Discovery of Thienoimidazole-Based HCV NS5A Genotype 1a and 1b Inhibitors.

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Supporting Information

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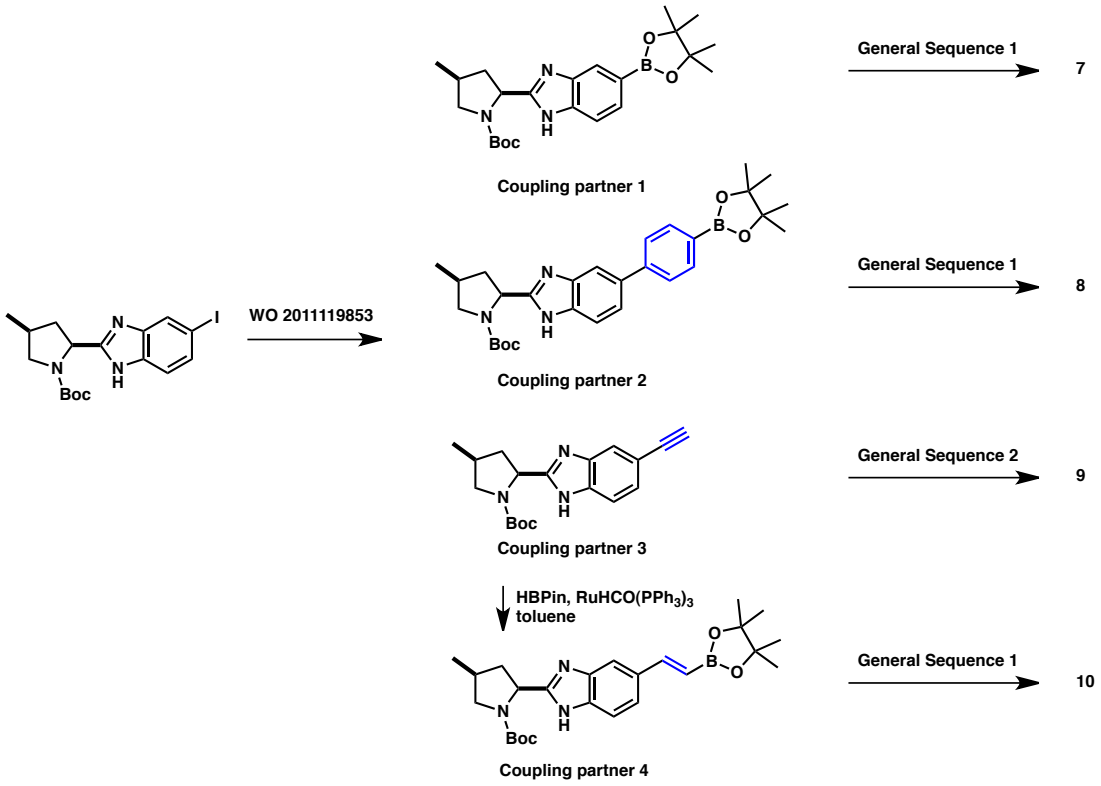
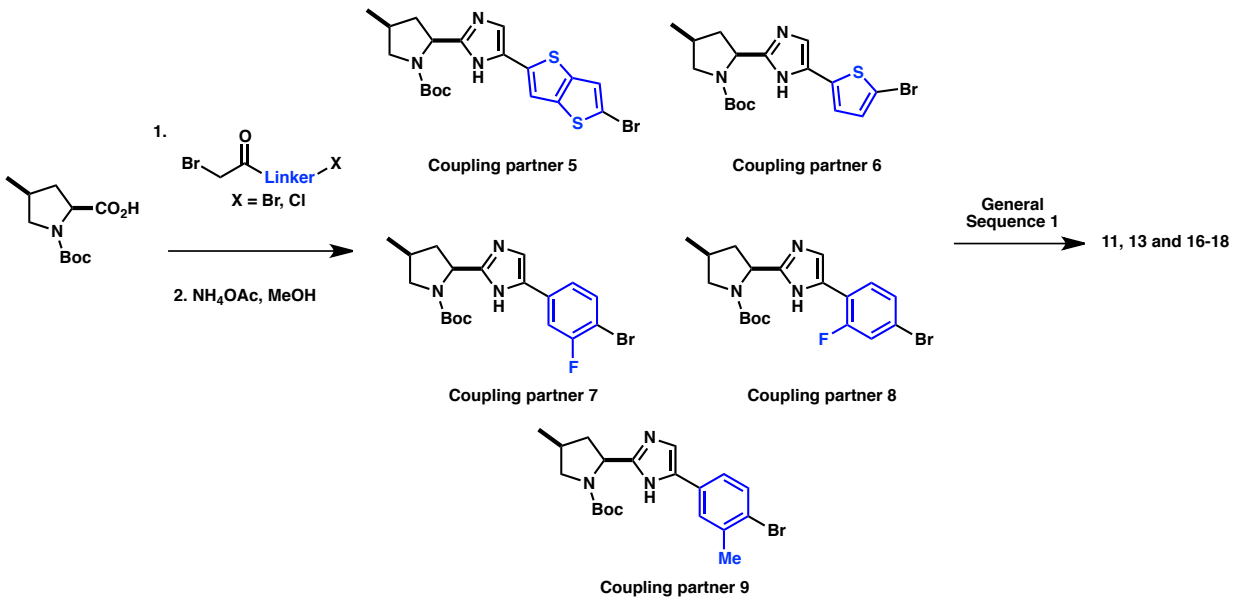
General experimental details

All commercial reagents and anhydrous solvents were obtained from commercial sources and were used without further purification, unless otherwise specified. Mass samples were analyzed on a MicroMass ZQ, ZMD, Quattro LC, or Quattro II mass spectrometer operated in a single MS mode with electrospray ionization. Samples were introduced into the mass spectrometer using flow injection (FIA) or chromatography. The mobile phase for all mass analysis consisted of acetonitrile-water mixtures with either 0.2% formic acid or ammonium formate. High-resolution mass spectra were measured using a 9.4T APEX III FTMS Bruker Daltonics instrument; chromatography was performed on a Waters Sunfire C18 5um, 4.6x50mm column with a 10-90% gradient over 3 minutes with a total run time of 5 minutes. ^{1}H NMR and ^{13}C NMR spectra were recorded either using a Bruker Avance 400 (400 MHz) or a Bruker Avance II-300 (300 MHz) instrument. Column chromatography was performed using an ISCO Combiflash or glass column packed with Merck silica gel 60 (0.040-0.063 mm). The final purity of all compounds tested was > 95% as determined by UV absorbance on HPLC. Typical HPLC conditions were as follows: chromatography was performed on a YMC Pack Pro C18 5um, 2x50mm column with a 0-100% (water/acetonitrile/0.1%TFA) gradient over 4 minutes with a total run time of 7.5 minutes. The flow rate is 0.8 ml/min. Preparative reversed phase chromatography was carried out using a Gilson 215 liquid handler coupled to a UV-VIS 156 Gilson detector, an Agilent Zorbax SB-C18 column, 21.2x100 mm, a linear gradient from 10% to 90% CH_3CN in H_2O over 10 min (0.1% TFA); flow rate 20 mL/min.

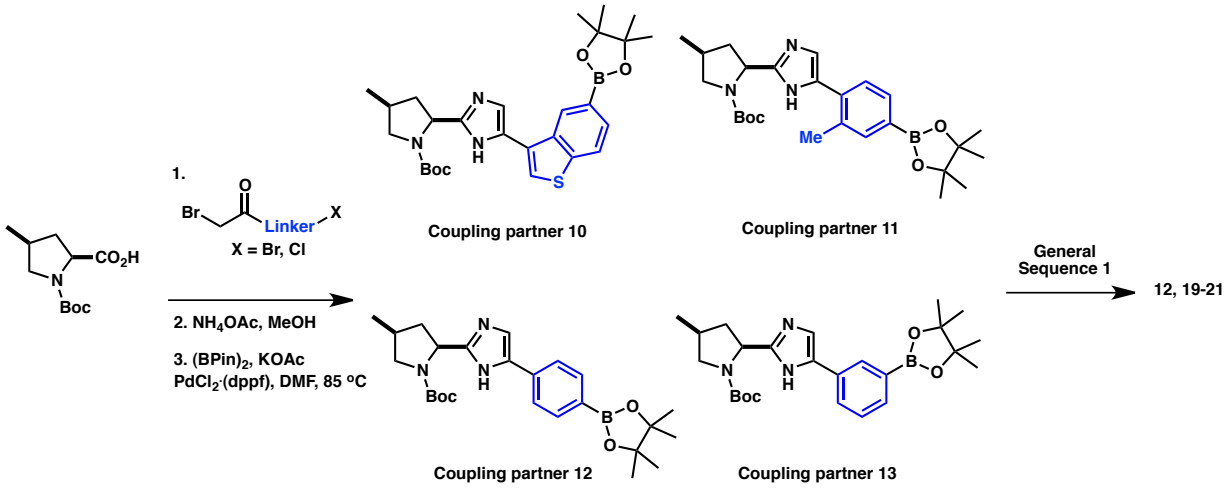
General scheme for the preparation of compounds **7-10**.



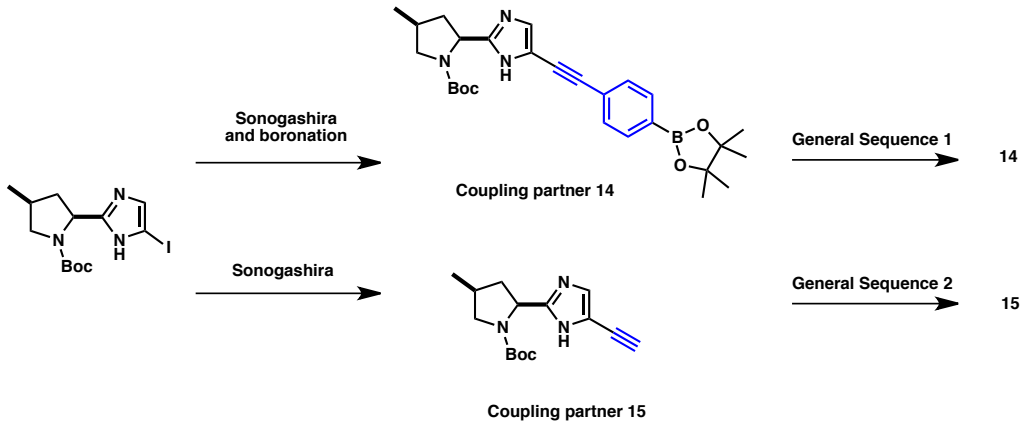
General scheme for the preparation of compounds **11**, **13** *and* **16-18**.



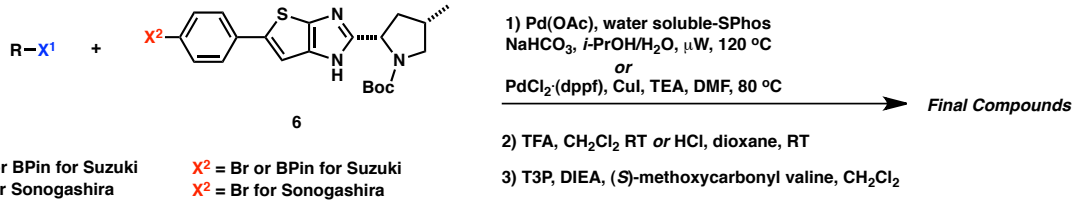
General scheme for the preparation of compounds **12** and **19-21**.



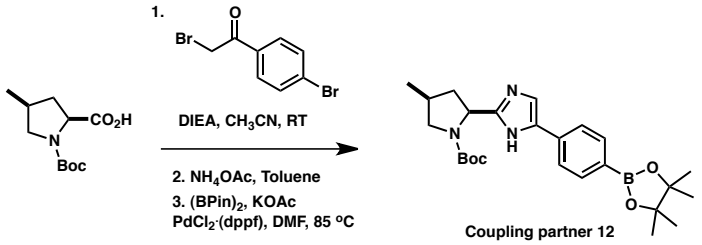
General scheme for the preparation of compounds **14** *and* **15**.



General sequences 1 and 2 for the preparation of all final compounds.



Example of a preparation of a coupling partner (coupling partner 12)



Step 1: A solution of (2*S*, 4*S*)-1-*tert*-butoxycarbonyl-4-methyl-pyrrolidine-2-carboxylic acid (2.0 g, 8.8 mmol), 2-bromo-1-(4-bromophenyl)ethanone (2.5 g, 8.0 mmol) and DIEA (1.13 g, 1.53 mL, 8.8 mmol) in CH_3CN (22 mL) was stirred at RT for 18h. The resulting mixture was diluted with H_2O and EtOAc. The aqueous phase was extracted with EtOAc and the organic phase was dried over Na_2SO_4. After evaporation, the residue was purified by flash chromatography on silica gel (10→25% EtOAc in hexanes) to give the corresponding ester (3.4 g, 99%) as a colorless oil.

^{1}H NMR (300 MHz, DMSO-d_6) δ 7.91 (dd, *J* = 8.5, 3.9 Hz, 2H), 7.78 (d, *J* = 8.5 Hz, 2H), 5.65-5.37 (m, 2H), 4.30 (t, *J* = 8.2 Hz, 1H), 3.62 (t, J = 8.7 Hz, 1H), 2.83 (dd, *J* = 20.2, 10.2 Hz, 1H), 2.48-2.07 (m, 2H), 1.64 (tdd, *J* = 15.5, 10.4, 5.5, 1H), 1.37 (d, *J* = 11.7 Hz, 9H), 1.02 (d, *J* = 6.5 Hz, 3H)

LC/MS: m/z = 426.38 ($M+H^+$), r.t. = 3.94 min

Step 2: A solution of the ester obtained from step 1 (40.7 g, 95.5 mmol), NH_4OAc (147.2 g, 1.91 mol) in toluene (500 mL) was heated to 100 °C for 5h. The solution was then cooled to RT and diluted with H_2O. The aqueous phase was extracted with EtOAc and the organic phase was dried over Na_2SO_4. After evaporation, the residue was purified by flash chromatography on silica gel (10→40% EtOAc in hexanes) to give the corresponding imidazole (24.8 g, 64%) as a light yellow solid.

^{1}H NMR (300 MHz, DMSO-d_6) δ 12.0 (s, 1H), 7.88-7.20 (m, 5H), 4.91-4.52 (m, 1H), 3.67 (dd, *J* = 17.6, 8.6 Hz, 1H), 3.02 (t, *J* = 10.1 Hz, 1H), 2.41-2.05 (m, 2H), 1.63 (dd, *J* = 24.3, 13.3 Hz, 1H), 1.37 (s, 3H), 1.20-0.89 (m, 9H)

LC/MS: m/z = 406.31 ($M+H^+$), r.t. = 1.90 min

Step 3: To a solution of the above imidazole (250 mg, 0.614 mmol), $(BPin)_2$ (623 mg, 2.45 mmol) and KOAc (301 mg, 3.07 mmol) in DMF (4 mL) was added $PdCl_2$·(dppf)

(50.1 mg, 0.0614 mmol). The reaction was then degassed by a N_2 flow using a needle. The solution was then sealed and heated at 90 ^oC in a microwave for 5 min. The solution was then cooled to RT and diluted with H_2O. The aqueous phase was extracted with EtOAc and the organic phase was dried over Na_2SO_4. After evaporation, the residue was purified by flash chromatography on silica gel (50% EtOAc in hexanes) to give the corresponding boronate (coupling partner 12) (223 mg, 80%) as a light yellow foam.

^{1}H NMR (300 MHz, DMSO-d_6) δ 7.84-7.50 (m, 5H), 4.68 (d, J = 7.5 Hz, 1H), 3.95 (s, 1H), 3.80-3.54 (m, 1H), 3.03 (t, J = 10.2 Hz, 1H), 2.44-2.10 (m, 2H), 1.37 (s, 3H), 1.29 (s, 9H), 1.06 (d, J = 5.9 Hz, 12H)

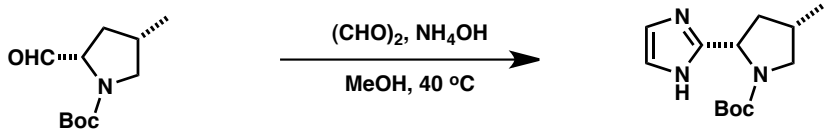
LC/MS: m/z = 454.00 ($M+H^+$), r.t. = 1.99 min

Additional characterization data for the coupling partners.

Coupling Partners	LCMS ($M+H^+$)	r.t. (min)
1	428.62	3.12
2	504.50	3.57
3	326.38	2.77
4	454.72	2.34
5	469.83	2.56
6	414.09	2.09
7	424.04	2.15
8	424.36	2.16
9	420.55	1.99
10	510.39	2.21
11	468.71	2.09
12	454.77	3.35
13	477.90	3.33
14	276.30	1.57

Experimental procedures for the preparation of key compound **20**.

(2*S*,4*S*)-*tert*-butyl 2-(1*H*-imidazol-2-yl)-4-methylpyrrolidine-1-carboxylate (3)



3

To a stirred solution of *tert*-butyl (2*S*,4*S*)-2-formyl-4-methyl-pyrrolidine-1-carboxylate[1] (6 g, 28.13 mmol) in methanol (12 mL) was added NH_4OH (12 mL, 28 % aqueous). The reaction mixture was warmed to 40 °C and glyoxal (12.24 mL of 40% w/v, 84.4 mmol) in H_2O (12 mL) was added over 30 min. The resulting reaction mixture was stirred for 30 min. at which point TLC analysis revealed consumption of the starting material. The reaction mixture was cooled to ambient temperature and H_2O (24 mL) and CH_2Cl_2 (24 mL) were added. The pH was adjusted to 2.0 using 6 *N* HCl (12 mL). The organic layer was separated. After evaporation, the residue was purified by flash chromatography on silica gel (0→100% EtOAc in hexanes) to give the corresponding imidazole **3** (4.1 g, 57%) as a white solid.

^{1}H NMR (300 MHz, DMSO-d_6) δ 11.79 (m, 1H), 6.86 (d, J = 59.1 Hz, 2H), 4.83-4.50 (m, 1H), 3.82-3.3.49 (m, 1H), 2.97 (t, J = 10.1 Hz, 1H), 2.38-2.05 (m, 2H), 1.81-1.46 (m, 1H), 1.37 (s, 3H), 1.21-0.77 (m, 9H)

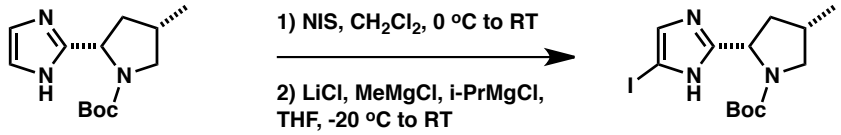
^{13}C NMR (125 MHz, $CDCl_3$) δ 156.3, 148.7, 80.2, 54.3, 54.2, 36.6, 32.8, 28.4, 16.8 ppm

$[\alpha]_D$: -270 (*c* = 1, $CHCl_3$)

LC/MS: m/z = 255.22 ($M+H^+$), r.t. = 1.29 min

m.p.: 190-192 °C

(2*S*,4*S*)-*tert*-butyl 2-(5-iodo-1*H*-imidazol-2-yl)-4-methylpyrrolidine-1-carboxylate (4)



3 **4**

Step 1: To a stirred solution of **3** (10 g, 39.8 mmol) in CH_2Cl_2 (100 mL) was slowly added *N*-iodosuccinimide (19.3 g, 85.6 mmol) at 0 °C. The reaction was allowed to warm to RT over 15 h. The reaction was quenched with with a 10% aq. Na_2SO_3 solution (40 mL), the organic phase was extracted with CH_2Cl_2 and dried over Na_2SO_4. After evaporation, the residue was purified by flash chromatography on silica gel (10→40% EtOAc in hexanes) to give the corresponding diiodoimidazole (19.1 g, 95%) as a white foam.

^{1}H NMR (300 MHz, CDCl3) δ 10.89 (s, 1H), 7.06 (s, 1H), 4.86 (m, 1H), 3.77 (dd, *J* = 10.3, 7.4 Hz, 1H), 2.83 (t, *J* = 10.4 Hz, 1H), 2.65 – 2.34 (m, 2H), 2.22 (qd, *J* = 13.3, 6.7 Hz, 1H), 1.43 (s, 9H), 1.10 (d, *J* = 6.5 Hz, 3H)

^{13}C NMR (125 MHz, $CDCl_3$) δ 156.7, 151.0, 122.6, 80.6, 77.2, 54.3, 54.1, 36.1, 32.8, 28.4, 16.6 ppm

$[\alpha]_D$: -74.1 (*c* = 0.5, $CHCl_3$)

LC/MS: m/z = 504.13 ($M+H^+$), r.t. = 2.96 min

HRMS calcd for $C_{13}H_{19}I_2N_3O_2$ (M+H+): 503.9645; found: 503.9652

m.p.: 179-180 °C

Step 2: A solution of the above diiodoimidazole (19 g, 37.8 mmol) and LiCl (1.6 g, 37.8 mmol) in THF (100 mL) was stirred at RT for 10 min and cooled down to -20 °C. Then, a solution of MeMgCl (12.6 mL, 3*M* in THF, 37.8 mmol) was added dropwise, keeping the internal temperature to -20 °C. After 30 min, a solution of *i*-PrMgCl (37.8 mL, 2*M* in THF, 75.5 mmol) was added dropwise, keeping the internal temperature to -20 °C. The reaction was then slowly warmed to RT over 3h and then cooled to 0 °C and quenched with saturated aq. NH_4Cl (50 mL). Water (200 mL) was added the organic phase was extracted with EtOAc and dried over Na_2SO_4. After evaporation, the residue was purified

by flash chromatography on silica gel (0→70% EtOAc in hexanes) to give **4** (10.9 g, 95%) as a white solid.

^{1}H NMR (300 MHz, $CDCl_3$) δ 11.07 (s, 1H), 4.86 (t, *J* = 8.3 Hz, 1H), 3.77 (dd, *J* = 10.6, 7.1 Hz, 1H), 2.83 (t, *J* = 10.4 Hz, 1H), 2.55 – 2.34 (m, 2H), 2.24 (dd, *J* = 10.2, 6.6 Hz, 1H), 1.50 (s, 9H), 1.09 (d, *J* = 6.4 Hz, 3H)

^{13}C NMR (125 MHz, $CDCl_3$) δ 156.7, 154.6, 81.0, 77.2, 54.2, 35.9, 32.8, 28.4, 16.6 ppm

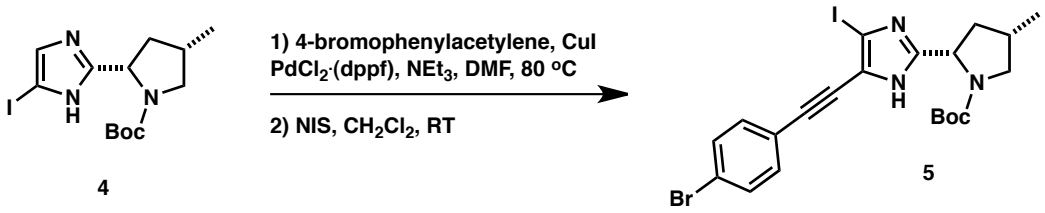
LC/MS: m/z = 378.00 ($M+H^+$), r.t. = 2.59 min

HRMS calcd for $C_{13}H_{20}IN_3O_2$ (M+H+): 378.0679; found: 378.0686

IR: (film, cm^{-1}) 2975, 1684, 1407, 1163

m.p.: 177-178 °C

(2*S*,4*S*)-*tert*-butyl 2-(5-((4-bromophenyl)ethynyl)-4-iodo-1*H*-imidazol-2-yl)-4-methylpyrrolidine-1-carboxylate (5)



4 5

Step 1: A solution of 4-bromophenylacetylene (408 mg, 2.25 mmol), **4** (850 mg, 2.25 mmol) and triethylamine (1.14 g, 11.2 mmol) in DMF (22 mL) was degassed with a N_2 flow for 15 min. To this solution was added $PdCl_2$·(dppf) (92 mg, 0.113 mmol) and CuI (43 mg, 0.225 mmol). The resulting solution was stirred at 80 °C for 16h, cooled down to RT and diluted with EtOAc and water. The organic phase was extracted with EtOAc and dried over Na_2SO_4. After evaporation, the residue was purified by flash chromatography on silica gel (0→100% EtOAc in hexanes) to give the desired alkyne (810 mg, 84%) as a brown solid.

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.47 (d, *J* = 8.5 Hz, 2H), 7.38 (dd, *J* = 2.0, 6.6 Hz, 2H), 7.27 (s, 1H), 4.93 - 4.87 (m, 1H), 3.79 (dd, *J* = 7.4, 10.1 Hz, 1H), 2.87 (t, *J* = 10.3 Hz, 1H), 2.62 - 2.45 (m, 2H), 2.28 (s, 2H), 1.51 (s, 9H) and 1.12 (d, *J* = 6.4 Hz, 3H)

LC/MS: m/z = 432.0 ($M+H^+$), r.t. = 3.40 min

Step 2: To a solution of the above alkyne (3.3 g, 7.67 mmol) in CH_2Cl_2 (77 mL) was added *N*-iodosuccinimide (1.89 g, 8.44 mmol) in one portion at 0 °C. The resulting brownish solution was stirred at 0 °C while warming gradually to RT over 90 min. The solution was directly purified by flash chromatography on silica gel (0→100% EtOAc in hexanes) to give **5** (4.1g, 96%) as a yellow powder.

^{1}H NMR (300 MHz, $CDCl_3$) δ 11.02 (d, *J* = 5.8 Hz, 1H), 7.51 (d, *J* = 8.5 Hz, 2H), 7.41 (d, *J* = 8.5 Hz, 2H), 4.89 - 4.83 (m, 1H), 3.81 - 3.72 (m, 1H), 2.87 - 2.81 (m, 1H), 2.48 - 2.44 (m, 2H), 2.24 (d, *J* = 4.5 Hz, 1H), 1.51 (s, 9H) and 1.11 (d, *J* = 6.5 Hz, 3H)

^{13}C NMR (125 MHz, $CDCl_3$) δ 156.1, 151.6, 132.8, 131.5, 122.6, 121.6, 94.2, 80.8, 80.3, 77.3, 60.4, 54.2, 37.5, 32.9, 28.5, 16.5 ppm

LC/MS: m/z = 556.37 ($M+H^+$), r.t. = 3.81 min

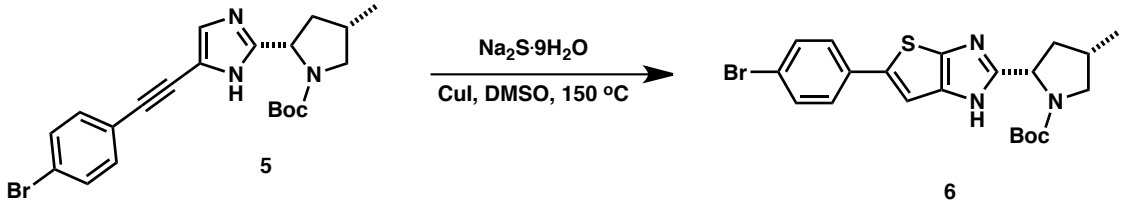
HRMS calcd for $C_{21}H_{23}BrIN_3O_2$ ($M+H^+$): 556.0097; found: 556.0091

$[\alpha]_D$: -40.8 (*c* = 1, $CHCl_3$)

IR: (film, cm^{-1}) 2975, 1667, 1408, 1214, 1161

m.p.: 107-109 °C

(2*S*,4*S*)-*tert*-butyl 2-(5-(4-bromophenyl)-1*H*-thieno[2,3-*d*]imidazol-2-yl)-4-methylpyrrolidine-1-carboxylate (6)



To a solution of **5** (7.9 g, 14.2 mmol) and CuI (3.2 g, 17.0 mmol) in DMSO (142 mL) was added $Na_2S{\cdot}9H_2O$ (6.8 g, 28.4 mmol) at r.t. The solution was then heated to 150 °C. After 3h, the solution is cooled to r.t. and dissolved with EtOAc and water, the aqueous phase was then extracted with EtOAc and dried over Na_2SO_4. After evaporation, the residue was purified by flash chromatography on silica gel (0 to 100% EtOAc in hexanes) to give **6** (2.56 g, 39%) as a pale yellow solid.

^{1}H NMR (400 MHz, CDCl3) δ 11.06 (s, 1H), 7.54 – 7.29 (m, 4H), 7.04 (s, 1H), 4.96 (t, *J* = 8.2 Hz, 1H), 3.74 (dd, *J* = 10.5, 7.1 Hz, 1H), 2.83 (t, *J* = 10.2 Hz, 1H), 2.63 – 2.36 (m, 2H), 2.32 – 2.12 (m, 1H), 1.43 (s, 9H), 1.06 (d, *J* = 6.5 Hz, 3H)

^{13}C NMR (125 MHz, $CDCl_3$) δ 156.1, 153.5, 139.4, 135.2, 134.3, 131.6, 126.5, 120.6, 106.2, 80.6, 55.4, 54.3, 38.0, 33.0, 28.5, 16.7 ppm

LC/MS: m/z = 462.65 ($M+H^+$), r.t. = 3.69 min

HRMS calcd for $C_{21}H_{24}BrN_3O_2S$ ($M+H^+$): 462.0851; found: 462.0863

$[\alpha]_D$: -45.4 (*c* = 1, $CHCl_3$)

IR: (film, cm^{-1}) 1671, 1393, 1214

m.p.: 116-118 ^oC

3-step conversion of **6** to **20** according to the general sequence 1:

Step 1: A solution of coupling partner 12 (981 mg, 2.16 mmol), **6** (1 g, 2.16 mmol), water-soluble S-Phos (333 mg, 0.65 mmol) in isopropanol (22 mL) / $NaHCO_3$ (11 mL of 1 *M*, 11 mmol) was degassed for 30 min with a flow of N_2. Then, $Pd(OAc)_2$ (39 mg, 0.17 mmol) was added and the solution was heated to 100 ^oC for 15h. The solution was cooled down to r.t. and diluted with EtOAc and water. The aqueous phase was extracted with EtOAc and the organic phase was dried over Na_2SO_4. After evaporation, the residue was purified by flash chromatography on silica gel (0 to 100% EtOAc in hexanes) to give the corresponding Suzuki adduct (880 mg, 57%) as a yellow solid which was taken directly to the next step.

LC/MS: m/z = 709.31 ($M+H^+$), r.t. = 2.15 min

Step 2: A solution of the above Suzuki adduct (58 mg, 0.097 mmol) in TFA (1 mL) and CH_2Cl_2 (1 mL) was stirred for 1h at RT. The solvent were removed *in vacuo* to afford the *N*-Boc-deprotected adduct which was taken directly to the next step.

LC/MS: m/z = 509.73 ($M+H^+$), r.t. = 1.53 min

Step 3: To a solution of the *N*-Boc-deprotected adduct (69.6 mg, 0.081 mmol), HATU (65.3, 0.17 mmol) in DMF (2 mL) was added DIEA (142.5 μL, 0.81 mmol). The solution was then stirred at RT for 3 h. The reaction was then diluted with H_2O and EtOAc. The aqueous phase was extracted with EtOAc. The combined organic phases were dried using

$MgSO_4$, evaporated and the residue was purified by flash chromatography to afford **20** (49.7 mg, 73%) as a white powder.

^{1}H NMR (300 MHz, DMSO) δ 12.30 (m, 2H), 7.97 – 7.62 (m, 8H), 7.51 (dd, *J* = 7.9, 3.8 Hz, 2H), 7.20 (m, 2H), 4.96 (dd, *J* = 18.7, 9.2 Hz, 2H), 4.18 – 3.95 (m, 4H), 3.54 (s, 6H), 3.23 (m, 2H), 2.46 – 2.09 (m, 4H), 1.95 – 1.63 (m, 4H), 1.10 (d, *J* = 6.3 Hz, 6H), 0.82 (dt, *J* = 19.3, 6.9 Hz, 12H)

^{13}C NMR (125 MHz, $CDCl_3$) δ 172.1, 172.0, 157.1, 152.1, 148.1, 142.8, 140.9, 135.0, 143.1, 126.8, 126.7, 125.4, 124.9, 105.7, 69.7, 57.9, 55.8, 55.1, 52.4, 33.7, 31.3, 19.2, 18.8, 17.5, 16.3 ppm

LC/MS: m/z = 823.09 ($M+H^+$), r.t. = 2.09 min

HRMS calcd for $C_{44}H_{54}N_8O_6S$ ($M+H^+$): 823.3965; found: 823.3956

[a]$_D$: -12.1 (*c* = 0.12, $CHCl_3$)

IR: (film, cm^{-1}) 1711, 1616, 1514, 1214

m.p.: 205 ^oC

Additional data for selected final compounds.

Compound 8:

^{1}H NMR (400 MHz, Acetone-d_6) δ 11.1 (m, 2H), 7.77 (m, 12H), 7.50 (m, 2H), 6.23 (s, 2H), 5.27 (m, 2H), 4.56 – 4.01 (m, 4H), 3.68 (m, 4H), 3.60 (s, 6H), 3.27 (m, 4H), 1.06 – 0.77 (m, 18H)

LC/MS: m/z = 873.34 ($M+H^+$), r.t. = 2.99 min

HRMS calcd for $C_{48}H_{56}N_8O_6S$: 873.4122; found: 873.4094

Compound 9:

^{1}H NMR (300 MHz, DMSO-d_6) δ 7.93 (s, 1H), 7.75 (dd, *J* = 8.2, 6.0 Hz, 3H), 7.67 – 7.49 (m, 4H), 7.27 – 7.08 (m, 2H), 5.20 – 5.08 (m, 1H), 5.01 (t, *J* = 8.6 Hz, 1H), 4.27 – 3.97 (m, 4H), 3.54 (s, 6H), 3.38 (d, *J* = 9.9 Hz, 1H), 2.65 – 2.18 (m, H), 2.01 –1.60 (m, 4H), 1.12 (dd, *J* = 8.1, 6.5 Hz, 6H), 0.80 (m, 12H)

LC/MS: m/z = 821.53 ($M+H^+$), r.t. = 3.07 min

HRMS calcd for $C_{44}H_{52}N_8O_6S$ ($M+H^+$): 821.3809; found: 821.3804

$[\alpha]_D$: 11.1 (*c* = 0.35, $CHCl_3$)

m.p.: 208-210 °C

Compound 10:

^{1}H NMR (300 MHz, DMSO-d_6) δ 7.91 (d, *J* = 1.4 Hz, 1H), 7.82 (d, *J* = 9.1 Hz, 1H), 7.76 (d, *J* = 8.6 Hz, 1H), 7.68 (d, *J* = 2.0 Hz, 4H), 7.56 (s, 1H), 7.48 (d, *J* = 16.3 Hz, 1H), 7.37 (d, *J* = 16.5 Hz, 1H), 7.25 (d, *J* = 8.3 Hz, 1H), 7.19 (d, *J* = 8.3 Hz, 1H), 5.15 (dd, *J* = 10.4, 7.0 Hz, 1H), 5.00 (t, *J* = 8.4 Hz, 1H), 4.39 – 3.98 (m, 4H), 3.54 (s, 6H), 3.42 (t, *J* = 10.1 Hz, 1H), 3.26 (t, *J* = 10.1 Hz, 1H), 2.61-2.23 (m, 4H), 2.04 – 1.57 (m, 4H), 1.15-1.09 (m, 6H), 0.97 – 0.41 (m, 12H)

LC/MS: m/z = 823.80 ($M+H^+$), r.t. = 2.25 min

HRMS calcd for $C_{44}H_{54}N_8O_6S$ ($M+H^+$) 823.3965; found: 8823.3953

$[\alpha]_D$: 11.0 (*c* = 0.35, $CHCl_3$)

m.p.: 213-215 °C

Compound 11:

^{1}H NMR (400 MHz, Acetone-d_6) δ 7.87-7.65 (m, 1H), 7.56-7.46 (m, 1H), 7.42 (s, 2H), 7.38 (s, 2H), 7.12-6.90 (m, 2H), 6.57-6.38 (m, 2H), 5.29 (s, 2H), 4.54 ? 4.13 (m, 4H), 3.63 (s, 6H), 3.52 (s, 1H), 2.68-2.53 (m, 2H), 2.53-2.40 (m, 2H), 2.40-2.26 (m, 1H), 2.27-2.09 (m, 5H), 1.21 (m, 6H), 1.08 (d, *J* = 6.8 Hz, 4H), 1.02-0.94 (m, 5H), 0.90 (d, *J* = 6.6 Hz, 4H)

LC/MS: m/z = 885.47 ($M+H^+$), r.t. = 2.40 min

HRMS calcd for $C_{44}H_{52}N_8O_6S_3$: 885.3250; found: 885.3237

m.p.: 198-200 °C

Compound 13:

^{1}H NMR (300 MHz, DMSO-d_6) δ 7.98 – 7.90 (m, 1H), 7.79 – 7.66 (m, 4H), 7.64 (d, *J* = 3.8 Hz, 1H), 7.59 (s, 1H), 7.50 (d, *J* = 3.8 Hz, 1H), 7.30 (d, *J* = 8.3 Hz, 1H), 7.24 (d, *J* = 8.3 Hz, 1H), 5.00 (td, *J* = 10.0, 7.0 Hz, 2H), 4.11 (m, 4H), 3.53 (s, 6H), 3.36 (t, *J* = 10.1 Hz, 1H), 3.22 (t, *J* = 10.1 Hz, 1H), 2.48 – 2.14 (m, 2H), 1.11 (t, *J* = 6.0 Hz, 6H), 1.01 – 0.60 (m, 12H).

LC/MS: m/z = 829.73 ($M+H^+$), r.t. = 2.14 min

Compound 17:

^{1}H NMR (400 MHz, Acetone-d_6) δ 11.52 (s, 1H), 11.23 (s, 1H), 8.21 (t, *J* = 8.2 Hz, 1H), 7.76 –7.55 (m, 5H), 7.49 (d, *J* = 8.2 Hz, 1H), 7.29 (d, *J* = 14.3 Hz, 1H), 7.16 (s, 2H), 6.29 (d, *J* = 8.5 Hz, 1H), 5.57 – 4.99 (m, 2H), 4.43 – 4.16 (m, 4H), 3.61 (s, 6H), 3.29 (d, *J* = 4.9 Hz, 2H), 2.83 (s, 2H), 2.52 (dd, *J* = 12.0, 6.8 Hz, 2H), 2.40 (d, *J* = 6.2 Hz, 2H), 2.34 – 2.22 (m, 2H), 0.94 – 0.82 (m, 18H)

^{19}F NMR (300 MHz, $CDCl_3$) δ -114.3

LC/MS: m/z = 841.29 ($M+H^+$), r.t. = 2.20 min

HRMS calcd for $C_{44}H_{53}FN_8O_6S$ ($M+H^+$): 841.3871; found: 841.3859

$[\alpha]_D$: -87.2 (*c* = 0.5, $CHCl_3$)

IR: (film, cm^{-1}) 2963, 1715, 1632, 1514, 1449, 1253

m.p.: 190-192 °C

Compound 18:

^{1}H NMR (400 MHz, Acetone-d_6) δ 11.47 (s, 1H), 11.07 (s, 1H), 7.97 – 7.49 (m, 4H), 7.42 – 7.07 (m, 5H), 6.25 (t, *J* = 8.9 Hz, 2H), 5.21 (dd, *J* = 22.6, 14.7 Hz, 2H), 4.43 – 4.08 (m, 4H), 3.61 (s, 6H), 3.25 (dd, *J* = 15.6, 9.8 Hz, 2H), 2.84 - 2.40 (m, 11H), 0.98 – 0.77 (m, 18H)

LC/MS: m/z = 837.71 ($M+H^+$), r.t. = 2.09 min

HRMS calcd for $C_{45}H_{56}N_8O_6S$ ($M+H^+$): 837.4122; found: 837.4099

$[\alpha]_D$: -63.4 (c = 0.5, $CHCl_3$)

m.p.: 191-192 °C

Compound 19:

^{1}H NMR (400 MHz, Acetone-d_6) δ 11.63 (s, 1H), 11.14 (s, 1H), 8.24 – 7.87 (m, 1H), 7.83 – 7.53 (m, 5H), 7.53 – 7.33 (m, 3H), 7.31 – 7.20 (m, 1H), 7.14 – 7.06 (m, 1H), 6.26 (m, 2H), 5.27 (m, 2H), 4.43 – 4.12 (m, 4H), 3.61 (s, 6H), 3.37 – 3.17 (m, 2H), 2.84 (s, 3H), 2.28 (m, 8H), 1.02 – 0.73 (m, 18H)

LC/MS: m/z = 837.66 ($M+H^+$), r.t. = 2.02 min

HRMS calcd for $C_{45}H_{56}N_8O_6S$ ($M+H^+$): 837.4122; found: 837.4188

$[\alpha]_D$: -47.0 (c = 0.5, $CHCl_3$)

IR: (film, cm^{-1}) 2960, 1720, 1629, 1514, 1445, 1261

m.p.: 185-187 °C

References

1) For synthesis see: WO2011119853 and WO2011079327

Protocols for the determination of IC_{50}'s

Activity determination using ELISA and a sub-genomic replicon 1a cell line

The cell line W11.8 containing the sub-genomic HCV replicon of genotype 1a was used to determine the potency of the drugs. The RNA replication in presence of different drug concentrations was indirectly measured in this cell line by the level of NS5A protein content upon drug treatment for four days. It was shown that the level of the NS5A protein correlated well with the level of HCV RNA in the replicon cell line. Cells were split twice a week in order to keep the confluence state below 85% of the culture flask surface area. The culture media used for cell passaging consisted of DMEM supplemented with a final concentration of 10% fetal bovine serum, 100 IU/mL penicillin,

100 μg/mL streptomycin, 2 mM L-glutamine, 1 mM sodium pyruvate, non-essential amino acids (1x) and 600 μg/mL of G418. Monolayer of the W11.8 cells was trypsinized and cells were counted. Cells were diluted at 50,000 cells/mL with complete DMEM without G418, then approximately 5,000 viable cells (100 μL) were plated per well in a white opaque 96-well microtiter plates. After an incubation period of 2 - 4 hours at 37 °C in a humidified incubator with 5% CO_2, compounds were added at various concentrations. Compounds were dissolved in DMSO at a stock concentration of 10 mM. Then, drugs were serially diluted at twice the final concentration in the same medium. One volume (100 μL) of each drug dilution was then added to each well containing cells. A control compound was used as an internal standard for each assay plate. Sixteen wells were used as control (0% inhibition) without drug. Eight wells were used as background control (100% inhibition) containing 2 μM (final concentration) of the control compound that was shown to inhibit the NS5A expression at 100% and is nontoxic to the cells. Values from wells with 100% inhibition were averaged and used as the background value. Cells were further incubated for four days at 37°C in a 5% CO_2 incubator. Following the incubation time of four days, the media was removed and wells were washed once with 150 μL of PBS at room temperature for five minutes. Cells were then fixed for five minutes using 150 μL per well of cold (-20°C) fixative solution (50% methanol /50% acetone mix). Cells were then washed twice with 150 μL of PBS (phosphate buffered saline) per well and following the addition of 150 μL of blocking solution, cells were incubated for one hour at 37 °C to block non-specific sites. The blocking solution was removed and cells were washed twice with 150 μL of PBS per well and once with 150 μL of PBSTS solution (PBS / 0.1% Triton X-100 / 0.02% SDS) per well. Then, 50 μL of mouse monoclonal anti-NS5A antibody (Santa Cruz, Cat. No. sc-52417) was added to each well, diluted 1 /1,000 in the blocking solution and incubated at 4°C overnight. Next day, media was removed and plates were washed five times with 150 μL of PBS per well with five-minute incubations at room temperature. Then 50 μL per well of peroxidase-conjugated donkey anti-mouse antibody (Jackson Immunoresearch, Cat. No. 715-036-150) diluted 1/10,000 in the blocking solution was added and incubated at room temperature for three hours on a shaker (500 rpm). Plates were washed four times with 150 μL of PBSTS solution per well and once with 150 μL of PBS. Then, substrate

solution (100 µL, SuperSignal ELISA Pico Chemiluminescent Substrate, Fisher Scientific, Cat. No.37069) was added to each well and plates were incubated 60 minutes at room temperature prior to reading the luminescence (relative light units) on the Analyst HT plate reader. The percentage of inhibition at each drug concentration tested (in duplicate) was calculated. The concentration of compound required for the reduction of viral replication by 50% (IC_{50}) was then determined from dose response curves using nonlinear regression analysis with the GraphPad Prism software, version 2.0 (GraphPad Software Inc., San Diego, CA, USA).

Cell-Based Luciferase Reporter HCV genotype lb RNA Replication Assay Cell Culture

The replicon cell line Huh-5.2 derived from the Huh-7 hepatocellular carcinoma cell line was maintained in culture as described previously (Krieger, N et al., *J. Virol.* **2001**, *75*, 4614-4624). The Huh-5.2 cells contain the highly cell culture-adapted replicon l_{389}luc-ubi-neo/NS3-375.1 construct that carries, in addition to the neomycin gene, an integrated copy to the firefly luciferase gene. This cell line allows measurement of HCV RNA replication and translation by measuring luciferase activity. It has been shown previously that the luciferase activity tightly followed the replicon RNA level in these cells. The Huh7-ET cell line has the same features as those mentioned for Huh-5.2 cell line, except that ET cells are more robust and contain an adaptive mutation in the HCV NS4B gene instead of NS5A. Both cell lines were maintained in cultures at a subconfluent level (<85%) as the level of replicon RNA is highest in actively proliferating cells. The culture media used for cell passaging consisted of DMEM (Gibco BRLLaboratories, Mississauga, ON, Canada) supplemented with a final concentration of 10% fetal bovine serum, 1 % penicillin/streptomycin, 1 % L-glutamine, 1 % sodium pyruvate, 1 % non-essential amino acids, and 180 mg/mL of G418. Cells are incubated at 37°C in a humidified incubator, in an atmosphere of 5% CO_2 and passaged twice a week to maintain sub-confluence. Approximately 3000 viable Huh- ET cells (100 µL) were plated per well in a white opaque 96-well microtiter plate. The cell culture media used for the assay was the same as described above except that it contained no G418 and no phenol red. After an incubation period of 3-4 hours at 37° C in a 5% CO_2 incubator, compounds (100 µL)

were added at various concentrations. Cells were then incubated further for 4 days at 37°C in a humidified incubator with 5% CO_2. Thereafter, the culture media was removed and cells were lysed by the addition of 95 μL of the luciferase buffer (luciferin substrate in buffered detergent). Cell lysates were incubated at room temperature and protected from direct light for at least 10 minutes. Plates were read for luciferase counts using a luminometer (Wallac MicroBeta Trilux, Perkin Elmer™, MA, USA). The 50% inhibitory concentrations (IC_{50} values) were determined from dose response curves using eleven concentrations per compound in duplicate. Curves were fitted to data points using nonlinear regression analysis, and IC_{50} values were interpolated from the resulting curves using GraphPad Prism software, version 2.0 (GraphPad Software Inc., San Diego, CA, USA)