Electronic supplementary materials *Mendeleev Commun*., 2013, **23**, 145−146

Ethynyl-equipped spirobenzopyrans as promising photochromic markers for nucleic acid fragments

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Experimental

Thin-layer chromatography was performed on $60F_{254}$ alumina plates (Merck, Germany) in dichloromethane as the eluent; the spots were visualized under UV light (254 nm). Preparative column adsorption chromatography was carried out on Brockmann II grade alumina (Reanal, Hungary). Spectroscopic studies were carried out at 25^0C. The NMR spectra were measured on a Bruker DPX-300 spectrometer (Germany) with an operating frequency of 300 MHz for 1H and 75.4 MHz for ^{13}C in $CDCl_3$. Chemical shifts (δ) with an accuracy of 0.01 ppm are given relative to a $CDCl_3$ internal standard δ (^{1}H NMR) - 7.26 ppm; the spin-spin coupling constant magnitudes J (Hz) were measured to within 0.1 Hz. The LC/MS were measured on an API-150EX mass spectrometer (Japan) with direct automatic injection Gilson-215, electrospray ionization M + 1, ELSD-Sedex-55 and UV-SCL-10A detectors (Shimadzu, Japan), an XBridge-C8 column /3.5 min x 50 mm in the acetonitrile + 0.1% trifluoracetic acid system. The melting points of the test compounds were determined on an Electrothermal MEL-TEMP instrument (the United States). The IR spectra were measured on a Perkin Elmer Spectrum 65 PC FT-IR spectrophotometer (the United States) as films on the surface of KBr windows.

The reagents of the following vendors were used: Merk (isobutyl chloroformate, 1,1'-carbonyldiimidazole (CDI), *N*,*N'*-Dicyclohexylcarbodiimide (DCC) and dimethylformamide); Acros (sodium borohydride, potassium *tert*-butoxide, and *N*-methylmorpholine); and Aldrich (triphenylphosphine, 60% suspension of sodium hydride in mineral oil, tetrakis(triphenylphosphine)palladium (0), copper(I) iodide, propargyl bromide, propargylamine, 1-amino-3-butyne, and 4-pentyne-1- amine). Domestically manufactured solvents were also used. (Bromomethyl)triphenylphosphonium bromide was obtained by heating of dibromomethane with triphenylphosphine in toluene.

5-Hydroxymethyl-6'-nitro-1,3,3-trimethylspiro(indolino-2,2'-[2*H*]chromene) (**2**). 110 mg (2.9 mmol) of sodium borohydride was added to a solution of 500 mg (1.4 mmol) 5-formyl-6 '-nitro-1,3,3-trimethylspiro(indolino-2,2'-[2*H*]chromene) (**1**) in a mixture of 25 ml of tetrahydrofuran and 25 ml of isopropyl alcohol with intense stirring. After stirring for 40 min, 10 ml of acetone was added.

The reaction mass was poured into 100 ml of distilled water and after 5 min the content was extracted with CH_2Cl_2 (2 x 50 ml). The extracts were dried with anhydrous sodium sulfate, and the solvent was removed in a vacuum. The target product was separated by column chromatography on aluminum oxide. The yield of reaction product (**2**) was 230 mg (0.65 mmol) (45%). R_f 0.15. Mp, 97-99 ^{0}C. ^{1}H NMR ($CDCl_3$, δ/ppm, *J*/Hz): 1.15 (3H, s, 3a–CH_3), 1.27 (3H, s, 3b–CH_3), 2.75 (3H, s, 1–CH_3), 4.61 (2H, s, $\underline{CH_2}$OH), 5.83 (1H, d, J 10.4, 3'–H), 6.51 (1H, d, J 8.3, 7–H), 6.75 (1H, d, J 9.0, 8'–H), 6.92 (1H, d, J 10.4, 4'–H), 7.12 (1H, s, 4–H), 7.19 (1H, dd, J 8.1/1.5, 6–H), 8.01 (1H, s, 5'-H), 8.02 (1H, dd, J 9.0/2.9, 7'–H).

5-Ethynyl-(6'-nitro-1,3,3-trimethylspiro(indolino-2,2'-[2*H*]chromene) (**4**). To a solution of 800 mg (2.3 mmol) of 5-formyl spiropyran (**1**) in 40 ml of anhydrous THF, 1.5 g (3.5 mmol) of (bromomethyl)triphenylphosphonium bromide and then 650 mg (5.8 mmol) of potassium tert-butoxide were introduced with stirring at a temperature of -70 ^{0}C under argon. The temperature was slowly raised to ~20 ^{0}C for 2 h; thereafter, 50 ml of distilled water was added, and the content was neutralized with 10% hydrochloric acid. The mixture was extracted with CH_2Cl_2; the organic phase was dried with sodium sulfate, and the solvent was removed *in vacuo*. Column chromatography on aluminum oxide gave product (**4**) which was additionally purified by crystallization from a mixture of ethanol and petroleum ether (1 : 10). The yield of product (**4**) was 400 mg (1.2 mmol) (50%). R_f 0.95. Mp, 184-186 ^{0}C. ^{1}H NMR ($CDCl_3$, δ/ppm, *J*/Hz): 1.16 (3H, s, 3a–CH_3), 1.27 (3H, s, 3b–CH_3), 2.74 (3H, s, 1–CH_3), 2.99 (1H, s, ≡CH), 5.82 (1H, d, J 10.3, 3'–H), 6.47 (1H, d, J 8.1, 7–H), 6.75 (1H, d, J 8.6, 8'–H), 6.93 (1H, d, J 10.3, 4'–H), 7.19 (1H, d, J 1.5, 4–H), 7.36 (1H, dd, J 8.0/1.6, 6–H), 8.0 (1H, s, 5'-H), 8.02 (1H, dd, J 8.7/2.8, 7'–H). ^{13}C NMR ($CDCl_3$, δ/ppm): 79.0 (HC≡), 88.7 (-C≡). IR (ν, sm^{-1}): 3300 (≡C-H); 3100-2800 (C-H); 2100 (C≡C); 1647 (spiro C=C); 1610,1575,1486 (Ar); 1514 (N-O, asym); 1331 (N-O, sym); 1263 (C-O-Car, asym); 1177 (C-N); 1120 (C-N); 1085 (C-O-Car, sym); 1015 (C-O); 947 (=C-H). LC/MS (*m/z*, $[M+1]^+$; UV254, min): 347.0 (2.19).

6'-Nitro-5-propargyloxymethyl-1,3,3-trimethylspiro(indolino-2,2'-[2*H*]chromene) (**5**). To a solution of 700 mg (2.0 mmol) of anhydrous 5-hydroxymethyl spiropyran (**2**) in 30 ml of anhydrous dimethylformamide, 120 mg (3.0 mmol) of a 60% suspension of sodium hydride (in mineral oil)

was added at an ice bath temperature in an atmosphere of argon with stirring on a magnetic stirrer. After 30 min, 0.4 ml (2.7 mmol) of a 80% solution of propargyl bromide in toluene was added to the reaction mass obtained (a transparent yellow solution). The mixture was stirred overnight and then poured into 100 ml of distilled water; the contents were extracted with CH_2Cl_2. The organic phase was dried with sodium sulfate, and the solvent was removed *in vacuo*. Column chromatography on aluminum oxide was used to separate the target product; compound (**5**) was additionally purified by crystallization from a mixture of ethanol and petroleum ether (1 : 1). The yield of reaction product (**5**) was 300 mg (0.77 mmol) (38%). R_f 0.85. Mp, 65-67 ^{0}C. ^{1}H NMR ($CDCl_3$, δ/ppm, *J*/Hz): 1.18 (3H, s, 3a–CH_3), 1.29 (3H, s, 3b–CH_3), 2.46 (1H, t, J 2.4, ≡CH), 2.73 (3H, s, 1–CH_3), 4.18 (2H, d, J 2.3, ≡C-$\underline{CH_2}$O), 4.55 (2H, s, Ph-$\underline{CH_2}$O), 5.84 (1H, d, J 10.3, 3'–H), 6.51 (1H, d, J 7.9, 7–H), 6.75 (1H, d, J 9.3, 8'–H), 6.92 (1H, d, J 10.3, 4'–H), 7.09 (1H, d, J 2.4, 4–H), 7.17 (1H, dd, J 7.8/1.7, 6–H), 7.99 (1H, s, 5'-H), 8.0 (1H, dd, J 9.1/2.7, 7'–H). ^{13}C NMR ($CDCl_3$, δ/ppm): 74.5 (HC≡), 79.9 (-C≡). IR (ν, sm^{-1}): 3290 (≡C-H); 3100-2800 (C-H); 2116 (C≡C); 1649 (spiro C=C); 1613,1577,1494 (Ar); 1517 (N-O, asym); 1336 (N-O, sym); 1272 (C-O-C, asym); 1184 (C-N); 1125 (C-N); 1087 (C-O-C, sym); 1017 (C-O); 951 (=C-H). LC/MS (*m/z*, $[M+1]^+$; UV254, min): 391.3 (2.14).

6'- Nitro-5-(*N*-propargylcarbamoyl)-1,3,3-trimethylspiro(indolino -2,2'-[2*H*]chromene) (**6**). (A) To a mixture of 1.0 g (2.7 mmol) of 5-carboxy-6'-nitro-1,3,3-trimethylspiro(indolino -2,2'[2*H*]chromene) (**3**) and 0.6 ml (5.0 mmol) of N-methylmorpholine in 50 ml of anhydrous tetrahydrofuran (THF), 0.42 ml (3.0 mmol) of isobutyl chloroformate was added dropwise at -70 ^{0}C in an atmosphere of argon with stirring on a magnetic stirrer. The temperature of the mixture was slowly raised to 0 ^{0}C within 30 min with stirring; then, 0.2 ml (4.0 mmol) of propargylamine was introduced. The mixture was allowed to stand at room temperature for 2 h; thereafter, 100 ml of water was added, and the reaction mass was neutralized with 10% hydrochloric acid. The product was extracted with CH_2Cl_2, and the extract was dried with anhydrous sodium sulfate. The target product was purified by column chromatography on aluminum oxide using the CH_2Cl_2/petroleum ether system (2 : 1). Compound (**6**) was additionally purified by crystallization from an ethanol/petroleum ether mixture (1 : 3). The yield of product (**6**) was 500 mg (1.2 mmol) (44%). (B) To a solution of 1.0 g (2.7 mmol) of compound (**3**) in 50 ml of anhydrous dimethylformamide, 0.53 g (3.3 mmol) of 1,1' -carbonyldiimidazole was added at room temperature in a atmosphere of argon with intense stirring on a magnetic stirrer. After 50 min, 0.2 ml (4.0 mmol) of propargylamine was added to the mixture, and the reaction mass was stirred overnight. The mixture was poured into 100 ml of water, and the product was extracted with methylene chloride. The subsequent isolation and purification were performed in accordance with procedure (A). The yield of product (**6**) was

530 mg (1.3 mmol) (46%).

R_f 0.44. Melting point,. 215-217 ^{0}C. ^{1}H NMR ($CDCl_3$, δ/ppm, *J*/Hz): 1.18 (3H, s, 3a–CH_3), 1.30 (3H, s, 3b–CH_3), 2.26 (1H, t, J 2.5, ≡CH), 2.78 (3H, s, 1–CH_3), 4.24 (2H, dd, J 5.1/2.5, ≡C-$\underline{CH_2}$NH), 5.83 (1H, d, J 10.3, 3'–H), 6.20 (1H, t, J 5.1, NH), 6.53 (1H, d, J 8.1, 7–H), 6.74 (1H, d, J 9.1, 8'–H), 6.94 (1H, d, J 10.3, 4'–H), 7.57 (1H, d, J 1.5, 4–H), 7.63 (1H, dd, J 8.1/1.5, 6–H), 8.0 (1H, s, 5'-H), 8.02 (1H, dd, J 9.1/2.6, 7'–H). ^{13}C NMR ($CDCl_3$, δ/ppm): 71.6 (HC≡), 79.9 (-C≡). IR (ν, sm^{-1}): 3300 (≡C-H); 3100-2800 (C-H); 2121 (C≡C); 1710 (C=O); 1635 (spiro C=C); 1613,1583,1488 (Ar); 1577 (N-H); 1518 (N-O, asym); 1336 (N-O, sym); 1303 (N-H); 1270 (C-O-Car, asym); 1181 (C-N); 1123 (C-N); 1089 (C-O-Car, sym); 1018 (C-O); 953 (=C-H). LC/MS (*m/z*, $[M+1]^+$; UV254, min): 404.4 (1.89).

5-[*N*-(but-3-ynylcarbamoyl)]-6'-nitro-1,3,3-trimethylspiro(indolino-2,2' -[2*H*]chromene) (**7**). Compound (**7**) was obtained analogously to (**6**) with the use of method B from 1.0 g (2.7 mmol) of compound (**3**) and 0.2 ml (2.9 mmol) of 1-amino-3-butyne with the use of 0.53 g (3.3 mmol) 1,1'-carbonyldiimidazole. Isolation and purification were performed analogously to those for (**6**). The yield of product (**7**) was 460 mg (1.1 mmol) (41%). R_f 0.42. Mp, 186-188 ^{0}C. ^{1}H NMR ($CDCl_3$, δ/ppm, *J*/Hz): 1.19 (3H, s, 3a–CH_3), 1.31 (3H, s, 3b–CH_3), 2.04 (1H, t, J 2.6, ≡CH), 2.52 (2H, td, J 6.3/2.6, ≡C-$\underline{CH_2}$), 2.78 (3H, s, 1–CH_3), 3.61 (2H, q, J 6.3, NH-$\underline{CH_2}$), 5.84 (1H, d, J 10.3, 3'–H), 6.37 (1H, t, J 6.3, NH), 6.52 (1H, d, J 8.1, 7–H), 6.75 (1H, d, J 8.6, 8'–H), 6.94 (1H, d, J 10.3, 4'–H), 7.58 (1H, d, J 1.5, 4–H), 7.61 (1H, dd, J 8.1/1.8, 6–H), 8.0 (1H, s, 5'-H), 8.02 (1H, dd, J 8.6/2.8, 7'–H). ^{13}C NMR ($CDCl_3$, δ/ppm): 70.0 (HC≡), 81.9 (-C≡). IR (ν, sm^{-1}): 3300 (≡C-H); 3100-2800 (C-H); 2119 (C≡C); 1725 (C=O); 1631 (spiro C=C); 1613,1583,1489 (Ar); 1577 (N-H); 1519 (N-O, asym); 1336 (N-O, sym); 1303 (N-H); 1270 (C-O-Car, asym); 1183 (C-N); 1123 (C-N); 1088 (C-O-Car, sym); 1016 (C-O); 953 (=C-H). LC/MS (*m/z*, $[M + 1]^+$; UV254, min): 418.4 (1.78).

6'-Nitro-5-[*N*-(pent-4-ynylcarbamoyl)]-1,3,3-trimethylspiro(indolino-2,2'-[2*H*]chromene) (**8**). Compound (**8**) was obtained analogously to (**6**) according to method (A) from 730 mg (2.0 mmol) of compound (3) and 0.2 ml (2.4 mmol) of 1-amino-4-pentyne with the use of 0.35 ml (2.7 mmol) of isobutyl chloroformate and 0.5 ml (5.0 mmol) of *N*-methylmorpholine. Isolation and purification were performed analogously to those for (**6**). The yield of product (**8**) was 330 mg (0.77 mmol) (38%). R_f 0.40. Mp, 71-73 ^{0}C. ^{1}H NMR ($CDCl_3$, δ/ppm, *J*/Hz): 1.19 (3H, s, 3a–CH_3), 1.31 (3H, s, 3b–CH_3), 1.86 (2H, p, J 6.8, CH_2-$\underline{CH_2}$-CH_2), 2.01 (1H, t, J 2.7, ≡CH), 2.32 (2H, td, J 6.8/2.7, ≡C-$\underline{CH_2}$), 2.78 (3H, s, 1–CH_3), 3.58 (2H, q, J 6.8, NH-$\underline{CH_2}$), 5.84 (1H, d, J 10.3, 3'–H), 6.26 (1H, t, J 6.3, NH), 6.52 (1H, d, J 8.1, 7–H), 6.75 (1H, d, J 8.5, 8'–H), 6.94 (1H, d, J 10.3, 4'–

H), 7.56 (1H, d, J 1.7, 4–H), 7.60 (1H, dd, J 8.1/1.7, 6–H), 8.0 (1H, s, 5'-H), 8.02 (1H, dd, J 8.5/2.7, 7'–H). ^{13}C NMR ($CDCl_3$, δ/ppm): 70.9 (HC≡), 83.8 (-C≡). IR (ν, sm^{-1}): 3298 (≡C-H); 3100-2800 (C-H); 2117 (C≡C); 1701 (C=O); 1629 (spiro C=C); 1614,1583,1490 (Ar); 1577 (N-H); 1518 (N-O, asym); 1337 (N-O, sym); 1304 (N-H); 1270 (C-O-Car, asym); 1186 (C-N); 1123 (C-N); 1088 (C-O-Car, sym); 1016 (C-O); 953 (=C-H). LC/MS (*m/z*, $[M + 1]^+$; UV254, min): 432.8 (1.70).

5-[2-(1,3-Dimethyluracil-5-yl)ethynyl]-2-[6'- nitro-1,3,3-trimethylspiro(indolino-2,2'-[2*H*]chromene)] (**10**). To a mixture of 20 mg (0.08 mmol) of 5-iodo-1,3-dimethyluracil (**9**) (1.5 equiv), 2 mg of copper(I) iodide (0.2 equiv), 1 mg of tetrakis(triphenylphosphine)palladium (0) (0.1 equiv) and 0.03 ml (0.3 mmol) of triethylamine (3 equiv) in dry dimethylformamide, 20 mg (0.06 mmol) of acetylene spiropyran derivative (**4**) (1.0 equiv) was added in an atmosphere of argon. The reaction mass was stirred for 24 h on a magnetic stirrer and then poured into water and extracted with CH_2Cl_2. The target product was isolated by preparative column chromatography on aluminum oxide; compound (**10**) was additionally purified by crystallization from an ethanol/petroleum ether mixture (5 : 1). The yield of compound (**10**) was 15 mg (0.03 mmol) (39%). R_f 0.55. Mp, 207−209 ^{0}C (decomp.). ^{1}H NMR ($CDCl_3$, δ/ppm, *J*/Hz): 1.17 (3H, s, 3a–CH_3), 1.27 (3H, s, 3b–CH_3), 2.75 (3H, s, 1–CH_3), 3.40 (3H, s, 3''-CH_3), 3.44 (3H, s, 1''-CH_3), 5.82 (1H, d, J 10.3, 3'–H), 6.48 (1H, d, J 8.1, 7–H), 6.76 (1H, d, J 8.6, 8'–H), 6.92 (1H, d, J 10.3, 4'–H), 7.22 (1H, d, J 1.6, 4–H), 7.35 (1H, dd, J 8.0/1.6, 6–H), 7.47 (1H, s, 4''-H), 8.0 (1H, s, 5'-H), 8.02 (1H, dd, J 8.6/2.7, 7'–H). IR (ν, sm^{-1}): 3100-2800 (C-H); 2210 (C≡C); 1707 (C=O); 1654 (C=C); 1611,1576,1478 (Ar); 1515 (N-O, asym); 1337 (N-O, sym); 1271 (C-O-Car, asym); 1210,1181,1123 (C-N); 1087 (C-O-Car, sym); 1017 (C-O); 954 (=C-H). LC/MS (*m/z*, $[M + 1]^+$; UV254, min): 485.5 (2.02).