3.6. Compounds 3b-e. Cu wire (small piece) and $CuSO_4$ (1M, 8.7 μL, 8.7 X10^{-3} mmol) were added to a mixture of alkyne **16** (20 mg, 0.087 mmol) and diazide **17a** (41 mg, 0.262 mmol) in CH_3CN (500 μL), and the mixture was stirred at 50 ˚C for 12 h. Solvents were removed and the

residue was purified using silica gel column chromatography affording compound **3b** (27 mg, 80% yield). ^{1}HNMR (400 MHz): δ 8.02 (d, *J* = 8.8 Hz, 2H), 7.79 (s, 1H), 7.05 (d, *J* = 8.8 Hz, 2H), 5.29 (s, 2H), 4.58 (d, *J* = 4.9 Hz, 2H), 3.88 (d, *J* = 4.8 Hz, 2H), 3.76 (t, *J* = 5.4 Hz, 2H), 3.60 (t, *J* = 4.8 Hz, 2H), 3.32 (t, *J* = 4.8 Hz, 2H), 3.09 (t, *J* = 5.4 Hz, 2H). LCMS-ESI: 386.3 (MH^+), 408.3 (MNa^+).

Similarly, compounds **3c**-**e** were prepared using compound **16** and diazides **17b**-**d**.

Compound 3c: ^{1}HNMR (400 MHz): δ 8.02 (d, *J* = 8.8 Hz, 2H), 7.88 (s, 1H), 7.05 (d, *J* = 8.8 Hz, 2H), 5.28 (s, 2H), 4.57 (d, *J* = 4.9 Hz, 2H), 3.89 (d, *J* = 4.8 Hz, 2H), 3.77 (t, *J* = 5.4 Hz, 2H), 3.63-3.59 (m, 6H), 3.36 (t, *J* = 4.8 Hz, 2H), 3.09 (t, *J* = 5.4 Hz, 2H). LCMS-ESI: 430.2 (MH^+), 452.2 (MNa^+).

Compound 3d: ^{1}HNMR (400 MHz): δ 8.02 (d, *J* = 7.2 Hz, 2H), 7.86 (s, 1H), 7.06 (d, *J* = 7.2 Hz, 2H), 5.28 (s, 2H), 4.56 (d, *J* = 4.4 Hz, 2H), 3.88 (d, *J* = 4.8 Hz, 2H), 3.77 (t, *J* = 5.4 Hz, 2H), 3.68-3.58 (m, 10H), 3.36 (t, *J* = 4.6 Hz, 2H), 3.09 (t, *J* = 5.4 Hz, 2H). LCMS-ESI: 474.3 (MH^+), 496.3 (MNa^+).

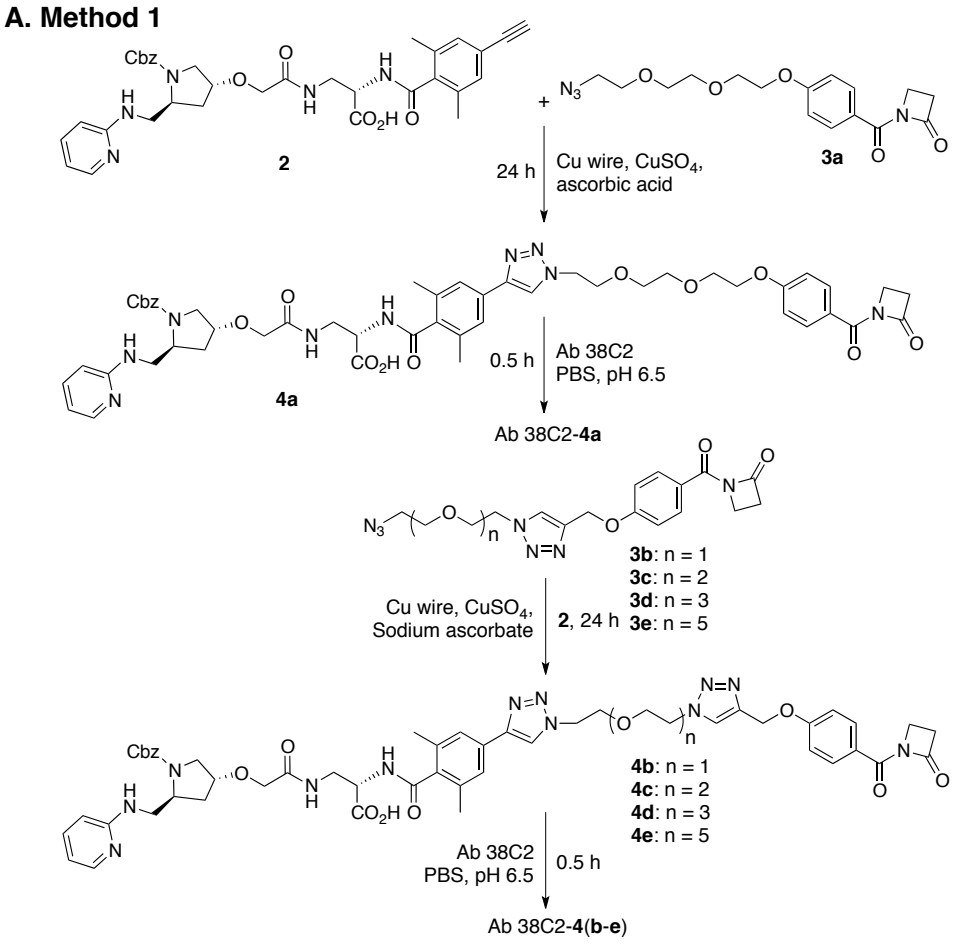
Compound 3e: ^{1}HNMR (400 MHz): δ 8.02 (d, *J* = 8.8 Hz, 2H), 7.88 (s, 1H), 7.06 (d, *J* = 8.8 Hz, 2H), 5.28 (s, 2H), 4.57 (d, *J* = 4.9 Hz, 2H), 3.88 (d, *J* = 4.8 Hz, 2H), 3.77 (t, *J* = 5.4 Hz, 2H), 3.70-3.60 (m, 18H), 3.36 (t, *J* = 4.8 Hz, 2H), 3.09 (t, *J* = 5.4 Hz, 2H). LCMS-ESI: 562.3 (MH^+), 584.3 (MNa^+).

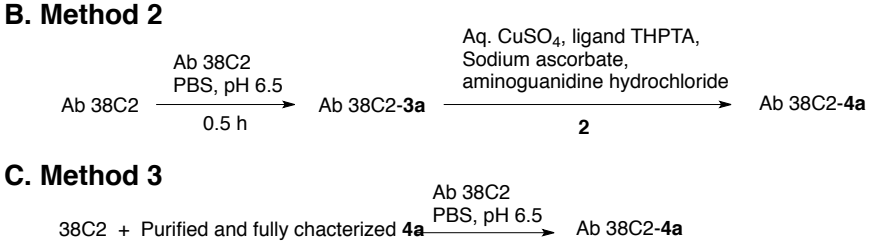
4. Construction of chem-Abs 38C2-4(a-e).

In method 1, compound **2** underwent Cu-ACC with **3a**, and the resulting product **4a** reacted with Ab 38C2 without any extensive purification of **4a** to give chem-Ab 38C2-**4a** (Scheme S-3A). Similarly, compound **2** reacted with **3b**-**e**, and the resulting products **4b**-**e** with Ab 38C2 giving 38C2-**4**(**b**-**e**).

In method 2, compound **3a** reacted with Ab first to give 38C2-**3a** and the latter product underwent Cu-ACC with compound **2** to give 38C2-**4a** (Scheme S-3B).

In method 3, compounds **2** and **3a** reacted to give compound **4a**. The latter was purified and fully characterized before reacting with Ab 38C2 to give cpAb 38C2-**4a** (Scheme S-3C).





Scheme S-3. Synthesis of chem-Abs using the (A) *in situ* convergent CP, and (B) classical methods starting with intermediates **2** and **3**'s.

4.1. *In situ* convergent CP method 1.

Step 1. A mixture of compound **2** (50 mM, 15 μl), linker **3a** (50 mM, 5 μl), Cu wire (small piece), and aqueous $CuSO_4$ (1 M, 1 μl) in CH_3CN (79 μl) was stirred at RT for 24 hrs. After all the linker **3a** had consumed to give compound **4a**, as analyzed using LCMS, the mixture was diluted with CH_3CN (400 μl) and treated with CupriSorb™ (10 mg) for 3 hrs, and subsequently

filtered using the nanopore filter. MS (ESI) of Compound **4a**: 976 $(M+H)^+$. Similarly, compound **2** reacted with linkers **3b**-**e** giving PAs **4b**-**e**.

Compound **4b**. MS (ESI): 1014.3 $(M+H)^+$.

Compound **4c**. MS (ESI): 1058.4 $(M+H)^+$.

Compound **4d**. MS (ESI): 1102.4 $(M+H)^+$.

Compound **4e**. MS (ESI): 1190.4 $(M+H)^+$.

Step 2. The above-described CH_3CN solution of compound **4a** (13 μl) was added to Ab 38C2 (4 μM, 200 μl) in PBS, pH 7.4, and the mixture was incubated at 37 ˚C for 2 h to give cpAb 38C2-**4a**. Concentration of the cpAb 38C2-**4a** solution, thus obtained, was determined using the nanoUV spectrophotometer for subsequent analysis. Similarly, PAs **4b**-**e** reacted with Ab 38C2 to afford cpAb 38C2-**4**(**b**-**e**).

4.2. Method 2.

Step 1. A mixture of Ab 38C2 (6.67 μM in PBS, pH 6.5, 0.1 ml) and linker **3a** (1.67 mM in CH_3CN, 2 μl) was incubated at 37 ˚C for 2 hrs. Complete Ab programming of the mixtures (giving 38C2-**3a**) was confirmed using the methodol assay. Excess linker was removed using Amicon spinning filter (10K) with an addition of PBS buffer (0.4 ml) and minimizing the volume to 0.1 ml (3x).

Step 2. Aq. $CuSO_4$ (20 mM, 0.5 μl) and tris(hydroxypropyltriazolyl)methylamine (THPTA) ligand (50 mM, 1 μl) were premixed and added to the above described cpAb 38C2-**3a** solution (100 μl), followed by sodium ascorbate (100 mM, 5 μl) and aminoguanidine.HCl (100 mM, 5 μl), and compound **2** (20 mM in DMSO, 2 μl). The mixtures were shaken at room temperature for 24 hrs, filtered using nano filter and dialyzed using 0.1% of the sodium salt of EDTA in PBS buffer to remove soluble copper, and then using PBS buffer (no EDTA), pH 7.4 giving cpAbs.

4.3. Method 3. Step 1 in method 1 was repeated using compound **2** (0.1 mmol), linker **3a** (0.1 mM), and the product **4a** was purified and analyzed before reacting with Ab 38C2.

5. Evaluation of chem-Abs 38C2-4's.

5.1. Binding of the chem-Abs 38C2-4(a-e) to U87 and/or 4T1 cells using flow cytometry.

U87 and 4T1 cells were detached by trypsinization with 0.25 %(wt/v) trypsin/1 mM EDTA and washed twice with PBS. The cells were resuspended at a concentration of 1.5×10^5 cells/ml and

distributed into each tube. The cells were incubated with different the chem-Abs 38C2-**4**(**a**-**e**), 15 μg/ml, in binding buffer (1% FCS and 100 nm $MnCl_2$ in PBS) for 2hr at 4 ˚C. Then the cells were washed three times, and incubated with FITC labeled anti mouse goat antibody (Ab) (1 μg/ml) for 1 hour at 4 ˚C. The cells were washed two times with PBS containing 1% FCS and analyzed using **Digital LSR II** scanner.

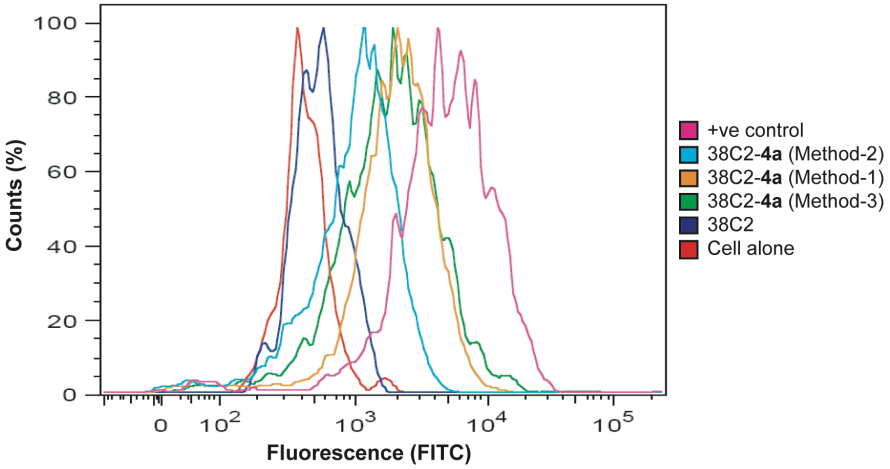


Figure S-1. Flow cytometry histograms showing binding of chem-Ab 38C2-**4a** (15 μg/ml), prepared by methods 1-3, to U87 human glioblastoma cells expressing integrin α5β1. Commercially available anti-α5β1 Ab (15 μg/ml) was used as a +ve control, and Ab38C2 (15 μg/ml) and cell alone were used as –ve controls. FITC-labeled anti-mouse goat secondary Abs (1 μg/ml) were used for detection. The y axis gives the number of events in linear scale, the x axis the fluorescence intensity in logarithmic scale.

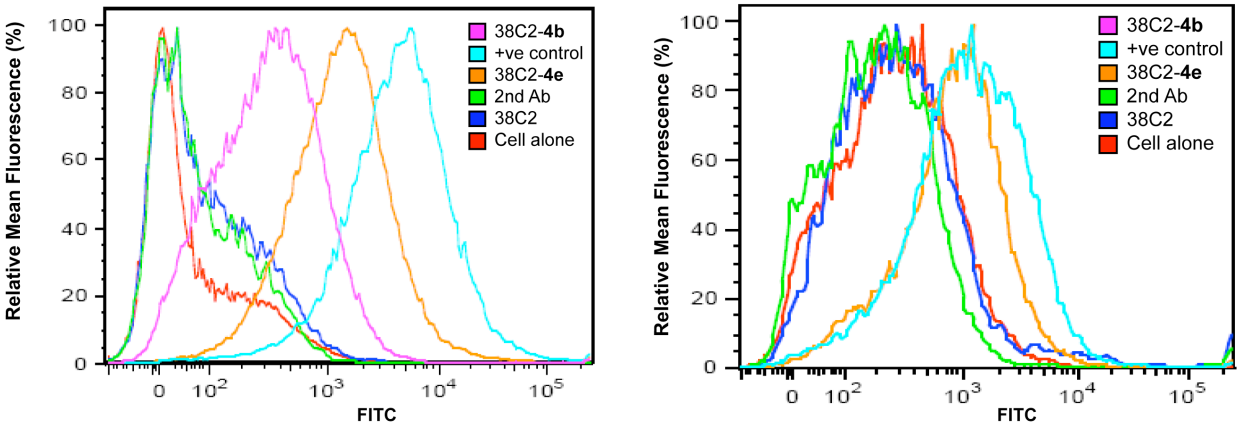


Figure S-2. FACS histograms showing binding of cpAbs 38C2-**4b** and 38C2-**4e** prepared by method 1 to (Left) U87 human glioblastoma, and (Right) 4T1 murine breast carcinoma cells

expressing integrin α5β1. Commercially available anti-α5β1 [MAB 1969, clone JBS5, available from Millipore Biosciences, Temecula, CA] Ab (15 μg/ml) was used as a +ve control, and secondary Ab and Ab 38C2 (15 μg/ml) were used as–ve controls. FITC-labeled anti-mouse goat secondary Abs (1 μg/ml) were used for detection. The y axis gives the number of events in linear scale, the x axis the fluorescence intensity in logarithmic scale.

5.2. Binding of the chem-Ab 38C2-4e to purified integrins. Immulon 2HB well plates (DYNE Technologies) were coated using the purified integrin α5β1 or αvβ3 protein overnight at 4 ˚C. The plates were washed 2 times with PBS and were blocked with the binding buffer (10% BSA in PBS) for 1hr. Ab 38C2 and the cpAb 38C2-**4e** were diluted with buffer, and the plates were incubated for 2 hrs at the room temperature. The plates were washed 3 times with washing buffer (2% BSA in PBS). Biotinylated anti-mouse Ab (Vector Lab, 1:500 dilution in binding buffer, 2 μg/ ml, 100 μl) was added to plate. After 1hr incubation, plates were washed 4 times with the washing buffer (10% BSA in PBS), and developed using avidin-horseradish peroxidase reagent and AEC (3-amino-9-ethylcarbazole). Results were obtained by measuring the relative absorptions at 405 nM using the UV spectrophotometer.

5.3. Cell proliferation assay. Human U87 cells were plated in 24-well plates in 2% serum containing medium and incubated until the cells adhered. Subsequently, the cells were treated with compound **2** (1 μM), cpAb 38C2-**4e** (1 μM), buffer, and Ab 38C2 alone at 37 ˚C. After 0, 48, 96, and 144 hrs incubation periods, cell proliferation was determined using the MTT (3-(4, 5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfo-phenyl)-2H-tetrazolium) assay as described in the manufacturer's instruction (Promega, San Deigo). Image from each well was scanned using a microscope on day 6.

5.4. Inhibition of angiogenesis by programmed Abs *in vitro* using the tube formation Assay. The wells of a 96-well microtiter plate were coated with 60 μl of the ice-cold matrigel (Collaborative Biomedical Products), and incubated overnight at 37°C. The next day, 12,000 single-donor human HUVEC cells were layered on top of the gel in EGM-2 complete growth medium with 2% serum supplements and the test compound **2b** (1 μM) or the cpAb 38C2-**4e** (1 μM). Buffer and Ab 38C2 alone were used as the –ve controls. After the plates were incubated for an additional 72 hrs, the image from each well was scanned using a microscope. Images were further analyzed by counting fragments of tubes. Each experiment was carried out in triplicate.

5.5. In vivo tumor growth and metastasis using mouse tumor models. Tumor induction was performed by s.c. injection of 5×10^5 4T1 cells in the right flank of six-week-old BALB/c mice. Four different groups of mice were treated with PBS (200 μl), Ab 38C2 (150 μg in 200 μl buffer), cpAb 38C2-**4e** (150 μg in 200 μl PBS), and equimolar concentration of compound **2b**, respectively starting on day 4 after the tumor induction. Each mouse was given i.p. injection every fifth day, total 7 injections per mouse. Tumor volumes were measured using micro caliper (Volume = $1/2(\text{Width})^2 \times$ length). When tumor volume reached 1500 mm^3 in the control groups, tumor was removed. All mice were euthanized on day 42, and lungs were removed and fixed into Bouin's solution. Spontaneous lung metastases were counted using the anatomy microscope. Tumor growth and signs of physical discomfort were monitored daily including for any gross evidence of tumor necrosis, local tumor ulceration, as well as evidence of toxicity including the mobility of animals, response to stimulus, piloerection, eating, and weight. These procedures have been reviewed and approved by the Institutional Animal Care and Use Committee at The Scripps Research Institute. The Scripps Research Institute maintains an assurance with the Public Health Service and is registered with the Department of Agriculture and is in compliance with all regulations relating to animal care and welfare.

Statistical analysis. Results are expressed as means. Student paired t-test was used to analyze the difference between two groups. Values were regarded significant at $P < 0.05$.

References.

(1) (a) Gavrilyuk, J. I.; Wuellner, U.; Salahuddin, S.; Goswami, R. K.; Sinha, S. C.; Barbas, C. F. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 3716. (b) Stokmaier, D.; Khorev, O.; Cutting, B.; Born, R.; Ricklin, D.; Ernst, T. O. G.; Boeni, F.; Schwingruber, K.; Gentner, M.; Wittwer, M.; Spreafico, M.; Vedani, A.; Rabbani, S.; Schwardt, O. Ernst, B. *Bioorg. Med. Chem.* **2009**, *17*, 7254.