Chemistry–A European Journal

Supporting Information

Designing a Red-Emitting Viscosity-Sensitive BODIPY Fluorophore for Intracellular Viscosity Imaging

Karolina Maleckaitė, Jelena Dodonova, Stepas Toliautas, Rugilė Žilėnaitė, Džiugas Jurgutis, Vitalijus Karabanovas, Sigitas Tumkevičius, and Aurimas Vyšniauskas*

Table of Contents

1. Quantum chemical calculations

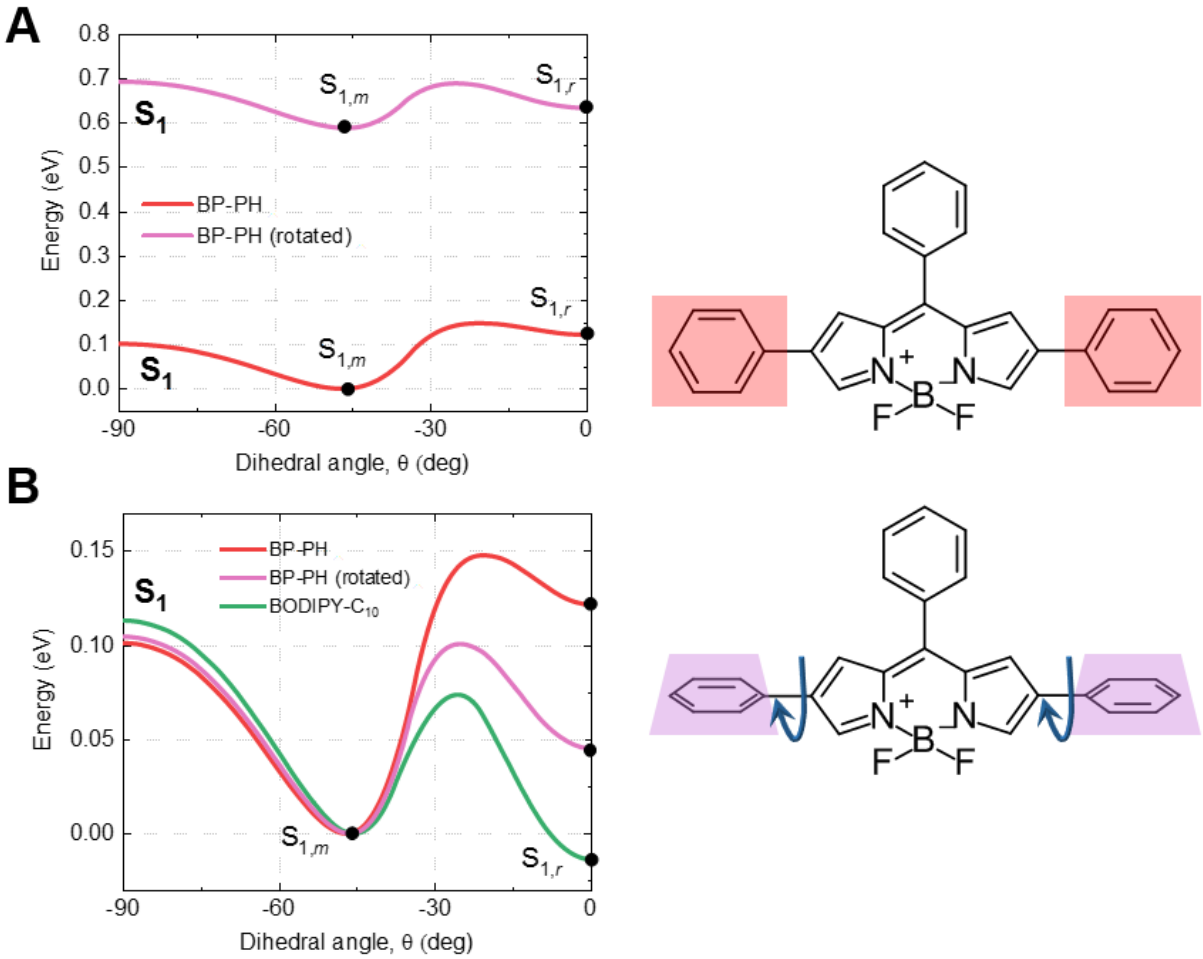


Figure S1. (A) Calculated potential energy surface (PES) curves of the first excited electronic state (S_1) of BP-PH when the β-phenyls are either in conjugation with the BODIPY core (red), or completely out of conjugation (pink). The molecular structures are shown on the right. The rotation of β-phenyls increases S_1 energy by 588 meV. (B) PES of the S_1 state, where the energy of the local minimum $S_{1,m}$ at approximately 45° is set to 0 eV as a reference point for easier comparison. PES of a well-known molecular rotor BODIPY-C_{10} is shown in green. When the β-phenyls are rotated out of conjugation, the barrier height for the nonradiative relaxation drops by 50 meV, approaching the barrier height of BODIPY-C_{10}. This demonstrates that the relative $S_{1,r}$ energy jump and the barrier-height increase going from BODIPY-C_{10} to BP-PH is mostly caused by the conjugation of β-phenyls to the BODIPY core. Therefore, the attempts to redshift the spectra of this type of BODIPY molecular rotors by enlarging conjugation length are likely to result in a higher barrier for non-radiative relaxation and subsequently in diminished viscosity sensitivity.

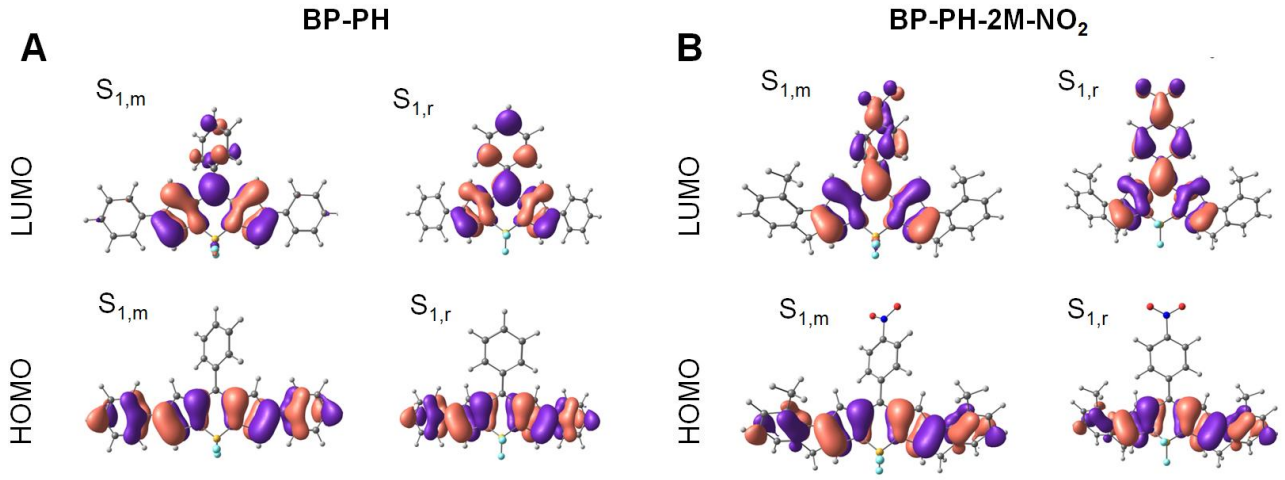


Figure S2. The HOMO and the LUMO of BP-PH (A) and BP-PH-2M-NO_2 (B) at the $S_{1,m}$ and $S_{1,r}$ geometries. The HOMO is localized on the BODIPY core and β-phenyls and shows no electron density on the *meso* phenyl ring. In contrast, the LUMO shows electron density on the core and the *meso*-phenyl ring but not on the β-phenyls. The addition of the -NO_2 group increases the electron density in the *meso*-phenyl group in the LUMO, which results in the extra force driving the *meso*-phenyl into the conjugation with the BODIPY core upon excitation.

Table S1. Values of local minimum $S_{1,R,}$ and transitional state TS energies relative to $S_{1,M,}$ minimum in the first excited state S_1 at the bottom-of-the-well, and when zero-point vibrational energy (ZPVE) is included.

	$S_{1,M}$, meV	**TS, meV**	**$S_{1,R}$, meV**
BP-PH, bottom-of-the-well	0	148	122
BP-PH, ZPVE included	0	152	135
BP-PH-2M-NO_2, bottom-of-the-well	0	103	79
BP-PH-2M-NO_2, ZPVE included	0	97	80

2. Spectroscopic characterisation, fluorescence lifetime measurements. DFT calculations of BP-PH-ISO.

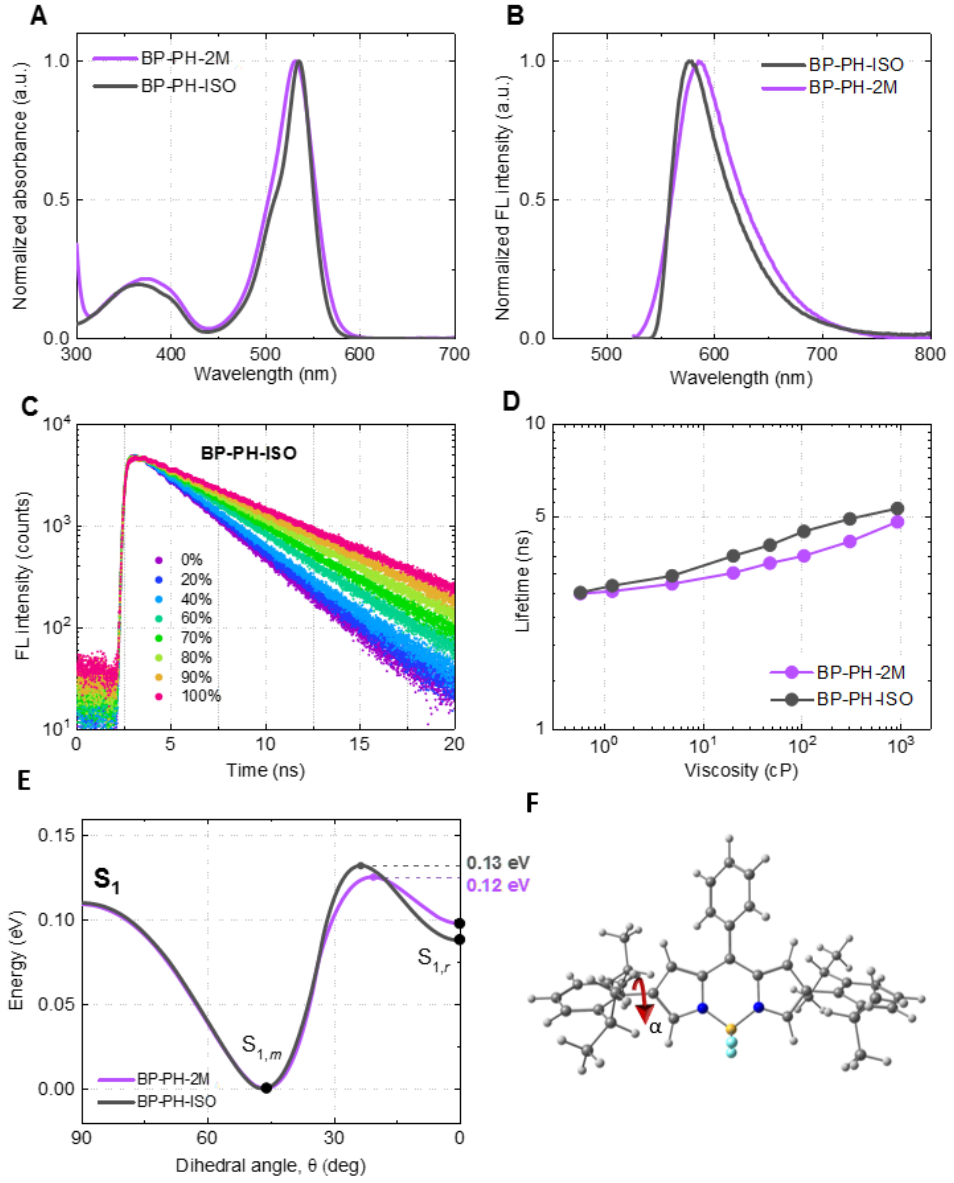


Figure S3. Comparison of BP-PH-2M (purple) and BP-PH-ISO (dark grey) conjugates. Absorbance (A) and fluorescence (B) spectra in toluene. (C) Time-resolved fluorescence decays of BP-PH-ISO obtained in toluene-castor oil mixtures. The legend indicates the volume fraction of castor oil in the mixture. (D) Fluorescence lifetimes of BP-PH-2M and BP-PH-2M-ISO obtained in toluene-castor oil mixtures. (E) PES curves of the first excited electronic state (S_1) of BP-PH-2M and BP-PH-ISO. The energy of the local minimum $S_{1,m}$ at approximately 45° is set to 0 eV as a reference point. Values on the right axis designate the height of activation energy

barrier. (F) Molecular structure of BP-PH-ISO at ground-state minimum $S_{0,m}$. Red arrow indicates rotation angle α.

According to the DFT calculations, average rotation angles α between the BODIPY core and β-phenyls (Fig. S3F) at excited-state minima $S_{1,m}$ are 42.8° and 60° for BP-PH-2M and BP-PH-ISO, respectively. Mid-range dihedral angles observed for these compounds lead to similar barrier heights that fall between in-conjugation and out-of-conjugation values calculated for BP-PH (cf. Fig. S1).

These results demonstrate that the steric hindrance caused by a larger isopropyl group compared to methyl groups on the β-phenyls does not have a strong effect on the viscosity sensitivity of the BODIPY fluorophore.

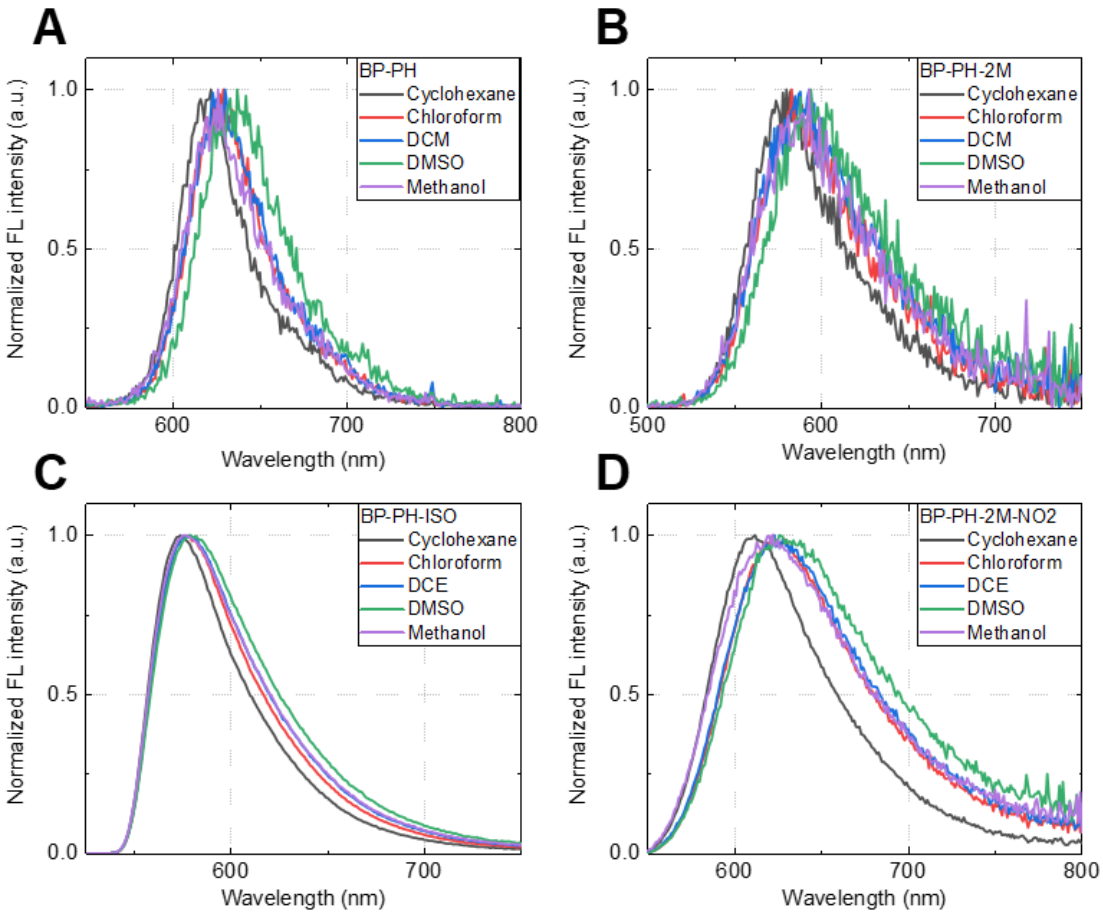


Figure S4. Fluorescence spectra for BP-PH (A), BP-PH-2M (B), BP-PH-ISO (C), and BP-PH-2M-NO_2 (D) in different polarity solvents (from non-polar to very polar: cyclohexane (dark grey), chloroform (red), dichloromethane (DCM) or dichloroethane (DCE) (blue), dimethyl sulfoxide (DMSO) (green), methanol (purple)). The conjugates are slightly polarity sensitive, showing the red shift of 14, 16, 8 and 19 nm for BP-PH, BP-PH-2M, BP-PH-ISO, and BP-PH-2M-NO_2, respectively, when going from the least polar to the most polar solvents.

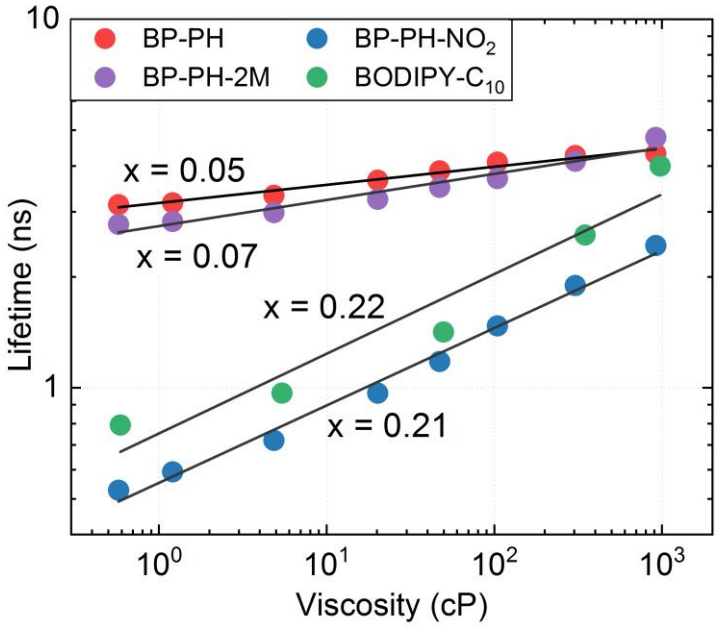


Figure S5 Förster-Hoffmann fits of lifetime-viscosity dependences of BP-PH (A), BP-PH-2M (B), BP-PH-2M-NO_2 (C), and BODIPY-C_{10} (D). The fit was performed using the Förster-Hoffmann equation[2-3] for fluorescence lifetime:

$$\tau = C\eta^x \tag{S1}$$

where τ is fluorescence lifetime, η is viscosity, C and x are constants. The latter constant x approximately shows the degree of sensitivity to viscosity. Black lines show linear fits with the constant x written for each fit. BP-PH and BP-PH-2M show low x values meaning very low viscosity sensitivity, while the sensitivity of BP-PH-2M-NO_2 is similar to the well-known viscosity sensor BODIPY-C_{10}. Therefore a nitro-substituted conjugate could be used as a viscosity probe. Fluorescence lifetime values were obtained in toluene-castor oil mixtures.

We note that fluorescent viscosity sensors typically show a sigmoidal dependence in the logarithmic lifetime-viscosity plots if the calibration is done over sufficiently wide range of viscosities.[4-5] While the Förster-Hoffmann equation describes lifetime-viscosity dependence well at intermediate viscosities, the discrepancies at low and high viscosities can be significant as can be seen for BODIPY-C_{10}.

Table S2. Lifetimes and amplitudes of fluorescent decays for BP-PH, BP-PH-2M, BP-PH-ISO, BP-PH-2M-NO_2 in toluene/castor oil mixtures. From left: the volume fraction of castor oil in the mixture, component of the kinetics exponent, lifetime (ns) and amplitudes (%) for each derivative, respectively.

Part of CO, %	Exponent Component	BP-PH		BP-PH-2M		BP-PH-ISO		BP-PH-2M-NO_2	
		τ, ns	Amplitude, %	τ, ns	Amplitude, %	τ, ns	Amplitude, %	τ, ns	Amplitude, %
0	1	3.19	100	0.51	4.8	2.80	100	0.53	100
	2			2.89	95.2				
20	1	3.18	100	0.91	6.67	2.95	100	0.57	98.1
	2			3.04	93.33			1.65	1.9
40	1	3.33	100	1.34	8.96	3.18	100	0.69	98.02
	2			3.15	91.04			2.11	1.98
60	1	3.66	100	1.00	1.56	3.70	100	0.90	91.23
	2			3.41	98.44			1.68	8.77
70	1	3.88	100	3.64	100	4.01	100	1.08	83.83
	2							1.73	16.17
80	1	4.1	100	0.38	1.02	4.45	100	1.23	57.35
	2			4.08	98.98			1.81	42.65
90	1	4.27	100	0.50	2.76	4.89	100	1.35	28.54
	2			4.22	97.24			2.11	71.46
100	1	4.32	100	4.53	100	5.29	100	1.58	19.2
	2							2.64	80.8

Some of the decays were biexponential and their intensity-weighted mean lifetimes are calculated using [Eq. (2), main text]. The second exponent results from the castor oil, which is itself fluorescent. However, the actual castor oil contribution to the total fluorescence is approximately 1%, much lower than the amplitudes shown in Table S2. The inaccurate individual amplitude and lifetime values are rather common results of biexponential fitting.[1] As a result, the lifetimes of BODIPY fluorophores are best reflected not by individual lifetimes with dominant amplitudes, but by intensity-weighted lifetimes, which are far less susceptible to pitfalls of biexponential fitting.

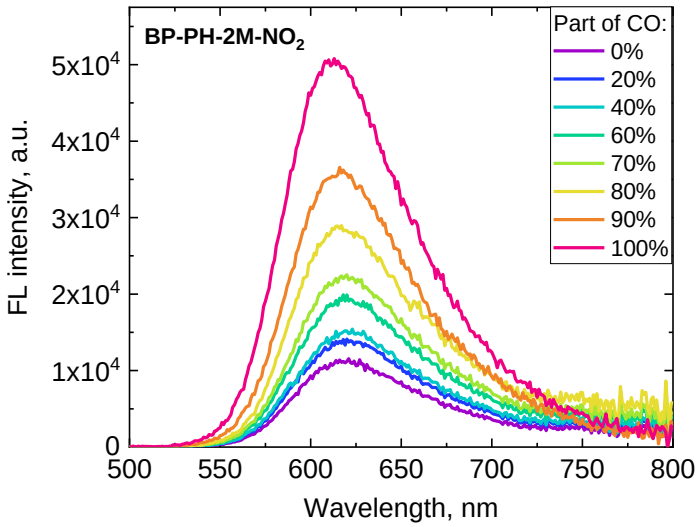


Figure S6. Fluorescence intensity of BP-PH-2M-NO_2 in toluene-castor oil mixtures. The legend indicate the volume fraction of castor oil (CO) in the mixture. Corrected spectra is shown after subtracting the spectrum of autofluorescent castor oil ($\lambda_{FL} \approx 675$ nm).

Fluorescence spectra of BP-PH-2M-NO_2 in various viscosity, non-polar toluene-castor oil mixtures shows that the fluorescence intensity rises with increasing solvent viscosity. Moreover, quantum yield values showed in Table S3 follow the same trend.

Table S3. Quantum yield (QY) values of BP-PH-2M-NO_2 in toluene-castor oil mixtures. The volume fraction of castor oil in the mixture is written in the left column (V_{CO}).

V_{CO}, %	QY, %
0	4.2
20	4.5
40	5.4
60	6.8
70	8.2
80	12.1
90	12.7
100	17.4

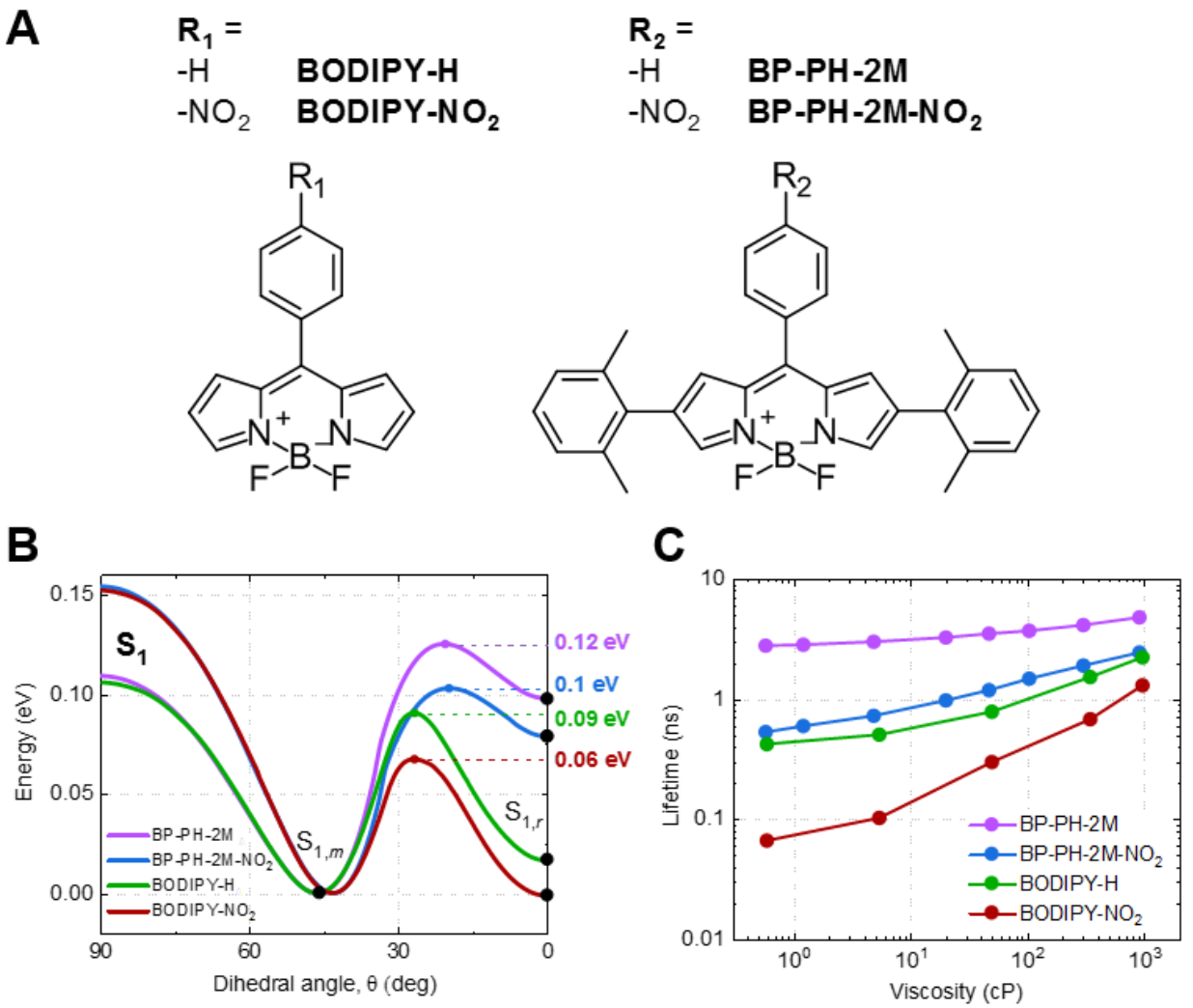


Figure S7. (A) The structures of previously reported BODIPY molecules[5] (left) and new BODIPY derivatives reported in this work (right). Potential energy surface (PES) curves of the first excited electronic state (B), and fluorescence lifetime dependenced on viscosity (C) of BP-PH-2M (purple), BP-PH-2M-NO_2 (blue), BODIPY-H (green), and BODIPY-NO_2 (dark red). X axis in picture B shows the dihedral angle θ between the BODIPY core and the meso-phenyl ring. The right side of the graph B shows energy barrier values for non-radiative relaxation for each molecule. $S_{1,m}$ denotes the minimum from where fluorescence takes place, while a fast non-radiative relaxation occurs from $S_{1,r}$. For both sets of fluorophores -NO_2 group reduced the energy barrier for non-radiative relaxation, which led to an improved sensitivity to viscosity.

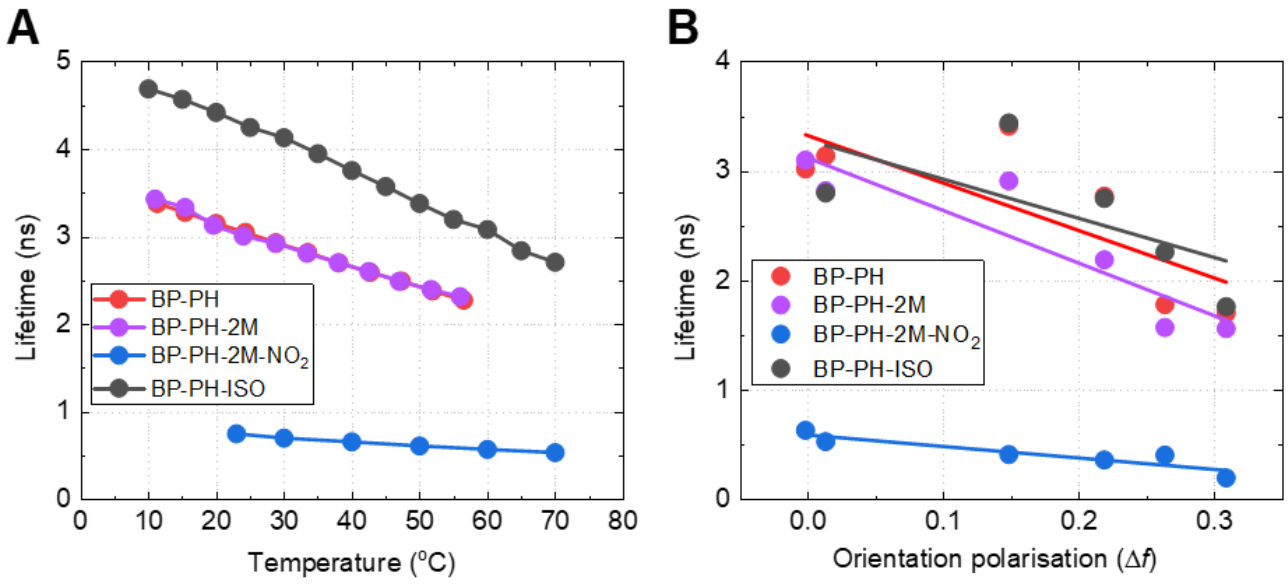


Figure S8. (A) Fluorescence lifetime dependence on temperature in cyclohexane for BP-PH (red), BP-PH-2M (purple), BP-PH-2M-NO_2 (blue) and BP-PH-ISO (dark grey). Weak temperature sensitivity is observed when the temperature increases in cyclohexane. BP-PH-ISO yields the highest values of fluorescence lifetime, while BP-PH and BP-PH-2M lifetimes coincide. BP-PH-2M-NO_2 shows much lower lifetimes because a non-viscous solvent determines a fast non-radiative relaxation.
(B) Fluorescence lifetime dependence on the orientation polarization,[1] defined as [Eq. (S2)]:

$$\Delta f = \frac{\varepsilon - 1}{2\varepsilon + 1} - \frac{n^2 - 1}{2n^2 + 1} \qquad (S2)$$

where ε is a relative permittivity and n is the refractive index of a pure solvent. Results are for BP-PH (red), BP-PH-2M (purple), BP-PH-2M-NO_2 (blue) and BP-PH-ISO (dark grey). Solvents range from non-polar (low Δf) to very polar (high Δf): cyclohexane, toluene, chloroform, DCM (or DCE interchangeably), DMSO, and methanol. The lifetimes decrease with increasing polarity, when the polarity of solvent is changed. BP-PH, BP-PH-2M and BP-PH-ISO demonstrate comparable lifetimes, while derivative with -NO_2 group showed lower lifetimes and less sensitivity to polarity.

The results displayed in (A) and (B) show that attaching the nitro group increases the viscosity sensitivity drastically, which then prevails over the observed temperature and solvent polarity sensitivities.

3. Cellular imaging

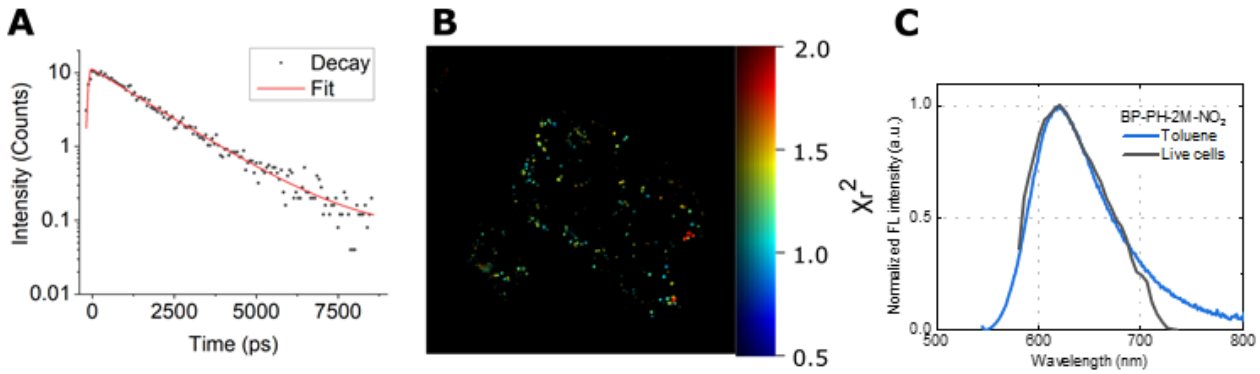


Figure S9. (A) An example of a single decay (black) with a monoexponential fit (red) from the FLIM image of BP-PH-2M-NO_2 in MCF-7 cells shown in Fig. 4B. The decay was obtained by averaging over 5x5 pixel area. (B) χ_r^2 image resulting from the analysis of the aforementioned FLIM image. The average χ_r^2 value was 1.26. (C) The fluorescence spectrum of BP-PH-2M-NO_2 in live cells (dark grey) in comparison with spectrum in toluene (blue).

Taken together, the monoexponential decay and the similarity of fluorescence spectrum of BP-PH-2M-NO_2 in live cells and toluene clearly demonstrate the absence of aggregation, degradation or other effects that could lead to different photophysical properties of BP-PH-2M-NO_2 in live cells. Therefore, the viscosity calibration in toluene-castor oil mixtures is valid method for estimating microviscosity in MCF-7 cells using BP-PH-2M-NO_2.

To determine BP-PH-2M-NO_2 localisation in MCF-7 cells, we utilised a green-emitting fluorophore – BODIPY-h. While the data regarding the localisation of BODIPY-h is not published, our prior experiments with Nile Red – commercial probe for lipid droplets – have proven that BODIPY-h stains lipid droplets in live MCF-7 cells (Figure S10, ESI). Colocalisation experiments with BP-PH-2M-NO_2 and BODIPY-h resulted in an overlap of both dyes (Figure S11C, ESI). Additionally, by measuring fluorescence spectra from the spherical structures in MCF-7 cells we observe two fluorescence peaks: 520 nm (BODIPY-h) and 617 nm (BP-PH-2M-NO_2) (Fig. S11D, ESI). This implies that both BDP-h and BP-PH-2M-NO_2 are localised within the same organelles. Therefore, we can conclude that BP-PH-2M-NO_2 is indeed staining lipid droplets in live MCF-7 cells.

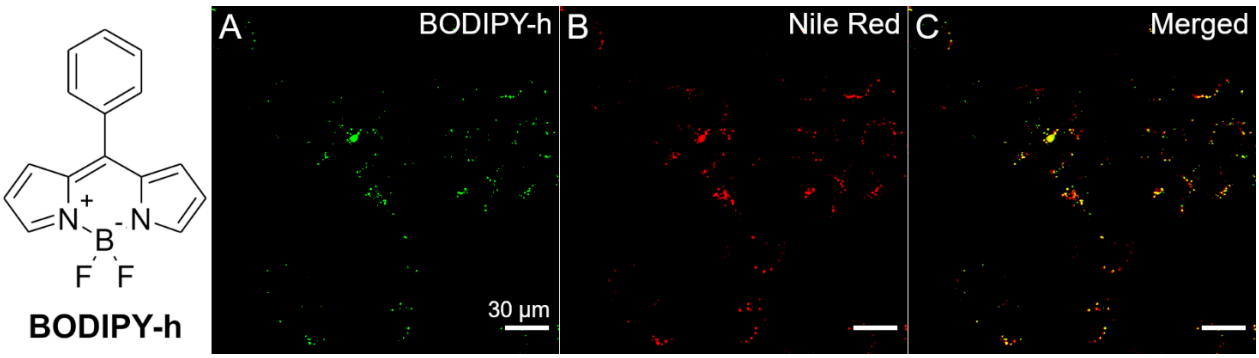


Figure S10. Live MCF-7 cells stained with BODIPY-h (A), Nile Red (B), and merged images (C). For BODIPY-h, the fluorescence was excited at 480 ±5 nm and detected over 501 nm – 590 nm range. For Nile Red, the fluorescence was excited at 480 ±5 nm and detected over 621 nm – 755 nm range.

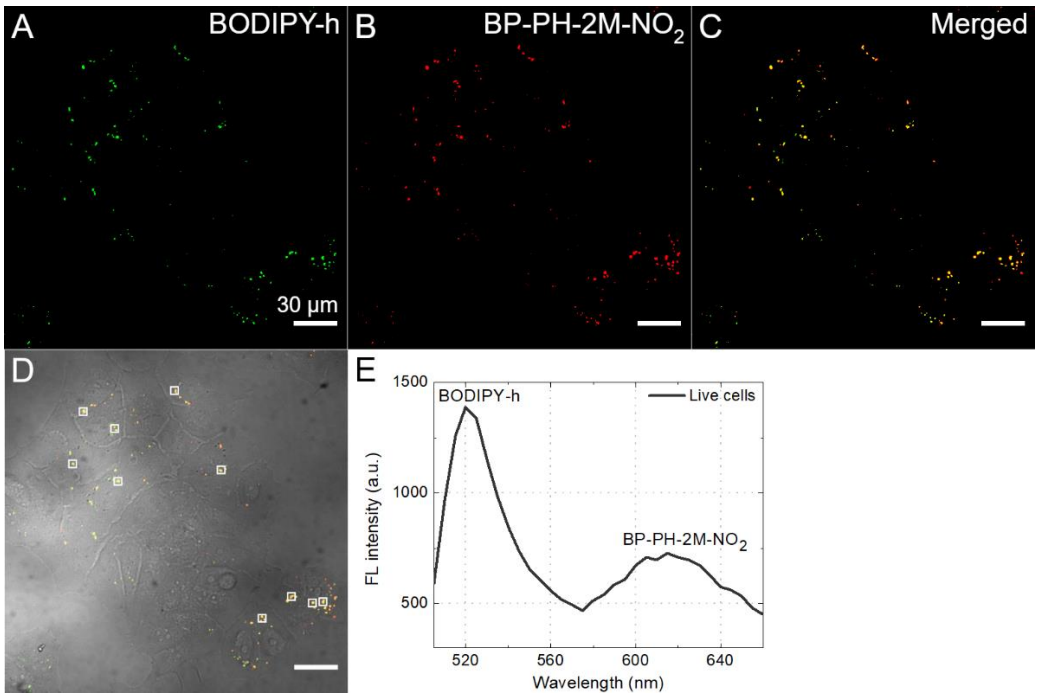


Figure S11. Live MCF-7 cells stained with BODIPY-h (A), BP-PH-2M-NO_2 (B), and merged images (C). (D) Brightfield image superimposed with a fluorescence image of BODIPY-h and BP-PH-2M-NO_2. White squares mark lipid droplets, where fluorescence was measured using 32-channel spectral detector. (E) The fluorescence spectrum from lipid droplets in live MCF-7 cells, that were stained with BP-PH-2M-NO_2 and BODIPY-h. For BODIPY-h, the fluorescence was excited at 480 ±5 nm and detected over 501 nm – 590 nm range. For BP-PH-2M-NO_2, the fluorescence was excited at 560 ±5 nm and detected over 621 nm – 755 nm range.

4. The Cartesian (XYZ) coordinates of the optimized ground and excited states for BP-PH, BP-PH-2M, BP-PH-ISO and BP-PH-2M-NO_2

BP-PH

=====

S_0

N	12.395811000	-9.430470000	-1.150557000
C	13.168782000	-9.796283000	-2.172500000
C	14.055571000	-8.747484000	-2.526599000
C	13.769149000	-7.697992000	-1.654043000
C	12.738539000	-8.134384000	-0.787097000
C	11.505250000	-5.110809000	0.774831000
C	11.873039000	-3.817651000	1.137842000
C	13.208484000	-3.518786000	1.408904000
C	14.178961000	-4.516210000	1.310414000
C	13.819421000	-5.807974000	0.934324000
C	12.477765000	-6.115595000	0.665446000
C	12.086797000	-7.487544000	0.275819000
C	11.069602000	-8.150425000	0.982211000
C	10.354497000	-7.778637000	2.146142000
C	9.521158000	-8.844419000	2.484251000
C	9.761846000	-9.846635000	1.509702000
N	10.672263000	-9.432927000	0.628926000
F	11.588215000	-11.510670000	-0.203172000
B	11.184504000	-10.242864000	-0.599361000
F	10.191729000	-10.320660000	-1.570082000
H	13.045490000	-10.774465000	-2.632987000
H	14.245705000	-6.722279000	-1.620790000
H	10.463965000	-5.345001000	0.549261000
H	11.112409000	-3.039591000	1.206786000
H	13.493434000	-2.506847000	1.698555000
H	15.222259000	-4.289380000	1.531182000

H	14.573676000	-6.593647000	0.873777000
H	10.483693000	-6.846251000	2.688930000
H	9.308246000	-10.830733000	1.411087000
C	15.051465000	-8.785296000	-3.609142000
C	15.528135000	-7.597821000	-4.183362000
C	15.544581000	-10.007298000	-4.089467000
C	16.472185000	-7.631546000	-5.206120000
C	16.484336000	-10.040295000	-5.116939000
C	16.953621000	-8.853044000	-5.678849000
H	15.140855000	-6.638307000	-3.836766000
H	15.202442000	-10.942714000	-3.643597000
H	16.828422000	-6.697575000	-5.642372000
H	16.857069000	-11.000412000	-5.475728000
H	17.690538000	-8.879213000	-6.482109000
C	8.583865000	-8.941845000	3.614265000
C	8.072495000	-7.784206000	4.218701000
C	8.183517000	-10.190659000	4.111655000
C	7.189865000	-7.872789000	5.291913000
C	7.295493000	-10.278404000	5.180985000
C	6.795919000	-9.120380000	5.776941000
H	8.356477000	-6.804399000	3.831154000
H	8.584389000	-11.104169000	3.669337000
H	6.800731000	-6.961319000	5.747235000
H	6.997561000	-11.258554000	5.555075000
H	6.101955000	-9.189622000	6.615141000

BP-PH

=====

$S_{1,m}$

N	12.623665000	-9.450596000	-0.988748000
C	13.637669000	-9.874871000	-1.738346000

C	14.376168000	-8.762702000	-2.232625000
C	13.732638000	-7.611554000	-1.726879000
C	12.657312000	-8.039898000	-0.934616000
C	10.509762000	-5.144387000	-0.159713000
C	10.495791000	-3.753987000	-0.099626000
C	11.687620000	-3.043892000	0.053677000
C	12.894234000	-3.737873000	0.150576000
C	12.912919000	-5.128692000	0.088397000
C	11.720226000	-5.855997000	-0.072835000
C	11.721025000	-7.324811000	-0.137039000
C	10.821572000	-8.074644000	0.673244000
C	9.976586000	-7.675934000	1.718207000
C	9.414160000	-8.843339000	2.280691000
C	9.947154000	-9.934331000	1.535849000
N	10.763890000	-9.481042000	0.588972000
F	11.944013000	-11.522445000	0.059407000
B	11.459866000	-10.340401000	-0.490356000
F	10.580863000	-10.615157000	-1.538872000
H	13.771891000	-10.937673000	-1.924725000
H	14.012812000	-6.577819000	-1.896038000
H	9.577919000	-5.694603000	-0.300059000
H	9.547882000	-3.220804000	-0.181776000
H	11.675617000	-1.954578000	0.100490000
H	13.829087000	-3.192818000	0.286742000
H	13.856322000	-5.664336000	0.200652000
H	9.847805000	-6.652964000	2.056443000
H	9.763407000	-11.001343000	1.634748000
C	15.540785000	-8.833660000	-3.099530000
C	16.091008000	-7.663397000	-3.659495000
C	16.150150000	-10.068828000	-3.400611000
C	17.205089000	-7.727397000	-4.487043000
C	17.263943000	-10.128573000	-4.228293000
C	17.797695000	-8.959125000	-4.776082000

H	15.631857000	-6.697333000	-3.448613000
H	15.752457000	-10.989504000	-2.972869000
H	17.614895000	-6.811069000	-4.912932000
H	17.722348000	-11.093338000	-4.447137000
H	18.672159000	-9.007739000	-5.425605000
C	8.483480000	-8.947606000	3.391821000
C	7.916284000	-7.790860000	3.963171000
C	8.125834000	-10.201918000	3.927436000
C	7.027976000	-7.886250000	5.026999000
C	7.237192000	-10.292953000	4.990815000
C	6.683492000	-9.136533000	5.546677000
H	8.170317000	-6.810533000	3.559370000
H	8.556554000	-11.113054000	3.511443000
H	6.597672000	-6.980098000	5.454432000
H	6.974761000	-11.272035000	5.392417000
H	5.985856000	-9.209812000	6.381445000

BP-PH

=====

$S_{1,r}$

N	12.945495000	-9.174412000	-0.679183000
C	14.092112000	-9.799842000	-0.436560000
C	14.921122000	-8.980550000	0.380030000
C	14.181846000	-7.803357000	0.627113000
C	12.955204000	-7.921139000	-0.037781000
C	10.550196000	-4.933348000	0.106292000
C	10.557751000	-3.548462000	0.203967000
C	11.762738000	-2.844497000	0.255645000
C	12.964232000	-3.551316000	0.170888000
C	12.965839000	-4.936211000	0.073125000

C	11.756912000	-5.674888000	0.074155000
C	11.753822000	-7.118209000	-0.027018000
C	10.550601000	-7.918360000	-0.006596000
C	9.341922000	-7.798056000	0.689929000
C	8.593491000	-8.973265000	0.461283000
C	9.399142000	-9.794053000	-0.376936000
N	10.540639000	-9.171330000	-0.648696000
F	11.729325000	-11.066300000	-1.557376000
B	11.731838000	-9.681291000	-1.499920000
F	11.716587000	-9.100669000	-2.761279000
H	14.279872000	-10.781493000	-0.863932000
H	14.465943000	-6.985240000	1.280090000
H	9.596038000	-5.447170000	0.016440000
H	9.609417000	-3.010424000	0.225776000
H	11.765089000	-1.757224000	0.333630000
H	13.914059000	-3.015500000	0.166440000
H	13.915973000	-5.452313000	-0.042773000
H	9.076724000	-6.979521000	1.350250000
H	9.198265000	-10.775168000	-0.799536000
C	16.261984000	-9.311568000	0.841745000
C	17.047392000	-8.354070000	1.511655000
C	16.804937000	-10.595026000	0.636028000
C	18.324017000	-8.669587000	1.960955000
C	18.081955000	-10.907098000	1.086208000
C	18.848294000	-9.947157000	1.751233000
H	16.654251000	-7.349732000	1.671466000
H	16.216128000	-11.358916000	0.127430000
H	18.917216000	-7.913511000	2.476214000
H	18.483020000	-11.907252000	0.919624000
H	19.850327000	-10.193439000	2.103589000
C	7.264162000	-9.301392000	0.957008000
C	6.710301000	-10.581301000	0.758278000
C	6.501042000	-8.344682000	1.653369000

C	5.444286000	-10.890665000	1.240246000
C	5.235403000	-8.657499000	2.134492000
C	4.700043000	-9.931480000	1.931050000
H	7.281854000	-11.344612000	0.229513000
H	6.902777000	-7.343117000	1.809108000
H	5.034503000	-11.888042000	1.078327000
H	4.659458000	-7.902060000	2.669901000
H	3.706572000	-10.175574000	2.308294000

BP-PH-2M-NO_2

=====

S_0

N	1.255517000	-1.756586000	-0.196074000
C	2.531083000	-2.146128000	-0.180623000
C	3.394316000	-1.026817000	-0.071560000
C	2.564956000	0.089566000	-0.029345000
C	1.224648000	-0.372466000	-0.095485000
C	-0.720895000	2.561788000	-0.880212000
C	-0.742138000	3.948118000	-0.776392000
C	-0.060140000	4.541126000	0.278846000
C	0.641396000	3.808188000	1.227847000
C	0.664259000	2.423145000	1.108050000
C	-0.015501000	1.791995000	0.056315000
C	0.003381000	0.315592000	-0.066037000
C	-1.207540000	-0.387777000	-0.133332000
C	-2.549701000	0.051345000	0.005001000
C	-3.365577000	-1.074274000	-0.051293000
C	-2.490909000	-2.177817000	-0.217862000
N	-1.222282000	-1.768848000	-0.265413000
F	0.003881000	-3.746163000	0.398911000

B	0.027196000	-2.675118000	-0.482565000
F	0.072100000	-3.115518000	-1.800882000
H	2.792127000	-3.199539000	-0.266123000
H	2.876246000	1.128348000	0.045611000
H	-1.237957000	2.069692000	-1.704305000
H	-1.271935000	4.569635000	-1.495179000
H	1.150901000	4.322464000	2.039942000
H	1.193683000	1.822418000	1.847614000
H	-2.867134000	1.079102000	0.162248000
H	-2.738098000	-3.233921000	-0.309698000
C	4.877429000	-1.069562000	-0.023194000
C	5.625755000	-0.583976000	-1.112983000
C	5.521057000	-1.597856000	1.112708000
C	7.021345000	-0.634807000	-1.048758000
C	6.918166000	-1.629943000	1.143829000
C	7.666293000	-1.152812000	0.071059000
H	7.605941000	-0.265426000	-1.893380000
H	7.421388000	-2.032968000	2.024684000
H	8.756043000	-1.185245000	0.107470000
C	-4.845013000	-1.137980000	0.046421000
C	-5.635718000	-0.585522000	-0.980172000
C	-5.444485000	-1.750079000	1.164500000
C	-7.027632000	-0.656736000	-0.872528000
C	-6.839408000	-1.799751000	1.240423000
C	-7.628748000	-1.258633000	0.229539000
H	-7.644431000	-0.236230000	-1.668950000
H	-7.308085000	-2.267118000	2.108430000
H	-8.716203000	-1.305910000	0.300773000
N	-0.083672000	6.013055000	0.398112000
O	0.517466000	6.507610000	1.327113000
O	-0.702701000	6.631522000	-0.440576000
C	4.945264000	-0.028037000	-2.338547000
H	4.162548000	-0.709433000	-2.702023000

H	4.456898000	0.934624000	-2.125524000
H	5.672453000	0.133082000	-3.144527000
C	4.725693000	-2.111259000	2.286584000
H	3.968930000	-1.379395000	2.604382000
H	4.188374000	-3.038188000	2.035014000
H	5.385048000	-2.325555000	3.137289000
C	-5.005342000	0.063584000	-2.186969000
H	-4.529732000	1.021223000	-1.926241000
H	-4.221982000	-0.575756000	-2.618994000
H	-5.761332000	0.261805000	-2.957429000
C	-4.606852000	-2.332443000	2.275287000
H	-4.092592000	-3.251184000	1.954724000
H	-3.829065000	-1.626008000	2.599221000
H	-5.233556000	-2.584852000	3.140169000

BP-PH-2M-NO_2

=====

$S_{1,m}$

N	-1.245436000	-1.803716000	0.424236000
C	-2.494096000	-2.251387000	0.327587000
C	-3.387814000	-1.168670000	0.078498000
C	-2.581808000	-0.012595000	0.029291000
C	-1.245911000	-0.411055000	0.204438000
C	0.924308000	2.553534000	0.694366000
C	0.982941000	3.926819000	0.512943000
C	0.139367000	4.513051000	-0.427416000
C	-0.753368000	3.763727000	-1.188290000
C	-0.807141000	2.391176000	-0.994170000
C	0.025415000	1.757599000	-0.048100000
C	-0.018615000	0.310647000	0.160420000

C	1.196157000	-0.429866000	0.264186000
C	2.523441000	-0.056604000	0.005335000
C	3.323907000	-1.214154000	0.096414000
C	2.428596000	-2.276002000	0.425428000
N	1.188744000	-1.809738000	0.540952000
F	-0.027323000	-3.893087000	0.419223000
B	-0.052242000	-2.621446000	0.977924000
F	-0.124897000	-2.693964000	2.368171000
H	-2.718793000	-3.298833000	0.512442000
H	-2.920917000	0.999948000	-0.154367000
H	1.559846000	2.083939000	1.445571000
H	1.658455000	4.551900000	1.093283000
H	-1.378019000	4.260611000	-1.927749000
H	-1.469874000	1.790864000	-1.616269000
H	2.845635000	0.930792000	-0.306448000
H	2.642690000	-3.322729000	0.624275000
C	-4.843680000	-1.274644000	-0.042638000
C	-5.685207000	-0.339627000	0.618269000
C	-5.418731000	-2.314477000	-0.822101000
C	-7.069023000	-0.470456000	0.496519000
C	-6.807158000	-2.393700000	-0.927859000
C	-7.633284000	-1.484506000	-0.272207000
H	-7.713955000	0.235906000	1.022018000
H	-7.245792000	-3.180523000	-1.543970000
H	-8.717218000	-1.566038000	-0.361415000
C	4.773230000	-1.329535000	-0.071766000
C	5.633124000	-0.345015000	0.487998000
C	5.326533000	-2.421608000	-0.793914000
C	7.012184000	-0.480113000	0.325954000
C	6.710648000	-2.503499000	-0.943688000
C	7.554323000	-1.546016000	-0.386653000
H	7.671764000	0.264632000	0.774559000
H	7.131719000	-3.331505000	-1.516539000

H	8.634796000	-1.630041000	-0.509263000
N	0.197605000	5.964690000	-0.626587000
O	-0.565822000	6.454408000	-1.434969000
O	1.006080000	6.591936000	0.028493000
C	-5.144216000	0.769483000	1.484978000
H	-4.277069000	0.443233000	2.073815000
H	-4.826075000	1.633228000	0.881623000
H	-5.924186000	1.121597000	2.171985000
C	-4.583811000	-3.318389000	-1.578136000
H	-3.687456000	-2.860525000	-2.016881000
H	-4.251473000	-4.140951000	-0.926596000
H	-5.176985000	-3.766739000	-2.385690000
C	5.119871000	0.822868000	1.293326000
H	4.766466000	1.637906000	0.643453000
H	4.283253000	0.534839000	1.943621000
H	5.926412000	1.229268000	1.916782000
C	4.472418000	-3.481313000	-1.444718000
H	4.176991000	-4.260187000	-0.725130000
H	3.554197000	-3.062170000	-1.876714000
H	5.037251000	-3.979174000	-2.243485000

BP-PH-2M-NO_2

=====

$S_{1,r}$

N	-1.202624000	-1.296006000	1.114570000
C	-2.347632000	-1.927557000	0.886633000
C	-3.172742000	-1.143159000	0.026370000
C	-2.428484000	0.016656000	-0.261647000
C	-1.202493000	-0.076393000	0.414189000
C	1.211800000	2.890087000	0.141630000

C	1.215665000	4.262338000	-0.033294000
C	-0.000880000	4.933609000	-0.141551000
C	-1.217075000	4.261889000	-0.032325000
C	-1.212529000	2.889615000	0.142556000
C	-0.000193000	2.152126000	0.194462000
C	0.000092000	0.719799000	0.369218000
C	1.202506000	-0.076238000	0.414043000
C	2.428975000	0.017220000	-0.261293000
C	3.173108000	-1.142519000	0.026538000
C	2.347886000	-1.927331000	0.886235000
N	1.202578000	-1.296164000	1.113864000
F	0.000116000	-3.141939000	2.101910000
B	0.000217000	-1.762980000	1.977488000
F	0.000636000	-1.116757000	3.204958000
H	-2.557775000	-2.877461000	1.370794000
H	-2.706019000	0.796284000	-0.962322000
H	2.164408000	2.384211000	0.275634000
H	2.145364000	4.825949000	-0.074720000
H	-2.147028000	4.825133000	-0.072918000
H	-2.164822000	2.383322000	0.277255000
H	2.706742000	0.796998000	-0.961708000
H	2.558146000	-2.877328000	1.370192000
C	-4.531701000	-1.480948000	-0.411728000
C	-5.551513000	-0.493967000	-0.385767000
C	-4.828971000	-2.793175000	-0.862692000
C	-6.838889000	-0.840300000	-0.799395000
C	-6.126560000	-3.087981000	-1.282385000
C	-7.130765000	-2.124501000	-1.249458000
H	-7.626563000	-0.085903000	-0.760509000
H	-6.347873000	-4.092152000	-1.648242000
H	-8.141100000	-2.374558000	-1.575367000
C	4.532356000	-1.480108000	-0.411232000
C	5.552457000	-0.493599000	-0.382641000

C	4.829347000	-2.791592000	-0.864284000
C	6.840077000	-0.839740000	-0.795713000
C	6.127194000	-3.086263000	-1.283350000
C	7.131788000	-2.123288000	-1.247754000
H	7.628032000	-0.085735000	-0.754852000
H	6.348388000	-4.089888000	-1.650786000
H	8.142313000	-2.373216000	-1.573176000
N	-0.001229000	6.382937000	-0.335658000
O	-1.077839000	6.942870000	-0.410956000
O	1.075105000	6.943278000	-0.411836000
C	-5.312080000	0.910882000	0.110257000
H	-4.645647000	0.928407000	0.983073000
H	-4.855048000	1.541365000	-0.667858000
H	-6.265696000	1.377486000	0.388178000
C	-3.783654000	-3.878476000	-0.942522000
H	-2.811077000	-3.488829000	-1.271361000
H	-3.633869000	-4.367836000	0.032096000
H	-4.103142000	-4.656451000	-1.647952000
C	5.312810000	0.910499000	0.115385000
H	4.856073000	1.542104000	-0.661986000
H	4.645875000	0.926665000	0.987851000
H	6.266257000	1.376748000	0.394481000
C	3.783449000	-3.876131000	-0.946812000
H	3.632469000	-4.366963000	0.026880000
H	2.811413000	-3.485285000	-1.275838000
H	4.103035000	-4.653171000	-1.653235000

========

BP-PH-2M

=====

S_0

N	-1.262715000	-1.893933000	0.232079000
C	-2.539753000	-2.242467000	0.404117000
C	-3.396052000	-1.148092000	0.131319000
C	-2.560957000	-0.089791000	-0.216969000
C	-1.225682000	-0.563820000	-0.161056000
C	0.731424000	2.472722000	-0.050893000
C	0.731306000	3.805896000	-0.453015000
C	0.022448000	4.195239000	-1.589825000
C	-0.694430000	3.248155000	-2.321455000
C	-0.709397000	1.915874000	-1.916332000
C	0.007871000	1.516850000	-0.779164000
C	-0.003494000	0.101387000	-0.350674000
C	1.204829000	-0.572848000	-0.112714000
C	2.551118000	-0.148058000	-0.240685000
C	3.365015000	-1.213614000	0.135268000
C	2.484773000	-2.271313000	0.470953000
N	1.214174000	-1.890362000	0.323360000
F	0.015371000	-3.796132000	-0.545560000
B	-0.029796000	-2.821131000	0.446697000
F	-0.080457000	-3.404373000	1.706321000
H	-2.803381000	-3.247138000	0.729790000
H	-2.862138000	0.922655000	-0.473223000
H	1.271248000	2.168406000	0.847043000
H	1.285181000	4.544588000	0.126862000
H	-1.243901000	3.546702000	-3.214533000
H	-1.257760000	1.170412000	-2.493860000
H	2.875565000	0.828506000	-0.590011000
H	2.726879000	-3.276790000	0.810373000

C	-4.877896000	-1.154613000	0.225127000
C	-5.516237000	-0.441508000	1.258220000
C	-5.631111000	-1.876696000	-0.720800000
C	-6.912283000	-0.461659000	1.330046000
C	-7.025472000	-1.872498000	-0.620520000
C	-7.664906000	-1.170803000	0.397825000
H	-7.411231000	0.084697000	2.132686000
H	-7.613163000	-2.426229000	-1.355161000
H	-8.753671000	-1.177134000	0.465626000
C	4.847383000	-1.257847000	0.185325000
C	5.539723000	-0.439632000	1.099929000
C	5.550448000	-2.116749000	-0.682543000
C	6.936129000	-0.495571000	1.134478000
C	6.946875000	-2.143700000	-0.622631000
C	7.638529000	-1.340611000	0.279766000
H	7.475553000	0.131395000	1.846951000
H	7.494897000	-2.802841000	-1.298545000
H	8.728275000	-1.373020000	0.316842000
C	-4.717272000	0.324496000	2.282319000
H	-3.915849000	-0.297498000	2.706621000
H	-4.233635000	1.206542000	1.836640000
H	-5.364409000	0.669116000	3.098983000
C	-4.953708000	-2.635126000	-1.834352000
H	-4.223910000	-2.001593000	-2.358990000
H	-4.401541000	-3.506316000	-1.450609000
H	-5.691472000	-2.997409000	-2.561632000
C	4.801101000	0.477612000	2.042658000
H	4.364553000	1.336067000	1.509816000
H	3.971837000	-0.046037000	2.539471000
H	5.480547000	0.869024000	2.810637000
C	4.822684000	-2.987040000	-1.676488000
H	4.302806000	-3.820361000	-1.180073000
H	4.060290000	-2.414453000	-2.223832000

H	5.526403000	-3.417098000	-2.400684000
H	0.028165000	5.238919000	-1.905628000

BP-PH-2M

=====

$S_{1,m}$

N	-1.243965000	-1.796775000	0.441425000
C	-2.496076000	-2.240924000	0.357077000
C	-3.384569000	-1.160551000	0.083417000
C	-2.574528000	-0.008888000	0.002654000
C	-1.239525000	-0.408761000	0.187726000
C	0.883424000	2.580906000	0.622977000
C	0.936045000	3.952523000	0.393683000
C	0.142853000	4.531619000	-0.598222000
C	-0.701344000	3.724760000	-1.361767000
C	-0.758907000	2.352360000	-1.133980000
C	0.029897000	1.756748000	-0.133649000
C	-0.013136000	0.308910000	0.115986000
C	1.198529000	-0.430864000	0.228002000
C	2.527776000	-0.059638000	-0.030822000
C	3.328101000	-1.215032000	0.083251000
C	2.434223000	-2.272285000	0.427537000
N	1.191563000	-1.806805000	0.530404000
F	-0.030915000	-3.889454000	0.443584000
B	-0.046440000	-2.609734000	0.988370000
F	-0.102183000	-2.673365000	2.381681000
H	-2.725744000	-3.283271000	0.563783000
H	-2.903518000	1.001140000	-0.212560000
H	1.490915000	2.135886000	1.413045000
H	1.595307000	4.575062000	0.999834000
H	-1.313039000	4.165212000	-2.150051000

H	-1.394114000	1.724787000	-1.760223000
H	2.846506000	0.925730000	-0.352686000
H	2.651248000	-3.314348000	0.647374000
C	-4.842289000	-1.264013000	-0.031902000
C	-5.678300000	-0.318832000	0.619984000
C	-5.423578000	-2.310495000	-0.796291000
C	-7.063319000	-0.445880000	0.504585000
C	-6.813033000	-2.386963000	-0.896237000
C	-7.634118000	-1.467250000	-0.249385000
H	-7.703660000	0.269578000	1.023578000
H	-7.255976000	-3.180486000	-1.500747000
H	-8.718672000	-1.546267000	-0.333747000
C	4.779839000	-1.332913000	-0.075004000
C	5.636908000	-0.348228000	0.486497000
C	5.336560000	-2.426959000	-0.789739000
C	7.017235000	-0.484326000	0.333106000
C	6.721933000	-2.511198000	-0.930848000
C	7.563319000	-1.552826000	-0.372403000
H	7.674277000	0.261994000	0.783113000
H	7.145248000	-3.342195000	-1.497882000
H	8.644492000	-1.638182000	-0.488155000
C	-5.128782000	0.798454000	1.470923000
H	-4.263607000	0.473104000	2.063220000
H	-4.802318000	1.650527000	0.855705000
H	-5.905701000	1.166296000	2.153326000
C	-4.593457000	-3.326289000	-1.541850000
H	-3.697030000	-2.875603000	-1.987921000
H	-4.260130000	-4.140733000	-0.880721000
H	-5.189772000	-3.783859000	-2.342043000
C	5.117612000	0.822278000	1.284273000
H	4.762703000	1.632649000	0.629544000
H	4.278595000	0.534481000	1.931580000
H	5.920477000	1.233927000	1.909263000

C	4.484083000	-3.488045000	-1.440696000
H	4.181171000	-4.262155000	-0.719104000
H	3.569278000	-3.068009000	-1.879168000
H	5.052230000	-3.991533000	-2.233736000
H	0.185118000	5.606358000	-0.777275000

BP-PH-2M

=====

$S_{1,r}$

N	-1.205516000	-0.612128000	1.136410000
C	-2.349180000	-1.261694000	0.947446000
C	-3.167772000	-0.545447000	0.025943000
C	-2.421487000	0.588365000	-0.347588000
C	-1.200606000	0.550693000	0.344256000
C	1.214274000	3.500418000	-0.098878000
C	1.214347000	4.865679000	-0.351075000
C	0.013542000	5.560441000	-0.511720000
C	-1.192514000	4.868592000	-0.380847000
C	-1.202192000	3.503294000	-0.128758000
C	0.003859000	2.769298000	-0.013424000
C	0.000389000	1.348011000	0.255987000
C	1.198811000	0.548141000	0.343832000
C	2.420692000	0.586592000	-0.346840000
C	3.164496000	-0.549580000	0.023308000
C	2.344460000	-1.267333000	0.942248000
N	1.201489000	-0.616666000	1.132552000
F	-0.003682000	-2.386848000	2.248676000
B	-0.001232000	-1.018719000	2.026002000
F	0.002172000	-0.290941000	3.208797000
H	-2.560902000	-2.174641000	1.497600000
H	-2.694495000	1.315054000	-1.104752000

H	2.164325000	2.997665000	0.069752000
H	2.164860000	5.397182000	-0.409381000
H	-2.139997000	5.402354000	-0.463058000
H	-2.157607000	3.003549000	0.015758000
H	2.695195000	1.317013000	-1.100049000
H	2.555096000	-2.181905000	1.490171000
C	-4.526642000	-0.915439000	-0.391927000
C	-5.549434000	0.066685000	-0.429468000
C	-4.818623000	-2.253084000	-0.761473000
C	-6.834955000	-0.308327000	-0.824783000
C	-6.114713000	-2.578628000	-1.164521000
C	-7.122174000	-1.618798000	-1.194798000
H	-7.624653000	0.445059000	-0.835239000
H	-6.331523000	-3.604838000	-1.466668000
H	-8.130869000	-1.892009000	-1.507099000
C	4.523112000	-0.921094000	-0.394983000
C	5.549170000	0.057657000	-0.422855000
C	4.810956000	-2.256753000	-0.774159000
C	6.834254000	-0.318750000	-0.818394000
C	6.106738000	-2.583833000	-1.177126000
C	7.117609000	-1.627349000	-1.197895000
H	7.626588000	0.431936000	-0.821474000
H	6.320559000	-3.608461000	-1.486694000
H	8.125995000	-1.901708000	-1.510199000
C	-5.313828000	1.500146000	-0.020549000
H	-4.650186000	1.571923000	0.851596000
H	-4.852544000	2.081781000	-0.833363000
H	-6.269149000	1.982027000	0.224046000
C	-3.768227000	-3.336551000	-0.771093000
H	-2.798153000	-2.963158000	-1.125492000
H	-3.613311000	-3.759261000	0.233376000
H	-4.084257000	-4.161059000	-1.423400000
C	5.316867000	1.488676000	-0.003649000

H	4.857384000	2.077323000	-0.812407000
H	4.652758000	1.555484000	0.868577000
H	6.273121000	1.966516000	0.245213000
C	3.756402000	-3.336050000	-0.793694000
H	3.597176000	-3.764849000	0.207522000
H	2.788702000	-2.956320000	-1.147891000
H	4.070745000	-4.157413000	-1.450776000
H	0.017294000	6.632166000	-0.710797000

========

BP-PH-ISO

=====

S_0

N	-0.451068000	2.953535000	-3.999839000
C	-1.489908000	3.186651000	-4.807760000
C	-1.202684000	4.251442000	-5.693882000
C	0.087261000	4.671575000	-5.377060000
C	0.555804000	3.850921000	-4.320480000
C	3.355007000	5.714722000	-2.992660000
C	4.351542000	6.620768000	-3.346468000
C	4.828632000	6.661639000	-4.656810000
C	4.300467000	5.798760000	-5.617326000
C	3.293968000	4.900071000	-5.272825000
C	2.816791000	4.847852000	-3.955108000
C	1.753253000	3.889080000	-3.585484000
C	1.929177000	3.029860000	-2.490402000
C	3.027204000	2.853032000	-1.608396000
C	2.670946000	1.874204000	-0.686397000
C	1.362175000	1.465342000	-1.043640000

N	0.930094000	2.146611000	-2.106677000
F	-0.341879000	0.598448000	-3.452540000
B	-0.392090000	1.868343000	-2.885435000
F	-1.488600000	1.986379000	-2.040865000
H	-2.401402000	2.597259000	-4.724172000
H	0.634527000	5.491763000	-5.834481000
H	2.967722000	5.692819000	-1.972923000
H	4.754675000	7.300065000	-2.595022000
H	4.675271000	5.822877000	-6.640826000
H	2.888354000	4.214523000	-6.018113000
H	3.973925000	3.384264000	-1.663202000
H	0.738862000	0.705161000	-0.576036000
C	-2.112889000	4.801281000	-6.736866000
C	-2.974956000	5.872574000	-6.417864000
C	-2.097306000	4.248710000	-8.035527000
C	-3.830008000	6.364523000	-7.409335000
C	-2.968787000	4.769463000	-8.997758000
C	-3.831188000	5.816220000	-8.688151000
H	-4.504943000	7.190029000	-7.180811000
H	-2.973361000	4.352901000	-10.005666000
H	-4.504397000	6.211659000	-9.450202000
C	3.470879000	1.345305000	0.453014000
C	3.423993000	2.004409000	1.699666000
C	4.255606000	0.185632000	0.276652000
C	4.191330000	1.500305000	2.755057000
C	5.006487000	-0.287066000	1.357644000
C	4.979064000	0.366163000	2.586040000
H	4.170303000	1.997597000	3.725414000
H	5.620573000	-1.180508000	1.240816000
H	5.570292000	-0.015944000	3.419580000
C	-3.020682000	6.453165000	-5.012356000
H	-2.038256000	6.280481000	-4.549536000
C	-1.192625000	3.075424000	-8.381278000

H	-0.334965000	3.102939000	-7.693463000
C	2.594175000	3.265195000	1.890773000
H	1.777103000	3.239613000	1.154625000
C	4.333172000	-0.509268000	-1.074451000
H	3.386864000	-0.318631000	-1.602162000
H	5.612933000	7.368160000	-4.930268000
C	-0.642092000	3.135571000	-9.807602000
H	0.113038000	2.348648000	-9.948709000
H	-1.430589000	2.966635000	-10.556580000
H	-0.172429000	4.107518000	-10.017334000
C	-1.935825000	1.753708000	-8.150189000
H	-2.819150000	1.694750000	-8.805909000
H	-1.282721000	0.896452000	-8.372542000
H	-2.279640000	1.663081000	-7.109599000
C	-3.284535000	7.959931000	-4.986754000
H	-3.157114000	8.342434000	-3.963683000
H	-2.592049000	8.501719000	-5.647161000
H	-4.313095000	8.199235000	-5.296810000
C	-4.069932000	5.712895000	-4.173239000
H	-5.070067000	5.839578000	-4.617504000
H	-3.855799000	4.635545000	-4.121269000
H	-4.095470000	6.108180000	-3.146600000
C	4.505296000	-2.025545000	-0.970285000
H	4.402983000	-2.480433000	-1.966051000
H	5.501694000	-2.296950000	-0.589563000
H	3.750500000	-2.472498000	-0.307312000
C	5.464454000	0.101156000	-1.911389000
H	6.432428000	-0.048208000	-1.406942000
H	5.514370000	-0.375169000	-2.902149000
H	5.317782000	1.181525000	-2.054112000
C	1.960220000	3.368203000	3.279403000
H	1.404961000	2.454779000	3.536686000
H	2.715819000	3.542775000	4.060368000

H	1.261334000	4.216733000	3.307725000
C	3.447313000	4.506197000	1.599727000
H	2.847774000	5.423222000	1.704977000
H	4.290416000	4.562572000	2.306707000
H	3.861851000	4.477015000	0.581437000

BP-PH-ISO

=====

$S_{1,m}$

N	-0.192002000	2.765822000	-4.280805000
C	-1.218506000	2.930355000	-5.114771000
C	-1.111264000	4.182030000	-5.789992000
C	0.061948000	4.780495000	-5.310041000
C	0.643759000	3.894641000	-4.376567000
C	3.101342000	5.938213000	-2.585282000
C	3.942196000	7.037562000	-2.734770000
C	4.387600000	7.416746000	-4.001651000
C	3.980625000	6.689283000	-5.121070000
C	3.138493000	5.590594000	-4.976324000
C	2.688223000	5.193486000	-3.704592000
C	1.800265000	4.030887000	-3.559072000
C	2.016808000	3.053097000	-2.549652000
C	3.003805000	2.952687000	-1.545237000
C	2.735248000	1.798227000	-0.796002000
C	1.573969000	1.212980000	-1.378513000
N	1.153740000	1.948239000	-2.408865000
F	0.509582000	0.451213000	-4.192817000
B	0.055023000	1.513328000	-3.407901000
F	-1.106933000	1.146005000	-2.735935000
H	-2.006066000	2.182173000	-5.190076000
H	0.465078000	5.747749000	-5.594217000

H	2.731732000	5.668202000	-1.594806000
H	4.243573000	7.607865000	-1.855232000
H	4.328365000	6.974089000	-6.114729000
H	2.841391000	5.011355000	-5.852139000
H	3.836431000	3.634328000	-1.403567000
H	1.035854000	0.315716000	-1.078479000
C	-2.088067000	4.694698000	-6.773144000
C	-2.797507000	5.898326000	-6.513167000
C	-2.322103000	3.967429000	-7.962387000
C	-3.731351000	6.337841000	-7.451791000
C	-3.273428000	4.445737000	-8.870647000
C	-3.972876000	5.618598000	-8.621664000
H	-4.290737000	7.254853000	-7.269897000
H	-3.459120000	3.893677000	-9.793952000
H	-4.708717000	5.980678000	-9.341011000
C	3.469841000	1.300604000	0.384203000
C	3.578788000	2.115367000	1.536174000
C	4.074937000	0.015831000	0.350761000
C	4.304732000	1.635267000	2.631653000
C	4.779059000	-0.423091000	1.472147000
C	4.899670000	0.380273000	2.604579000
H	4.396697000	2.253076000	3.526571000
H	5.253897000	-1.403765000	1.463706000
H	5.459461000	0.021847000	3.469556000
C	-2.628501000	6.641834000	-5.194595000
H	-1.560999000	6.638796000	-4.937131000
C	-1.548736000	2.706769000	-8.321325000
H	-0.809546000	2.516821000	-7.530338000
C	2.916505000	3.481780000	1.643405000
H	2.245185000	3.605562000	0.783671000
C	4.062151000	-0.822147000	-0.919954000
H	3.080135000	-0.700186000	-1.400146000
H	5.047651000	8.276988000	-4.116544000

C	-0.769528000	2.890557000	-9.628666000
H	-0.167889000	1.994577000	-9.841304000
H	-1.450159000	3.048730000	-10.479504000
H	-0.093756000	3.755731000	-9.567268000
C	-2.475527000	1.489858000	-8.403113000
H	-3.223890000	1.618152000	-9.200833000
H	-1.896710000	0.581271000	-8.625363000
H	-3.016151000	1.332385000	-7.458217000
C	-3.079280000	8.101353000	-5.246293000
H	-2.796128000	8.606654000	-4.312029000
H	-2.615181000	8.641534000	-6.084226000
H	-4.172324000	8.185815000	-5.344494000
C	-3.375271000	5.897770000	-4.078700000
H	-4.453630000	5.866559000	-4.301180000
H	-3.016891000	4.862956000	-3.975347000
H	-3.236017000	6.408907000	-3.114377000
C	4.267155000	-2.316862000	-0.678076000
H	4.097479000	-2.867930000	-1.613860000
H	5.295018000	-2.537580000	-0.351788000
H	3.573266000	-2.704895000	0.081607000
C	5.117791000	-0.289436000	-1.899203000
H	6.122426000	-0.383467000	-1.457400000
H	5.096649000	-0.863733000	-2.837332000
H	4.944141000	0.769797000	-2.137845000
C	2.058882000	3.604023000	2.907457000
H	1.316856000	2.794742000	2.962624000
H	2.672359000	3.569294000	3.820448000
H	1.522838000	4.564581000	2.905707000
C	3.964552000	4.599471000	1.590803000
H	3.478727000	5.586138000	1.633938000
H	4.656445000	4.523607000	2.444676000
H	4.564612000	4.548163000	0.670029000

BP-PH-ISO

=====

$S_{1,r}$

N	1.017681000	-1.499552000	0.365476000
C	2.214460000	-1.764742000	-0.153776000
C	3.133002000	-0.733052000	0.190523000
C	2.409832000	0.192786000	0.951882000
C	1.091087000	-0.296282000	1.082447000
C	-1.340958000	1.648197000	3.326234000
C	-1.360427000	2.430955000	4.472389000
C	-0.185978000	2.672297000	5.189155000
C	1.007667000	2.089047000	4.757094000
C	1.037163000	1.304526000	3.612468000
C	-0.133234000	1.085548000	2.845235000
C	-0.108133000	0.266613000	1.658095000
C	-1.253241000	0.035879000	0.806129000
C	-2.314206000	0.867695000	0.388077000
C	-3.078923000	0.148157000	-0.539026000
C	-2.430773000	-1.109468000	-0.694327000
N	-1.358430000	-1.167448000	0.092663000
F	-0.541016000	-3.068880000	1.348069000
B	-0.304110000	-2.301347000	0.214815000
F	-0.277935000	-3.064682000	-0.941055000
H	2.379411000	-2.657491000	-0.754278000
H	2.783286000	1.130227000	1.353266000
H	-2.277117000	1.440877000	2.811216000
H	-2.306630000	2.848661000	4.817942000
H	1.925524000	2.237365000	5.326963000
H	1.970585000	0.825475000	3.322994000
H	-2.488544000	1.889806000	0.711724000
H	-2.690393000	-1.939556000	-1.348238000

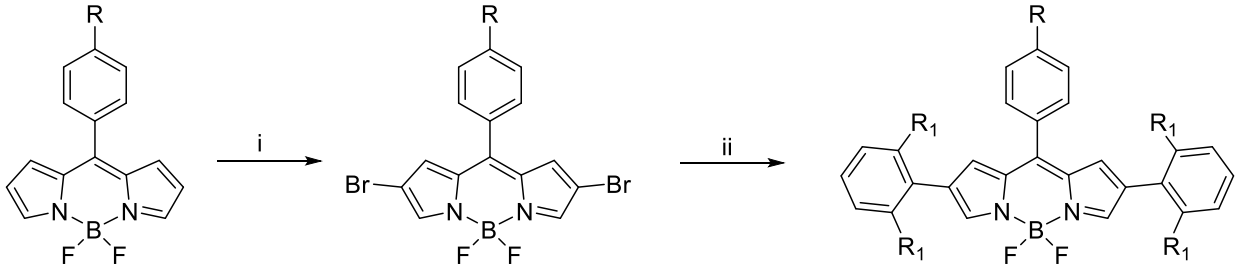
C	4.564202000	-0.695439000	-0.194401000
C	5.025103000	0.285512000	-1.108817000
C	5.453063000	-1.648137000	0.346400000
C	6.373773000	0.280740000	-1.468884000
C	6.795821000	-1.619265000	-0.049357000
C	7.254729000	-0.665669000	-0.947935000
H	6.748847000	1.023655000	-2.172366000
H	7.493065000	-2.351794000	0.361912000
H	8.304610000	-0.653055000	-1.243990000
C	-4.298875000	0.595805000	-1.250559000
C	-4.213656000	1.640555000	-2.196337000
C	-5.541909000	-0.027041000	-0.972744000
C	-5.383363000	2.058745000	-2.842478000
C	-6.679836000	0.416501000	-1.647864000
C	-6.605039000	1.455815000	-2.573715000
H	-5.331825000	2.865794000	-3.575811000
H	-7.644923000	-0.048054000	-1.446609000
H	-7.506040000	1.792028000	-3.088746000
C	4.058518000	1.267015000	-1.757903000
H	3.322182000	1.566693000	-0.999347000
C	5.012854000	-2.689904000	1.364214000
H	3.956178000	-2.508197000	1.606656000
C	-2.898027000	2.312763000	-2.559878000
H	-2.087415000	1.779887000	-2.045682000
C	-5.654111000	-1.091956000	0.108871000
H	-4.739646000	-1.703500000	0.075977000
H	-0.205013000	3.288988000	6.087840000
C	5.805088000	-2.562729000	2.669827000
H	5.429191000	-3.280551000	3.413744000
H	6.874028000	-2.773019000	2.510782000
H	5.715857000	-1.550165000	3.089204000
C	5.127310000	-4.105499000	0.789694000
H	6.173507000	-4.344960000	0.542267000

```
H        4.773773000    -4.847192000     1.521146000
H        4.531244000    -4.214143000    -0.128347000
C        4.727618000     2.540655000    -2.274287000
H        3.958308000     3.258417000    -2.592916000
H        5.345117000     3.018221000    -1.499746000
H        5.366060000     2.338538000    -3.147839000
C        3.295926000     0.571898000    -2.894158000
H        3.999120000     0.242158000    -3.675276000
H        2.750309000    -0.311179000    -2.530396000
H        2.570230000     1.261456000    -3.351229000
C       -6.841817000    -2.035986000    -0.074184000
H       -6.773484000    -2.860361000     0.649718000
H       -7.798823000    -1.522810000     0.105906000
H       -6.866451000    -2.465996000    -1.085943000
C       -5.711389000    -0.424331000     1.489957000
H       -6.609472000     0.209078000     1.565075000
H       -5.756253000    -1.184203000     2.284372000
H       -4.828894000     0.208026000     1.665618000
C       -2.618634000     2.225721000    -4.064054000
H       -2.632237000     1.181886000    -4.409214000
H       -3.365196000     2.787559000    -4.646031000
H       -1.629933000     2.651833000    -4.290449000
C       -2.880062000     3.769486000    -2.084678000
H       -1.914381000     4.240465000    -2.321600000
H       -3.672587000     4.352248000    -2.580276000
H       -3.041862000     3.838315000    -0.998814000
---
```

5. Synthesis and NMR spectra of BP-PH, BP-PH-2M, BP-PH-ISO and BP-PH-2M-NO_2

Reagents and solvents for the organic synthesis of the BODIPY molecules were purchased directly from commercial suppliers; solvents were purified by known procedures. Thin layer chromatography was performed using TLC-aluminum sheets with silica gel (Merck 60 F254). Visualization was accomplished by UV light. Column chromatography was performed using Silica gel 60 (0.040-0.063 mm) (Merck). NMR spectra were recorded on a Bruker Ascend 400 (400 MHz for ^{1}H, 100 MHz for ^{13}C, 128.4 MHz for ^{11}B, 376.5 MHz for ^{19}F). NMR spectra were referenced to residual solvent peaks. Melting points were determined in open capillaries with a digital melting point IA9100 series apparatus (Thermo Fischer Scientific) and were not corrected.

Reaction scheme



Reagents and conditions: i – NBS, CH_2Cl_2/DMF; ii – arylboronic acid, $Pd(OAc)_2$, SPhos, K_3PO_4, toluene, argon, 140 °C, 24h.

Compounds **BP1,2** were synthesized as previously reported.[4] **BP3,4** were synthesized using the analogical procedure.[5] To a solution of **BP1** (0.37 mmol) or **BP2** (0.96 mmol) in DMF/CH_2Cl_2 (10 mL /10 mL) a solution of NBS (2.4 eq. for **BP1** and 3.6 eq. for **BP2**) in CH_2Cl_2 (10 mL) was added dropwise at room temperature. The mixture was stirred at room temperature for 2 hours. After removal of solvents

under vacuum, the crude product was purified by column chromatography using $CHCl_3$-petroleum ether (2:1) as an eluent.

BP3, dark rose solid, yield 100 mg (63%), mp 215-216 °C. ^{1}H NMR ($CDCl_3$): δ (ppm) = 7.86 (s, 2H), 7.67-7.63 (m, 1H), 7.59-7.53 (m, 4H), 6.98 (s, 2H). ^{13}C NMR ($CDCl_3$): δ = 144.23, 134.66, 132.84, 131.79, 131.61, 130.44, 130.40, 128.92, 128.87. ^{11}B NMR ($CDCl_3$): δ = -0.27 (t, *J* =27.5 Hz). ^{19}F NMR ($CDCl_3$): δ = -144.72 (q, *J* = 30.2 Hz).

BP4, dark red, almost black solid, yield 160 mg (36%), mp 255-256 °C. ^{1}H NMR ($CDCl_3$): δ (ppm) = 8.36 (d, *J* = 8 Hz, 2H), 7.84 (s, 2H), 7.67 (d, *J* = 8 Hz, 2H), 6.81 (s, 2H). ^{13}C NMR ($CDCl_3$): δ = 149.48, 145.74, 138.67, 134.32, 131.26, 131.19, 124.08, 124.00, 108.24. ^{11}B NMR ($CDCl_3$): δ = -0.30 (t, *J* =27.5 Hz). ^{19}F NMR ($CDCl_3$): δ = -144.79 (q, *J* = 36.4 Hz).

General procedure for the synthesis of compounds BP-PH, BP-PH-2M, BP-PH-ISO and BP-PH-2M-NO_2.

To a solution of **BP3** (0.12 mmol) or **BP4** (0.106 mmol), corresponding arylboronic acid (2,4 eq.), K_3PO_4 (4.8 eq.), SPhos (20 mol%) in toluene (5 mL) Pd_2dba_3 (10 mol%) was added under an argon atmosphere. The mixture was heated at 140 °C for 24 hours, and then cooled to room temperature. Water (10 mL) was added and the mixture was extracted with $CHCl_3$ (2*20 mL), the combined organic layers were dried over Na_2SO_4 and concentrated under reduced pressure. Residue was purified by column chromatography using $CHCl_3$-petroleum ether (1:1) as an eluent.

BP-PH. Violet crystals, yield 50%, mp 91-92 °C. ^{1}H NMR ($CDCl_3$): δ (ppm) = 8.22 (s, 2H), 7.58-7.51 (m, 5H), 7.46-7.44 (m, 4H), 7.32-7.28 (m, 4H), 7.22-7.17 (m, 2H), 7.02 (s, 2H). ^{13}C NMR ($CDCl_3$): δ = 146.53, 142.12, 135.83, 134.37, 133.83, 132.55, 130.90, 130.52, 128.99, 128.67, 127.65, 125.67, 125.47. ^{11}B NMR ($CDCl_3$): δ = 0.23 (t, *J* =28.8 Hz). ^{19}F NMR ($CDCl_3$): δ = -145.13 (q, *J* = 30.2 Hz).

BP-PH-2M. Orange-red crystals, yield 60%, mp 247 °C. ^{1}H NMR ($CDCl_3$): δ (ppm) = 7.68 (s, 2H), 7.45-7.43 (m, 2H), 7.37-7.30 (m, 3H), 6.95-6.89 (m, 6H), 6.65 (s, 2H). ^{13}C NMR ($CDCl_3$): δ = 146.43, 144.35, 137.05, 135.10, 133.86, 132.98, 132.53, 130.86, 130.65, 128.53, 127.71, 127.57, 21.23. ^{11}B NMR ($CDCl_3$): δ = 0.35 (t, *J* =28.8 Hz). ^{19}F NMR ($CDCl_3$): δ = -144.74 (q, *J* = 30. Hz).

BP-PH-ISO. Dark rose crystals, yield 26%, mp 269-270 °C. ^{1}H NMR ($CDCl_3$): δ (ppm) = 7.77 (s, 2H), 7.56-7.54 (m, 2H), 7.47-7.40 (m, 3H), 7.26 (t, *J* = 8 Hz, 2H), 7.13-7.11 (m, 4H), 6.76 (s, 2H), 2.79-2.76 (m, 4H), 1.08 (d, *J* = 8 Hz, 12H), 1.02 (d, *J* = 8 Hz, 12H). ^{13}C NMR ($CDCl_3$): δ = 147.97, 146.50, 144.28, 135.06, 133.81, 132.13, 131.13, 130.91, 130.64, 130.61, 128.57, 128.54, 122.60, 30.66, 24.26, 24.15. ^{11}B NMR ($CDCl_3$): δ = 0.36 (t, *J* =27.5 Hz). ^{19}F NMR ($CDCl_3$): δ = -144.6 (q, *J* = 30.2 Hz).

BP-PH-2M-NO$_2$. Dark red crystals, yield 44%, mp 260 °C. ^{1}H NMR ($CDCl_3$): δ (ppm) = 8.42 (d, *J* = 8 Hz, 2H), 7.96 (s, 2H), 7.87 (d, *J* = 8 Hz, 2H), 7.19-7.12 (m, 6H), 6.77 (s, 2H), 2.21 (s, 12H). ^{13}C NMR ($CDCl_3$): δ = 149.16, 145.89, 142.69, 139.87, 136.89, 134.69, 133.49, 132.36, 131.36, 130.21, 129.05, 128.24, 127.95, 127.67, 126.74, 125.31, 123.74, 21.18. ^{11}B NMR ($CDCl_3$): δ = 0.28 (t, *J* =27.5 Hz). ^{19}F NMR ($CDCl_3$): δ = -144.66 (q, *J* = 30.2 Hz).

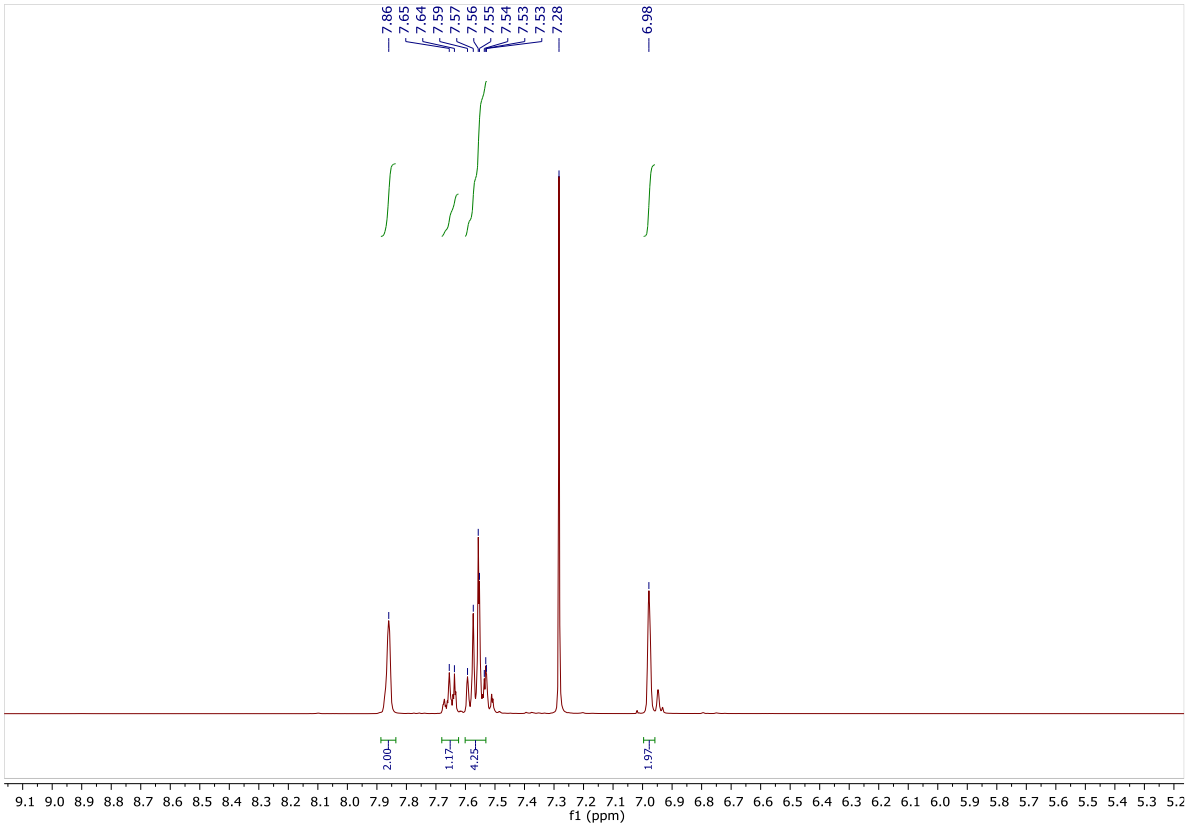


Figure S12. ^{1}H NMR spectrum of BP3.

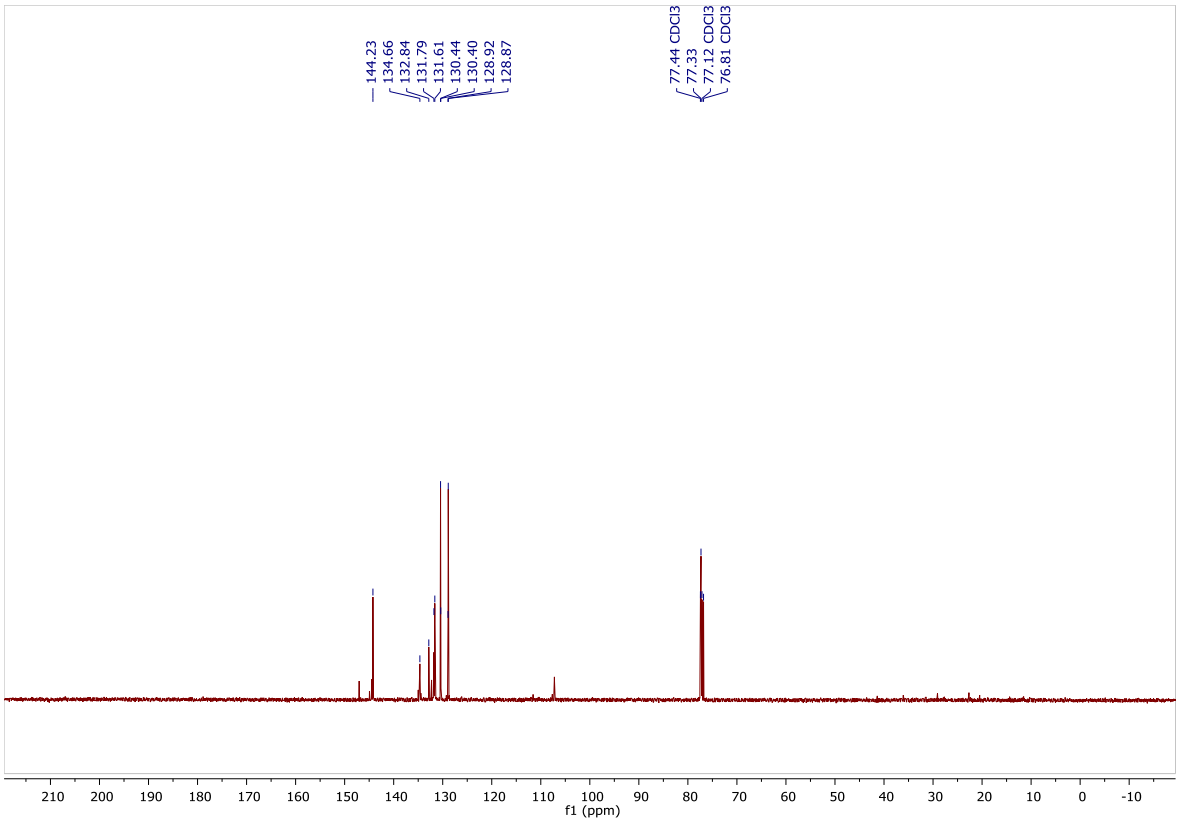


Figure S13. ^{13}C NMR spectrum of BP3.

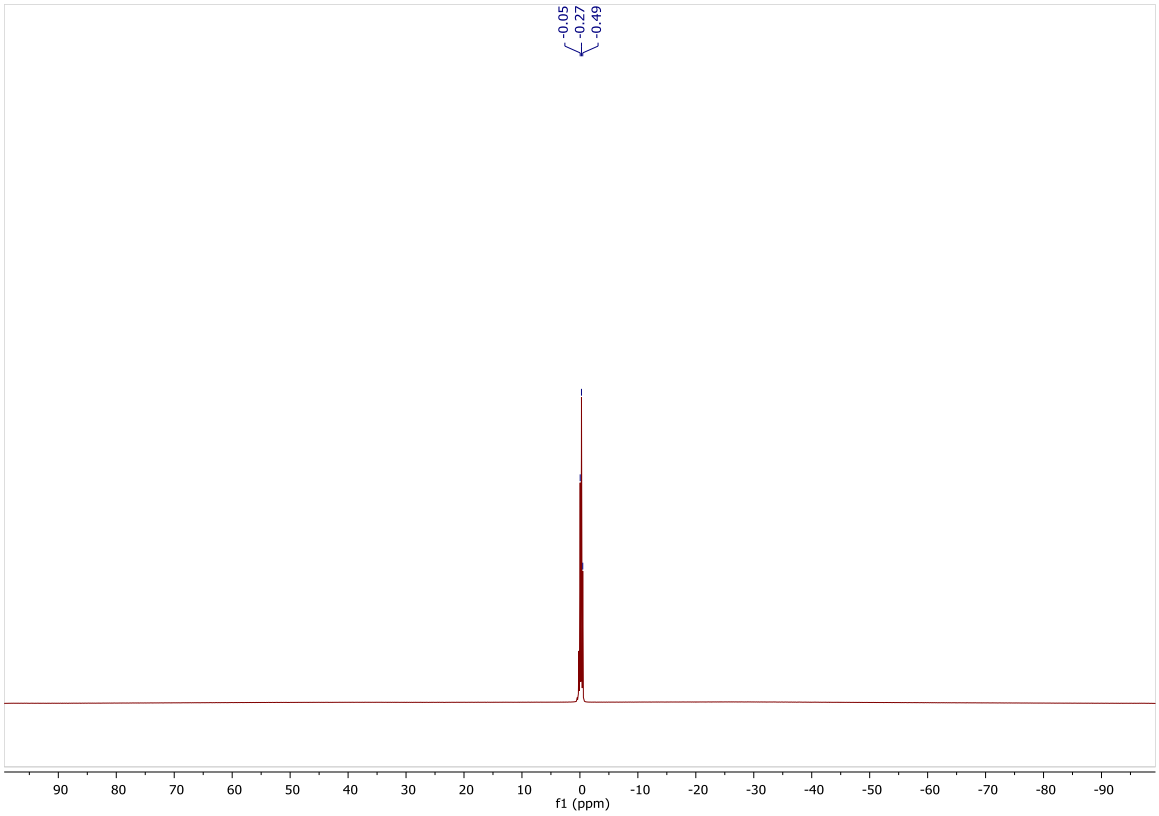


Figure S14. ^{11}B NMR spectrum of BP3.

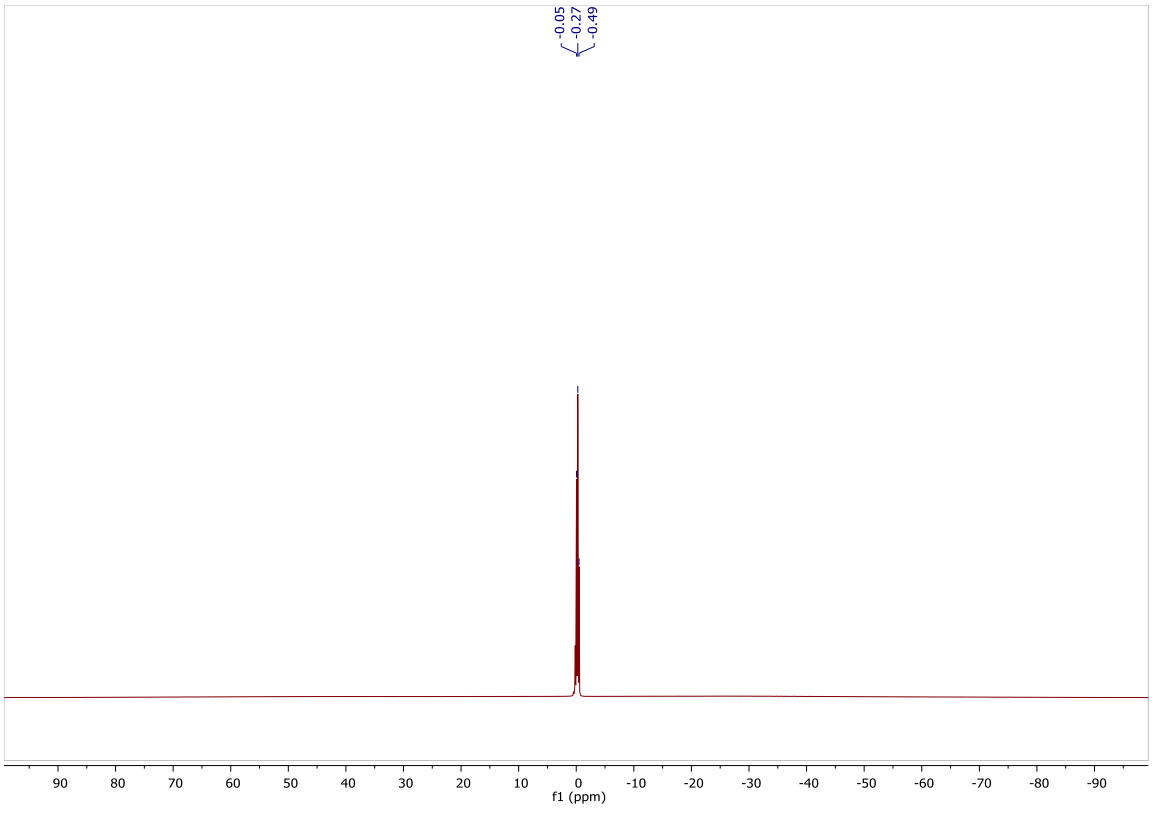


Figure S15. ^{19}F NMR spectrum of BP3.

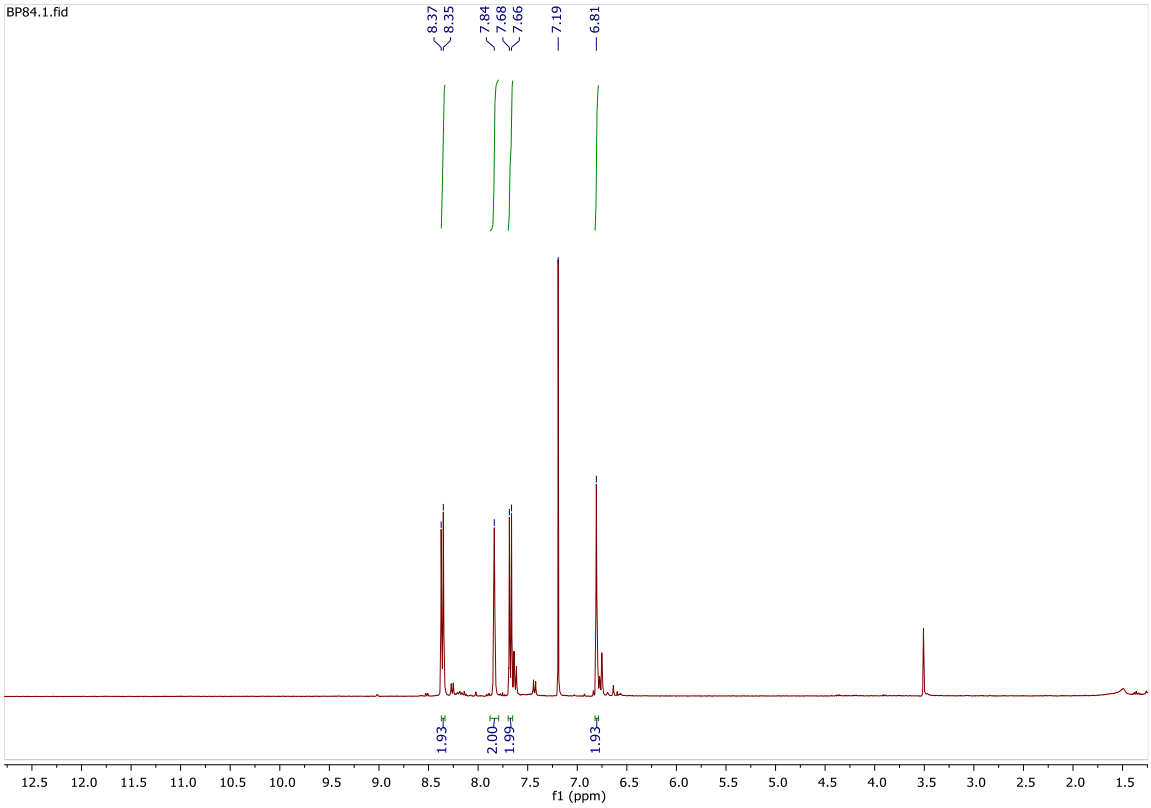


Figure S16. ^{1}H NMR spectrum of BP4.

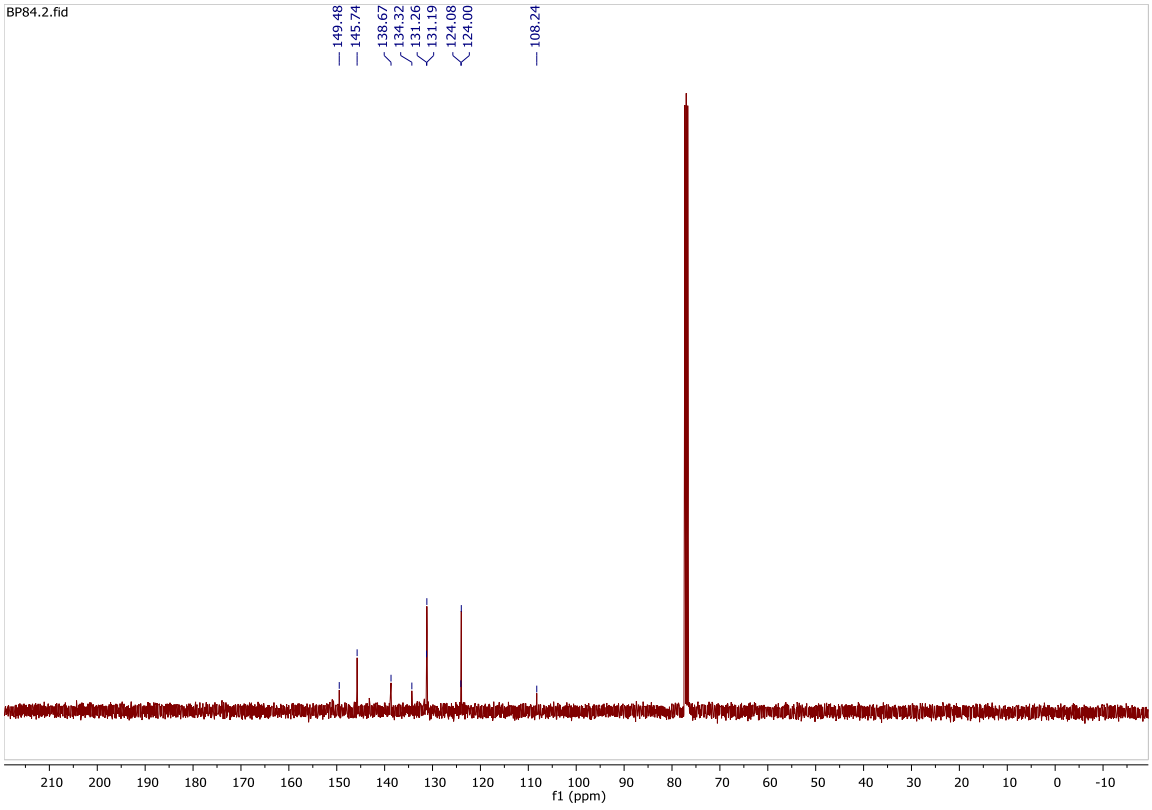


Figure S17. ^{13}C NMR spectrum of BP4.

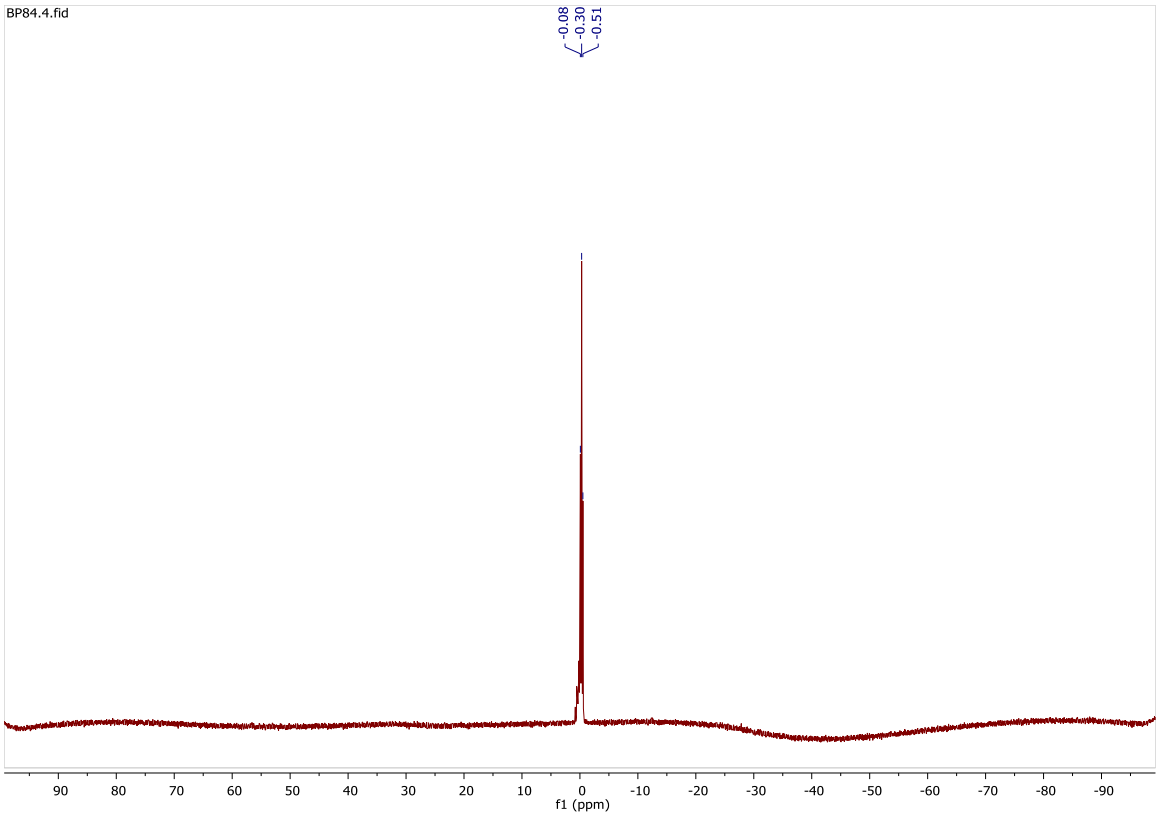


Figure S18. ^{11}B NMR spectrum of BP4.

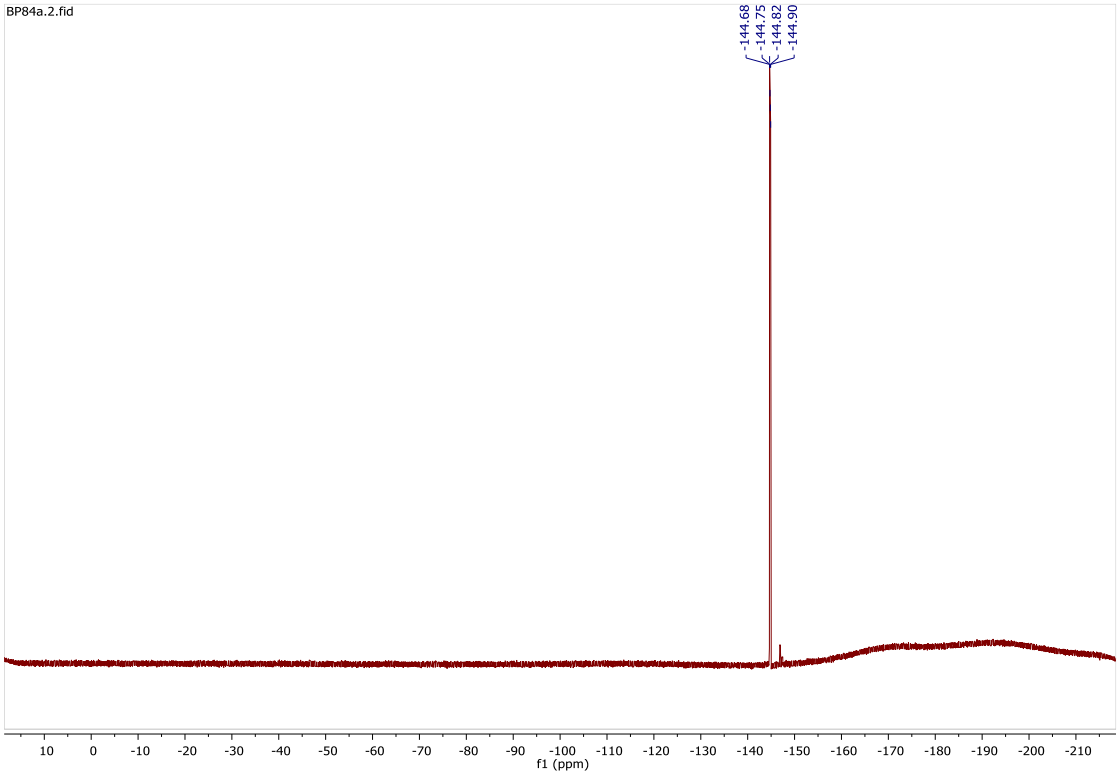


Figure S19. ^{19}F NMR spectrum of BP4.

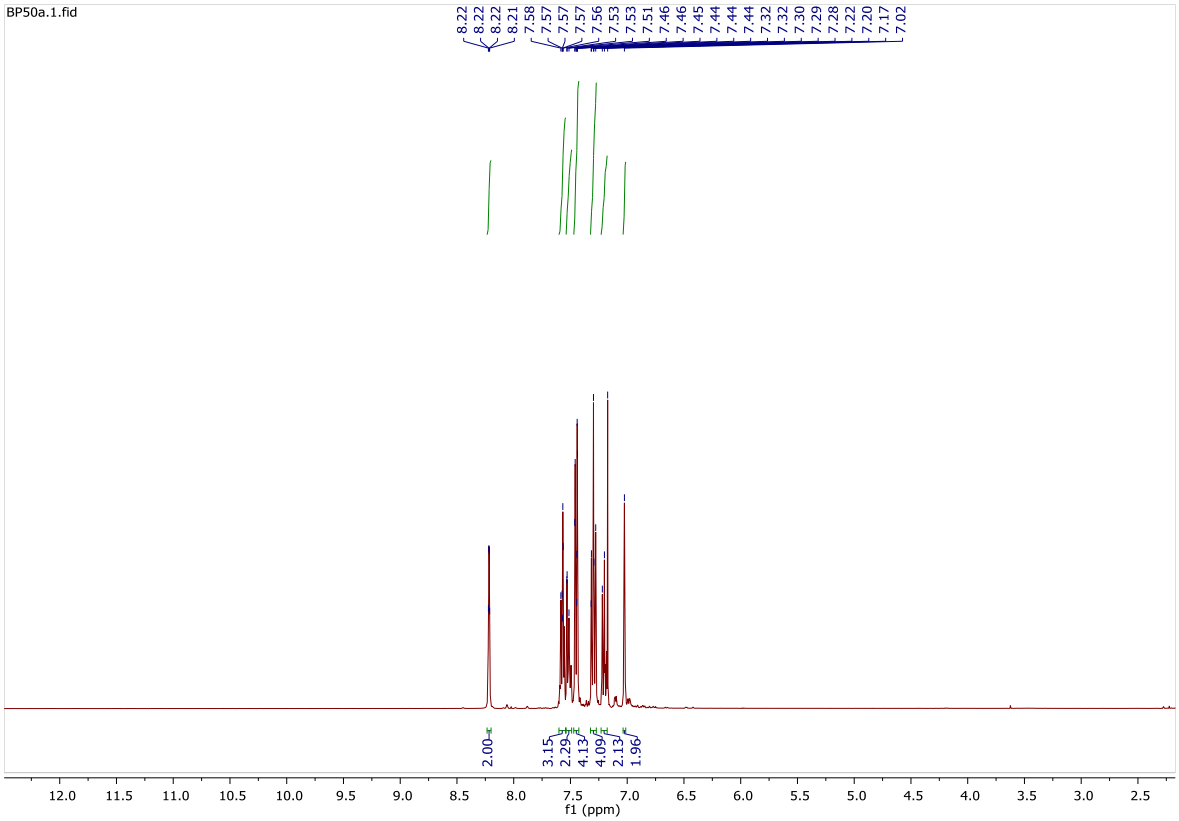


Figure S20. ^{1}H NMR spectrum of BP-PH.

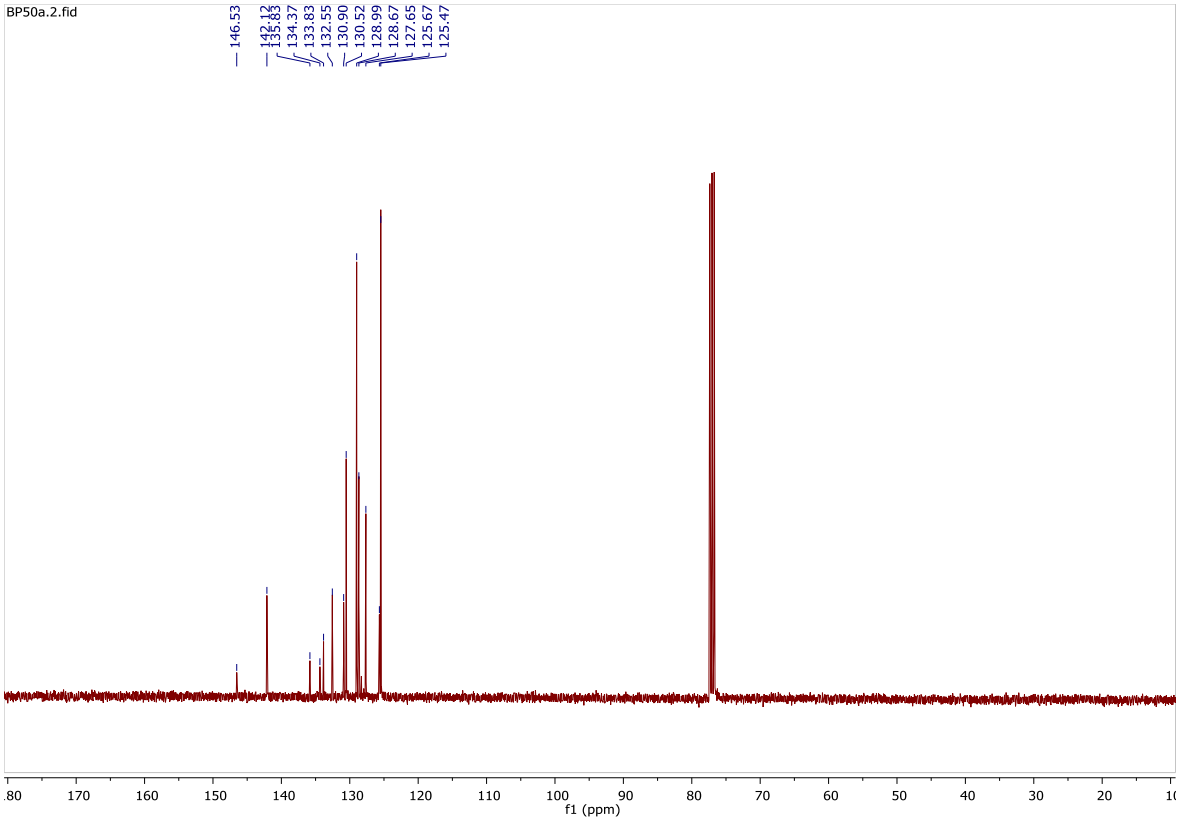


Figure S21. ^{13}C NMR spectrum of BP-PH.

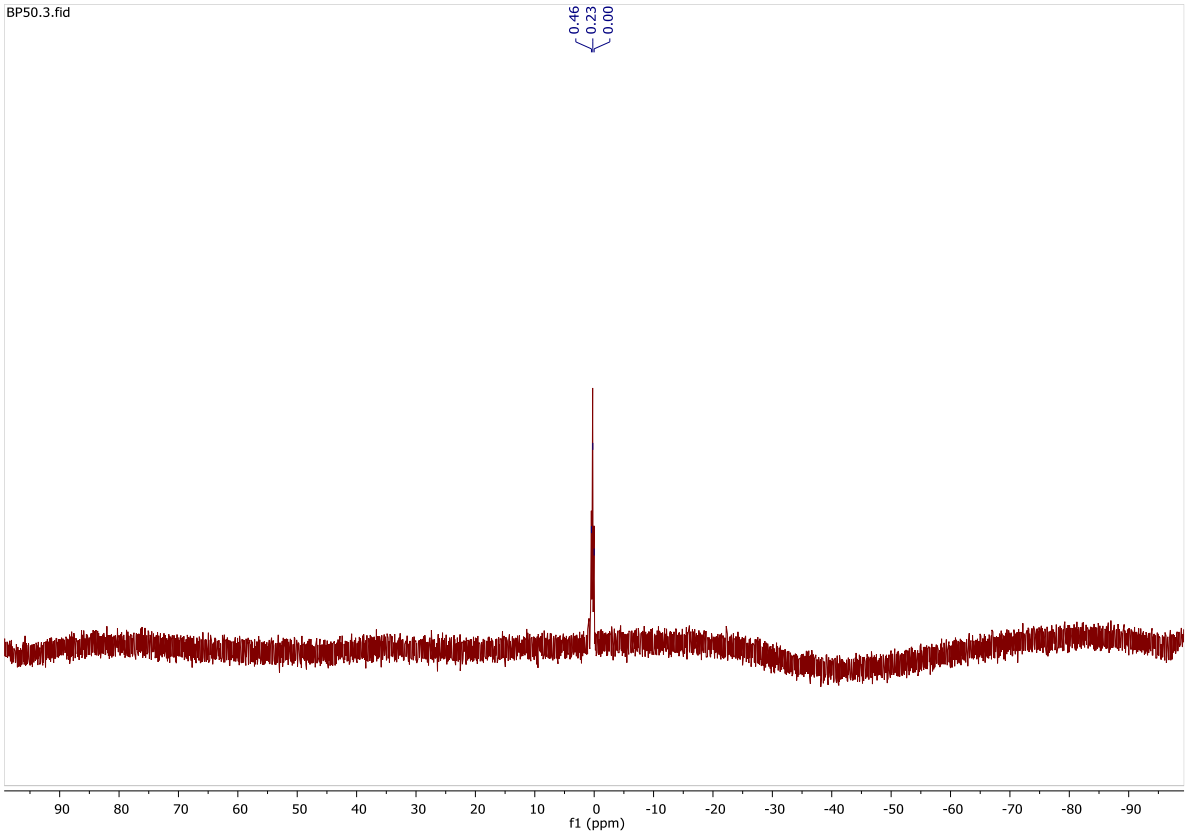


Figure S22. ^{11}B NMR spectrum of BP-PH.

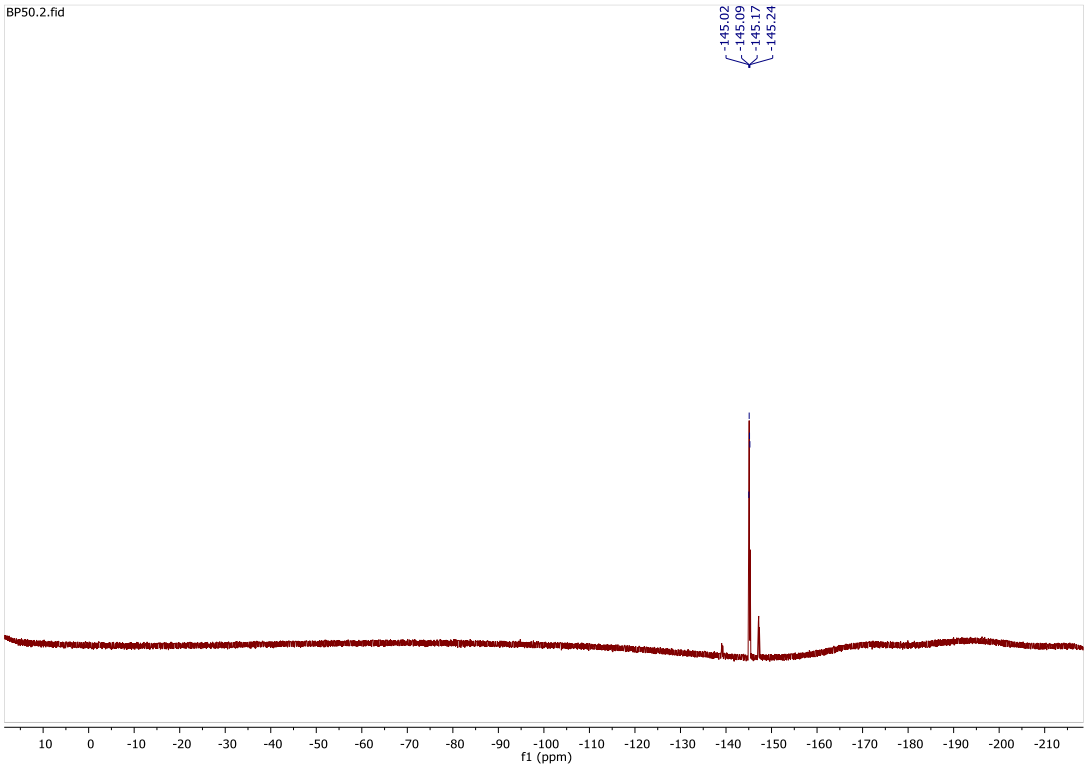


Figure S23. ^{19}F NMR spectrum of BP-PH.

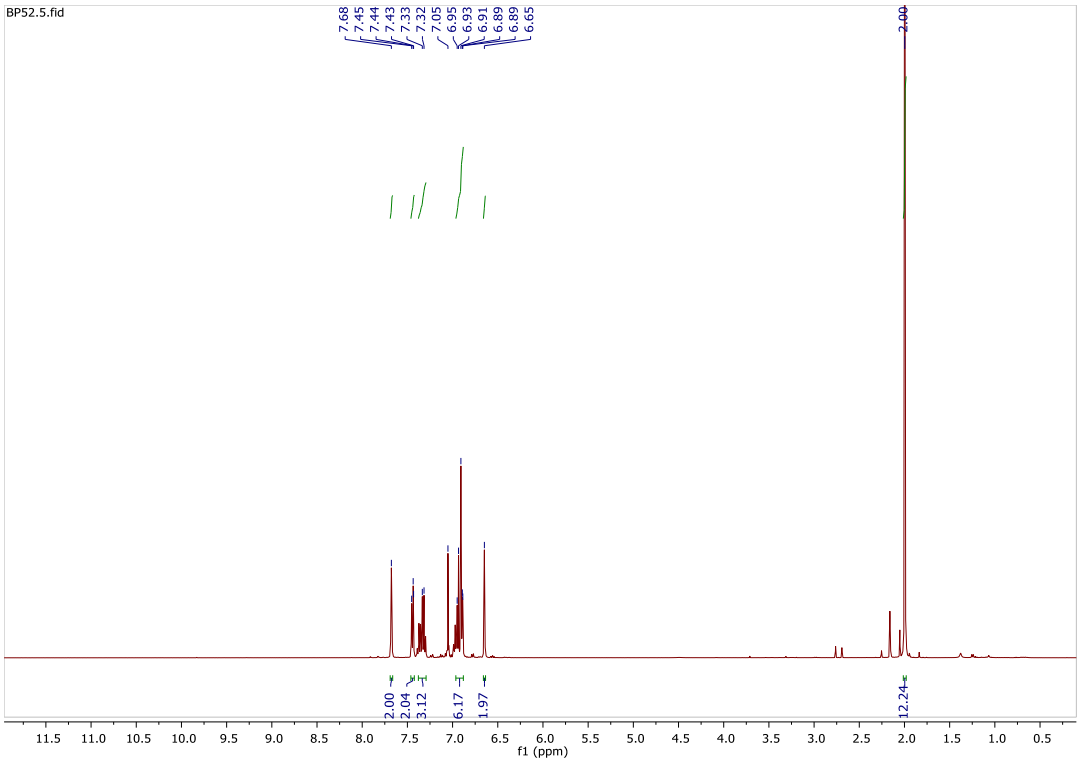


Figure S24. ^{1}H NMR spectrum of BP-PH-2M.

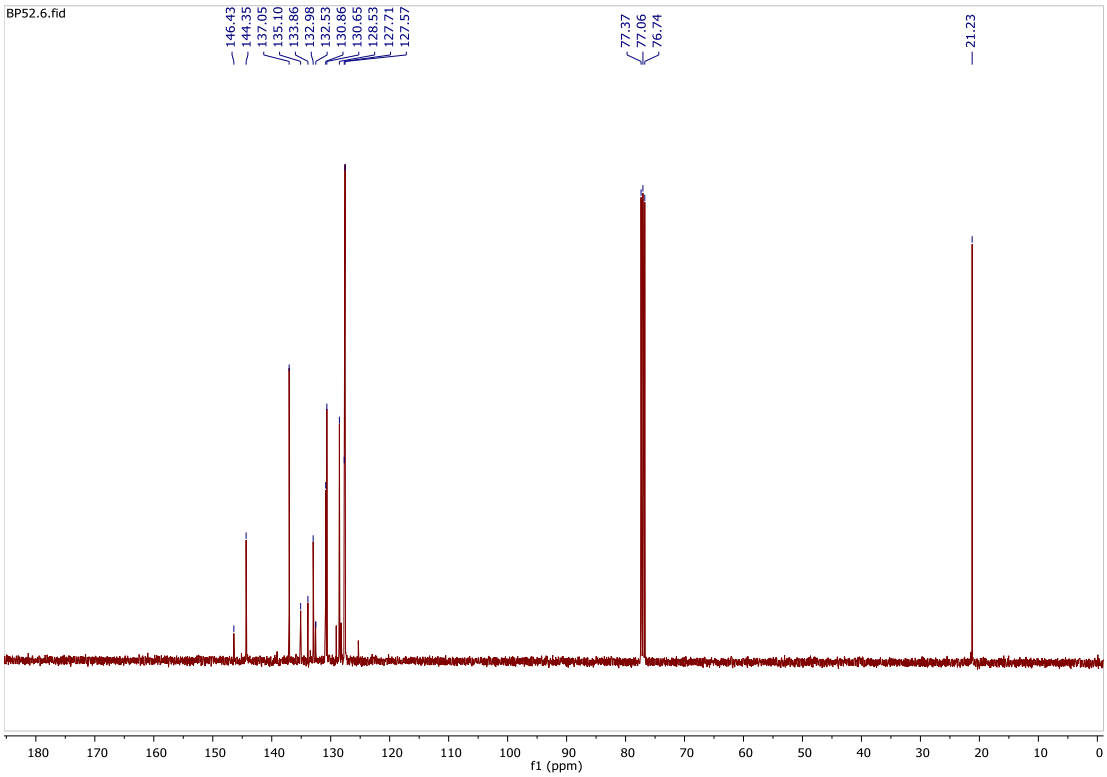


Figure S25. ^{13}C NMR spectrum of BP-PH-2M.

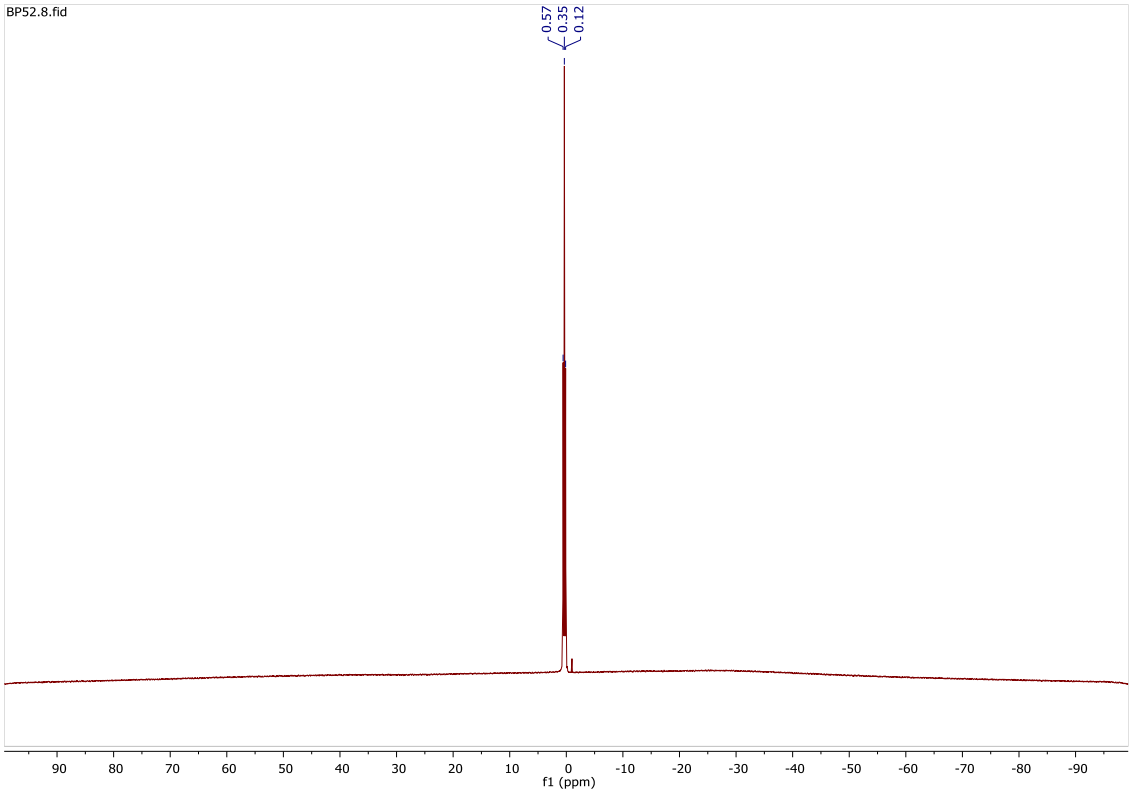


Figure S26. ^{11}B NMR spectrum of BP-PH-2M.

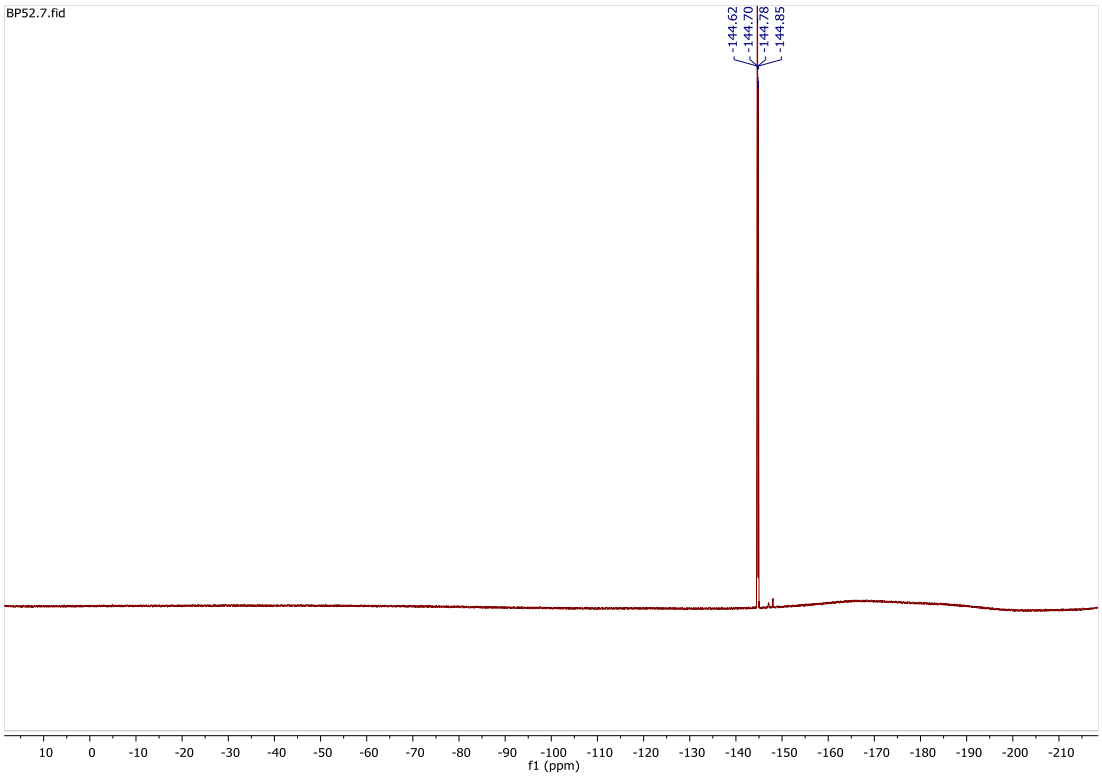


Figure S27. ^{19}F NMR spectrum of BP-PH-2M.

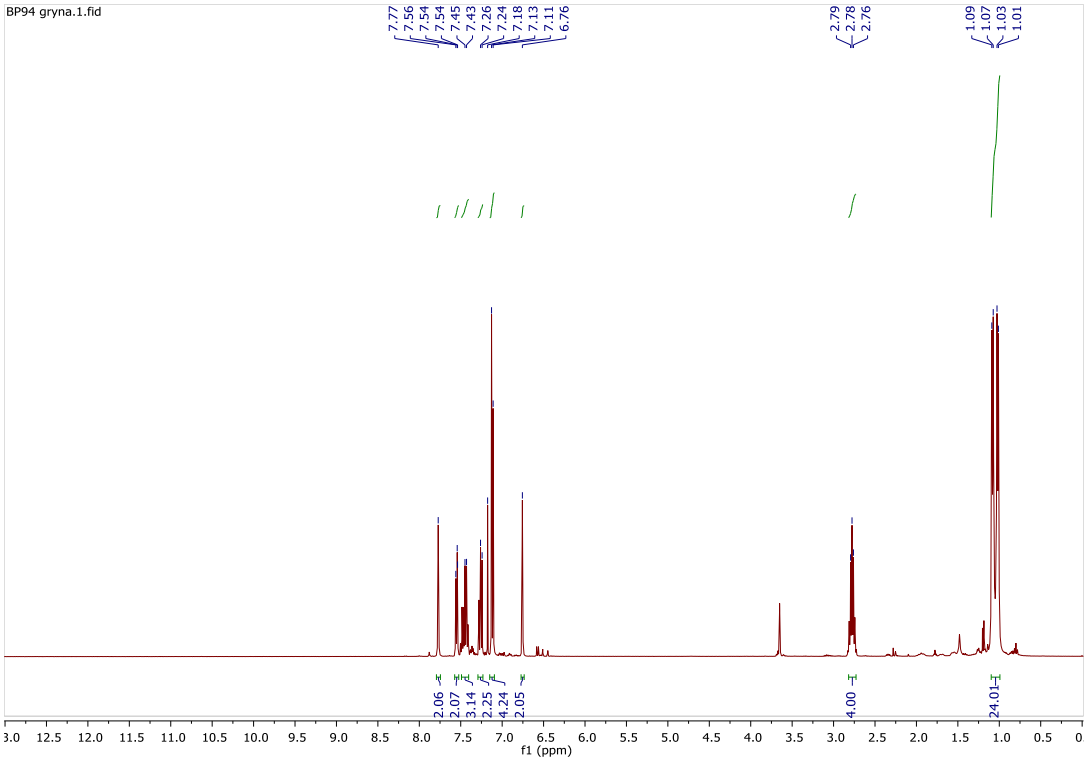


Figure S28. ^{1}H NMR spectrum of BP-PH-ISO.

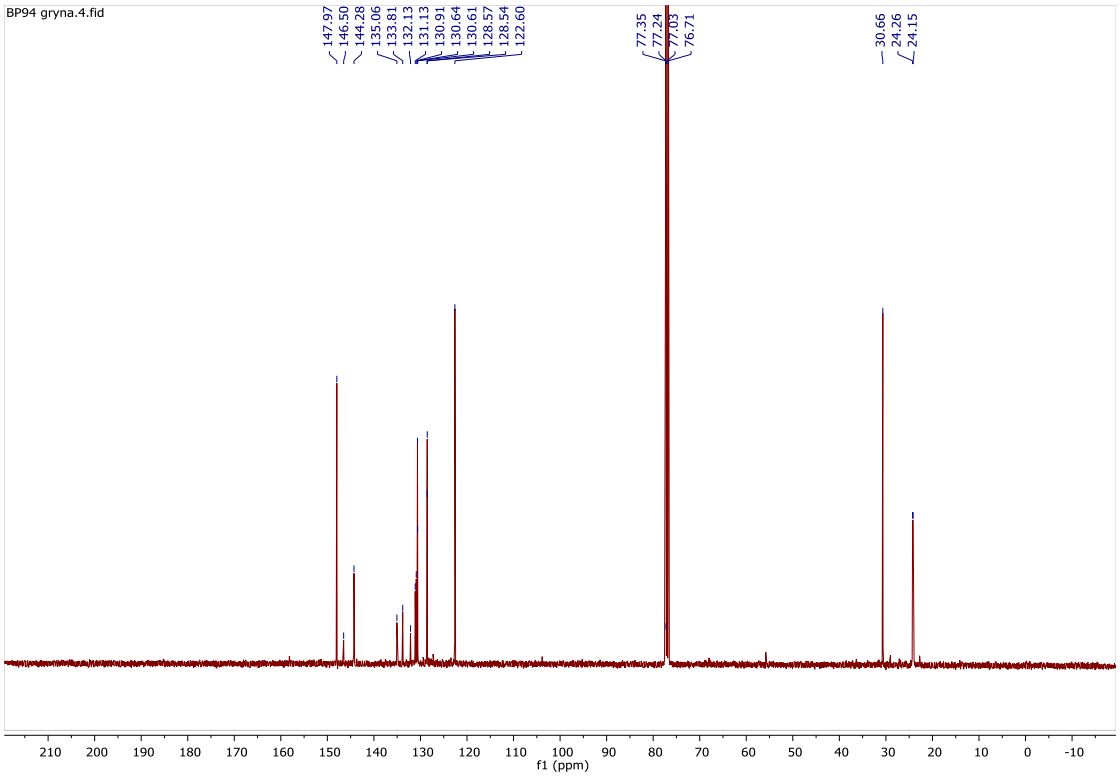


Figure S29. ^{13}C NMR spectrum of BP-PH-ISO.

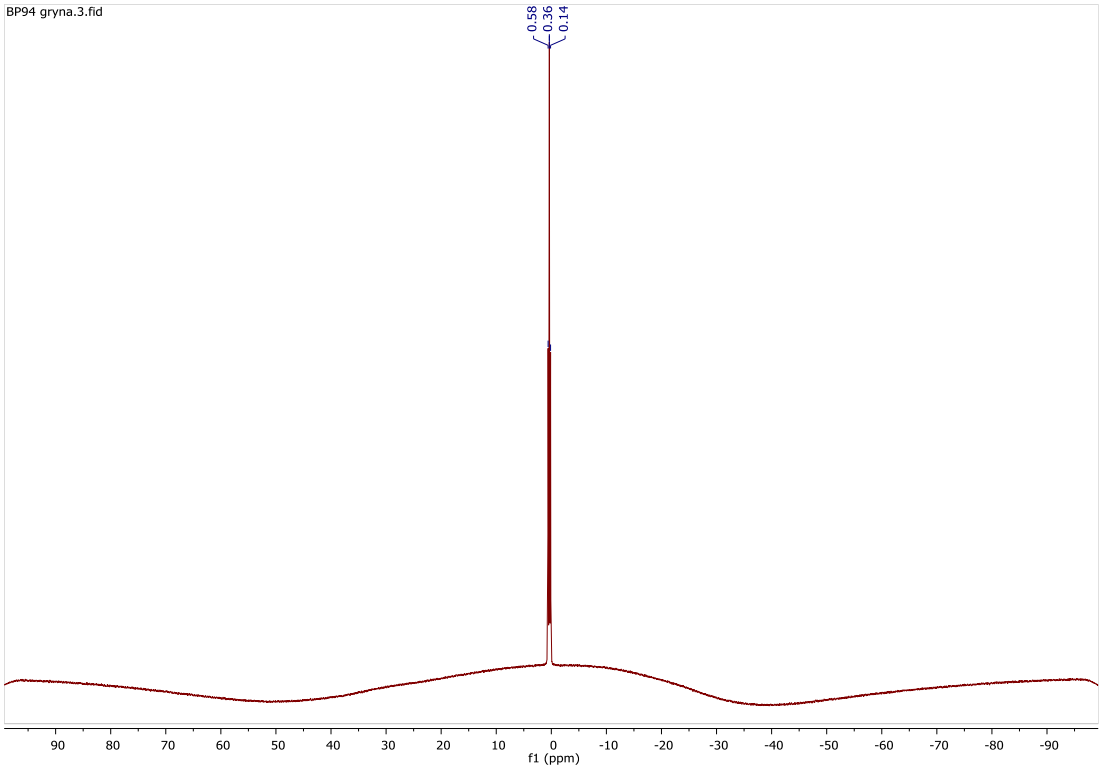


Figure S30. ^{11}B NMR spectrum of BP-PH-ISO.

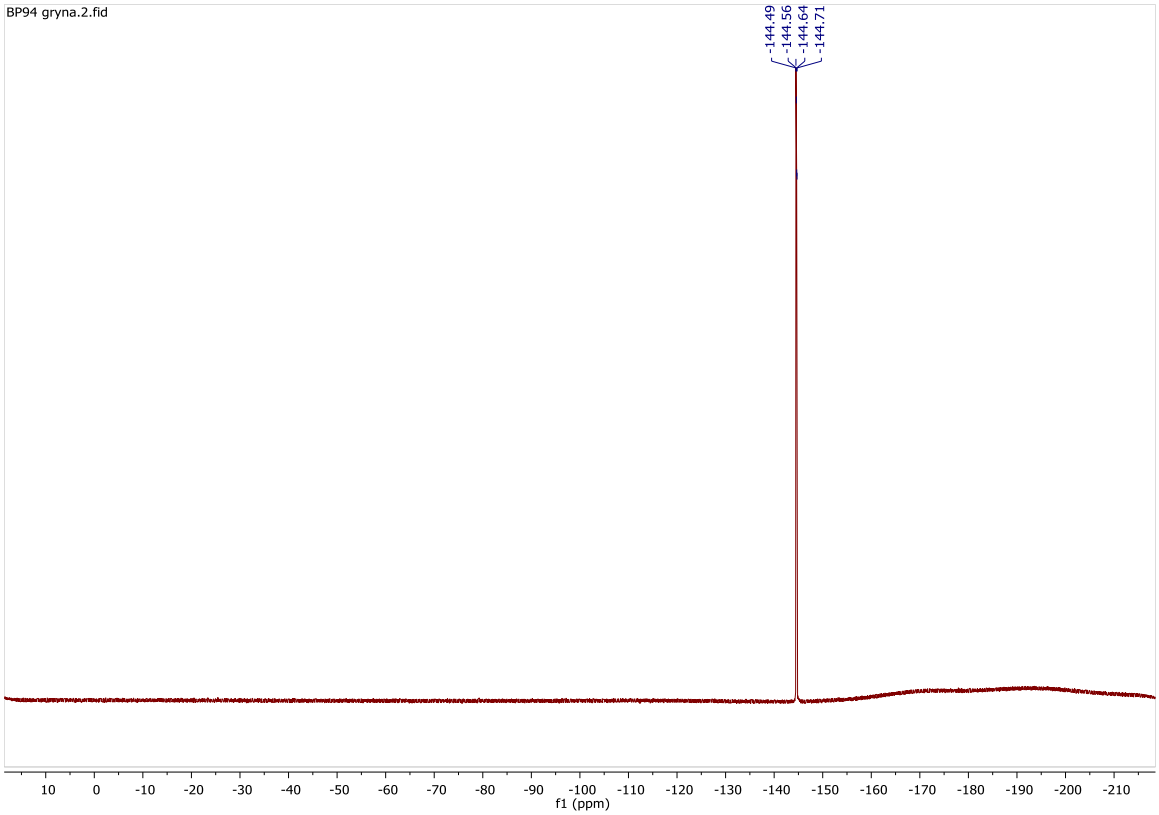


Figure S31. ^{19}F NMR spectrum of BP-PH-ISO.

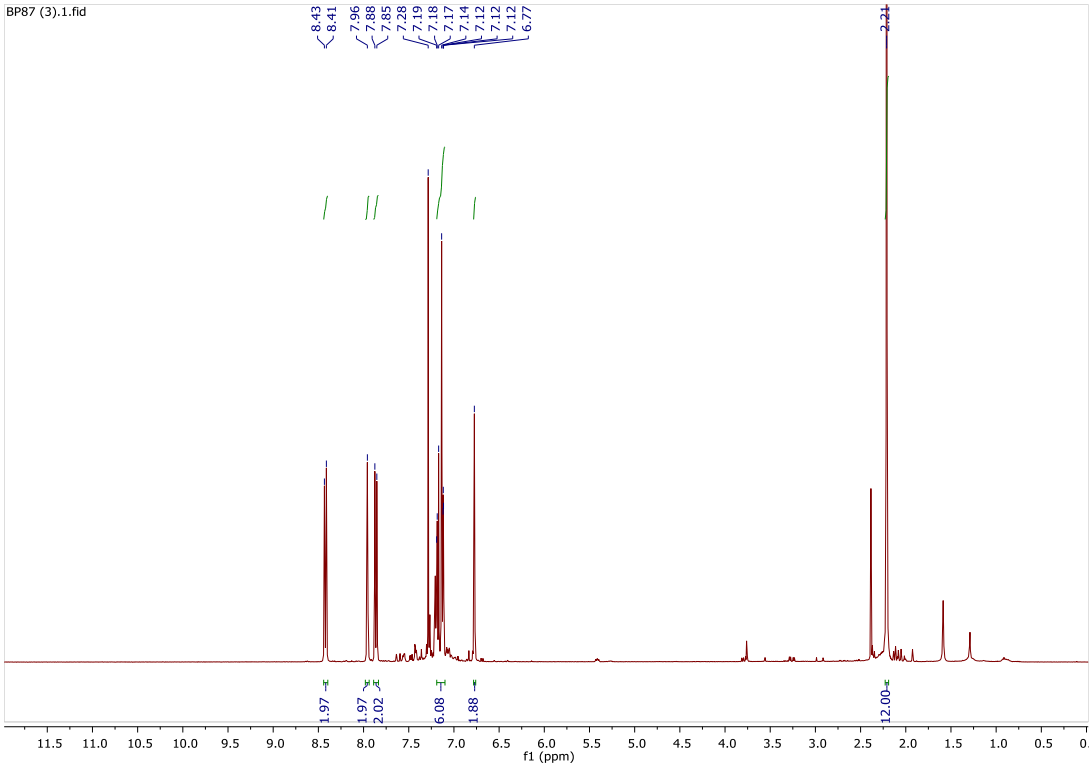


Figure S32. ^{1}H NMR spectrum of BP-PH-2M-NO_2.

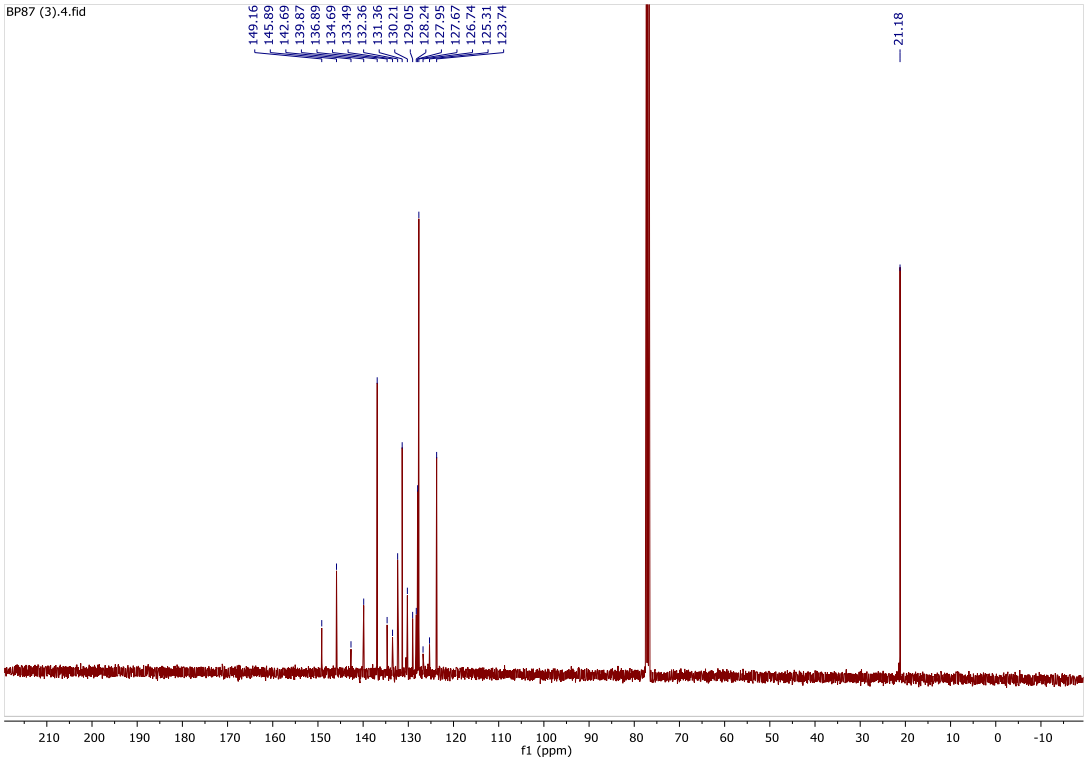


Figure S33. ^{13}C NMR spectrum of BP-PH-2M-NO_2.

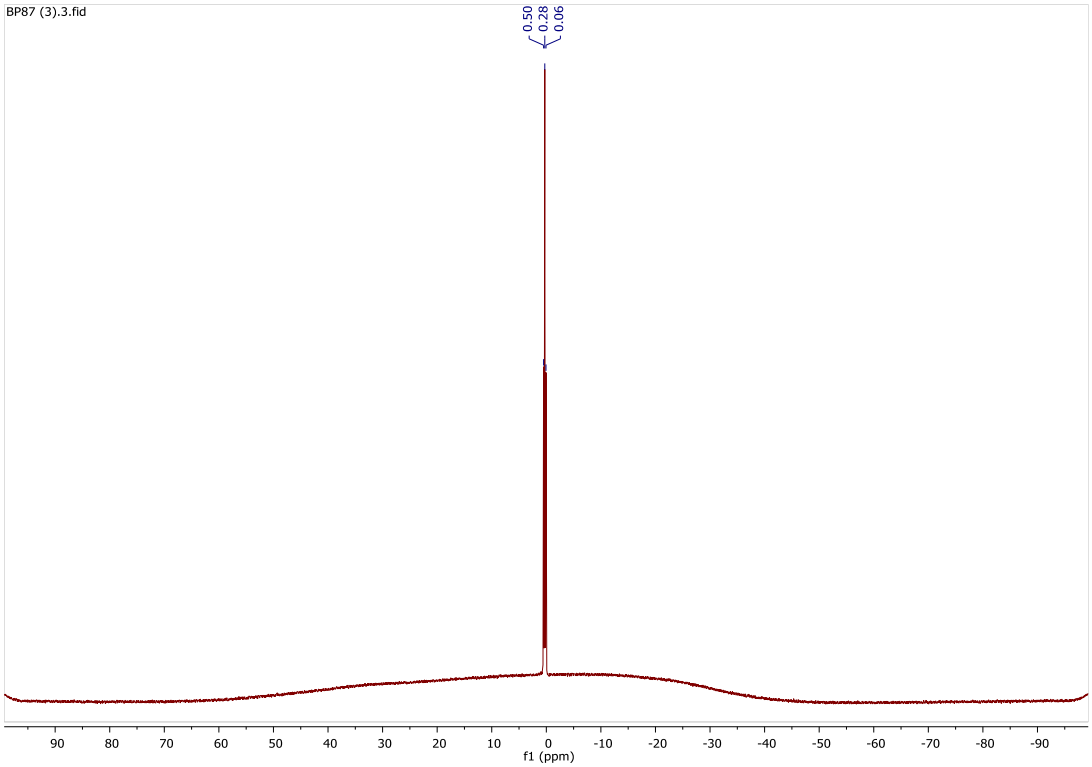


Figure S34. ^{11}B NMR spectrum of BP-PH-2M-NO_2.

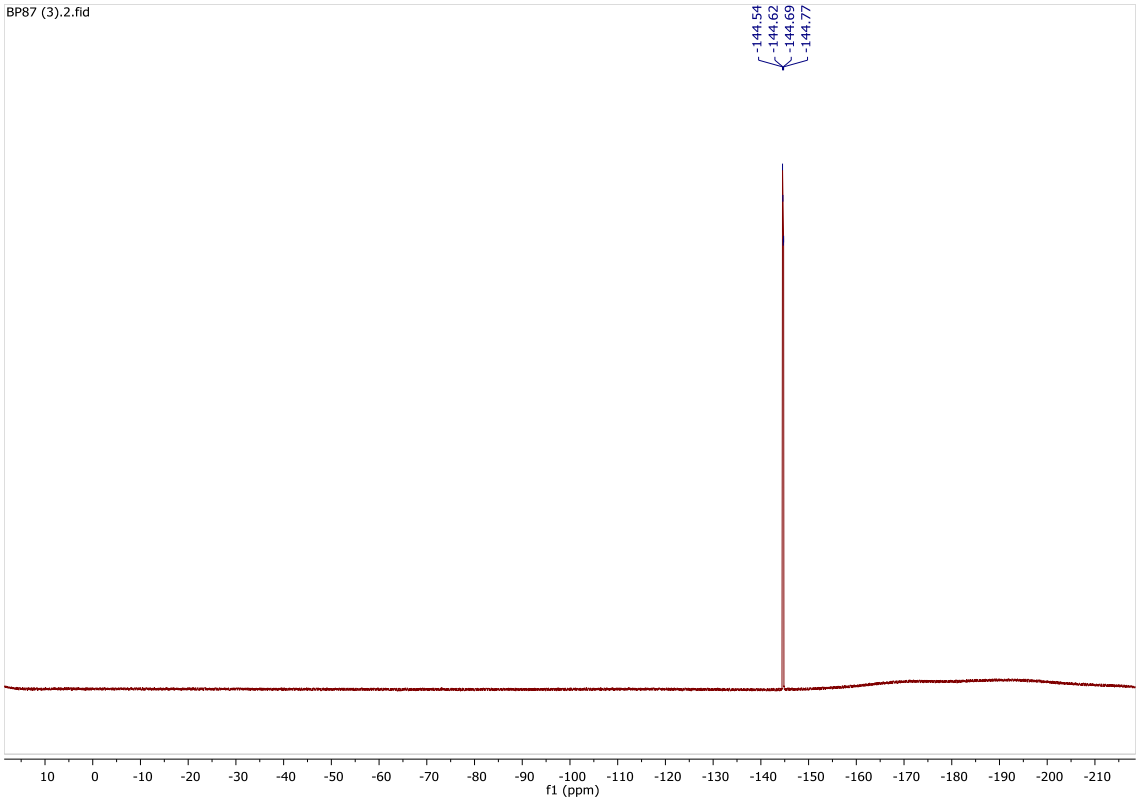


Figure S35. ^{19}F NMR spectrum of BP-PH-2M-NO_2.

List of references

1. Lakowicz, J. R. *Principles of Fluorescence Spectroscopy*, 3rd ed.; Springer: Boston, MA, **2006.**
2. Förster, T.; Hoffmann G. Effect of viscosity on the fluorescence quantum yield of some dye systems. *Zeitschrift fur physikalische chemie* **1971**, *75*, 63–76.
3. Kuimova, M. K. Mapping viscosity in cells using molecular rotors. *Physical Chemistry Chemical Physics*, **2012**, *14*, 12671–12686.
4. Toliautas, S.; Dodonova, J.; Žvirblis, A.; Čiplys, I.; Polita, A.; Devižis, A.; Tumkevičius, Šulskus, J.; Vyšniauskas, A. Enhancing the Viscosity-Sensitive Range of a BODIPY Molecular Rotor by Two Orders of Magnitude. *Chemistry - A European Journal*, **2019**, 25, 10342-10349.
5. Vyšniauskas, A.; López-Duarte, I.; Duchemin, N.; Vu, T. T.; Wu, Y.; Budynina, E. M.; Volkova, Y. A.; Peña Cabrera, E.; Ramírez-Ornelas, D. E.; Kuimova, M. K. Exploring viscosity, polarity and temperature sensitivity of BODIPY-based molecular rotors. *Physical Chemistry Chemical Physics*, **2017**, 19, 25252-25259.