Experimental

General: P450cam was expressed and purified as previously described.[1] Steady state UV-visible absorption spectra were measured on a Hewlett Packard 8452A diode array spectrophotometer. Steady state fluorescence spectra were measured using an ISS K2 fluorometer (λ_{ex}=340 nm). Absorption and emission spectra were collected in quartz cuvettes using 50 mM potassium phosphate buffer (pH 7.4) containing 100 mM KCl. NMR spectra were collected on an Oxford Instruments 300 MHz NMR and analyzed with Varian VNMR 6.1B software. Electrospray mass spectra were collected on a Finnigan LCQ ion trap mass spectrometer. Buried solvent accessible surface area was calculated using the solvation module of InsightII (1.4 Å radius probe). All reagents were purchased from the Aldrich chemical company and used as received. DMF and N,N-diisopropylethylamine were anhydrous, and used as received.

Syntheses:

Adamantane-1-carboxylic acid [4-(5-dimethylamino-naphthalene-1-sulfonylamino)-butyl]-amide (**1**): (D-4-Ad) 0.100 g (0.312 mmole) **3**, 74.5 mg (0.37 mmole) 1-adamantyl carbonyl chloride, and 0.11 mL (0.62 mmole) N,N-diisopropylethylamine were dissolved in 5 mL dry DMF under Ar and stirred overnight at ambient temperature. The reaction mixture was diluted with 25 mL CH_2Cl_2, washed twice with water, and the organic phase concentrated under reduced pressure. The crude product was purified via flash chromatography using 9:1 MeOH:CH_2Cl_2 as eluent to give the product as a pale yellow-green solid. Yield 35.6 mg (24 %) ^{1}H NMR ($CDCl_3$) 8.53 (1H, d, J=8.4 Hz) 8.31 (1H, d, J=8.4 Hz) 8.22 (1H, dd, J=0.9, 7.2 Hz) 7.55 (1H, dd, J= 7.5, 8.4 Hz) 7.51 (1H, dd, J= 7.2, 8.4 Hz) 7.18 (1H, d, J=7.5 Hz) 5.63 (1H, m) 5.30 (1H, t, J=6.0 Hz) 3.11 (2H, m) 2.89 (2H, m) 2.88 (6H, s) 2.00 (3H, m) 1.77 (6H, m) 1.68 (6H, m) 1.42 (4H, m) ^{13}C NMR ($CDCl_3$) 178.43, 152.16, 134.95, 130.58, 130.06, 129.81, 128.57, 123.44, 119.07, 115.41, 45.68, 43.10, 40.77, 39.45, 38.73, 36.72, 28.33, 26.99, 26.90. ESI-MS (m/z) 484.3 ($M+H^+$).

Adamantane-1-carboxylic acid [4-(5-dimethylamino-naphthalene-1-sulfonylamino)-octyl]-amide (**2**): Was prepared from **4** and 1-adamantyl carbonyl chloride in a manner identical to **1**. Yield 45%. ^{1}H NMR ($CDCl_3$) 8.53 (1H, d, J=8.4) 8.29 (1H, d, J=8.7) 8.24 (1H, dd, J=7.5, 1.2 Hz) 7.56 (1H, dd, J=7.5, 8.7 Hz) 7.52 (1H, dd, J=7.2, 8.4 Hz) 7.18 (1H, d, J=7.2 Hz) 5.58 (1H, m) 4.77 (1H, t, J=5.7 Hz) 3.17 (2H, m) 2.88 (6H, s) 2.87 (2H, m) 2.02 (3H, m) 1.90 (3H, m) 1.82 (3H, m) 1.70 (6H, m) 1.38 (4H, m) 1.14 (8H, m) ^{13}C NMR ($CDCl_3$) 178.17, 152.20, 134.98, 130.57, 130.07, 129.86, 123.45, 118.98, 115.40, 45.67, 43.48, 40.77, 39.50, 38.83, 36.75, 36.65, 29.73, 29.17, 28.99, 28.36, 28.06, 26.86, 26.50. ESI-MS (m/z) 540.3 ($M+H^+$). 5-Dimethylamino-naphthalene-1-sulfonic acid (4-amino-butyl)-amide (**3**): Following the preparation by Ikunaga *et al*.,[2] 200 mg (0.75 mmole) dansyl chloride and 1.49 mL 1,4-diaminobutane (14.8 mmole) were dissolved in 5 mL CH_2Cl_2 and stirred for 2 hours under argon. The reaction mixture was loaded directly onto a flash silica column, and eluted using 4:1:1 CH_2Cl_2:MeOH:Et_3N to give the product as a pale yellow-green oil. Yield 0.104 g (44 %) ^{1}H NMR ($CDCl_3$) 8.49 (1H, d, J=8.4 Hz) 8.36 (1 H, d, J=8.7 Hz) 8.20 (1H, d, J=7.5 Hz) 7.49 (1H, dd, J= 7.5, 8.7 Hz) 7.48 (1H, dd, J = 7.2, 8.4 Hz) 7.13 (1H, d, J=7.2 Hz) 5.3 (3H, overlapping m) 2.85 (6H, s) 2.84 (2H, m) 2.73 (2H, t, J=6.3 Hz) 1.52 (4H, m) ^{13}C NMR ($CDCl_3$) 152.00, 135.28, 130.25, 130.02, 129.81, 129.49, 128.32, 123.39, 119.28, 115.31, 45.61, 43.01, 40.61, 28.36, 27.22. ESI-MS (m/z) 322.2 ($M+H^+$).

5-Dimethylamino-naphthalene-1-sulfonic acid (4-amino-octyl)-amide (**4**):[3] Was prepared from 1,8-diaminooctane and dansyl chloride in an identical fashion to **3**. Yield 66%. ^{1}H NMR ($CDCl_3$) 8.49 (2H, d, J=8.4 Hz) 8.32 (2H, d, J=8.4 Hz) 8.20 (2H, dd, J=0.9, 7.2) 7.52 (2H, dd, J=8.4, 7.5 Hz) 7.48 (2H, dd, J=7.2, 8.4 Hz) 7.14 (2H, d, J=7.5 Hz) 5.5 (3H, overlapping m) 2.85 (6H, s) 2.82 (2H, m) 2.75 (2H, t, J=7.2 Hz) 1.49 (2H, m) 1.33 (2H, m) 1.11 (8H, m) ^{13}C NMR ($CDCl_3$) 152.09, 135.25, 130.42, 130.05, 129.87, 129.60, 128.51, 123.43, 119.18, 115.36, 45.65, 43.39, 40.99, 30.44, 29.64, 28.95, 28.82, 26.51, 26.35. ESI-MS (m/z) 378.3 ($M+H^+$).

Crystallization and Data Collection. The C334A P450cam:D-8-Ad complex was formed at a molar ratio of 1:1 (400 μM) at room temperature and crystallized by hanging drop vapor diffusion at 4° C. Crystals were obtained under 0.1 M citrate (pH 5.5), 200 mM KCl, 13% (wt/vol) polyethylene glycol (PEG; molecular weight = 8,000). For diffraction experiments, crystals were soaked in a solution containing 0.75 M citrate (pH 5.5), 150 mM KCl, 10% (wt/vol) PEG 8000, and 25% (wt/vol) PEG 400 for 1 minute and flash frozen in liquid nitrogen. Data were collected on an Raxis IV detector equipped with Osmic confocal mirrors and Xstream cryo-device (100K) using CuK_{α} radiation (λ = 1.5418 Å) from a Ru200 X-ray generator operated at 50 kV, 100 mA. Data were processed using DENZO and SCALEPACK.[4] The space group was $P2_12_12_1$ with cell dimensions: a = 64.95, b = 75.31, c = 93.17 $Å^3$ (Matthews coefficient (V_M) = 2.50; solvent content = 49.9%).

Structure Determination: The structure was solved by molecular replacement using the program AMoRE[5] with camphor-bound P450cam (PDB code 2cpp) as the initial model. After initial rigid body refinement in CNS,[6] further refinement was carried out by iterative cycles of simulated annealing and B factor refinement using CNS and manual fitting using XFIT.[7] The heme and D-8-Ad were located in $|F_o|-|F_c|$ electron density omit maps and further refined by simulated annealing and manual fitting. The difference electron density map ($|F_{obs}|-|F_{calc}|$) of the D-8-Ad is well defined and continuous, and the average B-factor for D-8-Ad is moderately low (38 $Å^2$) confirming the high occupancy of the ligand. The final model, which includes residues (11 – 414) of P450cam, D-8-Ad, heme, and 301 waters, gave R_{factor}/R_{free} values of 20.2 and 24.7.

Table 1: Diffraction and Refinement Statistics for P450cam complexed with D-8-Ad

Diffraction Data:	
PDB code	
Resolution (Å)	20 - 2.2
Unit Cell (Å)	a=64.95, b=75.31, c=93.17
Space Group	$P2_12_12_1$
Reflections (Total/Unique)	115720 / 21045
Multiplicity	5.2
Completeness (%)	93.3 (63.8)*
R_{sym}	0.102 (0.266)*
I/σ(I)	13.9 (2.5)*
Refinement Statistics:	
R_{factor} [§]	20.2 (28.5)*
R_{free} [¶]	24.7 (33.0)*
Average B (from Wilson plot, $Å^2$)	26.2
No. of protein atoms and Ave B, ($Å^2$)	3200, 25.4
No. of waters and Ave B, ($Å^2$)	301, 34.0
No. of heme atoms and Ave B, ($Å^2$)	43, 16.5
No. of D-8-Ad atoms and Ave B, ($Å^2$)	38, 38.9
Rms bonds, angles[†]	0.006 Å , 1.3°

* Outer shell statistics (2.30 – 2.20 Å)
§ $R = \sum||F_{obs}| - |F_{calc}||/\sum|F_{obs}|$ for all reflections (no σ cutoff).
¶ Free R calculated using 4.8% as test set.
† rms deviations from ideal bond and angle restraints.

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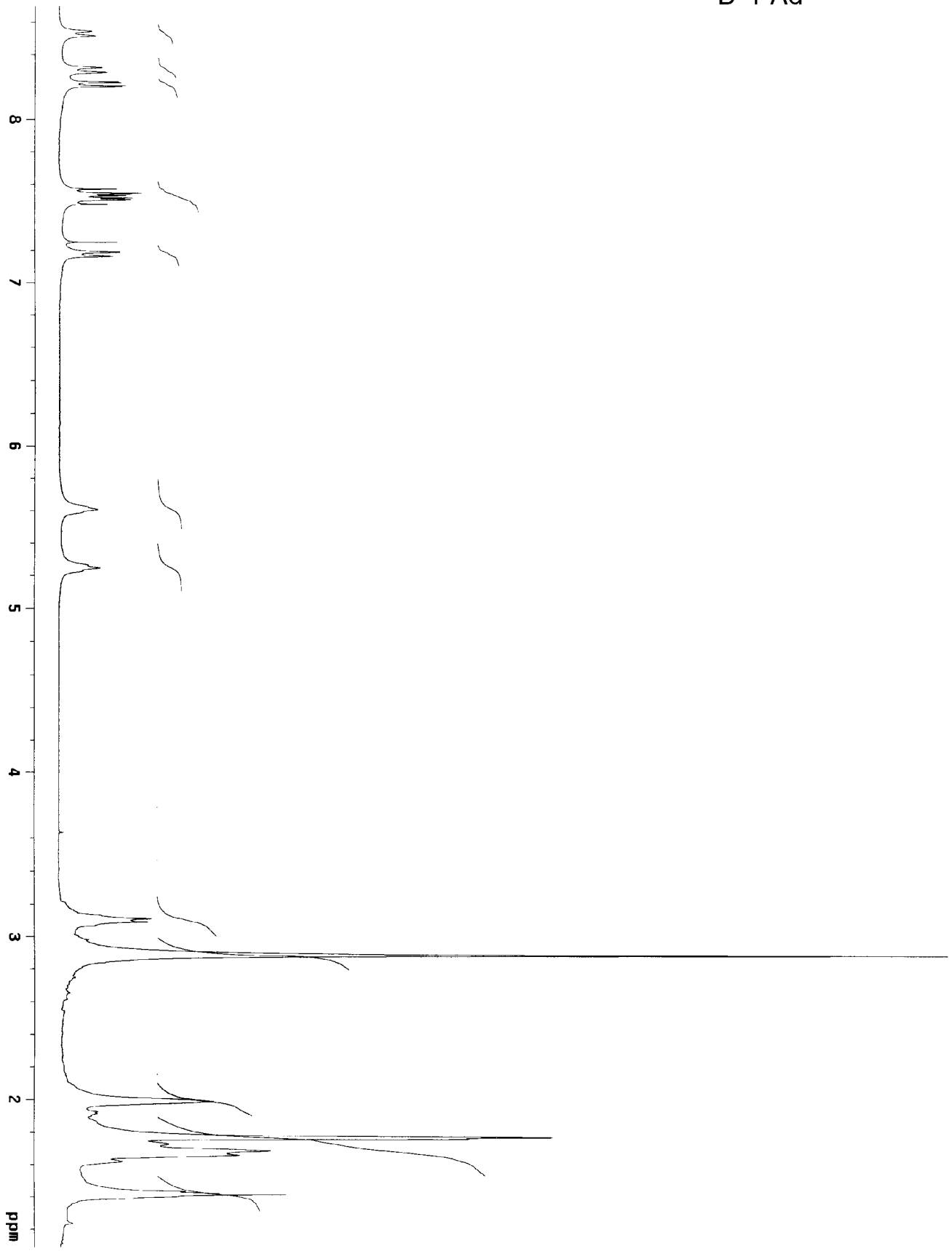
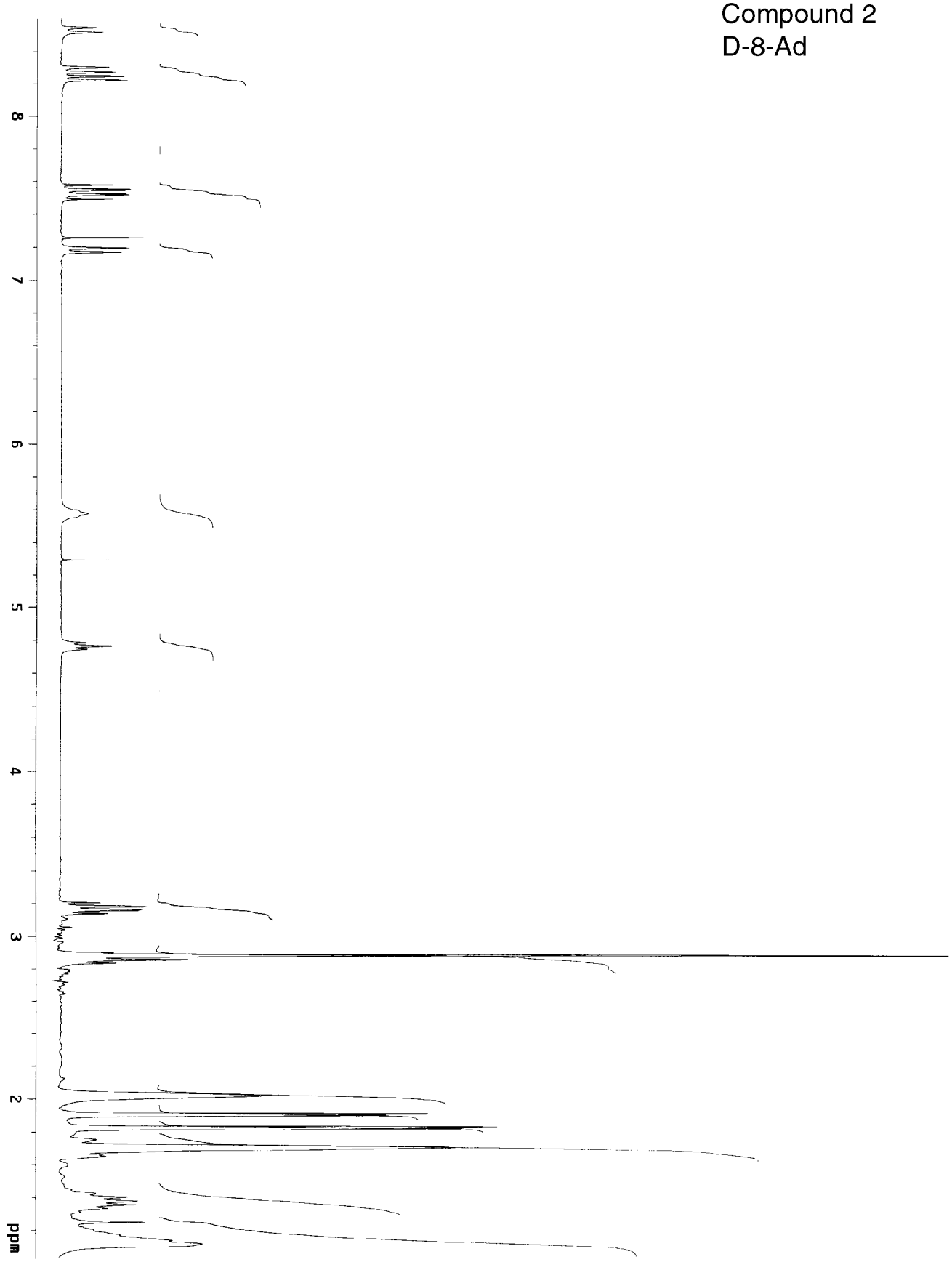
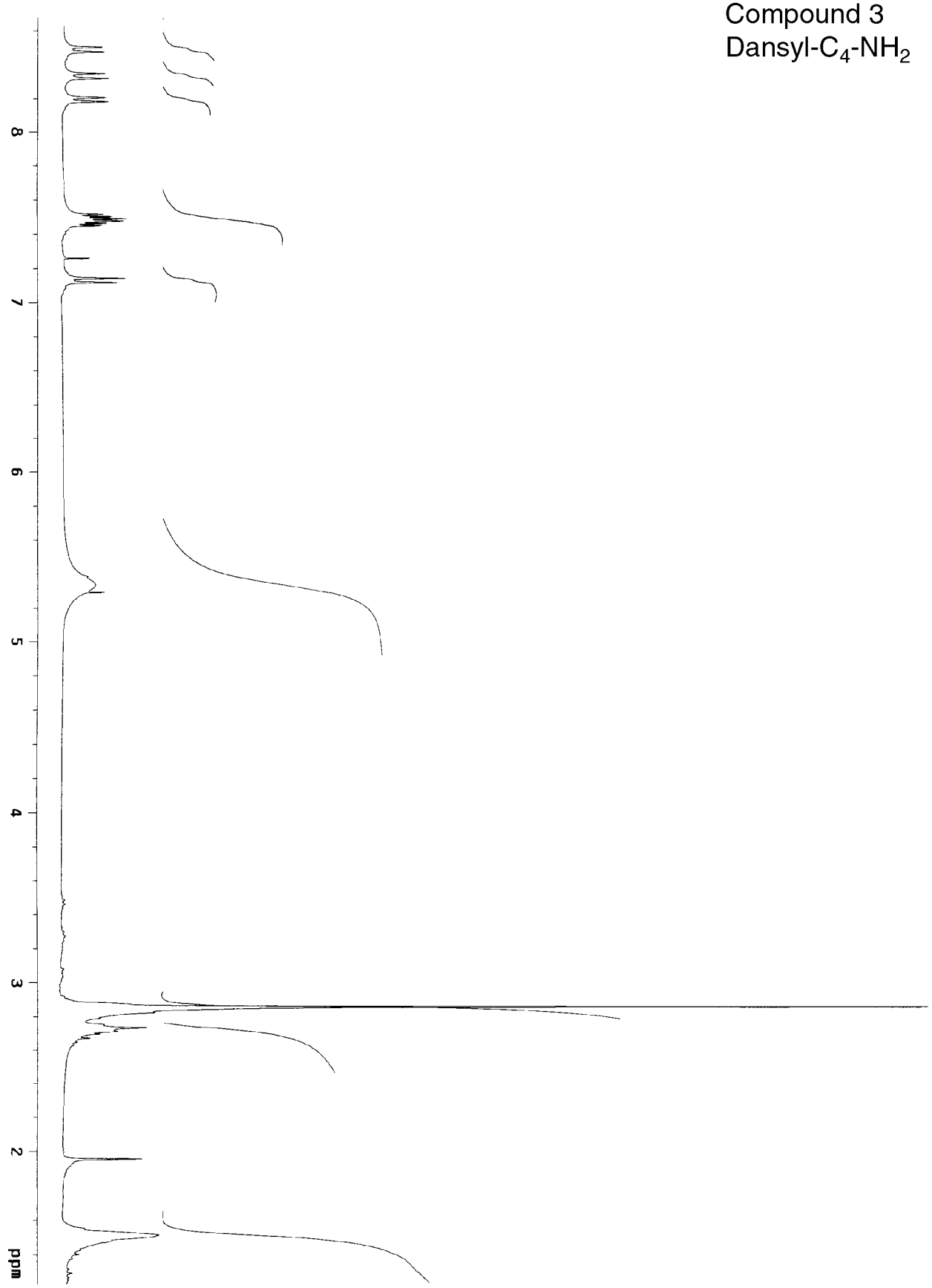
Compound 1
D-4-Ad



Compound 2
D-8-Ad



Compound 3
Dansyl-C_4-NH_2



Comound 4
Dansyl-C_8-NH_2