

PONy Dyes: Direct Addition of P(III) Nucleophiles to Organic Fluorophores

Alexey N. Butkevich^{a#}, Maksim V. Sednev^{b#}, Heydar Shojaei^a, Vladimir N. Belov^{a*}
and Stefan W. Hell^a

^a Max Planck Institute for Biophysical Chemistry, Department of NanoBiophotonics
Am Fassberg 11, 37077 Göttingen, Germany

^b Present address: Institut für Organische Chemie, Julius-Maximilians-Universität Würzburg
Am Hubland, Geb. C1, 97074 Würzburg, Germany

*Corresponding authors' e-mails: alexey.butkevich@mpibpc.mpg.de and vbelov@mpibpc.mpg.de.

A.N.B. and M.V.S. contributed equally to this work.

Table of Contents

Supplementary Figures	4
Figure S1. Examples of P(III) reagents capable of reacting with electrophilic fluorophores	4
Figure S2. Examples of electrophilic fluorophores of the present study	5
Figure S3. Spatial distributions of electron density (at isosurface value 0.02) of calculated HOMOs and LUMOs for the sample parent fluorophores.....	10
Figure S4. Additional examples of PONy dyes (not included in Figure 1).....	11
Figure S5. Sample absorption and fluorescence emission spectra of PONy dyes	14
Figure S6. Energies of frontier orbitals of pyronin-type fluorophores.....	15
Figure S7. Correlation of the calculated HOMO/LUMO energy gap values of the parent electrophilic fluorophores with the observed experimental lowest energy UV-Vis absorption maxima.....	16
Supplementary Tables.	17
Table S1. Photophysical properties of the parent electrophilic fluorophores and the derived PONy dyes (including the hydrolytically unstable examples).....	19
Table S2. Excited state lifetimes and the corresponding relative amplitudes for the PONy dyes showing biexponential fluorescence decay	20
Supplementary Methods	21
General experimental information and synthesis	21
Chemical synthesis of the PONy dyes	23
Coumarin derivatives (C1-C9).....	23
Dye C1	23
Dye C2	24
Dye C3	25
Dye C4	27
Dye C5	28
Dye C6	29
Dye C7	30
Dye C8	31
Dye C9	35
Pyronin derivatives (P1-P14).....	36
Dye P1	36
Dye P1-Halo	38
Dye P2	40
Dye P3	41

Dye P4	43
Dye P5	46
Dye P6	48
Dye P7	50
Dye P8	52
Dye P8 NHS ester	55
Dye P9	56
Dye P10	57
Dye P11	58
Dye P12	60
Dyes P13 and P14.....	62
Acridine derivatives (A1, A2)	63
Dye A1	63
Dye A1 NHS ester	65
Dye A2	66
Thiopyronin derivatives (SP1, SP2).....	67
Dye SP1	67
Dye SP2	69
BODIPY derivative BP1.....	70
Dye BP1	70
Carbopyronin derivative CP1	71
Dye CP1	71
Si-pyronin derivative SiP1	72
Dye SiP1	72
Benzanthriliun derivative BA1.....	74
Dye BA1	74
Supplementary references	79
NMR spectra	80

Supplementary Figures

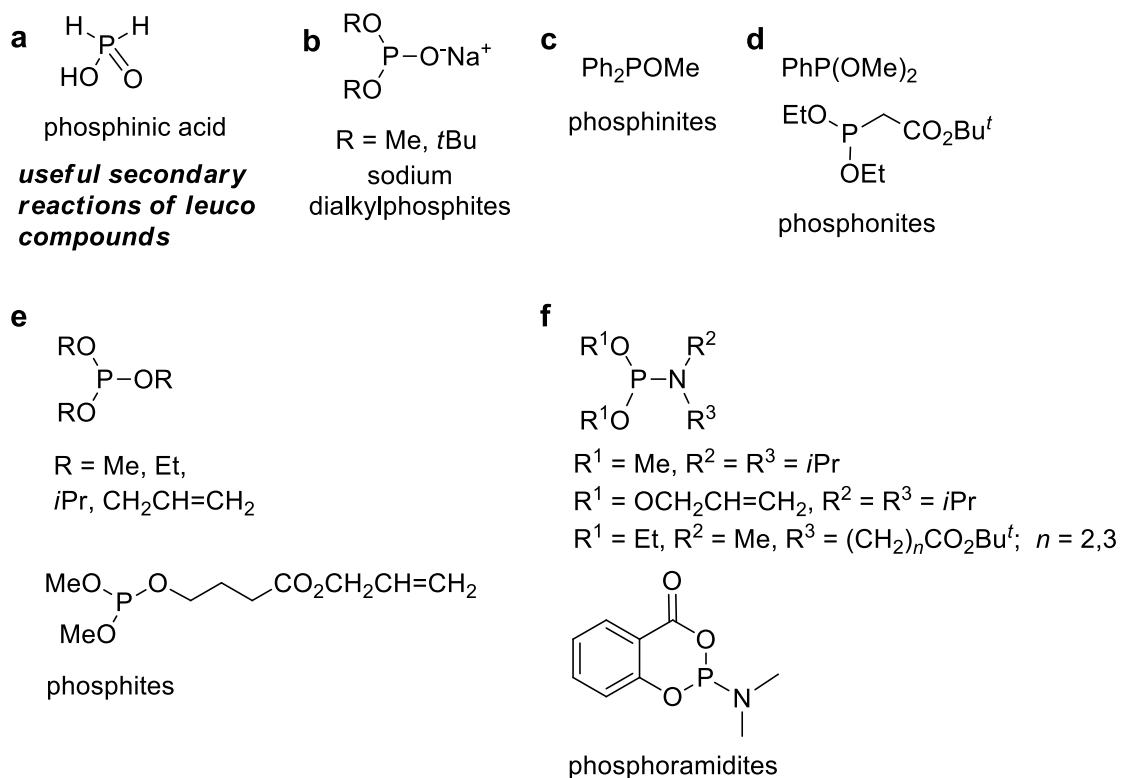


Figure S1. Examples of P(III) reagents capable of reacting with electrophilic fluorophores (Figure S1) according to Scheme 1: a) phosphinic (hypophosphorous) acid (H₃PO₂); b) sodium dialkylphosphites [(RO)₂PO⁻Na⁺]; c) phosphinites [R¹OPR'₂]; d) phosphonites [(RO)₂PR']; e) phosphites [(RO)₃P] and f) phosphoramidites [(RO)₂PNR'₂].

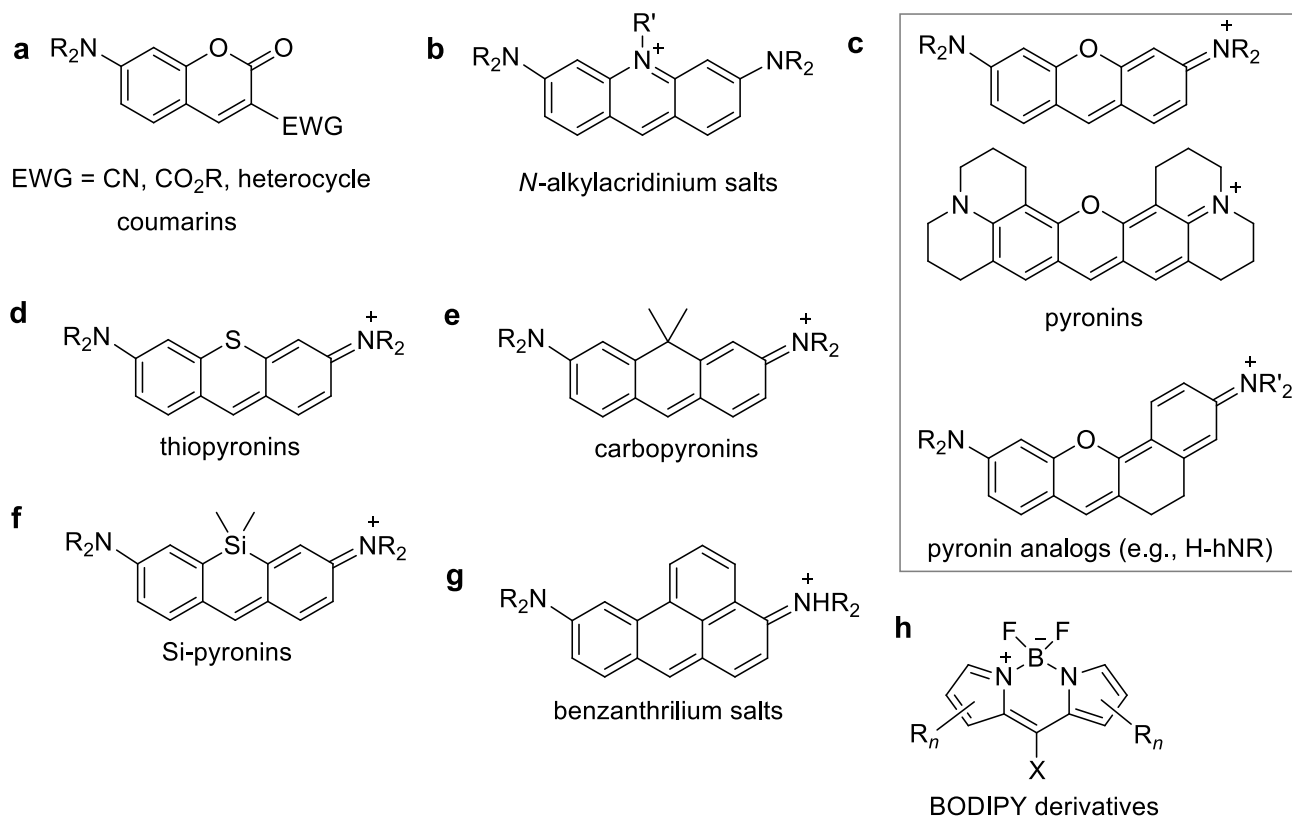
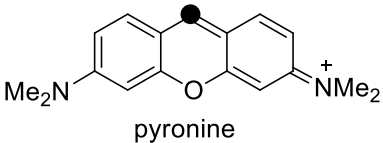
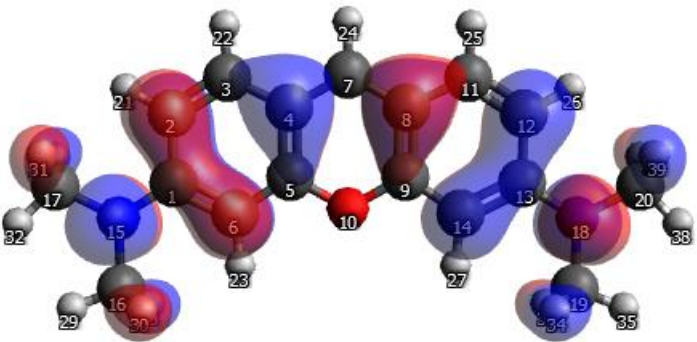
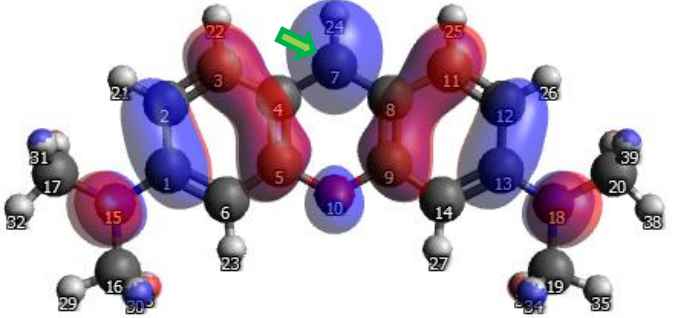
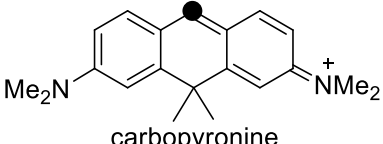
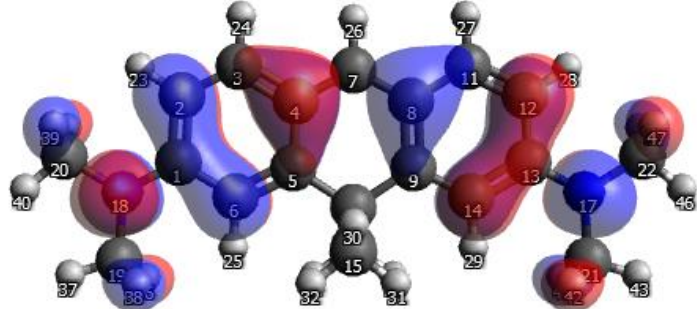
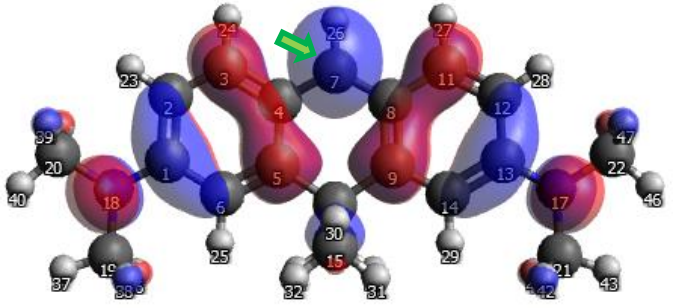
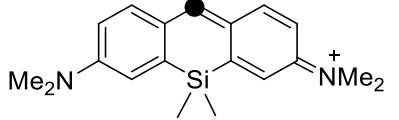
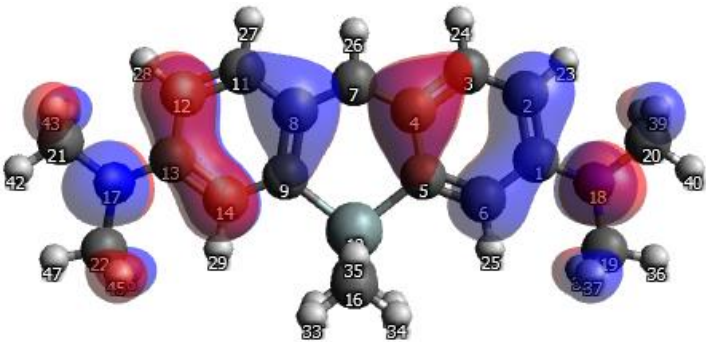
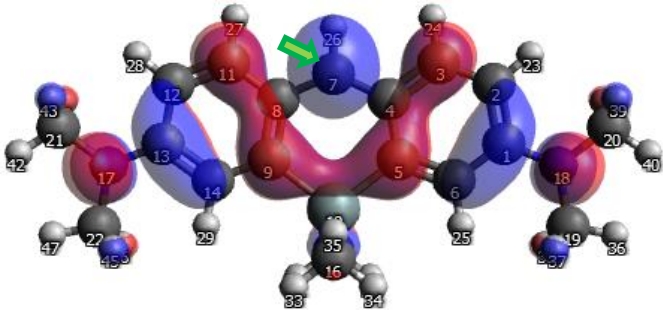
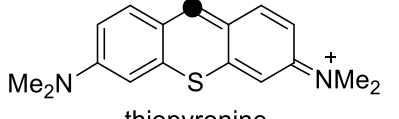
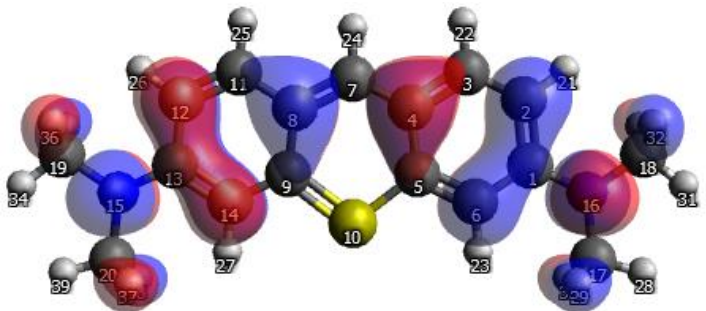
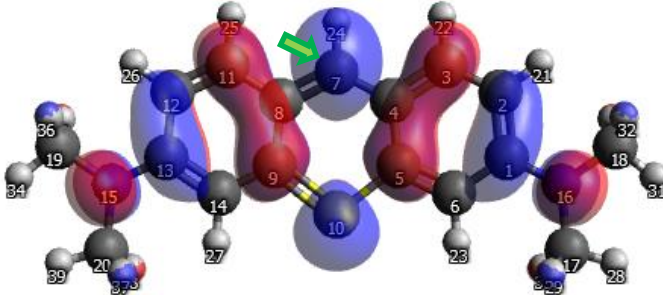
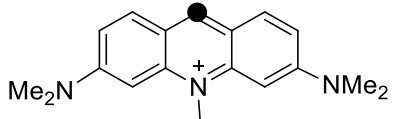
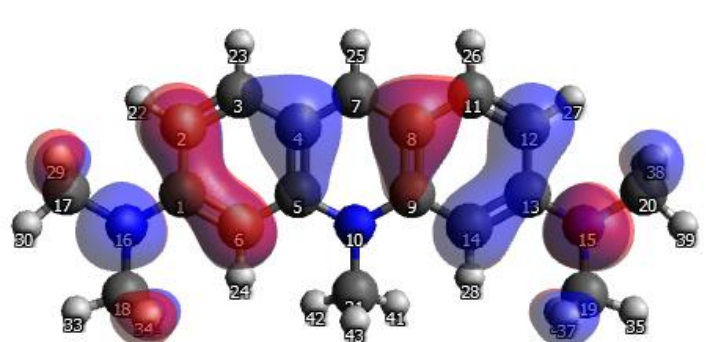
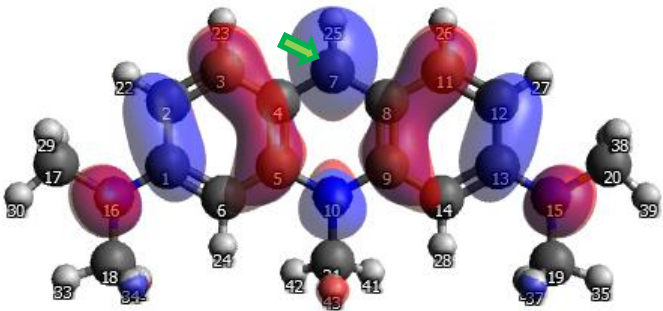
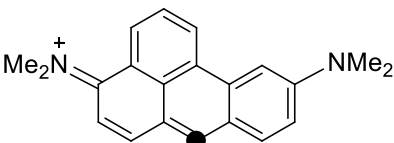
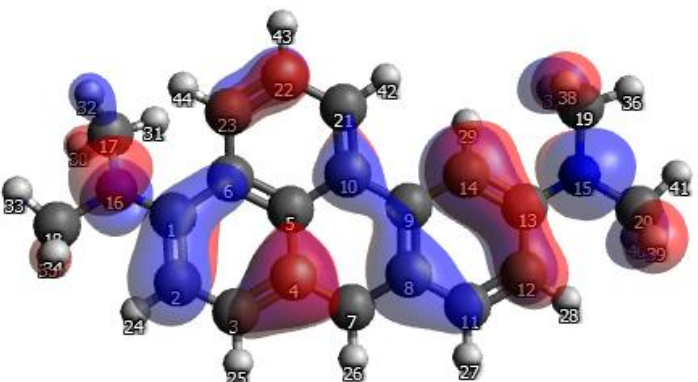
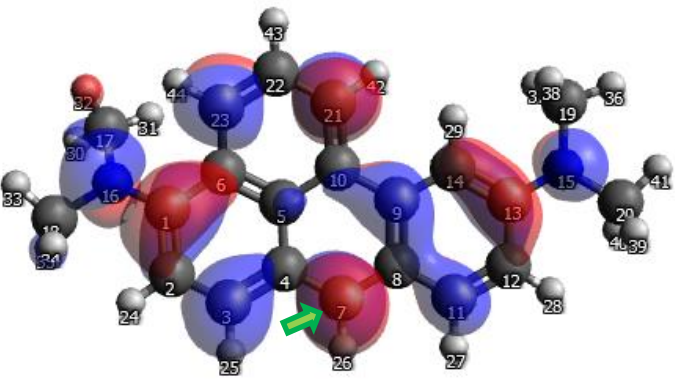
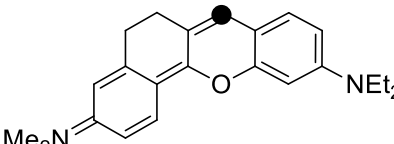
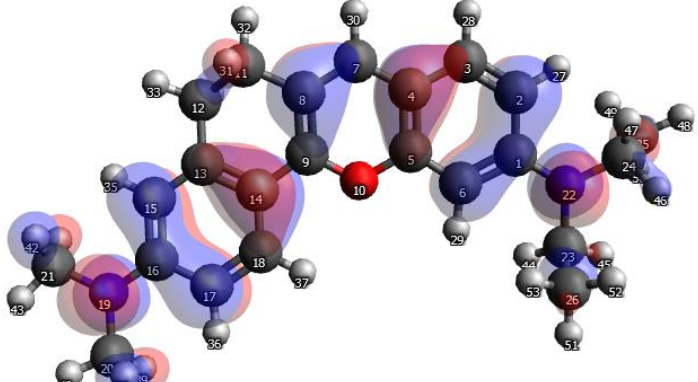
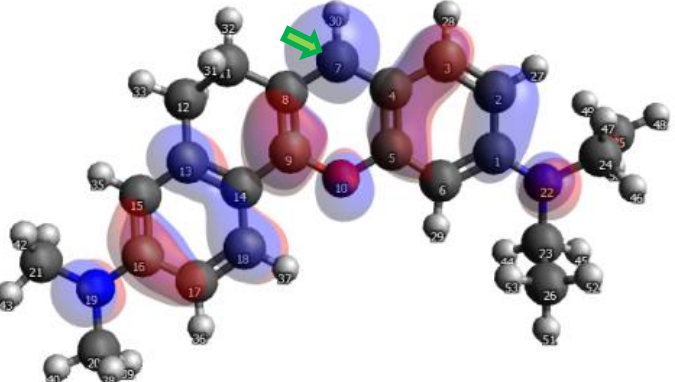
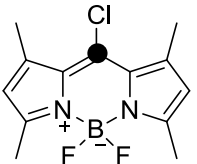
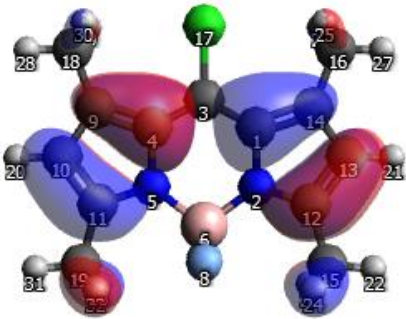
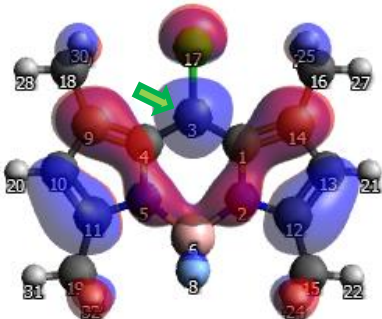
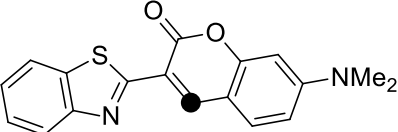
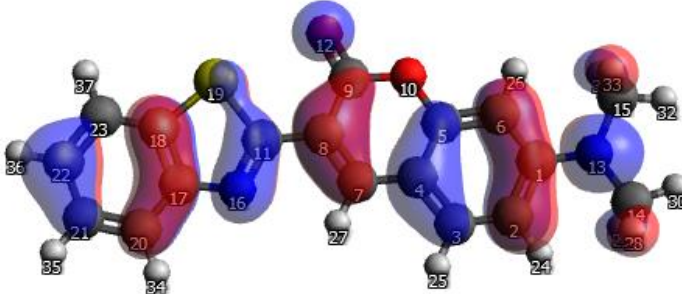
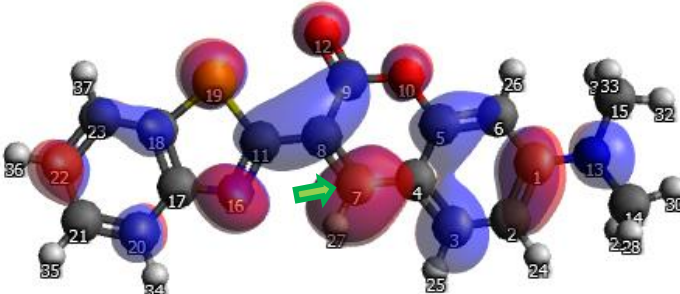


Figure S2. Examples of electrophilic fluorophores of the present study: a) coumarins with an electron-withdrawing group in position 3; b) *N*-alkylacridinium salts; c) pyronins and extended pyronin analogs (e.g., H-hNR); d) thiopyronins; e) carbopyronins; f) Si-pyronins; g) benzanthrillium salts; h) BODIPY derivatives (reactive when X = Cl).

Dye	HOMO 3D	LUMO 3D (atomic contributions to LUMO)
 <p>pyronine</p>		 <p>(C7-p = 0.29)</p>
 <p>carbopyronine</p>		 <p>(C7-p = 0.29)</p>

Dye	HOMO 3D	LUMO 3D (atomic contributions to LUMO)
 <p>Si-pyronine</p>		 <p>(C7-p = 0.27)</p>
 <p>thiopyronine</p>		 <p>(C7-p = 0.28)</p>
 <p>10-alkylacridinium</p>		 <p>(C7-p = 0.27)</p>

Dye	HOMO 3D	LUMO 3D (atomic contributions to LUMO)
 <p>4,10-bis(dimethylamino)-7H-benzo[de]anthracen-7-ylum</p>		 <p>(C7-p = 0.18, C1-p = 0.15, C3-p = 0.13)</p>
 <p>3,10-bis(dimethylamino)-5,6-dihydrobenzo[c]xanthen-12-ium</p>		 <p>(C7-p=0.23, C9-p=0.21)</p>

Dye	HOMO 3D	LUMO 3D (atomic contributions to LUMO)
 <p>BODIPY (9-Cl)</p>		 <p>(C3-p = 0.25, C11-p = 0.12, C12-p = 0.12, C9-p = 0.12, C14-p = 0.12)</p>
 <p>3-(2-benzothiazolyl)- 6-dimethylaminocoumarin</p>		 <p>(C7-p = 0.29)</p>

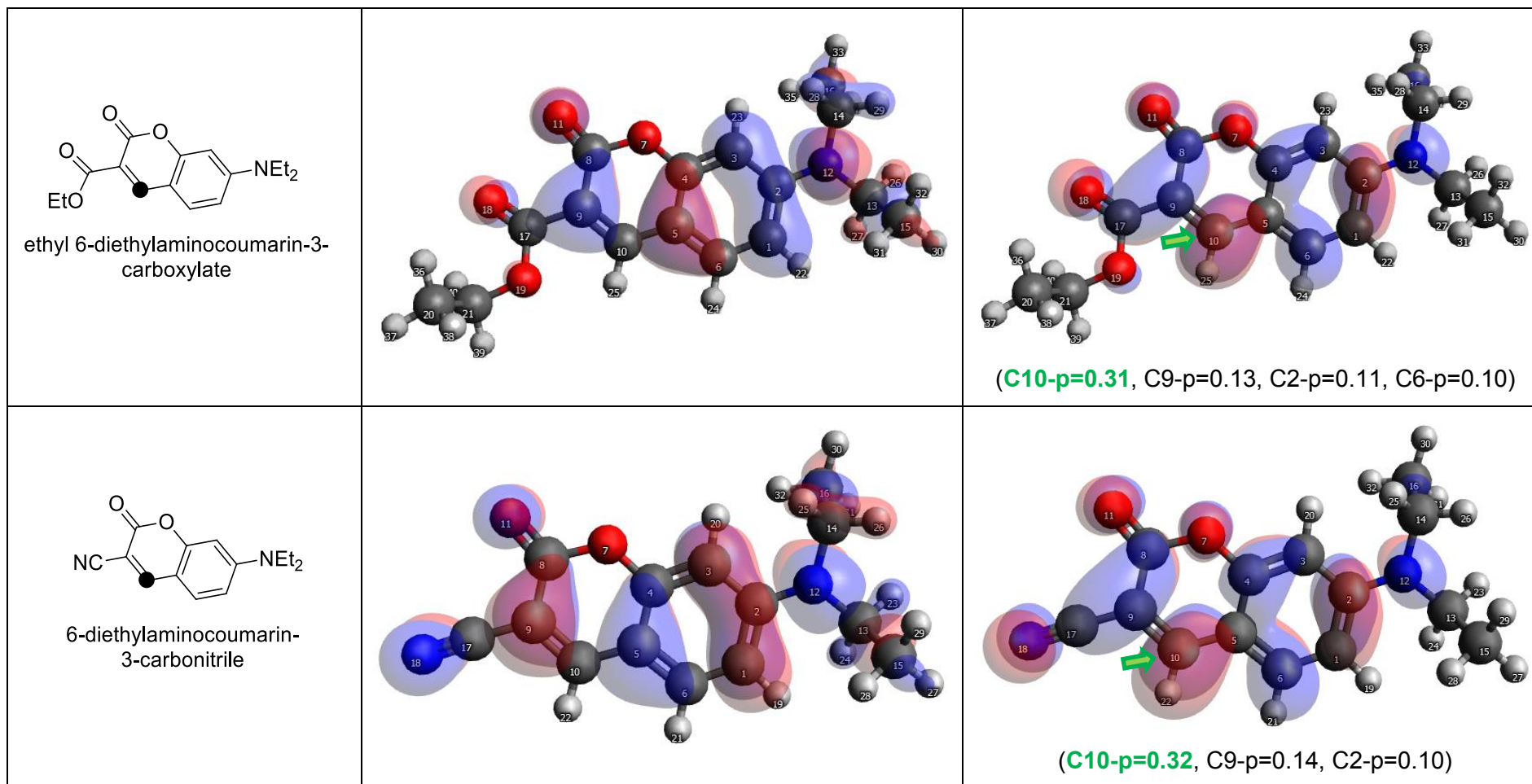


Figure S3. Spatial distributions of electron density (at isosurface value 0.02) of calculated HOMOs and LUMOs (see also Figure S6) for the sample parent fluorophores. For LUMOs, the highest contributions of atomic orbitals are listed. Note that these correspond to the observed regioselectivity (green arrow) of the nucleophilic addition of P(III) reagents in an orbital controlled reaction (as expected for the favorable soft/soft interactions according to Pearson; see Ref.3 in the main text). Calculated with Gaussian 09 (revision E.01)^[1] at the B3LYP/6-31+G(d) level of theory. The initial molecular geometries were generated using a built-in molecular mechanics method of ChemBio3D software (ChemBioOffice 12.0, CambridgeSoft) followed by additional refinement with the molecular mechanics method (force field: UFF, 4 steps per update, steepest descent algorithm) of Avogadro 1.1.1 software (<http://avogadro.cc/>).

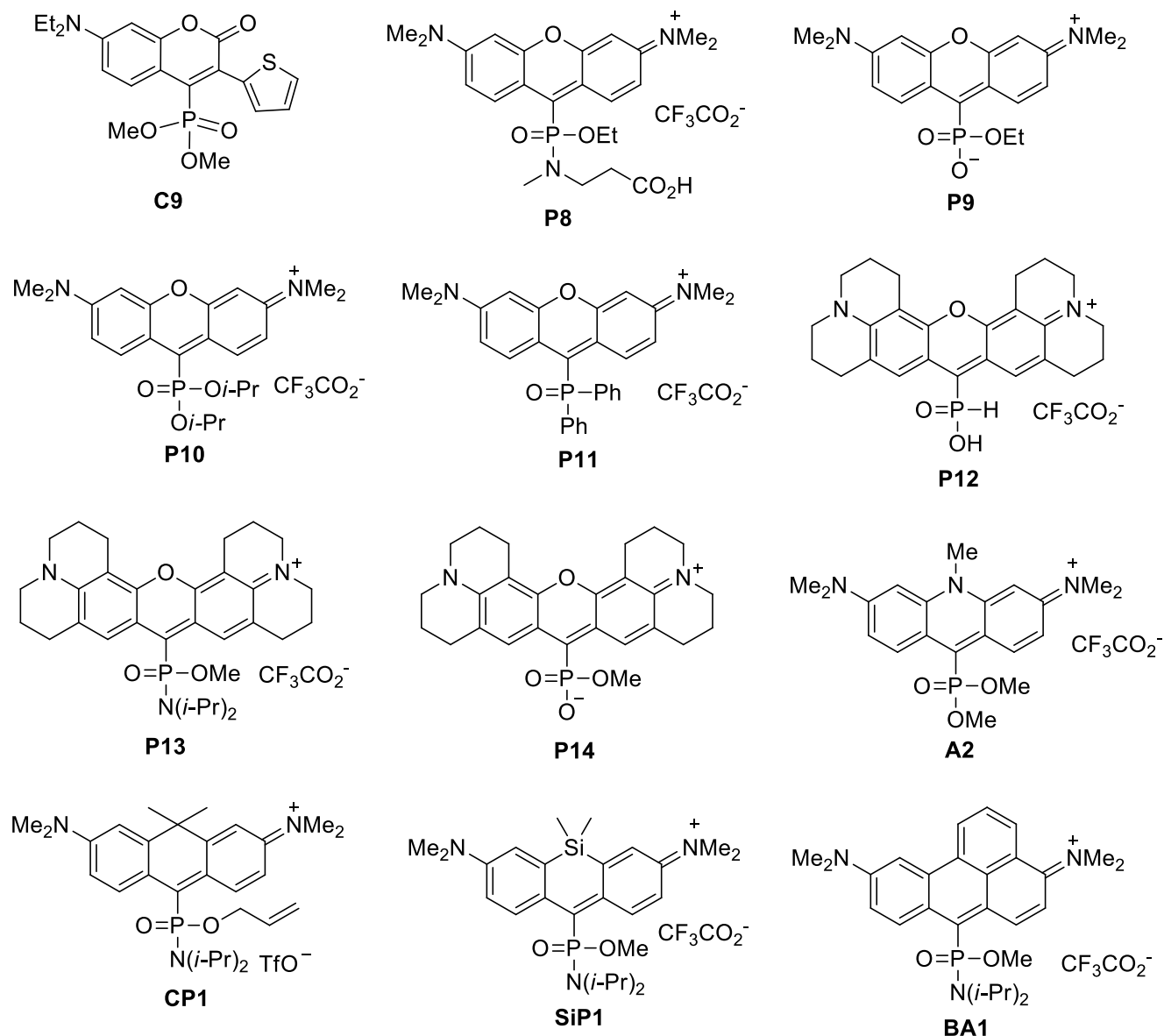
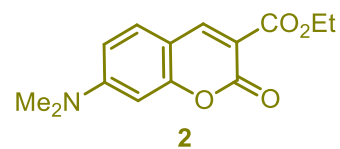
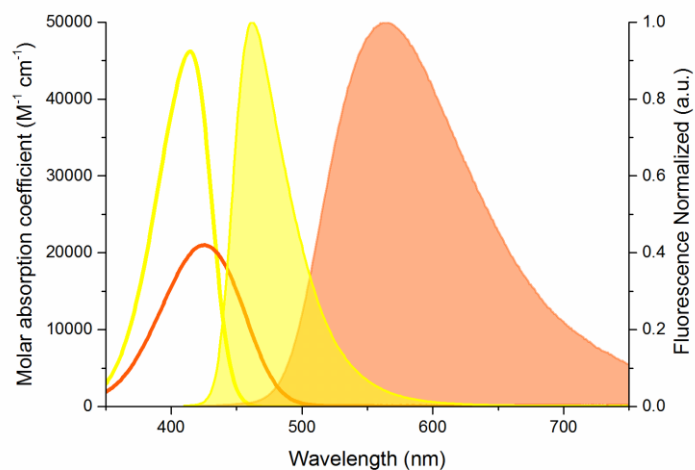
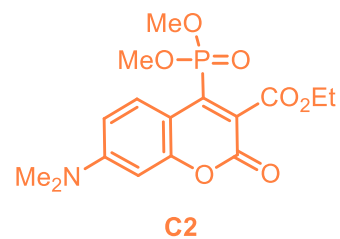


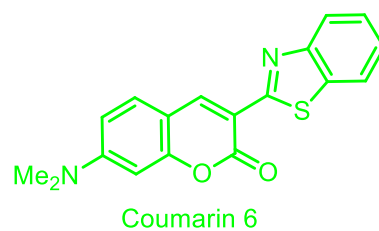
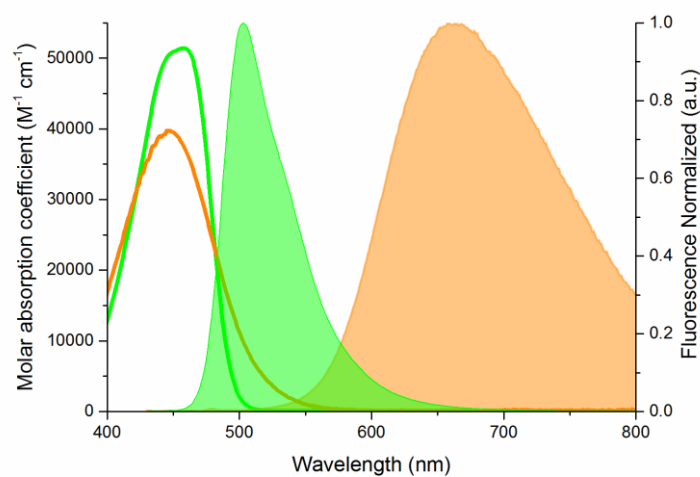
Figure S4. Additional examples of PONy dyes (not included in Figure 1). The compounds **P11**, **CP1** and in particular **SiP1** demonstrated poor hydrolytic stability (especially in protic solvents). For their comprehensive photophysical data, see Table S1.

a

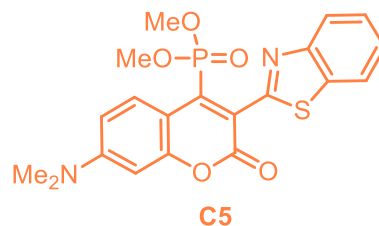
solvent: MeCN



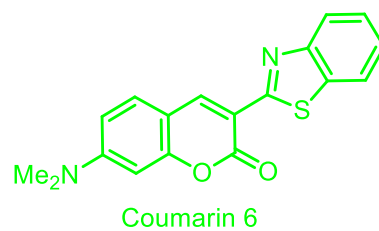
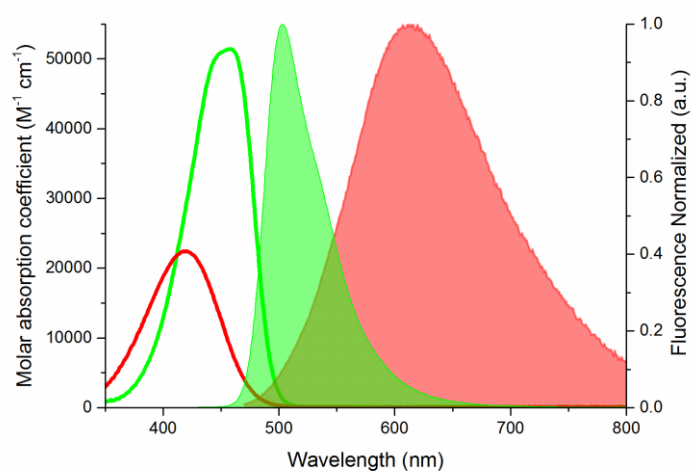
solvent: MeCN

b

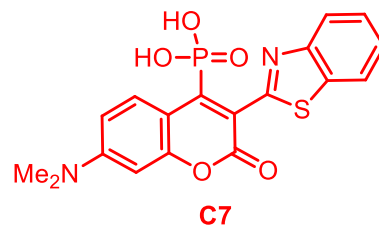
solvent: MeCN



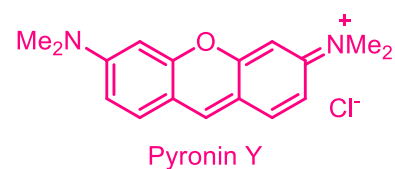
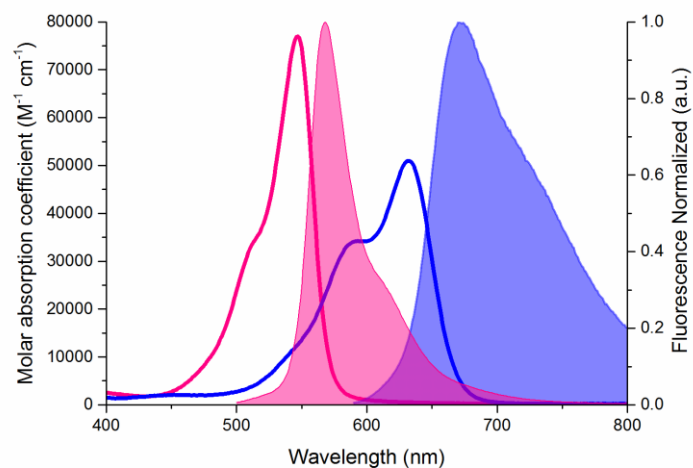
solvent: MeCN

c

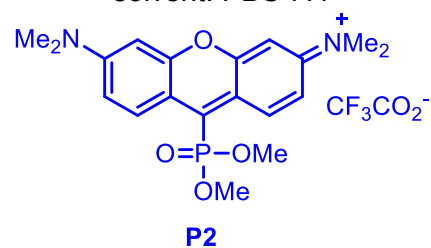
solvent: MeCN



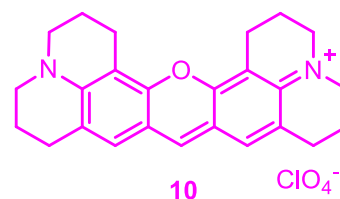
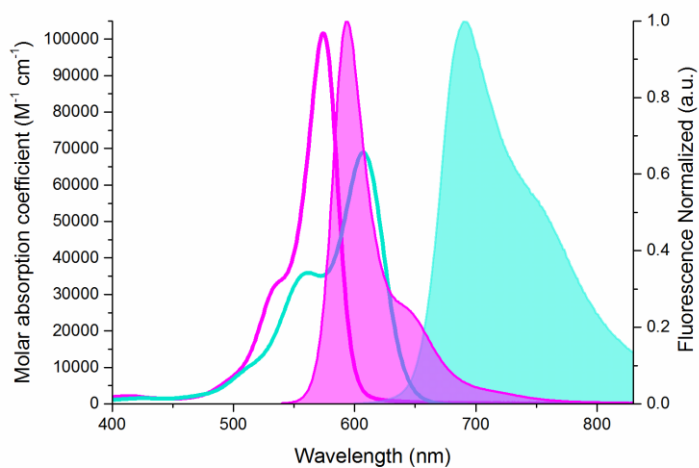
Solvent: PBS 7.4

d

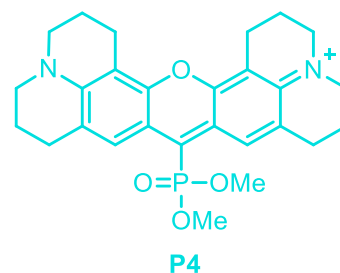
solvent: PBS 7.4



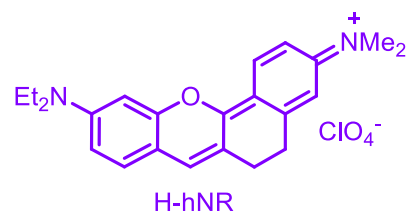
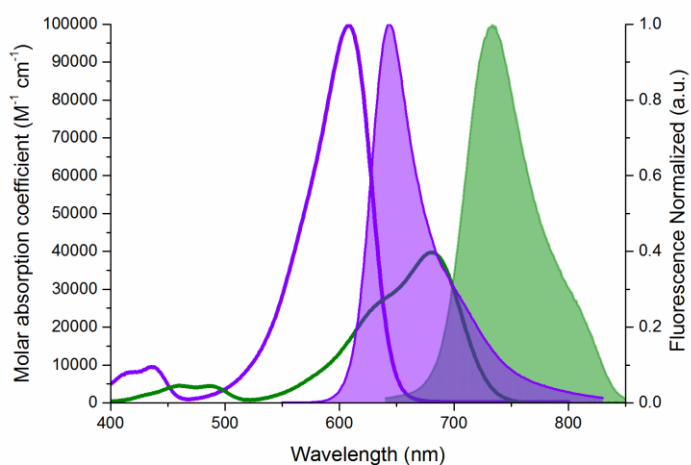
solvent: PBS 7.4

e

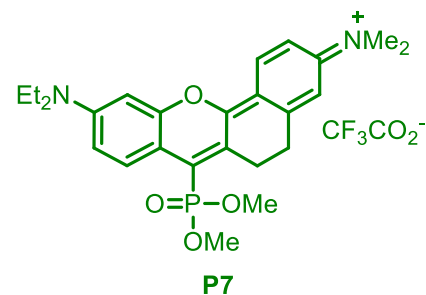
solvent: MeCN



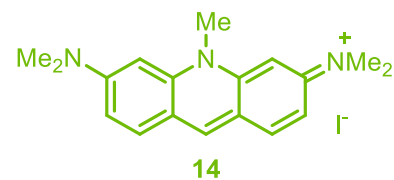
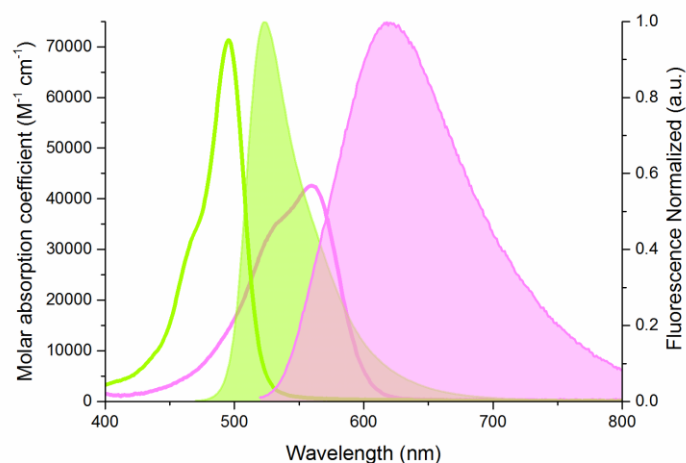
solvent: MeOH

f

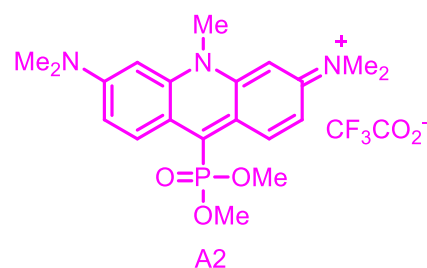
solvent: MeOH



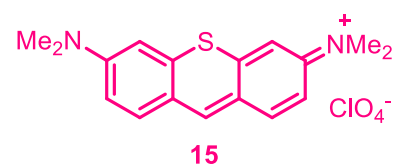
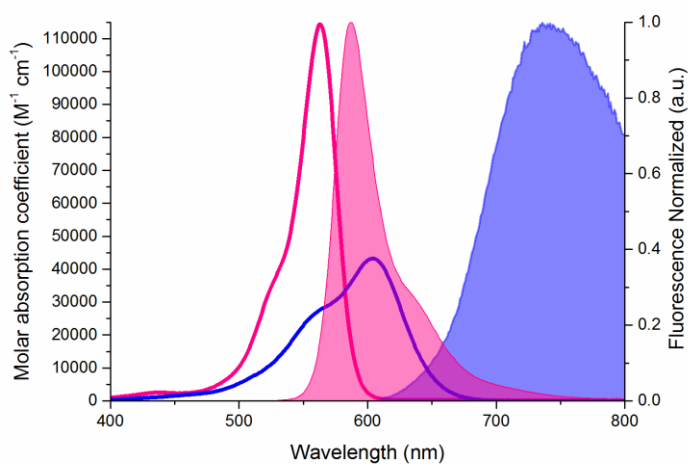
solvent: MeCN

g

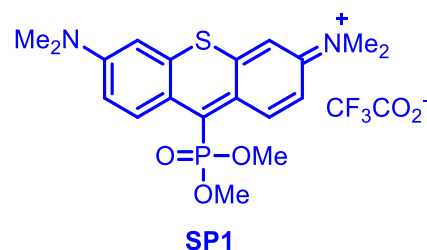
solvent: MeOH



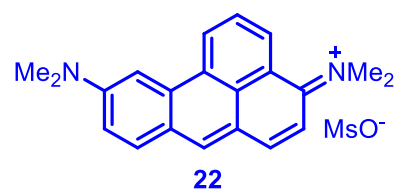
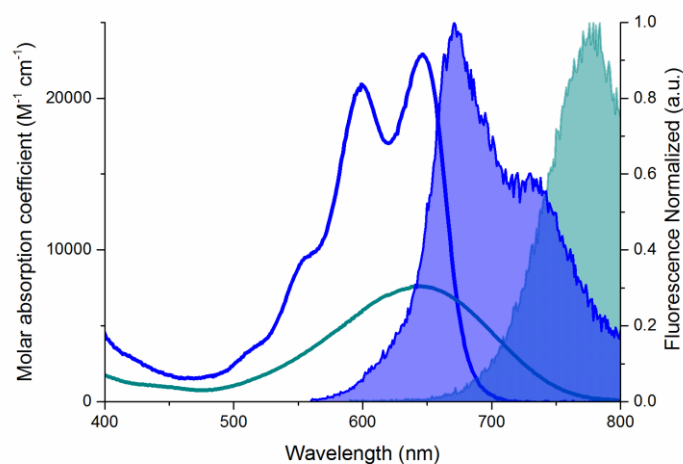
solvent: MeOH

h

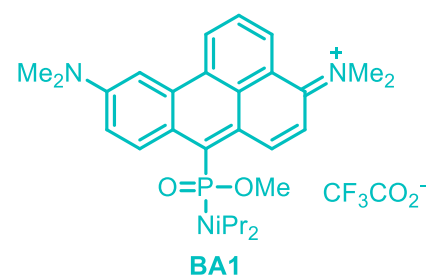
solvent: MeOH



solvent: MeOH

i

solvent: MeOH



solvent: PBS 7.4

Figure S5. Sample absorption and fluorescence emission spectra of PONy dyes derived from coumarins (**a-c**), pyronins (**d-f**), acridine (**g**), thiopyronin (**h**) and benzanthrylium dye (**i**) as compared to the parent fluorophores.

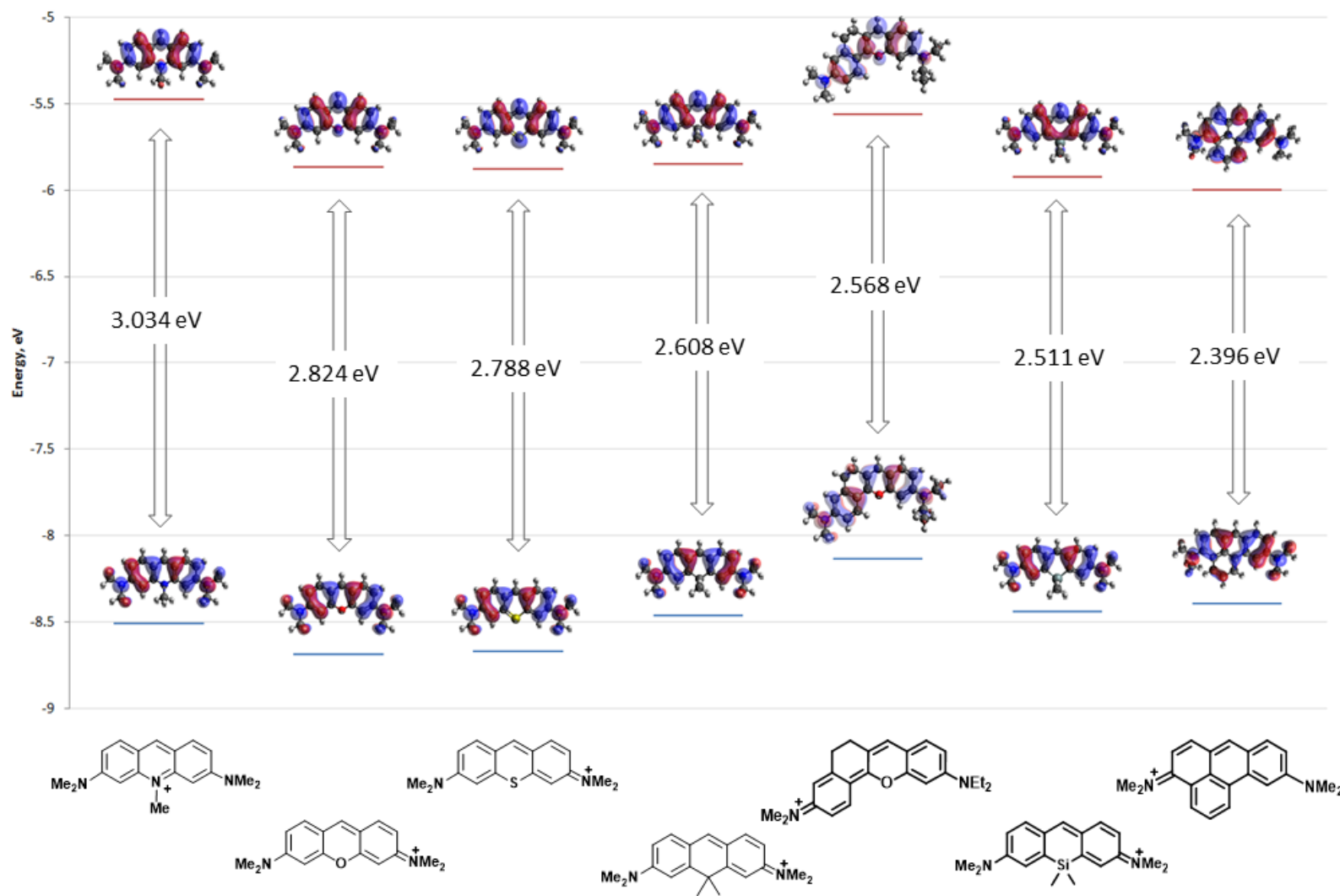


Figure S6. Energies of frontier orbitals of pyronin-type fluorophores in order of decreasing HOMO/LUMO energy gap values. Calculated with Gaussian 09 (revision E.01) at the B3LYP/6-31+G(d) level of theory.

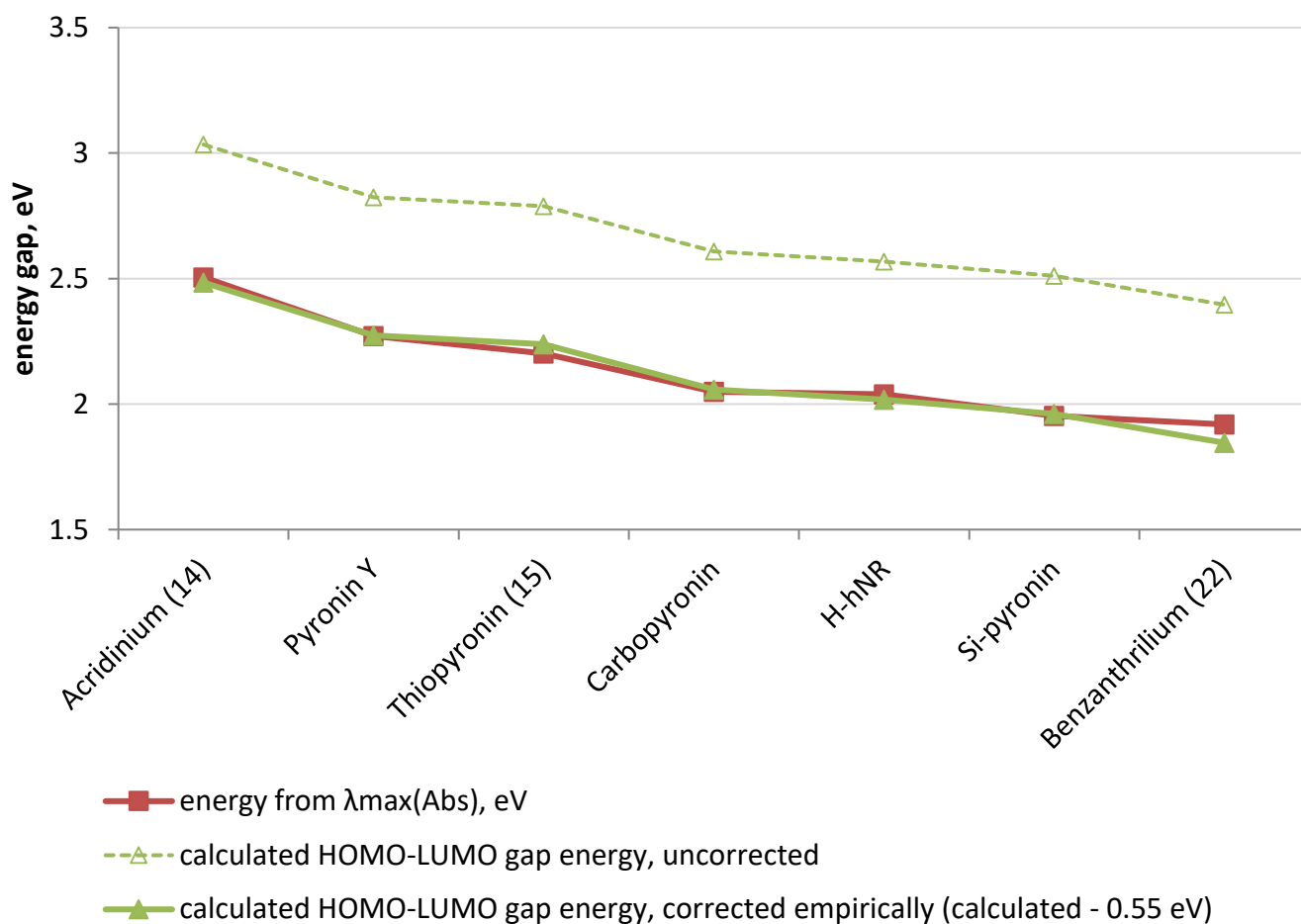


Figure S7. Correlation of the calculated HOMO/LUMO energy gap values of the parent electrophilic fluorophores (B3LYP/6-31+G(d); with an empirical correction: $\Delta E_{\text{theor}} = E_{\text{calc}}(\text{LUMO}) - E_{\text{calc}}(\text{HOMO}) - 0.55 \text{ eV}$) with the observed experimental lowest energy UV-Vis absorption maxima ($\Delta E_{\text{obs}} = hc / \lambda_{\max}(\text{Abs})$). All computations were performed at the High Performance Computing center for the Georg-August-Universität Göttingen (<https://www.gwdg.de/application-services/high-performance-computing>).

Supplementary Tables.

Dye (MW)	P(III) reagent	$\lambda_{\text{abs}}^{\text{max}}$ [nm] / ε [M ⁻¹ cm ⁻¹] ^a	$\lambda_{\text{em}}^{\text{max}}$ [nm], $\Phi_{\text{fl}}^{\text{b}}$	τ [ns] ^c	solvent	stability
1 (242)	-	424/48000	472, 0.02	n.d.	MeCN	good
C1 (350)	(MeO) ₂ PONa	468/32000	555, 0.03	n.d.	MeCN	good
2 (289)	-	414/46000	462, 0.04	n.d.	MeCN	good
C2 (397)	(MeO) ₂ PONa	425/21000	564, 0.10	0.9	MeCN	good
C3 (480)	(MeO) ₂ PONa	439/25000	642, 0.23	2.1	MeCN	good
C4 (455)	(MeO) ₂ PONa	431/22000	562, 0.39	2.6	MeCN	good
Coumarin 6 (350)	-	457/51000	501, 0.63	3.2	MeCN	good
C5 (458)	(MeO) ₂ PONa	439/24000	651, 0.20	2.6	MeCN	good
C6 (543)	(Bu ^t O) ₂ PONa	434/39000	658, 0.29	2.5	MeCN	good
C7 (430)	(Bu ^t O) ₂ PONa ^d	419/22000	613, 0.04	1.2, 0.3 ^e	PBS	good
7 (299)	-	422/34000	496, 0.88	3.1	MeCN	good
C8 (458)	(MeO) ₂ PONa	424/19000	651, 0.16	2.0	MeCN	good
C9 (407)	(MeO) ₂ PONa	430/23000	639, 0.21	2.2	MeCN	good
Pyronin Y (267)	-	546/77000	569, 0.36	1.8	PBS	good
		546/93000	568, 0.38	1.9	MeOH	
P1 (330)	H ₃ PO ₂	590/59000	627, 0.33	1.8	PBS	good
		579/67000	616, 0.44	2.7	MeOH	
		582/n.d.	615, 0.54	3.9	TFE	
		587/n.d.	620, 0.57	4.4	HFIP	
P1-Halo (608)	see Scheme 2 ^f	603/18000	635, 0.26	1.9	PBS	good
		588/73000	620, 0.50	3.1	MeOH	
P2 (375)	P(OMe) ₃	632/51000	672, 0.16	0.8	PBS	moderate
		624/62000	660, 0.22	1.6	MeOH	
		619/n.d.	653, 0.33	2.6	TFE	
		620/n.d.	656, 0.40	3.1	HFIP	
P3 (421)	PhP(OMe) ₂	629/62000	663, 0.21	1.6	MeOH	moderate
10 (371)	-	574/101000	594, 0.89	4.1	MeCN	good
P4 (480)	P(OMe) ₃	657/69000	691, 0.26	2.1	MeOH	good
P5 (593)	multistep ^f	660/38000	704, 0.10	1.7, 0.7 ^e	PBS	moderate
		651/46000	693, 0.32	2.7	MeCN ^h	
P6 (493)	multistep ^f	603/69000	638, 0.09	1.9	PBS	good
		589/49000	622, 0.36	3.0	MeOH	

Dye (MW)	P(III) reagent	$\lambda^{\max}_{\text{abs}}$ [nm] / ϵ [M ⁻¹ cm ⁻¹] ^a	$\lambda^{\max}_{\text{em}}$ [nm], Φ_{f} ^b	τ [ns] ^c	solvent	stability
H-hNR	-	608/100000	644, 0.70	3.9	MeOH	good
P7 (427)	P(OMe) ₃	693/29000 681/40000	754, 0.02 737, 0.17	n.d. 1.5	PBS MeCN	good
P8 (459)	multistep ^f	615/n.d. 625/n.d.	652, 0.46 670, 0.17	n.d. n.d.	MeCN PBS	moderate
P9 (374)	hydrolysis of P8 ^f	597/66000 565/64000	633, 0.22 595, 0.44	n.d. n.d.	PBS MeCN	moderate
P10 (431)	P(OPr ⁱ) ₃	629/52000 617/58000 621/57000 614/n.d. 614/n.d.	668, 0.12 649, 0.38 655, 0.24 645, 0.40 648, 0.46	0.9 2.5 1.8 3.1 3.6	PBS MeCN MeOH TFE HFIP	moderate
P11 (468)	Ph ₂ POMe	635/26000 ^g 627/32000 ^g	702, 0.06 697, 0.13	0.5 1.2	PBS MeOH	poor
P12 (434)	H ₃ PO ₂	608/48000	642, 0.61	3.9	MeOH	moderate
P13 (464)	(MeO) ₂ PNPr _{i2}	630/52000 596/50000	666, 0.17 623, n.d.	n.d. n.d.	PBS MeCN	moderate
P14 (593)	(MeO) ₂ PNPr _{i2}	654/64000	728, 0.10	0.8	MeCN	good
14 (280)	-	495/71000	524, 0.30	1.7	MeOH	good
A1 (416)	H ₃ PO ₂	535/38000 527/67000	595, 0.10 579, 0.32	1.4 2.6	PBS MeOH	good
A2 (388)	(MeO) ₂ PONa	560/43000	620, n.d.	n.d.	MeOH	poor
15 (283)	-	563/114000	587, 0.38	2.1	MeOH	good
SP1 (391)	P(OMe) ₃	654/43000	739, 0.04	1.3, 0.4 ^e	MeOH	good
SP2 (505)	(MeO) ₂ PNPr _{i2}	654/64000	728, 0.10	0.8	MeCN	good
Carbopyronin (293)	-	605	627, 0.71	n.d.	EtOH	good
CP1 (541)	(MeO) ₂ PNPr _{i2}	710/17000 698/41000	783, 0.19 775, 0.26	n.d. n.d.	PBS MeCN	poor
18 (310)	-	635/155000	654, 0.64	3.4	MeOH	good
SiP1 (488)	(MeO) ₂ PNPr _{i2}	752/n.d. ^g 743/43000	819, 0.03 809, 0.06	n.d. n.d.	PBS MeCN	poor
22 (301)	-	646/23000	673, 0.25	1.3	MeOH	good
BA1 (523)	(MeO) ₂ PNPr _{i2}	644/7600 ^g	782, n.d.	n.d.	PBS	poor
BODIPY 505/515 (248)	-	505/79000	515, 0.98	n.d.	CH ₂ Cl ₂	good
BP1 (412)	P(OPr ⁱ) ₃	574/33000	647, <0.002	–	MeCN	good

Table S1. Photophysical properties of the parent electrophilic fluorophores and the derived PONY dyes (including the hydrolytically unstable examples). a) lowest energy absorption peak; b) fluorescence quantum yield (absolute value); c) fluorescence lifetime; d) deprotection of **C7** with CF₃CO₂H (TFA); e) biexponential, see Table S2 below; f) multiple steps, see the Experimental section below; g) decomposes in solution at rt; h) with addition of 1% (v/v) TFA. MW – molecular mass (not including counterions). PBS – phosphate buffered saline (1×), pH 7.4; TFE – 2,2,2-trifluoroethanol; HFIP – 1,1,1,3,3,3-hexafluoro-2-propanol. n.d. – not determined.

Dye	Solvent	Fluorescence lifetime τ_i		Relative amplitude A_i	
		τ_1 , ns	τ_2 , ns	A_1	A_2
C7	PBS 7.4	1.2	0.3	0.28	0.72
P5	PBS 7.4	1.7	0.7	0.67	0.33
SP1	MeOH	1.2	0.4	0.06	0.94

Table S2. Excited state lifetimes and the corresponding relative amplitudes for the PONy dyes showing biexponential fluorescence decay (Table 1 and Table S1).

Supplementary Methods

General experimental information and synthesis

Thin layer chromatography: Analytical TLC (normal phase) was performed on Merck Millipore ready-to-use aluminum sheets coated with silica gel 60 (F₂₅₄) (Cat. No. 1.05554.0001). Analytical TLC on reversed phase (RP-C₁₈) was performed on Merck Millipore ready-to-use aluminum sheets coated with RP-18 60 (F_{254s}) (Cat. No. 1.05560.0001). Preparative TLC was performed on silica-precoated glass plates for high performance TLC (HPTLC Silica gel 60 F₂₅₄ 10×10 cm, layer thickness 150-200 µm, with concentrating zone 10 x 2.5 cm) from Merck Millipore (Cat. No. 1.13727.0001). Compounds were detected by exposing TLC plates to UV-light (254 or 366 nm) or by heating with vanillin stain (6 g vanillin and 1.5 mL conc. H₂SO₄ in 100 mL ethanol); leuco dyes were detected by staining with 1% DDQ in CH₂Cl₂.

Preparative flash column chromatography: Silica 60 (0.04 – 0.063 mm) for column chromatography was used (Macherey-Nagel, Germany; Cat. No. 815380.5). Reversed phase column chromatography was performed on POLYGOPREP 60-50 C₁₈ (Macherey-Nagel, Cat. No. 711500.1000). Automated separations were performed with an Isolera Spektra One system (Biotage AG, Sweden) using the type of cartridge and solvent gradient indicated.

High-performance liquid chromatography: Analytical HPLC was performed on a Knauer Azura liquid chromatography system with a binary P 6.1L pump (Article No. EPH35, Knauer), UV diode array detector DAD 6.1L (Article No. ADC11, Knauer), an injection valve with a 20 µL loop and two electrical switching valves V 2.1S with 6-port multiposition valve head (Article No. EWA10, Knauer). Analytical columns: Knauer Eurospher II 100-5 C₁₈, 5 µm, 150×4 mm (Article No. 15DE181E2J, Knauer) or Interchim Uptisphere Strategy C₁₈-HQ, 10 µm, 250×4.6 mm (Article No. US10C18HQ-250/P46, Interchim), typical flow rate: 1.2 mL/min, unless stated otherwise.

Preparative HPLC was performed on an Interchim puriFlash 4250 2X preparative HPLC/Flash hybrid system (Article No. 1I5140, Interchim) with a 2 mL injection loop, a 200-600 nm UV-Vis detector and an integrated ELSD detector (Article No. 1A3640, Interchim). Preparative column: Interchim Uptisphere Strategy C₁₈-HQ, 10 µm,

250×21.2 mm (Article No. US10C18HQ-250/212, Interchim), typical flow rate: 20 mL/min, unless specified otherwise.

Optical spectroscopy: Absorption spectra were recorded with a Varian Cary 4000 UV-Vis double-beam spectrophotometer (Agilent Technologies, USA). The emission spectra were recorded with a Varian Cary Eclipse fluorescence spectrophotometer (Agilent). The absorption and emission spectra were recorded in quartz cells (optical path length 1 cm). The fluorescence quantum yields (absolute values) were obtained with a Quantaaurus-QY absolute PL quantum yield spectrometer (model C11347-12, Hamamatsu) according to the manufacturer's instructions. Fluorescence lifetimes were measured with a Quantaaurus-Tau fluorescence lifetime spectrometer (model C11367-32, Hamamatsu) according to the manufacturer's instructions. All measurements were performed in air-saturated solvents at ambient temperature.

NMR spectra were recorded at 25 °C with an Agilent 400-MR spectrometer at 400.06 MHz (^1H), 376.40 MHz (^{19}F), 161.94 MHz (^{31}P) and 100.60 MHz (^{13}C) and are reported in ppm. All ^1H spectra are referenced to tetramethylsilane ($\delta = 0$ ppm) using the signals of the residual protons of CHCl_3 (7.26 ppm) in CDCl_3 , CHD_2CN (1.94 ppm) in CD_3CN , CHD_2OD (3.31 ppm) for CD_3OD , $\text{CHD}_2\text{COCD}_3$ (2.05 ppm) for acetone- d_6 , $\text{CHD}_2\text{CO}_2\text{D}$ (2.04 ppm) for acetic acid- d_4 or $\text{DMSO}-d_5$ (2.50 ppm) for $\text{DMSO}-d_6$. ^{13}C spectra are referenced to tetramethylsilane ($\delta = 0$ ppm) using the signals of the solvent: CDCl_3 (77.16 ppm), CD_3CN (1.32 ppm), CD_3OD (49.00 ppm), $(\text{CD}_3)_2\text{CO}$ (29.84 ppm), $\text{DMSO}-d_6$ (39.52 ppm) or CNO_2 signal (148.60 ppm) of nitrobenzene- d_5 . Multiplicities of signals are described as follows: s = singlet, d = doublet, t = triplet, q = quartet, p = pentet, m = multiplet or overlap of non-equivalent resonances; br = broad signal. Coupling constants (J) are given in Hz. For the ^{13}C chemical shifts obtained by indirect detection from HSQC experiments (minimum resolution in F1: $t_1 \geq 192$), only H-coupled C-nuclei are resolved.

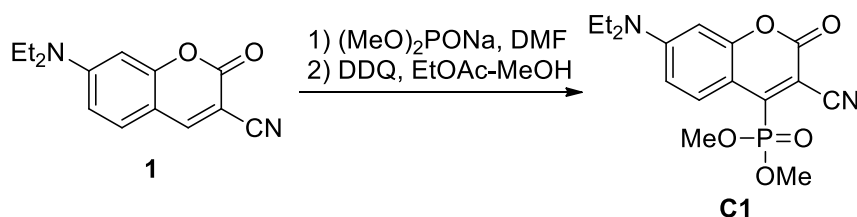
ESI-MS were recorded on a Varian 500-MS spectrometer (Agilent). **ESI-HRMS** were recorded on a MICROTOF spectrometer (Bruker) equipped with ESI ion source (Apollo) and direct injector with LC autosampler Agilent RR 1200.

Chemical synthesis of the PONy dyes

Coumarin derivatives (C1-C9)

Dye C1

dimethyl [3-cyano-7-diethylamino-2-oxo-2*H*-chromen-4-yl]phosphonate



To a stirred suspension of NaH (120 mg of 60 wt.% in mineral oil, 3.0 mmol) in dry DMF (3 mL), cooled in an ice-water bath, dimethyl phosphite (Sigma-Aldrich, 276 μ L, 3.0 mmol) was added dropwise. The suspension was warmed up to rt and stirred for 30 min, turning into a clear solution, which was added to a stirred suspension of 7-(diethylamino)coumarin-3-carbonitrile **1** (TCI Chemicals, 242 mg, 1.0 mmol) in DMF (1 mL). The mixture was stirred at rt for 30 min, and a clear colorless solution formed. It was poured into water (70 mL) and brine (10 mL), extracted with EtOAc (3 \times 25 mL); the combined organic solutions were washed with brine, dried over Na₂SO₄, filtered and evaporated. The residue was taken up in EtOAc (15 mL) and MeOH (5 mL), heated up to 70 $^{\circ}$ C, and a solution of DDQ (227 mg, 1.0 mmol) in EtOAc (3 mL) was added quickly dropwise. The mixture was stirred at 70 $^{\circ}$ C for 5 min, cooled down to rt and evaporated. The crude product was purified by flash chromatography on Biotage Isolera system twice (24 g RediSep Rf cartridge, gradient 50% to 100% EtOAc/hexane, and 12 g Sepacore Silica HP cartridge, gradient 40% to 100% EtOAc/hexane) and lyophilized from 1,4-dioxane. Bright orange fluffy solid, yield 23 mg (7%).

¹H NMR (400 MHz, CDCl₃): δ 8.38 (d, *J* = 9.5 Hz, 1H), 6.66 (dd, *J* = 9.5, 2.7 Hz, 1H), 6.45 (dd, *J* = 2.7, 1.7 Hz, 1H), 3.95 (s, 3H), 3.93 (s, 3H), 3.47 (q, *J* = 7.2 Hz, 4H), 1.24 (t, *J* = 7.1 Hz, 7H).

¹³C NMR (101 MHz, CDCl₃): δ 158.3 (d, *J* = 16.8 Hz), 157.2 (d, *J* = 15.2 Hz), 153.3, 149.1, 147.4, 131.5 (d, *J* = 3.0 Hz), 114.6 (d, *J* = 7.6 Hz), 110.7, 107.4 (d, *J* = 9.9 Hz), 97.5 (d, *J* = 2.4 Hz), 96.0 (d, *J* = 2.4 Hz), 54.2, 54.1, 45.4, 12.6.

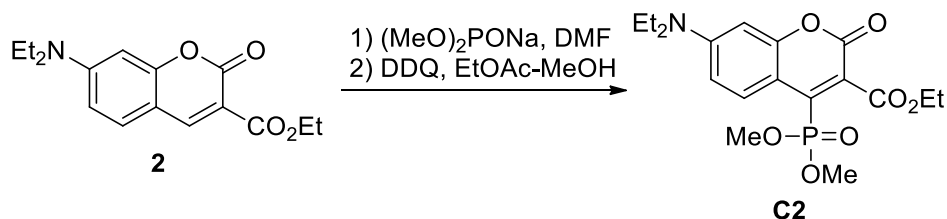
³¹P NMR (162 MHz, CDCl₃): δ 10.0.

MS (ESI): *m/z* (positive mode, rel. int., %) = 351.1 (100) [M+H]⁺.

HRMS (C₁₆H₁₉N₂O₅P): *m/z* (positive mode) = 351.1106 (found [M+H]⁺), 351.1104 (calc.).

Dye C2

ethyl 7-diethylamino-4-dimethoxyphosphoryl-2-oxo-2H-chromene-3-carboxylate



To a stirred suspension of NaH (120 mg of 60 wt.% in mineral oil, 3.0 mmol) in dry DMF (3 mL), cooled in an ice-water bath, dimethyl phosphite (Sigma-Aldrich, 276 μ L, 3.0 mmol) was added dropwise. The suspension was warmed up to rt and stirred for 30 min, turning into a clear solution, which was added to a stirred solution of ethyl 7-(diethylamino)coumarin-3-carboxylate **2** (TCI Chemicals, 289 mg, 1.0 mmol) in DMF (1 mL). The mixture was stirred at rt for 30 min, and a clear light yellow solution formed. It was poured into water (70 mL) and brine (10 mL), acidified with 1 N HCl to pH \sim 3, extracted with EtOAc (3 \times 20 mL); the combined organic solutions were washed with brine, dried over Na₂SO₄, filtered and evaporated. The residue was taken up in EtOAc (15 mL) and MeOH (5 mL), heated up to 70 $^{\circ}$ C, and a solution of DDQ (227 mg, 1 mmol) in EtOAc (3 mL) was added quickly dropwise. The mixture was stirred at 70 $^{\circ}$ C for 5 min, cooled down to rt and evaporated. The crude product was purified by flash chromatography on Biotage Isolera system twice (24 g RediSep Rf cartridge, gradient 40% to 100% EtOAc/hexane, and 12 g Sepacore Silica HP cartridge, gradient 40% to 100% EtOAc/hexane) and lyophilized from 1,4-dioxane. Yellow solid, yield 111 mg (28%).

¹H NMR (400 MHz, CDCl₃): δ 7.91 (d, J = 9.3 Hz, 1H), 6.60 (dd, J = 9.3, 2.7 Hz, 1H), 6.46 (dd, J = 2.7, 1.6 Hz, 1H), 4.38 (q, J = 7.2 Hz, 2H), 3.82 (s, 3H), 3.79 (s, 3H), 3.41 (q, J = 7.1 Hz, 4H), 1.36 (t, J = 7.2 Hz, 3H), 1.20 (t, J = 7.1 Hz, 6H).

¹³C NMR (101 MHz, CDCl₃): δ 165.0 (d, J = 7.6 Hz), 158.5 (d, J = 20.6 Hz), 156.1 (d, J = 13.8 Hz), 151.3, 138.3, 136.6, 129.6 (d, J = 2.7 Hz), 122.6 (d, J = 7.7 Hz), 109.6, 105.2 (d, J = 9.3 Hz), 97.5 (d, J = 2.6 Hz), 62.4, 53.6, 53.6, 45.0, 14.1, 12.5.

³¹P NMR (162 MHz, CDCl₃): δ 13.5.

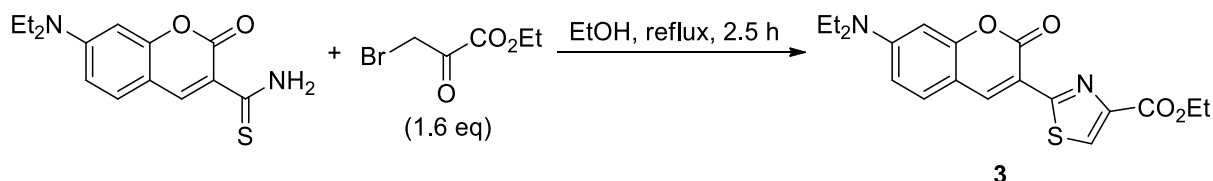
MS (ESI): m/z (positive mode, rel. int., %) = 420.1 (100) [M+Na]⁺.

HRMS (C₁₈H₂₄NO₇P): m/z (positive mode) = 420.1183 (found [M+Na]⁺), 420.1183 (calc.).

Dye C3

Compound 3

ethyl 2-(7-diethylamino-2-oxo-2*H*-chromen-3-yl)thiazole-4-carboxylate



Ethyl bromopyruvate (~90%, Sigma-Aldrich; 0.61 mL, 4.8 mmol, 1.2 eq) was added dropwise to a suspension of 7-diethylamino-2-oxo-2*H*-chromene-3-carbothioic acid amide^[2] (1.1 g, 4.0 mmol) in ethanol (40 mL). The flask was immersed in a 100 °C oil bath and the reaction mixture was refluxed for 1.5 h. TLC control (silica, 5% ethyl acetate – CH₂Cl₂) showed incomplete conversion, so that another portion of ethyl bromopyruvate (0.2 mL, 1.6 mmol, 0.4 eq) was added at rt, and the mixture was refluxed for further 1 h. The reaction mixture was then evaporated on Celite, and the product was isolated by flash chromatography on Biotage Isolera system (40 g Sepacore Silica HP cartridge, gradient 0% to 5% ethyl acetate/CH₂Cl₂) to give 421 mg (28%) of **3** as yellow solid.

¹H NMR (400 MHz, CDCl₃): δ 8.87 (s, 1H), 8.18 (s, 1H), 7.45 (d, *J* = 8.9 Hz, 1H), 6.65 (dd, *J* = 8.9, 2.5 Hz, 1H), 6.54 (d, *J* = 2.5 Hz, 1H), 4.44 (q, *J* = 7.1 Hz, 2H), 3.45 (q, *J* = 7.1 Hz, 4H), 1.42 (t, *J* = 7.1 Hz, 3H), 1.23 (t, *J* = 7.1 Hz, 6H).

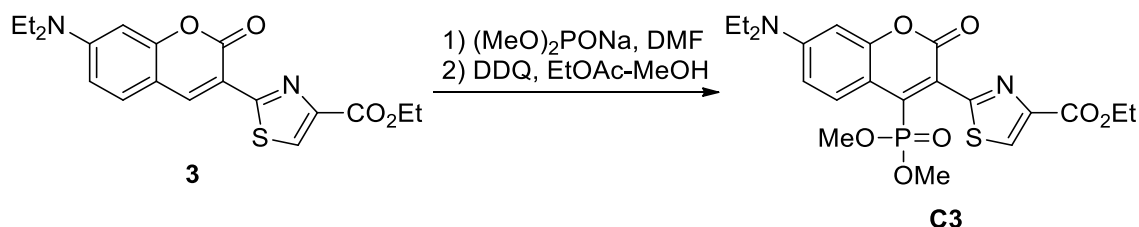
¹³C NMR (101 MHz, CDCl₃): δ 161.9, 161.8, 161.3, 156.9, 152.1, 146.6, 141.5, 130.8, 128.2, 111.9, 110.1, 108.6, 97.1, 61.5, 45.2, 14.5, 12.6.

MS (ESI): *m/z* (positive mode, rel. int., %) = 373.1 (100) [M+H]⁺.

HRMS (C₁₉H₂₀N₂O₄S): *m/z* (positive mode) = 373.1217 (found [M+H]⁺), 373.1217 (calc.).

Dye C3

ethyl 2-(7-diethylamino-4-dimethoxyphosphoryl-2-oxo-2*H*-chromen-3-yl)thiazole-4-carboxylate



To a stirred suspension of NaH (127 mg of 60 wt.% in mineral oil, 3.18 mmol) in dry DMF (3 mL), cooled in an ice-water bath, dimethyl phosphite (Sigma-Aldrich, 292 μL, 3.18 mmol) was added dropwise. The suspension was warmed up to rt and stirred for 30 min, turning into a clear solution, which was added to a stirred suspension of **3** (382 mg, 1.03 mmol) in DMF (3

mL). A clear red solution formed. The mixture was stirred at rt for 30 min, poured into water (30 mL), acidified with acetic acid to pH ~ 4, extracted with EtOAc (3×25 mL); the combined organic solutions were washed with brine, dried over Na₂SO₄, filtered and evaporated. The residue was taken up in EtOAc (15 mL) and MeOH (3 mL), heated up to 70 °C, and a solution of DDQ (234 mg, 1.03 mmol) in EtOAc (3 mL) was added quickly dropwise. The mixture was stirred at 70 °C for 5 min, cooled down to rt and evaporated. The crude product was purified by flash chromatography on Biotage Isolera system twice (24 g RediSep Rf cartridge, gradient 40% to 100% EtOAc/hexane, and 25 g Sepacore Silica HP cartridge, gradient 50% to 100% EtOAc/hexane) and lyophilized from 1,4-dioxane. Fluffy orange solid, yield 53 mg (11%).

¹H NMR (400 MHz, CDCl₃): δ 8.37 (s, 1H), 8.07 (d, *J* = 9.4 Hz, 1H), 6.63 (dd, *J* = 9.4, 2.7 Hz, 1H), 6.49 (dd, *J* = 2.7, 1.6 Hz, 1H), 4.39 (q, *J* = 7.1 Hz, 2H), 3.77 (s, 3H), 3.74 (s, 3H), 3.44 (q, *J* = 7.1 Hz, 4H), 1.37 (t, *J* = 7.1 Hz, 3H), 1.23 (t, *J* = 7.1 Hz, 6H).

¹³C NMR (101 MHz, CDCl₃): δ 161.8 (d, *J* = 7.2 Hz), 161.4, 160.2 (d, *J* = 19.8 Hz), 156.4 (d, *J* = 14.2 Hz), 151.5, 146.5, 143.4, 141.7, 130.51, 130.45 (d, *J* = 2.5 Hz), 118.9 (d, *J* = 6.6 Hz), 107.4 (d, *J* = 9.3 Hz), 109.7, 97.4 (d, *J* = 2.5 Hz), 67.2, 61.4, 53.6, 53.5, 45.0, 14.5, 12.6.

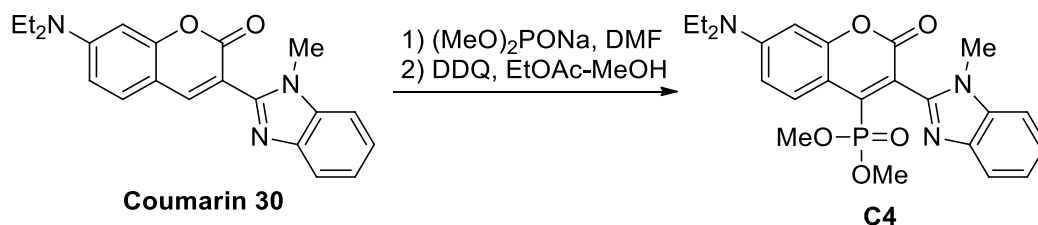
³¹P NMR (162 MHz, CDCl₃): δ 13.7.

MS (ESI): *m/z* (positive mode, rel. int., %) = 503.1 (100) [M+Na]⁺.

HRMS (C₂₁H₂₅N₂O₇PS): *m/z* (positive mode) = 481.1204 (found [M+H]⁺), 481.1193 (calc.).

Dye C4

dimethyl [7-(diethylamino)-3-(1-methyl-1*H*-benzo[*d*]imidazol-2-yl)-2-oxo-2*H*-chromen-4-yl]phosphonate



To a stirred suspension of NaH (60 mg of 60 wt.% in mineral oil, 1.5 mmol) in dry DMF (1.5 mL), cooled in an ice-water bath, dimethyl phosphite (Sigma-Aldrich, 138 μ L, 1.5 mmol) was added dropwise. The suspension was warmed up to rt and stirred for 30 min, turning into a clear solution, which was added to a stirred suspension of Coumarin 30 (Sigma-Aldrich, 174 mg, 0.5 mmol) in DMF (2 mL). The solid dissolved over 1 h, and a yellow clear solution formed. The mixture was poured into water (70 mL) and brine (20 mL), extracted with EtOAc (3 \times 30 mL); the combined organic solutions were dried over Na₂SO₄, filtered and evaporated. The residue was taken up in EtOAc (15 mL) and MeOH (5 mL), heated up to 70 $^{\circ}$ C, and a solution of DDQ (114 mg, 0.5 mmol) in EtOAc (2 mL) was added quickly dropwise. The mixture was stirred at 70 $^{\circ}$ C for 5 min, cooled down to rt and evaporated on Celite. The product was isolated by flash chromatography (first 24 g RediSep Rf, gradient 40% to 100% EtOAc/hexane; then 12 g Sepacore Silica HP, gradient 50% to 100% EtOAc/hexane) and lyophilized from 1,4-dioxane. Fluffy orange-yellow solid, yield 27 mg (12%); purity 90% (NMR).

¹H NMR (400 MHz, CDCl₃): δ 8.13 (d, J = 9.3 Hz, 1H), 7.74 (ddd, J = 7.7, 1.4, 0.7 Hz, 1H), 7.37 (ddd, J = 7.9, 1.5, 0.7 Hz, 1H), 7.29 (ddd, J = 7.9, 7.2, 1.4 Hz, 1H), 7.26 – 7.22 (m, 1H), 6.65 (dd, J = 9.3, 2.7 Hz, 1H), 6.52 (dd, J = 2.7, 1.7 Hz, 1H), 3.70 (s, 3H), 3.63 (d, J = 11.6 Hz, 3H), 3.60 (d, J = 11.6 Hz, 3H), 3.44 (q, J = 7.1 Hz, 4H), 1.23 (t, J = 7.1 Hz, 6H).

¹³C NMR (101 MHz, CDCl₃): δ 159.9 (d, J = 20.2 Hz), 156.8 (d, J = 14.3 Hz), 151.5, 148.5 (d, J = 7.0 Hz), 144.9, 143.19, 143.17, 109.64, 109.58, 106.8 (d, J = 10.3 Hz), 97.4 (d, J = 2.6 Hz), 53.8 (d, J = 5.9 Hz), 53.5 (d, J = 6.0 Hz), 45.0, 30.5, 12.6.

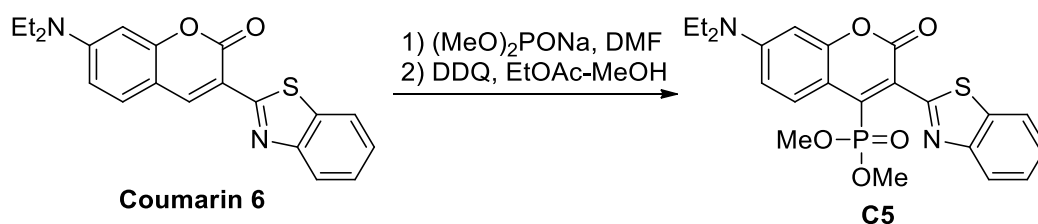
³¹P NMR (162 MHz, CDCl₃): δ 13.1.

MS (ESI): m/z (positive mode, rel. int., %) = 456.1 (100) [M+H]⁺.

HRMS (C₂₃H₂₆N₃O₅P): m/z (positive mode) = 456.1684 (found [M+H]⁺), 456.1683 (calc.).

Dye C5

dimethyl [3-(benzo[d]thiazol-2-yl)-7-(diethylamino)-2-oxo-2*H*-chromen-4-yl]phosphonate



To a stirred suspension of NaH (17 mg of 60 wt.% in mineral oil, 0.43 mmol) in dry DMF (0.5 mL), cooled in an ice-water bath, dimethyl phosphite (Sigma-Aldrich, 40 μ L, 0.43 mmol) was added in one portion. The suspension was warmed up to rt and stirred for 30 min, turning into a clear solution, which was added to a stirred suspension of Coumarin 6 (Sigma-Aldrich, 50 mg, 0.14 mmol) in DMF (0.5 mL). The orange solid dissolved immediately and clear red-orange solution formed. The mixture was stirred at rt for 1 h, and the pale orange solution was poured into water (30 mL) and brine (10 mL), extracted with EtOAc (4 \times 15 mL); the combined organic solutions were dried over Na₂SO₄, filtered and evaporated. The residue was taken up in EtOAc (20 mL) and MeOH (5 mL), heated up to 70 $^{\circ}$ C, and a solution of DDQ (32 mg, 0.14 mmol) in EtOAc (2 mL) was added quickly dropwise. The mixture was stirred at 70 $^{\circ}$ C for 5 min, cooled down to rt and evaporated. The crude product was purified by column chromatography twice (16 g SiO₂, gradient 50% to 100% EtOAc/hexane, and 17 g SiO₂, gradient 50% to 80% EtOAc/hexane) and lyophilized from 1,4-dioxane. Bright yellow solid, yield 34 mg (52%).

¹H NMR (400 MHz, CDCl₃): δ 8.18 (d, *J* = 9.3 Hz, 1H), 8.08 (ddd, *J* = 8.1, 1.3, 0.7 Hz, 1H), 7.92 (ddd, *J* = 8.0, 1.3, 0.7 Hz, 1H), 7.48 (ddd, *J* = 8.2, 7.2, 1.3 Hz, 1H), 7.41 (ddd, *J* = 8.3, 7.3, 1.2 Hz, 1H), 6.65 (dd, *J* = 9.4, 2.7 Hz, 1H), 6.52 (dd, *J* = 2.7, 1.7 Hz, 1H), 3.67 (s, 3H), 3.64 (s, 3H), 3.44 (q, *J* = 7.1 Hz, 4H), 1.23 (t, *J* = 7.1 Hz, 6H).

¹³C NMR (101 MHz, CDCl₃): δ 162.2 (d, *J* = 7.1 Hz), 160.0 (d, *J* = 19.9 Hz), 156.6 (d, *J* = 14.3 Hz), 152.8, 151.5, 142.3 (d, *J* = 172.0 Hz), 137.1, 130.5 (d, *J* = 2.7 Hz), 126.1, 125.6, 123.6, 121.7, 119.5 (d, *J* = 6.4 Hz), 109.7, 107.2 (d, *J* = 9.9 Hz), 97.4 (d, *J* = 2.5 Hz), 67.2, 53.6, 53.5, 45.1, 12.6.

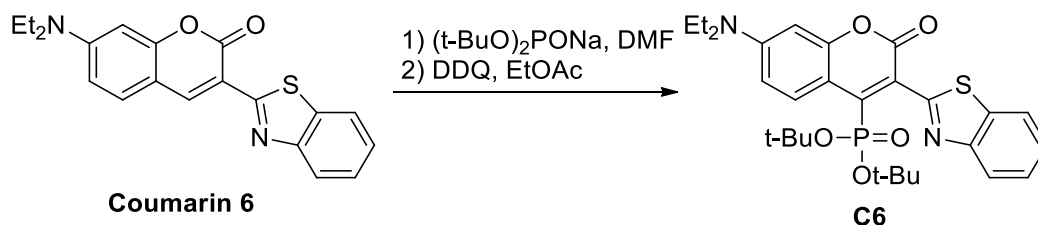
³¹P NMR (162 MHz, CDCl₃): δ 13.4.

MS (ESI): *m/z* (positive mode, rel. int., %) = 459.1 (100) [M+H]⁺, 497.1 (45) [M+K]⁺.

HRMS (C₂₂H₂₃N₂O₅PS): *m/z* (positive mode) = 459.1138 (found [M+H]⁺), 459.1138 (calc.).

Dye C6

di-*tert*-butyl [3-(benzo[*d*]thiazol-2-yl)-7-(diethylamino)-2-oxo-2*H*-chromen-4-yl]-phosphonate



To a stirred suspension of NaH (34 mg of 60 wt.% in mineral oil, 0.86 mmol) in dry DMF (1.5 mL), cooled in ice-water bath, di(*tert*-butyl) phosphite (Sigma-Aldrich; 170 μ L, 0.86 mmol) was added in one portion. The suspension was stirred for 2 h at rt and for 30 min at 55 $^{\circ}$ C. The resulting thin white suspension was added to a stirred suspension of Coumarin 6 (100 mg, 0.29 mmol) in DMF (0.8 mL). The solids dissolved immediately and clear light-orange solution formed. The mixture was stirred at rt for 1 h and then poured into sat. aq. NaHCO_3 (50 mL), extracted with EtOAc (4 \times 15 mL); the combined organic solutions were dried over Na_2SO_4 , filtered and evaporated. The residue was redissolved in EtOAc (20 mL), heated up to 75 $^{\circ}$ C, and a solution of DDQ (65 mg, 0.29 mmol) in EtOAc (3 mL) was added quickly dropwise. The mixture was stirred at 75 $^{\circ}$ C for 5 min, cooled down to rt and evaporated. The product was isolated by column chromatography (30 g SiO_2 , gradient 20% to 50% EtOAc/hexane) and lyophilized from 1,4-dioxane. Bright yellow-orange solid, yield 130 mg (84%).

^1H NMR (400 MHz, acetone- d_6): δ 8.25 (br.s, 1H), 8.08 – 8.03 (m, 1H), 7.99 (ddd, J = 8.1, 1.2, 0.6 Hz, 1H), 7.55 – 7.47 (m, 1H), 7.47 – 7.41 (m, 1H), 6.84 (dd, J = 9.4, 2.7 Hz, 1H), 6.58 (dd, J = 2.7, 1.5 Hz, 1H), 3.57 (q, J = 7.2 Hz, 4H), 1.45 (s, 18H), 1.26 (t, J = 7.1 Hz, 6H).

^{31}P NMR (162 MHz, acetone- d_6): δ 0.60; the small peak at -3.79 ppm corresponds to a trace amount of mono-de-*tert*-butylated impurity (easily formed on silica).

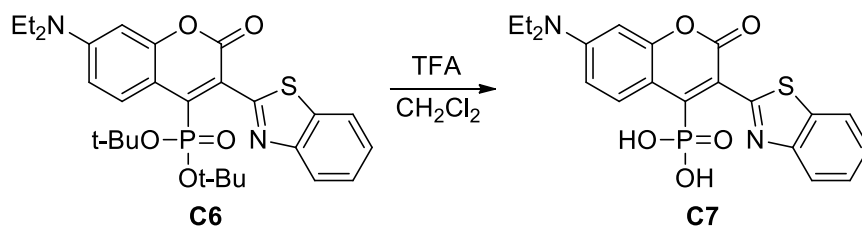
^{13}C NMR (101 MHz, acetone- d_6): δ 209.9, 163.7 (d, J = 7.0 Hz), 160.4, 157.5 (d, J = 13.4 Hz), 154.1, 152.1, 138.2, 132.1 (d, J = 2.0 Hz), 126.3, 125.7, 123.9, 122.3, 110.9, 109.8, 107.2 (d, J = 7.9 Hz), 97.6 (d, J = 2.6 Hz), 85.2 (d, J = 7.3 Hz), 67.6, 45.2, 12.8.

MS (ESI): m/z (positive mode, rel. int., %) = 543.2 (55) $[\text{M}+\text{H}]^+$, 565.2 (35) $[\text{M}+\text{Na}]^+$, 581.2 (100) $[\text{M}+\text{K}]^+$.

HRMS ($\text{C}_{28}\text{H}_{35}\text{N}_2\text{O}_5\text{PS}$): m/z (positive mode) = 543.2077 (found $[\text{M}+\text{H}]^+$), 543.2077 (calc.).

Dye C7

[3-(benzo[d]thiazol-2-yl)-7-(diethylamino)-2-oxo-2*H*-chromen-4-yl]phosphonic acid



Trifluoroacetic acid (TFA; 150 μ L) was added to a stirred solution of the dye **C6** (82 mg, 0.15 mmol) in CH₂Cl₂ (5 mL). The orange color of the solution turned violet upon addition of TFA and eventually deep purple. The mixture was stirred at rt for 30 min, evaporated to dryness, the residue was dissolved in acetic acid and lyophilized. Yield 64 mg (99%), red-brown solid.

¹H NMR (400 MHz, acetic acid-*d*₄): δ 9.06 (d, *J* = 9.6 Hz, 1H), 8.14 (t, *J* = 7.3 Hz, 2H), 7.76 (t, *J* = 7.6 Hz, 1H), 7.66 (t, *J* = 7.7 Hz, 1H), 6.91 (d, *J* = 9.2 Hz, 1H), 6.61 (s, 1H), 3.61 (q, *J* = 7.1 Hz, 4H), 1.30 (t, *J* = 7.0 Hz, 6H).

¹³C NMR not available due to low solubility of the compound.

³¹P NMR (162 MHz, acetic acid-*d*₄): δ 5.4.

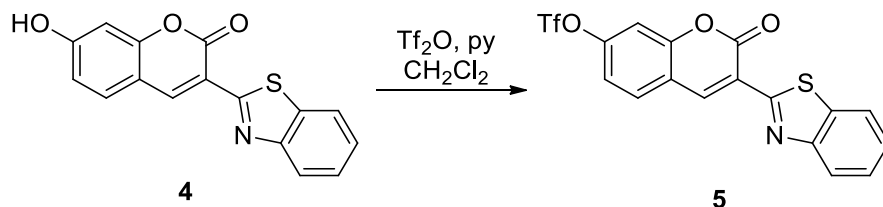
MS (ESI): *m/z* (negative mode, rel. int., %) = 429.1 (100) [M-H]⁻.

HRMS (C₂₀H₁₉N₂O₅PS): *m/z* (negative mode) = 429.0684 (found [M-H]⁻), 429.0680 (calc.).

Dye C8

Compound 5

3-(benzo[d]thiazol-2-yl)-2-oxo-2H-chromen-7-yl trifluoromethanesulfonate



Pyridine (0.55 mL, 6.8 mmol, 4.0 eq) was added to a suspension of 3-(2-benzothiazolyl)umbelliferone **4** (Sigma-Aldrich, 500 mg, 1.69 mmol) in CH_2Cl_2 (25 mL); a voluminous yellow precipitate formed. The suspension was cooled in an ice-water bath, and triflic anhydride (570 μL , 3.4 mmol, 2.0 eq) was added dropwise; the precipitate dissolved. The mixture was warmed up to rt, and the thin suspension was stirred at rt for 3 h. It was then cooled in an ice-water bath, diluted with water (30 mL), extracted with CH_2Cl_2 (2 \times 20 mL); and the combined organic solutions were washed with water and brine, dried over Na_2SO_4 . The product was isolated by flash chromatography on Biotage Isolera system (12 g Sepacore Silica HP cartridge, gradient 20% to 100% CH_2Cl_2 /hexane over 10 column volumes); the fractions containing the product were combined and evaporated to lemon-yellow solid, which was triturated with hexane, filtered off, washed with hexane and dried *in vacuo*. Yield 630 mg (87%).

^1H NMR (400 MHz, CDCl_3): δ 9.03 (s, 1H), 8.11 – 8.05 (m, 1H), 8.00 – 7.94 (m, 1H), 7.80 (d, J = 8.6 Hz, 1H), 7.54 (ddd, J = 8.3, 7.1, 1.3 Hz, 1H), 7.43 (ddd, J = 8.2, 7.1, 1.1 Hz, 1H), 7.39 – 7.35 (m, 1H), 7.31 (dd, J = 8.6, 2.4 Hz, 1H).

^{19}F NMR (376 MHz, CDCl_3): δ -72.5.

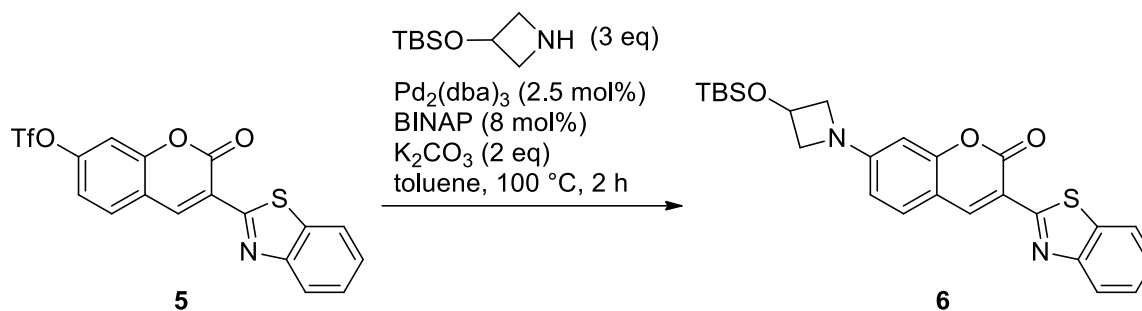
^{13}C NMR (101 MHz, CDCl_3): δ 158.9, 158.8, 154.2, 152.6, 151.6, 139.7, 137.1, 131.0, 126.9, 125.9, 123.3, 121.9, 121.5, 119.0, 118.8 (q, J = 321.0 Hz), 118.7, 110.6.

MS (ESI): m/z (positive mode, rel. int., %) = 428.2 (100) $[\text{M}+\text{H}]^+$.

HRMS ($\text{C}_{17}\text{H}_8\text{NO}_5\text{S}_2\text{F}_3$): m/z (positive mode) = 427.9867 (found $[\text{M}+\text{H}]^+$), 427.9869 (calc.).

Compound 6

3-(benzo[d]thiazol-2-yl)-7-[3-(*tert*-butyldimethylsilyloxy)azetidin-1-yl]-2*H*-chromen-2-one



A mixture of **5** (150 mg, 0.35 mmol), 3-(*tert*-butyldimethylsilyloxy)azetidine^[3] (196 mg, 1.05 mmol, 3.0 eq), Pd₂(dba)₃ (8 mg, 9 μmol, ~2.5 mol%), (±)-BINAP (18 mg, 28 μmol, 8 mol%) and K₂CO₃ (97 mg, 0.70 mmol, 2.0 eq) in toluene (2 mL) was sealed in a 10 mL vial capped with a septum, degassed on a Schlenk line and stirred under argon at 100 °C (bath temperature) for 2 h. Yellow solution gradually turned into an orange suspension. Upon cooling down to rt, acetic acid (1 mL) was added to the reaction mixture, it was diluted with CH₂Cl₂ (30 mL) and evaporated on Celite. The product was isolated by flash chromatography on Biotage Isolera system (12 g Sepacore Silica HP cartridge, gradient 50% to 100% CH₂Cl₂/hexane over 10 column volumes); the fractions containing the product **6** were combined and evaporated to bright orange solid, yield 98 mg (60%).

¹H NMR (400 MHz, CD₃CN + 1% TFA): δ 8.16 (ddd, *J* = 8.2, 1.2, 0.7 Hz, 1H), 8.09 (dt, *J* = 8.3, 0.9 Hz, 1H), 7.78 (ddd, *J* = 8.4, 7.3, 1.2 Hz, 1H), 7.72 – 7.62 (m, 2H), 7.67 (s, 1H), 6.58 (dd, *J* = 8.9, 2.1 Hz, 1H), 6.36 (dd, *J* = 2.1, 0.7 Hz, 1H), 4.89 (tt, *J* = 6.5, 4.2 Hz, 1H), 4.51 – 4.44 (m, 2H), 4.06 – 3.99 (m, 2H), 0.95 (s, 9H), 0.14 (s, 6H).

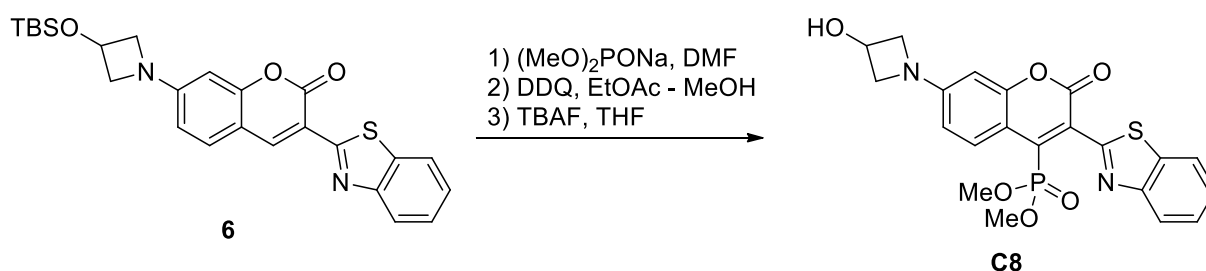
¹³C NMR (101 MHz, nitrobenzene-*d*₅): δ 162.1, 161.1, 157.2, 154.7, 153.6, 142.9, 137.2, 131.6, 126.8, 125.3, 123.2, 122.3, 113.3, 110.0, 109.9, 96.5, 62.8, 61.7, 26.0, 18.4, -4.9.

MS (ESI): *m/z* (positive mode, rel. int., %) = 465.3 (100) [M+H]⁺.

HRMS (C₂₅H₂₈N₂O₃SSi): *m/z* (positive mode) = 465.1653 (found [M+H]⁺), 465.1663 (calc.).

Dye C8

dimethyl [3-(benzo[d]thiazol-2-yl)-7-(3-hydroxyazetidin-1-yl)-2-oxo-2H-chromen-4-yl]phosphonate



To a stirred suspension of NaH (23 mg of 60 wt.% in mineral oil, 0.58 mmol, 3.0 eq) in dry DMF (0.5 mL), cooled in ice-water bath, dimethyl phosphite (53 μL , 0.58 mmol, 3 eq) was added in one portion. The resulting suspension was warmed up to rt and stirred for 30 min, turning into a clear solution, which was added to a stirred suspension of **3** (90 mg, 0.19 mmol) in DMF (3 mL). The orange solid dissolved rapidly, and a clear reddish-brown solution formed. The mixture was stirred at rt for 1 h, DMF was evaporated *in vacuo* at rt, and the residue was mixed with water (20 mL) and brine (20 mL). Acetic acid was added to pH \sim 3, and the mixture was extracted with EtOAc (3 \times 20 mL), the combined extracts were dried over Na_2SO_4 , filtered and evaporated. The residue was taken up in EtOAc (20 mL) and MeOH (5 mL), heated up to 70 $^\circ\text{C}$, and a solution of DDQ (44 mg, 0.194 mmol, 1 eq) in EtOAc (3 mL) was added quickly dropwise. The resulting red-orange mixture was stirred at 70 $^\circ\text{C}$ for 5 min, cooled down to rt and evaporated on Celite. The product was isolated by flash chromatography on Biotage Isolera system (10 g Biotage SNAP Ultra cartridge, gradient 0% to 10% methanol/ CH_2Cl_2 over 10 column volumes); two fractions were collected, containing the product and the TBS-protected product. Both fractions were pooled together, evaporated, and the mixture was used for complete deprotection. The material was dissolved in THF (7 mL), cooled in ice-water bath, and tetrabutylammonium fluoride trihydrate (92 mg, 0.291 mmol) was added. The resulting brown-yellow solution was allowed to warm up to rt and stirred for 1 h. The mixture was diluted with brine (15 mL), extracted with EtOAc (3 \times 20 mL), the combined organic solutions were dried over Na_2SO_4 , filtered and evaporated on Celite. The product was isolated by flash chromatography on Biotage Isolera system (12 g Sepacore Silica HP cartridge, gradient 0% to 50% methanol/ethyl acetate over 10 column volumes); the fractions containing the product were evaporated to brown-red solid, which was freeze-dried from aqueous dioxane to fluffy red solid, yield 20 mg (23% over 3 steps).

^1H NMR (400 MHz, acetic acid- d_4): δ 11.57 (s, 1H), 8.16 (ddd, J = 8.2, 1.2, 0.7 Hz, 1H), 8.10 (d, J = 9.1 Hz, 1H), 8.03 (ddd, J = 8.0, 1.3, 0.7 Hz, 1H), 7.57 (ddd, J = 8.3, 7.2, 1.3 Hz, 1H), 7.50 (ddd, J = 8.3, 7.2, 1.2 Hz, 1H), 6.48 (dd, J = 9.1, 2.4 Hz, 1H), 6.33 (dd, J = 2.4, 1.6

Hz, 1H), 4.86 (tt, $J = 6.6, 4.3$ Hz, 1H), 4.34 (ddd, $J = 9.2, 6.6, 1.3$ Hz, 2H), 3.97 (ddd, $J = 9.2, 4.4, 1.3$ Hz, 2H), 3.72 (s, 3H), 3.69 (s, 3H).

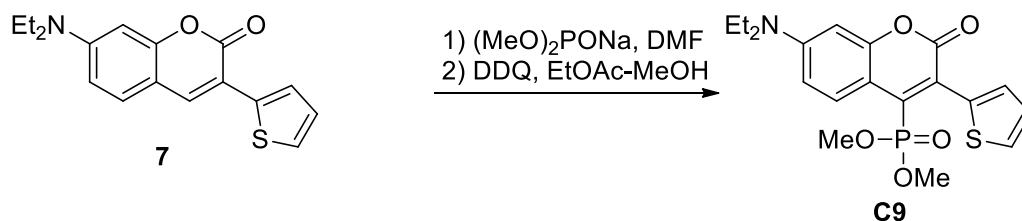
^{13}C and ^{31}P NMR not available due to low solubility of the compound.

MS (ESI): m/z (positive mode, rel. int., %) = 459.2 (100) $[\text{M}+\text{H}]^+$.

HRMS ($\text{C}_{21}\text{H}_{19}\text{N}_2\text{O}_6\text{PS}$): m/z (positive mode) = 459.0764 (found $[\text{M}+\text{H}]^+$), 459.0774 (calc.).

Dye C9

dimethyl [7-(diethylamino-2-oxo-3-(thiophen-2-yl)-2*H*-chromen-4-yl]phosphonate



To a stirred suspension of NaH (120 mg of 60 wt.% in mineral oil, 3 mmol) in dry DMF (3 mL), cooled in an ice-water bath, dimethyl phosphite (Sigma-Aldrich, 276 μL , 3 mmol) was added dropwise. The suspension was warmed up to rt and stirred for 30 min, turning into a clear solution, which was added to a stirred suspension of 7-(diethylamino)-3-(2-thienyl)coumarin **7** (TCI Chemicals, 299 mg, 1 mmol) in DMF (1 mL). The mixture was stirred at rt for 1 h, and a clear yellow-brown solution formed. It was poured into water (50 mL) and brine (10 mL), acidified with acetic acid to pH \sim 5, extracted with EtOAc (3 \times 25 mL); the combined organic solutions were washed with brine, dried over Na_2SO_4 , filtered and evaporated. The residue was taken up in EtOAc (15 mL) and MeOH (5 mL), heated up to 70 $^\circ\text{C}$, and a solution of DDQ (227 mg, 1 mmol) in EtOAc (3 mL) was added quickly dropwise. The mixture was stirred at 70 $^\circ\text{C}$ for 5 min, cooled down to rt and evaporated. The crude product was purified by flash chromatography on Biotage Isolera system (24 g RediSep Rf cartridge, gradient 30% to 100% EtOAc/hexane) and lyophilized from 1,4-dioxane. Fluffy orange solid, yield 36 mg (9%).

^1H NMR (400 MHz, CDCl_3): δ 8.17 (d, J = 9.3 Hz, 1H), 7.47 (dd, J = 5.1, 1.3 Hz, 1H), 7.16 (dd, J = 3.5, 1.3 Hz, 1H), 7.06 (dd, J = 5.1, 3.6 Hz, 1H), 6.62 (dd, J = 9.4, 2.7 Hz, 1H), 6.50 (dd, J = 2.7, 1.7 Hz, 1H), 3.58 (s, 3H), 3.55 (s, 3H), 3.42 (q, J = 7.1 Hz, 4H), 1.21 (t, J = 7.1 Hz, 6H).

^{13}C NMR (101 MHz, CDCl_3): δ 161.0 (d, J = 20.7 Hz), 155.7 (d, J = 14.9 Hz), 150.6, 140.3, 138.5, 136.0 (d, J = 6.5 Hz), 130.5 (d, J = 1.4 Hz), 129.9 (d, J = 2.9 Hz), 128.1, 126.4, 120.7 (d, J = 6.0 Hz), 109.3, 107.7 (d, J = 11.1 Hz), 97.4 (d, J = 2.7 Hz), 53.1, 53.0, 44.9, 12.6.

^{31}P NMR (162 MHz, CDCl_3): δ 14.6.

MS (ESI): m/z (positive mode, rel. int., %) = 430.1 (100) $[\text{M}+\text{Na}]^+$.

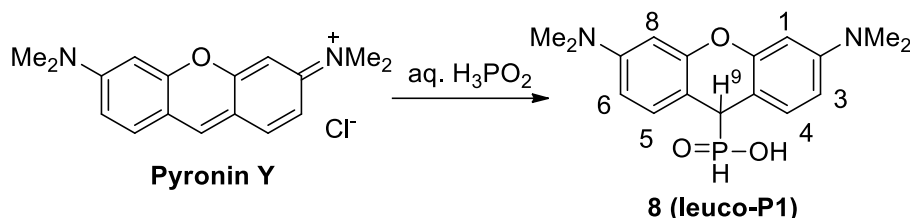
HRMS ($\text{C}_{19}\text{H}_{22}\text{NO}_5\text{PS}$): m/z (positive mode) = 408.1030 (found $[\text{M}+\text{H}]^+$), 408.1029 (calc.).

Pyronin derivatives (P1-P14)

Dye P1

Compound 4 (leuco-P1)

[3,6-bis(dimethylamino)-9*H*-xanthen-9-yl]phosphinic acid



A solution of Pyronin Y (Sigma-Aldrich; 1.0 g, 3.3 mmol) in aq. H_3PO_2 (50%, 4.4 g; 33 mmol) was stirred overnight at 100 °C under microwave irradiation. TLC (RP- C_{18}) showed complete conversion of the starting material. After cooling down to room temperature, the reaction mixture was diluted with H_2O (~25 mL) and applied onto RP cartridge (Biotage SNAP Ultra C18 30 g, HP-Sphere, 25 μM), which had been pre-equilibrated with deionized water. The cartridge was eluted with 400-500 mL deionized water (without TFA addition), until hypophosphorous acid was removed (pH of eluate reached 5-6). Further elution with $\text{H}_2\text{O}/\text{MeCN}$ (0.1 v/v% TFA in both components, 90:10 \rightarrow 50:50, 25 mL/min) followed by lyophilization afforded 1.03 g (94%) of **8**. It was additionally purified by preparative HPLC (Interchim Puriflash; see below): column 21 \times 250 mm, eluent: $\text{MeCN} / \text{H}_2\text{O} + 0.1$ v/v% TFA in H_2O , 3/97 – 25/75 over 25 min, flow 20 mL/min. The title *leuco*-compound **8** was isolated as a slightly purple solid soluble in water, methanol and acetonitrile; $R_f = 0.32$ (RP- C_{18} plates, eluent: $\text{H}_2\text{O}/\text{MeCN}$ 1:1, each with 0.1 v/v% TFA).

HPLC: $t_R = 10.1$ min (peak area 98%), gradient: $\text{MeCN} / \text{H}_2\text{O} + 0.1$ v/v% TFA, 2/98 – 50/50 in 20 min, detection at 580 nm, column US10C18HQ – 250/P46 (Interchim, France), 4 \times 250 mm, flow rate 1.2 mL/min.

^1H NMR (400 MHz, CD_3OD): δ 7.46 (d, $J = 8.0$ Hz, H^4 and H^5 , 2H), 7.14 (m, H^1 , H^3 , H^6 and H^8 , 4H), 6.97 (d, $^1J_{\text{H-P}} = 535$ Hz, PH, 1H), 4.35 (d, $^3J_{\text{H-P}} = 15.4$ Hz, H^9 , 1H), 3.11 (s, 12H, NMe_2) ppm. Due to the presence of an asymmetric P atom, the aromatic rings are diastereotopic.

^{13}C NMR (101 MHz, CD_3OD): δ 153.8 (« dd », $J = 4.2, 1.9$ Hz, C), 146.9 (« m », C), 132.0 (d, $J = 2.8$ Hz, C^4 and C^5), 116.7 (« m », C), 114.4 (d, $J = 10$ Hz, CH), 107.7 (d, $J = 15$ Hz, CH), 45.0 /45.1 (2 \times NMe_2), 44.4 (d, $J = 83$ Hz, C^9) ppm.

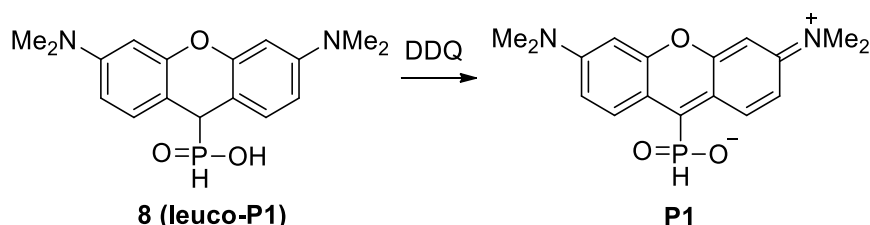
^{31}P NMR (162 MHz, CD_3OD): δ 25.8 ppm.

MS (ESI): m/z (negative mode, rel. int., %) = 331.1 (80) $[\text{M-H}]^-$, 663.4 (100) $[2\text{M-H}]^-$.

HR-MS ($C_{17}H_{21}N_2O_3P$): m/z (positive mode) = 333.1364 (found $[M+H]^+$), 333.1363 (calc. $[M+H]^+$).

Dye P1

[3,6-bis(dimethylamino)-9H-xanthylium-9-yl]phosphinate



To a solution of **8** (110 mg; 0.33 mmol) in MeCN (10 mL) and MeOH (4 mL), a solution of DDQ (89 mg, 0.4 mmol) in MeCN (5 mL) was added dropwise at -78°C with stirring. The dry ice-acetone bath was removed, and the reaction mixture was stirred at room temperature for 30 minutes. Celite was added, and the solvents were removed *in vacuo*. The dry residue was subjected to chromatography on RP-SiO₂ (Biotage SNAP Ultra C18 30 g, HP-Sphere 25 μm , 25 mL/min, MeCN/H₂O with 0.5 v/v% TFA in both components, 20 \rightarrow 60% MeCN over 10 column volumes, flow rate 25 mL/min). The fractions containing the title compound were collected and lyophilized to yield 109 mg (100%) of dark violet solid. This material was dissolved in H₂O/MeCN (10:1, 2 mL), and purified again by preparative HPLC (Interchim Puriflash, column 16 \times 250 mm with Eurospher II C18 SiO₂, 5 μ ; solvent MeCN / H₂O + 0.1 v/v TFA, 15/85 – 40/60, 14 mL/min). The violet colored fractions were pooled and lyophilized to yield 83 mg (76%) of the title dye as dark blue-violet solid; R_f = 0.32, RP-TLC, or R_f = 0.30, TLC on regular SiO₂, eluent MeCN/H₂O, 10:1, with 0.5 v/v% TFA in both components); moderately soluble in H₂O, MeOH. Analytical HPLC: t_R = 17.5 min (peak area 97%), solvent system MeCN/H₂O + 0.1 v/v% TFA in both components, gradient 2 – 50% ACN in 20 min, detection at 580 nm, column US10C18HQ-250/P46, 4 \times 250 mm (Interchim), flow rate 1.2 mL/min.

^1H NMR (400 MHz, CD₃OD): δ 8.71 (d, J = 9.8 Hz, 2H), 8.34 (d, $^1J_{P-H}$ = 548 Hz, PH), 7.15 (d, J = 9.3 Hz, 2H), 6.83 (s, 2H), 3.30 (s, 12H, NMe₂) ppm.

^{13}C NMR (101 MHz, CD₃OD, indirect detection from an HSQC experiment): δ 130.7 (CH), 113.8 (CH), 96.2 (CH), 39.4 (NMe₂) ppm.

^{31}P NMR (162 MHz, CD₃OD): δ 2.36 ppm.

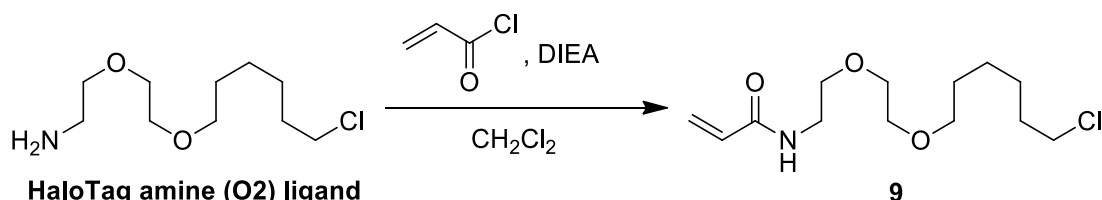
MS (ESI): m/z (positive mode), rel. int. (%) = 331.1 (100) $[M]^+$.

HR-MS ($C_{17}H_{20}N_2O_3P$): m/z (positive mode) = 331.1205 (found, $[M]^+$), 331.1206 (calc. for $[M]^+$).

Dye P1-Halo

Compound 9 (HaloTag O2 ligand acrylamide)

N-[2-(2-(6-chlorohexyloxy)ethoxy)ethyl]acrylamide



To a solution of HaloTag amine (O2) ligand^[4] (300 mg, 1.34 mmol) and ethyldiisopropylamine (DIEA, 350 μ L, 2 mmol) in dry CH_2Cl_2 (5 mL), cooled in ice-water bath, acryloyl chloride (131 μ L, 1.61 mmol) dissolved in dry CH_2Cl_2 (1 mL) was added dropwise. The reaction mixture was stirred at 0 $^\circ\text{C}$ for 30 min and at rt for 2 h. The mixture was then diluted with CH_2Cl_2 (40 mL), washed with sat. aq. NaHCO_3 , brine and dried over Na_2SO_4 . The product was isolated by column chromatography (20 g SiO_2 , gradient 0% to 5% methanol/EtOAc) and dried in vacuo to yield 325 mg (87%) of the product as colorless oil. The material contained ~30% of 3-hydroxypropionamide impurity and was used without further purification.

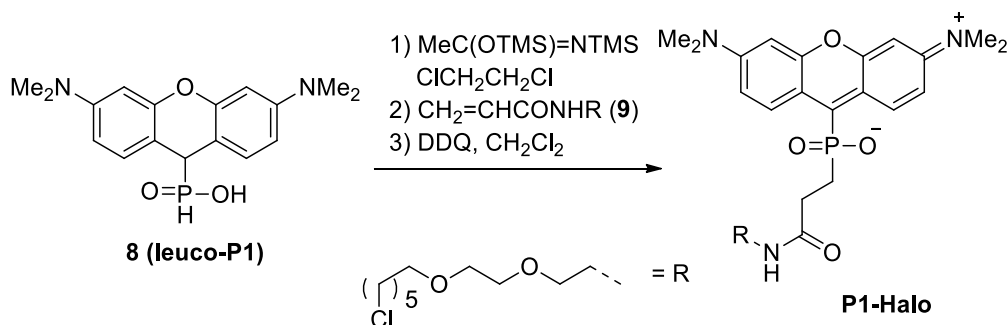
^1H NMR (400 MHz, $\text{DMSO}-d_6$): δ 8.15 (t, J = 5.8 Hz, 1H), 6.24 (dd, J = 17.1, 10.1 Hz, 1H), 6.07 (dd, J = 17.1, 2.3 Hz, 1H), 5.56 (dd, J = 10.1, 2.3 Hz, 1H), 3.61 (t, J = 6.6 Hz, 2H), 3.53 – 3.25 (m, 10H), 1.75 – 1.66 (m, 2H), 1.48 (tt, J = 8.0, 6.4 Hz, 2H), 1.43 – 1.24 (m, 4H).

^{13}C NMR (101 MHz, $\text{DMSO}-d_6$): δ 164.6, 131.7, 125.0, 70.2, 69.6, 69.4, 69.0, 45.3, 38.6, 32.0, 29.1, 26.1, 24.9.

MS (ESI): m/z (positive mode, rel. int., %) = 278.2 (29) $[\text{M}+\text{H}]^+$, 300.1 (100) $[\text{M}+\text{Na}]^+$, 316.1 (78) $[\text{M}+\text{K}]^+$.

HRMS ($\text{C}_{13}\text{H}_{24}\text{NO}_3\text{Cl}$): m/z (positive mode) = 278.1518 (found $[\text{M}+\text{H}]^+$), 278.1517 (calc.).

Dye P1-Halo



To a suspension of **8** (60 mg, 0.18 mmol) in 1,2-dichloroethane (2 mL), cooled in ice-water bath, *N,O*-bis(trimethylsilyl)acetamide (350 μ L, 1.44 mmol) was added quickly dropwise. The

resulting clear solution was stirred at 0 °C under N₂ atmosphere for 10 min, followed by addition of **9** (278 mg, purity ~70%, ~0.7 mmol) in 1,2-dichloroethane (1.5 mL). The mixture was stirred at 70 °C under N₂ atmosphere overnight, the solvent was evaporated, the residue was redissolved in CH₂Cl₂ (3 mL), cooled in dry ice-acetone bath followed by addition of DDQ (41 mg, 0.18 mmol) in CH₂Cl₂ (3 mL) quickly dropwise. The dark violet mixture was allowed to warm up to rt and stirred for 15 min. Trifluoroacetic acid (50 µL) was added, the mixture was evaporated to dryness and the product was isolated by column chromatography (30 g SiO₂, gradient 10% to 30% methanol/ CH₂Cl₂) and lyophilized from aqueous 1,4-dioxane. Dark violet crystalline solid, yield 53 mg (48%).

¹H NMR (400 MHz, CD₃OD): δ 9.43 (d, *J* = 9.9 Hz, 2H), 7.99 (t, *J* = 5.6 Hz, 1H), 7.13 (dd, *J* = 9.9, 2.7 Hz, 2H), 6.81 (dd, *J* = 2.7, 1.3 Hz, 2H), 3.56 – 3.51 (m, 6H), 3.43 (t, *J* = 6.5 Hz, 2H), 3.42 (t, *J* = 5.6 Hz, 2H), 3.31 (s, 12H), 3.20 (td, *J* = 5.6, 4.0 Hz, 2H), 2.43 – 2.34 (m, 2H), 2.16 – 2.06 (m, 2H), 1.78 – 1.68 (m, 2H), 1.54 (dq, *J* = 7.6, 6.6 Hz, 2H), 1.47 – 1.28 (m, 4H).

¹³C NMR (101 MHz, CD₃OD): δ 174.8 (d, *J* = 16.3 Hz), 158.5 (d, *J* = 8.8 Hz), 158.1, 135.1 (d, *J* = 2.2 Hz), 117.5 (d, *J* = 8.2 Hz), 114.6, 97.4, 72.1, 71.2 (d, *J* = 7.7 Hz), 70.5, 45.7, 40.9, 40.4, 33.7, 30.5, 27.7, 26.4.

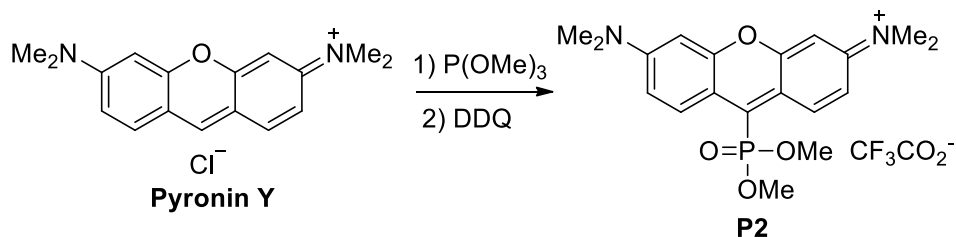
³¹P NMR (162 MHz, CD₃OD): δ 24.97.

MS (ESI): *m/z* (positive mode, rel. int., %) = 608.3 (48) [M+H]⁺, 630.3 (53) [M+Na]⁺, 646.2 (100) [M+K]⁺.

HRMS (C₁₃H₂₄NO₃Cl): *m/z* (positive mode) = 608.2653 (found [M+H]⁺), 608.2651 (calc.).

Dye P2

9-(dimethoxyphosphoryl)-3,6-bis(dimethylamino)-9*H*-xanthenylium trifluoroacetate



Trimethyl phosphite (Sigma-Aldrich; 175 μ L, 184 mg, 1.49 mmol) was added to a stirred solution of Pyronin Y (150 mg, 0.495 mmol) and tetrabutylammonium iodide (183 mg, 0.495 mmol) in dry CH₂Cl₂ (12 mL). The reaction mixture stirred at room temperature for 3 hours, until the red-violet color disappeared. The reaction mixture applied onto Celite, the solvent was evaporated to dryness, and the leuco dye was isolated by flash column chromatography (Biotage Isolera, cartridge RediSep Rf with 24 g of regular SiO₂, gradient: *n*-hexane/EtOAc 5:95 \rightarrow 100% of EtOAc) to yield 158 mg (85%) of the leuco dye as colorless oil (*R*_f = 0.21, regular SiO₂, 100% EtOAc). The leuco compound was dissolved in dry CH₂Cl₂ (2 mL), the solution cooled in a dry ice-acetone bath, and a solution of DDQ (95 mg, 0.42 mmol) in CH₂Cl₂ (5 mL) was added quickly dropwise. The dark green solution was allowed to warm-up to rt and stirred for 15 min. The mixture was applied onto Celite, and the product was isolated by chromatography (Biotage Isolera, cartridge Büchi Sepacore Silica HP 25 g, MeCN/H₂O + 0.1 v/v% of TFA, gradient: 100% of MeCN \rightarrow 90% of MeCN). The fractions containing the product were pooled and evaporated *in vacuo*. The residue was dissolved in 1,4-dioxane, filtered through a 0.2 μ m PTFE membrane filter and lyophilized to yield 205 mg (100%, or 85% over 2 steps) of the dye **P2** as dark green solid (TLC: MeCN/H₂O 10:1 + 0.1 v/v% of TFA, *R*_f = 0.35). HPLC (C₁₈): *t*_R = 8.4 min (peak area 97%), MeCN/H₂O + 0.1% TFA in both components: 20/80 – 100% of MeCN in 15 min, detection at 254 nm, column 4 \times 250 mm, flow rate 1.2 mL/min.

¹H NMR (400 MHz, CD₃CN): δ 8.72 (d, *J* = 9.9 Hz, 2H), 7.16 (dd, *J* = 9.9 and 2.6 Hz, 2H), 6.79 ("t", *J* = 2.4 Hz, 2H), 3.86 (d, ³*J*_{H-P} = 11.6 Hz, 6H, OMe), 3.32 (s, 12H, NMe₂) ppm.

¹³C NMR (101 MHz, CD₃CN): δ 158.2 (d, *J* = 13 Hz), 158.0, 141.7 (d, *J* = 167 Hz), 133.0 (d, *J* = 3 Hz), 116.8 (d, *J* = 10 Hz), 116.0 (CH), 97.4 (d, *J* = 2 Hz), 54.3 (d, *J* = 5 Hz, OMe), 41.4 (NMe₂) ppm.

¹⁹F NMR (376 MHz, CD₃CN): δ -76.5 ppm.

³¹P NMR (162 MHz, CD₃CN): δ 13.86 ppm.

MS (ESI): *m/z* (positive mode, rel. int., %) = 375.2 (100) [M]⁺.

HR-MS (ESI, positive mode): 375.1468 (found), 375.1468 (calculated for C₁₉H₂₄N₂O₄P⁺ as [M]⁺).

Dye P3

methyl [3,6-bis(dimethylamino)-9*H*-xanthenylium-9-yl](phenyl)phosphinite trifluoroacetate



Dimethyl phenylphosphinite (Sigma-Aldrich; 160 μ L, 170 mg, 1.0 mmol) was added at room temperature to a screw-cap test tube containing a stirred solution of Pyronin Y (100 mg, 0.33 mmol) and tetrabutylammonium iodide (122 mg, 0.33 mmol) in dry CH_2Cl_2 (3 mL). The reaction mixture was stirred at rt until the red-violet color disappeared (~ 3 h). TLC (SiO_2 , 100% EtOAc): $R_f = 0.28$ (**leuco-P3**; colorless to blue-purple upon exposure to air over several minutes). The reaction mixture was diluted with CH_2Cl_2 (5 mL), evaporated on Celite and subjected to flash chromatography (RediSep Rf cartridge, 24 g of regular SiO_2 ; gradient: hexane – EtOAc, 50:50 \rightarrow 0:100 over 10 column volumes). Yield 139 mg (quant.) of leuco-**P3** as a colorless oil. It was dissolved in CH_2Cl_2 (3 mL) and placed into a screw-cap test tube. The solution was cooled down to -70°C , and a solution of DDQ (75.0 mg, 0.33 mmol) in CH_2Cl_2 (5 mL) was added quickly. An instant color change to dark green was observed. After 15 min, the dry ice-acetone bath was removed, and the dark green solution was allowed to warm-up to room temperature and stirred additionally for 15 min. The reaction mixture was diluted with CH_2Cl_2 (8 mL) and evaporated on Celite. Flash chromatography conditions: Sepacore Silica HP cartridge, 25 g of 15 μm regular SiO_2 , gradient MeCN/ H_2O 100:0 \rightarrow 95:5; each component with 0.1 v/v% TFA, flow rate 40 mL/min. The fractions containing the product were pooled and evaporated *in vacuo*. The residue was dissolved in 1,4-dioxane, filtered through a 0.2 μm PTFE membrane filter and lyophilized. Yield 134 mg (76%) of dye **P3** ($R_f = 0.30$, TLC with MeCN/ H_2O 10:1 + 0.1 % TFA mixture as an eluent.) as a dark green solid well soluble in MeCN. HPLC: $t_R = 10.3$ min (area 99.8%), MeCN/ H_2O + 0.1% TFA in both components: 20 – 100% MeCN in 20 min, detection at 570 nm and 600 nm, column 4 \times 250 mm, flow rate 1.2 mL/min.

^1H NMR (400 MHz, CD_3CN): δ 8.85 (d, $J = 9.9$ Hz, 2H), 7.86 (ddd, $J = 13.5$, 8.3 and 1.3 Hz, 2H), 7.66 (m, 1H), 7.56 (m, 2H), 7.11 (dd, $J = 9.9$, 2.6 Hz, 2H), 6.75 (dd, $J = 2.7$, 1.7 Hz, 2H), 3.92 (d, $J = 11.4$ Hz, 3H, OMe), 3.29 (s, 12H, 2 \times NMe₂) ppm.

^{13}C NMR (101 MHz, CD_3CN): δ 158.2 (d, $J = 11$ Hz), 157.8, 134.4 (d, $J = 3$ Hz), 132.6 (d, $J = 3$ Hz), 131.4 (d, $J = 11$ Hz), 130.2 (d, $J = 14$ Hz), 117.0 (d, $J = 10$ Hz), 116., 97.4, 53.4 (d, $J = 6$ Hz, OMe), 41.4 (2 \times NMe₂) ppm.

^{19}F NMR (376 MHz, CD_3CN): δ -76.5 ppm.

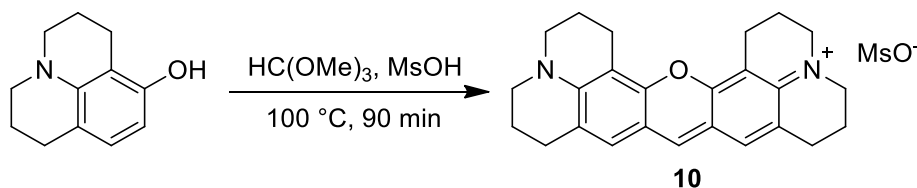
^{31}P NMR (162 MHz, CD_3CN): δ 29.2 ppm.

MS (ESI): m/z (positive mode, rel. int., %) = 421.2 (100) $[\text{M}]^+$.

HR-MS (ESI, positive mode): 421.1685 (found), 421.1676 (calculated for $\text{C}_{24}\text{H}_{26}\text{N}_2\text{O}_3\text{P}^+$).

Dye P4

Compound 10



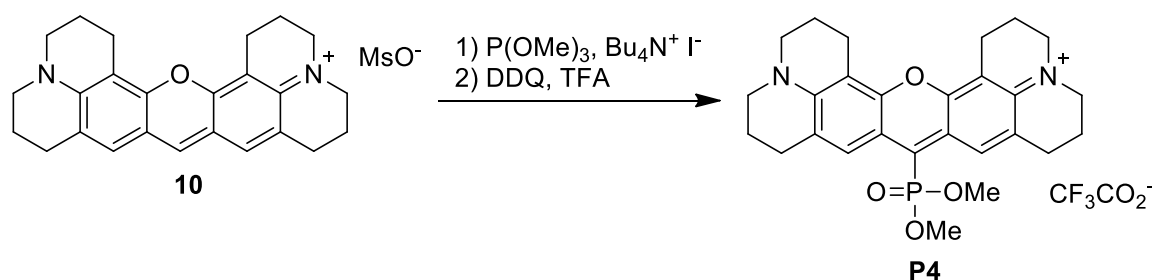
A mixture of 8-hydroxyjulolidine (1.57 g, 8.30 mmol), triethyl orthoformate (7.38 g, 8.24 mL, 49.8 mmol) and methanesulfonic acid (4.78 g, 3.23 mL, 49.8 mmol) was placed into a 20 mL microwave vial and stirred magnetically (mildly exothermic reaction started immediately upon addition of acid). The vial was sealed and heated at 100 °C in a Biotage Initiator+ microwave reactor for 1.5 h (MW absorption level: very high). TLC control: regular SiO₂ plate, 10% v/v H₂O in acetonitrile, R_f = 0.30 (product, bright pink, fluorescent), R_f = 0.63 (byproduct, violet, non-fluorescent). The purple reaction mixture was diluted with sat. aqueous NaCl (200 mL) and extracted with ethyl acetate – propanol-2 (1:1, 4×100 mL). The combined organic solutions were dried over Na₂SO₄ and evaporated in vacuo, yielding 3.36 g of raw product as a violet solid. It was dissolved in a mixture of H₂O (5 mL) and MeCN (10 mL), and the solution was injected on top of a cartridge (RediSep R_f 120 g of regular SiO₂). Flash chromatography (gradient 0% to 5% H₂O in acetonitrile) followed by evaporation of the fractions containing a bright pink-colored and fluorescent product afforded the solid material. It was dissolved in aqueous 1,4-dioxane, the solution was filtered through a 0.2 μm PTFE membrane filter and lyophilized. Yield 494 mg (25%) of **10** (methanesulfonate salt) as a violet solid soluble in water. HPLC: t_R = 13.6 min (area 100%), MeCN/H₂O + 0.1 v/v% TFA in both components: 20/80 – 100% MeCN in 15 min, detection at 560 nm and 600 nm, column 4×250 mm, flow rate 1.2 mL/min.

¹H NMR (400 MHz, DMSO-*d*₆): δ 8.37 (s, 1H), 7.41 (s, 2H), 3.50 (t, J = 5.1 Hz, 8H), 2.91 (t, J = 6.4 Hz, 4H), 2.80 (t, J = 6.0 Hz, 4H), 1.93 (m, J = 18.1 and 6.3 Hz, 8H) ppm.

¹³C NMR / APT (101 MHz, DMSO-*d*₆): δ 151.5 (C), 150.9 (C), 142.5 (CH), 127.9 (CH), 123.3 (C), 112.8 (C), 104.6 (C), 50.3 (NCH₂), 49.8 (NCH₂), 26.7, 20.1, 19.2 and 19.0 (all CH₂) ppm.

MS (ESI) m/z (positive mode, rel. int., %) = 371.2 (100) [M]⁺.

Dye P4



Trimethyl phosphite P(OMe)_3 (99 μL , 104 mg, 0.84 mmol), was added under argon and at room temperature to a suspension of **10** (130 mg, 0.28 mmol) and tetrabutylammonium iodide (103 mg, 0.28 mmol) in DCM (5 mL). The reaction mixture was stirred at room temperature overnight. TLC (silica, 100% EtOAc) showed complete conversion: $R_f = 0.35$ (product), colorless, turns green in the presence of the air. The solvent was removed under reduced pressure, and the crude product (about 250 mg) was evaporated on Celite and isolated by flash chromatography (Sepacore Silica HP 25 g; eluent: EtOAc:hexane 20:80 \rightarrow 100:0; flow rate 40 mL/min) yielded 134 mg (100%) of leuco-**P4** as a slightly greenish oil. A solution of DDQ (63 mg, 0.28 mmol) in DCM (5 mL) was added dropwise at -78°C to a solution of leuco-**P4** (134 mg, 0.28 mmol) in DCM (2 mL), and the reaction mixture was stirred at -78°C for 15 min. The color of the reaction mixture changed from slightly green to intense blue-green. After removing the cooling bath, the reaction was stirred for further 15 min at room temperature. TLC (MeCN / H_2O 2:1 + 0.1% TFA) revealed the colored spot of the product, $R_f = 0.52$. The reaction mixture was diluted with DCM (5 mL), applied on Celite, evaporated to dryness and subjected to flash chromatography on silica (Sepacore Silica HP 40 g, eluent: MeCN/ H_2O + 0.1% TFA for both components, gradient: 100:0 \rightarrow 98:2). After pooling and evaporating the fractions containing the dye, the dark green product was dissolved in 1,4-dioxane (250 μL) and H_2O (5 mL) and freeze-dried. Yield: 165 mg (100%) of **P4** (trifluoroacetate salt) as a dark green solid. HPLC: $t_R = 14.2$ min (peak area 79%), eluent: MeCN/ H_2O + 0.1% TFA in both components, gradient: 20 – 100% MeCN in 15 min, detection at 254 nm and 630 nm, column 4 \times 250 mm, flow rate 1.2 mL/min.

^1H NMR (400 MHz, CD_3OD): δ 8.26 (s, 2H), 3.86 (d, $J = 11.6$ Hz, 6H, OMe), 3.58 (t, $J = 5.8$ Hz, 8H, $4 \times \text{NCH}_2$), 3.01 and 2.89 (2 \times t, $J = 6.4$ Hz, $\Sigma = 8\text{H}$, $4 \times \text{CH}_2\text{C}_{ar}$), 2.07 (m, 8H, $4 \times \text{CH}_2$).

^{13}C NMR (101 MHz, CD_3OD): δ 152.7 (d, $J = 14$ Hz, C_qO), 152.5 (s, C_q), 137.3 (d, $J = 172$ Hz, C_q), 128.0 (d, $J = 4$ Hz, C_q), 126.2 (d, $J = 4$ Hz, CH), 116.3 (d, $J = 11$ Hz, C_q), 107.0 (d, $J = 2$ Hz, C_q), 54.0 (d, $J = 5.6$ Hz, OMe), 52.1, 51.5 ($4 \times \text{NCH}_2$), 28.9 (2 $\times \text{C}_q\text{CH}_2$), 21.8 (2 $\times \text{C}_q\text{CH}_2$), 20.9, 20.8 ($4 \times \text{CH}_2\text{CH}_2\text{CH}_2\text{N}$) ppm.

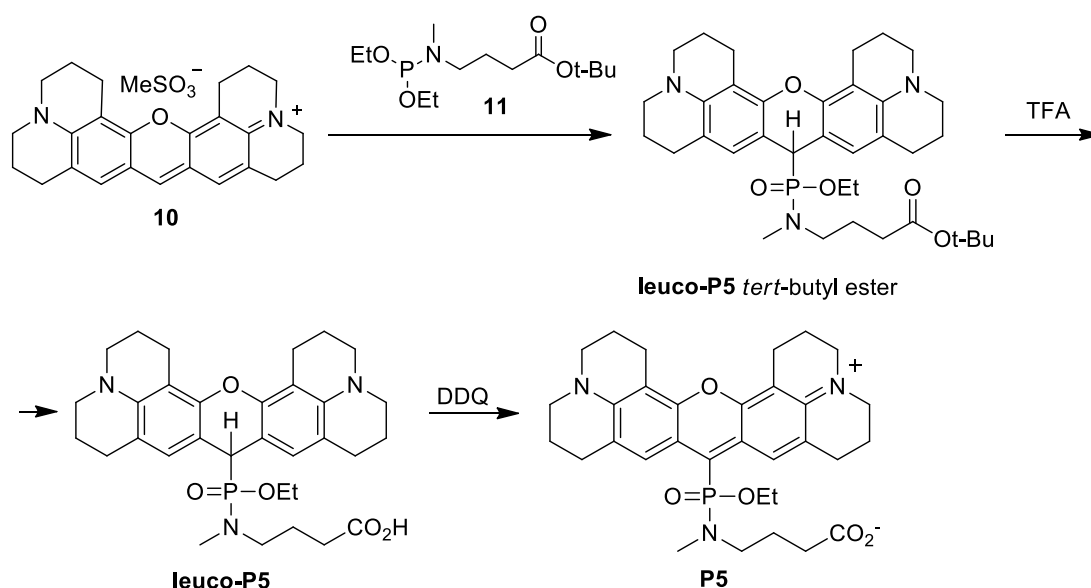
^{19}F NMR (376 MHz, CD_3OD): δ -77.0.

^{31}P NMR (162 MHz, CD_3OD): δ 15.9.

MS (ESI): m/z (positive mode, rel. int., %) = 479.3 (100) $[\text{M}]^+$.

HR-MS (ESI, positive mode): 479.2094 (found), 479,2094 (calculated for $\text{C}_{27}\text{H}_{32}\text{N}_2\text{O}_4\text{P}^+$ as $[\text{M}]^+$).

Dye P5



A solution of **10** (100 mg, 0.22 mmol) in acetonitrile (2 mL) was prepared in a test tube with a screw-cap, and **11** (126 mg, 0.43 mmol; prepared from $(\text{EtO})_2\text{PCl}$ and $\text{HN}(\text{Me})(\text{CH}_2)_3\text{CO}_2\text{Bu}^t$ according to the method described below for compound **13**) was added at room temperature under argon. The reaction mixture was stirred at room temperature for 16 h, diluted with CH_2Cl_2 (10 mL), and evaporated on Celite. Flash chromatography on silica (cartridge Interchim Puriflash 25 g of 15 μM SiO_2 , elution with CH_2Cl_2 / MeOH mixture; gradient: 1% \rightarrow 10 % MeOH/ CH_2Cl_2) afforded 126 mg (93%) of **leuco-P5 tert-butyl ester** as a slightly brown oil. TLC: regular SiO_2 , CH_2Cl_2 – MeOH 30:1; R_f = 0.26.

The intermediate *tert*-butyl ester (126 mg, 0.198 mmol) was dissolved in CH_2Cl_2 (3 mL) and TFA (3 mL) was added dropwise at 0 °C. The ice bath was removed, and the solution stirred at room temperature for 2 h. All volatiles were removed *in vacuo*, the residue (150 mg of a red brown oil) was dissolved in CH_2Cl_2 (10 mL) and evaporated on Celite. Flash chromatography (Interchim Puriflash 25 g of 15 μM SiO_2 ; solvent: CH_2Cl_2 – MeOH; gradient: 1 \rightarrow 40% MeOH/ CH_2Cl_2) afforded 61 mg (54%) of **leuco-P5** as a blue solid (TLC: CH_2Cl_2 – MeOH 5:1, R_f = 0.25) soluble in acetonitrile. The spot of **leuco-P5** on silica compound turns blue under UV lamp or in presence of air. HPLC: t_R = 9.1 min (peak area 82%); solvent: MeCN – H_2O + 0.1 v/v% TFA in both components; gradient: 30 \rightarrow 100% MeCN in 15 min, detection at 254 nm; column: 250 \times 4 mm, flow rate 1.2 mL/min. $\text{C}_{33}\text{H}_{42}\text{N}_3\text{O}_5\text{P}$. MS (ESI): m/z (negative mode, rel. int., %) = 578.8 (100) $[\text{M}-\text{H}]^-$.

The compound **leuco-P5** (30 mg, 52 μmol) was dissolved in MeCN (2 mL), and the solution was cooled down in ice bath to 0 °C. DDQ (18 mg, 78 μmol) was added, and the reaction mixture was stirred for 10 min at 0 °C, warmed up to room temperature, stirred 15 min, diluted with MeCN (10 mL) and evaporated on Celite. The product was isolated by flash

chromatography (cartridge Reveleris HP 24 g of SiO₂; solvent: MeCN – H₂O + 0.2 v/v% of HCOOH in both components; gradient: 1 → 15% v/v H₂O, 32 mL/min). The blue fractions were collected and freeze-dried immediately without evaporating MeCN. Yield 10 mg (33%) of dye **P5** as dark blue solid. TLC; MeCN – H₂O 10:1 + 0.2 v/v% of HCOOH in both component, *R_f* (dye) = 0.20. HPLC: *t_R* = 5.4 min (peak area 100%, abs. max. 654 nm), solvent: MeCN – H₂O + 0.5% v/v TFA in H₂O; gradient: 20 → 100% MeCN in 10 min, diode array detection; column: Kinetex 2.6 μ, 75 × 4.6 mm, flow rate 1.0 mL/min.

¹H NMR (400 MHz, CD₃CN): δ 8.34 (s, 2H), 4.24 – 4.06 (m, 2H, OCH₂), 3.53 (t, *J* = 5.8 Hz, 8H, 4 × NCH₂), 3.16 – 3.00 (m, 2H, MeNCH₂), 2.97 (t, *J* = 6.4 Hz, 4H, 2 × C_qCH₂), 2.87 (t, *J* = 6.3 Hz, 4H, 2 × C_qCH₂), 2.65 (d, *J* = 10.5 Hz, 3H, NMe), 2.26 (m, overlaps with H₂O signal, CH₂CH₂CH₂ in julolidine), 2.03 (m, 4H, CH₂CH₂CH₂ in julolidine), 1.77 (m, 2H, MeNCH₂CH₂CH₂), 1.37 (t, *J* = 7.1 Hz, 3H, OCH₂CH₃) ppm.

¹³C NMR (101 MHz, CD₃CN, ¹³C(¹H) correlation): δ 128.5 (8.3) (CH), 63.1 (4.15) (CH₂O), 51.3 (3.52) (CH₂N), 48.0 (3.04) (CH₂N), 32.9 (2.62) (NMe), 31.1 (2.26) (CH₂), 28.6 (2.86) (CH₂), 23.5 (1.76) (CH₂), 21.6 (2.00) (CH₂), 20.6 (2.96) (CH₂), 20.5 (2.04) (CH₂), 16.6 (1.36) (CH₃).

³¹P NMR (162 MHz, CD₃CN): δ 18.4 ppm.

¹⁹F NMR (376 MHz, CD₃CN): δ -76.2 ppm.

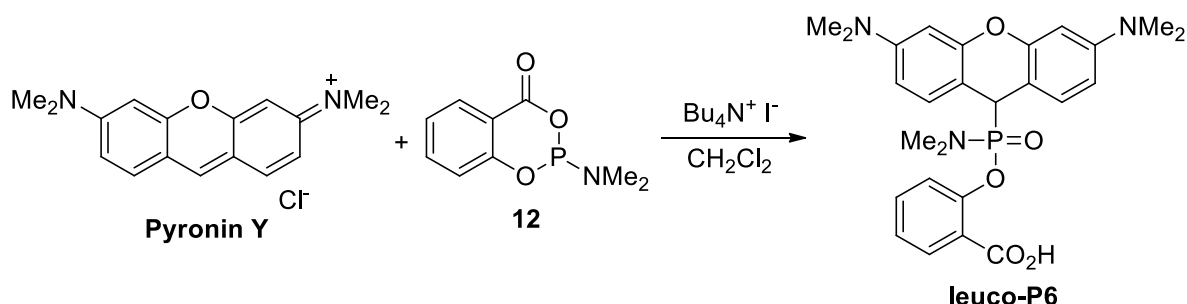
MS (ESI): *m/z* (positive mode, rel. int., %) = 578.3 (100) [M+H]⁺.

HRMS (C₃₂H₄₀N₃O₅P): *m/z* (positive mode) = 578.2782 (found [M+H]⁺), 578.2778 (calc.).

Dye P6

leuco-P6

2-[[3,6-bis(dimethylamino)-9*H*-xanthen-9-yl](dimethylamino)phosphoryloxy]benzoic acid



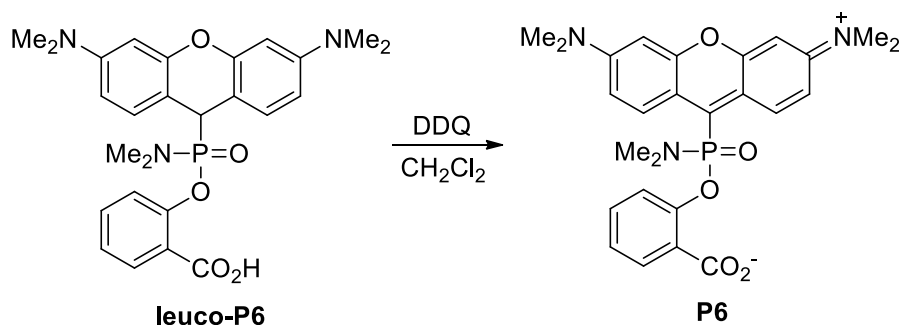
A suspension of Pyronin Y (151 mg; 0.50 mmol) and tetrabutylammonium iodide (185 mg; 0.50 mmol) in CH_2Cl_2 was sonicated briefly, and 2-dimethylamino-4*H*-1,3,2-benzodioxaphosphorin-4-one^[5] **12** (211 mg, 1.0 mmol) was added. The resulting mixture was sonicated for 2 min and stirred vigorously for 1 h, during which time a bright pink solution turned into a deep purple thin suspension. Sodium hydroxide (1 mL of 10% in MeOH/ H_2O 1:1) was added followed by just enough MeOH to homogenize the mixture. After stirring for 10 min, AcOH (2 mL) was added, and the mixture was evaporated to dryness. The residue was re-evaporated several times with acetone and subjected to column chromatography (45 g of SiO_2 , gradient 10% to 40% MeOH/ CH_2Cl_2). Fractions containing the product were pooled, evaporated to dryness, redissolved in 1,4-dioxane (20 mL), filtered through a 0.45 μm PTFE membrane filter and freeze-dried, yielding the title compound **leuco-P6** (106 mg, 43% yield) as a fluffy violet solid, which was used directly in the next step without further characterization.

MS (ESI): m/z (negative mode, rel. int., %) = 494.3 (100) $[\text{M}-\text{H}]^+$.

HRMS ($\text{C}_{27}\text{H}_{30}\text{N}_3\text{O}_5\text{P}$): m/z (negative mode) = 494.1850 (found $[\text{M}-\text{H}]^-$), 494.1850 (calc.).

Dye P6

2-[[3,6-bis(dimethylamino)-9*H*-xanthenylium-9-yl](dimethylamino)phosphoryloxy]-benzoate



The **leuco-P6** compound from the previous step (52 mg, 0.11 mmol) was dissolved in CH₂Cl₂ (5 mL), the solution was cooled in a dry ice-acetone bath, and DDQ (24 mg, 0.11 mmol) in CH₂Cl₂ (3 mL) was added quickly dropwise. The resulting dark violet solution was allowed to warm up to rt and evaporated to dryness. The residue was subjected to column chromatography (30 g of SiO₂, gradient 5% to 50% MeOH/CH₂Cl₂); the fractions containing the product were evaporated and re-purified by reversed-phase chromatography (15 g of RP-C₁₈, gradient 10% to 30% H₂O/MeCN). The pure fractions were evaporated to yield the product **P6** as a bronze solid (40 mg, 77%).

¹H NMR (400 MHz, CD₃OD): δ 9.14 (d, *J* = 9.8 Hz, 2H), 7.43 (d, *J* = 8.4 Hz, 1H), 7.32 (ddd, *J* = 8.5, 6.4, 2.8 Hz, 1H), 7.14 – 7.04 (m, 4H), 6.72 – 6.68 (m, 2H), 3.28 (s, 12H), 2.75 (s, 3H), 2.64 (s, 3H) ppm.

¹³C NMR (126 MHz, CD₃OD): δ 170.3, 158.6 (d, *J* = 11.7 Hz), 158.1, 153.8 (d, *J* = 156 Hz), 149.2 (d, *J* = 7.4 Hz), 135.3 (d, *J* = 2.9 Hz), 131.3, 129.6 (d, *J* = 6.2 Hz), 128.8, 124.8, 122.3 (d, *J* = 3.0 Hz), 116.8, 116.7 (d, *J* = 9.4 Hz), 97.2 (d, *J* = 1.4 Hz), 40.9, 39.0, 34.9.

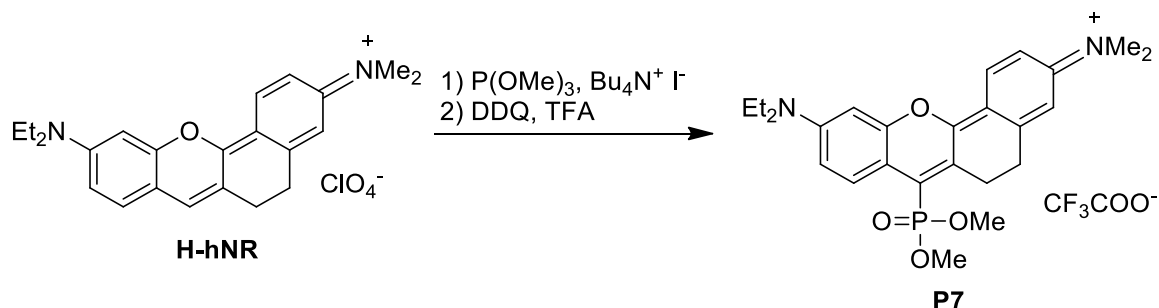
³¹P NMR (162 MHz, CD₃OD): δ -1.56 ppm.

MS (ESI): *m/z* (positive mode, rel. int., %) = 494.2 (100) [M+H]⁺, 516.2 (91) [M+Na]⁺.

HRMS (C₂₆H₂₈N₃O₅P): *m/z* (positive mode) = 494.1841 (found [M+H]⁺), 494.1839 (calc.).

Dye P7

7-(dimethoxyphosphoryl)-3,10-bis(dimethylamino)-5,6-dihydrobenzo[c]xanthen-12-ium trifluoroacetate



Trimethyl phosphite (Sigma-Aldrich; 88 μ L, 0.75 mmol, 3 eq) was added at room temperature to a screw-cap test tube containing a stirred solution of **H-hNR** dye^[6] (perchlorate salt; 112 mg, 0.25 mmol) and tetrabutylammonium iodide (92 mg, 0.25 mmol, 1 eq) in dry CH₂Cl₂ (6 mL). The reaction mixture was evaporated on Celite and the leuco dye was isolated by flash chromatography on Biotage Isolera system (Sepacore Silica HP cartridge, 12 g SiO₂; gradient 50% to 100% EtOAc – hexane over 10 column volumes). The entire amount of the leuco dye was used directly in the next step.

The material was dissolved in CH₂Cl₂ (5 mL) and cooled down to -78°C, and a solution of DDQ (57 mg, 0.25 mmol) in CH₂Cl₂ (5 mL) was added dropwise. The resulting dark green solution was allowed to warm-up to room temperature and stirred for 15 min. The reaction mixture was evaporated on Celite and the product was isolated by flash chromatography on Biotage Isolera system (Sepacore Silica HP cartridge, 12 g SiO₂; gradient 0% to 100% A:B over 10 column volumes, A – acetonitrile-water 95:5 + 0.1% (v/v) TFA, B – acetonitrile over 10 column volumes). The fractions containing the product were pooled and evaporated, the residue was dissolved in 1,4-dioxane, microfiltered through a 0.2 μ m PTFE membrane filter and lyophilized to give 152 mg (100%, remainder dioxane) of the dye as fluffy black hygroscopic solid.

¹H NMR (400 MHz, DMSO-*d*₆): δ 8.57 (d, *J* = 9.6 Hz, 1H), 8.14 (d, *J* = 9.3 Hz, 1H), 7.22 (dd, *J* = 9.7, 2.7 Hz, 1H), 7.16 (t, *J* = 2.4 Hz, 1H), 6.91 (dd, *J* = 9.3, 2.4 Hz, 1H), 6.75 (d, *J* = 2.3 Hz, 1H), 3.82 (s, 3H), 3.79 (s, 3H), 3.61 (q, *J* = 6.8 Hz, 4H), 3.30 – 3.24 (m, 2H), 3.22 (s, 6H), 2.98 – 2.91 (m, 2H), 1.21 (t, *J* = 7.0 Hz, 6H).

¹³C NMR (101 MHz, DMSO-*d*₆): δ 164.1 (d, *J* = 15.3 Hz), 158.3 (q, *J* = 35.2 Hz, CF₃), 155.7, 155.5 (d, *J* = 12.9 Hz), 152.5, 146.7, 139.3, 137.6, 130.3, 129.5 (d, *J* = 2.1 Hz), 125.6 (d, *J* = 10.6 Hz), 115.0, 114.8 (d, *J* = 12.1 Hz), 113.5 (d, *J* = 1.5 Hz), 113.0, 110.5, 96.3, 53.31, 53.26, 44.8, 40.2, 27.0, 25.0, 12.5.

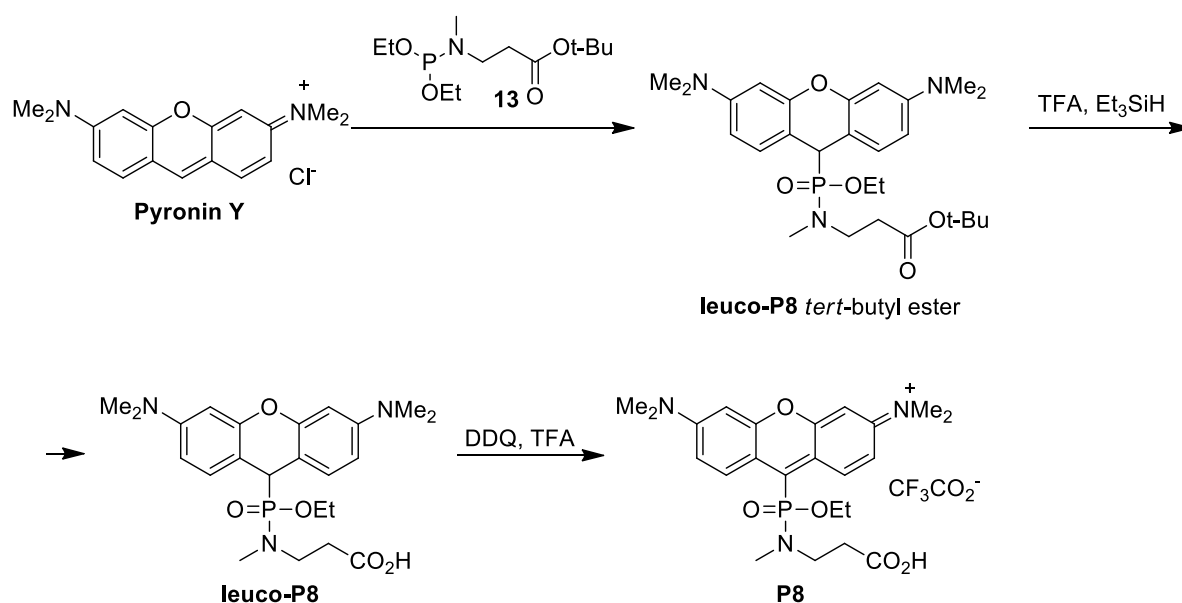
^{19}F NMR (376 MHz, $\text{DMSO-}d_6$): δ -74.6 ppm.

^{31}P NMR (162 MHz, $\text{DMSO-}d_6$): δ 14.6 ppm.

MS (ESI): m/z (positive mode, rel. int., %) = 455.2 (100) $[\text{M}]^+$.

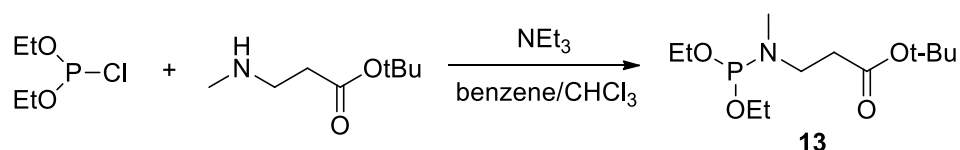
HR-MS (ESI, positive mode): 455.2099 (found), 455.2094 (calculated for $\text{C}_{25}\text{H}_{32}\text{N}_2\text{O}_4\text{P}^+$).

Dye P8



Compound 8

tert-butyl 3-[(diethoxyphosphino)(methyl)amino]propanoate



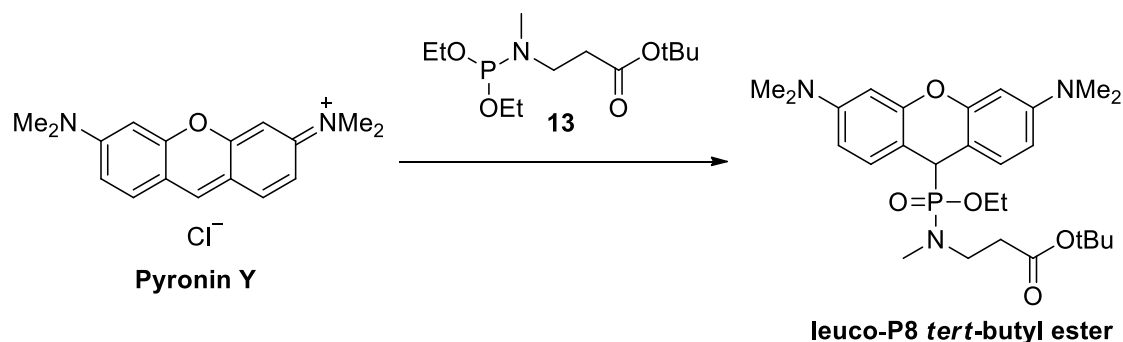
To a solution of *tert*-butyl 3-(methylamino)propanoate^[7] (500 mg; 3.14 mmol) and triethylamine (380 mg; 3.77 mmol) in a mixture of benzene and CHCl_3 (20 mL; 3:1), a solution of diethyl chlorophosphite (Alfa Aesar, 490 mg; 3.14 mmol) in benzene (2 mL) was added dropwise at 0 °C. The reaction mixture was refluxed for 2 h. After cooling down to r.t., the reaction mixture was diluted with *n*-hexane (~20 mL) and filtered through a glass filter. The filtrate was evaporated and subjected to column chromatography (30 g of SiO_2 , Hex/EtOAc 1:3 + 0.1 v/v% of NEt_3) to afford 392 mg (50%) of **13** as an air-sensitive colorless oil, which was used in the following step without additional purification.

^1H NMR (400 MHz, CD_3CN): δ = 1.18 (t, $J_{\text{H-H}}$ = 7.0 Hz, 6H, 2×OEt), 1.43 (s, 9H, *t*Bu), 2.37 (t, $J_{\text{H-H}}$ = 7.0 Hz, 2H, CH_2), 2.52 (d, $J_{\text{H-P}}$ = 6.6 Hz, 3H, NMe), 3.21 (dt, $J_{\text{H-P}}$ = 9.7 Hz, $J_{\text{H-H}}$ = 7.0 Hz, 2H, NCH_2), 3.57–3.72 (m, 4H, 2×OEt) ppm.

^{31}P NMR (162 MHz, CD_3CN): δ = 144.9 ppm.

leuco-P8 *tert*-butyl ester

tert-butyl 3-[[[3,6-bis(dimethylamino)-9*H*-xanthen-9-yl](ethoxy)phosphoryl](methyl)amino]propanoate



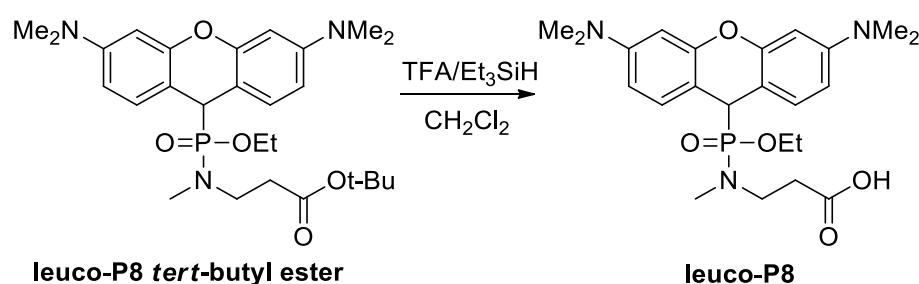
Pyronin Y (50 mg; 0.17 mmol) was suspended in MeCN (1 mL) in a screw-cap test tube, and **13** (62 mg; 0.247 mmol) was added at r.t. under argon. The mixture was stirred for 2 h at 60 °C. After cooling down to r.t., the reaction mixture was diluted with CH₂Cl₂ (~3 mL) and subjected to column chromatography (30 g of SiO₂, CH₂Cl₂/MeOH 30:1) to yield 37 mg (43%) of **leuco-P8 *tert*-butyl ester** as brown oil. HPLC: *t_R* = 7.8 min (HPLC area 84%), B/A = 30/70–100/0 in 25 min, column 4×250 mm, 1.2 mL/min, detection at 254 nm.

¹H NMR (400 MHz, CD₃CN): δ = 1.21 (t, *J*_{H-H} = 7.0 Hz, 3H, OEt), 1.38 (s, 9H, *t*Bu), 2.05–2.25 (m, 2H, CH₂), 2.39 (d, *J*_{H-P} = 8.2 Hz, 3H, NMe), 2.78–2.94 (m, 2H, NCH₂), 2.93 (s, 12H, 2×NMe₂), 3.74–3.98 (m, 2H, OEt), 4.25 (d, *J*_{H-P} = 20.9 Hz, 1H), 6.37 (m, *J*_{H-H} = 2.2 Hz, 2H_{ar}), 6.50 (dd, *J*_{H-H} = 8.7 Hz and 2.6 Hz, 2H_{ar}), 7.05 (dd, *J*_{H-H} = 8.6 Hz and 2.4 Hz, 1H_{ar}), 7.15 (dd, *J*_{H-H} = 8.5 Hz and 2.5 Hz, 1H_{ar}) ppm.

³¹P NMR (162 MHz, CD₃CN): δ = 26.9 ppm.

leuco-P8

3-[[[3,6-bis(dimethylamino)-9*H*-xanthen-9-yl](ethoxy)phosphoryl](methyl)amino]propanoic acid



To a solution of **leuco-P8 *tert*-butyl ester** (33 mg, 0.064 mmol) in CH₂Cl₂ (1 mL), triethylsilane (37 mg; 0.32 mmol) and TFA (1 mL) were added dropwise. The resulting reaction mixture stirred for 1.5 h at r.t., and all volatiles were removed *in vacuo*. The residue was subjected to column chromatography (25 g of SiO₂, CH₂Cl₂/MeOH 10:1) to afford 27 mg (92%) of **leuco-P8** as bluish solid.

¹H NMR (400 MHz, CD₃CN): δ 1.20 (t, *J*_{H-H} = 7.0 Hz, 3H, OEt), 2.09–2.24 (m, 2H, CH₂), 2.39 (d, *J*_{H-P} = 8.1 Hz, 3H, NMe), 2.75–3.07 (m, 2H, NCH₂), 2.93 (s, 12H, NMe₂), 3.74–3.97

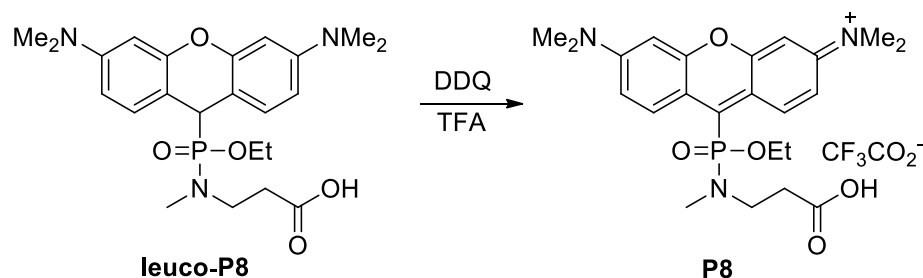
(m, 2H, OEt), 4.31 (d, $J_{\text{H-P}} = 21.1$ Hz, 1H), 6.38–6.43 (m, 2H_{ar}), 6.49–6.55 (m, 2H_{ar}), 7.02–7.09 (m, 1H_{ar}), 7.12–7.18 (m, 1H_{ar}) ppm.

^{31}P NMR (162 MHz, CD_3CN): δ 27.3 ppm.

MS (ESI): m/z (negative mode, rel. int., %) = 460.4 (100) $[\text{M-H}]^-$.

Dye P8

9-[[[(2-carboxyethyl)(methyl)amino](ethoxy)phosphoryl]-3,6-bis(dimethylamino)-9H-xanthenylium trifluoroacetate



To a solution of **leuco-P8** (25 mg, 0.054 mmol) in MeCN (1 mL), DDQ (12 mg; 0.054 mmol) was added at 0 °C. The mixture was stirred for 30 min at r.t. and then subjected to column chromatography directly (applied onto 15 g of SiO_2 , eluted with MeCN \rightarrow MeCN/ H_2O 1:1 + 0.1 v/v% of TFA to yield 22 mg (80%) of the dye **P8** as a dark violet solid. HPLC: $t_R = 16.0$ min (HPLC area 95%), B/A = 20/80–100/0 in 25 min, column 4×250 mm, 1.2 mL/min, detection at 636 nm.

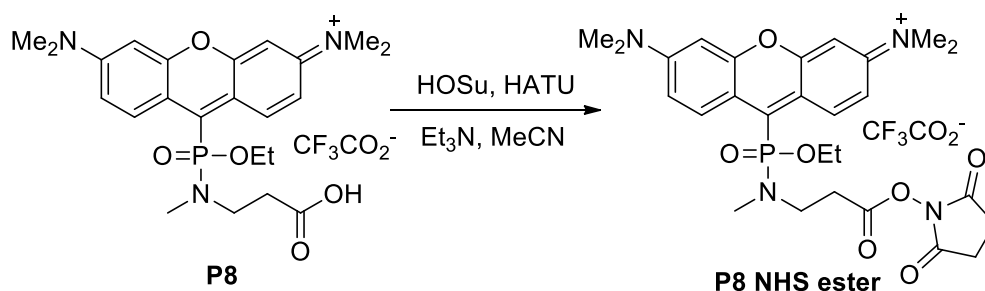
^1H NMR (400 MHz, CDCl_3): δ = 1.41 (t, $J_{\text{H-H}} = 7.3$ Hz, 3H, OEt), 2.54–2.73 (m, 2H, CH_2), 2.80 (d, $J_{\text{H-P}} = 10.8$ Hz, 3H, NMe), 3.27–3.50 (m, 2H, NCH_2), 3.37 (s, 12H, $2\times\text{NMe}_2$), 4.18–4.37 (m, 2H, OEt), 6.74 (s, broad, 2H_{ar}), 7.18 (dd, $J_{\text{H-H}} = 9.9$ Hz, $J_{\text{H-P}} = 2.3$ Hz, 2H_{ar}), 8.86 (d, $J_{\text{H-H}} = 9.9$ Hz, 2H_{ar}) ppm.

^{31}P NMR (162 MHz, CDCl_3): δ = 17.7 ppm.

MS (ESI): m/z (positive mode, rel. int., %) = 460.2 (100) $[\text{M}]^+$.

Dye P8 NHS ester

9-[[[(2-carboxyethyl)(methyl)amino](ethoxy)phosphoryl]-3,6-bis(dimethylamino)-9*H*-xanthenylium trifluoroacetate *N*-hydroxysuccinimide ester

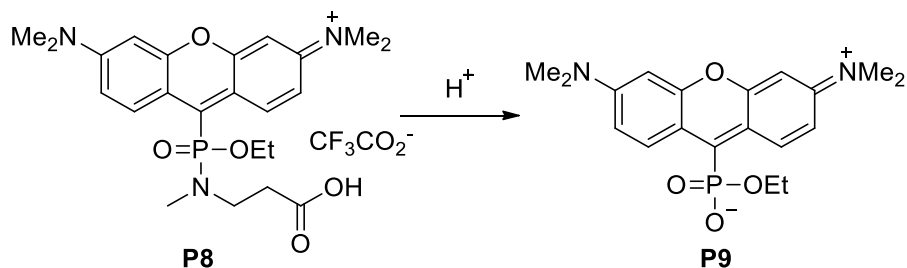


To a solution of **P8** (10 mg, 0.020 mmol) in MeCN (1 mL), *N*-hydroxysuccinimide (35 mg; 0.30 mmol), HATU (30 mg; 0.08 mmol) and Et₃N (36 mg; 0.36 mmol) were added at r.t. under Ar. After stirring for 30 min, AcOH (21 μL) was added; the reaction mixture diluted with CH₂Cl₂ and washed with water (2×). The organic layer was separated, dried with Na₂SO₄ and evaporated to give 10 mg (85%) of the crude product as blue material. HPLC (B/A = 30/70–100/0 in 25 min, column 4×250 mm, 1.2 mL/min, detection at 636 nm) showed the presence of two substances with *t*_R = 6.6 min (area 10%; the starting material) and *t*_R = 8.6 min (area 90%; NHS ester). After purification by preparative HPLC followed by freeze-drying, 3 mg (25%) of a violet solid was isolated. HPLC: *t*_R = 8.6 min (area 94%), B/A = 30/70–100/0 in 25 min, column 4×250 mm, 1.2 mL/min, detection at 254 nm.

MS (ESI): *m/z* (positive mode, rel. int., %) = 557.2 (100) [M-Cl]⁺.

Dye P9

ethyl [3,6-bis(dimethylamino)-9*H*-xanthylium-9-yl]phosphonate



Solutions of dye **P8** in protic solvents (MeOH, H_2O) hydrolyze (particularly in the presence of acids) with formation of the compound **P9**.

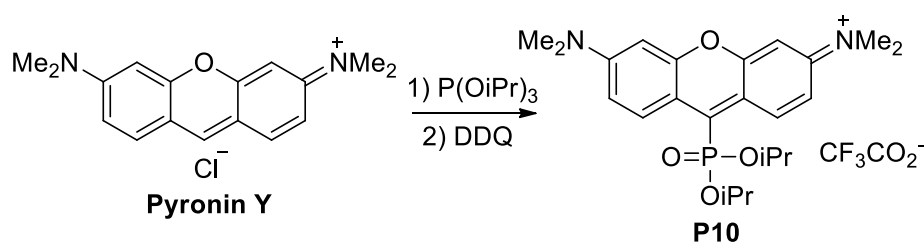
^1H NMR (400 MHz, CD_3CN): δ 1.04 (t, $J_{\text{H-H}} = 7.0$ Hz, 3H, OEt), 3.27 (s, 12H, $2 \times \text{NMe}_2$), 3.79 (dt, $J_{\text{H-P}} = 14.2$ Hz, $J_{\text{H-H}} = 7.2$ Hz, 2H, OEt), 6.77–6.80 (m, 2H_{ar}), 7.18 (dd, $J_{\text{H-H}} = 9.8$ Hz, $J_{\text{H-H}} = 2.6$ Hz, 2H_{ar}), 9.04 (d, $J_{\text{H-H}} = 9.8$ Hz, 2H_{ar}) ppm.

^{31}P NMR (162 MHz, CD_3CN): δ 3.7 ppm.

MS (ESI): m/z (positive mode, rel. int., %) = 375.1 (100) $[\text{M}+\text{H}]^+$, 397.2 (47) $[\text{M}+\text{Na}]^+$.

Dye P10

9-(diisopropoxyphosphoryl)-3,6-bis(dimethylamino)-9*H*-xanthenylium trifluoroacetate



Triisopropyl phosphite (Sigma-Aldrich; 244 μL , 206 mg, 0.99 mmol) was added to a stirred solution of Pyronin Y (100 mg, 0.33 mmol,) and tetrabutylammonium iodide (122 mg, 0.33 mmol,) in dry CH_2Cl_2 (12 mL). The reaction mixture was stirred for 3 h at rt, until the red-violet color disappeared and then was evaporated on Celite. The leuco dye was isolated by flash column chromatography (cartridge RediSep Rf with 24 g of SiO_2 , gradient *n*-hexane/EtOAc 70:30 \rightarrow 30:70) to yield 108 mg (76%) of the bluish viscous oil. It was dissolved in dry CH_2Cl_2 (3 mL), and the solution was cooled in a dry ice-acetone bath. A solution of DDQ (60 mg, 0.25 mmol) in CH_2Cl_2 (5 mL) was added quickly dropwise. The dark green reaction mixture was allowed to warm up to rt and stirred for additional 15 min. The mixture was evaporated on Celite and the product was isolated by flash column chromatography (cartridge Büchi Sepacore Silica HP, 25 g of SiO_2 , gradient: MeCN/ H_2O + 0.1 v/v% TFA, 100:0 \rightarrow 90:10). The fractions containing the product were pooled and evaporated *in vacuo*. The residue was dissolved in 1,4-dioxane, filtered through a 0.2 μm PTFE membrane filter and lyophilized to yield 136 mg (100%, 76% over 2 steps) of the dye **P10** as a dark green solid (TLC: eluent MeCN/ H_2O 10:1 + 0.1 v/v% TFA, R_f = 0.36). HPLC: t_R = 11.4 min (peak area 98%), MeCN/ H_2O + 0.1% TFA in both components: 20/80 – 100% MeCN in 20 min, detection at 570 nm and 600 nm, column 4 \times 250 mm, flow rate 1.2 mL/min.

^1H NMR (400 MHz, CD_3CN) δ 8.86 (d, J = 9.9 Hz, 2H), 7.18 (dd, J = 9.9 and 2.6 Hz, 2H), 6.79 (« t », J = 2.4 Hz, 2H), 4.86 (dp, J = 7.6 and 6.1 Hz, 2H, CHO), 3.32 (s, 12H, NMe_2), 1.45 (d, J = 6.1 Hz, 6H, diastereotopic methyl groups in *i*Pr), 1.19 (d, J = 6.1 Hz, 6H, diastereotopic methyl groups in *i*Pr).

^{13}C NMR (101 MHz, CD_3CN) δ 158.4 (d, J = 13 Hz), 158.0, 133.4 (d, J = 3 Hz), 116.5 (d, J = 10 Hz), 115.8, 97.3 (d, J = 2 Hz), 74.3 (d, J = 5.6 Hz), 41.4, 24.2 (d, J = 4 Hz), 23.9 (d, J = 5 Hz) ppm.

^{19}F NMR (376 MHz, CD_3CN) δ -76.6 ppm.

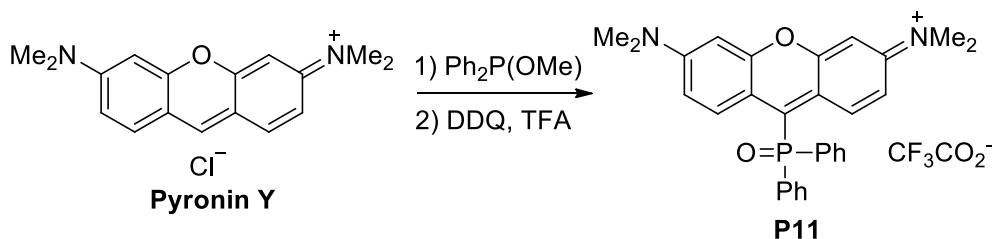
^{31}P NMR (162 MHz, CD_3CN) δ 8.0 ppm.

MS (ESI): m/z (positive mode, rel. int., %) = 431.2 (100) $[\text{M}]^+$.

HR-MS (ESI, positive mode): 431.2094 (found), 431,2094 (calculated for $C_{23}H_{32}N_2O_4P^+$ as $[M]^+$).

Dye P11

P-[3,6-bis(dimethylamino)-9*H*-xanthenylium-9-yl]-*P,P*-diphenylphosphine oxide trifluoroacetate



To a stirred solution of Pyronin Y (100 mg, 0.33 mmol) and tetrabutylammonium iodide (122 mg, 0.33 mmol) in dry CH_2Cl_2 (8 mL), methyl diphenylphosphinite (Sigma-Aldrich, 200 μ L, 214 mg, 1.0 mmol) was added at room temperature. The reaction mixture was stirred at room temperature, until the red-violet color disappeared (ca. 3 h) and Pyronin Y could not be detected by TLC (100% EtOAc, R_f = 0.21 of the product). The reaction mixture was diluted with CH_2Cl_2 (10 mL), evaporated on Celite and subjected to flash chromatography (cartridge RediSep Rf with 24 g of SiO_2 , gradient: EtOAc/Hexane 95:5 \rightarrow 100:0, flowrate 35 mL/min). Yield - 154 mg (99.6%) of **leuco-P11** (R_f = 0.21, 100% EtOAc) as a slightly blue solid, which was oxidized without further characterization. The entire amount (154 mg) was dissolved in CH_2Cl_2 (3 mL), the solution was cooled in a dry ice - acetone bath (ca. $-70^\circ C$), and a solution of DDQ (76 mg, 0.33 mmol) in CH_2Cl_2 (5 mL) was added quickly dropwise. The reaction mixture was stirred 15 min at $-70^\circ C$; a change in color from colorless to dark green was observed. The cooling bath was removed, the dark green solution was allowed to warm up to room temperature and stirred for 15 min. The reaction mixture was diluted with CH_2Cl_2 (8 mL), evaporated to dryness with Celite and subjected to flash chromatography (cartridge Sepacore Silica HP 25 g 15 μ m silica, gradient MeCN/ H_2O 100:0 \rightarrow 98:2 + 0.1 v/v% TFA). The fractions containing the product were pooled and evaporated *in vacuo*. The residue was dissolved in 1,4-dioxane, filtered through a 0.2 μ m PTFE membrane and lyophilized to give 160 mg (84%) of **P11** as a dark brown solid. This impure material was subjected to preparative HPLC (column Interchim 25QE181E2J, 21 mm \times 25 cm, RP-C18 10 μ m, eluents: H_2O + 0.1 v/v % of TFA, MeCN (TFA free); gradient 30 \rightarrow 60% MeCN in 20 min; 20 mL/min). The fractions containing the product were pooled and freeze-dried; the residue was dissolved in 1,4-dioxane, filtered through a 0.2 μ m PTFE membrane filter and freeze-dried. Yield 103 mg (54%, 54% over 2 steps) of the dye **P11** as a dark brown solid; TLC: regular SiO_2 , MeCN/ H_2O 10:1 + 0.1 v/v% of TFA) revealed a colored spot with R_f = 0.36. 1H ^{13}C and ^{31}P NMR spectra indicate the presence of 2 forms. HPLC: t_R = 11.9 min (peak area 97%),

MeCN/H₂O + 0.1% v/v% TFA in both components: 20/80 – 100% MeCN in 15 min, detection at 570 nm – 600 nm, column 4×250 mm, flow rate 1.2 mL/min.

¹H NMR (400 MHz, CD₃CN) δ 8.22 (d, *J* = 9.9 Hz, 2H), 7.82 and 7.79 (2×dd, *J* = 8.3 and 1.3 Hz, Σ 4H) 7.73 (m, 2H), 7.61 (m, 4H), 6.84 (dd, *J* = 9.8, 2.7 Hz, 2H), 6.71 (dd, *J* = 2.6, 1.4 Hz, 2H), 3.23 (s, 12H, 2 × NMe₂) ppm.

¹³C NMR (101 MHz, CD₃CN) δ 157.9 (d, *J* = 9 Hz, C), 157.6 (C), 134.2 (d, *J* = 2.8 Hz, *p*-CH in Ph₂PO), 132.5 and 132.3 (*m*-CH in Ph₂PO and C^{4,5}), 130.4 (d, *J* = 13 Hz, *o*-CH Ph₂PO), 117.4 (d, *J* = 7.6 Hz), 115.2 (C^{3,6}), 97.4 (C^{1,8}), 41.3 (2 × NMe₂) ppm.

¹⁹F NMR (376 MHz, CD₃CN) δ -76.6 ppm.

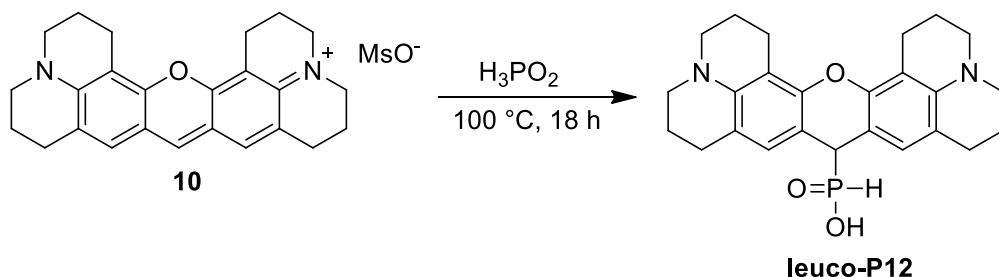
³¹P NMR (162 MHz, CD₃CN) δ 28.04 ppm.

MS (ESI): *m/z* (positive mode, rel. int., %) = 467.3 (100) [M]⁺.

HR-MS (ESI, positive mode): 467.1883 (found), 467,1883 (calculated for C₂₉H₂₈N₂O₂P⁺).

Dye P12

leuco-P12



A solution of **10** (300 mg, 0.77 mmol) in aqueous hypophosphorous acid (50% w/w, Alfa Aesar; 3 mL) was placed in a 5 mL microwave vial and flushed with Argon. The vial was sealed and heated under microwave irradiation at 100 °C for 18 h (MW absorption level: very high). After cooling down to room temperature, the cherry-red reaction mixture was diluted with water (5 mL) and applied on top of the reversed-phase cartridge (Biotage Isolera SNAP Ultra RP C18, 30 g, 30 μM) primed with MeCN (200 mL) and H₂O (200 mL). Excess H₃PO₂ was eluted with water (40–50 mL) until the pH of the eluate reached 4–5 and then eluted with MeCN/H₂O (+ 0.1 v/v TFA in the both components), gradient: 10 \rightarrow 90% MeCN. The product elutes as a yellow band and gradually oxidizes in air during concentration and to a light blue solution. Acetonitrile was removed under reduced pressure, and the residual aqueous solution was freeze-dried; yield 278 mg (99%) of **leuco-P12** as a dark blue solid (TLC: SiO₂, MeCN/H₂O 1:1 + 0.1 v/v% TFA, with DDQ as a staining reagent revealed a blue spot of the product). HPLC: t_{R} = 15.4 min (peak area 94%), MeCN (TFA free) / H₂O + 0.1 v/v% TFA: 2/98 – 50/50 in 20 min, detection at 580 nm, column, 4 \times 250 mm, flow rate 1.2 mL/min. C₂₅H₂₉N₂O₃P (436.1916).

¹H NMR (400 MHz, CD₃OD): δ 6.88 (s, 2H), 6.65 (d, J_{HP} = 543 Hz, 1H, HP), 4.14 (d, J = 16.4 Hz, 1H, HPCH), 3.30 (m, J = 4.6, 2.9 and 2.3 Hz, 8H, 2 \times N(CH₂)₂), 3.03–2.61 (m, 8H, 4 \times CH₂), 2.16 – 1.86 (m, 8H, CH₂) ppm.

¹³C NMR (101 MHz, CD₃OD, gHSQC : only H-coupled carbons are resolved): δ 129.2 (CH), 43.7 (d, CHP), 53.0, 27.6, 22.6, 22.4, 22.0 (all CH₂) ppm.

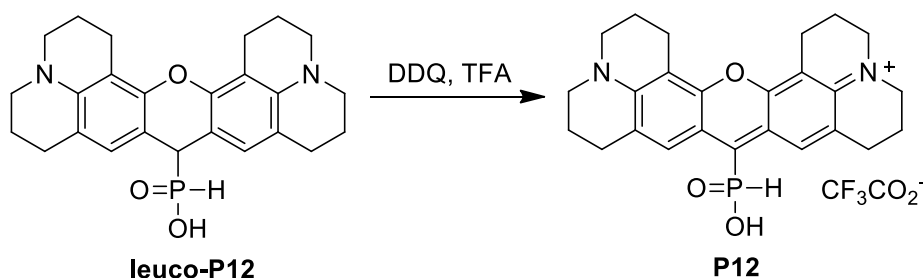
¹⁹F NMR (376 MHz, CD₃OD): δ -77.5 ppm.

³¹P NMR (162 MHz, CD₃OD): δ 29.0 ppm.

MS (ESI): m/z (negative mode, rel. int., %) = 435.3 (100) [M-H]⁻, 871.5 [2M-H]⁻; m/z (positive mode, rel. int., %) = 437.2 (100) [M+H]⁺, 873.4 (8) [2M+H]⁺.

HR MS (ESI): m/z (positive mode) = 437.1987 (found for C₂₅H₃₀N₂O₃P, [M+H]⁺), 437.1994 (calc. for C₂₅H₃₀N₂O₃P); 459.1806 (found for C₂₅H₂₉N₂NaO₃P, [M+Na]⁺), 459.1813 (calc. for C₂₅H₂₉N₂NaO₃P, [M+Na]⁺).

Dye P12



A solution of **leuco-P12** (20.0 mg, 45.9 μmol) in MeCN and MeOH (1.2 and 0.4 mL) was flushed with argon, cooled to 0 $^{\circ}\text{C}$, and a solution of DDQ (11 mg, 50 μmol) in MeCN (2 mL) was added. The reaction mixture was stirred at 0 $^{\circ}\text{C}$ for 30 minutes, evaporated *in vacuo*, the residue was dissolved in MeOH (~ 10 mL) and applied to a cartridge with regular SiO_2 . Isolation with Biotage Isolera: cartridge Sepacore Silica HP, 25 g; gradient MeOH/ CH_2Cl_2 : 100/0 \rightarrow 95/5, then MeCN/ H_2O 70:30 (+0.1 v/v% of TFA in both components). The blue colored fractions were collected, acetonitrile was removed under reduced pressure. The residue was freeze-dried. The solid material was dissolved in aqueous 1,4-dioxane, and the solution was filtered through a 0.2 μm PTFE membrane filter and lyophilized to yield 12 mg (58%) of dark blue dye **P12** soluble in methanol; R_f = 0.4 on regular SiO_2 , MeCN/ H_2O 10:1 + 0.1 v/v% TFA in each component, red fluorescent spot. HPLC: t_R = 14.5 min (peak area 85%), eluent: MeCN / H_2O + 0.1 v/v% TFA in both components; gradient: 20 \rightarrow 100% MeCN in 15 min, detection at 254 nm, column, 4 \times 250 mm, flow rate 1.2 mL/min.

^1H NMR (400 MHz, CD_3OD): δ 8.29 (d, J_{HP} = 544 Hz, 1H), 8.29 (s, 2H, CH), 3.52 (m, 8H, $\text{N}(\text{CH}_2)_2$), 2.95 (t, J = 6.2 Hz, 4H), 2.67 (t, J = 6.2 Hz, 4H), 2.13–1.95 (m, 8H) ppm.

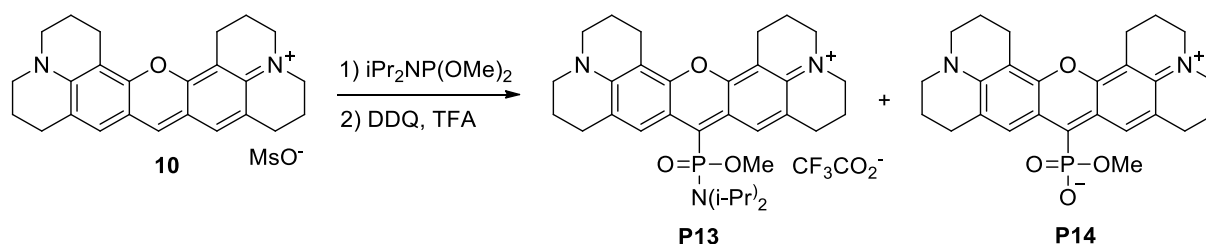
^{19}F NMR (376 MHz, CD_3OD): δ -77.2 ppm.

^{31}P NMR (162 MHz, CD_3OD): δ 3.3 ppm.

MS (ESI): m/z (negative mode, %) = 914.6 (100) [$2\text{M} + \text{HCOOH}$] $^-$; 1349.6 (53) [$3\text{M} + \text{HCOOH}$] $^-$; m/z (positive mode, %) = 435.3 (100) [$\text{M} + \text{H}$] $^+$.

HR-MS (ESI, positive mode): 435.1832 (found), 435.1838 (calculated for $\text{C}_{25}\text{H}_{28}\text{N}_2\text{O}_3\text{P}$ as [$\text{M} + \text{H}$] $^+$); 457.1655 (found), 457.1657 (calculated for $\text{C}_{25}\text{H}_{27}\text{N}_2\text{NaO}_3\text{P}$, [$\text{M} + \text{Na}$] $^+$).

Dyes P13 and P14



In a screw-cap test tube, dimethyl *N,N*-diisopropylphosphoramidite (Sigma-Aldrich; 100 mg, 0.52 mmol) was added to a suspension of **10** (55 mg; 0.13 mmol) in MeCN (1 mL) at rt under Ar. The reaction mixture was warmed up to 60 °C and stirred for 2.5 h at this temperature. After cooling down to 0 °C, DDQ (116 mg, 0.52 mmol) was added, and the reaction mixture was stirred for additional 10 min at 0 °C. After warming up to rt, the reaction mixture was diluted with MeCN (10 ml) and directly subjected to column chromatography on regular SiO_2 (100 g; MeCN \rightarrow MeCN/ H_2O 20:1 + 0.1 v/v% of TFA). Fractions containing the dye were evaporated to dryness, dissolved in water and extracted with CH_2Cl_2 (3 \times). Combined organic solutions were dried with Na_2SO_4 and evaporated to yield 40 mg of a dark blue solid. HPLC analysis (B/A = 50/50–100/0 in 25 min, column 4 \times 250 mm, 1.2 mL/min, detection at 254 nm) showed the presence of two colored substances: **P13** with t_R = 10.7 min (area 58%) and **P14** with t_R = 18.3 min (area 42%). This mixture was subjected to reverse-phase column chromatography (30 g of RP- SiO_2 , MeCN/ H_2O 2:1 + 0.1% v/v TFA \rightarrow MeCN + 0.1% TFA \rightarrow MeOH) to yield 8 mg (10%) of **P13** and 7 mg (12%) of **P14**.

Dye P13:

MS (ESI): m/z (positive mode, rel. int., %) = 548 (100) $[\text{M}]^+$.

HR-MS ($\text{C}_{32}\text{H}_{43}\text{N}_3\text{O}_3\text{P}^+$): m/z (positive mode) = 548.3046 (found M^+), 548.3037 (calc.).

Dye P14:

^1H NMR (400 MHz, CDCl_3): δ 1.94–2.02 (m, 4H, 2 \times CH_2), 2.03–2.11 (m, 4H, 2 \times CH_2), 2.84–2.90 (m, 4H, 2 \times CH_2), 2.91–2.98 (m, 4H, 2 \times CH_2), 3.43–3.52 (m, 8H, 4 \times CH_2), 3.59 (d, $J_{\text{H-P}}$ = 11.5 Hz, 3H, OMe), 8.95 (s, 2 H_{ar}) ppm.

^{31}P NMR (162 MHz, CD_3CN): δ 5.0 ppm.

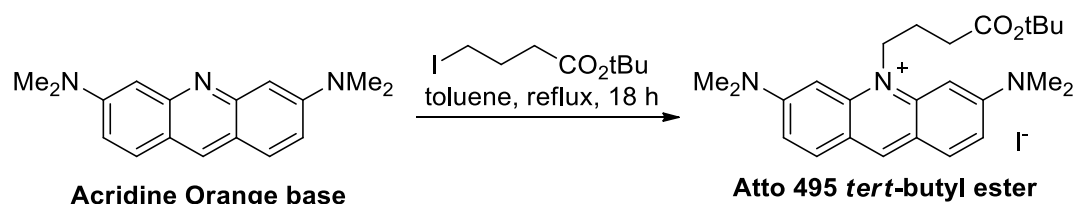
HR-MS ($\text{C}_{26}\text{H}_{29}\text{N}_2\text{O}_4\text{P}$): m/z (positive mode) = 465.1935 (found $[\text{M}+\text{H}]^+$), 465.1938 (calc.).

Acridine derivatives (A1, A2)

Dye A1

Atto 495 *tert*-butyl ester

10-(4-*tert*-butoxy-4-oxobutyl)-3,6-bis(dimethylamino)acridinium iodide



A suspension of Acridine Orange base (Sigma-Aldrich, 265 mg, 1.0 mmol) and *tert*-butyl 4-iodobutyrate^[8] in toluene (15 mL) was refluxed for 18 h. The reaction mixture was evaporated to dryness, and the residue was subjected to column chromatography (40 g of SiO₂, gradient 5% to 10% EtOH/CH₂Cl₂), eluting the fluorescent band. The eluate was evaporated and the product was isolated by reversed-phase column chromatography (30 g RP-C₁₈, gradient 50% to 20% H₂O/MeCN + 1 v/v% TFA). The fractions containing the product were pooled, and the residue was lyophilized from H₂O/MeCN (2:1). Orange solid, yield 165 mg (31%).

¹H NMR (400 MHz, CD₃OD) : δ 8.46 (d, J = 3.3 Hz, 1H), 7.76 (dd, J = 9.3, 2.3 Hz, 2H), 7.15 (dt, J = 9.3, 2.2 Hz, 2H), 6.79 (br.s, 2H), 4.59 – 4.51 (m, 2H), 3.29 (s, 12H), 2.64 (dd, J = 6.9, 4.9 Hz, 2H), 2.05 (dq, J = 11.8, 6.4 Hz, 2H), 1.49 (s, 9H) ppm.

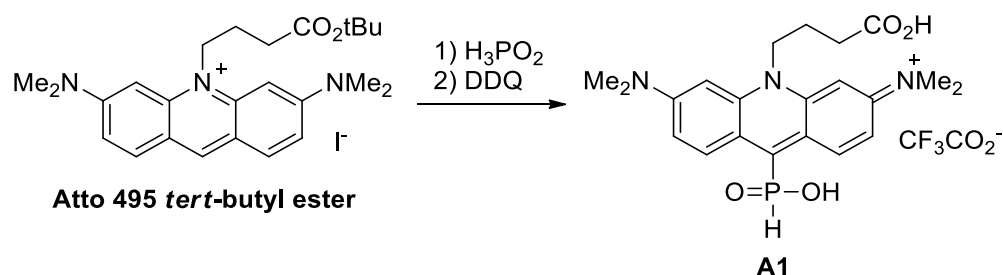
¹³C NMR (101 MHz, CD₃OD): δ 174.4, 157.5, 144.1, 144.0, 134.2, 118.3, 115.4, 93.7, 82.2, 48.1, 41.0, 31.9, 28.5, 21.4 ppm.

MS (ESI): m/z (positive mode, rel. int., %) = 408.3 (100) [M]⁺.

HRMS (C₂₅H₃₄N₃O₂): m/z (positive mode) = 408.2643 (found [M]⁺), 408.2646 (calc.).

Dye A1

10-(3-carboxypropyl)-3,6-bis(dimethylamino)-9-(hydroxyhydrophosphoryl)acridin-10-ium trifluoroacetate



A suspension of **Atto 495 tert-butyl ester** (165 mg, 0.31 mmol) in 50% aq. H_3PO_2 (2 mL) was stirred at 100 °C for 4 days. The resulting solution was cooled down to rt and transferred directly on top of a reversed-phase column (15 g RP-C₁₈). The excess of H_3PO_2 was removed first by elution with water; then 50% to 30% $\text{H}_2\text{O}/\text{MeCN}$ gradient was applied. Fractions containing the leuco dye were pooled, MeCN was evaporated and the residue was lyophilized, giving 100 mg of the leuco dye as a red solid. MS (ESI): m/z (negative mode, rel. int., %) = 416.2 (100) $[\text{M}-\text{H}]^-$.

The leuco acid was dissolved in a mixture of CH_2Cl_2 (3 mL) and MeOH (3 mL), the solution was cooled in dry ice-acetone bath, and DDQ (54 mg; 0.24 mmol) in CH_2Cl_2 (4 mL) was added quickly dropwise. The resulting bright-pink suspension was allowed to warm up to rt and stirred for 15 min. The mixture was evaporated to dryness, and the residue was subjected to column chromatography (30 g of SiO_2 , gradient 20% to 50% MeCN in water, then 50% $\text{H}_2\text{O}/\text{MeCN}$ + 1 v/v% TFA); the fractions containing the product were pooled and evaporated. Further purification was done by reversed-phase column chromatography (15 g RP-C₁₈, gradient 10% to 40% MeCN in 5 v/v% 0.1 M $\text{Et}_3\text{NH}^+ \text{HCO}_3^-$ in H_2O). Pure fractions containing the product were evaporated to dryness, the residue was dissolved in acetic acid (~50 mL), centrifuged, the supernatant was filtered through 0.2 μm PTFE membrane filter and freeze-dried. The impure fractions were re-chromatographed and treated again as described, giving the combined yield of 100 mg (61% over 2 steps) as a red solid.

^1H NMR (400 MHz, acetic acid- d_4): δ 9.02 (d, J = 9.8 Hz, 2H), 8.57 (d, J = 568 Hz, 1H), 7.23 (dd, J = 9.5, 1.9 Hz, 2H), 6.73 (s, 2H), 4.72 – 4.54 (m, 2H), 3.30 (s, 12H), 2.80 (t, J = 6.1 Hz, 2H), 2.27 (td, J = 11.9, 5.6 Hz, 1H).

^{13}C NMR not available due to low solubility of the compound.

^{31}P NMR (162 MHz, acetic acid- d_4): δ 5.8.

^{19}F NMR (376 MHz, acetic acid- d_4): δ -76.7.

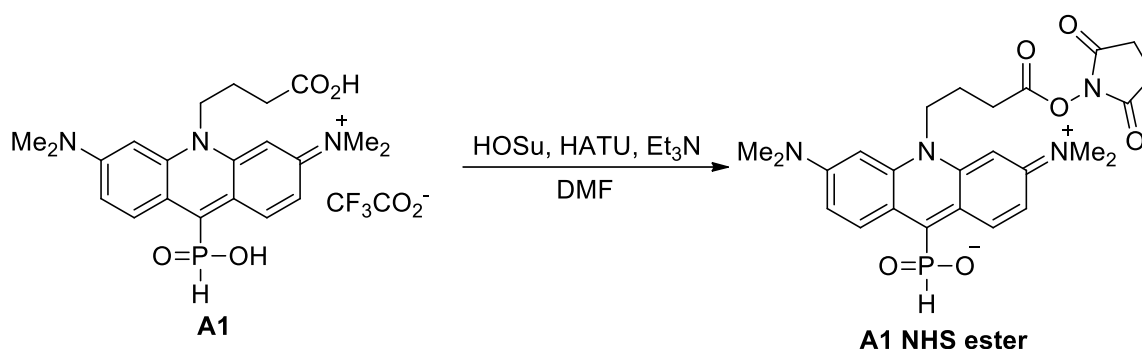
MS (ESI): m/z (positive mode, rel. int., %) = 416.2 (100) $[\text{M}]^+$, 438.2 (48) $[\text{M}-\text{H}+\text{Na}]^+$, 454.1 (34) $[\text{M}-\text{H}+\text{K}]^+$.

HRMS ($\text{C}_{21}\text{H}_{27}\text{N}_3\text{O}_4\text{P}$): m/z (positive mode) = 416.1732 (found $[\text{M}]^+$), 416.1734 (calc.).

Dye A1 NHS ester

[10-(3-carboxypropyl)-3,6-bis(dimethylamino)acridinium-9-yl]phosphinate

N-hydroxysuccinimide ester

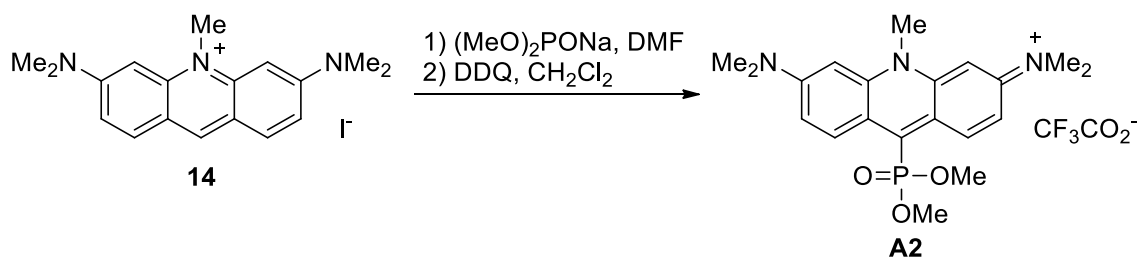


Triethylamine (20 μ L, \sim 140 μ mol), *N*-hydroxysuccinimide (50 μ L of a 1.13 M stock solution in DMF, 57 μ mol) and HATU (50 μ L of a 0.76 M stock solution in DMF, 38 μ mol) were added to a suspension of **A1** (2.0 mg, 3.8 μ mol) in DMF (0.1 mL). A clear bright-pink solution was formed and stirred at rt for 1 h. The solvent was evaporated to dryness at rt in vacuo, and the product was isolated from the residue by column chromatography (15 g of SiO₂, gradient 10% to 25% H₂O/MeCN in 5% increments). The fractions containing the product were pooled, evaporated at rt; the residue was dissolved in dioxane (with minimal amount of water added to dissolve the solids), and centrifuged off the silica dust. The supernatant was filtered through 0.2 μ m PTFE membrane filter and lyophilized. Yield 1.2 mg (62%), HPLC area 87%; red solid. HPLC: t_R = 9.0 min (87%), B/A = 30/70–100/0 in 25 min, column 4 \times 250 mm, flow 1.2 mL/min, detection at 254 nm.

MS (ESI): m/z (positive mode, rel. int., %) = 513.2 (100) [M+H]⁺, 535.2 (51) [M+Na]⁺.

Dye A2

9-(dimethoxyphosphoryl)-3,6-bis(dimethylamino)-10-methylacridinium trifluoroacetate



To a stirred suspension of NaH (33 mg of 60 wt.% in mineral oil, 0.83 mmol) in dry DMF (1.0 mL), cooled in an ice-water bath, dimethyl phosphite (78 μL , 0.83 mmol) was added in one portion. The resulting suspension was warmed up to rt and stirred for 30 min, turning into a clear solution. A suspension of 3,6-bis(dimethylamino)-10-methylacridinium iodide^[9] **14** (Methylacridine Orange; 100 mg, 0.25 mmol) in DMF (1 mL) was added, and the resulting clear orange-brown solution was stirred at rt for 1 h and at 100 °C for 1 h. The reaction mixture was evaporated *in vacuo* to dryness (bath temperature 60 °C) and re-evaporated with acetone. The intermediate leuco dye was isolated by column chromatography (15 g of SiO_2 , gradient 0% to 5% MeOH in EtOAc) and used directly in the next step.

The material was dissolved in CH_2Cl_2 (3 mL), the solution was cooled in a bath with acetone and dry ice, and then a solution of DDQ (30 mg; 0.13 mmol) in CH_2Cl_2 (2 mL) was added quickly dropwise. The resulting bright red-purple mixture was allowed to warm up to rt and stirred for 15 min. The mixture was evaporated to dryness, and the residue was subjected to column chromatography (15 g of SiO_2 , gradient 0% to 5% $\text{H}_2\text{O}/\text{MeCN}$, then 5% to 10% $\text{H}_2\text{O}/\text{MeCN}$ + 0.5 v/v% TFA); the fractions containing the product were pooled, evaporated and re-purified by column chromatography (18 g of SiO_2 , 5% $\text{H}_2\text{O}/\text{MeCN}$ + 0.2 v/v% TFA). The residue after evaporation was dissolved in 1,4-dioxane (with addition of minimal amount of water to dissolve the solids), centrifuged, the supernatant was filtered through 0.2 μM PTFE membrane filter and lyophilized. Purple solid, yield 65 mg (52%).

^1H NMR (300 MHz, CD_3OD): δ 8.94 (d, J = 10.0 Hz, 2H), 7.33 (dd, J = 9.7, 2.1 Hz, 2H), 6.70 (t, J = 2.4 Hz, 2H), 4.17 (s, 3H), 3.93 (s, 3H), 3.89 (s, 3H), 3.34 (s, 12H).

^{13}C NMR (126 MHz, CD_3OD): δ 156.2 (d, J = 1.8 Hz), 145.0 (dd, J = 14.0, 2.9 Hz), 137.3 (d, J = 173 Hz), 132.4 (d, J = 4.1 Hz), 120.2 (dd, J = 11.2, 2.2 Hz), 116.5, 94.3, 68.1, 54.1 (d, J = 5.9 Hz), 40.7, 38.3.

^{31}P NMR (122 MHz, CD_3OD): δ 16.45.

^{19}F NMR (282 MHz, CD_3OD): δ -72.94.

MS (ESI): m/z (positive mode, rel. int., %) = 388.2 (100) $[\text{M}]^+$.

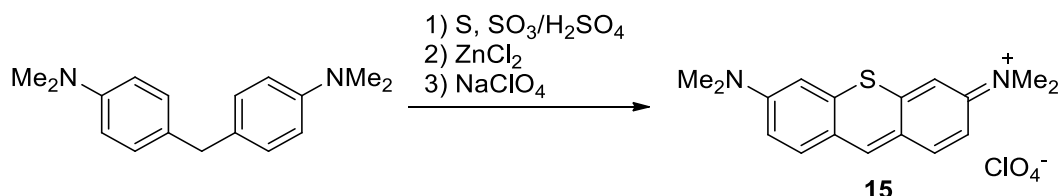
HRMS ($\text{C}_{20}\text{H}_{27}\text{N}_3\text{O}_3\text{P}$): m/z (positive mode) = 388.1785 (found $[\text{M}]^+$), 388.1785 (calc.).

Thiopyronin derivatives (SP1, SP2)

Dye SP1

Compound 15

3,6-bis(dimethylamino)-9*H*-thioxanthenylium perchlorate



Powdered sulfur (10 g, 0.31 mol) was added in portions over 15 min to 30% SO₃ - H₂SO₄ (25 mL), the brown-yellow suspension was cooled in an ice-water bath and 4,4'-bis(dimethylamino)diphenylmethane (9.5 g, 37 mmol) was added in portions at such a rate that the temperature of the reaction mixture remained below 20 °C (over ~10 min). The yellow suspension was stirred at rt for 1.5 h. The mixture was then poured on ice (~250 mL), the dark purple mixture was allowed to warm up to rt, transferred into a 500 mL round-bottom flask and refluxed for 1 h. The resulting suspension was cooled down to rt, filtered through a layer of Celite, a solution of ZnCl₂ (80 g in 150 mL water) was added and the mixture was left at 4 °C overnight. A dark red oil, containing the crystals of 3,6-bis(dimethylamino)thioxanthylium trichlorozincate,^[10] separated. The colorless supernatant was decanted off, the residue was dissolved in boiling water (150 mL) and NaClO₄ solution (5 g in 10 mL water) was added. The resulting suspension was allowed to cool down to rt and then left in ice-water bath to complete crystallization. The crystals of **15** were filtered off, washed with water, Et₂O/hexane (1:1) and Et₂O, dried *in vacuo*. Small brown crystals, yield 606 mg (4%).

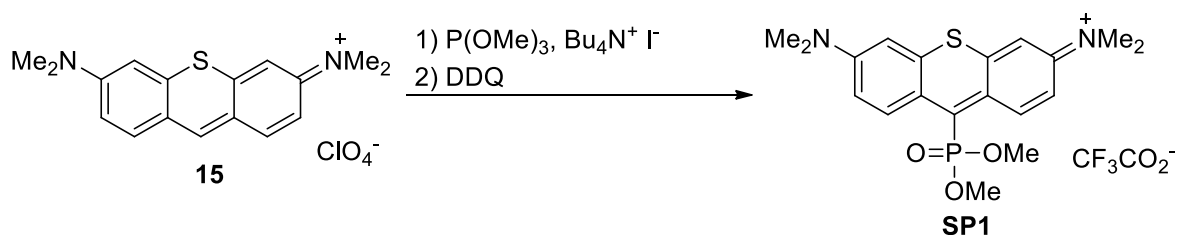
¹H NMR (400 MHz, CDCl₃ + DMSO-*d*₆ 1:5): δ 8.56 (s, 1H), 7.92 (d, *J* = 9.3 Hz, 2H), 7.26 (d, *J* = 2.4 Hz, 2H), 7.20 (dd, *J* = 9.3, 2.5 Hz, 2H), 3.25 (s, 12H).

¹³C NMR (101 MHz, CDCl₃ + DMSO-*d*₆ 1:5) δ 153.88, 148.72, 143.07, 137.39, 118.41, 115.43, 105.66, 40.33.

MS (ESI): *m/z* (positive mode, rel. int., %) = 283.1 (100) [M]⁺.

Dye SP1

9-(dimethoxyphosphoryl)-3,6-bis(dimethylamino)-9*H*-thioxanthen-9-ylum trifluoroacetate



To a solution of **15** (100 mg, 0.262 mmol) and tetrabutylammonium iodide (96.7 mg, 0.26 mmol) in dry CH_2Cl_2 (5 mL), trimethyl phosphite (93 μL , 97 mg, 0.79 mmol) was added at room temperature. The reaction mixture was stirred at rt for 3 h, turning light brown. TLC (100% EtOAc) displayed full conversion to the product with $R_f = 0.22$; greenish colored spot gradually appeared on a TLC plate (air, UV light). The reaction mixture was diluted with CH_2Cl_2 (8 mL) and evaporated on Celite. The leuco dye was isolated by flash chromatography (cartridge RediSep Rf 24 g of SiO_2 , gradient EtOAc/hexane, 20:80 \rightarrow 100:0) giving 102 mg (99%) of the leuco-dye as a colorless oil which gradually solidifies. The entire amount (102 mg, 0.26 mmol) was dissolved in CH_2Cl_2 (5 mL), the solution was cooled in a dry ice-acetone bath (-70°C), and a solution of DDQ (89 mg, 0.39 mmol) in CH_2Cl_2 (10 mL) was added quickly dropwise. Upon stirring for 15 min at -70°C , the solution became dark green. The reaction mixture was allowed to warm-up to room temperature and stirred for 15 min. The reaction mixture was diluted with CH_2Cl_2 (10 mL), evaporated to dryness on Celite and subjected to flash chromatography (Interchim PuriFlash, 25 g of 15 μm SiO_2 , gradient: MeCN/ H_2O + 0.1 v/v% of TFA, 100% of MeCN \rightarrow 90% of MeCN). The fractions containing the product were pooled and evaporated. The residue was dissolved in 1,4-dioxane, filtered through a 0.2 μm PTFE membrane filter and lyophilized. Yield 130 mg (99%) of **SP1** dye as a dark green TFA salt well soluble in MeCN. TLC on regular SiO_2 , MeCN/ H_2O 10:1 + 0.1 v/v% TFA in each component, $R_f = 0.23$. HPLC: $t_R = 12.2$ min (peak area 98%), MeCN/ H_2O + 0.1% v/v TFA in both components: 20/80 – 80/20% MeCN in 20 min, detection at 580 nm, column 4 \times 250 mm, flow rate 1.2 mL/min.

^1H NMR (400 MHz, CD_3CN): δ 9.02 (dd, $J = 15.3$ and 5.4 Hz, 2H), 7.3 – 7.1 (m, 4H), 3.86 (dt, $J = 11.6$ and 2.1 Hz, 6H, OMe), 3.28 (s, 12H, NMe_2) ppm.

^{13}C NMR (101 MHz, CD_3CN): δ 153.8 (s), 144.8 (d, $J = 20$ Hz), 136.9 (d, $J = 4.5$ Hz), 122.1 (d, $J = 11$ Hz), 116.9 (s), 106.8 (s), 54.2 (d, $J = 6$ Hz), 41.1 ppm.

^{19}F NMR (376 MHz, CD_3CN): δ -76.6 ppm.

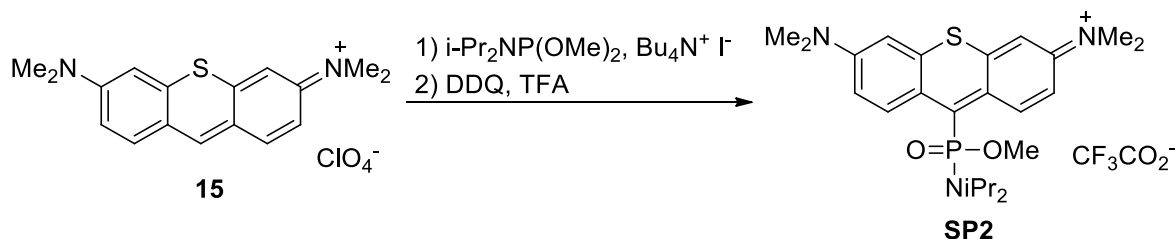
^{31}P NMR (162 MHz, CD_3CN): δ 16.53 ppm.

MS (ESI): m/z (positive mode, rel. int., %) = 391.1 (100) $[\text{M}]^+$.

HR-MS (ESI, positive mode): 391.1247 (found), 391.1240 (calculated for $C_{19}H_{24}N_2O_3PS^+$ as $[M]^+$).

Dye SP2

9-[(diisopropylamino)(methoxy)phosphoryl]-3,6-bis(dimethylamino)-9*H*-thioxanthen-9-ylum trifluoroacetate



Dimethyl *N,N*-diisopropylphosphoramidite (Sigma-Aldrich, 181 μL ; 0.786 mmol) was added to a stirred suspension of **15** (100 mg, 0.262 mmol) and tetrabutylammonium iodide (97 mg, 0.262 mmol) in dry CH_2Cl_2 (4 mL). The reaction mixture, which quickly turned into a light-brown clear solution, was stirred at rt for 30 min. After evaporation to dryness, the leuco dye was isolated by column chromatography (18 g SiO_2 , gradient 50% to 100% EtOAc/hexane) and used directly in the next step. The material was dissolved in CH_2Cl_2 (3 mL), the solution was cooled in a dry ice-acetone bath, and DDQ (59 mg, 0.26 mmol) in CH_2Cl_2 (3 mL) was added quickly dropwise. The resulting turquoise-blue solution was allowed to warm up to rt and stirred for 15 min. The mixture was evaporated to dryness, and the residue was subjected to column chromatography (20 g of SiO_2 , gradient 0% to 5% $\text{H}_2\text{O}/\text{MeCN}$, then 5% $\text{H}_2\text{O}/\text{MeCN}$ + 0.5 v/v% TFA); the fractions containing the product were pooled and evaporated. The residue was dissolved in 1,4-dioxane (with addition of minimal amount of water to dissolve solids), centrifuged, the supernatant was filtered through 0.2 μm PTFE membrane filter and lyophilized. Blue solid, yield 145 mg (97%).

^1H NMR (400 MHz, CD_3CN): δ 9.14 (d, J = 10.0 Hz, 2H), 7.21 (dd, J = 10.0, 2.8 Hz, 2H), 7.20 – 7.13 (m, 2H), 3.69 – 3.61 (m, 2H), 3.56 (d, J = 11.5 Hz, 3H), 3.27 (s, 12H), 1.32 (d, J = 5.3 Hz, 6H), 1.31 (d, J = 5.4 Hz, 6H).

^{13}C NMR (101 MHz, CD_3CN): δ 153.8, 137.1, 116.5, 106.7, 48.33, 48.27, 41.1, 40.3, 23.10, 23.07, 22.76, 22.74.

^{31}P NMR (122 MHz, CD_3OD): δ 23.32.

^{19}F NMR (282 MHz, CD_3OD): δ -76.11.

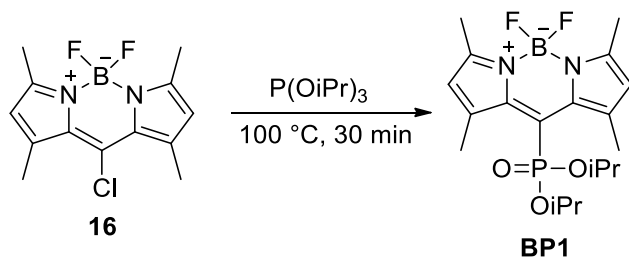
MS (ESI): m/z (positive mode, rel. int., %) = 460.2 (100) $[M]^+$.

HRMS ($\text{C}_{24}\text{H}_{35}\text{N}_3\text{O}_2\text{PS}$): m/z (positive mode) = 460.2184 (found $[M]^+$), 460.2182 (calc.).

BODIPY derivative BP1

Dye BP1

10-(diisopropoxyphosphoryl)-5,5-difluoro-1,3,7,9-tetramethyl-5*H*-dipyrrolo[1,2-*c*:2',1'-*f*][1,3,2]diazaborinin-4-ium-5-uide



A solution of 10-chloro-5,5-difluoro-1,3,7,9-tetramethyl-5*H*-dipyrrolo[1,2-*c*:2',1'-*f*][1,3,2]diazaborinin-4-ium-5-uide^[11] **16** (20 mg, 0.07 mmol) in triisopropyl phosphite (0.5 mL) was stirred under argon at 100 °C for 30 min. After cooling down to r.t., the violet reaction mixture was diluted with *n*-hexane (~5 mL) and subjected to column chromatography (30 g of SiO₂, hexane/EtOAc 1:1) to yield 28 mg (96%) of **BP1** as violet solid.

¹H NMR (400 MHz, CDCl₃): δ 1.26 (d, *J*_{H-H} = 6.2 Hz, 6H, *i*Pr), 1.39 (d, *J*_{H-H} = 6.2 Hz, 6H, *i*Pr), 2.45 (s, 6H, 2Me), 2.49 (s, 6H, 2Me), 4.84 (d.hept, 2H, *J*_{H-P} = 12.4 Hz, *J*_{H-H} = 6.2 Hz, 2×CHO), 6.08 (s, 2H_{ar}) ppm.

¹³C NMR (101 MHz, CDCl₃): δ 157.6, 144.5, 137.1 (d, *J* = 13.9 Hz), 129.6 (d, *J* = 182.3 Hz), 123.5 (br.d, *J* = 2.6 Hz), 72.8 (d, *J* = 6.7 Hz), 24.0 (d, *J* = 4.4 Hz), 23.6 (d, *J* = 5.0 Hz), 16.6, 15.2 (app.td, *J* = 3.2, 1.2 Hz).

³¹P NMR (162 MHz, CDCl₃): δ = 9.8 ppm.

¹⁹F NMR (376 MHz, CDCl₃): δ -146.6 (app.q 1:1:1:1, ¹*J*_{B-F} = 32.2 Hz).

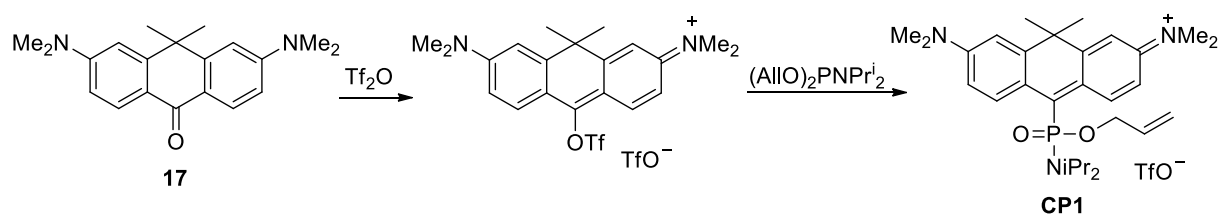
MS (ESI): *m/z* (positive mode, rel. int., %) = 413.2 (100) [M+H]⁺.

HR-MS (C₁₉H₂₈BF₂N₂O₃P): *m/z* (positive mode) = 413.1979 (found [M+H]⁺), 413.1975 (calc.).

Carbopyronin derivative CP1

Dye CP1

9-[(allyloxy)(diisopropylamino)phosphoryl]-3,6-bis(dimethylamino)-10,10-dimethyl-9,10-dihydroanthracenylium trifluoromethanesulfonate



In a Schlenk flask, to a solution of 3,6-bis(dimethylamino)-10,10-dimethylanthrone^[12] **17** (50 mg, 0.16 mmol) in CH_2Cl_2 (2 mL) triflic anhydride (46 mg, 0.16 mmol) was injected under argon. The blue colored reaction mixture stirred for 10 min at r.t., and then diallyl *N,N*-diisopropylphosphoramidite (40 mg, 0.16 mmol) was injected. After stirring overnight at r.t., the reaction mixture was diluted with MeCN (~5 mL) and subjected to column chromatography (30 g of SiO_2 , MeCN \rightarrow MeCN/ H_2O , 20:1 \rightarrow 10:1 \rightarrow 5:1 \rightarrow 2:1). The green-colored fractions were collected, combined and evaporated. After additional RP chromatography (20 g of RP- SiO_2 , MeCN/ H_2O 1:1 \rightarrow 5:1 + 0.1% TFA), 18 mg (21%) of **CP1** as a dark green solid were obtained; purity ~85% (NMR). HPLC: t_R = 15.7 min (peak area 96%), B/A = 30/70–100/0 in 25 min, column 4 \times 250 mm, 1.2 mL/min, detection at 635 nm.

^1H NMR (400 MHz, CDCl_3): δ 1.28 (d, $J_{\text{H-H}}$ = 6.7 Hz, 6H, diastereotopic $\text{CH}(\text{CH}_3)_2$), 1.33 (d, $J_{\text{H-H}}$ = 6.8 Hz, 6H, diastereotopic $\text{CH}(\text{CH}_3)_2$), 1.69 (s, 6H, 2 \times Me), 3.35 (s, 12H, 2 \times NMe₂), 3.47–3.58 (m, 2H, NPr₂), 4.27–4.37 (m, 1H, $\text{CH}^{\text{A}}\text{H}^{\text{B}}\text{O}$), 4.52–4.61 (m, 1H, $\text{CH}^{\text{A}}\text{H}^{\text{B}}\text{O}$), 5.09–5.14 (m, $J_{\text{H-H}}$ = 10.3 and 1.1 Hz, 1H, $\text{CH}_2=$), 5.19–5.26 (m, $J_{\text{H-H}}$ = 17.1 and 1.5 Hz, 1H, $\text{CH}_2=$), 5.75–5.86 (m, 1H, CH=), 6.75 (t, $J_{\text{H-H}}$ = 9.8 Hz, $J_{\text{H-P}}$ = 2.5 Hz, 2H_{ar}), 7.11 (t, $J_{\text{H-H}}$ = 2.5 Hz, $J_{\text{H-P}}$ = 2.5 Hz, 2H_{ar}), 8.84 (d, $J_{\text{H-H}}$ = 9.8 Hz, 2H_{ar}) ppm.

^{13}C NMR (100 MHz, CDCl_3 , APT): δ 19.1 (+), 22.6 (+, d, $J_{\text{C-P}}$ = 3.1 Hz), 22.8 (+, d, $J_{\text{C-P}}$ = 2.7 Hz), 41.2 (+), 47.7 (+, d, $J_{\text{C-P}}$ = 6.0 Hz), 67.5 (–, d, $J_{\text{C-P}}$ = 5.3 Hz), 111.2 (+), 112.6 (+), 119.1 (–), 124.1 (–, d, $J_{\text{C-P}}$ = 8.5 Hz), 129.7 (+), 132.5 (+, d, $J_{\text{C-P}}$ = 6.7 Hz), 138.6 (d, +, $J_{\text{C-P}}$ = 4.2 Hz), 155.7 (–), 156.9 (–, d, $J_{\text{C-P}}$ = 12.1 Hz) ppm.

^{31}P NMR (162 MHz, CDCl_3): δ 22.3 ppm.

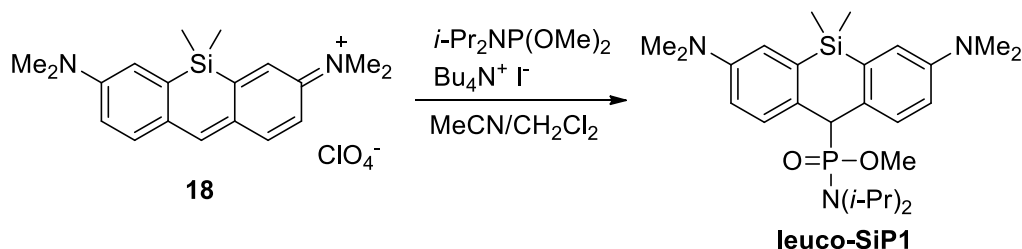
MS (ESI): m/z (positive mode, rel. int., %) = 496.4 (100) $[\text{M}]^+$.

Si-pyronin derivative SiP1

Dye SiP1

leuco-SiP1

methyl *P*-[3,7-bis(dimethylamino)-5,5-dimethyl-5,10-dihydrodibenzo[*b,e*]silin-10-yl]-*N,N*-diisopropylphosphoramidate



Dimethyl *N,N*-diisopropylphosphoramidite (84 μ L, 0.37 mmol) was added to a solution of 3,7-bis(dimethylamino)-5,5-dimethyl-5,10-dihydrodibenzo[*b,e*]silinium perchlorate^[13] **18** (50 mg, 0.12 mmol) and tetrabutylammonium iodide (45 mg, 0.12 mmol) in MeCN (2 mL) and DCM (2 mL), and the resulting mixture was stirred at rt for 20 min. The clear colorless solution was evaporated to dryness and the product was isolated by column chromatography (25 g of SiO₂, gradient 33% to 50% EtOAc in hexane). The fractions containing the product were pooled, evaporated and dried *in vacuo* to yield 55 mg (92%) of **leuco-SiP1** as a viscous colorless oil.

¹H NMR (400 MHz, CD₃CN): δ 7.18 (ddd, *J* = 8.6, 3.7, 2.5 Hz, 2H), 6.97 (dd, *J* = 16.1, 2.9 Hz, 2H), 6.73 (dddd, *J* = 11.4, 8.5, 3.0, 0.9 Hz, 2H), 4.49 (d, *J* = 24.2 Hz, 1H), 3.41 (dp, *J* = 17.6, 6.7 Hz, 2H), 3.16 (d, *J* = 10.6 Hz, 3H), 2.92 (s, 6H), 2.90 (s, 6H), 1.17 (d, *J* = 6.6 Hz, 6H), 0.93 (d, *J* = 6.7 Hz, 6H), 0.61 (s, 3H), 0.37 (s, 3H) ppm.

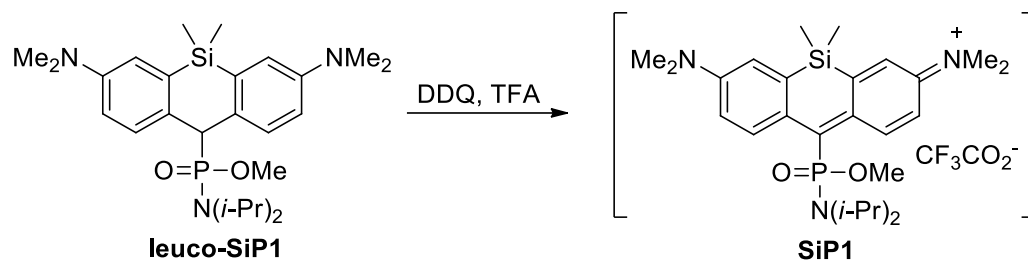
¹³C NMR (101 MHz, CD₃CN): δ 150.1 (d, *J* = 3.0 Hz), 149.8 (d, *J* = 2.9 Hz), 138.3 (d, *J* = 4.8 Hz), 137.8 (d, *J* = 4.6 Hz), 132.5 (d, *J* = 8.5 Hz), 132.1 (d, *J* = 5.4 Hz), 131.5 (d, *J* = 6.9 Hz), 118.7 (d, *J* = 3.4 Hz), 118.1 (d, *J* = 3.2 Hz), 114.3 (d, *J* = 3.0 Hz), 113.8 (d, *J* = 3.3 Hz), 52.1 (d, *J* = 120.9 Hz), 51.2 (d, *J* = 7.6 Hz), 46.7 (d, *J* = 3.6 Hz), 41.0 (d, *J* = 1.1 Hz), 24.2, 22.6 (d, *J* = 2.3 Hz), 0.2 (d, *J* = 1.0 Hz), -0.1 (d, *J* = 4.2 Hz) ppm.

³¹P NMR (162 MHz, CD₃CN): δ 31.20 ppm.

MS (ESI): *m/z* (positive mode, rel. int., %) = 488.3 (100) [M+H]⁺.

HRMS (C₂₆H₄₂N₃O₂PSi): *m/z* (positive mode) = 488.2860 (found [M+H]⁺), 488.2857 (calc.).

Dye SiP1



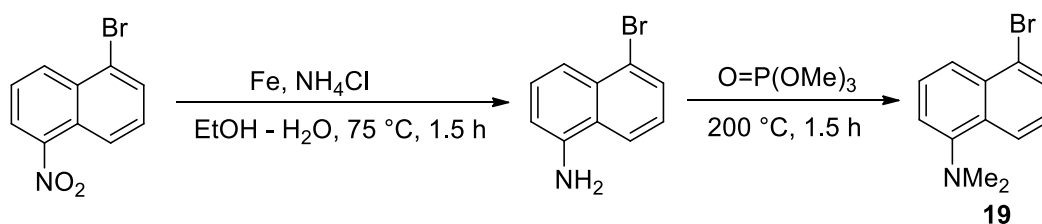
Oxidation of **leuco-SiP1** (DDQ in CH_2Cl_2) as follows led to formation of an unstable silaxanthylum dye **SiP1** which easily hydrolyzes to the starting **18**. 22 mg (0.045 mmol) of the leuco dye was dissolved in CH_2Cl_2 (2 mL), and the solution was cooled to -78°C . A solution of DDQ (10 mg, 0.044 mmol) in CH_2Cl_2 (1 mL) was then added. The reaction mixture was allowed to warm up to rt and subjected to column chromatography (30 g of SiO_2 , $\text{MeCN} \rightarrow \text{MeCN} + 0.1\% \text{ TFA} \rightarrow \text{MeCN} / \text{H}_2\text{O} 40:1 + 0.1\% \text{ of TFA}$), the green-colored fractions were pooled and evaporated to provide the hydrolytically unstable material used for recording the absorption and fluorescence emission spectra.

Benzanthriliium derivative BA1

Dye BA1

Compound 19

1-bromo-5-(dimethylamino)naphthalene



To a suspension of 1-bromo-5-nitronaphthalene (Apollo Scientific; 2.0 g, 7.9 mmol) in ethanol (50 mL), a solution of NH₄Cl (2.2 g, 41 mmol) in water (20 mL) was added, followed by iron powder (1.33 g, 23.8 mmol). The resulting mixture was stirred for 1.5 h at 75 °C (bath temperature). Celite (3 g) was added, and the mixture was allowed to cool down to rt, diluted with DCM (100 mL), filtered through a plug of Celite, washing with DCM (150 mL). The filtrate was washed with brine and dried over Na₂SO₄. Upon evaporation of the filtrate, the crude material was redissolved in DCM (20 mL), applied onto a column with 80 g SiO₂, and ran with 20% to 80% EtOAc/hexane gradient. The fractions containing the product were evaporated to viscous light brown oil that quickly crystallized. Yield of 5-bromo-1-aminonaphthalene^[14] 1.48 g (84%).

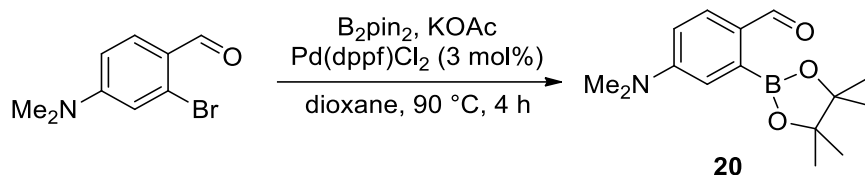
5-Bromo-1-aminonaphthalene (1.37 g, 6.17 mmol) was dissolved in trimethyl phosphate (760 µL, 6.5 mmol) in a 50 mL round-bottom flask, equipped with an air condenser and a CaCl₂ drying tube, the apparatus was flushed with nitrogen, and the mixture was heated at 200 °C (bath temperature) for 1.5 h. The flask was then allowed to cool below 100 °C, 1 M aq. NaOH (20 mL) was added, the resulting suspension was sonicated briefly and stirred at rt overnight. The mixture was diluted with brine, extracted with DCM (3×50 mL), the combined extracts were dried over Na₂SO₄. The product was isolated by column chromatography (100 g of SiO₂, gradient 10% to 50% CH₂Cl₂ in hexane) to yield 1-bromo-5-(dimethylamino)naphthalene^[14] **19** as a light-orange viscous oil (1.29 g, 84%).

¹H NMR (301 MHz, CDCl₃): δ 8.26 (dt, *J* = 8.6, 1.0 Hz, 1H), 7.95 (dt, *J* = 8.6, 0.9 Hz, 1H), 7.78 (dt, *J* = 7.4, 1.0 Hz, 1H), 7.51 (ddd, *J* = 8.5, 7.5, 0.7 Hz, 1H), 7.32 (ddd, *J* = 8.4, 7.3, 0.7 Hz, 1H), 7.14 (dd, *J* = 7.6, 1.0 Hz, 1H), 2.90 (s, 6H) ppm.

¹³C NMR (76 MHz, CDCl₃): δ 151.3, 133.4, 130.4, 130.1, 127.3, 125.4, 124.3, 123.3, 122.0, 115.0, 45.5.

Compound 20

4-(dimethylamino)-2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzaldehyde



1,4-Dioxane (25 mL) was added to the solid 2-bromo-4-(dimethylamino)benzaldehyde^[15] (684 mg, 3.0 mmol), bis(pinacolato)diboron (840 mg, 3.3 mmol), KOAc (880 mg, 9.0 mmol) and $Pd(dppf)Cl_2$ (66 mg, 0.09 mmol), the mixture was deoxygenated on a Schlenk line and stirred under N_2 at 90 °C (bath temperature) for 4 h. Upon cooling to rt, the reaction mixture was filtered through a 1.5 cm pad of Celite washing with EtOAc (100 mL). The filtrate was evaporated; the residue was dissolved in DCM and applied onto a column with 30 g SiO_2 . Elution with 10% to 50% EtOAc in hexane followed by recrystallized from DCM – hexane (with cooling in -78 °C bath) afforded 536 mg (65%) of the compound **13** as light-orange crystals.

1H NMR (500 MHz, $CDCl_3$): δ 10.20 (s, 1H), 7.84 (d, J = 8.8 Hz, 1H), 7.01 (d, J = 2.7 Hz, 1H), 6.77 (dd, J = 8.8, 2.7 Hz, 1H), 3.08 (s, 6H), 1.39 (s, 12H).

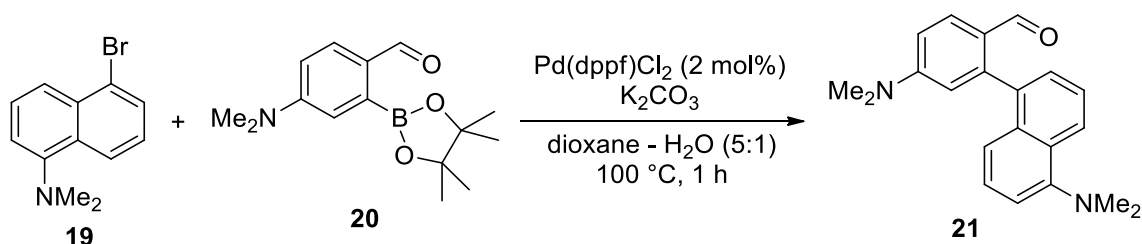
^{13}C NMR (126 MHz, $CDCl_3$): δ 192.22, 192.20, 152.9, 130.8, 130.1, 117.3, 112.9, 84.3, 40.4, 25.1.

MS (ESI): m/z (positive mode, rel. int., %) = 244.1 (100), 298.2 (3) $[M+Na]^+$.

HR-MS ($C_{15}H_{22}NO_3B$): m/z (positive mode) = 298.1578 (found $[M+Na]^+$), 298.1588 (calc.).

Compound 21

4-(dimethylamino)-2-[5-(dimethylamino)naphthalen-1-yl]benzaldehyde



1,4-Dioxane (15 mL) and water (3 mL) were added to a mixture of **19** (414 mg, 1.65 mmol), **20** (500 mg, 1.82 mmol), K_2CO_3 (455 mg, 3.3 mmol) and $Pd(dppf)Cl_2$ (24 mg, 0.033 mmol). The mixture was deoxygenated on a Schlenk line and stirred under N_2 at 100 °C (bath temperature) for 1 h. Upon cooling down to rt, the mixture was diluted with sat. aq. $NaHCO_3$ (50 mL), extracted with EtOAc (3×40 mL), washed with brine and dried over Na_2SO_4 . The product was isolated by column chromatography (40 g of SiO_2 , gradient 10% to 30%

EtOAc/hexane) and dried *in vacuo* to yield the title compound (524 mg, 99%) as yellowish foam.

^1H NMR (400 MHz, CDCl_3): δ 9.39 (s, 1H), 8.38 (d, J = 8.5 Hz, 1H), 8.06 (d, J = 8.9 Hz, 1H), 7.56 (dd, J = 8.6, 6.9 Hz, 1H), 7.45 (d, J = 6.9 Hz, 1H), 7.37 – 7.25 (m, 2H), 7.11 (dd, J = 6.7, 1.8 Hz, 1H), 6.83 (dd, J = 8.9, 2.7 Hz, 1H), 6.61 (d, J = 2.7 Hz, 1H), 3.09 (s, 6H), 2.96 (s, 6H).

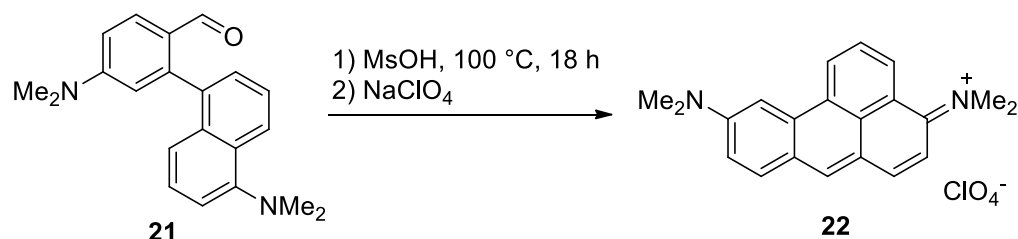
^{13}C NMR (101 MHz, CDCl_3): δ 190.4, 153.5, 151.0, 147.2, 137.2, 134.2, 129.0, 128.8, 127.5, 126.3, 124.4, 124.2, 124.1, 121.2, 114.2, 113.1, 111.0, 45.4, 40.1.

MS (ESI): m/z (positive mode, rel. int., %) = 319.2 (100) $[\text{M}+\text{H}]^+$, 341.2 (29) $[\text{M}+\text{Na}]^+$.

HR-MS ($\text{C}_{21}\text{H}_{22}\text{N}_2\text{O}$): m/z (positive mode) = 319.1810 (found $[\text{M}+\text{H}]^+$), 319.1805 (calc.).

Compound 22

4,10-bis(dimethylamino)-7H-benzo[de]anthracenylum perchlorate



A solution of **21** (440 mg, 1.38 mmol) in methanesulfonic acid (1 mL) was heated at 100 °C (bath temperature) overnight. The viscous mixture was diluted with methanesulfonic acid (2 mL) and poured into 150 mL of ice-water mixture, containing 5 g NaClO_4 . The resulting blue suspension was stirred until all ice melted; the dark solid was filtered off, washed with water and dried on filter. The crude solid was recrystallized from MeOH/DCM, adding hexane to complete precipitation, filtered off, washed with hexane and dried *in vacuo*. Small black crystals; yield 520 mg (94%).

^1H NMR (400 MHz, $\text{DMSO}-d_6$): δ 9.28 (d, J = 8.0 Hz, 1H), 8.68 (d, J = 8.0 Hz, 1H), 8.42 (s, 1H), 8.11 (d, J = 9.5 Hz, 1H), 7.94 (t, J = 8.1 Hz, 1H), 7.92 (d, J = 9.1 Hz, 1H), 7.81 (d, J = 2.4 Hz, 1H), 7.38 (d, J = 9.5 Hz, 1H), 7.26 (dd, J = 9.1, 2.4 Hz, 1H), 3.73 (s, 6H), 3.27 (s, 6H).

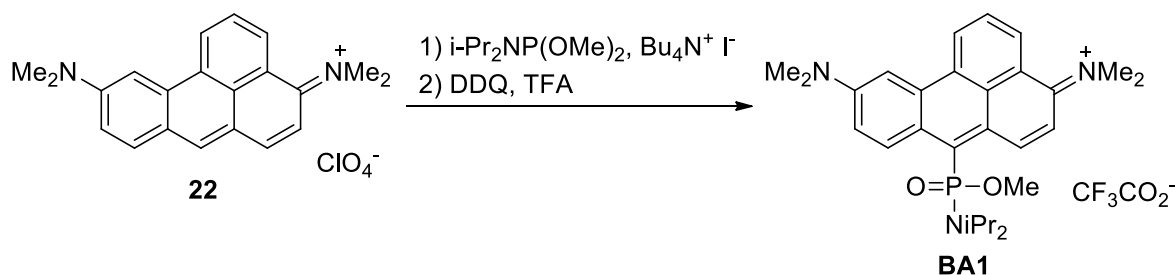
^{13}C NMR (101 MHz, $\text{DMSO}-d_6$): δ 163.2, 153.0, 142.9, 141.6, 135.8, 133.7, 133.2, 131.2, 128.3, 126.2, 125.9, 122.3, 121.9, 120.4, 116.2, 115.5, 103.3, 46.3, 40.2.

MS (ESI): m/z (positive mode, rel. int., %) = 288.2 (100) $[\text{M}-\text{CH}_3]^+$, 301.2 (1) $[\text{M}]^+$.

HRMS ($\text{C}_{21}\text{H}_{21}\text{N}_2$): m/z (positive mode) = 301.1690 (found $[\text{M}]^+$), 301.1699 (calc.).

Dye BA1

7-[(diisopropylamino)(methoxy)phosphoryl]-4,10-bis(dimethylamino)-7*H*-benzo[*de*]anthracen-7-ylum trifluoroacetate



Dimethyl *N,N*-diisopropylphosphoramidite (170 μL , 0.75 mmol) was added to a stirred solution of **22** (100 mg, 0.25 mmol) and tetrabutylammonium iodide (92 mg; 0.25 mmol) in dry DCM (5 mL). The vial was flushed with argon and the blue suspension was stirred at rt for 30 min, turning into a clear brown solution. The mixture was evaporated to dryness, and the intermediate 7*H*-benz[*de*]anthracene adduct (**leuco-BA1**) was isolated by column chromatography (25 g of SiO_2 , gradient 50% to 100% EtOAc in hexane). The compound was used immediately in the next step.

The material was dissolved in DCM (10 mL), the solution was cooled in dry ice-acetone bath, and DDQ (57 mg, 0.11 mmol) in DCM (3 mL) was added quickly dropwise. The resulting blue-green solution was allowed to warm up to rt, stirred for 15 min, and trifluoroacetic acid (100 μL) was added. The mixture was evaporated to dryness, and the residue was subjected to column chromatography (35 g of SiO_2 , gradient 0% to 50% H_2O in MeCN); the fractions containing the product were pooled, trifluoroacetic acid (200 μL) was added, MeCN was evaporated (bath temperature $\leq 25^\circ\text{C}$) and the aqueous solution was freeze-dried. The residue was dissolved in 1,4-dioxane (with addition of minimal amount of water to dissolve the solids), filtered through 0.2 μm PTFE membrane filter and lyophilized. Blue solid, yield 88 mg (61%).

^1H NMR (400 MHz, CD_3OD): δ 9.49 (d, $J = 10.3$ Hz, 1H), 9.35 (d, $J = 8.2$ Hz, 1H), 9.19 (d, $J = 9.8$ Hz, 1H), 8.73 (d, $J = 7.9$ Hz, 1H), 8.06 (t, $J = 8.0$ Hz, 1H), 8.01 (t, $J = 2.0$ Hz, 1H), 7.58 (d, $J = 10.3$ Hz, 1H), 7.41 (dd, $J = 9.8, 2.7$ Hz, 1H), 4.99 (d, $J = 1.7$ Hz, 6H), 3.85 (s, 6H), 3.68 (d, $J = 11.5$ Hz, 2H), 3.34 (s, 3H), 1.35 (dd, $J = 6.8, 3.2$ Hz, 12H) ppm.

^{13}C NMR (101 MHz, CD_3OD): δ 165.9, 153.6, 143.1 (d, $J = 4.9$ Hz), 139.6, 138.1, 137.8 (d, $J = 11.5$ Hz), 135.3, 133.4 (d, $J = 4.2$ Hz), 132.5, 131.4 (d, $J = 2.4$ Hz), 128.3, 127.9 (d, $J = 13.7$ Hz), 126.3 (d, $J = 9.4$ Hz), 125.3 (d, $J = 9.1$ Hz), 124.0 (d, $J = 1.2$ Hz), 104.4 (d, $J = 1.2$ Hz), 52.9 (d, $J = 5.9$ Hz), 48.7 (d, $J = 5.8$ Hz), 46.7, 40.5, 23.2 (d, $J = 2.8$ Hz), 22.8 (d, $J = 2.8$ Hz) ppm.

^{19}F NMR (376 MHz, CD_3OD): δ -77.3 ppm.

^{31}P NMR (162 MHz, CD_3OD): δ 26.0 ppm.

MS (ESI): m/z (positive mode, rel. int., %) = 478.3 (100) $[\text{M}]^+$.

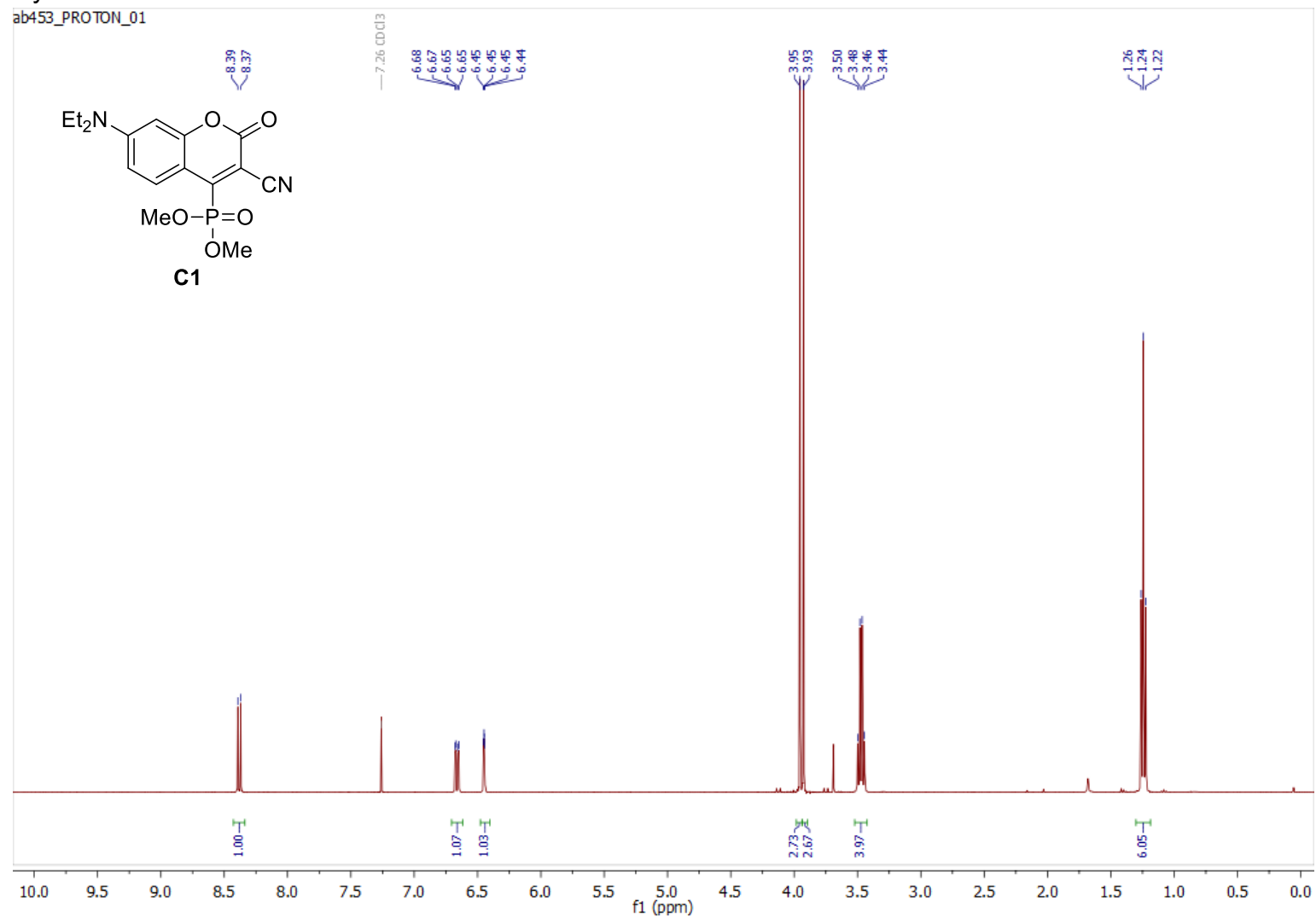
HRMS ($\text{C}_{28}\text{H}_{37}\text{N}_3\text{O}_2\text{P}$): m/z (positive mode) = 478.2621 (found $[\text{M}]^+$), 478.2618 (calc.).

Supplementary references

- [1] Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Scalmani, G.; Barone, V.; Petersson, G. A.; Nakatsuji, H.; Li, X.; Caricato, M.; Marenich, A. V.; Bloino, J.; Janesko, B. G.; Gomperts, R.; Mennucci, B.; Hratchian, H. P.; Ortiz, J. V.; Izmaylov, A. F.; Sonnenberg, J. L.; Williams; Ding, F.; Lipparini, F.; Egidi, F.; Goings, J.; Peng, B.; Petrone, A.; Henderson, T.; Ranasinghe, D.; Zakrzewski, V. G.; Gao, J.; Rega, N.; Zheng, G.; Liang, W.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Vreven, T.; Throssell, K.; Montgomery Jr., J. A.; Peralta, J. E.; Ogliaro, F.; Bearpark, M. J.; Heyd, J. J.; Brothers, E. N.; Kudin, K. N.; Staroverov, V. N.; Keith, T. A.; Kobayashi, R.; Normand, J.; Raghavachari, K.; Rendell, A. P.; Burant, J. C.; Iyengar, S. S.; Tomasi, J.; Cossi, M.; Millam, J. M.; Klene, M.; Adamo, C.; Cammi, R.; Ochterski, J. W.; Martin, R. L.; Morokuma, K.; Farkas, O.; Foresman, J. B.; Fox, D. J. Wallingford, CT, 2016.
- [2] Menta, E.; Da Re, G.; Grugni, M. Patent WO 2003105842 A1, 24 Dec 2003.
- [3] Bennett, S. N. L.; Bonn, P. G.; Brink, M. D.; Butlin, R. J.; Campbell, L.; Davies, R. D. M.; Faegerhag, J. R.; Jurva, U.; Pointon, H. C.; Robb, G. R.; Schnecke, V.; Svensson Henriksson, A. M.; Walker, R. P.; Waring, M. J.; Christer, R. Patent WO 2010015849 A2, 11 Feb 2010.
- [4] Singh, V.; Wang, S.; Kool, E. T. *J. Am. Chem. Soc.* **2013**, *135*, 6184-6191.
- [5] Gast, R.; Kaukorat, T.; Neda, I.; Schmutzler, R. *Z. Naturforsch.* **1993**, *48b*, 867-874.
- [6] Niu, G.; Liu, W.; Zhou, B.; Xiao, H.; Zhang, H.; Wu, J.; Ge, J.; Wang, P. *J. Org. Chem.* **2016**, *81*, 7393-7399.
- [7] Egorov, M.; Fortun, Y.; Heymann, D.; Lebreton, J.; Methe, M.; Padrines, M.; Redini, F. Patent WO 2009083614 A1, 9 Jul 2009.
- [8] Hardouin, C.; Kelso, M. J.; Romero, F. A.; Rayl, T. J.; Leung, D.; Hwang, I.; Cravatt, B. F.; Boger, D. L. *J. Med. Chem.* **2007**, *50*, 3359-3368.
- [9] Rodriguez, M. E.; Azizuddin, K.; Zhang, P.; Chiu, S.; Lam, M.; Kenney, M. E.; Burda, C.; Oleinick, N. L. *Mitochondrion* **2008**, *8*, 237-246.
- [10] a) Clunas, S.; Storry, J. M. D.; Rickard, J. E.; Horsley, D.; Harrington, C. R.; Wischik, C. M. Patent WO 2010067078 A2, 17 Jun 2010; b) Nealey, R. H.; Driscoll, J. S. *Heterocyclic Chemistry*, **1966**, *3*, 228-229.
- [11] Leen, V.; Yuan, P.; Wang, L.; Boens, N.; Dehaen, W. *Org. Lett.* **2012**, *14*(24), 6150-6153.
- [12] Pastierik, T.; Sebej, P.; Medalova, J.; Stacko, P.; Klan, P. *J. Org. Chem.* **2014**, *79*, 3374-3382.
- [13] Koide, Y.; Urano, Y.; Hanaoka, K.; Terai, T.; Nagano, T. *ACS Chem. Biol.* **2011**, *6*, 600-608.
- [14] West, R. W. *J. Chem. Soc.* **1925**, *127*, 494-495.
- [15] Meyer, W. E.; Tomcufcik, A. S.; Chan, P. S.; Emma, J. E. *J. Med. Chem.* **1984**, *27*, 1705-1710.

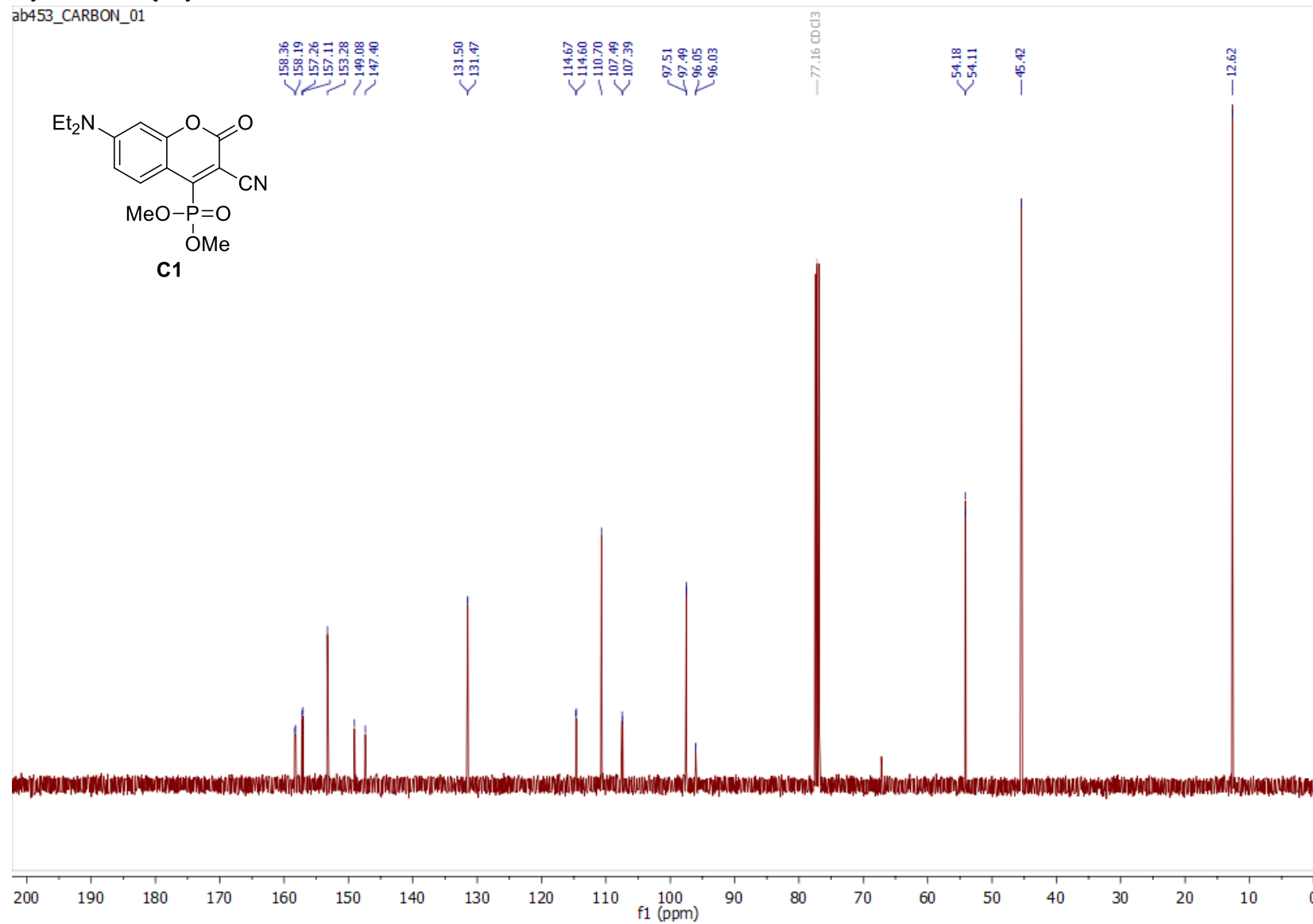
NMR spectra

Dye C1: ^1H



Dye **C1**: $^{13}\text{C}\{^1\text{H}\}$

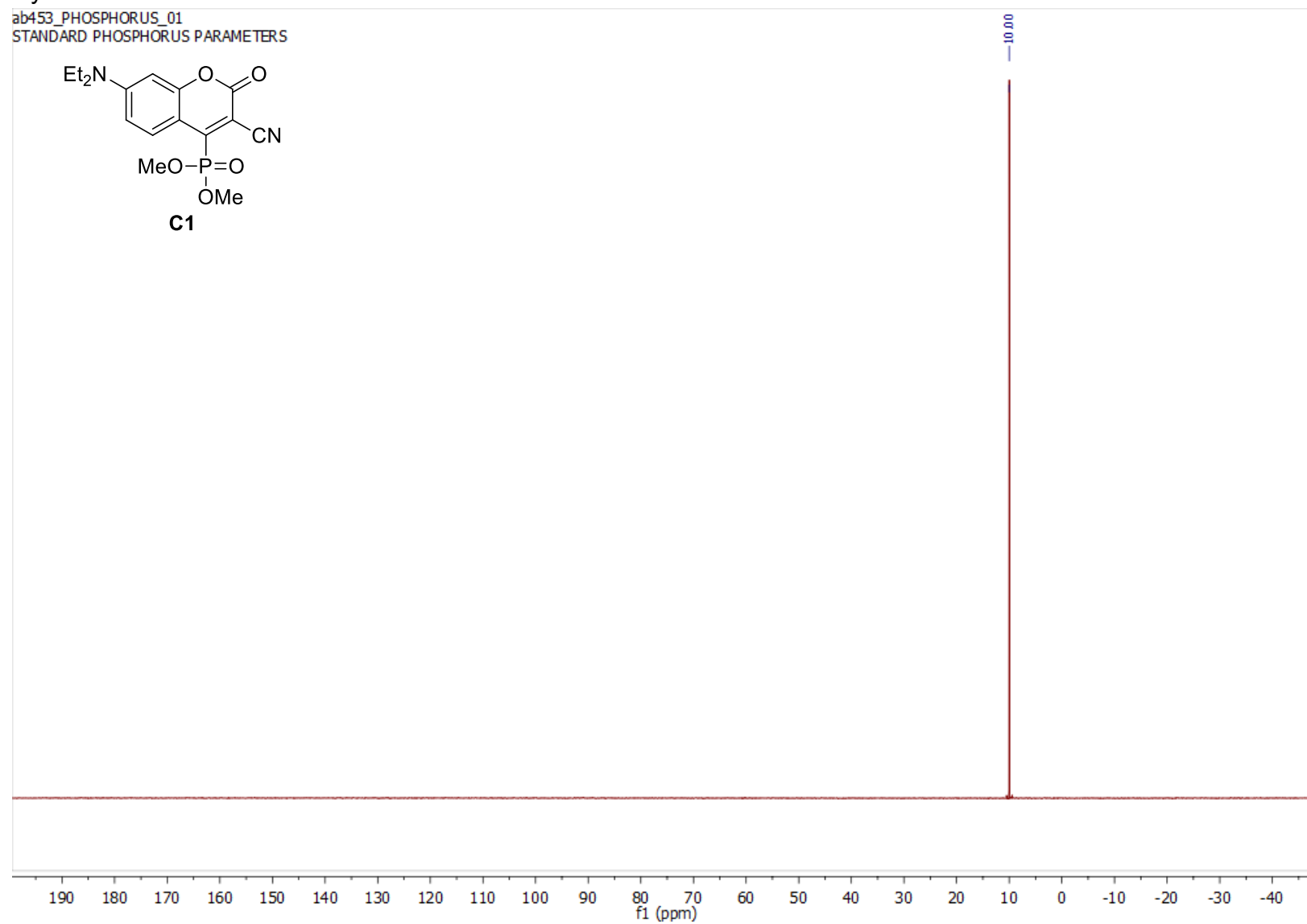
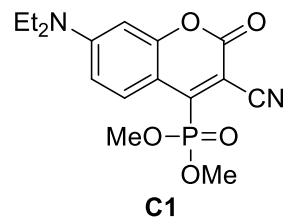
ab453_CARBON_01



Dye **C1**: ^{31}P

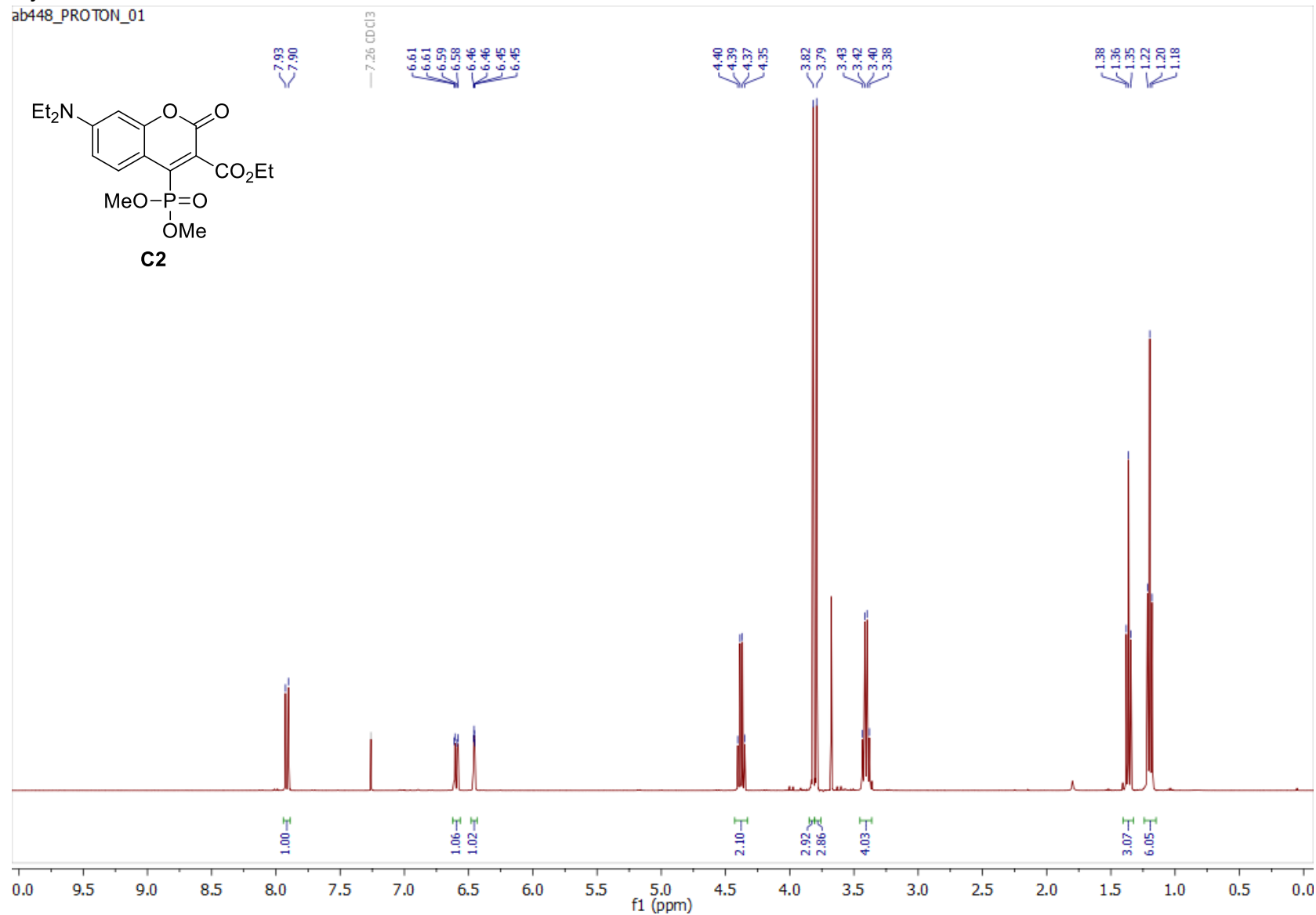
ab453_PHOSPHORUS_01

STANDARD PHOSPHORUS PARAMETERS



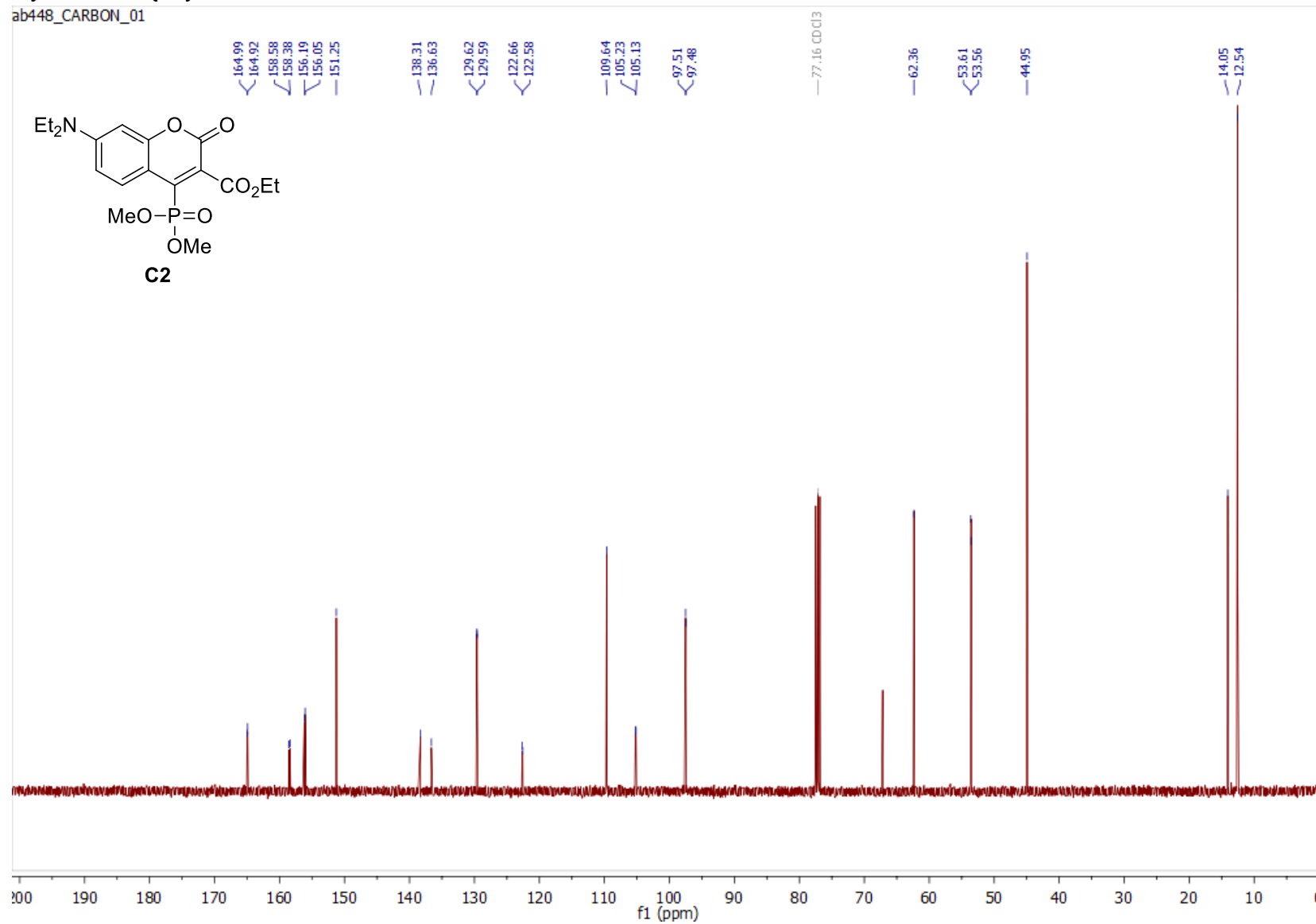
Dye C2: ^1H

ab448_PROTON_01



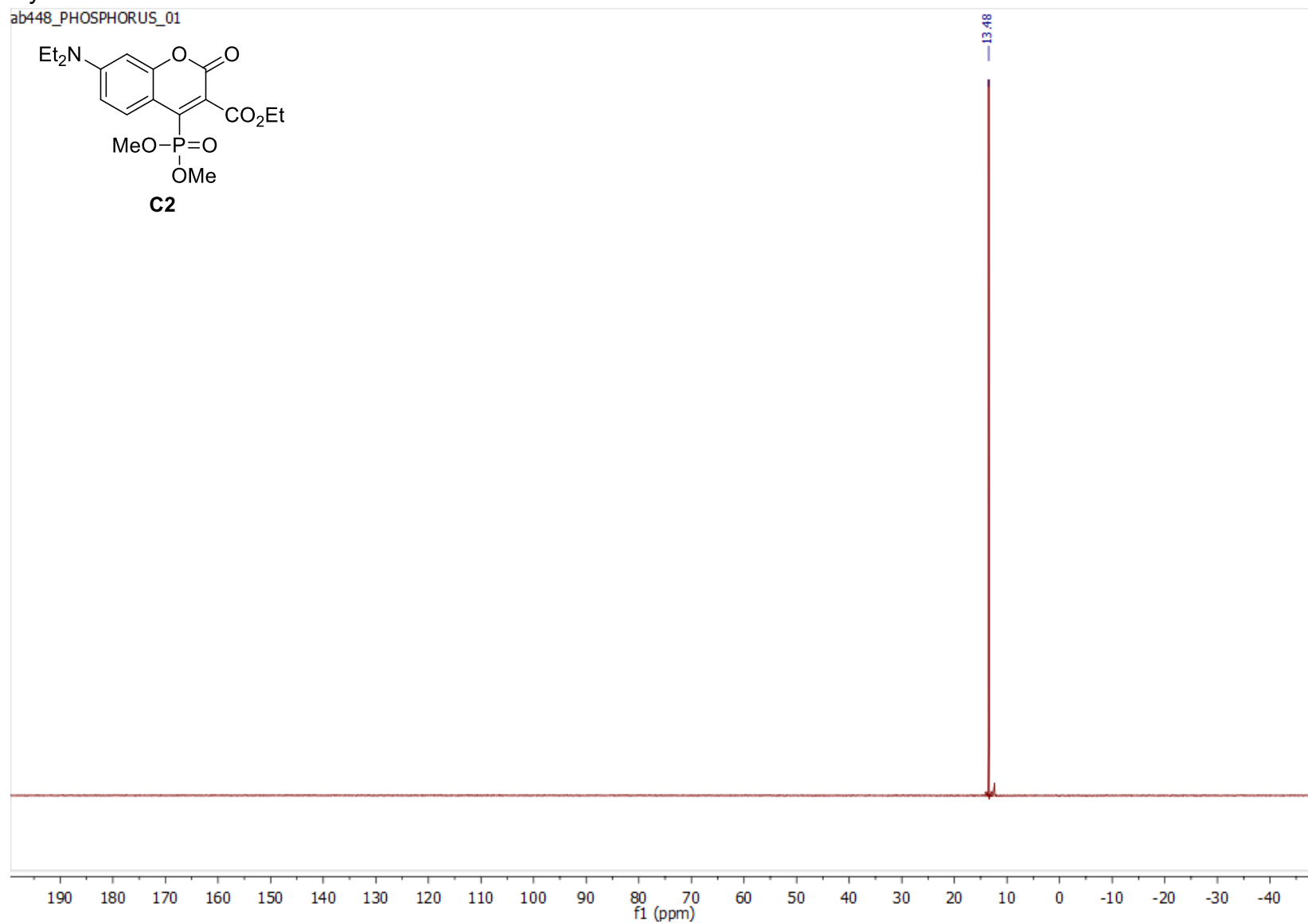
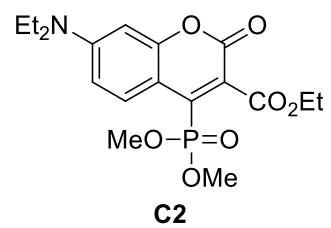
Dye **C2**: $^{13}\text{C}\{^1\text{H}\}$

ab448_CARBON_01



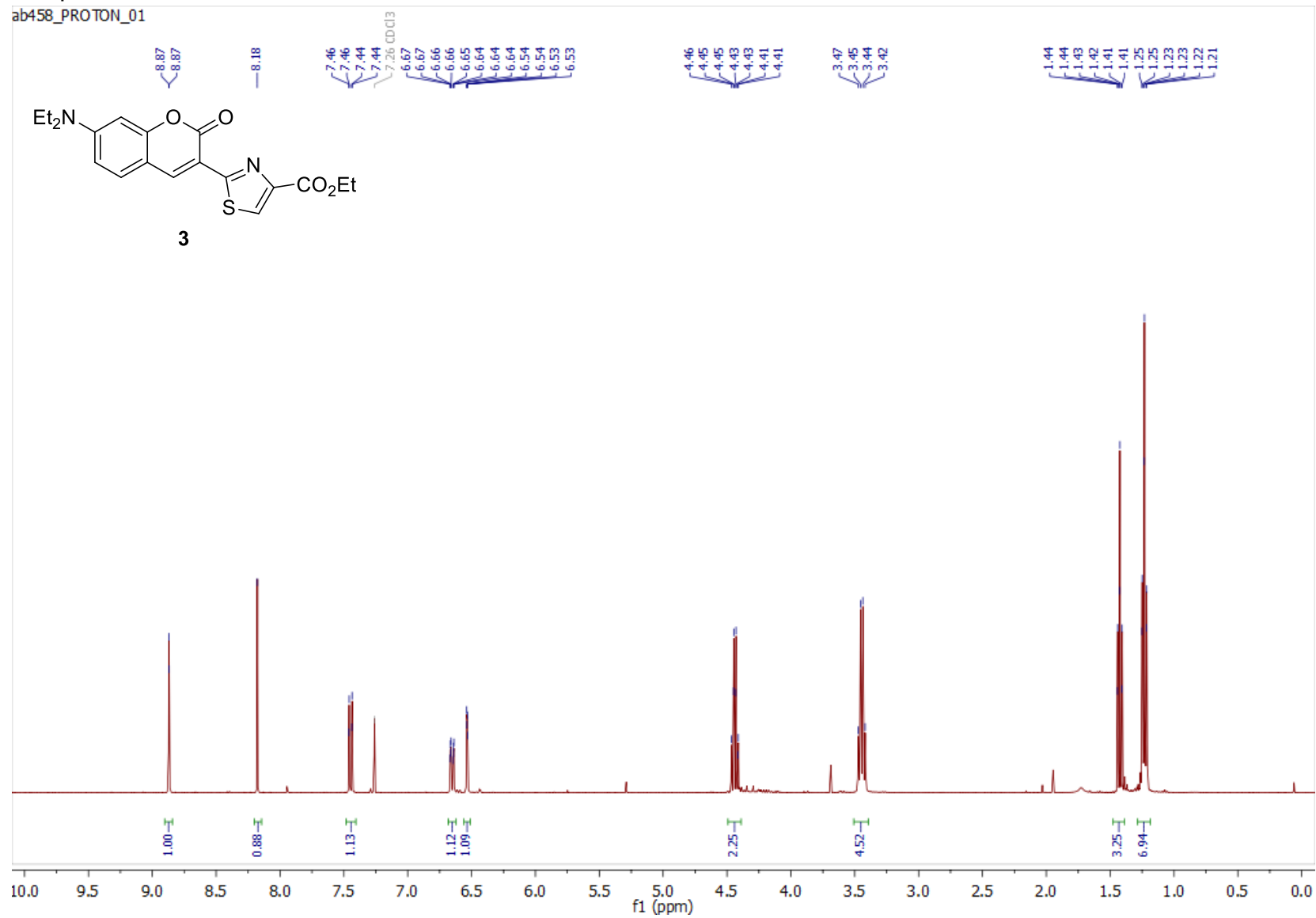
Dye **C2**: ^{31}P

ab448_PHOSPHORUS_01



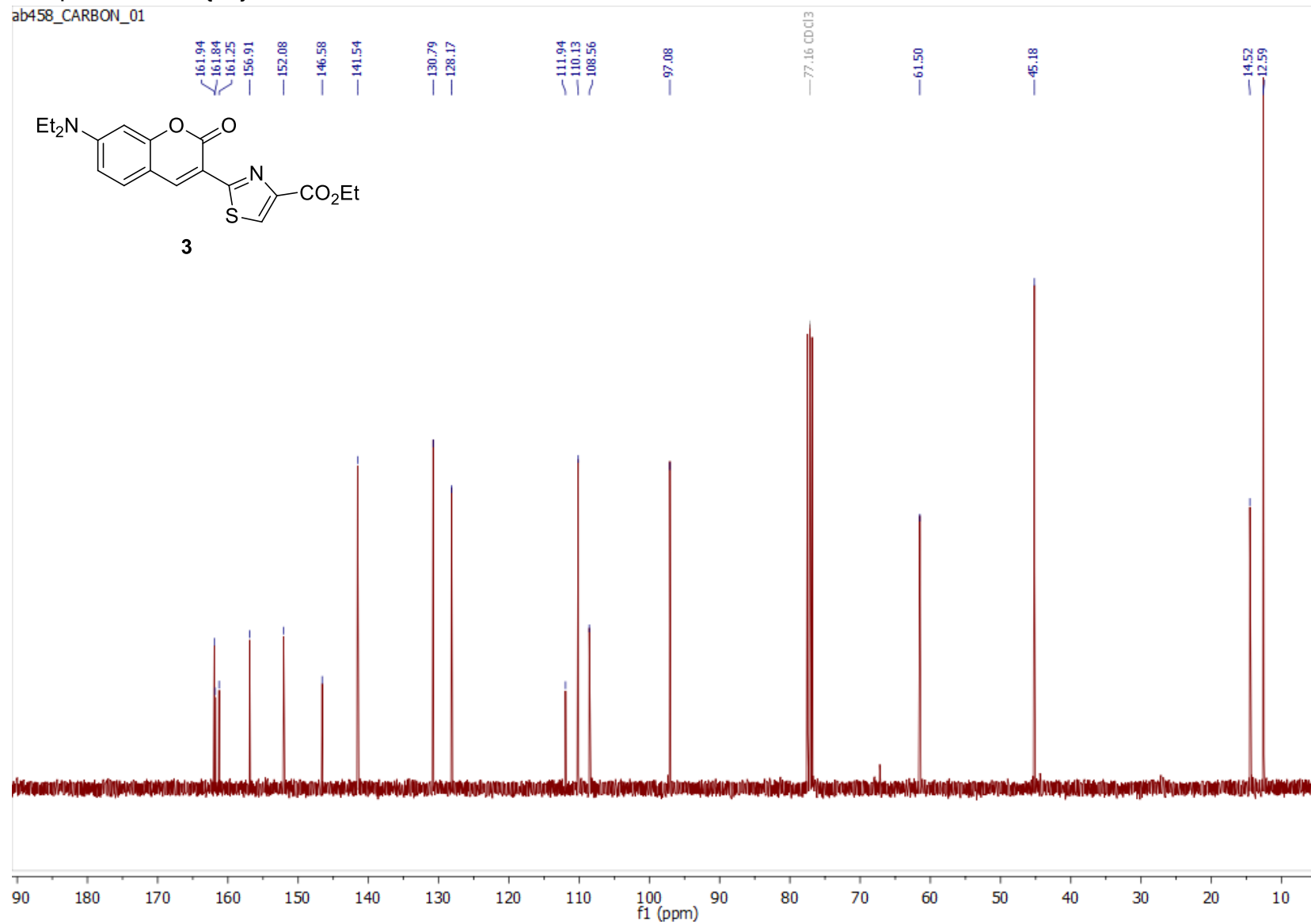
compound **3**: ^1H

ab458_PROTON_01



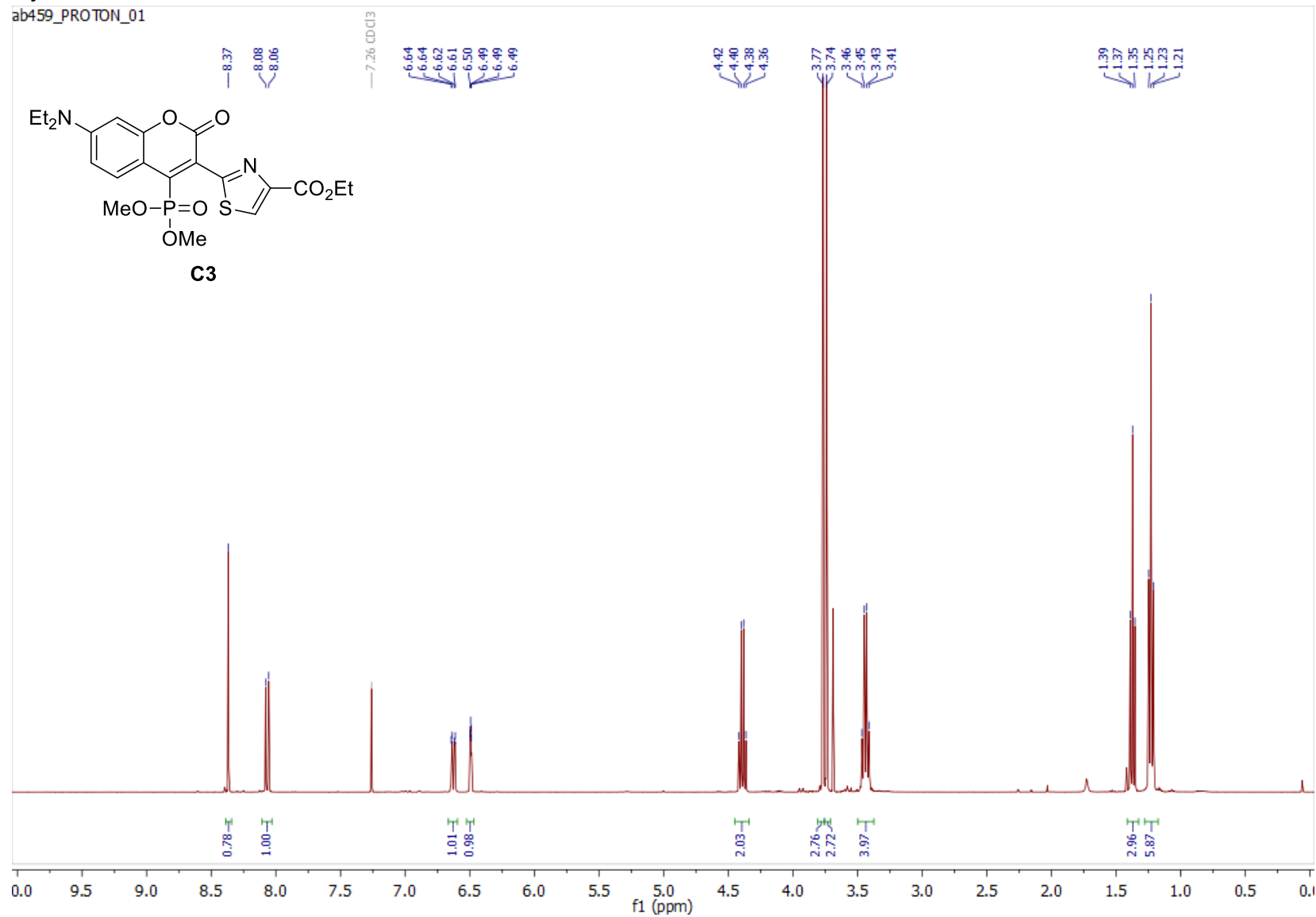
compound **3**: $^{13}\text{C}\{^1\text{H}\}$

ab458_CARBON_01



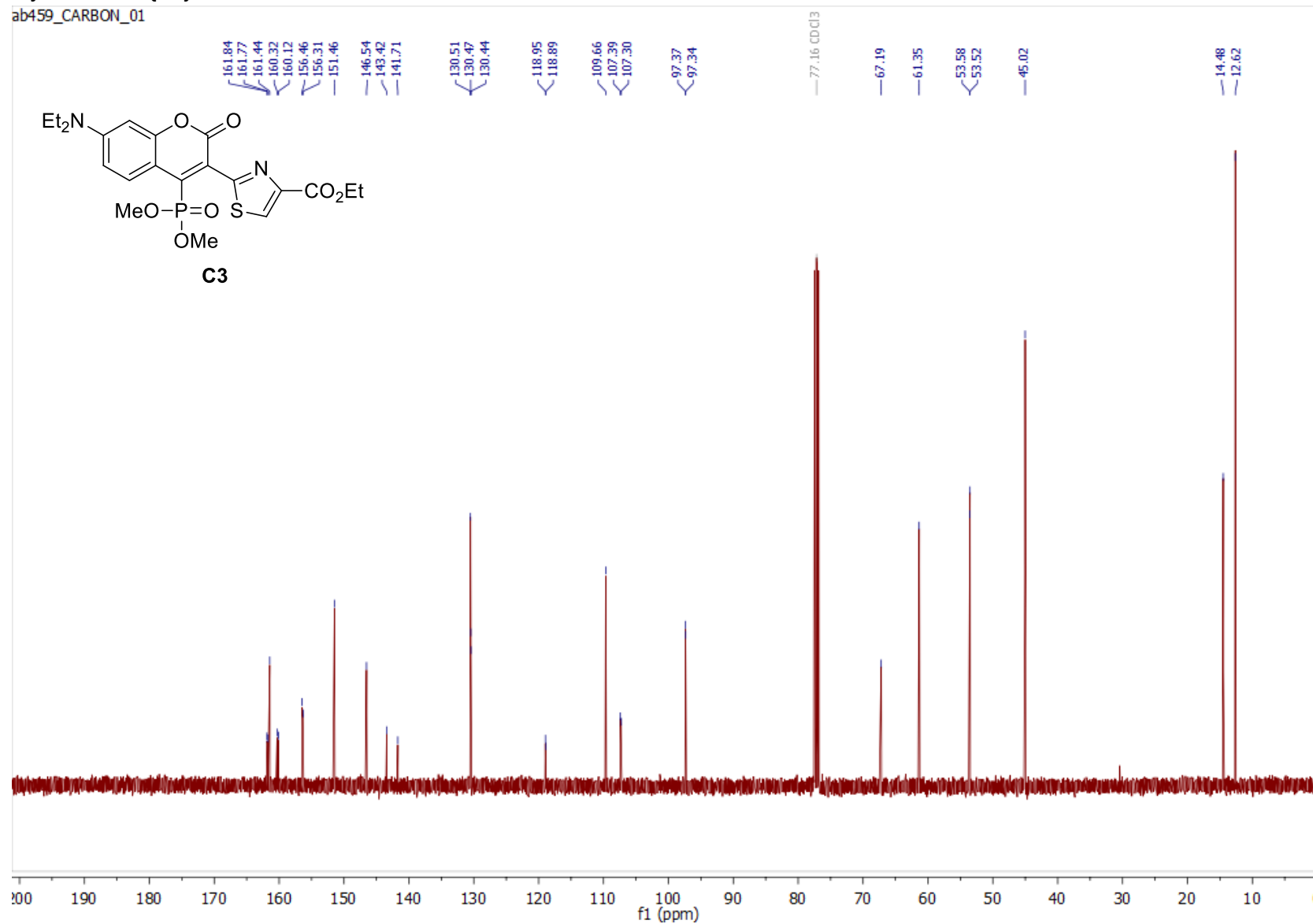
Dye C3: ¹H

ab459_PROTON_01



Dye **C3**: $^{13}\text{C}\{^1\text{H}\}$

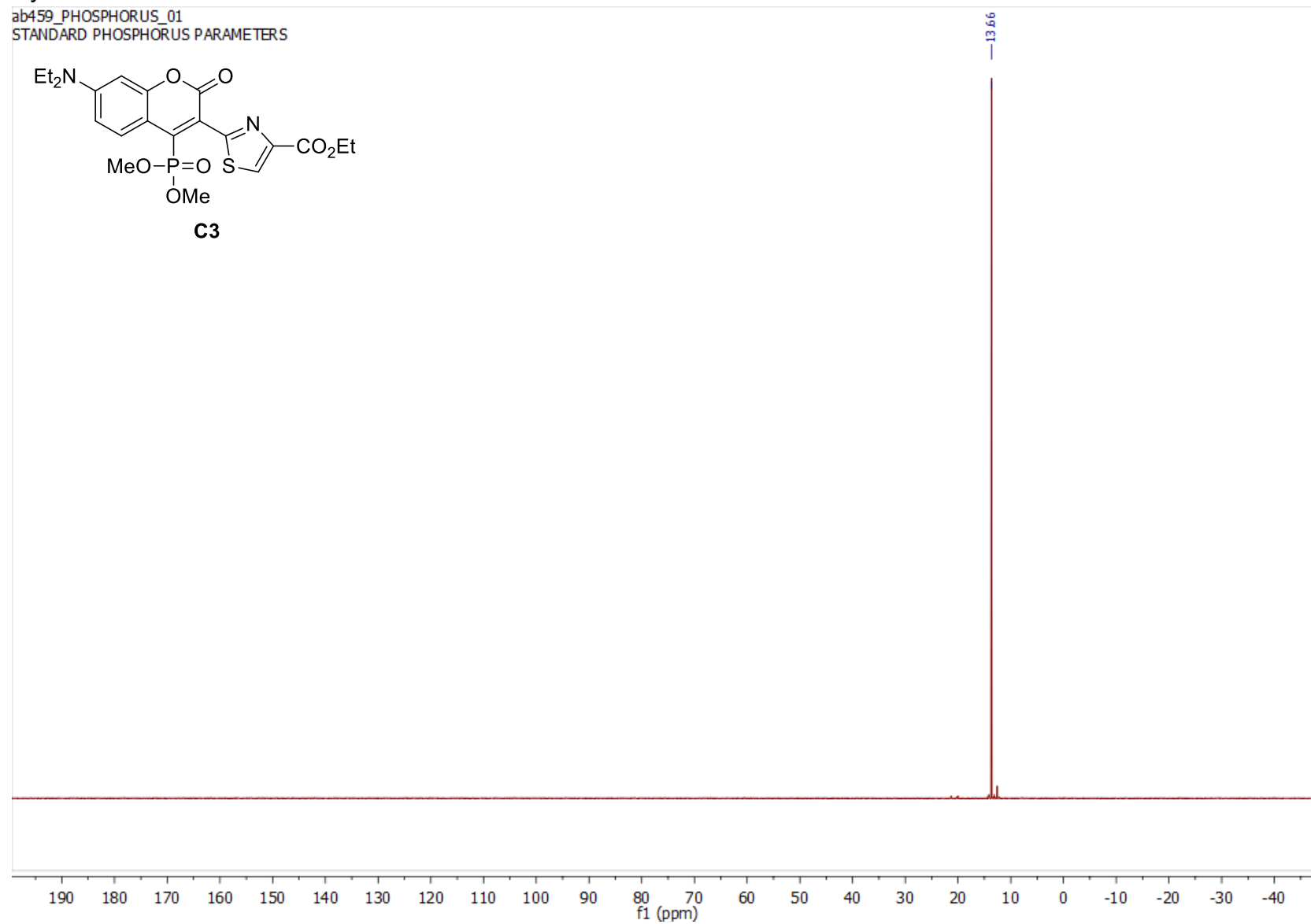
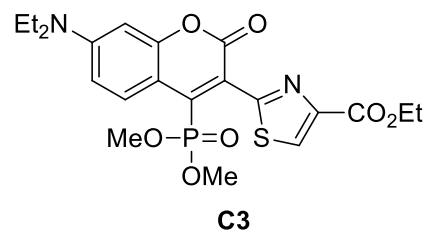
ab459 CARBON_01



Dye **C3**: ^{31}P

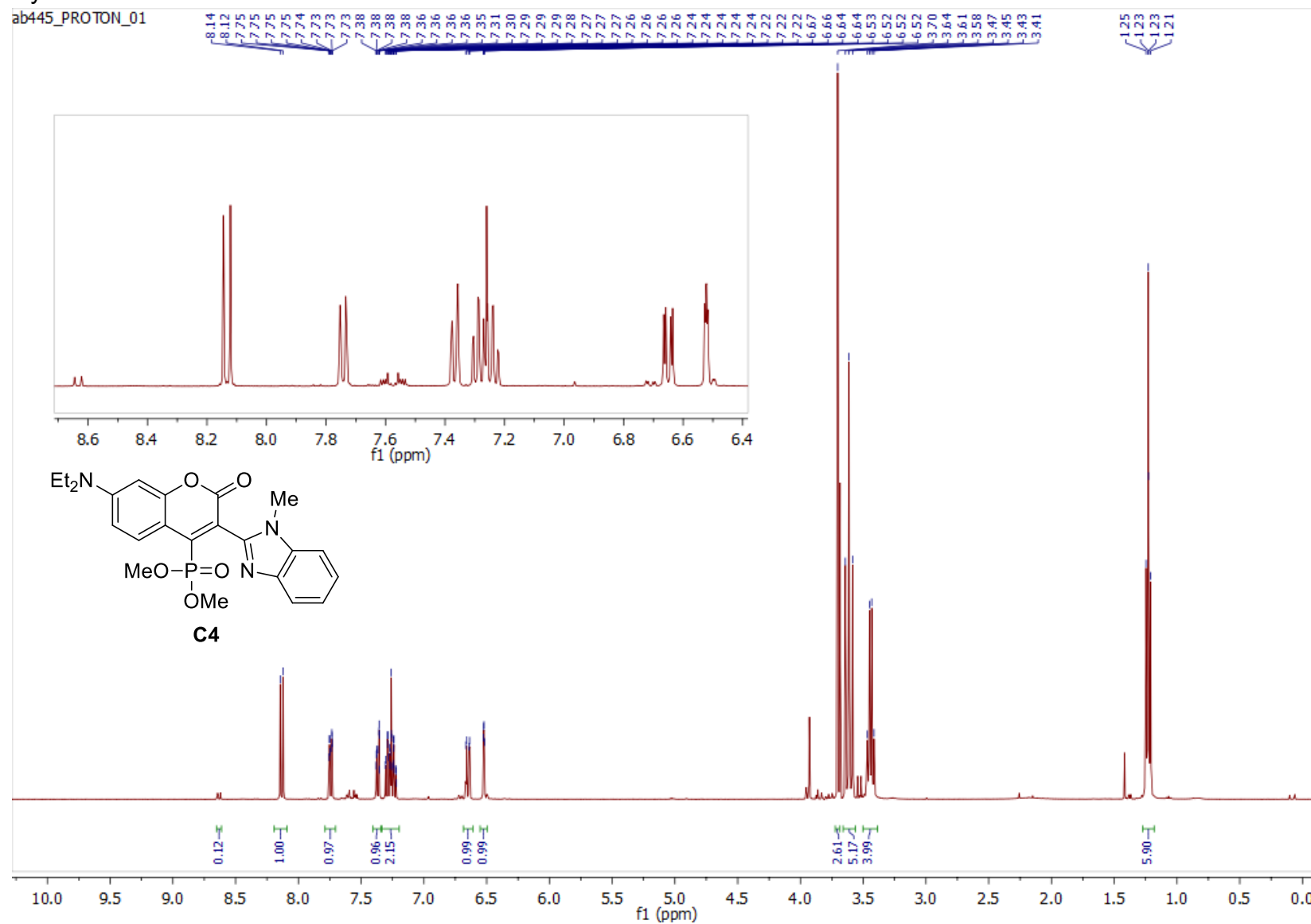
ab459_PHOSPHORUS_01

STANDARD PHOSPHORUS PARAMETERS



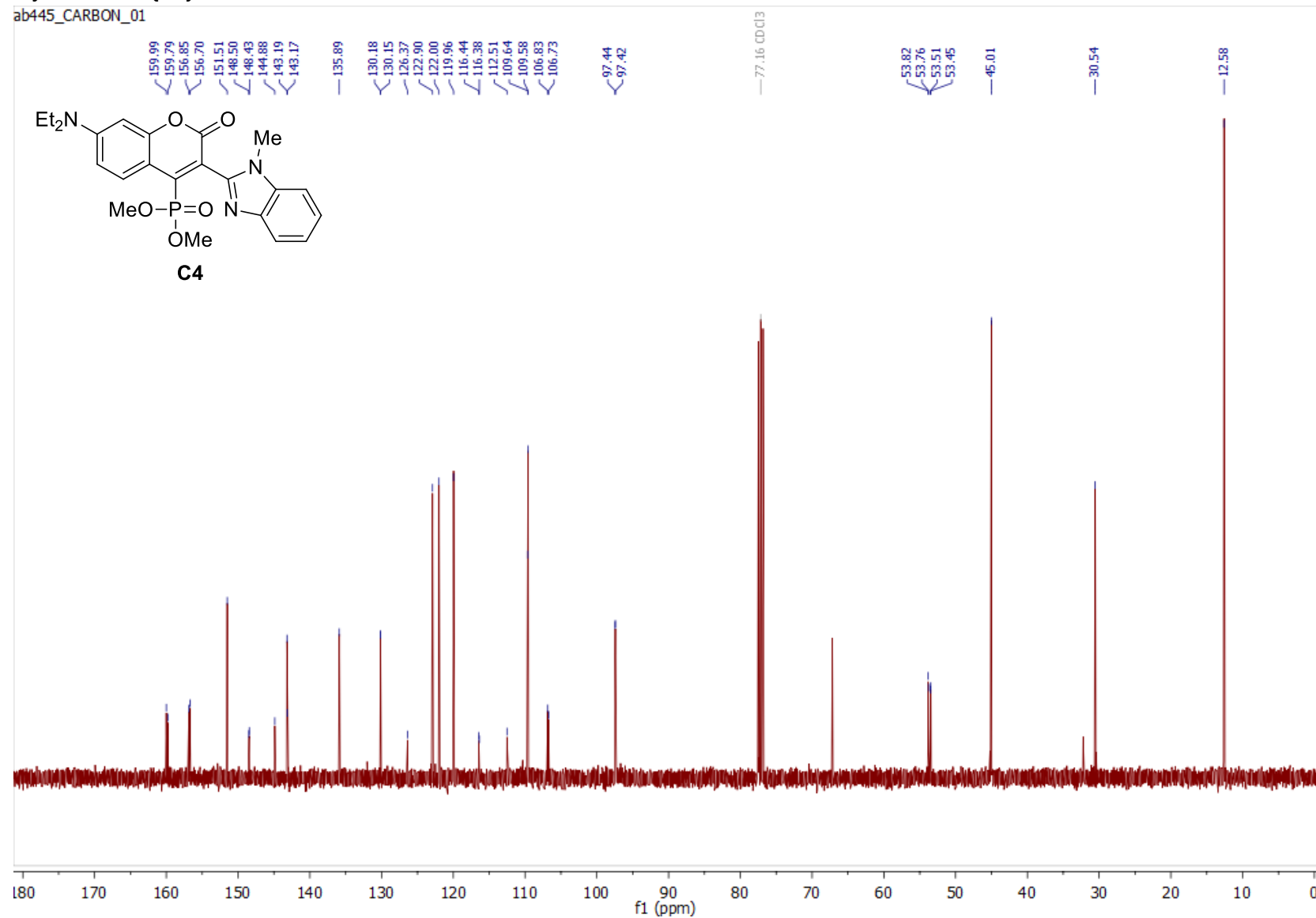
Dye C4: ¹H

ab445_PROTON_01



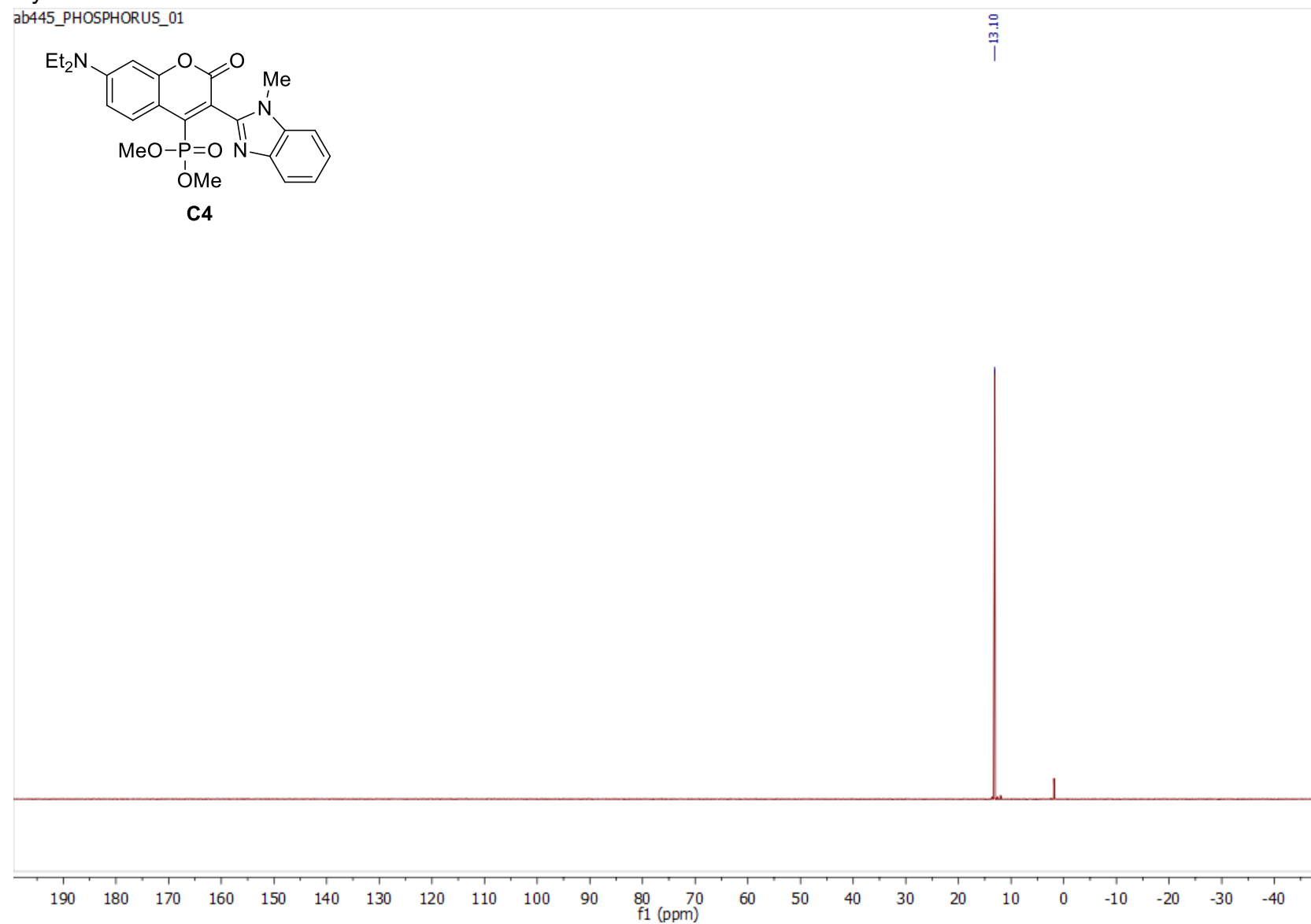
Dye **C4**: $^{13}\text{C}\{^1\text{H}\}$

ab445_CARBON_01



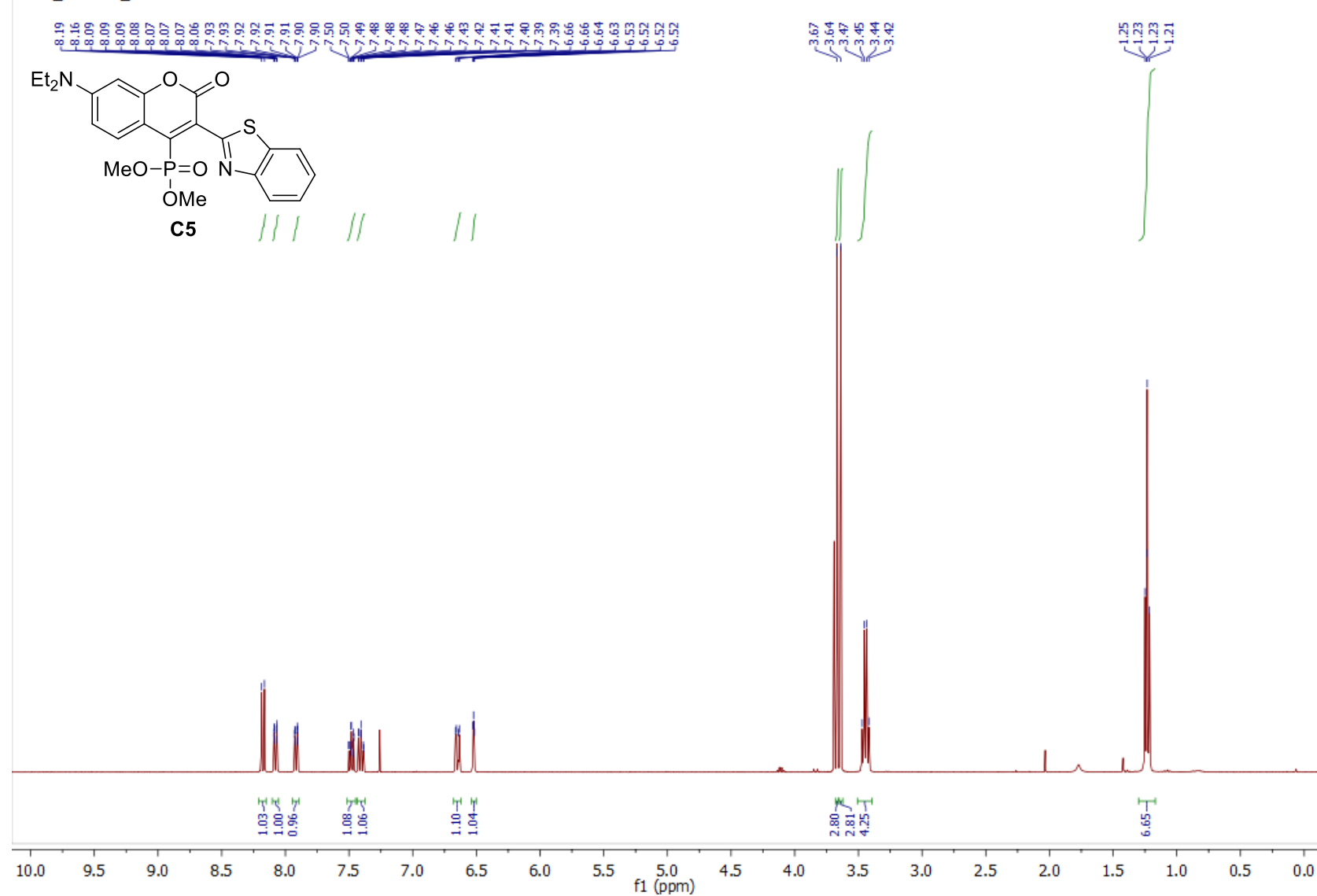
Dye **C4**: ^{31}P

ab445_PHOSPHORUS_01



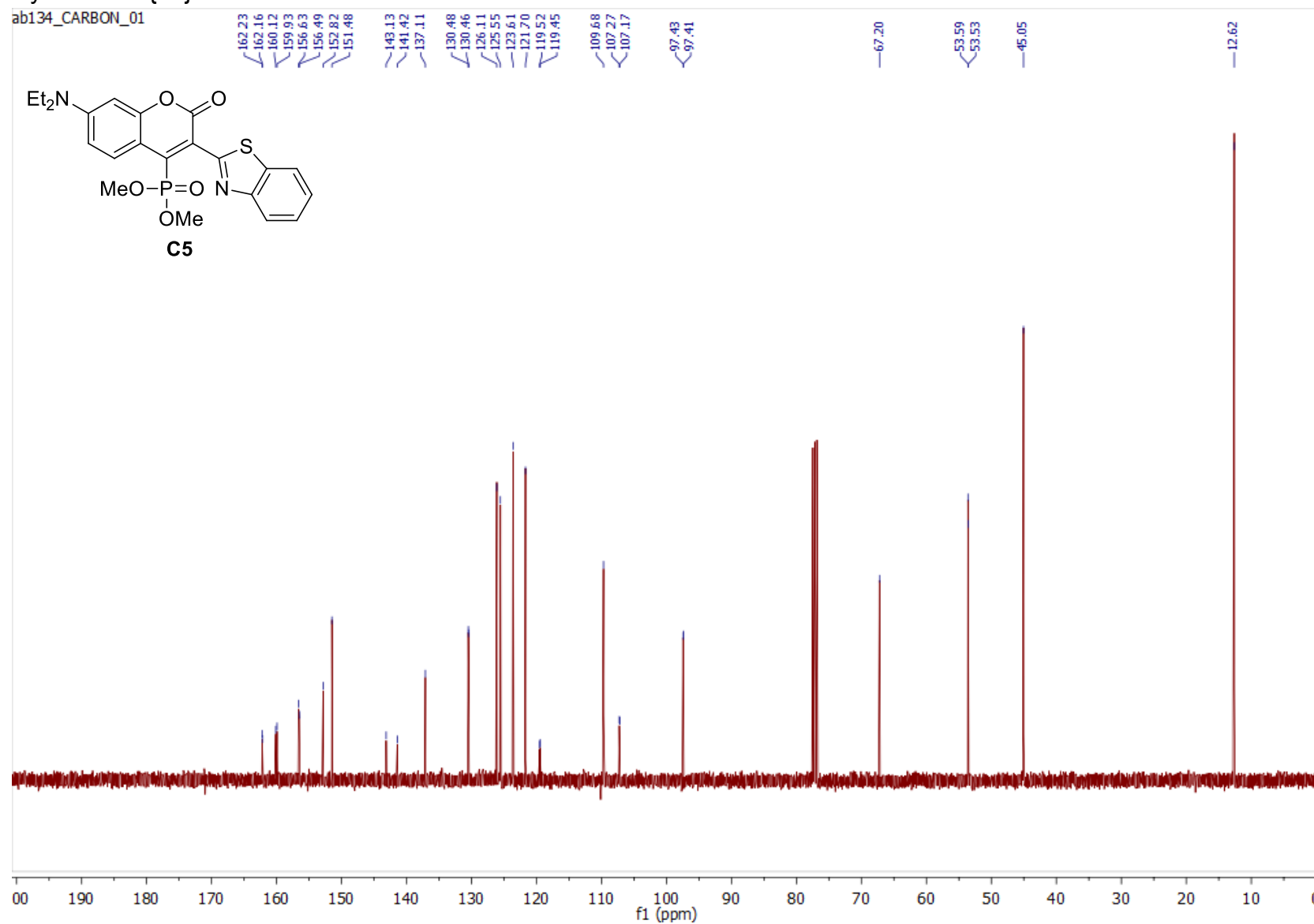
Dye C5: ¹H

ab134_PROTON_01



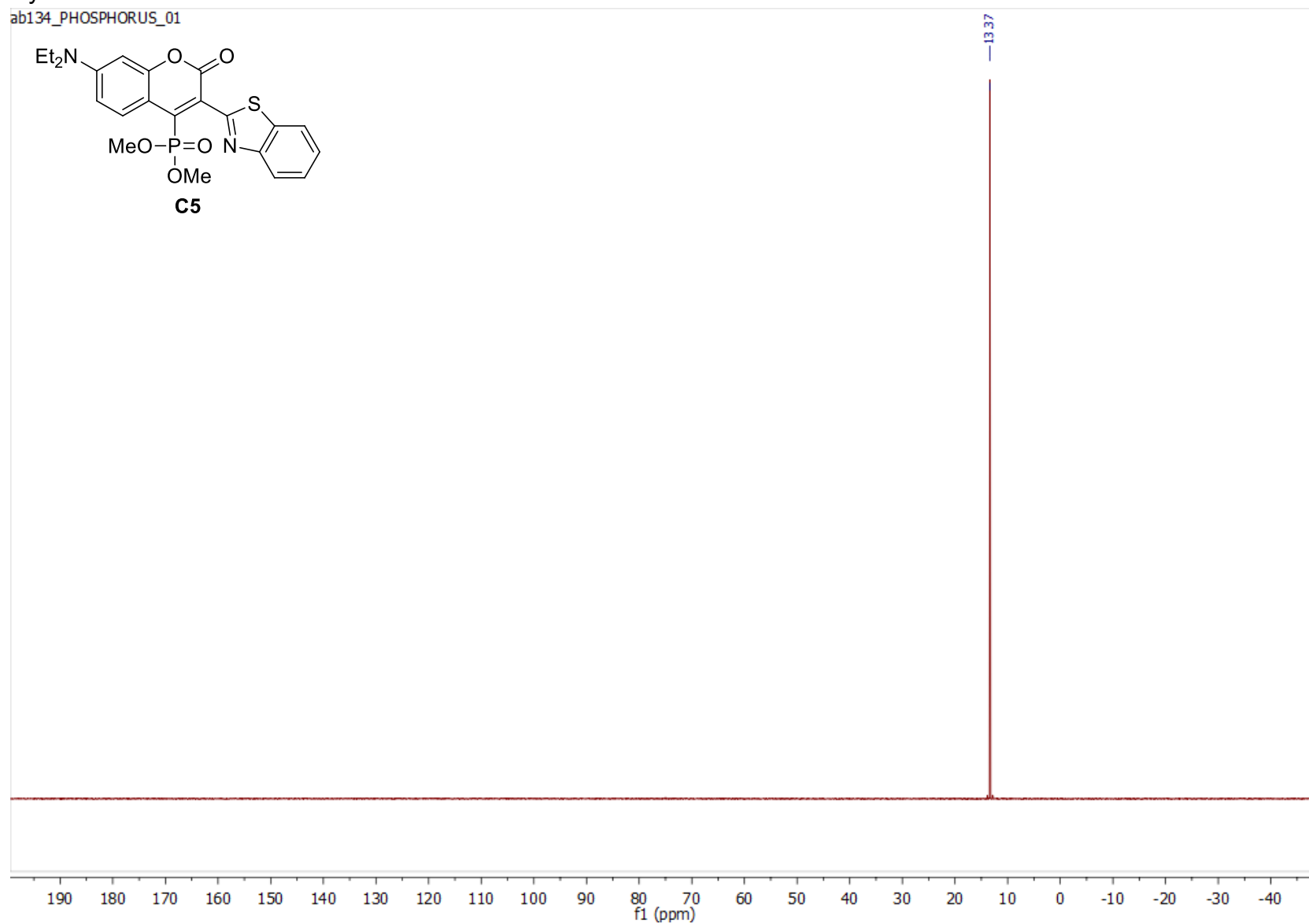
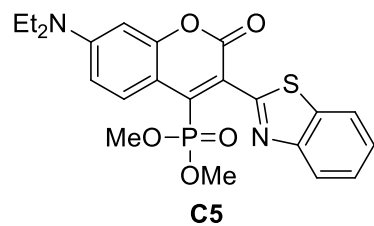
Dye **C5**: $^{13}\text{C}\{^1\text{H}\}$

ab134_CARBON_01



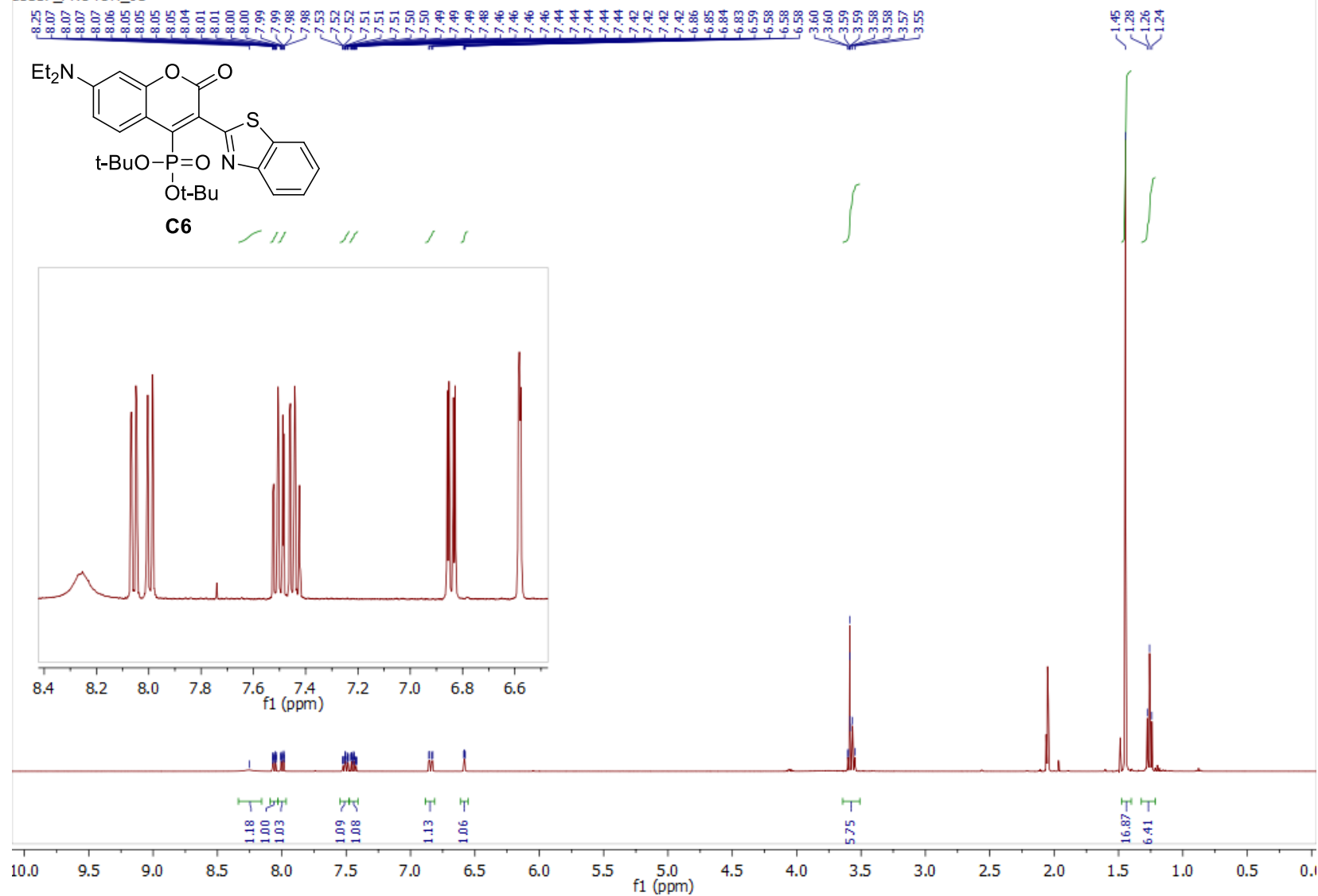
Dye **C5**: ^{31}P

ab134_PHOSPHORUS_01

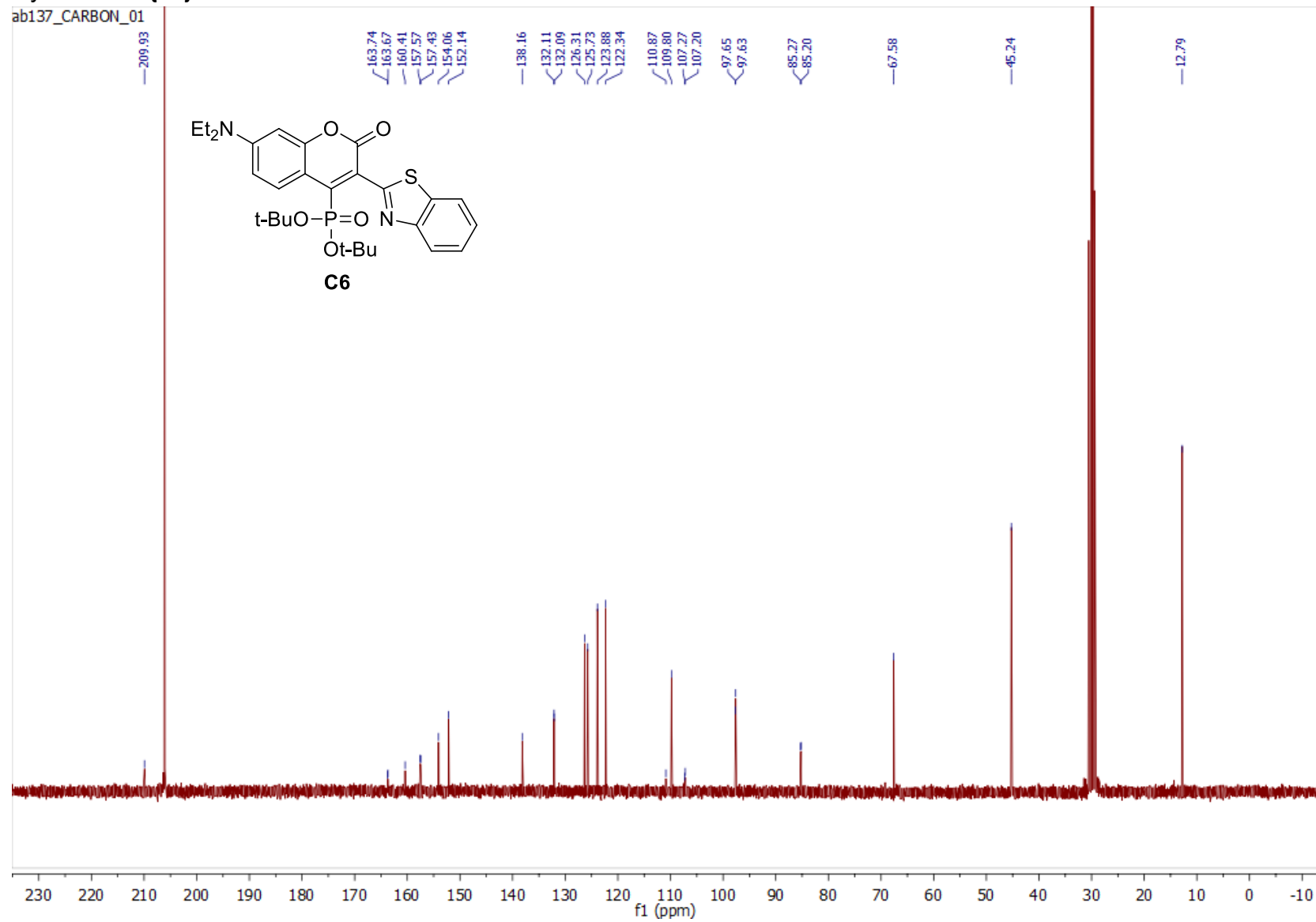


Dye C6: ¹H

ab137_PROTON_01

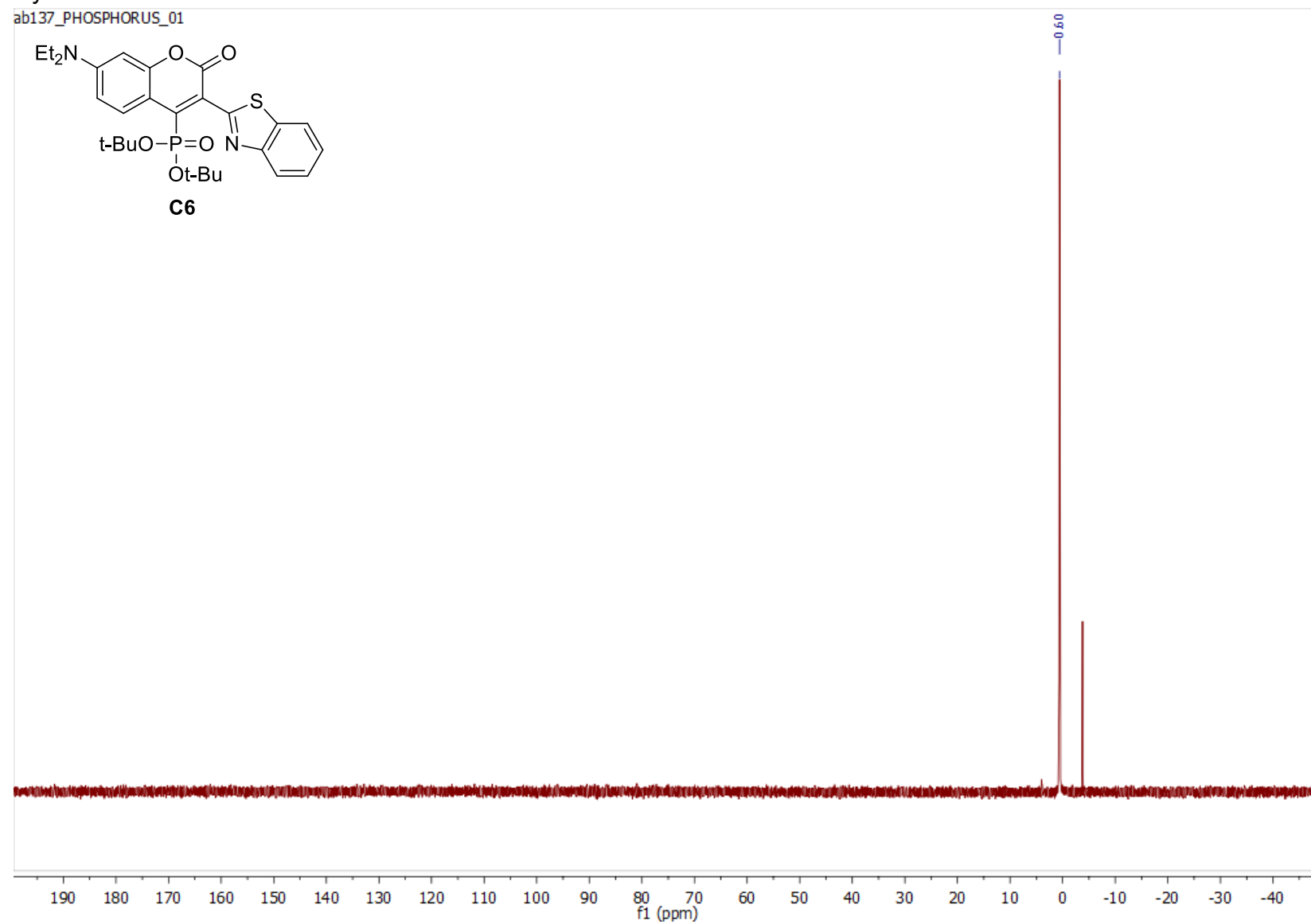


Dye **C6**: $^{13}\text{C}\{^1\text{H}\}$

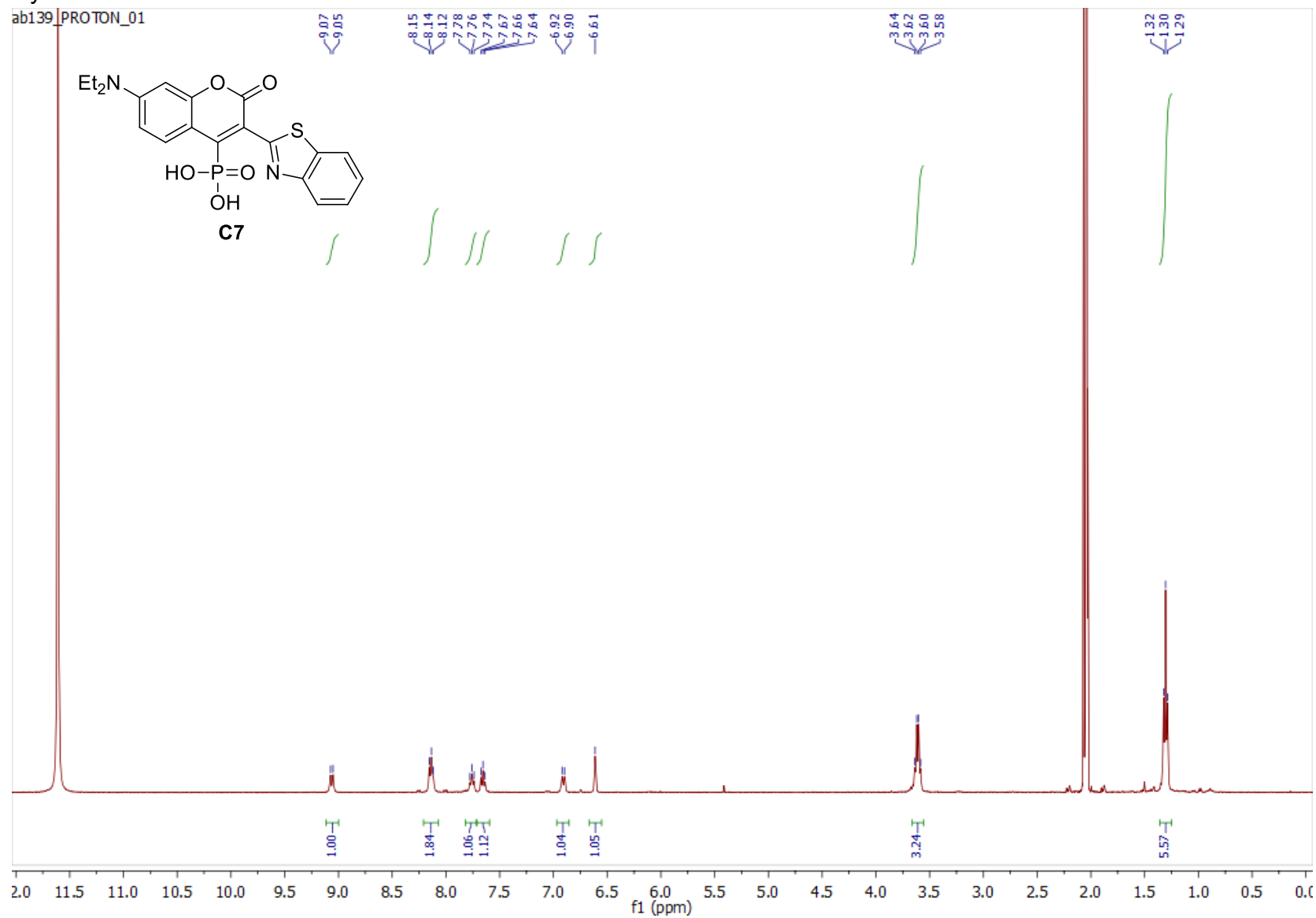


Dye **C6**: ^{31}P

ab137_PHOSPHORUS_01

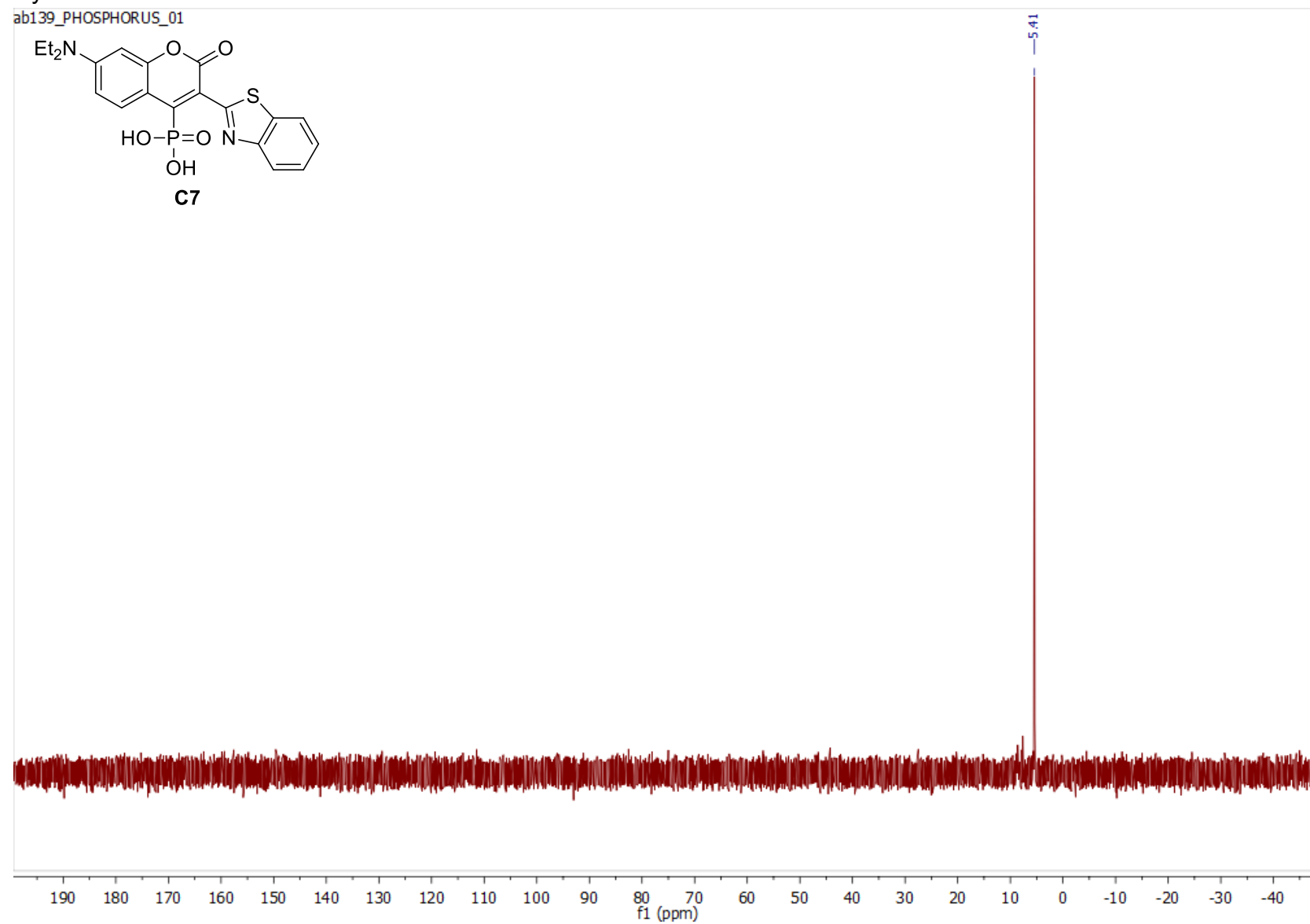


Dye **C7**: ^1H



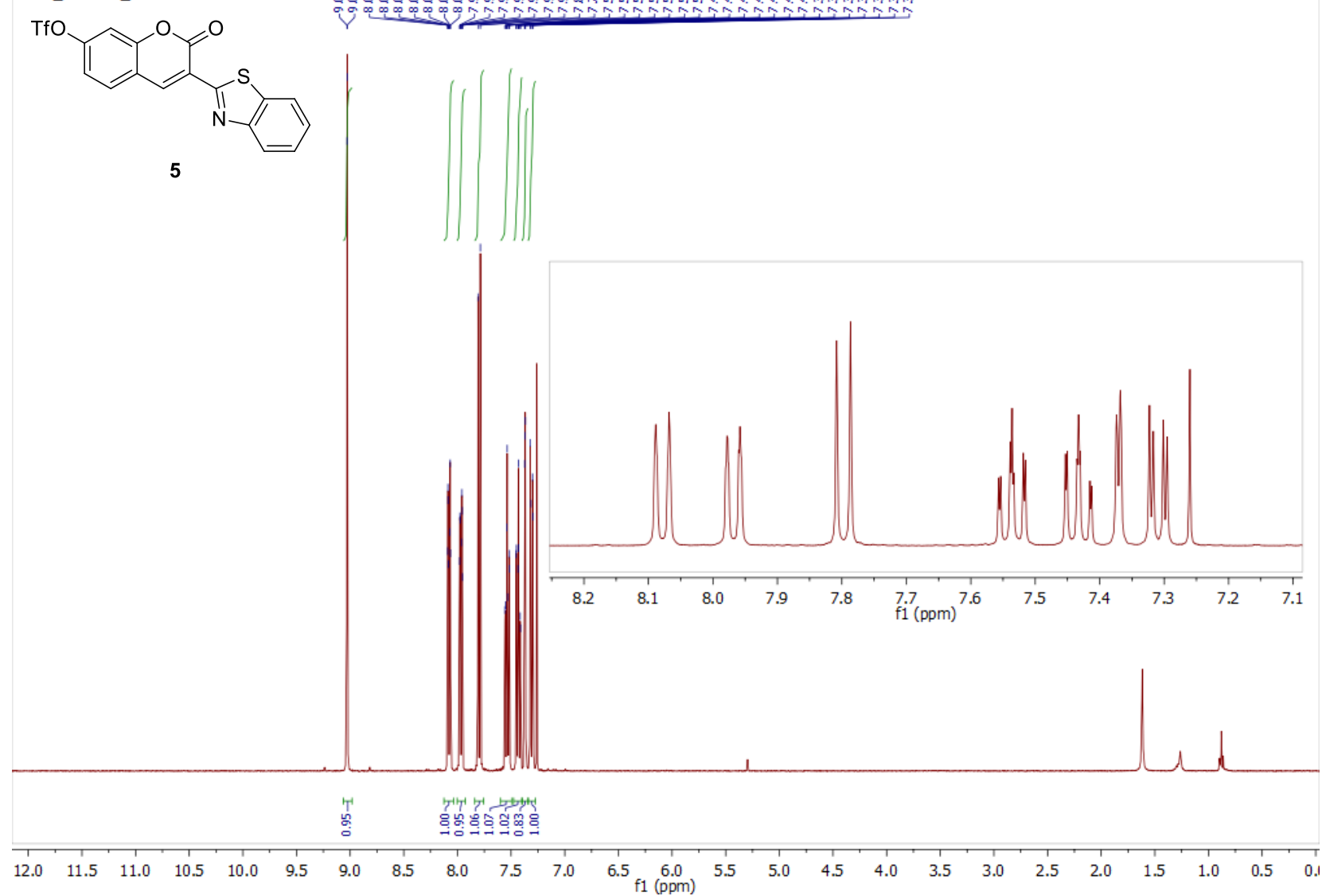
Dye **C7**: ^{31}P

ab139_PHOSPHORUS_01

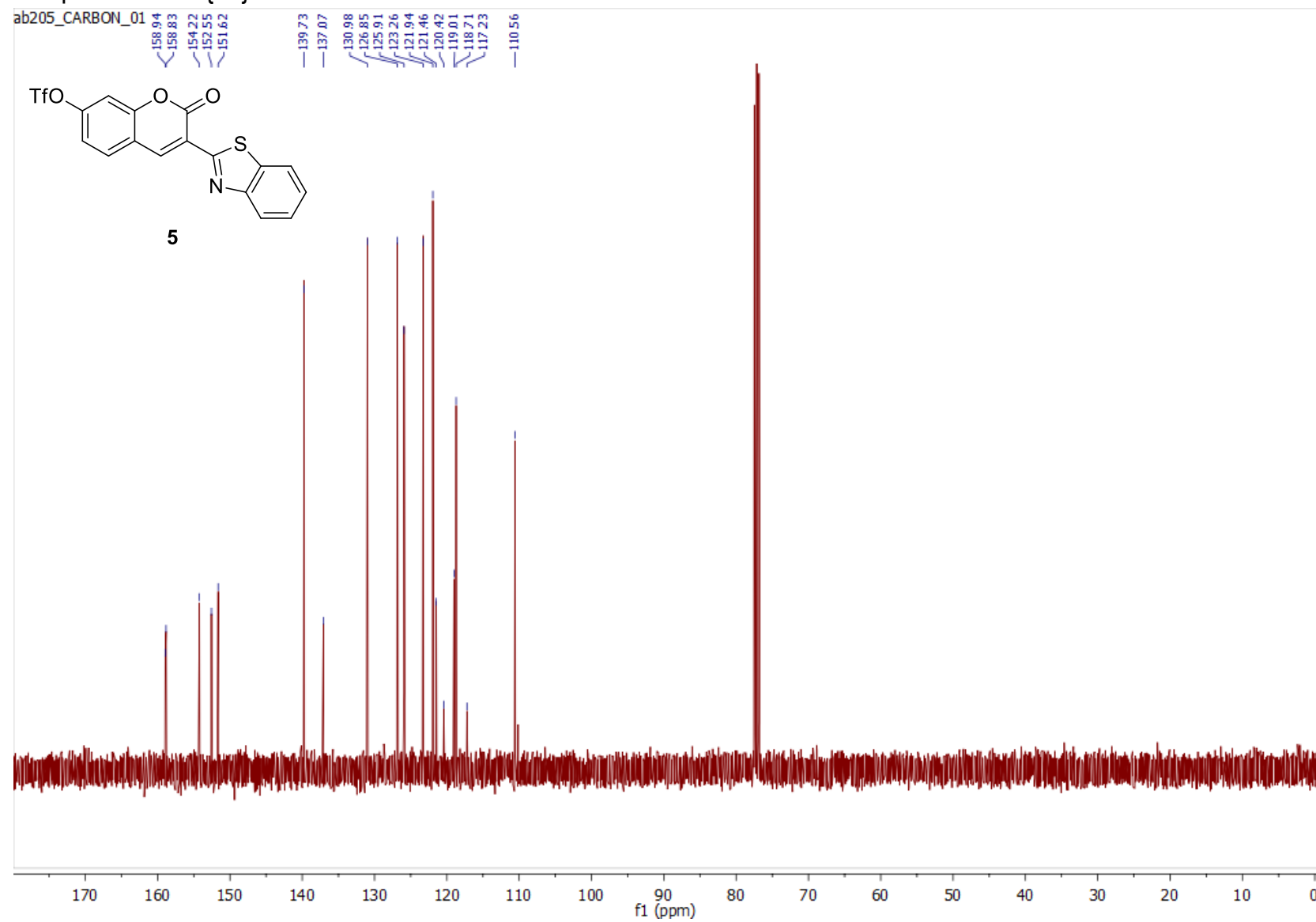


compound **5**: ^1H

ab205_PROTON_01

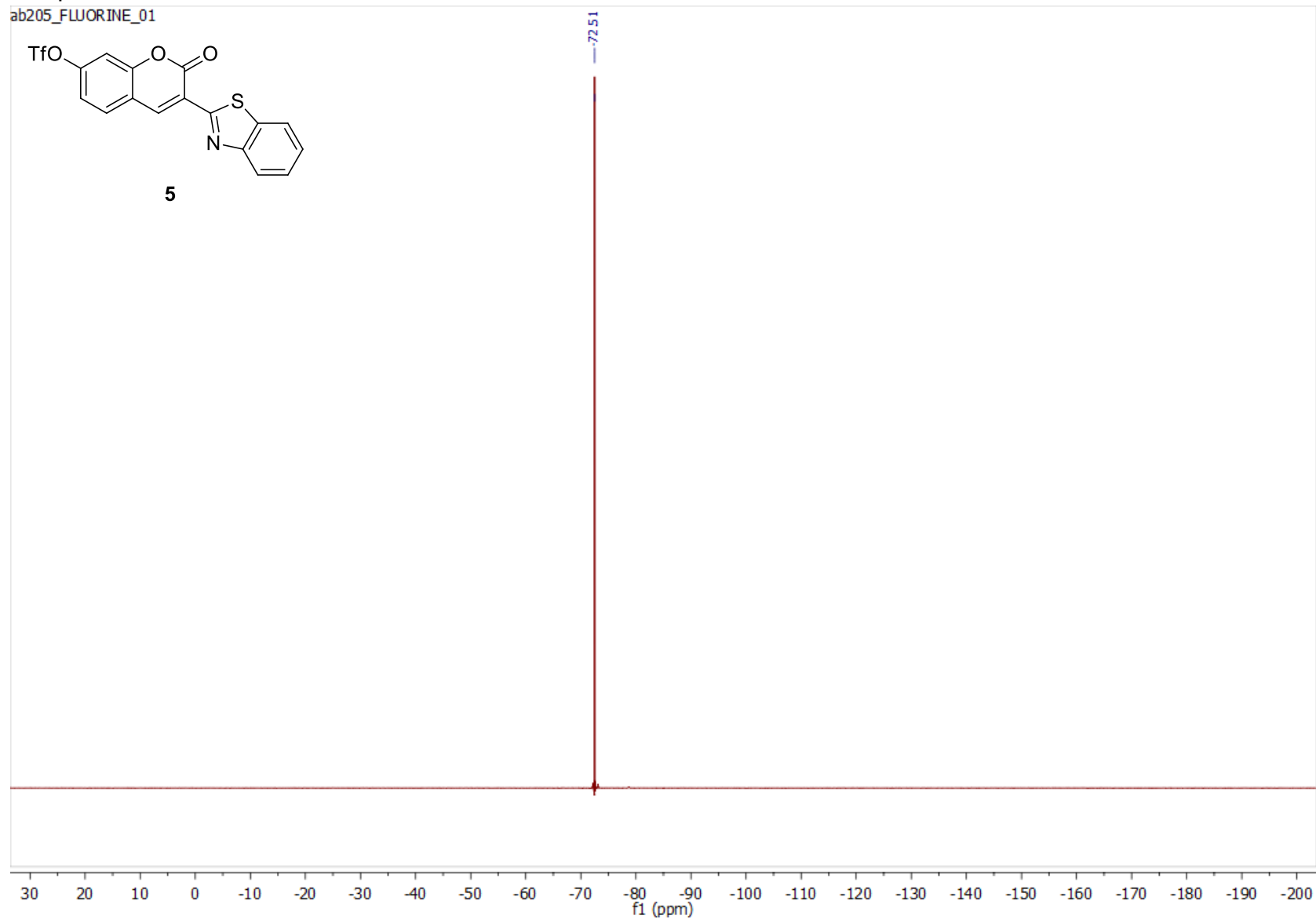
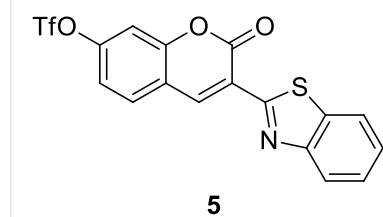


compound **5**: $^{13}\text{C}\{^1\text{H}\}$

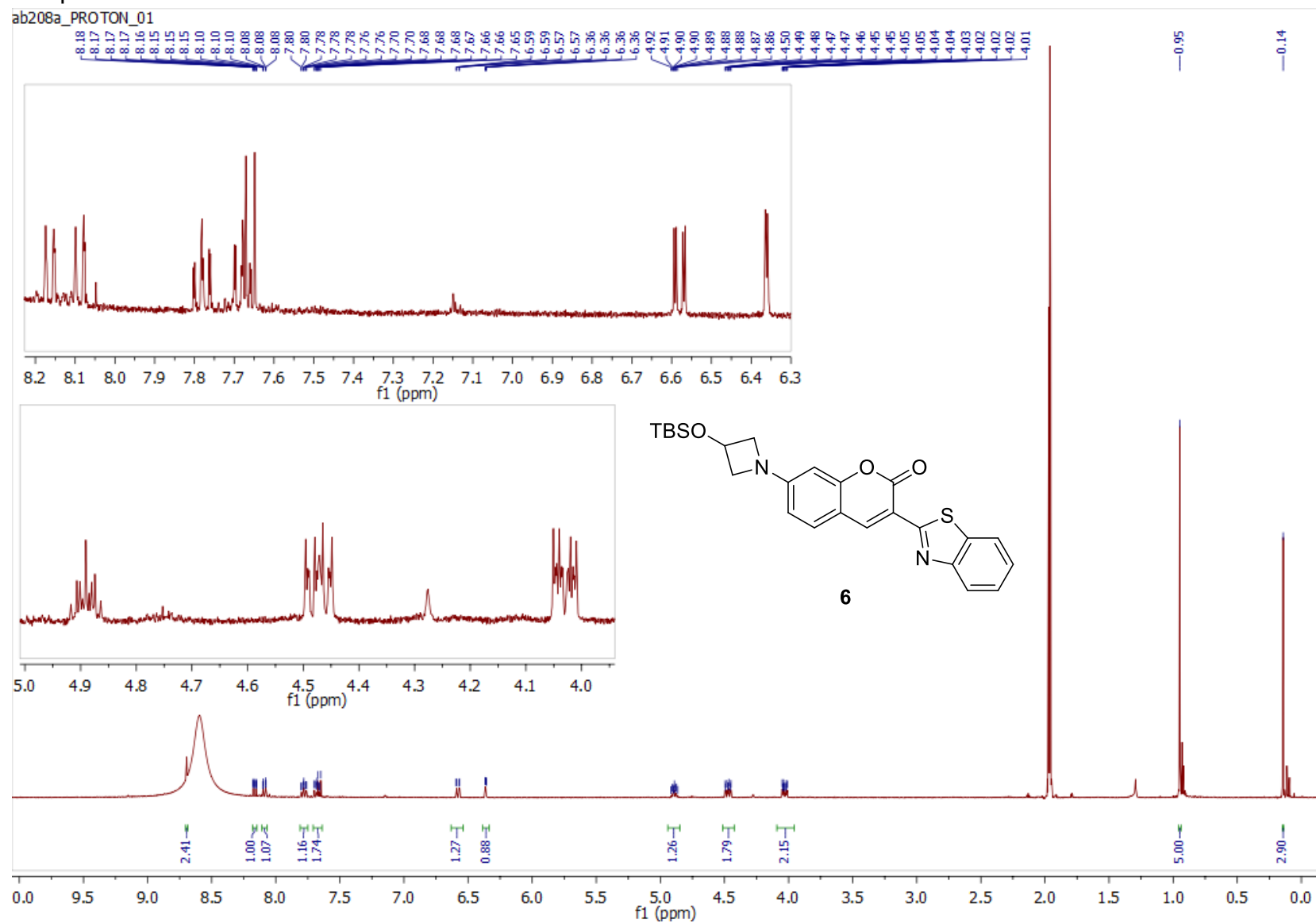


compound **5**: ^{19}F

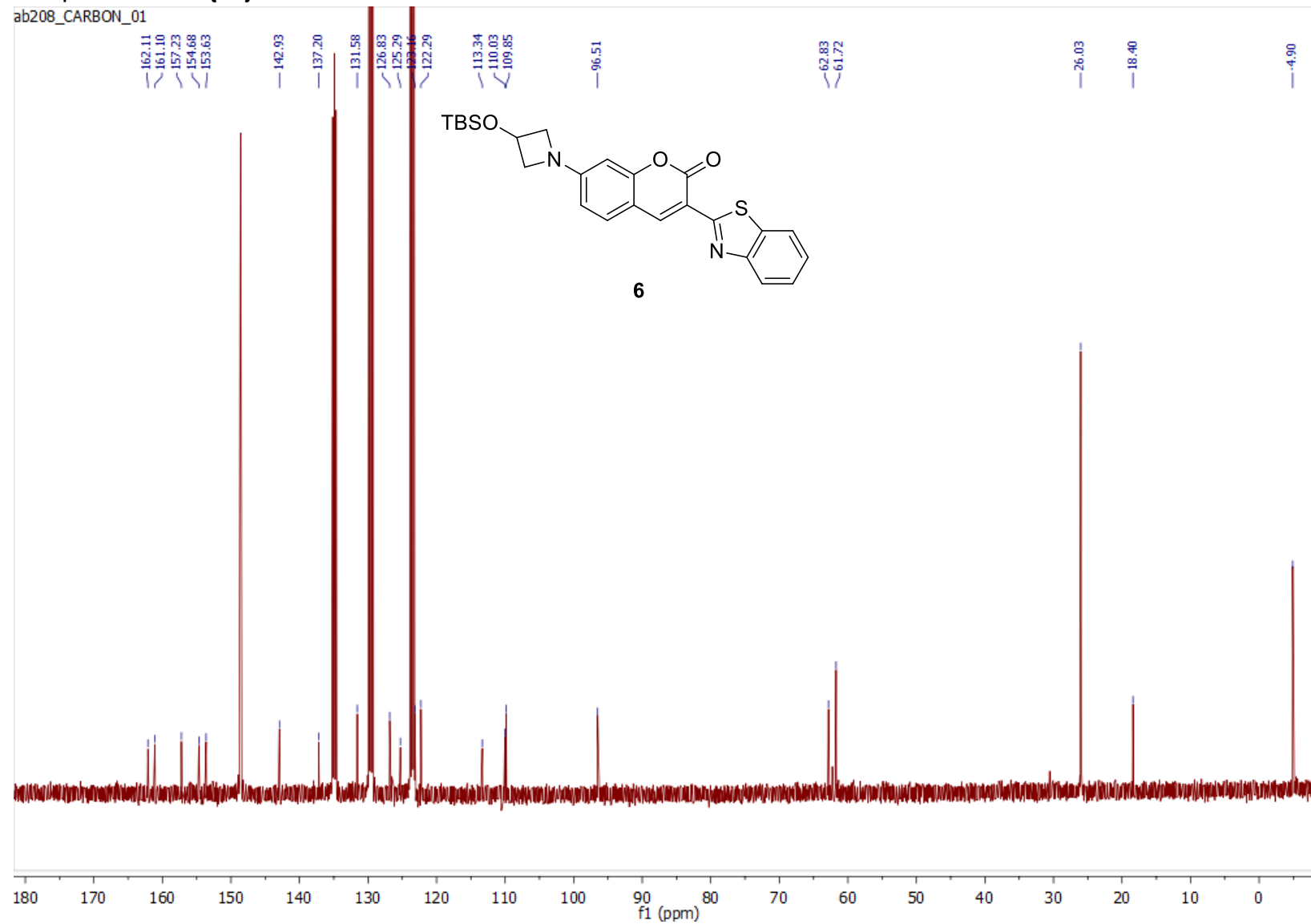
ab205_FLUORINE_01



compound **6**: ^1H

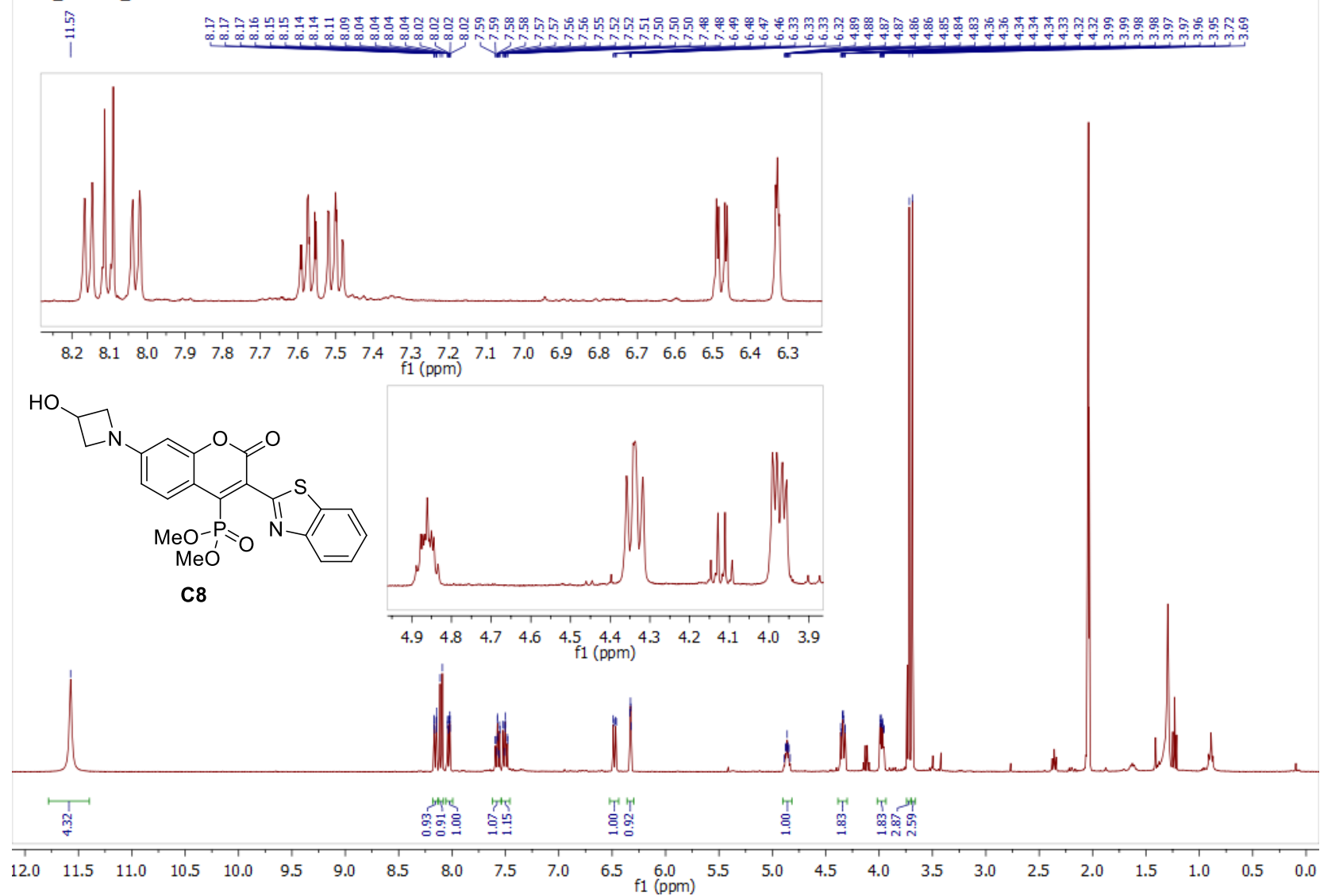


compound **6**: $^{13}\text{C}\{^1\text{H}\}$



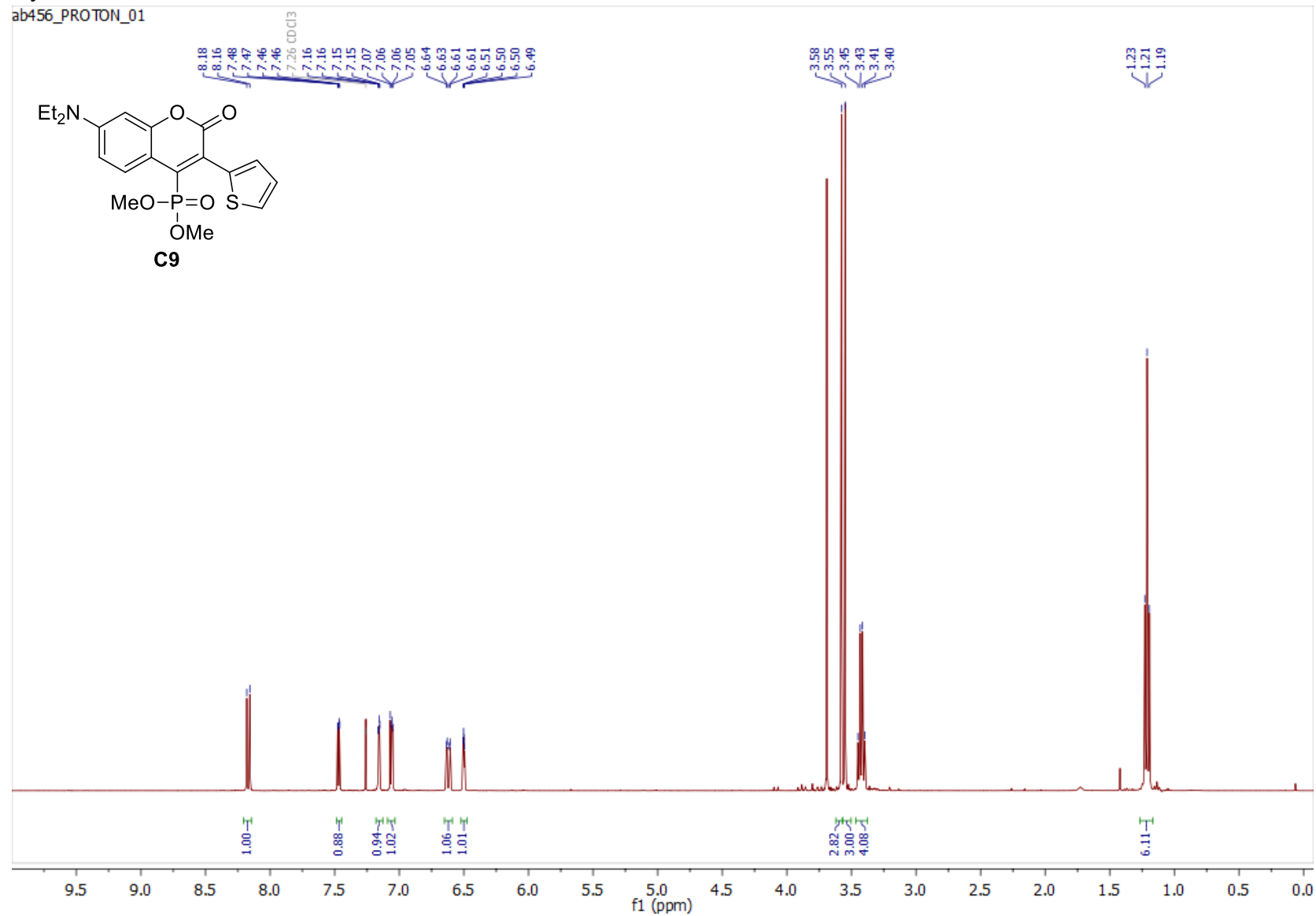
Dye C8: ¹H

ab211_PROTON_01



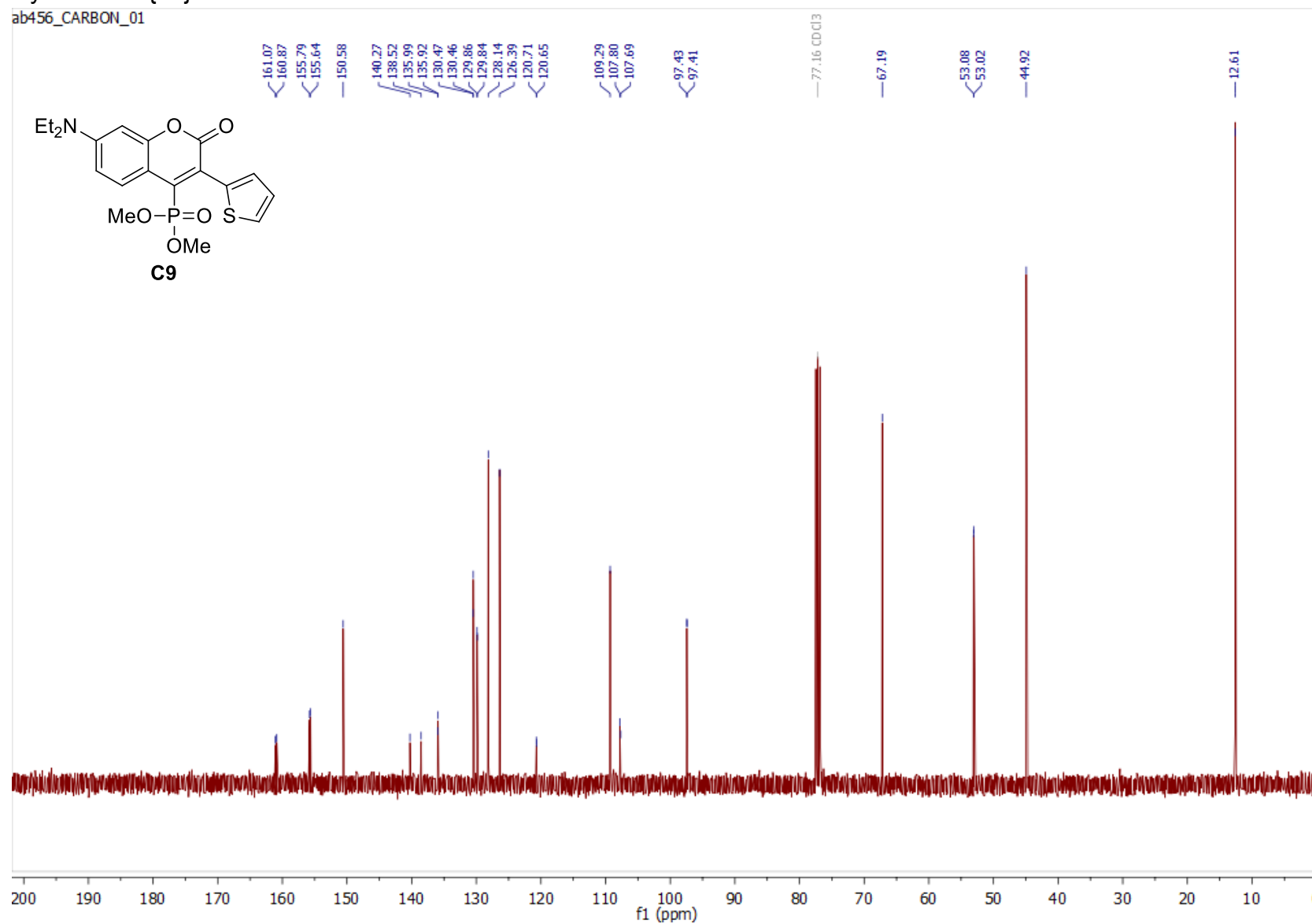
Dye C9: ¹H

ab456_PROTON_01



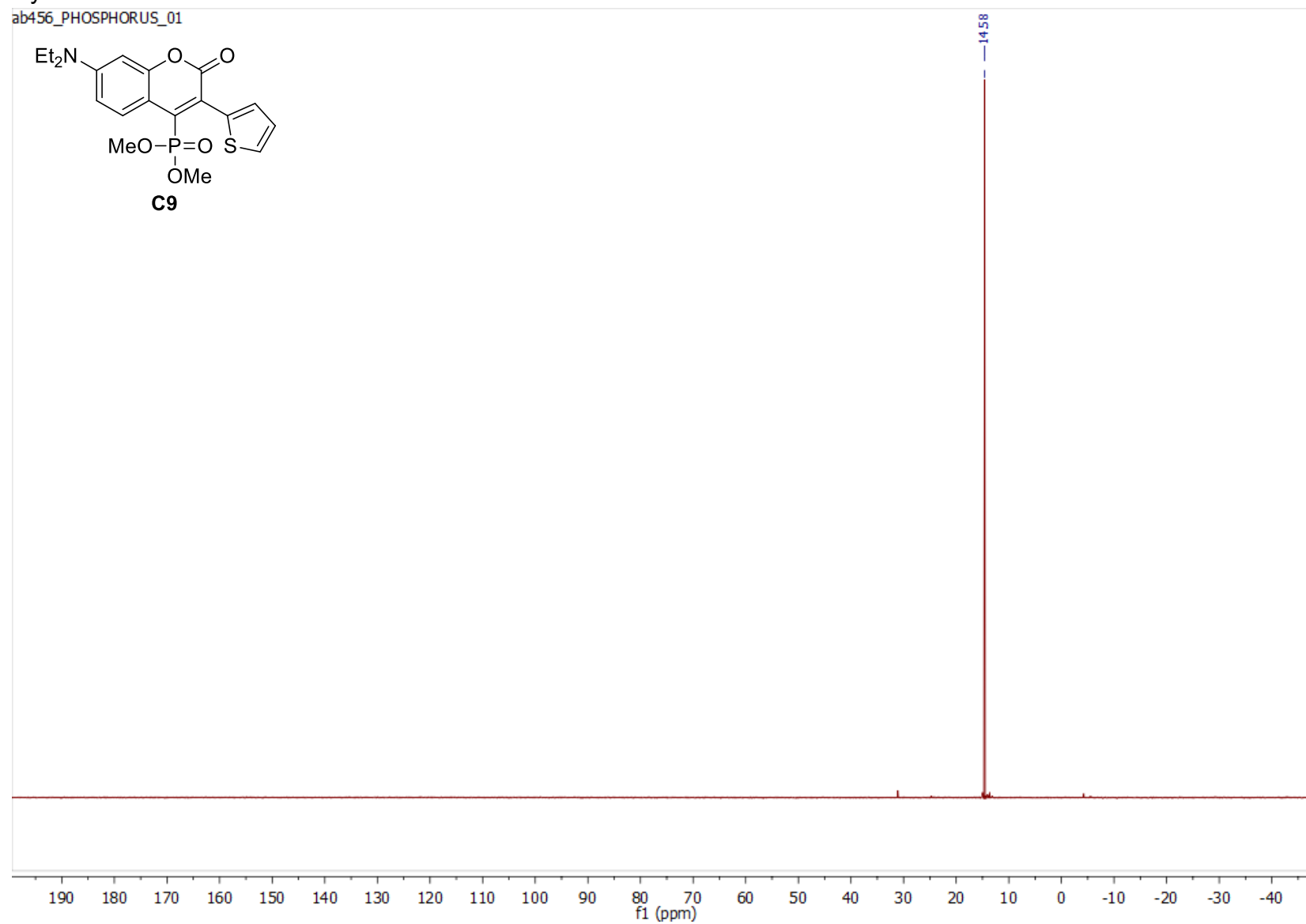
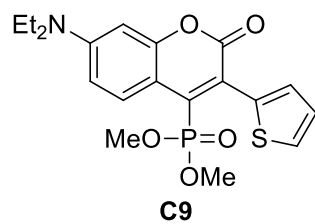
Dye **C9**: $^{13}\text{C}\{^1\text{H}\}$

ab456_CARBON_01



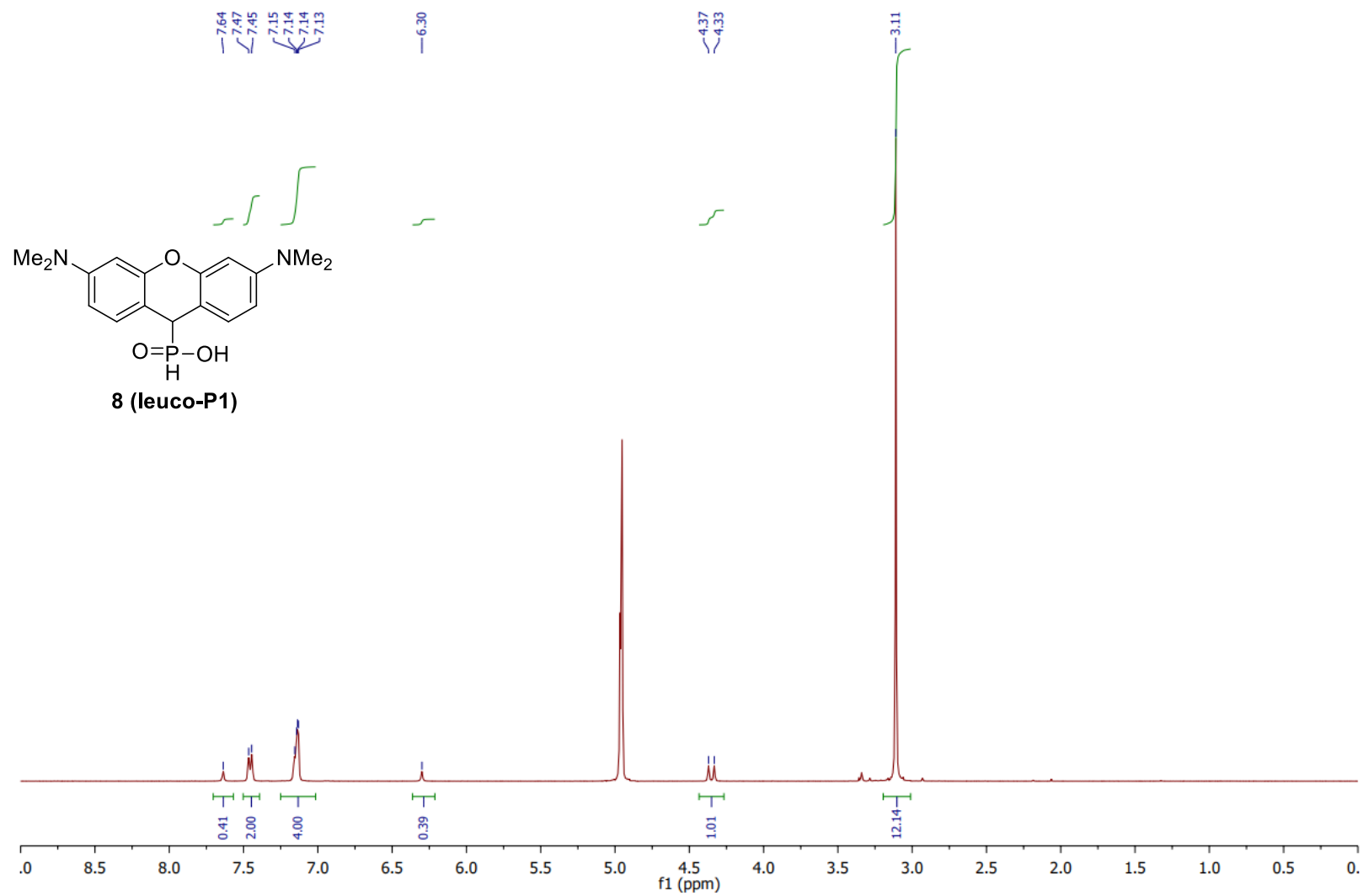
Dye **C9**: ^{31}P

ab456_PHOSPHORUS_01



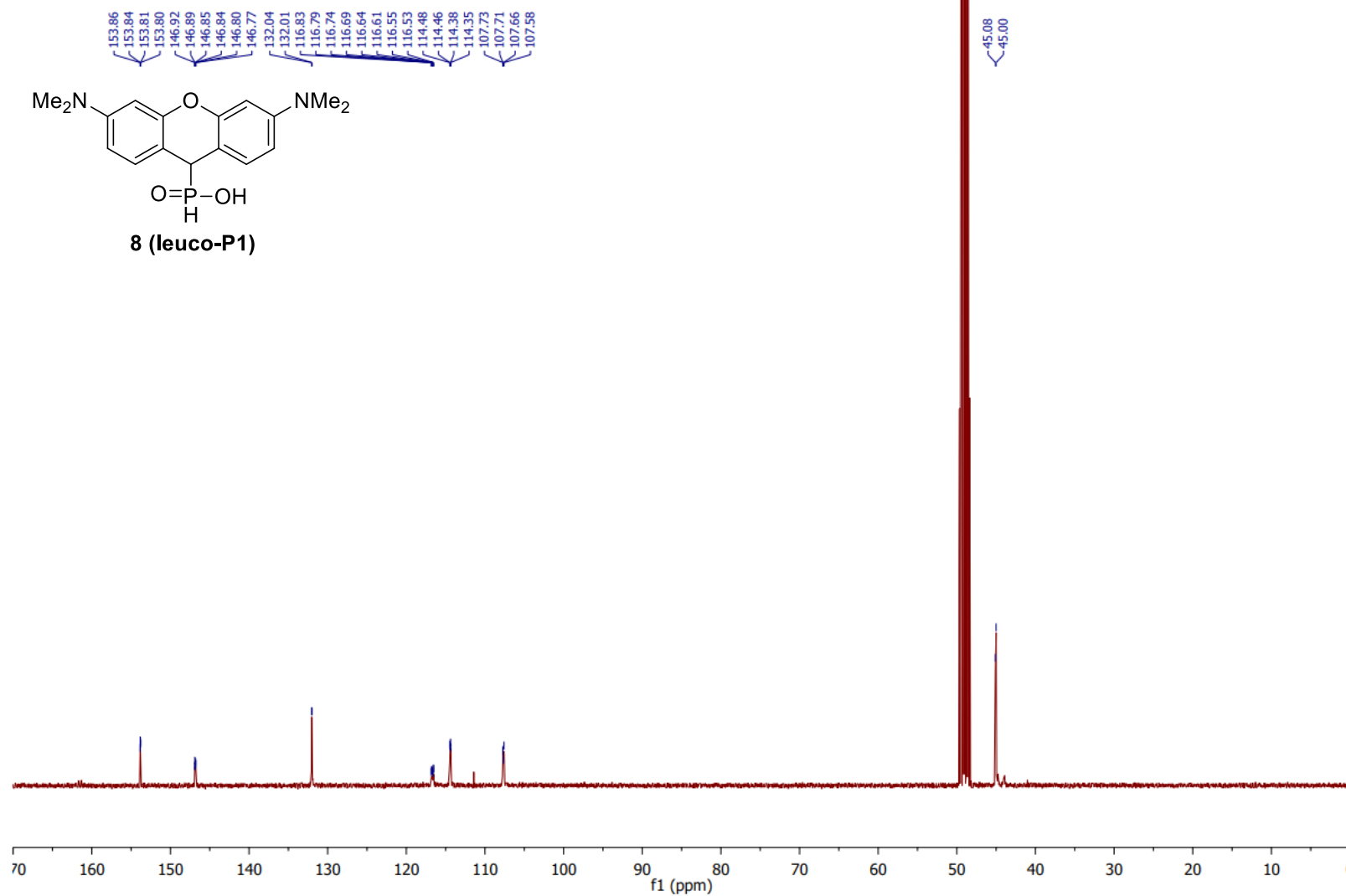
compound **8**: ^1H

shs398-3-iso-1_PROTON_01

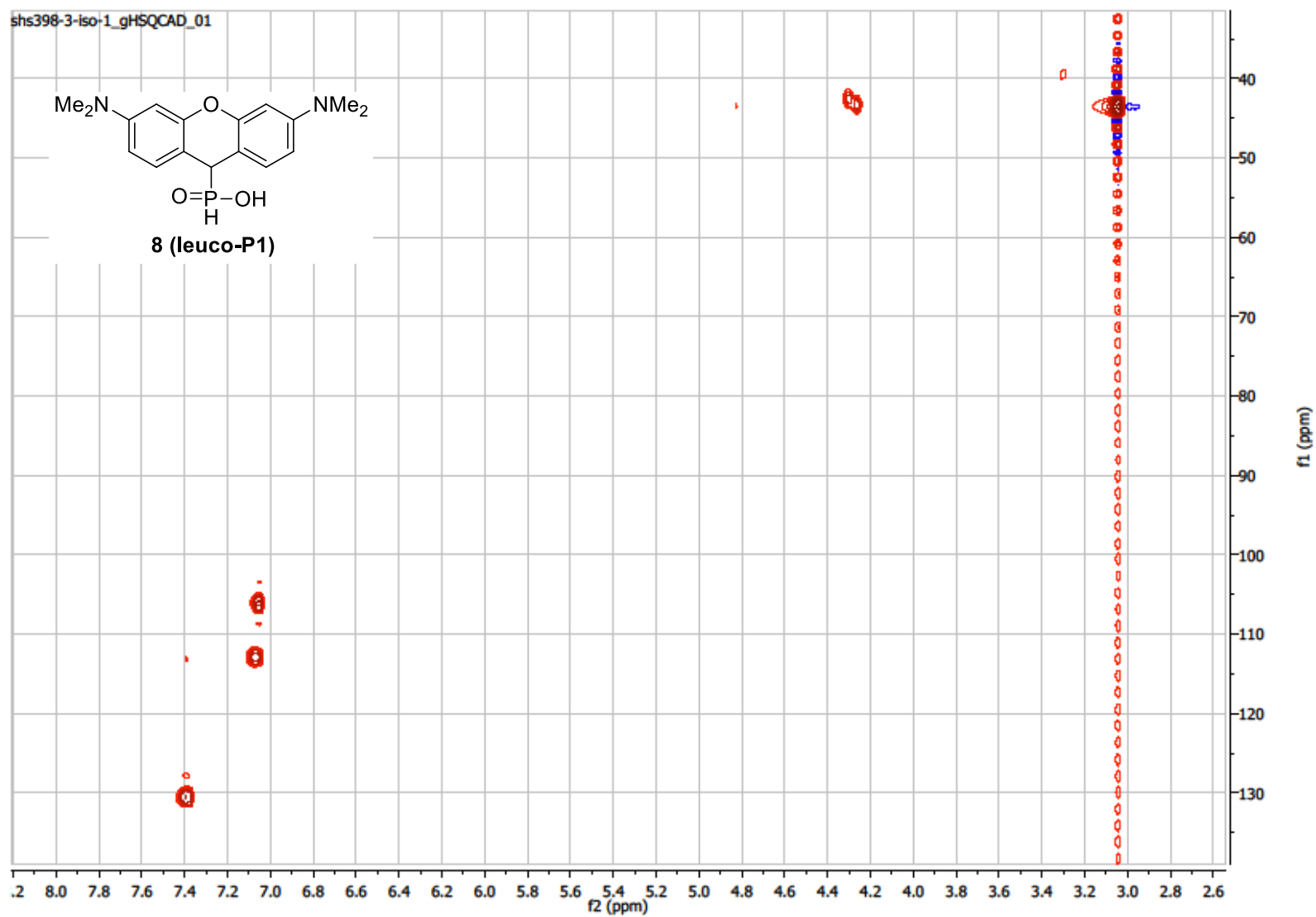


compound **8**: $^{13}\text{C}\{^1\text{H}\}$

shs398-3-iso-1_CARBON_02

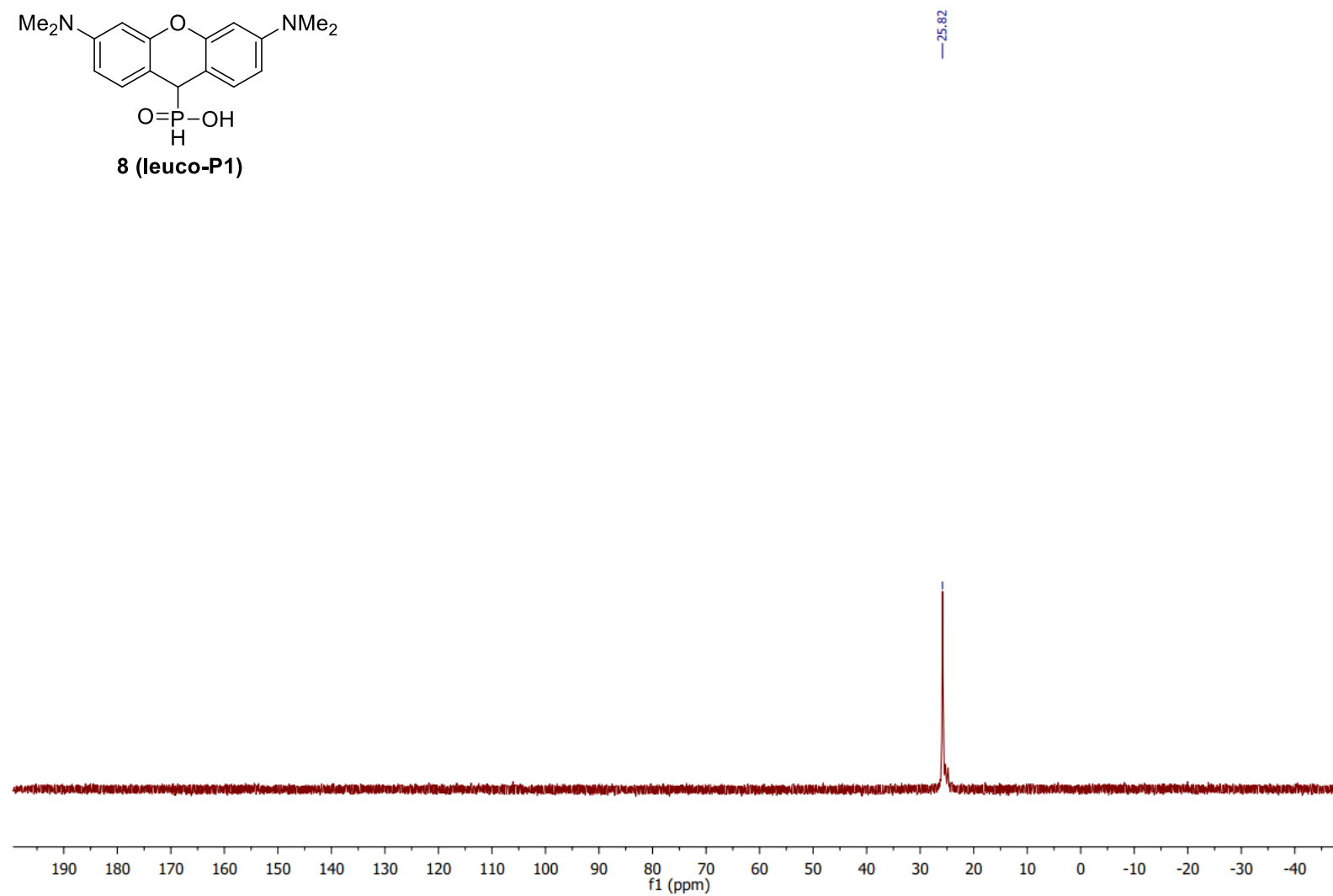
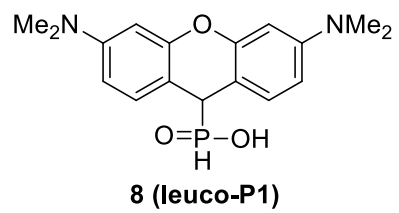


compound **8**: gHSQCad



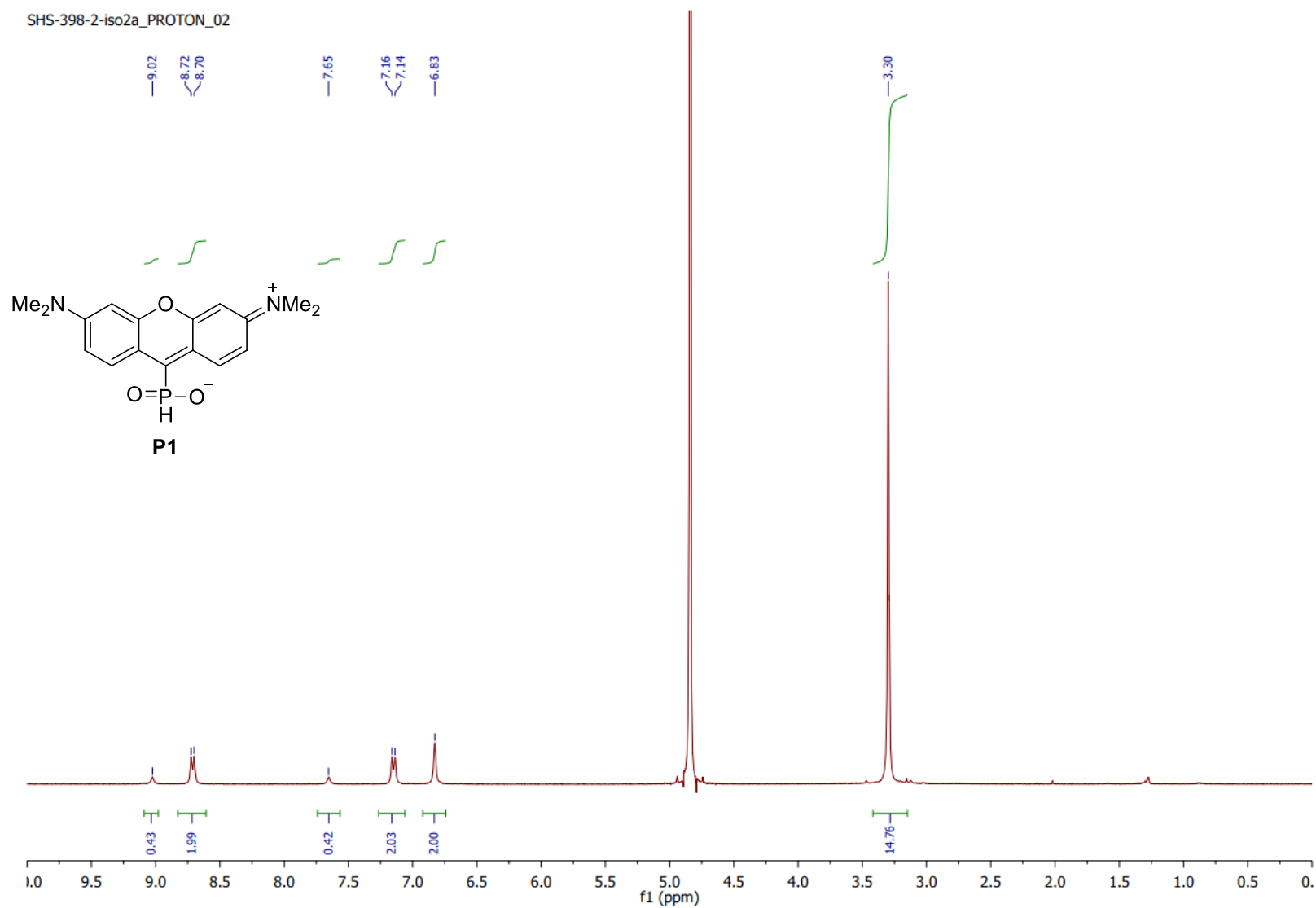
compound **8**: ^{31}P

shs398-3-iso-1_PHOSPHORUS_01

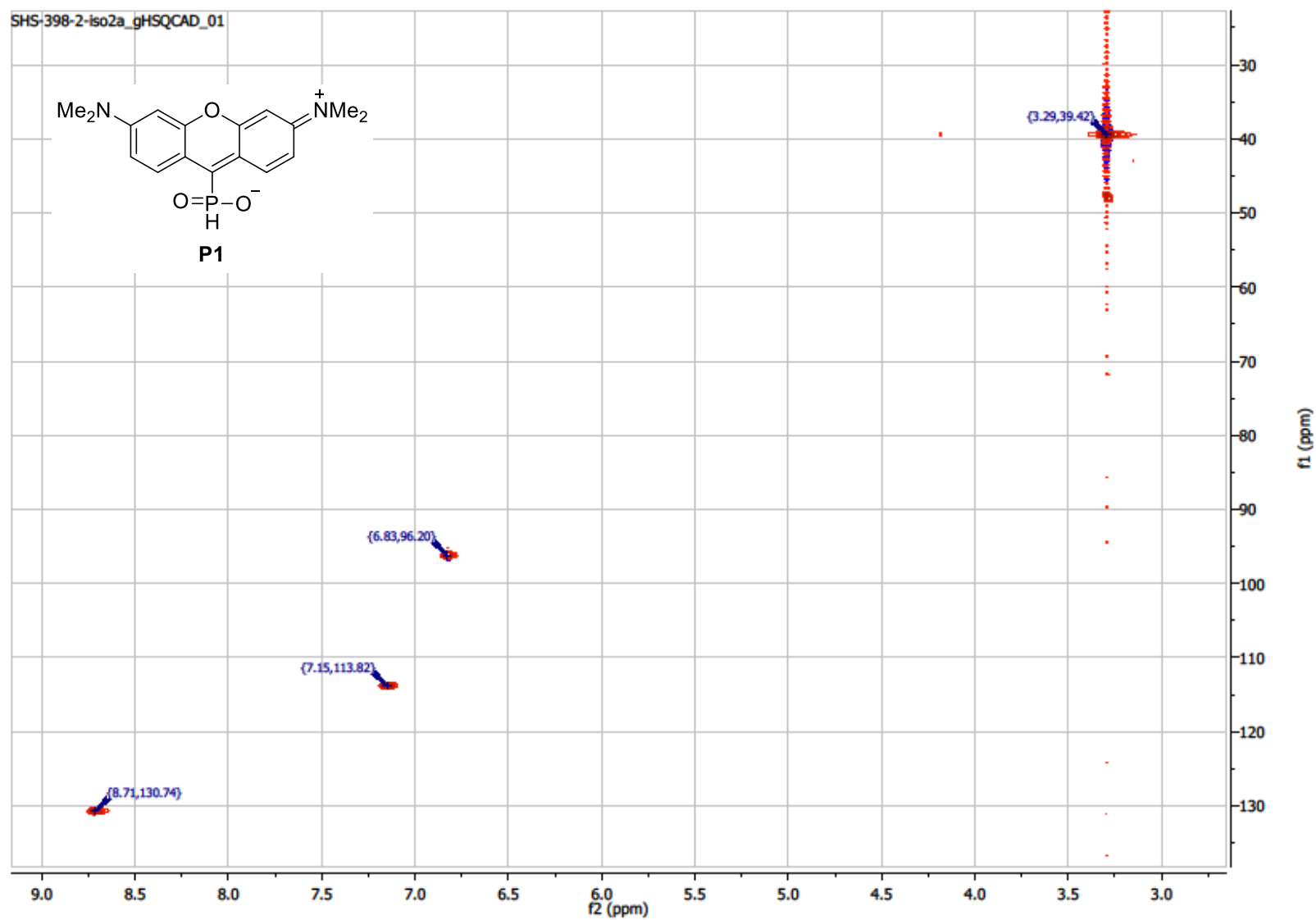


Dye P1: ¹H

SHS-398-2-iso2a_PROTON_02

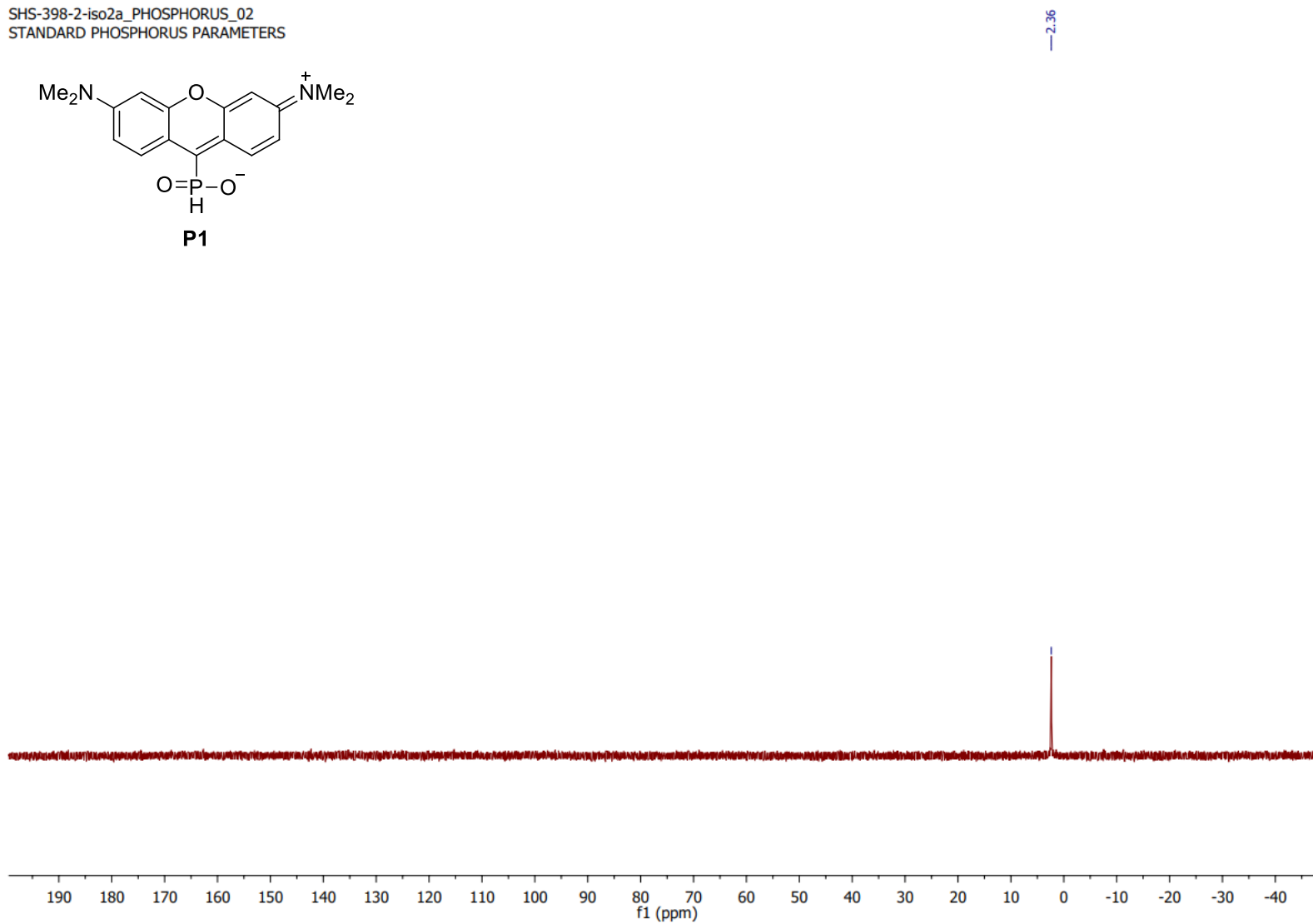
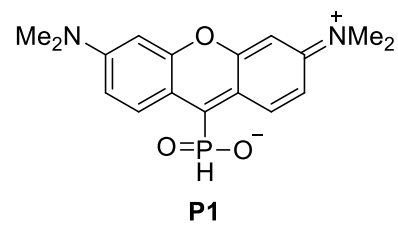


Dye **P1**: gHSQCAD

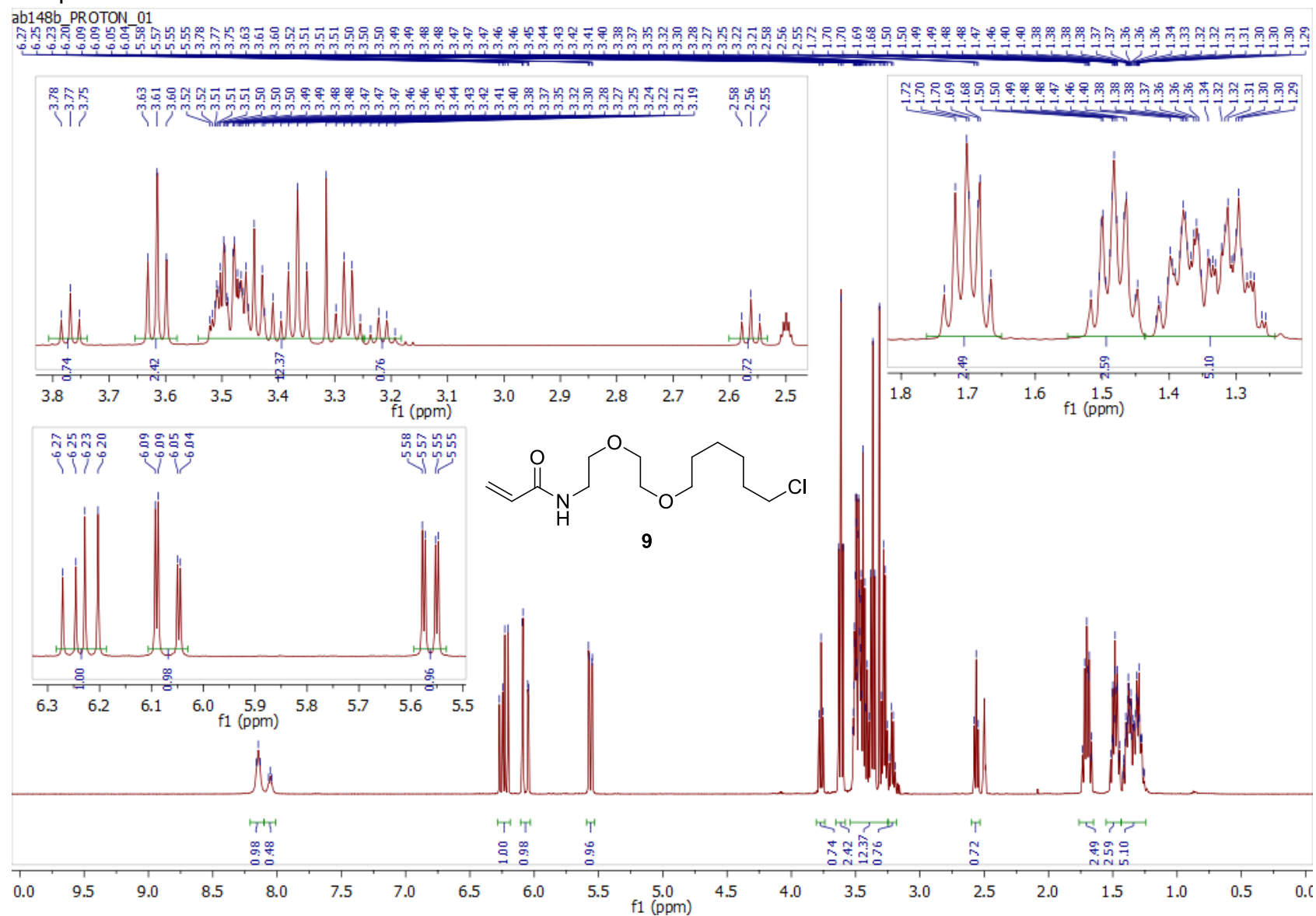


Dye P1: ^{31}P

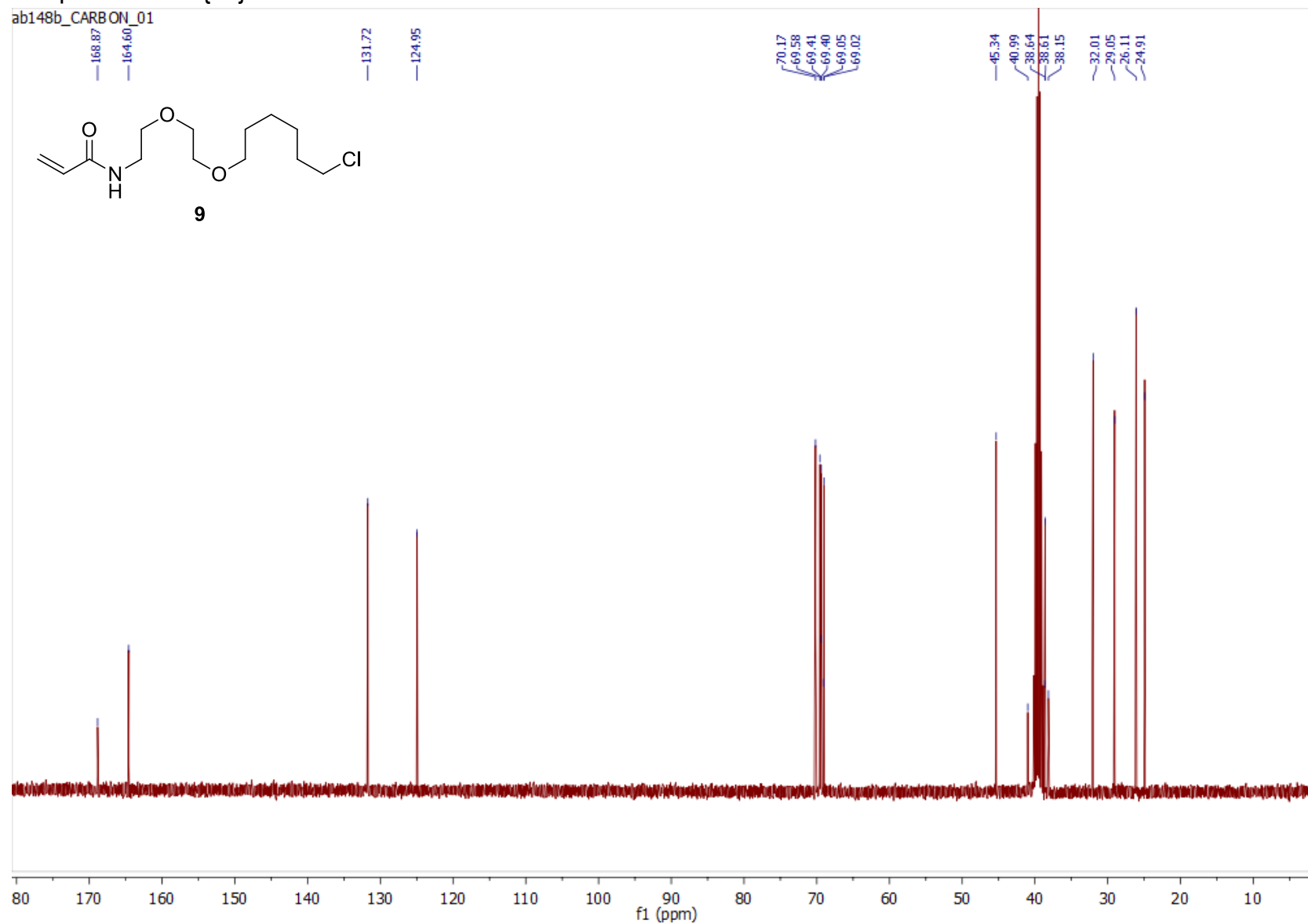
SHS-398-2-iso2a_PHOSPHORUS_02
STANDARD PHOSPHORUS PARAMETERS



compound **9**: ^1H

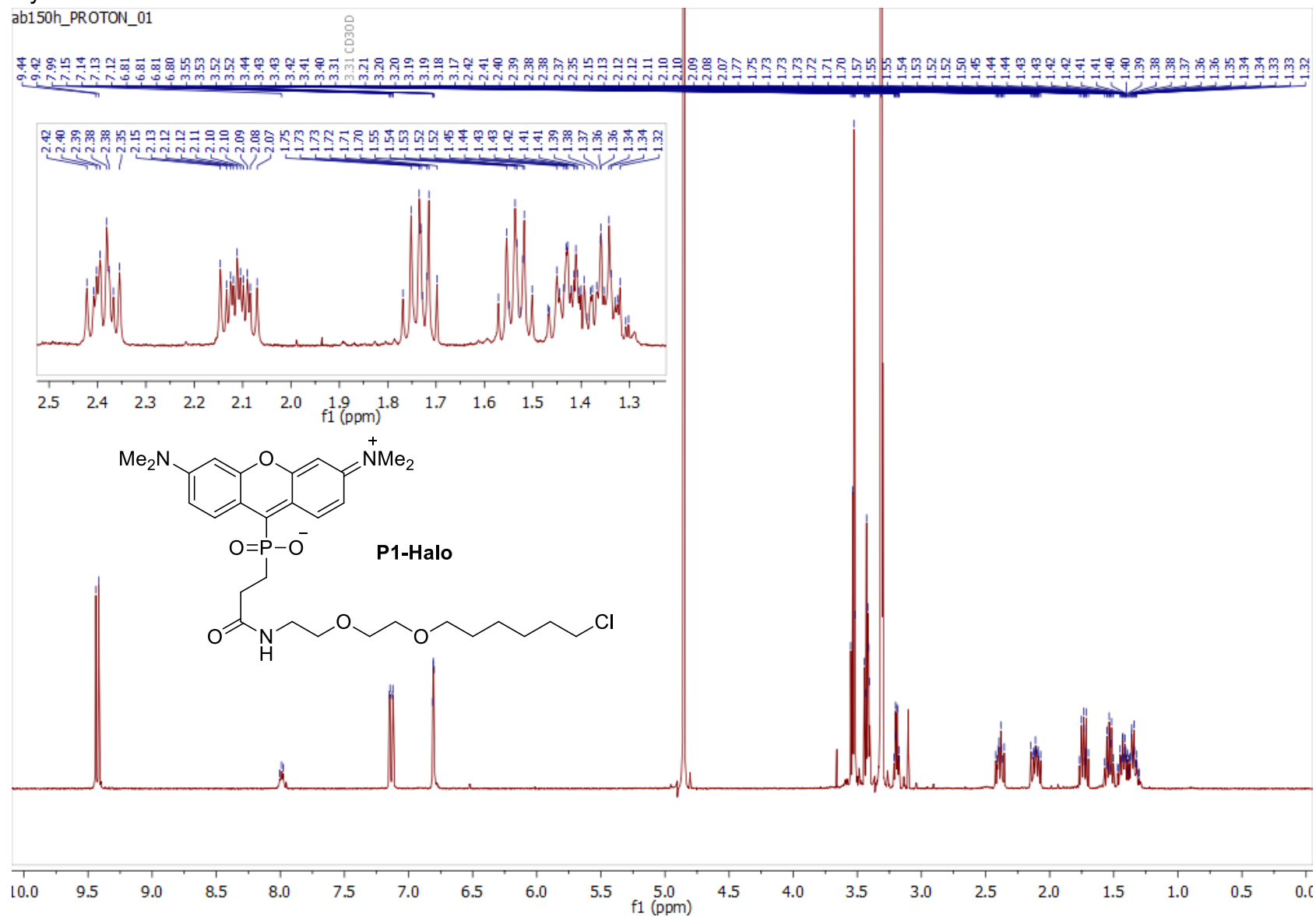


ab148b_CARBON_01

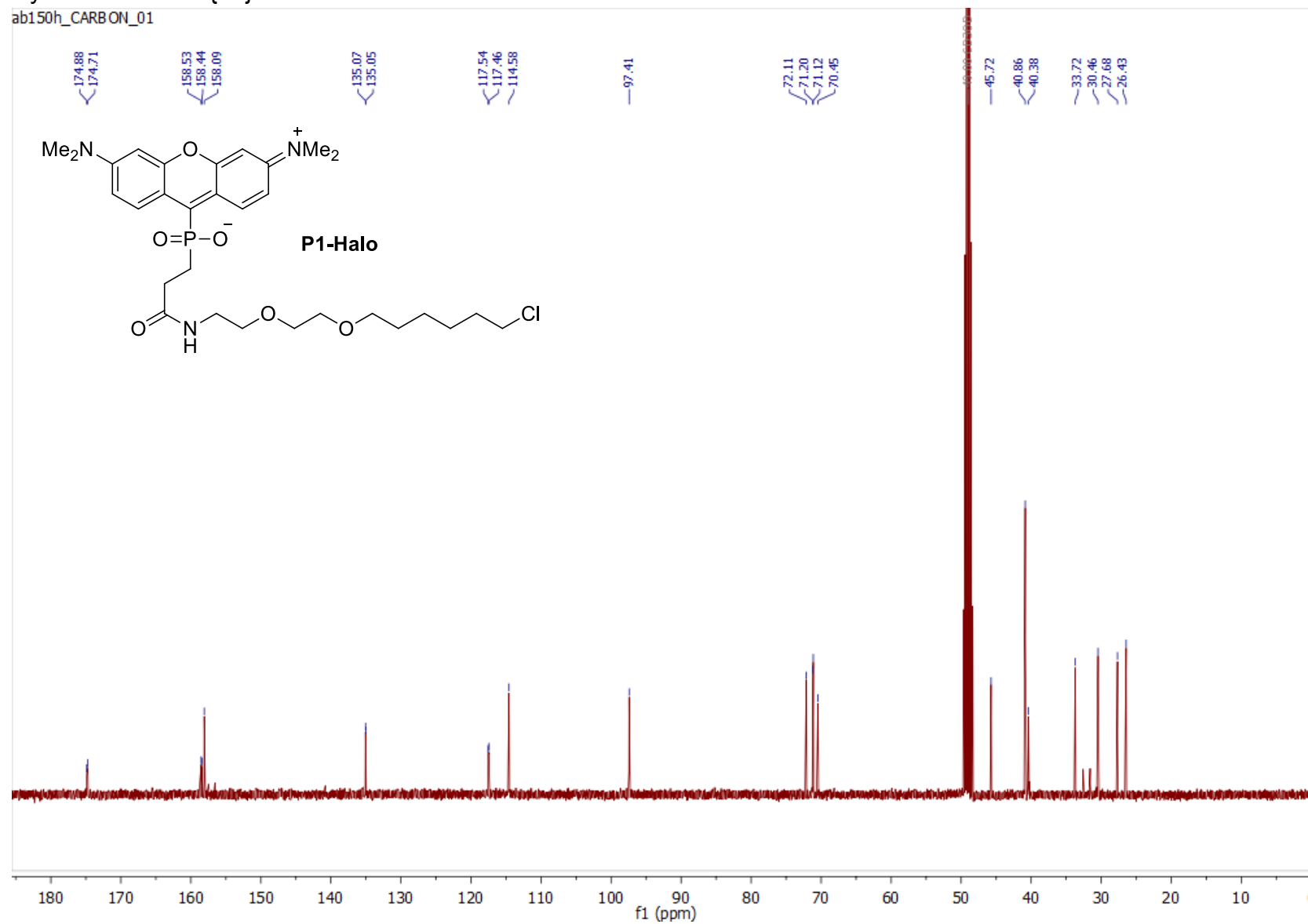


Dye P1-Halo: ¹H

ab150h_PROTON_01

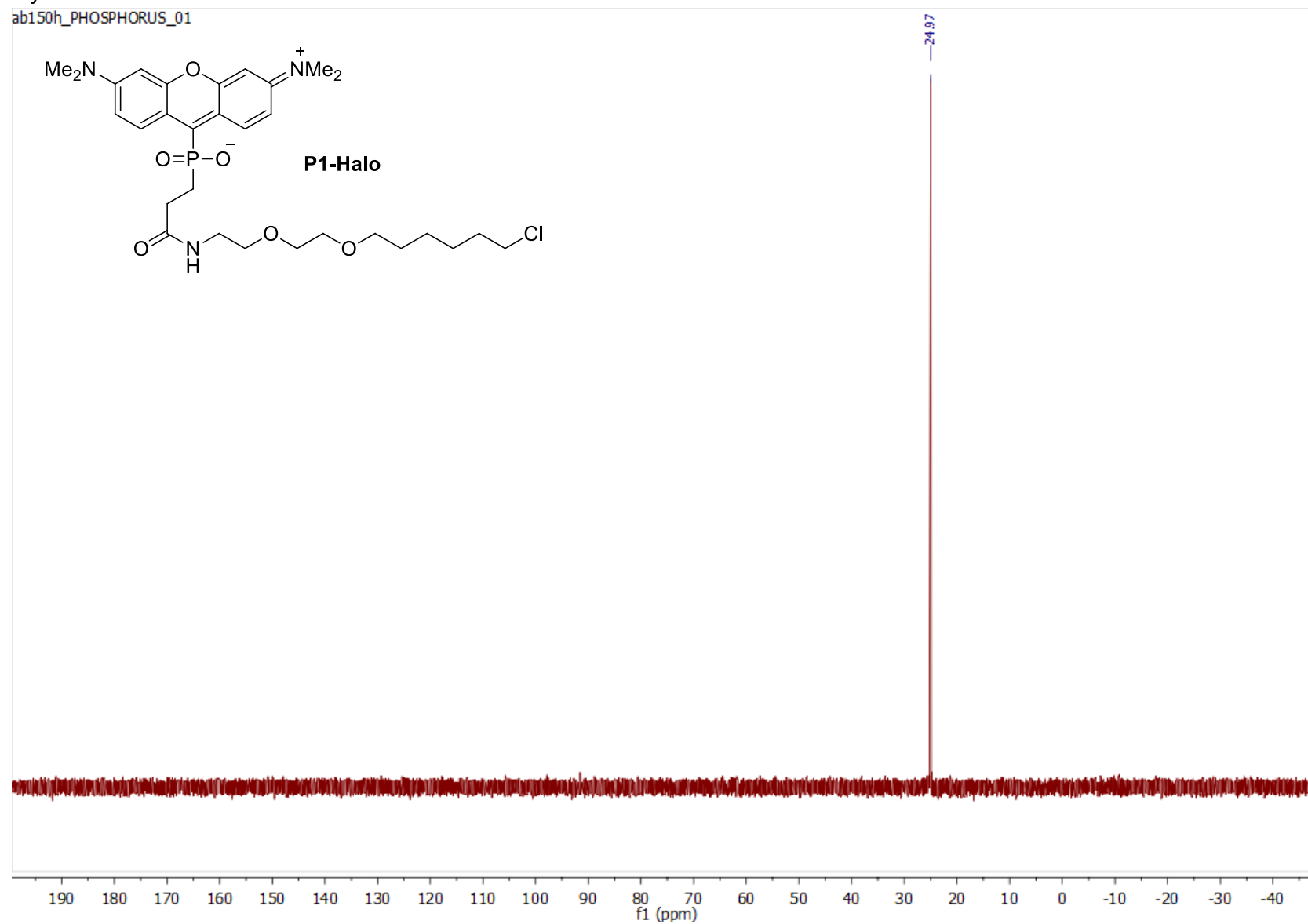


ab150h_CARBON_01



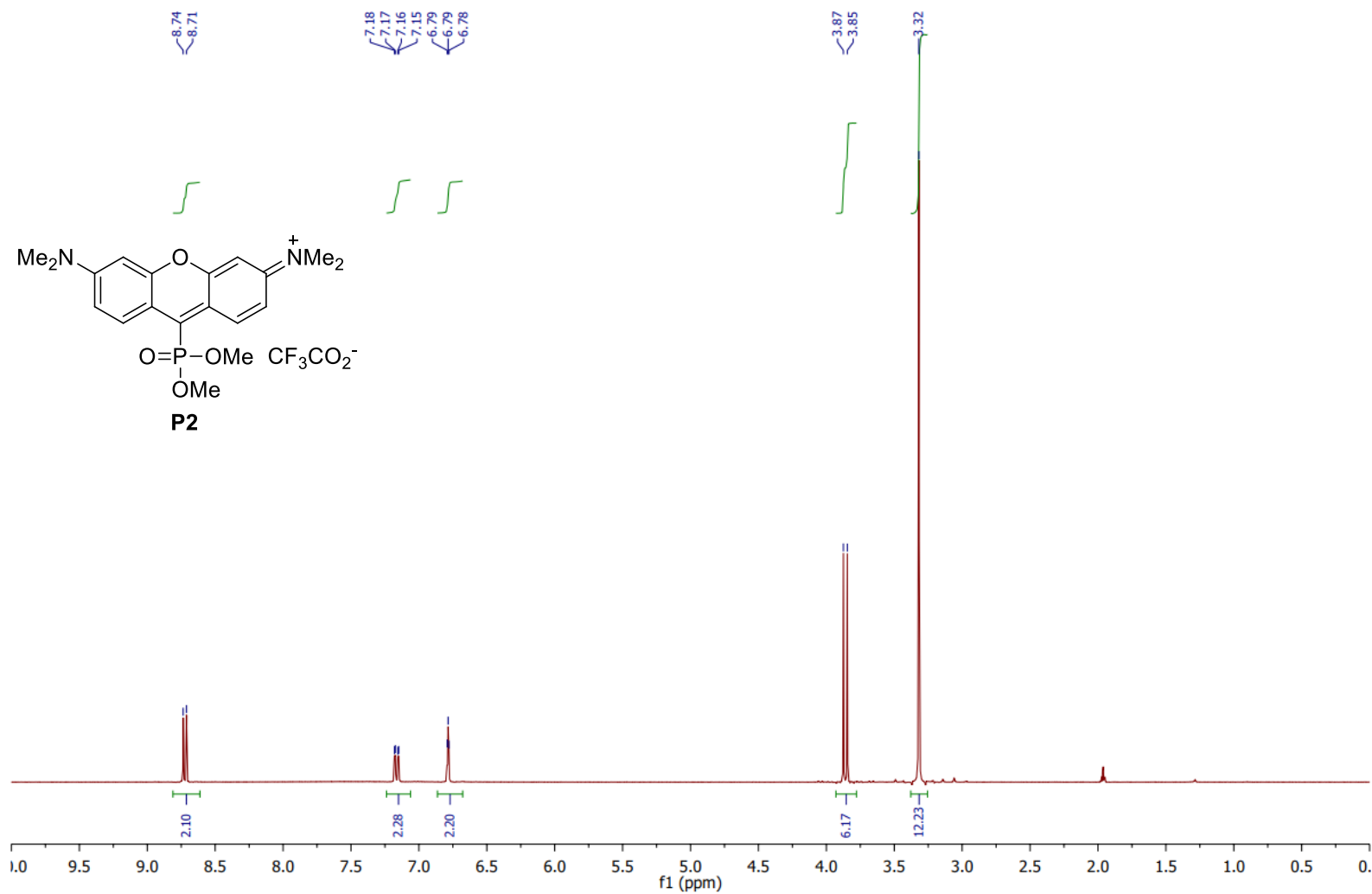
Dye **P1-Halo**: ^{31}P

ab150h_PHOSPHORUS_01



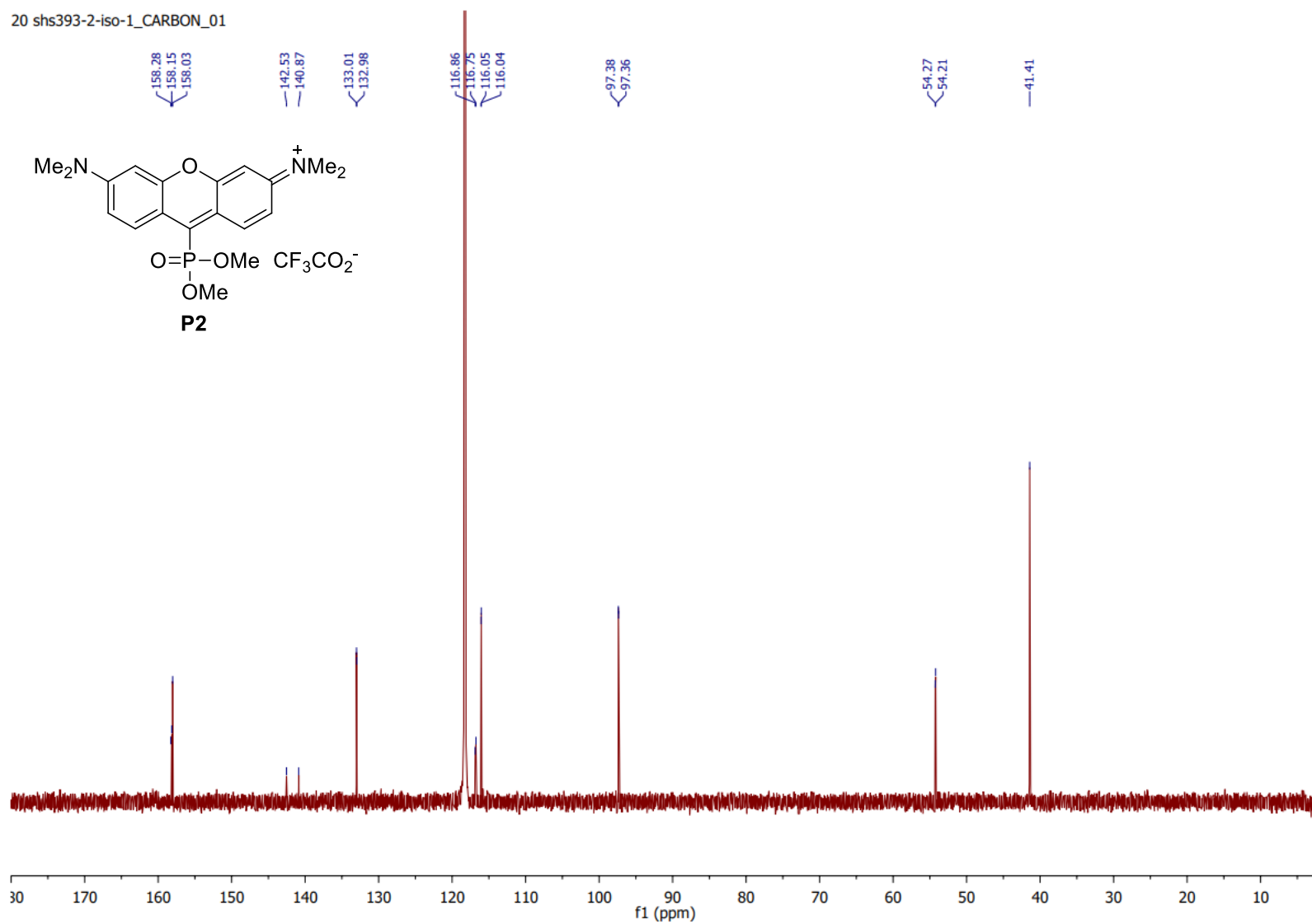
Dye P2: ^1H

20 shs393-2-iso-1_PROTON_01



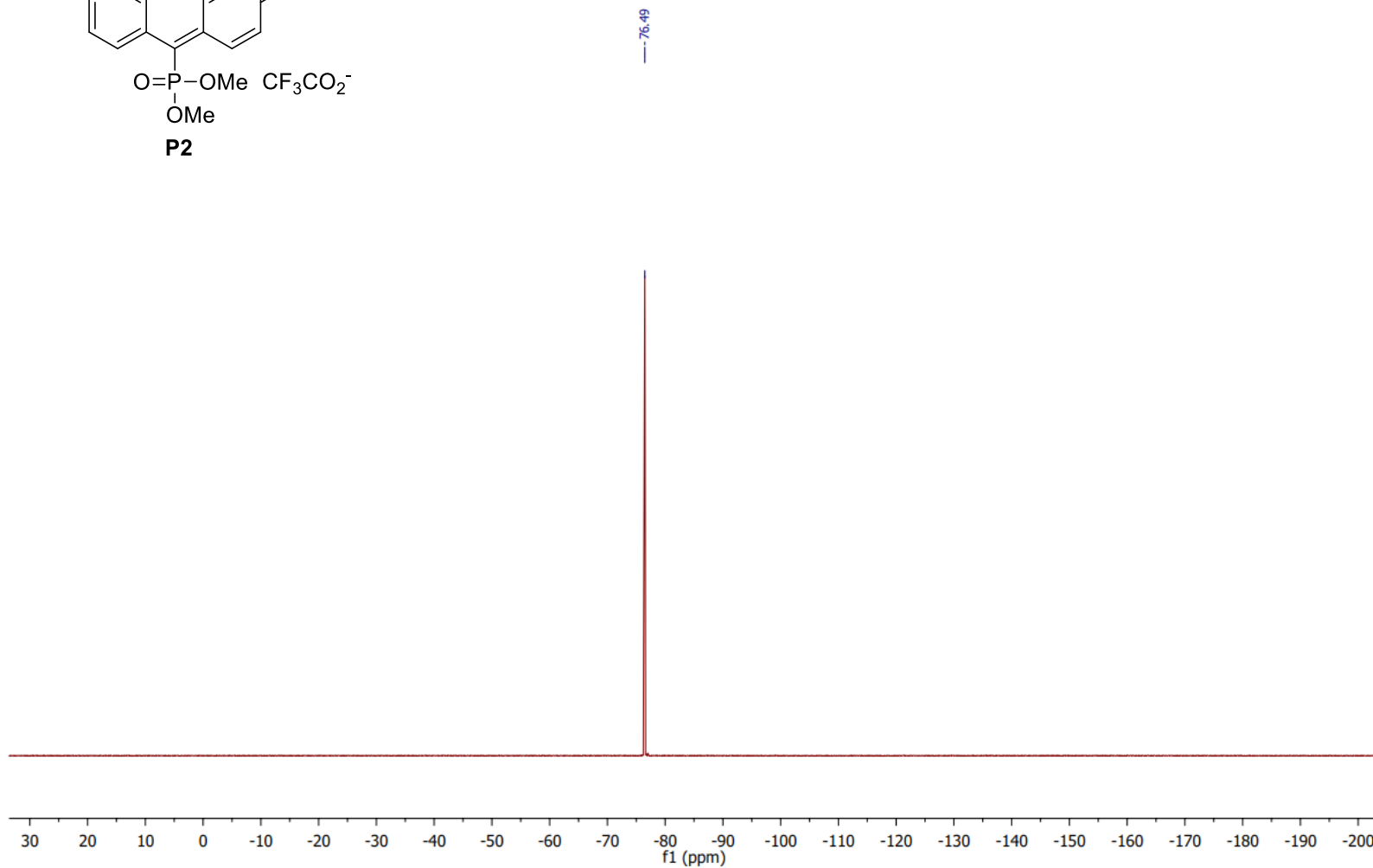
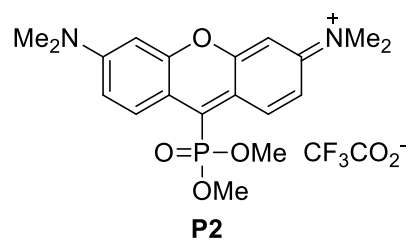
Dye **P2**: $^{13}\text{C}\{^1\text{H}\}$

20 shs393-2-iso-1_CARBON_01



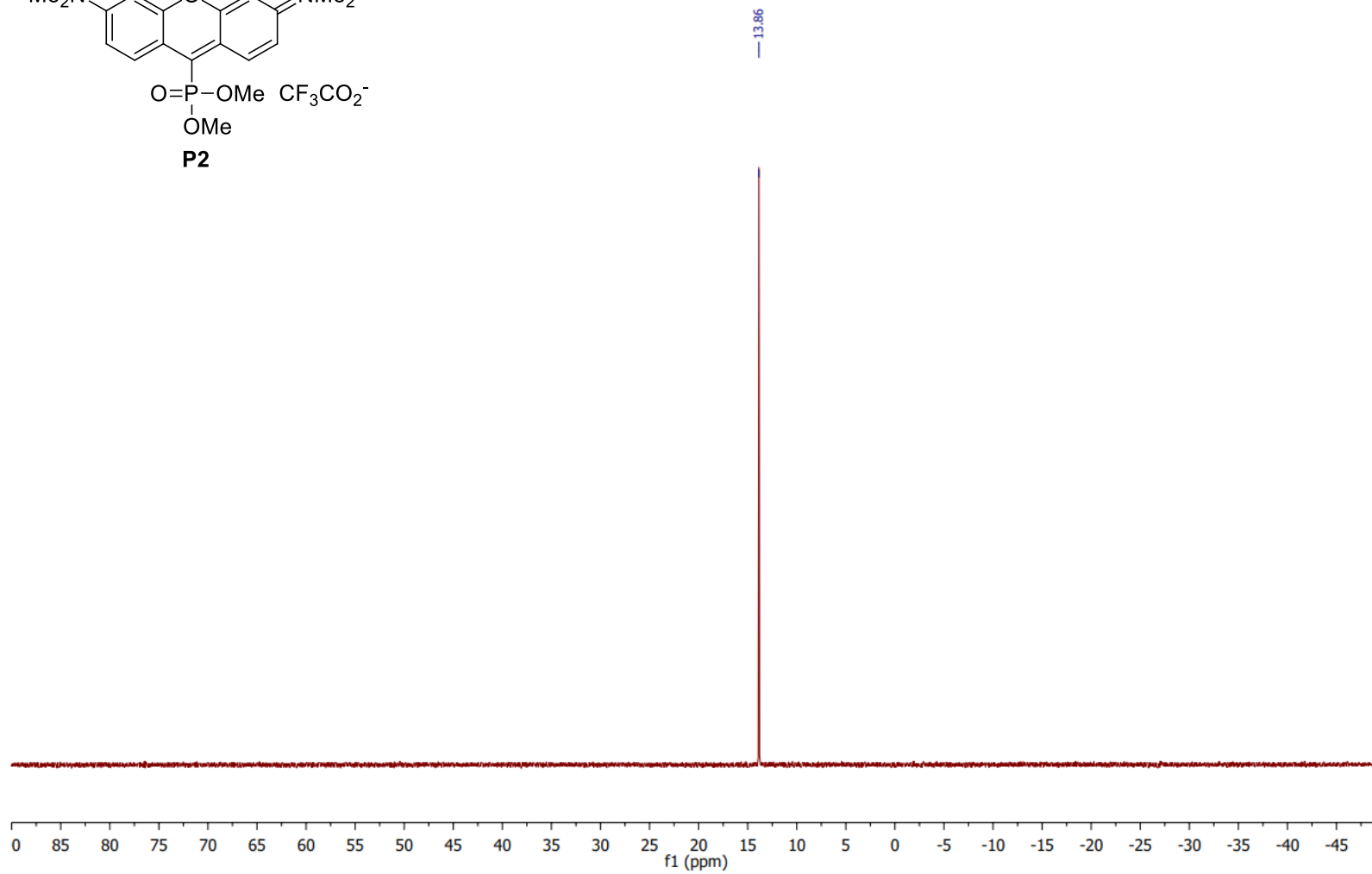
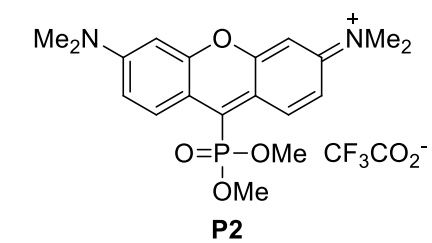
Dye **P2**: ^{19}F

20 shs393-2-iso-1_FLUORINE_01



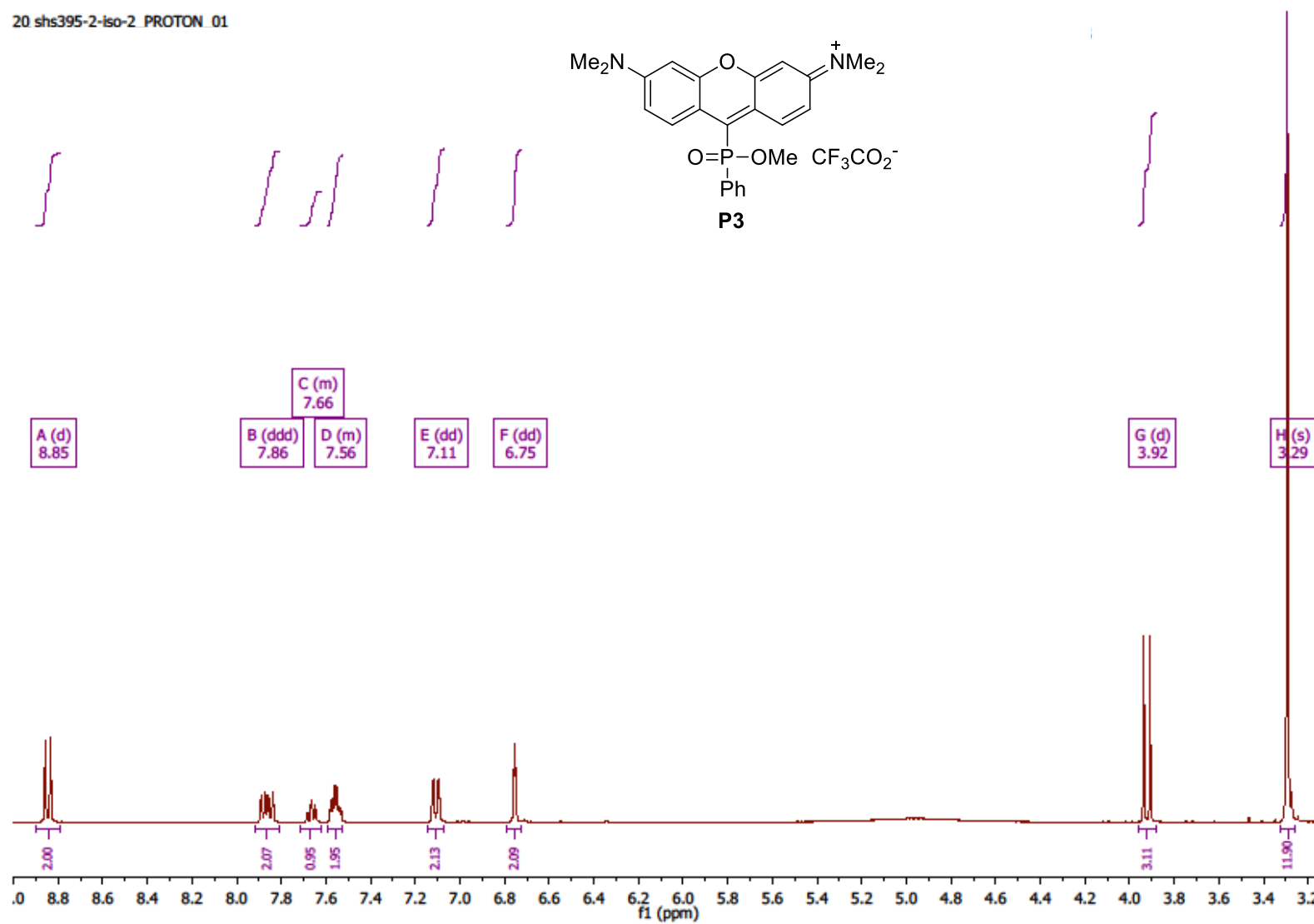
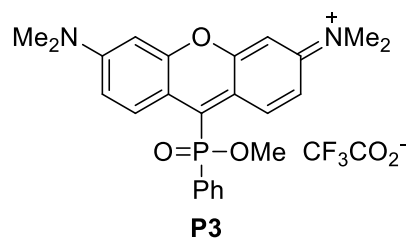
Dye P2: ^{31}P

20 shs393-2-iso-1_PHOSPHORUS_01

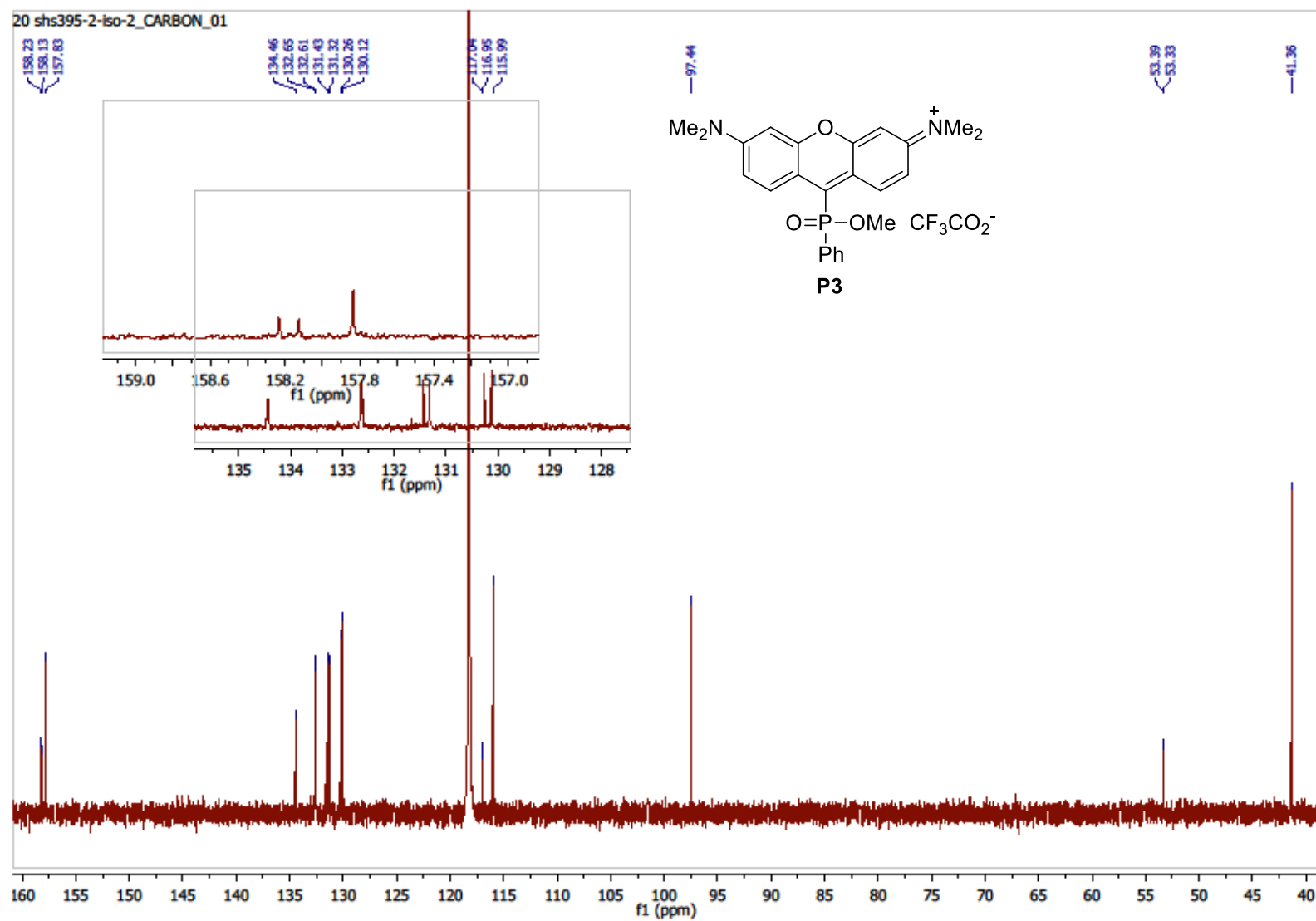


Dye P3: ¹H

20 shs395-2-iso-2 PROTON .01

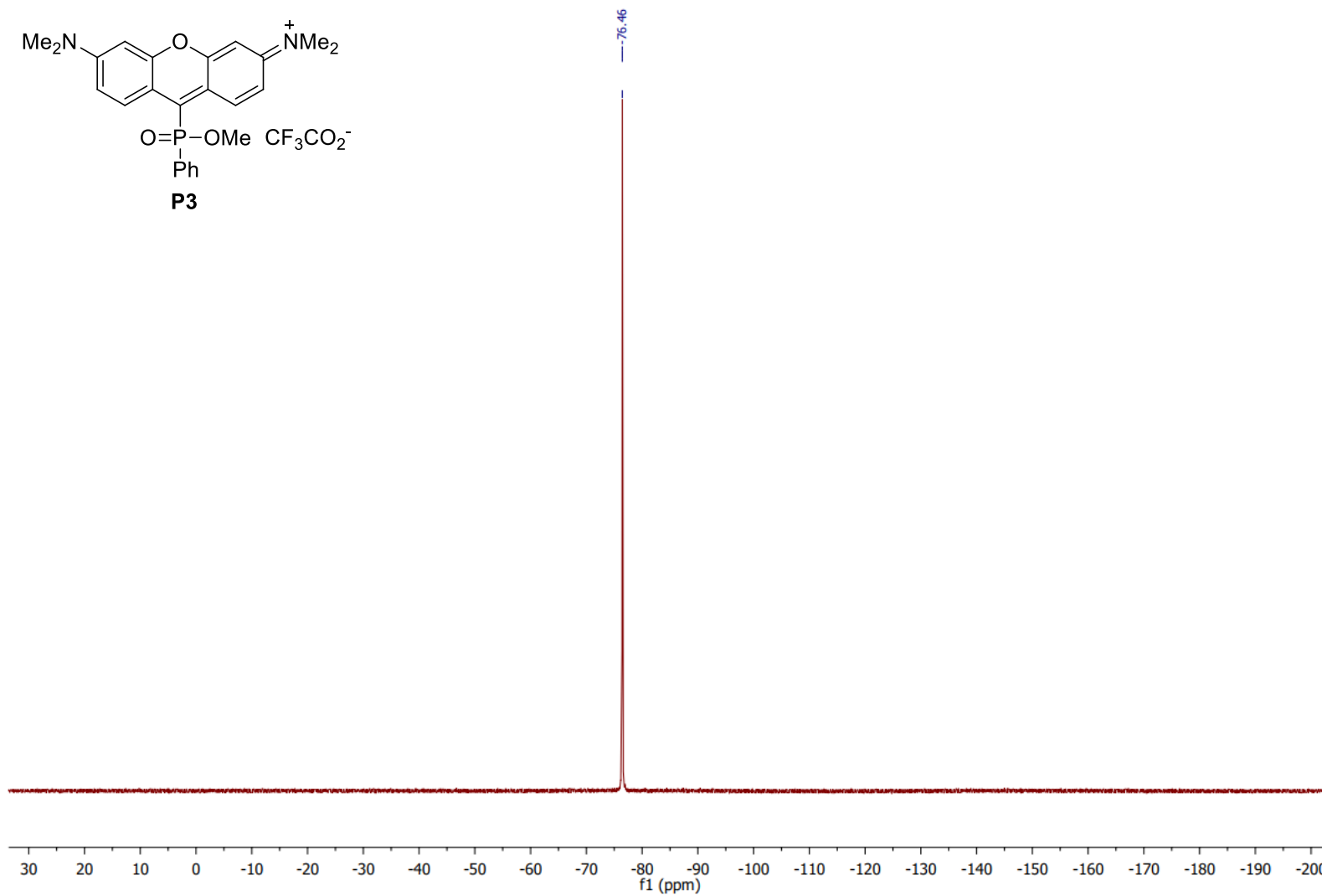
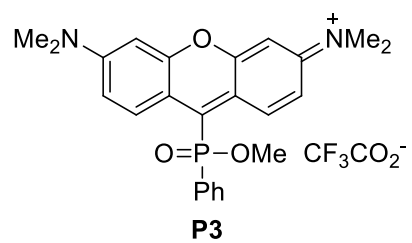


Dye **P3**: $^{13}\text{C}\{^1\text{H}\}$



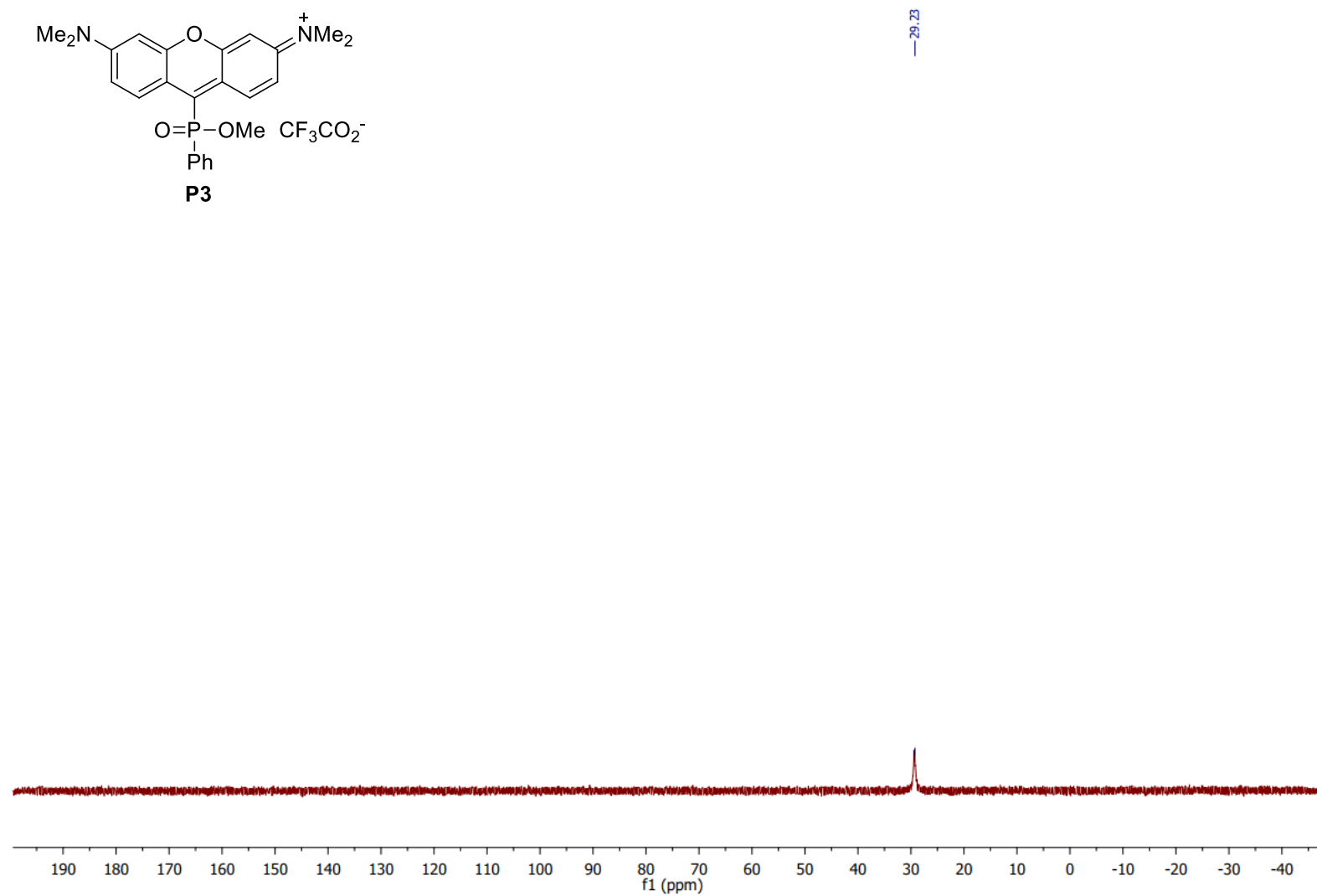
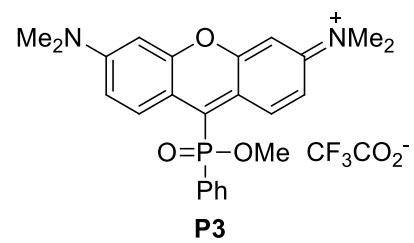
Dye **P3**: ^{19}F

20 shs395-2-iso-2_FLUORINE_01



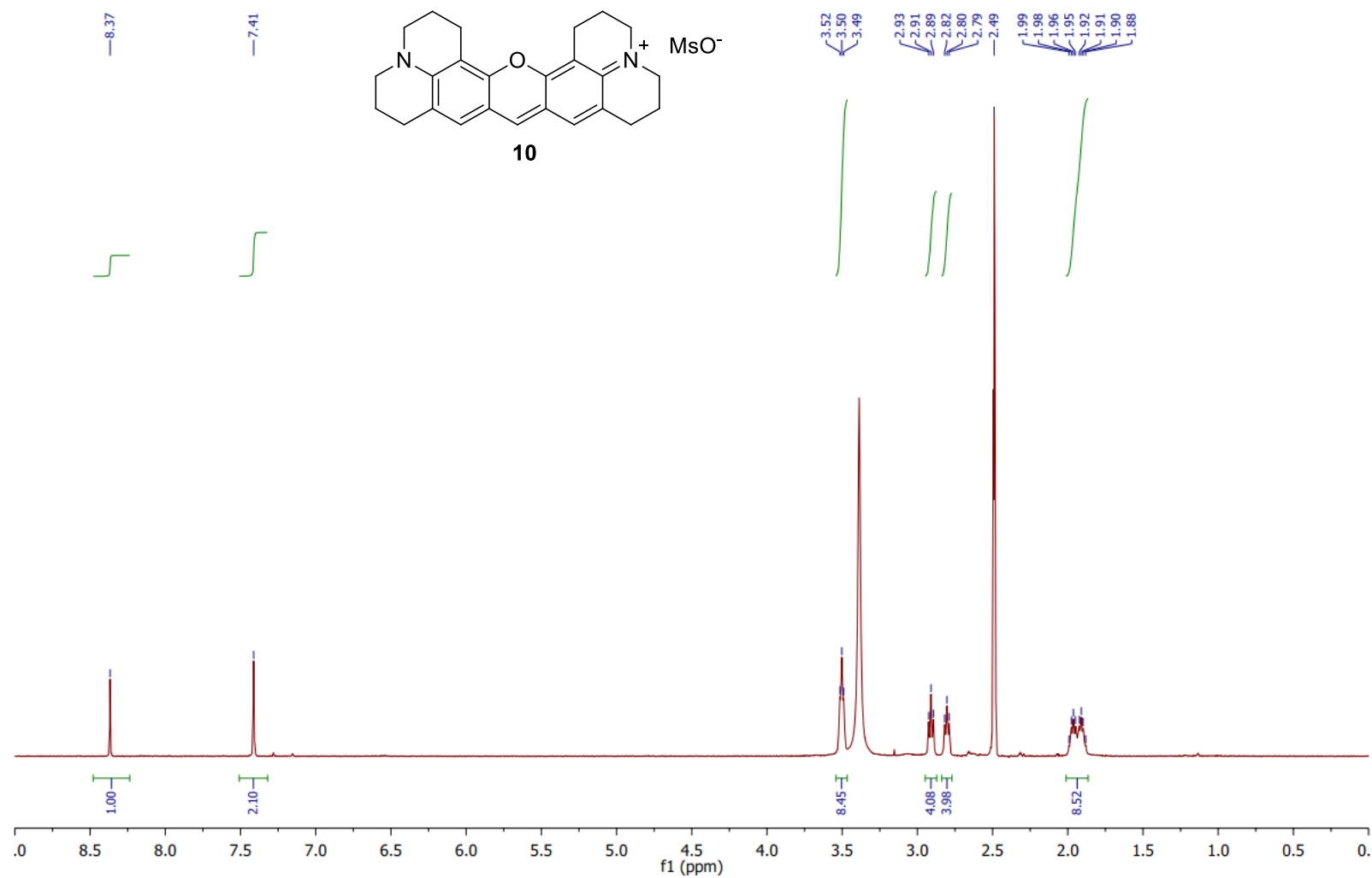
Dye **P3**: ^{31}P

20 shs395-2-iso-2_PHOSPHORUS_01



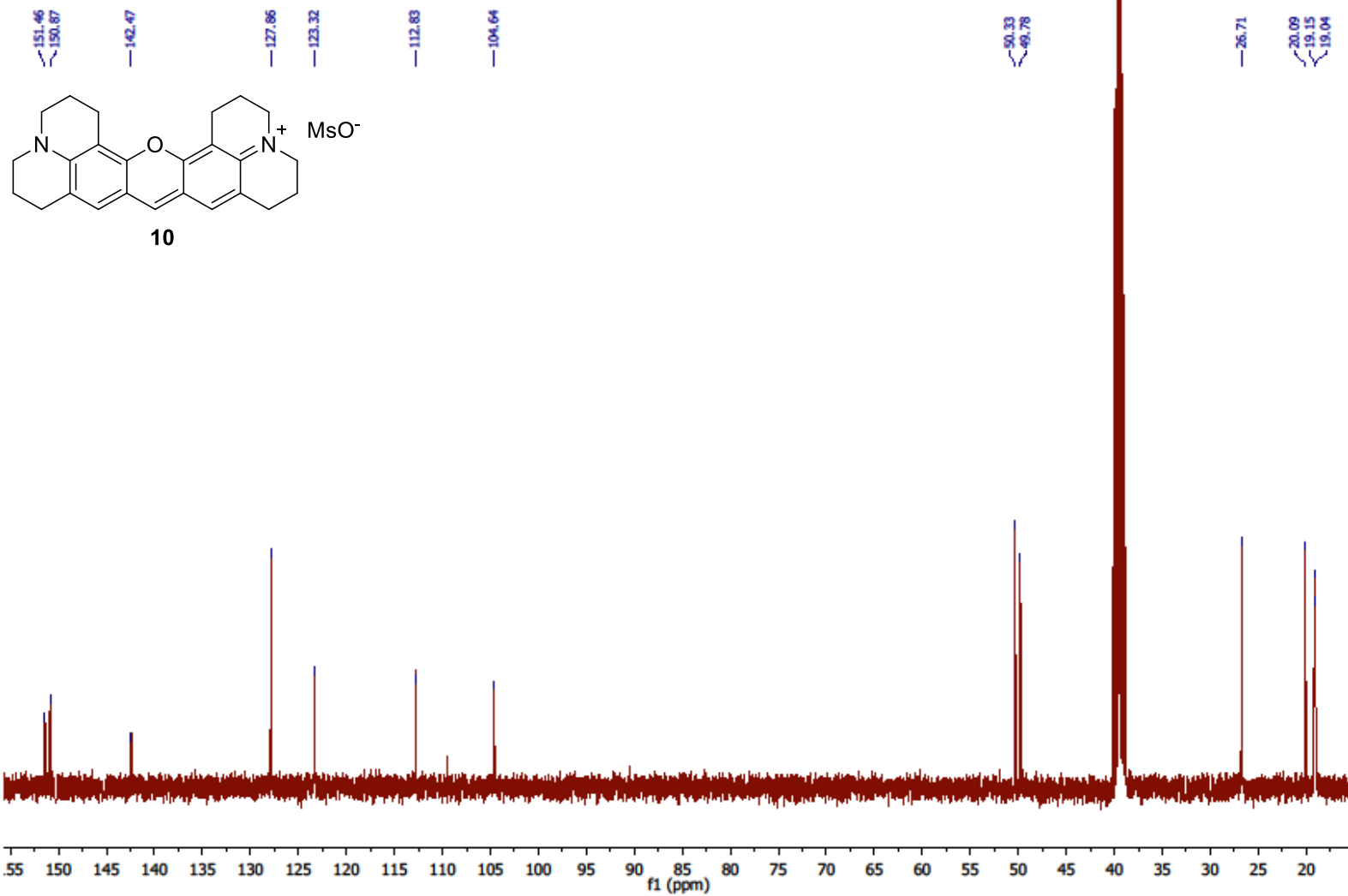
compound **10**: ^1H

shs385-3-iso2b_PROTON_01



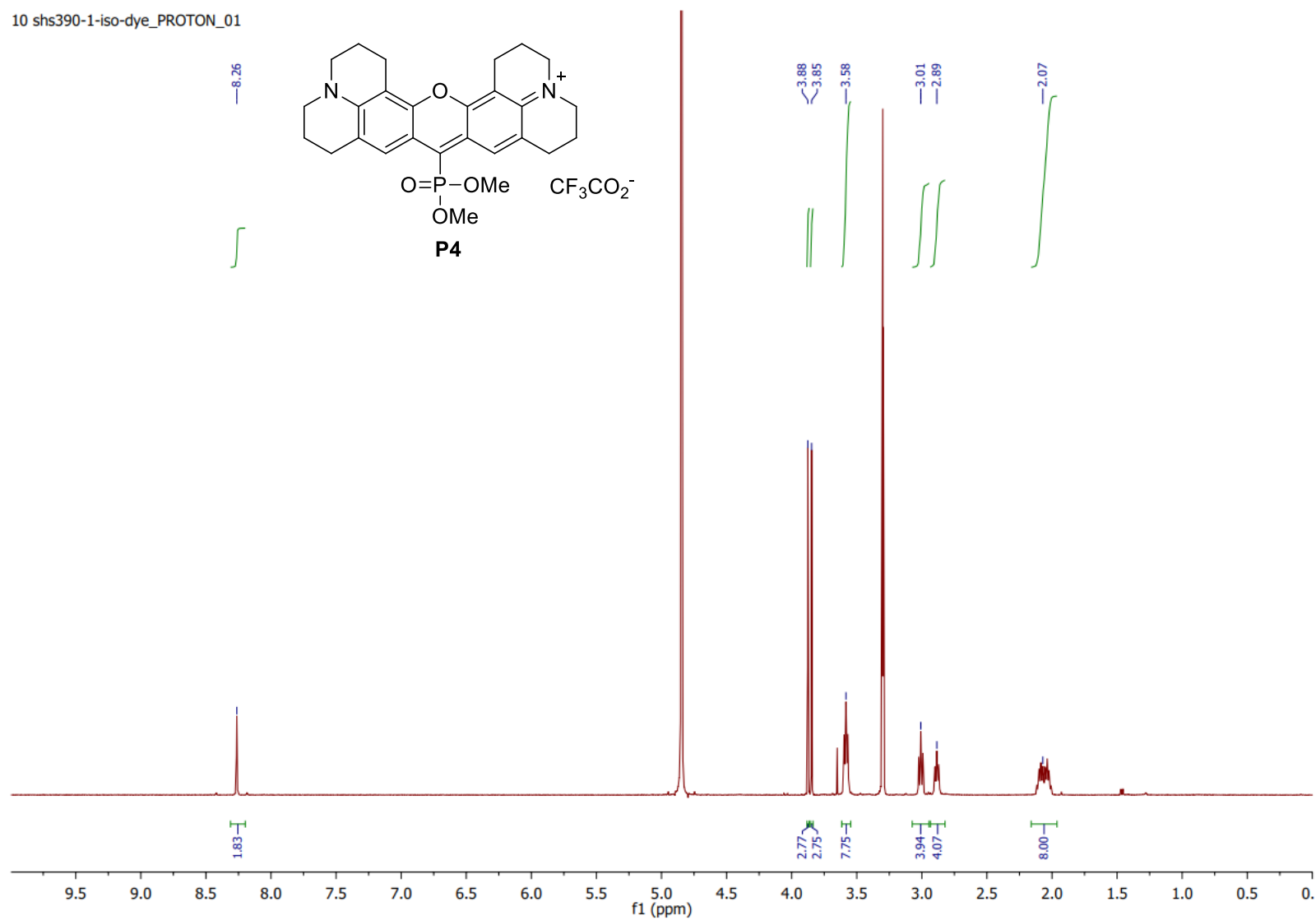
compound **10**: $^{13}\text{C}\{^1\text{H}\}$

shs385-4-iso_CARBON_01



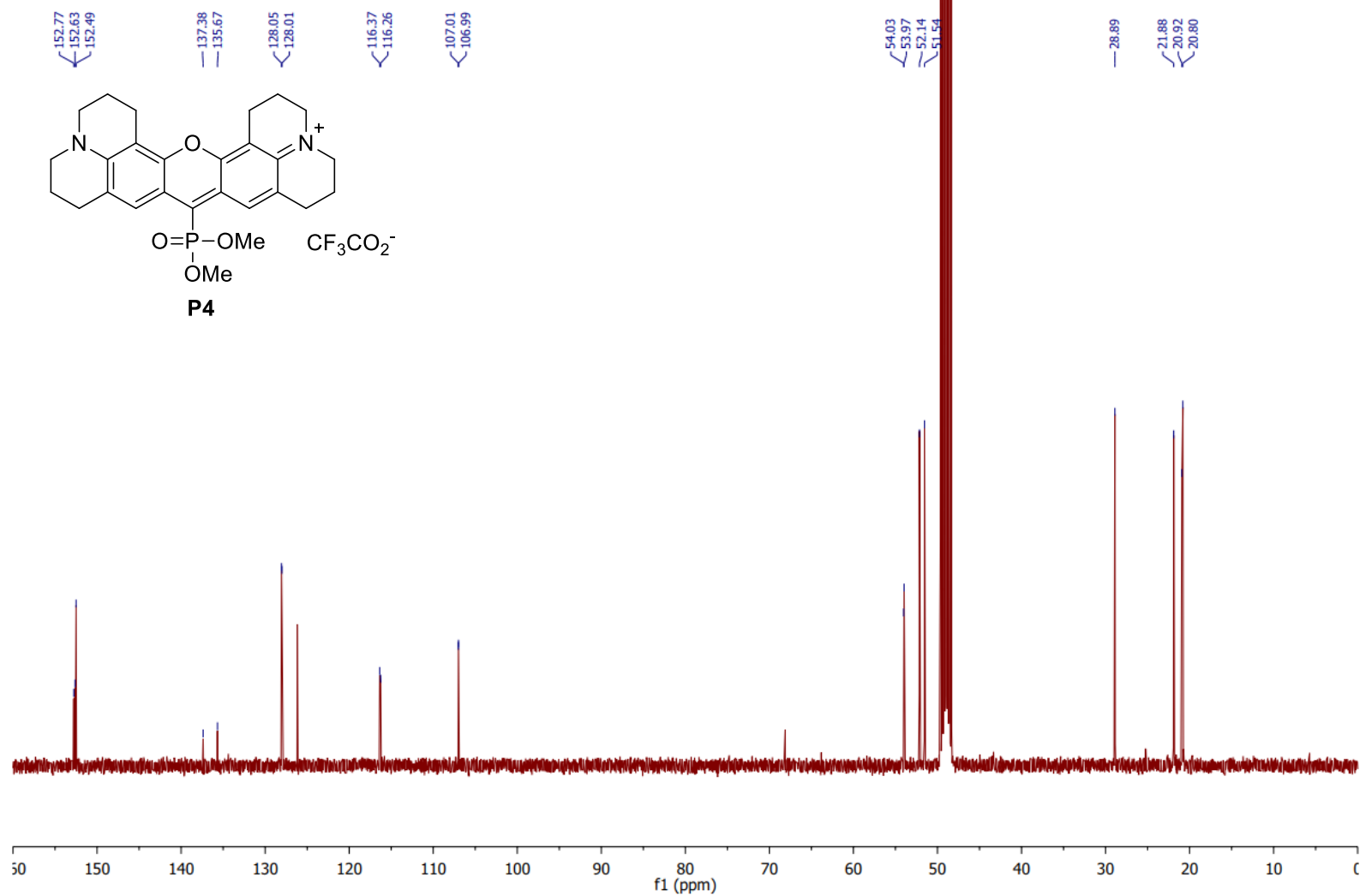
Dye P4: ^1H

10 shs390-1-iso-dye_PROTON_01



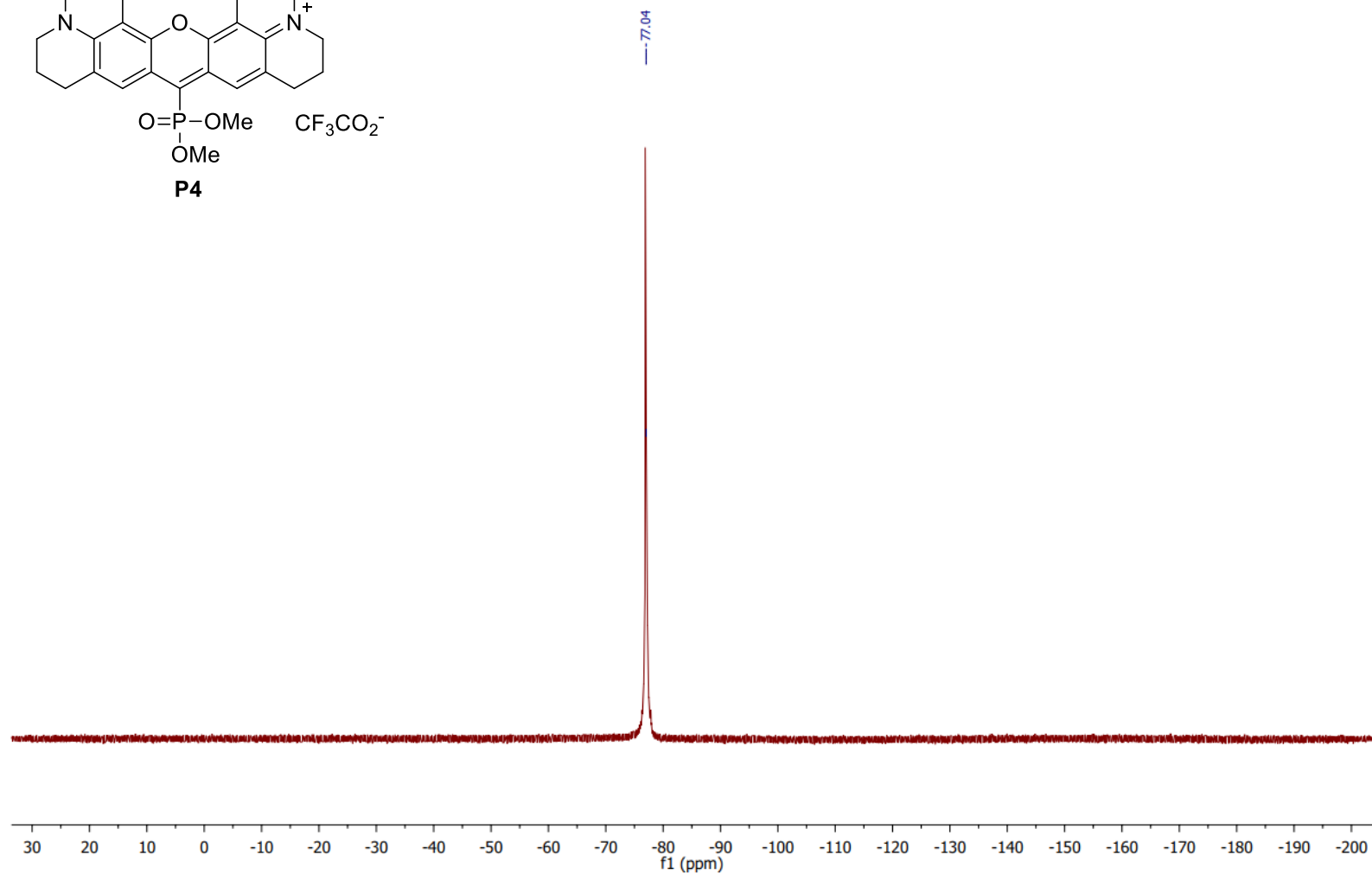
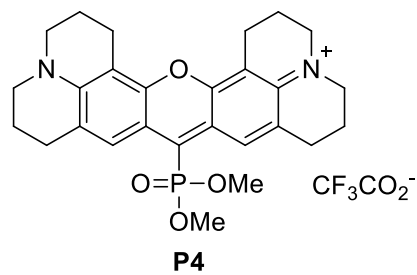
Dye **P4**: $^{13}\text{C}\{^1\text{H}\}$

10 shs390-1-iso-dye_CARBON_01



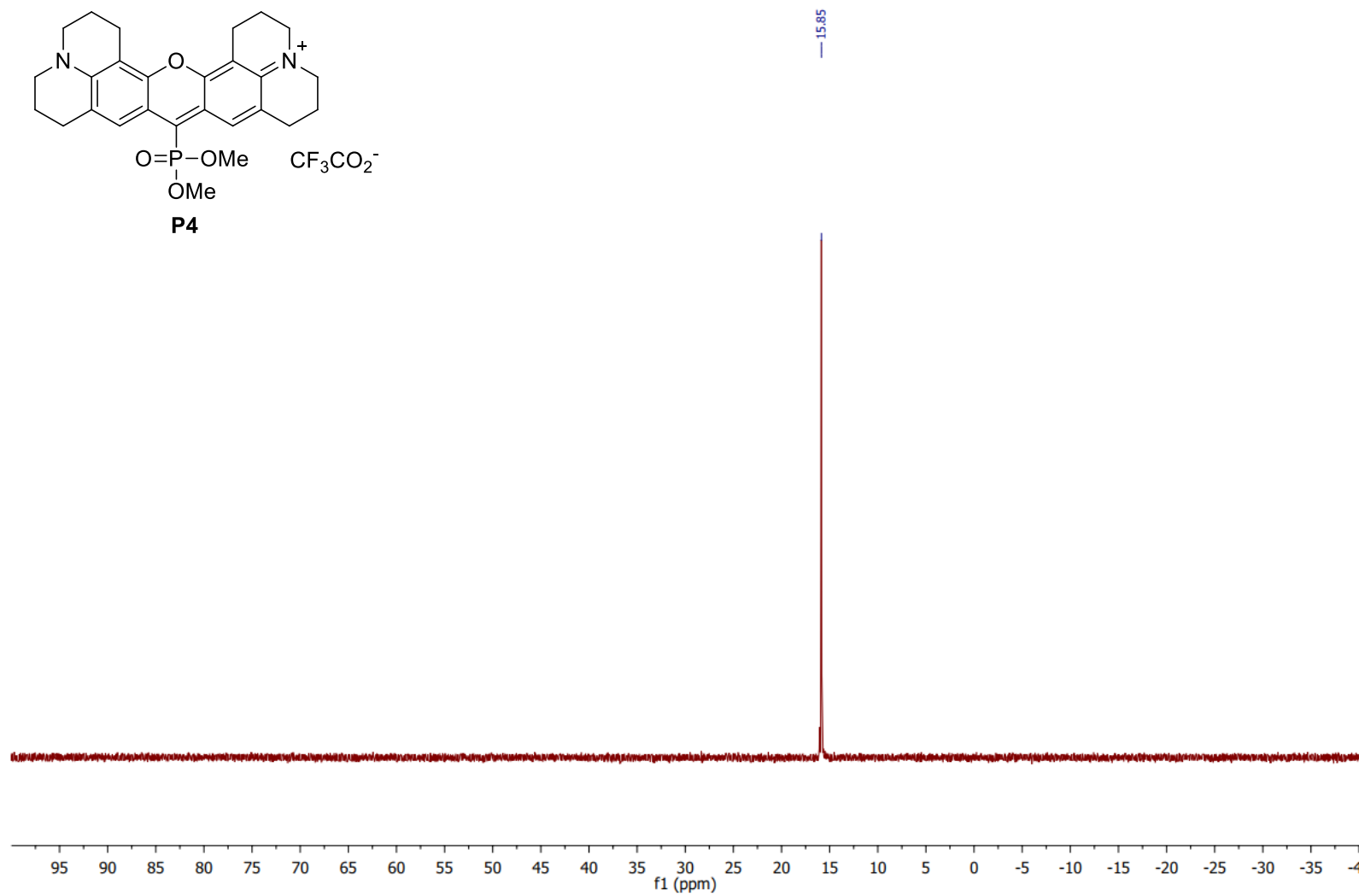
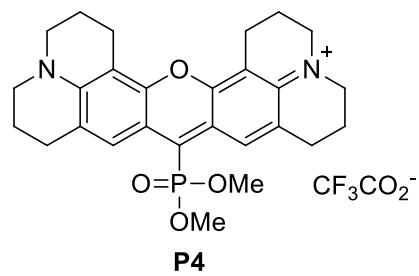
Dye **P4**: ^{19}F

10 shs390-1-iso-dye_FLUORINE_01



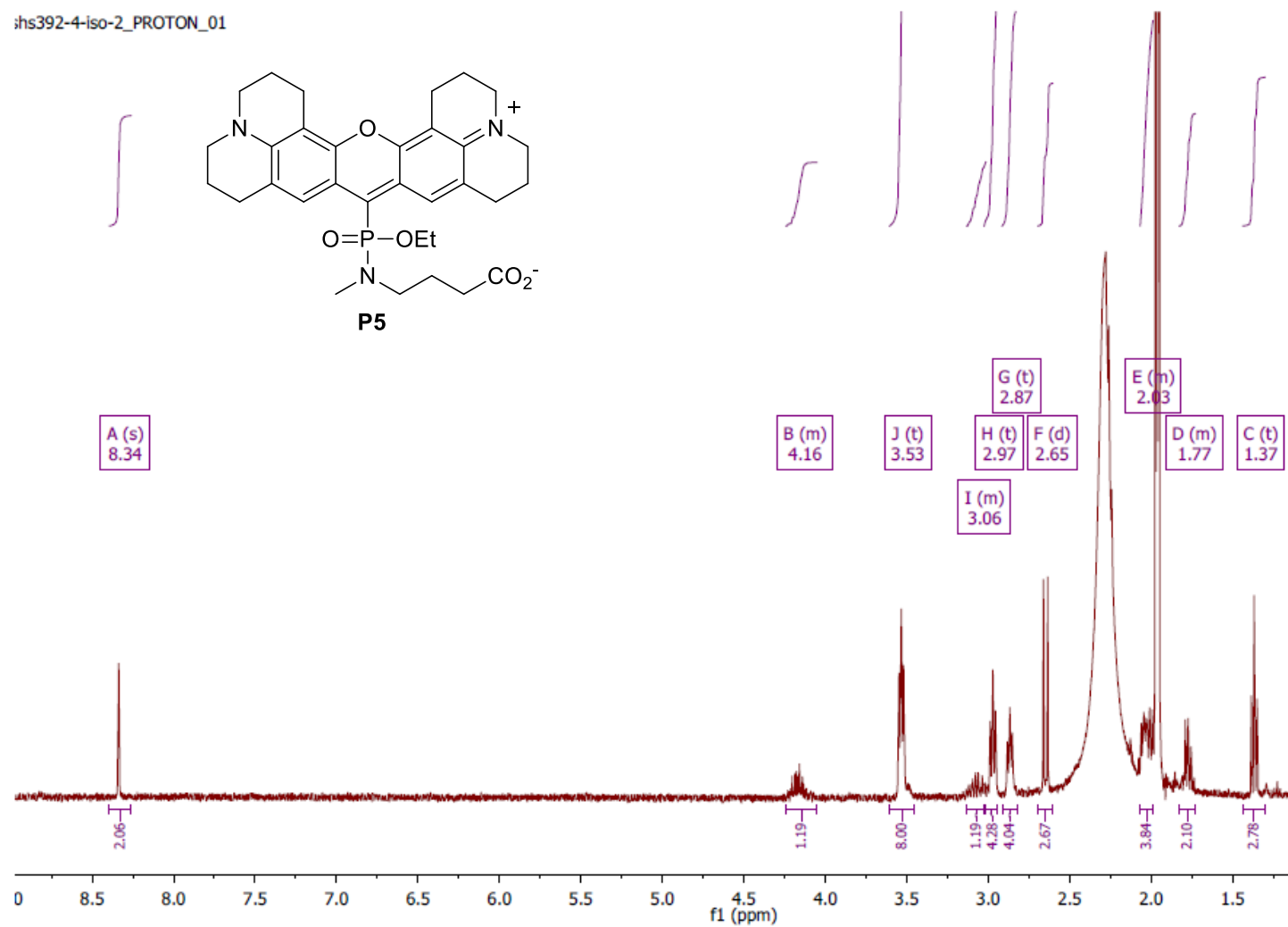
Dye **P4**: ^{31}P

10 shs390-1-iso-dye_PHOSPHORUS_01

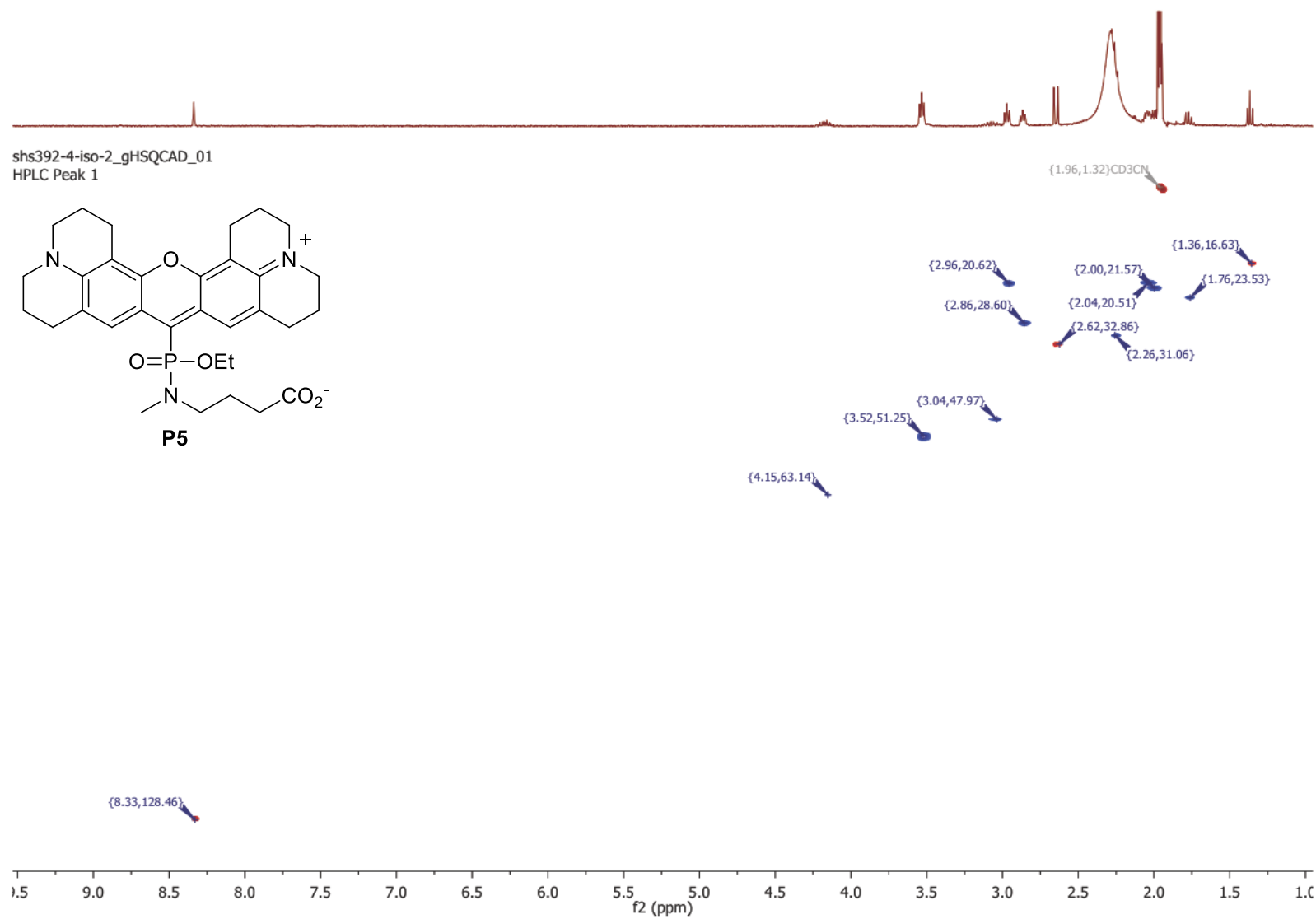


Dye **P5**: ^1H

hs392-4-iso-2_PROTON_01

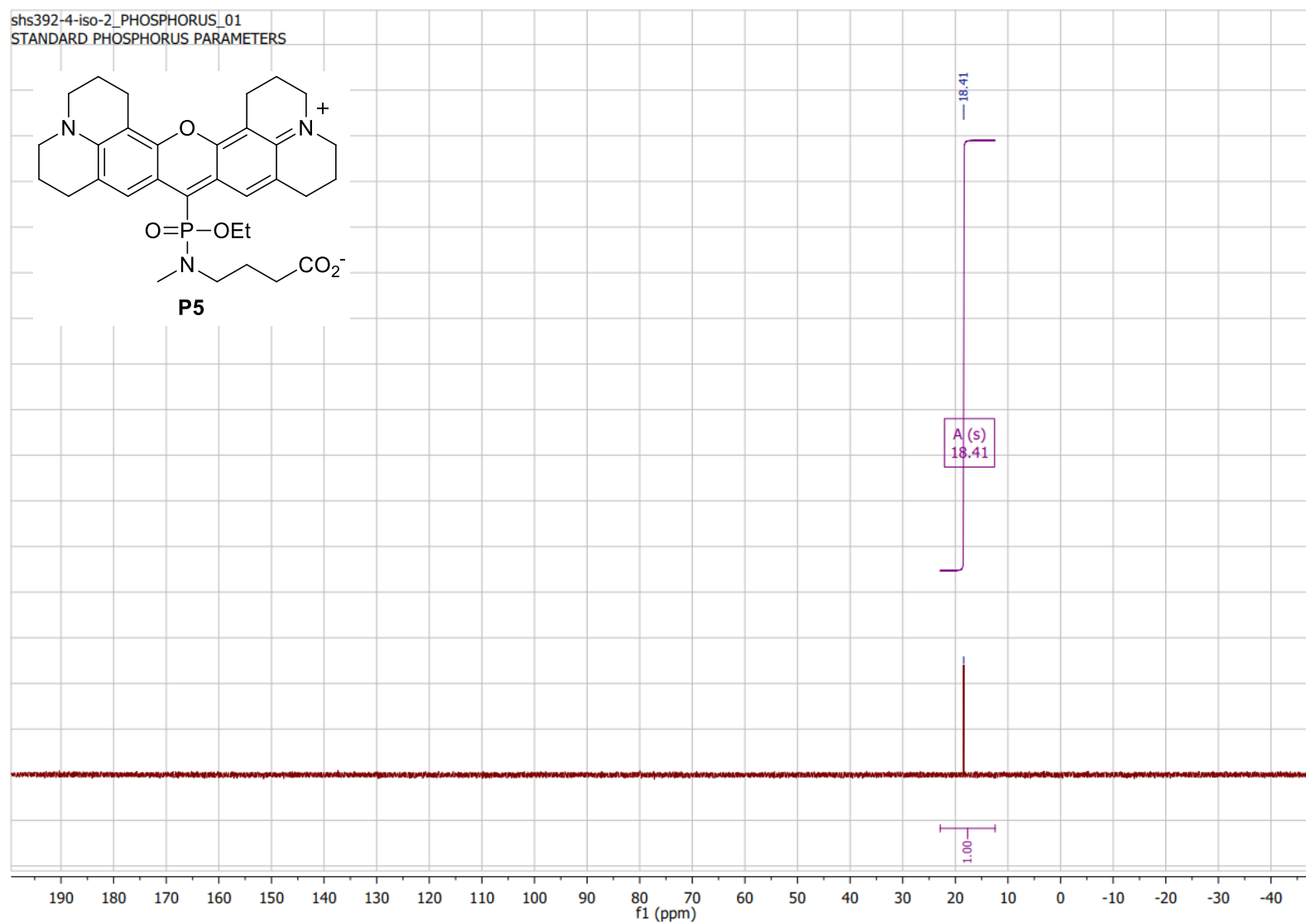
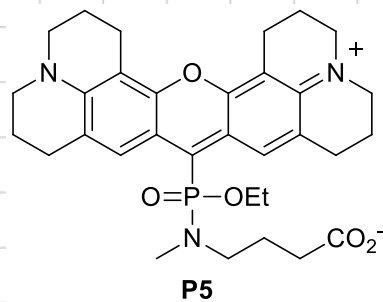


Dye **P5**: gHSQCad

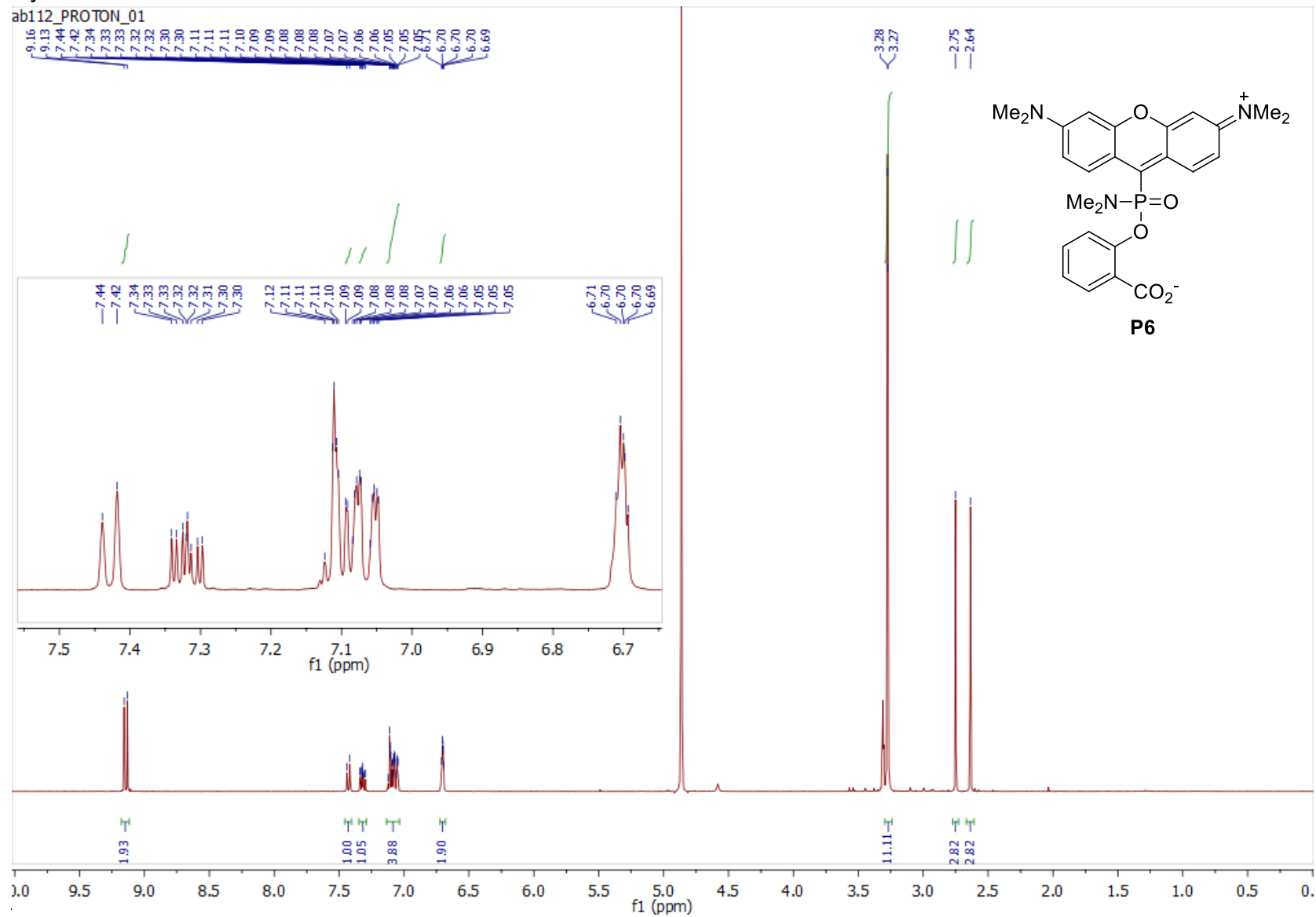


Dye P5: ^{31}P

shs392-4-iso-2_PHOSPHORUS_01
STANDARD PHOSPHORUS PARAMETERS



Dye **P6**: ^1H

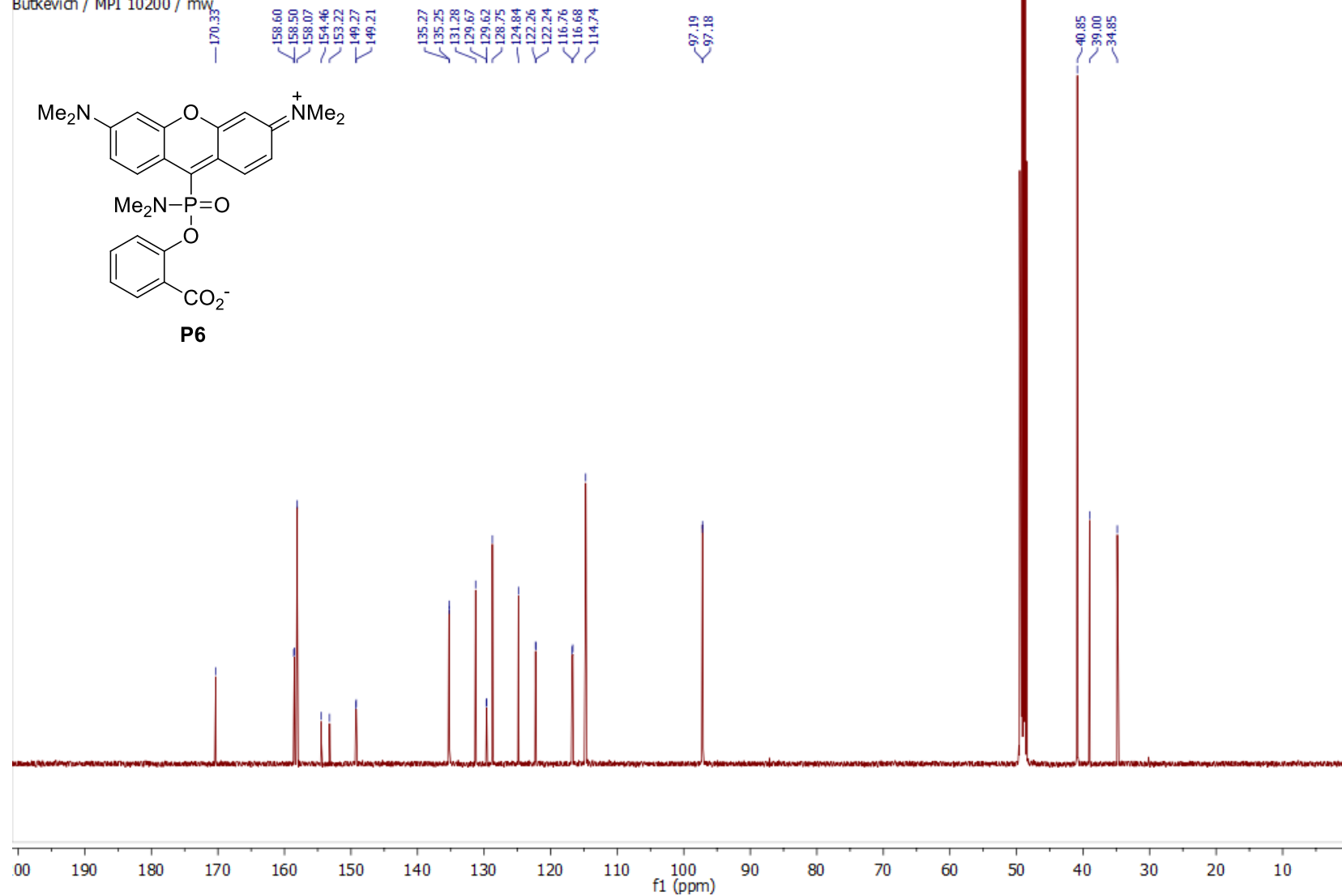


Dye **P6**: $^{13}\text{C}\{^1\text{H}\}$

ab112_5c

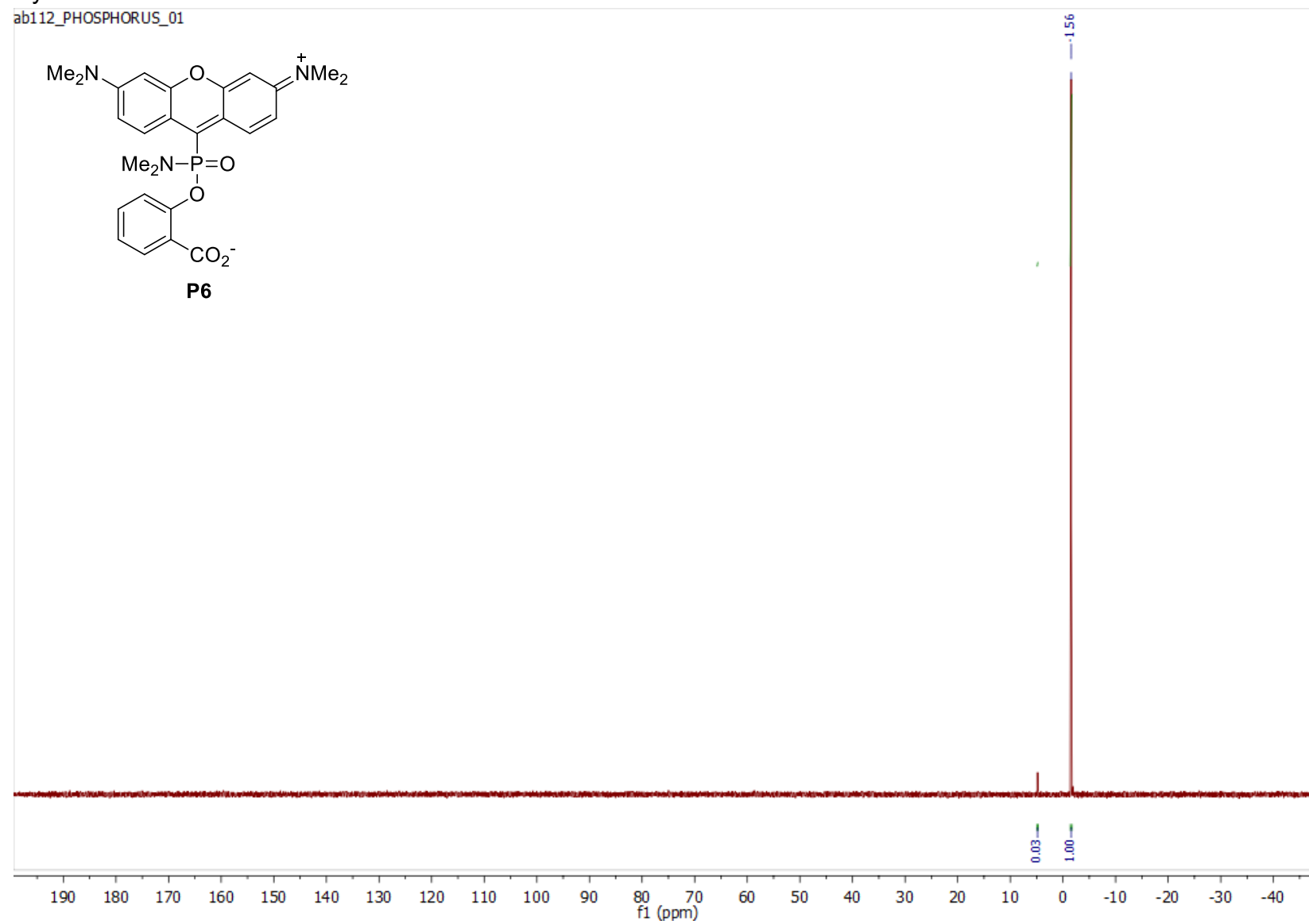
AB112 cd3od

Butkevich / MPI 10200 / mw



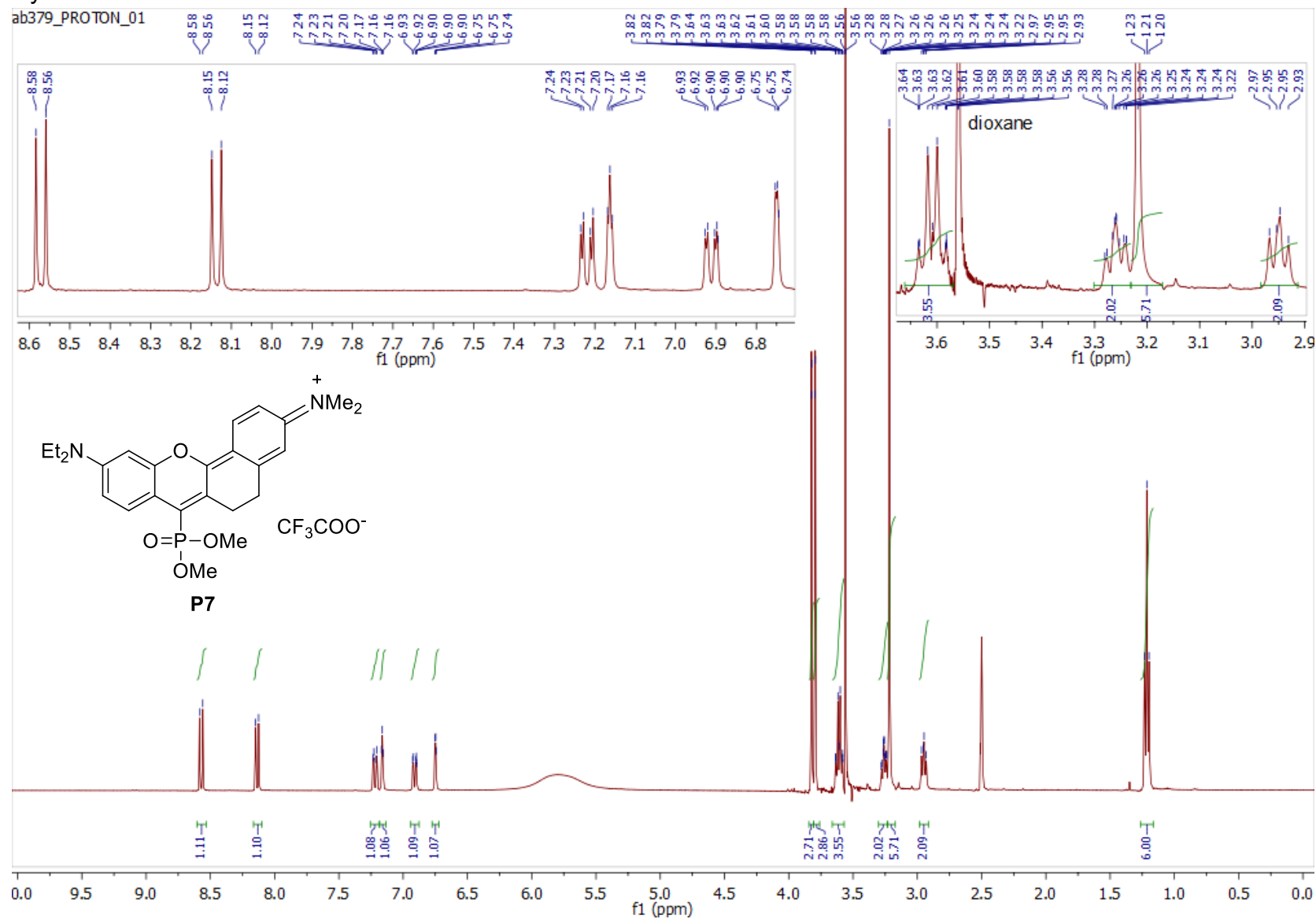
Dye **P6**: ^{31}P

ab112_PHOSPHORUS_01

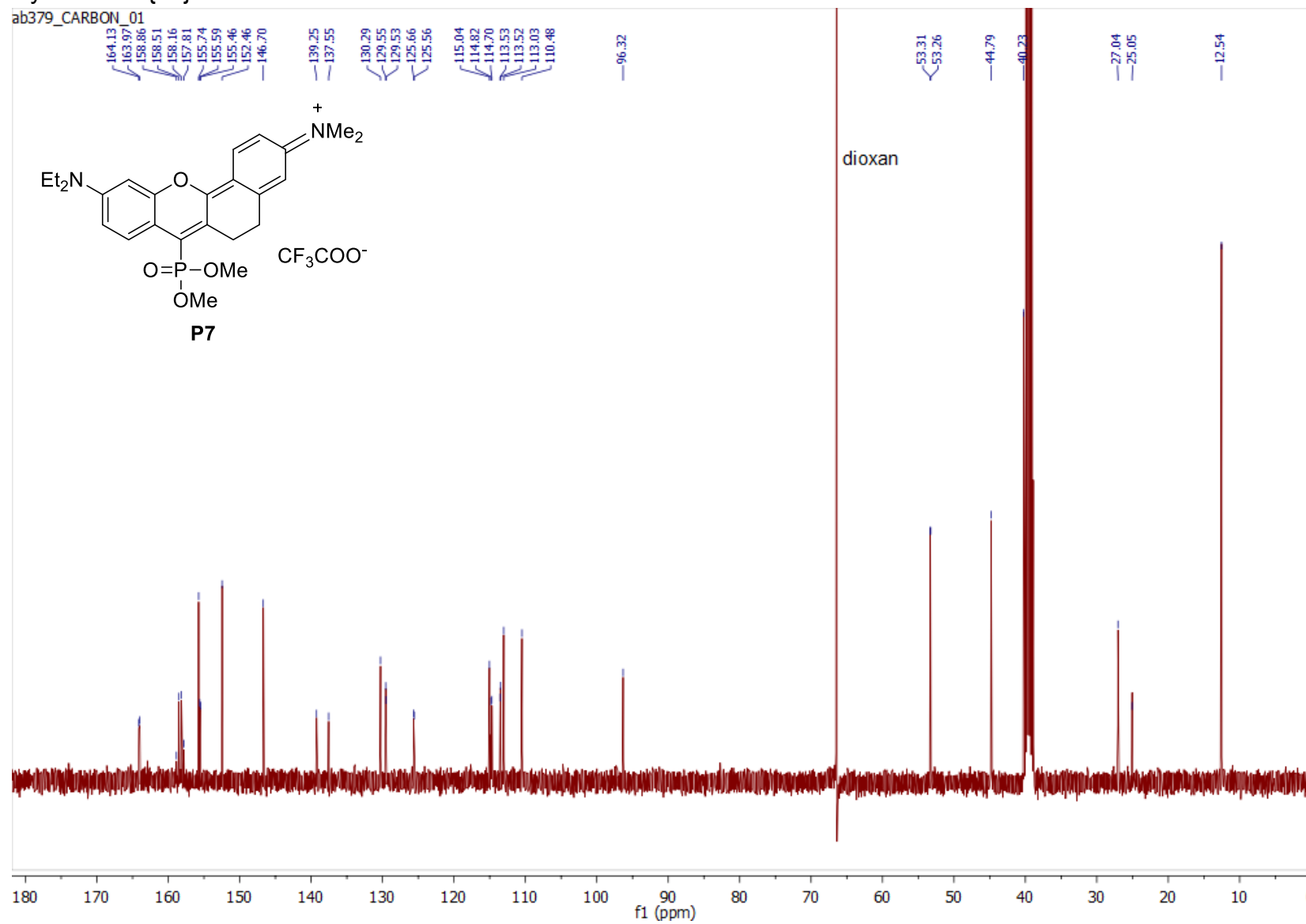


Dye P7: ¹H

ab379_PROTON_01

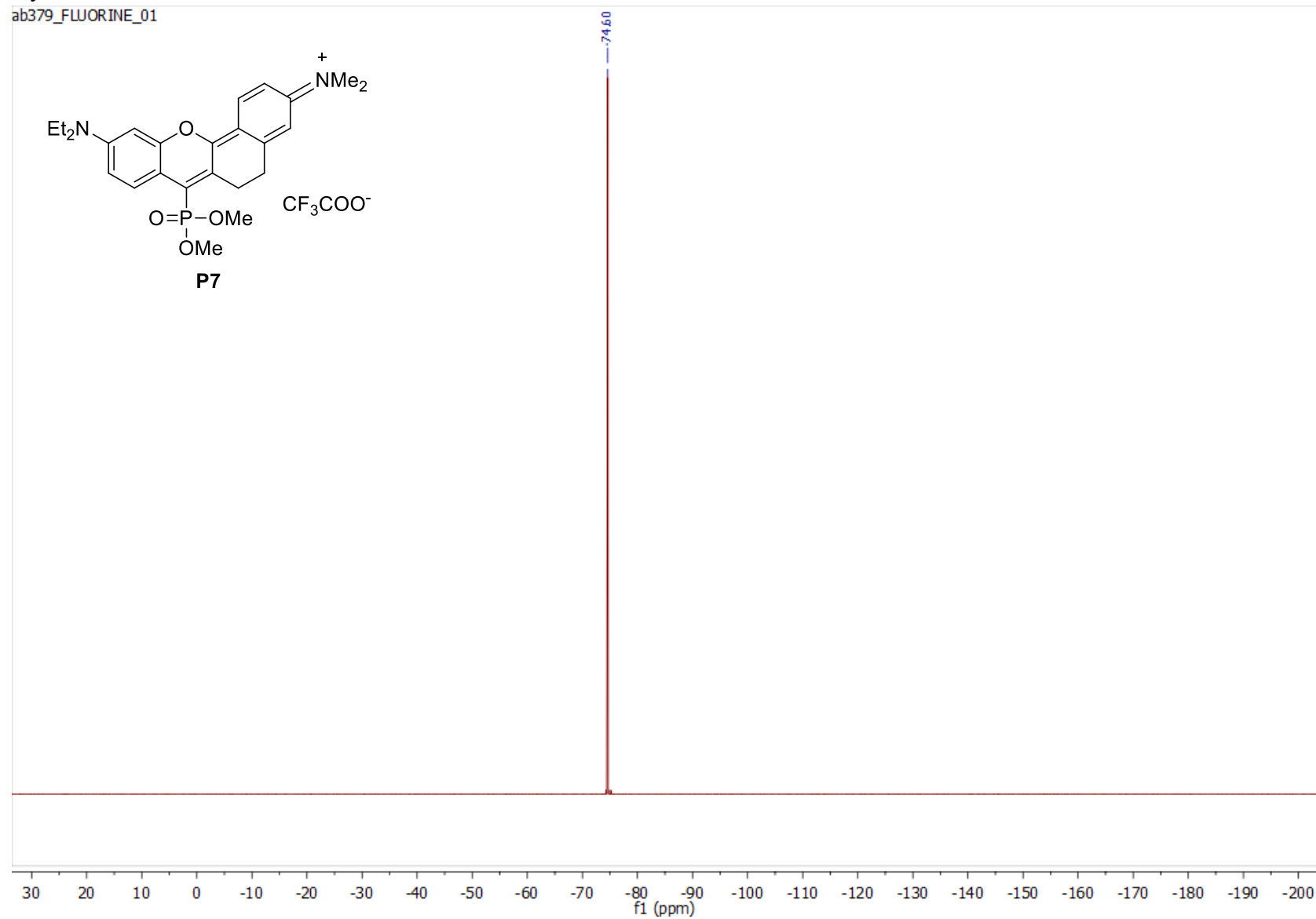


Dye **P7**: $^{13}\text{C}\{^1\text{H}\}$



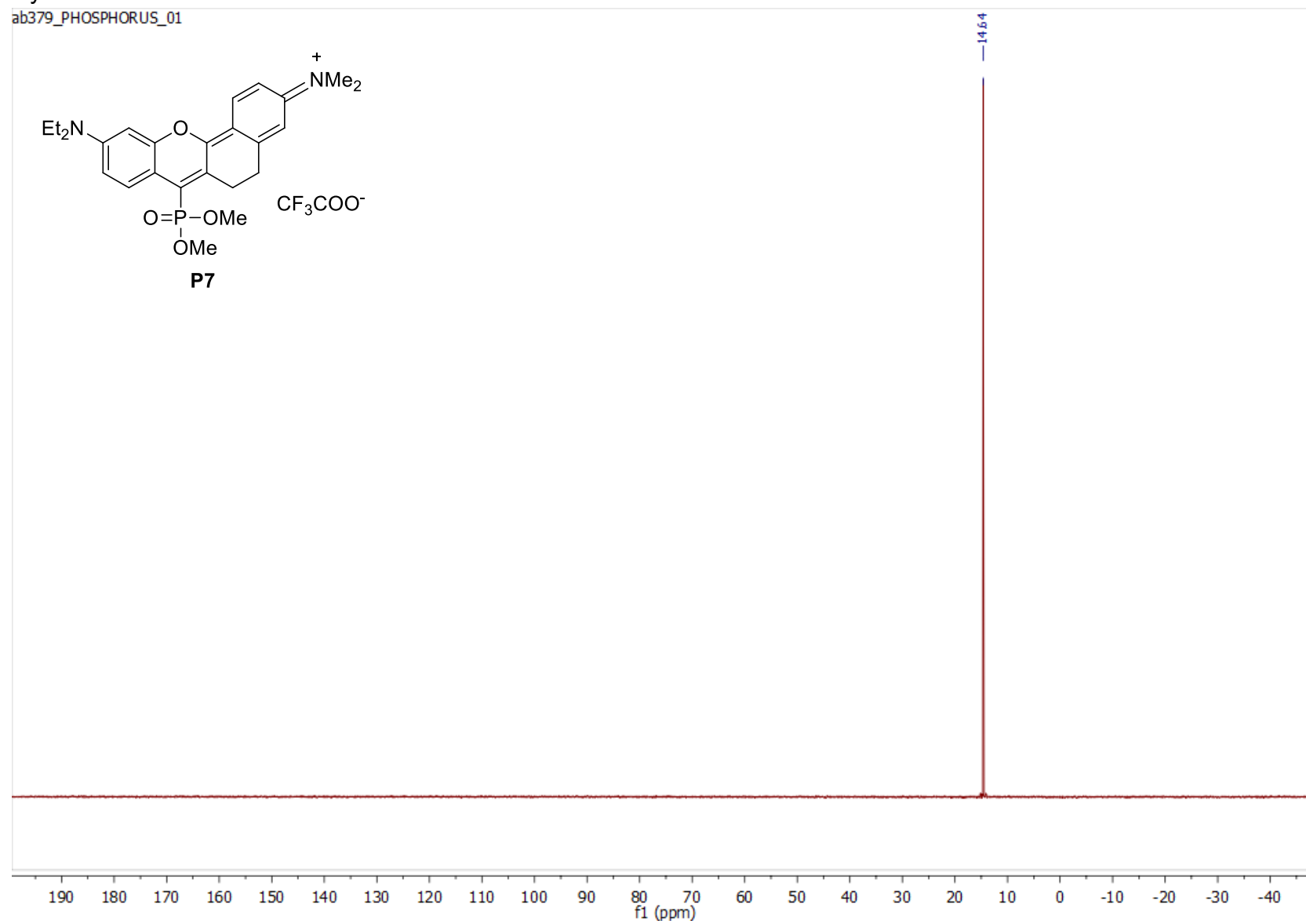
Dye **P7**: ^{19}F

ab379_FLUORINE_01

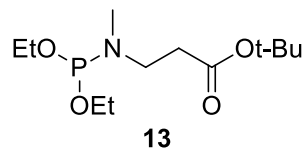


Dye **P7**: ^{31}P

ab379_PHOSPHORUS_01

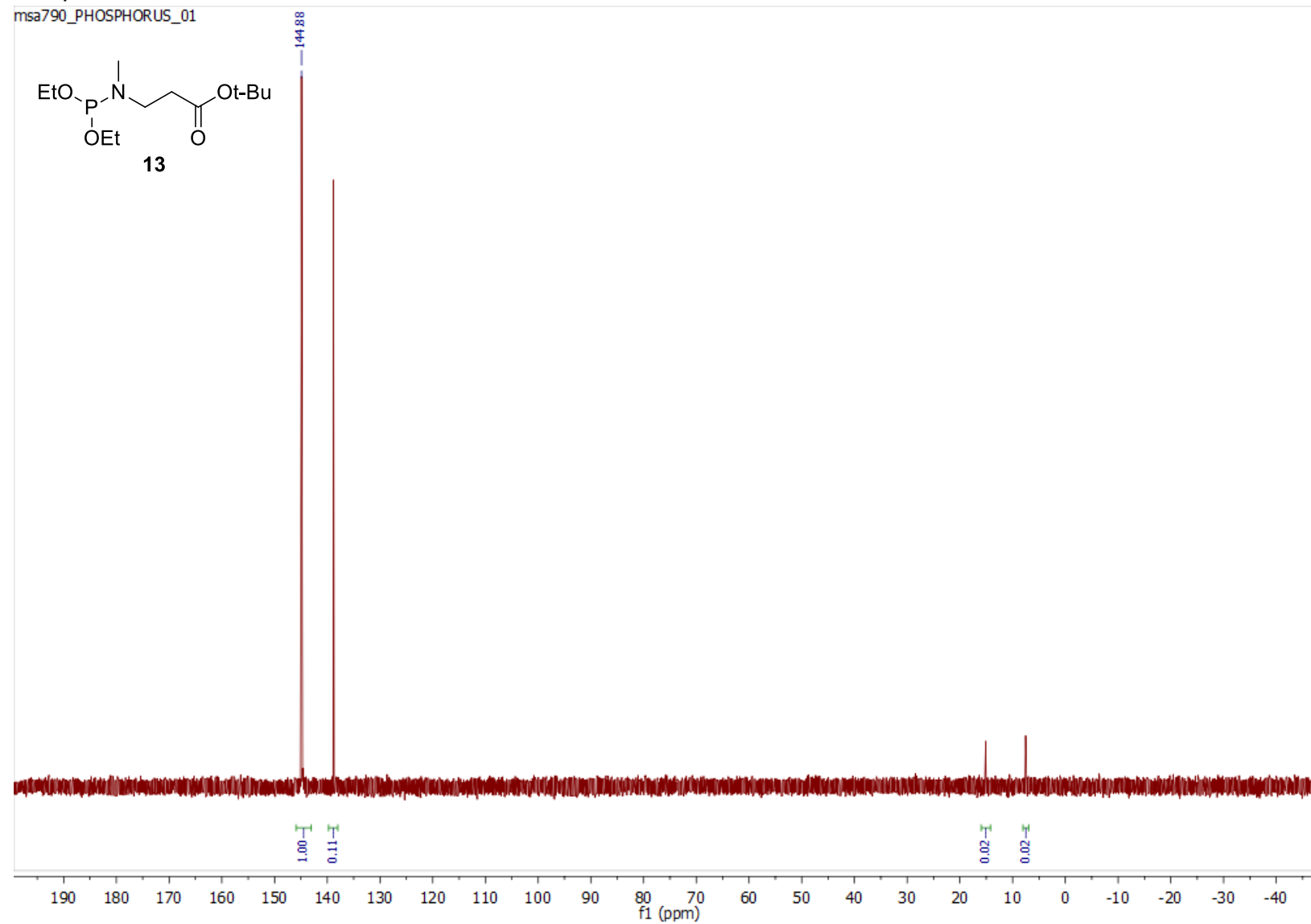


msa790_PROTON_01



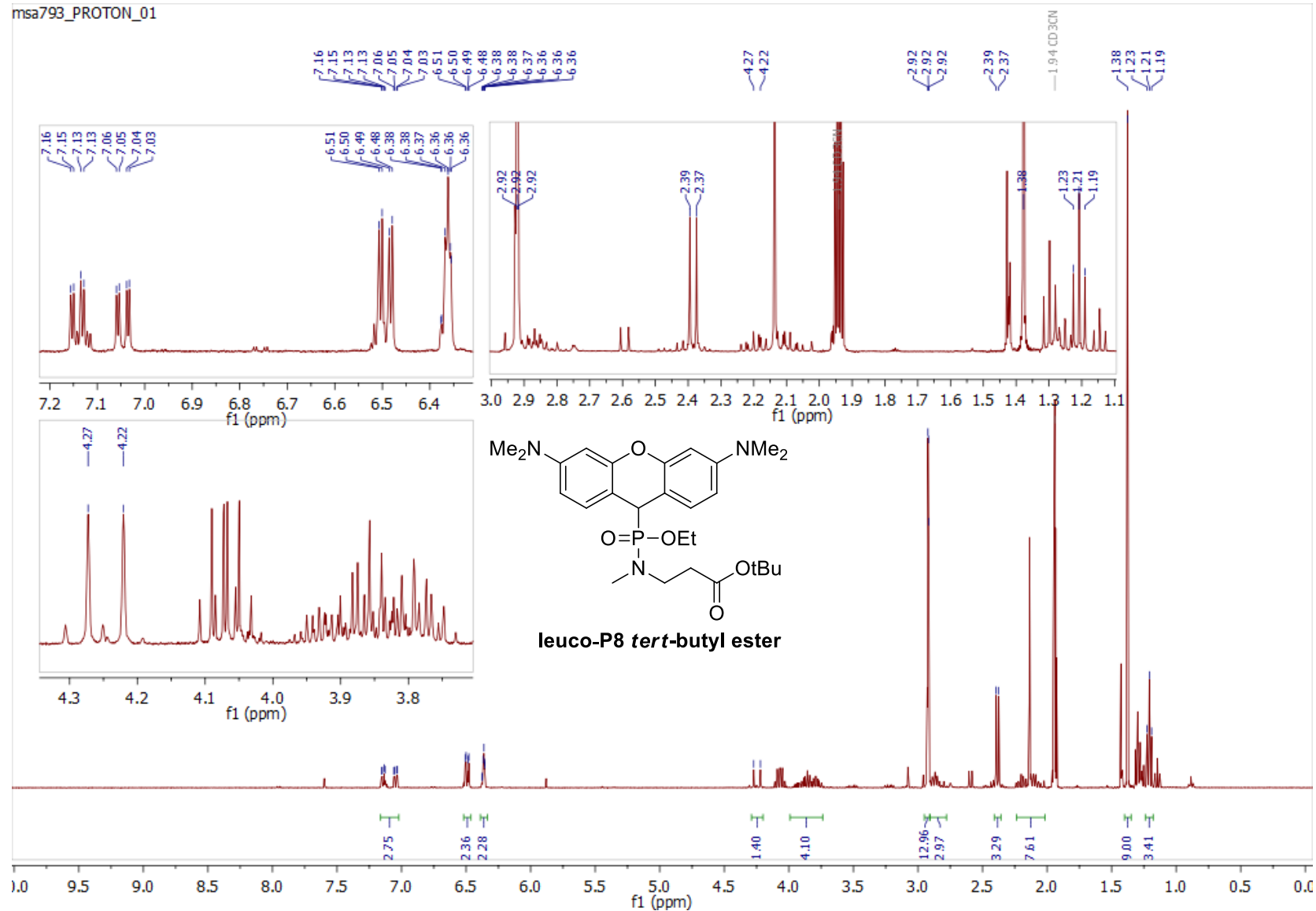
compound **13**: ^{31}P

msa790_PHOSPHORUS_01



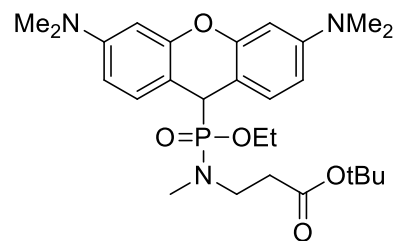
leuco-P8 *tert*-butyl ester: ^1H

msa793_PROTON_01

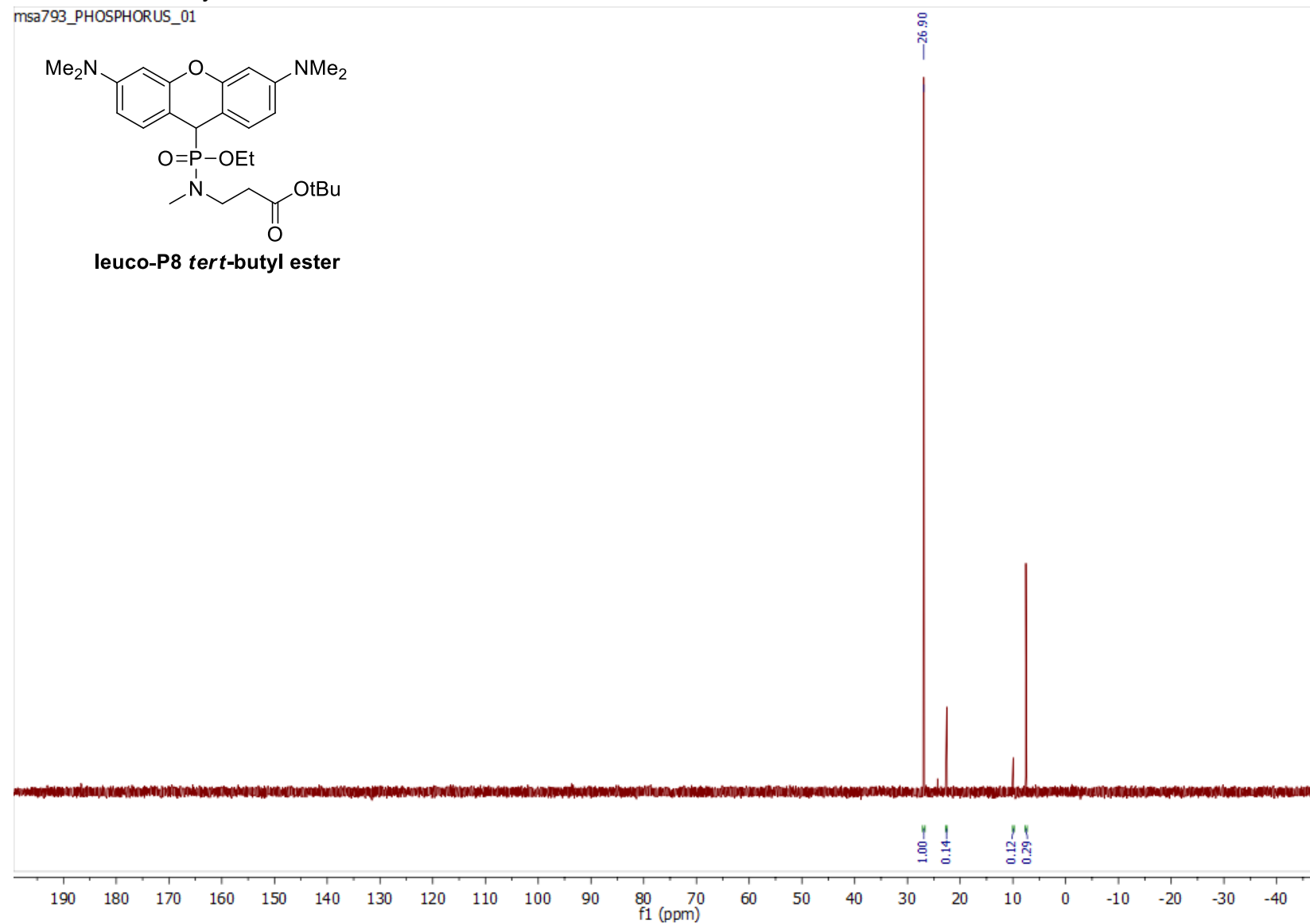


leuco-P8 *tert*-butyl ester: ^{31}P

msa793_PHOSPHORUS_01

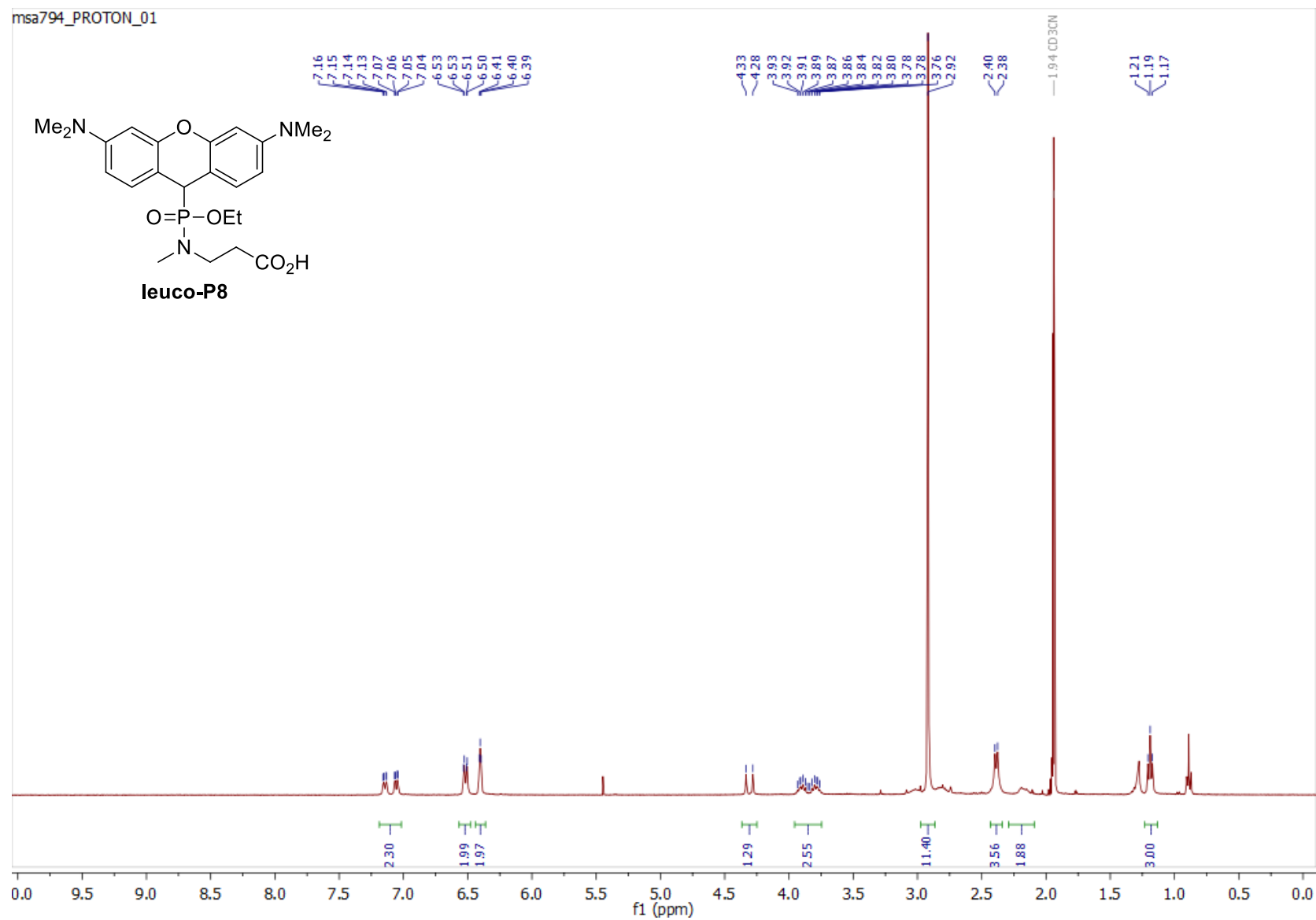


leuco-P8 *tert*-butyl ester



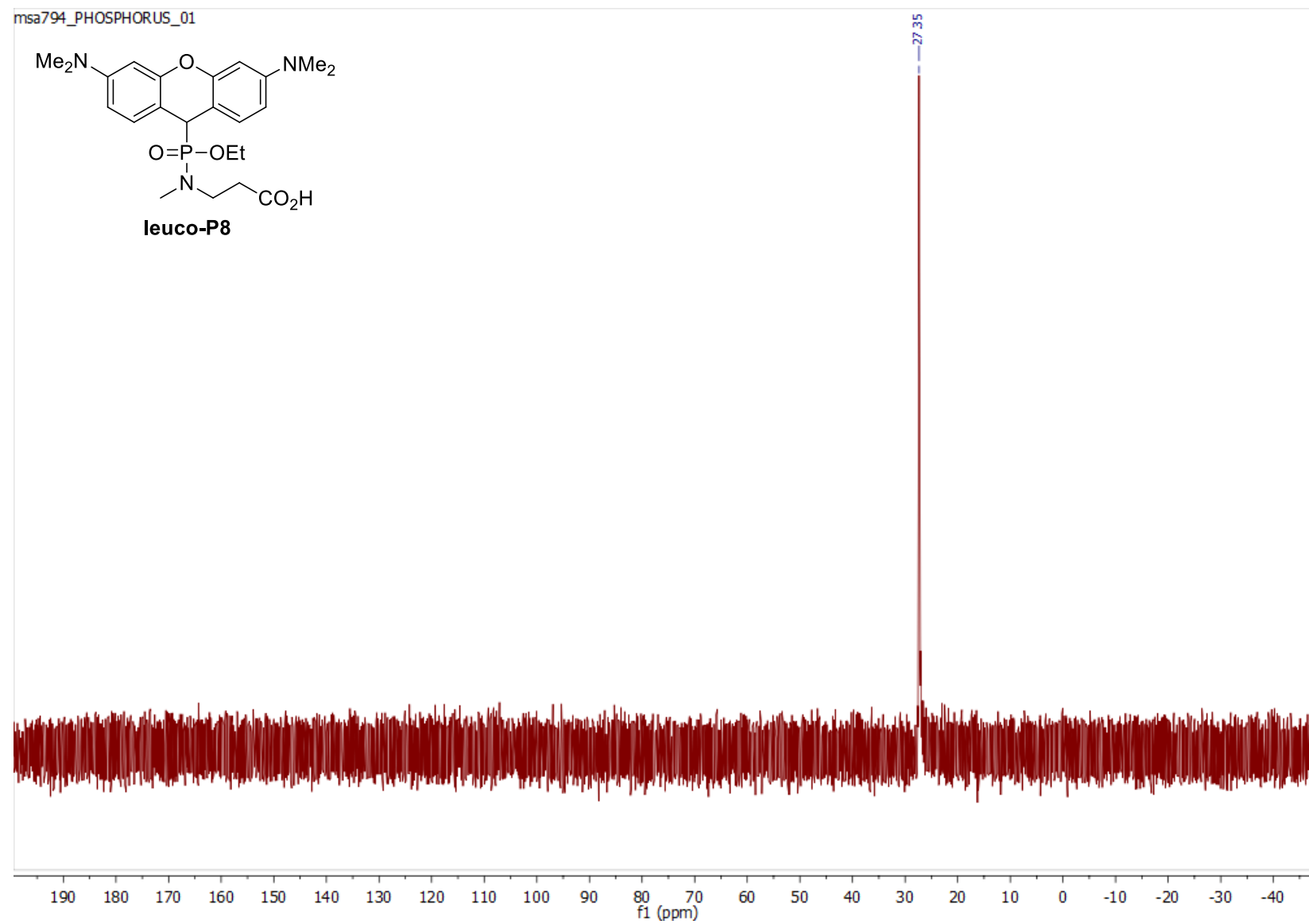
leuco-P8: ^1H

msa794_PROTON_01



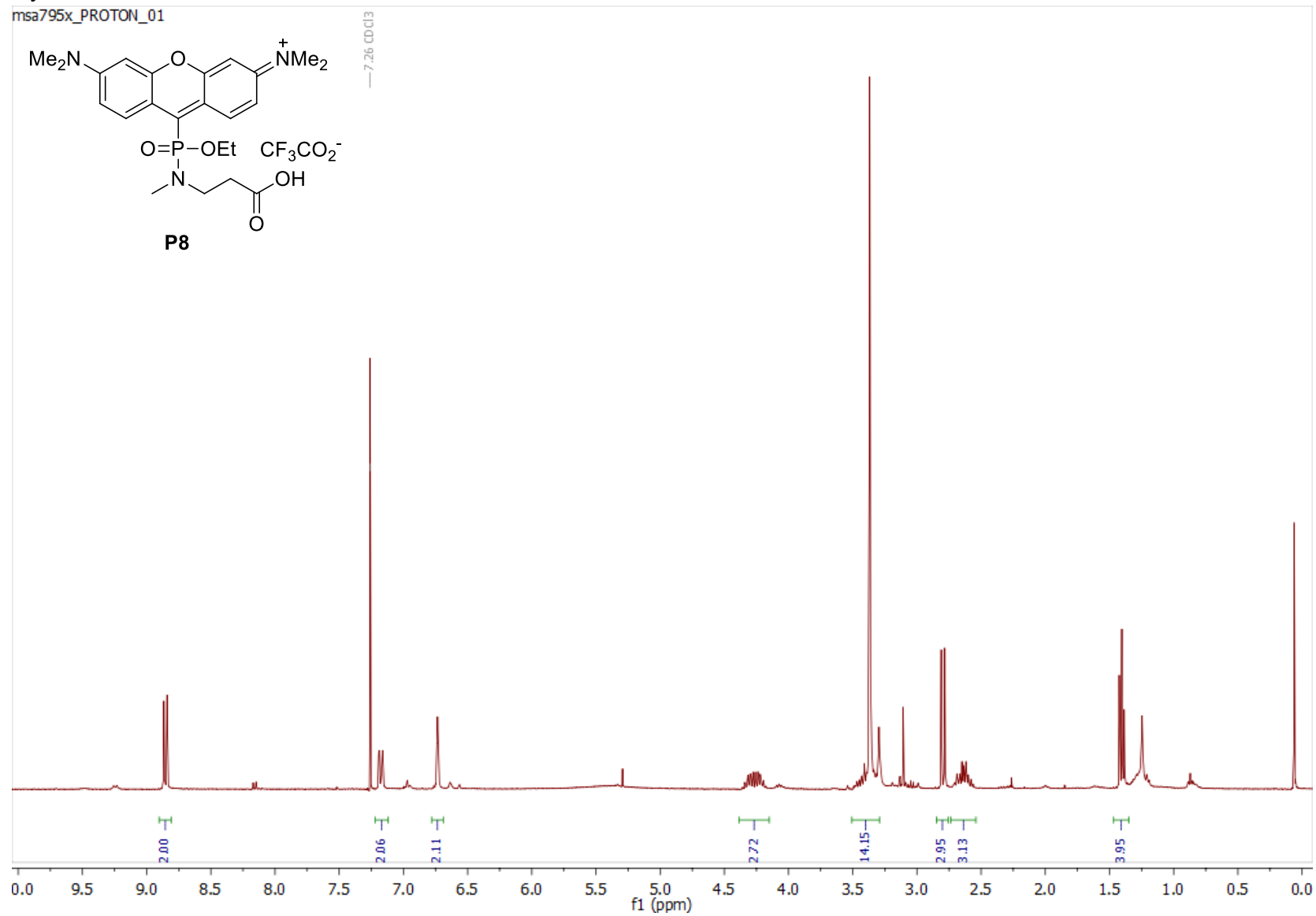
leuco-P8: ^{31}P

msa794_PHOSPHORUS_01



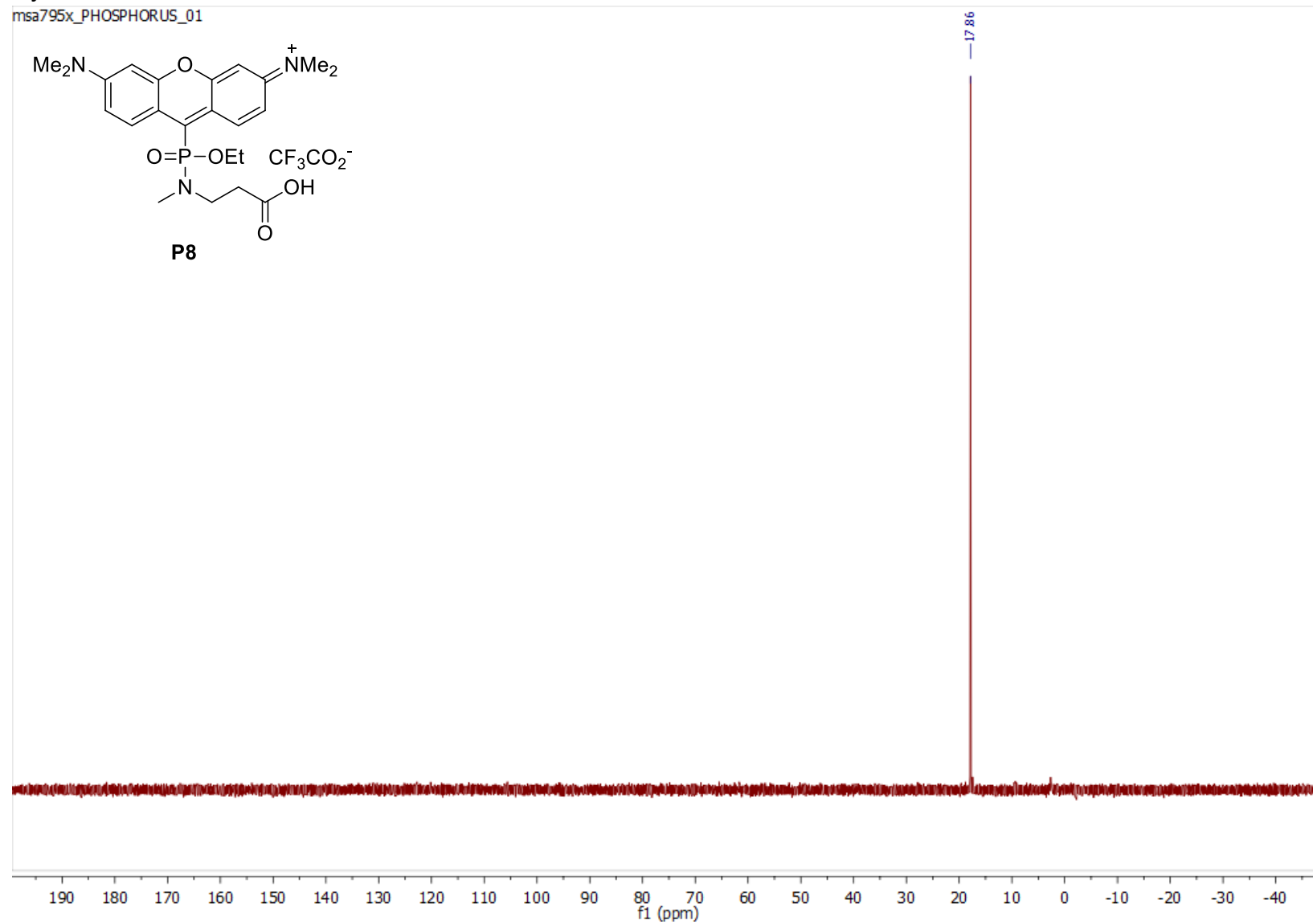
Dye P8: ^1H

msa795x_PROTON_01



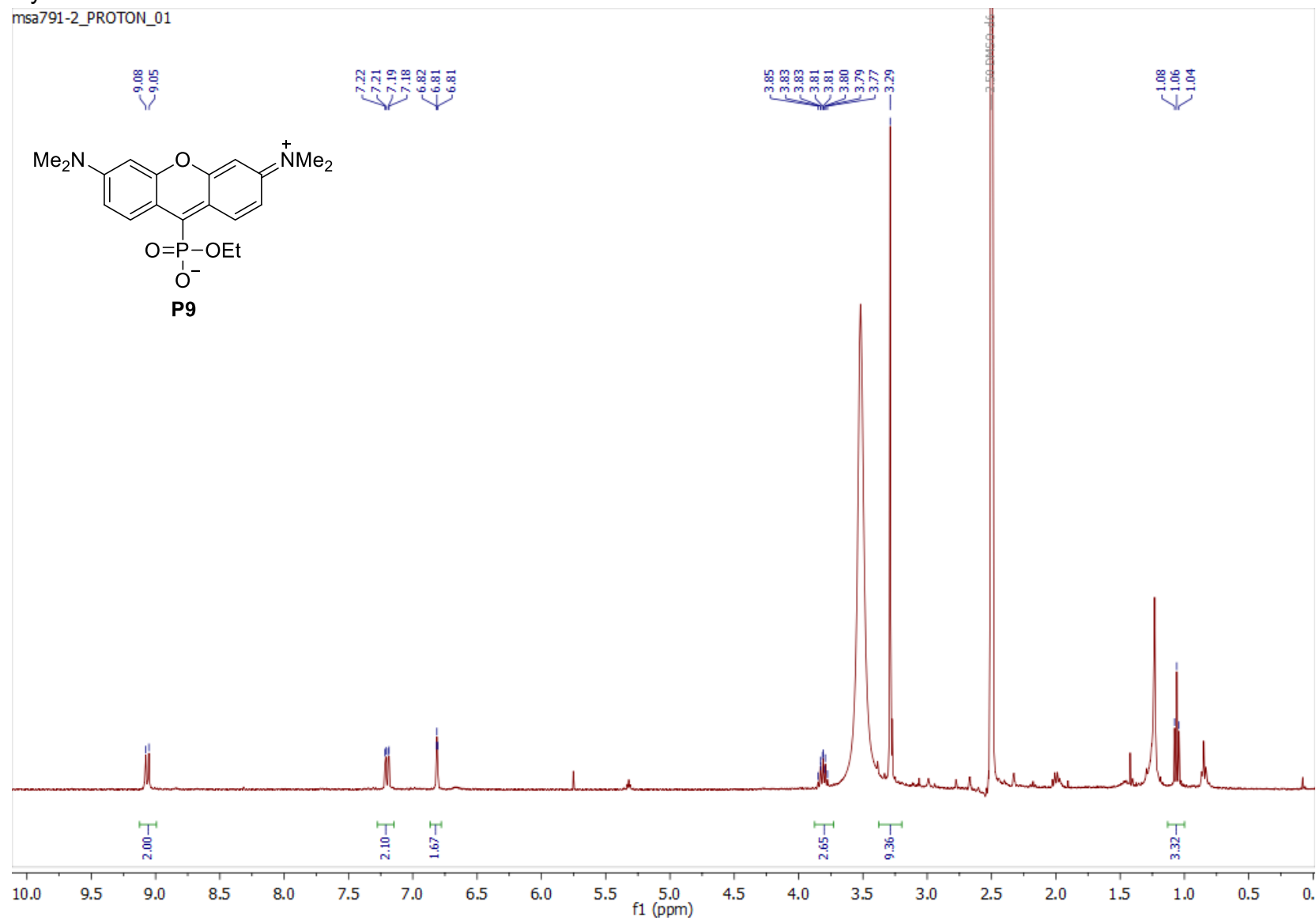
Dye P8: ^{31}P

msa795x_PHOSPHORUS_01



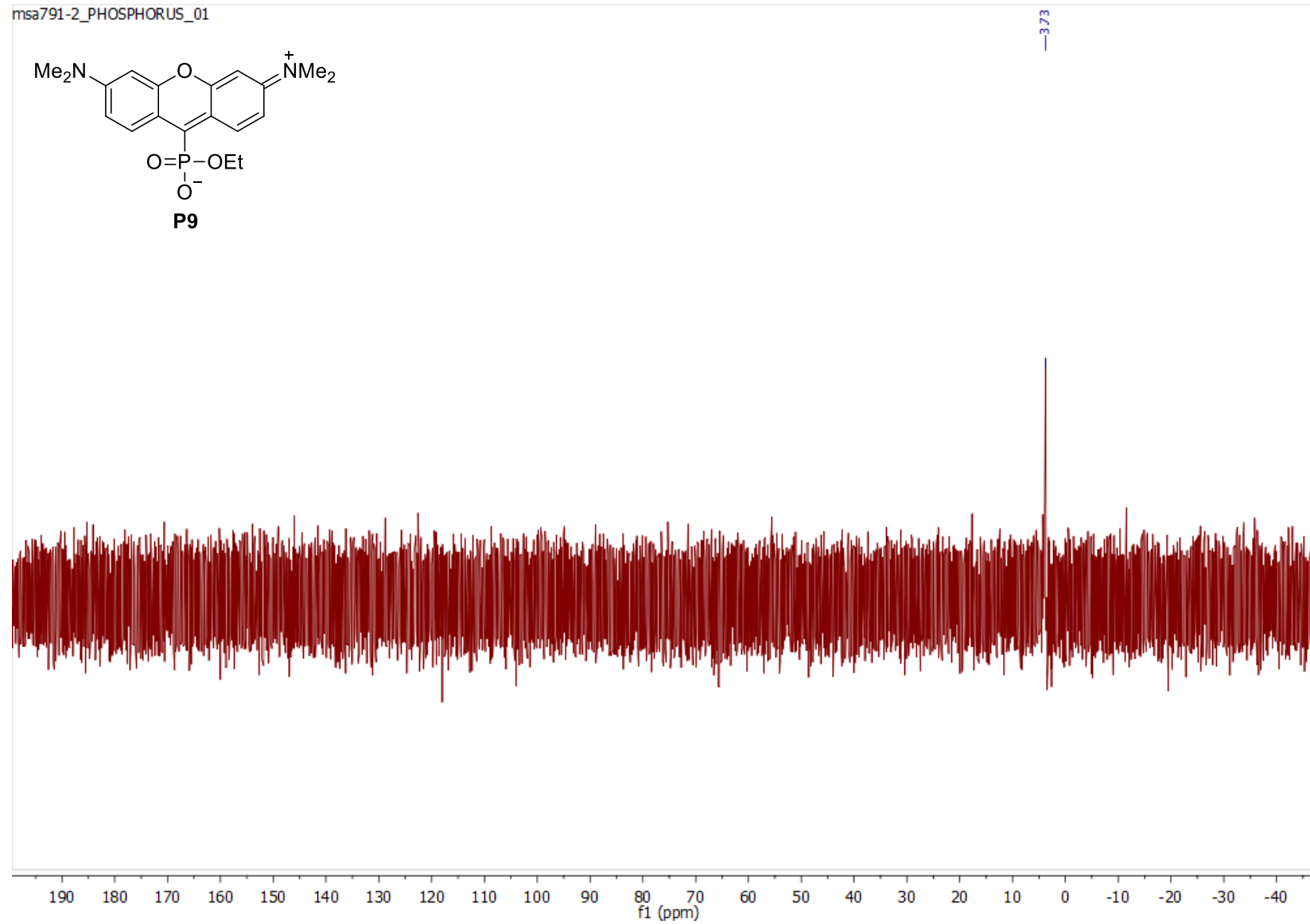
Dye P9: ^1H

msa791-2_PROTON_01



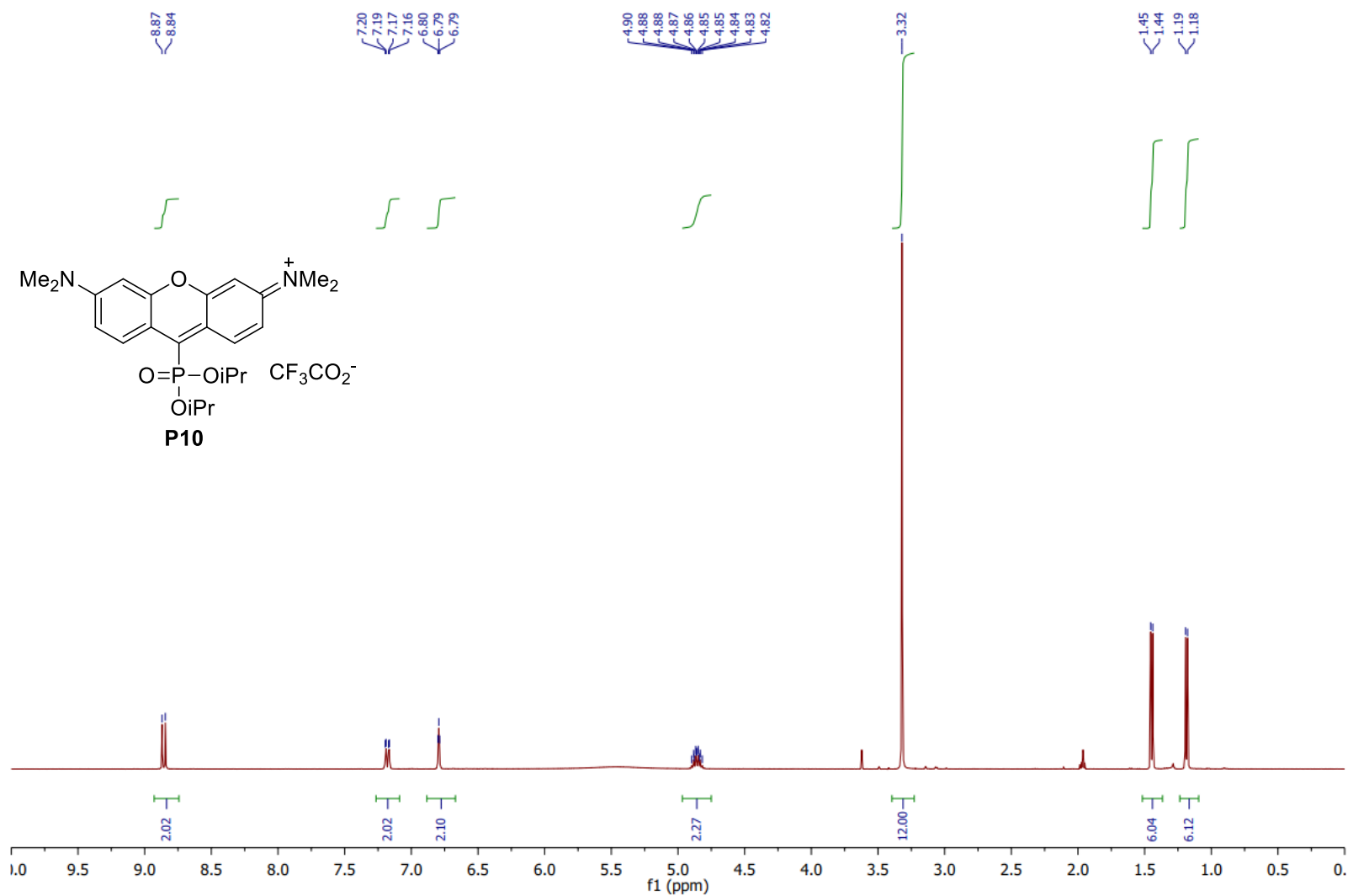
Dye **P9**: ^{31}P

msa791-2_PHOSPHORUS_01



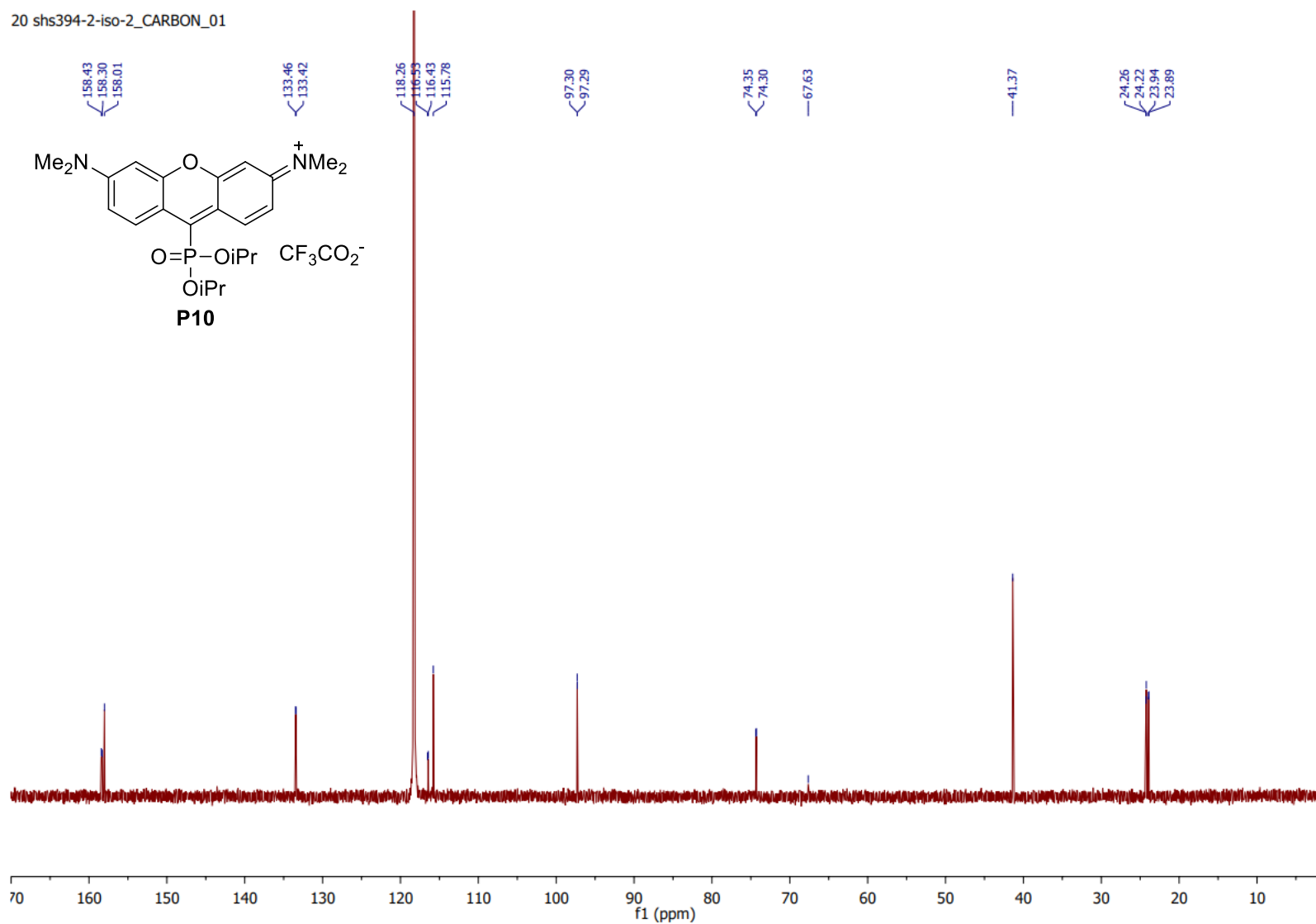
Dye P10: ^1H

20 shs394-2-iso-2_PROTON_01



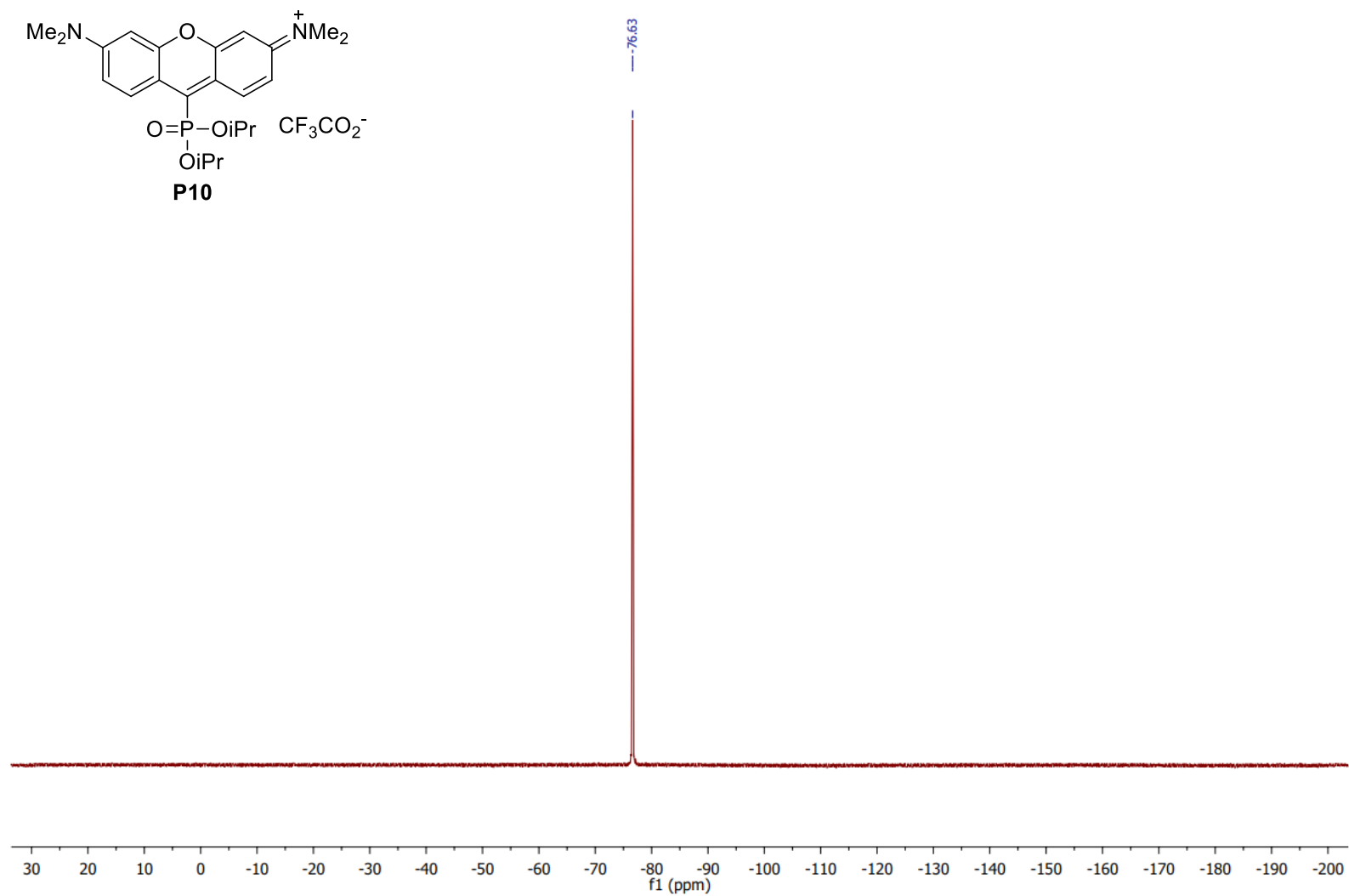
Dye P10: $^{13}\text{C}\{^1\text{H}\}$

20 shs394-2-iso-2_CARBON_01



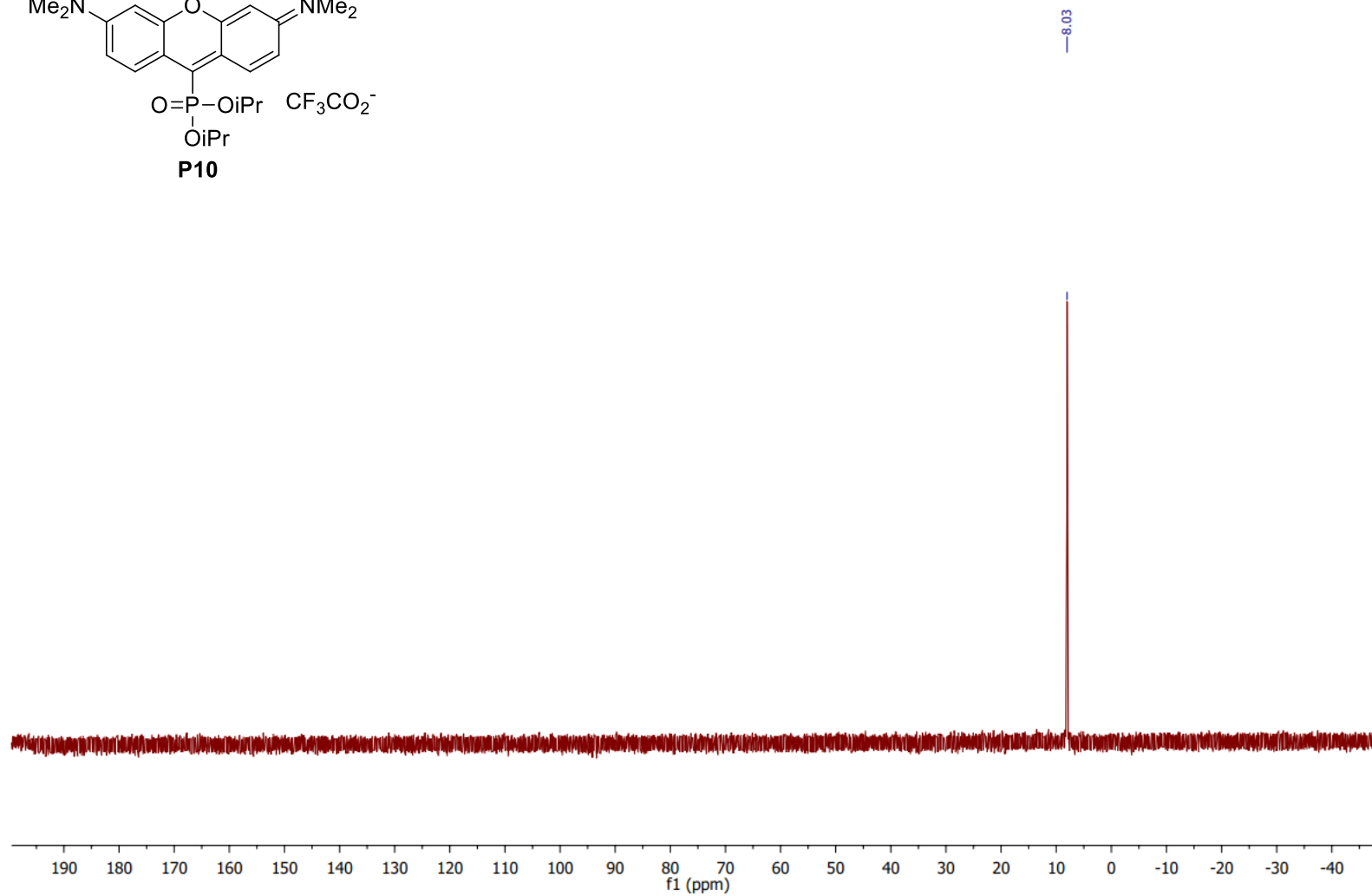
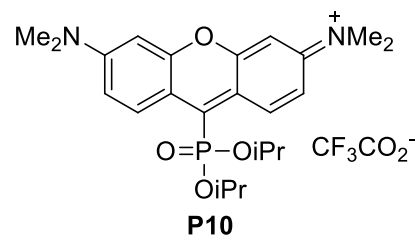
Dye **P10**: ^{19}F

20 shs394-2-iso-2_FLUORINE_01

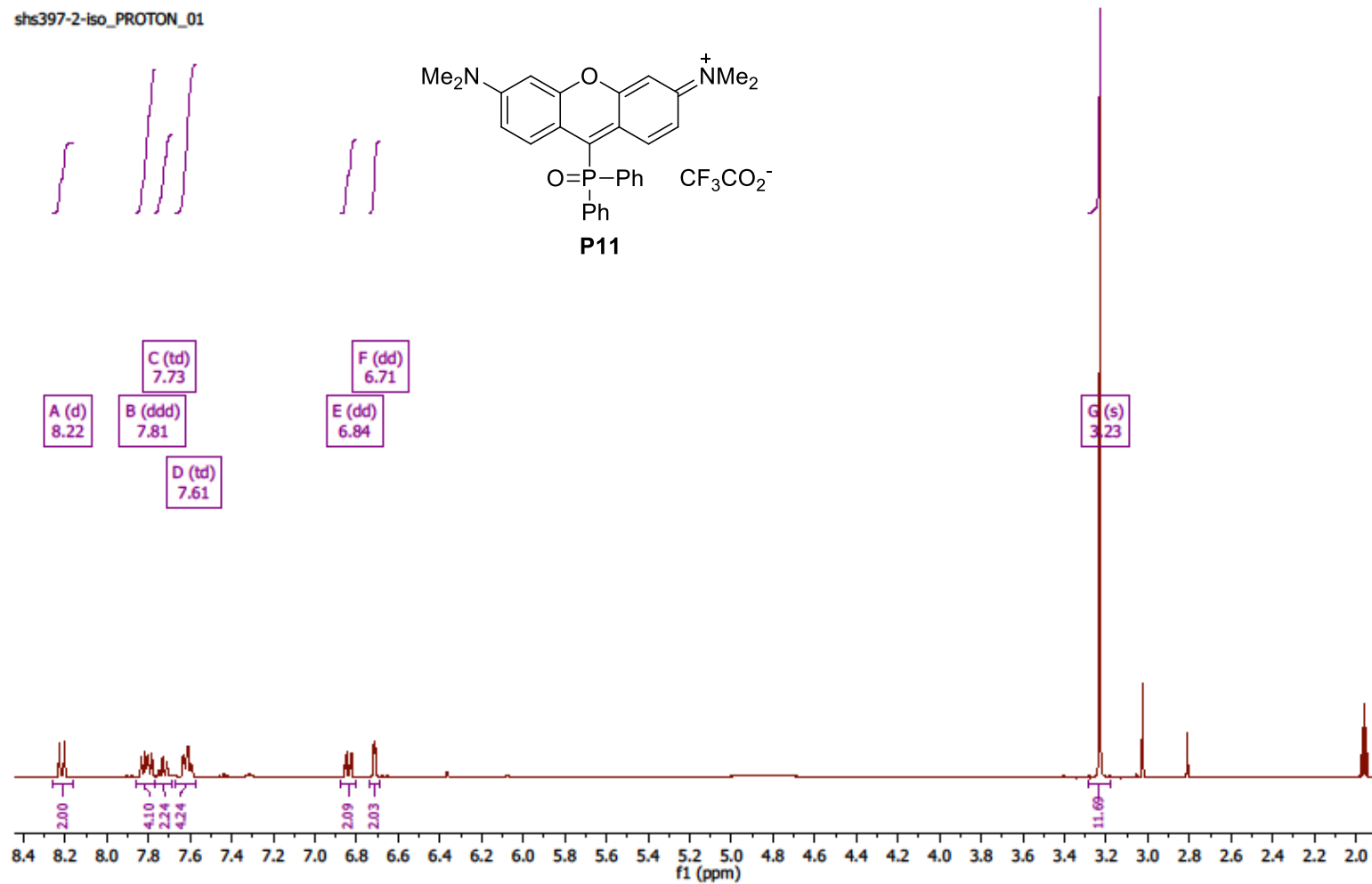


Dye **P10**: ^{31}P

20 shs394-2-iso-2_PHOSPHORUS_01

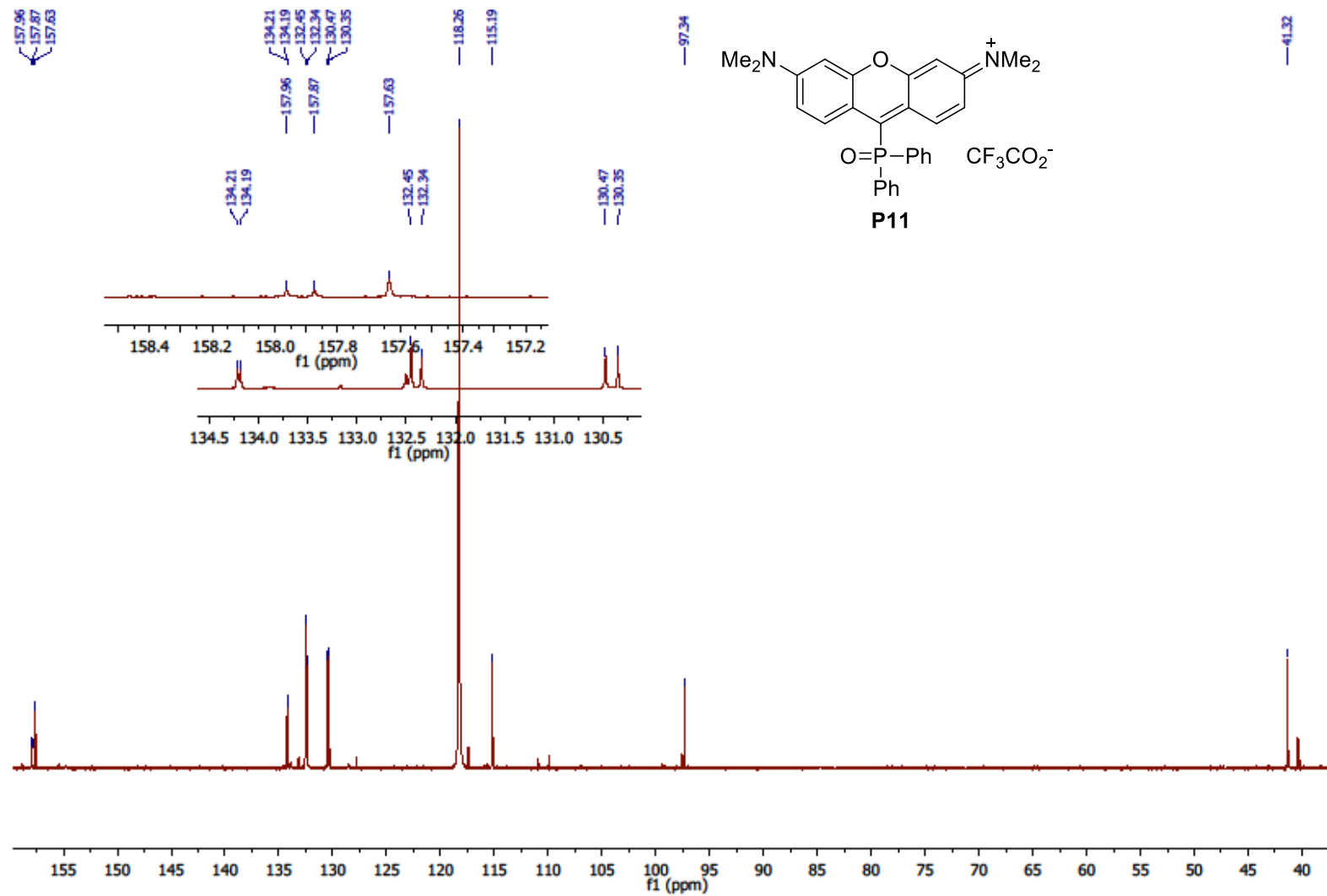


Dye **P11**: ^1H

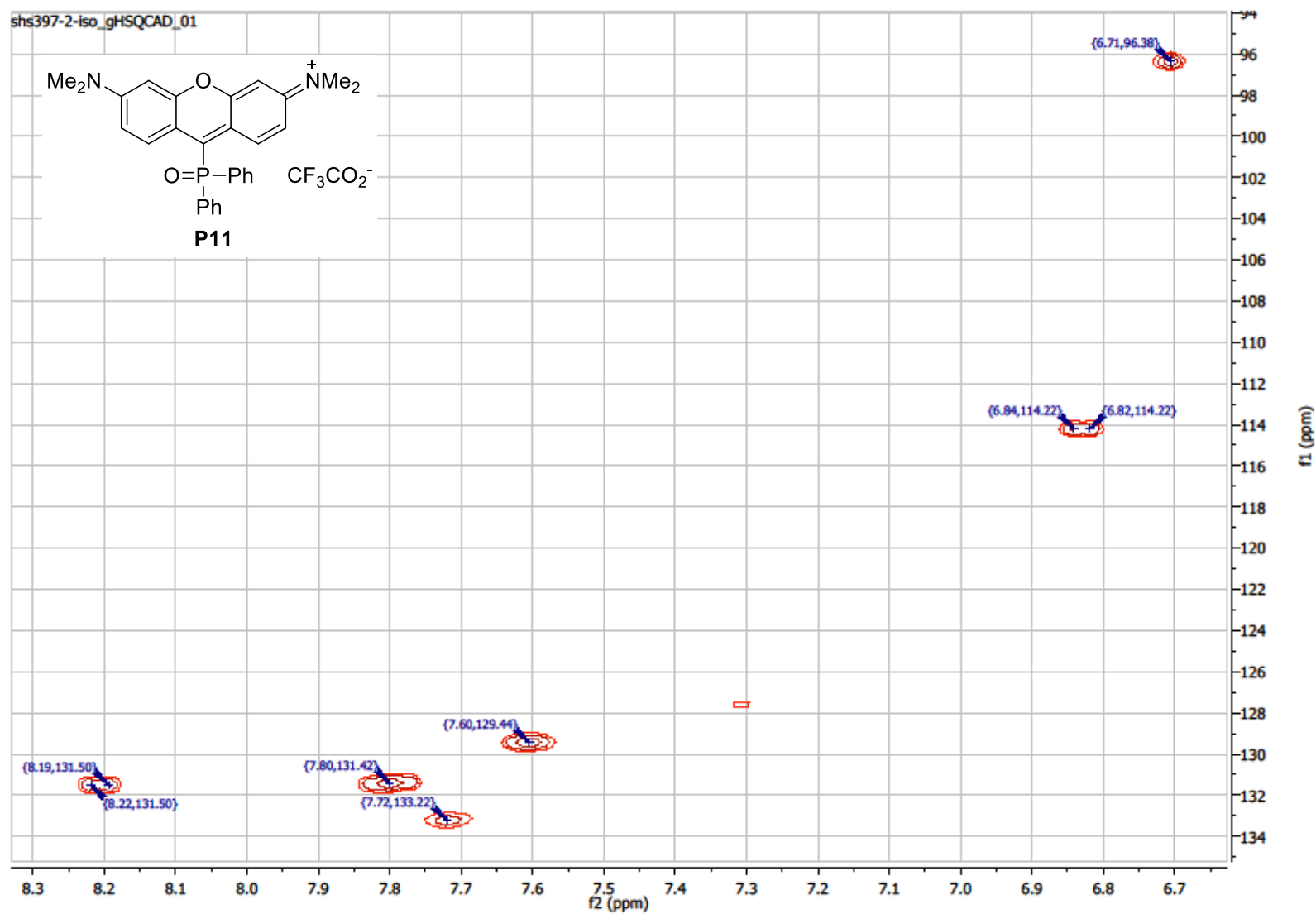


Dye **P11**: $^{13}\text{C}\{^1\text{H}\}$

shs397-2-iso_CARBON_01

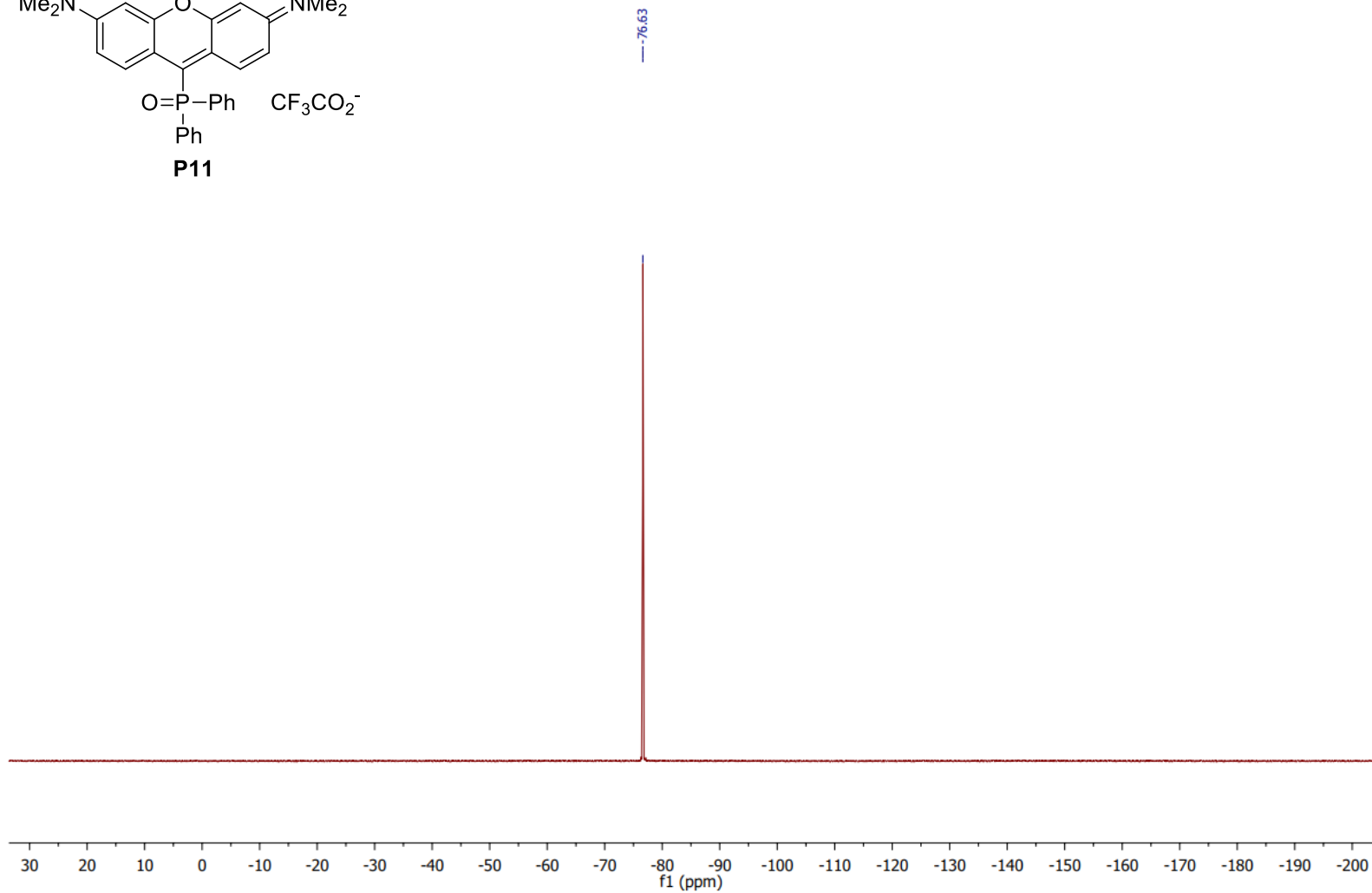
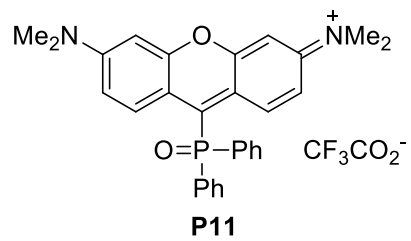


Dye **P11**: gHSQCad



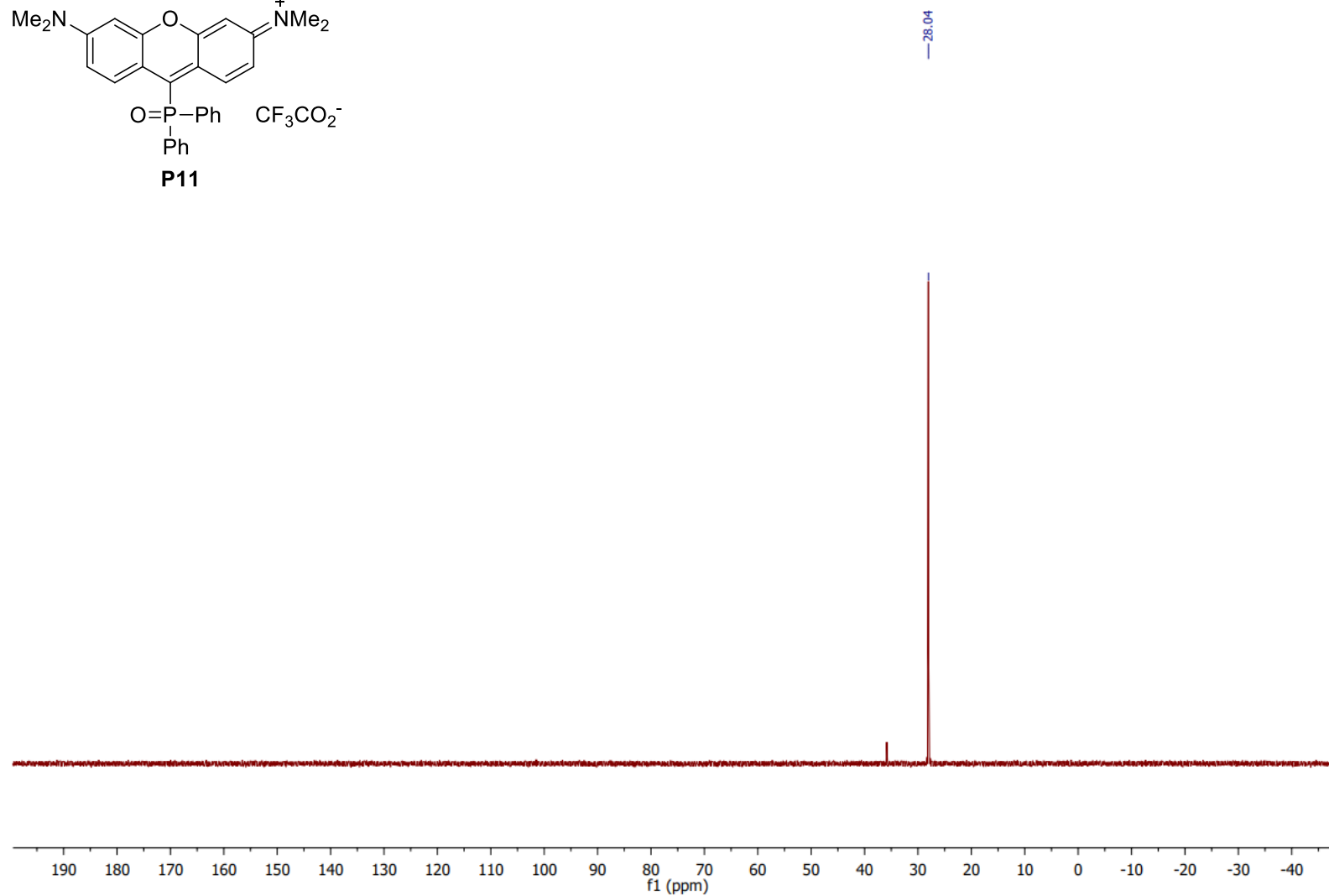
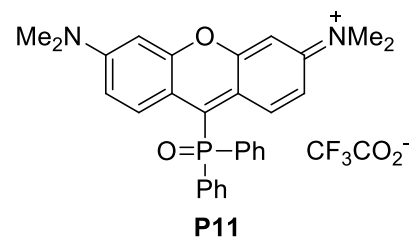
Dye **P11**: ^{19}F

shs397-2-iso_FLUORINE_01



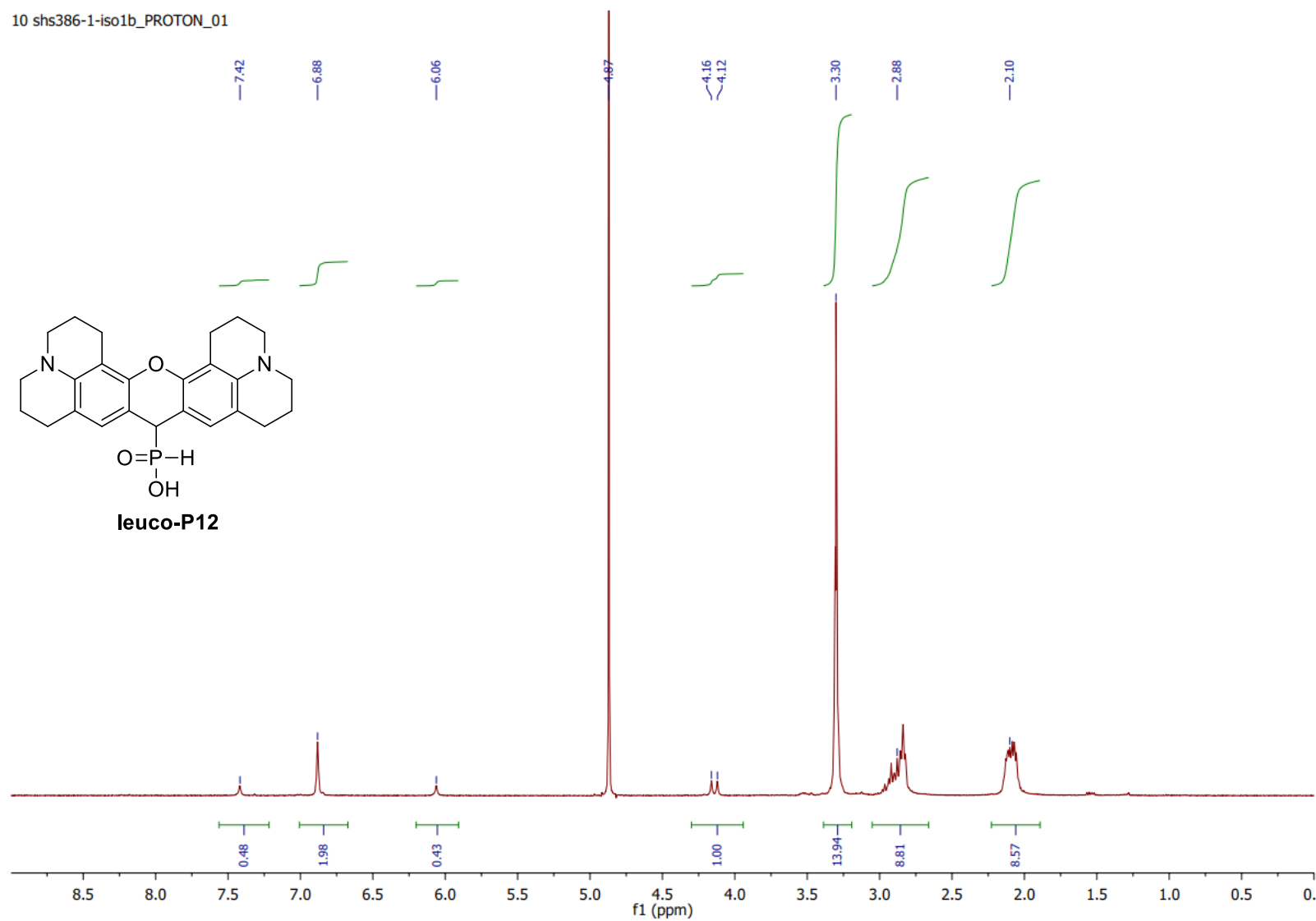
Dye P11: ^{31}P

shs397-2-iso-2b_PHOSPHORUS_01

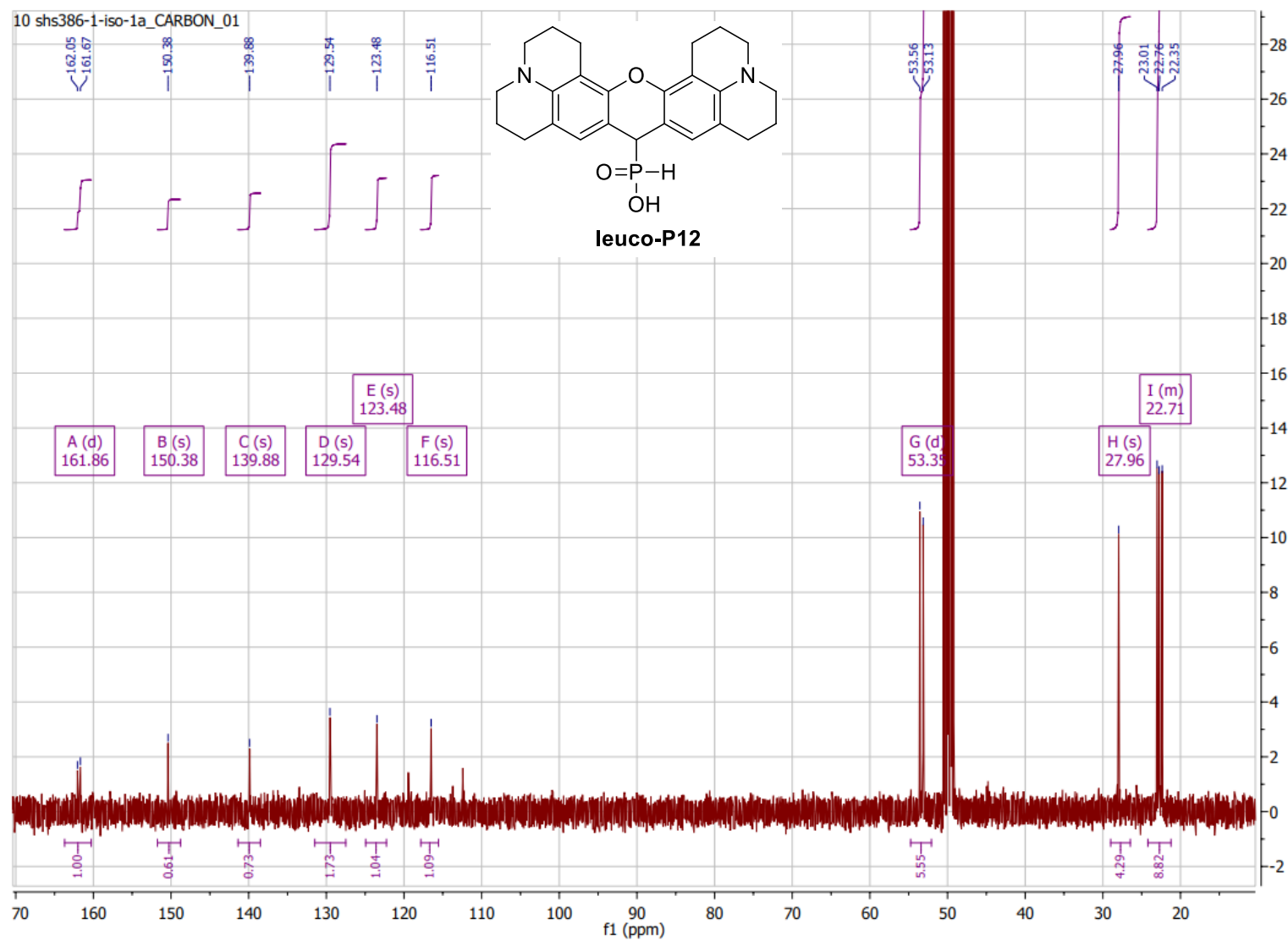


leuco-P12: ^1H

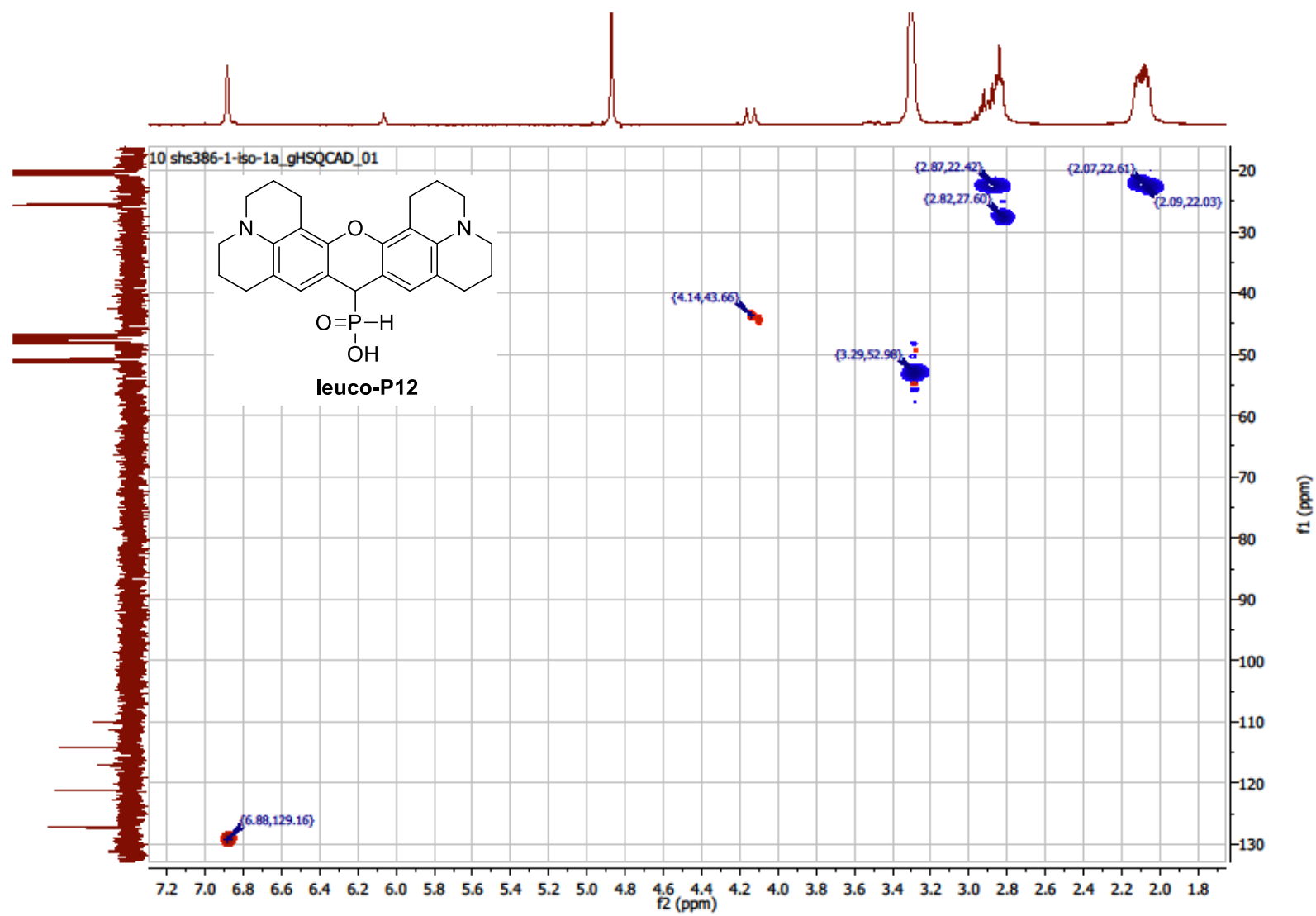
10 shs386-1-iso1b_PROTON_01



leuco-P12: $^{13}\text{C}\{^1\text{H}\}$

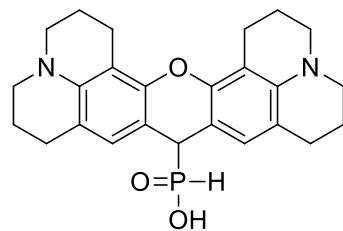


leuco-P12: gHSQCad

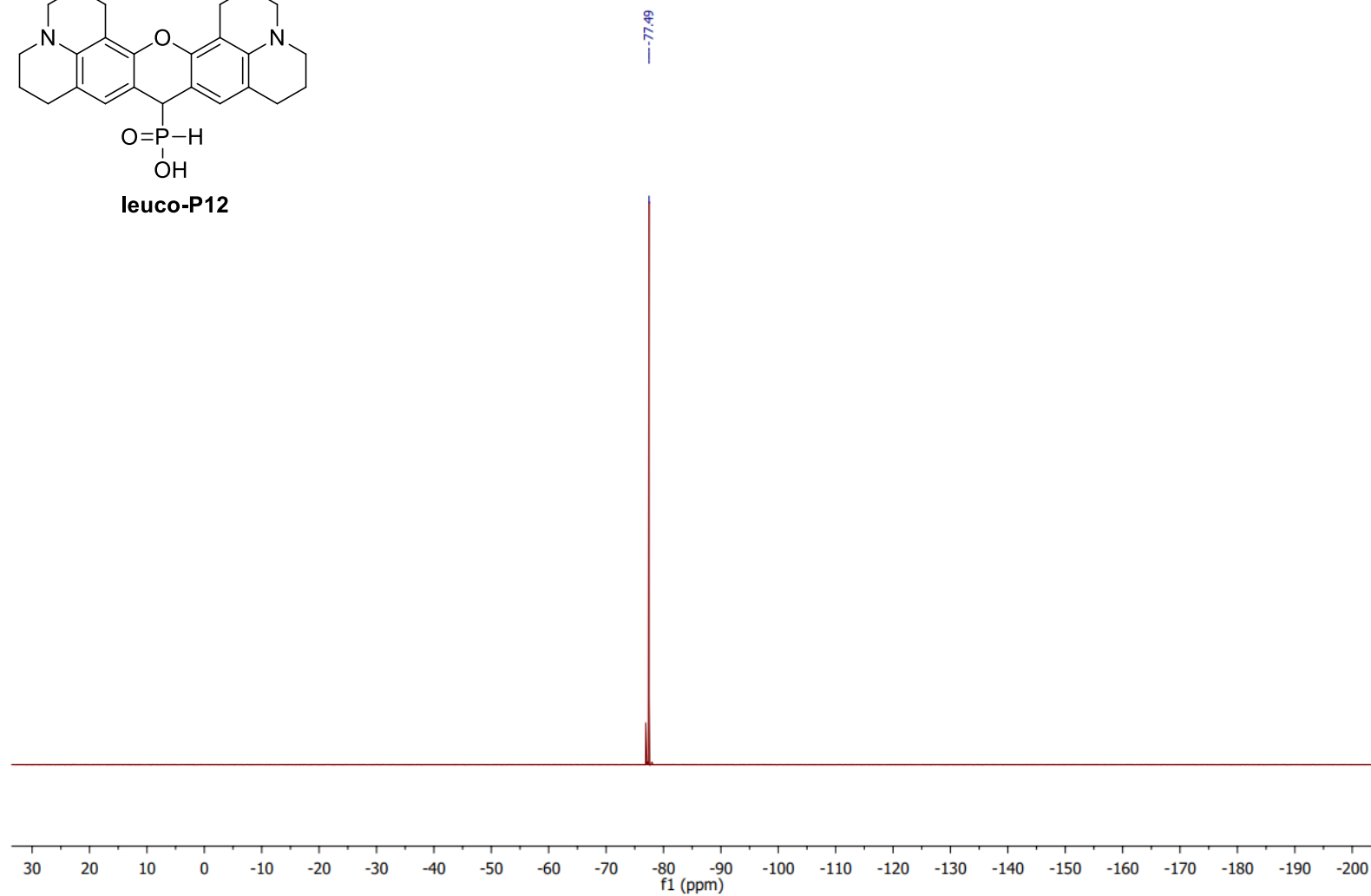


leuco-P12: ^{19}F

10 shs386-1-iso-1a_FLUORINE_01

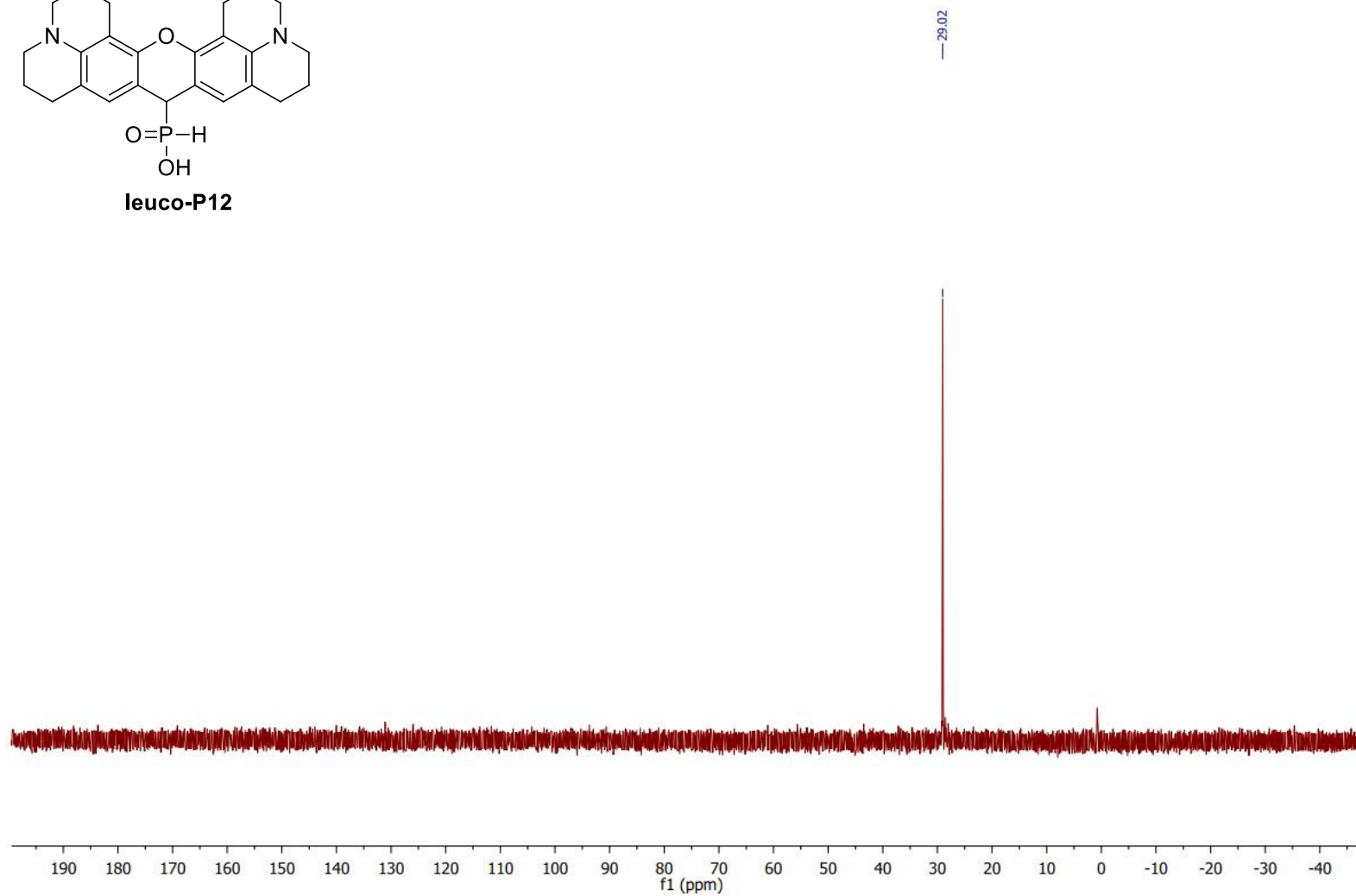
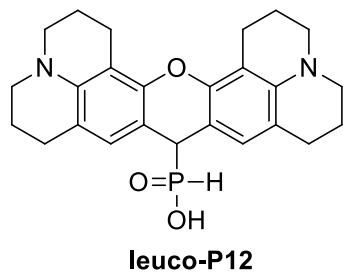


leuco-P12



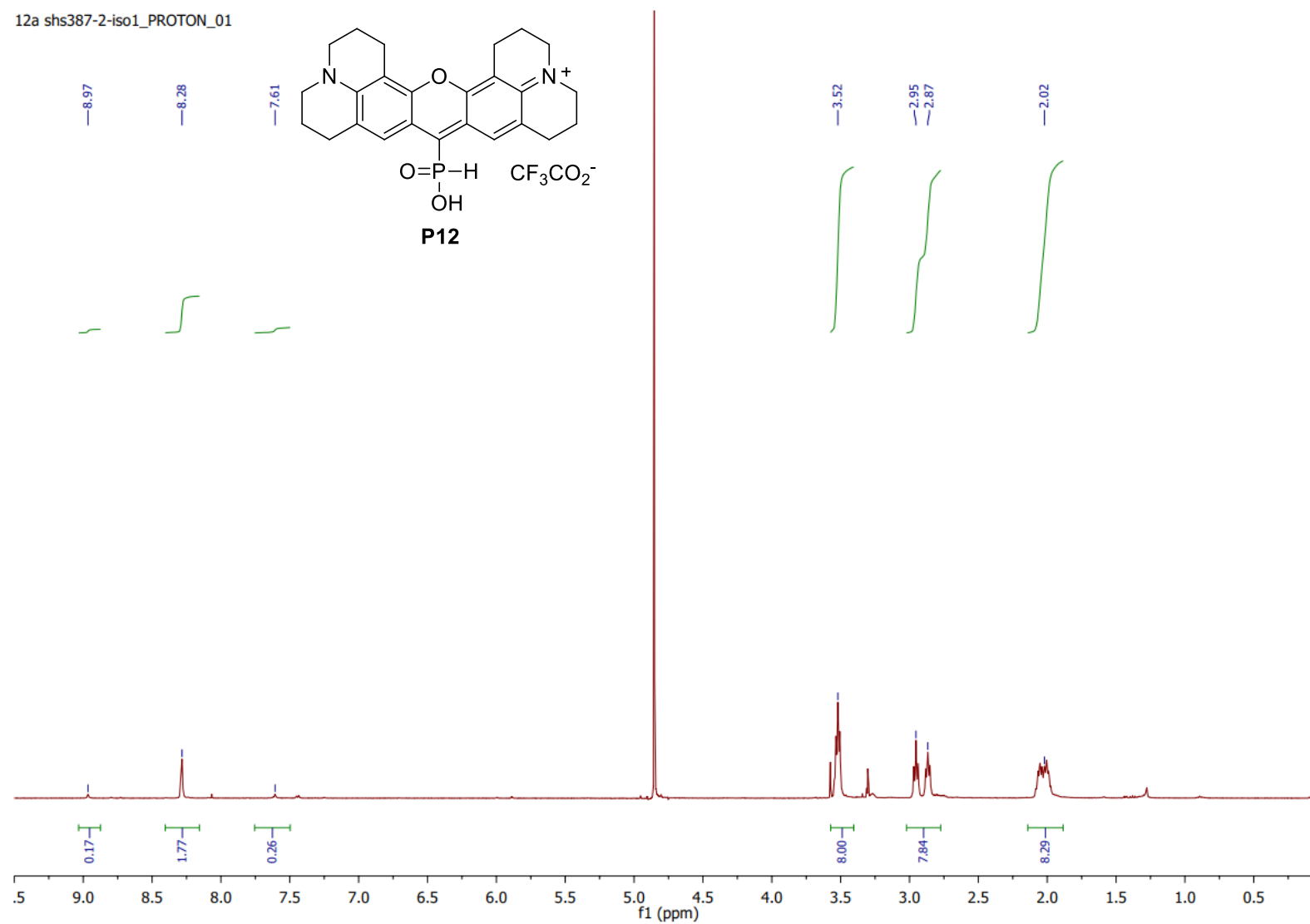
leuco-P12: ^{31}P

10 shs386-1-iso-1a_PHOSPHORUS_01



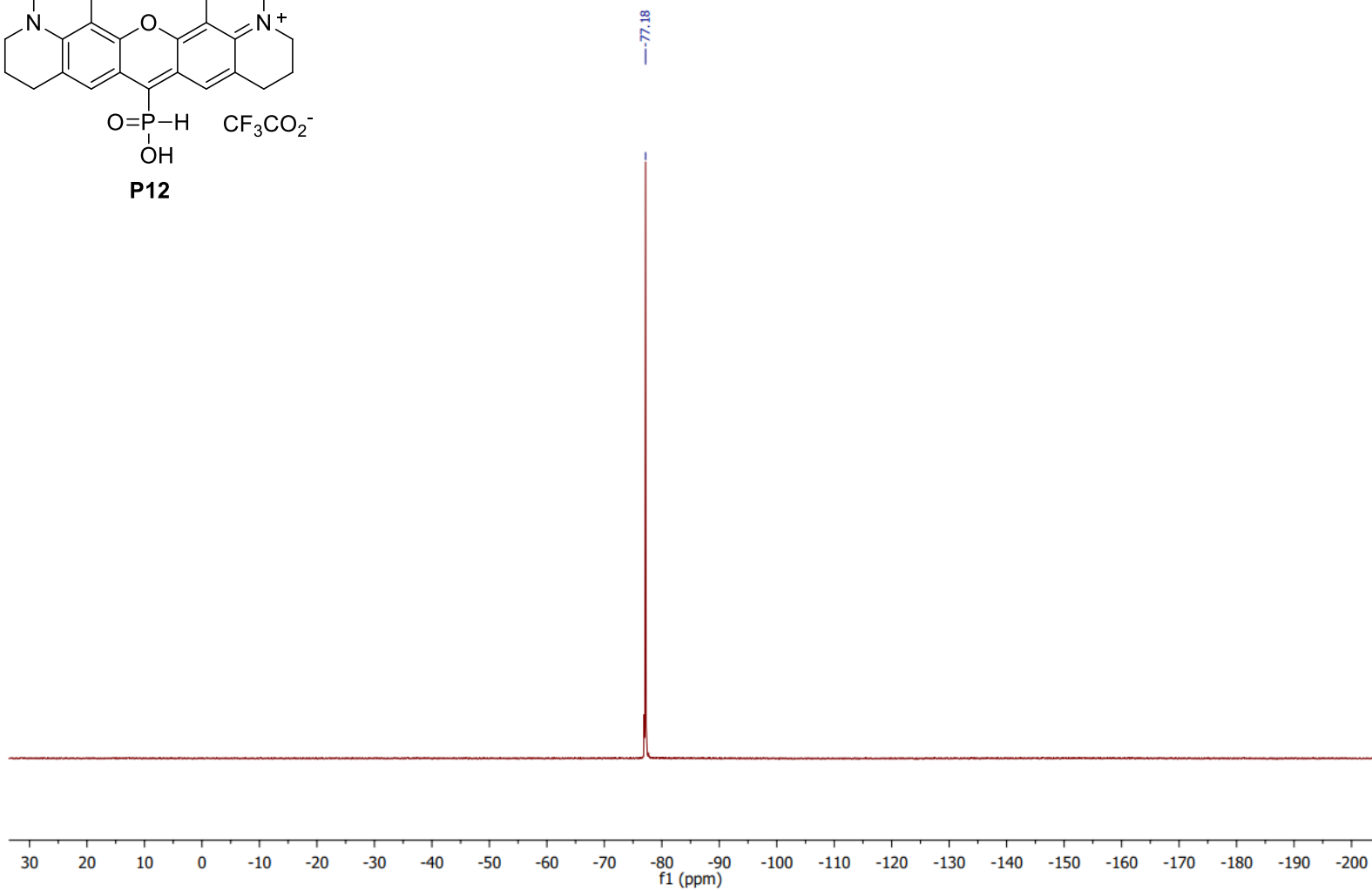
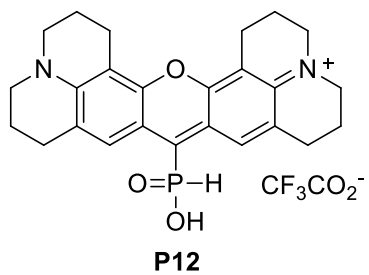
Dye P12: ^1H

12a shs387-2-iso1_PROTON_01



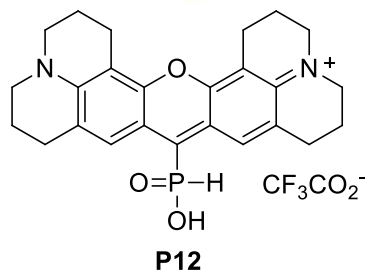
Dye **P12**: ^{19}F

shs387-4-iso-2_FLUORINE_01

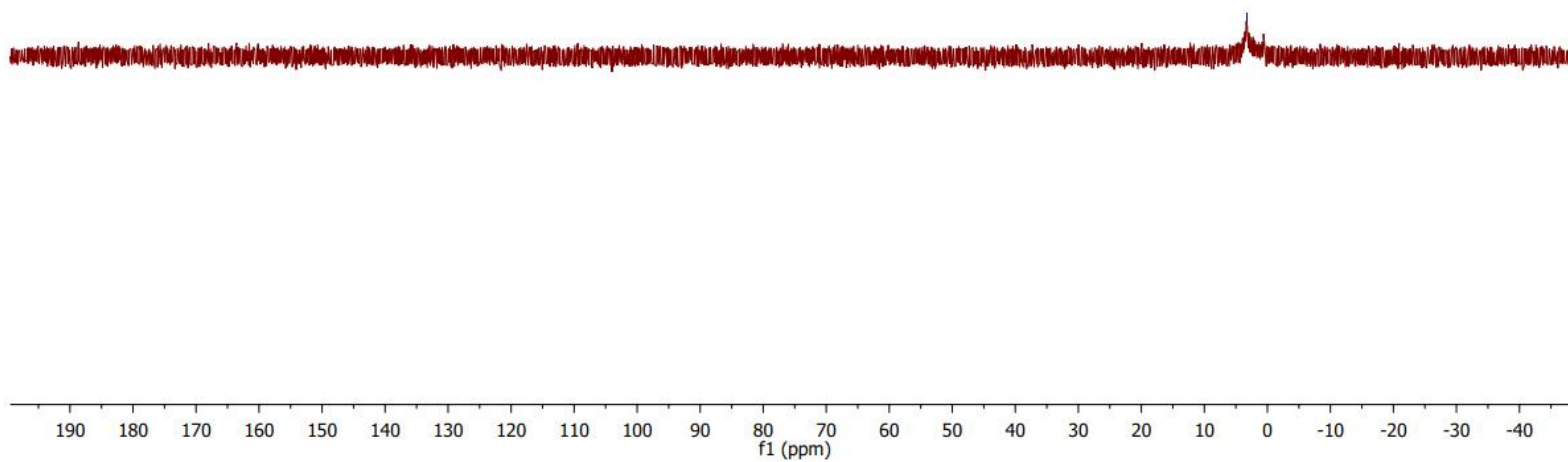


Dye P12: ^{31}P

shs387-4-iso-2_PHOSPHORUS_01

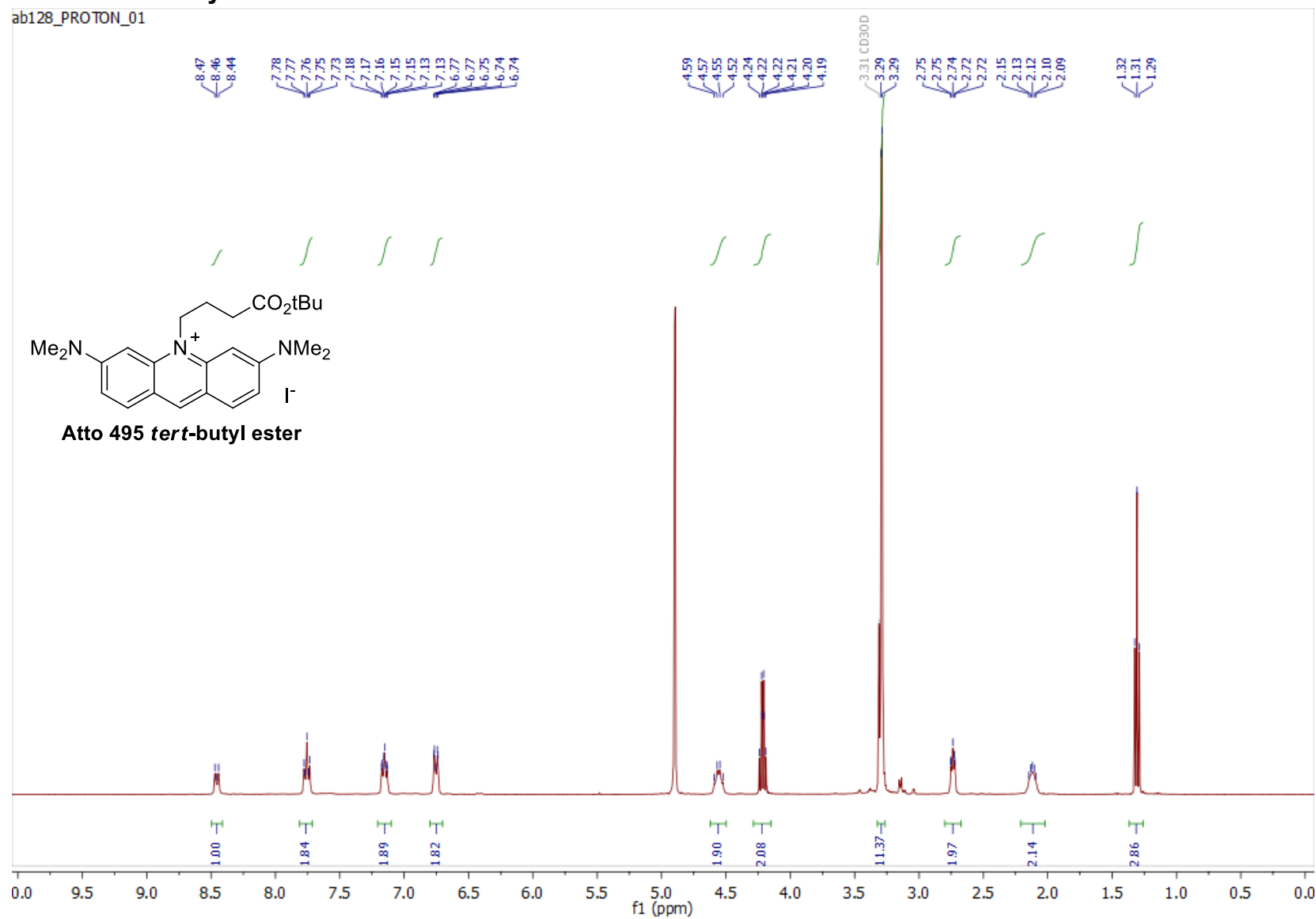


—3.25



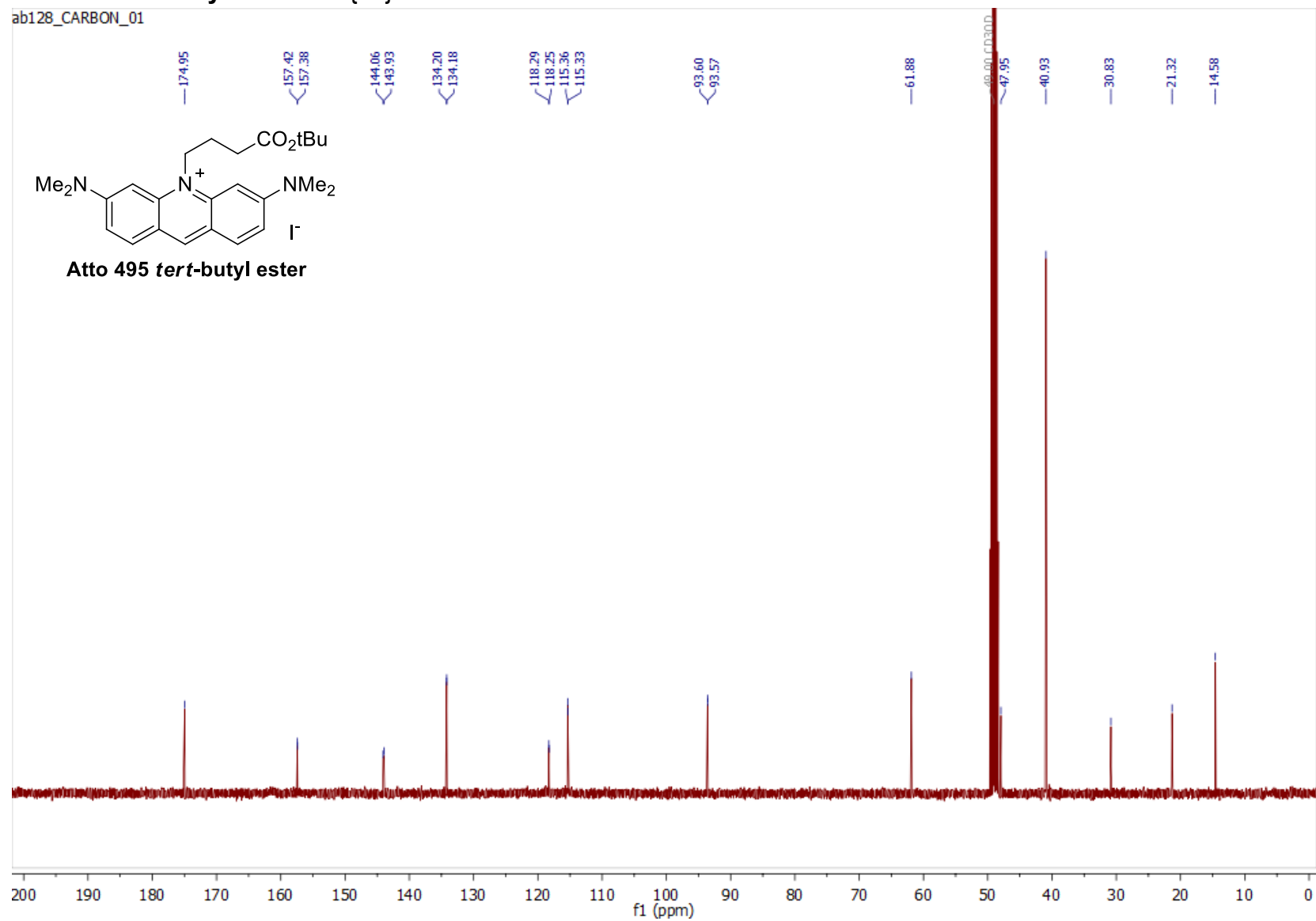
Atto 495 *tert*-butyl ester: ^1H

ab128_PROTON_01



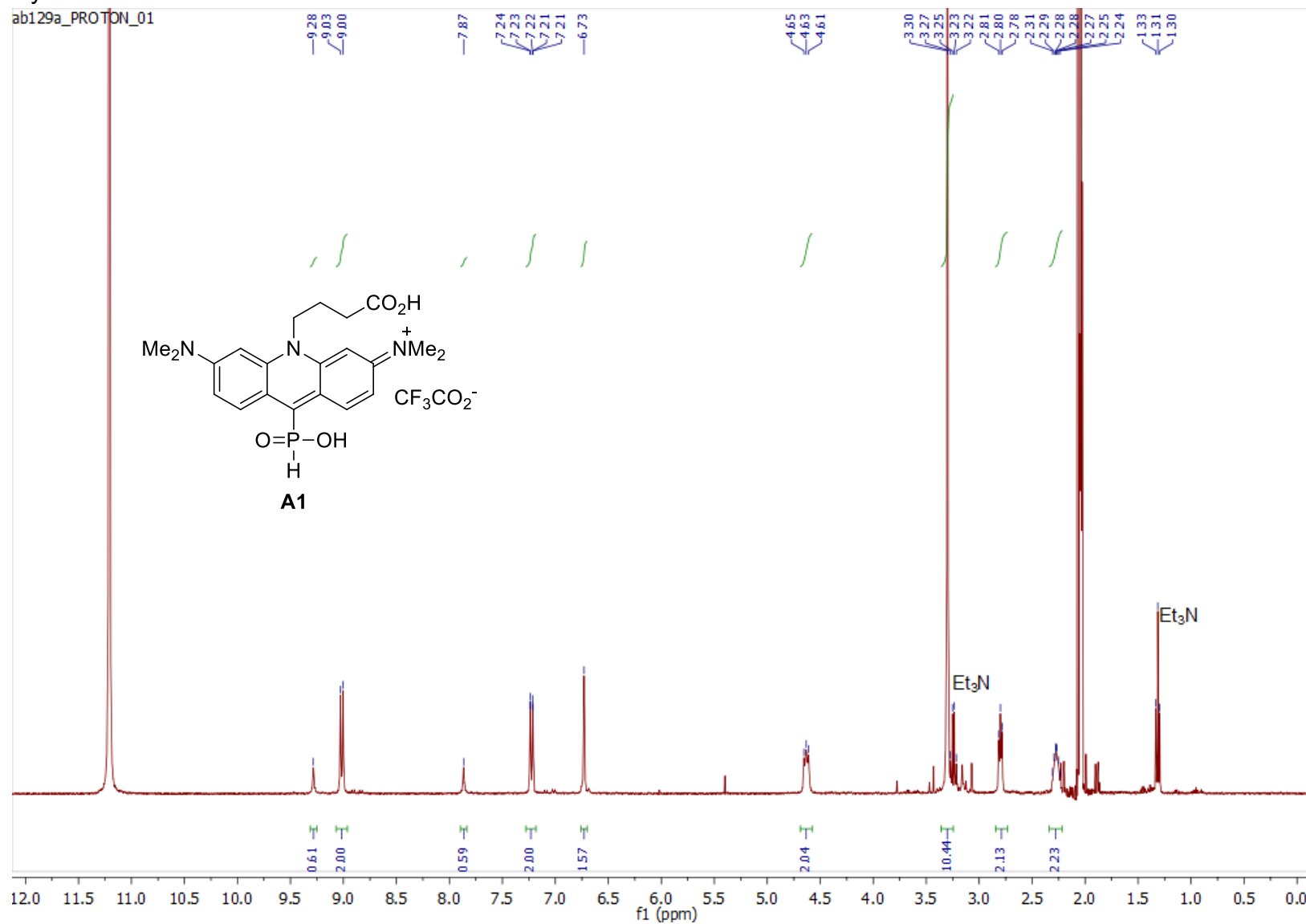
Atto 495 *tert*-butyl ester: $^{13}\text{C}\{^1\text{H}\}$

ab128_CARBON_01



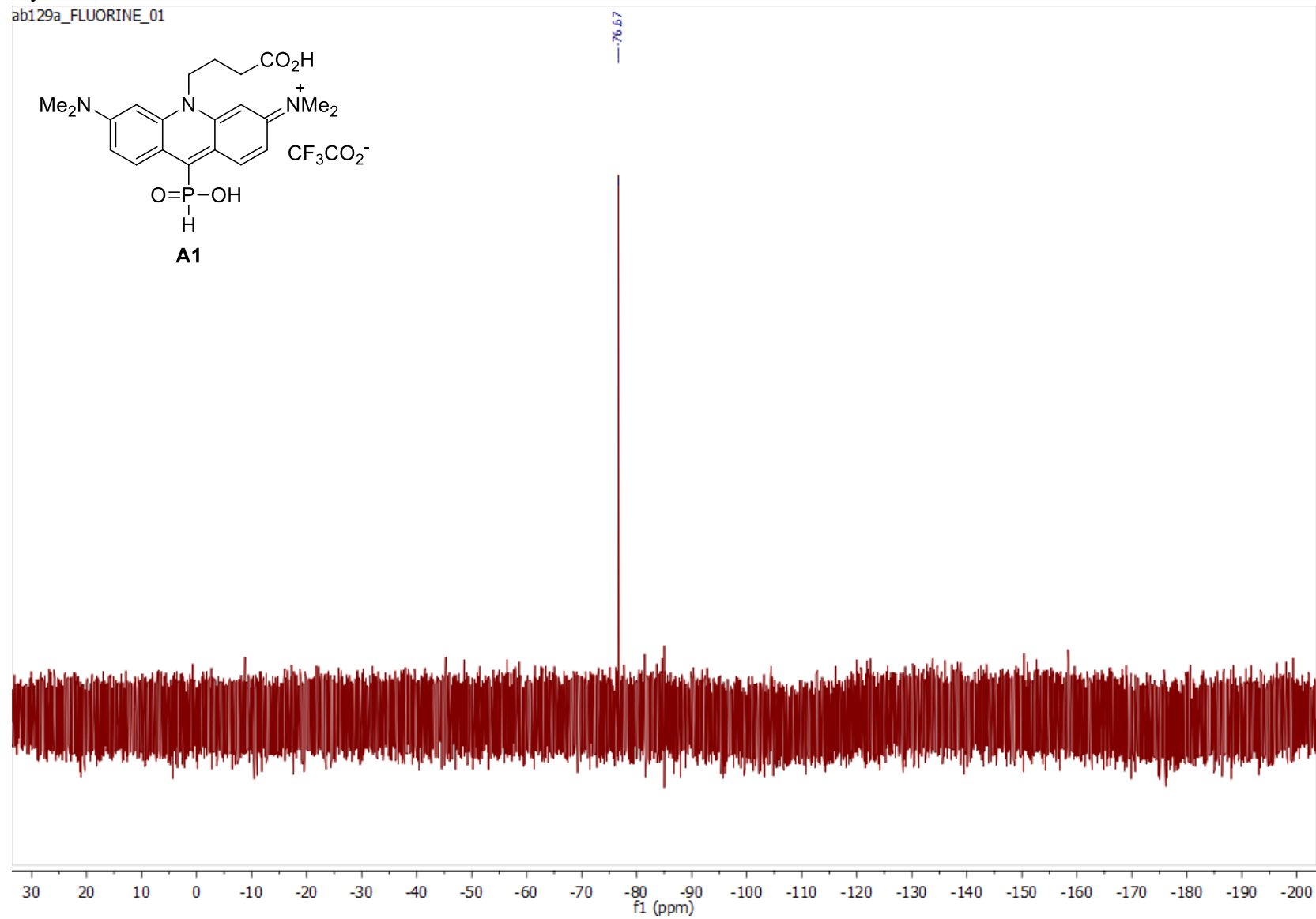
Dye A1: ¹H

ab129a_PROTON_01



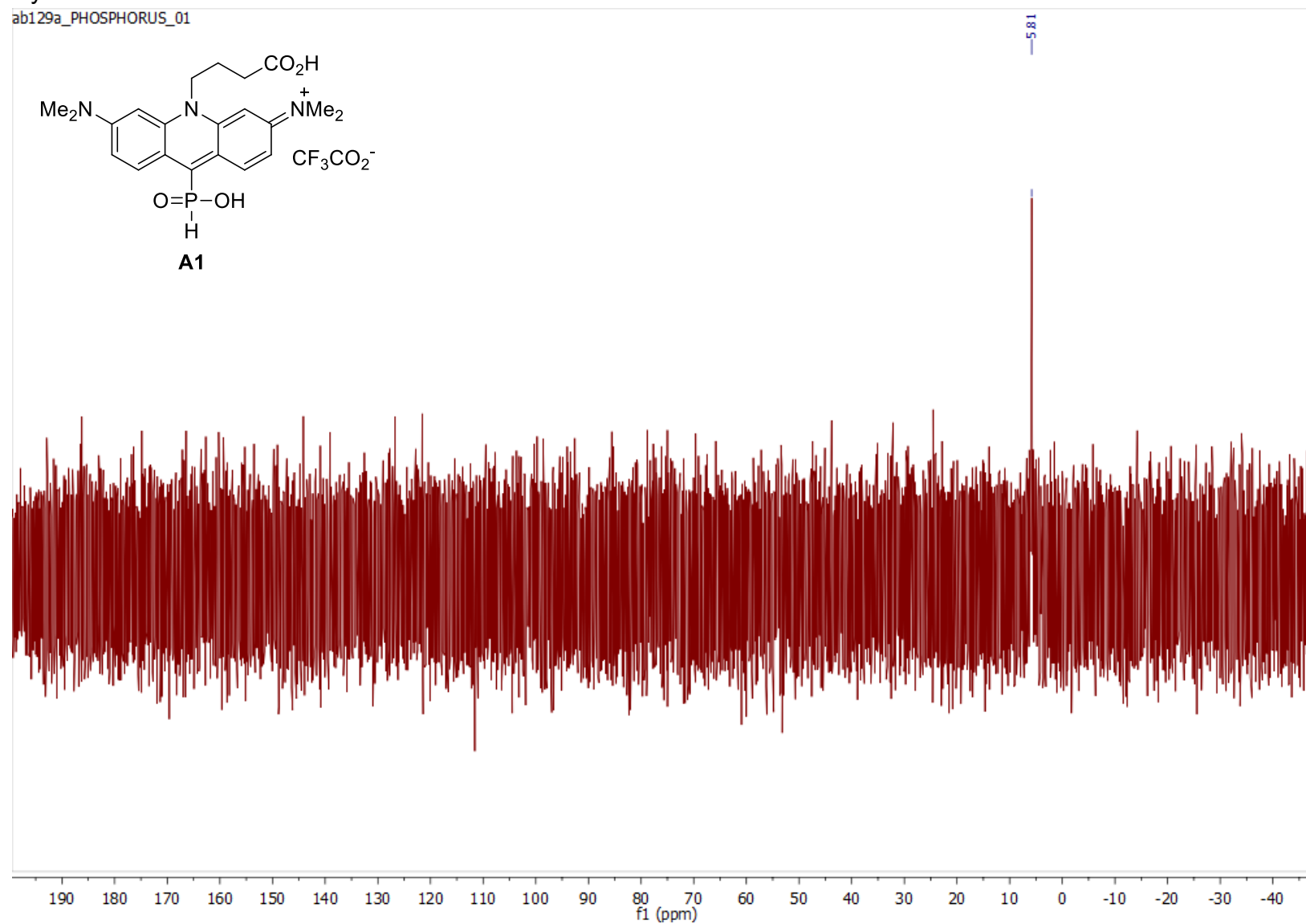
Dye A1: ^{19}F

ab129a_FLUORINE_01



Dye A1: ^{31}P

ab129a_PHOSPHORUS_01

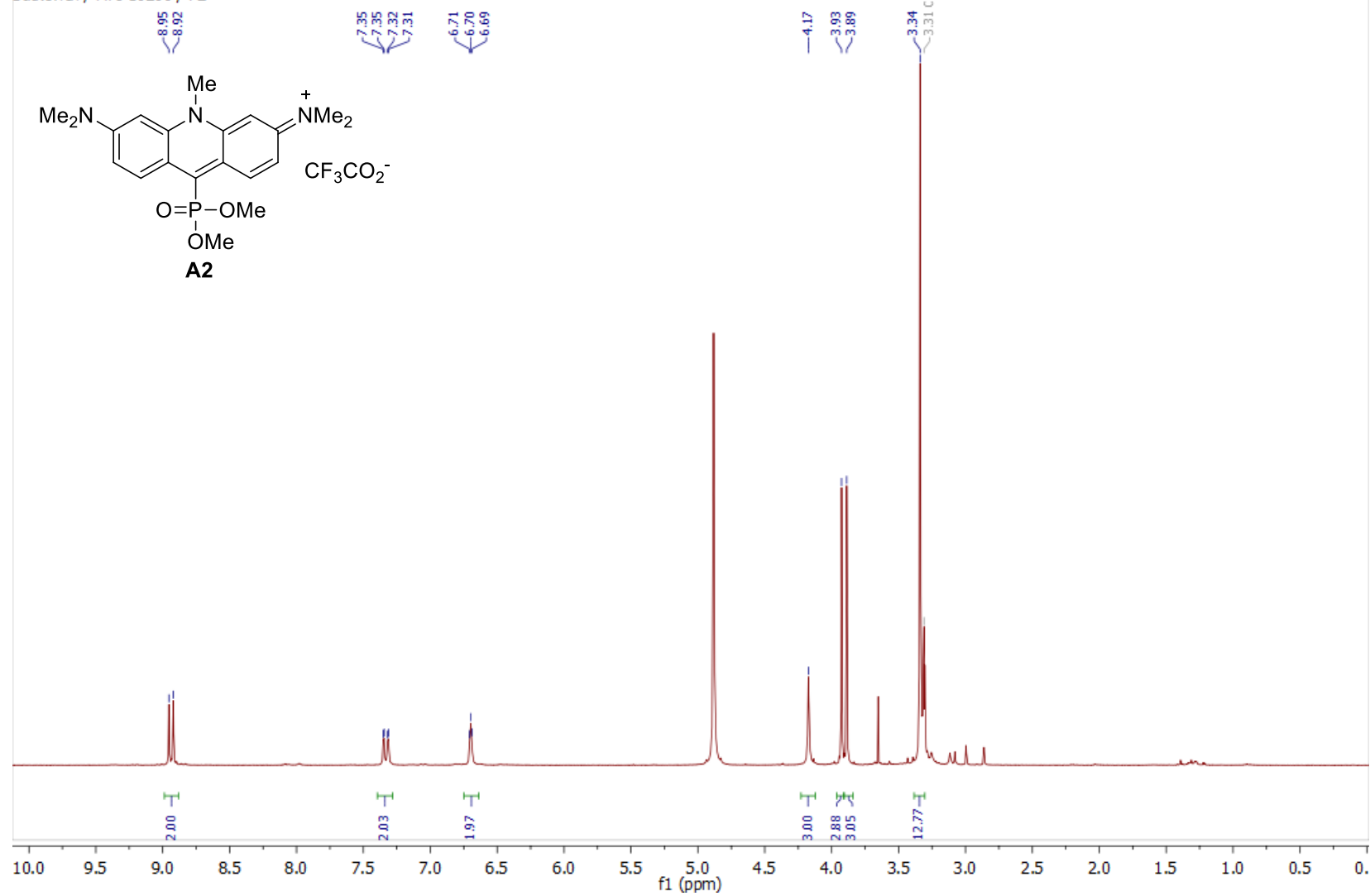


Dye A2: ^1H

ab132_3h

AB132 cd30d

Butkevich / MPI 10200 / PE

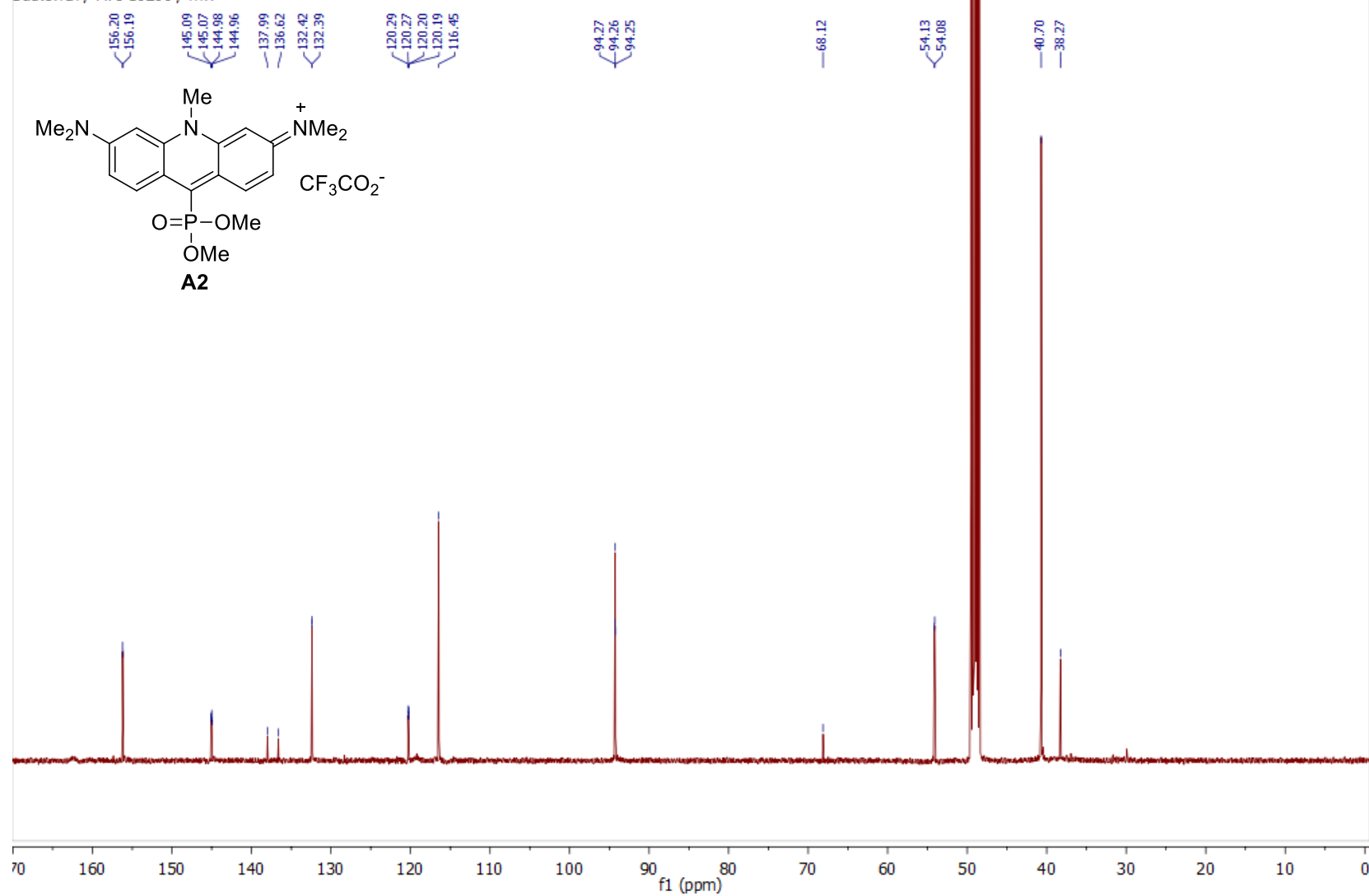


Dye **A2**: $^{13}\text{C}\{^1\text{H}\}$

ab132_5c

AB132 cd3od

Butkevich / MPI 10200 / mw

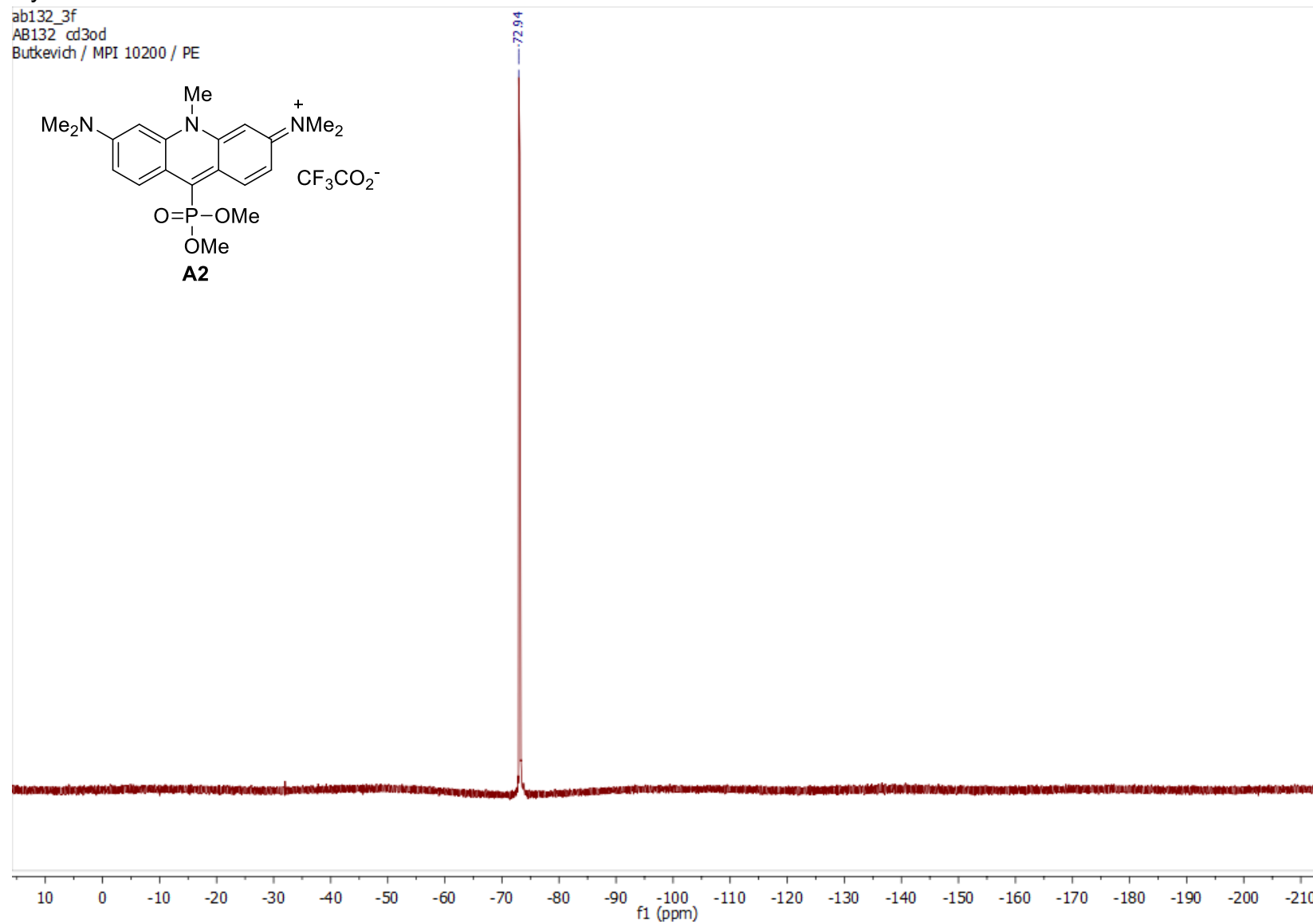


Dye **A2**: ^{19}F

ab132_3f

AB132 cd3od

Butkevich / MPI 10200 / PE

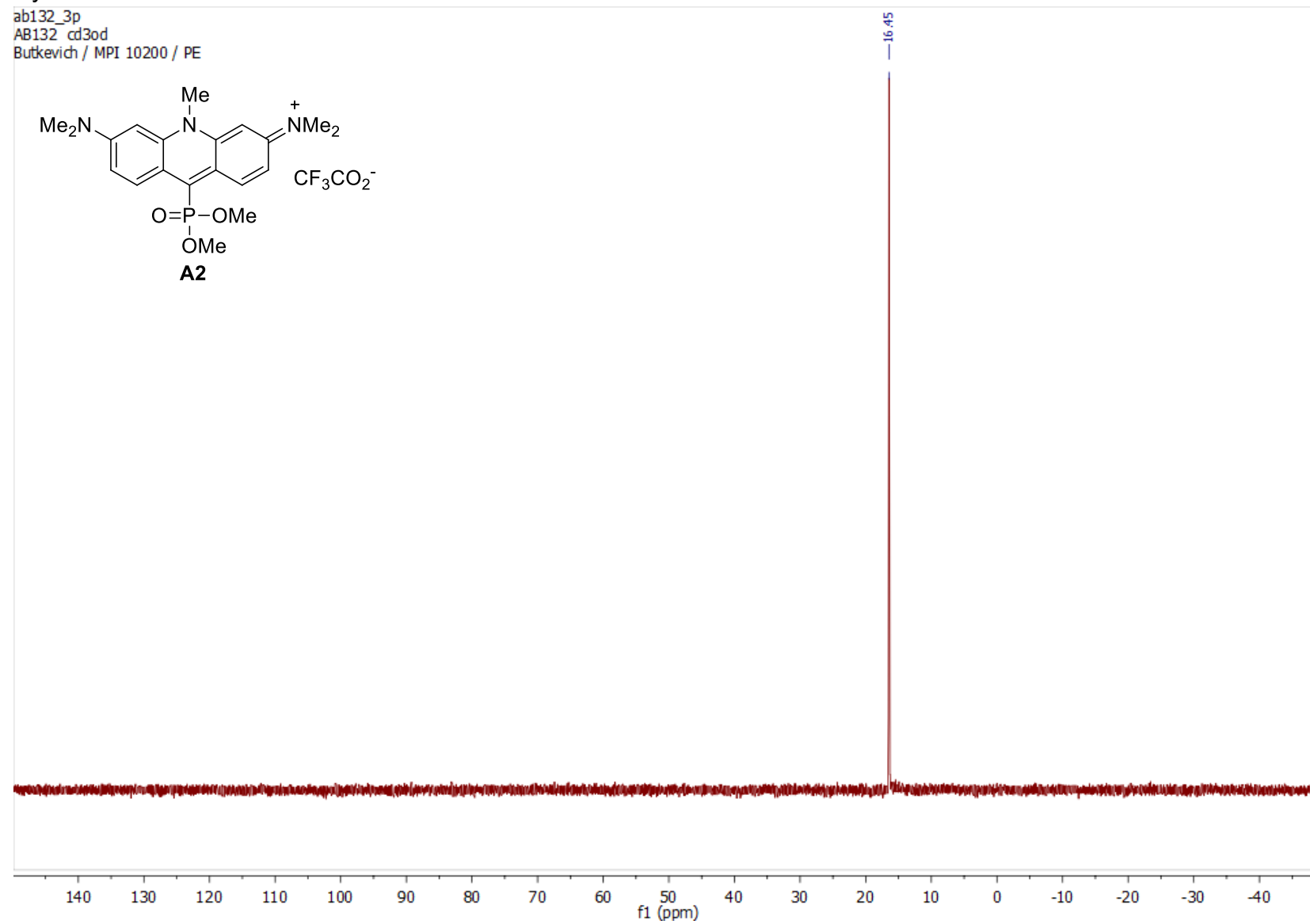
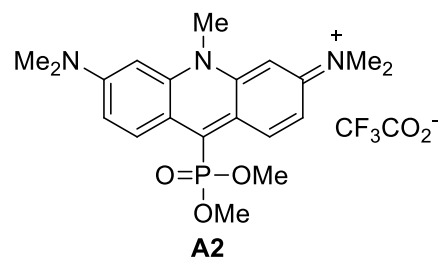


Dye A2: ^{31}P

ab132_3p

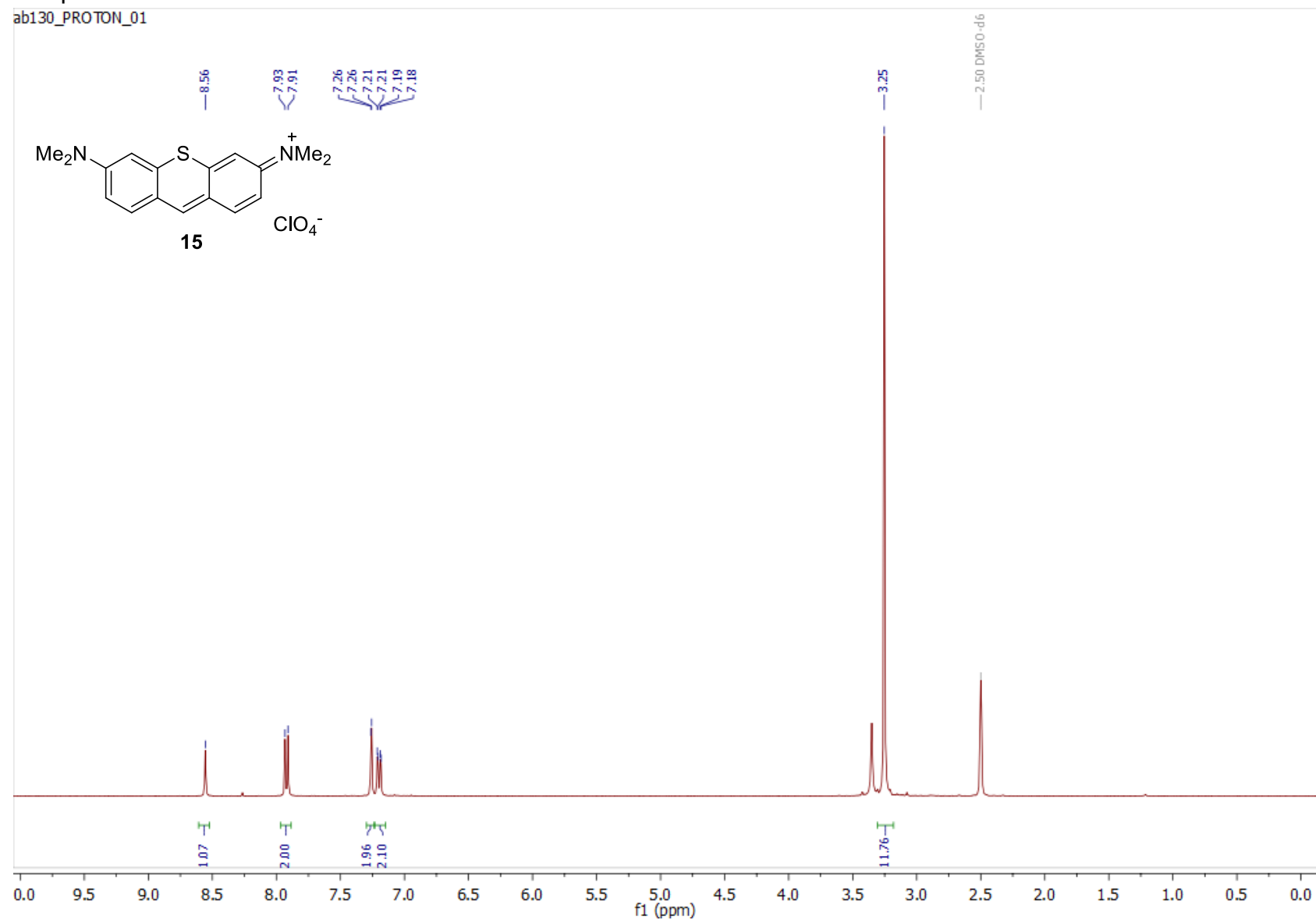
AB132 cd3od

Butkevich / MPI 10200 / PE



compound **15**: ^1H

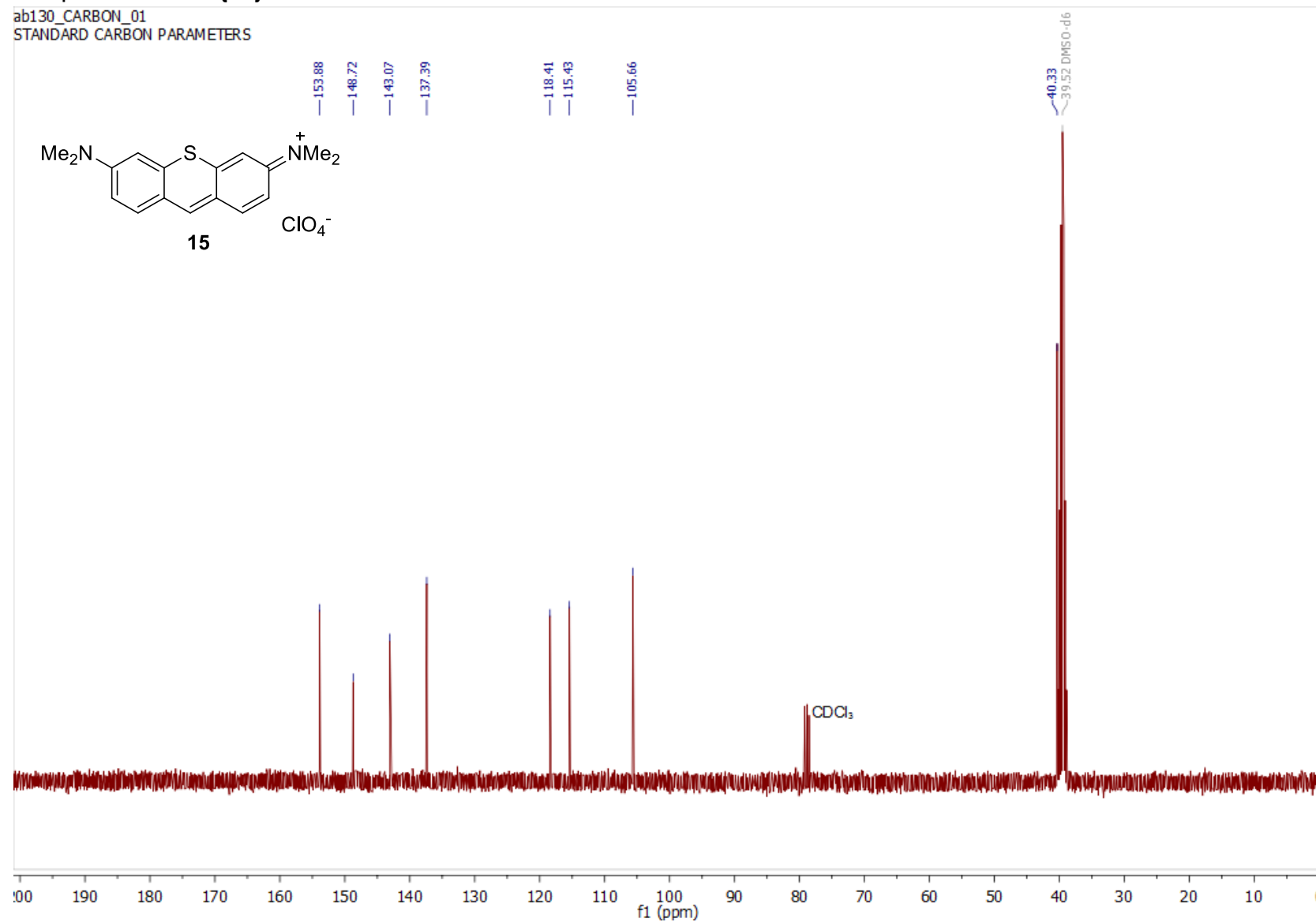
ab130_PROTON_01



compound **15**: $^{13}\text{C}\{^1\text{H}\}$

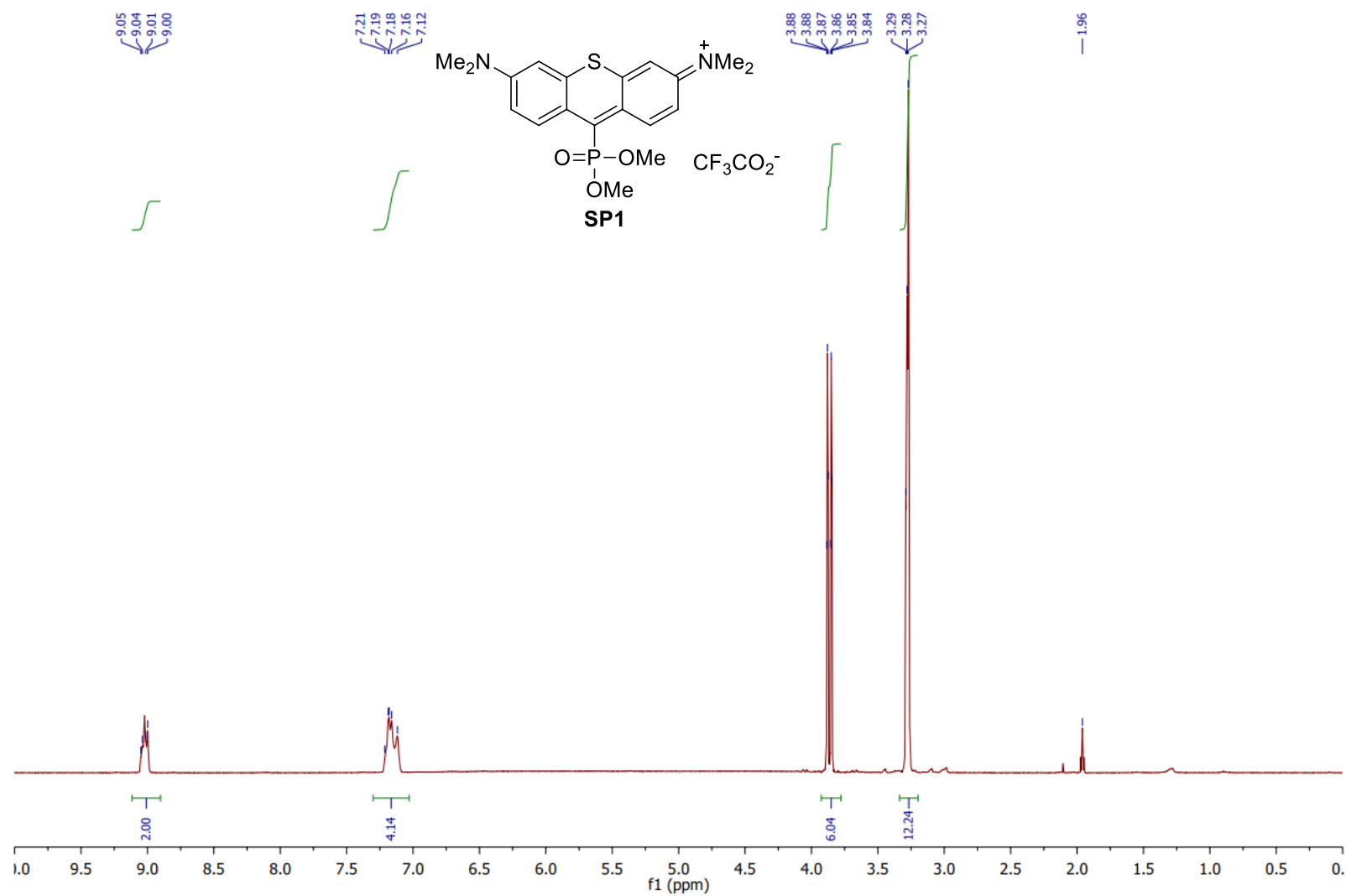
ab130_CARBON_01

STANDARD CARBON PARAMETERS



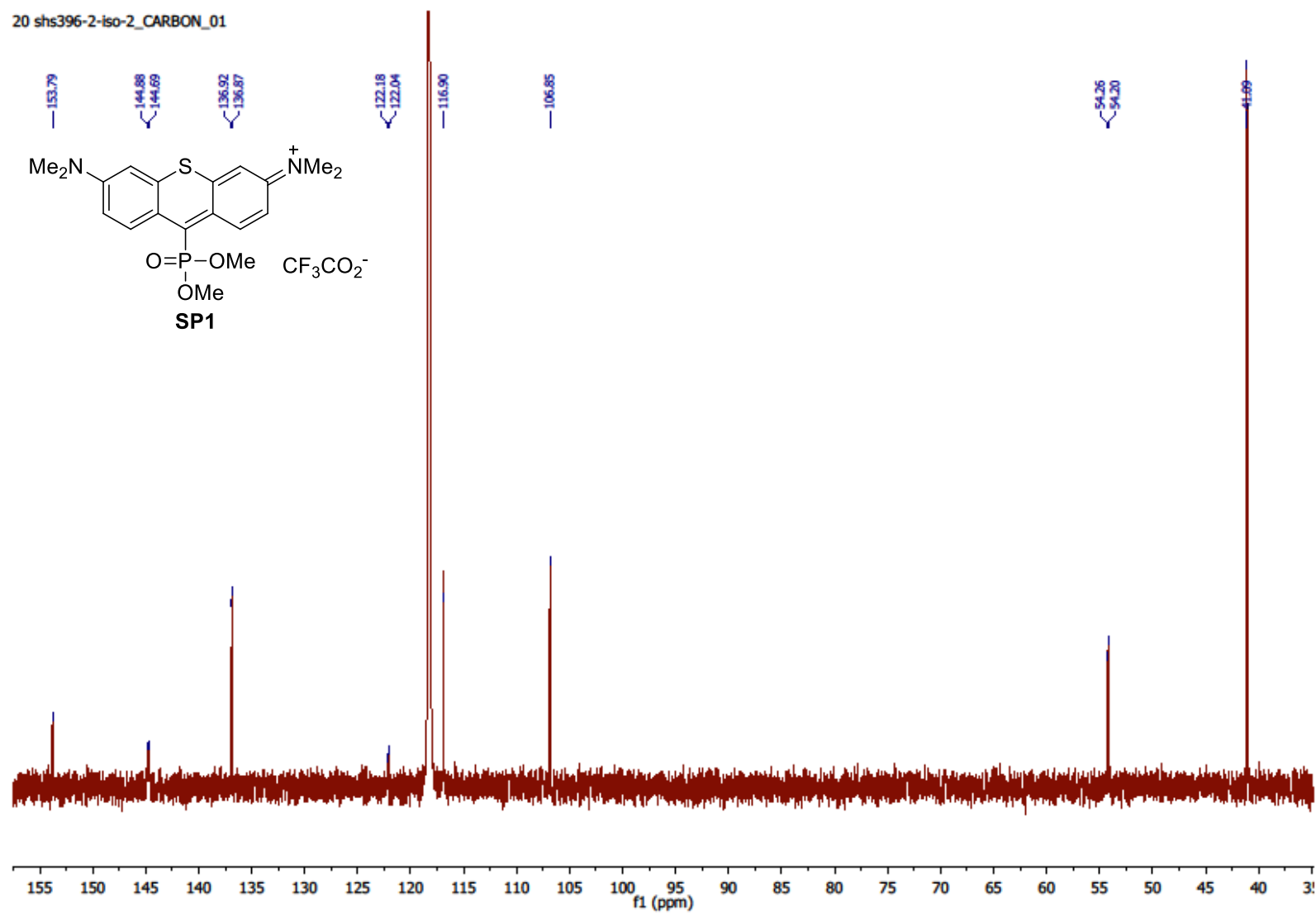
Dye SP1: ^1H

20 shs396-2-iso-2_PROTON_01



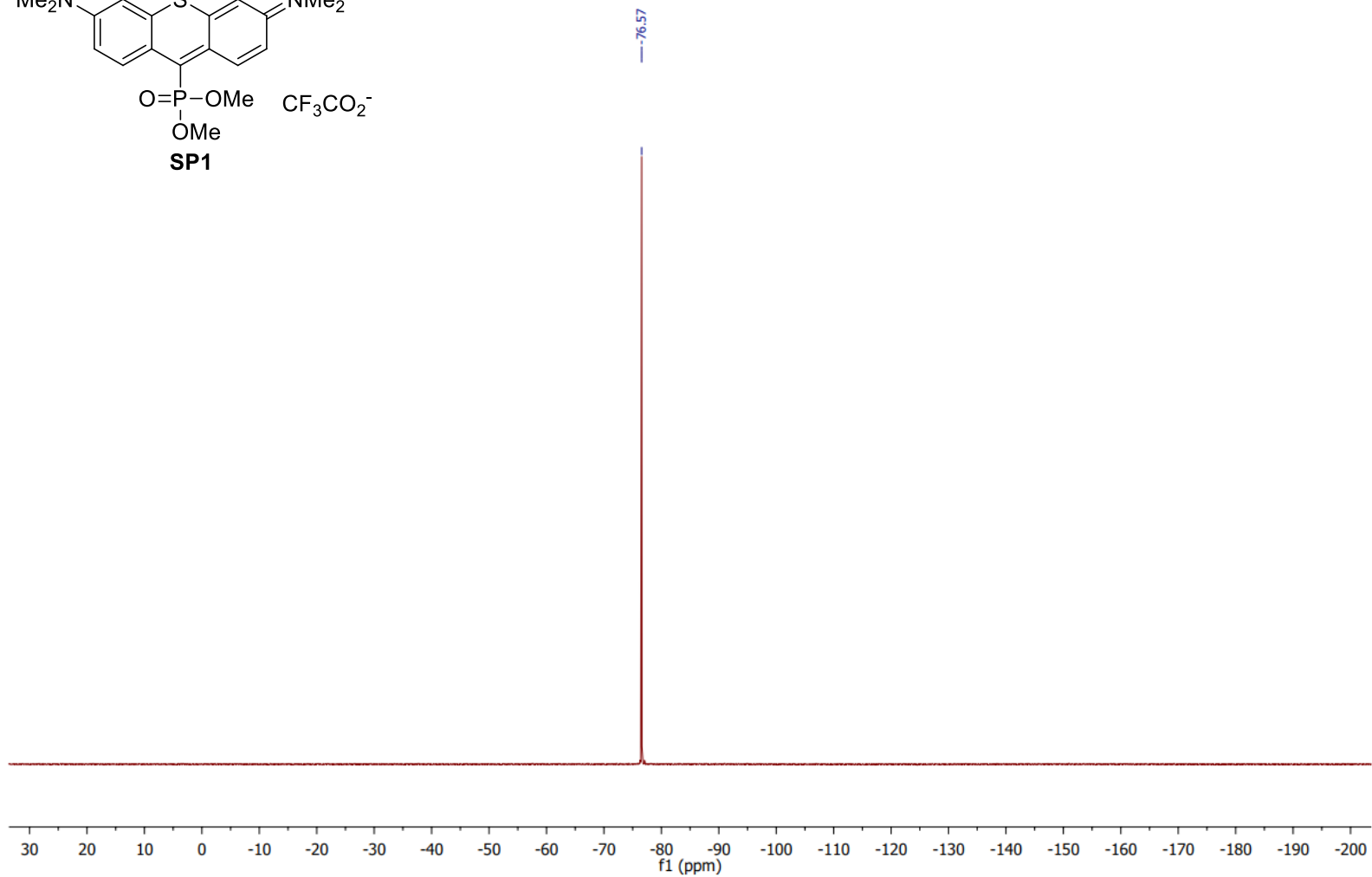
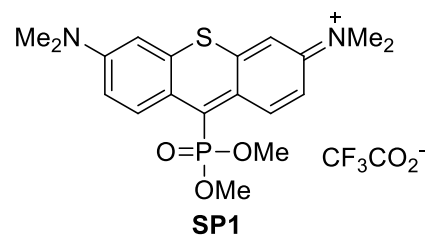
Dye **SP1**: $^{13}\text{C}\{^1\text{H}\}$

20 shs396-2-iso-2_CARBON_01



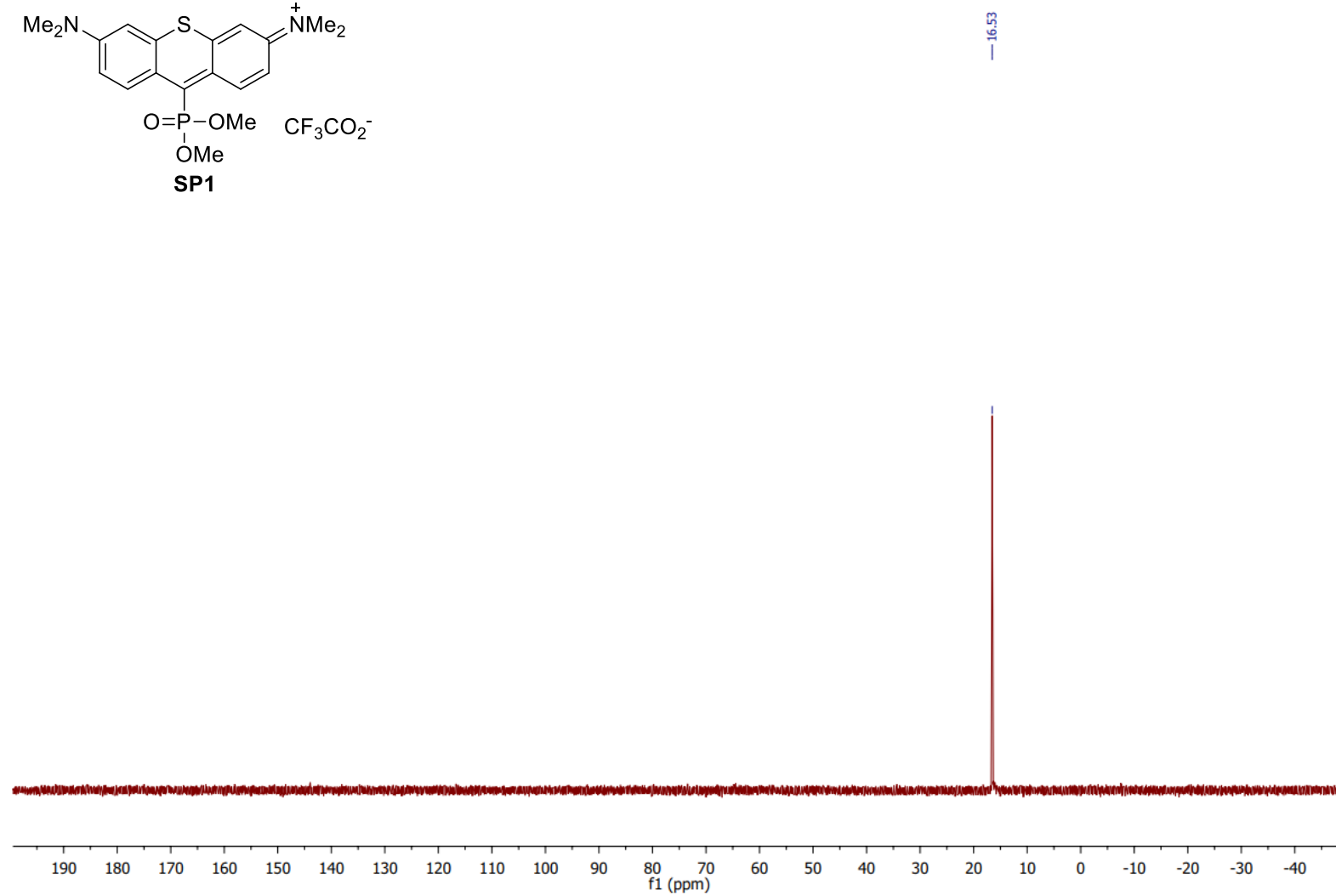
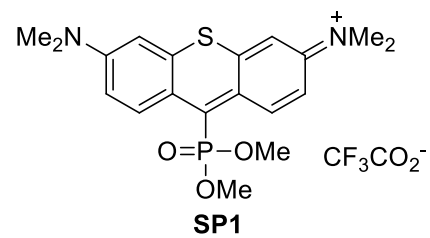
Dye **SP1**: ^{19}F

20 shs396-2-iso-2_FLUORINE_01



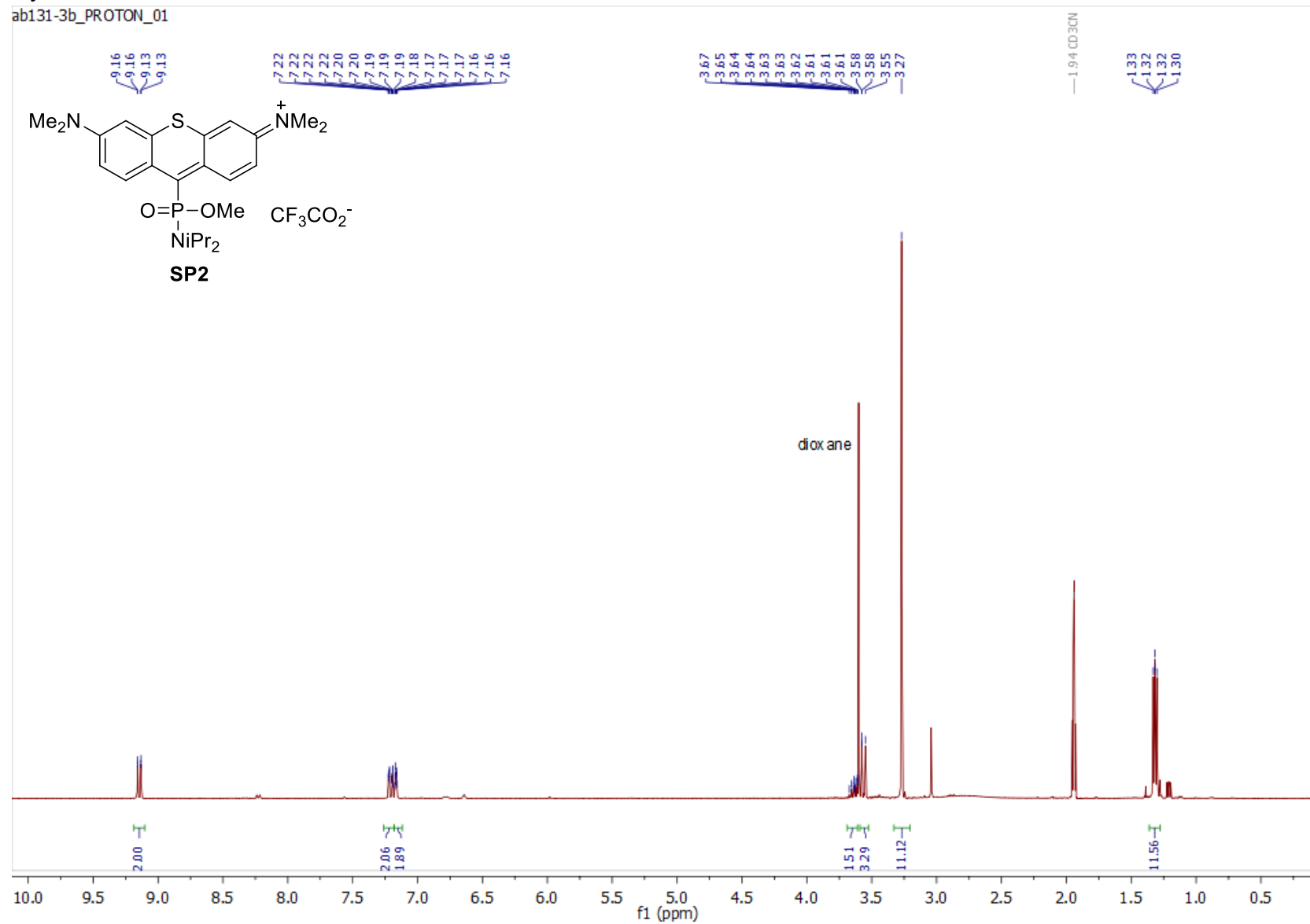
Dye **SP1**: ^{31}P

20 shs396-2-iso-2_PHOSPHORUS_01



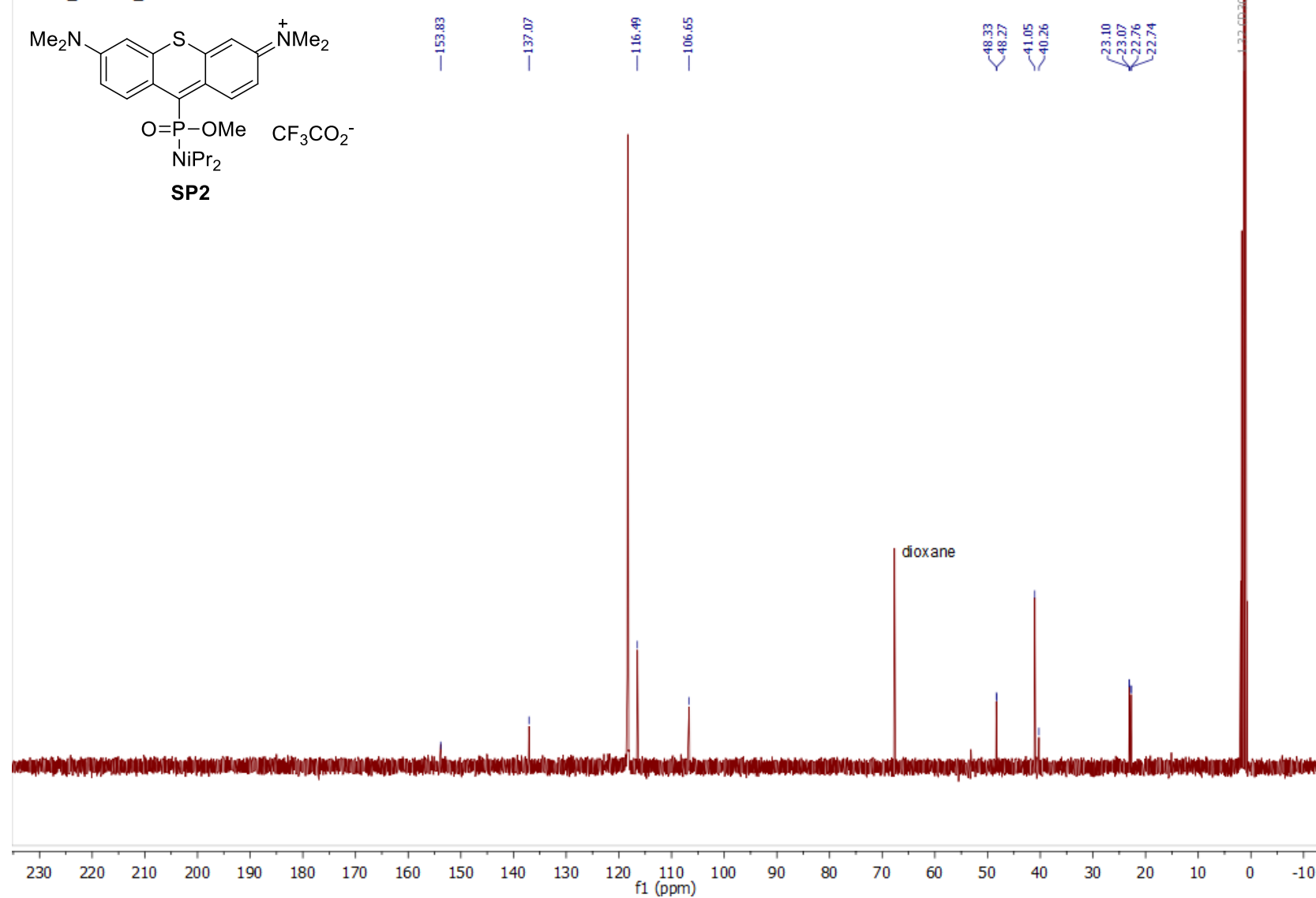
Dye SP2: ¹H

ab131-3b_PROTON_01



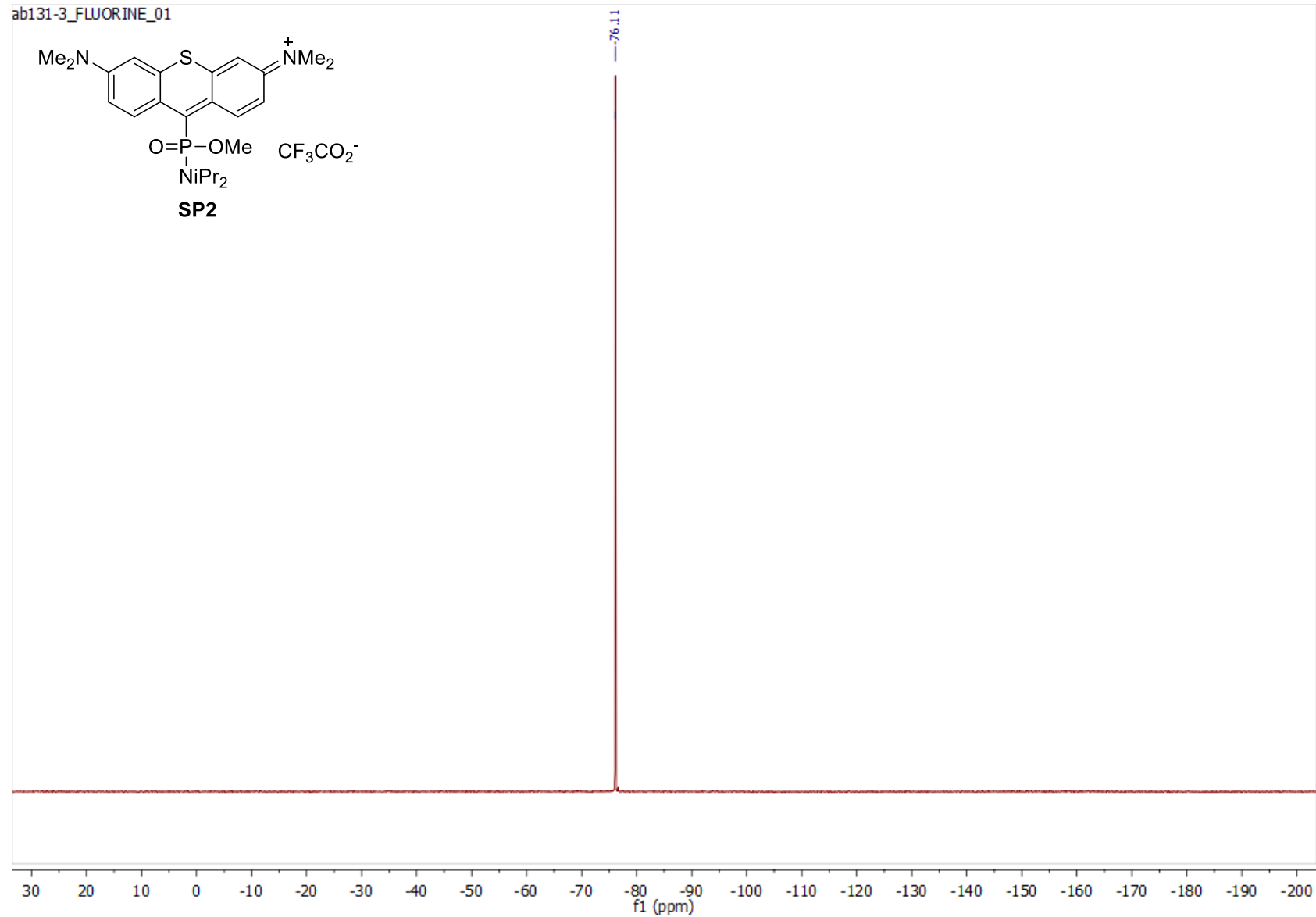
Dye **SP2**: $^{13}\text{C}\{^1\text{H}\}$

ab131-3_CARBON_01



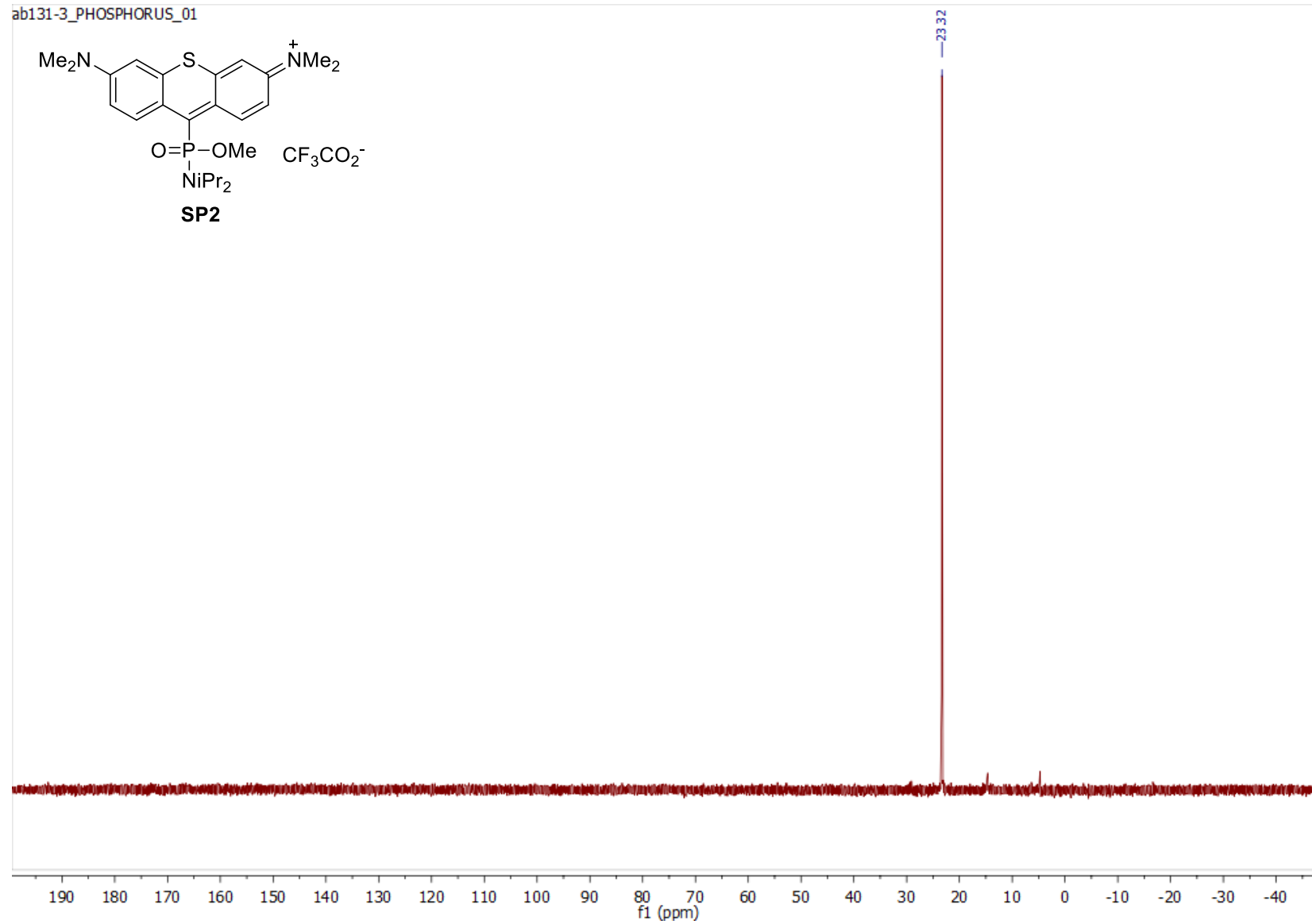
Dye **SP2**: ^{19}F

ab131-3_FLUORINE_01



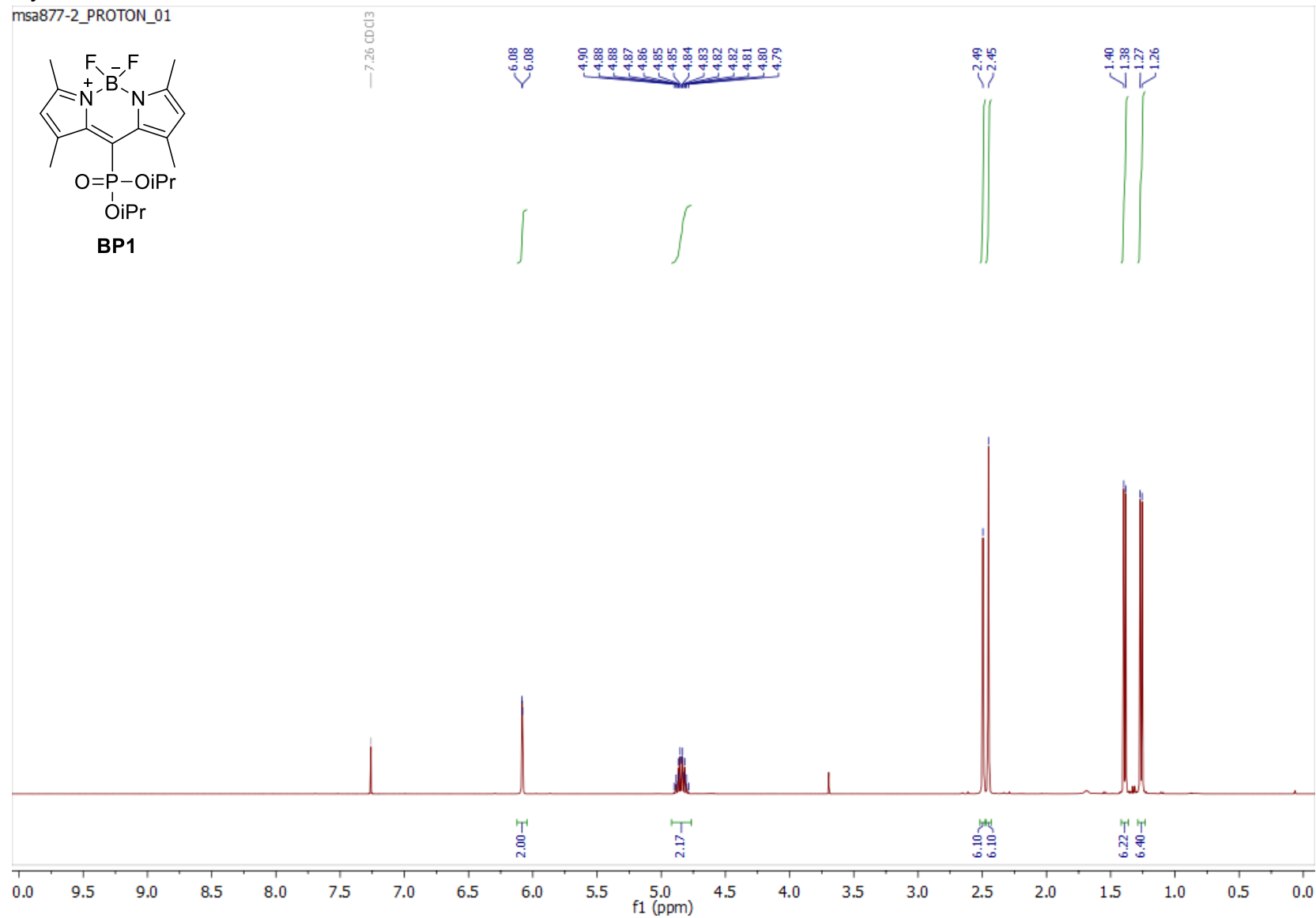
Dye **SP2**: ^{31}P

ab131-3_PHOSPHORUS_01



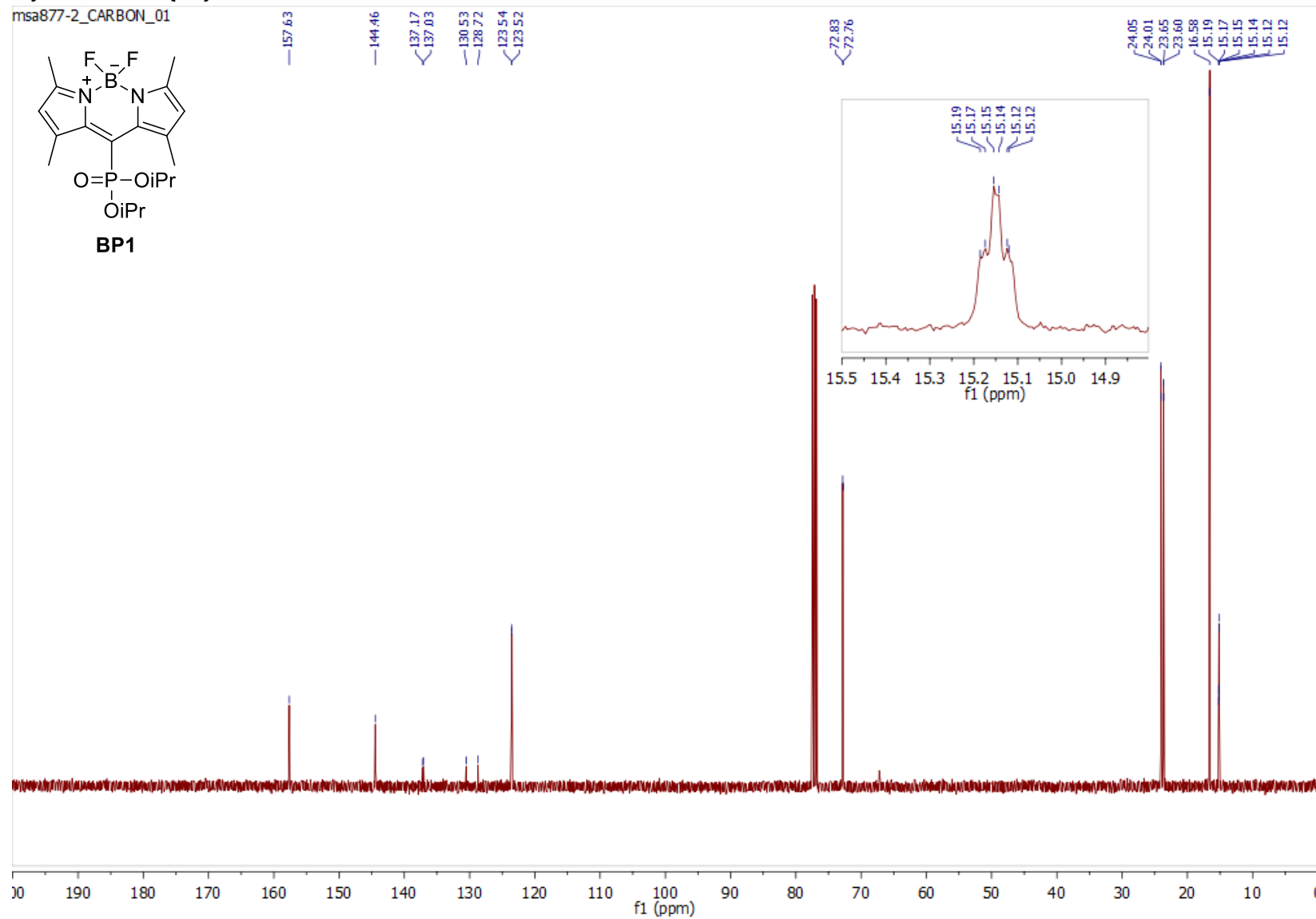
Dye BP1: ^1H

msa877-2_PROTON_01



Dye **BP1**: $^{13}\text{C}\{^1\text{H}\}$

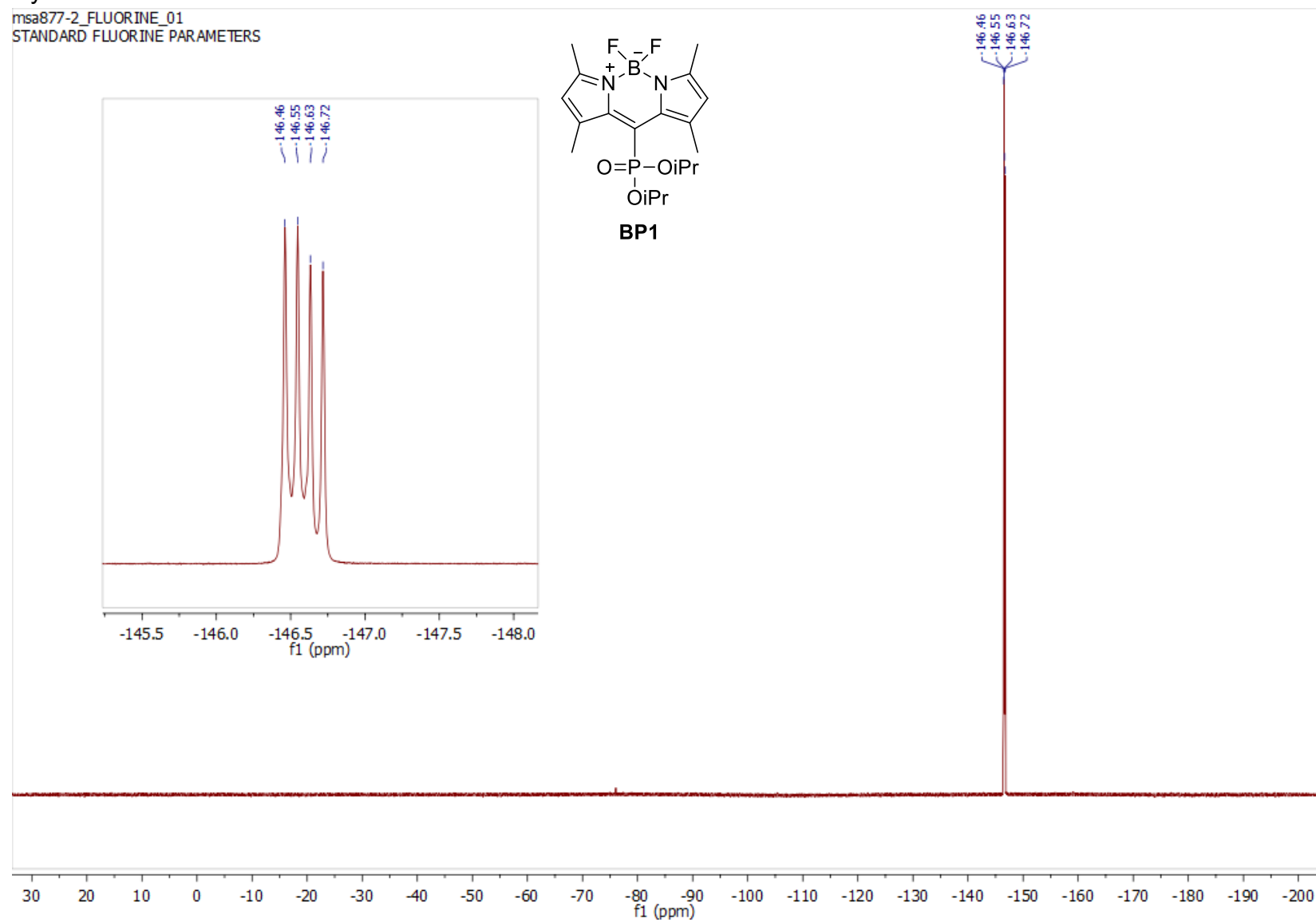
msa877-2_CARBON_01



Dye BP1: ¹⁹F

msa877-2_FLUORINE_01

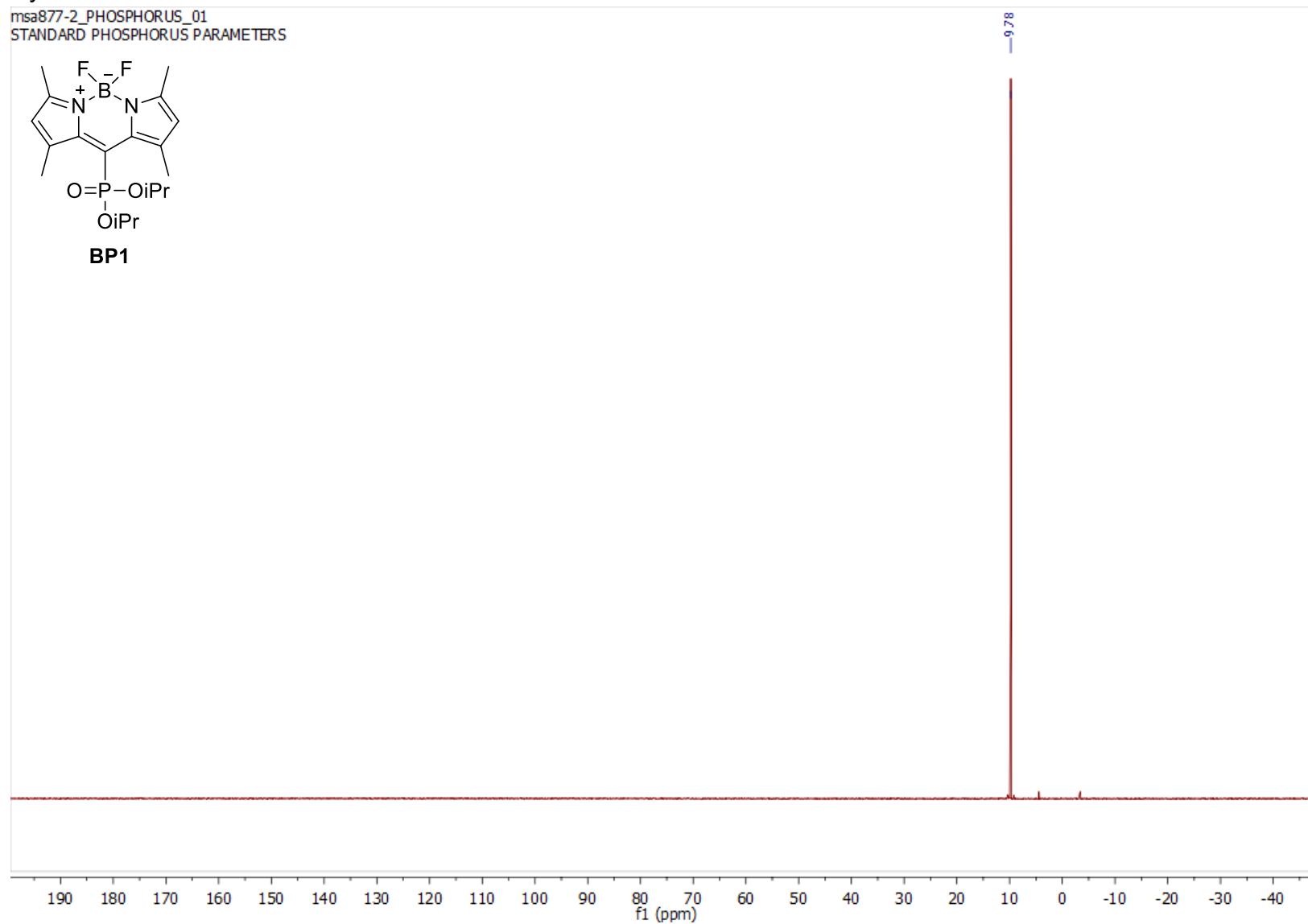
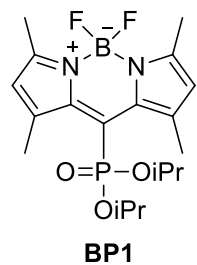
STANDARD FLUORINE PARAMETERS



Dye BP1: ³¹P

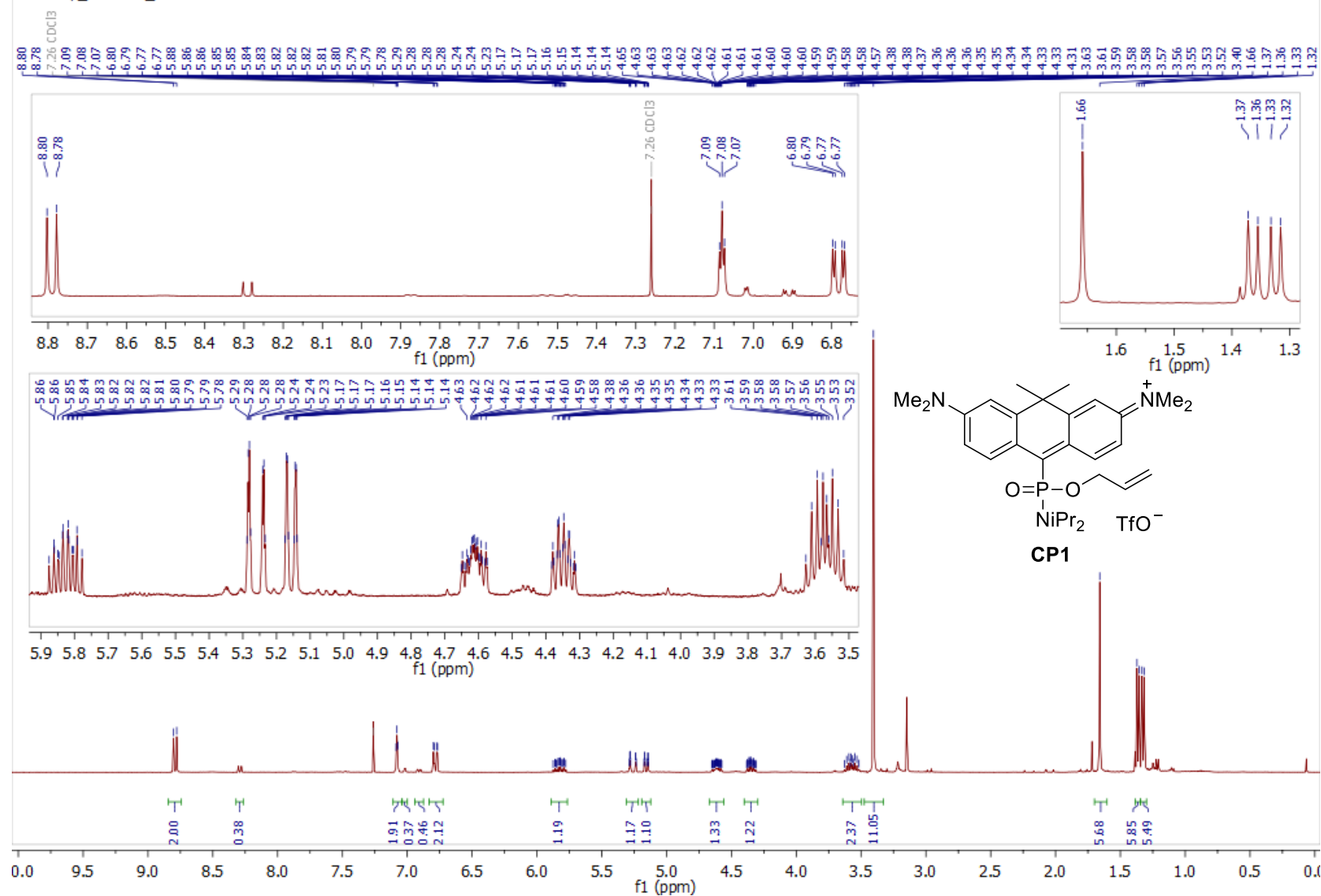
msa877-2_PHOSPHORUS_01

STANDARD PHOSPHORUS PARAMETERS



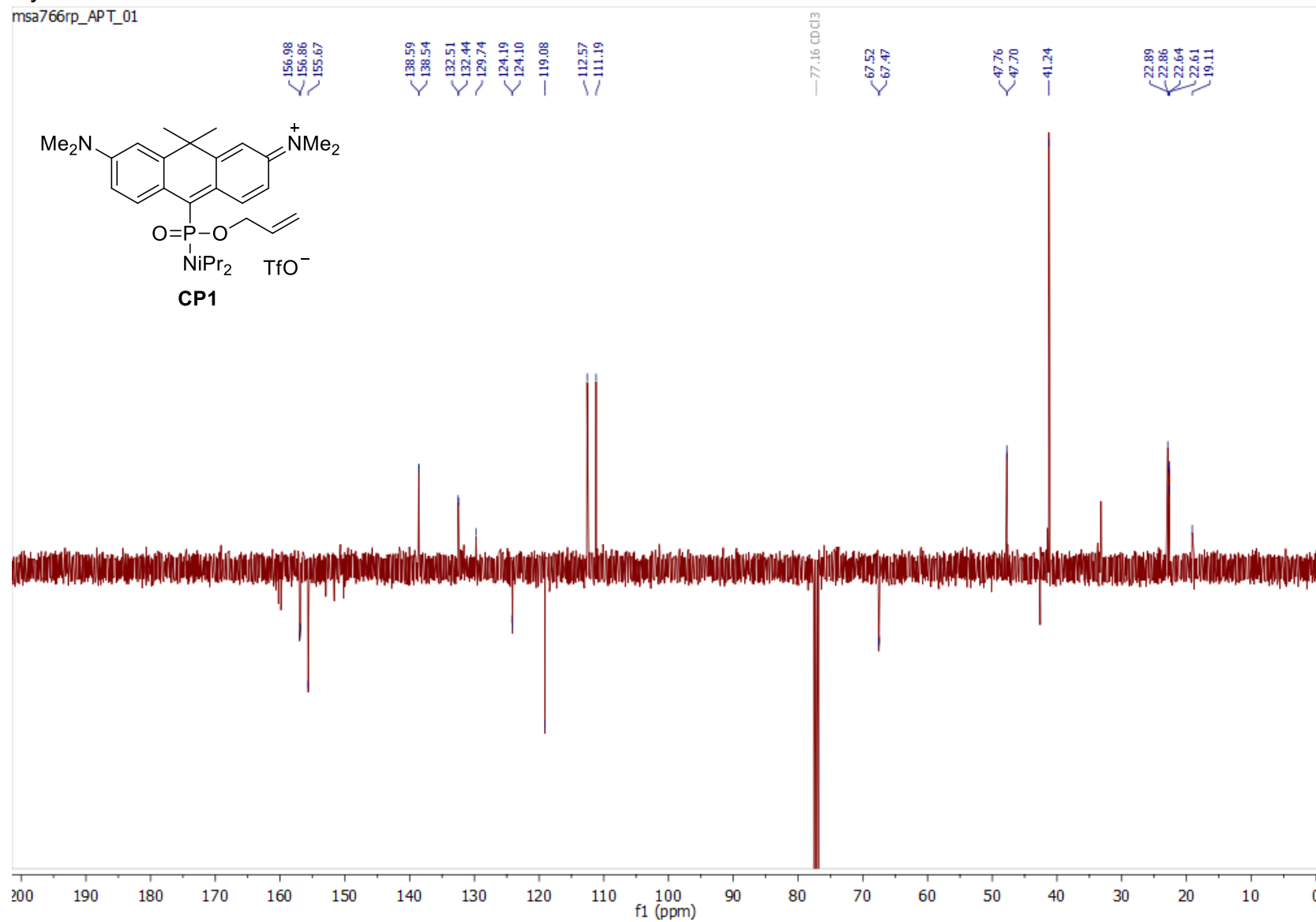
Dye CP1: ^1H

msa766rp_PROTON_01



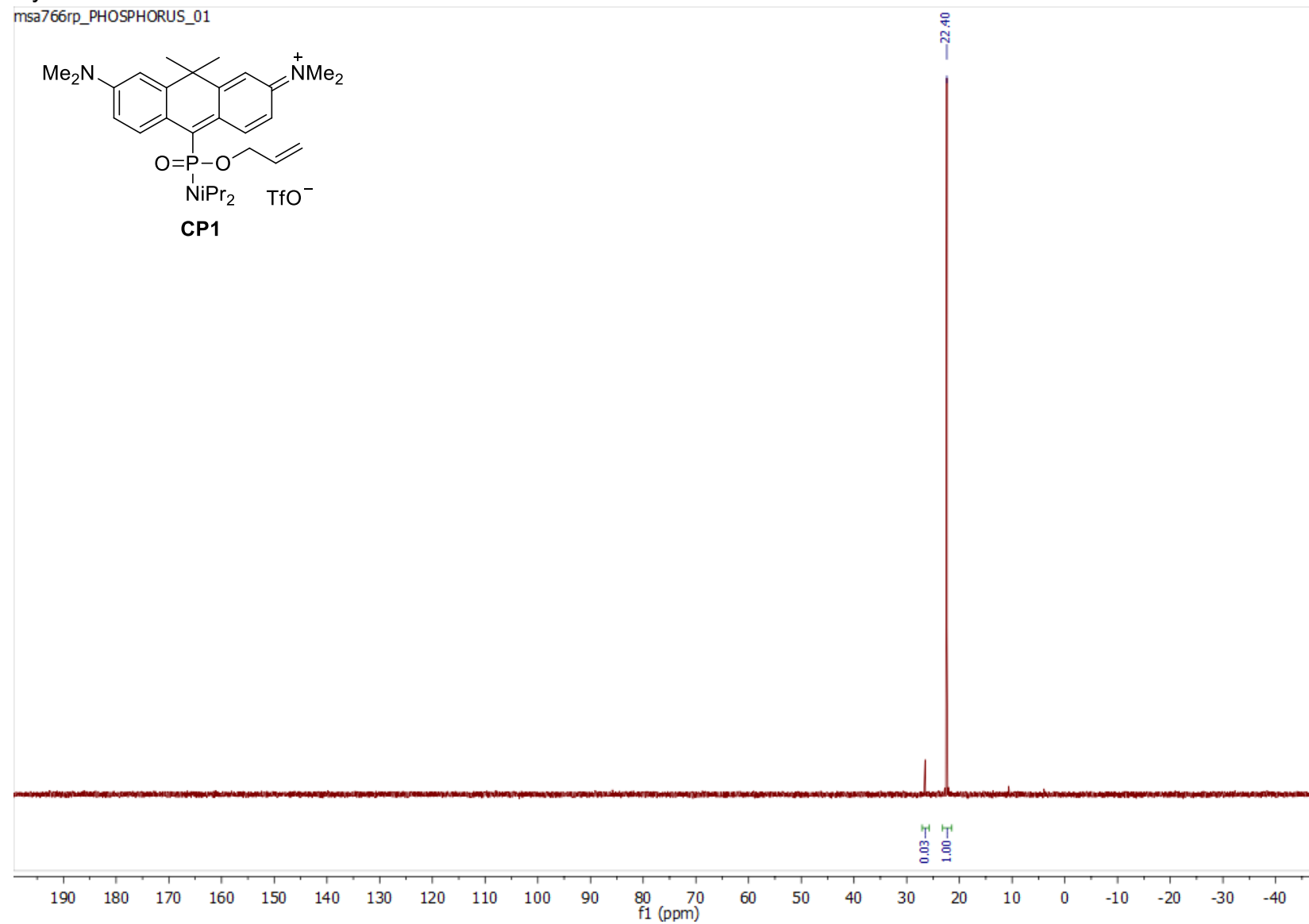
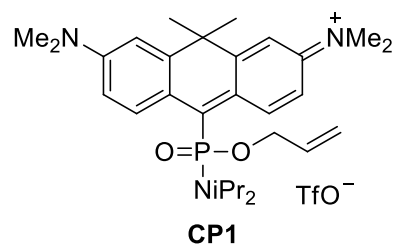
Dye CP1: APT

msa766rp_AP T_01



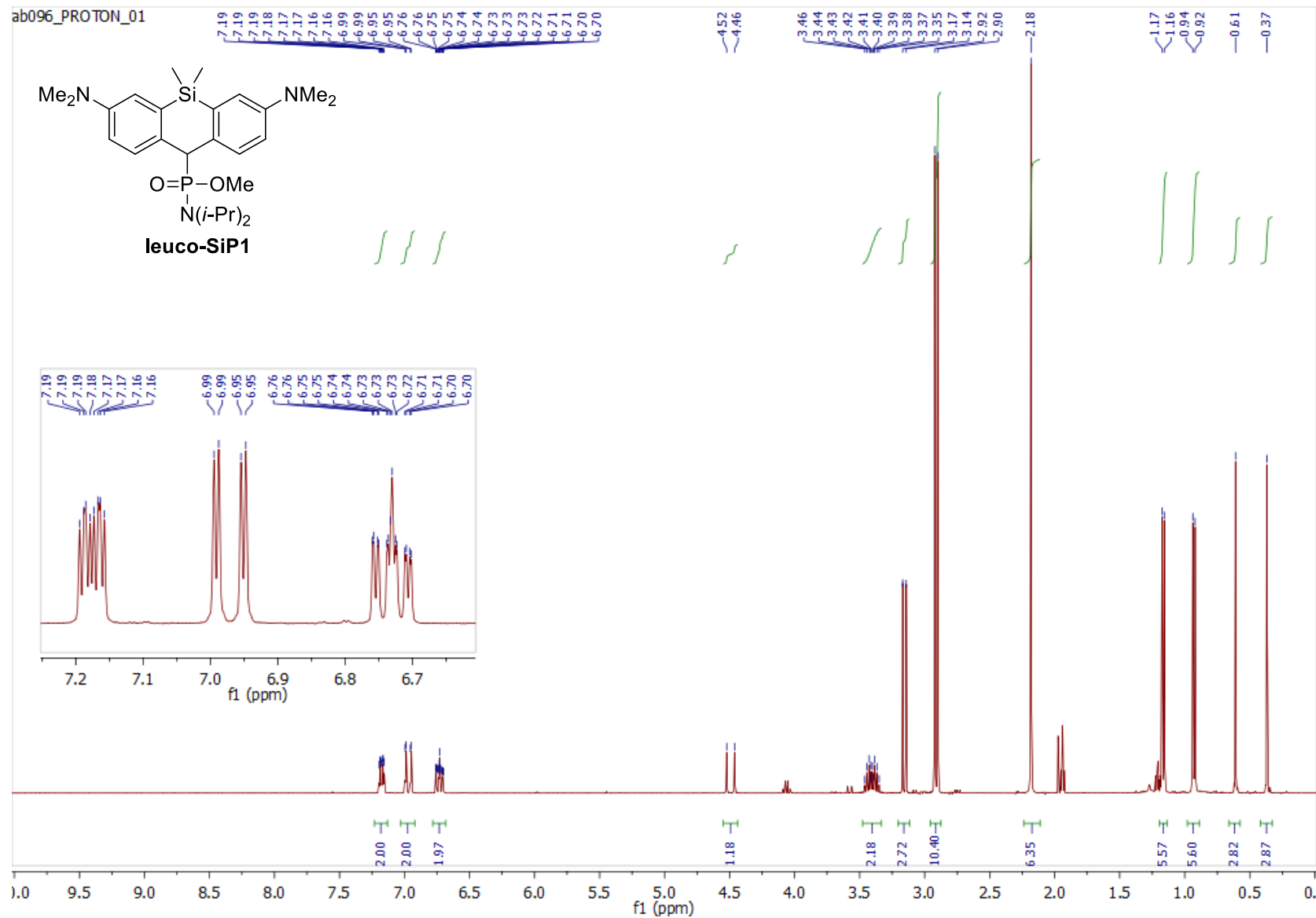
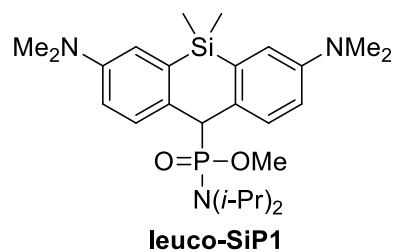
Dye **CP1**: ^{31}P

msa766rp_PHOSPHORUS_01



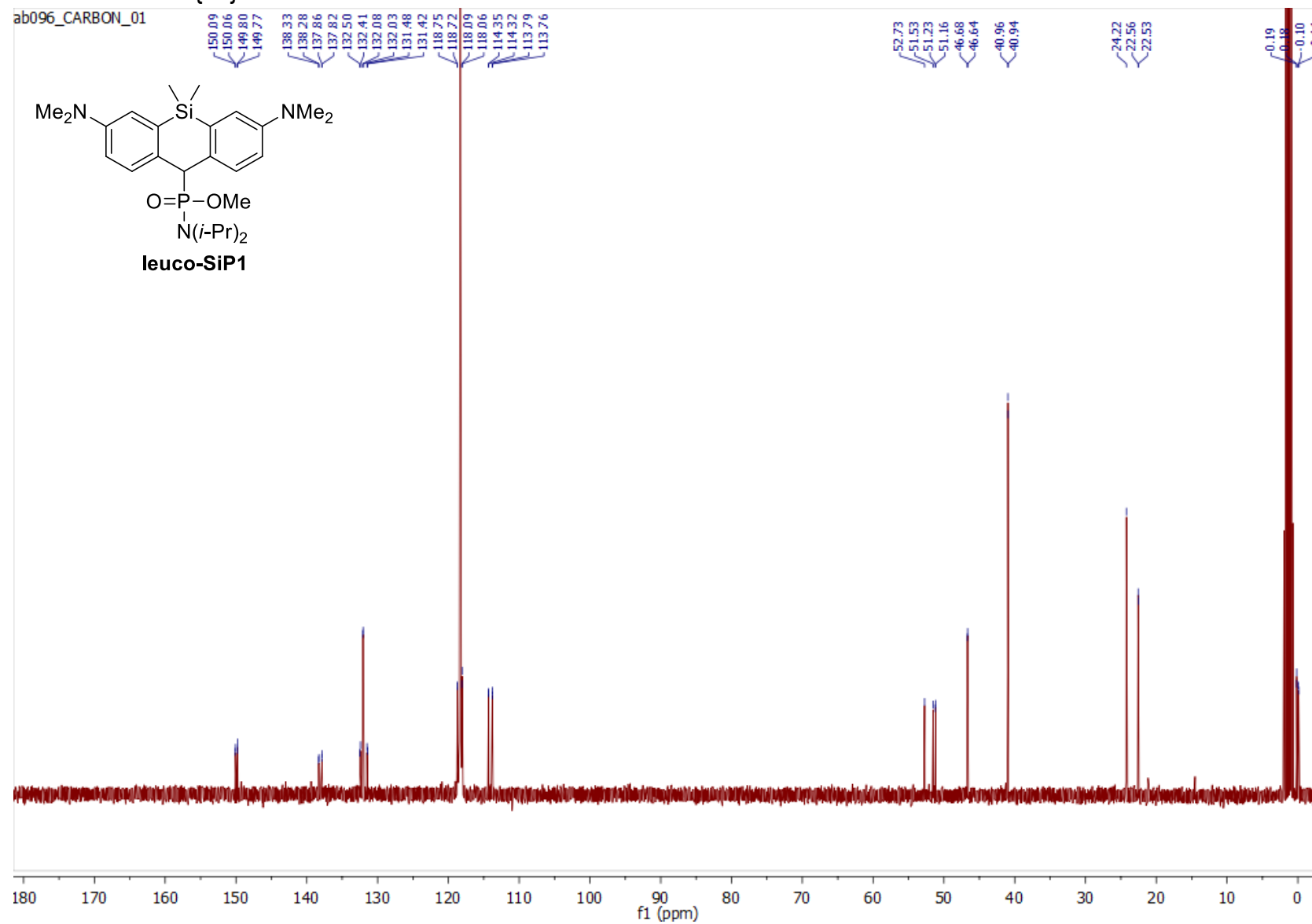
leuco-SiP1: ^1H

ab096_PROTON_01



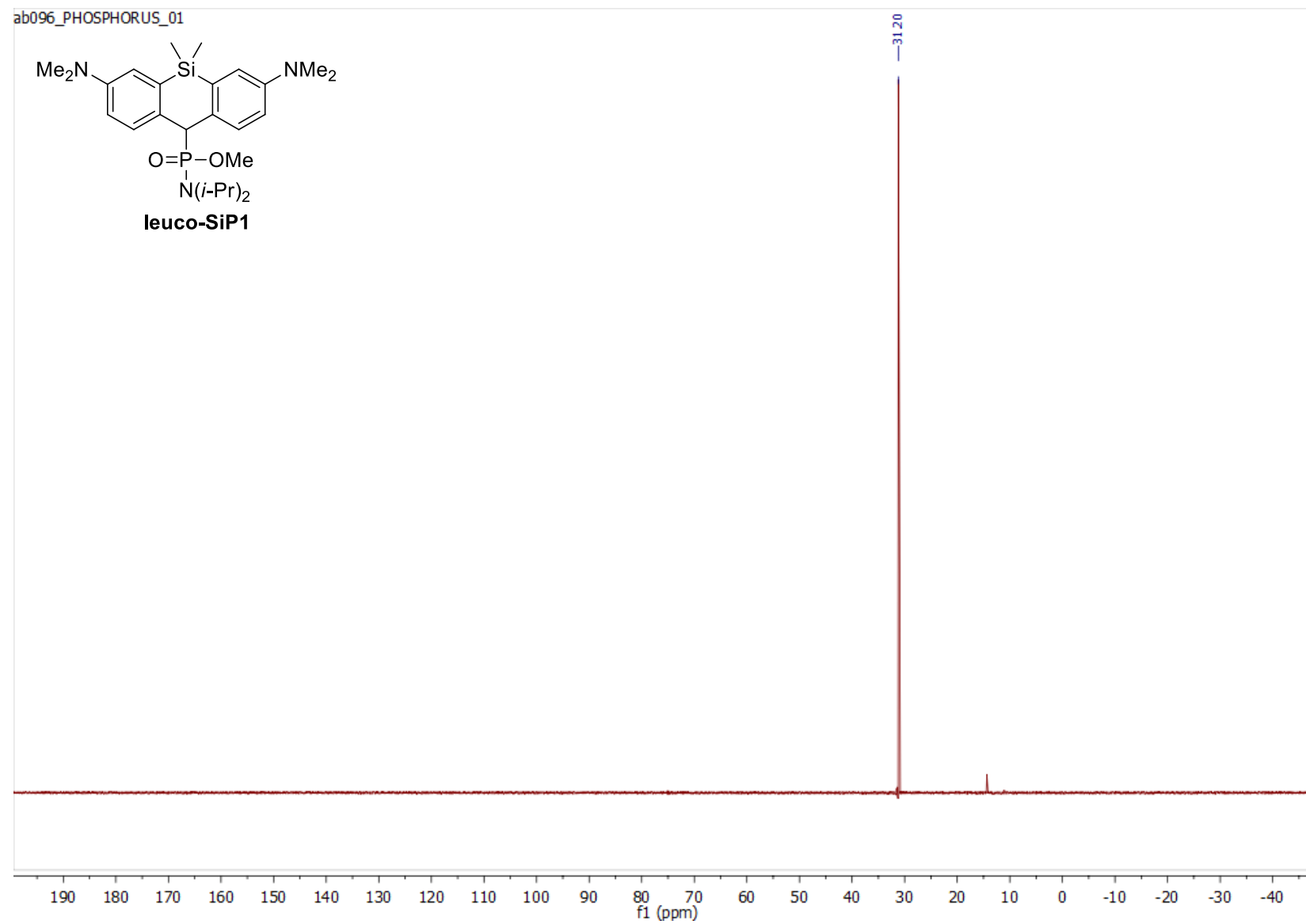
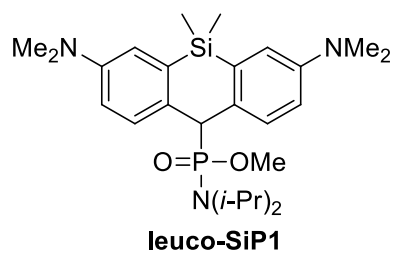
leuco-SiP1: $^{13}\text{C}\{^1\text{H}\}$

ab096_CARBON_01

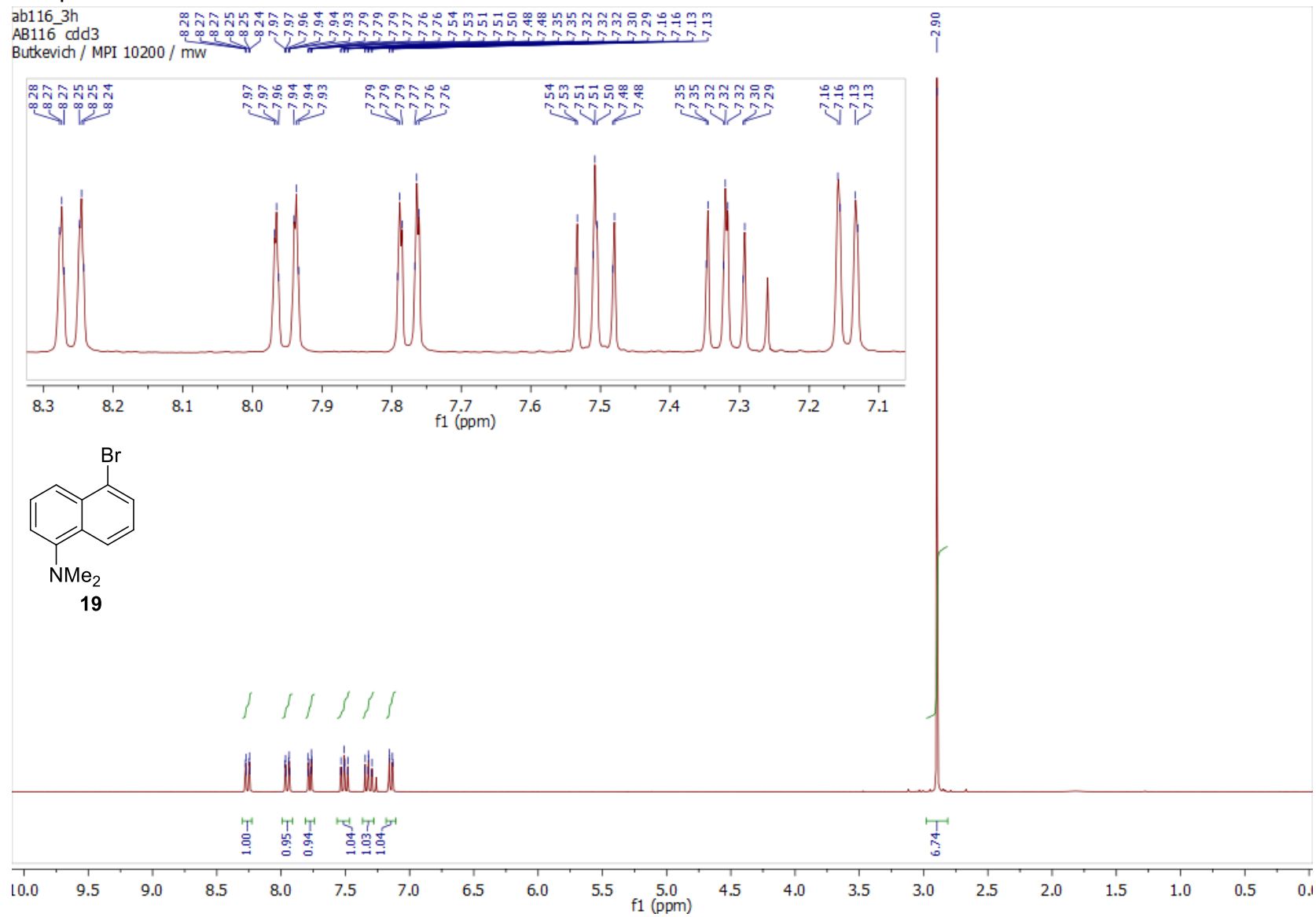


leuco-SiP1: ^{31}P

ab096_PHOSPHORUS_01



compound **19**: ^1H

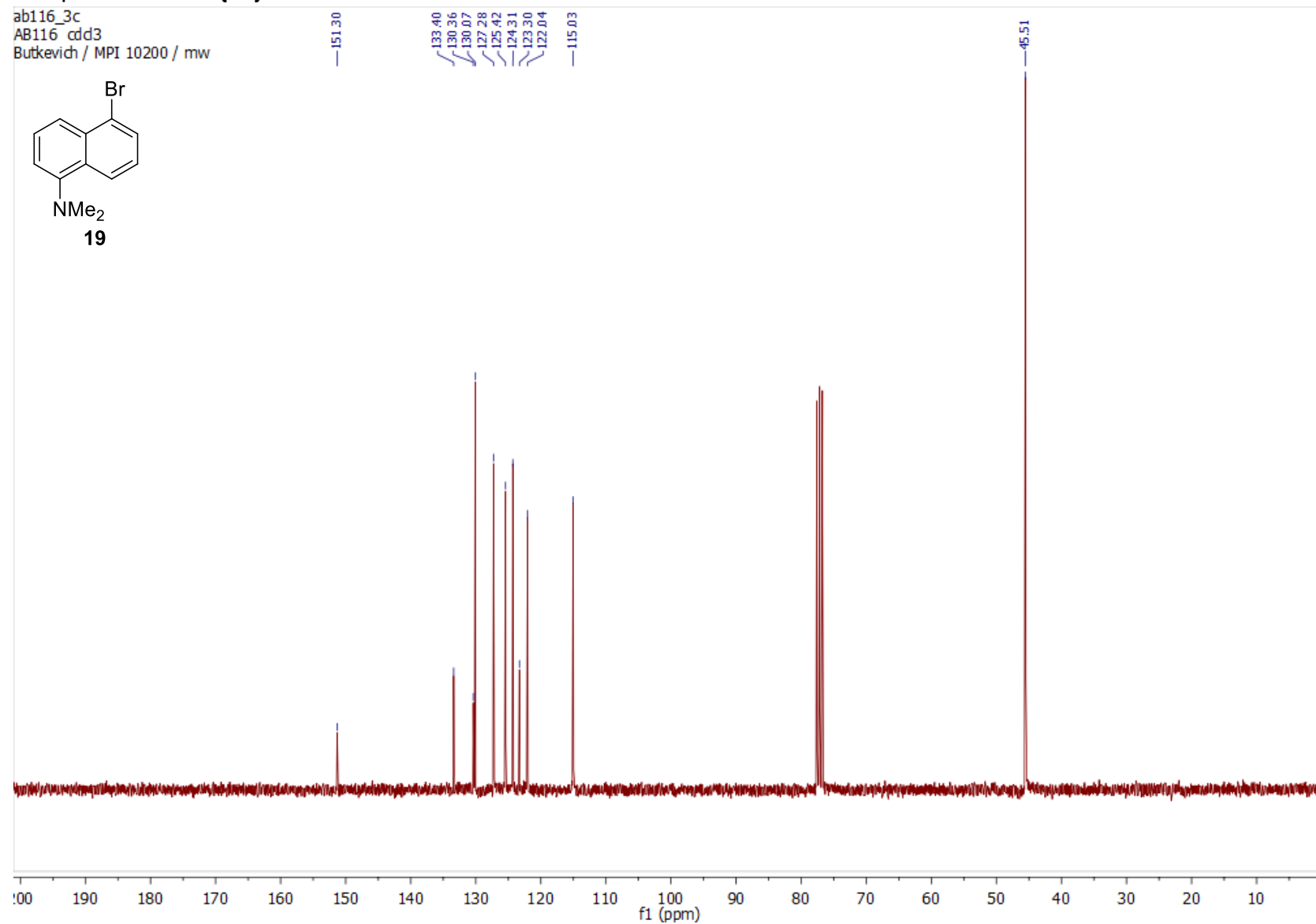
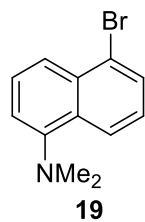


compound **19**: $^{13}\text{C}\{^1\text{H}\}$

ab116_3c

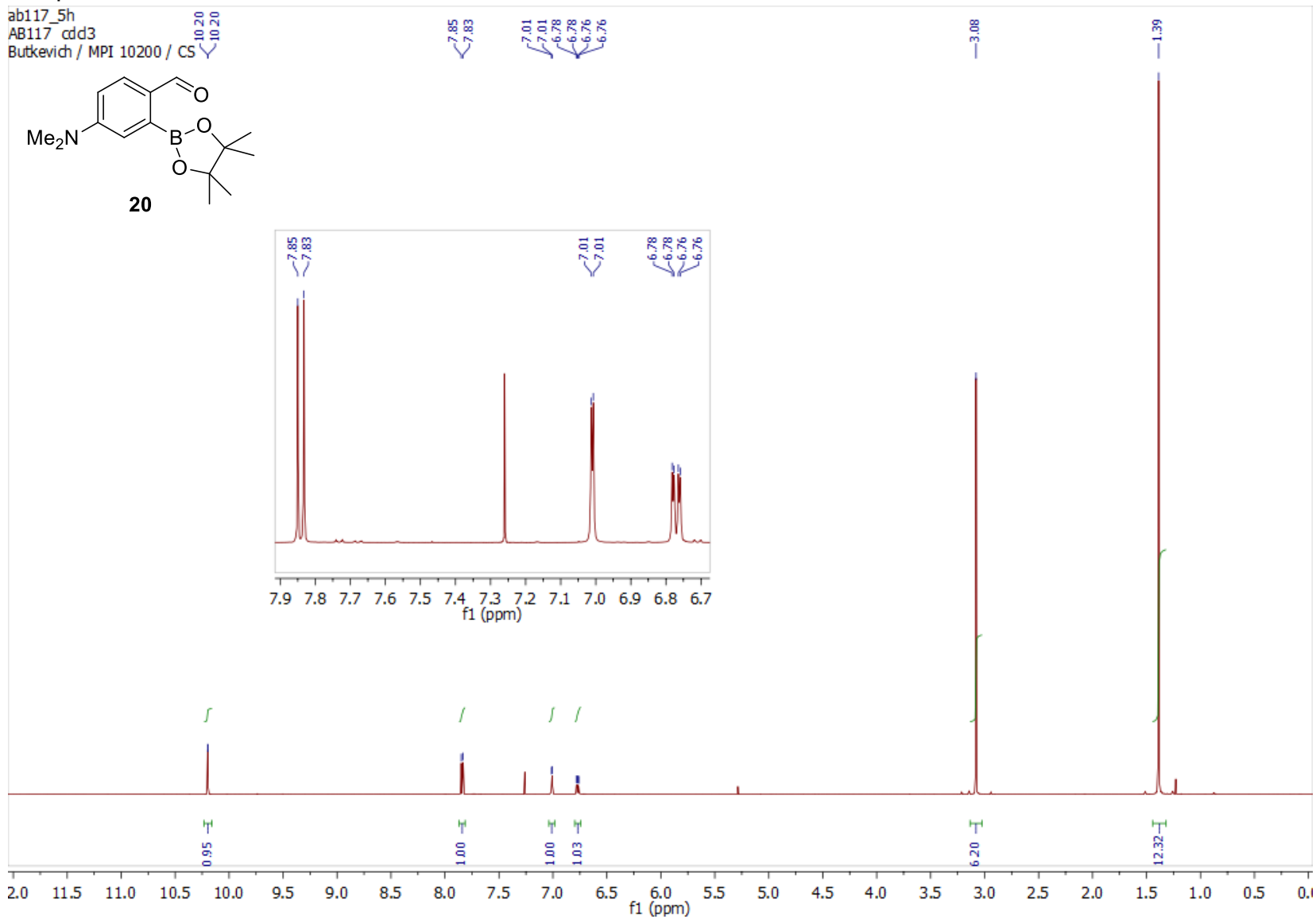
AB116 cdd3

Butkevich / MPI 10200 / mw



compound **20**: ^1H

ab117_5h
AB117 cdd3
Butkevich / MPI 10200 / CS

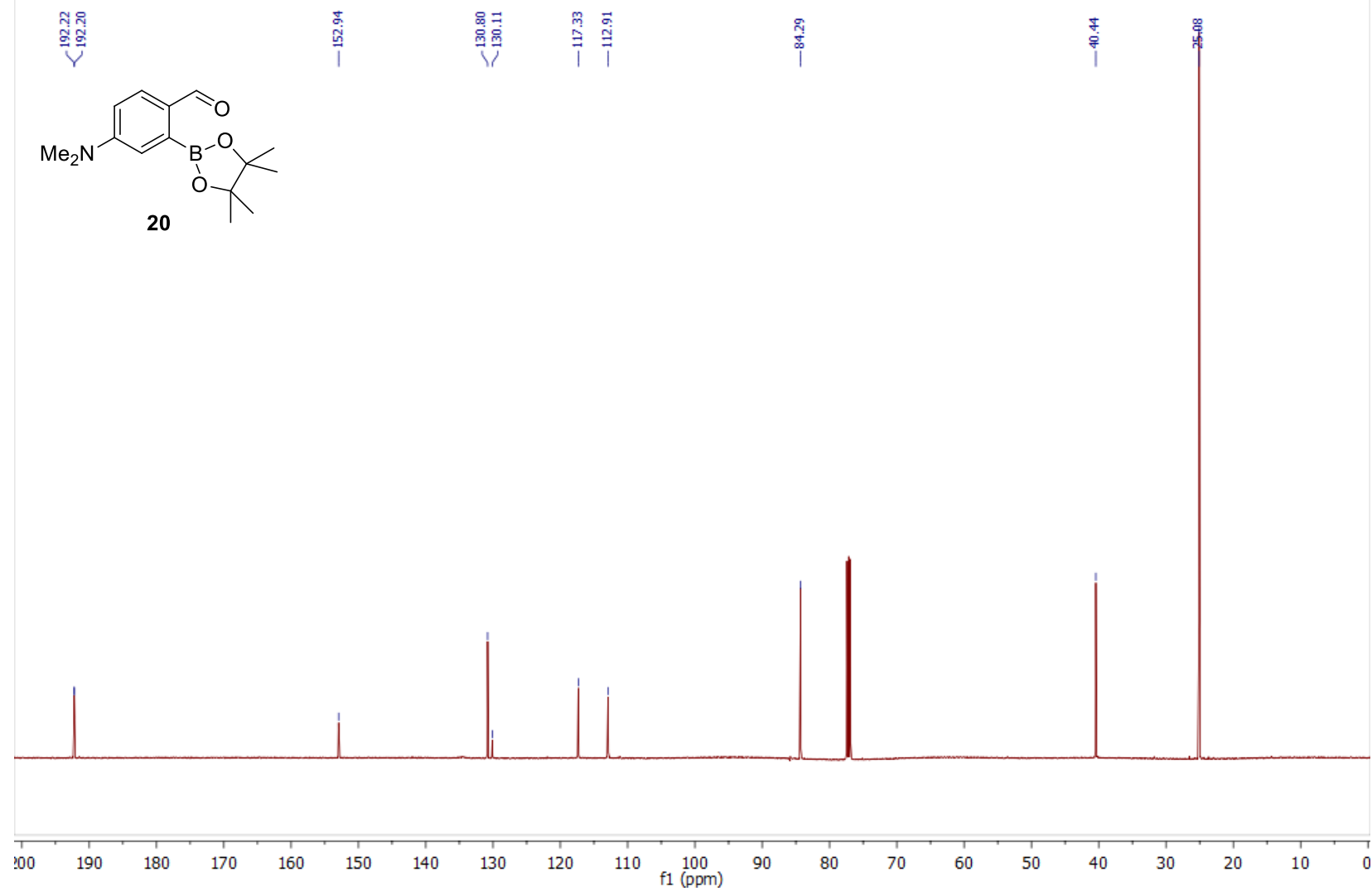


compound **20**: $^{13}\text{C}\{^1\text{H}\}$

ab117_5c

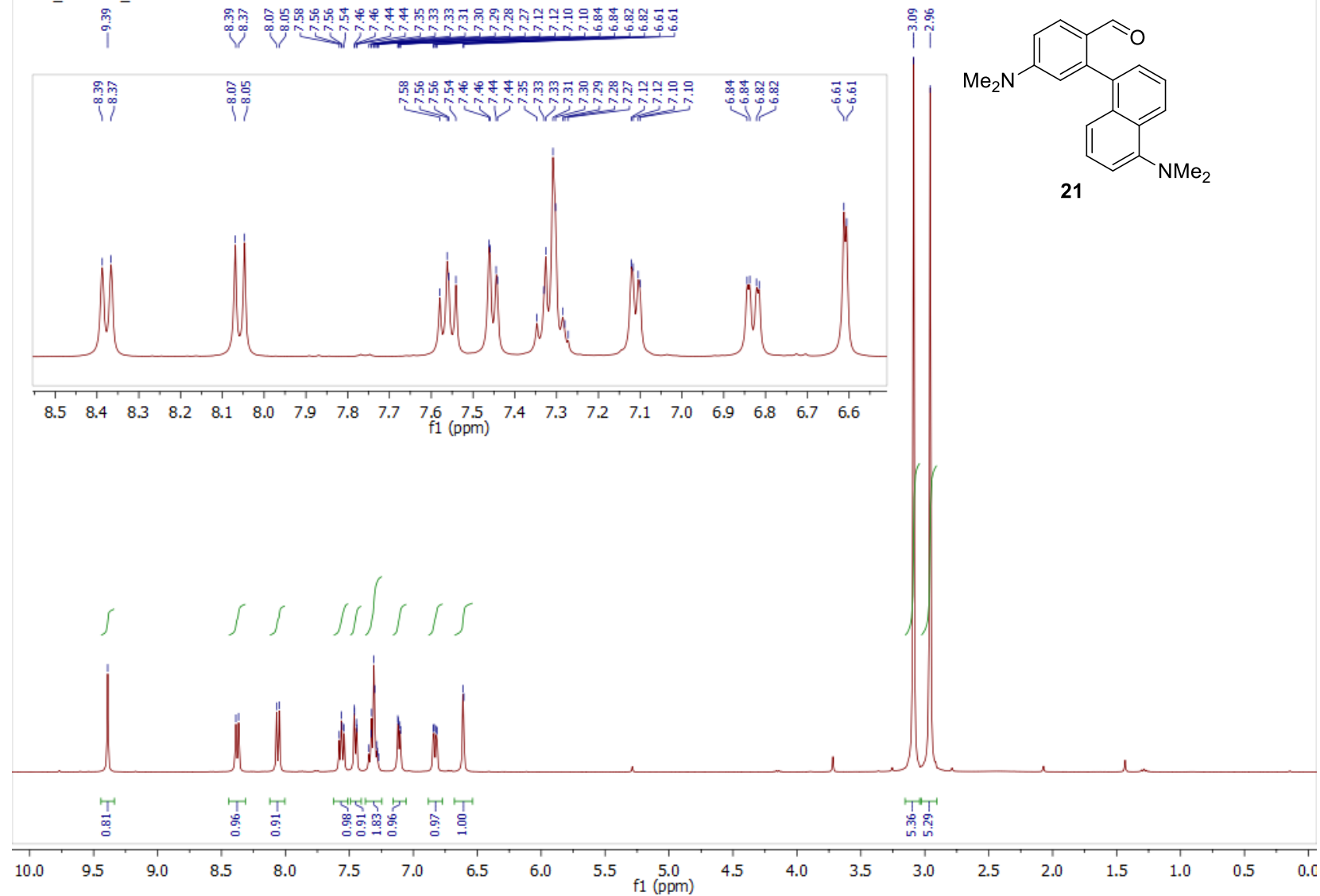
AB117 cdd3

Butkevich / MPI 10200 / CS



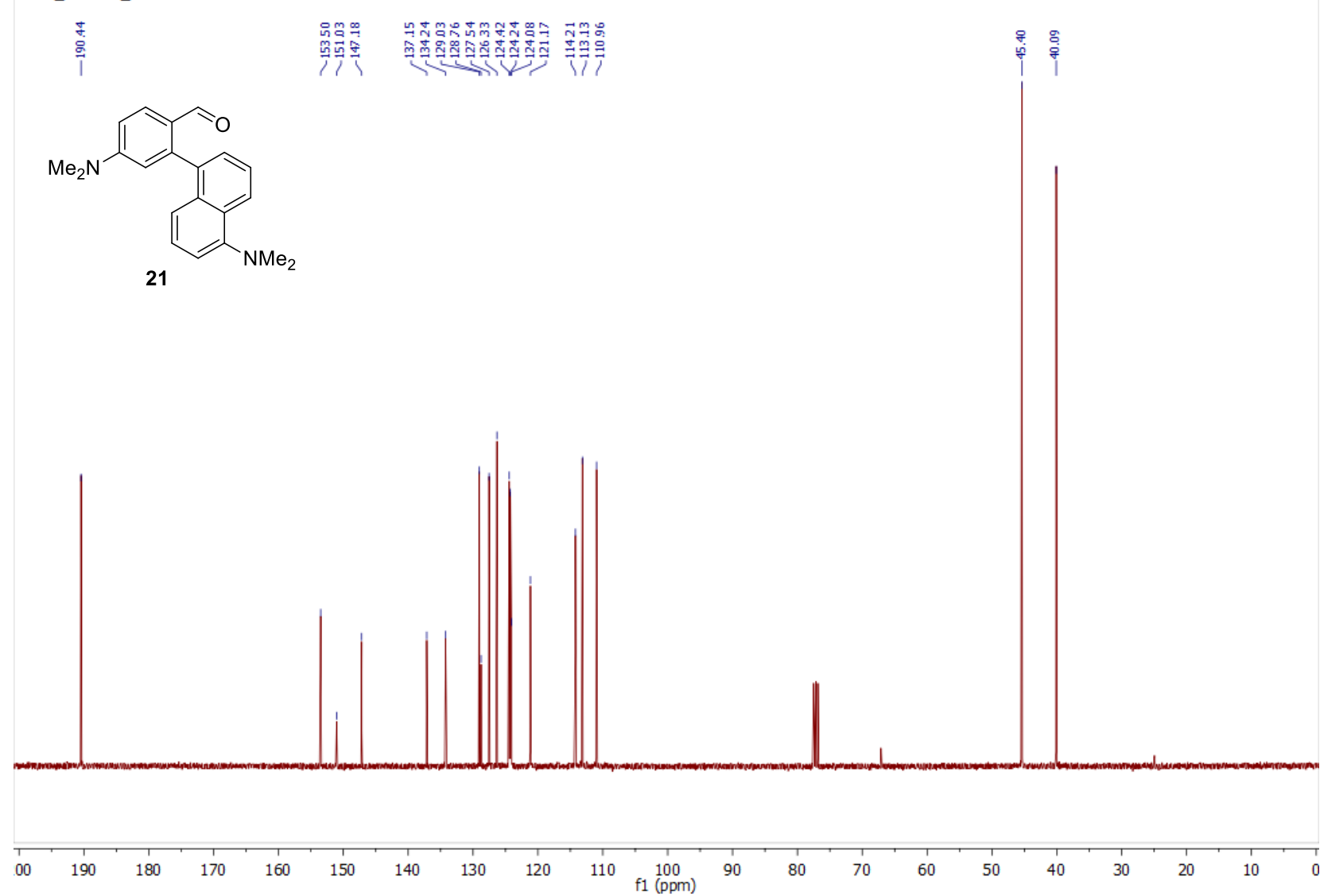
compound **21**: ^1H

ab118_PROTON_01



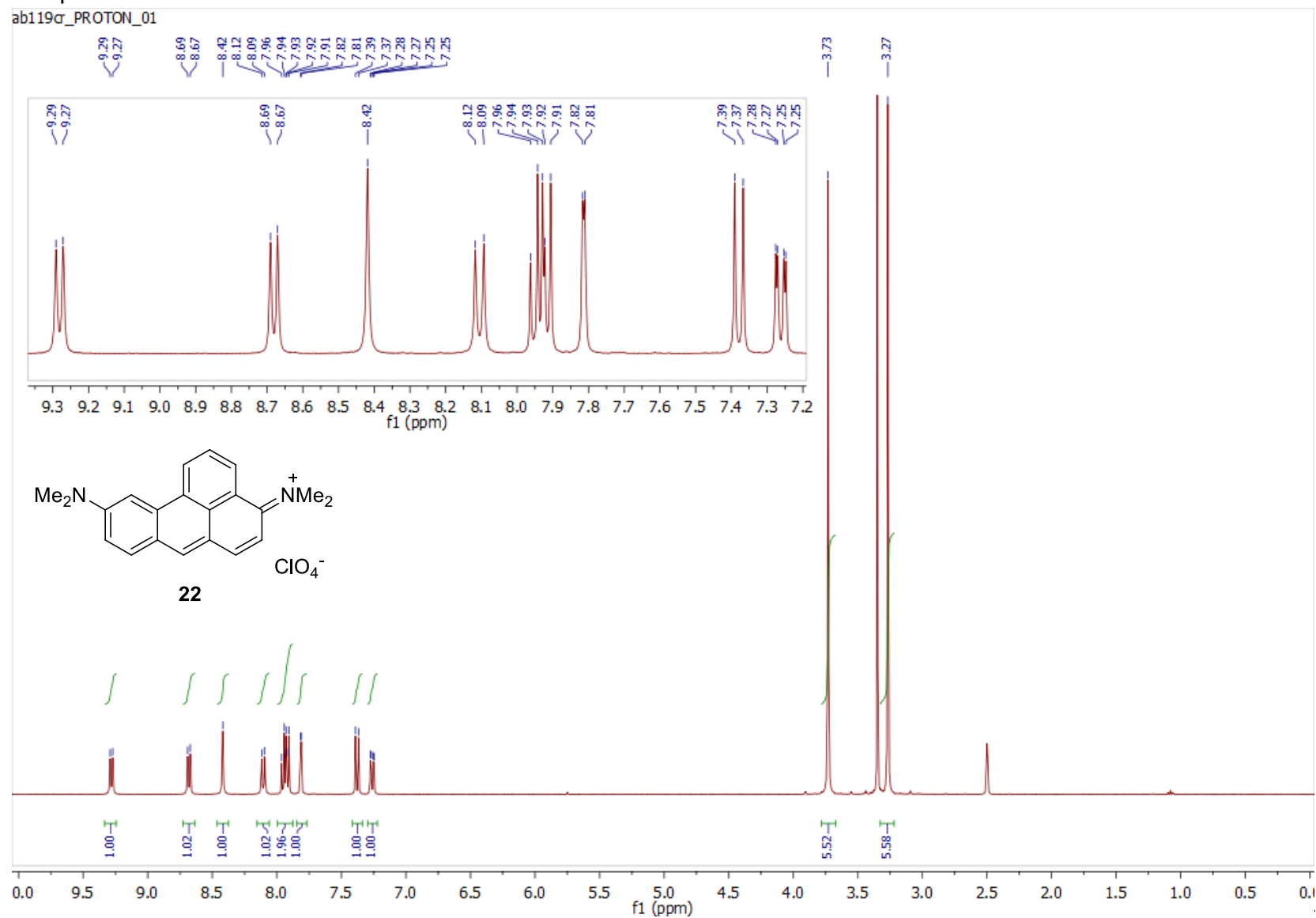
compound **21**: $^{13}\text{C}\{^1\text{H}\}$

ab118_CARBON_01



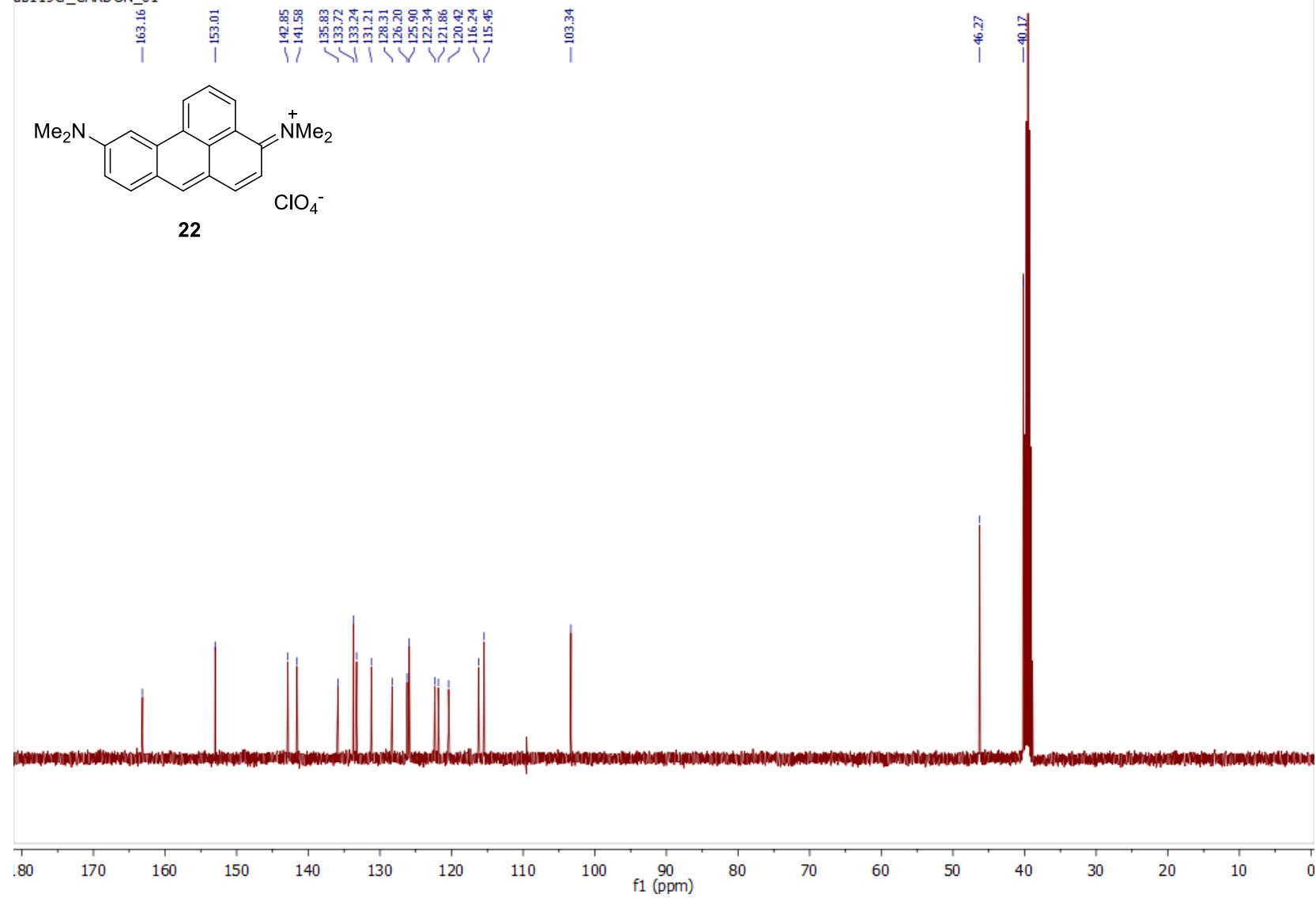
compound **22**: ^1H

ab119 σ _PROTON_01

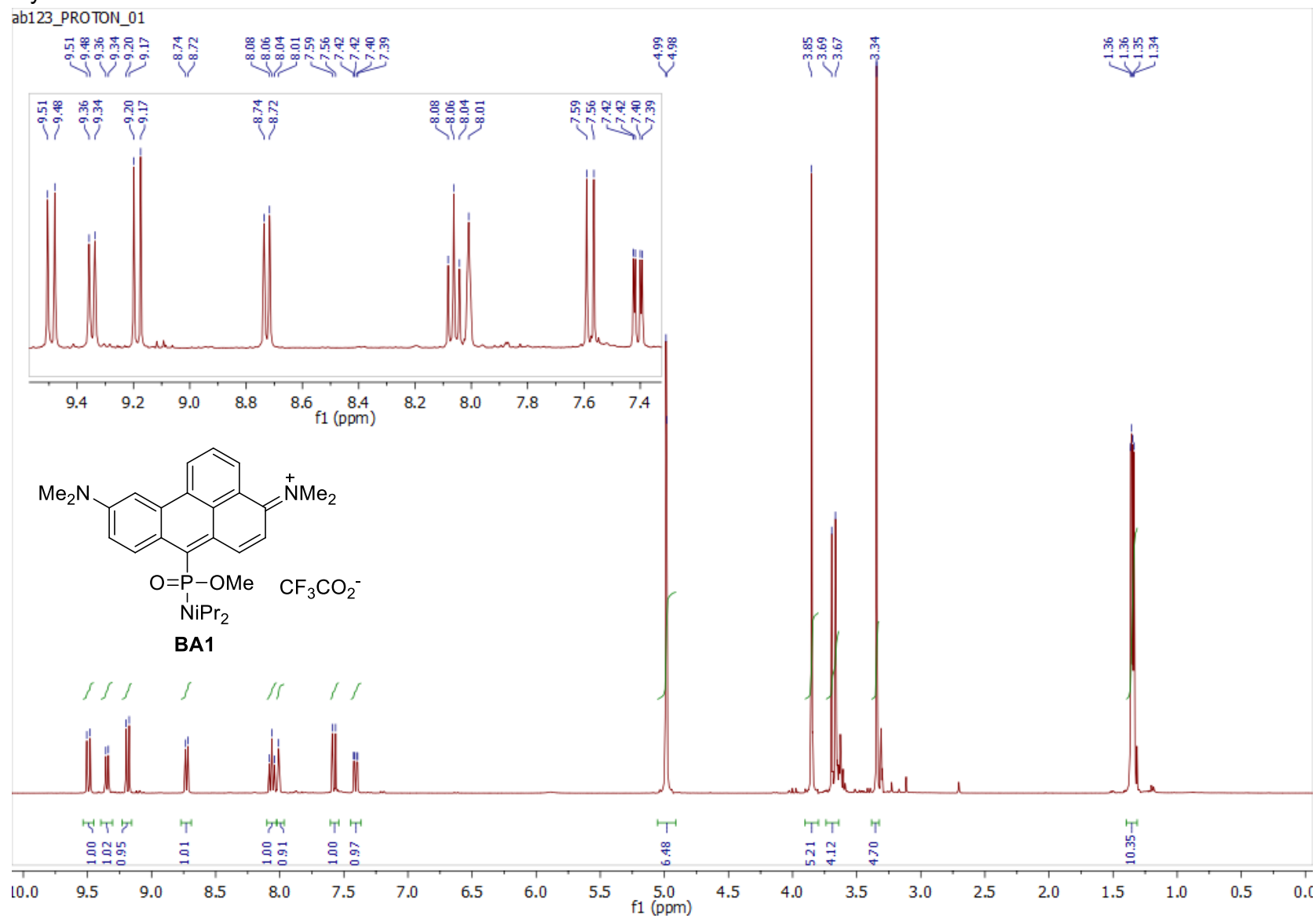


compound **22**: $^{13}\text{C}\{^1\text{H}\}$

ab119σ_CARBON_01

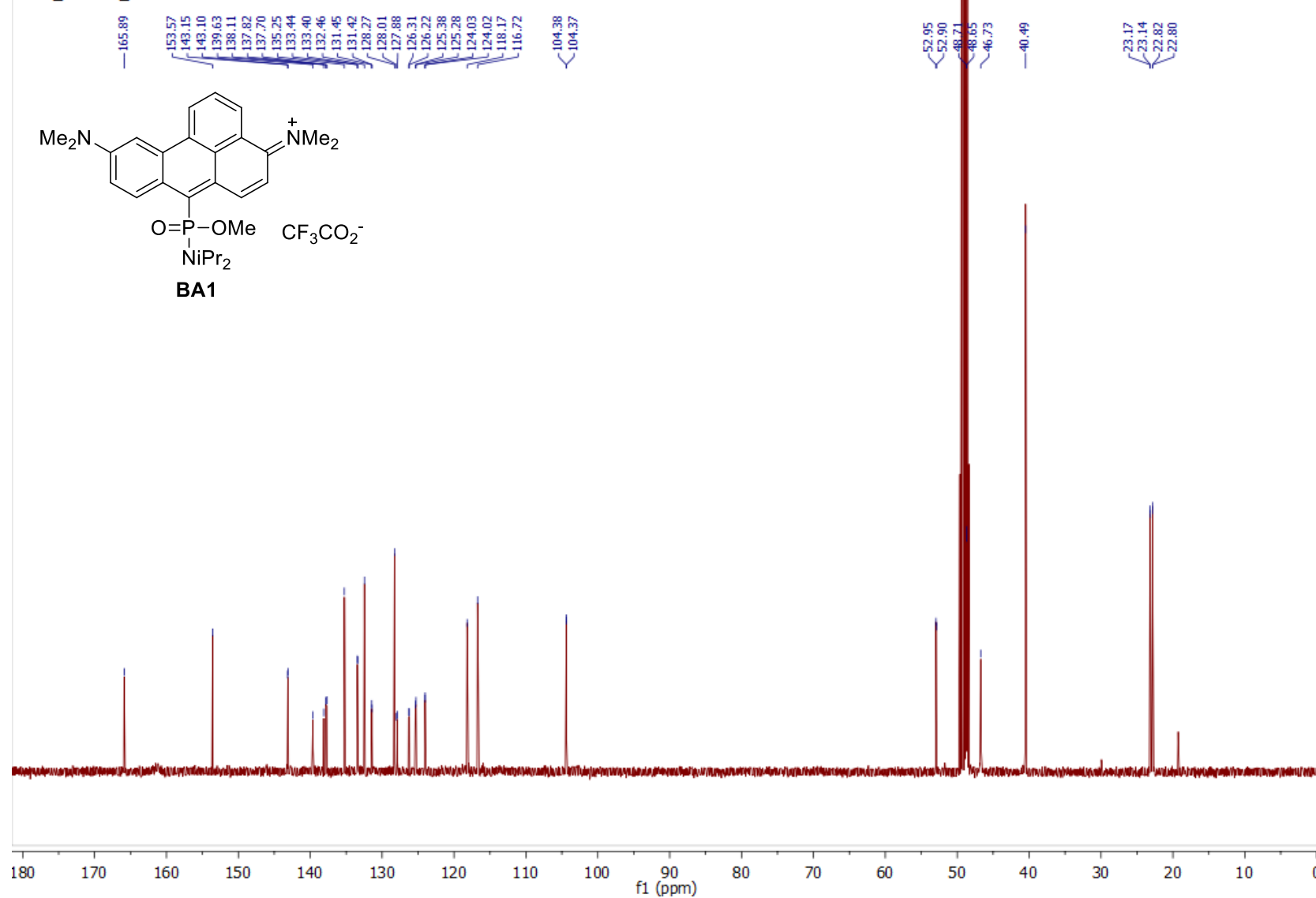


Dye BA1: ^1H



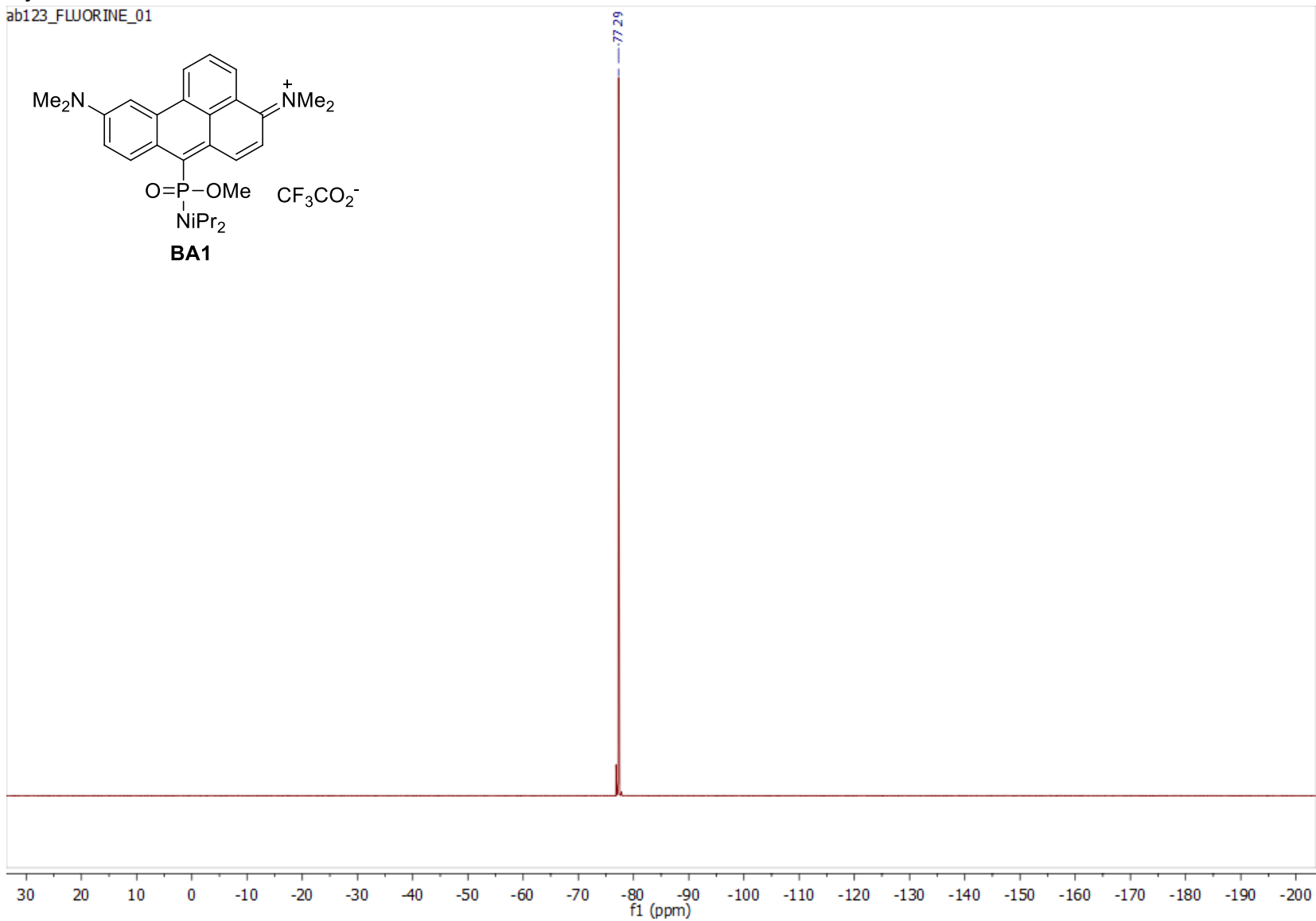
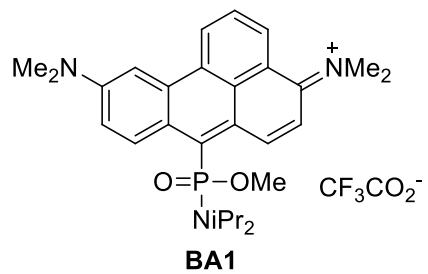
Dye **BA1**: $^{13}\text{C}\{^1\text{H}\}$

ab123_CARBON_01



Dye **BA1**: ^{19}F

ab123_FLUORINE_01



Dye **BA1**: ^{31}P

ab123_PHOSPHORUS_01

