Supporting Information

Discovery of a Potent Dual Inhibitor of Wild-Type and Mutant Respiratory Syncytial Virus Fusion Proteins

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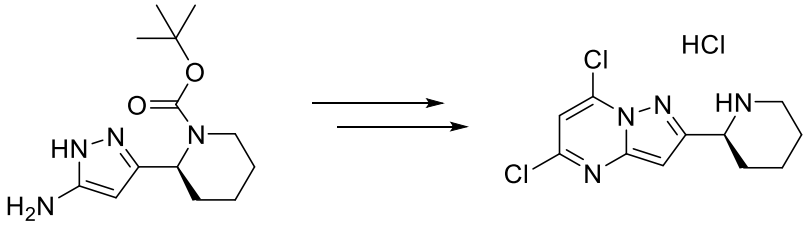
Synthetic Procedures

General details

All solvents and reagents were purchased from commercial suppliers and were used without purification or were prepared according to published procedures. The ^{1}H-NMR and ^{13}C-NMR spectra of the compounds synthesized in this study were recorded on a JNM-ECA600, JNM-ECA500 (JEOL Ltd., Tokyo, Japan), or Avance III HD 400 (Bruker Corp., Billerica, MA, USA), and the chemical shifts were expressed as δ (:) values, with trimethylsilane as the internal standard (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, and brs = broad singlet). Two sets of NMR signals were observed due to structural variations of the cis- and trans-amide rotamers. Mass spectra were recorded on a Micromass Platform LC (Micromass Ltd., Manchester, UK) or Shimadzu LCMS-2010EV (Shimadzu Corp., Kyoto, Japan). High-resolution (HR) mass spectral data were acquired using an LCMS-IT-TOF equipped with an electrospray ionization (ESI)/atmospheric pressure chemical ionization dual ion source (Shimadzu Corp.). Intermediates and final compounds were purified using preparative HPLC on an Agilent 1260 Infinity/Agilent 6130 (Agilent Technologies Inc., Santa Clara, CA, USA) or a GX-281, UV/VIS-155, 331 PUMP, 332 PUMP, and SOFTA Model 300S ELSD (Gilson Inc., Middleton, WI, USA) under the following conditions: column, Sunfire prep C18 OBD (5.0 μm, 30 mm × 50 mm) (Waters Corp., Milford, MA, USA), YMC-Actus Triart C18 (5.0 μm, 30 mm × 50 mm) (YMC Co., Ltd., Kyoto, Japan), Xbridge Prep C18 OBD (5.0 μm, 30 mm × 50 mm) (Waters Corp.), or XSelect CSH C18 (5.0 μm, 30 mm × 50 mm) (Waters Corp.); flow, 50 mL/min; linear gradient, 10%–95% acetonitrile in water containing 0.1% formic acid for 7.5–11.5 min; detection wavelength, 254 nm. The purity of synthesized compounds was determined using an LC-MS system (Agilent 1290 Infinity, Agilent Technologies Inc.) under the following conditions: column, ACQUITY UPLC CSH C18 (1.7 μm, 2.1 × 50 mm) (Waters Corp.); flow, 0.8 mL/min; linear gradient, 20%–99% acetonitrile in water containing 0.1% formic acid in 1.2 min; detection wavelength, 254 nm. All final compounds were obtained with purity of ≥95%.



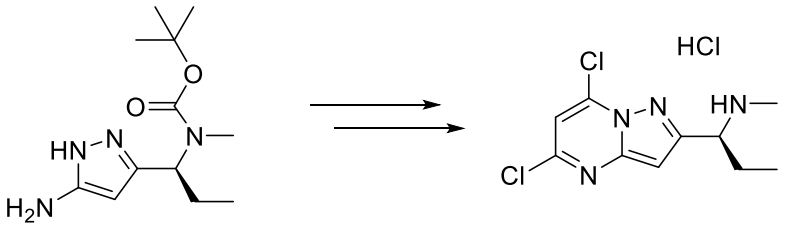
5,7-Dichloro-2-[(2*S*)-piperidin-2-yl]pyrazolo[1,5-a]pyrimidine hydrochloride (8c)

To a solution of *tert*-butyl (2*S*)-2-(5-amino-1*H*-pyrazol-3-yl)piperidine-1-carboxylate (15.0 g, 56.3

mmol) in ethanol (280 mL) was added diethyl malonate (13 mL, 84.5 mmol, 1.5 eq.) and 20% sodium ethoxide in ethanol (120 mL, 282 mmol, 5.0 eq.), and the mixture was stirred at 90°C for 18 h. The reaction mixture was acidified using 1M hydrogen chloride aq. and extracted with chloroform. The organic layer was washed with brine, dried over magnesium sulfate, filtered, and concentrated under reduced pressure to obtain *tert*-butyl (2*S*)-2-(7-hydroxy-5-oxo-4,5-dihydropyrazolo[1,5-a]pyrimidin-2-yl)piperidine-1-carboxylate (21 g, 61.3 mmol, quant.) as a pale-yellow amorphous, which was used for the next reaction without further purification. MS (ESI/APCI dual) m/z: 303 $[M-H]^-$.

A solution of *tert*-butyl (2*S*)-2-(7-hydroxy-5-oxo-4,5-dihydropyrazolo[1,5-a]pyrimidin-2-yl)piperidine-1-carboxylate (21 g, 61.3 mmol) in 4M hydrogen chloride in 1,4-dioxane (150 mL) was stirred for 3 h at room temperature. Then, the reaction mixture was concentrated under reduced pressure to obtain 7-hydroxy-2-[(2*S*)-2-piperidyl]-4*H*-pyrazolo[1,5-a]pyrimidin-5-one hydrochloride (17 g, 63.0 mmol, quant.) as a yellow powder, which was used for the next reaction without further purification. MS (ESI/APCI dual) m/z: 235 $[M+H]^+$.

The mixture of 7-hydroxy-2-[(2*S*)-2-piperidyl]-4*H*-pyrazolo[1,5-a]pyrimidin-5-one hydrochloride (8.5 g, 31.0 mmol) and phosphorus oxychloride (83 mL, 910 mmol, 29 eq.) was stirred at 90°C overnight. Then, the reaction mixture was concentrated under reduced pressure and washed with 2-propanol/diisopropyl ether (1:4) to obtain **8c** (8.5 g, 28.0 mmol, 88%) as a brown powder. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.37–9.59 (m, 2H), 7.11 (s, 1H), 7.77 (s, 1H), 4.57–4.70 (m, 1H), 3.28–3.41 (m, 1H), 2.99–3.17 (m, 1H), 2.13–2.25 (m, 1H), 1.58–1.95 (m, 5H). MS (ESI/APCI dual) m/z: 271 $[M+H]^+$.

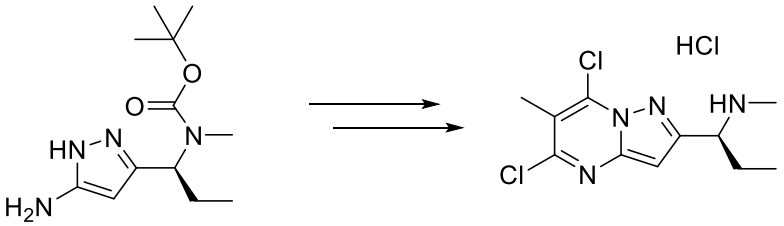


(1*S*)-1-(5,7-Dichloropyrazolo[1,5-a]pyrimidin-2-yl)-*N*-methylpropan-1-amine hydrochloride (8a)

To a solution of *tert*-butyl [(1*S*)-1-(5-amino-1*H*-pyrazol-3-yl)propyl]methylcarbamate (6.1 g, 23.8 mmol) in methanol (45 mL) was added dimethyl propanedioate (4.1 mL, 35.7 mmol, 1.5 eq.) and 28% sodium methoxide in methanol (24 mL, 119 mmol, 5.0 eq.), and the mixture was stirred at 90°C for 4 h. The reaction mixture was acidified using 1M hydrogen chloride aq. and extracted with chloroform. The organic layer was dried over a phase separator and concentrated under reduced pressure to obtain *tert*-butyl *N*-[(1*S*)-1-(7-hydroxy-5-oxo-4*H*-pyrazolo[1,5-a]pyrimidin-2-yl)propyl]-*N*-methyl

carbamate (7.6 g, 23.5 mmol, 99%) as a pale-yellow amorphous, which was used for the next reaction without further purification. MS (ESI/APCI dual) m/z: 323 $[M+H]^+$.

The mixture of *tert*-butyl *N*-[(1*S*)-1-(7-hydroxy-5-oxo-4*H*-pyrazolo[1,5-a]pyrimidin-2-yl)propyl]-*N*-methyl carbamate (1.1 g, 3.46 mmol) and phosphorus oxychloride (83 mL, 910 mmol, 29 eq.) was stirred at 110°C for 2 h. Then, the reaction mixture was concentrated under reduced pressure to obtain **8a** (2.3 g, 3.53 mmol, quant.) as a brown oil. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.28–9.67 (m, 2H), 7.76 (s, 1H), 7.10 (s, 1H), 4.32–4.51 (m, 1H), 2.42–2.48 (m, 3H), 1.94–2.21 (m, 2H), 0.77–0.86 (m, 3H). MS (ESI/APCI dual) m/z: 259 $[M+H]^+$.

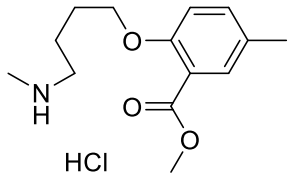


(1*S*)-1-(5,7-Dichloro-6-methylpyrazolo[1,5-a]pyrimidin-2-yl)-*N*-methylpropan-1-amine hydrochloride (8b)

To a solution of *tert*-butyl [(1*S*)-1-(5-amino-1*H*-pyrazol-3-yl)propyl]methyl carbamate (5.0 g, 19.7 mmol) in ethanol (66 mL) was added diethyl 2-methylmalonate (5.0 mL, 29.5 mmol, 1.5 eq.) and 20% sodium ethoxide in ethanol (33 mL, 98.3 mmol, 5.0 eq.), and the mixture was stirred at 90°C for 3 h. The reaction mixture was acidified using 1M hydrogen chloride aq. and extracted with chloroform. The organic layer was washed with brine, dried over a phase separator and concentrated under reduced pressure. The residue was washed with diethyl ether to obtain *tert*-butyl (*S*)-(1-(7-hydroxy-6-methyl-5-oxo-4,5-dihydropyrazolo[1,5-a]pyrimidin-2-yl)propyl)(methyl) carbamate (4.8 g, 13.3 mmol, 68%) as a yellow powder. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.30–11.89 (m, 1H), 5.50–5.75 (m, 1H), 4.87–5.23 (m, 1H), 2.59 (brs, 3H), 1.70–2.05 (m, 5H), 1.41 (s, 9H), 0.80–0.95 (m, 3H). MS (ESI/APCI dual) m/z: 337 $[M+H]^+$.

The mixture of *tert*-butyl (*S*)-[1-(7-hydroxy-6-methyl-5-oxo-4,5-dihydropyrazolo[1,5-a]pyrimidin-2-yl)propyl](methyl) carbamate (4.6 g, 13.6 mmol) and phosphorus oxychloride (13 mL, 136 mmol, 10 eq.) was stirred at 110°C for 3 h. Then, the reaction mixture was concentrated under reduced pressure. The residue was purified using silica gel column chromatography (NH 1%–5% methanol in chloroform). The collected fraction was added to 4M hydrogen chloride in ethyl acetate and concentrated under reduced pressure. The precipitate was washed with acetonitrile to obtain **8b** (1.8 g, 5.77 mmol, 42%) as a colorless powder. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.39 (brs, 2H), 7.05 (s, 1H), 4.33–4.45 (m, 1H), 2.48 (s, 3H), 2.45 (s, 3H), 1.93–2.19 (m, 2H), 0.77–0.85 (m, 3H). MS (ESI/APCI

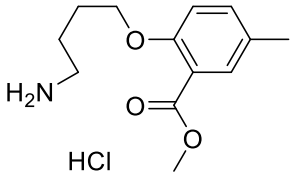
dual) m/z: 273 $[M+H]^+$.



Methyl 5-methyl-2-[4-(methylamino)butoxy]benzoate hydrochloride (9b)

To a solution of *tert*-butyl *N*-(4-hydroxybutyl)-*N*-methyl carbamate (1.0 g, 4.92 mmol) in chloroform (4.9 mL) was added triethylamine (2.1 mL, 14.8 mmol, 3.0 eq.) and methanesulfonyl chloride (0.57 mL, 7.38 mmol, 1.5 eq.) at 0°C. After stirring at room temperature for 1 h, the reaction mixture was poured into water and extracted with chloroform. The organic layer was dried over a phase separator and concentrated under reduced pressure. To a solution of the residue in *N*,*N*-dimethylformamide (9.8 mL) was added methyl 5-methylsalicylate (1.4 mL, 9.83 mmol, 2.0 eq.) and potassium carbonate (2.7 g, 19.7 mmol, 4.0 eq.). After stirring at 90°C for 2 h, the reaction mixture was poured into water and extracted with ethyl acetate. The organic layer was washed with brine and dried over magnesium sulfate, filtered, and concentrated under reduced pressure. The residue was purified using silica gel column chromatography (OH 5%–30% ethyl acetate in hexane) to obtain methyl 2-{4-[*tert*-butoxycarbonyl(methyl)amino]butoxy}-5-methyl benzoate (1.5 g, 4.15 mmol, 84%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.56–7.60 (m, 1H), 7.20–7.25 (m, 1H), 6.82–6.88 (m, 1H), 3.98–4.07 (m, 2H), 3.87 (s, 3H), 3.24–3.33 (m, 2H), 2.86 (s, 3H), 2.30 (s, 3H), 1.67–1.85 (m, 4H), 1.45 (s, 9H). MS (ESI/APCI dual) m/z: 374 $[M+Na]^+$.

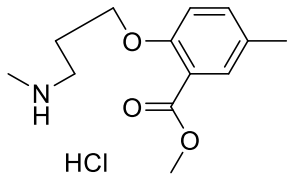
To a solution of methyl 2-{4-[*tert*-butoxycarbonyl(methyl)amino]butoxy}-5-methyl benzoate (1.46 g, 3.07 mmol) in 1,4-dioxane (5.0 mL) was added 4M hydrogen chloride in 1,4-dioxane (5.0 mL) and the mixture was stirred for 20 h at room temperature. Then, the reaction mixture was concentrated under reduced pressure to obtain **9b** (1.09 g, 3.78 mmol, 91%) as a colorless powder. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.58 (brs, 2H), 7.46 (s, 1H), 7.29–7.37 (m, 1H), 7.01–7.07 (m, 1H), 3.97–4.07 (m, 2H), 3.79 (s, 3H), 2.89–3.01 (m, 2H), 2.54 (s, 3H), 2.26 (s, 3H), 1.71–1.81 (m, 4H). MS (ESI/APCI dual) m/z: 252 $[M+H]^+$.



Methyl 2-(4-aminobutoxy)-5-methylbenzoate hydrochloride (9e)

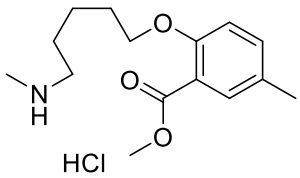
According to the procedure described for **9b**, the title compound was obtained as a colorless powder using *tert*-butyl *N*-(4-hydroxybutyl) carbamate instead of *tert*-butyl *N*-(4-hydroxybutyl)-*N*-methyl

carbamate. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.61–7.68 (m, 1H), 7.24–7.29 (m, 1H), 6.83–6.87 (m, 1 H), 4.09 (t, J = 5.1 Hz, 2H), 3.88 (s, 3H), 3.20 (t, J = 6.3 Hz, 2H), 2.29 (s, 3H), 2.00–2.14 (m, 4H). MS (ESI/APCI dual) m/z: 238 $[M+H]^+$.



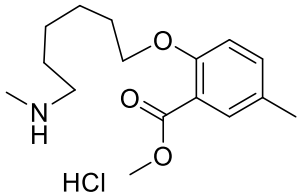
Methyl 5-methyl-2-[3-(methylamino)propoxy]benzoate hydrochloride (9a)

According to the procedure described for **9b**, the title compound was obtained as a colorless oil using *tert*-butyl *N*-(3-hydroxypropyl)-*N*-methyl carbamate instead of *tert*-butyl *N*-(4-hydroxybutyl)-*N*-methyl carbamate. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.84 (brs, 2H), 7.77 (d, J = 1.9 Hz, 1H), 7.34 (dd, J = 8.5, 1.9 Hz, 1H), 6.86 (d, J = 8.5 Hz, 1H), 4.16–4.23 (m, 2H), 3.87 (s, 3H), 3.70 (s, 3H), 3.25 (brs, 2H), 2.76–2.85 (m, 3H), 2.41–2.50 (m, 2H), 2.33 (s, 3H). MS (ESI/APCI dual) m/z: 238 $[M+H]^+$.



Methyl 5-methyl-2-{[5-(methylamino)pentyl]oxy}benzoate hydrochloride (9c)

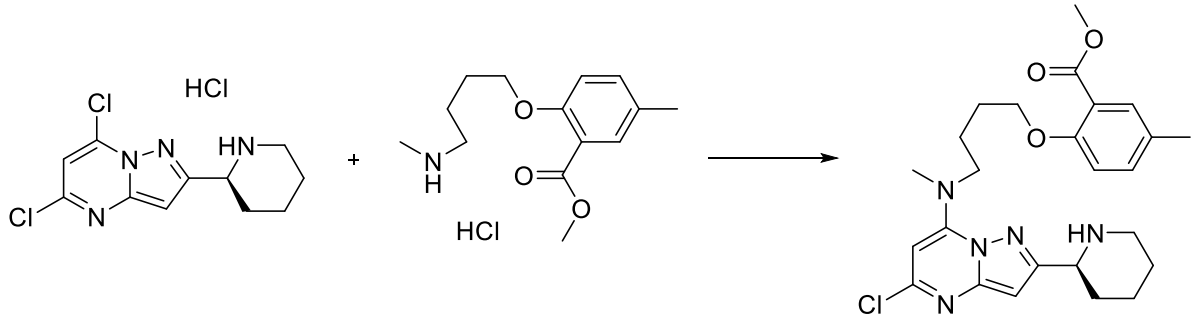
According to the procedure described for **9c**, the title compound was obtained as a colorless powder using *tert*-butyl (5-hydroxypentyl)(methyl) carbamate instead of *tert*-butyl *N*-(4-hydroxybutyl)-*N*-methyl carbamate. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.48 (brs, 2H), 7.44 (s, 1H), 7.28–7.35 (m, 1H), 6.99–7.07 (m, 1H), 3.95–4.04 (m, 2H), 3.77 (s, 3H), 2.82–2.92 (m, 2H), 2.53 (s, 3H), 2.25 (s, 3H), 1.58–1.78 (m, 4H), 1.43–1.53 (m, 2H). MS (ESI/APCI dual) m/z: 266 $[M+H]^+$.



Methyl 5-methyl-2-{[6-(methylamino)hexyl]oxy}benzoate hydrochloride (9d)

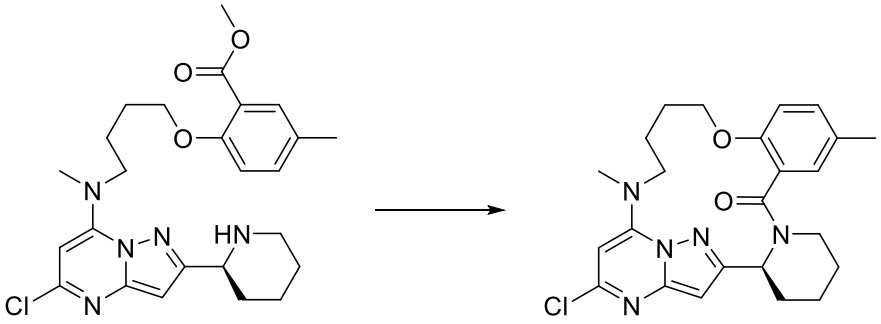
According to the procedure described for **9d**, the title compound was obtained as a colorless powder using *tert*-butyl *N*-(6-hydroxyhexyl)-*N*-methyl carbamate instead of *tert*-butyl *N*-(4-hydroxybutyl)-*N*-methyl carbamate. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.69 (brs, 2H), 7.43 (d, J = 1.6 Hz, 1H), 7.31 (dd, J = 8.5, 1.6 Hz, 1H), 7.02 (d, J = 8.5 Hz, 1H), 3.99 (t, J = 6.2 Hz, 2H), 3.77 (s, 3H), 3.31 (s, 3H), 2.84 (t, J = 7.6 Hz, 2H), 2.25 (s, 3H), 1.55–1.73 (m, 4H), 1.31–1.50 (m, 4H). MS (ESI/APCI dual) m/z: 280

$[M+H]^+$.



Methyl 2-[4-({5-chloro-2-[(2*S*)-2-piperidyl]pyrazolo[1,5-a]pyrimidin-7-yl}-methyl-amino)butoxy]-5-methylbenzoate (10h)

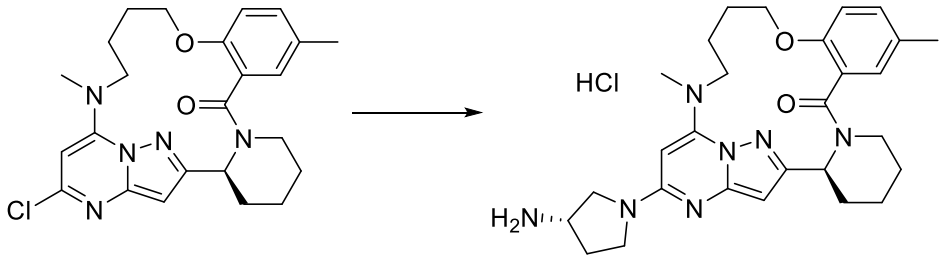
To a solution of **8c** (1.0 g, 2.93 mmol) in ethanol (29 mL) was added **9b** (1.0 g, 3.51 mmol, 1.2 eq.) and triethylamine (4.1 mL, 29.3 mmol, 10 eq.), and the mixture was stirred at 65°C for 0.5 h. The reaction mixture was poured into water and extracted with chloroform. The organic layer was washed with brine, dried over magnesium sulfate, filtered, and concentrated under reduced pressure. The residue was purified using silica gel column chromatography (NH 50%–100% ethyl acetate in hexane) to obtain **10h** (1.2 g, 2.43 mmol, 83%) as a colorless amorphous. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.56–7.63 (m, 1H), 7.20–7.25 (m, 1H), 6.78–6.86 (m, 1H), 6.33 (s, 1H), 5.87 (s, 1H), 4.07–4.17 (m, 2H), 3.99–4.05 (m, 2H), 3.79–3.87 (m, 4H), 3.23 (s, 3H), 3.12–3.19 (m, 1H), 2.73–2.83 (m, 1H), 2.30 (s, 3H), 1.79–2.03 (m, 6H), 1.45–1.71 (m, 4H). MS (ESI/APCI dual) m/z: 486 $[M+H]^+$.



(23a*S*)-18-Chloro-8,16-dimethyl-1,3,4,13,14,15,16,23a-octahydro-2*H*,6*H*,12*H*-23,20-(metheno)pyrido[2,1-k]pyrimido[6,1-g][1,6,8,9,12]benzoxatetraazacyclopentadecin-6-one (11h)

To a solution of **10h** (1.2 g, 2.43 mmol) in methanol (10 mL) and tetrahydrofuran (10 mL) was added 1M sodium hydroxide aq. (15 mL) , and the mixture was stirred at 65°C for 0.5 h. The reaction mixture was acidified using 1M hydrogen chloride aq. and extracted with chloroform. The organic layer was washed with brine, dried over magnesium sulfate, filtered, and concentrated under reduced pressure to obtain 2-[4-({5-chloro-2-[(2*S*)-2-piperidyl]pyrazolo[1,5-a]pyrimidin-7-yl}-methylamino)butoxy]-5-methylbenzoic acid (1.3 g, 2.75 mmol, quant.) as a colorless amorphous, which was used for the next reaction without further purification.

To a solution of 2-[4-({5-chloro-2-[(2*S*)-2-piperidyl]pyrazolo[1,5-a]pyrimidin-7-yl}-methyl-amino)butoxy]-5-methylbenzoic acid (1.2 g, 2.44 mmol) in *N*,*N*-dimethylformamide (120 mL, 0.020 M) was added trimethylamine (2.7 mL, 19.5 mmol, 8.0 eq.) and 1-[bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-b]pyridinium 3-oxide hexafluorophosphate (1.9 g, 4.87 mmol, 2.0 eq.). After stirring at room temperature overnight, the reaction mixture was poured into water and extracted with ethyl acetate. The organic layer was washed with brine, dried over magnesium sulfate, filtered, and concentrated under reduced pressure. The residue was purified using silica gel column chromatography (NH 50%–100% ethyl acetate in hexane) to obtain **11h** (1.5 g, 3.22 mmol, quant.) as a colorless amorphous. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.02–7.15 (m, 2H), 6.76–6.82 (m, 0.7H), 6.68–6.74 (m, 0.3H), 6.34–6.39 (m, 0.7H), 6.30 (s, 0.7H), 6.16 (s, 0.3H), 5.86 (s, 1H), 5.07–5.13 (m, 0.3H), 4.60–4.71 (m, 0.6H), 4.19–4.31 (m, 0.7H), 4.01–4.14 (m, 1H), 3.92–4.01 (m, 0.7H), 3.80–3.92 (m, 0.3H), 3.63–3.73 (m, 0.3H), 3.37–3.52 (m, 2.1H), 3.08 (s, 0.9H), 3.05 (s, 2.1H), 2.74–2.85 (m, 0.3H), 2.34–2.50 (m, 1H), 2.12–2.33 (m, 4H), 1.89–2.08 (m, 2H), 1.31–1.87 (m, 6H). MS (ESI/APCI dual) m/z: 454 $[M+H]^+$.

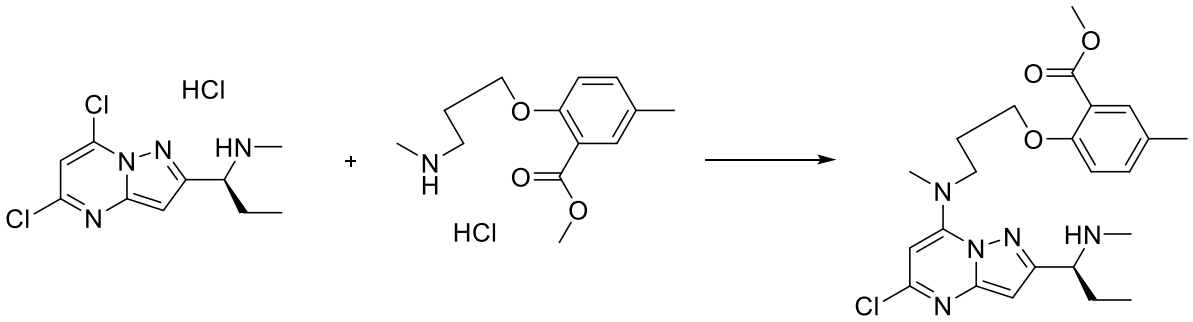


(23a*S*)-18-[(3*S*)-3-Aminopyrrolidin-1-yl]-8,16-dimethyl-1,3,4,13,14,15,16,23a-octahydro-2*H*,6*H*,12*H*-23,20-(metheno)pyrido[2,1-k]pyrimido[6,1-g][1,6,8,9,12]benzoxatetraazacyclopentadecin-6-one hydrochloride (12h)

To a solution of **11h** (0.20 g, 0.441 mmol) in 1-methyl-2-pyrrolidone (2.2 mL) was added trimethylamine (0.74 mL, 5.29 mmol, 12.0 eq.) and *tert*-butyl *N*-[(*3S*)-pyrrolidin-3-yl]carbamate (0.49 g, 2.64 mmol, 6.0 eq.). After stirring at 150°C under microwave irradiation for 1 h, the reaction mixture was poured into water and extracted with ethyl acetate. The organic layer was washed with brine, dried over magnesium sulfate, filtered, and concentrated under reduced pressure. The residue was purified using silica gel column chromatography (50%–100% ethyl acetate in hexane) to obtain *tert*-butyl {(3*S*)-1-[(23a*S*)-8,16-dimethyl-6-oxo-1,3,4,13,14,15,16,23a-octahydro-2*H*,6*H*,12*H*-23,20-(metheno)pyrido[2,1-k]pyrimido[6,1-g][1,6,8,9,12]benzoxatetraazacyclopentadecin-18-yl]pyrrolidin-3-yl}carbamate (0.18 g, 0.298 mmol, 68%) as a colorless amorphous. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.07–7.13 (m, 1.6H), 7.01–7.06 (m, 0.4H), 6.74–6.80 (m, 0.8H), 6.67–6.73 (m, 0.2H), 6.27–6.35 (m, 0.8H), 5.96 (s, 0.8H), 5.82 (s, 0.2H), 5.10 (s, 0.8H), 5.06 (s, 0.2H), 4.99–5.04 (m, 0.2H), 4.60–4.76 (m,

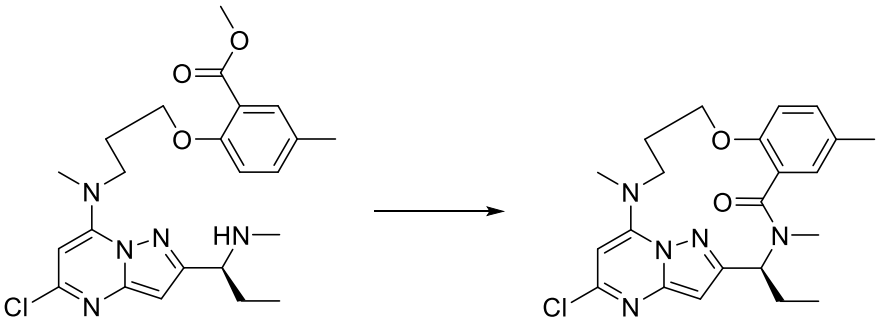
1H), 4.26–4.39 (m, 1H), 4.00–4.16 (m, 2H), 3.87–3.99 (m, 1H), 3.73–3.84 (m, 1H), 3.54–3.70 (m, 3H), 3.32–3.46 (m, 2H), 3.01–3.13 (m, 0.8H), 2.84–2.99 (m, 3.2H), 2.41–2.57 (m, 1H), 2.10–2.33 (m, 5H), 1.28–2.02 (m, 18H). MS (ESI/APCI dual) m/z: 604 $[M+H]^+$.

The mixture of *tert*-butyl {(3*S*)-1-[(23a*S*)-8,16-dimethyl-6-oxo-1,3,4,13,14,15,16,23a-octahydro-2*H*,6*H*,12*H*-23,20-(metheno)pyrido[2,1-k]pyrimido[6,1-g][1,6,8,9,12]benzoxatetraazacyclopentadecin-18-yl]pyrrolidin-3-yl}carbamate (0.18 g, 0.298 mmol) and 4M hydrogen chloride in 1,4-dioxane (3.0 mL) was stirred for 1 h at room temperature, and the reaction mixture was concentrated under reduced pressure to obtain **12h** (0.16 g, 0.296 mmol, 99%) as a colorless powder. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.68 (brs, 3H), 7.15–7.18 (m, 0.8H), 7.10–7.13 (m, 0.2H), 7.07–7.09 (m, 0.2H), 7.04–7.07 (m, 0.8H), 6.95–6.98 (m, 0.8H), 6.84–6.87 (m, 0.2H), 6.34 (brs, 0.8H), 6.19 (brs, 0.2H), 6.04–6.09 (m, 0.8H), 5.22–5.26 (m, 1H), 4.85–4.88 (m, 0.2H), 3.95–4.06 (m, 4H), 3.44–3.94 (m, 5H), 3.37–3.43 (m, 1H), 3.25–3.32 (m, 1H), 3.15 (s, 3H), 2.33–2.41 (m, 1H), 2.26 (s, 3H), 2.12–2.24 (m, 2H), 2.01–2.11 (m, 1H), 1.83–1.92 (m, 1H), 1.36–1.79 (m, 5H), 1.20–1.34 (m, 2H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 167.43, 155.94, 151.92, 150.33, 150.11, 130.39, 129.45, 127.68, 126.23, 125.11, 112.60, 88.51, 66.68, 66.30, 62.76, 54.26, 51.91, 49.18, 47.17, 46.52, 43.71, 29.36, 28.74, 26.04, 25.44, 23.49, 19.93, 19.77. HRMS ESI/APCI dual m/z calcd. for $C_{28}H_{37}N_7O_2$ $[M+H]^+$: 504.3082, found: 504.3059.



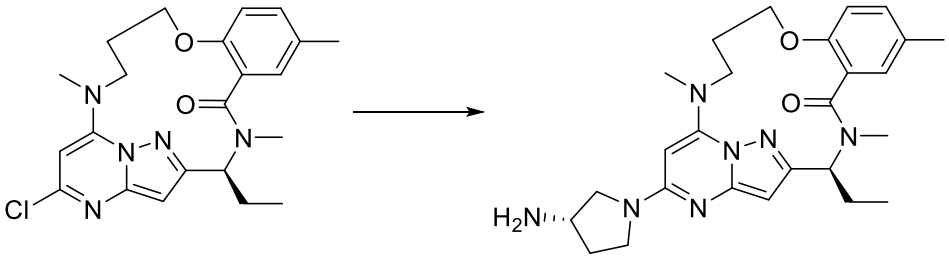
Methyl 2-{3-[{5-chloro-2-[(1*S*)-1-(methylamino)propyl]pyrazolo[1,5-a]pyrimidin-7-yl}(methyl)amino]propoxy}-5-methylbenzoate (10a)

According to the procedure described for **10h**, **8a** (0.41 g, 1.0 eq.) and **9a** (0.23 g, 1.2 eq.) were reacted together. After workup, the residue was purified using silica gel column chromatography (NH 30%–100% ethyl acetate in hexane) to obtain **10a** (0.15 g, 48%) as a pale yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.60–7.65 (m, 1H), 7.21–7.27 (m, 1H), 6.79–6.85 (m, 1H), 6.28 (s, 1H), 5.90 (s, 1H), 4.23–4.31 (m, 2H), 4.06–4.12 (m, 2H), 3.88 (s, 3H), 3.59–3.65 (m, 1H), 3.30 (s, 3H), 2.35 (s, 3H), 2.22–2.33 (m, 5H), 1.70–1.90 (m, 2H), 0.84–0.91 (m, 3H). MS (ESI/APCI dual) m/z: 460 $[M+H]^+$.



(16*S*)-3-Chloro-16-ethyl-5,12,15-trimethyl-5,6,7,8,15,16-hexahydro-14*H*-17,1-(metheno)pyrimido[6,1-f][1,5,7,8,11]benzoxatetraazacyclotetradecin-14-one (11a)

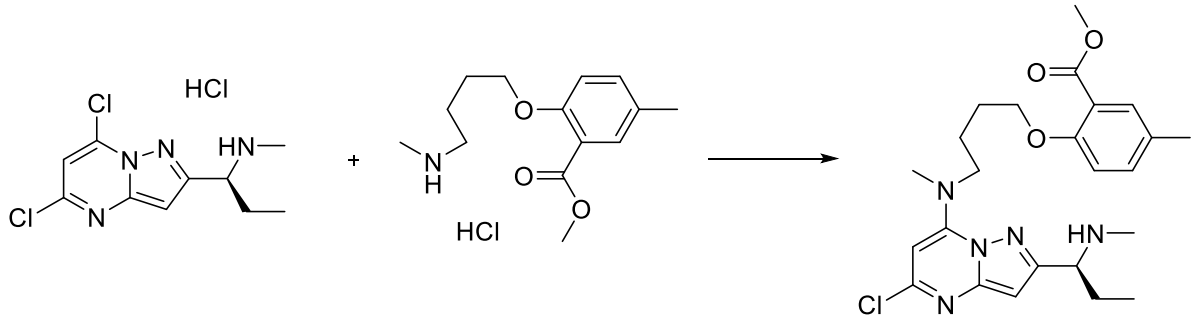
According to the procedure described for **11h**, the title compound **11a** was obtained (0.11 g, 87%) as a colorless powder. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.08–7.16 (m, 2H), 6.68–6.75 (m, 1H), 6.30 (s, 1H), 6.11–6.19 (m, 1H), 5.82 (s, 1H), 5.01–5.13 (m, 1H), 4.18–4.30 (m, 1H), 4.03–4.11 (m, 1H), 3.89–3.97 (m, 1H), 3.13 (s, 3H), 2.69 (s, 3H), 2.30 (s, 3H), 1.84–2.27 (m, 4H), 1.10–1.16 (m, 3H). MS (ESI/APCI dual) m/z: 428 $[M+H]^+$.



(16*S*)-3-[(3*S*)-3-Aminopyrrolidin-1-yl]-16-ethyl-5,12,15-trimethyl-5,6,7,8,15,16-hexahydro-14*H*-17,1-(metheno)pyrimido[6,1-f][1,5,7,8,11]benzoxatetraazacyclotetradecin-14-one (12a)

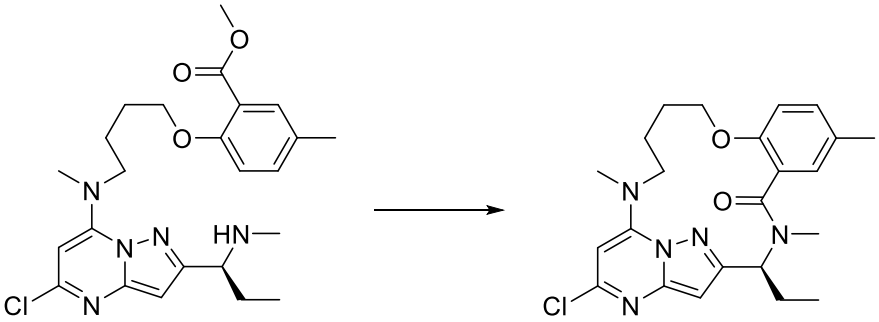
To a solution of **11a** (40 mg, 0.093 mmol) in 1-methyl-2-pyrrolidone (0.93 mL) was added trimethylamine (0.13 mL, 0.935 mmol, 10.0 eq.) and (*S*)-3-aminopyrolidine (0.041 mL, 0.467 mmol, 5.0 eq.). After stirring at 150°C under microwave irradiation for 1 h, the reaction mixture was poured into sat. sodium bicarbonate aq. and extracted with ethyl acetate. The organic layer was washed with brine, dried over magnesium sulfate, filtered, and concentrated under reduced pressure. The residue was purified using silica gel column chromatography (NH 50%–100% ethyl acetate in hexane to 5% methanol in chloroform) to obtain **12a** (12 mg, 0.025 mmol, 27%) as a colorless amorphous. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.06–7.13 (m, 2H), 6.84–6.90 (m, 0.1H), 6.67–6.73 (m, 0.9H), 6.04–6.10 (m, 1H), 5.99 (s, 0.9H), 5.96 (s, 0.1H), 5.26 (s, 0.1H), 5.06 (s, 0.9H), 4.56–4.72 (m, 1H), 4.09–4.21 (m, 1H), 3.97–4.05 (m, 1H), 3.86–3.96 (m, 1H), 3.63–3.79 (m, 3H), 3.50-3.61 (m, 1H), 3.23–3.33 (m, 1H), 3.03 (s, 2.7H), 2.89 (s, 0.3H), 2.80 (s, 0.3H), 2.63 (s, 2.7H), 2.26–2.32 (m, 3H), 1.75–2.26 (m, 6H), 1.08–1.15 (m, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 169.18, 155.27, 154.19, 152.14, 151.43, 148.72,

130.11, 129.14, 127.68, 126.73, 111.06, 88.27, 76.79, 64.85, 54.97, 51.81, 50.66, 49.42, 44.96, 38.16, 33.98, 30.10, 28.38, 22.71, 19.89, 10.51. HRMS ESI/APCI dual m/z calcd. for $C_{26}H_{35}N_7O_2$ $[M+H]^+$: 478.2925, found: 478.2915.



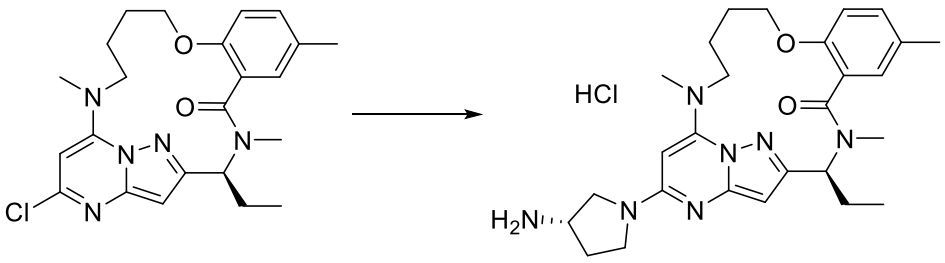
Methyl 2-{4-[{5-chloro-2-[(1*S*)-1-(methylamino)propyl]pyrazolo[1,5-a]pyrimidin-7-yl}(methyl)amino]butoxy}-5-methylbenzoate (10b)

According to the procedure described for **10h**, **8a** (0.80 g, 1.0 eq.) and **9b** (0.94 g, 1.2 eq.) were reacted together. After workup, the residue was purified using silica gel column chromatography (NH 50%–100% ethyl acetate in hexane) to obtain **10b** (0.60 g, 47%) as a colorless amorphous. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.57–7.61 (m, 1H), 7.20–7.25 (m, 1H), 6.80–6.84 (m, 1H), 6.28 (s, 1H), 5.86 (s, 1H), 4.10–4.18 (m, 2H), 3.99–4.05 (m, 2H), 3.84 (s, 3H), 3.58–3.65 (m, 1H), 3.24 (s, 3H), 2.34 (s, 3H), 2.30 (s, 3H), 1.91–2.01 (m, 2H), 1.72–1.89 (m, 4H), 0.84–0.90 (m, 3H). MS (ESI/APCI dual) m/z: 474 $[M+H]^+$.



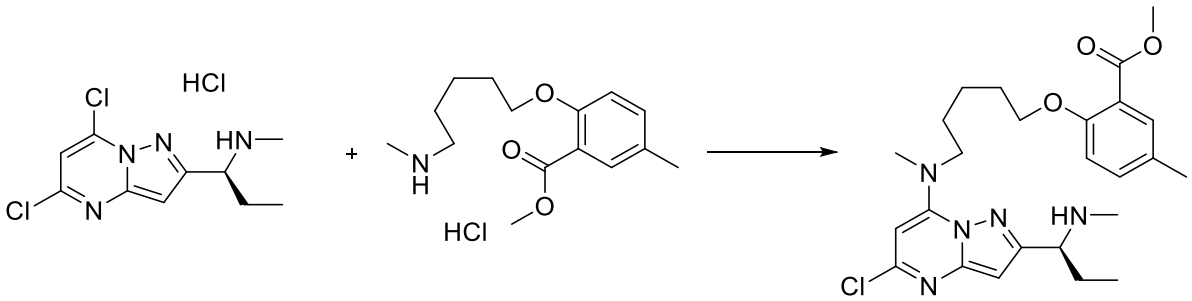
(17*S*)-3-Chloro-17-ethyl-5,13,16-trimethyl-6,7,8,9,16,17-hexahydro-5*H*,15*H*-18,1-(metheno)pyrimido[6,1-g][1,6,8,9,12]benzoxatetraazacyclopentadecin-15-one (11b)

According to the procedure described for **11h**, the title compound **11b** was obtained (0.59 g, quant.) as a colorless amorphous. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.01–7.13 (m, 2H), 6.73–6.81 (m, 1H), 6.34 (s, 0.6H), 6.26 (s, 0.4H), 6.12–6.21 (m, 1H), 5.80–5.85 (m, 1H), 4.67–4.79 (m, 1H), 4.53–4.64 (m, 0.4H), 4.02–4.12 (m, 1H), 3.87–3.96 (m, 0.6H), 3.77–3.87 (m, 0.4H), 3.64–3.74 (m, 0.4H), 3.20–3.30 (m, 0.6H), 3.10 (s, 1H), 3.08 (s, 2H), 2.80 (s, 1H), 2.60 (s, 2H), 2.32–2.53 (m, 1H), 2.28–2.31 (m, 3H), 1.64–2.26 (m, 5H), 1.10–1.21 (m, 2H), 0.95–1.04 (m, 1H). MS (ESI/APCI dual) m/z: 442 $[M+H]^+$.



(17*S*)-3-[(3*S*)-3-Aminopyrrolidin-1-yl]-17-ethyl-5,13,16-trimethyl-6,7,8,9,16,17-hexahydro-5*H*,15*H*-18,1-(metheno)pyrimido[6,1-g][1,6,8,9,12]benzoxatetraazacyclopentadecin-15-one hydrochloride (12b)

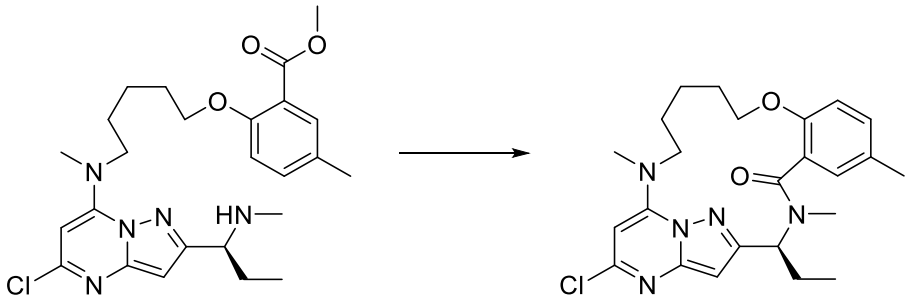
According to the procedure described for **12h**, the title compound **12b** was obtained (0.17 g, 98%) as a colorless amorphous. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.62 (brs, 3H), 7.12–7.19 (m, 1H), 6.90–7.02 (m, 2H), 6.29–6.37 (m, 1H), 5.83–5.92 (m, 0.6H), 5.22 (s, 0.6H), 5.18 (s, 0.4H), 4.54–4.67 (m, 0.4H), 4.45–4.53 (m, 0.4H), 4.16–4.30 (m, 1H), 3.24–4.10 (m, 7.6H), 3.13–3.23 (m, 3H), 2.66 (s, 1H), 2.54 (s, 2H), 2.07–2.43 (m, 7H), 1.45–2.05 (m, 4H), 0.98–1.06 (m, 2H), 0.79–0.88 (m, 1H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 169.40, 168.35, 156.02, 154.57, 152.54, 151.88, 149.91, 149.24, 130.21, 130.05, 129.61, 128.64, 127.66, 127.20, 127.01, 125.57, 112.71, 111.72, 89.05, 88.87, 66.86, 64.97, 62.78, 57.24, 54.20, 51.87, 51.37, 49.20, 46.46, 30.91, 28.76, 27.17, 26.00, 24.03, 23.97, 23.51, 23.43, 19.92, 19.89, 10.93, 10.35. HRMS ESI/APCI dual m/z calcd. for $C_{27}H_{37}N_7O_2$ $[M+H]^+$: 492.3082, found: 492.3058.



Methyl 2-({5-[{5-chloro-2-[(1*S*)-1-(methylamino)propyl]pyrazolo[1,5-a]pyrimidin-7-yl}(methyl)amino]pentyl}oxy)-5-methylbenzoate (10c)

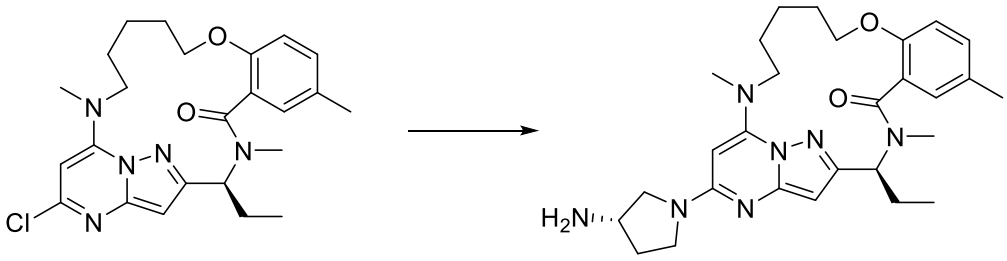
According to the procedure described for **10h**, **8a** (0.60 g, 1.0 eq.) and **9c** (0.22 g, 1.2 eq.) were reacted together. After workup, the residue was purified using silica gel column chromatography (NH 20%–100% ethyl acetate in hexane to 10% methanol in chloroform) to obtain **10c** (0.26 g, 86%) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.56–7.61 (m, 1H), 7.20–7.25 (m, 1H), 6.80–6.87 (m, 1H), 6.30 (s, 1H), 5.86 (s, 1H), 4.03–4.10 (m, 2H), 3.96–4.02 (m, 2H), 3.86 (s, 3H), 3.60–3.68 (m, 1H), 3.22 (s, 3H), 2.35 (s, 3H), 2.30 (s, 3H), 1.74–1.91 (m, 6H), 1.47–1.70 (m, 2H), 0.84–0.89 (m, 3H). MS

(ESI/APCI dual) m/z: 488 $[M+H]^+$.



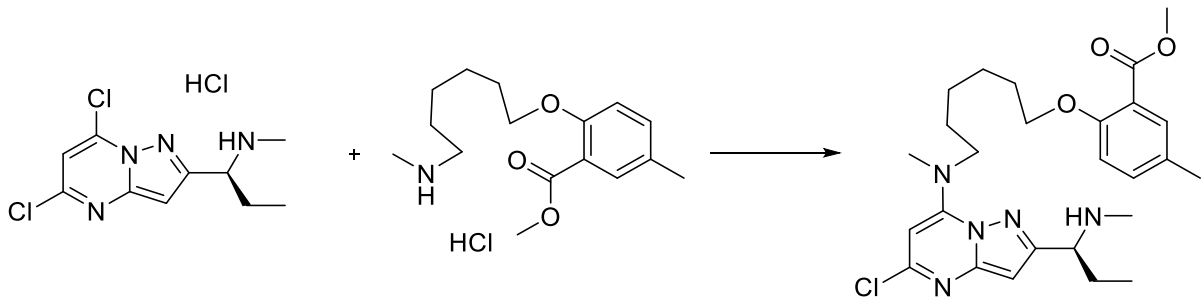
(18*S*)-3-Chloro-18-ethyl-5,14,17-trimethyl-5,6,7,8,9,10,17,18-octahydro-16*H*-19,1-(metheno)pyrimido[6,1-h][1,7,9,10,13]benzoxatetraazacyclohexadecin-16-one (11c)

According to the procedure described for **11h**, the title compound **11c** was obtained (0.20 g, 85%) as a colorless amorphous. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.01–7.12 (m, 2H), 6.76–6.82 (m, 1H), 6.39 (s, 0.8H), 6.26 (s, 0.2H), 6.09–6.17 (m, 0.8H), 5.88 (s, 1H), 4.90–5.00 (m, 0.8H), 4.69–4.76 (m, 0.2H), 4.54–4.64 (m, 0.2H), 4.02–4.12 (m, 1H), 3.86–3.95 (m, 1H), 3.57–3.68 (m, 0.8H), 3.17–3.28 (m, 0.2H), 3.08 (s, 2.4H), 3.07 (s, 0.6H), 2.93 (s, 0.6H), 2.59 (s, 2.4H), 2.29 (s, 3H), 1.83–2.25 (m, 3H), 1.49–1.82 (m, 3H), 1.08–1.15 (m, 2.4H), 0.91-0.97 (m, 0.6H). MS (ESI/APCI dual) m/z: 456 $[M+H]^+$.



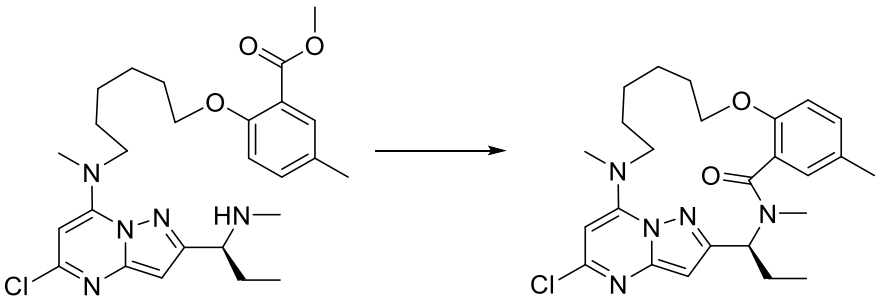
(18*S*)-3-[(3*S*)-3-Aminopyrrolidin-1-yl]-18-ethyl-5,14,17-trimethyl-5,6,7,8,9,10,17,18-octahydro-16*H*-19,1-(metheno)pyrimido[6,1-h][1,7,9,10,13]benzoxatetraazacyclohexadecin-16-one (12c)

According to the procedure described for **12a**, the title compound **12c** was obtained (45 mg, 68%) as a colorless powder. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.00–7.12 (m, 2H), 6.79–6.84 (m, 0.3H), 6.73–6.78 (m, 0.7H), 6.00–6.10 (m, 1H), 5.19 (s, 0.3H), 5.12 (s, 0.7H), 4.71–4.83 (m, 0.7H), 4.59–4.66 (m, 0.3H), 4.07–4.16 (m, 0.3H), 3.92–4.07 (m, 1.3H), 3.81–3.91 (m, 0.7H), 3.65–3.79 (m, 3H), 3.49–3.62 (m, 1H), 3.22–3.44 (m, 1.7H), 2.98 (s, 2H), 2.93 (s, 1H), 2.88 (s, 1H), 2.59 (s, 2H), 2.28 (s, 3H), 1.35–2.25 (m, 10H), 1.06–1.15 (m, 2H), 0.79–0.86 (m, 1H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 168.54, 155.17, 153.78, 151.73, 151.17, 149.68, 129.97, 129.29, 127.50, 127.28, 112.58, 89.39, 78.18, 66.32, 58.08, 54.88, 51.83, 50.64, 44.94, 37.92, 33.88, 30.97, 27.71, 26.06, 23.71, 21.84, 19.91, 10.79. HRMS ESI/APCI dual m/z calcd. for $C_{28}H_{39}N_7O_2$ $[M+H]^+$: 506.3238, found: 506.3227.



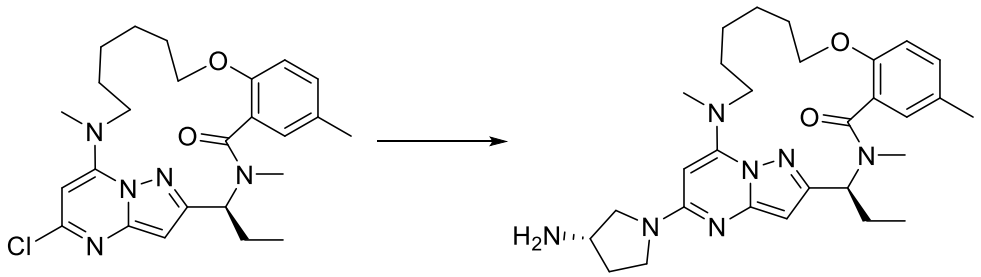
Methyl 2-({6-[{5-chloro-2-[(1*S*)-1-(methylamino)propyl]pyrazolo[1,5-a]pyrimidin-7-yl}(methyl)amino]hexyl}oxy)-5-methylbenzoate (10d)

According to the procedure described for **10h**, **8a** (0.33 g, 1.0 eq.) and **9d** (0.21 g, 1.2 eq.) were reacted together. After workup, the residue was purified using silica gel column chromatography (NH 20%–100% ethyl acetate in hexane) to obtain **10d** (0.12 g, 42%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.56–7.60 (m, 1H), 7.20–7.25 (m, 1H), 6.81–6.85 (m, 1H), 6.28 (s, 1H), 5.84 (s, 1H), 4.01–4.07 (m, 2H), 3.95–4.01 (m, 2H), 3.86 (s, 3H), 3.60–3.65 (m, 1H), 3.21 (s, 3H), 2.34 (s, 3H), 2.29 (s, 3H), 1.69–1.90 (m, 6H), 1.47–1.57 (m, 2H), 1.33–1.43 (m, 2H), 0.84–0.91 (m, 3H). MS (ESI/APCI dual) m/z: 502 $[M+H]^+$.



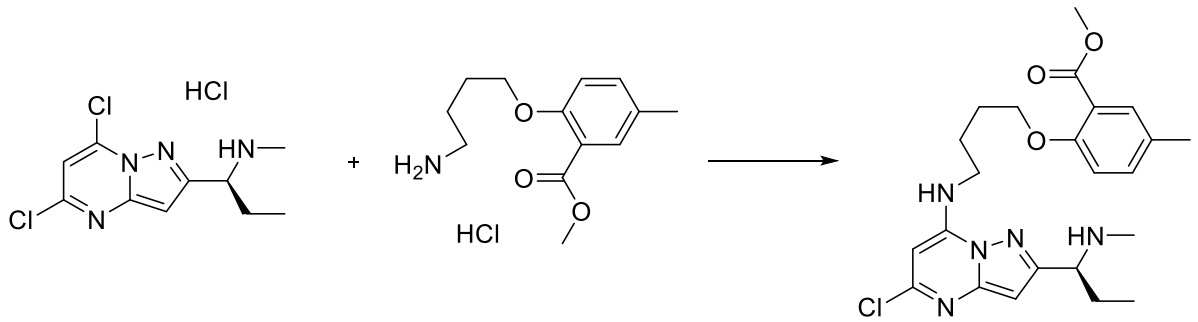
(19*S*)-3-Chloro-19-ethyl-5,15,18-trimethyl-6,7,8,9,10,11,18,19-octahydro-5*H*,17*H*-20,1-(metheno)pyrimido[6,1-i][1,8,10,11,14]benzoxatetraazacycloheptadecin-17-one (11d)

According to the procedure described for **11h**, the title compound **11d** was obtained (96 mg, 70%) as a colorless amorphous. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.02–7.12 (m, 2H), 6.65–6.79 (m, 1H), 6.45 (s, 0.5H), 6.04–6.13 (m, 0.8H), 5.85–5.93 (m, 1H), 5.49–5.60 (m, 0.5H), 4.82–4.94 (m, 0.2H), 3.52–4.13 (m, 4H), 3.06–3.12 (m, 3H), 2.80 (s, 1.5H), 2.69 (s, 1.5H), 2.25–2.32 (m, 3H), 1.05–2.10 (m, 10H), 0.97–1.03 (m, 2H), 0.91–0.96 (m, 1H). MS (ESI/APCI dual) m/z: 470 $[M+H]^+$.



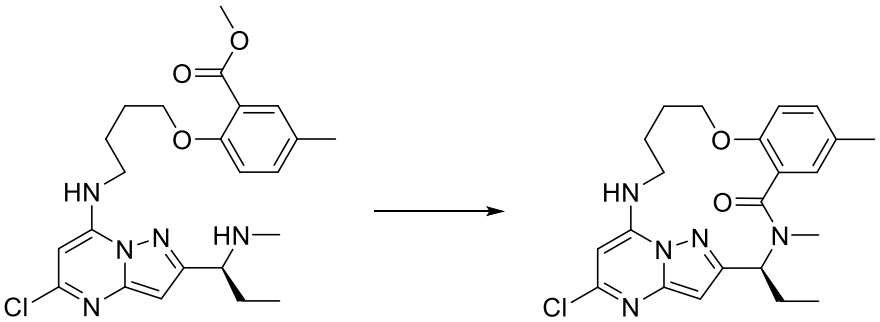
(19*S*)-3-[(3*S*)-3-Aminopyrrolidin-1-yl]-19-ethyl-5,15,18-trimethyl-6,7,8,9,10,11,18,19-octahydro-5*H*,17*H*-20,1-(metheno)pyrimido[6,1-i][1,8,10,11,14]benzoxatetraazacycloheptadecin-17-one (12d)

According to the procedure described for **12a**, the title compound **12d** was obtained (16 mg, 44%) as a colorless powder. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.02–7.11 (m, 2H), 6.63–6.72 (m, 1H), 6.11 (s, 0.8H), 5.95–6.04 (m, 0.8H), 5.69 (s, 0.2H), 5.48–5.62 (m, 0.2H), 5.31–5.45 (m, 0.8H), 5.06–5.15 (m, 1H), 4.75–4.84 (m, 0.2H), 3.82–3.91 (m, 1H), 3.67–3.81 (m, 3H), 3.24–3.62 (m, 4H), 2.98–3.05 (m, 3H), 2.84 (s, 1H), 2.69 (s, 2H), 2.34–2.42 (m, 1H), 2.25–2.31 (m, 3H), 2.14–2.24 (m, 1H), 1.92–2.11 (m, 3H), 1.04–1.90 (m, 7H), 0.94–1.03 (m, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 167.95, 155.03, 153.08, 151.96, 150.97, 149.93, 130.11, 129.21, 127.66, 126.75, 111.50, 89.85, 78.74, 67.89, 54.04, 52.15, 49.38, 48.43, 44.78, 38.38, 33.02, 30.75, 28.82, 25.52, 24.60, 23.85, 19.87, 17.18, 10.85. HRMS ESI/APCI dual m/z calcd. for $C_{29}H_{41}N_7O_2[M+H]^+$: 520.3395, found: 520.3389



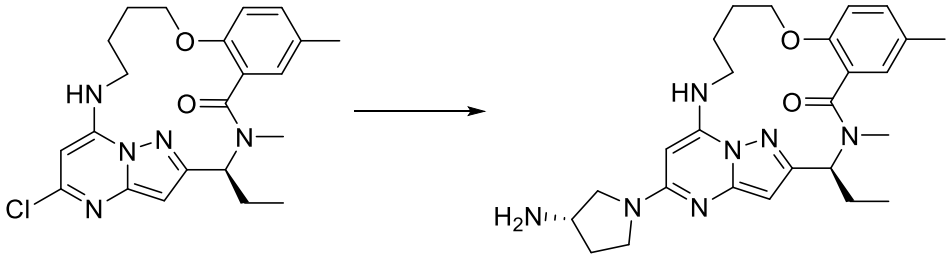
Methyl 2-[4-({5-chloro-2-[(1*S*)-1-(methylamino)propyl]pyrazolo[1,5-a]pyrimidin-7-yl}amino)butoxy]-5-methylbenzoate (10e)

According to the procedure described for **10h**, **8a** (0.15 g, 1.0 eq.) and **9e** (0.12 g, 1.1 eq.) were reacted together. After workup, the residue was purified using silica gel column chromatography (NH 20%–80% ethyl acetate in hexane) and reverse-phase preparative HPLC to obtain **10e** (0.11 g, 56%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.62–7.66 (m, 1H), 7.22–7.28 (m, 1H), 6.82–6.88 (m, 1H), 6.63–6.71 (m, 1H), 6.27 (s, 1H), 5.95 (s, 1H), 4.06–4.12 (m, 2H), 3.88 (s, 3H), 3.51–3.63 (m, 3H), 2.33 (s, 3H), 2.31 (s, 3H), 1.95–2.08 (m, 4H), 1.71–1.87 (m, 2H), 0.83–0.90 (m, 3H). MS (ESI/APCI dual) m/z: 460 $[M+H]^+$.



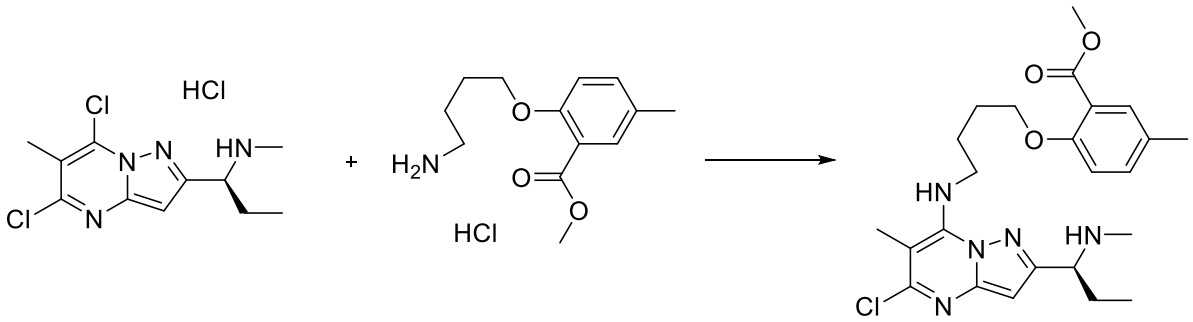
(17*S*)-3-Chloro-17-ethyl-13,16-dimethyl-6,7,8,9,16,17-hexahydro-5*H*,15*H*-18,1-(metheno)pyrimido[6,1-g][1,6,8,9,12]benzoxatetraazacyclopentadecin-15-one (11e)

According to the procedure described for **11h**, the title compound **11e** was obtained (9.0 mg, 16%) as a colorless amorphous. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.49–8.56 (m, 0.5H), 7.10–7.18 (m, 2H), 7.01–7.09 (m, 1H), 6.88–6.95 (m, 0.5H), 6.26 (s, 0.5H), 6.14 (s, 0.5H), 5.89–5.97 (m, 0.5H), 5.86 (s, 0.5H), 5.82 (s, 0.5H), 5.04–5.12 (m, 0.5H), 4.30–4.38 (m, 0.5H), 4.07–4.15 (m, 1.5H), 3.30–3.49 (m, 1.5H), 3.14–3.26 (m, 0.5H), 2.69 (s, 1.5H), 2.60 (s, 1.5H), 2.29–2.34 (m, 3H), 1.85–2.28 (m, 6H), 1.14–1.24 (m, 3H). MS (ESI/APCI dual) m/z: 428 $[M+H]^+$.



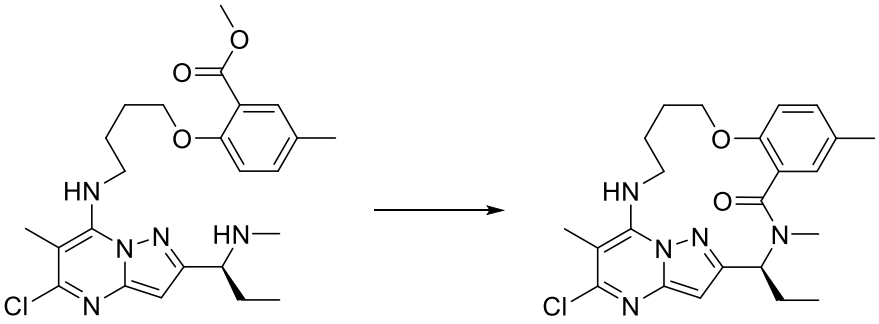
(17*S*)-3-[(3*S*)-3-Aminopyrrolidin-1-yl]-17-ethyl-13,16-dimethyl-6,7,8,9,16,17-hexahydro-5*H*,15*H*-18,1-(metheno)pyrimido[6,1-g][1,6,8,9,12]benzoxatetraazacyclopentadecin-15-one (12e)

According to the procedure described for **12a**, the title compound **12e** was obtained (4.3 mg, 43%) as a pale pink amorphous. ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.42–7.47 (m, 0.4H), 6.92–7.18 (m, 3H), 6.78–6.83 (m, 0.6H), 5.84 (s, 0.6H), 5.80 (s, 0.4H), 5.67–5.72 (m, 0.6H), 5.26 (s, 0.6H), 5.17 (s, 0.4H), 4.60–4.66 (m, 0.4H), 3.69–4.03 (m, 3H), 2.99–3.57 (m, 8H), 2.63 (s, 1H), 2.44 (s, 2H), 2.27 (s, 1H), 2.25 (s, 2H), 1.58–2.19 (m, 8H), 0.99–1.05 (m, 2H), 0.92–0.97 (m, 1H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 169.40, 155.61, 154.15, 153.60, 152.62, 147.74, 130.01, 129.61, 127.74, 127.58, 127.42, 113.43, 88.37, 87.86, 79.12, 73.33, 68.49, 55.01, 52.19, 50.66, 44.94, 43.41, 42.30, 34.02, 30.59, 27.69, 26.39, 26.13, 26.04, 22.79, 22.57, 19.96, 19.87, 10.99, 10.69. HRMS ESI/APCI dual m/z calcd. for $C_{26}H_{35}N_7O_2$ $[M+H]^+$: 478.2925, found: 478.2927.



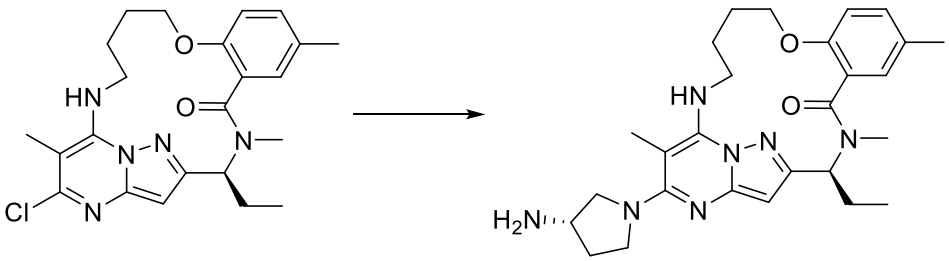
Methyl 2-[4-({5-chloro-6-methyl-2-[(1*S*)-1-(methylamino)propyl]pyrazolo[1,5-a]pyrimidin-7-yl}amino)butoxy]-5-methylbenzoate (10f)

According to the procedure described for **10h**, **8b** (0.10 g, 1.0 eq.) and **9e** (92 mg, 1.2 eq.) were reacted together. After workup, the residue was purified using silica gel column chromatography (NH 80%–100% ethyl acetate in hexane) and reverse-phase preparative HPLC to obtain **10f** (98 mg, 64%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.59–7.64 (m, 1H), 7.21–7.28 (m, 1H), 6.80–6.87 (m, 1H), 6.54–6.62 (m, 1H), 6.24 (s, 1H), 4.05–4.10 (m, 2H), 3.82–3.90 (m, 5H), 3.59–3.66 (m, 1H), 2.45 (s, 3H), 2.35 (s, 3H), 2.30 (s, 3H), 1.90–2.02 (m, 4H), 1.72–1.90 (m, 2H), 0.83–0.90 (m, 3H). MS (ESI/APCI dual) m/z: 475 $[M+H]^+$.



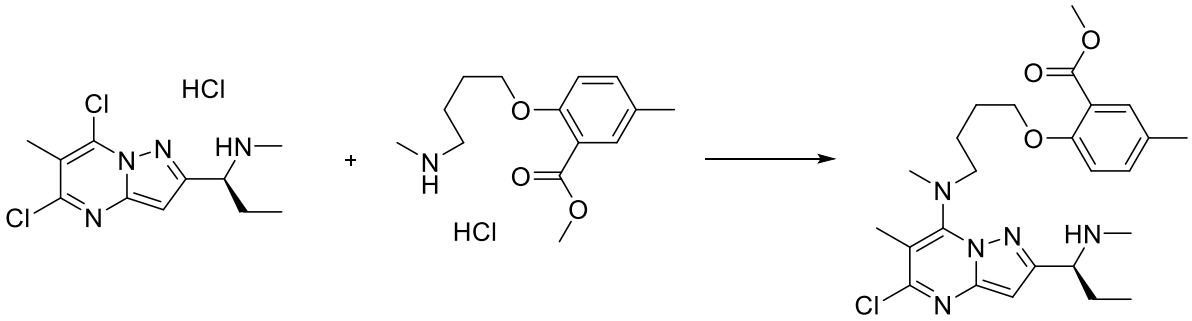
(17*S*)-3-Chloro-17-ethyl-4,13,16-trimethyl-6,7,8,9,16,17-hexahydro-5*H*,15*H*-18,1-(metheno)pyrimido[6,1-g][1,6,8,9,12]benzoxatetraazacyclopentadecin-15-one (11f)

According to the procedure described for **11h**, the title compound **11f** was obtained (61 mg, 71%) as a colorless powder. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.33–7.42 (m, 1H), 7.11–7.17 (m, 1H), 6.89–6.97 (m, 2H), 6.32 (s, 1H), 5.78–5.89 (m, 1H), 4.44–4.58 (m, 0.5H), 4.21–4.36 (m, 1H), 3.80–3.99 (m, 1.5H), 3.51–3.67 (m, 1H), 2.42 (s, 3H), 2.26 (s, 3H), 2.20 (s, 3H), 1.47–2.16 (m, 6H), 0.99–1.07 (m, 3H). MS (ESI/APCI dual) m/z: 442 $[M+H]^+$.



(17*S*)-3-[(3*S*)-3-Aminopyrrolidin-1-yl]-17-ethyl-4,13,16-trimethyl-6,7,8,9,16,17-hexahydro-5*H*,15*H*-18,1-(metheno)pyrimido[6,1-g][1,6,8,9,12]benzoxatetraazacyclopentadecin-15-one (12f)

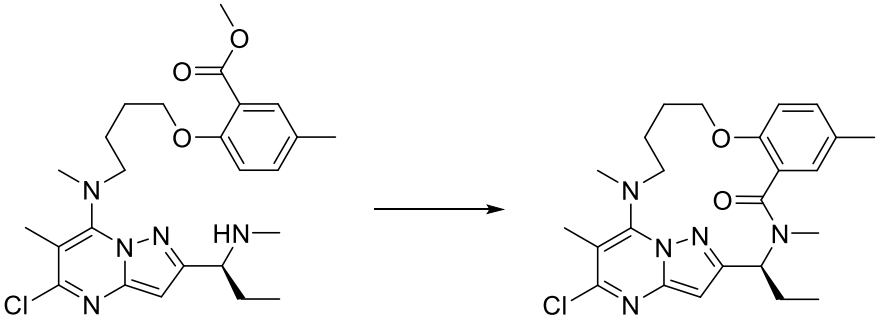
According to the procedure described for **12a**, the title compound **12f** was obtained (21 mg, 50%) as a pink amorphous. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.99–7.12 (m, 2H), 6.80–6.86 (m, 0.2H), 6.70–6.77 (m, 0.8H), 6.03–6.14 (m, 1.6H), 5.94 (s, 0.2H), 5.50–5.65 (m, 1H), 4.78–4.87 (m, 0.2H), 4.05–4.13 (m, 0.2H), 3.91–4.00 (m, 1H), 3.72–3.85 (m, 1.8H), 3.37–3.72 (m, 5H), 3.25–3.34 (m, 0.2H), 3.13–3.25 (m, 0.8H), 2.85 (s, 0.6H), 2.43 (s, 2.4H), 2.28 (s, 3H), 2.06–2.25 (m, 5H), 1.51–2.06 (m, 6H), 1.08–1.16 (m, 2.4H), 1.01–1.07 (m, 0.6H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 168.88, 168.25, 158.85, 158.81, 153.96, 152.84, 152.36, 148.58, 147.77, 147.61, 146.61, 130.03, 129.53, 127.48, 127.30, 126.07, 112.89, 111.90, 89.69, 88.81, 86.56, 67.89, 65.31, 58.24, 58.12, 57.94, 51.71, 50.87, 50.78, 48.17, 48.05, 45.76, 43.79, 34.06, 33.92, 30.33, 29.12, 27.57, 27.05, 26.33, 25.62, 24.24, 22.75, 19.91, 13.99, 13.54, 11.09, 10.63. HRMS ESI/APCI dual m/z calcd. for $C_{27}H_{37}N_7O_2$ $[M+H]^+$: 492.3082, found: 492.3065.



Methyl 2-{4-[{5-chloro-6-methyl-2-[(1*S*)-1-(methylamino)propyl]pyrazolo[1,5-a]pyrimidin-7-yl}(methyl)amino]butoxy}-5-methylbenzoate (10g)

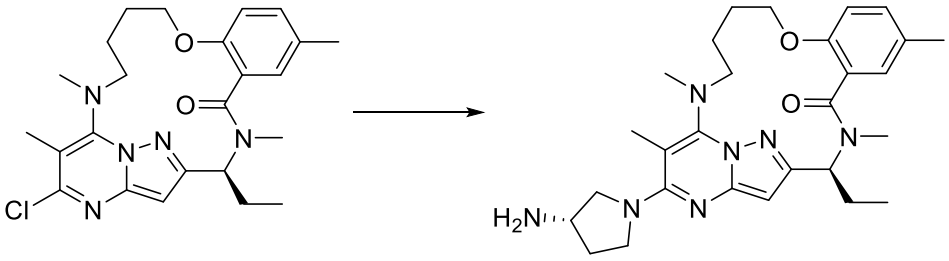
According to the procedure described for **10h**, **8b** (0.10 g, 1.0 eq.) and **9b** (0.11 g, 1.2 eq.) were reacted together. After workup, the residue was purified using silica gel column chromatography (NH 80%–100% ethyl acetate in hexane) and reverse-phase preparative HPLC to obtain **10g** (0.16 g, quant.) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.54–7.60 (m, 1H), 7.17–7.23 (m, 1H), 6.73–6.81 (m, 1H), 6.36 (s, 1H), 3.93–4.00 (m, 2H), 3.84 (s, 3H), 3.64–3.70 (m, 1H), 3.57–3.64 (m, 2H), 3.15 (s, 3H), 2.35 (s, 3H), 2.33 (s, 3H), 2.29 (s, 3H), 1.72–1.92 (m, 6H), 0.83–0.91 (m, 3H). MS (ESI/APCI dual)

m/z: 488 $[M+H]^+$.



(17*S*)-3-Chloro-17-ethyl-4,5,13,16-tetramethyl-6,7,8,9,16,17-hexahydro-5*H*,15*H*-18,1-(metheno)pyrimido[6,1-g][1,6,8,9,12]benzoxatetraazacyclopentadecin-15-one (11g)

According to the procedure described for **11h**, the title compound **11g** was obtained (0.14 g, 88%) as a colorless powder. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.01–7.12 (m, 2H), 6.68–6.79 (m, 1H), 6.41 (s, 0.7H), 6.26 (s, 0.3H), 6.17–6.24 (m, 0.7H), 4.79–4.87 (m, 0.3H), 3.91–4.05 (m, 1H), 3.73–3.84 (m, 1H), 3.57–3.70 (m, 1.3H), 3.23–3.34 (m, 0.7H), 3.14 (s, 3H), 2.95 (s, 0.9H), 2.80 (s, 3H), 2.58 (s, 2.1H), 2.33 (s, 3H), 2.29 (s, 3H), 1.75–2.21 (m, 4H), 1.42–1.68 (m, 2H), 1.09–1.18 (m, 2.1H), 0.99–1.07 (m, 0.9H). MS (ESI/APCI dual) m/z: 456 $[M+H]^+$.



(17*S*)-3-[(3*S*)-3-Aminopyrrolidin-1-yl]-17-ethyl-4,5,13,16-tetramethyl-6,7,8,9,16,17-hexahydro-5*H*,15*H*-18,1-(metheno)pyrimido[6,1-g][1,6,8,9,12]benzoxatetraazacyclopentadecin-15-one (12g)

According to the procedure described for **12a**, the title compound **12g** was obtained (0.12 g, 77%) as a colorless powder. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.02–7.10 (m, 2H), 6.72–6.76 (m, 1H), 6.11–6.15 (m, 0.7H), 6.09 (s, 0.7H), 5.97 (s, 0.3H), 4.75–4.80 (m, 0.3H), 3.59–4.05 (m, 5.3H), 3.34–3.46 (m, 1.7H), 3.04–3.22 (m, 5H), 2.95 (s, 1H), 2.61 (s, 2H), 2.28 (s, 3H), 1.54–2.23 (m, 11H), 1.07–1.15 (m, 2.1H), 0.96–1.02 (m, 0.9H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 169.02, 159.39, 153.98, 152.90, 152.14, 149.64, 148.18, 130.03, 129.77, 129.39, 128.36, 127.78, 127.44, 127.24, 112.56, 111.80, 95.72, 89.95, 79.12, 67.55, 65.42, 58.12, 58.04, 52.92, 51.71, 51.03, 50.95, 50.89, 48.15, 48.05, 47.91, 34.20, 34.14, 32.80, 30.67, 26.69, 25.56, 24.72, 24.01, 23.81, 23.55, 22.49, 19.90, 15.90, 15.69, 11.03, 10.83, 10.61. HRMS ESI/APCI dual m/z calcd. for $C_{28}H_{39}N_7O_2$ $[M+H]^+$ 506.3238, found: 506.3228

Computational details

Molecular docking simulations were performed using the CDOCKER algorithm in Discovery Studio 2017 R2.[1] The input coordinates for wild-type and mutant (D486N) RSV F protein were obtained from the PDB entries 5EA3. The "Input Site Sphere" parameter for CDOCKER was defined using the inhibitor JNJ-2408068 in the PDB entries. Hydrogen atoms were added and the ionization states were assigned using the Protonate-3D function of the Molecular Operating Environment program (MOE)[2]; the positions of the hydrogen atoms were then optimized using the Amber10 forcefield implemented in MOE. For D486N, after manually correcting the coordinates from wild-type to D486N, the positions of the amino acid residues within 4.5 angstroms of JNJ-2408068 were optimized using the Amber10 forcefield implemented in MOE.

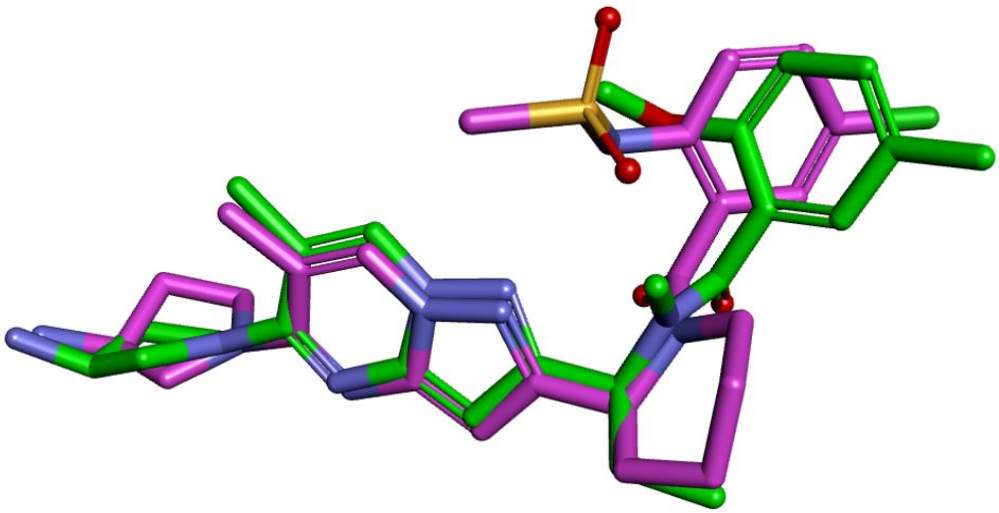


Figure S1. Conformation of **5** (green) docked on the RSV A2 F protein superposed with **1** (magenta) binding to the A2 protein by docking simulation, indicating that the two binding conformations are similar to each other.

Biological assay protocols

Cells and viruses

HEp-2 cells were purchased from DS Pharma Biomedical Co., Ltd. (Osaka, Japan) and cultured in minimum essential medium (MEM) supplemented with 10% fetal bovine serum (FBS), 50 μg/mL gentamicin and 600 μg/mL L-glutamine. RSV A2 (ATCC VR-1540) was purchased from the American Type Culture Collection (Manassas, VA, USA). RSV A2 with the D486N mutation in F protein was selected by serial passage in the presence of a pyrazolo[1,5-a]pyrimidine derivative **9c** (*N*-[(1*S*)-1-{5-

[(3*S*)-3-aminopyrrolidin-1-yl]-6-methylpyrazolo[1,5-a]pyrimidin-2-yl}propyl]-5-chloro-2-[(methanesulfonyl)amino]-*N*-methylbenzamide), as described in our previous report.[3] The mutant was confirmed to have no other mutations in F gene by genotypic analysis.

Antiviral assay

HEp-2 cells were cultured in 96-well plates overnight, and the test compounds were added after dilution with MEM supplemented with 2% FBS, 100 units/mL penicillin, 100 μg/mL streptomycin and 300 μg/mL L-glutamine. The cells were then infected with RSV A2 or D486N. After incubation at 37°C, 5% CO_2 for 4 d, the RSV-induced CPE was determined by adding XTT reagent. The concentration of the test compound required to inhibit the CPE by 50% (EC_{50}) was calculated using the least squares method.

References and notes

(1) *Discovery Studio Modeling Environment*, Dassault Systèmes BIOVIA, Release 2017, San Diego: Dassault Systèmes, 2017.

(2) *Molecular Operating Environment, MOE 2019.01;* Chemical Computing Group Inc.: 1010 Sherbooke St. West, Suite #910, Montreal, QC, Canada, H3A 2R7, 2019.

(3) Yamaguchi-Sasaki, T.; Tamura, Y.; Ogata, Y.; Kawaguchi, T.; Kurosaka, J.; Sugaya, Y.; Iwakiri, K.; Takahashi, R.; Sugiyama, H.; Kanuma, K. Design and Synthesis of 2-(1-Alkylaminoalkyl)pyrazolo[1,5-a]pyrimidines as New Respiratory Syncytial Virus Fusion Protein Inhibitors. *Chem. Pharm. Bull.* **2020**, *68*, 345–362.