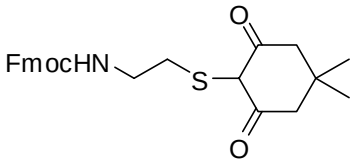
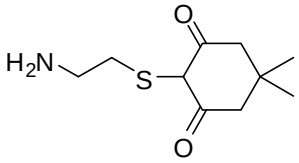
SI Appendix

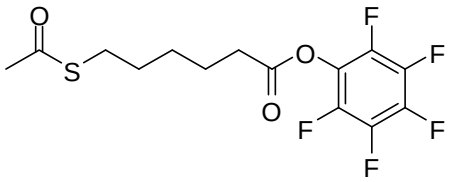
Chemical Synthesis of Hapten. 6-Acetylthiohexanoic acid (6) and pentafluorophenyl trifluoroacetate (7, TFAPfp) were purchased from Aldrich. 2-bromodimedone (2) (2) and *N*-Fmoc-2-aminoethanethiol (3) (3) were synthesized as previously described.



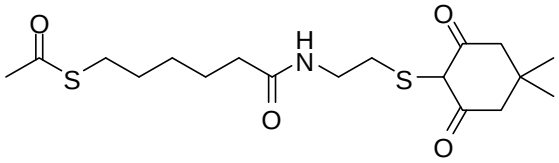
To a solution of 2-bromodimedone (0.146 g, 0.669 mmol) in THF (2 mL) was added *N*-Fmoc-2-aminoethanethiol (0.3 g, 1.0 mmol) followed by $NaHCO_3$ (56 mg, 0.669 mmol) at rt. The reaction was stirred for 2 h at rt. The reaction mixture was diluted with ethyl acetate (40 mL), washed with water (20 mL), dried over $MgSO_4$, concentrated *in vacuo* and purified by silica gel chromatography, eluting with ethyl acetate to 9:1 ethyl acetate: methanol to provide the title compound (0.204 g, 0.467 mmol) in 70% yield. R_f: 0.32 (ethyl acetate). ^1H NMR ($CDCl_3$, 300 MHz): δ 7.79 (d, *J* = 7.2, 2H), 7.66 (d, *J* = 7.2, 2H), 7.43 (t, *J* = 7.0, 2H), 7.34 (t, *J* = 7.0, 2H), 5.92 (s, 1H), 4.43 (d, *J* = 7.5, 2H), 4.27 (t, *J* = 7.2, 1H), 3.32 (q, *J* = 5.4, 2H), 2.69 (t, *J* = 5.4, 2H), 2.48 (s, 4H), 1.12 (s, 6H). ESI-LRMS calcd. For $C_{25}H_{27}NNaO_4S$ ($M+Na^+$) 460.2, found 460.2.



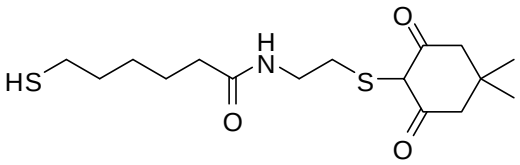
To a solution of compound 4 (15 mg, 0.034 mmol) in DMF (1 mL) was added piperidine (0.2 mL) at rt. The reaction was stirred for 20 min at rt. The reaction mixture was directly loaded to silica gel chromatography and purified, eluting with ethyl acetate to 1:1 ethyl acetate: methanol to provide the title compound (25 mg, 0.012 mmol) in 35% yield. R_f: 0.11 (3:1 ethyl acetate: methanol). ^1H NMR (CD_3OD, 400 MHz): δ 2.93 (t, *J* = 6.0, 2H), 2.45 (t, *J* = 6.0, 2H), 2.46 (s, 4H), 1.06 (s, 6H). ESI-LRMS calcd. For $C_{10}H_{17}NNaO_2S$ ($M+H^+$) 238.1, found 238.1.



To a solution of 6-acetylthiohexanoic acid (0.276 g, 1.45 mmol) in CH_2Cl_2 (10 mL) was added pyridine (0.130 mL, 1.60 mmol) followed by TFAPfp (0.30 mL, 1.74 mmol) at rt. After being stirred for 30 min ar rt, the reaction mixture was diluted with CH_2Cl_2 (50 mL) and washed with sat. $NaHCO_3$. The organic layer was dried over Na_2SO_4, concentrated *in vacuo* at rt and purified by silica gel chromatography, eluting with hexanes to 1:9 ethyl acetate: hexanes to provide the title compound (0.418 g, 1.17 mmol) in 81% yield. R_f: 0.55 (1:9 ethyl acetate: hexanes). ^1H NMR ($CDCl_3$, 300 MHz): δ 2.89 (t, *J* = 7.2, 2H), 2.67 (t, *J* = 7.2, 2H), 2.33 (s, 3H), 1.90-1.74 (m, 2H), 1.70-1.58 (m, 2H), 1.54-1.45 (m, 2H).



To a solution of compound 8 (43 mg, 0.120 mmol) in DMF (2 mL) was added *N*,*N*-diisopropylethylamine (25 μL, 0.144 mmol) followed by amine 5 (31 μL, 0.144 mmol) at rt under argon. The reaction mixture was warmed to 50 °C. After being stirred for 1.5 h at 50 °C, compound 8 (43 mg, 0.120 mmol) in DMF (2 mL) was added. The reaction was stirred for 2.5 more hours at 50 °C under argon. The reaction mixture was concentrated *in vacuo* at 30 °C, diluted with CH_2Cl_2 (80 mL), washed with H_2O (80 mL), dried over Na_2SO_4, concentrated *in vacuo* at rt and purified by silica gel chromatography, eluting with ethyl acetate to 5:1 ethyl acetate: methanol to provide the title compound (41 mg, 0.106 mmol) in 88% yield. R_f: 0.29 (3:1 ethyl acetate: methanol). ^{1}H NMR (acetone-d_6, 400 MHz): δ 7.12 (s, 1H), 3.30 (q, J = 4.5, 2H), 2.84 (t, J = 6.9, 2H), 2.60 (t, J = 4.5, 2H), 2.43 (s, 4H), 2.30 (s, 3H), 2.25 (t, J = 6.9, 2H), 2.30 (s, 3H), 1.69-1.52 (m, 5H), 1.48-1.35 (m, 2H) , 1.07 (s, 6H). ESI-LRMS calcd. For $C_{18}H_{29}NNaO_4S_2$ ($M+Na^+$) 410.1, found 410.1.



To a solution of compound 9 (64 mg, 0.165 mmol) in THF (2 mL) was added 1 N NaOH (2 mL) at rt. After being stirred for 2 h at rt, the reaction was neutralized with 1 N HCl, then diluted with CH_2Cl_2 (100 mL), washed with brine (50 mL), dried over Na_2SO_4 and concentrated *in vacuo*. The crude mixture was incubated with DTT (40 mg) in 1 mL of PBS (pH 8) and 1 mL of MeCN for 1 h at rt and then purified by C18 reverse-phase HPLC (10 μm, 21.2×150 mm, Beckman Coulter) with a gradient of 0% B to 50 % B in 50 min (buffer A: 0.1% TFA in H_2O; buffer B: 0.1% TFA in CH_3CN) at a flow rate of 10 mL/min to provide the title compound (39 mg, 0.113 mmol) in 68% yield. ^{1}H NMR (acetone-d_6, 400 MHz): δ 7.61 (s, 1H), 3.28 (q, J = 6.0, 2H), 2.57 (t, J = 6.0, 2H), 2.51 (q, J = 7.2, 2H), 2.42 (s, 4H), 2.24 (t, J = 7.2, 2H), 1.69-1.58 (m, 5H), 1.48-1.40 (m, 2H) , 1.07 (s, 6H). ^{13}C NMR (acetone-d_6, 100 MHz): δ174.62, 106.21, 39.97, 36.91, 36.78, 34.60, 31.98, 30.50, 28.58, 28.32, 25.91, 24.77. ESI-LRMS calcd. For $C_{16}H_{28}NO_3S_2$ ($M+H^+$) 346.2, found 346.2.