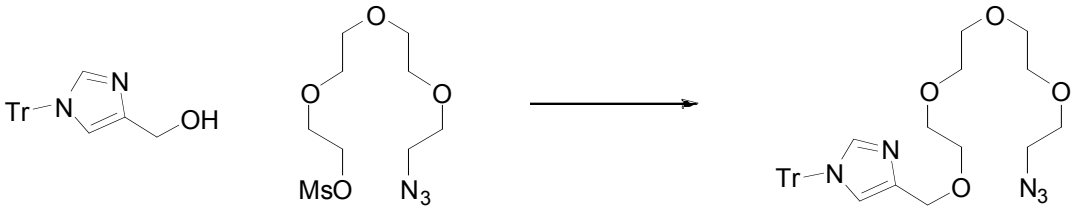
Supporting Information (SI)

Synthesis and characterization of Bodipy-FL-Tasigna® and its intermediates:

General Methods. All air or moisture sensitive reactions were performed under positive pressure of nitrogen with oven-dried glassware. Anhydrous solvents such as tetrahydrofuran (THF), toluene, dichloromethane, *N,N*-dimethylforamide (DMF), acetonitrile, methanol and triethylamine were obtained by purchasing from Sigma-Aldrich. Preparative purification was performed on a Waters semi-preparative HPLC. The column used was a Phenomenex Luna C18 (5 micron, 30 x 75 mm) at a flow rate of 45 mL/min. Method A: The mobile phase consisted of acetonitrile and water (each containing 0.1% trifluoroacetic acid). A gradient of 10% to 50% acetonitrile over 8 minutes was used during the purification. Method B: The mobile phase consisted of water (0.1% ammonium hydroxide) and acetonitrile. A gradient of 20% to 70% acetonitrile over 8 minutes was used during the purification. Fraction collection for both methods was triggered by UV detection (220 nM). Analytical analysis and purity determination were performed on an Agilent LC/MS (Agilent Technologies, Santa Clara, CA). A 7 minute gradient of 4% to 100% Acetonitrile (containing 0.025% trifluoroacetic acid) in water (containing 0.05% trifluoroacetic acid) was used with an 8 minute run time at a flow rate of 1 mL/min. A Phenomenex Luna C18 column (3 micron, 3 x 75 mm) was used at a temperature of 50°C. Mass determination was performed using an Agilent 6130 mass spectrometer with electrospray ionization in the positive mode. ^{1}H NMR spectra were recorded on Varian 400 MHz spectrometers. Chemical Shifts are reported in ppm with residual $CHCl_3$ (7.27 ppm) as internal standard for $CDCl_3$ solutions or residual DMSO-h6 (2.50 ppm) for DMSO-d6 solutions. All of the analogs for assay have purity

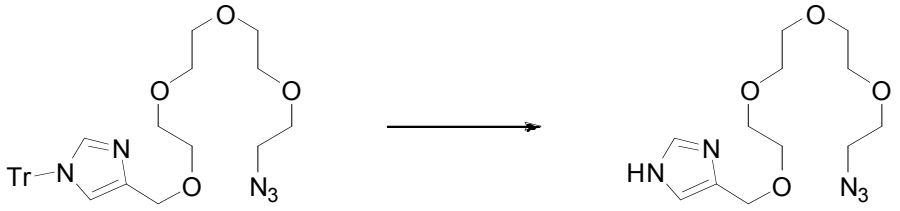
greater than 95% based on both analytical methods. High resolution mass spectrometry was recorded on Agilent 6210 Time-of-Flight LC/MS system. Confirmation of molecular formula was accomplished using electrospray ionization in the positive mode with the Agilent Masshunter software (version B.02).



S1 **S2** **S3**

To a solution of (1-trityl-1H-imidazol-4-yl)methanol (**S1**, 10 g, 29.4 mmol) in THF (226 mL) was added NaH (60% dispersion in mineral oil, 1.410 g, 35.3 mmol) at ambient temperature. The resulting solution was stirred for 10 minutes at which point a solution of 2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)ethyl methanesulfonate[1] (**S2**, 9.61 g, 32.3 mmol) in THF (45.2 mL) was added, followed by a THF (22.60 mL) wash. The reaction was fitted with a reflux condenser and heated to 60 °C for 12 h. Additional NaH (60% dispersion in mineral oil, 1.410 g, 35.3 mmol) was added and the reaction was stirred at 60 °C for an additional 12h. The reaction was cooled and poured onto ice water and extracted with CH_2Cl_2 (3 x 300 mL). Combined organics were dried over anhydrous Na_2SO_4. Filtration and concentration *in vacuo* provided a crude oil that was purified via silica gel chromatography (gradient 0 to 20% MeOH/CH_2Cl_2) to afford **S3** (13.24 g, 90%) as a viscous oil. An analytical sample was purified via reverse phase preparative HPLC (Method A).

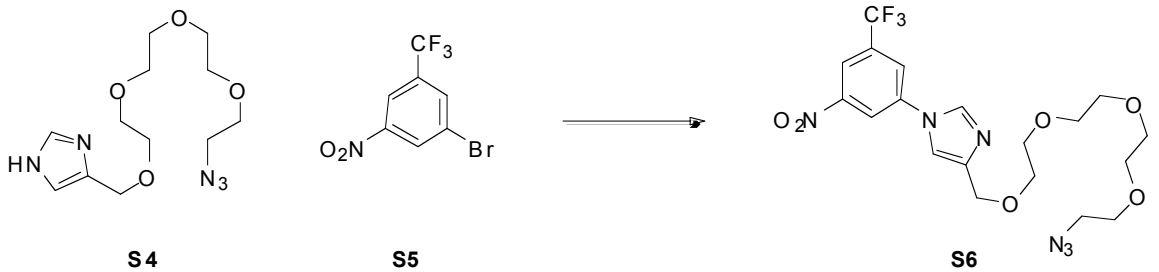
4-(13-azido-2,5,8,11-tetraoxatridecyl)-1-trityl-1H-imidazole (S3): 1H NMR (400 MHz, DMSO-d_6) δ ppm 9.07 (s, 1 H), 7.67 (s, 1 H), 6.97 - 7.57 (m, 15 H), 4.55 (s, 2 H), 3.25 - 3.71 (m, 16 H); LC-MS: RT (min) = 5.14; HRMS calculated for $C_{31}H_{36}N_5O_4$ (M + H) 542.2771, found 542.2768.



S3 **S4**

To a solution of 4-(13-azido-2,5,8,11-tetraoxatridecyl)-1-trityl-1H-imidazole (**S3**, 11.1 g, 20.49 mmol) in CH_2Cl_2 (427 mL) was added TFA (85 mL). The reaction was stirred for 12 h at ambient temperature and was then concentrated *in vacuo*. The crude material was taken up in CH_2Cl_2 (300 mL) and washed twice with saturated aqueous $NaHCO_3$. The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo*. Filtration and concentration *in vacuo* provided a crude oil that was purified via silica gel chromatography (gradient 0 to 30% MeOH/EtOAc) to provide **S4** (5.98 g, 97%) as a clear, viscous oil that solidified upon standing. An analytical sample was purified via reverse phase preparative HPLC (Method A).

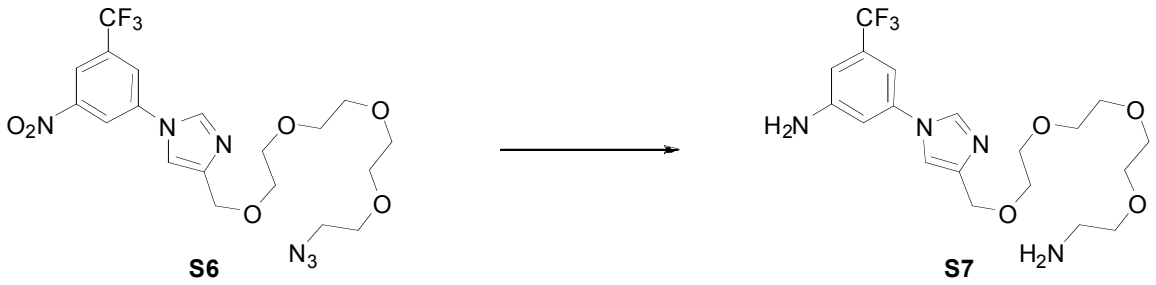
4-(13-azido-2,5,8,11-tetraoxatridecyl)-1H-imidazole (S4): 1H NMR (400 MHz, CHLOROFORM-*d*) δ ppm 10.59 (br. s., 1 H), 8.55 (s, 1 H), 7.24 (s, 1 H), 4.63 (s, 2 H), 3.60 - 3.76 (m, 14 H), 3.39 (t, *J* = 5.0 Hz, 2 H); LC-MS: RT (min) = 2.97; HRMS calculated for $C_{12}H_{22}N_5O_4$ (M + H) 300.1666, found 300.1669.



S4 **S5** **S6**

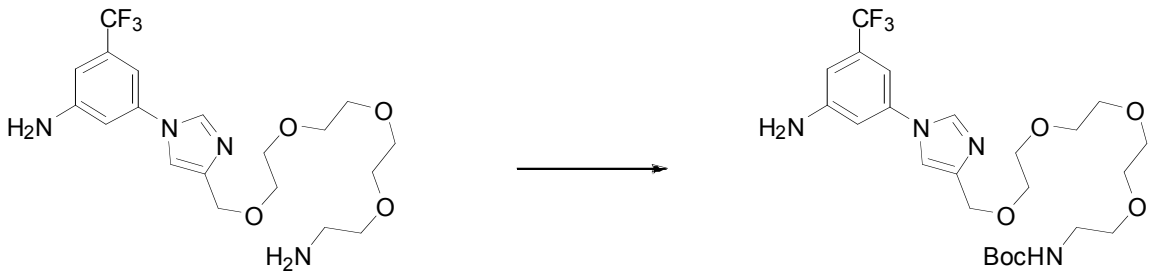
To a suspension of 4-(13-azido-2,5,8,11-tetraoxatridecyl)-1H-imidazole (**S4**, 3.76 g, 12.56 mmol), commercially-available 1-bromo-3-nitro-5-(trifluoromethyl)benzene (**S5**, 2.83 g, 10.47 mmol), and K_2CO_3 (2.60 g, 18.84 mmol) in DMF (6.98 mL) in an oven-dried microwave vial were added ethylenediamine (0.106 mL, 1.570 mmol) and copper(I) iodide (0.199 g, 1.047 mmol). Reaction was capped and heated to 110 °C for 24 h. After cooling, the reaction was diluted in EtOAc (100 mL) and brine (50mL). The aqueous layer was extracted with EtOAc (3 x 100 mL) and the combined organics were dried over anhydrous Na_2SO_4. Filtration and concentration *in vacuo* provided a crude oil that was purified via silica gel chromatography (gradient 0 to 20% MeOH/CH_2Cl_2) to provide **S6** (3.20 g, 63%) as an orange oil. An analytical sample was purified via reverse phase preparative HPLC (Method A).

4-(13-azido-2,5,8,11-tetraoxatridecyl)-1-(3-nitro-5-(trifluoromethyl)phenyl)-1H-imidazole (S6): 1H NMR (400 MHz, CHLOROFORM-*d*) δ ppm 8.57 (s, 2 H), 8.52 (br. s., 1 H), 8.11 (s, 1 H), 7.59 (br. s., 1 H), 4.68 (s, 2 H), 3.75 - 3.81 (m, 2 H), 3.70 - 3.75 (m, 2 H), 3.62 - 3.70 (m, 10 H), 3.36 (t, *J* = 4.99 Hz, 2 H); LC-MS: RT (min) = 4.59; HRMS calculated for $C_{19}H_{24}F_3N_6O_6$ (M + H) 489.1718, found 489.1720.



To an oven-dried round-bottom flask under nitrogen were added 4-(13-azido-2,5,8,11-tetraoxatridecyl)-1-(3-nitro-5-(trifluoromethyl)phenyl)-1H-imidazole (**S6**, 1.00 g, 2.047 mmol), anhydrous MeOH (10.24 mL), and $Pd(OH)_2$ (0.029 g, 0.205 mmol), sequentially. The reaction flask was fitted with a balloon of H_2 and was left to stir at ambient pressure. After 12 h, additional catalyst was added and a new balloon of H_2 was attached. After an additional 12 h, N_2 was bubbled through the solution for 5 min and Celite (~200 mg) and thiol-impregnated silica gel (~200 mg) were added. Filtration through Celite (MeOH wash) and concentration of the filtrate *in vacuo* provided crude **S7** (0.65 g, 74%) that was used in subsequent reactions without further purification. An analytical sample was purified via reverse phase preparative HPLC (Method A).

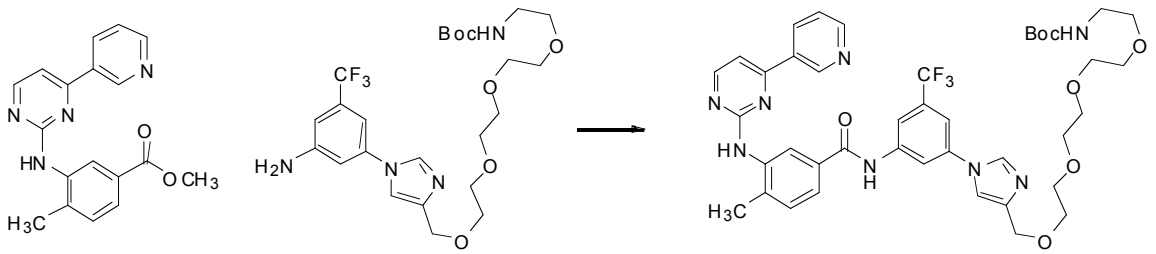
1-(1-(3-amino-5-(trifluoromethyl)phenyl)-1H-imidazol-4-yl)-2,5,8,11-tetraoxatridecan-13-amine (S7): 1H NMR (400 MHz, DMSO-d_6) δ ppm 8.95 (s, 1 H), 7.94 (s, 1 H), 7.90 (br. s, 2 H), 7.11 (s, 1 H), 7.04 (s, 1 H), 6.94 (s, 1 H), 4.50 (s, 2 H), 3.39 - 3.75 (m, 16 H), 2.97 (m, 2 H); LC-MS: RT (min) = 3.24; HRMS calculated for $C_{19}H_{28}F_3N_4O_4$ (M + H) 433.2057, found 433.2068.



S7 **S8**

To a solution of 1-(1-(3-amino-5-(trifluoromethyl)phenyl)-1H-imidazol-4-yl)-2,5,8,11-tetraoxatridecan-13-amine (**S7**, 0.600 g, 1.39 mmol) in MeOH (13.9 mL) at ambient temperature were added Et_3N (0.39 mL, 2.77 mmol) and Boc_2O (0.34 mL, 1.46 mmol). The reaction was allowed to stir for 4 h and was then diluted in EtOAc (50 mL) and H_2O (50 mL). The aqueous layer was extracted with EtOAc (3 x 50 mL) and the organic extracts were dried over anhydrous Na_2SO_4. Filtration and concentration *in vacuo* provided a crude orange oil that was purified via silica gel chromatography (gradient 0 to 20% MeOH/CH_2Cl_2) to provide **S8** (695 mg, 94%) as an orange oil. An analytical sample was purified via reverse phase preparative HPLC (Method A).

tert-butyl 1-(1-(3-amino-5-(trifluoromethyl)phenyl)-1H-imidazol-4-yl)-2,5,8,11-tetraoxatridecan-13-ylcarbamate (S8): 1H NMR (400 MHz, CHLOROFORM-*d*) δ ppm 8.53 (br. s., 1 H), 7.49 (s, 1 H), 6.89 - 7.03 (m, 3 H), 5.14 (br. s., 1 H), 4.69 (s, 2 H), 3.58 - 3.80 (m, 14 H), 3.52 (t, *J* = 5.1 Hz, 2 H), 3.29 (br. s., 2 H), 1.43 (s, 9 H); LC-MS: RT (min) = 4.48; HRMS calculated for $C_{24}H_{36}F_3N_4O_6$ (M + H) 533.2604, found 533.2603.

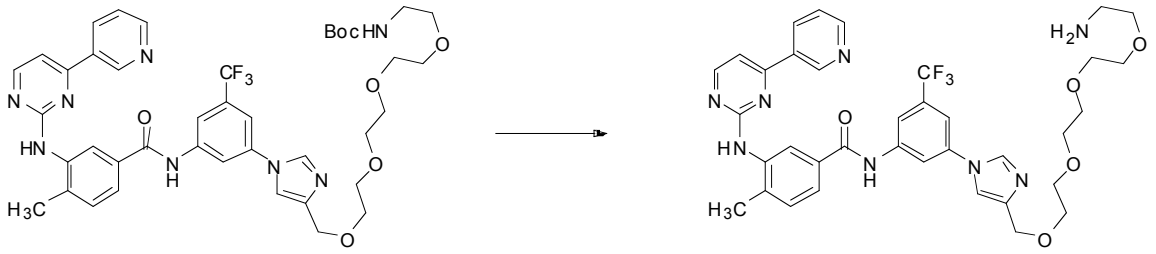


S9 **S8** **S10**

To an oven dried round-bottom flask equipped with a stir bar were added tert-butyl 1-(1-(3-amino-5-(trifluoromethyl)phenyl)-1H-imidazol-4-yl)-2,5,8,11-tetraoxatridecan-13-ylcarbamate (**S8**, 0.033 g, 0.062 mmol), THF (0.416 mL) and methyl 4-methyl-3-(4-(pyridin-3-yl)pyrimidin-2-ylamino)benzoate[2] (**S9**, 0.020 g, 0.062 mmol), sequentially. ~20 mg of 4Å molecular sieves were added and mixture was allowed to stir for 15 min at ambient temperature before being cooled to 0 °C. Potassium *tert*-butoxide (0.039 g, 0.343 mmol) was added in a single portion and the reaction was allowed to return to ambient temperature. After 4 h, brine (2 mL) was added and the aqueous layer was extracted with EtOAc (3 x 10 mL). The combined organic extracts were washed with brine (2 x 10 mL) and water (2 x 10 mL) and then dried over anhydrous Na_2SO_4. Filtration and concentration *in vacuo* provided a crude orange oil that was purified via reverse phase preparative HPLC (Method B) to provide **S10** (5.4 mg, 10.5%) as a fluffy pale orange powder.

tert-butyl 1-(1-(3-(4-methyl-3-(4-(pyridin-3-yl)pyrimidin-2-ylamino)benzamido)-5-(trifluoromethyl)phenyl)-1H-imidazol-4-yl)-2,5,8,11-tetraoxatridecan-13-ylcarbamate (S10): 1H NMR (400 MHz, CHLOROFORM-*d*) δ ppm 9.31 (s, 1 H), 8.97 (br. s., 1 H), 8.84 (s, 1 H), 8.70 (d, *J* = 3.9 Hz, 1 H), 8.54 (d, *J* = 5.3 Hz, 1 H), 8.39 (d, *J* = 8.0 Hz, 1 H), 8.19 (s, 1 H), 8.05 (s, 1 H), 7.95 (s, 1 H), 7.66 (d, *J* = 7.8 Hz, 1 H), 7.38 -

7.45 (m, 2 H), 7.33 - 7.38 (m, 2 H), 7.19 - 7.25 (m, 2 H), 5.19 (br. s., 1 H), 4.59 (s, 2 H), 3.54 - 3.83 (m, 14 H), 3.49 (t, *J* = 5.1 Hz, 2 H), 2.44 (s, 3 H), 1.37 - 1.49 (m, 9 H); LC-MS: RT (min) = 4.72; HRMS calculated for $C_{41}H_{48}F_3N_8O_7$ (M + H) 821.3593, found 821.3593.

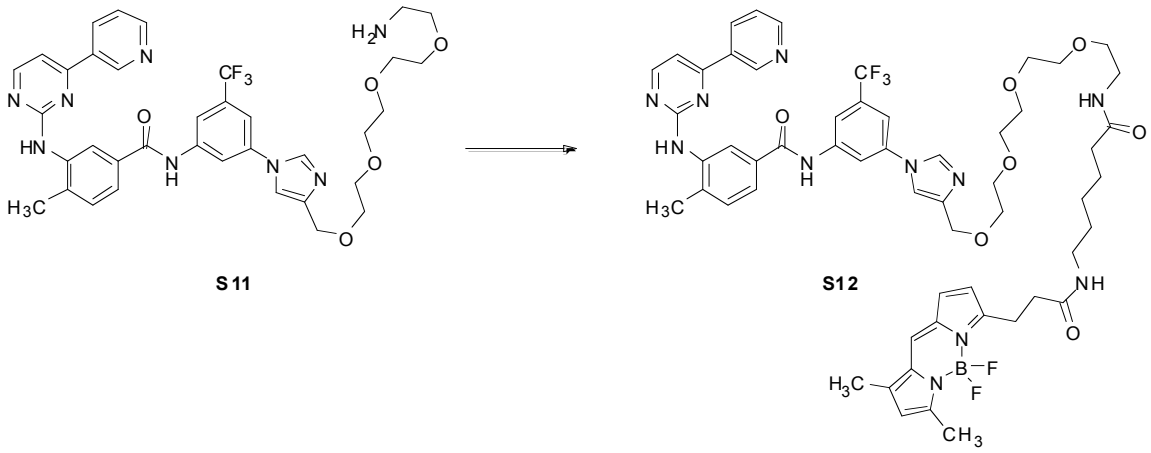


S10 **S11**

To a solution of tert-butyl 1-(1-(3-(4-methyl-3-(4-(pyridin-3-yl)pyrimidin-2-ylamino)benzamido)-5-(trifluoromethyl)phenyl)-1H-imidazol-4-yl)-2,5,8,11-tetraoxatridecan-13-ylcarbamate (**S10**, 0.005 g, 6.09 µmol) in CH_2Cl_2 (0.609 mL) at ambient temperature was added TFA (9.39 µL, 0.122 mmol). After 2 h, additional TFA (9.39 µL, 0.122 mmol) was added and the reaction was allowed to stir an additional 3 h. The reaction was concentrated *in vacuo* to provide **S11** (4.2 mg, 96%) as an orange oil that was used in subsequent reactions without further purification. An analytical sample was purified via reverse phase preparative HPLC (Method A).

N-(3-(4-(13-amino-2,5,8,11-tetraoxatridecyl)-1H-imidazol-1-yl)-5-(trifluoromethyl)phenyl)-4-methyl-3-(4-(pyridin-3-yl)pyrimidin-2-ylamino)benzamide (S11): 1H NMR (400 MHz, DMSO-d_6) δ ppm 10.78 (s, 1 H), 9.31 (br. s., 1 H), 9.25 (s, 1 H), 9.21 (s, 1 H), 8.73 (d, *J* = 4.11 Hz, 1 H), 8.49 - 8.62 (m, 2 H), 8.33 (s, 1 H), 8.23 (s, 1 H), 8.12 (s, 1 H), 7.90 (s, 1 H), 7.76 – 7.84 (m, 2 H), 7.61 (dd, *J* =

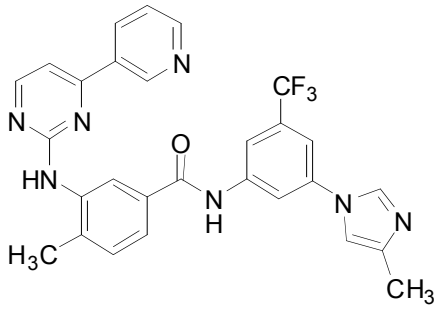
7.83, 4.89 Hz, 1 H), 7.41 - 7.54 (m, 2 H), 4.56 (s, 2 H), 3.43 - 3.70 (m, 16 H), 2.88 - 3.04 (m, 2 H), 2.36 (s, 3 H); LC-MS: RT (min) = 3.86; HRMS calculated for $C_{36}H_{40}F_3N_8O_5$ (M + H) 721.3091, found 721.3090.



A solution of 6-((4,4-difluoro-5,7-dimethyl-4-bora-3a,4a-diaza-s-indacene-3-propionyl)amino)hexanoic acid, succinimidyl ester (BODIPY® FL-X, SE, 5.0 mg, 11 μmol) in DMF (0.2 mL) was added to a solution of N-(3-(4-(13-amino-2,5,8,11-tetraoxatridecyl)-1H-imidazol-1-yl)-5-(trifluoromethyl)phenyl)-4-methyl-3-(4-(pyridin-3-yl)pyrimidin-2-ylamino) benzamide (**S12**, 8.0 mg, 9.95 μmol) in DMF (0.2 mL) in a foil-covered vial. The reaction solution was stirred at room temperature for 20 minutes, followed by immediate purification via reverse phase preparative HPLC (Method A) to provide **S12** (5.0 mg, 42%).

5,5-difluoro-7,9-dimethyl-3-(1-(1-(3-(4-methyl-3-(4-(pyridin-3-yl)pyrimidin-2-ylamino)benzamido)-5-(trifluoromethyl)phenyl)-1*H*-imidazol-4-yl)-15,22-dioxo-2,5,8,11-tetraoxo-14,21-diazatetracosan-24-yl)-5*H*-dipyrrolo[1,2-*c*:1',2'-*f*][1,3,2]diazaborinin-4-ium-5-uide (**S12**, BODIPY® FL Tasigna): ^{1}H NMR (d6-DMSO,

400 MHz) δ 10.73 (s, 1H), 9.29 (s, 1H), 9.19 (app. s, 2H), 8.70 (d, *J* = 3.91 Hz, 1H), 8.55 (d, *J* = 5.09 Hz, 1H), 8.52 (s, 1H), 8.49 (dd, *J* = 8.02, 1.76 Hz, 1H), 8.32 (s, 1H), 8.22 (s, 1H), 8.09 (s, 1H), 7.85-7.90 (m, 2H), 7.76-7.81 (m, 2H), 7.67 (s, 1H), 7.55 (dd, *J* = 8.02, 4.89 Hz, 1H), 7.49 (d, *J* = 5.09 Hz, 1H), 7.46 (d, *J* = 8.22 Hz, 1H), 7.07 (d, *J* = 3.91 Hz, 1H), 6.32 (d, *J* = 3.91 Hz, 1H), 6.28 (s, 1H), 4.54 (s, 2H), 3.60-3.64 (m, 2H), 3.55-3.58 (m, 2H), 3.47-3.52 (m, 8H), 3.36 (t, *J* = 6.06 Hz, 2H), 3.16 (q, *J* = 5.87 Hz, 2H), 3.00-3.08 (m, 4H), 2.43-2.47 (app. t, 2H), 2.45 (s, 3H), 2.36 (s, 3H), 2.25 (s, 3H), 2.03 (t, *J* = 7.43 Hz, 2H), 1.45 (q, *J* = 7.43 Hz, 2H), 1.36 (q, *J* = 7.43 Hz, 2H), 1.24-1.18 (m, 2H); LC-MS: RT (min) = 5.08; $[M/2]^+$ 554.5; HRMS calculated for $C_{56}H_{64}BF_5N_{11}O_7$ (M + H) 1108.4925, found 1108.4961.



S13

The synthesis of Tasigna (**S13**) was accomplished according to published methods.[2]

4-methyl-*N*-(3-(4-methyl-1*H*-imidazol-1-yl)-5-(trifluoromethyl)phenyl)-3-(4-(pyridine-3-yl)pyrimidin-2-ylamino)benzamide (**S13**, Tasigna, **AMN-107**): ^{1}H NMR (d6-DMSO, 400 MHz) δ 10.61 (s, 1H), 9.28 (d, *J* = 1.57 Hz, 1H), 9.16 (s, 1H), 8.67 (d, *J* = 3.52 Hz, 1H), 8.54 (d, *J* = 5.48 Hz, 1H), 8.44 (ddd, *J* = 8.22, 1.96, 1.57 Hz, 1H), 8.32 (d, *J* = 1.96 Hz, 1H), 8.29 (t, *J* = 1.96 Hz, 1H), 8.20 (s, 1H), 8.15 (s, 1H), 7.76 (dd, *J* = 7.83, 1.57 Hz, 1H), 7.71 (s, 1H), 7.45-7.53 (m, 4H), 2.36 (s, 3H), 2.17 (s, 3H); LC-MS:

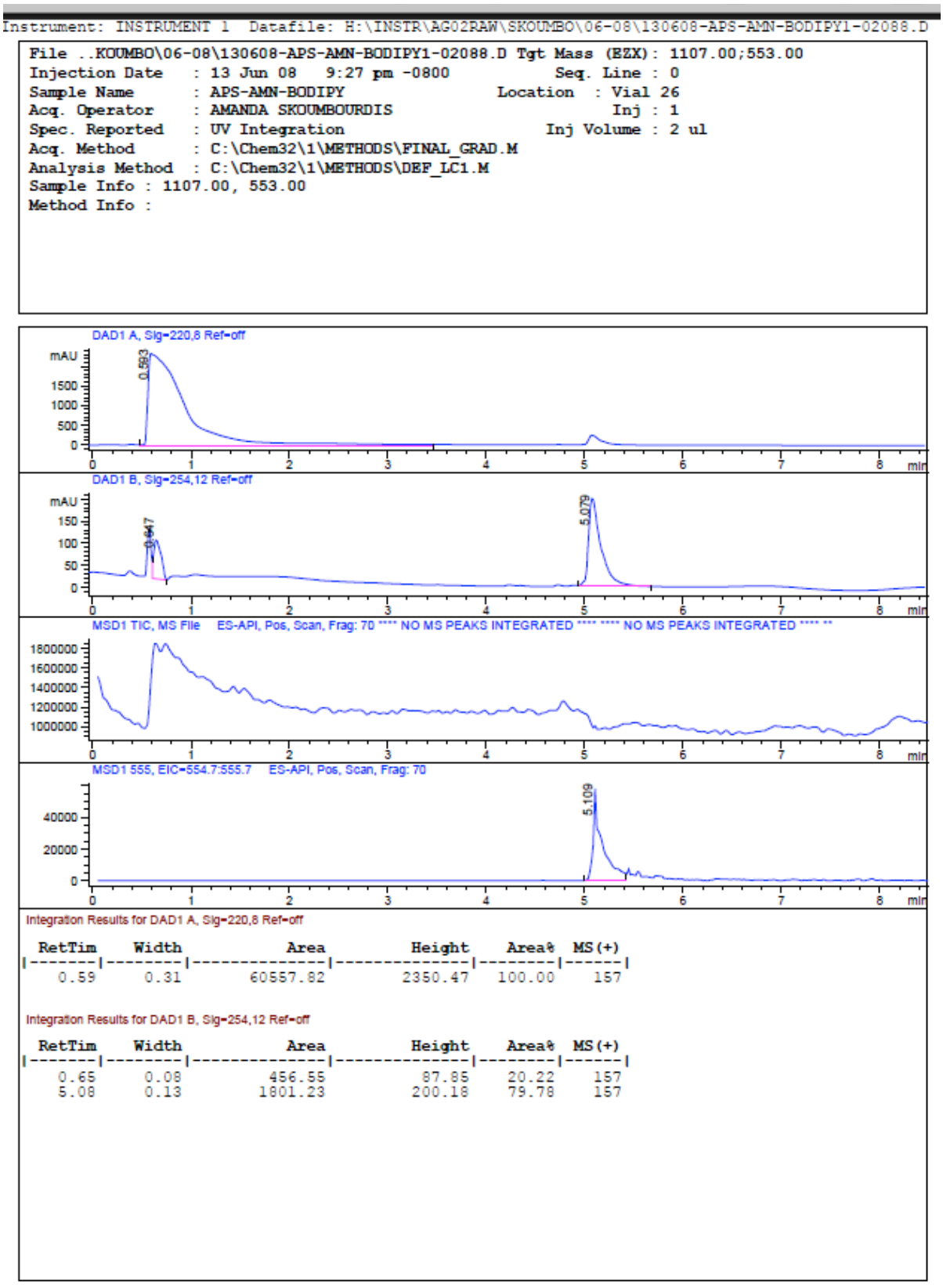
RT (min) = 4.27; $[M + H]^+$ 530.0; HRMS calculated for $C_{28}H_{23}F_3N_7O$ (M + H) 530.1838, found 530.1917.

References:

1) Svedhem, S.; Hollander, C.-A.; Shi, J.; Konradsson, P.; Liedberg, B.; Svensson, S. C. T. *J. Org. Chem.* **2001**, *66*, 4494–4503.

2) Wei-Sheng, H., and William, C. S. (2007) An Efficient Synthesis of Nilotinib (AMN107), *Synthesis 14*, 2121–2124.

Analytical trace demonstrating purity of Bodipy FL Tasigna:



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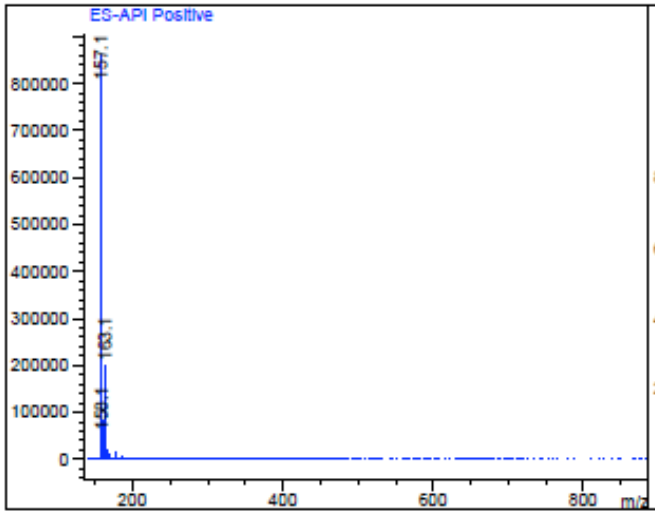

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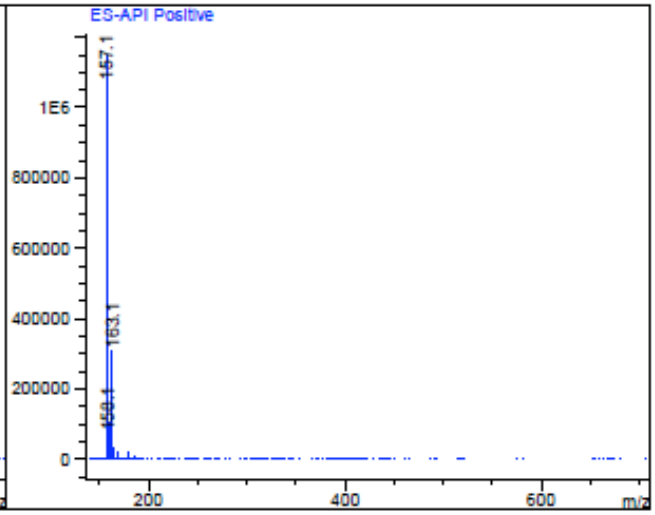

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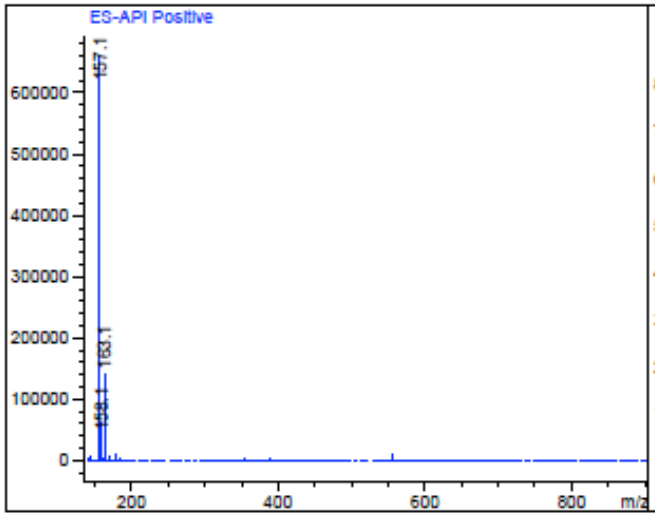

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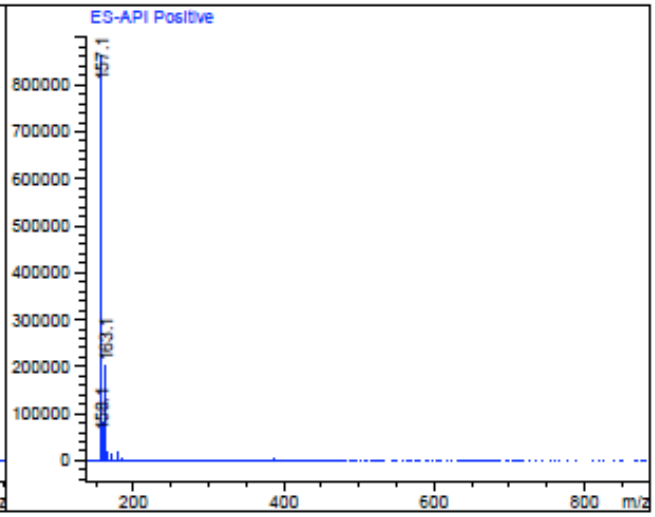

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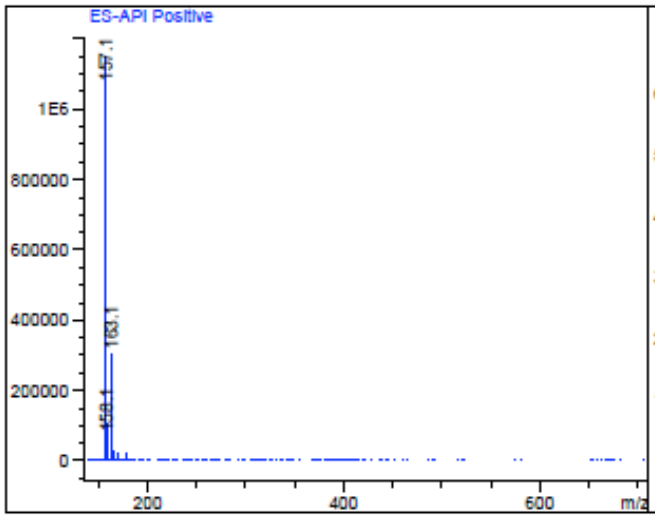

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