Spectral Characteristics of Highly Fluorescent 2-(*N,N*-dimethylamino)tryptanthrinJun Kawakami^{1*}, Takuma Kadowaki¹, Mari Ikeda², Yoichi Habata³, Shunji Ito¹,
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We previously reported that 2-aminotryptanthrin (**T2NH₂**) possesses excellent photophysical properties such as wide-wavelength absorption and emission in the visible region and a high fluorescence quantum yield. It also exhibits large positive fluorescent solvatochromism. In this study, we synthesized 2-(*N,N*-dimethylamino)tryptanthrin (**T2NMe₂**), from which we expected a substantial bathochromic shift of the emission band to the near-infrared (NIR) region by acceleration of intramolecular charge transfer. As such, the emission maxima ($\lambda_{f, \max}$) of **T2NMe₂** in some solvents were observed in the NIR region.

Key words: Tryptanthrin, Near-infrared fluorescent dye, Solvatochromism, Intramolecular charge transfer

1. INTRODUCTION

Tryptanthrin is a weakly basic alkaloid found in a number of plant species [1] that exhibits antimicrobial activity against various pathogenic bacteria and fungi [2]. In particular, it demonstrates a significant antifungal activity against *Malassezia furfur*, which causes atopic dermatitis [3], and is also effective in the treatment of contact dermatitis (delayed-type allergy) [4]. Therefore, it has a potential use as a therapeutic drug for various conditions as well as applications in cosmetics. In previous studies, we synthesized various tryptanthrin derivatives and investigated their antimicrobial properties against a fungus (*M. furfur*) and gram-positive bacterium (methicillin-resistant *Staphylococcus aureus*, MRSA). The antifungal and antibacterial activities of the derivatives were influenced by the tryptanthrin substituents, with the halogen-substituted tryptanthrin derivatives exhibiting the highest potency against *M. furfur* and MRSA [5, 6].

Some tryptanthrin derivatives were found to exhibit fluorescence on being synthesized as antimicrobial agents. We found that 2-aminotryptanthrin (**T2NH₂**) had especially excellent photophysical properties such as wide-wavelength absorption and emission in the visible region and a high fluorescence quantum yield [7]. The fluorescence spectra of **T2NH₂** in solvents with different polarities demonstrated large positive fluorescent solvatochromism. The emission maxima ($\lambda_{f, \max}$) were red-shifted with increasing polarity in both protic and aprotic solvents, but the shift was more pronounced in the samples dissolved in protic alcohols. The emitted colors changed from green to red with increasing solvent polarity. **T2NH₂** possesses a planar, polar structure with effective intramolecular charge transfer (ICT) between the carbonyl group of the five-membered ring and the amino group. The large fluorescent solvatochromism is attributable to the influence of the ICT, although stabilization by hydrogen bonding would also contribute in the case of protic solvents [8]. **T2NH₂** and its derivatives have

widespread potential applications in highly diverse fields, such as labeling reagents, chemosensors, laser dyes, photosensitizers, and fluorescent organic devices. With regard to their various applications, it is necessary for these dyes to absorb and emit at longer wavelengths. We expect that they could be of particular interest as organic fluorescent dyes for fluorescent imaging probes in biological research.

The near-infrared (NIR) region (650–900 nm) is referred to as the “optical window” of cells and tissues due to the lack of efficient endogenous absorbers in this spectral range and the subsequent high penetration depth (of the order of a few millimeters) in most tissues [9]. Organic fluorescent dyes that emit NIR fluorescence are preferable for applications in biological systems as they can reduce auto-fluorescence and photodamage to the living cells. The absorption maxima ($\lambda_{a, \max}$) of **T2NH₂** are at 447 nm (in toluene) and 464 nm (in methanol), and the emission maxima ($\lambda_{f, \max}$) are at 516 nm (in toluene) and 632 nm (in methanol). Therefore, we planned to shift the $\lambda_{a, \max}$ and $\lambda_{f, \max}$ of **T2NH₂** to longer wavelengths by accelerating the ICT. As such, we synthesized 2-(*N,N*-dimethylamino)tryptanthrin (**T2NMe₂**), in which the dimethylamino group are better electron donor than amino group of **T2NH₂**. Herein, we report the details of our study on the spectral characteristics of compound **T2NMe₂** in comparison with compound **T2NH₂**.

2. EXPERIMENTAL

2.1. General

The ¹H and ¹³C NMR spectra were recorded on a JEOL JNM-ECA 500 spectrometer with tetramethylsilane (TMS) as the internal standard. The UV–visible absorption spectra were acquired on a JASCO-V670 spectrometer. The high-resolution mass spectra (HRMS) were obtained using electrospray ionization (ESI) with a HITACHI NanoFrontier LD spectrometer. The fluorescence spectra were acquired on a Hitachi F-4500 fluorophotometer.

Measurement of the absorption and fluorescence spectra were conducted in 13 solvents with different relative permittivities (ϵ) at room temperature, where the concentrations of all of the sample solutions were 1.0×10^{-5} mol dm $^{-3}$ and no intermolecular interactions were found. The 13 solvents were toluene (PhMe, $\epsilon = 2.43$), chloroform (CHCl $_3$, $\epsilon = 4.89$), ethyl acetate (EtOAc, $\epsilon = 6.03$), tetrahydrofuran (THF, $\epsilon = 7.47$), dichloromethane (DCM, $\epsilon = 9.02$), dichloroethane (DCE, $\epsilon = 10.74$), benzonitrile (PhCN, $\epsilon = 25.30$), acetonitrile (MeCN, $\epsilon = 36.00$), *N,N*-dimethylformamide (DMF, $\epsilon = 37.06$), dimethylsulfoxide (DMSO, $\epsilon = 47.00$), 2-propanol (2-PrOH, $\epsilon = 19.92$), ethanol (EtOH, $\epsilon = 25.00$), and methanol (MeOH, $\epsilon = 32.35$). The absolute fluorescence quantum yield (Φ_f) of **T2NMe $_2$** in DCM was determined to be 0.89 using a HAMAMATSU Quantaurus-QY system. The other Φ_f values were determined at room temperature relative to that of **T2NMe $_2$** in DCM ($\Phi_f = 0.89$) using solutions of matched absorbance ($0.1 \text{ L mol}^{-1} \text{ cm}^{-1}$) at the excitation wavelength. In order to gain better understanding of the molecular geometries, molecular orbitals, optical properties, and electronic properties of compounds **T2NH $_2$** and **T2NMe $_2$** , density functional theory (DFT) calculations at the B3LYP/6-31G* level were performed using the SPARTAN '10 software package (Wavefunction Inc., Irvine, CA, 2000).

2.2 Synthesis

T2NMe $_2$ was obtained from **T2NH $_2$** (synthesized according to Ref. [5]) by Eschweiler–Clarke reaction (Scheme 1) [10, 11].

Scheme 1 Synthesis of 2-(*N,N*-dimethylamino)tryptanthrin (**T2NMe $_2$**)

Sodium triacetoxyborohydride (0.326 g, 1.54 mmol), formic acid (5 mL), and 37% aqueous formaldehyde solution (formalin, 10 mL) were added to a stirred solution of **T2NH $_2$** (0.0842 g, 0.320 mmol) in dry THF (15 mL). The mixture was refluxed for 15 h under an argon atmosphere and cooled to room temperature. The reaction mixture was neutralized with saturated aqueous sodium hydrogen carbonate solution, extracted with DCM, washed with distilled water, dried over anhydrous magnesium sulfate, and concentrated under a vacuum to afford the crude product. Column chromatography on alumina with DCM as the eluent yielded pure **T2NMe $_2$** as a reddish brown solid (0.0929 g, 50.6%).

T2NMe $_2$: ^1H NMR δ_{H} (DMSO- d_6 , 500 MHz, Me $_4$ Si): 8.50(1H, d, $J = 8.6$ Hz, H $_{10}$), 7.80–7.90 (2H, m, H $_7$ and H $_9$), 7.77 (1H, d, $J = 9.2$ Hz, H $_4$), 7.46 (1H, t, $J = 7.5$ Hz, H $_8$), 7.39(1H, d, $J = 3.2$ Hz, H $_1$), 7.34 (1H, dd, $J = 9.2$ Hz, 3.2 Hz, H $_3$), 3.13

(6H, s, CH $_3$). ^{13}C NMR δ_{C} (CDCl $_3$, 125 MHz, Me $_4$ Si): 182.26 (C=O), 158.34 (C=O), 151.34, 145.81, 140.53, 137.44, 136.62, 132.31, 126.78, 125.04, 124.94, 122.91, 119.03, 117.86, 107.18, 40.42 (CH $_3$). HRMS (ESIMS, m/z) calcd: $[\text{M} + \text{H}]^+$ 292.1086, $[\text{M} + \text{Na}]^+$ 314.0905; found: $[\text{M} + \text{H}]^+$ 292.1174, $[\text{M} + \text{Na}]^+$ 314.1002. FT-IR [wavenumbers (cm $^{-1}$)]: $\nu_{\text{C=O}}$: 1670; $\nu_{\text{C=O}}$: 1640.

3. RESULTS AND DISCUSSION

3.1 Spectral characteristics of **T2NH $_2$** and **T2NMe $_2$**

We investigated the solvent effects of **T2NH $_2$** and **T2NMe $_2$** in the 13 solvents with different ϵ . The absorption and fluorescence spectra of **T2NMe $_2$** and the photophysical properties of **T2NH $_2$** and **T2NMe $_2$** are shown in Figure 1 and Table 1, respectively. The absorption and fluorescence spectra of **T2NH $_2$** are shown in Figures 2, 3, and 4 of Ref. [8].

Figure 1. (a) UV-vis absorption and (b) fluorescence spectra of **T2NMe $_2$** in solvents with different polarities.

In comparison with **T2NH $_2$** , **T2NMe $_2$** , in which a dimethylamino group was installed instead of an amino group, exhibited red-shifted $\lambda_{a, \text{max}}$ and $\lambda_{f, \text{max}}$ values. The longest $\lambda_{a, \text{max}}$ and $\lambda_{f, \text{max}}$ of **T2NMe $_2$** were 508 nm in CHCl $_3$ and 674 nm in MeOH, respectively. The $\lambda_{f, \text{max}}$ of **T2NH $_2$** are not in the NIR region, but those of **T2NMe $_2$** are in the NIR region for one polar aprotic solvent (DMSO) and three protic solvents (2-PrOH, EtOH and MeOH). **T2NMe $_2$** shows Φ_f values higher than those of **T2NH $_2$** and between 0.51 and 0.96 in the seven different aprotic solvents (PhMe, CHCl $_3$, EtOAc, THF, DCM, DCE

Table 1. Absorption maxima ($\lambda_{a, \max}$), molar absorption coefficients ($\epsilon_{a, \max}$), emission maxima ($\lambda_{f, \max}$), fluorescence quantum yields (Φ_f), and Stokes shifts of **T2NH₂** and **T2NMe₂**.

Solvents	$\lambda_{a, \max} / \text{nm}$		$\log(\epsilon_{a, \max} / \text{dm}^{-3} \text{mol}^{-1} \text{cm}^{-1})$		$\lambda_{f, \max} / \text{nm}$		Φ_f		Stokes shifts / cm^{-1}	
	T2NH₂	T2NMe₂	T2NH₂	T2NMe₂	T2NH₂	T2NMe₂	T2NH₂	T2NMe₂	T2NH₂	T2NMe₂
PhMe	447	488	3.97	4.34	516	551	0.18	0.90	2992	2343
CHCl ₃	450	508	4.03	4.34	560	592	0.60	0.96	4365	2793
EtOAc	452	481	4.12	4.29	553	596	0.67	0.79	4041	4011
THF	457	481	4.08	4.27	558	595	0.59	0.82	3961	3983
DCM	447	503	4.07	4.35	545	604	0.64	0.89	4023	3324
DCE	447	499	4.05	4.18	547	608	0.64	0.88	4090	3593
PhCN	462	504	4.16	4.32	581	624	0.51	0.51	4433	3816
MeCN	451	490	4.13	4.29	588	637	0.56	0.28	5166	4710
DMF	472	492	4.02	4.26	602	641	0.31	0.17	4575	4725
DMSO	477	496	4.06	4.21	615	654	0.30	0.12	4704	4871
2-PrOH	481	496	4.21	4.24	616	650	0.28	0.15	4556	4777
EtOH	475	497	4.24	4.22	624	660	0.17	0.08	5027	4969
MeOH	464	493	4.19	4.25	632	674	0.12	0.04	5729	5447

and PhCN). The Φ_f values of **T2NMe₂** are lower than those of **T2NH₂** in the three polar aprotic solvents (MeCN, DMF, and DMSO) and three protic solvents (2-PrOH, EtOH, and MeOH). The $\lambda_{f, \max}$ of **T2NMe₂** are at more than 637 nm in these solvents. Small Φ_f values of **T2NMe₂** in comparison with **T2NH₂** could be due to the smallness of the HOMO-LUMO energy gaps. The molar absorption coefficients ($\epsilon_{a, \max}$) of **T2NMe₂** are larger than those of **T2NH₂**, with exception of that of EtOH. Brightness is given by the product of $\epsilon_{a, \max}$ and Φ_f . Therefore, **T2NMe₂** can shine more brightly than **T2NH₂** in many solvents. Photographs taken of **T2NH₂** and **T2NMe₂** under 365 nm UV light in different solvents are shown in Figure 2.

Figure 2. Photographs of **T2NH₂** and **T2NMe₂** taken under 365 nm UV light in different solvents.

The fluorescence of **T2NH₂** in solvents with different polarities demonstrated large positive fluorescent solvatochromism. The emission maxima were red-shifted with increasing polarity in both protic and aprotic solvents, but the shift was more pronounced in the samples dissolved in the protic alcohols. The emitted colors changed from green (516 nm) to red (632 nm) with increasing solvent polarity. In the longer wavelength region, a similar phenomenon was also observed in **T2NMe₂**. The emitted colors of **T2NMe₂** changed from orange (592 nm) to red (674 nm) with increasing solvent polarity, with the exception of green (551 nm) in PhMe having the lowest solvent polarity. **T2NMe₂** showed a large bathochromic shift of the emission band to the NIR region. The $\lambda_{f, \max}$ values of **T2NMe₂** in some solvents were in the NIR region. **T2NH₂** and **T2NMe₂** possess planar, polar structures with effective ICT between the carbonyl group of the five-membered ring and the amino

group. The large fluorescent solvatochromism of **T2NH₂** and **T2NMe₂** may be attributed to the influence of ICT, although stabilization by hydrogen bonding would be also contribute in the case of protic solvents.

3.2 Strong ICT characteristics of **T2NH₂** and **T2NMe₂** in their excited states

The molecular structures of **T2NH₂** and **T2NMe₂** were calculated using DFT calculations. The molecular orbital surfaces of the corresponding HOMO and LUMO surfaces of **T2NH₂** and **T2NMe₂** and their dipole moments in the ground state (μ_G) are shown in Figure 3.

Figure 3. HOMO and LUMO surfaces and μ_G values of **T2NH₂** and **T2NMe₂** according to DFT calculations.

In **T2NH₂** and **T2NMe₂**, the electrons are localized in the amino group in the HOMO and in the carbonyl group of the five-membered ring in the LUMO. Electronic transitions are $\pi-\pi^*$, originating from the amino or dimethylamino groups. These results show that **T2NH₂** and **T2NMe₂** have ICT characteristics. The μ_G values of **T2NH₂** and **T2NMe₂** were 4.9 Debye (D) and 5.4 D, respectively. The solvatochromic shift in the ground state is smaller than that in the excited state for both **T2NH₂** and **T2NMe₂**, as shown in Table 1. These results indicate that the dipole moments of the excited state (μ_E) are larger than those in μ_G . Solvent-induced spectral shifts are often interpreted in terms of the Lippert–Mataga equation (1) [12, 13, 14]:

$$\Delta\nu = 2(\mu_E - \mu_G)^2 \Delta f / hca^3 + \text{constant} \quad (1)$$

$$\Delta f = \{(\varepsilon - 1) / (2\varepsilon + 1)\} - \{(n^2 - 1) / (2n^2 + 1)\}$$

where h (6.6256×10^{-27} erg s) is Planck's constant, c (2.9979×10^{10} cm s $^{-1}$) is the speed of light, a is the radius of the cavity in which the fluorophore resides, ν_A and ν_F are the wavenumbers (cm $^{-1}$) of the absorption and emission bands, respectively. Equation (1) describes the Stokes shifts ($\Delta\nu = \nu_A - \nu_F$) in terms of the change in dipole moment of the fluorophore ($\Delta\mu = \mu_E - \mu_G$) and the dependence of the dipole energy on the ε values and refractive index (n) of the solvent. It also accounts for the general solvent effect, but it does not account for specific solvent–fluorophore interactions, such as those involved in hydrogen bonding [15]. The Lippert–Mataga plots for **T2NH₂** and **T2NMe₂** in aprotic solvents are shown in Figure 4.

Figure 4. Lippert–Mataga plots of **T2NH₂** and **T2NMe₂** showing $\Delta\nu$ versus Δf .

From the slopes in this plot, the change in the dipole moment ($\Delta\mu$) of the fluorophore upon electronic excitation ($\mu_E - \mu_G$) was estimated, assuming the molecular radius as the cavity radius [15]. The molecules under consideration are non-spherical; hence, the substitution of the molecular radius for the cavity radius is only approximate. The molecular radii for **T2NH₂** (6.03 Å) and **T2NMe₂** (6.66 Å) were obtained from DFT calculations. The $\Delta\mu$ values for **T2NH₂** and **T2NMe₂** were estimated to be 11.9 D and 16.5 D, respectively (Table 2).

Table 2. Molecular radii^{a)} and μ_G and μ_E values of **T2NH₂** and **T2NMe₂**.

	1	2
$a / \text{\AA}^a)$	6.03	6.66
$\Delta\mu (= \mu_E - \mu_G) / \text{D}$	11.9	16.5
$\mu_G / \text{D}^a)$	4.9	5.4
μ_E / D	16.8	21.9

a) Obtained from DFT calculations.

The μ_E values can be estimated from the $\Delta\mu$ and μ_G values obtained by the DFT calculations. The μ_E values of **T2NH₂** and **T2NMe₂** are 16.8 D and 21.9 D, respectively. The μ_E values would be qualitatively correct and these results can well explain the experimental results. **T2NH₂** and **T2NMe₂**, which have planar structures suitable for effective ICT, are strongly polarized in their excited states. The large fluorescent solvatochromisms of **T2NH₂** and

T2NMe₂ are attributed to the influence of strong ICT in their excited states. In particular, **T2NMe₂** is very strongly polarized in its excited state. Therefore, fluorescence of **T2NMe₂**, with the strong electron-donating dimethylamino group, was observed in the longer-wavelength NIR region due to the acceleration of the ICT.

4. CONCLUSION

We compared the photophysical properties of **T2NMe₂** with those of **T2NH₂**. Our results showed that **T2NMe₂** exhibited high Φ_F values and large $\varepsilon_{a, \text{max}}$ values in many of the solvents. It also exhibited a large positive fluorescent solvatochromism, as did **T2NH₂**. It is also more strongly polarized in the excited state than **T2NH₂** is. Therefore, fluorescence of **T2NMe₂**, with the strong electron-donating dimethylamino group, was observed in the longer-wavelength NIR region due to the acceleration of the ICT. Thus, we can conclude that **T2NMe** can be used as a fluorescent dye in the NIR region, which is referred to as the “optical window” of cells and tissues. As such, we expect that it can potentially be used as an organic fluorescent dye for fluorescent imaging probes in biological research.

5. ACKNOWLEDGEMENTS

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6. REFERENCES

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