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Supplementary Information for

Turn-on Mode Diarylethenes for Bioconjugation and Fluorescence Microscopy of Cellular Structures

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This PDF file includes:

SI Materials and Methods

Other supplementary materials for this manuscript include the following:

Movie S1

SI Materials and Methods

Abbreviations

acetonitrile (MeCN), antiparallel (ap), aqueous (aq.), argon (Ar), [bis(acetoxy)iodoso]benzene (BAIB), bis(pinacolato)diboron $(Bpin)_2$, bovine serum albumin (BSA), brine (aq. NaCl), catalyst/catalysis (cat.), closed form (CF), concentrated (conc.), diarylethene (DAE), dichloromethane (DCM), dimethyl sulfoxide (DMSO), equivalent (eq.), electrospray ionization (ESI), ethyl acetate (EtOAc), fluorescent diarylethene (fDAE), high performance liquid chromatography (HPLC), high resolution mass-spectrometry (HR-MS), methanol (MeOH), NBS (*N*-bromosuccinimide), *N*-hydroxysuccinimide (NHS), *N,N*-diisopropyl ethyl amine (DIPEA), *N,N*-dimethylformamide (DMF), nitrogen (N_2), nuclear magnetic resonance (NMR), open form (OF), parallel (p), phosphate buffer saline (PBS), photostationary state (PSS), potassium acetate (KOAc), reversed phase (RP), room temperature (r.t.), saturated (sat.), 2-dicyclohexylphosphino-2',6'-dimethoxybiphenyl (Sphos), tetrahydrofurane (THF), thin layer chromatography (TLC), triethylamine (TEA), (2,2,6,6-tetramethylpiperidin-1-yl)oxyl (TEMPO), trifluoroacetic acid (TFA), ultraviolet (UV), visible (Vis), volume ratio of two solvents (v/v).

High performance liquid chromatography

Preparative HPLC was performed on puriFlash 4250 2X preparative HPLC/Flash hybrid system (Interchim) with a 2 mL injection loop, a 200-600 nm UV-Vis detector and an integrated ELSD detector. Preparative column: Interchim Uptisphere Strategy C18-HQ, 10 µm, 250×21.2 mm (US10C18HQ-250/212, Interchim), flow rate 20 mL/min, unless specified otherwise. Analytical TLC was performed on Merck Millipore ready-to-use plates with silica gel 60 (F254). Flash chromatography was performed on Biotage Isolera 3.0 flash purification system using cartridges and solvent gradients indicated below. Analytical HPLC was performed on a KNAUER Azura system with a photodiode array detector, a 20 µL injection loop, and a 150 × 4 mm column (Knauer, Eurospher II 100-10 C18A with precolumn, Vertex Plus), at a flowrate of 1.2 mL/min with water/MeCN gradient; and both solvents containing 0.1% of TFA.

Starting materials

Et-Ox-2I, ***i*Bu-Ox-2I**, and 2-ethyl-3-(perfluorocyclopent-1-en-1-yl)benzo[b]thiophene (**C**) were synthesized as described in references 1, 2, and 3 at the end of this text, respectively. All other starting materials were purchased from TCI (Deutschland GmbH, Tokyo Chemical Industry Co.), Santa Cruz Biotechnology (SCBT) Inc., abcr GmbH, or MERCK (Sigma Aldrich) and used without further purification.

Nuclear Magnetic Resonance (NMR)

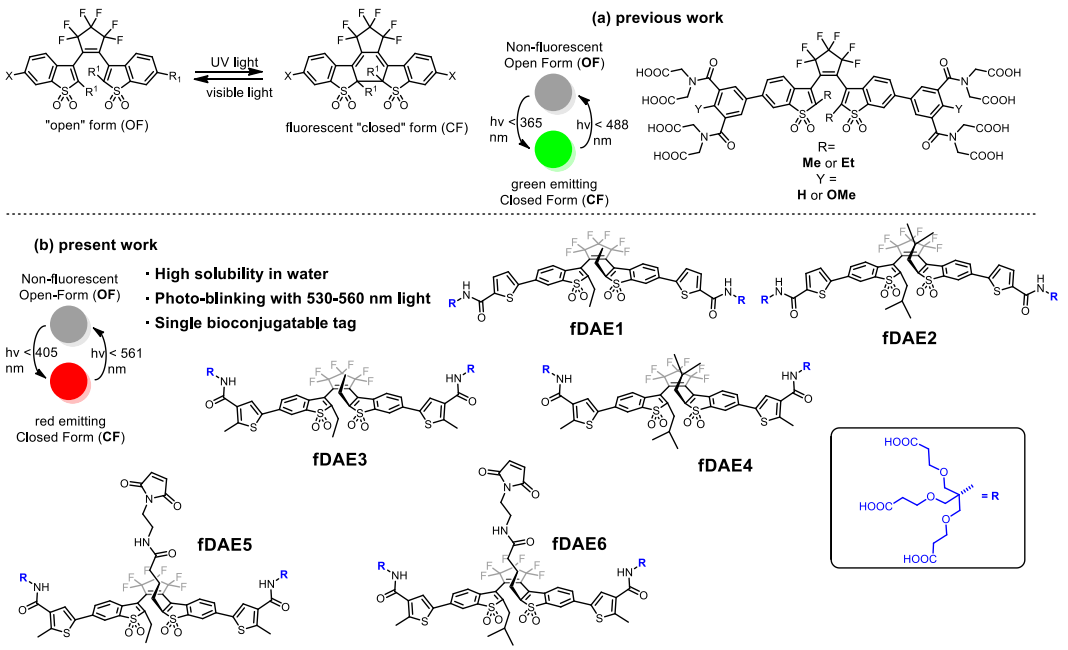
NMR Spectra (^{1}H, ^{13}C and ^{19}F) were recorded on an Agilent 400MR DD2 (400 MHz for ^{1}H) or Bruker Avance Neo 600 (av600, 600 MHz for ^{1}H) spectrometers. All ^{1}H- and ^{13}C- NMR spectra are referenced to the signals of the residual protons and ^{13}C in $CDCl_3$ (^{1}H: 7.26, ^{13}C: 77.00 ppm) and [D_7]DMF (^{1}H: 8.03, ^{13}C: 163.2). Multiplicities of the signals are described as follows: s = singlet, br = broad, d = doublet, t = triplet, m = multiplet. Coupling constants (*J*) are given in Hz.

High resolution mass spectrometry (HR-MS)

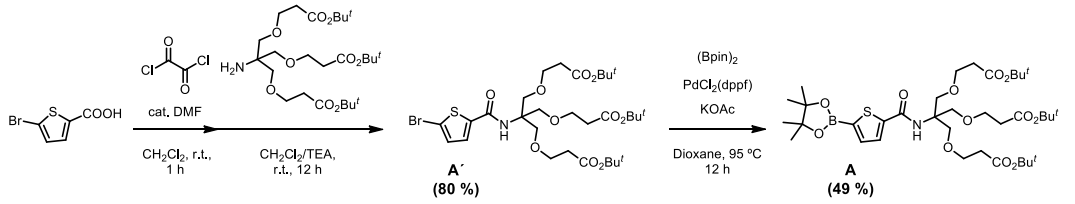
(ESI-MS) were recorded on a Varian 500MS spectrometer (Agilent). ESI-HRMS were recorded on a MICROTOF spectrometer (Bruker) equipped with an *Apollo* ion source and a direct injector with an LC-autosampler Agilent RR 1200.

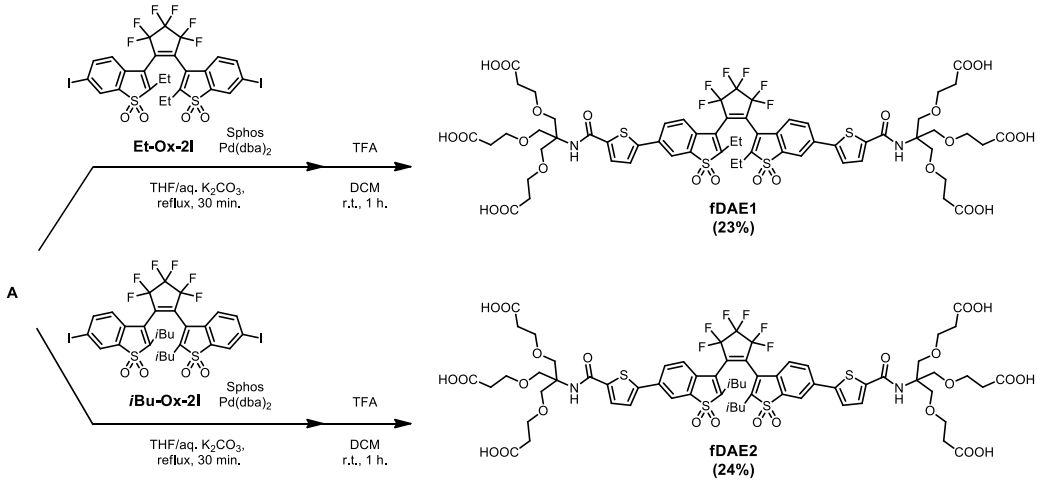
UV-Vis absorption and emission spectra

Absorption spectra were recorded in a Cary 5000 UV-Vis-NIR spectrometer (Agilent Technologies), and emission spectra (Figure S28) in a Cary Eclipse Fluorescence Spectrophotometer (Agilent Technologies), in 3 mL quartz cuvettes with magnetic stirring (model 119F-10-40, Hellma Analytics). The spectra of the OFs were obtained from non-irradiated solutions (ca. 2-5 μM). The absorption and emission spectra of the CF were measured at the photo-stationary state. To this end, solutions were irradiated in a custom-built setup [4], with 405 nm light (**fDAE3-6**) or 365 nm light (**fDAE1-2**). To confirm complete conversion to the CF, an aliquot was analyzed by HPLC and LC-MS. In all cases, no trace of the OF was observed.



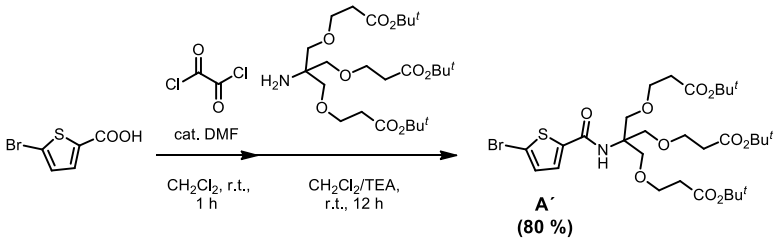
Scheme S1. Reversible photocyclization, main features and structures of fDAEs of the previous [5] and present work (fDAEs **1**-**6**).





Scheme S2a. Synthesis of compounds **fDAE1** and **fDAE2**.

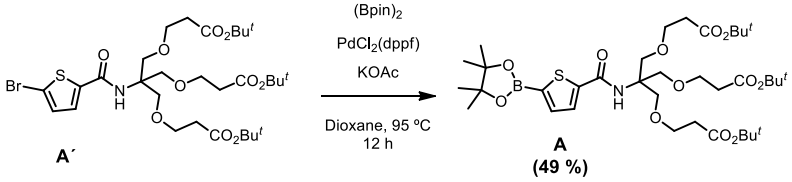
Synthesis of **compound A′**



To a suspension of 5-bromothiophene-2-carboxylic acid (0.41 g, 2.0 mmol) in DCM (20 mL) was slowly added oxalyl chroride (0.75 g, 5.9 mmol, 3.0 eq.) followed by DMF (5 drops, catalytic amount). After stirring for 2 h at r.t., DCM and DMF were removed under vacuum to give a yellow powder. It was dissolved in DCM (15 mL) to which DCM (10 mL) containing TEA (1.0 mL) and amino-tri-(t-butoxycarbonyl ethoxymethyl)-methane (1.0 g, 2.0 mmol, 1.0 eq.) was added at r.t. The reaction solution was stirred for 12 h. at r.t. under nitrogen. Then the mixture was washed with brine, dried over Na_2SO_4 and concentrated under vacuum. The residue was purified by chromatography on a silica gel with a gradient elution (*n*-hexane/EtOAc: 9/1 → 1/1), and compound **A´** was isolated as a pale yellow oil (1.1 g, 80%). R_f (*n*-hexane/EtOAc, 7/3, v/v) = 0.50. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) = 7.35 (d, J = 4.0 Hz, 1 H), 6.96 (d, J = 4.0 Hz, 1 H), 6.53 (s, 1 H), 3.77 (s, 6 H), 3.65 (t, J = 6.4 Hz, 6 H), 2.44 (t, J = 6.4 Hz ,6 H), 1.41 (s, 27 H). ^{13}C NMR (101 MHz, $CDCl_3$): δ (ppm) = 170.9, 160.8, 141.8, 130.3, 128.1, 117.6, 80.5, 69.1, 67.0, 60.3, 36.1, 28.1.

ESI-MS: positive mode, *m/z* 716.2080 [M+Na, ^{79}Br]$^+$ (found), 718.2064 [M+Na, ^{81}Br]$^+$ (found), 716.2075 (calculated for $C_{30}H_{48}BrNNaO_{10}S^+$, [M+Na, ^{79}Br]$^+$), 718.2054 (calculated for $C_{30}H_{48}BrNNaO_{10}S^+$, [M+Na, ^{81}Br]$^+$).

Synthesis of **compound A**



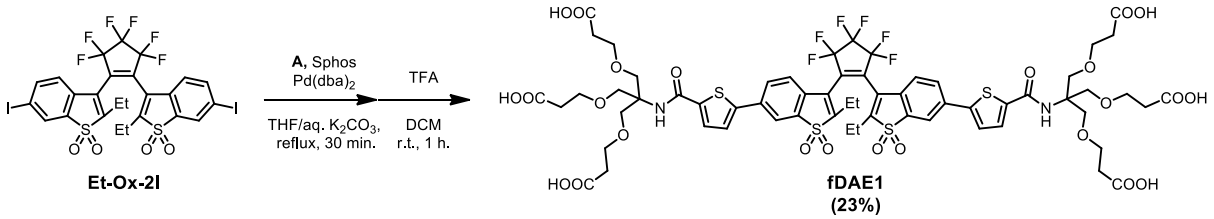
Into a flask flushed with N_2 was added compound **A'** (1.1 g, 1.6 mmol), $(Bpin)_2$ (0.60 g, 2.4 mmol, 1.5 eq.), KOAc (0.46 g, 4.7 mmol, 3.0 eq.), and $Pd(dppf)Cl_2$ (0.12 g, 0.16 mol, 0.1 eq.). Then 1,4-dioxan (15 mL) was added under N_2. The reaction mixture was heated to 95˚C and stirred for 12 h. To the reaction mixture was added saturated brine (50 mL), it was extracted with EtOAc (2×50 mL), and the organic solution was dried over Na_2SO_4. After concentrating under reduced pressure, the crude material was subjected to chromatography on silica gel with gradient

eluention (*n*-hexane/EtOAc: 90/10 → 60/40) to afford compound **A** as a pale yellow oil. (0.58 g, 49%). R_f (*n*-hexane/EtOAc, 7/3, v/v) = 0.33.

^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) = 7.60 (d, J = 4.0 Hz, 1 H), 7.50 (d, J = 3.6 Hz, 1 H), 6.53 (s, 1 H), 3.80 (s, 6 H), 3.67 (t, J = 6.4 Hz, 6 H), 2.45 (t, J = 6.4 Hz, 6 H), 1.42 (s, 27 H) , 1.33 (s, 12 H). ^{13}C NMR (101 MHz, $CDCl_3$): δ (ppm) = 170.8, 161.6, 146.0, 136.9, 129.2, 84.4, 80.4, 69.2, 67.0, 60.3, 36.2, 28.1, 24.9, 24.7.

ESI-MS: positive mode, *m/z* 764.3821 $[M+Na]^+$ (found), 764.3821 (calculated for $C_{36}H_{60}BNNaO_{12}S^+$, $[M+Na]^+$).

Synthesis of **fDAE1**



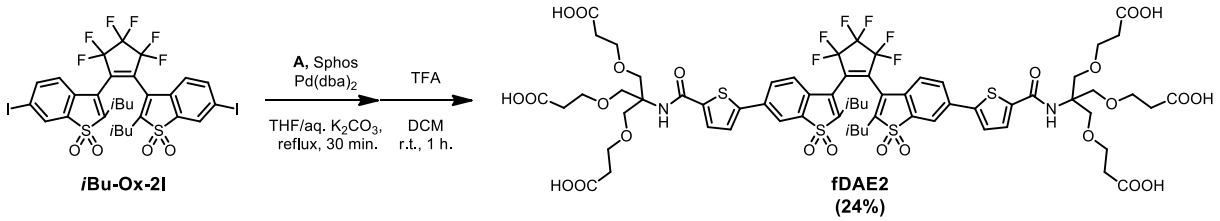
To THF (10 mL) containing **Et-Ox-2I** [1] (0.20 g, 0.25 mmol) and **A** (0.47 g, 0.64 mmol, 2.6 eq.) was added water (10 mL) containing K_2CO_3 (100 mg) under N_2 atmosphere. To the vigorously stirring reaction solution was added Sphos (20 mg, 49 μmol, 0.20 eq.) and $Pd(dba)_2$ (45 mg, 49 μmol, 0.20 eq.). The reaction mixture was heated to reflux for 30 min. After cooling down to r.t., brine was added, and the reaction mixture was extracted with DCM (2×100 mL). The organic solutions were combined, dried over Na_2SO_4, and concentrated under reduced pressure. The remaining dark solid was subjected to column chromatography (*n*-hexane/EtOAc, 8/2→2/8). The isolated crude product was added to a solution of DCM/TFA (5 mL/5 mL) and then stirred at r.t. for 1 h. DCM and excess TFA were removed under vacuum to give yellow solid. Purification was carried out by RP column chromatography (0.1% aq. TFA / CH_3CN, gradient from 7/3 to 3/7) followed by lyophilization (deionized H_2O and 1,4-dioxane) to give **fDAE1** as orange solid (82 mg, 23%).

^{1}H NMR (400 MHz, [D_7]DMF). δ (ppm) = 12.5 (br. s, 6 H, *ap/p*), 8.52 (d, J = 1.6 Hz, 1.2 H, *ap*), 8.41 (d, J = 2.0 Hz, 0.8 H, *p*), 8.16 (dd, J = 8.0 and 2.0 Hz, 1.3 H, *ap*), 8.00 (dd, J = 8.0 and 2.0 Hz, 0.8 H, *p*), 7.96–7.86 (m, 4.0 H, *ap/p*), 7.82–7.76 (m, 2.0 H, *ap/p*), 7.52 (s, 1.2 H, *ap*), 7.47 (s, 0.8 H, *p*), 3.83 (s, 7.2 H, *ap*), 3.81 (s, 4.8 H, *ap*), 3.77–3.67 (m, 12 H, *ap/p*), 2.86–2.76 (m, 2.4 H, *ap*), 2.69–2.59 (m, 1.6 H, *p*), 2.60–2.50 (m, 12 H, *ap/p*), 1.44–1.37 (m, 2.4 H, *p*), 1.07 (t, J = 7.6 Hz, 3.6 H, *ap*). ^{13}C NMR (101 MHz, dDMF): δ (ppm) = 173.8, 162.3, 150.1, 149.9, 145.5, 145.4, 143.6, 143.5, 141.1, 138.0 (2), 137.8, 137.6, 132.7, 132.4, 130.6, 130.5, 129.1, 128.9, 128.1,

128.1, 126.1, 126.0, 124.0, 120.4, 69.7, 68.2, 68.2, 62.2, 62.1, 20.1, 20.0, 12.8, 12.5. ^{19}F NMR (376 MHz, dDMF) δ (ppm) = -109.7 (m, 4.0 F, *p/ap*), -130.7 (m, 2.0 F, *p/ap*).

ESI-MS: negative mode, *m/z* = 1449.2558 $[M-H]^-$ (found), 1449.2563 (calculated for $C_{61}H_{63}F_6N_2O_{24}S_4^-$, $[M-H]^-$).

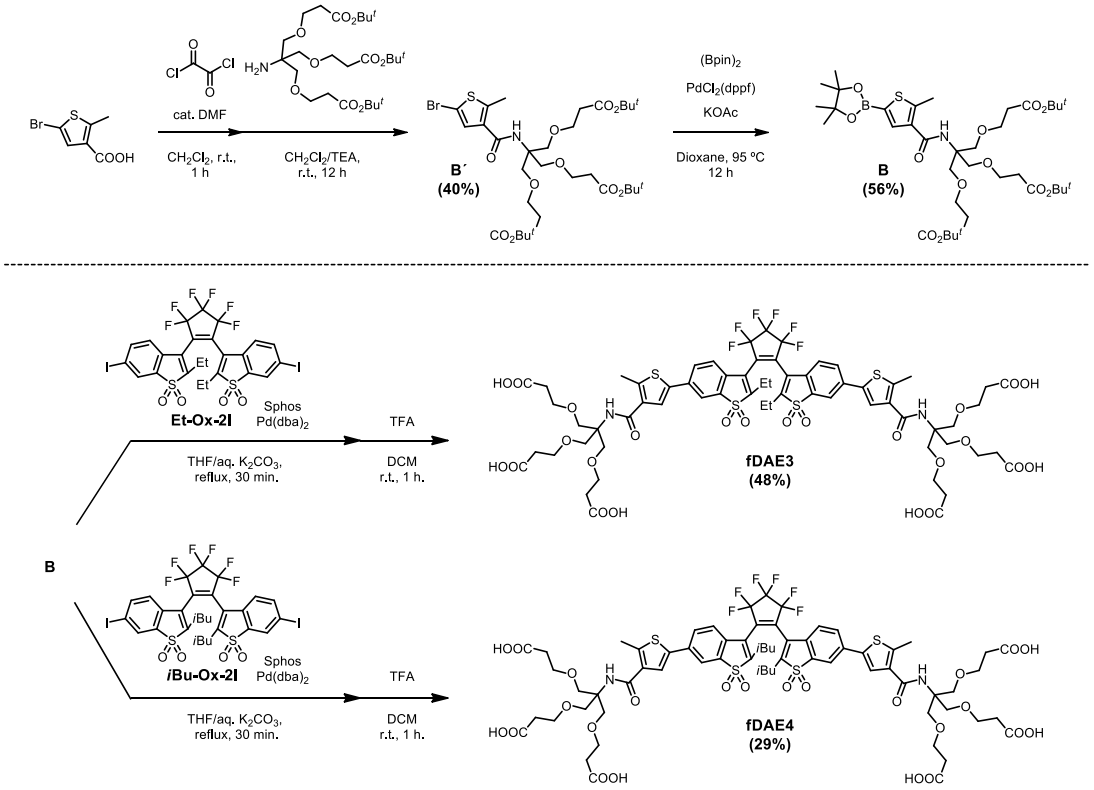
Synthesis of **fDAE2**



THF (10 mL) containing ***i*Bu-Ox-2I** [2] (80 mg, 92 µmol) and compound **A** (0.18 g, 0.24 mmol, 2.6 eq.) was diluted with water (10 mL) containing K_2CO_3 (100 mg) under N_2 atmosphere. To the vigorously stirring reaction mixture was added Sphos (7.5 mg, 18 µmol, 0.20 eq.) and $Pd(dba)_2$ (17 mg, 18 µmol, 0.20 eq.). The reaction mixture was heated to reflux for 30 min. After cooling down to r.t., brine was added, and the reaction mixture was extracted with DCM (2×100 mL). The organic solutions were combined, dried over Na_2SO_4, and concentrated under reduced pressure. The remaining dark solid was subjected to column chromatography (*n*-hexane/EtOAc, 8/2→5/5). The obtained crude product was added to a solution of DCM/TFA (5 mL/5 mL) and then stirred at r.t. for 1 h. DCM and excess TFA were removed under vacuum to give yellow solid. Purification was carried out by RP column chromatography (0.1% aq. TFA / CH_3CN, gradient from 7/3 to 3/7) followed by lyophilization (deionized H_2O and 1,4-dioxane) to give **fDAE2** as orange solid (32 mg, 24%).

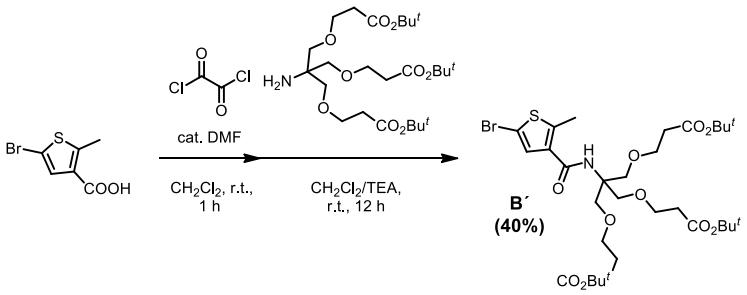
^{1}H NMR (400 MHz, dDMF): δ (ppm) = 12.5 (sbr, 6.0 H, *ap/p*), 8.54 (d, *J* = 1.6 Hz, 1.4 H, *ap*), 8.39 (d, *J* = 1.6 Hz, 0.6 H, *p*), 8.22 (dd, *J* = 8.0 and 2.0 Hz, 1.4 H, *ap*), 7.98–7.93 (m, 2.0 H, *ap/p*), 7.90 (d, *J* = 4.0 Hz, 1.4 H, *ap*), 7.86 (d, *J* = 4.4 Hz, 0.6 H, *p*), 7.85–7.78 (m, 2.0 H, *ap/p*), 7.76 (s, 0.6 H, *p*), 7.53 (s, 1.4 H, *ap*), 7.47 (s, 0.6 H, *p*), 3.83 (s, 8.4 H, *ap*), 3.81 (s, 3.6 H, *p*), 3.77–3.68 (m, 12 H, *ap/p*), 2.61–2.51 (m, 12 H, *ap/p*), 2.50–2.20 (m, 6.0 H, *ap/p*), 1.15 (d, *J* = 6.4 Hz, 1.8 H, *p*), 1.02 (d, *J* = 5.2 Hz, 1.8 H, *p*), 0.93 (d, *J* = 6.0 Hz, 4.2 H, *ap*), 0.77 (d, *J* = 6.4 Hz, 4.2 H, *ap*). ^{13}C NMR (101 MHz, $[D_7]$DMF): δ (ppm) = 173.8, 162.3, 162.2, 148.4, 148.4, 145.6, 145.4, 143.7, 143.5, 141.4, 141.2, 140.5, 138.2, 138.0, 137.9, 137.5, 132.6, 132.3, 130.7, 130.6, 128.9, 128.2, 128.1, 126.7, 126.7, 126.3, 126.2, 125.7, 125.7, 120.5, 120.3, 69.7, 68.3, 68.2, 62.2, 62.1, 27.3, 27.1, 23.9, 22.7, 22.3, 21.2. ^{19}F NMR (376 MHz, dDMF) δ (ppm) = -108.6 (m, 4.0 F, *p/ap*), -131.5 (m, 2.0 F, *p/ap*).

ESI-MS: positive mode, *m/z* = 1529.3123 $[M+Na]^+$ (found), 1529.3154 (calculated for $C_{65}H_{72}F_6N_2NaO_{24}S_4^+$, $[M+Na]^+$).



Scheme S2b. Synthesis of compounds **fDAE3**, and **fDAE4**

Synthesis of **compound B´**



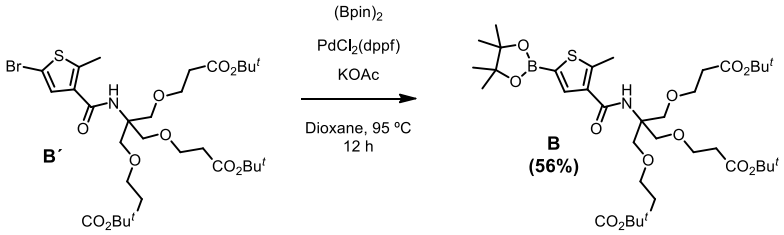
To DCM (15 mL) containing suspension of 5-bromo-2-methylthiophene-3-carboxylic acid (0.41 g, 1.9 mmol) was added oxalyl chroride (0.70 g, 5.6 mmol, 3.0 eq.) followed by DMF (5 drops) which were slowly added at r.t. After stirring for 1 h at r.t., DCM and DMF were removed under vacuum to give a yellow powder. It was dissolved in DCM (15 mL) to which DCM (10 mL) containing TEA (1.0 mL) and amino-tri-(*t*-butoxycarbonyl ethoxymethyl)-methane (0.94 g, 1.90 mmol, 1.0 eq.) was added at r.t. The reaction mixture was stirred for 12 h at r.t. under nitrogen atmosphere. Then it was washed with brine, dried over Na_2SO_4 and concentrated under vacuum. The residue was

purified by chromatography on silica gel with a gradient eluention with *n*-hexane/EtOAc (9/1 → 1/1). Compound **B´** was isolated as a pale yellow oil (0.52 g, 40% yield). R_f (*n*-hexane/EtOAc, 7/3, v/v) = 0.43.

^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) = 7.12 (s, 1 H), 6.25 (s, 1 H), 3.77 (s, 6 H), 3.65 (t, J = 6.4 Hz, 6 H), 2.57 (s, 3 H), 2.43 (t, J = 6.4 Hz, 6 H), 1.41 (m, 27 H). ^{13}C NMR (101 MHz, $CDCl_3$): δ (ppm) = 170.8, 163.4, 144.5, 134.0, 129.8, 107.5, 80.5, 69.2, 67.0, 60.1, 36.1, 28.0.

ESI-MS: positive mode, m/z 730.2230 [M+Na, ^{79}Br]$^+$ (found), 732.2211 [M+Na, ^{81}Br]$^+$ (found), 730.2231 (calculated for $C_{31}H_{50}BrNNaO_{10}S^+$, [M+Na, ^{79}Br]$^+$), 732.2211 (calculated for $C_{31}H_{50}BrNNaO_{10}S^+$, [M+Na, ^{81}Br]$^+$).

Synthesis of **compound B**

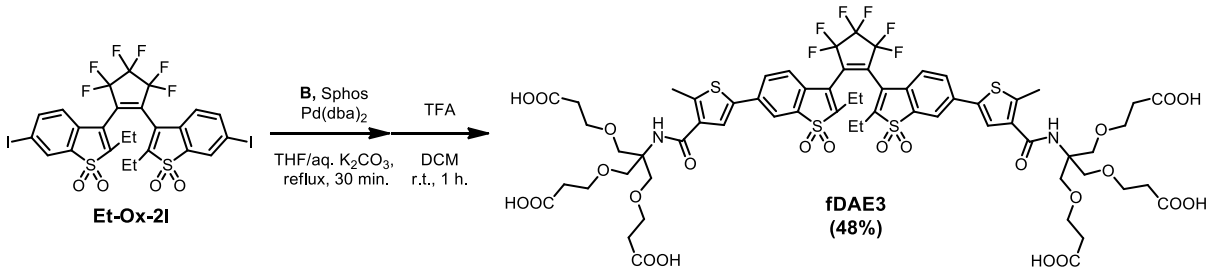


Into a flask flushed with N_2 were added compound **B´** (0.52 g, 0.73 mmol), $(Bpin)_2$ (0.30 g, 1.2 mmol, 1.6 eq.), KOAc (0.22 g, 2.2 mmol, 3.0 eq.) and $Pd(dppf)Cl_2$ (54 mg, 0.073 mmol, 0.1 eq.). 1,4-dioxan (15 mL) was added under N_2, and the reaction mixture was heated to 95˚C and stirred for 12 h. To the reaction mixture was added saturated brine (50 mL); it was extracted with EtOAc (2×50 mL), and then the organic solution was dried over Na_2SO_4. After concentrating under reduced pressure, the crude material was subjected to chromatography on silica gel with gradient eluention (*n*-hexane/EtOAc: 9/1 → 1/1) to afford **B** as a pale yellow oil. (0.31 g, 56% yield). R_f (*n*-hexane/EtOAc, 7/3) = 0.40

^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) = 7.62 (s, 1 H), 6.17 (s, 1 H), 3.79 (s, 6 H), 3.66 (t, J = 6.4 Hz, 6 H), 2.66 (s, 3 H), 2.44 (t, J = 6.4 Hz, 6 H), 1.40 (s, 27 H), 1.31 (s, 12 H). ^{13}C NMR (101 MHz, $CDCl_3$): δ (ppm) = 170.8, 164.6, 150.6, 137.1, 134.9, 84.1, 80.4, 69.2, 67.1, 60.1, 36.2, 28.0, 24.7, 15.1.

ESI-MS: positive mode, *m/z* = 778.3982 [M+Na]$^+$ (found), 778.3978 (calculated for $C_{37}H_{62}BNNaO_{12}S^+$, [M+Na]$^+$).

Synthesis of **fDAE3**

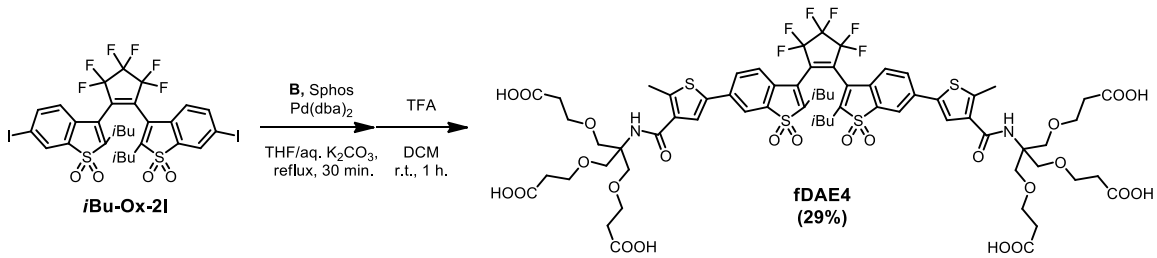


THF (10 mL) containing **Et-Ox-2I** [1] (75 mg, 92 μmol) and **B** (0.18 g, 0.24 mmol, 2.6 eq.) was diluted with water (10 mL) containing K_2CO_3 (100 mg) under N_2 atmosphere. To the vigorously stirring reaction solution was added Sphos (7.6 mg, 18 μmol, 0.20 eq.) and $Pd(dba)_2$ (17 mg, 18 μmol, 0.20 eq.). The reaction mixture was heated to reflux for 30 min. After cooling down to r.t., brine was added, and the reaction mixture was extracted with DCM (2×100 mL). The organic solutions were collected, dried over Na_2SO_4, and concentrated under reduced pressure. The remaining dark solid was subjected to column chromatography (*n*-hexane/EtOAc, 8/2→2/8). The obtained crude product was added to a solution of DCM/TFA (5 mL/5 mL) and then stirred at r.t. for 1h. DCM and excess TFA were removed under vacuum to give yellow solid. Purification was carried out by RP column chromatography (0.1% aq. TFA / CH_3CN, gradient from 7/3 to 3/7) followed by lyophilization (deionized H_2O and 1,4-dioxane) to give **fDAE3** as orange solid (65 mg, 48%).

^{1}H NMR (400 MHz, [D_7]DMF). δ (ppm) = 12.4 (sbr. 6 H, *ap/p*), 8.37 (d, *J* = 2.0 Hz, 1.2 H, *ap*), 8.30 (d, *J* = 1.6 Hz, 0.8 H, *p*), 8.05 (dd, *J* = 8.4 and 2.0 Hz, 1.2 H, *ap*), 7.99 (s, 1.2 H, *ap*), 7.90 (s, 0.8 H, *p*), 7.87 (dd, *J* = 8.4 and 2.0 Hz, 0.8 H, *p*), 7.83 (d, *J* = 8.4 Hz, 0.8 H, *p*), 7.74 (d, *J* = 8.0 Hz, 1.2 H, *ap*), 7.15 (s, 1.2 H, *ap*), 7.09 (s, 0.8 H, *p*), 3.83 (s, 7.2 H, *ap*), 3.81 (s, 4.8 H, *p*), 3.76–3.69 (m, 12.0 H, *ap/p*), 2.84–2.76 (m, 2.4 H, *ap*), 2.71(s, 3.6 H, *ap*), 2.66 (s, 2.4 H, *p*), 2.64–2.58 (m, 1.6 H, *ap*), 2.58–2.52 (m, 12.0 H, *ap/p*), 1.40 (t, *J* = 7.6 Hz, 2.4 H, *p*), 1.06 (t, *J* = 7.6 Hz, 3.6 H, *ap*). ^{13}C NMR (101 MHz, [D_7]DMF): δ (ppm) = 173.8, 165.0, 164.9, 163.4, 149.7, 149.4, 146.1, 146.0, 138.2, 138.1, 137.8, 137.6, 137.0, 136.9, 136.8, 131.9, 131.5, 128.3, 128.1, 127.9, 127.8, 126.1, 125.9, 124.1, 119.8, 119.7, 69.8, 68.3 (2), 67.9, 61.9, 61.8, 35.9, 20.1, 20.0, 15.4, 15.4, 12.8, 12. 6. ^{19}F NMR (376 MHz, [D_7]DMF) δ (ppm) = -109.7 (m, 4.0 F, *p/ap*), -130.8 (m, 2.0 F, *p/ap*).

ESI-MS: positive mode, *m/z* = 1501.2835 $[M+Na]^+$ (found), 1501.2841 (calculated for $C_{63}H_{68}F_6N_2NaO_{24}S_4^+$, $[M+Na]^+$).

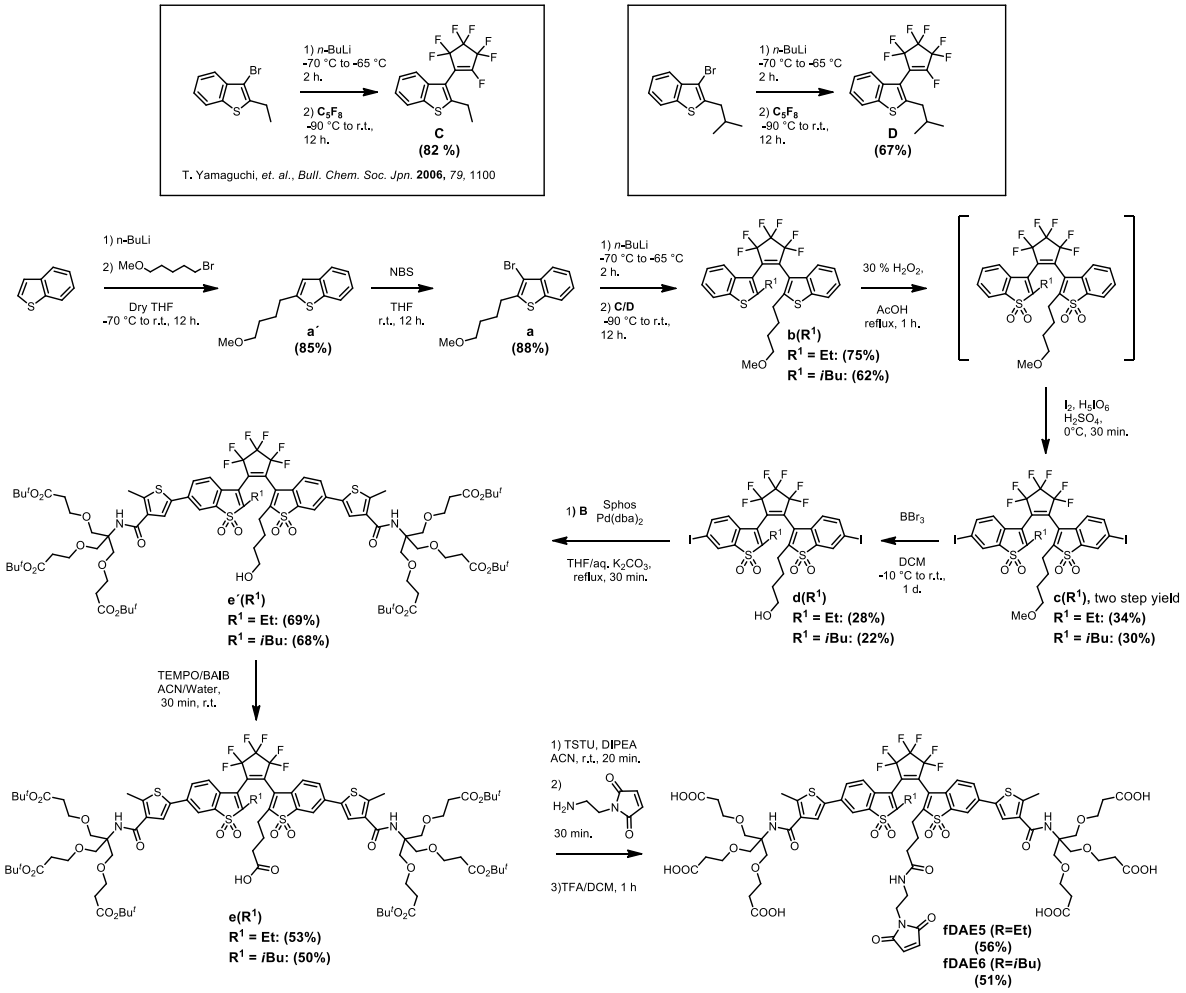
Synthesis of **fDAE4**



THF (10 mL) containing ***i*Bu-Ox-2I** [2] (62 mg, 71 μmol) and **B** (0.17 g, 0.23 mmol, 3.2 eq.) was diluted water (10 mL) containing K_2CO_3 (100 mg) under N_2 atmosphere. To the vigorously stirring reaction mixture was added Sphos (5.9 mg, 18 μmol, 0.20 eq.) and $Pd(dba)_2$ (13 mg, 14 μmol, 0.20 eq.). The reaction mixture was heated to reflux for 30 min. After cooling down to r.t., brine was added, and the reaction mixture was extracted with DCM (2×100 mL). The organic solutions were combined, dried over Na_2SO_4, and concentrated under reduced pressure. The remaining dark solid was subjected to column chromatography (*n*-hexane/EtOAc, 85/15→2/3). The obtained crude product was added to a solution of DCM/TFA (5 mL/5 mL) and then stirred at r.t. for 1 h. DCM and excess TFA were removed under vacuum to give yellow solid. Purification was carried out by RP column chromatography (0.1% aq. TFA / CH_3CN, gradient from 7/3 to 3/7) followed by lyophilization (deionized H_2O and 1,4-dioxane) to give **fDAE4** as orange solid (32 mg, 29%).

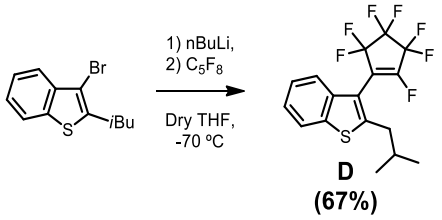
^{1}H NMR (400 MHz, [D_7]DMF). δ (ppm) = 12.58 (sbr. 6 H, *ap/p*), 8.54 (d, *J* = 2.0 Hz, 1.4 H, *ap*), 8.41 (d, *J* = 1.6 Hz, 0.6 H, *p*), 8.25 (dd, *J* = 8.4 and 2.0 Hz, 1.4 H, *ap*), 8.19–8.13 (m, 4.0 H, *ap/p*), 7.96 (dd, *J* = 8.0 and 2.0 Hz, 0.6 H, *p*), 7.90 (dd, *J* = 8.0 and 2.0 Hz, 1.4 H, *ap*), 7.83 (d, *J* = 8.0 Hz, 0.6 H, *p*), 7.28 (s, 1.4 H, *ap*), 7.23 (s, 0.6 H, *p*), 3.97 (s, 8.4 H, *ap*), 3.95 (s, 3.6 H, *p*), 3.90–3.83 (m, 12.0 H, *ap/p*), 2.85 (s, 4.2 H, *ap*), 2.79 (s, 1.8 H, *p*), 2.73–2.66 (m, 12.0 H, *ap/p*), 1.28 (d, *J* = 6.0 Hz, 1.8 H, *p*), 1.15 (d, *J* = 5.2 Hz, 1.8 H, *p*), 1.06 (d, *J* = 6.0 Hz, 4.2 H, *ap*), 0.90 (d, *J* = 6.4 Hz, 4.2 H, *ap*). ^{13}C NMR (101 MHz, [D_7]DMF): δ (ppm) = 173.8, 164.9 (2), 147.9 (2), 146.1, 145.9, 138.3, 138.1, 137.8, 137.5, 137.0, 136.9, 136.8 (2), 131.8, 131.4, 128.2, 128.1, 127.9, 127.8, 126.6, 125.8, 119.8, 117.5, 69.8, 68.3, 67.9, 61.9, 61.8, 35.9, 27.1, 23.9, 22.7, 22.3 (2), 21.7 (2), 15.4 (2).^{19}F NMR (376 MHz, dDMF) δ (ppm) = -109.5 (m, 4.0 F, *p/ap*), -131.7 (m, 2.0 F, *p/ap*).

ESI-MS: positive mode, *m/z* = 1533.3508 $[M-H]^-$ (found), 1533.3502 (calculated for $C_{67}H_{75}F_6N_2O_{24}S_4^-$, $[M-H]^-$).



Scheme S3. Synthesis of compounds **fDAE5** and **fDAE6.**

Synthesis of 2-isobutyl-3-(perfluorocyclopent-1-en-1-yl)benzo[b]thiophene **(D)**



3-bromo-2-isobutylbenzo[b]thiophene (4.8 g, 17.8 mmol) was dissolved in THF (75 mL) under N_2. *n*-BuLi (1.6 M in hexanes, 12 mL, 1.9 mmol, 1.1 eq.) was added at temperature between -70 to -65˚C with stirring, and the reaction solution was stirred for 1 h. THF (4 mL) containing octafluorocyclopentene (9.6 g, 45 mmol, 2.5 eq.) was added in one portion at -95˚C, and the reaction mixture was warmed to r.t. over 1 h. Hydrochloric acid aqueous solution (1 M, 30 mL) and brine (200 mL) were added sequentially to the reaction mixture, and it was extracted with

ethyl acetate (2×150 mL). The organic phase was separated, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The obtained crude product was purified by column chromatography (eluent: hexane) to afford **D** as colorless solid (4.6 g, 67%).

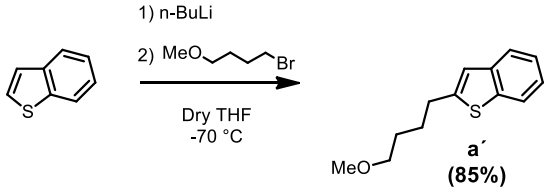
R_f (*n*-hexane) = 0.57.

^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) = 7.86–7.79 (m, 1 H), 7.49 (d, *J* = 7.6 Hz 1 H), 7.43–7.34 (m, 2 H), 2.70 (d, *J* = 7.2 Hz 2 H), 7.04 (quin, *J* = 6.8 Hz, 1 H), 0.99 (d, *J* = 6.8 Hz 6 H).

^{13}C NMR (101 MHz, $CDCl_3$): δ (ppm) = 149.3, 138.4, 138.0, 125.1, 124.8, 122.3, 121.6, 121.6, 121.6, 114.1, 38.6, 30.7, 22.2. ^{19}F NMR (367 MHz, $CDCl_3$) δ (ppm) = -107.8 (br, 2 F), -119.1 (s, 2.0 F), 125.0 (m, 1.0F), 130.4 (s, 2.0F).

ESI-MS: positive mode, m/z 382.0631 $[M]^+$ (found), 382.0621 (calculated for $C_{17}H_{13}F_7S$, $[M]^+$).

Synthesis of 2-(4-methoxybutyl)benzo[b]thiophene **(a´)**



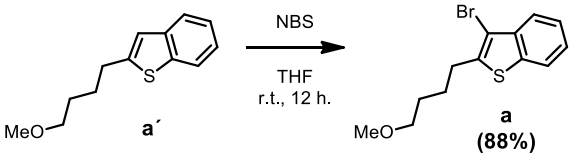
Benzo[b]thiophene (15 g, 0.11 mol) was dissolved in dry THF (100 mL) under N_2. *n*-BuLi (1.6 M in hexanes, 75 ml, 0.12 mol, 1.1 equiv.) was added at temperature between -75 to -70˚C with stirring, and the reaction solution was stirred for 2 h. 1-Bromo-4-methoxybutane (19 g, 0.11 mol, 1.0 equiv.) was slowly added, and the reaction mixture was warmed to r.t. over 12 h. Water (50 mL) was added to the reaction solution, and it was extracted with diethyl ether (2×200 mL). The organic layer was separated, dried over Na_2SO_4 and concentrated under reduced pressure. The crude material was subjected to chromatography on silica gel with gradient eluention (*n*-hexane/EtOAc: 100/0 → 10/1) to yield compound **a´** (21 g, 85%) as a pale yellow oil.

R_f (*n*-hexane/ EtOAc = 9/1) = 0.37.

^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) = 7.79–7.74 (m, 1.0 H), 7.69–7.64 (m, 1.0 H), 7.34–7.22 (m, 2.0 H), 7.03–6.99 (m, 1.0 H), 3.42 (t, *J* = 6.4 Hz, 2.0 H), 3.34 (s, 3.0 H), 2.94 (dt, *J* = 1.2 and 7.6 Hz, 2.0 H), 1.88–1.78 (m, 2.0 H), 1.73–1.64 (m, 2 H). ^{13}C NMR (101 MHz, $CDCl_3$): δ (ppm) = 146.3, 140.2, 139.3, 124.0, 123.3, 122.6, 122.1, 120.6, 72.4, 58.6, 30.6, 29.1, 27.7.

ESI-MS: positive mode, *m/z* 243.0815 $[M+Na]^+$ (found), 243.0814 (calculated for $C_{13}H_{16}NaOS$, $[M+Na]^+$).

Synthesis of 3-bromo-2-(4-methoxybutyl)benzo[b]thiophene **(a)**

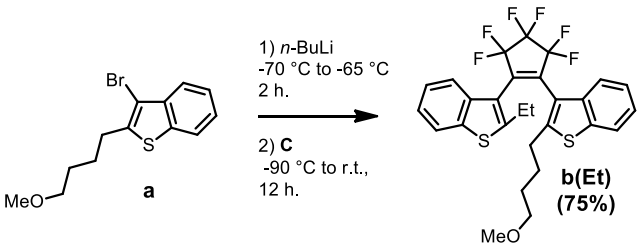


Compound **a´** (21 g, 95 mmol) was dissolved in THF (200 mL) under N_2. NBS (18 g, 0.10 mol, 1.1 equiv.) was added to the reaction mixture in one portion at 0˚C. The reaction temperature was gradually increased to r.t., and the reaction mixture stirred for 12 h. THF was removed under reduced pressure; 1 M NaOH aq. (200 mL) added to the residue, and the mixture was extracted with ether (2×200 mL). The combined organic solutions were dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude material was subjected to chromatography on silica gel with gradient eluention (*n*-hexane/EtOAc: 100/0 → 10/1) to give compound **a** (25 g, 88% yield) as a pale yellow oil. R_f (*n*-hexane/ EtOAc = 9/1) = 0.37.

^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) = 7.77–7.71 (m, 2 H), 7.44–7.39 (m, 1 H), 7.36–7.30 (m, 1 H), 3.43 (t, *J* = 6.4 Hz, 2 H), 3.34 (s, 3 H), 2.99 (t, *J* = 7.6 Hz, 2 H), 1.87–1.78 (m, 2 H), 1.74–1.65 (m, 2 H). ^{13}C NMR (101 MHz, $CDCl_3$): δ (ppm) = 140.4, 138.3, 137.1, 124.9, 124.7, 122.6, 122.2, 105.9, 72.3, 58.6, 29.7, 29.0, 27.0.

ESI-MS: positive mode, *m/z* 320.9920 [M+Na, ^{79}Br]$^+$ (found), 322.9899 [M+Na, ^{81}Br]$^+$ (found), 320.9934 (calculated for $C_{13}H_{15}BrNaOS^+$, [M+Na, ^{79}Br]$^+$), 322.9898 (calculated for $C_{13}H_{15}BrNaOS^+$, [M+Na, ^{81}Br]$^+$).

Synthesis of compound **b(Et)**



Compound **a** (4.40 g, 14.7 mmol) was dissolved in dry THF (22 mL) under N_2. *n*-BuLi (1.6 M in hexanes, 10 mL, 16 mmol, 1.1 equiv.) was added at temperature between -75 to -70˚C with stirring, and the reaction solution was stirred for 2 h. THF (5 mL) containing compound **C** [3] (5.21 g, 14.7 mmol. 1.0 equiv.) was slowly added at temperature below -90°C, and the reaction mixture was warmed to r.t. over 12 h. Then 1 M aq. hydrochroric acid (30 mL) and brine were added sequentially to the reaction solution, and it was extracted with ether (2×200 mL). The organic phase was separated, dried over Na_2SO_4, and concentrated under reduced pressure. The crude

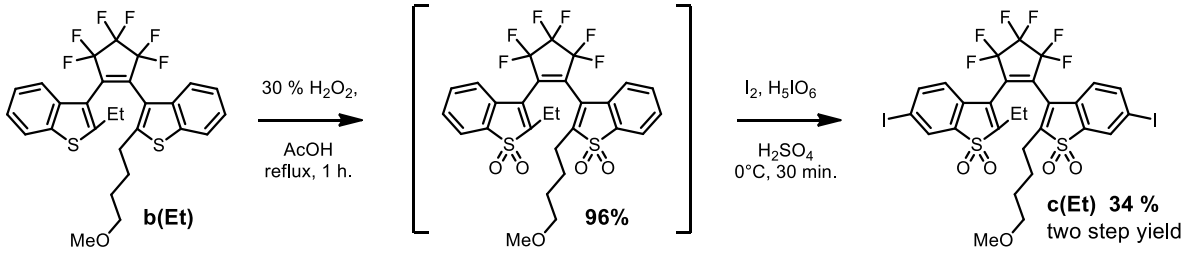
material was subjected to chromatography on silica gel with gradient eluention (*n*-hexane/EtOAc: 95/5 → 10/1) to yield pale yellow oil. Recrystallization from hexane give compound **b(Et)** as a white solid (6.1 g, 75%). R_f (*n*-hexane/EtOAc = 4/1) = 0.47.

^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) = 7.75–7.68 (m, 3.0 H, *ap*), 7.64–7.54 (m, 1.0 H, *p*), 7.43–7.36 (m, 1.5 H, *ap*), 7.35–6.28 (m, 1.5 H, *ap*), 7.22–7.14 (m, 1.0 H, *p*), 3.46–3.39 (m, 0.5 H, *p*), 3.36 (s, 0.75 H, *p*), 3.28 (s, 2.25 H, *ap*), 3.19–3.12 (m, 1.5 H, *ap*), 2.99–2.87 (m, 0.5 H, *p*), 2.84–2 .64 (m, 2.0 H, *ap/p*), 2.48–2.29 (m, 1.5 H, *ap*), 1.84–1.74 (m, 0.5 H, *p*), 1.74–1.65 (m, 0.5 H, *p*), 1.58–1.35 (m, 1.50 H, *ap*). 1.32 (t, J = 7.6 Hz, 0.75 H, *p*), 1.29–1.07 (m, 1.50 H, *ap*), 0.76 (t, J = 7.6 Hz, 2.25 H, *ap*). ^{13}C NMR (101 MHz, $CDCl_3$): δ (ppm) = 150.7, 150.0, 148.8, 148.3 (2), 142.0, 141.7, 138.3, 138.3, 138.1 (2), 138.0, 137.9, 124.7 (2), 124.4, 124.3 (2), 122.4 (2), 122.3 (2), 122.2 (2), 122.1, 122.0, 121.9, 118.9, 118.6 (2), 118.3, 118.0, 117.9, 116.3, 116.1, 115.8, 114.5, 114.2, 113.8, 113.5, 113.3, 111.8, 111.5, 111.3, 109.1, 108.8, 108.6, 72.2, 72.1, 58.6, 58.5, 29.7, 29.4, 29.4, 28.9, 28.3, 27.9, 23.3, 22.9, 16.0, 15.3.

^{19}F NMR (376 MHz, $CDCl_3$) δ (ppm) = -110.1 (m, 4.0 F, *p/ap*), -132.8 (m, 2.0 F, *p/ap*).

ESI-MS: positive mode, *m/z* = 577.1066 $[M+Na]^+$ (found), 577.1065 (calculated for $C_{28}H_{24}F_6NaOS_2^+$, $[M+Na]^+$).

Synthesis of compound **c(Et)**



Acetic acid (60 mL) containing **b(Et)** (3.0 g, 5.4 mmol) was heated to reflux (oil bath temperature 130˚C). 30% H_2O_2 (20 mL, 176 mmol, 33 equiv.) was added carefully to the solution, and the mixture was refluxed for 2 h. After cooling to r.t., water (500 mL) was added and the reaction mixture extracted with EtOAc (2×200 mL. The combined organic solutions were washed with 1 M NaOH (2×200 mL) and concentrated to give the di-sulfone intermediate as a yellow oil (3.2 g, 96%) which was used in the next reaction without further purification. ESI-MS: positive mode, *m/z* 641.0856 $[M+Na]^+$ (found), 641.0862 (calculated for $C_{28}H_{24}F_6NaO_5S_2^+$, $[M+Na]^+$).

The crude compound was dissolved in sulfuric acid (100 mL). I_2 (3.2 g, 12 mmol, 2.4 equiv.) and H_5IO_6 (1.6 g, 7.0 mmol, 1.4 equiv) were mixed and ground to a fine powder which was added to the reaction solution at 0˚C in one portion. The reaction mixture was stirred at 0˚C (± 1˚C) for 30 min., while monitoring the progress of reaction by TLC. After the reaction was complete, the

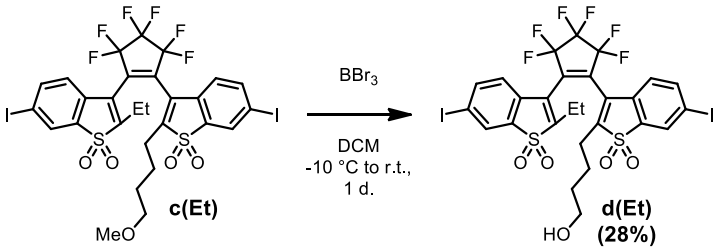
reaction mixture was poured onto ice (500 g). The mixture was warmed-up to r.t. and extracted with ethyl acetate (2×500 mL). The combined organic solutions were washed with 1.0 M NaOH aq. (2×300 mL), dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The crude material was subjected to chromatography on regular silica gel with gradient elution (*n*-hexane/EtOAc: 90/10 → 80/20) which afforded mixture of **OF** and **CF** of compound **c(Et)** as a yellow solid. Recrystallization from a mixture of hexane and DCM (1/1) gave pure compound **c(Et)** as white solid (1.6 g, 34% yield). R_f (n-hexane/EtOAc, 7/3, v/v) = 0.40.

^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) = 8.06 (s, 1.3 H, *ap*), 7.99 (d, *J* = 1.6 Hz, 0.7 H, *p*), 7.98–7.91 (m, 1.3 H, *ap*), 7.81–7.73 (m, 0.7 H, *p*), 6.99–6.86 (m, 1.3 H, *ap*), 6.86–6.74 (m, 0.7 H, *p*), 3.41 (t, *J* = 6.0 Hz, 0.7 H, *p*), 3.32 (s, 3.0 H, *ap/p*), 3.31–3.22 (m, 1.3 H, *ap*), 2.70–2.20 (m, 4.0 H, *ap/p*), 2.02–1.42 (m, 4.0 H, *ap/p*), 1.38 (t, *J* = 7.6 Hz, 1.05 H, *p*), 1.04 (t, *J* = 7.6 Hz, 1.95 H, *ap*). ^{13}C NMR (101 MHz, $CDCl_3$): δ (ppm) = 148.5, 147.9, 147.5, 147.0, 142.8, 142.7, 142.4, 142.3, 139.8 (2), 139.6 (2), 136.9, 136.8 (3), 131.3 (2), 131.2, 128.8, 128.7, 128.6, 124.2, 124.1, 123.9, 123.8 (2), 123.5 (2), 123.4, 123.2, 123.1, 122.8, 122.6, 117.8, 117.5, 117.3, 115.2, 114.9, 114.7, 113.6, 113.4, 113.1, 112.6, 112.4, 112.1, 111.1, 110.9, 110.6, 110.4, 108.2, 107.9, 107. 7, 96.4 (3), 72.1, 72.0, 58.7, 58.6, 29.6, 29.5, 25.8, 25.7, 24.2, 24.0, 19.3, 19.1, 11.8, 11.4.

^{19}F NMR (376 MHz, $CDCl_3$) δ (ppm) = -109.9 (m, 4.0 F, *p/ap*), -132.2 (m, 2.0 F, *p/ap*).

ESI-MS: positive mode, *m/z* 892.8795 $[M+Na]^+$ (found), 892.8794 (calculated for $C_{28}H_{22}F_6I_2NaO_5S_2^+$, $[M+Na]^+$).

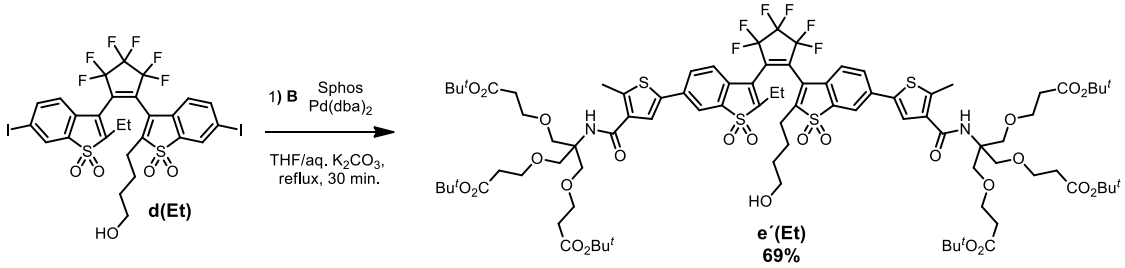
Synthesis of compound **d(Et)**



To DCM (25 mL) containing compound **c(Et)** (1.35 g, 1.55 mmol) was added BBr_3 (1 M in DCM, 15 mL) at -10°C under N_2 atmosphere. The solution was allowed to warm-up to r.t. within 24 h. The reaction mixture was poured into brine and extracted with DCM (2×100 mL). The organic layers were combined, dried over Na_2SO_4 and concentrated under reduced pressure. The crude material was subjected to chromatography on silica gel with gradient eluention (*n*-hexane/EtOAc: 85/15 → 40/60) to yield a mixture of **OF** and **CF** of **d(Et)**. Recrystallization from hexane/DCM give pure **OF** of **d(Et)** as white solid (0.37 g, 28%). R_f (n-hexane/EtOAc, 3/2, v/v) = 0.39.

^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) = 8.06 (s, 1.3 H,*2ap*), 8.00 (s, 0.7 H, *p*), 8.00–7.91 (m, 1.3 H, *ap*), 7.80 (d, *J* = 7.6 Hz, 0.35 H, *p*), 7.75 (d, *J* = 7.6 Hz, 0.35 H, *p*), 7.05–6.86 (m, 1.3 H, *ap*), 6.84 (d, *J* = 8.0 Hz, 0.35 H, *p*), 6.77 (d, *J* = 8.0 Hz, 0.35 H, *p*), 3.78–3.42 (m, 2.0 H, *ap/p*), 2.68–2.22 (m, 4.0 H, *ap/p*), 2.02–1.52 (m, 4.0 H, *ap/p*), 1.46 (s, 1.0 H, *ap/p*), 1.38 (t, *J* = 7.2 Hz, 1.05 H, *p*), 1.02 (t, 1.95 H, *ap*). ^{13}C NMR (101 MHz, $CDCl_3$): δ (ppm) = 148.4, 147.9, 147.3, 146.8, 142.9, 142.8, 142.5, 142.4, 139.7, 136.8 (2), 136.7 (2), 131.3 (2), 131.2, 128.8, 128.6, 128.5, 124.2 (2), 123.9 (2), 123.6 (2), 123.4 (2), 123.2, 122.8, 122.6, 117.7, 117.5, 117.2, 115.1, 114.9, 114.6, 112.5, 112.3, 112.0, 110.9, 110.6, 110.4, 96.5, 96.40 (2), 62.1, 62.0, 32.5, 32.1, 30.9, 25.7, 26.6, 23.5, 23.4, 19.4, 19.0, 11.8, 11.4. ^{19}F NMR (376 MHz, $CDCl_3$) δ (ppm) = -109.8 (m, 4.0 F, *p/ap*), -132.2 (m, 2.0 F, *p/ap*). ESI-MS: positive mode, *m/z* 878.8674 $[M]^+$ (found), 878.8638 (calculated for $C_{27}H_{20}F_6I_2NaO_5S_2^+$, $[M+Na]^+$).

Synthesis of compound **e´(Et)**



A Schlenk flask was charged with **d**(**Et)** (55 mg, 64 µmol), **B** (0.12 g, 0.16 mmol, 2.5 eq.), Sphos (5.3 mg, 19 µmol, 0.20 eq.), and $Pd(dba)_2$ (12 mg, 13 µmol, 0.20 eq.) under N_2 atmosphere. Anhydrous THF (7 mL) and water (7 mL) containing K_2CO_3 (70 mg) were added, and the reaction mixture was heated to reflux for 30 min. After cooling down to r.t., brine was added, and the reaction mixture was extracted with EtOAc (2×100 mL). The organic solutions were combined, dried over Na_2SO_4, and concentrated under reduced pressure. The crude product was subjected to column chromatography (*n*-hexane/EtOAc, 8/2→2/8) followed by lyophilization (1,4-dioxane) to give compound **e´(Et)** orange solid (82 mg, 69%). R_f (*n*-hexane/EtOAc = 1/1) = 0.37.

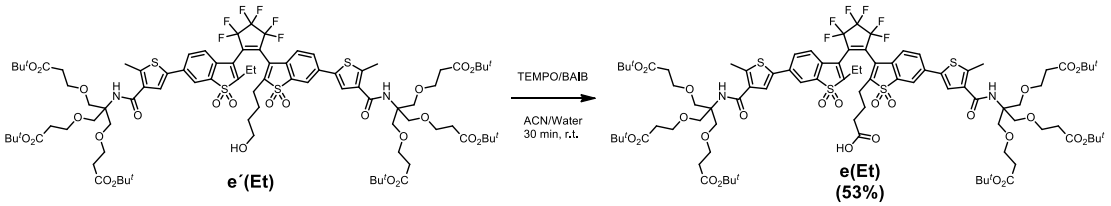
^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) = 7.96–7.85 (m, 2.75 H, *ap/p*), 7.85–7.78 (m, 0.75 H, *p*), 7.69–7.60 (m, 0.5 H, *p*), 7.60–7.54 (m, 1.5 H, *ap*), 7.54–7.47 (m, 0.5 H, *p*), 7.21 (d, *J* = 8.0 Hz, 0.75 H, *ap*), 7.17 (d, *J* = 8.0 Hz, 0.75 H, *ap*), 7.11 (d, *J* = 8.0 Hz, 0.25 H, *p*), 7.05 (d, 8.0 H, *p*), 6.45–6.30 (m, 2.0 H, *ap/p*), 3.70–3.64 (m, 12.5 H, *ap/p*), 3.53–3.31 (m, 1.5 H, *ap*), 4.50 (s, 4.5 H, *ap*), 2.67–2.63 (m, 1.5 H, *p*), 2.60–2.47 (m, 2.0 H, *ap/p*), 2.47–2.40 (m, 12.0 H, *ap/p*), 2.39–2.26 (m, 2.0 H, *ap/p*), 2.67–2.63 (m, 1.5 H, *p*), 1.98–1.63 (m, 2.0 H, *ap/p*), 1.98–1.63 (m, 2.0 H, *ap/p*), 1.40–1.34 (m, 54.0 H, *ap/p*), 1.26- 1.19 (m,3.75 H, *ap/p*). 2.25 (t, *J* = 7.6 Hz, 2.25 H, *ap*). ^{13}C NMR

(101 MHz, $CDCl_3$): δ (ppm) = 171.0, 170.9, 170.8, 164.1, 164.0, 164.0, 148.2, 147.7, 147.0, 146.6, 145.5, 145.3, 145.0, 144.9, 139.9, 139.7, 137.2, 137.1, 137.0 (2), 136.5 (3) , 136.1, 136.0, 135.2, 135.0, 130.1, 130.0, 129.7, 129.7, 127.5, 127.3, 127.2, 126.0, 125.8, 123.6, 123.3 (2), 123.2, 123.1, 122.9, 122.8, 119.2, 119.1, 115.2, 115.0, 114.8, 80.5, 80.4, 80.4, 69.2, 69.1, 62.1, 61.8, 60.2, 36.2 (2), 32.6, 32.5, 28.0, 25.7, 24.8, 23.6, 23.3, 19.4, 19.0, 14.9 (0), 12.0, 11.6.

^{19}F NMR (376 MHz, $CDCl_3$) δ (ppm) = -109.9 (m, 4.0 F, *p/ap*), -132.3 (m, 2.0 F, *p/ap*).

ESI-MS: positive mode, *m/z* = 1881.6896 $[M+H]^+$ (found), 1881.6859 (calculated for $C_{89}H_{120}F_6N_2NaO_{25}S_4^+$, $[M+H]^+$).

Synthesis of compound **e(Et)**

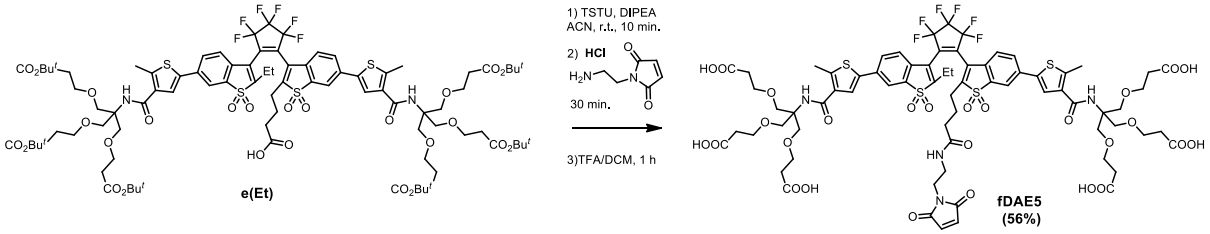


To a mixture of ACN (0.8 mL) and water (0.8 mL) containing compound **e´(Et)** (80 mg, 43 µmol) was added TEMPO (34 mg, 0.22 mmol, 5.0 eq) and PIDA (0.14 g, 0.43 mmol, 10 eq.), and the reaction mixture was stirred at r.t. for 30 min. Then the reaction mixture was poured into brine and extracted with EtOAc (2×100 mL). The organic solutions were collected, dried over Na_2SO_4, and concentrated under reduced pressure. The remaining crude material was subjected to column chromatography (*n*-hexane/EtOAc, 8/2→2/8) followed by lyophilization (1,4-dioxane) to give as compound **e(Et)** as orange solid (43 mg, 53%).

^1H NMR (400 MHz, $CDCl_3$). δ (ppm) = 8.05–7.90 (m. 2.0 H, *ap/p*), 7.90–7.78 (m, 2.0 H, *ap/p*), 7.63–7.47 (m, 2.0 H, *ap/p*), 7.41–7.29 (m, 1.0 H, *ap/p*),7.24–7.15 (m , 1.0 H, *ap/p*), 6.47–7.27 (m, 2.0 H, *ap/p*), 3.87–3.67 (m, 12.0 H, *ap/p*), 3.74–3.64 (m, 12.0 H, *ap/p*), 2.73–2.65 (m, 6.0 H, *ap/p*), 2.65–2.51 (m, 2.0 H, *ap/p*), 2.51–2.41 (m, 12.0 H, *ap*), 2.40–2.20 (m, 4.0 H, *ap/p*), 2.20–1.70 (m, 2.0 H, *ap/p*), 1.41–1.35 (m, 54.0 H, *ap/p*),1.07–0.97 (m, 3.0 H, *ap/p*). ^{13}C NMR (101 MHz, $CDCl_3$): δ (ppm) = 174.0 (2), 170.9 (2), 164.2, 164.1, 147.6, 146.1, 146.0, 145.3, 137.1, 137.0, 136.5, 136.4, 136.3, 136.1, 135.1, 134.3, 130.3, 130.0, 127.5, 127.4, 125.9, 125.6, 124.1, 124.0, 123.3, 123.2, 119.2, 119.1, 80.9, 80.5, 69.2 (2), 67.1, 60.3, 60.2, 36.3, 36.2, 32.8, 30.3, 29.7, 29.3, 28.0 (2), 24.6, 22.2, 19.0, 15.0, 11.7. ^{19}F NMR (376 MHz, $CDCl_3$) δ (ppm) = -109.8 (m, 4.0 F, *p/ap*), -132.3 (m, 2.0 F, *p/ap*).

ESI-MS: positive mode, *m/z* = 1895.6689 $[M+Na]^+$ (found), 1895.6652 (calculated for $C_{89}H_{118}F_6N_2NaO_{26}S_4^+$, $[M+Na]^+$).

Synthesis of **fDAE5**

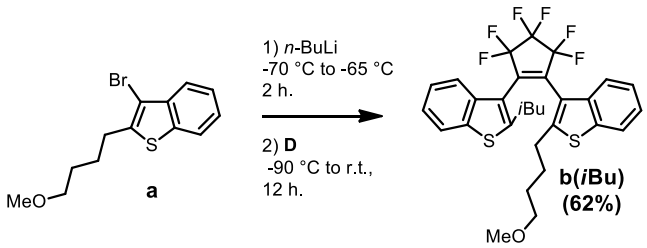


To a solution in ACN (1.6 mL) containing **e(Et)** (40 mg, 21 μmol) and TSTU (13 mg, 43 μmol, 2.0 eq.) was added DIPEA (11 mg, 0.85 μmol, 4.0 eq). The reaction mixture was stirred at r.t. for 10 min. 1-(2-Aminoethyl)maleimide hydrochloride (**E**) (15 mg, 85 μmol, 4.0 eq.) and DIPEA (11 mg, 0.85 μmol, 4.0 eq) were added to the reaction mixture which was then stirred for 30 min at r.t. The reaction mixture was added to a solution of TFA (10 mL) and DCM (10 mL) and was stirred at r.t. for 1 h. DCM and excess TFA were removed under reduced pressure. Purification was carried out by reverse phase column chromatography (0.1% aq. TFA / CH_3CN, gradient from 7/3 to 3/7) followed by lyophilization (deionized H_2O and 1,4-dioxane) to give **fDAE5** as orange solid (20 mg, 56%).

^{1}H NMR (600 MHz, [D$_7$]DMF). δ (ppm) = 12.5 (sbr, 6 H, *ap/p*), 8.38 (d. *J* = 1.8 Hz, 0.60 H, *ap*), 8.36 (d, *J* = 2.4 Hz, 0.60 H, *ap*), 8.31–8.28 (m, 0.80 H, *p*), 8.06–8.03 (m, 1.0 H, *ap/p*), 8.02–8.01 (d, *J* = 2.4 Hz, 0.40 H, *p*), 8.00 (s, 0.6 H, *ap*), 7.99–7.96 (m, 1.2 H, *ap/p*), 7.91 (s, 0.4 H, *p*), 7.90 (s, 0.4 H, *p*), 7.88–7.83 (m, 1.0 H, *ap/p*), 7.83–7.77 (m, 0.80 H, *p*), 7.77–7.70 (m, 1.2 H, *ap*), 7.16 (s, 0.6 H, *ap*), 7.13 (s, 0.6 H, *ap*), 7.12–7.09 (m, 0.8 H, *p*), 7.03–6.99 (ss, 2.0 H, *ap/p*), 3.86–3.79 (m, 12.0 H, *ap/p*), 3.75–3.70 (m, 12.0 H, *ap/p*), 3.60–3.57 (m, 2.0 H, *ap/p*), 3.40–3.33 (m, 2.0 H, *ap/p*), 2.84–2.76 (m, 2.0 H, *ap/p*), 2.72 –2.69 (ss, 3.6 H, *ap*), 2.68–2.65 (ss, 2.4 H, *p*), 2.64–2.57 (m, 2.0 H, *ap/p*), 2.57–2.50 (m, 12.0 H, *ap/p*), 2.35–2.31 (m, 0.8 H, *p*), 2.26–2.21 (m, 1.2 H, *ap*), 2.19–2.10 (m, 0.4 H, *p*), 2.01–1.90 (m, 1.0 H, *ap/p*), 1.80 –1.70 (m, 0.6 H, *ap*), 1.39 (t, *J* = 9.0 Hz, 1.2 H, *a*), 1.04 (t, *J* = 9.0 Hz, 1.8 H, *ap*). ^{13}C NMR (151 MHz, [D$_7$]DMF): δ (ppm) = 173.8, 172.9, 172.8, 172.3, 165.0, 149.7, 149.3, 148.5, 148.2, 146.1, 146.0, 145.9, 141.2, 141.0, 140.9, 138.2, 138.1, 138.0, 137.7, 137.6, 137.5, 137.0 (2), 136.8, 136.8, 135.7, 131.9, 131.5, 131.4, 128.2, 128.1, 127.9, 126.3, 126.1, 126.0, 125.9, 124.7, 124.1, 124.0, 119.8, 119.7, 119.7, 118.8, 118.5, 118.4, 116.7, 116.5, 116.3, 114.6, 114.4, 114.2, 112.2, 112.0, 111.8, 69.7, 68.3, 61.8, 38.5, 26.1, 24.5, 24.3, 20.2, 20.0, 15.4, 15.4, 14.8, 12.9, 12.5. ^{19}F NMR (565 MHz, [D$_7$]DMF) δ (ppm) = -109.5 (m, 4.0 F, *p/ap*), -130.5 (m, 2.0 F, *p/ap*).

ESI-MS: positive mode, *m/z* = 1681.3385 $[M+Na]^+$ (found), 1681.3376 (calculated for $C_{71}H_{76}F_6N_4NaO_{27}S_4^+$, $[M+Na]^+$).

Synthesis of compound **b(*i*Bu)**

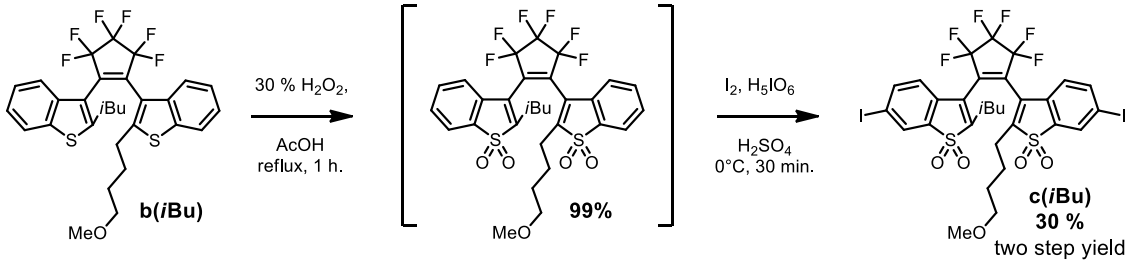


Compound **a** (3.5 g, 11.7 mmol) was dissolved in dry THF (17 mL) under N_2. *n*-BuLi (1.6 M in hexanes, 8.0 mL, 12.8 mmol, 1.1 equiv.) was added at temperature between -75 to -70˚C with stirring, and the reaction solution was stirred for 2 h. THF (5 mL) containing compound **D** (4.5 g, 12 mmol. 1.0 equiv.) was slowly added at temperature below -95˚C, and the reaction mixture was warmed up to r.t. over 12 h. 1 M aq. HCl(30 mL) and brine (200 mL) were added sequentially to the reaction mixture, and it was extracted with ether (2×200 mL). The organic solutions were combined, dried over Na_2SO_4 and concentrated under reduced pressure. The crude material was subjected to chromatography on silica gel with gradient eluention (*n*-hexane/EtOAc: 9/1) to yield pale yellow oil. Recrystallization from hexane give compound **b(*i*Bu)** as a white solid (4.2 g, 62%). R_f (*n*-hexane/EtOAc = 7/3) = 0.60.

^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) = 7.80–7.71 (m, 3.2 H, *ap*), 7.69 (d, *J* = 8.0 Hz, 0.2 H, *p*), 7.60 (dd, *J* = 8.0 and 5.2 Hz, 0.4 H, *p*), 7.45 (d, *J* = 8.0 Hz, 0.2 H, *p*), 7.43–7.37 (m, 1.6 H, *ap*), 7.36–7.29 (m, 1.6 H, *ap*), 7.29–7.17 (m, 0.4 H, *p*), 7.17–7.04 (m, 0.4 H, *p*), 3.48–3.41 (m, 0.4 H, *p*), 3.36 (s, 0.6 H, *p*), 3.27 (s, 2.4 H, *ap*), 3.16–3.06 (m, 1.6 H, *ap*), 3.03–2.92 (m, 0.2 H, *p*), 2.85–2.75 (m, 0.2 H, *p*), 2.70–2.60 (m, 1.0 H, *ap/p*), 2.57–2.44 (m, 1.0 H, *ap/p*), 2.32–2.23 (m, 0.8 H, *ap*), 2.09–1.99 (m, 1.0 H, *ap/p*), 1.88–1.68 (m, 1.6 H, *ap/p*), 1.53–1.40 (m, 0.8 H, *ap*), 1.39–1.28 (m, 0.8 H, *ap*), 1.25–1.14 (m, 0.8 H, *ap*), 1.12–1.00 (m, 1.2 H, *ap/p*), 0.93 (d, *J* = 6.4 Hz, 0.6 H, *p*), 0.78 (d, *J* = 6.4 Hz, 2.4 H, *ap*), 0.53 (d, *J* = 6.4 Hz, 2.4 H, *ap*). ^{13}C NMR (101 MHz, $CDCl_3$): δ (ppm) = 149.0, 148.6, 148.1, 147.8, 142.0, 141.8, 138.3, 138.2, 138.1 (2), 138.0 (2), 137.9, 124.7, 124.5, 124.4 (2), 124.3 (3), 122.9, 122.8 (3), 122.5, 122.4 (2), 122.2 (2), 122.0 (2), 121.9 (2), 119.3, 119.1, 118.9, 118.7 (2), 118.6, 118.4, 116.3, 116.1, 115.9, 114.5, 114.2, 114.0, 113.8, 113.5, 113.3, 111.8, 111.5, 111.3, 109.1, 108.8, 108.6, 72.2, 72.1, 58.6, 58.5, 38.7, 38.4, 30.5, 30.3 (2), 29.8, 29.5, 29.3, 28.9, 28.6, 27.8, 23.0, 21.7 (2), 21.5 (2). ^{19}F NMR (376 MHz, $CDCl_3$) δ (ppm) = -110.0 (m, 4.0 F, *p/ap*), -133.1 (m, 2.0 F, *p/ap*).

ESI-MS: positive mode, *m/z* 605.1371 $[M+Na]^+$ (found), 605.1378 (calculated for $C_{30}H_{28}F_6NaOS_2^+$, $[M+Na]^+$).

Synthesis of compound **c(*i*Bu)**



Acetic acid (60 mL) containing compound **b(*i*Bu)** (3.0 g, 5.4 mmol) was heated to reflux (oil bath temperature 130˚C). 30% H_2O_2 (20 mL, 176 mmol, 33 equiv.) was added to the solution, and the mixture was refluxed for 2 h. After cooling to r.t., water (500 mL) was added, and the mixture was extracted with EtOAc (2×200 mL). The combined organic solutions were washed with 1 M aq. NaOH (2×200 mL), and concentrated to give the crude di-sulfone intermediate as a yellow oil (3.3 g, 99%) which was used in the next reaction without further purification.

MS: positive mode, *m/z* = 669.1180 [M+Na]+ (found), 669.1175 (calculated for $C_{30}H_{28}F_6NaO_5S_2^+$, $[M+Na]^+$)

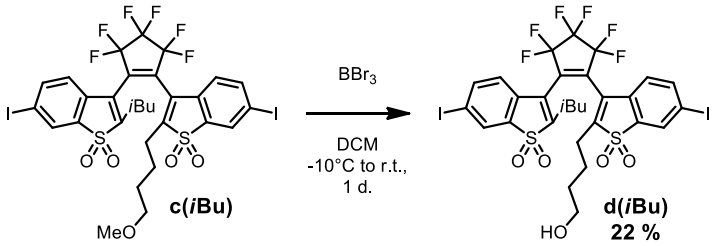
The crude compound was dissolved in sulfuric acid (100 mL). I_2 (3.2 g, 12 mmol, 2.4 equiv.) and H_5IO_6 (1.6 g, 7.0 mmol, 1.4 equiv) were mixed and grounded to a fine powder which was added to the reaction solution at 0˚C in one portion. The reaction mixture was stirred at 0˚C (± 1˚C) for 30 min., while monitoring the progress of the reaction by TLC. After the reaction was complete, the mixture was poured into ice (500 g). The mixture was warmed-up to r.t. and extracted with ethyl acetate (2×500 mL). The combined organic solutions were washed with 1.0 M NaOH aq. (2×300 mL), dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude material was subjected to chromatography on regular silica gel with gradient elution (*n*-hexane/EtOAc: 90/10 → 80/20) which afforded mixture of **OF** and **CF** of **c(iBu)** as a yellow solid. Recrystallization from hexane/DCM (1/1) gave pure **OF** of **c(*i*Bu)** as a white solid (1.4 g, 31% yield). R_f (n-hexane/EtOAc, 7/3, v/v) = 0.50.

^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) = 8.10–8.04 (m, 1.33 H, *ap*), 8.01–7.97 (m, 0.67 H, *p*), 7.97–7.91 (m, 1.33 H, *ap*), 7.84 (dd, *J* = 8.0 and 1.6 Hz, 0.33 H, *p*), 7.65 (dd, *J* = 8.0 and 1.6 Hz, 0.33 H, *p*), 6.99 (dd, *J* = 8.0 and 2.0 Hz, 0.67 H, *ap*), 6.97–6.88 (m, 1.0 H, *ap/p*), 6.63 (d, *J* = 8.0 Hz, 0.33 H, *p*), 3.42 (t, *J* = 6.0 Hz, 0.67 H, *p*), 3.33 (s, 1.0 H, *p*), 3.31 (s, 2.0 H, *ap*), 2.71–1.97 (m, 5.0 H, *ap/p*), 1.88–1.63 (m, 2.0 H, *ap/p*), 1.49–1.30 (m, 2.0 H, *ap/p*), 1.04 (d, *J* = 6.4 Hz, 1.0 H, *p*), 0.94 (dd, *J* = 6.4 and 1.2 Hz, 1.0 H, *p*), 0.88 (d, *J* = 6.4 Hz, 2.0 H, *ap*), 0.77 (d, *J* = 6.4 Hz, 2.0 H, *ap*). ^{13}C NMR (101 MHz, $CDCl_3$): δ (ppm) = 147.4, 147.3, 147.2, 146.7, 142.8, 142.4 (2), 142.2, 134.0, 139.8, 137.0, 136.8 (3), 131.3 (2), 131.2, 128.8, 128.7, 128.6, 128.5, 124.5 (2), 124.4,

124.3, 124.2, 124.1, 123.9, 123.8, 123.3, 123.1 (2), 123.0, 117.5, 117.2, 115.1, 114.9, 112.3, 112.0, 110.7, 110.46, 96.40, 96.34, 96.30, 72.09, 71.96, 58.72, 58.63, 34.98, 34.96, 29.67, 29.49, 26.51, 26.49, 26.01, 25.88, 25.6, 24.4, 23.9, 23.5, 22.6, 21.8, 21.7, 21.3, 21.2. ^{19}F NMR (376 MHz, $CDCl_3$) δ (ppm) = -109.7 (m, 4.0 F, *p/ap*), -132.6 (m, 2.0 F, *p/ap*).

ESI-MS: positive mode, m/z 920.9089 $[M+Na]^+$ (found), 920.9107 (calculated for $C_{30}H_{26}F_6I_2NaO_5S_2^+$, $[M+Na]^+$).

Synthesis of compound **d(*i*Bu)**

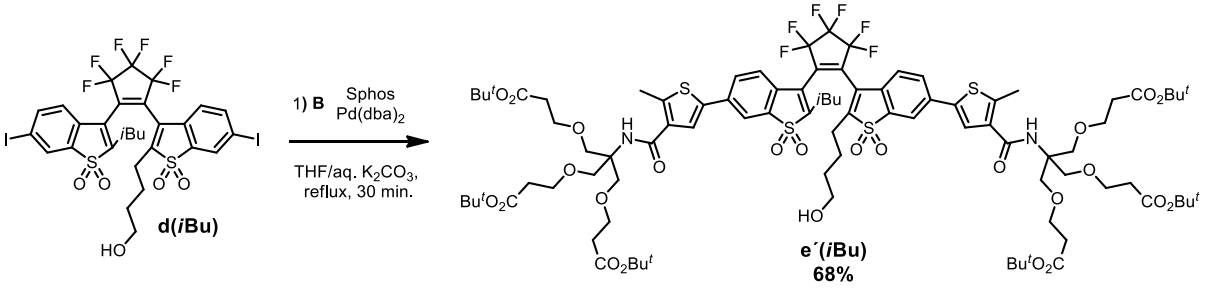


To DCM (25 mL) containing **c(*i*Bu)** (1.30 g, 1.45 mmol) was added BBr_3 (1 M in DCM, 15 mL) at -10°C under N_2 atmosphere. The solution was allowed to warm-up to r.t. over 24 h. The reaction mixture was poured into brine and extracted with DCM (2×100 mL). The organic solutions were combined, dried over Na_2SO_4 and concentrated under reduced pressure. The crude material was subjected to chromatography on silica gel with gradient eluention (*n*-hexane/EtOAc: 85/15 → 40/60) to yield a mixture of **OF** and **CF** of **d(*i*Bu)** as a yellow solid. Recrystallization from hexane/DCM give pure **OF** of **d(*i*Bu)** as white solid (0.28 g, 22%). R_f (n-hexane/EtOAc, 3/2, v/v) = 0.42.

^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) = 8.10–8.05 (m, 1.34 H, *ap*), 8.01–7.97 (m, 1.0 H, *ap/p*), 7.97–7.93 (m, 1.0 H, *ap/p*), 7.83 (dd, *J* = 8.0 and 1.2 Hz, 0.33 H, *p*), 7.66 (dd, *J* = 8.0 and 1.2 Hz, 0.33 H, *p*), 7.01 (dd, *J* = 8.0 and 1.6 Hz, 0.67 H, *ap*), 6.95 (d, *J* = 8.4 Hz, 0.67 H, *ap*), 6.90 (dd, *J* = 8.0 and 1.6 Hz, 0.33 H, *p*), 6.65 (d, *J* = 8.0 Hz, 0.33 H, *p*), 3.70 (t, *J* = 6.4 Hz, 0.67 H, *p*), 3.56–3.45 (m, 1.33 H, *ap*), 2.72–1.97 (m, 5.0 H, *ap/p*), 1.92–1.62 (m, 2.0 H, *ap/p*), 1.46 (s, 1.0 H, *ap/p*), 1.42–1.24 (m, 2.0 H, *ap/p*), 1.46 (s, 1.0 H, *ap/p*), 1.04 (d, *J* = 6.4 Hz, 1.0 H, *p*), 0.94 (dd, *J* = 6.4 and 1.2 Hz, 1.0 H, *p*), 0.88 (d, *J* = 6.4 Hz, 2.0 H, *ap*), 0.76 (d, *J* = 6.4 Hz, 2.0 H, *ap*). ^{13}C NMR (101 MHz, $CDCl_3$): δ (ppm) = 147.3, 147.1, 147.0, 146.7, 142.8, 142.5, 142.4, 142.2, 140.0, 139.7, 136.9, 136.8, 136.7, 131.3 (3), 131.2, 128.8, 128.5 (2), 124.6, 124.5 (2), 124.3, 124.2, 123.8 (2), 123.4, 123.2 (2), 117.7, 117.5, 115.1, 114.9, 112.5, 112.3, 111.0, 110.7, 110. 5, 96.4 (3), 62.1, 62.0, 34.9, 32.5, 32.1, 26.4, 26.3, 26.0, 25.9, 25.5, 23.7, 23.5, 23.3, 22.6, 21.8, 21.3, 21.2. ^{19}F NMR (376 MHz, $CDCl_3$) δ (ppm) = -109.6 (m, 4.0 F, *p/ap*), -132.6 (m, 2.0 F, *p/ap*).

ESI-MS: positive mode, *m/z* 906.8932 $[M+Na]^+$ (found), 906.8951 (calculated for $C_{29}H_{24}F_6I_2NaO_5S_2^+$, $[M+Na]^+$).

Synthesis of compound **e´(*i*Bu)**



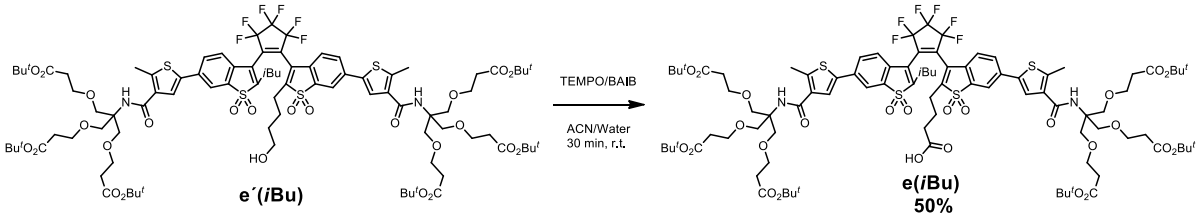
Schlenk flask was charged with **d(*i*Bu)** (55 mg, 62 μmol), **B** (0.12 g, 0.16 mmol, 2.5 eq.), Sphos (5.1 mg, 12 μmol, 0.20 eq.), and $Pd(dba)_2$ (11 mg, 12 μmol, 0.20 eq.) under N_2 atmosphere. THF (7 mL) and water (7 mL) containing K_2CO_3 (70 mg) were added, and the reaction mixture was heated to reflux for 30 min. After cooling down to r.t., brine was added, and the reaction mixture was extracted with EtOAc (2×100 mL). The combined organic solutions were dried over Na_2SO_4 and concentrated under reduced pressure. The crude product was subjected to column chromatography (*n*-hexane/EtOAc, 8/2→2/8) followed by lyophilization (1,4-dioxane) to give compound **e´(*i*Bu)** as orange solid (80 mg, 68%). R_f (*n*-hexane/EtOAc, 1/1, v/v) = 0.33.

^{1}H NMR (400 MHz, $CDCl_3$). δ (ppm) = 7.98–7.92 (d, J = 2.0 Hz, 0.8 H, *ap*), 7.92–7.76 (m, 2.8 H, *ap/p*), 7.68 (dd, J = 8.0 and 2.0 Hz, 0.2 H, *p*), 7.59 (s, 0.8 H, *ap*), 7.57 (s , 0.8 H, *ap*), 7.52 (s, 0.2 H, *p*), 7.50–7.46 (m, 0.4 H, *p*), 7.29–7.24 (m, 0.8 H, *ap*), 7.23–7.16 (m, 1.0 H, *ap/p*), 6.95 (d, J = 8.0 Hz, 0.2 H, *p*), 6.42 (s, 0.8 H, *ap*), 6.38 (s, 0.8 H, *ap*), 6.34 (s, 0.2 H, *p*), 6.33 (s, 0.2 H, *p*), 3.86–3.77 (m, 12.0 H, *ap/p*), 3.73–3.64 (m, 12.0 H, *ap/p*), 3.53–3.27 (m, 2.0 H, *ap/p*), 2.73–2.68 (m, 4.8 H, *ap*), 2.67 (s, 0.6 H, *p*), 2.65 (s, 0.6 H, *p*), 2.49–2.41 (m, 12.0 H, *ap/p*), 2.35–2.05 (m, 4.0 H, *ap/p*), 1.85–1.65 (m, 6.0 H, *ap/p*), 1.41–1.35 (m, 54.0 H, *ap/p*), 1.05 (d, J = 6.8 Hz, 0.6 H, *p*), 0.97 (d, J = 6.4 Hz, 0.6 H, *p*), 0.89 (d, J = 6.4 Hz, 2.4 H, *ap*), 0.76 (d, J = 6.4 Hz, 2.4 H, *ap*). ^{13}C NMR (101 MHz, $CDCl_3$): δ (ppm) = 171.0, 170.9 (2), 164.1 (2), 146.8, 146.5, 145.6, 145.1, 144.9, 137.2 (2), 136.7, 136.5, 136.2, 136.1, 135.3, 135.0, 130.2, 129.7, 127.7, 127.3 (2), 126.1, 125.9, 125.8, 124.7, 123.9, 123.8, 123.7, 123.5, 123.4, 119.3, 119.2, 119.1 (2), 80.6, 80.5 (2), 69.2 (2), 62.2, 61.8, 60.3, 60.3, 36.3, 36.2, 35.0, 32.6, 32.5, 30.3, 29.7, 28.0, 26.4, 26.1, 26.0, 25.6, 24.8, 23.5, 23.3, 22.5, 21.8, 21.3, 15.0 (2).

. ^{19}F NMR (376 MHz, $CDCl_3$) δ (ppm) = -109.4 (m, 4.0 F, *p/ap*), -132.6 (m, 2.0 F, *p/ap*).

ESI-MS: positive mode, *m/z* 1923.6964 $[M+Na]^+$ (found), 1923.6965 (calculated for $C_{91}H_{122}F_6N_2NaO_{26}S_4^+$, $[M+Na]^+$).

Synthesis of compound **e(*i*Bu)**



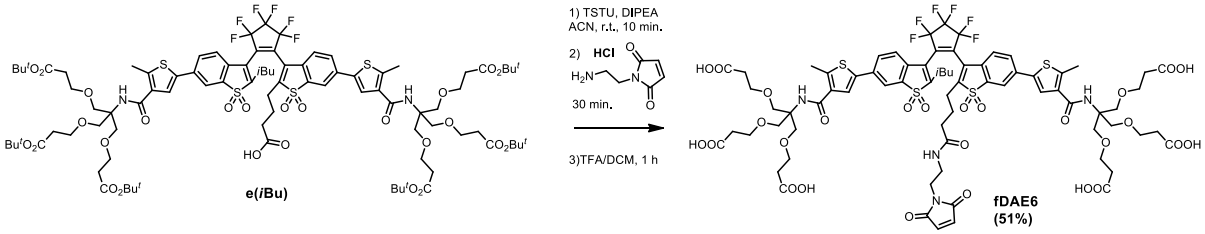
To a mixture of ACN (1.0 mL) and water (1.0 mL) containing **e´(*i*Bu)** (0.11 mg, 58 µmol) was added TEMPO (45 mg, 0.29 mmol, 5.0 eq) and BAIB (0.19 g, 0.58 mmol, 10 eq.), and the reaction mixture was stirred at r.t. for 10 min. Then, the reaction mixture was poured into brine and extracted with EtOAc (2×100 mL). The organic solutions were ccombined, dried over Na_2SO_4, and concentrated under reduced pressure. The remaining crude material was subjected to column chromatography (*n*-hexane/EtOAc, 8/2→2/8) followed by liophilization (1,4-dioxane) to give compound **e(*i*Bu)** as orange solid (55 mg, 50%).

^{1}H NMR (400 MHz, $CDCl_3$). δ (ppm) = 8.10–7.95 (m, 1.0 H, *ap/p*), 7.94 (s, 0.8 H, *ap*), 7.88 (s, 0.2 H, *p*), 7.85 (s, 0.2 H, *p*), 7.83–7.75 (m, 1.6 H, *ap*), 7.67 (d, J = 7.2 Hz, 0.2 H, *p*), 7.57 (s, 0.8 H, *ap*), 7.55–7.45 (m, 1.2 H, *ap/p*), 7.38 (d, *J* = 8.0 Hz, 0.8 H, *ap*), 7.22 (d, *J* = 8.0 Hz, 0.8 H, *ap*), 7.16 (d, *J* = 8.0 Hz, 0.2 H, *p*), 7.01–6.93 (m, 0.2 H, *p*), 6.98–6.27 (m, 2.0 H, *ap/p*), 3.89–3.75 (m, 12.0 H, *ap/p*), 3.72–3.63 (m, 12.0 H, *ap/p*), 3.76–2.63 (m, 6.0 H, *ap/p*), 2.50–2.40 (m, 12.0 H, *ap/p*), 2.37–2.06 (m, 6.0 H, *ap/p*), 2.03–1.91 (m, 1.0 H, *ap/p*), 1.80–1.64 (m, 2.0 H, *ap/p*), 1.43–1.32 (m, *5*4.0 H, *ap/p*), 1.04 (d, *J* = 6.4 Hz, 0.6 H, *p*), 0.96 (d, *J* = 6.4 Hz, 0.6 H, *p*), 0.89 (d, *J* = 6.4 Hz, 2.4 H, *ap*), 0.73 (d, *J* = 6.4 Hz, 2.4 H, *ap*). ^{13}C NMR (101 MHz, $CDCl_3$): δ (ppm) = 173.8, 171.5, 170.9 (3), 164.1 (2), 146.3, 146.2, 146.1, 145.1, 145.0, 137.2, 137.0, 136.6, 136.4, 136.3, 136.2, 135.4, 134.4, 130.3, 129.7, 127.5, 127.3, 125.9, 125.4, 124.7, 124.3, 124.2, 124.1, 123.9, 119.2, 119.1, 80.9, 80.5, 69.2 (2), 60.3, 60.2 (2), 36.4, 36.2, 35.0, 32.7, 30.3, 28.0 (2), 25.9, 24.5, 22.4, 22.2, 21.9, 15.0 (2).

. ^{19}F NMR (376 MHz, $CDCl_3$) δ (ppm) = -109.7 (m, 4.0 F, *p/ap*), -132.4 (m, 2.0 F, *p/ap*).

ESI-MS: positive mode, *m/z* 1923.6954 $[M+Na]^+$ (found), 1923.6965 (calculated for $C_{91}H_{122}F_6N_2NaO_{26}S_4^+$, $[M+Na]^+$).

Synthesis of compound **fDAE6**



To ACN (1.4 mL) containing **e(*i*Bu)** (35 mg, 21 µmol) and TSTU (11 mg, 37 µmol, 2.0 eq.) DIPEA (9.5 mg, 0.74 µmol, 4.0 eq) was added with stirring. The reaction mixture was stirred at r.t. for 10 min. 1-(2-Aminoethyl)maleimide hydrochloride (**E**) (13 mg, 0.74 µmol, 4.0 eq.) and DIPEA (9.5 mg, 0.74 µmol, 4.0 eq) were added, and the reaction mixture was stirred for 30 min at r.t. The reaction mixture was added to a solution of TFA (10 mL) and DCM (10 mL) and stirred at r.t. for 1 h. DCM and excess TFA were removed under reduced pressure. Purification was carried out by RP column chromatography (0.1% aq. TFA / CH_3CN, gradient from 7/3 to 3/7) followed by liophilization (deionized H_2O and 1,4-dioxane) to give **fDAE6** as a reddish solid (15 mg, 51%).

^{1}H NMR (600 MHz, [D$_7$]DMF). δ (ppm) = 12.5 (sbr, 6 H, *ap/p*), 8.39 (d. *J* = 1.8 Hz, 0.67 H, *ap*), 8.37 (d, *J* = 1.8 Hz, 0.67 H, *ap*), 8.29 (d, *J* = 1.8 Hz, 0.33 H, *p*), 8.27 (d, *J* = 1.2 Hz, 0.33 H, *p*), 8.10 (d, *J* = 1.8 Hz, 0.33 H, *p*), 8.09 (d, *J* = 1.8 Hz, 0.33 H, *p*), 8.05–8.02 (m, 1.33 H, *ap*), 8.01–7.98 (m , 1.0 H, *ap/p*), 7.94 (t, *J* = 6.0 Hz, 0.67 H, *ap*), 7.92–7.88 (m, 1.0 H, *ap/p*), 7.83 (dd, *J* = 7.8 and 1.8 Hz, 0.33 H, *p*), 7.79–7.72 (m, 1.67 H, *ap/p*), 7.64 (d, *J* = 7.8 Hz, 0.33 H, *p*), 7.14 (d, *J* = 11.4 Hz, 1.33 H, *ap*), 7.10 (d, *J* = 4.8 Hz, 12.0 H, *p*), 7.01 (s, 0.67 H, *p*), 7.00 (s, 1.33 H, *ap*), 3.85–3.79 (m, 12.0 H, *ap/p*), 3.76–3.70 (m, 12 H, *ap/p*), 3.62–3.56 (m, 2.0 H, *ap/p*), 3.40–3.31 (m, 2.0 H, *ap/p*), 2.84–2.79 (m, 0.67 H, *p*), 2.71 (d, *J* = 0.6 Hz, 4.0 H, *ap*), 2.68–2.63 (m, 2.0 H, *p*), 2.59–2.53 (m, 12.0 H, *ap/p*),), 2.50–2.39 (m, 2.0 H, *ap/p*), 2.39 –2.15 (m, 4.0 H, *ap/p*), 2.02 –1.85 (m, 1.0 H, *ap/p*), 1.72 –1.53 (m, 1.0 H, *ap/p*), 1.12 (d, *J* = 6.6 Hz, 1.0 H, *a*), 1.00 (d, *J* = 6.0 Hz, 1.0 H, *a*), 0.94 (d, *J* = 6.0 Hz, 2.0 H, *ap*), 0.82 (d, *J* = 6.6 Hz, 2.0 H, *ap*). ^{13}C NMR (151 MHz, [D$_7$]DMF): δ (ppm) = 173.8, 172.8, 172.7, 172.3, 164.9, 164.9, 148.3, 148.1, 147.8, 146.1, 146.0 (2), 145.9, 141.2, 141.1, 140.9, 138.3, 138.2, 138.1, 138.0, 137.6, 137.5 (2), 137.0 (2), 136.9 (2), 136.8 (3), 135.7, 131.9, 131.7, 131.5, 131.3, 128.1 (2), 128.0, 127.9 (2), 127.8, 126.5, 126.4, 126.3, 126.2, 126.1 (2), 125.8, 125.7, 124.8, 124.6, 119.8 (2), 119.7, 119.6, 69.7, 68.3, 61.9, 61.8, 38.5 (3), 31.2, 27.3, 27.0, 26.8, 26.1, 26.0, 24.6, 24.3, 23.9, 23.6, 22.9, 22.2 (2), 21.9 (2), 15.4, 15.3.^{19}F NMR (565 MHz, dDMF) δ (ppm) = -109.2 (m, 4.0 F, *p/ap*), -131.1 (m, 2.0 F, *p/ap*).

ESI-MS: positive mode, *m/z* 1709.3669 $[M+Na]^+$ (found), 1709.3689 (calculated for $C_{73}H_{80}F_6N_4NaO_{27}S_4^+$, $[M+Na]^+$).

NMR spectra of compound A´

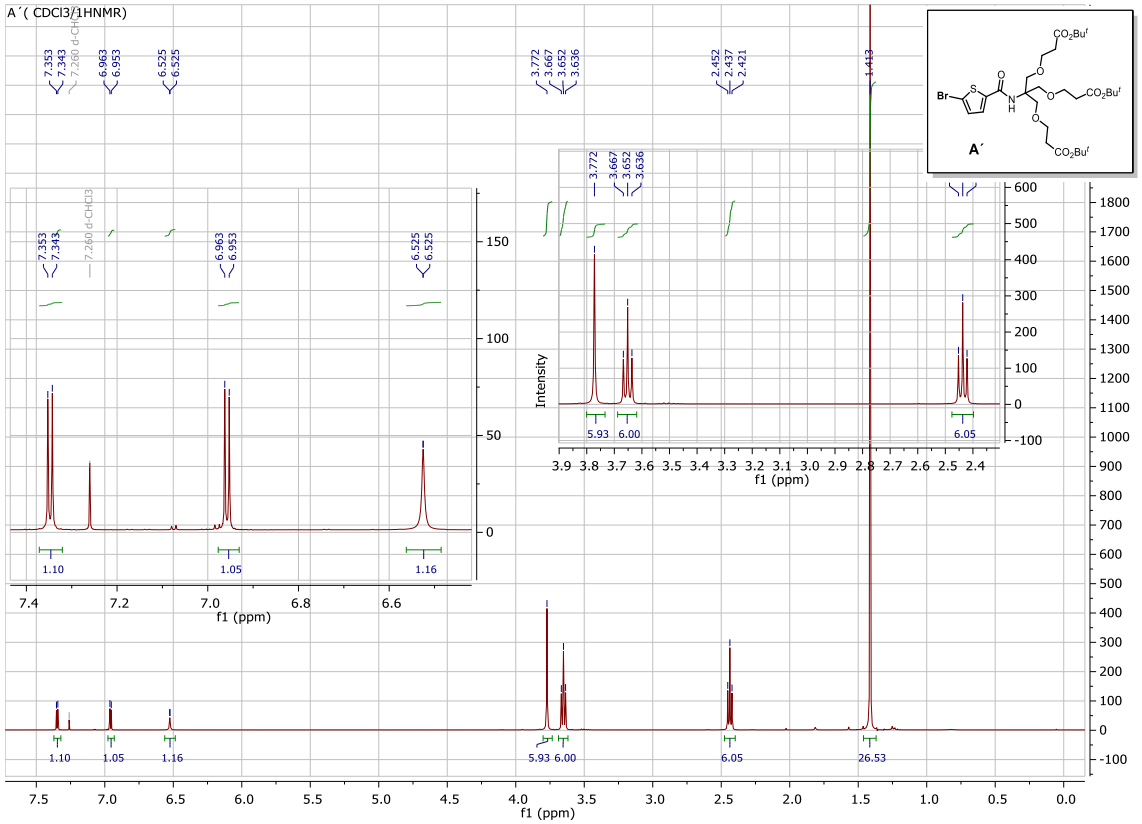


Figure S1a. ^{1}H-NMR (400 MHz) spectrum of compound **A'** in $CDCl_3$

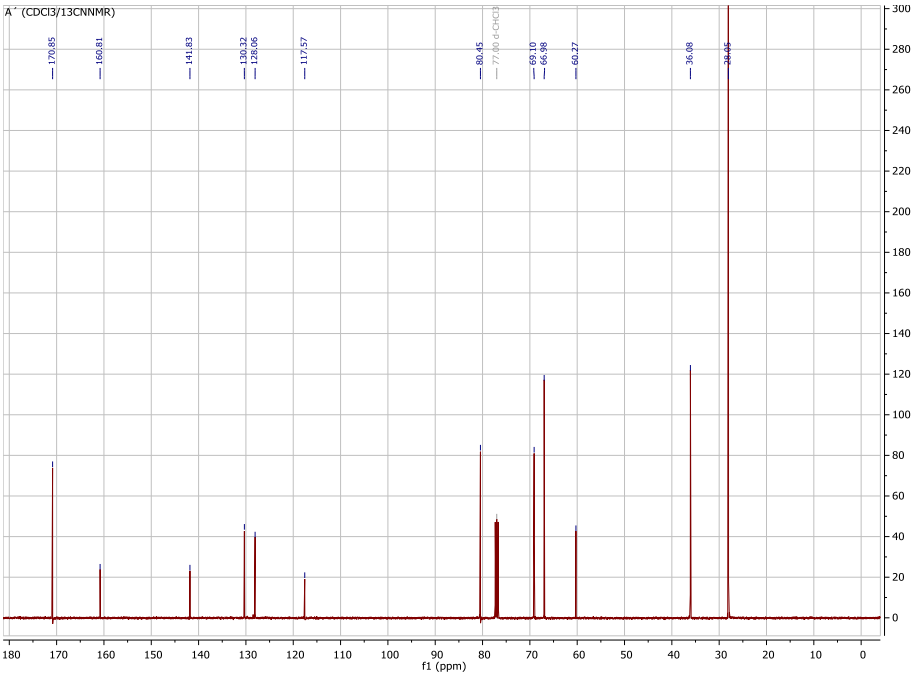


Figure S1b. ^{13}C-NMR (101 MHz) spectrum of compound **A'** in $CDCl_3$

NMR spectra of compound A

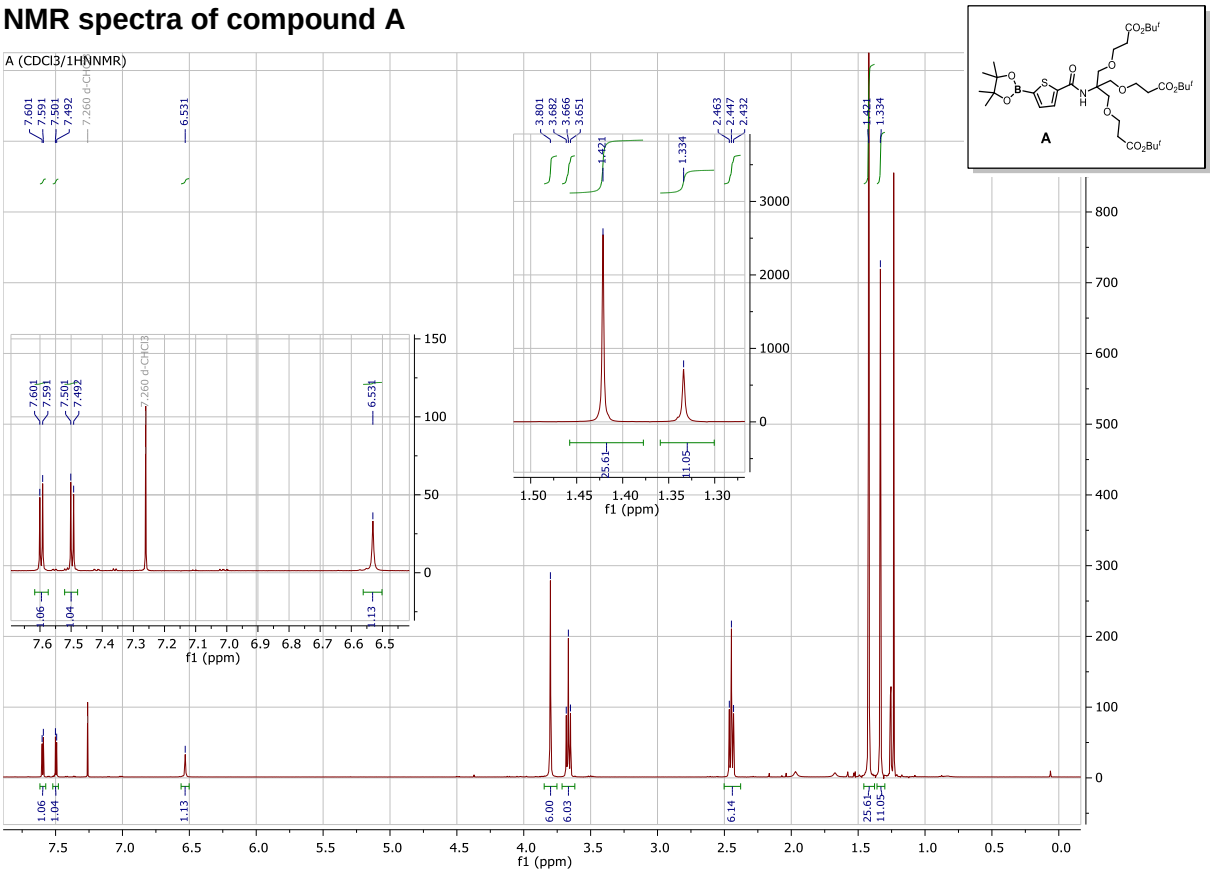


Figure S2a. ^{1}H-NMR (400 MHz) spectrum of **A** in $CDCl_3$

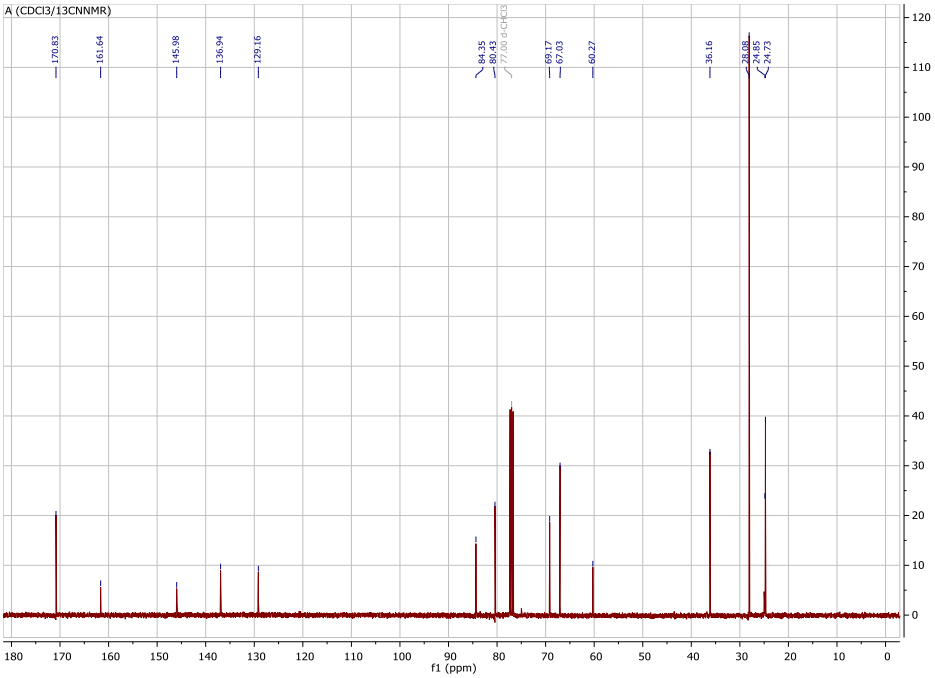


Figure S2b. ^{13}C-NMR (101 MHz) spectrum of **A** in $CDCl_3$

NMR spectra of fDAE1

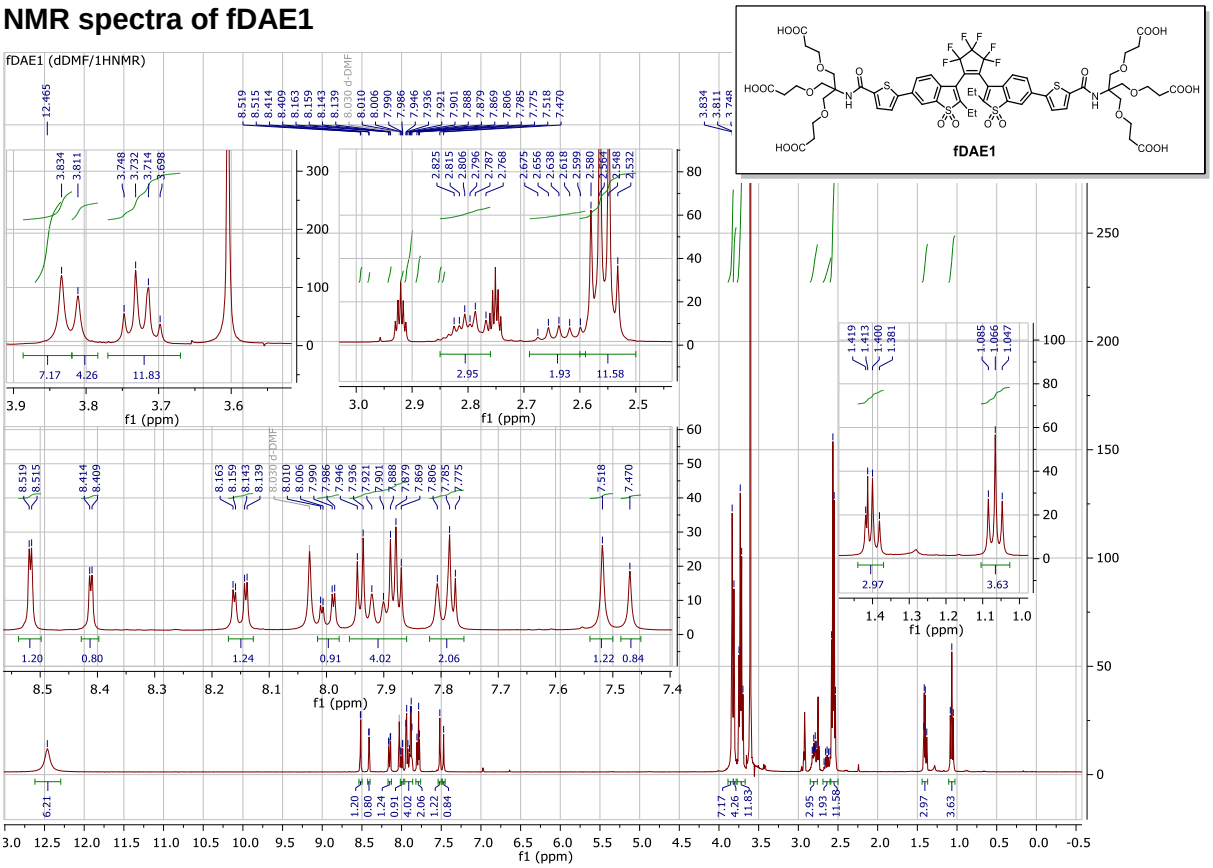


Figure S3a. ^{1}H-NMR (400 MHz) spectrum of **fDAE1** in [D_7]DMF

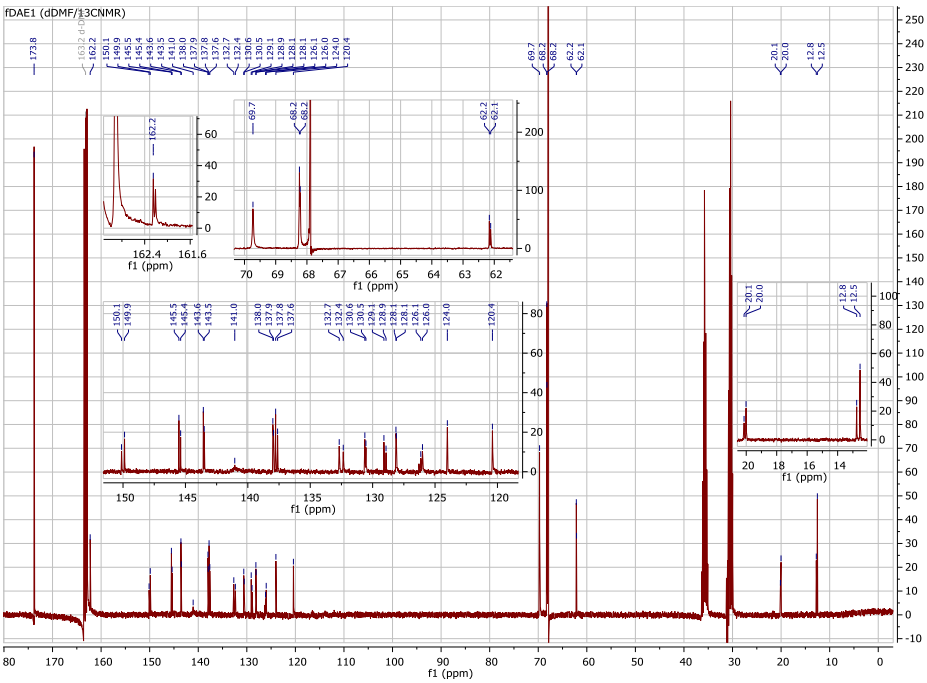


Figure S3b. ^{13}C-NMR (101 MHz) spectrum of **fDAE1** in [D_7]DMF

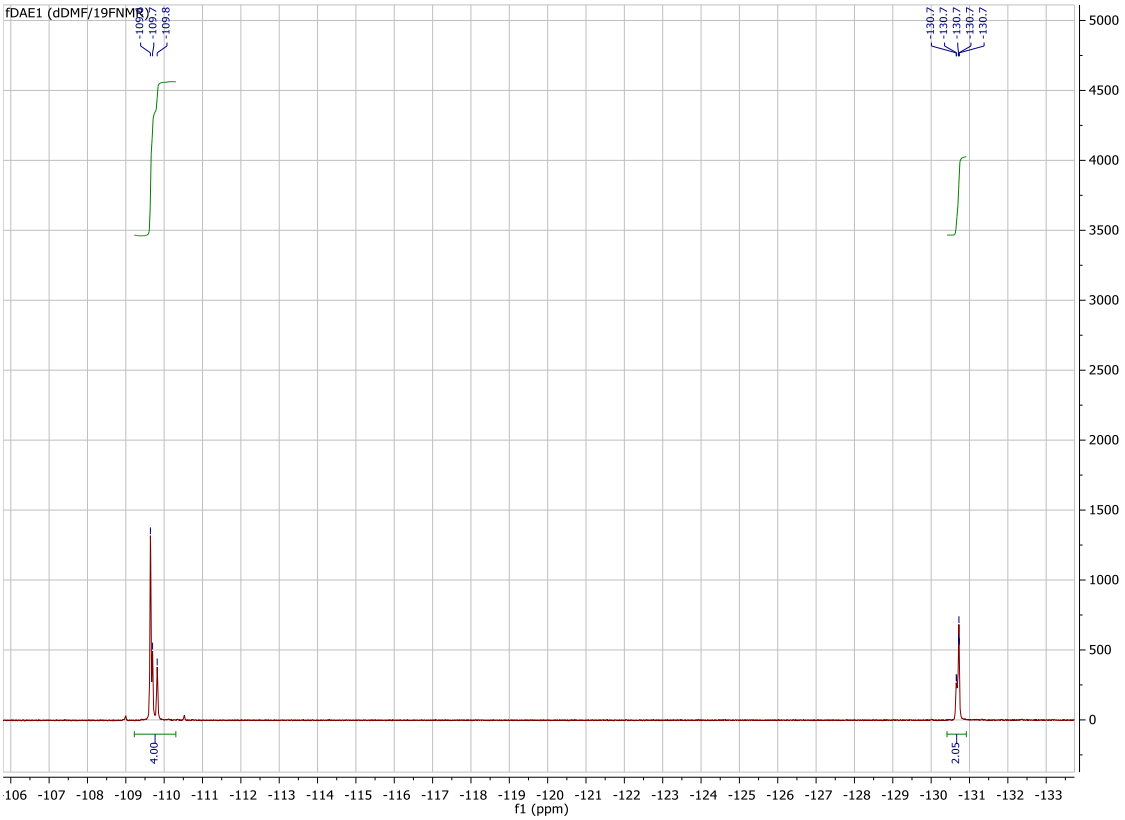


Figure S3c. ^{19}F-NMR (367 MHz) spectrum of **fDAE1** in [D_7]DMF

NMR spectra of fDAE2

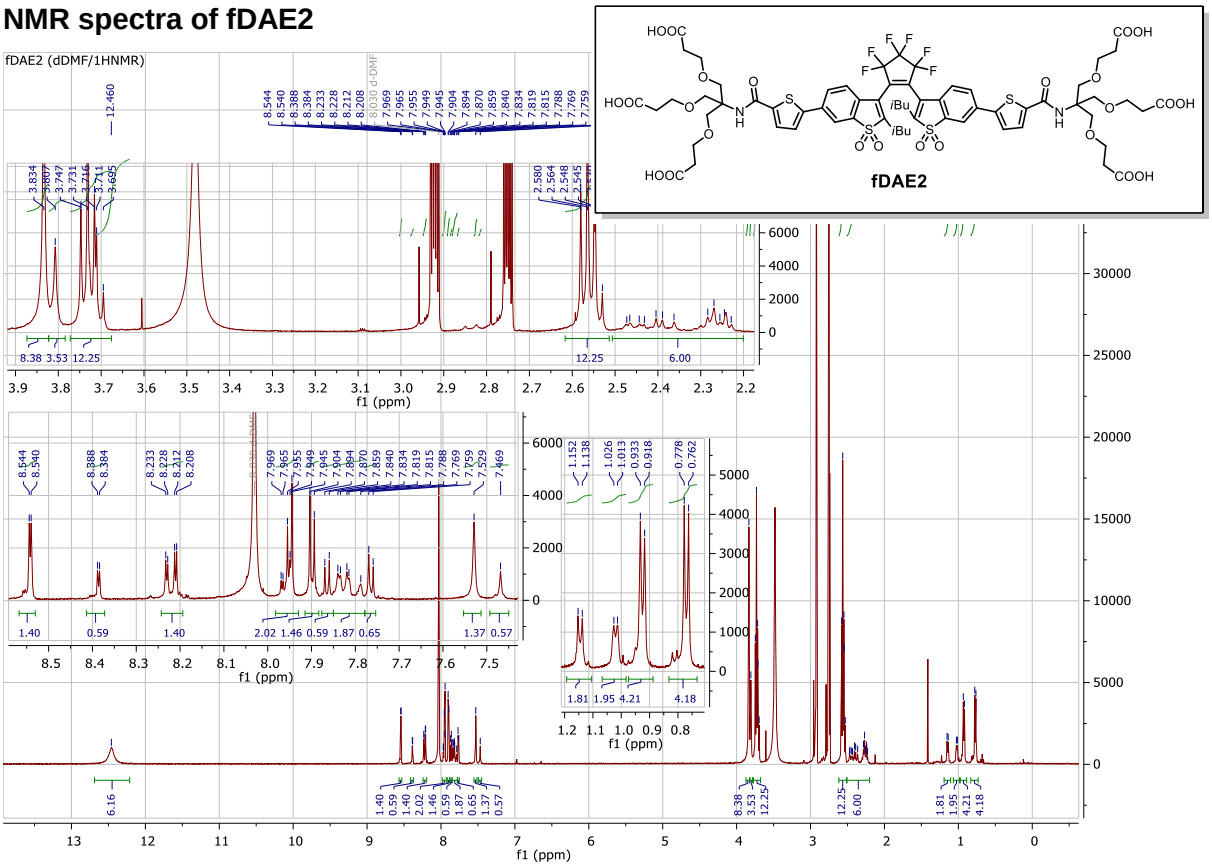


Figure S4a. ^{1}H-NMR (400 MHz) spectrum of **fDAE2** in [D_7]DMF

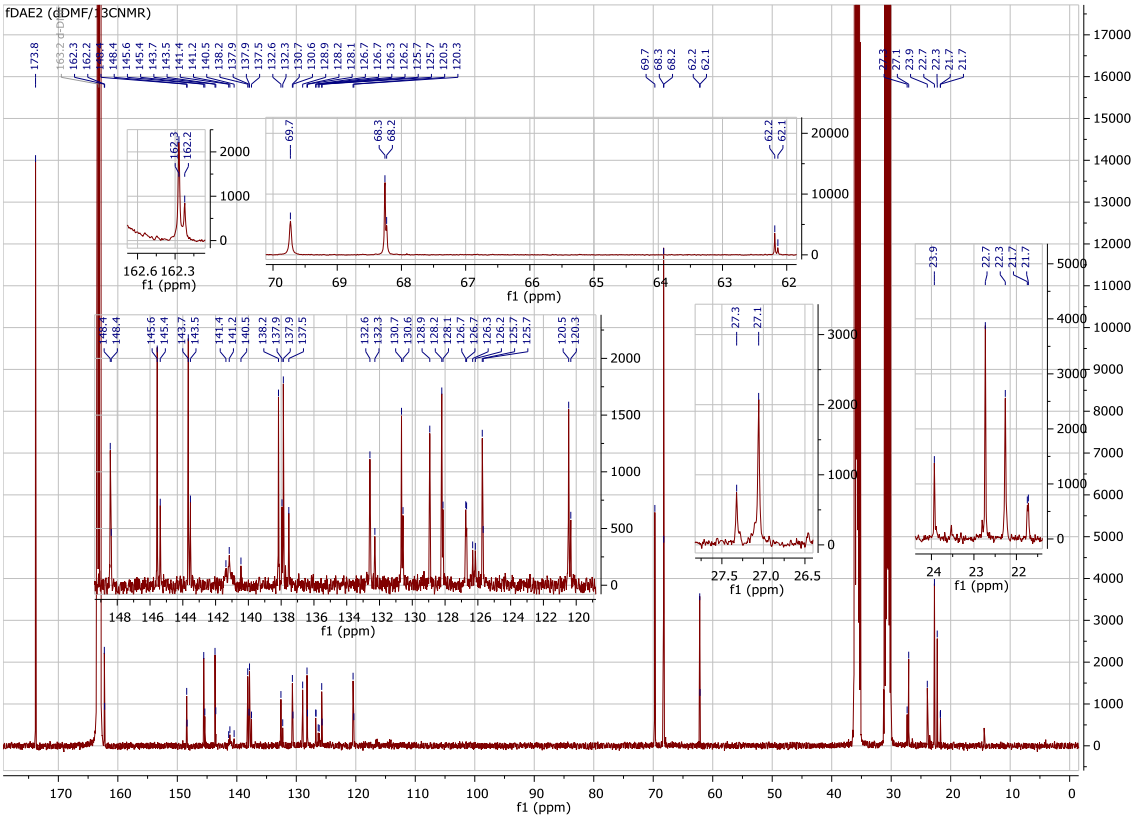


Figure S4b. ^{13}C-NMR (101 MHz) spectrum of **fDAE2** in [D_7]DMF

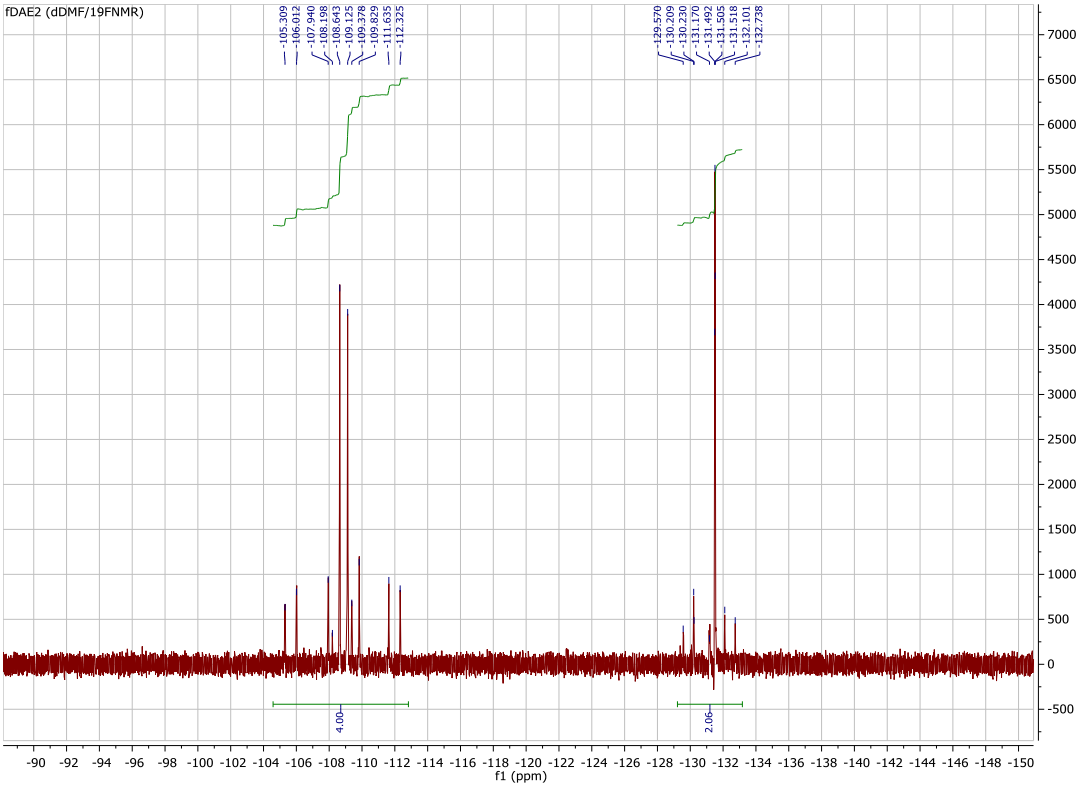


Figure S4c. ^{19}F-NMR (367 MHz) spectrum of **fDAE2** in [D_7]DMF

NMR spectra of compound B´

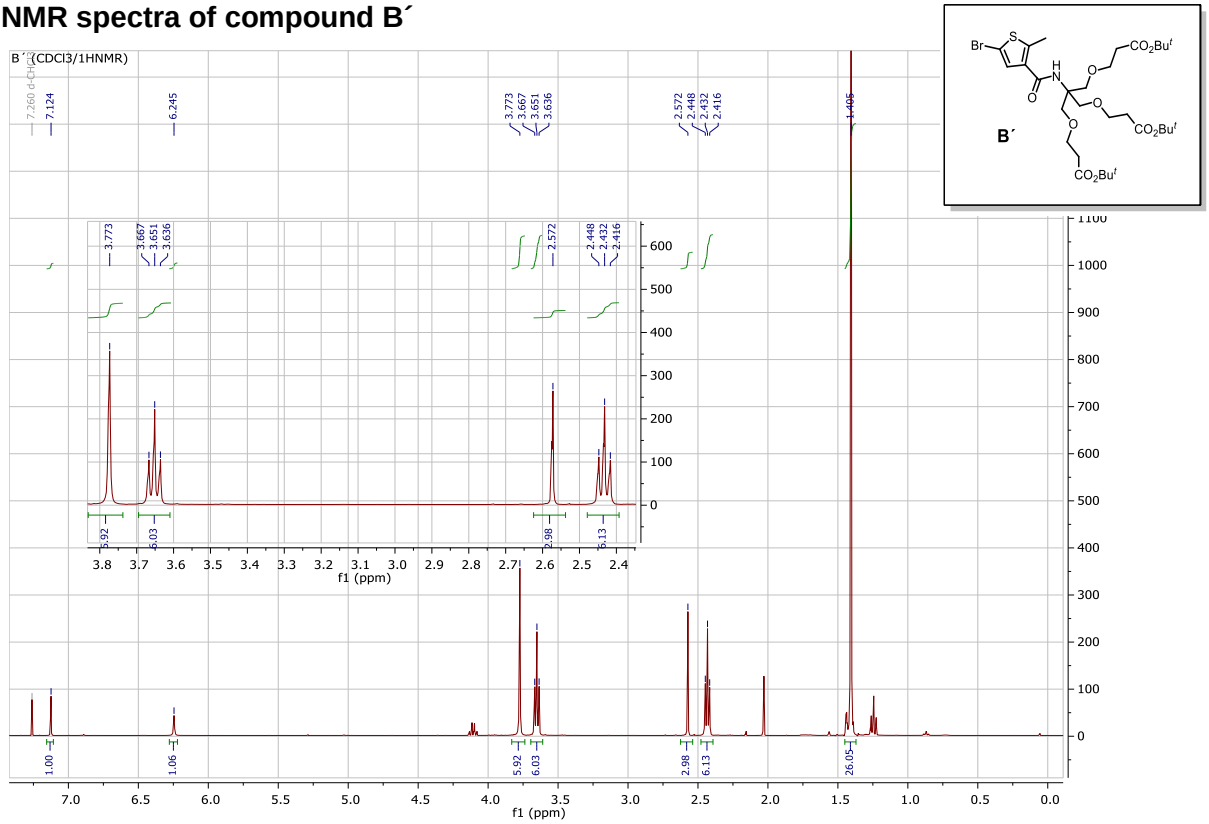


Figure S5a. ^{1}H-NMR (400 MHz) spectrum of **B´** in $CDCl_3$

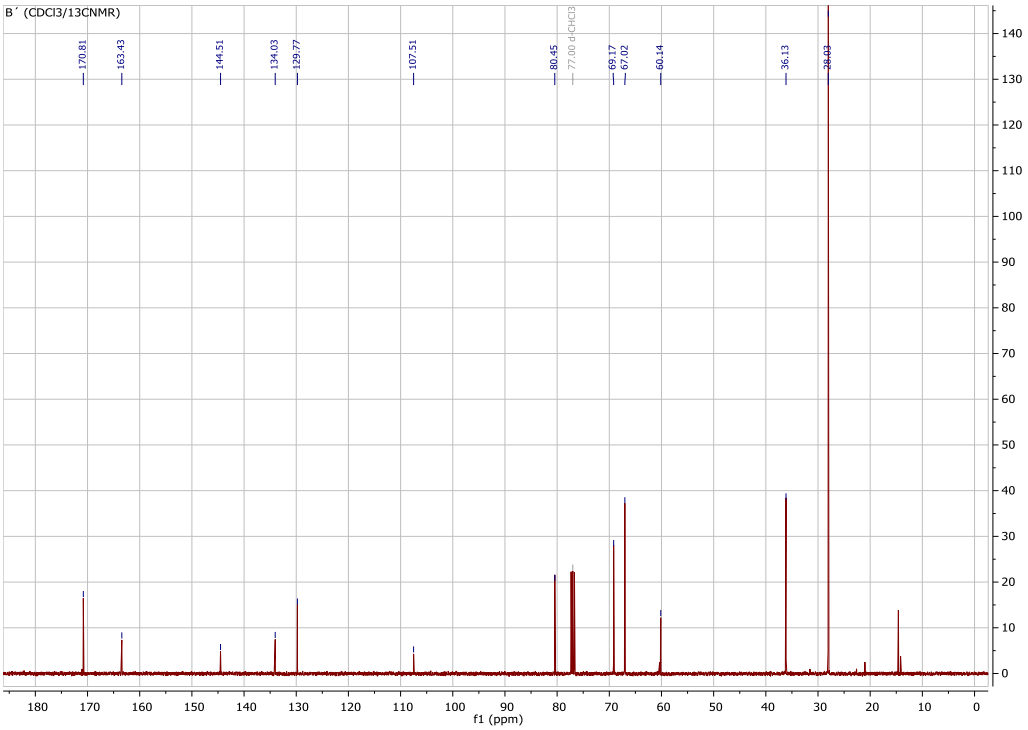


Figure S5b. ^{13}C-NMR (101 MHz) spectrum of **B´** in $CDCl_3$

NMR spectra of compound B

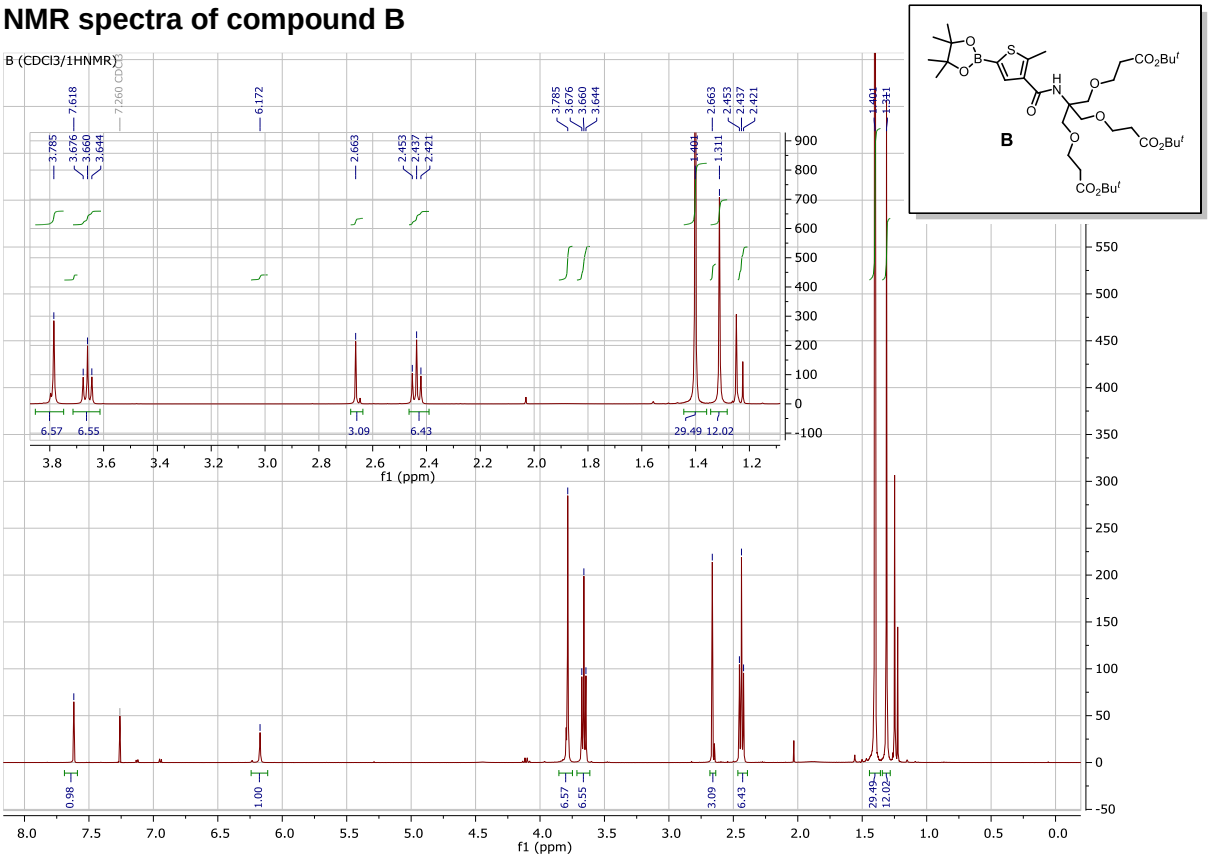


Figure S6a. ^{1}H-NMR (400 MHz) spectrum of **B** in $CDCl_3$

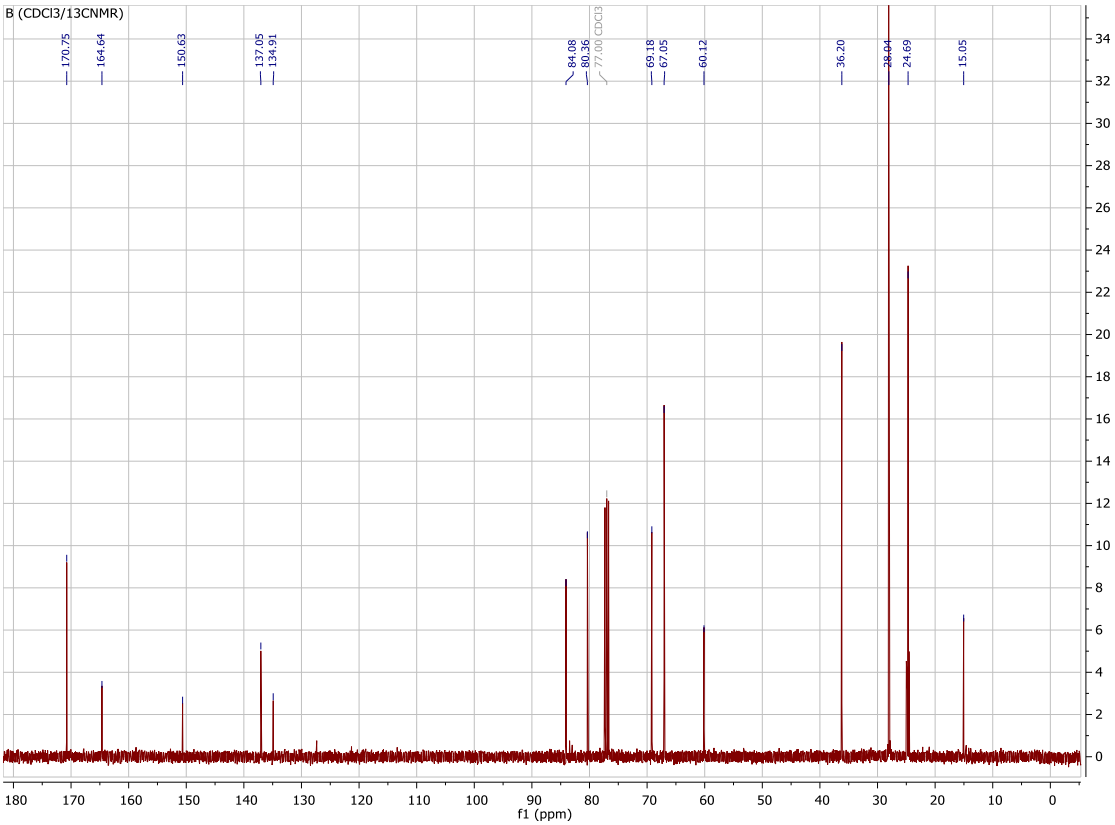


Figure S6b. ^{13}C-NMR (101 MHz) spectrum of **B** in $CDCl_3$

NMR spectra of D

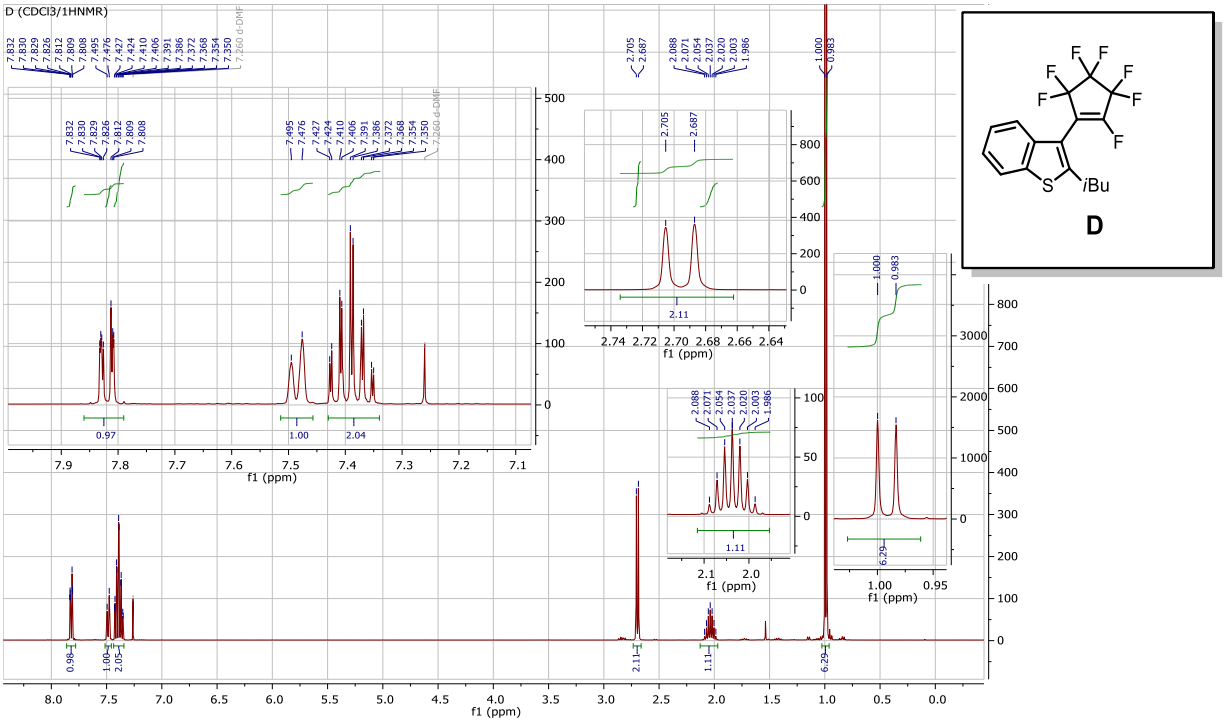


Figure S7a. ^{1}H-NMR (400 MHz) spectrum of **D** in $CDCl_3$

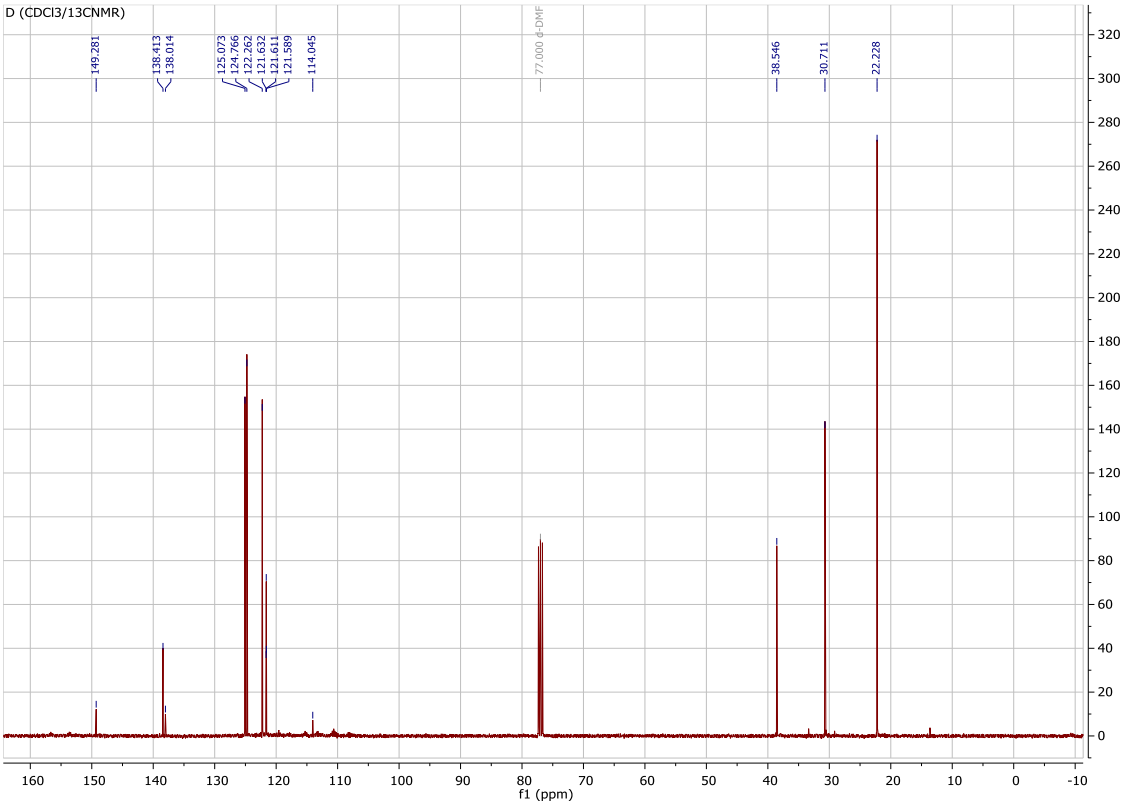


Figure S7b. ^{13}C-NMR (101 MHz) spectrum of **D** in $CDCl_3$

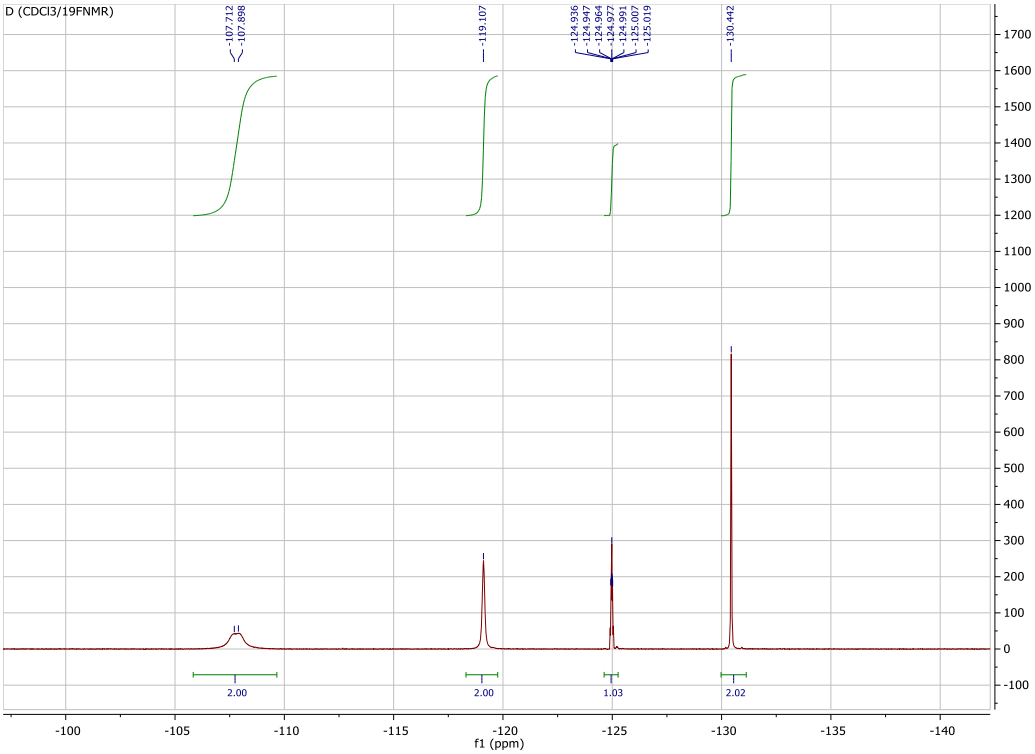


Figure S7c. ^{19}F-NMR (376 MHz) spectrum of **D** in $CDCl_3$

NMR spectra of fDAE3

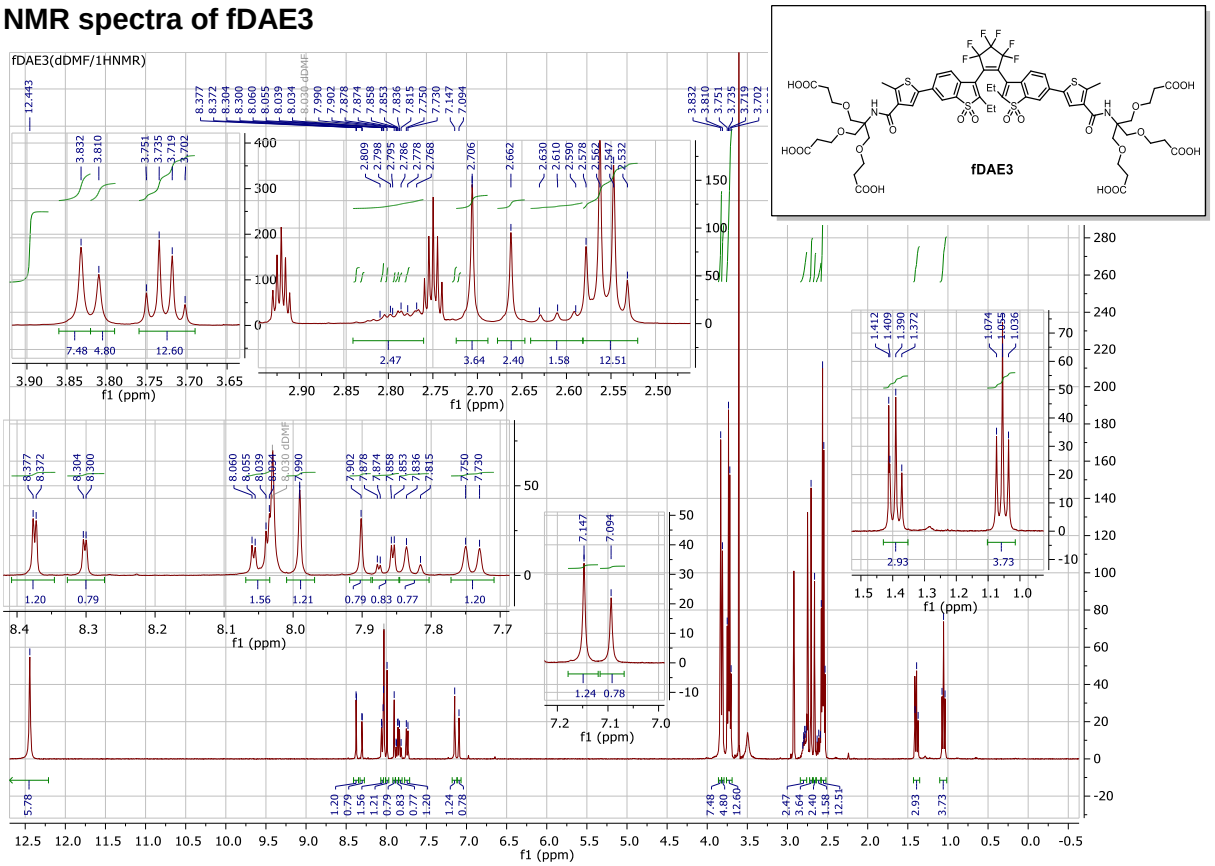


Figure S8a. ^{1}H-NMR (400 MHz) spectrum of **fDAE3**in [D_7]DMF

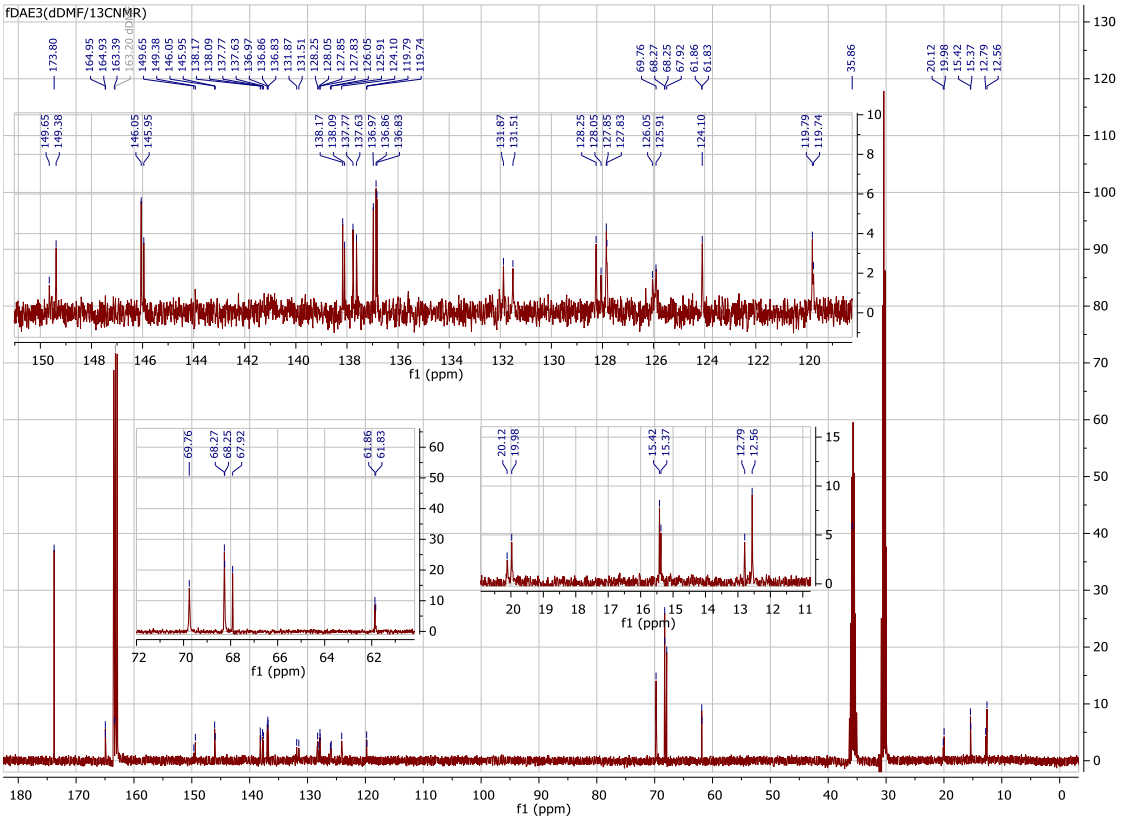


Figure S8b. ^{13}C-NMR (101 MHz) spectrum of **fDAE3**in [D_7]DMF

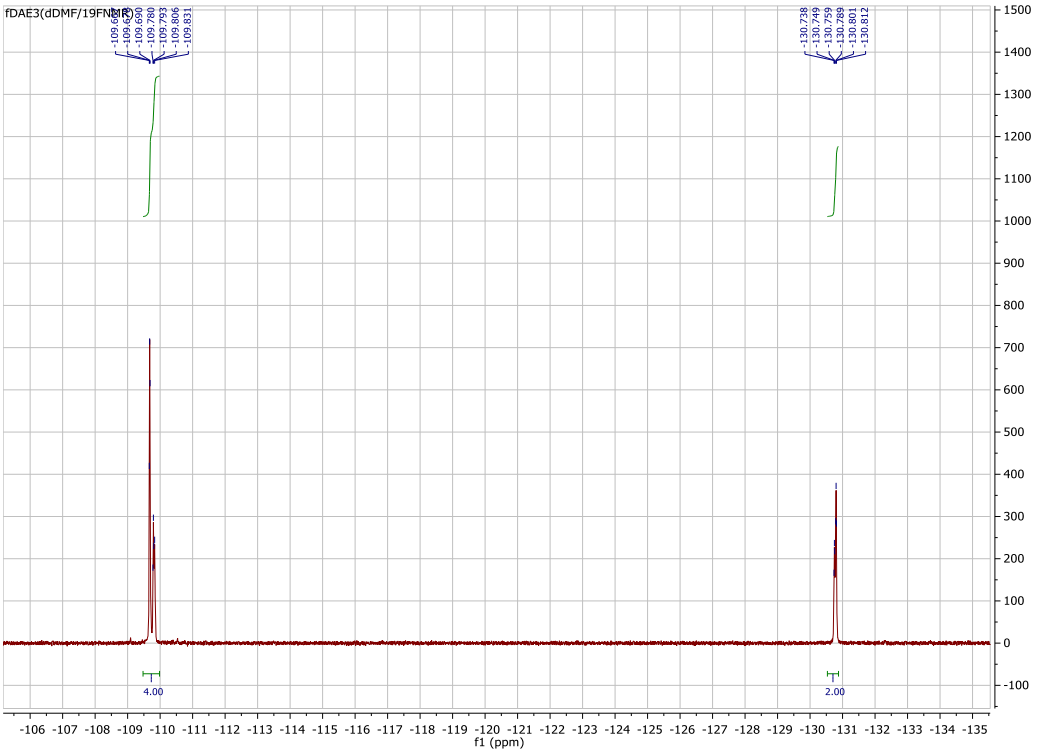


Figure S8c. ^{19}F-NMR (367 MHz) spectrum of **fDAE3** in [D_7]DMF

NMR spectra of fDAE4

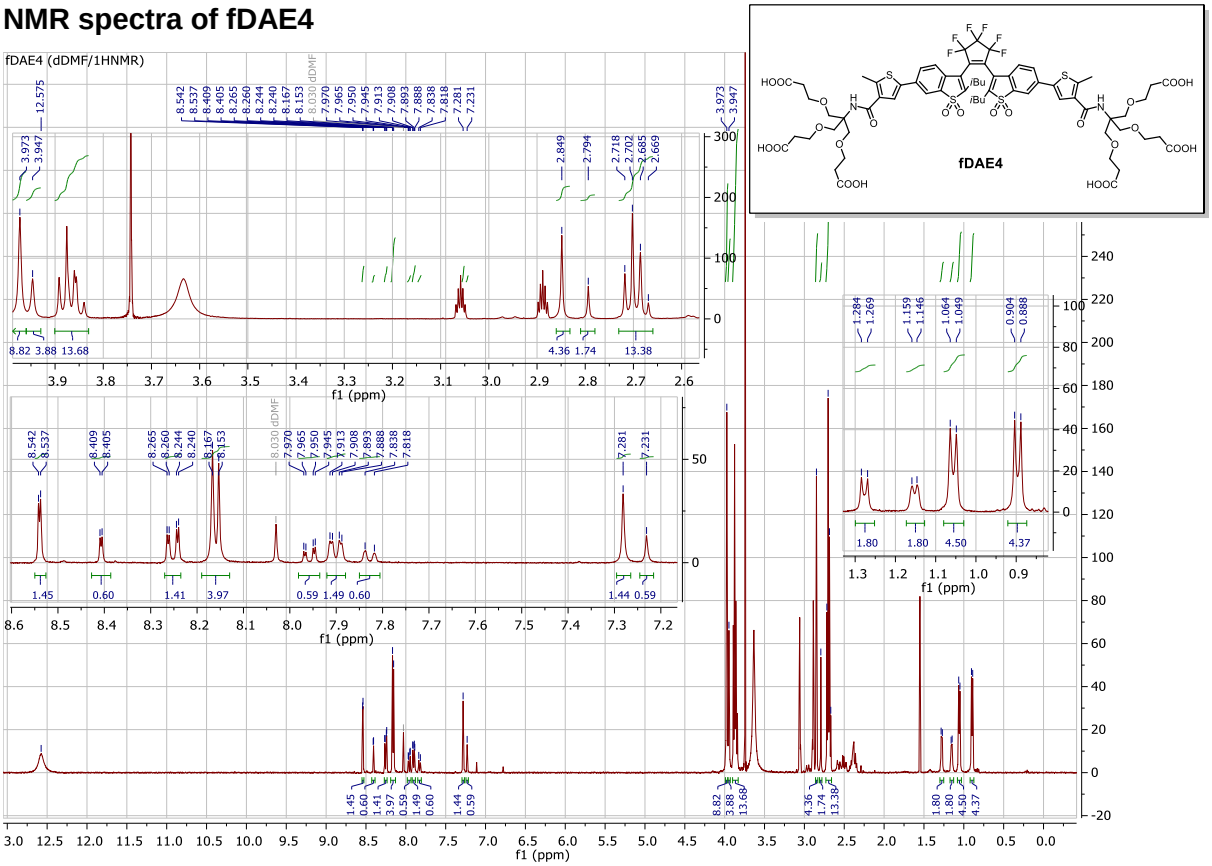


Figure S9a. ^{1}H-NMR (400 MHz) spectrum of **fDAE4**in [D_7]DMF

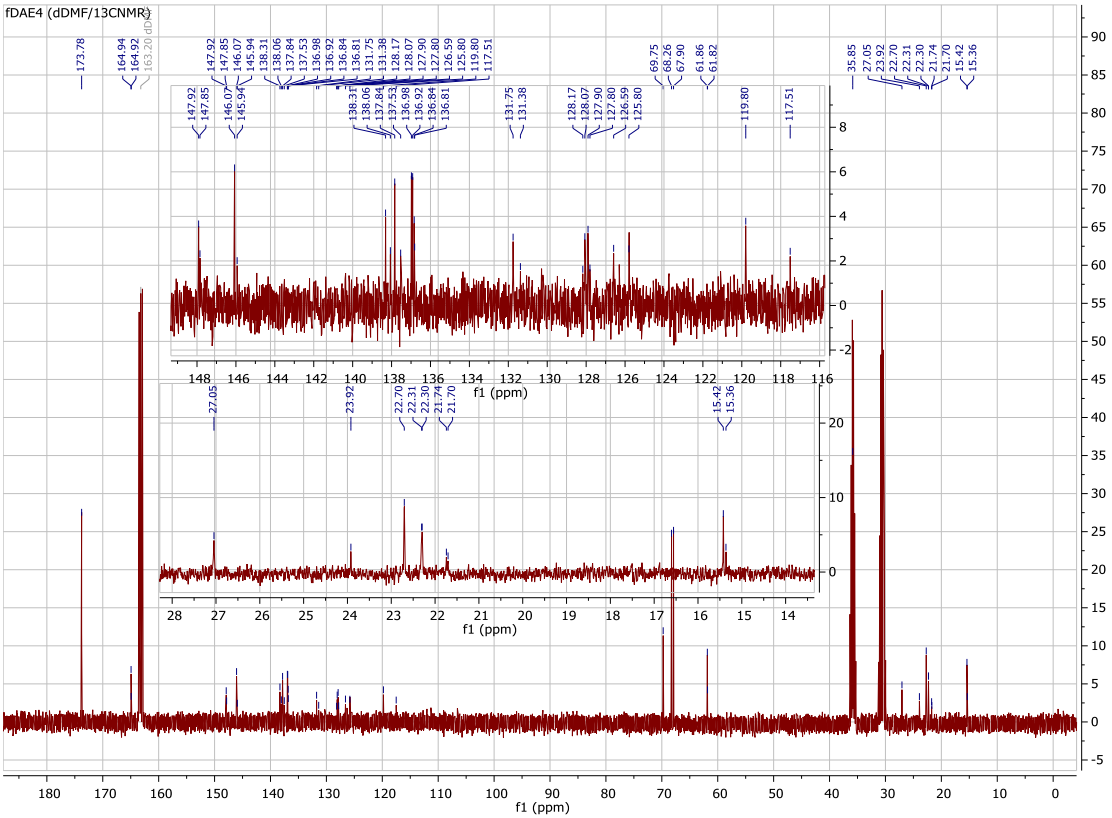


Figure S9b. ^{13}C-NMR (101 MHz) spectrum of **fDAE4** in [D_7]DMF

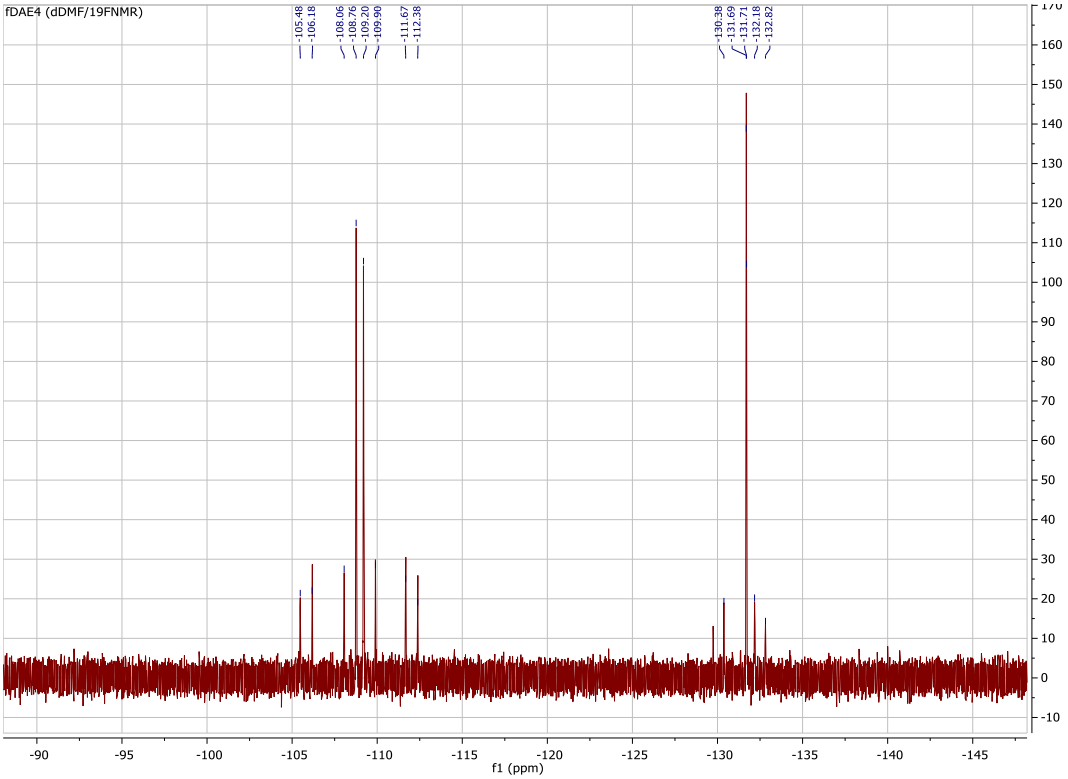


Figure S9c. ^{19}F-NMR (367 MHz) spectrum of **fDAE4** in [D_7]DMF

NMR spectra of a´

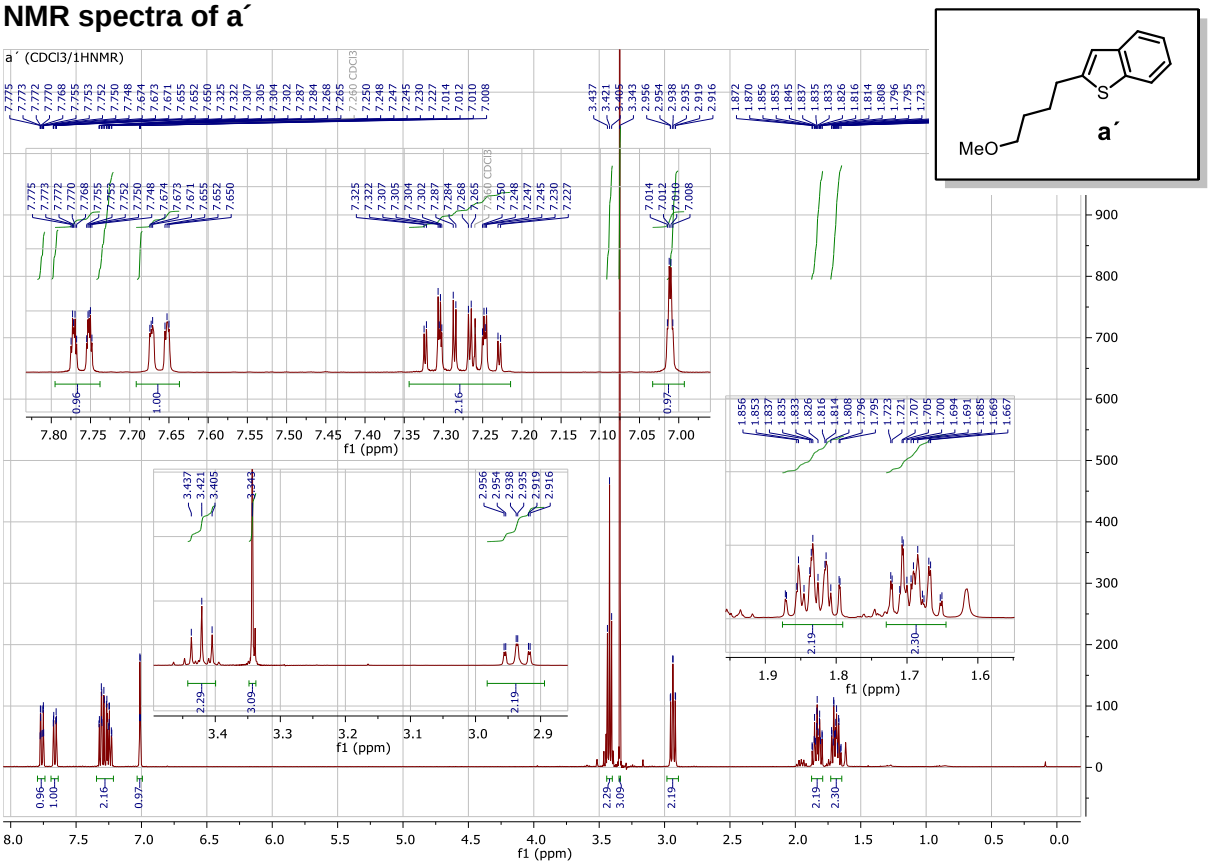


Figure S10a. ^{1}H-NMR (400 MHz) spectrum of **a´** in $CDCl_3$

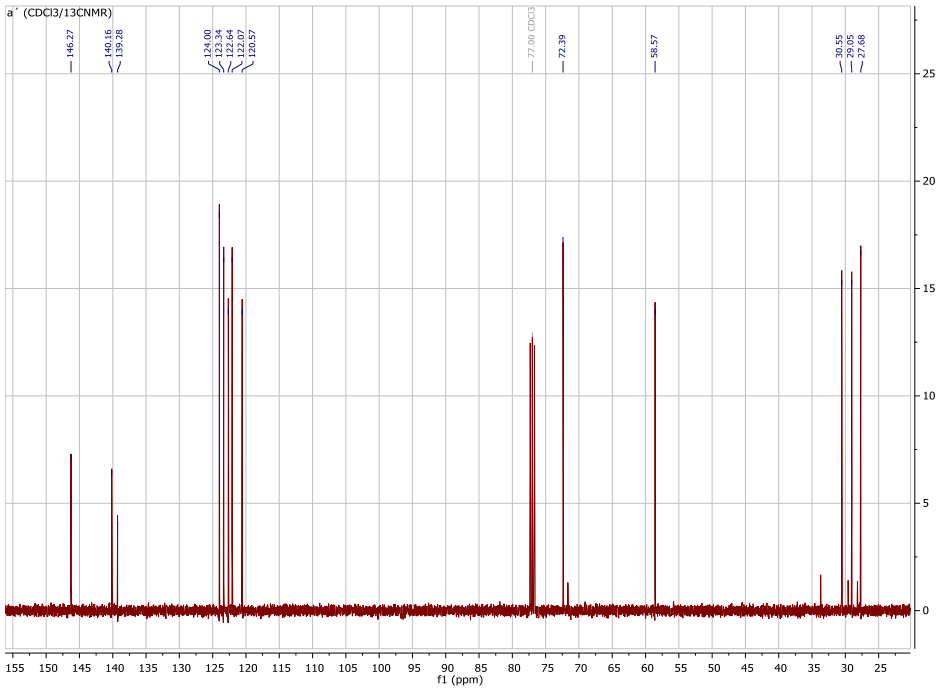


Figure S10b. ^{13}C-NMR (101 MHz) spectrum of **a´** in $CDCl_3$

NMR spectra of a

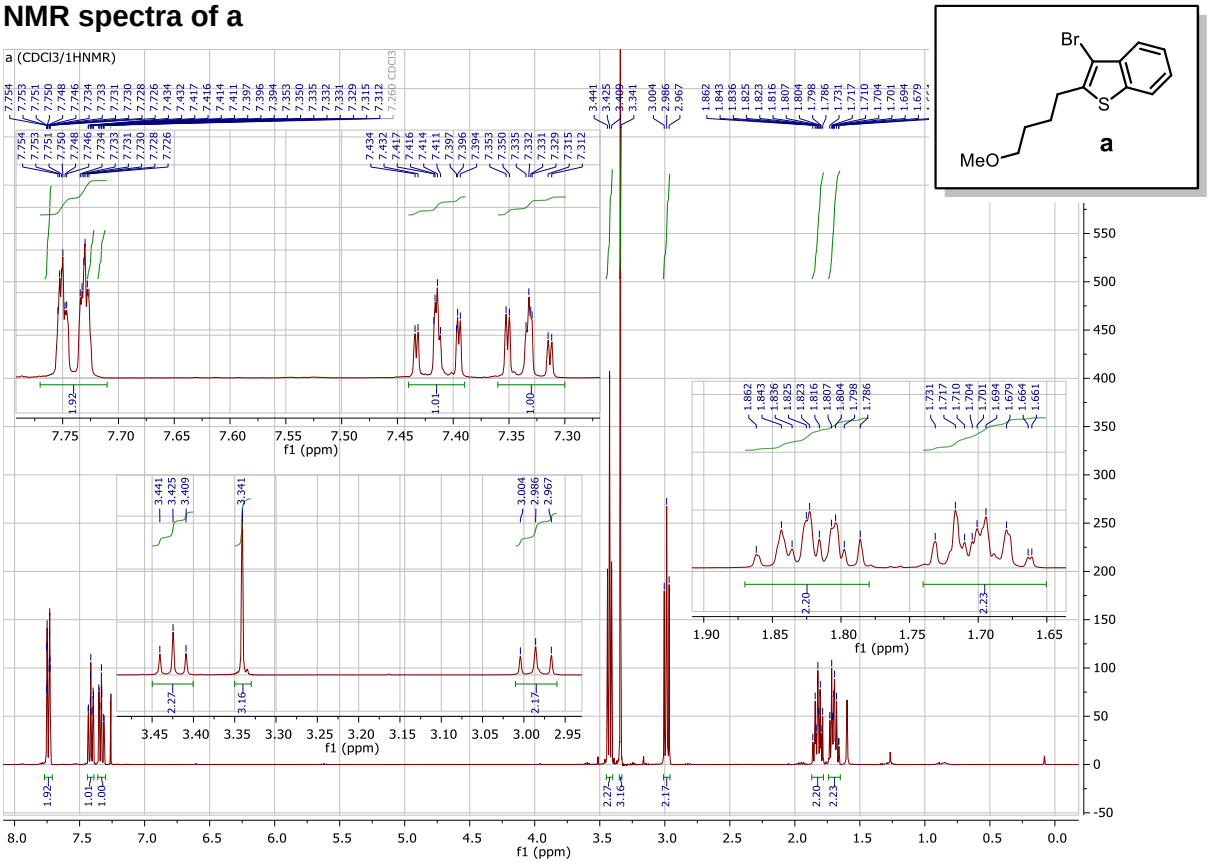


Figure S11a. ^{1}H-NMR (400 MHz) spectrum of **a** in $CDCl_3$

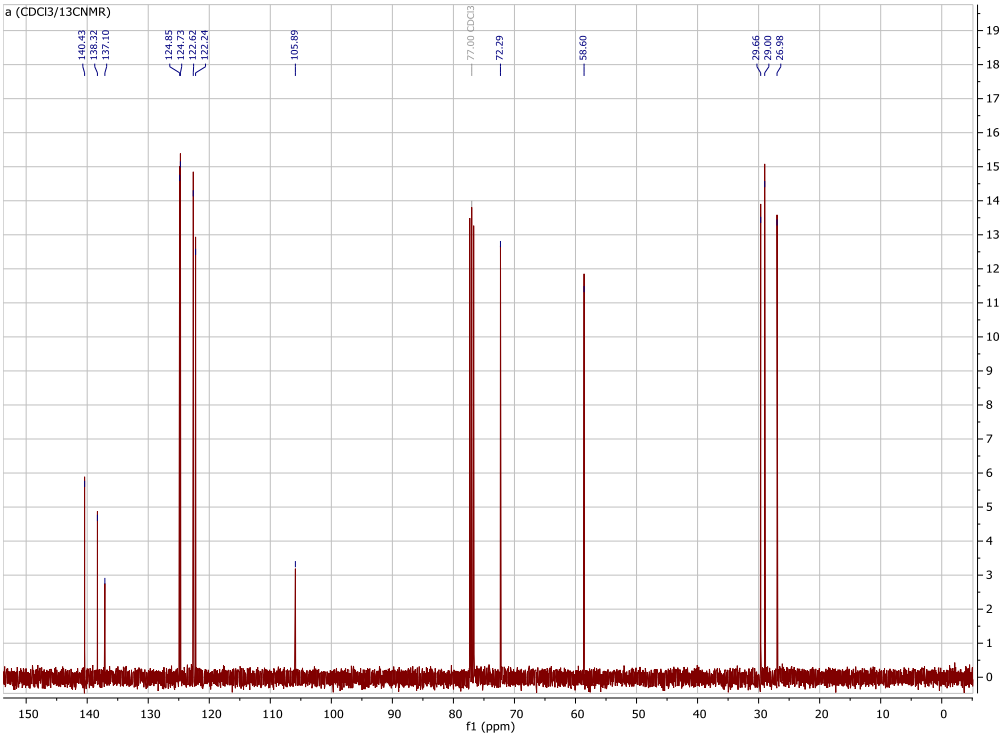


Figure S11b. ^{13}C-NMR (101 MHz) spectrum of **a** in $CDCl_3$

NMR spectra of b(Et)

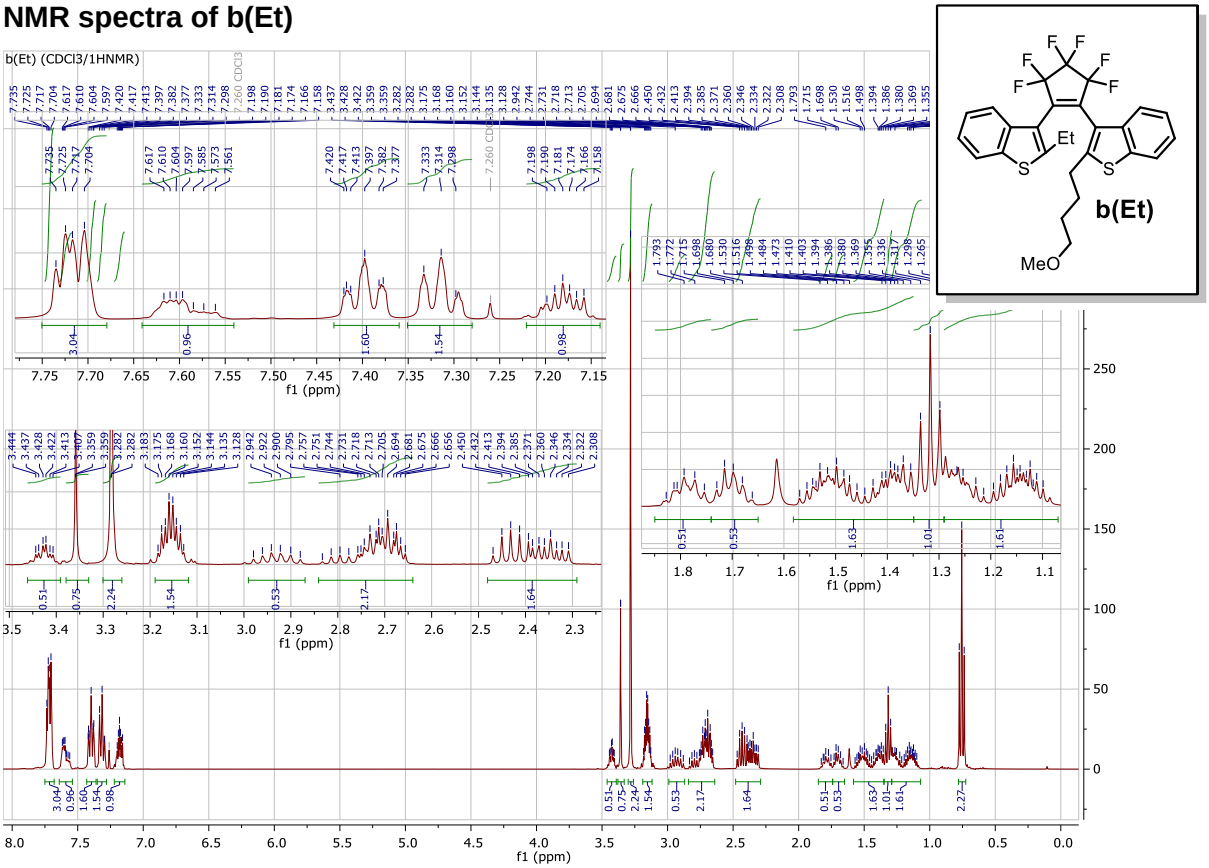


Figure S11a. ^{1}H-NMR (400 MHz) spectrum of **b(Et)** in $CDCl_3$

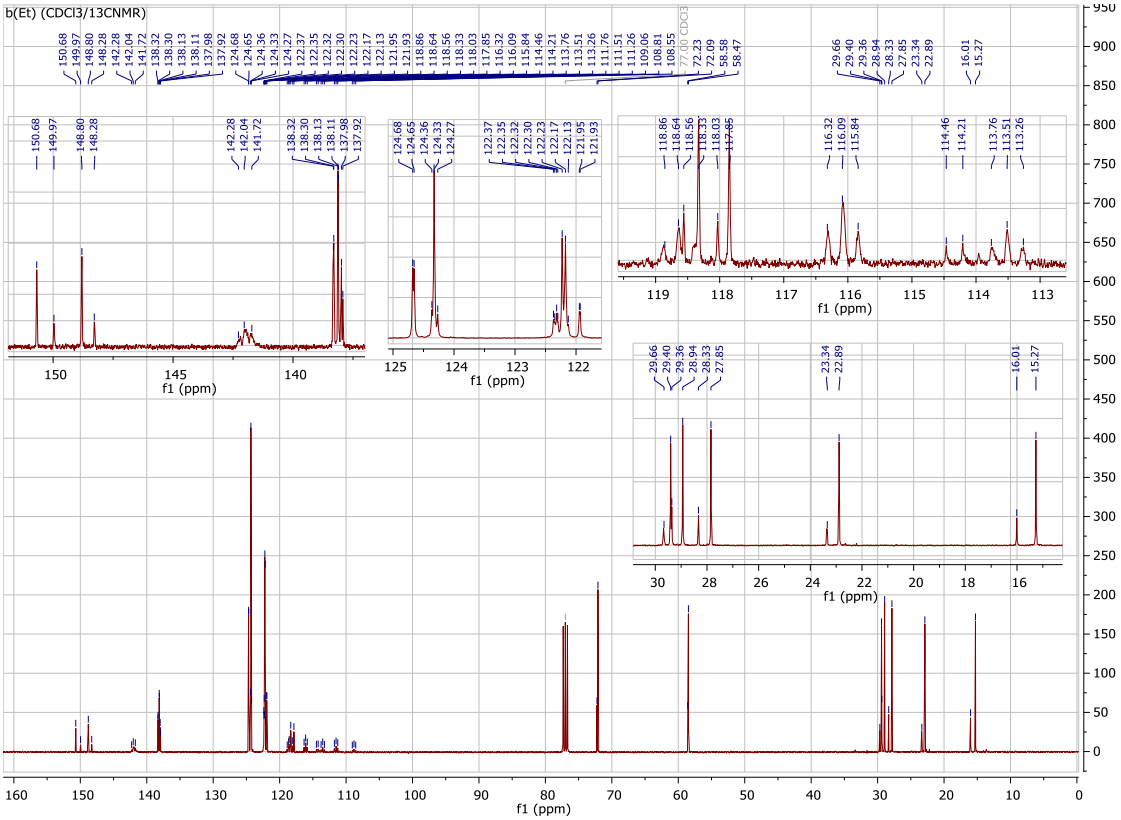


Figure S11b. ^{13}C-NMR (101 MHz) spectrum of **b(Et)** in $CDCl_3$

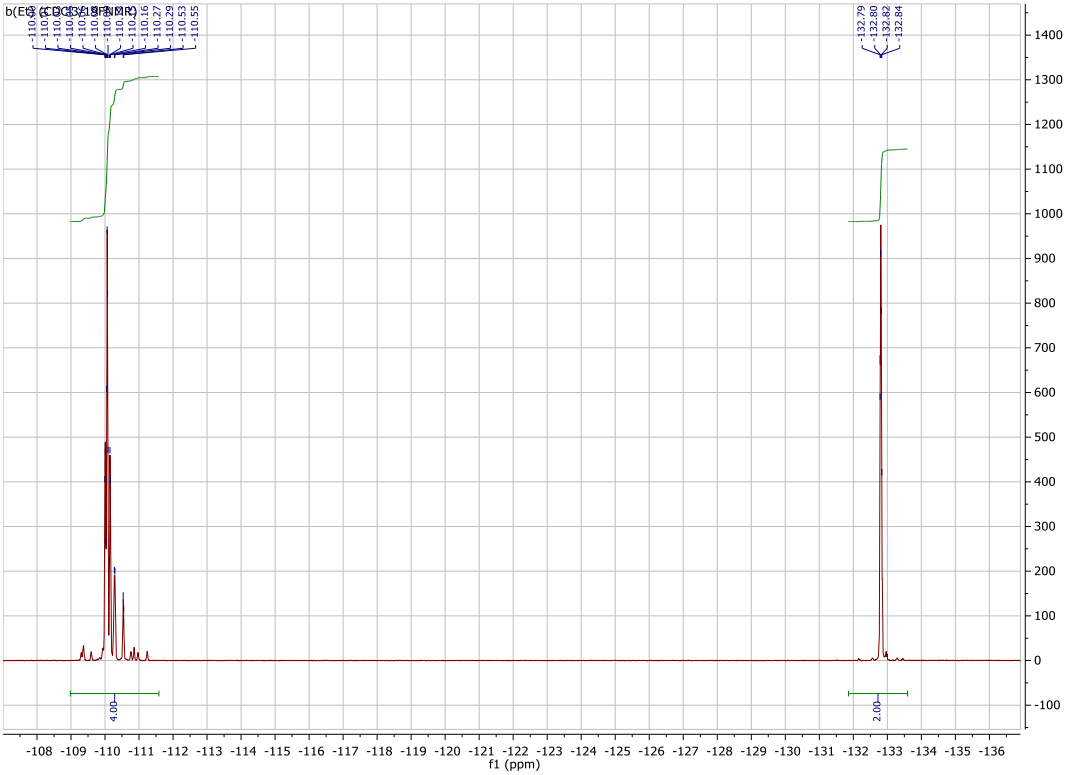


Figure S11c. ^{19}F-NMR (367 MHz) spectrum of **b(Et)** in $CDCl_3$

NMR spectra of c(Et)

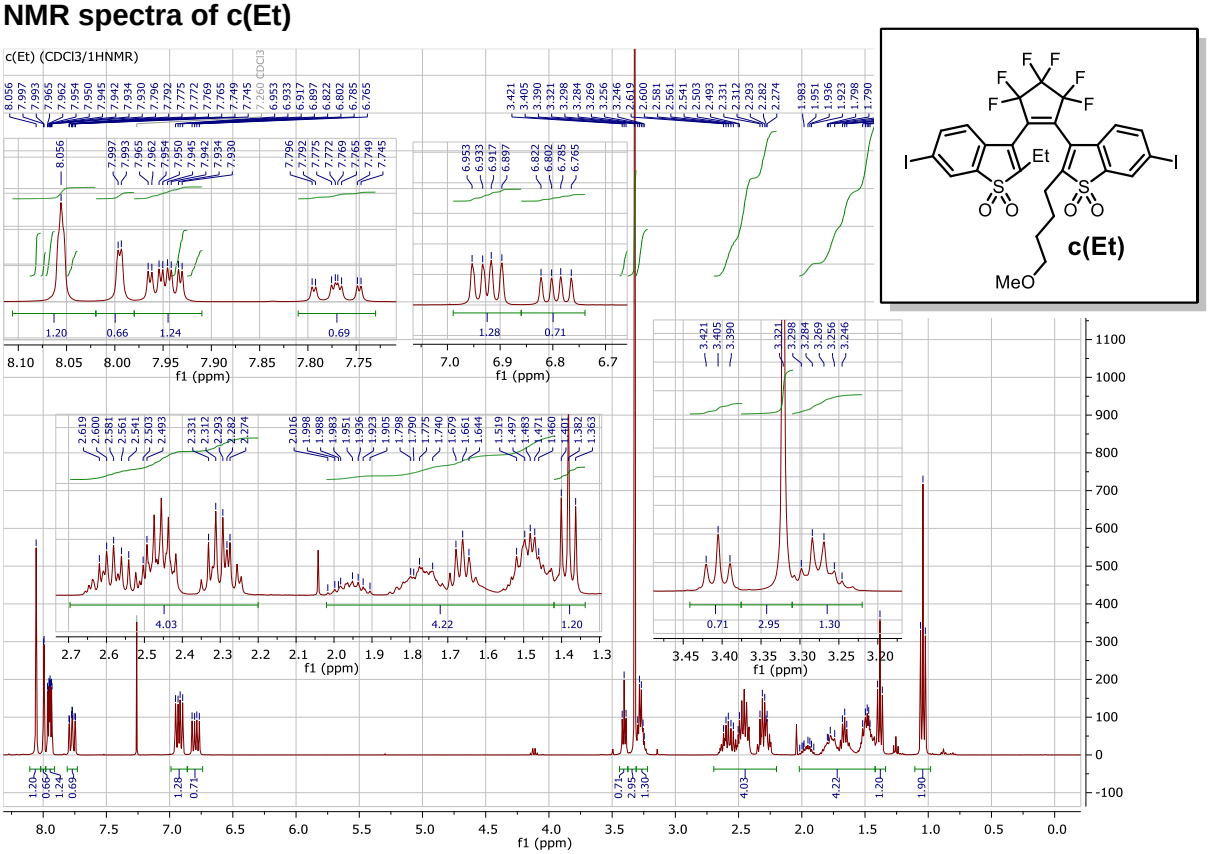


Figure S12a. ^{1}H-NMR (400 MHz) spectrum of **b(Et)** in $CDCl_3$

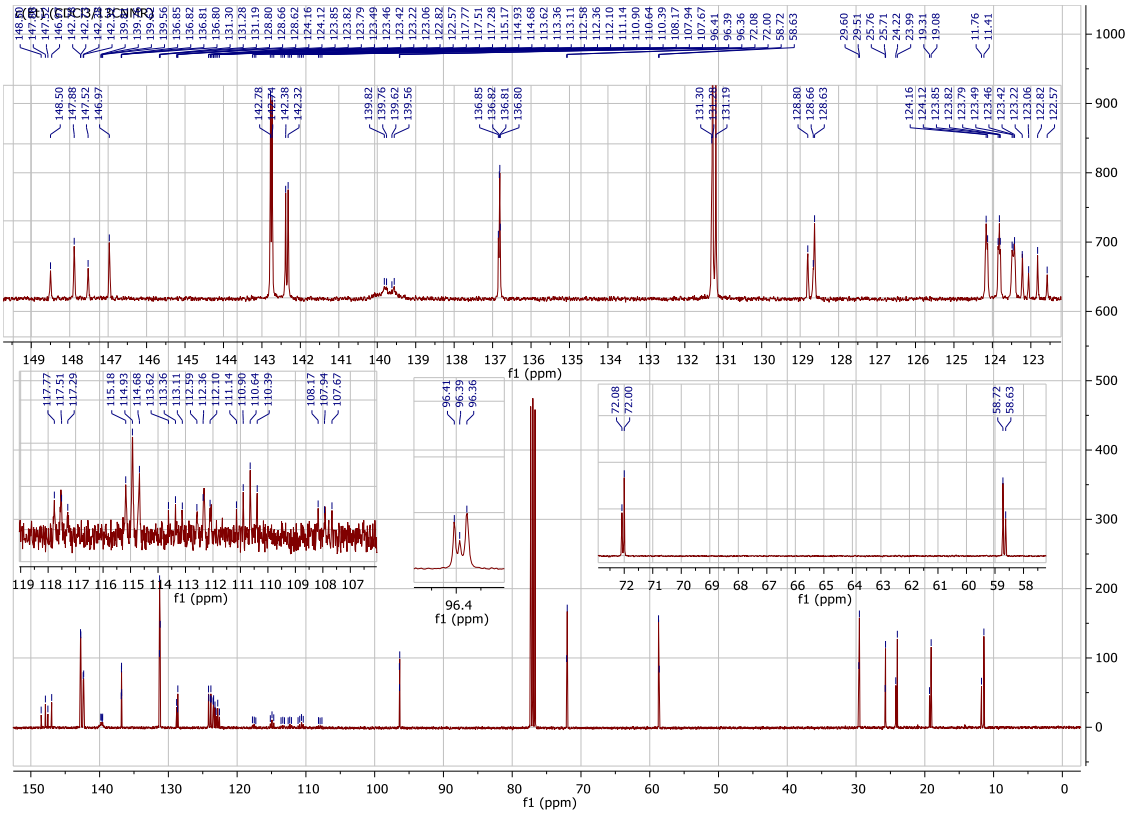


Figure S12b. ^{13}C-NMR (101 MHz) spectrum of **c(Et)** in $CDCl_3$

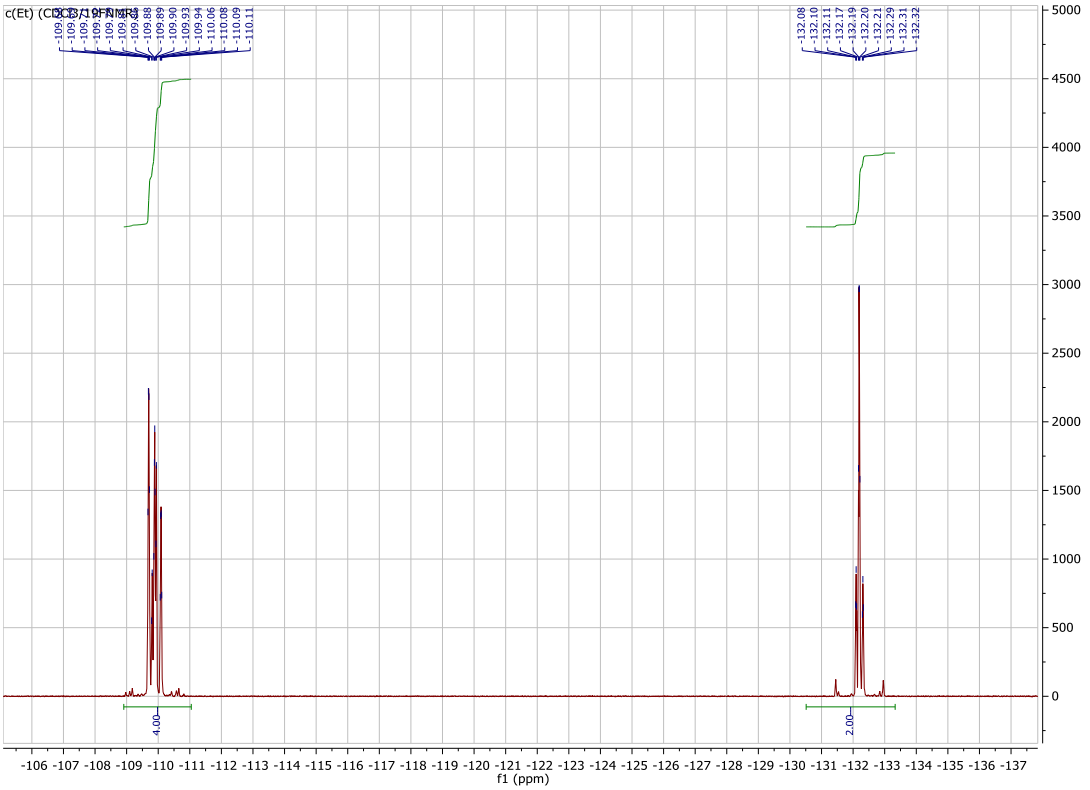


Figure S12c. ^{19}F-NMR (367 MHz) spectrum of **c(Et)** in $CDCl_3$

NMR spectra of d(Et)

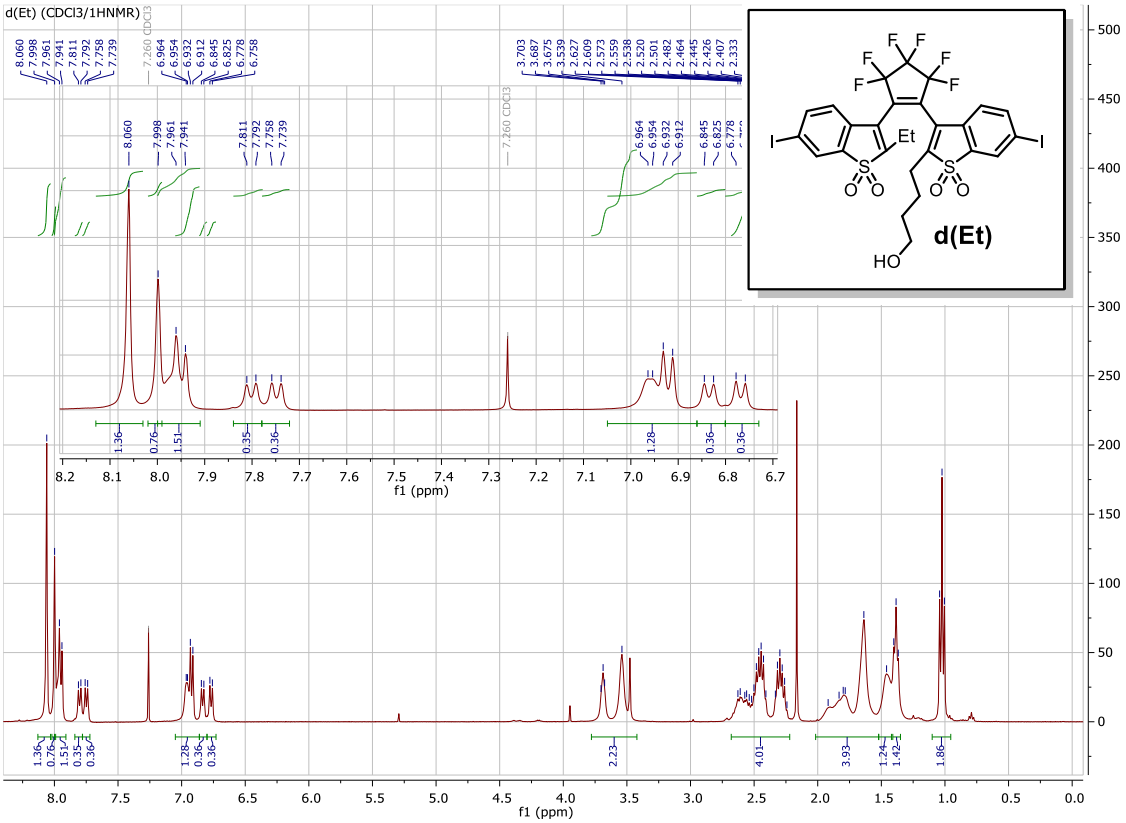


Figure S13a. ^{1}H-NMR (400 MHz) spectrum of **d(Et)** in $CDCl_3$

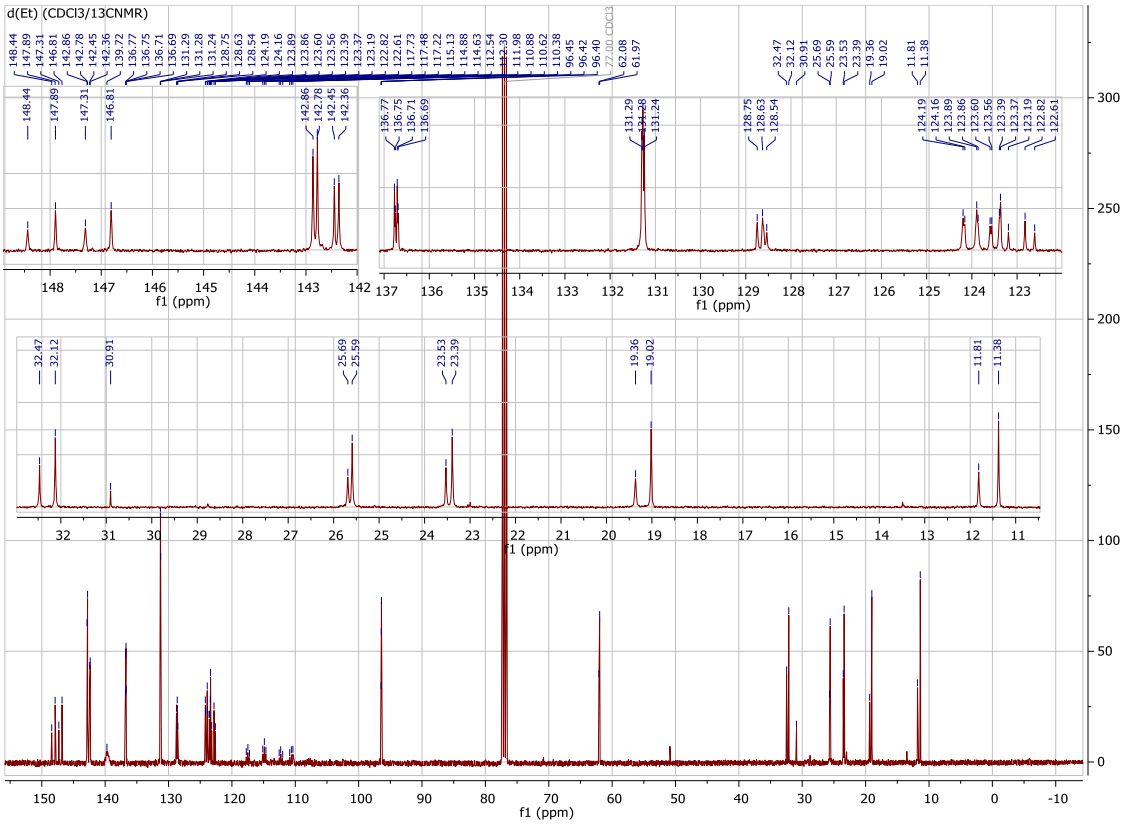


Figure S13b. ^{13}C-NMR (101 MHz) spectrum of **d(Et)** in $CDCl_3$

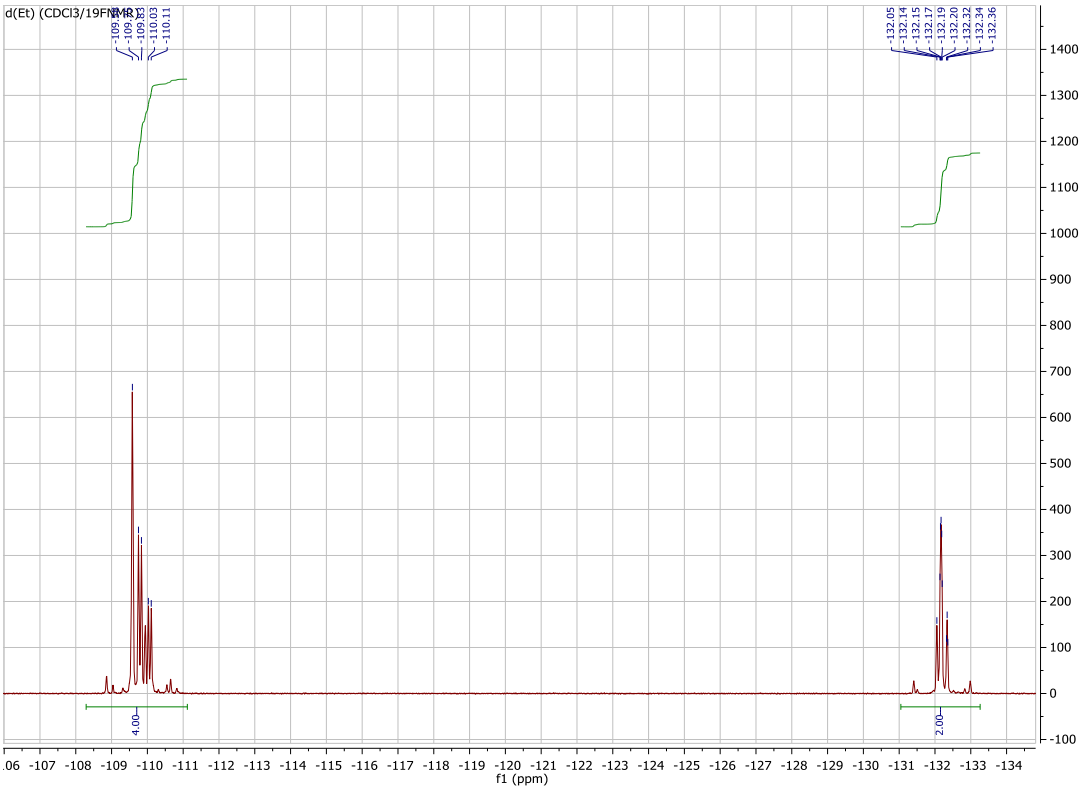


Figure S13c. ^{19}F-NMR (367 MHz) spectrum of **d(Et)** in $CDCl_3$

NMR spectra of e´(Et)

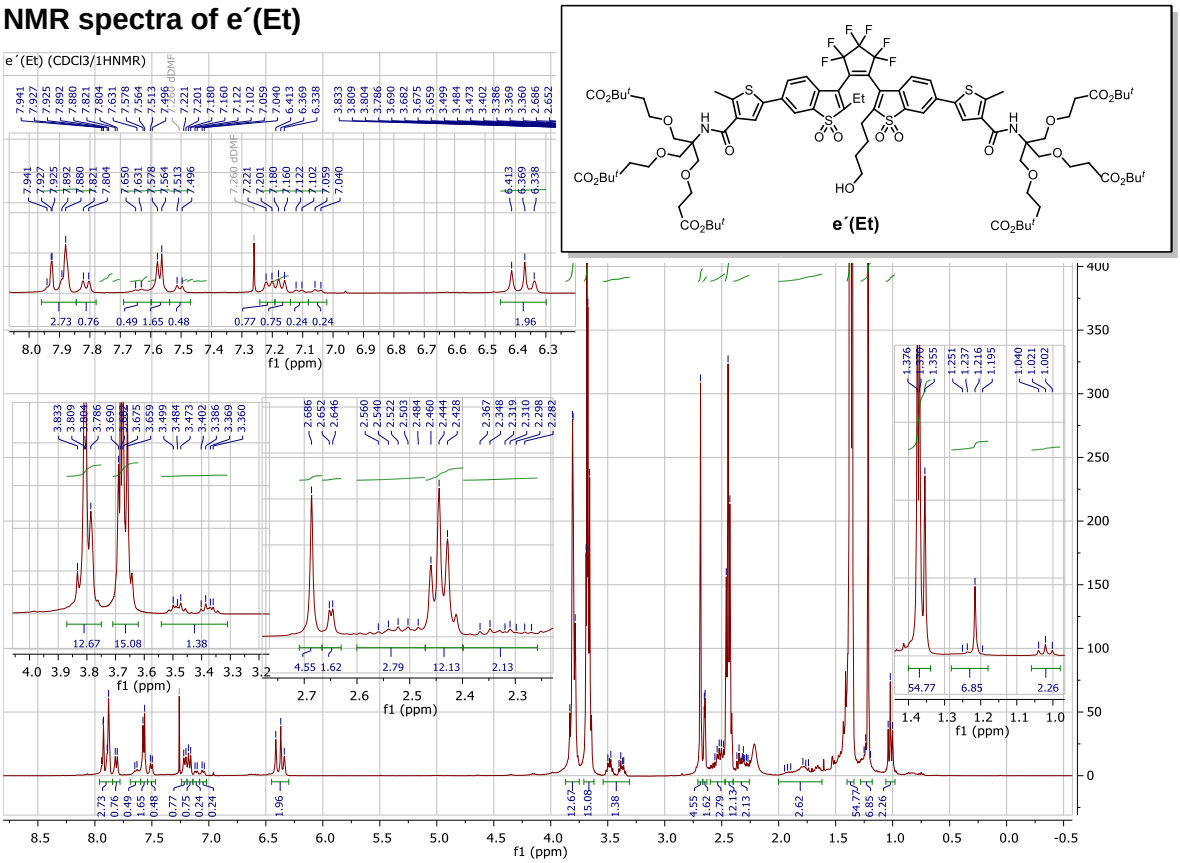


Figure S14a. ^{1}H-NMR (400 MHz) spectrum of **e´(Et)** in $CDCl_3$

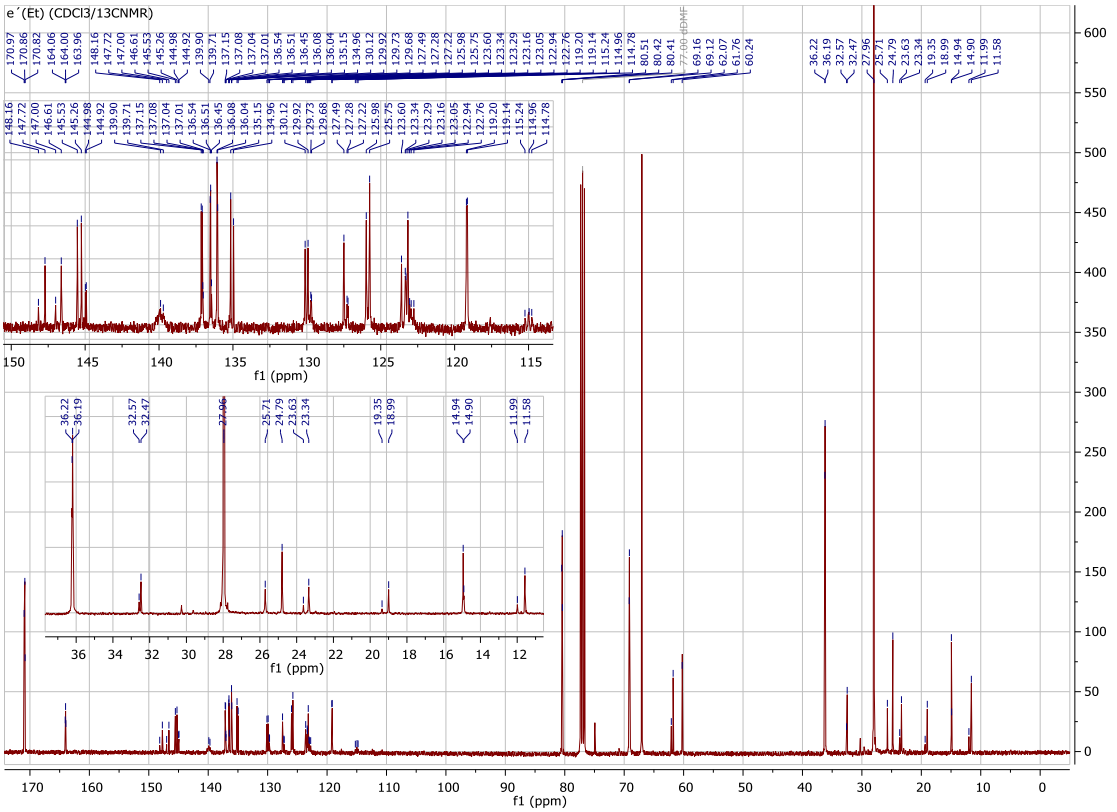


Figure S14b. ^{13}C-NMR (101 MHz) spectrum of **e´(Et)** in $CDCl_3$

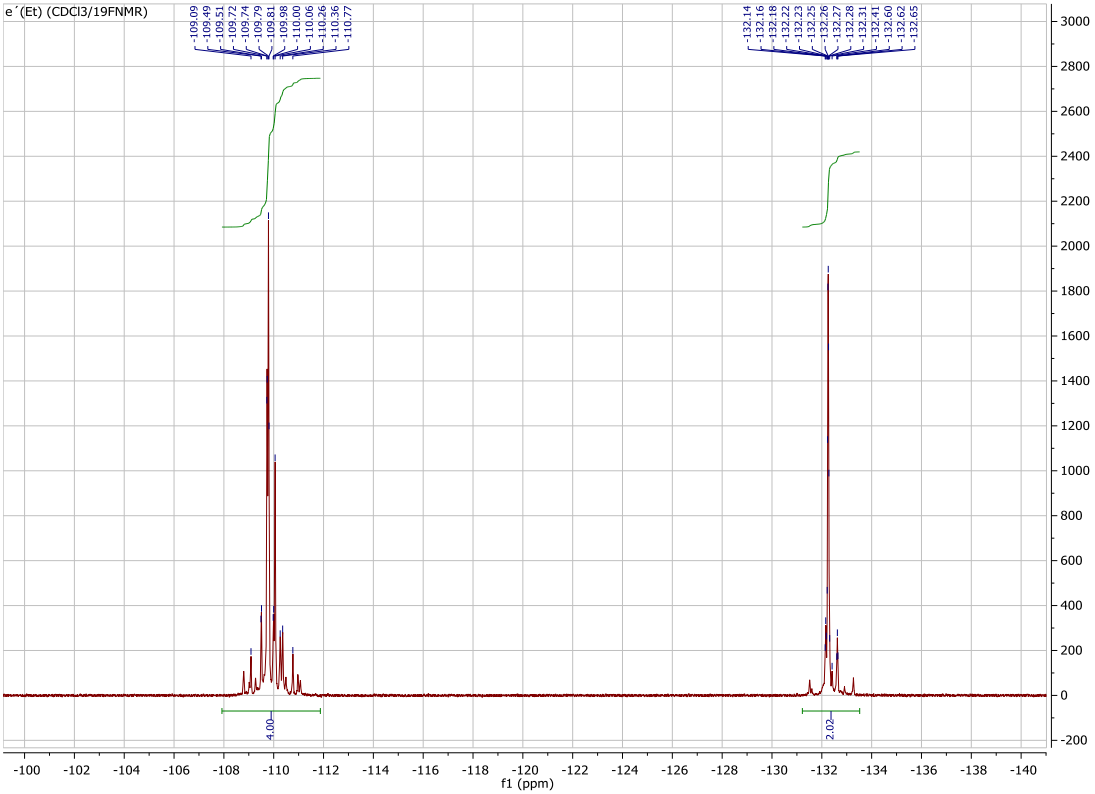


Figure S14c. ^{19}F-NMR (367 MHz) spectrum of **e´(Et)** in $CDCl_3$

NMR spectra of e(Et)

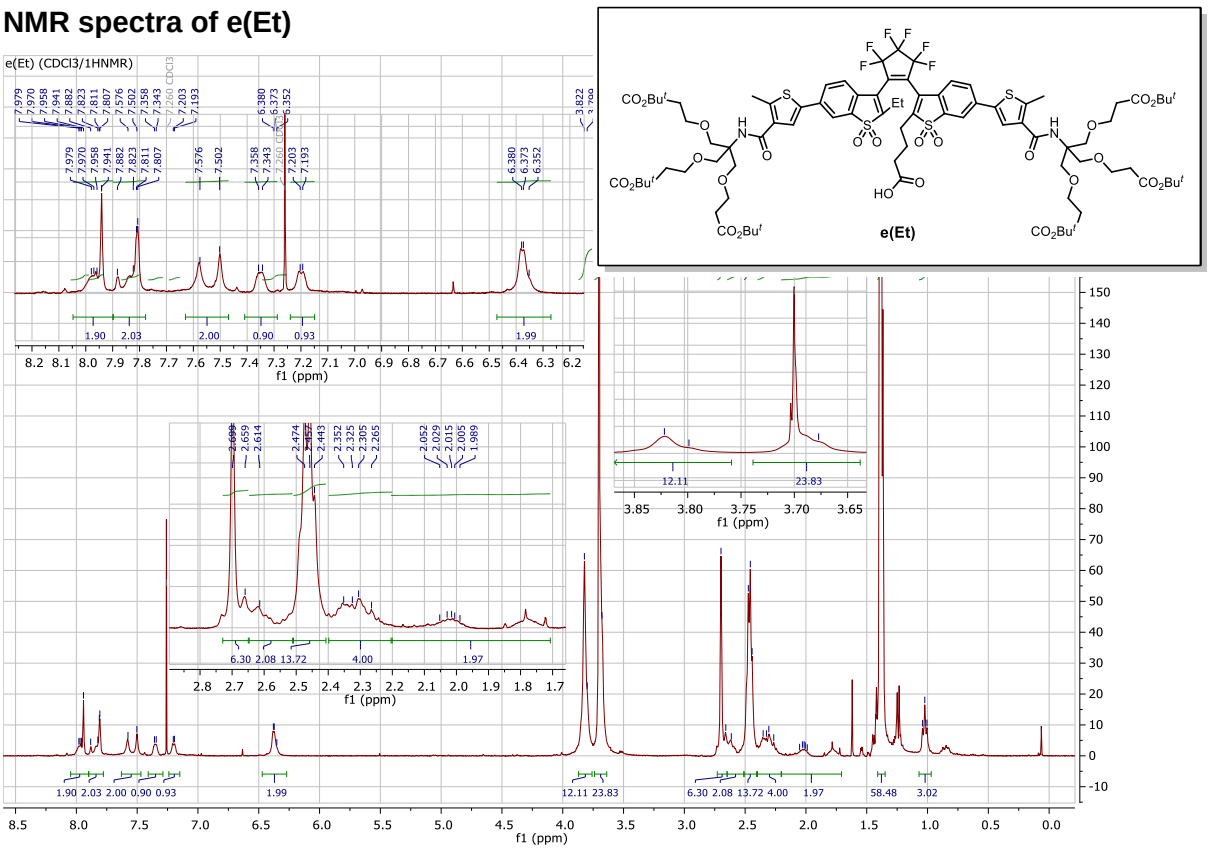


Figure S15a. ^{1}H-NMR (400 MHz) spectrum of **e(Et)** in $CDCl_3$

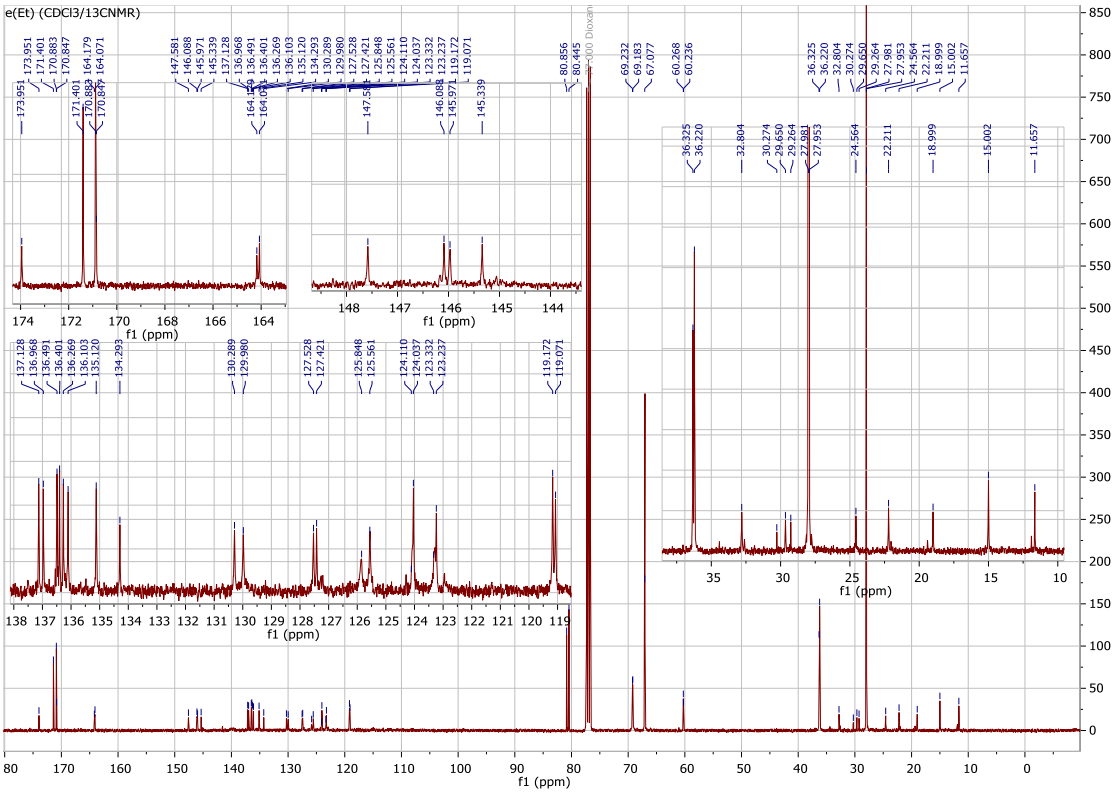


Figure S15b. ^{13}C-NMR (101 MHz) spectrum of **e(Et)** in $CDCl_3$

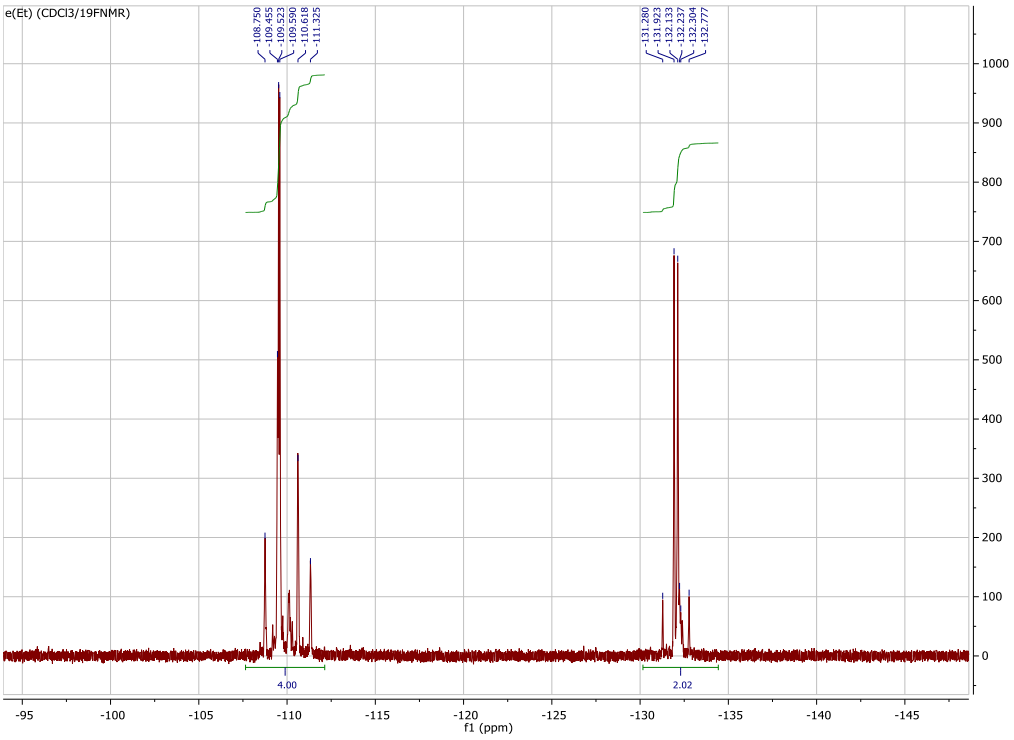


Figure S15c. ^{19}F-NMR (367 MHz) spectrum of **e(Et)** in $CDCl_3$

NMR spectra of fDAE5

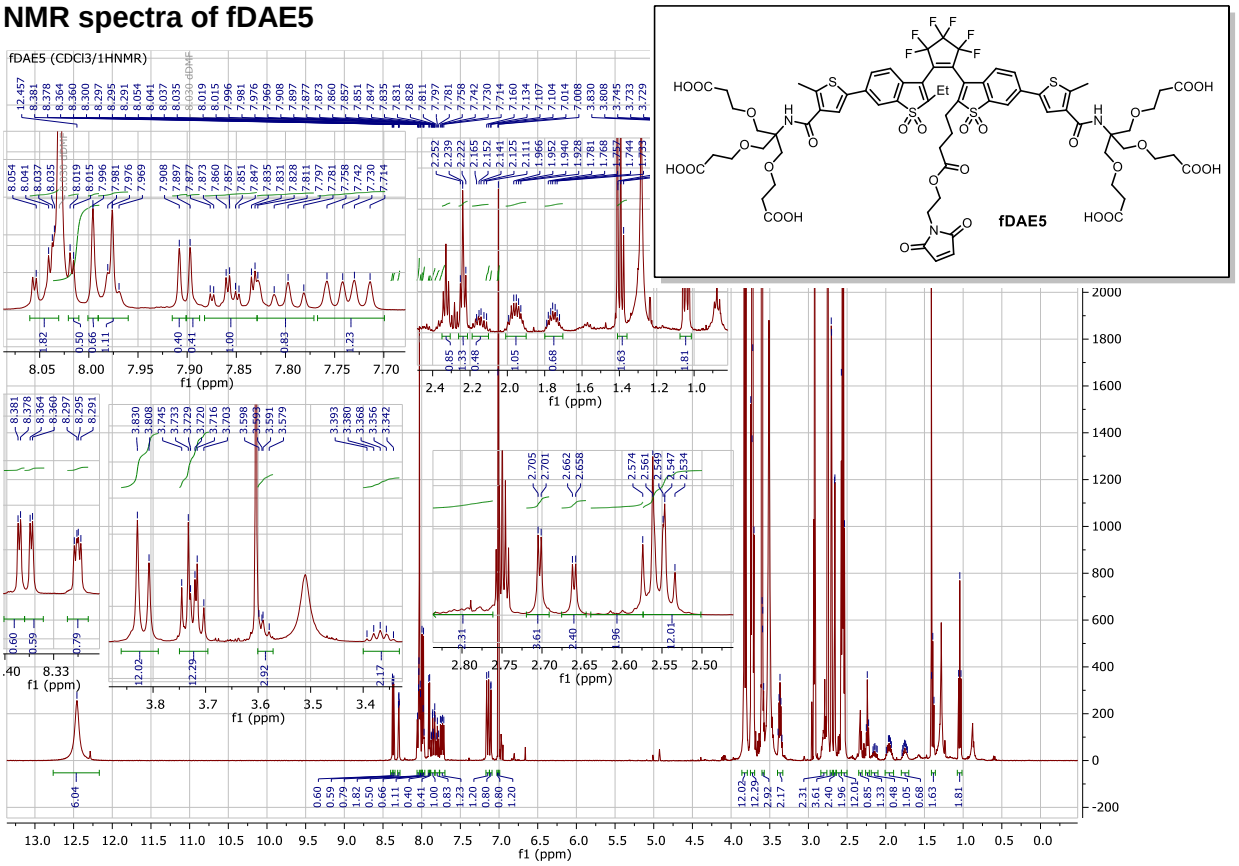


Figure S16a. ^{1}H-NMR (600 MHz) spectrum of **fDAE5** in [D_7]DMF

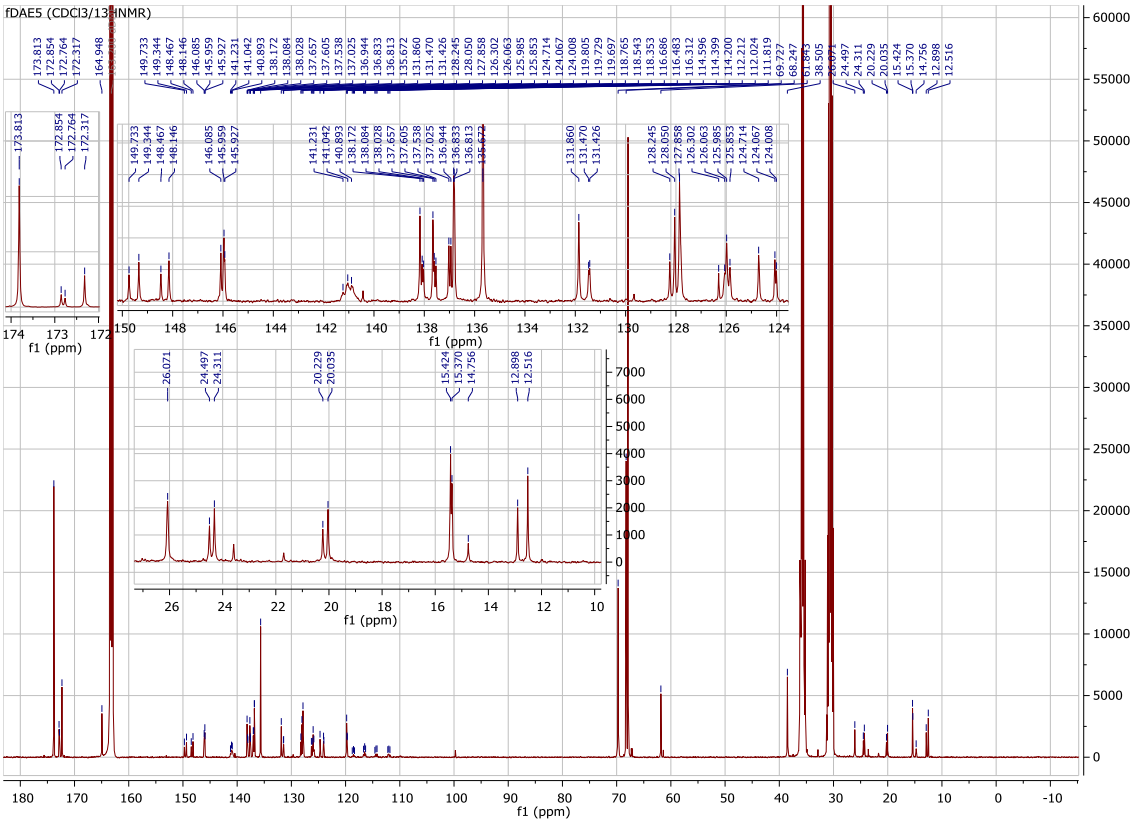


Figure S16b. ^{13}C-NMR (151 MHz) spectrum of **fDAE5** in [D_7]DMF

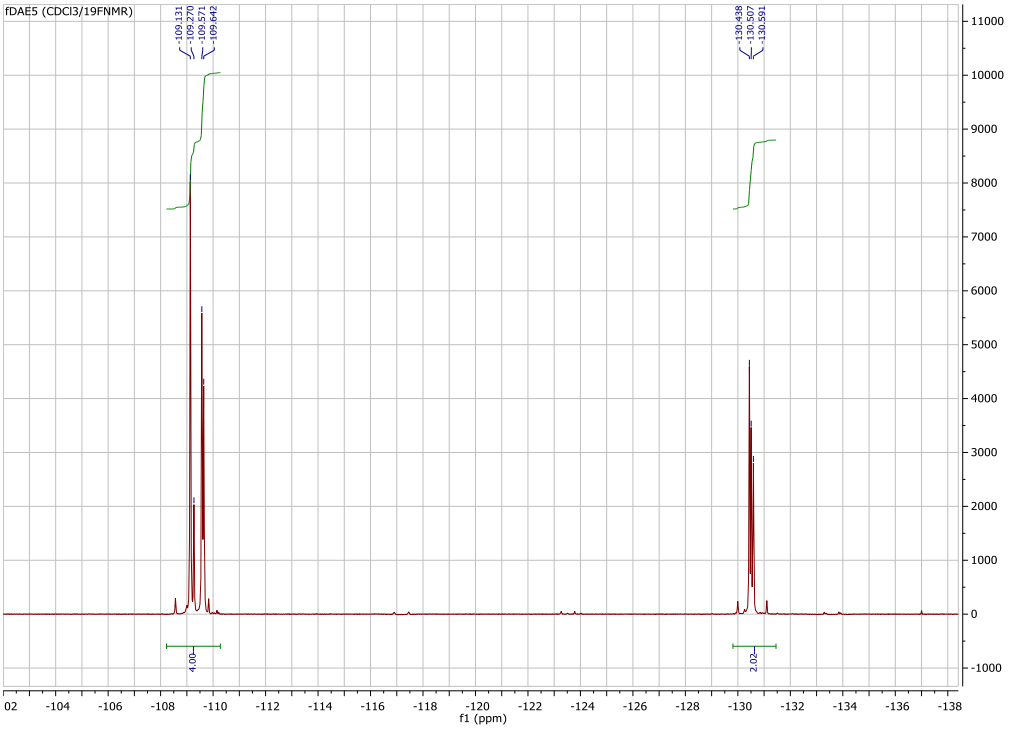


Figure S16c. ^{19}F-NMR (565 MHz) spectrum of **fDAE5** in [D_7]DMF

NMR spectra of b(*i*Bu)

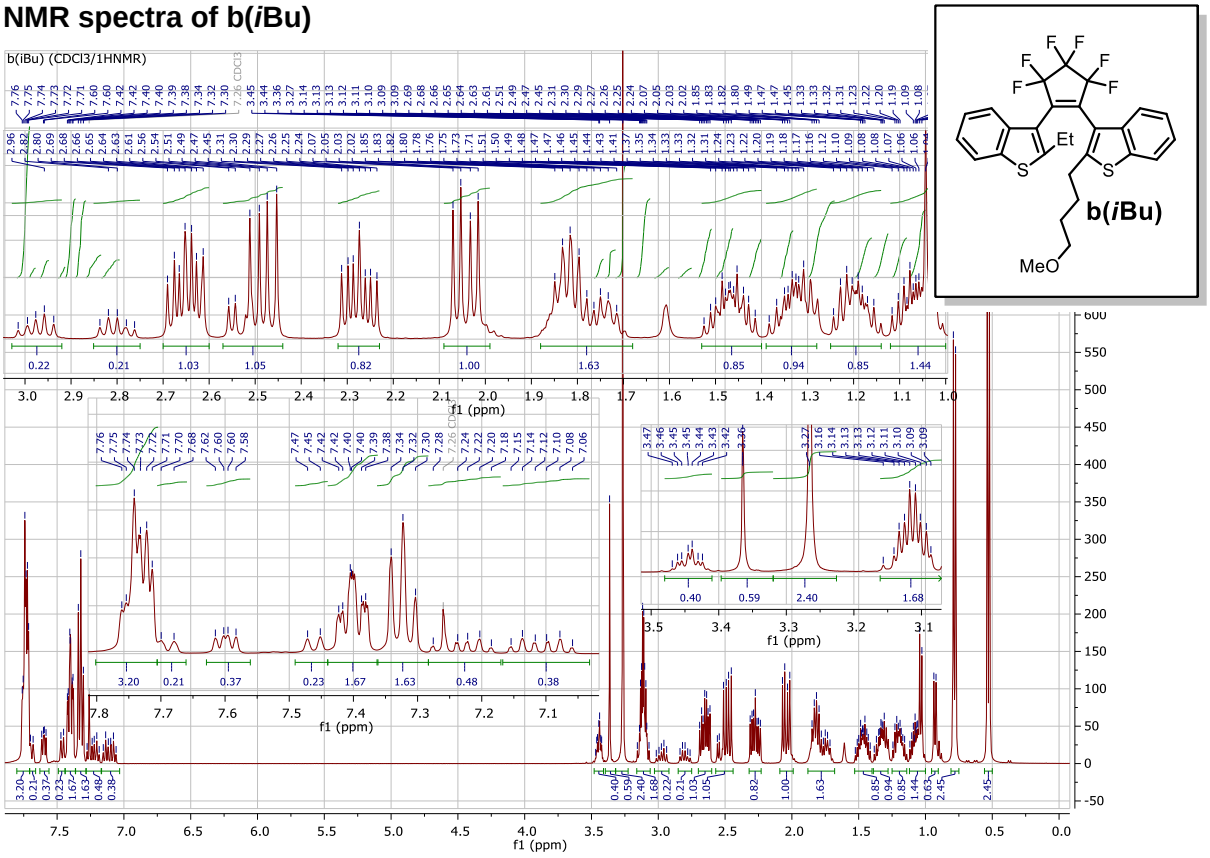


Figure S17a. ^{1}H-NMR (400 MHz) spectrum of **b(*i*Bu)** in $CDCl_3$

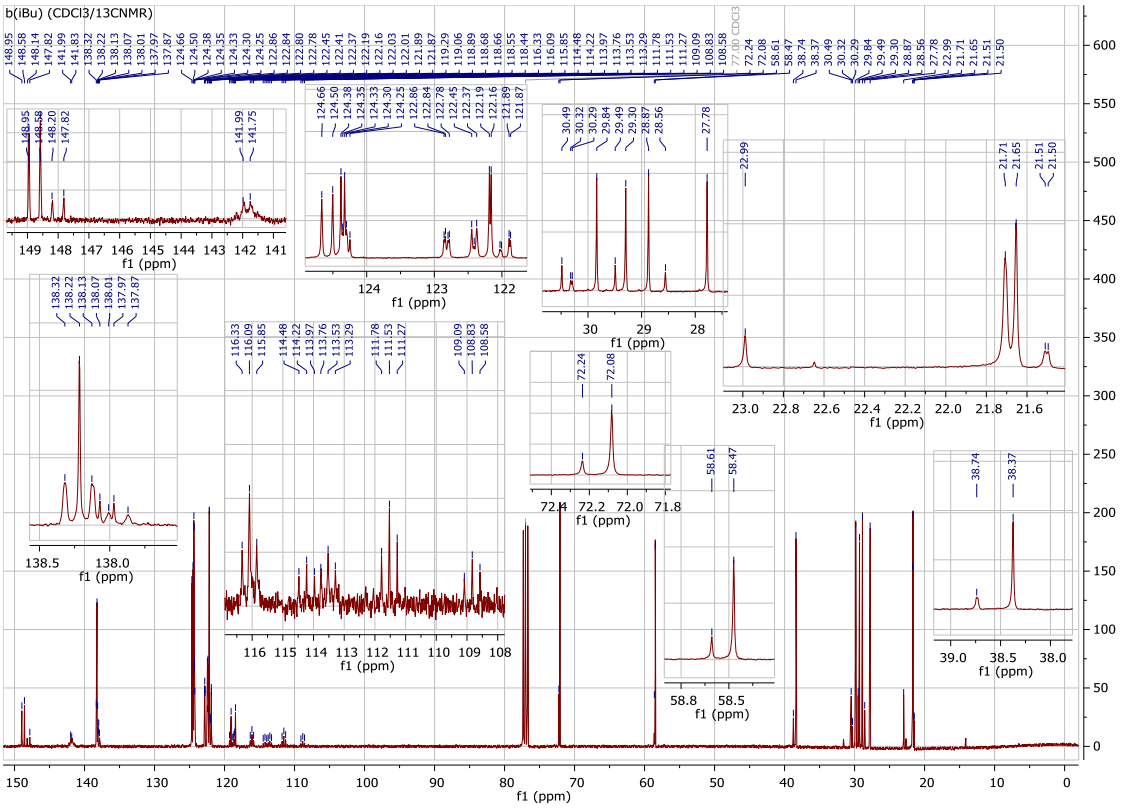


Figure S17b. ^{13}C-NMR (101 MHz) spectrum of **b(*i*Bu)** in $CDCl_3$

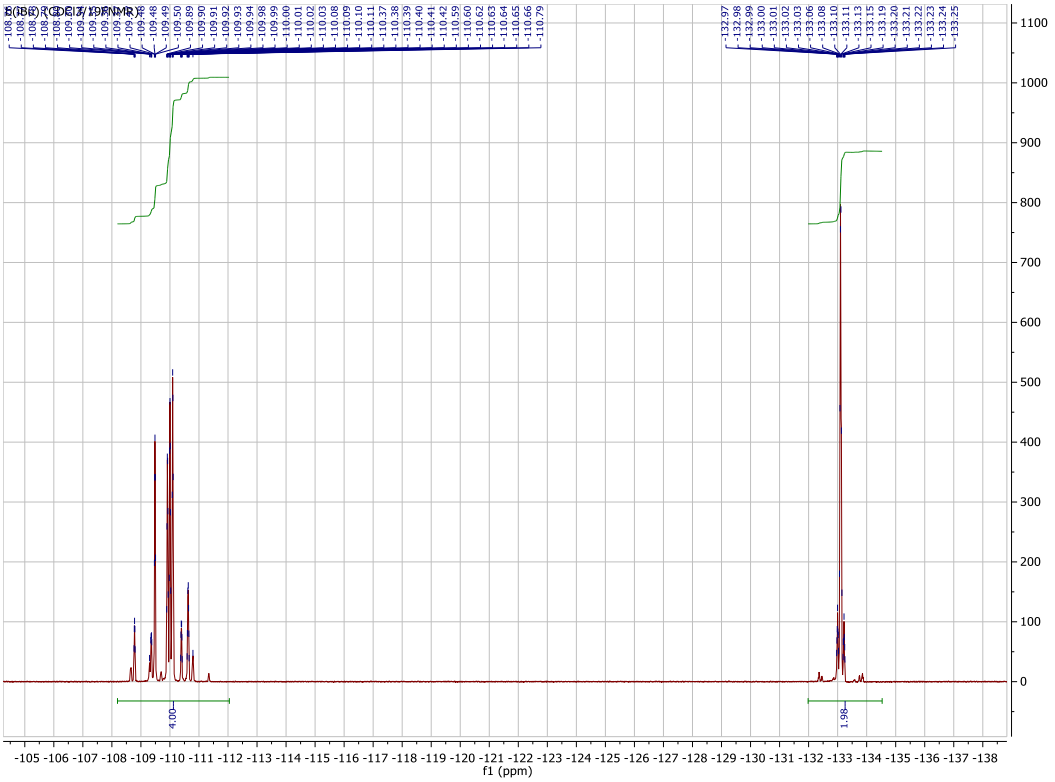


Figure S17c. ^{19}F-NMR (367 MHz) spectrum of **b(*i*Bu)** in $CDCl_3$

NMR spectra of c(*i*Bu)

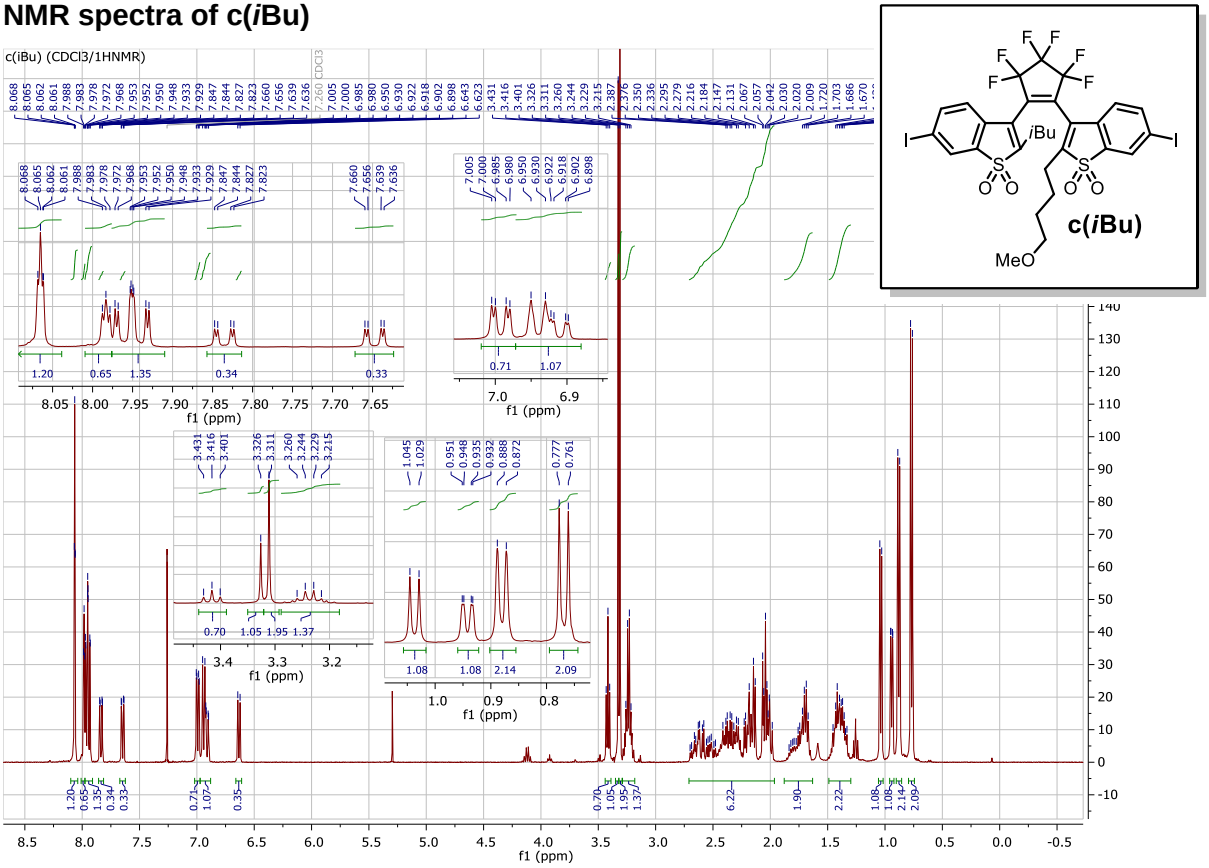


Figure S18a. ^{1}H-NMR (400 MHz) spectrum of **c(*i*Bu)** in $CDCl_3$

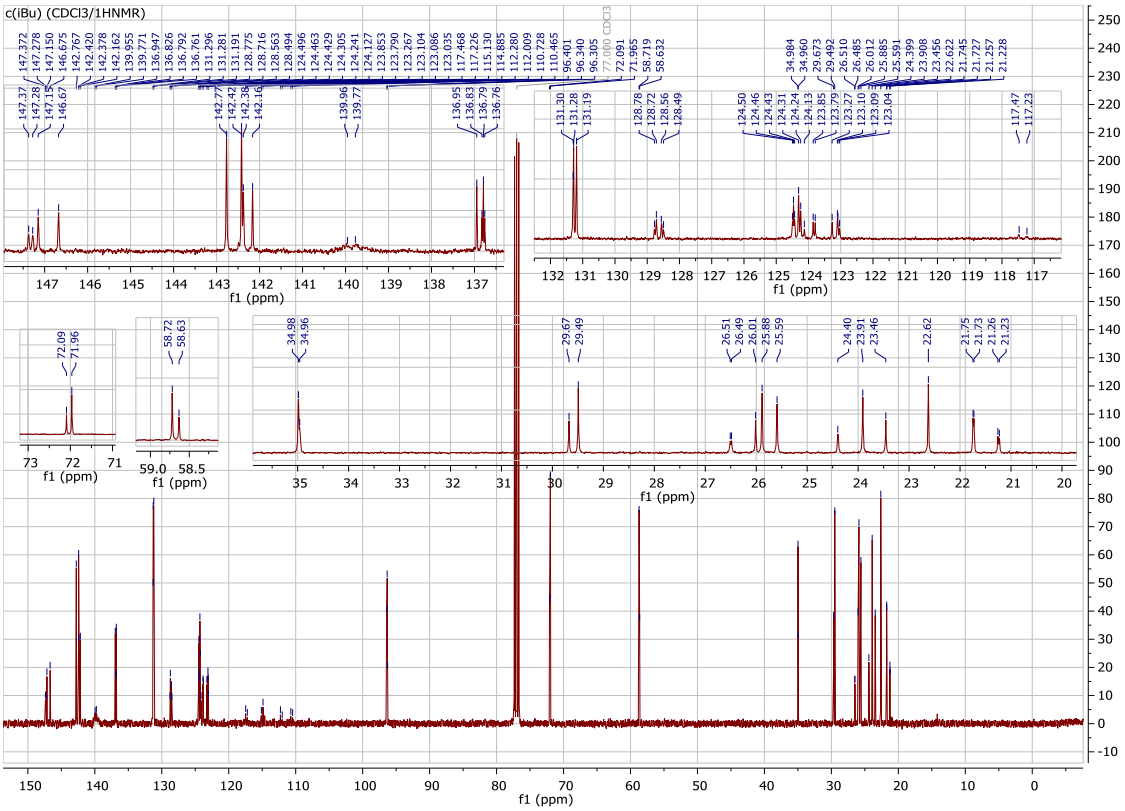


Figure S18b. ^{13}C-NMR (101 MHz) spectrum of **c(*i*Bu)** in $CDCl_3$

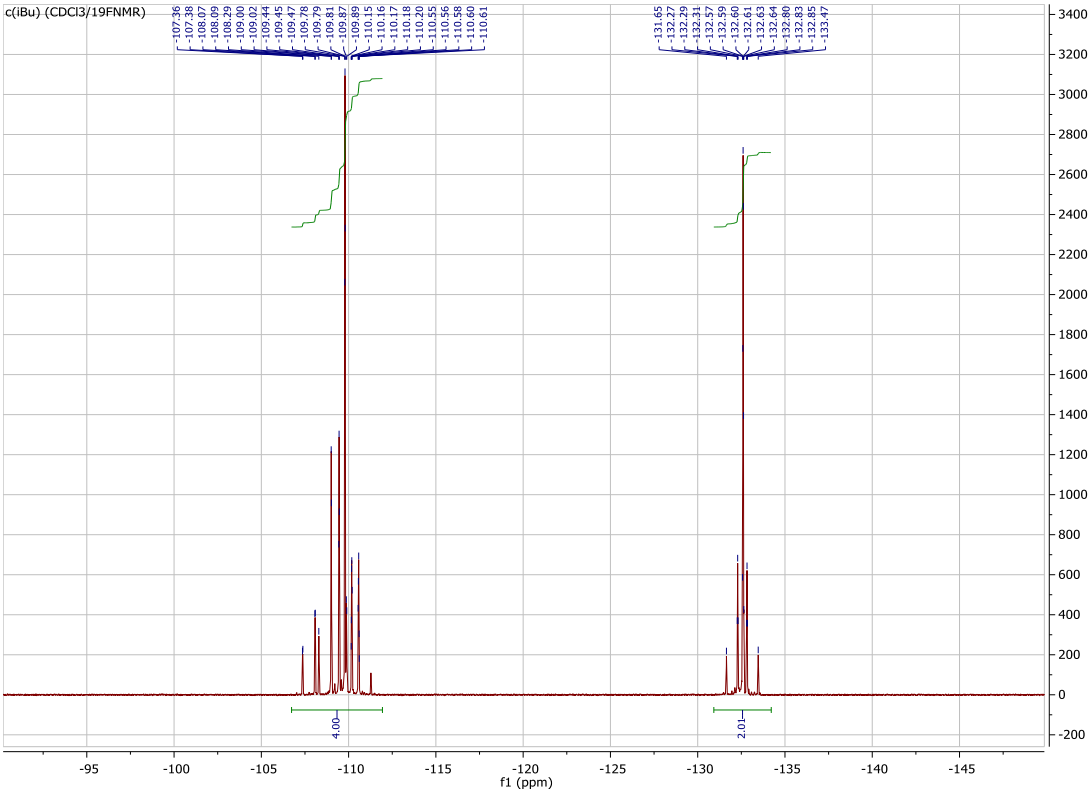


Figure S18c. ^{19}F-NMR (367 MHz) spectrum of **c(*i*Bu)** in $CDCl_3$

NMR spectra of d(*i*Bu)

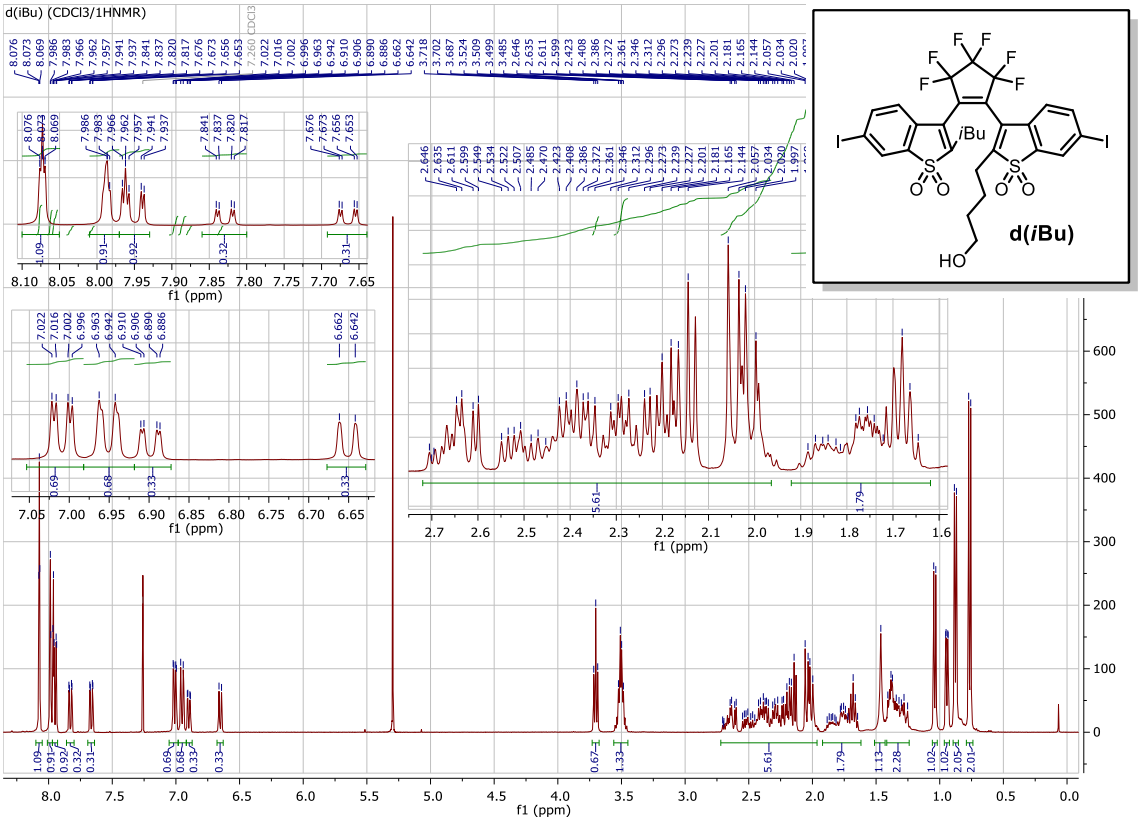


Figure S19a. ^{1}H-NMR (400 MHz) spectrum of **d(*i*Bu)** in $CDCl_3$

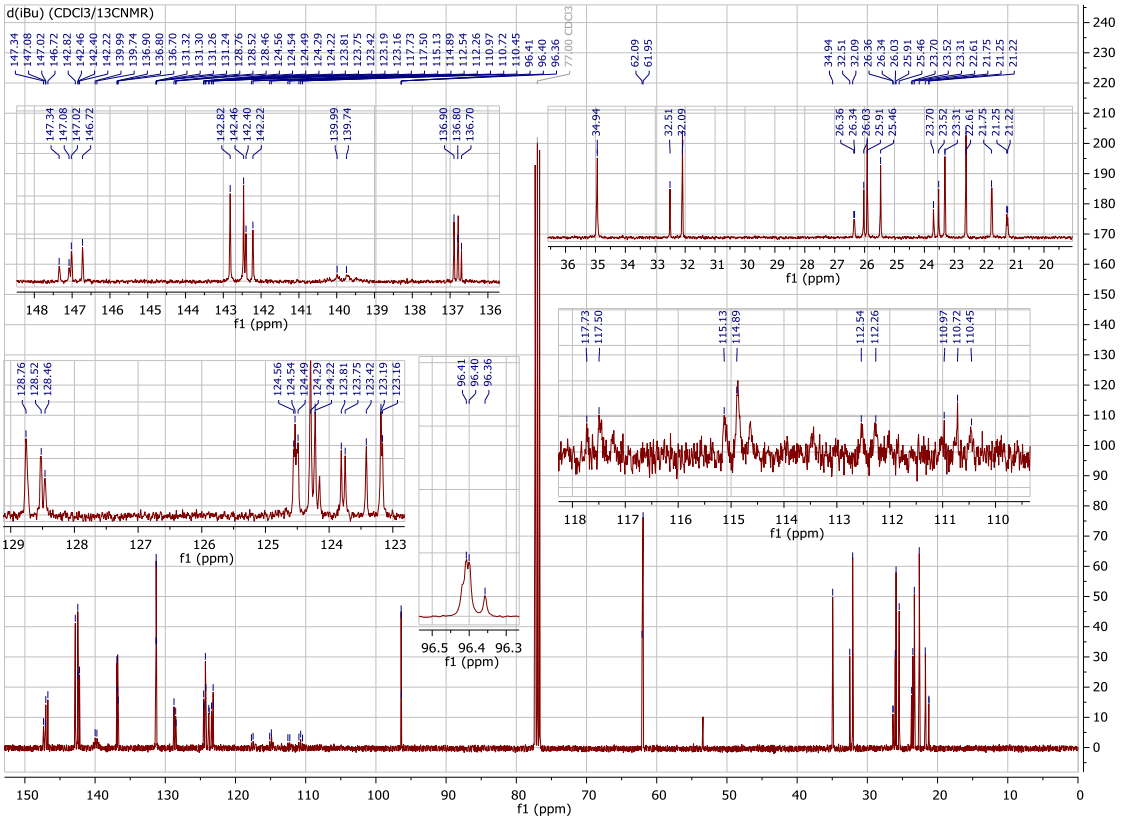


Figure S19b. ^{13}C-NMR (101 MHz) spectrum of **d(*i*Bu)** in $CDCl_3$

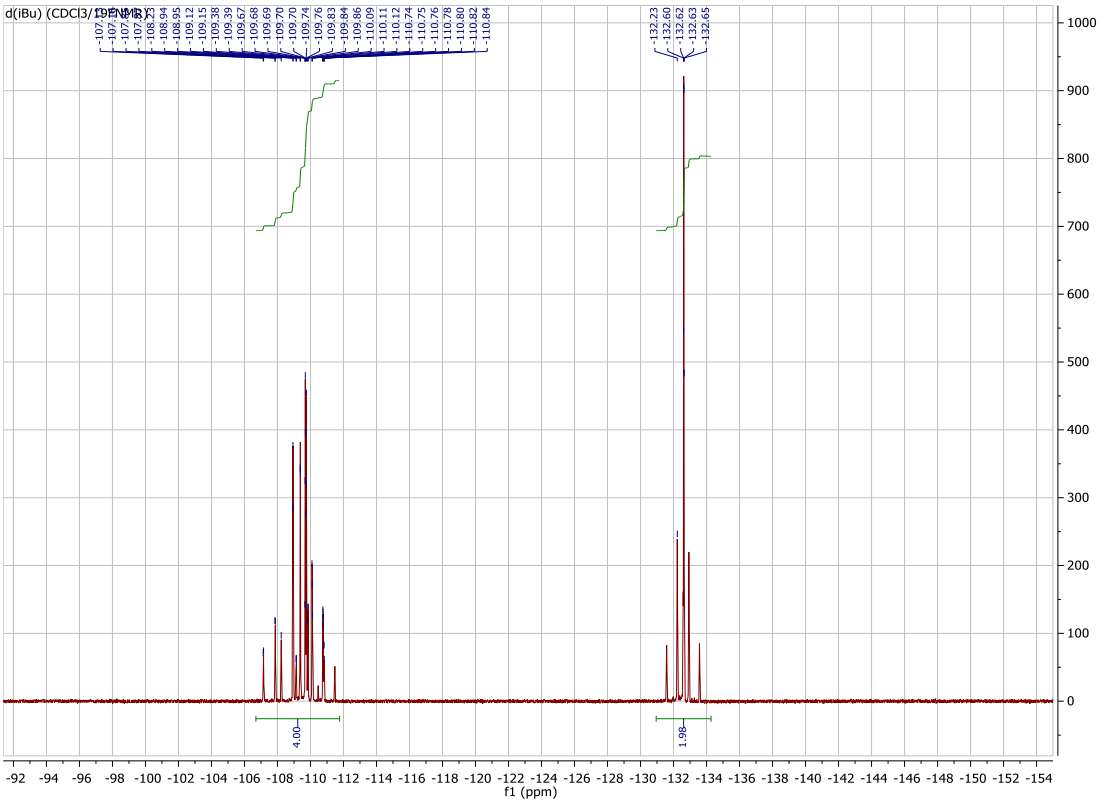


Figure S19c. ^{19}F-NMR (367 MHz) spectrum of **d(*i*Bu)** in $CDCl_3$

NMR spectra of e´(*i*Bu)

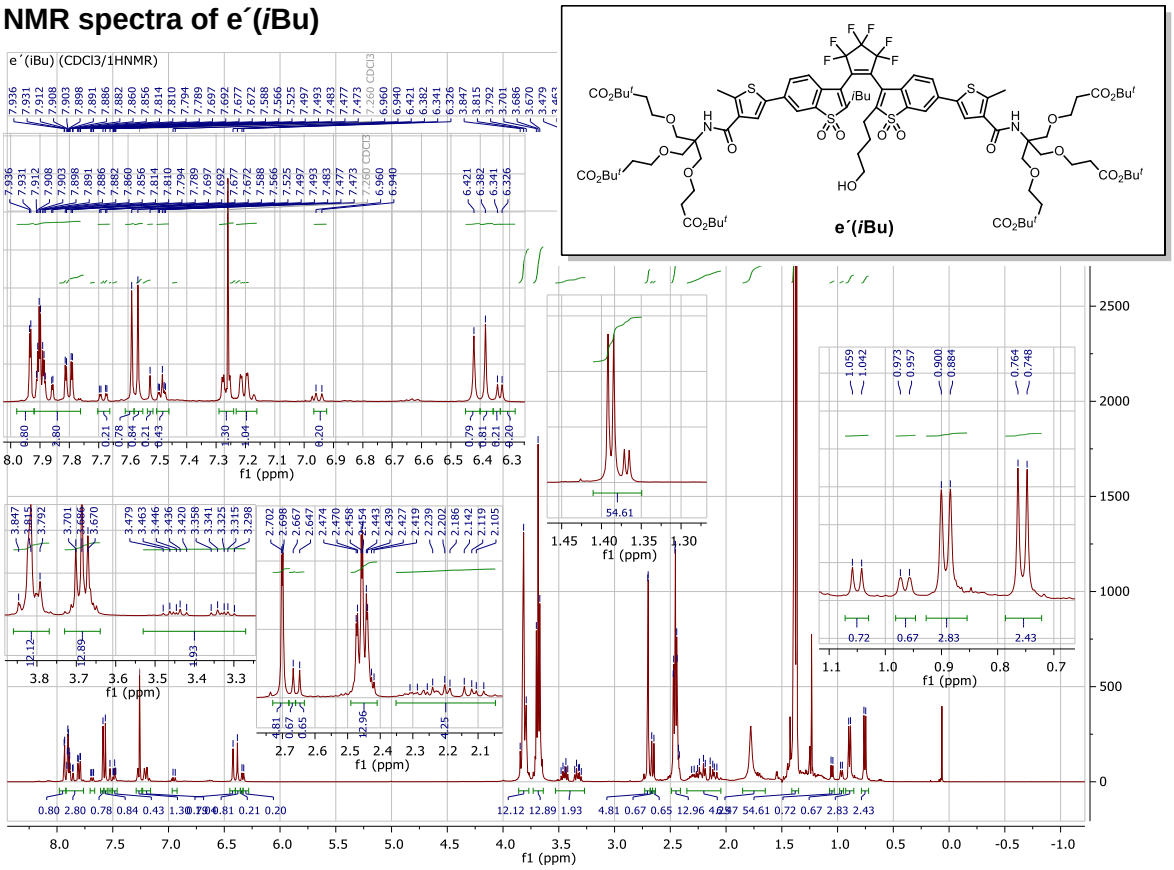


Figure S20a. ^{1}H-NMR (400 MHz) spectrum of **e´(*i*Bu)** in $CDCl_3$

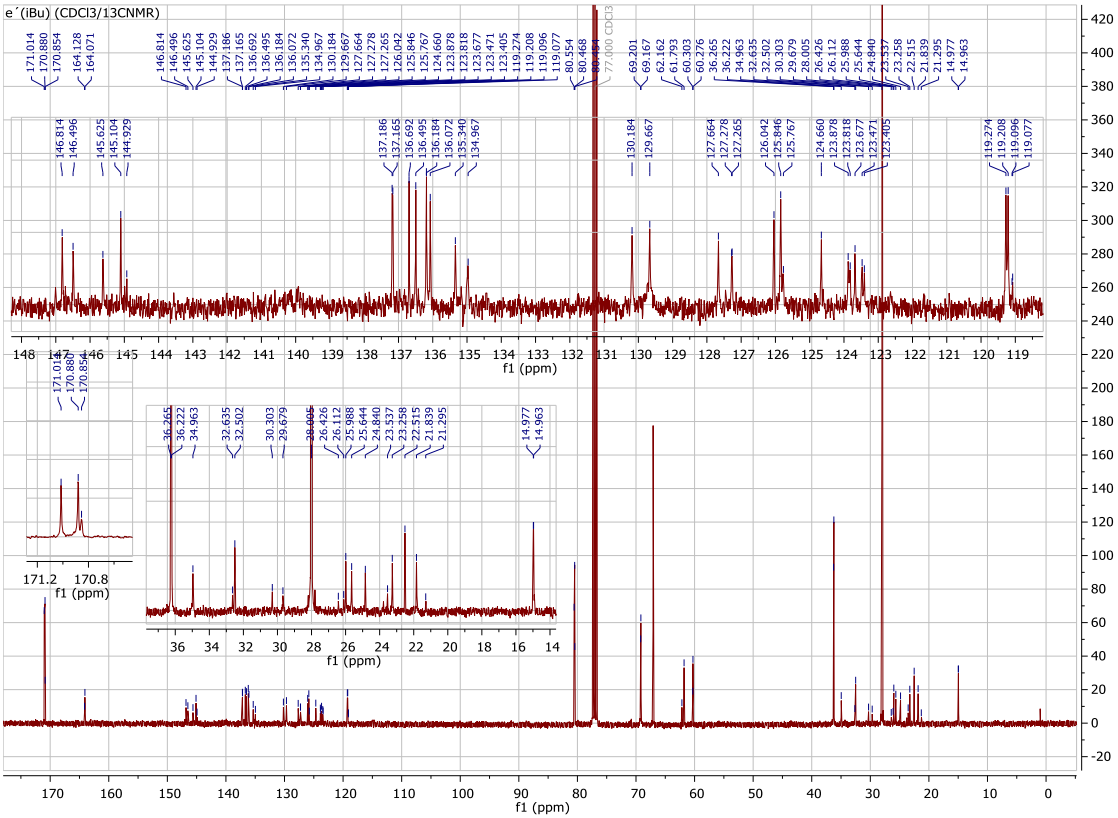


Figure S20b. ^{13}C-NMR (101 MHz) spectrum of **e´(*i*Bu)** in $CDCl_3$

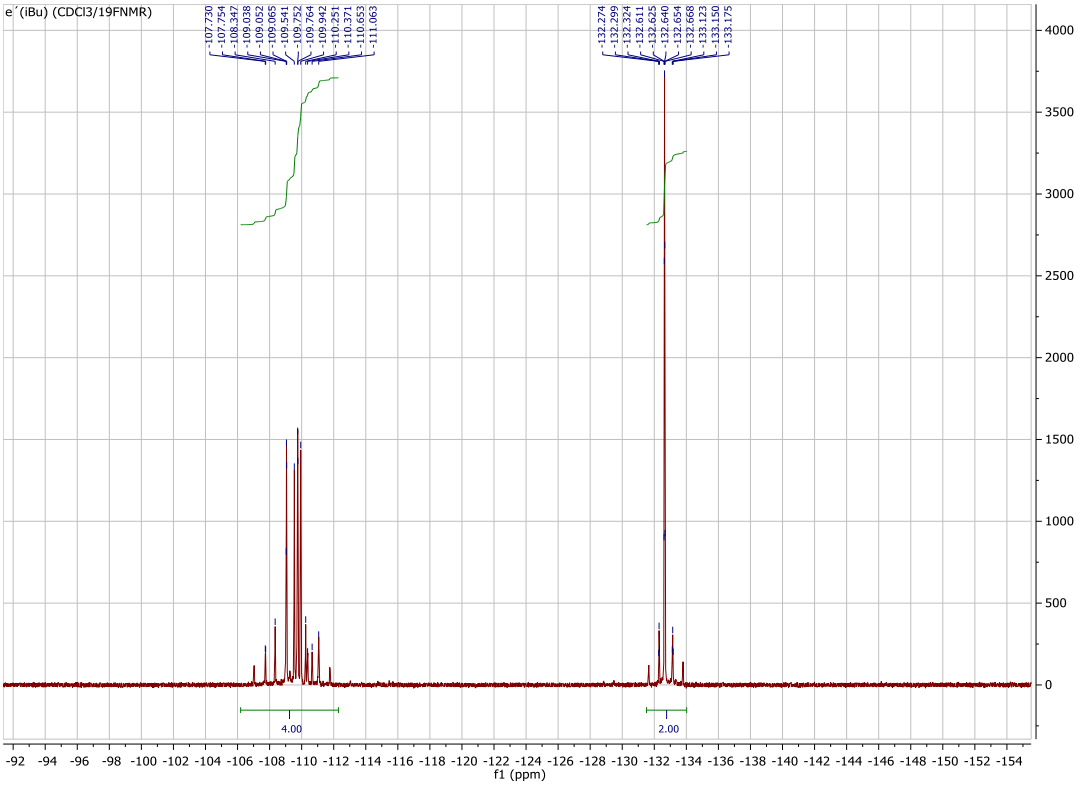


Figure S20c. ^{19}F-NMR (367 MHz) spectrum of **e´(*i*Bu)** in $CDCl_3$

NMR spectra of e(*i*Bu)

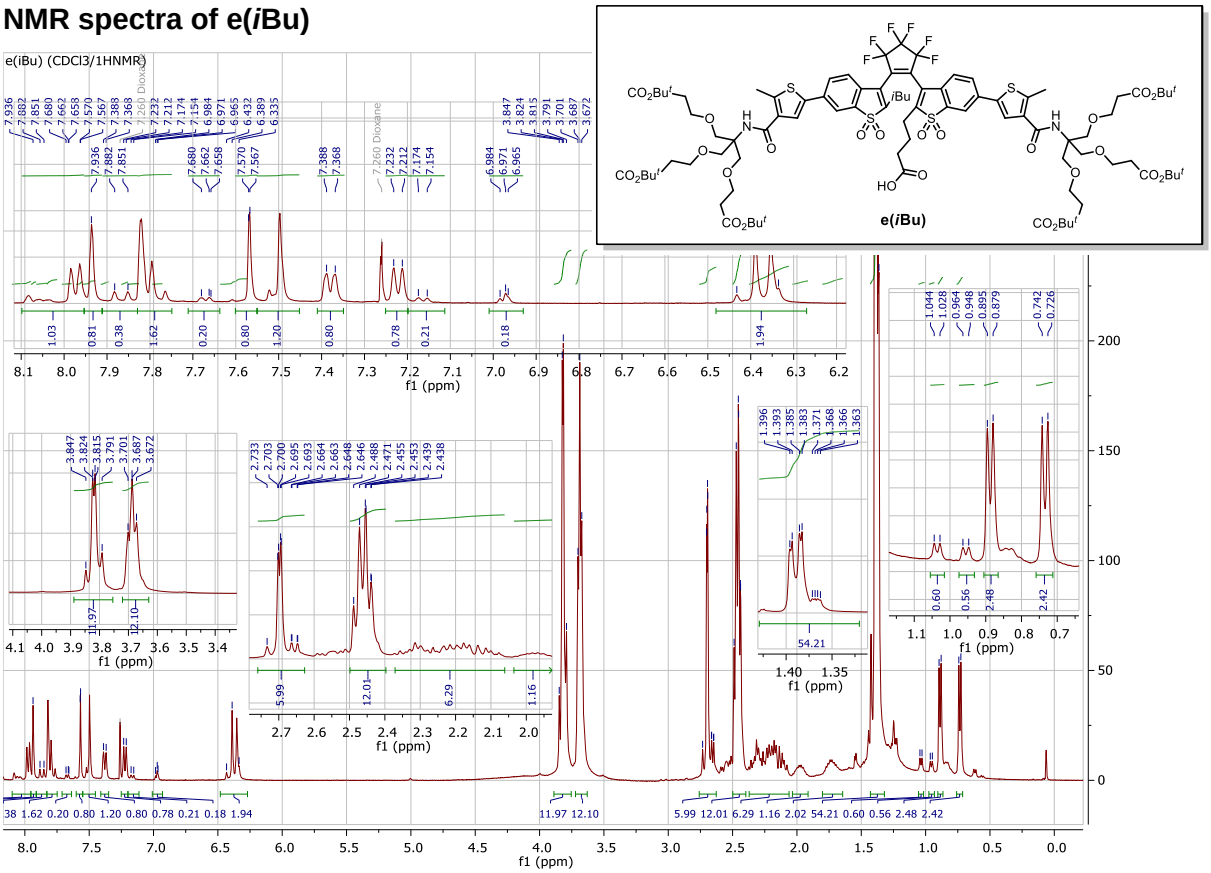


Figure S21a. ^{1}H-NMR (400 MHz) spectrum of **e(*i*Bu)** in $CDCl_3$

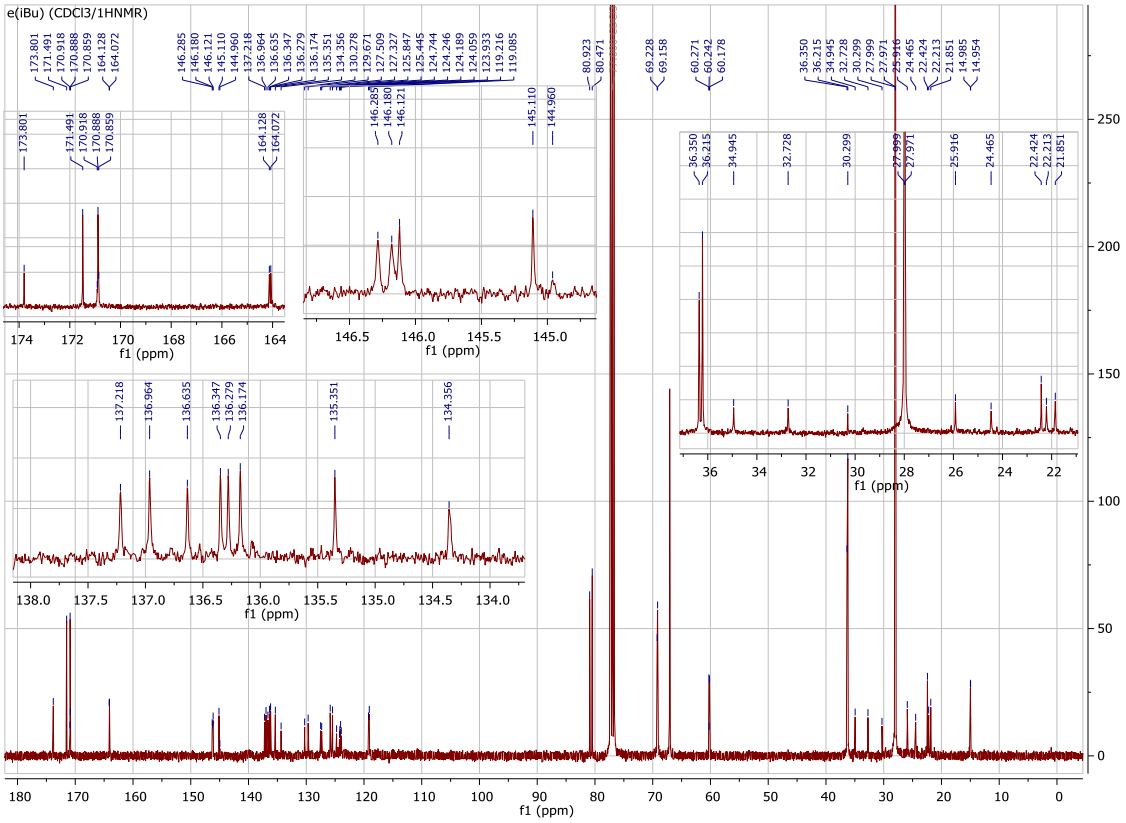


Figure S21b. ^{13}C-NMR (101 MHz) spectrum of **e(*i*Bu)** in $CDCl_3$

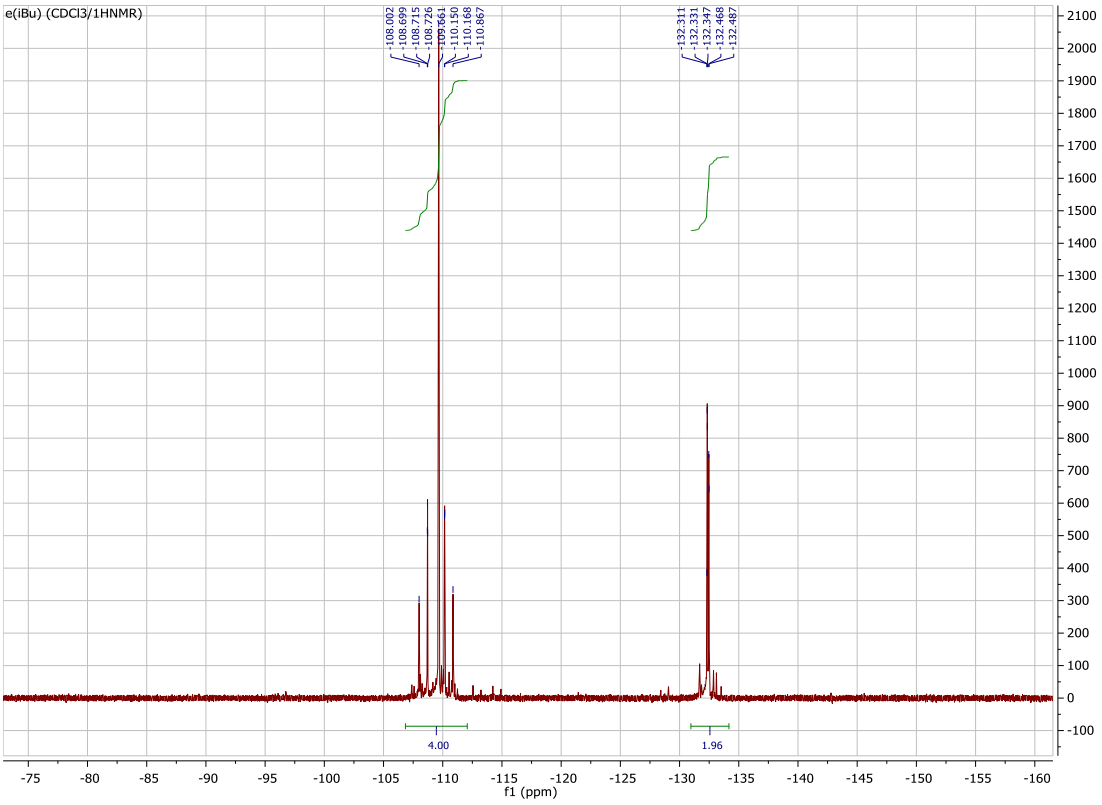


Figure S21c. ^{19}F-NMR (367 MHz) spectrum of **e´(*i*Bu)** in $CDCl_3$

NMR spectra of fDAE6

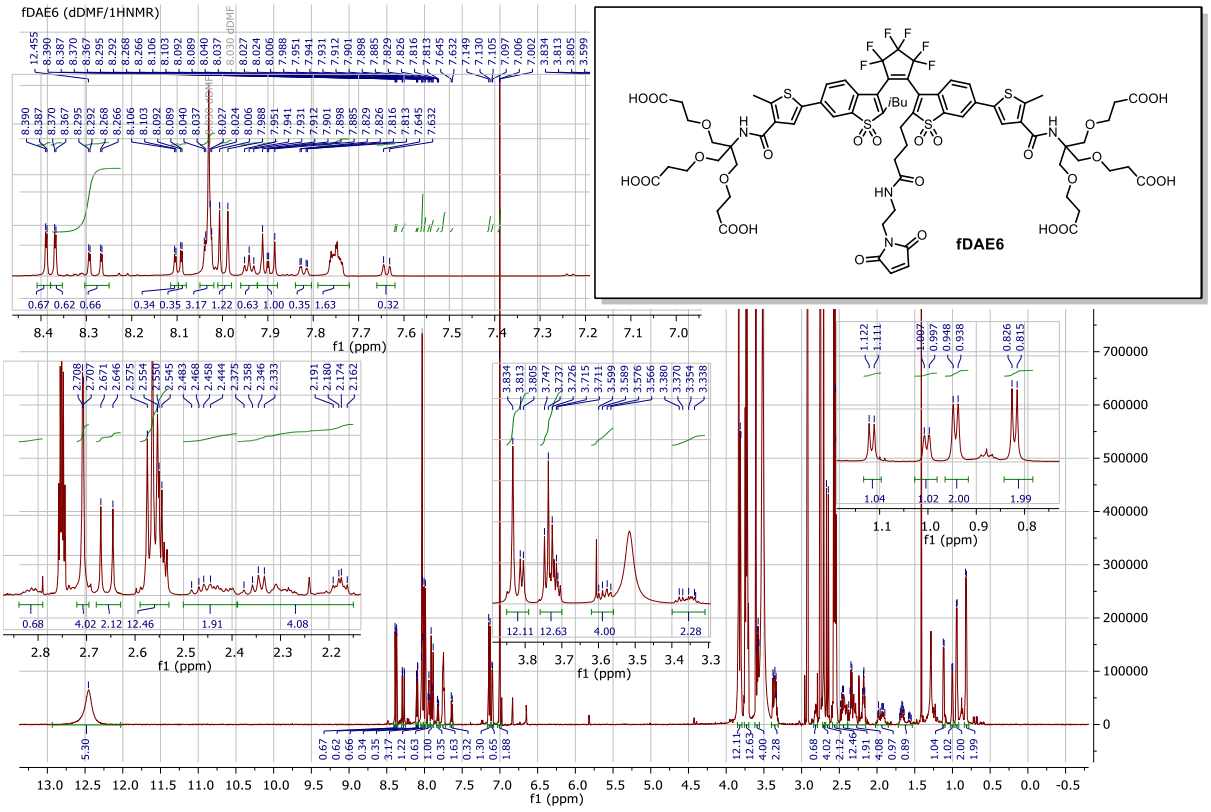


Figure S22a. ^{1}H-NMR (600 MHz) spectrum of **fDAE6** in [D_7]DMF

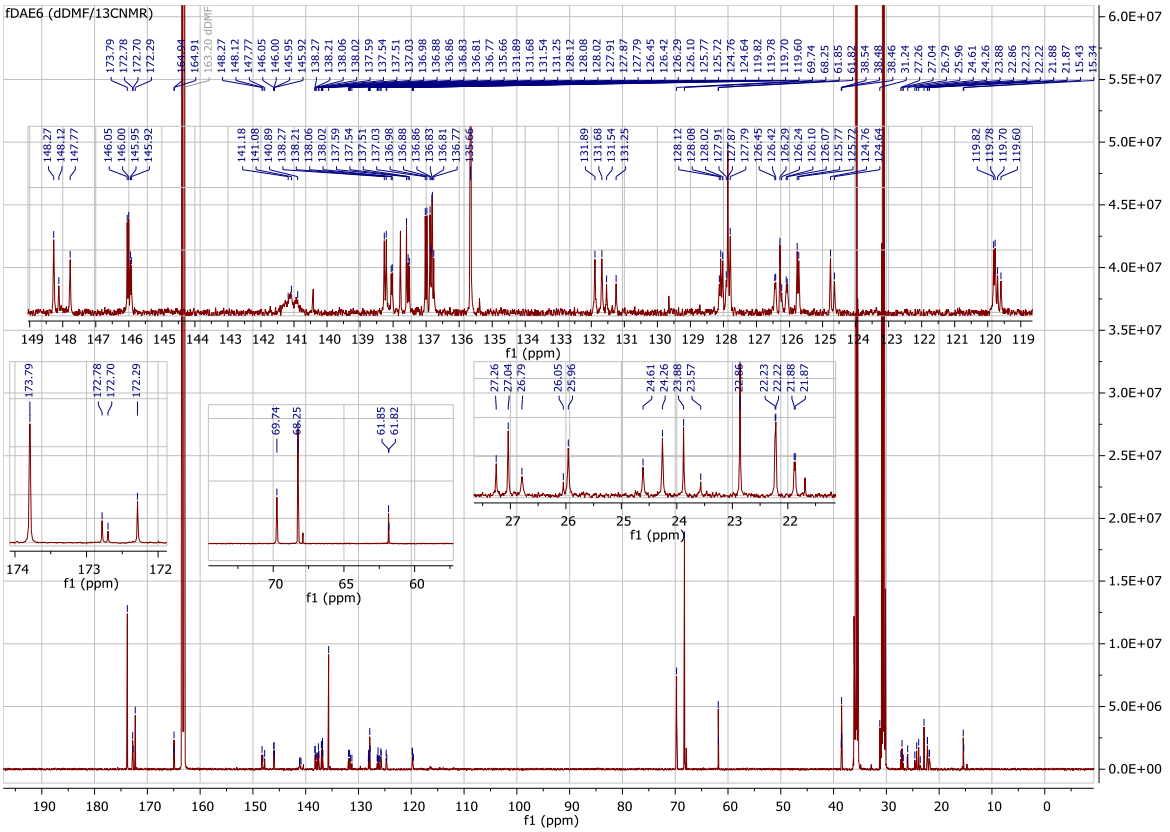


Figure S22b. ^{13}C-NMR (151 MHz) spectrum of **fDAE6** in [D_7]DMF

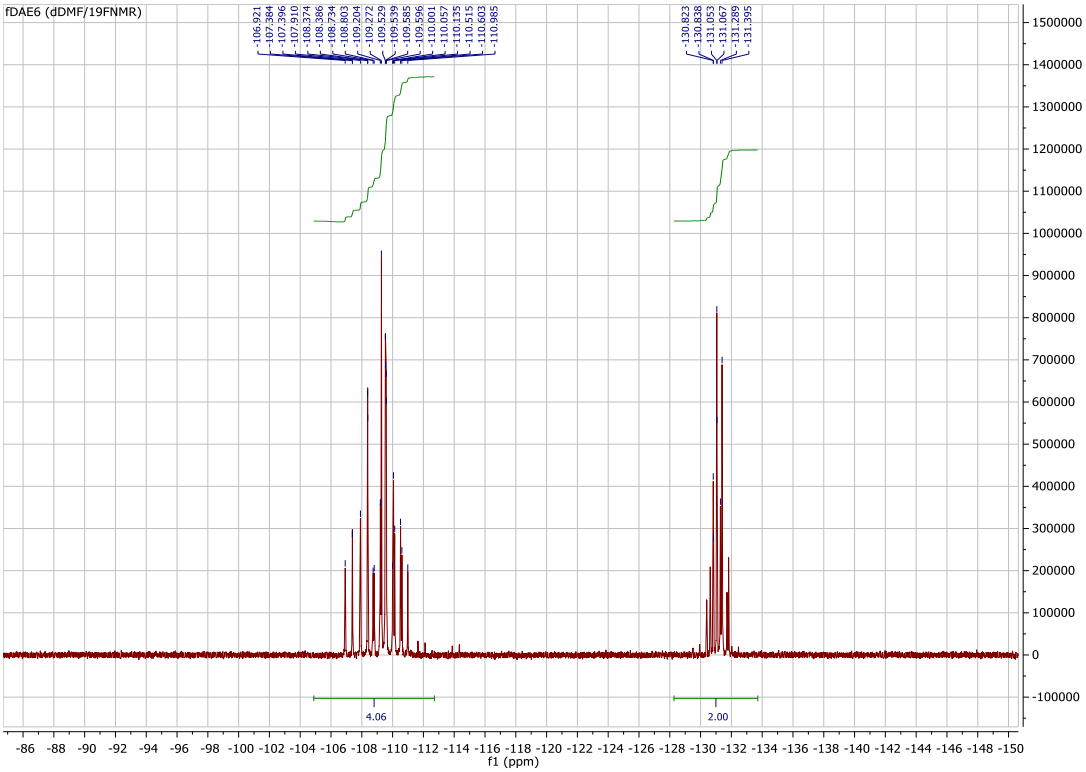


Figure S22c. ^{19}F-NMR (565 MHz) spectrum of **fDAE6** in [D_7]DMF

Conjugation of antibodies via amino groups. The pH of the solutions of unconjugated secondary antibodies (AffiniPure Goat anti-Rabbit Polyclonal IgG (H+L) (111-005-003 Jackson ImmunoResearch) or AffiniPure Goat anti-Mouse Polyclonal IgG (H+L) (115-005-003 Jackson ImmunoResearch)) was adjusted to pH ≈ 8.0-8.2 by addition of 1/10th volume of aq. $NaHCO_3$ (1 M). A concentrated solution (approx. 4 mM) of the dye in DMF was prepared. The dye solution was treated with 1.1-1.4 equivalents of *N*-hydroxysuccinimide (NHS) (approx. 43 mM in DMF) and 10 equivalents of 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC) (approx. 23 mM in DMF), and stirred for 15 min at room temperature to generate the NHS-ester derivative(s). The mixture was added to the antibody [M~150 000] solution in a 5 to 10-fold molar excess and stirred at room temperature for 1 h. The labeled antibody was separated from the unreacted dye using a pre-packed Sephadex G-25 column (PD-MiniTrap G25, GE Healthcare). UV-Vis measurements were used to determine the fractions containing the protein, and for the spectroscopic determination of the degree of labeling (DOL). Additional MALDI-MS measurements were performed for the determination of the DOL values.

Conjugation of nanobodies via thiol groups. Tris(2-carboxyethyl)phosphine (TCEP) was removed from the received unconjugated nanobody solution (NanoTag Biotechnologies N1202, N2402, N0302 or N0303 with site-specific cysteine residues) using a desalting column (7K MWCO Zeba Spin Desalting Columns, Thermo Scientific), and the buffer was exchanged to 10 mM potassium phosphate buffer (vacuum filtrated, pH 6.0) with additional NaCl (300 mM) and EDTA (2 mM). Then the pH of the solution was adjusted by the addition of 1/10 volume of 1 M Tris/HCl buffer (pH 8.0), and 1.5- to 2-fold molar excess (in respect to the number of cysteines per nanobody) of maleimide-DAE derivative (approx. 4 mM in DMF) was immediately added. The reaction mixture was vortexed, overlaid with argon and incubated on ice for 1.5 h. The unreacted excess dye was removed, and the buffer was exchanged to PBS (2×) using a desalting column (7K MWCO Zeba Spin Desalting Columns, Thermo Scientific). The DOL value of the resulting conjugate was determined by ESI-MS.

Degrees of labelling: absorption spectroscopy. The DOL values of the antibody conjugates were calculated using the equation given below, considering the amount of OF and CF of the dye (typically, 2-5% of CF is formed during labelling, purification and manipulation). As only the CF absorbs at 504 nm, the absorption $A_{CF,504}$ and the extinction coefficient $\varepsilon_{CF,504}$ at this wavelength was used to calculate the concentration $c_{CF.}$ This concentration was used to subtract the absorption of the CF $A_{CF,373}$ from the total absorption at 373 nm $A_{tot,373}$, to yield the absorption of

the OF at this wavelength $A_{OF,373}$ and thereby the concentration c_{OF} of the OF using the extinction coefficient $\varepsilon_{OF,373}$. Finally, the absorption of the dye ($A_{CF,280}$+ $A_{OF,280}$) was subtracted from the total absorption at 280 nm $A_{tot,280}$, to yield the absorption of the antibody $A_{AB,280}$, from which the concentration of the antibody c_{AB} was calculated using the theoretical extinction coefficient $\varepsilon_{AB,280}$.

$$DOL = \frac{N_{dye}}{N_{AB}} = \frac{c_{CF} + c_{OF}}{c_{AB}} = \frac{\frac{A_{CF,504}}{\varepsilon_{CF,504}} + \frac{A_{tot,373} - A_{CF,373}}{\varepsilon_{OF,373}}}{\frac{A_{tot,280} - \left(A_{CF,280} + A_{OF,280}\right)}{\varepsilon_{AB,280}}}$$

$$DOL = \frac{\frac{A_{CF,504}}{\varepsilon_{CF,504}} + \frac{A_{tot,373} - \frac{A_{CF,504}}{\varepsilon_{CF,504}}\varepsilon_{CF,373}}{\varepsilon_{OF,373}}}{\frac{A_{tot,280} - \left(\frac{A_{CF,504}}{\varepsilon_{CF,504}}\varepsilon_{CF,280} + \frac{A_{tot,373} - \frac{A_{CF,504}}{\varepsilon_{CF,504}}\varepsilon_{CF,373}}{\varepsilon_{OF,373}}\varepsilon_{OF,280}\right)}{\varepsilon_{AB,280}}}$$

A list of DOL values (spectroscopy) for antibodies labeled with all fDAEs, as well as DOL values (mass-spectrometry; see next section) are presented in Table S1.

Table S1. DOL values of secondary antibodies, determined by the indicated method.

Compound	Spectroscopic	MS-MALDI
fDAE1	4.5	3.2
fDAE2	2.2	1.2
fDAE3	4.5	2.1
fDAE4	4.5	0.7

Degrees of labelling: mass-spectrometry. The DOL values of conjugated antibodies were determined from the data obtained from the matrix-assisted laser desorption ionization mass-spectroscopy (MALDI-MS) measurements analyzed with a MATLAB routine. The routine creates several replicas (R_n) of the reference spectrum (unlabeled antibody) shifted an integer number of times (***n***) by the molecular mass of the corresponding dye. Typically, ***n*** spans from 0 to 3 times of the expected DOL. The measured spectrum is assumed to by a linear combination of such replicas ($S_{fit} = \sum_n A_n \times R_n$). The routine obtains the best set of amplitudes for each replica, using a standard fitting method, minimizing the sum or errors (difference between the measured spectrum S_{exp} and the calculated one S_{fit}). The amplitudes provide a distribution of the DOL values, and a weighted average produces the mean DOL ($\langle DOL \rangle = \{\sum_n n \times A_n\}/\{\sum_n A_n\}$). The

mass spectra obtained for antibodies labeled with compounds fDAE1-fDAE4 are presented in Figure S23.

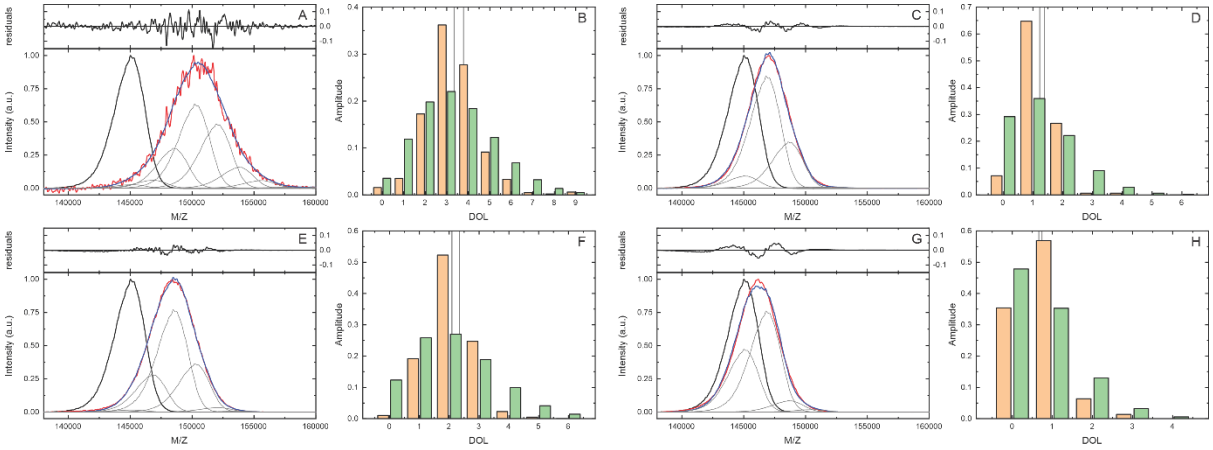


Figure S23. Mass-spectrometry data (MALDI) of secondary antibodies labelled with compounds **fDAE1** (A,B), **fDAE2** (C,D), **fDAE3** (E,F) and **fDAE4** (G,H). The spectrum (A, C, E and G) of the unlabeled antibody (black) and the labelled (red) are shown, along with a fit (blue) to a distribution of the shifted curves with various amplitudes (grey lines). The residuals of the fit are presented on each case. The fitted amplitudes (green) are shown in (B, D, F, H), superimposed with a Poissonian distribution (beige) with expected value of 2. The vertical lines are the fitted average DOL, and the DOL estimated from the difference in the maxima of the unlabeled and labeled antibody.

The DOL values of nanobody conjugates were determined from the electrospray ionization mass-spectrometry (ESI-MS) measurements. The spectra were used to calculate the DOL values directly by subtracting the detected mass of the nanobody conjugate from the reference (unlabeled nanobody); no further analysis was required. ES-MS spectra of the labeled nanobodies are presented in Figure S24.

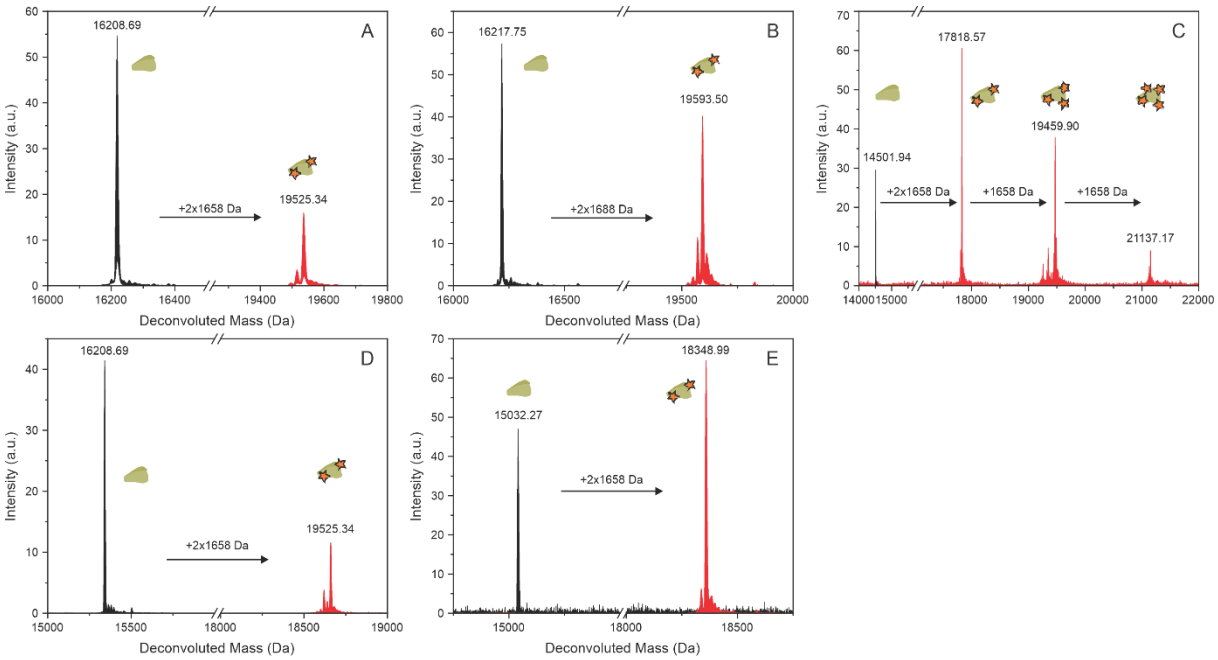


Figure S24. Mass-spectrometry data (ESI-MS) of all labeled nanobodies NBm-**fDAE5** (A), NBm-**fDAE6** (B), NBr-**fDAE5** (C), NBg2b-**fDAE5** (D), NBg2a-**fDAE5** (E).

Immunofluorescence with antibodies or nanobodies. U2OS or U2OS-Vim-rsEGFP2 cells were grown for 12-72 h on glass coverslips. After washing twice with PBS (pH 7.4) the cells were fixed with methanol (–20 °C) for 10 min on ice, and finally washed twice with PBS. To reduce unspecific binding blocking buffer (2% BSA in PBS) was added and incubated for 30 to 60 min at room temperature. The coverslips were overlaid with the primary antibody solution diluted in a 1:1 solution of blocking buffer and PBS and incubated in a humid chamber for 1 h at room temperature, or overnight at 4 °C. Following this, the coverslips were washed with PBS (3×5 min). The coverslips were overlaid with the secondary antibody/nanobody solution diluted in a 1:1 solution of blocking buffer and PBS and incubated in a humid chamber for 45-60 min at room temperature. Lastly, coverslips were washed (3×5 min) and mounted with PBS (no additives were used), and sealed with a two-component silicone resin (Picodent Twinsil, Picodent Dental-Produktions- und Vertriebs-GmbH). The same procedure was used for anti-GFP nanobodies, on cell line expressing GFP, omitting the step with the primary antibody.

Depending on the target species of the secondary antibody/nanobody, immunostainings of the microtubules were carried out with a 1:20 dilution of anti-alpha tubulin primary antibody from rabbit (Abcam ab18251) or 1:500 dilution of anti-alpha tubulin primary antibody from mouse (Synaptic Systems 302 211). Immunostaining of the vimentin filaments was performed with anti-vimentin EPR3776 primary antibody from rabbit (Abcam ab92547).

Confocal microscopy. Confocal images were acquired on an Abberior STED microscope (Expert Line, Abberior Instruments) at the Optical Microscopy Facility of the Max Planck Institute for Medical Research. Imaging was performed with 405 nm activation and 561 nm excitation with a detection window of 580-800 nm. Illumination times were adjusted according to the samples and switching properties of the dyes between 10-50 µs for activation, and 100-1000µs for excitation.

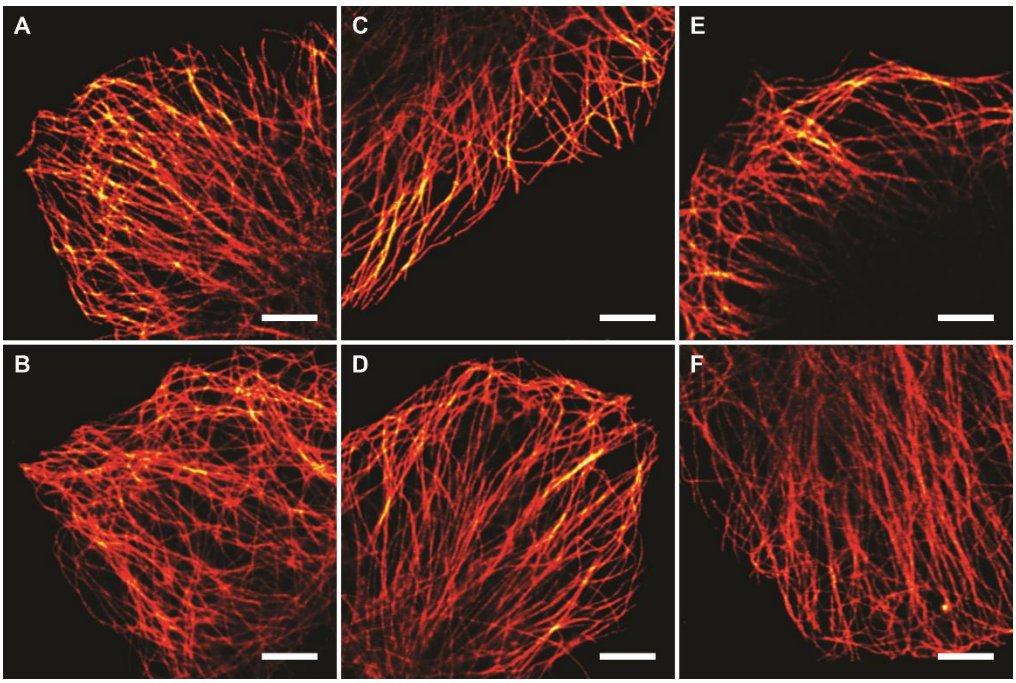


Figure S25. Confocal images of U2OS labeled with primary antibodies against tubulin, and secondary antibodies labelled with **fDAE1** (A), **fDAE2** (B), **fDAE3** (C), and **fDAE4** (D), and secondary nanobodies labelled with **fDAE5** (NBr) (E), **fDAE6** (NBm) (F). Samples were mounted in PBS, without any additives. Scale bars: 5 μm.

Superresolution (single molecule localization) microscopy. Images were acquired on a custom-built setup, equipped with a 532 and a 560 nm (1 W) laser for excitation, a 405 nm (300 mW) laser for activation (when necessary), a back illuminated EMCCD camera (Andor iXon 897 / 512×512 sensor), and a Leica HCX PL APO CS 100x/1.46 oil lens. Emission light was separated from the excitation and activation light with a dichroic mirror (Semrock Di02-R561), and further filtered with two consecutive emission filters (Semrock FF01-665/150), placed in front of the camera. A movable mirror was used to switch between wide field, highly inclined and laminated optical sheet (HILO) and total internal reflection fluorescence (TIRF) illumination modes. Images were acquired with a 20-50 ms exposure time, and actual excitation laser powers

in the back focal plane of 10-150 mW, depending on the dye and sample properties. Images were acquired without activation for thousands of frames; when the numbers of activation events per frame were too low (Figure S26, S27), the 405 nm activation laser was incorporated as 200-500 µs pulsed, in between frames with a power of 0.1-1 mW in the back focal plane.

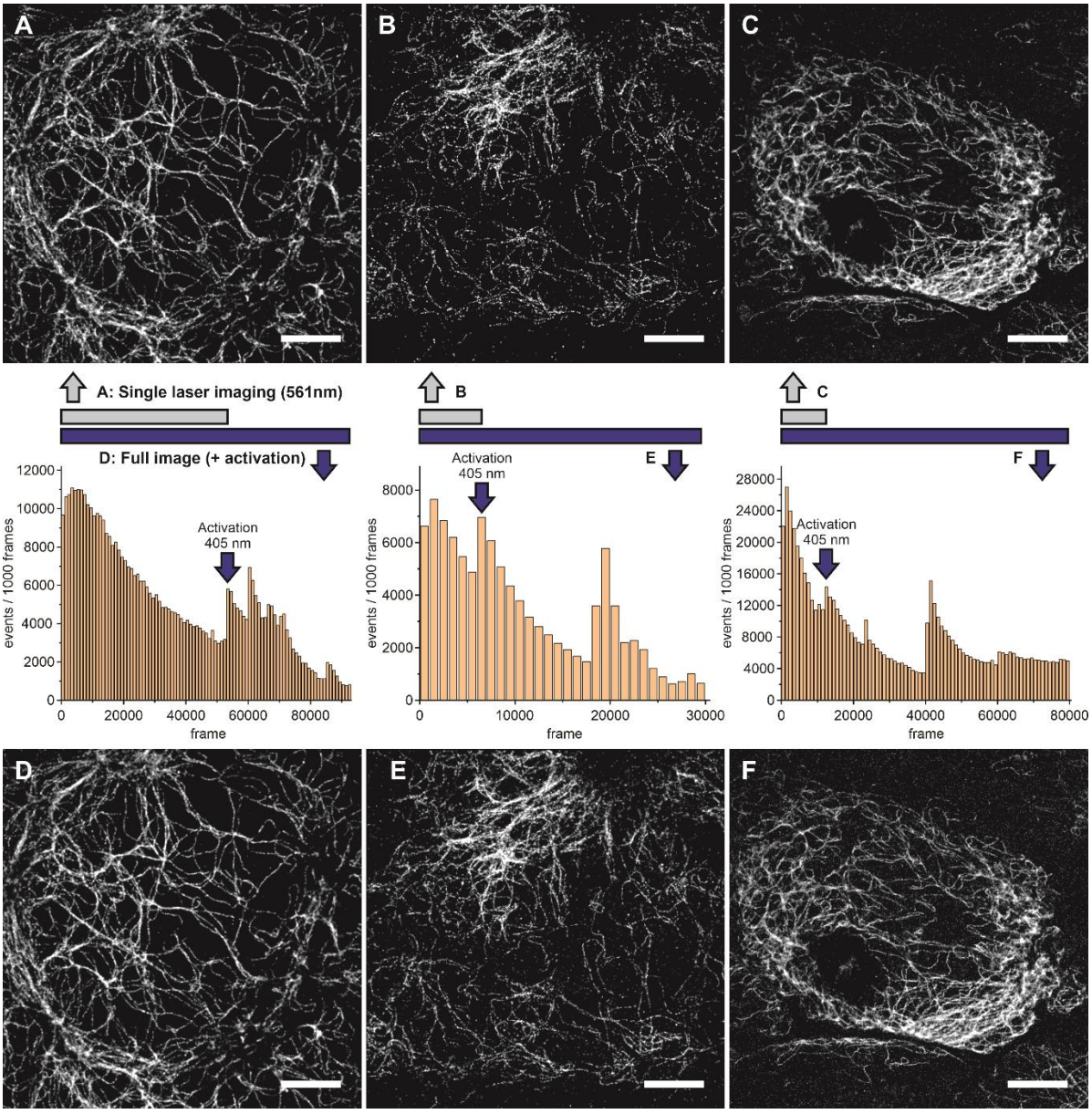


Figure S26. Superresolution images of U2OS cells expressing rsEGFP2 in vimentin and labelled with a primary and a secondary antibody (A, D), a primary antibody and a secondary (NBr) nanobody (B, E), and with two anti-GFP nanobodies (NBg2a and NBg2b, 1:1 mixture, C, F). Samples were mounted in PBS, without any additives. Images D-F are the same as in Figure 2C-E, and shown again here for a fair comparisson with the images renderd without activation with 405 nm. The number of localization over the measurments are shown in the middle, with a mark

at the time the violet laser (405 nm) was switched on to increase activation. Images on top (A-C) were renderd only with localized events activated by Urbach-tail effect (single laser activation/excitation, 561 nm), and images on the bottom (D-F) were renderd using all events. Following spikes (increase in localized events) correspond to an increase on the 405 nm laser. Scale bars: 4 μm.

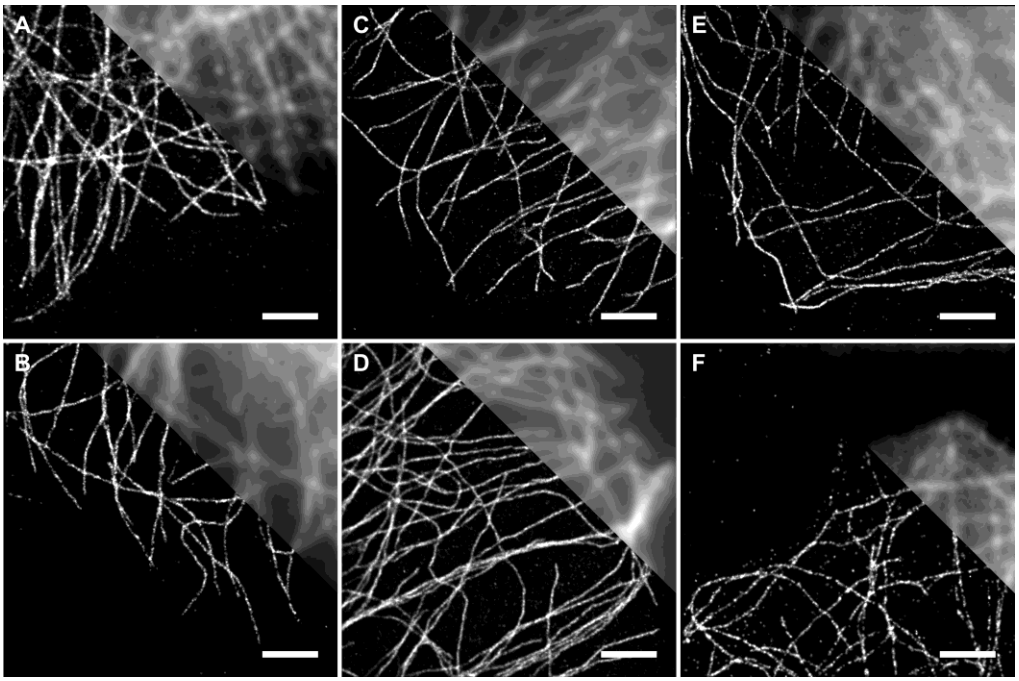


Figure S27. Superresolution images of U2OS labeled with primary antibodies against tubulin, and secondary antibodies labelled with **fDAE1** (A), **fDAE2** (B), **fDAE3** (C), and **fDAE4** (D), and secondary nanobodies labelled with **fDAE5** (NBm) (E), **fDAE6** (NBm) (F). The upper-right corner shows a pseudo wide-field image (all frames add-up). Samples were mounted in PBS, without any additives. Excitation was performed at 532 nm (A, B, D, F) or at 561 nm (C, E). Scale bars: 2 μm.

Image analysis and post-processing. All images were processed using ImageJ (1.52p). For the analysis and post-processing of single molecule localization microscopy images the ThunderSTORM plugin was used. All images are displayed as normalized Gaussians with a magnification factor of 25.0. Camera settings were set to 100 nm pixel size, 7.25 photoelectrons per A/D count and 1.0 quantum efficiency. EM gain (50-300) and the base level were adjusted according to the corresponding measurement conditions. STORM images were filtered using a wavelet filter (B-spline) with an order of 3 and a scale of 2. For the approximate localization of the molecules the local maximum approach with 8-connected neighborhoods was used and the

intensity threshold was set to 1.6 times the standard deviation of the 1st wavelet. For sub-pixel localization of molecules, an integrated Gaussian PSF model with a fitting radius of 3 pixels and the maximum likelihood fitting method was used. To determine the approximate initial sigma, ThunderSTORM analysis was carried out with the default settings on multiple images and the mean value of 1.3 pixels was set as the initial sigma for further analysis. As the first step of post-processing, a drift correction based on cross correlation with a number of bins according to the acquired number of frames (10000 to 80000) was applied. The next step was merging localizations within 1 pixel with maximum 2 off frames allowed. A filter was applied to the sigma to converge to a normal Gaussian distribution function and any localization with an uncertainty of above half a pixel was neglected. As the final step, a density filter was applied to remove noise caused by isolated localizations within 50 nm according to the sample density.

Full width on half maximun (FWHM) of vimentin filaments. Line-profiles were traced on reconstructed single molecule localization microscopy images displayed as histograms with a pixel size of 4 nm. For each image, 20 ROIs containing a single filament were manually selected, with approximate sizes of 400 nm x 400 nm. The line-profile was traced transversal to the filament, averaged through the whole ROI/filament, and fitted to a 1D-Gaussian function to calculate the FWHM.

Urbach tail absorption of the OF

Acetonitrile solutions were irradiated in a home-made setup [1], with variable LED sources, in combination with 10 nm band-pass filters, centered at 530 nm, 515 nm, 508 nm, 470 nm, 460 nm, and 450 nm. After the corresponding photo-stationary state was reached, the absorption and emission was recorded. At the end of the experiment, complete conversion to the CF was obtained by irradiation with 405 nm (**fDAE3-6**), or 365 nm (for **fDAE1** and f**DAE2**), and verified by HPLC measurements. During the irradiation, samples were kept at 20 °C and continuously stirred. The conversion at each irradiation wavelength, was calculated by the ratio of the absorption (or emission) of the CF in the maximum, with respect to full conversion obtained with violet/UV light.

Isomerization quantum yields

Acetonitrile or aqueous buffered solutions were irradiated in a home-made setup[4], with 405 nm or 365 nm light, for the ring-closing reaction, and with 530 nm or 505 nm light, for the ring-opening reaction. Absorption and emission spectra were recorded at set irradiation intervals (ca. 1-120 s),

and the isomerization quantum yields were calculated from numerical fits of the transients obtained at the absorption maxima of the CF. During the irradiation, samples were kept at 20 °C and continuously stirred with a Peltier-based temperature control (Luma 40, Quantum Northwest, Inc.).

Absorption and emission spectra

Absorption spectra (Cary Series UV-Vis-NIR Spectrophotometer, Agilent Technologies) were recorded in diluted acetonitrile solutions (2-5 μM). The samples were irradiated until the photo-stationary state was reached, with 365 nm light (**fDAE1-2**) or with 405 nm light for the rest of the compounds, to record the absorption of the CF. Full conversion was confirmed by HPLC experiments. The emission of the CF was recorded at low conversion (i.e. absorption at the maximum of the CF < 0.05).

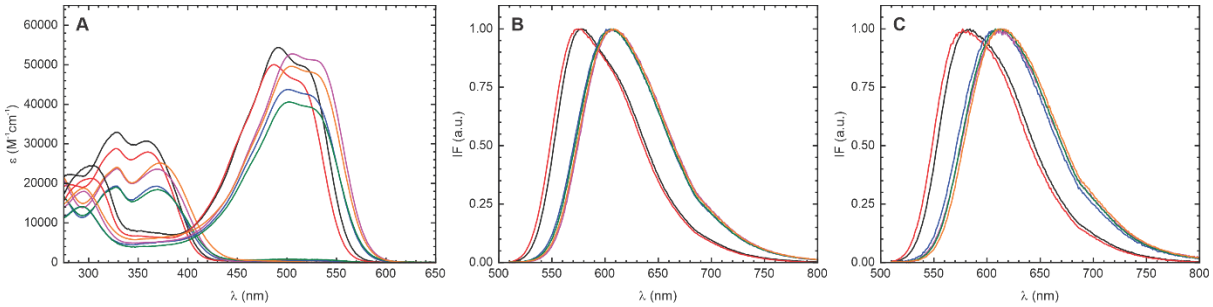


Figure S28. (A) Absorption coefficients in MeCN and emission of the close forms in (B) MeCN solution, and (C) in aqueous buffered solutions (phosphate buffer, 100 mM, pH = 7.0). Color code: **fDAE1** (red), **fDAE2** (black), **fDAE3** (blue), **fDAE4** (purple), **fDAE5** (green) and **fDAE6** (orange).

Movie S1 Legend

Wide-Filed imaging of vimentin filaments stained with primary/secondary antibodies labelled with fDAE5. The sample is illuminated only with a 561 nm laser. Initially, pre-activated markers are observed. They are progressively switched off, until a single molecule regime is observed under activation induced by 561 nm light (Urbach-tail effect). In the last frame, an add-up of the following 10000 frames is shown. Figure 2C was reconstructed from the localizations in the data set used to create this movie. The movie is displayed at a speed of 100 frames/second.

Supplementary References

[1] Uno, K.; Niikura, H.; Morimoto, M.; Ishibashi, Y.; Miyasaka, H.; Irie, M. In Situ Preparation of Highly Fluorescent Dyes upon Photoirradiation. *J. Am. Chem. Soc.,* **2011**, *133*, 13558-13564.

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[3] Yamaguchi, T.; Irie, M. Photochromism of Diarylethene Derivatives Having n-Alkylbenzothiophene and n-Alkylbenzofuran Units. *Bull. Chem. Soc. Jpn.* **2006**, *79,* 1100–1105.

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