CHIRAL PRODYES. ETHERS AND ESTERS OF DIHYDROFLUORESCEIN, PART 1: DIBENZYLDIHYDROFLUORESCEIN (DBDF) A NEW REAGENT

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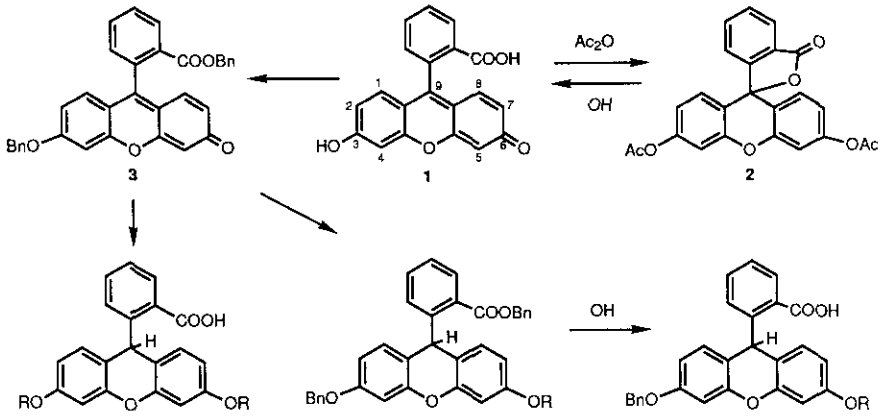
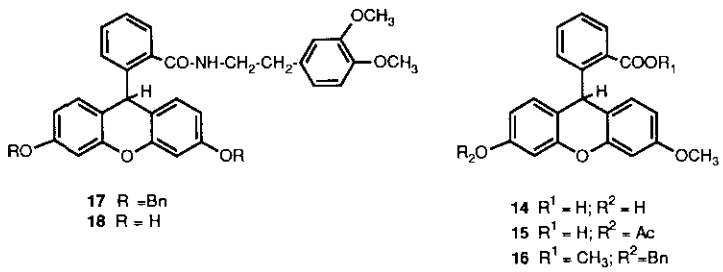
Abstracts - Ethers and esters of dihydrofluorescein (4) were prepared by chemical modification of the benzyl ester (6), obtained by benzylation of fluorescein (1), and reduction of benzyl ester (3) with sodium borohydride.

In attempts to develop highly sensitive reagents that would allow the detection of minute amounts of biogenic amines, metabolically derived alcohols, and biopolymers, we reinvestigated dihydrofluoresceinfluorescein diacetate (5), prepared by Liebig in 1915.[1] Compound (5), made by an improved process,[2] is now commercially available,[3] and has been used, under the code name DADF, to derivatize amines such as mescaline, and alcohols such as codeine.[2] DADF-derivatives convert on tlc-plates, after exposure to ammonia and iodine, into red colored dyes of erythrosine structure. They show a uv maximum at 540-545 nm and are visible on tlc plates in subnanomolar quantities.[4] DADF has proved useful in measuring metabolites of the antimalarial drug artemisinin in fluids and tissues.[5] To further explore the lead provided by DADF we thought it worthwhile to examine the possibility of making optically active analogs of DADF which would allow detection and analysis of optically active substrates. Chirality would be expressed by carbon atom C(9) in analogs of dihydrofluorescein having two differently substituted benzenoid rings. We now present the basic chemistry developed for making some of the key compounds. Chemically pure fluorescein (1) was obtained from commercially available material by acetylation to lactone (2), and hydrolysis of 2 with ethanolic potassium hydroxide, a procedure already used by Fischer and Hepp.[6] We report this purification here since the compounds are now characterized by spectral data. Treatment of 1 with benzyl chloride in DMF in the presence of anhydrous K_2CO_3 afforded the

benzyl ester (3), a key compound in this project. Its structure was ascertained by spectral data, by its catalytic reduction over $Pd(OH)_2$-catalyst, and by its in situ acetylation of the intermediate (4) to afford the known diacetate (5). Reduction of 3, with sodium borohydride in methanol, afforded ester (6) used for O-alkylation reaction under standard conditions. This afforded, with methyl iodide, the ester (7), with benzyl chloride the ester (8), and with methoxyethoxymethyl chloride (MEM chloride) the ester (9). These esters are yellowish oils which, on exposure to oxygen, slowly oxidize back to the corresponding fluoresceins. Hydrolysis of esters (7) and (8) with ethanolic sodium hyroxide afforded, after acidification, the crystalline acids (11) and (12) respectively.





The amorphous analogs, (10) and (13), were obtained under similar conditions from the corresponding esters (6) and (9). Acid (11) was further characterized by catalytic debenzylation over $Pd(OH)_2$-catalyst to give 14, followed by acetylation to give 15 as well as by its conversion to give the methyl ester (16). The dibenzyl ether acid (DBDF) (12) emerged from our investigation as an interesting analog of DADF, useful for the derivatization of amines and alcohols. The benzyl ether groups are more stable towards hydrolysis than the acetate groups in DADF. The potential use of DBDF is illustrated by its conversion into the homoveratrylamide (17), and its catalytic debenzylation to the amide (18) which occurred smoothly in ethyl acetate over $Pd(OH)_2$-catalyst. Amide (18) afforded, when exposed on tlc plates to iodine vapors, red colored materials showing, after extraction with ethanol, a uv maximum at 545 nm. Esters (6) and (7) are now further explored for the synthesis of chiral representatives of this series of compounds.

EXPERIMENTAL

Melting points were taken on a Fisher-Johns Apparatus and are uncorrected. Elemental analyses were performed by Dr. F. Scheidl, Hoffmann La-Roche, Inc., Nutley, N.J. Ir spectra (KBr) were recorded on a Beckman ir 4230 instrument. Uv spectra were recorded on the Hewlett-Packard 8450 uv spectrophotometer, and they were, if not otherwise noted, measured in EtOH (λ max, nm). Nmr spectra were determined by using a Varian XL-300 spectrometer with $(CH_3)_4$ Si as the internal reference and were measured in $CDCl_3$ (ppm). Chemical ionization (CI) mass spectra were obtained by using a Finnigan 1015 D spectrometer with a model 6000 data collection system. Thin layer chromatography (tlc) plates were purchased from Analtech, Inc. The solvent systems used for the tlc analysis were the following: CH_2Cl_2; CH_2Cl_2/MeOH (5%), CH_2Cl_2/MeOH (10%), and iodine vapors were used for the detection. Preparative chromatography colums were packed with silica gel 60 (0.015-0.040 mm).

Diacetylfluorescein (2):According to the procedure described by H.V. Liebig [1] (2) was obtained in 51% yield as beige crystals: mp 205°C; ms (m/z): 417 (M^++1), 311, 269; ir 1770 cm^{-1}; uv: 234, 281, 290; (EtOH + NaOH) 281, 287, 324, 363, 480.

Fluorescein (1) from (2): Prepared according to the procedure described by H.V. Liebig[1] as dark

red crystalline solid: mp > 300°C; ms (m/z): 333 (M^++1); ir: 1600 cm^{-1}; uv: 233, 277, 425, 456, 484; (EtOH+NaOH) 233, 293, 324,376, 500; (EtOH+HCl) 233, 310, 448.

O-Benzylfluorescein benzyl ester (3): 1 (10 g, 0.03 mol) was dissolved in 150 ml of DMF, and than K_2CO_3 (18 g) was added to the solution. The reaction mixture was heated to 80°C and benzyl chloride (10.4 ml, 0.09 mol) was added dropwise. After stirring for 24 h, the hot solution was filtered and left to crystallize over night. The compound (3) (12.64 g, 81%) was obtained as a bright yellow crystallline solid: mp 193-194°C; ms (m/z): 513 (M^++1), 423,174; ir:1730, 1650, 1600 cm^{-1}; uv: 235, 256, 311, 363, 410, 434, 460, 486; ^{1}H-nmr: δ 4.92 (m, 2H), 5.17 (s, 2H), 6.29 (d, J = 2 Hz, 1H), 6.49 (dd, Jo = 10 Hz, Jm = 2 Hz, 1H), 6.80 (m, 7H), 7.2 (m, 3H), 7.4 (m, 5H), 7.7 (m, 2H), 8.29 (dd, Jo = 7.5 Hz, Jm= 1.5 Hz, 1H). Anal. Calcd for $C_{34}H_{24}O_5$: C, 79.67; H, 4.72. Found: C, 79.98; H, 4.69.

Dihydrofluoresceindiacetate (DADF) (5) from (3): 200 mg (0.39 mmol) of 3 were dissolved in EtOH (10 ml), the excess of $Pd(OH)_2$ on carbon was added, and the reaction mixture was hydrogenated at room temperature for 30 min. The reaction mixture was filtered and evaporated to dryness. The remaining solid was dissolved in 1N HCL and extracted with CH_2Cl_2. The organic phase was dryed over Na_2SO_4, evaporated, dissolved in pyridine (10 ml), and then acetic anhydride (4 ml) was added. The reaction mixture was stirred at room temperature overnight, evaporated to dryness, and chromatographed on silica gel (flash chromatography CH_2Cl_2/MeOH, 1%) to give 5 (20 mg), which was crystallized from isopropyl ether: mp 205-206°C (lit., mp 213°C); ir: 1730, 1770, 1620 cm^{-1}; uv: 208, 281.

O-Benzyldihydrofluorescein benzyl ester (6): 3 (4.75 g, 9.2 mmol) was dissolved in 250 ml of MeOH, cooled to 0°C in an ice bath while stirring, and the excess of $NaBH_4$ was rapidly added. The reaction mixture was sttired at room temperature for 1h. After removal of the solvent under reduced pressure, the remaining solid was dissolved in ether and washed two times with water. The organic layer was dryed over anhydrous Na_2SO_4 and evaporated to dryness. The compound (6) (4.62 g, 97%) was obtained as a bright, yellowish amorphous solid: ms (m/z): 515 (M^++1), 497, 423, 407, 331, 240; uv: 222, 281; ^{1}H-nmr: δ 4.93 (s, 1H), 5.04 (s, 2H), 6.20 (s, 1H), 6.41 (dd, Jo = 8.5 Hz, Jm = 2.5 Hz, 1H), 6.57 (m, 2H), 6.72 (d, Jm = 2.5 Hz, 1H), 6.91 (m, 2H), 7.15 (m, 2H), 7.35 (m, 11H), 7.83 (s, 1H).

O-Benzyl-O-methyldihydrofluorescein benzyl ester (7): 6 (4.62 g, 8.9 mmol) was dissolved in 160 ml of acetone, and CH_3I (1.37 g, 9.6 mmol) was added while stirring. The reaction mixture was refluxed for 24 h. After filtration and removal of the solvent under reduced pressure, the remaining solid was dissolved in ether and washed two times with water. The organic layer was dried over anhydrous Na_2SO_4 and evaporated to dryness. The compound (7) (4.61 g, 97 %) was obtained as a yellowish oil: ms (m/z): 546 ($M^+ + NH_3$), 529 (M^++1), 511, 437, 421; uv: 231, 280.

O-Benzyldihydrofluorescein (10): To a solution of 6 (850 mg, 1.6 mmol) in 20 ml of ethanol, was 10 ml of 20% aqueous NaOH added while stirring. The reaction mixture was refluxed under argon for 45 min. After the elimination of ethanol under reduced pressure, the remaining alkaline solution was diluted with water, acidified with 10% aqueous HCl and extracted with ethyl acetate. The organic layer was dried over anhydrous Na_2SO_4 and evaporated to dryness. The resulting residue was chromatographed on silica gel (column chromatography, CH_2Cl_2/MeOH, 5%) and the unstable compound (10) was obtained as a yellowish, sticky solid (440 mg, 62%): ms (m/z): 442 ($M^+ + NH_4$), 424, 352; ir: 1695 cm^{-1}; uv: 240, 251, 287.

O-Benzyl-O-methyldihydrofluorescein (11): To a solution of 7 (4.61 g, 8.73 mmol) in 100 ml of ethanol, was added 80 ml of 20% aqueous NaOH while stirring. The reaction mixture was refluxed for 2 h, then cooled. After elimination of ethanol under reduced pressure, the remaining alkaline solution was acidified with 10% aqueous HCl and extracted with ethyl acetate. The organic layer was dried over anhydrous Na_2SO_4 and evaporated to dryness. The resulting residue was dissolved in 100 ml of glacial acetic acid, and crystallization was initiated by the addition of several drops of water. 11 (3 g, 55%) was obtained as a white crystalline solid: mp 107-108°C; ms (m/z): 439 (M^++1), 437, 421; ir: 1685, 1505 cm^{-1}; uv: 232, 280, 291; ^{1}H-nmr: δ 3.79 (s, 3H), 5.04 (s, 2H), 6.45 (s, 1H), 6.55 (dd, Jo = 8.5 Hz, Jm = 2.5 Hz, 1H), 6.61 (dd, Jo = 8.5 Hz, Jm = 2.5 Hz, 1H), 6.65 (d, Jm = 2.5 Hz, 1H), 6.73 (d, Jm = 2.5 Hz, 1H), 6.97 (m, 2H), 7.14 (d, Jo = 8.0 Hz, 1H), 7.23 (td, Jo = 7.8 Hz, Jm = 1.3 Hz, 1H), 7.4 (m, 6H), 8.00 (dd, Jo = 7.8 Hz, Jm= 1.3Hz, 1H).

O,O-Dibenzyldihydrofluorescein (DBDF) (12): To a mixture of 670 mg of 6 and K_2CO_3 (500 mg) in 50 ml of DMF, benzyl chloride (0.3 ml, 2 mmol) was added. The reaction mixture was heated to

80-90°C and stirred for 3 h. The hot solution was filtered and the solvent was evaporated under reduced pressure. The remaining residue was dissolved in ethyl acetate and the organic phase was washed with water, dried over anhydrous Na2SO4 and evaporated to yield 770 mg (98%) of 8 as a slightly yellow oil. To a solution of 8 (770 mg, 12.7 mmol) in ETOH (80ml), 50 ml of 20% aqueous NaOH was added, and the reaction mixture was refluxed for 2 h. The work-up was done in a similar way as in the case of 11 and compound (12) was obtained as a light beige crystaline solid (400 mg, 61.5%) from glacial acetic acid: mp 186-187°C; ms (m/z): 514, 468, 423, 393, 377; ir: 1695, 1510 cm^{-1}; uv: 230, 280; ^{1}H-nmr: δ 4.96 (s, 4H), 6.42 (s, 1H), 6.51 (br s, 2H), 6.69 (s, 2H), 6.93 (d, Jo = 8.0 Hz, 2H), 7.12 (m, 2H), 7.3 (m, 10H), 7.94 (d, Jo = 8.0 Hz, 1H).

O-Benzyl-O-Methoxyethoxymethyldihydrofluorescein (13): NaH (100 mg) was added to a solution of 6 (2.1 g, 4 mmol) in dry THF (100 ml). After stirring at 0°C for 15 min, MEM chloride (0.45 ml, 4 mmol) was added and the stirring was continiued under N_2 for 3 h at room temperature until the reaction was complete. The reaction mixture was acidified with 10% aqueous HCl and extracted with ether. The organic layer was washed with water, dryed over anhydrous Na_2SO_4 and evaporated to dryness to give compound (9) as a yellowish thick oil (2.44 g, 98%). This material was dissolved in 100 ml of ethanol, 20% aquous NaOH (80 ml) was added. and then the reaction mixture was refluxed for 2 h. The work-up was done in a similar way as in the case of 11. After column chromatography, 13 (1.4 g, 69%) was obtained as a yellow, sticky solid: ir 1710, 1610, 1500 cm^{-1}; ms (m/z): 513 (M^++1, minor), 512, 511 (major). Acid (13) shows a different fragmentation pattern than acids (10), (11), and (12) which showed the M^++1 peak as the major peak.

O-Methyl-O-acetylfluorescein (15): $Pd(OH)_2$ on carbon (50 mg) was added to a solution of 11 (100 mg, 0.2 mmol) in ethyl acetate (10 ml). The reaction mixture was hydrogenated at room temperature while stirring for 2 h, filtered and evaporated to dryness to give the instable compound (14) as a yellowish, amorphous solid, (80 mg). This material was immediately dissolved in pyridine (10 ml) and then was added 5 ml of acetic anhydride. The reaction mixture was stirred at room temperature over night. After the removal of the solvents under reduced pressure, the chromatography on silica gel (preparative tlc, CH_2Cl_2/MeOH, 10%) afforded 15 (60 mg, 67%) as a yellowish, amorphous solid: ms (m/z): 390 (M+), 347, 331,301; ir: 1760, 1610, 1500 cm^{-1}; uv: 234, 278; ^{1}H-nmr (DMSO): δ 2.24 (s, 3H), 3.73 (s, 3H), 6.5-7.3 (m, 10H), 7.68 (d, J = 7Hz, 1H).

O-Benzyl-O-methyldihydrofluorescein methyl ester (16): A mixture of 11 (100 mg, 0.23 mmol) and CH_3I (32 mg, 0.23 mmol) and K_2CO_3 (100 mg) was refluxed in acetone (10 ml) for 3 h, then filtered. After the removal of the solvent under reduced pressure, the remaining solid was dissolved in ethyl acetate and washed two times with 1N NaOH and water. The organic layer was dried over anhydrous Na_2SO_4 and evaporated. The chromatography on silica gel (preparative tlc, CH_2Cl_2) afforded 16 (90 mg, 97%) as yellowish, amorphous solid: ms (m/z) 453 (M^++1), 437, 421, 361; ir: 1720, 1610, 1500 cm^{-1}; uv: 232, 280; ^{1}H-nmr: δ 3.74 (s, 3H), 3.90 (s, 3H), 4.99 (s, 2H), 6.15 (s, 1H), 6.48 (dd, Jo = 8Hz, Jm = 2.5 Hz, 1H), 6.55 (dd, Jo = 8 Hz, Jm = 2.5 Hz, 1H), 6.60 (d, Jm = 2.5 Hz, 1H), 6.68 (d, Jm = 2.5 Hz, 1H), 6.89 (dd, 2H, Jo = 9 Hz, Jm = 3.0 Hz), 7.06 (d, Jo = 8 Hz, 1H), 7.14 (t, Jo = 8 Hz, 1H), 7.30 (m, 6H), 7.75 (d, Jo =8Hz, 1H).

O,O-Dibenzyldihydrofluoresceylhomoveratrylamide (17): A mixture of 12 (180 mg, 0.35 mmol), homoveratrylamine (63 mg, 0.34 mmol), DCC (72 mg), and dimethylaminopyridine (49 mg) in dry CH_2Cl_2 (10 ml) was stirred at room temperature for 18 h. The reaction mixture was evaporated to dryness, and the residue was dissolved in a mixture of ethyl acetate and ether (1:1). The organic layer was washed two times with 1N HCl, two times with 1N NaOH and water, dried over anhydrous Na_2SO_4, and evaporated. The compound (17) (100 mg, 42%) was obtained as a white, crystaline solid: mp 144^{0}C (ethyl acetate - ether); ms (m/z) 677 (M^+), 586, 524, 496; ir: 1630, 1500 cm^{-1}.

Dihydrofluoresceylhomoveratrylamide (18): To a solution of 17 (45 mg, 0.07 mmol) in 10 ml of ethyl acetate was added 20 mg of $Pd(OH)_2$ on carbon. The reaction mixture was hydrogenated at room temperature while stirring for 30 min, filtered, and the organic solution evaporated. After the chromatography on silica gel, preparative tlc, CH_2Cl_2/MeOH 10%), 18 (25 mg, 75%) was obtained as white crystals: mp 236-237^{0}C (acetone - isopropyl ether); ms (m/z): 497 (M^+), 316, 287. Tlc analysis of 18 afforded, after exposure to iodine vapors, red colored spots. The dye, after extraction with ethanol, had a uv maximum at 545 nm.

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