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Chemically Modified Dansyl Probes: A Fluorescent Diagnostic for Ion and Proton Detection in Solution and in Polymers

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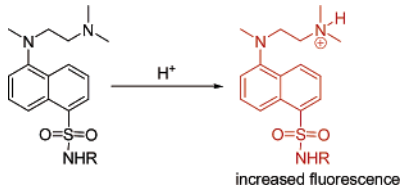
ABSTRACT

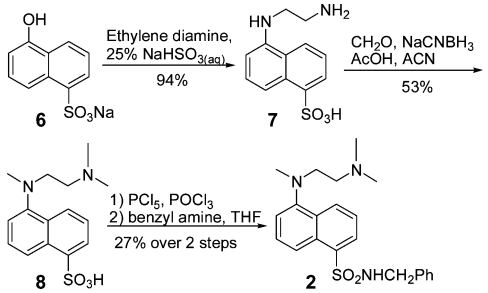


The fluorescence emission intensity of the dansyl group is significantly diminished upon appending an ethyldimethylamino group to the N1 nitrogen substituent. Addition of acids and metal ions (i.e., Zn^{2+}) to solutions of trimethylethylenediamine naphthalene sulfonamide (trinsyl) 2 produces a >25-fold increase in fluorescence intensity. Trinsyl probe 2 has been used as a diagnostic for the diffusion of protons and metal ions in a network polymer as well as an optical reporter for the glass transition temperature.

The fluorescence emission of 1,5-dimethylaminonaphthalene sulfonamide (dansyl) derivatives (i.e., **1**) serves as an important probe in both biological[1] and synthetic polymers.[2] Dansyl chloride is a widely used derivatization reagent for end-group analysis of proteins and amino acid detection. Dansyl derivatives are environmentally sensitive and are known to exhibit large Stokes shifts along with varying fluorescence quantum yields due to changes in the local environment.

We report that the fluorescence characteristics of this probe can be profoundly modified by appending an ethyldimethylamino group to the N1 nitrogen substituent. The modified probe **2** (trinsyl) exhibits significantly reduced fluorescence emission intensity when compared with the parent dansyl (**1**).[1a] However, upon addition of 1 equiv of acid or 4 equiv of Zn^{2+} ion to **2**, a >25-fold *enhancement* of fluorescence emission intensity is exhibited. The location and substitution pattern of the aminoethyl group is crucial in producing this effect. The synthesis of trinsyl **2** is shown in Scheme 1.

Scheme 1. Synthesis of Trinsyl **2**



Plots of the fluorescence emission intensity of a 10^{-5} M solution (CH_3CN) of **2** in the presence and absence of CF_3-

(1) (a) Haugland, R. P. *Molecular Probes*, 6th ed.; Molecular Probes: Eugene, OR, 1996. (b) Pfleiderer, W.; Matysiak, S.; Bergmann, F.; Schnell, R. *Acta Biochim. Pol.* **1996**, *43*, 37−44. (c) Walker, J. M. *Methods Mol. Biol.* **1997**, *64*, 189−195. (d) Tolosa, L.; Nowaczyk, K.; Lakowicz, J. In *Introduction to Laser Spectroscopy*, 2nd ed.; Andrews, D. L., Demidov, A. A., Eds.; Kluwer Academic/Plenum: New York, 2002; pp 139−170.

(2) (a) Chu, D. Y.; Thomas, J. K. In *Photochemistry and Photophysics*; Rabek, J. F., Ed.; CRC: Boca Raton, FL, 1991; Vol. 3. (b) Guillet, J. *Photophysics and Photochemistry*; Cambridge University Press: Cambridge, UK, 1985. (c) Hollaender, A. *Surf. Interface Anal.* **2004**, *36*, 1023−1026. (d) Shea, K. J.; Sasaki, D. Y.; Stoddard, G. J. *Macromolecules* **1989**, *22*, 1722−1730.

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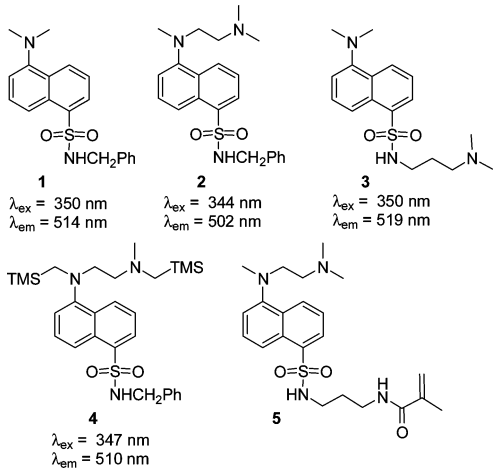


Figure 1. Dansyl derivatives and their fluorescence excitation and emission maxima in acetonitrile (10^{-5} M).

CO_2H are shown in Figure 2a. An analogous plot of fluorescence intensity as a function of added Zn^{2+} (CH_3CN) is shown in Figure 2b. In both cases, added electrophile

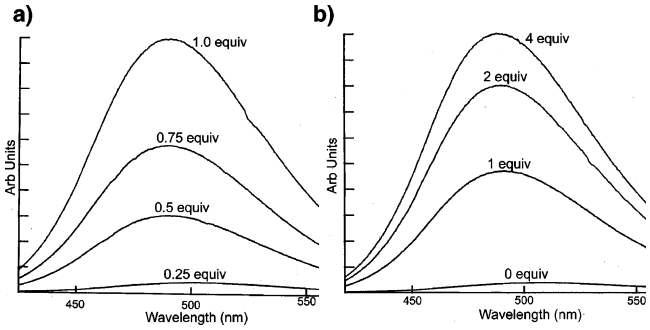


Figure 2. (a) Emission fluorescence spectra of 10^{-5} trinsyl **2** in CH_3CN, titrated with CF_3CO_2H: 0, 0.25, 0.50, and 1 equiv. (b) Emission fluorescence spectra of 10^{-5} trinsyl **2** in CH_3CN, titrated with $ZnCl_2$: 0, 1, 2, and 4 equiv (λ_{ex} = 332 nm).

produces a >25-fold *increase* in fluorescence emission intensity. The intensity maximum is achieved at 1 equiv in the case of protic acid and with 4 equiv in that of Zn^{2+}. The difference in stoichiometry needed for each electrophile can be attributed to their respective binding constants to the dimethylaminoethyl group. The binding constant of ethylenediamine with $ZnCl_2$ is on the order of 10^5.[3] The binding constant of $ZnCl_2$ for trinsyl **2** is expected to be lower because of the reduced basicity of N1 due to conjugation with an electron poor aromatic ring.[4]

By using this binding constant and a trinsyl **2** concentration of 10^{-5} M, calculation of the equilibrium concentration of Zn complexed trinsyl **2** in a 1:1 mixture is estimated to be less than 33%. The binding constant of a 3° aliphatic amine for a proton is much higher, on the order of 10^{10} (pK_a of 10). In 1:1 mixture of protic acid and aliphatic amine at a concentration of 10^{-5} M, approximately 99% of the amine will be protonated. This agrees with our observations that approximately 4 equiv of $ZnCl_2$ are needed for complete fluorescence enhancement versus 1 equiv of CF_3CO_2H. Intramolecular quenching of the fluorescence of aromatic chromophores by amines by photoinduced electron transfer (PET) is well-precedented[5] and has been demonstrated recently in striking examples of metal-enhanced intensity of fluorescence emission.[6]

The fluorescence enhancement accompanying protonation or coordination of metal of the aliphatic amine (N2) prevents quenching by the pendant amine. Interestingly the position of this pendant amine was found to be important. For example, the fluorescence emission intensity of *N*,*N*-dimethylaminoethyl sulfonamide derivative **3** is essentially identical with that of dansyl benzylamine **1**. Upon addition of 1 equiv of CF_3CO_2H, the fluorescence emission intensity *decreases* 30%. Under similar conditions, the fluorescence emission intensity of dansyl probe **1** also experiences a slight (3%) *decrease*. The >25-fold *increase* in fluorescene emission intensity of trinsyl probe **2** upon treatment with 1 equiv of CF_3CO_2H arises from protonation of the most basic nitrogen (N2), which removes the amine as a source for intramolecular quenching. Similar regiochemical effects of PET have been observed by de Silva et al.[7]

Indeed, their explanation for the regiochemical effect on quenching is consistent with the results of this study. The donor−acceptor substitution pattern on the naphthalene ring produces a strong dipole moment (internal charge transfer, ITC) in the excited state. In models proposed by both de Silva and Lewis (Figure 3), PET requires overlap of the ground-state dimethylamino lone pair (N2) with the positive end of the molecular dipole at N1. This position corresponds to the LUMO of the excited state. Overlap (and PET) occurs with facility in **2** but in **3,** because of poor overlap of the N2 lone pair or repulsive interactions of N2 with the induced electric field of the ITC, PET is not observed.[7,8]

Addition of CF_3CO_2H also produces a blue shift from 339 to 318 nm in the UV spectrum of trinsyl probe **2**. This

(3) McIntyre, G. H. J.; Block, B. P.; Fernelius, W. C. *J. Am. Chem. Soc.* **1959**, *81*, 529−535.
(4) Whidby, J. F.; Leyden, D. E.; Himel, C. M.; Mayer, R. T. *J. Phys. Chem.* **1971**, *75*, 4056−4059.

(5) (a) Callan, J. F.; de Silva, A. P.; Ferguson, J.; Huxley, A. J. M.; O'Brien, A. M. *Tetrahedron* **2004**, *60*, 11125−11131. (b) Pischel, U.; Abad, S.; Miranda, M. A. *Chem. Commun.* (*Cambridge, U.K.*) **2003**, 1088−1089. (c) Griesbeck, A. G.; Schieffer, S. *Photochem. Photobiol. Sci.* **2003**, *2*, 113−117. (d) Lewis, F. D.; Wagner-Brennan, J. M.; Denari, J. M. *J. Phys. Chem. A* **1998**, *102*, 519−525. (e) Huston, M. E.; Haider, E. W.; Czarnik, A. W. *J. Am. Chem. Soc.* **1988**, *110*, 4460−4462. (f) de Silva, A. P.; Nimal Gunaratne, H. Q. *J. Chem. Soc., Chem. Commun.* **1985**, *23*, 1669−1670. (g) Chandross, E. A.; Thomas, H. T. *Chem. Phys. Lett.* **1971**, *9*, 393−397.
(6) (a) Lewis, F. D.; Kultgen, S. G. *J. Photochem. Photobiol., A* **1998**, *112*, 159−164. (b) Kimura, E.; Koike, T. *Chem. Soc. Rev.* **1998**, *27*, 179−184.
(7) de Silva, A. P.; Nimal Gunaratne, H. Q.; Habib-Jiwan, J.; McCoy, C. P.; Rice, T. E.; Soumillion, J. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 1728−1731.
(8) (a) Lewis, F. D.; Li, L.; Kurth, T. L.; Kalgutkar, R. S. *J. Am. Chem. Soc.* **2000**, *122*, 8573−8574. (b) Lewis, F. D.; Ahrens, A.; Kurth, T. L. *Photochem. Photobiol. Sci.* **2004**, *3*, 341−347. (c) Lewis, F. D.; Kalgutkar, R. S.; Kurth, T. L. *J. Phys. Chem. A* **2004**, *108*, 1425−1434.

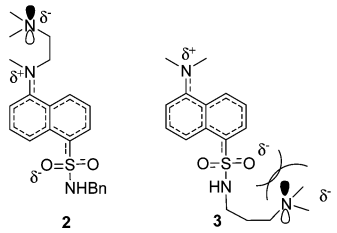


Figure 3. Regiochemical effect of pendant amine on intramolecular quenching of the ICT excited state.

spectral shift may be attributed to the destablilization of the ICT excited singlet state of the fluorophore by the interaction of the protonated amine with the positive dipole of the fluorphore. Similar observations are seen in the UV spectrum of trinsyl probe **2** when it is titrated with $ZnCl_2$.

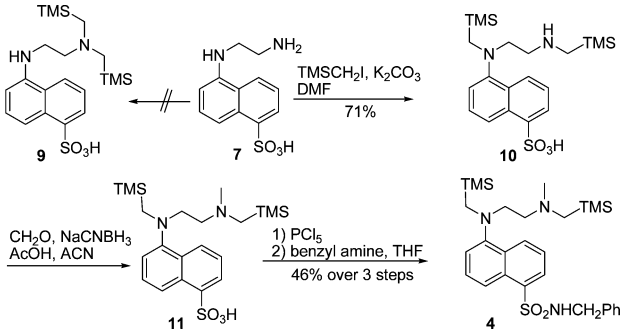
The substitution pattern on the aliphatic amine (N2) is also important. Dansyl derivatives incorporating a simple primary ethylamine are fluorescent, and protonation of this nitrogen does not affect fluorescence. Quenching ability is attributed to the lowered oxidation potential of tertiary amines compared to that of primary amines.[9]

We attempted the synthesis of a trimethylsilyl-substituted trinsyl derivative **9** with the goal of obtaining even greater fluorescence quenching. The C−Si δ orbitals are higher in energy than C−H or C−C δ orbitals and can interact with the neighboring nonbonding orbital of the nitrogen.[10−12] The result of this hyperconjugation is a decrease in oxidation potential[9,10,13] and an increase in basicity.[14] It was anticipated this would increase the quenching efficiency of the aliphatic amine and thus upon protonation effect a larger fluorescence change.

Attempts at bis alkylation of the primary amine were, however, unsuccessful; we observed alkylation on both nitrogens to obtain methylenetrimethylsilyl derivative **10** (Scheme 2). This derivative was carried on to trinsyl analogue **4** (TMS-trinsyl). The addition of CF_3CO_2H or $ZnCl_2$ to TMS-trinsyl **4** had no effect on its fluoresence emission intensity.

TMS-trinsyl **4** had a similar pH profile to trinsyl **2**. Titration of 10^{-5} M trinsyl probe **2** and TMS-trinsyl **4** in aqueous acidic solution with 10 N NaOH is shown in Figure 4. Titrations starting at approximately pH 2 and ending at pH 12 resulted in a 12.5-fold increase fluorescence intensity for trinsyl **2** while only a 2-fold increase for TMS-trinsyl **4**. Both exhibit the opposite behavior to dansyl derivative **1**, which has a lowered fluorescence at acidic pH. The pH values at the midpoints of the titration curve for trinsyl **2**

Scheme 2. Synthesis of TMS-Trinsyl **4**



and TMS-trinsyl **4** are 9.0 and 12.0, respectively. This corresponds to a pK_a value in aqueous solution for trinsyl **2**

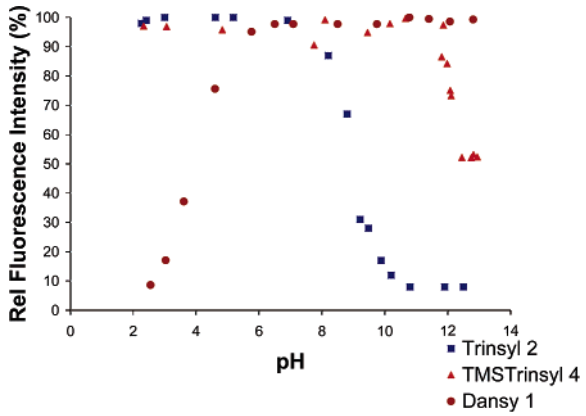


Figure 4. Relative fluorescence intensity (%) vs pH value of 10^{-5} M trinsyl **2** in aqueous solution and TMS-trinsyl **4**. Titration of TMS-trinsyl **4** was performed in 30% MeOH aqueous solution due to decreased solubility in H_2O. The excitation λ was set at 332 nm and the fluorescence emission maximum was recorded at each pH value.

of 9.0, which is quite close to the pK_{a1} value of tetraethylenediamine (pK_{a1} = 9.2 and pK_{a2} = 5.2 in water). A pK_a value of 12.0 for TMS-trinsyl **4** also follows our expectations of an increased basicity. The reduced quenching efficiency of TMS-trinsyl **4** as compared to that of trinsyl **2** may be a result of steric hindrance in exciplex formation caused by the increased size of the trimethylsilyl substituents. It has been shown that steric bulk can adversely affect the quenching efficiency of amines.[15]

Trinsyl probe **2** offers a sensitive diagnostic for protons and metal ions. An example of its utility is given below. The differential functionality of the dansyl molecule allows for synthesis of probes that can be covalently incorporated into synthetic polymers or attached to proteins. Fluorescence intensity measurements can then be used to monitor proton

(9) Beeson, J. C.; Huston, M. E.; Pollard, D. A.; Venkatachalam, T. K.; Czarnik, A. W. *J. Fluoresc.* **1993**, *3*, 65−68.
(10) Yoshida, J.; Isoe, S. *Tetrahedron Lett.* **1987**, *28*, 6621−6624.
(11) Yoshida, J.; Nishiwaki, K. *Dalton Trans.* **1998**, *16*, 2589−2596.
(12) Cooper, B. E.; Owen, W. J. *J. Organomet. Chem.* **1971**, *29*, 33−40.
(13) Yoon, U. C. *Acc. Chem. Res.* **1992**, *25*, 233−240.
(14) (a) Sommer, L. H.; Rockett, J. *J. Am. Chem. Soc.* **1951**, *73*, 5130−5134. (b) Abel, E. W.; Farrow, G. W. *J. Inorg. Nucl. Chem.* **1980**, *42*, 541−545.
(15) (a) Cheung, S. T.; Ware, W. R. *J. Phys. Chem.* **1983**, *87*, 466−473. (b) Wang, Y. *Chem. Phys. Lett.* **1985**, *116*, 286−291.

or ion flux.[16] We demonstrate this by synthesis and copolymerization of methacrylamide probe **5**. To ensure compatibility with free radical polymerization the trifluoroacetic acid salt **5** was covalently incorporated into a macroporous cross-linked copolymer by copolymerization with ethyleneglycol dimethacrylate and methyl methacrylate (10^{-4}:7:3) in acetonitrile. The resulting polymer particles were suspended in CH_2C1_2 and treated with 4 equiv of diethylamine, which resulted in a *decrease* in fluorescence emission intensity. Addition of 4 equiv of trifluoroacetic acid resulted in recovery of fluorescence intensity (Figure 5). Similar results

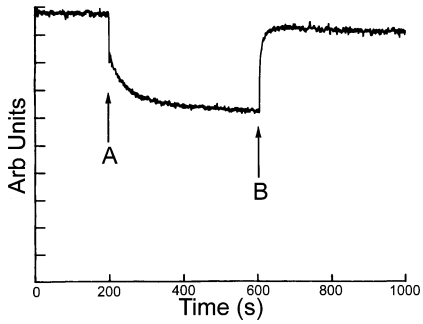


Figure 5. Plot of fluorescence emission intensity ($\lambda_{em} = 472$ nm; $\lambda_{ex} = 332$ nm) vs time for a stirred suspension of macroporous cross-linked poly(ethylene glycol)dimethacrylate doped with probe **5** in CH_2C1_2. Time A (arrow) corresponds to addition of diethylamine (4:1 amine:probe) and time B (arrow) corresponds to addition of CF_3CO_2H (4:1 acid:probe).

are obtained upon addition of Zn^{2+} ions at the second stage. These changes in fluorescence intensity were completely reversible. The rates of recovery and diminution of fluorescence emission intensity are related to the rate at which these reagents diffuse throughout the bulk material.

Fluorescent molecules have been shown to be useful nondestructive probes for the determination of physical properties of polymeric materials.[17] Blending trinsyl **2** with commercial PMMA provides a method to determine the T_g and changes in free volume of the polymer.[18]

Trinsyl molecule **2** and PMMA ($M_w = 3 \times 10^5$) were dissolved in chloroform. After evaporation the solid was ground into small particles. The fluorescence emission intensity was measured at different temperatures. Figure 6

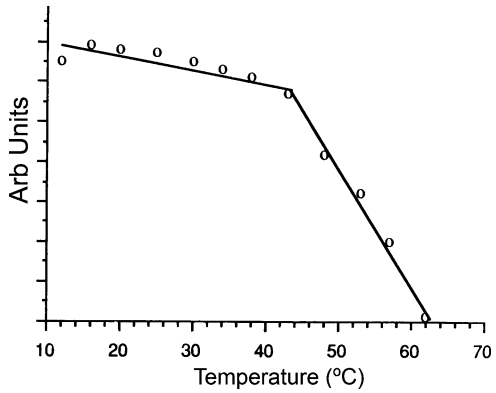


Figure 6. Fluorescence emission intensity of PMMA doped with trinsyl **2** as a function of temperature ($\lambda_{em} = 472$ nm; $\lambda_{ex} = 332$ nm).

shows the plot of fluorescence emission intensity versus temperature. As the temperature increased a *decrease* in fluorescence intensity is observed. This is attributed to a change in free volume of the polymer.[19] The excited state of the probe can return to the ground state by either fluorescing or internal conversion (dissipation of excess energy by friction or momentum transfer to the polymer matrix). As the polymer is heated, its free volume *increases* and the probe is more free to rotate and vibrate, causing an increase in the occurrence of internal conversion. A transition temperature at approximately 43 °C can be observed in the plot. This correlates to the glass transition temperature (T_g) of 42 °C for the material, as measured by DSC.

In summary, we have developed a simple and sensitive fluorescent probe, trinsyl **2**. Unlike dansyl, trinsyl **2** is sensitive to the presence of Zn^{2+} ions and protic acids. We have demonstated its use as a diagnostic of proton and ion diffusion in solids as well as the determination of T_g. The application of this probe to the development of sensors for acidic molecules is currently underway.

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Supporting Information Available: Experimental details and characterizations for all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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(16) (a) Klok, H.; Möller, M. *Macromol. Chem. Phys.* **1996**, *197*, 1395−1409. (b) Shea, K. J.; Stoddard, G. J.; Sasaki, D. Y. *Macromolecules* **1989**, *22*, 4303−4308.

(17) Bosch, P.; Catalina, T. C.; Peinado, C. *Chem. Eur. J.* **2005**, *11*, 4314−4325.

(18) Loutfy, R. O. *Macromolecules* **1981**, *14*, 270−275.

(19) Meyer, E. F.; Jamieson, A. M.; Shima, R.; Palmen, J. H. M.; Booij, H. C.; Maurer, F. H. J. *Polymer* **1990**, *31*, 243−247.