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Supporting Information

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Phosphorylated 3-heteroarylcoumarines and Their Use in Fluorescence Microscopy and Nanoscopy

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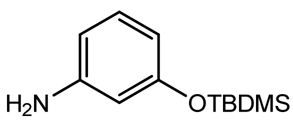
[a] Department of NanoBiophotonics, Max Planck Institute for Biophysical Chemistry, Am Fassberg 11, 37077 Göttingen, Germany
Fax: +49 551 2012505; E-mail: vbelov@gwdg.de, shell @gwdg.de

General remarks

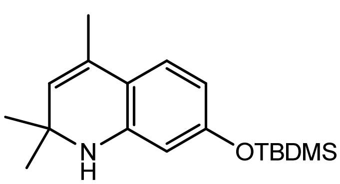
UV-visible absorption spectra were recorded on a Varian Cary 4000 UV-Vis spectrophotometer, and the fluorescence spectra on a Varian Cary Eclipse fluorescence spectrophotometer. Following dyes were used as standards for the determination of the fluorescence quantum yields: Coumarin 6 with $\Phi_{fl.}$ = 0.77 (EtOH) for compound **5**-Et; Coumarin 334 with $\Phi_{fl.}$ = 0.60 (EtOH) for compounds **6**-H, **6**-NHS (antibody conjugates labeled with compound **6**-H), **11**-H,H, **11**-H,NHS (antibody conjugates labeled with compound **11**-H,H), and **21a**; Oxazin 4 with $\Phi_{fl.}$ = 0.63 (MeOH) for compound **7**-H; Atto 425 with $\Phi_{fl.}$ = 0.90 (PBS 7.4) for compound **11**-Bu^t,Bu^t; RDC with $\Phi_{fl.}$ = 0.38 (1,4-dioxane) for compound **12**-Bu^t,Bu^t; Coumarin 307 with $\Phi_{fl.}$ = 0.56 (EtOH) for compound **13**-Cl,Me; Coumarin 522 with $\Phi_{fl.}$ = 0.65 (EtOH) for compounds **20a**, **21c**, **22a**, **22b**, and **22c**. Reactions were carried out upon magnetic stirring in Schlenk flasks equipped with septa or reflux condensers with bubble-counters under argon using a standard manifold with vacuum and argon lines. The MICROTOF spectrometer equipped with ESI ion source Apollo and direct injector with LC autosampler Agilent RR 1200 was used for obtaining high resolution mass spectra (ESI-HRMS). ESI-HRMS were obtained also on APEX IV spectrometer (Bruker). HPLC system (Knauer): Smartline pump 1000 (2×), UV detector 2500, column thermostat 4000 (25 °C), mixing chamber, injection valve with 20 and 100 µL loop for the analytical and preparative columns, respectively; 6-port-3-channel switching valve; analytical column: Eurospher-100 C18, 5 µm, 250×4 mm, 1.1 mL/min; solvent A: water + 0.1 % v/v trifluoroacetic acid (TFA); solvent B: CH_3CN + 0.1 % v/v TFA; detection at 254 nm or as specified. Analytical TLC was performed on MERCK ready-to-use plates with regular silica gel 60 (F_{254}) and UV-detector (unless specified otherwise). Preparative column chromatography was performed on silica gel 60 (40–63 µ) from Macherey-Nagel (Germany). Freeze-drying of the dye solutions in aqueous acetonitrile was perfomed with ALPHA 2- 4 LD plus device with the cooler maintained at –80°C (Martin Christ, Germany). Coupling constants (*J*) are given in Hz. In the DEPT mode, the ^{13}C signals of the methyl (CH_3) and methyne (CH) groups are "positive" (+), while the signals of methylene groups (CH_2) are negative (–).

Table S1. Properties of the lipophilic coumarin dyes in methanol.

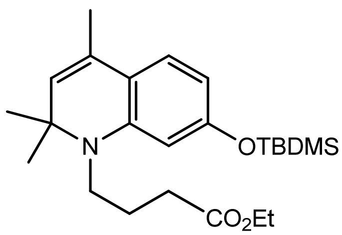
Dye	Absorption λ_{max} (nm)	Emission λ_{max} (nm)	$\varepsilon \times 10^{-5}$ ($M^{-1}cm^{-1}$)	$\Phi_{fl.}$ (%)
5-Et	437	507	0.33	77
17-H	451	535	0.40	8
20a	433	504	0.14	76
11-Bu^t,Et	430	501	0.31	82
21a	431	498	0.32	67
12-Bu^t,Bu^t	516	614	0.56	10



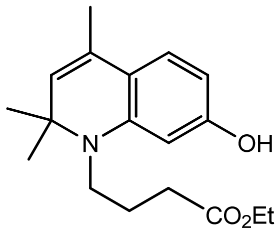
3-Amino-*O*-(*tert*-butyldimethylsilyl)phenol (1-TBDMS):[1] 3-Aminophenol (21.8 g, 0.20 mol) and imidazole (34 g, 0.50 mol) were dissolved in dry DMF (200 mL), the solution was cooled in an ice-water bath, and TBDMSCl (36.1 g, 0.24 mol) was added in one portion. The cooling bath was removed, the reaction mixture was allowed to warm-up to room temperature, and stirred for 1 h. DMF (*ca.* 150 mL) was evaporated *in vacuo* at +55 °C, the residue was diluted with AcOEt (250 mL), washed with sat. aq. $NaHCO_3$ (twice), water (several times), brine and dried over $MgSO_4$. After evaporation of solvents, the oily residue was dried *in vacuo* (0.5 Torr) to a constant weight. Purification by column chromatography (gradient elution with hexane to hexane/ether = 5/1) afforded compound **1**-TBDMS (R_f = 0.24 in hexane/ether = 8/1) as a clear oil (34.1 g, 76%). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.21 (s, 6 H, Si$\underline{Me_2}$$Bu^t$), 0.99 (s, 9 H, $SiMe_2\underline{Bu^t}$) , 3.60 (br. s , 2 H, NH_2) , 6.18 (m, 1 H) , 6.25 (m , 2 H), 6.98 (m, 1 H) ppm.



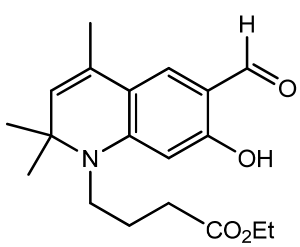
7-(*tert*-Butyldimethylsilyl)oxy-1,2-dihydro-2,2,4-trimethylquinoline (2): Anhydrous ytterbium(III) triflate (4.2 g, 6.8 mmol, 6.5% mol %, freshly dried *in vacuo* at 130 °C for 4 h) was added in one portion to a solution of compound **1**-TBDMS (23.3 g, 0.105 mol) in dry acetone (300 mL). The reaction mixture was stirred for 16 h at room temperature. Acetone was evaporated *in vacuo*, the residue was dissolved in AcOEt, washed with sat. aq. $NaHCO_3$ (twice), water, brine and dried over $MgSO_4$. After evaporation of solvents, the oily residue was dried *in vacuo* (0.5 Torr) to a constant weight. Purification by column chromatography (gradient elution with hexane to hexane/ether = 10/1) afforded compound **2** (R_f = 0.86, hexane/ether = 8/1) as a clear oil (19.85 g, 63% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.20 (s, 6 H, Si<u>Me</u>$_2$Bu^t), 0.99 (s, 9 H, SiMe$_2$<u>Bu</u>^{t}), 1.27 (s, 6 H, 2×Me), 1.97 (d, *J* = 1.2, 3 H, Me), 3.63 (br. s, 1 H, NH), 5.20 (q, *J* = 1.2, 1 H, <u>H</u>C=), 5.99 (d, *J* = 2.4, 1 H) , 6.15 (dd, *J* = 8.2 and 2.4, 1 H), 6.92 (d, *J* = 8.2, 1 H) ppm. ^{13}C NMR (125.7 MHz, $CDCl_3$): δ = -4.4, 18.2, 18.6, 25.7, 30.9, 51.9, 104.7, 108.9, 115.9, 124.5, 126.1, 128.3, 144.5, 156.1 ppm. MS (ESI): *m/z* (negative mode, rel. int., %) = 302.2 (100), $[M–H]^-$; HRMS ($C_{18}H_{29}NOSi$): 302.1940 (found $[M–H]^-$), 302.1946 (calc.); *m/z* (positive mode, rel. int., %) = 605.5 (5) $[2M+H]^+$, 326.2 (10) $[M+Na]^+$, 304.2 (100) $[M+H]^+$; HRMS ($C_{18}H_{29}NOSi$): 304.2096 (found $[M+H]^+$), 304.2091 (calc.).



Ethyl [7-(*tert*-butyldimethylsilyl)oxy-1,2-dihydro-2,2,4-trimethylquinoline]-1-butanoate (3-TBDMS,Et): *N,N*-diisopropyl-*N*-ethyl amine (DIEA, 11.8 g, 91.7 mmol) was added to a mixture of compound **3** (13.9 g, 45.9 mmol) and ethyl 3-iodobutyrate (13.3 g, 55.1 mmol) placed in a screw-cup bottle, and the reaction mixture was stirred with heating (+110 °C) for 2 days. After cooling, it was diluted with diethyl ether, passed through a plug of silica gel (eluting with ether), and the filtrate evaporated *in vacuo*. The residue was dissolved in hexane/ether (3/1) mixture, washed with water, brine and dried over $MgSO_4$. The product **3**-TBDMS,Et was isolated by a short path column chromatography (hexane→hexane/ether 10/1; R_f = 0.59 in hexane/ether = 10/1); yield 18.8 g (98%) of a clear oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.21 (s, 6 H, Si<u>Me</u>$_2$Bu^t), 0.99 (s, 9 H, SiMe$_2$<u>Bu</u>^{t}), 1.27 (t, *J* = 7.2, 3 H, CO$_2$CH$_2$C<u>H</u>$_3$), 1.28 (s, 6 H, 2×Me), 1.91 (m, 2 H, NCH$_2$C<u>H</u>$_2$CH$_2$CO$_2$Et), 1.94 (d, *J* = 1.2, 3 H, Me), 2.38 (m, 2 H, NCH$_2$CH$_2$C<u>H</u>$_2$CO$_2$Et), 3.21 (m, 2 H, NC<u>H</u>$_2$CH$_2$CH$_2$CO$_2$Et), 4.16 (q, *J* = 7.2, 2 H, CO$_2$C<u>H</u>$_2$CH$_3$), 5.11 (q, *J* = 1.2, 1 H, <u>H</u>C=), 6.03 (d, *J* = 2.4, 1 H), 6.12 (dd, *J* = 8.2 and 2.4, 1 H), 6.90 (d, *J* = 8.2, 1 H) ppm. ^{13}C NMR (125.7 MHz, $CDCl_3$): δ = -4.3, 14.3, 18.3, 18.7, 23.5, 25.8, 28.2, 31.8, 43.3, 56.8, 60.4, 103.1, 107.1, 117.2, 124.4, 127.1, 127.6, 145.0, 156.4, 173.1 ppm. MS (ESI): *m/z* (positive mode, rel. int., %) = 857.5 (45) $[2M+H]^+$, 440.3 (100) $[M+Na]^+$, 418.3 (51) $[M+H]^+$; HRMS ($C_{24}H_{39}NO_3Si$): 440.2598 (found $[M+Na]^+$), 440.2591 (calc.); 418.2767 (found $[M+H]^+$), 418.2772 (calc.).

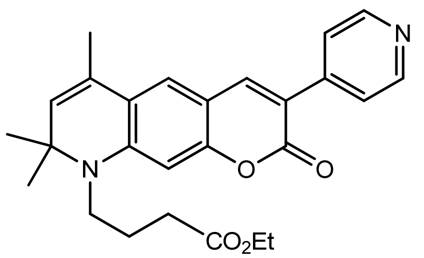


Ethyl (1,2-dihydro-7-hydroxy-2,2,4-trimethylquinoline)-1-butanoate (3-H,Et): A solution of TBAF·3H_2O (7.66 g, 24.3 mmol, 0.6 eq) in THF (50 mL) was added to a solution of ester **3**-TBDMS,Et (16.9 g, 40.5 mmol) in THF (60 mL) at +5 °C. After 5 min, the reaction mixture was diluted with ether (200 mL), washed with water (2×) and brine. The combined aqueous layers were extracted with ether, and the combined organic solutions were dried ($MgSO_4$). After evaporation of solvents, the residue was purified by column chromatography (ether/hexane, 2/1→4/1). Phenol **3**-H,Et (R_f = 0.07 in hexane/ether, 1/5) was isolated in 99% yield (12.2 g). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.27 (s, 6 H, 2×Me), 1.28 (t, J = 7.2, 3 H, $CO_2CH_2C\underline{H}_3$), 1.92 (m, 2 H, $NCH_2C\underline{H}_2CH_2CO_2Et$), 1.93 (d, J = 1.2, 3 H, Me), 2.39 (m, 2 H, $NCH_2CH_2C\underline{H}_2CO_2Et$), 3.20 (m, 2 H, $NC\underline{H}_2CH_2CH_2CO_2Et$), 4.18 (q, J = 7.2, 2 H, $CO_2C\underline{H}_2CH_3$), 5.08 (q, J = 1.2, 1 H, $\underline{H}C$=), 6.13 (m, 2 H), 6.54 (bs, 1 H, OH), 6.90 (d, J = 8.6, 1 H) ppm. ^{13}C NMR (100.7 MHz, $CDCl_3$): δ = 14.2, 18.7, 23.4, 28.3, 31.6, 43.4, 56.8, 60.8, 98.3, 102.4, 116.2, 124.8, 126.5, 127.6, 145.3, 156.9, 174.0 ppm. MS (ESI): m/z (negative mode, rel. int., %) = 605.4 (100), $[2M–H]^-$, 302.2 (63), $[M–H]^-$; m/z (positive mode, rel. int., %) = 629.4 (100) $[2M+Na]^+$, 326.2 (79) $[M+Na]^+$, 304.2 (28) $[M+H]^+$; HRMS ($C_{18}H_{25}NO_3$): 326.1727 (found $[M+Na]^+$), 326.1727 (calc.); 304.1905 (found $[M+H]^+$), 304.1907 (calc.).

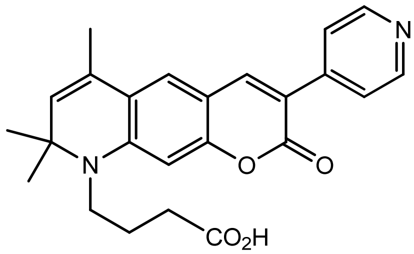


Ethyl (1,2-dihydro-6-formyl-7-hydroxy-2,2,4-trimethylquinoline)-1-butanoate (4-Et): $POCl_3$ (3.90 g, 25.5 mmol) was added to DMF (30 mL) at +5°C, and the mixture was allowed to warm to room temperature. After stirring for 15 min at room temperature, it was cooled down (+5 °C), and a solution of phenol **3**-H,Et (5.15 g, 17.0 mmol) in DMF (15 mL) was added slowly. The cooling bath was removed, and the reaction mixture was allowed to warm up to room temperature, stirred for 0.5 h, and finally heated at 50 °C for 15 min. The TLC control of this reaction is difficult, because the product was found to have the same R_f value, as the starting material (in most solvent systems). After cooling, the reaction was "quenched" by adding 1 mL of sat. aq. $NaHCO_3$, and the product **4**-Et was extracted with dichloromethane. The organic layer was separated, dried ($MgSO_4$), and, after evaporation of solvents, the residue was purified by column chromatography (gradient elution with hexane/ether mixture, 1/1 to 1/4).

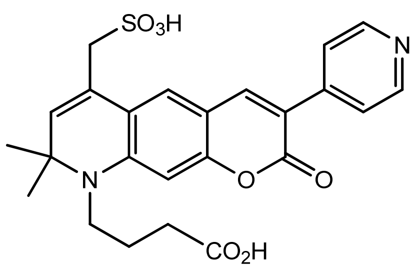
Yield 3.59 g (64 %). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.29 (t, *J* = 7.2, 3 H, $CO_2CH_2C\underline{H}_3$), 1.31 (s, 6 H, 2×Me), 1.93 (m, 2 H, $NCH_2C\underline{H}_2CH_2CO_2Et$), 1.96 (d, *J* = 1.2, 3 H, Me), 2.40 (m, 2 H, $NCH_2CH_2C\underline{H}_2CO_2Et$), 3.33 (m, 2 H, $NC\underline{H}_2CH_2CH_2CO_2Et$), 4.18 (q, *J* = 7.2, 2 H, $CO_2C\underline{H}_2CH_3$), 5.19 (q, *J* = 1.2, 1 H, $\underline{H}C$=), 5.98 (s, 1 H, Ar), 7.03 (s, 1 H, Ar), 9.48 (s, 1 H, HC=O), 11.76 (s, 1 H, OH) ppm. ^{13}C NMR (100.7 MHz, $CDCl_3$): δ = 14.2(+), 18.6(+), 22.8(-), 29.3(+), 31.4(-), 44.0(-), 58.3(q), 60.7(-), 96.4(+), 110.9(q), 115.9(q), 126.1(q), 127.4(+), 128.1(+), 151.1(q), 164.7(q), 172.7(q), 191.9(+) ppm. MS (ESI): *m/z* (negative mode, rel. int., %) = 661.3 (10), $[2M–H]^-$, 330.2 (100), $[M-H]^-$; HRMS ($C_{19}H_{25}NO_4$): 330.1715 (found $[M–H]^-$), 330.1711 (calc.); *m/z* (positive mode, rel. int., %) = 685.4 (100) $[2M+Na]^+$, 354.2 (34) $[M+Na]^+$, 332.2 (76) $[M+H]^+$; HRMS ($C_{19}H_{25}NO_4$): 354.1671 (found $[M+Na]^+$), 354.1676 (calc.); 332.1859 (found $[M+H]^+$), 332.1856 (calc.).



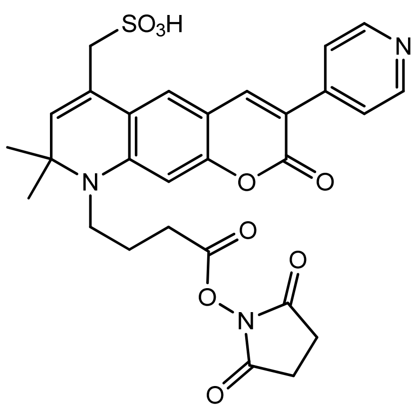
Ethyl [8,9-dihydro-6,8,8-trimethyl-3-(pyridin-4-yl)-2*H*-pyrano[3,2-*g*]quinolin-2-one]-9-butanoate (5-Et): 4-Pyridylacetic acid hydrochloride (1.79 g, 10.3 mmol) was added with stirring to a solution of compound **4-Et** (3.41 g, 10.3 mmol) in dichloromethane (50 mL). Then Et_3N (2.08 g, 20.6 mmol) was added, and, after 10 min, *N,N´*-dicyclohexylcarbodiimide (2.12 g, 10.3 mmol) and 4-dimethylaminopyridine (126 mg, 1.03 mmol) were added. The reaction mixture was stirred for 48 h, and filtered through a plug of silica gel using dichloromethane/ether mixture (2/1) as an eluent. After evaporation of solvents, product **5**-Et was isolated by column chromatography (eluting with dichloromethane to dichloromethane/ether mixture, 2/1). After evaporation of solvents, compound **5**-Et was precipitated from ether with hexane. Yield: 2.44 g (52 %) of yellow crystals. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.27 (t, *J* = 7.2, 3 H, $CO_2CH_2C\underline{H}_3$), 1.38 (s, 6 H, 2×Me), 1.93 (m, 2 H, $NCH_2C\underline{H}_2CH_2CO_2Et$), 1.98 (d, *J* = 1.2, 3 H, Me), 2.40 (m, 2 H, $NCH_2CH_2C\underline{H}_2CO_2Et$), 3.33 (m, 2 H, $NC\underline{H}_2CH_2CH_2CO_2Et$), 4.18 (q, *J* = 7.2, 2 H, $CO_2C\underline{H}_2CH_3$), 5.30 (q, *J* = 1.2, 1 H, $\underline{H}C$=), 6.38 (s, 1 H, Ar), 7.08 (s, 1 H, Ar), 7.65 (m, 2 H, AA´ part of AA´BB´ system), 7.82 (s, 1H, Ar), 8.60 (m, 2 H, BB´ part of AA´BB´ system) ppm. ^{13}C NMR (125.7 MHz, $CDCl_3$): δ = 14.3(+), 18.8(+), 22.7(-), 29.3(+), 31.4(-), 44.1(-), 58.2(q), 60.8(-), 96.5(+), 108.6(q), 117.1(q), 120.5(q), 122.1(+), 122.6(+), 127.4(+), 125.9(q), 129.9(+), 141.8(+), 143.2 (q), 148.1(q), 149.7(+), 156.5(q), 160.6(q), 172.6(q) ppm. MS (ESI): *m/z* (positive mode, rel. int., %) = 865.5 (45) $[2M+H]^+$, 433.3 (100) $[M+H]^+$; HRMS ($C_{26}H_{28}N_2O_4$): 433.2124 (found $[M+H]^+$), 433.2122 (calc.). λ_{abs} = 437 nm, λ_{em} = 507 nm, ε = 33400 $M^{-1}cm^{-1}$, $\Phi_{fl.}$ = 0.77 (MeOH); Coumarin 6 ($\Phi_{fl.}$ = 0.77 in EtOH) as a standard.



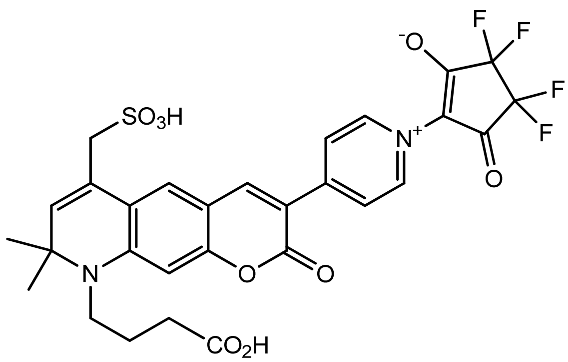
[8,9-Dihydro-6,8,8-trimethyl-3-(pyridin-4-yl)-2*H*-pyrano[3,2-*g*]quinolin-2-one]-9-butanoic acid (5-H): 1 M aq. NaOH (5.4 mL, 5.4 mmol) was added to a warmed (50 °C) solution of coumarin **5**-Et (1.54 g, 3.56 mmol) in THF / MeOH mixture (100 mL / 100 mL), and the reaction mixture was stirred at this temperature until saponification was complete (1 h; TLC-control). Volatile solvents (MeOH, THF) were evaporated *in vacuo*, the residue was dissolved in water (30 mL), and neutralized with 6 M aq. HCl to pH = 6.0. Then NEt_3 (2 mL) was added, the mixture was evaporated to dryness *in vacuo,* and the product was isolated by column chromatography on a regular silica gel with acetonitrile / water (4 / 1) mixture, giving 1.24 g (86% yield) of the title acid. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 1.34 (s, 6 H, 2×Me), 1.73 (m, 2 H, $NCH_2C\underline{H_2}CH_2CO_2H$), 1.93 (d, *J* = 1.2, 3 H, Me), 2.38 (m, 2 H, $NCH_2CH_2C\underline{H_2}CO_2H$), 3.34 (m, 2 H, $NC\underline{H_2}CH_2CH_2CO_2H$), 5.42 (d, *J* = 1.2, 1 H, <u>H</u>C=), 6.60 (s, 1 H, Ar), 7.30 (s, 1 H, Ar), 7.73 (m, 2 H, AA' part of AA'XX' system), 8.32 (s, 1 H, Ar), 8.54 (m, 2 H, XX' part of AA'XX' system) ppm. ^{13}C NMR (125.7 MHz, DMSO-d_6): δ = 18.7(+), 22.9(-), 29.3(+), 31.0(-), 43.9(-), 58.4(q), 96.3(+), 108.7(q), 110.0(q), 115.6(q), 120.0(+), 122.3(+), 123.7(q), 125.7(+), 130.5(q), 143.3(+), 148.6(q), 150.0(+), 156.8(q), 160.2(q), 174.9(q) ppm. MS (ESI): *m/z* (negative mode, rel. int., %) = 403.2 (100), $[M–H]^–$; HRMS ($C_{24}H_{24}N_2O_4$): 403.1664 (found $[M–H]^–$), 403.1663 (calc.); *m/z* (positive mode, rel. int., %) = 427.2 (40) $[M+Na]^+$, 405.2 (100) $[M+H]^+$; HRMS ($C_{24}H_{24}N_2O_4$): 427.1611 (found $[M+Na]^+$), 427.1628 (calc.); 405.1799 (found $[M+H]^+$), 405.1809 (calc.).



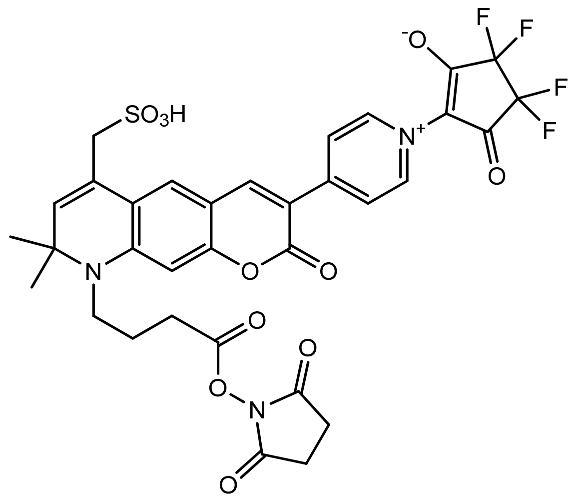
[8,9-Dihydro-8,8-dimethyl-3-(pyridin-4-yl)-6-sulfomethyl-2*H*-pyrano[3,2-*g*]-quinolin-2-one]-9-butanoic acid (6-H): 98% H_2SO_4 (2 mL) was added to acid **5**-H (190 mg, 0.47 mmol). The reaction mixture was heated at 35 °C with stirring for 16 h. After cooling, it was added dropwise to the stirred ether/dioxane mixture (100mL/10 mL). The precipitate was separated by decantation, washed with ether and dried in a flow of nitrogen. The solid material was dissolved in acetone/acetonitrile/water (2.5/2.5/1) mixture, and a red solution was obtained. It was neutralized with a saturated aq. solution of Na_2HPO_4, until a green-yellow color appeared. The lower layer (aqueous solution of inorganic salts) was discarded, and the organic solution was subjected to reversed phase chromatography on Polygogrep 60-50 C_{18} (50 g). The column was eluted with CH_3CN/water (4/1) mixture. The fractions containing the first fluorescent compound were evaporated, and the residue was purified additionally by column chromatography on a regular silica gel (acetone/CH_3CN/water; 2.5 /2.5 / 1; the sulfonated product **6**-H had R_f = 0.75, while the starting acid **5**-H - R_f = 0.82). After evaporation of solvents, the residue was precipitated from acetone giving compound **6**-H (101 mg, 44%, HPLC area 97%). For NMR measurements, a sample of compound **6**-H was dissolved in NEt_3 and evaporated *in vacuo*. ^{1}H NMR (300 MHz, DMSO-d$_6$): δ = 1.10 (t, *J* = 7.0, CH_3 in NEt_3), 1.38 (s, 6 H, 2×Me), 1.79 (m, 2 H, $NCH_2C\underline{H_2}CH_2CO_2H$), 2.41 (m, 2 H, $NCH_2CH_2C\underline{H_2}CO_2H$), 2.90 (q, *J* = 7.0, CH_2N in NEt_3), 3.40 (m, 2 H, $NC\underline{H_2}CH_2CH_2CO_2H$), 3.52 (s, 2H), 5.58 (s, 1 H, $\underline{H}C$=), 6.60 (s, 1 H, Ar), 7.62 (s, 1 H, Ar), 7.80 (m, 2 H, AA' part of AA'XX' system), 8.30 (s, 1 H, Ar), 8.56 (m, 2 H, XX' part of AA'XX' system) ppm. ^{13}C NMR (125.7 MHz, DMSO-d$_6$): δ = 9.5(+, CH_3 in NEt_3), 22.5(-), 28.6(+), 30.5(-), 43.5 (-), 45.7(-, CH_2 in NEt_3), 53.5(-), 57.9(q), 95.6(+), 108.0(q), 114.9(q), 119.0(q), 121.8(+), 124.2(q), 124.9(+), 132.9(+), 142.8(q), 142.9(+), 148.5(q), 149.4(+), 155.9(q), 159.8(q), 174.3(q) ppm. MS (ESI): *m/z* (negative mode, rel. int., %) = 483.1 (100), $[M{-}H]^-$; HRMS ($C_{24}H_{24}N_2O_7S$): 483.1233 (found $[M{-}H]^-$), 483.1231 (calc.); *m/z* (positive mode, rel. int., %) = 507.1 (100) $[M+Na]^+$, 485.1 (36) $[M+H]^+$; HRMS ($C_{24}H_{24}N_2O_7S$): 507.1185 (found $[M+Na]^+$), 507.1196 (calc.); 485.1364 (found $[M+H]^+$), 485.1377 (calc.). HPLC: t_R = 9.4 min (B/A = 20/80 – 50/50 in 25 min, column 4×250 mm, 1.2 mL/min, detection at 433 nm). λ_{abs} = 436 nm, λ_{em} = 510 nm, ε = 25900 $M^{-1}cm^{-1}$, $\Phi_{fl.}$ = 0.56 (MeOH); λ_{abs} = 436 nm, λ_{em} = 515 nm, ε = 31200 $M^{-1}cm^{-1}$, $\Phi_{fl.}$ = 0.57 (in aqueous PBS buffer at pH 7.4). Standard: Coumarin 334, $\Phi_{fl.}$ = 0.69 (EtOH).



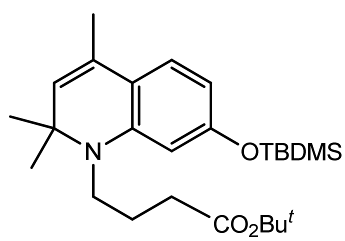
***N*-Succinimidyl [8,9-dihydro-8,8-dimethyl-3-(pyridin-4-yl)-6-sulfomethyl-2*H*-pyrano-[3,2-*g*]quinolin-2-one]-9-butanoate (6-NHS):** acid **6**-H (4.8 mg, 10 μmol) was dissolved in DMF (0.5 mL), *N*-hydroxysuccinimid (3.3 mg, 20 μmol) was added at room temperature followed by HATU (7.6 mg, 20 μmol) and NEt_3 (3.0 mg, 30 μmol). The reaction mixture was stirred at room temperature for 16 h. After that, it was subjected to chromatography on a regular silica gel without any additional work-up (with acetone to acetone / acetonitrile / water = 4 / 4 / 1 as an eluent), and 2.9 mg (50% yield) of the title compound as an orange solid was isolated. MS (ESI): *m/z* (negative mode, rel. int., %) = 580.1 (90), $[M{-}H]^-$; HRMS ($C_{28}H_{27}N_3O_9S$): 580.1387 (found $[M{-}H]^-$), 580.1395 (calc.). HPLC: t_R = 12.2 min (B/A = 20/80 – 50/50 in 25 min, column 4×250 mm, 1.2 mL/min, detection at 433 nm). The conjugate with sheep anti-mouse IG had the following properties in PBS buffer at pH 7.4: λ_{abs} = 437 nm, λ_{em} = 512 nm, $\Phi_{fl.}$ = 0.18, degree of labeling (DOL) = 2.7. Standard: Coumarin 334, $\Phi_{fl.}$ = 0.69 (EtOH).



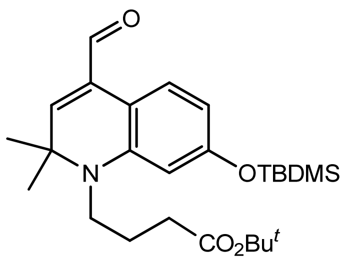
(9-Carboxypropyl-8,9-dihydro-8,8-dimethyl-6-sulfomethyl-2*H*-pyrano[3,2-*g*]quinolin-2-one)-3-[4-(pyrid-1-ium)-(3´,3´,4´,4´-tetrafluoro-2´-oxido-5´-oxo-1-cyclopentene-1-yl) betaine] (7-H): Perfluorocyclopentene (50 μL) was added to a cold (ice-water bath) solution of compound **6**-H (4.8 mg, 0.1 mmol) in aqueous *i*-PrOH (95% v/v) in a screw-cap bottle. The reaction vessel was closed, and the mixture was heated at 70 °C for 2 h. After cooling, all volatile materials were evaporated *in vacuo,* and the residue was purified by column chromatography (acetonitrile/water = 4/1) afforded a title compound as a red solid: 3.1 mg (47% yield). ^{1}H NMR (300 MHz, DMSO-d$_6$): δ = 1.40 (s, 6 H, 2×Me), 1.80 (m, 2 H, $NCH_2C\underline{H_2}CH_2CO_2H$), 2.40 (m, 2 H, $NCH_2CH_2C\underline{H_2}CO_2H$), 3.30 (m, 2 H, $NC\underline{H_2}CH_2CH_2CO_2H$), 5.62 (s, 1 H, $\underline{H}$C=), 6.72 (s, 1 H, Ar), 7.72 (s, 1 H, Ar), 8.65 (m, 2 H, AA' part of AA'XX' system), 8.81 (s, 1H, Ar), 9.08 (m, 2 H, XX' part of AA'XX' system) ppm. ^{13}C NMR (125.7 MHz, DMSO-d$_6$, signals of perfluorocyclopentadione residue were not detected): δ = 22.7(-), 28.8(+), 30.9(-), 44.1(-), 53.5(-), 58.8(q), 95.7(+), 108.7(q), 109.6(q), 119.5(q), 122.6(+), 123.9(q), 126.0(+), 133.4(+), 141.0(+), 146.5(+), 150.4(q), 150.8(q), 157.4(q), 159.3(q), 171.5(q) ppm. ^{19}F NMR (282.7 MHz, CDCl$_3$): δ = 126.0. MS (ESI): *m/z* (negative mode, rel. int., %) = 651.1 (100), [M–H]$^-$; HRMS ($C_{29}H_{24}F_4N_2O_9S$): 651.1067 (found [M–H]$^-$), 651.1066 (calc.). HPLC: t_R = 18.27 min (B/A = 20/80 – 50/50 in 25 min, column 4×250 mm, 1.2 mL/min, detection at 552 nm). λ_{abs} = 526 nm, λ_{em} = 629 nm, ε = 45500 M^{-1}cm^{-1}, $\Phi_{fl.}$ = 0.09 (MeOH). Standard: Oxazin 4, $\Phi_{fl.}$ = 0.63 (MeOH).



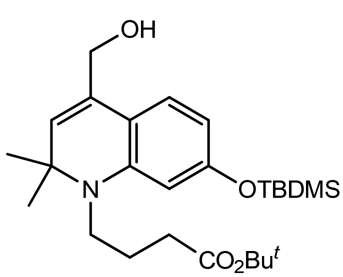
(8,9-Dihydro-8,8-dimethyl-9-(*N*-succinimidyl)oxycarbonylpropyl-6-sulfomethyl-2*H*-pyrano[3,2-*g*]-quinolin-2-one)-3-[4-(pyridin-1-ium)-(3´,3´,4´,4´-tetrafluoro-2´-oxido-5´-oxo-1-cyclopentene-1-yl) betaine] (7-NHS): The title compound was obtained according to procedure described for **6**-NHS. From the acid 7-H (3.1 mg, 4.75 μmol) NHS-ester **7**-NHS was obtained in 71% yield (2.5 mg). MS (ESI): *m/z* (negative mode, rel. int., %) = 748.1 (100), $[M–H]^–$; HRMS ($C_{33}H_{27}F_4N_3O_{11}S$): 748.1228 (found $[M–H]^–$), 748.1230 (calc.). HPLC: t_R = 21.2 min (B/A = 20/80 – 50/50 in 25 min, column 4×250 mm, 1.2 mL/min, detection at 552 nm).



***tert*-Butyl [7-(*tert*-butyldimethylsilyl)oxy-1,2-dihydro-2,2,4-trimethylquinoline]-1-butanoate (3-TBDMS,Bu^t):** *N,N*-Diisopropyl-*N*-ethylamine (7.80 g, 60.5 mmol) was added to a mixture of compound **2** (9.17 g, 30.3 mmol) and freshly prepared *t*-butyl 3-iodobutyrate (9.8 g, 39.3 mmol),[2] which was additionally purified by column chromatography and placed into a screw-cap bottle. The reaction mixture was stirred at 110°C (bath temp.) for 2 days. After cooling, it was diluted with diethyl ether, passed through a plug of silica gel (eluting with ether), and the solvents were evaporated *in vacuo*. The residue was dissolved in a hexane/ether (3/1) mixture, washed with sat. aq. $NaHCO_3$, and the solution was passed again through a plug of silica gel (eluting with hexane/ether (3/1) mixture). According to TLC (hexane/ether = 10/1), two compounds were detected in the eluate: compound **2** (R_f = 0.48) and compound **3**-TBDMS,Bu^t (R_f = 0.43). After evaporation of solvents *in vacuo,* the oily residue was distilled in the Kugelrohr distillation apparatus. The compound **3**-TBDMS,Bu^t (9.20 g, 68% yield) was collected at 180 °C (0.6 Torr). The first fraction (2.75 g, b. p. 150 °C at 0.6 Torr) was shown to be compound **2** (ca. 95% pure; 29% recovered), and the second one (0.21 g, b. p. 150–180 °C at 0.6 Torr) – a mixture of compounds **2** and **3**-TBDMS,Bu^t in *ca.* 1/3 ratio. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.19 (s, 6 H, Si$\underline{Me_2}$Bu^t), 0.97 (s, 9 H, $SiMe_2\underline{Bu^t}$), 1.26 (s, 6 H, 2×Me), 1.43 (s, 9 H, $CO_2\underline{Bu^t}$), 1.83 (m, 2 H, $NCH_2C\underline{H_2}CH_2CO_2Bu^t$), 1.92 (d, *J* = 1.2, 3 H, Me), 2.27 (m, 2 H, $NCH_2CH_2C\underline{H_2}CO_2Bu^t$), 3.17 (m, 2 H, $NC\underline{H_2}CH_2CH_2CO_2Bu^t$), 5.08 (d, *J* = 1.2, 1 H, $\underline{H}$C=), 6.02 (d, *J* = 1.8, 1 H), 6.09 (dd, *J* = 8.4 and 1.8, 1H), 6.88 (d, *J* = 8.4, 1 H) ppm. ^{13}C NMR (125.7 MHz, $CDCl_3$): δ = -4.2(+), 18.3(q), 18.8(+), 23.8(-), 25.8(+), 28.2(+), 28.3(+), 33.1(-), 43.4(-), 56.8(-), 80.3(q), 103.9(+), 107.0(+), 117.1(q), 124.3(+), 127.0(+), 127.5(q), 145.0(q), 156.3(q), 172.3(q) ppm. MS (ESI): *m/z* (positive mode, rel. int., %) = 468.3 (6) $[M+Na]^+$, 446.3 (100) $[M+H]^+$; HRMS ($C_{26}H_{43}NO_3Si$): 468.2891 (found $[M+Na]^+$), 468.2904 (calc.); 446.3082 (found $[M+H]^+$), 446.3085 (calc.).

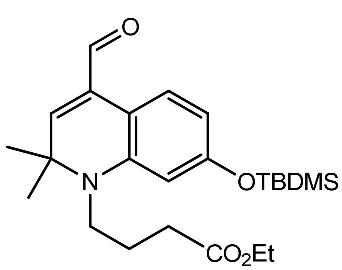


***tert*-Butyl [7-(*tert*-butyldimethylsilyl)oxy-1,2-dihydro-4-formyl-2,2-dimethylquinoline]-1-butanoate (8-TBDMS,Bu^t):** SeO_2 (88 mg, 0.793 mmol) was added to a hot (70°C) solution of compound **3**-TBDMS,Bu^t (255 mg, 0.573 mmol) in dioxane (15 mL). Then the reaction mixture was stirred at this temperature for 0.5 h. After cooling, it was diluted with ether, and the organic layer was washed with sat. aq. $NaHCO_3$ and dried over $MgSO_4$. Solvents were evaporated, and the residue was purified twice by column chromatography (the first one with Et_2O and the second one with hexane/ether/dichloromethane [10/3/1] as an eluent). In hexane/ether = 10/1 mixture the starting compound **3**-TBDMS,Bu^t showed R_f = 0.84, while the product **8**-TBDMS,Bu^t has R_f = 0.42. Yield 81 mg (31%) of light yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.21 (s, 6 H, Si$\underline{Me_2}$Bu^t), 0.99 (s, 9 H, $SiMe_2Bu^t$), 1.41 (s, 6 H, 2×Me), 1.46 (s, 9 H, $CO_2\underline{Bu^t}$), 1.85 (m, 2 H, $NCH_2C\underline{H_2}CH_2CO_2Et$), 2.29 (m, 2 H, $NCH_2CH_2C\underline{H_2}CO_2Et$), 3.23 (m, 2 H, $NC\underline{H_2}CH_2CH_2CO_2Et$), 6.05 (s, 1 H, $\underline{H}$C=), 6.14 (d, *J* = 2.4, 1 H), 6.20 (dd, *J* = 2.4 and 8.4), 8.07 (d, *J* = 8.4, 1 H), 9.57 (s, 1 H, C$\underline{H}$O) ppm. ^{13}C NMR (125.7 MHz, $CDCl_3$): δ = -4.3, 18.3, 23.6, 25.7, 27.0, 28.1, 32.8, 43.4, 57.0, 80.4, 104.2, 108.4, 111.1, 127.0, 132.4, 145.2, 149.6, 157.6, 172.3, 192.4 ppm. MS (ESI): *m/z* (positive mode, rel. int., %) = 941.6 (55) $[2M+Na]^+$, 482.3 (65) $[M+Na]^+$, 460.3 (100) $[M+H]^+$; HRMS ($C_{26}H_{41}NO_4Si$): 460.29 (found $[M+H]^+$), 460.2878 (calc.).



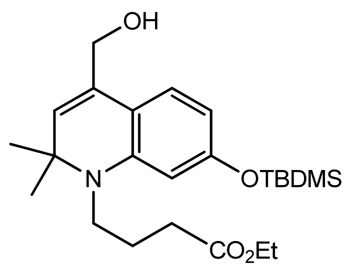
***tert*-Butyl [7-(*tert*-butyldimethylsilyl)oxy-1,2-dihydro-4-hydroxymethyl-2,2-dimethylquinoline]-1-butanoate (9-H,TBDMS,Bu^t):** SeO_2 (99.9%, 1.50 g, 13.5 mmol) was added in portions to a hot (60°C) solution of **3**-TBDMS,Bu^t (3.24 g, 7.28 mmol) in dioxane (50 mL). Then the reaction mixture was stirred at this temperature for 1 h. After cooling, it was diluted with ether, and the organic layer was washed with sat. aq. $NaHCO_3$ and dried over $MgSO_4$. Solvents were evaporated, and the residue was purified by column chromatography (with hexane/ether/dichloromethane [10/3/1] as an eluent). After evaporation of the solvents, the crude compound **8**-TBDMS,Bu^t (1.3 g; the preparation and spectra are given above) was dissolved in THF/MeOH (20 mL / 1 mL), and $NaBH_4$ (100 mg, 2.6 mmol) was added in portions at room temp. After 5 min, the reaction was complete (TLC). The excess of $NaBH_4$ was destroyed by adding acetone (0.5 mL); all volatile materials were evaporated *in vacuo,* and alcohol **9**-H,TBDMS,Bu^t was isolated by column chromatography (with hexane/ether/dichloromethane/MeOH mixture (10/3/4/0.3) as an eluent; R_f = 0.42); yield 0.745 g (22%) of light yellow oil.

If the corresponding aldehyde was isolated after oxidation with SeO_2 (see above), it could be reduced according to the following method: $NaBH_4$ (5.8 mg, 0.15 mmol) was added in one portion to a cold (5°C) solution of aldehyde (70 mg, 0.15 mmol) in THF / MeOH = 3mL / 1mL. The reaction was stirred for 5 min at RT, several drops of acetone were added and the reaction mixture was evaporated to dryness in vacuo. After column chromatography, the alcohol (66 mg, 94% yield) was obtained as light yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.21 (s, 6 H, Si$\underline{Me_2}$Bu^t), 0.99 (s, 9 H, SiMe$_2$Bu^t), 1.31 (s, 6 H, 2×Me), 1.42 (t, J = 6.0, 1 H, CH$_2$O$\underline{H}$), 1.46 (s, 9 H, CO$_2\underline{Bu^t}$), 1.85 (m, 2 H, NCH$_2$C$\underline{H_2}$CH$_2$CO$_2$Bu^t), 2.29 (m, 2 H, NCH$_2$CH$_2$C$\underline{H_2}$CO$_2$Bu^t), 3.20 (m, 2 H, NC$\underline{H_2}$CH$_2$CH$_2$CO$_2$Bu^t), 4.43 (m, 2 H, C$\underline{H_2}$OH), 5.35 (t, J = 1.2, 1 H, $\underline{H}$C=), 6.08 (d, J = 2.4, 1 H), 6.11 (dd, J = 2.4 and 8.4), 6.98 (d, J = 8.4, 1 H) ppm. ^{13}C NMR (125.7 MHz, $CDCl_3$): δ = -4.3, 18.3, 23.7, 25.7, 28.05, 28.1, 32.9, 43.4, 56.6, 63.2, 80.3, 103.6, 107.4, 114.2, 124.0, 127.0, 131.2, 145.4, 156.8, 172.4 ppm. MS (ESI): *m/z* (positive mode, rel. int., %) = 945.6 (27) [2M+Na]$^+$, 484.3 (20) [M+Na]$^+$, 462.3 (100) [M+H]$^+$; HRMS ($C_{26}H_{43}NO_4Si$): 484.2850 (found [M+Na]$^+$), 484.2854 (calc.); 462.3039 (found [M+H]$^+$), 462.3034 (calc.).



Ethyl [7-(*tert*-butyldimethylsilyl)oxy-1,2-dihydro-4-formyl-2,2-dimethylquinoline]-1-butanoate (8-TBDMS,Et): SeO_2 (33 mg, 0.3 mmol) was added to a hot (65°C) solution of **3**-TBDMS,Et (100 mg, 0.24 mmol) in dioxane (5 mL). Then the reaction mixture was stirred at this temperature for 0.5 h. After cooling, it was diluted with ether, and the organic layer was washed with sat. aq. $NaHCO_3$ and dried over $MgSO_4$.

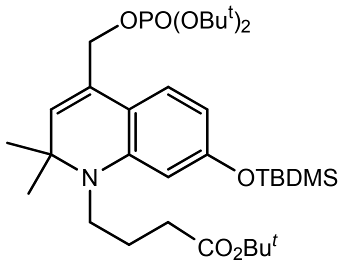
Solvents were evaporated, and the residue was purified twice by column chromatography (the first one using Et_2O and the second one – with hexane/ether/dichloromethane mixture [10/3/1] as an eluent). Yield 31 mg (30%) of light yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.21 (s, 6 H, Si$\underline{Me_2}$Bu^t), 0.98 (s, 9 H, SiMe$_2$Bu^t), 1.26 (t, J = 7.2, 3 H, $CO_2CH_2C\underline{H_3}$), 1.40 (s, 6 H, 2×Me), 1.90 (m, 2 H, $NCH_2C\underline{H_2}CH_2CO_2Et$), 2.38 (m, 2 H, $NCH_2CH_2C\underline{H_2}CO_2Et$), 3.25 (m, 2 H, $NC\underline{H_2}CH_2CH_2CO_2Et$), 4.15 (q, J = 7.2, 2 H, $CO_2C\underline{H_2}CH_3$), 6.05 (s, 1 H, $\underline{H}$C=), 6.12 (d, J = 2.4, 1 H), 6.20 (dd, J = 2.4 and 8.4, 1 H), 8.07 (d, J = 8.4, 1 H), 9.56 (s, 1 H, C$\underline{H}$O) ppm. ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = -4.3(+), 14.2(+), 18.3(q), 23.5(-), 25.7(+), 27.0(+), 31.6(-), 43.3(-), 57.0(q), 60.5(-), 104.2(+), 108.5(+), 111.2(q), 127.0(+), 132.4(q), 145.1(q), 149.6(+), 157.5, 173.0, 192.4(+) ppm. MS (ESI): *m/z* (negative mode, rel. int., %) = 430.2 (9), $[M–H]^–$, 316.1 (100), $[M\text{-}TBDMS]^-$; *m/z* (positive mode, rel. int., %) = 885.6 (22) $[2M+Na]^+$, 454.3 (71) $[M+Na]^+$, 432.3 (100) $[M+H]^+$; HRMS ($C_{24}H_{37}NO_4Si$): 454.2378 (found $[M+Na]^+$), 454.2384 (calc.); 432.2560 (found $[M+H]^+$), 432.2565 (calc.).



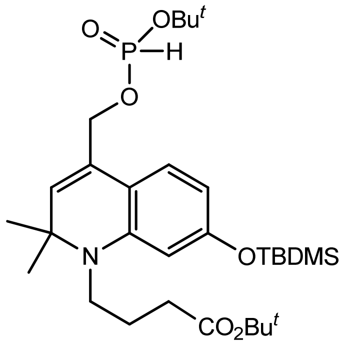
Ethyl [7-(*tert*-butyldimethylsilyl)oxy-1,2-dihydro-2,2-dimethyl-4-hydroxymethylquinoline]-1-butanoate (9-H,TBDMS,Et): SeO_2 (2.46 g, 22.2 mmol) was added in portions to a hot (65°C) solution of **3**-TBDMS,Et (7.4 g, 17.8 mmol) in dioxane (50 mL). Then the reaction mixture was stirred at this temperature for 15 min. After cooling, it was diluted with ether, and the organic layer was washed with sat. aq. $NaHCO_3$ and dried over $MgSO_4$. Solvents were evaporated, and the residue was purified by chromatography in a short column (with hexane/ether/dichloromethane mixture [10/3/1] as an eluent). After evaporation of solvents, the crude compound **8**-TBDMS,Et (3.1 g) was dissolved in THF/MeOH (40 mL / 5 mL), cooled (0 ^{o}C) and $NaBH_4$ (675 mg, 17.8 mmol) was added in portions. After 5 min at RT, the reaction was complete (TLC). The excess of $NaBH_4$ was destroyed by adding acetone (1.0 mL); all volatile materials were evaporated *in vacuo,* and alcohol **9**-H,TBDMS,Et was isolated by column chromatography (with hexane/ether/dichloromethane/MeOH mixture (10/3/4/0.3) as an eluent; R_f = 0.40); yield 1.31 g (17%) of light yellow oil.

Aldehyde **8**-TBDMS,Et (43 mg, 0.1 mmol) could also be reduced to **9**-H,TBDMS,Et according to the procedure described above for compound **9**-H,TBDMS,Bu^t; **9**-H,TBDMS,Et was obtained in 95% yield (41 mg). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.21 (s, 6 H, Si$\underline{Me_2}$Bu^t), 0.99 (s, 9 H, SiMe$_2\underline{Bu}^t$), 1.27 (t, J = 7.2 Hz, 3 H, $CO_2CH_2C\underline{H_3}$), 1.31 (s, 6 H, 2×Me), 1.90 (m, 2 H, $NCH_2C\underline{H_2}CH_2CO_2Et$), 2.38 (m, 2 H, $NCH_2CH_2C\underline{H_2}CO_2Et$), 3.22 (m, 2 H, $NC\underline{H_2}CH_2CH_2CO_2Et$), 4.15 (q, J = 7.2, 2 H, $CO_2C\underline{H_2}CH_3$), 4.42 (m, 2H, $C\underline{H_2}OH$), 5.35 (t, J = 1.2, 1 H, $\underline{H}$C=), 6.05 (d, J = 2.4, 1 H, Ar), 6.11 (dd, J = 2,4 and 8.4, 1 H, Ar), 6.97

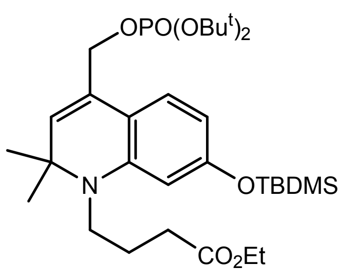
(d, J = 8.4, 1 H, Ar) ppm. ^{13}C NMR (100.5 MHz, $CDCl_3$): δ = -3.8, 14.8, 18.8, 24.0, 26.3, 28.5, 32.2, 43.8, 57.1, 60.9, 63.6, 104.0, 107.8, 114.7, 124.3, 127.4, 131.5, 145.6, 157.0, 173.3 ppm. MS (ESI): *m/z* (positive mode, rel. int., %) = 456.3 (96) $[M+Na]^+$, 434.3 (100) $[M+H]^+$; HRMS ($C_{24}H_{39}NO_4Si$): 456.2549 (found $[M+Na]^+$), 456.2541 (calc.); 434.2720 (found $[M+H]^+$), 434.2721 (calc.).



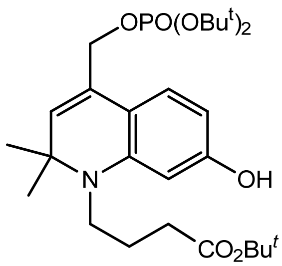
Di-(*tert*-butyl) [1-*tert*-butoxycarbonylpropyl-7-(*tert*-butyldimethylsilyl)oxy-1,2-dihydro-2,2-dimethylquinoline]-4-methyl phosphate (9-PO(OBu^t)$_2$,TBDMS, Bu^t): A solution of di-*tert*-butyl *N,N*-diisopropyl phosphoramidite (33 mg, 0.12 mmol) in THF (2 mL) was added to a solution of **9**-H,TBDMS, Bu^t (60 mg, 0.13 mmol) in THF (2 mL) followed by 1*H*-tetrazole (0.45 M in acetonitrile, 0.80 mL, 0.36 mmol). The reaction mixture was stirred at 40 °C for 0.5 h, and TLC showed 50% conversion: the starting alcohol **9**-H,TBDMS, Bu^t has R_f = 0.31, while phosphite **9**-X has R_f = 0.91 in hexane / ether = 2 / 1. Then additionally di-*tert*-butyl *N,N*-diisopropyl phosphoramidite (33 mg, 0.12 mmol) and 1*H*-tetrazole (0.45 M in acetonitrile, 0.8 mL, 0.36 mmol) were added. The reaction was stirred at 40 ^{o}C for 2 h, uintil it was complete, cooled in an ice-water bath, and a solution of 70% *m*-chloroperoxybenzoic acid (78 mg, 0.314 mmol) in dichloromethane (2 mL) was added in one portion. The color changed immediately from pale yellow to brown, the spot of intermediate **9**-X disappeared, and a new polar spot formed (R_f = 0.32 in hexane/ether = 1/3). After 5 min, the reaction was quenched by adding 10% aq. Na_2SO_3 (1 mL). Diethyl ether (20 mL) was added; the organic layer was separated and washed with sat. $NaHCO_3$, water, brine and dried over $MgSO_4$. After evaporation of solvents, the oily residue was purified by column chromatography (gradient elution with hexane/ether, 2/1 $\rightarrow$ 1/1). The title compound was obtained as viscous oil; yield 45 mg (53 %). ^1H NMR (300 MHz, $CDCl_3$): δ = 0.18 (s, 6 H, Si$\underline{Me_2}$Bu^t), 0.95 (s, 9 H, SiMe$_2\underline{Bu^t}$), 1.28 (s, 6 H, 2$\times$Me), 1.41 (s, 9 H, CO$_2\underline{Bu^t}$), 1.45 (s, 18 H, OPO(O$\underline{Bu^t}$)$_2$), 1.81 (m, 2 H, NCH$_2$C$\underline{H_2}$CH$_2$CO$_2$Bu^t), 2.26 (m, 2 H, NCH$_2$CH$_2$C$\underline{H_2}$CO$_2$Bu^t), 3.17 (m, 2 H, NC$\underline{H_2}$CH$_2$CH$_2$CO$_2$Bu^t), 4.68 (m, 2 H, C$\underline{H_2}$OPO(OBu^t)$_2$), 5.39 (m, 1 H, $\underline{H}$C=), 6.05 (m, 2 H), 6.92 (m, 1 H) ppm. ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = -4.4(+), 18.2(q), 23.6(-), 25.7(+), 27.9(+), 28.1(+), 29.8(+, $J_{C,P}$ = 4.5), 32.9(-) 43.3(-), 56.6(g), 66.4 (-, $J_{C,P}$=6.0), 80.3(q), 82.4(q, $J_{C,P}$=7.5), 103.4(+), 107.3(+), 114.1(q), 124.2(+), 127.4(q, $J_{C,P}$ = 7.5), 128.5(+), 145.1(q), 156.7(q), 172.4(q) ppm. MS (ESI): *m/z* (positive mode, rel. int., %) = 676.4 (100) $[M+Na]^+$, 654.4 (60) $[M+H]^+$; HRMS ($C_{34}H_{60}NO_7PSi$): 676.3771 (found $[M+Na]^+$), 676.3769 (calc.); 654.3953 (found $[M+H]^+$), 654.3949 (calc.).



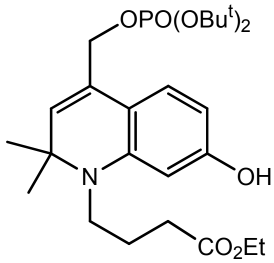
[1-(*tert*-Butoxycarbonylpropyl)-7-(*tert*-butyldimethylsilyloxy)-1,2-dihydro-2,2-dimethylquinoline]-4-methyl *tert*-butyl phosphonate (9-PHO(OBu^t),TBDMS, Bu^t): A solution of di-*tert*-butyl *N,N*-diisopropyl phosphoramidite (744 mg, 3.04 mmol) in THF (5 mL) was added to a solution of compound **9**-H,TBDMS, Bu^t (700 mg, 1.52 mmol) in THF (10 mL) followed by 1*H*-tetrazole (0.45 M in acetonitrile, 6.8 mL, 3.04 mmol). The reaction mixture was stirred at RT for 16 h, cooled in an ice-water bath, and a solution of 70% *m*-chloroperoxybenzoic acid (748 mg, 3.04 mmol) in dichloromethane (6 mL) was added in one portion. After 5 min, the reaction was quenched by adding 10% aq. Na_2SO_3 (2 mL). Diethyl ether (60 mL) was added; the organic layer was separated and washed with sat. $NaHCO_3$, water, brine and dried over $MgSO_4$. After evaporation of solvents, the oily residue was purified by column chromatography (gradient elution with hexane/ether, 2/1 $\rightarrow$ 1/1) and the product **9**-PO(OBu^t)$_2$,TBDMS,Bu^t was obtained as viscous oil (R_f = 0.32 in hexane/ether = 1/3); yield 410 mg (41 %). Compound **9**-PHO(OBu^t),TBDMS,Bu^t (R_f = 0.25 in hexane/ether = 1/3) was isolated as a by-product in 10% yield (88 mg): ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.19 (s, 6 H, Si<u>Me$_2$</u>Bu^t), 0.97 (s, 9 H, SiMe$_2$<u>Bu^t</u>), 1.28 (s, 6 H, 2×Me), 1.42 (s, 9 H, CO$_2$<u>Bu^t</u>), 1.50 (s, 9 H, OPH(O)O<u>Bu^t</u>), 1.82 (m, 2 H, NCH$_2$C<u>H$_2$</u>CH$_2$CO$_2$Bu^t), 2.25 (m, 2 H, NCH$_2$CH$_2$C<u>H$_2$</u>CO$_2$Bu^t), 3.16 (m, 2 H, NC<u>H$_2$</u>CH$_2$CH$_2$CO$_2$Bu^t), 4.77 (m , 2 H, C<u>H$_2$</u>OPH(O)OBu^t), 5.40 (m, 1 H, <u>H</u>C=), 6.08 (m, 2 H, Ar), 6.87 (d, $J_{H,P}$ = 696, 1 H, P-<u>H</u>), 6.92 (d, J = 8.4, 1 H, Ar) ppm. ^{13}C NMR (125.7 MHz, $CDCl_3$): δ = -4.2(+), 18.3(q), 23.7(-), 25.8(+), 27.9(+, $J_{C,P}$ = 5.0), 28.1(+), 30.4(+, $J_{C,P}$ = 3.8), 32.9(-) 43.4(-), 56.7(g), 65.3 (-, $J_{C,P}$ = 5.0), 80.3(q), 83.9(q, $J_{C,P}$ = 8.8), 103.5(+), 107.4(+), 113.7(q), 124.1(+), 127.0(q, $J_{C,P}$ = 6.3), 129.5(+), 145.0(q), 156.8(q), 172.2(q) ppm. MS (ESI): *m/z* (positive mode, rel. int., %) = 604.3 (23) [M+Na]$^+$, 582.4 (100) [M+H]$^+$; HRMS ($C_{30}H_{52}NO_6PSi$): 604.3189 (found [M+Na]$^+$), 604.3194 (calc.); 582.3372 (found [M+H]$^+$), 582.3374 (calc.).



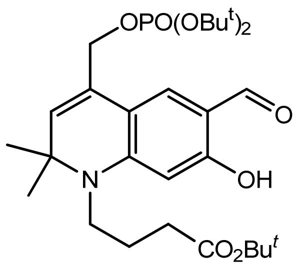
Di-(*tert*-butyl) [1-ethoxycarbonylpropyl-7-(*tert*-butyldimethylsilyl)oxy-1,2-dihydro-2,2-dimethyl-quinoline]-4-methyl phosphate (9-PO(OBu^t)$_2$,TBDMS,Bu^t): From alcohol **9**-H,TBDMS,Et (1.05 g, 2.41 mmol), according to procedure described above for **9**-PO(OBu^t)$_2$,TBDMS,Bu^t, compound **9**-PO(OBu^t)$_2$,TBDMS,Et was obtained in 52% yield (785 mg). ^{1}H NMR (400 MHz, CDCl$_3$): δ = 0.21 (s, 6 H, Si$\underline{Me_2}$Bu^t), 0.98 (s, 9 H, SiMe$_2$$\underline{Bu^t}$), 1.27 (t, J = 7.2, 3 H, CO$_2$CH$_2$C$\underline{H_3}$), 1.34 (s, 6 H, 2×Me), 1.47 (s, 18 H, OPO(O$\underline{Bu^t}$)$_2$), 1.85 (m, 2 H, NCH$_2$C$\underline{H_2}$CH$_2$CO$_2$Bu^t), 2.28 (m, 2 H, NCH$_2$CH$_2$C$\underline{H_2}$CO$_2$Bu^t), 3.20 (m, 2 H, NC$\underline{H_2}$CH$_2$CH$_2$CO$_2$Bu^t), 4.15 (q, J = 7.2, 2 H, CO$_2$C$\underline{H_2}$CH$_3$), 4.72 (m , 2 H, C$\underline{H_2}$OPO(OBu^t)$_2$), 5.40 (m, 1 H, $\underline{H}$C=), 6.05 (m, 2 H), 6.94 (m, 1 H) ppm. ^{13}C NMR (75.5 MHz, CDCl$_3$): δ = -4.4(+), 14.2(+), 18.2(q), 23.4(-), 25.7(+), 27.8(+), 29.8(+, $J_{C,P}$ = 4.5), 31.6(-) 43.2(-), 56.6(g), 60.3(-), 66.4 (-, $J_{C,P}$=5.3), 82.2(q, $J_{C,P}$=7.5), 103.4(+), 107.3(+), 114.1(q), 124.2(+), 127.4(q, $J_{C,P}$ = 8.2), 128.5(+), 145.0(q), 156.6(q), 173.0(q) ppm. MS (ESI): *m/z* (positive mode, rel. int., %) = 648.4 (100) [M+Na]$^+$, 626.4 (50) [M+H]$^+$; HRMS (C$_{32}$H$_{56}$NO$_7$PSi): 626.3637 (found [M+Na]$^+$), 626.3636 (calc.); 648.3461 (found [M+H]$^+$), 648.3456 (calc.).



Di-(*tert*-butyl) (1-*tert*-butoxycarbonylpropyl-1,2-dihydro-7-hydroxy-2,2-dimethylquinoline)-4-methyl phosphate (9-PO(OBu^t)$_2$,H,Bu^t): a solution of TBAF·3H$_2$O (173 mg, 0.551 mmol) in THF (0.5 mL) was added to a solution of compound **9**-PO(OBu^t)$_2$,TBDMS,Bu^t (360 mg, 0.551 mmol) in THF at +5°C. After 5 min, the reaction mixture was diluted with ether (10 mL), washed with water (twice) and dried over MgSO$_4$. After evaporation of solvents, the residue was purified by column chromatography (gradient elution with ether/hexane mixture, 2/1 to 4/1). Phenol **9**-PO(OBu^t)$_2$,H,Bu^t (R_f = 0.24 in hexane/ether, 1/4) was isolated in 84% yield (249 mg). ^{1}H NMR (300 MHz, CDCl$_3$): δ = 1.27 (s, 6 H, 2×Me), 1.43 (s, 9 H, CO$_2$$\underline{Bu^t}$), 1.47 (s, 18 H, OPO(O$\underline{Bu^t}$)$_2$), 1.82 (m, 2 H, NCH$_2C\underline{H_2}CH_2CO_2$Bu^t), 2.24 (m, 2 H, NCH$_2CH_2C\underline{H_2}CO_2$Bu^t), 3.18 (m, 2 H, NC$\underline{H_2}CH_2CH_2CO_2$Bu^t), 4.68 (d , $^3J_{H\text{-}P}$ = 7.2, 2 H, C$\underline{H_2}$OP), 5.32 (s, 1 H, $\underline{H}$C=), 6.07 (d, J = 1.8, 1 H), 6.23 (dd, J = 8.4 and 1.8, 1 H), 6.92 (d, J = 8.4, 1 H) ppm. ^{13}C NMR (75.5 MHz, CDCl$_3$): δ = 24.0(-), 28.4(+), 28.5(+), 30.3(+, $J_{C,P}$ = 4.5 Hz), 33.4(-) 43.8(-), 57.1(g), 67.3 (-, $J_{C,P}$=5.3), 80.8(q), 83.3(q, $J_{C,P}$=7.5), 99.2(+), 103.4(+), 113.0(q), 124.9(+), 127.9(q, $J_{C,P}$ = 7.5), 128.3(+), 145.8(q), 158.6(q), 173.2(q) ppm. MS (ESI): *m/z* (negative mode, rel. int., %) = 1077.5 [2M–H]$^-$, 538.3 [M–H]$^-$; HRMS (C$_{28}$H$_{45}$NO$_7$P): 538.2936 (found [M–H]$^-$), 538.2939 (calc.); *m/z* (positive mode, rel. int., %) = 1101.6 (100) [2M+Na]$^+$, 562.3 (93) [M+Na]$^+$, 540.3 (11) [M+H]$^+$; HRMS (C$_{28}$H$_{47}$NO$_7$P): 540.3075 (found [M+H]$^+$), 540.3085 (calc.).

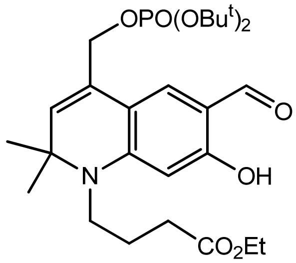


Di-(*tert*-butyl) (1,2-dihydro-1-ethoxycarbonylpropyl-7-hydroxy-2,2-dimethylquinoline)-4-methyl phosphate (9-PO(OBu^t)$_2$,H,Et): From the compound **9**-PO(OBu^t)$_2$,TBDMS,Et (781 mg, 2.25 mmol), according to procedure described above for **9**-PO(OBu^t)$_2$,H,Bu^t, the product **9**-PO(OBu^t)$_2$,H,Et was obtained in 90% yield (580 mg). ^{1}H NMR (400 MHz, $CDCl_3$): δ =1.27 (t, J = 7.2, 3 H, $CO_2CH_2C\underline{H}_3$), 1.29 (s, 6 H, 2×Me), 1.47 (s, 18 H, OPO(O$\underline{Bu}^t$)$_2$), 1.89 (m, 2 H, $NCH_2C\underline{H}_2CH_2CO_2Bu^t$), 2.35 (m, 2 H, $NCH_2CH_2C\underline{H}_2CO_2Bu^t$), 3.20 (m, 2 H, $NC\underline{H}_2CH_2CH_2CO_2Bu^t$), 4.13 (q, J = 7.2, 2 H, $CO_2C\underline{H}_2CH_3$), 4.71 (m , 2 H, C$\underline{H}_2$OPO(OBu^t)$_2$), 5.33 (t, J = 1.2, 1 H, $\underline{H}$C=), 6.10 (d, J = 2.4, 1 H, Ar), 6.25 (dd, J = 2,4 and 8.4, 1 H, Ar), 6.92 (d, J = 8.4, 1 H, Ar), 8.30 (br. s, 1 H, O$\underline{H}$) ppm. ^{13}C NMR (125.7 MHz, $CDCl_3$): δ = 14.8(+), 24.0(-), 28.4(+), 30.4 (+, $J_{C,P}$ = 4.5), 32.2(-), 43.8(-), 57.1(q), 60.9(-), 67.4 (-, $J_{C,P}$=5.3), 83.3(q, $J_{C,P}$=7.5), 99.2(+), 103.5(+), 112.9(q), 124.8(+), 127.8 (q, $J_{C,P}$ = 7.5), 128.2(+), 145.6(q), 158.6(q), 173.7(q) ppm. MS (ESI): *m/z* (negative mode, rel. int., %) = 510.3 (100), [M–H]$^-$; HRMS ($C_{26}H_{42}NO_7P$): 510.2631 (found [M-H]$^-$), 510.2626 (calc.); *m/z* (positive mode, rel. int., %) = 534.3 (100) [M+Na]$^+$, 512.3 (58) [M+H]$^+$; HRMS ($C_{26}H_{42}NO_7P$): 534.2592 (found [M+Na]$^+$), 534.2591 (calc.); 512.2775 (found [M+H]$^+$), 512.2772 (calc.).

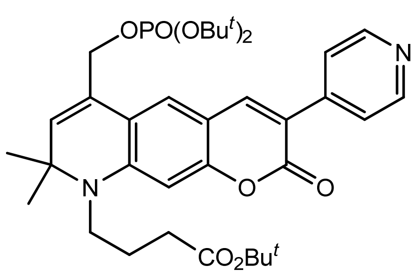


Di-(*tert*-butyl) (1-*tert*-butoxycarbonylpropyl-1,2-dihydro-6-formyl-7-hydroxy-2,2-dimethylquinoline)-4-methyl phosphate (10-Bu^t,Bu^t): $POCl_3$ (62 mg, 0.40 mmol) was added to DMF (2 mL) at +5°C, and the mixture was allowed to warm to room temperature. After stirring for 15 min at room temperature, it was cooled down (+5 °C), and a solution of phenol **9**-PO(OBu^t)$_2$,H,Bu^t (145 mg, 0.269 mmol) in DMF (1 mL) was added slowly. The cooling bath was removed, and the reaction mixture was allowed to warm up to room temperature, stirred for 4 h, and finally heated at 50 °C for 15 min. The TLC control of this reaction is difficult, because the product was found to have the same R_f value, as the starting material (in most solvent mixtures). After cooling, the reaction was "quenched" by adding 1 mL of sat. aq. $NaHCO_3$, and the product **10**-Bu^t,Bu^t was extracted with dichloromethane. The organic layer was dried ($MgSO_4$), and, after evaporation of solvents, the residue was purified by column chromatography (gradient elution with hexane/ether mixture, 1/1 to 1/4). Yield 102 mg (67%). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.38 (s, 9 H,

$CO_2\underline{Bu}^t$), 1.40 (s, 6 H, 2×Me), 1.45 (s, 18 H, $OPO(O\underline{Bu}^t)_2$), 1.86 (m, 2 H, $NCH_2C\underline{H}_2CH_2CO_2Bu^t$), 2.30 (m, 2 H, $NCH_2CH_2C\underline{H}_2CO_2Bu^t$), 3.30 (m, 2 H, $NC\underline{H}_2CH_2CH_2CO_2Bu^t$), 4.70 (d, $^3J_{H\text{-}P}$ = 7.2, 2 H, $C\underline{H}_2OP$), 5.46 (s, 1 H, $\underline{H}C$=), 5.95 (s, 1 H, Ar), 7.16 (s, 1 H, Ar), 9.44 (s, 1 H, $C\underline{H}O$), 11.72 (s, 1 H, $O\underline{H}$) ppm. ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 23.0(-), 28.1(+), 29.0(+), 29.9 (+, $J_{C,P}$ = 4.5), 32.7(-), 44.1(-), 58.2(q), 66.1 (-, $J_{C,P}$ = 5.3), 80.8(q), 82.6 (q, $J_{C,P}$ = 7.5), 96.8(+), 111.0(q), 112.7(q), 126.15 (q, $J_{C,P}$ = 8.3), 128.4(+), 129.2(+), 151.0(q), 164.7(q), 172.0(q), 192.1(+) ppm. MS (ESI): *m/z* (negative mode, rel. int., %) = 566.3 (100), $[M–H]^–$; HRMS ($C_{29}H_{46}NO_8P$): 566.2885 (found $[M–H]^–$), 566.2888 (calc.); *m/z* (positive mode, rel. int., %) = 1157.6 (98) $[2M+Na]^+$, 590.3 (100) $[M+Na]^+$; HRMS ($C_{29}H_{46}NO_8PNa$): 590.2857 (found $[M+Na]^+$), 590.2853 (calc.).

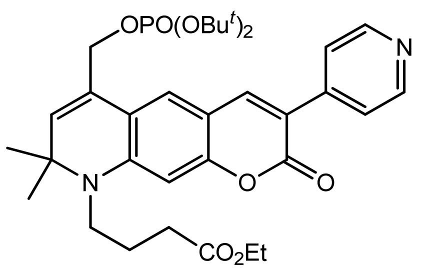


Di-(*tert*-butyl) (1,2-dihydro-1-ethoxycarbonylpropyl-6-formyl-7-hydroxy-2,2-dimethylquinoline)-4-methyl phosphate (10-Bu^t,Et): from phenol **9**-$PO(OBu^t)_2$,H,Et (540 mg, 1.06 mmol), according to the procedure described for **10**-Bu^t,Bu^t, compound **10**-Bu^t,Et was obtained in 69% yield (391 mg). ^1H NMR (300 MHz, $CDCl_3$): δ = 1.25 (t, J = 7.2 Hz, 3 H, $CO_2CH_2C\underline{H}_3$), 1.38 (s, 6 H, 2×Me), 1.45 (s, 18 H, $OPO(O\underline{Bu}^t)_2$), 1.90 (m, 2 H, $NCH_2C\underline{H}_2CH_2CO_2Bu^t$), 2.37 (m, 2 H, $NCH_2CH_2C\underline{H}_2CO_2Bu^t$), 3.30 (m, 2 H, $NC\underline{H}_2CH_2CH_2CO_2Bu^t$), 4.15 (q, J = 7.2 Hz, 2 H, $CO_2C\underline{H}_2CH_3$), 4.69 (d, $J_{H\text{-}P}$ = 7.2 Hz, 2 H, $C\underline{H}_2OP$), 5.43 (t, J = 1.2, 1 H, $\underline{H}C$=), 5.90 (s, 1 H, Ar), 7.14 (s, 1 H, Ar), 9.44 (s, 1 H, $C\underline{H}O$), 11.72 (s, 1 H, $O\underline{H}$) ppm. ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 14.2(+), 22.8(-), 29.0(+), 29.9(+, $J_{C,P}$ = 4.5), 31.3(-), 44.0(-), 58.2(-), 60.7(-), 66.1(-,$J_{C,P}$ = 5.3), 82.7(q, $J_{C,P}$ = 7.5), 96.8(+), 111.0(q), 112.7(q), 126.1(q, $J_{C,P}$ = 8.3), 128.4(+), 129.2(+), 151.0(q), 164.7(q), 172.7(q), 192.1(+) ppm. MS (ESI): *m/z* (negative mode, rel. int., %) = 538.3 (100), $[M–H]^–$; HRMS ($C_{27}H_{42}NO_8P$): 538.2582 (found $[M–H]^–$), 538.2575 (calc.); *m/z* (positive mode, rel. int., %) = 1101.5 (53) $[2M+Na]^+$, 562.3 (100) $[M+Na]^+$; HRMS ($C_{27}H_{42}NO_8P$): 562.2541 (found $[M+Na]^+$), 562.2540 (calc.).



Di-(*tert*-butyl) [9-*tert*-butoxycarbonylpropyl-8,9-dihydro-8,8-dimethyl-3-(pyridin-4-yl)-2*H*-pyrano[3,2-*g*]quinolin-2-one]-6-methyl phosphate (11-Bu^t,Bu^t): 4-Pyridylacetic acid hydrochloride (58 mg, 0.34

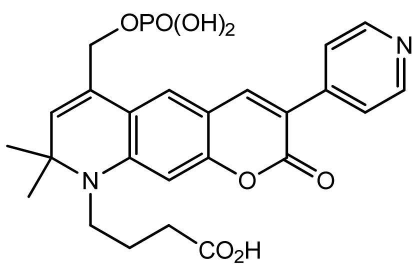
mmol) was added to a stirred solution of compound **10**-Bu^t,Bu^t (127 mg, 0.224 mmol) in dichloromethane (10 mL). Then Et_3N (59 mg, 0.58 mmol) was added, and, after 10 min, *N,N´*-dicyclohexylcarbodiimide (92 mg, 0.45 mmol) and 4-dimethylaminopyridine (3.0 mg, 0.022 mmol) were added. The reaction mixture was stirred for 16 h, filtered through a plug of silica gel (using dichloromethane/ether mixture (2/1) as eluent). After evaporation of solvents, the product **11**-Bu^t,Bu^t was isolated by column chromatography (eluting with dichloromethane to dichloromethane/ether mixture, 2/1) followed by precipitation from ether with hexane. Yield: 101 mg (67%) of light-yellow crystals. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.38 (s, 9 H, $CO_2\underline{Bu^t}$), 1.40 (s, 6 H, 2×Me), 1.45 (s, 18 H, OPO(O$\underline{Bu^t}$)$_2$), 2.10 (m, 2 H, $NCH_2C\underline{H_2}CH_2CO_2Bu^t$), 2.30 (m, 2 H, $NCH_2CH_2C\underline{H_2}CO_2Bu^t$), 3.30 (m, 2 H, $NC\underline{H_2}CH_2CH_2CO_2Bu^t$), 4.70 (d, $J_{H\text{-}P}$ = 7.2, 2 H, $C\underline{H_2}OP$), 5.60 (s, 1 H, $\underline{H}$C=), 6.38 (s, 1 H, Ar), 7.28 (s, 1H, Ar), 7.65 (m, 2 H, AA' part of AA'XX' system), 7.81 (s, 1 H, Ar), 8.60 (m, 2 H, XX' part of AA'XX' system) ppm. ^{13}C NMR (125.7 MHz, $CDCl_3$): δ = 22.9(-), 28.2(+), 30.0(+, $J_{C,P}$=5.0 Hz), 31.0(+), 32.7(-), 44.2(-), 58.1(q), 66.1(-, $J_{C,P}$ = 4.5), 80.9(q), 82.8(q, $J_{C,P}$ = 7.5), 96.9(+), 108.8(q), 117.4(q), 117.6(q), 122.2 (+), 122.9(+), 126.1(q, $J_{C,P}$ = 8.3), 131.9 (+), 141.7(+), 143.1(q), 148.1(q), 149.7(+), 156.5(q), 160.5(q), 171.9(q) ppm. MS (ESI): *m/z* (positive mode, rel. int., %) = 669.4 (100) [M+H]$^+$. λ_{abs} = 430 nm, λ_{em} = 501 nm, ε = 30800 M^{-1}cm^{-1}, $\Phi_{fl.}$ = 0.82 (MeOH). Standard: Atto 425, $\Phi_{fl.}$ = 0.90 (PBS 7.4).



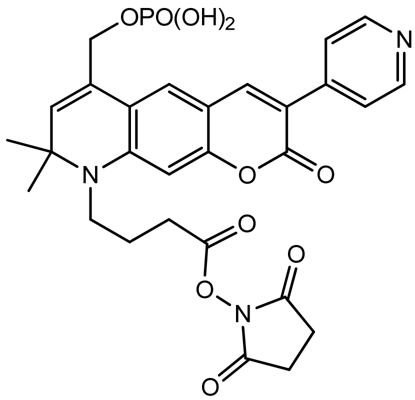
Di-(*tert*-butyl) [9-ethoxycarbonylpropyl-8,9-dihydro-8,8-dimethyl-3-(pyridin-4-yl)-2*H*-pyrano[3,2-*g*]quinolin-2-one]-6-methyl phosphate (11-Bu^t,Et): From the salicylic aldehyde **10-**Bu^t,Et (150 mg, 0.278 mmol), according to procedure described for **11**-Bu^t,Bu^t, compound **11**-Bu^t,Et was prepared and isolated as yellow solid with 90% yield (160 mg).

*Preparation of compound **11**-Bu^t,Et from **20a**:* 1*H*-Tetrazole (114 mg, 1.61 mmol) and di-*tert*-butyl *N,N*-diisopropyl phosphoramidite (370 mg, 1.49 mmol) were added in two equal portions at an interval of 20 min to a stirred and hot (40ºC) solution of compound **20a** (222 mg, 0.5 mmol) in CH_2Cl_2 (30 mL). After 10 min, the reaction mixture was cooled with an ice bath, and a solution of 70% MCPBA (366 mg, 1.49 mmol) in CH_2Cl_2 (5 mL) was added dropwise. The cooling bath was removed, the reaction mixture was allowed to warm up to RT, and 10% aq. Na_2SO_3 (10 mL) and aq. sat. $NaHCO_3$ (15 mL) were added. The organic layer was separated, and the aqueous solution was extracted with CH_2Cl_2 (2×20 mL). The combined organic solutions were dried ($MgSO_4$), volatile materials were evaporated *in vacuo*, and the titled compound was isolated by column chromatography (30 g of SiO_2, dichloromethane / ether / MeOH = 10 / 5 / 0.1) as a yellow amorphous solid (215 mg, 65 %).

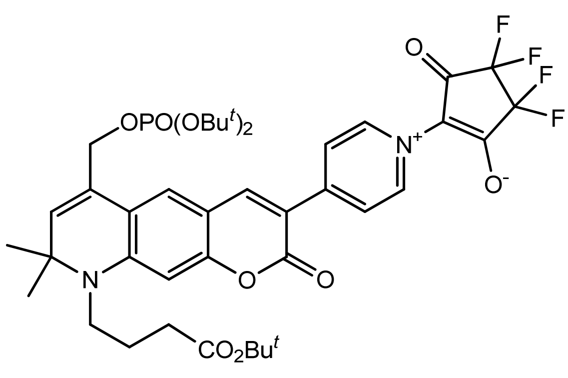
^{1}H NMR (300 MHz, $CDCl_3$): δ =1.28 (t, J = 7.2, 3 H, $CO_2CH_2C\underline{H}_3$), 1.40 (s, 6 H, 2×Me), 1.46 (s, 18 H, $OPO(O\underline{Bu}^t)_2$), 1.90 (m, 2 H, $NCH_2C\underline{H}_2CH_2CO_2Bu^t$), 2.40 (m, 2 H, $NCH_2CH_2C\underline{H}_2CO_2Bu^t$), 3.32 (m, 2 H, $NC\underline{H}_2CH_2CH_2CO_2Bu^t$), 4.17 (q, J = 7.2, 2 H, $CO_2C\underline{H}_2CH_3$), 4.73 (d, $J_{H\text{-}P}$ = 7.2, 2 H, $C\underline{H}_2OP$), 5.60 (d, J = 0.8, 1 H, $\underline{H}C$=), 6.40 (s, 1 H, Ar), 7.27 (s, 1H, Ar), 7.63 (m, 2 H, AA' part of AA'XX' system), 7.81 (s, 1 H, Ar), 8.60 (m, 2 H, XX' part of AA'XX' system) ppm. ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 14.2 (+), 22.6(-), 28.9(+), 29.9 (+, $J_{C,P}$=4.5), 31.2(-), 44.0(-), 58.1(q), 60.8(-), 66.1(-, $J_{C,P}$ = 5.3), 82.8(q, $J_{C,P}$ = 7.6), 96.9(+), 108.8(q), 117.4(q), 117.6(q), 122.2 (+), 123.0(+), 126.1(q, $J_{C,P}$ = 8.3), 131.9 (+), 141.8(+), 143.1(q), 148.1(q), 149.8(+), 156.6(q), 160.6(q), 172.7(q) ppm. MS (ESI): *m/z* (positive mode, rel. int., %) = 663.3 (100) $[M+Na]^+$; HRMS ($C_{34}H_{45}N_2O_8P$): found 663.2803 $[M+Na]^+$, calc. 663.2806.



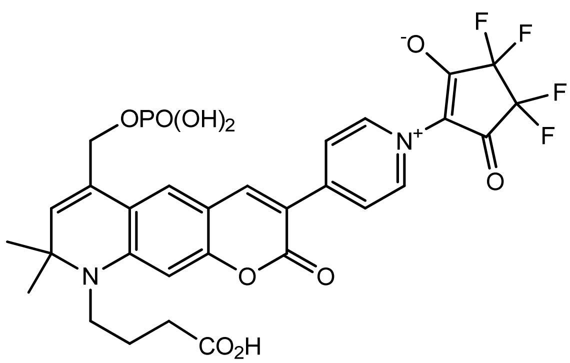
6-{8,9-Dihydro-8,8-dimethyl-9-[3-(hydroxycarbonyl)propyl]-3-(4-pyridyl)-2*H*-pyrano[3,2-*g*]quinolin-2-one}methyl phosphoric acid (11-H,H): Compound **11-Bu^t,Bu^t** (12 mg, 0.018 mmol) was dissolved in TFA (0.5 mL). After 4 h, TFA was evaporated and the title product was triturated with ether giving 7.8 mg (87%) of dye **11**-H,H as a brown solid. *From the ethyl ester **11**-Bu^t,Et*: the compound **11**-Bu^t,Et (170 mg, 0.266 mmol) was dissolved in THF/MeOH (4mL/4mL) mixture and a 5-fold excess of 1 M aq. NaOH (~1.5 mL) was added. The reaction mixture was stirred at RT for 16 h up to completion (TLC). Then it was evaporated *in vacuo* to dryness, and CF_3COOH (2 mL) was added. After 4 h, it was diluted with dichloromethane/ether mixture (1/1), the precipitate was filtered off, dried in air, dissolved in the minimal volume of DMF and subjected to column chromatography (acetonitrile / water, 4 / 1). After evaporation of solvents, the residue was dissolved in CF_3COOH (0.5 mL), and added to ether (10 mL). The precipitate was filtered off, washed with ether and dried giving 128 mg (96% yield) of **11**-H,H. For NMR measurements, a sample of compound **11**-H,H was dissolved in NEt_3 and evaporated *in vacuo*. ^{1}H NMR (400 MHz, DMSO-d$_6$): δ = 1.14 (t, J = 7.0, CH_3 in NEt_3), 1.34 (s, 6 H, 2×Me), 1.71 (m, 2 H, $NCH_2C\underline{H}_2CH_2CO_2H$), 2.38 (m, 2 H, $NCH_2CH_2C\underline{H}_2CO_2H$), 3.06 (q, J = 7.0, CH_2N in NEt_3), 3.32 (m, 2 H, $NC\underline{H}_2CH_2CH_2CO_2H$), 4.61 (m, 2H), 5.66 (m, 1 H, $\underline{H}C$=), 6.61 (s, 1 H, Ar), 7.35 (s, 1 H, Ar), 7.75 (m, 2 H, AA' part of AA'XX' system), 8.22 (s, 1 H, Ar), 8.54 (m, 2 H, XX' part of AA'XX' system) ppm. ^{13}C NMR (125.7 MHz, DMSO-d$_6$): δ= 8.6 (+, NEt_3), 22.4(-), 28.5(+), 30.4 (-), 43.4 (-), 45.7 (-, NEt_3), 57.8 (q), 64.2 (-, $J_{C,P}$ = 5.0), 96.0(+), 108.1(q), 115.2(q), 116.7(q), 121.8 (+), 123.0(+), 125.8(q, $J_{C,P}$ = 8.8), 130.9 (+), 142.6(+), 142.9(q), 148.0(q), 148.9(+), 156.0(q), 159.4(q), 174.0(q) ppm. MS (ESI): *m/z* (negative mode, rel. int., %) = 521.2 (30) $[M+Na-2H]^-$, 499 (57) [M–H]–. HRMS ($C_{24}H_{25}N_2O_8P$): found 499.1280 $[M-H]^-$, calc. 499.1276; HPLC: t_R = 9.2 min (MeCN/H_2O (+0.1% v/v TFA in H_2O and MeCN): 20/80 – 50/50 in 25 min, column 4×250 mm, 1.2 mL/min, detection at 433 nm); λ_{abs} = 436 nm, λ_{em} = 515 nm, ε = 22700 $M^{-1}cm^{-1}$, $\Phi_{fl.}$= 0.68 (in PBS buffer at pH 7.4). Standard: Coumarin 334, $\Phi_{fl.}$ = 0.69 (EtOH).



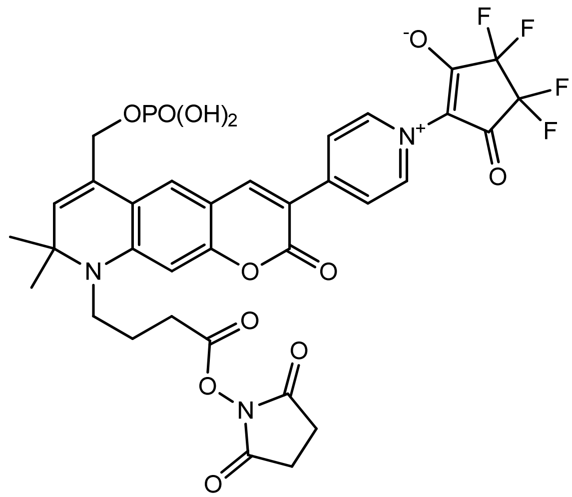
{8,9-Dihydro-8,8-dimethyl-3-(pyridin-4-yl)-9-[(*N*-succinimidyl)oxycarbonylpropyl]-2*H*-pyrano-[3,2-*g*]quinolin-2-one}-6-methyl phosphoric acid (11-H,NHS): Acid **11**-H,H (10 mg, 20 μmol) was dissolved in DMF (1 mL), *N*-hydroxysuccinimid (3.5 mg, 30 μmol) was added at room temperature followed by HATU (11.4 mg, 30 μmol) and NEt_3 (3.0 mg, 30 μmol). The reaction mixture was stirred at room temperature for 16 h. After that, the reaction mixture was subjected to chromatography (without additional work-up). Gradient elution with acetone→acetone/acetonitrile/water = 4/4/1 afforded NHS ester **11**-H,NHS (7.2 mg, 60% yield). MS (ESI in MeOH): *m/z* (negative mode, rel. int. %) = 628.2 (100) $[M+CH_3OH–H]^–$, 596.2 (55) $[M–H]^–$; HRMS ($C_{29}H_{27}N_3O_{10}P$): 596.1438 (found for $[M–H]^–$), 596.1440 (calc.). HPLC: t_R = 12.4 min (B/A = 20/80 – 50/50 in 25 min, column 4×250 mm, 1.2 mL/min, detection at 433 nm). The conjugate with anti sheep anti-mouse IG had the following properties in PBS buffer at pH 7.4: λ_{abs} = 439 nm, λ_{em} = 513 nm, $\Phi_{fl.}$ = 0.30, degree of labeling (DOL) = 2.6. Standard: Coumarin 334, $\Phi_{fl.}$ = 0.69 (EtOH).



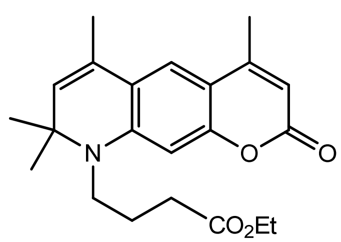
(9-*tert*-Butoxycarbonylpropyl-8,9-dihydro-8,8-dimethyl-2*H*-pyrano[3,2-*g*]quinolin-2-one)-3-{4-[pyridin-1-ium-(3´,3´,4´,4´-tetrafluoro-2´-oxido-5´-oxo-1-cyclopentene-1-yl) betaine]} (12-Bu^t,Bu^t): Perfluorocyclopentene (0.2 mL) was added to a cold (ice-water bath) solution of compound **11**-Bu^t,Bu^t (12 mg, 0.018 mmol) in aqueous EtOH (95% v/v) in a screw-cap bottle. The reaction vessel was closed, and the mixture was heated at 60 °C for 3 h. After cooling, all volatile materials were evaporated *in vacuo,* and the residue was purified by column chromatography (AcOEt→AcOEt/acetone/dichloromethane, 6/3/1; R_f = 0.84 in AcOEt/acetone/dichloromethane = 6/3/1.5). The red-colored fractions were collected, and, after evaporation of solvents, the residue was triturated with ether/hexane mixture affording 7.2 mg (48%) of the title compound. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.38–1.44 (s, 9 H, $CO_2\underline{Bu^t}$; s, 6 H, 2×Me; s, 18 H, $OPO(O\underline{Bu^t})_2$), 2.10 (m, 2 H, $NCH_2C\underline{H_2}CH_2CO_2Bu^t$), 2.35 (m, 2 H, $NCH_2CH_2C\underline{H_2}CO_2Bu^t$), 3.40 (m, 2 H, $NC\underline{H_2}CH_2CH_2CO_2Bu^t$), 4.74 (d, $J_{H\text{-}P}$ = 7.2, 2 H, $C\underline{H_2}OP$), 5.67 (s, 1 H, $\underline{H}C$=), 6.42 (s, 1 H, Ar), 7.38 (s, 1 H, Ar), 8.20 (s, 1 H, Ar), 8.42 (m, 2 H, AA' part of AA'XX' system), 9.55 (m, 2 H, XX' part of AA'XX' system) ppm. ^{13}C NMR (125.7 MHz, $CDCl_3$, signals of perfluorocylopentadione residue were not detected): δ = 22.8(-), 28.2(+), 30.0 (+, $J_{C,P}$=4.5 Hz), 31.0(+), 32.5 (-), 44.6 (-), 59.1 (q), 65.9 (-, $J_{C,P}$ = 5.0), 81.2 (q), 83.0 (q, $J_{C,P}$ = 7.5), 96.7(+), 109.0(q), 111.1(q), 118.2(q), 122.9(+), 124.2(+), 125.7(q, $J_{C,P}$ = 8.8), 132.6 (+), 139.7(+), 144.4(+), 149.3(q), 150.6(q), 158.1(q), 159.3(q), 171.7(q) ppm. MS (ESI): *m/z* (positive mode, rel. int. %) = 859.3 (70) $[M+Na]^+$, 837.3 (100) $[M+H]^+$; HRMS ($C_{41}H_{49}F_4N_2O_{10}P$): 859.2960 (found for $[M+Na]^+$), 859.2953 (calc.), 837.3124 (found for $[M+H]^+$), 837.3134 (calc.). λ_{abs} = 516 nm, λ_{em} = 614 nm, ε = 55700 $M^{-1}cm^{-1}$, $\Phi_{fl.}$ = 0.10 (in MeOH). Standard: RDC, $\Phi_{fl.}$ = 0.38 (1,4-dioxane).



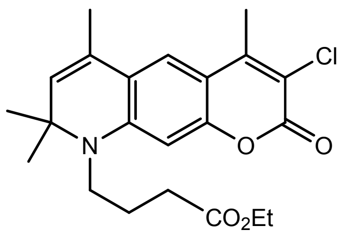
(9-Carboxypropyl-8,9-dihydro-8,8-dimethyl-3-{4-[pyridin-1-ium-(3',3',4',4'-tetrafluoro-2'-oxido-5'-oxo-1-cylopenten-1-yl)betaine]}-2*H*-pyrano[3,2-g]quinolin-2-one)-6-methyl phosphoric acid (12-H,H): Ester **12**-Bu^t,Bu^t (6 mg, 7.2 μmol) was dissolved in TFA (0.5 mL). After keeping at room temperature for 16 h, TFA was evaporated, and the residue was triturated with ether affording 4.5 mg (93%) of the title compound as a red solid. ^{1}H NMR (300 MHz, DMSO-d_6): δ = 1.42 (s, 6 H, 2×Me), 1.80 (m, 2 H, $NCH_2C\underline{H_2}CH_2CO_2H$), 2.40 (m, 2 H, $NCH_2CH_2C\underline{H_2}CO_2H$), 3.33 (m, 2 H, $NC\underline{H_2}CH_2CH_2CO_2H$), 4.82 (m, 2 H, $C\underline{H_2}OP$), 5.60 (m, 1 H, $\underline{H}C$=), 6.72 (s, 1 H), 7.68 (s, 1 H, Ar), 8.70 (s, 1 H, Ar), 8.72 (m, 2 H, AA' part of AA'XX' system), 9.10 (m, 2 H, XX' part of AA'XX' system) ppm. ^{13}C NMR (125.7 MHz, DMSO-d_6, signals of perfluorocyclopentadione part were not detected): δ = 22.8(-), 28.5(+), 31.5(-), 44.1(-), 58.3(q), 63.6(-), 95.7(+), 108.9(q), 109.3(q), 117.9(q), 122.7(+), 125.4(q), 127.2(+), 140.5(+), 141.0(+), 147.3(+), 150.3(q), 157.3(q), 159.2(q), 171.5(q) ppm. MS (ESI): *m/z* (negative mode, rel. int., %) = 667.1 $[M-H]^-$; *m/z* (positive mode, rel. int. %) = 735.5 (42) $[M+3Na-2H]^+$; HPLC: t_R = 21.2 min (B/A = 20/80 – 50/50 in 25 min, column 4×250 mm, 1.2 mL/min, detection at 552 nm).



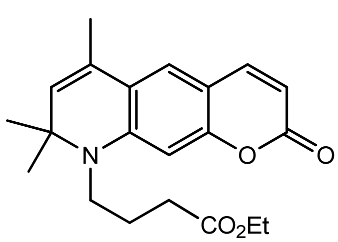
(8,9-Dihydro-8,8-dimethyl-9-(*N*-succinimidyl)oxycarbonylpropyl-3-{4-[pyridin-1-ium-(3',3',4',4'-tetrafluoro-2'-oxido-5'-oxo-1-cylopenten-1-yl)betaine]}-2*H*-pyrano[3,2-g]quinolin-2-one)-6-methyl phosphoric acid (12-H,NHS): The acid **12**-H,H (3.4 mg, 5 μmol) was dissolved in DMF (1 mL), *N*-hydroxysuccinimide (0.9 mg, 7.5 μmol) was added at room temperature followed by HATU (2 mg, 7.5 μmol) and NEt_3 (1.5 mg, 15 μmol). The reaction mixture was stirred at room temperature for 16 h. Then it was subjected to chromatography without an additional work-up (with acetone→acetone/acetonitrile/water = 10/10/1) giving **12**-H,NHS (2.4 mg, 62% yield). HRMS: found 764.1300 $[M–H]^-$; calcd. 764.1274 ($C_{33}H_{28}N_3O_{12}PF_4$). HPLC: B/A = 20/80 to 50/50 in 25 min, 552 nm, t_R = 24.0 min (100 %).



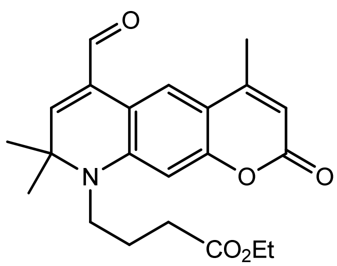
9-(Ethoxycarbonylpropyl)-8,9-dihydro-4,6,8,8-tetramethyl-2*H*-pyrano[3,2-*g*]quinolin-2-one (13-H,Me): Ethyl acetoacetate (1.50 g, 11.5 mmol) was added to a solution of phenol **3**-H,Et (1.95 g, 6.44 mmol) in ethanol (4 mL) followed by addition of dry $ZnCl_2$ (1.3 g, 9.6 mmol). The reaction mixture was heated in an open flask (90 °C) for 20 h. The obtained green-grey slurry was cooled, dissolved in dichloromethane (50 mL) and shaken with 2% aq. ammonia solution (50 mL). The organic layer was separated and passed through a plug of silica gel (dichloromethane / ether = 1 / 1 eluent). After evaporation of solvents, the oily residue was purified by column chromatography (cyclohexane / dichloromethane / ether = 4 / 4 / 1 eluent). Finally, the product was precipitated from hexane giving 1.52 g (64%) of pale-yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.28 (t, *J* = 7.2, 3 H, CO_2Et), 1.35 (s, 6 H, 2×Me), 1.90 (m, 2 H, $NCH_2C\underline{H}_2CH_2CO_2Bu^t$), 1.98 (d, 3 H, *J* = 0.8, 3 H, Me), 2.31 (d, 3 H, *J* = 0.8, 3 H, Me), 2.38 (m, 2 H, $NCH_2CH_2C\underline{H}_2CO_2Bu^t$), 3.29 (m, 2 H, $NC\underline{H}_2CH_2CH_2CO_2Bu^t$), 4.16 (q, J = 7.2, 2 H, CO_2Et), 5.28 (q, J = 0.8, 1 H, $\underline{H}$C=), 5.91 (q, 3H, J = 0.8, 1 H, $\underline{H}$C=), 6.34 (s, 1 H, Ar), 7.09 (s, 1 H, Ar) ppm. ^{13}C NMR (100.7 MHz, $CDCl_3$): δ = 14.2, 18.5, 18.7, 22.7, 29.0, 31.4, 43.8, 57.7, 60.6, 97.2, 108.8, 109.0, 118.8, 119.8, 126.3, 129.8, 147.3, 152.9, 155.8, 162.0, 172.8 ppm. MS (ESI): *m/z* (positive mode, rel. int., %) = 761.4 (100) $[2M+Na]^+$, 392.3 (70) $[M+Na]^+$, 370.3 (18) $[M+H]^+$.



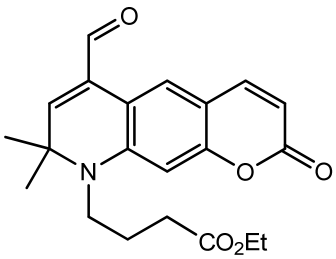
Ethyl (3-chloro-8,9-dihydro-4,6,8,8-tetramethyl-2*H*-pyrano[3,2-*g*]quinolin-2-one)-9-butanoate (13-Cl,Me): Ethyl 2-chloroacetoacetate (3.26 g, 19.8 mmol) was added to a solution of phenol **3**-H,Et (5.0 g, 16.5 mmol) in ethanol (4 mL) followed by addition of dry $ZnCl_2$ (2.7 g, 19.8 mmol). The reaction mixture was heated in opened flask (90^{o}C) for 20 h. The obtained green-grey slurry was cooled, dissolved in dichloromethane (50 mL) and shaked with 2% aq. ammonia solution (50 mL). The organic layer was separated, passed through a plug of silica gel (dichloromethane / ether = 1 / 1 eluent). After evaporation of solvents the oily residue was purified by column chromatography (cyclohexane / dichloromethane / ether = 4 / 4 / 1 eluent). Finally, the product was precipitated from ether giving 1.31 g (20% yield) of pale-yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.28 (t, J = 7.2, 3 H, CO_2Et), 1.38 (s, 6 H, 2$\times$Me), 1.92 (m, 2 H, $NCH_2C\underline{H}_2CH_2CO_2Bu^t$), 2.02 (d, 3 H, J = 0.8, 3 H, Me), 2.40 (m, 2 H, $NCH_2CH_2C\underline{H}_2CO_2Bu^t$), 2.51 (s, 3 H, Me), 3.30 (m, 2 H, $NC\underline{H}_2CH_2CH_2CO_2Bu^t$), 4.19 (q, J = 7.2, 2 H, CO_2Et), 5.28 (q, J = 0.8, 1 H, $\underline{H}$C=), 6.38 (s, 1 H, Ar), 7.10 (s, 1 H, Ar) ppm. ^{13}C NMR (125.7 MHz, $CDCl_3$): δ = 14.2(+), 15.9(+), 18.7(+), 22.6(-), 29.1(+), 31.4(-), 43.9(-), 57.8(q), 60.7(-), 96.9(+), 108.7(q), 114.2(q), 119.1(+), 120.4(q), 126.2(q), 130.2(+), 147.2(q), 148.4(q), 153.8(q), 158.1(q), 172.8(q) ppm. MS (ESI): *m/z* (negative mode, rel. int., %) = 404.1/402.1 (^{37}Cl/^{35}Cl, 17/48)$[M-H]^-$; HRMS ($C_{22}H_{26}ClNO_4$): 404.1449/402.1482 (found $[M-H]^-$), 404.1450/402.1478 (calc.); *m/z* (positive mode, rel. int., %) = 831.3/829.3 (^{37}Cl/^{35}Cl, 36/100) $[2M+Na]^+$, 438.1/426.1 (^{37}Cl/^{35}Cl, 13/36) $[M+Na]^+$; HRMS ($C_{22}H_{26}ClNO_4$): 428.1410/426.1436 (found $[M+Na]^+$), 428.1415/426.1443 (calc.).



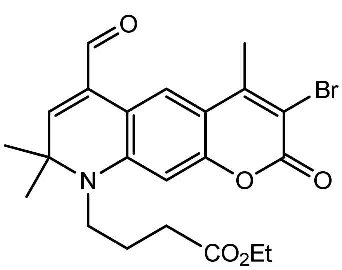
Ethyl (8,9-dihydro-6,8,8-trimethyl-2*H*-pyrano[3,2-g]quinolin-2-one)-9-butanoate (13-H,H): Compound **4-**Et and ethyl (triphenylphosphoranylidene)acetate were dissolved in *p*-xylene, and the obtained solution was refluxed for 3 h under Ar. After cooling to RT, the reaction mixture was filtered to remove triphenylphosphine oxide, and the solvent evaporated *in vacuo*. The residue was subjected to column chromatography (100 g SiO_2; cyclohexane/EtOAc, 4:1 → 2:1) to furnish 2.4 g (73 %) of the title product. ^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ = 1.28 (t, J = 7.2, 3 H, $CO_2CH_2C\underline{H}_3$), 1.35 (s, 6 H, 2×CH_3), 1.87 (m, 2 H, $NCH_2C\underline{H}_2CH_2CO_2Et$), 1.96 (d, J = 1.4, 3 H, CH_3), 2.39 (m, 2 H, $NCH_2CH_2C\underline{H}_2CO_2Et$), 3.30 (m, 2 H, $NC\underline{H}_2CH_2CH_2CO_2Et$), 4.17 (q, J = 7.2, 2 H, $CO_2C\underline{H}_2CH_3$), 5.26 (q, J = 1.4, 1 H, HC=), 6.01 (d, J = 9.3, 1 H), 6.34 (s, 1 H, Ar), 6.99 (s, 1 H, Ar), 7.49 (d, J = 9.3, 1 H). ^{13}C NMR (125.7 MHz, $CDCl_3$): δ = 14.2 (+), 18.6 (+), 22.6 (-), 29.0 (+), 31.3 (-), 43.8 (-), 57.8 (q), 60.6 (-), 97.1 (+), 108.3 (q), 109.4 (+), 120.1 (q), 122.2 (+), 126.3 (q), 129.8 (+), 143.8 (+), 147.5 (q), 156.6 (q), 162.2 (q), 172.8 (q). MS (ESI+): m/z = 356 $[M+H]^+$, 378 $[M+Na]^+$. HRMS ($C_{21}H_{24}NO_4$): 356.1852 (found $[M+H]^+$), 356.1856 (calc.).



Ethyl (8,9-dihydro-6-formyl-4,8,8-trimethyl-2*H*-pyrano[3,2-*g*]quinolin-2-one)-9-butanoate (14-H,Me): Finely powdered SeO_2 (676 mg, 6.09 mmol) was added to a hot (90°C) solution of **13**-H,Me (1.50 g, 4.06 mmol) in dioxane (30 mL). Then the reaction mixture was stirred at 100 °C for 1 h, until it was complete. After cooling, dioxane was evaporated *in vacuo*, the residue was diluted with dichloromethane (50 mL) and the organic layer was washed with sat. aq. $NaHCO_3$ and dried over $MgSO_4$. Solvents were evaporated, and the residue was purified by column chromatography with cyclohexane / dichloromethane / ether (2 / 2 / 0.6) as an eluent. In cyclohexane / dichloromethane / ether mixture (1 / 1 / 1), the starting compound **13**-H,Me had R_f = 0.56 (blue fluorescent spot), and the non-fluorescence spot of product **14**-H,Me had R_f = 0.39. Fractions containing the product were collected and, after evaporation of solvents, the residue was triturated with ether affording 794 mg (51%) of a yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.28 (t, J = 7.2, 3 H, CO_2Et), 1.50 (s, 6 H, 2×Me), 1.90 (m, 2 H, $NCH_2C\underline{H}_2CH_2CO_2Et$), 2.37 (d, 3 H, J = 0.8, 3 H, Me), 2.39 (m, 2 H, $NCH_2CH_2C\underline{H}_2CO_2Et$), 3.32 (m, 2 H, $NC\underline{H}_2CH_2CH_2CO_2Et$), 4.17 (q, J = 7.2, 2 H, CO_2Et), 5.97 (q, J = 0.8, 1 H, $\underline{H}$C=), 6.21 (s, 1 H, Ar), 6.43 (s, 1 H, Ar), 8.54 (s, 1 H, $\underline{H}$C=), 9.58 (s, 1 H, HC=O) ppm. ^{13}C NMR (125.7 MHz, $CDCl_3$): δ = 14.3(+), 18.6(+), 22.9(-), 27.9(+), 31.3(-), 43.9(-), 58.0(q), 60.7(-), 98.3(+), 109.7(+), 109.9(q), 113.4(q), 122.3(+), 130.8(q), 146.9(q), 152.0(+), 153.4(q), 156.0(q), 161.6(q), 172.6(q), 191.8(+) ppm. MS (ESI): *m/z* (positive mode, rel. int., %) = 761.4 (100) $[2M+Na]^+$, 392.3 (70) $[M+Na]^+$, 370.3 (18) $[M+H]^+$.

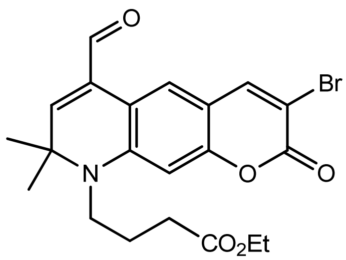


Ethyl (8,9-dihydro-6-formyl-8,8-dimethyl-2*H*-pyrano[3,2-g]quinolin-2-one)-9-butanoate (14-H,H): In a typical experiment, a 10 ml flask was charged with solution of compound **13**-H,H (300 mg; 0.845 mmol) in dioxane (4 ml) and finely powdered SeO_2 (117 mg; 1.06 mmol). The resulted suspension was refluxed for 3.5 h, then water (0.5 ml) was added and the reaction mixture was allowed to cool to RT. All volatile materials were evaporated *in vacuo*, the residue was dissolved in CH_2Cl_2 and washed with saturated aq. $NaHCO_3$. Organic extract was dried with Na_2SO_4, evaporated to give a crude product. The title compound was isolated as a yellow powder (235 mg; 75 %) by column chromatography (30 g SiO_2, hexane/EtOAc, 1:1). ^{1}H NMR (300 MHz, $CDCl_3$, ppm): δ = 1.28 (t, *J* = 7.1, 3 H, CH_3), 1.50 (s, 6 H, 2×CH_3), 1.85–1.97 (m, 2 H, CH_2), 2.40 (t, *J* = 6.8, 2 H, CH_2), 3.29–3.37 (m, 2 H, NCH_2), 4.16 (q, *J* = 7.1, 2 H, CH_2), 6.07 (d, *J* = 9.3, 1 H_{ar}), 6.21 (s, 1 H), 6.44 (s, 1 H_{ar}), 7.56 (d, *J* = 9.3, 1 H_{ar}), 8.39 (s, 1 H_{ar}), 9.56 (s, 1 H, CHO). ^{13}C NMR (125 MHz, $CDCl_3$): δ = 14.3 (CH_3), 22.9 (CH_2), 28.0 (2×CH_3), 31.3 (CH_2), 44.0 (CH_2), 58.1 (C), 60.8 (CH_2), 98.3 (CH), 109.0 (C), 110.5 (CH), 113.5 (C), 125.4 (CH), 130.6 (C), 143.9 (CH), 147.0 (C), 151.9 (CH), 156.6 (C), 161.6 (C), 172.6 (C=O), 191.6 (CHO). MS (ESI+): m/z = 370 $[M+H]^+$, 392 $[M+Na]^+$. HRMS: calcd. for $C_{21}H_{23}NO_5$ $[M+H]^+$ 370.1649; found 370.1640.

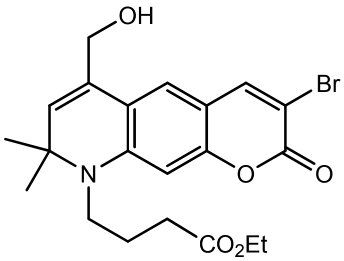


Ethyl (3-bromo-6-formyl-8,9-dihydro-4,8,8-trimethyl-2*H*-pyrano[3,2-*g*]quinolin-2-one)-9-butanoate (14-Br,Me): A solution of bromine (184 mg, 1.15 mmol) in AcOH (1 mL) was added to a solution of **14-**H,Me (421 mg, 1.09 mg) in AcOH (8 mL). The reaction mixture was stirred at RT for 10 min and left in refrigerator (5 ^{o}C) for 1 h. The precipitate was filtered, washed with cold ether and dried; yield – 571 mg of the crude product. It was dissolved in dichloromethane, washed with sat. aq. $NaHCO_3$ and passed through a plug of silica gel with dichloromethane / ether = 1 / 1 as an eluent. After evaporation of solvents, the title compound was precipitated from ether with hexane; 530 mg (corresponds to 96 % yield) of yellow crystals we obtained; solvate with CH_2Cl_2 according to ^{1}H NMR (**14**-Br,Me·1/2CH_2Cl_2). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.28 (t, *J* = 7.2, 3 H, CO_2Et), 1.50 (s, 6 H, 2×Me), 1.90 (m, 2 H, $NCH_2C\underline{H_2}CH_2CO_2Et$), 2.40 (m, 2 H, $NCH_2CH_2C\underline{H_2}CO_2Et$), 2.55 (s, 3H, Me), 3.32 (m, 2 H, $NC\underline{H_2}CH_2CH_2CO_2Et$), 4.17 (q, *J* = 7.2, 2 H,

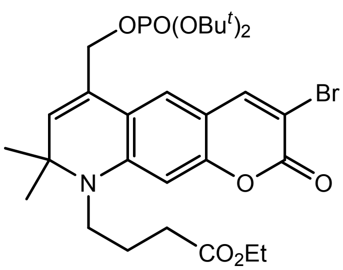
CO_2Et), 5.27 (1 H, ½·CH_2Cl_2), 6.23 (s, 1 H, Ar), 6.44 (s, 1 H, Ar), 8.62 (s, 1 H, <u>H</u>C=), 9.49 (s, 1 H, HC=O) ppm. ^{13}C NMR (125.7 MHz, $CDCl_3$): δ = 14.3(+), 19.4(+), 22.8(-), 28.1(+), 31.2(-), 44.0(-), 58.2(q), 60.8(-), 98.0(+), 106.7(q), 109.8(q), 113.8(q), 122.8(+), 130.5(q), 147.0(q), 151.9(q), 152.3(+), 154.4(q), 157.6(q), 172.6(q), 191.7(+) ppm. MS (ESI): *m/z* (negative mode, rel. int., %) = 492.1/490.1 ($^{81}Br/^{79}Br$, 100) $[M+MeOH–H]^-$, 462.1/460.1 ($^{81}Br/^{79}Br$, 75) $[M–H]^-$; HRMS ($C_{22}H_{24}NO_5Br$): 462.0754/460.0752 (found $[M–H]^-$), 462.0746/460.0765 (calc.); *m/z* (positive mode, rel. int., %) = 949.1/947.1/945.1 (50/100/50) $[2M+Na]^+$, 486.1/484.1 ($^{81}Br/^{79}Br$, 59) $[M+Na]^+$, 464.1/462.1 ($^{81}Br/^{79}Br$, 59) $[M+H]^+$; HRMS ($C_{22}H_{24}NO_5Br$): 486.0697/484.0725 (found $[M+Na]^-$), 486.0711/484.0730 (calc.); 464.0888/462.0897 (found $[M+H]^-$), 464.0891/464.0911 (calc.).



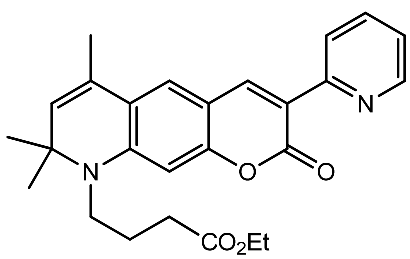
Ethyl (3-bromo-8,9-dihydro-6-formyl-8,8-dimethyl-2*H*-pyrano[3,2-*g*]quinolin-2-one)-9-butanoate (14-Br,H): To a solution of compound **14**-H,H (1.03 g, 2.8 mmol) in acetic acid (10 ml), bromine solution (537 mg; 3.36 mmol in 5 ml acetic acid) was added dropwise with stirring. After 1 h, the reaction mixture was poured into water, and the resulted slurry was extracted with CH_2Cl_2 (3×20 mL). The combined organic solutions were dried with Na_2SO_4 and evaporated. The crude product was purified by column chromatography (100 g SiO_2, hexane/EtOAc, 1:1) to yield 1.24 g (90%) of title compound as a yellow powder; solvate with CH_2Cl_2 (according to ^1H NMR): **14**-Br,H·1/2CH_2Cl_2. ^1H NMR (400 MHz, $CDCl_3$, ppm): δ = 1.28 (t, J = 7.1, 3 H, CH_3), 1.51 (s, 6 H, 2×CH_3), 1.85–1.95 (m, 2 H, CH_2), 2.40 (t, J = 6.8, 2 H, CH_2), 3.29–3.37 (m, 2 H, NCH_2), 4.17 (q, J = 7.1, 2 H, CH_2), 5.27 (1 H, ½CH_2Cl_2), 6.23 (s, 1 H), 6.44 (s, 1 H_{ar}), 7.92 (s, 1 H_{ar}), 8.38 (s, 1 H_{ar}), 9.56 (s, 1 H, CHO). ^{13}C NMR (125.7 MHz, $CDCl_3$): δ = 14.3 (CH_3), 22.8 (CH_2), 28.1 (2×CH_3), 31.2 (CH_2), 44.1 (CH_2), 58.3 (C), 60.8 (CH_2), 98.0 (CH), 104.4 (C), 109.5 (C), 113.9 (C), 124.7 (CH), 130.3 (C), 144.9 (CH), 147.2 (C), 152.0 (CH), 155.9 (C), 157.8 (C), 172.5 (C=O), 191.4 (CHO). MS (ESI+): m/z = 448 $[M+H]^+$, 470 $[M+Na]^+$. HRMS: calcd. for $C_{21}H_{22}BrNO_5$ $[M+H]^+$ 448.0754; found 448.0741.



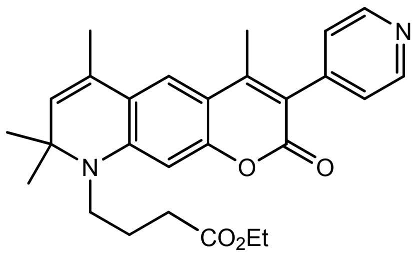
Ethyl (3-bromo-6-hydroxymethyl-8,8-dimethyl-2*H*-pyrano[3,2-g]quinoline-2-one)-9-butanoate (15-Br,H,H): To an ice-cooled solution of **14**-Br,H (100 mg, 0.22 mmol) in a solvent mixture (5 mL, THF/MeOH, 1:1), $CeCl_3$ (54 mg, 0.22 mmol) was added with stirring. After its dissolution, $NaBH_4$ (8.5 mg, 0.22 mmol) was added in one portion. The reaction mixture was stirred for 5 min, acetone (5 mL) was added, and reaction mixture was allowed to warm up to RT. All volatile materials were evaporated *in vacuo*, the residue was taken-up in water (10 mL) and extracted with $CHCl_3$ (3×10 mL). The combined organic extracts were dried (Na_2SO_4) and concentrated to give crude product. The title compound was purified by column chromatography (25 g of SiO_2, CH_2Cl_2/MeOH = 25/1) and isolated as a yellow amorphous solid (96 mg, 95 %).^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ = 1.30 (t, J = 7.1, 3 H, CH_3), 1.42 (s, 6 H, 2×CH_3), 1.87–1.96 (m, 2 H, CH_2), 2.41 (t, J = 6.9, 2 H, CH_2), 3.29–3.35 (m, 2 H, NCH_2), 4.19 (q, J = 7.1, 2 H, CH_2), 4.45 (broad s, 2 H, CH_2), 5.55 (t, J = 1.2, 1 H), 7.1 (s, 1 H_{ar}), 7.87 (s, 1 H_{ar}). ^{13}C NMR (125.7 MHz, $CDCl_3$): δ = 14.2 (CH_3), 22.6 (CH_2), 29.0 (2×CH_3), 31.3 (CH_2), 44.0 (CH_2), 57.9 (C), 60.7 (C), 63.0 (CH_2), 97.2 (CH), 103.4 (C), 109.1 (C), 117.8 (C), 121.5 (CH), 129.5 (C), 130.2 (CH), 144.7 (CH), 147.8 (C), 155.9 (C), 172.7 (C=O). MS (ESI+): m/z = 449 $[M+H]^+$, 471 $[M+Na]^+$. HRMS: calcd. for $C_{26}H_{28}N_2O_5$ $[M+H]^+$ 449.2071; found 449.2071.



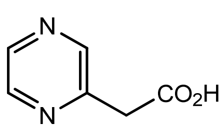
(3-Bromo-8,9-dihydro-9-ethoxycarbonylpropyl-8,8-dimethyl-2*H*-pyrano[3,2-g]quinolin-2-one)-6-methyl di-*tert*-butyl phosphate (15-Br,PO(OBu^t)$_2$,H): To a stirred and preheated (40 ºC) solution of compound **15-**Br,H,H (130 mg; 0.29 mmol) in CH_2Cl_2 (10 mL), di-*tert*-butyl *N,N*-diisopropyl phosphoramidite (240 mg, 0.87 mmol) and 1*H*-tetrazole (65 mg, 0.93 mmol) were added in two equal portions at interval of 20 min under Ar. After another 20 min, the reaction mixture was cooled with an ice bath, and the solution of MCPBA (214 mg, 70% content, 0.87 mmol) in CH_2Cl_2 was added. After stirring for additional 30 min, aqueous solutions of 10% Na_2SO_3 (4 mL) and sat. $NaHCO_3$ (5 mL) were added, and the reaction mixture was allowed to warm up to RT. The organic layer was separated and the aqueous phase was extracted with CH_2Cl_2 (3×20 mL). The combined organic solutions were dried (Na_2SO_4), evaporated, and the titled compound was isolated by column chromatography (30 g SiO_2, hexane/EtOAc, 1:1) as a yellow amorphous solid (162 mg, 87 %). ^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ = 1.29 (t, J = 7.1, 3 H, CH_3), 1.41 (s, 6 H, 2×CH_3), 1.48 (s, 18 H, 2×*t*Bu), 1.86–1.95 (m, 2 H, CH_2), 2.40 (t, J = 6.8, 2 H, CH_2), 3.28–3.34 (m, 2 H, NCH_2), 4.18 (q, J = 7.1, 2 H, CH_2), 4.72 (d, $^3J_{HP}$ = 7.4, 2 H, CH_2), 5.61 (s, 2 H, CH_2), 6.38 (s, 1 H_{ar}), 7.13 (s, 1 H_{ar}), 7.86 (s, 1 H_{ar}). ^{13}C NMR (125.7 MHz, $CDCl_3$): δ = 14.2 (CH_3), 22.6 (CH_2), 28.9 (2×CH_3), 29.9 (d, $^3J_{CP}$ = 4.3, 6CH_3, 2×*t*Bu), 31.3 (CH_2), 44.0 (CH_2), 58.0 (C), 60.7 (C), 66.0 (d, $^2J_{CP}$ = 5.5, CH_2), 82.7 (d, $^2J_{CP}$ = 7.4, 2×C, 2×*t*Bu), 97.2 (CH), 103.5 (C), 109.1 (C), 117.4 (C), 121.6 (CH), 126.1 (d, $^3J_{CP}$ = 7.8, C), 132.0 (CH), 144.7 (CH), 147.6 (C), 155.9 (C), 158.1 (C), 172.7 (C=O). MS (ESI+): m/z = 642 $[M+H]^+$, 664 $[M+Na]^+$. HRMS: calcd. for $C_{29}H_{41}BrNO_8P$ $[M+H]^+$ 642.1826; found 642.1818.



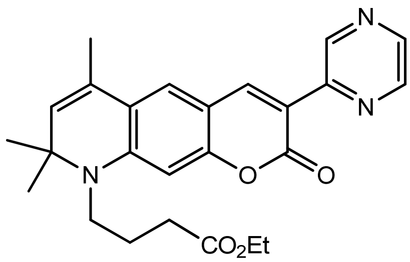
Ethyl (6,8,8-trimethyl-3-(pyridin-2-yl)-2*H*-pyrano[3,2-g]quinolin-2-one)-9-butanoate (16-H): To a suspension of 2-pyridylacetic acid hydrochloride (394 mg, 2.27 mmol) in DMF (5 mL), NEt_3 (400 µL) was added. The resulted mixture was stirred for 5 min, then the solution of aldehyde **4**-Et (500 mg, 1.51 mmol) in CH_2Cl_2 (10 mL), EDC·HCl (436 mg, 2.27 mmol), NEt_3 (800 µL), DMAP (18.3 mg, 0.15 mmol) were added in a given order. The reaction mixture was stirred overnight at RT, water (15 mL) was added, and the organic phase was separated. The aqueous layer was extracted with CH_2Cl_2. Combined organic solutions were dried with Na_2SO_4 and evaporated. After a column chromatography (100 g SiO_2, hexane/EtOAc, 2:1), the titled product was isolated in 35 % yield (227 mg) as an orange solid. ^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ = 1.29 (t, J = 7.2, 3 H, CH_3), 1.38 (s, 6 H, 2×CH_3), 1.89–1.97 (m, 2 H, CH_2, overlapped), 1.97 (d, J = 1.3, 3 H, CH_3), 2.40 (t, J = 6.9, 2 H, CH_2), 3.29–3.37 (m, 2 H, NCH_2), 4.18 (q, J = 7.2, 2H, CH_2), 5.28 (q, J = 1.3, 1 H), 6.37 (s, 1 H_{ar}), 7.16 (s, 1 H_{ar}), 7.17 (ddd, J = 7.8, 4.8, and 1.0, 1 $H_{ar,}$ overlapped), 7.70 (ddd, J = 8.1, 7.8 and 1.9, 1 H_{ar}), 8.40 (ddd, J = 8.1, 1.0 and 1.0 Hz, 1 H_{ar}), 8.60 (ddd, J = 4.8, 1.9, and 1.0, 1 H_{ar}), 8.64 (s, 1 H_{ar}). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 14.2 (CH_3), 18.7 (2×CH_3), 22.7 (CH_2), 29.2 (CH_3), 31.4 (CH_2), 44.0 (CH_2), 58.1 (C), 60.7 (CH_2), 96.3 (CH), 109.1 (C), 117.8 (C), 120.4 (C), 122.1 (CH), 123.2 (2×CH), 126.2 (C), 129.6 (CH), 136.4 (CH), 142.9 (CH), 148.0 (C), 149.0 (CH), 152.5 (C), 156.7 (C), 161.3 (C=O), 172.7 (C=O). MS (ESI+): m/z = 356 $[M+H]^+$. HRMS: calcd. for $C_{26}H_{28}N_2O_4$ $[M+H]^+$ 433.2122; found 433.2123.



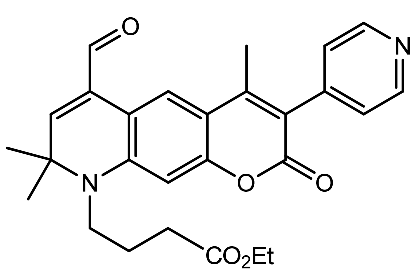
Ethyl (4,6,8,8-tetramethyl-3-(pyridin-4-yl)-2*H*-pyrano[3,2-g]quinolin-2-one)-9-butanoate (16-Me): compound **13**-Cl,Me (500 mg, 1.24 mmol), $Pd(dba)_2$ (36 mg, 0.062 mmol) and toluene (6 mL) were placed into a screw-cap bottle and closed with a septum. The mixture was purded with argon, before a dioxane solution of PBu^t_3 (0.395 M, 0.47 mL, 0.186 mmol) was added. The reaction mixture was stirred for 5 min, then 4-tributylstannylpyridyne (543 mg, 1.49 mmol) was added. The flask (bottle) was closed with a screw-cap, and the reaction mixture was stirred at 110°C for 20 h. After cooling, the reaction mixture was separated by column chromatography (with dichloromethane / ether = 2 / 1 → dichloromethane / ether / acetone = 3 / 2 / 1 as an eluent). Precipitation from cyclohexane afforded the pure compound **16**-Me as orange crystals (185 mg, 33% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.30 (t, *J* = 7.2, 3 H, CO_2Et), 1.40 (s, 6 H, 2×Me), 1.95 (m, 2 H, $NCH_2C\underline{H_2}CH_2CO_2Bu^t$), 2.02 (d, 3 H, *J* = 0.8, 3 H, Me), 2.24 (s, 3H, Me), 2.41 (m, 2 H, $NCH_2CH_2C\underline{H_2}CO_2Bu^t$), 3.33 (m, 2 H, $NC\underline{H_2}CH_2CH_2CO_2Bu^t$), 4.20 (q, *J* = 7.2, 2 H, CO_2Et), 5.32 (q, *J* = 0.8, 1 H, $\underline{H}$C=), 6.40 (s, 1 H, Ar), 7.19 (s, 1 H, Ar), 7.24 (m, 2 H, AA´ part of AA´BB´ system), 8.65 (m, 2 H, BB´ part of AA´BB´ system) ppm. ^{13}C NMR (125.7 MHz, $CDCl_3$): δ = 14.3(+), 16.3(+), 18.8(+), 22.7(-), 29.1(+), 31.4(-), 43.9(-), 57.9(q), 60.7(-), 96.9(+), 109.0(q), 118.4(q), 119.5(+), 120.2(q), 125.7(+), 126.3(q), 130.1(+), 143.7(q), 147.6(q), 149.2(q), 149.7(+), 155.1(q), 161.0(q), 172.8(q) ppm. MS (ESI): *m/z* (negative mode, rel. int., %) = 445.2 (76) $[M-H]^-$; HRMS ($C_{27}H_{30}N_2O_4$): 445.2129 (found $[M-H]^-$), 445.2133 (calc.); *m/z* (positive mode, rel. int., %) = 915.4 (100) $[2M+Na]^+$, 469.2 (64) $[M+Na]^+$, 447.2 (32) $[M+H]^+$; HRMS ($C_{27}H_{30}N_2O_4$): 469.2091 (found $[M+Na]^+$), 469.2098 (calc.). λ_{abs} = 410 nm, λ_{em} = 502 nm, ε = 34200 $M^{-1}cm^{-1}$, $\Phi_{fl.}$ = 0.50 (MeOH). Standard: Coumarin 307, $\Phi_{fl.}$ = 0.56 (EtOH).



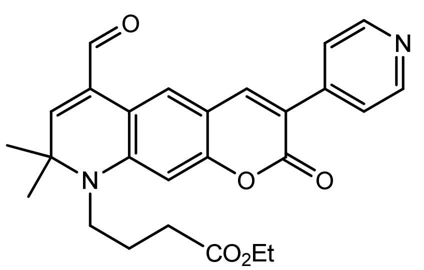
(Pyrazin-2-yl)acetic acid. A 100 mL Schlenk flask was charged with a solution of *i*Pr_2NH (1.29 g, 12.8 mmol) in THF (15 mL), cooled down to –78 °C, and a solution of BuLi (1.6 M in hexanes, 8 mL, 12.8 mmol) was added dropwise. After stirring for 30 min at this temperature, 2-methylpyrazine (1.0 g, 10.6 mmol) was added. The reaction mixture was stirred for 1 h, and quenched with an excess of solid CO_2. The cooling bath was removed, the mixture was allowed to warm to RT, and water was added, until the liquid phases separated. A pH-value was adjusted to 3 with conc. aq. HCl which was added with stirring and cooling with ice-water. The product was extracted with EtOAc (8×50 mL); combined organic solutions were dried over Na_2SO_4. Solvents were evaporated *in vacuo* at RT to furnish 915 mg (62 %) of orange powder. This crude product was used directly in the next step without further purification. ^{1}H NMR (300 MHz, CD_3OD, ppm): δ = 3.89 (s, 2 H, CH_2), 8.49 (d, *J* = 2.6, 1 H_{ar}), 8.54 (dd, *J* = 2.6 and 1.5, 1 H_{ar}), 8.62 (d, *J* = 1.5, 1 H_{ar}).



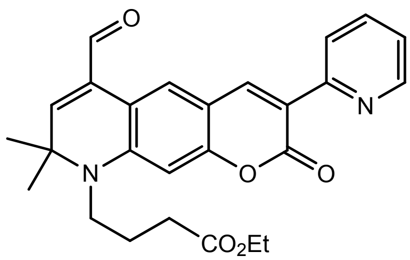
Ethyl (8,9-dihydro-6,8,8-trimethyl-3-(pyrazin-2-yl)-2*H*-pyrano[3,2-g]quinolin-2-one)-9-butanoate (17-H): To a solution of **4-**Et (166 mg, 0.500 mmol) in CH_2Cl_2 (3 mL), (pyrazin-2-yl)acetic acid (69 mg, 0.50 mmol), NEt_3 (106 mg, 1.05 mmol), DCC (103 mg, 0.5 mmol) and DMAP (6 mg, 10 mol %) were added in a given sequence. The resulting mixture was stirred overnight. The precipitate was filtered off, and the filtrate evaporated under reduced pressure. The residue was subjected to column chromatography (25 g of SiO_2; CH_2Cl_2/MeOH, 30:1) to yield 100 mg (46%) of the title product as an orange powder. ^{1}H NMR (300 MHz, $CDCl_3$, ppm): δ = 1.29 (t, *J* = 7.1, 3 H, CH_3), 1.39 (s, 6 H, 2×CH_3), 1.89-1.97 (m, 2 H, CH_2), 1.98 (d, *J* = 1.4, 3 H, CH_3), 2.41 (t, *J* = 6.9, 2 H, CH_2), 3.30–3.39 (m, 2 H, CH_2), 4.18 (q, *J* = 7.1, 2 H, CH_2), 5.30 (q, *J* = 1.4, 1 H), 6.39 (s, 1 H_{ar}), 7.15 (s, 1 H_{ar}), 8.45 (d, *J* = 2.5, 1 H_{ar}), 8.54 (dd, *J* = 2.5 and 1.5, 1 H_{ar}), 8.64 (s, 1 H_{ar}), 9.66 (d, *J* = 1.5, 1 H_{ar}). ^{13}C NMR (125 MHz, $CDCl_3$): δ = 14.2 (CH_3), 18.6 (2×CH_3), 22.5 (CH_2), 29.2 (CH_3), 19.6 (C), 31.3 (CH_2), 44.1 (CH_2), 58.3 (C), 60.7 (CH_2), 96.3 (CH), 109.1 (C), 114.9 (C), 120.6 (C), 123.4 (CH), 126.1 (C), 129.9 (CH), 142.3 (CH), 143.6 (CH), 144.0 (CH), 144.4 (CH), 148.8 (C), 157.3 (C), 160.8 (C), 172.8 (C=O). MS (ESI+): m/z = 434 $[M+H]^+$, 456 $[M+Na]^+$. HRMS: calcd. for $C_{25}H_{27}N_3O_4$ $[M+H]^+$ 456.1894; found 456.1878.



Ethyl (8,9-dihydro-6-formyl-4,8,8-trimethyl-3-(pyridin-4-yl)-2*H*-pyrano[3,2-*g*]quinolin-2-one)-9-butanoate (18-Me). *1. The Suzuki coupling*: Into a screw-cap bottle closed with a septum, compound **14-**Br,Me (316 mg, 0.628 mmol) and 4-pyridylboronic acid (93 mg, 0.7532 mmol) were placed; toluene (6 mL) and EtOH (1.5 mL) were added, and the mixture was purged with argon, before $Pd(PPh_3)_4$ (36 mg, 0.031 mmol) and 2 M aq. Na_2CO_3 (1.25 mL) were added. The flask (bottle) was closed with screw-cap, and the reaction mixture was stirred at 110 ^oC for 6 h. After cooling, it was diluted with water and extracted with dichloromethane. Purification by column chromatography (with dichloromethane / ether = 2 / 1 → dichloromethane / ether / acetone = 3 / 2 / 1 as an eluent) followed by precipitation from ether afforded the pure compound **18**-Me (194 mg, 67%). *2) By oxidation with selenium dioxide:* Finely powdered SeO_2 (125 mg, 1.13 mmol) was added to a hot (90°C) solution of compound **16**-Me (335 mg, 0.73 mmol) in dioxane (20 mL). Then the reaction mixture was stirred at 110 ^oC (oil bath temperature) for 2 h, until the reaction was complete. After cooling, dioxane was evaporated *in vacuo*, the residue was diluted with dichloromethane (50 mL), and the organic layer was washed with sat. aq. $NaHCO_3$ and dried over $MgSO_4$. Solvents were evaporated, and the residue was purified by column chromatography (with cyclohexane / dichloromethane / ether = 2 / 2 / 1 as an eluent). After evaporation of solvents, the product was precipitated from ether to yield 251 mg (75%) of a yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.28 (t, J = 7.2, 3 H, CO_2Et), 1.53 (s, 6 H, 2×Me), 1.92 (m, 2 H, $NCH_2C\underline{H}_2CH_2CO_2Et$), 2.27 (s, 3 H, Me), 2.40 (m, 2 H, $NCH_2CH_2C\underline{H}_2CO_2Et$), 3.35 (m, 2 H, $NC\underline{H}_2CH_2CH_2CO_2Et$), 4.18 (q, J = 7.2, 2 H, CO_2Et), 6.24 (s, 1 H, Ar), 6.47 (s, 1 H, Ar), 7.23 (m, 2 H, AA´ part of AA´XX´ system), 8.65 (m, 2 H, XX´ part of AA´XX´ system), 8.67 (s, 1H, $\underline{H}$C=) 9.68 (s, 1 H, $\underline{H}$C=O) ppm. ^{13}C NMR (125.7 MHz, $CDCl_3$): δ = 14.3(+), 16.5(+), 22.9(-), 28.0(+), 31.3(-), 44.0(-), 58.2(q), 60.8(-), 98.1(+), 109.9(q), 113.7(q), 119.3(q), 123.1(+), 125.5(+), 130.6(q), 143.2(q), 147.1(q), 149.7(+), 152.2(+), 154.4(q), 155.3(q), 160.7(q), 172.6(q), 191.7(+) ppm. MS (ESI): *m/z* (negative mode, rel. int., %) = 491.2 (76) $[M+MeOH–H]^-$, 459.2 (100) $[M-H]^-$; HRMS ($C_{27}H_{28}N_2O_5$): 459.1914 (found $[M-H]^-$), 459.1925 (calc.); *m/z* (positive mode, rel. int., %) = 953.4 (24) $[2M+Na]^+$, 921.4 (70) $[2M+H]^+$, 461.2 (100) $[M+H]^+$; HRMS ($C_{27}H_{28}N_2O_5$): 461.2072 (found $[M+H]^+$), 461.2071 (calc.).

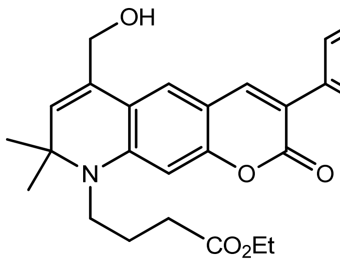


Ethyl (6-formyl-8,8-dimethyl-3-(pyridin-4-yl)-2*H*-pyrano[3,2-g]quinolin-2-one)-9-butanoate (18-H): According to procedures *1)* and *2)* described above for compound **18**-Me, the title product was obtained in 76% yield (170 mg from 224 mg of **14**-Br,H) and 90% yield (228 mg from 245 mg of **5**-Et), respectively. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.30 (t, J = 7.2, 3 H, CO_2Et), 1.56 (s, 6 H, 2×Me), 1.93 (m, 2 H, $NCH_2C\underline{H}_2CH_2CO_2Et$), 2.43 (m, 2 H, $NCH_2CH_2C\underline{H}_2CO_2Et$), 3.40 (m, 2 H, $NC\underline{H}_2CH_2CH_2CO_2Et$), 4.21 (q, J = 7.2, 2 H, CO_2Et), 6.27 (s, 1 H, Ar), 6.52 (s, 1 H, Ar), 7.67 (m, 2 H, AA´ part of AA´XX´ system), 8.52 (s, 1H, $\underline{H}$C=), 8.65 (m, 2 H, XX´ part of AA´XX´ system), 9.60 (s, 1 H, $\underline{H}$C=O) ppm. ^{13}C NMR (125.7 MHz, $CDCl_3$): δ = 14.3(+), 22.8(-), 28.2(+), 31.2(-), 44.1(-), 58.4(q), 60.8(-), 97.8(+), 109.4(q), 114.0(q), 118.7(q), 122.3(+), 126.1(+), 130.4(q), 142.2(+), 143.0(q), 147.9(q), 149.9(+), 152.0(+), 156.8(q), 160.4(q), 172.7(q), 191.7(+) ppm. MS (ESI): *m/z* (positive mode, rel. int., %) = 915.4 (100) $[2M+Na]^+$, 469.2 (99) $[M+Na]^+$, 447.2 (22) $[M+H]^+$; HRMS ($C_{26}H_{26}N_2O_5$): 469.1727 (found $[M+Na]^+$), 469.1734 (calc.); 447.1909 (found $[M+H]^+$), 447.1914 (calc.).

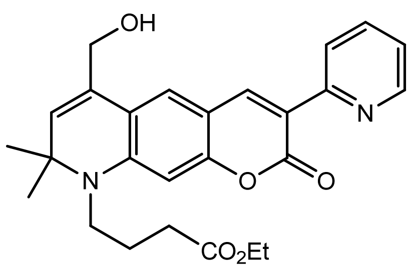


Ethyl (6-formyl-8,8-dimethyl-3-(pyridin-2-yl)-2*H*-pyrano[3,2-g]quinolin-2-one)-9-butanoate (19-H). *1. The Stille coupling:* In a screw-cap tube, compound **14**-Br,H (50 mg, 0.11 mmol), 2-(tributylstannyl)pyridine (43 mg, 0.12 mmol), and $Pd(PPh_3)_4$ (6.3 mg, 5 mol %) in dioxane (1 mL) were purged with a stream of argon. The mixture was heated up to 110°C and stirred overnight at this temperature. Then the reaction mixture was allowed to cool down to RT, diluted with CH_2Cl_2 (10 mL), and water (5 mL) was added. The organic layer was separated; the aqueous phase was extracted with CH_2Cl_2 (3×10 mL). The combined organic extracts were dried and concentrated *in vacuo* to give a crude product. Column chromatography (25 g SiO_2; hexane/EtOAc, 1:1) furnished the desired product as a yellow solid (22 mg, 45 %). *2. Oxidation with selenium dioxide:* a round bottom flask was charged with the solution of compound **16**-H (208 mg, 0.48 mmol) in dioxane (5 mL) and finely powdered SeO_2 (67 mg, 0.60 mmol). The suspension was refluxed for ca. 3.5 h, then water (1 mL) was added, and the reaction mixture was allowed to cool down to RT. All volatile materials were evaporated *in vacuo*; the residue was dissolved in CH_2Cl_2, washed with saturated aq. $NaHCO_3$, dried, and evaporated *in vacuo*. The title compound was isolated as a yellow solid (183 mg, 85 %) by column chromatography (40 g SiO_2, CH_2Cl_2/ether = 10/1).

^{1}H NMR (300 MHz, $CDCl_3$, ppm): δ = 1.29 (t, J = 7.1, 3 H, CH_3), 1.52 (s, 6 H, 2×CH_3), 1.88–1.99 (m, 2 H, CH_2), 2.42 (t, J = 6.8, 2 H, CH_2), 3.32–3.40 (m, 2 H, NCH_2), 4.18 (q, J = 7.1, 2 H, CH_2), 6.22 (s, 1 H), 6.47 (s, 1 H_{ar}), 7.22 (ddd, J = 7.5, 4.8 and 1.0, 1 H_{ar}), 7.74 (ddd, J = 8.1, 7.5 and 1.9, 1 H_{ar}), 8.37 (dt, J = 8.1, 1.0 and 1.0 Hz, 1 H_{ar}), 8.58 (s, 1 H_{ar}), 8.63 (ddd, J = 4.8, 1.9 and 1.0, 1 H_{ar}), 8.71 (s, 1 H_{ar}) 9.58 (s, 1 H, CHO). ^{13}C NMR (125.7 MHz, $CDCl_3$): δ = 14.3 (CH_3), 22.9 (CH_2), 28.2 (2×CH_3), 31.3 (CH_2), 44.2 (CH_2), 58.4 (C), 60.8 (CH_2), 97.5 (CH), 109.7 (C), 113.9 (C), 122.3 (CH), 123.4 (CH), 126.6 (CH), 130.5 (C), 136.7 (CH), 143.5 (CH), 147.6 (C), 148.7 (CH), 151.4 (CH), 151.9 (C), 156.8 (C), 160.8 (C), 172.6 (C=O), 191.3 (CHO). MS (ESI+): m/z = 447 $[M+H]^+$, 469 $[M+Na]^+$. HRMS: calcd. for $C_{26}H_{26}NO_5$ $[M+H]^+$ 447.1914; found 447.1906.

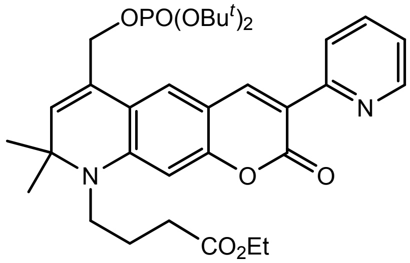


Ethyl (6-hydroxymethyl-8,8-dimethyl-3-(pyridin-4-yl)-2*H*-pyrano[3,2-g]quinolin-2-one)-9-butanoate (20a): From **18**-H (250 mg, 0.558 mmol), according to the procedure described for **15**-Br,H,H, the title compound was obtained in 95% yield (238 mg). ^{1}H NMR (300 MHz, $CDCl_3$, ppm): δ = 1.30 (t, *J* = 7.2, 3 H, CO_2Et), 1.44 (s, 6 H, 2×Me), 1.95 (m, H, $NCH_2C\underline{H}_2CH_2CO_2Et$), 2.00 (br. s, 1 H, $C\underline{H}_2OH$), 2.42 (m, 2 H, $NCH_2CH_2C\underline{H}_2CO_2Et$), 3.40 (m, 2 H, $NC\underline{H}_2CH_2CH_2CO_2Et$), 4.21 (q, *J* = 7.2, 2 H, CO_2Et), 4.48 (d, *J* = 0.8, 2 H, $C\underline{H}_2OH$), 5.60 (d, *J* = 0.8, 1 H, $\underline{H}$C=), 6.42 (s, 1 H, Ar), 7.30 (s, 1 H, Ar), 7.65 (m, 2 H, AA´ part of AA´XX´ system), 7.83 (s, 1 H, Ar), 8.62 (m, 2 H, XX´ part of AA´XX´ system) ppm. ^{13}C NMR (125.7 MHz, $CDCl_3$): δ = 14.3(+), 22.7(-), 29.1(+), 31.3(-), 44.1(-), 58.1(q), 60.8(-), 63.0(-), 97.0(+), 108.9(q), 117.5(q), 117.8(q), 122.3(+), 122.8(+), 129.5(q), 130.2(+), 141.9(+), 143.3(q), 148.4(q), 149.8(+), 156.6(q), 160.6(q), 172.7(q) ppm. MS (ESI): *m/z* (positive mode, rel. int., %) = 449.2 (100) $[M+H]^+$; HRMS ($C_{26}H_{28}N_2O_5$): 449.2074 (found $[M+H]^+$), 449.2071 (calc.).λ_{abs} = 433 nm, λ_{em} = 504 nm, ε = 14100 $M^{-1}cm^{-1}$, $\Phi_{fl.}$ = 0.76 (in MeOH). Standard: Coumarin 522, $\Phi_{fl.}$ = 0.65 (EtOH).



Ethyl (6-hydroxymethyl-8,8-dimethyl-3-(pyridin-2-yl)-2*H*-pyrano[3,2-g]quinolin-2-one)-9-butanoate (21a). To a cooled solution (0 °C) of compound **19**-H (67 mg, 0.15 mmol) in the mixture of THF and MeOH (1:1, total volume 5 mL), powdered $CeCl_3$ (37 mg, 0.15 mmol) was added. The suspension was stirred, until $CeCl_3$ dissolved, and $NaBH_4$ (6 mg, 0.15 mmol) was added in one portion. The bright green fluorescence appeared immediately, and after 5 min, saturated aq. NH_4Cl (5 mL) and water (5 mL) were added. The reaction mixture was extracted with CH_2Cl_2 (4×10 mL), the combined organic extracts were dried with Na_2SO_4 and evaporated. The residue was purified by column chromatography (30 g SiO_2, CH_2Cl_2/MeOH = 25/1) to furnish the title compound as an orange solid (27 mg, 85 %). ^{1}H NMR (300 MHz, $CDCl_3$, ppm): δ = 1.29 (t, *J* = 7.1, 3 H, CH_3), 1.36 (s, 6 H, 2×CH_3), 1.87–1.99 (m, 2 H, CH_2), 2.41 (t, *J* = 6.9, 2 H, CH_2), 3.27–3.36 (m, 2 H, NCH_2), 4.20 (q, *J* = 7.1, 2 H, CH_2), 4.45 (d, *J* = 1.2, 2 H, CH_2), 5.47 (t, *J* = 1.2, 1 H), 6.37 (s, 1 H_{ar}), 7.18 (ddd, *J* = 7.5, 4.8 and 1.0, 1 H_{ar}), 7.25 (s, 1 H_{ar}), 7.71 (ddd, *J* = 8.1, 7.5 and 1.9, 1 H_{ar}), 8.37 (ddd, *J* = 8.1, 1.0 and 1.0, 1 H_{ar}), 8.56 (s, 1 H_{ar}), 8.59 (ddd, *J* = 4.8, 1.9 and 1.0, 1

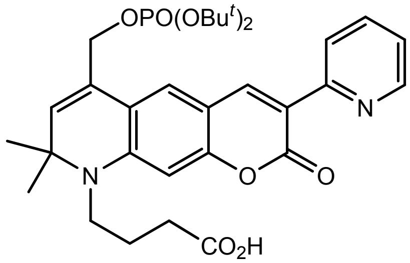
H_{ar}). ^{13}C NMR (125 MHz, $CDCl_3$): δ = 14.4 (CH_3), 22.7 (CH_2), 29.1 (2×CH_3), 31.4 (CH_2), 44.2 (CH_2), 58.0 (C), 60.7 (CH_2), 62.8 (CH_2), 96.7 (CH), 109.1 (C), 117.6 (C), 117.9 (C), 122.1 (CH), 123.0 (CH), 123.3 (CH), 129.3 (CH), 129.5 (C), 136.4 (CH), 142.9 (CH), 148.0 (C), 148.8 (CH), 152.3 (C), 156.6 (C), 161.1 (C), 172.6 (C=O). MS (ESI+): m/z = 449 $[M+H]^+$, 471 $[M+Na]^+$. HRMS: calcd. for $C_{26}H_{28}NO_5$ $[M+H]^+$ 449.2071; found 449.2071. λ_{abs} = 431 nm, λ_{em} = 498 nm, ε = 32000 $M^{-1}cm^{-1}$, $\Phi_{fl.}$ = 0.67 (MeOH). Standard: coumarin 334, $\Phi_{fl.}$ = 0.69 (EtOH).



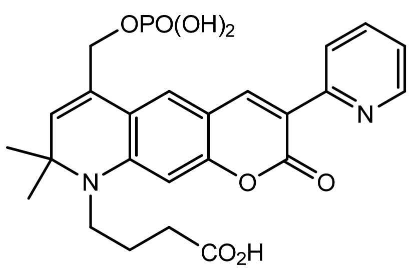
Di-(*tert*-butyl) [9-ethoxycarbonylpropyl-8,9-dihydro-8,8-dimethyl-3-(pyridin-2-yl)-2*H*-pyrano[3,2-*g*]quinolin-2-one]-6-methyl phosphate (21b): To a stirred and warm (40 ºC) solution of compound **21a** (70 mg, 0.16 mmol) in CH_2Cl_2 (10 mL), di-*t*-butyl N,N-diisopropyl phosphoramidite (130 mg, 0.47 mmol) and 1*H*-tetrazole (35 mg, 0.5 mmol) were added under argon in two equal portions at an interval of 20 min. After further 20 min, the reaction mixture was cooled with an ice bath (0 ºC), and solution of MCPBA (115 mg, 70% content, 0.47 mmol) in CH_2Cl_2 was added. After stirring for additional 30 min, 10% aq. solutions of Na_2SO_3 (2 mL) and sat. $NaHCO_3$ (2 mL) were added, and the reaction mixture was allowed to warm up to RT. The organic layer was separated, and the aqueous phase was extracted with CH_2Cl_2 (3×10 mL). The combined organic extracts were dried, the solvents were evaporated, and the titled compound was isolated by column chromatography (30 g of SiO_2, CH_2Cl_2/MeOH = 25/1) as an orange amorphous solid (88 mg, 88 %).

*The preparation of compound **21b** from **15-**Br,PO(OBu^t)$_2$,H:* A 10 mL Schlenk flask was flushed with argon and charged with toluene (0.5 mL), $Pd(OAc)_2$ (1 mg, $4.5 \cdot 10^{-3}$ mmol), the solution of P(*t*-Bu)$_3$ in dioxane (0.395 M, 23 µL, $9 \cdot 10^{-3}$ mmol), the solution of bromide **15-**Br,PO(OBu^t)$_2$,H (48 mg, 75 µmol) in toluene (1mL) and 2-(tributylstannyl)pyridine (30 mg, 82 µmol) added in the given order. The reaction mixtire was stirred at 110 ºC for 2 h, cooled to RT, and water (5 mL) and CH_2Cl_2 were added. The organic phase was separated, and the aqueous layer was extracted with CH_2Cl_2 (3×10 mL). The combined organic extracts were dried with Na_2SO_4 and evaporated. A purification of the crude product by chromatography (30 g of SiO_2, CH_2Cl_2/MeOH = 25/1) give 27 mg (56 %) of the title product as a red solid. ^1H NMR (400 MHz, $CDCl_3$, ppm): δ = 1.31 (t, J = 7.1, 3 H, CH_3), 1.43 (s, 6 H, 2×CH_3), 1.50 (s, 18 H, 2×*t*Bu), 1.90–1.99 (m, 2 H, CH_2), 2.42 (t, J = 6.9, 2 H, CH_2), 3.33–3.38 (m, 2 H, NCH_2), 4.20 (q, J = 7.1, 2 H, CH_2), 4.75 (dd, $^3J_{HP}$ = 7.3, $^4J_{HH}$ = 1.2, 2 H, CH_2), 5.64 (t, J = 1.2, 1 H), 6.42 (s, 1 H_{ar}), 7.19 (ddd, J = 7.5, 4.8 and 1.0, 1 H_{ar}), 7.26 (s, 1 H_{ar}), 7.72 (ddd, J = 8.1, 7.5 and 1.9 Hz, 1 H_{ar}), 8.39 (ddd, J = 8.1, 1.0 and 1.0 Hz, 1 H_{ar}), 8.62 (ddd, J = 4.8, 1.9 and 1.0 Hz, 1 H_{ar}), 8.65 (s, 1 H_{ar}). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 14.3 (CH_3), 22.7 (CH_2), 28.9 (2×CH_3), 29.9 (d, $^3J_{CP}$ = 4.3, CH_3 in 2×*t*Bu), 31.3 (CH_2), 44.1

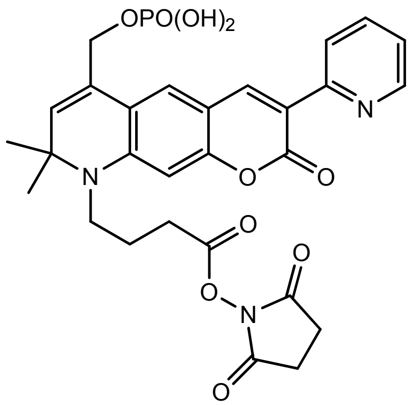
(CH_2), 58.0 (C), 60.7 (CH_2), 65.8 (d, $^2J_{CP}$ = 5.5, CH_2), 82.6 (d, $^2J_{CP}$ = 7.4, 2×C, 2×*t*Bu), 96.7 (CH), 109.3 (C), 117.4 (C), 118.4 (C), 122.2 (CH), 123.1 (CH), 123.2 (CH), 126.2 (d, $^3J_{CP}$ = 7.8, C), 131.1 (CH), 136.4 (CH), 142.9 (CH), 147.9 (C), 149.1 (CH), 152.4 (C), 156.7 (C), 161.2 (C), 172.7 (C=O). MS (ESI+): m/z = 641 $[M+H]^+$, 663 $[M+Na]^+$. HRMS: calcd. for $C_{34}H_{45}N_2O_8P$ $[M+H]^+$ 641.2986; found 641.2986.



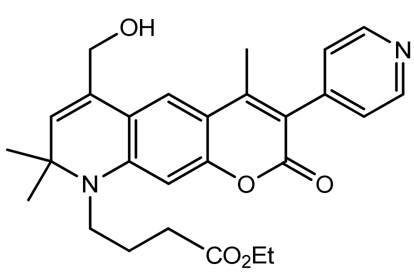
Di-(*tert*-butyl) [9-carboxypropyl-8,9-dihydro-8,8-dimethyl-3-(pyridin-2-yl)-2*H*-pyrano[3,2-*g*]quinolin-2-one]-6-methyl phosphate (21-PO(OBu^t)$_2$,H,H,CH,CH,N): To a solution of **21b** (98 mg, 0.15 mmol) in the solvent mixture (20 mL, THF/water, 3:2), 1 M aq. NaOH (0.6 mL, 0.6 mmol) was added. The reaction mixture was stirred overnight at RT, and acidified to pH 4 with sat. aq. $KHSO_4$. The resulted solution was extracted with EtOAc (5×25 mL), the combined organic solutions were dried and concentrated *in vacuo*. The titled compound was isolated by column chromatography (30 g of SiO_2, CH_2Cl_2/MeOH, 15:1) as a red solid (60 mg, 65 %). ^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ = 1.40 (s, 6 H, 2×CH_3), 1.50 (s, 18 H, 2×*t*Bu), 1.90–2.00 (m, 2 H, CH_2), 2.47 (t, J = 6.8, 2 H, CH_2), 3.33–3.39 (m, 2 H, CH_2), 4.78 (dd, J_{HP} = 7.5, J_{HH} = 1.1, 2 H, CH_2), 5.64 (d, J = 1.1, 1 H), 6.43 (s, 1 H_{ar}), 7.21 (ddd, J = 7.5, 4.9 and 1.1, 1 H_{ar}), 7.73 (ddd, J = 8.0, 7.5 and 1.9, 1 H_{ar}), 8.35 (ddd, J = 8.0, 1.1 and 1.1, 1 H_{ar}), 8.59 (s, 1 H_{ar}), 8.64 (ddd, J = 4.9, 1.9 and 1.1, 1H_{ar}). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 14.1 (CH_3), 22.7 (CH_2), 28.8 (2×CH_3), 29.9 (d, J_{CP} = 4.3, 6×CH_3, 2×*t*Bu), 31.1 (CH_2), 43.9 (CH_2), 58.0 (C), 66.0 (d, J_{CP} = 5.5, CH_2), 83.1 (d, J_{CP} = 7.6, 2×C, 2×*t*Bu), 96.9 (CH), 109.3 (C), 117.5 (C), 118.2 (C), 122.3 (CH), 123.1 (CH), 123.4 (CH), 126.2 (d, J_{CP} = 7.7, C), 131.4 (CH), 136.6 (CH), 143.1 (CH), 148.0 (C), 148.9 (CH), 152.4 (C), 156.7 (C), 161.2 (C), 176.7 (C=O). MS (ESI, negative mode): m/z = 611 $[M-H]^-$. HRMS: calcd. for $C_{32}H_{41}N_2O_8P$ $[M-H]^-$ 611.2528; found 611.2517.



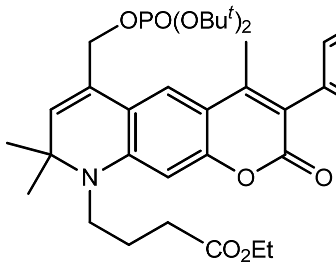
[9-Carboxypropyl-8,9-dihydro-8,8-dimethyl-3-(pyridin-2-yl)-2*H*-pyrano[3,2-*g*]quinolin-2-one]-6-methyl phosphoric acid (21c): To a solution of **21**-PO(OBu^t)$_2$,H,H,CH,CH,N (60 mg, 0.10 mmol) in CH_2Cl_2 (5 mL), trifluoroacetic acid (0.3 mL) was added. The reaction mixture was stirred for 30 min. Then all volatile substances were evaporated *in vacuo*, and the residue was subjected to a column chromatography (20 g SiO_2, MeCN/water, 2:1 + 0.1% NEt_3) to furnish 56 mg (78%) of the title compound as a red amorphous solid (**21c**·3NEt$_3$). ^{1}H NMR (400 MHz, CD$_3$OD, ppm): δ = 1.29 (t, J = 7.3, 27 H, 9×CH$_3$), 1.41 (s, 6 H, 2×CH$_3$), 1.84–1.93 (m, 2 H, CH$_2$), 2.44 (t, J = 6.7, 2 H, CH$_2$), 3.17 (q, J = 7.3, 18 H, 9×CH$_2$), 3.37–3.44 (m, 2 H, CH$_2$), 4.69 (d, J_{HP} = 4.8, 2 H, CH$_2$), 5.71 (s, 1 H), 6.58 (s, 1 H$_{ar}$), 7.28 (ddd, J = 7.5, 4.9 and 1.1, 1 H$_{ar}$), 7.48 (s, 1 H$_{ar}$), 7.81 (ddd, J = 8.0, 7.5 and 1.9, 1 H$_{ar}$), 8.15–8.20 (m, 1 H$_{ar}$), 8.52 (s, 1 H$_{ar}$), 8.53–8.57 (m, 1 H$_{ar}$). ^{13}C NMR (125.7 MHz, CD$_3$OD): δ = 22.6 (CH$_2$), 27.7 (2×CH$_3$), 30.4 (CH$_2$), 43.7 (CH$_2$), 58.0 (C), 64.4 (d, J_{CP} = 5.5, CH$_2$), 96.2 (CH), 108.9 (C), 110.0 (C), 116.7 (C), 118.1 (C), 122.2 (CH), 123.2 (CH), 123.5 (CH), 127.3 (d, J_{CP} = 7.7, C), 131.1 (CH), 136.8 (CH), 143.8 (CH), 148.3 (CH), 148.7 (C), 152.5 (C), 156.6 (C), 161.6 (C), 175.4 (C=O). MS (ESI, negative mode): m/z = 499 [M-H]$^-$. HRMS: calcd. for $C_{24}H_{25}N_2O_8P$ [M-H]$^-$ 499.1276; found 499.1266. λ_{abs} = 432 nm, λ_{em} = 512 nm, ε = 20417 M^{-1}cm^{-1}, $\Phi_{fl.}$ = 0.81 (all data in PBS buffer at pH 7.4). Standard: Coumarin 522, $\Phi_{fl.}$ = 0.65 (EtOH).



(8,9-Dihydro-8,8-dimethyl-3-(pyridin-2-yl)-9-(*N*-succinimidyl)oxycarbonylpropyl-2*H*-pyrano[3,2-g]quinolin-2-one)-6-methyl phosphate (21d). The solution of **21c**·3NEt$_3$ (10 mg, 12 μmol), *N*-hydroxysuccinimide (2.8 mg, 24 μmol), HATU (11.4 mg, 30 μmol) and NEt$_3$ (12 mg, 120 μmol) in DMF (1.5 mL) was stirred overnight at RT. Then DMF was evaporated at RT, and the residue was subjected to column chromatography (15 g of SiO$_2$, MeCN/water = 4/1) to give 3.0 mg of the title compound as a red solid (42% yield). HPLC: B/A = 20/80 to 50/50 in 25 min, 433 nm, t_R = 12.6 min (100 %). MS (ESI, negative mode): m/z = 596 [M-H]$^-$. HRMS: calcd. for $C_{28}H_{28}N_3O_{10}P$ [M-H]$^-$ 596.1440; found 596.1428.

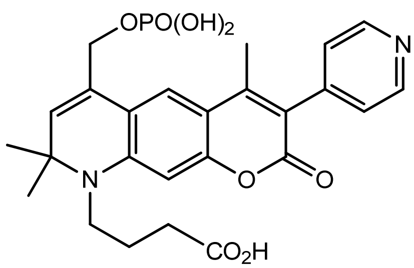


Ethyl (8,9-dihydro-6-hydroxymethyl-4,8,8-trimethyl-3-(pyridin-4-yl)-2*H*-pyrano[3,2-*g*]quinolin-2-one)-9-butanoate (22a): CeCl$_3$ (97 mg, 0.391 mmol) was added to a cooled solution (0 °C) of compound **18**-Me (180 mg, 0.391 mmol) in the mixture of THF and MeOH (5 mL+5mL). The resulted suspension was stirred, until CeCl$_3$ dissolved, and NaBH$_4$ (15 mg, 0.391 mmol) was added in two portions. Bright green fluorescence appeared immediately, and after 5 min, acetone (0.5 mL) was added. Solvents were evaporated *in vacuo,* and the residue was purified by column chromatography (CH_2Cl_2/ether/acetone/MeOH = 8/4/4/1 eluent) to furnish compound **22a** (163 mg, 90 % yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.28 (t, J = 7.2, 3 H, CO_2Et), 1.40 (s, 6 H, 2×Me), 1.93 (m, 2 H, $NCH_2C\underline{H}_2CH_2CO_2Et$), 2.22 (s, 3 H, Me), 2.40 (m, 2 H, $NCH_2CH_2C\underline{H}_2CO_2Et$), 3.32 (m, 2 H, $NC\underline{H}_2CH_2CH_2CO_2Et$), 4.17 (q, J = 7.2, 2 H, CO_2Et), 4.48 (m, 2 H), 5.55 (d, J = 0.8, 1 H, $\underline{H}$C=), 6.41 (s, 1 H, Ar), 7.23 (m, 2 H, AA' part of AA'XX' system), 7.37 (s, 1 H, Ar), 8.64 (m, 2 H, XX' part of AA'XX' system) ppm. ^{13}C NMR (125.7 MHz, $CDCl_3$): δ = 14.2(+), 16.3(+), 22.7(-), 28.9(+), 31.3(-), 43.9(-), 57.7(q), 60.7(-), 63.0, 97.3(+), 109.2(q), 117.5(q), 118.6(q), 119.7(+), 125.7(+), 129.9(q), 130.2(q), 143.6(q), 147.7(+), 149.3(+), 149.6(q), 155.1(q), 160.4(q), 172.8(q) ppm. MS (ESI): *m/z* (positive mode, rel. int., %) = 463.4 (100) $[M+H]^+$. λ_{abs} = 410 nm, λ_{em} = 499 nm, ε = 39200 $M^{-1}cm^{-1}$, $\Phi_{fl.}$ = 0.68 (all data in MeOH). Standard: Coumarin 522, $\Phi_{fl.}$ = 0.65 (EtOH).

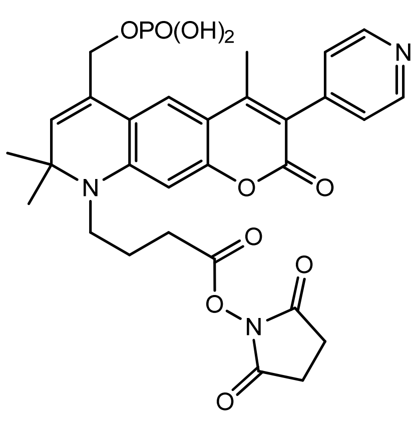


Di-(*tert*-butyl) [9-ethoxycarbonylpropyl-8,9-dihydro-4,8,8-trimethyl-3-(pyridin-4-yl)-2*H*-pyrano[3,2-*g*]quinolin-2-one]-6-methyl phosphate (22b): To a stirred and warm (40 ºC) solution of **22a** (165 mg, 0.357 mmol) in CH_2Cl_2 (7 mL). di-*t*-butyl *N,N*-(di-isopropyl) phosphoramidite (267 mg, 1.07 mmol) and 1*H*-tetrazole (75 mg, 1.07 mmol) were added under argon in two equal portions at an interval of 20 min. After further 20 min, the reaction mixture was cooled with an ice bath (0 ºC), and the solution of 70% MCPBA (246 mg, 1.07 mmol) in CH_2Cl_2 (5 mL) was added dropwise. The reaction mixture was allowed to warm up to RT (within ca. 5 min), and 10% aq. Na_2SO_3 (2 mL) and aq. sat. $NaHCO_3$ solutions (2 mL) were added. The organic layer was separated, and the aqueous phase was extracted with CH_2Cl_2 (3×10 mL). The combined organic extracts were dried, solvents were evaporated, and the residue (231 mg) was purified by column chromatography (with CH_2Cl_2/ether/MeOH mixture [60/20/1] as an eluent) followed by precipitation from hexane with ether giving pure **22b** (182 mg, 78 %yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.28 (t, J = 7.2, 3 H, CO_2Et), 1.40 (s, 6 H, 2×Me), 1.45 (d, J = 0.5, 18 H, 2xOBu^t), 1.92 (m, 2 H, $NCH_2C\underline{H}_2CH_2CO_2Et$), 2.24 (s, 3 H, Me), 2.40 (m, 2 H, $NCH_2CH_2C\underline{H}_2CO_2Et$), 3.32 (m, 2 H, $NC\underline{H}_2CH_2CH_2CO_2Et$), 4.17 (q, J = 7.2, 2 H, CO_2Et), 4.76 (dd, 2 H), 5.60 (d, J = 0.8, 1 H, $\underline{H}$C=), 6.41 (s, 1 H, Ar), 7.22 (m, 2 H, AA' part of AA'XX' system), 7.36 (s, 1 H, Ar), 8.64 (m, 2 H, XX' part of AA'XX' system) ppm. ^{13}C NMR (125.7 MHz, $CDCl_3$): δ = 14.2(+), 16.4(+), 22.7(-), 28.7(+), 29.8 (+), 31.3(-), 43.9(-), 57.7(q), 60.7(-), 66.2 (-, $J_{C\text{-}P}$ = 5.5), 82.7 (q, $J_{C\text{-}P}$ = 7.5), 97.4(+), 109.3(q), 117.2(q), 118.7(q), 120.0(+), 125.6(+), 126.6(q, $J_{C\text{-}P}$ =), 132.2(q), 143.5(q), 147.5(q), 149.4(q), 149.7(+), 155.1(q), 160.9(q), 172.8(q) ppm. MS (ESI): *m/z* (positive mode, rel. int., %) = 677.5 (17%) $[M+Na]^+$, 655.5 (100) $[M+H]^+$. HRMS:

found 655.3144 $[M+H]^+$; calcd. 655.3143 ($C_{35}H_{47}N_2O_8P$). λ_{abs} = 403 nm, λ_{em} = 494 nm, ε = 25394 $M^{-1}cm^{-1}$, $\Phi_{fl.}$ = 0.62 (in MeOH). Standard: Coumarin 522, $\Phi_{fl.}$ = 0.65 (EtOH).



[9-Carboxypropyl-8,9-dihydro-4,8,8-trimethyl-3-(pyridin-4-yl)-2*H*-pyrano[3,2-*g*]quinolin-2-one]-6-methyl phosphoric acid (22c): Compound **22b** (60 mg, 92 μmol) was dissolved in THF/MeOH mixture (1mL/1mL), and a 4-fold excess of 1 M NaOH (0.37 mL) was added. The reaction mixture was stirred at RT for 16 h with a TLC-control. Then it was evaporated to dryness *in vacuo,* and CF_3COOH (1 mL) was added. After 4 h, it was diluted with dichloromethane/ether mixture (1/1), the precipitate was filtered off, dried in air, dissolved in the minimal volume of DMF and was subjected to column chromatography on SiO_2 (acetonitrile / water = 8 / 1). After evaporation of solvents, the solid residue was dissolved in CF_3COOH (0.5 mL) and poured into ether (10 mL). The precipitate was filtered, washed with ether and dried leaving 43 mg (90%) of **22c** as a red solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 1.30 (s, 6 H, 2×Me), 1.72 (m, 2 H, $NCH_2C\underline{H_2}CH_2CO_2H$), 2.13 (s, 3 H, Me), 2.37 (m, 2 H, $NCH_2CH_2C\underline{H_2}CO_2H$), 3.29 (m, 2 H, $NC\underline{H_2}CH_2CH_2CO_2H$), 4.52 (d, $J_{H\text{-}P}$ = 5, 2 H), 5.60 (s, 1 H, $\underline{H}$C=), 6.58 (s, 1 H, Ar), 7.25 (m, 2 H, AA' part of AA'XX' system), 7.48 (s, 1 H, Ar), 8.57 (m, 2 H, XX' part of AA'XX' system) ppm. MS (ESI): *m/z* (negative mode, rel. int. %) = 513.2 (64) $[M-H]^-$. HRMS: 515.1577 $[M+H]^+$, calcd. 515.1578 ($C_{25}H_{27}N_2O_8P$); HPLC: B/A = 20/80 to 50/50 in 25 min, 433 nm, t_R = 9.0 min (100 %). λ_{abs} = 410 nm, λ_{em} = 503 nm, ε = 13900 $M^{-1}cm^{-1}$, $\Phi_{fl.}$= 0.44 (all data in PBS buffer at pH 7.4). Standard: Coumarin 522, $\Phi_{fl.}$ = 0.65 (EtOH).



(8,9-Dihydro-4,8,8-trimethyl-3-(pyridin-4-yl)-9-(*N*-succinimidyl)oxycarbonylpropyl-2*H*-pyrano[3,2-g]quinolin-2-one)-6-methyl phosphate (22d): A solution of **22c** (14 mg, 27 μmol), *N*-hydroxysuccinimide (6.3 mg, 54 μmol), HATU (21 mg, 54 μmol) and NEt_3 (5.5 mg, 54 μmol) in DMF (2 mL) was stirred overnight at RT. The reaction mixture was subjected to a column chromatography on a regular SiO_2 without work-up (MeCN/water = 4/1; **22c** has R_f = 0.34, while **22d** has R_f = 0.53). Yield -14.2 mg of **22d** as a red solid (86 %). HRMS: 610.1597 [M–H]$^-$; calcd. 610.1596 ($C_{29}H_{30}N_3O_{10}P$); HPLC: B/A = 20/80 to 50/50 in 25 min, 433 nm, t_R = 12.1 min (77 %).

Immunofluorescence labeling and mounting of the samples

Labeling of the secondary antibodies (1–2 mg of protein in ca. 1–2 mL of PBS buffer) with *N*-hydroxysuccinimidyl esters of the dyes **6**-H, **7**-H, **11**-H,H, **21c** and **22c** (0.2–0.4 mg) was performed according to the standard protocols[4] in the presence of aq. $NaHCO_3$ at pH 8–8.5, followed by gel-filtration through the Sephadex G25 (PD-10) column (ø=1.7 cm, L = 7 cm) (in order to remove excess unreacted dye) and determination of the degree of labeling (DOL, average amount of the dye residues attached to one protein molecule).[4b,c]

For the preparation of cell samples, PtK2 cells were grown on cover slips. Cells were fixed with anhydrous methanol for 5 min at –20°C and blocked with 5% (w/v) BSA in PBS. Then the cells were incubated with a monoclonal mouse antiserum directed against the alpha-tubulin (Sigma-Aldrich, St. Louis, MO, USA). The primary antibodies were detected with secondary antibodies (sheep anti-mouse; Jackson ImmunoResearch Laboratories, West Grove; PA; USA) custom labeled with the fluorescent dyes. After several washing steps with PBS the samples were mounted in Mowiol. Staining and sample preparation were carried out according to the standard protocols, described by *C. A. Wurm et al.*[5]

Supplementary figures:

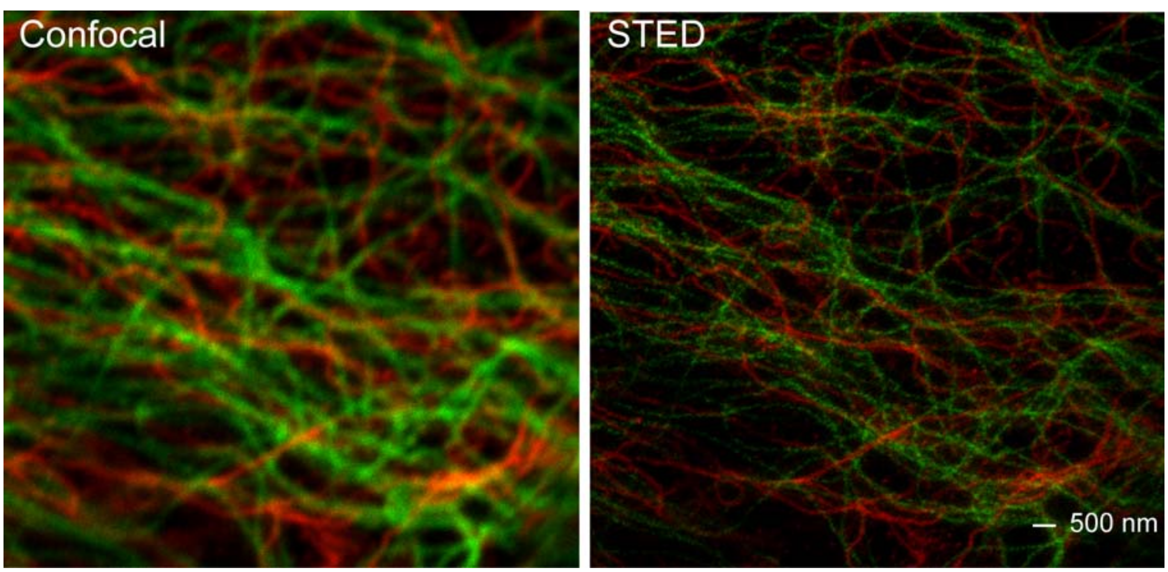


Figure S1. Confocal (left) and STED (right) microscopy images of Vimentin filaments stained with BD Horizon™ V500 dye (BD Biosciences; abs. 415 nm, emission 500 nm, excitation with 405 nm light) (red) and microtubule stained with Oregon Green™ 488 (abs. 490 nm, emission 510 nm, excitation with 490 nm) (green). The mammalian cell was fixed and immunolabeled via a primary and secondary antibodies (the latter were conjugated with the corresponding dyes). Detection at 510-560 nm for both dyes; STED at 590 nm for both dyes (STED power ca. 84 mW at the back focal plane).

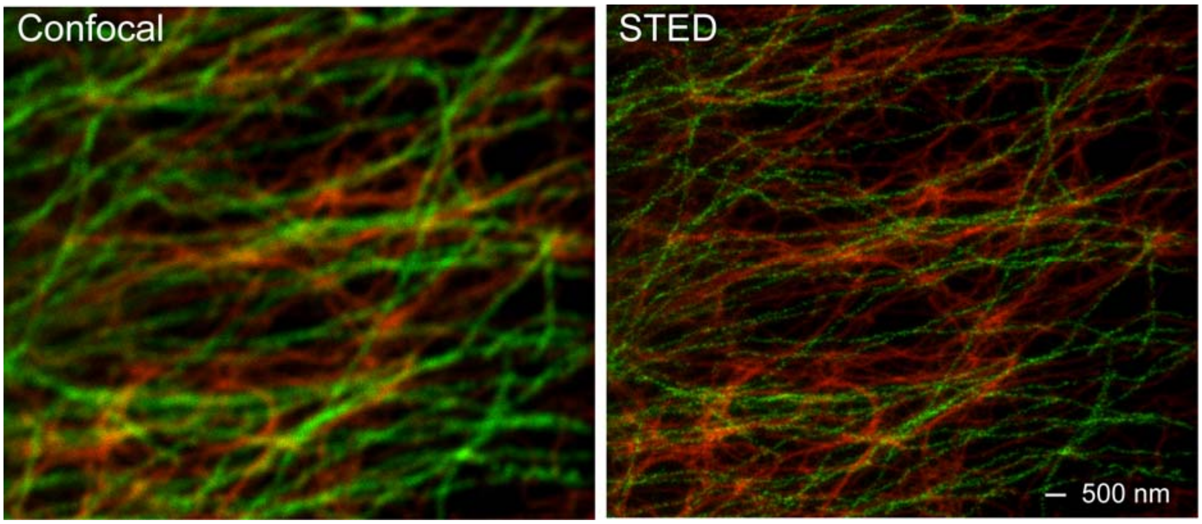


Figure S2. Confocal (left) and STED (right) microscopy images of Vimentin filaments stained with compound **6**-NHS (abs. 437 nm, emission 512 nm) (red) and microtubule stained with Oregon Green^{TM} 488 (abs. 490 nm, emission 510 nm) (green). The mammalian cell was fixed and immunolabeled via a primary and secondary antibodies (the latter were conjugated with the corresponding dyes). For microscopy settings, see the legend to Figure S1.

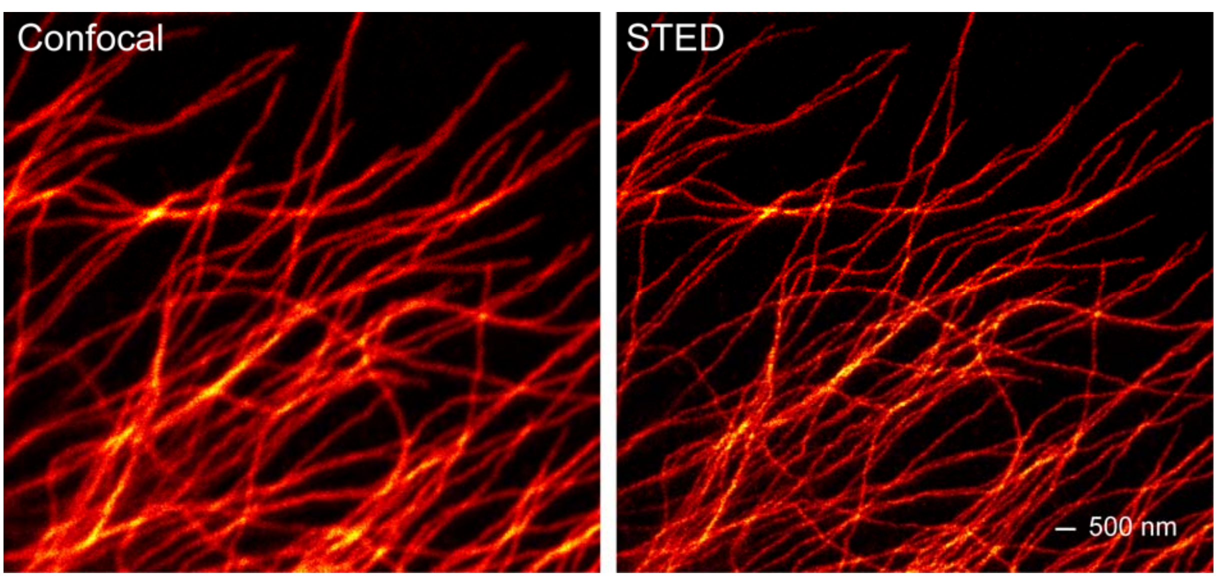


Figure S3. Confocal and STED microscopy image of microtubule stained with compound **21c**. The mammalian cell was fixed and immunolabeled via a primary and secondary antibody (the latter was conjugated with compound **21c**). For microscopy settings, see the legend to Figure S1

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[5] C. A. Wurm, D. Neumann, R. Schmidt, A. Egner and S. Jakobs in *Methods in Molecular Biology* **2010**, *591*, Part 2, 185–199, DOI: 10.1007/978-1-60761-404-3_11 (Sample Preparation for STED Microscopy).