Supporting Information for:

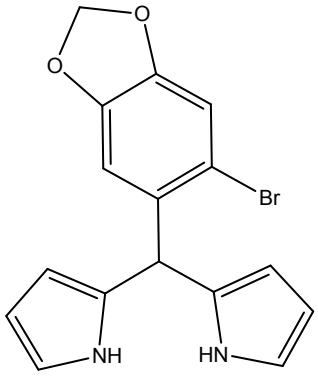
A Bodipy as Luminescent Probe for Detection of the G Protein Estrogen Receptor (GPER)

Teresa Papalia,[#] Rosamaria Lappano,[‡] Anna Barattucci,[†] Assunta Pisano,[‡] Giuseppe Bruno,[†] Maria Francesca Santolla,[‡] Sebastiano Campagna,[†] Paola De Marco,[‡] Fausto Puntoriero,[†] Ernestina Marianna De Francesco,[‡] Camillo Rosano,[§] Marcello Maggiolini,*[,‡] and Paola Bonaccorsi*[,†]

[#] Department of "Scienze del Farmaco e Prodotti per la Salute", v. Annunziata, Università degli Studi di Messina, Polo Universitario, 98168 Messina, Italy, [†]Department of "Scienze Chimiche", v. F. Stagno d'Alcontres, 31, Università degli Studi di Messina, 98166 Messina, Italy, [‡]Department of "Farmacia e Scienze della Salute e della Nutrizione", Università della Calabria, 87036 Rende, Italy, [§]IRCCS Azienda Ospedaliera Universitaria San Martino IST - Istituto Nazionale per la Ricerca sul Cancro, Largo R. Benzi 10 – 16132 Genova, Italy.

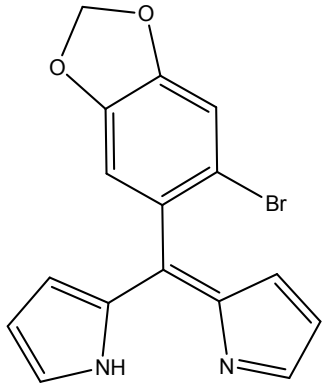
Molecular Modeling and Docking Simulations.
Docking simulations were performed using the program GOLD [The Cambridge Crystallographic Data Center, CCDC, UK]. The three dimensional structure of GPER, previously modeled(1) and employed with success in different cases,(2) was used as a target. The protein active site was described as a sphere of 20Å radius centered on the O atom of Phe 208. The default GOLD parameters were used in order to run the simulations. Some of the protein side chains were considered as flexible, particularly Tyr 123, Gln 138, Phe 206, Phe 208, Glu 275, Phe 278 and His 282. Ligands have been built and energy minimized using the program MarvinSketch [ChemAxon ltd, Budapest, Hu], figures have been drawn with Chimera.(3)

Synthesis and Characterization.
General methods and Materials. Pyrrole was distilled before use. All other chemicals were of standard reagent grade and were used without further purification. All air-sensitive and/or moisture-sensitive reactions were conducted under a dry argon atmosphere. Analytical TLC was performed on Aldrich silica gel 60 F_{254} plates. Compounds were visualized with vanillin [1 g dissolved in MeOH (60 mL) and conc. H_2SO_4 (0.6 mL)] or by examination under UV light. Column chromatography was performed on Aldrich 60 silica gel (40-63 μm). ^{1}H and ^{13}C NMR measurements were performed in $CDCl_3$ solutions at 500.1 and 125.7 MHz respectively on a Varian 500 spectrometer. All chemical shifts are reported in parts per million (δ/ppm) and downfield to tetramethylsilane (Me_4Si) as an internal standard (δ = 0.00 ppm), or referenced to the residual solvent $CDCl_3$ (^{1}H NMR 7.27 ppm and ^{13}C NMR 77.0 ppm). Abbreviations used in the description of resonances are: s (single), d (doublet), br (broad), m (multiplet or overlap of non equivalent resonances). Coupling constants *J* are given in Hz and quoted to the nearest 0.1 Hz. Melting points were determined on a Kofler hot-stage apparatus and are uncorrected. Combustion analyses were carried out on a FISONS EA1108 elemental analyzer.



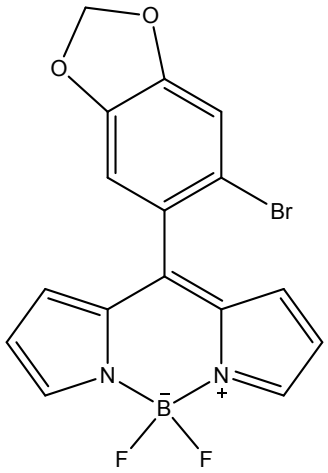
3

2-[(6-Bromobenzo[1,3]dioxol-5-yl)(1H-pyrrol-2-yl)methyl]-1H-pyrrole (3). To a solution of 6-bromo-1,3-benzodioxole-5-carboxaldehyde **2** (2.0 g, 8.7 mmol, 1 equiv.) in freshly distilled pyrrole (15.1 mL, 218.3 mmol, 25 equiv.), sheltered from light and purged with a stream of Ar for 5 min, was added dropwise TFA (100 µL, 1.4 mmol, 0.15 equiv.); the resulting mixture was stirred at room temperature for 1 h. TEA (304 µL, 2.2 mmol, 0.25 equiv.) was then added, and the reaction continued to stir for 15 min. The mixture was poured into toluene (48 mL) and washed with brine (3 x 50 mL). The organic layers were combined, dried over Na_2SO_4, filtered, concentrated under vacuum. The crude was purified by flash chromatography (Hexane/EtOAc 90:10) to give **3** as a white solid (1.9 g, 63%). TLC *Rf* (Hexane/EtOAc 80:20) 0.36; mp 161-163 °C. **^{1}H NMR** (500 MHz, $CDCl_3$): δ 8,01 (brs, 2H), 7.02 (s, 1H), 6.72 (bd, *J* = 5.9, 2H), 6.62 (s, 1H), 6.17 (bdd, *J* = 5.9, 3.0, 2H), 5.95 (s, 2H), 5.92 (brd, *J* = 3.0, 2H), 5.83 (s, 1H); **^{13}C NMR** (125 MHz, $CDCl_3$): δ 147.6, 147.2, 134.9, 131.4, 117.3, 114.4, 112.6, 109.6, 108.6, 107.4, 101.7, 43.2. Anal. Calcd for $C_{16}H_{13}BrN_2O_2$: C, 55.67; H, 3.80; N, 8.12; Found: C, 55.77; H, 3.79; N, 8.10.



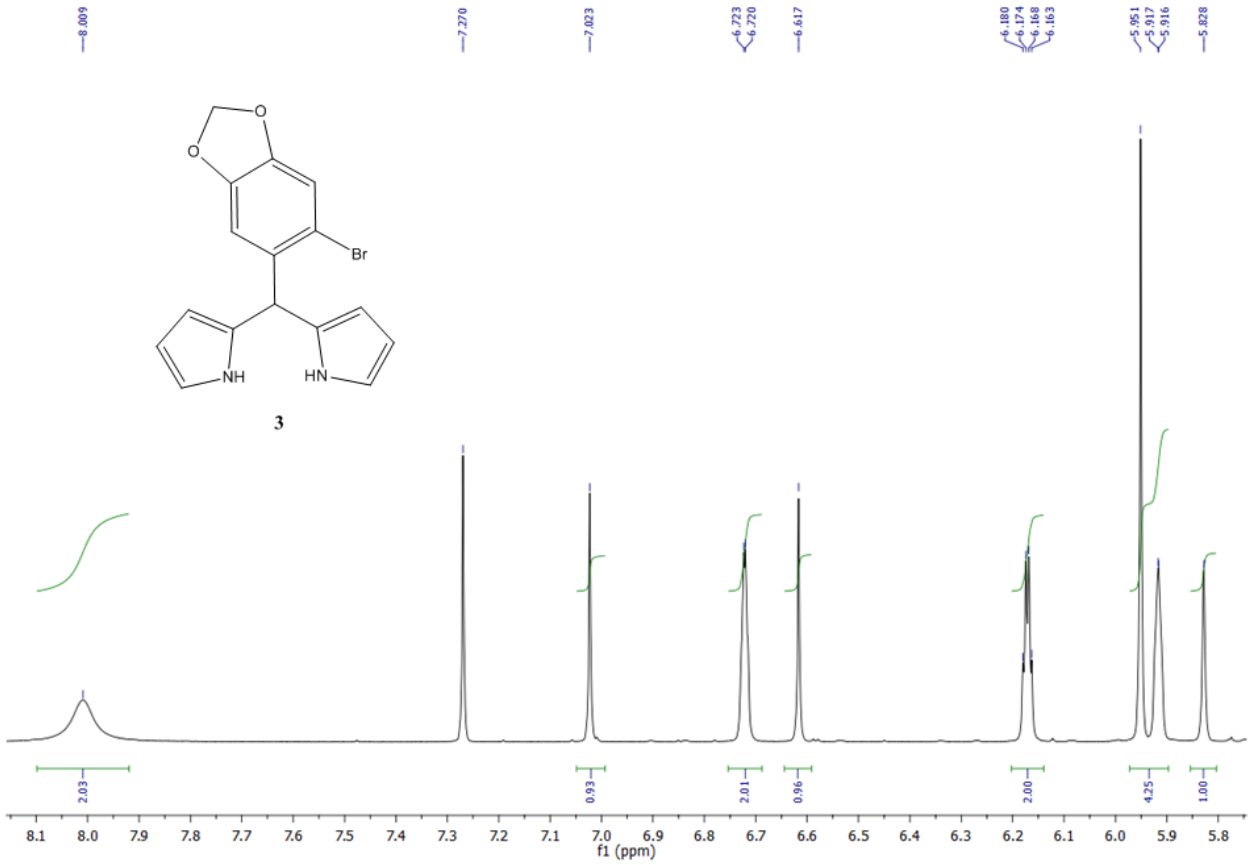
4

2-[(6-Bromobenzo[1,3]dioxol-5-yl)1H-pyrrol-2-ylidenemethyl]-1H-pyrrole (4). *p*-Chloranil (1.45 g, 5.9 mmol, 2.04 equiv.) was added to a solution of **3** (1.0 g, 2.9 mmol, 1 equiv.) in DCM (20 mL); the reaction was stirred overnight at room temperature. The mixture was concentrated under vacuum and the orange-yellow obtained residue was purified by flash chromatography (Hexane/EtOAc 90:10). **4** was isolated as a dark yellow solid (0.66 g, 65%). TLC *Rf* (Hexane/EtOAc 80:20) 0.40; mp 147-150 °C. **^{1}H NMR** (500 MHz, $CDCl_3$): δ 7.72 (bd, *J* = 1.0, 2H), 7.12 (s, 1H), 6.88 (s, 1H), 6.53 (d, *J* = 4.4, 2H), 6.42 (dd, *J* = 4.4, 1.0, 2H), 6.09 (s, 2H); **^{13}C NMR** (125 MHz, $CDCl_3$): δ 146.9, 144.3, 140.8, 131.0, 130.5, 117.8, 114.7, 112.7, 111.5, 102.3. Anal. Calcd for $C_{16}H_{11}BrN_2O_2$ (343,17): C, 56.00; H, 3.23; N, 8.16; Found C, 55.94; H, 3.23; N, 8.15.

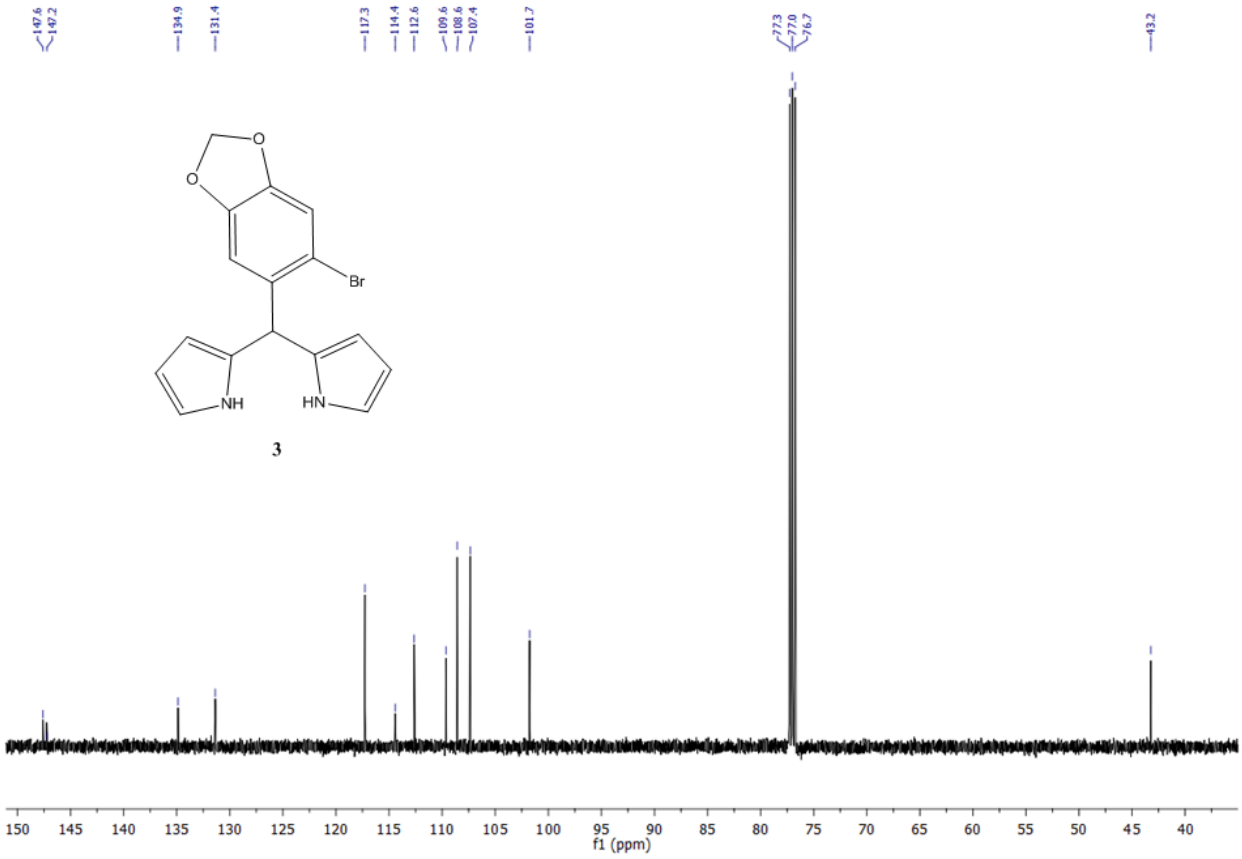


1

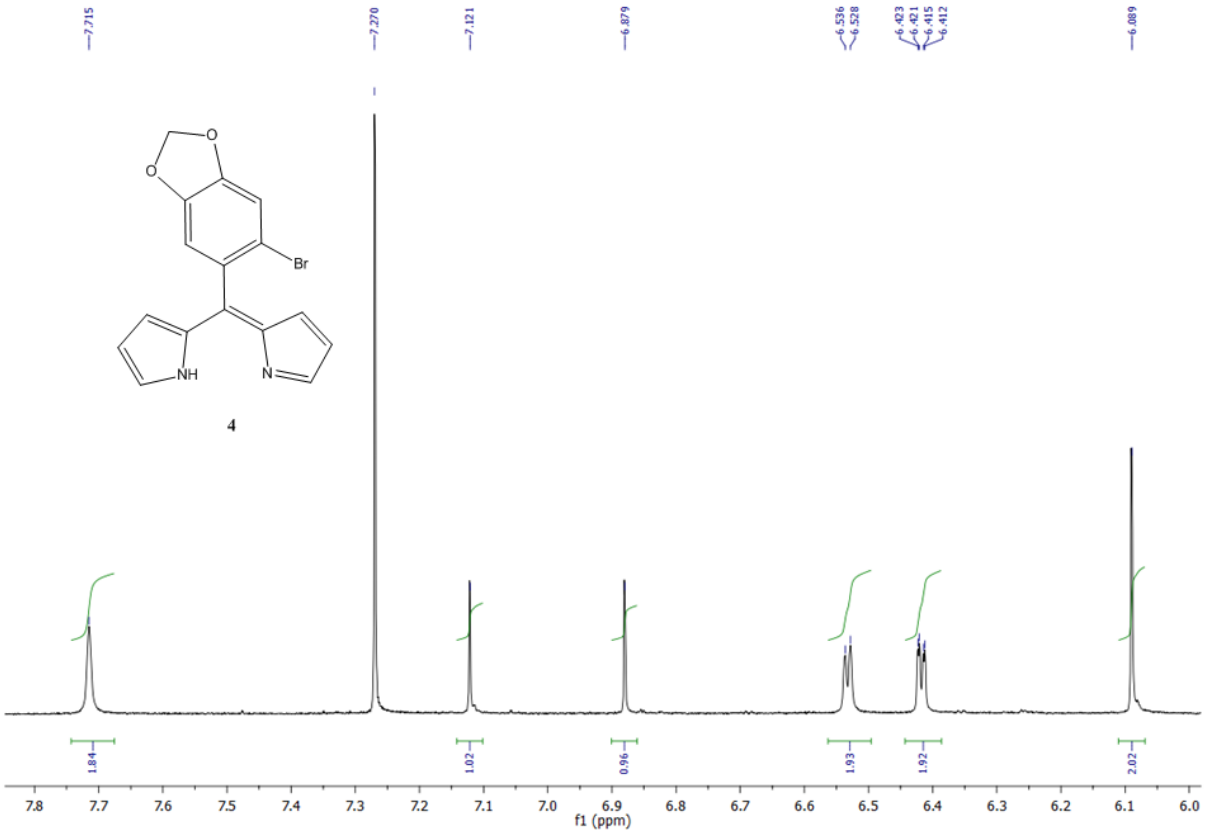
Bodipy 1. Anhydrous TEA (0.47 mL, 3.2 mmol, 4.4 equiv.) was added dropwise to a solution of **4** (0.25 g, 0.73 mmol, 1 equiv.) in anhydrous DCM (8 mL); the resulting mixture was stirred for 20 min. $BF_3 \cdot OEt_2$ (0.80mL, 6.49 mmol, 8.9 equiv.) was then added dropwise, and the reaction was stirred for 1h at room temperature. The mixture was quenched with water (5 mL) and then extracted with DCM (3 x 15 mL). The combined organic layers were dried over Na_2SO_4. After filtration of the inorganic solid, the solvent was removed under reduced pressure. Purification of the crude by flash chromatography (Hexane/EtOAc 90:10) gave **1** as a orange-red solid (0.17 g, 60%). TLC *Rf* (Hexane/EtOAc 70:30) 0.54; mp 160-163 °C. **^{1}H NMR** (500 MHz, $CDCl_3$): δ 7.94 (bs, 2H), 7.16 (s, 1H), 6.88 (s, 1H), 6.79 (d, J = 4.4, 2H), 6.52 (bd, J = 4.4, 2H), 6.11 (s, 2H); **^{13}C NMR** (125 MHz, $CDCl_3$): δ 149.7, 147.2, 144.9, 135.4, 130.9, 127.2, 118.8, 113.8, 113.3, 111.1, 102.5. Anal. Calcd for. $C_{16}H_{10}BBrF_2N_2O_2$ (390,97): C, 49.15; H, 2.58; N, 7.17; Found: C, 49.12; H, 2.58; N, 7.18.



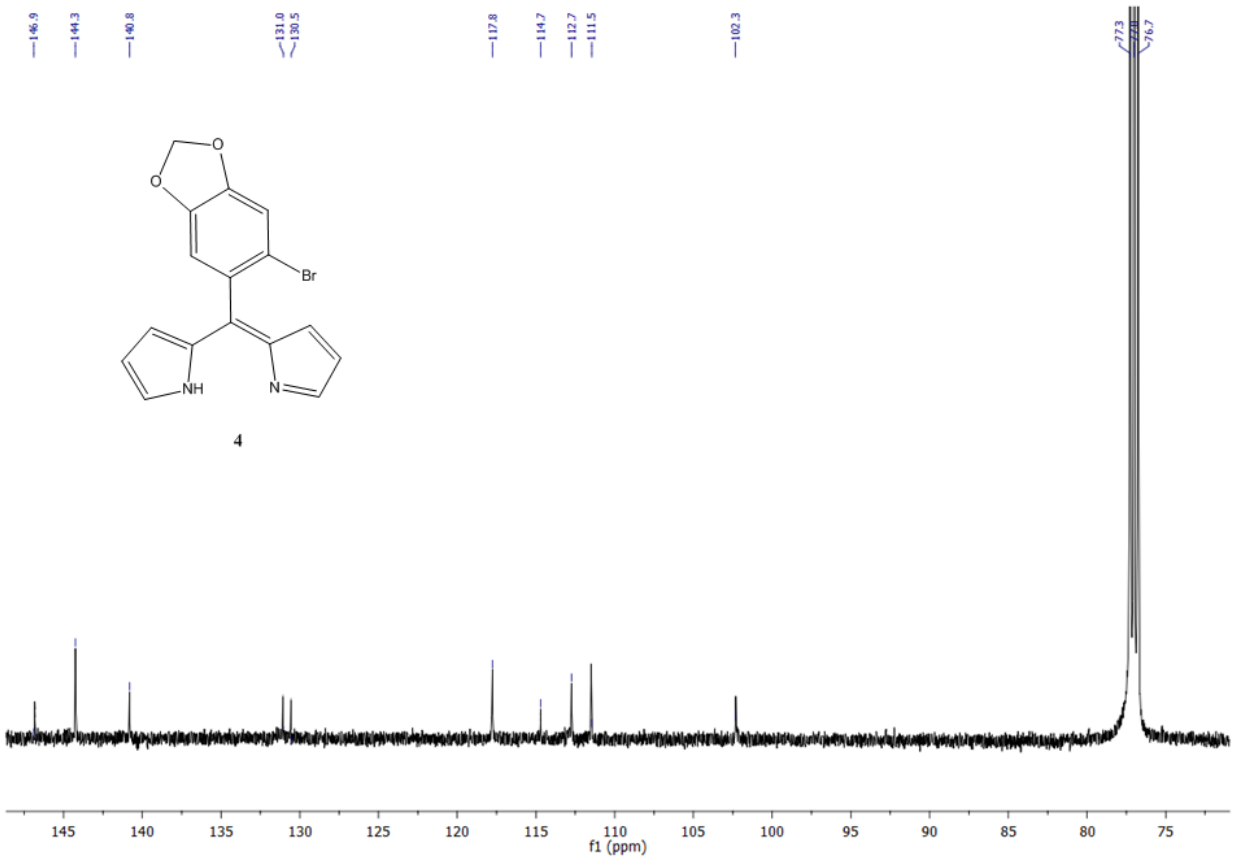
^{1}H NMR (500 MHz, $CDCl_3$) of Compound **3**



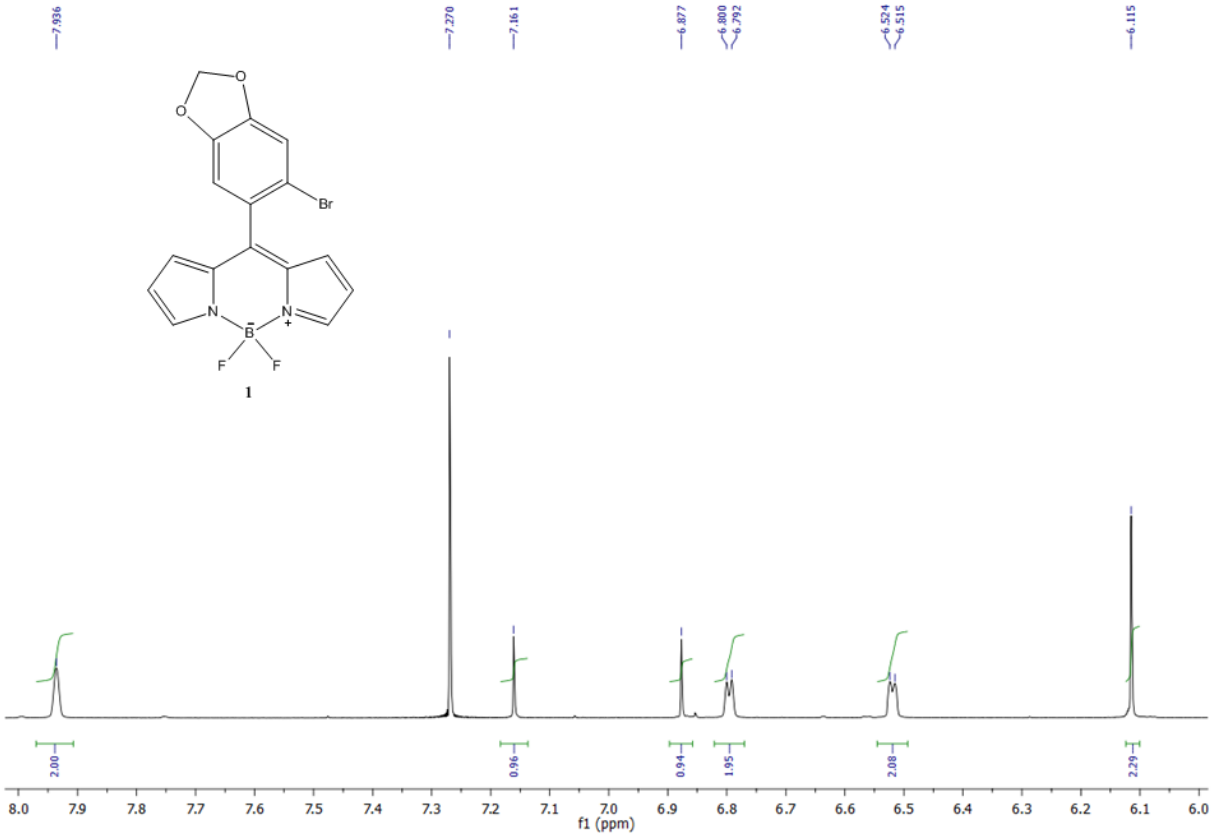
^{13}C NMR (125 MHz, $CDCl_3$) of Compound **3**



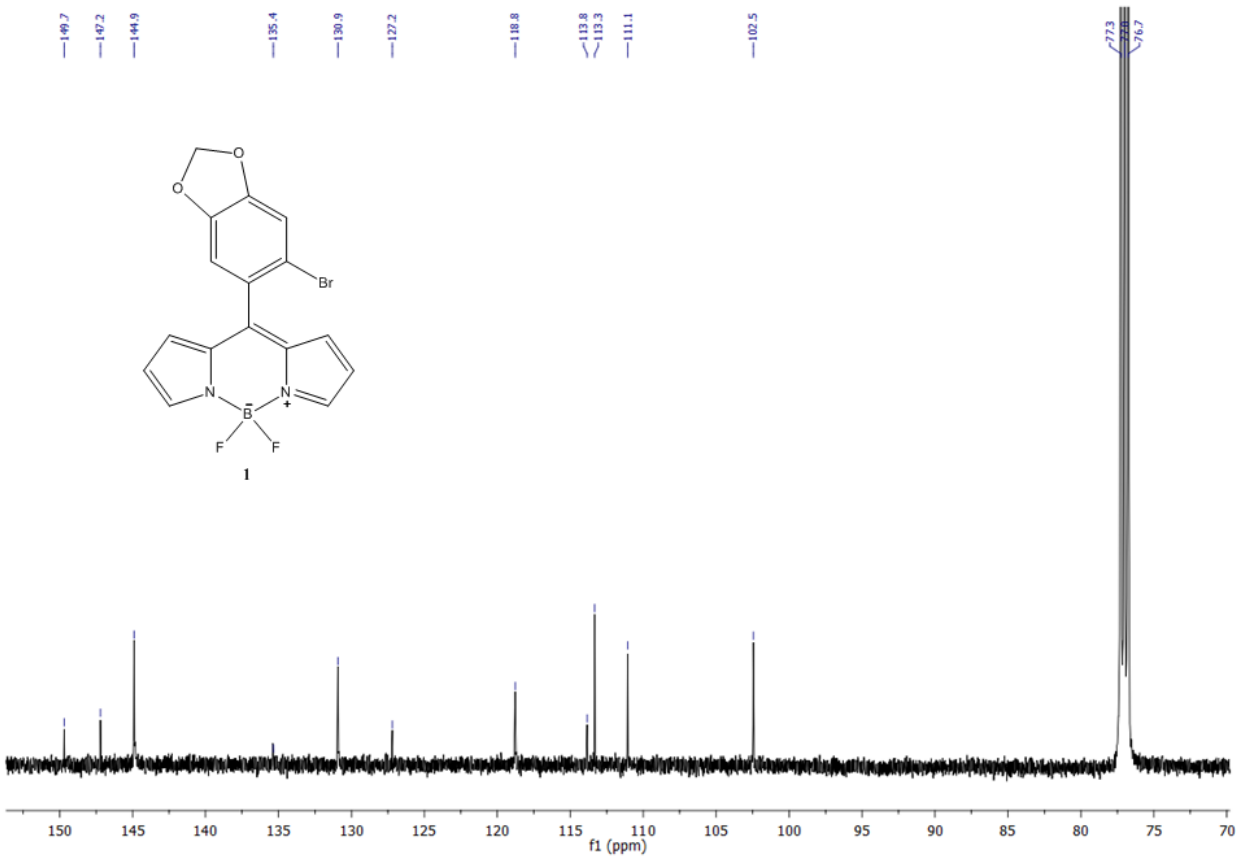
^{1}H NMR (500 MHz, $CDCl_3$) of Compound **4**



^{13}C NMR (125 MHz, $CDCl_3$) of Compound **4**



^{1}H NMR (500 MHz, $CDCl_3$) of Compound **1**



^{13}C NMR (125 MHz, $CDCl_3$) of Compound **1**

Crystal structure determination.

Red-orange crystals of suitable quality for X-ray analysis were selected from the crystals formed in acetone of Bodipy **1**.

Data were collected at room temperature with a Bruker APEX II CCD area-detector diffractometer using MoKα radiation (λ = 0.71073 Å). Data collection, cell refinement, data reduction and absorption correction were performed using multiscan methods with Bruker software.(4) The structures were solved by direct methods using SIR2004.(5)

The non hydrogen atoms were refined anisotropically by the full matrix least squares method on F^2 using SHELXL.(6) All the hydrogen (H) atoms were placed at the calculated positions and constrained to ride on their parent atoms. The most relevant crystallographic data are reported in Tables Sn.

SUPPLEMENTARY TABLES for BODIPY1

Table SI. Crystal data

Crystal data

a =	6.7070(0.0001)	alpha=	110.62(0.00)
b =	9.4550(0.0002)	beta =	95.01(0.00)
c =	12.9340(0.0003)	gamma=	95.59(0.00)
V =	757.49(0.03) Å3		

Niggli reduced cell: 6.707 9.455 12.934 110.62 95.01 95.59

Niggli matrix:

44.9838	89.3970	167.2884
-43.0590	-7.5712	-6.1816

Transformation matrix:

1.00	0.00	0.00
0.00	1.00	0.00
0.00	0.00	1.00

C 16. H 10. B 1. N 2. O 2. F 2. Br 1.

M	= 390.978	(Atomic weights 1977)
Z	= 2.00	
D(calc.)	= 1.7142	Mg/m**3
F(000)	= 388.0	
mu	= 27.470	cm**-1 (Int.Tab. Vol.C, Table 4.2.4.2, p.193)
Lambda	= 0.7107300	Angstrom

Table SII

Fractional atomic coordinates and isotropic temperature factors (Angstrom squared), with standard deviations in the least significant digits in parentheses. For anisotropic atoms, the equivalent isotropic temperature factors are shown.

	x/a	y/b	z/c	U
BR(1)	0.92985(3)	0.07007(2)	0.29878(2)	0.04091
O(2)	0.4891(2)	-0.2902(2)	-0.1537(1)	0.04889
F(2)	0.6156(2)	-0.5346(1)	0.4017(1)	0.06438
O(1)	0.6812(2)	-0.0607(2)	-0.1278(1)	0.04237
C(5)	0.7969(3)	0.0031(2)	0.0709(1)	0.03359
H(1D)	0.86999	0.09711	0.08179	0.04030
F(1)	0.8265(2)	-0.3259(2)	0.5108(1)	0.07460
N(2)	0.5551(2)	-0.2930(2)	0.3912(1)	0.04128

C(1)	0.6758(3)	-0.1918(2)	0.1428(1)	0.03262
C(2)	0.5658(3)	-0.2790(2)	0.0382(2)	0.03892
H(2)	0.48568	-0.37049	0.02692	0.04671
N(1)	0.8448(3)	-0.4189(2)	0.3138(1)	0.04261
C(4)	0.6943(3)	-0.0880(2)	-0.0302(1)	0.03241
C(6)	0.7862(2)	-0.0529(2)	0.1577(1)	0.03122
C(15)	0.3883(3)	-0.1395(3)	0.3240(2)	0.04955
H(7)	0.34919	-0.08374	0.28098	0.05946
C(8)	0.6829(3)	-0.2585(2)	0.2319(1)	0.03438
C(9)	0.8271(3)	-0.3554(2)	0.2323(2)	0.03994
C(16)	0.5490(3)	-0.2278(2)	0.3095(2)	0.03765
C(13)	0.4066(3)	-0.2460(3)	0.4518(2)	0.05470
H(11)	0.37763	-0.27312	0.51193	0.06564
B(1)	0.7116(4)	-0.3958(3)	0.4083(2)	0.04249
C(7)	0.5767(3)	-0.1994(2)	-0.2110(2)	0.04383
H(13A)	0.47206	-0.17690	-0.25721	0.05260
H(13B)	0.67025	-0.25376	-0.25834	0.05260
C(3)	0.5803(3)	-0.2250(2)	-0.0461(1)	0.03536
C(14)	0.3005(4)	-0.1507(3)	0.4129(2)	0.06177
H(15)	0.19142	-0.10401	0.44183	0.07412
C(12)	0.9929(4)	-0.5063(3)	0.2926(2)	0.06246
H(16)	1.03597	-0.56190	0.33497	0.07495
C(10)	0.9710(4)	-0.4074(3)	0.1606(2)	0.05667
H(1C)	0.99320	-0.38227	0.09881	0.06800
C(11)	1.0728(4)	-0.5019(3)	0.1983(2)	0.07389
H(1B)	1.17643	-0.55358	0.16663	0.08866

Table SIII

Vibration parameters (Angstrom squared) in the expression:
-2(pi squared)(U11((h.a*)squared) + U22((k.b*)squared) +
U33((l.c*)squared) + 2.U12.h.k.a*.b* + 2.U13.h.l.a*.c* +
2.U23.k.l.b*.c*)

	U11	U22	U33	U12	U13	U23
BR(1)	0.0441(1)	0.0458(1)	0.0317(1)	0.0009(1)	0.0004(1)	0.0152(1)
O(2)	0.0643(9)	0.0485(8)	0.0295(7)	-.0041(7)	-.0035(6)	0.0141(6)
F(2)	0.108(1)	0.042(1)	0.055(1)	0.005(1)	0.026(1)	0.030(1)
O(1)	0.0534(8)	0.0489(8)	0.0308(6)	0.0042(6)	0.0056(6)	0.0225(6)
C(5)	0.0345(8)	0.0350(9)	0.0366(9)	0.0038(7)	0.0080(7)	0.0190(8)
F(1)	0.092(1)	0.077(1)	0.042(1)	0.019(1)	-0.022(1)	0.010(1)
N(2)	0.0527(9)	0.0443(9)	0.0364(8)	0.0059(7)	0.0073(7)	0.0262(7)
C(1)	0.0401(9)	0.0326(9)	0.0314(9)	0.0097(7)	0.0077(7)	0.0172(7)
C(2)	0.050(1)	0.031(1)	0.037(1)	0.002(1)	0.004(1)	0.016(1)
N(1)	0.0519(9)	0.0376(9)	0.0476(9)	0.0094(7)	0.0038(7)	0.0265(8)
C(4)	0.0352(8)	0.0402(10)	0.0298(8)	0.0108(7)	0.0085(7)	0.0200(8)
C(6)	0.0331(8)	0.0354(9)	0.0282(8)	0.0074(7)	0.0052(6)	0.0143(7)
C(15)	0.055(1)	0.058(1)	0.052(1)	0.019(1)	0.017(1)	0.036(1)
C(8)	0.0443(9)	0.0302(9)	0.0320(9)	0.0032(7)	0.0023(7)	0.0166(7)
C(9)	0.050(1)	0.036(1)	0.040(1)	0.009(1)	0.006(1)	0.021(1)
C(16)	0.047(1)	0.040(1)	0.035(1)	0.008(1)	0.007(1)	0.023(1)
C(13)	0.064(1)	0.069(1)	0.046(1)	0.014(1)	0.020(1)	0.036(1)
B(1)	0.063(1)	0.038(1)	0.029(1)	0.006(1)	-0.002(1)	0.018(1)
C(7)	0.054(1)	0.052(1)	0.031(1)	0.014(1)	0.007(1)	0.019(1)
C(3)	0.0413(9)	0.0366(10)	0.0295(9)	0.0081(7)	0.0048(7)	0.0130(8)
C(14)	0.066(1)	0.081(2)	0.061(1)	0.030(1)	0.030(1)	0.042(1)
C(12)	0.072(1)	0.057(1)	0.080(2)	0.028(1)	0.014(1)	0.044(1)
C(10)	0.071(1)	0.056(1)	0.061(1)	0.028(1)	0.027(1)	0.034(1)
C(11)	0.084(2)	0.073(2)	0.092(2)	0.047(1)	0.037(2)	0.047(2)

Table SIV

Complete listing of torsion angles

H(1D) - C(5) - C(4) - O(1)	0.3
H(1D) - C(5) - C(4) - C(3)	-177.8
H(1D) - C(5) - C(6) - BR(1)	-0.7
H(1D) - C(5) - C(6) - C(1)	179.2
C(4) - C(5) - C(6) - BR(1)	179.3
C(6) - C(5) - C(4) - O(1)	-179.7
C(4) - C(5) - C(6) - C(1)	-0.8
C(6) - C(5) - C(4) - C(3)	2.2
C(13) - N(2) - C(16) - C(15)	-0.2
C(13) - N(2) - C(16) - C(8)	-178.6
C(16) - N(2) - C(13) - H(11)	180.0
C(16) - N(2) - C(13) - C(14)	0.0
C(16) - N(2) - B(1) - F(2)	-122.9
C(16) - N(2) - B(1) - F(1)	116.3
C(16) - N(2) - B(1) - N(1)	-3.8
B(1) - N(2) - C(16) - C(15)	-178.4
B(1) - N(2) - C(16) - C(8)	3.2
C(13) - N(2) - B(1) - F(2)	59.3
C(13) - N(2) - B(1) - F(1)	-61.5
C(13) - N(2) - B(1) - N(1)	178.4
B(1) - N(2) - C(13) - H(11)	-1.9
B(1) - N(2) - C(13) - C(14)	178.1
C(2) - C(1) - C(6) - BR(1)	178.2
C(2) - C(1) - C(6) - C(5)	-1.7
C(6) - C(1) - C(2) - H(2)	-177.2
C(6) - C(1) - C(2) - C(3)	2.8
C(8) - C(1) - C(2) - H(2)	7.7
C(2) - C(1) - C(8) - C(9)	83.9
C(2) - C(1) - C(8) - C(16)	-94.7
C(8) - C(1) - C(2) - C(3)	-172.3
C(8) - C(1) - C(6) - BR(1)	-6.9
C(8) - C(1) - C(6) - C(5)	173.2
C(6) - C(1) - C(8) - C(9)	-91.0
C(6) - C(1) - C(8) - C(16)	90.4
C(1) - C(2) - C(3) - O(2)	179.5
C(1) - C(2) - C(3) - C(4)	-1.6
H(2) - C(2) - C(3) - O(2)	-0.5
H(2) - C(2) - C(3) - C(4)	178.4
C(9) - N(1) - B(1) - F(2)	121.0
C(9) - N(1) - B(1) - F(1)	-118.2
C(9) - N(1) - B(1) - N(2)	1.4
B(1) - N(1) - C(9) - C(8)	1.6
B(1) - N(1) - C(9) - C(10)	-178.3
C(12) - N(1) - C(9) - C(8)	179.7
C(9) - N(1) - C(12) - H(16)	179.8
C(12) - N(1) - C(9) - C(10)	-0.1
C(9) - N(1) - C(12) - C(11)	-0.2
C(12) - N(1) - B(1) - F(2)	-56.7
C(12) - N(1) - B(1) - F(1)	64.1
C(12) - N(1) - B(1) - N(2)	-176.3
B(1) - N(1) - C(12) - H(16)	-2.1
B(1) - N(1) - C(12) - C(11)	177.9
O(1) - C(4) - C(3) - O(2)	-0.4
O(1) - C(4) - C(3) - C(2)	-179.5
C(5) - C(4) - C(3) - O(2)	178.1
C(5) - C(4) - C(3) - C(2)	-1.0

H(7) - C(15) - C(16) - N(2)	-179.7
H(7) - C(15) - C(16) - C(8)	-1.5
H(7) - C(15) - C(14) - C(13)	179.7
H(7) - C(15) - C(14) - H(15)	-0.3
C(14) - C(15) - C(16) - N(2)	0.3
C(14) - C(15) - C(16) - C(8)	178.5
C(16) - C(15) - C(14) - C(13)	-0.3
C(16) - C(15) - C(14) - H(15)	179.7
C(1) - C(8) - C(9) - N(1)	178.7
C(1) - C(8) - C(9) - C(10)	-1.5
C(1) - C(8) - C(16) - N(2)	178.9
C(1) - C(8) - C(16) - C(15)	0.9
C(9) - C(8) - C(16) - N(2)	0.4
C(16) - C(8) - C(9) - N(1)	-2.6
C(9) - C(8) - C(16) - C(15)	-177.7
C(16) - C(8) - C(9) - C(10)	177.1
N(1) - C(9) - C(10) - H(1C)	-179.6
N(1) - C(9) - C(10) - C(11)	0.4
C(8) - C(9) - C(10) - H(1C)	0.6
C(8) - C(9) - C(10) - C(11)	-179.4
N(2) - C(13) - C(14) - C(15)	0.2
N(2) - C(13) - C(14) - H(15)	-179.8
H(11) - C(13) - C(14) - C(15)	-179.8
H(11) - C(13) - C(14) - H(15)	0.2
N(1) - C(12) - C(11) - C(10)	0.4
N(1) - C(12) - C(11) - H(1B)	-179.6
H(16) - C(12) - C(11) - C(10)	-179.6
H(16) - C(12) - C(11) - H(1B)	0.4
C(9) - C(10) - C(11) - C(12)	-0.5
C(9) - C(10) - C(11) - H(1B)	179.5
H(1C) - C(10) - C(11) - C(12)	179.5
H(1C) - C(10) - C(11) - H(1B)	-0.5

Table SV
Complete listing of bond distances (Angstroms)

BR(1) - C(6)	1.895(2)	O(2) - C(3)	1.365(3)
F(2) - B(1)	1.375(3)	O(1) - C(4)	1.372(3)
C(5) - H(1D)	0.930(2)	C(5) - C(4)	1.365(3)
C(5) - C(6)	1.403(3)	F(1) - B(1)	1.375(3)
N(2) - C(16)	1.398(3)	N(2) - C(13)	1.335(3)
N(2) - B(1)	1.552(3)	C(1) - C(2)	1.406(3)
C(1) - C(6)	1.385(3)	C(1) - C(8)	1.495(3)
C(2) - H(2)	0.930(2)	C(2) - C(3)	1.365(3)
N(1) - C(9)	1.388(3)	N(1) - B(1)	1.543(3)
N(1) - C(12)	1.339(4)	C(4) - C(3)	1.377(3)
C(15) - H(7)	0.930(3)	C(15) - C(16)	1.412(3)
C(15) - C(14)	1.368(4)	C(8) - C(9)	1.396(3)
C(8) - C(16)	1.378(3)	C(9) - C(10)	1.400(4)
C(13) - H(11)	0.930(3)	C(13) - C(14)	1.398(4)
C(7) - H(13A)	0.970(2)	C(7) - H(13B)	0.970(3)
C(14) - H(15)	0.930(3)	C(12) - H(16)	0.930(3)
C(12) - C(11)	1.386(4)	C(10) - H(1C)	0.930(3)
C(10) - C(11)	1.368(4)	C(11) - H(1B)	0.930(3)

Table SVI
Complete listing of bond angles (degrees)

H(1D)-C(5)-C(4)	121.8(2)	H(1D)-C(5)-C(6)	121.8(2)
C(4)-C(5)-C(6)	116.3(2)	C(16)-N(2)-C(13)	107.0(2)
C(16)-N(2)-B(1)	125.4(2)	C(13)-N(2)-B(1)	127.5(2)
C(2)-C(1)-C(6)	119.4(2)	C(2)-C(1)-C(8)	118.5(2)
C(6)-C(1)-C(8)	122.0(2)	C(1)-C(2)-H(2)	121.1(2)
C(1)-C(2)-C(3)	117.8(2)	H(2)-C(2)-C(3)	121.1(2)
C(9)-N(1)-B(1)	125.5(2)	C(9)-N(1)-C(12)	107.3(2)
B(1)-N(1)-C(12)	127.1(2)	O(1)-C(4)-C(5)	127.9(2)
O(1)-C(4)-C(3)	109.9(2)	C(5)-C(4)-C(3)	122.2(2)
BR(1)-C(6)-C(5)	117.3(2)	BR(1)-C(6)-C(1)	120.4(2)
C(5)-C(6)-C(1)	122.3(2)	H(7)-C(15)-C(16)	126.2(3)
H(7)-C(15)-C(14)	126.2(3)	C(16)-C(15)-C(14)	107.7(2)
C(1)-C(8)-C(9)	117.6(2)	C(1)-C(8)-C(16)	121.1(2)
C(9)-C(8)-C(16)	121.3(2)	N(1)-C(9)-C(8)	120.7(2)
N(1)-C(9)-C(10)	107.9(2)	C(8)-C(9)-C(10)	131.4(2)
N(2)-C(16)-C(15)	107.7(2)	N(2)-C(16)-C(8)	120.5(2)
C(15)-C(16)-C(8)	131.8(2)	N(2)-C(13)-H(11)	124.5(3)
N(2)-C(13)-C(14)	111.0(2)	H(11)-C(13)-C(14)	124.5(3)
F(2)-B(1)-F(1)	109.3(2)	F(2)-B(1)-N(2)	110.5(2)
F(2)-B(1)-N(1)	109.7(2)	F(1)-B(1)-N(2)	110.0(2)
F(1)-B(1)-N(1)	110.8(2)	N(2)-B(1)-N(1)	106.5(2)
H(13A)-C(7)-H(13B)	108.5(2)	O(2)-C(3)-C(2)	128.0(2)
O(2)-C(3)-C(4)	110.1(2)	C(2)-C(3)-C(4)	121.9(2)
C(15)-C(14)-C(13)	106.7(3)	C(15)-C(14)-H(15)	126.7(3)
C(13)-C(14)-H(15)	126.7(3)	N(1)-C(12)-H(16)	124.9(3)
N(1)-C(12)-C(11)	110.2(3)	H(16)-C(12)-C(11)	124.9(3)
C(9)-C(10)-H(1C)	126.3(3)	C(9)-C(10)-C(11)	107.3(3)
H(1C)-C(10)-C(11)	126.3(3)	C(12)-C(11)-C(10)	107.3(3)
C(12)-C(11)-H(1B)	126.4(3)	C(10)-C(11)-H(1B)	126.4(3)

Table SVII
Possible hydrogen bonds

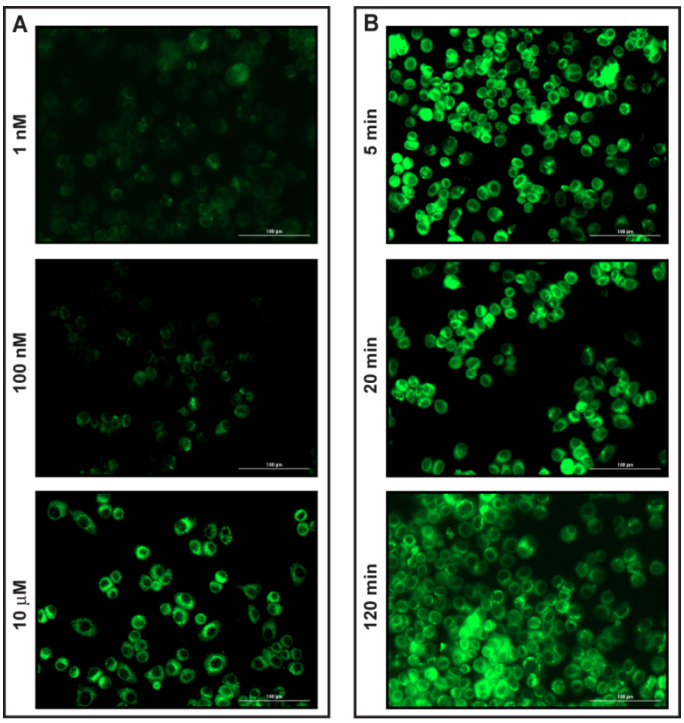
Donor-H	Donor...Acceptor	H...Acceptor	Donor-H.....Acceptor
C15 -H7	C15 ...O1 (1)	H7 ...O1 (1)	C15 -H7 ...O1 (1)
0.930(.003)	3.686(.003)	2.778(.002)	165.53(0.15)
1.080		2.633	164.72 (**)
C13 -H11	C13 ...F2 (2)	H11 ...F2 (2)	C13 -H11 ...F2 (2)
0.930(.003)	3.267(.003)	2.448(.002)	146.88(0.16)
1.080		2.324	144.85 (**)
C7 -H13A	C7 ...F2 (3)	H13A ...F2 (3)	C7 -H13A ...F2 (3)
0.970(.002)	2.918(.002)	2.680(.001)	94.30(0.12)
1.080		2.674	91.95 (**)
C7 -H13B	C7 ...F2 (3)	H13B ...F2 (3)	C7 -H13B ...F2 (3)
0.970(.002)	2.918(.002)	2.671(.001)	94.84(0.12)
1.080		2.664	92.49 (**)
C12 -H16	C12 ...F1 (4)	H16 ...F1 (4)	C12 -H16 ...F1 (4)
0.930(.003)	3.623(.004)	2.705(.002)	169.57(0.17)
1.080		2.557	168.96 (**)
C11 -H1B	C11 ...O2 (5)	H1B ...O2 (5)	C11 -H1B ...O2 (5)

```
0.930(.003)      3.668(.003)          2.789(.002)          157.99( 0.19)
1.080                                 2.651                156.77       (**)


Equivalent positions:
( 1)    -x+1,-y,-z
( 2)    -x+1,-y-1,-z+1
( 3)    -x+1,-y-1,-z
( 4)    -x+2,-y-1,-z+1
( 5)    -x+2,-y-1,-z
```

Fluorescence properties of Bodipy 1.

Fluorescence micrographs of live SkBr3 cells treated with Bodipy 1. (A) Cells were treated for 20 min with increasing concentrations of Bodipy 1, as indicated. (B) Cells were treated with 10 µM Bodipy 1 and monitored up to 2 h, as indicated. Each experiment shown is representative of 20 random fields of three independent experiments.



Photophysical Data.

UV/Vis absorption spectra in solution were recorded with a Jasco V-560 spectrophotometer. For steady-state luminescence measurements, a Jobin Yvon-Spex Fluoromax P spectrofluorimeter equipped with a Hamamatsu R3896 photomultiplier was used. The spectra were corrected for the photomultiplier response using a program purchased with the fluorimeter. For the luminescence lifetimes, an Edinburgh OB 900 time-correlated single-photon-counting spectrometer was used.

Hamamatsu PLP 2 laser diode (59 ps pulse width at 408 nm) and/or nitrogen discharge (pulse width 2 ns at 337 nm) were employed as excitation sources. Emission quantum yields for deaerated solutions were determined by the optically diluted method with Rhodamine 6G in air equilibrated ethanolic solution as quantum yield standard (Φ = 0.91).(7)

Biological Data.

Materials and Methods

Reagents and cell culture. 17β-Estradiol (E2) was purchased from Sigma-Aldrich Srl (Milan, Italy) and solubilized in ethanol. G-1 (1-[4-(-6-bromobenzol[1,3]diodo-5-yl)-3a,4,5,9b-tetrahidro3H5cyclopenta[c]quinolin-8yl]-ethanone) and G-15 (3a*S**,4*R**,9b*R**)-4-(6-Bromo-1,3-benzodioxol-5-yl)-3a,4,5,9b-3H-cyclopenta[c]quinoline were bought from Tocris Bioscience (Bristol, United Kingdom) and dissolved in dimethyl sulfoxide (DMSO). Nicotinic acid (pyridine-3-carboxylic acid) was purchased from Sigma-Aldrich Srl. (Milan, Italy) and solubilized in water. Bodipy 1 was dissolved in DMSO. SkBr3 breast cancer cells were maintained in RPMI 1640 without phenol red supplemented with 10% FBS and 100 mg/mL penicillin/streptomycin (Life Technologies, Milan, Italy).

Ligand binding assays. In ligand binding assays, SkBr3 cells were grown in 10-cm cell culture dishes, washed two times and incubated either with 1 nM [2,4,6,7-3H]E2 (89 Ci/ mmol; Amersham Bioscience, GE Healthcare, Milan, Italy) or with 50 nM [5,6-3H] nicotinic acid (50–60 Ci/mmol; BIOTREND, Chemikalien GmbH Technologiezentrum Köln Eupener Str. 157 D-50933 Köln) in the presence or absence of increasing concentration of nonlabeled competitors, as indicated. Then, cells were incubated for 2 h at 37°C and washed three times with ice-cold PBS; the radioactivity collected by 100% ethanol extraction was measured by liquid scintillation counting. Competitor binding was expressed as a percentage of maximal specific binding.

Fluorescent microscopy experiments. Cells were seeded in Lab-Tek II chamber slides at a density of 1 x 10^5 per well and incubated for 24 h in the maintenance media. For immunofluorescence staining, cells were transfected, using X-tremeGENE 9 DNA Transfection Reagent (Roche Molecular Biochemicals, Milan, Italy), with a recombinant GPER-FLAG for 36 h in serum free medium, treated for 20 min with Bodipy 1, fixed in 4% paraformaldehyde, permeabilized with 0.2% Triton X-100, washed 3 times with PBS and incubated overnight with a primary monoclonal anti-flag antibody (10 µg/mL, from Sigma-Aldrich, Milan, Italy). After incubation, the slides were extensively washed with PBS and incubated with donkey anti-mouse IgG-R (1:200, purchased from Santa Cruz Santa Cruz Biotechnology, DBA, Milan, Italy) and 40,6-diamidino-2-phenylindole dihydrochloride (DAPI) (1:1000, Sigma-Aldrich, Milan, Italy). As it concerns the in vivo fluorescent microscopy experiments, cells (1 x 10^5) were seeded in 24-well plates in regular growth medium. After 24 h, cells were incubated with the indicated treatments at 37 °C in an atmosphere containing 5% CO_2. A Cytation 3 Cell Imaging Multi-Mode Reader (BioTek, Thermo Fisher Scientific Inc., Waltham, USA) was used for experiment evaluation.

Statistical analysis. Statistical analysis was done using ANOVA followed by Newman-Keuls' testing to determine differences in means. $P<0.05$ was considered as statistically significant.

References

(1) a) R. Lappano, C. Rosano, P. De Marco, E. M. De Francesco, V. Pezzi and M. Maggiolini, *Mol Cell Endocrinol*., 2010, **320**, 162; b) C. Rosano, R. Lappano, M.F. Santolla, M. Ponassi, A. Donadini and M. Maggiolini, *Curr Med Chem*., 2012, **19**, 6199.

(2) a) R. Lappano, M. F. Santolla, M. Pupo, M. S. Sinicropi, A. Caruso, C. Rosano and M. Maggiolini, *Breast Cancer Res*., 2012, **14**, R12; b) R. Lappano, C. Rosano, M. F. Santolla, M. Pupo, E. M. De Francesco, P. De Marco, M. Ponassi, A. Spallarossa, A. Ranise and M. Maggiolini, *Curr Cancer Drug Targets*, 2012, **12**, 531; c) A. Chimento, I. Casaburi, C. Rosano, P. Avena, A. De Luca, C. Campana, E. Martire, M. F. Santolla, M. Maggiolini, V. Pezzi and R. Sirianni, *Mol Nutr Food Res*., 2014, **58**, 478.

(3) E. F. Pettersen, T. D. Goddard, C. C. Huang, G. S. Couch, D. M. Greenblatt, E. C. Meng and T. E. Ferrin, *J Comput Chem*., 2004, **25**, 1605.

(4) (a) COSMO, Version 1.60, Bruker AXS Inc., Madison, Wisconsin, 2005.; (b) SAINT, Version 7.06A, Bruker AXS Inc., Madison, Wisconsin, 2005; (c) SADABS, Version 2.10, Bruker AXS Inc., Madison, Wisconsin, 2005.

(5) M. C. Burla, R. Caliandro, M. Camalli, B. Carrozzini, G. L. Cascarano, L. De Caro, C. Giacovazzo, G. Polidori and R. Spagna, *J. Appl. Crystallogr.,* 2005, **38**, 381.

(6) G.M. Sheldrick, SHELXL-97, University of Göttingen, Göttingen, Germany, 1997.

(7) C. Würth, M. Grabolle, J. Pauli, M. Spieles and U. Resch-Genger, *Nature Protocols,* 2013, **8**, 1535.