Supplementary Information

Structure-guided enhancement of selectivity of chemical probe inhibitors targeting bacterial seryl-tRNA synthetase

Ricky Cain,[a]+ Ramya Salimraj,[a]+ Avinash S. Punekar,[a] Dom Bellini,[a] Colin W. G. Fishwick ,[b] Lloyd Czaplewski,[c] David J. Scott,[d,e] Gemma Harris,[e] Christopher G. Dowson,[a] Adrian J. Lloyd,[a] David I. Roper[a]

[a] School of Life Sciences, University of Warwick, Gibbet Hill Road, Coventry, CV4 7AL, United Kingdom.

[b] School of Chemistry, University of Leeds, Leeds, LS2 9JT, United Kingdom.

[c] Chemical Biology Ventures Limited, Abingdon, OX14 1XD, United Kingdom

[d] School of Biosciences, University of Nottingham, Nottingham, LE12 5RD, United Kingdom

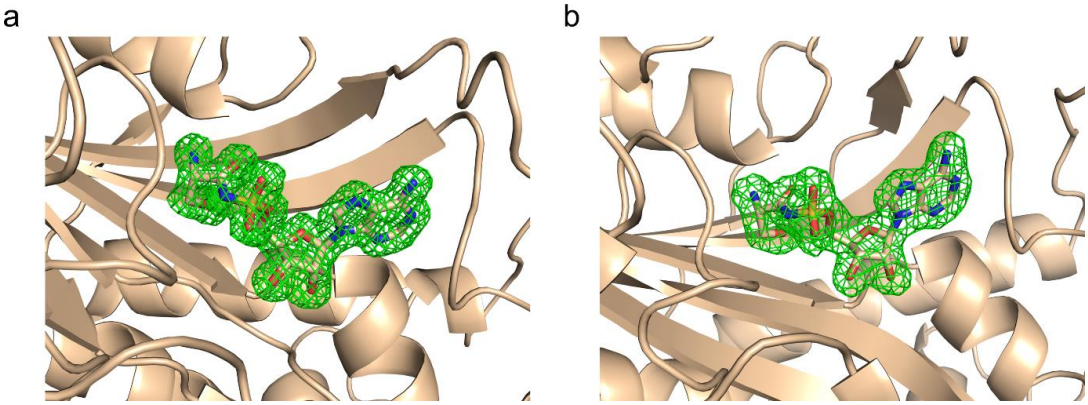
[e] ISIS Spallation Neutron and Muon Source and the Research Complex at Harwell, Rutherford Appleton Laboratory, Oxfordshire OX11 0FA, United Kingdom

+ These authors contributed equally to this work

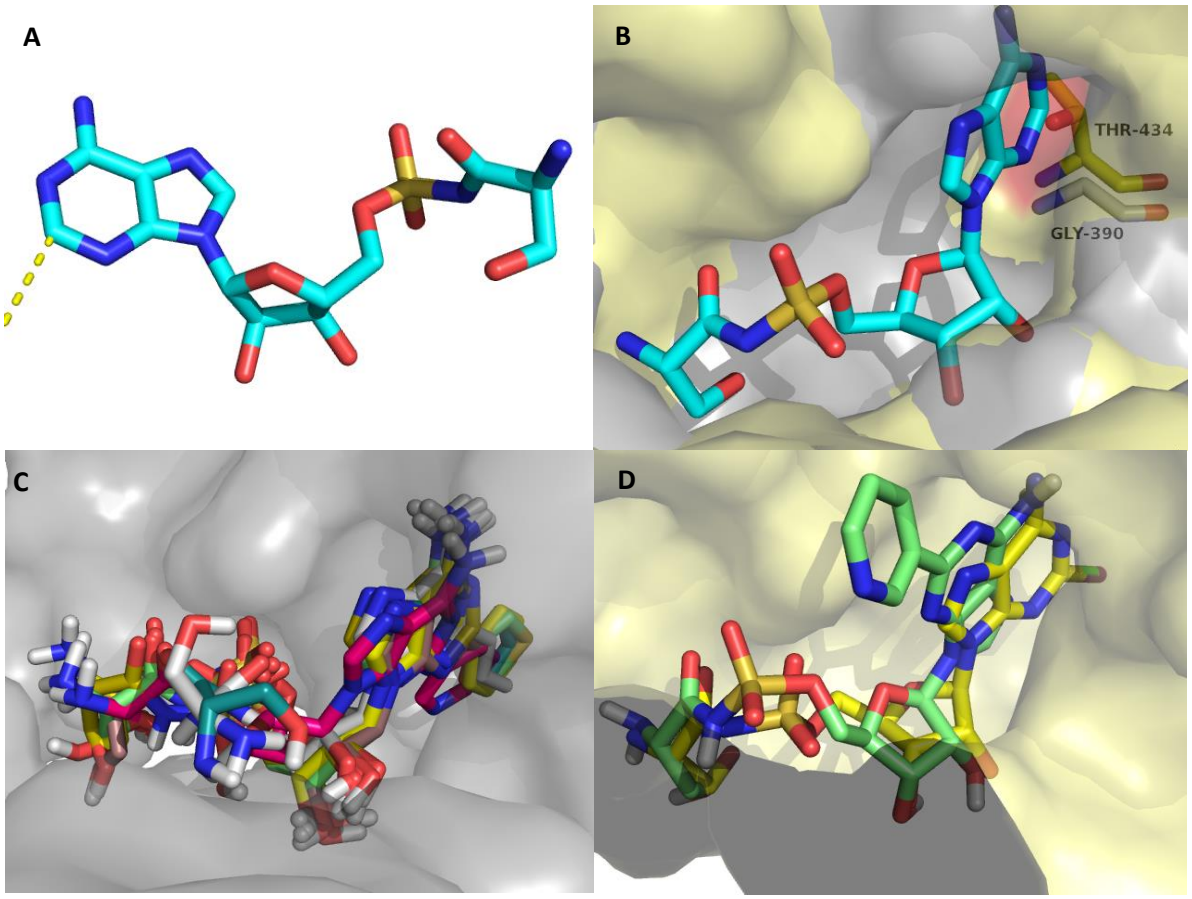
E-mail: david.roper@warwick.ac.uk

A) List of Figures and Schemes

***Supplementary Figure 1*:** Overlay of F_o-F_c omit map of SerSA in *Ec*SerRS chain A (a) and *Sa*SerRS (b) active site contoured at σ 3.



***Supplementary Figure 2*:** Binding modes of designed seryl sulfamoyl adenylate selectivity probes. (a) 3D spatial representation of the seryl sulfamoyl adenylate derivatives. (b) Structural overlay of *S. aureus* SerRS (Yellow, PDB ID 6R1N) and human cytoplasmic SerRS (Grey, PDB ID: 4L87) active site showing the key residue change near the 2 position of the sulfamoyl adenylate inhibitor from Gly390 in the bacterial form to Thr434 in the human form. (c) Predicted binding modes of seryl sulfamoyl adenylates to *E. coli* SerRS (PDB ID 6R1M) using AutoDock 4.2. (d) Predicted binding modes of seryl sulfamoyl adenylates to human cytoplasmic SerRS using AutoDock 4.2.



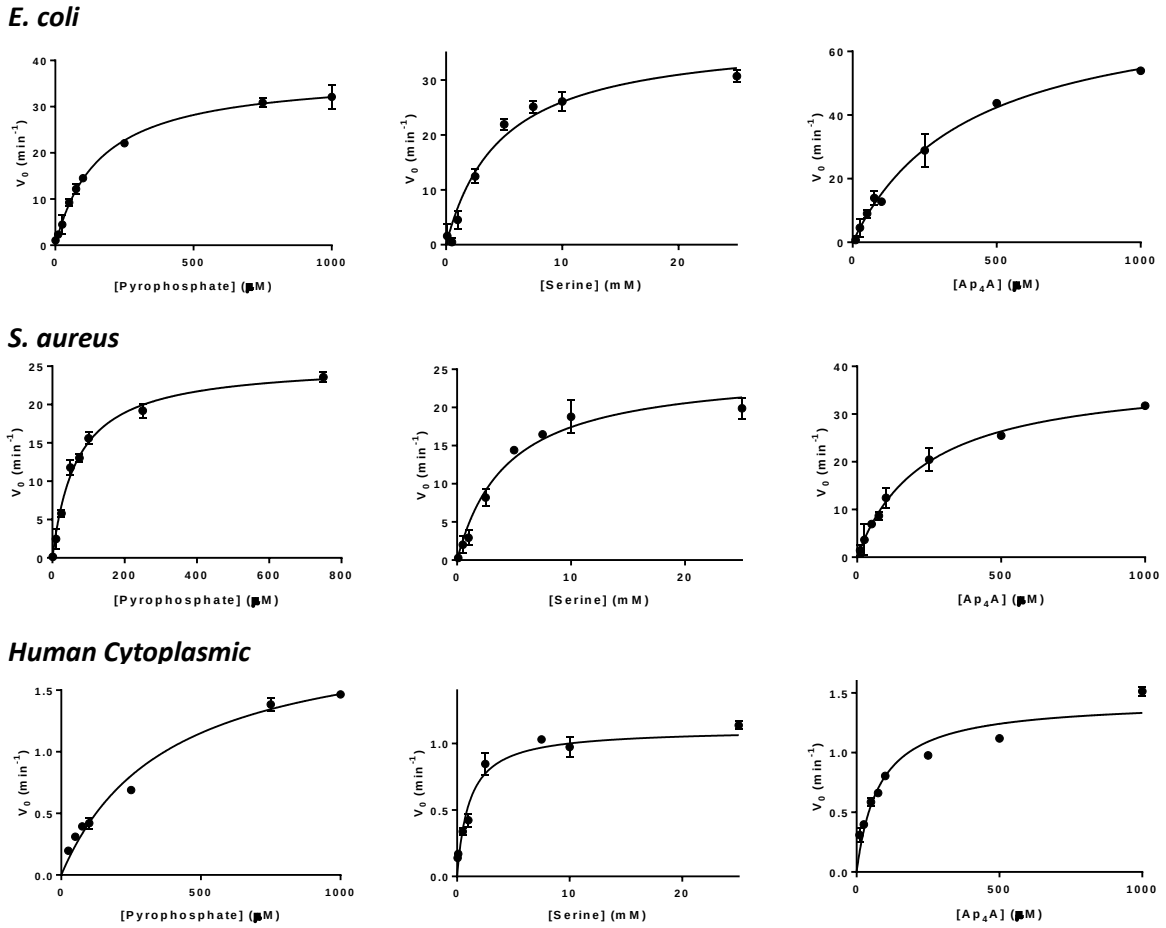
Supplementary Figure 3a: Sequence alignment prepared by EMBL-EBI MUSCLE[1] of representative Gram-positive and Gram-negative bacterial pathogens against cytoplasmic and mitochondrial variants of the human, bovine and mouse SerRS enzymes. The blue boxed feature indicates the conserved glycine (396 in *Ec*SerRS) found in the bacterial enzymes which is replaced by threonine (434 in the cytoplasmic *Hs*SerRS) and proline (458 in the mitochondrial *Hs*SerRS) in the cytoplasmic and mitochondrial eukaryotic enzymes respectively. A consensus sequence across all SerRS enzymes in this region is indicated.

```
Homo sapiens(cyto)          KVEFVHMLNATMCATTRTICAILENYQTEKG-ITV
Homo sapiens(mito)          ELQFAHTVNATACAVPRLLIALLESNQQKDGSVLV
Bos taurus(cyto)            KVEFVHMLNATMCATTRTICAILENYQTEKG-ILV
Bos taurus(mito)            ELQFAHTVNATGCAVPRLLIALLESYQQKDGSVLV
Mus musculus(cyto)          KVEFVHMLNATMCATTRTICAILENYQAEKG-IAV
Mus musculus(mito)          ELQFAHTVNATACAVPRVLIALLESNQQKDGSVLV

Escherichia coli            KTRLVHTLNGSGLAVGRTLVAVMENYQQADGRIEV
Acinetobacter baumannii     KTELVHTLNGSGLAVGRTLLAVMENYQREDGSIEI
Pseudomonas aeruginosa      KPELVHTLNGSGLAVGRTLVAVLENYQQADGSIRV
Haemophilus influenzae      KTRLVHTLNGSGLAVGRTLVAVLENYQNADGSITV
Neisseria gonorrhoeae       KNRLVHTLNGSGLAVGRTLVAVLENHQNADGSINI

Staphylococcus aureus       KPELAHTLNGSGLAVGRTFAAIVENYQNEDGTVTI
Streptococcus pneumoniae    KVKLLHTLNGSGLAVGRTVAAILENYQNEDGSVTI
Enterococcus faecium        KVQYTHTLNGSGLAVGRTVTAILENYQNEDGSVTI
Clostridium difficile       KAEYVHTLNGSGLAIGRCLAAILENYQQADGSVVV
Listeria monocytogenes      KPEYVHTLNGSGLALGRTVAAILENYQDADGSVRI
                            :      :*.:  *  * . *::*. *  .* : :
                                   NxxxxAxxR
```

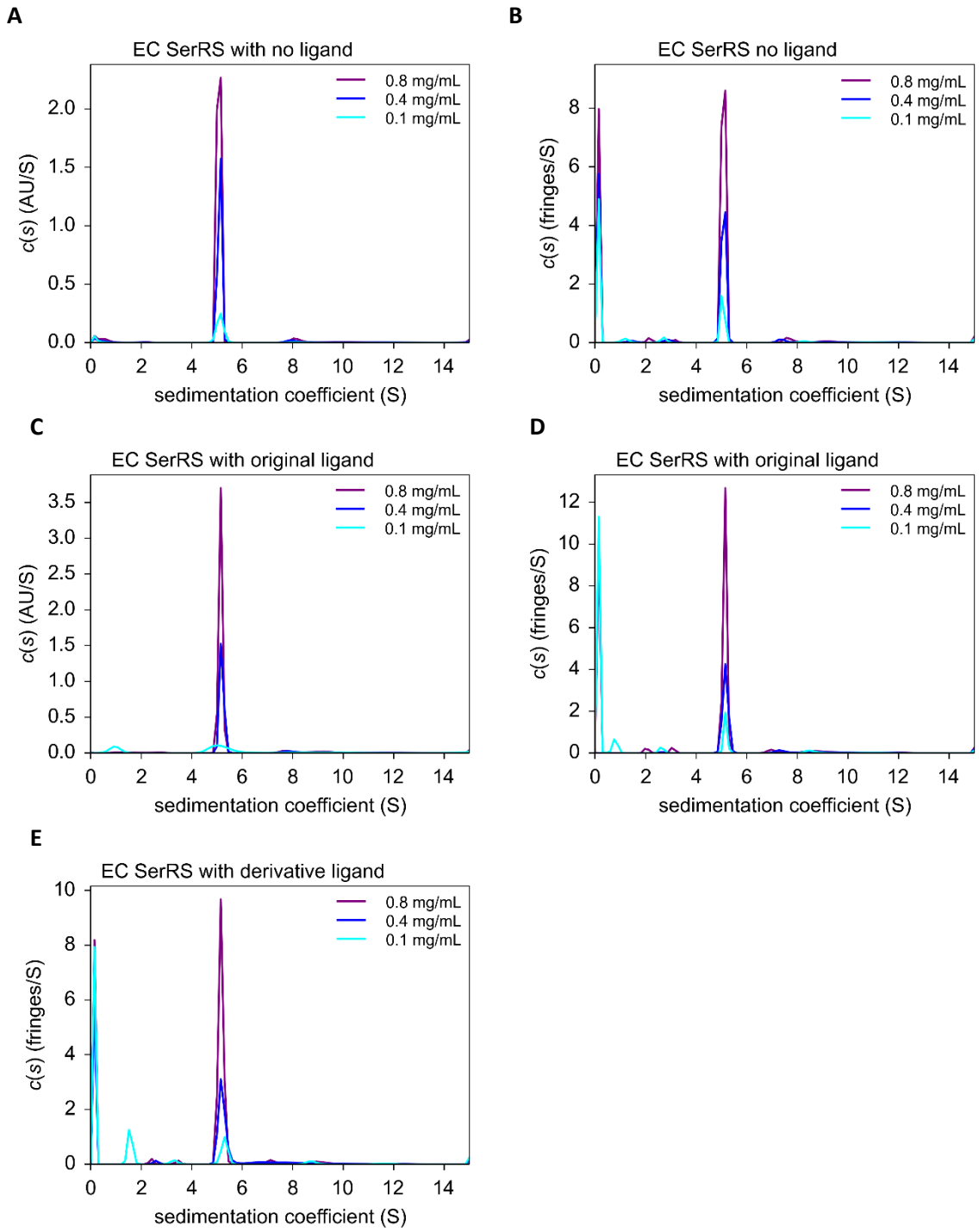
Supplementary Figure 3b: Sequence alignment prepared by EMBL-EBI MUSCLE[1] of representative Gram-positive and Gram-negative bacterial pathogens. The blue box indicate a 12 amino acid sequence within the adenylate binding pocket. The glycine indicated by the arrow at the 11th position in this sequence is replaced by threonine and proline in the cytoplasmic and mitochondrial eukaryotic enzymes as described in Supplementary Figure 2a. As described in the main text the glycine is at position 396 in *Ec*SerRS.

```
Escherichia coli            SSCSNVWDFQARRMQARCRSKSDKKTRLVHTLNGSGLAVGRTLVAVMENYQQADGRIEVP
Acinetobacter baumannii     SSCSNMGDFQARRMKARFRMD-QKKTELVHTLNGSGLAVGRTLLAVMENYQREDGSIEIP
Pseudomonas aeruginosa      SSCSNCGDFQARRMQARYRNPETGKPELVHTLNGSGLAVGRTLVAVLENYQQADGSIRVP
Haemophilus influenzae      SSCSNMWDFQARRMQARCKAKGDKKTRLVHTLNGSGLAVGRTLVAVLENYQNADGSITVP
Neisseria gonorrhoeae       SSCSNCEDFQARRMKARFKDE-NGKNRLVHTLNGSGLAVGRTLVAVLENHQNADGSINIP

Staphylococcus aureus       SSCSNCTDFQARRANIRFKRDKAAKPELAHTLNGSGLAVGRTFAAIVENYQNEDGTVTIP
Streptococcus pneumoniae    SSCSNTEDFQARRAQIRYRDEADGKVKLLHTLNGSGLAVGRTVAAILENYQNEDGSVTIP
Enterococcus faecium        SSCSNCEDFQARRAMIRYRNE-EGKVQYTHTLNGSGLAVGRTVTAILENYQNEDGSVTIP
Clostridium difficile       SSCSNFEDFQARRAGIRFKRDKKSKAEYVHTLNGSGLAIGRCLAAILENYQQADGSVVVP
Listeria monocytogenes      SSCSNFESFQARRANIRFRREPGSKPEYVHTLNGSGLALGRTVAAILENYQDADGSVRIP
                            *****   *****   * .     *    ************ . *::****. ***: :*
```

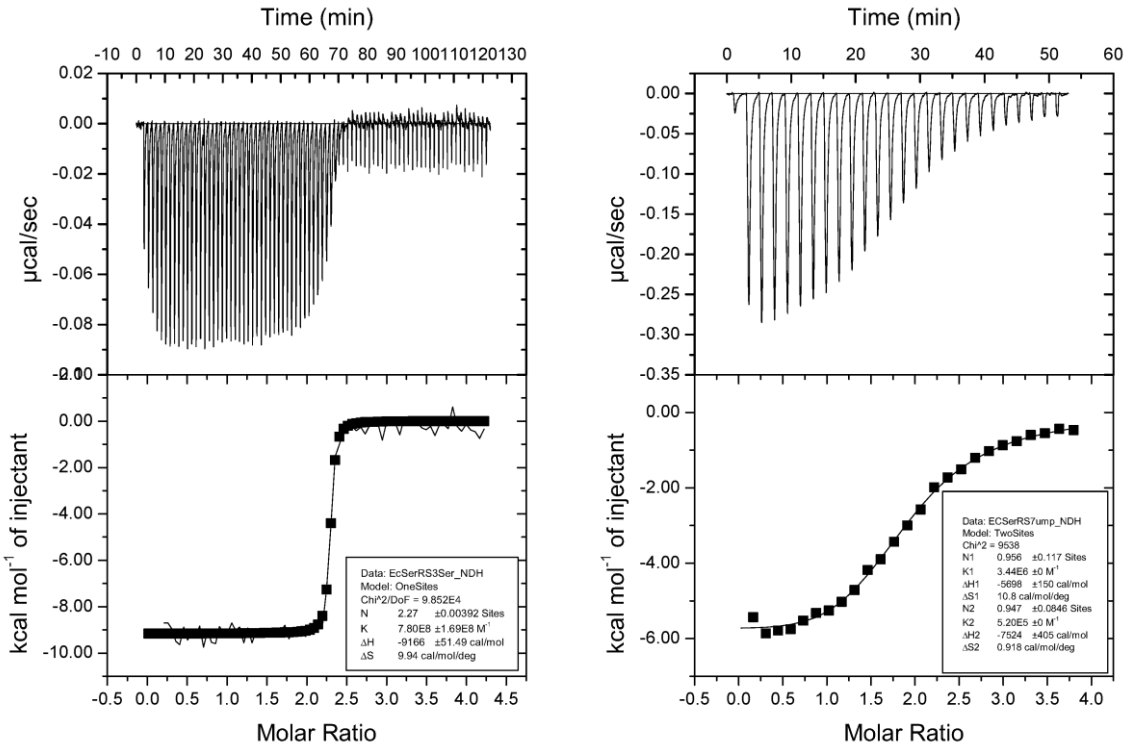
Supplementary Figure 4: Dependence of *E. coli* SerRS, *S. aureus* SerRS and human cytoplasmic SerRS on a) [Pyrophosphate], b) [Serine], and c) [Ap_4A].



Supplementary Figure 5: **Sedimentation coefficient distributions of *Ec*SerRS. A:** Absorbance data **B:** Interference data **C:** Absorbance data **D:** Interference data **E:** Interference data.



Supplementary Figure 6: **Isothermal Titration Calorimetry (ITC) curves. A:** Titration of 70 µM SerSA **1** into 3 µM *Ec*SerRS **B:** Titration of 140 µM compound **8** into 7 µM *Ec*SerRS.



Supplementary Tables

***Supplementary Table 1*:** Data collection and refinement statistics of SerSA **1** bound bacterial SerRS structures and **8** bound to *E. coli* SerRS

Data Collection	*E. coli* SerRS: SerSA	*S. aureus* SerRS: SerSA	*E. coli* SerRS: compound 8
X-ray source	DLS (I03)	DLS (I04)	DLS (I04)
Wavelength	0.9762	0.9795	0.9795
Space Group	*P*1	$C222_1$	$P6_122$
Cell Dimensions			
a, *b*, *c* (Å)	63.07, 63.52, 75.89	94.44 116.41 91.61	85.41, 85.41, 317.72
α, β, γ	75.44, 70.25, 89.20	90, 90, 90	90, 90, 120
Molecules/ asymmetric unit	2	1	1
Resolution (Å)	61.29 - 1.50 (1.53 - 1.5)	91.61 - 2.03 (2.06 - 2.03)	158.86 - 2.60 (2.64 - 2.60)
Redundancy	2.4 (2.5)	11.8 (7.7)	28.5 (30.3)
R_{merge}	0.048 (0.269)	0.106 (0.807)	0.179 (1.876)
$CC_{1/2}$	0.997 (0.829)	0.999 (0.856)	0.999 (0.949)
I/σ	9.3 (2.5)	16.1 (2.3)	17.4 (2.0)
Completeness (%)	95.5 (93.8)	99.8 (97.7)	100 (100)
Refinement			
Resolution (Å)	59.18 - 1.50	57.25 - 2.03	27.96 - 2.60
No. of reflections	163577	33037	22215
R_{work}/R_{free}	0.162/0.180	0.173/0.221	0.182/0.215
Atoms			
Protein	6831	3332	3310
Ligand	58	29	82
Solvent	1000	416	192
B factor (Å^2)			
Protein	24.18	35.51	63.01
Ligand	15.04	23.12	62.37
Solvent	38.63	45.79	70.74
RMSD			
Bonds (Å)	0.010	0.010	0.010
Angles (°)	1.03	1.05	1.09
Ramachandran (%)			
Favoured	99	99	97
Allowed	1	1	3
Outlier	0	0	0

***Supplementary Table 2*:** RMSD scores generated using PDBeFOLD[2] when comparing the full-length SerRS structures from *E. coli* (PDB ID: 6R1M), *S. aureus* (PDB ID: 6R1N), and human (PDB ID: 4L87). *E. coli* SerRS (chain A) was used as a reference for superposing other structures. Number of matched residues shown in parentheses.

	***Ec*SerRS (chain A)**	***Ec*SerRS (chain B)**	***Sa*SerRS**	***Hs*SerRS**
***Ec*SerRS (chain A)**	-	1.18 (426)	1.37 (410)	1.66 (407)

a. Lower score is better

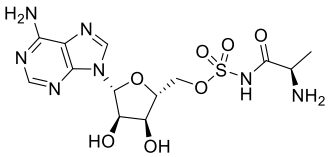
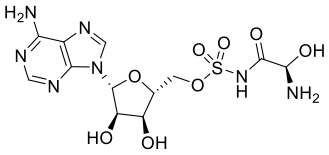
***Supplementary Table 3*:** Components of the phosphate exchange assay

Reagent	***Sa*SerRS Final Concentrations**	***Ec*SerRS Final Concentrations**	***Hs*SerRS Final Concentrations**
0.25 M HEPES, 50 mM $MgCl_2$, adjusted to pH 7.6	50 mM HEPES, 10 mM $MgCl_2$	50 mM HEPES, 10 mM $MgCl_2$	50 mM HEPES, 10 mM $MgCl_2$
0.1 M DTT	1 mM	1 mM	1 mM
2 M KCl	50 mM	50 mM	50 mM
1 M D-Glucose	10 mM	10 mM	10 mM
10 mM $NADP^+$	0.5 mM	0.5 mM	0.5 mM
Coupling Enzymes (Leuconostoc mesenteroides hexokinase & glucose 6-Phosphate dehydrogenase)	1.7 and 0.85 µM/min/mL respectively	1.7 and 0.85 µM/min/mL respectively	1.7 and 0.85 µM/min/mL respectively
DMSO (+inhibitor)	10 % (v/v)	10% (v/v)	10% (v/v)
Enzyme (aaRS)	0.343 µM	0.284 µM	7.62 µM
10 mM PPi	1mM	1 mM	1 mM
1M Serine	25 mM	25 mM	25 mM
AP4A	250 µM	250 µM	250 µM
Triton X-100 (10% v/v)	2 µl	2 µl	2 µl
Water	85 µl	85 µl	40 µl

***Supplementary Table 4*:** Km and Kcat Values

AaRS	$[AaRS]_{assay}$ (μM)	nonvaried substrate	[nonvaried substrate] (mM)	varied substrate	K_m^{app} (μM)	K_{cat}^{app} (min^{-1})	K_{cat}^{app}/K_m^{app} (min^{-1} μM^{-1})	goodness of fit (r^2)
*Ec*SerRS	0.284	L-serine	25.0	L-serine	4706 ± 706	38.2 ± 2.10	0.00812	0.964
	0.284	Ap_4A	0.250	Ap4A	402 ± 43.1	76.5 ± 3.71	0.190	0.984
	0.284	PPi	1.00	PPi	159 ± 10.2	37.2 ± 0.810	0.234	0.990
*Sa*SerRS	0.343	L-serine	25.0	L-serine	4390 ± 654	25.1 ± 1.33	0.00572	0.963
	0.343	Ap_4A	0.250	Ap4A	231 ± 23.5	38.6 ± 1.53	0.167	0.979
	0.343	PPi	1.00	PPi	68.7 ± 5.30	25.4 ± 0.648	0.370	0.986
*Hs*SerRS	7.62	L-serine	25.0	L-serine	1083 ± 156	1.11 ± 0.0334	0.00102	0.947
	7.62	Ap_4A	0.250	Ap4A	79.5 ± 11.2	1.49 ± 0.0620	0.0187	0.916
	7.62	PPi	1.00	PPi	379 ± 43.6	2.03 ± 0.0963	0.00535	0.980

***Supplementary Table 5*: Enzymatic screening results for AlaSA **9** and ThrSA **10**. Assays were conducted as reported.[3]**

No.	Structure	*IC₅₀ Ec*SerRS (μM)	*IC₅₀ Sa*SerRS (μM)	IC₅₀ *Hs*SerRS (μM)
9 (Ala)		>1000 ± >100	>1000 ± >100	>1000 ± >100
10 (Thr)		285 ± 29.3	231 ± 19.0	>1000 ± >100

***Supplementary Table 6*: Species estimated molecular weights from the c(s) analysis.** For each sample concentration the signal-weighted sedimentation co-efficient and the estimated molecular weight of each species is shown, together with the best-fit frictional ratio for the distribution.

Monomer MW (kDa)	Detection method	Concentration (mg/mL)	Major species		f/f_0
			MW (kDa)	Sed. Co (S)	
EC SerRS with no ligand					
49.2	Absorbance	0.8	97.5	5.08	1.40
		0.4	96.9	5.11	1.39
		0.1	93.8	5.13	1.36
	Interference	0.8	102	5.08	1.45
		0.4	104	5.06	1.46
		0.1	76.4	5.05	1.20
EC SerRS with original ligand					
49.2	Absorbance	0.8	100	5.15	1.41
		0.4	92.6	5.19	1.33
		0.1	67.8	5.16	1.08[#]
	Interference	0.8	105	5.14	1.46
		0.4	99.9	5.16	1.41
		0.1	70.5	5.16	1.11
EC SerRS with 8					
49.2	Absorbance	0.8	Absorbance too high		-
		0.4			-
		0.1			-
	Interference	0.8	103	5.16	1.43
		0.4	96.7	5.22	1.36
		0.1	41.9	5.30	0.77[#]

[#] Very poor data fit

Supplementary Methods

Chemicals were from commonly used suppliers and used without further purification. Solvents (including dry solvents) for chemical transformations, work-up and chromatography were from Sigma-Aldrich (Dorset, UK) at HPLC grade, and used without further distillation. Silica gel 60 F254 analytical thin layer chromatography (TLC) plates were from Merck (Darmstadt, Germany) and visualized under UV light and/or with potassium permanganate stain. Chromatographic purifications were performed using Merck Geduran 60 silica (40-63 μm) or prepacked SNAP columns on a Biotage Isolera Purification system (Uppsala, Sweden). Deuterated solvents were from Sigma-Aldrich, Chambridge Isotopes and Apollo Scientific Ltd. All ^{1}H and ^{13}C NMR spectra were recorded using a Bruker Avance 500 MHz spectrometer. All chemical shifts are in ppm relative to the solvent peak, and coupling constants (J) are reported in Hz to the nearest 0.5. High Resolution (HR) mass spectrometry data (m/z) were obtained using a Bruker MaXis Impact instrument with an ESI source and Time of Flight (TOF) analyzer. Fourier transform Infrared (FT-IR) spectra were recorded on a Bruker Alpha Platinum instrument. Melting points were obtained from a Reichert Hot Stage melting point apparatus. HPLC analysis was run on an Agilent 1290 Infinity system equipped with a Supelco Ascentis Express 2.7 μM C18 column (50 x 2.1 mm) using a gradient of 95% solvent A → 95% solvent B (solvent A: H_2O containing 0.1% formic acid; solvent B: 100% MeCN containing 0.1% formic acid), flow rate = 0.5 mL/min and UV detection at 254 nm. Specific rotation measurements were recorded using a Schmidt and Haensch Polartronic H532 polarimeter, using a 100 mm cell and the Sodium D line (589 nm). $[\alpha]_D$ are reported in units of 10^{-1} deg dm^2g^{-1}.

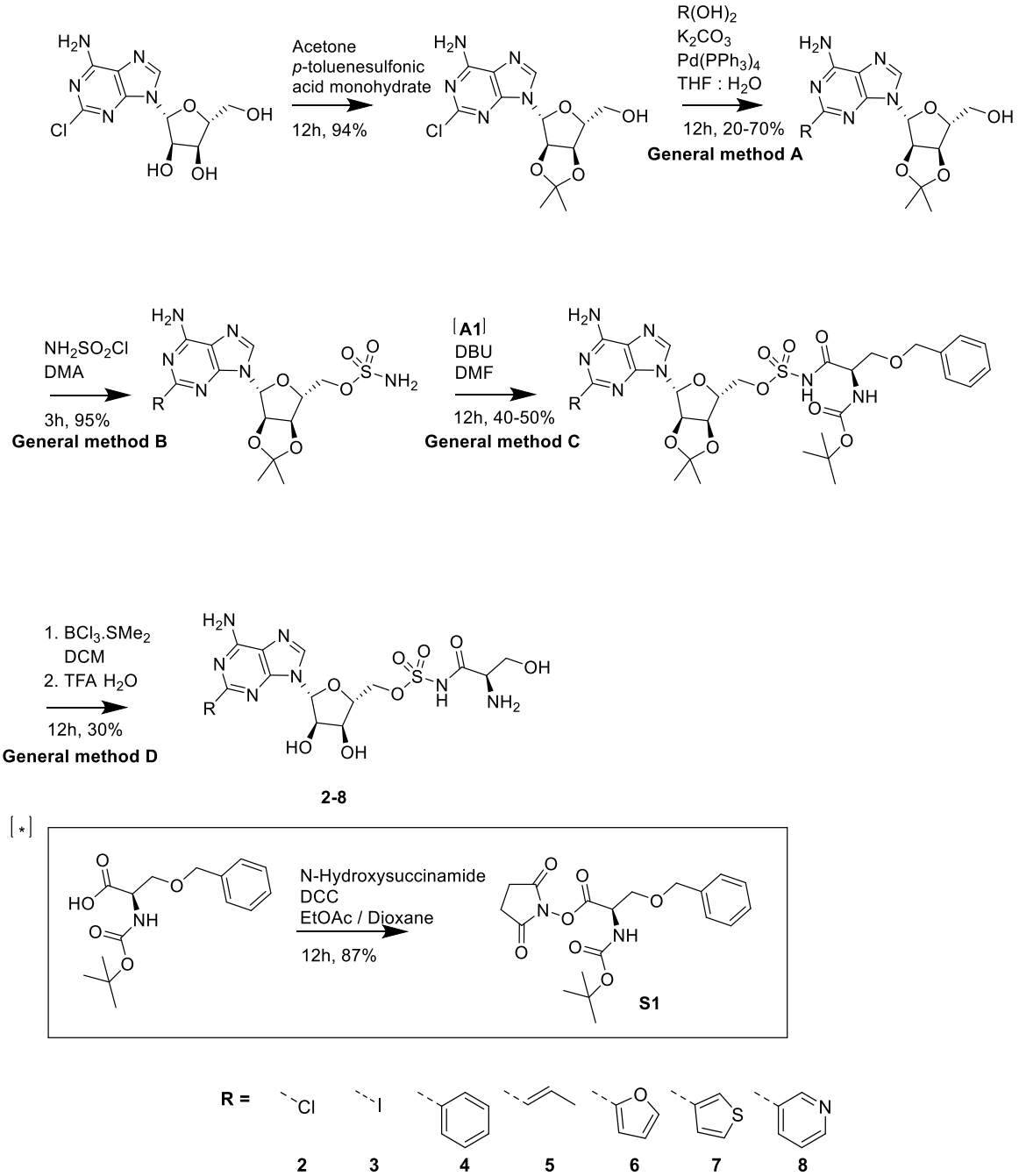
AutoDock Docking protocol

The "*in silico"* docking studies were conducted using AutoDock 4.2[4] as described in the steps below:

1. The crystal structure of the target protein *Ec*SerRS was derived from the study as described in the main text (PDB ID: 6R1M).
2. The active site was defined using maestro.[5] A 20 Å spherical 'cut' of the protein surrounding the active site was designated as the protein receptor.
3. Each ligand was constructed in maestro.[5] The resulting structures were then fully energy minimised using the multiple minimisation tool (MM).
4. Each compound was then docked into the active site of the target synthetase using AutoDock 4.2 which utilises a Lamarckian genetic algorithm. Twenty dockings were conducted for each compound.
5. The resulting docking 'poses' were scored using the AutoDock scoring function.
6. The process was repeated for each synthetase (*Sa*SerRS (PDB ID: 6R1N) and Human cytoplasmic SerRS (4L87)).

Supplementary synthesis

Synthetic routes



General method A: Suzuki coupling reactions

The boronic acid (4.0 equiv.) was added to a stirred solution of 2-chloroadenosine (1.0 equiv.), potassium carbonate (2.00 equiv.) and tetrakis (triphenylphosphine) palladium (0) (0.20 equiv.) in THF (8 mL) and water (4 mL). The reaction mixture was heated to reflux for 12 h. The reaction mixture was filtered through Celite® and concentrated *in vacuo*. The residue was diluted with water (20 mL) and extracted in EtOAc (3 × 20 mL). The combined organics were washed with water (3 × 10 mL) and brine (3 × 10 mL), dried ($MgSO_4$) and concentrated *in vacuo* to give an off-white/yellow solid, which was purified using flash column chromatography to afford the coupled products which were used without further purification.

General method B: Introduction of sulfonyl group

Generation of sulfanoyl chloride:

Chlorosulfonyl isocyanate (1 mL, 11.5 mmol, 1.0 equiv.) was cooled to 0 °C under an atmosphere of nitrogen. Formic acid (0.43 mL, 11.5 mmol, 1.0 equiv.) was added dropwise and the mixture stirred at room temperature overnight. Gas evolution was observed. The resulting colourless solid was dried *in vacuo*. The colourless solid was used without further purification / characterisation.

Sulfanoyl chloride (2.0 equiv.) in DMA (2 mL) was added dropwise to a stirred mixture of acetyl protected adenolate (1.0 equiv.) in DMA (3 mL) at 0 °C under an atmosphere of nitrogen. The mixture was then stirred at room temperature for 3 h. The reaction mixture was quenched with Et_3N (1.5 mL) then MeOH (5 mL). The resulting solution was concentrated *in vacuo* before EtOAc (50 mL) was added. The mixture was extracted with 5% $NaHCO_3$ (3 × 15 mL), brine (3 × 10 mL), dried ($MgSO_4$) and concentrated *in vacuo* to afford the desired product as a colourless glassy solid.

General method C: Amide coupling reaction

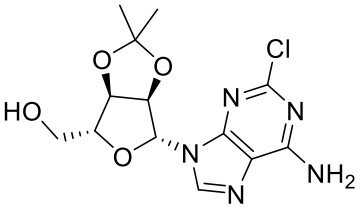
Sulfonamide adenylate (1.0 equiv.) was dissolved in DMF (10 mL). After addition of DBU (1.1 equiv.), N-Boc-Ser(bzl)-OSu (1.1 equiv.) was added to the reaction mixture. After stirring for 16 h at room temperature, the mixture was concentrated *in vacuo* and the residue taken up in water (50 mL) and extracted with dichloromethane (50 mL). The organic layers were dried ($MgSO_4$) concentrated *in vacuo* and purified by flash chromatography (EtOAc) to afford the desired product as a colourless powder.

General method D: Global deprotection

Seryl-sulfonamide adenylate (1.0 equiv.) was dissolved in DCM (5 mL) under an atmosphere of nitrogen. To this was added $BCl_3.SMe_2$ (2M in DCM, 7.00 equiv.) and the reaction was stirred at room

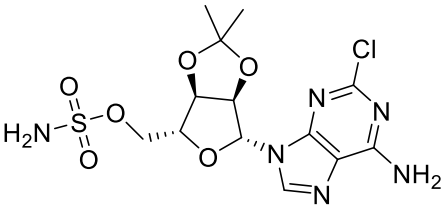
temperature for 8 h. The mixture was concentrated *in vacuo*. The residue was re-suspended in TFA:H_2O (3:1, 4 mL) and the reaction was stirred overnight at room temperature. The mixture was concentrated *in vacuo*. The crude product was purified by preparative HPLC to afford the desired product as a colourless solid.

Preparation of 2'3'-O-Isopropylidene-2-chloroadenosine



2-Chloroadenosine (0.20 g, 0.66 mmol, 1.0 equiv.) and p-toluenesulfonic acid mono hydrate (1.26 g, 6.6 mmol, 10 equiv.) were dissolved in acetone (100 mL) under an atmosphere of nitrogen. The reaction was stirred at room temperature overnight. While cooling in an ice bath a saturated $NaHCO_3$ solution (100 mL) was added to the reaction mixture until the pH of the solution was slightly basic. The acetone was removed *in vacuo*. The remaining aqueous solution was extracted with EtOAc (3 × 50 mL). The combined organics were dried ($MgSO_4$) and concentrated *in vacuo*. The desired product was isolated as a colourless solid (203 mg, 0.59 mmol, 90%); m.p.: 184.2-185.6 °C; R_f: 0.67 (9:1 Chloroform-MeOH); δ_H (500 MHz, DMSO-d6): 8.37 (1H, s, 6-HAr), 7.87 (2H, brs, NH_2), 6.07 (1H, d, *J* 2.8 2-HFuryl), 5.29 (1H, dd, *J* 6.2 and 2.8, 3-HFuryl), 5.09 (1H, app t, *J* 5.4, OH), 4.95 (1H, dd, *J* 6.2 and 2.8, 4-HFuryl), 4.22 (1H, dd, *J* 6.2 and 5.4, 5-HFuryl), 3.65-3.50 (2H, m, CH_2), 1.56 (3H, s, CH_3a), 1.34 (3H, s, CH_3b); δ_C (125 MHz, DMSO-d6): 158.1 (C4Ar), 153.0 (C2Ar), 149.9 (C8Ar), 139.9 (C6Ar), 128.2 (C5Ar), 119.0 (C9Ar), 113.1 (Acetyl C), 89.4 (C2Furyl), 86.7 (C5Furyl), 83.4 (C3Furyl), 81.2 (C4Furyl), 61.5 (CH_2), 37.0 (CH_3a), 25.2 (CH_3b); ν_{max}/ cm^{-1} (solid): 3473, 3299, 1761, 1651, 1381; HPLC: T_r= 2.26 (100% rel. area); *m/z* (ES): (Found: $[M+H]^+$, 342.0965. $C_{13}H_{16}ClN_5O_4$ requires *[M+H]*, 342.0964. $[\alpha]_D$ = -115.5° (c 0.1, MeOH).

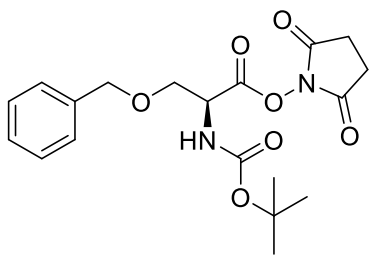
Preperation of [(3aR, 4R, 6R, 6aR)-6-(6-amino-2-chloro-9H-purin-9-yl)-2,2-dimethyl-tetrahydro-2H-furo[3,4-d][1,3]dioxol-4-yl]methyl sulfamate



Preparation was *via* general method B using 2'3'-O-Isopropylidene-2-chloroadenosine (0.17 g, 0.49 mmol, 1.0 equiv.) to afford the desired product as a colourless glassy solid (0.19 g, 0.46 mmol, 94%) m.p.: 69.4-71.7 °C; R_f: 0.46 (9:1 Chloroform-MeOH); δ_H (500 MHz, DMSO-d6): 8.33 (1H, s, 6-HAr), 7.90 (2H, brs, NH_2), 7.60 (2H, brs, SNH_2), 6.18 (1H, d, *J* 2.5, 2-HFuryl), 5.36 (1H, dd, *J* 6.2 and 2.3, 3-HFuryl), 5.03 (1H, dd, *J* 6.2 and 3.4, 4-HFuryl), 4.43 (1H, dd, *J* 9.0 and 3.4, 5-HFuryl), 4.23 (2H, ddd, *J* 17.2, 9.0 and 3.4, CH_2), 1.57 (3H, s, CH_3a), 1.36 (3H, s, CH_3b); δ_C (125 MHz, DMSO-d6): 156.9 (C4Ar), 153.2 (C2Ar), 149.8 (C8Ar), 139.9 (C6Ar), 118.1 (C9Ar), 113.7 (C Acetyl), 88.8 (C2Furyl), 83.7 (C4Furyl), 83.4 (C3Furyl), 80.9 (C4Furyl), 68.1 (CH_2), 26.9 (CH_3a), 25.2 (CH_3b); ν_{max}/

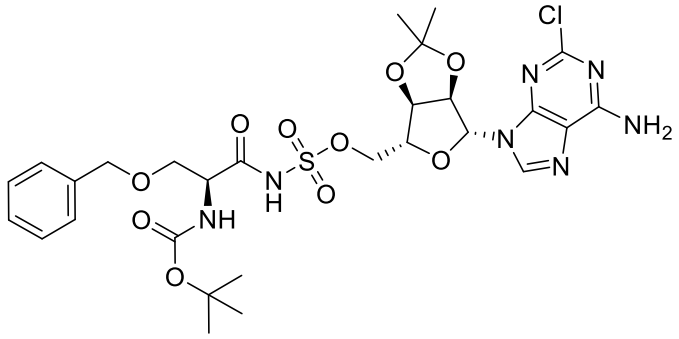
cm^{-1} (solid): 3321, 3171, 1594, 1206; HPLC: T_r= 2.30 (95% rel. area); *m/z* (ES): (Found: $[M+H]^+$, 421.0695. $C_{13}H_{17}ClN_6O_6S$ requires *[M+H]*, 421.0692. $[\alpha]_D$ = -20.2° (c 0.1, MeOH).

Preparation of N-Boc-Ser(bzl)-OSu (S1)



To a stirred solution of N-Boc-(Bzl)-Ser-OH (0.50 g, 1.69 mmol, 1.0 equiv.) in EtOAc/Dioxane (1:1, 10 mL) cooled to 0 °C were added N-hydroxysuccinimide (0.21 g, 1.78 mmol, 1.05 equiv.) and DCC (0.37 g, 1.78 mmol, 1.05 equiv.). The resulting mixture was stirred at room temperature overnight. The reaction mixture was filtered through Celite® and the filtrate concentrated *in vacuo*. The residue was re-suspended in EtOAc (35 mL), washed with 5% $NaHCO_3$ (3 × 5 mL), water (2 × 10 mL), and brine (10 mL). The organic phase was dried ($MgSO_4$) and concentrated in vacuo to afford the desired product as a colourless solid which was used without further purification. (0.35 g, 0.90 mmol, 53 %) m.p.:98.3-99.2 °C; R_f: 0.5 (9:1 DCM-MeOH); δ_H (500 MHz, DMSO-d6): 7.45-7.28 (5H, m, H-Bzl), 4.69 (1H, d, J 5.7, H-Chiral), 4.55 (2H, s, CH_2Bzl), 3.80 (2H, d, J 5.7, CH2), 2.82 (4H, brs, CH_2C=O), 1.41 (9H, s, BOC); δ_C (125 MHz, DMSO-d6): 169.8 (Succinimide C=O), 166.8 (C=O), 155.2 (BOC C=O), 137.8 (C1Ar), 128.2 (C3 and C5), 127.5 (C4), 127.5 (C2 and C6), 78.9 (C BOC), 72.3 (CH_2Bzl), 68.6 (CH_2C), 52.3 (Chiral C), 28.1 (CH_3 × 3), 25.4 (CH_2 × 2); ν_{max}/ cm^{-1} (solid): 3366, 2970, 1741, 1518, 1366; HPLC: T_r= 2.61 (83% rel. area); *m/z* (ES): (Found: $[M+H]^+$, 393.1663. $C_{19}H_{24}N_2O_7$ requires *[M+H]*, 393.1656. $[\alpha]_D$ = -7.6° (c 0.1, MeOH).

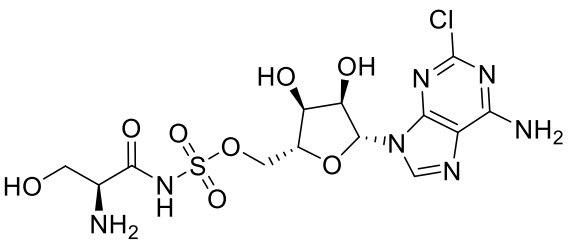
Preparation of tert-butyl N-[(2S)-1-[(([(3aR, 4R, 6R, 6aR)-6-(6-amino-2-chloro-9H-purin-9-yl)-2,2-dimethyl-tetrahydro-2H-furo[3,4-d][1,3]dioxol-4-yl]methoxy)sulfonyl)amino]-3-(benzyloxy)-1-oxopropan-2-yl]carbamate



Preparation was *via* general method C using [(3aR, 4R, 6R, 6aR)-6-(6-amino-2-chloro-9H-purin-9-yl)-2,2-dimethyl-tetrahydro-2H-furo[3,4-d][1,3]dioxol-4-yl]methyl sulfamate (0.15 g, 0.36 mmol) to afford the desired product as a colourless glassy solid (0.19 g, 0.46 mmol, 94%) m.p.: 168.6-170.2 °C; R_f: 0.51 (9:1 DCM-MeOH); δ_H (500 MHz, DMSO-d6): 8.43 (1H, s, 6-HAr), 7.83 (2H, brs, NH2), 7.30-7.20 (5H, m, Bzl), 6.10 (1H, d, *J* 5.7, 2-HFuryl), 6.04 (1H, brs, NH), 5.25 (1H, d, *J* 5.7, 3-HFuryl), 4.94 (1H, d, *J* 5.7, 4-HFuryl), 4.47 (2H, s, CH_2 Bzl), 4.35 (1H, d, *J* 5.7, 5-HFuryl), 4.07-4.01 (3H, m, CH_2Chiral and Chiral H), 3.69-3.67 (2H, m, CH_2), 1.55 (3H, s, CH_3a), 1.29 (3H, s, CH_3b); δ_C (125 MHz, DMSO-d6): 171.6 (C=O), 157.8 (C4Ar), 155.4 (C=O BOC), 153.9 (C2Ar), 151.0 (C8Ar),

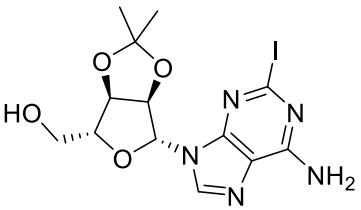
140.3 (C6Ar), 138.0 (C1Bzl), 128.7 (C3Bzl and C5Bzl), 128.3 (C2Bzl and C6Bzl), 127.9 (C4Bzl), 119.1 (C9Ar), 114.4 (C acetate), 90.6 (C2Furyl) 84.4 (C5Furyl), 83.3 (C3Furyl), 81.4 (C4Furyl), 79.5 (BOC C), 73.7 (CH_2 Bzl), 70.5 (CH_2O), 69.8 (CH_2C), 54.9 (Chiral C), 28.3 (CH3 × 3), 26.7 (CH_3a), 24.9 (CH_3b); ν_{max}/ cm^{-1} (solid):3330, 1691, 1637, 1304; HPLC: T_r= 2.59 (100% rel. area); *m/z* (ES): (Found: $[M+H]^+$, 698.2012. $C_{28}H_{36}ClN_7O_{10}S$ requires *[M+H]*, 698.2006. $[\alpha]_D$ = -50.4° (c 0.1, MeOH).

Preparation of (2S)-2-amino-1-[([[(2R, 3S, 4R, 5R)-5-(6-amino-2-chloro-9H-purin-9-yl)-3,4-dihydroxyoxolan-2-yl]methoxy)sulfonyl)amino]-3-hydroxypropan-1-one (2)



Preparation was via general method D using tert-butyl N-[(2S)-1-[(([(3aR, 4R, 6R, 6aR)-6-(6-amino-2-chloro-9H-purin-9-yl)-2,2-dimethyl-tetrahydro-2H-furo[3,4-d][1,3]dioxol-4-yl]methoxy)sulfonyl)amino]-3-(benzyloxy)-1-oxopropan-2-yl]carbamate (0.30 g, 0.43 mmol) to afford the desired product as a colourless powder (50.4 mg, 0.11 mmol, 25%) m.p.: 121.3 °C (Decomp); R_f: Baseline (9:1 DCM-MeOH); δ_H (500 MHz, DMSO-d6): 8.04 (1H, s, 6-HAr), 7.65 (2H, brs, NH_2), 5.97 (1H, d, *J* 8.2, 2-HFuryl), 4.60 (1H, apps, 3-HFuryl), 4.31 (1H, dd, *J* 10.1 and 5.9, CH_2*O), 4.19-4.15 (2H, m, CH_2*O and 4-HFuryl), 4.13 (1H, app s, 5-HFuryl), 3.81 (1H, dd, *J* 10.2 and 7.3, CH_2*chiral), 3.76 (1H, d, *J* 7.3, CHChiral), 3.65 (1H, dd, *J* 10.2 and 7.3, CH_2*chiral); δ_C (125 MHz, DMSO-d6): 172.1 (C=O), 157.8 (C4Ar), 154.1 (C2Ar), 151.0 (C9Ar), 140.1 (C6Ar), 119.1 (C10Ar), 89.4 (C2Furyl), 84.4 (C5Furyl), 73.9 (C3Furyl), 71.5 (C4Furyl), 70.3 (CH_2O), 64.4 (CH_2Chiral), 56.9 (CHChiral); ν_{max}/ cm^{-1} (solid): 3321, 3125, 2784, 1673, 1592, 1358; *m/z* (ES): No mass ion found $[\alpha]_D$ = 28.8° (c 0.1, MeOH).

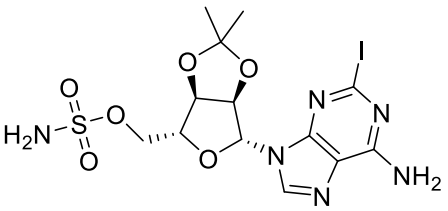
Preparation of 2'3'-O-Isopropylidene-2-iodoadenosine



2-iodoadenosine (1.00 g, 2.54 mmol, 1.0 equiv.) and p-toluenesulfonic acid mono hydrate (4.83 g, 25.4 mmol, 10 equiv.) were dissolved in acetone (200 mL) under an atmosphere of nitrogen. The reaction was stirred at room temperature overnight. While cooling in an ice bath a saturated $NaHCO_3$ solution (200 mL) was added to the reaction mixture until the pH of the solution was slightly basic. The acetone was removed *in vacuo*. The remaining aqueous solution was extracted with EtOAc (3 × 100 mL). The combined organics were dried ($MgSO_4$) and concentrated *in vacuo*. The desired product was isolated as a colourless solid (0.94 g, 2.17 mmol, 86%); m.p.: 182.4-184.1 °C; R_f: 0.69 (9:1 Chloroform-MeOH); δ_H (400 MHz, DMSO-d6): 8.34 (1H, s, 6-HAr), 7.83 (2H, brs, NH_2), 6.06 (1H, d, *J* 4.0, 2-HFuryl), 5.30 (1H, dd, J 6.2 and 4.0, 3-HFuryl), 5.09 (1H, app t, *J* 5.2, OH), 4.96 (1H, dd, *J* 6.2 and 3.9, 4-HFuryl), 4.20 (1H, d, *J* 3.9, 5-HFuryl), 3.52 (2H, app s, CH_2), 1.56 (3H, s, CH_3a), 1.37 (3H, s, CH_3b); δ_C (100 MHz, DMSO-d6):

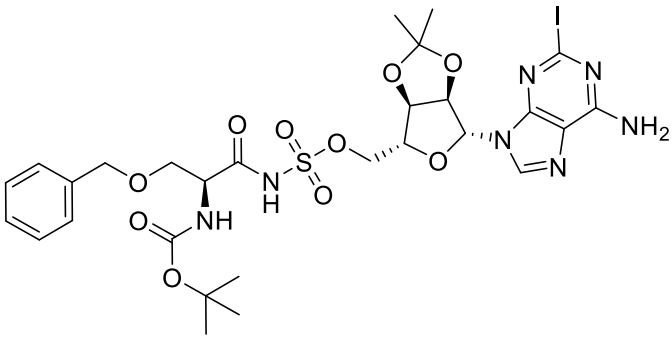
158.2 (C4Ar), 156.4 (C8Ar), 149.8 (C6Ar), 121.4 (C8Ar), 119.3 (C2Ar) 113.6 (Acetyl C), 89.6 (C2Furyl), 87.3 (C5Furyl), 84.0 (C3Furyl), 81.7 (C4Furyl), 62.0 (CH_2), 27.5 (CH_3a), 25.7 (CH_3b); ν_{max}/ cm^{-1} (solid): 3473, 3299, 1761, 1651, 1381; HPLC: T_r= 2.30 (100% rel. area); *m/z* (ES): (Found: $[M+Na]^+$, 455.8. $C_{13}H_{16}IN_5O_4$ requires *[M+Na]*, 455.8. $[\alpha]_D$ = -102.0° (c 0.1, MeOH).

Preperation of [(3aR, 4R, 6R, 6aR)-6-(6-amino-2-iodo-9H-purin-9-yl)-2,2-dimethyl-tetrahydro-2H-furo[3,4-d][1,3]dioxol-4-yl]methyl sulfamate



Preparation was *via* general method B using 2'3'-O-Isopropylidene-2-iodoadenosine (0.20 g, 0.46 mmol) to afford the desired product as a colourless glassy solid (0.20 g, 0.39 mmol, 85%) m.p.: 70.2-71.7 °C; R_f: 0.48 (9:1 Chloroform-MeOH); δ_H (500 MHz, DMSO-d6): 8.24 (1H, s, 6-HAr), 7.80 (2H, brs, NH_2), 7.59 (2H, brs, SNH_2), 6.18 (1H, d, *J* 4.0, 2-HFuryl), 5.33 (1H, dd, *J* 6.1 and 4.0, 3-HFuryl), 5.02 (1H, dd, *J* 6.1 and 3.8), 4.43 (1H, app d, *J* 3.8, 5-HFuryl), 4.28-4.12 (2H, m, CH_2), 1.63 (3H, s, CH_3a), 1.38 (3H, s, CH_3b); δ_C (100 MHz, DMSO-d6): 156.5 (C4Ar), 156.4 (C8Ar), 149.7 (C6Ar), 121.5 (C9Ar), 119.4 (C2Ar), 114.2 (Acetyl C), 89.1 (C2Furyl), 84.3 (C5Furyl), 84.1 (C3Furyl), 81.4 (C4Furyl), 68.5 (CH_2), 27.4 (CH_3a), 25.7 (CH_3b); ν_{max}/ cm^{-1} (solid): 3321, 3171, 1594, 1206; HPLC: T_r= 2.35 (100% rel. area); *m/z* (ES): (Found: $[M+Na]^+$, 442.9. $C_{13}H_{17}IN_6O_6S$ requires *[M+Na]*, 442.9. $[\alpha]_D$ = -21.9 ° (c 0.1, MeOH).

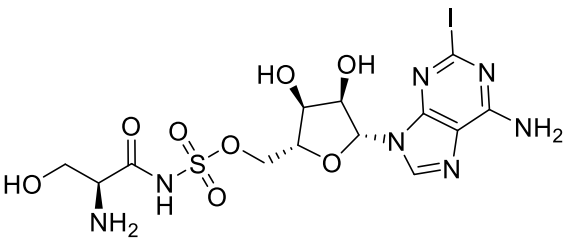
Preparation of tert-butyl N-[(2S)-1-[(([(3aR, 4R, 6R, 6aR)-6-(6-amino-2-iodo-9H-purin-9-yl)-2,2-dimethyl-tetrahydro-2H-furo[3,4-d][1,3]dioxol-4-yl]methoxy)sulfonyl)amino]-3-(benzyloxy)-1-oxopropan-2-yl]carbamate



Preparation was *via* general method C using [(3aR, 4R, 6R, 6aR)-6-(6-amino-2-iodo-9H-purin-9-yl)-2,2-dimethyl-tetrahydro-2H-furo[3,4-d][1,3]dioxol-4-yl]methyl sulfamate (0.20 g, 0.39 mmol) to afford the desired product as a colourless glassy solid (40.4 mg, 0.05 mmol, 13%) m.p.: 166.2-168.9°C; R_f: 0.54 (DCM-MeOH); δ_H (400 MHz, DMSO-d6): 8.38 (1H, s, 6-HAr), 7.75 (2H, brs, NH_2), 7.30-7.20 (5H, m, Bzl), 6.07 (1H, app s, 2-HFuryl), 5.24 (1H, app s, 3-HFuryl), 4.92 (1H, app s, 4-HFuryl), 4.42 (2H, app s, CH_2 Bzl), 4.35 (1H, app s, 5-HFuryl), 4.08-4.01 (3H, m, CH_2 Chiral and Chiral H) 3.70-3.62 (2H, m, CH_2), 1.54 (3H, s, CH_3a), 1.38 (9H, s, CH3 × 3), 1.20 (3H, s, CH_3b); δ_C (100 MHz, DMSO-d6): 174.5 (C=O), 157.3 (C4Ar), 155.5 (C=O BOC), 152.3 (C8Ar), 139.0 (C6Ar), 137.7 (C1Bzl), 128.7 (C5Bzl and C3Bzl), 128.1 (C4Bzl), 127.8 (C2'Ar and C6'Ar), 118.5 (C9Ar),

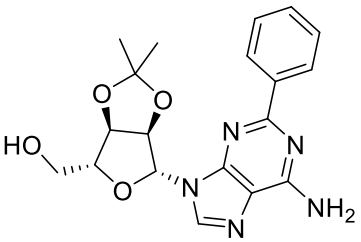
116.4 (C2Ar), 114.4 (Acetyl C), 88.3 (C2Furyl), 82.8 C5Furyl), 81.5 (C3Furyl), 79.6 (BOC C), 79.0 (C4Furyl), 73.5 (CH_2 Bzl); 69.9 (CH_2O), 67.4 (CH_2C), 56.4 (Chiral C), 28.3 (CH_3 × 3), 26.4 (CH_3a), 24.9 (CH_3b); ν_{max}/ cm^{-1} (solid): 3330, 1691, 1637, 1304; HPLC: T_r= 2.67 (100% rel. area); *m/z* (ES): (Found: $[M+Na]^+$, 812.1. $C_{28}H_{36}IN_7O_{10}S$ requires *[M+Na]*, 812.1. $[\alpha]_D$ = -52.2° (c 0.1, MeOH).

Preparation of (2S)-2-amino-1-[([[(2R, 3S, 4R, 5R)-5-(6-amino-2-iodo-9H-purin-9-yl)-3,4-dihydroxyoxolan-2-yl]methoxy)sulfonyl)amino]-3-hydroxypropan-1-one (3)



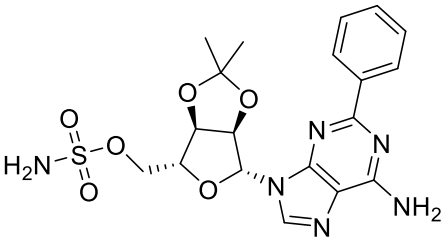
Preparation was via general method D using tert-butyl N-[(2S)-1-[(([(3aR, 4R, 6R, 6aR)-6-(6-amino-2-iodo-9H-purin-9-yl)-2,2-dimethyl-tetrahydro-2H-furo[3,4-d][1,3]dioxol-4-yl]methoxy)sulfonyl)amino]-3-(benzyloxy)-1-oxopropan-2-yl]carbamate (50 mg, 0.06 mmol) to afford the desired product as a colourless powder (4.0 mg, 0.07 mmol, 12%) m.p.: 135.2 °C (Decomp); R_f: Baseline (9:1 DCM-MeOH); δ_H (500 MHz, DMSO-d6): 8.36 (1H, s, 6-HAr), 7.68 (2H, brs, NH_2), 5.88 (1H, d, J 5.4, 2-HFuryl), 4.60 (1H, apps, 3-HFuryl), 4.31 (1H, dd, *J* 10.1 and 5.9, CH_2*O), 4.19-4.15 (2H, m, CH_2*O and 4-HFuryl), 4.13 (1H, app s, 5-HFuryl), 3.81 (1H, dd, *J* 10.2 and 7.3, CH_2*chiral), 3.76 (1H, d, *J* 7.3, CH Chiral), 3.65 (1H, dd, *J* 10.2 and 7.3, CH_2*chiral); ν_{max}/ cm^{-1} (solid): 3321, 3125, 2784, 1673, 1592, 1358; *m/z* (ES): (Found: $[M-H]^-$, 558.0. $C_{13}H_{18}IN_7O_8S$ requires *[M-H]*, 558.0. $[\alpha]_D$ = 28.4° (c 0.1, MeOH).

Preparation of 2'3'-O-Isopropylidene-2-phenyladenosine



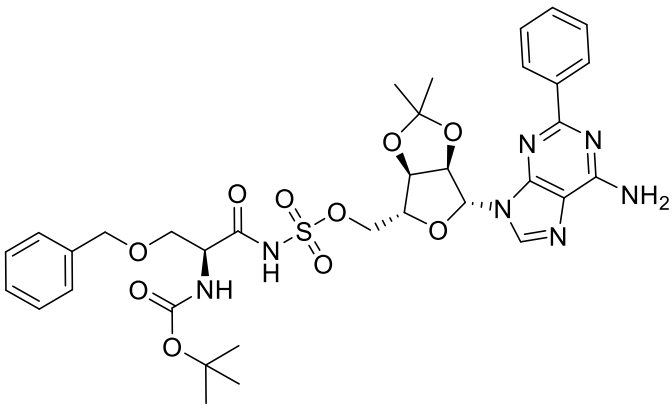
Preparation was via general method A using 2'3'-O-Isopropylidene-2-chloroadenosine (0.50 g, 1.46 mmol) and phenyl boronic acid (0.71 g, 5.85 mmol) to afford the desired product as a colourless powder (0.43 g, 1.12 mmol, 76%); m.p.: 166.2-168.1 °C; R_f: 0.58 (9:1 Chloroform-MeOH); δ_H (500 MHz, DMSO-d6): 8.36 (3H, app s, 6-HAr, 2'-HAr and 6'-HAr), 7.46 (3H, app s, 4'-HAr, 3'-HAr and 5'-HAr), 7.39 (2H, brs, NH_2), 6.26 (1H, app s, 2-HFuryl), 5.52 (1H, app s, 3-HFuryl), 5.10 (1H, app s, 4-HFuryl), 5.04 (1H, app s, OH), 4.22 (1H, app s, 4-HFuryl), 3.69-3.51 (2H, m, CH_2), 1.59 (3H, s, CH_3a), 1.37 (3H, s, CH_3b); δ_C (125 MHz, DMSO-d6): 158.5 (C2Ar), 156.4 (C4Ar), 150.4 (C8Ar), 140.8 (C6Ar), 138.8 (C1'Ar), 134.4 (C4'Ar), 128.7 (C3'Ar and C5'Ar), 128.2 (C2' Ar and C6'Ar), 118.7 (C9Ar), 113.6 (Acetyl C), 89.5 (C2Furyl), 87.2 (C5Furyl), 83.8 (C3Furyl), 81.9 (C4Furyl), 62.0 (CH_2), 27.6 (CH_3a), 25.7 (CH_3b); ν_{max}/ cm^{-1} (solid): 3314, 3157, 1655, 1596, 1372; HPLC: T_r= 2.24 (100% rel. area); *m/z* (ES): (Found: $[M+H]^+$, 384.1676. $C_{19}H_{21}N_5O_4$ requires *[M+H]*, 384.1666. $[\alpha]_D$ = -1.4° (c 0.1, MeOH).

Preperation of [(3aR, 4R, 6R, 6aR)-6-(6-amino-2-phenyl-9H-purin-9-yl)-2,2-dimethyl-tetrahydro-2H-furo[3,4-d][1,3]dioxol-4-yl]methyl sulfamate



Preparation was *via* general method B using 2'3'-O-Isopropylidene-2-phenyladenosine (0.40 g, 1.04 mmol) to afford the desired product as a colourless glassy solid (0.23 g, 0.50 mmol, 48%) m.p.: 89.9-91.2 °C; R_f: 0.50 (9:1 Chloroform-MeOH); δ_H (500 MHz, DMSO-d6): 8.38-8.33 (3H, m, 6-HAr, 2'-HAr and 6'-HAr), 7.60 2H, brs, SNH_2), 7.54-7.45 (3H, m, 3'-HAr, 4'-HAr and 5'-HAr), 7.42 2H, brs, NH_2), 6.34 (1H, app s, 2-HFuryl), 5.36 1H, d, J 6.3, 3-HFuryl), 5.20 (1H, app s, 4-HFuryl), 4.46 (1H, app s, 5-HFuryl), 4.25-4.15 (2H, m, CH_2), 1.60 (3H, s, CH_3a), 1.38 (3H, s, CH_3b); δ_C (125 MHz, DMSO-d6): 158.6 (C2Ar), 156.4 (C4Ar), 150.3 (C8Ar), 140.8 (C6Ar), 138.7 (C1'Ar), 130.2 (C4'Ar), 129.2 (C3'Ar and C5'Ar), 128.7 (C6'Ar and C2'Ar), 89.3 (C2Furyl), 84.1 (C5Furyl), 83.8 (C3Furyl), 81.6 (C4Furyl), 68.6 (CH_2), 27.5 (CH_3a), 25.7 (CH_3b); ν_{max}/ cm^{-1} (solid): 3354, 2936, 1628, 1376; HPLC: T_r= 2.27 (100% rel. area); *m/z* (ES): (Found: $[M+H]^+$, 463.1401. $C_{19}H_{22}N_6O_6S$ requires *[M+H]*, 463.1394. $[\alpha]_D$ = 17.6° (c 0.1, MeOH).

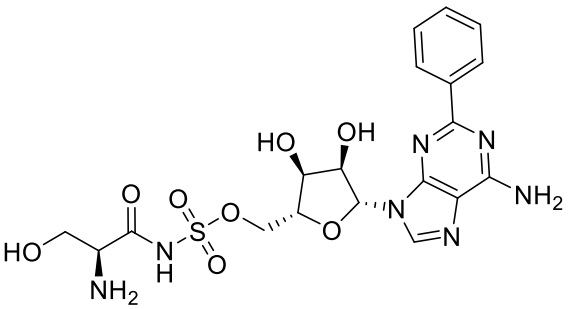
Preparation of tert-butyl N-[(2S)-1-[(([(3aR, 4R, 6R, 6aR)-6-(6-amino-2-phenyl-9H-purin-9-yl)-2,2-dimethyl-tetrahydro-2H-furo[3,4-d][1,3]dioxol-4-yl]methoxy)sulfonyl)amino]-3-(benzyloxy)-1-oxopropan-2-yl]carbamate



Preparation was *via* general method C using [(3aR, 4R, 6R, 6aR)-6-(6-amino-2-phenyl-9H-purin-9-yl)-2,2-dimethyl-tetrahydro-2H-furo[3,4-d][1,3]dioxol-4-yl]methyl sulfamate (0.20 g, 0.43 mmol) to afford the desired product as a colourless glassy solid (0.21 g, 0.29 mmol, 67%) m.p.: 155.8-157.7 °C; R_f: 0.64 (9:1 DCM-MeOH); δ_H (500 MHz, DMSO-d6): 8.42(1H, s, 6-HAr), 8.37 (2H, d, J 6.9 6'-HAr and 2'-HAr), 7.57-7.41 (3H, m, 5'-HAr, 4'-HAr and 3'-HAr), 7.38 (2H, brs, NH_2), 7.26-7.24 (5H, m, 2-HBzl, 3-HBzl, 4-HBzl, 5-HBzl and 6-HBzl), 6.27 (1H, d, J 3.0, 2-HFuryl), 6.06 (1H, d J 8.1, NH), 5.46 (1H, d, J 3.0, 3-HFuryl), 5.07 (1H, app s, 4-HFuryl), 4.45-4.41 (3H, m, BzlCH2, 5-HFuryl), 4.05 (2H, d, J 4.9, CH2O), 3.96-3.92 (1H, m, Chiral H), 3.67-3.58 (2H, m, Chiral CH_2), 1.59 (3H, s, CH_3a), 1.39 (9H, s, CH_3 × 3), 1.35 (3H, s, CH_3b); δ_C (125 MHz, DMSO-d6): 173.5 (C=ONH), 158.7 (C2Ar), 156.8 (C4Ar), 156.4 (C=O BOC), 150.6 (C8Ar), 140.0 (C6Ar), 138.5 (C1'Ar and C1Bzl), 130.4 (C4'Ar), 129.6-127.1 (C2Bzl, C3Bzl, C4Bzl, C5Bzl, C6Bzl,

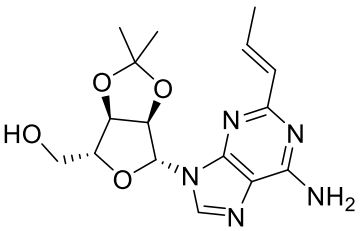
C2'Ar, C3'Ar, C5'Ar and C6'Ar), 118.0 (C9Ar), 112.5 (Acetyl C), 88.8 (C2Furyl), 83.5 (C5Furyl), 83.4 (C3Furyl), 81.6 (C4Furyl), 79.5 (BOC C), 71.8 (CH_2 Bzl), 71.1 (Chiral CH_2), 67.1 (CH_2), 57.1 (Chiral C), 28.2 (CH_3 × 3), 27.1 (CH_3a), 25.2 (CH_3b); ν_{max}/ cm^{-1} (solid): 3358, 3193, 2980, 1629, 1575, 1438; HPLC: T_r= 2.54 (100% rel. area); *m/z* (ES): (Found: $[M+H]^+$, 740.2718. $C_{34}H_{41}N_7O_{10}S$ requires *[M+H]*, 740.2708. $[\alpha]_D$ = 17.9° (c 0.1, MeOH).

Preparation of (2S)-2-amino-1-[([[(2R, 3S, 4R, 5R)-5-(6-amino-2-phenyl-9H-purin-9-yl)-3,4-dihydroxyoxolan-2-yl]methoxy)sulfonyl)amino]-3-hydroxypropan-1-one (4)



Preparation was via general method D using tert-butyl N-[(2S)-1-[(([(3aR, 4R, 6R, 6aR)-6-(6-amino-2-phenyl-9H-purin-9-yl)-2,2-dimethyl-tetrahydro-2H-furo[3,4-d][1,3]dioxol-4-yl]methoxy)sulfonyl)amino]-3-(benzyloxy)-1-oxopropan-2-yl]carbamate (0.20 g, 0.27 mmol) to afford the desired product as a colourless powder (54.0 mg, 0.11 mmol, 39%) m.p.: 143.2 (Decomp); R_f: Baseline (9:1 DCM-MeOH); δ_H (500 MHz, DMSO-d6): 8.47 (1H, s, 6-HAr), 8.43 (2H, dd, J 8.0 and 1.6, 6'-HAr and 2'-HAr), 8.20 (1H, s, NH), 7.91 (2H, brs, NH2), 7.58-7.48 (3H, m, 3'-HAr, 4'-HAr and 5'-HAr), 6.09 (1H, d, J 5.9, 2-HFuryl), 4.77-4.70 (1H, m, 3-HFuryl), 4.34-4.28 (2H, m, 4-HFuryl and CH2*O), 4.22-4.16 2H, m, 5-HFuryl and CH2*O), 3.87 (1H, dd, J 11.2 and 3.7, CH2*Chiral) 3.69 (1H, dd, J 11.2 and 7.3, CH2*Chiral), 3.60-3.56 (1H, m, ChiralH); δ_C (125 MHz, DMSO-d6): 172.0 (C=O), 163.5 (C2Ar), 155.9 (C4Ar), 150.8 (C8Ar), 140.5 (C6Ar), 137.8 (C1'Ar), 130.1 (C4'Ar), 128.7 (C5'Ar and C3'Ar), 128.2 (C6'Ar and C2'Ar), 19.1 (C9Ar), 87.1 (C2Furyl), 82.8 (C5Furyl), 73.8 (C3Furyl), 71.3 (C4Furyl), 68.5 (CH_2O), 61.0 (CH_2Chiral), 57.6 (Chiral H); ν_{max}/ cm^{-1} (solid): 3367, 3070, 1673, 1598; HPLC: T_r= 1.89 (100% rel. area); *m/z* (ES): (Found: $[M+H]^+$, 510.1405. $C_{19}H_{23}N_7O_8S$ requires *[M+H]*, 510.1402). $[\alpha]_D$ = 25.7° (c 0.1, MeOH).

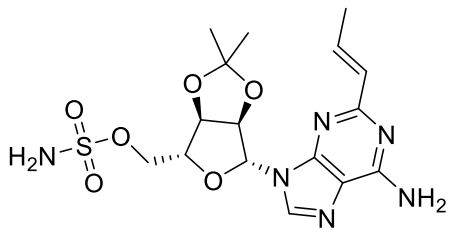
Preparation of 2'3'-O-Isopropylidene-2-propenyladenosine



Preparation was via general method A using 2'3'-O-Isopropylidene-2-chloroadenosine (0.50 g, 1.46 mmol) and transpropenyl boronic acid (0.50 g, 5.85 mmol) to afford the desired product as a colourless powder (0.17 g, 0.50 mmol, 34%); m.p.: 81.6-83.4 °C; R_f: 0.58 (9:1 Chloroform-MeOH); δ_H (500 MHz, DMSO-d6): 8.29 (1H, s, 6-HAr), 7.22 (2H, brs, NH_2), 6.92 (1H, dd, J 15.2 and 7.2, 2-Hpropyl), 6.33 (1H, d, J 15.2, 1-Hpropyl), 6.13 (1H, app s, 2-HFuryl), 5.34 (1H, app s, 3-HFuryl), 5.26 (1H, t, J 5.4, OH), 5.02 (1H, app s, 4-HFuryl), 4.22 (1H, app s, 5-HFuryl), 3.62-3.49 (2H, m, CH_2), 1.90 (3H, d, J 7.2, CH_3propyl), 1.56 (3H, s, CH_3a),

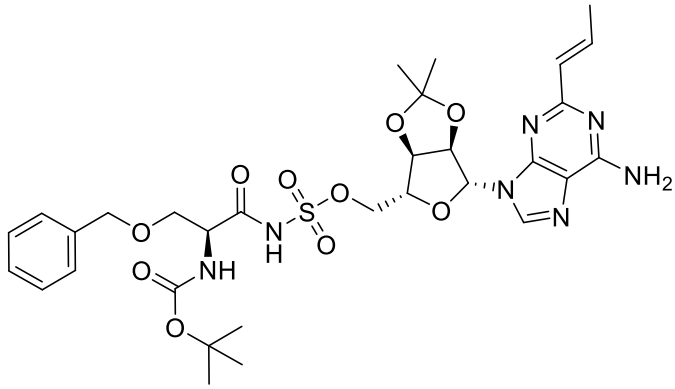
1.35 (3H, s, CH_3b); δ_C (125 MHz, DMSO-d6): 158.8 (C2Ar), 156.2 (C4Ar), 149.9 (C8Ar), 140.3 (C6Ar), 134.0 (C2Pro), 131.9 (C1Pro), 89.9 (C2Furyl), 86.9 (C5Furyl), 83.6 (C3Furyl), 81.9 (C4Furyl), 62.2 (CH_2), 27.6 (CH_3a), 25.7 (CH_3b), 18.3 (CH_3Pro); ν_{max}/ cm^{-1} (solid): 3451, 3310, 3162, 1657, 1575, 1373; HPLC: T_r= 2.11 (72% rel. area); *m/z* (ES): (Found: $[M+H]^+$, 348.3. $C_{16}H_{21}N_5O_4$ requires *[M+H]*, 348.5. $[\alpha]_D$ = -50.2° (c 0.1, MeOH).

Preperation of [(3aR, 4R, 6R, 6aR)-6-(6-amino-2-propenyl-9H-purin-9-yl)-2,2-dimethyl-tetrahydro-2H-furo[3,4-d][1,3]dioxol-4-yl]methyl sulfamate



Preparation was *via* general method B using 2'3'-O-Isopropylidene-2-propenyladenosine (0.15 g, 0.43 mmol) to afford the desired product as a pale yellow oil (0.14 g, 0.33 mmol, 77%) R_f: 0.47 (9:1 Chloroform-MeOH); δ_H (500 MHz, DMSO-d6): 8.25 (1H, s, 6-HAr), 7.61 2H, brs, SNH_2), 7.23 (2H, brs, NH_2), 6.90 (1H, dd, J 13.4 and 6.6, 2-HPropyl), 6.36 (1H, d, J 13.4, 1-HPropyl), 6.24 (1H, app s, 2-HFuryl), 5.42 (1H, app s, 3-HFuryl), 5.15 (1H, app s, 4-HFuryl), 4.40 (1H, app s, 5-HFuryl), 4.35-4.25 (2H, m, CH_2), 1.90 (3H, d, J 6.6, CH_3 Propyl), 1.57 (3H, s, CH_3a), 1.36 (3H, s, CH_3b); δ_C (125 MHz, DMSO-d6): 171.0 (C2Ar), 156.1 (C4Ar), 150.9 (C8Ar), 140.0 (C6ar), 125.5 (C2Pro), 119.4 (C9Ar), 117.7 (C1Pro), 112.4 (Acetyl C), 88.2 (C2Furyl), 83.4 (C5Furyl), 83.1 (C3Furyl), 81.4 (C4Furyl), 70.5 (CH_2), 26.7 (CH_3a), 25.0 (CH_3b), 18.8 (CH_3Pro); ν_{max}/ cm^{-1} (solid): 3326, 3182, 2987, 2938, 1622, 1585, 1374; HPLC: T_r= 2.14 (67% rel. area); *m/z* (ES): (Found: $[M+H]^+$, 427.1400. $C_{16}H_{22}N_6O_6S$ requires *[M+H]*, 427.1394. $[\alpha]_D$ = 16.5° (c 0.1, MeOH).

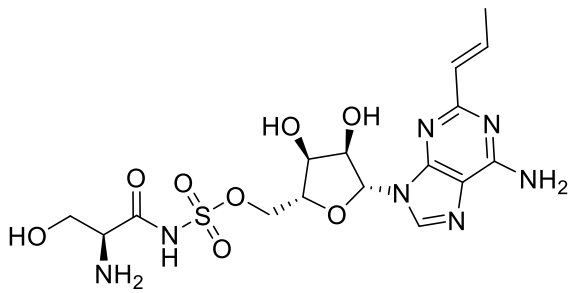
Preparation of tert-butyl N-[(2S)-1-[(([(3aR, 4R, 6R, 6aR)-6-(6-amino-2-propenyl-9H-purin-9-yl)-2,2-dimethyl-tetrahydro-2H-furo[3,4-d][1,3]dioxol-4-yl]methoxy)sulfonyl)amino]-3-(benzyloxy)-1-oxopropan-2-yl]carbamate



Preparation was *via* general method C using [(3aR, 4R, 6R, 6aR)-6-(6-amino-2-propenyl-9H-purin-9-yl)-2,2-dimethyl-tetrahydro-2H-furo[3,4-d][1,3]dioxol-4-yl]methyl sulfamate (0.14 g, 0.33 mmol) to afford the desired product as a colourless glassy solid (64.9 mg, 0.09 mmol, 28%) m.p.: 142.9-144.3 °C; R_f: 0.47 (9:1 DCM-MeOH); δ_H (500 MHz, DMSO-d6): 8.33 (1H, s, 6-HAr), 7.30-7.26 (5H, m, 2'-HAr, 3'-HAr, 4'-HAr, 5'-HAr and 6'-HAr), 7.18 (2H, brs, NH_2), 6.92 (1H, dd, J 15.3 and 7.0, 2-HPro), 6.35 (1H, d, J 15.3, 1-HPro), 6.15 (1H, d, J 2.8, 2-HFuryl), 6.06 (1H, d, J 7.7 NH), 5.33 (1H, dd, J 6.3 and 2.8, 3-HFuryl),

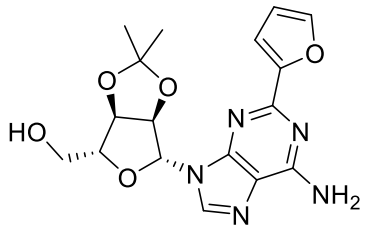
5.04 (1H, app t, J 6.3, 4-HFuryl), 4.42 (2H, d, J 9.9, CH_2 Bzl), 4.36 (1H, d J 2.4, 5-HFuryl), 4.15-3.95 (3H, m, CH_2O and ChiralH), 3.73-3.54 (2H, m, CH_2 Chiral), 1.89 (3H, dd, J 7.0 and 1.5, Me), 1.56 (3H, s, CH_3a), 1.39 (9H, s, CH_3 × 3), 1.33 (3H, s, CH_3b); δ_C (125 MHz, DMSO-d6): 172.5 (C2Ar), 170.1 (C=ONH), 156.1 (C4Ar), 155.4 (C=OBOC), 150.8 (C8Ar), 140.3 (C6Ar), 137.8 (C1'Ar), 128.7-127.5 (Aromatics), 125.2 (C2pro), 119.1 (C9Ar), 117.7 (C1Pro), 114.3 (Acetyl C), 88.9 (C2Furyl), 83.7 (C5Furyl), 83.4 (C3Furyl), 81.7 (C4Furyl), 79.5 (BOC C), 71.8 (CH_2 Bzl), 71.1 (CH_2O), 67.1 (CH_2 Chiral), 56.5 (Chiral C), 28.2 (CH_3 × 3), 27.1 (CH_3a), 25.2 (CH_3b), 17.8 (CH_3Pro); ν_{max}/ cm^{-1} (solid): 3359, 2968, 1627, 1579, 1345; HPLC: T_r= 2.45 (78% rel. area); *m/z* (ES): (Found: $[M+H]^+$, 704.2719. $C_{31}H_{41}N_7O_{10}S$ requires *[M+H]*, 704.2708. $[\alpha]_D$ = -8.6° (c 0.1, MeOH).

Preparation of (2S)-2-amino-1-[([[(2R, 3S, 4R, 5R)-5-(6-amino-2-propenyl-9H-purin-9-yl)-3,4-dihydroxyoxolan-2-yl]methoxy)sulfonyl)amino]-3-hydroxypropan-1-one (5)



Preparation was via general method D using tert-butyl N-[(2S)-1-[(([(3aR, 4R, 6R, 6aR)-6-(6-amino-2-propenyl-9H-purin-9-yl)-2,2-dimethyl-tetrahydro-2H-furo[3,4-d][1,3]dioxol-4-yl]methoxy)sulfonyl)amino]-3-(benzyloxy)-1-oxopropan-2-yl]carbamate (65 mg, 0.09 mmol) to afford the desired product as a colourless powder (22.5 mg, 0.05 mmol, 53%) m.p.: 115.3 (Decomp); R_f: Baseline (9:1 DCM-MeOH); δ_H (500 MHz, DMSO-d6): 8.50 (1H, s, 6-HAr), 8.15 (1H, s, NH), 7.88 (2H, brs, NH_2), 7.09 (1H, dd, J 15.4 and 6.7, 2-HPropyl), 6.41 (1H, d, J 15.4, 1-HPropyl), 5.95 (1H, d, J 5.9, 2-HFuryl), 4.63 (1H, app t, J 5.9, 3-HFuryl), 4.28-4.09 (4H, m, 4-HFuryl, 5-HFuryl and CH_2O), 3.83 (1H, dd, J 11.1 and 3.6, CH_2* Chiral), 3.65 (1H, dd, 11.1 and 7.0, CH_2* Chiral), 3.56-3.50 (1H, m, Chiral H), 2.88 (2H, app d, J 5.3, NH_2), 1.98 (3H, d, J 6.7, CH_3); δ_C (125 MHz, DMSO-d6): 172.2 (C=O), 163.5 (C2Ar), 153.8 (C4Ar), 150.9 (C8Ar), 145.9 (C6Ar), 122.3 (C2Pro), 119.4 (C9Ar), 117.9 (C1Pro), 93.7 (C2furyl), 83.4 (C5Furyl), 75.4 (C3Furyl), 70.5 (C4Furyl), 70.2 (CH_2O), 57.3 (CH_2 Chiral), 54.8 (Chiral H), 19.1 (CH_3); ν_{max}/ cm^{-1} (solid): 3106, 2942, 1668, 1595, 1182; HPLC: T_r= 0.81 (100% rel. area); *m/z* (ES): (Found: $[M+H]^+$, 474.1406. $C_{16}H_{23}N_7O_8S$ requires *[M+H]*, 474.1402). $[\alpha]_D$ = 27.0° (c 0.1, MeOH).

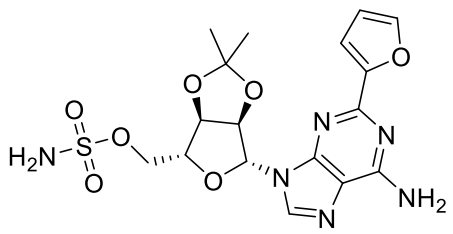
Preparation of 2'3'-O-Isopropylidene-2-(furan-2-yl)adenosine



Preparation was via general method A using 2'3'-O-Isopropylidene-2-chloroadenosine (0.50 g, 1.46 mmol) and 2-furyl boronic acid (0.66 g, 5.85 mmol) to afford the desired product as a colourless powder (0.25 g, 0.67 mmol, 46%); m.p.: 96.3-98.2 °C; R_f: 0.59 (9:1 Chloroform-MeOH); δ_H (500 MHz, DMSO-d6): 8.35 (1H, s, 6-HAr),

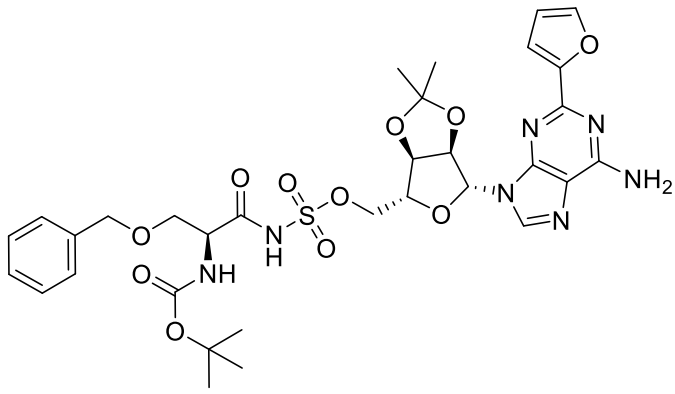
7.83 (1H, 5'-HAr), 7.44 (2H, brs, NH2), 7.13 (1H, s, 3'-HAr), 6.65 (1H, s, 4'-HAr), 6.20 (1H, app s, 2-HFuryl), 5.40 (1H, app s, 3-HFuryl), 5.10 (1H, app s, 4-HFuryl), 5.05 (1H, app s, OH), 4.22 (1H, app s, 5-HFuryl), 3.67-3.52 (2H, m, CH_2), 1.55 (3H, s, CH_3a), 1.35 (3H, s, CH_3b); δ_C (125 MHz, DMSO-d6): 156.8 (C2Ar), 156.0 (C4Ar), 152.6 (C2'Ar), 149.3 (C8Ar), 144.2 (C5'Ar), 140.2 (C6Ar), 177.9 (C9Ar), 113.0 (Acetyl C), 112.0 (C3'Ar), 111.4 (C4'Ar), 89.0 (C2Furyl), 86.8 (C5Furyl), 83.3 (C3Furyl), 81.5 (C4Furyl), 61.6 (CH_2), 27.9 (CH_3a), 25.2 (CH_3b); ν_{max}/ cm^{-1} (solid): 3419, 3311, 2987, 2938, 1639, 1547, 1369; HPLC: T_r= 2.20 (57% rel. area); *m/z* (ES): (Found: $[M+H]^+$, 374.1462. $C_{17}H_{19}N_5O_5$ requires *[M+H]*, 374.1459. $[\alpha]_D$ = -44.8° (c 0.1, MeOH).

Preperation of [(3aR, 4R, 6R, 6aR)-6-(6-amino-2-(furan-2-yl)-9H-purin-9-yl)-2,2-dimethyl-tetrahydro-2H-furo[3,4-d][1,3]dioxol-4-yl]methyl sulfamate



Preparation was *via* general method B using 2'3'-O-Isopropylidene-2-(furan-2-yl)adenosine (0.25g, 0.67 mmol) to afford the desired product as a colourless glassy solid (0.20 g, 0.45 mmol, 67%) m.p.: 83.3-85.2 °C; R_f: 0.46 (9:1 Chloroform-MeOH); δ_H (500 MHz, DMSO-d6): 8.31 (1H, s, 6-HAr), 7.83 (1H, s, 3'-HAr), 7.56 (2H, brs, SNH_2), 7.47 (2H, brs, NH_2), 7.12 (1H, d, J 3.2, 5'-HAr), 6.65 (1H, dd, J 3.2 and 1.8, 4'-HAr), 6.30 (1H, d, J 1.9, 2-HFuryl), 5.44 (1H, d, J 6.2 3-HFuryl), 5.23 (1H, dd, J 6.2 and 3.5, 4-HFuryl), 4.43 (1H, app s, 5-HFuryl), 4.35-4.15 (2H, m, CH_2), 1.58 (3H, s, CH_3a), 1.36 (3H, s, CH_3b); δ_C (125 MHz, DMSO-d6): 159.5 (C2Ar), 155.8 (C4Ar), 152.3 (C1'Ar), 150.6 (C8Ar), 145.0 (C3'Ar), 140.0 (C6Ar), 119.4 (C9Ar), 114.4 (Acetyl C), 113.3 (C5'Ar), 112.1 (C4'Ar), 90.7 (C2Furyl), 84.4 (C5Furyl), 83.3 (C3Furyl), 81.1 (C4Furyl), 70.5 (CH_2), 26.7 (CH_3a), 24.5 (CH_3b); ν_{max}/ cm^{-1} (solid): 3319, 3161, 2988, 1628, 1577, 1361; HPLC: T_r= 2.27 (51% rel. area); *m/z* (ES): (Found: $[M+H]^+$, 453.1189. $C_{17}H_{20}N_6O_7S$ requires *[M+H]*, 453.1187. $[\alpha]_D$ = 1.4° (c 0.1, MeOH).

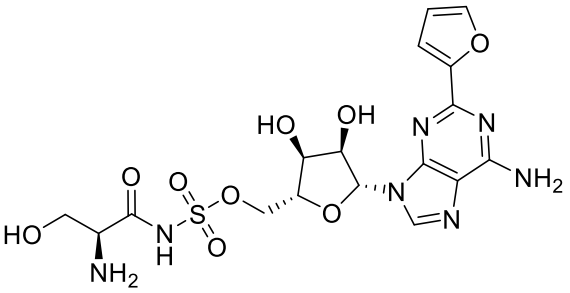
Preparation of tert-butyl N-[(2S)-1-[(([(3aR, 4R, 6R, 6aR)-6-(6-amino-2-(furan-2-yl)-9H-purin-9-yl)-2,2-dimethyl-tetrahydro-2H-furo[3,4-d][1,3]dioxol-4-yl]methoxy)sulfonyl)amino]-3-(benzyloxy)-1-oxopropan-2-yl]carbamate



Preparation was *via* general method C using [(3aR, 4R, 6R, 6aR)-6-(6-amino-2-(furan-2-yl)-9H-purin-9-yl)-2,2-dimethyl-tetrahydro-2H-furo[3,4-d][1,3]dioxol-4-yl]methyl sulfamate (0.20 g, 0.44 mmol) to afford the desired product as a colourless glassy solid (0.10 g, 0.14 mmol, 32%) m.p.:157.7-159.4 °C; R_f: 0.50 (9:1 DCM-

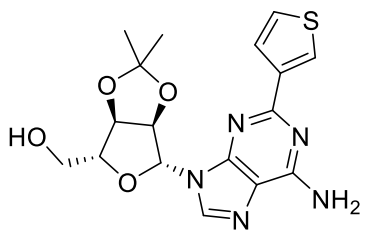
MeOH); δ_H (500 MHz, DMSO-d6): 8.40 (1H, s, 6-HAr), 7.81 (1h, s, 3'-HAr), 7.42 (2H, brs, NH2), 7.32-7.28 (5H, m, Bzl), 7.24 (1H, d, J 4.1, 5'-HAr), 6.62 (1H, dd, J 4.1 and 1.87, 4'-HAr), 6.21 (1H, d, J 2.8, 2-HFuryl), 6.06 (1H, app s, NH), 5.36 (1H, app s, 3-HFuryl), 5.08 (1H, app s, 4-HFuryl), 4.48-4.32 (3H, m, CH_2 Bzl and 5-HFuryl), 4.15-3.90 (3H, m, CH_2O and Chiral H), 3.70-3.50 (2H, m, CH_2Chiral), 1.57 (3H, s, CH_3a), 1.38 (9H, s, CH_3 × 3), 1.33 (3H, s, CH_3b); δ_C (125 MHz, DMSO-d6): 170.0 (C=ONH), 158.3 (C2Ar), 156.4 (C4Ar), 155.3 (C=OBOC), 152.5 (C1Furan), 150.6 (C8Ar), 144.7 (C3Furan), 140.2 (C6Ar), 137.7 (C1Bzl), 128.8-127.7 (Aromatics), 119.3 (C9Ar), 114.2 (Acetyl C), 112.4 (C5Furan), 111.9 (C4Furan), 89.2 (C2Furyl), 84.4 (C5Furyl), 84.1 (C3Furyl), 82.1 (C4Furyl), 79.6 (BOC C), 72.2 (CH2 Bzl), 71.0 (CH2O), 67.7 (CH2 Chiral), 56.9 (Chiral C), 28.7 (CH3 × 3), 27.6 (CH3a), 25.7 (CH3b); ν_{max}/ cm^{-1} (solid): 3342, 1674, 1572, 1364; HPLC: T_r= 2.51 (73% rel. area); *m/z* (ES): (Found: $[M+H]^+$, 730.2513. $C_{32}H_{39}N_7O_{11}S$ requires *[M+H]*, 730.2501. $[\alpha]_D$ = -24.8° (c 0.1, MeOH).

Preparation of (2S)-2-amino-1-[([[(2R, 3S, 4R, 5R)-5-(6-amino-2-(furan-2-yl)-9H-purin-9-yl)-3,4-dihydroxyoxolan-2-yl]methoxy)sulfonyl)amino]-3-hydroxypropan-1-one (6)



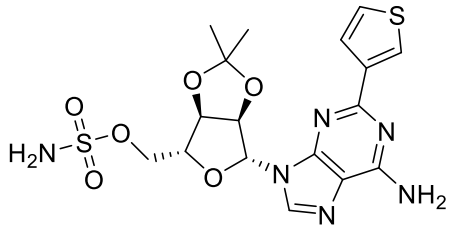
Preparation was via general method D using tert-butyl N-[(2S)-1-[(([(3aR, 4R, 6R, 6aR)-6-(6-amino-2-(furan-2-yl)-9H-purin-9-yl)-2,2-dimethyl-tetrahydro-2H-furo[3,4-d][1,3]dioxol-4-yl]methoxy)sulfonyl)amino]-3-(benzyloxy)-1-oxopropan-2-yl]carbamate (100 mg, 0.14 mmol) to afford the desired product as a colourless powder (14.6 mg, 0.03 mmol, 21%) m.p.: 117.2 (Decomp); R_f: Baseline (9:1 DCM-MeOH); δ_H (500 MHz, DMSO-d6): 8.54 (1H, brs, NH), 8.34 (2H, brs, NH_2), 8.14 (1H, s, 6-HAr), 7.96 (1H, app s, 3'-HAr), 7.46 (1H, app t, J 10.1, 5'-HAr), 6.76 (1H, dd, J 10.1 and 8.5, 4'-HAr), 6.02 (1H, d, J 5.2, 2-HFuryl), 4.65 (1H, d, J 5.2, 3-HFuryl), 4.52-4.45 (2H, m, CH_2O), 4.29 (1H, app s, 4-HFuryl), 4.20 (1H, app s, 5-HFuryl), 3.89-3.75 (3H, m, CH_2 Chiral and Chiral H); δ_C (125 MHz, DMSO-d6): 172.0 (C=O), 158.1 (C2Ar), 156.1 (C4Ar), 152.5 (C1'Ar), 150.6 (C8Ar), 145.3 (C3'Ar), 141.2 (C6Ar), 117.9 (C9Ar), 113.9 (C5'Ar), 113.2 (C4'Ar), 88.2 (C2Furyl), 84.6 (C5furyl), 73.4 (C3Furyl), 71.8 (C4Furyl), 60.6 (CH_2O), 54.9 (Chiral C), 45.2 (CH_2 Chiral); ν_{max}/ cm^{-1} (solid): 3089, 1689, 1595, 1477, 1383; *m/z* (ES): (Found: $[M+H]^+$,. $C_{17}H_{21}N_7O_9S$ requires *[M+H]*, 500.1194.) $[\alpha]_D$ = 22.9° (c 0.1, MeOH).

Preparation of 2’3’-O-Isopropylidene-2-(thiophen-3-yl)adenosine



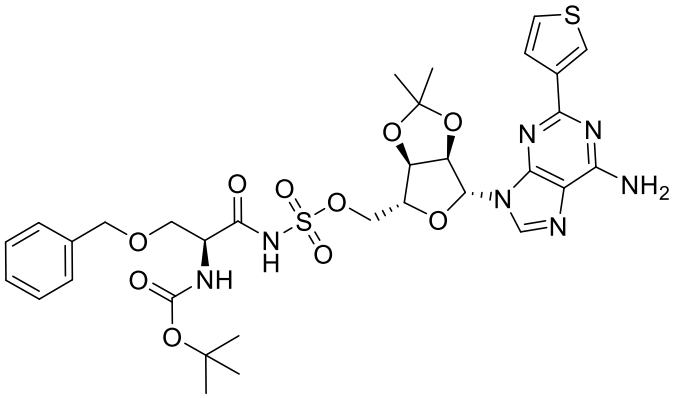
Preparation was via general method A using 2’3’-O-Isopropylidene-2-chloroadenosine (0.50 g, 1.46 mmol) and 3-thienyl boronic acid (0.75 g, 5.85 mmol) to afford the desired product as a colourless powder (0.46 g, 1.17 mmol, 80%); m.p.: 100.1-102.0 °C; R_f: 0.62 (9:1 Chloroform-MeOH); δ_H (500 MHz, DMSO-d6): 8.34 (1H, s, 6-HAr), 8.18 (1H, dd, J 3.1 and 1.2, 2’-HAr), 7.79 (1H, dd, J 5.0 and 1.2, 4’-HAr), 7.42 (1H, dd, J 5.0 and 3.1, 5’-H), 7.36 (2H, brs, NH_2), 6.23 (1H, d, J 6.2, 2-HFuryl), 5.49 (1H, dd, J 6.2 and 2.7, 3-HFuryl), 5.11 (1H, dd, J 6.2 and 2.7, 4-HFuryl), 4.22 (1H, t, J 5.5 and 2.7, 5-HFuryl), 3.59 (2H, dd, J 11.5 and 5.5, CH_2), 1.57 (3H, s, CH_3a), 1.36 (3H, s, CH_3b); δ_C (125 MHz, DMSO-d6): 173.2 (C2Ar), 155.8 (C4Ar), 140.3 (C6Ar), 127.4 (C4’Ar), 125.1 (C5’Ar), 120.0 (C2’Ar), 119.2 (C9Ar), 116.7 (C1’Ar), 114.2 (AcetylC), 90.6 (C2Furyl), 87.3 (C5Furyl), 83.6 (C3Furyl), 81.2 (C4Furyl), 61.8 (CH_2), 26.7 (CH_3a), 25.0 (CH_3b); ν_{max}/ cm^{-1} (solid): 3253, 2939, 1633, 1586, 1344; HPLC: T_r= 2.19 (79% rel. area); *m/z* (ES): (Found: $[M+H]^+$, 365.1058. $C_{17}H_{19}N_5O_4S$ requires *[M+H]*, 365.1051. $[\alpha]_D$ = -6.6° (c 0.1, MeOH).

Preperation of [(3aR, 4R, 6R, 6aR)-6-(6-amino-2-(thiophen-3-yl)-9H-purin-9-yl)-2,2-dimethyl-tetrahydro-2H-furo[3,4-d][1,3]dioxol-4-yl]methyl sulfamate



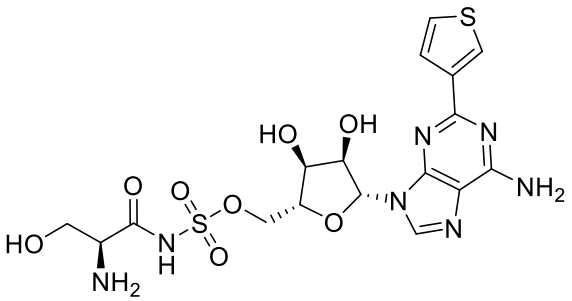
Preparation was *via* general method B using 2’3’-O-Isopropylidene-2-(thiophen-3-yl)adenosine (0.45 g, 1.16 mmol) to afford the desired product as a pale brown oil (0.51 g, 1.09 mmol, 94%) R_f: 0.48 (9:1 Chloroform-MeOH); δ_H (500 MHz, DMSO-d6): 8.31 91H, s, 6-HAr), 8.18 (1H, d, J 2.0, 2’-HAr), 7.77 (1H, dd, J 6.1 and 2.0, 5’-HAr), 7.58 (2H, brs, SNH_2), 7.42 (1H, d, J 6.1, 4’-H), 7.38 (2H, brs, NH_2), 6.31 (1H, d, J 2.2, 2-HFuryl), 5.54 (1H, d, J 4.0, 3-HFuryl), 5.20 (1H, d, J 4.0, 4-H Furyl), 4.44 (1H, app s, 5-HFuryl), 4.35-4.15 (2H, m, CH_2), 1.60 (3H, s, CH_3a), 1.37 (3H, s, CH_3b); δ_C (125 MHz, DMSO-d6): 169.5 C2Ar), 155.9 (C4Ar), 149.4 (C8Ar), 142.2 (C6Ar), 134.8 (C4’Ar), 132.4 (C5’Ar), 126.4 (C9Ar), 125.0 (C2’Ar), 117.9 (C1’Ar), 113.5 (Acetyl C), 88.9 (C2Furyl), 83.7 (C5Furyl), 83.2 (C3Furyl), 81.2 (C4Furyl), 68.1 (CH_2), 26.7 (CH_3a), 25.1 (CH_3b); ν_{max}/ cm^{-1} (solid): 3324, 3200, 2988, 1619, 1398; HPLC: T_r= 2.23 (82% rel. area); *m/z* (ES): (Found: $[M+H]^+$, 469.0958. $C_{17}H_{20}N_6O_6S_2$ requires *[M+H]*, 469.0959. $[\alpha]_D$ = 6.0° (c 0.1, MeOH).

Preparation of tert-butyl N-[(2S)-1-[(([(3aR, 4R, 6R, 6aR)-6-(6-amino-2-(thiophen-3-yl)-9H-purin-9-yl)-2,2-dimethyl-tetrahydro-2H-furo[3,4-d][1,3]dioxol-4-yl]methoxy)sulfonyl)amino]-3-(benzyloxy)-1-oxopropan-2-yl]carbamate



Preparation was *via* general method C using [(3aR, 4R, 6R, 6aR)-6-(6-amino-2-(thiophen-3-yl)-9H-purin-9-yl)-2,2-dimethyl-tetrahydro-2H-furo[3,4-d][1,3]dioxol-4-yl]methyl sulfamate (0.50 g, 1.06 mmol) to afford the desired product as a colourless glassy solid (0.33 g, 0.45 mmol, 42%) m.p.: 156.2-158.0 °C; R_f: 0.58 (9:1 DCM-MeOH); δ_H (500 MHz, DMSO-d6): 8.38 (1H, s, 6-HAr), 8.19 (1H, dd, J 3.1 and 1.1, 2'-HAr), 7.78 (1h, dd, J 5.1 and 1.1 4'-HAr), 7.57 (1H, dd, J 5.0 and 3.1, 5'-HAr), 7.30 (2H, brs, NH_2), 7.29-7.25 (5H, m, bzl), 6.23 (1H, d, J 3.0, 2-HFuryl), 6.07 (1H, app s, NH), 5.41 (1H, d, J 2.8, 3-HFuryl), 5.07 (1H, d, J 2.8, 4-HFuryl), 4.41 (3H, app d, J 9.9, CH_2Bzl) and 5-HFuryl, 4.10-4.00 (2H, m, CH_2O), 3.98-3.94 (1H, m, Chiral H), 3.70-3.50 (2H, m, CH_2Chiral), 1.58 (3H, s, CH_3a), 1.39 (9H, s, $CH_3 \times 3$), 1.35 (3H, s, CH_3b); δ_C (125 MHz, DMSO-d6): 173.2 (C2Ar), 170.1 (C=ONH), 156.1 (C4Ar), 155.9 (C=OBOC), 150.8 (C8Ar), 142.8 (C6Ar), 138.5 (C1Bzl), 128.0 (C5Bzl and C3Bzl), 127.3 (C6Bzl and C2Bzl), 127.1 (C4Bzl), 126.1 (C4Thio), 125.1 (C5Thio), 120.0 (C2Thio), 119.4 (C9Ar), 117.0 (C1Thio), 113.1 (Acetyl C), 88.7 (C2Furyl), 83.6 (C5Furyl), 83.3 (C3Furyl), 81.6 (C4Furyl), 79.7 (BOC C), 73.4 (CH_2 Bzl), 71.7 (CH_2O), 68.9 (CH_2 Chiral), 56.2 (Chiral C), 28.2 ($CH_3 \times 3$), 27.1 (CH_3a), 25.2 (CH_3b); ν_{max}/ cm^{-1} (solid): 3250, 3052, 1675, 1526, 1374; HPLC: T_r= 2.53 (100% rel. area); *m/z* (ES): (Found: $[M+H]^+$, 746.2286. $C_{32}H_{39}N_7O_{10}S_2$ requires *[M+H]*, 746.2273. $[\alpha]_D$ = 28.9° (c 0.1, MeOH).

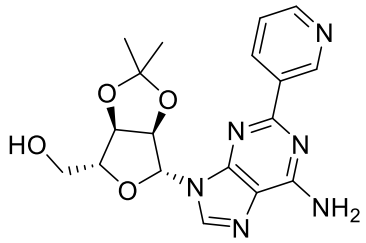
Preparation of (2S)-2-amino-1-[([[(2R, 3S, 4R, 5R)-5-(6-amino-2-(thiophen-3-yl)-9H-purin-9-yl)-3,4-dihydroxyoxolan-2-yl]methoxy)sulfonyl)amino]-3-hydroxypropan-1-one (7)



Preparation was via general method D using tert-butyl N-[(2S)-1-[(([(3aR, 4R, 6R, 6aR)-6-(6-amino-2-(thiophen-3-yl)-9H-purin-9-yl)-2,2-dimethyl-tetrahydro-2H-furo[3,4-d][1,3]dioxol-4-yl]methoxy)sulfonyl)amino]-3-(benzyloxy)-1-oxopropan-2-yl]carbamate (0.30 g, 0.40 mmol) to afford the desired product as a colourless powder (27.5 mg, 0.05 mmol, 13%) m.p.: 108.3 °C (Decomp); R_f: Baseline (9:1 DCM-MeOH); δ_H (500 MHz,

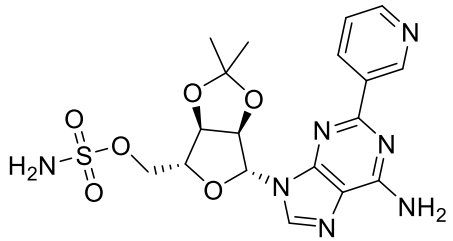
DMSO-d6): 8.46 (1H, brs, NH), 8.34 (1H, s, 2'-HAr), 8.14 (1H, s, 6-HAr), 8.00 (2H, brs, NH2), 7.87-7.78 (1H, m, 4'-HAr), 7.68-7.59 (1H, m, 5'-HAr), 6.00 (1H, d, J 5.5, 2-HFuryl), 4.70 (1H, t, J 5.5, 3-HFuryl), 4.37-4.22 (4H, m, CH_2O, 4-HFuryl and 5-HFuryl), 3.83-3.65 (3H, m, CH_2 chiral and chiral H); δ_C (125 MHz, DMSO-d6): 172.0 (C=O), 163.5 (C2Ar), 156.0 C4Ar), 150.7 C8Ar), 140.1 (C6Ar), 127.8 (C4'Ar), 127.1 (C5'Ar), 120.0 (C2'Ar), 119.2 (C9Ar), 116.8 (C1'Ar), 88.2 (C2Furyl), 84.2 (C5furyl), 73.7 (C3Furyl), 71.8 (C4Furyl), 60.8 (CH_2O), 57.2 (Chiral C), 45.4 (CH_2 chiral); ν_{max}/ cm^{-1} (solid): 3096, 1685, 1588, 1402, 1275; *m/z* (ES): (Found: $[M+H]^+$, 516.0972. $C_{17}H_{21}N_7O_8S_2$ requires *[M+H]*, 516.0966.) $[\alpha]_D$ = 20.7° (c 0.1, MeOH).

Preparation of 2'3'-O-Isopropylidene-2-(pyridine-3-yl)adenosine



Preparation was via general method A using 2'3'-O-Isopropylidene-2-chloroadenosine (0.50 g, 1.46 mmol) and 3-pyridine boronic acid (0.71 g, 5.85 mmol) to afford the desired product as a colourless powder (0.29 g, 0.75 mmol, 51%); m.p.: 122.4-124.2 °C; R_f: 0.53 (9:1 Chloroform-MeOH); δ_H (500 MHz, DMSO-d6): 9.49 (1h, s, 2'-HAr), 8.67-8.61 (2H, m, 6'-HAr and 4'-HAr), 8.40 (1H, s, 6-HAr), 7.52 (3H, brs, NH_2 and 5'-HAr), 6.26 (1H, d, J 2.7, 2-HFuryl), 5.52 (1H, dd, J 6.1 and 2.7, 3-HFuryl), 5.10 (1H, dd, J 6.1 and 2.9, 4-HFuryl), 5.05 (1H, t, J 5.5, OH), 4.23 (1H, dd, J 8.2 and 5.3, 5-HFuryl), 3.62-3.51 2H, m, CH_2), 1.57 (3H, s, CH_3a), 1.36 (3H, s, CH_3b); δ_C (125 MHz, DMSO-d6): 160.3 (C2Ar), 156.5 (C4Ar), 150.8 (C4Pip), 150.2 (C8Ar), 149.5 (C2Pip), 141.1 (C6Ar), 134.5 (C6Pip), 134.1 (C1Pip), 124.0 (C5Pip), 119.0 (C9Ar), 113.5 (Acetyl C), 89.7 (C2Furyl), 87.2 (C5Furyl), 83.8 (C3furyl), 81.9 (C4Furyl), 62.0 (CH_2), 27.6 (CH_3a), 25.7 (CH_3b); ν_{max}/ cm^{-1} (solid): 3322, 1632, 1572, 1375; HPLC: T_r= 1.99 (100% rel. area); *m/z* (ES): (Found: $[M+H]^+$, 385.1626. $C_{18}H_{20}N_6O_4$ requires *[M+H]*, 385.1619. $[\alpha]_D$ = -1.4° (c 0.1, MeOH).

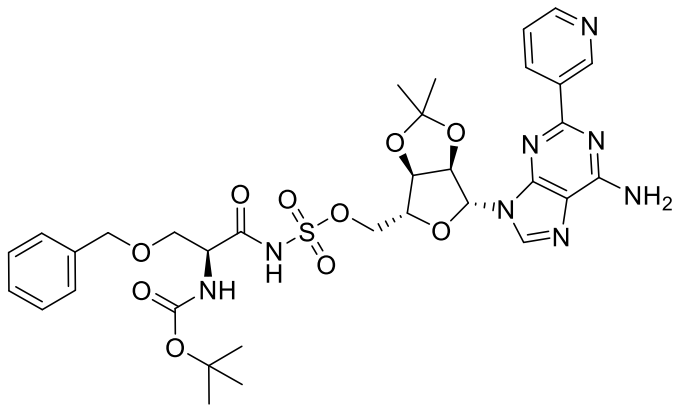
Preperation of [(3aR, 4R, 6R, 6aR)-6-(6-amino-2-(pyridine-3-yl)-9H-purin-9-yl)-2,2-dimethyl-tetrahydro-2H-furo[3,4-d][1,3]dioxol-4-yl]methyl sulfamate



Preparation was *via* general method B using 2'3'-O-Isopropylidene-2-(pyridine-3-yl)adenosine (0.29 g, 0.75 mmol) to afford the desired product as a colourless glassy solid (0.25 g, 0.54 mmol, 72%) m.p.: 93.1-94.7 °C; R_f: 0.39 (9:1 Chloroform-MeOH); δ_H (500 MHz, DMSO-d6):9.48 (1H, s, 2'-HAr), 8.67-8.59 (2H, m, 6'-H and 4'-H), 8.37 (1h, s, 6-HAr), 7.65-7.50 (5H, m, SNH_2, NH_2 and 3'-HAr), 6.34 (1H, d, J 2.2, 2-HFuryl), 5.58 (1H, d, J 3.7 3-HFuryl), 5.19 (1H, dd, J 6.0 and 3.7, 4-HFuryl), 4.45 (1H, app s, 5-HFuryl), 4.30-4.20 (2H, m, CH_2), 1.60 (3H, s, CH_3a), 1.39 (3H, s, CH_3b) ; δ_C (125 MHz, DMSO-d6): 161.7 (C2Ar), 155.8 C4Ar), 150.8 (C4'Ar), 150.6 (C8Ar), 149.5 (C2'Ar), 141.1

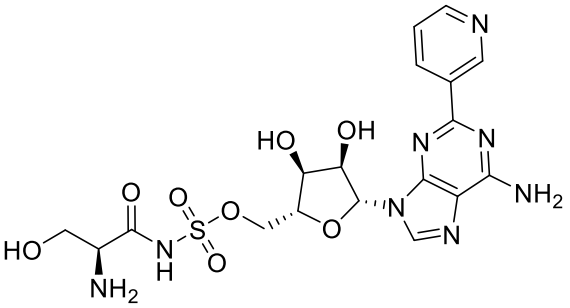
(C6Ar), 135.4 (C6'Ar), 133.2 (C1'Ar), 124.0 (C5'Ar), 119.1 (C9Ar), 114.2 (Acetyl C), 89.4 (C2Furyl), 84.0 (C5Furyl), 83.7 (C3Furyl), 81.6 (C4Furyl), 68.0 (CH_2), 27.5 (CH_3a), 25.7 (CH_3b); ν_{max}/ cm^{-1} (solid): 3345, 3180, 2985, 1632, 1580, 1374; HPLC: T_r= 2.04 (100% rel. area); *m/z* (ES): (Found: $[M+H]^+$, 464.1357. $C_{18}H_{21}N_7O_6S$ requires *[M+H]*, 464.1347. $[\alpha]_D$ = 53.5° (c 0.1, MeOH).

Preparation of tert-butyl N-[(2S)-1-[(([(3aR, 4R, 6R, 6aR)-6-(6-amino-2-(pyridine-3-yl)-9H-purin-9-yl)-2,2-dimethyl-tetrahydro-2H-furo[3,4-d][1,3]dioxol-4-yl]methoxy)^{6}sulfonyl)amino]-3-(benzyloxy)-1-oxopropan-2-yl]carbamate



Preparation was *via* general method C using [(3aR, 4R, 6R, 6aR)-6-(6-amino-2-(pyridine-3-yl)-9H-purin-9-yl)-2,2-dimethyl-tetrahydro-2H-furo[3,4-d][1,3]dioxol-4-yl]methyl sulfamate (0.25 g, 0.54 mmol) to afford the desired product as a colourless glassy solid (0.12 g, 0.16 mmol, 29%) m.p.: 167.2-164.5 °C; R_f: 0.39 (9:1 DCM-MeOH); δ_H (500 MHz, DMSO-d6): 9.49 (1H, s, 2'-HAr), 8.64 (2H, d, J 5.7, 6'-HAr and 4'-HAr), 8.46 (1H, s, 6-HAr), 7.52 (3H, brs, NH_2 and 5'-HAr), 7.27-7.24 (5H, m, Bzl), 6.27 (1H, d, J 3.0, 2-HFuryl), 6.08 (1H, d, J 7.8, NH), 5.43 (1H, d, J 2.7, 3-HFuryl), 5.07 (1H, app s, 4-Hfuryl), 4.41 (3H, app, s, CH_2 Bzl and 5-HFuryl), 4.05 (2H, d, J 4.6, CH_2O), 3.94 (1H, s, Chiral H), 3.62 (2H, d J 6.6, CH_2 Chiral), 1.59 (3H, s, CH_3a), 1.36 (9H, s, CH_3 × 3), 1.35 (3H, s, CH_3b); δ_C (125 MHz, DMSO-d6): 173.7 (C=ONH), 160.5 (C2Ar), 156.1 (C4Ar), 155.4 (C=OBOC), 150.2 (C4Pip), 150.1 (C8Ar), 148.9 (C2Pip), 140.1 (C6Ar), 137.7 (C1Bzl), 134.9 (C6Pip), 133.2 (C1Pip), 128.0 (C5Bzl and C3Bzl), 127.3 (C6Bzl and C2Bzl), 127.1 (C4Bzl), 123.5 (C5Pip), 119.4 (C9Ar), 114.2 (Acetyl C), 89.0 (C2Furyl), 83.5 (C5Furyl), 83.4 (C3Furyl), 81.5 (C4Furyl), 79.6 (BOC C), 73.7 (CH_2 Bzl), 71.7 (CH_2O), 68.2 (CH_2 Chiral), 54.7 (Chiral C), 28.2 (CH_3 × 3), 27.1 (CH_3a), 25.2 (CH_3b); ν_{max}/ cm^{-1} (solid): 3377, 2976, 1662, 1573, 1369; HPLC: T_r= 2.37 (100% rel. area); *m/z* (ES): (Found: $[M+H]^+$, 741.2674. $C_{33}H_{40}N_8O_{10}S$ requires *[M+H]*, 741.2661. $[\alpha]_D$ = -13.6° (c 0.1, MeOH).

Preparation of (2S)-2-amino-1-[([[(2R, 3S, 4R, 5R)-5-(6-amino-2-(pyridine-3-yl)-9H-purin-9-yl)-3,4-dihydroxyoxolan-2-yl]methoxy)sulfonyl)amino]-3-hydroxypropan-1-one (8)



Preparation was via general method D using tert-butyl N-[(2S)-1-[(([(3aR, 4R, 6R, 6aR)-6-(6-amino-2-(pyridine-3-yl)-9H-purin-9-yl)-2,2-dimethyl-tetrahydro-2H-furo[3,4-d][1,3]dioxol-4-yl]methoxy)sulfonyl)amino]-3-(benzyloxy)-1-oxopropan-2-yl]carbamate (100 mg, 0.13 mmol) to afford the desired product as a colourless powder (23.8 mg, 0.05 mmol, 36%) m.p.: 120.2 °C (Decomp); R_f: Baseline (9:1 DCM-MeOH); δ_H (500 MHz, DMSO-d6): 9.50 (1H, s, 2'-HAr), 8.95-8.75 (2H, m, 6'-HAr and 4'-HAr), 8.47 (1H, s, 6-HAr), 7.93 (2H, brs, NH_2), 7.54-7.50 (1H, m, 5'-HAr), 6.04 (1H, d, J 6.0 2-HFuryl), 4.82-4.72 (1H, m, 3-HFuryl), 4.37-4.23 2H, m, CH_2*O and 4-HFuryl), 4.18 (2H, app d, J 8.9, 5-HFuryl and CH2*O), 3.82 (1H, d, J 8.0 CH_2 chiral), 3.73-3.60 (1H, m, CH_2 chiral), 3.58-3.54 (1H, m, Chiral H); δ_C (125 MHz, DMSO-d6): 171.9 (C=O), 160.3 (C2Ar), 156.1 (C4Ar), 151.0 (C4'Ar), 150.8 (C8Ar), 150.7 (C2'Ar), 141.2 (C6Ar), 138.6 (C2'Ar), 135.6 (C6'Ar), 133.4 (C1'Ar), 125.2 (C5'Ar), 119.4 (C9Ar), 87.9 (C2Furyl), 82.9 (C5Furyl), 73.7 (C3Furyl), 71.2 (C4Furyl), 68.7 (CH_2O), 60.8 (CH_2 chiral), 57.5 (Chiral C); ν_{max}/ cm^{-1} (solid): 3317, 3118, 1633, 1587, 1587, 1382; *m/z* (ES): (Found: $[M+H]^+$, 511.1356. $C_{18}H_{22}N_8O_8S$ requires *[M+H]*, 511.1356. $[\alpha]_D$ = 19.3° (c 0.1, MeOH).

Supplementary References

1. Edgar, R. C., MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Res.* **2004,** *32* (5), 1792-1797.
2. Krissinel, E.; Henrick, K., Secondary-structure matching (SSM), a new tool for fast protein structure alignment in three dimensions. *Acta Crystallographica Section D* **2004,** *60* (12 Part 1), 2256-2268.
3. Lloyd, A. J.; Potter, N. J.; Fishwick, C. W. G.; Roper, D. I.; Dowson, C. G., Adenosine Tetraphosphoadenosine Drives a Continuous ATP-Release Assay for Aminoacyl-tRNA Synthetases and Other Adenylate-Forming Enzymes. *ACS Chem. Biol.* **2013,** *8* (10), 2157-2163.
4. Morris, G. M.; Huey, R.; Lindstrom, W.; Sanner, M. F.; Belew, R. K.; Goodsell, D. S.; Olson, A. J., AutoDock4 and AutoDockTools4: Automated Docking with Selective Receptor Flexibility. *J. Comput. Chem.* **2009,** *30* (16), 2785-2791.
5. Maestro, version 9.3, Schrödinger, LLC, New York, NY.
6. F C Neidhardt; J Parker, a.; McKeever, W. G., Function and Regulation of Aminoacyl-tRNA Synthetases in Prokaryotic and Eukaryotic Cells. *Annu. Rev. Microbiol.* **1975,** *29* (1), 215-250.