[Chem. Pharm. Bull. 28(6)1722—1729(1980)]

Fluorescence Reagents as an Analytical Tool for Studies of Thio-nucleosides. Their Reactions with Thiouridines[1]

Eisuke Sato, Minoru Machida[2a] and Yuichi Kanaoka[2]

Faculty of Pharmaceutical Sciences, Hokkaido University[2]

(Received December 3, 1979)

The fluorescent reagents, N-(4-dimethylamino-1-naphthyl)bromoacetamide (**2a**) and N-(4-anilino-1-naphthyl)bromoacetamide (**2b**), were covalently introduced into 4- and 2-thiouridines at the sulfur site as a model fluorescent probe. The fluorescence properties of the products are discussed.

Keywords——fluorescent probe; 4- and 2-thiouridines; dimethylaminonaphthalene; anilinonaphthalene; emission spectra

The principle of using small ligands as spectroscopic probes for studies of the structural and functional properties of biological macromolecules is well established. However, fluorescence spectroscopy is a recent addition to the techniques that have been used to study nucleic acids. In contrast to the field of protein chemistry ,where various applications of fluorescence techniques have been reported,[3,4c,4d] there are relatively few published data on the possible applications to nucleic acid research.[4a,b,d] For example, although it is well known that certain fluorescent dyes such as ethidium bromide bind noncovalently to nucleic acids as spectroscopic probes,[5] there are not many reports of "extrinsic" fluorescence reagents which have been covalently introduced into nucleic acids. Fluorescent labeling of chromosomal DNA with quinacrine mustard[6] is one of the few examples.

The large number of unusual bases present in tRNA affords an opportunity for chemical modification at a particular site.[7] If selective modification of particular bases could be achieved in conjunction with the attachement of a spectroscopic probe, this would represent a powerful tool for studies of the structure and function of tRNA. We have earlier established[8] that 4- and 2-thiouridines react specifically with certain primary bromides under mild conditions, employing 2-hydroxy-5-nitrobenzyl bromide (Koshland's reagent), a familiar "reporter" in protein chemistry, as a representative example. Since a fluorescent spectrum

1) *a*) Heterocycles Related to Nucleotides. IX. Part VIII: H. Nakamura, H. Wada, E. Sato, and Y. Kanaoka, *J. Carbohydrates. Nucleosides. Nucleotides*, **4**, 241 (1977); *b*) Organic Fluorescence Reagents. V. Part IV: Y. Kanaoka, T. Takahashi, H. Nakayama, K. Takada, T. Kimura, and S. Sakakibara, *Chem. Pharm. Bull.*, **25**, 3126 (1977).

2) Location: *Kita-12, Nishi-6, Kitaku, Sapporo, 060, Japan*; *a*) Present address: *Faculty of Pharmaceutical Sciences, Higashi Nippon Gakuen University, Ishikari-tobetsu, Hokkaido, 061-02, Japan.*

3) For a leading reference see: Y. Kanaoka, *Angew. Chem. Intl. Edn.*, **16**, 137 (1977).

4) *a*) C.R. Cantor and T. Tao, "Procedures of Nucleic Acid Research," ed. G.L. Cantoni and D.R. Davis, Vol. 2, Harper and Row, New York, 1971, p. 31; *b*) K. Imakubo, "Fluorescence Spectroscopy: Principles and Application to Biological Systems." ed. Y. Kanaoka, K. Shibata, T. Sekine, and T. Takagi, Kyoritsu-Shuppan, Tokyo, 1974, p. 206; *c*) Y. Kanaoka, *ibid.*, p. 109; *d*) T. Sekine, T. Ohyashiki, Y. Kanaoka, and M. Machida, *ibid.*, p. 120; *d*) M. Saneyoshi, "Seikagaku Jikken Koza," Vol. 2-III, Tokyo Kagaku Dojin, Tokyo, 1977, p. 299.

5) *a*) J.B. LePecq and C. Paoletti, *J. Mol. Biol.*, **27**, 87 (1967); *b*) T. Tao, J.H. Nelson and C.R. Cantor, *Biochemistry*, **9**, 3514 (1970).

6) T. Caspersson, L. Zech, and E.J. Modest, *Science*, **170**, 762 (1971).

7) *a*) M. Saneyoshi, *Tanpakushitsu-Kakusan-Koso*, **15**, 251 (1970); *b*) E. Sato and Y. Kanaoka, *Chem. Pharm. Bull.*, **22**, 799 (1974).

8) E. Sato and Y. Kanaoka, *Biochim. Biophys. Acta*, **232**, 213 (1971).

is generally more affected by the environment[3,4,9] and can be measured at lower concentration than the absorption spectrum,[3,4,10] the covalent attachment of a fluorescent probe seemed promising. In fact, this approach was followed by Leonard *et al.*,[11] with a fluorescent coumarin derivative, which was further used for labeling of tRNA.^{fMet}[12] Covalent attachment of fluorescent groups to the 5′-end of tRNA was also reported.[13] In our screening search for new fluorophores, we have examined the fluorescence properties of dimethylaminonaphthalenes as one of the simpler fluorogenic arenes.[14] In the present paper we report the results of a model study with such simple fluorescence reagents; *e. g.*, reactions of 4-thiouridine and 2-thiouridine with N-(4-dimethylamino-1-naphthyl)bromoacetamide **2a** and N-(4-anilino-1-naphthyl)bromoacetamide **2b,** and the spectroscopic properties of the modified thiobase derivatives.

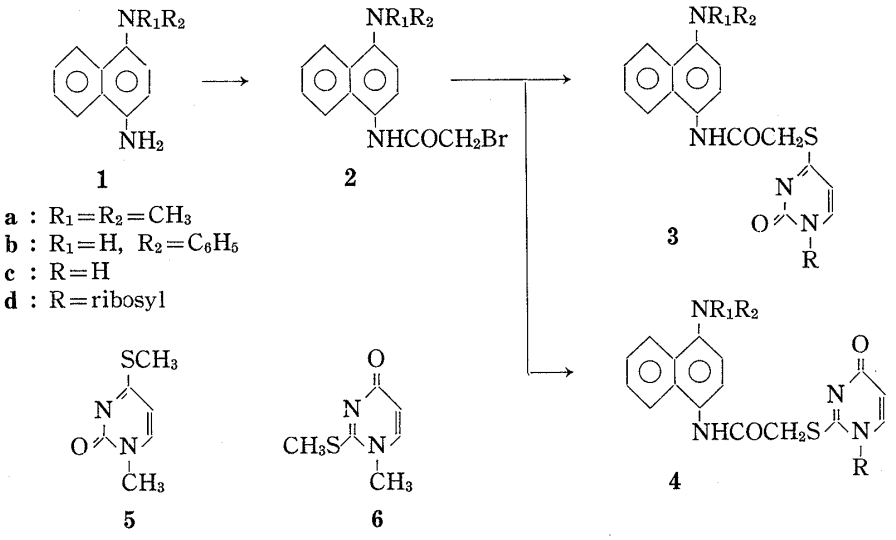

Chart 1

Experimental

All melting points are uncorrected. IR spectra were recorded on a Kogaku Kenkyusho DS-301 spectrometer in nujol mulls. UV spectra were measured with a Shimadzu UV-200 spectrometer. NMR spectra were obtained with a Hitachi H-60 spectrometer. Chemical shifts were recorded in ppm units using tetramethylsilane as an internal reference. Mass spectra were recorded on a Hitachi RMU-7E spectrometer. Fluorescence spectra were measured with a Hitachi MPF-2A spectrophotofluorometer.

Quantum yields were determined from corrected emission spectra by comparison with the fluorescence of quinine in 0.5 N sulfuric acid. Quinine has been assumed to fluoresce with a quantum efficiency of 0.55.[15] In general, during the course of reactions of the reagent with the substrate, the pH of the solution was maintained with a Radiometer pH-stat titrator, model TTTlc.

Thin-layer chroatography (TLC) was performed with silica gel (Merck, Kieselgel GF_{254}).

4-Dimethylamino-1-naphthylamine (1a)——The hydrochloride of **1a** was prepared as described previously,[14] mp 212—214° (lit.,[14] mp 211—213°).

N-(4-Dimethylamino-1-naphthyl)bromoacetamide (2a)——The hydrochloride of **1a** (650 mg; 2.1 mmol) was suspended in benzene (100 ml), and neutralized with 10% Na_2CO_3. The whole was extracted with benzene, and the extract was dried over anhydrous K_2CO_3. Removal of the solvent left a crude base (451

9) Y. Kanaoka, M. Machida, M.I. Machida, and T. Sekine, *Biochim. Biophys. Acta*, **317**, 563 (1973).
10) H. Nakayama and Y. Kanaoka, *Chem. Pharm. Bull.*, **21**, 2904 (1973).
11) J.A. Secrist, J.R. Barrio, and N.J. Leonard, *Biochem. Biophys. Res. Commun.*, **45**, 1262 (1971).
12) C.H. Yang and D. Soll, *J. Biochem.*, **73**, 1243 (1973).
13) C.H. Yang and D. Soll, *Arch. Biochem. Biophys.*, **155**, 70 (1973).
14) M. Machida, M. Bando, M.I. Machida, Y. Migita, and Y. Kanaoka, *Chem. Pharm. Bull.*, **24**, 3045 (1976).
15) C.P. Parker and W.T. Rees, *Analyst*, **85**, 587 (1960).

mg). To a benzene (10 ml) solution of this base (451 mg), a solution of bromoacetyl bromide (510 mg; 212 mmol) in benzene (10 ml) was added dropwise. Precipitated hydrobromide was collected by suction, washed with benzene, and suspended in H_2O (100 ml). The benzene extract was dried over Na_2SO_4. Removal of the solvent *in vacuo* left a colorless solid, which was recrystallized from benzene to provide colorless fine needles (**2a**) of mp 135—137°; 572 mg or 59%. IR cm^{-1}; $\nu_{C=O}$ 1650 (nujol); ν_{N-H} 1530 (nujol). UV λ_{max}^{EtOH} nm (log ε): 323 (3.87), 231 (4.13), 217 (4.63). NMR ($CDCl_3$) δ: 2.87 (6H, singlet, $N(CH_3)_2$), 4.10 (2H, singlet, $-CH_2-$). MS *m/e*: 308, 306 (M^+). *Anal.* Calcd for $C_{14}H_{15}BrN_2O$: C, 54.73; H, 4.92; Br, 26.02; N, 9.12. Found: C, 54.68; H, 4.92; Br. 25.96; N, 8.94.

4-Anilino-1-naphthylamine (1b)——**1b** was obtained from α-anilinonaphthalene in the manner described for **1a**; mp 143—145° (lit.,[13] mp 148°).

N-(4-Anilino-1-naphthyl)bromoacetamide (2b)——The hydrobromide of **2b**, prepared from **1b** as described for **2a**, (900 mg; 2.1 mmol) was suspended in H_2O (50 ml). The whole was extracted with CH_2Cl_2, and the extract was dried over anhydrous Na_2SO_4. After removal of the solvent, the residual solid was recrystallized from benzene to provide pale green leaflets of mp 124—126°; 375 mg or 46%. IR cm^{-1}: $\nu_{C=O}$ 1660 (nujol). UV λ_{max}^{EtOH} nm (log ε): 352 (3.97), 255 (4.23), 218 (4.69). NMR (DMSO-d_6) δ: 4.17 (2H, singlet, $-CH_2-$). MS *m/e*: 356, 354 (M^+). *Anal.* Calcd for $C_{21}H_{18}BrN_2O \cdot 1/2\ C_6H_6$: C, 63.97; H, 4.60; Br, 20.27; N, 7.11. Found: C, 64.22; H, 4.49; Br, 20.31; N, 6.91.

Reactions of Thiouracil Derivatives with the Fluorescent Reagents: General Procedure:

A solution of the reagent in MeOH was added to an aqueous solution of a thiouracil derivative. The pH of the solution was maintained constant at 9.0 by addition of 0.5 N NaOH with a pH-stat until the consumption of alkali essentially ceased after 4—8 hr. After addition of a small piece of dry ice, the whole solution was concentrated to 1—20 ml under reduced pressure. Precipitates were collected by suction, dried *in vacuo*, and, after isolation of the product by TLC as required, recrystallized.

S-[N-(4-Dimethylamino-1-naphthyl)carbamylmethyl]-4-thiouracil (3ac)——Prepared from 4-thiouracil (64 mg; 0.50 mmol) in H_2O (100 ml) and **2a** (184 mg; 0.60 mmol) in MeOH (500 ml). Recrystallized from MeOH, 142 mg or 80%; Colorless needles, mp 213—214°. IR cm^{-1}: $\nu_{C=O}$ 1655 (nujol); ν_{N-H} 1550. UV λ_{max}^{EtOH} nm (log ε): 305 (4.14), 245 (4.24), 216 (4.74). NMR (DMSO-d_6) δ: 2.79 (6H, singlet, $N(CH_3)_2$), 4.19 (2H, singlet, $-CH_2-$), MS *m/e*: 354 (M^+). *Anal.* Calcd for $C_{18}H_{18}N_4O_2S$: C, 61.01; H, 5.12; N, 15.81; S, 9.04. Found: C, 61.28; H, 5.02; N, 15.84; S, 9.04.

S-[N-(4-Dimethylamino-1-naphthyl)carbamylmethyl]-4-thiouridine (3ad)——Prepared from 4-thiouridine (52 mg; 0.20 mol) in H_2O (10 ml) and **2a** (70 mg, 0.22 mmol) in MeOH (5 ml); TLC (AcOEt: EtOH (5: 1, v/v)), crystalline powder from EtOH–EtOEt, 56 mg or 57%, mp 130—134°. IR cm^{-1}: $\nu_{C=O}$ 1645 (nujol), UV λ_{max}^{EtOH} nm (log ε): 306 (4.21), 247 (4.18), 215 (4.66), NMR (DMSO-d_6) δ: 3.77 (6H, singlet, $N(CH_3)_2$), 4.19 (2H, singlet, $-CH_2-$). *Anal.* Calcd for $C_{23}H_{26}N_4O_6S \cdot H_2O$: C, 54.75; H, 5.59; N, 11.11; S, 6.37. Found: C, 55.04; H, 5.03; N, 10.90; S, 6.58.

S-[N-(4-Dimethylamino-1-naphthyl)carbamylmethyl]-2-thiouracil (4ac)——Prepared from 2-thiouracil (64mg; 0.50 mmol) in H_2O/(100 ml) and **2a** (230 mg; 0.73 mmol) in MeOH (20 ml); recrystallized from MeOH, 161 mg or 91%, colorless needles, mp 215—217°. IR cm^{-1}: $\nu_{C=O}$ 1660 (nujol). UV λ_{max}^{EtOH} nm (log ε): 295 (3.98), 217 (4.73). NMR (DMSO-d_6) δ: 2.78 (6H, singlet, $N(CH_3)_2$), 4.17 (2H, singlet, $-CH_2-$). MS *m/e*: 354 (M^+). *Anal.* Calcd for $C_{18}H_{18}N_4O_2S$: C, 61.01; H, 5.12; N, 15.81; S, 9.03. Found: C, 61.13; H, 5.05; N, 15.78; S, 9.05.

S-[N-(4-Dimethylamino-1-naphthyl)carbamylmethyl]-2-thiouridine (4ad)——Prepared from 2-thiouridine (52 mg; 0.20 mmol) in H_2O (10 ml) and **2a** (82 mg; 0.25 mmol) in MeOH (5 ml); TLC (AcOEt: EtOH (3: 1, v/v)), crystalline powder from EtOH–EtOEt, 60 mg or 61%, mp 173—177°, IR cm^{-1}: $\nu_{C=O}$ 1645 (nujol). UV λ_{max}^{EtOH} nm (log ε): 318 (3.89), 217 (4.74). NMR (DMSO-d_6) δ: 2.79 (6H, singlet, $N(CH_3)_2$), 4.25 (2H, singlet, $-CH_2-$). *Anal.* Calcd for $C_{23}H_{26}N_4O_6S \cdot 1/2H_2O$: C, 55.74; H, 5.49; N, 11.31; S, 6.47. Found: C, 55.45; H, 5.28; N, 11.37; S, 6.17.

S-[N-(4-Anilino-1-naphthyl)carbamylmethyl]-4-thiouracil (3bc)——Prepared from 4-thiouracil (32 mg; 0.25 mmol) in H_2O (100 ml) and **2b** (118 mg; 0.30 mmol) in MeOH (50 ml); recrystallized from MeOH, 82 mg or 82% pale yellow prisms, mp 224—226°. IR cm^{-1}: $\nu_{C=O}$ 1635 (nujol). UV λ_{max}^{EtOH} nm (log ε): 3.48 (4.05), 295 (4.16), 256 (4.39), 217 (4.78). NMR (DMSO-d_6) δ: 4.18 (2H, singlet, $-CH_2-$). MS *m/e*: 402 (M^+). *Anal.* Calcd for $C_{22}H_{18}N_4O_2S$: C, 65.66; H, 4.51; N, 13.92; S, 7.95. Found: C, 65.46; H, 4.38; N, 13.67; S, 7.91.

S-[N-(4-Anilino-1-naphthyl)carbamylmethyl]-4-thiouridine (3bd)——Prepared from 4-thiouridine (52 mg; 0.20 mmol) in H_2O (7 ml) and **2b** (87 mg; 0.22 mmol) in MeOH (15 ml); TLC (AcOEt: EtOH (10: 1, v/v)), crystalline powder from EtOH–EtOEt, 66 mg or 62%, mp 168—171°. IR cm^{-1}: $\nu_{C=O}$ 1630 (nujol). UV λ_{max}^{EtOH} nm (log ε): 350 (3.97), 300 (4.18), 256 (4.31), 217 (4.69). NMR (DMSO-d_6) δ: 4.23 (2H, singlet, $-CH_2-$). *Anal.* Calcd for $C_{27}H_{26}N_4O_6S \cdot H_2O$: C, 58.68; H, 5.11; N, 10.14; S, 5.79. Found: C, 58.47; H, 4.91; N, 10.03; S, 5.49.

S-[N-(4-Anilino-1-naphthyl)carbamylmethyl]-2-thiouracil (4bc)——Prepared from 2-thiouracil (32 mg; 0.25 mmol) in H_2O (50 ml) and **2b** (148 mg; 0.38 mmol) in MeOH (50 ml); recrystallized from EtOH,

16) L. Wacker, *Ann.*, **243**, 305 (1888).

69 mg or 69%. Colorless fine prisms, mp 204—206°. IR cm^{-1}: $\nu_{C=O}$ 1650 (nujol). UV λ_{max}^{EtOH} nm (log ε): 350 (4.03), 253 (4.34), 218 (4.78). NMR (DMSO-d_6) δ: 4.20 (2H, singlet, $-CH_2-$). MS *m/e*: 402 (M^+). *Anal.* Calcd for $C_{22}H_{18}N_4O_2S$: C, 65.66; H, 4.51; N, 13.92; S, 7.95. Found: C, 65.53; H, 4.40; N, 13.77; S, 7.79.

S-[N-(4-Anilino-1-naphthyl)carbamylmethyl]-2-thiouridine (4b d)——Prepared from 2-thiouridine (52 mg; 0.20 mmol) in H_2O (15 ml) and **2b** (103 mg; 0.26 mmol) in MeOH (30 ml); TLC (AcOEt–EtOH (5: 1, v/v), crystalline powder from EtOH–EtOEt, 76 mg or 71%. IR cm^{-1}: $\nu_{C=O}$ 1630 (nujol). UV λ_{max}^{EtOH} nm (log ε): 350 (4.00), 250 (4.43), 219 (4.76). NMR (DMSO-d_6) δ: 4.27 (2H, singlet, $-CH_2-$). *Anal.* Calcd for $C_{27}H_{26}N_4O_6S \cdot 1/2H_2O$: C, 59.66; H, 4.01; N, 10.31; S, 5.90. Found: C, 59.62; H, 4.90; N, 10.25; S, 5.77.

N-(4-Dimethylamino-1-naphthyl)acetamide 2a: Br=H)——Prepared from **1a** by acetylation with acetic anhydride, mp 195—196° (lit.,[17] mp 195°). UV λ_{max}^{EtOH} nm (log ε). 320 (3.83), 242 (4.16), 212 (4.63).

N-(4-Anilino-1-naphthyl)acetamide (2b: Br=H)——Prepared from **1b** by acetylation with acetic anhydride, mp 191—192° (lit.,[18] mp 192°). UV λ_{max}^{EtOH} nm (log ε), 350 (3.92), 254 (4.20), 219 (4.62).

1,4-Dimethyl-4-thiouracil (5)——Prepared from 4-thiouracil by dimethylation with dimethyl sulfate, mp 123—124° (lit.,[19] 124°).

1,2-Dimethyl-2-thiouracil (6)——Prepared from 2-thiouracil by dimethylation with dimethyl sulfate, mp 160—162° (lit.,[20] 166—167°).

Reactions of 2a and 2b with the Major Nucleosides——A monoglyme solution of **2b** (8 mM; 0.5 ml) was added to an aqueous solution of 4- or 2-thiouridine, uridine, cytidine, adenosine, or guanosine (2 mM; 2 ml) in a pH-stat. After 2 hr, the reaction mixture was analyzed by paper chromatography [Toyo Roshi No. 51A; isopropanol–H_2O–28% NH_3 (70: 20: 10, v/v); *n*-butanol–H_2O (86: 14, v/v); methanol–c. HCl–H_2O (70: 20: 10, v/v; *n*-butanol–H_2O–28% NH_3 (86: 14: 5, v/v); *n*-butanol–H_2O–HCO_2H (77: 33: 10, v/v)] and TLC [EtOAc–EtOH (10: 1 or 20: 1, v/v); EtOAc–benzene (1: 1, v/v)]. 4-Thiouridine and 2-thiouridine reacted to the extents of 60% and 23%, respectively, while other major nucleosides were recovered unchanged.

Results and Discussion

When the reagents (**2a**, **2b**) were mixed with thiouracils or thiouridines in aqueous solution at pH 9.0, a reaction readily occurred in each case to give the desired product (**3** or **4**), in which the fluorophore is introduced at the sulfur atom of the heterocycle. Héléne *et al.* described phosphorescence of 4-thiouracil at 77°K in a glass.[22] Favre *et al.* found a photoreaction of a 4-thiouridine residue in *E. coli* $tRNA_1^{Val}$ and subsequently studied the luminescence of 4-thiouridine.[23] Tsuboi *et al.* have also recently studied the emission spectra of the thiopyrimidines.[24] However, emission spectra of 4- and 2-alkylthiopyrimidines have not previously been reported. Before studying the fluorescence characteristics of these products (**3**, **4**), the fluorescence of the reagents (**2a**, **2b**) was examined. However, the reagents (**2a**, **2b**) were found to be unstable to ultraviolet light, so the acetyl derivatives (**2a**, **2b** (Br=H)), which have the same fluorophore, were investigated as models. The fluorescence spectra of the models (**2a**, **2b** (Br=H)) and the reaction products (**3**, **4**) are shown in Figs. 1—3, while the emission maxima and quantum yields of these compounds are summarized in Table I. Both the fluorescence and excitation spectra of the reaction products (**3**, **4**) in ethanol are superimposable on those of the acetyl compounds (**2a**, **2b** (Br=H)) (Fig. 2). S-Alkylthiopyrimidines (**5**, **6**) showed no fluorescence. From thses results, it is clear that the fluorescence of the reaction products (**3**, **4**) can be ascribed to the introduced fluorophore, *i. e.*, the dimethylaminonaphthalene or anilinonaphthalene moiety.

As shown in Table I and Fig. 1, the smaller the dielectric constant of the solvent, the more intense the fluorescence of the acetyl derivatives (**2a**, **3b** (Br–H)) is. Decrease of dielectric constant and increase of viscosity of the solvent generally tend to increase the fluorescence

17) H.H. Hodgson and J.H. Crook, *J. Chem. Soc.*, **1936**, 1500.
18) W. Hofmann, *Ann.*, **286**, 182 (1895).
19) H.L. Wheeler and T.B. Johnson, *Ann. Chem. J.*, **42**, 301 (1909).
20) D.G. Brown, E. Hoerger, and S.F. Mason, *J. Chem. Soc.*, **1955**, 211.
21) E. Lippert, W. Nagel, I. Seibold-Blankenstein, V. Staiger and W. Voss, *Z. Anal. Chem.*, **170**, 1 (1959).
22) C. Héléne, M. Yaniv, and J.W. Elder, *Biochem. Biophys. Res. Comm.*, **31**, 660 (1968).
23) A. Favre, *Photochem. Photobiol.*, **19**, 15 (1974) and papers cited therein.
24) H. Todoroki, Y. Nishimura, A. Hirakawa, M. Tsuboi, N. Takita, and M. Ito, 98th Annual Meeting of the Pharmaceutical Society of Japan, Okayama, April, 1978, Abstracts of Papers p. 367.

quantum yield of **2a** and **2b** (Br=H). It is worth noting that there is large difference in the fluorescent quantum yield of **2b** (Br=H) on going from an aqueous solution to a solution in a solvent with a smaller dielectric constant. Such a solvent dependency is appropriate for a reporter group. The reaction products (**3**, **4**), which possess the same fluorophore, are similar to the corresponding acetyl compounds (**2a**, **2b** (Br=H)) in their fluorescence quantum yields.

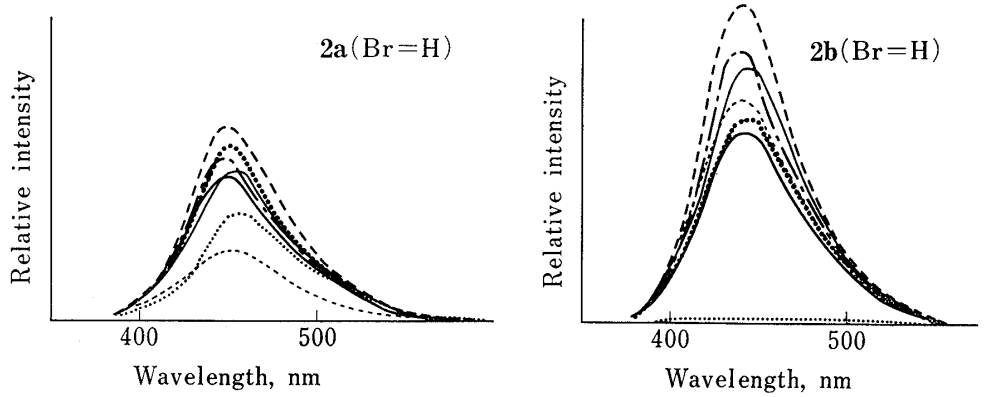

Fig. 1. Relative Fluorescence Intensities of **2a** and **2b** (Br=H) in Various Solvents

2a (Br=H) (2.00×10^{-5} M) was excited at 325 nm and **2b** (Br=H) (2.00×10^{-5} M) at 355 nm.
——: glycerol, ——: tetrahydrofuran, ——: dioxane,
----------: ethylene glycol, ——: acetonitrile, – – – –: ethanol,
----------: water.

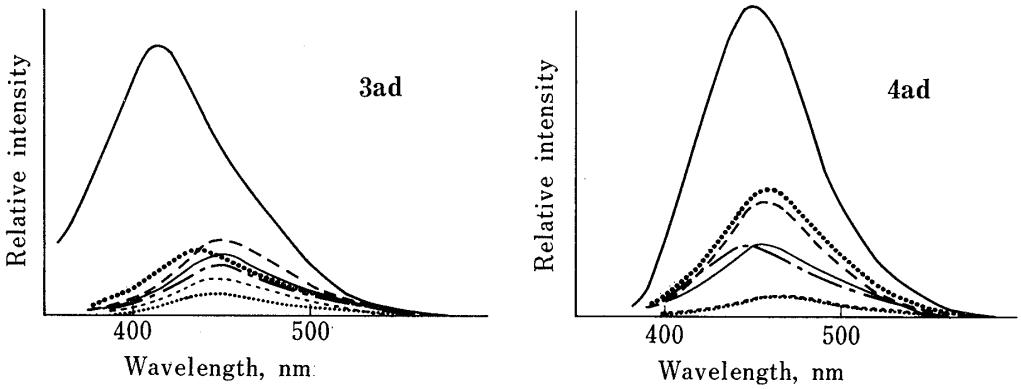

Fig. 2. Relative Fluorescence Intensities of **3ad** and **4ad** in Various Solvents

3ad (2.14×10^{-5} M) and **4ad** (2.13×10^{-5} M) were excited at 325 nm. Solvents are the same as in Fig. 1.

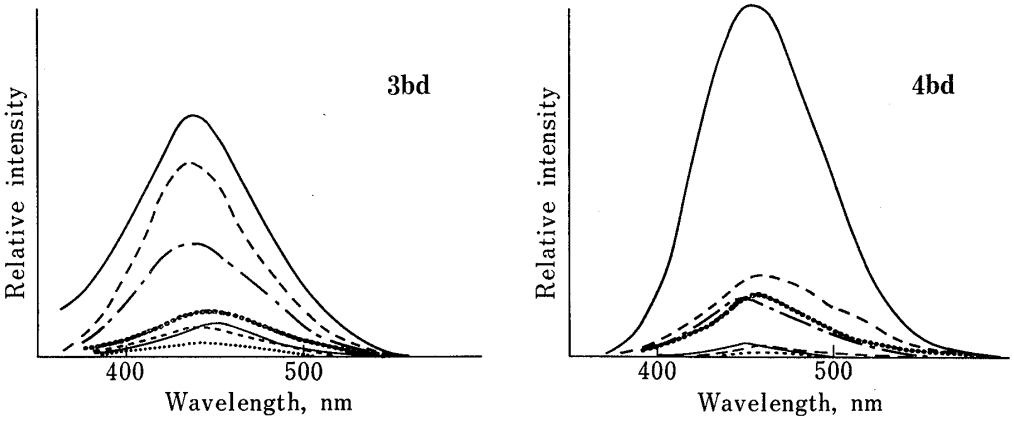

Fig. 3. Relative Fluorescence Intensities of **3bd** and **4bd** in Various Solvents

3bd (2.12×10^{-5} M) and **4bd** (2.00×10^{-5} M) were excited at 355 nm. Solvents are the same as in Fig. 1.

TABLE I. Emission Maxima and Quantum Yields (Φ) of **2a**, **2b** (Br=H), **3ad**, **4ad**, **3bd** and **4bd** in Various Solvents

Solvent	**2a**(Br=H)[a]		**2b**(Br=H)[b]		**3ad**[a]		**4ad**[b]		**3bd**[a]		**4bd**[b]	
	λ_{em}^{max} (nm)	Φ[c]	λ_{em}^{max} (nm)	Φ[c]	λ_{em}^{max} (nm)	$\Phi \times 10^{3}$[c]	λ_{em}^{max} (nm)	$\Phi \times 10^{2}$[c]	λ_{em}^{max} (nm)	$\Phi \times 10^{2}$[c]	λ_{em}^{max} (nm)	$\Phi \times 10$[c]
Dioxane	445	0.44	440	0.73	440	0.8	448	0.5	435	0.5	440	0.2
Tetrahydrofuran	445	0.35	440	0.61	440	0.4	445	0.3	435	0.3	443	0.1
Ethanol	444	0.15	440	0.51	440	0.4	448	0.2	442	0.07	440	0.03
Acetonitrile	453	0.32	447	0.58	452	0.5	460	0.3	447	0.09	450	0.05
Ethyleneglycol	448	0.38	448	0.48	445	0.5	455	0.5	448	0.1	450	0.2
Glycol	450	0.34	447	0.43	410	3.0	450	1.0	435	0.6	457	0.8
Water	460	0.25	470	0.7×10^{-2}	435	0.2	478	0.2	475	0.02	480	0.02

a) excited at 325 nm.
b) excited at 355 nm.
c) Fluorescence quantum yields were calculated by the method of Parker and Ree,[15] based on a value of 0.55 for quinine sulfate. The correction factor at each wavelength was obtained by the method of Lippert.[21]

Unexpectedly, the fluorescence intensities of the reaction products (**3**, **4**) were weaker than those of the corresponding acetyl compounds (**2a**, **2b** (Br=H)). The fluorescences of the 2-thiouridine derivatives (**4ad**, **4bd**) were relatively more intense than those of 4-thouridines (**3ad**, **3bd**). The finding that the introduction of the fluorophore into thiouridines is accompanied by a decrease of fluorescence quantum yield is suggestive of an energy transfer from the fluorophore of the reagent to the alkylthiopyrimidine moiety. The decrease of fluorescence intensities of the acetyl compounds (**2a**, **2b** (Br=H)) in the presence of alkylthiopyrimidines is illustrated in Fig. 4. Since a Stern-Volmer plot of the quenching of the fluorescence of **2a** (Br=H) with the alkylthiopyrimidine (**5**) or 4-thiouridine was linear, the fluorescence of **2a** (Br=H) is quenched by **5** or 4-thiouridine, probably by a single process. In contrast, in the case of the quenching of the fluorescence of **2a** (Br=H) by 2-thiouridine, or that of **2b** (Br=H) by 4-thiouridine, quenching does take place but not by a single process. In the quenching of **2a** (Br=H) by an alkylthiopyrimidine (**6**), and that of **2b** (Br=H) by the alkylthiopyrimidines (**5**, **6**) or 2-thiouridine, addition of the thiopyrimidine derivatives at low concentrations (2.0×10^{-4} M) did not affect the fluorescence.

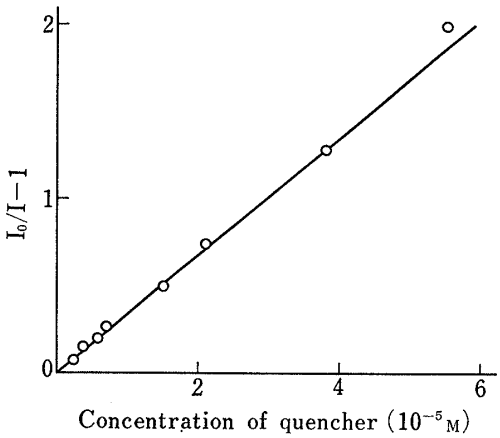

Fig. 4. Stern-Volmer Plot; Quenching of the Fluorescence of 4-(N-Dimethylamino-1-naphthyl)acetamide (**2a**, Br=H) by 1,4-Dimethyl-4-thiouracil (**5**)

Excited at 325 nm. Emission was recorded at 444 nm. The solvent was ethanol.

As shown in Table I and Figs. 2 and 3, the higher the viscosity of the solvent, the higher the fluorescence quantum yields of **3** and **4**, whereas the thiopyrimidine derivatives decrease the fluorescence of the acetyl compounds (**2a**, **2b** (Br=H)). These results suggest that the emission of the reaction products is more intense at low temperature. Table II shows the relative intensity of the emission at 77°K in liquid nitrogen. The emission intensities of the 2-thiouridine derivatives (**4ad**, **4bd**) are almost equal to those of the acetyl compounds (**3ad**, **3bd**) (**2a**, **2b** (Br=H)) at low temperature, although the emission intensities of the 4-thiouridine derivatives (**3ad**, **3bd**) are lower than those of the acetyl compounds (**2a**, **2b** (Br=H)). These

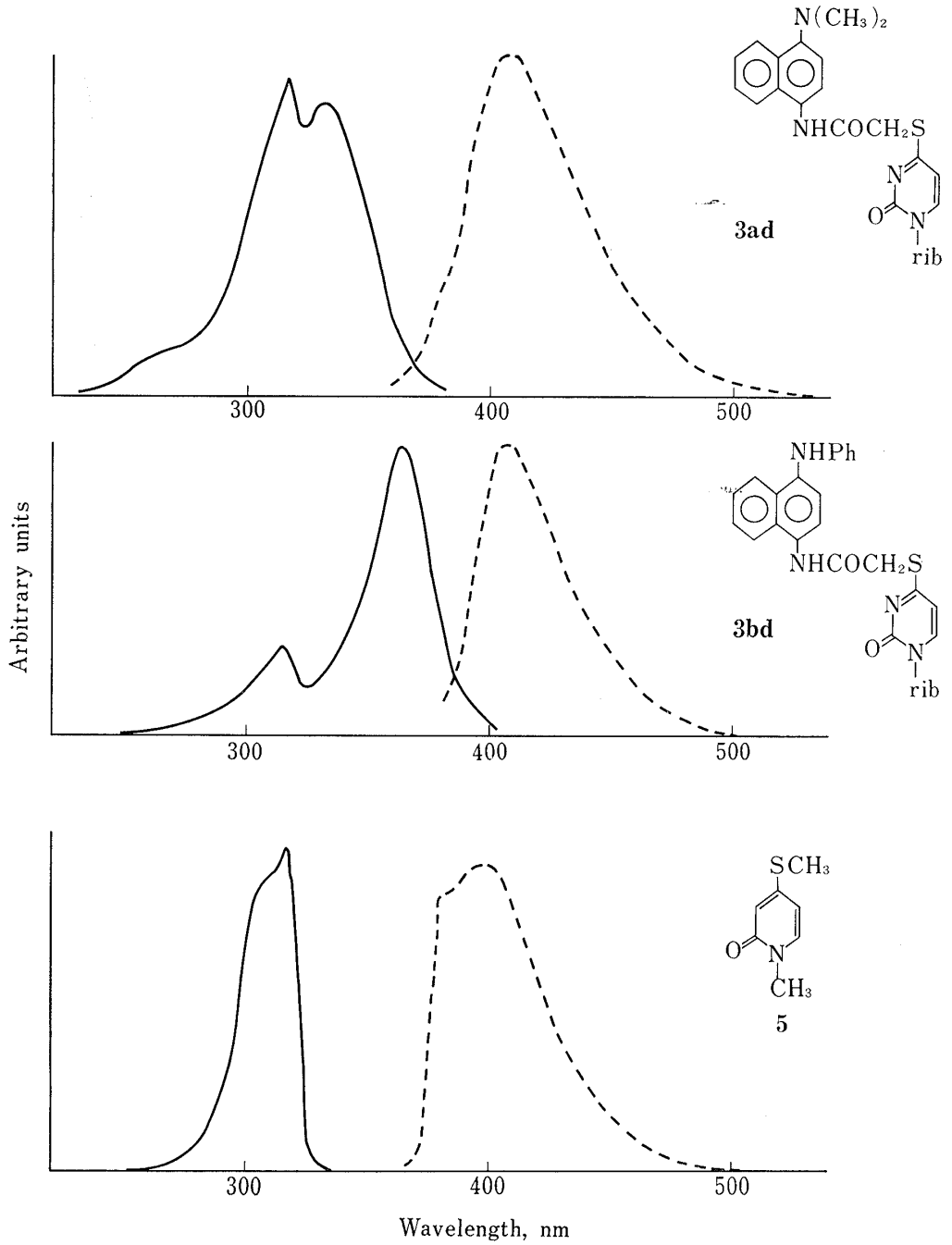

Fig. 5. Emission and Excitation Spectra at 77°K

3ad (2.14×10^{-5} M), and **3bd** (2.12×10^{-5} M) and **5** (2.00×10^{-5} M) were excited at 330, 370 and 315 nm respectively, and the excitation curves were recorded with emission at 408, 410 and 400 nm. The solvent was ethanol.

TABLE II. Relative Intensities of Emission Spectra at 77°K

Compound	Relative intensity	Compound	Relative intensity
2a (Br=H)	1.0	**2b** (Br=H)	1.0
3ad	0.13	**3bd**	0.31
4ad	0.98	**4bd**	1.0

emission spectra were concluded to be due not to phosphorescence but to fluorescence, because the emission disappeared on intermittent blocking of a light path with a chopper, and the maximum wavelengths of the emission spectra at low temperature were very close to those observed at room temperature.

New maxima at around 315 nm were observed in the excitation spectra of **3ad** and **3bd** at low temperature, as shown in Fig. 5. Since **5** had an intense phosphorescence maximum at 315 nm,[25] the excitation spectra of the reaction products (**3ad** and **3bd**) at low temperature must be additive spectra corresponding to the fluoresecence excitation of the reagent moiety and the phosphorescence excitation of the alkylthiopyrimidine moiety.

Fluorescence emission studies of nucleic acids and polynucleotides are extremely complex because of the various interactions of a number of emitting species involved.[26] In the present work, a simple fluorophore (**2**) was introduced into simple substrates, thiopyrimidines, as a model of a possible target for the modification of tRNA. Interactions of the introduced fluorophore and the modified thiopyrimidine were studied in terms of fluorescence quenching. It was also shown that the fluorescence intensity of the labeled compounds at room temperature depends strongly on the dielectric constant and viscosity of the solvent. This solvent effect indicates that the fluorescence label should be useful as a reporter group to study local conformations of these biopolymers.

Acknowledgement This work was supported in part by Grat-in-Aid for Scientific Research (No. 447120) from the Ministry of Education, Science and Culture.

25) F. Pochon, C. Balny, K.H. Schelt, and A.E. Michelson, *Biochim. Biophys. Acta*, **228**, 49 (1971).
26) *a*) M. Gueron, J. Eisinger, and K.G. Shulman, *J. Chem. Phys.*, **47**, 4077 (1967); *b*) D.T. Browne, J. Eisinger, and N.J. Leonard, *J. Am. Chem. Soc.*, **90**, 7302 (1968).