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Supporting Information

Bimodal Fluorescence/Magnetic Resonance Molecular Probes with Extended Spin Lifetimes

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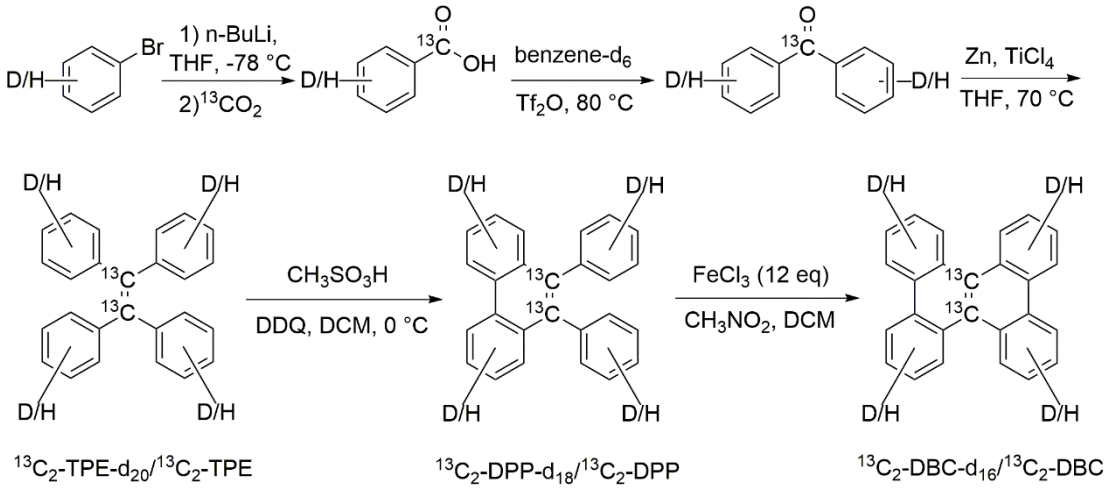
Materials and instruments

All the chemicals used in the reaction were purchased from commercial suppliers without further treatment. Thin-layer chromatography (TLC) analysis was carried out on pre-coated silica plates. Column chromatography was performed using silica gel by using the eluents in the indicated v/v ratio. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker ultrashield 300 MHz spectrometer at 25 °C. Chemical shifts (δ) are given in parts per million (ppm) and referenced with respect to the residual $^{1}H/^{13}C$ resonances of the deuterated solvent. High-resolution mass spectrometry (HR MS-ESI) spectra were taken on Thermo Scitific LTQ Orbitrap XL. Fluorescence spectra were recorded on an Agilent Cary Eclipse fluorescence spectrophotometer (with temperature controller) at 25 ℃. $^{15}N,^{15}N$-Azobenzene-d_{10} has been synthesized according to literature proceedings[1] with the exception that Anilin-d_5 has been used instead of the protonated starting material.



Scheme S1: Synthetic route for the molecules.

1.3 Synthesis of 1-^{13}C-benzoic acid-d_5[2]

This is compound was synthesized by the reported procedure with a slightly modification. To a stirred solution of bromobenzene-d_5 (4.8 g, 30 mmol) in anhydrous THF (35 mL) at -78 °C in two-necked round-bottom flask, *n*-butyl lithium (25 mL, 1.6 M in hexane, 40 mmol) was slowly added under N_2 atmosphere. Upon completion, the reaction mixture was stirred for 0.5 h and subsequently bubbled by $^{13}CO_2$ for another 0.5 h at the same temperature. Then reaction mixture was allowed to warm to room temperature. After stirred overnight, the reaction mixture was quenched by saturated aqueous ammonium chloride solution, adjusted to pH = 1.0 by addition of hydrochloride aqueous solution (1.0 M) and extracted with ethyl acetate (25 mL× 3). The organic layer was combined and washed by brine (20 mL× 2), dried by anhydrous Na_2SO_4. After filtration, the filtrate was concentrated under reduced pressure to the product of 1-^{13}C-benzoic acid-d_5. ^{1}H NMR (300 MHZ, $(CD_3)_2CO$): δ 11.03 (s, 1 H). ^{2}H NMR (46.1 MHZ, $(CD_3)_2CO$): δ 7.54 (s, 2 ^{2}H), 7.66 (s, 1 ^{2}H), 8.10 (s, 2 ^{2}H). ^{13}C {^{1}H} NMR (75 MHz, $(CD_3)_2CO$) : δ 168.01 (s), 133.30 (t, $^{13}C–^{2}H$, ^{1}J = 24.46 Hz), 131.17(d, $^{13}C–^{13}C$, ^{1}J = 72.35 Hz), 130.05 (t, $^{13}C–^{2}H$, ^{1}J = 24.60 Hz), 128.80 (td, $^{13}C–^{2}H$, $^{13}C–^{13}C$, ^{1}J = 24.60 Hz, ^{2}J = 4.35 Hz).

Synthesis of 1-^{13}C-benzophenone-d_{10}[3]

To a 50 mL round bottom flask, 1-^{13}C-benzoic acid-d_5 (384 mg, 3.0 mmol), benzene-d_6 (6.0 mL) and trifluoromethanesulfonic anhydride (0.71 mL) was subsequently charged. The reaction mixture was stirred at 80 °C for 4 h. After cooling down to room temperature, the reaction solution was diluted by $CHCl_3$ (20 mL), washed by 0.2 M aqueous NaOH solution (20 mL × 3), brine (20 mL), and dried by anhydrous Na_2SO_4. After filtration, the solvent was evaporated under reduced pressure. The resulting residue was subjected to column chromatography (eluent: ethyl acetate : petroleum ether = 1 : 15 to 1 : 5) to give the compound of 1-^{13}C-benzophenone-d_{10} (500 g, 86%). ^{2}H NMR (46.1 MHZ, $(CD_3)_2CO$): δ 7.54 (s, 2 ^{2}H), 7.66 (s, 1 ^{2}H), 8.10 (s, 2 ^{2}H). ^{13}C {^{1}H} NMR (75 MHz, $(CD_3)_2CO$) : δ 196.39 (s), 138.27 (d, ^{13}C–^{2}H, ^1J = 54.55 Hz), 132.67 (t, ^{13}C–^{2}H, ^1J = 24.45 Hz), 130.12 (t, ^{13}C–^{2}H, ^1J = 24.17 Hz), 128.67 (td, ^{13}C–^{2}H, ^{13}C–^{13}C, ^1J = 24.72 Hz, ^2J = 3.73 Hz).

Synthesis of $^{13}C_2$-TPE-d_{20}[4,5]

To a two-necked flask equipped with magnetic bar, preactivated zinc powder (1.0 g)[3] and anhydrous THF (25 mL) was charged under nitrogen atmosphere. The suspension cooled in an ice-water bath at 0 ℃, and $TiCl_4$ (0.9 mL) was added slowly by a syringe. Upon completion, the suspension was warmed up to room temperature and stirred for 0.5 h, then heated to 70 °C for another 2.5 h. The suspension was cooled in ice-water bath again and anhydrous pyridine (0.4 mL) was dropwise added and stirred for 10 min. The solution of 1-^{13}C-benzophenone-d_{10} (600 mg) in anhydrous THF (22 mL) was subsequently slowly added and the suspension was then refluxed at 70 °C. After 6 h, the reaction mixture was quenched by addition of 10% K_2CO_3 solution, and extracted with ethyl acetate (35 mL×3). The organic layer was collected and washed with brine (20 mL), followed by drying with anhydrous Na_2SO_4. After filtration, the filtrate was concentrated under reduced pressure. The resulting crude material was purified by column chromatography on silica gel (Eluent: petroleum ether : ethyl acetate = 50 : 1 to 10 : 1) to give a white solid (310 mg, 56%). ^{2}H NMR (46.1 MHz, THF-d_8): δ 7.07 (br, 20 ^{2}H). ^{13}C{^{1}H} NMR (75 MHz, THF-d_8) : δ 144.60 (dd, J = 29.67 Hz, J = 27.10 Hz), 141.82 (s, J = 71 Hz), 131.68 (t, J = 24.02 Hz), 127.88 (t, J = 24.70 Hz), 126.64 (t, J = 24.28 Hz). HR MS (ESI): m/z calcd for $C_{24}{}^{13}C_2D_{20}Na^+$ [M + Na^+] 377.2780, found 377.2771.

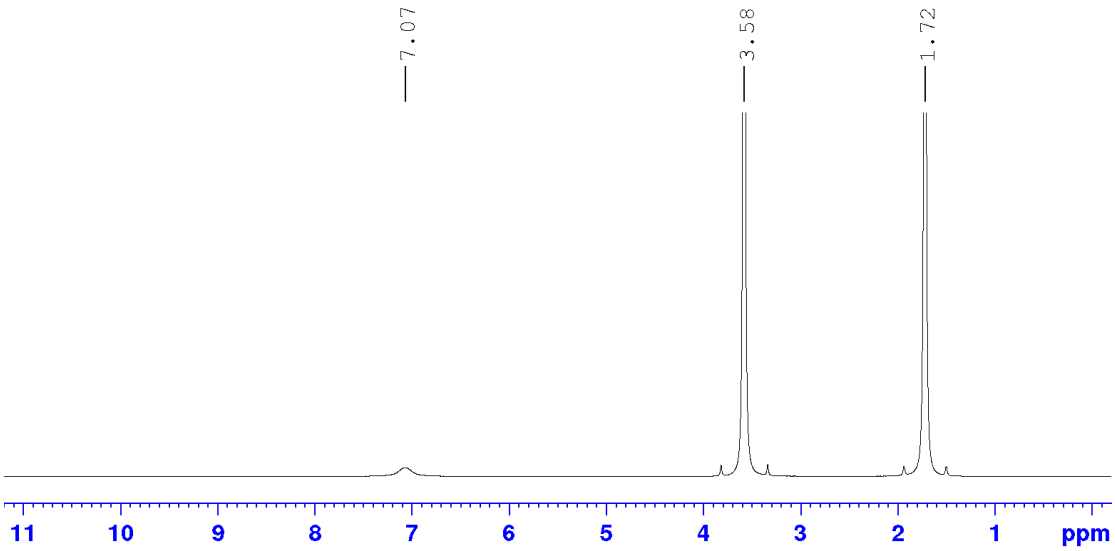


Figure S2: ^{2}H NMR of $^{13}C_2$-TPE-d_{20} recorded in THF-d_8.

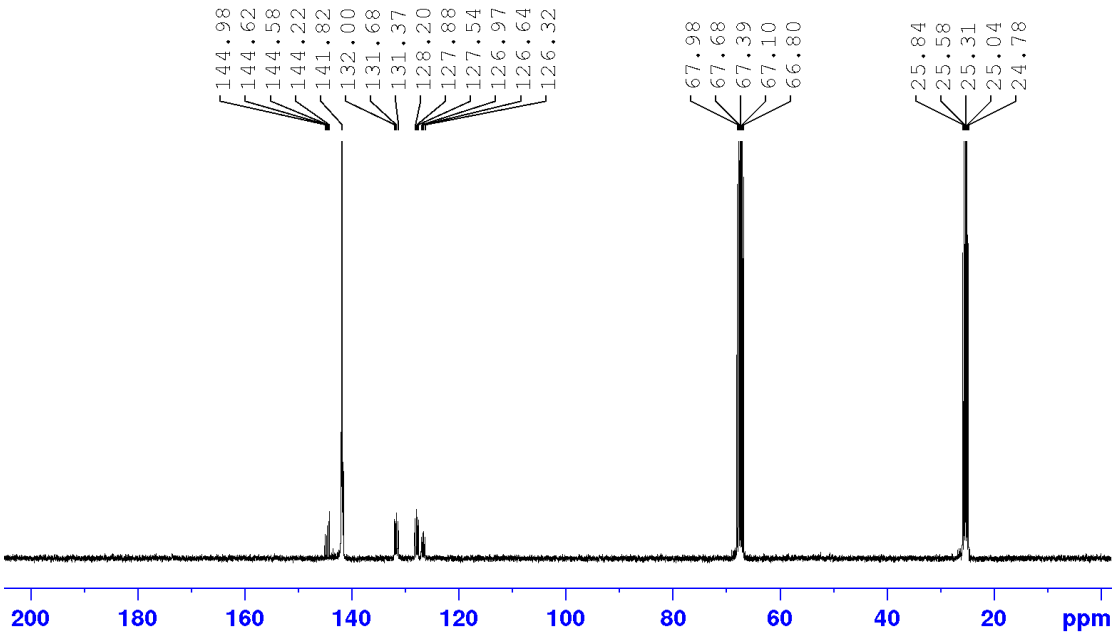


Figure S3: ^{13}C NMR of $^{13}C_2$-TPE-d_{20} recorded in THF-d_8.

Synthesis of $^{13}C_2$-DPP-d_{18}[6]

To the solution of $^{13}C_2$-TPE-d_{20} (142 mg, 0.4 mmol) under ice-water bath in dichloromethane (20 mL), methansonfonic acid (3.7 mL) and 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (99 mg) were added with stirring. After 0.5 h, the reaction mixture was quenched by slowly pouring into saturated aqueous $NaHCO_3$ solution with vigorously stirring. The resulting mixture was extracted by ethyl acetate (20 mL × 3). The organic layer was combined and washed with water (20 mL × 3), brine (20 mL) and dried over anhydrous Na_2SO_4. After filtration, the filtrate was concentrated under reduced repressure to give a crude product, which was further purified by column chromatography (petroleum ether : ethyl acetate = 10 : 1 to 30 : 1)) on silica gel to afford pure $^{13}C_2$-DPP-d_{18} (120 mg, 85%). ^2H NMR (46.1 MHz, THF-d_8): δ 8.91 (br, 2 ^2H), 7.53 (br, 6 ^2H), 7.21 (br, 10 ^2H). $^{13}C\{^2H\}$-NMR (75 MHz, THF-d_8) : δ 139.56 (dd, ^{13}C–^{13}C, ^{13}C–^{13}C, J = 30.89 Hz, J = 27.77 Hz), 137.02 (s, ^{13}C–^{13}C, J = 62 Hz), 131.76 (t, ^{13}C–^{13}C, J = 27.20 Hz), 130.47 (t, ^{13}C–^{13}C, J = 1.43 Hz), 130.06 (t, ^{13}C-^{13}C, J = 2.62 Hz), 127.08 (t, ^{13}C–^{13}C, J = 3.09 Hz), 126.87 (t, ^{13}C–^{13}H, J = 1.49 Hz), 125.78 (t, ^{13}C–^{13}C, J = 2.08 Hz), 125.73 (s), 125.67 (s), 122.03 (s). HR MS (ESI): m/z calcd for $C_{24}{}^{13}C_2D_{18}Na^+$ [M + Na^+] 373.2498, found 373.2497.

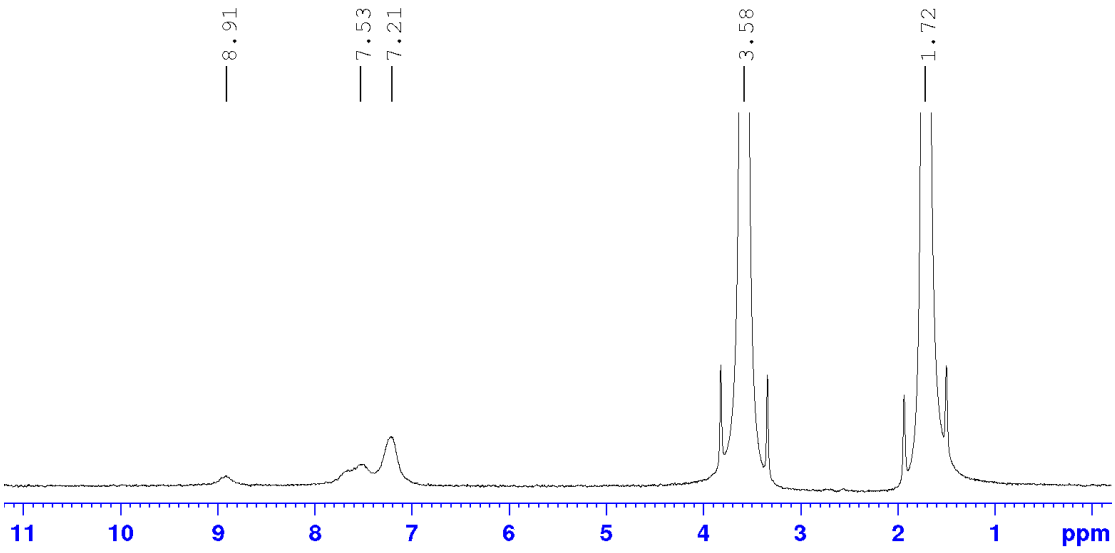


Figure S4: ^{2}H NMR of $^{13}C_2$-DPP-d_{18} recorded in THF-d_8.

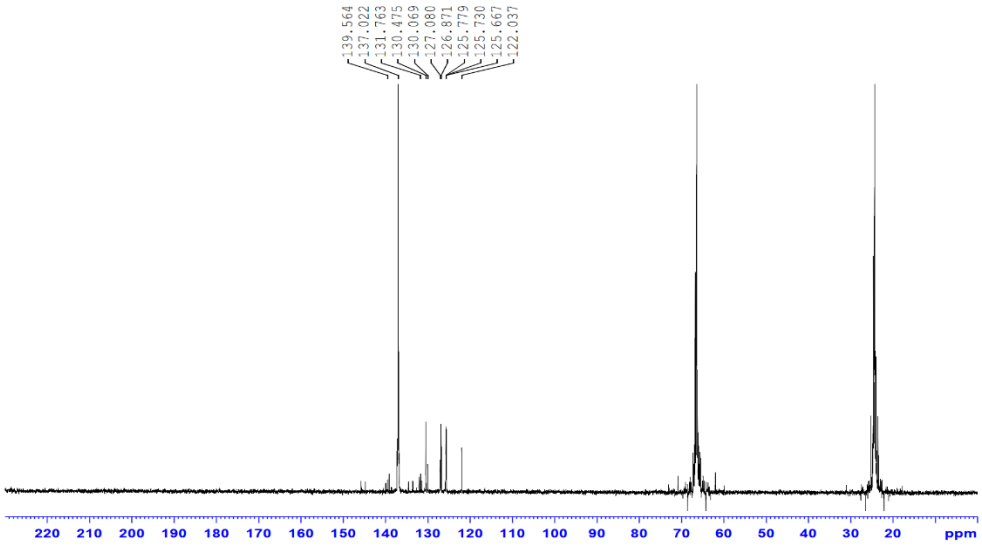


Figure S5: ^{13}C NMR of $^{13}C_2$-DPP-d_{18} recorded in THF-d_8.

Synthesis of $^{13}C_2$-DBC-d_{16}[7]

To a two-necked flask, $^{13}C_2$-DPP-d_{18} (120 mg, 0.34 mmol) was dissolved in dichloromethane (25 mL) and degassed by three freeze-pump-thaw cycles. To this solution, a solution of $FeCl_3$ (676 mg) in CH_3NO_2 (6.0 mL) was added under N_2 atmosphere with stirring at room temperature. After 10 min, the reaction mixture was quenched by methanol and washed by water (15 mL × 3), brine (20 mL). The organic layer was collected and dried with anhydrous Na_2SO_4. After filtration, the filtrate was concentrated under reduced pressure to give a residue, which was purified by column chromatography on silica gel to afford a white solid (41 mg, 34%). ^{2}H NMR (46.1 MHz, THF-d_8): δ 8.79 (br, 8 ^{2}H), 7.70 (br, 8 ^{2}H). $^{13}C\{^{2}H\}$-NMR (75 MHz, $CDCl_3$) : δ 130.86 (t, ^{13}C-^{13}C, *J* = 2.52 Hz), 129.09 (dd, ^{13}C-^{13}C,

^{13}C-^{13}C, *J* = 32.57 Hz, *J* = 26.30 Hz), 128.17 (t, ^{13}C–^{13}C, *J* = 2.31 Hz), 127.21 (s, ^{13}C–^{13}C, *J* = 57 Hz), 125.96 (s), 125.85 (t, ^{13}C–^{13}C, *J* = 1.82 Hz), 123.17 (s). HR MS (ESI): m/z calcd for $C_{24}{}^{13}C_2D_{16}Na^+$ [M + Na^+] 369.2216, found 369.2207.

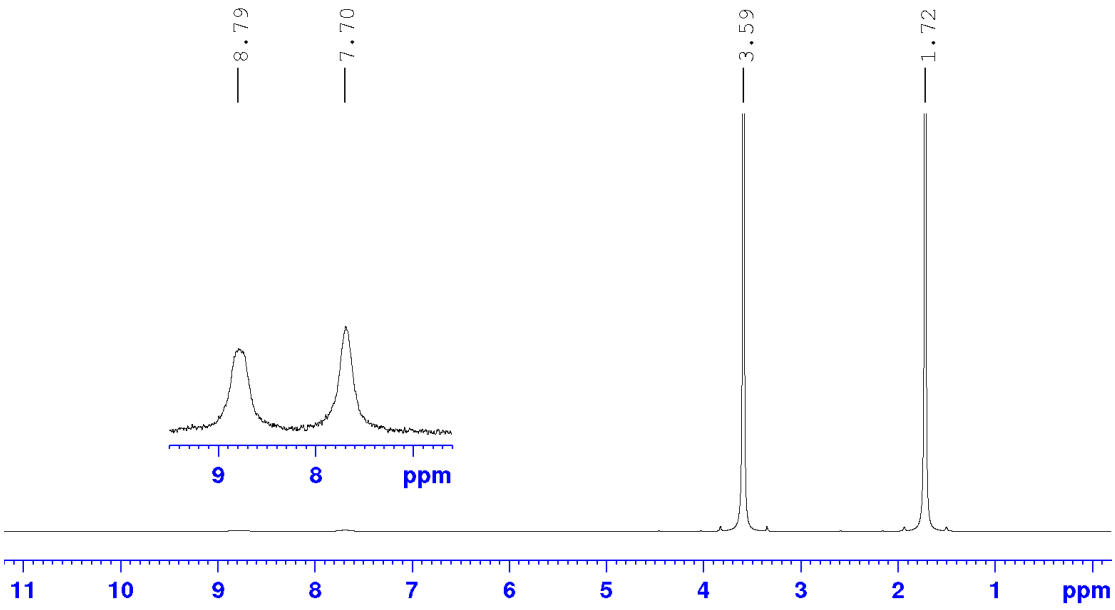


Figure S6: ^{2}H NMR of $^{13}C_2$-DBC-d_{16} recorded in THF-d_8.

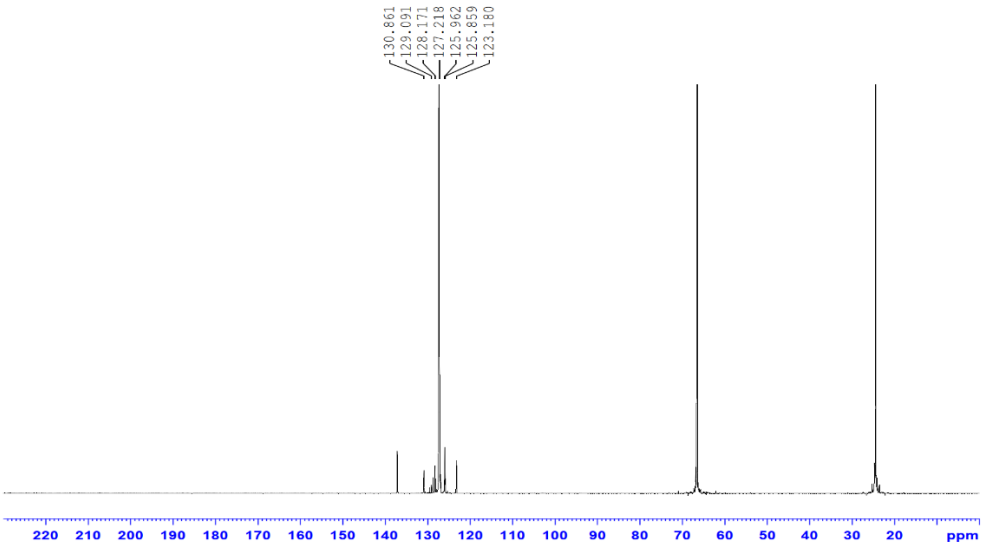


Figure S7: ^{13}C NMR of $^{13}C_2$-DBC-d_{16} recorded in $CDCl_3$.

Synthesis of $^{13}C_2$-TPE

This chemical agent was synthesized by using the same method described as $^{13}C_2$-TPE-d_{20}. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.06-7.11 (m, 20 H). $^{13}C\{^1H\}$ NMR (75 MHz, $CDCl_3$) : δ 143.73 (dd, $^{13}C–^{13}C$, ^1J = 27.19 Hz, ^2J = 26.98 Hz), 140.90 (singlet)[4], 131.30 (dd, $^{13}C–^{13}C$, ^2J = 1.90 Hz, ^3J = 1.84 Hz), 127.61 (dd, $^{13}C–^{13}C$, ^3J = 1.72 Hz, ^4J = 1.41 Hz), 126.38 (s).

Synthesis of $^{13}C_2$-DPP

This chemical agent was synthesized by using the same method described as $^{13}C_2$-DPP-d_{18}. ^{1}H NMR (300 MHz, THF-d_8): δ 8.87 (d, 2 H, J = 8.32 Hz), 7.63 (td, 2 H, J = 7.50 Hz, J = 1.28 Hz), 7.53-7.42 (m, 4 H), 7.23-7.14 (m, 10 H). $^{13}C\{^1H\}$ NMR (75 MHz, THF-d_8) : δ 140.70 (dd, $^{13}C–^{13}C$, ^1J = 30.96 Hz, ^2J = 27.52 Hz), 138.07 (singlet), 132.79 (dd, $^{13}C–^{13}C$, ^1J = 27.69 Hz, ^2J = 26.87 Hz), 131.88 (t, $^{13}C–^{13}C$, ^3J = 1.57 Hz), 131.11 (t, $^{13}C–^{13}C$, ^2J = 2.68 Hz), 128.47 (t, $^{13}C–^{13}C$, ^2J = 3.07 Hz), 128.35 (t, $^{13}C–^{13}C$, ^3J = 1.51 Hz), 127.28 (t, $^{13}C–^{13}C$, ^3J = 2.07 Hz), 127.22 (s), 127.16 (s), 123.39 (s). HR MS (ESI): m/z calcd for $C_{24}{}^{13}C_2H_{18}Na^+$ [M + Na^+] 355.1368, found 355.1369.

Synthesis of $^{13}C_2$-DBC

This chemical agent was synthesized by using the same method described as $^{13}C_2$-DBC-d_{16}. ^{1}H NMR (300 MHz, THF-d_8): δ 8.77 (d, 4 H, J = 7.65 Hz), 8.69 (d, 4 H, J = 7.98 Hz), 7.64 (m, 8 H). $^{13}C\{^1H\}$ NMR (75 MHz, $CDCl_3$) : δ 130.83 (t, $^{13}C–^{13}C$, ^2J = 2.60 Hz), 129.23 (s), 128.88 (t, $^{13}C–^{13}C$, ^2J = 2.24 Hz), 127.45 (singlet), 126.54 (s, 2 × C), 123.58 (s). HR MS (ESI): m/z calcd for $C_{24}{}^{13}C_2H_{16}Na^+$ [M + H^+] 331.1392, found 331.1401.

Singlet State Experiments

The NMR experiments were performed on a 7.05 T or 16.5 T NMR spectrometer (Bruker Biospin, DE) at 298 K. Data were acquired and analyzed via the proprietary spectrometer software TopSpin 3.5pl7 and TopSpin 4.0.3 (Bruker Biospin, DE), respectively.

The spin-lattice relaxation time T_1 for all molecule were measured by using inversion recovery method. The pulse program t1irig in the standard Bruker pulse sequence library was employed to measure the T_1 value of ^{13}C and ^{15}N in the molecules.

The spin density operator was converted from magnetization into singlet and back to magnetization for observation using the SLIC sequence[8], by exploiting the asymmetry in the J-couplings with the closest ^{2}H nuclei. During the sequence, radio-frequency pulses were applied on-resonance at the frequency of the spin pair bearing the singlet. CW decoupling was applied during the sustaining period d_{25}. The principal condition for spin order transfer is that the power level of the conversion blocks and matches the intra-nuclear J-coupling. The power level during the conversion blocks corresponded to a nutation frequency of 71 Hz, 62 Hz and 57 Hz for the $^{13}C_2$-TPE-d_{20}, $^{13}C_2$-DPP-d_{18} and $^{13}C_2$-DBC-d_{16} sample, respectively. The blocks had a duration of p11 = 1370,000 ms for $^{13}C_2$-TPE-d_{20}, 1150,000 ms for $^{13}C_2$-DPP-d_{18} and 1500,000 ms for $^{13}C_2$-DBC-d_{16}. The singlet relaxation time T_s was measured by

monitoring the NMR signal decay during the variable delay d_{25}. The T_1 value of the ^{15}N in the $^{15}N,^{15}N$-Azobenzene-d_{10} has been determined in the same way at 16 Hz nutation frequency with a duration of p11 = 1200 ms.

Singlet and thermal NMR spectra in THF-d_8 at different D_2O concentrations

Thermal and singlet NMR spectra of $^{13}C_2$-TPE-d_{20}, $^{13}C_2$-DPP and $^{13}C_2$-DBC-d_{16} (100 mM) in THF have been measured at different D2O concentrations. No linear correlation of singlet state population efficiency and D_2O content of the sample could be detected. However at concentrations of 50 % D_2O for $^{13}C_2$-TPE-d_{20}, 20 % D_2O for $^{13}C_2$-DPP and 35 % for $^{13}C_2$-DBC-d_{16} respectively, population of the singlet state was not possible anymore and no singlet state spectrum could be obtained while thermal NMR spectra could still be obtained.

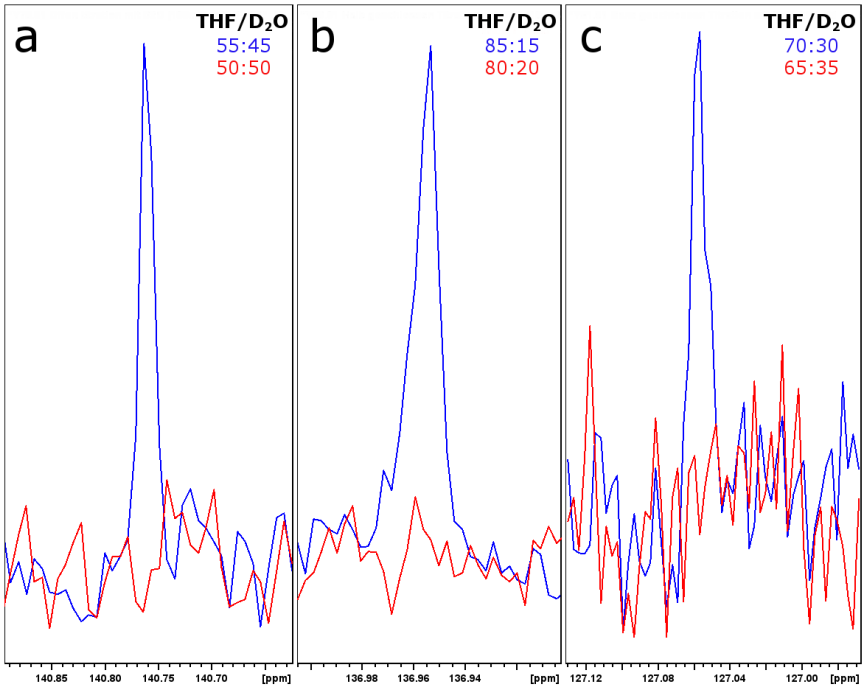


Figure S8: ^{13}C singlet NMR spectra of $^{13}C_2$-TPE-d_{20} (a), $^{13}C_2$-DPP (b) and $^{13}C_2$-DBC-d_{16} (c) at different THF/D_2O ratios. While the singlet state can be populated and a singlet NMR spectrum can be obtained up to a certain solvent ratio, after this threshold, no singlet NMR spectrum can be obtained.

Structure calculations

Geometry optimizations and NMR shielding computations were performed with Gaussian09.[9] Structures were optimized at B3LYP/6-31+G(d,p) level using a polarizable continuum model (IEFPCM)[10] for THF (ε = 7.4257). Harmonic vibrational frequencies were computed to confirm convergence into stationary states. Chemical shieldings were computed using the GIAO method[11] as implemented in Gaussian09. To convert chemical shieldings into chemical shifts referenced to TMS, a model for TMS was optimized at the same level of theory (point group: *T*), and the chemical shielding for the CH_3 groups in TMS were used as chemical shift reference.

Table S1: A summary of DFT calculation.

compounds	$^{13}C_2$-TPE-d_{20}	$^{13}C_2$-DDP-d_{18}	$^{13}C_2$-DBC-d_{16}
point group	D_2	C_2	D_2
δ (^{13}C) [ppm]	112.0	106.6	95.5
CSA (^{13}C) [ppm]	126.0	148.4	162.9
d (^{13}C, H) [Å]	2x 2.724, 2x 2.7330, 2x 2.954	4x (2.724 to 2.741), 2x 2.95	2 x 2.738
d (H, H) [Å]	3.54	3.098	2.123

Fluorescence spectroscopy:

Fluorescence measurements were carried out at an excitation wavelength of 320 nm at concentrations of 10 µM of $^{13}C_2$-TPE-d_{20}, $^{13}C_2$-DPP and $^{13}C_2$-DBC-d_{16} respectively. Samples have been measured at different ratios of THF to H_2O (THF/H_2O 100:0 to THF/H_2O 5:95 in steps of 5). Those measurements showed no linear correlation of the solvent ratio to the fluorescence effects but rather a rapid change at THF/H_2O (30:70) for $^{13}C_2$-TPE-d_{20}, THF/H_2O (20:80) for $^{13}C_2$-DPP and THF/H_2O (20:80) for $^{13}C_2$-DBC-d_{16}. At higher water concentrations minor changes could still be detected but up to the respective water concentrations hardly any change could be observed.

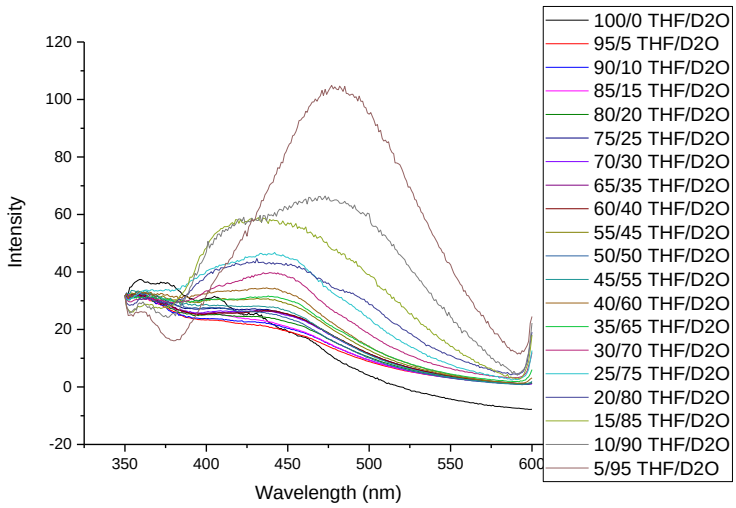


Figure S9: Fluorescence spectra of 10 µM of $^{13}C_2$-TPE-d_{20} in THF at H_2O concentrations of 0 % to 95 %.

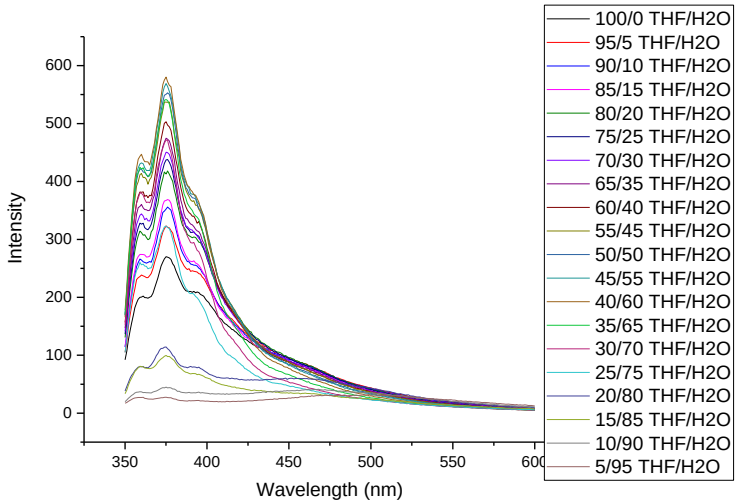


Figure S10: Fluorescence spectra of $^{13}C_2$-DPP in THF at H_2O concentrations of 0 % to 95 %.

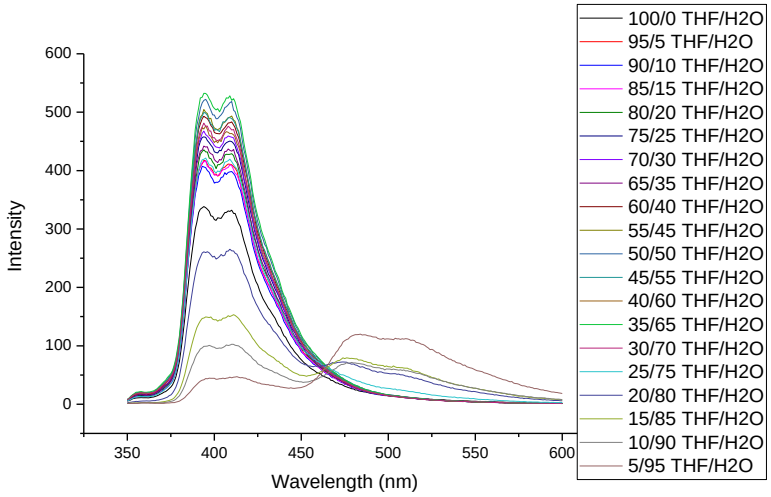


Figure S11: Fluorescence spectra of $^{13}C_2$-DBC-d_{16} in THF at H_2O concentrations of 0 % to 95 %.

UV/VIS measurements:

UV/VIS absorbance spectra have been recorded at an Eppendorf BioSpectrometer. Spectra have been measured in THF for $^{13}C_2$-DPP, and $^{13}C_2$-DBC-d_{16} and in THF/H_2O (5:95) for $^{13}C_2$-TPE-d_{20}. The extinction coefficients in the same solvents have been determined by determination of the absorption at concentrations of 10 µM, 20 µM and 30 µM.

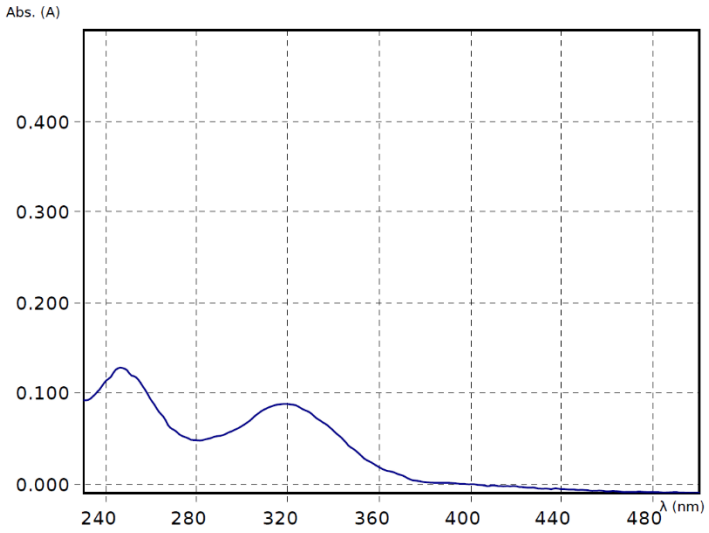


Figure S12: UV/VIS spectrum of $^{13}C_2$-TPE-d_{20} in THF/H_2O (5:95).

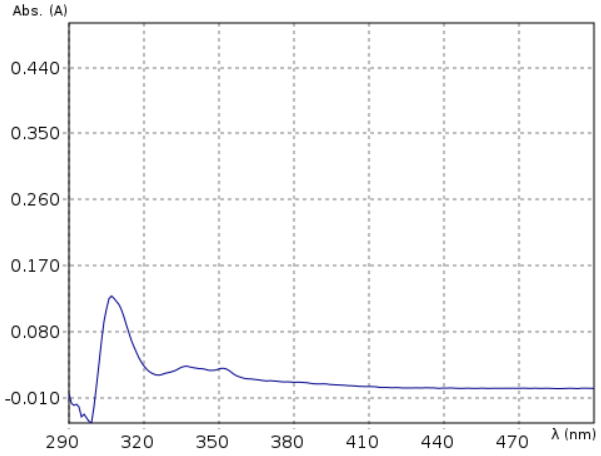


Figure S13: UV/VIS spectrum of $^{13}C_2$-DPP in THF.

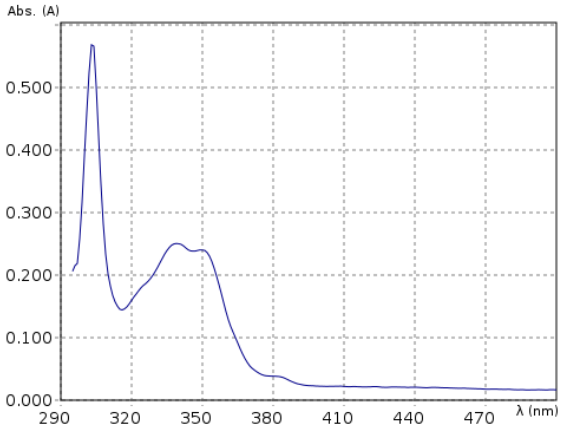


Figure S14: UV/VIS spectrum of $^{13}C_2$-DBC-d_{16} in THF.

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