Supplementary Material

Design and Development of a Fluorescent Imaging Agent for Vemurafenib to Probe Drug Activity in Melanoma

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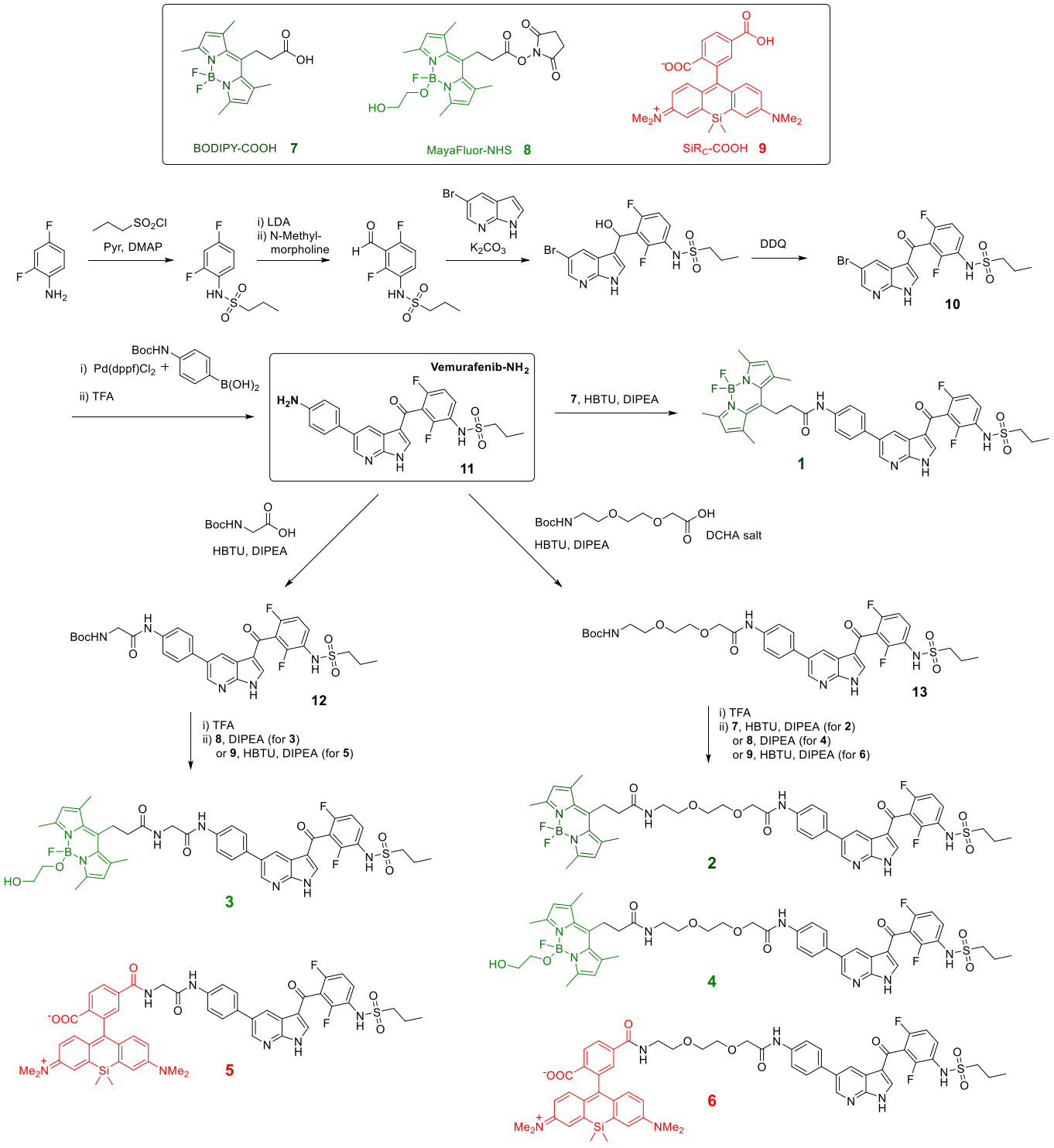
1. Chemical synthesis

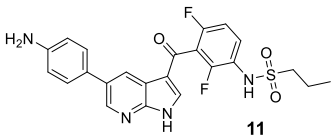
Synthetic Scheme



General Remarks

Unless otherwise noted, reactions were carried out under an atmosphere of nitrogen or argon in air-dried glassware with magnetic stirring. Air- and/or moisture-sensitive liquids were transferred *via* syringe. Analytical hin layer chromatography (TLC) was performed using plates cut from glass sheets (silica gel 60 F-254, Silicycle). Visualization was achieved under a 254 or 365 nm UV light and by immersion in an ethanolic solution of cerium sulfate, followed by treatment with a heat gun. Column chromatography was carried out using silica gel G-25 (40-63 µM) or C18 flash cartridges (Biotage). All reagents were obtained from commercial sources (Sigma Aldrich, Chem-Impex, Apollo Scientific) and used without further purification. Vemurafenib was obtained from LC Laboratories (Woburn, MA, USA). Dry solvents were obtained from Sigma Aldrich. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker 400 MHz spectrometer. Chemical shifts are reported in parts per million (δ) and calibrated using residual undeuterated solvent. Data are represented as follows: Chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, p = pentet, m = multiplet, b = broad), coupling constant (*J*, Hz) and integration. LC-ESI-MS analysis and HPLC-purifications were performed on a Waters (Milford, MA) LC-MS system using a Waters XTerra® C18 5 µm column (eluents 0.1% TFA in water and MeCN; gradient: 0-1.5 min, 5-100% B; 1.5-2.0 min 100% B).

N-(3-(5-(4-aminophenyl)-1H-pyrrolo[2,3-b]pyridine-3-carbonyl)-2,4-difluorophenyl)propane-1-sulfonamide (**11**, vemurafenib-NH_2)



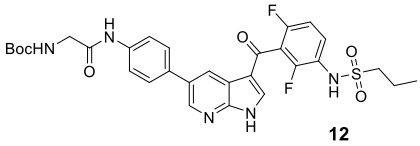
11

A solution of intermediate **10** [1, 2] (0.92 g, 2 mmol) and 4-(N-Boc-amino)phenylboronic acid (0.71 g, 3 mmol) in acetonitrile (15 mL) and aqueous Na_2CO_3 solution (2M, 4 mL) was placed in a microwave tube and degassed by passing argon through the solution for 5 min. $Pd(ddpf)Cl_2$ (163 mg, 0.2 mmol) was added and the mixture was irradiated in a microwave reactor (Biotage Initiator, Biotage, Uppsala, Sweden) at 160 °C for 30 min. The mixture was poured into water (200 mL) and extracted with EtOAc (3 x 200 mL). The combined organic layer was dried over Na_2SO_4 and concentrated. The residue was dissolved in dry dichloromethane (40 mL) and TFA (20 mL) was slowly added. The solution was stirred at room temperature for 3 h, then slowly poured into a mixture of saturated $NaHCO_3$ solution (300 mL) and 1M NaOH (100 mL) and extracted with EtOAc (3 x 250 mL). The combined organic layer was dried over Na_2SO_4 and concentrated onto silica (10 g). Reversed phase column chromatography (90 g silica, hexanes/EtOAc gradient elution, 10-40% EtOAc) afforded **11** as a beige solid (830 mg, 90%).

^{1}H NMR (400 MHz, DMSO-d_6) δ 12.85 (d, J=2.2 Hz, 1H), 9.75 (bs, 1H), 8.59 (d, J=2.2 Hz, 1H), 8.47 (bs, 1H), 8.14 (d, J=2.1 Hz, 1H), 7.58 (td, J = 9.0, 5.8 Hz, 1H), 7.41 (d, J=8.5 Hz, 2H), 7.31 – 7.24 (m, 1H), 6.70 (d, J=8.5 Hz, 2H), 5.27 (bs, 2H), 3.16 – 3.09 (m, 2H), 1.79 – 1.69 (m, 2H), 0.96 (t, J=7.6 Hz, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 181.0, 156.5 (dd, J_{CF}=246.3, 7.1 Hz), 152.8 (dd, J_{CF}=249.4, 8.6 Hz), 149.0, 148.5,

143.8, 138.7, 132.7, 129.2 (d, J_{CF}=9.6 Hz), 128.1 (2C), 125.9, 125.7, 122.4 (dd, J_{CF}=13.9, 4.0 Hz), 118.8 (t, J_{CF}=23.8 Hz), 118.0, 116.0, 114.9 (2C), 112.8 (dd, J_{CF}=22.9, 4.0 Hz), 53.9, 17.3, 13.1; ESI-MS $[M+Na]^+$ calcd. 493.1 for $C_{23}H_{20}F_2N_4NaO_3S^+$, found 493.0.

tert-butyl (2-((4-(3-(2,6-difluoro-3-(propylsulfonamido)benzoyl)-1H-pyrrolo[2,3-b]pyridin-5-yl)phenyl) amino)-2-oxoethyl)carbamate (**12**)

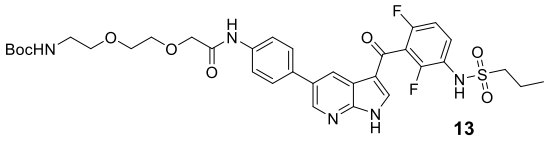


12

N-Boc-L-glycine (96.4 mg, 0.55 mmol), **11** (235.3 mg, 0.5 mmol) and DIPEA (161.6 mg, 1.25 mmol) were dissolved in dry DMF (3 mL) and HBTU (208.6 mg, 0.55 mmol) was added. The reaction mixture was stirred at room temperature for 24 h. LC/MS analysis indicated full conversion. The mixture was concentrated to ~1 mL and directly loaded onto a C18 column (Biotage, 30 g C18 silica). Reverse phase column chromatography (water/acetonitrile gradient elution, 2-60% acetonitrile, eluents containing 0.1% formic acid) afforded the desired product **12** as a white solid (236 mg, 75%).

^{1}H NMR (400 MHz, DMSO-d_6) δ 12.96 (bs, 1H), 10.07 (s, 1H), 9.78 (bs, 1H), 8.70 (d, J=2.2 Hz, 1H), 8.60 (bs, 1H), 8.20 (s, 1H), 7.75 (d, J=8.8 Hz, 2H), 7.71 (d, J=8.8 Hz, 2H), 7.59 (td, J = 9.0, 5.9 Hz, 1H), 7.31 – 7.25 (m, 1H), 7.06 (t, J=6.0 Hz, 1H), 3.81-3.68 (m, 2H), 3.16 – 3.09 (m, 2H), 1.79 – 1.69 (m, 2H), 1.41 (s, 9H), 0.96 (t, J=7.5 Hz, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 181.1, 168.8, 156.5 (dd, J_{CF}=246.5, 7.3 Hz), 156.4, 152.8 (dd, J_{CF}=249.4, 8.8 Hz), 149.1, 144.2, 139.0 (2C), 133.2, 131.6, 129.2 (d, J_{CF}=9.6 Hz), 127.9 (2C), 127.0, 122.5 (dd, J_{CF}=13.5, 3.8 Hz), 120.1 (2C), 118.7 (t, J_{CF}=23.8 Hz), 118.0, 116.1, 112.8 (dd, J_{CF}=22.7, 3.9 Hz), 78.5, 54.0, 44.3, 28.7 (3C), 17.3, 13.1; ESI-MS $[M+H]^+$ calcd. 628.2 for $C_{30}H_{32}F_2N_5O_6S^+$, found 628.1.

tert-butyl (2-(2-(2-((4-(3-(2,6-difluoro-3-(propylsulfonamido)benzoyl)-1H-pyrrolo[2,3-b]pyridin-5-yl)phenyl) amino)-2-oxoethoxy)ethoxy)ethyl)carbamate (**13**)



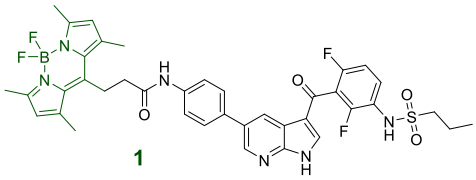
13

8-(Boc-amino)-3,6-dioxaoctanoic acid dicyclohexyl-amine salt (201.0 mg, 0.45 mmol), **11** (188.2 mg, 0.4 mmol) and DIPEA (175 µL, 1 mmol) were dissolved in dry DMF (4 mL) and HBTU (170.7 mg, 0.45 mmol) was added. The reaction mixture was stirred at room temperature for 12 h, concentrated to ~1 mL and directly loaded onto a C18 column (Biotage, 30 g C18 silica). Reversed phase column chromatography (water/acetonitrile gradient elution, 2-50% acetonitrile, eluents containing 0.1% formic acid) afforded the desired product **13** as a yellowish solid (222 mg, 78%).

^{1}H NMR (400 MHz, DMSO-d_6) δ 12.94 (bs, 1H), 9.87 (bs, 1H), 9.77 (s, 1H), 8.70 (d, J=2.2 Hz, 1H), 8.60 (bs, 1H), 8.21 (s, 1H), 7.81 (d, J=8.8 Hz, 2H), 7.72 (d, J=8.8 Hz, 2H), 7.59 (td, J = 9.0, 5.9 Hz, 1H), 7.31 – 7.25 (m, 1H), 6.80 (t, J=5.6 Hz, 1H), 4.12 (s, 2H), 3.71 – 3.66 (m, 2H), 3.64 – 3.59 (m, 2H), 3.45 (t, J=6.1 Hz, 2H), 3.15 – 3.08 (m, 4H), 1.80 – 1.69 (m, 2H), 1.37 (s, 9H), 0.96 (t, J=7.4 Hz, 3H); ^{13}C NMR (100 MHz,

DMSO-d_6) δ 181.1, 168.9, 156.5 (dd, J_{CF}=247.1, 7.5 Hz), 156.1, 152.8 (dd, J_{CF}=249.5, 8.9 Hz), 149.2, 144.3, 139.1, 138.4, 133.7, 131.6, 129.2 (d, J_{CF}=9.5 Hz), 127.8 (2C), 127.0, 122.6 (dd, J_{CF}=13.4, 3.7 Hz), 120.7 (2C), 118.7 (t, J_{CF}=23.6 Hz), 118.0, 116.1, 112.8 (dd, J_{CF}=22.8, 3.7 Hz), 78.1, 70.8, 70.7, 69.8, 69.7, 54.0, 40.1 (under DMSO signal), 28.7 (3C), 17.3, 13.1; ESI-MS $[M+H]^+$ calcd. 716.3 for $C_{34}H_{40}F_2N_5O_8S^+$, found 716.3.7.

Vemurafenib-BODIPY (**1**)

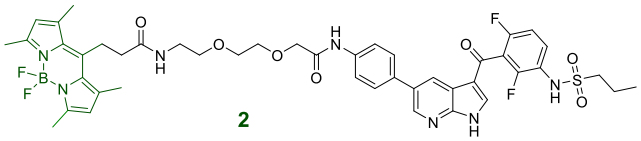


1

BODIPY-carboxylic acid **7** [3] (35.2 mg, 0.11 mmol), **11** (47.1 mg, 0.1 mmol) and DIPEA (43.5 µL, 0.25 mmol) were dissolved in dry DMF (1 mL) and HBTU (41.7 mg, 0.11 mmol) was added. The reaction mixture was stirred at room temperature for 12 h, directly loaded onto a C18 column (Biotage, 30 g C18 silica) and purified by reversed phase column chromatography (water/acetonitrile gradient elution, 2-50% acetonitrile, eluents containing 0.1% formic acid) to afford **1** as an orange solid (51 mg, 66%).

^{1}H NMR (400 MHz, DMSO-d_6) δ 11.1 (bs, 1H), 10.21 (s, 1H), 8.70 (d, J=2.2 Hz, 1H), 8.60 (bs, 1H), 8.32 – 8.12 (bs+s, 2H), 7.77 (d, J=8.7 Hz, 2H), 7.72 (d, J=8.7 Hz, 2H), 7.58 (td, J = 9.0, 6.0 Hz, 1H), 7.30 – 7.24 (m, 1H), 6.28 (s, 2H), 3.37 – 3.30 (m, 2H), 3.14 – 3.08 (m, 2H), 2.74 – 2.66 (m, 2H), 2.52 (s, 6H), 2.43, (s, 6H), 1.80 – 1.68 (m, 2H), 0.96 (t, J=7.5 Hz, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 180.7, 169.2, 155.7 (dd, J_{CF}=245.4, 6.8 Hz), 153.5, 152.3 (dd, J_{CF}=249.0, 8.5 Hz), 148.7, 145.4 (2C), 143.7, 141.1 (2C), 138.7, 138.5, 132.9, 131.1, 130.7 (2C), 128.5 (d, J_{CF}=9.7 Hz), 127.4 (2C), 126.5, 122.4 (dd, J_{CF}=13.8, 4.3 Hz), 121.8 (2C), 119.8 (2C), 118.2 (t, J_{CF}=23.6 Hz), 117.5, 115.6, 112.2 (dd, J_{CF}=23.0, 4.0 Hz), 53.5, 37.2, 23.7, 16.8, 16.0 (2C), 14.1 (2C), 12.6; ESI-MS $[M+H]^+$ calcd. 773.3 for $C_{39}H_{38}BF_4N_6O_4S^+$, found 773.2.

Vemurafenib-Linker-BODIPY (**2**)



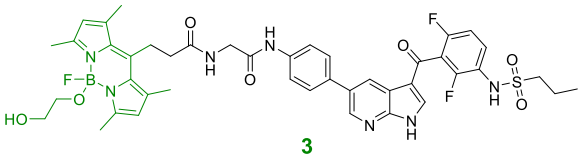
2

13 (35.8 mg, 0.05 mmol) was dissolved in dry CH_2Cl_2 (6 mL) and TFA (3 mL) was added. The mixture was stirred at room temperature for 1 h, concentrated and dried in vacuo (100 mtorr). The residue was dissolved in dry DMF (1.5 mL) and BODIPY-carboxylic acid **7**[3] (22.4 mg, 0.07 mmol), DIPEA (34.8 µL, 0.2 mmol) and HBTU (26.5 mg, 0.07 mmol) were added sequentially. The clear solution was stirred at room temperature for 1 h and then directly loaded onto a C18 column (Biotage, 12 g C18 silica). Conjugate **2** was obtained after reversed phase column chromatography (water/acetonitrile gradient elution, 2-60% acetonitrile, eluents containing 0.1% formic acid) as an orange solid (21 mg, 46%).

^{1}H NMR (400 MHz, DMSO-d_6) δ 12.98 (d, J=2.1 Hz, 1H), 9.81 (s, 1H), 9.77 (s, 1H), 8.68 (d, J=2.2 Hz, 1H), 8.61 (bs, 1H), 8.22 (d, J=3.0 Hz, 1H), 8.15 (t, J=5.6 Hz, 1H), 7.79 (d, J=8.7 Hz, 2H), 7.69 (d, J=8.7 Hz,

2H), 7.59 (td, J = 9.1, 6.1 Hz, 1H), 7.32 – 7.25 (m, 1H), 6.19 (s, 2H), 4.13 (s, 2H), 3.72 – 3.67 (m, 2H), 3.65 – 3.61 (m, 2H), 3.50 (t, J=5.8 Hz, 2H), 3.31 – 3.25 (m, 2H), 3.22 – 3.15 (m, 2H), 3.15 – 3.09 (m, 2H), 2.44 – 2.35 (m, 2H), 2.43 (s, 6H), 2.37 (s, 6H), 1.80 – 1.69 (m, 2H), 0.96 (t, J=7.4 Hz, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 180.6, 170.2, 168.4, 156.0 (dd, J_{CF}=246.9, 7.5 Hz), 153.3, 152.3 (dd, J_{CF}=249.6, 8.7 Hz), 148.7, 145.7 (2C), 143.8, 141.0 (2C), 138.7, 137.9, 133.2, 131.1, 130.6 (2C), 128.8 (d, J_{CF}=9.0 Hz), 127.3 (2C), 126.6, 121.9 (dd, J_{CF}=14.0, 3.9 Hz), 121.7 (2C), 120.2 (2C), 117.5, 118.2 (t, J_{CF}=23.8 Hz), 115.6, 112.3 (dd, J_{CF}=23.2, 4.0 Hz), 70.31, 70.25, 69.4, 69.1, 53.5, 38.6, 36.2, 24.0, 16.8, 15.9 (2C), 14.1 (2C), 12.6; ESI-MS $[M+H]^+$ calcd. 918.3 for $C_{45}H_{49}BF_4N_7O_7S^+$, found 918.3.

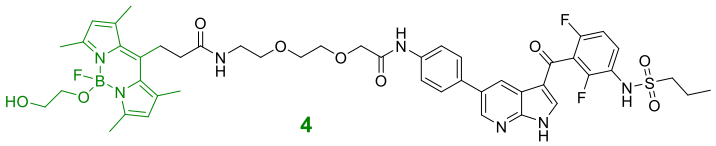
Vemurafenib-MayaFluor (**3**)



3

12 (31.4 mg, 0.05 mmol) was dissolved in dry CH_2Cl_2 (2 mL) and TFA (1 mL) was added. The mixture was stirred at room temperature for 3 h, concentrated and dried in vacuo (100 mtorr). The residue was dissolved in dry DMF (1.5 mL) and MayaFluor-NHS **8** [4] (27.6 mg, 0.06 mmol) was added followed by DIPEA (34.8 µL, 0.2 mmol). The reaction mixture was stirred at room temperature for 1 h and then directly loaded onto a C18 column (Biotage, 12 g C18 silica). Conjugate **3** was obtained after reversed phase column chromatography (water/acetonitrile gradient elution, 2-60% acetonitrile)[1] as an orange solid (20 mg, 46%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.96 (bs, 1H), 10.17 (s, 1H), 9.81 (s, 1H), 8.59 (d, J=2.2 Hz, 1H), 8.62 (bs, 1H), 8.44 (bs, 1H), 8.22 (s, 1H), 7.77 (d, J=8.7 Hz, 2H), 7.73 (d, J=8.7 Hz, 2H), 7.59 (td, J = 9.0, 5.9 Hz, 1H), 7.32 – 7.25 (m, 1H), 6.21 (s, 2H), 4.39 – 4.32 (m, 1H), 3.99 (d, J=5.6 Hz, 2H), 3.31 – 3.22 (m, 4H), 3.16 – 3.09 (m, 2H), 2.77 (t, J=6.0 Hz, 2H), 2.56 – 2.50 (m, 2H), 2.49 (s, 6H), 2.44 (s, 6H), 1.81 – 1.69 (m, 2H), 0.97 (t, J=7.5 Hz, 3H); ESI-MS $[M-OC_2H_4OH]^+$ calcd. 810.3 for $C_{41}H_{40}BF_3N_7O_5S^+$, found 810.2.

Vemurafenib-Linker-MayaFluor (**4**)



4

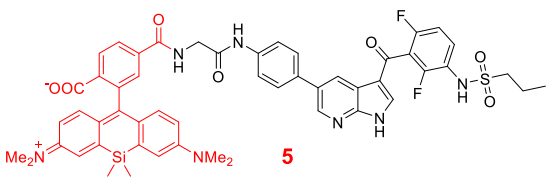
13 (35.8 mg, 0.05 mmol) was dissolved in dry CH_2Cl_2 (6 mL) and TFA (3 mL) was added. The mixture was stirred at room temperature for 1 h, concentrated and dried in vacuo (100 mtorr). The residue was dissolved in dry DMF (1.5 mL) and MayaFluor-NHS **8**[4] (27.6 mg, 0.06 mmol) was added followed by DIPEA (34.8 µL, 0.2 mmol). The reaction mixture was stirred at room temperature for 2 h and then directly loaded onto a C18 column (Biotage, 30 g

[1] No formic acid was used for chromatographic purification of MayaFluor-conjugates as the ethyleneglycol-modified BODIPY core structure is not sufficiently stable under acidic conditions (even though analytical HPLC analysis can be carried out using eluents containing 0.1% formic acid).

C18 silica). Conjugate **4** was obtained after reversed phase column chromatography (water/acetonitrile gradient elution, 2-60% acetonitrile)[1] as an orange solid (25 mg, 52%).

^{1}H NMR (400 MHz, DMSO-d_6) δ 12.88 (bs, 1H), 9.80 (s+bs, 2H), 8.69 (d, J=2.2 Hz, 1H), 8.61 (bs, 1H), 8.22 (s, 1H), 8.15 – 8.09 (m, 1H), 7.80 (d, J=8.7 Hz, 2H), 7.70 (d, J=8.7 Hz, 2H), 7.59 (td, J = 9.0, 6.0 Hz, 1H), 7.31 – 7.25 (m, 1H), 6.15 (s, 2H), 4.32 (bs, 1H), 4.13 (s, 2H), 3.73 – 3.68 (m, 2H), 3.66 – 3.62 (m, 2H), 3.50 (t, J=6.0 Hz, 2H), 3.32 – 3.28 (m, 2H), 3.27 – 3.22 (m, 2H), 3.15 – 3.09 (m, 2H), 2.73 (t, J=6.0 Hz, 2H), 2.45 – 2.36 (m, 2H), 2.43 (s, 6H), 2.40 (s, 6H), 1.80 – 1.69 (m, 2H), 0.97 (t, J=7.5 Hz, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 181.1, 170.7, 168.9, 156.3 (dd, J_{CF}=247.0, 7.8 Hz), 154.0, 152.7 (dd, J_{CF}=249.8, 9.0 Hz), 149.1, 145.7, 144.3, 140.3, 139.2, 138.4, 133.7, 131.8, 131.6, 129.1 (d, J_{CF}=9.6 Hz), 127.8, 127.0, 122.7 (dd, J_{CF}=13.7, 4.0 Hz), 122.0, 120.7, 118.7 (t, J_{CF}=23.7 Hz), 118.0, 116.1, 112.8 (dd, J_{CF}=22.7, 3.8 Hz), 70.8, 70.7, 69.9, 69.5, 63.33, 63.28, 63.1, 53.9, 36.9, 24.4, 17.3, 16.5, 14.66, 14.65, 13.1; ESI-MS $[M\text{-}OC_2H_4OH]^+$ calcd. 898.3 for $C_{45}H_{48}BF_3N_7O_7S^+$, found 898.3.

Vemurafenib-SiR$_C$ (**5**)

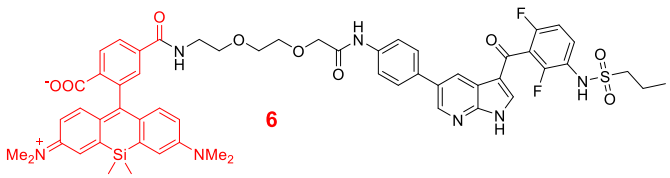


5

12 (31.4 mg, 0.05 mmol) was dissolved in dry CH_2Cl_2 (2 mL) and TFA (1 mL) was added. The mixture was stirred at room temperature for 3 h, concentrated and dried in vacuo (100 mtorr). The residue, SiR$_C$-COOH **9 [5]** (28.4 mg, 0.06 mg) and HBTU (22.8 mg, 0.06 mmol) were dissolved in dry DMF (1 mL) and DIPEA (15 µL) was added. The yellow solution was stirred at room temperature for 1 h after which a color change was observed. To the green solution was added DIPEA (5 µL) and stirring was continued for 30 min. After addition of further DIPEA (5 µL) and stirring for another 30 min full conversion was observed by LC/MS. The reaction mixture was directly loaded onto a C18 column (Biotage, 30 g C18 silica) and reversed phase column chromatography (water/acetonitrile gradient elution, 2-70% acetonitrile, eluents containing 0.1% formic acid) afforded **5** as a blue solid (33 mg, 67%).

^{1}H NMR (400 MHz, DMSO-d_6) δ 12.94 (bs, 1H), 10.22 (s, 1H), 9.19 (t, J=5.7 Hz, 1H), 8.70 (d, J=2.2 Hz, 1H), 8.60 (bs, 1H), 8.21 (s, 1H), 8.17 (d, J=8.0 Hz, 1H), 8.08 (d, J=8.0 Hz, 1H), 7.79 – 7.68 (m, 5H), 7.58 (td, J = 8.9, 5.9 Hz, 1H), 7.28 (t, J=8.8 Hz, 1H), 7.03 (d, J=2.3 Hz, 2H), 6.72 – 6.63 (m, 4H), 4.08 (d, J=5.7 Hz, 2H), 3.16 – 3.09 (m, 2H), 2.94 (s, 12H), 1.81 – 1.68 (m, 2H), 0.97 (t, J=7.6 Hz, 3H), 0.66 (s, 3H), 0.53 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 181.5, 170.1, 168.3, 166.1, 156.7 (dd, J_{CF}=246.2, 7.2 Hz), 155.8, 153.0 (dd, J_{CF}=249.6, 8.9 Hz), 150.1, 149.5, 144.6, 140.2, 139.5, 139.3, 136.7, 133.7, 131.9, 131.1, 129.4 (d, J_{CF}=8.7 Hz), 129.2, 128.6, 128.4, 128.2, 127.3, 126.2, 123.7, 123.1 (dd, J_{CF}=13.8, 4.4 Hz), 120.5, 119.0 (t, J_{CF}=23.5 Hz), 118.3, 117.2, 116.4, 114.6, 113.1 (dd, J_{CF}=22.9, 4.2 Hz), 92.1, 54.3, 44.2, 17.6, 13.4, 0.9, -0.5; ESI-MS $[M+H]^+$ calcd. 982.3 for $C_{52}H_{50}F_2N_7O_7SSi^+$, found 982.5.

Vemurafenib-Linker-SiR$_C$ (**6**)

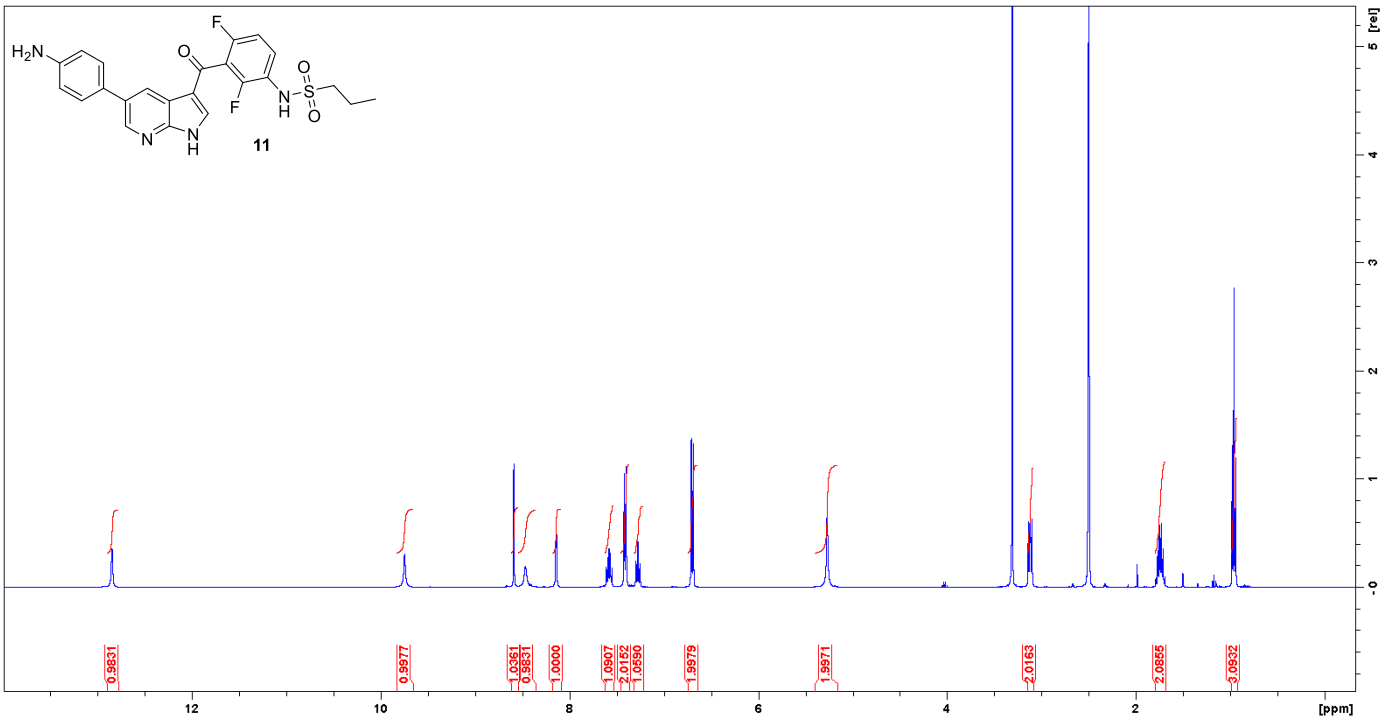


13 (35.8 mg, 0.05 mmol) was dissolved in dry CH_2Cl_2 (6 mL) and TFA (3 mL) was added. The mixture was stirred at room temperature for 2 h, concentrated and dried in vacuo (100 mtorr). The residue was dissolved in dry DMF (1 mL) and SiR_C-COOH **9**[5] (28.4 mg, 0.06 mg) and HBTU (22.8 mg, 0.06 mmol) were added. DIPEA (43.5 µL, 0.25 mmol) was added dropwise until a yellowish solution was obtained. The reaction mixture was stirred at room temperature for 4 h and then directly loaded onto a C18 column (Biotage, 30 g C18 silica). Reversed phase column chromatography (water/acetonitrile gradient elution, 2-70% acetonitrile, eluents containing 0.1% formic acid) afforded **6** as a light blue solid (28 mg, 52%).

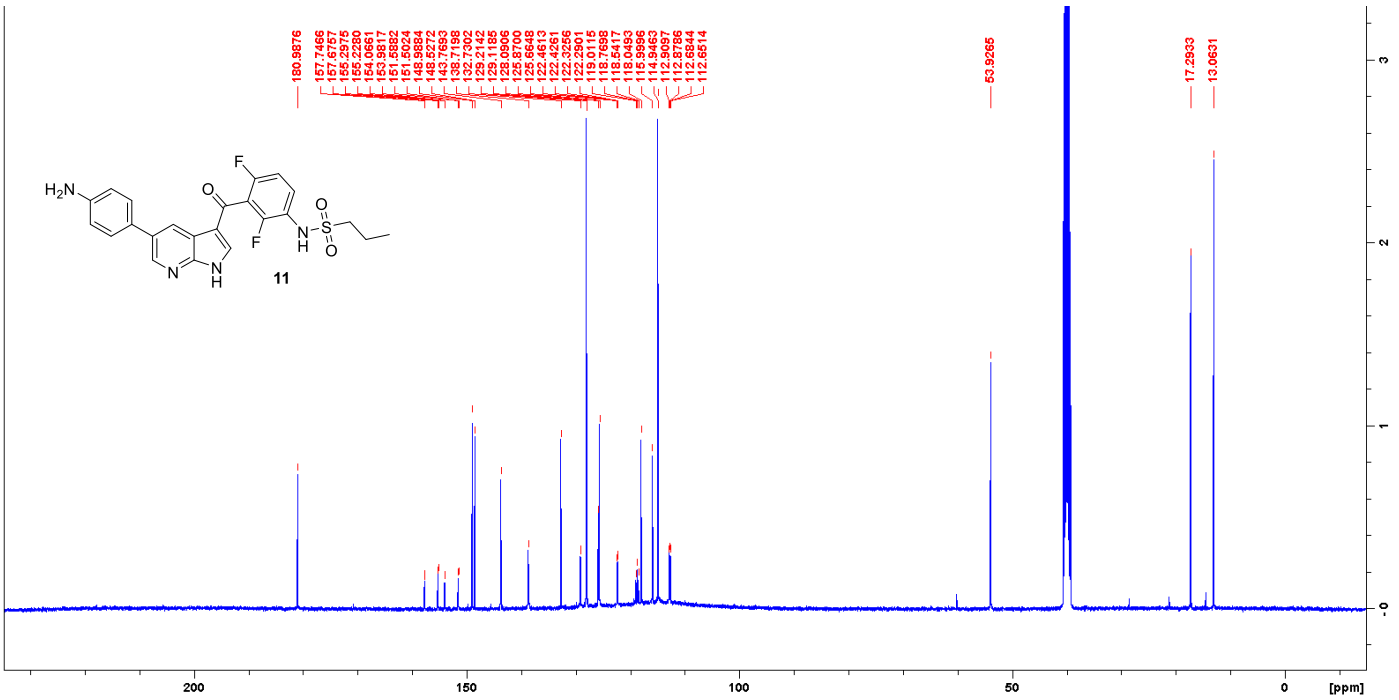
^{1}H NMR (400 MHz, DMSO-d_6) δ 12.98 (d, J=3.2 Hz, 1H), 9.78 (s, 1H), 9.75 (s, 1H), 8.84 (t, J=5.1 Hz, 1H), 8.68 (d, J=2.2 Hz, 1H), 8.62 (bs, 1H), 8.23 (d, J=3.0 Hz, 1H), 8.11 (dd, J=8.0, 1.2 Hz, 1H), 8.02 (d, J=8.0 Hz, 1H), 7.78 (d, J=8.7 Hz, 2H), 7.71 – 7.66 (m, 3H), 7.59 (td, J = 9.0, 5.9 Hz, 1H), 7.32 – 7.25 (m, 1H), 7.02 – 6.99 (m, 2H), 6.64 – 6.59 (m, 4H), 4.08 (s, 2H), 3.70 – 3.66 (m, 2H), 3.65 – 3.61 (m, 2H), 3.58 (t, J=5.6 Hz, 2H), 3.48 – 3.41 (m, 2H), 3.17 – 3.11 (m, 2H), 2.9 (s, 12H), 1.81 – 1.69 (m, 2H), 0.97 (t, J=7.6 Hz, 3H), 0.64 (s, 3H), 0.50 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 181.1, 170.8, 169.8, 168.8, 165.5, 156.5 (dd, J_{CF}=246.3, 7.3 Hz), 155.3, 152.8 (dd, J_{CF}=249.4, 8.7 Hz), 149.7, 149.2, 144.3, 140.3, 139.2, 138.4, 136.4, 133.7, 131.6, 130.9, 129.3 (d, J_{CF}=8.7 Hz), 128.7, 128.2, 127.9, 127.8, 127.0, 125.8, 123.2, 122.4 (dd, J_{CF}=13.6, 3.8 Hz), 120.7, 118.7 (t, J_{CF}=23.4 Hz), 118.0, 116.8, 116.1, 114.2, 112.8 (dd, J_{CF}=23.0, 3.8 Hz), 91.7, 70.8, 70.7, 69.8, 69.2, 60.2, 54.0, 21.2, 17.3, 14.6, 13.1, -0.5, -0.9; ESI-MS $[M+H]^+$ calcd. 1070.4 for $C_{56}H_{58}F_2N_7O_9SSi^+$, found 1070.5.

2. NMR spectra

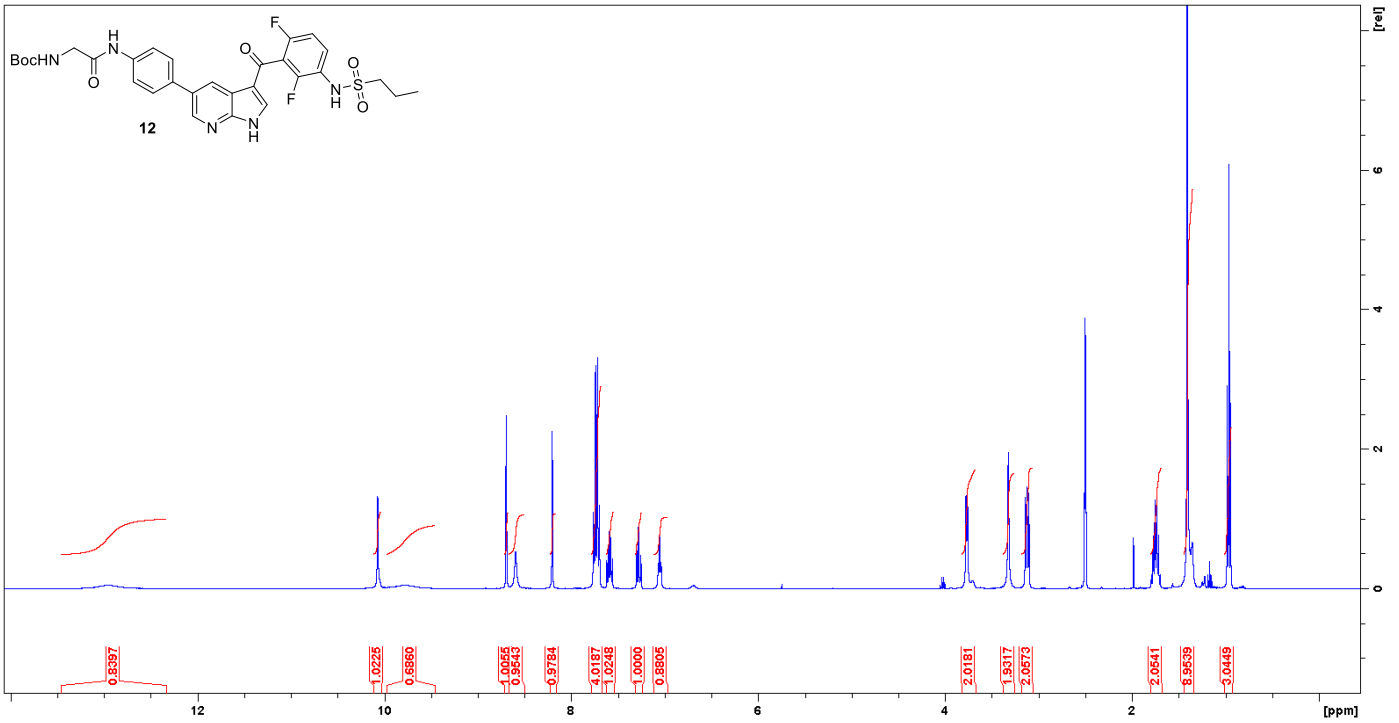
^{1}H NMR of compound **11**



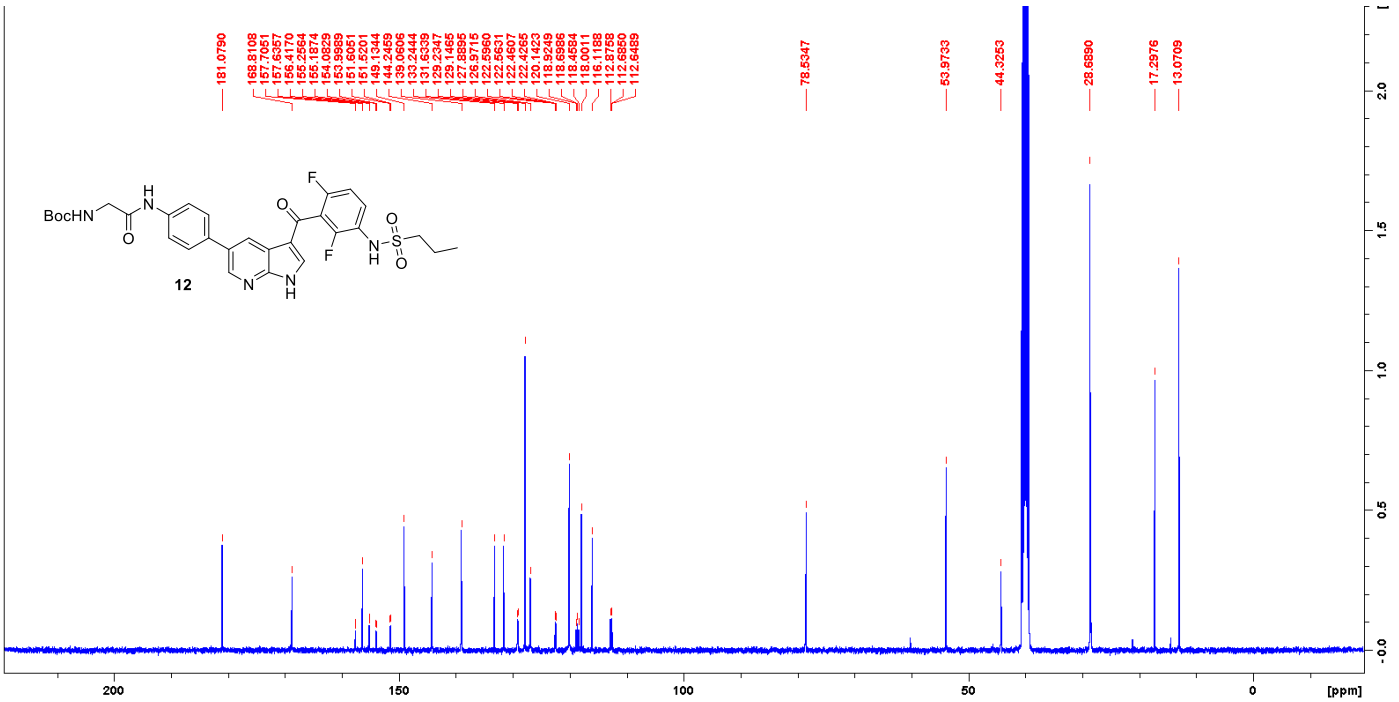
^{13}C NMR of compound **11**



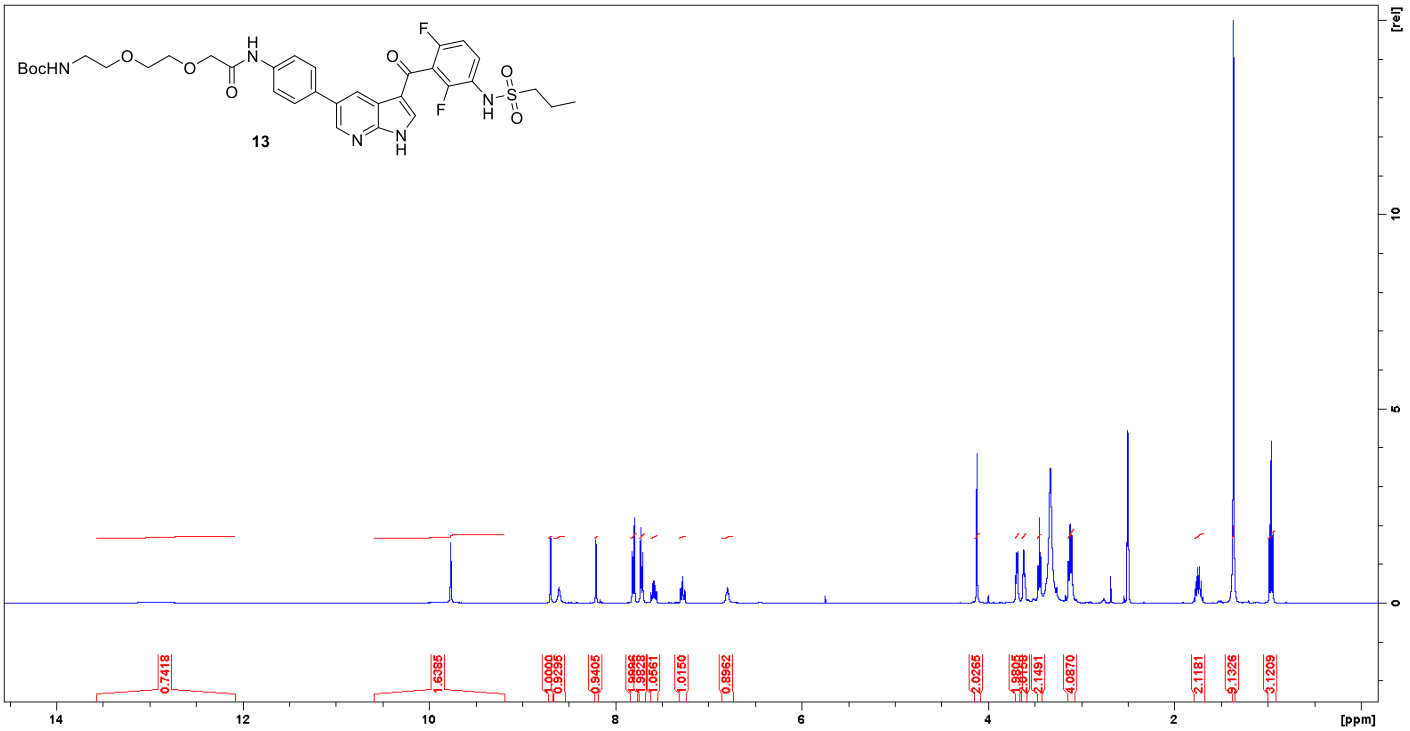
^{1}H NMR of compound **12**



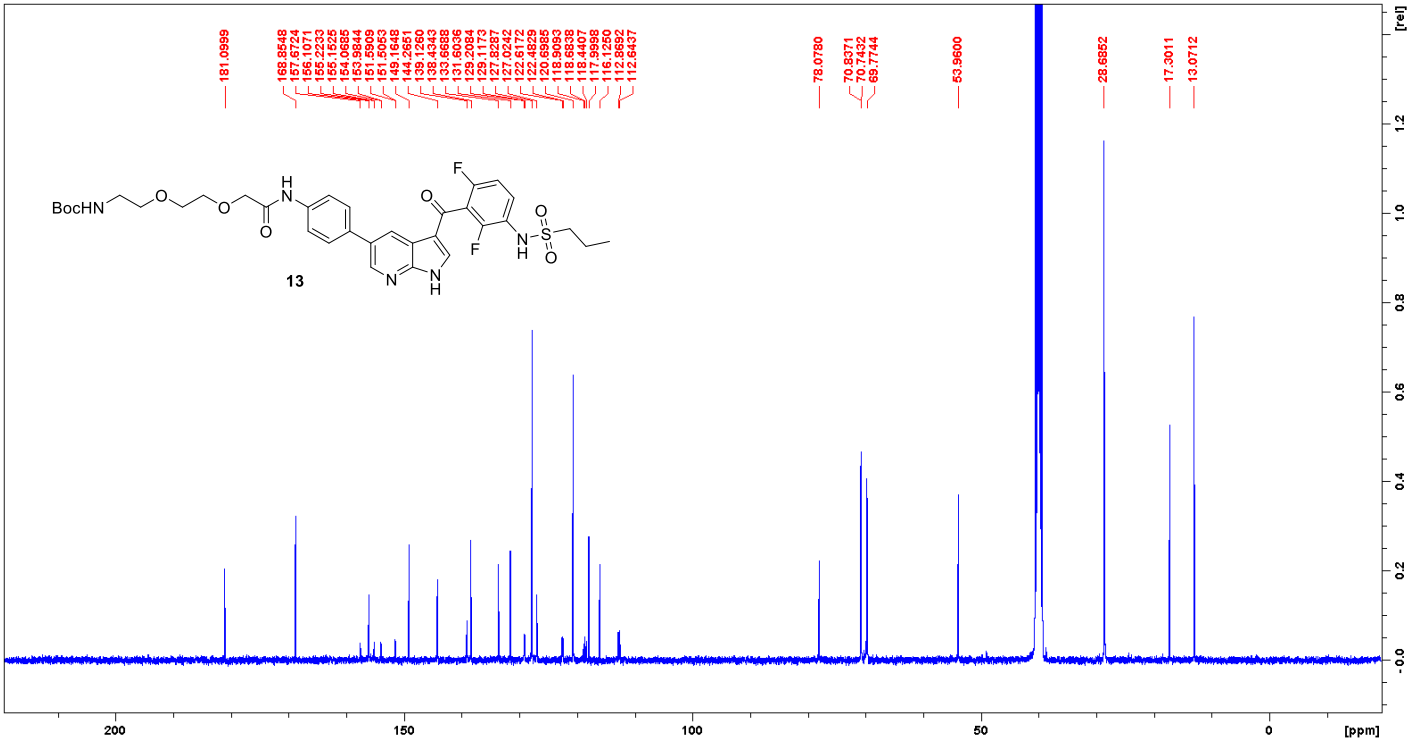
^{13}C NMR of compound **12**



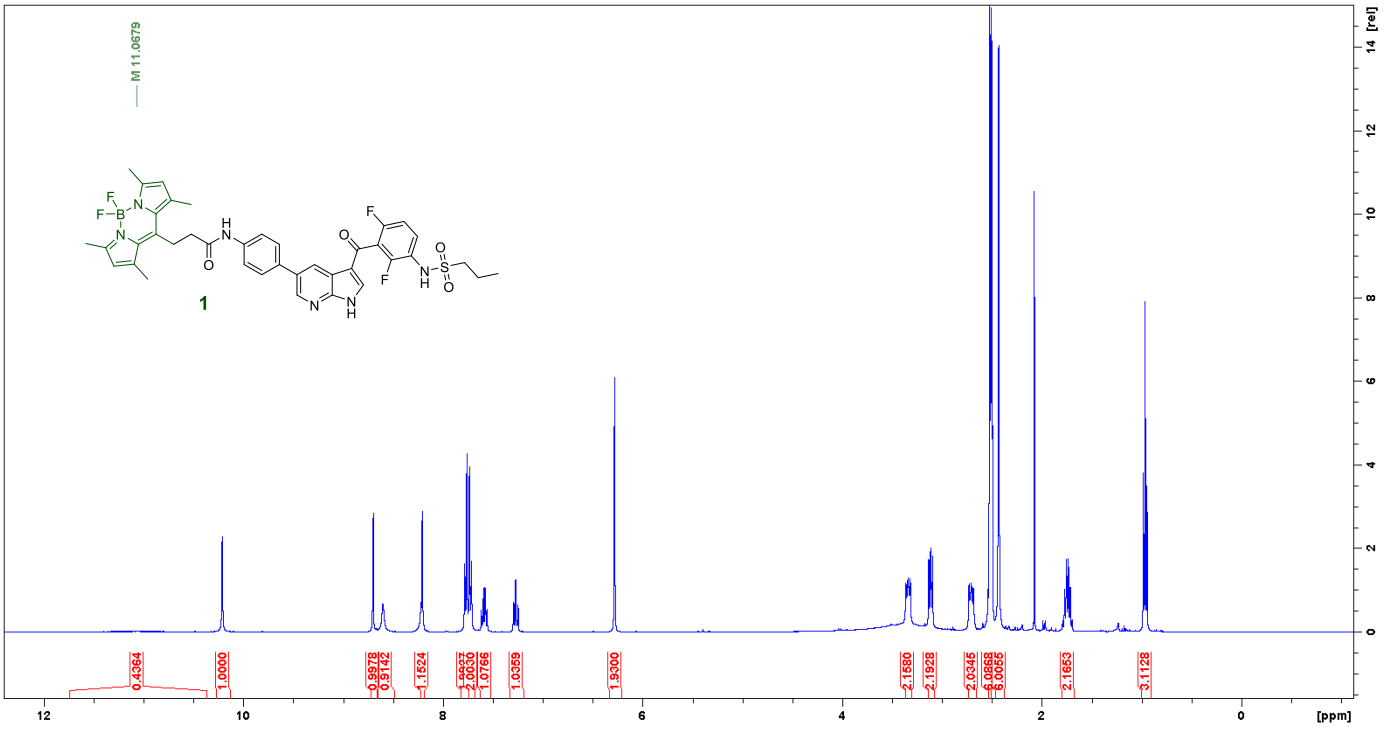
^{1}H NMR of compound **13**



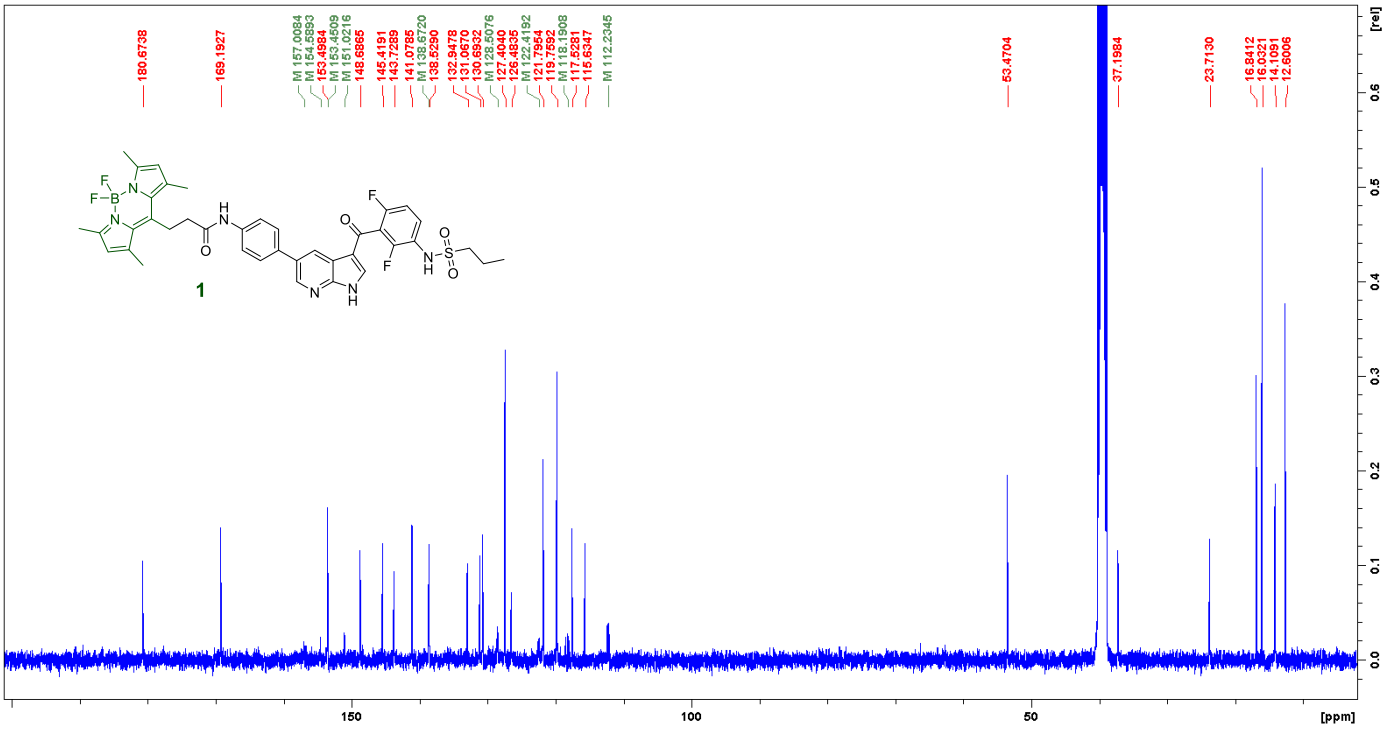
^{13}C NMR of compound **13**



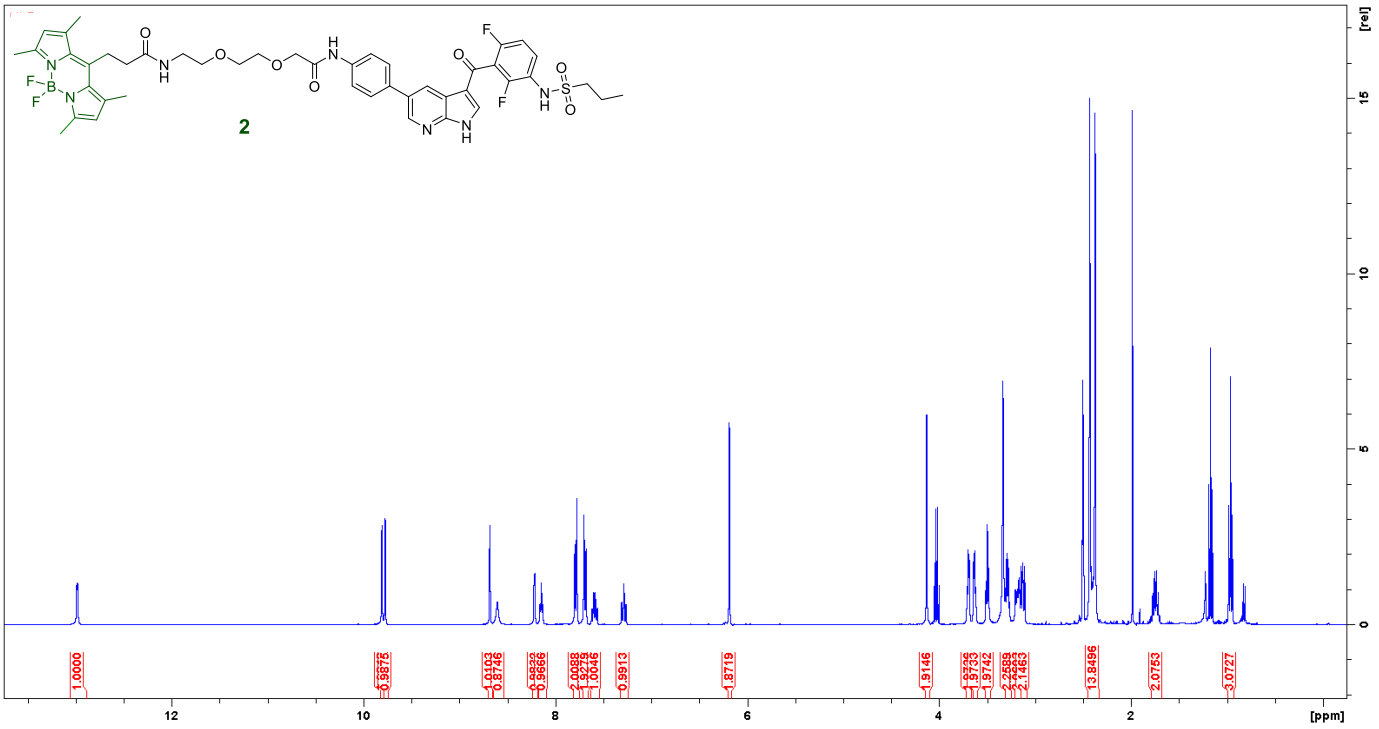
^{1}H NMR of compound **1**



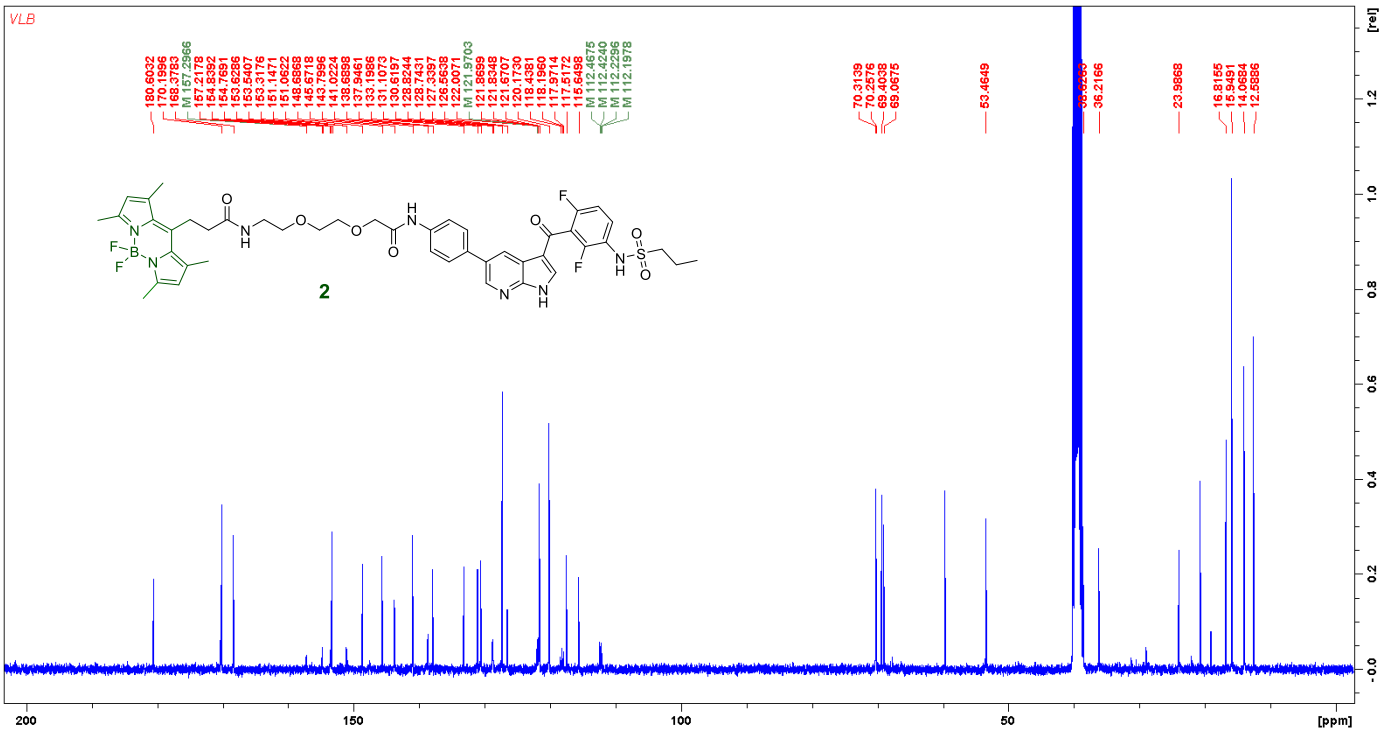
^{13}C NMR of compound **1**



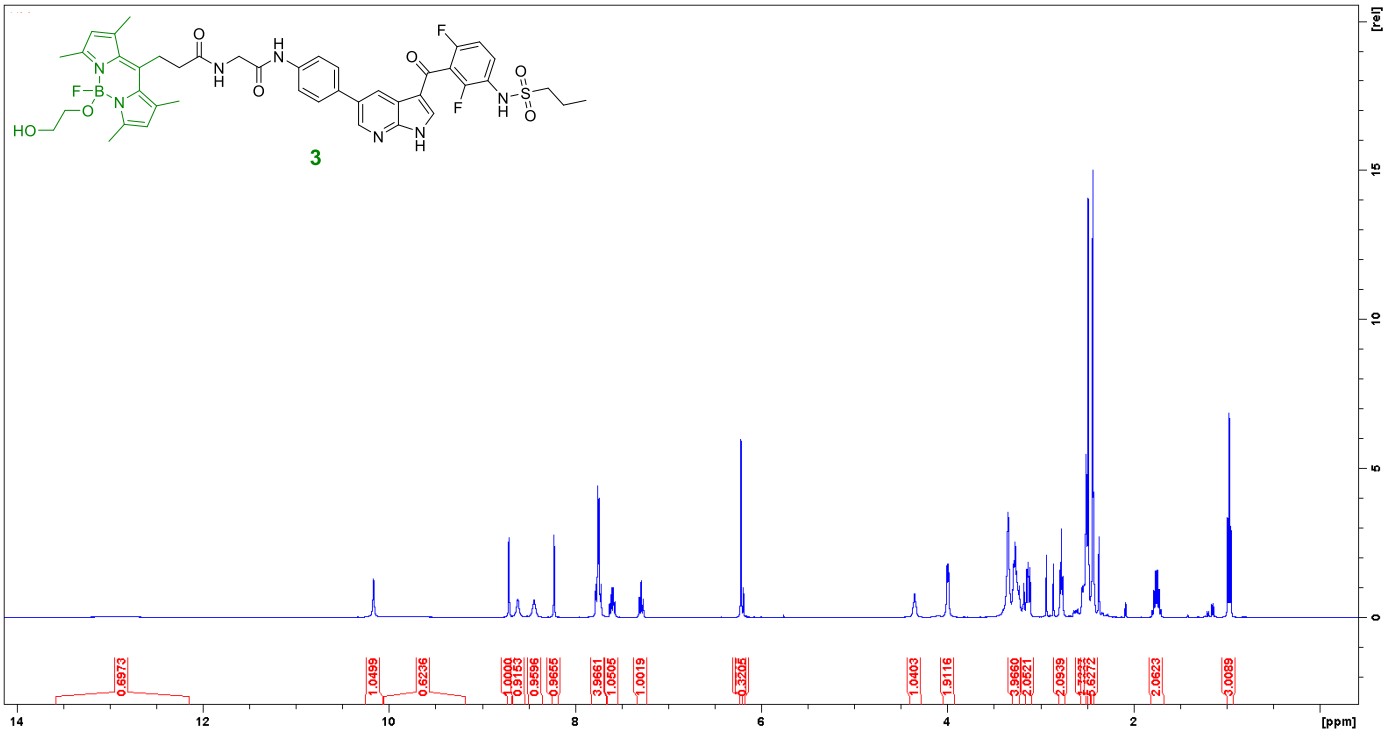
^{1}H NMR of compound **2**



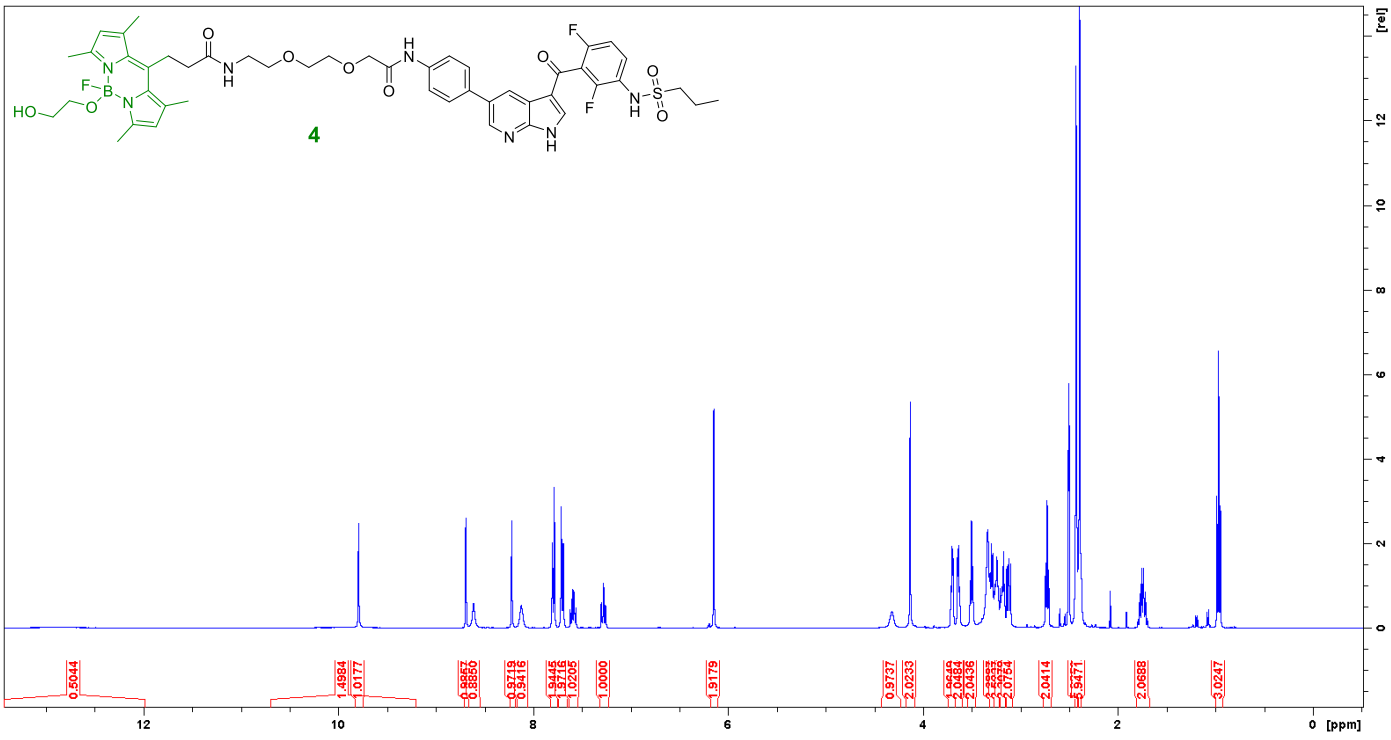
^{13}C NMR of compound **2**



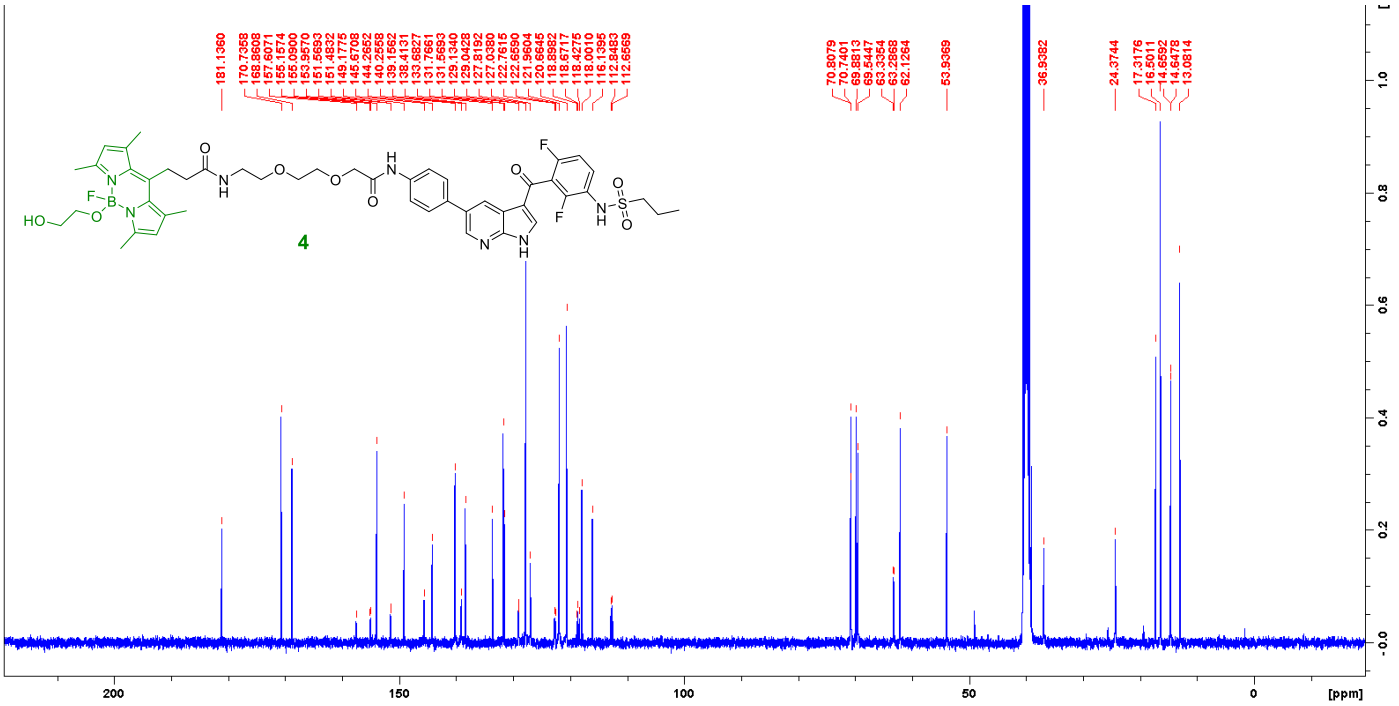
^{1}H NMR of compound **3**



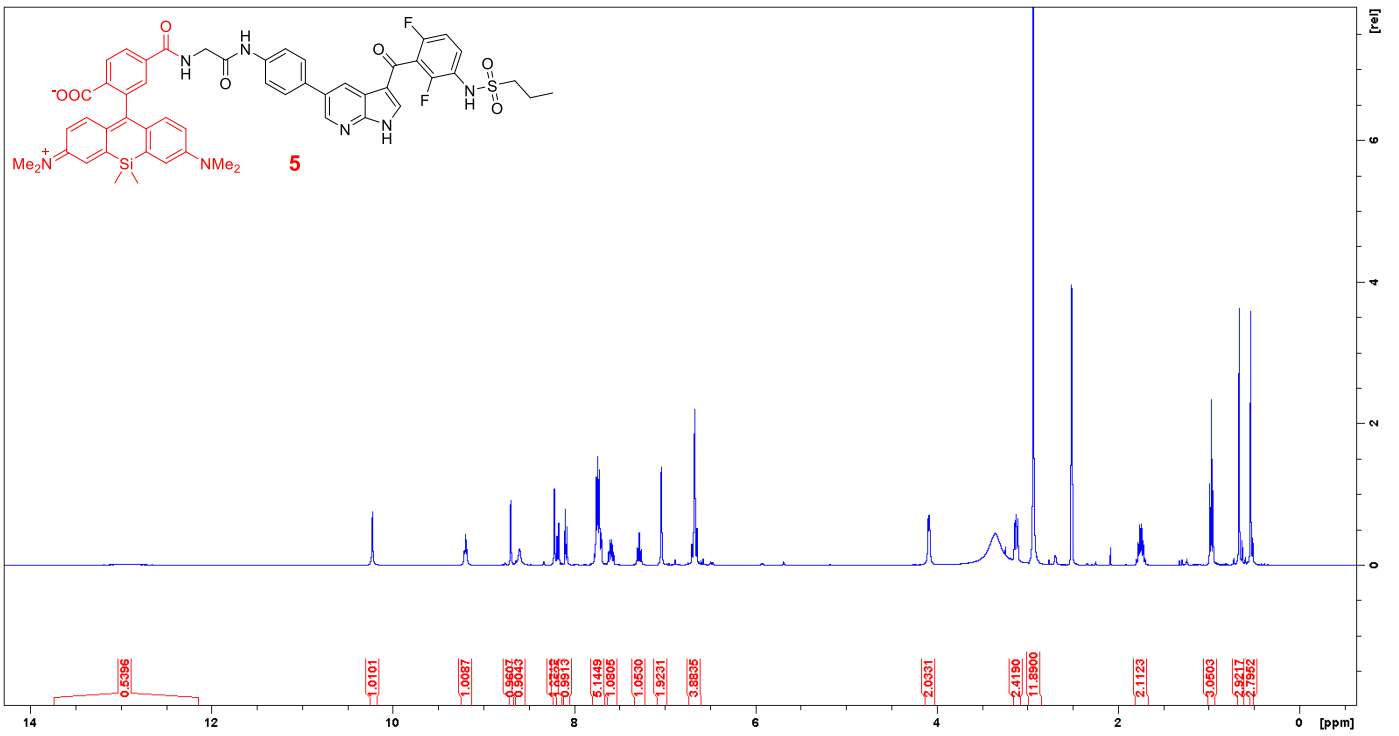
^{1}H NMR of compound **4**



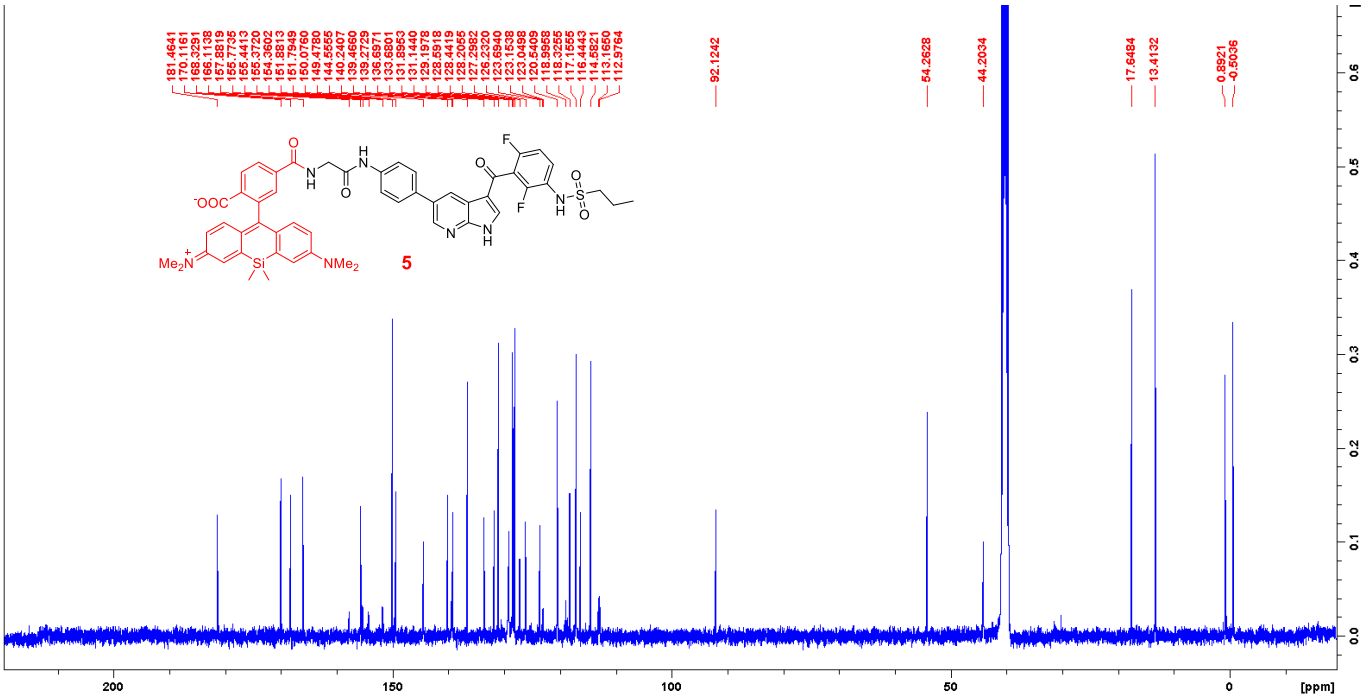
^{13}C NMR of compound **4**



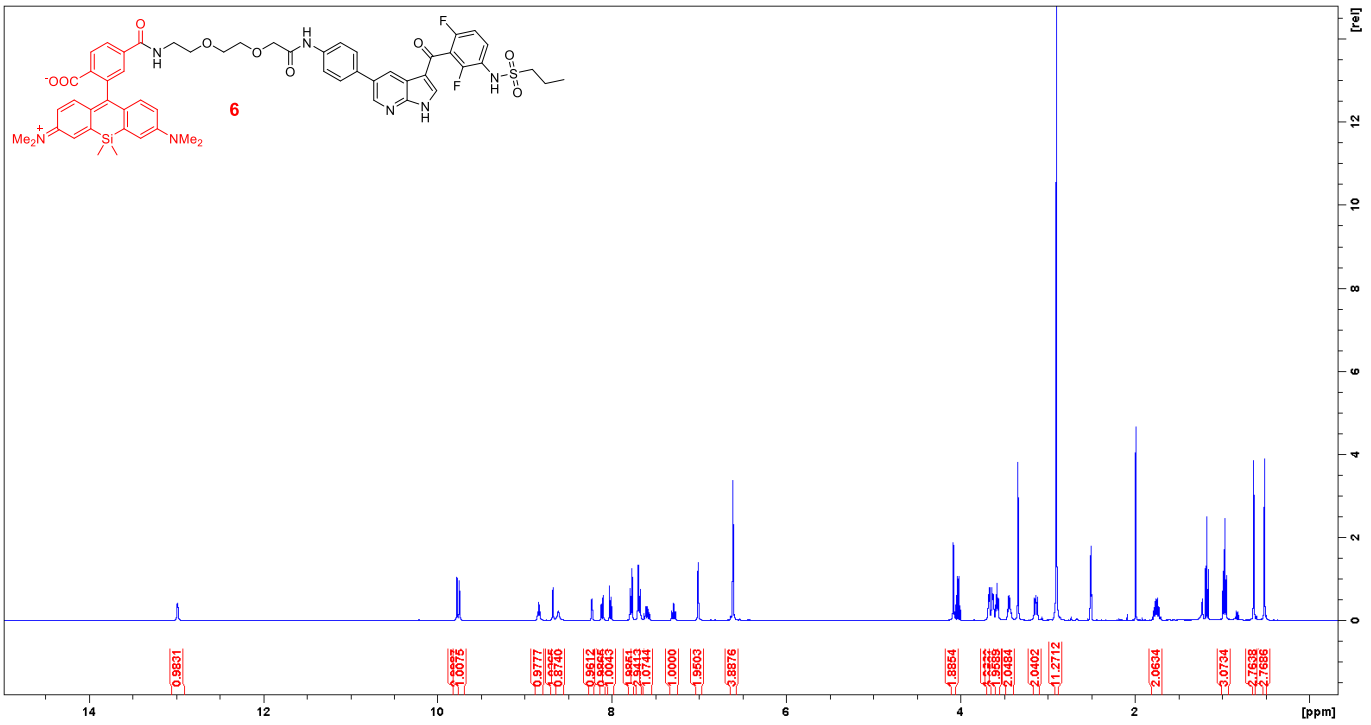
^{1}H NMR of compound **5**



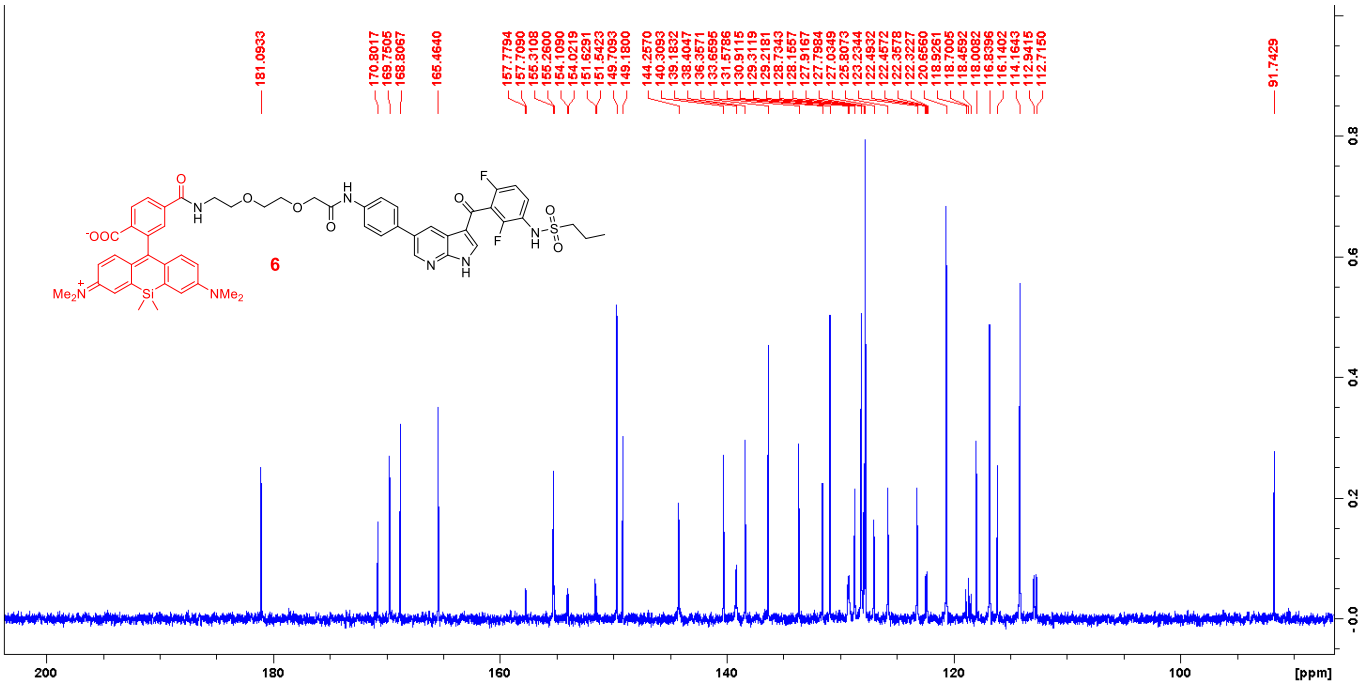
^{13}C NMR of compound **5**



^{1}H NMR of compound **6**



^{13}C NMR of compound **6**



3. References

1. Tsai J, Lee JT, Wang W, Zhang J, Cho H, Mamo S, et al. Discovery of a selective inhibitor of oncogenic B-Raf kinase with potent antimelanoma activity. Proc Natl Acad Sci. 2008; 105: 3041-6.
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