CHEMISTRY
A EUROPEAN JOURNAL

Supporting Information

4-Trifluoromethyl-Substituted Coumarins with Large Stokes Shifts: Synthesis, Bioconjugates, and Their Use in Super-Resolution Fluorescence Microscopy

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Supporting Information

4-Trifluoromethyl-Substituted Coumarins with Large Stokes Shifts: Synthesis, Bioconjugates and the Use in Super-resolution Fluorescence Microscopy

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Synthesis

General remarks: UV-visible absorption spectra were recorded on a Varian Cary 4000 UV-Vis spectrophotometer, and fluorescence spectra on a Varian Cary Eclipse fluorescence spectrophotometer. 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. Anhydrous THF was distilled under argon over sodium with addition of benzophenone; all other anhydrous solvents were purchased (acetonitrile, *N,N*-dimethylformamide, dimethylsulfoxide, ethanol, ether, dichloromethane, 1,2-dichloroethane, toluene). The MICROTOF spectrometer equipped with ESI ion source Apollo and direct injector with LC autosampler Agilent RR 1200 was used for obtaining mass spectra and high resolution mass spectra (ESI-HRMS). ESI-HRMS were obtained also on APEX IV spectrometer (Bruker), low resolution ESI spectra – on a Varian 500MS spectrometer. HPLC system (Knauer): Smartline pump 1000 (2×), UV detector 2500, column thermostat 4000 (25 °C), mixing chamber, injection valve with 20 and 100 µL loops for the analytical and semi-preparative columns, respectively; 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 Macherey Nagel ready-to-use plates with regular silica gel 60 (Alugram® Xtra SIL G/UV_{254}) and UV-detection (unless specified otherwise). Chromatographic separations were carried out on Merck silica gel 60 (40–63 µm or 63–200 µm) from Macherey-Nagel (Germany). Reversed phase chromatography was performed on Macherey-Nagel POLYGOPREP 60-50 C18 (40–63 µm) silica gel. 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*) in NMR spectra 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 (–). The following reagents were prepared according to the known methods: ethyl 4-[(7-hydroxy-2,2,4-trimethyl-1,2-dihydroquinolin-1-yl]butanoate[1] and 2-chloro-4,4,4-trifluoroacetoacetate (**5**-Cl).[2]

General Procedure A – Stille coupling of 3-chlorocoumarins (GPA): The starting 3-chlorocoumarin and the palladium catalyst were placed into a screw-cap tube under Ar (or argon), and a tributylstannane and toluene (1 mL) were added. The resulting solution was purged with argon by bubbling this gas through the solution, before the ligand for the catalyst was added. The closed tube was heated at the given temperature for a specified time (Table S1). After cooling, the reaction was quenched by adding 2.5% aqueous solution of potassium fluoride (4 mL) and ethyl acetate (4 mL). After vigorous stirring for 30–45 min, the phases were separated, and the aqueous solution was

extracted once with ethyl acetate. The combined organic solutions were dried over anhydrous magnesium sulfate, concentrated *in vacuo*, and the residue was purified as indicated.

General Procedure B – Quaternization of pyridines (GPB): The pyrido-coumarin and the alkylating agent were dissolved in acetonitrile (1 mL) and heated to 120 °C (bath temp.) in a screw-cap tube for the specified time (Table S2). The reaction mixture was concentrated and purified by column chromatography as decribed below for the individual compounds.

Table S1. Stille coupling reactions with 3-chlorocoumarins **6a,b** (Scheme 1, pathway ii)

Entry	Reactant	Group R^4	Catalyst[a]	Time (h)	Yield of **7** (%)[b]
1	**6a**	2-thienyl	A	16	64
2	**6a**	4-pyridyl	B	18	52
3	**6a**	2-pyridyl-*trans*-(CH=CH)-	B	18	59
4	**6a**	4-pyridyl-*trans*-(CH=CH)-	B	18	52
5	**6b**	4-pyridyl	B	18	79

[a] A: PEPPSI-SiPr (4 mol%). B: $Pd(dba)_2$ (3 mol%) + $P(Bu^t)_3$ (4.5 mol%);
[b] highest yield of the isolated product.

Table S2. Quaternization of the pyridine rings in coumarins **7a-c**,R^4 with 6-bromohexanoic acid (conditions: A), 6-iodohexanoic acid (conditions: B), and 1,3-propanesultone (conditions: C) in refluxing acetonitrile.

Entry	Reactant	Conditions[a]	Yield (%)[b]
1	**7a**,CH=CH-4-py	A (18 h)	64
2	**7a**,CH=CH-2-py	B (4 d)	55
3	**7a**,4-py	B (3 d)	59
4	**7b**,4-py	C (18 h)	96
5	**7c,**2-py	C (5 d)	100
6	**7c,**4-py	C (2 d)	68

[a] A, B: 6-halohexanoic acid (5 eq.), or C: 1,3-propanesultone (11 eq.), MeCN, 120 °C (bath temp.); [b] isolated compounds; for structures, see the main text.

General procedure C – Cyclization of keto-phenols with the substituted acetic acids (GPC): A solution of the keto-phenol, the substituted acetic acid, 4-(dimethylamino)pyridine (DMAP) and triethyl amine (TEA) in dichloromethane (2 mL) was placed into a screw-cap test-tube. *N,N'*-Dicyclohexyl carbodiimide (DCC) was added, and the closed tube was heated at 50 °C (bath temp.) for 17–19 h. Upon cooling, the reaction mixture was diluted with diethyl ether (4 mL) and filtered. The precipitate was washed with ether (10 mL), and the combined filtrate was concentrated. The solid residue was purified by column chromatography as indicated.

Table S3. Acylation–cyclization reactions of **8a-c** with aryl(ethenyl) acetic acids R^4CH_2COOH afford coumarins **7a-c**,R^4 (Scheme 1, pathway iv).

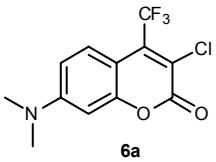
Entry	Reactant	Group R^4	Yield of **7**(%)[a]	Recovered **8** (%)[a,b]
1	**8a**	C_6H_5-*trans*-(CH=CH)-	32	n. d.
2	**8b**	4-pyridyl	25	n. d.
3	**8b**	2-pyridyl	54	n. d.
4	**8c**	2-pyridyl	54	46
5	**8c**	4-pyridyl	64	n. d.
6	**8c**	2-thienyl	57	16

7	**8c**	C_6H_5-*trans*-(CH=CH)-	31	55
8	**8c**	2-pyridyl-*trans*-(CH=CH)-	39	28
9	**8c**	4-pyridyl-*trans*-(CH=CH)-	35	31

[a] yield of the isolated compound; [b] recovered starting material; n. d. – not determined

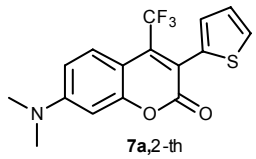
General procedure D – Acylation of phenols with trifluoroacetic acid anhydride (GPD): TFAA (7 molar equivalents) was slowly added to a solution of the appropriate phenol in diethyl ether (1 mL/mmol). The reaction mixture was heated for 5 h with reflux. The volatiles were distilled off under reduced pressure, the residue was dissolved in methanol (2 mL/mmol), and treated with sat. aq. $NaHCO_3$ solution (5 mL/mmol). The solution was extracted with ethyl acetate (2x5mL/mmol), and the combined organic solutions were dried over $MgSO_4$ and concentrated. The residue was separated by column chromatography as indicated below.

General procedure E – saponification of coumarins esters: 1 M NaOH (2 eq.) was added to a solution of coumarin ester in THF / MeOH (4 / 1) mixture, heated to 50 °C and stirred at this temperature for 0.5 h. All volatiles were evaporated; TFA was added to the residue and after 15 min was evaporated. The crude acid was dissolved in min. amount of NEt_3 and purified by column chromatography (MeCN / water = 4 / 1 eluent).



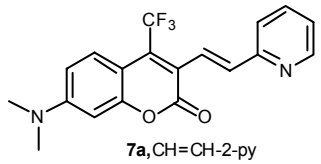
6a

Coumarin 6a: A mixture of 3-(dimethylamino)phenol (1.37 g, 9.99 mol), 2-chloro-4,4,4-trifluoroacetoacetate (2.51 g, 11.3 mmol) and anhydrous $ZnCl_2$ (1.64 g, 12 mmol) in ethanol (10 mL) was refluxed for 18 h. After cooling, ethanol was evaporated in vacuo, and the residue was dissolved in dichloromethane (100 mL). This solution was washed with water (100 mL) and dried (Na_2SO_4). After column chromatography on SiO_2 (hexane/CH_2Cl_2, 1:1), compound **6a** was isolated as light yellow solid (1.65 g, 56%). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.58 (dq, *J* = 9.4 and 2.3, 1 H), 6.63 (dd, *J* = 9.4 and 2.3, 1 H), 6.49 (d, *J* = 2.6, 1 H,), 3.06 (s, 6 H, NMe_2) ppm. ^{13}C NMR (75 MHz, $CDCl_3$, APT): δ = 157.7 154.2, 152.9, 136.9 (q, *J* = 30.1), 126.6 (+, q, $J_{C\text{-}F}$ = 5.4), 122.4 (q, *J* = 279, CF_3), 116.2, 110.2 (+), 104.1, 98.1 (+), 40.0 (+, NMe_2) ppm. MS (ESI): *m/z* (positive mode, rel. int., %) = 605 (50), $[2M + Na]^+$, 314 (100), $[M + Na]^+$; HRMS ($C_{12}H_9ClF_3NO_2$): *m/z* (positive mode, rel. int., %) = 314.0163 (found $[M + Na]^+$), 314.0166 (calc.).



7a,2-th

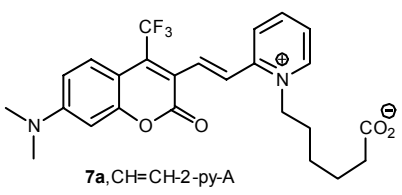
Coumarin 7a,2-th: According to GPA, 7-(dimethylamino)-3-chloro-4-(trifluoromethyl)coumarin (**6a**, 88 mg, 0.30 mmol), PEPPSI-SiPr (8 mg, 12 μmol) and 2-(tributylstannyl)thiophene (96 μL, 113 mg, 0.30 mmol) reacted at 120 °C for 18 h. Chromatography on silica gel (16 g, pentane/CH_2Cl_2, 1:1) gave the title compound as a yellow solid (66 mg, 0.20 mmol, 64% yield, 91% yield on the reacted starting material). R_f = 0.22 (pentane/DCM, 1:1); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.63 (dq, *J* = 9.4 and 2.3, 1 H, 5-H), 7.49 (dd, *J* = 4.7 and 1.6, 1 H, 5'-H), 7.04–7.10 (m, 2 H, 3',4'-H), 6.67 (dd, *J* = 9.4 and 2.3, 1 H, 6-H), 6.55 (d, *J* = 2.6, 1 H, 8-H), 3.10 (s, 6 H, NMe_2) ppm. ^{13}C NMR (75 MHz, $CDCl_3$, APT): δ = 160.9 (–, C-2), 155.4 (–, C-8a), 152.9 (–, C-7), 138.4 (–, q, *J* = 30, C-4), 133.1 (–, C-2'), 129.6 (+, q, *J* = 1.8, C-3'), 127.9 (+, C-5'), 127.1 (+, q, *J* = 4.1, C-5), 126.5 (+, C-4'), 122.5 (–, q, *J* = 279, CF_3), 116.1 (–, C-3), 109.7 (+, C-6), 104.3 (–, C-4a), 97.9 (+, C-8), 40.0 (+, NMe_2) ppm; ^{19}F-NMR (282.4 MHz, $CDCl_3$): δ = –55.4 (d, $J_{F\text{-}H}$ = 2.3) ppm. UV/Vis (EtOH): λ_{max} (ε) = 211 (20100), 257 (14200), 416 (24100 M^{-1} cm^{-1}); fluorescence (EtOH): $\lambda_{excit.}$ = 424 nm, $\lambda_{em.}$ = 611 nm; MS (ESI): *m/z* (positive mode, rel. int., %) = 362 (100), $[M + Na]^+$, 340 (15), $[M + H]^+$; HRMS ($C_{16}H_{12}F_3NO_2S$): 362.0430 (found $[M + Na]^+$), 362.0433 (calc.); 340.0610 (found $[M + H]^+$), 340.0614 (calc.).



7a,CH=CH-2-py

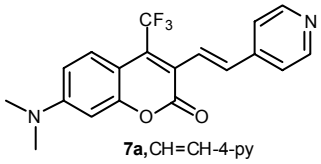
Coumarin 7a,CH=CH-2-py: According to GPA, 7-(dimethylamino)-3-chloro-4-(trifluoromethyl)coumarin (**6a**, 22 mg, 75 μmol), $Pd(dba)_2$ (1.3 mg, 2.3 μmol) 2-[(*E*)-2-(tributylstannyl)ethenyl]pyridine (30 mg, 76 μmol), and tris(*tert*-butyl)phosphine (0.26 M in dioxane, 13 μL, 3.4 μmol) reacted at 120 °C for 18 h. Chromatography on SiO_2

(10 g, CH_2Cl_2/MeOH, 25:1) gave the title compound as an orange solid (16 mg, 59%). R_f = 0.32 (DCM/MeOH, 25:1); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.63 (dd, *J* = 4.7 and 0.9, 1 H, 6''-H), 7.84 (dq, *J* = 9.4 and 2.3, 1 H, 5-H), 7.72 (d, *J* = 15.6, 1 H, 2'-H), 7.63–7.71 (m, 2 H, 5-H and 4''-H), 7.37 (d, *J* = 7.8, 1 H, 3''-H), 7.17 (ddd, *J* = 6.9, 5.5 and 1.1, 1 H, 5''-H), 6.66 (dd, J = 9.4 and 2.8, 1 H, 6-H), 6.52 (d, *J* = 2.8, 1 H, 8-H), 3.10 (s, 6 H, NMe_2) ppm. ^{13}C NMR (75 MHz, $CDCl_3$, APT): δ = 159.9 (–, C-2), 155.0 (–, C-2''), 154.4 (–, C-8a), 152.4 (–, C-7), 149.7 (+, C-6''), 136.4 (+, q, J_{C-F} = 1.5), 136.3 (+, C-4''), 135.7 (–, q, J_{C-F} = 30, C-4), 126.8 (+, q, J_{C-F} = 5, C-5), 123.5 (+, C-3''/C-5''), 123.4 (–, q, J_{C-F} = 279, CF_3), 123.2 (+, q, J_{C-F} = 4, C-1'), 122.5 (+, C-5''/C-3''), 118.2 (–, q, J_{C-F} = 2.1, C-3), 109.7 (+, C-6), 104.8 (–, C-4a), 97.8 (+, C-8), 40.0 (+, NMe_2) ppm; UV/Vis (EtOH): λ_{max} (ε) = 207 (41200), 253 (13200), 307 (12400), 444 (36600 M^{-1} cm^{-1}); fluorescence (EtOH): $\lambda_{excit.}$ = 465 nm, $\lambda_{em.}$ = 578 nm; MS (ESI): *m/z* (positive mode, rel. int., %) = 743 (100) $[2M + Na]^+$, 721 (13) $[2M + H]^+$, 383 (13) $[M + Na]^+$, 361 (6), $[M + H]^+$; HRMS ($C_{19}H_{15}F_3N_2O_2$): *m/z* (positive mode) = 361.1157 (found $[M + H]^+$), 361.1158 (calc.); 383.0977 (found $[M + Na^+]$), 383.0978 (calc.).



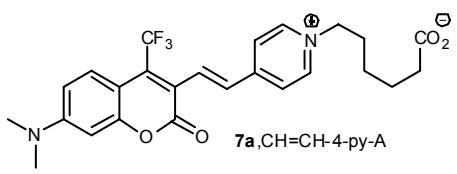
7a,CH=CH-2-py-A

7a,CH=CH-2-py-A: According to GPB, coumarin **7a,**CH=CH-2-py (23 mg, 64 μmol) and 6-iodohexanoic acid (77 mg, 320 μmol) reacted for 4 d. Chromatography on SiO_2 (3 g, CH_2Cl_2/MeOH, 25:1 → 0:1) gave two fractions. Fraction 1 (21 mg, 35 μmol, 55%) proved to be hydroiodide of the title compound; fraction 2 (13 mg, 27 μmol, 43%) was the title compound. Both of them possess identical MS-spectra. Fraction 1: R_f = 0.17 (DCM/MeOH, 4:1), fraction 2: R_f = 0.04 (DCM/MeOH, 4:1). ^{1}H NMR (300 MHz, CD_3OD): δ = 8.90 (m, 1 H), 8.50 (m, 1 H), 8.28 (m, 1 H), 7.92 (m, 2 H), 7.70 (m, 2 H), 6.86 (dd, *J* = 9.4 and 2.8, 1 H), 6.61 (d, *J* = 2.8, 1 H), 4.64 (m, 2 H), 3.14 (s, 6 H, NMe_2), 2.20 (m, 2 H), 2.00-1.40 (m, 6 H) ppm; ^{19}F-NMR (282.4 MHz, $CDCl_3$): δ = –53.7 (dd, J^1_{F-H} = J^2_{F-H} = 2.3) ppm. UV/Vis (EtOH): λ_{max} (ε) = 210 (20700), 255 (3750), 329 (2340), 475 (9900 M^{-1} cm^{-1}); fluorescence (EtOH): $\lambda_{excit.}$ = 470 nm, $\lambda_{em.}$ = 646 nm; MS (ESI): *m/z* (positive mode, rel. int., %) = 497 (2) $[M + Na]^+$, 475 (100), $[M + H]^+$; HRMS ($C_{25}H_{25}F_3N_2O_4$): *m/z* (positive mode) = 475.1842 (found $[M + H]^+$), 475.1839 (calc.); 497.1649 (found $[M + Na^+]$), 497.1659 (calc.).



7a,CH=CH-4-py

Coumarin 7a,CH=CH-4-py: According to GPA, 7-(dimethylamino)-3-chloro-4-(trifluoromethyl)coumarin (**6a**, 22 mg, 75 μmol), $Pd(dba)_2$ (1.3 mg, 2.3 μmol), 4-[(*E*)-2-(tributylstannyl)ethenyl]pyridine (30 mg, 76 μmol), and tris(*tert*-butyl)phosphine (0.26 M in dioxane, 13 μL, 3.4 μmol) reacted at 120 °C for 18 h. Chromatography on SiO_2 (10 g, CH_2Cl_2/MeOH, 25:1) gave the title compound as a light red solid (14 mg, 52%). R_f = 0.29 (DCM/MeOH, 25:1); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.60 (m, 2 H, 2'',6''-H), 7.67 (dq, *J* = 9.4 and 2.2, 1 H, 5-H), 7.60 (d, *J* = 16.2, 1 H, 2'-H), 7.37 (m, 2 H, 3'',5''-H), 7.33 (dq, *J* = 16.2 and 3.1, 1 H, 1'-H), 6.67 (dd, *J* = 9.4 and 2.8, 1 H, 6-H), 6.53 (d, *J* = 2.8, 1 H, 8-H), 3.11 (s, 6 H, NMe_2) ppm. ^{13}C NMR (126 MHz, $CDCl_3$, APT): δ = 159.7 (–, C-2), 154.6 (–, C-8a), 152.6 (–, C-7), 150.1 (+, C-2'',6''), 144.5 (–, C-4''), 136.1 (–, q, *J* = 29, C-4), 134.7 (+, q, *J* = 1, C-2'), 126.8 (+, q, *J* = 5, C-5), 123.6 (+, q, *J* = 4, C-1'), 123.3 (–, q, *J* = 279, CF_3), 121.1 (+, C-3'',5''), 117.3 (–, q, *J* = 2, C-3), 109.9 (+, C-6), 104.5 (–, C-4a), 97.7 (+, C-8), 40.0 (+, NMe_2) ppm; ^{19}F-NMR (282.4 MHz, $CDCl_3$): δ = –54.2 (dd, J^1_{F-H} = J^2_{F-H} = 2.3) ppm. UV/Vis (EtOH): λ_{max} (ε) = 211 (153000), 275 (21800), 449 (28500 M^{-1} cm^{-1}); fluorescence (EtOH): $\lambda_{excit.}$ = 505 nm, $\lambda_{em.}$ = 580 nm; MS (ESI): *m/z* (positive mode, rel. int., %) = 1103 (84) [3M + Na]+, 743 (100) $[2M + Na]^+$, 383 (35) $[M + Na]^+$, 361 (63), $[M + H]^+$; HRMS ($C_{19}H_{15}F_3N_2O_2$): *m/z* (positive mode) = 361.1166 (found $[M + H]^+$), 361.1158 (calc.); 383.0987 (found $[M + Na^+]$), 383.0978 (calc.).



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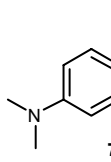
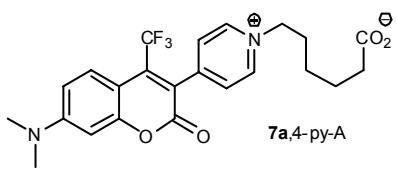
Coumarin 7a,CH=CH-4-py-A: According to GPB, coumarin **7a,**CH=CH-4-py (13 mg, 36 μmol) and 6-bromohexanoic acid (35 mg, 180 μmol) reacted for 18 h. Chromatography on SiO_2 (2 g, CH_2Cl_2/MeOH, 10:1) afforded the title compound (11 mg, 43%) as a red solid. R_f = 0.37

(DCM/MeOH, 10:1). ^{1}H NMR (300 MHz, CD_3OD): δ = 8.82 (m, 2 H, 2'',6''-H), 8.13 (m, 2 H, 3'',5''-H), 7.79 (m, 2 H, 1', 2'-H), 7.70 (dq, J_{H-H} = 9.4 and J_{H-F} = 2.2, 1 H, 5-H), 6.83 (dd, J = 9.4 and 2.8, 1 H, 6-H), 6.60 (d, J = 2.8, 1 H, 8-H), 4.53 (m, 2H), 3.13 (s, 6 H, NMe_2), 2.20 (m, 2 H), 2.01 (m, 2 H), 1.64 (m, 2 H), 1.40 (m, 2 H) ppm; ^{19}F-NMR (282.4 MHz, $CDCl_3$): δ = –53.5 (dd, J^1_{F-H} = J^2_{F-H} = 2.3) ppm. UV/Vis (EtOH): λ_{max} (ε) = 210 (49300), 255 (9980), 291 (4790), 317 (4480), 500 (13300 M^{-1} cm^{-1}); fluorescence (EtOH): $\lambda_{excit.}$ = 570 nm, $\lambda_{em.}$ = 668 nm; MS (ESI): *m/z* (positive mode, rel. int., %) = 949 (2) $[2M + H]^+$, 475 (100), $[M + H]^+$; HRMS ($C_{25}H_{25}F_3N_2O_4$): *m/z* (positive mode) = 475.1834 (found $[M+H]^+$), 475.1839 (calc.).

Coumarin 7a,4-py: According to GPA, 7-(dimethylamino)-3-chloro-4-(trifluoromethyl)coumarin (**6a**, 46 mg, 180 μmol), $Pd(dba)_2$ (2.6 mg, 4.5 μmol), 4-(tributylstannyl)pyridine (61 mg, 170 μmol), and tris(*tert*-butyl)phosphine (0.26 M in dioxane, 26 μL, 4.5 μmol) reacted at 120 °C for 18 h. Chromatography on SiO_2 (10 g, CH_2Cl_2/MeOH, 25:1) gave the title compound as a yellow solid (29 mg, 52%). R_f = 0.17 (DCM/MeOH, 25:1); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.68 (m, 2 H, 2',6'-H), 7.64 (dq, J_{H-H} = 9.4 and J_{H-F} = 2.3, 1 H, 5-H), 7.21–7.24 (m, 2 H, 3',5'-H), 6.70 (dd, J = 9.4 and 2.6, 1 H, 6-H), 6.57 (d, J = 2.6, 1 H, 8-H), 3.12 (s, 6 H, NMe_2) ppm. ^{13}C NMR (126 MHz, $CDCl_3$, APT): δ = 160.3 (–, C-2), 155.6 (–, C-8a), 153.0 (–, C-7), 149.4 (+, C-2'',6''), 142.2 (–, C-3), 137.7 (–, q, J = 30, C-4), 127.0 (+, q, J = 4, C-5), 124.3 (+, q, J = 1, C-3',5'), 122.2 (–, q, J = 279, CF_3), 119.8 (–, q, J = 3, C-4'), 108.9 (+, C-7), 103.5 (–, C-4a), 97.9 (+, C-8), 40.1 (+, NMe_2) ppm; ^{19}F-NMR (282.4 MHz, $CDCl_3$): δ = –54.9 (d, J_{F-H} = 2.3) ppm. UV/Vis (EtOH): λ_{max} (ε) = 206 (57800), 255 (16200), 413 (18800 M^{-1} cm^{-1}); fluorescence (EtOH): $\lambda_{excit.}$ = 418 nm, $\lambda_{em.}$ = 588 nm; MS (ESI): *m/z* (positive mode, rel. int., %) = 691 (100) $[2M + Na]^+$, 669 (17) $[2M + H]^+$, 357 (26) $[M - Na]^+$, 335 (12), $[M + H]^+$; HRMS ($C_{17}H_{13}F_3N_2O_2$): *m/z* (positive mode) = 335.0997 (found $[M + H]^+$), 335.1002 (calc.); 357.0817 (found $[M + Na]^+$), 357.0821 (calc.).

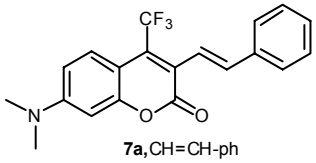


7a,4-py



7a,4-py-A

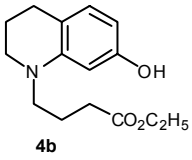
Coumarin 7a,4-py-A: According to GPB, coumarin **7a**,4-py (17 mg, 51 μmol) and 6-iodohexanoic acid (62 mg, 260 μmol) reacted for 3 d. Chromatography on SiO_2 (2 g, CH_2Cl_2/MeOH, 4:1) afforded the title compound (17 mg, 59%) as a red solid. R_f = 0.14 (DCM/MeOH, 4:1). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 9.09 (m, 2 H, 2',6'-H), 8.14 (m, 2 H, 3',5'-H), 7.70 (dq, J_{H-H} = 9,4 and J_{H-F} = 2.4, 1 H, H-5), 6.91 (dd, J = 9.5 and 2.6, 1 H, 6-H), 6.70 (d, J = 2.6, 1 H, 8-H), 4.71 (t, J = 7.5, 2 H, 1''-H), 3.17 (s, 6 H, NMe_2), 2.31 (t, J = 7.2, 2 H, 5''-H), 2.11 (m, 2 H, 2''-H), 1.72 (m, 2 H, 4''-H), 1.39–1.57 (m, 2 H, 3''-H) ppm; ^{19}F-NMR (282.4 MHz, $CDCl_3$): δ = –54.4 (d, J_{F-H} = 2.3) ppm. UV/Vis (EtOH): λ_{max} (ε) = 256 (10000), 439 (11800 M^{-1} cm^{-1}); fluorescence (EtOH): $\lambda_{excit.}$ = 450 nm, $\lambda_{em.}$ = 644 nm; MS (ESI): *m/z* (positive mode, rel. int., %) = 471 (22) $[M + Na – H]^+$, 449 (100) $[M]^+$; HRMS $[C_{23}H_{24}F_3N_2O_4]^+$: *m/z* (positive mode) = 449.1684 (found $[M]^+$), 449.1683 (calc.).



7a,CH=CH-ph

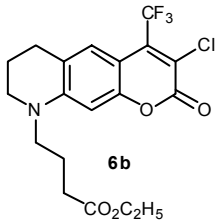
Coumarin 7a,CH=CH-ph: According to GPC, compound **8a** (70 mg, 0.30 mmol), styrylacetic acid (49 mg, 0.30 mmol), DMAP (4.0 mg, 33 μmol), triethylamine (63 μL, 45 mg, 0.45 mmol), and DCC (62 mg, 0.30 mmol) reacted for 4 h. Chromatography on SiO_2 (10 g, CH_2Cl_2) gave the title compound as a yellow solid (34 mg, 32%). R_f = 0.44 (CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.64 (dq, J_{H-H} = 9.7 and J_{H-F} = 2.2, 1 H, 5-H), 7.63 (d, J = 15.9, 1 H, 2'-H), 7.57–7.47 (m, 2 H, 3'',5''-H), 7.42–7.23 (m, 3 H, 2'',4'',6''-H), 7.15 (dq, J = 16.0 and 3.1, 1 H, 1'-H), 6.64 (dd, J = 9.4 and 2.5, 1 H, 6-H), 6.51 (d, J = 2.8, 1 H, 8-H), 3.07 (s, 6 H, NMe_2) ppm. ^{13}C NMR (126 MHz, $CDCl_3$, APT): δ = 160.0 (–, C-2), 154.2 (–, C-8a), 152.2 (–, C-7), 137.7 (+, q, J = 2, C-2'), 137.2 (–, C-1''), 134.5 (–, q, J = 29, C-4), 128.6 (+, C-3'',5''), 128.3 (+, C-4''), 126.9 (+, C-2'',6''), 126.5 (+, q, J = 5, 5-C), 123.5 (–, q, J = 278, CF_3), 119.4 (+, q, J = 3, C-1'), 118.9 (–, q, J = 2, C-3), 109.6 (+, C-6), 104.8 (–, C-4a), 97.8 (+, C-8), 40.0 (+, NMe_2) ppm; ^{19}F-NMR (282.4 MHz, $CDCl_3$): δ = –54.4 (dd, J^1_{F-H} = J^2_{F-H} = 2.3) ppm. UV/Vis (EtOH): λ_{max} (ε) = 208

(108000), 254 (11900), 293 (11500), 438 (27600 M^{-1} cm^{-1}); fluorescence (EtOH): $\lambda_{excit.}$ = 435 nm, $\lambda_{em.}$ = 576 nm; MS (ESI): *m/z* (positive mode, rel. int., %) = 1100 (47) $[3M + Na]^+$, 741 (100) $[2M + Na]^+$, 719 (13) $[2M + H]^+$, 382 (20) $[M + Na]^+$; HRMS ($C_{20}H_{16}F_3NO_2$): *m/z* (positive mode) = 382.1026 (found $[M + Na]^+$), 382.1025 (calc.).



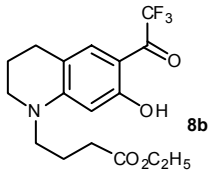
4b

Compound 4b: 1,2,3,4-Tetrahydroquinolin-7-ol (1.49 g, 9.99 mmol), $NaHCO_3$ (1.06 g, 12.6 mmol) and tetramethylammonium bromide (161 mg, 0.5 mmol) were suspended in water (15 mL), cooled to 0°C, and ethyl 4-bromobutyrate (2.38 g, 12.2 mmol) was added dropwise. When the addition was complete, the cooling bath was removed, and the reaction mixture was stirred at room temperature for 60 h. Then the product was extracted with EtOAc (2×35 mL), organic solutions were shaken with brine and dried ($MgSO_4$). Column chromatography on SiO_2 (hexane/EtOAc, 3:1) afforded 1.19 g (45%) of the title compound. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 6.76 (m, 1 H), 6.16 (d, *J* = 2.4, 1 H), 6.07 (dd, *J* = 8.0 and 2.4, 1 H), 5.81 (br. s., 1 H, OH), 4.05 (q, *J* = 7.1, 2 H), 3.27–3.20 (m, 4 H), 2.65 (m, 2 H), 2.36 (t, *J* = 7.3, 2 H), 1.97–1.86 (m, 4 H), 1.27 (t, *J* = 7.1, 3 H) ppm. ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 173.8, 155.2, 146.0, 129.7, 114.6, 102.5, 97.9, 60.7, 50.7, 49.4, 31.6, 27.3, 22.4, 21.5, 14.2 ppm. MS (ESI): *m/z* (positive mode, rel. int., %) = 286 (15) $[M + Na]^+$, 264 (100) $[M + H]^+$; HRMS ($C_{15}H_{21}NO_3$): *m/z* (positive mode) = 286.1416 (found $[M + Na]^+$), 286.1414 (calc.); 262.1448 (found $[M + H]^+$), 262.1449 (calc.).



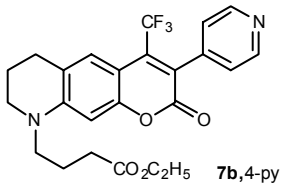
6b

Compound 6b: A solution of phenol **4b** (780 mg, 2.96 mmol), 2-chloro-4,4,4-trifluoroacetoacetate (890 mg, 3.70 mmol) and anhydrous $ZnCl_2$ (605 mg, 4.44 mmol) in EtOH (15 mL) was heated under reflux for 18 h. Ethanol was removed in vacuo, and the residue was subjected to column chromatography (CH_2Cl_2 to CH_2Cl_2/EtOAc 10:1) to afford the pure title compound (474 mg, 38%). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.28 (q, $^3J_{H\text{-}F}$ = 2.3, 1 H), 6.42 (s, 1 H), 4.10 (q, *J* = 7.2, 2 H), 3.22–3.40 (m, 4 H), 2.70–2.80 (m, 2 H), 2.36 (t, *J* = 7.3, 2 H), 1.85–1.98 (m, 4 H), 1.22 (t, *J* = 7.2, 3 H) ppm. ^{13}C NMR (75.5 MHz, $CDCl_3$, APT): δ = 172.6, 157.6, 153.2, 148.5, 136.4 (q, $J_{C\text{-}F}$ = 31.1), 124.8 (+, q, $J_{C\text{-}F}$ = 4.9), 124.4, 122.2 (q, $J_{C\text{-}F}$ = 282, CF_3), 120.8, 103.5, 96.5 (+), 60.7 (-), 50.6 (-), 49.3 (-), 31.2 (-), 27.9 (-), 21.4 (-), 21.2 (-), 14.2 (+) ppm; MS (ESI): *m/z* (positive mode, rel. int., %) = 440 (10) $[M + Na]^+$, 418 (100) $[M + H]^+$; HRMS ($C_{19}H_{19}ClF_3NO_4$): *m/z* (positive mode) = 440.0843 (found $[M+Na]^+$), 440.0847 (calc.); 418.1030 (found $[M + H]^+$), 418.1027 (calc.).



8b

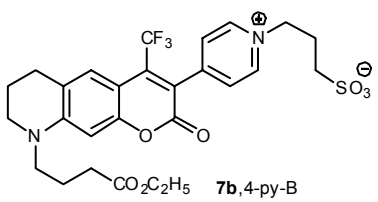
Compound 8b: Ethyl 4-(7-hydroxy-1,2,3,4-tetrahydroquinolin-1-yl)butanoate (527 mg, 2.00 mmol) and trifluoroacetic anhydride (2.0 mL, 3.0 g, 14 mmol) reacted according GPD. Chromatography on SiO_2 (80 g, hexane/EtOAc, 3:1) provided the title compound (529 mg, 74%) as a greenish oil which darkens quickly. R_f = 0.44 (hexane/EtOAc, 1:1). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 11.86 (s, 1 H, OH), 7.07–7.03 (m, 1 H, 5'-H), 6.09 (s, 1 H, 5'-H), 4.15 (q, *J* = 7.2, 2 H, CO_2CH_2), 3.30–3.43 (m, 4 H, 2'-H and 4-H), 2.67 (t, *J* = 6.2, 2 H), 2.36 (t, *J* = 7.3, 2 H), 2.01–1.89 (m, 4 H, 3-H and 3'-H), 1.26 (t, *J* = 7.2, 3 H, CH_3) ppm. ^{13}C NMR (75.5 MHz, $CDCl_3$, APT): δ = 179.9 (q, $J_{C\text{-}F}$ = 34.1, CO), 172.6, 166.6, 153.4, 149.9, 129.7 (+, q, $J_{C\text{-}F}$ = 3.1), 117.4 (q, $J_{C\text{-}F}$ = 289, CF_3), 114.2, 96.1, 60.6 (–, CH_2O), 50.7 (–), 49.8 (–), 31.2 (–), 27.4 (–), 21.6 (–), 21.4 (–), 14.2 (+, Me) ppm; MS (ESI): *m/z* (positive mode, rel. int., %) = 741 (15) $[2M + Na]^+$, 382 (12) $[M + Na]^+$, 360 (100), $[M + H]^+$; HRMS ($C_{17}H_{20}F_3NO_4$): *m/z* (positive mode) = 382.1230 (found $[M + Na]^+$), 382.1237 (calc.); 360.1419 (found $[M + H]^+$), 360.1417 (calc.).



7b,4-py

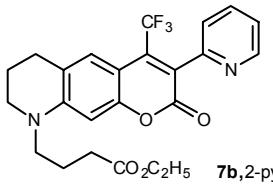
Coumarin 7b,4-py: According to GPC, compound **8b** (269 mg, 0.75 mmol), 4-pyridylacetic acid hydrochloride (195 mg, 1.12 mmol), DMAP (9 mg, 74 μmol), Et_3N (0.37 mL, 266 mg, 2.63 mmol) and DCC (230 mg, 1.11 mmol) were mixed in dichloromethane (10 mL). Chromatography on SiO_2 (20 g, hexane/EtOAc, 3:1 → 1:1)

provided the title compound (87 mg, 25%) as a yellow solid. R_f = 0.19 (hexane/EtOAc, 1:1); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.66 (m, 2 H, 2',6'-H), 7.32–7.27 (m, 1 H, 5-H), 7.23–7.18 (m, 2 H, 3',5'-H), 6.52 (s, 1 H, 10-H), 4.16 (q, J = 7.2, 2 H, CO_2Et), 4.45–3.34 (m, 4 H, 1'',8-H), 2.80 (t, J = 6.0, 2 H, 6-H), 2.38 (t, J = 7.2, 2 H, 3''-H), 2.06–1.83 (m, 4 H, 2'',7-H), 1.27 (t, J = 7.2, 3 H, CO_2Et) ppm. ^{13}C NMR (75.5 MHz, $CDCl_3$, APT): δ = 172.6 (–, C-4''), 160.5 (–, C-2), 156.8 (–, C-10a), 155.0 (–, C-9a), 149.3 (+, C-2',6'), 142.7 (–, C-4'), 137.6 (–, q, J = 30, C-4), 125.5 (+, q, J = 4, C-5), 124.5 (+, br. s, C-3',5'), 120.7 (–, C-3), 118.9 (–, C-5a), 122.3 (–, q, J = 279, CF_3), 103.2 (–, C-4a), 96.4 (+, C-10), 60.7 (–, CH_2O), 50.7 (–, C-1''), 49.4 (–, C-8), 31.2 (–, C-3''), 27.9 (–, C-6), 21.4 (–, C-2''), 21.2 (–, C-7), 14.2 (+, Me) ppm; ^{19}F-NMR (282.4 MHz, $CDCl_3$): δ = –54.2 (d, $J_{F\text{-}H}$ 2.3) ppm. UV/Vis (EtOH): λ_{max} (ε) = 211 (28900), 261 (8460), 430 (13700 M^{-1} cm^{-1}); fluorescence (EtOH): $\lambda_{excit.}$ = 432 nm, $\lambda_{em.}$ = 593 nm; MS (ESI): *m/z* (positive mode, rel. int., %) = 1403.5 (10) $[3M + Na]^+$, 943 (100) $[2M + Na]^+$, 921 (37) $[2M + H]^+$, 483 (73) $[M + Na]^+$, 461 (45), $[M + H]^+$; HRMS ($C_{24}H_{23}F_3N_2O_4$): *m/z* (positive mode) = 483.1491 (found $[M + Na]^+$), 483.1502 (calc.); 461.1670 (found [M + H]), 461.1683 (calc.).



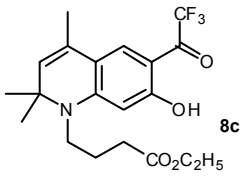
7b,4-py-B

Coumarin 7b,4-py-B: According to GPB, coumarin **7b**,4-py (21 mg, 45 μmol) and 1,3-propanesultone (62 mg, 0.51 mmol) reacted 18 h. Upon cooling, the reaction mixture was concentrated, and the solid residue was subjected to chromatography on SiO_2 (2 g, $CHCl_3$/MeOH, 4:1). The title compound (87 mg, 25%) was isolated as as a light red solid. R_f = 0.26 ($CHCl_3$/MeOH, 4:1); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 9.06 (m, 2 H, 2',6'-H), 8.11 (m, 2 H, 3',5'-H), 7.36 (d, J = 1.2, 1 H, 5-H), 6.70 (d, J = 1.2, 1 H, 10-H), 4.90 (t, J = 7.2, 2 H, 1'''-H), 4.16 (q, J = 7.2, 2 H, CO_2Et), 3.56–3.41 (m, 4 H, 1'',8-H), 2.95 (t, J = 6.8, 2 H, 3'''-H), 2.90 (t, J = 6.8, 2 H, 6-H), 2.51 (tt, J = 7.2 and 6.8, 2 H, 2'''-H), 2.44 (t, J = 6.9, 2 H, 3''-H), 2.05–1.93 (m, 4 H, 2'',7-H), 1.26 (t, J = 7.1, 3 H, CO_2Et) ppm. ^{13}C NMR (126 MHz, CD_3OD, APT): δ = 174.9 (C-4''), 161.4 (C-2), 157.4 (C-10a), 155.0 (C-9a), 151.8 (+, C-4'), 145.8 (C-2',6'), 139.7 (q, J = 31, C-4), 131.2 (C-3',5'), 126.6 (br. s, C-5), 123.9 (J = 279, CF_3), 123.2 (C-3), 115.8 (C-5a), 104.2 (C-4a), 97.6 (C-10), 61.9 (C-1'''), 61.3 (CH_2O), 51.8 (C-1''), 50.6 (C-8)), 48.3 (C-3'''), 32.0 (C-3''), 28.4 (C-2'''), 25.5 (C-6), 22.5 (C-2''), 22.3 (C-7), 14.7 (+, Me) ppm; ^{19}F-NMR (282.4 MHz, $CDCl_3$): δ = –54.1 (d, $J_{F\text{-}H}$ = 2.3) ppm. UV/Vis (EtOH): λ_{max} (ε) = 211 (28900), 261 (8460), 430 (13700 M^{-1} cm^{-1}); fluorescence (EtOH): $\lambda_{excit.}$ = 460 nm, $\lambda_{em.}$ = 655 nm; MS (ESI): *m/z* (positive mode, rel. int., %) = 1187 (6) $[2M + Na]^+$, 605.2 (100) $[M + Na]^+$; HRMS ($C_{27}H_{29}F_3N_2O_7S$): *m/z* (positive mode) = 605.1546 (found $[M + Na]^+$), 605.1540 (calc.).



7b,2-py

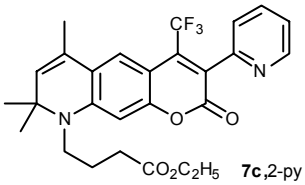
Coumarin 7b,2-py: According to GPA, compound **6b** (22 mg, 49 μmol), $Pd(dba)_2$ (1.0 mg, 1.7 μmol), 2-tributylstannylpyridine (19 mg, 52 μmol), and $P(Bu^t)_3$ (230 mg, 0.26 M indioxane, 10 μL, 2.6 μmol) reacted at 120°C for 18 h. Chromatography on SiO_2 (5 g, DCM/MeOH, 50:1) provided ethyl ester of the title compound (13 mg, 54%) as a dark yellow solid. R_f = 0.09 (DCM/MeOH, 50:1). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.64 (m, 1 H), 7.73 (m, 1 H), 7.38 - 7.27 (m, 3 H), 6.48 (s, 1 H), 4.16 (q, J = 7.2, 2 H, CO_2Et), 3.42 - 3.30 (m, 4 H), 2.78 (m, 2 H), 2.37 (t, J = 7.3, 2 H), 2.01-1.84 (m, 4 H), 1.22 (t, J = 7.2, 3 H, CO_2Et) ppm. ^{13}C NMR (126 MHz, $CDCl_3$, APT): δ = 172.6, 161.1, 154.9, 153.0, 149.2 (+), 148.7, 137.8 (q, $J_{C\text{-}F}$ = 30.6), 136.1 (+), 125.5 (+, q, $J_{C\text{-}F}$ = 3.6), 124.9 (+, q, $J_{C\text{-}F}$ = 1.6), 122.9 (+), 122.3 (q, $J_{C\text{-}F}$ = 278, CF_3), 120.8 (q, $J_{C\text{-}F}$ = 2.5), 120.4, 103.3, 96.4 (+), 60.7 (-), 50.7 (-), 49.5 (-), 31.4 (-), 28.0 (-), 21.6 (-), 21.3 (-), 14.3 (+);^{19}F-NMR (282.4 MHz, $CDCl_3$): δ = –55.9 (d, $J_{F\text{-}H}$ = 2.3) ppm. UV/Vis (EtOH): λ_{max} (ε) = 212 (44100), 262 (9960), 429 (16400 M^{-1} cm^{-1}); fluorescence (EtOH): $\lambda_{excit.}$ = 429 nm, $\lambda_{em.}$ = 578 nm; MS (ESI): *m/z* (positive mode, rel. int., %) = 483 (6) $[M + Na]^+$, 461 (100), $[M + H]^+$; HRMS ($C_{24}H_{23}F_3N_2O_4$): *m/z* (positive mode) = 483.1500 (found $[M + Na]^+$), 483.1502 (calc.); 461.1684 (found $[M + H]^+$), 461.1683 (calc.).



8c

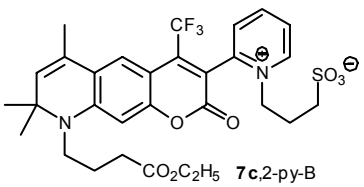
Compound 8c: A solution of TBAF trihydrate (662 mg, 2.10 mmol) in THF (3 mL) was added at 0°C to a solution of ethyl 4-[7-(*t*-butyldimethylsilyl)oxy]-2,2,4-trimethyl-1,2-

dihydroquinolin-1-yl]butanoate[1] (835 mg, 2.00 mmol) in THF (7 mL). The reaction mixture was stirred for 5 min at 0°C, quenched with brine (10 mL), and extracted with EtOAc (2×10 mL). The combined organic solutions were diluted with hexane (20 mL) and filtered through a pad of silica gel (10 g) without concentration. The fractions with product **4c** (TLC) were pooled and concentrated. According to GPD, the residue (crude **4c**) reacted with TFAA (2.0 mL, 3.0 g, 14 mmol). Chromatography on SiO_2 (80 g, hexane/EtOAc, 3:1→1:1) provided the title compound (500 mg, 65%) as a greenish oil which darkens quickly. R_f = 0.73 (hexane/EtOAc, 1:1); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 11.86 (s, 1 H, OH), 7.29–7.27 (m, 1 H, 5'-H), 6.09 (s, 1 H, 8'-H), 5.21 (br. s., 1 H, H-3'), 4.16 (q, J = 7.2, 2 H, CO_2Et), 3.33–3.39 (m, 2 H), 2.40 (t, J = 6.2, 2 H), 1.93–1.90 (m, 2 H), 1.89 (s, 3 H, MeCH=), 1.40 (s, 6 H, Me×2), 1.27 (t, J = 7.2, 3 H, CO_2Et) ppm. ^{13}C NMR (126 MHz, $CDCl_3$, APT): δ = 180.0 (q, J = 34, CO), 172.7 (–, C-1), 167.9 (–, C-7'), 152.3 (–, C-8'a), 128.3 (+, CH), 126.0 (–), 124.6 (+, q, J = 3.7, C-5'), 117.5 (–, q, J = 290, CF_3), 116.3 (–), 109.9 (–, C-6'), 96.7 (+, C-8'), 60.8 (–, CH_2O), 58.7 (–), 44.2 (–), 31.3 (–, C-2), 29.4 (+, CH_3), 22.7 (–), 18.4 (+, CH_3), 14.2 (+, CH_2Me) ppm; ^{19}F-NMR (282.4 MHz, $CDCl_3$): δ = –69.6 (d, J_{F-H} = 2.0) ppm. MS (ESI): *m/z* (positive mode, rel. int., %) = 422 (15) $[M + Na]^+$, 400 (100) $[M + H]^+$; MS (ESI): *m/z* (negative mode, rel. int., %) = 398 (100) $[M - H]^-$. HRMS ($C_{20}H_{24}F_3NO_4$): *m/z* (positive mode) = 422.1547 (found $[M + Na]^+$), 422.1550 (calc.); 400.1732 (found $[M + H]^+$), 400.1730 (calc.); *m/z* (negative mode) = 398.1577 (found $[M - H]^-$), 398.1585 (calc.).



7c,2-py

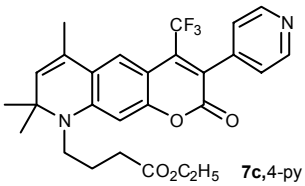
Coumarin 7c,2-py: According to GPC, compound **8c** (100 mg, 0.25 mmol), 2-pyridylacetic acid hydrochloride (66 mg, 0.38 mmol), DMAP (3.6 mg, 29 µmol), Et_3N (0.13 mL, 95 mg, 0.94 mmol) and DCC (78 mg, 0.38 mmol) reacted for 20 h. Chromatography on SiO_2 (10 g, hexane/EtOAc, 1:1) yielded the title compound (67 mg, 54%) as a yellow solid along with starting material (R_f = 0.59, 47 mg). R_f = 0.42 (hexane/EtOAc, 1:1); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.69 (m, 1 H, 6'-H), 7.77 (m, 1 H, 4'-H), 7.37 (m, 1 H, 3'-H), 7.35 (q, J = 2.2, 1 H, 5-H), 7.31 (ddd, J = 7.7, 5.0 and 1.2, 1 H, 5'-H), 6.42 (s, 1 H, 10-H), 5,35 (q, J =1.2, 1 H, 7-H), 4.20 (q, J = 7.2, 2 H, CO_2Et), 3.31–3.41 (m, 2 H, 1''-H), 2.43 (t, J = 6.8, 2 H, 3''-H), 2.00 (d, J = 1.2, 3 H, 6-Me), 1.88–1.99 (m, 2 H, 2''-H), 1.42 (s, 6 H, 8-Me), 1.31 (t, J = 7.2, 3 H, CO_2Et) ppm. ^{13}C NMR (75.5 MHz, $CDCl_3$, APT): δ = 172.6 (–, C-4''), 161.1 (–, C-2), 156.1 (–, C-10a), 153.0 (–, C-2'), 149.4 (+, C-6'), 147.9 (–, C-9a), 138.0 (–, q, J = 30, C-4), 136.1 (+, C-4), 130.2 (+, C-7), 126.2 (–, C-5a), 124.9 (+, C-5'), 123.0 (+, C-3'), 122.4 (–, q, J = 279, CF_3), 121.2 (–, q, J = 2, C-3), 120.6 (–, C-6), 120.5 (+, q, J = 4, C-5), 103.5 (–, C-4a), 96.9 (+, C-10), 60.7 (–, CH_2O), 58.2 (–, C-8), 44.0 (–, C-3'), 31.3 (–, C-1''), 29.3 (+, 10-Me), 22.6 (–, C-2''), 18.6 (+, 6-Me), 14.2 (+, Me) ppm; ^{19}F-NMR (282.4 MHz, $CDCl_3$): δ = –55.9 (d, J = 2.3) ppm. UV/Vis (EtOH): λ_{max} (ε) = 215 (40600), 238 (34100), 269 (9740), 358 (3510), 438 (17900 M^{-1} cm^{-1}); fluorescence (EtOH): $\lambda_{excit.}$ = 449 nm, $\lambda_{em.}$ = 591 nm; MS (ESI): *m/z* (positive mode, rel. int., %) = 1023 (25) $[2M + Na]^+$, 1001 (50) $[2M + H]^+$, 523 (58) $[M + Na]^+$, 501 (100) $[M + H]^+$; HRMS ($C_{27}H_{27}F_3N_2O_4$): *m/z* (positive mode) = 523.1805 (found $[M + Na]^+$), 523.1815 (calc.); 501.1987 (found $[M + H]^+$), 501.1996 (calc.).



7c,2-py-B

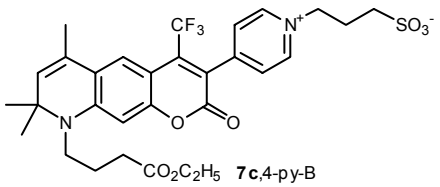
Coumarin 7c,2-py-B: According to GPB, coumarin **7c**,2-py (20 mg, 40 µmol) and 1,3-propanesultone (54 mg, 442 µmol) in MeCN (1 mL) were reacted for 5 d. Column chromatography (2 g SiO_2, $CHCl_3$/MeOH 4:1, R_f = 0.39) gave the title compound (25 mg, quant.) as a red solid. ^{1}H-NMR (300 MHz, CD_3OD): δ = 9.28 (m, 6'-H), 8.68 (m, 1 H, 4'-H), 8.23 (ddd, J = 7.6, 6.3, and 1.5, 1 H, 5'-H), 8.17 (m, 1 H, 3'-H), 7.37 (q, J = 1.9, 1 H, 5-H), 6.79 (s, 1 H, 10-H), 5.56 (s, 1 H, 7-H), 4.19 (q, J = 7.2, 2 H, CO_2Et), 3.46–3.59 (m, 2 H, 1''-H), 2.76–2.99 (m, 4 H, 1''',3'''-H), 2.52 (t, J = 6.6, 2 H, 3''-H), 2.27–2.47 (m, 2 H, 2'''-H), 2.02 (s, 3 H, 6-CH_3), 1.90–2.00 (m, 2 H, 2''-H), 1.47 (s, 3 H, 8-CH_3), 1.46 (s, 3 H, 8-CH_3), 1.29 (t, J = 7.2, 3 H, CO_2Et) ppm. ^{13}C-NMR (75.5 MHz, CD_3OD, APT) δ = 174.9 (–, C-4''), 161.0 (–, C-2), 159.6 (–, C-10a), 151.7 (–, C-2'), 151.4 (–, C-9a), 147.9 (+, C-6'), 147.6 (+, C-4'), 142.4 (–, q, J = 31.2, C-4), 132.8 (+, C-5'), 132.8 (+, br. s., C-7), 129.8 (+, C-3'), 126.9 (–, C-

5a), 123.6 (–, q, J = 278 Hz, CF_3), 122.8 (–, C-6), 121.3 (+, q, J = 3.1, C-5), 110.1 (–, q, J = 2 Hz, C-3), 104.5 (–, C-4a), 98.6 (+, C-10), 61.9 (–, C-1'''), 60.4 (–, CH_2O), 58.8 (–, C-8), 50.3 (–, C-3'''), 45.4 (–, C-3''), 31.8 (–, C-1''), 29.7 (+, 8-CH_3), 29.7 (+, 8-CH_3), 28.0 (–, C-2'''), 23.8 (–, C-2''), 18.7 (+, 6-CH_3), 14.8 (+, CH_3) ppm; ^{19}F-NMR (282.4 MHz, $CDCl_3$): δ = –56.2 (d, J_{F-H} = 2.3) ppm. UV/Vis (EtOH): λ_{max} (ε) = 213 (35000), 238 (38200), 271 (12200), 332 (3510), 356 (3030), 479 (22400 M^{-1} cm^{-1}); fluorescence (EtOH): $\lambda_{excit.}$ = 480 nm, $\lambda_{em.}$ = 632 nm; MS (ESI, MeOH, positive mode): 645 ([M + Na]$^+$, 100%); HRMS ($C_{30}H_{33}F_3N_2O_7S$): *m/z* (positive mode) = 645.1856 (found [M + Na]$^+$), 645.1853 (calc.).



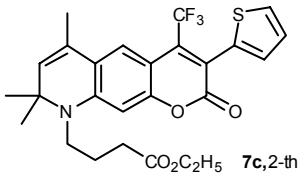
7c,4-py

Coumarin 7c,4-py: According to GPC, compound **8c** (100 mg, 0.25 mmol), 4-pyridylacetic acid hydrochloride (44 mg, 0.253 mmol), DMAP (3.1 mg, 25 µmol), Et_3N (0.13 mL, 95 mg, 0.94 mmol) and DCC (52 mg, 0.25 mmol) reacted for 20 h. Chromatography on SiO_2 (10 g, hexane/EtOAc, 1:1) yielded the title compound (80 mg, 64%) as a yellow solid, R_f = 0.36 (hexane/EtOAc, 1:1); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.65 (m, 2 H), 7.32 (q, J_{C-F} = 2.2, 1 H, 5-H), 7.18 (m, 2 H), 6.41 (s, 1 H), 5,35 (q, J = 1.2, 1 H, 7-H), 4.18 (q, J = 7.2, 2 H, CO_2Et), 3.34 (m, 2 H), 2.40 (t, J = 6.8, 2 H), 2.00 (d, J = 1.2, 3 H, 6-Me), 1.90 (m, 2 H), 1.40 (s, 6 H, 8-Me), 1.26 (t, J = 7.2, 3 H, CO_2Et) ppm; ^{13}C NMR (125.7 MHz, $CDCl_3$, APT): δ = 172.7, 160.5, 156.1, 149.5 (+), 148.1, 142.4, 137.7 (q, J_{C-F} = 30.3), 130.5 (+), 126.1, 124.5 (+), 122.3 (q, J_{C-F} = 279.1), 120.7, 120.3 (+, q, J_{C-F} = 4.1), 119.1 (q, J_{C-F} = 2.3), 103.3, 96.9 (+), 60.8, 58.2, 44.0, 31.3, 29.3 (+), 22.6, 18.6 (+), 14.2 (+) ppm; UV/Vis (EtOH): λ_{max} (ε) = 440 (18100 M^{-1} cm^{-1}); fluorescence (EtOH): $\lambda_{excit.}$ = 443 nm, $\lambda_{em.}$ = 611 nm; MS (ESI): *m/z* (positive mode, rel. int., %) = 1023 (14) [2M + Na]$^+$, 523 (60) [M + Na]$^+$, 501 (100) [M + H]$^+$; HRMS ($C_{27}H_{27}F_3N_2O_4$): *m/z* (positive mode) = 523.1812 (found [M + Na]$^+$), 523.1815 (calc.); 501.1994 (found [M + H]$^+$), 501.1996 (calc.).



7c,4-py-B

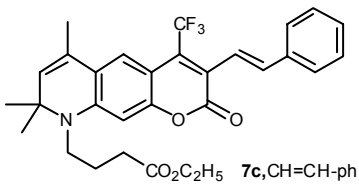
Coumarin 7c,4-py-B: According to GPB, coumarin **7c**,4-py (20 mg, 40 µmol) and 1,3-propanesultone (54 mg, 442 µmol) in MeCN (1 mL) were reacted for 2 d. Column chromatography (2 g SiO_2, $CHCl_3$/MeOH 4:1, R_f = 0.36) gave the title compound (17 mg, 68%) as a red solid. ^{1}H-NMR (300 MHz, CD_3OD): δ = 9.08 (m, 2 H), 8.15 (m, 2 H), 7.37 (q, J = 1.9, 1 H, 5-H), 6.80 (s, 1 H), 5.58 (q, J = 1.2, 1 H), 4.20 (q, J = 7.2, 2 H, CO_2Et), 3.50 (m, 2 H), 2.90–2.78 (m, 2 H), 2.55-2.30 (m, 4 H), 2.02 (d, J = 1.2, 3 H, Me), 2.00-1.89 (m, 4 H), 1.45 (s, 3 H, Me), 1.44 (s, 3 H, Me), 1.28 (t, J = 7.2, 3 H, CO_2Et) ppm. UV/Vis (EtOH): λ_{max} (ε) = 475 (23000 M^{-1} cm^{-1}); fluorescence (EtOH): $\lambda_{excit.}$ = 474 nm, $\lambda_{em.}$ = 687 nm; MS (ESI, MeOH, positive mode): 645 ([M + Na]$^+$, 100%); HRMS ($C_{30}H_{33}F_3N_2O_7S$): *m/z* (positive mode) = 645.1849 (found [M + Na]$^+$), 645.1853 (calc.).



7c,2-th

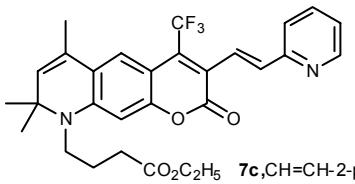
Coumarin 7c,2-th: According to GPC, ketophenol **8c** (50 mg, 125 µmol), thiophen-2-ylacetic acid (27 mg, 190 µmol), DMAP (1.6 mg, 13 µmol), triethylamine (44 µL, 31.7 mg, 313 µmol) and DCC (39 mg, 190 µmol) reacted for 18 h. Column chromatography (10 g SiO_2, DCM) provided the title compound (R_f = 0.14, 36 mg, 57%) as a yellow solid along with starting material (R_f = 0.29, 8 mg, 20 µmol). ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 7.49 (dd, J = 4.9, 1.1 Hz, 1 H, 5'-H), 7.36 (q, J = 2.0 Hz, 1 H, 5-H), 7.08 (dd, J = 4.9, 3.4 Hz, 1 H, 4'-H), 7.05 (dd, J = 3.4, 1.1 Hz, 1 H, 3'-H), 6.42 (s, 1 H, 10-H), 5.35 (s, 1 H, 7-H), 4.21 (q, J = 7.2 Hz, 2 H, CO_2Et), 3.30–3.42 (m, 2 H, 1''-H), 2.43 (t, J = 6.8 Hz, 2 H, 3''-H), 2.01 (s, 3 H, 6-CH_3), 1.95 (m, 2 H, 2''-H), 1.42 (s, 6 H, 8-CH_3), 1.31 (t, J = 7.2 Hz, 3 H, CO_2Et) ppm. ^{13}C-NMR (75.5 MHz, $CDCl_3$, APT): δ = 172.7 (–, C-4''), 160.9 (–, C-2), 155.8 (–, C-10a), 147.8 (–, C-9a), 138.4 (–, q, J = 30.2, C-4), 133.3 (–, C-2'), 130.3 (+, C-3'), 129.5 (+, q, J = 2.0, C-7), 127.8 (+, C-5'), 126.5 (+, C-4'), 126.2 (–, C-5a), 120.5 (–, C-6), 120.4 (+, q, J = 4.1, C-5), 122.5 (–, q, J = 279 Hz, CF_3), 115.4 (–, C-3), 104.0 (–, C-4a), 96.9 (+, C-10), 60.7 (–, CH_2O), 58.2 (–, C-8), 44.0 (–, C-1''), 31.3 (–, C-3''), 29.2 (+, 6-CH_3), 22.6 (–, C-2''), 18.6 (+, 8-CH_3), 14.3 (+, CO_2Et) ppm. ^{19}F-NMR (282.4 MHz, $CDCl_3$): δ = –55.5 (d, J_{F-H} = 2.3) ppm.

UV/Vis (EtOH): λ_{max} (ε) = 218 (26500), 238 (33600), 272 (67500), 322 (3080), 359 (3010), 445 (17400 M^{-1} cm^{-1}); fluorescence (EtOH): $\lambda_{excit.}$ = 454 nm, $\lambda_{em.}$ = 636 nm; MS (ESI, positive mode): 506 ($[M + H]^+$, 13%), 528 ($[M + Na]^+$, 70%), 1033 ($[2M + Na]^+$, 100%). HRMS (ESI, positive mode): $C_{26}H_{26}F_3NO_4S$, $[M + H]^+$ calcd. 506.1607, found 506.1599; $[M + Na]^+$ calcd. 528.1427, found 528.1423.



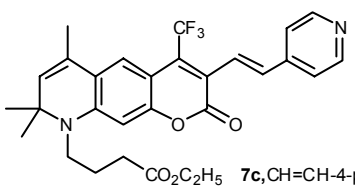
7c,CH=CH-ph

Coumarin 7c,CH=CH-ph: According to GPC, ketophenol **8c** (80 mg, 0.20 mmol), styrylacetic acid (36 mg, 0.22 mmol), DMAP (4 mg, 33 µmol), Et_3N (42 µL, 30 mg, 0.30 mmol) and DCC (41 mg, 0.20 mmol) reacted for 19 h. Column chromatography (20 g SiO_2, DCM) yielded the title compound (R_f = 0.44, 32 mg, 31%) as a yellow solid along with starting material (R_f = 0.46, 44 mg, 110 µmol). ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 7.64 (d, J = 16.2, 1 H, 2'-H), 7.53 (m, 3",5"-H), 7.25–7.42 (m, 4 H, 5,2",4",6"-H), 7.17 (dq, J_{H-H} = 16.2 and J_{H-F} = 2.8, 1 H, 1'-H), 6.39 (s, 1 H, 10-H), 5.34 (s, 1 H, 7-H), 4.21 (q, J = 7.0, 2 H, CO_2Et), 3.30–3.40 (m, 2 H, 1'"-H), 2.43 (t, J = 6.8 Hz, 2 H, 3'"-H), 2.01 (s, 3 H, 6-CH_3), 1.88–1.99 (m, 2 H, 2'"-H), 1.40 (s, 6 H, 8-CH_3), 1.32 (t, J = 7.2, 3 H, CO_2Et) ppm. ^{13}C-NMR (75.5 MHz, $CDCl_3$, APT): δ = 172.7 (–, C-4'"), 160.2 (–, C-2), 154.7 (–, C-10a), 147.2 (–, C-9a), 137.5 (+, q, J = 2, C-2'), 137.4 (–, C-1"), 134.5 (–, q, J = 30, C-4), 130.2 (+, C-7), 128.6 (+, C-3",5"), 128.3 (+, C-4"), 127.0 (+, C-2",6"), 126.4 (–, C-5a), 123.6 (–, q, J = 280, CF_3), 120.5 (–, C-6), 120.1 (+, q, J = 5, C-1'), 119.5 (+, q, J = 3, C-5), 118.4 (–, q, J = 2, C-3), 104.5 (–, C-4a), 96.8 (+, C-10), 60.7 (–, CH_2O), 58.1 (–, C-8), 43.9 (–, C-3'"), 31.4 (–, C-1'"), 29.2 (+, 6-CH_3), 22.7 (–, C-2'"), 18.6 (+, 8-CH_3), 14.3 (+, CH_3) ppm. ^{19}F-NMR (282.4 MHz, $CDCl_3$): δ = –54.2 (dd, J^1_{F-H} = J^2_{F-H} = 2.3) ppm. UV/Vis (EtOH): λ_{max} (ε) = 211 (54100), 240 (25600), 306 (10300), 467 (20000 M^{-1} cm^{-1}); fluorescence (EtOH): $\lambda_{excit.}$ = 477 nm, $\lambda_{em.}$ = 598 nm; MS (ESI, positive mode): 526 ($[M + H]^+$, 19%), 548 ($[M + Na]^+$, 80%), 1073 ($[2M + Na]^+$, 100%). HRMS (ESI, positive mode): $C_{30}H_{30}F_3NO_4$, $[M + H]^+$ calcd. 526.2200, found 526.2191; $[M + Na]^+$ calcd. 548.2019, found 548.2010.



7c,CH=CH-2-py

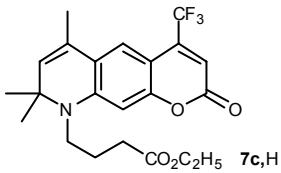
Coumarin 7c,CH=CH-2-py: According to GPC, ketophenol **8c** (60 mg, 150 µmol), 2-(2-pyridyl)ethenylacetic acid hydrochloride (45 mg, 225 µmol), DMAP (1.9 mg, 16 µmol), Et_3N (53 µL, 38 mg, 0.38 mmol) and DCC (46 mg, 0.22 mol) reacted for 20 h. Column chromatography (20 g SiO_2, DCM/MeOH, 50:1) yielded the title compound (R_f = 0.10, 31 mg, 39%) as a red solid along with starting material (R_f = 0.68, 17 mg, 42.6 µmol). ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 8.63 (m, 1 H, 6"-H), 7.85 (dq, J = 15.6 and 3.1, 1 H, 1'-H), 7.74 (d, J = 15.6, 1 H, 2'-H), 7.67 (m, 1 H, 4"-H), 7.40 (q, J = 1.9, 1 H, 5-H), 7.37 (m, 1 H, 3"-H), 7.17 (ddd, J = 7.5, 4.8 and 1.1, 1 H, 5"-H), 6.40 (s, 1 H, 10-H), 5.34 (q, J = 1.4, 1 H, 7-H), 4.21 (q, J = 7.1, 2 H, CO_2Et), 3.30–3.41 (m, 2 H, 1'"-H), 2.43 (t, J = 6.7, 2 H, 3'"-H), 2.01 (d, J = 1.2, 3 H, 6-CH_3), 1.89–2.00 (m, 2 H, 2'"-H), 1.41 (s, 6 H, 8-CH_3), 1.32 (t, J = 7.2, 3 H, CO_2Et) ppm. ^{13}C-NMR (125.7 MHz, $CDCl_3$, APT): δ = 172.6 (–, C-4'"), 159.9 (–, C-2), 155.1 (–, C-2"), 154.8 (–, C-10a), 149.6 (+, C-6"), 147.4 (–, C-9a), 136.5 (+, C-4"), 135.9 (+, C-2'), 135.7 (–, q, J = 29, C-4), 130.1 (+, C-7), 126.2 (–, C-5a), 123.5 (+, C-5"), 123.4 (–, q, J = 279, CF_3), 123.4 (+, C-5), 122.5 (+, C-3"), 120.5 (–, C-6), 120.2 (+, q, J = 5, C-1'), 117.4 (–, C-3), 104.5 (–, C-4a), 96.7 (+, C-10), 60.8 (–,CH_2O), 58.2 (–, C-8), 44.0 (–, C-3'"), 31.4 (–, C-1'"), 29.3 (+, 6-CH_3), 22.7 (–, C-2'"), 18.7 (+, 8-CH_3), 14.3 (+, CH_3) ppm. ^{19}F-NMR (282.4 MHz, $CDCl_3$): δ = –54.4 (dd, J^1_{F-H} = J^2_{F-H} = 2.3) ppm. UV/Vis (EtOH): λ_{max} (ε) = 212 (54000), 238 (32300), 313 (14300), 475 (30400 M^{-1} cm^{-1}); fluorescence (EtOH): $\lambda_{excit.}$ = 475 nm, $\lambda_{em.}$ = 602 nm; MS (ESI, positive mode): 527 ($[M + H]^+$, 51%), 549 ($[M + Na]^+$, 55%), 1053 ($[2M + H]^+$, 74%), 1075.4 ($[2M + Na]^+$, 100%). HRMS (ESI, positive mode): $C_{29}H_{29}F_3N_2O_4$, $[M + H]^+$ calcd. 527.2152, found 527.2139; $[M + Na]^+$ calcd. 549.1972, found 549.1966.



7c,CH=CH-4-py

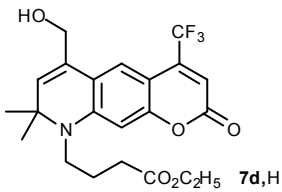
Coumarin 7c,CH=CH-4-py: According to GPC, keto-phenol **8c** (45 mg, 0.11 mmol), 2-(4-pyridyl)ethenylacetic acid trifluoroacetate (36 mg, 0.13 mmol), DMAP (2.2 mg, 18 µmol), Et_3N (69 µL, 46 mg, 0.46 mmol), and DCC (24 mg, 0.12 mmol) reacted for 18 h. Column chromatography (15 g SiO_2, DCM/MeOH,

50:1) provided the title compound (R_f = 0.09, 21 mg, 35%) as a red solid along with starting material (R_f = 0.68, 14 mg, 35 µmol). ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 8.50–8.61 (m, 2 H, 3'',5''-H), 7.63 (d, J = 15.9 , 1 H, 2'-H), 7.35–7.44 (m, 3 H, 5,2'',6''-H), 7.35 (dq, J = 16.1 and 3.1, 1 H, 1'-H), 6.40 (s, 1 H, 10-H), 5.35 (s, 1 H, 7-H), 4.20 (q, J = 7.0, 2 H, CO_2Et), 3.31–3.41 (m, 2 H, 1'''-H), 2.43 (t, J = 6.9, 2 H, 3'''-H), 2.00 (s, 3 H, 6-CH_3), 1.87–1.99 (m, 2 H, 2'''-H), 1.41 (s, 6 H, 8-CH_3), 1.31 (t, J = 7.2, 3 H, CO_2Et) ppm. ^{13}C-NMR (125.7 MHz, $CDCl_3$, APT): δ = 172.5 (–, C-4'''), 159.7 (–, C-2), 155.1 (–, C-10a), 149.4 (+, C-3'',5''), 147.8 (–, C-9a), 145.3 (–, C-4''), 136.2 (–, q, J = 30, C-4), 134.0 (+, C-2'), 130.3 (+, C-7), 126.1 (–, C-5a), 124.2 (+, q, J = 4, C-5), 123.4 (–, q, J = 280, CF_3), 121.2 (+, C-2'',6''), 120.7 (–, C-6), 120.2 (+, q, J = 5, C-1'), 116.2 (–, C-3), 104.3 (–, C-4a), 96.7 (+, C-10), 60.8 (–, CH_2O), 58.3 (–, C-8), 44.1 (–, C-3'''), 31.4 (–, C-1'''), 29.3 (+, 6-CH_3), 22.7 (–, C-2'''), 18.7 (+, 8-CH_3), 14.3 (+, CH_3) ppm. ^{19}F-NMR (282.4 MHz, $CDCl_3$): δ = –53.65 (dd, $J^1_{F\text{-}H}$ = $J^2_{F\text{-}H}$ = 2.3) ppm. UV/Vis (EtOH): λ_{max} (ε) = 213 (87000), 242 (43700), 308 (14800), 361 (4680), 480 (32100 M^{-1} cm^{-1}); fluorescence (EtOH): $\lambda_{excit.}$ = 480 nm, $\lambda_{em.}$ = 609 nm; MS (ESI, positive mode): 527 ([M + H]$^+$, 100%), 549 ([M + Na]$^+$, 6%), 1053 ([2M + H]$^+$, 22%). HRMS (ESI, positive mode): $C_{29}H_{29}F_3N_2O_4$, [M + H]$^+$ calcd. 527.2152, found 527.2152; [M + Na]$^+$ calcd. 549.1972, found 549.1961.



7c,H

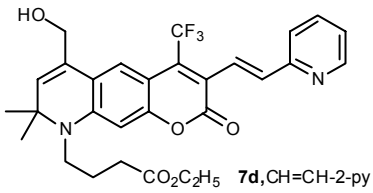
Coumarin 7c,H: Ethyl 4,4,4-trifluoroacetoacetate (0.390 mL, 491 mg, 2.67 mmol) and anhydrous zinc chloride (331 mg, 2.43 mmol) were added to a solution of phenol **4c** [20] (584 mg, 1.92 mmol) in ethanol (4 mL), the reaction mixture was heated at reflux under argon for 1 d and stirred at room temperature for further 2 d. The reaction mixture was concentrated, and the residue was purified by column chromatography (70 g SiO_2, hexane/EtOAc, 6:1) to yield the title compound (672 mg, 83%) as a yellow solid. R_f = 0.23 (hexane/EtOAc, 4:1); ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 7.21 (q, J = 2.0, 1 H, 5-H), 6.41 (s, 1 H, 3-H), 6.35 (s, 1 H, 9-H), 5.33 (br. s., 1 H, 7-H), 4.19 (q, J = 7.2, 2 H, CO_2Et), 3.29–3.38 (m, 2 H, 1'-H), 2.41 (t, J = 6.8, 2 H, 3'-H), 1.98 (s, 3 H, 6-Me), 1.85–1.98 (m, 2 H, 2'-H), 1.39 (s, 6 H, 8-Me), 1.30 (t, J = 7.2, 3 H, CO_2Et) ppm. ^{13}C-NMR (126 MHz, $CDCl_3$, APT): δ = 172.5 (–, C-4'), 160.3 (–, C-2), 156.9 (–, C-9a), 148.0 (–, C-8a), 141.5 (–, q, J = 32, C-4), 130.2 (+, C-7), 126.0 (–, C-5a) , 121.9 (–, q, J = 275, CF_3), 120.4 (–, C-6), 119.2 (+, C-5), 107.7 (+, q, J = 6, C-3), 102.6 (–, C-4a), 97.2 (+, C-9), 60.7 (–, CH_2O), 58.2 (–, C-8), 44.0 (–, C-3'), 31.4 (–, C-1'), 29.3 (+, 8-Me), 22.6 (–, C-2'), 18.6 (+, 6-Me), 14.3 (+, CH_3) ppm. ^{19}F-NMR (282.4 MHz, $CDCl_3$): δ = –64.6 (d, $J_{F\text{-}H}$ = 2.3) ppm. UV/Vis (EtOH): λ_{max} (ε) = 216 (44400), 237 (41600), 268 (8710), 357 (5250), 424 (19600 M^{-1} cm^{-1}); fluorescence (EtOH): $\lambda_{excit.}$ = 423 nm, $\lambda_{em.}$ = 527 nm; MS (ESI, positive mode): 424 ([M + H]$^+$, 7%), 446 ([M + Na]$^+$, 28%), 869 ([2M + Na]$^+$, 100%). HRMS (ESI, positive mode): $C_{22}H_{24}F_3NO_4$, [M + H]$^+$ calcd. 424.1730, found 424.1719; [M + Na]$^+$ calcd. 446.1550, found 446.1543.



7d,H

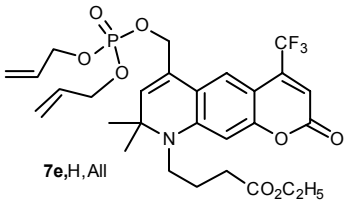
Coumarin 7d,H: Coumarin **7c**,H (127 mg, 0.3 mmol) and SeO_2 (83 mg, 0.75 mmol) were dissolved in a mixture of MeCN (2 mL) and water (0.2 mL), The reaction mixture was refluxed for 18 h, cooled down to room temperature, and evaporated. The residue was taken-up in EtOH (2 mL), and $NaBH_4$ (28 mg, 0.74 mmol) was added to this solution at room temperature in one portion. After stirring for 30 min, TLC showed complete consumption of the starting material. The reaction mixture was diluted with water (10 mL), DCM was added (10 mL), organic layer was separated, and the aqueous solution was extracted with DCM (3×5 mL). The combined organic solutions were dried over $MgSO_4$, concentrated, and the residue was purified by column chromatography (70 g SiO_2, hexane/EtOAc, 4:1→2:1→0:1) to yield the title compound (80 mg, 61%) as a yellow solid. R_f = 0.14 (hexane/EtOAc, 2:1); ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 7.32 (q, J = 1.7, 1 H, 5-H), 6.44 (s, 1 H, 10-H), 6.39–6.32 (m, 1 H, 3-H), 5.60 (s, 1 H, 7-H), 4.47 (d, J = 1.2, 2 H, CH_2OH), 4.19 (q, J = 7.2, 2 H, CO_2Et), 3.40–3.28 (m, 2 H, 1'-H), 2.41 (t, J = 6.8, 2 H, 3'-H), 1.97–1.87 (m, 2 H, 2'-H), 1.42 (s, 6 H, 8-Me), 1.30 (t, J = 7.2, 3 H, CO_2Et) ppm. ^{13}C-NMR (75.5 MHz, $CDCl_3$, APT): δ= 172.7 (–, C-4'), 160.4 (–, C-2), 156.9 (–, C-10a), 148.1 (–, C-9a), 141.6 (–, q, J = 32, C-4), 130.0 (+, C-7), 129.3 (–, C-6) , 121.9 (–, q, J = 276, CF_3), 119.0 (+, q, J = 2, C-5), 117.8 (–, C-5a), 108.0 (+, q, J = 6, C-3), 102.7 (–, C-4a), 97.7 (+, C-10), 62.4 (–, CH_2OH), 60.8 (–, CH_2O), 58.1 (–, C-8), 43.9 (–, C-3'), 31.2 (–, C-1'), 29.3 (+, 8-Me),

22.5 (–, C-2'), 14.2 (+, *C*H$_3$) ppm. ^{19}F-NMR (282.4 MHz, CDCl$_3$): δ = –64.6 (d, $J_{F\text{-}H}$ = 2.3) ppm.UV/Vis (EtOH): λ_{max} (ε) = 212 (45800), 237 (16000), 261 (5370), 357 (3400), 420 (8000 M^{-1} cm^{-1}); fluorescence (EtOH): $\lambda_{excit.}$ = 420 nm, $\lambda_{em.}$ = 523 nm; MS (ESI, positive mode): 901 (100) [M + H]$^+$, 462 (35) [M + Na]$^+$, 440 (2) [M + H]$^+$; HRMS (ESI, positive mode): $C_{22}H_{24}F_3NO_5$ [M + H]$^+$ found 440.1665, calcd. 440.1679; [M + Na]$^+$ found 462.1485, calcd. 446.1499.



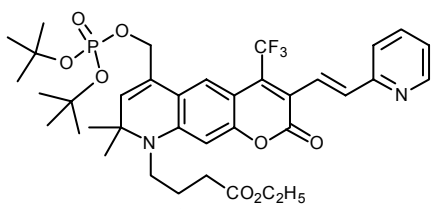
7d,CH=CH-2-py

Coumarin 7d,**CH=CH-2-py:** Finelly powdered SeO_2 (52 mg, 0.47 mmol) was added to a solution of coumarin **7c**,CH=CH-2-py (165 mg, 0.314 mmol) in dioxane (10 mL), The reaction mixture was heated (100 °C) for 1 h, cooled down to room temperature, and evaporated up to dryness. The residue was taken-up in EtOH / THF mixture (2 / 2 mL) cooled (0 °C) and $NaBH_4$ (20 mg, 0.526 mmol) was added to this solution in one portion. After stirring for 5 min, TLC showed complete consumption of the starting material. The reaction mixture was diluted with acetone (2 mL), evaporated and the residue was purified by column chromatography (hexane/dichloromethane/EtOAc, 4:0:1→2:1:1) with following precipitation from ether/ hexane to yield the title compound (80 mg, 47%) as a red solid.
^{1}H-NMR (300 MHz, CDCl$_3$): δ = 8.59 (m, 1 H,), 7.77 (dq, $J_{H\text{-}H}$ = 15.6, $J_{H\text{-}F}$ = 3.0, 1 H), 7.67 (d, J = 15.6, 1 H), 7.63 (ddd, J = 7.7, 7.7, 1.9, 1 H), 7.48 (q, J = 1.8, 1 H), 7.33 (m, 1 H), 7.14 (ddd, J = 7.7, 4.7, 1.1, 1 H), 6.39 (s, 1 H), 5.55 (s, 1 H), 4.47 (s, 2 H), 4.19 (q, J = 7.2, 2 H), 3.34 (m, 2 H), 2.41 (t, J = 6.8, 2 H), 1.93 (m, 2 H), 1.40 (s, 6 H), 1.28 (t, J = 7.2, 3 H). ^{13}C-NMR (125.7 MHz, CDCl$_3$, APT): δ = 172.8, 159.9, 155.2, 154.9, 149.8 (+), 147.6, 136.5 (+),136.2 (+), 135.6 (q, $J_{C\text{-}F}$ = 29.7), 129.9 (+), 129.6, 123.5 (+), 123.4 (q, $J_{C\text{-}F}$ = 279.5), 123.3 (+, q, $J_{C\text{-}F}$ = 3.7), 122.6 (+), 120.0 (+, q, $J_{C\text{-}F}$ = 5.6), 117.9, 117.7, 104.6, 97.2 (+), 62.5, 60.8, 58.1, 44.0, 31.3, 29.1(+), 22.7, 14.3 (+) ppm. ^{19}F-NMR (282.4 MHz, CDCl$_3$): δ = –54.2 (dd, $J^1_{F\text{-}H}$ = $J^2_{F\text{-}H}$ = 2.3) ppm. MS (ESI): *m/z* (positive mode, rel. int., %) = 1107 (17) [2M + Na]$^+$, 1085 (28) [2M + H]$^+$, 565 (34) [M + Na]$^+$, 543 (100) [M + H]$^+$. HRMS ($C_{29}H_{29}F_3N_2O_5$): *m/z* (positive mode) = 565.1923 (found [M + Na]$^+$), 565.1921 (calcd.); 543.2101 (found [M + H]$^+$), 543.2101 (calcd.).



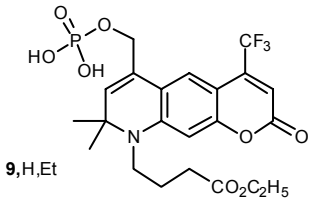
7e,H,All

Coumarin 7e,H,All: *N,N'*-Diisopropyldiallylphosphoramidite (24 μL, 22 mg, 91 μL) and 1*H*-tetrazole (0.45 M in MeCN, 0.20 mL, 91 μL) were added to a solution of coumarin **7d**,H (20 mg, 46 μmol) in THF (2 mL) under argon. The reaction mixture was stirred at 40°C, and monitored by TLC: After 60 min and 105 min, additional amounts of the phosphoramidite (24 μL each time) and 1*H*-tetrazole solution (0.20 mL each time) were added. After 195 min, the reaction mixture was cooled to room temperature, and MCPBA (69 mg, 0.28 mmol) was added in one portion. The reaction mixture was diluted with DCM (20 mL), washed with water, and sat. aq. $NaHCO_3$ (15 mL each), dried over $MgSO_4$ and concentrated. The residue was purified by column chromatography (15 g SiO_2, hexane/EtOAc, 2:1→0:1) to afford the title compound (29 mg, quant.) as a yellow solid. R_f = 0.13 (hexane/EtOAc, 1:1); ^{1}H-NMR (300 MHz, CDCl$_3$): δ = 7.32 (q, J = 1.8, 1 H, 5-H), 6.46 (s, 1 H, 10-H), 6.38 (s, 1 H, 3-H), 6.01–5.81 (m, 2 H, 2×OCH$_2$C<u>H</u>=), 5.65 (d, J = 1.0, 1 H, 7-H), 5.33 (m, 2 H, 2×=C<u>H</u>^{t}H^c), 5.23 (m, 2 H, 2×=CH^t<u>H</u>^{c}), 4.83 (dd, J = 7.8 and 1.0, 2 H, CH$_2$OP), 4.53 (ddd, J = 8.3, 5.6 and 1.4, 4 H, 2×OC<u>H</u>$_2$CH=), 4.19 (q, J = 7.1, 2 H, CH$_2$O), 3.37–3.29 (m, 2 H, 1'-H), 2.41 (t, J = 6.8, 2 H, 3'-H), 1.97–1.87 (m, 2 H, 2'-H), 1.42 (s, 6 H, 8-Me), 1.30 (t, J = 7.12, 3 H, CH$_3$) ppm. ^{13}C-NMR (75.5 MHz, CDCl$_3$, APT): δ = 172.6 (–, C-4'), 160.1 (–, C-2), 157.1 (–, C-10a), 148.0 (–, C-9a), 141.5 (–, q, J = 32, C-4), 133.2 (+, C-7), 132.3 (+, d, J = 7, CH= in allyl), 125.8 (–, d, J = 7, C-6), 121.8 (–, q, J = 276, CF$_3$), 119.4 (+, q, J = 2, C-5), 118.3 (+, CH$_2$= in allyl), 117.2 (–, C-5a), 108.4 (+, q, J = 6, C-3), 102.8 (–, C-4a), 97.9 (+, C-10), 68.3 (–, d, J = 6, OCH$_2$ in allyl), 66.8 (–, d, J = 5, 6-CH$_2$O), 60.8 (–, CH$_2$O), 58.1 (–, C-8), 43.9 (–, C-3'), 31.2 (–, C-1'), 28.9 (+, 8-Me), 22.5 (–, C-2'), 14.2 (+, *C*H$_3$) ppm. MS (ESI, positive mode): 1221 (100) [2M + Na]$^+$, 662 (62) [M + Na]$^+$; HRMS (ESI, positive mode): $C_{28}H_{33}F_3NO_8P$ [M + Na]$^+$ found 622.1788, calcd. 622.1788.



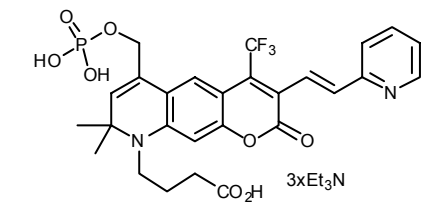
7e,CH=CH-2-py,Bu^t

Coumarin 7e,CH=CH-2-py,Bu^t: *N,N'*-Diisopropyl di-*tert*-butyl phosphoramidite (63 mg, 0.207 mmol) and 1*H*-tetrazole (15 mg, 0.207 mmol) were added to a preheated (40 °C) solution of coumarin **7d**,CH=CH,2-py (75 mg, 0.138 mmol) in dichloromethane (10 mL) under argon. The reaction mixture was stirred for 20 min in at this temperature and monitored by TLC. Phosphite intermediate has R_f = 0.78, whereas starting coumarin has R_f 0.33 (hexane/dichloromethane/EtOAc, 1:1:1). The reaction mixture was cooled (5°C) and a solution of 75% wet. MCPBA (46 mg, 0.2 mmol) in dichoromethane (2 mL) was added slowly. The reaction mixture was diluted with DCM (20 mL), washed with 10% $NaHSO_3$, sat. aq. $NaHCO_3$, brine, dried over $MgSO_4$ and concentrated. The crude product with R_f = 0.19 (hexane/dichloromethane/EtOAc, 1:1:1) was purified by column chromatography (hexane/dichloromethane, 2:1 → hexane/dichloromethane/EtOAc, 1:1:1) with following precipitation fom hexane/ether to afford the title compound (79 mg, 78% yield) as a red solid. ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 8.58 (m, 1 H,), 7.79 (dq, J_{H-H} = 15.6, J_{H-F} = 2.3, 1 H), 7.68 (d, J = 15.6, 1 H), 7.62 (ddd, J = 7.7, 7.7, 1.9, 1 H), 7.44 (q, J = 1.8, 1 H), 7.32 (m, 1 H), 7.12 (ddd, J = 7.7, 4.7, 1.1, 1 H), 6.39 (s, 1 H), 5.64 (d, J = 0.9, 1 H), 4.72 (dd, J_{H-P} = 7.5, J_{H-H} = 0.9, 2 H), 4.16 (q, J = 7.2, 2 H), 3.31 (m, 2 H), 2.39 (t, J = 6.8, 2 H), 1.90 (m, 2 H), 1.45 (d, J_{H-P} = 0.9, 18 H), 1.42 (s, 6 H), 1.27 (t, J = 7.2, 3 H). ^{13}C-NMR (125.7 MHz, $CDCl_3$, APT): δ = 172.6, 159.8, 155.1, 154.9, 149.8 (+), 147.4, 136.4 (+), 136.3 (+), 135.6 (q, J_{C-F} = 29.7), 132.0 (+), 126.3 (d, J_{C-P} = 7.6), 123.5 (+), 123.4 (q, J_{C-F} = 279.5), 123.2 (+, q, J_{C-F} = 3.7), 122.6 (+), 120.1 (+, q, J_{C-F} = 5.3), 117.9, 117.6, 104.6, 97.2 (+), 82.6 (d, J_{C-P} = 7.6), 65.7 (d, J_{C-P} = 5.6), 60.7, 58.0, 43.9, 31.2, 29.8(+, J_{C-P} = 4.4), 28.9(+), 22.6, 14.2 (+) ppm. ^{19}F-NMR (282.4 MHz, $CDCl_3$): δ = –54.2 (dd, $J^1_{F-H} = J^2_{F-H}$ = 2.3) ppm. MS (ESI): *m/z* (positive mode, rel. int., %) = 677 (100).



9,H,Et

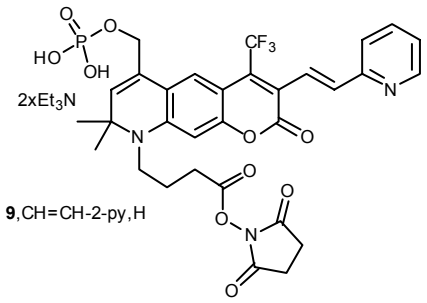
Coumarin 9-H,Et: Compound **7e**,H,All (14 mg, 23 μmol), Ph_3P (9.0 mg, 34 μmol), *n*-butylamine (2.7 μL, 28 μmol) and formic acid (1 μL, 1.3 mg, 27 μmol) in THF (2 mL) were purged with argon for 3 min, and then $Pd(dba)_2$ (4.0 mg, 7.0 μmol) was added. The reaction mixture was stirred in a closed vessel at room temperature for 19 h. A solution of HCl in dioxane (4 M, 0.2 mL, 0.8 mmol) was added, and the solvents were evaporated. The residue was purified by column chromatography successively on the RP silica gel (1.5 g RP-SiO_2, MeCN/H_2O, 1:1 +0.1%TFA) and common SiO_2 (1.5 g RP-SiO_2, MeCN/H_2O, 5:1→1:1) to give the title compound as a yellow solid (11.5 mg, 97%). R_f = 0.30 (MeCN/H_2O, 5:1, common SiO_2); ^{1}H-NMR (300 MHz, CD_3OD): δ = 7.10 (q, J_{C-F} = 1.8, 1 H), 6.63 (s, 1H), 6.41 (s, 1 H), 5.69 (d, J = 1.0, 1 H), 4.44 (m, 2H), 4.08 (q, J = 7,2, 2 H, CO_2Et), 3.32 (m, 2 H), 2.35 (m, 2 H), 1.73 (m, 2 H), 1.33 (s, 6 H), 1.18 (t, J = 7.2, 3H, CO_2Et); ^{19}F-NMR (282.4 MHz, $CDCl_3$): δ = –64.5 (d, J_{F-H} = 2.3) ppm. UV/Vis (EtOH): λ_{max} (ε) = 422 (10500 M^{-1} cm^{-1}); fluorescence (EtOH): $\lambda_{excit.}$ = 420 nm, $\lambda_{em.}$ = 526 nm; MS (ESI, negative mode): 518 (100) $[M - H]^-$; HRMS (ESI, negative mode): $C_{22}H_{25}F_3NO_8P$, $[M - H]^-$: found 518.1197, calcd. 518.1197.



9,CH=CH-2-py,H

Coumarin 9,CH=CH-2-py,H: According to GPE from 50 mg (0.068 mmol) **7e**,CH=CH-2-py,Bu^t the acid (37 mg, 91%) was obtained as an orange solid. For a measurement of NMR spectra the sample of acid was dissolved in NEt_3 and evaporated to dryness. ^{1}H-NMR (300 MHz, D_2O): δ = 8.37 (m, 1 H, py), 7.75 (m, 1 H, py), 7.40-7.16 (m, 5 H), 6.22 (s, 1 H), 5.76 (s, 1 H), 4.60 (m, 2H), 3.22 (q, J = 7.2, 6 H, NEt_3), 3.20 (m, 2H), 2.36 (m, 2H), 1.82 (m, 2 H), 1.39 (s, 6H), 1.31 (t, J = 7.2, 9 H, NEt_3) ppm. ^{13}C-NMR (125.7 MHz, D_2O, APT): δ = 163.3, 157.1, 155.2, 150.8, 149.1 (+), 142.4 (+), 139.2 (q, J_{C-F} = 29.6), 135.1 (+), 133.8 (+), 128.3 (d, J_{C-P} = 5.6), 127.1 (+), 126.1 (+), 125.9 (+, q, J_{C-F} = 3.7), 125.5 (q, J_{C-F} = 279.5), 121.9 (+, q, J_{C-F} = 5.3), 120.6, 116.2, 116.1, 106.5, 99.0 (+), 67.0, 60.9, 49.3 (NEt_3), 46.7, 30.9(+), 30.85, 26.1, 10.8 (+, NEt_3) ppm. MS (ESI): *m/z* (positive mode, rel. int., %) = 595 (100) $[M + H]^+$, 617 (31) $[M + Na]^+$; *m/z* (negative mode rel. int., %) = 593 (100) $[M - H]^-$. HRMS ($C_{27}H_{26}F_3N_2O_8P$): *m/z*

(positive mode) = 595.1446 (found $[M + H]^+$), 595.1452 (calc.), 617.1271 (found $[M + Na]^+$), 617.1271 (calcd.); HRMS ($C_{27}H_{26}F_3N_2O_8P$): *m/z* (negative mode) = 593.1306 (found $[M - H]^-$), 593.1306 (calcd.). UV/Vis (PBS 7.4): λ_{max} (ε) = 472 (4935 M^{-1} cm^{-1}); fluorescence (PBS 7.4): $\lambda_{excit.}$ = 430 nm, $\lambda_{em.}$ = 624 nm.



Coumarin 9,CH=CH-2-py,NHS: HATU (5.0 mg, 13.1 μmol) and HOSu (1.7 mg, 15.1 μmol) were added to a solution of acid **9**,CH=CH-2-py,H (6.0 mg, 10.1 μmol) in DMF (2 mL) followed by addition of NEt_3 (2.0 mg, 20.2 μmol). The reaction mixture was stirred at RT for 14 h, DMF was evaporated under reduced pressure at RT and the residue was purified by column chromatography using CH_3CN/water = 4/1 mixture as eluent (**9**,CH=CH-2-py,H: R_f = 0.47; **9**,CH=CH-2-py,NHS: R_f = 0.64 in CH_3CN/water = 4/1) giving the product 6.4 mg (91%) as an orange solid. MS (ESI): *m/z* (positive mode, rel. int., %) = 692 (100) $[M + H]^+$; *m/z* (negative mode rel. int., %): 690 (100) $[M - H]^-$. HRMS ($C_{31}H_{29}F_3N_3O_{10}P$): *m/z* (positive mode) = 692.1603 (found $[M + H]^+$), 692.1615 (calcd.); HRMS ($C_{31}H_{29}F_3N_3O_{10}P$): *m/z* (negative mode) = 690.1468 (found $[M - H]^-$), 690.1470 (calcd.).

Photostability measurements

The dyes where dissolved in aqueous PBS buffer solution (pH 7.4, 50 mM), containing 2.5% PVA [poly(vinyl alcohol); Mowiol® 40-88, Sigma-Aldrich] to give a 10 μM dye solution. 50 μL of this solution was placed on a coverslip and spincoated with 3000 U/min for 20 sec. The coverslips were dried and attached to an object holder. The bleaching experiments were performed on a custom made confocal microscope. The dyes were excited with a 488 nm pulsed laser diode with a repetition rate of 80 MHz (PicoQuant) and a power density of 50 W/cm^2 in the focal spot. For averaging, several traces were measured on each dye and background was subtracted.

Table S4. The radiative rates ($k_r = \phi/\tau$) non-radiative rates ($k_{nr} = (1-\phi)/\tau$) for coumarins (in ethanol) and their *conjugates with sheep anti-mouse antibodies* (in aqueous phosphate buffer at pH 7.4) calculated from the values of fluorescence quantum yields (ϕ) and lifetimes of the excited state (τ).

Compound[a]	Abs. nm	Em. nm	k_r ns^{-1}	k_{nr} ns^{-1}	ϕ	τ ns	Compound[a]	Abs. nm	Em. nm	k_r ns^{-1}	k_{nr} ns^{-1}	ϕ	τ ns
7a,2-th	416	611	0.11	0.73	0.13	1.2	**7c**,2-th	445	636	0.14	0.76	0.16	1.1
7a,CH=CH-2-py	444	578	0.22	0.12	0.64	2.9	**7c**,CH=CH-ph	467	598	0.28	0.20	0.58	2.1
7a,CH=CH-2-py-A[b]	475	646	0.26	0.45	0.37	1.4	**7c**,CH=CH-2-py	475	602	0.21	0.05	0.80	3.9
7a,CH=CH-4-py	449	580	0.24	0.10	0.71	3.0	***7c**,CH=CH-2-py-protein (DOL10)*[c,e]	*473*	*609*	*0.02*	*0.31*	*0.05*	*3.1*
7a,CH=CH-4-py-A[b]	500	668	0.29	0.33	0.47	1.6	**7c**,CH=CH-4-py	480	609	0.17	0.08	0.68	4.0
***7a**,CH=CH-4-py-A-protein (DOL 3.6)*[c]	*491*	*645*	*0.01*	*0.46*	*0.02*	*2.1*	**7c**,H	424	527	0.10	0.13	0.45	4.3
7a,4-py	413	588	0.43	0.48	0.47	1.1	***7c**,H-protein (DOL 3.5)*[c]	*423*	*544*	*0.03*	*0.29*	*0.08*	*3.2*
7a,4-py-A[b]	439	644	0.21	0.56	0.27	1.3	**7d**,H	420	523	0.10	0.12	0.46	4.5
7a,CH=CH-ph	438	576	0.12	0.13	0.49	4.0	***7d**,H-protein (DOL 14)*[c]	*426*	*539*	*0.03*	*0.26*	*0.10*	*3.5*
7b,4-py	430	593	0.19	0.81	0.19	1.0	**9**,H,Et	422	526	0.10	0.15	0.40	4.1
7b,4-py-B[d]	459	655	0.26	0.50	0.34	1.3	***9**,H,H-protein (DOL 1.3)*[c]	*425*	*541*	*0.07*	*0.26*	*0.21*	*3.0*
7b,2-py	429	578	0.13	0.50	0.20	1.6	**9**,CH=CH-2-py,H[f]	472	624	0.15	1.1	0.12	0.8
7c,2-py	438	591	0.17	0.73	0.19	1.1	***9**,CH=CH-2-py,H-protein (DOL 11.6)*[c]	*479*	*607*	*0.03*	*0.64*	*0.04*[g]	*1.5*[h]
7c,2-py-B[d]	479	632	0.08	0.82	0.09	1.1							

a) Structures are given above in the text of the synthetic part; b) A: pyridine nitrogen is alkylated with ω-(carboxy)pentyl residue; c) DOL: degree of labeling; d) B: pyridine nitrogen is alkylated with ω-(sulfo)propyl group; e) goat anti-rabbit; f) in aqueous phosphate buffer at pH 7.4; g) the same fluorescence quantum yield was found for the conjugate with goat anti-rabbit antibodies with DOL = 5.6; h) τ = 1.7 for the conjugate with goat anti-rabbit antibodies (DOL 5.6).

Immunofluorescence labeling

Labeling of the secondary antibodies (1–2 mg of protein in ca. 1–2 mL of PBS buffer) with an *N*-hydroxysuccinimidyl ester (0.2–0.4 mg) was performed according to the standard protocols[3] 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), elution with PBS buffer (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).[3b,c]

Cell culture and immunofluorescence

HeLa cells were maintained at 37°C in 5% CO_2 in DMEM supplemented with 10% FBS. In preparation to immunofluorescence, ca. 100,000 cells were plated in each well of a 24-wells plate on a #1.5 glass coverslip. Cells were then incubated overnight to adhere to the glass surface. Fixation was performed with 4% paraformaldehyde for

30 min. Cells were then permeabilized with a permeabilization buffer (0.3% NP40, 0.05% Triton X-100, 0.2% BSA in PBS) for 3 min. Blocking was then performed in a blocking buffer (0.05% NP40, 0.05% Triton X-100, 5% normal goat serum in PBS) for 1 hour. Primary antibodies against Gpp130 (Rabbit polyclonal, COVANCE) and p230 (Mouse monoclonal, BD biosciences) were then added into a blocking buffer. After incubation overnight with primary antibodies, cells were then incubated with Star635anti-Mouse and Star470SX+ anti-Rabbit secondary antibodies for 1 hour. Cells were then mounted with ProLong Gold Antifade reagent (Life Technologies).

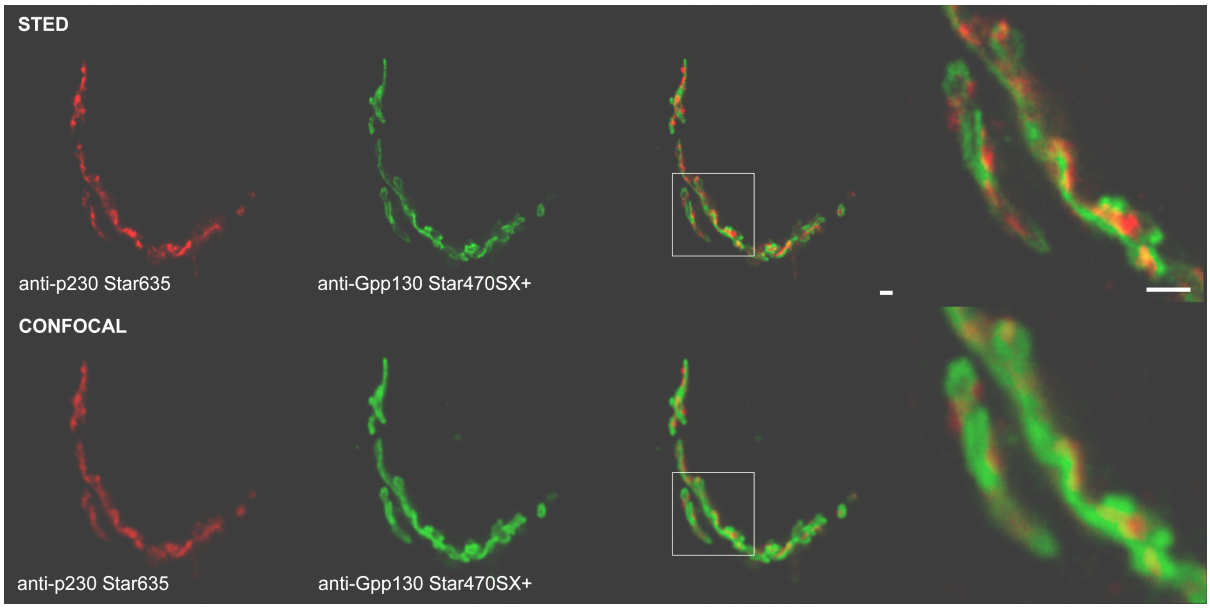


Figure S1. Two-color STED (upper panel) and confocal (lower panel) images of the Golgi apparatus. HeLa cells were fixed and probed with primary antibodies against Gpp130 and p230 to stain the *trans*- and *cis*-sides of the Golgi ribbon, respectively. Secondary antibodies conjugated with Star470SX+ and Star635 dyes were then applied. Two-color STED images were acquired using a commercial Leica TCS STED microscope. Scale bar: 1 μm.

Two-color STED microscopy

Two-color STED images were acquired using a commercial Leica TCS STED microscope. Star470SX+ and Star635 dyes were excited with 532 nm and 640 nm pulsed diode lasers, respectively. For depletion, a tunable, mode-locked Ti:Sapphire laser was used (750 nm for Star470SX+ and 770 nm for Star635). Imaging was performed with a 100X/1.4NA oil immersion objective lens. Fluorescence was split by a long-pass dichroic mirror (650 nm), band pass filtered (FF01-685/40 for 640 nm excitation or FF01-582/75 for 532 nm excitation) and detected by avalanche photodiodes (APD1 for STAR470SX+ and APD2 for STAR635). Images were then smoothed with a 0.7 pixel full width half-maximum Gaussian filter using imageJ software.[4]

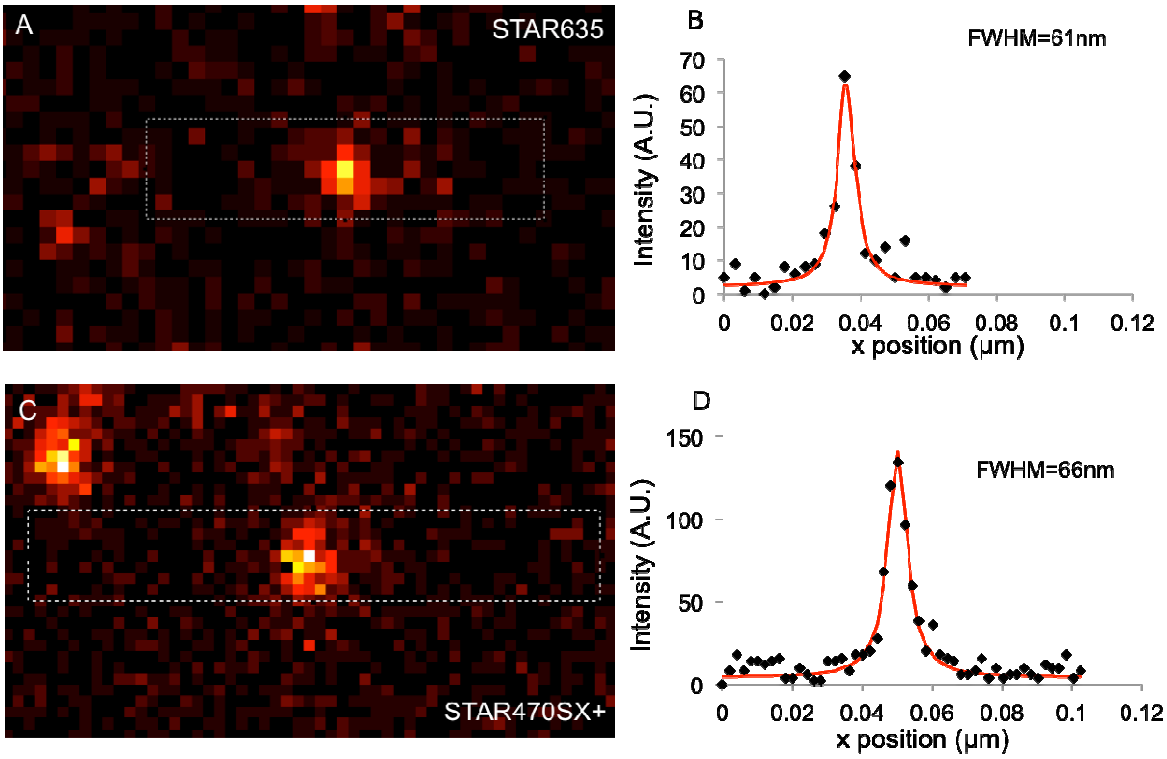


Figure S2. Lateral resolution of Star635 (top panels A and B) and Star470SX+ (bottom panels C and D) determined from antibodies clusters from Figure 4. Line profiles (highlighted in white; panels A and C) were averaged in the Y direction. A Lorentizian was fit to the line profiles and the resulting FWHM were found to be 61 nm for Star635 and 66 nm for Star470SX+.

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