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Synthesis of thiacyanine dyes containing coumarin moieties at benzothiazole rings

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New bischromophoric dyes (thiacarbocyanine and thiadicarbocyanine) containing coumarin moieties at each of the two benzothiazole systems, as well as monomethinecyanine containing coumarin moiety at only one heterocyclic system, manifest significant bathochromic shift compared to relative coumarin-free dyes.

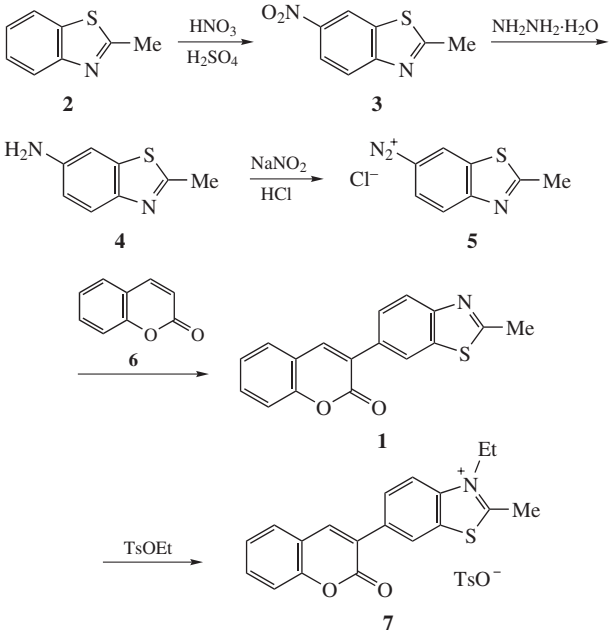
Coumarin derivatives constitute an important group of organic luminophores and laser dyes that efficiently generate radiation in the region of 400–560 nm.[1–3] Coumarin polymethines have efficient fluorescence that makes them promising probes in biochemistry and medicine.[4–7] Psoralens (furocoumarins) became important for photobiology and photochemotherapy.[8–10]

Currently, cyanine dyes that form strongly fluorescenting complexes with nucleic acids and proteins have found broad application in chemistry and biochemistry for identification of biomacromolecules.[11,12] It can be expected that cyanine dyes containing coumarin moieties would possess valuable properties.

The purpose of this study was to synthesize cyanine dyes containing coumarin moieties at heterocyclic systems. We intended to obtain symmetric bischromophoric dyes (thiacarbocyanine and thiadicarbocyanine) containing coumarin fragments at each of the two benzothiazole systems, as well as monomethinecyanine containing coumarin fragment at only one heterocyclic system. Both symmetric and unsymmetric thiacarbocyanine dyes became popular for identification of DNA molecules.

The key compounds were quaternary salts obtained from 2-methylbenzothiazole bearing one coumarin moiety. It is expedient to obtain such salts from the corresponding bases. Synthesis of the latter was an independent and rather complicated task. The key stage in their preparation supposed to be the Meerwein reaction between aryldiazonium halides of benzothiazole type with α,β-unsaturated carbonyl compounds (in our case coumarin). The Meerwein reaction is a complicated process and usually accompanied by strong resinification, hence purification and identification of the target product are often laborous. Despite these complications, the Meerwein reaction seems nearly the only method to incorporate a coumarin moiety into a heterocyclic system.

6-(Coumarin-2-yl)-3-ethyl-2-methylbenzothiazolium tosylate **1** was synthesized according to Scheme 1.† 2-Methylbenzo-

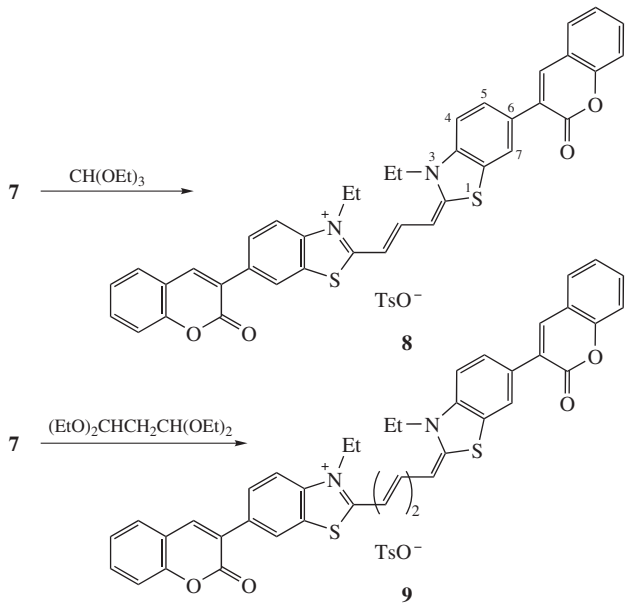


Scheme 1

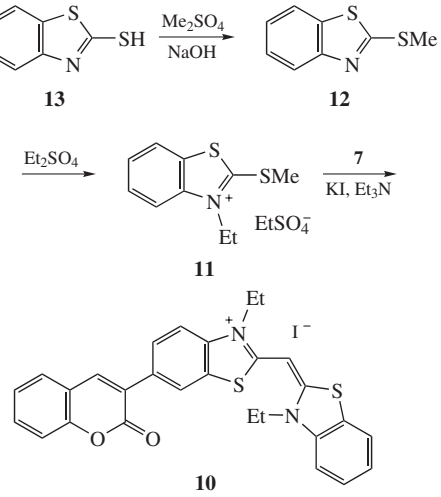
† ^{1}H NMR spectra were recorded using a Bruker WM-250 instrument (250 MHz). The absorption spectra of the dyes were measured on a Specord UV-VIS spectrophotometer. High resolution mass spectra were obtained on a Bruker microTOF instrument by means of electrospray ionization (ESI). The reactions were monitored using UV spectroscopy.

2-Methyl-6-nitrobenzothiazole **3** was obtained in 75% yield by nitration of 2-methylbenzothiazole **2** with nitrating mixture in conc. H_2SO_4.[13] A few methods were tried for the reduction of 2-methyl-6-nitrobenzothiazole **3** into amine **4**.[14–16] It was found to be most convenient to use the method suggested for the catalytic reduction of aromatic nitro compounds with hydrazine hydrate.[17] Reduction of 2-methyl-6-nitrobenzothiazole **3** with hydrazine hydrate in Pr^{i}OH in the presence of 'Norit' activated carbon and iron trichloride gave amine **4** in 80% yield, mp 123–124 °C. UV (EtOH, λ_{max}/nm): 223, 290. ^{1}H NMR ($CDCl_3$) δ: 2.75 (s, 3H, Me), 3.78 (br. s, 2H, NH_2), 6.78 (dd, 1H, H-5), 7.05 (br. s, 1H, H-7), 7.7 (d, 1H, H-4).

*3-(2-Methylbenzo[*d*]thiazol-6-yl)-2*H*-chromen-2-one* **1**. A solution of sodium nitrite (7.6 g) in water (12 ml) was added dropwise at –5 °C to a stirred mixture of hydrochloric acid (25 ml), water (20 ml) and 6-amino-2-methylbenzothiazole **4** (16.4 g) to give diazonium salt **5**. Then the solution of **5** was filtered and added dropwise to a vigorously stirred mixture of coumarin **6** (14.6 g), acetone (120 ml), $AcONa·3H_2O$ (27.5 g), $CuCl_2·2H_2O$ (5 g) and H_2O (10 ml) cooled to 0 °C. Nitrogen evolution continued for 1.5 h. Meanwhile, the reaction mixture reached room temperature. The reaction mixture was steam distilled to collect ~1 dm^3 of the distillate. After cooling, $CHCl_3$ (500 ml) was added to the residue in the distillation flask. The flask contents were stirred with a mechanical stirrer and a dark resinous precipitate was filtered off on a Büchner funnel. The precipitate was washed with $CHCl_3$. The filtrate was transferred into a separating funnel to separate the lower chloroform layer, which was concentrated to 1/3 *in vacuo*. The residue was subjected to column chromatography on Al_2O_3 using $CHCl_3$ as the eluent. According to UV spectroscopic data, the first portions of the



Scheme 2



Scheme 3

thiazole **2** was nitrated with a $HNO_3 + H_2SO_4$ mixture to afford nitro derivative **3** which was reduced to 6-amino-2-methylbenzothiazole **4**. Diazotization of amine **4** gave benzothiazolyldiazonium chloride **5**. The Meerwein reaction of the latter with coumarin **6** provided a small yield of 6-(coumarin-2-yl)-2-methylbenzothiazole **1** which was isolated in pure form by steam removal of other components, chromatography on Al_2O_3 and recrystallization from EtOH. The structure and purity of base **1** were confirmed by its ^{1}H NMR spectrum and high resolution mass spectrum (micrOTOF). The formation of base **1** during the synthesis and its purity were monitored by UV spectroscopy. Tosylate **7** was obtained in 53% yield from base **1** by treatment with ethyl *p*-toluenesulfonate.

Dyes **8**, **9** (Scheme 2) and **10** (Scheme 3) were synthesized from tosylate **7**.‡ Condensation of compound **7** with ethyl orthoformate in dry pyridine under reflux gave thiacarbocyanine **8** in 80% yield, whereas condensation of compound **7** with 1,1,3,3-tetraethoxypropane in dry pyridine under reflux led to thiadicarbocyanine **9** in 52% yield.

Reaction of compound **7** with salt **11** in anhydrous EtOH in the presence of dry pyridine and triethylamine followed by addition

eluate contained coumarin (λ_{max} = 215, 280 and 312 nm), while the next portions were yellow and contained compound **1**. These portions of the eluate were concentrated *in vacuo* and the residue was recrystallized from EtOH to give 2.1 g (7.2%) of **1** as cream-coloured crystals with mp 193–195 °C. UV spectrum [EtOH, λ_{max}/nm (ε)]: 215 (41320), 235 (sh, 19533), 270 (13147), 310 (sh, 21411), 335 (25167). ^{1}H NMR ($CDCl_3$) δ: 2.85 (s, 3H, Me), 7.7 (d, 1H, H-5, *J* 8 Hz), 7.9 (s, 1H, H-7), 8.0 (d, 1H, H-4, *J* 8 Hz), 8.25 (s, 1H, H-8), 7.2–7.6 (m, 4H, Ar). MS (ESI), *m*/*z*: 294.0610 $[M+H]^+$ (calc. for $C_{17}H_{11}NO_2S$: 294.059). Base **1** is insoluble in diethyl ether but soluble in ethanol on heating.

*3-Ethyl-2-methyl-6-(2-oxo-2*H*-chromen-3-yl)benzo[*d*]thiazolium tosylate* **7**. A mixture of **1** (0.18 g, 0.6 mmol) and ethyl *p*-toluenesulfonate (0.14 g, 0.7 mmol) was refluxed for 4 h at 140 °C. After cooling, the reaction mixture was ground into powder and dry acetone (1.5 ml) was added. The mixture was kept for 1 h at –5 °C and a grey precipitate was separated, which was repeatedly washed with acetone to give 0.16 g (53%) of tosylate **7**, mp 96–97 °C. UV [EtOH, λ_{max}/nm (ε)]: 315 (sh), 335. ^{1}H NMR ($CDCl_3$) δ: 2.2 (s, 3H, $MeC_6H_4SO_3^-$), 1.5 (t, 3H, NCH_2Me), 3.2 (s, 3H, Me), 4.8 (q, 2H, NCH_2Me), 7.0 (d, 2H, $MeC_6H_4SO_3^-$), 7.6 (d, 2H, $MeC_6H_4SO_3^-$), 7.3–8.3 (m, 6H, Ar), 7.9 (s, 1H, H-7), 8.65 (s, 1H, H-8).

‡ *3-Ethyl-2-{(1*E*,3*E*)-3-[3-ethyl-6-(2-oxo-2*H*-chromen-3-yl)benzo[*d*]-thiazol-2(3*H*)-ylidene]prop-1-enyl}-6-(2-oxo-2*H*-chromen-3-yl)benzo-[*d*]thiazolium tosylate* **8**. A mixture of tosylate **7** (0.45 g, 0.9 mmol), dry triethyl orthoformate (0.5 g, 3.4 mmol) and dry pyridine (3 ml) was heated for 15 min. After cooling, Et_2O (15 ml) was added to the reaction mixture containing a dark precipitate. The precipitate was separated and recrystallized from EtOH. Drying resulted in 0.3 g (81%) of dye **8** as dark grey crystals with mp 245–247 °C (lit.,[20] mp 230–232 °C). UV [λ_{max}/nm (ε)]: 585 (145412, EtOH), 600 (143304, $CHCl_3$). ^{1}H NMR (DMSO-d_6) δ: 1.3 (t, 6H, NCH_2Me), 2.28 (s, 3H, $MeC_6H_4SO_3^-$), 4.3 (q, 4H, NCH_2Me), 6.45 (d, 2H, α-H, γ-H, *J* 12.5 Hz), 7.4 (t, 1H, β-H, *J* 12.5 Hz), 7.15 (d, 2H, H-5 and H-5′), 7.2 (d, 2H, H-4″, TsO^-, *J* 7.5 Hz), 7.55 (d, 2H, H-3″, TsO^-, *J* 7.5 Hz), 8.02 (br. s, 2H, H-7 and H-7′), 8.18 (s, 2H, H-8 and H-8′), 7.6–7.8 (m, 10H, H-4,4′,10,10′,11,11′,12,12′,13,13′). Found (%): C, 66.75; H, 4.21; N, 3.18. Calc. for $C_{46}H_{36}N_2O_7S_3$ (%): C, 66.97; H, 4.40; N, 3.40.

*3-Ethyl-2-{(1*E*,3*E*,5*E*)-5-[3-ethyl-6-(2-oxo-2*H*-chromen-3-yl)benzo-[*d*]thiazol-2-(3*H*)-ylidene]penta-1,3-dienyl}-6-(2-oxo-2*H*-chromen-3-yl)-benzo[*d*]thiazolium tosylate* **9**. A mixture of tosylate **7** (0.06 g, 0.12 mmol), 1,1,3,3-tetraethoxypropane (0.03 g, 0.13 mmol) and dry pyridine (0.5 ml) was heated for 30 min to give a precipitate. Diethyl ether (10 ml) was added to the cooled reaction mixture, the precipitate was separated and washed with water, EtOH and Et_2O. Recrystallization from EtOH gave 0.026 g (52%) of dye **9** as black crystals, mp 240–243 °C (decomp.). UV [λ_{max}/nm (ε)]: 700 (97396, EtOH), 720 (90312, $CHCl_3$). ^{1}H NMR (DMSO-d_6) δ: 1.3 (t, 6H, NCH_2Me), 2.28 (s, 3H, $MeC_6H_4SO_3^-$), 4.35 (q, 4H, NCH_2Me), 6.30–6.62 (m, 3H, α,γ,ε-H), 7.12 (d, 2H, H-4″, TsO^-, *J* 7.5 Hz), 7.35 (d, 2H, H-3″, TsO^-, *J* 7.5 Hz), 7.41–7.80 (m, 16H, β,δ-H, H-5,5′,7,7′,4,4′,10,10′,11,11′,12,12′,13,13′). Found (%): C, 67.44; H, 4.62; N, 3.15. Calc. for $C_{48}H_{38}N_2O_7S_3$ (%): C, 67.74; H, 4.50; N, 3.29.

*3-Ethyl-2-{[3-ethylbenzo[*d*]thiazol-2(3*H*)-ylidene]methyl}-6-(2-oxo-2*H*-chromen-3-yl)benzo[*d*]thiazolium iodide* **10**. Dry pyridine (0.8 ml) and NEt_3 (0.04 ml) were added with stirring to a mixture of salts **7** (0.14 g, 0.28 mmol) and **11** (0.17 g, 0.51 mmol) in anhydrous EtOH (8.2 ml). The mixture was stirred for 1 h at 40 °C, then a 25% aqueous solution of KI (0.5 ml) was added to give a fine precipitate. The mixture was cooled in a refrigerator and the precipitate was separated, washed with Et_2O and recrystallized from MeOH to give 0.06 g (35%) of dye **10** as greenish-grey crystals, mp > 265 °C. UV [λ_{max}/nm (ε)]: 440 (50155, EtOH), 443 (56256, $CHCl_3$). ^{1}H NMR (DMSO-d_6) δ: 1.4 (t, 6H, NCH_2Me), 4.75 (q, 4H, NCH_2Me), 6.80 (s, 1H, α-H), 8.35 (s, 1H, H-7), 8.55 (s, 1H, H-8), 7.3–8.2 (m, 10H, Ar). Found (%): C, 55.32; H, 3.61; N, 4.25. Calc. for $C_{28}H_{23}IN_2O_2S_2$ (%): C, 55.08; H, 3.80; N, 4.59.

*Ethyl 3-ethyl-2-methylthiobenzo[*d*]thiazolium sulfate* **11** was obtained in 90% yield by heating of equimolar mixture of compound **12** and freshly distilled diethyl sulfate for 2 h in a bath (100 °C); mp 47–50 °C. ^{1}H NMR ($CDCl_3$) δ: 1.25 (t, 3H, OCH_2Me), 1.55 (t, 3H, NCH_2Me), 3.10 (s, 3H, SMe), 4.10 (q, 2H, OCH_2Me), 4.70 (q, 2H, NCH_2Me), 7.60 (t, 1H, H-5), 7.75 (t, 1H, H-6), 8.00 (d, 1H, H-7), 8.23 (d, 1H, H-4).

2-Methylmercaptobenzothiazole **12** was obtained from 2-mercaptobenzothiazole **13** using a reported procedure;[18] yield 60%, mp 43–45 °C (EtOH). ^{1}H NMR ($CDCl_3$) δ: 2.80 (s, 3H, Me), 7.28 (t, 1H, H-5, *J* 10 Hz), 7.42 (t, 1H, H-6, *J* 10 Hz), 7.75 (d, 1H, H-7, *J* 10 Hz), 7.88 (d, 1H, H-4, *J* 10 Hz), assignment to H-5/H-6 and to H-4/H-7 was made arbitrarily.

of 25% aqueous KI solution gave thiamethinecyanine **10** in 35% yield.

The structures of dyes **8**–**10** were confirmed by ^{1}H NMR and UV spectra and by elemental analyses. The maxima in the absorption spectra of dyes **8**–**10** have a long-wave shift in comparison with the non-substituted dyes, which indicates that conjugation exists between the coumarin moiety and the polymethine chain of the dyes. The absorption bands of the coumarin fragment of the dyes synthesized lie in the 280–300 nm region, and their intensity is much lower than that of the polymethine chain band for these dyes.

The formation of noncovalent complexes of the new bischromophoric cyanine dyes with nucleic acids can be used in nucleic acid identification by the fluorescent method. On the other hand, photochemical activation of the coumarin moiety in these dyes provides a unique opportunity for creating fluorescent covalent labels in nucleic acid molecules by a photochemical reaction with one of the bases in the structure of nucleic acids.[19] The results on photochemical and photophysical studies of the dyes **8**–**10** for fluorescent detection of biomacromolecules will be published elsewhere.

References

1 L. K. Denisov and B. M. Uzhinov, *Chem. Heterocycl. Compd*. (*Engl. Transl.*), 1980, **16**, 553 (*Khim. Geterotsikl. Soedin.*, 1980, 723).
2 V. I. Alekseeva, V. K. Shalaev and E. A. Luk'yanets, *Khim. Prom.*, 1981, 597 (in Russian).
3 B. M. Krasovitsky and G. M. Boloshin, *Organicheskie lyuminofory (Organic Luminophores)*, Khimiya, Moscow, 1984, p. 145 (in Russian).
4 S. Yudenfrend, *Fluorestsentnyi analiz v biologii i meditsine (Fluorescent Analysis in Biology and Medicine)*, Mir, Moscow, 1965, p. 304 (in Russian).
5 V. B. Kovalska, K. D. Volkova, A. V. Manaev, M. Yu. Losytskyy, J. N. Okhrimenko, V. F. Traven and S. M. Yarmoluk, *Dyes Pigm.*, 2010, **84**, 159.
6 L. Yuan, W. Lin and J. Song, *Chem. Commun.*, 2010, **46**, 7930.
7 K. Fujii, N. Iyi, H. Hashizume, S. Shimomura and T. Ando, *Chem. Mater*., 2009, **21**, 1179.
8 D. Bethea, B. Fullmer, S. Syed, G. Seltzer, J. Tiano, C. Rischko, L. Gillespie, D. Brown and F. P. Gasparro, *J. Dermatol. Sci.*, 1999, **19**, 78.
9 A. E. da Hora Machado, J. A. de Miranda, A. M. de Olivenia Campus, D. Severino and D. E. Nicodem, *J. Photochem. Photobiol. A: Chem.*, 2001, **146**, 75.
10 V. F. Traven, N. Ya. Podkhalyuzina and D. S. Kanevskii, *Chem. Heterocycl. Compd.* (*Engl. Transl*.), 2003, **39**, 866 (*Khim. Geterotsikl. Soedin*., 2003, 1006).
11 H. Zipper, H. Brunner, J. Bernhagen and F. Vitzthum, *Nucl. Acids Res.*, 2004, **32**, 103.
12 L. Bethge, D. V. Jarikote and O. Seitz, *Bioorg. Med. Chem.*, 2008, **16**, 114.
13 Y. Mizuno and K. Adachi, *J. Pharm. Soc. Jpn.*, 1952, **72**, 745.
14 J. Libeer, H. Depoorter and J. Nys, *Bull. Soc. Chim. Belg.*, 1971, **80**, 43.
15 S. Abuzar and S. Sharma, *Z. Naturforsch.*, 1981, **366**, 108.
16 A. I. Kiprianov, I. K. Ushenko and E. D. Sych, *Zh. Obshch. Khim.*, 1945, **15**, 200 (in Russian).
17 T. Hirashima and O. Manable, *Chem. Lett.*, 1975, 259.
18 A. I. Kiprianov and Z. N. Pazenko, *Zh. Obshch. Khim*., 1949, **19**, 1523 (in Russian).
19 G. D. Cimino, H. B. Gamper, S. T. Isaacs and J. E. Hearst, *Ann. Rev. Biochem*., 1985, **54**, 1151.
20 M. A. Al'perovich, I. K. Ushenko and L. N. Tyurina, *Zh. Obshch. Khim*., 1958, **28**, 2538 (in Russian).

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