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New maleimide spirobenzopyran derivatives as photochromic labels for macromolecules with sulfhydryl groups

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Two new photochromic labels of spiro[chromene-2,2′-indole] series supplied with 5-positioned maleimide moieties suitable for labelling macromolecules with sulfhydryl groups were synthesized.

The property of maleimide group to react with SH group is widely used for conjugation of natural proteins with additives.[1–3] As for additives, of special interest are photochromic probes that provide optical control of the activity of a biomolecule and/or the process in which it participates. Conjugate properties are controlled either due to close interaction of the probe with the active site or due to changes in the hydrophilic-hydrophobic environment of the object.[4] Marriott *et al*. reported a synthesis and applications of thiol-reactive spirobenzopyrans containing maleimidoethyl or maleimidomethyl moiety at indole 1- or 6-positions, respectively (Figure 1) and obtained bioconjugates of these compounds with bovine serum albumin.[5]

The present paper complements these studies and shows an alternative possibility for obtaining similar spirobenzopyran probes. This is the first use of indole 5-position in a photochrome molecule for the attachment of the maleimide moiety (Scheme 1).

We selected hydroxymethyl derivative **1** and carboxy derivative **3** of 1′,3′,3′-trimethyl-6-nitrospiro[chromene-2,2′-indole][6] as the starting compounds. Maleimidomethyl derivative **2** was synthesized from a hydroxymethyl precursor by Mitsunobu reaction[7] in an acceptable yield (29%).† Maleimidoethylcarbamoyl

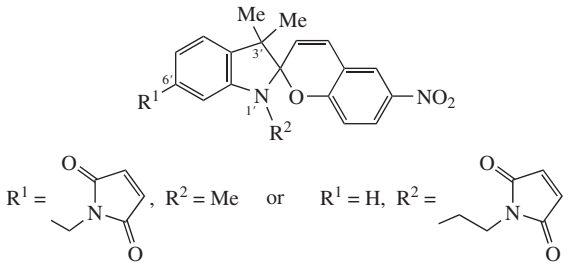
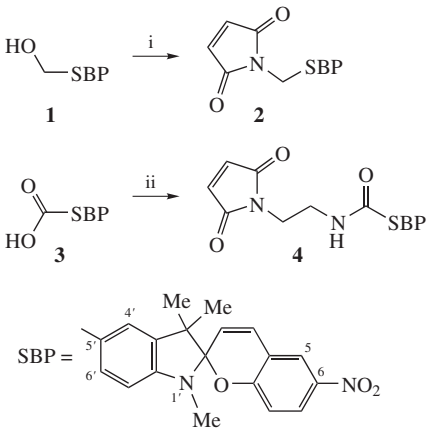


Figure 1



Scheme 1 *Reagents and conditions*: i, PPh_3, DIAD, neopentyl alcohol, maleimide, THF, –70 °C; ii, HBTU, *N*-(2-aminoethyl)maleimide, TFA, *N*-methylmorpholine, DMF, 0°C.

† TLC was carried out on Aluminiumoxid $60F_{254}$ neutral plates (Merck) in hexane–ethyl acetate (1:1) system. The spots were detected by UV illumination (254 nm) of the developed plates. Column chromatography was carried out on aluminium oxide (activity II according to Brockman, Reanal). ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ on a Bruker DPX-300 spectrometer with 300 and 75.5 MHz working frequencies, respectively. LC-MS spectra were obtained with an API-150EX mass spectrometer with a Gilson-215 direct automatic injector, M+1 electrospray ionization, ELSD-Sedex-55 and UV-SCL-10A detectors (Shimadzu), XBridge-C8/3.5min/4.6x50mm column, in the acetonitrile–0.1% trifluoroacetic acid system. Electronic spectra of solutions of the compounds in DMSO were measured in 10 mm thick quartz cells using a Shimadzu UV-1700 spectrophotometer. Irradiation of samples was performed with the continuous light of Vilber Lourmat VL-6.LC 6W lamps in 365 nm mode. Melting points of compounds were determined with an Electrothermal MEL-TEMP instrument. Elemental analysis was carried out with a Finnigan EA 1112 automatic C,H,N analyzer (Thermo).

5′-Maleimidomethyl-1′,3′,3′-trimethyl-6-nitro-1′,3′-dihydrospiro-[chromene-2,2′-indole] **2**. Diisopropyl azodicarboxylate (DIAD) (80 μl, 0.43 mmol) was added dropwise at –70 °C under argon, with agitation on a magnetic stirrer, to a solution of triphenylphosphine (112 mg, 0.43 mmol) in 20 ml of dry THF. After 5 min, a solution of 5′-hydroxomethyl-1′,3′,3′-trimethyl-6-nitro-1′,3′-dihydrospiro[chromene-2,2′-indole] **1** (140 mg, 0.40 mmol) in THF was added. After another 5 min, 2,2-dimethylpropan-1-ol (35 mg, 0.39 mmol) and maleimide (40 mg, 0.41 mmol) were added. The mixture was stirred overnight at room temperature. The solvent was then removed *in vacuo* and the residue was chromatographed in a column packed with alumina in a hexane–ethyl acetate system. The product was triturated in hexane to form yellow crystals, yield 50 mg (29%), R_f 0.8, mp 202–204 °C. UV-VIS [DMSO, λ_{max}/nm (lg ε)]: 260 (4.92), 347 (4.61); λ_{max}^{MC} = 561 nm. ^{1}H NMR ($CDCl_3$) δ: 1.15 (s, 3H, $C^{3'a}H_3$), 1.26 (s, 3H, $C^{3'b}H_3$), 2.71 (s, 3H, $C^{1'}H_3$), 4.62 (s, 2H, $C^{5'}H_2$), 5.81 (s, 1H, H^3, *J* 10.4 Hz), 6.46 (d, 1H, $H^{7'}$, *J* 7.9 Hz), 6.69 (s, 2H, $H_{maleimide}$), 6.75 (d, 1H, H^8, *J* 8.4 Hz), 6.90 (d, 1H, H^4, *J* 10.4 Hz), 7.08 (d, 1H, $H^{4'}$, *J* 1.6 Hz), 7.21 (dd, 1H, $H^{6'}$, *J* 7.9 and 1.8 Hz), 7.98 (s, 1H, H^5), 8.0 (dd, 1H, H^7, *J* 8.4 and 2.7 Hz). ^{13}C NMR ($CDCl_3$) δ: 19.89 (Me), 25.84 (Me), 28.91 (NMe), 41.45 (NCH_2), 52.26 ($C^{3'}$), 106.81 (C_{spiro}), 115.49 ($C^{7'}H$), 118.60 (C^8H), 121.46 (C^4H), 122.46 (C^5H), 122.79 ($C^{6'}H$), 125.90 (C^3H), 127.69 (C^7H), 128.33 ($C^{4'}H$), 128.73 ($C^{10}H$), 134.20 ($2CH_{maleimide}$), 134.48 ($C^{9'}H$), 136.62 ($C^{5'}H$), 140.95 (C^6H), 147.46 ($C^{8'}H$), 159.70 (C^9H), 170.63 (2C=O). LC-MS (UV_{254}), *m/z*: 432.3 $[M+1]^+$. Found (%): C, 67.22; H, 5.11; N, 9.38. Calc. for $C_{24}H_{21}N_3O_5$ (%): C, 66.81; H, 4.91; N, 9.74.

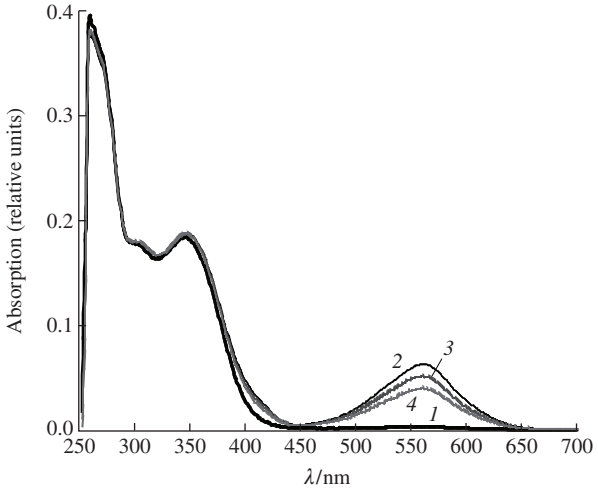


Figure 2 Absorption spectra of compound **2** in DMSO, 25 °C: (*1*) original solution, (*2*) after UV illumination at 365 nm, (*3*) after subsequent storage in the dark for 3 min and (*4*) after subsequent storage in the dark for 10 min.

analogue **4** was obtained by the reaction of HBTU-activated starting compound **3** with commercially available *N*-(2-aminoethyl)maleimide.‡ The low yield of the product (9%) may be explained by non-optimum conditions of product isolation.

Preliminary study of the photochromic properties of labels **2** and **4** showed that the principal behaviour of these compounds nearly did not differ from that of other 6′-nitro-substituted spirobenzopyrans.[8] UV irradiation (365 nm) of maleimides **2** and **4** in DMSO solutions resulted in colouring (a growth in the absorption mono band of the spiropyran merocyanine form at 560–570 nm). Upon storage of the resulting samples in the dark, the absorption curves slowly returned to the original positions (Figures 2 and 3).

As a result, hitherto unknown maleimide spirobenzopyrans **2** and **4** have been obtained as potential photochromic markers for macromolecules with sulfhydryl groups. The maleimide and

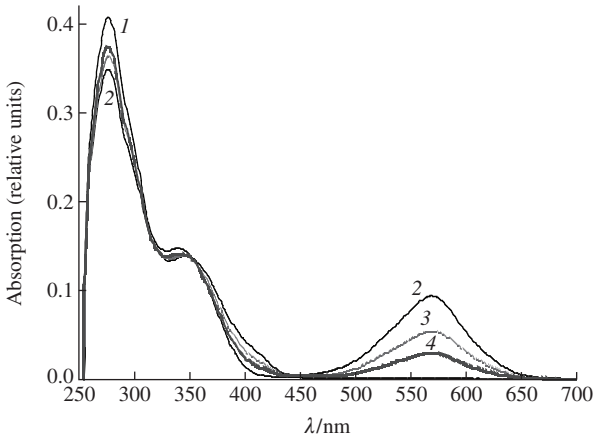


Figure 3 Absorption spectra of compound **4** in DMSO, 25 °C: (*1*) original solution, (*2*) after UV illumination at 365 nm (*3*) after subsequent storage in the dark for 1 min and (*4*) after subsequent storage in the dark for 5 min.

spiropyran moieties are separated by spacers that are stable under physiological conditions.

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References

1 (*a*) J. D. Gregory, *J. Am. Chem. Soc*., 1955, **77**, 3922; (*b*) D. G. Smyth, O. O. Blumenfeld and W. Konigsberg, *Biochem. J*., 1964, **91**, 589.
2 (*a*) N. Fujii, M. Kaneda, R. Misu, T. Noguchi, H. Ohno, S. Oishi, S. Setsuda, B. Evans, J.-M. Navenot and S. C. Peiper, *Bioorg. Med. Chem. Lett.*, 2013, **23**, 2628; (*b*) T. Ito, M. Inoue, K. Akamatsu, E. Kusaka, K. Tanabe and S.-I. Nishimoto, *Bioorg. Med. Chem. Lett.*, 2011, **21**, 3515; (*c*) C. Waengler, P. Bartenstein, B. Waengler, M. Schaefer and R. Schirrmacher, *Bioorg. Med. Chem.*, 2011, **19**, 3864; (*d*) P. D. Garimella, A. Datta, D. W. Romanini, K. N. Raymond and M. B. Francis, *J. Am. Chem. Soc*., 2011, **133**, 14704; (*e*) F. Liu, A.-S.-Y. Ni, Y. Lim, B. Xing, H. Mohanram and S. Bhattacharjya, *Bioconjugate Chem.*, 2012, **23**, 1639; (*f*) V. Tolmachev, M. Altai, A. Orlova, M. Sandstroem, A. Perols, A.-E. Karlstroem and F. Boschetti, *Bioconjugate Chem*., 2011, **22**, 894.
3 (*a*) W. W. Cleland, *Biochemistry*, 1964, **3**, 480; (*b*) S. Klueter, C. Gruetter, M. Rabiller, J. R. Simard, V. Pawar, M. Getlik, D. Rauh and T. Naqvi, *J. Med. Chem*., 2010, **53**, 357.
4 M. Harada, M. Sisido, J. Hirose and M. Nakanishi, *Bull. Chem. Soc. Jpn*., 1994, **67**, 1380.
5 (*a*) T. Sakata, Y. Yan and G. Marriott, *J. Org. Chem.*, 2005, **70**, 2009; (*b*) G. Marriott, T. Sakata and Y. Yan, *US Patent 0195309(A1)*, 2007.
6 A. V. Laptev, A. Yu. Lukin, N. E. Belikov, V. A. Barachevskii, O. V. Demina, A. A. Khodonov, S. D. Varfolomeev and V. I. Shvets, *Mendeleev Commun*., 2013, **23**, 145.
7 M. A. Walker, *J. Org. Chem*., 1995, **60**, 5352.
8 (*a*) *Photochromism: Molecules and Systems*, eds. H. Durr and H. Bouas-Laurent, Elsevier, Amsterdam, 2003; (*b*) E. R. Zakhs, V. M. Martynova and L. S. Efros, *Chem. Heterocycl. Compd*., 1979, **15**, 351 (*Khim. Geterotsikl. Soedin.*, 1979, 435); (*c*) O. V. Demina, P. P. Levin, N. E. Belikov, A. V. Laptev, A. Yu. Lukin, V. A. Barachevsky, V. I. Shvets, S. D. Varfolomeev and A. A. Khodonov, *J. Photochem. Photobiol. A: Chem.*, 2013, **270**, 60; (*d*) A. V. Laptev, A. Yu. Lukin, N. E. Belikov, R. V. Zemtsov, V. I. Shvets, O. V. Demina, S. D. Varfolomeev, V. A. Barachevskii and A. A. Khodonov, *High Energy Chem*., 2010, **44**, 211 (*Khim. Vys. Energ.*, 2010, **44**, 239).

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‡ *5′-[N-(2-Maleimidoethyl)carbamoyl]-1′,3′,3′-trimethyl-6-nitro-1′,3′-dihydrospiro[chromene-2,2′-indole]* **4**. *O*-(Benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (coupling reagent for peptide, HBTU) (200 mg, 0.85 mmol) was added to a solution of 1′,3′,3′-trimethyl-6-nitro-1′,3′-dihydrospiro[chromene-2,2′-indole]-5′-carboxylic acid **3** (400 mg, 1.09 mmol) in 20 ml of DMF cooled to 0 °C. The mixture was agitated for 10 min with a magnetic stirrer, then a solution of *N*-(2-aminoethyl)maleimide [freshly prepared from the corresponding hydrotrifluoroacetate salt (140 mg, 0.55 mmol) neutralized with *N*-methylmorpholine (200 μl, 2.83 mmol)] in 2 ml of dry DMF was added. The mixture was agitated overnight, then the solvent was removed *in vacuo*. The target spiropyran **4** was isolated using column chromatography on alumina in a hexane–ethyl acetate system. Yield 50 mg (9%). R_f 0.4. UV-VIS [DMSO, λ_{max}/nm (lg ε)]: 277 (4.85), 342 (4.42); λ_{max}^{MC} = 569 nm. ^{1}H NMR ($CDCl_3$) δ: 1.17 (s, 3H, $C^{3'a}H_3$), 1.30 (s, 3H, $C^{3'b}H_3$), 2.77 (s, 3H, $C^{1'}H_3$), 3.65 (m, 2H, $C^{1''}H_2$), 3.82 (m, 2H, $C^{2''}H_2$), 5.83 (d, 1H, H^3, *J* 10.4 Hz), 6.53 (d, 1H, $H^{7'}$, *J* 8.1 Hz), 6.57 (t, 1H, NH, *J* 4.0 Hz), 6.73 (s, 2H, $H_{maleimide}$), 6.76 (d, 1H, H^8, *J* 9.0 Hz), 6.92 (d, 1H, H^4, *J* 10.4 Hz), 7.56 (d, 1H, $H^{4'}$, *J* 1.6 Hz), 7.59 (dd, 1H, $H^{6'}$, *J* 8.1 and 1.8 Hz), 8.0 (s, 1H, H^5), 8.02 (d, 1H, H^7, *J* 9.0 and 2.7 Hz). ^{13}C NMR ($CDCl_3$) δ: 19.88 (Me), 25.87 (Me), 28.90 (NMe), 38.39 ($NHCH_2$), 40.90 ($C^{3'}$), 41.55 (NCH_2), 106.80 (C_{spiro}), 114.21 ($C^{7'}H$), 118.62 (C^8H), 121.43 (C^4H), 122.44 (C^5H), 123.67 ($C^{6'}H$), 125.88 (C^3H), 127.72 (C^7H), 127.90 ($C^{4'}H$), 128.75 ($C^{10}H$), 134.02 ($C^{9'}H$), 134.93 ($2CH_{maleimide}$), 135.95 ($C^{5'}H$), 140.92 (C^6H), 148.51 ($C^{8'}H$), 159.73 (C^9H), 163.69 (C=O), 168.24 ($2C{=}O_{maleimide}$). LC-MS (UV_{254}), *m*/*z*: 489.5 $[M+1]^+$. Found (%): C, 63.55; H, 5.30; N, 11.26. Calc. for $C_{26}H_{24}N_4O_6$ (%): C, 63.93; H, 4.95; N, 11.47.