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Supporting Information

Carboxylated Photoswitchable Diarylethenes for Biolabeling and Super-Resolution RESOLFT Microscopy

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Supporting information

1. Abbreviations

The following abbreviations are used in the text of the ESI file: anti-parallel (ap), aqueous (aq.), argon (Ar), bis(pinacolato)diboron ($b(pin)_2$), closed form (CF), degree of labeling (DOL), diarylethene (DE), dichloromethane (DCM), *N,N′*-dicyclohexylcarbodiimide (DCC), 2-dicyclohexylphosphino-2′,6′-dimethoxybiphenyl (SPhos), 4-(dimethylamino)pyridine (DMAP), dimethylformamide (DMF), dimethylsulfoxide (DMSO), electrospray ionization (ESI), ethyl acetate (EtOAc), high performance liquid chromatography (HPLC), high resolution mass spectrometry (HR-MS), Matrix-assisted laser desorption/ionization time of flight (MALDI-TOF), minutes (min), *N*-hydroxysuccinimide (NHS), nuclear magnetic resonance (NMR), open form (OF), parallel (p), phosphate buffer saline (PBS), potassium acetate (KOAc), reverse phase (RP), room temperature (r.t.), tetrahydrofuran (THF), triethylamine (TEA), trifluoroacetic acid (TFA), thin layer chromatography (TLC), ultra-violet (UV), visible (vis).

2. General remarks

2.1 Chemicals and reagents

Flash column chromatography was performed using cartridges from Interchim (PF-SIHC, 15 µM, 25 g or 40 g SiO_2) or Teledyne Isco (RediSep®Rf, 35 µM, 24 g or 40 g SiO_2). Analytical TLC was performed on Merck Millipore ready-to-use plates with silica gel 60 (F_{254}). The spots were visualized by illumination with a UV lamp (λ = 254 and 365 nm) and/or staining with aq. $KMnO_4$ solution. Anhydrous DMF were purchased from Sigma-Aldrich and stored over 4 Å molecular sieves. HPLC gradient-grade acetonitrile (CH_3CN) was obtained from Sigma-Aldrich. Aqueous buffers (PBS (pH 6.5) and $NaHCO_3$ (pH 8.3) used for the preparation of bioconjugates and in the fluorescence assay) and mobile phases for HPLC were prepared with water purified by means of an ELGA system. Unless stated otherwise, all chemicals were used as received from commercial sources without further purification. The following starting material were synthesized according to literature procedures: 1,2-bis(2-ethyl-6-iodobenz[a]thiophen-1,1-dioxide-3-yl)perfluorocyclo-pentene[1], 3,5-di(*tert*-butoxycarbonylphenyl)boronic acid pinacol ester[2] and *tert*-butyl iminodiacetate.[3] 3,5-dicarboxyphenylboronic acid pinacol ester, 5-boronoisophtalic acid and 3,5-diformylphenylboronic acid were respectively purchased from Fluorochem, Apollo Scientific and Sigma Aldrich. Secondary antibodies (AffiniPure Sheep Anti-Mouse IgG (H+L)) were obtained from Jackson ImmunoResearch Laboratories, Inc. PD-10 desalting columns used for the isolation of the conjugates with antibodies were purchased from GE Healthcare Europe GmbH.

2.2 Instruments and methods

^1H, ^{19}F and ^{13}C NMR spectra were recorded at 25 °C on an Agilent 400-MR or 500-MR spectrometer. Chemical shifts are given in parts per million (ppm) using the residual solvent peak(s) as a reference.[4] Multiplicities of the signals are described as follows: s = singlet, d =

doublet, t = triplet, q = quartet, m = multiplet or overlap of non-equivalent resonances. *J* values are expressed in Hz. The NMR spectra of hydrophilic diarylethenes **1** and **2** were recorded in DMF-d_7 due to poor solubility of these dyes in common solvents and their reaction with DMSO-d_6 and traces of water in it (leading to a compound with a -CF_2CF_2CO- fragment instead of the initial -$(CF_2)_3$- bridge; an analog of the transformation observed in the oxidation according to Swern). Each diarylethene derivatives **1**, **2**, **5**, **6** and **7** presented in this article was obtained as a mixture of three forms (two open forms (OF): anti-parallel (ap) and parallel (p); and the closed isomer (CF)). These isomers could be detected separately by NMR spectroscopy. The anti-parallel (ap) and parallel (p) forms of the "open" isomers gave one peak in HPLC (and could not be isolated separately due to rapid interconversion). We managed to isolate both closed isomers of **1** and **2** after irradiation at 365 nm (see experimental procedure in section 3 and NMR spectra in section 6). Analytical and preparative RP-HPLC were carried out with a Knauer Smartline system equipped with a Dionex Ultimate 3000 detector. Automated flash purifications on regular silica gel and reversed phase (RP-C18) cartridges were performed with a Biotage Isolera One device. Mass-spectra with electro-spray ionization (ESI-MS) were recorded on a Varian 500-MS spectrometer (Agilent). High-resolution ESI-MS (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. MALDI-TOF measurement were performed using an autoflex speed mass spectrometer (Bruker) and sinapinic acid as a matrix. The absorption spectra were recorded on a Varian Cary 4000 UV-Vis spectrophotometer. The emission spectra were recorded on a Varian Cary Eclipse fluorescence spectrophotometer. The relative fluorescence quantum yields were measured at 25 °C using fluorescein in NaOH (0.1 M) as the reference fluorescent dye with emission efficiency of 0.79.

Determination of the quantum efficiencies of the isomerization reactions were performed in a home-made setup. Irradiation was performed in 1 cm path quartz cuvettes under continuous stirring, using LEDs at 365 ± 4 nm and 470 ± 12 nm (Thorlabs) as irradiation sources. The intensities of the irradiation light were determined using Azobenzene in methanol (365 nm) and Aberchrome 670 in toluene (470 nm), as chemical actinometers. Absorption and emission of the solutions were monitored after each short irradiation step until the photostationary state was reached. Data analysis was performed as described in detail on pages 12-14 of *Supporting Information* to ref. [3] in the main text (http://www.wiley-vch.de/contents/jc_2002/2006/z602591_s.pdf).

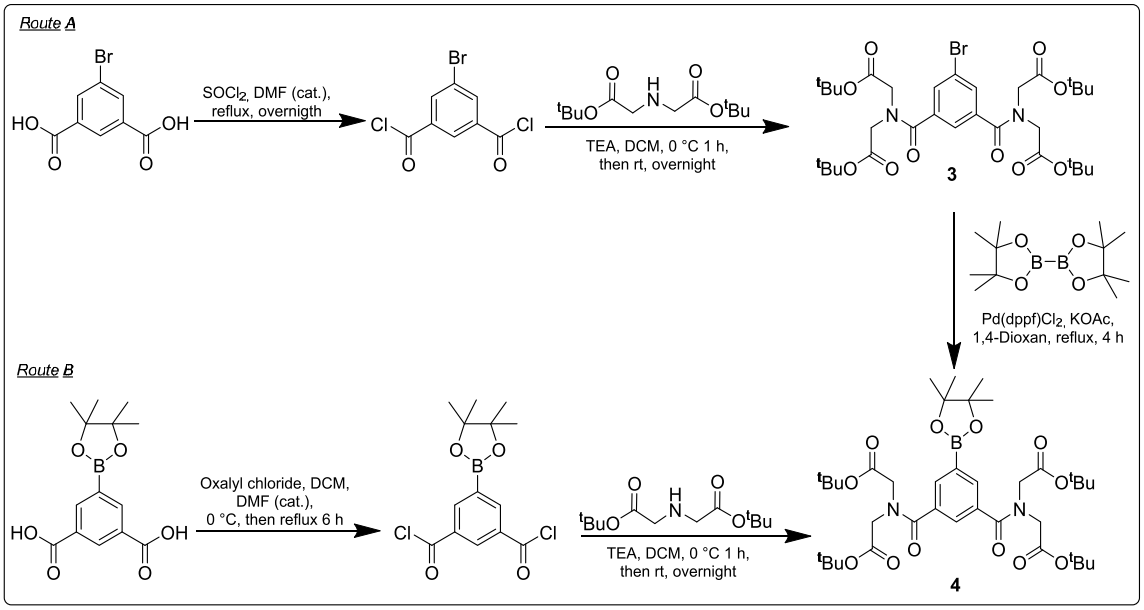
2.3 High-performance liquid chromatography separations

Several columns and solvent systems were used for the analytical and preparative HPLC separations. **System A:** RP-HPLC (Kinetec C_{18} 100 column, 5 µm, 4.6×250 mm) with CH_3CN and 0.05% aqueous trifluoroacetic acid (0.05% aq. TFA, pH ~ 2.0) as eluents [linear gradient from 40 % to 100% of CH_3CN in 20 min] at a flow rate of 1.2 mL/min; UV-vis detection with diode array and at 254 nm and 450 nm. **System B:** see System A; linear gradient from 30% to 100% of CH_3CN in 20 min. **System C:** preparative RP-HPLC (Kinetec C_{18} 100 column, 5 µm, 20×250 mm) with a linear gradient from 60% to 80% of CH_3CN in 20 min at a flow rate of 10 mL/min and detection at 254 nm. **System D:** automated flash

purification on Biotage Isolera One (ISO-1EW) device (cartridge PF-C_{18}-HC, 30 µM, with 20 g of RP silica gel) with the following eluent: 0.05% aq. TFA / CH_3CN, 3:2, at a flow rate of 20 mL/min for 15 min; UV detection at 254 nm. **System E:** see System C; linear gradient from 35% to 65% of CH_3CN in 20 min, dual detection at 254 nm (for detection of OF) and 450 nm (for detection of CF). **System F:** see System C; linear gradient from 50% to 75% of CH_3CN in 15 min, dual detection at 254 nm and 450 nm.

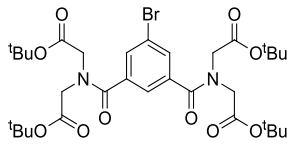
3. Synthesis

3.1 Peparation of boronic esters



Scheme S1. Preparation of 3,5-disubstituted phenyl boronates.

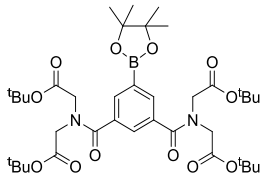
Tetra-*tert*-butyl 5-bromobenzene-1,3-bis-(*N*-iminodiacetate) (3)



To a solution of 5-bromoisophtalic acid (610 mg, 4.1 mmol), $SOCl_2$ (3 mL, 40.1 mmol, 10 equiv.) and few drops of DMF were added, and the reaction mixture was refluxed overnight with stirring. An excess of thionyl chloride was removed by distillation, the residue co-evaporated twice with DCM was used directly in the next step. This acyl chloride was dissolved in 35 mL of dry DCM, and a solution of TEA (1.65 mL, 12.2 mmol, 3 equiv.) and *tert*-butyl iminodiacetate[3] (2.1 g, 8.6 mmol, 2.1 equiv.) was added dropwise at 0 °C. After stirring overnight at r.t., the mixture was filtered, the filtrate was washed thrice with a saturated aq. solution of $NaHCO_3$ (3×35 mL), dried over $MgSO_4$, concentrated under reduced pressure and subjected to flash chromatography on silica gel (*n*-hexane/EtOAc, with a gradient from 100:0 to 80:20). Compound **3** was isolated as a white solid (1.74 g, 61% yield).

R_f (*n*-hexane/EtOAc, 4:1, v/v) = 0.42. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.64 (dd, J = 1.5 Hz, 0.7 Hz, 2 H), 7.43 (t, J = 1.4 Hz, 1 H), 4.15 (s, 4 H), 3.90 (s, 4 H), 1.48 (s, 18 H), 1.45 (s, 18 H). ^{13}C NMR (101 MHz, $CDCl_3$): δ = 169.7, 168.1, 167.7, 137.7, 131.4, 123.7, 123.1, 83.2, 82.4, 52.5, 48.5, 28.2, 28.1. ESI-MS, positive mode: m/z (rel. int., %) = 721.4 (100) [M+Na, ^{79}Br]$^+$, 723.4 (100) [M+Na, ^{81}Br]$^+$ (found), 722.6 (calculated for $C_{32}H_{47}BrN_2NaO_{10}$, [M+Na, ^{81}Br]$^+$).

Tetra-*tert*-butyl benzeneboronic acid pinacol ester 3,5-bis-(*N*-iminodiacetate) (4)



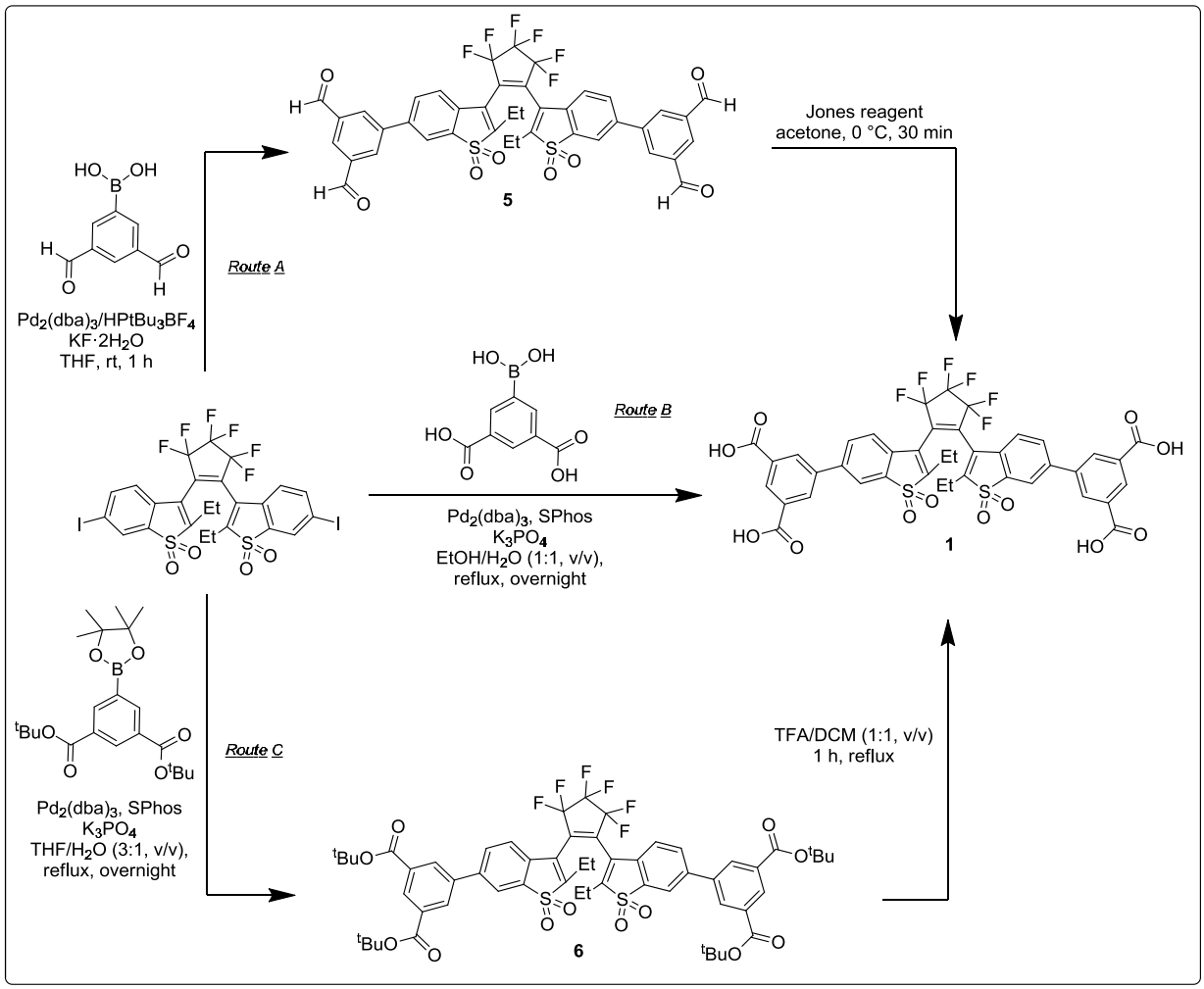
From tetra-*tert*-butyl 5-bromobenzene-1,3-bis-(*N*-iminodiacetate) (**3**)

In a sealed tube purged with Ar, compound **3** (200 mg, 0.29 mmol), bis-pinacolatodiboron ($b(pin)_2$; 87 mg, 0.34 mmol, 1.2 equiv.), KOAc (84 mg, 0.86 mmol, 3 equiv.), $PdCl_2$(dppf) (7 mg, 8.6 µmol, 0.03 equiv.) was combined, and dry 1,4-dioxan (4 mL) was added. The reaction mixture was purged with Ar for further 5 min (Ar bubbling) and stirred at reflux (bath temp. 120 °C) for 4 h. After removal of volatile materials in vacuum, EtOAc (30 mL) was added, and the reaction mixture was washed with brine (2×30 mL), dried over Na_2SO_4, concentrated under reduced pressure and subjected to column chromatography on silica gel (n-hexane/EtOAc, with a gradient from 95:5 to 50:50) to afford compound **4** as a white solid (80 mg, 37% yield).

From 3,5-dicarboxyphenylboronic acid, pinacol ester

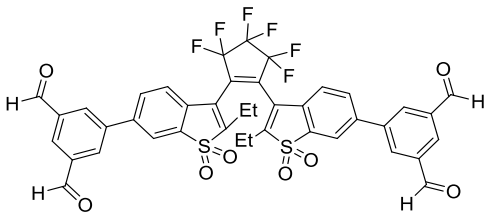
The synthesis of aromatic acyl chloride (route B in Scheme 1) was performed according to the published procedure.[5] A suspension of 3,5-dicarboxyphenylboronic acid, pinacol ester (200 mg, 0.69 mmol, Fluorochem) in 20 mL of dry DCM was treated with dry DMF (≈ 20 µL) and cooled at 0 °C. Then, oxalyl chloride (294 µL, 3.4 mmol, 5 equiv.) was added dropwise, and the reaction mixture was refluxed for 6 h. After concentration in vacuum, the acyl chloride was dissolved in dry DCM (20 mL), and a solution of TEA (475 µL, 3.4 mmol, 5 equiv.) and *tert*-butyl iminodiacetate[3] (352 mg, 1.44 mmol, 2.1 equiv.) was added dropwise at 0 °C under Ar. After stirring overnight at r.t., the reaction mixture was washed with brine (3×25 mL), dried over $MgSO_4$, concentrated in vacuum and purified by flash chromatography on silica gel (*n*-hexane/EtOAc, with a gradient from 95:5 to 50:50) to afford compound **4** as a white solid (164 mg, 32% yield). R_f (*n*-hexane/EtOAc, 4:1, v/v) = 0.35. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.92 (d, J = 1.7 Hz, 2 H), 7.57 (t, J = 1.7 Hz, 1 H), 4.17 (s, 4 H), 3.93 (s, 4 H), 1.49 (s, 18 H), 1.46 (s, 18 H), 1.28 (s, 12 H). ^{13}C NMR (101 MHz, $CDCl_3$): δ = 171.4, 168.3, 168.0, 135.3, 134.5, 127.7, 84.3, 82.9, 82.1, 52.5, 48.4, 28.3, 28.2, 24.9. ESI-MS, positive mode: m/z (rel. int., %) = 769.6 (100) [M+Na]$^+$ (found), 769.7 (calculated for $C_{38}H_{59}BN_2NaO_{12}$, [M+Na]$^+$).

3.2 Diarylethene derivatives – "tetra-acid" 1 and "octa-acid" 2



Scheme S2. Syntheses of "tetra-acid" **1**

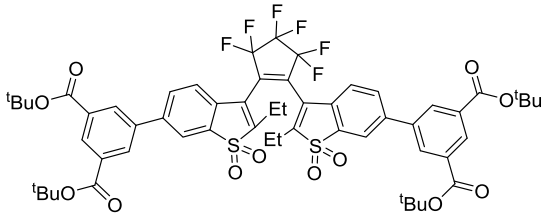
"Tetra-aldehyde" (5)



A suspension of $Pd_2(dba)_3$/P(*t*Bu)$_3$*HBF$_4$ (1:1.2, 74 mg, 61.5 μmol of $Pd_2(dba)_3$, 10 mol %), 3,5-diformylphenylboronic acid (Sigma Aldrich, 241 mg, 1.4 mmol, 2.2 equiv), and KF·2H$_2$O (382 mg, 4.1 mmol, 6.6 equiv.) in 10 ml of THF was purged with Ar for 3 min (Ar "bubbling"). Then 1,2-bis(2-ethyl-6-iodobenz[a]thiophen-1,1-dioxide-3-yl)perfluorocyclopentene[1] (500 mg, 0.62 mmol) was added, and the reaction mixture stirred at r.t. for 1 h. Then, the crude mixture was diluted with Et_2O (2 mL), filtered through a plug of silica gel and washed with Et_2O (2×50 mL). The filtrate was concentrated, and the residue was purified by flash chromatography on silica gel (*n*-hexane/EtOAc, with a gradient from

95:5 to 35:65) to afford compound **5** as a yellow solid (163 mg, 32% yield). R_f (*n*-hexane/EtOAc, 3:2, v/v) = 0.24. *ap:p* = 60:40. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 10.23 (s, 0.2 H, *p*), 10.22 (s, 0.5 H, *ap*), 10.20 (s, 2 H, *ap*), 10.16 (s, 1.3 H, *p*), 8.45 (t, *J* = 1.5 Hz, 1.2 H, *ap*), 8.40 (t, *J* = 1.5 Hz, 0.8 H, *p*), 8.37 (d, *J* = 1.5 Hz, 2.4 H, *ap*), 8.29 (d, *J* = 1.5 Hz, 1.6 H, *p*), 8.06 (d, *J* = 1.6 Hz, 1.2 H, *ap*), 8.01 (d, *J* = 1.6 Hz, 0.8 H, *p*), 7.92 (dd, *J* = 8.0 Hz, 1.7 Hz, 1.2 H, *ap*), 7.76 (dd, *J* = 8.0 Hz, 1.7 Hz, 0.8 H, *p*), 7.34 (d, *J* = 7.9 Hz, 1.2 H, *ap*), 7.30 (d, *J* = 7.9 Hz, 0.8 H, *p*), 2.65 (m, 2.8 H, *p/ap*), 2.50 (m, 1.2 H, *p*), 1.45 (t, *J* = 7.6 Hz, 2.4 H, *p*), 1.17 (t, *J* = 7.6 Hz, 3.6 H, *ap*). ^{13}C NMR (126 MHz, $CDCl_3$): δ = 192.6 *p*, 192.4 *ap*, 192.4 *p*, 192.3 *ap*, 149.9 *p*, 149.6 *ap*, 142.0 *ap*, 141.8 *p*, 139.9 *ap*, 139.8 *p*, 138.5 *p*, 138.4 *p/ap*, 138.3 *p*, 136.9 *ap*, 136.7 *p*, 134.1 *ap*, 134.0 *p*, 133.9 *p*, 133.7 *ap*, 133.6 *p*, 129.4 *p*, 129.2 *ap*, 129.1 *ap*, 129.0 *p*, 125.4 *p*, 125.2 *ap*, 123.1 *p/ap*, 121.5 *ap*, 121.4 *p*, 19.4 *p*, 19.3 *ap*, 12.0 *p*, 11.8 *ap*. ^{19}F NMR (376 MHz, $CDCl_3$): δ = –109.76 (t, *J* = 5.0 Hz, 1.15 F, *ap*), –109.81 (t, *J* = 4.6 Hz, 1.15 F, *ap*), –109.88 (t, *J* = 5.8 Hz, 1.7 F, *p*), –132.04 (m, 1.6 F, *p/ap*), –132.26 (m, 0.4 F, *p*). HR-MS (ESI, positive mode): 847.0852 $[M+Na]^+$ (found), 847.0865 (calculated for $C_{41}H_{26}F_6NaO_8S_2$, $[M+Na]^+$).

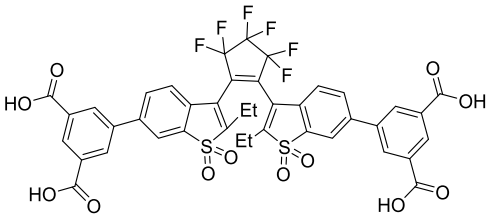
"Tetra-*tert*-butyl ester" (6)



To a solution of 1,2-bis(2-ethyl-6-iodobenz[a]thiophen-1,1-dioxide-3-yl)perfluorocyclopentene[1] (50 mg, 61.6 µmol) in a mixture of THF/H_2O (4 mL, 3:1, v/v), 3,5-di(tert-butycarboxyphenyl)boronic acid, pinacol ester[2] (52 mg, 0.13 mmol, 2.1 equiv.), K_3PO_4 (40 mg, 0.19 mmol, 3 equiv.), SPhos (1.5 mg, 3.7 µmol, 6 mol %) and $Pd(dba)_2$ (1.1 mg, 1.9 µmol, 3 mol %) were added. The suspension was purged for 5 min with Ar (Ar "bubbling") and stirred 4 h at reflux (≈ 80 °C). Then, the mixture was diluted in EtOAc (15 mL), washed with brine, dried over Na_2SO_4 and concentrated in vacuum. The residue was separated by flash chromatography on silica gel (*n*-hexane/EtOAc, with a gradient from 100:0 to 90:10) to afford compound **6** as an yellow solid (35 mg, 51% yield). R_f (*n*-hexane/EtOAc, 9:1, v/v) = 0.30. *ap:p* = 55:45. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.63 (t, *J* = 1.5 Hz, 1.1 H, *ap*), 8.57 (t, *J* = 1.5 Hz, 0.9 H, *p*), 8.35 (d, *J* = 1.5 Hz, 2.2 H, *ap*), 8.27 (d, *J* = 1.5 Hz, 1.8 H, *p*), 8.03 (d, *J* = 1.5 Hz, 1.1 H, *ap*), 7.97 (d, *J* = 1.5 Hz, 0.9 H, *p*), 7.89 (dd, *J* = 8.0, 1.7 Hz, 1.1 H, *ap*), 7.73 (dd, *J* = 8.0, 1.7 Hz, 0.9 H, *p*), 7.31 (d, *J* = 8.0 Hz, 1.1 H, *ap*), 7.26 (t, *J* = 4.0 Hz, 0.9 H, *p*), 2.63 (m, 2.9 H, *ap/p*), 2.45 (m, 1.1 H, *p*), 1.64 (s, 21 H, *ap/p*), 1.60 (s, 15 H, *ap/p*), 1.44 (t, *J* = 7.6 Hz, 2.7 H, *p*), 1.12 (t, *J* = 7.6 Hz, 3.3 H, *ap*). ^{13}C NMR (101 MHz, $CDCl_3$): δ = 164.6 *ap*, 164.6 *p*, 149.2 *p*, 148.8 *ap*, 142.8 *ap*, 142.8 *p*, 140.2 *ap/p*, 138.4 *ap*, 138.4 *p*, 136.8 *ap*, 136.8 *p*, 133.6 *ap*, 133.5 *p*, 132.5 *ap*, 132.3 *p*, 131.6 *ap*, 131.6 *p*, 130.8 *ap*, 130.8 *p*, 128.9 *ap*, 128.8 *p*, 123.4 *ap/p*, 123.2 *ap*, 123.1 *p*, 123.0 *ap/p*, 121.3 *p*, 121.3 *ap*, 82.4 *ap*, 82.3 *p*, 28.3 *ap*, 28.3 *p*, 19.5 *p*, 18.4 *ap*, 12.0 *p*, 11.8 *ap*.^{19}F NMR (376 MHz, $CDCl_3$): δ = -109.80 (t, *J* = 4.9 Hz, 2.3 F, *ap/p*), -109.98 (t, *J* = 4.9 Hz, 1.7 F, *ap/p*), -132.05 (t, *J* = 4.8 Hz, 0.4 F, *p*), -132.11 (t, *J* = 4.7 Hz, 1.2 F, *ap*), -132.27 (t, *J* = 4.9 Hz, 0.4 F, *p*). ESI-MS, negative mode:

m/z (rel. int., %) = 1111.6 (100) $[M-H]^-$ (found), 1112.2 (calculated for $C_{57}H_{57}FO_{12}S_2$, $[M-H]^-$).

"Tetra-acid" (1)



From compound **5**

To a solution of compound **5** (135 mg, 0.16 mmol) in 90 mL of acetone, Jones reagent (1.64 mL, 3.27 mmol, 2 M solution in aq. H_2SO_4, 21 equiv.) was added dropwise at 0 °C; the reaction mixture was stirred for 30 min and then allowed to warm to r.t. After removal of volatile materials, the residue was taken in EtOAc, washed successively with H_2O and saturated aq. NaCl solution, dried over Na_2SO_4, and concentrated in vacuum. Then the crude mixture was purified by flash chromatography on silica gel (*n*-hexane/EtOAc + 5% HCO_2H, with a gradient from 95:5 to 0:100) to afford compound **1** as an orange-yellow solid (106 mg, 73% yield).

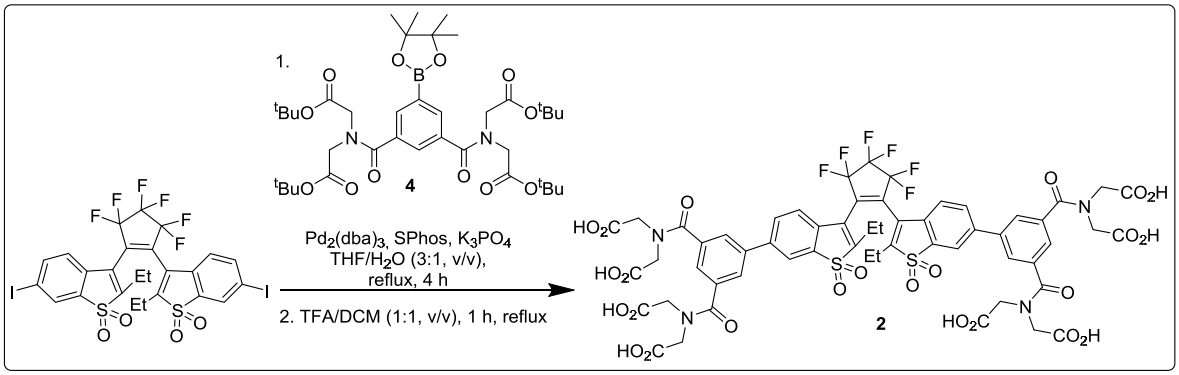
From compound **6**

Compound **6** (35 mg, 31.4 µmol) was dissolved in a mixture of TFA/DCM (5 mL, 1:1, v/v) and stirred for 1 h at reflux. Then the reaction mixture was concentrated in vacuum and subjected to flash chromatography using a RP-C18 cartridge (system D). The product-containing fractions were pooled and lyophilized to give compound **6** as an amorphous yellow solid (7 mg, 25% yield).

From 5-boronoisophtalic acid

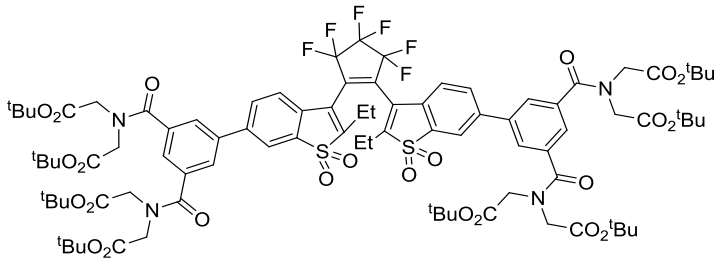
To a solution of 1,2-bis(2-ethyl-6-iodobenz[a]thiophen-1,1-dioxide-3-yl)perfluoro-cyclopentene[1] (50 mg, 61.6 µmol) in a mixture of EtOH/H_2O (8 mL, 1:1, v/v), 5-boronoisophtalic acid (Apollo Scientific, 27 mg, 0.13 mmol, 2.1 equiv.), K_3PO_4 (40 mg, 0.19 mmol, 3 equiv.), SPhos (1.5 mg, 3.7 µmol, 6 mol %) and $Pd(dba)_2$ (1.1 mg, 1.9 µmol, 3 mol %) were added. The suspension was purged for 5 min with Ar (Ar "bubbling") and stirred overnight at reflux (≈ 80 °C). Then, the mixture was concentrated in vacuum, and the residue was separated by flash chromatography on silica gel (*n*-hexane/EtOAc + 5% HCO_2H, with a gradient from 95:5 to 0:100) to afford compound **1** as an orange-yellow solid (25 mg, 46% yield). R_f (*n*-hexane/EtOAc + 5% HCOOH, 1:4, v/v) = 0.28. *ap:p* = 60:40. ^{1}H NMR (500 MHz, $CDCl_3$/CF_3COOD): δ = 8.89 (t, J = 1.5 Hz, 1.2 H, *ap*), 8.84 (t, J = 1.5 Hz, 0.8 H, *p*), 8.62 (d, J = 1.6 Hz, 2.4 H, *ap*), 8.51 (d, J = 1.6 Hz, 1.6 H, *p*), 8.12 (d, J = 1.7 Hz, 1.2 H, *ap*), 8.06 (d, 0.8 H, *p*), 7.96 (dd, J = 8.1 Hz, 1.7 Hz, 1.2 H, *ap*), 7.78 (dd, J = 8.0 Hz, 1.7 Hz, 0.8 H, *p*), 7.4 (d, J = 8.0 Hz, 1.2 H, *ap*), 7.33 (d, J = 8.0 Hz, 0.8 H, *p*), 2.60 (m, 2.7 H, *p/ap*), 2.49 (m, 1.3 H, *p*), 1.45 (t, J = 7.5 Hz, 2.3 H, *p*), 1.15 (t, J = 7.6 Hz, 3.6 H, *ap*). ^{13}C NMR (126 MHz, $CDCl_3$/CF_3COOD): δ = 170.8 *ap*, 170.7 *p*, 149.4 *ap*, 148.8 *p*, 141.8 *ap*, 141.7 *p*, 139.7 *ap*, 139.6 *p*, 136.6 *p*, 136.5 *ap*, 134.3 *ap*, 134.2 *p*, 133.1 *ap*, 132.6 *p*, 132.6 *ap*, 132.5 *p*, 130.6 *ap*,

130.5 *p*, 129.5 *p*, 129.5 *ap*, 125.0 *p/ap*, 123.9 *p*, 123.8 *ap*, 123.7 *p*, 123.5 *ap*, 121.7 *p/ap*, 19.6 *p*, 19.4 *ap*, 12.0 *p*, 11.8 *ap* (2 carbons are masked by solvent peaks). ^{19}F NMR (471 MHz, $CDCl_3$): δ = -109.86 (t, J = 4.5 Hz, 2.6 F, *ap*), -109.98 (t, J = 4.5 Hz, 0.7 F, *p*), -110.11 (t, J = 4.5 Hz, 0.7 F, *p*), -132.03 (m, 0.4 F, *p*), -132.19 (q, J = 4.5 Hz, 1.2 F, *ap*), -132.34 (m, 0.4 F, *p*). HR-MS (ESI, negative mode): 887.0698 $[M-H]^-$ (found), 887.0697 (calculated for $C_{41}H_{25}F_6O_{12}S_2$, $[M-H]^-$). HPLC (system A): t_R = 9.7 min (closed form) and 10.0 min (open form).



Scheme S3. The synthesis of "octa-acid" **2**

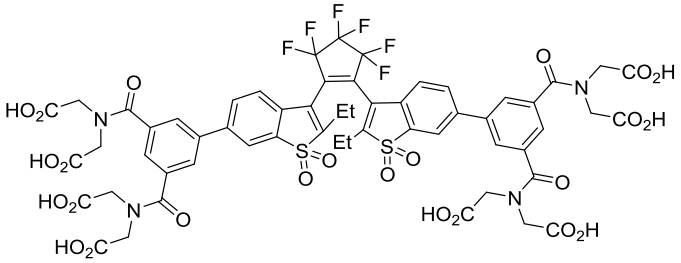
"Octa-acid" *tert*-butyl ester (7)



To a solution of 1,2-bis(2-ethyl-6-iodobenz[a]thiophen-1,1-dioxide-3-yl)perfluorocyclopentene[1] (80 mg, 0.099 mmol) in a mixture of THF/H_2O (4 mL, 3:1, v/v), arylboronic ester **4** (154 mg, 0.21 mmol, 2.1 equiv.), K_3PO_4 (63 mg, 0.30 mmol, 3 equiv.), SPhos (2.4 mg, 6 µmol, 6 mol %) and $Pd(dba)_2$ (1.7 mg, 3 µmol, 3 mol %) were added; the suspension was purged for 5 min with Ar (Ar "bubbling") and stirred 4 h at reflux. Then, the reaction mixture was diluted with EtOAc, washed with brine (3×25 mL), dried over Na_2SO_4 and concentrated. The residue was purified by flash chromatography on silica gel (*n*-hexane/EtOAc, with a gradient from 95:5 to 50:50) to afford compound **7** as a yellow solid (102 mg, 58% yield). R_f (*n*-hexane/EtOAc, 7:3, v/v) = 0.32. *ap:p* = 80:20. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.96 (d, J = 1.7 Hz, 1.6 H, *ap*), 7.89 (d, J = 1.7 Hz, 0.4 H, *p*), 7.83 (dd, J = 8.0, 1.7 Hz, 1.6 H, *ap*), 7.78 (d, J = 1.5 Hz, 3.2 H, *ap*), 7.71 (m, 1.2 H, *p/ap*), 7.57 (t, J = 1.4 Hz, 1.6 H, *ap*), 7.52 (t, J = 1.5 Hz, 0.4 H, *p*), 7.29 (d, J = 8.0 Hz, 1.6 H, *ap*), 7.20 (d, J = 8.0 Hz, 0.4 H, *p*), 4.24 (s, 0.7 H, *p/ap*), 4.21 (s, 2.5 H, *p/ap*), 4.18 (s, 2.6 H, *p/ap*), 4.15 (s, 1.7 H, *p/ap*), 4.14 (s, 0.8 H, *p/ap*), 3.95 (s, 6 H, *p/ap*), 3.91 (s, 1.7 H, *p/ap*), 2.58 (m, 2.4 H, *p/ap*), 2.35 (m, 1.6H, *p*), 1.50 (s, 14.4 H, *ap*), 1.50 (t, 1.4 H, *p*, masked by previous peak), 1.48 (s, 3.6 H, *p*), 1.38 (s, 14.4 H, *ap*), 1.33 (s, 3.6 H, *p*), 1.23 (s, 36 H, *p/ap*), 1.04 (t, J = 7.6 Hz, 4.6 H, *ap*). ^{13}C NMR (126 MHz, $CDCl_3$): δ = 170.6 *ap*, 170.5 *p*, 168.3 *p/ap*, 167.8 *p/ap*, 149.5 *p*, 149.1 *ap*, 142.2 *p/ap*, 139.0 *p*, 138.9 *ap*, 137.2 *ap*, 137.1 *p*, 137.0 *ap*, 136.9 *p*, 132.4 *ap*, 132.3 *p*, 129.2 *ap*, 128.9 *p*, 127.2 *p*,

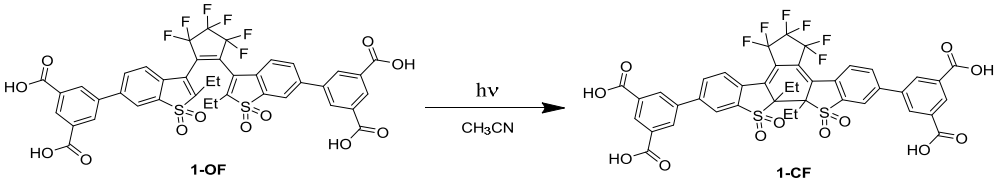
127.1 *ap*, 125.4 *p*, 125.4 *ap*, 123.5 *p/ap*, 123.0 *ap*, 122.9 *p*, 121.1 *p*, 120.9 *ap*, 83.3 *ap*, 83.1 *p*, 82.4 *ap*, 82.3 *p*, 75.2 *p/ap*, 52.6 *p/ap*, 48.6 *p/ap*, 28.3 *p/ap*, 28.1 *ap*, 28.0 *p*, 25.0 *p/ap*, 19.5 *p*, 19.2 *ap*, 12.0 *p*, 11.8 *ap*. ^{19}F NMR (376 MHz, $CDCl_3$): δ = -109.78 (t, J = 5.4 Hz, 1.6 F, *p/ap*), -109.85 (t, J = 5.3 Hz, 1.6 F, *p/ap*), -110.03 (t, J = 5.8 Hz, 0.8 F, *p/ap*), -132.19 (m, 2F, *p/ap*). ESI-MS, positive mode: m/z (rel. int., %) = 1796.3 (100) $[M+H]^+$ (found), 1797.0 (calculated for $C_{89}H_{109}F_6N_4O_{24}S_2$, $[M+H]^+$).

"Octa-acid" (2)



Compound **7** (102 mg, 57.8 µmol) was dissolved in a mixture of TFA/DCM (20 mL, 1:1, v/v) and stirred for 1 h at reflux. Then the reaction mixture was concentrated in vacuum and subjected to flash chromatography using a RP-C18 cartridge (system D). The product-containing fractions were pooled and lyophilized to give compound **2** as an amorphous yellow solid (54 mg, 71% yield). *ap:p* = 65:35. ^{1}H NMR (500 MHz, DMF-d_7): δ = 8.48 (d, J = 1.7 Hz, 1.3 H, *ap*), 8.38 (d, J = 1.7 Hz, 0.7 H, *p*), 8.23 (dd, J = 8.1, 1.8 Hz, 1.3 H, *ap*), 8.09 (dd, J = 8.1 Hz, 1.8 Hz, 0.7 H, *p*), 7.97 (d, J = 1.5 Hz, 2.4 H, *ap*), 7.92 (d, J = 8.1 Hz, 0.7 H, *p*), 7.88 (m, 2.7 H, *p/ap*), 7.55 (t, J = 1.5 Hz, 1.3 H, *ap*), 7.48 (t, J = 1.5 Hz, 0.8 H, *p*), 4.36 (s, 5.2 H, *ap*), 4.32 (s, 2.8 H, *p*), 4.30 (s, 5.2 H, *ap*), 4.24 (s, 2.8 H, *p*), 2.75 (m, 2 H, *p/ap*, masked partially by NMR solvent), 2.60 (m, 2 H, *p*), 1.42 (t, J = 7.6 Hz, 2.1 H, *p*), 1.03 (t, J = 7.6 Hz, 3.9 H, *ap*). ^{13}C NMR (126 MHz, DMF-d_7): δ = 171.6 *p*, 171.4 *ap*, 170.8 *ap*, 170.7 *p*, 170.6 *p/ap*, 149.6 *p*, 149.5 *ap*, 142.5 *ap*, 142.3 *p*, 138.8 *ap*, 138.5 *p*, 137.6 *ap*, 137.4 *p*, 136.7 *ap*, 136.5 *p*, 133.8 *ap*, 133.4 *p*, 128.8 *ap*, 128.5 *p*, 126.8 *ap*, 126.7 *p*, 125.7 *p/ap*, 125.0 *p/ap*, 123.1 *p/ap*, 121.2 *ap*, 121.0 *p*, 52.2 *p*, 52.1 *ap*, 48.4 *p*, 48.3 *ap*, 19.3 *p*, 19.1 *ap*, 11.9 *p*, 11.6 *ap*. ^{19}F NMR (376 MHz, DMF-d_7): δ = -110.82 (m, 2.7 F, *p/ap*), -110.98 (m, 1.3 F, *p*), -130.84 (m, 2 F, *p/ap*). HR-MS (ESI, negative mode): 673.0833 $[M-2H]^{2-}$ (found), 673.0851 (calculated for $C_{57}H_{45}F_6N_4O_{24}S_2$, $[M-2H]^{2-}$). HPLC (system B): t_R = 6.0 min (closed form) and 7.5 (open form).

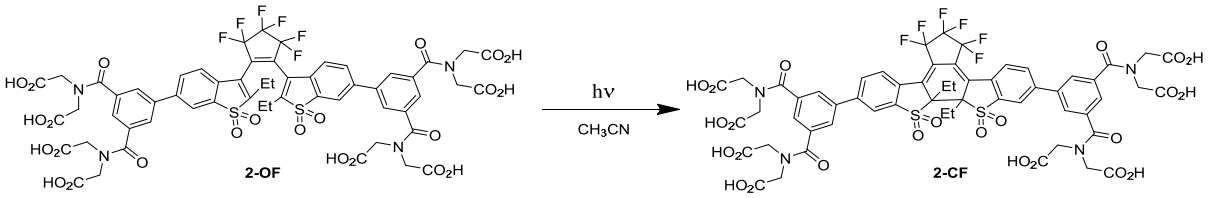
Preparation of pure closed isomer (1-CF)



A solution of compound **1-OF** (2 mg, 2.25 µmol) in CH_3CN (4 mL) was placed into a quartz cuvette for photolysis. Then the middle-pressure mercury lamp (150 W) was turned on, and the solution was irradiated at rt with stirring for 45 min, until compound **1-OF** was fully converted in compound **1-CF** (*checked for completion by RP-HPLC*). The solvent was

removed in vacuo and the residue was isolated by semi-preparative HPLC (system F). The product-containing fractions were pooled and lyophilized to give compound **1-CF** as an amorphous yellow solid (0.4 mg, 20% yield). ^{1}H NMR (500 MHz, DMF-d_7): δ = 8.64 (s, 2 H), 8.52 (dd, J = 8.7, 1.6 Hz, 2 H), 8.41 (d, J = 8.3 Hz, 2 H), 8.12 (d, J = 1.3 Hz, 4 H), 7.61 (s, 2 H), 4.37 (s, 8 H), 4.30 (s, 8 H), 2.45 (m, 4 H), 0.67 (t, J = 7.4 Hz, 6 H). 8.80 (t, J = 1.5 Hz, 1H), 8.72 (d, J = 1.5 Hz, 1H), 8.70 (d, J = 1.4 Hz, 2H), 8.60 (dd, J = 8.7, 1.8 Hz, 1H), 8.47 (d, J = 8.3 Hz, 1H), 2.53 – 2.40 (m, 2H), 0.69 (t, J = 7.4 Hz, 3H).

Preparation of pure closed isomer (2-CF)

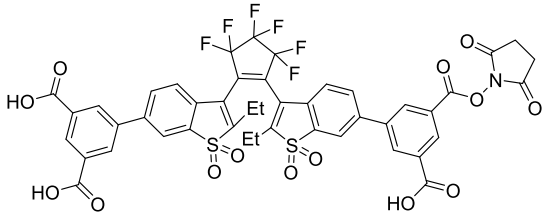


A solution of compound **2-OF** (2 mg, 1.48 µmol) in CH_3CN (4 mL) was placed into a quartz cuvette for photolysis. Then the middle-pressure mercury lamp (150 W) was turned on, and the solution was irradiated at rt with stirring for 45 min, until compound **2-OF** was fully converted in compound **2-CF** (*checked for completion by RP-HPLC*). The solvent was removed in vacuo and the residue was isolated by semi-preparative HPLC (system E). The product-containing fractions were pooled and lyophilized to give compound **2-CF** as an amorphous yellow solid (0.6 mg, 30% yield). ^{1}H NMR (500 MHz, DMF-d_7): δ = 8.64 (s, 2 H), 8.52 (dd, J = 8.7, 1.6 Hz, 2 H), 8.41 (d, J = 8.3 Hz, 2 H), 8.12 (d, J = 1.3 Hz, 4 H), 7.61 (s, 2 H), 4.37 (s, 8 H), 4.30 (s, 8 H), 2.45 (m, 4 H), 0.67 (t, J = 7.4 Hz, 6 H).

3.3 Conjugation of "tetra-" and "octa-acids" 1a,b with antibodies

Preparation of NHS ester from "Tetra-acid" (8) followed by conjugation with antibodies (IgG)

(a) Synthesis of *mono* NHS ester (8)



To a solution of **1** (10 mg, 11.3 µmol) in 200 µL of dry DMF, NHS (15.6 µL *of a stock solution in DMF; c = 0.87 M*, 13.6 µmol, 1.2 equiv.), DMAP (8.3 µL *of a stock solution in DMF; c = 0.16 M,* 1.4 µmol, 0.1 equiv.) and DCC (14 µL *of a stock solution in DMF; c = 0.97 M*, 13.6 µmol, 1.2 equiv.) were added, and the reaction mixture was stirred at r.t. for 2 h. The course of the reaction was monitored by HPLC. Additional amounts of NHS (6.5 µL, 5.7 µmol, 0.5 equiv.) and DCC (5.8 µL, 5.7 µmol, 0.5 equiv.) were added, and stirring was continued overnight. Then the reaction was "quenched" by adding acetic acid (5 µL). The solution was concentrated in vacuum, in order to remove DMF, and the products were

isolated by preparative HPLC (system C). The fractions containing compound **8** were lyophilized to give a yellow amorphous solid (2 mg, 18% yield). ESI-MS, negative mode: m/z (rel.int., %) = 984.6 (100) $[M-H]^-$ (found), 984.8 (calculated for $C_{45}H_{28}F_6NO_{14}S_2$, $[M-H]^-$). HPLC (system B): t_R = 12.8 min (closed form) and 13.0 (open form).

(b) Antibody labeling

A solution of *mono* NHS ester **8** (0.2 mg or 0.1 mg in 40 µL DMF) was added dropwise to a stirred solution of the secondary antibodies (2 mg in 1 mL of $NaHCO_3$ buffer; pH 8.3). The reaction mixture was protected from light and stirred at r.t. for 1 h. Then the solution was applied onto a Sephadex G-25 column (PD-10) pre-equilibrated with the elution buffer (PBS pH 6.5). Fractions of ca. 0.5 mL were collected, and the protein-content in each of them was evaluated by absorption spectroscopy and Bradford assay. The bioconjugate eluted with the front, with typical recovery efficiencies and dilution factors of ca. 90% and 1.5, respectively (calculated from UV-Vis spectroscopy measurements). Free (non-conjugated) diarylethene stays in the column and does not elute with protein.

In situ activation of the "octa-acid" 2 followed by conjugation with antibodies (IgG)

To a DMF solution of "octacid" **2** (0.2 mg, 0.1 mg, or 0.05 mg, in 50 µL DMF), NHS (1.4 equiv.) and EDC (10 equiv.) were added as stock solutions in DMF, and the reaction mixture was stirred at r.t. for 1 h. Then the reaction mixture containing the activated "octa-acid" **2** was added dropwise to the stirred solution of the secondary antibody (2 mg in 1 mL of $NaHCO_3$ buffer at pH = 8.3), protected from light and stirred at r.t. for 1 h. Then, the resulting mixture was purified by a sephadex column pre-equilibrated with PBS buffer (pH 6.5) and eluted with the same buffer (unlabeled DE and DE-bioconjugate are closely eluted). Fractions of ca. 0.5 mL were collected, and the protein-content in each of them was evaluated by absorption spectroscopy and Bradford assay.

Determination of the degrees of labeling of the bioconjugates

The degrees of labeling (DOL) of the different bioconjugates prepared were determined independently by two methods, optical spectroscopy (UV-vis absorption) and MALDI-TOF mass-spectrometry. In the first case, the total amount of the dye (OF+CF) had to be considered, since a small amount of the CF (ca. 5-10%) was present. The latter was possible formed during purification and handling of the bioconjugate solutions. As is the standard procedure, we assumed for the calculations that all absorption coefficients (for both isomers) in PBS are the same for the free dyes and their bioconjugates. Therefore, this method might introduce a considerable error in the DOL_{Abs} value calculated. Thus, an independent measurement of the DOL was performed by mass-spectrometry. Unfortunately, the values determined by this second method also have some errors, due to the lack of a calibration standard in the high-mass region (~150 kDa) of the antibodies. The DOL_{MALDI} was calculated from the difference of the mass measured for the unlabeled antibody (the exact same batch was used; a few microliters were saved for MALDI measurements prior to the labeling) and the corresponding bioconjugate. The results (DOL_{Abs} and DL_{MALDI}) obtained for the preparation of different bioconjugates are presented in Table S1. The differences encountered

within the two methods are in the range as previously reported ones.[6] Comparing both labeling protocols, we can estimate that the efficiency of the "*in situ*" labeling (3.3.2) yield approximately a ca. 40-50% lower efficiency than the protocol with the priory activated (NHS) dye (3.3.1).

Table S1. Degree of labeling (DOL) of diarylethene-bioconjugates prepared from compounds **1**, **2** and IgG as determined by UV-Vis spectroscopy and/or by MALDI-TOF mass-spectrometry.

Conjugate	Dye	Amount [mg][a]	Molecular mass [Da][b]	DOL Abs	DOL MALDI	τ_{Fluo} [ns]
AB[c]	---	---	146007 ± 93	---	---	---
BC1A	**1**[d]	0.20	150108 ± 288	8.1	4.6 ± 0.3	2.3 (84 %) / 1.0 (16 %)[e]
BC1B	**1**[d]	0.10	148300 ± 271	5.2	2.6 ± 0.3	2.3 (80 %) / 1.0 (20 %)[e]
BC2A	**2**[f]	0.20	149659 ± 126	5.3	2.7 ± 0.1	2.05 ± 0.5
BC2B	**2**[f]	0.10	147962 ± 277	4.0	1.5 ± 0.2	2.01 ± 0.5
BC2C	**2**[f]	0.05	146740 ± 301	2.4	0.5 ± 0.2	2.00 ± 0.5

(a) the mass of the protein was 2 mg in all cases; (b) determined from MALDI-TOF mass-spectrometry; (c) the unlabeled secondary antibody (IgG) of the same protein batch (few micrograms were separated before the dye addition); (d) compound 8 was used (see protocol 3.3.1); (e) biexponential decays (relative amplitudes are given in parenthesis); (f) compound 2 was used (see protocol 3.3.2).

4. Immunolabeling and fluorescence imaging

4.1 Immunolabeling protocol

Vero cell samples cells were grown on standard cover slips and then fixed with previously cooled (-20 °C) methanol for 5 min, and blocked with 5% (w/v) BSA in PBS pH = 7.4 (blocking buffer). Then the cells were incubated with an anti-α-tubulin mouse primary antibody (Sigma-Aldrich, St. Louis, MO, USA) at r.t. for 1 h, followed by three washing steps of 5 min each with blocking buffer. The cells were then incubated at r.t. for 1 h with our labeled bioconjugates from Table S1 (typical dilutions of 1:50 to 1:100 from the purified bioconjugate) and washed again (5 min each step) three times with blocking buffer and finally with the mounting media (PBS, pH 7.4). The samples were mounted in concave microscopy slides and sealed with a silicone resin (Picodent Twinsil) to prevent leakage.

4.2 Confocal images with two representative bioconjugates, BC1B and BC2A

Standard confocal imaging was performed in a commercial Leica TCS SP5 confocal microscope. Images (Figure S1) were recorded with 488 nm excitation, after a short (ca. 1 s) and low- intensity wide-field pre-activation with 366 nm light, from the mercury lamp. Detection was collected between 510 and 600 nm. A fading of the signal was observed after repeated scanning of the same area, due to the isomerization (CF→OF) induced by the excitation light (a process competing with fluorescence emission of the probe). The signal can

be recovered with another pre-activation pulse, and thus further imaging of the same area can be repeated several times.

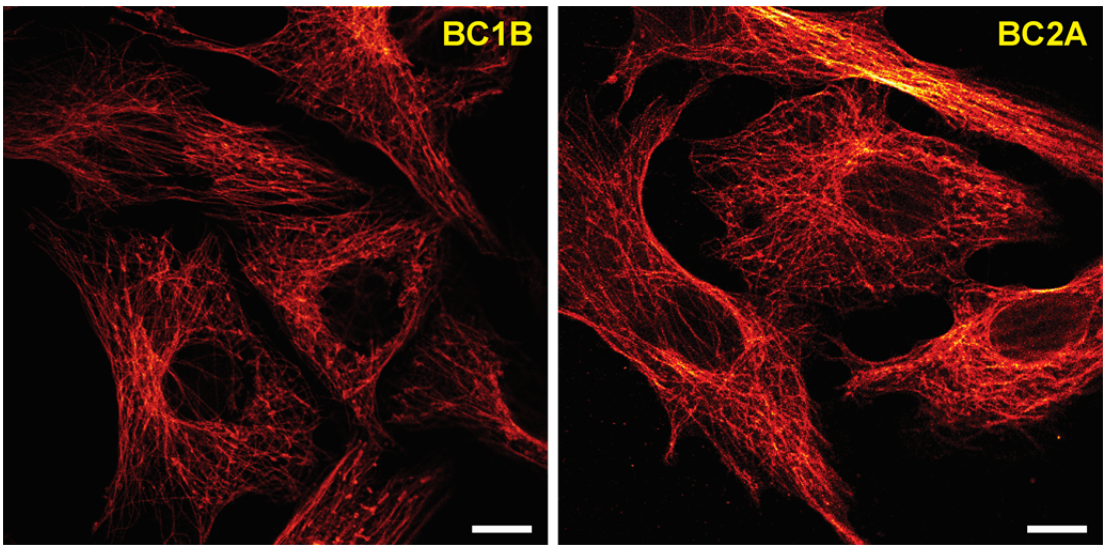


Figure S1. Confocal images of immunolabeled cytoskeletal protein tubulin in mammalian cells (Vero). Scale bars: 10 μm.

4.3 Fluorescence spectroscopy and RESOLFT imaging

The pump-probe fluorescence measurements were performed on a custom build confocal microscope with an additional widefield illumination (Figure S2). The intensities of this widefield illumination given in the main text were calculated from the observed illumination FWHMs of the corresponding laser spots on a fluorescent sample and their measured power in front of the sample ($I = P_{meas} / (\pi \cdot FWHM^2/4)$). The RESOLFT images were acquired with a modified 1C RESOLFT QUAD Scanning microscope (Abberior Instruments, Göttingen, Germany). The intensities of the different illumination beams were calculated from the measured size of their point spread functions (PSFs) of fluorescent beads and their measured power in the back focal plane of the objective ($I = P_{meas} / (\pi \cdot FWHM^2/4)$. For the intensity calculations of the doughnut-shaped beam it was taken into account, that the illuminated area is about 2.3 times bigger than by a corresponding Gaussian-shaped beam. The transmission of the 488 nm light through the objective was assumed to be 85% and the transmission of the 355 nm light to be 50%.

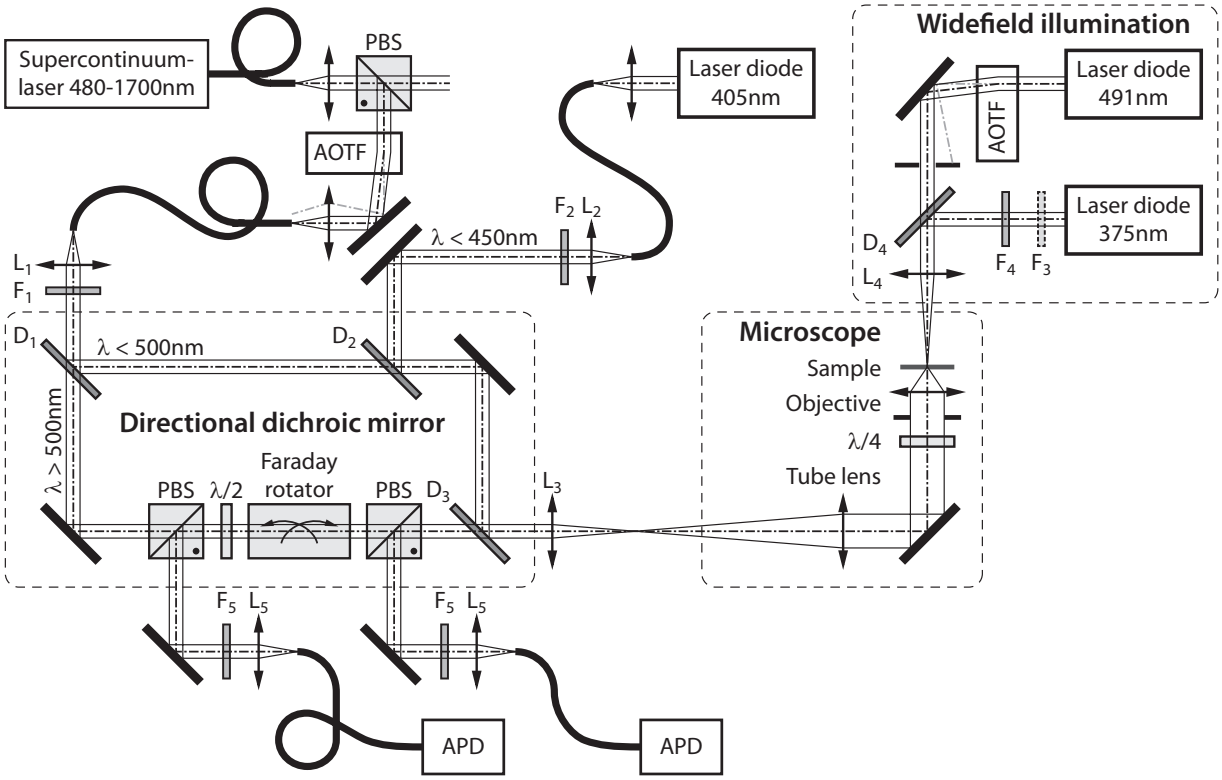


Figure S2. Outline of the microscope setup for fluorescence lifetime and photo-switching measurements.

A confocal illumination and detection was provided via the base port of a commercial microscope stand (DM IRBE, Leica Microsystems) and a 100× oil-immersion objective with 1.4 NA (HCX PL APO 100×/1.4-0.7 Oil CS, Leica). A broad-band supercontinuum laser (SuperK Extreme EXB-6, NKT Photonics) and an acousto-optic tunable filter (AOTFnC-VIS-TN head with MDS8C-B66-22-80.153 driver, AA OptoElectronic) provided pulsed excitation light (40 MHz repetition rate, ~100ps pulse width) at selectable wavelengths with ~3nm bandwidth and modulated power. A polarization-maintaining single-mode fiber (PM460-HP, Thorlabs) cleaned up the spatial mode profile and a bandpass filter F_1 (Z488/10x, Chroma) the spectrum. The beam was collimated by an achromatic lens L_1 (f_1 = 10 mm, AC080-010-A, Thorlabs).

Two polarizing beam splitters PBS (PBS251, Thorlabs), an achromatic half-wavelength retarder plate λ/2 (AHWP05M-600, Thorlabs) and a Faraday rotator (711A, Conoptics, used without polarizers) provided a directional broad-band beam splitting for the excitation and fluorescence light. The UV and blue light were fed separately via dichroic mirrors D_1 and D_3 (Z488RDC, Chroma) because the Faraday rotator showed poor performance at wavelengths λ < 500 nm. A power-modulated UV laser diode (405 nm wavelength, 30 mW, BCL-30-405-S, CrystaLaser), a polarization-maintaining single-mode fiber (P5-405BPM, Thorlabs), an achromatic lens L_2 (f_2 = 30 mm, AC254-030-A, Thorlabs) and a clean-up filter F_2 (Z405/10x, Chroma) provided a clean laser beam at 405 nm wavelength for switching. The illumination beams were combined by a dichroic mirror D_2 (Z405RDC, Chroma) and their beam diameters were magnified 4× by an achromatic lens L_3 (f_3 = 50 mm, AC254-050-A, Thorlabs) and the microscope tube lens (f_t = 200 mm). The confocal illumination polarization was circularized by a quarter-wave retarder plate λ/4 (AQWP05M-630, Thorlabs).

The fluorescence emission was selected by band-pass filters F_5 (FF03-525/50, Semrock), focused with achromatic lenses L_5 (f_5 = 30 mm, AC254-030-A, Thorlabs) into step-index multimode fibers (core Ø25 µm, M67L01, Thorlabs) and detected by avalanche photo-diodes APDs (PD-050-CTD-FC, MicroPhotonDevices). The detected photon events were recorded as photon streams with a TimeHarp260 NANO board (PicoQuant) and analyzed for fluorescence lifetime. Simultaneously, a hardware correlator (Flex02-08D/C, Correlator.com) was used for real-time monitoring of the detection count rates and for fluorescence correlation spectroscopy of fluorophore solutions, useful for quickly aligning the setup.

Piezo scanners allowed to position the sample and to focus on fluorescently labeled features. For finding cells quickly, a widefield illumination was set up. Laser diodes provided light at 491 nm wavelength (50 mW cw, Calypso 50, Cobolt) and 375 nm wavelength (20 mW cw, switchable up to 100 kHz, FBB-375-020-FS-E-1-0, RGBLase). An acousto-optic tunable filter AOTF (AOTFnC-TN head with MOD4C-VIS driver, AA OptoElectronic) was used to modulate the power of the 491 nm light. Neutral density filters F_3 adjusted the power and a band-pass filter F_4 (Z375/10x, Chroma) cleaned up the spectrum of the 375 nm laser beam. A dichroic mirror D_4 (Z375RDC, Chroma) combined the laser beams. A clean-up filter was not required for the 491 nm laser beam. An illumination field of 15–20 µm diameter was obtained by focusing the beams with an achromatic lens L_4 (f_4 = 45 mm, AC254-045-A, Thorlabs). *This widefield illumination allowed to characterize photo-switching of moving fluorophores because the fluorophores were switched synchronously regardless of their microscopic motion.*

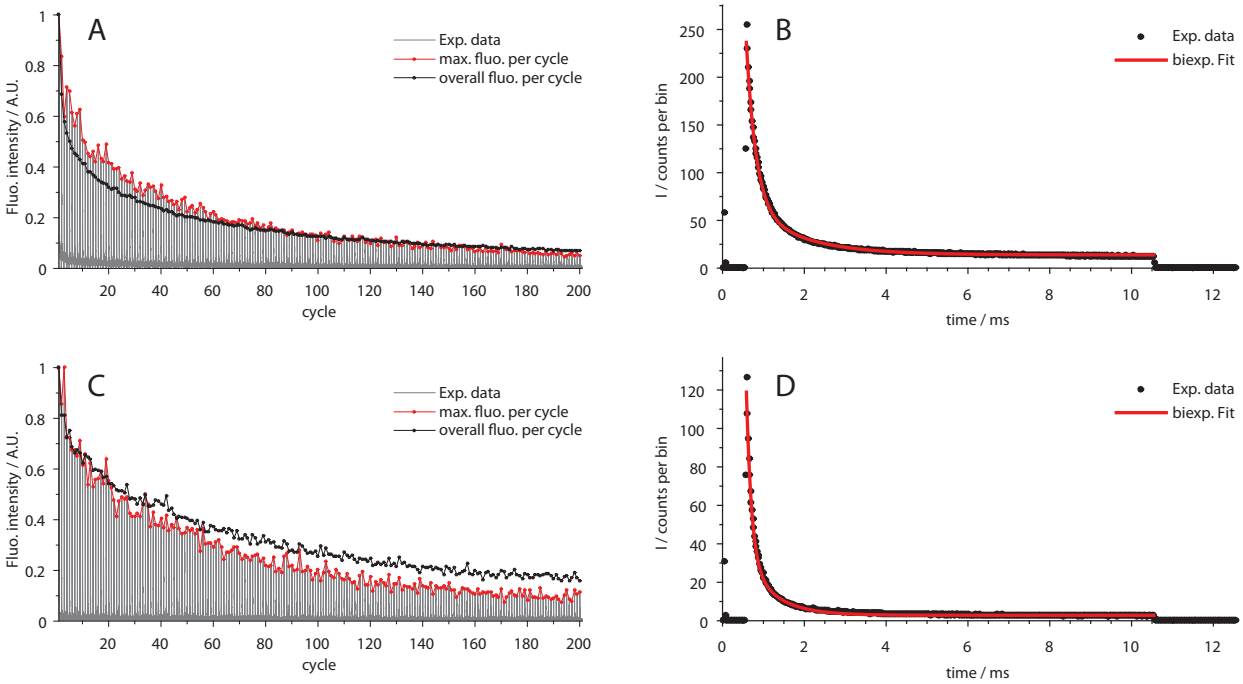


Figure S3. Fluorescence emission of cells immunolabeled with dyes **1** (A) and **2** (C) showing the on/off switching cycles upon irradiation with the light pulse sequence mentioned in the main text (gray bursts). The red line indicates the maximum fluorescence signal detected within a switching cycle, while the black line displays the overall fluorescence gained per cycle. Both plots were normalized to their maximum value. B and D show one switching cycle as the mean value of 200 cycles (black dots) of dye **1** and **2**, respectively. The bi-exponential fit used to determine the switching kinetics is shown as red solid line. All measurements were taken with 20 µs bins. Displayed intensities were corrected according to the dead-time of the sensors.

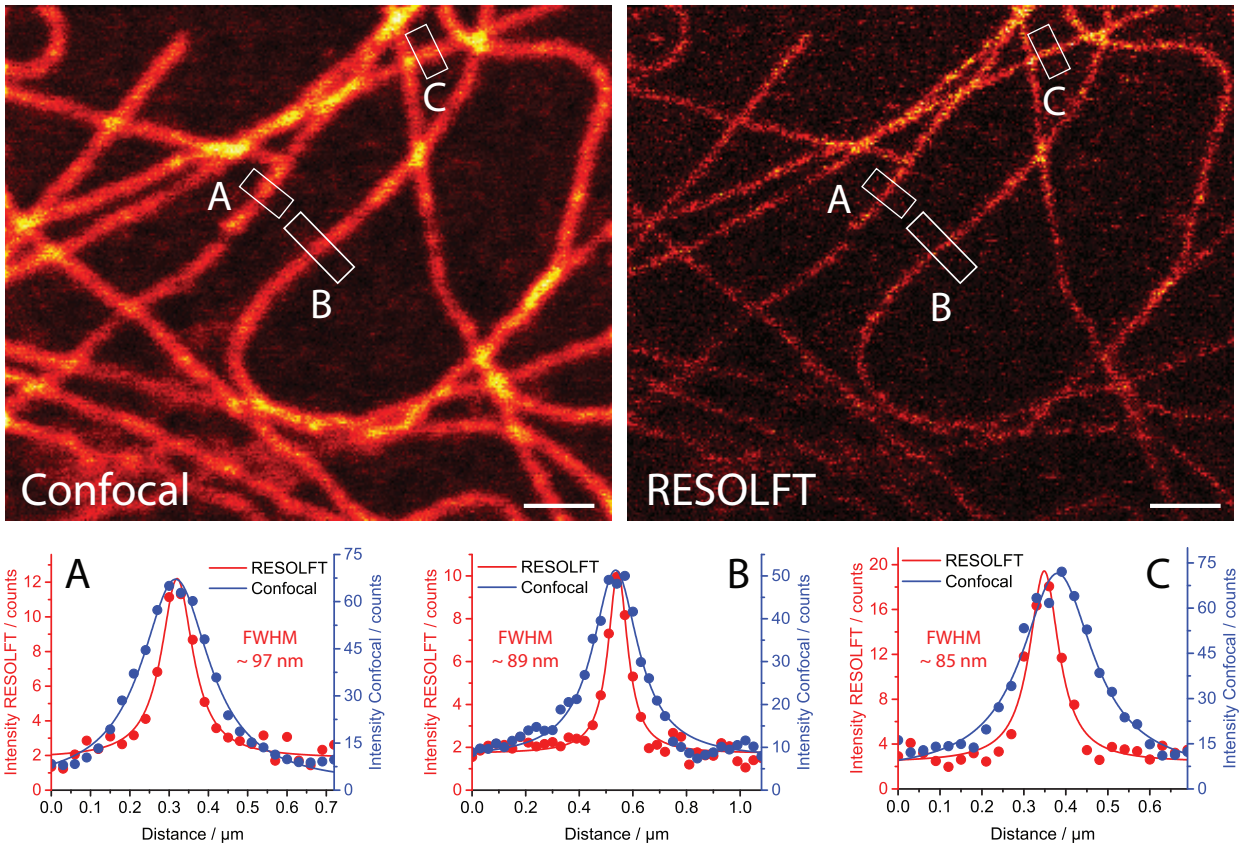


Figure S4. Confocal and corresponding RESOLFT image of the whole fixed Vero cells immunostained with primary antibodies against α-Tubulin and with the diarylethene **1** attached to the secondary antibodies. All images show raw data and were acquired with 30 nm pixel size. Scale bar: 1 µm. The following pulse pattern was used to acquire the RESOLFT image: 10 µs of 355 nm light (0.63 kW/cm^2), 200 µs illumination break, 7 ms of doughnut-shaped 488 nm beam(10.6 kW/cm^2), 80 µs of a Gaussian shaped 488 nm light beam (9.7 kW/cm^2) to probe the remaining fluorescence. The same pulse sequence was applied to record the confocal images but the off-switching with the doughnut-shaped beam was omitted. The line profiles A–C (averaged over ten adjacent lines) display the regions indicated in the images. The data (dots) was fitted with a Lorentzian function (solid line) for the RESOLFT (red) and the confocal (blue) image. The FWHM was determined on the fits A, B and C.

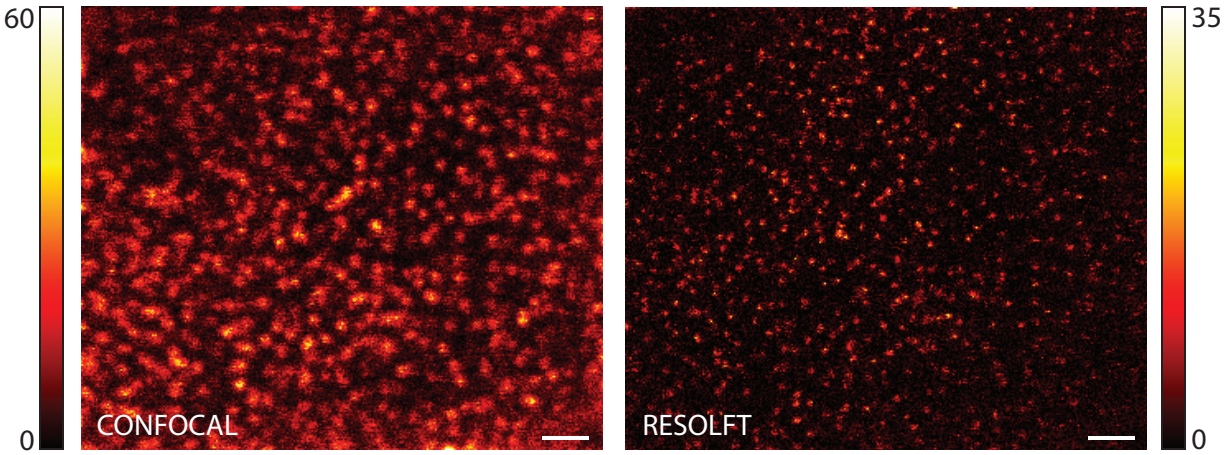
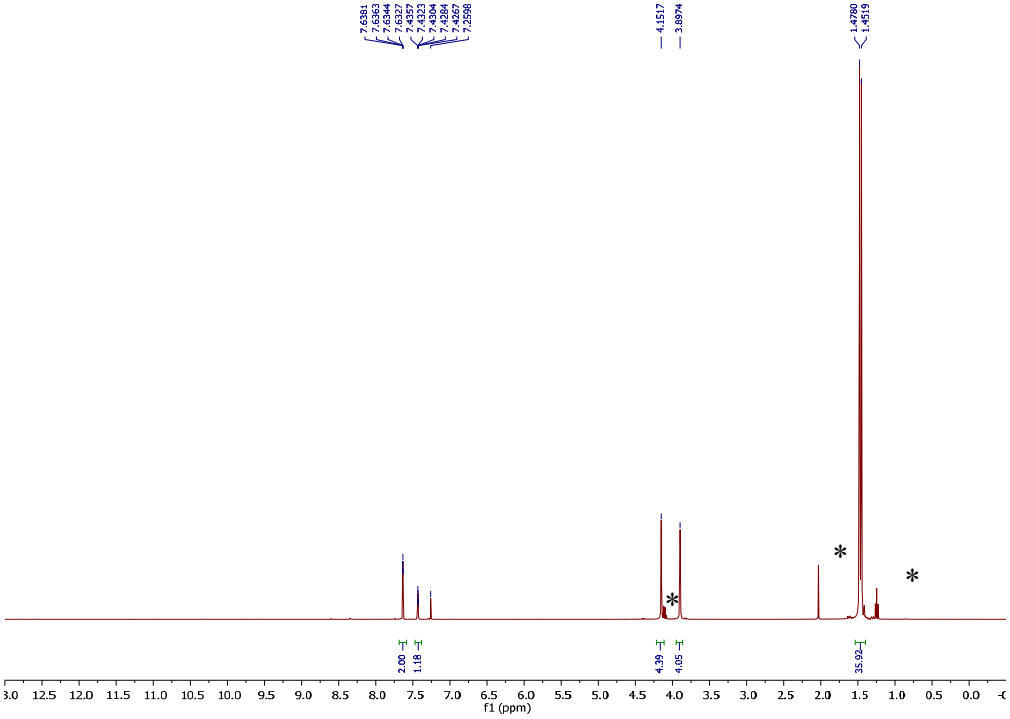


Figure S5. Confocal and corresponding RESOLFT image of the whole fixed Vero cells immunostained with primary antibodies against Nucleoporin 153 (Nup153) and with the diarylethene **2** attached to the secondary antibodies. All images show raw data and were acquired with 35 nm pixel size. The color –bars indicate the intensity of both images in counts.

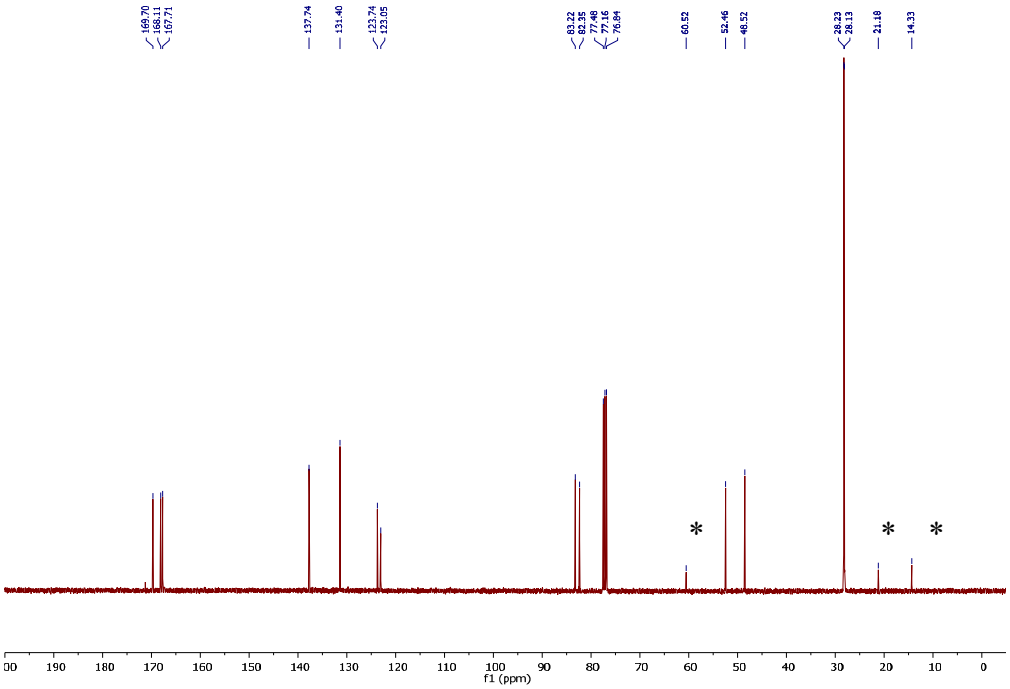
5. NMR spectra of 3,5-disubstituted phenyl boronates

Tetra-*tert*-butyl 5-bromobenzene-1,3-bis-(*N*-iminodiacetate) (3)

^{1}H NMR spectrum in $CDCl_3$ (400 MHz) * *peaks assigned to residual solvent peak (EtOAc)*

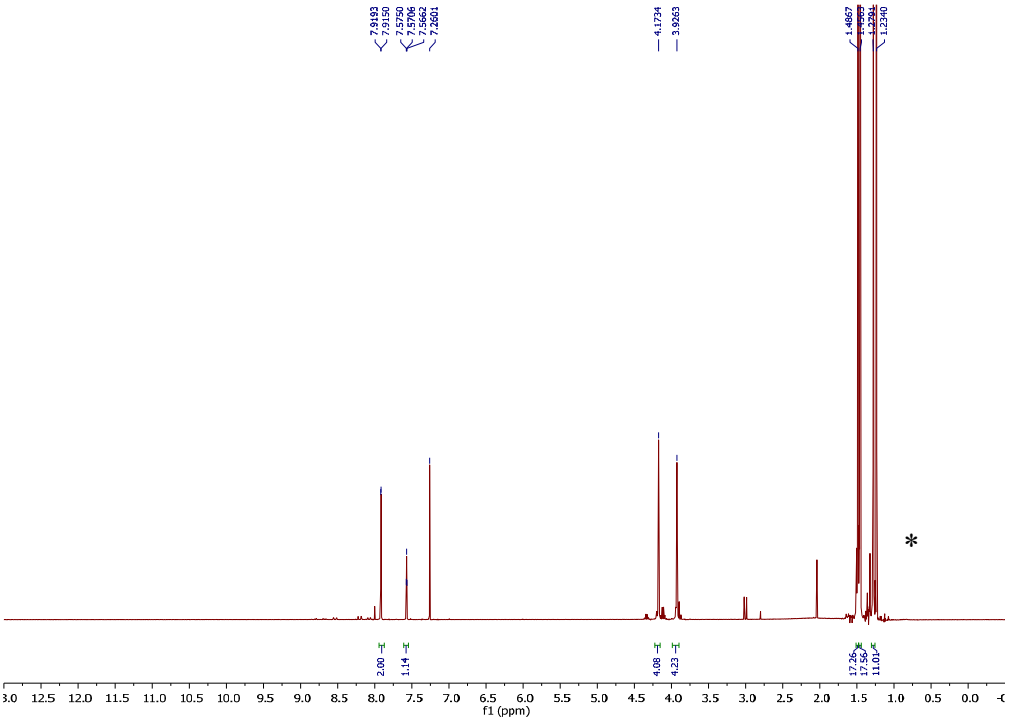


^{13}C NMR spectrum in $CDCl_3$ (101 MHz) * *residual solvent peaks (EtOAc)*

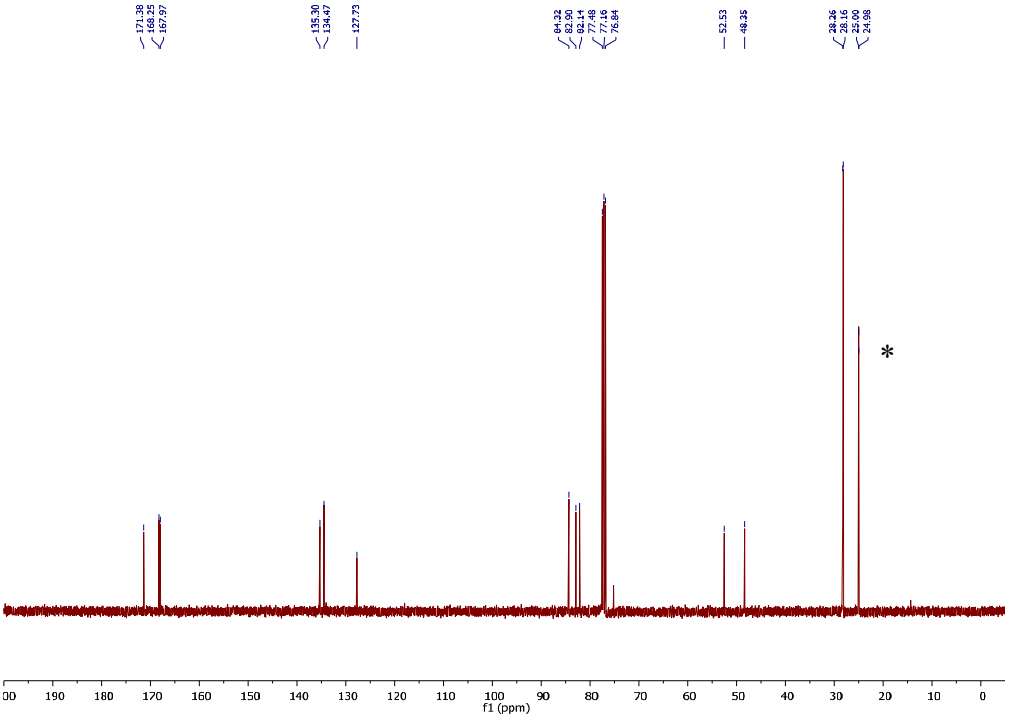


Tetra-*tert*-butyl benzeneboronic acid pinacol ester 3,5-bis-(N-iminodiacetate) (4)

^{1}H NMR spectrum in $CDCl_3$ (400 MHz) * *peak assigned to residual B(pin)$_2$*



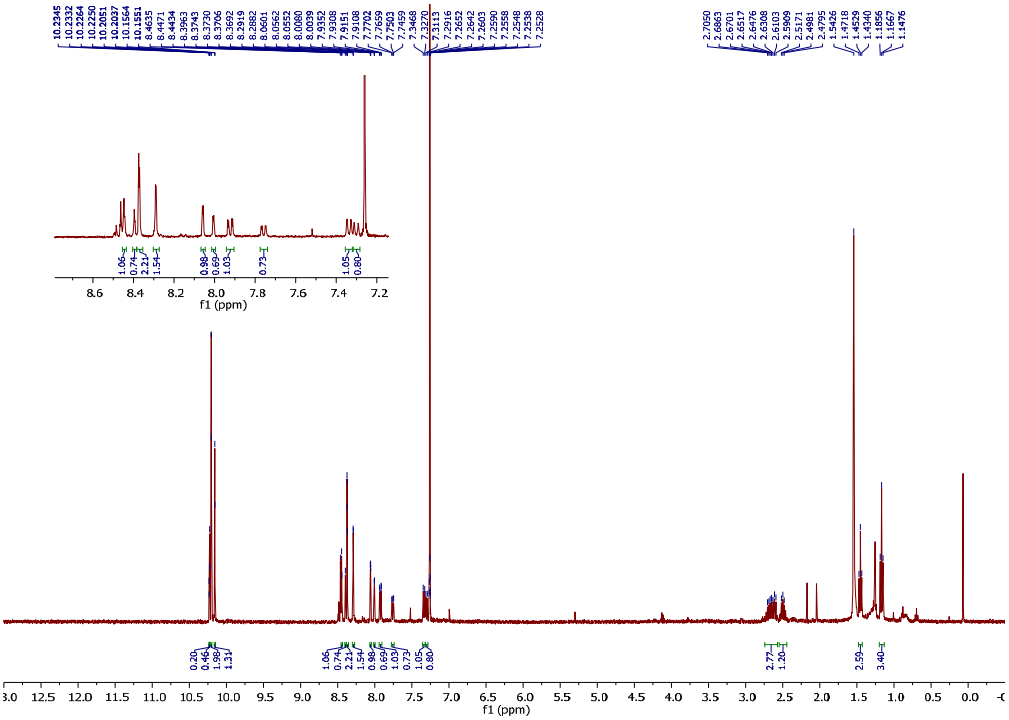
^{13}C NMR spectrum in $CDCl_3$ (101 MHz) * *peak assigned to residual B(pin)$_2$*



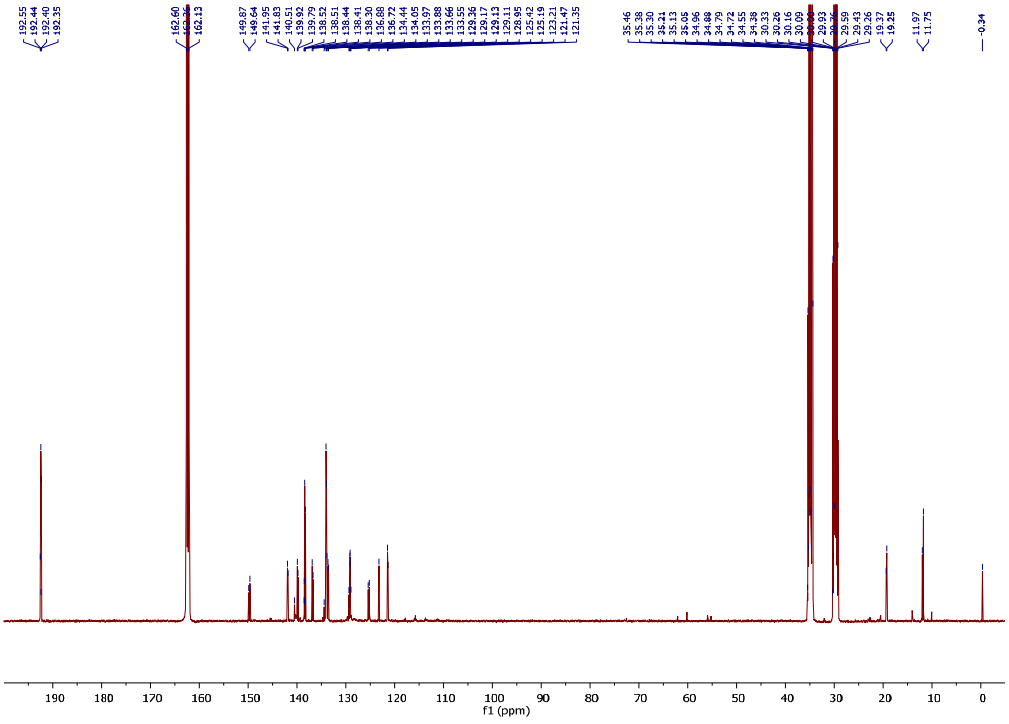
6. NMR, ESI-MS and RP-HPLC spectra of diarylethene derivatives

"Tetra-aldehyde" (5)

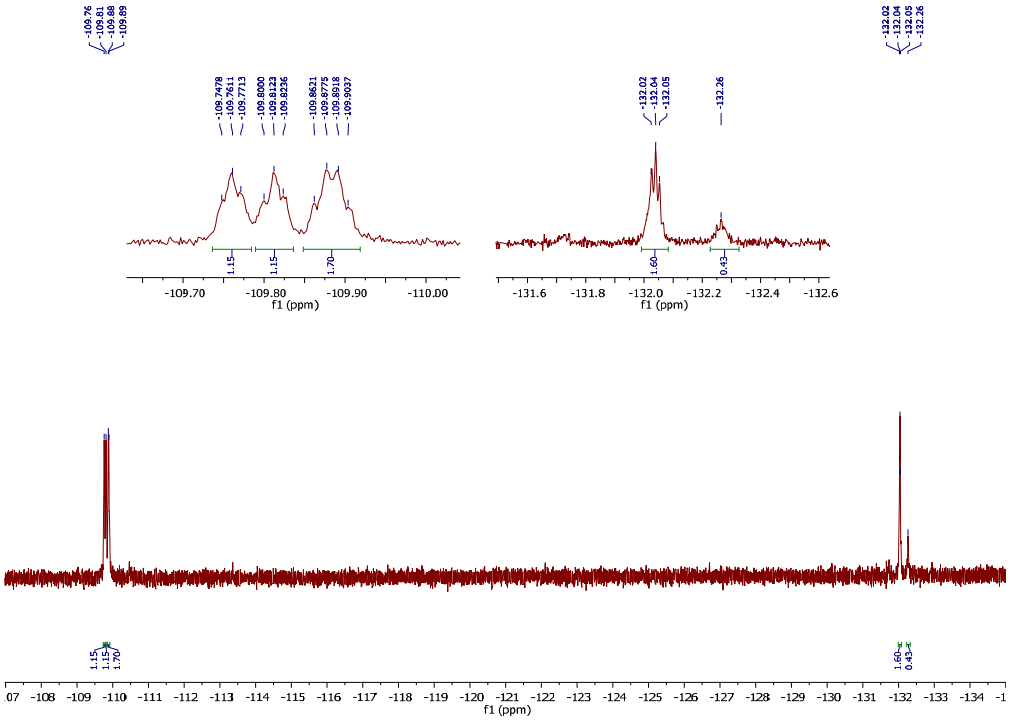
^{1}H NMR spectrum in $CDCl_3$ (400 MHz)



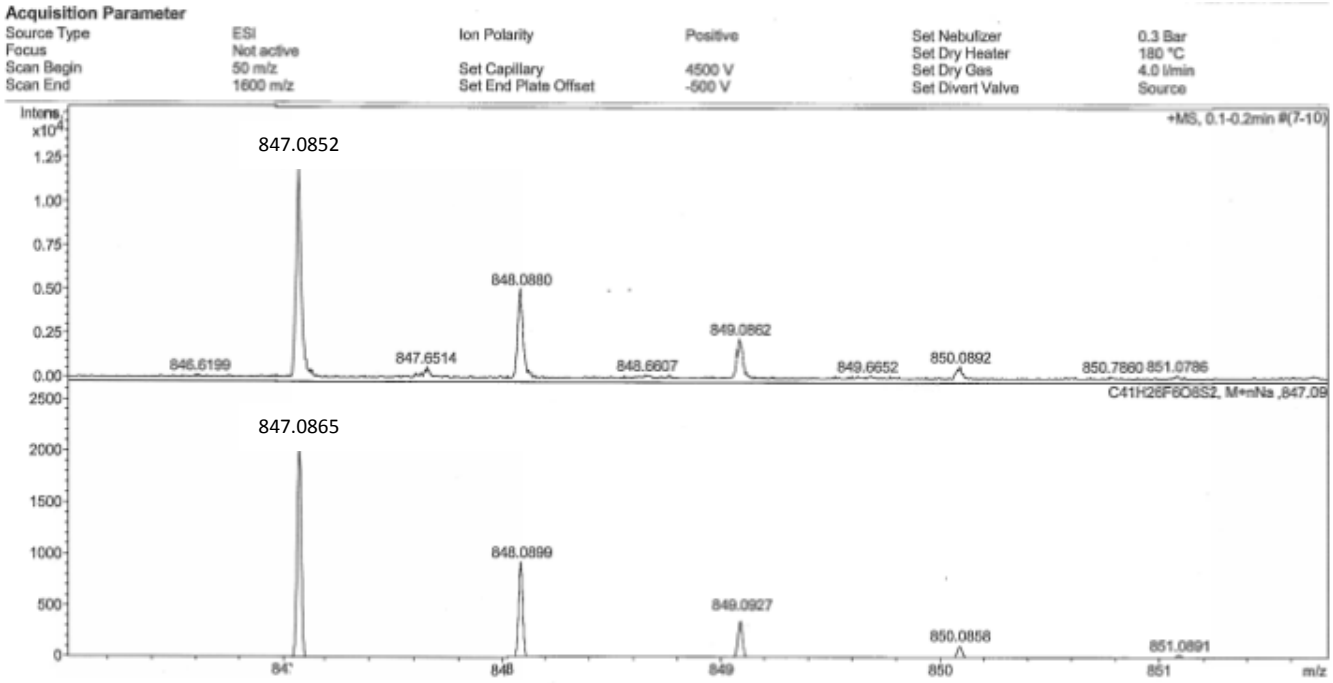
^{13}C NMR spectrum in $CDCl_3$ (126 MHz)



^{19}F NMR spectrum in $CDCl_3$ (376 MHz)

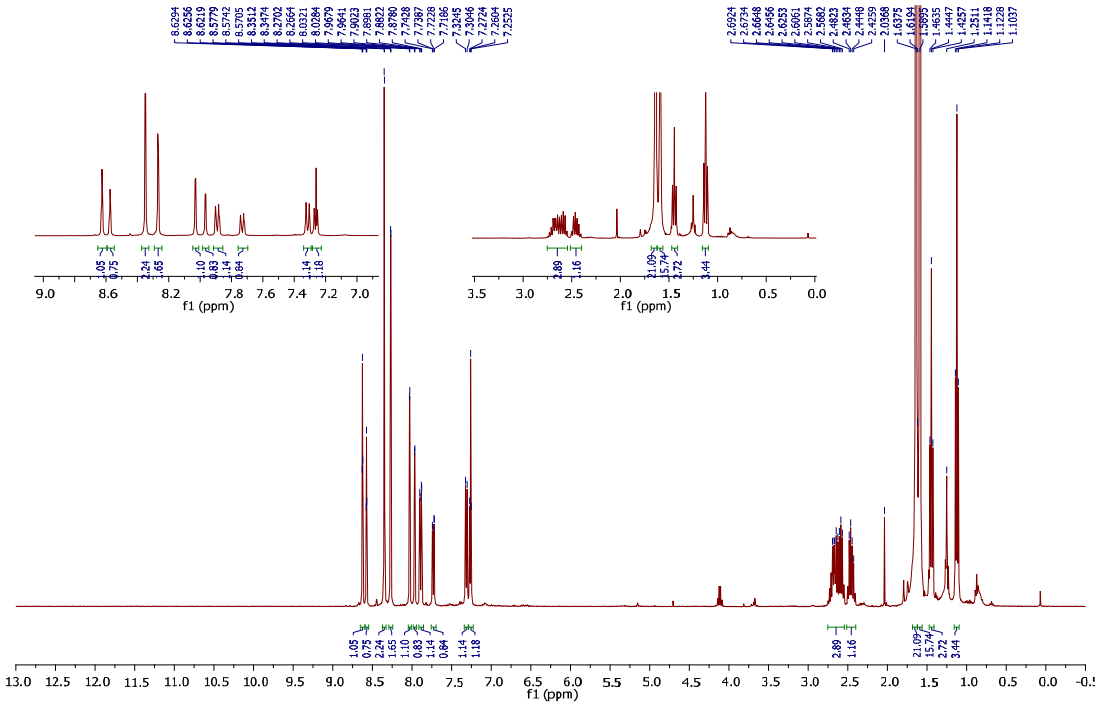


HRMS-ESI mass spectra (positive mode)

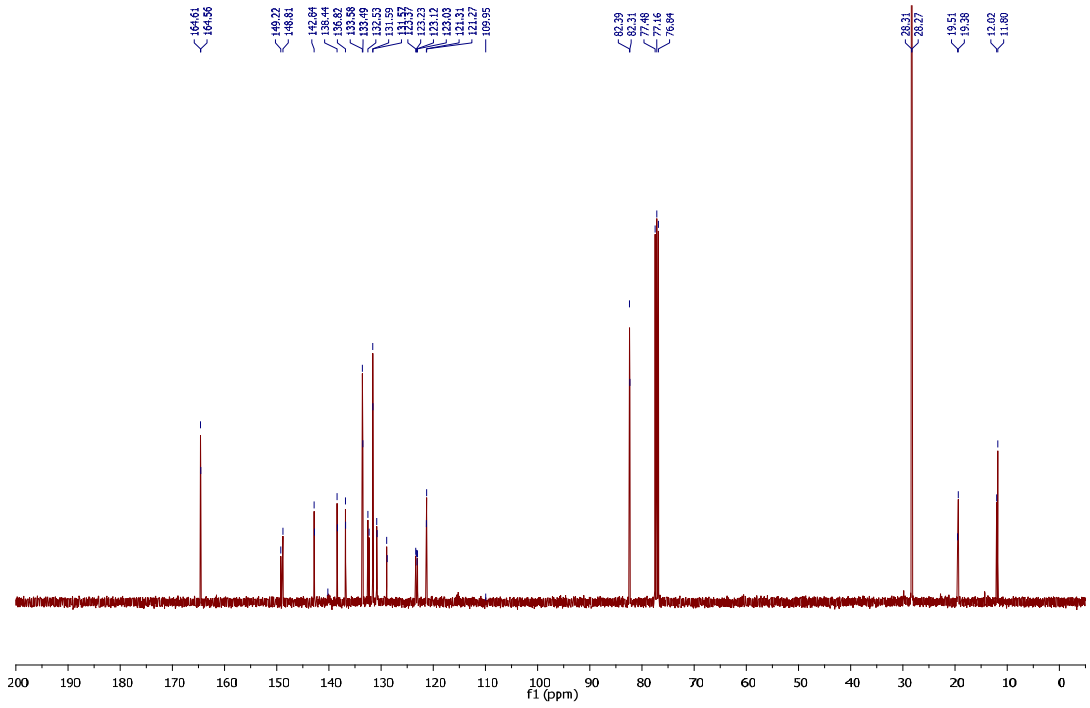


“Tetra-*tert*-butyl ester” (6)

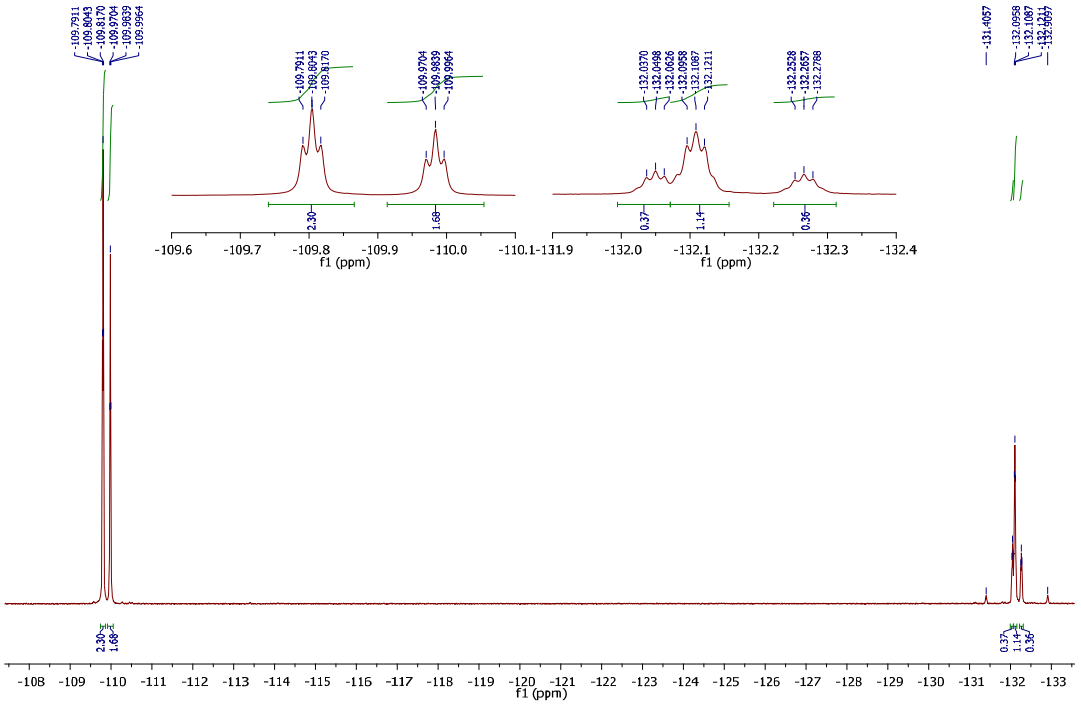
^{1}H NMR spectrum in $CDCl_3$ (400 MHz)



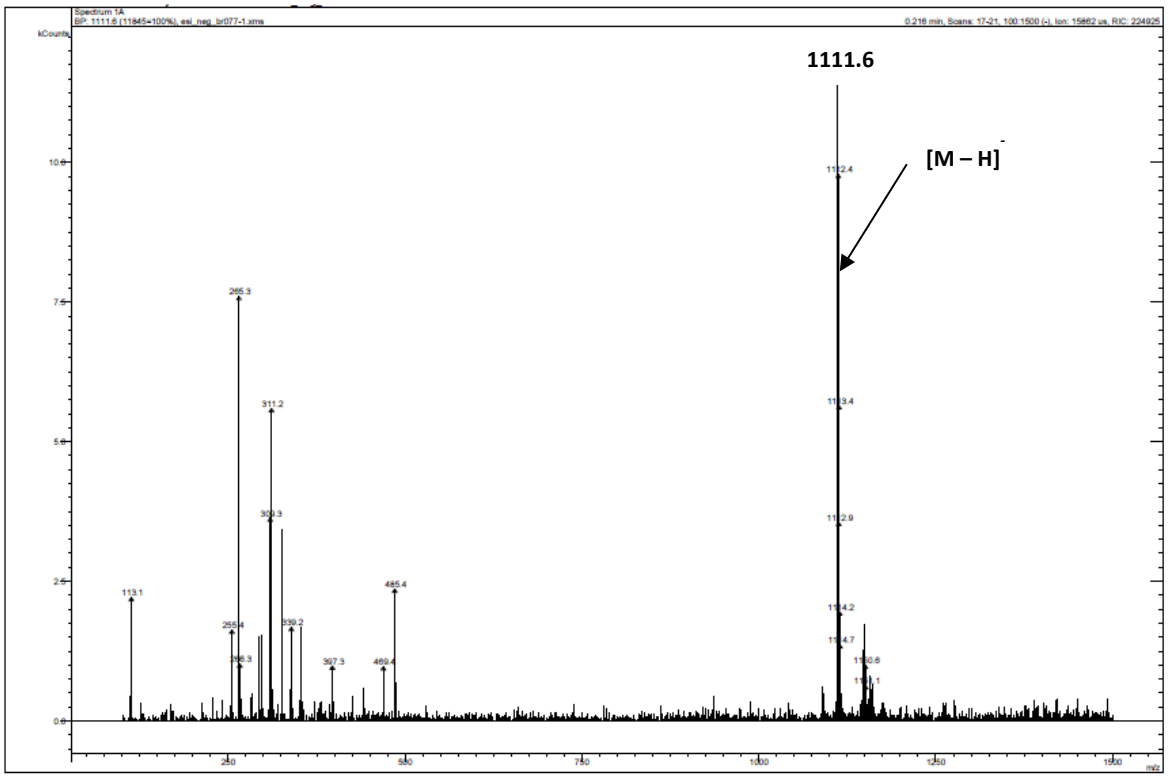
^{13}C NMR spectrum in $CDCl_3$ (101 MHz)



^{19}F NMR spectrum in $CDCl_3$ (376 MHz)

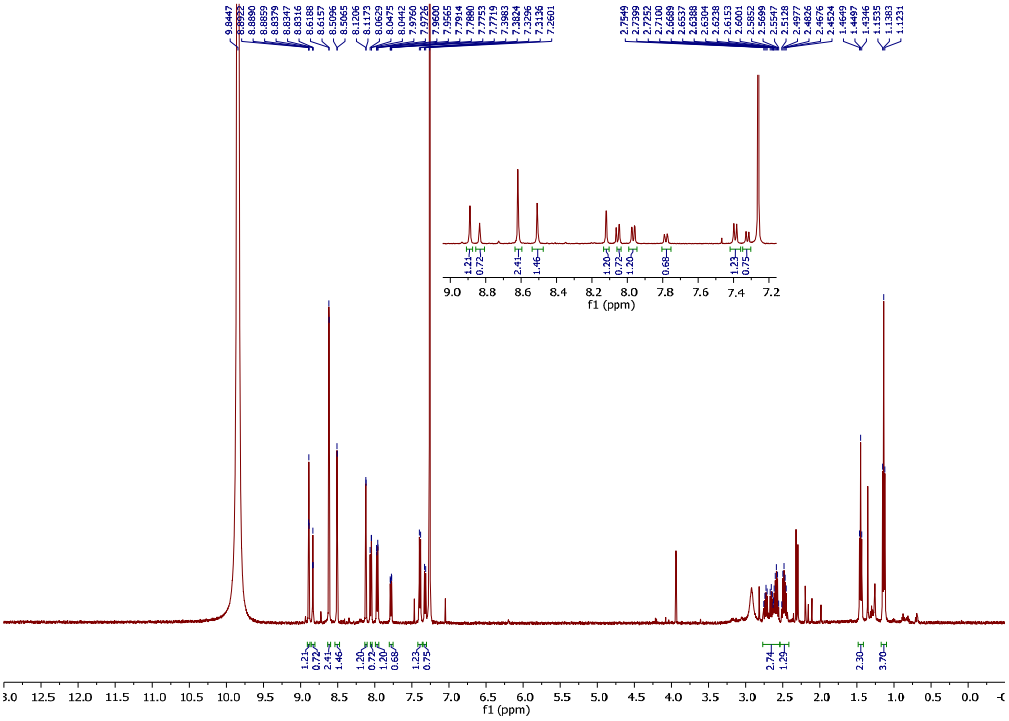


ESI mass spectra (negative mode)

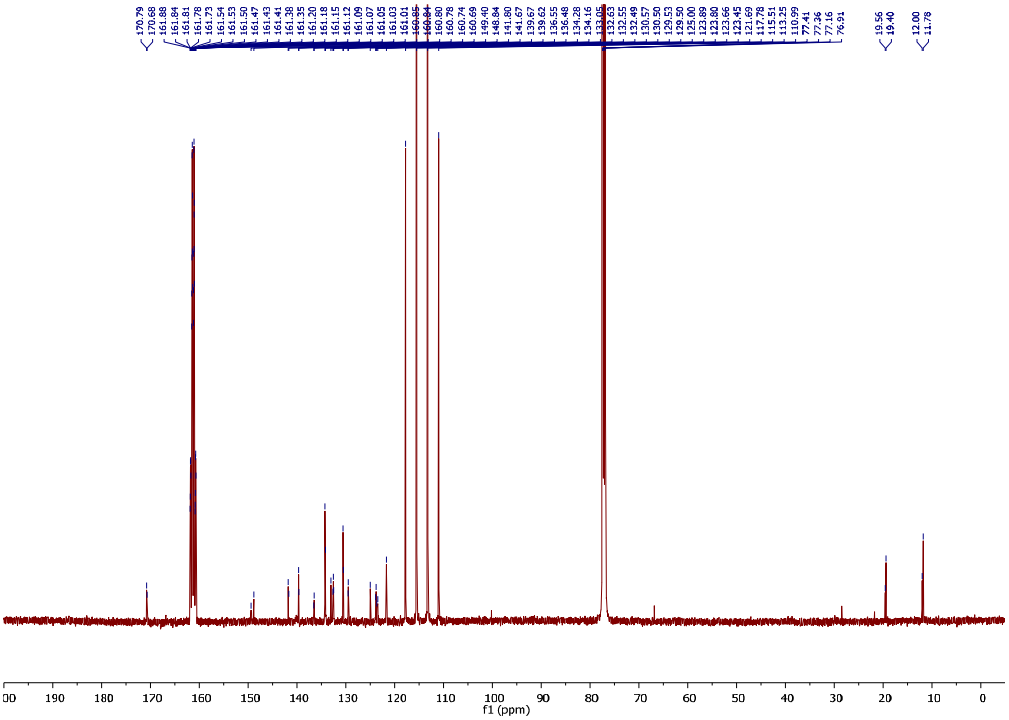


“Tetra-acid” (1)

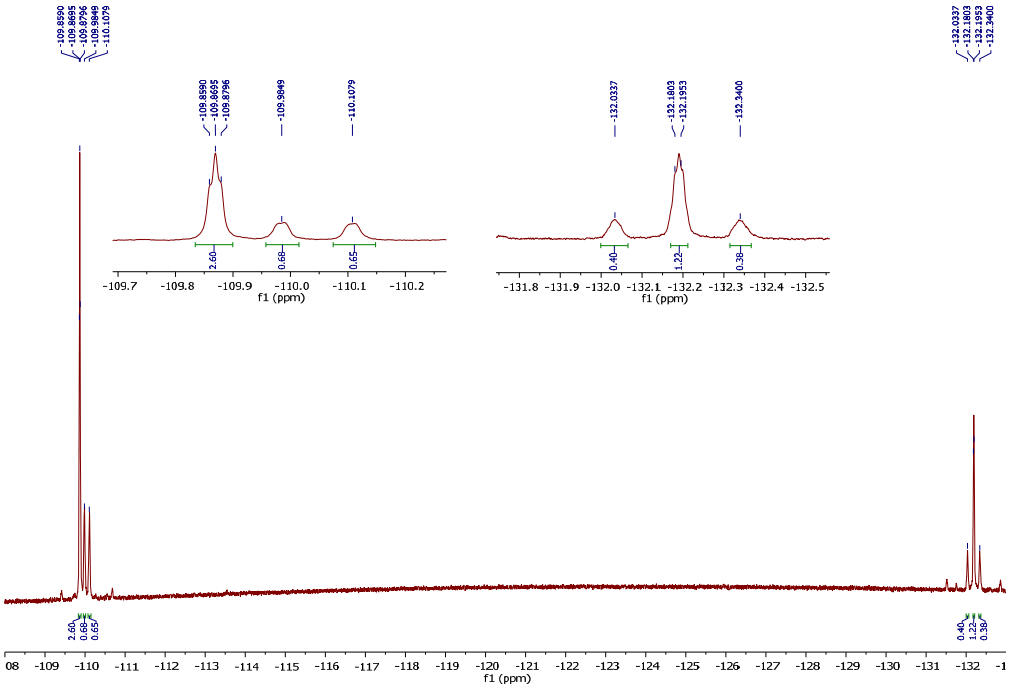
^{1}H NMR spectrum in $CDCl_3/CF_3COOD$ (500 MHz)



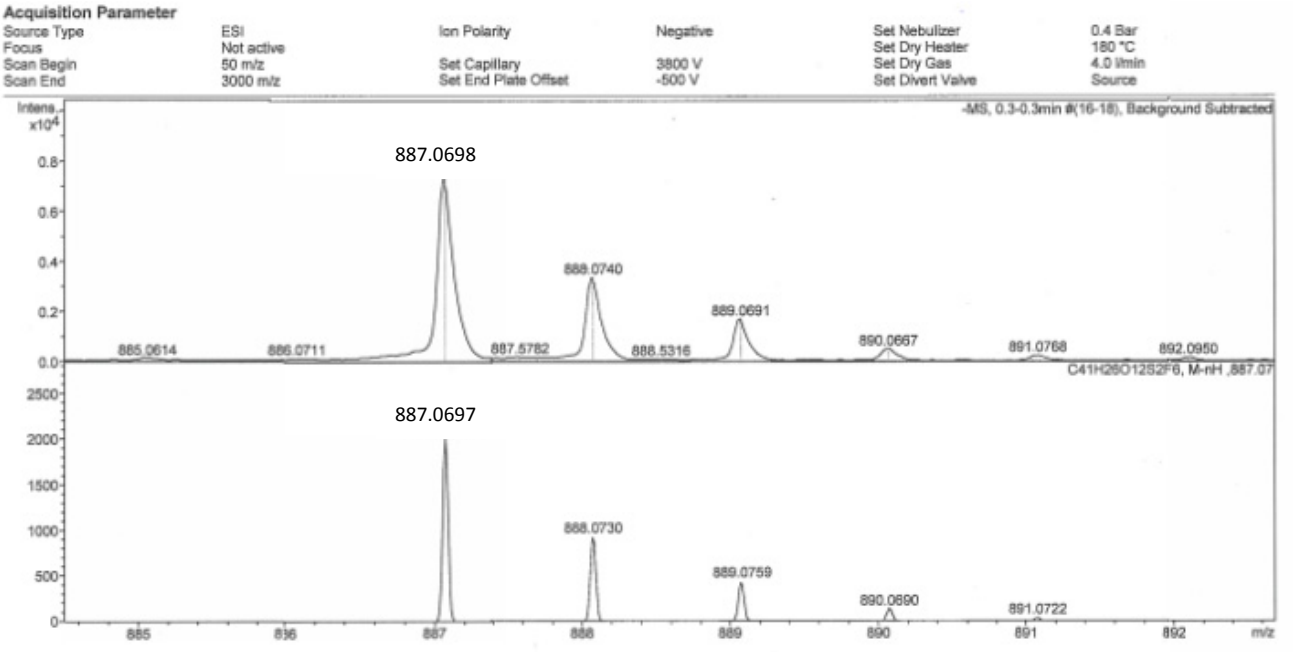
^{13}C NMR spectrum in $CDCl_3/CF_3COOD$ (126 MHz)



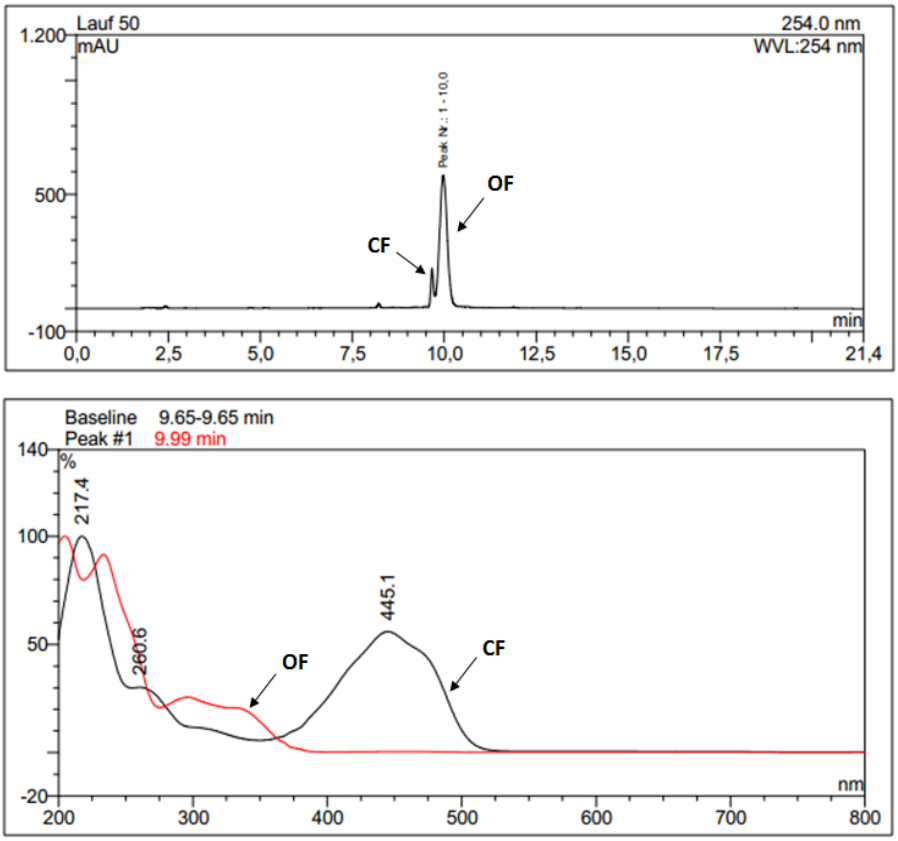
^{19}F NMR spectrum in $CDCl_3/CF_3COOD$ (471 MHz)



HRMS-ESI mass spectra (negative mode)

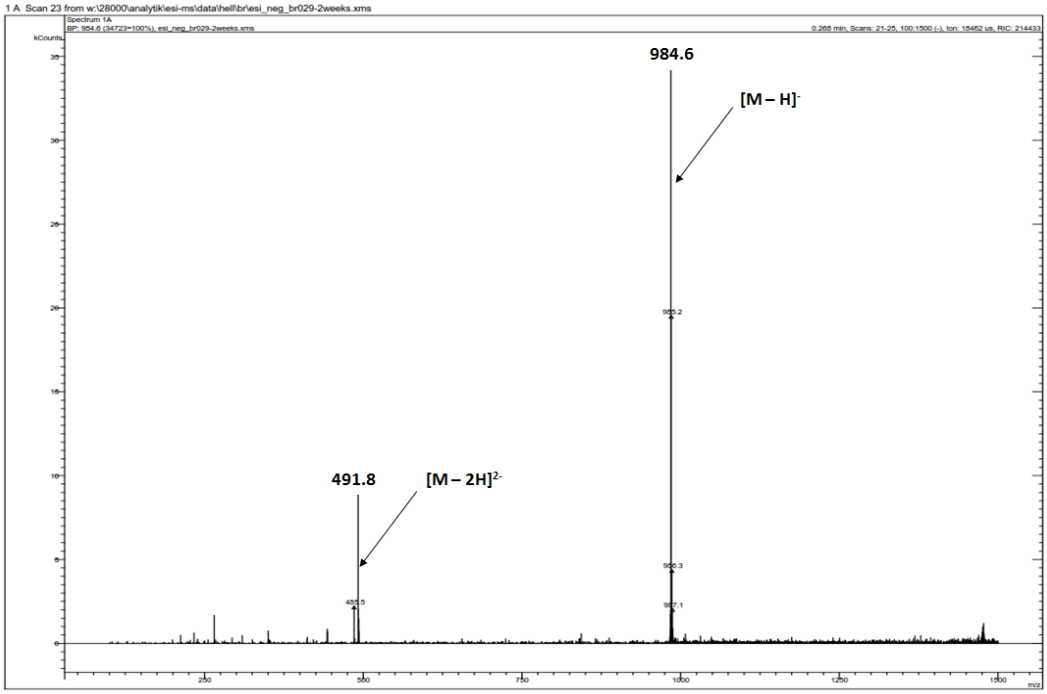


RP-HPLC elution profile (system A)

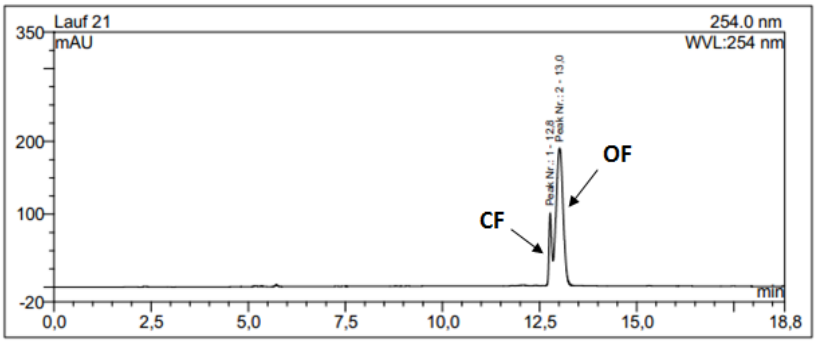


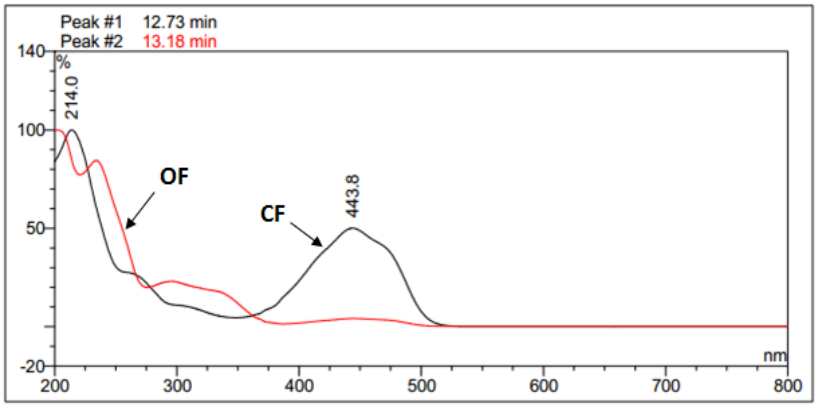
mono NHS ester (8)

HRMS-ESI mass spectra (negative mode)



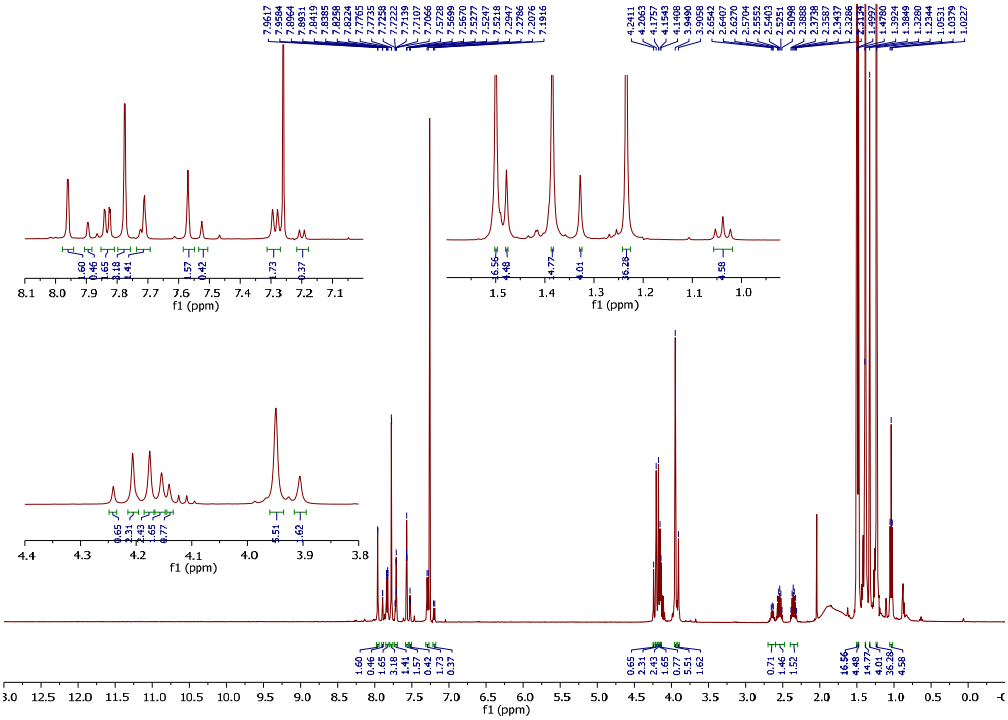
RP-HPLC elution profile (system B)



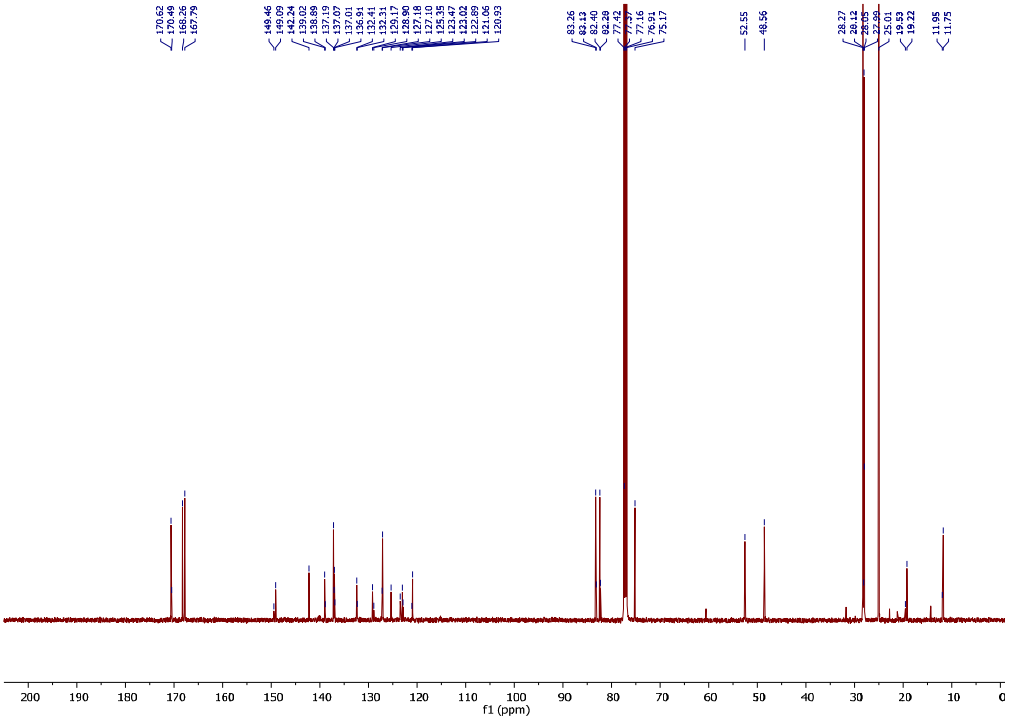


"Octa-acid" *tert*-butyl ester (7)

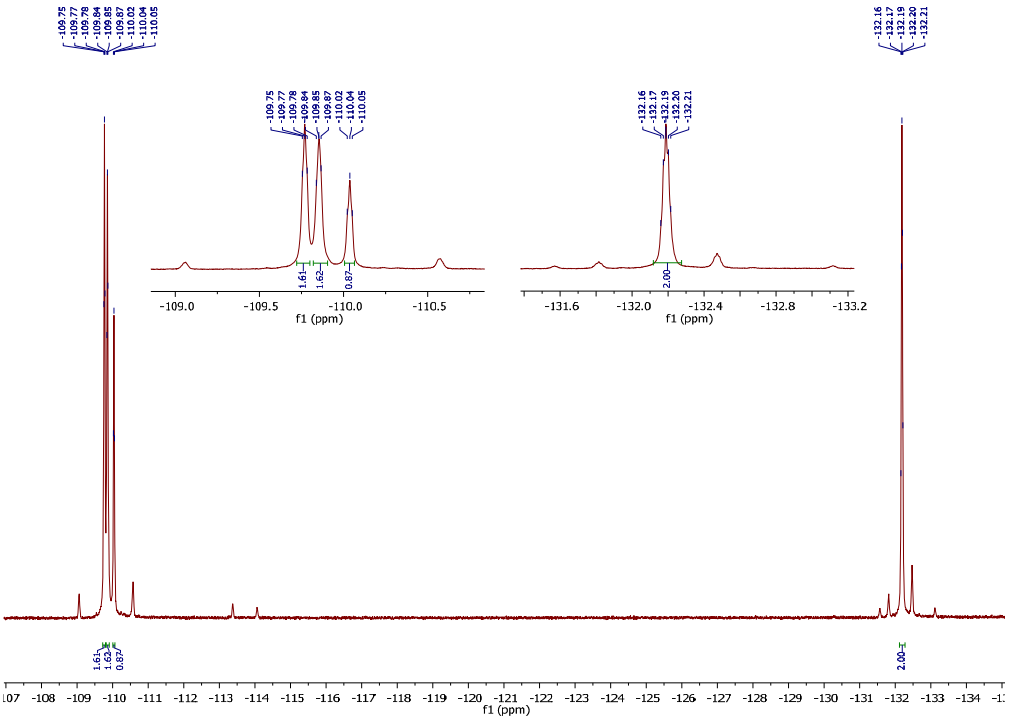
^{1}H NMR spectrum in $CDCl_3$ (500 MHz)



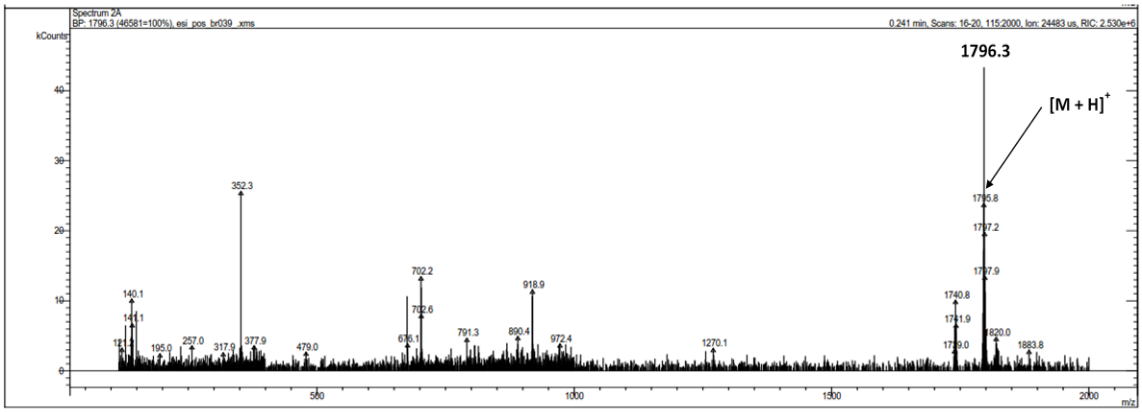
^{13}C NMR spectrum in $CDCl_3$ (126 MHz)



^{19}F NMR spectrum in $CDCl_3$ (376 MHz)

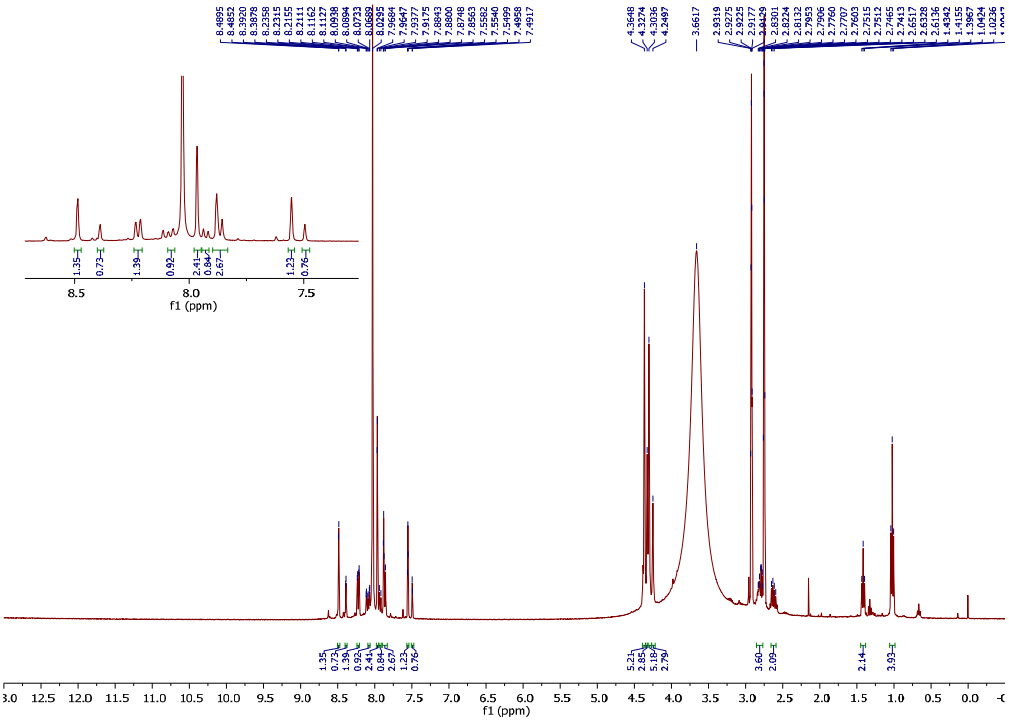


ESI mass spectra (positive mode)



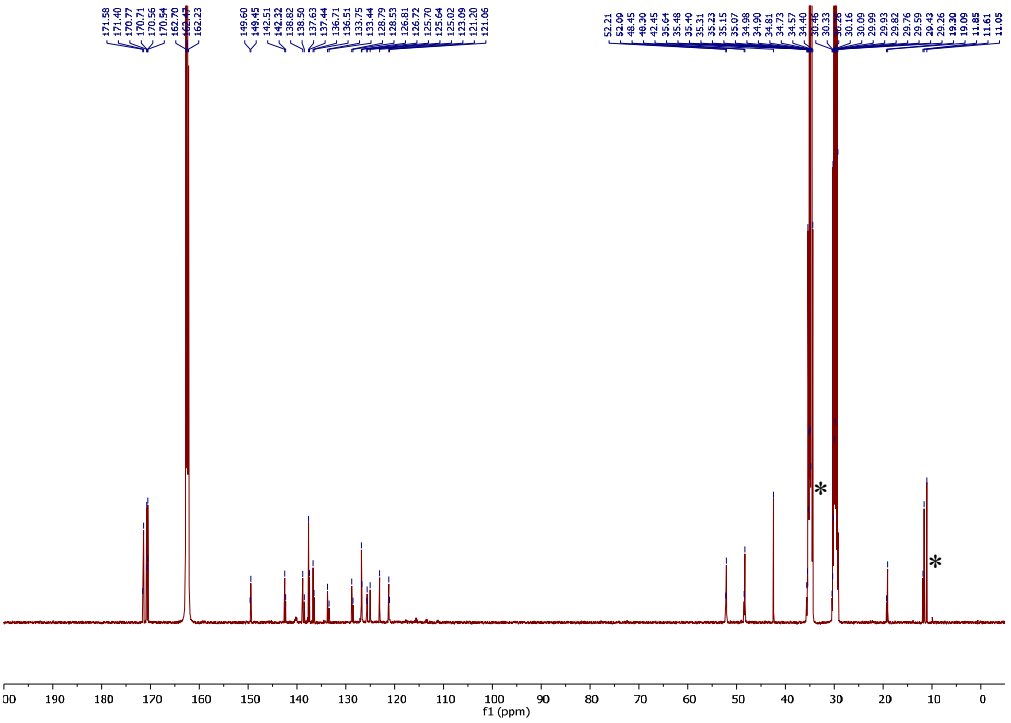
“Octa-acid” (2)

^{1}H NMR spectrum in DMF-d$_7$ (500 MHz)

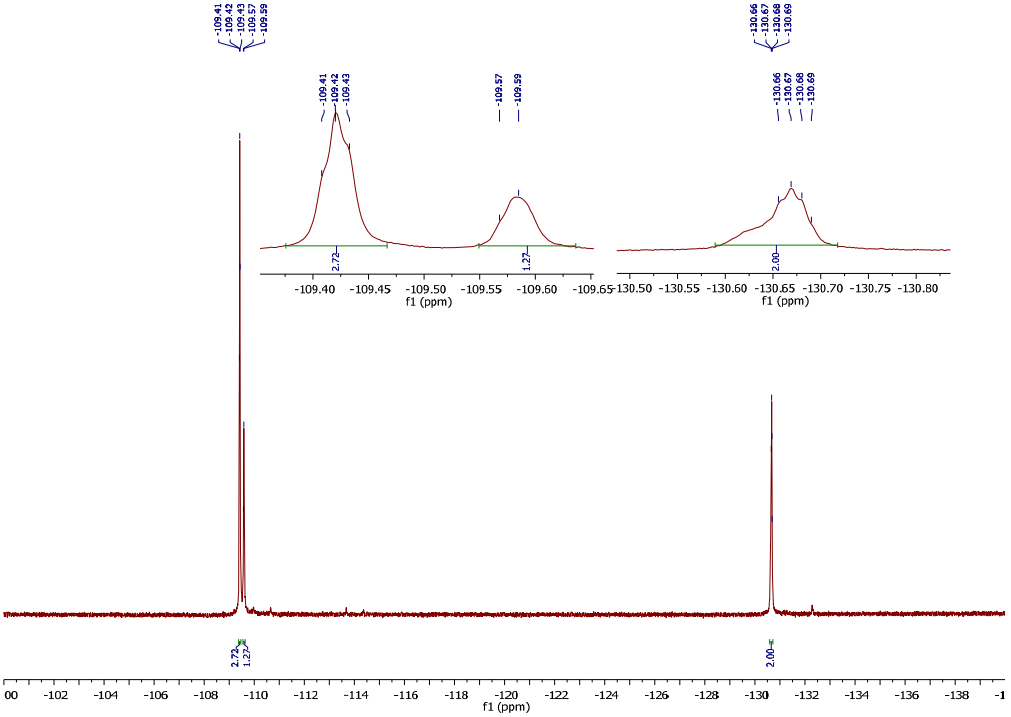


^{13}C NMR spectrum in DMF-d_7 (126 MHz)

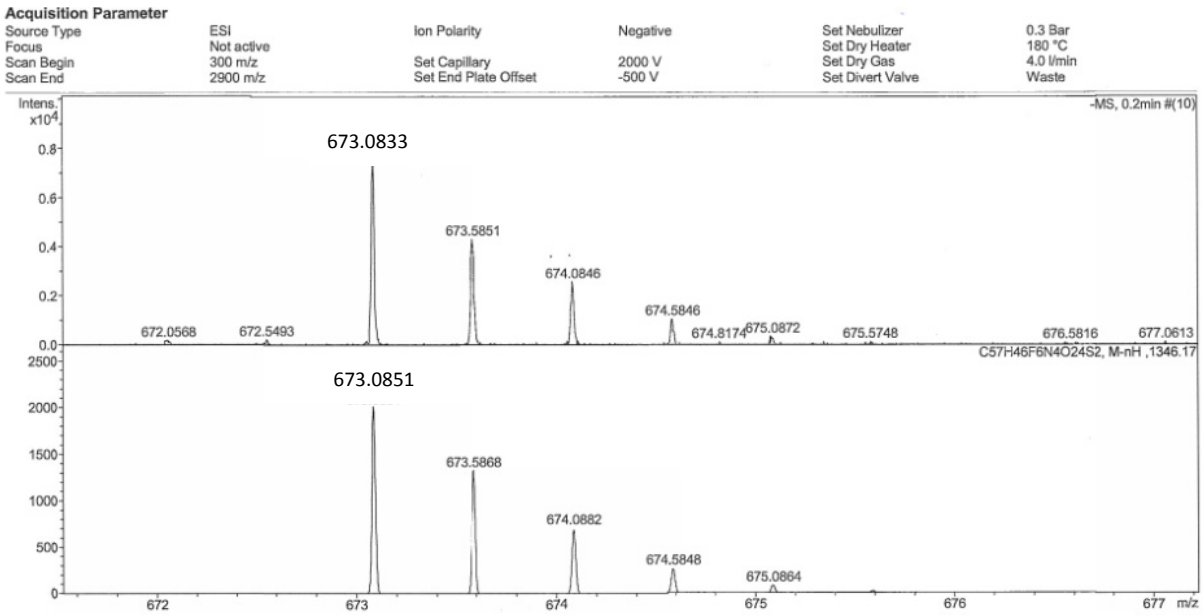
** peak assigned to residual triethyl amine left after lyophilisation*



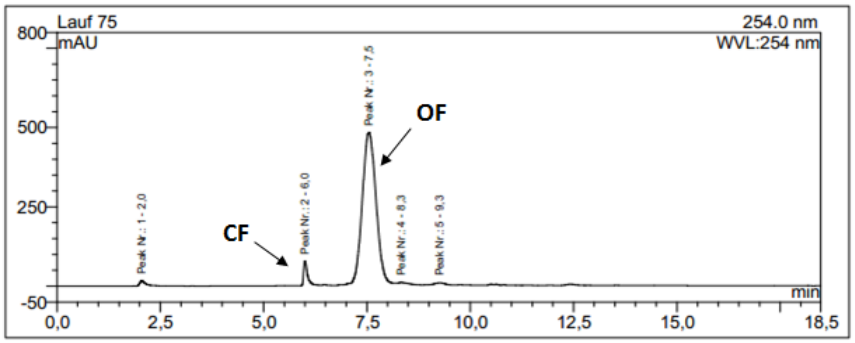
^{19}F NMR spectrum in DMF-d_7 (376 MHz)

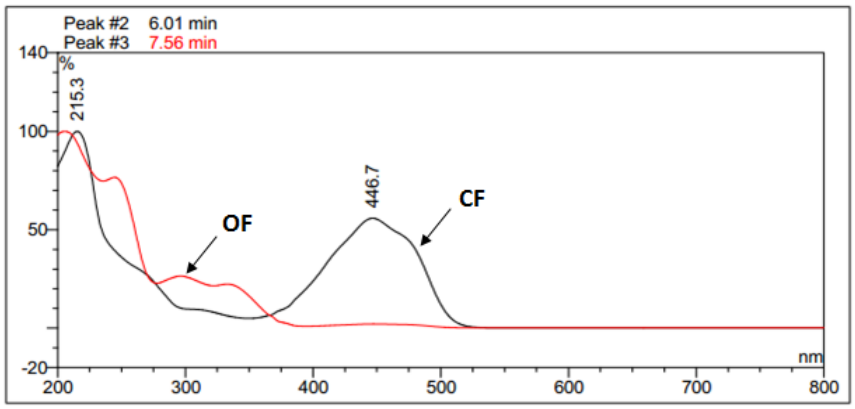


HRMS-ESI mass spectra (negative mode)



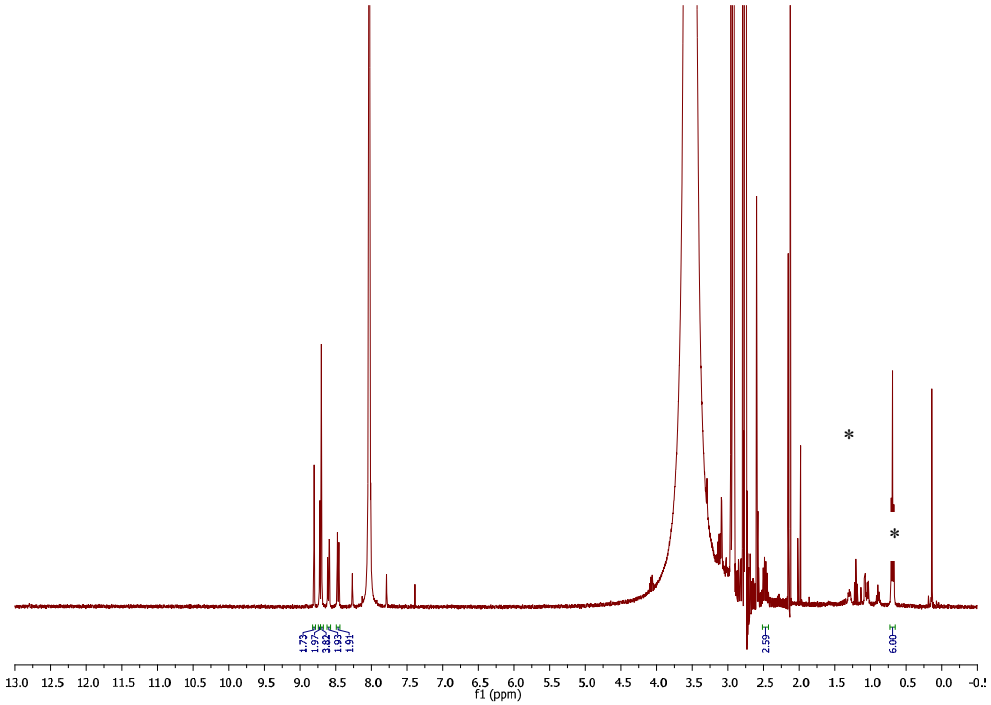
RP-HPLC elution profile (system B)





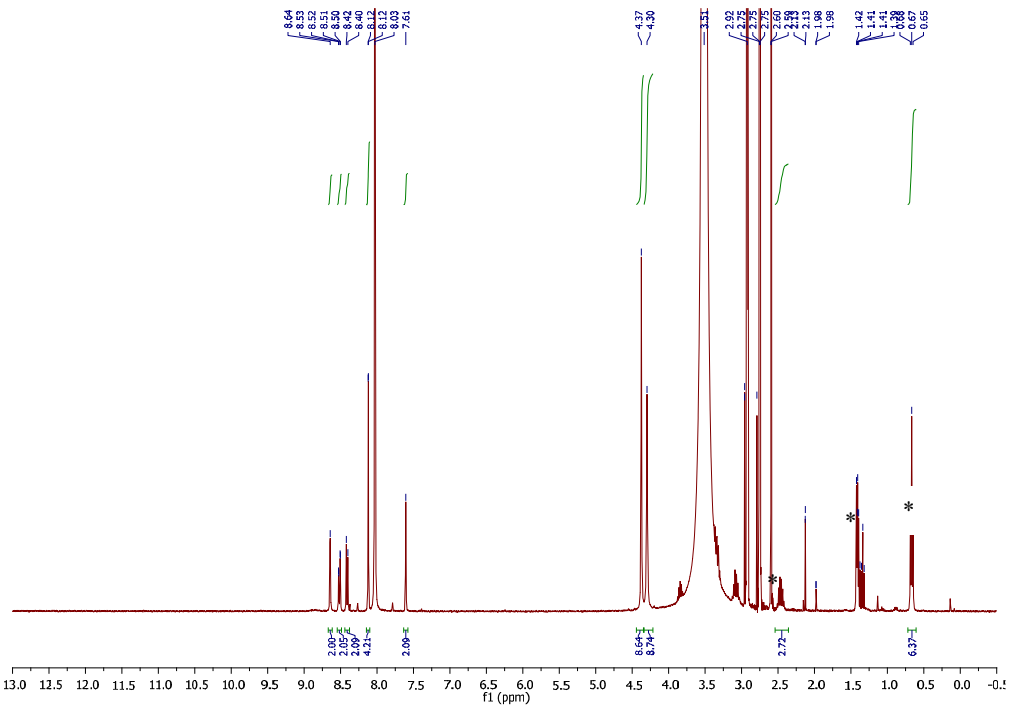
“Tetra-acid” (1-CF)

^{1}H NMR spectrum in DMF-d_7 (400 MHz) ** residual CH_3CN and solvents*



“Octa-acid” (2-CF)

^{1}H NMR spectrum in DMF-d_7 (400 MHz) ** residual CH_3CN and solvents*



7. Absorption/Emission spectra of dyes 1 and 2 and their bioconjugates in PBS and MeOH

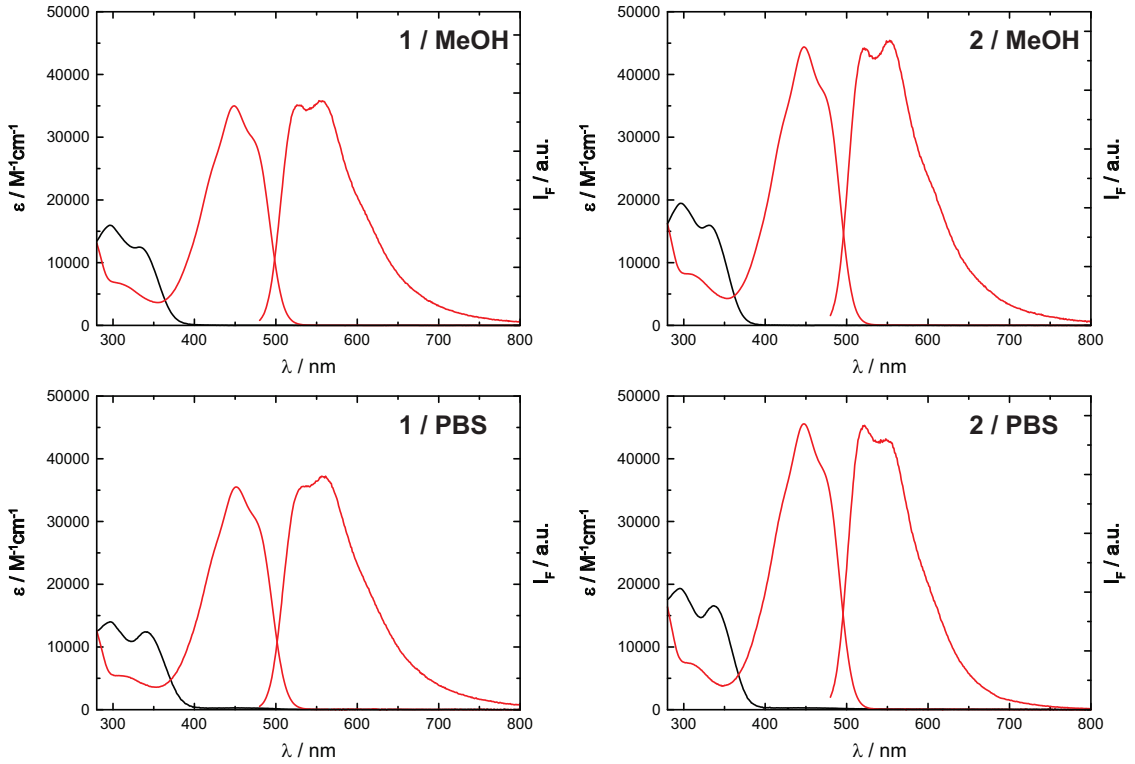


Figure S6. Absorption and emission spectra of free dyes **1** and **2** in MeOH and PBS (pH = 7.4). Absorption coefficients (left axes) of the open (black lines) and closed (red lines) and fluorescence emission (right axes). Main properties in methanol for both compounds are summarized in Table S2 (see below).

Table S2. Photophysical properties of free dyes **1** and **2** in methanol.

		1	**2**
$\lambda_{max\ abs}$ [nm] / ε [$M^{-1}cm^{-1}$]	OF[a]	330 / 12400	331 / 16000
$\lambda_{max\ abs}$ [nm] / ε [$M^{-1}cm^{-1}$]	CF	449 / 35000	448 / 44500
$\lambda_{max\ em}$ [nm]	CF	528; 556	523; 552
Φ_{fl} [b]	CF	0.69	0.70
τ [ns]	CF	2.52 ± 0.05	2.54 ± 0.05
$\Phi_{OF\rightarrow CF}$ [c]		0.29 ± 0.04	0.24 ± 0.04
$\Phi_{CF\rightarrow OF}$ [d]		2.8×10^{-3} ± 3×10^{-4}	2.6×10^{-3} ± 3×10^{-4}

[a] only the peak at the longest wavelength is reported; [b] fluorescein in 0.1 M NaOH (emission efficiency 0.79) was used as standard; [c] at 365 nm; [d] at 470 nm.

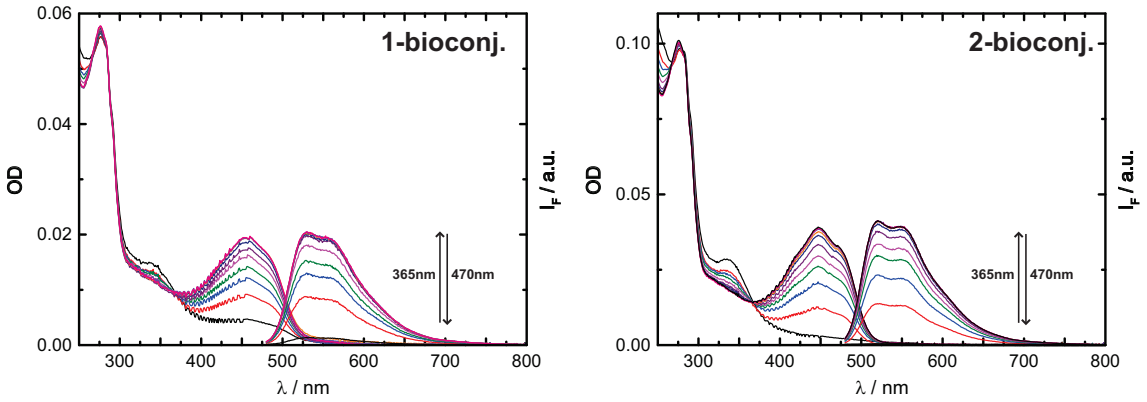


Figure S7. Absorption and emission spectra of two representative bioconjugates of **1** and **2** in PBS (pH = 7.4): changes observed upon irradiation. Diluted samples were placed in a 1 cm path quartz cuvette, and irradiated with 365 nm light or 470 nm light from collimated LEDs (M365L2 and M470L3, Thorlabs Inc., Germany) under continuous vigorous stirring. Typical irradiation light powers used were around 10-20 mW. Absorption and emission spectra were recorded after each irradiation step. Irradiation of bioconjugates of compounds **1** (BC1B) and **2** (BC2A) with a similar degree of labeling ($DOL_{Abs} \approx 5$), in diluted PBS (pH = 7.4) solutions. Absorption (left axes) and fluoresce emission (right axes) changes upon irradiation with UV and visible light.

8. References

[1] K. Uno, H. Niikura, M. Morimoto, Y. Ishibashi, H. Miyasaka, M. Irie, *J. Am. Chem. Soc.* **2011**, *133*, 13558-13564.

[2] J. Jiang, P. Vairaprakash, K. R. Reddy, T. Sahin, M. P. Pavan, E. Lubian, J. S. Lindsey, *Org. Biomol. Chem.* **2014**, *12*, 86-103.

[3] D. Paramelle, S. Cantel, C. Enjalbal, M. Amblard, E. Forest, M. Heymann, C. Geourjon, J. Martinez, G. Subra, *Proteomics* **2009**, *9*, 5384-5388.

[4] G. R. Fulmer, A. J. M. Miller, N. H. Sherden, H. E. Gottlieb, A. Nudelman, B. M. Stoltz, J. E. Bercaw, K. I. Goldberg, *Organometallics* **2010**, *29*, 2176-2179.

[5] C. Ruzié, M. Krayer, T. Balasubramanian, J. S. Lindsey, *J. Org. Chem.* **2008**, *73*, 5806-5820.

[6] B. Roubinet, C. Massif, M. Moreau, F. Boschetti, G. Ulrich, R. Ziessel, P. Y. Renard, A. Romieu, *Chem. Eur. J.* **2015**, *21*, 14589-14601.