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Synthesis of New Reagents for the Fluorescence Derivatisation of Thiols and Alcohols*

Synthesis of Fluorescent Dyes, 15[1]

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The synthesis of two selective thiol derivatisation reagents (**3**, **4b**) is described, starting from readily available 2-(4-aminophenyl)-6-methyl-benzothiazole (**1**). The isocyanate **6**, being a known alcohol derivatisation reagent, is obtained from **1** in a two step synthesis, in which the usual isocyanate synthesis using phosgen is avoided.

(Keywords: Alcohol reagent; Derivatisation; Fluorescence; Thiol reagent)

Synthese von neuen Reagentien zur Fluoreszenzderivatisierung von Thiolen und Alkoholen

Die Synthese zweier selektiver Thiolderivatisierungsreagentien (**3**, **4b**), ausgehend vom leicht zugänglichen 2-(4-Aminophenyl)-6-methylbenzthiazol (**1**), wird beschrieben. Das Isocyanat **6**, ein bekanntes Reagens zur Derivatisierung von Alkoholen, wird ebenfalls aus Verbindung **1** in zweistufiger Synthese erhalten, bei welcher die Isocyanatsynthese mit Hilfe von Phosgen umgangen wird.

Introduction

Thiol derivatisation reagents play an important role in environmental analytical chemistry due to the widespread occurence of thiolic compounds in industrial and urban wastes. Their use allows a highly sensitive and fairly selective determination of thiolic groups. In a

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This work is dedicated to Prof. Dr. *Erwin Schauenstein* on the occasion of his 65th birthday.

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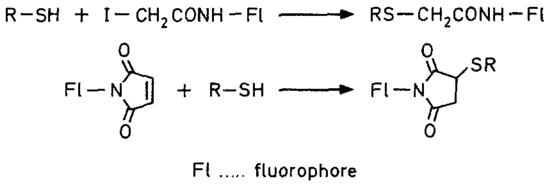
similar way, the detection of thiol groups by fluorescence techniques is a useful method to locate and analyse SH-groups in biological material[2,3].

A rather generally applicable method involves the derivatisation of non-fluorescent thiols using fluorescent iodoacetamides to give the corresponding conjugate thioethers. Reaction of thiols with non-fluorescent N-sustituted maleinimides to give highly fluorescent addition products under mild conditions (*pH* about 7.4)[2] seems to be a more elegant procedure. The reaction sequences are shown in Scheme **1**, wherein *Fl* means a(potential) fluorophor.

Scheme 1



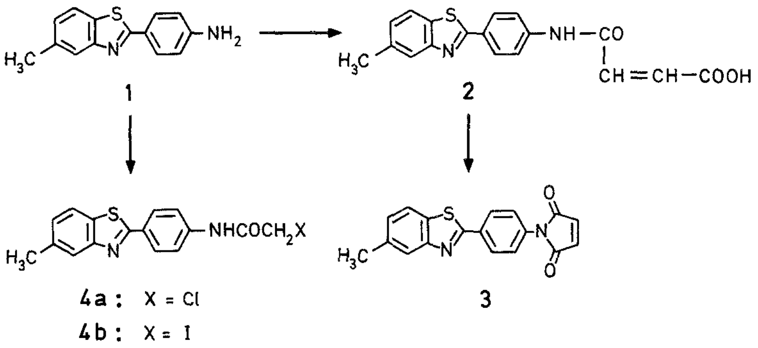
Unfortunately, most commercially available reagents of the above type are rather expensive[4]. We thought, that 2-(4-aminophenyl)-6-methylbenzothiazole (**1**), a strongly fluorescent system readily available from *p*-toluidine and elemental sulfur[5,6], might be a useful starting material for new reagents.

Results and Discussion

Compound **1**, the synthesis of which was modified, reacts with maleic acid anhydride to give the *cis*-monoamide **2**; its configuration was confirmed by ^{1}H-NMR data. Cyclisation of **2** with acetic acid anhydride yields the maleinimide **3**. In contrast to **2**, compound **3** is practically non-fluorescent in organic solvents, but forms highly fluorescent addition products with both aliphatic and aromatic thiols. They are maximally excited by light of wavelength 330 nm, and exhibit maximum fluorescence at about 390 nm.

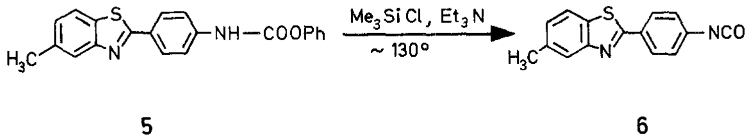
The fact, that maleinimide **3** is non-fluorescent, while both the precursor **2** and the addition compounds with thiols are fluorescent, can be interpreted in terms of energy separation of the first excited singlet and triplet state[7]. In **3**, the energy of the excited triplet state is so close to the energy of the excited singlet state, that intersystem crossing (isc) becomes effective to a very high degree.

Scheme 2



The iodoacetyl derivative **4b** was prepared by reaction of **1** with chloroacetylchloride, followed by halogen exchange of the resulting **4a** with potassium iodide to give the desired **4b** (Scheme 2). Despite of the presence of an iodine atom with its known adverse effect on fluorescence intensity (heavy atom effect), **4b** exhibits strong fluorescence. Like **3**, compound **4b** forms fluorescent conjugates with thiols under mild experimental conditions.

Scheme 3



Both **3** and **4b** react selectively with thiols and do not attack alcoholic or phenolic hydroxy groups. Table **1** summarizes the R_f values of the conjugates of compounds **3** and **4b** with some frequently encountered thiols. The reagent may likewise be applied to biogenic thiols like glutathione.

To derivatise alcohols, a more reactive function is required, such as an isocyanate group. To avoid working with phosgen, which is the usual reagent to transform amines in to isocyanates[8], we have applied a two step synthesis via an urethane intermediate[10,11]. Compound **1** was reacted at room temperature with phenyl chloroformate to give the urethane **5** which after silylation and thermal elimination of phenyltrimethylether afforded the reagent **6** (s. Scheme 3) by a less tedious procedure than the reported ones[8,9,12]. **6** is a useful reagent for the derivatisation of pharmaceutical drugs containing alcohol or amino groups[12].

Table 1. *R_f-values of* **3** *and* **4 b** *and their conjugates with selected thiols on Merck Kieselgel* 60 F_{254} tlc *plates*. 10 µl of either reagent (conc. 10^{-2} *M* in methanol) was spotted on the plate and 15 µl of the thiol solution (ca. 10^{-2} *M* in methanol) were added. Reaction was accomplished by warming to 60 °C for at least one minute. Development solvent cyclohexane/isoamylalcohol (1:3, *v*/*v*) for **3** and chloroform/ethylacetate (5:1, *v*/*v*) for **4 b**

Reagent	R_f-values					
	without thiol added	*n*-*Bu*SH	2-*Bu*SH	*Ph*-CH_2SH	*p*-Thiocresol	*p*-Chlorothiophenol
3	0.45	0.92	0.90	0.96	0.82	0.83[a]
4 b	0.38	0.68	0.72[a]	0.65	0.66	0.53

[a] Sample prepared by mixing the two stock solutions and leaving at 60 °C for at least 20 min.

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Experimental

The following instruments were used for the spectra: IR: Perkin Elmer 200; UV: Perkin Elmer Lamda 5; Fluorescence: Aminco SPF 500 (uncorrected); ^1H-NMR; Varian EM 360; MS: Varian Mat Gnom. The melting points are uncorrected.

2-(4-Aminophenyl)-6-methyl-benzothiazole (**1**)

100.0 g (0.9 mol) of *p*-toluidine and 30.0 g (0.9 mol) of powdered sulphur were heated for 18 h at 180 °C and then for 6 h at 220 °C. After cooling a mixture of 250 ml benzene and 250 ml of petrol ether (bp. 40–60 °C) was poured onto the warm molten mass. After cooling the resulting precipitate was filtered and dried at 100 °C. The crude product was distilled at reduced pressure (bp. 220 °C/0.02 Torr) and recrystallised from isopropanol to give yellow needles. Mp. 194 °C. Lit. mp.[5,9] 191 °C, 199 °C. Yield ca. 19 g (15%) of the purified product.

N-[4-(7-Methyl-2-benzothiazolyl)phenyl]-cis-butandioic acid monoamide (**2**)

3.0 g (0.03 mol) of maleic anhydride, dissolved in 30 ml of warm benzene was added to a solution of 2.4 g (0.01 mol) of compound **1** in 70 ml of warm acetone, resulting in an immediate precipitation of **2**. The crystals were collected by filtration and were analytically pure. Yield 3.3 g (97%), mp. 241–245 °C (dec.).

IR (KBr): 3290, 1705 and 1625 cm^{-1}.

^{1}H-NMR (NaOD): $\delta = 2.42$ (s, 3 H); 6.40 (dd, $J = 6$ Hz, 2 H); 7.31 (d, $J = 2$ Hz, 1 H); 8.10-8.80 (m, 6 H); 10.60 (s, 1 H).

MS (70 eV): 338 (M^+, 9%), 240 (100%).

$C_{18}H_{14}N_2O_3S$ (338.2). Calc. C 63.89 H 4.17 N 8.28 S 9.47.
Found C 63.88 H 4.23 N 8.19 S 9.59.

2-(4-Maleinimidophenyl)-6-methyl-benzothiazole (**3**)

1.0 g (3 mmol) of compound **2** and 1.0 g of sodium acetate were refluxed in 50 ml acetic acid anhydride for 1 h. After cooling to room temperature the crystals which had separated from the solution were collected. The mother liquor was treated with ice water to cause some more **3** to precipitate. Small yellow needles from toluene. Yield 0.85 g (89%), mp. 248 °C.

UV (*Me*OH): $\lambda_{max} = 329$ nm ($\varepsilon = 34400$).

IR (KBr): 3109, 1703, 1605, 1520, 1490 and 1400 cm^{-1}.

^{1}H-NMR ($CDCl_3$): $\delta = 2.50$ (s, 3 H), 6.84 (s, 2 H); 7.20-8.20 (m, 7 H).

MS (70 eV): 320 (M^+, 100%).

$C_{18}H_{12}N_2O_2S$ (320.2). Calc. C 67.48 H 3.78 N 8.74 S 10.01.
Found C 67.58 H 3.74 N 8.79 S 10.20.

2-(4-Chloroacetylaminophenyl)-6-methyl-benzothiazole (**4a**)

To 2.0 g (8.4 mmol) of compound **1** in 25 ml of dry *DMF*, 1.3 g (12.6 mmol) of triethylamine was added. Then a solution of 1.4 g (12.6 mmol) of chloroacetylchloride in 5 ml of dry *DMF* was added dropwise under cooling with ice water. After 3 h at room temperature the solvent was evaporated. Addition of water precipitated a greenish product, which was filtered and dried. Crystallisation from chlorobenzene gave almost colourless crystals. Yield 2.65 g (100%), mp. 221 °C.

UV (*Me*OH): $\lambda_{max} = 327$ nm ($\varepsilon = 35000$).

IR (KBr): 3325, 1665, 1605, 1590, 1530 and 1510 cm^{-1}.

^{1}H-NMR (*DMSO-d$_6$*): $\delta = 2.43$ (s, 3 H); 4.26 (s, 2 H); 7.00-8.20 (m, 7 H); 10.51 (br. s, 1 H).

MS (70 eV): 316 (M^+, 100%).

$C_{16}H_{13}ClN_2OS$ (316.8). Calc. C 60.66 H 4.14 Cl 11.19 N 8.84 S 10.12.
Found C 60.42 H 3.94 Cl 11.39 N 8.73 S 10.26.

2-(4-Iodoacetylaminophenyl)-6-methyl-benzothiazole (**4b**)

To 1.0 g (3.2 mmol) of compound **4a**, dissolved in 80 ml of acetone, a solution of 0.66 g (4.0 mmol) potassium iodide in a mixture of 80 ml acetone and 20 ml methanol was added. After stirring overnight the reaction mixture was filtered and evaporated to dryness. Crystallisation of the residue from toluene gave a colourless product of mp. 216 °C. Yield 0.96 g (74%).

UV (*Me*OH): $\lambda_{max} = 330$ nm ($\varepsilon = 37900$).

Fluorescence (*Me*OH): $\lambda_{max} = 385$ nm (excitation wavelength 340 nm).

IR (KBr): 3310, 1668, 1585 and 1512 cm^{-1}.

^{1}H-NMR (*DMSO-d$_6$*): $\delta = 2.42$ (s, 3 H); 3.88 (s, 2 H); 7.20-8.20 (m, 7 H); 10.58 (br. s, 1 H).

MS (70 eV): 408 (M^+, 12%), 240 (100%).

$C_{16}H_{13}IN_2OS$ (408.3). Calc. C 47.07 H 3.21 N 6.86.
Found C 47.34 H 3.14 N 6.86.

2-(4-Phenoxycarbonylaminophenyl)-6-methyl-benzothiazole (**5**)

To a solution of 2.0 g (8.3 mmol) of compound **1** in 5 ml of *DMF* and 0.5 ml of pyridine, 1.9 g (12.1 mmol) of phenyl chloroformate was added dropwise under cooling and stirring. After standing for several hours the solvent was removed in vacuo. The residue was treated with 10 ml of ether, filtered and washed with a little ether and then with cold water to remove ionic material. Crystallisation from chlorobenzene (under addition of charcoal) gave almost colourless plates of mp. 210-211 °C. Yield 2.3 g (77%).

IR (KBr): 3320, 1716, 1610 and 1539 cm^{-1}.

^1H-NMR (*DMSO*-d_6): $\delta = 2.48$ (s, 3 H); 7.20-8.20 (m, 12 H); 10.62 (br. s, 1 H).

MS (70 eV): 360 (M^+, 6%); 266 (100%).

$C_{21}H_{16}N_2O_2S$ (360.4). Calc. C 70.04 H 4.46 N 7.76 S 8.89.
Found C 70.29 H 4.55 N 7.69 S 8.92.

2-(4-Isocyanatophenyl)-6-methyl-benzothiazole (**6**)

A mixture of 1.0 g (2.7 mmol) of compound **5**, 0.33 g (3.2 mmol) of triethylamine and 0.35 g (3.2 mmol) of trimethylchlorosilane was refluxed in 40 ml chlorobenzene for 2.5 h. After filtering off triethylamine hydrochloride the solvent was evaporated. The residue was boiled with 70 ml of cyclohexane, filtered and then allowed to cool slowly. The product precipitated in small crystals of mp. 142 °C. Lit. mp.[8,9]. 143-144 °C and 139 °C. Yield 0.42 g (57%).

IR (KBr): 2280, 1635, 1600 and 1545 cm^{-1}.

MS (70 eV): 266 (M^+, 100%).

$C_{15}H_{10}N_2OS$ (266.3). Calc. C 67.64 H 3.79 N 10.52.
Found C 67.54 H 3.85 N 10.70.

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