Experimental

General Information

All reactions were run under nitrogen atmosphere. Commercial reagents were used as received, unless otherwise stated. Silica gel was used for chromatography, and silica gel plates with fluorescence F_{254} were used for thin-layer chromatography (TLC) analysis. Data for ^1H are reported as follows: chemical shift (ppm) relative to TMS at 0.00 ppm, coupling constant (J-value, Hz), and multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet). Data for ^{13}C NMR are reported as ppm relative to $CDCl_3$ 77.0 ppm. High (HRMS) resolution mass spectra were obtained by using electrospray ionization (ESI). Fluorescence measurements were performed on a FluoroMax-2 spectrofluorometer. Absorption spectra were measured on Cary 3E UV-Visible spectrophotometer.

Values of fluorescence quantum yields was determined in the reference to perylene in toluene that have a reported Φ_F of 0.75.[1] Images were made in a Leica stereo zoom macrofluorescence imager, with using 480 nm bp for excitation and 530 nm bp for emission (GFP Filter cube).

Synthesis of 5-Phenyl-4,6-dipyrrin[3]:

To a stirred solution of **1** (7.53 cm^3, 74.50 mmol) in **2** (230.0 cm^3, 3.32 mol) at 23 °C, under Ar (g), was added catalytic amount of trifluoroacetic acid (0.55 cm^3, 7.45 mmol). After 30 min. the reaction mixture was evaporated under reduced pressure. The residue was dissolved in dichloromethane (70 cm^3) and washed with a 1.0 M solution of NaOH (aq) (100 cm^3). The aqueous layer was back extracted with dichloromethane (70 cm^3) and the combined organic layers were dried ($MgSO_4$) and concentrated under reduced pressure. The resulting residue was purified by silica gel chromatography (EtOAc/Hexane/ Dichloromethane = 1/8/1) and gave as a yellow solid dipyrrole **3**[2] (8.75g, 53%). ^1H NMR (500 MHz, $CDCl_3$): δ 7.86 (br s, 2H), 7.31 (t, 2H, *J* = 7.5), 7.25 (t, 1H, *J* = 7.5), 7.20 (d, 2H, *J* = 7.5), 6.66 (d, 2H, *J* = 1.5), 6.15 (m, 2H), 5.91 (m, 2H), 5.45 (s, 1H).

To a stirred solution of **3** (467.2mg, 2.10 mmol) in THF (100 cm^3) at 0 °C, under Ar (g), was added *p*-chloranil (568.5 mg, 2.31 mmol). After 1 h the reaction mixture was heated to 23 °C. After 2 h the reaction mixture was evaporated under reduced pressure and the residue was purified by silica gel chromatography (EtOAc/Hexane/Dichloromethane = 1/8/1) and gave as a yellow-brown solid dipyrrin **4** (212.8 mg, 46%). Total yield was 24% for two steps. ^1H NMR (500 MHz, $CDCl_3$): δ 7.67 (s, 2H), 7.49-7.41 (m, 5H), 6.61 (d, 2H, *J* = 4.0 Hz), 6.41-6.39 (m, 2H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 143.6, 142.7, 139.9, 137.2, 130.8, 129.6, 129.0, 127.5, 117.5.

Synthesis of 5-(Anthracen-9-yl)-4,6-dipyrrin:

To a stirred solution of **5** (5.0000 g, 24.20 mmol) in **2** (84 cm^3, 1.2 mol) at 23 °C, under Ar (g), was added a catalytic amount of trifluoroacetic acid (0.180 cm^3, 2.40 mmol). After 30 min. the reaction mixure was evaporated under reduced pressure. The residue was dissolved in dichloromethane (70 cm^3) and washed with a 1.0 M solution of NaOH (aq) (100 cm^3). The aqueous layer was back extracted with dichloromethane (70 cm^3) and the combined organic layers were dried ($MgSO_4$) and concentrated under reduced pressure. The residue was partially purified by silica gel chromatography (EtOAc/Hexane = 1/4) to give a crude dipyrrole **6** (3.2800 g) as a yellow solid.

To a stirred solution of crude dipyrrole **6** (200.0 mg) in dichloromethane (30 cm^3) at 23 °C, under Ar (g), was added a solution of *p*-chloranil (182.9 mg, 0.74 mmol) in dichloromethane (100 cm^3). After 48 h the reaction mixture was evaporated under reduced pressure and the residue was then purified by silica gel chromatography (EtOAc/Hexane = 1/10) and gave as a yellow solid the dipyrrin **7** [72.6 mg, 16% (for both steps)]. ^1H NMR (500 MHz, $CDCl_3$): δ 8.55 (s, 1H), 8.03 (d, 2H, *J* = 9.5), 7.89 (d, 2H, *J* = 9.0), 7.67 (s, 2H), 7.46-7.42 (m, 2H), 7.37-7.33 (m, 2H), 6.22 (dd, 2H, *J* = 4.0, 1.0), 6.01 (dd, 2H, *J* = 4.0, 1.0). ^{13}C NMR (125 MHz, $CDCl_3$): δ 143.7, 142.3, 140.8, 131.1, 130.9, 130.8, 128.4, 128.2, 127.7, 126.8, 126.0, 125.3, 118.1. HRMS (ESI) calcd for $C_{23}H_{17}N_2$ [MH^+] 321.1392, found 321.1381.

Synthesis of 5-(Pyren-1-yl)-4,6-dipyrrin:

To a stirred mixture of **8** (0.8 g, 3.47 mmol) and **2** (18.0 cm^3, 260.40 mmol) at 25 °C, under Ar (g), was added a catalytic amount of trifluoroacetic acid (0.027 cm^3, 0.35 mmol). After 30 min. the reaction mixture was evaporated under reduced pressure. The residue was dissolved in dichloromethane (50 cm^3) and washed with a 1.0 M solution of NaOH (aq) (50 cm^3). The aqueous layer was back extracted with dichloromethane (50 cm^3) and the combined organic layers were dried ($MgSO_4$) and concentrated under reduced pressure. The residue was partially purified by silica gel chromatography (EtOAc/Hexane = 1/4) and gave as a yellow solid dipyrrole **9** (404.6 mg).

To a stirred solution of crude dipyrrole **9** (202.3 mg) in dichloromethane (30 cm^3) at 23 °C, under Ar (g), was added a solution of *p*-chloranil (172.3 mg, 0.70 mmol) in dichloromethane (100 cm^3). After 12 h the reaction mixture was

evaporated under reduced pressure and residue was purified by silica gel chromatography (EtOAc/Hexane/Dichloromethane = 1/8/1) to give as a dark red solid dipyrrin **10** [103.8 mg, 17% (for both steps)]. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.21-8.17 (m, 2H), 8.13-8.09 (m, 3H), 8.02-7.98 (m, 3H), 7.93 (d, 1H, *J* = 9.5), 7.69 (s, 2H), 6.28 (d, 2H, *J* = 4.5), 6.23 (d, 2H, *J* = 4.0). ^{13}C NMR (125 MHz, $CDCl_3$): δ 143.8, 141.6, 140.6, 131.8, 131.5, 131.3, 130.8, 130.5, 129.2, 128.2, 128.1, 127.8, 127.2, 126.2, 125.6, 125.5, 125.4, 124.4, 124.3, 123.7, 117.8. HRMS (ESI) calcd for $C_{25}H_{17}N_2$ [MH^+] 345.1392, found 345.1397.

Preparation and Staining of Tissue Section

A 10 mM stock solution of **4** (PyDPy1) (1 mg, 2.9 µmol) was prepared in DMSO (0.1 cm^3) and diluted into in 50 mM solution of HEPES (pH 7.4, 0.02/10 cm^3) (aq) to give a working concentration of 10 µM. Sections were cut fresh and unfixed on a freezing microtome, at 30 µm, and sections were thawed onto clean glass slides and allowed to dry for 10 min to 24 h at +4 °C. To stain, the section was laid flat and the **4**/DMSO/HEPES solution (1.2 cm^3) was pipetted onto the surface and left for 1 to 3 min. Excess was poured off and images were made while the sections were wet and without coverslips.

Preparation of acute hippocampal slices

Hippocampal slices were prepared from 25-30 days old Swiss Webster mice (from Hilltop labs). The mice were deeply anesthetized with an isofuran, and brains were removed and placed in an ice-cold preparation solution containing: Sucrose (220 mM), KCl (3 mM), NaH_2PO_4 (1.25 mM), $MgSO_4$ (6 mM), $NaHCO_3$ (26 mM), $CaCl_2$ (0.2 mM), Glucose (10 mM).

Slices (300 µm) were cut using a Leica vibratome (VT 1200) and equilibrated for 1 h at 35 ºC in artificial CSF (ACSF) containing: NaCl (126 mM), KCl (3 mM), NaH_2PO_4 1.25 (mM), $MgSO_4$ (1 mM), $NaHCO_3$ (26 mM), $CaCl_2$ (2 mM), Glucose (10 mM), 290 mOsm with Sucrose.

For recordings, the slices were placed in a flow-through chamber (RC-27L chamber with plastic slice anchor, Warner instruments) mounted on the stage of an upright microscope (BX51WI Olympus, Japan) and perfused with oxygenated ACSF (95% O_2 / 5% CO_2) at 2 cm^3/min. Experiments were performed at 32 ±0.5ºC.

The slice preparations were loaded with of the fluorescent indicator (**4**), (final concentration 70 µM) diluted in ACSF as follows: the ACSF perfusion was stopped and 1 cm^3 of dye-containing ACSF was added; 5 min. later the perfusion was restored for the next 5 min., the perfusion was stopped again and dye was re-introduced for 5 more min.

The indicator was excited at 482(20) nm via a 40x water-immersion objective. Emitted fluorescence was collected at 532 (40) nm using a Hamamatsu CCD camera. Images were acquired and analyzed with METAFLUOR 7.0 software (Universal Imaging).

Oxygen-glucose deprivation in slices

To modulate hypoxic-hypoglycemic conditions, ACSF bathing solution was changed to an identical solution lacking glucose and bubbled with 95% N_2 / 5% CO_2.

References

1. Olmsted, J., *J. Phys. Chem.* **1979,** 83, 2581-2584.
2. Lee, C.-H.; Lindsey, J. S., *Tetrahedron* **1994,** 50, 11427-11440.
3. Brückner, C.; Karunaratne, V.; Rettig, S. J.; Dolphin, D., *Can. J. Chem.* **1996,** 74, 2182-2193.