



Design and synthesis of Coenzyme A analogues as Aurora kinase A inhibitors: An exploration of the roles of the pyrophosphate and pantetheine moieties



Fiona Bellany^{a,1}, Yugo Tsuchiya^b, Trang M. Tran^a, A.W. Edith Chan^c, Helen Allan^a, Ivan Gout^b, Alethea B. Tabor^{a,*}

^a Department of Chemistry, UCL, Christopher Ingold Building, 20, Gordon Street, London WC1H 0AJ, UK

^b Department of Structural and Molecular Biology, UCL, Gower Street, London WC1E 6BT, UK

^c Wolfson Institute for Biomedical Research, University College London, London WC1E 6BT, UK

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ABSTRACT

Coenzyme A (CoA) is a highly selective inhibitor of the mitotic regulatory enzyme Aurora A kinase, with a novel mode of action. Herein we report the design and synthesis of analogues of CoA as inhibitors of Aurora A kinase. We have designed and synthesised modified CoA structures as potential inhibitors, combining dicarbonyl mimics of the pyrophosphate group with a conserved adenosine headgroup and different length pantetheine-based tail groups. An analogue with a -SH group at the end of the pantotheinate tail showed the best IC₅₀, probably due to the formation of a covalent bond with Aurora A kinase Cys290.

1. Introduction

The Aurora kinases are a family of highly homologous serine/threonine kinases,^{1–3} which have well-documented roles in the control of meiosis, mitosis and cell division.⁴ There are three distinct sub-families, Aurora A, Aurora B and Aurora C, which vary in their function and subcellular location. Since the first observation of the over-expression of Aurora kinases in cancer cell lines by Bischoff et al.⁵ they have been regarded as promising drug targets for cancer chemotherapy. Aurora A overexpression is frequently detected in leukemia, breast, prostate and colon cancers,^{2,5,6} with a lower overall survival rate seen in colorectal cancer patients with increased Aurora A levels.⁷ This has seen a number of Aurora kinase inhibitors progressing through different stages of clinical trials.^{3,8–11} The Aurora kinases are highly homologous, with a highly conserved C-terminal catalytic domain, a short N-terminal domain which varies in size, and an activation loop (Asp274-Glu299) which is conserved between the three family members.³ The kinase activity of Aurora A is dependent on autophosphorylation of Thr288 (and possibly Thr287), found in the activation loop. In addition, binding of Aurora A to microtubule-associated proteins, in particular TPX2, alters the structure of Aurora A by stabilisation of the activation loop, allowing ATP to bind and driving the kinase into the optimal conformation for catalysis.^{1,3,12} Inhibition of Aurora A results in

inhibition of this phosphorylation, giving delayed entry into mitosis and the failure of the centrosomes to assemble bipolar spindles, resulting in aneuploidy and mitotic arrest.¹³ Different conformational states of Aurora A can be induced or stabilised by different small molecule inhibitors,^{14–17} and in particular the conformation of the activation loop modulates the interaction of Aurora A with its binding partners.¹⁸ Coenzyme A (CoA) is an essential and ubiquitous cofactor made from vitamin B5 (pantothenate), ATP, and cysteine. CoA and its thioester derivatives (Acetyl CoA, Malonyl CoA, HMG CoA among others) are involved in diverse anabolic and catabolic pathways, biosynthesis of neurotransmitters and the regulation of gene expression.^{19,20} Dysregulation of CoA biosynthesis or CoA thioester homeostasis is associated with various human pathologies, including neurodegeneration, cancer and metabolic disorders.^{21–24} Recent studies have uncovered a novel function of CoA in redox regulation, involving covalent modification of cellular proteins by disulphide bond formation, termed CoAlation, in cellular response to oxidative and metabolic stress.^{25,26} Protein CoAlation is a widespread and reversible post-translational modification, which occurs in single-cell and multicellular organisms, and modulates catalytic activity, regulatory interactions, subcellular localization and the stability of modified proteins.^{27–30}

Recently, we have reported that CoA is a specific ATP-competitive Aurora A inhibitor *in vitro*.³¹ Using a combination of biochemical, cell

* Corresponding author.

E-mail address: a.b.tabor@ucl.ac.uk (A.B. Tabor).

¹ Present address: Drug Discovery Unit, Division of Biological Chemistry and Drug Discovery, University of Dundee, Dundee DD1 5EH, UK.

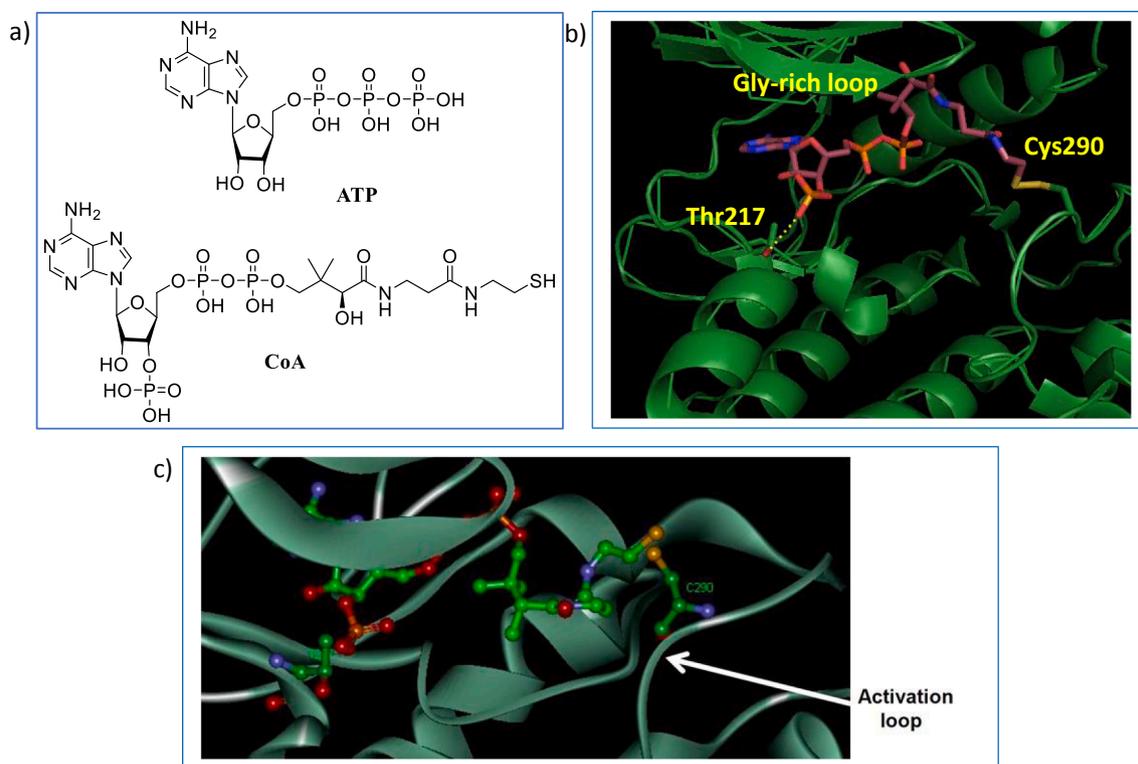


Fig. 1. (a) Structural similarities of ATP and CoA; (b) Crystal structure of Aurora A in complex with CoA highlighting the Thr217 and Cys290 side chains (PDB: 6I2U); (c) Model of CoA bound to Aurora A (PDB 1OL7). The location of Thr217 adjacent to the 3'-phospho-adenine of CoA, and Cys290 in the kinase activation segment, are both indicated.

biology and biophysical studies, combined with the determination of the X-ray crystal structure of CoA with Aurora A (PDB: 6I2U) we demonstrated that the ADP moiety of CoA occupies the canonical ATP binding site of Aurora A, while the extended CoA pantetheine tail stretches away from the ATP site towards the kinase activation loop, forming a disulfide bond with the side chain of Cys290 adjacent to the Thr288 phosphorylated residue (Fig. 1b). The pantoic acid moiety of CoA interacts with the tip of the Gly-rich loop (residues 142–145) and displaces it from the position found in Aurora A/ADP complexes. The 3'-phosphate group of CoA is also ideally positioned to form a H-bond with the side chain of Thr217. Both the phosphate ADP moiety and the thiol group of CoA are involved in mediating specificity determining interactions with Aurora A via targeting of Thr217 and Cys290 respectively. This dual-mode action of Aurora A inhibition by CoA traps the kinase in an inhibitory conformation, thus making CoA an irreversible inhibitor of Aurora A.

This unique interaction between CoA and Aurora A kinase presents an opportunity to design novel selective inhibitors of Aurora A kinase. In this paper, we have undertaken the design and synthesis of potential selective inhibitors of Aurora kinase A, based on the CoA structure, exploring in particular the structure-activity relationships of the pyrophosphate and pantetheine moieties of CoA.

2. Results and discussion

2.1. Design of pyrophosphate isostere analogues of CoA

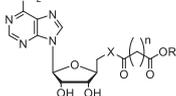
Based on the structural similarities between ATP and CoA (Fig. 1a), and detailed biochemical investigations of the interactions between CoA and Aurora kinase A,³¹ we were also able to predict the binding mode of CoA into Aurora A by molecular docking studies using GOLD (Genetic Optimisation for Ligand Docking).^{32,33} A PDB file (1OL7) that corresponds to the published crystal structure data of the active, phosphorylated form of human Aurora A kinase¹² was modified using

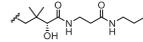
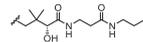
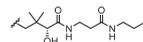
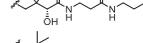
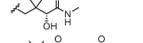
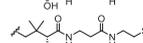
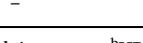
MOE³⁴ to include the cysteine residue Cys290, present in the activation loop of Aurora A (Fig. 1c). The results of the docking were in good agreement with the X-ray crystal structure of the complex. In particular, the 3'-phosphate group of CoA is able to interact with the side chain of Thr 217, and in the majority of the docking poses the pantetheine tail extends towards a front pocket of the active site in proximity to the side chain of Cys290. We were thus able to use this modified crystal structure as a basis for designing potential selective inhibitors or Aurora A kinase, based on modified CoA structures.

CoA has several limitations as a lead structure for drug design,^{35,36} in particular its large size, the presence of charged phosphate groups which would prevent it from crossing the cell membrane, and the possibility of off-target effects due to its use in many metabolic pathways. Initially therefore we elected to explore structures with the 3'-phosphate group removed and incorporating mimics of the pyrophosphate group, in order to make a more drug-like CoA structure. Several reviews of phosphate and pyrophosphate mimics have been published.^{37–39} Phosphate isosteres include phosphonates, sulfamoyl groups, carboxylate groups and heterocycles such as triazoles and thiazolidinones. Despite the literature, there is no clear “gold-standard” pyrophosphate mimic. Isosteres based on the carboxylate group have improved cell permeability when compared to the phosphate group,³⁹ and are frequently more synthetically accessible. In this work, we focused our attention on dicarbonyl mimics of the pyrophosphate group^{40,41} combining these with a conserved adenosine headgroup and a variety of pantetheine tail groups (Table 1). To assess the drug-likeness of these structures, as CoA and many of these analogues contain ionizable groups, we calculated the logD values using Stardrop⁴² and compared these, the MW, HBD and HBA with CoA. This showed a significant improvement in lipophilicity for **3**, **5** and **10** compared with CoA. However, the logD values of these compounds still lie below the desirable range of $1 < \log D < 3$ in which cell permeability and compound solubility are optimized.⁴³

The suitability of analogues bearing a dicarbonyl linker was initially

Table 1
Aurora A inhibitor analogues of CoA.



Compound	X	n	R	logD	MW	HBD ^a	HBA ^b
1	O	1		0.39	595.6	6	17
2	O	1	H	-2.34	353.3	4	12
3	N	1		0.59	594.6	7	17
4	N	1	H	-2.39	352.3	5	12
5	O	2		0.54	609.6	6	17
6	O	3		0.70	623.7	6	17
7	O	2		0.35	437.4	3	12
8	O	2		-0.28	510.5	5	15
9	O	2		0.18	581.6	6	17
10	O	2		0.57	627.7	6	17
CoA	-	-	-	-0.05	767.5	9	23

^a HBA: No. of H Bond Acceptors, ^bHBD: No. of H bond donors.

evaluated by docking these analogues into the Cys290-modified, active, phosphorylated form of human Aurora A kinase described above. Initial docking studies using analogue **1** suggest that the adenosine region binds to the ADP-binding pocket as expected. However, **1** does not have a particular preference for the position of the tail region, either extending towards the back pocket of Aurora A or potentially interacting with Cys290 (Fig. 2a,b), possibly because the analogue lacks the -SH warhead. Fig. 2c shows the highest CHEMPLP GOLD score result, the required hydrogen bonding between the Ala213 in the hinge region of the kinase and the adenine of **1** can be clearly seen. There is also a potential interaction between one of the carbonyl groups in the linker and either Glu260/Asn261. It is also apparent that the 3'OH is too far away to pick up an interaction with Thr217 to improve selectivity towards Aurora A. Although the result in Fig. 2 is shown for the binding mode where the tail extends into the back pocket, these interactions were consistent throughout all the results.

In addition to analogue **1** we aimed to investigate the SAR of varying the pyrophosphate and pantetheine moieties by synthesising analogues with substitution of an ester bond with an amide (**3**), different dicarbonyl linker lengths (**5**, **6**) and tail lengths (**2**, **4**, **7**, **8**, **9**). It is possible that with the addition of a suitable group to the terminal region of the tail, the preferred binding mode with interaction with Cys290 could be achieved. This interaction is believed to be an important requirement for potency and selectivity towards Aurora A and this could be exploited in this structure by switching the terminal CH₃ group to an SH group (**10**, as in CoA). It is also worth noting that the addition of an extra moiety to the 3' OH is required to pick up an interaction with Thr217 to improve the selectivity towards Aurora A. However, in the first instance, it was envisaged that compounds would be synthesised without the addition of these groups to identify simplified lead compounds for the inhibition of Aurora A.

2.2. Synthesis of pyrophosphate isostere analogues of Coenzyme A

Initially the synthesis of the dicarbonyl analogue **1** was attempted using adenosine protected at the 2'/3' positions with an acetonide protecting group, however, it was found that removal of this protecting group in the final step was not possible due to the degradation of the pantetheine tail under a range of acidic conditions. A range of different protecting groups were tried, and the desired dicarbonyl analogue **1**

was finally synthesised using adenosine protected with the tetra-isopropylidisilyl (TIPDS) group (Scheme 1).

Linker **12** was prepared in 71% yield via ring-opening of Meldrum's acid with benzyl alcohol.⁴⁴ Esterification^{45,46} of 2',3'-isopropylidene adenosine (**11**) with protected linker **12** was followed by the removal of the acetonide group using TFA to give **14**. This intermediate was protected with the TIPDS group to afford **15**, using the conditions reported by Sierzchala et al.⁴⁷ before removing the benzyl protecting group by hydrogenation. Once deprotected, the resulting acid **16** was then coupled to the pantetheine tail **17** (synthesised in 2 steps from pantothenic acid following a modified version of the procedure of Virga et al.⁴⁸) using EDC in moderate yield before removing the silyl protecting group. Due to the concern of the strong basicity of the F⁻ ion from TBAF causing unwanted side reactions,^{49,50} ammonium fluoride was successfully applied to the silyl deprotection instead.⁵¹ The synthetic route was repeated with the initial conversion of the adenosine 6'-OH to an -NH₂ group, using conditions developed by Yao et al.⁵² to afford **19**, which was then coupled with linker **12** to give **20**. The addition of the silyl protecting group to **21** to give **22** was found to be higher yielding when the conditions developed by Kim et al.⁵³ were used. Hydrogenation to give **23** was again followed by coupling of **17** to afford **24**, which was finally deprotected to give **3**. During the development of the synthetic routes to **1** and **3**, compounds **2** and **4**, lacking the pantetheine tail, were also synthesised as controls (Scheme 2).

A modified procedure was developed for the synthesis of the extended linker analogues **5** and **6**. In order to develop a more convergent approach, we wished to first prepare the TIPDS protected adenosine **26**. Direct protection of adenosine (**27**) with TIPDSCl₂ gave the 3',5'-protected adenosine derivative, as expected: unfortunately, attempted isomerisation to the required 2',3'-protected adenosine **26**, using previously reported conditions^{54,55} was unsuccessful. We therefore elected to carry out initial protection of the 5' position with the dimethoxytrityl (DMTr) protecting group, utilising the conditions developed by Matulic-Adamic et al.⁵⁶ to afford **28**. Subsequent silyl addition to give **29** was followed by trityl group removal with acetic acid,⁵⁷ which gave **26** (Scheme 3). The esterification proceeded well with linker lengths of 1 carbon (**12**)⁴⁴ 2 carbons (**30**)⁵⁸ and 3 carbons (**34**),⁵⁸ giving **15**, **31** and **35** respectively. The conversions of **31** to **32**, and **35** to **36**, were performed as before. As the coupling of **32** to the pantetheine tail **17** using EDC, and the equivalent coupling of **36** with **17**, gave products (**33** and **37**) that were difficult to purify, the silyl group was removed directly from each intermediate using ammonium fluoride to afford **5** and **6** respectively.

2.3. Role of the pantetheine tail

As our docking studies and the crystal structure of the Aurora A kinase/CoA complex had indicated the importance of the pantetheine tail, we wished to explore whether simplified analogues with shorter tail structures would also be effective inhibitors. Structural analogues of CoA bearing modified pantetheine tails have not previously been reported as enzyme inhibitors. However, pantetheine analogues are important lead compounds as inhibitors of pantothenate kinase,^{59,60} a key enzyme in the biosynthesis of CoA which is a validated target for antimicrobial chemotherapy. A range of structural mimics of pantetheine side chains have therefore been developed,^{45,48,61,62} and these were used as a starting point for the present study. Prior to the synthesis of further analogues, alternative esterification conditions for the coupling of the tail to the linker moiety were investigated, and it was found that the use of DIC as the coupling agent gave the highest yields. These conditions were then applied to the syntheses of truncated analogues **7**, **8**, and **9** (Scheme 4). Alcohol **40** was synthesised according to the literature procedure.⁶³ Alcohol **42** was synthesised by the ring opening of D-pantolactone with *N*-methyl-β-aminopropionamide under basic conditions.

Compound **10** was synthesised using alcohol **43** (the oxidised dimer

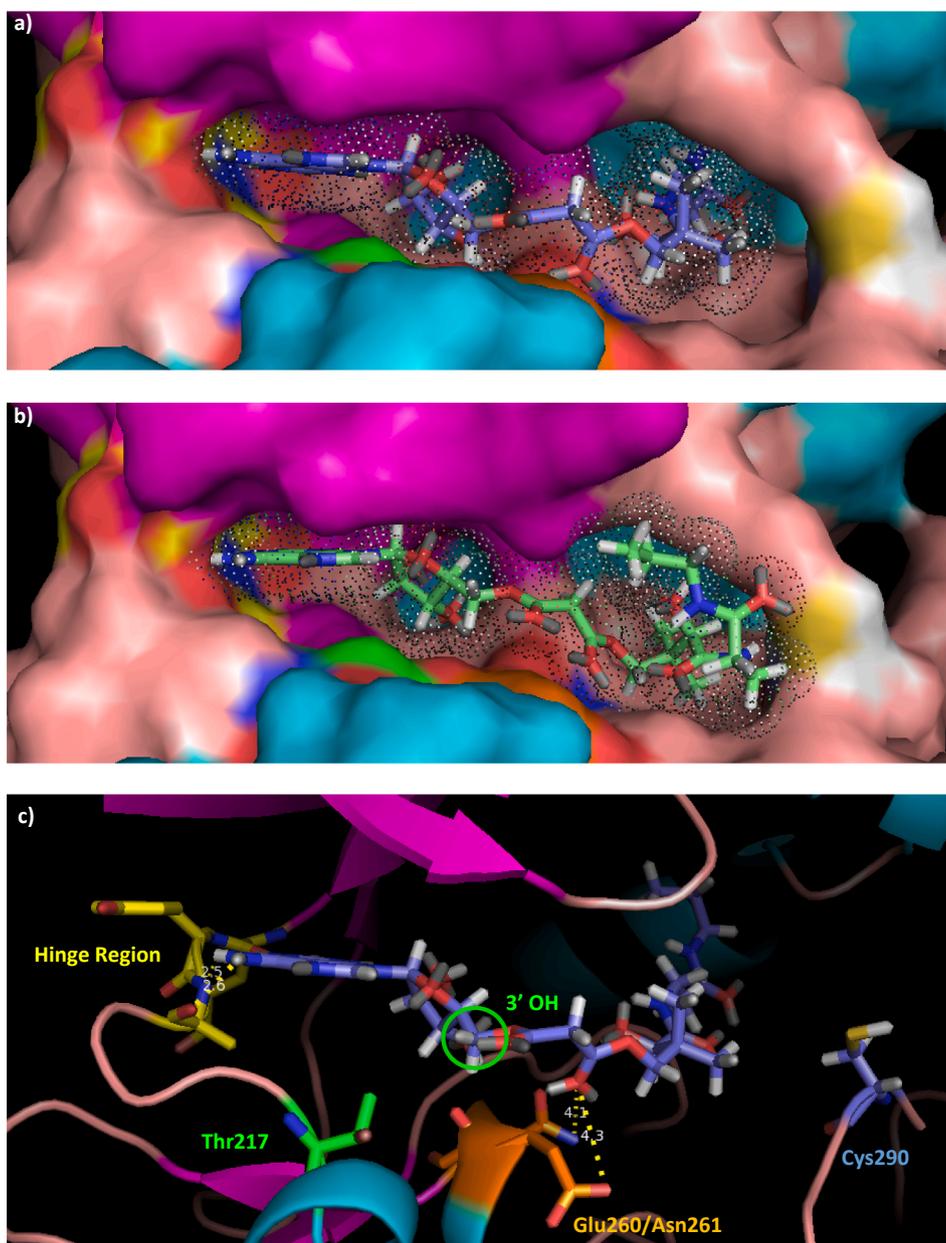


Fig. 2. Docking results for the binding of **1** to Aurora A, showing the two possible tail binding modes: (a) binding of the tail into the back pocket of the kinase and (b) the binding of the tail to the solvent front of the kinase where it is possible to pick up an interaction with Cys290, (c) This figure highlights the potential hydrogen bonding between the adenine of **1** and the hinge region of the kinase and the interactions between the linker moiety and Glu260/Asn261.

of pantothenic acid) which gave a mixture of mono-substitution and the adenosine dimer (**44**, **45**), the crude material was directly treated with TBAF deprotection which enabled isolation of the dimer (**46**). The disulfide bond was then cleaved using TCEP to give **10** (Scheme 5).

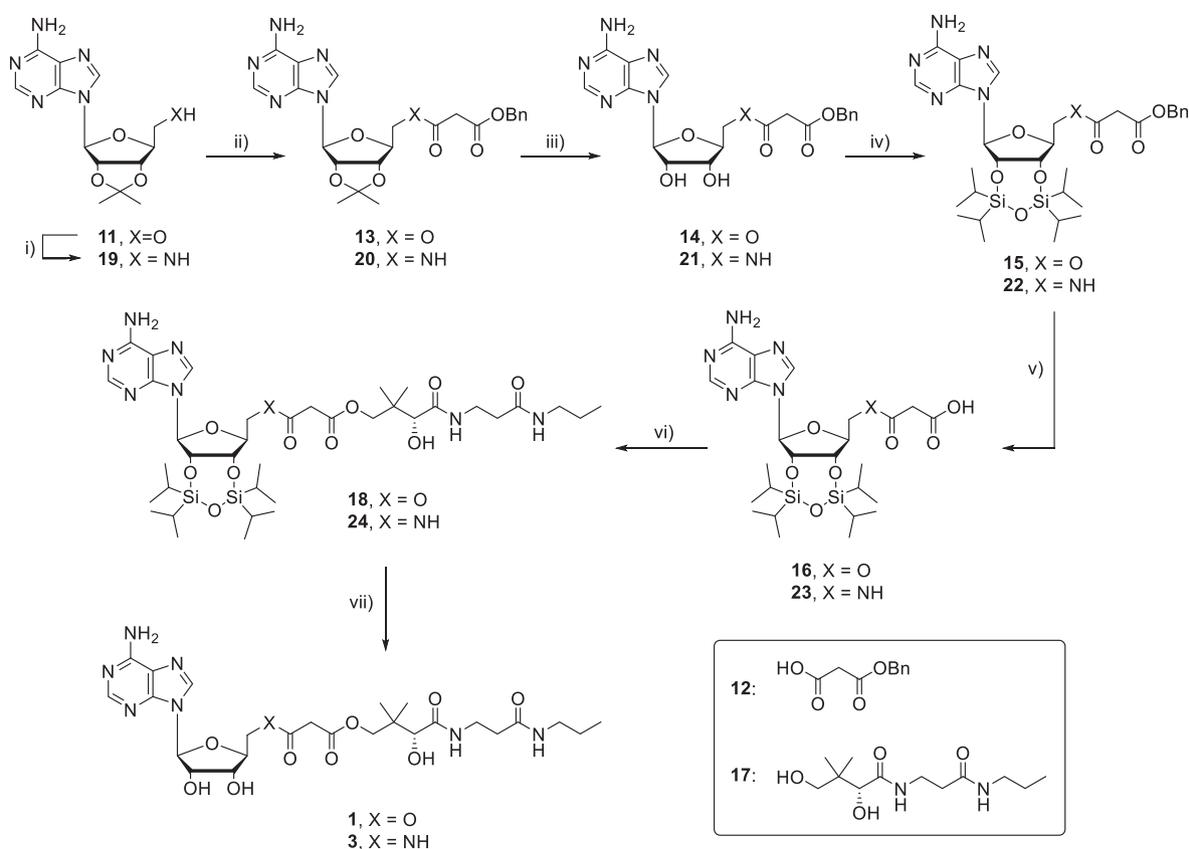
2.4. Biochemical and docking studies of Aurora A kinase inhibitors

With our analogues in hand we measured the IC₅₀ values against Aurora A using a radioactive filter-binding assay (Table 2).

Comparison of the IC₅₀ values allows us to define some of the features of these analogues that are important for Aurora A kinase inhibition. Analogues with ester linkages, such as **2**, showed a two-fold improvement in activity over equivalent analogues with amide linkages, e.g. **4**. Secondly, the use of a longer linker (**5,6**) led to a two-fold increase in activity compared to the initial dicarbonyl analogue **1**, indicating that in this case, the succinic or glutaric acid dicarbonyl moieties are better isosters of the pyrophosphate group in CoA.

Unfortunately, the docking results for these compounds are very similar to those obtained for **1** and do not explain the increase in potency. For both analogues, due to the flexibility of the tail and the linker, there is no clear preference to either binding mode available for the tail.

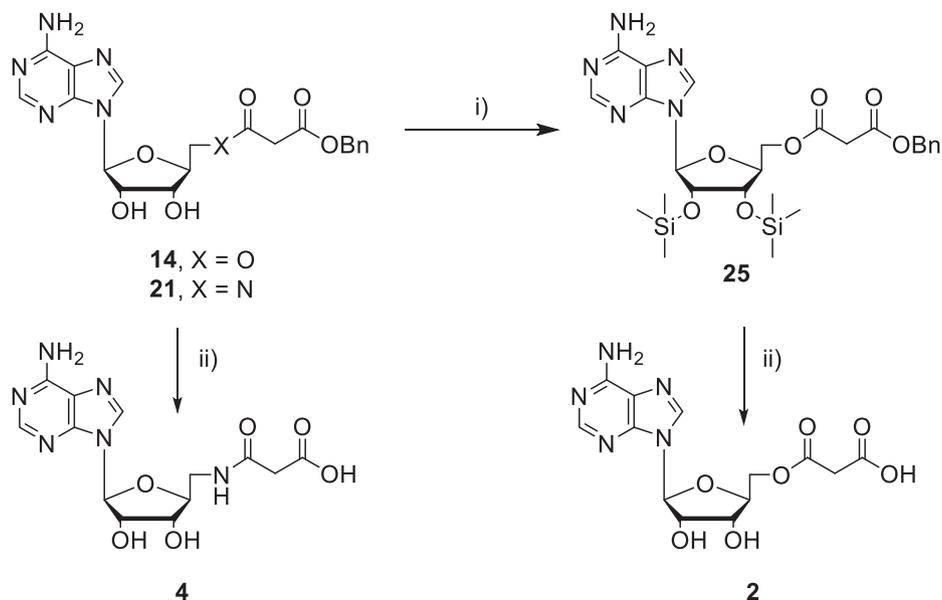
It is also clear that the terminal -SH group plays a crucial role in the recognition of CoA and analogues by Aurora A kinase. Comparison of the IC₅₀ of analogue **10** with those for dpCoA and desulfo-CoA suggests that the presence of the -SH group is more important in determining the binding to Aurora A kinase than the presence of the 3'-phosphate or the pyrophosphate moieties. This is consistent with our recent discovery³¹ that Aurora A is specifically CoAlated by the formation of a covalent bond between the -SH group and Cys290. In order to establish if a similar covalent interaction was present with appropriate CoA analogues, the activity assay of **10** was run in both the presence and absence of DTT. The inhibition of Aurora A by **10** was found to be DTT dependent, with the IC₅₀ value for DTT present 731 μM and the IC₅₀ value for no DTT present 117 μM.



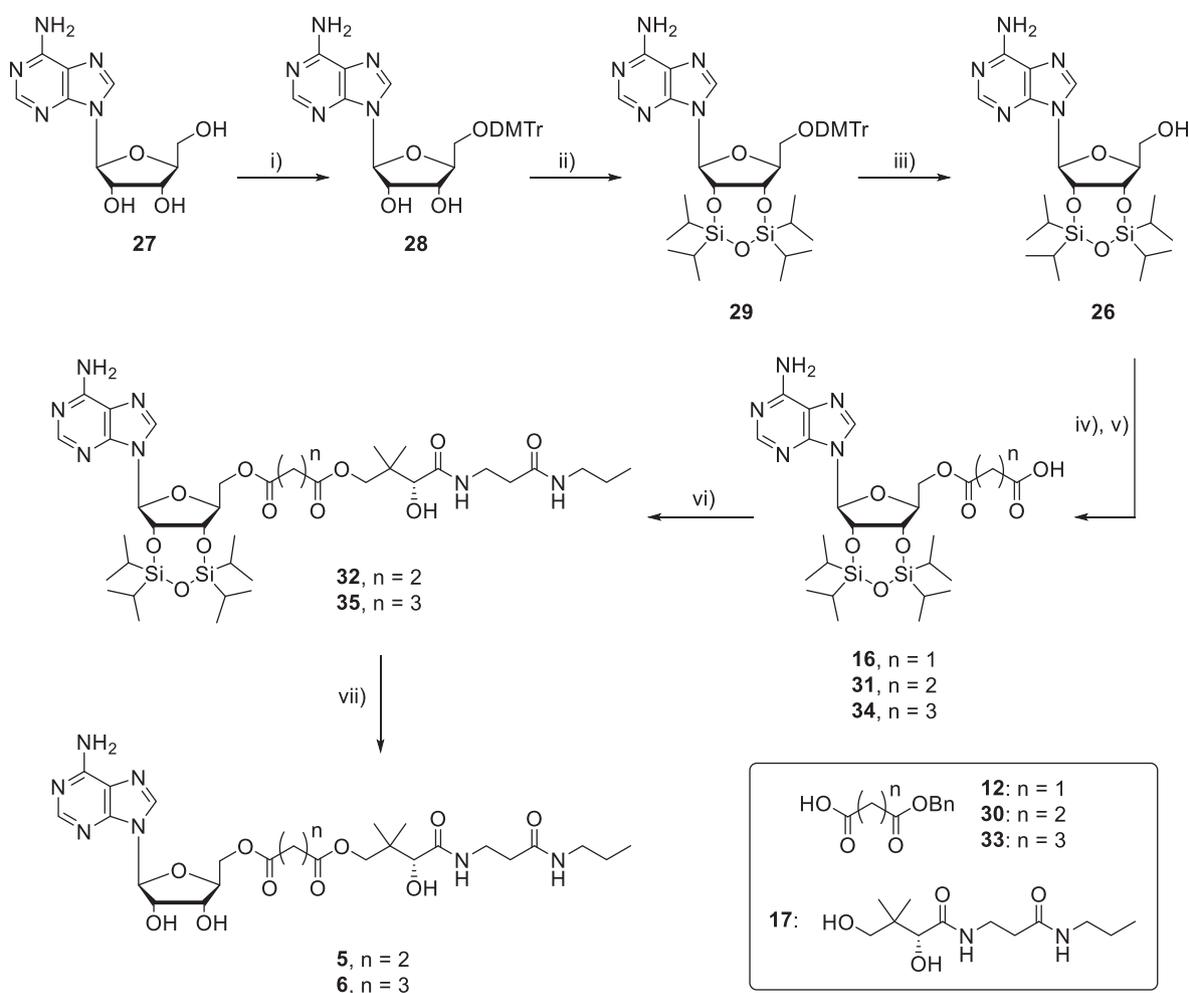
Scheme 1. Synthesis of **1** and **3** using the TIPDS protecting group. Conditions: (i) 1.1 eq. phthalimide, 1.1 eq. PPh₃, 1.1 eq. DIAD, THF, RT, 5 h then 5.0 eq. hydrazine hydrate, EtOH, reflux, 16 h, 44% over two steps; (ii) 2.0 eq. **12**, 25 mol% DMAP, 1.3 eq. DCC, CH₂Cl₂, RT, 19 h, **13**: 52%, **20**: 78%; (iii) TFA, H₂O, RT, 3–4 h, **14**: 80%, **21**: 90%; (iv) **15**: 1.1 eq. TIPDSCl₂, pyridine, RT, 18 h, 45%; **22**: 1.2 eq. TIPDSCl₂, 2.5 eq. imidazole, 25 mol% DMAP, DMF, RT, 17 h, 61%; (v) 10 mol% Pd/C, MeOH, RT, 3–5 h, **16**: 71%, **23**: 95%; (vi) 1.0 eq. **17**, 1.2 eq. EDC, 1.2 eq. DMAP, RT, 18–20 h, **18**: 25%, **24**: 14%; (vii) 8.5 eq. NH₄F, MeOH, RT, 1.5 h, **1**: 42%, **2**: 75%.

In the absence of a terminal –SH group, analogue **7**, with the shortest truncated tail structure, was found to be the most active. Comparison of the docking of **7** into the binding site of our model, Cys290-modified crystal structure of the active Aurora A kinase (Section 2.1) with the binding of the other truncated structures **8** and **9** provides some insight into these results (Fig. 3). Analogue **7** can be seen

to fit perfectly in the binding site between the hinge region and the activation loop (Fig. 3a) and therefore has good activity towards the kinase. By contrast, the longer pantetheine tail moieties of analogues **8** and **9** extend towards the back pocket of the kinase and thus cannot interact with either residues Thr 288 or Cys290 in the kinase activation loop, nor with the Gly-rich loop (Fig. 3b, c). Additional analysis of the



Scheme 2. Synthesis of **2** and **4**. Conditions: (i) 2.5 eq. TMSCl, 3.0 eq. NEt₃, CH₂Cl₂, RT, 16 h, 56%; (ii) 10 mol% Pd/C, MeOH, RT, 5–6 h, **2**: 87%, **4**: 98%.



Scheme 3. Conditions: (i) 1.1 eq. 4,4-dimethoxytrityl chloride, 2.0 eq. NEt_3 , 25 mol% DMAP, pyridine, RT, 20 h, 34%; (ii) 1.2 eq. TIPDSCl_2 , 2.5 eq. imidazole, 25 mol% DMAP, DMF, RT, 17 h, 61%; (iii) 80% AcOH, RT, 4 h, 74%; (iv) 2.0 eq. alcohol, 1.3 eq. DCC, 25 mol% DMAP, CH_2Cl_2 , RT, 18 h, alcohol **12** gave **15**: 90%; alcohol **30** gave **31**: 85%; alcohol **34** gave **35** 34%; (v) 10 mol% Pd/C, MeOH, RT, 3–5 h, **32**: 97%, **36**: 97%; (vi) 1.0 eq. **17**, 1.2 eq. EDC, 1.2 eq. DMAP, RT, 18 h; (vii) 8.5 eq. NH_4F , MeOH, RT, 1.5 h, **5**: 1% over two steps; **6**: 3% over two steps.

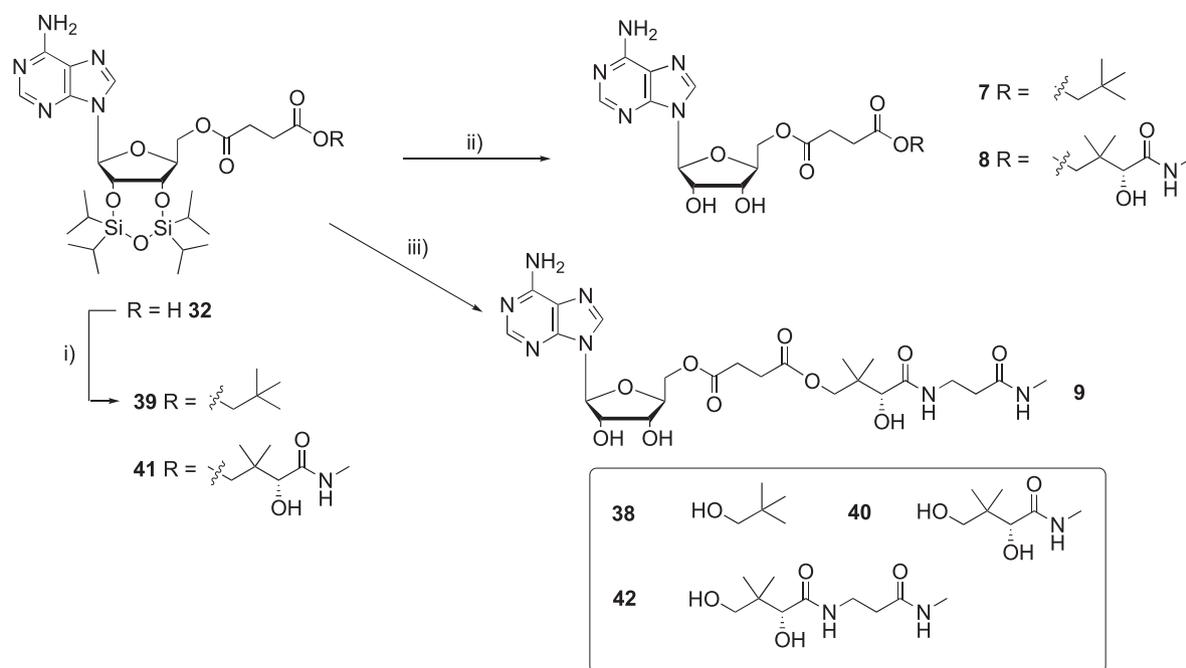
docking of **7** (Fig. 3d) highlights the possibility of extending the length of **7** by the addition of a group with the potential to interact with a cysteine residue within the active site to increase the activity of this analogue against Aurora A.

3. Conclusion

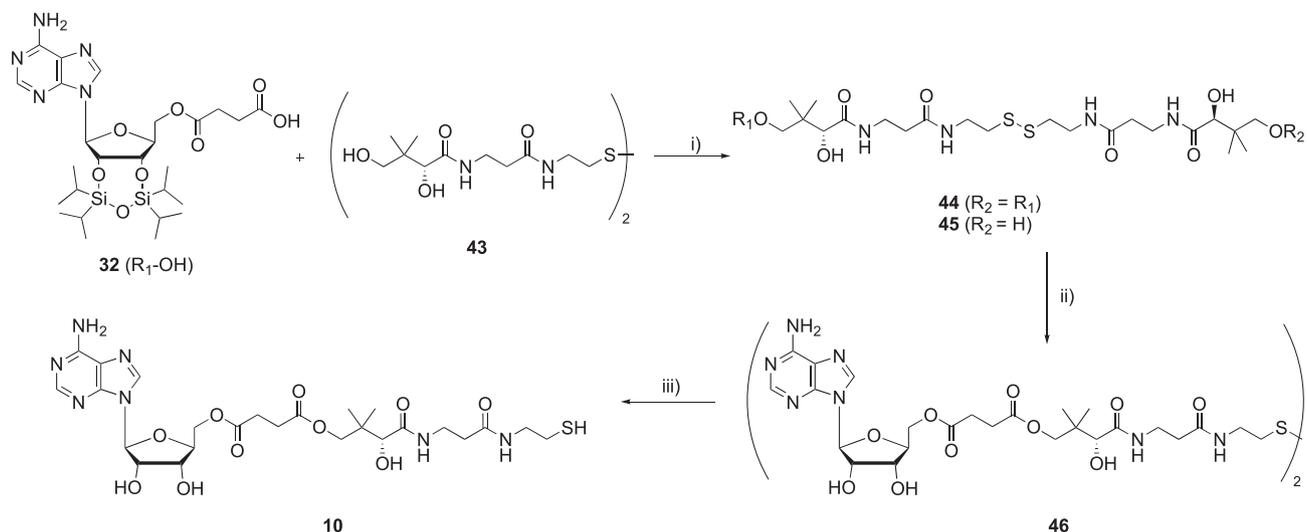
In this preliminary study, we have shown that understanding the structural basis for the unprecedented and highly selective inhibition of Aurora A kinase by CoA is an effective starting point for the design of inhibitors. We report the docking of CoA into the active site of a model structure of the active form of CoA, and have compared this with our recently published Aurora A kinase-CoA complex structure.³¹ We have then used the model structure as the basis for docking studies.

We have designed and synthesised a series of simplified CoA analogues to probe the structural requirements for the inhibition of Aurora A. In particular, our results highlight the importance of targeting interactions with the Cys290 residue in the kinase activation loop, and also illustrate that quite simple dicarbonyl isosteres can be useful mimics of the pyrophosphate group of CoA. Of our synthesised compounds, analogue **10** has the best IC_{50} value against Aurora A. It has improved drug likeness when compared to the parent molecule: the molecular weight has been reduced and the charged pyrophosphate group has been replaced with a neutral dicarbonyl linker, which should

allow improved cell permeability. The interaction between the terminal –SH and Aurora A was found to be crucially important for activity, probably due to the formation of a disulfide bond formed between the terminal –SH of CoA and the –SH of the cysteine residue contained in the activation loop. In the absence of the terminal –SH analogue **7** with the shortest pantetheine tail shows the best Aurora A activity, and we have rationalised this observation by docking studies. Further improvements in activity could come from truncated pantetheine tail analogues with –SH termini to interact with Cys290. These analogues would have a lower molecular weight and improved drug likeness when compared to CoA. In addition, extensive literature on alternative adenosine groups may allow this headgroup to be simplified. Combining these modifications with analogues that have an additional Thr217 interaction (such as H-bonding or a phosphate mimic) could further increase the potency of these compounds towards Aurora A. Finally, the majority of Aurora kinase inhibitors so far reported are competitive inhibitors which mimic the binding of ATP at the active site.^{3,8–11} This work opens up the possibility for the design of selective, irreversible (Type IV) covalent inhibitors based on analogue **10**, which have the potential both to block the ATP active site and to alter the Aurora A conformation to prevent the binding of TPX2. Inhibitors with such a novel mode of action thus represent an important new paradigm for developing anticancer agents with reduced off-target side effects.



Scheme 4. Conditions: (i) 1.5 eq. DIC, 25 mol% DMAP, CH₂Cl₂, RT, 20 h, 1.5 eq. alcohol. **38** gave **39**: 66%, **40** gave **41**: 46%; (ii) 8.5 eq. NH₄F, MeOH, RT, 1.5 h, **7**: 59%, **8**: 34%; (iii) 1.5 eq. DIC, 25 mol% DMAP, CH₂Cl₂, RT, 20 h, 1.5 eq. **42**, then 8.5 eq. NH₄F, MeOH, RT, 1.5 h, 18% over 2 steps.



Scheme 5. Conditions: (i) 1.5 eq. DIC, 25 mol% DMAP, CH₂Cl₂, RT, 20 h, 1.5 eq alcohol **43** gave a mixture of singly added (**44**) and doubly added (**45**) products, which was then treated (ii) with 8.5 eq. NH₄F, MeOH, RT, 1.5 h to give **46**: 4% over 2 steps; (iii) 10.0 eq. TCEP, H₂O, MeCN, RT, 1 h, **10**: 78%.

4. Experimental

4.1. Chemistry

¹H and ¹³C NMR spectra of novel compounds can be found in the [supporting information](#) along with full spectral assignments. Known compounds **12**,⁴⁴ **17**,⁴⁸ **19**,⁵⁴ **28**,⁶⁵ **30**,⁶⁶ **34**⁵⁸ and **40**⁶³ were synthesised according to the published literature procedures. Data and procedures for these compounds can be found in the [supporting information](#).

4.1.1. General

Unless otherwise stated, all reagents were purchased from commercial suppliers and used without purifications. All reactions were carried under Argon. Thin layer chromatography (TLC) was performed

on aluminium backed Sigma-Aldrich TLC plates with F₂₅₄ fluorescent indicator, flash column chromatography was carried out using silica gel (43–60 μm) supplied by Merck. Optical rotations were recorded using a Perkin Elmer 343 polarimeter and specific rotations are reported in 10⁻¹ deg cm² g⁻¹ and concentrations (c) are reported as 10 mg ml⁻¹. Melting points were recorded on a Gallenkamp Hot Stage apparatus and are uncorrected. Infrared spectra were recorded using a Perkin Elmer 100 FT-IR spectrometer and adsorption maxima are reported in wave-numbers (cm⁻¹). Electron ionisation (EI) and chemical ionisation (CI) massed were determined using Thermo Finnigan MAT900 XP instrumentation. Electrospray ionisation (ESI) accurate mass was determined using Waters LCT Premier XE instrumentation. ¹H NMR and ¹³C NMR spectra were obtained on 300, 500 and 600 MHz AMX Bruker Spectrometers using deuterated chloroform (CDCl₃), dimethylsulfoxide (d₆-DMSO) and methanol (CD₃OD) as solvents (as stated).

Table 2
IC₅₀ values against Aurora A.

Compound	Aurora A activity (IC ₅₀ , μM) ^a
1	1300
2	420
3	1100
4	910
5	497
6	542
7	430
8	1300
9	1400
10	83
CoA	5
dpCoA ^b	17
desulfo-CoA ^c	160

^a For this assay, CoA was used as a control and values are represented as the mean of at least two independent determinations and are within ± 30%.

^b dpCoA is an analogue of CoA lacking the 3'-phosphate.

^c desulfo-CoA is an analogue lacking the -SH group at the end of the pantetheine tail (data from reference 19)

4.1.2. Experimental procedures and data

4.1.2.1. ((3*aS*,4*S*,6*S*,6*aS*)-6-(6-Amino-9*H*-purin-9-yl)-2,2-dimethyltetrahydrofuro[3,4-*d*][1,3]dioxol-4-yl)methyl benzyl malonate (**13**). To a solution of acid **12** (3.79 g, 19.5 mmol) and protected adenosine **11** (3.00 g, 9.77 mmol) in CH₂Cl₂ (30 mL) was added DCC (2.54 g, 12.7 mmol) and DMAP (297 mg, 2.44 mmol). The reaction was stirred at RT for 19 h. The insoluble dicyclohexylurea was removed by filtration, washed with CH₂Cl₂ and the filtrate concentrated *in vacuo*. Purification using flash column chromatography (Gradient: 2–3% MeOH/CH₂Cl₂) gave **13** (2.47 g, 52%) as a colourless gum; R_f 0.51 (5% MeOH/CH₂Cl₂); [α]_D²⁰ –21.2 (c 0.7, MeOH); IR ν_{max} (solution in CH₂Cl₂) 3320 (NH₂), 3060–2930 (CH), 1730 (C=O) and 1600 (C=C) cm⁻¹; ¹H NMR (600 MHz, CDCl₃) δ 8.32 (1H, s), 7.89 (1H, s), 7.37–7.28 (5H, m), 6.27 (2H, br), 6.10 (1H, s), 5.42 (1H, d, *J* = 6.4 Hz), 5.15 (1H, d, *J* = 12.0 Hz), 5.13 (1H, d, *J* = 12.0 Hz), 5.04 (1H, dd, *J* = 6.4 and 3.4 Hz), 4.50–4.29 (3H, m), 3.41 (1H, d, *J* = 10.0 Hz), 3.38 (1H, d, *J* = 10.0 Hz), 1.60 (3H, s) and 1.38 (3H, s); ¹³C NMR (150 MHz, CDCl₃) δ 166.3, 166.0, 155.9, 153.3, 149.3, 139.8,

135.2, 128.5 (overlapping signals), 120.3, 102.7, 90.9, 84.8, 84.3, 81.6, 67.5, 64.9, 41.3, 27.3 and 25.5; HRMS calc'd for C₂₃H₂₆N₅O₇ expected 484.1832, found 484.1861.

4.1.2.2. ((2*S*,3*R*,4*S*,5*S*)-5-(6-Amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methylbenzyl malonate (**14**). To a suspension of adenosine analogue **13** (3.90 g, 8.07 mmol) in H₂O (120 mL) at 0 °C was added TFA (30 mL). The solution was stirred at 0 °C for 30 min before stirring at RT for 4 h. The reaction mixture was then concentrated *in vacuo*. Purification using flash column chromatography (Gradient: 2%, 5% then 7.5% MeOH/CH₂Cl₂) gave **14** (2.86 g, 80%) as an off-white solid; R_f 0.37 (10% MeOH/CH₂Cl₂); mp 131–133 °C; [α]_D²⁰ –40.5 (c 0.4, MeOH); IR ν_{max} (solid) 3320 (NH), 3280 (NH), 3120 (OH), 3050–2980 (CH), 1680 (C=O) and 1600 (C=C) cm⁻¹; ¹H NMR (600 MHz, CD₃OD) δ 8.34 (1H, s), 8.27 (1H, s), 7.38–7.24 (5H, m), 6.03 (1H, d, *J* = 4.9 Hz), 5.14 (2H, apparent s), 4.66 (1H, t, *J* = 4.9 Hz), 4.44 (2H, dd, *J* = 4.0 Hz, 2.5 Hz), 4.33 (1H, t, *J* = 4.9 Hz) and 4.27 (1H, q, *J* = 4.5 Hz); ¹³C NMR (150 MHz, CD₃OD) δ 168.0, 167.9, 155.1, 150.4, 142.3, 136.9, 129.5 (overlapping signals), 120.5, 90.3, 83.6, 75.3, 71.6, 68.2, 65.5 and 41.7; HRMS calc'd for C₂₀H₂₂N₅O₇ expected 444.1519, found 444.1509. Note: (CO)CH₂(CO) signal is not seen in ¹H NMR as it is exchangeable with NMR solvent.

4.1.2.3. ((5*aS*,6*S*,8*S*,8*aS*)-8-(6-Amino-9*H*-purin-9-yl)-2,2,4,4-tetraisopropyltetrahydrofuro[3,4-*f*][1,3,5,2,4]trioxadisilepin-6-yl)methyl benzyl malonate (**15**). **Route 1**: To adenosine diol **14** (2.20 g, 4.96 mmol) in anhydrous pyridine (27 mL) was added 1,3-dichloro-1,1,3,3-triisopropylsiloxane (1.75 mL, 5.46 mmol). The reaction was stirred at RT for 18 h. The reaction mixture was concentrated *in vacuo*. Purification using flash column chromatography (Gradient: 0–3% MeOH/CH₂Cl₂) gave **15** (1.55 g, 45%) as an off-white solid. **Route 2**: To a solution of alcohol **26** (650 mg, 1.28 mmol) and acid **12** (497 mg, 2.56 mmol) in CH₂Cl₂ (8 mL) was added DCC (333 mg, 1.66 mmol) and DMAP (39 mg, 0.32 mmol). The reaction was stirred at RT for 18 h. The insoluble dicyclohexylurea was removed by filtration, washed with CH₂Cl₂ and the filtrate concentrated *in vacuo*. Purification using flash column chromatography (Gradient: 0–3% MeOH/CH₂Cl₂) gave **15** (790 mg, 90%) as an off-white solid. R_f 0.73 (10% MeOH/CH₂Cl₂); mp 132–135 °C; [α]_D²⁰ –19.5 (c 0.6, MeOH); IR ν_{max} (solid) 3280 (NH), 3150 (NH), 2940–2870 (CH), 1740 (C=O) 1690 (C=O) and

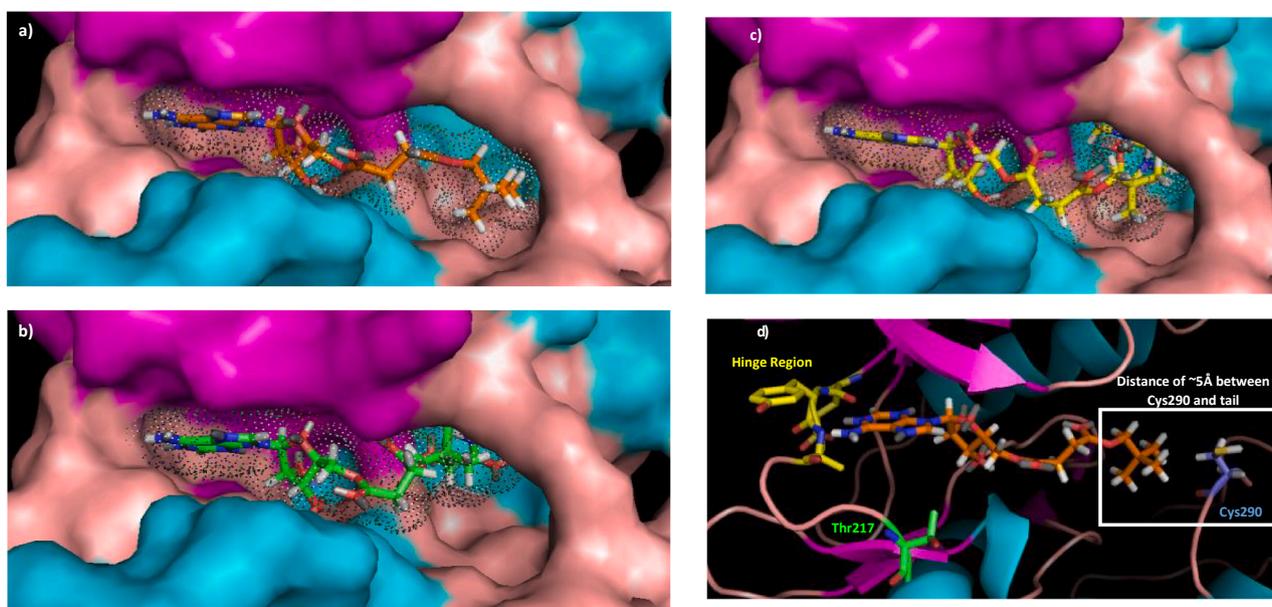


Fig. 3. Comparison of the docking of three tested analogues: (a) **7**, (b) **8** and (c) **9** (d) Docking of **7** in the Aurora A active site. This figure highlights the possibility of extending the tail when designing further analogues.

1570 (C=C) cm^{-1} ; ^1H NMR (600 MHz, CDCl_3) δ 8.32 (1H, s), 7.98 (1H, s), 7.36–7.29 (5H, m), 5.99 (1H, d, $J = 3.4$ Hz), 5.91 (2H, br), 5.17 (2H, apparent s), 5.09 (1H, dd, $J = 4.9$ and 3.4 Hz), 4.72 (1H, dd, $J = 6.4$ and 4.9 Hz), 4.53 (1H, dd, $J = 12.0$ and 3.2 Hz), 4.41 (1H, dd, $J = 12.0$ and 5.2 Hz), 4.33 (1H, ddd, $J = 6.4$, 5.2 and 3.2 Hz), 3.46 (2H, apparent s) and 1.10–0.99 (28H, m); ^{13}C NMR (150 MHz, CDCl_3) δ 166.2, 155.7, 153.2, 149.6, 140.2, 135.2, 128.6 (overlapping signals), 120.5, 90.6, 81.7, 75.7, 72.6, 67.5, 64.6, 41.4, 17.4 and 13.5; HRMS calc'd for $\text{C}_{32}\text{H}_{48}\text{O}_8\text{N}_5\text{Si}_2$ expected 686.3041, found 686.3073.

4.1.2.4. 3-(((5a*S*,6*S*,8*S*,8a*S*)-8-(6-Amino-9*H*-purin-9-yl)-2,2,4,4-tetraisopropyltetrahydrofuro[3,4-*f*][1,3,5,2,4]trioxadisilepin-6-yl)methoxy)-3-oxopropanoic acid (**16**). To protected adenosine analogue **15** (1.40 g, 2.04 mmol) and Pd/C (10 wt%, 55 mg, 0.51 mmol) was added MeOH (5 mL) before evacuating and filling the flask with hydrogen. The reaction was stirred at RT under a hydrogen atmosphere for 5 h. The reaction mixture was filtered through Celite®, washed with MeOH and the filtrate concentrated *in vacuo* to give **16** (860 mg, 71%) as an off-white solid; R_f 0.13 (8% MeOH/ CH_2Cl_2); mp 159–161 °C; $[\alpha]_D^{20} - 17.5$ (c 0.2, MeOH); IR ν_{max} (solid) 3320 (NH), 3280 (NH), 3170 (OH), 2940–2870 (CH), 1750 (C=O), 1670 (C=O) and 1620 (C=C) cm^{-1} ; ^1H NMR (600 MHz, d_6 -DMSO) δ 12.91 (1H, br), 8.38 (1H, s), 8.14 (1H, s), 7.36 (1H, br), 6.00 (1H, d, $J = 5.2$ Hz), 5.17 (1H, t, $J = 5.2$ Hz), 4.74 (1H, t, $J = 4.7$ Hz), 4.39 (1H, dd, $J = 11.9$ and 4.8 Hz), 4.28 (1H, dd, $J = 11.9$ and 5.6 Hz), 4.19 (1H, q, $J = 4.8$ Hz), 3.40 (2H, apparent s) and 1.09–0.92 (28H, m); ^{13}C NMR (150 MHz, d_6 -DMSO) δ 167.9, 166.8, 156.2, 152.8, 149.3, 139.7, 119.1, 81.7, 81.7, 74.8, 72.3, 63.9, 41.4, 17.2 and 12.5; HRMS calc'd for $\text{C}_{25}\text{H}_{42}\text{O}_8\text{N}_5\text{Si}_2$ expected 596.2572, found 596.2566

4.1.2.5. ((5a*S*,6*S*,8*S*,8a*S*)-8-(6-Amino-9*H*-purin-9-yl)-2,2,4,4-tetraisopropyltetrahydrofuro[3,4-*f*][1,3,5,2,4]trioxadisilepin-6-yl)methyl ((*R*)-3-hydroxy-2,2-dimethyl-4-oxo-4-((3-oxo-3-(propylamino)propyl)amino)butyl) malonate (**18**). To a solution of acid **16** (700 mg, 1.17 mmol) and alcohol **17** (337 mg, 1.30 mmol) in CH_2Cl_2 (30 mL) at 0 °C was added EDC (271 mg, 1.42 mmol) and DMAP (173 mg, 1.42 mmol). The reaction was stirred at RT for 16 h. The reaction mixture was diluted with CH_2Cl_2 (20 mL) and washed with 5% citric acid solution (40 mL), then sat. NaHCO_3 solution (40 mL). The organic layer was dried (MgSO_4), filtered and concentrated *in vacuo*. Purification using flash column chromatography (Gradient: 2%, 5% then 7.5% MeOH/ CH_2Cl_2) gave **18** (240 mg, 25%) as an off-white solid; R_f 0.28 (10% MeOH/ CH_2Cl_2); mp 84–86 °C; $[\alpha]_D^{20} + 2.4$ (c 0.2, MeOH); IR ν_{max} (solid) 3310 (NH), 3280 (NH), 2940–2860 (CH), 1730 (C=O), 1640 (C=O) and 1600 (C=C) cm^{-1} ; ^1H NMR (600 MHz, CDCl_3) δ 8.30 (1H, s), 8.09 (1H, s), 7.52 (1H, t, $J = 6.0$ Hz), 6.75 (1H, d, $J = 4.5$ Hz), 6.07 (2H, br), 6.04 (1H, s), 5.73 (1H, t, $J = 5.6$ Hz), 4.97 (1H, d, $J = 3.7$ Hz), 4.64 (1H, dd, $J = 12.4$ and 2.3 Hz), 4.49–4.42 (2H, m), 4.40–4.35 (2H, m), 4.15 (1H, d, $J = 4.9$ Hz), 3.84 (1H, d, $J = 10.9$ Hz), 3.67–3.59 (1H, m), 3.56–3.49 (1H, m), 3.48 (2H, apparent s), 3.29–3.16 (2H, m), 2.51–2.37 (2H, m), 1.74–1.56 (2H, m) and 1.17–0.89 (37H, m); ^{13}C NMR (150 MHz, CDCl_3) δ 172.5, 171.4, 166.7, 166.0, 155.3, 153.5, 149.0, 138.4, 119.6, 91.0, 81.1, 76.3, 73.8, 71.8, 71.5, 63.0, 41.4, 41.3, 38.5, 35.6, 35.2, 22.9, 21.9, 18.9, 14.5, 13.7 and 13.0; HRMS calc'd for $\text{C}_{37}\text{H}_{64}\text{O}_{11}\text{N}_7\text{Si}_2$ expected 838.4202, found 838.4238.

4.1.2.6. ((2*S*,3*R*,4*S*,5*S*)-5-(6-Amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl ((*R*)-3-hydroxy-2,2-dimethyl-4-oxo-4-((3-oxo-3-(propylamino)propyl)amino)butyl) malonate (**1**). To a solution of protected CoA analogue **18** (70.0 mg, 0.08 mmol) in MeOH (2 mL) was added ammonium fluoride (25 mg, 0.68 mmol) and the reaction was stirred at RT for 1.5 h. The reaction mixture was concentrated *in vacuo*. Purification using flash column chromatography (Gradient: 5%, 10% then 12.5% MeOH/ CH_2Cl_2) gave **1** (20 mg, 42%) as a colourless oil; R_f 0.40 (15% MeOH/ CH_2Cl_2); mp 96–98 °C;

$[\alpha]_D^{20} + 54.5$ (c 0.1, MeOH); IR ν_{max} (oil) 3320 (NH), 3280 (NH), 3080 (OH), 2960–2880 (CH), 1730 (C=O), 1640 (C=O) and 1570 (C=C) cm^{-1} ; ^1H NMR (600 MHz, CD_3OD) δ 8.28 (1H, s), 8.21 (1H, s), 6.02 (1H, d, $J = 4.5$ Hz), 4.72 (1H, t, $J = 4.5$ Hz), 4.45 (2H, dd, $J = 14.5$ and 4.0 Hz), 4.39 (1H, t, $J = 5.0$ Hz), 4.29–4.26 (1H, m), 4.11 (1H, d, $J = 10.5$ Hz), 3.95 (1H, d, $J = 10.5$ Hz), 3.86 (1H, s), 3.50–3.39 (2H, m), 3.10 (2H, t, $J = 6.5$ Hz), 2.40 (2H, t, $J = 6.8$ Hz), 1.54–1.45 (2H, m), 0.95 (3H, s) and 0.92–0.88 (6H, m); ^{13}C NMR (150 MHz, CD_3OD) δ 175.3, 173.6, 168.2, 168.0, 157.6, 153.4, 150.6, 142.0, 121.0, 90.1, 83.4, 79.3, 75.7, 72.7, 71.7, 65.9, 42.4, 41.6, 39.6, 36.4, 23.6, 20.7, 20.6 and 11.7; HRMS calc'd for $\text{C}_{25}\text{H}_{38}\text{N}_7\text{O}_{10}$ expected 596.2680, found 596.2684. Note: (CO) CH_2 (CO) signal is not seen in ^1H NMR as it is exchangeable with NMR solvent.

4.1.2.7. Benzyl 3-(((3a*S*,4*S*,6*S*,6a*S*)-6-(6-amino-9*H*-purin-9-yl)-2,2-dimethyltetrahydrofuro[3,4-*d*][1,3]dioxol-4-yl)methyl)amino)-3-oxopropanoate (**20**). To a solution of amine **19** (3.50 g, 11.4 mmol) and acid **12** (4.42 g, 22.8 mmol) in CH_2Cl_2 (35 mL) was added DCC (2.96 g, 14.8 mmol) and DMAP (347 mg, 2.85 mmol). The reaction was stirred at RT for 21 h. The insoluble dicyclohexylurea was removed by filtration, washed with CH_2Cl_2 and the filtrate concentrated *in vacuo*. Purification using flash column chromatography (Gradient: 0–3% MeOH/ CH_2Cl_2) gave **20** (4.26 g, 78%) as a white gum; R_f 0.63 (10% MeOH/ CH_2Cl_2); mp 77–79 °C; $[\alpha]_D^{20} - 68.2$ (c 0.5, MeOH); IR ν_{max} (solution in CH_2Cl_2) 3320 (NH), 3180 (NH), 3060–2920 (CH), 1730 (C=O), 1640 (C=O) and 1590 (C=C) cm^{-1} ; ^1H NMR (600 MHz, CDCl_3) δ 8.70 (1H, d, $J = 7.9$ Hz), 8.26 (1H, s), 7.85 (1H, s), 7.28–7.20 (5H, m), 6.12 (2H, br), 5.8 (1H, d, $J = 4.9$ Hz), 5.19 (1H, dd, $J = 6.0$ and 4.9 Hz), 5.13 (1H, d, $J = 12.5$ Hz), 5.10 (1H, d, $J = 12.5$ Hz), 4.89 (1H, dd, $J = 6.0$ and 2.0 Hz), 4.49 (1H, q, $J = 2.0$ Hz), 4.17 (1H, ddd, $J = 14.4$, 8.8 and 2.5 Hz), 3.48 (2H, apparent s), 3.29 (1H, dt, $J = 14.4$ and 2.5 Hz), 1.62 (3H, s) and 1.36 (3H, d); ^{13}C NMR (150 MHz, CDCl_3) δ 168.4, 166.0, 156.1, 152.6, 148.8, 140.6, 135.2, 128.6 (overlapping signals), 121.2, 114.8, 92.8, 83.7, 82.4, 81.7, 67.4, 42.9, 41.4, 27.6 and 25.3; HRMS calc'd for $\text{C}_{23}\text{H}_{19}\text{N}_6\text{O}_6$ expected 483.1992, found 483.1992.

4.1.2.8. Benzyl 3-(((2*S*,3*R*,4*S*,5*S*)-5-(6-amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl)amino)-3-oxopropanoate (**21**). To a cooled suspension of protected adenosine analogue **20** (4.20 g, 8.71 mmol) in H_2O (130 mL) was added TFA (33 mL) and the reaction stirred at 0 °C for 30 min before warming to RT for 3 h. The reaction mixture was then concentrated *in vacuo*. Purification using flash column chromatography (Gradient: 2%, 5% then 7.5% MeOH/ CH_2Cl_2) gave **21** (3.47 g, 90%) as an off-white solid; R_f 0.39 (10% MeOH/ CH_2Cl_2); mp 113–116 °C; $[\alpha]_D^{20} - 55.2$ (c 0.5, MeOH); IR ν_{max} (solid) 3350 (NH), 3290 (NH), 3100 (OH), 3070–2900 (CH), 1710 (C=O), 1650 (C=O) and 1580 (C=C) cm^{-1} ; ^1H NMR (600 MHz, d_6 -DMSO) δ 8.51 (1H, s), 8.45 (1H, t, $J = 5.8$ Hz), 8.33 (1H, s), 8.20 (2H, br), 7.37–7.29 (5H, m), 5.90 (1H, d, $J = 6.0$ Hz), 5.51 (2H, br), 5.11 (2H, apparent s), 4.64 (1H, t, $J = 6.0$ Hz), 4.09 (1H, t, $J = 4.7$ Hz), 3.97 (1H, dt, $J = 5.8$ and 4.7 Hz), 3.50–3.38 (2H, m) and 3.35 (2H, apparent s); ^{13}C NMR (150 MHz, d_6 -DMSO) δ 167.8, 165.4, 153.9, 149.7, 148.9, 141.3, 135.9, 128.4, 128.0, 127.8, 119.2, 87.7, 83.5, 73.0, 71.2, 65.9, 42.4 and 41.2; HRMS calc'd for $\text{C}_{20}\text{H}_{23}\text{O}_6\text{N}_6$ expected 443.1679, found 443.1672.

4.1.2.9. Benzyl 3-(((5a*S*,6*S*,8*S*,8a*S*)-8-(6-amino-9*H*-purin-9-yl)-2,2,4,4-tetraisopropyltetrahydrofuro[3,4-*f*][1,3,5,2,4]trioxadisilepin-6-yl)methyl)amino)-3-oxopropanoate (**22**). To adenosine diol **21** (350 mg, 0.79 mmol) in anhydrous DMF (3 mL) was added imidazole (135 mg, 1.98 mmol) and DMAP (24 mg, 0.20 mmol). Then, 1,3-dichloro-1,1,3,3-triisopropylidisiloxane (303 μL , 0.95 mmol) was added dropwise. The reaction was stirred at RT for 17 h. The reaction was concentrated *in vacuo*. The resulting residue was dissolved in CH_2Cl_2 (10 mL) and washed with sat. NaHCO_3 solution (10 mL). The aqueous layer was

extracted with CH_2Cl_2 (2 × 10 mL). The combined organics washed with brine (10 mL), dried (MgSO_4), filtered and concentrated *in vacuo*. Purification using flash column chromatography (Gradient: 0–3% MeOH in CH_2Cl_2) gave **22** (330 mg, 61%) as an off-white solid; R_f 0.62 (10% MeOH/ CH_2Cl_2); mp 66–68 °C; $[\alpha]_D^{20}$ –81.3 (c 0.2, MeOH); IR ν_{max} (solid) 3290 (NH), 3210 (NH), 2950–2870 (CH), 1740 (C=O), 1640 (C=O) and 1580 (C=C) cm^{-1} ; ^1H NMR (600 MHz, CDCl_3) δ 8.72 (1H, d, J = 6.8 Hz), 8.33 (1H, s), 7.89 (1H, s), 7.38–7.29 (5H, m), 5.96 (2H, br), 5.79 (1H, d, J = 7.0 Hz), 5.16 (2H, apparent s), 5.03 (1H, dd, J = 7.0 and 5.0 Hz), 4.53 (1H, dd, J = 5.0 and 2.3 Hz), 4.37 (1H, q, J = 3.0 Hz), 4.14 (1H, ddd, J = 14.5, 8.8 and 3.0 Hz), 3.47 (1H, d, J = 15.0 Hz), 3.40 (1H, d, J = 15.0 Hz), 3.31 (1H, dt, J = 14.3 and 3.0 Hz) and 1.13–0.87 (28H, m); ^{13}C NMR (150 MHz, CDCl_3) δ 168.0, 165.6, 155.8, 152.5, 149.2, 141.1, 135.3, 128.6 (overlapping signals), 121.3, 90.9, 85.8, 75.4, 73.6, 67.4, 47.8, 41.3, 17.5 and 13.2; HRMS calc'd for $\text{C}_{32}\text{H}_{49}\text{O}_7\text{N}_6\text{Si}_2$ expected 685.3201, found 685.3226.

4.1.2.10. 3-(((5*aS*,6*S*,8*S*,8*aS*)-8-(6-Amino-9*H*-purin-9-yl)-2,2,4,4-tetraisopropyltetrahydrofuro[3,4-*ff*][1,3,5,2,4]trioxadisilepin-6-yl)methyl)amino)-3-oxopropanoic acid (**23**). To protected adenosine analogue **22** (800 mg, 1.17 mmol) and Pd/C (10 wt%, 31 mg, 0.29 mmol) was added MeOH (3 mL) before evacuating and filling the flask with hydrogen. The reaction was stirred at RT under a hydrogen atmosphere for 3 h. The reaction mixture was filtered through Celite®, washed with MeOH and the filtrate concentrated *in vacuo* to give **23** (666 mg, 95%) as an off-white solid; R_f 0.10 (5% MeOH/ CH_2Cl_2); mp 129–131 °C; $[\alpha]_D^{20}$ –108.6 (c 0.2, MeOH); IR ν_{max} (solid) 3280 (NH), 3210 (NH), 3070 (OH), 2950–2870 (CH), 1700 (C=O), 1660 (C=O) and 1580 (C=C) cm^{-1} ; ^1H NMR (600 MHz, d_6 -DMSO) δ 8.66 (1H, br), 8.46 (1H, s), 8.16 (1H, s), 7.37 (2H, br), 5.94 (1H, d, J = 6.4 Hz), 5.15 (1H, dd, J = 6.4 and 4.9 Hz), 4.59 (1H, dd, J = 5.0 and 3.2 Hz), 4.05 (1H, dt, J = 5.0 and 3.0 Hz) 3.50–3.40 (2H, m), 3.34 (1H, br), 3.11 (2H, apparent s) and 1.13–0.83 (28H, m); ^{13}C NMR (150 MHz, d_6 -DMSO) δ 169.5, 166.8, 156.2, 152.7, 149.4, 140.1, 119.3, 87.2, 83.9, 74.7, 73.1, 42.9, 41.0, 17.3 and 13.2; HRMS calc'd for $\text{C}_{25}\text{H}_{43}\text{O}_7\text{N}_6\text{Si}_2$ expected 595.2732, found 595.2723.

4.1.2.11. (*R*)-3-Hydroxy-2,2-dimethyl-4-oxo-4-((3-oxo-3-(propylamino)propyl)amino)butyl 3-(((5*aS*,6*S*,8*S*,8*aS*)-8-(6-amino-9*H*-purin-9-yl)-2,2,4,4-tetraisopropyltetrahydrofuro[3,4-*ff*][1,3,5,2,4]trioxadisilepin-6-yl)methyl)amino)-3-oxopropanoate (**24**). To a solution of acid **23** (270 mg, 0.45 mmol) and alcohol **17** (140 mg, 0.54 mmol) in CH_2Cl_2 (13 mL) at 0 °C was added EDC (104 mg, 0.54 mmol) and DMAP (66 mg, 0.54 mmol). The reaction was stirred at RT for 18 h. The reaction mixture was diluted with CH_2Cl_2 (10 mL) and washed with 5% citric acid solution (10 mL), then sat. NaHCO_3 solution (10 mL). The organic layer was dried (MgSO_4), filtered and concentrated *in vacuo*. Purification using flash column chromatography (Gradient: 2%, 5% then 7.5% MeOH/ CH_2Cl_2) gave **24** (51 mg, 14%) as an off-white solid; R_f 0.59 (10% MeOH/ CH_2Cl_2); mp 106–107 °C; $[\alpha]_D^{20}$ –41.5 (c 0.1, MeOH); IR ν_{max} (solid) 3320 (NH), 3210 (NH), 3080 (OH), 2950–2870 (CH), 1740 (C=O), 1640 (C=O) and 1590 (C=C) cm^{-1} ; ^1H NMR (600 MHz, CDCl_3) δ 8.97 (1H, d, J = 8.2 Hz), 8.31 (1H, s), 7.90 (1H, d), 7.59 (1H, t, J = 5.8 Hz), 6.40 (2H, br), 6.09 (1H, br), 5.80 (1H, d, J = 6.6 Hz), 4.98 (1H, dd, J = 6.6 and 5.1 Hz), 4.48 (1H, dd, J = 5.1 and 2.5 Hz), 4.37 (1H, q, J = 2.5 Hz), 4.26 (1H, d, J = 10.6 Hz), 4.14 (1H, ddd, J = 14.4, 9.1 and 2.5 Hz), 4.02 (1H, s), 3.77 (1H, d, J = 10.6 Hz), 3.68–3.60 (1H, m), 3.54–3.37 (3H, m), 3.29 (1H, dt, J = 14.4 and 2.5 Hz), 3.21–3.13 (2H, m), 2.51–2.39 (2H, m), 1.53–1.45 (2H, m) and 1.12–0.86 (37H, m); ^{13}C NMR (150 MHz, CDCl_3) δ 173.1, 171.4, 168.0, 166.1, 156.7, 152.4, 148.9, 140.8, 121.1, 91.0, 85.6, 75.6, 74.3, 73.5, 71.8, 42.6, 41.5, 41.1, 38.3, 36.1, 35.5, 22.8, 21.8, 19.7, 17.5, 12.9 and 11.5; HRMS calc'd for $\text{C}_{37}\text{H}_{65}\text{O}_{10}\text{N}_8\text{Si}_2$ expected 837.4362, found 837.4365.

4.1.2.12. (*R*)-3-Hydroxy-2,2-dimethyl-4-oxo-4-((3-oxo-3-(propylamino)

propyl)amino)butyl 3-(((2*S*,3*R*,4*S*,5*S*)-5-(6-amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl)amino)-3-oxopropanoate (**3**). To a solution of protected CoA analogue **24** (35.0 mg, 0.04 mmol) in MeOH (1 mL) was added ammonium fluoride (13 mg, 0.36 mmol) and the reaction was stirred at RT for 1.5 h. The reaction mixture was concentrated *in vacuo*. Purification using flash column chromatography (Gradient: 5%, 10% then 15% MeOH/ CH_2Cl_2) gave **3** (8 mg, 75%) as a colourless oil; R_f 0.16 (15% MeOH/ CH_2Cl_2); mp 101–103 °C; $[\alpha]_D^{20}$ –38.5 (c 0.1, MeOH); IR ν_{max} (oil) 3310 (NH), 3280 (NH), 3180 (OH), 2960–2870 (CH), 1730 (C=O), 1640 (C=O) and 1600 (C=C) cm^{-1} ; ^1H NMR (600 MHz, CD_3OD) δ 8.26 (1H, s), 8.25 (1H, s), 5.92 (1H, d, J = 6.2 Hz), 4.80 (1H, t, J = 6.2 Hz), 4.25–4.18 (2H, m), 4.08 (1H, d, J = 10.6 Hz), 3.94 (1H, d, J = 10.6 Hz), 3.86–3.81 (2H, m), 3.49–3.39 (3H, m), 3.35 (2H, apparent s), 3.12–3.08 (2H, m), 2.42 (2H, t, J = 6.7 Hz), 1.53–1.45 (2H, m), 0.96 (3H, s), 0.93 (3H, s) and 0.90 (3H, t, J = 7.4 Hz); ^{13}C NMR (150 MHz, CD_3OD) δ 176.1, 175.3, 169.4, 168.8, 157.5, 153.8, 150.4, 142.2, 121.1, 90.8, 85.5, 77.2, 75.7, 74.5, 72.8, 49.5, 42.5, 42.4, 39.5, 36.9, 23.6, 21.6, 21.4 and 11.7; HRMS calc'd for $\text{C}_{25}\text{H}_{39}\text{N}_8\text{O}_9$ expected 595.2840, found 595.2857.

4.1.2.13. ((2*S*,3*S*,4*S*,5*S*)-5-(6-Amino-9*H*-purin-9-yl)-3,4-bis(trimethylsilyloxy)tetrahydrofuran-2-yl)methyl benzyl malonate (**25**). To a solution of adenosine diol **14** (800 mg, 1.80 mmol) and NEt_3 (750 μL , 5.40 mmol) in CH_2Cl_2 (9 mL) at 0 °C was added TMSCl (570 μL , 4.50 mmol) dropwise. The reaction was stirred at RT for 16 h. The reaction mixture was filtered, washed with Et_2O and the filtrate was concentrated *in vacuo*. Purification using flash column chromatography (Gradient: 50% then then 80% EtOAc/pet. ether) gave **25** (590 mg, 56%) as an off-white solid; R_f 0.57 (100% EtOAc); mp 103–105 °C; $[\alpha]_D^{20}$ –29.6 (c 0.2, MeOH); IR ν_{max} (solid) 3280 (NH), 3140 (NH), 2950–2900 (CH), 1730 (C=O), 1680 (C=O) and 1600 (C=C) cm^{-1} ; ^1H NMR (600 MHz, CDCl_3) δ 8.34 (1H, s), 7.96 (1H, s), 7.36–7.30 (5H, m), 6.02 (2H, br), 5.90 (1H, d, J = 4.1 Hz), 5.18 (2H, apparent s), 4.96 (1H, t, J = 4.5 Hz), 4.54 (1H, d, J = 3.8 Hz), 4.42–4.35 (2H, m), 4.29 (1H, q, J = 4.0 Hz), 3.48 (2H, apparent s), 0.16 (9H, s) and 0.01 (9H, s); ^{13}C NMR (150 MHz, CDCl_3) δ 166.2, 155.7, 153.0, 149.7, 140.2, 135.2, 128.6 (overlapping signals), 120.6, 89.8, 81.9, 74.2, 71.7, 67.5, 64.3, 41.4 and 0.26; HRMS calc'd for $\text{C}_{26}\text{H}_{38}\text{N}_5\text{O}_7\text{Si}$ expected 588.2310, found 588.2308.

4.1.2.14. 3-(((2*S*,3*R*,4*S*,5*S*)-5-(6-Amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methoxy)-3-oxopropanoic acid (**2**). To adenosine diol **25** (550 mg, 0.94 mmol) and Pd/C (10 wt%, 25 mg, 0.23 mmol) was added MeOH (3 mL) before evacuating and filling the flask with hydrogen. The reaction was stirred at RT under a hydrogen atmosphere for 5 h. The reaction mixture was filtered through Celite®, washed with MeOH and the filtrate concentrated *in vacuo*. The resulting white solid was triturated in Et_2O to give **2** (290 mg, 87%) as an off-white solid; R_f 0.60 (10% MeOH/ CH_2Cl_2); mp 148–150 °C; $[\alpha]_D^{20}$ –33.3 (c 0.7, MeOH); IR ν_{max} (solid) 3310 (NH), 3220 (NH), 3120 (OH), 2980–2950 (CH), 1740 (C=O), 1690 (C=O) and 1600 (C=C) cm^{-1} ; ^1H NMR (600 MHz, d_6 -DMSO) δ 8.34 (1H, s), 8.15 (1H, s), 7.32 (2H, br), 5.91 (1H, d, J = 5.3 Hz), 4.64 (1H, t, J = 5.3 Hz), 4.36 (1H, dd, J = 12.0 and 3.8 Hz), 4.25–4.20 (2H, m), 4.11–4.07 (1H, m) and 3.35 (2H, apparent s); ^{13}C NMR (150 MHz, d_6 -DMSO) δ 168.2, 167.3, 156.1, 152.7, 149.4, 139.8, 119.2, 87.7, 81.6, 72.9, 70.7, 64.0 and 42.1; HRMS calc'd for $\text{C}_{13}\text{H}_{16}\text{O}_7\text{N}_5$ expected 354.1050, found 354.1037.

4.1.2.15. 3-(((2*S*,3*R*,4*S*,5*S*)-5-(6-Amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl)amino)-3-oxopropanoic acid (**4**). To protected adenosine diol **21** (100 mg, 0.23 mmol) and Pd/C (10 wt%, 6 mg, 0.25 mmol) was added MeOH (2.5 mL) before evacuating and filling the flask with hydrogen. The reaction was stirred at RT under a hydrogen atmosphere for 5 h. The reaction mixture was filtered through Celite®, washed with MeOH and the

filtrate concentrated *in vacuo* to give **4** (80 mg, 98%) as an off-white solid; R_f 0.20 (20% MeOH/CH₂Cl₂); mp 105–108 °C; $[\alpha]_D^{20}$ –71.4 (c 0.2, MeOH); IR ν_{\max} (solid) 3290 (NH), 3260 (NH), 3210 (OH), 3180 (OH), 2960–2900 (CH), 1630 (C=O) and 1570 (C=C) cm⁻¹; ¹H NMR (600 MHz, CD₃OD) δ 8.49 (1H, s), 8.40 (1H, s), 6.02 (1H, d, J = 5.7 Hz), 4.74 (1H, t, J = 5.7 Hz), 4.27 (1H, dd, J = 5.1 and 4.1 Hz), 4.19–4.14 (1H, m), 3.72 (1H, dd, J = 14.2 and 5.6 Hz) and 3.56 (1H, dd, J = 14.2 and 4.3 Hz); ¹³C NMR (150 MHz, CD₃OD) δ 171.5, 169.4, 152.3, 150.1, 146.8, 144.2, 120.8, 90.5, 85.4, 75.2, 72.6, 49.5 and 42.5; HRMS calc'd for C₁₃H₁₇N₆O₆ expected 353.1210, found 353.1199. Note: (CO)CH₂(CO) signal is not seen in ¹H NMR as it is exchangeable with NMR solvent.

4.1.2.16. ((5*aS*,6*S*,8*S*,8*aS*)-8-(6-Amino-9*H*-purin-9-yl)-2,2,4,4-tetraisopropyltetrahydrofuro[3,4-*f*][1,3,5,2,4]trioxadisilepin-6-yl)methanol (**26**). Following a novel procedure. A solution of protected adenosine **29** (7.58 g, 9.34 mmol) in 80% acetic acid (140 mL) was stirred at RT for 4 h. The reaction mixture was diluted with H₂O, cooled to 0 °C and taken to pH 8–9 with 2 M NaOH solution. The aqueous solution was extracted with CH₂Cl₂ (2 × 500 mL). The combined organic layers were dried (MgSO₄), filtered and concentrated *in vacuo*. Purification using flash column chromatography (Gradient: 0–3% MeOH/CH₂Cl₂) gave **26** (3.50 mg, 74%) as an off-white solid; R_f 0.71 (10% MeOH/CH₂Cl₂); mp 217–219 °C; $[\alpha]_D^{20}$ –94.1 (c 0.1, MeOH); ¹H NMR (600 MHz, CDCl₃) δ 8.32 (1H, s), 7.83 (1H, s), 6.60 (1H, br), 5.94 (2H, br), 5.79 (1H, d, J = 7.7 Hz), 5.14 (1H, dd, J = 7.7 and 4.7 Hz), 4.68 (1H, d, J = 4.7 Hz), 4.34 (1H, d, J = 1.3 Hz), 3.97 (1H, dd, J = 13.0 and 1.3 Hz), 3.74–3.67 (1H, m) and 1.15–0.90 (28H, m); ¹³C NMR (150 MHz, CDCl₃) δ 156.0, 152.5, 148.7, 141.0, 121.4, 92.0, 89.3, 76.1, 73.3, 63.3, 17.3 and 13.4; HRMS calc'd for C₂₂H₄₀O₅N₅Si₂ expected 510.2568, found 510.2564. The data is in good agreement with the literature values.⁵⁴

4.1.2.17. 9-((5*aS*,6*S*,8*S*,8*aS*)-8-((Bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-2,2,4,4-tetraisopropyltetrahydrofuro[3,4-*f*][1,3,5,2,4]trioxadisilepin-6-yl)-9*H*-purin-6-amine (**29**). To protected adenosine **28** (8.70 g, 15.3 mmol) in anhydrous DMF (60 mL) was added imidazole (2.60 g, 38.3 mmol) and DMAP (466 mg, 3.83 mmol). Then, 1,3-dichloro-1,1,3,3-triisopropylsiloxane (5.87 mL, 18.3 mmol) was added dropwise. The reaction was stirred at RT for 17 h. The reaction was concentrated *in vacuo*. The resulting residue was dissolved in CH₂Cl₂ (400 mL) and washed with sat. NaHCO₃ solution (400 mL). The aqueous layer was extracted with CH₂Cl₂ (2 × 300 mL). The combined organics washed with brine (400 mL), dried (MgSO₄), filtered and concentrated *in vacuo*. Purification using flash column chromatography (Gradient: 0–2% MeOH in CH₂Cl₂) gave **29** (7.58 g, 61%) as an off-white solid; R_f 0.36 (5% MeOH/CH₂Cl₂); mp 67–69 °C; $[\alpha]_D^{20}$ –11.2 (c 0.6, MeOH); IR ν_{\max} (solid) 3330 (NH), 3280 (NH), 2950–2870 (CH) and 1580 (C=C) cm⁻¹; ¹H NMR (600 MHz, CDCl₃) δ 8.31 (1H, s), 8.11 (1H, s), 7.47–7.41 (2H, m), 7.36–7.31 (4H, m), 7.29–7.16 (3H, m), 6.83–6.78 (4H, m), 6.10 (2H, br), 6.06 (1H, d, J = 4.7 Hz), 5.11 (1H, d, J = 4.7 Hz), 4.78 (1H, t, J = 4.7 Hz), 4.29–4.25 (1H, m), 3.78 (6H, s), 3.50 (1H, dd, J = 10.7 and 3.4 Hz), 3.33 (1H, dd, J = 10.7 and 3.4 Hz) and 1.13–0.92 (28H, m); ¹³C NMR (150 MHz, CDCl₃) δ 158.7, 155.7, 153.2, 150.0, 144.8, 139.3, 135.9, 130.1, 128.3, 128.2, 127.0, 120.3, 113.5, 89.0, 86.6, 84.3, 76.2, 73.2, 63.3, 55.3, 17.2 and 13.7; HRMS calc'd for C₄₃H₅₈O₇N₅Si₂ expected 812.3875, found 812.3900.

4.1.2.18. ((5*aS*,6*S*,8*S*,8*aS*)-8-(6-Amino-9*H*-purin-9-yl)-2,2,4,4-tetraisopropyltetrahydrofuro[3,4-*f*][1,3,5,2,4]trioxadisilepin-6-yl)methyl benzyl succinate (**31**). To a solution of alcohol **26** (2.49 g, 4.89 mmol) and protected succinic acid **30** (1.22 g, 5.87 mmol) in CH₂Cl₂ (40 mL) was added DCC (1.27 g, 6.36 mmol) and DMAP (149 mg, 1.22 mmol). The reaction was stirred at RT for 16 h. The insoluble dicyclohexylurea was removed by filtration, washed with CH₂Cl₂. The organic filtrate was washed with sat. NaHCO₃ solution (3 × 100 mL), brine (150 mL),

dried (MgSO₄), filtered and concentrated *in vacuo*. Purification using flash column chromatography (Gradient: 0–2% MeOH/CH₂Cl₂) gave **31** (2.91 g, 85%) as an off-white solid; R_f 0.54 (5% MeOH/CH₂Cl₂); mp 135–137 °C; $[\alpha]_D^{20}$ –18.6 (c 0.4, MeOH); IR ν_{\max} (solid) 3300 (NH), 3150 (NH), 2950–2870 (CH), 1740 (C=O) and 1600 (C=C) cm⁻¹; ¹H NMR (600 MHz, CDCl₃) δ 8.32 (1H, s), 8.00 (1H, s), 7.38–7.29 (5H, m), 5.99 (1H, d, J = 3.2), 5.88 (2H, br), 5.13 (2H, s), 5.09 (1H, dd, J = 4.8 and 3.2 Hz), 4.70 (1H, dd, J = 6.1 and 4.8 Hz), 4.51–4.46 (1H, m), 4.36–4.31 (2H, m), 2.72–2.64 (4H, m) and 1.11–0.97 (28H, m); ¹³C NMR (150 MHz, CDCl₃) δ 172.1, 155.5, 152.9, 149.6, 139.7, 135.8, 128.5 (overlapping signals), 120.5, 90.5, 81.8, 75.8, 72.7, 66.8, 63.9, 29.1, 29.0, 17.3 and 13.6; HRMS calc'd for C₃₃H₅₀O₈N₅Si₂ expected 700.3198, found 700.3188.

4.1.2.19. 4-(((5*aS*,6*S*,8*S*,8*aS*)-8-(6-Amino-9*H*-purin-9-yl)-2,2,4,4-tetraisopropyltetrahydrofuro[3,4-*f*][1,3,5,2,4]trioxadisilepin-6-yl)methoxy)-4-oxobutanoic acid (**32**). To protected adenosine analogue **31** (1.50 g, 2.15 mmol) and Pd/C (10 wt%, 57 mg, 0.54 mmol) was added MeOH (32 mL) before evacuating and filling the flask with hydrogen. The reaction was stirred at RT under a hydrogen atmosphere for 5 h. The reaction mixture was filtered through Celite®, washed with MeOH and the filtrate concentrated *in vacuo* to give **32** (1.28 g, 97%) as an off-white solid; R_f 0.21 (8% MeOH/CH₂Cl₂); mp 126–129 °C; $[\alpha]_D^{20}$ –22.7 (c 0.2, MeOH); IR ν_{\max} (solid) 3330 (NH), 3280 (NH), 3190 (OH), 2950–2870 (CH), 1740 (C=O), 1710 (C=O) and 1610 (C=C) cm⁻¹; ¹H NMR (600 MHz, CD₃OD) δ 8.32 (1H, s), 8.19 (1H, s), 6.05 (1H, d, J = 4.5 Hz), 5.20 (1H, t, J = 4.5 Hz), 4.76 (1H, t, J = 5.2 Hz), 4.47–4.35 (2H, m), 4.32–4.27 (1H, m), 3.32–3.29 (4H, m) and 2.63–2.56 (28H, m); ¹³C NMR (150 MHz, CD₃OD) δ 176.0, 173.9, 157.3, 153.9, 150.6, 141.9, 120.6, 90.6, 83.9, 77.7, 73.8, 64.3, 30.1, 30.0, 17.9 and 14.6; HRMS calc'd for C₂₆H₄₄N₅O₈Si₂ expected 610.2728, found 610.2730.

4.1.2.20. ((2*S*,3*R*,4*S*,5*S*)-5-(6-Amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl ((*R*)-3-hydroxy-2,2-dimethyl-4-oxo-4-((3-oxo-3-(propylamino)propyl)amino)butyl) succinate (**5**). To a solution of acid **32** (480 mg, 0.79 mmol) and alcohol **17** (410 mg, 1.58 mmol) in CH₂Cl₂ (20 mL) at 0 °C was added EDC (181 mg, 0.95 mmol) and DMAP (116 mg, 0.95 mmol). The reaction was stirred at RT for 18 h. The reaction was diluted with CH₂Cl₂ (10 mL) and washed with 5% citric acid solution (20 mL), sat. NaHCO₃ solution (20 mL), dried (MgSO₄), filtered and concentrated *in vacuo* to give crude intermediate **33** (25 mg, 5%, 70% purity) as a colourless oil which was used without further purification. The crude oil was dissolved in MeOH (7 mL) before ammonium fluoride (8 mg, 0.22 mmol) was added and the reaction stirred at RT for 90 min. The reaction mixture was concentrated *in vacuo*. Purification using flash column chromatography (Gradient: 5, 10 then 14% MeOH/CH₂Cl₂) gave **5** (4.9 mg, 1% over two steps) as a colourless oil; R_f 0.63 (15% MeOH/CH₂Cl₂); $[\alpha]_D^{20}$ –3.8 (c 0.3, MeOH); IR ν_{\max} (oil) 3320 (NH), 3250 (NH), 3150 (OH), 2960–2880 (CH), 1730 (C=O), 1640 (C=O) and 1570 (C=C) cm⁻¹; ¹H NMR (600 MHz, CD₃OD) δ 8.29 (1H, s), 8.21 (1H, s), 6.01 (1H, d, J = 4.6 Hz), 4.74 (1H, t, J = 4.6 Hz), 4.44–4.35 (3H, m), 4.27–4.24 (1H, m), 4.03 (1H, d, J = 10.5), 3.89 (1H, d, J = 10.5 Hz), 3.86 (1H, s), 3.51–3.39 (2H, m), 3.13–3.08 (2H, m), 2.66–2.64 (4H, m), 2.43–2.38 (2H, m), 1.53–1.45 (2H, m), 0.96 (3H, s) and 0.93–0.89 (6H, m); ¹³C NMR (150 MHz, CD₃OD) δ 175.4, 173.9, 173.8, 173.6, 157.3, 153.9, 150.6, 141.3, 120.5, 90.3, 83.5, 75.8, 75.2, 71.7, 71.5, 65.1, 42.3, 39.3, 36.4, 30.3, 23.5, 21.6, 20.9 and 11.7; HRMS calc'd for C₂₆H₄₀N₇O₁₀ expected 610.2837, found 610.2830.

4.1.2.21. ((5*aS*,6*S*,8*S*,8*aS*)-8-(6-Amino-9*H*-purin-9-yl)-2,2,4,4-tetraisopropyltetrahydrofuro[3,4-*f*][1,3,5,2,4]trioxadisilepin-6-yl)methyl benzyl glutarate (**35**). To a solution of alcohol **26** (1.00 g, 1.96 mmol) and protected glutaric acid **34** (870 mg, 3.92 mmol) in CH₂Cl₂ (15 mL)

was added DCC (510 mg, 2.55 mmol) and DMAP (60 mg, 0.49 mmol). The reaction was stirred at RT for 16 h. The insoluble dicyclohexylurea was removed by filtration, washed with CH_2Cl_2 . The organic filtrate was washed with sat. NaHCO_3 solution (3×20 mL), brine (20 mL), dried (MgSO_4), filtered and concentrated *in vacuo*. Purification using flash column chromatography (Gradient: 0–2% $\text{MeOH}/\text{CH}_2\text{Cl}_2$) gave **35** (480 mg, 34%) as an off-white solid; R_f 0.54 (5% $\text{MeOH}/\text{CH}_2\text{Cl}_2$); mp 111–113 °C; $[\alpha]_D^{20}$ –24.2 (c 0.1, MeOH); IR ν_{max} (solid) 3310 (NH), 3180 (NH), 2940–2870 (CH), 1740 (C=O) and 1600 (C=C) cm^{-1} ; ^1H NMR (600 MHz, CDCl_3) δ 8.30 (1H, s), 7.97 (1H, s), 7.39–7.29 (5H, m), 6.01–5.97 (3H, m), 5.11 (2H, s), 5.09 (1H, dd, $J = 4.7$ and 3.3 Hz), 4.69 (1H, dd, $J = 6.4$ and 4.7 Hz), 4.48–4.44 (1H, m), 4.34–4.29 (2H, m), 2.44–2.38 (4H, m), 1.96 (2H, q, $J = 7.4$ Hz) and 1.10–1.00 (28H, m); ^{13}C NMR (150 MHz, CDCl_3) δ 172.7, 155.6, 152.9, 149.6, 139.8, 136.0, 128.7, 128.6, 128.5, 120.4, 90.5, 81.8, 75.7, 72.7, 66.4, 63.7, 33.6, 33.5, 20.2, 17.3 and 13.4; HRMS calc'd for $\text{C}_{34}\text{H}_{52}\text{O}_8\text{N}_5\text{Si}_2$ expected 714.3354, found 714.3351.

4.1.2.22. 5-(((5a*S*,6*S*,8*S*,8*aS*)-8-(6-Amino-9*H*-purin-9-yl)-2,2,4,4-tetraisopropyltetrahydrofuro[3,4-*f*][1,3,5,2,4]trioxadisilepin-6-yl)methoxy)-5-oxopentanoic acid (**36**). To protected adenosine analogue **35** (450 mg, 0.63 mmol) and Pd/C (10 wt%, 17 mg, 0.16 mmol) was added MeOH (10 mL) before evacuating and filling the flask with hydrogen. The reaction was stirred at RT under a hydrogen atmosphere for 3 h. The reaction mixture was filtered through Celite®, washed with MeOH and the filtrate concentrated *in vacuo* to give **36** (387 mg, 97%) as an off-white solid; R_f 0.31 (10% $\text{MeOH}/\text{CH}_2\text{Cl}_2$); mp 137–138 °C; $[\alpha]_D^{20}$ –17.6 (c 0.2, MeOH); IR ν_{max} (solid) 3320 (NH), 3280 (NH), 3150 (OH), 2950–2860 (CH), 1740 (C=O), 1700 (C=O) and 1610 (C=C) cm^{-1} ; ^1H NMR (600 MHz, CD_3OD) δ 8.29 (1H, s), 8.19 (1H, s), 6.04 (1H, d, $J = 4.4$ Hz), 5.26 (1H, t, $J = 4.7$ Hz), 4.78 (1H, t, $J = 5.1$ Hz), 4.45–4.32 (2H, m), 4.29 (1H, q, $J = 4.4$ Hz), 2.40–2.28 (4H, m), 1.89–1.81 (2H, m) and 1.17–0.89 (28H, m); ^{13}C NMR (150 MHz, CD_3OD) δ 177.0, 174.2, 157.4, 153.9, 150.6, 141.6, 120.6, 90.7, 83.7, 76.8, 74.0, 64.1, 34.1, 34.0, 21.4, 17.6 and 14.7; HRMS calc'd for $\text{C}_{28}\text{H}_{42}\text{N}_5\text{O}_8\text{Si}_2$ expected 624.2881, found 624.2908.

4.1.2.23. ((2*S*,3*R*,4*S*,5*S*)-5-(6-Amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl ((*R*)-3-hydroxy-2,2-dimethyl-4-oxo-4-((3-oxo-3-(propylamino)propyl)amino)butyl) glutarate (**6**). To a solution of acid **36** (350 mg, 0.56 mmol) and alcohol **17** (175 mg, 0.67 mmol) in CH_2Cl_2 (17 mL) at 0 °C was added EDC (128 mg, 0.67 mmol) and DMAP (82 mg, 0.67 mmol). The reaction was stirred at RT for 18 h. The reaction was diluted with CH_2Cl_2 (10 mL) and washed with 5% citric acid solution (20 mL), sat. NaHCO_3 solution (20 mL), dried (MgSO_4), filtered and concentrated *in vacuo* to give crude intermediate **37** (65 mg, 13%, 60% purity) as a colourless oil which was used without further purification. The crude oil was dissolved in MeOH (20 mL) before ammonium fluoride (23 mg, 0.64 mmol) was added and the reaction stirred at RT for 90 min. The reaction mixture was concentrated *in vacuo*. Purification using flash column chromatography (Gradient: 5, 10 then 14% $\text{MeOH}/\text{CH}_2\text{Cl}_2$) gave **6** (17 mg, 3% over two steps) as a colourless gum; R_f 0.31 (15% $\text{MeOH}/\text{CH}_2\text{Cl}_2$); $[\alpha]_D^{20}$ –0.7 (c 0.8, MeOH); IR ν_{max} (solid) 3340 (NH), 3260 (NH), 3090 (OH), 2970–2880 (CH), 1720 (C=O), 1650 (C=O) and 1590 (C=C) cm^{-1} ; ^1H NMR (600 MHz, CD_3OD) δ 8.27 (1H, s), 8.20 (1H, s), 6.01 (1H, d, $J = 4.4$ Hz), 4.75 (1H, t, $J = 4.8$ Hz), 4.43–4.35 (3H, m), 4.27–4.23 (1H, m), 4.02 (1H, d, $J = 9.5$ Hz), 3.90 (1H, d, $J = 9.5$ Hz), 3.86 (1H, s), 3.51–3.39 (2H, m), 3.12–3.08 (2H, m), 2.50–2.35 (6H, m), 1.92–1.85 (2H, m), 1.53–1.45 (2H, m), 0.96 (3H, s) and 0.94–0.88 (6H, m); ^{13}C NMR (150 MHz, CD_3OD) δ 175.4, 174.6, 173.9, 173.5, 157.3, 153.9, 150.6, 141.2, 120.6, 90.4, 83.4, 75.8, 75.1, 71.8, 71.3, 64.8, 42.3, 39.4, 36.8, 33.8, 23.6, 21.6, 21.1, 20.8 and 11.8; HRMS calc'd for $\text{C}_{27}\text{H}_{42}\text{N}_7\text{O}_{10}$ expected 624.2993, found 624.3005.

4.1.2.24. ((5*aS*,6*S*,8*S*,8*aS*)-8-(6-Amino-9*H*-purin-9-yl)-2,2,4,4-

tetraisopropyltetrahydrofuro[3,4-*f*][1,3,5,2,4]trioxadisilepin-6-yl)methyl neopentyl succinate (**39**). To a solution of acid **32** (330 mg, 0.54 mmol) in CH_2Cl_2 (10 mL) at 0 °C was added DIC (125 μL , 0.81 mmol) and DMAP (17 mg, 0.14 mmol). The reaction was stirred at 0 °C for 10 min before 2,2-dimethyl-1-propanol (71 mg, 0.81 mmol) in CH_2Cl_2 (5 mL) was added dropwise. The reaction was stirred at RT for 20 h. The insoluble material was filtered and washed with CH_2Cl_2 . The organic filtrate was washed with 5% citric acid solution (30 mL), then sat. NaHCO_3 solution (30 mL), dried (MgSO_4), filtered and concentrated *in vacuo*. Purification using flash column chromatography (Gradient: 1–4% $\text{MeOH}/\text{CH}_2\text{Cl}_2$) gave **39** (243 mg, 66%) as an off-white solid; R_f 0.31 (8% $\text{MeOH}/\text{CH}_2\text{Cl}_2$); mp 113–115 °C; $[\alpha]_D^{20}$ –17.5 (c 0.2, MeOH); IR ν_{max} (solid) 3340 (NH), 3180 (NH), 2950–2870 (CH), 1740 (C=O) and 1610 (C=C) cm^{-1} ; ^1H NMR (600 MHz, CDCl_3) δ 8.31 (1H, s), 8.00 (1H, s), 5.99 (1H, d, $J = 3.3$ Hz), 5.90 (2H, br), 5.08 (1H, dd, $J = 4.7$ and 3.3 Hz), 4.69 (1H, t, $J = 4.7$ Hz), 4.50–4.46 (1H, m), 4.35–4.31 (2H, m), 3.77 (2H, apparent s), 2.67–2.65 (4H, m), 1.09–1.01 (28H, m) and 0.91 (9H, s); ^{13}C NMR (150 MHz, CDCl_3) δ 172.3, 155.4, 152.7, 149.6, 139.7, 120.5, 90.4, 81.8, 75.8, 74.3, 72.7, 63.9, 31.4, 29.1, 26.5, 17.3 and 13.2; HRMS calc'd for $\text{C}_{31}\text{H}_{54}\text{O}_8\text{N}_5\text{Si}_2$ expected 679.3427, found 679.34269.

4.1.2.25. ((2*S*,3*R*,4*S*,5*S*)-5-(6-Amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl neopentyl succinate (**7**). To a solution of protected CoA analogue **39** (80.0 mg, 0.12 mmol) in MeOH (3 mL) was added ammonium fluoride (37 mg, 1.02 mmol). The reaction was stirred at RT for 1.5 h. The reaction was concentrated *in vacuo*. Purification using flash column chromatography (Gradient: 3, 5 then 7% $\text{MeOH}/\text{CH}_2\text{Cl}_2$) gave **7** (31 mg, 59%) as a colourless oil; R_f 0.45 (10% $\text{MeOH}/\text{CH}_2\text{Cl}_2$); mp 75–77 °C; $[\alpha]_D^{20}$ –5.8 (c 0.4, MeOH); IR ν_{max} (oil) 3330 (NH), 3270 (NH), 3130 (OH), 2960–2910 (CH), 1730 (C=O), 1650 (C=O) and 1610 (C=C) cm^{-1} ; ^1H NMR (600 MHz, CD_3OD) δ 8.28 (1H, s), 8.20 (1H, s), 6.01 (1H, d, $J = 4.5$ Hz), 4.74 (1H, t, $J = 4.9$ Hz), 4.44–4.33 (3H, m), 4.27–4.23 (1H, m), 3.76 (1H, d, $J = 10.5$ Hz), 3.73 (1H, d, $J = 10.5$ Hz), 3.32–3.29 (4H, m) and 2.65 (9H, s); ^{13}C NMR (150 MHz, CD_3OD) δ 174.0, 173.8, 157.3, 153.9, 150.6, 141.3, 120.5, 90.3, 83.5, 75.1, 75.0, 71.8, 65.1, 32.1, 29.9 and 26.7; HRMS calc'd for $\text{C}_{19}\text{H}_{28}\text{N}_8\text{O}_7$ expected 438.1983, found 438.1982.

4.1.2.26. ((5*aS*,6*S*,8*S*,8*aS*)-8-(6-Amino-9*H*-purin-9-yl)-2,2,4,4-tetraisopropyltetrahydrofuro[3,4-*f*][1,3,5,2,4]trioxadisilepin-6-yl)methyl ((*R*)-3-hydroxy-2,2-dimethyl-4-(methylamino)-4-oxobutyl) succinate (**41**). To a solution of acid **32** (310 mg, 0.51 mmol) in CH_2Cl_2 (10 mL) at 0 °C was added DIC (120 μL , 0.77 mmol) and DMAP (16 mg, 0.13 mmol). The reaction was stirred at 0 °C for 10 min before alcohol **40** (123 mg, 0.77 mmol) in CH_2Cl_2 (5 mL) was added dropwise. The reaction was stirred at RT for 20 h. The insoluble material was filtered and washed with CH_2Cl_2 . The organic filtrate was washed with 5% citric acid solution (30 mL), then sat. NaHCO_3 solution (30 mL), dried (MgSO_4), filtered and concentrated *in vacuo*. Purification using flash column chromatography (Gradient: 1–5% $\text{MeOH}/\text{CH}_2\text{Cl}_2$) gave **41** (175 mg, 46%) as an off-white solid; R_f 0.51 (10% $\text{MeOH}/\text{CH}_2\text{Cl}_2$); mp 109–111 °C; $[\alpha]_D^{20}$ –8.8 (c 0.1, MeOH); IR ν_{max} (solid) 3330 (NH), 3290 (NH), 3180 (OH), 2940–2870 (CH), 1730 (C=O), 1640 (C=O) and 1600 (C=C) cm^{-1} ; ^1H NMR (600 MHz, CDCl_3) δ 8.29 (1H, s), 8.09 (1H, s), 6.81 (1H, d, $J = 5.0$ Hz), 6.13 (2H, br), 6.02 (1H, d, $J = 2.6$ Hz), 5.06 (1H, dd, $J = 4.7$ and 2.6 Hz), 4.70 (1H, dd, $J = 6.8$ and 4.7 Hz), 4.56–4.50 (1H, m), 4.36–4.28 (2H, m), 4.23 (1H, d, $J = 10.8$ Hz), 4.05 (1H, s), 3.84 (1H, d, $J = 10.8$ Hz), 2.83 (3H, d, $J = 5.0$ Hz), 2.63–2.58 (4H, m), 1.10–1.04 (31H, m) and 0.93 (3H, s); ^{13}C NMR (150 MHz, CDCl_3) δ 173.2, 172.7, 172.2, 155.2, 152.6, 149.4, 139.8, 120.0, 90.6, 81.7, 76.2, 74.4, 72.2, 71.0, 63.0, 38.7, 29.5, 25.8, 21.5, 19.9, 17.5 and 13.6; HRMS calc'd for $\text{C}_{33}\text{H}_{57}\text{O}_{10}\text{N}_6\text{Si}_2$ expected 753.3675, found 753.3679.

4.1.2.27. ((2*S*,3*R*,4*S*,5*S*)-5-(6-Amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl ((*R*)-3-hydroxy-2,2-dimethyl-4-(methylamino)-4-oxobutyl) succinate (**8**). To a solution of protected CoA analogue **41** (60.0 mg, 0.08 mmol) in MeOH (2 mL) was added ammonium fluoride (25 mg, 0.68 mmol) and the reaction was stirred at RT for 1.5 h. The reaction mixture was concentrated *in vacuo*. Purification using flash column chromatography (Gradient: 5% then 10% MeOH/CH₂Cl₂) gave **8** (14 mg, 34%) as a colourless oil; R_f 0.17 (10% MeOH/CH₂Cl₂); mp 86–88 °C; [α]_D²⁰ –1.8 (c 0.9, MeOH); IR ν_{max} (solution in CH₂Cl₂) 3360 (NH), 3240 (NH), 3090 (OH), 2980–2860 (CH), 1740 (C=O), 1650 (C=O) and 1600 (C=C) cm⁻¹; ¹H NMR (600 MHz, CD₃OD) δ 8.29 (1H, s), 8.20 (1H, s), 6.02 (1H, d, *J* = 4.5 Hz), 4.74 (1H, t, *J* = 4.9 Hz), 4.55–4.35 (3H, m), 4.27–4.23 (1H, m), 4.03 (1H, d, *J* = 10.6 Hz), 3.90 (1H, d, *J* = 10.6 Hz), 3.86 (1H, s), 2.74 (3H, s), 2.66–2.63 (4H, m), 0.96 (3H, s) and 0.92 (3H, s); ¹³C NMR (150 MHz, CD₃OD) δ 176.1, 173.9, 173.8, 157.3, 153.9, 150.6, 141.2, 120.5, 90.3, 83.5, 76.0, 75.2, 71.8, 71.4, 65.1, 39.3, 29.9, 25.8, 21.5 and 20.8; HRMS calc'd for C₂₇H₃₁N₆O₉ expected 511.2147, found 511.2146.

4.1.2.28. (*R*)-2,4-Dihydroxy-3,3-dimethyl-*N*-(3-(methylamino)-3-oxopropyl)butanamide (**42**). To Boc protected *N*-methyl-β-aminopropionamide (2.11 g, 10.4 mmol) in CH₂Cl₂ (80 mL) was added TFA (20 mL). The reaction was stirred at RT for 4 h. The reaction was concentrated *in vacuo* to give *N*-methyl-β-aminopropionamide (3.50 g, quantitative) as the TFA salt which was used without any further purification. The crude oil was dissolved in MeOH (60 mL) to which was added NEt₃ (12 mL). The solution was heated at reflux for 90 min before *D*-pantolactone (1.40 g, 10.8 mmol) was added. The reaction was heated at reflux for 20 h. The reaction mixture was concentrated *in vacuo*. Purification using flash column chromatography (Gradient: 0, 2, 4 and 6% MeOH/CH₂Cl₂) gave the NEt₃ salt of **42** (835 mg, 34% over two steps) as a colourless oil; R_f 0.36 (10% MeOH/CH₂Cl₂); [α]_D²⁰ +12.0 (c 0.3, MeOH); IR ν_{max} (oil) 3300 (NH), 3250 (NH), 3080 (OH), 2950–2870 (CH), 1740 (C=O) and 1640 (C=O) cm⁻¹; ¹H NMR (600 MHz, CD₃OD) δ 3.89 (1H, s), 3.54–3.41 (3H, m), 3.38 (1H, d, *J* = 11.0 Hz), 2.71 (3H, s), 2.41 (2H, t, *J* = 6.7 Hz), 0.91 (3H, s) and 0.90 (3H, s); ¹³C NMR (150 MHz, CD₃OD) δ 176.1, 174.3, 77.3, 70.3, 40.3, 36.4, 26.5, 21.3 and 20.9; HRMS calc'd for C₁₀H₂₁N₂O₄ expected 233.1496, found 233.1495.

4.1.2.29. ((2*S*,3*R*,4*S*,5*S*)-5-(6-Amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl ((*R*)-3-hydroxy-2,2-dimethyl-4-((3-(methylamino)-3-oxopropyl)amino)-4-oxobutyl) succinate (**9**). To a solution of acid **32** (300 mg, 0.43 mmol) in CH₂Cl₂ (9 mL) at 0 °C was added DIC (100 μL, 0.65 mmol) and DMAP (13 mg, 0.11 mmol). The reaction was stirred at 0 °C for 10 min before alcohol **42** (200 mg, 0.86 mmol) in CH₂Cl₂ (5 mL) was added dropwise. The reaction was stirred at RT for 20 h. The resulting precipitate was filtered and washed with CH₂Cl₂. The organic filtrate was washed with 5% citric acid solution (20 mL), then sat. NaHCO₃ solution (20 mL), dried (MgSO₄), filtered and concentrated *in vacuo*. Purification using flash column chromatography (Gradient: 1–10% MeOH/CH₂Cl₂) gave the crude intermediate (182 mg, 46%, 75% purity) as a colourless oil which was used without further purification. The crude oil (85 mg) was dissolved in MeOH (20 mL) before ammonium fluoride (31 mg, 0.85 mmol) was added and the reaction stirred at RT for 1 h. The reaction mixture was concentrated *in vacuo*. Purification using flash column chromatography (Gradient: 10, 12 then 15% MeOH/CH₂Cl₂) gave **9** (22 mg, 18% over two steps) as a colourless gum; R_f 0.36 (15% MeOH/CH₂Cl₂); [α]_D²⁰ –1.5 (c 0.9, MeOH); IR ν_{max} (solid) 3340 (NH), 3290 (NH), 3100 (OH), 2980–2920 (CH), 1730 (C=O), 1690 (C=O) and 1550 (C=C) cm⁻¹; ¹H NMR (600 MHz, CD₃OD) δ 8.50 (1H, s), 8.41 (1H, s), 6.08 (1H, d, *J* = 4.5 Hz), 4.74 (1H, t, *J* = 4.8 Hz), 4.42–4.34 (3H, m), 4.30–4.27 (1H, m), 4.02 (1H, d, *J* = 10.6 Hz), 3.88 (1H, d, *J* = 10.6 Hz), 3.84 (1H, s), 3.52–3.39 (2H, m), 2.70–2.68 (4H,

m), 2.65 (3H, s), 2.41 (2H, t, *J* = 6.6 Hz), 0.95 (3H, s) and 0.92 (3H, s); ¹³C NMR (150 MHz, CD₃OD) δ 175.4, 174.0, 173.9, 173.8, 152.3, 150.0, 145.9, 143.9, 120.7, 90.7, 83.9, 75.9, 75.5, 71.7, 71.5, 64.9, 39.5, 36.4, 29.9, 26.4, 21.5 and 20.8; HRMS calc'd for C₂₄H₃₆N₇O₁₀ expected 582.2518, found 582.2516.

4.1.2.30. (1*0R*,2*9R*)-1-((2*R*,3*S*,4*R*,5*R*)-5-(6-Amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)-10,29-dihydroxy-9,9,30,30-tetramethyl-3,6,11,15,24,28-hexaoxo-2,7-dioxo-19,20-dithia-12,16,23,27-tetraazahentriacontan-31-yl (((2*S*,3*R*,4*S*,5*S*)-5-(6-amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl) succinate (**46**). To a solution of acid **32** (700 mg, 1.15 mmol) in THF (50 mL) at 0 °C was added DIC (180 μL, 1.15 mmol) and DMAP (56 mg, 0.46 mmol). The reaction was stirred at 0 °C for 10 min before *D*-pantethine **43** (255 mg, 0.46 mmol) in THF (25 mL) was added dropwise. The reaction was stirred at RT for 20 h. The reaction was concentrated *in vacuo*. The resulting residue was dissolved in CH₂Cl₂ (40 mL) and washed with 5% citric acid solution (40 mL), sat. NaHCO₃ solution (40 mL), dried (MgSO₄), filtered and concentrated *in vacuo*. Purification using flash column chromatography (Gradient: 1–10% MeOH/CH₂Cl₂) gave the crude mixture (130 mg, mixture of singly and doubly added products, **44** and **45**) as a colourless oil which was used without further purification. The crude oil was dissolved in MeOH (18 mL) before ammonium fluoride (28 mg, 0.77 mmol) was added and the reaction stirred at RT for 75 min. The reaction mixture was concentrated *in vacuo*. Purification using flash column chromatography (Gradient: 10, 15 then 20% MeOH/CH₂Cl₂) gave **46** (25 mg, 4% over two steps) as a colourless oil; R_f 0.23 (15% MeOH/CH₂Cl₂); [α]_D²⁰ –0.3 (c 1.2, MeOH); IR ν_{max} (oil) 3340 (NH), 3220 (NH), 3080 (OH), 2960–2930 (CH), 1790 (C=O), 1730 (C=O), 1640 (C=O) and 1600 (C=C) cm⁻¹; ¹H NMR (600 MHz, CD₃OD) δ 8.29 (1H, s), 8.20 (1H, s), 6.02 (1H, d, *J* = 4.5 Hz), 4.74 (1H, t, *J* = 5.0 Hz), 4.44–4.35 (3H, m), 4.28–4.24 (1H, m), 4.03 (1H, d, *J* = 10.5 Hz), 3.88 (1H, d, *J* = 10.5 Hz), 3.86 (1H, s), 3.50–3.44 (4H, m), 2.81–2.75 (2H, m), 2.66–2.63 (4H, m), 2.46–2.40 (2H, m), 0.95 (3H, s) and 0.92 (3H, s); ¹³C NMR (150 MHz, CD₃OD) δ 175.5, 174.0, 173.9, 173.8, 157.6, 153.9, 150.6, 141.3, 120.5, 90.3, 83.4, 75.9, 75.2, 71.8, 71.5, 65.2, 39.6, 39.3, 38.4, 36.8, 30.7, 21.7 and 20.8; HRMS calc'd for C₅₀H₇₃N₁₄O₂₀S₂ expected 1253.4561, found 1253.4565.

4.1.2.31. ((2*S*,3*R*,4*S*,5*S*)-5-(6-Amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl ((*R*)-3-hydroxy-4-((3-((2-mercaptoethyl)amino)-3-oxopropyl)amino)-2,2-dimethyl-4-oxobutyl) succinate (**10**). To disulfide **46** (15 mg, 0.012 mmol) in H₂O:MeCN (60:40, 1 mL) was added TCEP (35 mg, 0.12 mmol). The reaction was stirred at RT for 1 h. The reaction mixture was concentrated *in vacuo*. Purification using preparative HPLC (gradient 2, r_t 10 min) gave **10** (15 mg, 78%) as a colourless gum; R_f 0.48 (15% MeOH/CH₂Cl₂); [α]_D²⁰ –2.5 (c 0.6, MeOH); IR ν_{max} (oil) 3310 (NH), 3290 (NH), 3110 (OH), 2940–2880 (CH), 1730 (C=O), 1690 (C=O) and 1550 (C=C) cm⁻¹; ¹H NMR (600 MHz, CD₃OD) δ 8.49 (1H, s), 8.40 (1H, s), 6.08 (1H, d, *J* = 4.6 Hz), 4.74 (1H, t, *J* = 4.9 Hz), 4.42–4.35 (3H, m), 4.30–4.27 (1H, m), 4.01 (1H, d, *J* = 10.5 Hz), 3.89 (1H, d, *J* = 10.5 Hz), 3.85 (1H, s), 3.52–3.39 (2H, m), 3.33–3.28 (2H, m), 2.66–2.63 (2H, m), 2.60–2.55 (2H, m), 2.45–2.41 (2H, m), 0.96 (3H, s) and 0.93 (3H, s); ¹³C NMR (150 MHz, CD₃OD) δ 175.4, 174.0, 173.9, 173.8, 152.4, 150.0, 146.1, 143.9, 120.7, 90.7, 83.9, 76.0, 75.5, 71.7, 71.5, 65.9, 43.9, 39.3, 36.4, 29.9, 24.5, 21.6 and 20.8; HRMS calc'd for C₂₅H₃₈N₁₀O₇S expected 627.2395, found 627.2394.

4.2. Biological assay

Aurora A kinase activity was assayed by measuring incorporation of ³³P into myelin basic protein (Sigma). 100 ng of purified recombinant Aurora A was incubated at room temperature for 30 min in a total volume of 15 μL containing 50 mM HEPES pH 7.5, 10 mM MgCl₂, 1 mM EGTA, 0.05% Brij-35, 0.5 mg/ml myelin basic protein, and 5 μM

γ -³³P-ATP (100 dpm/pmol) in the presence or absence of 1 mM DTT. The reaction was stopped by spotting the reaction mixture onto squares of P81 phosphocellulose ion exchange paper (Whatmann), which were then immersed in 1% (v/v) phosphoric acid. After washing twice in 1% phosphoric acid followed by two washes in distilled water, the papers were air dried and radioactivity was counted by a scintillation counter. IC50 values were determined using GraphPad Prism version 6 (GraphPad Software, La Jolla California USA, www.graphpad.com).

4.3. GOLD modelling

For modelling the interaction between the enzyme and the inhibitors, the previously reported structure (PDB:1OL7¹²) of active, phosphorylated human Aurora kinase A was altered using MOE³⁴ to include the cysteine residue Cys290, present in the activation loop of Aurora A kinase. GOLD (Genetic Optimisation for Ligand Docking)^{32,33} was used for the *in silico* docking studies. All heteroatoms (H₂O and ligands) were removed from the PDB file and all polar hydrogens added. Ligand files were generated using MOE and used as .mol2 files. Each ligand structure was energy minimised using MOE prior to use in the GOLD docking. Since only interaction with the binding site was required, the existing ligand within the binding site was used (either AMP or ADP) to define the binding site and the default 10 genetic algorithm runs were run. The scoring function CHEMPLP⁶⁷ was used to rank each of the resulting poses. The solutions were then exported as a .mol2 file and viewed with PyMOL.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.bmc.2020.115740>.

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