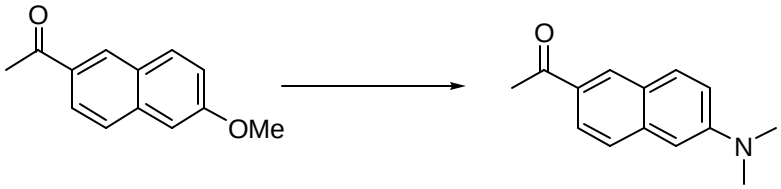
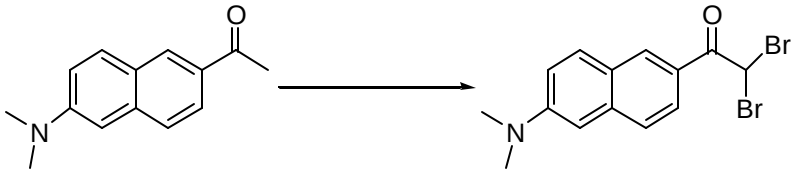
Supplementary material

Enantioselective synthesis and application of the highly fluorescent and environment-sensitive amino acid 6-(2-dimethylaminonaphthoyl) alanine (DANA)

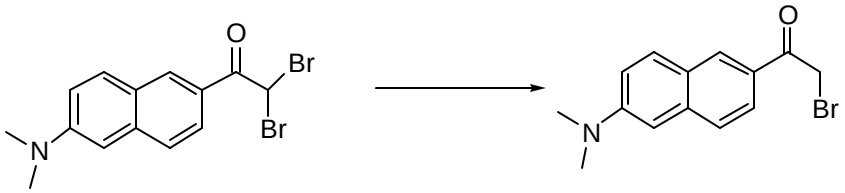
Mark Nitz, Adam R. Mezo, Mayssam H. Ali and Barbara Imperiali*
Department of Chemistry, Massachusetts Institute of Technology, 77 Massachusetts Avenue, Cambridge MA. 02139 E-mail: imper@mit.edu



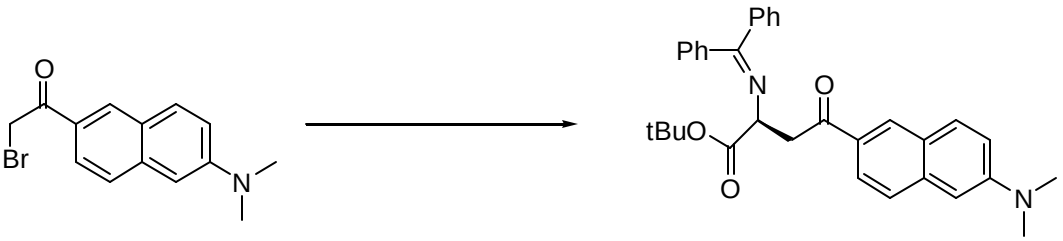
6-acetyl-2-(dimethylamino)naphthalene: To a solution of 6-acetyl-2-methoxy-1-naphthalene (Avacado) (5 g, 25.0 mmol) in 1,3-dimethyl-3,4,5,6-tetrahydro-2(1H)-pyrimidinone (DMPU) (100 mL) was added a 5% suspension of $LiN(Me)_2$ in hexane (200 mL) at ambient temperature. The mixture is allowed to stir under an inert atmosphere for 16 hours. The reaction was then quenched by slow addition of solid sodium sulfate decahydrate (5 g). The mixture was diluted with ethyl acetate (400 mL) and washed thrice with water (300 mL). The organics were dried over $MgSO_4$ and concentrated to a yellow solid that was recrystallized from ethanol to give pale yellow needles, with an overall yield of 86% (4.62 g). mp 152.5 °C; ^{1}H NMR (300 MHz, $CDCl_3$): 8.33 (d, 1H, J=1.6 Hz), 7.93 (dd, 1H, J =1.9 Hz, J=8.8 Hz), 7.81 (d, 1H, J=9.1 Hz), 7.64 (d, 1H, J=8.8 Hz), 7.18 (dd, 1H, J=2.5 Hz, J=9.1 Hz), 6.89 (d, 1H, J=2.5 Hz), 3.13 (s 6H) 2.69 (s, 3H) ppm; ESMS 214.4 (m+1)



6,6'-dibromoacetyl-2-(dimethylamino)naphthalene: To 6-acetyl-2-(dimethylamino)naphthalene (1.00 g, 4.69 mmol) in a 50 mL round bottom flask, was added concentrated sulfuric acid (15 mL) at room temperature. The mixture was stirred until complete dissolution had occurred resulting in a dark red solution. The solution was then cooled in an ice bath and an ice cold solution of bromine (312 μL in 2 mL THF) was added dropwise. The ice bath was removed and the flask sealed. After stirring for 4 hours at room temperature the solution was added drop-wise to a rapidly stirring solution of ice water (150 mL). The solid was collected by filtration and resulting red-orange powder was recrystallized from a ethyl acetate/hexane mixture to give orange needles (1.2 g, 68%) containing minor amounts of the tribrominated and monobrominated products; HRMS 369.9434, calc. 369.9450; ^{1}H NMR data are consistent with reported data.[15]

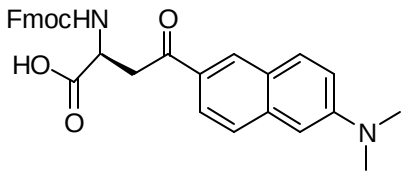


6-bromoacetyl-2-(dimethylamino)naphthalene 2. To a solution of 6,6'-dibromoacetyl-2-(dimethylamino)naphthalene (150 mg, 0.4 mmol) dissolved in THF (10 mL) was added diethyl phosphite (60 μL, 1.2 eq) and DIPEA (70 μL, 1.2 eq). The solution was stirred overnight at room temperature. When the reaction was judged complete by TLC (9:1 toluene: ethyl acetate) approximately 500 mg of silica gel was added to the solution and it was concentrated under vacuum to a free flowing solid which was applicated onto a silica column. After elution (9:1 tol: ethylacetate) the product was concentrated and crystallized from ethanol to give fine yellow needles (110 mg 86%). HRMS 292.0335, calc. 292.0332; mp 126-127 °C; ^{1}H NMR are consistent with reported data.[15]



PRODAN Schiff Base 3. *N*-(diphenylmethylene)glycine *tert*-butyl ester (200 mg 0.67 mmol) (Aldrich) and the phase transfer catalyst, *O*-(9)allyl-*N*-9-anthracenylmethylcinchonidinium bromide (86 mg, 25 mol%),[13] were dissolved in dichloromethane (2 mL) and cooled to –25 °C. 40% KOH (10 mL) was then added and the reaction was allowed to stir for 5 minutes. Compound **2** (95 mg, 3.25 mmol) as a solution in dichloromethane (1 mL) was slowly added to the biphasic mixture with a syringe pump over 3 hours. The reaction was stirred for 2 hours and then diluted with ethyl acetate (50 mL) and washed with water (50 mL). The crude reaction mixture was flashed through a 4 cm plug of silica gel, and the silica was washed with ethyl acetate (100 mL). Concentration yielded a pale yellow oil that was purified by chromatography on silica gel (7:2 hexanes:ethyl acetate) to yield the highly fluorescent compound **3** as a yellow oil (83%). $[\alpha]_D$ 55.0°; ^{1}H NMR (500 MHz, $CDCl_3$): 8.37 (d, 1H, J=1.4 Hz), 7.90 (dd, 1H, J=1.7 Hz, J=8.5 Hz), 7.78 (d, 1H, J=8.8 Hz), 7.60 (m, 3H), 7.44 (m 3H), 7.28-7.38 (m, 5H), 7.15 (dd, 1H, J=2.6 Hz, J=9.3 Hz), 6.85 (d, 1H, J=2.5 Hz), 4.72 (t, 1H, J=6.5 Hz), 3.78 (dd, 1H, J=6.0 Hz, J=16.8 Hz), 3.58-3.70 (bm, 1H), 3.10 (s, 6H), 1.46 (s, 9H) ppm; ^{13}C NMR (125 MHz) 197.3,

171.6, 171.2, 150.6, 140.2, 138.0, 136.9, 132.9, 131.2, 131.0, 130.7, 130.6, 130.5, 129.3, 129.2, 129.0, 128.7, 128.7, 128.4, 128.4, 126.5, 125.4, 125.0, 116.6, 105.7, 81.8, 63.4, 42.3, 40.9, 28.1; HRMS 507.2621, calc 507.2642.



Fmoc-DANA 4. (2-(*N*-(9-Fluorenylmethoxycarbonyl)amino-4-[6-(dimethylamino)-2-naphthyl]-4-oxo-butananoic acid**).** Schiff base **3** (225 mg, 0.44 mmol) was refluxed in 6 M HCl for 4 hours. The solution was concentrated under vacuum and taken up in a solution of DMF/water (1:1, 10 mL) and brough to pH 8 with the addition of sodium bicarbonate (~200 mg). 200 µl of this solution was set aside for determination of the enantiomeric excess of the amino acid (below). Fmoc-*O*Su (225 mg, 0.74 mmol) was added to the solution and the reaction was stirred at room temperature for 3 hours. The solution was diluted with ethyl acetate (100 mL) and acidified with 1M HCl (15 mL). The organic layer was extracted with water (3 x 50 mL), dried over $MgSO_4$ and concentrated to a yellow oil. Silica gel chromatography gave the desired protected amino acid as yellow solid (92%, 215 mg) that could be recrystallized from ethyl acetate/hexane. $[\alpha]_D$ = 59.5 °; mp 122-123 °C; ^{1}H NMR (500 MHz, $CDCl_3$): 8.37 (s, 1H), 7.89 (d, 1H, J=8.5 Hz), 7.78 (d, 1H, J=9 Hz), 7.74-7.71 (m, 2H), 7.63 (d, 1H, J=8.5 Hz), 7.58 (d, 2H, J=7.5 Hz), 7.4-7.1 (m, 5H), 7.16 (d, 1H, J=8.5 Hz), 6.87 (1H, s), 6.05 (d, 1H, J=8.0 Hz), 4.84 (p, 1H, J=4.0 Hz), 4.44-4.32 (m, 2H), 4.20 (t, 1H, J=7.5 Hz), 3.94 (dd, 1H, J=4 Hz, J=18.5 Hz), 3.62 (dd, 1H, J=4 Hz, J=18.5 Hz), 3.1 (6H, s); ^{13}C (125 MHz, DMSO): 195.50, 173.4, 155.9, 150.2, 143.8, 140.7, 137.3, 130.7, 130.3, 129.4, 127.6, 127.1, 125.9, 125.3, 124.5, 123.9, 120.1, 116.5, 104.7, 65.7, 54.9, 49.9, 46.6; HRMS 509.2071, calc 509.2063.

Determination of enantiomeric excess of DANA.

To 200 µl of the DMF/water solution (above) of the hydrolyzed salt was added Nα-(2,4-Dinitro-5-fluorophenyl)-L-alaninamide (Marfey's Reagent) (1.5mg) and the resulting solution was incubated at 40 °C for 1 hour. The solution was then acidified by addition of 2M HCl (20 µL). Analysis of 40 µL of this solution by reverse phase HPLC on a Beckman C18 column (5µM, 4.6mm x 15cm, ODS) using a 0 to 90% acetonitrile gradient over 25 minutes at 1 mL/min eluted the major diastereomer at 31 minutes (L) and the minor diasteromer at 33 minutes (D). The

identities of the peaks were confirmed by ESMS and integration of the absorbance peaks of these compounds at 280 nm was used to calculate the enantiomeric excess of the DANA free base.

Solvent dependence of DANA.

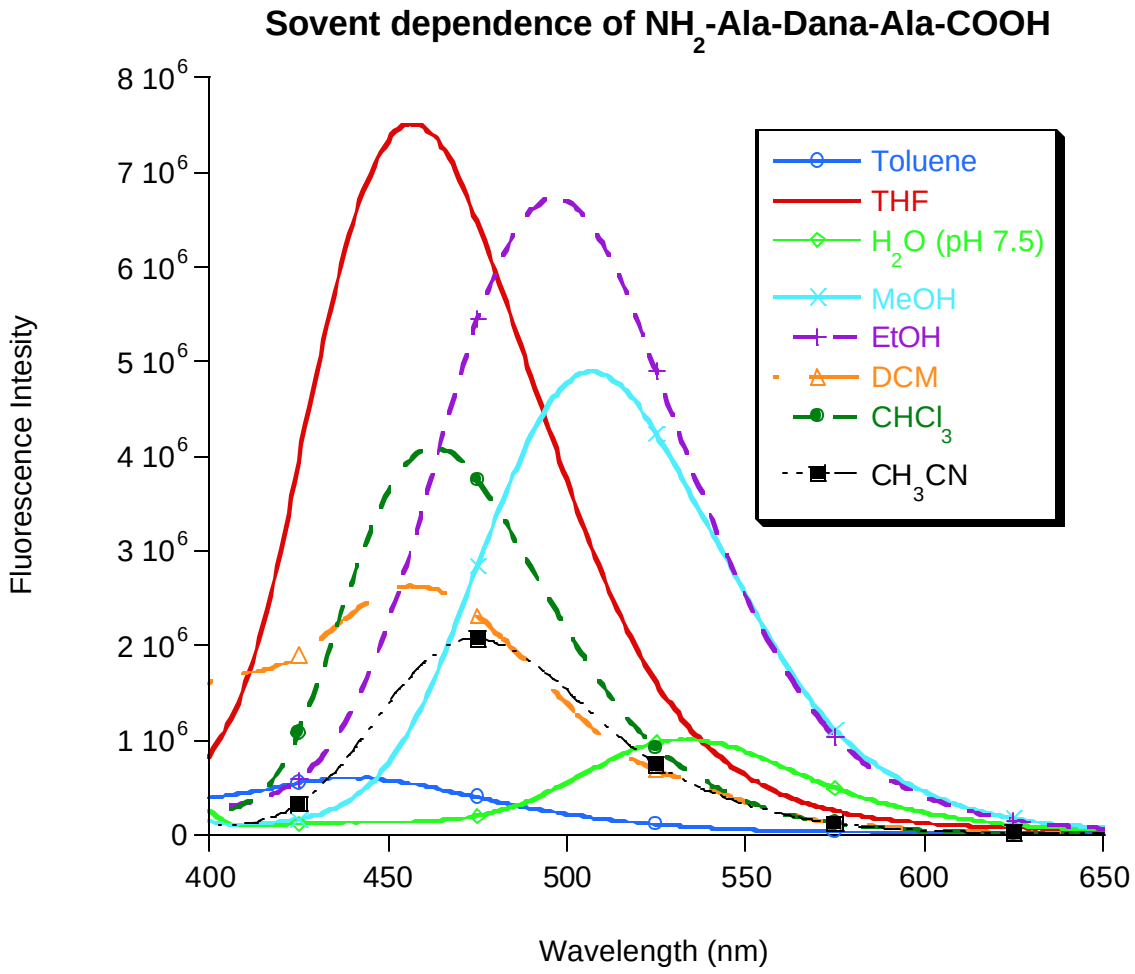
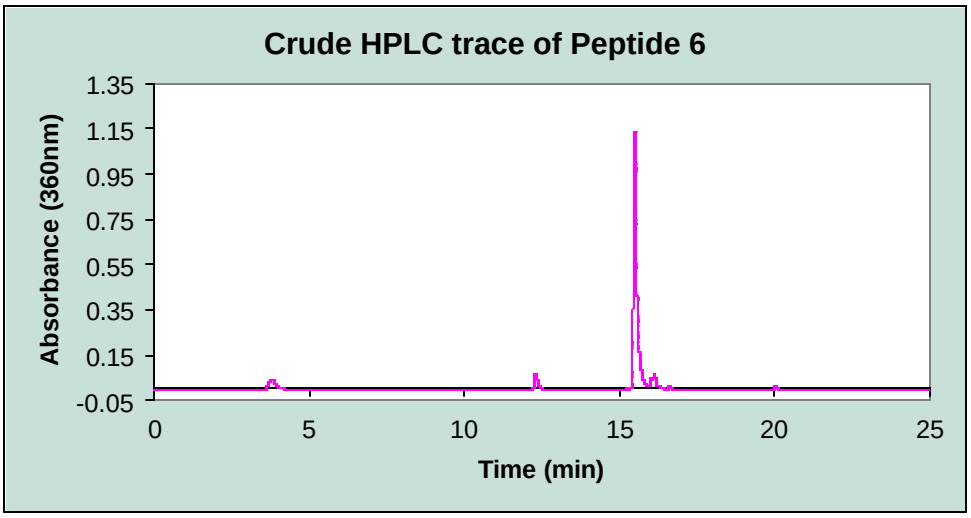


Figure 3. Solvent dependence of DANA

All samples at the same peptide concentration, diluted 1:100 from an acetonitrile stock solution and excited at 350nm.

General Peptide Synthesis: All peptides were synthesized using a Milligen 9050 peptide synthesizer on PAL-PEG-PS (Perseptive Biosystems, 0.2 mmol/g) resin using standard Fmoc-protected amino acids. Coupling cycles were performed with 2-(1H-Benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU) (1 eq) and *N*-Hydroxybenzotriazole (HOBt) (1 eq) with DIPEA (5 eq) as the base. Fmoc-DANA was coupled manually using 2.5 fold excess amino acid to resin loading using benzotriazole-1-yl-oxy-tris-pyrrolidino-phosphonium

hexafluorophosphate (PyBOP) (1 eq/eq amino acid) and DIPEA (5 eq/eq amino acid) for 1 hour. Peptides were cleaved with a solution of trifluoroacetic acid/triisopropylsilane/water/phenol/thioanisole (84:1:2.5:2.5:5:5) for 2 hours at room temperature. Precipitation from this solution with diethyl ether gave the desired peptide. Further purification was achieved by reverse phase HPLC on a C18 column. Purity was confirmed by analytical HPLC and electrospray mass spectrometry. Stock solutions were made and their concentrations determined by quantitative amino acid analysis. The HPLC trace of the crude peptide **6** is shown below.



Fluorescence Spectra: All fluorescence spectra were recorded on a Fluoromax 2 Fluorometer. Excitation was set at 367 nm and emission spectra were scanned from 400 to 600 nm with a 2 nm slit width, 0.1 s integration time over 1 nm increments. In cases where spectra were compared to another spectrum both were recorded within 12 hours to minimize fluctuations due to lamp intensity. All spectra were recorded in 10 mM Hepes pH 7.0 with 150 mM NaCl, at 20 °C. Titrations of peptides were performed with 2.8×10^{-6} M Peptide **5** and 2.5×10^{-6} M, Peptide **6**. Pro S protein was obtained by literature methods.[18] Data were analyzed using the global fitting program Spec Fit for a 1:1 complexation. Data points were extracted at 520 for Peptide 5 and at 520 and 450 nm for peptide 6 as an indication of the quality of the fit to the experimental data.

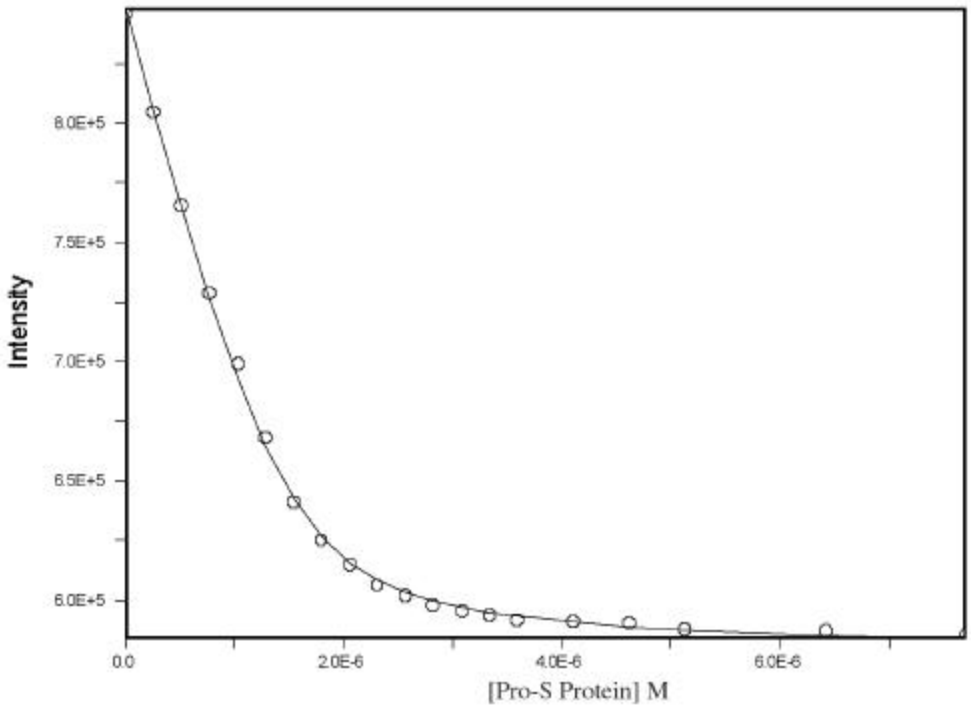
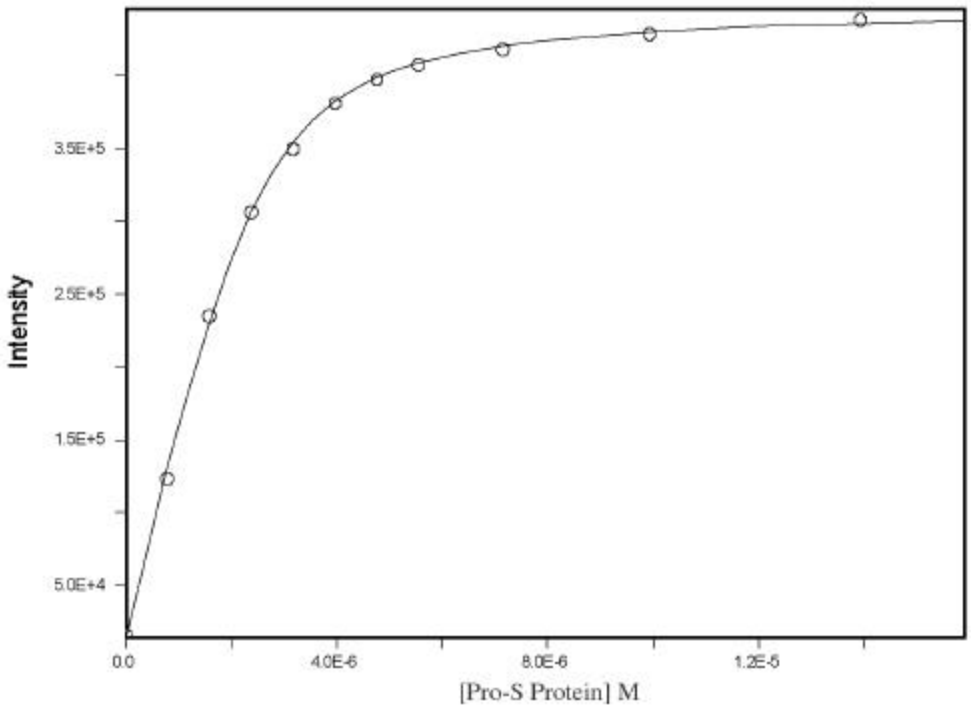


Figure 4. Curve fit extracted from global fit of fluorescence of Peptide 5 upon binding to S-Peptide at 520 nm. Fit gives K_a 1.6 x 10^7 M^{-1}.



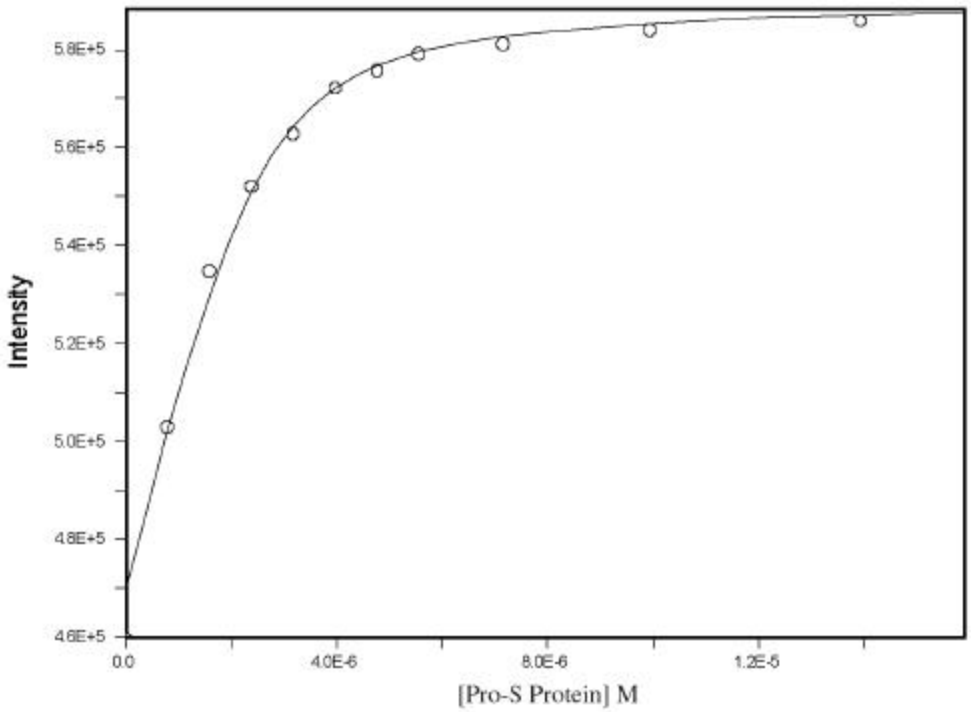


Figure 5. Fit extracted from global fit of fluorescence of Peptide 6 upon binding to S-Peptide at 450 nm(top) and 520 nm (bottom). Fit gives K_a 3.0 x 10^6 M^{-1}.