Supplementary information

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

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**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 [ROPR’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) benzanthrilium salts; h) BODIPY derivatives (reactive when X = Cl).
<table>
<thead>
<tr>
<th>Dye</th>
<th>HOMO 3D</th>
<th>LUMO 3D (atomic contributions to LUMO)</th>
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<tr>
<td><img src="image1" alt="Pyronine" /></td>
<td><img src="image2" alt="Pyronine HOMO" /></td>
<td><img src="image3" alt="Pyronine LUMO" /> (C7-p = 0.29)</td>
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<tr>
<td><img src="image4" alt="Carbopyronine" /></td>
<td><img src="image5" alt="Carbopyronine HOMO" /></td>
<td><img src="image6" alt="Carbopyronine LUMO" /> (C7-p = 0.29)</td>
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<tr>
<td>Dye</td>
<td>HOMO 3D</td>
<td>LUMO 3D (atomic contributions to LUMO)</td>
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<td>Si-pyronine</td>
<td><img src="image" alt="Si-pyronine HOMO 3D" /></td>
<td><img src="image" alt="Si-pyronine LUMO 3D" /></td>
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<td>(C7-p = 0.27)</td>
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<td>thiopyronine</td>
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<td>(C7-p = 0.28)</td>
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<td>10-alkylacridinium</td>
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<td>(C7-p = 0.27)</td>
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<tr>
<td>Dye</td>
<td>HOMO 3D</td>
<td>LUMO 3D (atomic contributions to LUMO)</td>
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<tr>
<td>4,10-bis(dimethylamino)-7H-benzo[de]anthracen-7-ylium</td>
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<td></td>
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<td>(C7-p = 0.18, C1-p = 0.15, C3-p = 0.13)</td>
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<td><img src="image6" alt="HOMO 3D" /></td>
<td>(C7-p=0.23, C9-p=0.21)</td>
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<td>Dye</td>
<td>HOMO 3D</td>
<td>LUMO 3D (atomic contributions to LUMO)</td>
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<td>---------------------------------------</td>
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<td>BODIPY (9-Cl)</td>
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<td><img src="image2" alt="LUMO 3D" /></td>
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<tr>
<td>C3 - p = 0.25, C11 - p = 0.12, C12 - p = 0.12, C9 - p = 0.12, C14 - p = 0.12</td>
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<td>3-(2-benzothiazolyl)-6-dimethylaminocoumarin</td>
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<td>C7 - p = 0.29</td>
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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

![Graph a](image)

solvent: MeCN

b

![Graph b](image)

solvent: MeCN

c

![Graph c](image)

solvent: MeCN

Solvent: PBS 7.4
d

Me₂N
O
NMe₂
+Cl⁻
Pyronin Y
solvent: PBS 7.4

Me₂N
O=PO(O)Me
NMe₂
OMe
P2
solvent: PBS 7.4

Me₂N
O
NMe₂
+CF₃CO₂⁻

solvent: MeCN

P4

solvent: MeOH

Me₂N
O
NMe₂
+ClO₄⁻

H-hNR
solvent: MeOH

P7
solvent: MeCN
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 ($\Delta E_{\text{obs}} = \frac{hc}{\lambda_{\text{max}}(\text{Abs})}$). 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.

<table>
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<tr>
<th>Dye (MW)</th>
<th>P(III) reagent</th>
<th>$\lambda_{\text{max abs}}$ [nm]</th>
<th>$\varepsilon$ [M$^{-1}$ cm$^{-1}$]$^a$</th>
<th>$\lambda_{\text{max em}}$ [nm], $\Phi_h$$^b$</th>
<th>$\tau$ [ns]$^c$</th>
<th>solvent</th>
<th>stability</th>
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<td>472, 0.02</td>
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<td>MeCN</td>
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<td>MeCN</td>
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<tr>
<td>P1-Halo (608)</td>
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<td>635, 0.26, 620, 0.50</td>
<td>1.9, 3.1</td>
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<tr>
<td>P2 (375)</td>
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<td>PBS</td>
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<td>P4 (480)</td>
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<td>MeOH</td>
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<td>P5 (593)</td>
<td>multistep$^f$</td>
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<td>603/69000, 589/49000</td>
<td>638, 0.09, 622, 0.36</td>
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<td>MeOH</td>
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<tr>
<td>Dye (MW)</td>
<td>P(III) reagent</td>
<td>$\lambda_{\text{max}}^{\text{abs}}$ [nm]</td>
<td>$\lambda_{\text{max}}^{\text{em}}$ [nm], $\Phi_{\text{b}}$</td>
<td>$\tau$ [ns]$^a$</td>
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<td>H-hNR</td>
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<td>MeCN</td>
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<td>CP1 (541)</td>
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<td>-</td>
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<td>673, 0.25</td>
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<td>644/7600$^g$</td>
<td>782, n.d.</td>
<td>n.d.</td>
<td>PBS</td>
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<td>647, &lt;0.002</td>
<td>–</td>
<td>MeCN</td>
<td>good</td>
<td></td>
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</table>
**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 CF$_3$CO$_2$H (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.
<table>
<thead>
<tr>
<th>Dye</th>
<th>Solvent</th>
<th>( \text{Fluorescence lifetime } \tau_i )</th>
<th>Relative amplitude ( A_i )</th>
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<tr>
<td></td>
<td></td>
<td>( \tau_1, \text{ ns} )</td>
<td>( \tau_2, \text{ ns} )</td>
</tr>
<tr>
<td>C7</td>
<td>PBS 7.4</td>
<td>1.2</td>
<td>0.3</td>
</tr>
<tr>
<td>P5</td>
<td>PBS 7.4</td>
<td>1.7</td>
<td>0.7</td>
</tr>
<tr>
<td>SP1</td>
<td>MeOH</td>
<td>1.2</td>
<td>0.4</td>
</tr>
</tbody>
</table>

**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 (F254s) (Cat. No. 1.05560.0001). Preparative TLC was performed on silica-precoated glass plates for high performance TLC (HPTLC Silica gel 60 F254 10x10 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, 150x4 mm (Article No. 15DE181E2J, Knauer) or Interchim Uptisphere Strategy C18-HQ, 10 μm, 250x4.6 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. 115140, 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 Quantaurus-QY absolute PL quantum yield spectrometer (model C11347-12, Hamamatsu) according to the manufacturer’s instructions. Fluorescence lifetimes were measured with a Quantaurus-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 (19F), 161.94 MHz (31P) and 100.60 MHz (13C) and are reported in ppm. All 1H spectra are referenced to tetramethylsilane (δ = 0 ppm) using the signals of the residual protons of CHCl₃ (7.26 ppm) in CDCl₃, CHD₂CN (1.94 ppm) in CD₃CN, CHD₂OD (3.31 ppm) for CD₃OD, CHD₂COCD₃ (2.05 ppm) for acetone-d₆, CHD₂CO₂D (2.04 ppm) for acetic acid-d₄ or DMSO-d₅ (2.50 ppm) for DMSO-d₆. ¹³C spectra are referenced to tetramethylsilane (δ = 0 ppm) using the signals of the solvent: CDCl₃ (77.16 ppm), CD₃CN (1.32 ppm), CD₃OD (49.00 ppm), (CD₃)₂CO (29.84 ppm), DMSO-d₆ (39.52 ppm) or CNO₂ signal (148.60 ppm) of nitrobenzene-d₅. 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 ¹³C chemical shifts obtained by indirect detection from HSQC experiments (minimum resolution in F1: t₁≥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-2H-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 µL, 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 (3×25 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 °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 °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

\[
\begin{align*}
\text{Et}_2\text{N} & \quad \text{O} \quad \text{O} \quad \text{CO}_2\text{Et} \\
\text{C2} & \quad \text{MeO} \quad \text{P} = \text{OMe} \quad \text{CO}_2\text{Et}
\end{align*}
\]

1) \((\text{MeO})_2\text{PONa, DMF}\)
2) DDQ, EtOAc-MeOH

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 µL, 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 ~ 3, extracted with EtOAc (3×20 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 °C, and a solution of DDQ (227 mg, 1 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 12 g Sepacore Silica HP cartridge, gradient 40% to 100% EtOAc/hexane) and lyophilized from 1,4-dioxane. Yellow solid, yield 111 mg (28%).

\(^1\)H 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).

\(^13\)C 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.

\(^{31}\)P 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-2H-chromen-3-yl)thiazole-4-carboxylate

\[
\text{Et}_2\text{N} \quad \text{O} \quad \text{NH}_2 + \text{Br-CO}_2\text{Et} \quad \text{EtOH, reflux, 2.5 h} \quad \text{Et}_2\text{N} \quad \text{O} \quad \text{N} \quad \text{CO}_2\text{Et}
\]

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-2H-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.

\(^1\)H 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).

\(^13\)C 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
eethyl 2-(7-diethylamino-4-dimethoxyphosphoryl-2-oxo-2H-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-1H-benzo[d]imidazol-2-yl)-2-oxo-2H-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 µL, 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 (3×30 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 °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 °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-2H-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 µL, 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 (4×15 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 °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 °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-2H-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 µL, 0.86 mmol) was added in one portion. The suspension was stirred for 2 h at rt and for 30 min at 55 °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. NaHCO₃ (50 mL), extracted with EtOAc (4×15 mL); the combined organic solutions were dried over Na₂SO₄, filtered and evaporated. The residue was redissolved in EtOAc (20 mL), heated up to 75 °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 °C for 5 min, cooled down to rt and evaporated. The product was isolated by column chromatography (30 g SiO₂, 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-d₆): 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).

³¹P NMR (162 MHz, acetone-d₆): 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).

¹³C NMR (101 MHz, acetone-d₆): 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) [M+H]+, 565.2 (35) [M+Na]+, 581.2 (100) [M+K]+.

HRMS (C₂₈H₃₅N₂O₅PS): m/z (positive mode) = 543.2077 (found [M+H]+), 543.2077 (calc.).
**Dye C7**

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

Trifluoroacetic acid (TFA; 150 µL) 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-d₄):** 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-d₄):** 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 CH₂Cl₂ (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 CH₂Cl₂ (2×20 mL); and the combined organic solutions were washed with water and brine, dried over Na₂SO₄. The product was isolated by flash chromatography on Biotage Isolera system (12 g Sepacore Silica HP cartridge, gradient 20% to 100% CH₂Cl₂/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, CDCl₃): 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).

¹⁹F NMR (376 MHz, CDCl₃): δ -72.5.

¹³C NMR (101 MHz, CDCl₃): 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) [M+H]⁺.

HRMS (C₁₇H₈NO₅S₂F₃): m/z (positive mode) = 427.9867 (found [M+H]⁺), 427.9869 (calc.).
Compound 6
3-(benzo[d]thiazol-2-yl)-7-[3-(tert-butyldimethylsilyloxy)azetidin-1-yl]-2H-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-d5): 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 ~ 3, and the mixture was extracted with EtOAc (3 × 20 mL), the combined extracts were dried over Na₂SO₄, filtered and evaporated. The residue was taken up in EtOAc (20 mL) and MeOH (5 mL), heated up to 70 °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 °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/CH₂Cl₂ 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 × 20 mL), the combined organic solutions were dried over Na₂SO₄, 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-d₆): 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) [M+H]$^+$. HRMS ($C_{21}H_{19}N_2O_6PS$): $m/z$ (positive mode) = 459.0764 (found [M+H]$^+$), 459.0774 (calc.).
Dye C9
dimethyl [7-(diethylamino-2-oxo-3-(thiophen-2-yl)-2H-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 ~ 5, 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 (5 mL), heated up to 70 °C, and a solution of DDQ (227 mg, 1 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 (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, CDCl3): 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).

13C NMR (101 MHz, CDCl3): 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.

31P NMR (162 MHz, CDCl3): δ 14.6.

MS (ESI): m/z (positive mode, rel. int., %) = 430.1 (100) [M+Na]+.

HRMS (C19H22NO5PS): m/z (positive mode) = 408.1030 (found [M+H]+), 408.1029 (calc.).
Pyronin derivatives (P1-P14)

Dye P1

Compound 4 (leuco-P1)

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

A solution of Pyronin Y (Sigma-Aldrich; 1.0 g, 3.3 mmol) in aq. H₃PO₂ (50%, 4.4 g; 33 mmol) was stirred overnight at 100 °C under microwave irradiation. TLC (RP-C₁₈) showed complete conversion of the starting material. After cooling down to room temperature, the reaction mixture was diluted with H₂O (~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 H₂O/MeCN (0.1 v/v% TFA in both components, 90:10 → 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 × 250 mm, eluent: MeCN / H₂O + 0.1 v/v% TFA in H₂O, 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; Rᵣ = 0.32 (RP-C₁₈ plates, eluent: H₂O/MeCN 1:1, each with 0.1 v/v% TFA).

HPLC: tᵣ = 10.1 min (peak area 98%), gradient: MeCN / H₂O + 0.1 v/v% TFA, 2/98 → 50/50 in 20 min, detection at 580 nm, column US10C18HQ – 250/P46 (Interchim, France), 4×250 mm, flow rate 1.2 mL/min.

¹H NMR (400 MHz, CD₃OD): δ 7.46 (d, J = 8.0 Hz, H⁴ and H⁵, 2H), 7.14 (m, H¹, H³, H⁶ and H⁸, 4H), 6.97 (d, ¹J_H,P = 535 Hz, PH, 1H), 4.35 (d, ³J_H,P = 15.4 Hz, H⁹, 1H), 3.11 (s, 12H, NMe₂) ppm. Due to the presence of an asymmetric P atom, the aromatic rings are diastereotopic.

¹³C NMR (101 MHz, CD₃OD): δ 153.8 (« dd », J = 4.2, 1.9 Hz, C), 146.9 (« m », C), 132.0 (d, J = 2.8 Hz, C⁴ and C⁵), 116.7 (« m », C), 114.4 (d, J = 10 Hz, CH), 107.7 (d, J = 15 Hz, CH), 45.0 /45.1 (2 × NMe₂), 44.4 (d, J = 83 Hz, C³) ppm.

³¹P NMR (162 MHz, CD₃OD): δ 25.8 ppm.

MS (ESI): m/z (negative mode, rel. int., %) = 331.1 (80) [M-H]⁻, 663.4 (100) [2M-H]⁻.
HR-MS (C_{17}H_{21}N_{2}O_{3}P): \( m/z \) (positive mode) = 333.1364 (found [M+H]^+), 333,1363 (calc. [M+H]^+).

**Dye P1**

[3,6-bis(dimethylamino)-9H-xanthenylium-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-SiO\(_2\) (Biotage SNAP Ultra C18 30 g, HP-Sphere 25 μM, 25 ml/min, MeCN/H\(_2\)O with 0.5 v/v% TFA in both components, 20 → 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 H\(_2\)O/MeCN (10:1, 2 mL), and purified again by preparative HPLC (Interchim Puriflash, column 16 × 250 mm with Eurospher II C18 SiO\(_2\), 5 μ; solvent MeCN / H\(_2\)O + 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 SiO\(_2\), eluent MeCN/H\(_2\)O, 10:1, with 0.5 v/v% TFA in both components; moderately soluble in H\(_2\)O, MeOH. Analytical HPLC: \( t_R = 17.5 \) min (peak area 97%), solvent system MeCN/H\(_2\)O + 0.1 v/v% TFA in both components, gradient 2 – 50% ACN in 20 min, detection at 580 nm, column US10C18HQ-250/P46, 4 × 250 mm (Interchim), flow rate 1.2 mL/min.

\(^1\)H NMR (400 MHz, CD\(_3\)OD): \( \delta \) 8.71 (d, \( J = 9.8 \) Hz, 2H), 8.34 (d, \( ^1J_{\text{PH}} = 548\) Hz, PH), 7.15 (d, \( J = 9.3 \) Hz, 2H), 6.83 (s, 2H), 3.30 (s, 12H, NMe\(_2\) ppm).

\(^{13}\)C NMR (101 MHz, CD\(_3\)OD, indirect detection from an HSQC experiment): \( \delta \) 130.7 (CH), 113.8 (CH), 96.2 (CH), 39.4 (NMe\(_2\) ppm).

\(^{31}\)P NMR (162 MHz, CD\(_3\)OD): \( \delta \) 2.36 ppm.

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

HR-MS (C\(_{17}\)H\(_{20}\)N\(_2\)O\(_3\)P): \( m/z \) (positive mode) = 331.1205 (found, [M]^+), 331.1206 (calc. for [M]^+).
**Dye P1-Halo**

**Compound 9 (HaloTag O2 ligand acrylamide)**

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

![Chemical structure](image)

To a solution of HaloTag amine (O2) ligand (300 mg, 1.34 mmol) and ethyldiisopropylamine (DIEA, 350 µL, 2 mmol) in dry CH\(_2\)Cl\(_2\) (5 mL), cooled in ice-water bath, acryloyl chloride (131 µL, 1.61 mmol) dissolved in dry CH\(_2\)Cl\(_2\) (1 mL) was added dropwise. The reaction mixture was stirred at 0 °C for 30 min and at rt for 2 h. The mixture was then diluted with CH\(_2\)Cl\(_2\) (40 mL), washed with sat. aq. NaHCO\(_3\), brine and dried over Na\(_2\)SO\(_4\). 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.

\(^1\)H NMR (400 MHz, 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, 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) [M+H]\(^+\), 300.1 (100) [M+Na]\(^+\), 316.1 (78) [M+K]\(^+\).

HRMS (C\(_{13}\)H\(_{24}\)NO\(_3\)Cl): m/z (positive mode) = 278.1518 (found [M+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 µL, 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)-9H-xanthenylium trifluoroacetate

![Chemical Structure]

Trimethyl phosphate (Sigma-Aldrich; 175 µL, 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 → 100% of EtOAc) to yield 158 mg (85%) of the leuco dye as colorless oil (Rf = 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 → 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 µm 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, Rf = 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 4×250 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, 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)-9H-xantherylium-9-yl](phenyl)phosphinite trifluoroacetate

Dimethyl phenylphosphinite (Sigma-Aldrich; 160 μL, 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$_2$Cl$_2$ (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-P$_3$; colorless to blue-purple upon exposure to air over several minutes). The reaction mixture was diluted with CH$_2$Cl$_2$ (5 mL), evaporated on Celite and subjected to flash chromatography (RediSep Rf cartridge, 24 g of regular SiO$_2$; gradient: hexane – EtOAc, 50:50 → 0:100 over 10 column volumes). Yield 139 mg (quant.) of leuco-P$_3$ as a colorless oil. It was dissolved in CH$_2$Cl$_2$ (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$_2$Cl$_2$ (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$_2$Cl$_2$ (8 mL) and evaporated on Celite. Flash chromatography conditions: Sepacore Silica HP cartridge, 25 g of 15 μm regular SiO$_2$, gradient MeCN/H$_2$O 100:0 → 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 P$_3$ ($R_f = 0.30$, TLC with MeCN/H$_2$O 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$_2$O + 0.1% TFA in both components: 20 → 100% MeCN in 20 min, detection at 570 nm and 600 nm, column 4×250 mm, flow rate 1.2 mL/min.

$^1$H NMR (400 MHz, CD$_3$CN): $\delta$ 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×NMe$_2$) ppm.

$^{13}$C NMR (101 MHz, CD$_3$CN): $\delta$ 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×NMe$_2$) ppm.

$^{19}$F NMR (376 MHz, CD$_3$CN): $\delta$ -76.5 ppm.
$^3$P NMR (162 MHz, CD$_3$CN): δ 29.2 ppm.

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

HR-MS (ESI, positive mode): 421.1685 (found), 421.1676 (calculated for C$_{24}$H$_{26}$N$_2$O$_3$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 SiO\(_2\) plate, 10% v/v \(\text{H}_2\text{O}\) 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 \(\times\) 100 mL). The combined organic solutions were dried over Na\(_2\)SO\(_4\) and evaporated in vacuo, yielding 3.36 g of raw product as a violet solid. It was dissolved in a mixture of \(\text{H}_2\text{O}\) (5 mL) and MeCN (10 mL), and the solution was injected on top of a cartridge (RediSep R\(_f\) 120 g of regular SiO\(_2\)). Flash chromatography (gradient 0% to 5% \(\text{H}_2\text{O}\) 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/\(\text{H}_2\text{O}\) + 0.1 v/v% TFA in both compomentes: 20/80 – 100% MeCN in 15 min, detection at 560 nm and 600 nm, column 4\(\times\)250 mm, flow rate 1.2 mL/min.

\(^1\)H 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.

\(^{13}\)C 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 (NCH\(_2\)), 49.8 (NCH\(_2\)), 26.7, 20.1, 19.2 and 19.0 (all CH\(_2\)) ppm.

MS (ESI) m/z (positive mode, rel. int., %) = 371.2 (100) [M]+.
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 → 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$_2$O 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$_2$O + 0.1% TFA for both components, gradient: 100:0 → 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$_2$O (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$_2$O + 0.1% TFA in both components, gradient: 20 → 100% MeCN in 15 min, detection at 254 nm and 630 nm, column 4×250 mm, flow rate 1.2 mL/min.

$^1$H NMR (400 MHz, CD$_3$OD): δ 8.26 (s, 2H), 3.86 (d, $J = 11.6$ Hz, 6H, OMe), 3.58 (t, $J = 5.8$ Hz, 8H, 4 × NCH$_2$), 3.01 and 2.89 (2 × t, $J = 6.4$ Hz, $\Sigma = 8$H, 4 × CH$_2$C$_{ar}$), 2.07 (m, 8H, 4 × CH$_2$).

$^{13}$C NMR (101 MHz, CD$_3$OD): δ 152.7 (d, $J = 14$ Hz, C$_{q}$O), 152.5 (s, C$_o$), 137.3 (d, $J = 172$ Hz, C$_q$), 128.0 (d, $J = 4$ Hz, C$_o$), 126.2 (d, $J = 4$ Hz, CH), 116.3 (d, $J = 11$ Hz, C$_q$), 107.0 (d, $J = 2$ Hz, C$_o$), 54.0 (d, $J = 5.6$ Hz, OMe), 52.1, 51.5 (4 × NCH$_2$), 28.9 (2 × C$_o$CH$_2$), 21.8 (2 × C$_q$CH$_2$), 20.9, 20.8 (4 × CH$_2$CH$_2$N) ppm.

$^{19}$F NMR (376 MHz, CD$_3$OD): δ -77.0.
$^{31}$P NMR (162 MHz, CD$_3$OD): $\delta$ 15.9.

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

HR-MS (ESI, positive mode): 479.2094 (found), 479.2094 (calculated for C$_{27}$H$_{32}$N$_2$O$_4$P$^+$ as [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 (EtO)₂PCl and HN(Me)(CH₂)₃CO₂Bu' 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 CH₂Cl₂ (10 mL), and evaporated on Celite. Flash chromatography on silica (cartridge Interchim Puriflash 25 g of 15 µM SiO₂; solvent: CH₂Cl₂ – MeOH mixture; gradient: 1% → 10 % MeOH/CH₂Cl₂) afforded 126 mg (93%) of leuco-P5 tert-butyl ester as a slightly brown oil. TLC: regular SiO₂, CH₂Cl₂ – MeOH 30:1; Rf = 0.26.

The intermediate tert-butyl ester (126 mg, 0.198 mmol) was dissolved in CH₂Cl₂ (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 CH₂Cl₂ (10 mL) and evaporated on Celite. Flash chromatography (Interchim Puriflash 25 g of 15 µM SiO₂; solvent: CH₂Cl₂ – MeOH; gradient: 1 → 40% MeOH/CH₂Cl₂) afforded 61 mg (54%) of leuco-P5 as a blue solid (TLC: CH₂Cl₂ – MeOH 5:1, Rf = 0.25) soluble in acetonitrile. The spot of leuco-P5 on silica compound turns blue under UV lamp or in presence of air. HPLC: tR = 9.1 min (peak area 82%); solvent: MeCN – H₂O + 0.1 v/v% TFA in both components; gradient: 30 → 100% MeCN in 15 min, detection at 254 nm; column: 250 x 4 mm, flow rate 1.2 mL/min. C₃₃H₄₂N₃O₅P. MS (ESI): m/z (negative mode, rel. int., %) = 578.8 (100) [M-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 SiO$_2$; solvent: MeCN – H$_2$O + 0.2 v/v% of HCOOH in both components; gradient: 1 → 15% v/v H$_2$O, 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 – H$_2$O 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 – H$_2$O + 0.5% v/v TFA in H$_2$O; 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.

$^1$H NMR (400 MHz, CD$_3$CN): $\delta$ 8.34 (s, 2H), 4.24 – 4.06 (m, 2H, OCH$_2$), 3.53 (t, $J$ = 5.8 Hz, 8H, 4 × NCH$_2$), 3.16 – 3.00 (m, 2H, MeNCH$_2$), 2.97 (t, $J$ = 6.4 Hz, 4H, 2 × C$_4$CH$_2$), 2.87 (t, $J$ = 6.3 Hz, 4H, 2 × C$_4$CH$_2$), 2.65 (d, $J$ = 10.5 Hz, 3H, NMe), 2.26 (m, overlaps with H$_2$O signal, CH$_2$CH$_2$CH$_2$ in julolidine), 2.03 (m, 4H, CH$_2$CH$_2$CH$_2$ in julolidine), 1.77 (m, 2H, MeNCH$_2$CH$_2$), 1.37 (t, $J$ = 7.1 Hz, 3H, OCH$_2$CH$_3$) ppm.

$^{13}$C NMR (101 MHz, CD$_3$CN, $^{13}$C($^1$H) correlation): $\delta$ 128.5 (8.3) (CH), 63.1 (4.15) (CH$_2$O), 51.3 (3.52) (CH$_3$N), 48.0 (3.04) (CH$_3$N), 32.9 (2.62) (NMe), 31.1 (2.26) (CH$_2$), 28.6 (2.86) (CH$_2$), 23.5 (1.76) (CH$_2$), 21.6 (2.00) (CH$_2$), 20.6 (2.96) (CH$_2$), 20.5 (2.04) (CH$_2$), 16.6 (1.36) (CH$_3$).

$^{31}$P NMR (162 MHz, CD$_3$CN): $\delta$ 18.4 ppm.

$^{19}$F NMR (376 MHz, CD$_3$CN): $\delta$ -76.2 ppm.

MS (ESI): $m/z$ (positive mode, rel. int., %) = 578.3 (100) [M+H]$^+$. HRMS (C$_{32}$H$_{40}$N$_3$O$_5$P): $m/z$ (positive mode) = 578.2782 (found [M+H]$^+$), 578.2778 (calc.).
Dye P6

leuco-P6

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

A suspension of Pyronin Y (151 mg; 0.50 mmol) and tetrabutylammonium iodide (185 mg; 0.50 mmol) in CH$_2$Cl$_2$ was sonicated briefly, and 2-dimethylamino-4H-1,3,2-benzodioxaphosphorin-4-one[6] 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$_2$O 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$_2$Cl$_2$). 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) [M–H]$^+$.

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

Dye P6

2-[[3,6-bis(dimethylamino)-9H-xanthenyl-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 µL, 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 µM 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-d₆): 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 = 2.4 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-d₆): 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, DMSO-$d_6$): $\delta$ -74.6 ppm.

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

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

HR-MS (ESI, positive mode): 455.2099 (found), 455.2094 (calculated for C$_{25}$H$_{32}$N$_2$O$_4$P$^+$).
Dye P8

Compound 8

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

To a solution of **tert-butyl 3-(methylamino)propionate**[^7] (500 mg; 3.14 mmol) and triethylamine (380 mg; 3.77 mmol) in a mixture of benzene and CHCl₃ (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 SiO₂, Hex/EtOAc 1:3 + 0.1 v/v% of NEt₃) to afford 392 mg (50%) of **13** as an air-sensitive colorless oil, which was used in the following step without additional purification.

[^7]: ³H NMR (400 MHz, CD₃CN): δ = 1.18 (t, J₃H = 7.0 Hz, 6H, 2×OEt), 1.43 (s, 9H, tBu), 2.37 (t, J₉H = 7.0 Hz, 2H, CH₂), 2.52 (d, J₉-P = 6.6 Hz, 3H, NMe), 3.21 (dt, J₉-P = 9.7 Hz, J₉-H = 7.0 Hz, 2H, NCH₂), 3.57−3.72 (m, 4H, 2×OEt) ppm.

[^31]: ³¹P NMR (162 MHz, CD₃CN): δ = 144.9 ppm.

**leuco-P8 tert-butyl ester**

**tert-butyl 3-[[3,6-bis(dimethylamino)-9H-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 CH$_2$Cl$_2$ (~3 mL) and subjected to column chromatography (30 g of SiO$_2$, CH$_2$Cl$_2$/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.

$^1$H NMR (400 MHz, CD$_3$CN): $\delta = 1.21$ (t, $J_{H-H} = 7.0$ Hz, 3H, OEt), 1.38 (s, 9H, tBu), 2.05–2.25 (m, 2H, CH$_2$), 2.39 (d, $J_{H-P} = 8.2$ Hz, 3H, NMe), 2.78–2.94 (m, 2H, NCH$_2$), 2.93 (s, 12H, 2×NMe$_2$), 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.

$^{31}$P NMR (162 MHz, CD$_3$CN): $\delta = 26.9$ ppm.

**leuco-P8**

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

To a solution of leuco-P8 tert-butyl ester (33 mg, 0.064 mmol) in CH$_2$Cl$_2$ (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 SiO$_2$, CH$_2$Cl$_2$/MeOH 10:1) to afford 27 mg (92%) of leuco-P8 as bluish solid.

$^1$H NMR (400 MHz, CD$_3$CN): $\delta = 1.20$ (t, $J_{H-H} = 7.0$ Hz, 3H, OEt), 2.09–2.24 (m, 2H, CH$_2$), 2.39 (d, $J_{H-P} = 8.1$ Hz, 3H, NMe), 2.75–3.07 (m, 2H, NCH$_2$), 2.93 (s, 12H, NMe$_2$), 3.74–3.97 ppm.
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 → MeCN/H$_2$O 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.

$^1$H NMR (400 MHz, CDCl$_3$): $\delta = 1.41$ (t, $J_{H-H} = 7.3$ Hz, 3H, OEt), 2.54–2.73 (m, 2H, CH$_2$), 2.80 (d, $J_{H-P} = 10.8$ Hz, 3H, NMe), 3.27–3.50 (m, 2H, NCH$_2$), 3.37 (s, 12H, 2×NMe$_2$), 4.18–4.37 (m, 2H, OEt), 6.74 (s, broad, 2H$_{ar}$), 7.18 (dd, $J_{H-H} = 9.9$ Hz, $J_{H-P} = 2.3$ Hz, 2H$_{ar}$), 8.86 (d, $J_{H-H} = 9.9$ Hz, 2H$_{ar}$) ppm.

$^{31}$P NMR (162 MHz, CDCl$_3$): $\delta = 17.7$ ppm.

MS (ESI): $m/z$ (positive mode, rel. int., %) = 460.2 (100) [M$^+$].
**Dye P8 NHS ester**

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

![Reaction Scheme]

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)-9H-xanthenylium-9-yl]phosphonate

Solutions of dye P8 in protic solvents (MeOH, H₂O) hydrolyze (particularly in the presence of acids) with formation of the compound P9.

1H NMR (400 MHz, CD₃CN): δ 1.04 (t, J_H-H = 7.0 Hz, 3H, OEt), 3.27 (s, 12H, 2×NMe₂), 3.79 (dt, J_H-P = 14.2 Hz, J_H-H = 7.2 Hz, 2H, OEt), 6.77−6.80 (m, 2H ary), 7.18 (dd, J_H-H = 9.8 Hz, J_H-H = 2.6 Hz, 2H ary), 9.04 (d, J_H-H = 9.8 Hz, 2H ary) ppm.

31P NMR (162 MHz, CD₃CN): δ 3.7 ppm.

MS (ESI): m/z (positive mode, rel. int., %) = 375.1 (100) [M+H]⁺, 397.2 (47) [M+Na]⁺.
**Dye P10**

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

![Chemical structure of Pyronin Y and P10](image)

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$_2$Cl$_2$ (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 → 30:70) to yield 108 mg (76%) of the bluish viscous oil. It was dissolved in dry CH$_2$Cl$_2$ (3 mL), and the solution was cooled in a dry ice-acetone bath. A solution of DDQ (60 mg, 0.25 mmol) in CH$_2$Cl$_2$ (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$_2$O + 0.1 v/v% TFA, 100:0 → 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$_2$O 10:1 + 0.1 v/v% TFA, $R_f$ = 0.36). HPLC: $t_R$ = 11.4 min (peak area 98%), MeCN/H$_2$O + 0.1% TFA in both components: 20/80 – 100% MeCN in 20 min, detection at 570 nm and 600 nm, column 4 × 250 mm, flow rate 1.2 mL/min.

$^1$H NMR (400 MHz, CD$_3$CN) 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 iPr), 1.19 (d, $J$ = 6.1 Hz, 6H, diastereotopic methyl groups in iPr).

$^{13}$C NMR (101 MHz, CD$_3$CN) δ 158.4 (d, $J$ = 13 Hz), 158.0, 133.4 (d, $J$ = 3Hz), 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$_3$CN) δ -76.6 ppm.

$^{31}$P NMR (162 MHz, CD$_3$CN) δ 8.0 ppm.

MS (ESI): $m/z$ (positive mode, rel. int., %) = 431.2 (100) [M$^+$].
HR-MS (ESI, positive mode): 431.2094 (found), 431,2094 (calculated for C\(_{23}\)H\(_{32}\)N\(_2\)O\(_4\)P\(^+\) as [M]\(^+\)).

Dye P11

\[P-[3,6-bis(dimethylamino)-9H-xanthenylium-9-yl]-P,\textit{P}-\text{ diphenylphosphine oxide trifluoroacetate}\]

\[
\text{Me}_2\text{N} \quad \text{Cl}^- 
\begin{array}{c}
\text{Pyronin Y} \\
1) \text{Ph}_2\text{P(OMe)} \\
2) \text{DDQ, TFA} \\
\text{Me}_2\text{N} \\
\text{O=P--Ph} \\
\text{Ph} \\
\text{CF}_3\text{CO}_2^- \\
P11
\end{array}
\]

To a stirred solution of Pyronin Y (100 mg, 0.33 mmol) and tetrabutylammonium iodide (122 mg, 0.33 mmol) in dry CH\(_2\)Cl\(_2\) (8 mL), methyl diphenylphosphinite (Sigma-Aldrich, 200 μL, 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\(_2\)Cl\(_2\) (10 mL), evaporated on Celite and subjected to flash chromatography (cartridge RediSep Rf with 24 g of SiO\(_2\), gradient: EtOAc/Hexane 95:5 → 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\(_2\)Cl\(_2\) (3 mL), the solution was cooled in a dry ice - acetone bath (ca. -70 °C), and a solution of DDQ (76 mg, 0.33 mmol) in CH\(_2\)Cl\(_2\) (5 mL) was added quickly dropwise. The reaction mixture was stirred 15 min at -70 °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\(_2\)Cl\(_2\) (8 mL), evaporated to dryness with Celite and subjected to flash chromatography (cartridge Sepacore Silica HP 25 g 15 μM silica, gradient MeCN/H\(_2\)O 100:0 → 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 μM 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 x 25 cm, RP-C18 10 μm, eluents: H\(_2\)O + 0.1 v/v % of TFA, MeCN (TFA free); gradient 30 → 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 μM 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\(_2\)O 10:1 + 0.1 v/v% of TFA) revealed a colored spot with \(R_f = 0.36\). \(^{1}H\) \(^{13}C\) and \(^{31}P\) NMR spectra indicate the presence of 2 forms. HPLC: \(t_R = 11.9\) min (peak area 97%).
MeCN/H_2O + 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, CD_3CN) 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×NMe_2) ppm.

^13C NMR (101 MHz, CD_3CN) δ 157.9 (d, J = 9 Hz, C), 157.6 (C), 134.2 (d, J = 2.8 Hz, p-CH in Ph_2PO), 132.5 and 132.3 (m-CH in Ph_2PO and C^4,5), 130.4 (d, J = 13 Hz, o-CH Ph_2PO), 117.4 (d, J = 7.6 Hz), 115.2 (C^3,6), 97.4 (C^1,8), 41.3 (2×NMe_2) ppm.

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

^31P NMR (162 MHz, CD_3CN) 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 C_{29}H_{28}N_2O_2P^+).
Dye P12

**leuco-P12**

![Chemical Structure](image)

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 → 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*<sub>R</sub> = 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 × 250 mm, flow rate 1.2 mL/min.

C<sub>25</sub>H<sub>29</sub>N<sub>2</sub>O<sub>3</sub>P (436.1916).

**¹H NMR** (400 MHz, CD₃OD): δ 6.88 (s, 2H), 6.65 (d, *J*<sub>HP</sub> = 543Hz, 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 × N(CH₂)₂), 3.03 – 2.61 (m, 8H, 4 × 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]<sup>−</sup>; 871.5 [2M-H]<sup>−</sup>; *m/z* (positive mode, rel. int., %) = 437.2 (100) [M+H]<sup>+</sup>; 873.4 (8) [2M+H]<sup>+</sup>.

**HR MS** (ESI): *m/z* (positive mode) = 437.1987 (found for C<sub>25</sub>H<sub>30</sub>N<sub>2</sub>O<sub>3</sub>P, [M+H]<sup>+</sup>), 437.1994 (calc. for C<sub>25</sub>H<sub>30</sub>N<sub>2</sub>O<sub>3</sub>P); 459.1806 (found for C<sub>25</sub>H<sub>28</sub>N<sub>2</sub>NaO<sub>3</sub>P, [M+Na]<sup>+</sup>), 459.1813 (calc. for C<sub>25</sub>H<sub>28</sub>N<sub>2</sub>NaO<sub>3</sub>P).
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 °C, and a solution of DDQ (11 mg, 50 µmol) in MeCN (2 mL) was added. The reaction mixture was stirred at 0 °C for 30 minutes, evaporated in vacuo, the residue was dissolved in MeOH (~10 mL) and applied to a cartridge with regular SiO₂. Isolation with Biotage Isolera: cartridge Sepacore Silica HP, 25 g; gradient MeOH/CH₂Cl₂: 100/0 → 95/5, then MeCN/H₂O 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; Rf = 0.4 on regular SiO₂, MeCN/H₂O 10:1 + 0.1 v/v% TFA in each component, red fluorescent spot. HPLC: tR = 14.5 min (peak area 85%), eluent: MeCN / H₂O + 0.1 v/v% TFA in both components; gradient: 20 → 100% MeCN in 15 min, detection at 254 nm, column, 4 × 250 mm, flow rate 1.2 mL/min.

1H NMR (400 MHz, CD₃OD): δ 8.29 (d, JHP = 544 Hz, 1H), 8.29 (s, 2H, CH), 3.52 (m, 8H, N(CH₂)₂), 2.95 (t, J = 6.2 Hz, 4H), 2.67 (t, J = 6.2 Hz, 4H), 2.13–1.95 (m, 8H) ppm.

19F NMR (376 MHz, CD₃OD): δ -77.2 ppm.

31P NMR (162 MHz, CD₃OD): δ 3.3 ppm.

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

HR-MS (ESI, positive mode): 435.1832 (found), 435,1838 (calculated for C₂₅H₂₈N₂O₃P as [M+H]⁺); 457.1655 (found), 457,1657 (calculated for C₂₃H₂₇N₂NaO₃P, [M+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 → MeCN/H\(_2\)O 20:1 + 0.1 v/v% of TFA). Fractions containing the dye were evaporated to dryness, dissolved in water and extracted with CH\(_2\)Cl\(_2\) (3×). Combined organic solutions were dried with Na\(_2\)SO\(_4\) and evaporated to yield 40 mg of a dark blue solid. HPLC analysis (B/A = 50/50−100/0 in 25 min, column 4×250 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\(_2\)O 2:1 + 0.1% v/v TFA → MeCN + 0.1% TFA → 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) [M]\(^+\).

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

Dye P14:

\(^1\)H NMR (400 MHz, CDCl\(_3\)): \(\delta\) 1.94−2.02 (m, 4H, 2×CH\(_2\)), 2.03−2.11 (m, 4H, 2×CH\(_2\)), 2.84−2.90 (m, 4H, 2×CH\(_2\)), 2.91−2.98 (m, 4H, 2×CH\(_2\)), 3.43−3.52 (m, 8H, 4×CH\(_2\)), 3.59 (d, \(J_{H-P} = 11.5\) Hz, 3H, OMe), 8.95 (s, 2H\(_ar\)) ppm.

\(^{31}\)P NMR (162 MHz, CD\(_3\)CN): \(\delta\) 5.0 ppm.

HR-MS (C\(_{26}\)H\(_{29}\)N\(_2\)O\(_4\)P): \(m/z\) (positive mode) = 465.1935 (found [M+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 SiO\(_2\), gradient 5% to 10% EtOH/CH\(_2\)Cl\(_2\)), eluting the fluorescent band. The eluate was evaporated and the product was isolated by reversed-phase column chromatography (30 g RP-C\(_{18}\), gradient 50% to 20% H\(_2\)O/MeCN + 1 v/v% TFA). The fractions containing the product were pooled, and the residue was lyophilized from H\(_2\)O/MeCN (2:1). Orange solid, yield 165 mg (31%).

\(^1\)H NMR (400 MHz, CD\(_3\)OD): \(\delta\) 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.

\(^13\)C NMR (101 MHz, CD\(_3\)OD): \(\delta\) 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 (C\(_{25}\)H\(_{34}\)N\(_3\)O\(_2\)): \(m/z\) (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$_3$PO$_2$ (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-C$_{18}$). The excess of H$_3$PO$_2$ was removed first by elution with water; then 50% to 30% H$_2$O/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) [M–H]$^-$.

The leuco acid was dissolved in a mixture of CH$_2$Cl$_2$ (3 mL) and MeOH (3 mL), the solution was cooled in dry ice-acetone bath, and DDQ (54 mg; 0.24 mmol) in CH$_2$Cl$_2$ (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% H$_2$O/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-C$_{18}$, gradient 10% to 40% MeCN in 5 v/v% 0.1 M Et$_3$NH$^+$ HCO$_3^-$ in H$_2$O). 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.

$^1$H NMR (400 MHz, acetic acid-$d_4$): $\delta$ 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-$d_4$): $\delta$ 5.8.

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

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

HRMS (C$_{21}$H$_{27}$N$_3$O$_4$P): m/z (positive mode) = 416.1732 (found [M]$^+$), 416.1734 (calc.).
Dye A1 NHS ester

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

N-hydroxysuccinimide ester

Triethylamine (20 µL, ~140 µmol), N-hydroxysuccinimide (50 µL of a 1.13 M stock solution in DMF, 57 µmol) and HATU (50 µL of a 0.76 M stock solution in DMF, 38 µmol) were added to a suspension of A1 (2.0 mg, 3.8 µmol) 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 µM PTFE membrane filter and lyophilized. Yield 1.2 mg (62%), HPLC area 87%; red solid. HPLC: tᵣ = 9.0 min (87%), B/A = 30/70−100/0 in 25 min, column 4×250 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

\[
\begin{align*}
\text{Me}_2\text{N} & \quad \text{Me}^+ \\
\text{NMe}_2 & \quad \text{NMe}_2 \\
\text{I} & \quad \text{OMe} \\
\to \quad \text{Me}_2\text{N} & \quad \text{Me}^+ \\
\text{NMe}_2 & \quad \text{NMe}_2 \\
\text{O=P} & \quad \text{O}\text{Me} \\
\text{CF}_3\text{CO}_2^- & \quad \text{OMe} \\
\end{align*}
\]

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 \(14\) (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\(_2\)Cl\(_2\) (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\(_2\)Cl\(_2\) (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% H\(_2\)O/MeCN, then 5% to 10% H\(_2\)O/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% H\(_2\)O/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%).

\(^{1}H\) NMR (300 MHz, CD\(_3\)OD): \(\delta\) 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\(_3\)OD): \(\delta\) 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\(_3\)OD): \(\delta\) 16.45.

\(^{19}F\) NMR (282 MHz, CD\(_3\)OD): \(\delta\) -72.94.

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

HRMS (C\(_{20}\)H\(_{27}\)N\(_3\)O\(_3\)P): \(m/z\) (positive mode) = 388.1785 (found [M\(^+\)], 388.1785 (calc.).
Thiopyronin derivatives (SP1, SP2)

Dye SP1

**Compound 15**

3,6-bis(dimethylamino)-9H-thioxanthenium 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-d₆ 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-d₆ 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)-9H-thioxanthen-9-ylium trifluoroacetate

To a solution of 15 (100 mg, 0.262 mmol) and tetrabutylammonium iodide (96.7 mg, 0.26 mmol) in dry CH₂Cl₂ (5 mL), trimethyl phosphite (93μL, 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 Rf = 0.22; greenish colored spot gradually appeared on a TLC plate (air, UV light). The reaction mixture was diluted with CH₂Cl₂ (8 mL) and evaporated on Celite. The leuco dye was isolated by flash chromatography (cartridge RediSep Rf 24 g of SiO₂, gradient EtOAc/hexane, 20:80 → 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 CH₂Cl₂ (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 CH₂Cl₂ (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 CH₂Cl₂ (10 mL), evaporated to dryness on Celite and subjected to flash chromatography (Interchim PuriFlash, 25 g of 15 μm SiO₂, gradient: MeCN/H₂O + 0.1 v/v% of TFA, 100% of MeCN → 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 SiO₂, MeCN/H₂O 10:1 + 0.1 v/v% TFA in each component, Rf = 0.23. HPLC: tr = 12.2 min (peak area 98%), MeCN/H₂O + 0.1% v/v TFA in both components: 20/80 – 80/20% MeCN in 20 min, detection at 580 nm, column 4×250 mm, flow rate 1.2 mL/min.

¹H NMR (400 MHz, CD₃CN): 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, NMe₂) ppm.

¹³C NMR (101 MHz, CD₃CN): 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.

¹⁹F NMR (376 MHz, CD₃CN): δ -76.6 ppm.

³¹P NMR (162 MHz, CD₃CN): δ 16.53 ppm.

MS (ESI): m/z (positive mode, rel. int., %) = 391.1 (100) [M]⁺.
HR-MS (ESI, positive mode): 391.1247 (found), 391.1240 (calculated for C_{19}H_{24}N_{2}O_{3}PS^{+} as [M]+).

**Dye SP2**

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

![Dye SP2 Reaction Scheme]

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_{2}Cl_{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_{2}Cl_{2} (3 mL), the solution was cooled in a dry ice-acetone bath, and DDQ (59 mg, 0.26 mmol) in CH_{2}Cl_{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% H_{2}O/MeCN, then 5% H_{2}O/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%).

{\textsuperscript{1}H NMR (400 MHz, CD_{3}CN):} 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).

{\textsuperscript{13}C NMR (101 MHz, CD_{3}CN):} 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.

{\textsuperscript{31}P NMR (122 MHz, CD_{3}OD):} δ 23.32.

{\textsuperscript{19}F NMR (282 MHz, CD_{3}OD):} δ -76.11.

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

HRMS (C_{24}H_{35}N_{3}O_{2}PS): \textit{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-5H-dipyrrlo[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-5H-dipyrrlo[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, iPr), 1.39 (d, J_H-H = 6.2 Hz, 6H, iPr), 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, J_F-F = 32.2 Hz).

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

HR-MS (C₁₉H₂₆BF₂N₂O₃P): 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-dihydroanthracenylum trifluoromethanesulfonate

In a Schlenk flask, to a solution of 3,6-bis(dimethylamino)-10,10-dimethylanthrone\textsuperscript{[12]} 17 (50 mg, 0.16 mmol) in CH\textsubscript{2}Cl\textsubscript{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\textsubscript{2}, MeCN \rightarrow MeCN/H\textsubscript{2}O, 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\textsubscript{2}, MeCN/H\textsubscript{2}O 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 4×250 mm, 1.2 mL/min, detection at 635 nm.

\textsuperscript{1}H NMR (400 MHz, CDCl\textsubscript{3}): $\delta$ 1.28 (d, $J_{\text{H-H}}$ = 6.7 Hz, 6H, diasteriotopic CH(CH\textsubscript{3})\textsubscript{2}), 1.33 (d, $J_{\text{H-H}}$ = 6.8 Hz, 6H, diasteriotopic CH(CH\textsubscript{3})\textsubscript{2}), 1.69 (s, 6H, 2×Me), 3.35 (s, 12H, 2×NMe\textsubscript{2}), 3.47–3.58 (m, 2H, NiPr\textsubscript{2}), 4.27–4.37 (m, 1H, CH\textsubscript{2}CH\textsubscript{2}O), 4.52–4.61 (m, 1H, CH\textsubscript{2}CH\textsubscript{2}O), 5.09–5.14 (m, $J_{\text{H-H}}$ = 10.3 and 1.1 Hz, 1H, CH=), 5.19–5.26 (m, $J_{\text{H-H}}$ = 17.1 and 1.5 Hz, 1H, CH=), 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\textsubscript{a}), 7.11 (t, $J_{\text{H-H}}$ = 2.5 Hz, $J_{\text{H-P}}$ = 2.5 Hz, 2H\textsubscript{a}), 8.84 (d, $J_{\text{H-H}}$ = 9.8 Hz, 2H\textsubscript{a}) ppm.

\textsuperscript{13}C NMR (100 MHz, CDCl\textsubscript{3}, APT): $\delta$ 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.

\textsuperscript{31}P NMR (162 MHz, CDCl\textsubscript{3}): $\delta$ 22.3 ppm.

MS (ESI): $m/z$ (positive mode, rel. int., %) = 496.4 (100) [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-diisopropylphosphonamidate

Dimethyl N,N-diisopropylphosphoramidite (84 µL, 0.37 mmol) was added to a solution of 3,7-bis(dimethylamino)-5,5-dimethyl-5,10-dihydrodibenzo[b,e]silinylium 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 SiO\(_2\), gradient 33% to 50% EtOAc in hexane). The fractions containing the product were pooled, evaporated and dried \textit{in vacuo} to yield 55 mg (92%) of \textit{leuco-SiP1} as a viscous colorless oil.

\(^1\text{H NMR (400 MHz, CD}_3\text{CN): }\delta\) 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.

\(^{13}\text{C NMR (101 MHz, CD}_3\text{CN): }\delta\) 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.

\(^{31}\text{P NMR (162 MHz, CD}_3\text{CN): }\delta\) 31.20 ppm.

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

HRMS (C\(_{26}\)H\(_{42}\)N\(_3\)O\(_2\)PSi): \(m/z\) (positive mode) = 488.2860 (found [M+H]\(^+\)), 488.2857 (calc.).
Oxidation of leuco-SiP1 (DDQ in CH$_2$Cl$_2$) as follows led to formation of an unstable silaxanthyl dye SiP1 which easily hydrolyzes to the starting material. 22 mg (0.045 mmol) of the leuco dye was dissolved in CH$_2$Cl$_2$ (2 mL), and the solution was cooled to -78 °C. A solution of DDQ (10 mg, 0.044 mmol) in CH$_2$Cl$_2$ (1 mL) was then added. The reaction mixture was allowed to warm up to rt and subjected to column chromatography (30 g of SiO$_2$, MeCN → MeCN + 0.1% TFA → MeCN /H$_2$O 40:1 + 0.1% 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.
Benzanthriline derivative BA1

Dye BA1

Compound 19
1-bromo-5-(dimethylamino)naphthalene

\[
\begin{array}{c}
 \text{Br} \\
 \text{NO}_2 \\
 \text{Fe, NH}_4\text{Cl} \\
 \text{EtOH - H}_2\text{O, 75 °C, 1.5 h} \\
 \text{Br} \\
 \text{NH}_2 \\
 \text{O=P(O\text{Me})}_3 \\
 \text{Br} \\
 \text{NMe}_2 \\
 \text{19}
\end{array}
\]

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

![Chemical Structure](image)

1,4-Dioxane (25 mL) was added to the solid 2-bromo-4-(dimethylamino)benzaldehyde \textsuperscript{[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.

\(^1\)H NMR (500 MHz, CDCl\(_3\)): \(\delta\) 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\(_3\)): \(\delta\) 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\(_3\)B): \(m/z\) (positive mode) = 298.1578 (found [M+Na]\(^+\)), 298.1588 (calc.).

**Compound 21**

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

![Chemical Structure](image)

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\(_2\)CO\(_3\) (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\(_2\)SO\(_4\). 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.

^1^H NMR (400 MHz, CDCl₃): 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, CDCl₃): 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, 121.2, 114.2, 113.1, 111.0, 45.4, 40.1.

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

HR-MS (C_{21}H_{22}N_{2}O): m/z (positive mode) = 319.1810 (found [M+H]^+), 319.1805 (calc.).

**Compound 22**

4,10-bis(dimethylamo)-7H-benzo[de]anthracenylium 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 NaClO₄. 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%).

^1^H NMR (400 MHz, DMSO-d₆): 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, DMSO-d₆): 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) [M–CH₃]^+, 301.2 (1) [M]^+.

HRMS (C_{21}H_{21}N_{2}): m/z (positive mode) = 301.1690 (found [M]^+), 301.1699 (calc.).
Dye BA1

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

![Chemical structure](image)

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 7H-benz[de]anthracene adduct (leuco-BA1) was isolated by column chromatography (25 g of SiO2, 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 SiO2, gradient 0% to 50% H2O in MeCN); the fractions containing the product were pooled, trifluoroacetic acid (200 µL) was added, MeCN was evaporated (bath temperature ≤25 °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, CD3OD): 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.

13C NMR (101 MHz, CD3OD): 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.

19F NMR (376 MHz, CD3OD): δ -77.3 ppm.
$^{31}\text{P NMR (162 MHz, CD}_3\text{OD)}: \delta 26.0 \text{ ppm.}$

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

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


NMR spectra

Dye C1: $^1\text{H}$

![NMR spectrum of dye C1 with chemical structure](image-url)
Dye C1: $^{13}\text{C}\{^1\text{H}\}$

![Dye C1: $^{13}\text{C}\{^1\text{H}\}$](image)
Dye C1: $^{31}$P

![Chemical Structure](image_url)

**STANDARD PHOSPHORUS PARAMETERS**

- δ (ppm) range: -40 to 160
- Scale: 10 ppm per unit
Dye C2: $^1$H
Dye C2: $^{13}\text{C}^{1}\text{H}$

![Chemical Structure of C2](image)

\[ \text{Et}_2\text{N} \]

\[ \text{CO}_2\text{Et} \]

\[ \text{MeO} - \text{P} = \text{O} \]

\[ \text{OMe} \]

\[ \text{C2} \]
Dye C2: $^{31}$P

The diagram shows the molecular structure of Dye C2 with labels indicating functional groups and atoms.

The chart or graph on the page represents the chemical shift in ppm (parts per million) on the x-axis and the intensity or intensity of the signal on the y-axis.
compound 3: $^1$H
compound 3: $^{13}$C$_1^1$H}

[Chemical Structure Image]

$^{13}$C NMR spectrum of compound 3.
Dye C3: $^1$H

C3
Dye C3: $^{13}\text{C}^{1}\text{H}$
Dye \textbf{C3}: $^{31}\text{P}$
Dye C4: $^1$H
Dye C4: $^{13}$C($^1$H)

![Chemical Structure of C4](image)

The image shows a $^{13}$C NMR spectrum for the compound C4. The spectrum includes peak assignments and chemical shifts in ppm. The structure of C4 is depicted with various atoms and bonds, indicating the presence of elements such as oxygen, nitrogen, and carbon. The spectrum is labeled with peak numbers and chemical shifts, which are crucial for identifying the molecular structure and properties of the compound.
Dye C4: $^{31}\text{P}$
Dye C5: $^{13}\text{C}^{1}\text{H}$

![Chemical structure](Image)
Dye C5: $^{31}$P
Dye C6: $^1$H

![Chemical Structure of C6]

![NMR Spectrum of C6]
Dye C6: $^{13}$C\$^1$H\$^1$ 

![Chemical Structure](image)
Dye C6: $^{31}\text{P}$

![Chemical Structure](image)
Dye C7: $^1$H
Dye C7: $^{31}$P

\begin{化学式}
Et_2N \quad \begin{array}{c}
\text{O} \\
\text{HO-P=O} \\
\text{OH} \\
\text{c7}
\end{array}
\end{化学式}
compound 5: $^1$H
compound 5: $^{13}$C($^1$H)
compound 5: $^{19}$F

![Chemical Structure](image_url)
compound 6: $^1$H
compound 6: $^{13}$C($^1$H)
Dye C8: $^1$H
Dye C9: $^1\text{H}$
Dye C9: $^{13}\text{C}^{1}\text{H}$
Dye C9: $^{31}$P

![Chemical Structure](image)

**C9**
compound 8: $^1$H

8 (leuco-P1)
compound 8: $^{13}\text{C}[^1\text{H}]$

8 (leuco-P1)
compound 8: gHSQCad
compound $\text{8}$: $^{31}\text{P}$

$\text{8 (leuco-P1)}$
Dye P1: $^1$H

SHS-398-2-is02a_PROTON_02

Me$_2$N
\[
\begin{array}{c}
\text{O} \\
\text{NMe}_2 \\
\text{O}^- \text{P} \text{O}^- \\
\text{H} \\
P1
\end{array}
\]
Dye P1: gHSQCad
Dye P1: $^{31}$P

SHS-396-2-ioc2a_PHOSPHORUS_02
STANDARD PHOSPHORUS PARAMETERS

Me$_2$N
\[\begin{array}{c}
\text{O} \\
\text{P} \\
\text{H}
\end{array}\]
\[\begin{array}{c}
\text{O} \\
\text{P} \\
\text{H}
\end{array}\]
\[\begin{array}{c}
\text{Me}_2\text{N} \\
\text{Me}_2
\end{array}\]

P1
compound 9: $^1$H
compound 9: $^{13}$C$^{1}$H
Dye P1-Halo: $^1$H
Dye P1-Halo: $^{13}{\text{C}}^1{\text{H}}$
Dye P1-Halo: $^{31}\text{P}$

![Chemical structure of P1-Halo](image)
Dye P2: $^1\text{H}$

Me$_2$N$_2$O$_2$O$^+$NMe$_2$

O=P=OMe CF$_3$CO$_2^-$

OMe

P2
Dye P2: $^{13}\text{C}^1\text{H}$
Dye P2: $^{19}\text{F}$

\[
\begin{array}{c}
\text{Me}_2\text{N} \\
\text{O} \\
\text{O}=\text{P} - \text{OMe} \\
\text{CF}_3\text{CO}_2^- \\
\text{OMe} \\
\text{P2}
\end{array}
\]
Dye P2: $^{31}\text{P}$
Dye P3: $^1$H

$$\text{Me}_2\text{N}$$

$$\text{O}$$

$$\text{O=PO-Me CF}_3\text{CO}_2^-$$

Ph

P3
Dye P3: \(^{13}C\{^1H\}\)
Dye P3: $^{19}\text{F}$

![Chemical Structure](image_url)
Dye P3: $^{31}$P

![Chemical Structure of Dye P3]

[Graph showing a 1D NMR spectrum with a peak at around 15 ppm]
compound 10: $^1$H

![Chemical structure of compound 10]
compound 10: $^{13}$C($^1$H)
Dye P4: $^1$H

[Chemical structure and NMR spectrum image]
Dye P4: \(^{13}\text{C}\{^1\text{H}\}\}}\\

O=P=\text{OMe} \quad \text{CF}_3\text{CO}_2^-
Dye P4: $^{19}$F

\[ \text{Dye P4: } ^{19}\text{F} \]
Dye P4: $^{31}$P

![Chemical Structure](image)

**Chemical Formula**
- O=P=OMe
- CF$_3$CO$_2^-$ (Tf$_2$N)

**NMR Spectrum**
- Peak at approximately 13.85 ppm

**10 shs390-1-iso-dye_PHOSPHORUS_01**

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Dye P5: $^1$H
Dye P5: \( gHSQCad \)
Dye P5: $^{31}$P
Dye P6: $^1$H

![NMR Spectrum]

![Chemical Structure]

$\text{Me}_2\text{N}$

$\text{Me}_2\text{P} = \text{O}$

$\text{Me}_2\text{N} - \text{O}$

$\text{CO}_2^{-}$

$\text{P6}$
Dye P6: $^{13}\text{C}^{11}\text{H}$

![Chemical Structure]

P6
Dye P6: $^{31}\text{P}$
Dye P7: $^1$H

![NMR Spectrum of Dye P7 with Chemical Structure and Peak Assignments]
Dye P7: $^{13}$C($^1$H)

![Diagram of dye P7 with chemical structures and carbon nuclei labeled with their ppm values in a carbon-13 NMR spectrum.]
Dye P7: $^{19}\text{F}$

![Chemical Structure](image)

P7

NMe$_2$
Dye P7: $^{31}\text{P}$

![Chemical Structure of Dye P7](image_url)
compound 13: $^1$H

EtO$_2$P-N-\(\text{Ot-Bu}\)

impurity
compound 13: $^{31}\text{P}$
leuco-P8 tert-butyl ester: $^1$H

![NMR Spectra](image_url)
leuco-P8 tert-butyl ester: $^{31}$P

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

Me$_2$N

O=P-OEt

NMe$_2$

O

N

leuco-P8

CO$_2$H
leuco-P8: $^{31}\text{P}$

![Chemical Structure]

leuco-P8
Dye P8: $^1$H

![Chemical Structure of P8](image)

- Me$_2$N
- O
- O=P-OEt
- CF$_3$CO$_2^-$
- NMe$_2$
- NH
- CO

The diagram shows a proton NMR spectrum with peaks labeled at various ppm values.
Dye P8: $^{31}$P
Dye P9: $^1$H

Ma791-2_PROTON_01

$\text{Me}_2\text{N} \quad \text{O} \quad \text{O}^\text{P} \quad \text{Et}$

$\text{P9}$
Dye P9: $^31P$

Me$_2$N

O$_2$

O

PO$_2$-OEt

P9
Dye P10: $^1$H
Dye P10: $^{13}\text{C}^{1\text{H}}$

\[
\text{Me}_2\text{N}^+\text{O}^\circ\text{Pr}\text{CF}_3\text{CO}_2^- \quad \text{P10}
\]
Dye P10: $^{19}$F

![Chemical Structure of Dye P10](image)
Dye P10: $^{31}\text{P}$
Dye P11: $^1$H

![Chemical Structure of P11](image)

The image shows a chemical structure of P11 with various peaks indicating proton signals at different chemical shifts. The peaks are labeled with their corresponding chemical shifts in parts per million (ppm).
Dye P11: $^{13}\text{C}^{1}\text{H}$
Dye P11: gHSQCad
Dye P11: $^{19}$F

\[
\text{Me}_2\text{N}\begin{array}{c}
\text{O} \\
\text{O=P-Ph} \\
\text{Ph} \\
\text{P11}
\end{array}\text{NMe}_2
\]

\[
\text{CF}_3\text{CO}_2^-
\]
Dye P11: $^{31}\text{P}$

![Chemical Structure of P11](image)

**Chart:**

![NMR Spectrum](image)

**Label:**

- **Chemical Formula:**
  - $\text{Me}_2\text{N}$
  - $\text{O}$
  - $\text{NMe}_2$
  - $\text{O} = \text{P} - \text{Ph}$
  - $\text{CF}_3\text{CO}_2^-$
  - $\text{Ph}$
  - $\text{P11}$

**NMR Parameters:**

- **Chemical Shift:**
  - 31 ppm

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leuco-P12: $^1$H

![NMR spectrum of leuco-P12]

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

![NMR Spectrogram](image_url)
leuco-P12: gHSQCad
leuco-P12: $^{19}\text{F}$

leuco-P12
leuco-P12: $^{31}\text{P}$

leuco-P12
Dye P12: $^1$H
Dye P12: $^{19}\text{F}$

![Chemical Structure of P12 with $^{19}\text{F}$](image)

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Dye P12: $^{31}\text{P}$

$\text{shc387-4-iso-2_PHOSPHORUS_01}$

$\text{P12}$
Atto 495 tert-butyl ester: $^{13}\text{C}^{1}\text{H}$

Atto 495 tert-butyl ester
Dye A1: $^1$H
Dye A1: $^{19}\text{F}$
Dye A1: $^{31}\text{P}$

![Chemical Structure of Dye A1](image)
Dye A2: $^1$H

![Chemical Structure of A2]

**Chemical Structure:**
- Me$_2$N
- N
- NMe$_2$
- CF$_3$CO$_2^-$
- O=P=OMe
- OMe

**NMR Spectrogram:**
- Peaks at various chemical shifts indicated.

**Additional Information:**
- AB132 cd3od
- Bukovich / MPI 10200 / PE
Dye A2: $^{13}\text{C}^{1}\text{H}$

\[
\begin{align*}
\text{Me}_2&\text{N} \\
\text{NMe}_2 & \text{CF}_3\text{CO}_2^- \\
\end{align*}
\]
Dye A2: $^{19}\text{F}$
Dye A2: $^{31}$P

\[
\text{Me}_2\text{N} \quad \text{N} \quad \text{NMe}_2 \\
\text{O} = \text{P} - \text{OMe} \\
\text{OMe} \\
\text{A2}
\]
compound 15: $^1$H
compound 15: $^{13}\text{C}^1\text{H}$
Dye SP1: $^1$H
Dye SP1: $^{13}$C{H}

![Chemical Structure](image)

**Chemical Structure**

- Dye SP1
- $^{13}$C{H

**Diagram**

- Spectral analysis showing chemical shifts in ppm
- Peaks at various ppm values
Dye SP1: $^{19}\text{F}$
Dye \textit{SP1}: $^{31}$P
Dye SP2: $^1H$

![Chemical Structure](image)
Dye SP2: $^{13}\text{C}^1\text{H}$

$\text{Me}_2\text{N}$

$\text{O=P-O\text{Me}}$

$\text{NiPr}_2$

$\text{CF}_3\text{CO}_2^-$

SP2

dioxane
Dye SP2: $^{19}$F

\[
\text{Me}_2\text{N} \quad \text{S} \quad \text{NMe}_2 \\
\text{O} = \text{P} \cdot \text{OMe} \quad \text{CF}_3\text{CO}_2^- \\
\text{NiPr}_2 \\
\text{SP2}
\]
Dye BP1: $^1$H

[Chemical Structure Image]

BP1
Dye BP1: $^{13}$C\{H\}
Dye BP1: $^{19}$F

STANDARD FLUORINE PARAMETERS

![Fluorine Spectrum](image)

BP1
Dye BP1: $^{31}\text{P}$

STANDARD PHOSPHORUS PARAMETERS

BP1
Dye CP1: \(^1\text{H}\)

![NMR Spectra and Structure](image)
Dye CP1: APT

![CP1 Chemical Structure]

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Dye CP1: $^{31}$P

![Chemical Structure]
leuco-SiP1: $^1$H

![NMR spectrum of leuco-SiP1](image)
leuco-SiP1: $^{13}\text{C}\left[{^1\text{H}}\right]$

leuco-SiP1
leuco-SiP1: $^{31}$P

![Chemical Structure](Image)

leuco-SiP1
compound 19: $^1$H
compound 19: $^{13}\text{C}^1\text{H}$

[Chemical structure of compound 19]
compound 20: $^1$H
compound 20: $^{13}\text{C}^{(1}\text{H})$
compound 21: $^1$H
compound 21: $^{13}\text{C}^1\text{H}$
compound 22: $^1$H
compound 22: $^{13}\text{C}^1\text{H}$

![](image_url)

$\text{Me}_2\text{N} - \text{NMe}_2$
Dye BA1: $^1$H

Me$_2$N

O=P-O\(_\text{Me}\)

\text{CF}_3\text{CO}_2^-

\text{NiPr}_2

BA1
Dye BA1: $^{13}\text{C}^{1}\text{H}$
Dye BA1: $^{19}$F

![Chemical Structure of BA1]
Dye BA1: $^{31}$P

[Chemical structure image]

BA1