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

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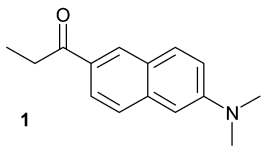
6-(2-Dimethylaminonaphthoyl) alanine (DANA) was prepared *via* an enantioselective synthesis and incorporated into the S-peptide of RNase S establishing the large changes in fluorescence that can occur upon peptide–protein interaction.

Fluorescent probes are exceptionally powerful tools for the investigation of biological events.[1] The utility of a fluorescent probe is amplified when the fluorescence emission spectrum is sensitive to environment. This allows the real time monitoring of binding events and conformational changes of the labeled molecule of interest. 6-Propionyl-2-(dimethylamino)naphthalene **1** (PRODAN), first introduced by Weber and Farris[2], is ideal for monitoring biological events as the fluorophore displays dramatic changes in fluorescence intensity and emission wavelength maxima with changes in environment. The fluorophore is also excited at wavelengths longer than those of most intrinsic biological chromophores and has a high quantum yield. Although, the source of the unique solvatochromism of PRODAN is a subject of debate, recent direct measurements of the excited state dipole moment have determined it to be likely caused by direct hydrogen bonding interactions with the solvent.[3]



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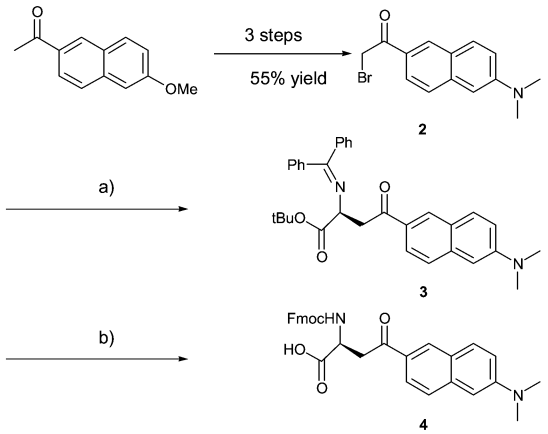
PRODAN has been used as a probe in many biological systems, perhaps most notably in the study of lipid bilayers[4] and in proteins which have a fortuitous binding site for the fluorophore.[5] Derivatives of PRODAN have also been attached covalently to proteins *via* reaction with amino acid side chain thiols with 6-acryloyl-2-(dimethylamino)naphthalene[6,7] or 6-bromoacetyl-2-dimethylaminonaphthalene (BADAN).[8] These methods provided useful information about the macromolecule of interest but are limited to systems with uniquely reacting amino acid side chains. Furthermore, the labeling of amino acid side chains results in significant distances and many degrees of freedom between the fluorophore and the protein backbone, thus reducing the environmental sensitivity of the probe.

We sought to develop an enantioselective synthesis of 6-(2-dimethylaminonaphthoyl) alanine (DANA), a fluorescent amino acid, in a form amenable to incorporation during solid phase peptide synthesis, simplifying the specific labeling of peptide substrates and minimizing the number of atoms between the fluorophore and the peptide backbone. The syntheses of many fluorescent amino acids have now been published including those containing the 7-nitrobenz-2-oxa-1,3-diazol-4-yl chromophore (NBD), but none have the same degree of sensitivity to the environment as PRODAN. [9,10]

† Electronic supplementary information (ESI) available: experimental details. See http://www.rsc.org/suppdata/cc/b2/b205224e/

Several strategies have been developed for the enantioselective synthesis of unnatural α-amino acids.[11] One of the most effective of these is the phase transfer catalysis mediated asymmetric alkylation of electrophiles with glycine benzophenone imines as first introduced by O'Donnell *et al.* and subsequently refined by Corey *et al*.[12,13] More than 25 different α-amino acids have been synthesized by this route but the electrophiles used have largely been derivatives of primary, allylic, or benzylic halides. Only one electrophile was an α-halo *tert*-butyl ester and conflicting values of the yield and ee have been reported.[14] This report demonstrates that this method can be extended to the implementation of aromatic α-halo ketones as the electrophilic species; both high yield and excellent enantioselectivity are achieved in the synthesis of DANA (Scheme 1).



Scheme 1 (a) *tert*-Butylglycinate benzophenone imine, *O*(9)allyl-*N*-9-anthracenylmethylcinchonidium bromide (25 mol%), 40% KOH, CH_2Cl_2, −25 °C, 83% yield, 87% ee, (b) i) 6 M HCl reflux, ii) Fmoc-OSu, DMF, $NaHCO_3$, 96%.

BADAN (**2**) was synthesized *via* literature reported methods, with minor modifications, from commercially available 6-acetyl-2-methoxynapthalene.[15,16] BADAN served as the electrophile for the key enantioselective alkylation reaction yielding the desired amino acid derivative in 83% yield and 87% ee. It was found that the choice of temperature was critical to the reaction, as at temperatures below −30 °C the alkylation reaction did not occur, and the α-bromoketone decomposed under the reaction conditions. At higher temperatures the enantioselectivity of the reaction eroded. Potassium hydroxide proved to be a superior base to cesium hydroxide in this reaction allowing a liquid/liquid biphasic mixture at the desired temperature.

The resulting benzophenone imine **3** was hydrolyzed and derivatized as the Fmoc carbamate to give the desired Fmoc-DANA (*N*-Fmoc-6-(2-dimethylaminonaphthoyl) alanine) in preparation for solid phase peptide synthesis. The enantiomeric excess of the amino acid was determined by reaction of the free amino acid with Marfey's reagent followed by reverse phase HPLC analysis.[16,17]

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Fmoc-DANA proved to be an excellent amino acid for solid phase synthesis using standard protocols. No loss in optical activity was observed upon incorporation of the amino acid into the peptide, elongation of the peptide, or cleavage of the peptide from the solid phase.[16] The solvent dependence of a short peptide (A-(DANA)-A) was evaluated and found to be similar to that of parent fluorophore, PRODAN, indicating that no significant change of its fluorescence properties had occurred as a result of its incorporation into a peptide scaffold (see ESI† for figure).[16]

To outline the utility of this amino acid, the well-characterized RNase S model system, consisting of a 15 amino acid S-peptide which associates tightly with S-protein (K_a 10^6–10^7 M^{-1}) to give catalytically active ribonuclease S (RNase S), was employed.[18,19] The amino acid DANA was incorporated into the S-Peptide and the changes in the fluorescence of DANA were observed upon binding to the S-Protein. These changes led directly to the dissociation constants for the newly synthesized peptides.

Two peptides which, based upon the crystal structure of RNase S and literature examples, would place the DANA amino acid in a highly hydrophilic (**5**)[20] or hydrophobic environment (**6**)[21] were synthesized by standard solid phase synthesis.

Peptide 5 KETAAAXFERQHLDS X = DANA
Peptide 6 KETAAAKFERQHLXS

Fig. 1 illustrates the large changes in fluorescence emission spectrum of DANA upon binding to the S-protein. Peptide **5**, which places DANA in a solvent exposed position upon binding, gave a 26% reduction in fluorescence intensity at 520 nm after saturation with Pro-S protein. Comparison of the titration data to a literature S-peptide containing a fluorescein labeled lysine at the equivalent position revealed DANA to have twice as large a fluorescence intensity change upon binding but 10 times weaker affinity for the Pro-S protein (K_a 1.6×10^7 M^{-1} *vs.* K_a 1.6×10^8 M^{-1}(lit)).[20] The decreased affinity is likely due to the closer proximity of the DANA fluorophore to the peptide backbone which could lead to steric congestion upon binding.

In contrast, peptide **6** exhibits a large increase in fluorescence upon binding to the Pro-S protein leading to the development of a fluorescence emission band at 450 nm that was completely absent before binding. The presence of two bands in the Pro-S protein saturated emission spectra may indicate that DANA is binding in two modes to the Pro-S protein, one of which places the fluorophore in a hydrophobic environment leading to the 450 nm emission band and a second band at 510–520 nm which places DANA in a more solvent exposed environment. Neither binding mode could be favored over a 5–45 °C temperature range suggesting both modes to be similar in energy. Analysis of the titration data leads directly to an affinity constant of 3.0×10^6 M^{-1}.

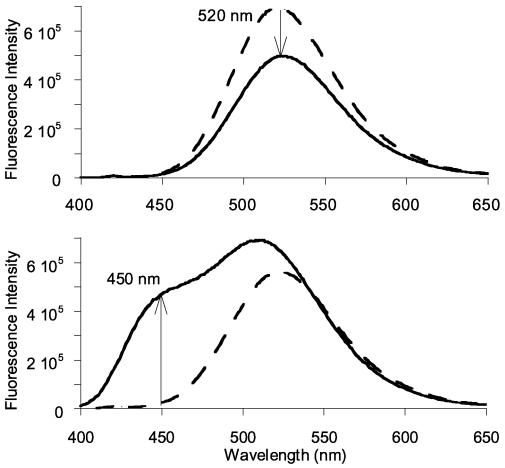

Fig. 1 Fluorescence emission spectra of peptides **5** and **6**. Top: peptide **5** (2.8 mM) in absence (hashed) and presence (12 mM) (bold) of saturating Pro-S protein. Bottom: peptide **6** (2.5 mM) in absence (hashed) and presence (16 mM) (bold) of saturating Pro-S protein. All spectra were collected in 10 mM HEPES pH 7.0, 150 mM NaCl at 20 °C with excitation at 367 nm.

The first facile synthesis of Fmoc-DANA has been reported in both high yield and high enantioselectivity. DANA was incorporated into peptides using solid phase synthesis and shown to retain similar fluorescent properties to PRODAN. Given the recent advances in protein labeling using *in vitro* biosynthesis[22] and chemical ligation[23] techniques this amino acid should prove to be a valuable tool for the study of protein–protein interactions.

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Note added at proof: after submission of this manuscript similar work appeared in Science web alerts 30/05/02.[24]

Notes and references

1 *Fluorescent and Luminescent Probes for Biological Activity*, ed. W. T. Mason, Academic Press, London, UK, 1999.
2 G. Weber and F. J. Farris, *Biochemistry*, 1979, **19**, 3075.
3 A. Samanta and R. W. Fessenden, *J. Phys. Chem. A*, 2000, **104**, 8972; F. M. Cerezo, S. C. Rocafort, P. S. Sierra, F. García-Bianco, C. D. Oliva and J. C. Sierra, *Helv. Chim. Acta*, 2001, **84**, 3306.
4 L.-G. P. Chong, *Biochemistry*, 1988, **27**, 399; J. Zeng and L.-G. P. Chong, *Biophys. J.*, 1995, **68**, 567; M. E. C. D. R. Olivera, G. Hungerford, M. G. Miguel and H. D. Burrows, *J. Mol. Struct.*, 2001, **563**, 443.
5 T. Hiratsuka, *Biochemistry*, 1998, **37**, 7167–7176.
6 S. S. Lehrer and Y. Ishii, *Biochemistry*, 1988, **27**, 5899.
7 K. G. Harikumar, D. I. Pinon, W. S. Wessels, F. G. Prendergast and J. L. Miller, *J. Biol. Chem.*, 2002, in press.
8 T. Hiratsuka, *J. Biol. Chem.*, 1999, **274**, 29159.
9 G. Sui, P. Kele, J. Orbulescu, Q. Hou and R. M. Leblanc, *Lett. Pept. Sci.*, 2002, **8**, 47; N. Jotterand, D. A. Pearce and B. Imperiali, *J. Org. Chem.*, 2001, **66**, 3224; A. Rzeska, J. Malicka, K. Guzow, M. Szabelski and W. J. Wiczk, *Photochem. Photobiol. A: Chem.*, 2001, **146**, 9; M. Taki, T. Hohsaka, H. Murakami, K. Taira and M. Sisido, *FEBS Lett.*, 2001, **507**, 35; K. Guzow, M. Szabelski, J. Malicka and W. Wiczk, *Helv. Chim. Acta*, 2001, **84**, 1086.
10 I. Dufau and H. Mararguil, *Tetrahedron Lett.*, 2000, **41**, 6063; S. Uchiyama, T. Santa and K. Imai, *J. Chem. Soc., Perkin Trans. 2*, 1999, 2524.
11 For recent reviews see (*a*) M. J. O'Donnell, *Aldrichim. Acta*, 2001, **34**, 3; (*b*) T. Abellán, R. Chinchilla, N. Galindo, G. Guillena, C. Nájera and J. M. Sansano, *Eur. J. Org. Chem.*, 2000, 2689.
12 M. J. O'Donnell, W. D. Bennet and S. Wu, *J. Am. Chem. Soc.*, 1989, **111**, 2353.
13 E. J. Corey, F. Xu and M. C. Noe, *J. Am. Chem. Soc.*, 1997, **119**, 12414.
14 (*a*) B. Lygo and P. G. Wainwright, *Tetrahedron Lett.*, 1997, **38**, 8595; (*b*) M. J. O'Donnell, F. Delgado and R. S. Pottof, *Tetrahedron*, 1999, **55**, 6347.
15 Z. Diwu, C. Beachdel and D. H. Klaubert, *Tetrahedron Lett.*, 1998, **39**, 4987.
16 Details contained in ESI†.
17 The absolute stereochemistry was deduced from the literature precedent of L-Cinchonidine derived catalyst producing the L-enantiomer and the literature precedent of the L-Marfey diastereomer of most amino acids eluting before the D-enantiomer when analyzed by reverse phase HPLC. K. Fugii, Y. Ikai, T. Mayumi, H. Oka, M. Suzuki and K. Harada, *Anal. Chem.*, 1997, **69**, 3346.
18 F. M. Richards and P. J. Vithayathil, *J. Biol. Chem.*, 1959, **234**, 1459.
19 P. R. Connelly, R. Varadarajan, J. M. Sturtevant and F. M. Richards, *Biochemistry*, 1990, **29**, 6108.
20 J. M. Goldberg and R. L. Baldwin, *Biochemistry*, 1998, **37**, 2546.
21 E. E. Kim, R. Varadarajan, H. W. Wyckoff and F. Richards, *Biochemistry*, 1992, **31**, 12304.
22 L. Wang and P. G. Schultz, *Chem. Commun.*, 2002, 1; L. Wang, A. Brock, B. Herberich and P. G. Schultz, *Science*, 2001, **292**, 498 and references therein.
23 T. W. Muir, *Synlett*, 2001, **6**, 733 and references therein.
24 B. E. Cohen, T. M. McAnaney, E. S. Park, N. Y. Jan, S. G. Boxer and L. Y. Jan, *Science*, 2002, **296**, 1700.