

# Supporting information

## Novel Peptidomimetic Hepatitis C Virus NS3/4A Protease Inhibitors Spanning the P2-P1' Region

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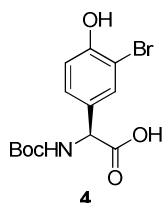
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## Experimental section

### General chemistry information

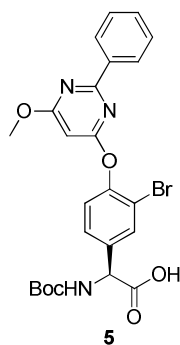
Reagents and solvents were obtained commercially and used without further purification. Thin layer chromatography (TLC) was performed on aluminium sheets precoated with silica gel 60 F<sub>254</sub> (0.2 mm). Chromatographic spots were visualized using UV-detection and/or 2% ninhydrin in ethanol solution followed by heating. Column chromatography was performed using silica gel 60 (40-63  $\mu$ m). Analytical HPLC-MS was performed on a Gilson-Finnigan ThermoQuest AQA system equipped with a C18 (Onyx Monolithic C18 (50  $\times$  4.6 mm)) or a C4 (Hichrom ACE C4 (5  $\mu$ m, 50  $\times$  4.6 mm)) column using MeCN/H<sub>2</sub>O (0.05% HCOOH) or MeOH/H<sub>2</sub>O (0.05% HCOOH) with UV (254 nm) and MS (ESI) detection or on a Dionex UltiMate 3000 equipped with a C18 (Phenomenex Kinetex C18 (50  $\times$  3.00 mm) column using MeCN/H<sub>2</sub>O (0.05% HCOOH) UV (254 nm) and MS (ESI IonTrap) detection or on a manual system equipped with a C8 (Zorbax SB-C8 (4.8  $\times$  50 mm)) column using UV (220 nm) detection. Preparative HPLC was performed on a Gilson-Finigan ThermoQuest AQA system equipped with a C8 (Zorbax SB-C8 (5  $\mu$ m, 150  $\times$  21.2 mm)) column using MeCN/H<sub>2</sub>O (0.05% HCOOH) as the mobile phase with UV (254 nm) detection or on a manual system equipped with a C8 (Zorbax SB-C8 (5  $\mu$ m, 150  $\times$  21.2 mm)) column using UV detection and MeCN/H<sub>2</sub>O (0.05% HCOOH) or MeCN/H<sub>2</sub>O with 25mM NH<sub>4</sub>OAc, pH 6.3 as the mobile phase. Purity determinations were done by RP-HPLC using the following conditions (UV detection at 254 nm): System 1 (ACE 5 C8, 50  $\times$  4.6 mm, MeCN/H<sub>2</sub>O with 0.1% TFA) and system 2 (Restec Allure Biphenyl, 50  $\times$  4.6 mm, MeCN/H<sub>2</sub>O with 0.1% TFA). Microwave reactions were carried out in a SmithSyntheSizer<sup>TM</sup> or in an Initiator<sup>TM</sup> single-mode microwave cavity producing controlled irradiation at 2450 MHz. NMR spectra were recorded on a Varian Mercury plus spectrometer (<sup>1</sup>H at 399.8 MHz, <sup>13</sup>C at 100.5 MHz) at ambient temperature. Chemical shifts ( $\delta$ ) are reported in ppm, indirectly referenced to tetrametylsilane (TMS) via the solvent signal (<sup>1</sup>H: CHCl<sub>3</sub>  $\delta$  7.26, CD<sub>2</sub>HOD  $\delta$  3.31; <sup>13</sup>C: CDCl<sub>3</sub>  $\delta$  77.16, CD<sub>3</sub>OD  $\delta$  49.00). Exact molecular masses were determined on Micromass Q-Tof2 mass spectrometer equipped with an electrospray ion source.

#### Compound 4 ((S)-2-(3-bromo-4-hydroxyphenyl)-2-((tert-butoxycarbonyl)amino)acetic acid).



Prepared as described by us previously.<sup>1</sup>

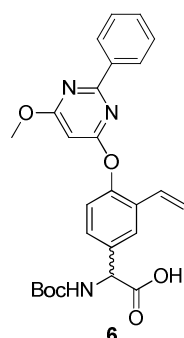
#### Compound 5 ((S)-2-(3-bromo-4-((6-methoxy-2-phenylpyrimidin-4-yl)oxy)phenyl)-2-((tert-butoxycarbonyl)amino)acetic acid).



Synthesis of the P2 substituent 4-chloro-6-methoxy-2-phenylpyrimidine<sup>2</sup> was performed by refluxing commercially available 4,6-dichloro-2-phenylpyrimidine with sodium methoxide in methanol until formation of the byproduct 4,6-dimethoxy-2-phenylpyrimidine was starting to be observed.

**4** (2.0 g, 5.7 mmol), 4-chloro-6-methoxy-2-phenylpyrimidine (1.9 g, 8.6 mmol) and KO<sup>t</sup>Bu (1.5 g, 2.2 mmol) were dried over night in vacuo over P<sub>2</sub>O<sub>5</sub>. Dry DMSO (H<sub>2</sub>O < 0.005%) (40 mL) was added to a vial containing **1** and KO<sup>t</sup>Bu. The vial was sealed under nitrogen and stirred for 40 min before 4-chloro-6-methoxy-2-phenylpyrimidine dissolved in DMSO (40 mL) was added. The reaction was heated at 64 °C for 2 days. The reaction mixture was filtered, diluted with water (500 mL), and extracted with petroleum ether. The aqueous phase was acidified with 1 M HCl to pH 3. Filtration of the precipitate yielded **5** (2.6 g, 87%) as a white solid. <sup>1</sup>H NMR (CD<sub>3</sub>OD) δ 8.17 (m, 2H), 7.78 (d, *J* = 2.2 Hz, 1H), 7.51 (dd, *J* = 2.1, 8.3 Hz, 1H), 7.42 (m, 1H), 7.38-7.33 (m, 2H), 7.29 (d, *J* = 8.4 Hz, 1H), 6.14 (s, 1H), 5.28 (s, 1H), 4.07 (s, 3H), 1.47 (s, 9H). <sup>13</sup>C NMR (CDCl<sub>3</sub>) δ: 172.8, 172.3, 170.4, 163.9, 156.9, 150.1, 137.3, 136.9, 132.4, 131.1, 128.5, 128.4, 127.3, 124.1, 116.7, 89.1, 82.4, 58.1, 54.3, 28.3. MS [M+H]<sup>+</sup>: 530.1, 532.1. MS calcd for C<sub>24</sub>H<sub>24</sub>BrN<sub>3</sub>O<sub>6</sub> [M+H]<sup>+</sup> 530.1, 532.1, found: 530.1, 532.1.

**Compound 6 (2-((*tert*-butoxycarbonyl)amino)-2-(4-((6-methoxy-2-phenylpyrimidin-4-yl)oxy)-3-vinylphenyl)acetic acid).**

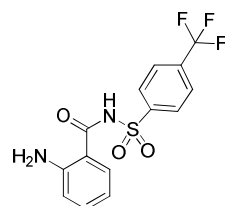


**5** (705.3 mg, 1.33 mmol), 2,4,6-trivinylcycloboroxane pyridine complex (591.5 mg, 2.66 mmol), Pd(OAc)<sub>2</sub> (32.2 mg, 0.133 mmol), [(*t*Bu)<sub>3</sub>PH]BF<sub>4</sub> (77 mg, 0.266 mmol), K<sub>2</sub>CO<sub>3</sub> (1.11 g, 8.1 mmol), DME (26.6 mL) and water (8 mL) were mixed in seven 5 mL microwave vials. The vials were sealed and exposed to microwave heating at 100 °C for 15 min. After centrifugal filtration, and pooling of the reaction mixtures, the DME was evaporated. Water (35 mL) was added and the reaction mixture was acidified with 5% HCl to pH 1. Filtration of the precipitate yielded **6** (518 mg, 82%) as a white solid. <sup>1</sup>H NMR (CD<sub>3</sub>OD) δ: 8.26-8.22 (m, 2H), 7.78 (d, *J* = 2.3 Hz, 1H), 7.47-7.36 (m, 4H), 7.15 (d, *J* = 8.3 Hz, 1H), 6.79 (dd, *J* = 11.4, 17.4 Hz, 1H), 6.00 (s, 1H), 5.86 (dd, *J* = 1.3, 17.8 Hz, 1H), 5.28 (dd, *J* = 1.3, 11.4 Hz, 1H), 5.20 (s, 1H), 4.06 (s, 3H), 1.46 (s, 9H). <sup>13</sup>C NMR (CD<sub>3</sub>OD) δ: 174.6, 173.8, 172.7, 165.3, 157.5, 151.0, 138.1, 137.5, 132.1, 132.0, 131.5, 129.3 (2 carbons), 129.1, 126.9, 123.8, 117.1, 89.5, 80.8, 59.3, 54.7, 28.7. MS calcd for C<sub>26</sub>H<sub>27</sub>N<sub>3</sub>O<sub>6</sub> [M+H]<sup>+</sup> 478.2, found: 478.2.

**General procedure A, for synthesis of compounds 7-9**

In a 5 mL microwave process vial, Herrmann's palladacycle<sup>4</sup>, [(*t*Bu)<sub>3</sub>PH]BF<sub>4</sub><sup>3</sup>, Mo(CO)<sub>6</sub>, 4-(trifluoromethyl)-benzene sulfonamide, bromoaniline and 1,4-dioxane was mixed. DBU was added just before the vial was sealed and irradiated by microwaves at 140 °C for 25 min. After cooling to room temperature a small amount of CH<sub>2</sub>Cl<sub>2</sub> was added, and the reaction mixture filtered before the solvents were evaporated.

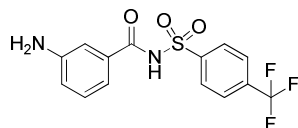
**Compound 7 (2-amino-*N*-((4-(trifluoromethyl)phenyl)sulfonyl)benzamide).**



Prepared according to the general procedure A described above using Herrmann's palladacycle (37.8 mg, 0.040 mmol), [(*t*Bu)<sub>3</sub>PH]BF<sub>4</sub> (24.5 mg, 0.084 mmol), Mo(CO)<sub>6</sub> (212 mg, 0.80 mmol), 4-(trifluoromethyl)-benzene sulfonamide (546 mg, 2.42 mmol), 2-bromoaniline (88 μL, 0.81 mmol), 1,4-dioxane (3 mL) and DBU (360 μL,

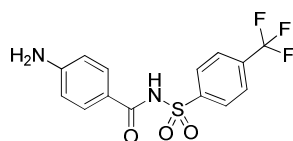
2.4 mmol). Purification on silica gel (CH<sub>2</sub>Cl<sub>2</sub>/MeOH 93:7 then CH<sub>2</sub>Cl<sub>2</sub>/MeOH 90:10 yielded **7** (136.3 mg, 50%) as a white solid. <sup>1</sup>H NMR (CDCl<sub>3</sub>/CD<sub>3</sub>OD): δ 8.17 (d, *J* = 8.4 Hz, 2H), 7.76-7.69 (m, 3H), 7.17 (ddd, *J* = 8.9, 7.2, 1.6 Hz, 1H), 6.64 (dd, *J* = 8.2, 1.0 Hz, 1H), 6.59 (ddd, *J* = 8.2, 7.3, 1.2 Hz, 1H). <sup>13</sup>C NMR (CDCl<sub>3</sub>/CD<sub>3</sub>OD): δ 171.8, 150.8, 145.9, 134.3 (q, *J* = 33.1 Hz), 134.1, 130.7, 128.7, 126.1 (q, *J* = 3.8 Hz), 124.0 (q, *J* = 270.2 Hz), 117.8, 116.8, 115.2. MS calcd for C<sub>14</sub>H<sub>11</sub>F<sub>3</sub>N<sub>2</sub>O<sub>3</sub>S [M+H]<sup>+</sup> 345.0, found: 345.1.

**Compound 8 (3-amino-*N*-((4-(trifluoromethyl)phenyl)sulfonyl)benzamide).**



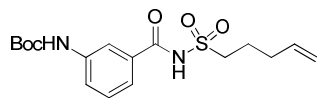
Prepared according to the general procedure A using Herrmann's palladacycle (37.5 mg, 0.04 mmol), [(*t*Bu)<sub>3</sub>PH]BF<sub>4</sub> (23.2 mg, 0.079 mmol), Mo(CO)<sub>6</sub> (211.2 mg, 0.80 mmol), 4-(trifluoromethyl)-benzene sulfonamide (541.2 mg, 2.40 mmol), 3-bromoaniline (88 μL, 0.81 mmol), 1,4-dioxane (3 mL) and DBU (360 μL, 2.4 mmol). Purification on silica gel (CH<sub>2</sub>Cl<sub>2</sub>/MeOH 97:3 then CH<sub>2</sub>Cl<sub>2</sub>/MeOH 93:7 then CH<sub>2</sub>Cl<sub>2</sub>/MeOH 90:10) followed by purification on aluminium oxide (CH<sub>2</sub>Cl<sub>2</sub>/MeOH 95:5 then CH<sub>2</sub>Cl<sub>2</sub>/MeOH/HCOOH 95:5:1) yielded **8** (176 mg, 64%) containing a small aliphatic impurity visible on NMR. <sup>1</sup>H NMR (CD<sub>3</sub>OD): δ 8.18 (d, *J* = 8.3 Hz, 2H), 7.73 (d, *J* = 8.3 Hz, 2H), 7.41-7.35 (m, 2H), 7.09 (t, *J* = 7.8 Hz, 1H), 6.85 (ddd, *J* = 1.0, 2.4, 7.9 Hz, 1H). <sup>13</sup>C NMR (CD<sub>3</sub>OD): δ 174.8, 148.4, 147.9, 138.5, 134.0 (q, *J* = 32.6 Hz), 129.7, 128.7, 126.5 (q, *J* = 3.7 Hz), 125.0 (q, *J* = 271.5 Hz), 120.1 (2 carbons), 117.0. MS calcd for C<sub>14</sub>H<sub>11</sub>F<sub>3</sub>N<sub>2</sub>O<sub>3</sub>S [M+H]<sup>+</sup> 345.0, found: 345.1.

**Compound 9 (4-amino-*N*-((4-(trifluoromethyl)phenyl)sulfonyl)benzamide).**

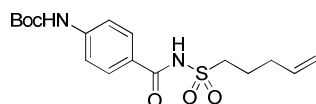


Prepared according to the general procedure A above using Herrmann's palladacycle (37.9 mg, 0.04 mmol), [(*t*Bu)<sub>3</sub>PH]BF<sub>4</sub> (23.9 mg, 0.082 mmol), Mo(CO)<sub>6</sub> (213 mg, 0.81 mmol), 4-(trifluoromethyl)-benzene sulfonamide (541.1 mg, 2.40 mmol), 4-bromoaniline (138 mg, 0.80 mmol), 1,4-dioxane (3 mL) and DBU (360 μL, 2.4 mmol). Purification was performed on silica gel (CH<sub>2</sub>Cl<sub>2</sub>/MeOH 97:3) and yielded **9** (171 mg, 62%) as a white solid. <sup>1</sup>H NMR (CD<sub>3</sub>OD): δ 8.15 (d, *J* = 8.1 Hz, 2H), 7.76-7.73 (m, 4H), 6.59 (m, 2H). <sup>13</sup>C NMR (CD<sub>3</sub>OD): δ 174.9, 153.2, 149.2, 133.7 (q, *J* = 32.9 Hz), 131.9, 128.9, 126.3 (q, *J* = 3.8 Hz), 126.0, 125.2 (q, *J* = 272.9 Hz), 114.4. MS calcd for C<sub>14</sub>H<sub>11</sub>F<sub>3</sub>N<sub>2</sub>O<sub>3</sub>S [M+H]<sup>+</sup> 345.0, found: 345.1.

**Compound 10 (tert-butyl (3-((pent-4-en-1-ylsulfonyl)carbamoyl)phenyl)carbamate).**



Boc-3-amino benzoic acid (237 mg, 1.0 mmol) was dried in vacuo over P<sub>2</sub>O<sub>5</sub> over night. CDI (324 mg, 2.0 mmol) and THF (20 mL) was added under N<sub>2</sub> atmosphere. The reaction mixture was stirred at 65 °C for 2 h. Thereafter, pent-4-ene sulfonamide (270 mg, 2.0 mmol) dissolved in THF (2mL) and DBU (450 μL, 3.0 mmol) was added and the reaction mixture was stirred at room temperature for 3 h. After evaporation of the solvent CH<sub>2</sub>Cl<sub>2</sub> (25 mL) was added and washed with 5% citric acid and brine. Evaporation of the organic phase followed by purification on silica gel (CH<sub>2</sub>Cl<sub>2</sub>/MeOH 96:4) followed by aluminium oxide (CH<sub>2</sub>Cl<sub>2</sub>/MeOH 94:6 then CH<sub>2</sub>Cl<sub>2</sub>/MeOH/HCOOH 95:5:1) yielded **10** (261 mg, 71%) as a white solid. <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ 7.77 (t, *J* = 1.9 Hz, 1H), 7.56 (ddd, *J* = 1.2, 1.8, 7.7 Hz, 1H), 7.41 (ddd, *J* = 1.2, 2.2, 8.1 Hz, 1H), 7.34 (m, 1H), 5.71 (m, 1H), 5.05-4.97 (m, 2H), 3.52-3.47 (m, 2H), 2.21-2.14 (m, 2H), 1.96-1.87 (m, 2H), 1.48 (s, 9H). <sup>13</sup>C NMR (CDCl<sub>3</sub>): δ 165.4, 152.8, 139.3, 136.2, 132.2, 129.9, 123.6, 122.7, 117.7, 116.9, 81.6, 53.0, 32.0, 28.4, 22.5. MS calcd for C<sub>17</sub>H<sub>24</sub>N<sub>2</sub>O<sub>5</sub>S [M+H]<sup>+</sup> 369.1, found: 369.3

**Compound 11 (tert-butyl (4-((pent-4-en-1-ylsulfonyl)carbamoyl)phenyl)carbamate)**

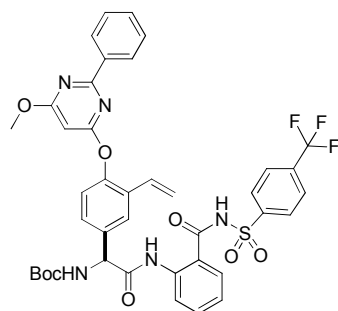
Boc-4-amino benzoic acid (237 mg, 1.0 mmol) was dried in vacuo over P<sub>2</sub>O<sub>5</sub> over night. CDI (324 mg, 2.0 mmol) and THF (20 mL) was added under N<sub>2</sub> atmosphere. The reaction was stirred at 65 °C for 2.5 h before addition of pent-4-ene sulfonamide (270 mg, 2.0 mmol) dissolved in THF (2 mL) and DBU (450 μL, 3.0 mmol). Thereafter, the reaction was stirred at room temperature for 3 days. The solvent was evaporated, CH<sub>2</sub>Cl<sub>2</sub> (25 mL) added and the mixture washed with 5% citric acid and brine. After evaporation of the organic phase purification was performed on silica gel (CH<sub>2</sub>Cl<sub>2</sub>/MeOH 95:5) and aluminium oxide (CH<sub>2</sub>Cl<sub>2</sub>/MeOH 95:5 then CH<sub>2</sub>Cl<sub>2</sub>/MeOH/HCOOH 95:5:1) yielded **11** (254 mg, 69%) as a white solid. <sup>1</sup>H NMR (CD<sub>3</sub>OD): δ 7.85-7.82 (m, 2H), 7.58-7.54 (m, 2H), 5.80 (m, 1H), 5.10-5.00 (m, 2H), 3.55-3.49 (m, 2H), 2.26-2.19 (m, 2H), 1.96-1.87 (m, 2H), 1.53 (s, 9H). <sup>13</sup>C NMR (CD<sub>3</sub>OD): δ 168.1, 154.6, 145.9, 138.0, 130.6, 126.5, 118.7, 116.6, 81.5, 53.4, 32.9, 28.6, 23.8. MS calcd for C<sub>17</sub>H<sub>24</sub>N<sub>2</sub>O<sub>5</sub>S [M+H]<sup>+</sup> 369.1, found: 369.2.

**General procedure B, for Boc deprotection of compounds 10-11 and 12-16**

The respective carbamate was dissolved in 4.0 M HCl in 1,4 dioxane (10 mL/mmol starting material) and stirred at room temperature until starting material could no longer be visualized by LC-MS. The solvent was thereafter evaporated and the hydrochloride salt of the *N*-deprotected product was used in forthcoming couplings without further purification.

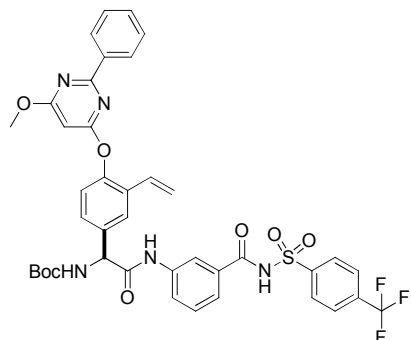
**General procedure C, for the preparation of compounds 12-16**

Building blocks **7-9** and the *N*-deprotected HCl salts of compounds **10-11** were mixed with P2 building block **6** and HATU in dry CH<sub>2</sub>Cl<sub>2</sub>. DIEA was added until the pH exceeded 10. The reactions were stirred at 45 °C. When starting material no longer could be visualized by LC-MS, CH<sub>2</sub>Cl<sub>2</sub> was added and the reaction mixture was washed with 0.1M NaHSO<sub>4</sub> followed by 0.035M NaHSO<sub>4</sub>. The organic phase was thereafter evaporated.

**Compound 12 (tert-butyl (1-(4-(((6-methoxy-2-phenylpyrimidin-4-yl)oxy)-3-vinylphenyl)-2-oxo-2-((4-(trifluoromethyl)phenyl)sulfonyl)carbamoyl)phenyl)amino)ethyl)carbamate).**

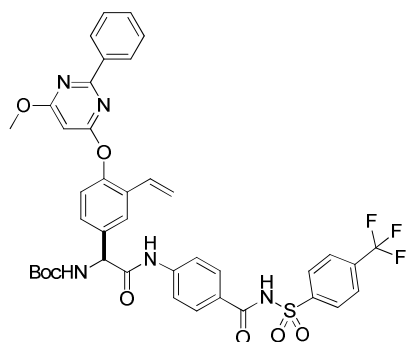
Prepared according to the general procedure C, described above using **6** (70 mg, 0.14 mmol), **7** (62 mg, 0.23 mmol), HATU (68 mg, 0.18 mmol), and DIEA (120 μL, 0.67 mmol). The reaction was stirred in CH<sub>2</sub>Cl<sub>2</sub> for 2h. Purification was performed on silica gel (CH<sub>2</sub>Cl<sub>2</sub>/MeOH 90:10) and yielded **12** (84.8 mg, 83%) as a white solid. <sup>1</sup>H NMR (CD<sub>3</sub>OD): δ 8.53 (d, *J* = 8.6 Hz, 1H), 8.14-8.07 (m, 5H), 7.96 (s, 1H), 7.64-7.57 (m, 3H), 7.38 (t, *J* = 7.4 Hz, 1H), 7.30 (dt, *J* = 1.4, 7.4 Hz, 1H), 7.20 (t, *J* = 7.4 Hz, 2H), 7.13 (d, 8.4 Hz, 1H), 7.03 (t, *J* = 7.4 Hz, 1H), 6.75 (dd, *J* = 11.0, 17.6 Hz, 1H), 6.02 (s, 1H), 5.89 (d, *J* = 17.6 Hz, 1H), 5.39 (s, 1H), 5.21 (d, 11.0 Hz, 1H), 4.01 (s, 3H), 1.47 (s, 9H). <sup>13</sup>C NMR (CD<sub>3</sub>OD): δ 173.7, 172.5, 171.1, 165.1, 157.5, 151.3, 148.9, 140.9, 137.9, 136.6, 133.6 (q, *J* = 32.3 Hz), 133.1, 132.3, 132.1, 132.0, 131.2, 129.2 (3 carbons), 128.9, 127.1, 126.4 (q, *J* = 3.9 Hz), 125.1 (q, *J* = 271.0), 124.9, 124.3, 123.8, 120.8, 117.7, 89.5, 81.1, 61.9, 54.7, 28.7. HRMS calcd for C<sub>40</sub>H<sub>36</sub>F<sub>3</sub>N<sub>5</sub>O<sub>8</sub>S [M+H]<sup>+</sup> 804.2315, found: 804.2313. HPLC purity (system 1: 99.4%, system 2: 98.1%).

**Compound 13** (*tert*-butyl (1-(4-((6-methoxy-2-phenylpyrimidin-4-yl)oxy)-3-vinylphenyl)-2-oxo-2-((3-(((4-(trifluoromethyl)phenyl)sulfonyl)carbamoyl)phenyl)amino)ethyl)carbamate).



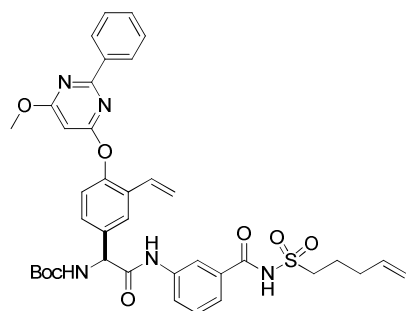
Prepared according to the general procedure C, using **6** (105 mg, 0.22 mmol), **8** (111 mg, 0.32 mmol), HATU (126 mg, 0.33 mmol), and DIEA (169  $\mu$ L, 0.97 mmol). The reaction was stirred in  $\text{CH}_2\text{Cl}_2$  for 3h. Purification on silica gel ( $\text{CH}_2\text{Cl}_2/\text{MeOH}$  95:5) yielded **13** (88 mg, 50%) as a white solid.  $^1\text{H}$  NMR ( $\text{CD}_3\text{OD}$ ):  $\delta$  8.21-8.12 (m, 4H), 8.06 (s, 1H), 7.86 (d,  $J = 2.2$  Hz, 1H), 7.83-7.77 (m, 3H), 7.68 (m, 1H), 7.51 (dd,  $J = 2.1, 7.5$  Hz, 1H), 7.37-7.31 (m, 3H), 7.28-7.23 (m, 2H), 7.16 (d,  $J = 8.4$  Hz, 1H), 6.78 (dd,  $J = 11.2, 17.7$  Hz, 1H), 6.02 (s, 1H), 5.85 (d,  $J = 17.7$  Hz, 1H), 5.27 (d,  $J = 11.2$  Hz, 1H), 4.03 (s, 3H), 1.46 (s, 9H).  $^{13}\text{C}$  NMR, containing small aliphatic impurities ( $\text{CD}_3\text{OD}$ ):  $\delta$  173.7, 172.6, 172.0, 171.2, 165.2, 157.5, 151.3, 147.7, 139.5, 138.0, 137.6, 136.9, 134.4 (q,  $J = 33.4$  Hz), 132.3, 132.1, 131.5, 129.8, 129.4, 129.2 (2 carbons), 129.1, 127.1, 126.6 (q,  $J = 3.9$  Hz), 125.6, 125.2, 124.8, 124.2, 121.4, 117.3, 89.6, 81.0, 60.1, 54.7, 28.7. HRMS calcd for  $\text{C}_{40}\text{H}_{36}\text{F}_3\text{N}_5\text{O}_8\text{S}$   $[\text{M}+\text{H}]^+$  804.2315, found: 804.2328.

**Compound 14** (*tert*-butyl (1-(4-((6-methoxy-2-phenylpyrimidin-4-yl)oxy)-3-vinylphenyl)-2-oxo-2-((4-(((4-(trifluoromethyl)phenyl)sulfonyl)carbamoyl)phenyl)amino)ethyl)carbamate).



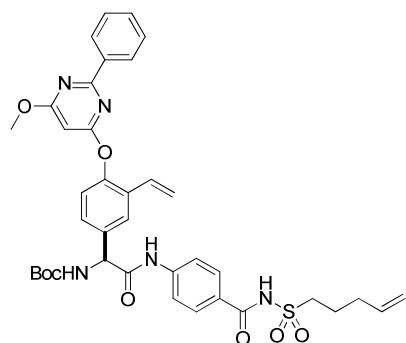
Prepared according to the general procedure C, using **6** (95.5 mg, 0.20 mmol), **9** (110 mg, 0.32 mmol), HATU (91.7 mg, 0.24 mmol), and DIEA (154  $\mu$ L, 0.88 mmol). The reaction was stirred in  $\text{CH}_2\text{Cl}_2$  for 3 h. Purification on silica gel ( $\text{CH}_2\text{Cl}_2/\text{MeOH}$  90:10) yielded **14** (87.9 mg, 56%) as a white solid.  $^1\text{H}$  NMR ( $\text{CD}_3\text{OD}$ )  $\delta$  8.19 (d,  $J = 8.4$  Hz, 2H), 8.14 (m, 2H), 7.91 (d,  $J = 8.7$  Hz, 2H), 7.85 (d,  $J = 2.2$  Hz, 1H), 7.81 (d,  $J = 8.6$  Hz, 2H), 7.64 (ddd,  $J = 2.3, 9.1$  Hz, 2H), 7.49 (dd,  $J = 2.3, 8.7$  Hz, 1H), 7.34 (dddd,  $J = 1.4, 6.5$  Hz, 1H), 7.25 (t,  $J = 7.8$  Hz, 2H), 7.16 (d,  $J = 8.4$  Hz, 1H), 6.78 (dd,  $J = 11.2, 17.7$  Hz, 1H), 6.04 (s, 1H), 5.85 (d,  $J = 17.6$  Hz, 1H), 5.39 (s, 1H), 5.28 (d,  $J = 11.2$  Hz, 1H), 4.04 (s, 3H), 1.47 (s, 9H).  $^{13}\text{C}$  NMR ( $\text{CD}_3\text{OD}$ ):  $\delta$  173.8, 172.6, 171.9, 171.3, 165.3, 157.5, 151.4, 147.8, 143.2, 138.0, 136.7, 134.4, 132.4, 132.3, 132.1, 131.5, 130.8, 129.5, 129.3 (2 carbons), 129.2, 127.1, 126.6, 124.2, 123.8, 120.1, 117.3, 89.6, 81.0, 60.1, 54.7, 28.7. HRMS calcd for  $\text{C}_{40}\text{H}_{36}\text{F}_3\text{N}_5\text{O}_8\text{S}$   $[\text{M}+\text{H}]^+$  804.2315, found: 804.2332.

**Compound 15** (*tert*-butyl (1-(4-((6-methoxy-2-phenylpyrimidin-4-yl)oxy)-3-vinylphenyl)-2-oxo-2-((3-((pent-4-en-1-ylsulfonyl)carbamoyl)phenyl)amino)ethyl)carbamate).



Prepared according to the general procedure C, using **6** (119 mg, 0.25 mmol), the *N*-deprotected HCl salt of **10** (114 mg, 0.38 mmol), HATU (114 mg, 0.30 mmol) and DIEA (273  $\mu$ L, 1.6 mmol). The reaction was stirred in  $\text{CH}_2\text{Cl}_2$  for 4 h. Purification on silica gel ( $\text{CH}_2\text{Cl}_2/\text{MeOH}$  95:5) yielded **15** (134 mg, 74%) as a white solid.  $^1\text{H}$  NMR ( $\text{CD}_3\text{OD}$ ):  $\delta$  8.18-8.15 (m, 2H), 8.13 (m, 1H), 7.87 (d,  $J$  = 2.4 Hz, 1H), 7.82 (m, 1H), 7.64 (ddd,  $J$  = 1.2, 1.8, 7.8 Hz, 1H), 7.52 (dd,  $J$  = 2.3, 8.4 Hz, 1H), 7.46 (t,  $J$  = 7.8 Hz, 1H), 7.40 (m, 1H), 7.32-7.27 (m, 2H), 7.19 (d,  $J$  = 8.5 Hz, 1H), 6.80 (dd,  $J$  = 11.4, 17.5 Hz, 1H), 6.06 (s, 1H), 5.87 (dd,  $J$  = 1.3, 17.7 Hz, 1H), 5.78 (m, 1H), 5.39 (s, 1H), 5.30 (dd,  $J$  = 1.2, 11.1 Hz, 1H), 5.08-4.98 (m, 1H), 4.05 (s, 3H), 3.53-3.48 (m, 2H), 2.24-2.17 (m, 2H), 1.94-1.86 (m, 2H), 1.48 (s, 9H).  $^{13}\text{C}$  NMR ( $\text{CD}_3\text{OD}$ ):  $\delta$  173.7, 172.5, 171.4, 170.2, 165.2, 157.5, 151.4, 139.9, 138.1, 138.0, 136.7, 135.7, 132.3 (2 carbons), 131.5, 130.2, 129.3, 129.2, 127.1, 125.4, 124.2, 121.3, 117.3, 116.5, 89.6, 81.0, 60.1, 54.7, 53.3, 33.0, 28.7. HRMS calcd for  $\text{C}_{38}\text{H}_{41}\text{N}_5\text{O}_8\text{S}$   $[\text{M}+\text{H}]^+$  728.2754, found: 728.2752. HPLC purity (system 1: 100%, system 2: 100%)

**Compound 16** (*tert*-butyl (2-((1-(4-((6-methoxy-2-phenylpyrimidin-4-yl)oxy)-3-vinylphenyl)-2-oxo-2-((4-((pent-4-en-1-ylsulfonyl)carbamoyl)phenyl)amino)ethyl)amino)-2-oxoethyl)carbamate).



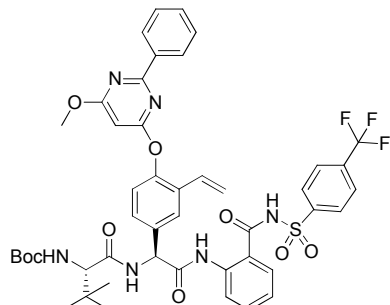
Prepared according to the general procedure C, using **6** (119 mg, 0.25 mmol), the *N*-deprotected HCl salt of **11** (114 mg, 0.38 mmol), HATU (114 mg, 0.3 mmol) and DIEA (273  $\mu$ L, 1.6 mmol). The reaction was stirred in  $\text{CH}_2\text{Cl}_2$  for 4 h. Purification on silica gel ( $\text{CH}_2\text{Cl}_2/\text{MeOH}$  94:6) followed by purification on preparative HPLC ( $\text{MeCN}/\text{H}_2\text{O}$  with 25 mM  $\text{NH}_4\text{OAc}$ , pH 6.3) yielded **16** as the  $\text{NH}_4^+$  salt.  $^1\text{H}$  NMR ( $\text{CD}_3\text{OD}$ ):  $\delta$  8.18-8.14 (m, 2H), 7.91-7.88 (m, 2H), 7.86 (m, 1H), 7.78-7.75 (m, 2H), 7.51 (dd,  $J$  = 2.4, 8.4 Hz, 1H), 7.39 (m, 1H), 7.30-7.25 (m, 2H), 7.18 (d,  $J$  = 8.4 Hz, 1H), 6.79 (dd,  $J$  = 11.2, 17.8 Hz, 1H), 6.06 (s, 1H), 5.87 (dd,  $J$  = 1.2, 17.6 Hz, 1H), 5.79 (m, 1H), 5.40 (s, 1H), 5.30 (dd,  $J$  = 1.2, 11.2 Hz, 1H), 5.10-4.99 (m, 2H), 4.05 (s, 3H), 3.55-3.50 (m, 2H), 2.25-2.19 (m, 2H), 1.95-1.87 (m, 2H), 1.48 (s, 9H).  $^{13}\text{C}$  NMR ( $\text{CD}_3\text{OD}$ ):  $\delta$  173.8, 172.6, 171.6, 168.2, 165.3, 157.6, 151.5, 144.5, 138.0 (2 carbons), 136.5, 132.4, 132.1, 131.5, 130.6, 129.3 (2 carbons), 129.2, 128.6, 127.2, 124.3, 120.5, 117.3, 116.6, 89.6, 81.1, 60.2, 54.7, 53.3, 32.9, 28.7, 23.8. HRMS calcd for  $\text{C}_{38}\text{H}_{41}\text{N}_5\text{O}_8\text{S}$   $[\text{M}+\text{H}]^+$  728.2754, found: 728.2749. HPLC purity (system 1: 100%, system 2: 100%).

#### General procedure D, for the preparation of compounds 17-21

The *N*-deprotected HCl-salts of **12-16** were mixed with HATU and Boc-L-*t*Leu in dry DMF. DIEA was added until the pH exceeded 10. The reactions were stirred in room temperature until starting material could no longer

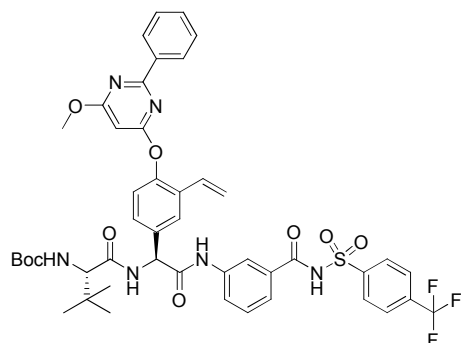
be detected by LC-MS. Ethyl acetate was added and the reaction mixture washed with sodium acetate buffer pH 4. The organic phase was evaporated before purification on preparative HPLC.

**Compound 17L ((S)-tert-butyl (1-((1-(4-((6-methoxy-2-phenylpyrimidin-4-yl)oxy)-3-vinylphenyl)-2-oxo-2-((3-((4-(trifluoromethyl)phenyl)sulfonyl)carbamoyl)phenyl)amino)ethyl)amino)-3,3-dimethyl-1-oxobutan-2-yl)carbamate).**



Prepared according to the general procedure D, using the *N*-deprotected HCl-salt of **12** (48.1 mg, 0.065 mmol), Boc-L-*t*Leu (38.8 mg, 0.168 mmol), HATU (88.9 mg, 0.234), and DIEA (92.8  $\mu$ L). The reaction was stirred in DMF for 4h. Purification on preparative HPLC (MeCN/H<sub>2</sub>O with 25 mM NH<sub>4</sub>OAc, pH 6.3) yielded **17L** (18.4 mg, 31%) as the NH<sub>4</sub><sup>+</sup> salt. Only the L-diastereoisomer could be isolated. <sup>1</sup>H NMR (CD<sub>3</sub>OD):  $\delta$  8.45 (dd, *J* = 1.2, 8.3 Hz, 1H), 8.12 (dd, *J* = 1.4, 8.3 Hz, 2H), 8.05 (d, *J* = 8.3 Hz, 2H), 8.01 (dd, *J* = 1.7, 8.0 Hz, 1H), 7.96 (d, *J* = 2.4 Hz, 1H), 7.65-7.57 (m, 3H), 7.42 (dt, *J* = 1.7, 7.5 Hz, 1H), 7.33 (m, 1H), 7.23-7.18 (m, 2H), 7.14 (m, 1H), 7.08 (m, 1H), 6.77 (dd, *J* = 11.5, 17.8 Hz, 1H), 6.07 (s, 1H), 5.91 (d, *J* = 17.8 Hz, 1H), 5.65 (s, 1H), 5.24 (d, *J* = 11.5 Hz, 1H), 5.24 (d, *J* = 11.5 Hz, 1H), 4.12 (s, 1H), 4.05 (s, 3H), 1.43 (s, 9H), 1.06 (s, 9H). <sup>13</sup>C NMR (CD<sub>3</sub>OD):  $\delta$  173.8, 173.2, 172.7, 172.6, 169.9, 165.2, 157.9, 151.5, 148.3, 140.8, 137.9, 136.2, 133.9 (q, *J* = 33.0 Hz), 133.3, 132.4, 132.0, 131.7, 131.2, 129.5, 129.3 (2 carbons), 129.2, 127.4, 126.5 (q, *J* = 3.6 Hz), 125.1 (q, *J* = 272.5 Hz), 124.4, 124.3, 124.0, 121.3, 117.7, 89.6, 80.6, 63.6, 60.4, 54.7, 28.7, 27.2. HRMS calcd for C<sub>46</sub>H<sub>47</sub>F<sub>3</sub>N<sub>6</sub>O<sub>9</sub>S [M+H]<sup>+</sup> 917.3156, found: 917.3162. HPLC purity (system 1: 100%, system 2: 100%).

**Compound 18L ((S)-tert-butyl (1-((1-(4-((6-methoxy-2-phenylpyrimidin-4-yl)oxy)-3-vinylphenyl)-2-oxo-2-((3-((4-(trifluoromethyl)phenyl)sulfonyl)carbamoyl)phenyl)amino)ethyl)amino)-3,3-dimethyl-1-oxobutan-2-yl)carbamate).**

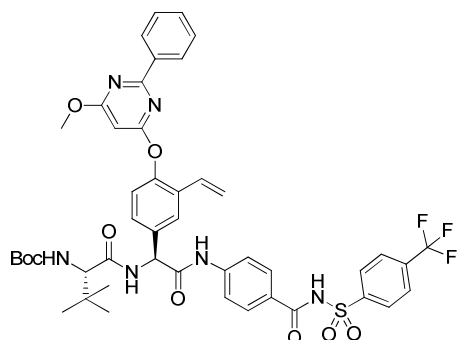


Prepared according to the general procedure D, using the *N*-deprotected HCl-salt of **13** (37.7 mg, 0.051 mmol), Boc-L-*t*Leu (19.0 mg, 0.082 mmol), HATU (23.4 mg, 0.062 mmol), and DIEA (48  $\mu$ L, 0.28 mmol). The reaction was stirred in DMF for 2.5 h. Purification on preparative HPLC (MeCN/H<sub>2</sub>O (0.1% TFA)) led to isolation of the L-isomer **18L** (13.8 mg, 29%). <sup>1</sup>H NMR (CD<sub>3</sub>OD):  $\delta$  8.27 (d, *J* = 8.7 Hz, 2H), 8.15 (dd, *J* = 1.3, 8.5 Hz, 2H), 8.07 (t, *J* = 1.9 Hz, 1H), 7.92 (d, *J* = 8.5 Hz, 2H), 7.89 (d, *J* = 2.3 Hz, 1H), 7.77 (d, *J* = 8.2 Hz, 1H), 7.53 (m, 2H), 7.43 (t, *J* = 7.6 Hz, 1H), 7.37 (dddd, *J* = 1.5, 6.4 Hz, 1H), 7.29 (t, *J* = 7.6 Hz, 2H), 7.18 (d, *J* = 8.5 Hz, 1H), 6.79 (dd, *J* = 11.6, 18.2 Hz, 1H), 6.05 (s, 1H), 5.87 (d, *J* = 17.6 Hz, 1H), 5.64 (s, 1H), 5.29 (d, *J* = 11.3 Hz, 1H), 4.06 (s, 3H), 4.05 (s, 1H), 1.42 (s, 9H), 1.05 (s, 9H). <sup>13</sup>C NMR (CD<sub>3</sub>OD):  $\delta$  173.8, 173.2, 172.6, 170.6, 167.5, 165.3, 158.0, 151.6, 144.7, 140.3, 138.0, 136.0 (q, *J* = 32.7 Hz), 135.9, 133.9, 132.5, 132.1, 131.4, 130.5, 130.3, 129.5, 129.2 (2 carbons), 127.5, 127.2 (q, *J* = 3.1 Hz), 125.9, 125.0, 124.9 (q, *J* = 271.2 Hz), 124.3, 120.8, 117.4,



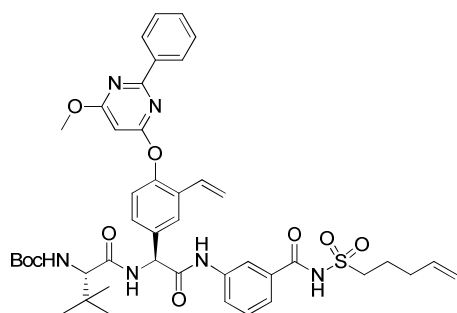
89.6, 80.7, 63.8, 59.0, 54.8, 35.4, 28.7, 27.3. HRMS calcd for  $C_{46}H_{47}F_3N_6O_9S$   $[M+H]^+$  917.3156, found: 917.3141. HPLC purity (system 1: 100%, system 2 100%)

**Compounds 19L** (*tert*-butyl ((S)-1-(((S)-1-(4-((6-methoxy-2-phenylpyrimidin-4-yl)oxy)-3-vinylphenyl)-2-oxo-2-((4-(((4-(trifluoromethyl)phenyl)sulfonyl)carbamoyl)phenyl)amino)ethyl)amino)-3,3-dimethyl-1-oxobutan-2-yl)carbamate) and **16D** (*tert*-butyl ((S)-1-(((R)-1-(4-((6-methoxy-2-phenylpyrimidin-4-yl)oxy)-3-vinylphenyl)-2-oxo-2-((4-(((4-(trifluoromethyl)phenyl)sulfonyl)carbamoyl)phenyl)amino)ethyl)amino)-3,3-dimethyl-1-oxobutan-2-yl)carbamate).



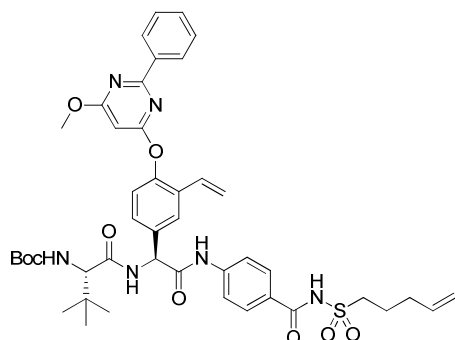
Prepared according to the general procedure D, using the *N*-deprotected HCl-salt of **14** (72 mg, 0.090 mmol), Boc-*L*-Leu (33.1 mg, 0.14 mmol), HATU (40.9 mg, 0.11 mmol), and DIEA (84  $\mu$ L, 0.48 mmol). The reaction was stirred in DMF for 3 h. Purification on preparative HPLC (MeCN/H<sub>2</sub>O with 25 mM NH<sub>4</sub>OAc, pH 6.3) led to isolation of **19L** (16.9 mg, 21%) and **19L** (3.66 mg, 5%) as NH<sub>4</sub><sup>+</sup>-salts. **19L** <sup>1</sup>H NMR (CD<sub>3</sub>OD):  $\delta$  8.25 (d,  $J$  = 9.0 Hz, 2H), 8.15 (m, 2H), 7.90 (s, 1H), 7.88 (m, 2H), 7.83 (m, 2H), 7.70 (m, 2H), 7.51 (dd,  $J$  = 2.1, 8.4 Hz, 1H), 7.36 (dddd,  $J$  = 1.5, 6.7 Hz, 1H), 7.27 (t,  $J$  = 8.3 Hz, 2H), 7.18 (d,  $J$  = 8.5 Hz, 1H), 6.78 (dd,  $J$  = 17.8, 11.5 Hz, 1H), 6.05 (s, 1H), 5.86 (d,  $J$  = 17.6 Hz, 1H), 5.65 (s, 1H), 5.28 (d,  $J$  = 11.3 Hz, 1H), 4.06 (s, 1H), 4.05 (s, 3H), 1.43 (s, 9H), 1.04 (s, 9H). <sup>13</sup>C NMR (CD<sub>3</sub>OD):  $\delta$  173.8, 173.2, 172.6, 170.5, 168.3, 165.3, 157.9, 151.6, 145.7, 144.3, 138.0, 135.9, 135.5 (q,  $J$  = 32.4 Hz), 132.5, 132.1, 131.4, 130.7, 130.1, 129.5, 129.3 (2 carbons), 129.2, 127.5, 127.0 (q,  $J$  = 4.0 Hz), 124.3, 123.6 (q,  $J$  = 271.3 Hz), 120.3, 117.4, 89.7, 80.7, 63.7, 59.1, 54.7, 35.4, 28.7, 27.2. HRMS calcd for  $C_{46}H_{47}F_3N_6O_9S$   $[M+H]^+$  917.3156, found: 917.3137. HPLC purity (system 1: 100%, system 2: 100%) **19D** <sup>1</sup>H NMR (CD<sub>3</sub>OD):  $\delta$  8.26-8.22 (m, 2H), 8.17-8.13 (m, 2H), 7.90-7.86 (m, 2H), 7.85 (m, 1H), 7.75 (m, 1H), 7.48 (dd,  $J$  = 2.3, 8.4 Hz, 1H), 7.37 (m, 1H), 7.28 (t,  $J$  = 7.9 Hz, 2H), 7.17 (d,  $J$  = 8.5 Hz, 1H), 6.78 (dd,  $J$  = 11.3 Hz, 17.6 Hz, 1H), 6.05 (s, 1H), 5.83 (dd,  $J$  = 1.2, 17.6 Hz, 1H), 5.70 (s, 1H), 5.28 (dd,  $J$  = 1.2, 11.0 Hz,  $J$  = 1H), 4.05 (s, 3H), 4.00 (s, 1H), 1.42 (s, 9H), 0.99 (s, 9H). <sup>13</sup>C NMR (CD<sub>3</sub>OD):  $\delta$  173.8, 173.4, 172.6, 170.6, 168.9, 165.3, 158.0, 151.5, 146.1, 144.0, 138.0, 136.1, 135.3 (q,  $J$  = 32.2 Hz), 132.5, 132.1, 131.5, 130.6, 130.0, 129.3, 127.6, 126.9 (q,  $J$  = 3.6 Hz), 124.9 (q,  $J$  = 271.0 Hz), 124.3, 120.5, 117.3, 111.4, 89.7, 80.7, 64.2, 58.8, 54.7, 35.2, 28.7, 27.2. HRMS calcd for  $C_{46}H_{47}F_3N_6O_9S$   $[M+H]^+$  917.3156, found: 917.3158. HPLC purity (system 1: 100%, system 2: 100%).

**Compounds 20L** (*tert*-butyl ((S)-1-(((S)-1-(4-((6-methoxy-2-phenylpyrimidin-4-yl)oxy)-3-vinylphenyl)-2-oxo-2-((3-((pent-4-en-1-ylsulfonyl)carbamoyl)phenyl)amino)ethyl)amino)-3,3-dimethyl-1-oxobutan-2-yl)carbamate) and **17D** (*tert*-butyl ((S)-1-(((R)-1-(4-((6-methoxy-2-phenylpyrimidin-4-yl)oxy)-3-vinylphenyl)-2-oxo-2-((3-((pent-4-en-1-ylsulfonyl)carbamoyl)phenyl)amino)ethyl)amino)-3,3-dimethyl-1-oxobutan-2-yl)carbamate).



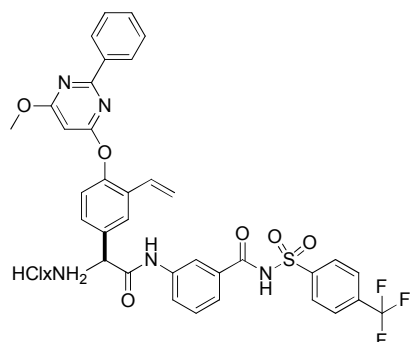
Prepared according to the general procedure D, using the *N*-deprotected HCl salt of **15** (63.1 mg, 0.095 mmol), Boc-*L*-*t*Leu (43.9 mg, 0.19 mmol), HATU (87 mg, 0.23 mmol) and DIEA (127  $\mu$ L, 0.74 mmol). The reaction was stirred in DMF for 3 h. Purification on preparative HPLC (MeCN/H<sub>2</sub>O with 25 mM NH<sub>4</sub>OAc, pH 6.3) gave **20L** (27 mg, 34%) and **20D** (7.7 mg, 10%) as NH<sub>4</sub><sup>+</sup> salts. **20L** <sup>1</sup>H NMR (CD<sub>3</sub>OD):  $\delta$  8.18-8.15 (m, 2H), 8.14 (m, 1H), 7.91 (d, *J* = 2.3 Hz, 1H), 7.79 (m, 1H), 7.62 (ddd, *J* = 1.0, 1.8, 7.8 Hz, 1H), 7.54 (dd, *J* = 2.2, 8.4 Hz, 1H), 7.45 (t, *J* = 8.0 Hz, 1H), 7.40 (m, 1H) 7.32-7.27 (m, 2H), 7.19 (d, *J* = 8.3 Hz, 1H), 6.79 (dd, *J* = 11.2, 17.7 Hz, 1H), 6.05 (s, 1H), 5.88 (dd, *J* = 1.2, 17.7 Hz, 1H), 5.77 (m, 1H), 5.67 (s, 1H), 5.29 (dd, *J* = 1.2, 11.2 Hz, 1H), 5.08-4.98 (m, 2H), 4.05 (s, 3H), 3.53-3.48 (m, 2H), 2.24-2.17 (m, 2H), 1.94-1.86 (m, 2H), 1.43 (s, 9H), 1.06 (s, 9H). <sup>13</sup>C NMR (CD<sub>3</sub>OD) 173.8, 173.2, 172.6, 170.6, 168.8, 165.3, 157.9, 151.5, 140.1, 138.0 (2 carbons), 135.9, 134.6, 132.5, 132.1, 131.4, 130.4, 129.5, 120.3, 127.5, 125.7, 125.1, 124.3, 120.9, 117.4, 116.6, 89.6, 80.7, 63.7, 59.0, 54.7, 53.2, 35.4, 32.9, 28.7, 27.2, 23.7. HRMS calcd for C<sub>44</sub>H<sub>52</sub>N<sub>6</sub>O<sub>9</sub>S [M+H]<sup>+</sup> 841.3595, found: 841.3593. HPLC purity (system 1:100%, system 2: 100%) **20D** <sup>1</sup>H NMR (CD<sub>3</sub>OD):  $\delta$  8.20-8.15 (m, 3H), 7.89-7.85 (m, 2H), 7.66 (ddd, *J* = 1.1, 1.8, 7.8 Hz, 1H), 7.51 (d, *J* = 8.5 Hz, 1H), 7.46 (t, *J* = 8.0 Hz, 1H), 7.42 (m, 1H), 7.34-7.29 (m, 2H), 7.20 (d, *J* = 8.5 Hz, 1H), 6.80 (dd, *J* = 11.2, 17.7 Hz, 1H), 6.07 (s, 1H), 5.86 (dd, *J* = 1.2, 17.7 Hz, 1H), 5.79 (m, 1H), 5.72 (s, 1H), 5.30 (dd, *J* = 1.2, 11.2 Hz, 1H), 5.09-4.99 (m, 2H), 4.06 (s, 3H), 3.52-3.47 (m, 2H), 2.24-2.18 (m, 2H), 1.94-1.87 (m, 2H), 1.42 (s, 9H), 1.01 (s, 9H). <sup>13</sup>C NMR (CD<sub>3</sub>OD) 173.8, 173.5, 172.6, 170.7, 169.4, 165.3, 158.0, 151.5, 139.9, 138.1 (2 carbons), 136.2, 135.1, 132.5, 132.1, 131.5, 130.3, 129.7, 129.3 (2 carbons), 127.6, 125.8, 125.3, 124.3, 121.4, 117.3, 116.5, 89.7, 80.8, 64.3, 58.8, 54.7, 53.2, 35.2, 33.0, 28.7, 27.2, 23.8. HRMS calcd for C<sub>44</sub>H<sub>52</sub>N<sub>6</sub>O<sub>9</sub>S [M+H]<sup>+</sup> 841.3595, found: 841.3597. HPLC purity (system 1:100%, system 2: 100%)

**Compound 21L** (*tert*-butyl ((*S*)-1-(((*S*)-1-(4-((6-methoxy-2-phenylpyrimidin-4-yl)oxy)-3-vinylphenyl)-2-oxo-2-(((4-((pent-4-en-1-ylsulfonyl)carbamoyl)phenyl)amino)ethyl)amino)-3,3-dimethyl-1-oxobutan-2-yl)carbamate) and **18D** (*tert*-butyl ((*S*)-1-(((*R*)-1-(4-((6-methoxy-2-phenylpyrimidin-4-yl)oxy)-3-vinylphenyl)-2-oxo-2-(((4-((pent-4-en-1-ylsulfonyl)carbamoyl)phenyl)amino)ethyl)amino)-3,3-dimethyl-1-oxobutan-2-yl)carbamate).



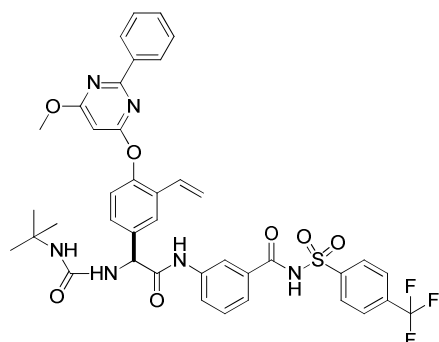
Prepared according to the general procedure D, using the *N*-deprotected HCl salt of **13** (73 mg, 0.11 mmol), Boc-*L*-*t*Leu (51 mg, 0.22 mmol), HATU (100 mg, 0.26 mmol) and DIEA (147  $\mu$ L, 0.86 mmol). The reaction was stirred in DMF for 2 h. Purification on preparative HPLC (MeCN/H<sub>2</sub>O with 25mM NH<sub>4</sub>OAc, pH 6.3) gave **21L** (20.6 mg, 22%) and **21D** (11.5 mg, 12%) as NH<sub>4</sub><sup>+</sup> salts. **21L** <sup>1</sup>H NMR (CD<sub>3</sub>OD):  $\delta$  8.17-8.14 (m, 2H), 7.90-7.88 (m, 3H), 7.76-7.74 (m, 2H), 7.53 (dd, *J* = 2.3, 8.5 Hz, 1H), 7.39 (m, 1H), 7.30-7.74 (m, 2H), 7.53 (dd, *J* = 2.3, 8.5 Hz, 1H), 7.39 (m, 1H), 7.30-7.27 (m, 2H), 7.18 (d, *J* = 8.4 Hz, 1H), 6.79 (dd, *J* = 11.2, 17.7 Hz, 1H), 6.04 (s, 1H), 5.87 (dd, *J* = 1.2, 17.6 Hz, 1H), 5.79 (m, 1H), 5.67 (s, 1H), 5.29 (dd, *J* = 1.1, 11.2 Hz, 1H), 5.08-4.99 (m, 2H), 4.08 (s, 1H), 4.05 (s, 3H), 3.53-3.49 (m, 2H), 2.24-2.19 (m, 2H), 1.95-1.89 (m, 2H), 1.43 (s, 9H), 1.05 (s, 9H). <sup>13</sup>C NMR (CD<sub>3</sub>OD):  $\delta$  173.7, 173.2, 172.6, 170.6, 168.4, 165.3, 157.9, 151.5, 144.5, 138.0, (2 carbons), 135.8, 132.5, 132.1, 131.4, 130.6, 129.6, 129.3 (2 carbons), 128.7, 127.5, 124.3, 120.3, 117.4, 116.6, 89.6, 80.7, 63.6, 59.1, 54.7, 53.3, 35.4, 33.0, 28.7, 27.2, 23.8. HRMS calcd for C<sub>44</sub>H<sub>52</sub>N<sub>6</sub>O<sub>9</sub>S [M+H]<sup>+</sup> 841.3595, found: 841.3596. HPLC purity (system 1: 100%, system 2: 100%). **21D** <sup>1</sup>H NMR (CD<sub>3</sub>OD):  $\delta$  8.18-8.16 (m, 2H), 7.91-7.89 (m, 2H), 7.85-7.80 (m, 3H), 7.50 (dd, *J* = 2.3, 8.5 Hz, 1H), 7.41 (m, 1H), 7.32-7.29 (m, 2H), 7.19 (d, *J* = 8.4 Hz, 1H), 1H), 6.79 (dd, *J* = 11.1, 17.7 Hz, 1H), 6.06 (s, 1H), 5.85 (dd, *J* = 1.2, 17.6 Hz, 1H), 5.79 (m, 1H), 5.73 (s, 1H), 5.29 (dd, *J* = 1.2, 11.2 Hz, 1H), 5.09-4.99 (m, 2H), 4.06 (s, 3H), 4.02 (s, 1H), 3.53-3.49 (m, 2H), 2.24-2.19 (m, 2H), 1.95-1.87 (m, 2H), 1.43 (s, 9H), 1.01 (s, 9H). <sup>13</sup>C NMR (CD<sub>3</sub>OD):  $\delta$  173.8, 173.4, 172.6, 170.7, 168.6, 165.3, 158.0, 151.5, 144.2, 138.1, 138.0, 136.1, 132.5, 132.1, 131.5, 130.6, 129.7, 129.3 (2 carbons), 129.1, 127.6, 124.3, 120.6, 117.3, 116.6, 89.7, 80.7, 64.2, 58.9, 54.7, 53.3, 35.3, 33.0, 28.7, 27.2, 23.8. HRMS calcd for C<sub>44</sub>H<sub>52</sub>N<sub>6</sub>O<sub>9</sub>S [M+H]<sup>+</sup> 841.3595, found: 841.3597. HPLC purity (system 1: 100%, system 2: 100%).

**Compound 22** (3-(2-amino-2-(4-(((6-methoxy-2-phenylpyrimidin-4-yl)oxy)-3-vinylphenyl)acetamido)-*N*-((4-(trifluoromethyl)phenyl)sulfonyl)benzamide).



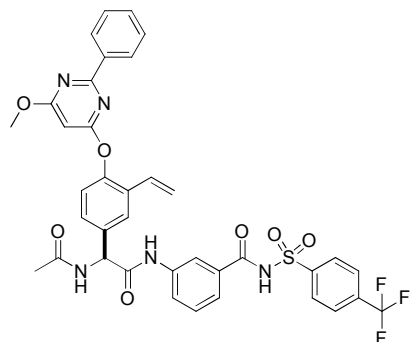
**13** (15 mg, 0.019 mmol) was *N*-deprotected according to the general procedure B. Purification on HPLC (MeCN/H<sub>2</sub>O (0.05% HCOOH)) yielded **22** (5.9 mg, 43%) as a white solid. <sup>1</sup>H NMR (d<sub>6</sub>DMSO):  $\delta$  10.66 (s, 1H), 8.77 (br s, 2H), 8.09-8.05 (m, 3H), 8.02-7.97 (m, 3H), 7.80 (m, 1H), 7.75 (d, *J* = 8.3 Hz, 2H), 7.64-7.59 (m, 2H), 7.43-7.38 (m, 2H), 7.36-7.28 (m, 3H), 6.77 (dd, *J* = 11.4, 17.7 Hz, 1H), 6.34 (s, 1H), 5.91 (d, *J* = 17.7 Hz, 1H), 5.41 (d, *J* = 11.4 Hz, 1H), 5.15 (s, 1H), 4.03 (s, 3H). <sup>13</sup>C NMR (d<sub>6</sub>DMSO):  $\delta$  171.9, 170.5, 169.1, 165.7, 162.9, 150.3, 150.1, 139.8, 137.3, 136.1, 132.1 (q, *J* = 34.5 Hz), 131.4, 131.2, 130.2, 129.7, 128.6, 128.5, 128.2, 127.8, 127.7, 126.3, 124.7 (q, *J* = 3.5 Hz), 124.2, 124.0 (q, *J* = 272.3 Hz), 123.6, 120.9, 119.9, 117.6, 89.1, 56.0, 54.3. HRMS calcd for C<sub>35</sub>H<sub>28</sub>F<sub>3</sub>N<sub>5</sub>O<sub>6</sub>S [M+H]<sup>+</sup> 704.1791, found: 704.1783. HPLC purity (system 1: 99.3%, system 2: 98.6%)

**Compound 23** (3-(2-(3-(*tert*-butyl)ureido)-2-(4-(((6-methoxy-2-phenylpyrimidin-4-yl)oxy)-3-vinylphenyl)acetamido)-*N*-((4-(trifluoromethyl)phenyl)sulfonyl)benzamide).



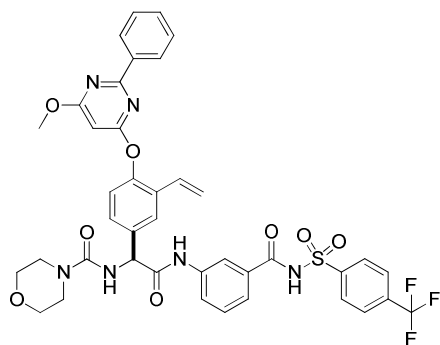
The *N*-deprotected HCl-salt of **13** (32.5 mg, 0.044 mmol) was dissolved in dry DMF (1 mL). DIEA (18  $\mu$ L, 0.132 mmol) was added and pH controlled to be >10 before *tert*-butylisocyanate (7.5  $\mu$ L, 0.066 mmol) was added. The reaction was stirred in room temperature over night. EtOAc (5 mL) was added and the reaction mixture was washed with sodium acetate buffer pH 4.0. After evaporation of the organic phase, purification was performed on silica gel (CH<sub>2</sub>Cl<sub>2</sub>/MeOH 95:5), yielding **23** (19.3 mg, 55%) as a white solid. <sup>1</sup>H NMR (CD<sub>3</sub>OD):  $\delta$  8.19 (d, *J* = 8.2 Hz, 2H), 8.16-8.11 (m, 3H), 7.84 (d, *J* = 2.2 Hz, 1H), 7.80 (d, *J* = 8.2 Hz, 2H), 7.76 (m, 1H), 7.67 (d, *J* = 8.0 Hz, 1H), 7.49 (dd, *J* = 2.3, 8.5 Hz, 1H), 7.38-7.31 (m, 2H), 7.29-7.24 (m, 2H), 7.15 (d, *J* = 8.4 Hz, 1H), 6.77 (dd, *J* = 11.1, 17.6 Hz, 1H), 6.02 (s, 1H), 5.84 (d, *J* = 17.6 Hz, 1H), 5.47 (s, 1H), 5.27 (d, *J* = 11.1 Hz, 1H), 4.04 (s, 3H), 1.31 (s, 9H). <sup>13</sup>C NMR (CD<sub>3</sub>OD):  $\delta$  173.7, 172.6, 172.0, 171.6, 165.2, 159.2, 151.2, 147.3, 139.6, 138.0, 137.8 (2 carbons), 137.1, 134.7 (q, *J* = 32.1 Hz), 132.4, 132.3, 132.1, 131.5, 129.8, 129.3 (2 carbons), 128.9, 126.8 (q, *J* = 3.7 Hz), 125.6, 125.1, 125.0 (q, *J* = 273.0 Hz), 124.2, 121.6, 117.2, 89.6, 59.1, 54.7, 51.0, 29.8. HRMS calcd for C<sub>40</sub>H<sub>37</sub>F<sub>3</sub>N<sub>6</sub>O<sub>7</sub>S [M+H]<sup>+</sup> 803.2475, found: 803.2469. HPLC purity (system 1: 100%, system 2: 97.7%).

**Compound 24 (3-(2-acetamido-2-(4-(((6-methoxy-2-phenylpyrimidin-4-yl)oxy)-3-vinylphenyl)acetamido)-N-((4-(trifluoromethyl)phenyl)sulfonyl)benzamide).**



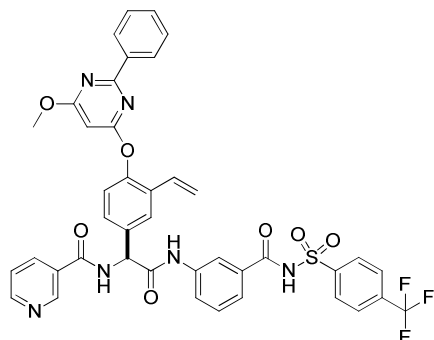
The *N*-deprotected HCl-salt of **13** (32.5 mg, 0.044 mmol) was dissolved in dry CH<sub>2</sub>Cl<sub>2</sub> (2 mL). Triethylamine (18 μL, 0.13 mmol) was added and pH controlled to be >10 before acetylchloride (4.6 μL, 0.066 mmol) was added. The reaction was stirred at 40 °C for 5 h. CH<sub>2</sub>Cl<sub>2</sub> (3 mL) was added and the reaction mixture washed with sodium acetate buffer pH 4.0. After evaporation of the organic phase, purification was performed on preparative HPLC (MeCN/H<sub>2</sub>O (0.05% HCOOH)) yielding **24** (11.2 mg, 34%) as a white solid. <sup>1</sup>H NMR (CD<sub>3</sub>OD): δ 8.29-8.25 (m, 2H), 8.17-8.14 (m, 2H), 8.07 (m, 1H), 7.93-7.90 (m, 2H), 7.87 (d, *J* = 2.3 Hz, 1H), 7.77 (m, 1H), 7.55 (m, 1H), 7.52 (dd, *J* = 2.3, 8.4 Hz, 1H), 7.43 (t, *J* = 8.0 Hz, 1H), 7.37 (m, 1H), 7.30-7.26 (m, 2H), 7.19 (d, *J* = 8.4 Hz, 1H), 6.79 (dd, *J* = 11.2, 17.7 Hz, 1H), 6.06 (s, 1H), 5.86 (dd, *J* = 1.2, 17.7 Hz, 1H), 5.62 (s, 1H), 5.30 (dd, *J* = 1.2, 11.2 Hz, 1H), 4.05 (s, 3H), 2.07 (s, 3H). <sup>13</sup>C NMR (CD<sub>3</sub>OD) δ 173.8, 173.2, 172.6, 171.1, 167.5, 165.3, 151.6, 144.7, 140.2, 138.0, 136.0 (q, *J* = 33.3 Hz), 135.9, 134.0, 132.5, 132.1, 131.4, 130.5, 130.3, 129.5, 129.3 (2 carbons), 127.4, 127.2 (q, *J* = 3.6 Hz), 125.9, 125.0, 124.7, 124.3, 121.0, 117.4, 89.6, 59.2, 54.7, 22.4. HRMS calcd for C<sub>37</sub>H<sub>30</sub>F<sub>3</sub>N<sub>5</sub>O<sub>7</sub>S [M+H]<sup>+</sup> 746.1896, found: 746.1901. HPLC purity (system 1: 100%, system 2: 97.7%).

**Compound 25 (N-(1-(4-(((6-methoxy-2-phenylpyrimidin-4-yl)oxy)-3-vinylphenyl)-2-oxo-2-(((4-(trifluoromethyl)phenyl)sulfonyl)carbamoyl)phenyl)amino)ethyl)morpholine-4-carboxamide).**



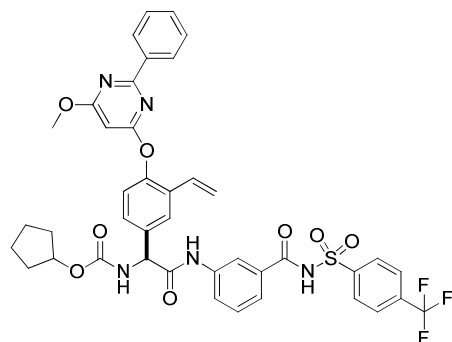
The *N*-deprotected HCl-salt of **13** (32.5 mg, 0.044 mmol) was dissolved in dry CH<sub>2</sub>Cl<sub>2</sub> (1 mL). Triethylamine (18 μL, 0.13 mmol) was added and pH controlled to be > 10 before 4-morpholinecarbonylchloride (7.7 μL, 0.066 mmol) was added. The reaction was stirred at 40 °C over night. CH<sub>2</sub>Cl<sub>2</sub> (3 mL) was added and the reaction mixture was washed with sodium acetate buffer pH 4.0. Purification was performed on preparative HPLC (MeCN/H<sub>2</sub>O (0.05% HCOOH)), yielding **25** (6.0 mg, 17%) as a white solid. <sup>1</sup>H NMR (CD<sub>3</sub>OD): δ 8.29-8.25 (m, 2H), 8.18-8.15 (m, 2H), 8.09 (m, 1H), 7.94-7.90 (m, 2H), 7.87 (d, *J* = 2.2 Hz, 1H), 7.79 (m, 1H), 7.57-7.51 (m, 2H), 7.43 (m, 1H), 7.38 (m, 1H), 7.31-7.26 (m, 2H), 7.19 (d, 8.3 Hz, 1H), 6.79 (dd, *J* = 11.2, 17.8 Hz, 1H), 6.05 (s, 1H), 5.86 (dd, *J* = 1.3, 17.8 Hz, 1H), 5.55 (s, 1H), 5.29 (dd, *J* = 1.3, 11.2 Hz, 1H), 4.06 (s, 3H), 3.69-3.65 (m, 4H), 3.48-3.44 (m, 4H). <sup>13</sup>C NMR (CD<sub>3</sub>OD): δ 173.8, 172.6, 172.1, 167.7, 165.3, 159.5, 151.5, 144.8, 140.2, 138.0, 136.6, 135.9 (q, *J* = 33.7 Hz), 134.1, 132.3, 132.1, 131.5, 130.4, 130.2, 129.6, 129.3 (2 carbons), 127.6, 127.2 (q, *J* = 3.8 Hz), 125.9, 125.0, 124.8 (q, *J* = 272.7 Hz), 124.2, 121.0, 117.3, 89.6, 67.6, 60.5, 54.7, 45.4. HRMS calcd for C<sub>40</sub>H<sub>35</sub>F<sub>3</sub>N<sub>6</sub>O<sub>8</sub>S [M+H]<sup>+</sup> 817.2267, found: 817.2266. HPLC purity (system 1: 100%, system 2: 100%).

**Compound 26** (*N*-(1-(4-((6-methoxy-2-phenylpyrimidin-4-yl)oxy)-3-vinylphenyl)-2-oxo-2-((3-(((4-(trifluoromethyl)phenyl)sulfonyl)carbamoyl)phenyl)amino)ethyl)picolinamide).



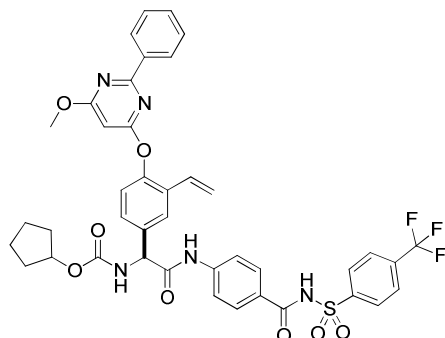
The *N*-deprotected HCl-salt of **13** (27.4 mg, 0.037 mmol) was dissolved in dry DMF (1 mL). DIEA (19  $\mu$ L, 0.11 mmol) was added and pH controlled to be > 10 before nicotinoylchloride hydrochloride (9.8 mg, 0.055 mmol) was added. The reaction was stirred in room temperature over night. EtOAc (5 mL) was added and the reaction mixture was washed with sodium acetate buffer pH 4.0. After evaporation of the organic phase purification was performed on preparative HPLC (MeCN/H<sub>2</sub>O (0.05% HCOOH)) yielding **26** (14.7 mg, 39%) as the HCOOH salt. <sup>1</sup>H NMR (CD<sub>3</sub>OD):  $\delta$  9.06 (s, 1H), 8.69 (m, 1H), 8.33 (dt, *J* = 2.0, 8.1 Hz, 1H), 8.28-8.24 (m, 2H), 8.16-8.13 (m, 2H), 8.11 (m, 1H), 7.96 (d, *J* = 2.3 Hz, 1H), 7.92-7.88 (m, 2H), 7.80 (m, 1H), 7.61 (dd, *J* = 2.3, 8.5 Hz, 1H), 7.57-7.52 (m, 2H), 7.43 (m, 1H), 7.35 (m, 1H), 7.28-7.23 (m, 2H), 7.21 (d, *J* = 8.4 Hz, 1H), 6.80 (dd, *J* = 11.2, 17.7 Hz, 1H), 6.06 (s, 1H), 5.88 (s, 1H), 5.86 (d, *J* = 17.5 Hz, 1H), 5.29 (d, *J* = 11.3 Hz, 1H), 4.04 (s, 3H). <sup>13</sup>C NMR (CD<sub>3</sub>OD):  $\delta$  173.8, 172.5, 170.8, 167.9, 167.6, 165.3, 152.7, 151.7, 149.5, 144.8 (2 carbons), 140.2, 138.0, 137.7, 135.8 (q, *J* = 32.7 Hz), 135.5, 134.1, 132.5, 132.1, 131.6, 131.4, 130.5, 130.2, 129.8, 129.3 (2 carbons), 127.8, 127.1 (q, *J* = 3.7 Hz), 125.8, 125.1 (2 carbons), 124.8 (q, *J* = 271.5), 124.4, 121.0, 117.4, 89.7, 59.7, 54.7. HRMS calcd for C<sub>41</sub>H<sub>31</sub>F<sub>3</sub>N<sub>6</sub>O<sub>7</sub>S [M+H]<sup>+</sup> 809.2005, found: 809.2006. HPLC purity (system 1: 100%, system 2: 99.0%).

**Compound 27** (cyclopentyl (1-(4-((6-methoxy-2-phenylpyrimidin-4-yl)oxy)-3-vinylphenyl)-2-oxo-2-((3-(((4-(trifluoromethyl)phenyl)sulfonyl)carbamoyl)phenyl)amino)ethyl)carbamate)



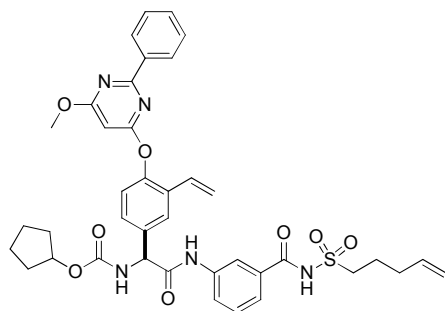
The *N*-deprotected HCl-salt of **13** (5.9 mg, 0.008 mmol) was dissolved in dry THF (0.5 mL). Cyclopentyl chloroformate (2.4 mg, 0.016 mmol) was added. The reaction was stirred on an ice bath and triethylamine (4.5  $\mu$ L, 0.032 mmol) was added so the pH exceeded 10, the reaction was thereafter stirred at 0 °C for 1 h. EtOAc (5 mL) was added and the reaction was washed with sodium acetate buffer pH 4. After evaporation of the organic phase purification was performed on preparative HPLC (MeCN/H<sub>2</sub>O with 25 mM NH<sub>4</sub>OAc, pH 6.3) yielding **27** (3.12 mg, 48%) as the NH<sub>4</sub><sup>+</sup> salt. <sup>1</sup>H NMR (CD<sub>3</sub>OD):  $\delta$  8.24-8.22 (m, 2H), 8.17-8.15 (m, 2H), 8.04 (s, 1H), 7.88-7.86 (m, 3H), 7.81 (m, 1H), 7.61 (m, 1H), 7.51 (dd, *J* = 2.3, 8.3 Hz, 1H), 7.41 (m, 1H), 7.37 (m, 1H), 7.30-7.26 (m, 2H), 7.18 (d, *J* = 8.4 Hz, 1H), 6.79 (dd, *J* = 11.1, 17.8 Hz, 1H), 6.06 (s, 1H), 5.87 (dd, *J* = 1.2, 17.7 Hz, 1H), 5.42 (s, 1H), 5.30 (dd, *J* = 1.1, 11.1 Hz, 1H), 5.09 (m, 1H), 4.06 (s, 3H), 1.86-1.61 (m, 8H). <sup>13</sup>C NMR (CD<sub>3</sub>OD):  $\delta$  173.8, 172.6, 171.3, 167.5, 165.3, 153.2, 151.5, 146.2, 139.9, 138.0, 136.6, 135.8, 135.3 (q), 132.4, 132.1, 131.5, 130.2, 129.9, 129.3 (2 signals), 129.2, 127.2, 126.9 (q, *J* = 3.8 Hz), 125.4, 125.3, 124.3, 123.7 (q), 121.1, 117.3, 89.6, 79.3, 60.3, 54.7, 33.8, 24.6. HRMS calcd for C<sub>41</sub>H<sub>36</sub>F<sub>3</sub>N<sub>5</sub>O<sub>8</sub>S [M+H]<sup>+</sup> 816.2315, found: 816.2313. HPLC purity (system 1: 99.1%, system 2: 99.2%).

**Compound 28 (cyclopentyl (1-(4-((6-methoxy-2-phenylpyrimidin-4-yl)oxy)-3-vinylphenyl)-2-oxo-2-((4-((4-(trifluoromethyl)phenyl)sulfonyl)carbamoyl)phenyl)amino)ethyl)carbamate)**



The *N*-deprotected HCl-salt of **14** (10.4 mg, 0.014 mmol) was dissolved in dry THF (0.5 mL). Cyclopentyl chloroformate (4.2 mg, 0.028 mmol) was added. The reaction was stirred on an ice bath and triethylamine (7.8  $\mu$ L, 0.056 mmol) was added so the pH exceeded 10, the reaction was thereafter stirred at 0 °C for 1 h. EtOAc (5 mL) was added and the reaction was washed with sodium acetate buffer pH 4. After evaporation of the organic phase purification was performed on preparative HPLC (MeCN/H<sub>2</sub>O with 25 mM NH<sub>4</sub>OAc, pH 6.3) yielded **28** as the NH<sub>4</sub><sup>+</sup> salt. <sup>1</sup>H NMR (CD<sub>3</sub>OD):  $\delta$  8.26-8.24 (m, 2H), 8.16-8.14 (m, 2H), 7.90-7.88 (m, 2H), 7.86-7.84 (m, 3H), 7.71-7.69 (m, 2H), 7.49 (dd, *J* = 2.3, 8.4 Hz, 1H), 7.35 (m, 1H), 7.28-7.24 (m, 2H), 7.18 (d, *J* = 8.4 Hz, 1H), 6.79 (dd, *J* = 11.3, 17.7 Hz, 1H), 6.06 (s, 1H), 5.86 (dd, *J* = 1.1, 17.7 Hz, 1H), 5.42 (s, 1H), 5.29 (dd, *J* = 1.1, 11.3 Hz, 1H), 5.08 (m, 1H), 4.06 (s, 3H), 1.86-1.61 (m, 4H). <sup>13</sup>C NMR (CD<sub>3</sub>OD):  $\delta$  173.8, 172.6, 171.3, 168.8, 165.3, 158.3, 151.5, 146.0, 144.1, 138.0, 136.4, 135.4 (q, *J* = 3.8 Hz), 132.4, 132.1, 131.5, 130.7, 130.0, 129.6, 129.3 (3 signals), 127.2, 126.9 (q, *J* = 3.7 Hz), 124.3, 123.6, 120.3, 117.3, 89.6, 79.4, 60.4, 54.7, 33.7, 24.6. HRMS calcd for C<sub>41</sub>H<sub>36</sub>F<sub>3</sub>N<sub>5</sub>O<sub>8</sub>S [M+H]<sup>+</sup> 816.2315, found: 816.2314. HPLC purity (system 1: 100%, system 2: 100%).

**Compound 29 (N-(1-(4-((6-methoxy-2-phenylpyrimidin-4-yl)oxy)-3-vinylphenyl)-2-oxo-2-((3-((4-(trifluoromethyl)phenyl)sulfonyl)carbamoyl)phenyl)amino)ethyl)nicotinamide).**



The *N*-deprotected HCl salt of **15** (33 mg, 0.05 mmol) was mixed with cyclopentyl chloroformate (14.4 mg, 0.1 mmol), and THF (2 mL). The reaction was placed on an ice bath whereafter triethylamine (27  $\mu$ L, 0.2 mmol) was added and the reaction was stirred for 1 h. EtOAc (6 mL) was added and the mixture washed with sodium acetate buffer pH 4.0. After evaporation of the organic phase purification was performed on preparative HPLC (MeCN/H<sub>2</sub>O with 25 mM NH<sub>4</sub>OAc, pH 6.3) yielded **29** (18.4 mg, 51%) as the NH<sub>4</sub><sup>+</sup> salt. <sup>1</sup>H NMR (CD<sub>3</sub>OD):  $\delta$  8.17-8.14 (m, 2H), 8.13 (m, 1H), 7.88 (d, *J* = 2.3 Hz, 1H), 7.81 (m, 1H), 7.63 (m, 1H), 7.52 (dd, *J* = 2.3, 8.5 Hz, 1H), 7.45 (t, *J* = 8.0 Hz, 1H), 7.39 (m, 1H), 7.31-7.26 (m, 2H), 7.18 (d, *J* = 8.4 Hz, 1H), 6.79 (dd, *J* = 11.2, 17.6 Hz, 1H), 6.04 (s, 1H), 5.87 (dd, *J* = 1.2, 17.7 Hz, 1H), 5.77 (m, 1H), 5.45 (s, 1H), 5.29 (dd, *J* = 1.2, 11.1 Hz, 1H), 5.09 (m, 1H), 5.07-4.98 (m, 2H), 4.04 (s, 3H), 3.52-3.47 (m, 2H), 2.23-2.16 (m, 2H), 1.94-1.85 (m, 2H), 1.82-1.69 (m, 5H), 1.64-1.52 (m, 3H). <sup>13</sup>C NMR (CD<sub>3</sub>OD):  $\delta$  173.7, 172.6, 171.3, 169.0, 165.2, 158.3, 151.5, 140.1, 138.0 (2 carbons), 136.5, 134.8, 132.4, 132.1, 131.5, 130.4, 129.3 (3 carbons), 127.2, 125.7, 125.1, 124.3, 121.1, 117.3, 116.6, 89.6, 79.4, 60.4, 54.7, 53.2, 33.8, 32.9, 24.6, 23.8. HRMS calcd for C<sub>39</sub>H<sub>41</sub>N<sub>5</sub>O<sub>7</sub>S [M+H]<sup>+</sup> 740.2754, found: 740.2756. HPLC purity (system 1: 100%, system 2: 100%).

## Enzyme Inhibition

The expression and purification of all enzyme variants were performed according to previously published methods.<sup>5,6</sup> An R155K variant (NS3<sub>fl</sub> R155K<sup>1a</sup>), in which the arginine (R) to lysine (K) mutation was introduced by PCR at position 155 was cloned in a similar way as has been described previously.<sup>6</sup> The protease activity of the full-length HCV NS3 protein (protease–helicase/NTPase from genotype 1a and the substituted variants A156T, D168T and R155K respectively) were measured using a FRET-assay as described previously.<sup>5,6</sup> In short, 1nM enzyme was incubated for 10 min at 30 °C in 50 mM HEPES, pH 7.5, 10 mM DTT, 40% glycerol, 0.1% *n*-octyl- $\beta$ -D-glucoside, 3.3% DMSO with 25  $\mu$ M of the peptide cofactor 2K-NS4A (KKGSVVIVGRIVLSGK), and inhibitor. The reaction was started by the addition of 0.5  $\mu$ M substrate (Ac-DED(Edans)EEAbu $\psi$ [COO]ASK(Dabcyl)-NH<sub>2</sub>) obtained from AnaSpec Inc. (San Jose, USA). Non-linear regression analysis of the data was made using Grafit 5.0.13 (Erithacus software limited).

## Molecular Modeling

The crystal structure (pdb code 4A92)<sup>7</sup> was prepared using the *Protein Preparation Wizard* implemented in Maestro (Schrödinger 2011). The A subunit was deleted and the B subunit was used in all modeling studies. Hydrogen atoms were added, correct bond orders assigned and crystallographic water molecules were deleted. The structure was minimized using restrained minimization with the OPLS-2005 force field and normal BatchMin cutoffs. Finally, a truncated protein structure that included only amino acids within 20 Å from ligand was created for docking studies.

To account for the conformational changes in the protein arising from the substitutions, induced fit docking was carried out using FLO (QXP 200605).<sup>8</sup> Protein flexibility was accounted for by allowing crucial amino acid residues in the binding pocket to move freely up to 0.2 Å. Movements larger than 0.2 Å were penalized by 20.0 kJ/mol/Å<sup>2</sup>. Flexible residues occupying the active site, i.e. Arg155, Gln526 and Lys136 were given full conformational freedom. Hydrogen bond constraints were applied between the inhibitors and the protein to restrict their translation away from the active site. These constraints mimic the hydrogen bond pattern found between the bound cleavage product and the protein. Four hydrogen bond constraints were used: between NH of Ala157 and the backbone carbonyl oxygen of P3 substituent, between the backbone carbonyl oxygen of Arg155 and NH of the P1 substituent, and between the NHs of Gly137 and Ser139 and the corresponding carbonyl oxygen of the P1. For each inhibitor, 10 unique binding poses were generated using 6000 Monte Carlo perturbation cycles (mcdock). These poses were further subjected to 20 steps of simulated annealing followed by energy minimization. Each cycle involved 400 rapid Monte Carlo steps generating unique conformations within an energy window of 50 kJ/mol/Å and an RMSD > 0.5 Å. Each of the complexes was submitted to a 3 fs dynamics at 600 K after 3000 fs of equilibration steps. The maximum movement of an atom in any single step was limited to 0.1 Å. Hydrogen vibrations were damped by assigning an atomic weight of 10 to the hydrogens. Among the 10 best poses generated, the most plausible binding mode conformation for each compound was selected based on visual inspection

## *In silico* predictions

The predicted  $pK_a$  and  $\log D_{7.4}$  values for compounds **2**, **12**, **13**, **14**, **18L** and **23** were calculated using ADMET predictor v.5.5 and are presented in Table 4 in the manuscript.

## *In vitro* preclinical profiling

### Metabolic Stability

The  $Cl_{int}$  can be used predictively as measurement of the *in vivo* metabolic stability when the total clearance mechanism can be described as hepatic and metabolic, and when oxidative metabolism dominates. Compounds **2**, **12**, **13**, **14**, **18L** and **23** (1  $\mu$ M) were pre-incubated for 5 min at 37 °C with pooled human liver microsomes (0.5 mg/mL; Xenotech, Kansas, KS) in 0.1 M potassium phosphate buffer pH 7.4 prior to the addition of NADPH (1 mM) to initiate the reaction. The reaction was then incubated for 0, 5, 15 and 40 min and at each time point the reaction was quenched by addition of ice-cold acetonitrile (35% final concentration) containing 0.1 nM Warfarin as an internal standard. Plates were centrifuged at 3,500 rpm for 20 min at 4 °C, and the supernatants were subjected to LC/MS/MS. The natural logarithm of the analytical peak area ratio (relative to 0 min sample which

was considered as 100%) was plotted against time and analyzed by linear regression. *In vitro* half-life ( $t_{1/2}$ ) and *in vitro* intrinsic clearance ( $Cl_{int}$ ) were calculated on the basis of first-order reaction kinetics of the percentage of remaining compound according to previously published models.<sup>9,10</sup> Dextromethorphan (3  $\mu$ M) and Midazolam (5  $\mu$ M) were used as positive controls for cytochrome P450 enzymes (CYP) isoforms CYP2D6 and CYP3A4, respectively. The cut-off values that were used to classify the compounds regarding metabolic stability follows:  $Cl_{int} < 47$  ( $\mu$ l/min/mg) indicates a low risk for high first metabolism *in vivo*,  $47 < Cl_{int} < 92$  a moderate risk, and  $Cl_{int} > 92$  a high risk.

### Solubility determinations

Determination of solubility was performed by addition of an aliquot of DMSO stock (10 mM) to phosphate buffered saline (PBS) (final DMSO concentration 1%). Incubation with rotation in HPLC glass vials for 1 h at 37 °C. The samples were then centrifuged at 16 000 x g for 20 min and the supernatant analyzed with LC-MS/MS. This medium throughput method can thus only detect solubilities below 100  $\mu$ M.

### Cell Culture

Caco-2 cells, obtained from American Tissue Collection, Rockville, MD, were maintained in an atmosphere of 90% air and 10% CO<sub>2</sub>, as described previously.<sup>11</sup> For transport experiments,  $3.0 \times 10^5$  cells were seeded on polycarbonate filter inserts (12 mm diameter; pore size 0.4  $\mu$ m; Costar, Cambridge, MA) and allowed to grow and differentiate for 21-24 days. The monolayers integrity was assessed by measuring the paracellular marker [<sup>14</sup>C]-Mannitol (1.0  $\mu$ Ci/mmol; Perkin-Elmer Life Sciences, Boston, MA) transport and the transepithelial electrical resistance (TEER) before and after the experiments.

### Permeability Experiments

Stock solutions (10 mM) of the compounds were prepared in DMSO and diluted to 10  $\mu$ M (final DMSO concentration of 0.1%) in fasted state simulated intestinal fluid (FASSIF) at pH 7.4. HBSS pH 7.4 supplemented with 1 % (w/v) BSA was prepared to be used in the receiving, basolateral, compartment.

The Caco-2 study was performed in accordance with published protocols.<sup>11</sup> Caco-2 cell monolayers (passage 94-105) were grown on permeable filter support and used for transport study on day 21 after seeding. Prior to the experiment a drug solution of 10  $\mu$ M was prepared and warmed to 37 °C. The Caco-2 filters were washed with pre-warmed Hank's balanced salt solution (HBSS) prior to the experiment, and thereafter the experiment was started by applying the donor solution on the apical side. The experiments were performed at 37 °C and with a stirring rate of 500 rpm. The receiver compartment was sampled at 15, 30 and 60 minutes, and at 60 minutes also a final sample from the donor chamber was taken in order to calculate the mass balance of the compound. Directly after the termination of the experiment the filter inserts were washed with pre-warmed HBSS and the membrane integrity was checked. This was performed by transepithelial electrical resistance (TEER) measurement and by measurement of Mannitol permeability, which is a paracellular marker used for integrity measurements.

For transport studies performed under sink conditions, where less than 10% of the compound was transported across the Caco-2 cell monolayers, the apparent permeability coefficients ( $P_{app}$ ) were calculated from the equation

$$P_{app} = \frac{\Delta Q}{\Delta t} \times \frac{1}{AC_0}$$

Where  $\frac{\Delta Q}{\Delta t}$  is the steady-state flux (mol/s),  $C_0$  is the initial concentration in the donor chamber at each time interval (mol/mL), and  $A$  is the surface area of the filter (cm<sup>2</sup>).  $P_{app}$  was obtained from nonlinear regression of the accumulated dose in the receiver compartment over time, minimizing the sum of squared residuals in the equation.

A  $P_{app}$  value below  $0.2 \times 10^{-6}$  cm/s indicates low permeability, a  $P_{app}$  value between  $0.2 \times 10^{-6}$  cm/s to  $1.6 \times 10^{-6}$  cm/s indicates moderate permeability, and a  $P_{app}$  value above  $1.6 \times 10^{-6}$  cm/s indicates high permeability.<sup>12</sup>



## Data Analysis.

All experiments were performed in, at least, triplicates, and samples were subjected to liquid chromatography/mass spectrometry analysis with a Waters XEVO TQ triple-quadrupole mass spectrometer (electrospray ionization, ESI) coupled to a Waters Acquity UPLC (Waters Corp.). For chromatographic separation a general gradient was used (1% mobile phase B to 90% over 2.5 min total run) on a C18 BEH 1.7  $\mu\text{m}$  column 2x50mm (Waters Corp.). Mobile phase A consisted of 5% acetonitrile 0.1% formic acid and mobile phase B 100% acetonitrile 0.1% formic acid. The flow rate was 0.5 mL/min. 5  $\mu\text{L}$  of the sample were injected onto a 10 $\mu\text{L}$  loop in partial injection mode using the MS settings in Table 1. In general, a standard curve between 1-1000 nM was prepared for quantization. Warfarin was used as an internal standard in all samples.

**Table S1. Mass spectrometric specific settings used for detection.**

Compound	ESI (+/-)	m/z (parent)	m/z (product)	Cone Voltage (V)	Collision energy (V)
<b>12</b>	+	804.4	748.3	24	16
<b>13</b>	+	804.4	748.3	28	20
<b>14</b>	+	804.4	748.3	28	18
<b>18L</b>	+	917.5	817.4	22	18
<b>23</b>	+	803.4	687.2	22	26
<b>2</b>	+	728.4	672.3	24	16

Radioactive samples ( $^{14}\text{C}$ -Mannitol) were analyzed with a liquid scintillation counter (TopCount NXT, Perkin-Elmer Life Sciences, Boston, MA).

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