Electronic Supplementary Information

NIR Fluorescence Labelled Carbon Nano-Onion:

Synthesis, Analysis and Cellular Imaging

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Synthetic Procedures

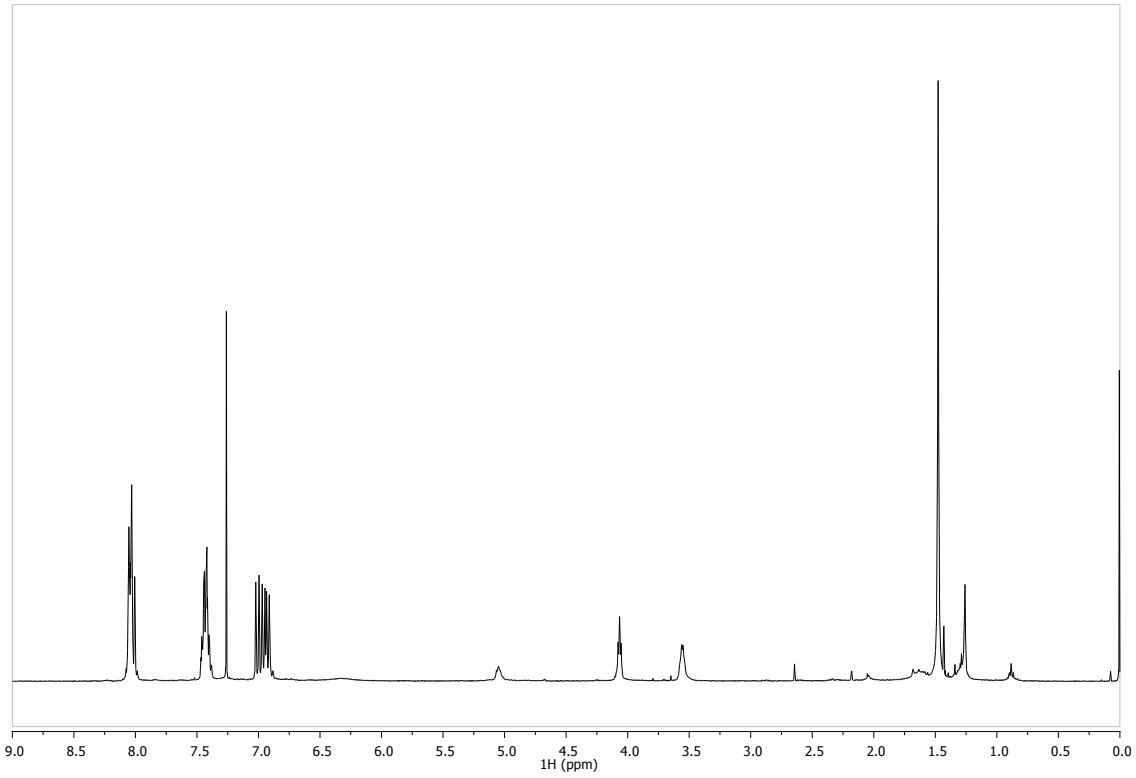
Materials:

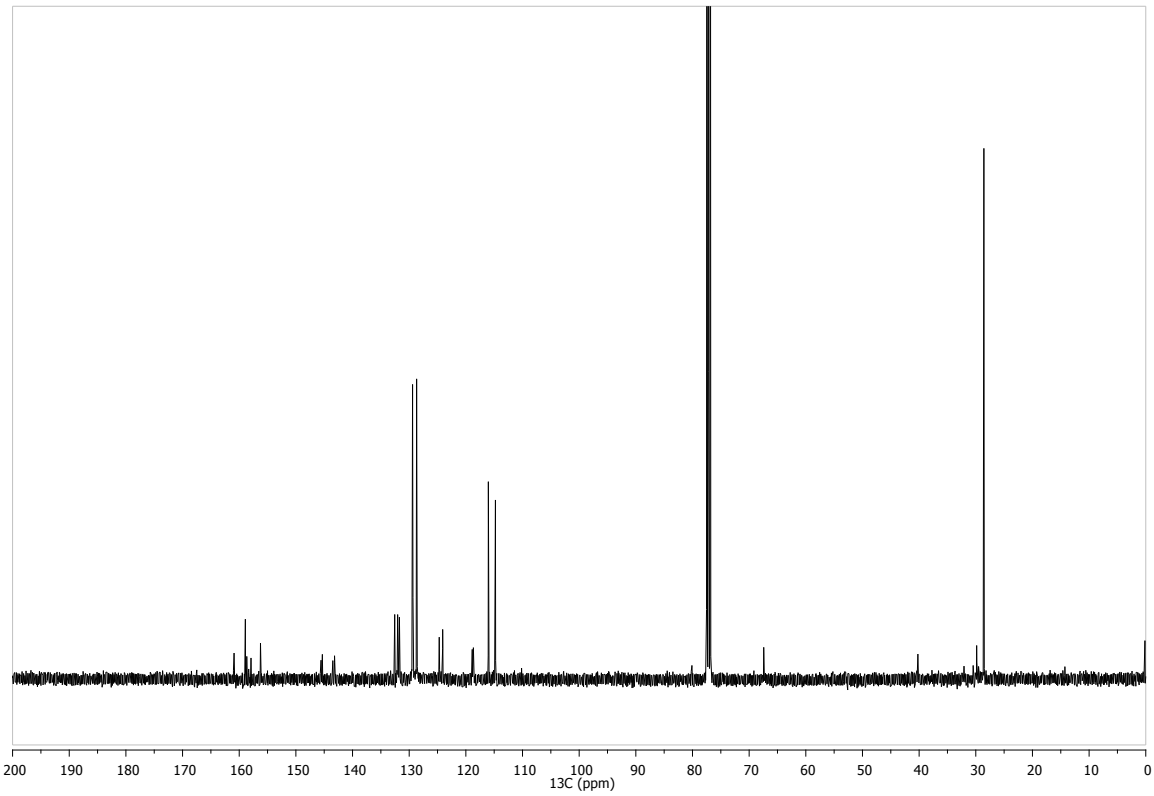
Reagents and solvents were purchased as reagent/spectroscopic-grade from Fisher Scientific Ireland Ltd., or Sigma–Aldrich Ireland and used without further purification. The synthesis of Boc-**1a** was previously described.[1]

Synthesis of Boc-1b:

A mixture of BF_2 chelated 4-(2-((5-(4-hydroxyphenyl)-3-phenyl-1*H*-pyrrol-2-yl)imino)-3-phenyl-2*H*-pyrrol-5-yl)phenol[2] (200 mg, 0.38 mmol), 2-((*t*-butoxycarbonyl)amino)ethyl 4-methylbenzenesulfonate (238 mg, 0.58 mmol) and cesium fluoride (0.58 g, 3.78 mmol) was heated under an inert atmosphere in THF (6.5 mL) at reflux for 4 h. An additional amount of 2-((*t*-butoxycarbonyl)amino)ethyl 4-methylbenzenesulfonate (119 mg, 0.38 mmol) was added to the mixture and heating continued for 2 h. The solution was cooled to rt, the solvent was removed under vacuum and the crude material was partitioned between ethyl acetate (200 mL) and water (200 mL). The organic phase was extracted with water (200 mL), brine (200 mL), dried over anhydrous Na_2SO_4, filtered and evaporated to dryness. The crude was separated by silica gel column chromatography (DCM - DCM/AcOEt 8:2), followed by alumina column chromatography (DCM - DCM/AcOEt 1:1) to obtain product Boc-**1b** (40 mg, 16%) as a green solid, mp 146-147 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.04-7.99 (m, 8H), 7.43-7.39 (m, 6H), 7.00 (s, 1H), 6.98 (s, 1H), 6.94 (d, J = 8.8 Hz, 2H), 6.93 (d, J = 8.8 Hz, 2H), 5.03 (br, s, 1H), 4.08 (t, J = 5.0 Hz, 2H), 3.63-3.50 (m, 2H), 1.47 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 160.72, 158.73, 158.48, 157.73, 156.06, 145.37, 145.15, 143.30, 142.99, 132.44, 132.38, 131.86, 131.54, 129.25, 129.22, 129.15, 128.51, 128.50, 128.48, 124.52, 123.91, 118.69, 118.48, 115.85, 114.60, 79.92, 67.24, 40.02, 28.39. ES-HRMS $[M-H]^-$: 671.2631, $C_{39}H_{34}N_4O_4BF_2$ requires 671.2641.

^{1}H and ^{13}C spectra of Boc-1b





Deprotection of Boc-1a and Boc-1b:

50 mg of boc-protected fluorophore Boc-**1a** or Boc-**1b** was dissolved in dichloromethane (6 ml). An aliquot of trifluoroacetic acid (0.5 ml) was added slowly and the reaction mixture was stirred at room temperature for 2 h. Saturated aqueous $NaHCO_3$ was added and the resulting suspension extracted with dichloromethane (2 x 15 ml). The combined organic phases were washed with water, dried over Na_2SO_4, evaporated to dryness and boron difluoride azadipyrromethene derivatives **1a** and **1b** were directly used for conjugation to **CNO-AE**.

Synthesis of p-CNO:

Purified CNOs were prepared by annealing of nanodiamonds as previously described.[3,4,5] Briefly, high purity ultradispersed nanodiamonds (Molto Ig, Inc.) were annealed in a graphite crucible at 1650°C in an Astro carbonization furnace under a partial pressure of helium (approx. 150mm). The temperature was maintained for 1 h and the sample was cooled to RT over a period of 1h. Annealing of the sample in air at 400 °C was performed to remove amorphous carbon and the recovered material was composed mainly of purified CNOs, 95% recovered mass.

Synthesis of CNO-CO_2H:

40 mg of **p-CNO** was dispersed in DMF (100 mL) by sonication. To this dispersion was added firstly the 4-aminobenzoic acid (3.3 g) and subsequently isoamyl nitrite (5.36 mL) under a nitrogen atmosphere. The reaction was stirred overnight at 60 °C and subsequently allowed to cool to room temperature. The CNOs were separated from the reaction solution by centrifugation. Fresh DMF was added following removal of the supernatant. This procedure was repeatedly conducted until the supernatant solution remained colourless. This was repeated with toluene and methanol and the black solid was finally dried under reduced pressure to afford 38 mg of black solid (yield: 95%).

Synthesis of CNO-AE:

In a 50 ml round-bottomed flask 30 mg of **CNO-CO_2H** were dispersed in 20 ml of anhydrous DMF and the solution immersed in an ultrasonic bath for 10 minutes. Afterwards, 40 mg of NHS and 67 mg of EDC HCl were added, and the reaction was vigorously stirred under an inert atmosphere at rt for 2 days. The product was purified by centrifugation with DMF (12,000 rpm for 15 minutes, then removal of the supernatant, addition to the product of 10 ml of DMF, dispersion of the product in DMF for 10 minutes by ultrasonic bath) until the organic layer was colourless, then the last centrifugation was performed with MeOH. Afterwards, the solvent was removed by rotavapor and the product dried under vacuum overnight.

Synthesis of CNO-1a:

To a 50 ml round-bottomed flask 20 mg of activated **CNO-AE** were dispersed in 20 ml of DMF by sonication in an ultrasonic bath for 5 minutes, then 16 mg of **1a** previously dissolved in 2 ml of DMF were transferred into the reaction flask. After addition of 0.1 ml of DIPEA, the reaction was stirred at rt for 3 days under an inert atmosphere. The product was purified by centrifugation in DMF (12000 rpm for 10 minutes, then removal of the supernatant, addition to the product of 10 ml of DMF, dispersion of the product in DMF for 10 minutes by ultrasonic bath) until the organic layer was colourless, then the last centrifugation was performed with MeOH. Afterwards, the solvent was removed by rotavapor and the product dried under vacuum overnight.

Synthesis of CNO-1b:

To a 50 ml round-bottomed flask 20 mg of activated **CNO-AE** were dispersed in 20 ml of DMF by sonication in an ultrasonic bath for 5 minutes, then 10 mg of **1b** previously dissolved in 2 ml of DMF were transferred into the reaction flask. After addition of 0.1 ml of DIPEA, the reaction was stirred at rt for 3 days under an inert atmosphere. The product was purified by centrifugation in DMF (12000 rpm for 10 minutes, then removal of the supernatant, addition to the product of 10 ml of DMF, dispersion of the product in DMF for 10 minutes by ultrasonic bath) until the organic layer was colourless, then the last centrifugation was performed with MeOH. Afterwards, the solvent was removed by rotavapor and the product dried under vacuum overnight.

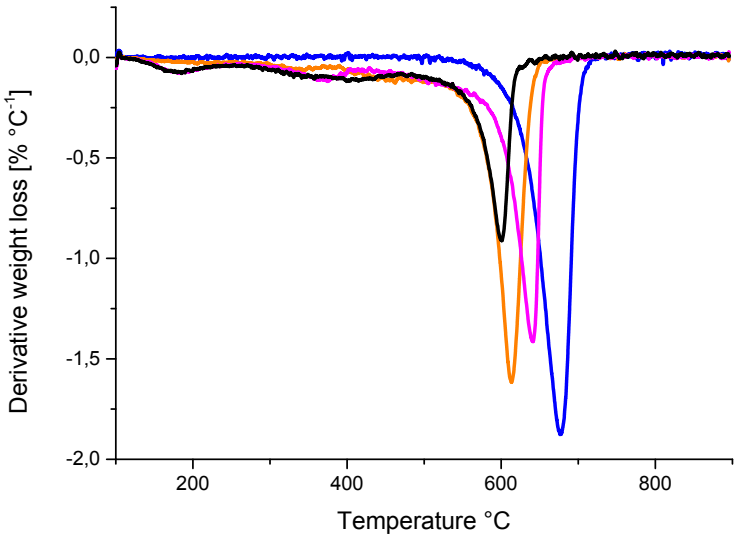
Thermogravimetric Analysis (TGA)

Methods:

The samples were deposited on a Pt pan. After an initial isothermal equilibration at 30 °C (5 min), the sample was heated under air to 100 °C with 10 °C min^{-1} and then equilibrated for another 20 min. Following this, the sample was heated to 900 °C with 10 °C min^{-1}.

Results:

Figure S1. Derivatives (% $°C^{-1}$) of the weight loss of **p-CNO** (blue), **CNO-CO_2H** (orange), **ANO-AE** (magenta) and **CNO-1a** (black) derived from TGA experiments.



Results:

The plot of the derivatives of the weight loss (in % $°C^{-1}$), derived from TGA experiments in air are shown in Figure S1. The **p-CNO**s decompose at higher temperatures than the functionalized CNO derivatives, which decompose in the order **CNO-AE** > **CNO-CO_2H** > **CNO-1a**. At lower temperatures, the decomposition of **CNO-1a** is the easiest, verifying the presence of additional organic functionalities, the boron difluoride azadipyrromethene dye.

Spectroscopic characterization

UV-Vis Absorption and Fluorescence Spectroscopy

Materials and Methods:

Chloroform was of ACS spectrosphotometric grade (Sigma-Aldrich), dimethylsulfoxide (DMSO) was for UV-spectroscopy (Fluka analytical), methanol (MeOH) was of ACS spectrosphotometric grade (Sigma-Aldrich). All solvents were purchased from Sigma-Aldrich and used as received. CNO samples were sonicated before measuring for 10 minutes in an ultrasonic bath. All measurements were carried out under ambient conditions at room temperature. Absorption spectra were recorded on a Varian Cary 300scan UV-Visible spectrophotometer and the corresponding fluorescence spectra on a Varian Cary Eclipse fluorescence spectrophotometer in 1.00 x 1.00 cm quartz glass cells. Fluorescence lifetimes were measured on an ISS Chronos - frequency-domain fluorescence lifetime spectrometer with the BF_2 chelate of [5-(4-methoxyphenyl)-3-phenyl-1H-pyrrol-2-yl]-[5-(4-methoxyphenyl)-3-phenylpyrrol-2-ylidene]amine[8] as reference, for which the reported fluorescence lifetime is 2.2 ns in acetonitrile.[6] The samples were excited with an ISS laser diode at 635 nm. Spectra for the pH dependency measurements were recorded on a Perkin-Elmer UV-Vis Spectrometer Lambda 35 and corresponding fluorescence measurements were performed on a HORIBA- Jobin Yvon Fluorolog-3 spectrofluorometer.

Fluorescence quantum yields were determined by the comparative method of Williams *et al*.[7] Toward this, the integrated fluorescence intensities of a known dye and the tested compound were compared and the fluorescence quantum yields were calculated using the following equation:

$$\Phi_x = (\Phi_{st}) \, (Grad_x / Grad_{st}) \, (\eta^2_x / \eta^2_{st})$$

st and *x* denotes the standard and test respectively, while Φ is the fluorescence quantum yield. *Grad* is the gradient obtained from the plot of integrated fluorescence intensity *vs.* absorbance of the dye at the excitation wavelength. η represents the refractive index of the used solvents. The fluorescence quantum yields of Boc-protected boron difluoride azadipyrromethene derivatives Boc-**1a** and Boc-**1b** were measured relative to the BF_2 chelate of [5-(4-methoxyphenyl)-3-phenyl-1H-pyrrol-2-yl]-[5-(4-methoxyphenyl)-3-phenylpyrrol-2-ylidene]amine with $\Phi_{St} = 0.36$ in chloroform at an excitation wavelength of 630 nm.[8] All

measurements were performed with similar instrumental settings at room temperature and ambient conditions.

Results:

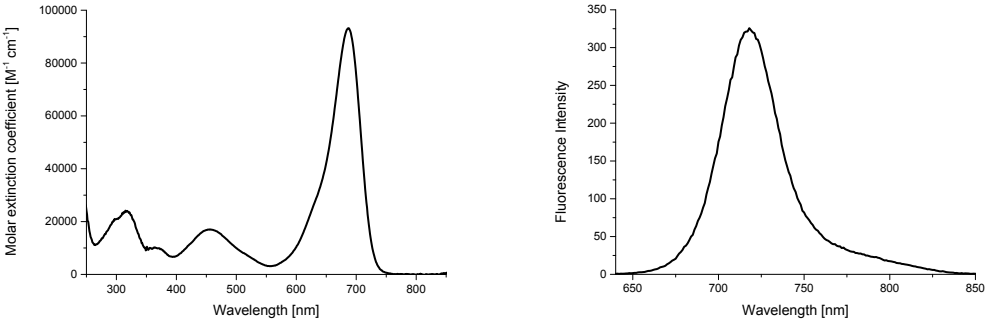
Table S1 Photophysical properties of boron difluoride azadipyrromethene derivatives Boc-**1a** and Boc-**1b** in different solvents

Compound	Solvent	λ_{Abs} [nm]	$\varepsilon \times 10^3$ [M^{-1} cm^{-1}]	λ_{Em} [nm]	Stokes Shift [nm]	Φ_F*	τ_F [ns] (%)
Boc-**1a**	chloroform	687	93.2	718	31	0.35	3.1
Boc-**1b**	chloroform	682	97.6	715	33	0.29	2.5
Boc-**1a**	methanol	684	96.6	715	31	0.21	1.9
Boc-**1b**	methanol	687	91.6	719	32	0.17	1.7
Boc-**1a**	DMSO	703	63.0	735	32	0.25	2.7
Boc-**1b**	DMSO	712	62.7	743	31	n.a.	1.8 (77) 3.3 (23)
Boc-**1b**	DMSO + 0.1 vol% TFA	708	94.4	740	32	0.21	2.1

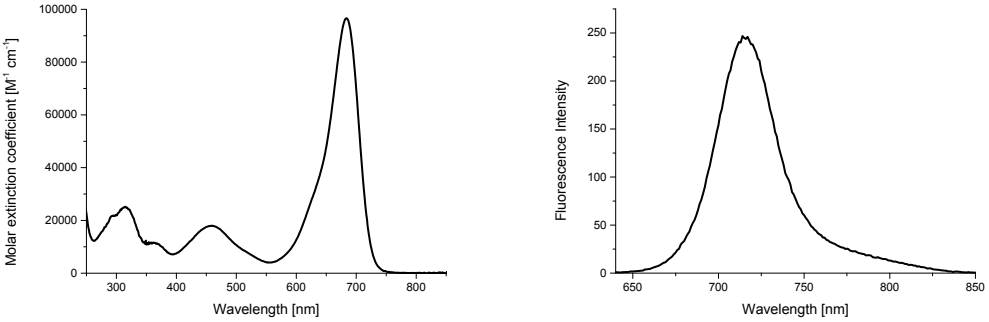
*relative to reference [5-(4-methoxyphenyl)-3-phenyl-1H-pyrrol-2-yl]-[5-(4-methoxyphenyl)-3-phenylpyrrol-2-ylidene]amine); 0.36 in chloroform, λ_{ex} = 630nm.[8]

Figure S2. Absorption and corresponding fluorescence spectra (excitation wavelength 630 nm) of Boc-**1a**.

- Chloroform



- Methanol



- DMSO

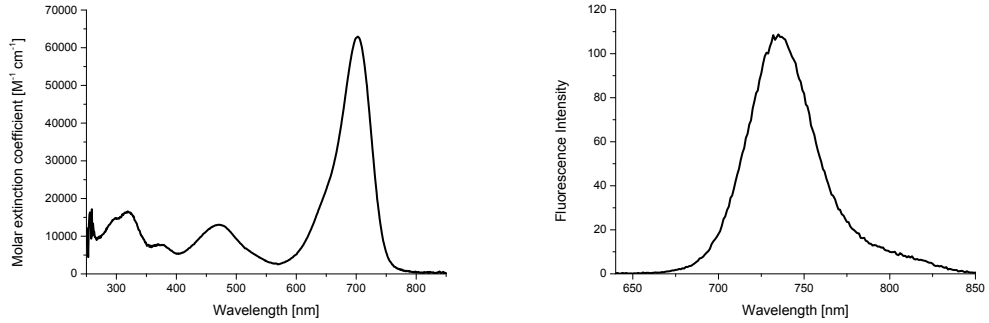
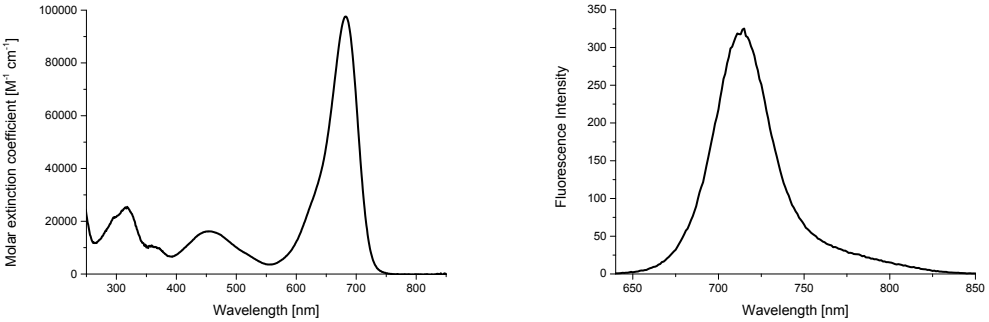
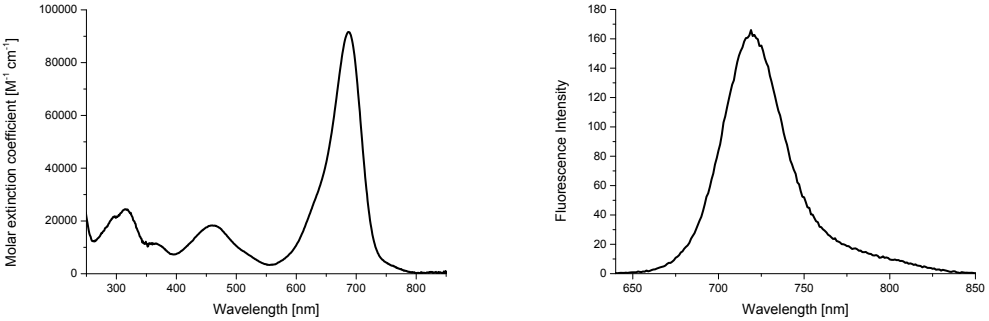


Figure S3. Absorption and corresponding fluorescence spectra (excitation wavelength 630 nm) of Boc-**1b**.

- Chloroform



- Methanol



- DMSO

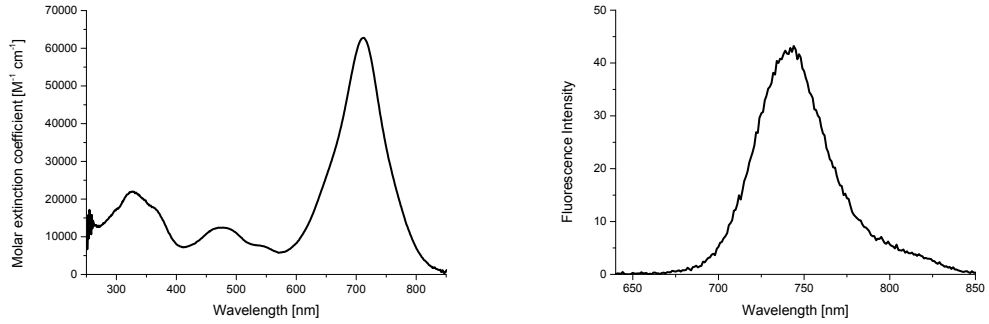


Figure S4. Excitation spectrum of **CNO-1a** in chloroform at an emission wavelength of 750 nm, excitation wavelength was varied.

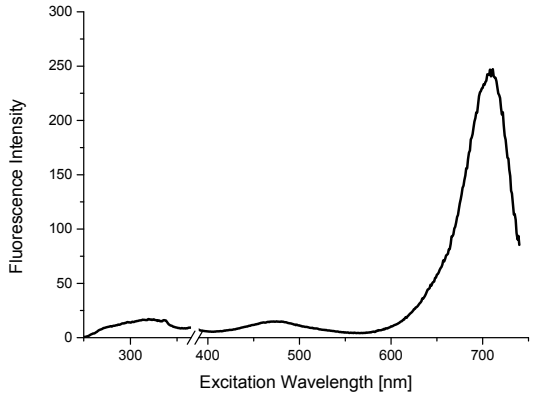
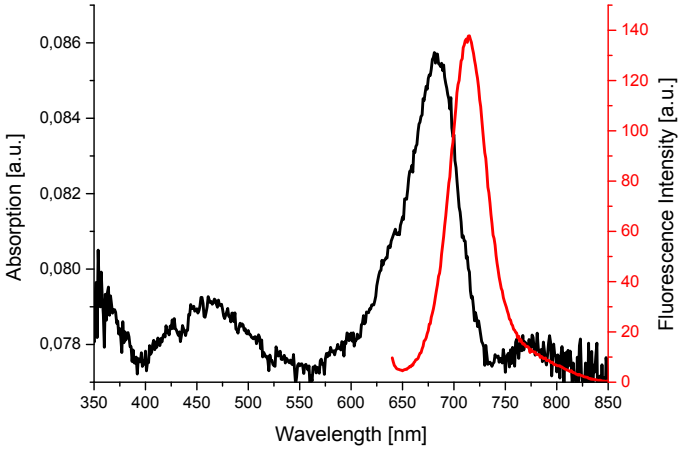


Figure S5. Absorption (black) and corresponding emission spectrum (red) of **CNO-1a** in chloroform. Emission spectra recorded upon excitation at 630 nm.



Additional Discussion:

Boron difluoride azadipyrromethene derivatives Boc-**1a** and Boc-**1b** show a moderate solvent dependency of their spectroscopic features. In chloroform, derivative Boc-**1b** has an absorption maximum of 682 nm, which shifts bathochromically in methanol to 687 nm. Compound Boc-**1a** has an absorption maximum of 687 nm in chloroform, which shifts hypsochromically in methanol to 684 nm (Table S1 and Figure S3). The fluorescence maxima shift in line with the absorption maxima with solvent independent Stokes Shifts of about 31 to 32 nm. All molar extinction coefficients measured in chloroform and methanol are between 90.000 and 100.000 M^{-1} cm^{-1}. In DMSO, the molar extinction coefficients are significantly lower with values of about 63.000 M^{-1} cm^{-1}. The absorption and emission maxima of both boron difluoride azadipyrromethene derivatives are largely shifted to higher wavelengths, to 703 nm for Boc-**1a** and 712 for Boc-**1b**. Fluorescence quantum yields appear to be lower for Boc-**1b** compared to Boc-**1a** in all investigated solvents potentially attributable to the phenolic substituent. The highest fluorescence quantum yields are observed in chloroform, followed by DMSO (for Boc-**1a**) and methanol. The fluorescence lifetimes of both boron difluoride azadipyrromethene derivatives Boc-**1a** and Boc-**1b** show a moderate solvent dependency. The longest fluorescence lifetimes were measured in chloroform, followed by DMSO and methanol, so increasing solvent polarity decreases the fluorescence lifetimes.

The excitation spectra of **CNO-1a** (Figure S5) resembles the absorption spectrum of a boron difluoride azadipyrromethene chromophore and thus reveals that the emission of **CNO-1a** is caused exclusively by the covalently connected boron difluoride azadipyrromethene. No contribution from CNO centered absorptions to the emission of the boron difluoride azadipyrromethene is observed.

Raman Spectroscopy

Experimental:

Raman spectra were measured on a Horiba Jobin Yvon HR 800 UV LabRam Raman microscope. The samples were deposited on a glass slide by adding the dry compound to a drop of methanol on the glass slide. The slides were dried in air for two hours. For the Raman measurements, the samples were excited with a built-in 632 nm laser. For comparison of the Raman intensities, all spectra in Figure S7 were normalized for the CNO G-band at around 1580 cm^{-1}.

Results:

Figure S6. Raman spectra (excitation at 632 nm) of **p-CNO** (blue), **CNO-CO_2H** (orange) and **CNO-AE** (magenta).

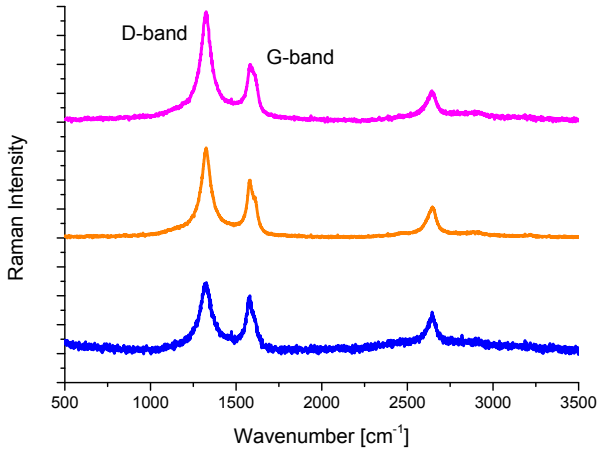
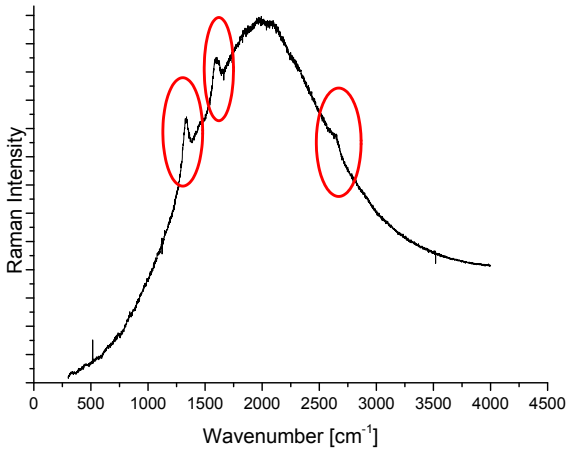


Figure S7. Typical Raman spectrum (excitation at 632 nm) of **CNO-1a** - no baseline correction was carried out. Encircled are CNO centred Raman bands.



Additional Discussion:

The Raman spectra of the boron difluoride azadipyrromethene functionalized **CNO-1a** is dominated by a strong fluorescence emission of the boron difluoride azadipyrromethene fluorophore, which is due to the excitation of the boron difluoride azadipyrromethene by the laser at 632 nm. This effect further corroborates the successful functionalization of the CNO material with boron difluoride azadipyrromethene. Next to the boron difluoride azadipyrromethene fluorescence, the aforementioned CNO centered Raman features are clearly observable. (encircled in the Figure S7).

FTIR spectroscopy

Experimental:

FTIR spectra were recorded on a Bruker Vertex 70v spectrometer, equipped with a ATR accessory for solid materials. Baseline correction using the instruments OPUS software was done, if noted in the figure caption.

Results:

Figure S8. ATR-FTIR spectra of **p-CNO**s (blue), **CNO-CO_2H** (orange) and **CNO-AE** (magenta) – no baseline correction was carried out to illustrate the background absorption of the CNO bulk material.

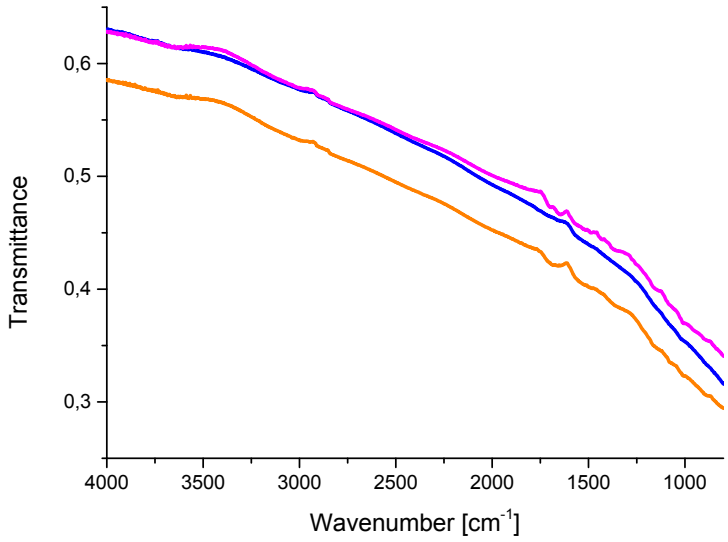
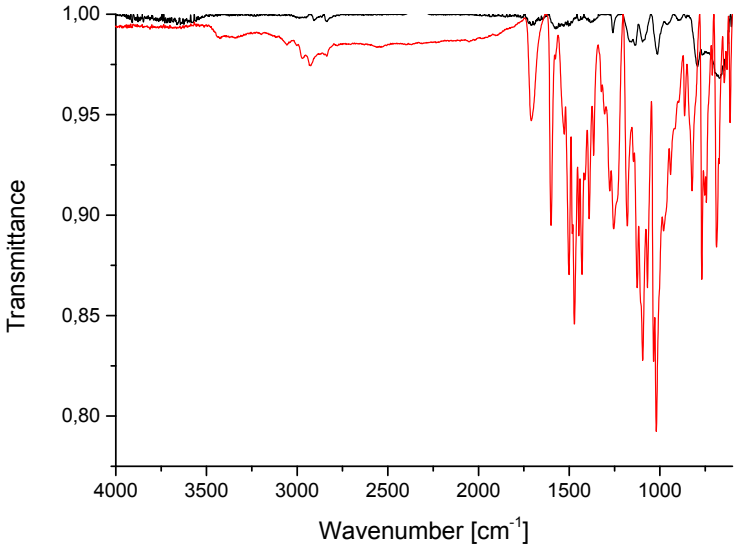


Figure S9. Typical ATR-FTIR spectra of boron difluoride azadipyrromethene derivative Boc-**1a** (red) and **CNO-1a** (black) – baseline corrected.



Additional Discussion:

The IR spectra of **p-CNO**s (blue), **CNO-CO_2H** (orange) and **CNO-AE** (Figure S8) are dominated by a large absorption of the CNO material over the whole observed spectral range, comparable to the UV-Vis spectra. When comparing the results for the **p-CNO** starting material with those of **CNO-CO_2H** and the **CNO-AE**, some weak new bands are observed. Most distinctive was a new IR absorption band in the area of 1700 cm^{-1}, which can be assigned to the carbonyl group of the benzoic acid functionality. Upon reaction with *N*-hydroxysuccinimide, leading to the activated **CNO-AE**, the carbonyl band remained, but changed from one broad band to two bands in the same area. Other, very small bands were observed at lower wavenumbers in the spectra of **CNO-CO_2H** and **CNO-AE**, further indicating a successful CNO functionalization. The weak appearance of the bands, however, makes it impossible to assign them to certain functionalities.

Microscopic characterization

Methods:

Atomic Force microscopy

Topographic images were collected in semi-contact mode with an NTEGRA Spectra inverted configuration system (NT-MDT). Silicon tips with reflectance gold coating on the back, tip apex radius 10 nm, force constant 2 N/m and frequency 170 kHz were used (NSG01 (NT-MDT)). The data were collected with NT-MDT Nova software. CNO samples, dispersed in DMF by ultrasonication, were spray coated onto freshly cleaved mica substrates and dried in an oven at 100 °C overnight.

High Resolution Transmission Electron Microscopy

HR-TEM imaging was carried out on a Jeol JEM 2200FS equipped with a Schottky FEG source operated at 200 kV, a CEOS image aberration corrector and an in-column energy filter (Ω-type). CNO samples, suspended in spectroscopic grade methanol, mildly sonicated, were deposited onto Cu grids covered with a holey C film.

Biological Imaging

Cell cultures:

HeLa Kyoto cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS, Invitrogen) and 1% *L*-glutamine (Invitrogen). Cells were maintained at 37˚C and 5% CO_2 in a humidified environment during culture.

Carbon Nano-Onion Imaging

10,000 HeLa Kyoto were seeded onto an eight well chamber slide (Millipore) and incubated overnight in 200 µL cell culture medium. The **CNO-1a** and **CNO-1b** samples were prepared by suspending 2 mg of the CNO in 1mL of DMSO followed by 90 min sonication. The required amount was then added an Eppendorf containing the required amount of DMEM. Eppendorf tubes were prepared with 190: 10 µL DMEM: **CNO-1a** or **CNO-1b** to give a CNO solution 100 µg/mL. 195:5 µL DMEM: **CNO-1a** or **CNO-1b** = 50 µg/mL. 198:2 µL DMEM: **CNO-1a** or **CNO-1b** = 20 µg/mL. CNO: DMEM solutions were pre-prepared in

Eppendorf tubes so they could be sonicated before addition to cells. The media was removed and replaced with one of these solutions to modify the amount of CNO in each well. The control consisted of 200 μL DMEM. After 120 min incubation the media was removed from the wells and the cells were washed with PBS three times to remove any remaining media. The cells were fixed by adding 100 μL of 3% para-formaldehyde for 25 min followed by three washings with PBS. The nucleus was stained by immersion in 1:5000 dilution of Hoechst 33342 for 25 min followed by three washings with PBS. The chambers were removed and 5 μL of Mowiol was added to each well and a coverslip was added.

All CNO imaging was performed on Leica DMI6000B wide-field epi-fluorescent microscope coupled to a Leica DFC340 FX Digital CCD camera and mercury arc lamp. The objectives were Objectives PL S-APO 10x/0.30, PL S-APO 20x/0.50, PL APO 40x/1.25 (oil), PL APO 63x/1.4 (oil). All images were acquired at 63X with an exposure of 929 ms and gain at 4.0X. Confocal imaging was performed using an Olympus FV1000 laser scanning confocal microscope (LSCM). The objective was an UPLSAPO 60x/1.35 (oil) which was paired with 405 nm and 635 nm laser lines and FluoView FV1000 scan unit.

pH Switching of CNO-OH

To determine if the **CNO-1b** internalised by the cells had retained its ability to switch "on" and "off" in response to pH the chambers were not removed from the slides. Cells were incubated with 50 μg/mL CNO. The fixing and staining procedure was described in the previous section. An acidic PBS solution was prepared by lowering the pH to 4.5 using 0.1 M hydrochloric acid. A basic PBS solution was prepared by increasing the pH to 8.5 using 0.1M sodium hydroxide. Fluorescence was switched "off" by adding 200 μL PBS pH 8.5 for one hour before imaging. Fluorescence was switched "on" by removing the PBS pH 8.5, washing with unmodified PBS, and replacing it with PBS pH 4.5 for one hour before imaging.

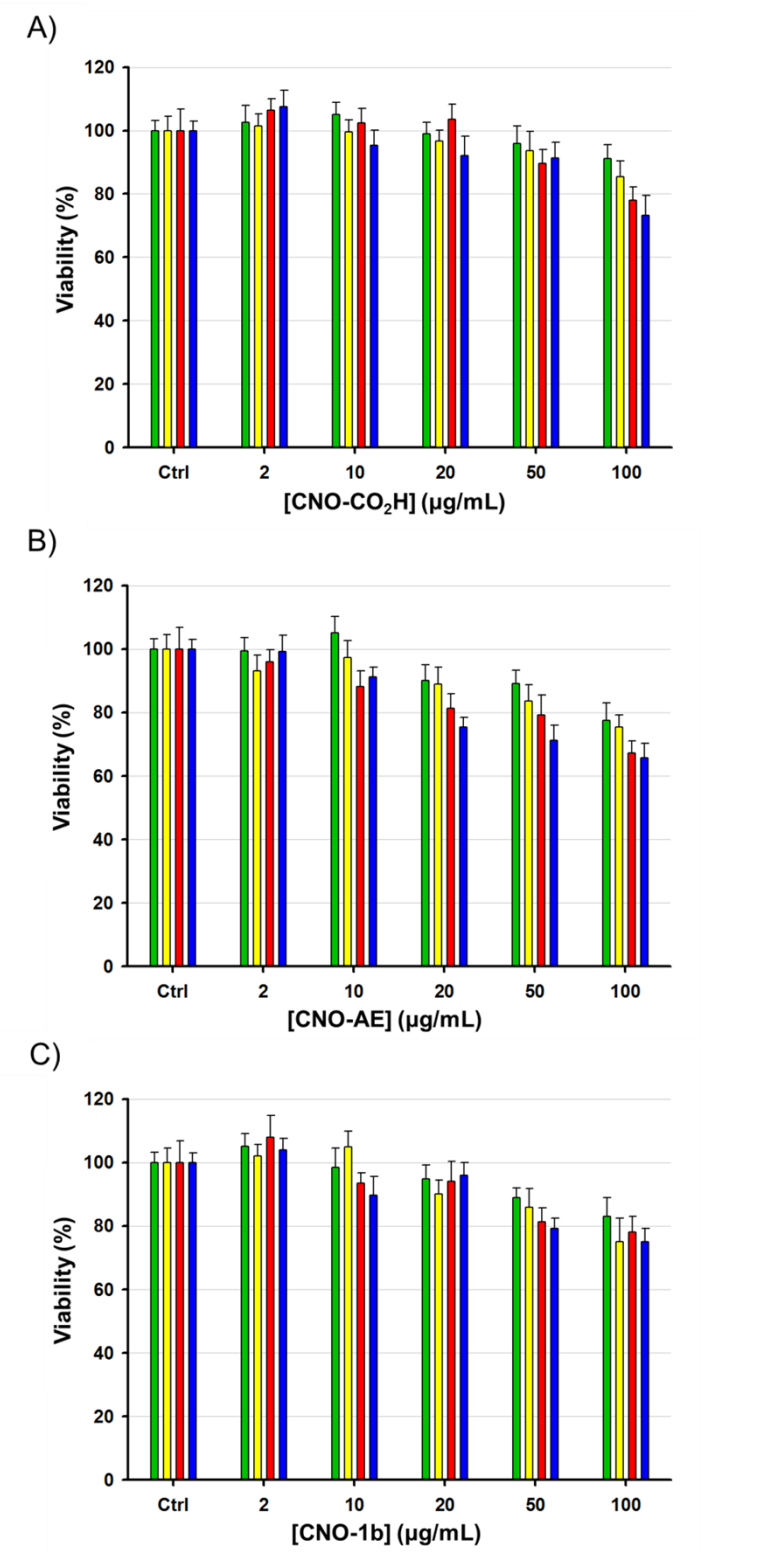
CNO Image processing

Image processing, analysis and enhancement were performed using ImageJ (NIH). Images obtained from the Leica DMI6000B Olympus FV1000 were all normalised by contrast by applying background subtraction (300 pixel filter size), min/max intensity set to 0-82, and a red LUT added for NIR fluorophores and blue LUT for Hoechst 33342. All images were 8-bit grayscale

Toxicity

Toxicity assays were performed by pleating 50,000 HeLa cells onto 24-well chambers slide (Millipore) and incubated in 500 µL cell culture medium for 48 h at 37 °C. The **CNO-CO_2H**, **CNO-AE**, **CNO-1b** and **CNO-1a** were prepared by suspending 2 mg of sample in 1ml of DMSO followed by sonication for 90 min. The samples were then dispersed in the cell culture media (DMEM) to attain CNO suspensions at final concentrations of 2, 10, 20, 50 and 100 µg/mL. The media was removed from the plates and replaced with 500 µL of suspensions of CNO samples. The metabolic activity of HeLa cells was measured after 12, 24, 48 and 72 h of exposure to the samples of CNO, utilizing PrestoBlue™ cell viability assay (Life Technologies). The colorimetric reagent was diluted in DMEM media at 1:10 ratio and 500 µL of solution was added to each well. The 24-wells chamber slide was were placed in a humidified atmosphere of 5% CO_2 at 37 °C and incubated for 20 min. Subsequently, the pink PrestoBlue™ product was measured on a microplate reader at a wavelength of 570 nm. Each measurement was normalized with the average signal of untreated wells to determine the percent cell viability.

Figure S10. Viability of HeLa cells after expose to increases doses (2; 10; 20; 50; 100 µg/mL) of **CNO-CO_2H** (A), **CNO-AE** (B) and **CNO-1a** (C) at different times, 12h (green bar), 24h (yellow bar), 48h (red bar) and 72h (blue bar) of incubation. The data are presented as the mean and the standard error of the mean of three experiments.



References

1 A. Palma, L. A. Alvarez, D. Scholz, D. O. Frimannsson, M. Grossi, S. J. Quinn, D. F. O'Shea *J. Am. Chem. Soc.* 2011, **133**, 19618.

2 J. Murtagh, D. O. Frimannsson, D. F. O'Shea *Org. Lett.* 2009, **11**, 5386.

3 V. L. Kuznetsov, M. N. Aleksandrov, I. V. Zagoruiko, A. L. Chuvilin, E. M. Moroz and V. N. Kolomiichuk *Carbon* 1991, **29**, 665.

4 V. L. Kuznetsov, A. L. Chuvilin, Y. V. Butenko, I. Y. Mal'kov and V. M. Titov *Chem. Phys. Lett.* 1994, **222**, 343.

5 A. Palkar, F. Melin, C. M. Cardona, B. Elliott, A. K. Naskar, D. D. Edie, A. Kumbhar and L. Echegoyen *Chem. Asian J.* 2007, **2**, 625.

6 P. Batat, M. Cantuel, G. Jonusauskas, L. Scarpantonio, A. Palma, D. F. O'Shea, N. D. McGlenaghan *J. Phys. Chem. A* 2011, **115**, 14034.

7 A. T. R. Williams, S. A. Winfield, J. N. Miller *Analyst* 1983, **108**, 1067.

8 A. Gorman, J. Killoran, C. O'Shea, T. Kenna, W. M. Gallagher, D. F. O'Shea *J. Am. Chem. Soc.* 2004, **126**, 10619.