Bioorganic & Medicinal Chemistry 19 (2011) 6949–6965

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Design, synthesis and biological evaluation of 6-pyridylmethylaminopurines as CDK inhibitors

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ARTICLE INFO

Article history:
Received 27 May 2011
Revised 22 August 2011
Accepted 23 August 2011
Available online 31 August 2011

Keywords:
Cyclin
Kinase
Inhibitor
Purine
CDK

ABSTRACT

The cyclin-dependent kinase (CDK) inhibitor seliciclib (**1**, CYC202) is in phase II clinical development for the treatment of cancer. Here we describe the synthesis of novel purines with greater solubility, lower metabolic clearance, and enhanced potency versus CDKs. These compounds exhibit novel selectivity profiles versus CDK isoforms. Compound **αSβR-21** inhibits CDK2/cyclin E with IC_{50} = 30 nM, CDK7-cyclin H with IC_{50} = 1.3 μM, and CDK9-cyclinT with IC_{50} = 0.11 μM; it (CCT68127) inhibits growth of HCT116 colon cancer cells in vitro with GI_{50} = 0.7 μM; and shows antitumour activity when dosed p.o. at 50 mg/kg to mice bearing HCT116 solid human tumour xenografts.

1. Introduction

The cyclin dependent kinases (CDKs) form heterodimeric complexes with their cyclin partners, and these complexes play important roles in regulating cellular processes, especially in the progression of the cell cycle.[1] For example, CDK2-cyclin E and CDK4-cyclin D facilitate the G_1 to S phase transition by phosphorylating RB protein: CDK5 is involved in the regulation of glucose uptake[2]; whilst CDK7-cyclin H and CDK9-cyclin T regulate transcription by phosphorylating RNA polymerase II.[3] Regulation of the cell cycle is governed by the status of CDK-cyclin complexes, the levels of which depend upon the balance between protein synthesis and proteasomal degradation.[4] The enzymic activity of CDKs is modulated by their phosphorylation state[5] and affected by the presence of endogenous protein inhibitors. However, in cancer cells CDK-cyclin activity is often deregulated[6,7]; diverse mechanisms have been reported including upstream activation of signal transduction, overexpression of cyclins,[8] amplification of CDKs, and inactivation or loss of endogenous protein inhibitors, particularly p16.[9] These observations have fuelled considerable activity in the quest for CDK inhibitors as therapeutic agents for treatment of various cancers.[10,11]

The earliest CDK inhibitors based upon staurosporine[12] were active against a wide range of kinases and therefore had considerable potential for undesirable off-target effects. Meijer and colleagues discovered purines with selectivity for the CDK family,[13] especially for CDK2, while other groups pursued compounds with selectivity for CDK4 and CDK6.[14] Since these pioneering efforts diverse classes of CDK inhibitors have been described and reviewed[15] and additional reports have recently appeared, notably those describing purines,[16] purine isosteres,[17] and biaryl purines.[18,19] Observations that CDK2-knockout mice were viable, and that cellular inhibition of CDK2 by overexpression of p27 did not affect proliferation of colon cancer cells,[20,21] suggested that blockade of several CDKs may be necessary to halt aberrant cell cycle progression in cancer, but it is not yet clear what pharmacological profile would be ideal.[9,22–26] Several CDK inhibitors, including flavopiridol (Sanofi-Aventis), SNS032 (BMS) and Ro4584820 (Roche) (Fig. 1), and seliciclib (**1**, R-roscovitine, CYC202 Cyclacel), have entered clinical trials and the sparse published data have been reviewed.[27–32] Additional drug candidate structures have been reported more recently for AZD5438 (Astra Zeneca)[33] and AT519 (Astex).[34]

Seliciclib **1** is primarily an inhibitor of the CDK2–cyclin E complex. It is a less potent inhibitor of other CDK–cyclin complexes,[35] and a weak inhibitor of non-target kinases.[36] Seliciclib

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doi:10.1016/j.bmc.2011.08.051

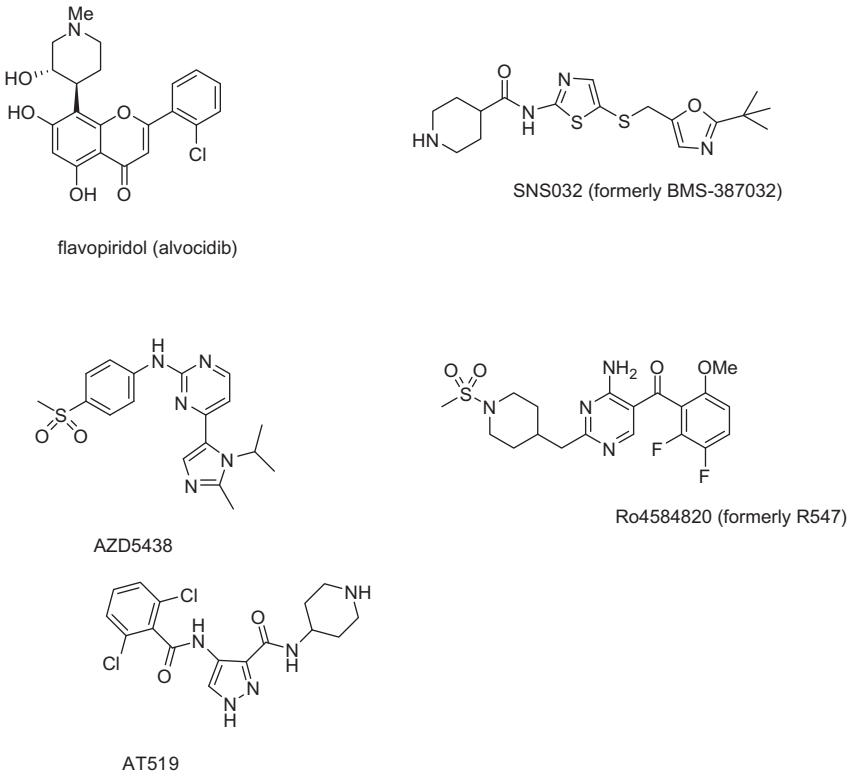
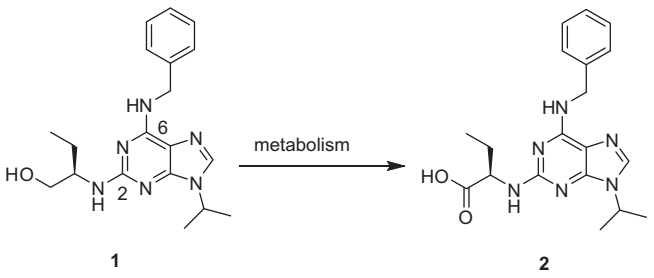


Figure 1. Examples of CDK inhibitors progressed to the clinic.

has antiproliferative activity on cancer cells coincident with loss of phospho RB[37] and cyclin D1,[38] and has antitumour activity in vivo.[39,40] It has moderate solubility but it is well absorbed when administered orally. The principal metabolite of seliciclib is the carboxylic acid **2** which is rapidly formed during oxidative first pass metabolism[40] (Scheme 1).

Carboxylic acid **2** has low potency as a CDK inhibitor (IC_{50} = 1.3 μM versus CDK2/cyclin E) and, with likely poor cell penetration, it is unlikely to contribute significantly to the observed efficacy in vivo of seliciclib (IC_{50} = 0.22 μM vs CDK2/cyclin E). A successor to seliciclib would ideally have greater metabolic stability, with enhanced potency and cellular activity such that in vivo drug levels exceed the GI_{50} for several hours after oral dosing, thereby acting on cancer cells at varying stages of the cell cycle. In efforts to achieve this profile we have prepared and characterised analogues, based upon the purine template, designed to have increased aqueous solubility (by introduction of a pyridine substituent), and to prevent side-chain oxidation to the level of carboxylic acid (by modification of the primary alcohol present in seliciclib).



Scheme 1. Metabolism of seliciclib.

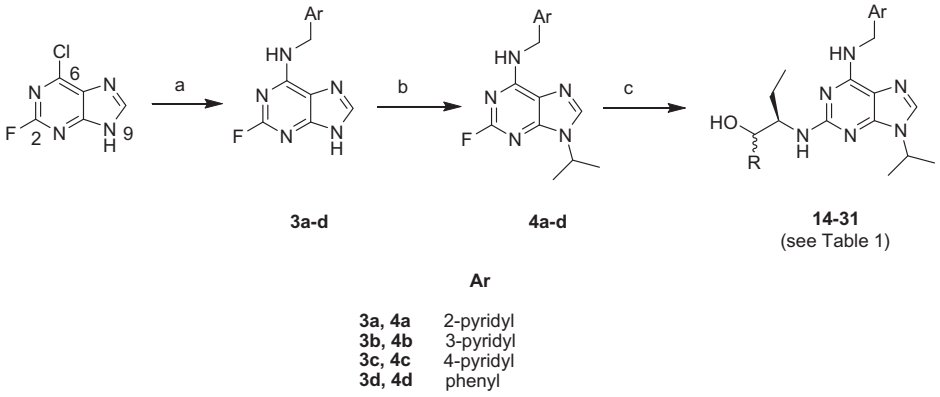
2. Chemistry

Tri-substituted purines were prepared from 2,6-dihalopurines by sequential amination at C-6, alkylation at N-9 and amination at C-2. Reaction of 6-chloro-2-fluoropurine with 2, 3 and 4-aminomethylpyridine in *n*-butanol in presence of Hunig's base at 25 °C afforded the isomeric 6-pyridylmethylamino intermediates **3a**–**c**. These compounds and the 6-benzylamino analogue **3d** (included as a control substituent since it is present in seliciclib) were treated with 2-bromopropane in DMA with K_2CO_3 as base, and the resultant 9-isopropyl derivatives **4a**–**d** were reacted with amino-alcohols **5**, **8e**–**h** and **11** to afford a set of purine analogues (Scheme 2); the product structures **14**–**31** are shown in detail in Table 1.

The (**αR**)-aminoalcohol reagents **8e**–**h** were prepared from (**αR**)-2-aminobutan-1-ol **5** by: (a) N-protection using tritylchloride in dichloromethane with Hunig's base; (b) Swern oxidation (DMSO/oxalyl chloride in dichloromethane) to aldehyde **6**; (c) reaction with methyl, iso-propyl or tert-butyl lithium in the presence of copper bromide/dimethylsulphide complex in ether, conditions reported[41] to favour the nonchelation-controlled alkyl addition; or reaction with neat ethyl magnesium bromide; (d) trityl deprotection using TFA (Scheme 3).

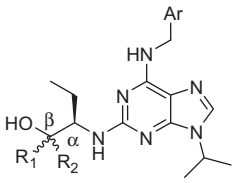
The reactions with ethyl, *i*-propyl and *t*-butyl Grignard reagents, were not significantly stereoselective and, afforded ca. 1:1 mixtures of diastereomers **7f**–**h** which were taken forward without separation. In contrast, methyl addition afforded 80% of the (**α-R**, **β-S**) isomer **7e**. The tertiary alcohol intermediate **11** was prepared by oxidation of **7e** to the methyl ketone **9** followed by reaction with methylmagnesium iodide in refluxing ether and subsequent deprotection (Scheme 4).

Aminoalcohols **8e**–**h** as mixtures of diastereomers, **5** and **11** were reacted with the foregoing 2-fluoropurines **4a**–**d** in DMSO/*n*-BuOH/Hunig's base at 140 °C for 72 h. The **α-R** diaminopurine products **14**–**31** are numbered as set out in Table 1.



Scheme 2. Reagents and conditions: (a) $ArCH_2NH_2$, $EtNPr_2^i$, *n*-BuOH, 0–25 °C; (b) 2-bromopropane, K_2CO_3, DMA, 25 °C; (c) aminoalcohols **5**, **8e**–**h**, **11**, $EtNPr_2^i$, *n*-BuOH/DMSO, 140 °C.

Table 1
Numbering scheme for **αR-**purine structures



αR-Series

R1	R2	Ar 2-Pyridyl	3-Pyridyl	4-Pyridyl	Phenyl
H	H	**αR-14**	**αR-20**	**αR-26**	**αR-1**
Me	H	**αR-15**[b]	**αR-21**[b]		**αR-27**[b]
Et	H	**αR-16**[a]	**αR-22**[a]		**αR-28**[a]
i-Pr	H	**αR-17**[a]	**αR-23**[a]		**αR-29**[a]
t-Bu	H	**αR-18**[a]	**αR-24**[a]		**αR-30**[a]
Me	Me	**αR-19**	**αR-25**		**αR-31**

[a] These compounds were prepared as ca. 1:1 mixtures of **β-R** and **β-S** diastereomers.
[b] These compounds were prepared as ca. 4:1 mixtures of **β-S**:**β-R** diastereomers.

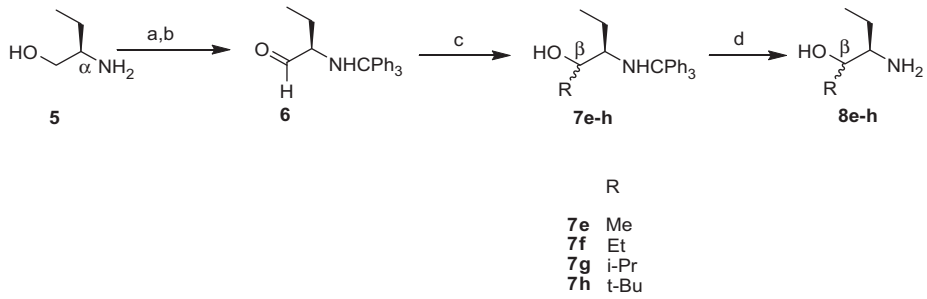
An identical series of synthetic transformations was carried out starting with the **α-S** enantiomer of butanol **5** to afford the complementary series of **α-S** aminopurines shown in Table 2.

Diastereomeric mixtures were not separated prior to initial screening for inhibition of CDK enzymes.

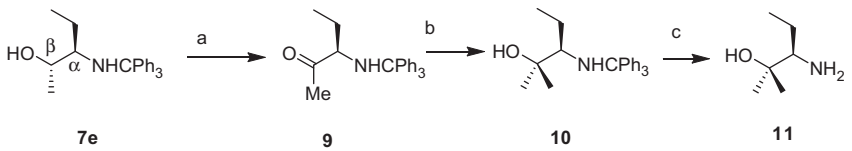
3. Results and discussion

To select the most promising compounds for further progression, the foregoing analogues were assayed for inhibition versus a panel of six kinases (Table 3); for effects on growth of a panel of 7 cancer cell lines (Table 4); and for rates of metabolic conversion by mouse liver microsomes (also Table 4). Table 3 reveals that all the compounds are potent inhibitors of both CDK2/cyclin E and CDK7/cyclin H, with scarcely any significant inhibition of PKA or ERK2.

The IC_{50}s for inhibition of CDK2/cyclin E by the 36 alcohols in Table 3 fall in the range 30 nM to 3.4 μM (cf. seliciclib = 0.22 μM). Consistently, regardless of aryl substituent or configuration at the alpha-carbon, the β-methyl-substituted secondary alcohols **15**, **21** and **27**; primary alcohols **1**, **14**, **20** and **26**; and tertiary alcohols **19**, **25** and **31** have similar activities versus CDK2/cyclin E and are the most potent analogues. The β-*iso*-propyl compounds **17**, **23** and **29** and β-*tert*-butyl compounds **18**, **24** and **30** are ca. 10-fold less potent; while the β-ethyl substituted compounds **16**, **22** and

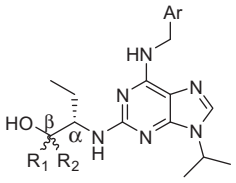


Scheme 3. Reagents and conditions: (a) trityl chloride, $EtNPr_2^i$, DCM, 25 °C: (b) $(COCl)_2$, DMSO, DCM, −45 °C; (c) RLi, CuBr·SMe_2, Et_2O, −70 °C; (d) TFA, DCM, 25 °C.



Scheme 4. Reagents and conditions: (a) $(COCl)_2$, DMSO, DCM, −45 °C; (b); (c) MeMgI, Et_2O, 25–45 °C; (d) TFA, DCM, 25 °C.

Table 2
Numbering scheme for **αS-**purine structures



αS series

R1	R2	Ar	2-Pyridyl	3-Pyridyl	Phenyl
Me	H		**αS-15**[b]	**αS-21**[b]	**αS-27**[b]
Et	H		**αS-16**[a]	**αS-22**[a]	**αS-28**[a]
i-Pr	H		**αS-17**[a]	**αS-23**[a]	**αS-29**[a]
t-Bu	H		**αS-18**[a]	**αS-24**[a]	**αS-30**[a]
Me	Me		**αS-19**	**αS-25**	**αS-31**

[a] These compounds were prepared as ca. 1:1 mixtures of **β-R** and **β-S** diastereomers.
[b] These compounds were prepared as ca. 4:1 mixtures of **β-R**:**β-S** diastereomers.

28 are of intermediate potency. Kinase inhibitory activity is consistently highest for the 3-pyridyl compounds **20**–**25** with 2-pyridyl **14**–**19**, phenyl **1**, **27**–**30** and the 4-pyridyl compound **26** all generally 2–4-fold less active. The X-ray structure of seliciclib (R-roscovitine) bound to CDK2 shows[42] that the hydroxyethylamine sidechain attached at C-2 of the purine template is buried within a cleft, but there is sufficient room to accommodate short substituents of either configuration at the alpha carbon. The observed ranking of substituent preferences is therefore consistent with the view that the current compounds interact with CDK2 in a similar manner to seliciclib. Furthermore, the benzyl substituent points out towards solvent, and the overlaid 3-pyridyl nitrogen appears close enough (3.9 Å in a simple replacement model) to Glutamate-8 for an energetically favourable interaction.

The compounds in Table 3 are on average 80-fold selective for CDK2–cyclin E versus CDK4–cyclinD1, and 60-fold selective versus CDK1 but they are potent inhibitors of CDK7-cyclin H with IC_{50} values ranging from 40 nM to 5.8 μM. Against this enzyme the SAR trends are less pronounced, but, the *i*-Pr and *t*-Bu substituted analogues are generally the least potent as was found for CDK2/cyclin E. Overall, compounds with the **αS** configuration are marginally more potent than those with the **αR** configuration of seliciclib.

Table 4 shows mean GI_{50} values (ranging from 1.0 to 43 μM, with 2 compounds >50 μM) for inhibition of human cancer cell growth; differences between the seven individual human cancer cell lines were small (Supplementary data). GI_{50} values show trends similar to those observed for CDK2 IC_{50} values, but were less sensitive to structural change. The analogues with best potency in both kinase and cellular assays are the secondary alcohols **15**, **21** and **27** having a methyl substituent at C-β, and the tertiary alcohols **19**, **25** and **21**. The most notable compounds are **αR-21**, **αS-21**, **αS-27** and **αS-25**.

In the microsomal stability assay, for both the **αR** and **αS** series, the 3-pyridyl compounds **20**–**25** and 4-pyridyl analogue **26** were significantly more stable than the 2-pyridyl isomers **15**–**19** while the phenyl compounds **1** and **27**–**31** had intermediate stability. Although the structures of the secondary and tertiary alcohols

Table 3
Kinase Inhibitory Activity and Kinase Selectivity Profile of Arylmethylaminopurines

Compound	Kinase inhibitory activity IC_{50}[a] (μM)					
	CDK1–cyclin B	CDK2–cyclin E	CDK4–cyclin D1	CDK7–cyclin H	PKA	ERK-2
αR-1	17	0.22	27	0.52	>50	>25
αR-14	3.9	0.06	3.8	0.04	>50	9.5
αR-15	39	0.52	48	0.55	>200	73
αR-16	35	0.77	20	1.2	>200	230
αR-17	84	3.4	32	3.9	>200	>200
αR-18	200	2.8	38	2.4	>200	>200
αR-19	44	0.48	18	4.2	>200	>200
αR-20	7.4	0.17	20	0.18	>50	24
αR-21	4.9	0.09	14	0.5	>200	23
αR-22	12	0.22	25	1.1	>200	37
αR-23	20	0.69	21	2.2	>200	111
αR-24	23	1.0	24	1.6	>200	51
αR-25	6.2	0.2	7.5	3.1	>200	91
αR-26	28	0.48	30	0.74	>50	32
αR-27	18	0.23	23	0.39	>50	43
αR-28	22	0.73	34	1.2	139	103
αR-29	28	2.4	28	4.4	92	>200
αR-30	30	3.1	200	3.7	87	>200
αR-31	17	0.38	12	3.7	191	149
αS-15	8.9	0.05	18	2.6	>200	77
αS-16	40	0.42	24	2.6	>200	80
αS-17	200	3.1	49	3.3	>200	>200
αS-18	200	1.0	22	5.8	>200	137
αS-19	24	0.31	20	4.3	>200	>200
αS-21	2.2	0.03	6.8	1.3	>200	20
αS-22	13	0.11	11	1.5	>200	17
αS-23	22	1.1	31	3.5	>200	67
αS-24	21	0.39	13	3.5	>200	23
αS-25	4.4	0.07	12	2.5	>200	35
αS-27	9.3	0.03	9	0.69	>50	29
αS-28	19	0.24	20	1.1	>200	22
αS-29	34	1.8	33	3	83	88
αS-30	45	1.5	60	4.3	>200	>200
αS-31	9.1	0.17	8.5	2	200	52

[a] IC_{50} values are means from three determinations. Standard deviations were <25% of the mean.

Table 4
Anti proliferative activity and metabolic stability of arylmethylaminopurines

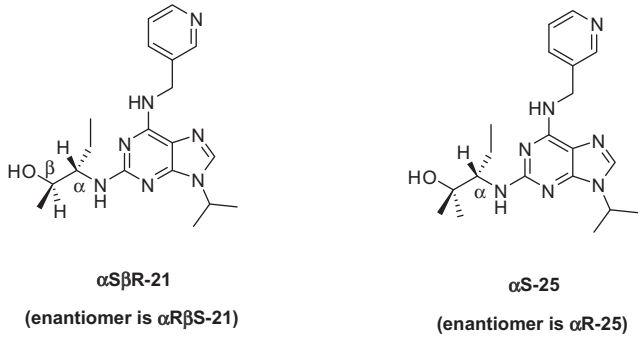
Compound	Mean cellular GI_{50}[a,c] (μM)	% Metabolised[b,c]
αR-1	7.4	67
αR-14	31.0	ND
αR-15	25.5	97
αR-16	34.0	88
αR-17	50.0	94
αR-18	34.6	93
αR-19	>50	73
αR-20	7.0	54
αR-21	4.2	10
αR-22	14.5	30
αR-23	29.0	36
αR-24	21.1	46
αR-25	16.7	16
αR-26	17.3	3
αR-27	6.9	91
αR-28	21.2	63
αR-29	20.3	83
αR-30	19.9	79
αR-31	20.6	52
αS-15	16.6	67
αS-16	33.0	85
αS-17	50.0	23
αS-18	42.7	97
αS-19	36	53
αS-21	1.0	33
αS-22	9.0	27
αS-23	22.6	62
αS-24	20.9	50
αS-25	5.2	22
αS-27	2.6	67
αS-28	21.1	67
αS-29	23.0	76
αS-30	22.8	74
αS-31	19.4	73

*R-1 is seliciclib.
[a] Values are averages of concentrations required to inhibit growth of seven different cancer cell lines. Full data are provided in Supplementary data.
[b] Values are % of compound consumed after 30 min incubation with mouse liver microsomes.
[c] Determinations were performed in triplicate and variations were <25% of the mean.

preclude oxidation of the side chain to carboxylic acid (as observed for seliciclib) other modes of metabolism remain possible, and improvement in overall metabolic stability is generally modest. However, consideration of the combined data on enzyme inhibition, growth proliferation, and metabolism, allows the compounds to be ranked for their potential to be further optimised towards a drug candidate.

Based on the foregoing data, the secondary alcohols **αR-21** and **αS-21** and the tertiary dimethylcarbinols **αR-25** and **αS-25** (Scheme 5) were selected for additional biochemical assays, PK profiling and efficacy studies in vivo. Whereas initial screening of the combinatorial set of purines had been conducted with mixtures of epimers at the β-stereocentre, the major diastereomers **αRβS-21** and **αSβR-21** were now isolated and it was confirmed that these isomers are primarily responsible for the observed biological activity. The minor **αSβS** and **αRβR** compounds are significantly less potent as inhibitors of CDK2 (data not shown).

Additional enzyme assays revealed that the four compounds **αRβS-21**, **αSβR-21**, **αR-25** and **αS-25** have significant inhibitory potency versus CDK9/cyclin T1, with IC_{50}s of 0.26, 0.11, 0.21 and 0.16 μM, respectively. The compounds were sufficiently soluble to be administered by intravenous bolus to mice at >50 mg/kg, and plasma drug levels were determined at varying time intervals. Seliciclib **1** was included as a positive control in this study. Table 5 shows that initial plasma clearance of the four new compounds is reduced by 2–5-fold relative to seliciclib. Administration at the same doses via the oral route gave oral bioavailability values for



Scheme 5. Compounds selected for in vivo evaluation.

the novel pyridylmethyl compounds in the range 15–46%, consistent with low to moderate in vivo clearance.

The four pyridylmethyl compounds **αRβS-21**, **αSβR-21**, **αR-25**, **αS-25** and seliciclib **1** were dosed orally twice daily at 12–14 h intervals at 75, 50, 100, 100 and 200 mg/kg, respectively, to athymic mice bearing established solid tumours derived from HCT116 human colon carcinoma cells. These doses were well tolerated and the greatest efficacy was observed for compound **αSβR-21** which effected the greatest growth delay and a 49% reduction in tumour weight compared to the vehicle control after 12 days of dosing (Figs. 2 and 3). This outcome is consistent with the PK data (Table 5) which show this compound to have the lowest clearance and longest half-life of the five compounds tested.

Having identified **αSβR-21** as a prime candidate for further evaluation, an improved synthetic route was developed (Scheme 6) and solubility studies were conducted on a series of salts. When the trityl protection of aminoaldehyde **6** was replaced by *N*,*N*-dibenzyl, the addition of methyl lithium to aldehyde **35** proceeded with very high stereoselectivity to produce the protected **αSβR** diastereomer **36**. Deprotection to **37** was achieved by catalytic hydrogenolysis and the resulting amine was reacted with purine **4b** to afford **αSβR-21** (CCT68127). The X-ray crystal structure (Fig. 4) of this compound confirmed the stereochemical assignment.

From **αSβR-21** 1:1 salts were prepared with HCl, $MeSO_3H$, TFA, (+) and (−) tartaric acid. The TFA salt is the most highly crystalline of these and its solubility in water exceeds 25 mg/ml to afford a solution of pH 3.

αSβR-21 (CCT68127) induced loss of RB phosphorylation at Ser 780 as shown by Western blots (Fig. 5A) and also decreased expression of RNA polymerase 2 (data not shown). The latter effect may be due to inhibition of CDK7 (IC_{50} = 1.30 μm) and/or CDK9 (IC_{50} = 107 nM), and combinatorial inhibition of CDK isoforms may contribute significantly to the antitumour activity of this set of compounds. Cell cycle analysis of HT29 human colon cancer cells, using flow cytometry to determine the distribution of bromodeoxyuridine incorporation, showed that **αSβR-21** causes a dramatic reduction in the proportion of cells synthesising DNA in S-phase, with a corresponding increase in cells held at G2/M phase during the first 16 h drug treatment (Fig. 5B). The foregoing effects are closely similar to those observed by treating the same cells with a ca. 15-fold higher concentration of seliciclib.[43]

In summary we have identified a water soluble purine CDK inhibitor **αSβR-21** (CCT68127) with improved potency versus CDK2/cyclin E (IC_{50} = 30 nM), good potency versus CDK9 (IC_{50} = 107 nM), enhanced anti-proliferative activity against cancer cells (mean GI_{50} = 700 nM), increased stability towards mouse microsomes (67% remaining at 30 min), promising in vivo PK properties and efficacy in vivo following oral administration. Further biological profiling of this compound involving cDNA gene expression microarrays and other mechanistic studies in cancer cells will be described elsewhere.

Table 5
PK parameters for 5 purines after iv administration to mice

Compound*	C_{max} (μmol/L)	Area under curve (h μmol/L)	Half-life (h)	Volume of distribution (L)	Clearance (L/h)
αR-25	275.4	223.3	0.6	0.010	0.012
αS-25	214.7	109.4	0.4	0.012	0.024
1	93.7	53.4	1.4	0.073	0.051
αRβS-21	377.2	158.0	1.7	0.084	0.017
αSβR-21	268.0	298.0	2.8	0.013	0.009

* All compounds were administered at doses of 50 mg/kg at concentration of 50 mM in aqueous HCl.

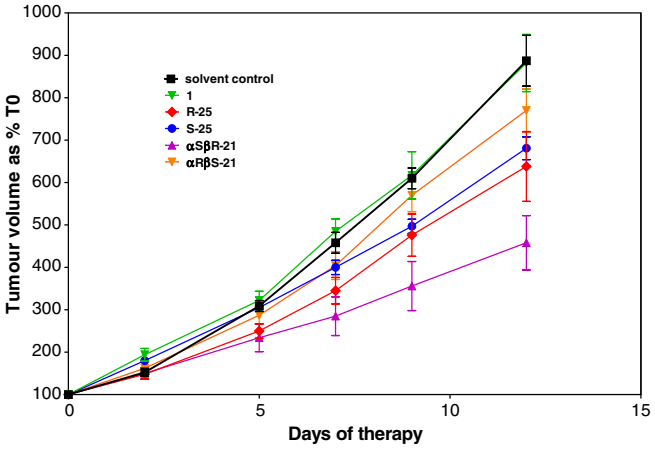


Figure 2. Response of established subcutaneous HCT116 human colon carcinoma xenografts in nude mice to therapy by arylmethylaminopurines: relative tumour volumes (expressed as % tumour volumes at start of therapy).

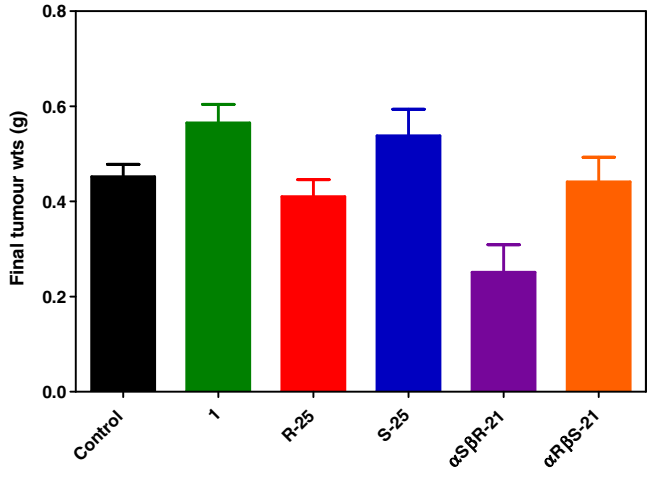


Figure 3. Response of HCT116 human colon carcinoma xenografts in nude mice to therapy by arylmethylaminopurines: final tumour weights after 12 days dosing.

4. Experimental

4.1. General

Chemicals and solvents (including anhydrous solvents) were purchased from commercial sources and were used as received unless otherwise stated. Microwave heated reactions were carried out in a CEM Discover® instrument. Reactions were followed by thin layer chromatography (TLC) using pre-coated aluminium sheets of silica 60 F_{254} (Merck Art. 5735), or by LC–MS using a Waters/Micromass LCT Time of Flight mass analyser with UV-Vis detector and Alliance 2795 LC. Flash column chromatography was carried out using BDH 'silica gel for flash chromatography' (purchased from VWR International Ltd) NMR spectra were recorded on Bruker Avance 500 MHz instruments or formerly on a Bruker 250 MHz instrument. Experimental details are reported in full for the **αR**- series of compounds, and additionally for the preparation of **αSβR-21** (CCT68127). Compounds in the enantiomeric series were prepared in an identical manner, and all spectral data were identical to those observed for their enantiomeric counterparts.

4.2. General procedure for amination at C6 of purines

4.2.1. 6-Benzylamino-2-fluoropurine (3d)

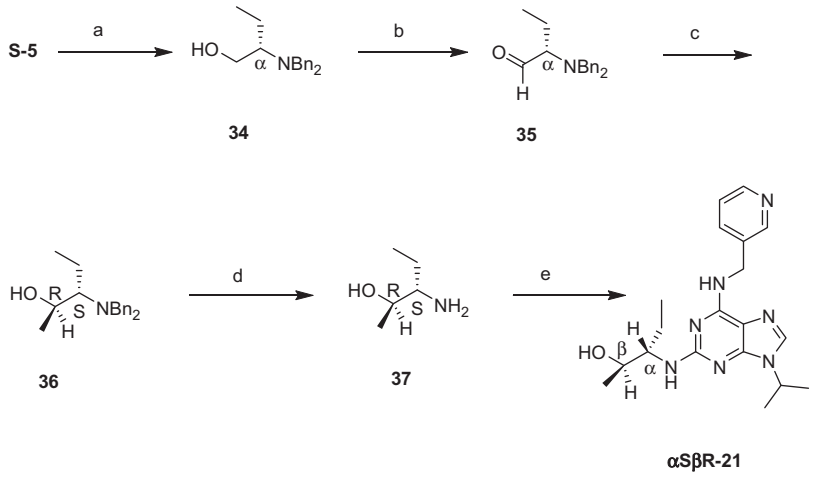
To a stirred solution of 6-chloro-2-fluoropurine[1] (1.5 g, 1 equiv, 8.69 mmol) in *n*-BuOH (100 mL) under an argon atmosphere, cooled to 0 °C, was added DIEA (4.0 mL, 2.64 equiv, 22.96 mmol) followed by benzylamine (1.15 mL, 1.21 equiv, 10.53 mmol). The reaction mixture was stirred at 0 °C for 3 h, and then allowed to return to room temperature over 30 min. and stirred at this temperature for 16 h, when TLC (DCM/ether/MeOH; 55:43:2) indicated that the reaction had gone to completion. The solvent was evaporated in vacuo and the residue purified by gradient column chromatography on silica gel, eluted with DCM/ether/MeOH (55:45:0→55:43:2), to afford 6-benzylamino-2-fluoropurine (**3d**) as a white solid; yield: 1.36 g (64%). Mp 240–241 °C. ^{1}H NMR (DMSO-d_6, 250 MHz): δ 4.62 (d, 2H, J = 5.60 Hz, –HNCH_2-Bz), 7.25–7.33 (m, 5H, Bz), 8.10 (s, 1H, –N=*CH*–NH–), 8.81 (br s, 1H, –*HN*CH_2-Bz), 13.06 (br s, 1H, –N=CH–*NH*–). FABMS *m/z* (relative intensity): 244 ([M+H]$^+$, 100), 180 (15), 166 (9), 136 (5), 91 (10). Accurate mass (M+H): actual: 244.0998, measured: 244.1002. Microanalysis (expected: measured) $C_{12}H_{10}N_5F$: C; 59.25: 59.12, H; 4.14: 4.06, N; 28.79: 28.47.

4.2.2. 2-Fluoro-6-[(pyridin-2-ylmethyl)-amino]purine (3a)

Prepared as described above but using 2-(aminomethyl)pyridine; reaction monitoring by TLC ($CHCl_3$/MeOH; 90:10) and purification by gradient column chromatography on silica gel, eluting with $CHCl_3$/MeOH (95:5→85:15), to afford 2-fluoro-6-[(pyridin-2-ylmethyl)-amino]purine (**3a**) as a light yellow solid; yield: 0.40 g (71%). Mp 217–220 °C. ^{1}H NMR (DMSO-d_6, 250 MHz): δ 4.58 (d, 2H, J = 5.68 Hz, –HNCH_2-Pyr), 7.29, 7.71, 8.49 (3 × m, 4H, Pyr), 8.10 (s, 1H, –N=*CH*–NH–), 8.69 (br s, 1H, –*HN*CH_2-Pyr), 13.07 (br s, 1H, –N=CH–*NH*–). FABMS *m/z* (relative intensity): 245 ([M+H]$^+$, 55), 176 (30), 154 (100), 136 (85). Accurate mass (M+H): Actual: 245.0951, measured: 245.0942. Microanalysis (expected: measured) $C_{11}H_9N_6F$ .0.4H_2O: C; 52.55: 52.91, H; 3.93: 3.49, N; 33.42: 33.26.

4.2.3. 2-Fluoro-6-[(pyridin-3-ylmethyl)-amino]purine (3b)

Prepared as described above but using 3-(aminomethyl)pyridine and conducting the reaction at −20 °C rather than 0 °C; and purification by gradient column chromatography on silica gel, eluting with $CHCl_3$/MeOH (97:3→90:10) to afford 2-fluoro-6-[(pyridin-3-ylmethyl)-amino]purine (**3b**) as a white solid; yield: 1.08 g (85%). Mp 218–220 °C. ^{1}H NMR (DMSO-d_6, 250 MHz): δ 4.65 (d,



Scheme 6. Reagents and conditions: (a) benzyl chloride, $EtNPr^i_2$, DCM, 25 °C: (b) $(COCl)_2$, DMSO, DCM, −45 °C; (c) MeLi, CuBr·SMe_2, Et_2O, −70 °C; (d) H_2/Pd on carbon, (e) purine **4b**, $EtNPr^i_2$, *n*-BuOH/DMSO, 140 °C.

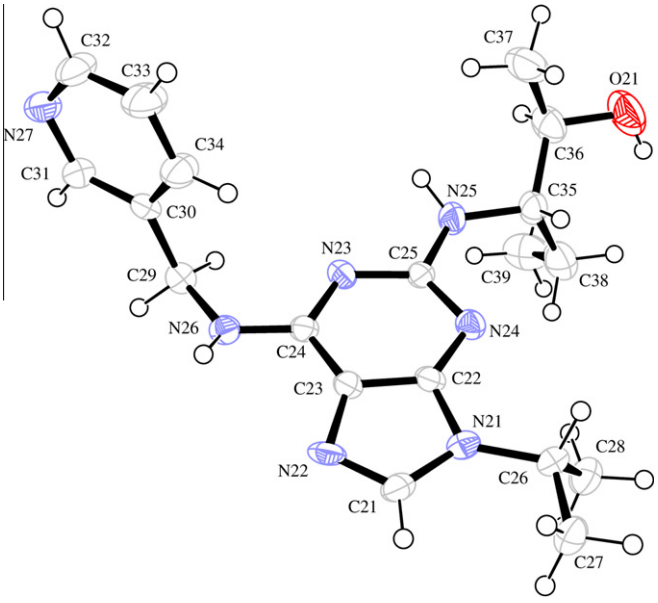


Figure 4. Structure of **αSβR-21** (CCT68127) derived from X-ray crystallography.

2H, *J* = 5.37 Hz, –HNC*H*$_2$-Pyr), 7.34, 8.43, 8.57 (3 × m, 4H, Pyr), 8.10 (s, 1H, –N=*CH*–NH–), 8.83 (br s, 1H, –*HN*CH$_2$-Pyr), 13.07 (br s, 1H, –N=CH–*NH*–). FABMS *m/z* (relative intensity): 245 ([M+H]$^+$, 40), 176 (27), 154 (100), 136 (74). Accurate mass (M+H): actual: 245.0951, measured: 245.0942. Microanalysis (expected: measured) $C_{11}H_9N_6F$ .0.2H_2O: C; 53.31: 54.03, H; 3.82: 3.83, N; 33.91: 32.74.

4.2.4. 2-Fluoro-6-[(pyridin-4-ylmethyl)-amino]purine (3c)

Prepared as described above but using 4-(aminomethyl)pyridine and conducting the reaction at −20 °C rather than 0 °C; and purification by gradient column chromatography on silica gel, eluting with $CHCl_3$/MeOH (100:0→90:10) to afford 2-fluoro-6-[(pyridin-4-ylmethyl)-amino]purine (**3c**) as a white solid; yield: 0.69 g (54%). Mp >340 °C. ^{1}H NMR (DMSO-d_6, 250 MHz): δ 4.67 (d, 2H, *J* = 6.35 Hz, –HNC*H*$_2$-Pyr), 7.34, 8.50 (2 × m, 4H, Pyr), 8.14 (s, 1H, –N=*CH*–NH–), 8.84 (br s, 1H, –*HN*CH$_2$-Pyr), 13.13 (br s, 1H, –N=CH–*NH*–). FABMS *m/z* (relative intensity): 245 ([M+H]$^+$, 27), 176 (15), 154 (100), 136 (77). Accurate mass (M+H): actual: 245.0951, measured: 245.0942. Microanalysis (expected: measured) $C_{11}H_9N_6F$ .0.6H_2O: C; 51.80: 52.08, H; 4.03: 3.84, N; 32.95: 31.29.

4.3. General procedure for alkylation at purine N9

4.3.1. 6-Benzylamino-2-fluoro-9-isopropylpurine (4d)

To a stirred solution of 6-benzylamino-2-fluoropurine (**3d**, 0.83 g, 1 equiv, 3.41 mmol) in DMA (10 mL) at room temperature

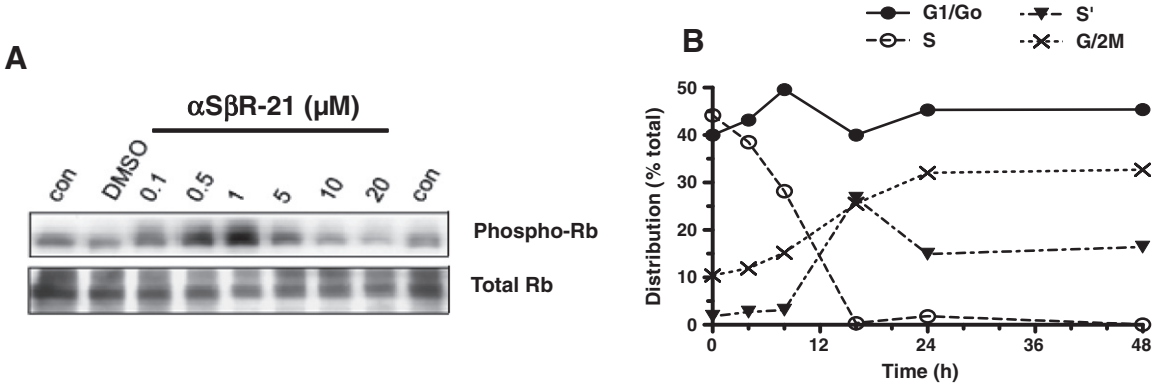


Figure 5. (A) Western blots showing the effect on protein levels of incubating HT29 human colon cancer cells with purine **αSβR-21** (CCT68127) for 24 h at concentrations ranging from 0.1 to 20 μM. Con and DMSO are drug-free controls. (B) Effect on cell cycle status of treating HT29 cells for 48 h with **αSβR-21** (CCT68127) (3.3 μM).

under an argon atmosphere, was added powdered, anhydrous K_2CO_3 (2.35 g, 5 equiv, 17.00 mmol), followed by 2-bromopropane (3.2 mL, 10 equiv, 34.08 mmol). The reaction mixture stirred at room temperature for 48 h, when DCM/ether/MeOH (55:40:5), indicated that the reaction had gone to completion. The solvent was evaporated in vacuo and the residue partitioned between EtOAc (100 mL) and water (200 mL). The aqueous phase was extracted with more EtOAc (2 × 50 mL) and the combined organic phase washed with brine (50 mL), dried ($MgSO_4$) and evaporated in vacuo. The residue was purified by silica gel column chromatography, eluted with $CHCl_3$ to afford 6-benzylamino-2-fluoro-9-isopropylpurine (**4d**) as a white solid; yield: 0.78 g (80%). Mp 158–160 °C. ^{1}H NMR (DMSO-d_6, 250 MHz): δ 1.49 (2 × s, 6H, CH(CH_3)$_2$), 4.64 (m, 3H, –CH(CH_3)$_2$ + –HNCH_2-Bz), 7.26 (m, 5H, Bz), 8.25 (s, 1H, –N=CH–N–), 8.90 (br s, 1H, –$HN$$CH_2$-Bz). FABMS *m/z* (relative intensity): 286 ([M+H]$^+$, 100), 242 (17), 176 (22), 154 (65), 136 (60). Accurate mass (M+H): actual: 286.1468, measured: 286.1462. Microanalysis (expected: measured) $C_{15}H_{16}N_5F$: C; 63.14: 63.08, H; 5.65: 5.58, N; 24.54: 24.46.

4.3.2. 2-Fluoro-9-isopropyl-6-[(pyridin-2-ylmethyl)-amino] purine (4a)

Prepared as in the foregoing example but using 2-fluoro-6-[(pyridin-2-ylmethyl)-amino]purine (**3a**), monitored by TLC ($CHCl_3$/MeOH; 90:10) and purified by gradient column chromatography on silica gel, eluted with $CHCl_3$/MeOH (100:0→95:5), to afford 2-fluoro-9-isopropyl-6-[(pyridin-2-ylmethyl)-amino]purine (**4a**) as a white solid; yield: 0.27 g (58%). Mp 150–152 °C. ^{1}H NMR (DMSO-d_6, 250 MHz): δ 1.49 (2 × s, 6H, CH(CH_3)$_2$), 4.63 (m, 1H, –CH(CH_3)$_2$), 4.71 (d, 2H, J = 5.76 Hz, –HNCH_2–Pyr), 7.26, 7.71, 8.49 (3 × m, 4H, Pyr), 8.26 (s, 1H, –N=CH–N–), 8.78 (br s, 1H, –$HN$$CH_2$-Pyr). FABMS *m/z* (relative intensity): 287 ([M+H]$^+$, 100), 245 (10), 154 (22), 136 (17). Accurate mass (M+H): actual: 287.1420, measured: 287.1412. Microanalysis (expected: measured) $C_{14}H_{15}N_6F$: C; 58.73: 58.38, H; 5.28: 5.13, N; 29.35: 29.36.

4.3.3. 2-Fluoro-9-isopropyl-6-[(pyridin-3-ylmethyl)-amino] purine (4b)

Prepared as in the foregoing example but using 2-fluoro-6-[(pyridin-3-ylmethyl)-amino]purine (**3b**), purified by gradient column chromatography on silica gel, eluted with $CHCl_3$/MeOH (100:0→95:5), to afford 2-fluoro-9-isopropyl-6-[(pyridin-3-ylmethyl)-amino]purine (**3b**) as a white solid; yield: 0.73 g (62%). Mp 131–133 °C. ^{1}H NMR (DMSO-d_6, 250 MHz): δ 1.49 (2 × s, 6H, –CH(CH_3)$_2$), 4.63 (m, 3H, –CH(CH_3)$_2$ + –HNCH_2-Pyr), 7.34, 7.24, 8.44, 8.58 (4 × m, 4H, Pyr), 8.26 (s, 1H, –N=CH–N–), 8.95 (br s, 1H, –$HN$$CH_2$-Pyr). FABMS *m/z* (relative intensity): 287 ([M+H]$^+$, 100), 245 (8), 154 (23), 136 (18). Accurate mass (M+H): actual: 287.1420, measured: 287.1412. Microanalysis (expected: measured) $C_{14}H_{15}N_6F$: C; 58.73: 58.57, H; 5.28: 5.21, N; 29.35: 29.27.

4.3.4. 2-Fluoro-9-isopropyl-6-[(pyridin-4-ylmethyl)-amino] purine (4c)

Prepared as in the foregoing example but using 2-fluoro-6-[(pyridin-2-ylmethyl)-amino]purine (**3c**), purified by gradient column chromatography on silica gel, eluted with $CHCl_3$/MeOH (100:0→95:5), to afford 2-fluoro-9-isopropyl-6-[(pyridin-4-ylmethyl)-amino]purine (**4c**) as a white solid; yield: 0.40 g (57%). Mp 270–273 °C. ^{1}H NMR (DMSO-d_6, 250 MHz): δ 1.49 (2 × s, 6H, –CH(CH_3)$_2$), 4.63 (m, 3H, –CH(CH_3)$_2$ + –HNCH_2-Pyr), 7.30, 8.47 (2 × m, 4H, Pyr), 8.28 (s, 1H, –N=CH–N–), 8.97 (br s, 1H, –$HN$$CH_2$-Pyr). FABMS *m/z* (relative intensity): 287 ([M+H]$^+$, 100), 245 (8), 154 (23), 136 (18). Accurate mass (M+H): actual: 287.1420, measured: 287.1412. Microanalysis (expected: measured) $C_{14}H_{15}N_6F$: C; 58.73: 58.57, H; 5.28: 5.21, N; 29.35: 29.27.

4.3.5. (*R*)-2-(Trityl-amino)-butan-1-ol

To a stirred solution of (*R*)-(−)-2-aminobutan-1-ol (**(*R*)-5**, 10 g, 1 equiv, 112.18 mmol) in DCM (500 mL) under an argon atmosphere at room temperature, was added DIEA (30 mL, 1.54 equiv, 172.22 mmol) followed by trityl chloride (35.4 mL, 1.13 equiv, 126.98 mmol). The reaction mixture was stirred at room temperature for 48 h, when TLC (hexane/ether/MeOH; 55:40:5) indicated that the reaction had gone to completion. The solvent was evaporated in vacuo and the residue precipitated from acetone (50 mL) with hexane (900 mL) with stirring, the precipitate was removed by filtration and the filtrate was evaporated in vacuo. The residue was dissolved in hexane (1 L), filtered, and the filtrate was evaporated in vacuo to afford (***R***)-2-(trityl-amino)-butan-1-ol as a light yellow oil; yield: 32 g (86%). ^{1}H NMR (DMSO-d_6, 250 MHz): δ 0.56 (t, 3H, J = 7.41 Hz, –NHCH(CH_2CH_3)CH_2OH), 1.10 (m, 2H, –NHCH(CH_2CH_3)CH_2OH), 2.22 (m, 1H, –NHCH(CH_2CH_3)CH_2OH), 2.38 (m, 1H, –NHCH(CH_2CH_3)CH_2OH), 2.72 + 3.00 (2 × m, 2H, –NHCH(CH_2CH_3)CH_2OH), 4.28 (t, 1H, J = 5.26 Hz, –NHCH(CH_2CH_3)CH_2OH), 7.14–7.49 (m, 15H, 3 × Bz).

4.3.6. (*R*)-2-(Trityl-amino)-butyraldehyde (R-6)

To a stirred solution of DMSO (3.0 mL, 2.8 equiv, 42.28 mmol) in DCM (30 mL) under an argon atmosphere at −45 °C, was added oxalyl chloride (2 M in DCM, 10.56 mL, 1.40 equiv, 21.12 mmol), dropwise. The reaction mixture was stirred at −45 °C for 1 h, after which time a solution of (*R*)-2-(trityl-amino)-butan-1-ol (5 g, 1 equiv, 15.08 mmol) in DCM (30 mL) was added dropwise with stirring. The reaction mixture was stirred at this temperature for 3 h, when TLC (hexane/ether; 80:20) indicated that the reaction had gone to completion. To the reaction mixture was added a solution of TEA (10.5 mL, 5 equiv, 75.33 mmol) in DCM (30 mL), and the solution allowed to warm to room temperature over 16 h. The reaction mixture was diluted with more DCM (200 mL) and washed with water (250 mL). The aqueous phase was extracted with DCM (3 × 50 mL), and the combined organic phase washed with brine (50 mL), dried ($MgSO_4$) and evaporated in vacuo. The residue was dissolved in ether (30 mL), the solid precipitate removed by filtration and the filtrate was evaporated in vacuo. The residue was dissolved in hexane (50 mL), the solid precipitate removed by filtration and the filtrate was evaporated in vacuo to afford (*R*)-2-(trityl-amino)-butyraldehyde (**R-6**) as a light yellow oil; yield: 2.59 g (52%). ^{1}H NMR (DMSO-d_6, 250 MHz): δ 0.77 (t, 3H, J = 7.42 Hz, –NHCH(CH_2CH_3)CHO), 1.34–1.61 (m, 2H, –NHCH(CH_2CH_3)CHO), 2.92 (m, 1H, –NHCH(CH_2CH_3)CHO), 3.62 (d, 1H, J = 8.21 Hz, –NHCH(CH_2CH_3)CHO), 7.16–7.46 (m, 15H, 3 × Bz), 8.77 (d, 1H, J = 3.00 Hz, –NHCH(CH_2CH_3)CHO). HMRS: $C_{23}H_{23}NO$ requires (M+H) 330.1852; found *m/z* 330.1861.

4.3.7. (2*RS*,3*R*)-3-(Trityl-amino)-pentan-2-ol (R-7e)

To a stirred suspension of CuBr.SMe_2 (2.74 g, 2.2 equiv, 13.33 mmol) in ether (100 mL) under an argon atmosphere at −70 °C, was added methyllithium (1.6 M in ether, 16.6 mL, 4.4 equiv, 26.56 mmol) dropwise, and the solution allowed to warm to room temperature. The mixture was recooled to −70 °C, to which was added a solution of (*R*)-2-(trityl-amino)-butyraldehyde (**R-6**), 2 g, 1 equiv, 6.05 mmol) in ether (25 mL) dropwise with stirring. The reaction mixture was stirred at this temperature for 2 h, when TLC (hexane/ether; 80:20) indicated that the reaction had gone to completion. To the reaction mixture was added a saturated aqueous solution of NH_4Cl (100 mL) and allowed to warm to room temperature over 16 h. The reaction mixture was extracted with ether (2 × 200 mL), and the combined organic phase washed with brine (50 mL), dried ($MgSO_4$) and evaporated in vacuo. The residue was purified by silica gel column chromatography, eluted with hexane/ether (80:20) to afford (3*R*)-3-(trityl-amino)-pentan-2-ol (**R-7e**) as a light yellow oil; yield: 1.91 g (91%) (80% 2*S*,3*R*:

20% 2*R*,3*R*). ^{1}H NMR (DMSO-d_6, 250 MHz): δ 0.47 + 0.55 (2 × t, *J* = 7.43 + 7.27 Hz, –NHCH(CH_2CH_3)CH(CH_3)OH), 0.99–1.12 (m, 5H, –NHCH(CH_2CH_3)CH(CH_3)OH), 2.03 (m, 1H, –NH*CH*(CH_2CH_3)CH(CH_3)OH), 3.32–3.51 (m, 1H, –NHCH(CH_2CH_3)*CH*(CH_3)OH), 4.40 (d, 1H, *J* = 3.79 Hz, –*NH*CH(CH_2CH_3)CH(CH_3)OH), 7.14–7.51 (m, 15H, 3 × Bz). HMRS: $C_{24}H_{27}NO$ requires (M+H) 346.2165; found *m*/*z* 346.2161.

4.3.8. (2R*S*,3*R*)-3-Amino-pentan-2-ol (R-8e)

To a stirred solution of (2R*S*,3*R*)-3-(trityl-amino)-pentan-2-ol ((**R-7e**), 1.32 g, 1 equiv, 3.83 mmol) in DCM (50 mL) under an argon atmosphere at room temperature, was added trifluoroacetic acid (10 mL) dropwise, and the solution was stirred at this temperature for 1 h. The solvent was evaporated in vacuo and the residue was precipitated from ether (15 mL) with hexane (300 mL) with stirring to give a yellow oil. The solvent was decanted from the oil, and the oil was washed with hexane (30 mL) and dried in vacuo to afford (3*R*)-3-amino-pentan-2-ol (**R-8e**) as a light yellow oil; yield: 0.30 g (99%) (80% 2*S*,3*R*: 20% 2*R*,3*R*). ^{1}H NMR (DMSO-d_6, 250 MHz): δ 0.915 + 0.924 (2 × t, 3H, *J* = 7.50 + 7.58 Hz, NH_2CH(CH_2CH_3)CH(CH_3)OH), 1.06 + 1.13 (2 × d, *J* = 6.48 + 6.32 Hz), NH_2CH(CH_2CH_3)CH(*CH*$_3$)OH), 1.41–1.59 (m, 2H, NH_2CH(*CH*$_2$CH$_3$)CH(CH_3)OH), 2.77 + 2.93 (2 × m, 1H, NH_2*CH*(CH_2CH_3)CH(CH_3)OH), 3.62–3.72 + 3.80–3.90 (2 × m, 1H, NH_2CH(CH_2CH_3)*CH*(CH_3)OH), 7.75 (br s, 2H, *NH*$_2$). HMRS: $C_5H_{13}NO$ requires (M+H) 104.1070; found *m*/*z* 104.1071.

4.3.9. (3*R*)-3-Amino-2-methyl-pentan-2-ol (R-11)

To a stirred solution of DMSO (2.19 mL, 2.8 equiv, 30.86 mmol) in DCM (30 mL) under an argon atmosphere at −45 °C, was added oxalyl chloride (2 M in DCM, 7.69 mL, 1.4 equiv, 15.38 mmol) dropwise. The reaction mixture was stirred at −45 °C for 1 h, after which time a solution (2*S*,3*R*)-3-(trityl-amino)-pentan-2-ol ((**R-7e**), 3.81 g, 1 equiv, 11.04 mmol) in DCM (20 mL) was added dropwise with stirring. The reaction mixture was stirred at this temperature for 4 h, when TLC (hexane/ether; 80:20) indicated that the reaction had gone to completion. To the reaction mixture was added *N*-ethylpiperidine (7.54 mL, 5 equiv, 54.88 mmol), and the solution allowed to warm to room temperature over 16 h. The reaction mixture was diluted with more DCM (50 mL) and washed with water (200 mL). The aqueous phase was extracted with DCM (2 × 50 mL), and the combined organic phase washed with brine (50 mL), dried ($MgSO_4$) and evaporated in vacuo. The residue was dissolved in ether (100 mL), the solid precipitate removed by filtration and the filtrate was evaporated in vacuo. The residue was dissolved in hexane (50 mL), the solid precipitate removed by filtration and the filtrate was evaporated in vacuo to (3*R*)-3-(trityl-amino)-pentan-2-one (**R-9**) as a light yellow oil; yield: 3.78 g (100%) (80% 2*S*,3*R*: 20% 2*R*,3*R*). ^{1}H NMR (DMSO-d_6, 250 MHz): δ 0.73 (t, 3H, *J* = 7.35 Hz, –NHCH(CH_2CH_3)C(CH_3)O), 1.47–1.60 (m, 5H, -NHCH(*CH*$_2$CH$_3$)C(*CH*$_3$)O), 3.12 (d, 1H, *J* = 8.38 Hz, –*NH*CH(CH_2CH_3)C(CH_3)O), 3.32 (m, 1H, –NH*CH*(CH_2CH_3)C(CH_3)O), 7.16–7.49 (m, 15H, 3 × Bz). To a stirred solution of the foregoing ((**R-9**), 0.87 g, 1 equiv, 2.54 mmol) in ether (100 mL) under an argon atmosphere at room temperature, was added methylmagnesium iodide (3 M in ether, 2.54 mL, 3 equiv, 7.62 mmol) dropwise. The solution was placed in a preheated oil bath at 45 °C and refluxed at this temperature for 16 h. The mixture was recooled to 0 °C, H_2O (100 mL) added, the solution filtered through Celite, and the Celite washed with more ether (50 mL). The combined organic phase was separated, the aqueous phase was extracted with ether (2 × 50 mL), and the combined organic phase washed with brine (50 mL), dried ($MgSO_4$) and evaporated in vacuo. The residue was purified by silica gel column chromatography, eluted with hexane/ether (100:0→90:10) to afford (3*R*)-2-methyl-3-(trityl-amino)-pentan-2-ol (**R-10**) as a light yellow oil; yield: 0.21 g (23%). ^{1}H NMR (DMSO-d_6, 250 MHz): δ 0.26 (t, *J* = 7.42 Hz, –NHCH(CH_2CH_3)CH(CH_3)OH), 1.00 + 1.25 (2 × s, 6H, –NHCH-(CH_2CH_3)C(*CH*$_3$)$_2$OH), 0.72–1.43 (m, 2H, –NHCH(*CH*$_2$CH$_3$)-C(CH_3)$_2$OH), 1.84 (m, 1H, –NHCH(CH_2CH_3)C(CH_3)$_2$*OH*), 2.90 (m, 1H, –NH*CH*(CH_2CH_3)C(CH_3)$_2$OH), 4.32 (s, 1H, –*NH*CH-(CH_2CH_3)C(CH_3)$_2$OH), 7.17–7.46 (m, 15H, 3 × Bz). To a stirred solution of the foregoing ((**R-10**), 0.21 g, 1 equiv, 0.60 mmol) in DCM (5 mL) under an argon atmosphere at room temperature, was added trifluoroacetic acid (2.5 mL) dropwise, and the solution was stirred at this temperature for 1 h. The solvent was evaporated in vacuo and the residue was precipitated from ether (15 mL) with hexane (300 mL) with stirring to give a yellow oil. The solvent was decanted from the oil, and the oil was washed with hexane (30 mL) and dried in vacuo to afford (3*R*)-3-amino-2-methyl-pentan-2-ol (**R-11**) as a light yellow oil; yield: 0.07 g (100%). ^{1}H NMR (DMSO-d_6, 250 MHz): δ 0.97 (t, 3H, *J* = 7.42 Hz, NH_2CH(CH_2*CH*$_3$)-C(CH_3)$_2$OH), 1.06 + 1.19 (2 × s, 6H, NH_2CH(CH_2CH_3)C(*CH*$_3$)$_2$OH), 1.28–1.71 (m, 2H, NH_2CH(*CH*$_2$CH$_3$)C(CH_3)$_2$OH), 2.72 (m, 1H, NH_2*CH*(CH_2CH_3)C(CH_3)$_2$OH), 5.21 (s, 1H, NH_2CH(CH_2CH_3)-C(CH_3)$_2$*OH*), 7.63 (br s, 2H, *NH*$_2$CH(CH_2CH_3)C(CH_3)$_2$OH). HMRS: $C_6H_{15}NO$ requires (M+H) 118.1226; found m/z 118.1226.

4.3.10. 2-[(*R*)-1-Ethyl-2-hydroxyethylamino]-9-isopropyl-6-[(pyridin-2-ylmethyl)-amino]purine (αR-14)

To a stirred solution of 2-fluoro-9-isopropyl-6-[(pyridin-2a-ylmethyl)-amino]purine **4a**, 0.25 g, 1 equiv, 0.87 mmol) in *n*-BuOH/DMSO (5 mL, 4:1) at room temperature under an argon atmosphere was added DIEA (1.5 mL, 9.85 equiv, 8.61 mmol) followed by (*R*)-2-aminobutan-1-ol (**5**, 0.82 mL, 9.97 equiv, 8.71 mmol). The reaction mixture was placed in a preheated oil bath at 140 °C and stirred at this temperature for 72 h, when TLC ($CHCl_3$/MeOH; 90:10) indicated that the reaction had gone to completion. The reaction mixture was allowed to cool to room temperature and the solvent was evaporated in vacuo. The residue was partitioned between EtOAc (100 mL) and water (150 mL), the aqueous phase was extracted with more EtOAc (2 × 50 mL), and the combined organic phase was washed with brine (50 mL), dried ($MgSO_4$) and evaporated in vacuo. The residue was purified by gradient column chromatography on silica gel eluted with $CHCl_3$/MeOH (100:0→97:3), to afford 2-[(*R*)-1-ethyl-2-hydroxyethylamino]-9-isopropyl-6-[(pyridin-2-ylmethyl)-amino]purine (**αR-14**) as a white solid; yield: 0.29 g (95%). Mp 30–35 °C. ^{1}H NMR (DMSO-d_6, 250 MHz): δ 0.77 (m, 3H, –NHCH(CH_2*CH*$_3$)CH$_2$OH), 1.24–1.48 (m, 8H, –NHCH(*CH*$_2$CH$_3$)CH$_2$OH + CH(*CH*$_3$)$_2$), 3.38 (m, 2H, –NHCH(CH_2CH_3)*CH*$_2$OH), 3.70 (m, 1H, –NH*CH*(CH_2CH_3)CH$_2$OH), 4.52 (m, 2H, –HN*CH*$_2$-Pyr), 4.75 (m, 2H, OH + *CH*(CH_3)$_2$), 5.81 (d, 2H, *J* = 8.33 Hz, –N*H*CH(CH_2CH_3)CH$_2$OH), 7.24, 7.67, 8.48 (3 × m, 4H, Pyr), 7.79 (br s, 2H, –N=*CH*–N + –*HN*CH$_2$-Pyr). FABMS *m*/*z* (relative intensity): 356 ([M+H]$^+$, 100), 324 (28), 154 (26), 136 (22). Accurate Mass (M+H): actual: 356.2199, measured: 356.2208. Microanalysis (expected: measured) $C_{18}H_{25}N_7O$: C; 60.83: 61.81, H; 7.09: 7.29, N; 27.58: 27.09.

4.3.11. 2-[(*R*)-1-Ethyl-2-hydroxyethylamino]-9-isopropyl-6-[(pyridin-3-ylmethyl)-amino]purine (αR-20)

To a stirred solution of 2-fluoro-9-isopropyl-6-[(pyridin-3-ylmethyl)-amino]purine (**4b**, 0.40 g, 1 equiv, 1.40 mmol) in *n*-BuOH/DMSO (5 mL, 4:1) at room temperature under an argon atmosphere was added DIEA (2.4 mL, 9.85 equiv, 13.78 mmol) followed by (*R*)-2-aminobutan-1-ol (**5**, 1.31 mL, 9.95 equiv, 13.91 mmol). The reaction mixture was placed in a preheated oil bath at 140 °C and stirred at this temperature for 48 h, when TLC ($CHCl_3$/MeOH; 90:10) indicated that the reaction had gone to completion. The reaction mixture was allowed to cool to room temperature and the solvent was evaporated in vacuo. The residue was partitioned between EtOAc (100 mL) and water (150 mL), the

aqueous phase was extracted with more EtOAc (2 × 50 mL), and the combined organic phase was washed with brine (50 mL), dried ($MgSO_4$) and evaporated in vacuo. The residue was purified by gradient column chromatography on silica gel eluted with $CHCl_3$/MeOH (100:0→97:3), to afford 2-[(*R*)-1-ethyl-2-hydroxyethylamino]-9-isopropyl-6-[(pyridin-3-ylmethyl)-amino]purine (**αR-20**) as a white solid; yield: 0.48 g (97%). Mp 128–129 °C. ^{1}H NMR (DMSO-d_6, 250 MHz): δ 0.82 (m, 3H, –NHCH(CH_2CH_3)CH_2OH), 1.44–1.60 (m, 8H, –NHCH(CH_2CH_3)CH_2OH + –CH(CH_3)$_2$), 3.34, 3.48 (2 × m, 2H, –NHCH(CH_2CH_3)CH_2OH), 3.78 (m, 1H, –NHCH(CH_2CH_3)CH_2OH), 4.52 (m, 4H, –HNCH_2-Pyr + –CH(CH_3)$_2$ + OH), 5.90 (d, 2H, *J* = 8.55 Hz, –N*H*CH(CH_2CH_3)CH_2OH), 7.31, 7.75, 8.41 (3 × m, 4H, Pyr), 7.79 (s, 1H, –N=*CH*–N–), 8.58 (br s, 1H, –*HN*CH_2-Pyr). FABMS *m/z* (relative intensity): 356 ([M+H]$^+$, 100), 324 (32), 154 (15), 136 (16). Accurate Mass (M+H): actual: 356.2199, measured: 356.2208. Microanalysis (expected: measured) $C_{18}H_{25}N_7O$: C; 60.83: 60.67, H; 7.09: 7.06, N; 27.58: 27.49.

4.3.12. 2-[(*R*)-1-Ethyl-2-hydroxyethylamino]-9-isopropyl-6-[(pyridin-4-ylmethyl)-amino]purine (αR-26)

To a stirred solution of 2-fluoro-9-isopropyl-6-[(pyridin-4-ylmethyl)-amino]purine (**4c**, 0.27 g, 1 equiv, 0.94 mmol) in *n*-BuOH/DMSO (5 mL, 4:1) at room temperature under an argon atmosphere was added DIEA (1.63 mL, 9.91 equiv, 9.36 mmol) followed by (*R*)-(−)-2-aminobutan-1-ol (**5**, 0.89 mL, 10 equiv, 9.45 mmol). The reaction mixture was placed in a preheated oil bath at 140 °C and stirred at this temperature for 48 h, when TLC ($CHCl_3$/MeOH; 90:10) indicated that the reaction had gone to completion. The reaction mixture was allowed to cool to room temperature and the solvent was evaporated in vacuo. The residue was partitioned between EtOAc (100 mL) and water (150 mL), the aqueous phase was saturated with NaCl and extracted with more EtOAc (2 × 50 mL), and the combined organic phase was washed with brine (50 mL), dried ($MgSO_4$) and evaporated in vacuo. The residue was purified by gradient column chromatography on silica gel eluted with $CHCl_3$/MeOH (100:0→97:3), to afford 2-[(*R*)-1-ethyl-2-hydroxyethylamino]-9-isopropyl-6-[(pyridin-4-ylmethyl)-amino]purine (**αR-26**) as a white solid; yield: 0.30 g (89%). Mp 105–106 °C. ^{1}H NMR (DMSO-d_6, 250 MHz): δ 0.78 (m, 3H, –NHCH(CH_2CH_3)CH_2OH), 1.39–1.55 (m, 8H, –NHCH(CH_2CH_3)CH_2OH + –CH(CH_3)$_2$), 3.32, 3.40 (2 × m, 2H, –NHCH(CH_2CH_3)CH_2OH), 3.70 (m, 1H, –NHCH(CH_2CH_3)CH_2OH), 4.50–4.64 (m, 4H, –HNCH_2-Pyr + –CH(CH_3)$_2$ + OH), 5.83 (d, 2H, *J* = 8.15 Hz, –N*H*CH(CH_2CH_3)CH_2OH), 7.30, 8.45 (2 × m, 4H, Pyr), 7.80 (s, 1H, –N=*CH*–N–), 7.88 (br s, 1H, –*HN*CH_2-Pyr). FABMS *m/z* (relative intensity): 356 ([M+H]$^+$, 100), 324 (28), 154 (47), 136 (38). Accurate mass (M+H): actual: 356.2199, measured: 356.2208. Microanalysis (expected: measured) $C_{18}H_{25}N_7O$: C; 60.83: 60.99, H; 7.09: 7.25, N; 27.58: 26.94.

4.3.13. 6-Benzylamino-2-[(1*R*,2*RS*)-1-ethyl-2-hydroxypropylamino]-9-isopropylpurine (αR-27)

To a stirred solution of 6-benzylamino-2-fluoro-9-isopropylpurine (**4d**, 0.20 g, 1 equiv, 0.70 mmol) in *n*-BuOH/DMSO (5 mL, 4:1) at room temperature under an argon atmosphere was added DIEA (1.2 mL, 9.8 equiv, 6.88 mmol) followed by (2*RS*,3*R*)-3-amino-pentan-2-ol ((**R-8e**), 0.18 g, 2.5 equiv, 1.74 mmol). The reaction mixture was placed in a preheated oil bath at 140 °C and stirred at this temperature for 72 h, when TLC DCM/ether/MeOH (55:40:5) indicated that the reaction had gone to completion. The reaction mixture was allowed to cool to room temperature and the solvent was evaporated in vacuo. The residue was partitioned between DCM (100 mL) and water (200 mL), the aqueous phase was extracted with more DCM (3 × 50 mL), and the combined organic phase was washed with brine (50 mL), dried ($MgSO_4$) and evaporated in vacuo. The residue was purified by gradient column chromatography on silica gel eluted with DCM/ether/MeOH (60:40:0→60:40:2) to afford 6-benzylamino-2-[(1*R*)-1-ethyl-2-hydroxypropylamino]-9-isopropylpurine (**αR-27**) as a white solid; yield: 0.11 g (43%). Mp 42–44 °C (80% 1*R*,2*S*: 20% 1*R*,2*R*). ^{1}H NMR (DMSO-d_6, 250 MHz): δ 0.81 (m, 3H, –NHCH(CH_2CH_3)CH(CH_3)OH), 1.02 (d, 3H, *J* = 6.16 Hz, –NHCH(CH_2CH_3)CH(CH_3)OH) 1.45 (m, 8H, –NHCH(CH_2CH_3)CH(CH_3)OH + –CH(CH_3)$_2$), 3.62 + 3.73 (2 × m, 2H, –NHCH(CH_2CH_3)CH(CH_3)OH), 4.49–4.63 (m, 4H, –HNCH_2-Bz + –NHCH(CH_2CH_3)CH(CH_3)OH + –CH(CH_3)$_2$), 5.59 + 5.90 (2 × d, 1H, *J* = 9.00 + 8.85 Hz, –N*H*CH(CH_2CH_3)CH(CH_3)OH), 7.19–7.37 (m, 5H, Bz), 7.77 (br s, 2H, –N=*CH*–N– + –*HN*CH_2-Bz). FABMS *m/z* (relative intensity): 369 ([M+H]$^+$, 100), 323 (70), 134 (15). Accurate mass (M+H): actual: 369.2403, measured: 369.2418.

4.3.14. 2-[(1*R*,2*RS*)-1-Ethyl-2-hydroxypropylamino]-9-isopropyl-6-[(pyridin-2-ylmethyl)-amino]purine (αR-15)

To a stirred solution of 2-fluoro-9-isopropyl-6-[(pyridin-2-ylmethyl)-amino]purine (**4a**, 30 mg, 1 equiv, 0.10 mmol) in *n*-BuOH/DMSO (2.5 mL, 4:1) at room temperature under an argon atmosphere was added DIEA (0.2 mL, 10.96 equiv, 1.14 mmol) followed by (2*RS*,3*R*)-3-amino-pentan-2-ol ((**R-8e**), 60 mg, 5.5 equiv, 0.58 mmol). The reaction mixture was placed in a preheated oil bath at 160 °C and stirred at this temperature for 72 h. The reaction mixture was allowed to cool to room temperature and the solvent was evaporated in vacuo. The residue was partitioned between EtOAc (50 mL) and water (50 mL), the aqueous phase was extracted with more EtOAc (2 × 25 mL), and the combined organic phase was washed with brine (50 mL), dried ($MgSO_4$) and evaporated in vacuo. The residue was purified by gradient column chromatography on silica gel eluted with $CHCl_3$/MeOH (100:0→98:2), to afford 2-[(1*R*)-1-ethyl-2-hydroxypropylamino]-9-isopropyl-6-[(pyridin-2-ylmethyl)-amino]purine (**αR-15**) as a white solid; yield: 36.1 mg (93%) (80% 1*R*,2*S*: 20% 1*R*,2*R*).[1] ^{1}H NMR ($CDCl_3$, 250 MHz): δ 0.91 + 1.06 (2 × t, 3H, *J* = 7.11 + 7.42 Hz, –NHCH(CH_2CH_3)CH(CH_3)OH), 1.16 + 1.29 (2 × d, 3H, *J* = 6.48 + 3.48 Hz, –NHCH(CH_2CH_3)CH(CH_3)OH) 1.57 (d, 6H, *J* = 6.79 Hz, –CH(CH_3)$_2$), 1.71–2.01 (m, 2H, –NHCH(CH_2CH_3)CH(CH_3)OH), 3.98 (m, 2H, –NHCH(CH_2CH_3)CH(CH_3)OH), 4.58–4.69 (m, 1H, –CH(CH_3)$_2$), 4.83–5.00 (m, 2H, –HNCH_2-Pyr), 6.75–6.91 (m, 1H, –*HN*CH_2-Pyr), 7.19–7.25 (m, 1H, Pyr-H), 7.37 (d, 1H, *J* = 8.05 Hz, Pyr-H), 7.57 (s, 1H, –N=*CH*–N–), 7.64–7.71 (m, 1H, Pyr-H), 8.61 (d, 1H, *J* = 4.58 Hz, Pyr-H). FABMS *m/z* (relative intensity): 370 ([M+H]$^+$, 100), 324 (40). Accurate mass (M+H): actual: 370.2355, measured: 370.2347.

4.3.15. 2-[(1*R*,2*RS*)-1-Ethyl-2-hydroxypropylamino]-9-isopropyl-6-[(pyridin-3-ylmethyl)-amino]purine (αR-21)

To a stirred solution of 2-fluoro-9-isopropyl-6-[(pyridin-3-ylmethyl)-amino]purine (**4b**, 30 mg, 1 equiv, 0.10 mmol) in *n*-BuOH/DMSO (2.5 mL, 4:1) at room temperature under an argon atmosphere was added DIEA (0.2 mL, 10.96 equiv, 1.14 mmol) followed by (2*RS*,3*R*)-3-amino-pentan-2-ol ((**R-8e**), 60 mg, 5.5 equiv, 0.58 mmol). The reaction mixture was placed in a preheated oil bath at 160 °C and stirred at this temperature for 72 h. The reaction mixture was allowed to cool to room temperature and the solvent was evaporated in vacuo. The residue was partitioned between EtOAc (50 mL) and water (50 mL), the aqueous phase was extracted with more EtOAc (2 × 25 mL), and the combined organic phase was washed with brine (50 mL), dried ($MgSO_4$) and evaporated in vacuo. The residue was purified by gradient column chromatography on silica gel eluted with $CHCl_3$/MeOH (100:0→98:2), to afford 2-[(1*R*)-1-ethyl-2-hydroxypropylamino]-9-isopropyl-6-[(pyridin-3-ylmethyl)-amino]purine (**αR-21**) as a white solid; yield: 22 mg (57%) (80% 1*R*,2*S*: 20% 1*R*,2*R*). ^{1}H NMR ($CDCl_3$, 250 MHz): δ 0.90 + 1.05 (2 × t, 3H, *J* = 6.95+7.42 Hz, –NHCH(CH_2CH_3)CH(CH_3)OH),

1.17 + 1.25 (2 × d, 3H, J = 6.32+6.16 Hz, –NHCH(CH_2CH_3)CH(CH_3)OH) 1.57 (d, 6H, J = 6.79 Hz, –CH)$CH_3)_2$), 1.77–2.09 (m, 2H, –NHCH(CH_2CH_3)CH(CH_3)OH), 3.91–4.03 (m, 2H, –NHCH(CH_2CH_3)CH(CH_3)OH), 4.58–4.69 (m, 1H, –CH($CH_3)_2$), 4.83–5.00 (m, 3H, –HNCH_2-Pyr + OH), 6.16 (m, 1H, –NHCH(CH_2CH_3)CH(CH_3)OH), 7.24–7.33 (m, 3H, 2 × Pyr-H + –N=CH–N–), 7.55 (m, 1H, Pyr-H), 7.74 (d, 1H, J = 7.42 Hz, Pyr-H), 8.67 (m, 1H, $HN$$CH_2$-Pyr). FABMS m/z (relative intensity): 370 ([M+H]$^+$, 100), 324 (35), 289 (37), 243 (65), 199 (85). Accurate mass (M+H): actual: 370.2355, measured: 370.2347.

4.3.16. 6-Benzylamino-2-[(1*R*,2*RS*)-1-ethyl-2-hydroxybutylamino]-9-isopropylpurine (αR-28)

To a stirred solution of (*R*)-2-(trityl-amino)-butyraldehyde (**R-6**), 1.5 g, 1 equiv, 4.53 mmol) in ether (150 mL) under an argon atmosphere at −78 °C, was added ethylmagnesium bromide (3 M in ether, 1.51 mL, 1 equiv, 4.53 mmol) dropwise. The solution was stirred at −78 °C for 2 h, then allowed to warm to room temperature over 16 h. The mixture was recooled to 0 °C, H_2O (150 mL) added, and the organic phase separated. The aqueous phase was extracted with more ether (2 × 50 mL), and the combined organic phase washed with brine (50 mL), dried ($MgSO_4$) and evaporated in vacuo. The residue was purified by silica gel column chromatography, eluted with hexane/ether (90:10) to afford (4*R*)-4-(trityl-amino)-hexan-3-ol (**R-7f**) as a light yellow oil; yield: 1.13 g (69%) (57% 3*S*,4*R*: 43% 3*R*,4*R*). ^{1}H NMR (DMSO-d_6, 250 MHz): δ 0.45 + 0.69 (t + m, 6H, J = 7.43 Hz, –NHCH(CH_2CH_3)CH(CH_2CH_3)OH), 1.12–1.29 (m, 4H, –NHCH(CH_2CH_3)CH(CH_2CH_3)OH), 2.16 (m, 1H, –NHCH(CH_2CH_3)CH(CH_2CH_3)OH), 2.54 (m, 1H, –NHCH(CH_2CH_3)-CH(CH_2CH_3)OH), 3.21–3.40 (m, 1H, –NHCH(CH_2CH_3)CH(CH_2CH_3)OH), 4.29 + 4.39 (2 × d, 1H, J = 4.42+5.37 Hz, –NHCH(CH_2CH_3)CH(CH_2CH_3)OH), 7.15–7.52 (m, 15H, 3 × Bz). To a stirred solution of the foregoing (**R-7f**), 1.13 g, 1 equiv, 3.14 mmol) in DCM (15 mL) under an argon atmosphere at room temperature, was added trifluoroacetic acid (7 mL) dropwise, and the solution was stirred at this temperature for 4 h. The solvent was evaporated in vacuo, EtOH (20 mL) added, and removed in vacuo, and this process repeated a further two times. The residue was precipitated from ether (5 mL) with hexane (40 mL) with stirring to give a yellow oil. The solvent was decanted from the oil, and the oil was washed with hexane (30 mL) and dried in vacuo to afford (4*R*)-4-amino-hexan-3-ol (**R-8f**) as a light yellow oil; yield: 0.37 g (100%) (57% 3*S*,4*R*: 43% 3*R*,4*R*). ^{1}H NMR (DMSO-d_6, 250 MHz): δ 0.79 + 0.92 (t + m, 6H, J = 7.42 Hz, NH_2CH(CH_2CH_3)CH(CH_2CH_3)OH), 1.30–1.67 (m, 4H, NH_2CH(CH_2CH_3)CH(CH_2CH_3)OH), 2.70 (m, 1H, NH_2CH(CH_2CH_3)CH(CH_2CH_3)OH), 2.84 + 2.96 (2 × m, 1H, NH_2CH(CH_2CH_3)CH(CH_2CH_3)OH), 3.41 + 3.56 (2 × m, 1H, NH_2CH(CH_2CH_3)CH(CH_2CH_3)OH), 7.71 (br s, 2H, NH_2CH(CH_2CH_3)CH(CH_2CH_3)OH). To a stirred solution of 6-benzylamino-2-fluoro-9-isopropylpurine (**4d**, 40 mg, 1 equiv, 0.14 mmol) in *n*-BuOH/DMSO (3.75 mL, 4:1) at room temperature under an argon atmosphere was added DIEA (0.24 mL, 9.8 equiv, 1.38 mmol) followed by the foregoing ((**R-8f**), 110 mg, 6.7 equiv, 0.93 mmol). The reaction mixture was placed in a preheated oil bath at 140 °C and stirred at this temperature for 72 h, when TLC DCM/ether/MeOH (55:40:5) indicated that the reaction had gone to completion. The reaction mixture was allowed to cool to room temperature and the solvent was evaporated in vacuo. The residue was partitioned between EtOAc (50 mL) and brine/water (1:1, 100 mL), the aqueous phase was extracted with more EtOAc (2 × 50 mL), and the combined organic phase was washed with brine (50 mL), dried ($MgSO_4$) and evaporated in vacuo. The residue was purified by gradient column chromatography on silica gel eluted with hexane/ether/MeOH (50:50:0→50:50:2) to afford 6-benzylamino-2-[(1*R*)-1-ethyl-2-hydroxybutylamino]-9-isopropylpurine (**αR-28**) as a white solid; yield: 31 mg (58%).(57% 1*R*,2*S*: 43% 1*R*,2*R*). ^{1}H NMR ($CDCl_3$, 250 MHz): δ 0.92–1.08 (m, 6H, –NHCH(CH_2CH_3)CH(CH_2CH_3)OH), 1.56 (d, 6H, J = 6.79 Hz, –CH)$CH_3)_2$), 1.44–1.69 (m, 4H, –NHCH(CH_2CH_3)CH(CH_2CH_3)OH), 3.45 (d, 1H, J = 6.32 Hz, OH), 3.56–3.70 (m, 1H, –NHCH(CH_2CH_3)CH(CH_2CH_3)OH), 3.91–4.06 (m, 1H, –NHCH(CH_2CH_3)CH(CH_2CH_3)OH), 4.58–4.69 (m, 1H, –CH($CH_3)_2$), 4.73–5.01 (m, 2H, –HNCH_2-Bz), 5.16–5.32 + 6.01–6.22 (2 × m, 1H, –NHCH(CH_2CH_3)CH(CH_2CH_3)OH), 7.22–7.43 (m, 6H, 5 × Bz-H + $HN$$CH_2$-Bz), 7.52 (s, 1H, –N=$CH$–N). FABMS m/z (relative intensity): 383 ([M+H]$^+$, 100), 323 (55), 296 (21). Accurate mass (M+H): actual: 383.2559, measured: 383.2542.

4.3.17. 2-[(1*R*,2*RS*)-1-Ethyl-2-hydroxybutylamino]-9-isopropyl-6-[(pyridin-2-ylmethyl)-amino]purine (αR-16)

To a stirred solution of 2-fluoro-9-isopropyl-6-[(pyridin-2-ylmethyl)-amino]purine (**4a**, 20 mg, 1 equiv, 0.07 mmol) in *n*-BuOH/DMSO (3.75 mL, 4:1) at room temperature under an argon atmosphere was added DIEA (0.18 mL, 15 equiv, 1.03 mmol) followed by (3*RS*,4*R*)-4-amino-hexan-3-ol ((**R-8f**), 110 mg, 13 equiv, 0.94 mmol) (prepared as described for (**αR-28**): see above). The reaction mixture was placed in a preheated oil bath at 140 °C and stirred at this temperature for 72 h. The reaction mixture was allowed to cool to room temperature and the solvent was evaporated in vacuo. The residue was partitioned between EtOAc (50 mL) and brine/water (1:1, 100 mL), the aqueous phase was extracted with more EtOAc (2 × 25 mL), and the combined organic phase was washed with brine (50 mL), dried ($MgSO_4$) and evaporated in vacuo. The residue was purified by gradient column chromatography on silica gel eluted with $CHCl_3$/MeOH (100:0→98:2), to afford 2-[(1*R*)-1-ethyl-2-hydroxypropylamino]-9-isopropyl-6-[(pyridin-2-ylmethyl)-amino]purine (**αR-16**) as a white solid; yield: 11 mg (41%) (57% 1*R*,2*S*: 43% 1*R*,2*R*). ^{1}H NMR ($CDCl_3$, 250 MHz): δ 0.85–1.06 (m, 6H, –NHCH(CH_2CH_3)CH(CH_2CH_3)OH), 1.57 (d, 6H, J = 6.79 Hz, –CH)$CH_3)_2$), 1.42–1.65 (m, 4H, –NHCH(CH_2CH_3)CH(CH_2CH_3)OH), 3.45 (d, 1H, J = 6.31 Hz, OH), 3.57–3.70 (m, 1H, –NHCH(CH_2CH_3)CH(CH_2CH_3)OH), 3.91–4.03 (m, 1H, –NHCH(CH_2CH_3)CH(CH_2CH_3)OH), 4.57–4.76 (m, 1H, –CH($CH_3)_2$), 4.86–4.98 (m, 2H, –HNCH_2-Pyr), 5.18–5.29 (m, 1H, –NHCH(CH_2CH_3)CH(CH_2CH_3)OH), 6.73–6.89 (m, 1H, –$HN$$CH_2$-Pyr), 7.15–7.25 (m, 1H, Pyr-H), 7.38 (d, 1H, J = 7.90 Hz, Pyr-H), 7.56 (s, 1H, –N=CH–N–), 7.63–7.70 (m, 1H, Pyr-H), 8.60 (d, 1H, J = 4.42 Hz, Pyr-H). FABMS m/z (relative intensity): 384 ([M+H]$^+$, 100), 324 (35), 307 (37), 297 (25), 289 (20). Accurate Mass (M+H): actual: 384.2512, measured: 384.2523.

4.3.18. 2-[(1*R*,2*RS*)-1-Ethyl-2-hydroxybutylamino]-9-isopropyl-6-[(pyridin-3-ylmethyl)-amino]purine (αR-22)

To a stirred solution of 2-fluoro-9-isopropyl-6-[(pyridin-3-ylmethyl)-amino]purine (**4b**, 20 mg, 1 equiv, 0.07 mmol) in *n*-BuOH/DMSO (3.75 mL, 4:1) at room temperature under an argon atmosphere was added DIEA (0.18 mL, 15 equiv, 1.03 mmol) followed by (3*RS*,4*R*)-4-amino-hexan-3-ol ((**R-8f**), 110 mg, 13 equiv, 0.94 mmol) (prepared as described for (**αR-28**): see above). The reaction mixture was placed in a preheated oil bath at 140 °C and stirred at this temperature for 72 h. The reaction mixture was allowed to cool to room temperature and the solvent was evaporated in vacuo. The residue was partitioned between EtOAc (50 mL) and brine/water (1:1, 100 mL), the aqueous phase was extracted with more EtOAc (2 × 25 mL), and the combined organic phase was washed with brine (50 mL), dried ($MgSO_4$) and evaporated in vacuo. The residue was purified by gradient column chromatography on silica gel eluted with $CHCl_3$/MeOH (100:0→98:2), to afford 2-[(1*R*)-1-ethyl-2-hydroxypropylamino]-9-isopropyl-6-[(pyridin-3-ylmethyl)-amino]purine (**αR-22**) as a white solid; yield: 23 mg (75%) (57% 1*R*,2*S*: 43% 1*R*,2*R*). ^{1}H NMR ($CDCl_3$, 250 MHz): δ 0.87–1.07 (m, 6H, –NHCH(CH_2CH_3)CH(CH_2CH_3)OH), 1.56 (d, 6H, J = 6.63 Hz, –CH)$CH_3)_2$), 1.43–1.63 (m, 4H, –

NHCH(CH_2CH_3)CH(CH_2CH_3)OH), 3.44 (d, 1H, *J* = 6.31 Hz, *OH*), 3.58–3.71 (m, 1H, –NHCH(CH_2CH_3)*CH*(CH_2CH_3)OH), 3.89–4.01 (m, 1H, –NHCH(CH_2CH_3)*CH*(CH_2CH_3)OH), 4.56–4.70 (m, 1H, –*CH*(CH_3)$_2$), 4.76–4.95 (m, 2H, –HN*CH*$_2$-Pyr), 5.20–5.29 + 6.17–6.34 (m, 1H, –*NH*CH(CH_2CH_3)CH(CH_2CH_3)OH), 7.19–7.38 (m, 3H, 2 × Pyr-H + –N=*CH*–N–), 7.48–7.60 (m, 1H, Pyr-H), 7.72 (d, 1H, *J* = 7.74 Hz, Pyr-H), 8.67 (m, 1H, *HN*CH$_2$-Pyr). FABMS *m/z* (relative intensity): 384 ([M+H]$^+$, 100), 324 (50), 297 (30). Accurate mass (M+H): actual: 384.2512, measured: 384.2500.

4.3.19. 6-Benzylamino-2-[(1*R*,2*RS*)-1-ethyl-2-hydroxy-3-methylbutylamino]-9-isopropylpurine (αR-29)

To a stirred suspension of CuBr·SMe$_2$ (1.37 g, 2.2 equiv, 6.66 mmol) in ether (100 mL) under an argon atmosphere at −78 °C, was added isopropyllithium (0.7 M in pentane, 17.29 mL, 4 equiv, 12.1 mmol) dropwise, and the solution allowed to warm to room temperature. The mixture was recooled to −70 °C, to which was added a solution of (*R*)-2-(trityl-amino)-butyraldehyde (**R-6**) (1 g, 1 equiv, 3.03 mmol) in ether (25 mL) dropwise with stirring. The reaction mixture was stirred at this temperature for 1 h, then allowed to warm to −55 °C and stirred at this temperature for 3 h. To the reaction mixture was added a saturated aqueous solution of NH_4Cl (100 mL) and allowed to warm to room temperature over 16 h. The reaction mixture was extracted with ether (2 × 200 mL), and the combined organic phase washed with brine (50 mL), dried ($MgSO_4$) and evaporated in vacuo. The residue was purified by silica gel gradient column chromatography, eluted with hexane/ether (100:0→90:10) to afford (3*RS*,4*R*)-2-methyl-4-(trityl-amino)-hexan-3-ol (**R-7g**) as a colourless oil; yield: 0.53 g (47%) (50% 3*S*,4*R*: 50% 3*R*,4*R*). ^{1}H NMR (DMSO-d_6, 250 MHz): δ 0.44 (t, 3H, *J* = 7.03 Hz, –NHCH(CH_2CH_3)CH(CH(CH_3)$_2$)OH), 0.52 + 0.77 (2 × d, 6H, *J* = 6.48 Hz, –NHCH(CH_2CH_3)CH(CH(CH_3)$_2$)OH), 0.79–1.13 (m, 2H, –NHCH(CH_2CH_3)CH(CH(CH_3)$_2$)OH), 1.72 (m, 1H, –NHCH(CH_2CH_3)CH(CH(CH_3)$_2$)*OH*), 2.11 (m, 1H, –NH*CH*(CH_2CH_3)-CH(CH(CH_3)$_2$)OH), 2.77 (m, 1H, –NHCH(CH_2CH_3)CH(*CH*(CH_3)$_2$)OH), 2.99 (m, 1H, –NHCH(CH_2CH_3)*CH*(CH(CH_3)$_2$)OH), 4.55 (d, 1H, *J* = 5.21 Hz, –*NH*CH(CH_2CH_3)CH(CH(CH_3)$_2$)OH), 7.15–7.46 (m, 15H, 3 × Bz). To a stirred solution of the foregoing ((**R-7g**), 0.53 g, 1 equiv, 1.41 mmol) in DCM (20 mL) under an argon atmosphere at room temperature, was added trifluoroacetic acid (5 mL) dropwise, and the solution was stirred at this temperature for 1 h. The solvent was evaporated in vacuo, the residue was precipitated from ether (10 mL) with hexane (90 mL) with stirring to give a yellow oil. The solvent was decanted from the oil, and the oil was washed with hexane (20 mL) and dried in vacuo to afford (4*R*)-4-amino-2-methyl-hexan-3-ol (**R-8g**) as a light yellow oil; yield: 0.18 g (100%) (50% 3*S*,4*R*: 50% 3*R*,4*R*). ^{1}H NMR (DMSO-d_6, 250 MHz): δ 0.85–0.99 (m, 9H, NH_2CH(CH_2CH_3)CH(CH)CH_3)$_2$)OH), 1.42–1.79 (m, 2H, NH_2CH(*CH*$_2$CH$_3$)CH(CH(CH_3)$_2$)OH), 2.95 (m, 1H, NH_2*CH*(CH_2CH_3)CH(CH(CH_3)$_2$)OH), 3.18 (m, 1H, NH_2CH(CH_2CH_3)-CH(*CH*(CH_3)$_2$)OH), 3.37 (m, 1H, NH_2CH(CH_2CH_3)*CH*(CH(CH_3)$_2$)OH), 7.58 (br s, 2H, *NH*$_2$CH(CH_2CH_3)CH(CH(CH_3)$_2$)OH). To a stirred solution of 6-benzylamino-2-fluoro-9-isopropylpurine (**4d**, 40 mg, 1 equiv, 0.14 mmol) in *n*-BuOH/DMSO (2.5 mL, 4:1) at room temperature under an argon atmosphere was added DIEA (0.12 mL, 4.9 equiv, 0.69 mmol) followed by the foregoing ((**R-8g**), 54 mg, 2.9 equiv, 0.41 mmol). The reaction mixture was placed in a preheated oil bath at 140 °C and stirred at this temperature for 72 h. The reaction mixture was allowed to cool to room temperature and the solvent was evaporated in vacuo. The residue was partitioned between EtOAc (50 mL) and brine/water (1:1, 100 mL), the aqueous phase was extracted with more EtOAc (2 × 50 mL), and the combined organic phase was washed with brine (50 mL), dried ($MgSO_4$) and evaporated in vacuo. The residue was purified by gradient column chromatography on silica gel eluted with hexane/ether/MeOH (50:50:0→50:50:2) to afford 6-benzylamino-2-[(1*R*)-1-ethyl-2-hydroxy-3-methylbutylamino]-9-isopropylpurine (**αR-29**) as a white solid; yield: 8.8 mg (16%) (50% 1*R*,2*S*: 50% 1*R*,2*R*). ^{1}H NMR ($CDCl_3$, 250 MHz): δ 0.96–1.04 (m, 9H, –NHCH(CH_2CH_3)CH(CH)CH_3)$_2$)OH), 1.57 (d, 6H, *J* = 6.63 Hz, –CH)CH_3)$_2$), 1.68–2.19 (m, 4H, –NHCH(*CH*$_2$CH$_3$)CH(*CH*(CH$_3$)$_2$)*OH*), 3.24–3.32 (m, 1H, –NH*CH*(CH_2CH_3)CH(CH(CH_3)$_2$)OH), 3.86–4.01 (m, 1H, –NHCH(CH_2CH_3)*CH*(CH(CH_3)$_2$)OH), 4.57–4.70 (m, 1H, –*CH*(CH_3)$_2$), 4.76–4.93 (m, 2H, –HN*CH*$_2$-Bz), 5.33–5.60 (m, 1H, –*NH*CH(CH_2CH_3)CH(CH(CH_3)$_2$)OH), 7.24–7.44 (m, 6H, 5 × Bz-*H* + *HN*CH$_2$-Bz), 7.52 (s, 1H, –N=*CH*–N). FABMS *m/z* (relative intensity): 397 ([M+H]$^+$, 100), 323 (70). Accurate mass (M+H): actual: 397.2716, measured: 393.2724.

4.3.20. 2-[(1*R*,2*RS*)-1-Ethyl-2-hydroxy-3-methylbutylamino]-9-isopropyl-6-[(pyridin-2-ylmethyl)-amino]purine (αR-17)

To a stirred solution of 2-fluoro-9-isopropyl-6-[(pyridin-2-yl-methyl)-amino]purine (**4a**, 30 mg, 1 equiv, 0.10 mmol) in *n*-BuOH/DMSO (2.5 mL, 4:1) at room temperature under an argon atmosphere was added DIEA (0.10 mL, 5.5 equiv, 0.57 mmol) followed by (3*RS*,4*R*)-4-amino-2-methyl-hexan-3-ol ((**R-8g**), 42 mg, 3.0 equiv, 0.32 mmol) (prepared as described for (**αR-29**): see above). The reaction mixture was placed in a preheated oil bath at 140 °C and stirred at this temperature for 72 h. The reaction mixture was allowed to cool to room temperature and the solvent was evaporated in vacuo. The residue was partitioned between EtOAc (50 mL) and brine/water (1:1, 100 mL), the aqueous phase was extracted with more EtOAc (2 × 25 mL), and the combined organic phase was washed with brine (50 mL), dried ($MgSO_4$) and evaporated in vacuo. The residue was purified by gradient column chromatography on silica gel eluted with $CHCl_3$/MeOH (100:0→98:2), to afford 2-[(1*R*)-1-ethyl-2-hydroxy-3-methylbutylamino]-9-isopropyl-6-[(pyridin-2-ylmethyl)-amino]purine (**αR-17**) as a white solid; yield: 7.8 mg (19%). 50% 1*R*,2*S*: 50% 1*R*,2*R*). ^{1}H NMR ($CDCl_3$, 250 MHz): δ 0.91–1.04 (m, 9H, –NHCH(CH_2CH_3)CH(CH)CH_3)$_2$)OH), 1.57 (d, 6H, *J* = 6.79 Hz, –CH)CH_3)$_2$), 1.66–1.94 (m, 4H, –NHCH(*CH*$_2$CH$_3$)CH(*CH*(CH$_3$)$_2$)*OH*), 3.22–3.34 (m, 1H, –NH*CH*(CH_2CH_3)CH(CH(CH_3)$_2$)OH), 3.79–3.93 (m, 1H, –NHCH(CH_2CH_3)*CH*(CH(CH_3)$_2$)OH), 4.57–4.71 (m, 1H, -*CH*(CH_3)$_2$), 4.85–4.97 (m, 2H, –HN*CH*$_2$-Pyr), 5.13–5.24 (m, 1H, –*NH*CH(CH_2CH_3)CH(CH(CH_3)$_2$)OH), 6.65–6.79 (m, 1H, –*HN*CH$_2$-Pyr), 7.13–7.24 (m, 1H, Pyr-H), 7.32–7.42 (m, 1H, Pyr-H), 7.56 (s, 1H, –N=*CH*–N–), 7.58–7.73 (m, 1H, Pyr-H), 8.60 (d, 1H, *J* = 4.42 Hz, Pyr-H). FABMS *m/z* (relative intensity): 398 ([M+H]$^+$, 100), 324 (50). Accurate mass (M+H): actual: 398.2668, measured: 398.2654.

4.3.21. 2-[(1*R*,2*RS*)-1-Ethyl-2-hydroxy-3-methylbutylamino]-9-isopropyl-6-[(pyridin-3-ylmethyl)-amino]purine (αR-23)

To a stirred solution of 2-fluoro-9-isopropyl-6-[(pyridin-3-yl-methyl)-amino]purine (**4b**, 30 mg, 1 equiv, 0.10 mmol) in *n*-BuOH/DMSO (2.5 mL, 4:1) at room temperature under an argon atmosphere was added DIEA (0.10 mL, 5.5 equiv, 0.57 mmol) followed by (3*RS*,4*R*)-4-amino-2-methyl-hexan-3-ol ((**R-8g**), 42 mg, 3.0 equiv, 0.32 mmol) (prepared as described for (**αR-29**): see above). The reaction mixture was placed in a preheated oil bath at 140 °C and stirred at this temperature for 72 h. The reaction mixture was allowed to cool to room temperature and the solvent was evaporated in vacuo. The residue was partitioned between EtOAc (50 mL) and brine/water (1:1, 100 mL), the aqueous phase was extracted with more EtOAc (2 × 25 mL), and the combined organic phase was washed with brine (50 mL), dried ($MgSO_4$) and evaporated in vacuo. The residue was purified by gradient column chromatography on silica gel eluted with $CHCl_3$/MeOH (100:0→98:2), to afford 2-[(1*R*)-1-ethyl-2-hydroxy-3-methylbutylamino]-9-isopropyl-6-[(pyridin-3-ylmethyl)-amino]purine (**αR-23**) as a white solid; yield: 6.3 mg (15%). 50% 1*R*,2*S*: 50% 1*R*,2*R*). ^{1}H-^{1}H

NMR ($CDCl_3$, 250 MHz): δ 0.93–1.04 (m, 9H, –NHCH(CH_2CH_3)CH(CH)$CH_3)_2$)OH), 1.57 (d, 6H, J = 6.95 Hz, –CH)$CH_3)_2$), 1.65–1.89 (m, 4H, –NHCH(CH_2CH_3)CH(CH($CH_3)_2$)OH), 3.22–3.32 (m, 1H, –NHCH(CH_2CH_3)CH(CH($CH_3)_2$)OH), 3.82–3.97 (m, 1H, –NHCH (CH_2CH_3)CH(CH($CH_3)_2$)OH), 4.54–4.70 (m, 1H, –CH($CH_3)_2$), 4.78–4.88 (m, 2H, –HNCH_2-Pyr), 5.03–5.14 + 6.08–6.20 (m, 1H, -NHCH (CH_2CH_3)CH(CH_2CH_3)OH), 7.22–7.36 (m, 3H, 2 × Pyr-H + –N=CH–N–), 7.49–7.58 (m, 1H, Pyr-H), 7.71 (d, 1H, J = 7.90 Hz, Pyr-H), 8.47–8.73 (m, 1H, $HN$$CH_2$-Pyr). FABMS m/z (relative intensity): 398 ($[M+H]^+$, 100), 324 (50). Accurate mass (M+H): actual: 398.2668, measured: 398.2654.

4.3.22. 6-Benzylamino-2-[(1*R*,2*RS*)-1-ethyl-2-hydroxy-3,3-dimethylbutylamino]-9-isopropylpurine (αR-30)

To a stirred suspension of $CuBr{\cdot}SMe_2$ (1.37 g, 2.2 equiv, 6.66 mmol) in ether (100 mL) under an argon atmosphere at −78 °C, was added *tert*-butyllithium (1.5 M in pentane, 8.0 mL, 4 equiv, 12.0 mmol) dropwise and the solution allowed to warm to room temperature. The mixture was recooled to −55 °C, to which was added a solution of (*R*)-2-(trityl-amino)-butyraldehyde (**R-6**) (1 g, 1 equiv, 3.03 mmol) in ether (25 mL) dropwise with stirring, and stirred at this temperature for 3 h. To the reaction mixture was added a saturated aqueous solution of NH_4Cl (100 mL) and allowed to warm to room temperature over 16 h. The reaction mixture was extracted with ether (2 × 200 mL), and the combined organic phase washed with brine (50 mL), dried ($MgSO_4$) and evaporated in vacuo. The residue was purified by silica gel gradient column chromatography, eluted with hexane/ether (100:0→90:10) to afford (3*RS*,4*R*)-2,2-dimethyl-4-(trityl-amino)-hexan-3-ol (**R-7h**) as a light yellow oil; yield: 0.57 g (49%) (55% 3*S*,4*R*: 45% 3*R*,4*R*). ^{1}H NMR (DMSO-d_6, 250 MHz): δ 0.36 + 0.86 (2 × t, 3H, J = 7.42 Hz, –NHCH(CH_2CH_3)CH(C($CH_3)_3$)OH), 0.57 + 0.71 (2 × s, 9H, –NHCH(CH_2CH_3)CH(C($CH_3)_3$)OH), 1.38–1.52 (m, 2H, –NHCH (CH_2CH_3)CH(C($CH_3)_3$)OH), 1.99 (m, 1H, –NHCH(CH_2CH_3)-CH(C($CH_3)_3$)OH), 2.27 (m, 1H, –NHCH(CH_2CH_3)CH(C($CH_3)_3$)OH), 2.95 (m, 1H, –NHCH(CH_2CH_3)CH(C($CH_3)_3$)OH), 4.22 + 4.77 (2 × d, 1H, J = 4.42 + 5.21 Hz, –NHCH(CH_2CH_3)CH(C($CH_3)_3$)OH), 7.14–7.52 (m, 15H, 3 × Bz). To a stirred solution of the foregoing (**R-7h**), 0.57 g, 1 equiv, 1.47 mmol) in DCM (10 mL) under an argon atmosphere at room temperature, was added trifluoroacetic acid (5 mL) dropwise, and the solution was stirred at this temperature for 1 h. The solvent was evaporated in vacuo, the residue was precipitated from ether (3 mL) with hexane (20 mL) with stirring to give a yellow oil. The solvent was decanted from the oil, and the oil was washed with hexane (20 mL) and dried in vacuo to afford (3*RS*,4*R*)-4-amino-2, 2-dimethyl-hexan-3-ol (**R-8h**) as a light yellow oil; yield: 0.21 g (100%) (55% 3*S*,4*R*: 45% 3*R*,4*R*). ^{1}H NMR (DMSO-d_6, 250 MHz): δ 0.84–0.99 (m, 3H, NH_2CH(CH_2CH_3)-CH(C($CH_3)_3$)OH), 1.25–1.29 (m, 9H, NH_2CH(CH_2CH_3)CH(C($CH_3)_3$) OH), 1.20–1.72 (m, 2H, NH_2CH(CH_2CH_3)CH(C($CH_3)_3$)OH), 3.14 (m, 1H, NH_2CH(CH_2CH_3)CH(C($CH_3)_3$)OH), 3.39 (m, 1H, NH_2CH (CH_2CH_3)CH(C($CH_3)_3$)OH), 3.65 (m, 1H, NH_2CH(CH_2CH_3)CH (C($CH_3)_3$)OH), 7.43, 7.77 + 8.54 (3 × br s, 2H, NH_2CH(CH_2CH_3)-CH(CH($CH_3)_2$)OH). To a stirred solution of 6-benzylamino-2-fluoro-9-isopropylpurine (**4d**, 40 mg, 1 equiv, 0.14 mmol) in *n*-BuOH/DMSO (5 mL, 4:1) at room temperature under an argon atmosphere was added DIEA (0.12 mL, 4.9 equiv, 0.69 mmol) followed by the foregoing (**R-8h**), 69 mg, 3.4 equiv, 0.48 mmol). The reaction mixture was placed in a preheated oil bath at 140 °C and stirred at this temperature for 72 h. The reaction mixture was allowed to cool to room temperature and the solvent was evaporated in vacuo. The residue was partitioned between EtOAc (50 mL) and brine/water (1:1, 100 mL), the aqueous phase was extracted with more EtOAc (2 × 50 mL), and the combined organic phase was washed with brine (50 mL), dried ($MgSO_4$) and evaporated in vacuo. The residue was purified by gradient column chromatography on silica gel eluted with hexane/ether/MeOH (50:50:0→50:50:2) to afford 6-benzylamino-2-[(1*R*)-1-ethyl-2-hydroxy-3,3-dimethylbutylamino]-9-isopropylpurine (**αR-30**) as a white solid; yield: 13 mg (23%) (55% 1*R*,2*S*: 45% 1*R*,2*R*). ^{1}H NMR ($CDCl_3$, 250 MHz): δ 1.00–1.04 (m, 12H, –NHCH(CH_2CH_3)-CH(C)$CH_3)_3$)OH), 1.56 + 1.58 (2 × d, 6H, J = 6.63 + 6.79 Hz, –CH)$CH_3)_2$), 1.63–1.87 (m, 2H, –NHCH(CH_2CH_3)CH(C($CH_3)_3$)OH), 3.57 (d, 1H, J = 1.42 Hz, –NHCH(CH_2CH_3)CH(C($CH_3)_3$)OH), 3.73–3.87 (m, 1H, –NHCH(CH_2CH_3)CH(C($CH_3)_3$)OH), 4.57–4.70 (m, 1H, –CH($CH_3)_2$), 4.77–4.86 (m, 2H, –HNCH_2-Bz), 5.22–5.36 + 5.93–6.09 (2 × m, 1H, –NHCH(CH_2CH_3)CH(C($CH_3)_3$)OH), 7.25–7.44 (m, 6H, 5 × Bz-H + $HN$$CH_2$-Bz), 7.53 (s, 1H, –N=$CH$–N). FABMS m/z (relative intensity): 411 ($[M+H]^+$, 90), 323 (100). Accurate mass (M+H): actual: 411.2872, measured: 411.2860.

4.3.23. 2-[(1*R*,2*RS*)-1-Ethyl-2-hydroxy-3,3-dimethylbutylamino]-9-isopropyl-6-[(pyridin-2-ylmethyl)-amino]purine (αR-18)

To a stirred solution of 2-fluoro-9-isopropyl-6-[(pyridin-2-ylmethyl)-amino]purine (**4a**, 30 mg, 1 equiv, 0.10 mmol) in *n*-BuOH/DMSO (5 mL, 4:1) at room temperature under an argon atmosphere was added DIEA (0.10 mL, 5.5 equiv, 0.57 mmol) followed by (3*RS*,4*R*)-4-amino-2,2-dimethyl-hexan-3-ol ((**R-8h**), 52 mg, 3.41 equiv, 0.36 mmol) (prepared as described for (**αR-30**): see above). The reaction mixture was placed in a preheated oil bath at 140 °C and stirred at this temperature for 72 h. The reaction mixture was allowed to cool to room temperature and the solvent was evaporated in vacuo. The residue was partitioned between EtOAc (50 mL) and brine/water (1:1, 100 mL), the aqueous phase was extracted with more EtOAc (2 × 25 mL), and the combined organic phase was washed with brine (50 mL), dried ($MgSO_4$) and evaporated in vacuo. The residue was purified by gradient column chromatography on silica gel eluted with $CHCl_3$/MeOH (100:0→98:2), to afford 2-[(1*R*)-1-ethyl-2-hydroxy-3,3-dimethylbutylamino]-9-isopropyl-6-[(pyridin-2-ylmethyl)-amino] purine (**αR-18**) as a white solid; yield: 6.3 mg (15%) (55% 1*R*,2*S*: 45% 1*R*,2*R*). ^{1}H NMR ($CDCl_3$, 250 MHz): δ 1.00–1.03 (m, 12H, –NHCH(CH_2CH_3)CH(C)$CH_3)_3$)OH), 1.56 + 1.58 (2 × d, 6H, J = 6.63 + 6.63 Hz, –CH)$CH_3)_2$), 1.69–1.89 (m, 2H, –NHCH(CH_2CH_3)CH (C($CH_3)_3$)OH), 3.56 (d, 1H, J = 1.89 Hz, –NHCH(CH_2CH_3CH(C($CH_3)_3$) OH), 3.72–3.84 (m, 1H, –NHCH(CH_2CH_3)CH(C($CH_3)_3$)OH), 4.58–4.70 (m, 1H, –CH($CH_3)_2$), 4.88–4.98 (m, 2H, –HNCH_2-Pyr), 5.22–5.39 (m, 1H, –NHCH(CH_2CH_3)CH(C($CH_3)_3$)OH), 6.70–6.80 (m, 1H, –$HN$$CH_2$-Pyr), 7.18–7.24 (m, 1H, Pyr-H), 7.38 (d, 1H, J = 7.90, Pyr-H), 7.57 (s, 1H, –N=CH–N–), 7.63–7.70 (m, 1H, Pyr-H), 8.61 (d, 1H, J = 4.90 Hz, Pyr-H). FABMS m/z (relative intensity): 412 ($[M+H]^+$, 100), 324 (70). Accurate mass (M+H): actual: 412.2825, measured: 412.2835.

4.3.24. 2-[(1*R*,2*RS*)-1-Ethyl-2-hydroxy-3,3-dimethylbutylamino]-9-isopropyl-6-[(pyridin-3-ylmethyl)-amino]purine (αR-24)

To a stirred solution of 2-fluoro-9-isopropyl-6-[(pyridin-3-ylmethyl)-amino]purine (**4b**, 30 mg, 1 equiv, 0.10 mmol) in *n*-BuOH/DMSO (5 mL, 4:1) at room temperature under an argon atmosphere was added DIEA (0.10 mL, 5.5 equiv, 0.57 mmol) followed by (3*RS*,4*R*)-4-amino-2,2-dimethyl-hexan-3-ol ((**R-8h**), 52 mg, 3.41 equiv, 0.36 mmol) (prepared as described for (**αR-30**): see above). The reaction mixture was placed in a preheated oil bath at 140 °C and stirred at this temperature for 72 h. The reaction mixture was allowed to cool to room temperature and the solvent was evaporated in vacuo. The residue was partitioned between EtOAc (50 mL) and brine/water (1:1, 100 mL), the aqueous phase was extracted with more EtOAc (2 × 25 mL), and the combined organic phase was washed with brine (50 mL), dried ($MgSO_4$) and evaporated in vacuo. The residue was purified by gradient column chromatography on silica gel eluted with

$CHCl_3$/MeOH (100:0→98:2), to afford 2-[(1*R*)-1-ethyl-2-hydroxy-3,3-dimethylbutylamino]-9-isopropyl-6-[(pyridin-3-ylmethyl)-amino]purine (**αR-24**) as a white solid; yield: 7.3 mg (17%) (55% 1*R*,2*S*: 45% 1*R*,2*R*). ^{1}H NMR ($CDCl_3$, 250 MHz): δ 0.99–1.03 (m, 12H, –NHCH(CH_2CH_3)CH(C)CH_3)$_3$OH), 1.56 + 1.58 (2 × d, 6H, *J* = 6.63 + 6.79 Hz, –CH)CH_3)$_2$), 1.69–1.91 (m, 2H, –NHCH(CH_2CH_3)CH(C(CH_3)$_3$)OH), 3.55 (d, 1H, *J* = 1.74 Hz, –NHCH(CH_2CH_3)*CH*(C(CH_3)$_3$)OH), 3.76–3.87 (m, 1H, –NH*CH*(CH_2CH_3)CH(C(CH_3)$_3$)OH), 4.57–4.70 (m, 1H, –*CH*(CH_3)$_2$), 4.80–4.89 (m, 2H, –HNCH_2-Pyr), 5.22–5.35 + 6.07–6.23 (m, 1H, –*NH*CH(CH_2CH_3)CH(C(CH_3)$_3$)OH), 7.24–7.36 (m, 3H, 2 × Pyr-H + –N=*CH*–N–), 7.55 (s, 1H, Pyr-H), 7.74 (d, 1H, *J* = 7.58 Hz, Pyr-H), 8.50–8.74 (m, 1H, *HN*CH_2-Pyr). FABMS *m/z* (relative intensity): 412 ([M+H]$^+$, 100), 324 (80). Accurate mass (M+H): actual: 412.2825, measured: 412.2835.

4.3.25. 6-Benzylamino-2-[(1*R*)-1-ethyl-2-hydroxy-2-methylpropylamino]-9-isopropylpurine (αR-31)

To a stirred solution of 6-benzylamino-2-fluoro-9-isopropylpurine (**4d**, 20 mg, 1 equiv, 0.07 mmol) in *n*-BuOH/DMSO (1.25 mL, 4:1) at room temperature under an argon atmosphere was added DIEA (0.25 mL, 20.4 equiv, 1.43 mmol) followed by (3*R*)-3-amino-2-methyl-pentan-2-ol ((**R-11**), 22 mg, 2.68 equiv, 0.19 mmol). The reaction mixture was placed in a preheated oil bath at 140 °C and stirred at this temperature for 72 h. The reaction mixture was allowed to cool to room temperature and the solvent was evaporated in vacuo. The residue was partitioned between EtOAc (50 mL) and brine/water (1:1, 100 mL), the aqueous phase was extracted with more EtOAc (2 × 50 mL), and the combined organic phase was washed with brine (50 mL), dried ($MgSO_4$) and evaporated in vacuo. The residue was purified by gradient column chromatography on silica gel eluted with hexane/ether/MeOH (50:50:0→50:50:2) to afford 6-benzylamino-2-[(1*R*)-1-ethyl-2-hydroxy-2-methylpropylamino]-9-isopropylpurine (**αR-31**) as a white solid; yield: 4.2 mg (16%). ^{1}H NMR ($CDCl_3$, 250 MHz): δ 1.01 (t, 3H, *J* = 7.50 Hz, –NHCH(CH_2CH_3)C(CH_3)$_2$OH), 1.23 + 1.30 (2 × s, 6H, –NHCH(CH_2CH_3)C)CH_3)$_2$OH), 1.57 (d, 6H, *J* = 6.79 Hz, –CH)CH_3)$_2$), 1.68–1.89 (m, 2H, –NHCH(CH_2CH_3)C(CH_3)$_2$OH), 3.69–3.86 (m, 1H, –NH*CH*(CH_2CH_3)C(CH_3)$_2$OH), 4.58–4.74 (m, 1H, –*CH*(CH_3)$_2$), 4.75–4.94 (m, 2H, –HNCH_2-Bz), 7.22–7.45 (m, 6H, 5 × Bz-*H* + *HN*CH_2-Bz), 7.57 (s, 1H, –N=*CH*–N). FABMS *m/z* (relative intensity): 383 ([M+H]$^+$, 75), 323 (60), 308 (20), 246 (20), 192 (60), 176 (100), 165 (40). Accurate mass (M+H): actual: 383.2559, measured: 383.2544.

4.3.26. 2-[(1*R*)-1-Ethyl-2-hydroxy-2-methylpropylamino]-9-isopropyl-6-[(pyridin-2-ylmethyl)-amino]purine (αR-19)

To a stirred solution of 2-fluoro-9-isopropyl-6-[(pyridin-2-ylmethyl)-amino]purine (**4a**, 20 mg, 1 equiv, 0.07 mmol) in n-BuOH/DMSO (1.25 mL, 4:1) at room temperature under an argon atmosphere was added DIEA (0.25 mL, 20.5 equiv, 1.44 mmol) followed by (3*R*)-3-amino-2-methyl-pentan-2-ol ((**R-11**), 22 mg, 2.7 equiv, 0.19 mmol). The reaction mixture was placed in a preheated oil bath at 140 °C and stirred at this temperature for 72 h. The reaction mixture was allowed to cool to room temperature and the solvent was evaporated in vacuo. The residue was partitioned between EtOAc (50 mL) and brine/water (1:1, 100 mL), the aqueous phase was extracted with more EtOAc (2 × 25 mL), and the combined organic phase was washed with brine (50 mL), dried ($MgSO_4$) and evaporated in vacuo. The residue was purified by gradient column chromatography on silica gel eluted with $CHCl_3$/MeOH (100:0→98:2), to afford 2-[(1*R*)-1-ethyl-2-hydroxy-2-methylpropylamino]-9-isopropyl-6-[(pyridin-2-ylmethyl)-amino]purine (**αR-19**) as a white solid; yield: 3.7 mg (14%). ^{1}H NMR ($CDCl_3$, 250 MHz): δ 1.01 (t, 3H, *J* = 7.35 Hz, –NHCH(CH_2CH_3)C(CH_3)$_2$OH), 1.22 + 1.30 (2 × s, 6H, –NHCH(CH_2CH_3)C)CH_3)$_2$OH), 1.57 (d, 6H, *J* = 6.79 Hz, –CH)CH_3)$_2$), 1.69–1.88 (m, 2H, –NHCH(CH_2CH_3)-C(CH_3)$_2$OH), 3.68–3.82 (m, 1H, –NH*CH*(CH_2CH_3)C(CH_3)$_2$OH), 4.59–4.72 (m, 1H, –*CH*(CH_3)$_2$), 4.86–5.03 (m, 2H, –HNCH_2-Pyr), 6.88–7.09 (m, 1H, –*HN*CH_2-Pyr), 7.20–7.25 (m, 1H, Pyr-H), 7.40 (d, 1H, *J* = 7.74, Pyr-H), 7.59 (s, 1H, –N=*CH*–N–), 7.65–7.72 (m, 1H, Pyr-H), 8.61 (d, 1H, *J* = 4.42 Hz, Pyr-H). FABMS *m/z* (relative intensity): 384 ([M+H]$^+$, 100), 324 (80), 307 (30), 193 (50), 176 (90), 165 (35). Accurate mass (M+H): actual: 384.2512, measured: 384.2494.

4.3.27. 2-[(1*R*)-1-Ethyl-2-hydroxy-2-methylpropylamino]-9-isopropyl-6-[(pyridin-3-ylmethyl)-amino]purine (αR-25)

To a stirred solution of 2-fluoro-9-isopropyl-6-[(pyridin-3-ylmethyl)-amino]purine (**4b**, 20 mg, 1 equiv, 0.07 mmol) in *n*-BuOH/DMSO (1.25 mL, 4:1) at room temperature under an argon atmosphere was added DIEA (0.25 mL, 20.5 equiv, 1.44 mmol) followed by (3*R*)-3-amino-2-methyl-pentan-2-ol ((**R-11**), 22 mg, 2.7 equiv, 0.19 mmol). The reaction mixture was placed in a preheated oil bath at 140 °C and stirred at this temperature for 72 h. The reaction mixture was allowed to cool to room temperature and the solvent was evaporated in vacuo. The residue was partitioned between EtOAc (50 mL) and brine/water (1:1, 100 mL), the aqueous phase was extracted with more EtOAc (2 × 25 mL), and the combined organic phase was washed with brine (50 mL), dried ($MgSO_4$) and evaporated in vacuo. The residue was purified by gradient column chromatography on silica gel eluted with $CHCl_3$/MeOH (100:0→98:2), to afford 2-[(1*R*)-1-ethyl-2-hydroxy-2-methylpropylamino]-9-isopropyl-6-[(pyridin-3-ylmethyl)-amino]purine (**αR-25**) as a white solid; yield: 3.7 mg (14%). ^{1}H NMR ($CDCl_3$, 250 MHz): δ 1.00 (t, 3H, *J* = 7.27 Hz, –NHCH(CH_2CH_3)C(CH_3)$_2$OH), 1.21 + 1.31 (2 × s, 6H, –NHCH(CH_2CH_3)C)CH_3)$_2$OH), 1.57 (d, 6H, *J* = 6.63 Hz, –CH)CH_3)$_2$), 1.69–1.89 (m, 2H, –NHCH(CH_2CH_3)-C(CH_3)$_2$OH), 3.66–3.83 (m, 1H, –NH*CH*(CH_2CH_3)C(CH_3)$_2$OH), 4.59–4.73 (m, 1H, –*CH*(CH_3)$_2$), 4.77–5.00 (m, 2H, –HNCH_2-Pyr), 7.26–7.46 (m, 3H, 2 × Pyr-H + –N=*CH*–N–), 7.49–7.69 (m, 1H, Pyr-H), 7.78 (d, 1H, *J* = 7.10 Hz, Pyr-H). FABMS *m/z* (relative intensity): 384 ([M+H]$^+$, 65), 366 (23), 324 (100), 217 (35), 192 (55). Accurate mass (M+H): actual: 384.2512, measured: 384.2494.

4.3.28. 6-Benzylamino-2-[(*R*)-1-ethyl-2-hydroxyethylamino]-9-isopropylpurine (αR-1)

To a stirred solution of 6-benzylamino-2-fluoro-9-isopropylpurine (**4d**, 0.50 g, 1 equiv, 1.75 mmol) in *n*-BuOH/DMSO (10 mL, 4:1) at room temperature under an argon atmosphere was added DIEA (3.0 mL, 9.82 equiv, 17.22 mmol) followed by (*R*)-(−)-2-aminobutan-1-ol (**R-5**) (1.6 mL, 10 equiv, 17.0 mmol). The reaction mixture was placed in a preheated oil bath at 140 °C and stirred at this temperature for 48 h, when TLC DCM/ether/MeOH (55:40:5) indicated that the reaction had gone to completion. The reaction mixture was allowed to cool to room temperature and the solvent was evaporated in vacuo. The residue was partitioned between DCM (100 mL) and water (150 mL), the aqueous phase was extracted with more DCM (2 × 50 mL), and the combined organic phase was washed with brine (50 mL), dried ($MgSO_4$) and evaporated in vacuo. The residue was purified by gradient column chromatography on silica gel eluted with DCM/ether/MeOH (50:50:0→50:50:1) to afford 6-benzylamino-2-[(*R*)-1-ethyl-2-hydroxyethylamino]-9-isopropylpurine (**αR-1**) as a white solid; yield: 0.58 g (93%). Mp 104–105 °C. ^{1}H NMR (DMSO-d_6, 250 MHz): δ 0.82 (t, 3H, *J* = 7.33 Hz, –NHCH(CH_2CH_3)CH_2OH), 1.45 (m, 8H, –NHCH(CH_2CH_3)CH_2OH + –CH)CH_3)$_2$), 3.30–3.42 (m, 2H, –NHCH(CH_2CH_3)CH_2OH), 3.70 (m, 1H, –NH*CH*(CH_2CH_3)CH_2OH), 4.51 (m, 4H, –HNCH_2-Bz, OH + –*CH*(CH_3)$_2$), 5.80 (d, 2H, *J* = 8.37 Hz, –N*H*CH(CH_2CH_3)CH_2OH), 7.17–7.35 (m, 5H, Bz), 7.76

(b s, 2H, –N=*CH*–N– + –*HN*CH_2-Bz). FABMS *m/z* (relative intensity): 355 ($[M+H]^+$, 100), 323 (52), 154 (10), 134 (14). Accurate mass (M+H): actual: 355.2246, measured: 355.2260. Microanalysis (expected: measured) $C_{19}H_{26}N_6O$: C; 64.38: 64.21, H; 7.39: 7.44, N; 23.71: 23.37.

4.3.29. (2*R*,3*S*)-3-(Dibenzylamino)pentan-2-ol (S-36)

To a stirred solution of (*S*)-(+)-2-aminobutan-1-ol **S-5** (5 g, 56.18 mmol) in dry acetonitrile (100 ml) was added dry powdered potassium carbonate (31 g, 224.72 mmol) followed by benzyl bromide (19 g, 111.11 mmol). The reaction was stirred at room temperature for 24 h. The solvent was removed under vaccuo and the residue was taken up in ethyl acetate (100 ml) and water (100 ml). The organic phase was washed again with water, dried (Na_2SO_4) and concentrated to provide (*S*)-2-(dibenzylamino)butan-1-ol (**S-34**) as slightly yellow oil (14.5 g, 97.3%). δ_H (250 MHz, $CDCl_3$) 0.98 (3 H, t, *J* = 7.5, $CHCH_2CH_3$), 1.38–1.2 (1H, m, $CHCHHCH_3$), 1.94–1.78 (1H, m, $CHHCH_3$), 2.83–2.71 (1H, m, $CHCHHCH_3$), 3.22 (1 H, s, br, O*H*), 3.65–3.4 (2H, m, CH_2OH), 3.47 (2 H, d, *J* = 17.5, 2 × CH*H*Ph), 3.94 (2 H, d, J 17.5, 2 × CH*H*Ph), 7.46–7.26 (10 H, m, 2 × C_6H_5); δ_C (250 MHz, $CDCl_3$) 139.42 (2 × C), 129.1 (2 × CH), 128.52 (2 × CH), 127.25 (2 × CH), 61.97 (CH), 60.67 (CH_2), 53.23 (CH_2), 17.92 (CH_2), 11.83 (CH_3); m/z 270.2 (M+H). A 2 M solution of oxalyl chloride in dichloromethane (3.18 ml, 6.36 mmol) was cooled to −78 0 °C and diluted with dry dichloromethane (20 ml) under dry nitrogen. A solution of dimethylsulfoxide (1 g, 12.72 mmol) in anhydrous dichloromethane was added dropwise to the cooled stirred solution. The reaction was stirred for a further 1 h after completion of addition. A solution of the foregoing **S-34** (1.43 g, 5.3 mmol) in dichloromethane was added over 5 min. After 10 min, diisopropylethylamine (2.73 g, 21.2 mmol) was added. The reaction was allowed to warm to room temperature and left stirring for 1 h. It was cooled to 0 °C and ethyl acetate/water (50 ml: 50 ml) was added. The organic layer was washed with water (50 ml), brine (50 ml) dried ($MgSO_4$) and concentrated. The product was purified by flash silica column chromatography (ethyl acetate/hexane 1:4) to provide (*S*)-2-(dibenzylamino)butanal (**S-35**) (1.28 g, 90.5%). δ_H (250 MHz, $CDCl_3$) 0.88 (3H, t, *J* = 7.5, $CHCH_2CH_3$), 1.77–1.54 (2H, m, CH_2CH_3), 2.99 (1H, t, *J* = 7.5, $CHCH_2CH_3$), 3.74–3.57 (4H, m, 2 × CH_2Ph), 7.31–7.11 (10H, m, 2 × C_6H_5) 9.64 (1H, s, C*H*O); δ_C (250 MHz, $CDCl_3$) 203.9 (CO), 139.33 (2 × C), 128.99 (4 × CH), 128.45 (4 × CH), 127.3 (2 × CH), 68.46 (CH), 54.85 (CH_2), 17.44 (CH_2), 11.83 (CH_3); *m/z* 268.2 (M+H). To a stirred suspension of CuBr·SMe_2 (1.54 g, 7.5 mmol) in anhydrous ether under an argon atmosphere at −78 °C, was added methyllithium (1.6 M in ether, 9.4 ml, 15 mmol) dropwise. After the addition was complete, the reaction was allowed to warm to room temperature. The reaction was recooled to −78 °C and a solution of the foregoing **S-35** (1 g, 3.75 mmol) in ether (20 ml) was added dropwise. After the addition, continued stirring for 2 h The reaction was then quenched with a saturated aqueous solution of NH_4Cl (10 ml). The reaction mixture was extracted with ether (2 × 30 ml) and the combined organic phase washed with brine (20 ml), dried ($MgSO_4$) and evaporated in vacuo. The residue was purified by flash silica gel gradient column chromatography, eluted with hexane/ethyl acetate (100:0→80:20) to afford the product **S-36** as a light yellow oil (0.95 g, 89%) as the only isomer. δ_H (250 MHz, $CDCl_3$) 1.05 (3H, t, *J* = 7.5, $CHCH_2CH_3$), 1.25 (3H, d, *J* = 7.5, $CH(CH_3)OH$), 1.6–1.49 (1H, m, $CHHCH_3$), 1.88–1.73 (1H, m, $CHHCH_3$), 2.41 (1H, s, br, OH), 2.66–2.59 (1H, m, $CHCH_2CH_3$), 3.85–3.65 (4H, m, 2 × CH_2Ph), 4.05–3.9 (1H, m, C*H*OH), 7.41–7.25 (10H, m, ArH) δ_C (250 MHz, $CDCl_3$) 140.05 (2 × C), 128.98 (4 × CH), 128.37 (4 × CH), 127.3 (2 × CH), 66.81 (CH), 63.65 (CH), 55.41 (CH_2), 20.63 (CH_3) 18.44 (CH_2), 12.5 (CH_3). HMRS: $C_{19}H_{25}NO$ requires (M+H) 283.1936; found m/z 283.1966

4.3.30. (2R,3S)-3-aminopentan-2-ol (S-37)

To a solution of the foregoing dibenzylamine **S-36** (1 g, 3.53 mmol) in methanol (15 ml) was added 20% palladium hydroxide on carbon (200 mg). The suspension was stirred under hydrogen gas at atmospheric pressure for 72 h. when TLC analysis indicated that debenzylation was complete. After filtration through Celite and evaporation of solvents the amine **S-37** was isolated as a single isomer in 61% yield. ^1H NMR (DMSO-d_6, 250 MHz): δ 0.915 (t, 3H, *J* = 7.50 Hz, $NH_2CH(CH_2CH_3)CH(CH_3)OH$), 1.06 (d, *J* = 6.5 Hz), $NH_2CH(CH_2CH_3)CH(CH_3)OH$), 1.5 (m, 2H, NH_2CH-$(CH_2CH_3)CH(CH_3)OH$), 2.85 (m, 1H, $NH_2CH(CH_2CH_3)CH(CH_3)OH$), 3.75 (m, 1H, $NH_2CH(CH_2CH_3)CH(CH_3)OH$), 7.75 (br s, 2H, NH_2). T-Boc derivative: HMRS: $C_{10}H_{21}NO_3$ requires (M+H) 203.1521; found m/z 203.1534.

4.3.31. 2-[(1*S*,2*R*)-1-Ethyl-2-hydroxypropylamino]-9-isopropyl-6-[(pyridin-3-ylmethyl)-amino]purine (αSβR-21; CCT68127)

To a stirred solution of 2-fluoro-9-isopropyl-6-[(pyridin-3-ylmethyl)-amino]purine (2.48 g, 8.67 mmol) in *n*-BuOH/DMSO (50 ml, 4:1) at room temperature under an argon atmosphere was added diisopropylethylamine (16.5 ml, 10 equiv, 86.7 mmol) followed by (2*R*,3*S*)-3-amino-pentan-2-ol (4.91 g, 5.5 equiv, 47.7 mmol). The flask was fitted with a condenser and the reaction mixture was placed in a preheated oil bath at 140 °C and stirred at this temperature for 72 h. The reaction mixture was allowed to cool to room temperature and the solvent was evaporated in vacuo. The residue was partitioned between ethyl acetate (100 ml) and water (50 ml), the aqueous phase was extracted with more ethyl acetate (2 × 25 ml), and the combined organic phase was washed with brine (50 ml), dried ($MgSO_4$) and evaporated in vacuo. The residue was purified by flash column chromatography on silica gel eluted with $CHCl_3$/MeOH (97:3), to afford the pure product as a white powder (1.7 g, 53%). ^1H NMR (DMSO-d_6, 250 MHz/$CDCl_3$: δ 0.81/1.02 (3H, t, *J* = 6.9, $CHCH_2CH_3$), 1.01/1.13 (3H, d, *J* = 6.2, $CHCH_3OH$), 1.43–1.38/1.47 (m, 1H, $NHCHCHHCH_3$), 1.45/1.52 (6H, d, *J* = 6.7, $CH)CH_3)_2$), 1.7–1.65/1.57 (m, 1H, –$NHCHCHHCH_3$), 3.6/3.8 (1H, s, br, $NHCHCH_2CH_3$), 3.78–3.7/3.9 (m, 1H, –C*H*OH), 4.58–4.5/4.6 (1H, m, br, –$NHCH(CH_3)_2$), 4.62/4.8 (2H, s, br, $NHCH_2$Py), 5.88/4.8 (1H, d, *J* = 8.9 $CH(CH_3)OH$), 7.31–7.28/7.2 (1 H, m, Pyr-H5), 7.74/7.7 (1H, s, Pyr-H4), 7.76/7.4 (1H, s, pyrim-H8), 7.77/6.5 (1H, s, br, $HNCH_2$-Pyr), 8.4/8.5 (1H, d, *J* = 6.3, Pyr-H6), 8.59/8.6 (1 H, d, *J* = 1.6, Pyr-H2); δ_C (DMSO-d_6, 250 MHz) 159.93 (C), 154.71 (C), 149.51 (CH), 148.23 (CH), 136.51 (C), 135.65 (CH), 135.57 (CH), 123.7 (CH), 114.55 (C), 69.41 (CH), 58.45 (CH), 46.14 (CH_2), 23.19 (CH_2), 22.46 (CH_3), 20.86 (2 × CH_3), 11.35 (CH_3); δ_C ($CDCl_3$) 11.6 (CH_3CH_2C), 17.3 (CH_3CHO), 22.5 $(CH_3)_2CHN$, 25 (CH_3CH_2C), 42, weak signal, NCH_2py, 46.5 $(CH_3)_2CHN$, 59.6 (NCHCHO), 71.6 (NCHCHO), 114.6 (pyrimC5), 123.4 (pyC5), 134.5 (pyrimC3), 134.7 (pyrimC8), 135.3 (pyC4), 148.7 (pyC2), 149.3 (pyC6)150 (pyrimC2) 154.7 (pyrimC6) 160.1 (pyrimC4). *m/z* 370 ($[M+H]^+$. HMRS: $C_{19}H_{27}N_7O$ requires (M+H) 369.2277; found m/z 369.2282. Optical rotation $[\alpha]_D = -78$ (*c*1.08, chloroform).

4.3.32. 2-[(1*R*,2*S*)-1-Ethyl-2-hydroxypropylamino]-9-isopropyl-6-[(pyridin-3-ylmethyl)-amino]purine (αRβS-21)

The enantiomer of the foregoing compound was made by multistep synthesis as above but starting from **R-5**. The spectral data for the two enantiomers were essentially identical but optical rotation was equal and opposite $[\alpha]_D = +77.3$ (*c* 1.11, chloroform).

4.4. Kinase assays

CDK and other kinase assays were carried out as previously described.[44,45]

4.5. Cellular assays

Cellular assays were carried out as previously described.[37,38,43]

Pharmacological studies were carried out as previously described.[31]

4.6. Pharmacokinetic assays

Microsomal incubations consisted of mouse microsomal protein 0.2 mg (microsomes obtained from Totem Biologicals, Northampton, UK), 20 mM NADPH, 10 mM $MgCl_2$, 1.5 mM EDTA (all from Sigma, Poole, Dorset, UK) and 10 μM investigational compound in a total volume of 100 μl. Samples were incubated for 30 min and the protein precipitated with 300 μl of ice cold methanol containing olomoucine as an internal standard. Calibration curves were prepared at 0 and 10 μM in microsomes preincubated for 30 min and these were also treated with 300 μl methanol containing olomoucine. All samples were then centrifuged and the supernatant analysed by LC–MS.

Female BALB/c mice were supplied by Charles River UK Ltd (Margate, Kent, UK) and maintained on SDA Expanded Rodent diet and water ad libitum. All experiments complied with the UKCCR guidelines for animal welfare in experimental neoplasia (Workman et al., 1997).

Compounds were formulated in 0.1 M HCl in 10%DMSO 1% tween. Control animals received the vehicle alone. Groups of three mice were injected per time point. Blood was collected by cardiac puncture following transient anaesthesia with halothane at 0.083, 0.25, 0.5, 1, 2, 4, 6, and 24 h post administration. Following centrifugation at 15,000*g* for 2 min to obtain plasma, samples were stored at −20 °C until analysis. For urinary excretion studies, all analogues were administered at 50 mg/kg iv (0.1 ml/10 g).

LCMS used a Supelco (Poole, Dorset, UK) LC-ABZ column with a mobile phase of methanol (mobile phase A) and 0.1% formic acid (mobile phase B). The gradient started with initial conditions of 10% A and 90% B held for 0.5 min after sample injection, then changing in a linear fashion to 90% A and 10% B over 6 min. These conditions were held for a further 4 min and then the column returned to initial conditions for subsequent injections. The HPLC eluant flow rate was set to 1 ml/min for all analyses.

The compounds were detected using an LCQ ion trap mass spectrometer. Source parameters were: mode of operation positive ion electrospray ionisation (analytes are detected as protonated ionised species MH^+), the heated capillary voltage was set to +4 to 4.5 kV and the heated capillary temperature was set to 250 to 280 °C. The mass spectrometer scan range was set to 50–750 m/z with a scan speed of 100 ms. Specific ions of interest can be displayed using instrumental software as selected ion traces once samples are analysed.

To analyse the results, selected ion traces of the MH^+ ions of the investigational compound and internal standard were extracted and the area of the relevant peaks obtained. The peak area ratio (investigational compound/internal standard) of the test incubation was then compared with the peak area ratios obtained for the calibration curve of the investigational compound. From these values the concentration of investigational compound left at the end of the incubation could be determined (e.g. XuM) and therefore the amount of investigational compound metabolised obtained (e.g. 10-XuM). From this the percentage metabolism was obtained (e.g. $Y\% = ((10 - \mathrm{XuM}) \times 100)/10\ \mu M$). Compounds were then compared as the % metabolised in 30 min to determine which compounds showed the greatest metabolic stability. Metabolite identification was performed by LC–MS. Fragmentation patterns were determined manually and position of metabolism deduced by interpretation of the fragmentation pattern of the parent and the metabolite.

Chromatography was performed using a 50 mm × 4.6 mm ID 5 μm zwitterionic ABZ+ column (Supelco, Poole, Dorset, UK) and a gradient of 20% MeOH in formic acid to 100% MeOH over 3 min, followed by an isocratic period at a flow rate of 0.6 ml/min. The total run time was 7 min. Detection was done by MS–MS by multiple reaction monitoring performed on a TSQ700 triple quadrupole mass spectrometer equipped with an electrospray source and operated in positive mode (Thermoquest Ltd, Hemel Hempstead, Herts, UK). The heated capillary was maintained at 280 °C and at 4.5 kV voltage. Optimisation of the fragmentation was performed and the most intense ions were monitored for each analytical transition. Calibration standards (10, 50, 100, 200, 500, 1000, 2000, 5000, 10,000 nM) were prepared in blank mouse plasma. Following the addition of internal standard (30 μL of 2 μM solution of Olomoucine) plasma proteins were precipitated with 300 μL methanol. Samples were then centrifuged and 20 μL of supernatant was injected onto the column. Peak area was plotted against concentration and unknown concentrations derived from the linear curve (analysed with Prism 2.01, Graphpad Software, San Diego, CA, USA).

Pharmacokinetic parameters were evaluated by non-compartmental analysis (model 200 for po and ip administration and 201 for iv administration) using WinNonlin®Professional Version 3.2 (Pharsight Corporation, Mountain View, California, USA). For simulation studies, parameters were derived from compartmental analysis.

4.7. In vivo efficacy studies

Female NCR athymic mice 6–8 weeks of age were implanted bilaterally with 2 million cells on day 0. Once the tumours had reached a mean diameter of 4–5 mm (day 5) dosing commenced with CDK inhibitors at previously determined, well-tolerated oral doses. Compounds were dissolved in water as HCl salts or DMSO/water/HCl (**1** only) and frozen as daily aliquots which were thawed and used as required. All compounds dissolved to clear solution. 0.1 ml of solution was administered per 10 g body weight. Mice were weighed and tumours measured three times weekly and tumour volumes calculated.[37] The study was terminated on day 12, when tumours were excised and weighed. Studies were carried out in accordance with the NCRI guidelines.[46]

We thank Sarah Langdon for assistance with protein modelling and Dr Maggie Liu for assistance with NMR spectra. The work of the Cancer Research UK Cancer Therapeutics Unit is funded primarily by Cancer Research UK [CUK] Grant C309/A2187. Paul Workman is a Cancer Research UK Life Fellow. We thank Professor Julian Blagg (ICR) and Dr. David Blake (Cyclacel) for helpful discussions, and Dr. Peter Horton at the EPSRC National Crystallography Service, University of Southampton for deriving the structure shown in Figure 4.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2011.08.051.

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