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SYNTHESIS OF 3-ARYL-7-DIETHYLAMINOCOUMARIN DERIVATIVES: REACTION WITH ISATIN AND THEIR FLUORESCENT PROPERTIES[1]

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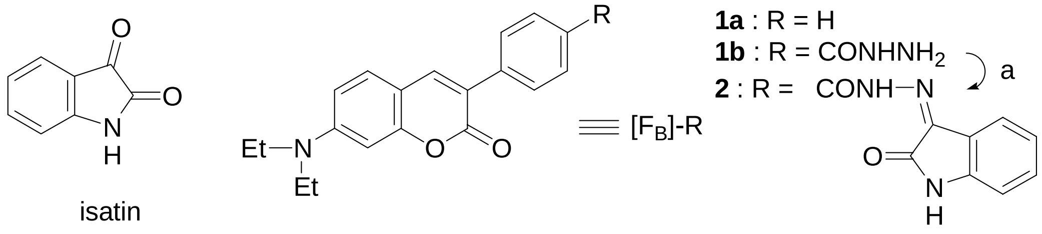
Abstract – Fluorescent derivatization reagent, 3-aryl-7-diethylaminocoumarin derivative was synthesized and reacted with isatin. The fluorescent properties were described.

Very recently, isatin has received considerable attention, because it plays physiologically important roles, for example, as a marker of stress and anxiety, an inhibitor of enzymes such as an endogenous monoamine oxidase (MAO) inhibitor, an antiseizure agent, and so forth.[2] Therefore, chemical tools for measuring isatin in various biological systems and human biological specimens are required. Although there are a few analytical methods for isatin,[3] little is known about more sensitive and practically useful analytical methods.

In the previous papers, we have shown that 3-phenyl-7-diethylaminocoumarin (**1a**) was one of the most promising candidates as a fluorophore, and developed the most sensitive and practically useful fluorescent derivatization reagents for carboxylic acids (MPAC-Br)[4, 5] and alcohols (MACB-CN[6] and

DACB-NCO[1]) in HPLC analysis. This paper deals with a fundamental study on development of fluorescent derivatization reagent, 3-aryl-7-diethylaminocoumarin having a reacting moiety toward a carbonyl group of isatin.

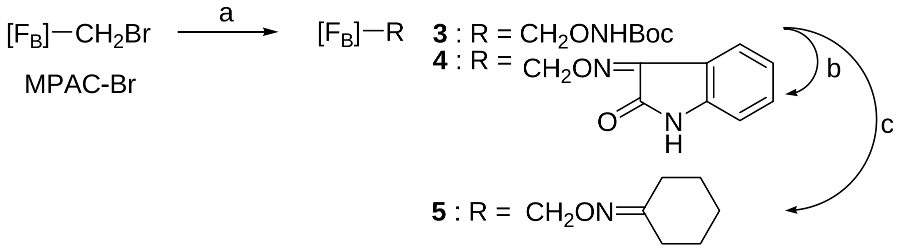


Reagents and conditions : a) isatin, TFA, MeOH, $CHCl_3$

Scheme 1

First, coumarin derivative (**1b**) with hydrazinocarbonyl group at *para*-position of phenyl group was synthesized (Scheme 1).[1] The reaction of 4-(7-diethylaminocoumarin-3-yl)benzenecarbohydrazide (DACB-HZ, **1b**) with isatin under room temperature afforded DACB-labeled isatin (**2**) in good yield. Unfortunately, DACB-labeled isatin was nonfluorescent.

Furthermore, as a reacting moiety, *N*-Boc-*O*-alkylhydroxylamine type in the place of hydrazinocarbonyl group was introduced. Derivatization reagent (**3**) of hydroxylamine type was prepared from 3-(4-bromomethylphenyl)-7-diethylamino-2*H*-1-benzopyran-2-one (MPAC-Br)[4] and *N*-*tert*-butyloxycarbonylhydroxylamine (BocNHOH). Reaction of **3** with isatin was carried out in the presence of trifluoroacetic acid. The resulting labeled isatin (**4**) fluoresced very weakly in comparison with the fluorescence intensity of reagent (**3**) (Table 1).



Reagents and conditions : a) BocNHOH, K_2CO_3, MeCN; b) isatin, TFA, CH_2Cl_2; c) cyclohexanone, TFA, CH_2Cl_2

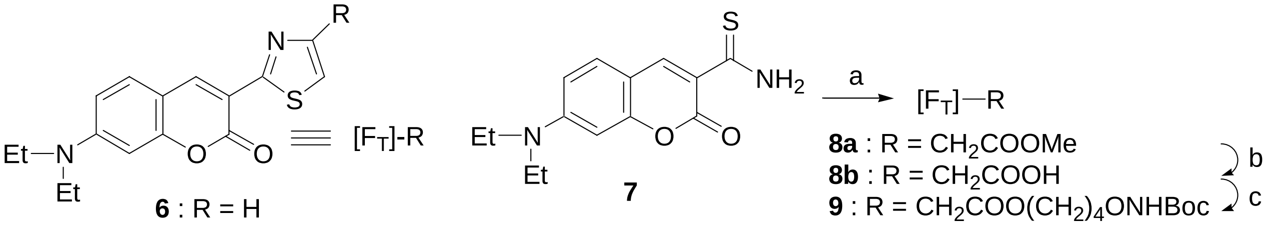
Scheme 2

In order to investigate the utility as a fluorescent labeling reagent for carbonyl compounds, cyclohexanone, which has not an absorption band at longer wavelengths, reacted with the reagent (**3**). The reaction proceeded quantitatively under mild conditions. It was suggested that *N*-Boc-hydroxylamine type is useful as a reacting moiety for carbonyl group. As expected, the resulting labeled cyclohexanone (**5**) strongly fluoresced (Table 1). This suggests that the decreased fluorescence intensity of **4** results in some interactions between fluorophore and isatin moiety.

It is known that isatin has a weak band (ε=708) at λmax 416 nm, which arises from the *n*, *π** transition.[7] If an *n*, *π** excited state is lower in energy than the lowest *π*, *π** excited state, a nonfluorescent species will result. Analogously, it seems that the significantly decreased intensity of **4** results in conversion to nonfluorescent species associated with spectral overlapping of absorption band near λmax 400 nm of 7-diethylamino-3-phenylcoumarin[4] and absorption band at longer wavelength region of isatin moiety.

Next, in order to diminish the spectral overlapping of isatin and fluorophore, fluorophore having excitation maximum at longer wavelength region, 7-diethylamino-3-thiazolylcoumarin [(7-diethylamino)coumarin-3-yl-2-thiazol-4-yl group = DACT, **6**][8] was selected.

Thiazolylcoumarin of hydroxylamine type was designed by considering the ease of synthesis. As shown in Scheme 3, the reaction of coumarin-3-carbothioamide (**7**) with methyl 4-chloroacetoacetate gave in good yield DACT-4-ester (**8a**), which was hydrolyzed to DACT-4-acetic acid (**8b**). DACT-4-acetic acid (**8b**) was esterified with 4-(*N*-Boc-aminooxy)butan-1-ol in the presence of triphenylphosphine, diisopropyl azodicarboxylate (DIAD), and imidazole, to give labeling reagent (DACT-ONH; **9**) in good yield.

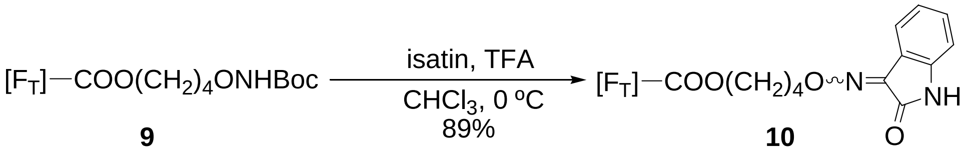


Reagents and conditions : a) methyl 4-chloroacetoacetate, K_2CO_3, DMF; b) AcOH, c. HCl, reflux; c) 4-(*N*-Boc aminooxy)butan-1-ol, DIAD, PPh_3, imidazole, THF

Scheme 3

The reaction of **9** with isatin in chloroform was carried out in the presence of trifluoroacetic acid under ice cooling (Scheme 4). The reaction proceeded rapidly to give DACT-labeled isatin (**10**) in good yield. This reactivity suggests that DACT-ONH is promising as fluorescent derivatization reagent for isatin in HPLC.

Structural assignments for **9** and **10** were made on the basis of spectral data and elemental analyses. In the NMR spectrum, two stereoisomers of **10** were obtained as a mixture in the ratio of 10-15 to 1, but the stereochemistry was not clear. The HPLC of **10** showed two peaks whose intensity ratios varied by changing of mixed solvent ratio (MeOH : H_2O) used in HPLC. Two peaks seem to be due to two conformers of main isomer, and minor isomer was not detected in HPLC under this condition.[9]



Scheme 4

Absorption and fluorescence properties of DACT-ONH (**9**) and related compounds were shown in Table 1. Both the absorption and fluorescence maxima of DACT-labeled isatin (**10**) showed the same values as those of **9**, whereas the molar absorptivity and relative fluorescence intensity decreased to 92% and 38%, respectively. Probably, this decreased intensity results in the spectral overlapping of isatin moiety and fluorophore, although it is difficult to rationalize the reasons.

In conclusion, the RFI of **10** was 50% in comparison with one of **1a**, whose related reagents MPAC-Br,[4, 5] DACB-CN,[6] and DACB-NCO[1] were used for the trace analysis by HPLC at a femtomol level. Therefore, this reagent (**9**) might be used as derivatization reagent for isatin although the question for sensitivity still remains. In addition, DACT-ONH (**9**) could serve as a fluorescent labeled reagent for

	Absorption[a)]		Fluorescence[b)]		
Compound	λmax (nm)	ε	Ex (nm)	F·λmax (nm)	RFI[c)]
3	404	36,000	404	466	1.17
4	405	38,900	405	476	0.02
5	402	36.900	402	466	1.12
8a	435	46,100	435	482	1.30
9	437	44,900	437	483	1.34
10	437	41,500	437	483	0.51
1a	398	34,200	398	478	1.00

a) Concentration in $CHCl_3 = 1.5 \times 10^{-5}$
b) Concentration in $CHCl_3 = 3.5 \times 10^{-6}$
c) Relative fluorescence intensity : the fluorescence intensity of **1a** is arbitrarily taken as 1.00.

Table 1

carbonyl compounds since the reagent is superior in respect of stability, high sensitivity, and reactivity.

EXPERIMENTAL

All melting points were determined on a Yamato melting point apparatus (model MP-2) and are uncorrected. NMR spectra were taken on JEOL JNM-LA-300 spectrometers. Chemical shifts are reported in ppm (δ) relative to TMS (0.0 ppm) as an internal standard. MS spectra were obtained on JEOL JMS-HX110 and JEOL JMS-700TZ. Column chromatography was conducted using silica gel (Merck, Kieselgel 60, 70-230 mesh). Absorption and fluorescence properties were obtained using the instruments and methods described in a previous paper.[8] Compounds (**1a**)[4] and (**1b**)[1] ware prepared according to the procedures described in previous papers.

4-(7-Diethylamino-2-oxochroman-3-yl)-benzoic acid (2-oxo-1,2-dihydroindol-3-ylidene)hydrazide (2)

A mixture of **1b** (70 mg, 0.2 mmol), isatin (29 mg, 0.2 mmol), and a drop of trifluoroacetic acid in methanol (20 mL) and chloroform (5 mL) was stirred at rt for 1 h. The precipitates of **2** were collected by suction filter to give yellow crystals (91 mg) as almost pure form in ^{1}H-NMR spectrum. The recrystallization of the crude product from DMSO gave yellow needles (67 mg, 70%). mp >300 ºC. ^{1}H NMR (DMSO-d_6) δ = 13.96 (br s, 1H, NH), 11.37 (br s, 1 H, NH), 8.24 (s, 1), 7.96 (d, 2 H, *J* = 8.3 Hz), 7.92 (d, 2 H, *J* = 8.3 Hz), 7.61 (d, 1 H, *J* = 7.3 Hz), 7.53 (d, 1 H, *J* = 8.8 Hz), 7.11 (t, 1 H, *J* = 7.3 Hz), 6.96 (d, 1 H, *J* = 7.8 Hz), 6.75 (dd, 1 H, *J* = 2.0 and 8.8 Hz), 6.57 (d, 1 H, *J* = 2.0 Hz), 3.45 (q, 4 H, *J* = 6.8 Hz), 1.14 (t, 6 H, *J* = 6.8 Hz). ^{13}C NMR ($CDCl_3$) δ = 163.1, 160.2, 156.1, 150.9, 142.4, 142.4, 140.1, 131.8, 130.4, 130.1, 128.3, 127.3, 122.8, 121.0, 119.8, 117.0, 111.2, 109.4, 108.3, 96.1, 44.1, 12.3. *Anal.* Calcd for $C_{28}H_{24}N_4O_4$: C, 69.99; H, 5.03; N, 11.66. Found: C, 69.77; H, 5.00; N, 11.65. FAB-LRMS *m/z* 481[MH^+].

3-{4-[*N*-(1-*tert*-Butyloxycarbonyl)aminooxymethyl]phenyl}-7-diethylaminochromen-2-one (3)

To a suspension of MPAC-Br (403 mg, 1.2 mmol) and *N*-hydroxycarbamic acid *tert*-butyl ester (210 mg, 1.5 mmol) in acetonitrile (30 mL) was added anhydrous potassium carbonate (686 mg, 4.9 mmol), the resulting mixture was stirred at rt for 28 h. The mixture was partitioned between water (50 mL) and chloroform (50 mL). The organic phase was washed with brine (30 mL), dried over Na_2SO_4, and the solvents were removed under reduced pressure. The residue was purified by column chromatography (hexane : ethyl acetate = 2 : 1) to give **3** (339 mg, 63%) as yellow needles. mp 132-133 ºC (hexane-AcOEt). ^{1}H NMR ($CDCl_3$) δ = 7.71-7.69 (m, 3 H), 7.44-7.27 (m, 3 H), 7.15 (s, 1 H), 6.59 (dd, 1 H, J = 2.3 and 8.8 Hz), 6.52 (d, 1 H, J = 2.3 Hz), 4.88 (s, 2 H), 3.42 (q, 4 H, J = 7.0 Hz), 1.49 (s, 9 H), 1.22 (t, 6 H, J = 7.0 Hz). ^{13}C NMR ($CDCl_3$) δ = 161.5, 156.6, 156.2, 150.5, 140.6, 136.0, 135.1, 129.0, 128.9, 128.3, 120.2, 109.0, 108.9, 97.0, 81.6, 78.0, 44.8, 28.2, 12.4. *Anal*. Calcd for $C_{25}H_{30}N_2O_5$: C, 68.47; H, 6.90; N, 6.39. Found: C, 68.43; H, 6.94; N, 6.35. FAB-LRMS *m/z* (%) 461 [MNa^+] (2.6). FAB-HRMS Calcd for $C_{25}H_{30}N_2O_5Na$ 461.2052. Found 461.2082.

1*H*-Indole-2,3-dione 3-{*O*-[4-(7-diethylamino-2-oxo-2*H*-chromen-3-yl)benzyl]oxime} (4)

To a solution of **3** (82 mg, 0.19 mmol) and isatin (28 mg, 0.19 mmol) in CH_2Cl_2 (2.0 mL) was added trifluoroacetic acid (1.0 mL, 13.0 mmol) at 0 ºC, and the mixture was stirred for 1 h. The mixture was poured into saturated $NaHCO_3$ (10 mL) and extracted with $CHCl_3$ (10 mL). The organic phase was washed with brine (5 mL), dried over Na_2SO_4, and the solvents were removed under reduced pressure. The residue was purified by column chromatography (hexane : ethyl acetate = 1 : 1) to give **4** (55 mg, 62%) as yellow needles. mp 229-230 ºC (hexane-AcOEt). ^{1}H NMR ($CDCl_3$) δ = 8.28 (br s, NH), 7.94 (d, 1 H, J = 7.5 Hz), 7.75-7.70 (m, 3 H), 7.52-7.31 (m, 4 H), 7.03 (m, 1 H), 6.88 (d, 1 H, J = 7.7 Hz), 6.60 (dd, 1 H, J = 2.5 and 8.8 Hz), 6.54 (d, 1 H, J = 2.5 Hz), 5.55 (s, 2 H), 3.43 (q, 4 H, J = 7.1 Hz), 1.23 (t, 6 H, J = 7.1 Hz). ^{13}C NMR (DMSO-d_6) δ = 163.6, 160.4, 155.8, 150.5, 144.4, 143.3, 141.3, 135.8, 135.6, 133.1, 129.7, 128.1, 128.0, 127.5, 122.3, 118.3, 115.6, 110.5, 109.1, 108.4, 96.1, 77.7, 40.1, 12.3. *Anal*. Calcd for $C_{28}H_{25}N_3O_4$: C, 71.93; H, 5.39; N, 8.99. Found: C, 71.83; H, 5.57; N, 8.92. FAB-LRMS *m/z* (%) 490 [MNa^+] (1.5). FAB-HRMS Calcd for $C_{28}H_{25}N_3O_4Na$ 490.1743. Found 490.1756.

3-(4-Cyclohexylideneaminooxymethylphenyl)-7-diethylaminochromen-2-one (5)

To a solution of **3** (35 mg, 0.08 mmol) and cyclohexanone (9 μL, 0.08 mmol) in CH_2Cl_2 (1.0 mL) was added trifluoroacetic acid (1.0 mL, 13.0 mmol) at 0 ºC, and the mixture was stirred for 1 h. The mixture was poured into saturated $NaHCO_3$ (5 mL) and extracted with ethyl acetate (10 mL). The organic phase was washed with brine (5 mL), dried over Na_2SO_4, and the solvents were removed under reduced pressure. The residue was purified by column chromatography (hexane : ethyl acetate = 2 : 1) to give **5** (31 mg, 93%) as yellow needles. mp 104-105 ºC (hexane-AcOEt). ^{1}H NMR ($CDCl_3$) δ = 7.69-7.66 (m, 3 H), 7.41-7.26 (m, 3 H), 6.59 (dd, 1 H, *J* = 2.3 and 8.8 Hz), 6.53 (d, 1 H, *J* = 2.3 Hz), 5.08 (s, 2 H), 3.43 (q, 4 H, *J* = 7.1 Hz), 2.51 (m, 2 H), 2.21 (m, 2 H), 1.68-1.58 (m, 6 H), 1.22 (t, 6 H, *J* = 7.1 Hz). ^{13}C NMR($CDCl_3$) δ = 161.7, 160.9, 156.2, 150.5, 140.4, 137.9, 135.1, 128.9, 128.1, 127.9, 120.7, 109.1, 108.9, 97.2, 74.8, 44.9, 32.2, 27.0, 25.8, 25.7, 25.5, 12.5. *Anal*. Calcd for $C_{26}H_{30}N_2O_3$: C, 74.61; H, 7.22; N, 6.69. Found: C, 74.52; H, 7.22; N, 6.61. EI-LHMS *m/z* (%) 418 [M$^+$] (26.9). FAB-HRMS Calcd for $C_{26}H_{30}N_2O_3$ Na 441.2154. Found 441.2166.

Methyl [2-(7-diethylamino-2-oxo-2*H*-chromen-3-yl)thiazol-4-yl]acetate (8a)

To a suspension of **7**[8] (600 mg, 2.2 mmol) and anhydrous potassium carbonate (1.9 g, 13.7 mmol) in DMF (10 mL) was added methyl 4-chloroacetoacetate (780 μL, 6.5 mmol) at rt, the mixture was stirred for 22 h. The mixture was diluted with ethyl acetate (850 mL) and washed with water (30 mL x 2) and brine (30 mL). The organic phase was dried over Na_2SO_4, and the solvents were removed under reduced pressure. The residue was purified by column chromatography (hexane : ethyl acetate = 2 : 1) to give **8a** (572 mg, 70%) as yellow needles. mp 143-145 ºC (hexane-AcOEt). ^{1}H NMR ($CDCl_3$) δ = 8.67 (s, 1 H), 7.41 (d, 1 H, *J* = 8.9 Hz), 7.23 (s, 1 H), 6.63 (dd, 1 H, *J* = 2.4 and 8.9 Hz), 6.53 (d, 1 H, *J* = 2.4 Hz), 3.90 (s, 2 H), 3.76 (s, 3 H), 3.43 (q, 4 H, *J* = 7.2 Hz), 1.23 (t, 6 H, *J* = 7.2 Hz). ^{13}C NMR ($CDCl_3$) δ = 170.9, 160.8, 160.6, 156.4, 151.5, 148.2, 139.9, 130.2, 117.5, 112.7, 109.7, 108.5, 96.9, 52.1, 44.9, 37.0, 12.4. *Anal*. Calcd for $C_{19}H_{20}N_2O_4S$: C, 61.27; H, 5.41; N, 7.52. Found: C, 61.43; H, 5.51; N, 7.52. FAB-LRMS *m/z* (%) 395 [MNa$^+$] (1.3). FAB-HRMS Calcd for $C_{19}H_{20}N_2O_4S$ Na 395.1041. Found

395.1060.

4-*tert*-Butyloxycarbonylaminooxybutyl [2-(7-diethylamino-2-oxo-2*H*-chromen-3-yl)thiazol-4-yl]-acetate (9)

A solution of **8a** (504 mg, 1.4 mmol) in acetic acid (5.0 mL) and conc. HCl (5.0 mL) was refluxed for 19 h. The solvents were removed under reduced pressure, and the residue was co-evaporated with 1,4-dioxane to give crude **8b**. To a suspension of crude **8b**, 4-(*N*-Boc-aminooxy)butan-1-ol (289 mg, 1.4 mmol), and triphenylphophine (747 mg, 2.8 mmol) in THF (4.0 mL) was added a solution of DIAD (933 μL, 2.8 mmol) in THF (4.0 mL) at rt, and the mixture was stirred for 4 h. The mixture was diluted with ethyl acetate (100 mL) and washed with water (50 mL) and brine (50 mL). The organic phase was dried over Na_2SO_4, and the solvents were removed under reduced pressure. The residue was purified by column chromatography (hexane : ethyl acetate = 1 : 1) to give **10** (425 mg, 78%) as orange crystals. mp 102-103 ºC (hexane-AcOEt). ^{1}H NMR ($CDCl_3$) δ = 8.69 (s, 1 H), 7.44 (d, 1 H, *J* = 9.0 Hz), 7.23 (s, 1 H), 7.16 (br s, 1 H, NH), 6.55 (dd, 1 H, *J* = 2.4 and 9.0 Hz), 6.55 (d, 1 H, *J* = 2.4 Hz), 4.19 (t, 2 H, *J* = 6.1 Hz), 3.88-3.85 (m, 4 H), 3.45 (q, 4 H, *J* = 7.1 Hz), 1.78-1.69 (m, 4 H), 1.47 (s, 9 H), 1.24 (t, 6 H, *J* = 7.1 Hz). *Anal*. Calcd for $C_{27}H_{35}N_3O_7S$: C, 59.43; H, 6.47; N, 7.70. Found: C, 59.18; H, 6.34; N, 7.91. FAB-LRMS *m/z* (%) 395 [M^+] (4.5). FAB-HRMS Calcd for $C_{27}H_{35}N_3O_7S$ 545.2196. Found 545.2171.

4-(2-Oxo-1,2-dihydroindol-3-ylideneaminooxy)butyl [2-(7-diethylamino-2-oxo-2*H*-chromen-3-yl)-thiazol-4-yl]acetate (10)

To a solution of **9** (66 mg, 0.13 mmol) and isatin (20 mg, 0.13 mmol) in CH_2Cl_2 (1.0 mL) was added trifluoroacetic acid (0.5 mL, 6.5 mmol) at 0 ºC, and the mixture was stirred for 1 h. The mixture was poured into saturated $NaHCO_3$ (5 mL) and extracted with $CHCl_3$ (10 mL). The organic phase was washed with brine (5 mL), dried over Na_2SO_4, and the solvents were removed under reduced pressure. The residue was purified by column chromatography (hexane : ethyl acetate = 3 : 7) to give **10** (66 mg, 89%) as yellow crystals. mp 163-165 ºC (hexane-AcOEt). ^{1}H NMR ($CDCl_3$) δ = 8.59 (s, 1 H), 8.51

(br s, 1 H, NH), 7.79 (d, 1 H, *J* = 7.5 Hz), 7.33-7.15 (m, 3 H), 6.96 (t, 1 H, *J* = 7.7 Hz), 6.79 (d, 1 H, *J* = 7.5 Hz), 7.55 (dd, 1 H, *J* = 2.4 and 8.8 Hz), 6.43 (d, 1 H, *J* = 2.4 Hz), 4.45 (t, 2 H, *J* = 6.2 Hz), 4.16 (t, 2 H, *J* = 6.2 Hz), 3.82 (s, 2 H), 3.36 (q, 4 H, *J* = 7.0 Hz), 1.88-1.74 (m, 4 H), 1.16 (t, 6 H, *J* = 7.0 Hz). ^{13}C NMR ($CDCl_3$) δ = 170.5, 165.8, 160.9, 160.8, 156.4, 151.5, 148.3, 144.0, 141.8, 140.0, 132.6, 130.3, 127.7, 123.0, 117.5, 116.1, 112.6, 110.7, 109.7, 108.4, 96.8, 16.8, 64.5, 44.9, 37.3, 25.8, 25.1, 12.4. *Anal*. Calcd for $C_{30}H_{30}N_4O_6S \cdot 0.1H_2O$: C, 62.51; H, 5.28; N, 9.72. Found: C, 62.25; H, 5.38; N, 9.89. FAB-LRMS *m/z* (%) 575 [MH^+] (1.7). FAB-HRMS Calcd for $C_{30}H_{31}N_4O_6S$ 575.1964. Found 575.1942.

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REFERENCES AND NOTES

1. Fluorescent Labeling Reagents. Part 5. Part 4. H. Takechi, Y. Goto, H. Takahashi, and M. Machida, *J. Heterocycl. Chem.*, 2001, **38**, 333.
2. N. Hamaue, M. Minami, M. Hirafuji, M. Terado, M. Machida, N. Yamazaki, M. Yoshioka, A. Ogata, and K. Tashiro, *CNS Drug Reviews*, 1999, **5**, 331.
3. a) K. Mawatari, M. Segawa, R. Masatsuka, Y. Hanawa, F. Iinuma, and M. Watanabe, *Analyst*, 2001, **126**, 33. b) S. Manabe, Q. Gao, J. Yuan, T. Takahashi, and A. Ueki, *J. Chromatogr. B*, 1997, **691**, 197.
4. H. Takechi, S. Kamada, and M. Machida, *Chem. Pharm. Bull.*, 1996, **44**, 763.
5. T. Kurosawa, H. Sato, M. Sato, H. Takechi, M. Machida, and M. Tohma, *J. Pharm. Biomed. Anal.*, 1997, **15**, 1375.
6. H. Takechi, Y. Goto, and M. Machida, *Chem. Pharm. Bull.*, 1998, **46**, 159.

7. V. Galasso and G. C. Pappalardo, *J. Chem. Soc., Perkin Trans. II*, 1976, 574.

8. H. Takechi, Y. Oda, N. Nishizono, K. Oda, and M. Machida, *Chem. Pharm. Bull.*, 2000, **48**, 1702.

9. The column was Inertsil ODS-2 (150 x 4.6 mm i.d.; particle size, 5 μm; Gasukuro Kogyo, Tokyo). The concentration of **10** : 1.5 x 10^{-10} M. The retention times of two peaks: 5.8 and 7.4 min.