Medicinal Chemistry Communications

Supporting Information

Small Structural Modifications of the Imidazopyridine Diacylglycerol Acyltransferase 2 (DGAT2) Inhibitors Produce an Improved Safety Profile

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Table S1. Selectivity data for compounds **1**, **3** and **5**

Target	Compound **1**		Compound **3**		Compound **5**	
	% inhibition or binding at 10 μM	IC_{50} or Ki (μM)	% inhibition or binding at 10 μM	IC_{50} or Ki (μM)	% inhibition or binding at 10 μM	IC_{50} or Ki (μM)
Kinases						
ABL1 (c-abl oncogene 1, receptor tyrosine kinase)[a]	<10		<10		<10	
AKT1 (RAC-beta serine/threonine-protein kinase 1)[a]	<10		<10		<10	
AKT2 (RAC-beta serine/threonine-protein kinase 2)[g]	<10		<10		<10	
ARK1 (Aurora-related kinase 1 or Aurora A)[a]	<10		<10		<10	
ARK2 (Aurora-related kinase 2 or Aurora B)[h]	<10		<10		<10	
BTK (Bruton's tyrosine kinase)[a]	<10		25		<10	
CAMK1 (Calcium/calmodulin-dependent protein kinase 1)[e]	<10		<10		<10	
CAMK2A (Calcium/calmodulin-dependent protein kinase 2 alpha)[a]	<10		<10		<10	
CAMKK2 (Calcium/calmodulin-dependent protein kinase kinase 2 beta)[e]	<10		<10		<10	
CDK2 (Cyclin-dependent kinase 2)[a]	<10		<10		<10	
CHK1 (Checkpoint kinase 1)[a]	<10		<10		<10	
CHK2 (Checkpoint kinase 2)[a]	<10		<10		<10	
CHKA (Choline kinase alpha)[h]		>10		>10		>10
CK1a1 (Casein kinase 1, alpha 1)[a]	<10		<10		<10	
CK2a2 (Casein kinase 2, alpha 2)[a]	<10		<10		<10	
CSK (C-terminal Src kinase)[e]	13		11		<10	
DGKB (Diacylglycerol kinase beta)[i]	<10		<10		<10	
DYRK2 (Dual specificity tyrosine phosphorylation-regulated kinase 2)[g]	<10		<10		<10	
EEF2K (eukaryotic elongation factor-2 kinase)[f]	20		20		<10	
EGFR (Epidermal growth factor receptor)[a]	<10		<10		<10	
EPHA2 (Ephrin type-A receptor 2)[a]	<10		<10		<10	
FGFR1 (Fibroblast growth factor receptor 1)[a]	<10		<10		<10	
GSK3B (Glycogen synthase kinase-3 beta)[a]	<10		<10		<10	
HGFR (Hepatocyte growth factor receptor)[a]	<10		<10		<10	
IKKbeta (Inhibitor of nuclear factor kappa-B kinase subunit B)[f]	<10		<10		<10	
IR (Insulin receptor)[a]	<10		<10		<10	
IRAK4 (Interleukin-1 receptor associated kinase 4)[e]	19		<10		<10	
JAK3 (Janus kinase 3)[a]	<10		<10		<10	
KDR (kinase insert domain receptor)[a]	<10		<10		16	
LCK (Lymphocyte-specific tyrosine kinase)[a]	<10		<10		10	
LKB1 (liver kinase B1)[e]	<10		<10		<10	
MAP3K7 (Mitogen-activated protein kinase kinase kinase 7)[f]	20		15		<10	
MAP3K9 (Mitogen-activated protein kinase kinase kinase 9)[e]	15		<10		<10	

MAP3K11 (Mitogen-activated protein kinase kinase kinase 11)[e]	<10		<10		<10	
MAP4K2 (Mitogen-activated protein kinase kinase kinase kinase 2)[e]	11		<10		<10	
MAP4K4 (Mitogen-activated protein kinase kinase kinase kinase 4)[a]	<10		<10		<10	
MAPK1 (Mitogen-activated protein kinase 1)[a]	<10		<10		<10	
MAPK8 (Mitogen-activated protein kinase 8)[e]	<10		<10		<10	
MAPK9 (Mitogen-activated protein kinase 9)[e]	<10		<10		<10	
MAPK12 (Mitogen-activated protein kinase 12)[f]	<10		<10		30	
MAPK13 (Mitogen-activated protein kinase 13)[f]	29	>10	15	>10	<10	
MAPK14 (Mitogen-activated protein kinase 14)[l]	<10		<10		<10	
MAPK15 (Mitogen-activated protein kinase 15)[f]	18		16		18	
MAPKAPK1b (MAP kinase activated protein kinase 1b)[g]	<10		13		<10	
MAPKAPK2 (MAP kinase activated protein kinase 2)[a]	<10		<10		<10	
MAPKAPK5 (MAP kinase activated protein kinase 5)[e]	11		<10		<10	
MARK1 (Microtubule affinity-regulating kinase 1)[a]	<10		<10		<10	
MARK3 (Microtubule affinity-regulating kinase 3)[f]	<10		<10		<10	
MNK1 (MAP kinase interacting serine/threonine kinase 1)[g]	<10		<10		<10	
MNK2 (MAP kinase interacting serine/threonine kinase 2)[g]	<10		<10		<10	
MSK1 (Mitogen and stress-activated protein kinase 1)[e]	<10		<10		<10	
MST2 (Mammalian STE20-like protein kinase 2)[a]	<10		<10		<10	
MST4 (Mammalian STE20-like protein kinase 4)[a]	<10		<10		<10	
MYLK2 (Myosin light chain kinase)[a]	<10		<10		<10	
NEK2 (Serine/threonine protein kinase 2)[a]	<10		<10		<10	
NEK6 (Serine/threonine protein kinase 6)[g]	<10		<10		<10	
NUAK-1 (SNF1-like kinase)[e]	<10		<10		<10	
PAK4 (P21 activated kinase 4)[a]	<10		<10		<10	
PBK (Lymphocyte-activated killer T-cell-originated protein kinase)[g]	10		<10		<10	
PDK1 (3-phosphoinositide-dependent protein kinase-1)[a]	<10		13		<10	
PI3KA (Phosphatidylinositol 3-kinase catalytic subunit alpha)[i]	<10	>10	<10	>10	<10	
PI3KB (Phosphatidylinositol 3-kinase catalytic subunit beta)[i]	<10		<10		<10	
PI3KG (Phosphatidylinositol 3-kinase catalytic subunit gamma)[i]	<10		<10		<10	
PI4KA (Phosphatidylinositol 4-kinase catalytic subunit alpha)[i]	<10		<10		<10	
PI4KB (Phosphatidylinositol 4-kinase catalytic subunit beta)[i]	<10		10		<10	
PIM1 (Proto-oncogene serine/threonine-protein kinase 1)[e]	<10		11		<10	
PIM2 (Proto-oncogene serine/threonine-protein kinase 2)[a]	<10		<10		<10	
PIM3 (Proto-oncogene serine/threonine-protein kinase 3)[e]	<10		17		<10	

PKA (Protein kinase A)[a]	<10		<10		<10	
PKB (Protein kinase B)[f]	<10		<10		<10	
PKC-alpha (Protein kinase C alpha)[e]	<10		<10		<10	
PKCB2 (Protein kinase C beta II)[a]	11		<10		<10	
PKD1 (Protein kinase C mu type)[g]	<10		<10		<10	
PKN2 (Protein kinase N2)[f]	10		<10		<10	
ROCK1 (Rho-associated, coiled-coil-containing protein kinase 1)[a]	<10		<10		<10	
P70S6K (Ribosomal protein S6 kinase, 70kDa, polypeptide 1)[e]	<10		<10		<10	
SGK (Serum glucocorticoid-regulated kinase)[a]	<10		<10		<10	
SPHK1 (Sphingosine kinase 1)[i]	<10		18		15	
SPHK2 (Sphingosine kinase 2)[h]	<10		<10		<10	
SRPK1 (serine/threonine kinase K1)[g]	10		<10		<10	
TAOK2 (TAO kinase 2)[a]	<10		<10		<10	
TEK (Angiopoietin 1 receptor)[a]	<10		<10		<10	
TRKA (High affinity nerve growth factor receptor)[a]	10		<10		20	
TTK (Dual specificity protein kinase)[e]	18		24		<10	
ZAP-70 (Zeta-chain-associated protein kinase 70)[a]	<10		<10		<10	
Receptors						
A1 (Adenosine receptor)[j]	<10		<10		11	
A2A (Adenosine receptor)[j]	<10		<10		10	
AChR (Nicotinic acetylcholine receptor complex)[k]	<10		<10		14	
ADRA1A (Adrenergic, alpha 1A receptor)[b]		>10		>30		>30
ADRA2B (Adrenergic, alpha 2B receptor)[l]	<10		<10		<10	
ADRB1 (Adrenergic, beta 1 receptor)[j]	<10		<10		<10	
ADRB2 (Adrenergic, beta 2 receptor)[b]		>30		>30		>30
AR (Androgen receptor)[j]	<10		<10		<10	
CB1 (Cannabinoid receptor 1)[b]		>10		>30		>30
CB2 (Cannabinoid receptor 2)[j]	14		<10		13	
CCKA (Cholecystokinin A receptor)[j]	<10		10		<10	
CCKB (Cholecystokinin B receptor)[j]	<10		35		<10	
D1 (Dopamine receptor 1)[b]		>10		>30		>30
D2 (Dopamine receptor 2)[j]	<10		<10		<10	
DOP1 (Delta-opioid receptor 1) [j]	<10		<10		<10	
Endothelin ETA receptor[l]	<10		<10		<10	
GR (Glucocorticoid receptor)[j]	<10		<10		<10	
H1 (Histamine 1 receptor)[b]		>10		>30		>30
H2 (Histamine 2 receptor)[l]	<10		<10		<10	
H3 (Histamine 3 receptor)[j]	<10		<10		<10	
5HTR1A (5-hydroxytryptamine (serotonin) receptor 1A)[j]	<10		<10		<10	

5HTR1B (5-hydroxytryptamine (serotonin) receptor 1B)[k]	<10		<10		<10	
5HTR2A (5-hydroxytryptamine (serotonin) receptor 2A)[j]	<10		<10		<10	
5HTR2B (5-hydroxytryptamine (serotonin) receptor 2B)[c]		>10		>30		>30
5HTR3 (5-hydroxytryptamine (serotonin) receptor 3)[k]	<10		<10		<10	
5HTR4 (5-hydroxytryptamine (serotonin) receptor 4)[l]	<10		<10		<10	
5HTR7 (5-hydroxytryptamine (serotonin) receptor 7)[j]	<10		<10		<10	
KOP1 (Kappa-opioid receptor 1)[j]	<10		<10		11	
M1 (Muscarinic receptor 1)[b]		>10		>30		>30
M2 (Muscarinic receptor 2)[j]	<10		<10		<10	
M3 (Muscarinic receptor 3)[k]	<10		<10		<10	
MOP (Mu-opioid receptor)[b]		>10		>30		>30
nAc (Nicotinic acetylcholine receptor)[j,k]	<10		<10		14	
NK1 (Neurokinin receptor 1)[l]	<10		<10		<10	
NK2 (Neurokinin receptor 2)[j]	14		14		<10	
PPAR-gamma (Peroxisome proliferator activated receptor gamma)[j]	<10		<10		<10	
SOP (Sigma-opioid receptor 1)[j]	13		<10		15	
UT-1 (Urotensin 1 receptor)[j]	<10		16		<10	
PDEs and Other Enzymes						
ACHE (Acetylcholinesterase)[l]	<10		<10		<10	
COX1 (Cyclooxygenase 1)[l]	<10		<10		13	
COX2 (Cyclooxygenase 2)[l]	11		<10		29	
CYP1A2 (Cytochrome P450 1A2)[d]		>30		>30		>30
CYP2C19 (Cytochrome P450 2C19)[d]		>30		>30		29
CYP2C8 (Cytochrome P450 2C8)[d]		15		21		29
CYP2C9 (Cytochrome P450 2C9)[d]		>30		>30		29
CYP2D6 (Cytochrome P450 2D6)[d]		>30		>30		>30
CYP3A4 (Cytochrome P450 3A4)[d]		>30		>30		>30
PDE1A (Phosphodiesterase 1A)[d]	<10		<10		<10	
PDE1B (Phosphodiesterase 1B)[d]		>20		>30		>30
PDE2A3 (Phosphodiesterase 2A3)[d]		>30		>30		>30
PDE3A (Phosphodiesterase 3A)[d]		>30	<10			>30
PDE3A1 (Phosphodiesterase 3A1)[d]		>200		>30		>30
PDE3B (Phosphodiesterase 3B)[d]	<10		<10		<10	
PDE4A (Phosphodiesterase 4A)[d]		>30		>30		>30
PDE4B (Phosphodiesterase 4B)[d]		27		>30		>30
PDE4C (Phosphodiesterase 4C)[d]		>30		>30		>30
PDE4D (Phosphodiesterase 4D)[d]		>30		>30		>30
PDE4D3 (Phosphodiesterase 4D3)[d]		>200		>30		>30
PDE5A (Phosphodiesterase 5A)[d]		>30		>9		>30

PDE5A1 (Phosphodiesterase 5A1)[d]		>200		>30		>30
PDE6A (Phosphodiesterase 6A)[d]		>30		>30		>30
PDE7B (Phosphodiesterase 7B)[d]		>30		>30		>30
PDE8B (Phosphodiesterase 8B)[d]		>30		>30		>30
PDE9A (Phosphodiesterase 9A)[d]	<10		<10		<10	
PDE10A (Phosphodiesterase 10A)[d]		>30		>30		>30
PDE11 (Phosphodiesterase 11)[d]		>200		>30		>30
Ion channels and Transporters						
CHT1 (High-affinity choline transporter, SLC5A7)[k]	<10		<10		<10	
DAT (Sodium-dependent dopamine transporter)[d]		>10		>30		>30
GABAa chloride channel (alpha 1 beta 1 gamma 2 subunit)[d]		ND		>10		>10
hERG[d]		95		>100		85
L-type calcium channel[d]		>6.4		>30		>30
NAT (Sodium-dependent noradrenaline transporter)[d]		>10		>30		>30
NaV1.1 (Sodium Channel Protein, neuronal alpha subunit)[d]		ND		>10		>10
NaV1.5 (Sodium Channel, voltage-gated, type V, alpha subunit)[d]		ND		<10		<10
SERT (Sodium-dependent serotonin transporter)[d]		>10		>30		>30

[a]Functional assay from Invitrogen panel, ATP = Km. [b]In-house functional assay in agonist and antagonist modes. [c]In-house functional assay in agonist mode. [d]In-house functional assay in antagonist/inhibitor mode. [e]Functional assay from Dundee panel, ATP = 20 μM. [f]Functional assay from Dundee panel, ATP = 5 μM. [g]Functional assay from Dundee panel, ATP = 50 μM. [h]Functional assay from Dundee panel, ATP = 1 μM. [i]Functional assay from Dundee panel, ATP = 10 μM. [j]Binding assay from CEREP panel using an agonist radioligand. [k]Binding assay from CEREP using an antagonist radioligand. [l]Functional assay from CEREP panel.

Table S2. Rat pharmacokinetic properties for compounds **3** and **5**

Compound	Dose (mg/kg)	route	$T_{1/2}$ (h)	CL,p (mL/min/kg)	Vdss (L/kg)	F (%)
3	1	iv[a]	1.4	23	1.7	NA[d]
	5	po[b]	1.3	NA[d]	NA[d]	61
5	1	Iv[c]	0.8	26	0.88	NA[d]
	5	po[b]	1.3	NA[d]	NA[d]	25

Male Wistar-Han rats were utilized for PK studies. All data reported here are means of two experiments. The compounds were dosed as the parent. [a]Vehicle was 10% DMSO/90% of 20% SBECD in water. [b]Vehicle was 0.5% methylcellulose. [c]Vehicle was 10% DMSO / 30% PEG400/60% water. [d]NA = not applicable.

Protocol for pharmacokinetic studies in rat and dog

Rat pharmacokinetic studies (oral, intravenous bolus) were conducted at BioDuro contract laboratories (Shanghai, China). Male Wistar-Han rats were used for the pharmacokinetics analysis. For oral (p.o.) studies, (n=2) rats were fasted overnight and allowed access to food 4 hr post dose, admittance to water was allowed ad libitum. Compound **3** or **5** was formulated in 0.5% methylcellulose and administered orally at a dose of 5 mg/kg via gavage needle. For intravenous (i.v.) bolus studies, (n=2), compound **3** or **5** was formulated in 10% DMSO / 90% sulfobutylether-β-cyclodextrin (for compound **3**) or 10% DMSO / 30% polyethylene glycol 400 / 60% water (for compound **5**) and administered i.v. at a dose of 1 mg/kg via tail vein. After dosing p.o. or i.v. serial blood samples were collected at 0, 0.03, 0.083, 0.25, 0.5, 1, 2, 4, 7, and 24 hr time points (0.03 hr for i.v. dose group only) via jugular vein into K2EDTA vacutainers. The blood was kept cold throughout the collection process until they were centrifuged to obtain plasma. The plasma samples was kept frozen at -20°C or -80°C until LC-MS/MS analysis. Dog pharmacokinetic studies (oral, intravenous bolus) were conducted in-house at Pfizer (Groton, CT). Male beagle dogs were used for the pharmacokinetics analysis. For p.o. studies, (n=2) dogs were fasted overnight and allowed access to food 4 hr post dose, admittance to water was allowed ad libitum. Compound **3** or **5** was formulated in 20% sulfobutylether-β-cyclodextrin in 0.5% methylcellulose and administered orally at a dose of 5 mg/kg via gavage tube. For intravenous (i.v.) bolus studies, (n=2),

compound **3** or **5** was formulated in 10% polyethylene glycol 200 / 90% of 12% sulfobutylether-β-cyclodextrin and sterile filtered using a 0.22µm Durapore® syringe end filter and administered i.v. at a dose of 1 mg/kg via cephalic vein. After dosing p.o. or i.v. serial blood samples were collected at 0, 0.083, 0.25, 0.5, 1, 2, 4, 7, and 24 hr time points (0.083 hr for i.v. dose group only) via jugular vein into K3EDTA vacutainers. The blood was kept cold throughout the collection process until they were centrifuged to obtain plasma. The plasma samples was kept frozen at -20°C or -80°C until LC-MS/MS analysis. All animal care and in vivo procedures were conducted in accordance with guidelines of the Pfizer Animal Care and Use Committee as well as the current guidelines for animal welfare (National Research Council Guide for the Use of Laboratory Animals, 2011; Animal Welfare Act, 1966, as amended in 1970, 1976, 1985, and 1990, and the Animal Welfare Act implementing regulations in Title 9, Code of Federal Regulations, Chapter 1, Subchapter A, Parts 1-3).

Protocols for safety pharmacology and toxicology studies

Animals and Husbandry Wistar Han IGS (Crl:WI[Han]) (8-10 weeks of age) were obtained from Charles River Laboratories (Kingston, NY). Beagle dogs (8-12 months of age) were obtained from Marshall Bioresources (North Rose, NY). Animals were randomly assigned to treatment groups. The animal room environment was controlled (21 ± 3°C, humidity 66-79%, 12 hour light/dark). Animals received appropriate certified laboratory diets. The studies were conducted in accordance with guidelines of the Pfizer Animal Care and Use Committee as well as the current guidelines for animal welfare (National Research Council Guide for the Use of Laboratory Animals, 2011; Animal Welfare Act, 1966, as amended in 1970, 1976, 1985, and 1990, and the Animal Welfare Act implementing regulations in Title 9, Code of Federal Regulations, Chapter 1, Subchapter A, Parts 1-3).

Experimental Design, Observations, and Measurements Observations and measurements performed in all 1-month studies included clinical signs, body weight, food consumption, ophthalmic examination, standard hematology, coagulation, clinical chemistry and urinalysis, toxicokinetics (TK), electrocardiogram (dog), complete necropsy, organ weights, and microscopic tissue examination. Studies designated as exploratory toxicity studies utilized smaller numbers of animals and an abbreviated tissue collection and evaluation list relative to full toxicity studies. Urinalysis, ophthalmic exams, and electrocardiograms were not performed in exploratory studies.

Blood samples for hematology, coagulation and serum chemistry determinations were collected from animals at necropsy. Standard hematology parameters were assessed using an Advia 120 automated analyzer (Siemens Diagnostics, Tarrytown, NY). Prothrombin and activated partial thromboplastin times that were measured using the STA Compact Automated Coagulation System (Stago) analyzer (Diagnostica Stago, Parsippany, NJ). Standard serum clinical chemistry assessments were measured on the Hitachi chemistry analyzer (Roche Diagnostics, Indianapolis, IN).

Compound 1 The preclinical safety of compound **1** was evaluated in rats and dogs. In the 14-day exploratory rat study (5/sex/dose main, 3/sex/dose TK) doses evaluated were 0, 10, 100, or 500 mg/kg/day. In the 1-month rat toxicity study (10/sex/dose main, 4/sex/dose TK) doses evaluated were 0, 30, 60, 300 mg/kg/day. The 14-day exploratory dog study (1 or 2/sex/dose) evaluated doses of 75 or 300 mg/kg/day and the 1-month dog toxicity studies (3/sex/dose) evaluated doses of 2, 5, 10, 50, or 150/100 mg/kg/day. The dosing vehicle for all studies was 0.5% (w/v) methylcellulose.

Compounds 3 and 5 Due to findings of arteriopathy in dogs administered compound **1**, compounds **3** and **5** were evaluated in a 1-month exploratory toxicity study. In this study, dogs (2/sex/dose) were administered vehicle (20% (w/v) sulfobutyl ether beta cyclodextrin in 0.5% (w/v) methylcellulose), compound **3** (3, 10, or 30 mg/kg/day), or compound **5** (3, 10, or 30 mg/kg/day). The study duration, extended tissue collection and evaluation list, and doses were selected to maximize the likelihood of detecting arteriopathy with a limited supply of each drug.

Protocol for cardiovascular studies in telemetry instrumented dogs

The objective of these studies was to evaluate the potential cardiovascular effects of orally administered test compounds at multiple dose levels in a balanced crossover study design in telemetry instrumented male beagle dogs. The cardiovascular data acquired and analysed included electrocardiographic (ECG) intervals, heart rate, arterial and left ventricular pressures and activity level and were collected starting approximately 1 hour predose through at least 24 hours post dose.

Compound **1** was administered by oral gavage to dogs (n = 8) implanted with radio-telemetry transmitters, at doses of 0, 5, 10, 25, and 75 mg/kg. The test item was administered as a solution in 0.5% methylcellulose at a dose volume of 5 mL/kg. In order to obtain a full pharmacokinetic (PK) profile for the study, all eight dogs received single doses of compound **1** at 5 mg/kg and 25 mg/kg on different treatment days and blood samples were collected predose and at 1, 2, 4, 6, 8, and 24 hour postdose.

In a separate study, Compound **1** was also administered by intravenous bolus administration to male dogs (n = 4) at a single dose level of 20 mg/kg. Animals received a single intravenous bolus dose over 2 minutes of either vehicle (sterile water) or compound in a crossover design. Telemetered data were obtained from each animal in the same manner as previously described.

Compound **3** was administered by oral gavage in the same dog telemetry model (n = 4). The dose levels selected for this study were 5, 25, and 50 mg/kg via oral gavage (PO). Animals received a single oral dose of either vehicle (0.5% methylcellulose) or test article in a cross-over design. One of the four dogs displayed significant premature ventricular contractions (PVC) in the control phase and was excluded from the subsequent analysis leaving an n=3 study.

Synthesis and characterization of compounds 1-6

General Experimental Methods. All chemicals, reagents, and solvents were purchased from commercial sources and were used without further purification unless otherwise noted. Silica gel chromatography was performed using a medium pressure Biotage or ISCO system and columns pre-packaged by various commercial vendors including Biotage and ISCO. The terms "concentrated" and "evaporated" refer to the removal of solvent at reduced pressure on a rotary evaporator with a water bath temperature not exceeding 60 °C. ^{1}H NMR data are referenced relative to residual solvent signals, and are reported as follows: chemical shift (δ ppm), multiplicity, integration, and coupling constant (J value in Hz). The multiplicities are denoted as follows: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br s, broad singlet; app, apparent. Optical rotation $[\alpha]_D$ values are reported in units of 10^{-1} deg cm^2 g^{-1}. Purity of final compounds was assessed by reversed-phase HPLC with UV detection at 215 nM**.** The preparation of compound **1** (PF-06424439) was previously reported.[1] Alternatively, PF-06424439 (**1**) was made available to order from Aldrich # PZ0233. *Safety note*: Sodium hydride and DMSO can react explosively, please take appropriate precautions in using this reagent/solvent combination.

(*R*)-(1-(2-(2-(4-Chloro-1*H*-pyrazol-1-yl)propan-2-yl)-3H-imidazo[4,5-b]pyridin-5-yl)piperidin-3-yl)(pyrrolidin-1-yl)methanone (2).

Step 1. 2-(4-Chloro-1H-pyrazol-1-yl)-2-methylpropanenitrile. Safety note: Sodium hydride and DMSO can react explosively, please take appropriate precautions in using this reagent/solvent combination. DMSO (30 mL) was added to sodium hydride (60% oil dispersion, 2.52 g, 64 mmol, 4.6 equiv) and the resulting suspension was cooled to 0 °C. A solution of 2-(4-chloro-1*H*-pyrazol-1-yl)acetonitrile (2.0 g, 14 mmol, 1 equiv) and methyl iodide (2.6 mL, 40 mmol, 2.9 equiv) in DMSO (30 mL) was added over a period of 30 min. The mixture was warmed to ambient temperature and was held at that temperature for 18 h. Excess base was quenched with saturated aqueous NH_4Cl solution and the mixture was extracted twice with EtOAc. The combined organic layers were washed with brine and were dried over sodium sulfate, then were concentrated. Purification by silica gel chromatography (0-20% EtOAc in petroleum ether) afforded the product (1.8 g, 76%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.67 (s, 1H), 7.54 (s, 1H), 1.99 (s, 6H). m/z 169.0 $(M)^+$.

Step 2. Ethyl 2-(4-chloro-1H-pyrazol-1-yl)-2-methylpropanimidate. Sodium metal (95 mg, 4.1 mmol, 0.7 equiv) was added to EtOH (5 mL). After 5 min, a solution of 2-(4-chloro-1*H*-pyrazol-1-yl)-2-methylpropanenitrile (1.0 g, 5.9 mmol, 1 equiv) in EtOH (2 mL) was added, and the solution was heated at reflux for 90 min. The solution was cooled to ambient temperature and was used directly in Step 4. Analytical data from a smaller scale reaction (with residual EtOH): ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.56 (s, 1H), 7.54 (s, 1H), 4.18 (q, J=7.2, 2H), 1.81 (s, 6H), 1.28 (q, 3H, obscured by residual EtOH).

*Step 3. (R)-(1-(5,6-Diaminopyridin-2-yl)piperidin-3-yl)(pyrrolidin-1-yl)methanone (**IIa**).* A solution of (*R*)-(1-(6-amino-5-nitropyridin-2-yl)piperidin-3-yl)(pyrrolidin-1-yl)methanone[1] (1.5 g, 4.7 mmol) in ethanol (20 mL) was added to a suspension of 10% palladium-on-carbon (800 mg) at ambient temperature. The mixture reacted under hydrogen gas for 4 h, then was filtered through Celite and washed with ethanol. The air-sensitive filtrate was used immediately in Step 4 without further purification.

*Step 4. (R)-(1-(2-(2-(4-Chloro-1H-pyrazol-1-yl)propan-2-yl)-3H-imidazo[4,5-b]pyridin-5-yl)piperidin-3-yl)(pyrrolidin-1-yl)methanone (**2**).* Acetic acid (2.9 mL, 49 mmol, 10 equiv) and a solution of ethyl 2-(4-chloro-1*H*-pyrazol-1-yl)-2-methylpropanimidate (from Step 2, 5.9 mmol, 1.3 equiv) were added sequentially to a solution of (*R*)-(1-(5,6-diaminopyridin-2-yl)piperidin-3-yl)(pyrrolidin-1-yl)methanone in ethanol (from Step 3, 4.7 mmol, 1 equiv). The mixture was heated at reflux for 18 h. The mixture was concentrated and the residue was partitioned between EtOAc and water. The organic layer was dried over sodium sulfate, filtered, and concentrated. The crude material was purified by silica gel chromatography (5% methanol in ethyl acetate) to afford **2** (530 mg, 25% over 2 steps). ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.08 (br s, 1H), 7.79 (d, J=8.8, 1H), 7.59 (s, 1H), 7.55 (s, 1H), 6.63 (d, J=8.8, 1H), 4.43 (d, J=12.9, 1H), 4.22 (d, J=13.6, 1H), 3.55-3.63 (m, 1H), 3.40-3.50 (m, 3H), 3.01-3.09 (m, 1H), 2.88-2.93 (m, 1H), 2.58-2.70 (m, 1H), 2.10 (s, 6H), 1.74-2.00 (m, 7H), 1.53-1.65 (m, 1H). m/z 442.2 $(M+H)^+$. HPLC purity, >99%.

((*R*)-1-(2-((*S*)-1-(4-chloro-1*H*-pyrazol-1-yl)ethyl)-3*H*-imidazo[4,5-b]pyridin-5-yl)piperidin-3-yl)(pyrrolidin-1-yl)methanone (3).

Step 1. Methyl (S)-2-(4-chloro-1H-pyrazol-1-yl)propanoate. Diisopropylazodicarboxylate (157 g, 777 mmol, 1.3 equiv) was added to a solution of methyl (*R*)-2-hydroxypropanoate (60 g, 576 mmol, 1 equiv), 4-chloro-1*H*-pyrazole (67 g, 634 mmol, 1.1 equiv) and PPh_3 (205 g, 782 mmol, 1.4 equiv) in THF (1.5 L) at 0 °C. The mixture was stirred at ambient temperature overnight, then was concentrated. The solids were removed by filtration and were rinsed with EtOAc, and the filtrate was concentrated. The crude product was purified by chromatography (20:1 petroleum ether:EtOAc). Three batches were run on this scale and were combined to afford a yellow oil (260 g, 80%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.53 (s, 1H), 7.45 (s, 1H), 5.04 (q, J=7.6, 1H), 3.75 (s, 3H), 1.76 (d, J=7.6, 3H).

*Step 2. (S)-2-(4-chloro-1H-pyrazol-1-yl)propanoic acid (**Va**).* A suspension of methyl (*S*)-2-(4-chloro-1*H*-pyrazol-1-yl)propanoate (160 g, 1.4 mol) in 6N HCl (2 L) was heated at reflux for 4 h. The solution was cooled to 0 °C, then was extracted with EtOAc (2 ×2 L). The combined organic layers were concentrated to afford a solid. Recrystallization (10:1 petroleum ether:EtOAc) afforded a white solid (49 g, 33%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.58 (br s, 1H), 7.54 (s, 1H), 7.51 (s, 1H), 5.11 (q, J=7.4, 1H), 1.80 (d, J=7.4, 3H). m/z 174.8 $(M)^+$. Chiral SFC: Chiralpak AD-3 150 × 4.6mm, 3 µ, 10% MeOH-CO_2, 2.5 mL/min, 220 nm; retention time 2.32 min; (*R*)-2-(4-chloro-1*H*-pyrazol-1-yl)propanoic acid retention time 3.58 min; 99% e.e.

*Step 3. tert-Butyl (R)-(2-amino-6-(3-(pyrrolidine-1-carbonyl)piperidin-1-yl)pyridin-3-yl)carbamate (**IVa**).* To a solution of (*R*)-(1-(6-amino-5-nitropyridin-2-yl)piperidin-3-yl)(pyrrolidin-1-yl)methanone[1] (50 g, 156 mmol, 1 equiv) and di-tert-butyl dicarbonate (37.6 g, 172 mmol, 1.1 equiv) in ethanol (600 mL) was added 10% Pd/C (5.0 g). The mixture was placed under a hydrogen atmosphere (45 psi) at 25 °C for 4 days. The mixture was filtered through Celite, the filtrate was concentrated, and the crude product was purified by silica gel chromatography (25-100% EtOAc:petroleum ether). Four batches on this scale were combined to afford a yellow solid (159 g, 65%). ^{1}H NMR (400 MHz, MeOH-d_4) δ = 7.22 (br d, J=8.2, 1H), 6.11 (d, J=8.2, 1H), 4.44 (br d, J=12.8, 1H), 4.13 (d, J=13.1, 1H), 3.71-3.74 (m, 1H), 3.53-3.55 (m, 1H), 3.41-3.45 (m, 2H), 2.76-2.91 (m, 2H), 2.63-2.75 (m, 1H), 1.69-2.09 (m, 7H), 1.52-1.62 (m, 1H), 1.51 (s, 9H). m/z 390.0 $(M+H)^+$.

*Step 4. tert-Butyl (2-((S)-2-(4-chloro-1H-pyrazol-1-yl)propanamido)-6-((R)-3-(pyrrolidine-1-carbonyl)piperidin-1-yl)pyridin-3-yl)carbamate (**VI**).* A solution of tert-butyl (*R*)-(2-amino-6-(3-(pyrrolidine-1-carbonyl)piperidin-1-yl)pyridin-3-yl)carbamate (118 g, 304 mmol, 1 equiv), (*S*)-2-(4-chloro-1*H*-pyrazol-1-yl)propanoic acid (60.9 g, 349 mmol, 1.15 equiv), and pyridine (113 mL, 1.40 mol, 4.6 equiv) in EtOAc (100 mL) was cooled in an ice bath. A solution of T3P (50% in EtOAc, 210 mL, 698 mmol, 2.3 equiv) was added with stirring at a rate to maintain internal temperature below 6.0 °C. As the ice bath melted, the mixture was allowed to warm to 20 °C and was stirred overnight. The resulting slurry was cooled to 0 °C and then the solids were collected by filtration, rinsing with cold EtOAc (0.5 L), to afford a white solid (115 g, 69%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.47 (br s, 1H), 7.76 (app br s, 1H), 7.61 (s, 1H), 7.59 (s, 1H), 7.25 (app br s, 1H), 6.58 (d, J=9.0, 1H), 5.08 (br q, J=7.0, 1H), 4.34 (app br d, J=13.3, 1H), 4.15 (app br d, J=11.7, 1H), 3.43-3.59 (m, 4H), 3.00 (dd , J=13.1, 11.1, 1H), 2.87 (td, J=13.0, 13.0, 2.5, 1H), 2.52-2.60 (m, 1H), 1.83-2.04 (m, 6H), 1.89 (d, J=7.0, 3H), 1.73-1.78 (m, 1H), 1.52-1.57 (m, 1H), 1.49 (s, 9H). m/z 546.5 $(M+H)^+$.

*Step 5. ((R)-1-(2-((S)-1-(4-chloro-1H-pyrazol-1-yl)ethyl)-3H-imidazo[4,5-b]pyridin-5-yl)piperidin-3-yl)(pyrrolidin-1-yl)methanone (**3**).* A solution of tert-butyl (2-((*S*)-2-(4-chloro-1*H*-pyrazol-1-yl)propanamido)-6-((*R*)-3-(pyrrolidine-1-carbonyl)piperidin-1-yl)pyridin-3-yl)carbamate (115 g, 210 mmol, 1 equiv) in CH_2Cl_2 (421 mL) was cooled to 0 °C. Methanesulfonic acid (68.3 mL, 1.05 mol, 5.0 equiv) was added with stirring at a rate to maintain internal temperature below 5.5 °C. The resulting solution was stirred overnight at 21 °C. The solution was then cooled to 0 °C and a solution of ammonium acetate (97.4 g, 1.26 mmol, 6.0 equiv) in water (180 mL) was added dropwise at a rate to maintain internal temperature below 7 °C. The biphasic solution was warmed to ambient temperature and was stirred an additional 1 h. The layers were separated, and the organic layer was diluted with EtOAc (1 L) and was cooled to 6 °C. Saturated aqueous $NaHCO_3$ (1.5 L) and brine (1 L) were added, and the layers were separated. The organic layer was dried over Na_2SO_4, was filtered, and was concentrated. The resulting residue was purified by silica gel chromatography (0-7% MeOH:CH_2Cl_2) to afford a foam that was concentrated from ethanol (2×). Further drying in a vacuum oven afforded **3** as a foam (83.5 g, 92%; with residual ethanol). Analytical data from a sample purified to be free of residual ethanol: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.78 (d, J=8.9, 1H), 7.60 (s, 1H), 7.53 (s, 1H), 6.68 (d, J=8.9, 1H), 5.76 (q, J=7.1, 1H), 4.46 (br d, J=13.2, 1H), 4.23 (br d, J=12.4, 1H), 3.58-3.63 (m, 1H), 3.42-3.54 (m, 3H), 3.08 (m, 1H), 2.89-3.00 (m, 1H), 2.59-2.70 (m, 1H), 2.04 (d, J=7.6, 3H), 1.75-2.00 (m, 7H), 1.54-1.66 (m, 1H). m/z 428.3 $(M+H)^+$. Chiral HPLC: Lux Cellulose-2, 250 × 4.6 mm, 5µ; Mobile phase A, heptane; Mobile phase B, ethanol; Gradient (time (min)/%B), 0.00/5, 1.00/5, 10.0/100, 11.0/100, 12.5/5; 1.5 mL/min; UV 210 nM; retention time 8.9 min; diastereomer **4** retention time 10.8 min; d.e. of 83.5 g batch, >99%.

*Step 6. ((R)-1-(2-((S)-1-(4-Chloro-1H-pyrazol-1-yl)ethyl)-3H-imidazo[4,5-b]pyridin-5-yl)piperidin-3-yl)(pyrrolidin-1-yl)methanone hydrochloride hydrate (**3**) .* To prepare a crystalline sample of **3**-hydrochloride-hydrate, **3** (32.4 g, 75.7 mmol, 1 equiv) was dissolved in THF (0.84 L). A solution of concentrated HCl (6.62 mL, 79.5 mmol, 1.05 equiv) in THF (0.42 L) was added dropwise with stirring; a precipitate formed, then water (13.6 mL, 0.755 mol, 10 equiv) was added. The resulting mixture was

stirred for 3.5 h, then the solids were collected by filtration and were dried under vacuum (32.7 g, 93%). Another portion of ((*R*)-1-(2-((*S*)-1-(4-chloro-1*H*-pyrazol-1-yl)ethyl)-3*H*-imidazo[4,5-b]pyridin-5-yl)piperidin-3-yl)(pyrrolidin-1-yl)methanone (76.5 g, 179 mmol, 1 equiv) was dissolved in THF (2.0 L) and water (32.2 mL, 1.79 mol, 10 equiv) with stirring. A solution of concentrated HCl (15.6 mL, 188 mmol, 1.05 equiv) in THF (1.0 L) was added dropwise with stirring. The resulting red solution became cloudy and then formed a granular precipitate. After complete addition of the HCl solution, the mixture was seeded with the first batch of crystalline material described above (32.6 g, 67.6 mmol). After an additional 2 h, the solids were collected by filtration and were dried under vacuum to afford **3**-hydrochloride-hydrate as a crystalline solid (109.7 g, 92%). mp 154-157 °C. $[\alpha]_D$ = -236.5°(c 0.049 in MeOH). Elemental analysis Found: C, 52.08; H 6.05; N, 20.13; Cl, 14.52; Calc for $C_{21}H_{29}Cl_2N_7O_2$, C, 52.29; H, 6.06; N, 20.32; Cl, 14.70. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.22 (s, 1H), 7.90 (d, J=9.2, 1H), 7.64 (s, 1H), 7.07 (d, J=9.2, 1H), 5.99 (br q, 1H), 4.28-4.38 (m, 2H), 3.50-3.55 (m, 1H), 3.41-3.46 (m, 1H), 3.26-3.33 (m, 2H), 2.96-3.06 (m, 2H), 2.57-2.65 (m, 1H), 1.95 (d, J=7.4, 3H), 1.62-1.92 (m, 7H), 1.48-1.56 (m, 1H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 171.3, 157.0, 149.0, 144.8, 138.6, 128.6, 126.2, 117.7, 109.2, 107.7, 54.4, 48.6, 46.8, 46.3, 45.8, 27.6, 26.2, 24.3, 24.0, 18.5. HPLC purity, 99%.

((*R*)-1-(2-((*R*)-1-(4-Chloro-1*H*-pyrazol-1-yl)ethyl)-3*H*-imidazo[4,5-b]pyridin-5-yl)piperidin-3-yl)(pyrrolidin-1-yl)methanone (4). Compound **4** was prepared from (*R*)-2-(4-chloro-1*H*-pyrazol-1-yl)propanoic acid by a procedure analogous to that described for Compound **3**, Steps 4-5. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.78 (d, J=8.9, 1H), 7.60 (s, 1H), 7.52 (s, 1H), 6.66 (d, J=8.9, 1H), 5.74 (q, J=7.1, 1H), 4.47 (br d, J=13.2, 1H), 4.23 (br d, J=13.2, 1H), 3.58-3.62 (m, 1H), 3.43-3.50 (m, 3H), 3.04-3.09 (m, 1H), 2.91-2.96 (m, 1H), 2.62-2.68 (m, 1H), 2.03 (d, J=7.1, 3H), 1.76-1.99 (m, 7H), 1.55-1.63 (m, 1H). m/z 428.3 $(M+H)^+$. Chiral HPLC: Lux Cellulose-2, 250 × 4.6 mm, 5μ; Mobile phase A, heptane; Mobile phase B, ethanol; Gradient (time (min)/%B), 0.00/5, 1.00/5, 10.0/100, 11.0/100, 12.5/5; 1.5 mL/min; UV 210 nM; retention time 10.8 min; diastereomer **3** retention time 8.9 min. HPLC purity, 99%.

((3*R*)-1-(8-(1-(4-Chloro-1*H*-pyrazol-1-yl)ethyl)-9*H*-purin-2-yl)piperidin-3-yl)(pyrrolidin-1-yl)methanone; diastereomers at methyl stereocenter (absolute configurations undetermined) (5 and 6).

Step 1. 2-(4-Chloro-1H-pyrazol-1-yl)propanenitrile. 4-Chloro-1*H*-pyrazole (50 g, 0.49 mol, 1 equiv), 2-chloropropanenitrile (45 g, 0.50 mol, 1.0 equiv), and Cs_2CO_3 (193 g, 0.55 mol, 1.1 equiv) were charged to a flask containing 2-Me-THF (1 L). The mixture was heated at reflux overnight. The solids were removed by filtration and were rinsed with ether (3 × 0.5 L), and the filtrate was concentrated to afford 2-(4-chloro-1*H*-pyrazol-1-yl)propanenitrile as a brown oil (75.7 g, 99%) that was used in Step 2 without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.59 (s, 1H), 7.51 (s, 1H), 5.25 (q, J=7.2, 1H), 1.89 (d, J=7.2, 3H). m/z 156.1 $(M+H)^+$.

Step 2. Ethyl 2-(4-chloro-1H-pyrazol-1-yl)propanimidate. To a solution of NaOEt (110 g, 1.62 mol, 1.4 equiv) in EtOH (1 L) was added 2-(4-chloro-1*H*-pyrazol-1-yl)propanenitrile (180 g, 1.16 mol, 1 equiv) in EtOH (2 L). The mixture was stirred at 70 °C for 12 h, then was cooled and was concentrated to near dryness to afford ethyl 2-(4-chloro-1*H*-pyrazol-1-yl)propanimidate (271 g, >100%) as a brown oil that was used without further purification. A second batch of material was combined for use in Step 6. Analytical data from a smaller scale reaction (with residual EtOH): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.54 (s, 1H), 7.48 (s, 1H), 4.88 (q, J=7.2, 1H), 4.20 (q, J=7.0, 2H), 1.75 (d, J=7.2, 3H), 1.30 (q, 3H, obscured by residual EtOH).

*Step 3. (R)-(1-(4-Amino-5-nitropyrimidin-2-yl)piperidin-3-yl)(pyrrolidin-1-yl)methanone (**Ib**).* A mixture of 2-chloro-5-nitropyrimidin-4-amine (79.8 g, 80% purity, 365 mmol, 1.0 equiv), (*R*)-piperidin-3-yl(pyrrolidin-1-yl)methanone hydrochloride[1] (80.0 g, 365 mmol, 1 equiv) and Et_3N (81.4 g, 804 mmol, 2.2 equiv) in CH_3CN (1.5 L) was heated at 80 °C for 4 h. The mixture was diluted with EtOAc (250 mL) and was washed sequentially with water and brine, then was dried over Na_2SO_4. The organic layer was concentrated to afford a crude product. Two batches on this scale were combined and were recrystallized from EtOAc/MTBE (200 mL/2500 mL) to afford a yellow solid (210 g, 90%). ^{1}H NMR (400 MHz, MeOH-d_4) δ 8.95 (s, 1H), 4.9 (m, obscured by solvent signals), 3.63-3.72 (m, 1H), 3.51-3.61 (m, 1H), 3.44 (app t, J=6.9, 2H), 2.89-3.15 (m, 2H), 2.67-2.69 (m, 1H), 2.10-1.72 (m, 7H), 1.47-1.65 (m, 1H). m/z 321.1 $(M+H)^+$.

*Step 4. tert-Butyl (R)-(4-amino-2-(3-(pyrrolidine-1-carbonyl)piperidin-1-yl)pyrimidin-5-yl)carbamate (**IVb**).* To a stirred solution of (*R*)-(1-(4-amino-5-nitropyrimidin-2-yl)piperidin-3-yl)(pyrrolidin-1-yl)methanone (50.0 g, 156 mmol, 1 equiv) and di-tert-butyl dicarbonate (37.5g, 172 mmol, 1.1 equiv) in EtOH (600 mL) was added 10% Pd/C (8.0 g). The mixture was reacted under hydrogen atmosphere (45 psi) at 25 °C for 2 days. The mixture was filtered through Celite and the filtrate was concentrated. Four reactions on this scale were combined to afford a yellow solid (280 g, >100%, with residual EtOH) which was used in Step 5 without further purification. ^{1}H NMR (400 MHz, MeOH-d_4) δ 7.72 (br s, 1H), 4.62-4.17 (m, 2H), 3.71 (m, 1H), 3.61-3.63 (m, 1H), 3.53-3.54 (m, 1H), 3.41-3.45 (m, 1H), 2.87-2.91 (m, 2H), 2.61-2.65 (m, 1H), 1.86-2.06 (m, 5H), 1.68-1.85 (m, 2H), 1.47-1.53 (m, 10H). m/z 391.2 $(M+H)^+$.

*Step 5. (R)-(1-(4,5-Diaminopyrimidin-2-yl)piperidin-3-yl)(pyrrolidin-1-yl)methanone dihydrochloride (**IIb**).* A solution of 4M HCl in dioxane (1.5 L) was added to tert-butyl (*R*)-(4-amino-2-(3-(pyrrolidine-1-carbonyl)piperidin-1-yl)pyrimidin-5-yl)carbamate (93.0

g, 238 mmol, 1 equiv). The mixture was stirred at ambient temperature for 3 h, then was filtered to collect the precipitated solids. Products from three reactions on this scale were combined, MTBE (1 L) was added, and the suspension was stirred for 2 h. The solids were collected by filtration and then were dissolved with MeOH (400 mL) and CH_2Cl_2 (100 mL). MTBE (2 L) was added to the solution. The resulting precipitate was collected by filtration to afford a white solid (186 g, 71%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.55 (br s, 1H), 8.18 (br s, 1H), 7.52 (s, 1H), 4.35 (br s, residual water obscuring signals), 3.57-3.59 (m, 1H), 3.40-3.43 (m, 1H), 3.24-3.28 (m, 2H), 2.99-3.17 (m, 2H), 2.63-3.66 (m, 1H), 1.68-1.96 (m, 6H), 1.41-1.81 (m, 2H). m/z 291.1 $(M+H)^+$.

Step 6. ((3R)-1-(8-(1-(4-Chloro-1H-pyrazol-1-yl)ethyl)-9H-purin-2-yl)piperidin-3-yl)(pyrrolidin-1-yl)methanone. EtOH (2 L) and acetic acid (524.2 mL, 9.17 mol, 20 equiv) were added to a flask containing (*R*)-(1-(4,5-diaminopyrimidin-2-yl)piperidin-3-yl)(pyrrolidin-1-yl)methanone dihydrochloride (164 g, 0.45 mol, 1 equiv) under a nitrogen atmosphere. A solution of ethyl 2-(4-chloro-1*H*-pyrazol-1-yl)propanimidate (from Step 2, 384 g, 1.91 mol, 4.2 equiv) in EtOH (2.8 L), and triethylamine (393 mL, 2.84 mol, 6.3 equiv) were then added sequentially. The resulting mixture was purged with nitrogen, and then was heated at 70 °C overnight. The mixture was cooled to ambient temperature and was concentrated to near dryness. The residue was partitioned between aqueous NH_4Cl solution (1 L) and CH_2Cl_2 (1 L) and the aqueous layer was extracted with another portion of CH_2Cl_2 (1 L). The combined organic layers were washed with saturated aqueous Na_2CO_3 solution (2 × 1 L). The base washes were extracted with CH_2Cl_2 (1 L). The combined organic layers were washed with brine (0.5 L), were dried over Na_2SO_4, and were concentrated to afford a residue that was purified by silica gel chromatography (MeOH:CH_2Cl_2, 1:99 then 5:95). The resulting residue was chromatographed a second time (20-80% acetone:hexane) to afford a mixture of diastereomers **5** and **6** as a yellow solid (130 g, 67%). The diastereomers were separated by preparative SFC: Lux Cellulose-3, 250 mm × 21.2 mm, 5 μ; 10% MeOH-CO_2 isocratic; 80 mL/min; UV 210 nM. Analytical SFC: Lux Cellulose-3, 250 mm × 4.6 mm, 5 μ; Gradient MeOH-CO_2, (time (min)/%CO_2): 0.0/95, 1.0/95, 9.0/40, 9.5/40, 10.0/95; 3.0 mL/min; UV 210 nM; compound **5** retention time 5.5 min, 98% d.e., 55.7 g, 29%; compound **6** retention time 5.0 min, 97% d.e., 54.0 g, 28%.

*((3R)-1-(8-(1-(4-Chloro-1H-pyrazol-1-yl)ethyl)-9H-purin-2-yl)piperidin-3-yl)(pyrrolidin-1-yl)methanone, single diastereomer of undetermined absolute configuration at methyl stereocenter (**5**).* A slurry of **5** (54 g, 126 mmol) was heated to 60 °C in iPrOH (180 mL) and was stirred at that temperature for 1 h, then the resulting slurry was cooled to ambient temperature and was stirred overnight. The solids were collected by filtration and were rinsed with cold iPrOH, then were dried overnight at 45 °C in a vacuum oven with nitrogen bleed, to afford **5** as a crystalline solid (46.6 g, 24%). mp 172-174 °C. ^{1}H NMR (400 MHz, DMSO-d_6) 12.78 (br s, 1H), 8.64 (s, 1H), 8.11 (s, 1H), 7.57 (s, 1H), 5.75 (q, J=7.0, 1H), 4.66 (app br d, J=13.1, 2H), 3.49-3.55 (m, 1H), 3.41-3.45 (m, 1H), 3.24-3.33 (m, 2H), 2.84-2.94 (m, 2H), 2.51 (m partially obscured by solvent, 1H), 1.86 (d, J=7.0, 3H), 1.62-1.94 (m, 7H), 1.38-1.48 (m, 1H). m/z 429.4 $(M+H)^+$. Chiral SFC purity, 99% d.e. HPLC purity, >99%.

*((3R)-1-(8-(1-(4-Chloro-1H-pyrazol-1-yl)ethyl)-9H-purin-2-yl)piperidin-3-yl)(pyrrolidin-1-yl)methanone, single diastereomer of undetermined absolute configuration at methyl stereocenter (**6**).* From a smaller scale reaction analogous to that described, an analytical sample of **6** was characterized. ^{1}H NMR (400 MHz, DMSO-d_6) 12.76 (br s, 1H), 8.64 (s, 1H), 8.12 (s, 1H), 7.58 (s, 1H), 5.75 (q, J = 7.0, 1H), 4.67 (app br d, J = 12.5, 2H), 3.40-3.55 (m, 2H), 3.28-3.33 (m, 2H), 2.84-2.95 (m, 2H), 2.51 (m partially obscured by solvent, 1H), 1.86 (d, J = 7.0, 3H), 1.62-1.94 (m, 7H), 1.39-1.49 (m, 1H). m/z 429.3 $(M+H)^+$. HPLC purity, >99%.

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