Supporting Information

Rhodamine F: A Novel Class of Fluorous Ponytailed Dyes for Bioconjugation

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General

UV/Vis absorption spectra were recorded by using a Varian Cary 300 scan UV/Vis spectrophotometer and fluorescence spectra were recorded by using a Varian Cary Eclipse fluorescence spectrophotometer. Closed quartz cuvettes with a 1 cm path length were used in all experiments. Fluorescence quantum yield measurements were performed on the previously mentioned fluorometer and UV/Vis instrument. The slit width was 5 nm for both excitation and emission. Relative quantum efficiencies were obtained by comparing the absorption values and the areas under the emission spectrum for the unknown substance with a standard. The following equation was used to calculate quantum yields:

\[
\Phi_x = \Phi_s \times \frac{F_x}{F_s} \times \frac{n_x}{n_s} \times \frac{A_s}{A_x}
\]

\(\Phi_s\) is the reported quantum yield of the standard, \(F\) is the integrated emission spectrum, \(A\) is the absorbance at the extinction wavelength, and \(n\) is the refractive index of the solvents used. The subscript \(x\) denotes unknown and \(s\) denotes standard. 5(6)-Carboxyfluorescein in 0.1 M aqueous NaOH (\(\Phi_F = 0.95\)) or rhodamine 101 in methanol (\(\Phi_F = 0.99\)) were used as standards. All reactions were carried out under stirring. Reactions under inert gas were carried out in flasks equipped with septa under argon (supplied by using a standard manifold with vacuum and argon lines). NMR spectra were recorded at 25 °C by using Bruker Avance 300 (300 (\(^1\)H) and 75 MHz (\(^13\)C)), Bruker AM 400 (400 (\(^1\)H), 100 (\(^13\)C) and 376.5 MHz (\(^19\)F)) and Bruker DRX 500 (500 (\(^1\)H) and 125 MHz (\(^13\)C)) spectrometer. All spectra are referenced to tetramethylsilane as the internal standard (\(\delta = 0\) ppm) by using the signals of the residual protons of CHCl\(_3\) (7.26 ppm (\(^1\)H) or 77.0 ppm (\(^13\)C)) in CDCl\(_3\), or CHD\(_2\)OD (3.31 ppm (\(^1\)H) or 49.1 ppm (\(^13\)C)) in CD\(_3\)OD. Multiplicities of signals are described as follows: s = singlet, bs = broad singlet, d = doublet, t = triplet, q = quartet, m = multiplet. Coupling constants (\(J\)) are given in Hz. Multiplicities in the \(^13\)C NMR spectra were determined by DEPT (distortionless enhancement by polarization transfer) measurements. Perfluorinated carbon atoms were not
analyzed by $^{13}\text{C}$ NMR spectroscopy due to their weak and complex signals. Mass spectra (EI or FAB) were obtained by using a Finnigan MAT 90 spectrometer. High resolution mass spectra of molecules with molecular masses >1000 g/mol were obtained by using an Agilent 6230 TOF LC/MS. MALDI-TOF mass spectra from the peptoids were obtained by using a Bruker Biflex IV spectrometer with a pulsed ultraviolet nitrogen laser, 200 μJ at 337 nm and a time-of-flight mass analyzer with a 125 cm linear flight path. Reversed phase analytical HPLC was performed using Agilent Series 1100, equipped with a C18 PerfectSil Target (MZ Analytik, 3–5 μm, 4.0 × 250 mm). Reversed phase semi-preparative HPLC was performed using Agilent Series 1200, equipped with a C8 Zorbax 300SB-C8 column (Agilent, 5 μm, 9.4 × 250 mm). Flow rate: 1 mL/min; solvent A: 0.1% TFA in water; solvent B: 0.1% TFA in MeCN. Analytical TLC was performed on MERCK ready-to-use plates with silica gel 60 (F254). Column chromatography: MERCK silica gel 60, 0.04–0.063 mm. F-SPE was performed on SIGMA-ALDRICH FluoroFlash SPE cartridges (2 g, 8 cm$^3$ tube). For microwave assisted peptoid synthesis the single mode CEM Discover microwave was used.

**Experimental**

**N-Ethyl-m-methoxyaniline (4-Et)**

The preparation and properties of compound 4-Et have been reported in reference 3.

**General method 1 for the preparation of N-acyl-m-methoxyanilines 5-R_6-H, 5-R_6-Et and 5-R_7-H**

$m$-Anisidine (4-H) or $N$-ethyl-m-anisidine (4-Et) (1 equiv.) and dry pyridine (1.2 equiv.) were dissolved in dry CH$_2$Cl$_2$. Perfluoroheptanoyl or perfluorooctanoyl chloride (1.2 equiv.) was then added dropwise with stirring. The mixture was stirred overnight at RT and then CH$_2$Cl$_2$ (10 mL) was added. The mixture was washed with water (5 mL), aqueous HCl (1 M, 5 mL), and saturated aqueous NaHCO$_3$ (5 mL). After drying over Na$_2$SO$_4$, the solvent was evaporated under reduced pressure and the crude product was purified by using column chromatography.

**N-Perfluoroheptanoyl-m-methoxyaniline (5-R_6-H)**

After purification (chromatography with eluent cyclohexane/EtOAc 4:1) the title compound was obtained as colorless crystals from $m$-anisidine (4-H) (244 μL, 2.18 mmol) and perfluoroheptanoyl chloride (578 μL, 261 mmol) according to general method 1: yield 826 mg (81%).

$R_f = 0.50$ (cyclohexane/EtOAc 4:1); mp: 104 °C; $^1$H NMR (500 MHz, CDCl$_3$): $\delta = 3.83$ (s, 3H), $6.80$ (dd, $^3$J(H,H) = 8.3 Hz, $^4$J(H,H) = 2.1 Hz, 1H), 7.04 (dd, $^3$J(H,H) = 8.0 Hz, $^4$J(H,H) = 1.5 Hz, 1H), 7.28–7.31 (m, 2H), 7.86 (bs, 1H, NH); $^{13}$C NMR (125 MHz, CDCl$_3$): $\delta = 55.4$ (CH$_3$), 106.1 (CH$_{ar}$), 112.4 (CH$_{ar}$), 112.4 (CH$_{ar}$), 130.1 (CH$_{ar}$), 136.2 (C$_{ar}$), 155.1 (t, $^2$J(C,F) = 26 Hz, C), 160.3 (C$_{ar}$); $^{19}$F NMR (376.5 MHz, CDCl$_3$): $\delta = -126.0$ (m, CF$_2$), -122.7 (m, CF$_2$), -122.2 (m, CF$_2$), -121.6 (m, CF$_2$), -119.2 (tt, $^3$J(F,F) = 12.8 Hz, $^4$J(F,F) = 2.9 Hz, CF$_2$), -80.7 (tt, $^3$J(F,F) = 9.8 Hz,
After purification (chromatography with eluent cyclohexane/EtOAc 8:1) the title compound was obtained as colorless oil from N-ethyl-m-anisidine (4-Et) (498 mg, 3.30 mmol) and perfluoroheptanoyl chloride (875 µL, 3.96 mmol) according to general method 1: yield 1.14 g (70%).

**N-Perfluoroheptanoyl-m-methoxyaniline (5-Rf6-Et)***

After purification (chromatography with eluent cyclohexane/EtOAc 6:1) the title compound was obtained as white solid from m-anisidine (4-H) (323 µL, 2.89 mmol) and perfluoroheptanoyl chloride (862 µL, 3.47 mmol) according to general method 1: yield 1.32 g (88%).

**General method 2 for the reduction of amides 5-Rf6-H, 5-Rf6-Et, 5-Rf7-H and 8***

A solution of BH3 in THF (1 m) (2 equiv.) was added at RT to amide 5-Rf-H, 5-Rf-Et or 8 (1 equiv.) in dry THF (3 mL), and the mixture was heated at reflux overnight before being cooled to 0 °C. Excess BH3 was carefully neutralized by adding MeOH (1 mL), and then aqueous NaOH (1 m, 10 mL) was added. After stirring at RT for 20 min, the mixture was diluted with diethyl ether (10 mL) and the organic layer was separated. The aqueous layer was extracted with diethyl ether (3 × 3 mL),
then the combined organic layers were washed with saturated aqueous NaHCO₃ (3 mL) and brine (3 mL), then dried and evaporated. The crude product was purified by using column chromatography.

**N-(1H,1H-Perfluoroheptyl)-m-methoxyaniline (6-Rf₅-H)**

After purification (chromatography with eluent cyclohexane/EtOAc 6:1) the title compound was obtained as colorless oil from compound 5-Rf₅-H (811 mg, 1.73 mmol) according to general method 2: yield 479 mg (61%).

Rᵡ = 0.23 (cyclohexane/EtOAc 6:1); ¹H NMR (400 MHz, CDCl₃): δ = 3.78 (s, 3H), 3.87 (t, 3J(H,F) = 14.6 Hz, 2H), 3.90 (bs, 1H, NH), 6.24 (t, ⁴J(H,H) = 2.3 Hz, 1H), 6.30 (dd, ³J(H,H) = 8.1 Hz, ⁴J(H,H) = 2.3 Hz, 1H), 6.37 (dd, ³J(H,H) = 8.1 Hz, ⁴J(H,H) = 2.3 Hz, 1H), 7.12 (t, ³J(H,H) = 8.1 Hz, 1H). ¹³C NMR (100 MHz, CDCl₃): δ = 44.4 (t, ²J(C,F) = 23 Hz, CH₂), 55.1 (CH₃), 99.6 (CHₚ), 104.1 (CHₚ), 106.1 (CHₚ), 130.2 (CHₚ), 147.8 (Cₚ), 160.9 (Cₚ); ¹⁹F NMR (376.5 MHz, CDCl₃): δ = −126.1 (m, CF₂), −123.3 (m, CF₂), −122.8 (m, CF₂), −121.9 (m, CF₂), −118.1 (m, CF₂), −80.7 (tt, ³J(F,F) = 9.8 Hz, ⁴J(F,F) = 1.9 Hz, CF₃); El MS: m/z (%): 455 (90) [M]+, 436 (13) [M–F]+, 185 (17) [M–C₆F₁₁]+, 136 (100) [M–C₆F₁₃]+, 108 (24) [M–C₆HF₁₃NO]+, 77 (14) [M–C₆H₅F₁₃NO]+; HRMS: m/z calcd for C₁₄H₁₀F₁₃NO: 455.0555; found: 455.0557 [M]+.

**N-Ethyl-N-(1H,1H-perfluoroheptyl)-m-methoxyaniline (6-Rf₅-Et)**

After purification (chromatography with eluent cyclohexane/EtOAc 20:1) the title compound was obtained as white solid from compound 5-Rf₅-Et (1.06 g, 2.13 mmol) according to general method 2: yield 888 mg (91%).

Rᵡ = 0.33 (cyclohexane/EtOAc 20:1); mp: 43 °C; ¹H NMR (400 MHz, CDCl₃): δ = 1.21 (t, ³J(H,H) = 7.0 Hz, 3H), 3.49 (q, ³J(H,H) = 7.0 Hz, 2H), 3.80 (s, 3H), 3.95 (t, ³J(H,F) = 16.3 Hz, 2H), 6.33–6.34 (m, 1H), 6.36–6.41 (m, 2H), 7.17 (t, ³J(H,H) = 8.2 Hz, 1H); ¹³C NMR (100 MHz, CDCl₃): δ = 11.4 (CH₃), 46.3 (CH₂), 50.7 (t, ²J(C,F) = 21 Hz, CH₂), 55.1 (CH₃), 99.9 (CHₚ), 102.6 (CHₚ), 106.0 (CHₚ), 148.9 (Cₚ), 160.8 (Cₚ); ¹⁹F NMR (376.5 MHz, CDCl₃): δ = −126.1 (m, CF₂), −123.7 (m, CF₂), −122.8 (m, CF₂), −121.8 (m, CF₂), −116.6 (m, CF₂), −80.7 (t, ³J(F,F) = 9.9 Hz, CF₃); El MS: m/z (%): 483 (30) [M]+, 468 (12) [M–C₆H₅]+, 464 (10) [M–F]+, 164 (100) [M–C₆F₁₃]+, 77 (3) [M–C₁₀H₉F₁₃NO]+; HRMS: m/z calcd for C₁₆H₁₄F₁₃NO: 483.0868; found: 483.0870 [M]+; elemental analysis calcd (%) for C₁₆H₁₄F₁₃NO: C 39.76, H 2.92, N 2.90; found: C 39.55, H 2.91, N 2.72.

**N-(1H,1H-Perfluoroctyl)-m-methoxyaniline (6-Rf₇-H)**

After purification (chromatography with eluent cyclohexane/EtOAc 20:1) the title compound was obtained as white solid from 5-Rf₇-H (1.73 g, 3.33 mmol) according to general method 2: yield 1.07 g (64%).

Rᵡ = 0.20 (cyclohexane/EtOAc 20:1); mp: 53 °C; ¹H NMR (400 MHz, CDCl₃): δ = 3.78 (s, 3H), 3.80–3.93 (m, 3H), 6.24 (t, ⁴J(H,H) = 2.2 Hz, 1H), 6.30 (dd, ³J(H,H) = 8.0 Hz, ⁴J(H,H) = 2.2 Hz,
1H), 6.37 (dd, $^3J$(H,H) = 8.0 Hz, $^4J$(H,H) = 2.2 Hz, 1H), 7.12 (t, $^3J$(H,H) = 8.0 Hz, 1H); $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$ = 44.4 (t, $^2J$(C,F) = 23 Hz, CH$_2$), 55.2 (CH$_3$), 99.6 (CH$_{ar}$), 104.1 (CH$_{ar}$), 106.1 (CH$_{ar}$), 130.2 (CH$_{ar}$), 147.8 (C$_{ar}$), 160.9 (C$_{ar}$); $^{19}$F NMR (376.5 MHz, CDCl$_3$): $\delta$ = −126.0 (m, CF$_2$), −123.3 (m, CF$_2$), −122.7 (m, CF$_2$), −122.0 (m, CF$_2$), −121.7 (m, CF$_2$), −118.1 (t, $^3J$(F,F) = 12.5 Hz, CF$_2$), −80.7 (t, $^3J$(F,F) = 10.1 Hz, CF$_3$); EI MS: m/z (%): 505 (92) [M]$^+$, 486 (73) [M−F$^+$], 185 (16) [M−C$_6$HF$_{13}$]$^+$, 136 (100) [M−C$_7$F$_{15}$]$^+$, 77 (5) [M−C$_9$H$_5$F$_{15}$NO]$^+$; HRMS: m/z calcld for C$_{15}$H$_{10}$F$_{15}$NO: 505.0523; found: 505.0525 [M]$^+$; elemental analysis calcld (%) for C$_{15}$H$_{10}$F$_{15}$NO: C 35.66, H 2.00, N 2.77; found: C 35.56, H 1.81, N 2.59.

7-Hydroxy-1,2,3,4-tetrahydroquinoline (9)

The preparation and properties of compound 9 have been reported in reference 4.

$\text{N-(1H,1H-Perfluoroheptyl)-m-hydroxyaniline (7-R_{f6}-H)}$

Compound 6-R$_{f6}$-H (316 mg, 964 µmol) was dissolved in glacial AcOH (400 µL), then 48% aqueous HBr (475 µL) was added and the mixture was heated at reflux for 6 h. After cooling, CHCl$_3$ (3 mL) was added and the solution was carefully neutralized to about pH 5-6 with aqueous NaOH (30%). The organic phase was separated and the aqueous phase was extracted with CHCl$_3$ (3 × 1.5 mL). The combined organic fractions were washed with saturated aqueous NaHCO$_3$ (4 mL), dried, and evaporated. The crude product was purified by using column chromatography (elucent cyclohexane/EtOAc 5:1) to give a white solid: yield 179 mg (58%).

$R_f$ = 0.17 (cyclohexane/EtOAc 5:1); mp: 85 °C; $^1$H NMR (500 MHz, CDCl$_3$): $\delta$ = 3.81–3.91 (m, 2H), 3.89 (bs, 1H, NH), 4.66 (bs, 1H, OH), 6.19 (t, $^4J$(H,H) = 2.3 Hz, 1H), 6.26–6.29 (m, 2H), 7.06 (t, $^3J$(H,H) = 8.1 Hz, 1H); $^{13}$C NMR (125 MHz, CDCl$_3$): $\delta$ = 44.3 (t, $^2J$(C,F) = 23 Hz, CH$_2$), 100.1 (CH$_{ar}$), 106.1 (CH$_{ar}$), 106.1 (CH$_{ar}$), 130.4 (CH$_{ar}$), 148.0 (C$_{ar}$), 156.7 (C$_{ar}$); $^{19}$F NMR (376.5 MHz, CDCl$_3$): $\delta$ = −126.1 (m, CF$_2$), −123.3 (m, CF$_2$), −122.8 (m, CF$_2$), −121.9 (m, CF$_2$), −80.7 (tt, $^3J$(F,F) = 10.2 Hz, $^4J$(F,F) = 2.1 Hz, CF$_3$); EI MS: m/z (%): 441 (69) [M]$^+$, 422 (16) [M−F]$^+$, 122 (100) [M−C$_6$F$_{13}$]$^+$; HRMS: m/z calcld for C$_{13}$H$_8$F$_{13}$NO: 441.0398; found: 441.0396 [M]$^+$.

General method 3 for the demethylation of amines 6-R$_{f6}$-Et, 6-R$_{f7}$-H, 6-CH$_2$R$_{f6}$-H, 6-CH$_2$R$_{f6}$-Et and 6-(CH$_2$)$_2$R$_{f8}$-H

A solution of BBr$_3$ in CH$_2$Cl$_2$ (1 m) was added at RT to amine 6-R$_1$-R$_2$ in dry CH$_2$Cl$_2$ (20 mL), and the mixture was stirred overnight at RT. Afterwards water (20 mL) was carefully added. The organic layer was washed with saturated aqueous NaHCO$_3$ (10 mL) and brine (10 mL), then dried and evaporated. The crude product was purified by using column chromatography.
**N-Ethyl-N-(1H,1H-perfluoroheptyl)-m-hydroxyaniline (7-R_{f6}^-Et).**

After purification (chromatography with eluent cyclohexane/EtOAc 5:1) the title compound was obtained as red solid from compound 6-R_{f6}^-Et (824 mg, 1.70 mmol) and 5 equiv. BBr₃ (8.50 mL, 8.50 mmol) according to general method 3: yield 592 mg (74%).

$R_t = 0.38$ (cyclohexane/EtOAc 5:1); mp: 42 °C; ¹H NMR (400 MHz, CDCl₃): $\delta = 1.20$ (t, $^3J(H,H) = 7.0$ Hz, 3H), 3.48 (q, $^3J(H,H) = 7.0$ Hz, 2H), 3.94 (t, $^3J(H,F) = 16.3$ Hz, 2H), 4.65 (bs, 1H, OH), 6.24–6.30 (m, 2H), 6.36 (d, $^3J(H,H) = 9.2$ Hz, 1H), 7.07–7.13 (m, 1H); ¹³C NMR (100 MHz, CDCl₃): $\delta = 11.4$ (CH₃), 46.3 (CH₂), 50.6 (t, $^2J(C,F) = 20$ Hz, CH₂), 100.2 (CH₂), 104.9 (CH₂), 105.8 (CH₂), 130.2 (CH₂), 149.2 (C₂), 156.6 (C₃); ¹⁹F NMR (376.5 MHz, CDCl₃): $\delta = -126.1$ (m, CF₂), $-123.6$ (m, CF₂), $-122.8$ (m, CF₂), $-121.8$ (m, CF₂), $-116.7$ (m, CF₂), $-80.7$ (t, $^3J(F,F) = 9.9$ Hz, CF₃); El MS: m/z (%): 469 (100) [M]⁺, 454 (30) [M–CH₃]⁺, 440 (16) [M–C₂H₅]⁺, 150 (96) [M–C₆F₁₃]⁺; HRMS: m/z calcd for C₁₅H₃₁F₁₃NO: 469.0711; found: 469.0710 [M]⁺.

**N-(1H,1H-Perfluoroctyl)-m-hydroxyaniline (7-R_{f7}^-H)**

After purification (chromatography with eluent cyclohexane/EtOAc 4:1) the title compound was obtained as white solid from compound 6-R_{f7}^-H (1.05 g, 2.09 mmol) and 5 equiv. BBr₃ (10.4 mL, 10.4 mmol) according to general method 3: yield 735 mg (71%).

$R_t = 0.26$ (cyclohexane/EtOAc 4:1); mp: 92 °C; ¹H NMR (400 MHz, CDCl₃): $\delta = 3.85$ (t, $^3J(H,F) = 15.6$ Hz, 2H), 3.89 (bs, 1H, NH), 4.72 (bs, 1H, OH), 6.19 (t, $^4J(H,H) = 2.3$ Hz, 1H), 6.28 (d, $^3J(H,H) = 8.0$ Hz, 1H), 6.28 (dd, $^3J(H,H) = 8.0$ Hz, $^4J(H,H) = 4.9$ Hz, 1H), 7.06 (t, $^3J(H,H) = 8.1$ Hz, 1H); ¹³C NMR (100 MHz, CDCl₃): $\delta = 44.3$ (t, $^2J(C,F) = 24$ Hz, CH₂), 100.2 (CH₂), 106.1 (CH₂), 130.4 (CH₂), 148.0 (C₂), 156.7 (C₃); ¹⁹F NMR (376.5 MHz, CDCl₃): $\delta = -126.1$ (m, CF₂), $-123.3$ (m, CF₂), $-122.7$ (m, CF₂), $-122.0$ (m, CF₂), $-121.7$ (m, CF₂), $-118.1$ (m, CF₂), $-80.7$ (t, $^3J(F,F) = 9.8$ Hz, $^4J(F,F) = 2.0$ Hz, CF₃); El MS: m/z (%): 491 (100) [M]⁺, 472 (22) [M–F]⁺, 122 (76) [M–C₇F₁₅]⁺; HRMS: m/z calcd for C₁₄H₃₈F₂₁NO: 491.0366; found: 491.0363 [M]⁺.

**N-(1H,1H,2H,2H-Perfluoroctyl)-m-hydroxyaniline (7-Ch₂R_{f6}^-H)**

After purification (chromatography with eluent cyclohexane/EtOAc 2:3) the title compound was obtained as colorless oil from compound 6-CH₂R_{f6}^-H (1.45 g, 3.09 mmol) and 2.2 equiv. BBr₃ (6.80 mL, 6.80 mmol) according to general method 3: yield 860 mg (61%).

$R_t = 0.80$ (cyclohexane/EtOAc 2:3); ¹H NMR (400 MHz, CDCl₃): $\delta = 2.38$ (tt, $^3J(H,F) = 19.0$ Hz, $^3J(H,H) = 7.1$ Hz, 2H), 3.50 (t, $^3J(H,H) = 7.1$ Hz, 2H), 3.79 (bs, 1H, NH), 4.64 (bs, 1H, OH), 6.10 (t, $^4J(H,H) = 2.1$ Hz, 1H), 6.17–6.22 (m, 2H), 7.03 (t, $^3J(H,H) = 8.1$ Hz, 1H); ¹³C NMR (100 MHz, CDCl₃): $\delta = 30.6$ (t, $^2J(C,F) = 22$ Hz, CH₂), 35.8 (t, $^3J(C,F) = 5$ Hz, CH₂), 99.6 (CH₂), 105.2 (CH₂), 105.9 (CH₂), 130.5 (CH₂), 148.5 (C₂), 156.8 (C₃); ¹⁹F NMR (376.5 MHz, CDCl₃): $\delta = -126.1$ (m, CF₂), $-123.4$ (m, CF₂), $-122.8$ (m, CF₂), $-121.8$ (m, CF₂), $-113.8$ (m, CF₂), $-80.7$ (t, $^3J(F,F) = 9.8$ Hz, CF₃); El MS: m/z (%): 455 (100) [M]⁺, 436 (42) [M–F]⁺, 122 (68) [M–C₇H₂F₁₃]⁺; HRMS: m/z calcd for C₁₄H₁₀F₁₃NO: 455.0555; found: 455.0553 [M]⁺.
N-Ethyl-N-(1H,1H,2H,2H-Perfluoroctyl)-m-hydroxyaniline (7-CH$_2$R$_{16}$-Et)

The preparation and properties of compound 7-CH$_2$R$_{16}$-Et have been reported in reference 5.

N-(1H,1H,2H,2H,3H,3H-Perfluoroundecyl)-m-hydroxyaniline (7-(CH$_2$)$_2$R$_{16}$-H)

After purification (chromatography with eluent cyclohexane/EtOAc 3:1) the title compound was obtained as white solid from compound 6-(CH$_2$)$_2$R$_{16}$-H (1.27 g, 2.18 mmol) and 2.2 equiv. BB$_3$ (4.80 mL, 4.80 mmol) according to general method 3; yield 1.13 g (91%).

$R_f = 0.29$ (cyclohexane/EtOAc 3:1); mp: 67 °C; $^1$H NMR (400 MHz, CDCl$_3$): $\delta = 1.93$ (tt, $^3$J(H,H) = 7.8 Hz, $^3$J(H,H) = 7.0 Hz, 2H), 2.13–2.26 (m, 2H), 3.22 (t, $^3$J(H,H) = 6.9 Hz, 2H), 3.77 (bs, 1H, NH), 4.58 (bs, 1H, OH), 6.11 (t, $^4$J(H,H) = 2.3 Hz, 1H), 6.18–6.22 (m, 2H), 7.03 (t, $^3$J(H,H) = 8.0 Hz, 1H); $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta = 20.6$ (CH$_2$), 28.6 (t, $^2$J(C,F) = 23 Hz, CH$_2$), 43.0 (CH$_2$), 99.6 (CH$_{ar}$), 104.7 (CH$_{ar}$), 105.9 (CH$_{ar}$), 130.3 (CH$_{ar}$), 149.4 (C$_{ar}$); 19F NMR (376.5 MHz, CDCl$_3$): $\delta = -126.1$ (m, CF$_2$), -123.3 (m, CF$_2$), -122.7 (m, CF$_2$), -121.9 (m, 2 × CF$_2$), -121.7 (m, CF$_2$), -114.1 (t, $^3$J(F,F) = 13 Hz, CF$_2$), -80.7 (t, $^3$J(F,F) = 9.8 Hz, CF$_3$); EI MS: m/z (%): 569 (100) [M$^+$], 550 (20) [M–F$^+$], 122 (87) [M–C$_{10}$H$_4$F$_{17}$]$^+$; HRMS: m/z calcd for C$_{17}$H$_{12}$F$_{17}$NO: 569.0647; found: 569.0646 [M$^+$]; elemental analysis calcd (%) for C$_{17}$H$_{12}$F$_{17}$NO: C 35.87, H 2.12, N 2.46; found: C 35.57, H 1.88, N 2.35.

General method 4 for the preparation of rhodamine F dyes 1a–1f, 2 and 3

A solution of phenol 7-R$_1$-R$_2$, 10 or 13 (2 equiv.) and phthalic anhydride (1.6 equiv.) in propionic acid (18 equiv.) was heated with p-toluenesulfonic acid monohydrate (0.15 equiv.) at 160 °C for 24 h. After cooling to RT MeOH (10 mL) and CH$_2$Cl$_2$ (10 mL) were added. The organic layer was washed with aqueous NaOH (0.3 mL, 15 mL). Afterwards the aqueous layer was repeatedly extracted with CH$_2$Cl$_2$ until the organic layer remained colorless. Hydrochloric acid in MeOH (0.5 mL, 2 mL) was added to the combined organic layers and afterwards the solvent was evaporated under reduced pressure. The crude product was purified by using column chromatography. After purification the product was converted into the hydrochloride by adding hydrochloric acid in MeOH (0.5 mL, 2 mL).

Compound 1a

After purification (chromatography with eluent cyclohexane/EtOAc 2:1) the title compound was obtained as orange solid from compound 7-R$_{16}$-H (120 mg, 272 µmol) according to general method 4; yield 35.2 g (25%).

$R_f = 0.18$ (cyclohexane/EtOAc 2:1); mp: 192 °C; $^1$H NMR (500 MHz, CD$_3$OD): $\delta = 3.78$ (t, $^3$J(H,F) = 15.6 Hz, 4H), 6.59 (dd, $^3$J(H,H) = 8.8 Hz, $^4$J(H,H) = 2.3 Hz, 2H), 6.69–6.71 (m, 4H), 7.24 (d, $^3$J(H,H) = 7.4 Hz, 1H), 7.68 (td, $^3$J(H,H) = 7.5 Hz, $^4$J(H,H) = 0.9 Hz, 1H), 7.73 (td, $^3$J(H,H) = 7.5 Hz, $^4$J(H,H) = 1.2 Hz, 1H), 8.02 (d, $^3$J(H,H) = 7.6 Hz, 1H); $^{13}$C NMR (125 MHz, CD$_3$OD): $\delta = 44.3$ (t, $^2$J(C,F) = 24 Hz, CH$_2$), 99.1 (CH$_{ar}$), 111.2 (C$_{ar}$), 112.7 (CH$_{ar}$), 126.7 (CH$_{ar}$), 127.0 (CH$_{ar}$), 130.7 (CH$_{ar}$), 131.0 (CH$_{ar}$), 135.1 (CH$_{ar}$), 153.6 (C$_{ar}$), 153.6 (C$_{ar}$), 155.8 (C$_{ar}$), 155.8 (C$_{ar}$), 155.8 (C$_{ar}$), 155.8 (C$_{ar}$).
172.2 (C_ar), 19F NMR (376.5 MHz, CD3OD): δ = −127.3 (m, 2 × CF2), −124.2 (m, 2 × CF2), −123.8 (m, 2 × CF2), −122.9 (m, 2 × CF2), −118.6 (m, 2 × CF2), −82.4 (t, 3J(F,F) = 9.9 Hz, 2 × CF3); FAB MS: m/z (%): 995 (100) [M]^+, 951 (6) [M–CO2]^+, 875 (2) [M–C6HF5]^+, 825 (1) [M–C3HF7]^+, 775 (2) [M–C4HF9]^+, 725 (6) [M–C5HF11]^+, 675 (6) [M–C6HF13]^+; HRMS: m/z calcd for C34H26F26N2O3: 995.0824; found: 995.0816 [M]^+.

**Compound 1b**

After purification (chromatography with eluent cyclohexane/EtOAc 3:1) the title compound was obtained as red solid from 7-R16-Et (453 mg, 965 μmol) according to general method 4: yield 198 mg (38%).

Rf = 0.33 (cyclohexane/EtOAc 3:1); mp: 141 °C; 1H NMR (500 MHz, CD3OD): δ = 1.35 (t, 3J(H,H) = 7.1 Hz, 6H), 3.87 (q, 3J(H,H) = 7.1 Hz, 4H), 4.64 (t, 3J(H,F) = 16.3 Hz, 4H), 7.23–7.26 (m, 4H), 7.34–7.36 (m, 2H), 7.47 (dd, 3J(H,H) = 7.4 Hz, 4J(H,H) = 0.9 Hz, 1H), 7.85 (td, 3J(H,H) = 7.6 Hz, 4J(H,H) = 1.3 Hz, 1H), 7.89 (td, 3J(H,H) = 7.5 Hz, 4J(H,H) = 1.4 Hz, 1H), 8.39 (dd, 3J(H,H) = 7.8 Hz, 4J(H,H) = 1.2 Hz, 1H); 13C NMR (125 MHz, CD3OD): δ = 12.0 (CH3), 49.3 (CH2), 50.8 (t, 2J(C,F) = 21 Hz, CH2), 99.5 (C_ar), 116.5 (C_ar), 116.7 (CH_ar), 131.4 (CH_ar), 131.9 (CH_ar), 132.2 (C_ar), 132.7 (CH_ar), 132.8 (CH_ar), 134.1 (CH_ar), 134.4 (C_ar), 159.0 (C_ar), 159.6 (C_ar), 165.2 (C_ar), 168.1 (C); 19F NMR (376.5 MHz, CD3OD): δ = −127.2 (m, 2 × CF2), −124.1 (m, 2 × CF2), −123.8 (m, 2 × CF2), −122.8 (m, 2 × CF2), −116.4 (m, 2 × CF2), −82.3 (t, 3J(F,F) = 10.2 Hz, 2 × CF3); FAB MS: m/z (%): 1051 (100) [M]^+, 931 (2) [M–C2HF5]^+, 781 (1) [M–C5HF11]^+, 732 (2) [M–C6HF13]^+, 717 (5) [M–C7H3F13]^+; HRMS: m/z calcd for C38H25F26N2O3: 1051.1445; found: 1051.1394 [M]^+.

**Compound 1c**

After purification (chromatography with eluent cyclohexane/EtOAc 2:1) the title compound was obtained as red solid from 7-R17-H (500 mg, 1.02 mmol) according to general method 4: yield 110 mg (19%).

Rf = 0.17 (cyclohexane/EtOAc 2:1); mp: 229 °C; 1H NMR (400 MHz, CD3OD): δ = 4.38 (t, 3J(H,F) = 15.6 Hz, 4H), 7.05 (d, 3J(H,H) = 9.3 Hz, 2H), 7.16 (d, 4J(H,H) = 1.9 Hz, 2H), 7.23 (d, 3J(H,H) = 9.3 Hz, 2H), 7.45 (dd, 3J(H,H) = 7.3 Hz, 4J(H,H) = 1.4 Hz, 1H), 7.83 (td, 3J(H,H) = 7.6 Hz, 4J(H,H) = 1.4 Hz, 1H), 7.88 (td, 3J(H,H) = 7.6 Hz, 4J(H,H) = 1.5 Hz, 1H), 8.37 (dd, 3J(H,H) = 7.5 Hz, 4J(H,H) = 1.4 Hz, 1H); 13C NMR was not obtained due to poor signal-to-noise ratio; 19F NMR (376.5 MHz, CD3OD): δ = −127.2 (m, 2 × CF2), −123.9 (m, 2 × CF2), −123.7 (m, 2 × CF2), −123.0 (m, 2 × CF2), −122.7 (m, 2 × CF2), −118.1 (m, 2 × CF2), −82.3 (t, 3J(F,F) = 10.2 Hz, 2 × CF3); FAB MS: m/z (%): 1095 (100) [M]^+, 875 (3) [M–C4HF3]^+, 775 (8) [M–C5HF3]^+, 725 (6) [M–C7HF15]^+; HRMS: m/z calcd for C36H17F30N2O3: 1095.0755; found: 1095.0790 [M]^+. 
Compound 1d

After purification (chromatography with eluent cyclohexane/EtOAc 1:1 then EtOAc) the title compound was obtained as red solid from compound 7-CH₂R₁₆-H (678 mg, 1.49 mmol) according to general method 4: yield 115 mg (15%).

Rᵣ = 0.12 (cyclohexane/EtOAc 1:1); mp: 253 °C; ¹H NMR (300 MHz, CD₃OD): δ = 2.63 (tt, ³J(H,F) = 18.8 Hz, ³J(H,H) = 6.8 Hz, 4H), 3.79 (t, ³J(H,H) = 6.8 Hz, 4H), 6.89 (dd, ³J(H,H) = 9.2 Hz, ⁴J(H,H) = 2.2 Hz, 2H), 6.95 (d, ⁴J(H,H) = 2.2 Hz, 2H), 7.12 (d, ³J(H,H) = 9.2 Hz, 2H), 7.41 (dd, ³J(H,H) = 7.2 Hz, ⁴J(H,H) = 1.6 Hz, 1H), 7.75–7.87 (m, 2H), 8.34 (dd, ³J(H,H) = 7.2 Hz, ⁴J(H,H) = 1.6 Hz, 1H); ¹³C NMR was not obtained due to poor signal-to-noise ratio; ¹⁹F NMR (376.5 MHz, CD₃OD): δ = −127.3 (m, 2 × CF₂), −124.5 (m, 2 × CF₂), −123.8 (m, 2 × CF₂), −122.8 (m, 2 × CF₂), −115.1 (m, 2 × CF₂), −82.4 (t, ³J(F,F) = 10.2 Hz, 2 × CF₃); FAB MS: m/z (%): 1023 (100) [M⁺]; HRMS: m/z calcd for C₃₆H₂₁F₂₆N₂O₃: 1023.1132; found: 1023.1119 [M⁺].

Compound 1e

After purification (chromatography with eluent cyclohexane/EtOAc 2:1 then EtOAc) the title compound was obtained as red solid from compound 7-CH₂R₁₆-Et (1.14 g, 2.36 mmol) according to general method 4: yield 112 mg (8.5%).

Rᵣ = 0.19 (cyclohexane/EtOAc 2:1); mp: 92 °C; ¹H NMR (400 MHz, CD₃OD): δ = 1.33 (t, ³J(H,H) = 7.0 Hz, 6H), 2.61–2.74 (m, 4H), 3.74 (q, ³J(H,H) = 7.0 Hz, 4H), 4.02 (t, ³J(H,H) = 7.0 Hz, 4H), 7.04–7.09 (m, 2H), 7.12 (dd, ³J(H,H) = 9.4 Hz, ⁴J(H,H) = 1.9 Hz, 2H), 7.25 (d, ³J(H,H) = 9.4 Hz, 2H), 7.44 (d, ³J(H,H) = 7.4 Hz, 1H), 7.82 (t, ³J(H,H) = 7.7 Hz, 1H), 7.88 (t, ³J(H,H) = 7.4 Hz, 1H), 8.37 (d, ³J(H,H) = 7.7 Hz, 1H); ¹³C NMR (100 MHz, CD₃OD): δ = 12.6 (CH₃), 29.5 (t, ²J(C,F) = 21 Hz, CH₂), 44.0 (CH₂), 47.3 (CH₂), 97.8 (CH₃), 115.6 (C₁₉), 115.7 (CH₂), 131.5 (CHAr), 131.7 (CHAr), 132.2 (C₁₉), 132.7 (CH₃), 132.9 (C₁₉), 134.0 (CH₂), 135.2 (C₁₉), 157.4 (C₁₉), 159.6 (C₁₉), 163.1 (C₁₉), 168.1 (C); ¹⁹F NMR (376.5 MHz, CD₃OD): δ = −127.3 (m, 2 × CF₂), −124.2 (m, 2 × CF₂), −123.8 (m, 2 × CF₂), −122.8 (m, 2 × CF₂), −115.0 (m, 2 × CF₂), −82.4 (t, ³J(F,F) = 10.1 Hz, 2 × CF₃); FAB MS: m/z (%): 1079 (100) [M⁺]; HRMS: m/z calcd for C₄₀H₂₉F₂₆N₂O₃: 1079.1758; found: 1079.1871 [M⁺].

Compound 1f

After purification (chromatography with eluent EtOAc then MeOH/EtOAc 3:100) the title compound was obtained as red solid from compound 7-(CH₂)₂R₁₈-H (1.10 g, 1.94 mmol) according to general method 4: yield 125 mg (10%).

Rᵣ = 0.35 (EtOAc); mp: 224 °C; ¹H NMR (400 MHz, CD₃OD): δ = 1.97–2.10 (m, 4H), 2.37 (tt, ³J(H,F) = 18.5 Hz, ³J(H,H) = 8.3 Hz, 4H), 3.52 (t, ³J(H,H) = 6.9 Hz, 4H), 6.84–6.91 (m, 4H), 7.00–7.11 (m, 2H), 7.41 (d, ³J(H,H) = 7.3 Hz, 1H), 7.78–7.90 (m, 2H), 8.34 (d, ³J(H,H) = 7.7 Hz, 1H); ¹³C NMR was not obtained due to poor signal-to-noise ratio; ¹⁹F NMR (376.5 MHz, CD₃OD): δ = −127.2 (m, 2 × CF₂), −124.3 (m, 2 × CF₂), −123.7 (m, 2 × CF₂), −122.9 (m, 4 × CF₂), −122.6 (m, 2 × CF₂); FAB MS:
m/z (%): 1251 (100) [M]+; HRMS: m/z calcd for C_{42}H_{25}F_{34}N_{2}O_{3}: 1251.1317; found: 1251.1411 [M]+.

**Compound 2**

After purification (chromatography with eluent EtOAc) the title compound was obtained as purple solid from compound 10 (262 mg, 529 µmol) according to general method 4: yield 71.7 mg (24%).

R$_f$ = 0.17 (EtOAc); mp: 153 ºC; $^1$H NMR (400 MHz, CD$_3$OD): $\delta$ = 1.92–2.02 (m, 4H), 2.63–2.81 (m, 8H), 3.62–3.97 (m, 4H), 4.00 (t, $^3$J(H,H) = 7.0 Hz, 4H), 6.85 (s, 2H), 6.97 (s, 2H), 7.39 (d, $^3$J(H,H) = 7.2 Hz, 1H), 7.79–7.82 (m, 1H), 7.84–7.88 (m, 1H), 8.34 (d, $^3$J(H,H) = 8.6 Hz, 1H); $^{13}$C NMR (100 MHz, CD$_3$OD): $\delta$ = 21.9 (CH$_2$), 28.4 (t, $^2$J(C,F) = 21 Hz, CH$_2$), 28.5 (CH$_2$), 45.3 (CH$_2$), 51.3 (CH$_2$), 96.3 (CH$_{ar}$), 115.6 (C$_{ar}$), 127.5 (C$_{ar}$), 129.2 (CH$_{ar}$), 131.5 (CH$_{ar}$), 132.2 (C$_{ar}$), 132.6 (CH$_{ar}$), 134.0 (CH$_{ar}$), 135.4 (C$_{ar}$), 155.1 (C$_{ar}$), 156.3 (C$_{ar}$), 160.5 (C$_{ar}$), 168.2 (C); $^{19}$F NMR (376.5 MHz, CD$_3$OD): $\delta$ = −127.3 (m, 2 × CF$_2$), −124.2 (m, 2 × CF$_2$), −123.8 (m, 2 × CF$_2$), −122.8 (m, 2 × CF$_2$), −114.9 (t, $^3$J(F,F) = 13 Hz, 2 × CF$_2$), −82.4 (t, $^3$J(F,F) = 10.2 Hz, 2 × CF$_3$); FAB MS: m/z (%): 1103 (100) [M]+; HRMS: m/z calcd for C$_{42}$H$_{29}$F$_{26}$N$_{2}$O$_{3}$: 1103.1758; found: 1103.1709 [M]+.

**Compound 3**

After purification (chromatography with eluent cyclohexane/EtOAc 3:2 then EtOAc) the title compound was obtained as purple solid from compound 13 (285 mg, 532 µmol) according to general method 4: yield 134 mg (41%).

R$_f$ = 0.24 (cyclohexane/EtOAc 3:2); mp: 145 ºC; $^1$H NMR (400 MHz, CD$_3$OD): $\delta$ = 1.55 (s, 12H), 1.75 (s, 6H), 2.61–2.81 (m, 4H), 4.05 (t, $^3$J(H,H) = 7.7 Hz, 4H), 5.60 (s, 2H), 6.83 (s, 2H), 6.84 (s, 2H), 7.47 (d, $^3$J(H,H) = 7.3 Hz, 1H), 7.84 (t, $^3$J(H,H) = 7.6 Hz, 1H), 7.90 (t, $^3$J(H,H) = 7.1 Hz, 1H), 8.35 (dd, $^3$J(H,H) = 7.7 Hz, $^4$J(H,H) = 0.9 Hz, 1H); $^{13}$C NMR (100 MHz, CD$_3$OD): $\delta$ = 18.2 (CH$_3$), 29.3 (CH$_3$), 30.0 (t, $^2$J(C,F) = 21 Hz, CH$_2$), 38.6 (CH$_2$), 62.1 (C), 96.7 (CH$_{ar}$), 116.0 (C$_{ar}$), 123.3 (CH$_{ar}$), 125.7 (C$_{ar}$), 126.5 (C$_{ar}$), 131.5 (CH$_{ar}$), 131.8 (CH$_{ar}$), 132.5 (CH$_{ar}$), 132.7 (C$_{ar}$), 134.1 (CH$_{ar}$), 134.4 (CH$_{ar}$), 134.7 (C$_{ar}$), 153.9 (C$_{ar}$), 159.7 (C$_{ar}$), 159.9 (C$_{ar}$), 168.4 (C); $^{19}$F NMR (376.5 MHz, CD$_3$OD): $\delta$ = −127.3 (m, 2 × CF$_2$), −124.1 (m, 2 × CF$_2$), −123.9 (m, 2 × CF$_2$), −122.8 (m, 2 × CF$_2$), −115.2 (m, 2 × CF$_2$), −82.4 (t, $^3$J(F,F) = 9.8 Hz, 2 × CF$_3$); FAB MS: m/z (%): 1183 (100) [M]+, 1168 (28) [M−CH$_3$]+; HRMS: m/z calcd for C$_{48}$H$_{37}$F$_{26}$N$_{2}$O$_{3}$: 1183.2384; found: 1183.2325 [M]+.

$N$-(1H,1H,2H,2H-Perfluorooctyl)-$m$-methoxyaniline (6-CH$_2$R$_{f6}$-H)

The preparation and properties of compound 6-CH$_2$R$_{f6}$-H have been reported in reference 5.
N-Ethyl-N-(1H,1H,2H,2H-perfluoroctyl)-m-methoxyaniline (6-CH₂R₁₆-Et)

The preparation and properties of compound 6-CH₂R₁₆-Et have been reported in reference 5.

N-(1H,1H,2H,2H,3H,3H-Perfluoroundecyl)-m-methoxyaniline (6-(CH₂)₂R₁₈-H)

1H,1H,2H,2H,3H,3H-Perfluoroundecyl iodide (1.82 g, 3.09 mmol) was added dropwise to m-anisidine (4-H) (1.73 mL, 15.4 mmol) at 90 °C. After complete addition, the mixture was stirred at 140 °C for 3 h. After cooling, diethyl ether (15 mL) was added, the organic layer was washed with aqueous NaOH (2 mL, 15 mL), and the aqueous layer was extracted with diethyl ether (15 mL). Then, the organic layer was dried with sodium sulfate. The solvent was removed under reduced pressure. The crude product was purified by using column chromatography (elucent cyclohexane/EtOAc 5:1) to give a white solid: yield 1.34 g (74%).

R_f = 0.38 (cyclohexane/EtOAc 5:1); mp: 57 °C; ¹H NMR (400 MHz, CDCl₃): δ = 1.90–1.97 (m, 2H), 2.14–2.27 (m, 2H), 3.23 (t, 3J(H,H) = 6.9 Hz, 2H), 3.78 (s, 3H), 6.18 (t, 4J(H,H) = 2.2 Hz, 1H), 6.24 (dd, 3J(H,H) = 8.1 Hz, 4J(H,H) = 2.2 Hz, 1H), 6.31 (dd, 3J(H,H) = 8.1 Hz, 4J(H,H) = 2.2 Hz, 1H), 7.10 (t, 3J(H,H) = 8.1 Hz, 1H); ¹³C NMR (100 MHz, CDCl₃): δ = 20.6 (CH₂), 28.6 (t, 2J(C,F) = 22 Hz, CH₂), 43.1 (CH₂), 55.1 (CH₃), 99.0 (CH₃), 102.9 (CH₃), 106.0 (CH₃), 130.1 (CH₃), 149.1 (C₆H₅), 160.9 (C₆H₅); ¹⁹F NMR (376.5 MHz, CDCl₃): δ = −126.1 (m, CF₂), −123.4 (m, CF₂), −122.7 (m, CF₂), −121.9 (m, 2 × CF₂), −121.7 (m, CF₂), −114.1 (t, 3J(F,F) = 14 Hz, CF₂), −80.7 (t, 3J(F,F) = 10 Hz, CF₃); EI MS: m/z (%): 583 (88) [M⁺], 564 (53) [M⁻F⁺], 136 (100) [M⁻C₆H₄F₁₇⁺]; HRMS: m/z calcd for C₁₈H₁₄F₁₇NO: 583.0804; found: 583.0802 [M⁺]; elemental analysis calcld (%) for C₁₈H₁₄F₁₇NO: C 37.06, H 2.42, N 2.40; found: C 37.01, H 2.23, N 2.13.

7-Hydroxy-N-(1H,1H,2H,2H-perfluoroctyl)-1,2,3,4-tetrahydroquinoline (10).

1H,1H,2H,2H-Perfluoroctyl iodide (823 µL, 3.35 mmol) was added dropwise to a solution of compound 9 (500 mg, 3.35 mmol) in DMF (1.8 mL) at 90 °C. After complete addition, the mixture was stirred at 140 °C for 2 h. After cooling, EtOAc (40 mL) and aqueous NaOH (2 mL, 20 mL) were added. The organic layer was separated, washed with brine (10 mL) and the solvent was removed under reduced pressure. The crude product was purified by using column chromatography (elucent cyclohexane/EtOAc 9:1) to give a white solid: yield 592 mg (36%).

R_f = 0.18 (cyclohexane/EtOAc 9:1); mp: 82 °C; ¹H NMR (400 MHz, CDCl₃): δ = 1.92–1.98 (m, 2H), 2.37 (tt, 3J(H,F) = 19.0 Hz, 3J(H,H) = 7.6 Hz, 2H), 2.69 (t, 3J(H,H) = 6.3 Hz, 2H), 3.27 (t, 3J(H,H) = 5.6 Hz, 2H), 3.58–3.61 (m, 2H), 4.76 (bs, OH), 6.08 (d, 4J(H,H) = 2.3 Hz, 1H), 6.11 (dd, 3J(H,H) = 7.9 Hz, 4J(H,H) = 2.3 Hz, 1H), 6.82 (d, 3J(H,H) = 7.9 Hz, 1H); ¹³C NMR (100 MHz, CDCl₃): δ = 22.3 (CH₂), 27.1 (CH₂), 27.2 (t, 2J(C,F) = 22 Hz, CH₂), 43.1 (CH₂), 49.2 (CH₂), 97.4 (CH₃), 103.1 (CH₃), 115.4 (C₆H₅), 130.1 (CH₃), 145.0 (C₆H₅), 155.1 (C₆H₅); ¹⁹F NMR (376.5 MHz, CDCl₃): δ = −126.1 (m, CF₂), −123.3 (m, CF₂), −122.8 (m, CF₂), −121.8 (m, CF₂), −114.3 (t, 3J(F,F) = 14.1 Hz, CF₂), −80.7 (t, 3J(F,F) = 9.9 Hz, CF₃); EI MS: m/z (%): 495 (90) [M⁺], 476 (11) [M⁻F⁺], 162 (100) [M⁻C₇H₅F₁₃⁺]; HRMS: m/z calcd for C₁₇H₁₄F₁₃NO: 495.0868; found: 495.0863 [M⁺].
7-Methoxy-2,2,4-trimethyl-1,2-dihydroquinoline (11)

The preparation and properties of compound 11 have been reported in reference 3.

7-Hydroxy-2,2,4-trimethyl-1,2-dihydroquinoline (12)

Compound 11 (2.34 g, 11.5 mmol) was dissolved in glacial AcOH (6.6 mL), then 48% aqueous HBr (7.9 mL) was added and the mixture was heated at reflux overnight. After cooling, CHCl₃ (3 mL) was added and the solution was carefully neutralized to about pH 5–6 with aqueous NaOH (30%). The organic phase was separated and the aqueous phase was extracted with CHCl₃ (3 × 20 mL). The combined organic fractions were washed with saturated aqueous NaHCO₃ (50 mL), dried, and evaporated. The crude product was purified by using column chromatography (eluent cyclohexane/EtOAc 4:1) to give a yellow solid: yield 1.12 g (51%). The obtained analytical data are identical to the published values.⁶

7-Hydroxy-2,2,4-trimethyl-N-(1H,1H,2H,2H-perfluoroocetyl)-1,2-dihydroquinoline (13)

1H,1H,2H,2H-Perfluoroocetyl iodide (463 µL, 1.88 mmol) was added dropwise to a solution of compound 12 (891 mg, 4.71 mmol) in DMF (2.5 mL) at 90 °C. After complete addition, the mixture was stirred at 140 °C for 3 h. After cooling, EtOAc (40 mL), aqueous NaOH (2 m, 20 mL) and brine (30 mL) were added. The organic layer was separated, washed with brine (20 mL) and the solvent was removed under reduced pressure. The crude product was purified by using column chromatography (eluent cyclohexane/EtOAc 10:1) to give a yellow oil: yield 302 mg (30%). Note: The product is not stable and decomposes within hours.

General method 5 for the solid phase synthesis of rhodamine F labeled peptoids 14a–d, 15 and 16

Fmoc-protected Rink amide resin (0.67 mmol/g, 50 mg) was swollen in DMF (1 mL) for 1 h. Multiple washing steps using DMF were performed between each step as described below. Fmoc deprotection was completed by adding piperidine (20% in DMF, 1 mL) (3 × 5 min). Following, the monomer 2-(((9H-fluoren-9-yl)methoxy)carbonyl)(6-((tert-butoxycarbonyl)amino)hexyl)amino)acetic acid was coupled to the resin. To achieve this, the monomer (50.2 mg, 101 µmol), diisopropylcarbodiimide (15.7 µL, 101 µmol) and 1-hydroxybenzotriazole hydrate (15.5 mg, 101 µmol) were dissolved in DMF biograde (1 mL) and added to the resin. The
reaction vessel was subjected to microwave irradiation to keep the constant temperature at 60 °C (max. 20 W) for 30 min while being stirred. The reaction solution was filtered and the resin was treated a second time with freshly prepared reaction solution under the same conditions as described above (double coupling). Afterwards, the resin was thoroughly washed with DMF (5 × 3 mL). This process of Fmoc deprotection and monomer coupling was repeated six times in total, so that a resin bound hexamer was obtained. Then another Fmoc deprotection step was carried out under the previously described conditions. Subsequently, rhodamine F 1b, 1d–f, 2 or 3 (0.5 equiv.), diisopropylcarbodiimide (15.7 µL, 101 µmol) and 1-hydroxybenzotriazole hydrate (15.5 mg, 101 µmol) dissolved in DMF biograde (1 mL) were added to the washed resin. The reaction vessel was shaken for 48 h at RT. Afterwards, the resin was thoroughly washed with DMF until the washing solution remained colorless. For the final cleavage the resin was incubated at RT overnight with TFA (95% in CH₂Cl₂, 1.5 mL). The solution was filtered and the resin was washed one more time with TFA (95% in CH₂Cl₂, 1.5 mL), followed by MeOH until the washing solution remained colorless. The crude product was lyophilized and purified using a FluoroFlash column (2 g, 8 cm³ tube). To achieve this, a new cartridge was loaded with DMF (1 mL). Afterwards, MeOH/H₂O (60:40, 4 mL) was passed to condition the cartridge. The preconditioning solution was discarded. The crude product was dissolved in H₂O (250 µL) and loaded onto the cartridge. The cartridge was washed with MeOH/H₂O (60:40, 10 mL) to remove non-fluorous compounds. Then it was washed with hydrochloric acid in MeOH (0.1 M, 10 mL) to obtain the product. The purified product was isolated after removing the solvent under reduced pressure. If necessary the prepurified peptoid was purified again by semi-preparative HPLC.

2-(((9H-Fluoren-9-yl)methoxy)carbonyl)(6-((tert-butoxycarbonyl)-amino)hexyl)amino)acetic acid

The preparation and properties of the peptoid monomer have been reported in reference 8.

Compound 14a

After F-SPE and HPLC purification the title compound was obtained as red solid from compound 1b (18.3 mg, 16.8 µmol) according to general method 5: yield 0.42 mg (HPLC purity: 98%).


Compound 14b

After F-SPE and HPLC purification the title compound was obtained as red solid from compound 1d (17.8 mg, 16.8 µmol) according to general method 5: yield 1.69 mg (HPLC purity: 95%).

Compound 14c
After F-SPE purification the title compound was obtained as dark red solid from compound 1e (18.7 mg, 16.8 µmol) according to general method 5: yield 4.09 mg (HPLC purity: 96%).

Compound 14d
After F-SPE and HPLC purification the title compound was obtained as red solid from compound 1f (21.6 mg, 16.8 µmol) according to general method 5: yield 1.28 mg (HPLC purity: 96%).
MALDI-TOF MS: m/z: 2187 [M]+.

Compound 15
After F-SPE purification the title compound was obtained as dark red solid from compound 2 (19.1 mg, 16.8 µmol) according to general method 5: yield 5.33 mg (HPLC purity: 96%).
MALDI-TOF MS: m/z: 2040 [M]+.

Compound 16
After F-SPE and HPLC purification the title compound was obtained as violet solid from compound 3 (20.5 mg, 16.8 µmol) according to general method 5: yield 0.65 mg (HPLC purity: 93%).
MALDI-TOF MS: m/z: 2119 [M]+.

Biological Methods

Cell culture techniques for mammalian cells
All procedures with mammalian cells were carried out under sterile conditions. 1 × 10⁴ HeLa (human cervix carcinoma) cells were plated into each well of an 8-well µ-slide from IBIDI (Ibitreat), Germany, and cultured in 200 µL of Dulbecco’s modified Eagle’s medium, high glucose, (DMEM, Sigma Taufkirchen) supplemented with 10% fetal calf serum (FCS, PAA), and 1 u/mL Penicillin/Streptomycin at 37 °C, 5% CO₂.

Treatment of HeLa cells with the rhodamine F dye coupled peptoids
The peptoids were dissolved in bidistilled water to yield a 2 mM stock solution and were further diluted with 10% DMEM to yield the respective incubation media. The cells cultured as described above were incubated with the different peptoids at final concentrations of 0.1, 1, 5, 1, 20, 50 or 100 µM, respectively. Cellular uptake of the peptoids was measured by live-cell imaging after 24 and 48 h as fixation would alter the intracellular distribution as described for other polycationic species.
Subcellular Localization

For the intracellular localization of the peptoids, the cells were co-incubated with fluorescent probes specific for different organelles (Molecular Probes, Karlsruhe). For mitochondria labeling the cells were treated with 100 nM MitoTracker® Green FM for 15 min, according to the manufacturer’s manual, and washed three times with PBS. For the staining of the nuclei, the cells were eventually treated with Hoechst 33342 dye (2 µg/mL) after washing of the MitoTracker treated cells with PBS according to the manufacturer’s instructions. The cells were covered with DMEM and subjected to live confocal microscopy at 37 °C and 5% CO₂ atmosphere.

Live imaging by confocal microscopy

Simultaneous visualization of the colocalization of the peptoids and mitochondria and nuclei was achieved by confocal microscopy using Leica TCS-SP5 II, equipped with a DMI6000 microscope. MitoTracker® Green FM was excited using the 488 nm line of an argon ion laser, the nuclei were excited with a UV laser at 364 nm, the peptoids were excited at 514 nm using an argon laser (14a), 561 nm using a DPSS laser(15) and 594 nm using a HeNe laser (16). The objective was a HCX PL APO CS 63.0x1.2 Water UV. The exposure was set to minimize oversaturated pixels in the final images. Fluorescence emission was measured at 400–461 nm (for Hoechst 33342), 503–538 nm (for MitoTracker® Green FM), 522–600 nm (for 14a), 567–646 nm (for 15), and 600–650 nm (for 16) using a simultaneous detection of the MitoTracker® Green FM and the respective peptoid. Image acquisition was conducted at a lateral resolution of 1024 × 1024 pixels and 8 bit depth using LAS-AF 2.0.2.4647 acquisition software.
Spectral Data

![Chemical Structure](image)

**ppm (δ)**

- 7.21 (1H, s)
- 7.00 (2H, d)
- 6.98 (2H, d)
- 13.88 (1H, s)
- 12.85 (1H, s)
- 8.19 (1H, d)
- 7.94 (1H, d)

**ppm (δ)**

- 172.21
- 137.98
- 137.98
- 135.27
- 132.74
- 128.67
- 115.15
- 99.99

**ppm (δ)**

- 44.54
- 44.69
- 44.70

![Additional Spectral Data](image)
Supporting Figures

**Fig. SI-1** HPLC trace of crude peptoid 14a after cleavage from solid supports. Signals were detected at 218 nm. Retention time of 14a: 15.1 min.

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**Fig. SI-2** HPLC trace of peptoid 14a after F-SPE. Signals were detected at 218 nm. Retention time of 14a: 15.3 min.

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Fig. SI-3 HPLC trace of peptoid 14a after HPLC purification. Signals were detected at 218 nm. Retention time of 14a: 15.1 min.

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Fig. SI-4 MALDI-TOF-mass spectrum of crude peptoid 14a after cleavage from solid supports.
**Fig. S1-5** MALDI-TOF-mass spectrum of peptoid 14a after F-SPE.

**Fig. S1-6** MALDI-TOF-mass spectrum of peptoid 14a after HPLC purification.
**Fig. SI-7** HPLC trace of crude peptoid 14b after cleavage from solid supports. Signals were detected at 218 nm. Retention time of 14b: 14.8 min.

**Fig. SI-8** HPLC trace of peptoid 14b after F-SPE. Signals were detected at 218 nm. Retention time of 14b: 15.1 min.
Fig. SI-9 HPLC trace of peptoid 14b after HPLC purification. Signals were detected at 218 nm. Retention time of 14b: 14.3 min.

Fig. SI-10 MALDI-TOF-mass spectrum of crude peptoid 14b after cleavage from solid supports.
Fig. SI-11 MALDI-TOF-mass spectrum of peptoid 14b after F-SPE.

Fig. SI-12 MALDI-TOF-mass spectrum of peptoid 14b after HPLC purification.
Fig. SI-13 HPLC trace of crude peptoid 14c after cleavage from solid supports. Signals were detected at 218 nm. Retention time of 14c: 15.0 min.

Fig. SI-14 HPLC trace of peptoid 14c after F-SPE. Signals were detected at 218 nm. Retention time of 14c: 15.5 min.
**Fig. SI-15** MALDI-TOF-mass spectrum of crude peptoid 14c after cleavage from solid supports.

**Fig. SI-16** MALDI-TOF-mass spectrum of peptoid 14c after F-SPE.
Fig. SI-17 HPLC trace of crude peptoid 14d after cleavage from solid supports. Signals were detected at 218 nm. Retention time of 14d: 16.9 min.

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Fig. SI-18 HPLC trace of peptoid 14d after F-SPE. Signals were detected at 218 nm. Retention time of 14d: 17.2 min.

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Fig. SI-19 HPLC trace of peptoid 14d after HPLC purification. Signals were detected at 218 nm. Retention time of 14d: 15.9 min.

Fig. SI-20 MALDI-TOF-mass spectrum of crude peptoid 14d after cleavage from solid supports.
**Fig. SI-21** MALDI-TOF-mass spectrum of peptoid 14d after F-SPE.

**Fig. SI-22** MALDI-TOF-mass spectrum of peptoid 14d after HPLC purification.
Fig. SI-23 HPLC trace of crude peptoid 15 after cleavage from solid supports. Signals were detected at 218 nm. Retention time of 15: 15.3 min.

Fig. SI-24 HPLC trace of peptoid 15 after F-SPE. Signals were detected at 218 nm. Retention time of 15: 15.7 min.
**Fig. SI-25** MALDI-TOF-mass spectrum of crude peptoid 15 after cleavage from solid supports.

**Fig. SI-26** MALDI-TOF-mass spectrum of peptoid 15 after F-SPE.
Fig. SI-27 HPLC trace of crude peptoid 16 after cleavage from solid supports. Signals were detected at 218 nm. Retention time of 16: 16.6 min.

Fig. SI-28 HPLC trace of peptoid 16 after F-SPE. Signals were detected at 218 nm. Retention time of 16: 16.8 min.
Fig. SI-29 HPLC trace of peptoid 16 after HPLC purification. Signals were detected at 218 nm. Retention time of 16: 18.7 min.

Fig. SI-30. MALDI-TOF-mass spectrum of crude peptoid 16 after cleavage from solid supports.
Fig. SI-31 MALDI-TOF-mass spectrum of peptoid 16 after F-SPE.

Fig. SI-32 MALDI-TOF-mass spectrum of peptoid 16 after HPLC purification.

References