

1 Chemistry Experimental Part

2 General

3 The following compounds were provided for testing by Enamine from (Enamine catalog screening/building
4 block collection IDs, see www.enaminestore.com): BPin-Thal-1 (Z4581577146/EN300-27105946), Bromo-
5 Thal-1 S (Z1269641829/EN300-7427158), Thalidomide (Z905162656/EN300-60005), BPin-NG-1
6 (Z7783943767/EN300-39926894), NG-1 (Z1269641791/EN300-33336997), BPin-EM12-1
7 (Z3660952592/EN300-7351981), Bromo-EM12-1 (Z3016698179/EN300-1272469), BPin-EM12-2
8 (Z3657566458/EN300-6767106), Bromo-EM12-2 (Z3205599917/EN300-6478894), BPin-EM12-3
9 (Z3660953199/EN300-7351983), Bromo-EM12-3 (Z3034847935/EN300-1608373), BPin-PDU-1
10 (Z5482475828/EN300-45215355), Bromo-PDU-1 (Z1343760689/EN300-4348253) Bromo-PDU-2
11 (Z1343760276/EN300-24851518), BPin-PDU-3 (Z8420004747/EN300-45481641), Bromo-PDU-3
12 (Z1343759533/EN300-2100570), PDU-1 (Z594217640/EN300-66200), PDU-2 (Z594217692/EN300-
13 935614), BPin-PG-1 (Z5194258336/EN300-35156530), Bromo-PG-1 (Z1509606371/EN300-25124578),
14 PG-1 (Z1251362416/EN300-258494), Bromo-PS-1 (Z1269121136/EN300-24812792), PS-1
15 (Z104341276/EN300-14419), Bromo-PH-1 (Z57138525/EN300-1168543), PH-1 (Z57138521/EN300-
16 30724)

17 EM12, Lenalidomide, FS-EM12, ARV-825 were purchased from MedChemExpress.

18 Starting reagents and building blocks including JQ1 carboxylic acid (EN300-7445162), (4-
19 Acetamidophenyl)(fluorosulfonyl)sulfamoyl fluoride or AISF (EN300-6745900), methyl 3-amino-5-bromo-
20 2-methylbenzoate (EN300-255346), 3-aminopiperidine-2,6-dione hydrochloride (EN300-113633), 3-
21 aminopiperidine-2,6-dione hydrochloride (EN300-113633), 4-{{4-(bromomethyl)phenyl}methyl}morpholine
22 hydrobromide (EN300-7494548), 5-bromo-1H,3H-benzo[de]isochromene-1,3-dione (EN300-118621), *tert*-
23 butyl (2-(2-(2-(2-bromoethoxy)ethoxy)ethoxy)ethyl)carbamate (EN300-6493693), 6-bromo-2-(2,6-
24 dioxopiperidin-3-yl)-4-fluoroisindoline-1,3-dione (EN300-47314144) were used as purchased from
25 Enamine Ltd. N-Me-Thal was synthesized by known procedure¹.

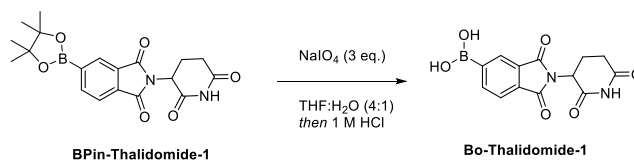
26 The products were purified by automated flash chromatography using Biotage Selekt or Interchim PuriFlash
27 XS520 chromatography systems and prepacked Biotage Sfär, Interchim PuriFlash or Büchi FlashPure Select
28 columns. Preparative HPLC was performed on an Agilent 1290 Infinity II Preparative LC System equipped
29 with an Agilent 6120 Quadrupole LC/MS mass detector using Chromatorex SMB C18 (100 x 19 mm, 5 µm)
30 and XBridge BEH C18 (100 x 20 mm, 5 µm) columns. Analytical LC/MS was performed on Agilent 1260
31 Infinity and Agilent 1260 Infinity II systems equipped with an Agilent 6120 Quadrupole LC/MS mass detector
32 and an Agilent 380-ELSD detector using Avantor ACE UltraCore 2.5 SuperC18 (50 × 4.6 mm, 2.5 µm),
33 Agilent Poroshell 120 SB C18 (30 x 4.6 mm, 2.7 µm) and Interchim US5C18HQ-050/046 (50 × 4.6 mm, 5
34 µm) columns. Analytical TLC was performed on pre-coated silica gel plates (Macherey-Nagel, Polygram®
35 SIL G/UV254). Visualization was accomplished with UV light, KMnO₄ solution, cerium(IV)/ ammonium

36 molybdate solution or ninhydrin solution. ^1H and ^{19}F NMR spectra were recorded with a Bruker Avance Neo
37 400 spectrometer [400 MHz (^1H), 377 MHz (^{19}F)]. Chemical shifts are reported in ppm (δ) with respect to
38 TMS, and the not fully deuterated solvent peak was used for referencing.

39

40 1. Synthesis of Br/Bo/Bpin derivatives of Thalidomide, N-Me-Thal, Naphthalimide
 41 glutarimide (NG) and EM12

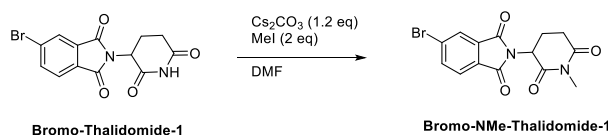
42
 43 1.1. (2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisindolin-5-yl)boronic acid (**Bo-Thal-1**)



45 To a solution of 2-(2,6-dioxopiperidin-3-yl)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)isoindoline-1,3-
 46 dione (**BPIN-Thal-1** or EN300-7427158, 300 mg, 1 eq, 0.78 mmol), in a mixture of tetrahydrofuran (2 mL)
 47 and water (0.5 mL) was added sodium periodate (500 mg, 3 eq, 2.34 mmol) and the mixture was stirred at rt.
 48 After 2 h, 6 M solution of HCl (1 eq, 0.78 mmol, 0.13 mL) was added and the resulting mixture was left at rt
 49 under stirring overnight. The reaction mixture was concentrated under reduced pressure, taken up in
 50 dichloromethane (6 mL) and water (2 mL) and stirred for 1 h. The resulting precipitate was filtered, washed
 51 with water, and dried to afford pure (2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisindolin-5-yl)boronic acid (**Bo-
 52 Thal-1**). Yield: 160 mg (67%) as a beige solid.

53 ¹H NMR (400 MHz, DMSO) δ 11.12 (s, 1H), 8.56 (s, 1H), 8.29 (s, 1H), 8.25 (d, J = 7.4 Hz, 1H), 7.9 (d, J =
 54 7.4 Hz, 1H), 5.16 (dd, J = 12.9, 5.4 Hz, 1H), 2.99 - 2.79 (m, 1H), 2.69 - 2.53 (m, 2H), 2.19 - 1.99 (m, 1H).
 55 LCMS: [M+H]⁺ m/z: calcd 303.07; found 303.0; R_t = 0.599 min.

56
 57 1.2. Synthesis of 5-bromo-2-(1-methyl-2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione (**Bromo-NMe-
 58 Thal-1**)

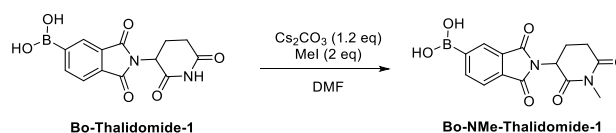


60
 61 To a solution of 5-bromo-2-(2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione (**Bromo-Thal-1** or EN300-
 62 7427158, 0.5 g, 1 eq, 1.49 mmol) in N,N-dimethylformamide (10 mL) was added Methyl iodide (0.32 g, 1.5
 63 eq, 2.23 mmol, 0.14 mL) and Cesium carbonate (0.58 g, 1.2 Eq, 1.79 mmol, 0.14 mL). The mixture was stirred
 64 at room temperature for 3 h. Then the additional amount of Methyl iodide (0.11 g, 0.5 eq, 0.74 mmol, 46.32
 65 μL) was added and the mixture was stirred at rt overnight. The resulted mixture was concentrated under
 66 vacuum and the crude product was purified over reverse phase column chromatography (40 g C18 silica,
 67 CH₃CN/H₂O + 0.1% Formic acid, 10-100% CH₃CN) giving pure 5-bromo-2-(1-methyl-2,6-dioxopiperidin-3-
 68 yl)isoindoline-1,3-dione (**Bromo-NMe-Thal**) as colorless solid. Yield: 0.135 g (26%).

¹H NMR (400 MHz, DMSO) δ 8.17 - 8.12 (m, 1H), 8.12 - 8.07 (m, 1H), 7.86 (d, J = 8 Hz, 1H), 5.23 (dd, J = 13.1, 5.4 Hz, 1H), 3.02 (s, 4H), 2.82 - 2.73 (m, 1H), 2.61 - 2.51 (m, 1H), 2.13 - 2.03 (m, 1H)

LCMS: [M+H]⁺ m/z: calcd 350.99; found 350.80; R_t = 1.235 min.

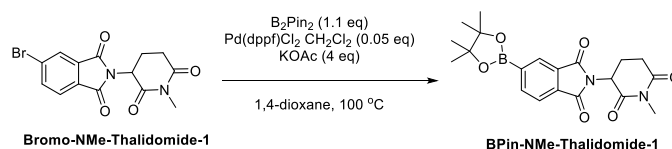
1.3. Synthesis of (2-(1-methyl-2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-5-yl)boronic acid (**Bo-NMe-Thal-1**)



To a solution of (2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-5-yl)boronic acid (**Bo-Thal-1**, 0.1 g, 0.33 mmol, 1 eq) in N,N-dimethylformamide (6 mL) was added Methyl iodide (70.48 mg, 0.5 mmol, 30.91 μL, 1.5 eq) and Cesium carbonate (0.13 g, 0.4 mmol, 1.2 eq, 31.78 μL). The mixture was stirred at room temperature for 3 h. Then the additional portion of Methyl iodide (23.49 mg, 0.17 mmol, 0.17 mL, 1 eq) was added and the mixture was stirred at rt overnight. The mixture was concentrated under vacuum and the crude mixture was purified over reverse phase column chromatography (40 g C18 silica, CH₃CN/H₂O + 0.1% formic acid, 10-100% CH₃CN) to lead to (2-(1-methyl-2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-5-yl)boronic acid **Bo-NMe-Thal-1** as colorless solid. Yield: 63 mg (60%).

¹H NMR (400 MHz, DMSO) δ 8.87 - 8.34 (m, 2H), 8.34 - 8.21 (m, 2H), 7.95 - 7.85 (m, 1H), 5.22 (dd, J = 13, 5.4 Hz, 1H), 3.02 (s, 3H), 3.01 - 2.89 (m, 1H), 2.83 - 2.72 (m, 1H), 2.65 - 2.51 (m, 1H), 2.15 - 2.01 (m, 1H)
LCMS: [M+H]⁺ m/z: calcd 317.09; found 316.8; R_t = 0.898 min.

1.4. Synthesis of 2-(1-methyl-2,6-dioxopiperidin-3-yl)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)isoindoline-1,3-dione (**BPin-NMe-Thal-1**)



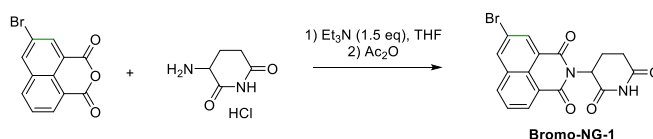
Into a crimped vial 5-bromo-2-(1-methyl-2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione (**Bromo-NMe-Thal**, 0.06 g, 1 eq, 0.17 mmol), Bis(pinacolato)diboron (65.15 mg, 1.1 eq, 0.26 mmol), potassium acetate (66.97 mg, 4 Eq, 0.68 mmol) and Pd(dppf)Cl₂ · CH₂Cl₂ (7.18 mg, 0.05 eq, 8.54 μmol) were added, the atmosphere was evacuated and backfilled with nitrogen three times and under inert atmosphere 1,4-dioxane (1 mL) was added. The mixture was degassed with nitrogen for three minutes then stirred at 100 °C overnight: The mixture was cooled down to room temperature, filtered over celite and washed with DCM, and concentrated under

reduced pressure. The resulted crude mixture was purified over normal phase column chromatography (12 g, ethyl acetate/cyclohexane, 20-70% ethyl acetate) to lead to 2-(1-methyl-2,6-dioxopiperidin-3-yl)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)isoindoline-1,3-dione (**BPin-NMe-Thal**) as colorless solid. Yield: 19 mg (29%).

¹H NMR (400 MHz, CDCl₃) δ 8.31 (s, 1H), 8.19 (dd, J = 7.5, 0.9 Hz, 1H), 7.86 (dd, J = 7.4, 0.7 Hz, 1H), 5.04 - 4.93 (m, 1H), 3.21 (s, 3H), 2.99 (dd, J = 13, 2.6 Hz, 1H), 2.89 - 2.7 (m, 2H), 2.18 - 2.07 (m, 1H), 1.37 (s, 12H).

LCMS: [M+H]⁺ m/z: calcd 399.16; found 399.20; R_t = 1.452 min.

1.5. Synthesis of 5-bromo-2-(2,6-dioxopiperidin-3-yl)-1H-benzo[de]isoquinoline-1,3(2H)-dione (**Bromo-NG-1**)

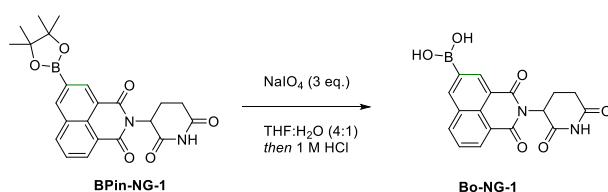


A mixture of 3-aminopiperidine-2,6-dione hydrochloride EN300-113633 (59.45 mg, 1 eq, 0.36 mmol), 5-bromo-1H,3H-benzo[de]isochromene-1,3-dione EN300-118621 (100 mg, 1 eq, 0.36 mmol) and triethylamine (54.97 mg, 1.5 eq, 0.54 mmol, 75.71 μL) in THF (1.5 mL) was refluxed for 20 h. The solvent was removed and the residue was suspended in acetic anhydride (1 mL) and refluxed for 20 minutes. Ethanol (5 mL) was added at 80°C and the mixture was stirred for 30 min. After the cooling the product was collected by filtration, washed with EtOAc, and recrystallized from hot DMSO to give 5-bromo-2-(2,6-dioxopiperidin-3-yl)-1H-benzo[de]isoquinoline-1,3(2H)-dione (**Bromo-NG-1**) as colorless solid. Yield: 14 mg (10%).

¹H NMR (400 MHz, DMSO) δ 11.03 (s, 1H), 8.82 (s, 1H), 8.62 - 8.38 (m, 3H), 7.94 (q, J = 7.6 Hz, 1H), 5.93 - 5.72 (m, 1H), 3.03 - 2.84 (m, 1H), 2.69 - 2.51 (m, 3H), 2.07 - 1.98 (m, 1H)

LCMS: [M+H]⁺ m/z: calcd 386.99; found 386.60; R_t = 1.234 min.

1.6. Synthesis of (2-(2,6-dioxopiperidin-3-yl)-1,3-dioxo-2,3-dihydro-1H-benzo[de]isoquinolin-5-yl)boronic acid (**Bo-NG-1**)



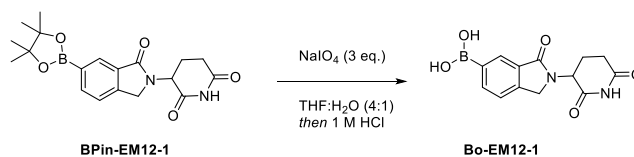
2-(2,6-dioxopiperidin-3-yl)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1H-benzo[de]isoquinoline-1,3(2H)-dione (**BPin-NG-1** or EN300-39926894, 50 mg, 1 eq, 0.12 mmol) with sodium periodate (73.89 mg, 3

eq, 0.35 mmol, 19.14 μ L) was stirred in a mixture of tetrahydrofuran (2 mL) and water (0.5 mL) for 30 min, then 6M HCl (4.14 mg, 1 eq, 0.12 mmol, 19.19 μ L) was added to the suspension. The reaction mixture was stirred for 24 h at room temperature. When reaction was completed, the mixture was diluted with water (10 mL) and extracted with EtOAc (3 \times 5 mL). The combined organic layers were washed with water (5 mL) and then brine (5 mL). The organic phase was dried using anhydrous Na₂SO₄ and concentrated in vacuum. The crude product was purified by C18 reverse phase column chromatography (eluent: CH₃CN:water 10%>100%) to obtain (2-(2,6-dioxopiperidin-3-yl)-1,3-dioxo-2,3-dihydro-1H-benzo[de]isoquinolin-5-yl)boronic acid **Bo-NG-1** as a beige solid. Yield: 18 mg (44%).

¹H NMR (400 MHz, DMSO) δ 11.01 (s, 1H), 8.98 (s, 0.5H), 8.89 (d, J = 6 Hz, 1H), 8.86 (s, 0.5H), 8.65 - 8.56 (m, 2H), 8.53 (d, J = 8 Hz, 1H), 8.48 (d, J = 7 Hz, 1H), 7.98 - 7.8 (m, 1H), 5.92 - 5.77 (m, 1H), 2.93 (d, J = 15.9 Hz, 1H), 2.67 - 2.53 (m, 2H), 2.11 - 1.99 (m, 1H).

LCMS: [M+H]⁺ m/z: calcd 353.09; found 352.8; R_t = 0.934 min

1.7. Synthesis of (2-(2,6-dioxopiperidin-3-yl)-3-oxoisindolin-5-yl)boronic acid (Bo-EM12-1)

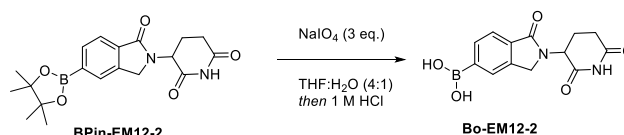


A mixture of 3-(1-oxo-6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)isindolin-2-yl)piperidine-2,6-dione (**BPin-EM12-1** or EN300-7351981, 300 mg, 1 eq, 0.81 mmol) and sodium periodate (0.52 g, 3 eq, 2.43 mmol) was stirred in the mixture of THF (2 mL) and water (0.5 mL) for 30 min. Then 6M HCl (29.16 mg, 1 Eq, 0.81 mmol, 0.14 mL) was added to the suspension and the mixture was left under stirring at rt overnight. When reaction was completed, the mixture was diluted with water and extracted with EtOAc (3 \times 5 mL). The combined organic layers were washed with water (5 mL) and brine (5 mL). The organic phase was dried using anhydrous Na₂SO₄, and the crude product was obtained by vacuum evaporation of solvent and was purified by C18 reverse phase column chromatography (eluent: CH₃CN:H₂O, 10% to 100%) giving (2-(2,6-dioxopiperidin-3-yl)-3-oxoisindolin-5-yl)boronic acid **Bo-EM12-1** as a beige solid. Yield: 0.16 g (68%).

¹H NMR (400 MHz, DMSO) δ 10.98 (s, 1H), 8.24 (s, 2H), 8.21 (s, 1H), 8.02 (dd, J = 7.6, 1 Hz, 1H), 7.57 (d, J = 7.6 Hz, 1H), 5.12 (dd, J = 13.4, 5.1 Hz, 1H), 4.53 - 4.42 (m, 1H), 4.4 - 4.28 (m, 1H), 2.97 - 2.82 (m, 1H), 2.6 (d, J = 16.8 Hz, 1H), 2.46 - 2.33 (m, 1H), 2.08 - 1.91 (m, 1H).

LCMS: [M+H]⁺ m/z: calcd 289.099; found 289.0; R_t = 0.514 min.

1.8. Synthesis of (2-(2,6-dioxopiperidin-3-yl)-3-oxoisindolin-5-yl)boronic acid (Bo-EM12-2)

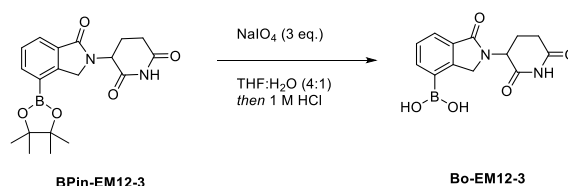


3-(1-Oxo-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)isoindolin-2-yl)piperidine-2,6-dione (**BPin-EM12-2** or EN300-6767106, 50 mg, 1 eq, 0.14 mmol) with sodium periodate (86.66 mg, 3 eq, 0.41 mmol, 22.45 μ L) was stirred in the mixture of tetrahydrofuran (1 mL) and water (0.25 mL) for 30 min, then 6M HCl (4.86 mg, 1 eq, 0.14 mmol, 22.51 μ L) was added to the suspension and the reaction mixture was stirred at rt overnight. When reaction was completed, the mixture was diluted with water (10 mL) and extracted with EtOAc (3 \times 10 mL). The combined organic layers were washed with water and then washed with brine. The organic phase was dried using anhydrous Na₂SO₄, and the crude product was purified by C18 reverse phase column chromatography (eluent: CH₃CN:water, 10 to 100%) to obtain (2-(2,6-dioxopiperidin-3-yl)-1-oxoisoindolin-5-yl)boronic acid **Bo-EM12-2** as a beige solid. Yield: 13 mg (33%).

¹H NMR (400 MHz, DMSO) δ 10.99 (s, 1H), 8.28 (s, 2H), 7.97 (s, 1H), 7.92 (d, J = 7.5 Hz, 1H), 7.69 (d, J = 7.6 Hz, 1H), 5.12 (dd, J = 13.2, 4.9 Hz, 1H), 4.54 - 4.39 (m, 1H), 4.39 - 4.26 (m, 1H), 3.01 - 2.78 (m, 1H), 2.6 (d, J = 16.7 Hz, 1H), 2.44 - 2.32 (m, 1H), 2.03 (d, J = 3 Hz, 1H).

LCMS: [M+H]⁺ m/z: calcd 289.099; found 289.0; R_t = 0.713 min.

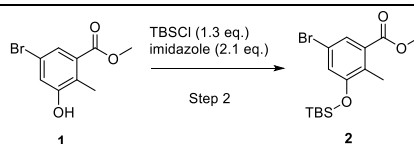
1.9. Synthesis of (2-(2,6-dioxopiperidin-3-yl)-1-oxoisoindolin-4-yl)boronic acid (**Bo-EM12-3**)



To a solution of 3-(1-oxo-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)isoindolin-2-yl)piperidine-2,6-dione (**BPin-EM12-3** or EN300-7351983, 50 mg, 0.135 mmol) in a mixture THF:H₂O (4:1, 1.5 mL) was added NaIO₄ (86 mg, 0.405 mmol) and the mixture was stirred at rt for 2 h. Then, 1 M aq. HCl (0.1 mL) was added, the resulting mixture was left under stirring overnight, concentrated under reduced pressure. The crude product was treated with CH₂Cl₂ (3 mL) and water (1 mL) and stirred for 1 h. The resulting precipitate was filtered, washed with water and dried giving pure (2-(2,6-dioxopiperidin-3-yl)-1-oxoisoindolin-4-yl)boronic acid **Bo-EM12-3** as a beige solid. Yield: 22 mg (56.55%).

¹H NMR (400 MHz, DMSO) δ 10.97 (s, 1H), 8.33 (s, 2H), 8.03 (d, J = 7.2 Hz, 1H), 7.76 (d, J = 6.8 Hz, 1H), 7.5 (t, J = 7.5 Hz, 1H), 5.13 (dd, J = 13.2, 5 Hz, 1H), 4.61 - 4.35 (m, 2H), 3 - 2.8 (m, 1H), 2.65 - 2.55 (m, 1H), 2.42 (d, J = 13.1 Hz, 1H), 2.08 - 1.93 (m, 1H).

LCMS: [M+H]⁺ m/z: calcd 289.099; found 289.0; R_t = 0.7921 min.

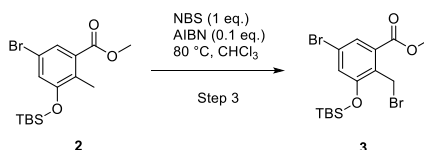


To a solution of methyl 5-bromo-3-hydroxy-2-methylbenzoate **1** (1 g, 4.1 mmol) in DMF (10 mL) was added 1H-imidazole (0.59 g, 8.61 mmol) at 0 °C. Then TBSCl (0.8 g, 5.33 mmol, 0.85 mL) was added dropwise and the mixture was stirred at rt. After 16 h, the reaction mixture was diluted with water (50 mL) and extracted with EtOAc (3×50 mL), the combined organic extracts were washed with brine (20 mL), dried under Na₂SO₄, filtered and concentrated under reduced pressure giving methyl 5-bromo-3-((*tert*-butyldimethylsilyl)oxy)-2-methylbenzoate **2** as a yellow oil, which was used for the next step without further purification. Yield: 1.47 g (4.07 mmol, quant).

¹H NMR (400 MHz, DMSO) δ 7.49 (d, J = 1.8 Hz, 1H), 7.13 (s, 1H), 3.82 (s, 3H), 2.26 (s, 3H), 0.98 (s, 9H), 0.23 (s, 6H).

LCMS: [M+H]⁺ m/z: calcd 359.067; found 358.8; R_t = 1.6680 min.

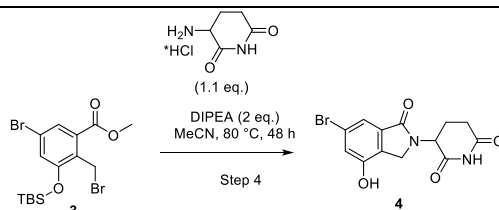
2.3. Step 3: The synthesis of methyl 5-bromo-2-(bromomethyl)-3-((*tert*-butyldimethylsilyl)oxy)-benzoate (**3**)



To a solution of methyl 5-bromo-3-((*tert*-butyldimethylsilyl)oxy)-2-methylbenzoate **2** (0.9 g, 2.50 mmol) in CHCl₃ (10 mL) was added *N*-Bromosuccinimide (0.44 g, 2.50 mmol) and 2,2'-(diazene-1,2-diyl)bis(2-methylpropanenitrile) (41.1 mg, 0.250 mmol) and the mixture was stirred at 80 °C. After 2 h, the reaction mixture was quenched with saturated aq. Na₂S₂O₃ (5 mL) and extracted with CH₂Cl₂ (3×10 mL). The organic extract was dried under Na₂SO₄, filtered and concentrated under reduced pressure to afford methyl 5-bromo-2-(bromomethyl)-3-((*tert*-butyldimethylsilyl)oxy)benzoate **3** as a yellow oil, which was used immediately into the next step. Yield: 0.9 g (81%).

¹H NMR (400 MHz, DMSO) δ 7.6 (d, J = 1.8 Hz, 1H), 7.23 (d, J = 1.9 Hz, 1H), 4.86 (s, 2H), 3.87 (s, 3H), 1.02 (s, 9H), 0.31 (s, 6H).

2.4. Step 4: The synthesis of 3-(6-bromo-4-hydroxy-1-oxoisindolin-2-yl)piperidine-2,6-dione (**4**)

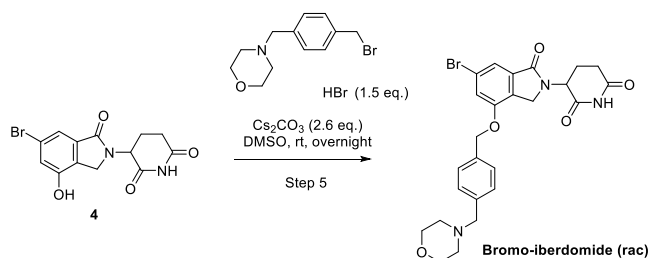


To a solution of methyl 5-bromo-2-(bromomethyl)-3-((tert-butyldimethylsilyl)oxy)benzoate **3** (0.9 g, 2.05 mmol) in MeCN (20 mL) were added DIPEA (0.53 g, 4.11 mmol, 0.71 mL) and 3-aminopiperidine-2,6-dione hydrochloride **EN300-113633** (0.37 g, 2.26 mmol) and the reaction mixture was stirred at 80 °C. After 48 h, water (30 mL) was added and the resulting grey precipitate was filtered, washed with water (3×10 mL) and dried on air to afford pure 3-(6-bromo-4-hydroxy-1-oxoisindolin-2-yl)piperidine-2,6-dione **4** as a light grey solid. Yield 0.60 g (86%).

¹H NMR (400 MHz, DMSO) δ 10.98 (s, 1H), 10.74 (s, 1H), 7.3 (d, J = 1.5 Hz, 1H), 7.19 (d, J = 1.4 Hz, 1H), 5.08 (dd, J = 13.3, 5.1 Hz, 1H), 4.38 - 4.24 (m, 1H), 4.21 - 4.1 (m, 1H), 3.04 - 2.75 (m, 1H), 2.65 - 2.55 (m, 1H), 2.47 - 2.29 (m, 1H), 2.04 - 1.89 (m, 1H).

LCMS: [M+H]⁺ m/z: calcd 338.997; found 339.0; R_t = 0.7794 min.

2.5. Step 5: The synthesis of 3-(6-bromo-4-((4-(morpholinomethyl)benzyl)oxy)-1-oxoisindolin-2-yl)piperidine-2,6-dione (**Bromo-iberdomide (rac)**)

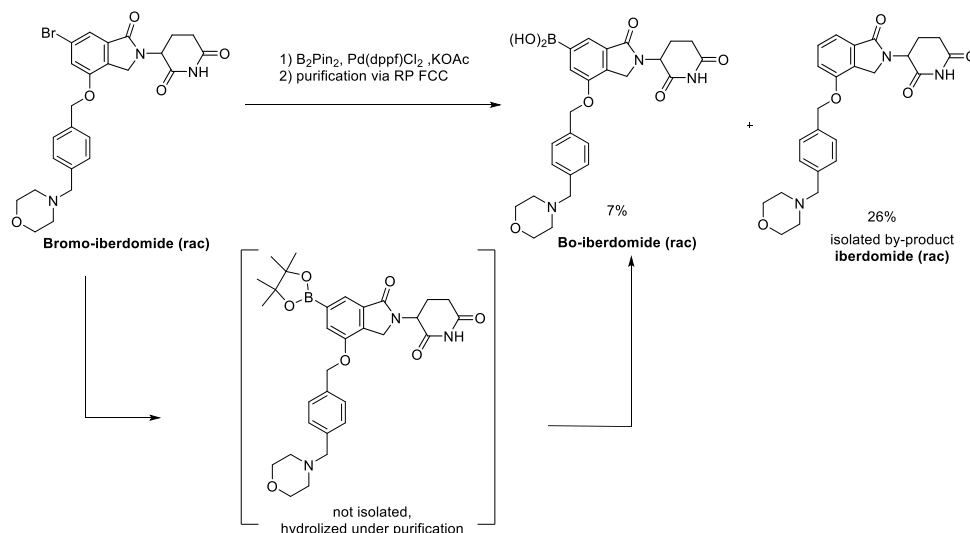


To a solution of 3-(6-bromo-4-hydroxy-1-oxoisindolin-2-yl)piperidine-2,6-dione **4** (0.15 g, 0.44 mmol) in dimethyl sulfoxide (3 mL) was added cesium carbonate (0.37 g, 1.15 mmol, 92 μL) and 4-[[4-(bromomethyl)phenyl]methyl]morpholine hydrobromide **EN300-7494548** (0.23 g, 0.66 mmol) and stirred at rt. After 16 h, the reaction mixture was directly purified with flash column chromatography (AQ C18, MeCN:H₂O (0.1% formic acid) 0-100%) to afford 3-(6-bromo-4-((4-(morpholinomethyl)benzyl)oxy)-1-oxoisindolin-2-yl)piperidine-2,6-dione **Bromo-iberdomide (rac)** as a white solid. Yield: 117 mg (50%).

¹H NMR (400 MHz, DMSO) δ 10.98 (s, 1H), 7.55 (d, J = 1.4 Hz, 1H), 7.47 (d, J = 1.4 Hz, 1H), 7.45 (s, 2H), 7.37 (s, 2H), 5.27 (s, 2H), 5.15 - 5.05 (m, 1H), 4.43 - 4.34 (m, 1H), 4.27 - 4.16 (m, 1H), 3.6 - 3.45 (m, 4H), 3.4 - 3.22 (m, 3H), 2.95 - 2.81 (m, 1H), 2.54 (s, 1H), 2.45 (s, 4H), 2.03 - 1.93 (m, 1H).

LCMS: [M+H]⁺ m/z: calcd 528.113; found 529.8; R_t = 0.9803 min.

265
266 **2.6. Step 6: The synthesis of (2-(2,6-dioxopiperidin-3-yl)-7-((4-(morpholinomethyl)benzyl)oxy)-3-**
267 **oxoisoindolin-5-yl)boronic acid (Bo-iberdomide (rac)) and 3-(4-((4-(morpholinomethyl)benzyl)oxy)-1-**
268 **oxoisoindolin-2-yl)piperidine-2,6-dione (iberdomide (rac))**



269
270
271 A solution of 3-(6-bromo-4-((4-(morpholinomethyl)benzyl)oxy)-1-oxoisoindolin-2-yl)piperidine-2,6-dione
272 **Bromo-iberdomide (rac)** (0.112 g, 0.21 mmol), potassium acetate (62.3 mg, 0.64 mmol),
273 bis(pinacolato)diboron (0.16 g, 0.64 mmol), $Pd(dppf)Cl_2 \cdot CH_2Cl_2$ (8.91 mg, 10.6 μ mol) in DMSO (2 mL)
274 was sealed and stirred at 80 °C. After 3 h, the reaction mixture was directly purified with flash column
275 chromatography (AQ C18, MeCN:H₂O (0.1% formic acid) 5-60%) to afford (2-(2,6-dioxopiperidin-3-yl)-7-
276 ((4-(morpholinomethyl)benzyl)oxy)-3-oxoisoindolin-5-yl)boronic acid **Bo-iberdomide (rac)** as a white solid
277 (yield: 7 mg, 7%) and 3-(4-((4-(morpholinomethyl)benzyl)oxy)-1-oxoisoindolin-2-yl)piperidine-2,6-dione
278 **iberdomide (rac)** as a white solid (yield: 25 mg, 26%).

279 **Bo-iberdomide (rac):** 1H NMR (400 MHz, DMSO) δ 10.96 (s, 1H), 8.26 (s, 2H), 7.84 (s, 1H), 7.72 (s, 1H),
280 7.45 (d, $J = 8$ Hz, 2H), 7.33 (d, $J = 8.1$ Hz, 2H), 5.22 (s, 2H), 5.11 (dd, $J = 13.2, 5.1$ Hz, 1H), 4.45 - 4.37 (m,
281 1H), 4.29 - 4.22 (m, 1H), 3.59 - 3.54 (m, 4H), 3.47 (s, 2H), 2.88 (d, $J = 12.9$ Hz, 1H), 2.58 (d, $J = 15.3$ Hz,
282 1H), 2.43 (s, 1H), 2.35 (s, 4H), 1.99 (s, 1H).

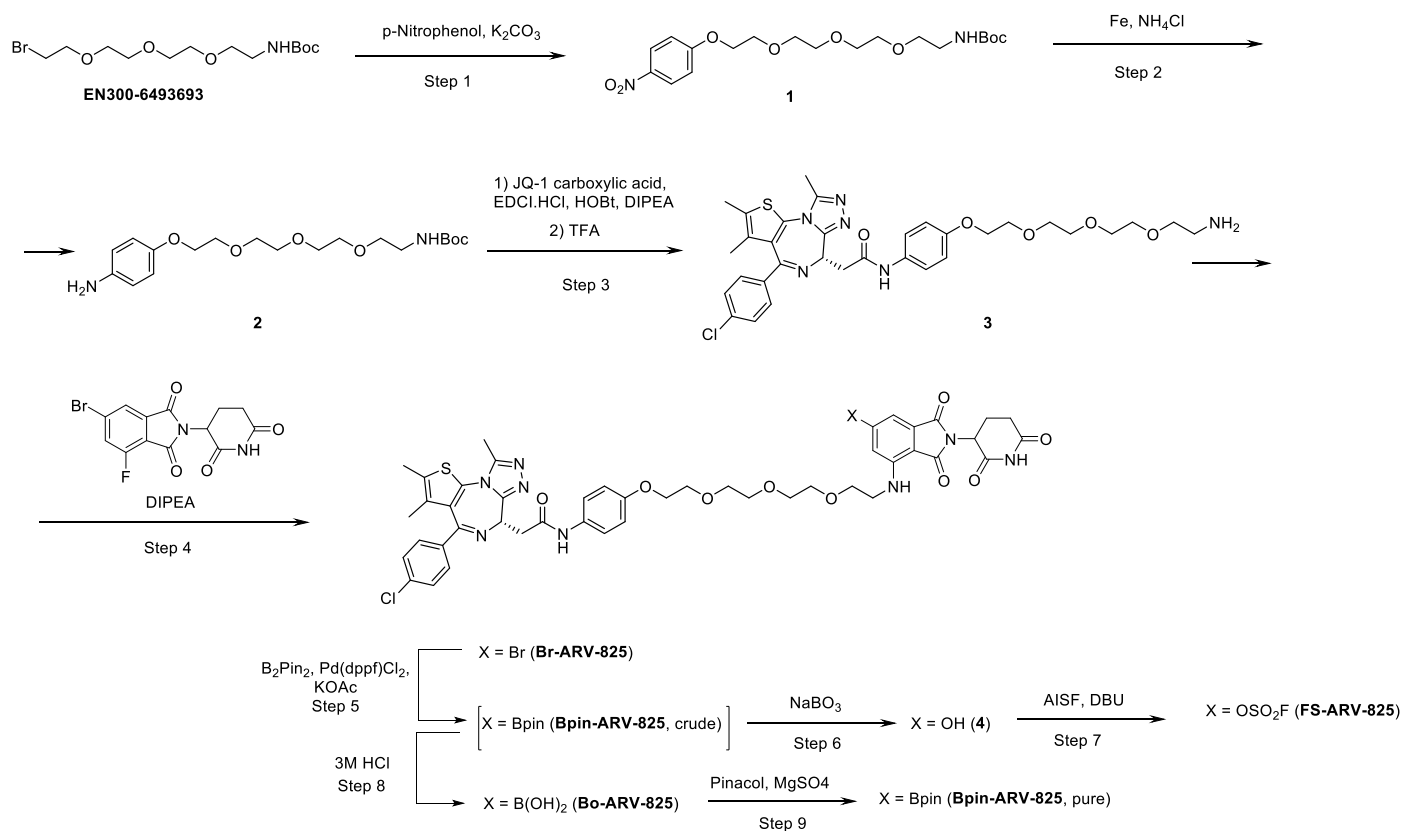
283 LCMS: $[M+H]^+$ m/z : calcd 494.209; found 493.8; $R_t = 0.8227$ min.

284 **Iberdomide (rac):** 1H NMR (400 MHz, DMSO) δ 10.96 (s, 1H), 7.52 - 7.41 (m, 3H), 7.33 (d, $J = 8.2$ Hz,
285 4H), 5.22 (s, 2H), 5.1 (s, 1H), 4.46 - 4.2 (m, 2H), 3.63 - 3.52 (m, 4H), 3.46 (s, 2H), 3 - 2.83 (m, 1H), 2.62 -
286 2.53 (m, 1H), 2.48 - 2.41 (m, 1H), 2.34 (d, $J = 4.4$ Hz, 4H), 2.03 - 1.91 (m, 1H).

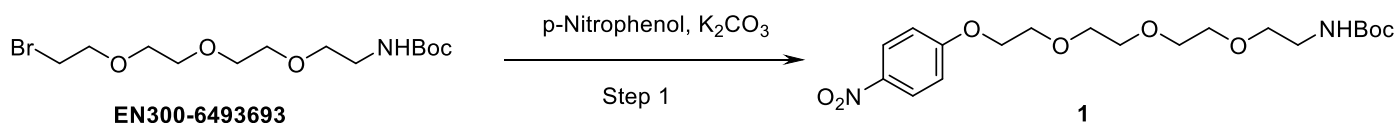
287 LCMS: $[M+H]^+$ m/z : calcd 450.202; found 449.8; $R_t = 0.8782$ min.

288 The Spectral data for **Iberdomide (rac)** coincides with the literature data³

3. The synthesis of Bromo (Br-), Pinacolborate (BPin-), Boronate (Bo-) and fluorosulfate (FS) substituted analogs of ARV-825



3.1. Step 1: the synthesis of tert-butyl (2-(2-(2-(2-(4-nitrophenoxy)ethoxy)ethoxy)ethoxy)ethyl)carbamate (1)

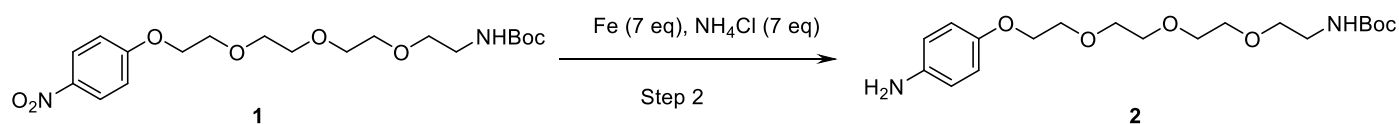


A mixture of 4-nitrophenol (0.47 g, 1.2 eq, 3.38 mmol, 0.32 mL), *tert*-butyl (2-(2-(2-(2-bromoethoxy)ethoxy)ethoxy)ethyl)carbamate EN300-6493693 (1 g, 1 eq, 2.82 mmol), potassium carbonate (0.78 g, 2 eq, 5.63 mmol, 0.78 mL) and DMF (14 mL) was stirred at 60°C overnight. The reaction mixture was diluted with ethyl acetate (20 mL) and water (10 mL), the organic layer was separated, the aqueous layer was extracted one more time with ethyl acetate (20 mL), the combined organics were washed with brine (3 × 25 mL), dried over Na₂SO₄, filtered and concentrated in vacuo to give *tert*-butyl (2-(2-(2-(2-(4-nitrophenoxy)ethoxy)ethoxy)ethoxy)ethyl)carbamate **1** as a yellow oil. Yield: 1.2 g (98 %).

¹H NMR (600 MHz, CDCl₃) δ 8.23 - 8.15 (m, 2H), 7.02 - 6.94 (m, 2H), 5.08 - 4.83 (m, 1H), 4.24 - 4.2 (m, 2H), 3.91 - 3.88 (m, 2H), 3.74 - 3.71 (m, 2H), 3.69 - 3.66 (m, 2H), 3.65 - 3.59 (m, 4H), 3.55 - 3.51 (m, 2H), 3.38 - 3.16 (m, 2H), 1.43 (s, 9H).

LCMS: $[M-Boc+H]^+$ m/z: calcd 315.34; found 315.0; $R_t = 1.3778$ min.

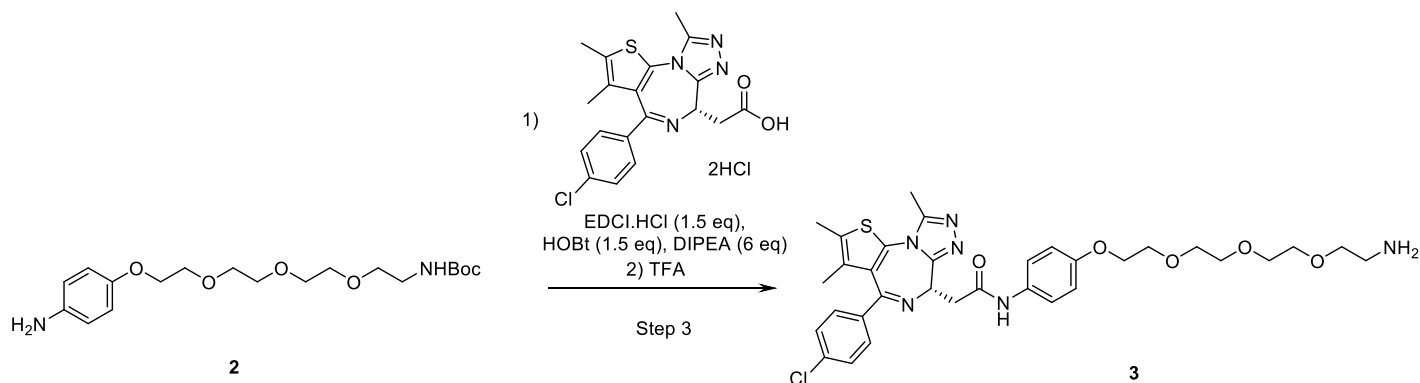
3.2. Step 2: the synthesis of *tert*-butyl (2-(2-(2-(2-(4-aminophenoxy)ethoxy)ethoxy)ethoxy)ethyl)carbamate



To a solution of *tert*-butyl (2-(2-(2-(2-(4-nitrophenoxy)ethoxy)ethoxy)ethoxy)ethyl)carbamate **1** (1.2 g, 1 eq, 2.9 mmol) in a mixture of ethanol (23 mL) and water (5.8 mL) was added ammonium chloride (1.07 g, 7 eq, 20.3 mmol) followed by iron (1.13 g, 7 eq, 20.3 mmol). The mixture was stirred at 80°C and monitored by LCMS until the full conversion (~ 1h). The reaction mixture was filtered, and the filtrate was concentrated under vacuum to remove ethanol. The obtained aqueous phase was extracted with EtOAc (3 × 25 mL), the combined organics were washed with brine, dried over Na₂SO₄, filtered and concentrated to give *tert*-butyl (2-(2-(2-(2-(4-aminophenoxy)ethoxy)ethoxy)ethoxy)ethyl)carbamate **2** as a brown oil, which was used in the next step without further purification. Yield: 0.92 g (83 %).

LCMS: $[M-Boc+H]^+$ m/z: calcd 285.36; found 285.0; $R_t = 0.9659$ min.

3.3. Step 3: *tert*-butyl (*S*)-(2-(2-(2-(2-(4-(2-(4-(4-chlorophenyl)-2,3,9-trimethyl-6*H*-thieno[3,2-*f*][1,2,4]triazolo[4,3-*a*][1,4]diazepin-6-yl)acetamido)phenoxy)ethoxy)ethoxy)ethoxy)ethyl)carbamate (**3**)



To a solution of (*R*)-2-(4-(4-chlorophenyl)-2,3,9-trimethyl-6*H*-thieno[3,2-*f*][1,2,4]triazolo[4,3-*a*][1,4]diazepin-6-yl)acetic acid dihydrochloride (JQ1-carboxylic acid dihydrochloride, 250 mg, 1.0 eq, 0.528 mmol) and *tert*-butyl (2-(2-(2-(2-(4-aminophenoxy)ethoxy)ethoxy)ethoxy)ethyl)carbamate **2** (243 mg, 1.2 eq, 0.634 mmol) in CH₂Cl₂ (5 mL) was added DIPEA (0.55 mL, 6 eq, 3.16 mmol), HOBT (107 mg, 1.5 eq, 0.792 mmol) and EDCI.HCl (151 mg, 1.5 eq, 0.792 mmol) and stirred at rt. After 16 h, the crude mixture was quenched with aq. NH₄Cl (10 mL) and extracted with CH₂Cl₂ (3×10 mL). The combined organic extract was dried under Na₂SO₄, filtered and concentrated under reduced pressure to afford crude product of acylation as yellow oil (594 mg), which was used into the next step without further purification.

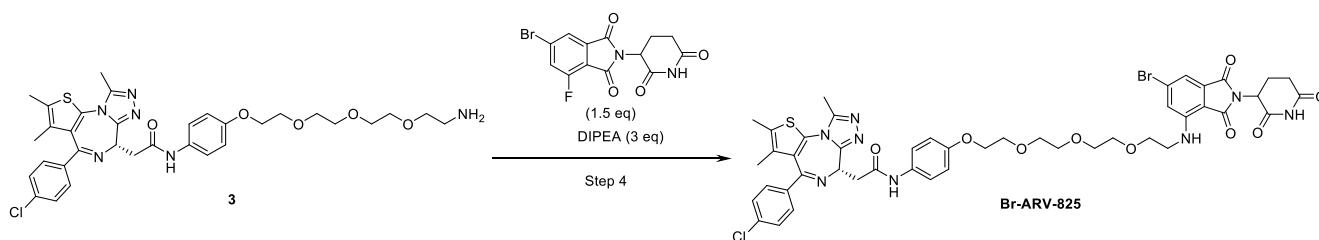
LCMS: $[M+H]^+$ m/z: calcd 767.299; found 767.20; $R_t = 1.0641$ min.

To a solution of the obtained crude product of acylation (590 mg) in DCM (5 mL) was added TFA (1 mL) and the mixture was stirred at rt. After 16 h, the reaction mixture was concentrated under reduced pressure and the crude residue was directly purified with flash column chromatography (C18, MeCN:H₂O, 30-80%) to afford (*R*)-*N*-(4-(2-(2-(2-(2-aminoethoxy)ethoxy)ethoxy)ethoxy)phenyl)-2-(4-(4-chlorophenyl)-2,3,9-trimethyl-6*H*-thieno[3,2-*f*][1,2,4]triazolo[4,3-*a*][1,4]diazepin-6-yl)acetamide **3** as a yellow oil. Yield: 369 mg (91%).

¹H NMR (400 MHz, DMSO) δ 10.18 (s, 1H), 8.23 (s, 2H), 7.56 - 7.51 (m, 2H), 7.51 - 7.46 (m, 2H), 7.45 - 7.39 (m, 2H), 6.92 - 6.86 (m, 2H), 4.6 - 4.57 (m, 1H), 4.06 (d, *J* = 4.4 Hz, 2H), 3.74 - 3.71 (m, 2H), 3.6 - 3.54 (m, 10H), 3.47 (d, *J* = 7 Hz, 2H), 2.95 (t, *J* = 5.3 Hz, 2H), 2.6 (s, 3H), 2.42 (d, *J* = 0.6 Hz, 3H), 1.63 (d, *J* = 0.6 Hz, 3H).

LCMS: $[M+H]^+$ m/z: calcd 667.246; found 667.2; $R_t = 1.2836$ min.

3.4. Step 4: *rac*-*N*-(4-(2-(2-(2-(2-((6-bromo-2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisindolin-4-yl)-amino)ethoxy)ethoxy)ethoxy)ethoxy)phenyl)-2-((*R*)-4-(4-chlorophenyl)-2,3,9-trimethyl-6*H*-thieno[3,2-*f*][1,2,4]triazolo[4,3-*a*][1,4]diazepin-6-yl)acetamide (Br-ARV-825)

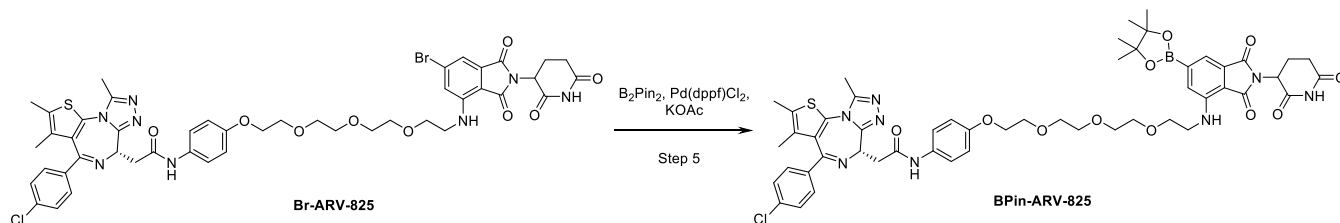


To a solution of *rac*-(*R*)-*N*-(4-(2-(2-(2-(2-aminoethoxy)ethoxy)ethoxy)ethoxy)phenyl)-2-(4-(4-chlorophenyl)-2,3,9-trimethyl-6*H*-thieno[3,2-*f*][1,2,4]triazolo[4,3-*a*][1,4]diazepin-6-yl)acetamide **3** (150 mg, 1 eq, 0.225 mmol) in DMF (3 mL) was added 6-bromo-2-(2,6-dioxopiperidin-3-yl)-4-fluoroisindoline-1,3-dione **EN300-47314144** (120 mg, 0.338 mmol) and DIPEA (0.118 μ L, 0.675 mmol) and the mixture was stirred at 80 °C for 4 h. After cooling the mixture was directly purified with flash column chromatography (C18, MeCN:H₂O (0.1% formic acid), 40-100%), affording *rac*-*N*-(4-(2-(2-(2-(2-((6-bromo-2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisindolin-4-yl)amino)-ethoxy)ethoxy)ethoxy)ethoxy)phenyl)-2-((*R*)-4-(4-chlorophenyl)-2,3,9-trimethyl-6*H*-thieno[3,2-*f*][1,2,4]triazolo[4,3-*a*][1,4]diazepin-6-yl)acetamide **Br-ARV-825** as a light yellow solid. Yield: 83 mg (37%).

¹H NMR (400 MHz, DMSO) δ 11.11 (s, 1H), 10.16 (s, 1H), 7.56 - 7.34 (m, 7H), 7.15 (d, *J* = 1.3 Hz, 1H), 6.88 (d, *J* = 9.2 Hz, 2H), 6.82 - 6.7 (m, 1H), 5.12 - 4.98 (m, 1H), 4.69 - 4.52 (m, 1H), 4.08 - 3.94 (m, 2H), 3.71 (d, *J* = 3.2 Hz, 2H), 3.64 - 3.42 (m, 15H), 3.29 - 3.24 (m, 1H), 2.94 - 2.79 (m, 1H), 2.61 (s, 3H), 2.42 (s, 3H), 2.08 - 1.97 (m, 1H), 1.64 (s, 3H).

LCMS: $[M+H]^+$ m/z: calcd 1001.205; found 1001.2; $R_t = 1.6113$ min.

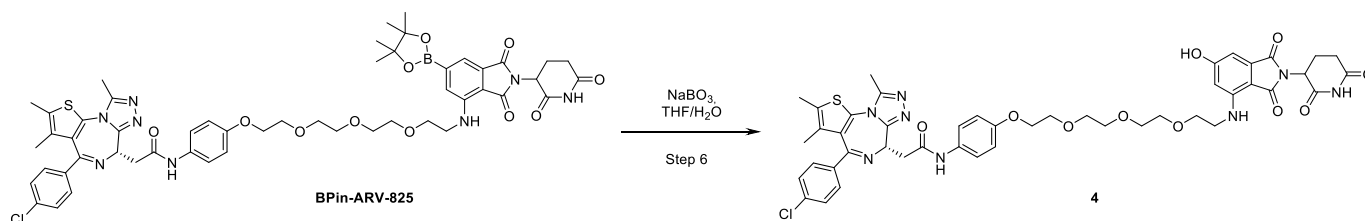
369 **3.5. Step 5: *rac*-2-((*R*)-4-(4-chlorophenyl)-2,3,9-trimethyl-6*H*-thieno[3,2-*f*][1,2,4]triazolo[4,3-
370 *a*][1,4]diazepin-6-yl)-*N*-(4-(2-(2-(2-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxo-6-(4,4,5,5-tetramethyl-1,3,2-
371 dioxaborolan-2-yl)isoindolin-4-yl)amino)ethoxy)ethoxy)ethoxy)ethoxy)phenyl)acetamide (**BPin-ARV-
372 825, crude**)**



373
374
375 A solution of *rac*-*N*-(4-(2-(2-(2-(2-((6-bromo-2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-
376 yl)amino)ethoxy)ethoxy)ethoxy)ethoxy)phenyl)-2-((*R*)-4-(4-chlorophenyl)-2,3,9-trimethyl-6*H*-thieno[3,2-
377 *f*][1,2,4]triazolo[4,3-*a*][1,4]diazepin-6-yl)acetamide **Br-ARV-825** (80 mg, 1 eq, 0.08 mmol),
378 bis(pinacolato)diboron (61 mg, 3 eq, 0.24 mmol), Pd(dppf)Cl₂ (4 mg, 0.1 eq, 0.008 mmol), KOAc (16 mg, 2
379 eq, 0.16 mmol) in degassed 1,4-dioxane (0.5 mL) was stirred at 100 °C for 1.5 h. The reaction mixture was
380 filtered and concentrated under reduced pressure to afford a brown oil (80 mg) of *rac*-2-((*R*)-4-(4-
381 chlorophenyl)-2,3,9-trimethyl-6*H*-thieno[3,2-*f*][1,2,4]triazolo[4,3-*a*][1,4]diazepin-6-yl)-*N*-(4-(2-(2-(2-(2-
382 ((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxo-6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)isoindolin-4-
383 yl)amino)ethoxy)ethoxy)ethoxy)ethoxy)phenyl)acetamide (**BPin-ARV-825**), which was used into the next
384 step without further purification.

385 LCMS: [M+H]⁺ m/z: calcd 1049.380; found 1049.4; R_t = 1.6340 min.

386
387 **3.6. Step 6: *rac*-2-((*R*)-4-(4-chlorophenyl)-2,3,9-trimethyl-6*H*-thieno[3,2-*f*][1,2,4]triazolo[4,3-
388 *a*][1,4]diazepin-6-yl)-*N*-(4-(2-(2-(2-(2-((2-(2,6-dioxopiperidin-3-yl)-6-hydroxy-1,3-dioxoisoindolin-4-
389 yl)amino)ethoxy)ethoxy)ethoxy)ethoxy)phenyl) (4)**

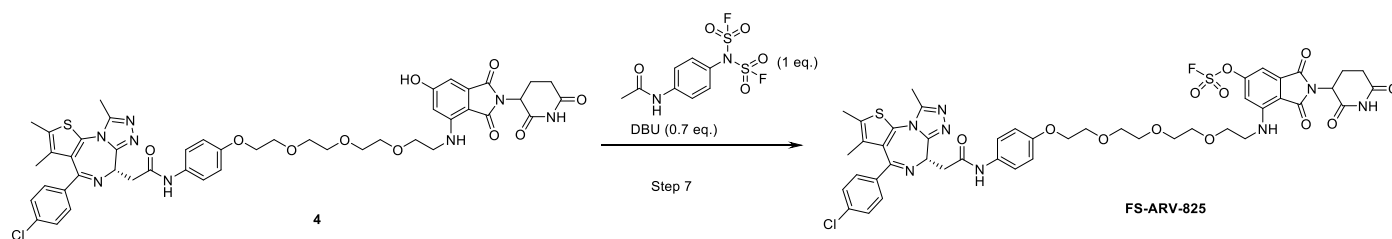


390
391
392 To a solution of crude **BPin-ARV-825** (80 mg) in THF:H₂O (2:1, 0.3 mL) was added NaBO₃ tetrahydrate (22
393 mg, 3 eq, 0.144 mmol). After 2 h, the reaction mixture was concentrated under reduced pressure and directly
394 purified with flash column chromatography (C18, MeCN:H₂O (0.1% formic acid), 10-100%), affording *rac*-
395 2-((*R*)-4-(4-chlorophenyl)-2,3,9-trimethyl-6*H*-thieno[3,2-*f*][1,2,4]triazolo[4,3-*a*][1,4]diazepin-6-yl)-*N*-(4-(2-
396 (2-(2-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxo-6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)isoindolin-
397 4-yl)amino)ethoxy)ethoxy)ethoxy)ethoxy)phenyl)acetamide **4** as a yellow solid. Yield: 26 mg (58%).

¹H NMR (400 MHz, DMSO) δ 11.08 - 11.02 (m, 1H), 10.73 - 10.59 (m, 1H), 10.19 - 10.12 (m, 1H), 7.53 (d, J = 8.9 Hz, 2H), 7.51 - 7.47 (m, 2H), 7.44 (s, 2H), 6.9 (s, 2H), 6.47 (s, 1H), 6.46 - 6.42 (m, 1H), 6.36 - 6.34 (m, 1H), 5.02 - 4.96 (m, 1H), 4.59 (s, 1H), 4.05 - 4.01 (m, 2H), 3.73 - 3.69 (m, 2H), 3.62 - 3.59 (m, 2H), 3.57 (s, 8H), 3.48 - 3.44 (m, 2H), 3.39 - 3.34 (m, 2H), 2.61 (s, 3H), 2.59 - 2.54 (m, 1H), 2.48 - 2.46 (m, 1H), 2.42 (s, 4H), 2.03 - 1.94 (m, 1H), 1.64 (s, 3H)

LCMS: [M+H]⁺ m/z: calcd 939.290; found 939.4; R_t = 1.5332 min.

3.7. Step 7: *rac*-7-((2-(2-(2-(2-(4-(2-((*R*)-4-(4-chlorophenyl)-2,3,9-trimethyl-6*H*-thieno[3,2-*f*][1,2,4]-triazolo[4,3-*a*][1,4]diazepin-6-yl)acetamido)phenoxy)ethoxy)ethoxy)ethoxy)ethyl)amino)-2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisindolin-5-yl sulfurofluoridate (FS-ARV-825)



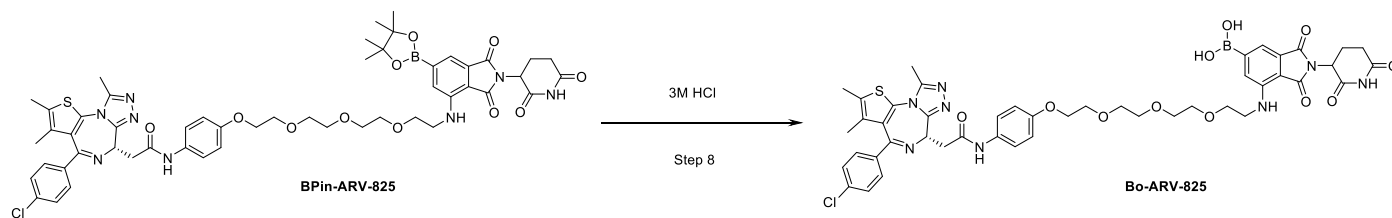
To a solution of *rac*-2-((*R*)-4-(4-chlorophenyl)-2,3,9-trimethyl-6*H*-thieno[3,2-*f*][1,2,4]triazolo[4,3-*a*][1,4]diazepin-6-yl)-*N*-(4-(2-(2-(2-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxo-6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)isoindolin-4-yl)amino)ethoxy)ethoxy)ethoxy)ethoxy)phenyl)acetamide **4** (26 mg, 0.028 mmol) in THF (0.5 mL) was added DBU (3 mg, 0.02 mmol) and (4-acetamidophenyl)-(fluorosulfonyl)sulfamoyl fluoride (8.8 mg, 0.028 mmol) and the mixture was stirred at rt. After 2 h, the reaction mixture concentrated under reduced pressure and directly purified with flash column chromatography (C18, MeCN:H₂O (0.1% formic acid) 50-100%), affording *rac*-7-((2-(2-(2-(2-(4-(2-((*R*)-4-(4-chlorophenyl)-2,3,9-trimethyl-6*H*-thieno[3,2-*f*][1,2,4]triazolo[4,3-*a*][1,4]diazepin-6-yl)acetamido)phenoxy)ethoxy)-ethoxy)ethoxy)ethyl)amino)-2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisindolin-5-yl sulfurofluoridate (**FS-ARV-825**) as a yellow solid. Yield: 9 mg (32%). Spectral data coincide with the literature

¹H NMR (400 MHz, DMSO) δ 11.11 - 11 (m, 1H), 10.08 (s, 1H), 7.48 - 7.43 (m, 2H), 7.43 - 7.39 (m, 2H), 7.35 (d, J = 8.5 Hz, 3H), 7.14 (s, 1H), 6.93 - 6.88 (m, 1H), 6.81 (d, J = 9.2 Hz, 2H), 5.03 (d, J = 7.6 Hz, 1H), 4.52 (s, 1H), 3.96 (d, J = 4.1 Hz, 2H), 3.67 - 3.59 (m, 2H), 3.58 - 3.52 (m, 2H), 3.5 - 3.4 (m, 10H), 3.39 (d, J = 7.3 Hz, 2H), 2.86 - 2.71 (m, 1H), 2.53 (s, 3H), 2.52 - 2.47 (m, 1H), 2.41 - 2.36 (m, 1H), 2.35 (s, 3H), 2 (s, 1H), 1.56 (s, 3H).

¹⁹F NMR (376 MHz, DMSO) δ 40.28 (s, 1F).

LCMS: [M+H]⁺ m/z: calcd 1021.242; found 1021.6; R_t = 1.4744 min.

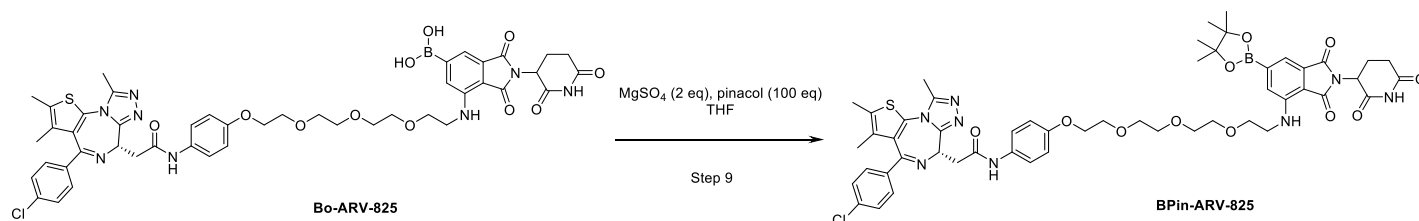
428 **3.8. Step 8:** (7-((2-(2-(2-(2-(4-(2-((S)-4-(4-chlorophenyl)-2,3,9-trimethyl-6H-thieno[3,2-f][1,2,4]-
429 *ptriazolo[4,3-a][1,4]diazepin-6-yl)acetamido)phenoxy)ethoxy)ethoxy)ethoxy)ethyl)amino)-2-(2,6-
430 *dioxopiperidin-3-yl)-1,3-dioxoisindolin-5-yl)boronic acid (Bo-ARV-825).**



433 A suspension of crude 2-((S)-4-(4-chlorophenyl)-2,3,9-trimethyl-6H-thieno[3,2-f][1,2,4]triazolo[4,3-
434 a][1,4]diazepin-6-yl)-N-(4-(2-(2-(2-(2-((2-(2,6-di (50 mg, 1 eq, 47.65 μmol) in 3M aq HCl (3mL) was stirred
435 overnight and then filtered. The resulted solid was purified by reverse phase flash chromatography (C18,
436 MeCN:H₂O (0.1% formic acid), 10-100%) to afford (7-((2-(2-(2-(2-(4-(2-((S)-4-(4-chlorophenyl)-2,3,9-
437 trimethyl-6H-thieno[3,2-f][1,2,4]triazolo[4,3-a][1,4]diazepin-6-yl)acetamidophenoxy)ethoxy)ethoxy)-
438 ethoxy)ethyl)amino)-2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisindolin-5-yl)boronic acid **Bo-ARV-825** as a
439 light yellow solid after lyophilization. Yield: 25 mg (54%).

440 ¹H NMR (400 MHz, DMSO) δ 11.09 (s, 1H), 10.16 (s, 1H), 8.59 - 8.16 (m, 2H), 7.62 - 7.36 (m, 8H), 6.89 (d,
441 $J = 9.1$ Hz, 2H), 6.6 - 6.47 (m, 1H), 5.05 (dd, $J = 12.9, 5.4$ Hz, 1H), 4.59 (t, $J = 7.1$ Hz, 1H), 4.09 - 3.92 (m,
442 2H), 3.75 - 3.45 (m, 18H), 2.93 - 2.82 (m, 1H), 2.61 (s, 3H), 2.42 (s, 3H), 2.09 - 1.97 (m, 1H), 1.63 (s, 3H).
443 LCMS: $[M+H]^+$ m/z : calcd 967.3; found 966.8; $R_t = 1.3175$ min.

444
445 **3.9. Step 9:** *rac-2-((R)-4-(4-chlorophenyl)-2,3,9-trimethyl-6H-thieno[3,2-f][1,2,4]triazolo[4,3-a][1,4]-*
446 *diazepin-6-yl)-N-(4-(2-(2-(2-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxo-6-(4,4,5,5-tetramethyl-1,3,2-*
447 *dioxaborolan-2-yl)isoindolin-4-yl)amino)ethoxy)ethoxy)ethoxy)ethoxy)phenyl)acetamide (BPin-ARV-*
448 *825, pure)*



451 A suspension of (7-((2-(2-(2-(2-(4-(2-((S)-4-(4-chlorophenyl)-2,3,9-trimethyl-6H-thieno[3,2-f][1,2,4]-
452 triazolo[4,3-a][1,4]diazepin-6-yl)acetamidophenoxy)ethoxy)ethoxy)ethoxy)ethyl)amino)-2-(2,6-
453 dioxopiperidin-3-yl)-1,3-dioxoisindolin-5-yl)boronic acid **Bo-ARV-825** (10 mg, 1 eq, 10.34 μmol), 2,3-
454 dimethylbutane-2,3-diol (0.12 g, 100 eq, 1.03 mmol, 0.13 mL) and magnesium sulfate (2.48 mg, 2 eq, 20.68
455 μmol) in THF (1 mL) was stirred overnight and then filtered and concentrated in vacuum. The crude material
456 was purified by flash chromatography (EtOAc/MeOH 0-20%) to afford 2-((S)-4-(4-chlorophenyl)-2,3,9-

457 trimethyl-6H-thieno[3,2-f][1,2,4]triazolo[4,3-a][1,4]diazepin-6-yl)-N-(4-(2-(2-(2-(2-((2-(2,6-dioxopiperidin-
458 3-yl)-1,3-dioxo-6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)isoindolin-4-yl)amino)ethoxy)ethoxy)-
459 ethoxy)ethoxy)phenyl)acetamide (**BPin-ARV-825**) as a yellow solid after lyophilization. Yield: 5 mg (46%).
460 ¹H NMR (400 MHz, CDCl₃) δ 8.91 (s, 1H), 8.58 - 8.37 (m, 1H), 7.52 - 7.41 (m, 5H), 7.37 - 7.31 (m, 3H), 6.84
461 (d, J = 8.9 Hz, 2H), 6.51 - 6.33 (m, 1H), 4.95 - 4.8 (m, 1H), 4.75 - 4.65 (m, 1H), 4.15 - 4.03 (m, 2H), 3.86 -
462 3.66 (m, 13H), 3.6 - 3.48 (m, 3H), 2.85 - 2.63 (m, 6H), 2.41 (s, 3H), 2.12 - 2.02 (m, 1H), 1.67 (s, 3H), 1.35 (s,
463 12H).

464 LCMS spectra contain peaks of both **BPin-ARV-825** and **Bo-ARV-825**:

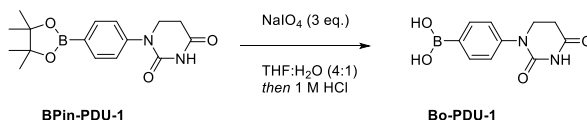
465 **BPin-ARV-825**: LCMS: [M+H]⁺ m/z: calcd 1049.4; found 1048.8; R_t = 1.555 min.

466 **Bo-ARV-825**: LCMS: [M+H]⁺ m/z: calcd 967.3; found 966.8; R_t = 1.3236 min.

467

4. Synthesis of Pinacolborate (BPin-) and Boronate (Bo-) derivatives of Phenyl Dehydrouracyl (PDU), Phenyl Glutarimide (PG), Phenyl Hydantoin (PH) and Phenyl Succinimide (PS).

4.1. (4-(2,4-Dioxotetrahydropyrimidin-1(2H)-yl)phenyl)boronic acid (Bo-PDU-1)

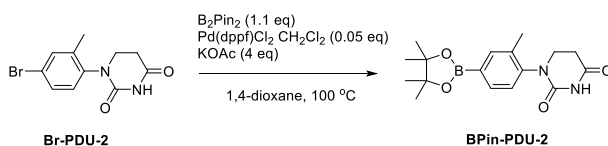


1-(4-(4,4,5,5-Tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)dihydropyrimidine-2,4(1H,3H)-dione (**BPin-PDU-1** or EN300-45215355, 100 mg, 1 eq, 0.32 mmol) with sodium periodate (0.2 g, 3 eq, 0.95 mmol, 52.57 μ L) was stirred in a mixture of THF (2 mL) and water (0.5 mL) for 30 min, then 1M HCl (11.38 mg, 1 eq, 0.32 mmol, 0.32 mL) was added to the suspension and the reaction mixture was stirred at rt for 24 h. Then the reaction mixture was diluted with water (10 mL) and extracted with EtOAc (3 \times 10 mL). The combined organic phases were washed with water (10 mL) and then with brine (10 mL), dried under Na₂SO₄ and concentrated in vacuum. The resulted crude product was purified by silica gel column chromatography (eluent: CH₂Cl₂ / methanol, 98:2) to obtain (4-(2,4-dioxotetrahydropyrimidin-1(2H)-yl)phenyl)boronic acid **Bo-PDU-1** as colorless solid after lyophilization. Yield: 20 mg (27%).

¹H NMR (400 MHz, DMSO) δ 10.37 (s, 1H), 8.04 (s, 2H), 7.78 (d, J = 8.3 Hz, 2H), 7.29 (d, J = 8.5 Hz, 2H), 3.8 (t, J = 6.7 Hz, 2H), 2.7 (t, J = 6.7 Hz, 2H).

LCMS: [M+H]⁺ m/z: calcd 235.08; found 235.0; R_t = 0.726 min.

4.2. 1-(2-Methyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)dihydro-pyrimidine-2,4(1H,3H)-dione (BPin-PDU-2)

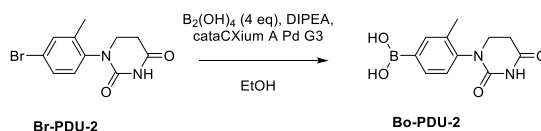


To a solution of 1-(4-bromo-2-methylphenyl)dihydropyrimidine-2,4(1H,3H)-dione (**Bromo-PDU-2** or EN300-24851518, 100 mg, 1 eq, 0.35 mmol) in 1,4-dioxane (2 mL) was added bis(pinacolato)diboron (0.11 g, 1.2 eq, 0.43 mmol), KOAc (41.69 mg, 1.2 eq, 0.43 mmol) and Pd(dppf)Cl₂ (12.94 mg, 0.05 eq, 17.73 μ mol). The reaction was stirred at 80 °C for 12 h under N₂ atmosphere. When the reaction was completed, the mixture was quenched with H₂O (5 mL) at 25 °C and then extracted with EtOAc (3 \times 10 mL). The combined organic layers were washed with brine (2 \times 10 mL), dried over Na₂SO₄, filtered and concentrated under reduced pressure. The residue was purified by column chromatography (SiO₂, Petroleum ether/Ethyl acetate, 10:1 to 1:1) to give 1-(2-methyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)dihydropyrimidine-2,4(1H,3H)-dione **BPin-PDU-2** as colorless solid after lyophilization. Yield: 39 mg, (34%).

¹H NMR (400 MHz, CDCl₃) δ 7.76 (s, 1H), 7.71 (d, J = 7 Hz, 1H), 7.48 (s, 1H), 7.19 (d, J = 7 Hz, 1H), 3.88 (s, 1H), 3.75 - 3.54 (m, 1H), 2.84 (s, 2H), 2.28 (s, 3H).

LCMS: [M+H]⁺ m/z: calcd 331.18; found 331.2; R_t = 1.277 min.

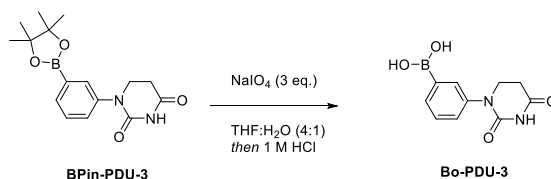
4.3. (4-(2,4-Dioxotetrahydropyrimidin-1(2H)-yl)-3-methylphenyl)boronic acid (**Bo-PDU-2**)



To a solution of 1-(4-bromo-2-methylphenyl)dihydropyrimidine-2,4(1H,3H)-dione (**Bromo-PDU-2** or EN300-24851518, 100 mg, 1 eq, 0.35 mmol) in ethanol (3 mL) was added hypodiboric acid (0.13 g, 4 eq, 1.42 mmol), N-ethyl-N-isopropylpropan-2-amine (0.14 g, 3 eq, 1.06 mmol, 0.19 mL) and cataCXium A Pd G3 (12.89 mg, 0.05 eq, 17.7 μmol). The mixture was stirred at r.t. for 16 h under N₂ atmosphere and then concentrated in vacuo. The residue was purified by flash silica gel chromatography (0-20% (EtOAc:EtOH, 3:1) / petroleum ether) to give (4-(2,4-dioxotetrahydropyrimidin-1(2H)-yl)-3-methylphenyl)boronic acid **Bo-PDU-2** as colorless solid after lyophilization. Yiled: 46 mg (52%).

¹H NMR (400 MHz, DMSO) δ 10.33 (s, 1H), 8.04 (s, 2H), 7.68 (s, 1H), 7.64 (d, J = 7.9 Hz, 1H), 7.2 (d, J = 7.7 Hz, 1H), 3.85 - 3.69 (m, 1H), 3.56 - 3.43 (m, 1H), 2.84 - 2.71 (m, 1H), 2.71 - 2.61 (m, 1H), 2.18 (s, 3H).
LCMS: [M+H]⁺ m/z: calcd 249.10; found 249.0; R_t = 0.750 min.

4.4. (3-(2,4-Dioxotetrahydropyrimidin-1(2H)-yl)phenyl)boronic acid (**Bo-PDU-3**)

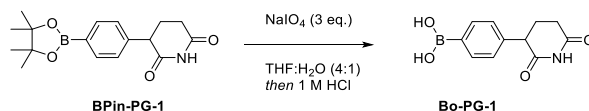


1-(3-(4,4,5,5-Tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)dihydropyrimidine-2,4(1H,3H)-dione (**BPin-PDU-3** or EN300-45481641, 100 mg, 1 Eq, 0.32 mmol) with sodium periodate (0.2 g, 3 eq, 0.95 mmol, 52.57 μL) is stirred in the mixture of THF (2 mL) and water (0.5 mL) for 30 min, then 1M HCl (11.38 mg, 1 eq, 0.32 mmol, 0.32 mL) was added to the suspension. The reaction mixture was stirred for 24 h at room temperature. After the end of the reaction, the reaction mixture is diluted with water and extracted with EtOAc. The combined organic phase is washed with water and then washed with saline. The organic phase was dried using anhydrous Na₂SO₄, and the crude product was obtained by vacuum evaporation solvent, and the crude product was purified by C18 reverse phase flash chromatography (eluent: water:CH₃CN, gradient 10 to 100%) to obtain (3-(2,4-dioxotetrahydropyrimidin-1(2H)-yl)phenyl)boronic acid **Bo-PDU-3** as colorless solid after lyophilization. Yield: 25 mg (34%).

¹H NMR (400 MHz, DMSO) δ 10.38 (s, 1H), 8.15 (s, 2H), 7.73 (d, J = 1.5 Hz, 1H), 7.71 - 7.63 (m, 1H), 7.44 - 7.33 (m, 2H), 3.82 (t, J = 6.7 Hz, 2H), 2.75 (t, J = 6.7 Hz, 2H).

LCMS: [M+H]⁺ m/z: calcd 235.08; found 235.2; R_t = 0.713 min.

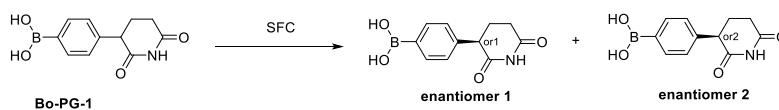
4.5. (4-(2,6-Dioxopiperidin-3-yl)phenyl)boronic acid (**Bo-PG-1**)



3-(4-(4,4,5,5-Tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)piperidine-2,6-dione (**BPin-PG-1** or EN300-35156530, 100 mg, 1 eq, 0.32 mmol) with sodium periodate (0.2 g, 3 eq, 0.95 mmol, 52.74 μL) was stirred in the mixture of THF (2 mL) and water (0.5 mL) for 30 min, then 1M HCl (11.42 mg, 1 eq, 0.32 mmol, 0.32 mL) was added to the suspension and the reaction mixture was stirred at rt for 24 h. Then the reaction mixture was diluted with water (10 mL) and extracted with EtOAc (3×10 mL). The combined organic phases were washed with water (10 mL) and then with brine (10 mL). The combined organic phases were dried under Na₂SO₄, and the solution was concentrated in vacuum. The obtained crude product was purified by silica gel column chromatography (eluent: CH₂Cl₂/methanol, 98:2) to obtain (4-(2,6-dioxopiperidin-3-yl)phenyl)boronic acid **Bo-PG-1** as colorless solid colorless solid after lyophilization. Yield: 23 mg (31%).

¹H NMR (400 MHz, DMSO) δ 10.83 (s, 1H), 8 (s, 2H), 7.74 (d, J = 8.1 Hz, 2H), 7.18 (d, J = 8 Hz, 2H), 3.84 (dd, J = 11.3, 5 Hz, 1H), 2.72 - 2.6 (m, 1H), 2.46 (t, J = 4.4 Hz, 1H), 2.27 - 2.11 (m, 1H), 2.07 - 1.96 (m, 1H)
LCMS: [M+H]⁺ m/z: calcd 234.09; found 234.0; R_t = 0.780 min.

4.6 Separation of enantiomers of (4-(2,6-dioxopiperidin-3-yl)phenyl)boronic acid (**Bo-PG-1**)



(4-(2,6-Dioxopiperidin-3-yl)phenyl)boronic acid (**Bo-PG-1**, 20.3 mg, 1 eq, 87.11 μmol) was separated under SFC conditions (absolute configuration of enantiomers was not established) using (S,S) Whelk-O[®]1 column (250×21 mm, 5 μm); mobile phase : CO₂:*i*-PrOH:CH₃CN, 85:7.5:7.5; Flow Rate: 50 mL/min. Add-on: 15 mL/min, column temperature:40 °C; wavelength: 210 nm.

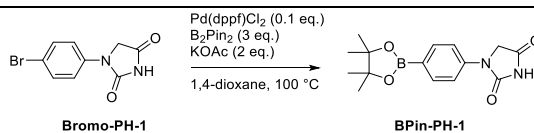
Enantiomer 1: colorless solid colorless solid after lyophilization, R_{t1} = 10.28 min. Yield: 9.2 mg (45%).

LCMS: [M+H]⁺ m/z: calcd 234.09; found 234.0; R_t = 0.823 min.

Enantiomer 2: colorless solid colorless solid after lyophilization, R_{t2} = 16.43 min. Yield: 9.6 mg (47%).

LCMS: [M+H]⁺ m/z: calcd 234.09; found 234.0; R_t = 0.822 min.

4.7. 1-(4-(4,4,5,5-Tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)imidazolidine-2,4-dione (**BPin-PH-1**).

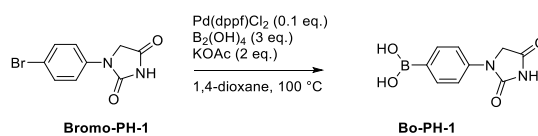


A solution of 1-(4-bromophenyl)imidazolidine-2,4-dione (**Bromo-PH-1** or EN300-1168543, 100 mg, 0.392 mmol), bis(pinacolato)diboron (299 mg, 3 eq, 1.176 mmol), Pd(dppf)Cl₂ (20 mg, 0.039 mmol), KOAc (77 mg, 2 eq, 0.784 mmol) in degassed 1,4-dioxane (2 mL) was sealed and heated at 100 °C. After 2 h, the crude mixture was concentrated under reduced pressure. The residue was purified with flash column chromatography (SiO₂, ethyl acetate/c-Hexane, 10-100%), affording 1-(4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)imidazolidine-2,4-dione **BPin-PH-1** as a beige solid after lyophilization. Yield: 32 mg (27.04%).

¹H NMR (400 MHz, DMSO) δ 11.25 (s, 1H), 7.73 - 7.49 (m, 4H), 4.44 (s, 2H), 1.29 (s, 12H).

LCMS: [M-H]⁻ m/z: calcd 301.137; found 301.0; R_t = 1.0953 min.

4.8. (4-(2,4-Dioxoimidazolidin-1-yl)phenyl)boronic acid (**Bo-PH-1**)

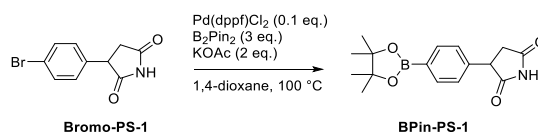


A solution of 1-(4-bromophenyl)imidazolidine-2,4-dione (**Bromo-PH-1** or EN300-1168543, 200 mg, 0.784 mmol), tetrahydroxydiboron (211 mg, 3 eq, 2.35 mmol), Pd(dppf)Cl₂ (40 mg, 0.1 eq, 0.078 mmol), potassium acetate (154 mg, 1.57 mmol) in degassed 1,4-dioxane (2 mL) was sealed and heated at 100 °C. After 2 h, the crude mixture was concentrated under reduced pressure. The residue was purified with flash column chromatography (C18, MeCN:H₂O (0.1% formic acid) 10-60%), affording (4-(2,4-dioxoimidazolidin-1-yl)phenyl)boronic acid **Bo-PH-1** as a white solid after lyophilization. Yield: 23 mg (13.39%).

¹H NMR (400 MHz, DMSO) δ 11.19 (s, 1H), 7.96 (s, 2H), 7.78 (d, J = 8.6 Hz, 2H), 7.56 (d, J = 8.7 Hz, 2H), 4.44 (s, 2H).

LCMS: [M-H]⁻ m/z: calcd 219.058; found 219.0; R_t = 0.4739 min.

4.9 3-(4-(4,4,5,5-Tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)pyrrolidine-2,5-dione (**BPin-PS**)



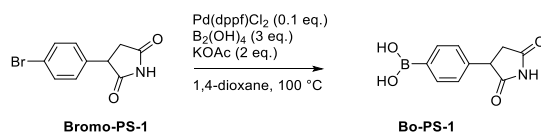
A solution of 3-(4-bromophenyl)pyrrolidine-2,5-dione (**Bromo-PS-1** or EN300-24812792, 100 mg, 0.394 mmol), bis(pinacolato)diboron (300 mg, 3 eq, 1.182 mmol), Pd(dppf)Cl₂ (20 mg, 0.1 eq, 0.039 mmol), KOAc

(77 mg, 2 eq, 0.788 mmol) in degassed 1,4-dioxane (2 mL) was sealed and heated at 100 °C. After 2 h, the crude mixture was concentrated under reduced pressure. The residue was purified with flash column chromatography (C18, MeCN:H₂O 10-60%), giving 3-(4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)pyrrolidine-2,5-dione **BPin-PS-1** as a white solid after lyophilization. Yield: 40 mg (34%).

¹H NMR (400 MHz, DMSO) δ 11.35 (s, 1H), 7.65 (d, J = 8.1 Hz, 2H), 7.30 (d, J = 8.1 Hz, 2H), 4.15 (dd, J = 9.5, 5.3 Hz, 1H), 3.13 (dd, J = 18, 9.5 Hz, 1H), 2.70 (dd, J = 18.1, 5.4 Hz, 1H), 1.29 (s, 12H).

LCMS: [M-H]⁻ m/z: calcd 300.141; found 300.0; R_t = 1.0890 min.

4.10. (4-(2,5-Dioxopyrrolidin-3-yl)phenyl)boronic acid (**Bo-PS-1**)



A solution of 3-(4-bromophenyl)pyrrolidine-2,5-dione (**Bromo-PS-1** or EN300-24812792, 200 mg, 0.787 mmol), tetrahydroxydiboron (212 mg, 3 eq, 2.36 mmol), Pd(dppf)Cl₂ (40 mg, 0.1eq, 0.079 mmol), KOAc (154 mg, 2 eq, 1.57 mmol) in degassed 1,4-dioxane (2 mL) was sealed and heated at 100 °C. After 2 h, the crude mixture was concentrated under reduced pressure. The residue was purified with flash column chromatography (C18, MeCN:H₂O (0.1% formic acid), 10-60%), affording (4-(2,5-dioxopyrrolidin-3-yl)phenyl)boronic acid **Bo-PS-1** as a white solid after lyophilization. Yield: 23 mg (13%).

¹H NMR (400 MHz, DMSO) δ 11.32 (s, 1H), 8.02 (s, 2H), 7.75 (d, J = 8 Hz, 2H), 7.24 (d, J = 8.1 Hz, 2H), 4.11 (dd, J = 9.5, 5.3 Hz, 1H), 3.12 (dd, J = 18.1, 9.5 Hz, 1H), 2.71 (dd, J = 18.1, 5.3 Hz, 1H).

LCMS: [M-H]⁻ m/z: calcd 218.063; found 218.0; R_t = 0.4462 min.