

# Discovery and Pre-Clinical Characterization of Third-Generation 4-H Heteroaryldihydropyrimidine (HAP) Analogues as Hepatitis B Virus (HBV) Capsid Inhibitors

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## Supporting Information

### Contents

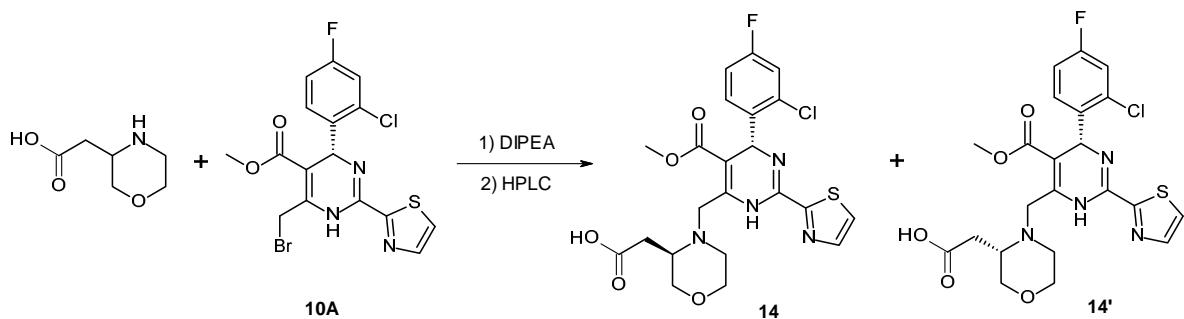
1. Detailed experimental procedures for the synthesis of analogues **14**, **16**, **17**, **18**, **20**, **26**, **32**, **33** and **34**, amine intermediates **15-a** and **22-a**, amidine intermediates **39-a** and **41-a**, and aldehyde intermediate **66-a**.

2. X-Ray Crystal Structure of **(R)-10A-2**.
3.  $^1\text{H}$  NMR and  $^{13}\text{C}$  NMR Spectrum of Compound **10**.
4. Distinct Antiviral Effect of Compound **10** Compared to entecavir.

### **General Experimental Conditions Used in Synthesis and Purification**

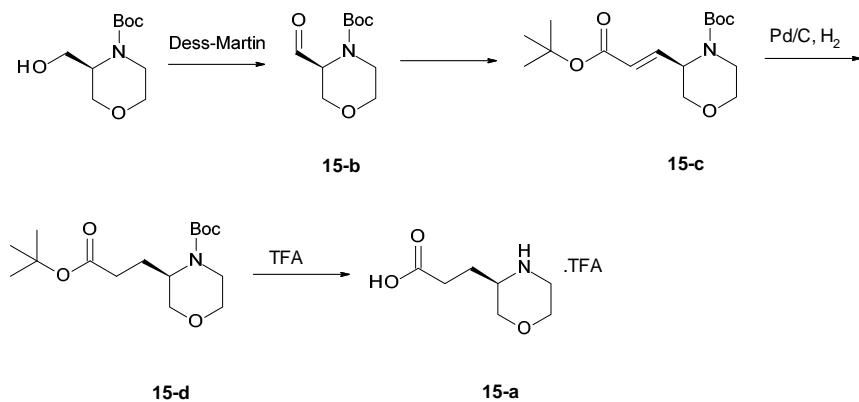
All of the intermediates were purified by silica gel chromatography using either a Biotage SP1 system or an ISCO CombiFlash chromatography instrument. All of the final compounds were purified by preparative HPLC (prep-HPLC) on a reversed-phase column using a Waters XBridge OBD Phenyl (30 mm  $\times$  100 mm, 5  $\mu\text{m}$ ) or OBD RP18 (30 mm  $\times$  100 mm, 5  $\mu\text{m}$ ) column under acidic conditions (A, 0.1% formic acid in  $\text{H}_2\text{O}$ ; B, 0.1% formic acid in acetonitrile) or basic conditions (A, 0.1% ammonia in  $\text{H}_2\text{O}$ ; B, acetonitrile). For SFC chiral separation, the intermediates were separated using a chiral column (Daicel Chiraldak IC, 30 mm  $\times$  250 mm, 5  $\mu\text{m}$ ) on a Mettler Teledo SFC-Multigram system (solvent system of 95%  $\text{CO}_2$  and 5% IPA (0.5% TEA in IPA), backpressure of 100 bar, UV detection at 254 nm). Optical rotation was measured using a Rudolph Autopol V automatic polarimeter at a wavelength of 589 nm. LC-MS spectra were obtained using a MicroMass Platform LC (Waters Alliance 2795-ZQ2000). NMR spectra were obtained using Bruker AVANCE 400 MHz spectrometer, operating at 400.13 MHz ( $^1\text{H}$ ) and 100.62 MHz ( $^{13}\text{C}$ ).  $^1\text{H}$  NMR spectra were obtained using the single pulse zg30.  $^{13}\text{C}\{^1\text{H}\}$  NMR spectra were obtained using composite pulse zgpg30 for proton decoupling. The chemical shifts were referenced against internal TMS( $^1\text{H}$ ,  $^{13}\text{C}$ ). All of the starting materials were obtained commercially. All of the final compounds had purities greater than 95% based upon HPLC, LC-MS and  $^1\text{H}$  NMR analyses. All of the reported yields are for isolated products and are not optimized.

**2-[(3*R*)-4-[(4*R*)-4-(2-Chloro-4-fluoro-phenyl)-5-methoxycarbonyl-2-thiazol-2-yl]-1,4-dihydropyrimidin-6-yl]methyl]morpholin-3-yl]acetic acid (14).**



2-(Morpholin-3-yl)acetic acid (BePharm, cas: 86236-84-2) was reacted with bromide **10A** as described for the preparation of **10** to give the coupling product as a crude mixture of two diastereomers after evaporation of solvent, which was then subjected to HPLC separation to give compound **14** and its diastereomer **14'**. The stereochemistry of **14** was tentatively assigned based on its better antiviral activity in HepG2.2.15 cells in analogy to the case of **10** vs **11**. MS: calc'd ( $\text{MH}^+$ ) 509, measured ( $\text{MH}^+$ ) 509.  $^1\text{H}$  NMR (Methanol-d<sub>4</sub>, 400 MHz):  $\delta$  ppm 7.97 (d,  $J$  = 3.01 Hz, 1H), 7.77 (d,  $J$  = 3.14 Hz, 1H), 7.44 (dd,  $J$  = 8.60, 6.21 Hz, 1H), 7.24 (dd,  $J$  = 8.78, 2.64 Hz, 1H), 7.06 (td,  $J$  = 8.38, 2.57 Hz, 1H), 6.17 (s, 1H), 4.32 (br d,  $J$  = 17.44 Hz, 1H), 4.07 (br s, 1H), 3.97 (br s, 1H), 3.67–3.92 (m, 4H), 3.63 (s, 3H), 3.24 (br s, 1H), 2.85–3.09 (m, 1H), 2.60 (br d,  $J$  = 4.77 Hz, 2H).

**3-[(3*R*)-Morpholin-3-yl]propanoic acid trifluoroacetic acid salt (intermediate **15-a**).**



To a stirred solution of (*R*)-4-boc-3-hydroxymethylmorpholine (1.86 g, 8.57 mmol, cas: 215917-99-0) in DCM (50 mL) was added Dess-Martin reagent (4.36 g, 10.3 mol) in portions at 0 °C. Then the reaction mixture was stirred at r.t. for 3 hrs. The undissolved material was filtered and the filtrate was concentrated

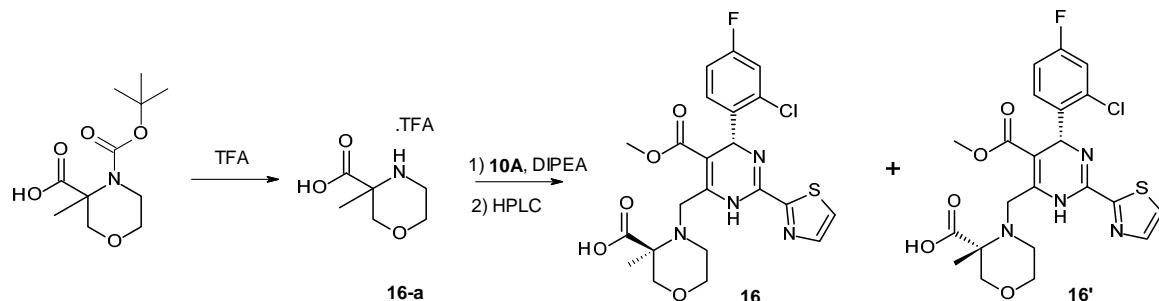
*in vacuo*. The residue was purified by column chromatography ( EA/PE: 1/4 to 1/2) to afford compound **15-b** as a viscous oil (1.5 g, yield: 81%).

To a solution of methyl *tert*-butyl 2-dimethoxyphosphorylacetate (0.45 g, 2.0 mmol) in THF (10 mL) was added KO*t*Bu (0.27 g, 2.4 mol) at 0 °C. After the mixture was stirred for 30 min, compound **15-b** (0.43 g, 20 mmol) was added. The mixture was heated to reflux for 3 hr before it was quenched by aqueous NH<sub>4</sub>Cl. The mixture was diluted with EA (20 mL) and washed with water (10 mL). The organic layer was separated and dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>. After filtration, the filtrate was concentrated and the residue was purified by column chromatography ( EA/PE 1/4 to 1/3) to afford compound **15-c** as a viscous oil (0.26 g, yield: 42%).

A mixture of compound **15-c** (0.26 g, 0.83 mmol) and Pd/C (20 mg) in MeOH (10 mL) was hydrogenated with aa hydrogen balloon at r.t. overnight. After filtration of undissolved material, the filtrate was concentrated to afford compound **15-d** as a viscous oil (0.24 g, yield: 92%).

To a stirred solution of compound **15-d** (0.24 g, 0.76 mmol) in DCM (2 mL) was added TFA (1 mL) at r.t. The reaction mixture was stirred overnight. Removal of solvent gave the crude product **15-a** as a viscous oil (0.25 g, as TFA salt) which was used directly in the next step without further purification.

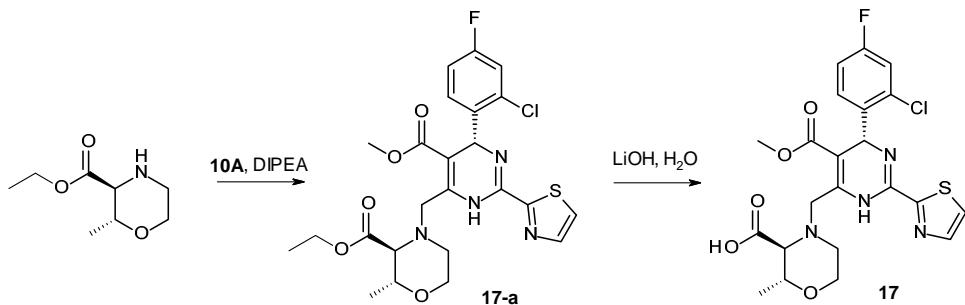
**(3S)-4-[[4R)-4-(2-Chloro-4-fluoro-phenyl)-5-methoxycarbonyl-2-thiazol-2-yl-1,4-dihydropyrimidin-6-yl]methyl]-3-methyl-morpholine-3-carboxylic acid (16).**



To a stirred solution of 3-methyl-4-[(2-methylpropan-2-yl)oxycarbonyl]morpholine-3-carboxylic acid (Accela ChemBio Co., Ltd., CAS: 1052680-53-1, 50 mg, 0.20 mmol) in DCM (1 mL) was added TFA (1 mL) dropwise at room temperature. After the resulting mixture was stirred at rt for 1h, the solvent was

removed *in vacuo* to give the crude product **16-a** without further purification. Intermediate **16-a** was then reacted with bromide **10A** as described for the preparation of **10** to give the coupling product as a crude mixture of two diastereomers after evaporation of solvent, which was then subjected to HPLC separation to give compound **16** and its diastereomer **16'**. The stereochemistry of **16** was tentatively assigned based on its better antiviral activity in HepG2.2.15 cells in analogy to the case of **10** vs **11**. MS: calc'd (MH<sup>+</sup>) 509, measured (MH<sup>+</sup>) 509. <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400MHz):  $\delta$  ppm 8.06 (d, *J* = 3.0 Hz, 1H), 7.85 (d, *J* = 3.0 Hz, 1H), 7.36 (dd, *J* = 8.8, 5.8 Hz, 1H), 7.28 (s, 1H), 7.20 (dd, *J* = 8.3, 2.5 Hz, 1H), 6.30 (s, 1H), 4.46 (d, *J* = 17.1 Hz, 1H), 4.21 (*br* d, *J* = 17.1 Hz, 1H), 3.93–4.09 (m, 2H), 3.78–3.89 (m, 2H), 3.66 (s, 3H), 3.18–3.31 (m, 1H), 2.97–3.10 (m, 1H), 1.58 (s, 3H).

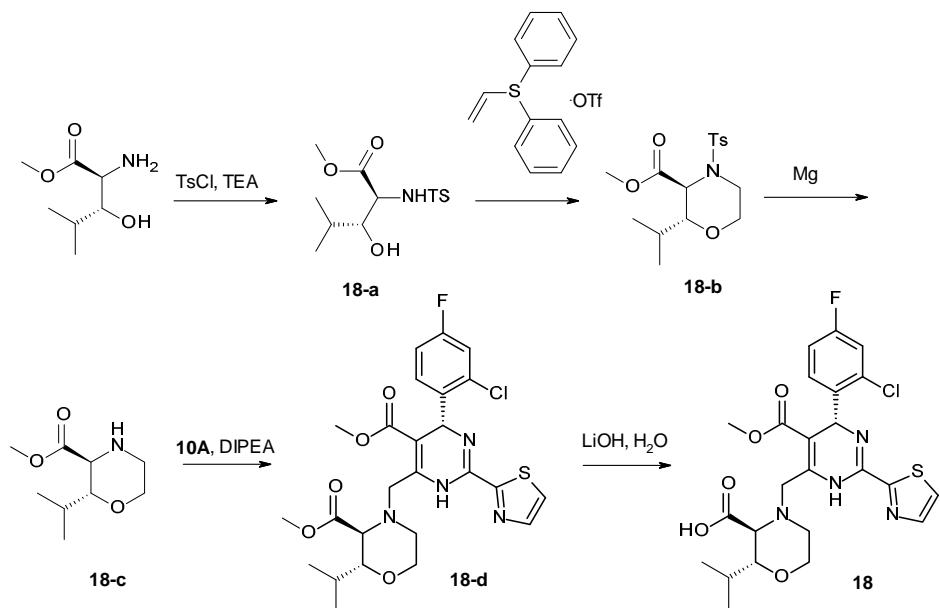
**(2R,3S)-4-[(4R)-4-(2-Chloro-4-fluoro-phenyl)-5-methoxycarbonyl-2-thiazol-2-yl-1,4-dihydropyrimidin-6-yl]methyl]-2-methyl-morpholine-3-carboxylic acid (17).**



Compound *(2R,3S)-2-methyl-morpholine-3-carboxylic acid ethyl ester* (50 mg, 0.29 mmol, for its synthesis, see: WO2011025889) was reacted with bromide **10A** (84 mg, 0.19 mmol) as the same as described for the preparation of **10** to give the crude coupling product **17-a** after evaporation of solvent, which was then dissolved in a mixed solvent of H<sub>2</sub>O (0.5 mL) and MeOH (2 mL). Lithium hydroxide monohydrate (78 mg, 1.9 mmol) was then added and the mixture was stirred for 12 hours at room temperature. The mixture was treated slowly with hydrochloric acid (10%) to adjust pH to ca. 4. After filtration and evaporation of solvent *in vacuo*, the residue was purified by preparative HPLC to give **17** as a yellow solid (58 mg, 60%). MS: calc'd (MH<sup>+</sup>) 509, measured (MH<sup>+</sup>) 509. <sup>1</sup>H NMR (Methanol-d<sub>4</sub>, 400 MHz)  $\delta$  ppm 8.03 (d, *J* = 3.14 Hz, 1H), 7.90 (d, *J* = 3.14 Hz, 1H), 7.51 (dd, *J* = 5.96, 8.72 Hz, 1H), 7.28

(dd,  $J = 2.64, 8.66$  Hz, 1H), 7.11 (dt,  $J = 2.64, 8.34$  Hz, 1H), 6.19 (s, 1H), 4.41 (d,  $J = 16.56$  Hz, 1H), 4.20 (d,  $J = 16.44$  Hz, 1H), 4.00–4.13 (m, 1H), 3.75–3.98 (m, 3H), 3.63 (s, 3H), 3.51 (d,  $J = 8.91$  Hz, 1H), 3.06 (dt,  $J = 3.51, 11.80$  Hz, 1H), 1.36 (d,  $J = 6.27$  Hz, 3H).

**(2*R*,3*S*)-4-[(4*R*)-4-(2-Chloro-4-fluoro-phenyl)-5-methoxycarbonyl-2-thiazol-2-yl-1,4-dihydropyrimidin-6-yl]methyl]-2-isopropyl-morpholine-3-carboxylic acid (18).**



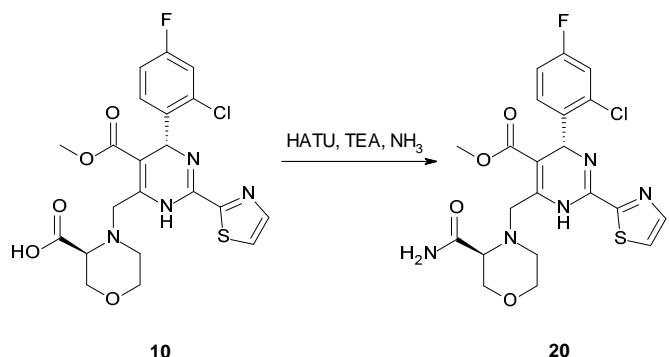
A mixture of (2*S*,3*R*)-2-amino-3-hydroxy-4-methyl-pentanoic acid methyl ester (0.50 g, 3.4 mmol) and triethylamine (0.69 g, 6.8 mmol) in dichloromethane (10 mL) was added TsCl (0.71 g, 3.7 mmol) at 0 °C. After stirring for 2 hours at room temperature, the mixture was washed with water and the organic layer was separated, dried over sodium sulfate. Removal of solvent gave the crude product **18-a** which was used in next step without purification. MS: calc'd (MH<sup>+</sup>) 316, measured (MH<sup>+</sup>) 316.

A mixture of **18-a** (0.40 g, 1.27 mmol) and diphenylvinylsulfonium triflate (0.50 g, 1.4 mmol) in dichloromethane (10 mL) was added triethylamine dropwise at 0 °C. After stirring for 12 hours, the mixture was diluted with water and extracted with ethyl acetate. The organic solvent was dried over sodium sulfate. After removal of solvent, the residue was purified by flash chromatography (eluted with ethyl acetate/hexane = 1:4) to afford the product **18-b** as a white solid (0.75 g, 65%). MS: calc'd (MH<sup>+</sup>) 342, measured (MH<sup>+</sup>) 342.

A mixture of **18-b** (0.20 g, 0.59 mmol) and magnesium powder (70 mg, 2.9 mmol) in anhydrous Methanol (5 mL) was stirred for 3 hours at 60 °C. The solid was removed by filtration, and the filtrate was concentrated *in vacuo* to give crude product **18-c** which was used in next step without purification. MS: calc'd (MH<sup>+</sup>) 188, measured (MH<sup>+</sup>) 188.

The crude **18-c** was reacted with bromide **10A** (84 mg, 0.19 mmol) as described for the preparation of **10** to give the crude product **18-d** after evaporation of solvent, which was then hydrolyzed with LiOH to give the desired product **18** as a yellow solid (41 mg, 40%). MS: calc'd (MH<sup>+</sup>) 537, measured (MH<sup>+</sup>) 537. <sup>1</sup>H NMR (Methanol-d<sub>4</sub>, 400 MHz) δ ppm 8.01 (d, *J* = 3.14 Hz, 1H), 7.70–7.95 (m, 1H), 7.50(dd, *J* = 5.96, 8.72 Hz, 1H), 7.28 (dd, *J* = 2.64, 8.78 Hz, 1H), 7.10 (dt, *J* = 2.70, 8.38 Hz, 1H), 6.02–6.27 (m, 1H), 4.35–4.61 (m, 1H), 4.21–4.35 (m, 1H), 4.11 (td, *J* = 3.00, 12.20 Hz, 1H), 3.72–3.97 (m, 2H), 3.67–3.71 (m, 1H), 3.65 (s, 3H), 3.35–3.46 (m, 1H), 2.95–3.17 (m, 1H), 2.10 (m, 1H), 1.07 (dd, *J* = 4.83, 6.84 Hz, 6H).

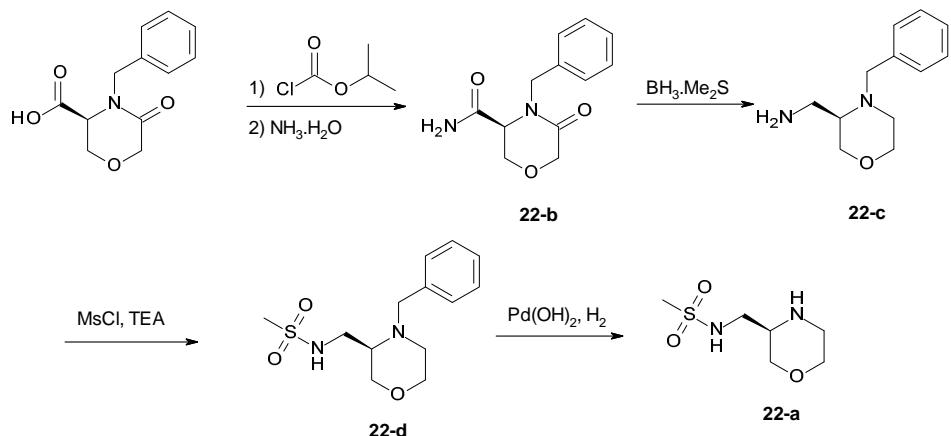
**Methyl (4*R*)-6-[(3*S*)-3-carbamoylmorpholin-4-yl]methyl]-4-(2-chloro-4-fluoro-phenyl)-2-thiazol-2-yl-1,4-dihydropyrimidine-5-carboxylate (20).**



To a stirred solution of compound **10** (49 mg, 0.11 mmol), HATU (57 mg, 0.15 mmol), TEA (0.042 mL, 0.3 mmol) in DCM (2 mL) was added ammonia solution (1 M in dioxane, 0.5 mL) at r.t. After the reaction mixture was stirred for 12 hours at r.t., water (5 mL) was added. The mixture was extracted with DCM (10 mL). The organic layer was dried over sodium sulfate. After removal of solvent, the residue was purified with HPLC to give product **20** as a yellow solid (18 mg, 33%). MS: calc'd (MH<sup>+</sup>) 494, measured (MH<sup>+</sup>) 494. <sup>1</sup>H NMR (Methanol-d<sub>4</sub>, 400 MHz) δ ppm 7.97 (d, *J* = 3.01 Hz, 1H), 7.71–7.81 (m,

1H), 7.42(t,  $J = 6.76$  Hz, 1H), 7.23 (dd,  $J = 2.32, 8.85$  Hz, 1H), 7.06 (dt,  $J = 2.70, 8.38$  Hz, 1H), 6.08–6.20 (m, 1H), 4.08–4.27 (m, 1H), 4.01 (dd,  $J = 3.26, 11.29$  Hz, 1H), 3.67–3.92 (m, 4H), 3.57–3.64 (s, 3H), 3.35–3.43 (m, 1H), 3.00–3.09 (m, 1H), 2.50–2.68 (m, 1H).

**Synthesis of *N*-[(3*R*)-morpholin-3-yl]methyl]methanesulfonamide (intermediate **22-a**).**



To a stirred solution of (3*S*)-4-benzyl-5-oxo-morpholine-3-carboxylic acid (1.0 g, 4.25 mmol, for its synthesis, see WO 2004054514) and TEA (0.60 g, 5.53 mmol) in THF (300 mL) was added isopropyl carbonochloridate (0.63 g, 5.10 mmol) at 0 °C. After the reaction mixture was stirred at 0 °C for 1h, NH<sub>3</sub>.H<sub>2</sub>O (2.90 g, 42.6 mmol) was added. The reaction mixture was further stirred at room temperature overnight. The solvent was removed *in vacuo*, and the crude product was washed with Methanol to give (3*S*)-4-benzyl-5-oxo-morpholine-3-carboxamide (**22-b**) (0.8 g, 80%) as a white solid. <sup>1</sup>H NMR (Methanol-d<sub>4</sub>, 400 MHz): δ ppm 7.54 (s, 1H), 7.34–7.20 (m, 6H), 5.35 (d,  $J = 15.2$ , 1H), 4.12 (dd,  $J = 7.6, J = 65.6$ , 2H), 3.87 (dd,  $J = 3.6, J = 12.0$ , 2H), 3.72 (s, 1H), 3.57 (d,  $J = 15.6$ , 1H).

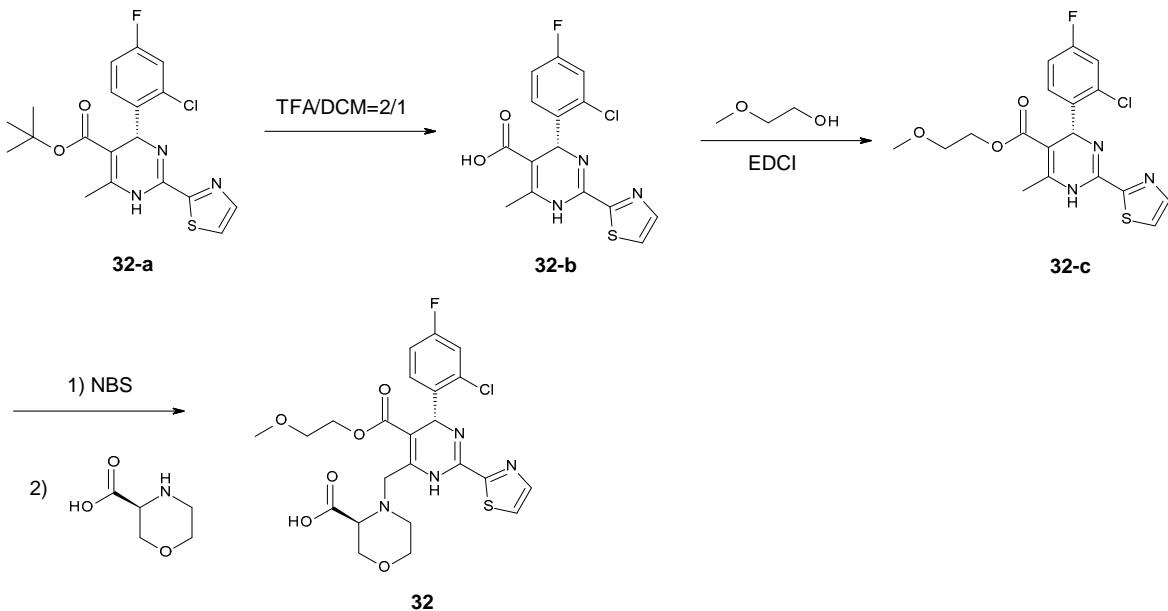
BH<sub>3</sub>.Me<sub>2</sub>S (3.4 mL, 34.2 mmol) was added to a suspension of compound **22-b** (0.80 g, 3.42 mmol) in THF (300 mL) at 0 °C. After the mixture was heated to reflux for 30 hrs, the reaction mixture was quenched with Methanol (1 mL) slowly 0 °C. 1M HCl solution (10 mL) was then added and the mixture was heated to reflux for 2 hrs. The mixture was neutralized with 1 M NaOH solution (10 mL) and extracted with EtOAc, concentrated *in vacuo* to give [(3*R*)-4-benzylmorpholin-3-yl]methanamine (**22-c**) as a crude product which was used directly in the next step.

To a mixture of **22-c** (600 mg, 2.91 mmol) and TEA (442 mg, 4.36 mmol) in DCM (50 mL) at 0 °C was added MsCl (366 mg, 3.20 mmol). After the mixture was stirred at 25 °C for 2 hrs, it was diluted with aq. NH<sub>4</sub>Cl. The organic layer was separated and the aqueous layer was extracted with EtOAc. The combined organic layers were washed with H<sub>2</sub>O, dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated *in vacuo*. The residue was purified by pre-HPLC to give *N*-[(3*R*)-4-benzylmorpholin-3-yl]methyl]methanesulfonamide (**22-d**) (300 mg, 36%) as a white solid. <sup>1</sup>H NMR (Methanol-d4, 400 MHz): δ ppm 7.58–7.49 (m, 5H), 5.09 (d, 1H), 4.13 (m, 2H), 4.05 (m, 2H), 3.71 (m, 2H), 3.50 (m, 2H), 3.22 (m, 1H), 3.15 (m, 1H), 3.05 (s, 3H). A mixture of compound **22-d** (300 mg, 1.05 mmol) and Pd(OH)<sub>2</sub> (100 mg) in MeOH (100 mL) was hydrogenated at room temperature with 40 psi hydrogen. The solution was filtered and the filter was concentrated *in vacuo* to give *N*-[(3*R*)-morpholin-3-yl]methyl]methanesulfonamide (**22-a**) (200 mg, 97%) as a white solid. <sup>1</sup>H NMR (Methanol-d4, 400 MHz): δ ppm 4.03 (m, 2H), 3.76 (m, 1H), 3.59 (m, 1H), 3.43–3.34 (m, 4H), 3.30 (m, 1H), 2.99 (s, 3H).

**(3*S*)-4-[(4*R*)-4-(2-Chloro-4-fluoro-phenyl)-5-methoxycarbonyl-2-thiazol-2-yl-1,4-dihydropyrimidin-6-yl]methyl]thiomorpholine-3-carboxylic acid (**26**).**

The title compound was prepared in analogy to **16** by using racemic thiomorpholine-3-carboxylic acid (PharmaBlock, cas: 20960-92-3) instead of **16-a**. Compound **26** was obtained as a yellow solid. MS: calc'd (MH<sup>+</sup>) 511, measured (MH<sup>+</sup>) 511. <sup>1</sup>H NMR (Methanol-d4, 400 MHz) δ ppm 8.02–8.09 (m, 1H), 7.86–8.00 (m, 1H), 7.46–7.60 (m, 1H), 7.24–7.36 (m, 1H), 6.99–7.19 (m, 1H), 6.12–6.31 (m, 1H), 4.80–4.87 (m, 1H), 4.38–4.52 (m, 2H), 3.80–3.99 (m, 1H), 3.66 (s, 3H), 3.36–3.51 (m, 2H), 3.10–3.29 (m, 2H), 2.76–2.89 (m, 1H).

**(3*S*)-4-[(4*R*)-4-(2-Chloro-4-fluoro-phenyl)-5-(2-methoxyethoxycarbonyl)-2-thiazol-2-yl-1,4-dihydropyrimidin-6-yl]methyl]morpholine-3-carboxylic acid (**32**).**



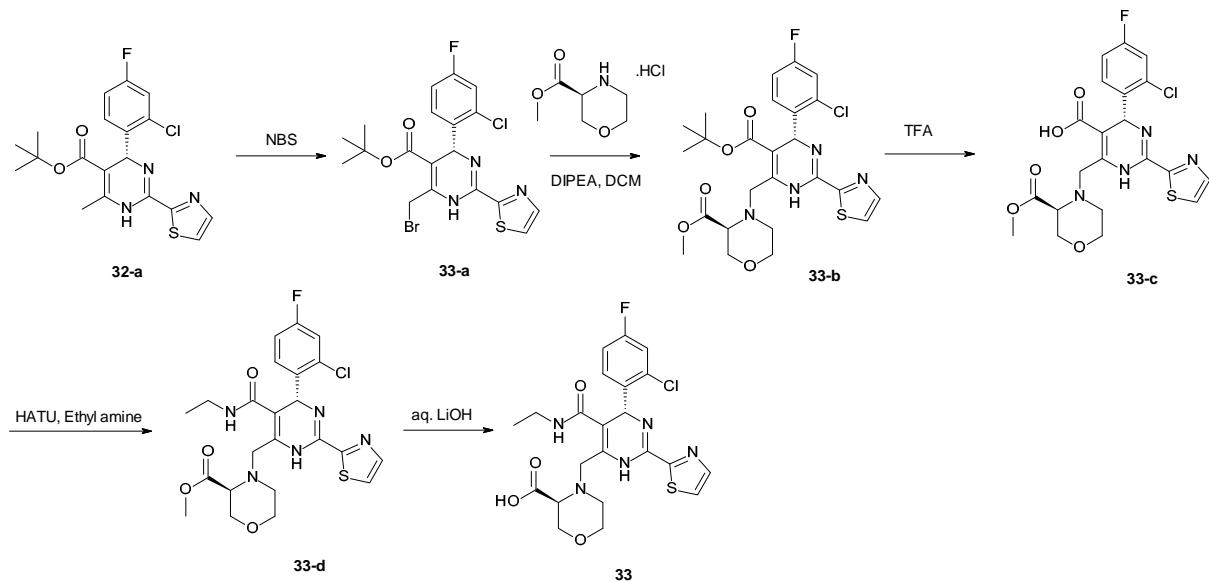
Compound **32-a** was an intermediate from **31** and was prepared in analogy to **(R)-10A-2** by using *tert*-butyl 3-oxobutanoate instead of methyl 3-oxobutanoate. MS: calc'd ( $\text{MH}^+$ ) 408, measured ( $\text{MH}^+$ ) 408.  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz)  $\delta$ : 7.83 (d,  $J$  = 3.0 Hz, 1H), 7.49 (*br s*, 1H), 7.35 (dd,  $J$  = 6.1, 8.7 Hz, 1H), 7.16 (dd,  $J$  = 2.8, 8.5 Hz, 1H), 6.96 (dt,  $J$  = 2.5, 8.3 Hz, 1H), 6.14 (s, 1H), 2.54 (s, 3H), 1.32 (s, 9H).

The mixture of compound **32-a** (3.5 g, 8.6 mmol) and TFA/DCM (10 mL/5 mL) was stirred at rt for 3 hours. The reaction mixture was concentrated. The residue was diluted with EA and ice-water. The mixture was neutralized to pH 6.0 with 1 N NaOH solution. The mixture was extracted with EA three times. The combined organic phase was dried over  $\text{Na}_2\text{SO}_4$ , filtered and concentrated to give the crude product **32-b** (2.4 g, 79%). MS: calc'd ( $\text{MH}^+$ ) 352, measured ( $\text{MH}^+$ ) 352.

To a solution of **32-b** (505 mg, 1.4 mmol) in DMF (2.0 mL) was added 2-methoxyethanol (228 mg, 3.0 mmol), EDCI (414 mg, 2.2 mmol) and DMAP (527 mg, 4.3 mmol). The reaction mixture was stirred at 60 °C for 3 hours. The reaction mixture was cooled down and quenched by adding ice-water. The mixture was extracted with PE/EA (2/1) three times. The combined organic phase was dried over  $\text{Na}_2\text{SO}_4$ , filtrated and concentrated. The residue was purified by column to give compound **32-c** (360 mg, 63%). MS: calc'd ( $\text{MH}^+$ ) 410, measured ( $\text{MH}^+$ ) 410.

From **32-c**, compound **32** was prepared in analogy to **10** and was obtained as a yellowish solid. MS: calc'd (MH<sup>+</sup>) 539, measured (MH<sup>+</sup>) 539. <sup>1</sup>H NMR (Methanol-d4, 400 MHz) δ ppm 7.97 (d, *J* = 3.3 Hz, 1H), 7.79 (d, *J* = 3.0 Hz, 1H), 7.48 (dd, *J* = 6.3, 8.8 Hz, 1H), 7.25 (dd, *J* = 2.5, 8.8 Hz, 1H), 7.07 (dt, *J* = 2.8, 8.4 Hz, 1H), 6.20 (s, 1H), 4.50 (*br* s, 1H), 4.39–4.22 (m, 1H), 4.22–4.06 (m, 4H), 3.97–3.81 (m, 2H), 3.68 (*br* s, 1H), 3.55–3.48 (m, 2H), 3.48–3.35 (m, 1H), 3.29 (s, 3H), 2.77 (*br* s, 1H).

**(3S)-4-[(4R)-4-(2-Chloro-4-fluoro-phenyl)-5-(ethylcarbamoyl)-2-thiazol-2-yl-1,4-dihydropyrimidin-6-yl]methylmorpholine-3-carboxylic acid (33).**



(3S)-4-[(4R)-5-tert-butoxycarbonyl-4-(2-chloro-4-fluoro-phenyl)-2-thiazol-2-yl-1,4-dihydropyrimidin-6-yl]methylmorpholine-3-carboxylate (**33-b**) was prepared in analogy to compound **10** by using *tert*-butyl (4R)-4-(2-chloro-4-fluoro-phenyl)-6-methyl-2-thiazol-2-yl-1,4-dihydropyrimidine-5-carboxylate (**32-a**) and methyl (3S)-morpholine-3-carboxylate hydrochloride salt instead of methyl (4R)-4-(2-chloro-4-fluoro-phenyl)-6-methyl-2-thiazol-2-yl-1,4-dihydropyrimidine-5-carboxylate (**(R)-10A-2**) and (3S)-morpholine-3-carboxylic acid hydrochloride salt.

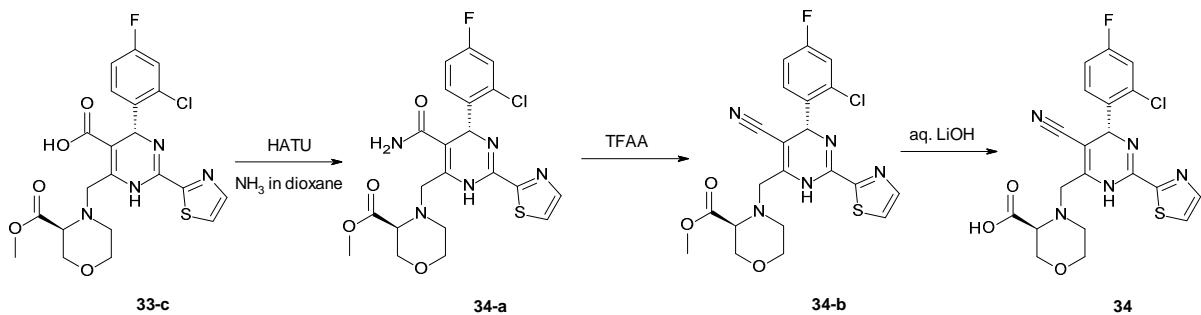
A solution of compound **33-b** (550 mg, 1 mmol) in DCM (5 mL) was added TFA (2 mL). After stirring for 2 hours at room temperature, the mixture was concentrated *in vacuo* to give 4-(2-chloro-4-fluoro-

phenyl)-6-[(3*S*)-3-methoxycarbonylmorpholin-4-yl]methyl]-2-thiazol-2-yl-1,4-dihydropyrimidine-5-carboxylic acid;2,2,2-trifluoroacetic acid (**33-c**) as yellow solid, yield 100%.

A mixture of compound **33-c** (305 mg, 0.5 mmol), HATU (285 mg, 0.75 mmol), TEA (202 mg, 2 mmol) and ethyl amine (45 mg, 1 mmol) in DCM (4 mL) was stirred for 12 hours at room temperature. Then H<sub>2</sub>O (4 mL) was added and the mixture was extracted with DCM (10 mL). The organic layer was dried over sodium sulfate. After removal of solvent, the residue was purified by chromatography (EtOAc: Hexane=1:3) to afford the product methyl (3*S*)-4-[(4-(2-chloro-4-fluoro-phenyl)-5-(ethylcarbamoyl)-2-thiazol-2-yl-1,4-dihydropyrimidin-6-yl)methyl]morpholine-3-carboxylate (**33-d**) as yellow solid. Yield: 80%.

A solution of compound **33-d** (130 mg, 0.25 mmol) in Methanol (2 mL) was added aq. LiOH (1 M, 2 mL). After the reaction mixture was stirred for 12 hours at room temperature, the pH value of the solution was adjusted to 3 with 1 M aq. HCl. The mixture was then extracted with ethyl acetate (10 mL x 3). The organic layers were dried over sodium sulfate and concentrated *in vacuo* to give the crude product, which was purified by HPLC to afford (3*S*)-4-[(4*R*)-4-(2-chloro-4-fluoro-phenyl)-5-(ethylcarbamoyl)-2-thiazol-2-yl-1,4-dihydropyrimidin-6-yl)methyl]morpholine-3-carboxylic acid (**33**) as yellow solid Yield: 20%. MS: calc'd (MH<sup>+</sup>) 508, measured (MH<sup>+</sup>) 508. <sup>1</sup>H NMR (Methanol-d4, 400 MHz) δ ppm 7.96 (d, *J* = 3.14 Hz, 1H), 7.79 (*br* d, *J* = 2.89 Hz, 1H), 7.49 (dd, *J* = 6.15, 8.53 Hz, 1H), 7.25 (dd, *J* = 2.51, 8.66 Hz, 1H), 7.09 (dt, *J* = 2.64, 8.28 Hz, 1H), 6.13 (s, 1H), 4.05–4.31 (m, 2H), 4.01 (*br* d, *J* = 8.91 Hz, 1H), 3.81 (*br* s, 3H), 3.61(*br* s, 1H), 3.02–3.29 (m, 3H), 2.70(m, 1H), 1.09 (t, *J* = 7.22 Hz, 3H).

**(3*S*)-4-[(4*R*)-4-(2-Chloro-4-fluoro-phenyl)-5-cyano-2-thiazol-2-yl-1,4-dihydropyrimidin-6-yl)methyl]morpholine-3-carboxylic acid (34).**



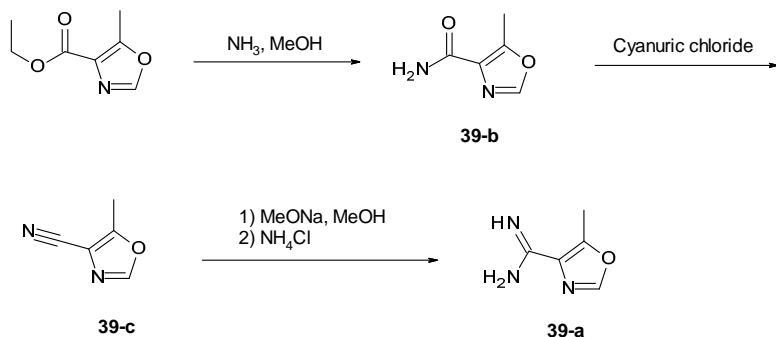
A mixture of (4*R*)-4-(2-chloro-4-fluoro-phenyl)-6-[(3*S*)-3-methoxycarbonylmorpholin-4-yl]methyl]-2-thiazol-2-yl-1,4-dihydropyrimidine-5-carboxylic acid (**33-c**, 305 mg, 0.5 mmol), HATU (285 mg, 0.75 mmol), TEA (202 mg, 2 mmol), ammonia solution (1 M in dioxane, 1.5 mL) in DCM (4 mL) was stirred for 12 hours at room temperature. Then H<sub>2</sub>O (4 mL) was added and the mixture was extracted with DCM (10 mL). The organic layer was dried over sodium sulfate. After removal of solvent, the residue was purified by chromatography flash (EA:PE = 1:3) to afford the product methyl (3*S*)-4-[(5-carbamoyl-4-(2-chloro-4-fluoro-phenyl)-2-thiazol-2-yl-1,4-dihydropyrimidin-6-yl)methyl]morpholine-3-carboxylate (**34-a**) as a yellow solid. Yield: 50%.

A solution of compound **34-a** (123 mg, 0.25 mmol) in THF (2 mL) was added TFAA (0.25 mL). After the reaction mixture was stirred for 3 hrs at r.t., the solvent was removed *in vacuo* and the residue was re-dissolved in MeOH (3 mL). Then K<sub>2</sub>CO<sub>3</sub> (0.25 g, 1.8 mmol) was added to this solution and the mixture was stirred at r.t. for 3 hr. The undissolved material was filtered and the solid was washed with EtOAc (10 mL × 2). The combined filtrate was concentrated and the residue was purified by column chromatography (EA:PE = 1/3 to 1/2) to afford product methyl (3*S*)-4-[(4-(2-chloro-4-fluoro-phenyl)-5-cyano-2-thiazol-2-yl-1,4-dihydropyrimidin-6-yl)methyl]morpholine-3-carboxylate **34-b** as yellow solid. Yield: 30%.

A solution of compound **34-b** (35 mg, 0.075 mmol) in Methanol (1 mL) was added aq. LiOH (0.5 M, 1 mL). After the reaction mixture was stirred for 12 hours at r.t., the pH value of the solution was adjusted to 3 with 1 M aq. HCl. The mixture was then extracted with ethyl acetate (5 mL x 3). The combined organic layers were dried over sodium sulfate and concentrated *in vacuo* to give the crude product, which was purified by HPLC to give (3*S*)-4-[(4*R*)-4-(2-chloro-4-fluoro-phenyl)-5-cyano-2-thiazol-2-yl-1,4-

dihydropyrimidin-6-yl]methyl]morpholine-3-carboxylic acid **34** as yellow solid, Yield: 20%. MS: calc'd (MH<sup>+</sup>) 462, measured (MH<sup>+</sup>) 462. <sup>1</sup>H NMR (Methanol-d4, 400 MHz) δ ppm 8.01 (d, *J* = 4.0 Hz, 1H), 7.80 (br d, *J* = 3.01 Hz, 1H), 7.53 (br dd, *J* = 5.96, 8.60 Hz, 1H), 7.32 (br dd, *J* = 2.57, 8.72 Hz, 1H), 7.18–7.22 (m, 1H), 5.93 (s, 1H), 4.02(m, 2H), 3.71–3.90 (m, 3H), 3.44–3.51 (m, 2H), 2.97(m, 1H), 2.51(m, 1H).

**5-Methyloxazole-4-carboxamidine (intermediate **39-a**).**

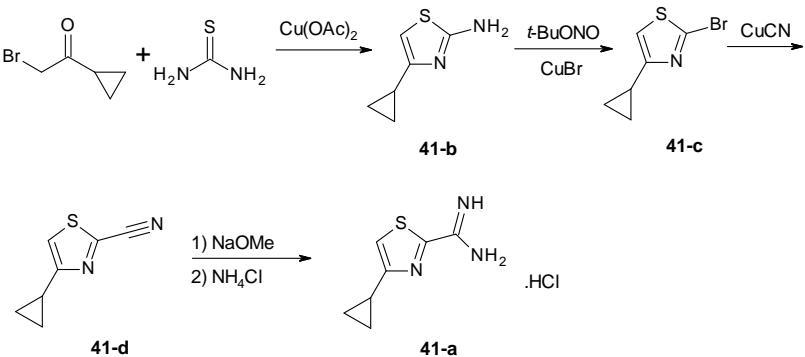


A mixture of ethyl 5-methyloxazole-4-carboxylate (CAS: 32968-44-8) (2.5 g, 16 mmol) and ammonia (100 mL, 7M in MeOH) was stirred at 65 °C in a sealed tube for 48 hours. The solvent was removed under reduced pressure to give **39-b** (1.94 g, 98%). <sup>1</sup>H NMR (DMSO- d<sub>6</sub>, 400 MHz) δ ppm 8.29 (s, 1H), 7.47 (s, 2H), 2.55 (s, 3H). MS: calc'd (MH<sup>+</sup>) 127, measured (MH<sup>+</sup>) 127.

To a white suspension of **39-b** (1.3 g, 10 mmol) in dry DMF (10 mL) was added cyanuric chloride (3.8 g, 21 mmol) at 0 °C. After the reaction was stirred at 0 °C for 2 hours, it was quenched with ice water (20 mL) carefully and then basified with 2N NaOH solution to pH 10. The mixture was extracted with EtOAc (50 mL × 3). The combined organic layers were washed with water (30 mL × 2), brine (30 mL × 1), dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated *in vacuo* to give a residue, which was purified by flash chromatography to afford colorless oil **39-c** (0.89 g, 79%). <sup>1</sup>H NMR (DMSO-d<sub>6</sub>, 400 MHz) δ ppm: 8.55 (s, 1H), 2.52 (s, 3H). To a solution of **39-c** (0.76 g, 7.0 mmol) in anhydrous MeOH (30 mL) was added MeONa (0.57 g, 10 mmol). After the mixture was stirred at 35 °C under nitrogen for 3h, NH<sub>4</sub>Cl (0.75 g, 14 mmol) was added and the mixture was refluxed for 2 hours. The solvent was removed and the residue was purified by flash

chromatography to afford a brown solid **39-a** (587 mg, 67%).  $^1\text{H}$  NMR (DMSO-d<sub>6</sub>, 400 MHz)  $\delta$  ppm: 9.11–9.19 (m, 3H), 8.60 (s, 1H), 2.59 (s, 3H). MS: calc'd (MH<sup>+</sup>) 126, measured (MH<sup>+</sup>) 126.

**4-Cyclopropyl-thiazole-2-carboxamidine (intermediate **41-a**)**



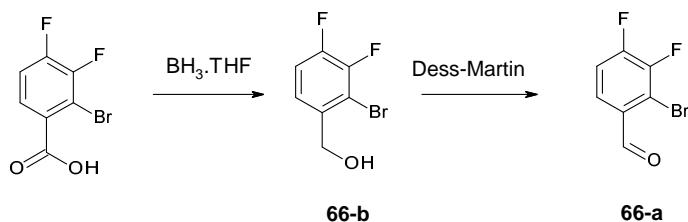
To a stirred solution of 2-bromo-1-cyclopropyl-ethanone (16 g, 100 mmol) in EtOH (150 mL) was added successively thiourea (8.0 g, 105 mmol) and Cu(OAc)<sub>2</sub> (0.90 g, 5.0 mmol) at room temperature. After the mixture was heated at 80 °C for 1 hour, the solvent was removed under reduced pressure. The residue was neutralized with saturated NaHCO<sub>3</sub> (100 mL) to pH = 8-9, and then DCM (200 mL) was added. The organic layer was separated and the aqueous layer was further extracted with DCM (200 mL x 2). The combined organic layers were washed with sat. NaHCO<sub>3</sub> (100 mL), brine (100 mL) and dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>. After filtration and concentration, the residue was purified by column chromatography (eluent: EA: PE = 1:4) to afford **41-b** (11 g, 78%) as a yellow solid.  $^1\text{H}$  NMR (CDCl<sub>3</sub>-d, 400 MHz):  $\delta$  ppm 6.06 (s, 1H), 4.86 (br s, 2H), 1.85–1.79 (m, 1H), 0.84–0.74 (m, 4H).

To a stirred solution of **41-b** (5.6 g, 40 mmol) and CuBr (8.5 g, 60 mmol) in CH<sub>3</sub>CN (100 mL) was added dropwise *t*-BuONO (6.2 g, 7.2 mL, 60 mmol) at 0 °C. Then the reaction mixture was warmed to room temperature and further stirred for additional 30 mins. After that, the precipitate was filtered and the solvent was removed under reduced pressure. The residue was purified by column chromatography (eluent: 100% petroleum ether) to afford **41-c** (3.5 g, 43%) as a light yellow oil containing small amount of petroleum ether.  $^1\text{H}$  NMR (400 MHz, CDCl<sub>3</sub>):  $\delta$  ppm 6.77 (s, 1H), 2.00–1.95 (m, 1H), 0.94–0.85 (m, 4H).

A mixture of **41-c** (3.3 g, 16 mmol) and CuCN (4.3 g, 49 mmol) in DMAc (15 mL) was heated to 135 °C for 3 hours. The reaction mixture was extracted with PE and dried over Na<sub>2</sub>SO<sub>4</sub>. The solvent was removed under reduced pressure to afford the crude product **41-d** (0.9 g, 38%) as a yellow oil, which was used as it in the next step.

From **41-d**, compound **41-a** was prepared in analogy to thiazole-2-carboxamidine **10A-1** as a brown solid.  
<sup>1</sup>H NMR (CDCl<sub>3</sub>-d, 400 MHz):  $\delta$  ppm 9.68 (br, 1H), 9.56 (br, 1H), 2.23–2.19 (m, 1H), 1.01–0.92 (m, 4H).

### 2-Bromo-3,4-difluoro-benzaldehyd (intermediate 66-a):

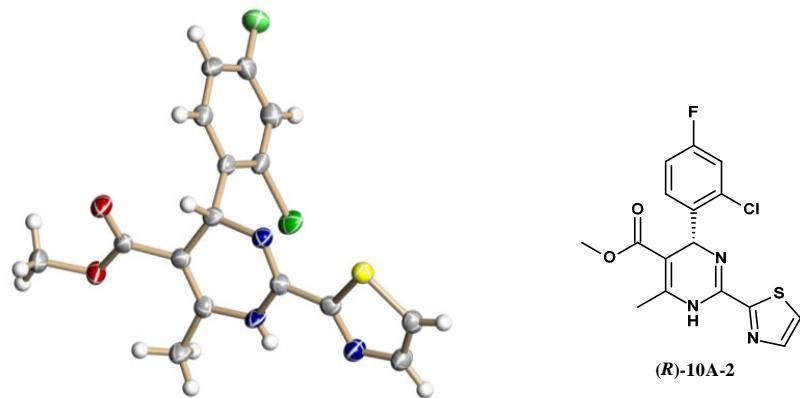


2-Bromo-3,4-difluorobenzoic acid (3.7 g, 16 mmol, Eq: 1.00) was dissolved in anhydrous THF (20 mL) and the solution was cooled to 0 °C.  $\text{BH}_3\text{-THF}$  (62.6 ml, 62.6 mmol, Eq: 4) was added dropwise. The resulting solution was allowed to warm up to room temperature and stirred overnight. The solution was cooled with an ice bath, and then 10% aqueous  $\text{Na}_2\text{CO}_3$  (30 mL) was added slowly. The suspension was concentrated *in vacuo* to give a white solid. The residue was acidified with 3M aqueous HCl solution (100 mL), diluted with dichloromethane (50 mL), and the mixture was filtered through Celite. The organic layers were separated and dried over sodium sulfate, and then followed by a filtration. Resulting filtrate was concentrated *in vacuo* to give 2-bromo-3,4-difluorophenyl)Methanol (**66-b**) as an off-white solid (2.55 g, 71%).  $^1\text{H}$  NMR (DMSO-d<sub>6</sub>, 400 MHz) δ ppm 7.46-7.53 (m, 1H), 7.35-7.41 (m, 1H), 4.51 (s, 2H), 4.44-4.58 (m, 2H).

Compound **66-b** (2.55 g, 11.4 mmol, Eq: 1.00) was dissolved in dichloromethane (15ml). Dess-Martin periodinane (4.85 g, 11.4 mmol, Eq: 1.00) was added. The reaction mixture was stirred for 2 hrs at room temperature. The reaction mixture was filtered over a plug of celite and washed with DCM (30 mL). Filtrate was concentrated to give a semi solid. Crude product was dissolved in EtOAc/DCM and loaded

into a silica gel column (4g). Silica gel column was flushed with EtOAc to give **66-a** as a light yellow solid (2.1 g, 85%). <sup>1</sup>H NMR (DMSO-d<sub>6</sub>, 400 MHz) δ ppm 10.13 (s, 1H), 7.74-7.82 (m, 1H), 7.70 (ddd, *J* = 9.5, 7.3, 0.8 Hz, 1H).

**X-ray crystal structure of (*R*)-10A-2.**



**Figure S1.** X-ray crystal structure of (*R*)-10A-2.

**Empirical formula** C16 H13 Cl F N3 O2 S

**Formula weight** 365.80

**Temperature** 150(2) K

**Wavelength** 1.54178 Å

**Crystal system, space group** Orthorhombic, P2(1)2(1)2(1)

**Unit cell dimensions**

*a* = 7.3344(2) Å *alpha* = 90 deg.

*b* = 13.5506(4) Å *beta* = 90 deg.

*c* = 15.7557(6) Å *gamma* = 90 deg.

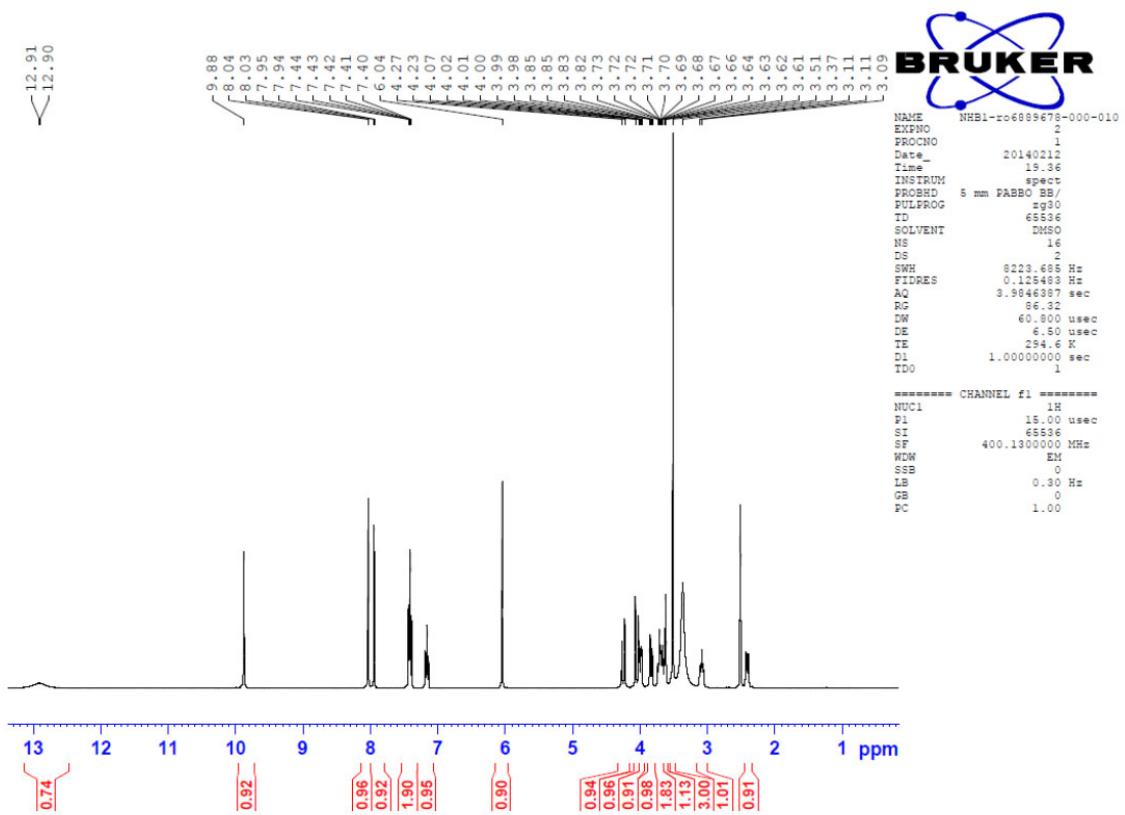
**Volume** 1565.89(9) Å<sup>3</sup>

**Z, Calculated density** 4, 1.552 Mg/m<sup>3</sup>

**Absorption coefficient** 3.648 mm<sup>-1</sup>

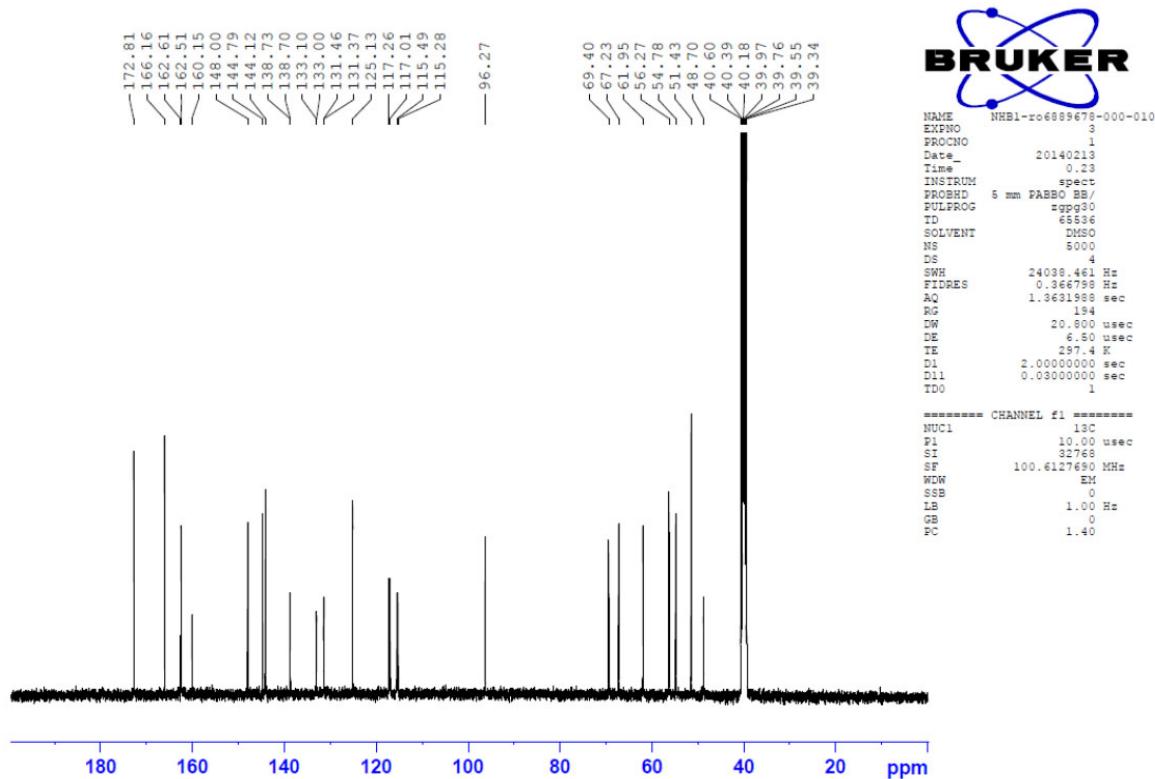
<b>F(000)</b>	752
<b>Crystal size</b>	0.4 x 0.3 x 0.2 mm
<b>Theta range for data collection</b>	4.30 to 58.91 deg.
<b>Limiting indices</b>	4<=h<=8, -12<=k<=15, -17<=l<=15
<b>Reflections collected / unique</b>	4420 / 2237 [R(int) = 0.0247]
<b>Completeness to theta = 58.91</b>	99.8 %
<b>Absorption correction</b>	Semi-empirical from equivalents
<b>Max. and min. transmission</b>	1.00000 and 0.80353
<b>Refinement method</b>	Full-matrix least-squares on F <sup>2</sup>
<b>Data / restraints / parameters</b>	2237 / 0 / 223
<b>Goodness-of-fit on F<sup>2</sup></b>	1.078
<b>Final R indices [I&gt;2sigma(I)]</b>	R1 = 0.0272, wR2 = 0.0722
<b>R indices (all data)</b>	R1 = 0.0275, wR2 = 0.0725
<b>Absolute structure parameter</b>	0.000(13)
<b>Largest diff. peak and hole</b>	0.198 and -0.227 e.Å <sup>-3</sup>

### **<sup>1</sup>H NMR of compound 10**



**Figure S2.**  $^1\text{H}$  NMR of compound **10**.

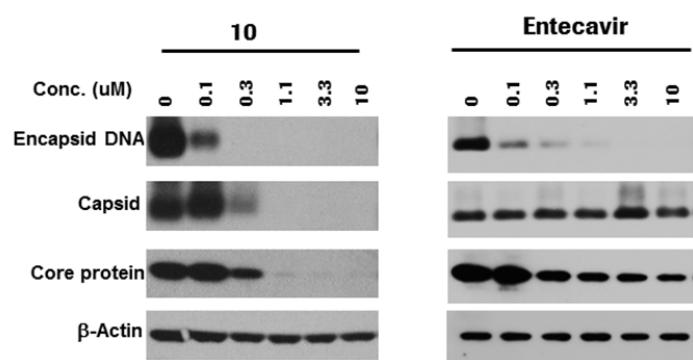
**$^{13}\text{C}$  NMR of compound 10**



**Figure S3.**  $^{13}\text{C}$  NMR of compound **10**.

#### Distinct Antiviral Effect of Compound **10** Compared to Entecavir.

To compare antiviral effect of capsid inhibitor with DNA polymerase inhibitor, the levels of core protein, capsid and encapsidated viral DNA in HepDE19 cells were examined by electrophoresis after the treatment with compound **10** and entecavir. As shown in Figure S4, both compounds can dose dependently reduce the encapsidated viral DNA. However, entecavir has no effect on either capsid or core protein at concentrations up to 10  $\mu\text{M}$ . In contrast, compound **10** at 0.1  $\mu\text{M}$  concentration induces significant degradation of both capsid and total core protein. This additional antiviral effect might indicate a better therapeutic potential of capsid inhibitor for HBV infection treatment as capsid is an important component of virus life cycle.



**Figure S4.** Antiviral effect comparison of capsid inhibitor **10** and polymerase inhibitor **Entecavir**.