

Small molecule inhibitors of Nicotinamide N-Methyltransferase (NNMT)

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Chapter 3

Potent inhibition of nicotinamide *N*methyltransferase by alkene-linked bisubstrate mimics bearing electron-deficient aromatics

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Abstract

Nicotinamide *N*-methyltransferase (NNMT) methylates nicotinamide (vitamin B3) to generate 1-methylnicotinamide (MNA). NNMT overexpression has been linked to a variety of diseases, most prominently human cancers, indicating its potential as a therapeutic target. The development of small molecule NNMT inhibitors has gained interest in recent years with the most potent inhibitors sharing many similar structural features based on the structures of the nicotinamide substrate and the *S*-adenosyl-L-methionine (SAM) cofactor. We here report the development of a series of inhibitors that depart from some of these conserved structural features through the introduction of alternative electron deficient aromatic groups to mimic the nicotinamide moiety. In addition, the identification of an optimal trans-alkene linker differs from the previously reported alkyl and alkynyl linkers used to connect the substrate and cofactor mimics in these inhibitors. The most potent NNMT inhibitor identified in our study exhibits an IC_{50} value of 3.7 nM placing it among the most active NNMT inhibitors reported to date. Complementary analytical techniques, modeling studies, and cell-based assays provide insight into the binding mode, affinity, and selectivity of these inhibitors.

1. Introduction

The enzyme nicotinamide *N*-methyltransferase (NNMT) catalyzes the methylation of nicotinamide using *S*-adenosyl-L-methionine (SAM) as cofactor and produces *S*-adenosyl-L-homocysteine (SAH) as byproduct (Figure 1). Since its discovery in 1952, its role was considered to be exclusively associated with cell detoxification through the metabolism of xenobiotics.¹ This function is carried out thanks to NNMT's broad substrate recognition that allows for the methylation of pyridines, quinolines and other related heterocyclic metabolites, followed by their excretion.² However, the view that NNMT is primarily involved in detoxification has recently changed as a result of numerous studies implicating NNMT in a variety of other critical metabolic pathways.^{3,4} For example, NNMT's substrate nicotinamide is the precursor of NAD⁺, a compound heavily involved in redox processes and energy management.⁵ In addition, while NNMT does not play an epigenetic role *per se*, its influence on the SAM/SAH balance has an indirect impact on gene expression.^{6,7} The involvement of NNMT in epigenetic reprogramming as well as in the cell's energetic balance and detoxification pathways provides a broader appreciation of its role in the development and progression of cancer,^{3,6,8-12} diabetes,^{5,13,14} obesity,^{5,14} and neurodegenerative disorders.¹⁵⁻¹⁸

Improving our understanding of NNMT and its role in disease hinges in significant part on the availability of potent, selective, and cell-active small-molecule inhibitors. Such chemical tools can both lead the way to validate NNMT as a drug target and at the same time be used as templates for the development of new medicines for treating NNMT-driven conditions. At present, the most potent NNMT inhibitors described in the literature are bisubstrate analogues, comprising two covalently linked moieties that mimic the cofactor and substrate, SAM and nicotinamide, respectively. Following our initial reports describing such bisubstrate mimics as NNMT inhibitors,^{19,20} significant progress has been made by other groups also working in the



Figure 1. Methylation of nicotinamide (NA) by NNMT using *S*-adenosyl-L-methionine (SAM) as the methyl donor forming *N*-methyl nicotinamide (MNA) and *S*-adenosyl-L-homocysteine (SAH).

field (Figure 2) ^{21–24} Notably, the potency of bisubstrate NNMT inhibitors has improved from our first reported compounds with IC₅₀ values in the micromolar range^{19,20,25} to those more recently described by the groups of Shair and Huang with IC₅₀ values in the low nanomolar range.^{26,27} Collectively, these studies have shown that bisubstrate inhibitor potency is heavily dependent on the relative spacing and spatial orientation of the adenosine, amino acid, and nicotinamide mimicking moieties.^{19,20,25-27} Notable in this regard are the different linkers that have been used to connect the SAM and nicotinamide groups, amongst which alkynyl species have been shown to achieve the highest levels of inhibition (Figure 2). Building on our previous endeavors in designing inhibitors for NNMT^{19,20} and bisubstrate inhibitors for other methyltransferases containing alkene-based linkers,^{28,29} we here describe our most recent efforts at developing novel NNMT inhibitors characterized by innovative design, improved potency, and ease of synthesis. These investigations have culminated in the discovery of a novel styrene scaffold with substitutions in the nicotinamide mimetic that get away from the amide functionality present in the majority of bisubstrate inhibitors reported to date. Our results with this new scaffold also revealed interesting structure-activity relationships of electron-withdrawing substitutions with para-cyano compound 17u (Figure 2) being the most potent inhibitor identified with an IC₅₀ value of 3.7 ± 0.2 nM. The extensive SAR results presented here were further corroborated by insights in the compounds' binding mode to NNMT as predicted by molecular modeling. Compound 17u was further characterized by means of isothermal titration calorimetry (ITC) experiments, biochemical assays to assess selectivity against other methyltransferases, and cellbased studies to assess investigate effects on the viability of several cancer cell lines.



Figure 2. Chemical structures, inhibition data, and publication dates of bisubstrate inhibitors of NNMT.

2. Results and Discussion

Design. The crystal structures reported for NNMT consistently reveal π - π stacking interactions between tyrosine residue Y204 and either the pyridine ring of the natural nicotinamide substrate³⁰ or the aromatic group that mimics it in the bisubstrate inhibitors.^{25–27} In order to capitalize on these interactions and improve the potency of our previously disclosed NNMT ligand **3**,²⁰ we first undertook a systematic exploration of its naphthalene portion (Figure 3A) where a selection of bicyclic (hetero)aromatics was incorporated. In addition, prompted by the desire for an approach which would allow for the introduction of a wider range of nicotinamide mimics with different shapes and electronic features, a novel styrene-based scaffold was devised. This scaffold-hopping approach, which was based on a naphthalene truncation strategy (Figure 3A), presents two key advantages: i) it allows for the expeditious synthesis of a diverse library of NNMT inhibitors starting from readily available building blocks; and ii) it provides insights into a novel alkenyl linker connecting the SAM-like portion and the nicotinamide mimic moiety. The latter feature is relevant because the resulting ligands complement the published bisubstrate inhibitors (Figure 2), which are generally linked by alkyl or alkynyl spacers.^{25–27} In addition, a selection of compounds was designed to assess the importance



Figure 3. (A) Strategy for the modification and optimization of inhibitor **3** through introduction of a variety of aromatics and the truncation of the naphthalene moiety resulting in the introduction of the alkenyl linker; **(B)** General synthetic route for the preparation of NNMT inhibitors, based on a double reductive amination approach followed by a single deprotection step.

of both the amino acid and adenosine moieties for NNMT active site binding.

Synthesis. The synthesis of the NNMT inhibitors here pursued was based on a convenient, modular strategy that provides access to a wide range of chemically different ligands. Starting from the known adenosine amine building block **9**, all bisubstrate analogues were obtained via a sequential double reductive amination process followed by global deprotection (Figure 3B). The required bicyclic (hetero)aromatic aldehydes **8a-1** used in the reductive amination steps were either commercially available or prepared through formation of the Weinreb amide and subsequent DIBAL-H reduction (Scheme 1). Phenylpropenaldehydes **15a-y** were either commercially available or prepared through a Wittig reaction coupling the corresponding benzaldehydes to (triphenyl-phosphoranylidene)acetaldehyde as shown in Scheme 2. The aldehydes were subsequently coupled to compound **11** (prepared by reductive amination of adenosine amine building block **9** with the appropriate l-Asp derived aldehyde building block **10**). These reductive aminations were found to proceed cleanly using sodium triacetoxyborohydride and acetic acid after which the final compounds were obtained by global deprotection of the acid-labile protecting groups using TFA/CH₂Cl₂, with isopropylidene group cleavage facilitated by the addition of water (Scheme 1 and 2).



Scheme 1. Representative synthetic scheme for the preparation of bicyclic aromatic compounds **13a-l**, shown for quinoline-containing compound **13a**.^a The variable group for compounds **6b-l**, **7b-l**, **8b-l**, **12b-l** and **13b-l** is indicated in blue. Reagents and conditions: (a) CH₃NHOCH₃·HCl, BOP, Et₃N, CH₂Cl₂, rt, 2 h (88%); (b) DIBAL-H in hexanes, THF, -78 °C, 2 h (assumed quant.); (c) NaBH(OAc)₃, AcOH, DCE, rt, overnight (47%); (d) TFA, CH₂Cl₂, H₂O, rt, 2 h, (86%).



Scheme 2. Representative synthetic scheme for the preparation of substituted cinnamaldehydes **15a-y** and resulting alkenyl linked aromatic compounds **17a-y**. Reagents and conditions: (a) PPh₃=CHCHO, toluene, 80°C, overnight (45-77%); (b) aldehyde **15a-y**, NaBH(OAc)₃, AcOH, DCE, rt, overnight (43-81%); (c) TFA, CH₂Cl₂, H₂O, rt, 2 h, (27-86%).

In order to investigate different substitutions of the amino acid moiety, building block 18 containing the *para*-cyano-substituted phenylpropenyl side chain, was prepared through coupling of 4-cyano-phenylpropenaldehyde 15u to the adenosine amine starting material 9 (Scheme 3). A variety of aldehydes were then coupled to probe the amino acid pocket as exemplified for compound 21a in which the amino acid linker was extended with an extra carbon. Compounds 24a and 24b lacking the adenosine unit were also synthesized in a similar fashion



Scheme 3. Representative synthetic scheme for the preparation of 4-cyano-phenylpropenyl compounds with different substitutions of the amino acid side chain, shown for compound **21a** bearing an extended linker to the amino acid moiety. The variable group in compounds **19b-k**, **20b-k** and **21b-k** is indicated in blue. Reagents and conditions: (a) aldehyde **15u**, NaBH(OAc)₃, AcOH, DCE, rt, overnight (81%); (b) aldehyde **19a**, NaBH(OAc)₃, AcOH, DCE, rt, overnight (81%); (c) TFA, CH₂Cl₂, H₂O, rt, 2 h, (86%).

through coupling of amino acid aldehyde **10** to 4-cyano-phenylpropenylamine **22a** or its methylated analogue **22b** (Scheme 4). The crude products were purified by preparative high-performance liquid chromatography (HPLC) to yield the desired bisubstrate analogues.



Scheme 4. Synthetic scheme for the preparation of 4-cyano-phenylpropenyl compounds **24a-b** lacking the adenosine unit ^a Reagents and conditions: (a) methylamine in MeOH (33% w/w), NaBH(OAc)₃, AcOH, DCE, rt, overnight (42%); (b) aldehyde **22**, NaBH(OAc)₃, AcOH, DCE, rt, overnight (48-77%); (c) TFA, CH₂Cl₂, H₂O, rt, 2 h, (75-87%)

Inhibition Studies. All bisubstrate analogues prepared were tested for NNMT inhibitory activity using a method recently developed in our group.² This assay employs hydrophilic liquid interaction chromatography (HILIC) coupled with tandem mass spectrometry (MS/MS) to rapidly and efficiently assess NNMT inhibition through direct analysis of the formation of MNA. For each compound, NNMT inhibition was initially screened at a fixed inhibitor concentration of 25 μ M. In cases where at least 50% inhibition was detected at this concentration, full inhibition curves were measured in triplicate to determine the corresponding half-maximal inhibitory concentration (IC₅₀) values. As reference compounds, we included our previously described NNMT inhibitor **3** and the recently described NNMT inhibitor **5**. The structures of these reference compounds are provided above in Figure 2 and the IC₅₀ values obtained in our assay were found to be in line with published values.^{20,27}

Structure–Activity Relationships (SAR): β -naphthalene modification. As previously mentioned, we aimed at improving the potency of our previously reported inhibitor **3** through further exploitation of the π - π stacking interactions between Y204 and the ligand's nicotinamide mimicking motif. To this end, a small library of compounds was made, in which the naphthalene moiety of compound **3** was replaced with other (hetero)aromatic groups (compounds **13a-l**, Figure 4). The introduction of electron-poor quinolines, which could potentially complement Y204 in a productive π - π stacking interaction, was met with poor results as the IC₅₀ values of



Figure 4. Structure—activity relationship (SAR) studies of bisubstrate NNMT inhibitors **13a-1** carrying bicyclic (hetero)aromatic side-chains to replace the naphthalene group of compound **3.** IC_{50} values (μ M) and s.e.m. are shown in red.

compounds **13a** and **13b** were above the 25 μ M threshold, with only compound **13c** showing moderate inhibition (IC₅₀ = 7.36 μ M). Although the incorporation of an α -naphthalene led to good inhibition (**13g**, IC₅₀ = 1.48 μ M), the addition of an extra carbon to the linker portion abrogated it (**13h**, IC₅₀ = 19.54 μ M) and switching to biphenyl resulted in a considerable drop in potency (**13d-f**, IC₅₀ > 25 μ M). A similar trend was observed with the introduction of an indole moiety, with inhibitors **13i** and **13j** failing to display IC₅₀ values below 25 μ M. Improved potency was achieved when a benzothiophene ring was incorporated (**13k** and **13l**) and especially when the branching point was at its C-3 position. Notable in this regard is compound **13l** which was found to inhibit NNMT with an IC₅₀ value of 0.57 μ M (Figure 4).

Scaffold hopping to styrene inhibitors. In light of the only moderate level of success obtained by introduction of other bicyclic (hetero)aromatic groups, we next shifted our focus to a different approach. Specifically, we applied a scaffold-hopping/truncation strategy to compound **3**, in which the naphthalene moiety was simplified into styrene derivatives **17a-y** (Figure 3A). Notably, this structural alteration and accompanying synthetic route, along with the wide availability of substituted benzaldehydes, allowed for ready access to a wide range of novel bisubstrate analogues (Figure 5).

The various styrene analogues thus prepared (**17a-y**) bear different electron-donating and electron-withdrawing substituents at *ortho*, *meta* and *para*-positions, and were evaluated for their *in vitro* activity against NNMT. *Ortho* methyl compound **17a** (IC₅₀ = 1.16 μ M) showed better



Figure 5. SAR studies of bisubstrate NNMT inhibitors **17a-y** carrying alkenyl linked substituted aromatics. IC_{50} values (μ M) and s.e.m. are shown in red and the substitutions are highlighted in blue

activity than the corresponding *meta* (17b, IC₅₀ = 4.60 μ M) and *para* (17c, IC₅₀ = 6.44 μ M) analogues. Methoxy-substituted compounds 17d-f all showed a somewhat lower potency (IC₅₀ \geq 4 μ M). A clear improvement was observed when electron-withdrawing substituents were introduced on the styrene ring. In addition, the orientation of the electron-withdrawing group was directly correlated to its activity with the potency of the compounds increasing from *ortho*- to *meta*- to *para*-substituted compound displaying an IC₅₀ value of 8.98 μ M for *ortho*-F, to 3.78 μ M for *meta*-F and the most potent activity observed for the *para*-F substituted compound displaying an IC₅₀ value of 0.19 μ M. The introduction of a chlorine atom in the same styrene scaffold resulted in a similar trend in NNMT inhibitory activity. In this instance, the IC₅₀ values for the *ortho*-Cl and *meta*-Cl compounds were 1.34 μ M and 0.64 μ M, respectively (17j and 17k, Figure 5), while *para*-analogue 17l (IC₅₀ = 0.24 μ M) was again the most active. Switching chlorine for bromine did not cause any major change in activity for the *ortho*-Br and *meta*-Br analogues (17m and 17n, IC₅₀ = 1.45 and 0.38 μ M, respectively), but positively impacted NNMT inhibition in the case of the *para*-Br compound 17o, which displayed

nanomolar activity (IC₅₀ = 0.061 μ M, Figure 5). Even more striking was the case of nitrosubstituted compounds **17p-r**: while the *para*-nitro-substituted analogue was found to be a highly potent inhibitor (**17r**, IC₅₀ = 0.010 μ M), both *ortho*-nitro and *meta*-nitro compounds failed to show any appreciable activity (**17p** and **17q**, IC₅₀ >25 μ M). Finally, introduction of nitrile functionality on the styrene core caused yet further improvements in potency, especially when situated at the *para* position. Whereas *ortho*-cyano analogue **17s** did not show inhibition at 25 μ M, *meta*-cyano analogue **17t** displayed good inhibition with an IC₅₀ of 0.86 μ M with another leap in activity for *para*-cyano compound **17u** which exhibits the most potent inhibition of all compounds prepared in the present study with a single digit nanomolar IC₅₀ value (IC₅₀ = 3.7 nM).

We next assessed the potential for combining structural features of these new NNMT inhibitors with known potent inhibitors **4** and **5** (Figure 2). In doing so, we generated two styrene-based compounds inspired by **17u** in which the nitrile functionality was replaced by a *meta-* or *para-*substituted primary amide (**17v** and **17w**). Notably, the *para-*amide showed a marked decrease in potency ($IC_{50} = 10.77 \mu M$) while the *meta-*amide proved to be an active NNMT inhibitor ($IC_{50} = 0.013 \mu M$). The behaviour of these two analogues highlighted an interesting trend: whereas for the cyano substituent the *para-*arrangement is superior to the *meta* one, for amides the contrary holds true. Interestingly, unsubstituted compound **17x** exhibited only very modest potency ($IC_{50} = 13.63 \mu M$). Finally, it is worth noting that *para-*alkynyl substituted compound **17y**, in which the nitrile group of **17u** was replaced by an acetylene, was completely inactive with an $IC_{50} > 25 \mu M$. This result clearly indicates as specific role for the nitrile functionality in facilitating productive binding interactions between the inhibitor and the NNMT active site.

From the data presented above, it can be inferred that a strongly electron-rich styrene moiety is not beneficial for NNMT inhibition. Also, it is clear that electron-withdrawing substituents like nitro or cyano are most effective when located at the *para* position on the aromatic ring. The origin of these trends is likely a combination of structural complementarity and electronics. For example, the geometric constraints of the binding pocket could be favouring the *para* substitution pattern, while a particularly effective π - π stacking between NNMT's tyrosine residue Y204 and the electron-poor styrene of compounds **170**, **17r** and **17u** might lie behind these ligands' potency.

Linker modifications. After having established compound 17u as our lead inhibitor, we turned our attention to the role of the linker bridging the SAM-derived motif and the nicotinamide mimicking moiety. Our own work in the field had already highlighted the importance of the

correct spacing for achieving potent NNMT inhibition.²⁰ Moreover, reports by other groups have reinforced the notion that a carefully judged linker, in terms of both length and rigidity, is required for potency (see compounds **2**, **4** and **5**, Figure 2).^{25–27} In order to compare our own alkenyl linker with the alternatives devised by others, a series of analogues of inhibitor **17u** were designed featuring a truncated linker (**25**), a fully saturated linker (**26**) and a propargylic linker (**27** and **28**, Figure 6). Additionally, compound **29** was prepared to assess the impact of replacing the core amine functionality with an amide linkage.

Both the truncated analogue 25 and amide-linked compound 29 displayed a clear drop in



Figure 6. SAR studies of bisubstrate NNMT inhibitors 5 and 25-31 carrying different linkers. IC_{50} values (μM) and s.e.m. are shown in red. Changes introduced relative to the lead inhibitor 17u are indicated in blue.

activity against NNMT (IC₅₀ = 2.78 μ M and > 25 μ M respectively). When the carbon-carbon double bond of inhibitor **17u** was reduced to a saturated three carbon linker, the IC₅₀ value increased more than 10-fold (**26**, IC₅₀ = 0.054 μ M), but the resulting compound still showed high potency. A similar outcome was observed when a propargyl spacer was introduced (**28**, IC₅₀ = 0.069 μ M).

In recently reported studies involving propargyl linked bisubstrate inhibitors of NNMT, the benzamide fragment featured prominently as the favoured nicotinamide mimic.^{25–27} Of note in this regard is the importance of the position of the amide group on the aromatic ring with the *para* substituted amide (**31**) displaying a clear lack of potency (IC₅₀ >25 μ M) relative to the *meta* compound (**5**²⁷) which was measured to have an IC₅₀ value of 0.010 μ M in our assay. Notably, a similar effect is also observed for the alkenyl linked amides **17v** and **17w** reported in our present study (Figure 5) with the *meta*-substituted analogue displaying a nearly 1000-fold increase in NNMT inhibition. Also of note was the observation that this trend is reversed for the corresponding propargyl-linked *meta*- and *para*-cyano analogues: in this case the *meta* isomer **27**

was a much weaker inhibitor (IC₅₀ = 1.43 μ M) than the *para* isomer **28** (IC₅₀ = 0.069 μ M, Figure 6). Finally, as also observed for the fully reduced *para*-cyano analogue **26**, replacement of the unsaturated linker in potent literature inhibitor **5** with a fully saturated alkyl linker led to compound **30** which exhibits reduced activity but retains nanomolar inhibition (IC₅₀ = 0.083 μ M).

The exploration of different linkers in conjunction with optimized nicotinamide mimicking moieties revealed that nitrile- and amide-substituted aromatics confer high level of NNMT inhibition, with the former narrowly outperforming the latter in our hands. Similarly, our newly developed unsaturated linker compared favourably with the alkyne-based linkers previously described.^{26,27} Taking a closer look at this finding, the potency of tight binding alkenyl and alkynyl linked *para*-cyano (**17u** and **28**) and *meta*-amide (**17v** and **5**) inhibitors was reevaluated in the presence of elevated concentrations of cofactor SAM to increase their IC₅₀ value, magnifying their differences in potency.³¹ The four compounds tested bear the same SAM-mimicking motif and are assumed to be equally SAM-competitive and thus similarly affected by increased levels of the cofactor. Increasing the concentration of SAM to 85 μ M (10 times its *K*_M value) in the biochemical assay, resulted in a 2- to 4-fold increase in IC₅₀, confirming the trend observed under standard assay conditions. In addition, the apparent *K_i* values were calculated using Morrison's equation for tight binding inhibitors³² and found to be similar under both SAM concentrations tested (Table 1). These studies confirm compound **17u** as the most potent NNMT inhibitor evaluated in the present study.

Table 1. IC_{50} and Ki_{app} values in nM with standard error of the mean (s.e.m.) for para-cyano compounds **17u** and **28** and meta-amide compounds **17v** and **5** with either propenyl or propargyl linkers. Compounds were tested in the presence of SAM at its K_M value of 8.5 μ M or at 10 times its K_M value (85 μ M)

	IC ₅₀ in nM		Ki _{app} in nM	
Compound	8.5 µM SAM	85 µM SAM	8.5 µM SAM	85 µM SAM
17u (alkene p-CN)	3.69 ± 0.17	16.00 ± 1.48	1.70 ± 0.12	1.49 ± 0.22
28 (alkyne p-CN)	69.29 ± 4.42	258.25 ± 26.21	34.90 ± 2.58	35.38 ± 0.96
17v (alkene m-CONH ₂)	12.76 ± 0.78	39.53 ± 4.52	6.93 ± 1.15	5.23 ± 4.52
5 (alkyne m-CONH ₂)	10.23 ± 0.90	21.66 ± 1.61	5.11 ± 0.44	2.48 ± 0.32

Amino acid and adenosine modifications. After having identified an optimal nicotinamide mimic/linker combination for potent NNMT inhibition, a small selection of ligands with modifications to other parts of the scaffold was next investigated. Structural alterations of the amino acid portion of 17u (Figure 7) revealed a very steep SAR with all analogues exhibiting IC₅₀ values several orders of magnitude higher than the parent compound. Compound **21a**, an

extended three-carbon homolog of **17u**, was significantly less active compared to the parent compound, but still showed submicromolar potency ($IC_{50} = 0.36 \mu M$). It is also clear that the amino group of the amino acid moiety is critical for inhibition, as compounds **21f** and **21g** lost all activity. Removal of the carboxylic acid was tolerated slightly better, with amine **21e** showing an IC_{50} value in the low micromolar range (0.96 μ M). Amino amide analogue **21b** showed a strong decrease in potency (1.90μ M), which was further diminished upon removal of the primary amine (**21c** and **21d**, $IC_{50} > 25 \mu$ M). Replacement of the amino acid moiety with a pyridinone mimic³³ (**21h**) was also not tolerated. When the entire amino acid chain was swapped for a lipophilic methyl or isopropyl group as in compounds **21j** and **21k**, all activity against NNMT was lost (both $IC_{50} > 25 \mu$ M). Notable, however, is the fully truncated, secondary amine **21i** that was surprisingly found to be active, albeit in the low micromolar range. Taken together, the results presented here demonstrate the crucial role the amino acid motif plays in the interaction of these bisubstrate inhibitors in the NNMT binding pocket. Similarly, two truncated analogues of inhibitor **17u**, lacking the adenosine unit (**24a** and **24b**, see Figure 7) or lacking the nicotinamide mimicking side-chain (AzaAdoMet **32**), displayed a complete loss of potency ($IC_{50} > 25 \mu$ M).

NNMT Inhibitor Binding Studies

The binding of the most potent inhibitor **17u** with NNMT was further characterized using



Figure 7. SAR studies of bisubstrate NNMT inhibitors **21a-k** bearing different amino acid substitutions and compounds **24a**, **24b** and **32** lacking either the adenosine unit or the nicotinamide mimicking aromatic sidechain. IC_{50} values (μ M) and s.e.m. are shown in red. Changes introduced relative to the lead inhibitor **17u** indicated in blue.

keeping with the bisubstrate inhibitor's capacity to simultaneously compete with both cofactor SAM and substrate NA, the ITC experiment also confirmed a 1:1 stoichiometry between ligand and enzyme. Details and additional thermograms of compound **17u** and NNMT as well as control titrations are provided in the Supplementary Information.



Figure 8. ITC thermogram of compound **17u** including the thermodynamic binding parameters obtained from three independent titration experiments with human wild-type NNMT.

NNMT Inhibitor Modeling

To learn more about the driving force of the *ortho-meta-para* effect observed for the electronwithdrawing (EWG)-substitutions in the styrene compounds, computational studies were performed on nitrile-substituted compounds **17s**, **17t** and **17u**. These studies were specifically aimed at estimating the relative binding affinity shifts, via free energy perturbation (FEP), due to the inclusion of the *ortho*, *meta*, or *para*-nitrile substituent in the unsubstituted reference compound **17x** (Figure 9). From these calculations, it becomes apparent that serine residues S201 and S213 in the nicotinamide binding pocket of NNMT play a crucial role in the potency of compound **17u**. The model predicts hydrogen bonding interactions with the *para*-cyano substituent of compound **17u** involving the sidechains of both S201 and S213. These interactions result in an estimated improvement of the binding affinity due to the *p*-CN substitution of more than 4 kcal/mol, relative to the unsubstituted analogue **17x** in agreement with the experimental data. For the *meta*-cyano compound **17t**, these interactions seem to be much weaker (less frequent), resulting in only a moderate improvement in the predicted affinity shift arising from the introduction of the *meta*-cyano substitution again in line with the biochemical experiments. Conversely, the *ortho*-cyano compound **17s** cannot reach the serine residues and instead seems to introduce a counterproductive steric hindrance in the binding site, as reflected by the weaker binding affinity predicted relative to the unsubstituted compound **17x**.



Figure 9. Results of modeling of compounds **17s-u** bearing the *ortho-*, *meta-* and *para-*cyano substituent. The results indicate strong hydrogen bonding of para-cyano compound **17u** with serine residues S201 and S213, which are not present in the models of compounds **17s** and **17t**. The modelled predictions are supported by the similarity in the difference in Gibbs free energy ($\Delta\Delta G$) compared to unsubstituted compound **17x** from the biochemical assay and the MD simulations as displayed in Table 2.

Inhibitor Selectivity Studies and Cell-based Assays

To evaluate the NNMT selectivity of the most potent bisubstrate inhibitor, compound 17u was tested for its activity against 12 representative SAM-dependent methyltransferases. For this

experiment, we selected protein methyltransferases G9a, SETDB1, SETD2, MLL1, SMYD2, PRMT1, CARM1, PRMT5, PRMT7, DNMT1 and DOT1L and small molecule methyltransferase phenylethanolamine *N*-methyltransferase (PNMT). Notably, PNMT has high structural similarity to NNMT sharing 39% sequence identity.³⁰ Compound **17u** showed good selectivity against all methyltransferases tested. Against PNMT, less than 50% inhibition was observed for compound **17u** at 10 μ M. Against PRMT5 and DOT1L, **17u** exhibited more than 50% inhibition at 10 μ M, but this activity was abolished at 1 μ M. The highest percentage inhibition was observed against lysine methyltransferase SMYD-2, with 19% and 39% activity remaining at the concentrations of 10 μ M and 1 μ M, respectively. Based on this data, compound **17u** inhibits NNMT with excellent selectivity over other methyltransferases.

To investigate whether the potent activity observed in the biochemical inhibition assays translates to cellular activity, compound 17u was also tested against human cancer cell lines. In addition to the human oral cancer cell line HSC-2 previously used for assessing the cell-based activity of naphthalene compound 3,²⁰ we here also tested compound 17u against a human lung cancer cell line (A549) and bladder cancer cell line (T24). The results of these studies reveal a clear inhibition of cell viability for the different cancer cell lines upon treatment with compound 17u at a concentration of 100μ M. However, this effect was absent at the lower concentrations tested. As the difference between the biochemical inhibition and the cellular activity spans several orders of magnitude, we investigated the cell permeability of compound 17u by means of a Parallel Artificial Membrane Permeability Assay (PAMPA). The data revealed very poor cell permeability for 17u, which is likely to be the explanation for the discrepancy between the nanomolar potency in the biochemical assay.

3. Conclusion

To date, the majority of bisubstrate NNMT inhibitors have logically employed benzamide groups to mimic the nicotinamide moiety. In addition, recent reports have highlighted the benefit of utilizing alkyne-based linkers to connect the benzamide group to the SAM mimicking moiety. We here report notable departures from both of these strategies to generate novel and potent NNMT inhibitors that: a) include non-benzamide aromatic mimics of the nicotinamide group and b) employ a 3-carbon trans-alkene linker to connect these aromatic groups to the SAM unit. This approach was enabled by a convenient and robust synthetic route utilizing a double reductive amination procedure that allowed for the preparation of a number of novel bisubstrate inhibitors. Biochemical evaluation of the inhibitors thus prepared revealed a striking effect for EWG groups present on the aromatic ring, predominantly when introduced at the position *para* to the linker.

Among these compounds, the *para*-cyano substituted styrene-based inhibitor **17u** was identified as the most potent NNMT inhibitor with an IC₅₀ value of 3.7 nM. This compound was subsequently used to further investigate the possibilities of altering and/or replacing the amino acid and adenosine moieties. These studies showed that subtle changes in the amino acid side chain resulted in dramatic decreases in activity. While the removal of the carboxylic acid moiety still yielded a low µM inhibitor, elimination of the primary amine led to inactive compounds. Similarly, the novel para-cyano side-chain could not compensate the loss of binding interactions when the adenosine moiety was eliminated. The results from the ITC experiments confirmed compound 17u to be a tight binder of NNMT with a dissociation constant of 21 nM and a 1:1 stoichiometry. In addition, modelling studies predict the presence of hydrogen bonding interactions of the *para*-cyano group with two active site serine residues in the substrate pocket of NNMT, providing a plausible explanation for the potency of compound 17u. The low nanomolar potency exhibited in biochemical assays was not maintained in cell-based assays and a significant decrease in cell viability was observed only when compound 17u was tested at 100 μ M against oral, lung, and bladder cancer cell lines. This discrepancy is likely to be explained by the poor cell permeability of compound 17u found in the PAMPA assay. Taken together, our findings provide valuable new insights towards the design and further optimisation of potent NNMT inhibitors.

EXPERIMENTAL PROCEDURES

General Procedures. All reagents employed were of American Chemical Society grade or finer and were used without further purification unless otherwise stated. For compound characterization, ¹H NMR spectra were recorded at 400, 500 or 600 MHz with chemical shifts reported in parts per million downfield relative to H₂O (δ 4.79), CH₃OH (δ 3.31), CHCl₃ (δ 7.26), or DMSO (δ 2.50). ¹H NMR data are reported in the following order: multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; and m, multiplet), coupling constant (*J*) in hertz (Hz), and the number of protons. Where appropriate, the multiplicity is preceded by br, indicating that the signal was broad. ¹³C NMR spectra were recorded at 101, 126 or 151 MHz with chemical shifts reported relative to CDCl₃ (δ 77.16), methanol (δ 49.00), or DMSO (δ 39.52). The ¹³C NMR spectra of the compounds recorded in D₂O could not be referenced. Compounds 5,²⁷ 9,³⁴ 10,²⁰ 19a,²⁰ 19b,³⁵ 19c-d,²⁰ 19e,³⁶ 19f-g,²⁰ 22a,³⁷ 30²⁷ and 32³⁸ were prepared as previously described and had NMR spectra and mass spectra consistent with the assigned structures. Purity was confirmed to be \geq 95% by LCMS performed on a Shimadzu LC-20AD system with a Shimadzu Shim-Pack GISS-HP C18 column (3.0 x 150 mm, 3 µm) at 30 °C and equipped with a UV detector monitoring at 214 and 254 nm. The following solvent system, at a flow rate of 0.5 mL/min, was used: solvent A, 0.1 % formic acid in water; solvent B, acetonitrile. Gradient elution was as follows: 95:5 (A/B) for 2 min, 95:5 to 0:100 (A/B) over 13 min, 0:100 (A/B) for 2 min, then reversion back to 95:5 (A/B) over 1 min, 95:5 (A/B) for 2 min. This system was connected to a Shimadzu 8040 triple quadrupole mass spectrometer (ESI ionisation).

The final compounds were purified via preparative HPLC performed on a BESTA-Technik system with a Dr. Maisch Reprosil Gold 120 C18 column (25×250 mm, 10μ m) and equipped with a ECOM Flash UV detector monitoring at 214 nm. The following solvent system, at a flow rate of 12 mL/min, was used: solvent A: 0.1 % TFA in water/acetonitrile 95/5; solvent B: 0.1 % TFA in water/acetonitrile 5/95. Gradient elution was as follows: 95:5 (A/B) for 5 min, 95:5 to 0:100 (A/B) over 40 min, 0:100 (A/B) for 5 min, then reversion back to 95:5 (A/B) over 2 min, 95:5 (A/B) for 8 min.

HRMS analyses were performed on a Shimadzu Nexera X2 UHPLC system with a Waters Acquity HSS C18 column (2.1×100 mm, 1.8μ m) at 30 °C and equipped with a diode array detector. The following solvent system, at a flow rate of 0.5 mL/min, was used: solvent A, 0.1 % formic acid in water; solvent B, 0.1 % formic acid in acetonitrile. Gradient elution was as follows: 95:5 (A/B) for 1 min, 95:5 to 15:85 (A/B) over 6 min, 15:85 to 0:100 (A/B) over 1 min, 0:100 (A/B) for 3 min, then reversion back to 95:5 (A/B) for 3 min. This system was connected to a Shimadzu 9030 QTOF mass spectrometer (ESI ionisation) calibrated internally with Agilent's API-TOF reference mass solution kit (5.0 mM purine, 100.0 mM ammonium trifluoroacetate and 2.5 mM hexakis(1H,1H,3H-tetrafluoropropoxy)phosphazine) diluted to achieve a mass count of 10000.

tert-butyl (*S*)-4-(((((3a*R*,4*R*,6*R*,6a*R*)-6-(6-amino-9*H*-purin-9-yl)-2,2dimethyltetrahydrofuro[3,4-d][1,3]dioxol-4-yl)methyl)amino)-2-((*tert*-

butoxycarbonyl)amino)butanoate (11). 9-((3aR, 4R, 6R, 6aR)-6-(aminomethyl)-2,2dimethyltetrahydrofuro[3,4-d][1,3]dioxol-4-yl)-9*H*-purin-6-amine **9** (7.3 g, 24 mmol), *tert*-butyl (*S*)-2-((*tert*-butoxycarbonyl)amino)-4-oxobutanoate **10** (5.5 g, 20 mmol), NaBH(OAc)₃ (6.4 g, 30 mmol) and AcOH (1 mL) were added to 1,2-dichloroethane (DCE, 100 mL) in a 250 mL roundbottom flask (RBF) and the mixture was stirred at room temperature under N₂ atmosphere overnight. The reaction was quenched by adding 1 N NaOH (20 mL), and the product was extracted with CH₂Cl₂. The combined organic layers were washed with brine and dried over Na₂SO₄. The solvent was evaporated, and the crude product was purified by column chromatography (10% MeOH in EtOAc) to give compound **11** as a white powder (6.4 g, 57% yield). ¹H NMR (400 MHz, CDCl₃) δ 8.31 (s, 1H), 7.90 (s, 1H), 6.04 – 5.76 (m, 4H), 5.49 (s, 1H), 5.29 (s, 1H), 5.09 – 5.05 (m, 1H), 4.36 (s, 1H), 4.28 (s, 1H), 2.95 (d, J = 9.5 Hz, 1H), 2.85 – 2.70 (m, 2H), 2.63 (s, 1H), 1.93 (br s, 1H), 1.81 (br, 1H), 1.60 (s, 3H), 1.41 (br d, J = 26.4 Hz, 21H). ¹³C NMR (101 MHz, CDCl₃) δ 170.8, 156.0, 155.1, 153.0, 149.2, 140.4, 120.2, 113.3, 90.9, 84.9, 83.0, 82.1, 81.5, 79.2, 77.9, 77.3, 77.1, 76.8, 52.9, 50.3, 46.2, 32.1, 28.2, 27.8, 27.2, 25.4. HRMS (ESI): calculated for C₂₆H₄₂N₇O₇ [M+H]⁺ 564.3146, found 564.3150.

tert-butyl (*S*)-4-((((3a*R*,4*R*,6*R*,6a*R*)-6-(6-amino-9*H*-purin-9-yl)-2,2dimethyltetrahydrofuro[3,4-d][1,3]dioxol-4-yl)methyl)(quinolin-6-ylmethyl)amino)-2-((*tert*-

butoxycarbonyl)amino)butanoate (12a). Compound 11 (112 mg, 0.20 mmol), 1-quinoline-6carbaldehyde 8a (38 mg, 0.24 mmol), NaBH(OAc)₃ (11 mg, 0.30 mmol) and AcOH (one drop) were added to 1,2- dichloroethane (DCE, 10 mL) in a 50 mL round-bottom flask (RBF) and the mixture was stirred at room temperature under N₂ atmosphere overnight. The reaction was quenched by adding 1 N NaOH (10 mL), and the product was extracted with CH₂Cl₂. The combined organic layers were washed with brine and dried over Na₂SO₄. The solvent was evaporated, and the crude product was purified by column chromatography (5% MeOH in EtOAc) to give compound **12a** as a white powder (66 mg, 47% yield). ¹H NMR (400 MHz, CDCl₃) δ 8.81 (d, J = 3.9 Hz, 1H), 8.02 (s, 1H), 7.95 (t, J = 9.2 Hz, 2H), 7.78 (s, 1H), 7.62 (d, J = 8.5 Hz, 1H), 7.55 (s, 1H), 7.29 (dd, *J* = 8.1, 4.2 Hz, 1H), 6.50 (s, 2H), 5.97 (s, 1H), 5.67 (d, *J* = 7.8 Hz, 1H), 5.28 (d, J = 5.4 Hz, 1H), 4.85 – 4.80 (m, 1H), 4.30 (d, J = 6.0 Hz, 1H), 4.20 – 4.12 (m, 1H), 3.78 (d, J = 8.1, 1H), 3.59 (br d, J = 12.0 Hz, 2H), 2.81 - 2.75 (m, 1H), 2.68 - 2.59 (m, 2H), 2.54 - 2.542.48 (m, 1H), 1.96 (br, 1H), 1.77 (br, 1H), 1.51 (s, 3H), 1.33 – 1.27 (br m, 21H). ¹³C NMR (101 MHz, CDCl₃) δ 171.7, 155.8, 155.4, 152.8, 150.0, 148.9, 139.7, 137.2, 135.7, 130.6, 129.2, 121.1, 120.1, 114.3, 90.6, 85.3, 83.3, 81.6, 58.9, 55.8, 52.8, 50.8, 29.4, 28.3, 27.8, 27.0, 25.3. HRMS (ESI): calculated for C₃₆H₄₉N₈O₇ [M+H]⁺ 705.3724, found 705.3728.

tert-butyl (*S*)-4-(((((3aR,4R,6R,6aR)-6-(6-amino-9H-purin-9-yl)-2,2dimethyltetrahydrofuro[3,4-d][1,3]dioxol-4-yl)methyl)(quinolin-2-ylmethyl)amino)-2-((*tert*butoxycarbonyl)amino)butanoate (12b). Following the procedure described for compound 12a, coupling compound 11 (112 mg, 0.20 mmol) with quinoline-2-carbaldehyde **8b** (38 mg, 0.24 mmol) afforded compound 12b, which was used in the next step without further purification.

tert-butyl (*S*)-4-(((((3*aR*,4*R*,6*R*,6*aR*)-6-(6-amino-9*H*-purin-9-yl)-2,2dimethyltetrahydrofuro[3,4-d][1,3]dioxol-4-yl)methyl)(isoquinolin-6-ylmethyl)amino)-2-((*tert*butoxycarbonyl)amino) butanoate (12c). Following the procedure described for compound 12a, coupling compound 11 (112 mg, 0.20 mmol) with isoquinoline-6-carbaldehyde 8c (38 mg, 0.24 mmol) afforded compound 12c as a white powder (77 mg, 55% yield). ¹H NMR (400 MHz, CDCl₃) δ 8.07 (s, 1H), 7.98 (dd, *J* = 8.3, 2.0 Hz, 2H), 7.86 (s, 1H), 7.73 (d, *J* = 8.1 Hz, 1H), 7.66 – 7.62 (m, 1H), 7.55 (d, *J* = 8.5 Hz, 1H), 7.49 – 7.45 (m, 1H), 6.04 (br, 3H), 5.57 (d, *J* = 7.7 Hz, 1H), 5.34 (d, *J* = 5.6 Hz, 1H), 4.94 – 4.89 (m, 1H), 4.43 – 4.36 (m, 1H), 4.20 – 4.16 (br, 1H), 3.96 (br, 1H), 3.86 (s, 1H), 2.92 – 2.84 (m, 1H), 2.81 – 2.66 (m, 2H), 2.61 (br, 1H), 2.06 – 1.92 (m, 1H), 1.77 (br, 1H), 1.56 (s, 3H), 1.41 – 1.31 (br m, 21H). ¹³C NMR (101 MHz, CDCl₃) δ 171.8, 159.9, 155.6, 152.9, 149.1, 147.4, 139.9, 136.2, 129.4, 129.0, 127.5, 127.3, 126.2, 124.8, 121.1, 120.2, 114.3, 90.7, 85.5, 83.9, 83.4, 81.6, 79.4, 77.3, 61.6, 56.4, 52.8, 51.2, 30.3, 28.4, 27.9, 27.2, 25.5. HRMS (ESI): calculated for C₃₆H₄₉N₈O₇ [M+H]⁺ 705.3724, found 705.3733.

tert-butyl (2*S*)-4-(([1,1'-biphenyl]-4-ylmethyl)(((3a*R*,4*R*,6*R*,6a*R*)-6-(6-amino-9*H*-purin-9-yl)-2,2-dimethyltetrahydrofuro[3,4-d][1,3]dioxol-4-yl)methyl)amino)-2-((*tert*-

butoxycarbonyl)amino) butanoate (12d). Following the procedure described for compound **12a**, coupling compound **11** (112 mg, 0.20 mmol) with [1,1'-biphenyl]-4-carbaldehyde **8d** (44 mg, 0.24 mmol) afforded compound **12d** as a white powder (103 mg, 71% yield). ¹H NMR (400 MHz, CDCl₃) δ 8.20 (s, 1H), 7.85 (s, 1H), 7.55 (d, *J* = 7.6 Hz, 2H), 7.46 (d, *J* = 7.9 Hz, 2H), 7.40 (t, *J* = 7.6 Hz, 2H), 7.30 (d, *J* = 7.9 Hz, 3H), 6.36 (s, 2H), 6.03 (s, 1H), 5.75 (d, *J* = 7.7 Hz, 1H), 5.37 (d, *J* = 5.4 Hz, 1H), 4.92 – 4.87 (m, 1H), 4.41 – 4.34 (m, 1H), 4.24 – 4.16 (m, 1H), 3.72 (br d, *J* = 12.0 1H), 3.49 (br d, *J* = 12.0 1H), 2.81 (br d, *J* = 19.7 Hz, 1H), 2.71 – 2.60 (m, 2H), 2.52 (d, *J* = 7.0 Hz, 1H), 2.06 – 1.93 (m, 1H), 1.86 – 1.74 (m, 1H), 1.59 (s, 3H), 1.41 – 1.36 (br m, 21H). ¹³C NMR (101 MHz, CDCl₃) δ 171.8, 155.5, 153.1, 149.3, 140.9, 137.6, 129.4, 128.8, 127.2, 127.0, 120.4, 58.7, 55.8, 53.0, 50.7, 30.4, 29.8, 29.4, 28.4, 28.0, 27.2, 25.5. HRMS (ESI): calculated for C₃₉H₅₂NrO7 [M+H]⁺ 730.3928, found 730.3956.

tert-butyl (2S)-4-(([1,1'-biphenyl]-2-ylmethyl)((((3*aR*,4*R*,6*R*,6*aR*)-6-(6-amino-9*H*-purin-9yl)-2,2-dimethyltetrahydrofuro[3,4-d][1,3]dioxol-4-yl)methyl)amino)-2-((*tert*butoxycarbonyl)amino) butanoate (12e). Following the procedure described for compound 12a, coupling compound 11 (112 mg, 0.20 mmol) with [1,1'-biphenyl]-2-carbaldehyde 8e (44 mg, 0.24 mmol) afforded compound 12e as a white powder (99 mg, 69% yield). ¹H NMR (400 MHz, CDCl₃) δ 8.22 (s, 1H), 7.79 (s, 1H), 7.57 – 7.51 (m, 1H), 7.39 – 7.11 (m, 8H), 5.97 (br d, *J* = 12.0 Hz, 3H), 5.34 (br, 2H), 4.75 (dd, *J* = 6.4, 3.3 Hz, 1H), 4.22 – 4.17 (m, 1H), 4.07–3.98 (m, 1H), 3.61 (br d, *J* = 12.0, 1H), 3.44 (br d, *J* = 16.0 1H), 2.64 – 2.59 (m, 1H), 2.50 – 2.44 (m, 2H), 2.37 – 2.30 (m, 2H), 1.83 – 1.72 (m, 1H), 1.57 (s, 3H), 1.42 – 1.36 (br m, 21H). ¹³C NMR (101 MHz, CDCl₃) δ 155.4, 153.1, 141.3, 136.1, 130.0, 129.7, 129.4, 128.1, 127.3, 127.0, 126.8, 114.3, 90.8, 85.4, 83.8, 83.3, 56.2, 55.9, 52.8, 50.8, 29.3, 28.4, 28.0, 27.2, 25.5. HRMS (ESI): calculated for C₃₉H₅₁NrOrNa [M+ Na]+ 752.3748, found 730.3759. *tert*-butyl (2S)-4-(([1,1'-biphenyl]-3-ylmethyl)(((3a*R*,4*R*,6*R*,6a*R*)-6-(6-amino-9*H*-purin-9-yl)-2,2-dimethyltetrahydrofuro[3,4-d][1,3]dioxol-4-yl)methyl)amino)-2-((*tert*-

butoxycarbonyl)amino) butanoate (12f). Following the procedure described for compound **12a**, coupling compound **11** (112 mg, 0.20 mmol) with [1,1'-biphenyl]-3-carbaldehyde **8f** (44 mg, 0.24 mmol) afforded compound **12f** as a white powder (108 mg, 74% yield). ¹H NMR (400 MHz, CDCl₃) δ 8.17 (s, 1H), 7.81 (s, 1H), 7.59–7.51 (m, 3H), 7.44 (d, *J* = 7.6 Hz, 1H), 7.38 (t, *J* = 7.5 Hz, 2H), 7.33 – 7.27 (m, 2H), 7.22 (d, *J* = 7.4 Hz, 1H), 6.51 (s, 2H), 6.02 (s, 1H), 5.68 (d, *J* = 6.6 Hz, 1H), 5.35 (d, *J* = 5.3 Hz, 1H), 4.93 – 4.89 (m, 1H), 4.39 – 4.32 (m, 1H), 4.22 – 4.15 (m, 1H), 3.75 (br, 1H), 3.52 (br, 1H), 2.84 – 2.79 (m, 1H), 2.71–2.60 (m, 2H), 2.59 – 2.49 (m, 1H), 2.06 – 1.94 (m, 1H), 1.83 (br s, 1H), 1.57 (s, 3H), 1.39 – 1.32 (br m, 21H). ¹³C NMR (101 MHz, CDCl₃) δ 171.7, 155.5, 153.1, 141.2, 141.1, 128.8, 127.9, 127.8, 127.3, 127.2, 126.0, 114.4, 90.8, 85.4, 83.9, 83.5, 59.1, 55.7, 52.9, 50.8, 29.5, 28.4, 28.0, 27.2, 25.4. HRMS (ESI): calculated for C₃₉H₅₂N₇O₇ [M+H]⁺730.3928, found 730.3938.

tert-butyl (2*S*)-4-((((3*aR*,4*R*,6*R*,6*aR*)-6-(6-amino-9*H*-purin-9-yl)-2,2dimethyltetrahydrofuro[3,4-d][1,3]dioxol-4-yl)methyl)(naphthalen-1-ylmethyl)amino)-2-((tertbutoxycarbonyl)amino) butanoate (12g). Following the procedure described for compound 12a, coupling compound 11 (112 mg, 0.20 mmol) with 1-naphthaldehyde 8g (37 mg, 0.24 mmol), afforded compound 12g as a white powder (94 mg, 67% yield). ¹H NMR (600 MHz, CDCl₃) δ 8.14 (d, *J* = 7.7 Hz, 1H), 8.10 (s, 1H), 7.73 (d, *J* = 7.7 Hz, 1H), 7.69 – 7.60 (m, 2H), 7.40 – 7.34 (m, 2H), 7.27–7.19 (m, 2H), 6.24 (br s, 2H), 5.88 (s, 1H), 5.32 (d, *J* = 7.8 Hz, 1H), 5.06 (d, *J* = 5.1 Hz, 1H), 4.54 (s, 1H), 4.30 (s, 1H), 4.10 – 4.05 (m, 2H), 3.78 – 3.73 (m, 1H), 2.72 – 2.64 (m, 2H), 2.60 – 2.56 (m, 1H), 2.53 – 2.47 (m, 1H), 2.02 – 1.93 (m, 1H), 1.86 – 1.73 (m, 1H), 1.46 (s, 3H), 1.33 – 1.29 (br m, 18H), 1.13 (s, 3H). ¹³C NMR (151 MHz, CDCl₃) δ 171.7, 155.7, 155.4, 153.0, 149.1, 139.6, 134.2, 133.76, 132.2, 128.5, 128.1, 127.7, 125.8, 125.6, 125.0, 124.57, 120.18, 91.0, 85.10, 83.5, 83.3, 81.7, 57.6, 55.4, 53.5, 52.8, 51.0, 29.1, 28.4, 27.9, 27.0, 25.1. HRMS (ESI): calculated for C₃₇H₅₀N₇O₇ [M+H]+704.3772, found 704.3775.

tert-butyl (2*S*)-4-(((((3*aR*,4*R*,6*R*,6*aR*)-6-(6-amino-9*H*-purin-9-yl)-2,2dimethyltetrahydrofuro[3,4-d][1,3]dioxol-4-yl)methyl)(2-(naphthalen-2-yl)ethyl)amino)-2-((*tert*-butoxycarbonyl)amino) butanoate (12h). Following the procedure described for compound 12a, coupling compound 11 (112 mg, 0.20 mmol) with 2-(naphthalen-2-yl)acetaldehyde 8h (38 mg, 0.24 mmol) afforded compound 12h as a white powder (99 mg, 69% yield). ¹H NMR (400 MHz, CDCl₃) δ 8.36 (s, 1H), 7.90 (s, 1H), 7.79 – 7.69 (m, 3H), 7.52 (s, 1H), 7.45 – 7.36 (m, 2H), 7.21 (dd, *J* = 8.4, 1.5 Hz, 1H), 6.15 (s, 2H), 6.03 (d, *J* = 1.7 Hz, 1H), 5.68 (d, *J* = 8.0 Hz, 1H), 5.48 – 5.46 (d, *J* = 8.0, 1H) 4.96 – 4.93 (m, 1H), 4.39 – 4.31 (m, 1H), 4.20 – 4.15 (m, 1H), 2.90 - 2.50 (m, 8H), 2.05 – 1.97 (m, 1H), 1.70 – 1.75 (m, 1H), 1.59 (s, 3H), 1.43 (d, *J* = 3.4 Hz, 18H), 1.33 (s, 3H). ¹³C NMR (101 MHz, CDCl₃) δ 172.4, 156.6, 153.1, 147.1, 140.2, 138.8, 134.4, 132.5, 128.0, 127.6, 127.4, 126.9, 126.0, 125.3, 120.4, 114.4, 90.2, 85.7, 83.8, 83.3, 81.7, 79.5, 52.9, 50.1, 28.4, 28.1, 27.2, 25.4. HRMS (ESI): calculated for C₃₈H₅₂N₇O₇ [M+H]+ 718.3928, found 718.3932.

tert-butyl (2*S*)-4-(((1*H*-indol-2-yl)methyl)(((3a*R*,4*R*,6*R*,6a*R*)-6-(6-amino-9*H*-purin-9-yl)-2,2-dimethyltetrahydrofuro[3,4-d][1,3]dioxol-4-yl)methyl)amino)-2-((*tert*-

butoxycarbonyl)amino) butanoate (12i). Following the procedure described for compound **12a**, coupling compound **11** (112 mg, 0.20 mmol) with 1*H*-indole-2-carbaldehyde **8i** (35 mg, 0.18 mmol) afforded compound **12i** as a white powder (77 mg, 56% yield). ¹H NMR (600 MHz, CDCl₃) δ 9.41 (s, 1H), 8.20 (s, 1H), 7.81 (s, 1H), 7.50 (d, *J* = 7.8 Hz, 1H), 7.28 – 7.23 (m, 1H), 7.10 (t, *J* = 7.5 Hz, 1H), 7.03 (t, *J* = 7.4 Hz, 1H), 6.25 (s, 1H), 6.00 (s, 3H), 5.46 (d, *J* = 8.4 Hz, 1H), 5.30 (d, *J* = 5.3 Hz, 1H), 4.90 (d, *J* = 4.9 Hz, 1H), 4.44 – 4.37 (m, 1H), 4.2 (m 1H), 3.76 (dd, *J* = 8.0, 2H), 2.87 – 2.84 (m, 1H), 2.78 – 2.75 (m, 6.8 Hz, 1H), 2.72 – 2.60 (m, 2H), 2.02 – 1.94 (m, 1H), 1.79 – 1.75 (m, 1H), 1.54 (s, 3H), 1.47 – 1.32 (br m, 21H). ¹³C NMR (151 MHz, CDCl₃) δ 172.1, 155.6, 153.0, 149.1, 139.8, 136.4, 128.2, 121.3, 120.2, 120.0, 119.2, 114.6, 110.8, 101.0, 90.2, 84.8, 83.9, 83.4, 82.0, 79.8, 55.9, 52.4, 52.1, 51.2, 30.5, 28.4, 27.9, 27.1, 25.5. HRMS (ESI): calculated for C₃₅H₄₉N₈O7 [M+H]⁺ 693.3724, found 693.3732.

tert-butyl 3-(((((3a*R*,4*R*,6*R*,6a*R*)-6-(6-amino-9*H*-purin-9-yl)-2,2dimethyltetrahydrofuro[3,4-d][1,3]dioxol-4-yl)methyl)((*S*)-4-(*tert*-butoxy)-3-((*tert*-

butoxycarbonyl)amino)-4-oxobutyl)amino) methyl)-1*H*-indole-1-carboxylate (12j). Following the procedure described for compound **12a**, coupling compound **11** (112 mg, 0.20 mmol) with *tert*-butyl 3-formyl-1*H*-indole-1-carboxylate **8j** (58 mg, 0.24 mmol) afforded compound **12j** as a white powder (79 mg, 50% yield). ¹H NMR (600 MHz, CDCl₃) δ 8.24 (s, 1H), 8.09 (s, 1H), 7.82 (s, 1H), 7.68 (d, *J* = 7.8 Hz, 1H), 7.44 (s, 1H), 7.28 (d, *J* = 7.4 Hz, 1H), 7.18 (t, *J* = 7.5 Hz, 1H), 5.97 (br d, *J* = 39.0 Hz, 3H), 5.37 – 5.32 (m, 2H), 4.81 (dd, *J* = 6.4, 3.2 Hz, 1H), 4.40 – 4.37 (m, 1H), 4.19 – 4.10 (m, 1H), 3.82 (br d, *J* = 13.7 Hz, 1H), 3.61 – 3.57 (br d, *J* = 13.8 Hz, 1H), 2.85 – 2.82 (br m, 1H), 2.71 – 2.58 (m, 2H), 2.52 – 2.48 (m, 1H), 2.02 – 1.99 (br m, 1H), 1.89 – 1.79 (m, 1H), 1.66 (s, 9H), 1.57 (s, 3H), 1.38 (br d, *J* = 27.7 Hz, 18H), 1.29 (s, 3H).¹³C NMR (151 MHz, CDCl₃) δ 170.7, 154.8, 154.4, 152.0, 148.6, 148.1, 138.7, 134.6, 129.3, 123.7, 123.4, 121.5, 119.2, 119.1, 114.1, 113.3, 89.7, 84.2, 82.6, 82.3, 80.6, 54.6, 52.4, 51.7, 49.7, 49.0, 28.5, 27.3, 27.2, 26.9, 26.1, 24.2. HRMS (ESI): calculated for C₄₀H₅₇N₈O₉ [M+H]⁺ 793.4249, found 793.4256.

tert-butyl (2*S*)-4-((((3*aR*,4*R*,6*R*,6*aR*)-6-(6-amino-9*H*-purin-9-yl)-2,2dimethyltetrahydrofuro[3,4-d][1,3]dioxol-4-yl)methyl)(benzo[*b*]thiophen-2-ylmethyl)amino)-2-((*tert*-butoxycarbonyl)amino) butanoate (12*k*). Following the procedure described for compound 12*a*, coupling compound 11 (112 mg, 0.20 mmol) with benzo[*b*]thiophene-2-carbaldehyde 8*k* (39 mg, 0.24 mmol) afforded compound 12*k* as a white powder (89 mg, 63% yield). ¹H NMR (400 MHz, CDCl₃) δ 8.10 (s, 1H), 7.86 (s, 1H), 7.72 (d, *J* = 7.7 Hz, 1H), 7.61 (d, *J* = 7.4 Hz, 1H), 7.28 (d, *J* = 7.1 Hz, 1H), 7.22 (d, *J* = 7.2 Hz, 1H), 6.99 (s, 1H), 6.27 (s, 2H), 6.04 (s, 1H), 5.61 (d, *J* = 7.8 Hz, 1H), 5.40 (d, *J* = 5.4 Hz, 1H), 5.00 (br s, 1H), 4.42 – 4.36 (m, 1H), 4.23 – 4.15 (m, 1H), 3.95 – 3.91 (br d, *J* = 16.0 Hz, 1H), 3.85 – 3.81 (br d, *J* = 16.0 Hz, 1H), 2.89 – 2.84 (m, 1H), 2.76 – 2.64 (m, 2H), 2.60 – 2.52 (m, 1H), 2.02 – 1.99 (br d, *J* = 12.0 Hz, 1H), 1.83 – 1.81 (d, *J* = 8.0 Hz, 1H), 1.59 (s, 3H), 1.40 – 1.36 (br m, 21H). ¹³C NMR (101 MHz, CDCl₃) δ 171.7, 155.8, 155.4, 153.0, 149.1, 143.1, 139.9, 139.5, 124.1, 123.9, 123.1, 122.2, 120.2, 114.4, 90.6, 85.5, 83.8, 83.2, 81.7, 79.4, 55.3, 54.0, 52.7, 50.3, 29.6, 28.3, 27.9, 27.1, 25.4. HRMS (ESI): calculated for C₃₅H₄₈N₇O₇S [M+H]⁺ 710.3336, found 710.3348.

tert-butyl (2*S*)-4-((((3*aR*,4*R*,6*R*,6*aR*)-6-(6-amino-9*H*-purin-9-yl)-2,2dimethyltetrahydrofuro[3,4-d][1,3]dioxol-4-yl)methyl)(benzo[*b*]thiophen-3-ylmethyl)amino)-2-((*tert*-butoxycarbonyl)amino) butanoate (12l). Following the procedure described for compound 12a, coupling compound 11 (112 mg, 0.20 mmol) with benzo[*b*]thiophene-3-carbaldehyde 8l (39 mg, 0.24 mmol) afforded compound 12l as a white powder (79 mg, 50% yield). ¹H NMR (400 MHz, CDCl₃) δ 8.63 (s, 1H), 8.30 – 8.23 (br d, *J* = 28.0 Hz, 3H), 7.73 – 7.67 (br d, *J* = 24.0 Hz, 3H), 6.94 (s, 2H), 6.44 (s, 1H), 6.06 (s, 1H), 5.72 (s, 1H), 5.20 (s, 1H), 4.81 (s, 1H), 4.63 (s, 1H), 4.35 – 4.32 (br d, *J* = 8.0 Hz, 1H), 4.16 – 4.13 (br d, *J* = 12.0 Hz, 1H), 3.34 – 2.89 (m, 4H), 2.46 (s, 1H), 2.27 (s, 1H), 2.00 (s, 3H), 1.85 – 1.81 (br d, *J* = 16.0 Hz, 18H), 1.71 (s, 3H). ¹³C NMR (101 MHz, CDCl₃) δ 171.7, 155.9, 155.4, 153.0, 149.0, 140.5, 139.6, 138.6, 133.4, 124.6, 124.3, 123.9, 122.6, 122.5, 120.2, 114.2, 90.8, 85.2, 83.6, 83.3, 81.7, 79.4, 77.4, 77.3, 77.1, 76.8, 55.7, 52.9, 52.8, 50.9, 29.3, 28.3, 27.9, 27.0, 25.2. HRMS (ESI): calculated for C₃₅H₄₈N₇O₇S [M+H]+ 710.3336, found 710.3355.

(*S*)-2-amino-4-((((2*R*,3*S*,4*R*,5*R*)-5-(6-amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl)(quinolin-6-ylmethyl)amino)butanoic acid (13a). To a solution of compound 12a (50 mg, 0.071 mmol) in 1 mL of CH₂Cl₂ was added a mixture of 9 mL TFA and 1 mL H₂O, and the solution was stirred for 2 h at room temperature. The mixture was concentrated, and the crude product was purified by preparative HPLC affording compound 13a as a white powder (33 mg, 74% yield). ¹H NMR (400 MHz, D₂O) δ 8.34 (d, *J* = 1.2 Hz, 1H), 8.10 (s, 1H), 7.79 (s, 1H), 7.39 (s, 2H), 7.28 (d, *J* = 8.2 Hz, 1H), 7.06 (t, *J* = 7.6 Hz, 1H), 6.92 (s, 1H), 6.05 (d, *J* = 5.0 Hz, 1H), 4.79 (t, J = 5.0 Hz, 1H), 4.56 - 4.49 (m, 2H), 4.38 (d, J = 9.9 Hz, 1H), 3.76 - 3.69 (m, 1H), 3.60 - 3.50 (m, 4H), 3.25 (t, J = 7.1 Hz, 1H), 2.43 - 2.34 (m, 1H), 2.24 (br s, 1H), 2.14 - 2.08 (m, 1H).¹³C NMR (101 MHz, D₂O) δ 169.9, 146.8, 143.6, 126.8, 122.8, 122.7, 120.3, 118.6, 109.0, 108.8, 73.5, 71.7, 52.2, 24.8. HRMS (ESI): calculated for C₂₄H₂₉N₈O₅ [M+H]⁺ 509.2261, found 509.2266.

(*S*)-2-amino-4-((((2*R*,3*S*,4*R*,5*R*)-5-(6-amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl)(quinolin-2-ylmethyl)amino)butanoic acid (13b). Following the procedure described for compound 13a, compound 12b (50 mg, 0.071 mmol) was deprotected and purified, affording compound 13b as a white powder (8 mg, 17% yield over two steps). ¹H NMR (400 MHz, D₂O) δ 8.45 (d, *J* = 8.6 Hz, 1H), 8.14 (s, 1H), 7.88 – 7.81 (m, 1H), 7.62 – 7.56 (m, 3H), 7.53 (s, 1H), 7.40 (d, *J* = 9.7 Hz, 1H), 5.93 (d, *J* = 4.5 Hz, 1H), 4.58 – 4.48 (m, 3H), 4.46 – 4.41 (m, 1H), 4.29 (t, *J* = 5.1 Hz, 1H), 4.06 (dd, *J* = 7.8, 5.3 Hz, 1H), 3.48 – 3.28 (m, 4H), 2.37 – 2.18 (m, 2H). ¹³C NMR (101 MHz, D₂O) δ 145.8, 142.4, 133.2, 132.1, 131.6, 129.8, 127.2, 123.0, 120.4, 92.3, 81.3, 80.4, 76.5, 74.4, 71.2, 54.2, 53.1, 27.5. HRMS (ESI): calculated for C_{24H29}N₈O₅ [M+H]+ 509.2261, found 509.2265.

(*S*)-2-amino-4-((((2*R*,3*S*,4*R*,5*R*)-5-(6-amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl)(isoquinolin-6-ylmethyl)amino)butanoic acid (13c). Following the procedure described for compound 13a, compound 12c (50 mg, 0.071 mmol) was deprotected and purified, affording compound 13c as a white powder (21 mg, 47% yield). ¹H NMR (400 MHz, D₂O) δ 8.00 (s, 1H), 7.83 – 7.70 (m, 3H), 7.49 – 7.32 (m, 3H), 6.99 (s, 1H), 5.81 (s, 1H), 4.88 (br d, *J* = 13.7 Hz, 1H), 4.64 (br d, *J* = 14.1 Hz, 1H), 4.44 (dd, *J* = 7.2, 5.7 Hz, 1H), 4.32 (dd, *J* = 5.4, 2.0 Hz, 2H), 3.94 (dd, *J* = 9.1, 4.1 Hz, 1H), 3.71 (t, *J* = 7.0 Hz, 2H), 3.59 (br d, *J* = 12.9 Hz, 1H), 2.50 – 2.45 (m, 1H), 2.38 – 2.28 (m, 1H). ¹³C NMR (101 MHz, D₂O) δ 171.5, 163.0, 162.6, 153.9, 148.9, 146.8, 144.2, 143.3, 142.9, 139.6, 133.3, 129.1, 128.3, 121.9, 120.8, 118.6, 117.7, 114.8, 90.4, 80.4, 72.9, 71.6, 56.8, 56.5, 51.0, 50.6, 25.9. HRMS (ESI): calculated for C₂₄H₂₉N₈O₅ [M+H]+ 509.2261, found 509.2273.

(*S*)-4-(([1,1'-biphenyl]-4-ylmethyl)(((2*R*,3*S*,4*R*,5*R*)-5-(6-amino-9*H*-purin-9-yl)-3,4dihydroxy-tetrahydrofuran-2-yl)methyl)amino)-2-aminobutanoic acid (13d). Following the procedure described for compound 13a, compound 12d (50 mg, 0.068 mmol) was deprotected and purified, affording compound 13d as a white powder (30 mg, 68% yield). ¹H NMR (400 MHz, D₂O) δ 8.13 (br s, 1H), 7.94 (s, 1H), 7.40 – 7.29 (m, 5H), 7.19 (br s, 4H), 5.88 (s, 1H), 4.53 – 4.48 (m, 1H), 4.31 (s, 3H), 4.06 (dd, *J* = 8.3, 4.8 Hz, 1H), 3.69 – 3.49 (m, 4H), 2.49 – 2.37 (br d, *J* = 48.0 Hz, 2H). ¹³C NMR (101 MHz, D₂O) δ 171.1, 163.0, 162.6, 162.2, 143.6, 140.2, 137.8, 131.1,

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129.2, 128.4, 126.0, 118.4, 117.7, 114.8, 111.9, 90.5, 77.7, 73.9, 71.4, 51.0, 24.6. HRMS (ESI): calculated for C₂₇H₃₃N₇O₅ [M+H]⁺ 534.2465, found 534.2474.

(*S*)-4-(([1,1'-biphenyl]-2-ylmethyl)(((2*R*,3*S*,4*R*,5*R*)-5-(6-amino-9*H*-purin-9-yl)-3,4dihydroxy-tetrahydrofuran-2-yl)methyl)amino)-2-aminobutanoic acid (13e). Following the procedure described for compound 13a, compound 12e (50 mg, 0.068 mmol) was deprotected and purified, affording compound 13e as a white powder (35 mg, 79% yield). ¹H NMR (400 MHz, D₂O) δ 8.31 (s, 1H), 8.24 (s, 1H), 7.51 – 7.29 (m, 6H), 7.25 – 7.17 (m, 3H), 5.98 (d, *J* = 3.4 Hz, 1H), 4.63 – 4.53 (m, 2H), 4.48 (d, *J* = 13.8 Hz, 1H), 4.40 (s, 1H), 4.27 – 4.21 (m, 1H), 3.71 (s, 1H), 3.48 – 3.23 (m, 4H), 2.19 – 2.11 (m, 1H), 2.03 – 1.95 (m, 1H). ¹³C NMR (101 MHz, D₂O) δ 171.2, 149.9, 147.6, 144.2, 143.6, 138.9, 131.2, 130.9, 130.1, 129.32, 128.9, 128.3, 126.2, 119.3, 117.7, 114.8, 90.3, 77.9, 73.3, 71.7, 55.3, 51.1, 24.3. HRMS (ESI): calculated for C₂₇H₃₃N₇O₅ [M+H]+ 534.2465, found 534.2472.

(*S*)-4-(([1,1'-biphenyl]-3-ylmethyl)(((2*R*,3*S*,4*R*,5*R*)-5-(6-amino-9*H*-purin-9-yl)-3,4dihydroxy-tetrahydrofuran-2-yl)methyl)amino)-2-aminobutanoic acid (13f). Following the procedure described for compound 13a, compound 12f (50 mg, 0.068 mmol) was deprotected and purified, affording compound 13f as a white powder (34 mg, 77% yield). ¹H NMR (400 MHz, D₂O) δ 7.98 (s, 1H), 7.67 (s, 1H), 7.23 – 7.14 (m, 8H), 7.03 (d, *J* = 6.9 Hz, 2H), 5.86 (s, 1H), 4.38 – 4.32 (br m, 3H), 4.25 – 4.13 (m, 2H), 3.96 (dd, *J* = 8.6, 4.6 Hz, 1H), 3.61 – 3.39 (m, 4H), 2.48 – 2.42 (m, 1H), 2.39 – 2.23 (m, 1H). ¹³C NMR (101 MHz, D₂O) δ 171.9, 163.3, 162.9, 162.2, 149.0, 146.9, 143.6, 143.0, 139.6, 137.6, 129.5, 129.0, 128.1, 127.2, 125.5, 120.7, 118.5, 117.8, 90.4, 73.6, 71.5, 51.8, 24.7. HRMS (ESI): calculated for C₂₇H₃₃N₇O₅ [M+H]⁺ 534.2465, found 534.2468.

(*S*)-2-amino-4-((((2*R*,3*S*,4*R*,5*R*)-5-(6-amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl)(naphthalen-2-ylmethyl)amino)butanoic acid (13g). Following the procedure described for compound 13a, compound 12g (50 mg, 0.071 mmol) was deprotected and purified, affording compound 13g as a white powder (33 mg, 74% yield). ¹H NMR (400 MHz, D₂O) δ 7.94 (s, 1H), 7.55 (d, *J* = 8.3 Hz, 4H), 7.39 (d, *J* = 6.9 Hz, 1H), 7.24 (s, 3H), 5.79 (s, 1H), 4.56 (br d, *J* = 12.0, 1H), 4.42 – 4.37 (m, 1H), 4.36 – 4.21 (m, 2H), 3.97 (dd, *J* = 8.6, 4.4 Hz, 1H), 3.76 – 3.42 (m, 4H), 2.53 – 2.25 (m, 2H). ¹³C NMR (101 MHz, D₂O) δ 171.4, 163.0, 162.7, 149.3, 146.7, 143.4, 143.3, 132.8, 130.1, 128.4, 126.4, 122.2, 118.5, 117.7, 90.7, 73.5, 71.6, 51.4. HRMS (ESI): calculated for C₂₅H₃₀N₇O₅ [M+H]+ 508.2308, found 508.2314.

(*S*)-2-amino-4-((((2*R*,3*S*,4*R*,5*R*)-5-(6-amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl)(2-(naphthalen-2-yl)ethyl)amino)butanoic acid (13h). Following the procedure described for compound 13a, compound 12h (50 mg, 0.069 mmol) was deprotected and purified, affording compound **13h** as a white powder (33 mg, 76% yield). ¹H NMR (400 MHz, CD₃OD) δ 8.45 (s, 1H), 8.23 (s, 1H), 7.78 – 7.63 (m, 3H), 7.58 (s, 1H), 7.46 – 7.39 (m, 2H), 7.26 (d, *J* = 8.4 Hz, 1H), 6.13 (d, *J* = 4.6 Hz, 1H), 4.71 (d, *J* = 9.6 Hz, 1H), 4.62 – 4.55 (m, 1H), 4.44 (t, *J* = 5.1 Hz, 1H), 4.11 (dd, *J* = 8.3, 4.7 Hz, 1H), 3.86 – 3.54 (m, 6H), 3.21 (t, *J* = 8.1 Hz, 2H), 2.56 – 2.46 (m, 1H), 2.36 – 2.28 (m, 1H). ¹³C NMR (101 MHz, CD₃OD) δ 170.3, 161.6, 161.2, 151.5, 148.1, 133.5, 133.1, 132.5, 119.7, 118.0, 115.1, 90.6, 79.8, 74.2, 68.7, 54.8, 52.0, 51.0, 29.4, 24.5. HRMS (ESI): calculated for C₂₆H₃₂N₇O₅ [M+H]⁺ 522.2465, found 522.2477.

(*S*)-4-(((1*H*-indol-2-yl)methyl)(((2*R*,3*S*,4*R*,5*R*)-5-(6-amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl)amino)-2-aminobutanoic acid (13i). Following the procedure described for compound 13a, compound 12i (50 mg, 0.072 mmol) was deprotected and purified, affording compound 13i as a white powder (27 mg, 61% yield). ¹H NMR (400 MHz, D₂O) δ 8.30 (s, 1H), 7.68 (s, 1H), 7.42 (d, *J* = 7.8 Hz, 1H), 7.13 (t, *J* = 7.5 Hz, 1H), 7.06 (t, *J* = 6.9 Hz, 1H), 6.97 (d, *J* = 8.1 Hz, 1H), 6.08 (s, 1H), 4.69 – 4.64 (m, 1H), 4.61 – 4.45 (m, 4H), 4.03 – 4.00 (m, 2H), 3.70 (t, *J* = 7.3 Hz, 2H), 3.63 – 3.60 (br d, *J* = 12.0, 1H), 2.57 – 2.45 (m, 1H), 2.38 – 2.33 (m, 1H). ¹³C NMR (101 MHz, D₂O) δ 170.7, 149.2, 146.7, 143.9, 143.0, 123.0, 120.4, 120.1, 111.0, 91.2, 73.8, 72.0, 25.0. HRMS (ESI): calculated for C₂₃H₂₉N₈O₅ [M+H]+ 497.2261, found 497.2263.

(*S*)-4-(((1*H*-indol-3-yl)methyl)(((2*R*,3*S*,4*R*,5*R*)-5-(6-amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl)amino)-2-aminobutanoic acid (13j). Following the procedure described for compound 13a, compound 12j (50 mg, 0.063 mmol) was deprotected and purified, affording compound 13j as a pink powder (23 mg, 61% yield). ¹H NMR (500 MHz, CD₃OD) δ 8.56 – 8.31 (m, 1H), 7.64 (d, *J* = 7.0 Hz, 1H), 7.55 (s, 1H), 7.42 (d, *J* = 8.2 Hz, 1H), 7.18 (t, *J* = 8.2 Hz, 1H), 7.07 (t, *J* = 7.5 Hz, 1H), 6.14 (dd, *J* = 9.4, 4.3 Hz, 1H), 4.75 – 4.56 (m, 3H), 4.51 – 4.38 (m, 1H), 4.02 (dd, *J* = 8.4, 4.7 Hz, 1H), 3.81 – 3.74 (m, 1H), 3.71 – 3.59 (m, 2H), 3.56 – 3.49 (m, 1H), 3.37 (s, 4H), 2.58 – 2.48 (m, 1H), 2.42 – 2.31 (m, 1H). ¹³C NMR (126 MHz, CD₃OD) δ 170.3, 160.4, 150.5, 148.1, 134.7, 128.0, 127.2, 122.2, 120.1, 116.8, 111.7, 101.8, 91.5, 90.3, 81.0, 78.8, 74.6, 66.4, 49.9, 48.5, 44.6, 26.1, 23.1. HRMS (ESI): calculated for C₂₃H₂₉N₈O₅ [M+H]⁺ 497.2261, found 497.2268.

(*S*)-2-amino-4-((((2*R*,3*S*,4*R*,5*R*)-5-(6-amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl)(benzo[*b*]thiophen-2-ylmethyl)amino)butanoic acid (13k). Following the procedure described for compound 13a, compound 12k (50 mg, 0.070 mmol) was deprotected and purified, affording compound 13k as a white powder (34 mg, 78% yield). ¹H NMR (400 MHz, D₂O) δ 8.26 (s, 1H), 7.69 (s, 1H), 7.64 – 7.58 (m, 1H), 7.42 – 7.35 (m, 1H), 7.34 – 7.27 (m, 2H), 7.13 (s, 1H), 6.04 (d, *J* = 2.3 Hz, 1H), 4.70 – 4.57 (m, 3H), 4.49 – 4.42 (m, 2H), 4.07 (dd, *J* = 8.7, *J* = 4.5 Hz, 1H), 3.92 – 3.86 (br t, *J* = 12.0 Hz, 1H), 3.73 – 3.67 (m, 2H), 3.63 – 3.59 (br d, *J* = 16.0, 1H), 2.57 – 2.47 (m, 1H), 2.41 – 2.33 (m, 1H). ¹³C NMR (101 MHz, D₂O) δ 172.7, 162.7, 144.0, 143.0, 128.7, 125.7, 125.0, 123.7, 122.1, 91.2, 78.0, 73.9, 71.9, 53.1, 51.45, 24.1. HRMS (ESI): calculated for C₂₃H₂₈N₇O₅S [M+H]⁺ 514.1873, found 514.1875.

(*S*)-2-amino-4-((((2*R*,3*S*,4*R*,5*R*)-5-(6-amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl)(benzo[*b*]thiophen-3-ylmethyl)amino)butanoic acid (13l). Following the procedure described for compound 13a, compound 12l (50 mg, 0.070 mmol) was deprotected and purified, affording compound 13l as a white powder (29 mg, 67% yield). ¹H NMR (400 MHz, CD₃OD) δ 8.37 (s, 1H), 8.06 (s, 1H), 7.93 (s, 1H), 7.85 – 7.80 (m, 2H), 7.35 – 7.26 (m, 2H), 6.12 (d, *J* = 3.0 Hz, 1H), 4.72 (s, 2H), 4.61 – 4.53 (m, 2H), 4.50 – 4.46 (m, 1H), 4.00 (dd, *J* = 8.5, 4.4 Hz, 1H), 3.84 – 3.60 (m, 4H), 2.55 – 2.46 (m, 1H), 2.37 – 2.31 (m, 1H). ¹³C NMR (101 MHz, CD₃OD) δ 170.8, 162.1, 161.8, 161.4, 161.1, 151.1, 147.8, 140.0, 137.8, 124.8, 120.9, 119.5, 118.0, 115.1, 112.2, 54.7, 51.80, 25.1. HRMS (ESI): calculated for C₂₃H₂₈N₇O₅S [M+H]+ 514.1873, found 514.1877.

(*E*)-3-(4-((trimethylsilyl)ethynyl)phenyl)acrylaldehyde (15y). To a solution of 4-((trimethylsilyl)ethynyl)benzaldehyde 14y (1.81 g, 8.0 mmol) in THF (40 ml), (triphenyl phosphoramylidene)acetaldehyde (2.20 g, 7.2 mmol) was added. The suspension was stirred at 50°C under N₂ for overnight and concentrated to dryness under vacuum. The crude product was purified by flash chromatography on silica gel (0–90% CH₂Cl₂ in petroleum ether) to give compound 15y (1.2 g, 73%) as a white solid. ¹H NMR (400 MHz, CDCl₃) δ 9.72 (d, *J* = 7.7 Hz, 1H), 7.54 – 7.50 (m, 4H), 7.45 (br d, *J* = 12.0 Hz, 1H), 6.75 – 6.69 (m, 1H), 0.28 (s, 9H). ¹³C NMR (101 MHz, CDCl₃) δ 193.5, 151.6, 132.9, 132.6, 128.3, 126.1, 104.3, 97.6. HRMS (ESI): calculated for C₁₄H₁₇OSi [M+H]⁺ 229.3740, found 229.3744.

tert-butyl (2*S*)-4-(((((3*aR*,4*R*,6*R*,6*aR*)-6-(6-amino-9*H*-purin-9-yl)-2,2dimethyltetrahydrofuro[3,4-d][1,3]dioxol-4-yl)methyl)((*E*)-3-(o-tolyl)allyl)amino)-2-((*tert*butoxycarbonyl)amino)butanoate (16a). Following the procedure described for compound 12a, coupling compound 11 (112 mg, 0.20 mmol) with (*E*)-3-(o-tolyl)acrylaldehyde 15a (35 mg, 0.24 mmol) afforded compound 16a as a white powder (100 mg, 72% yield) ¹H NMR (400 MHz, CDCl₃) δ 8.28 (s, 1H), 7.95 (s, 1H), 7.41 – 7.35 (m, 1H), 7.29 (s, 1H), 7.14 (dd, *J* = 5.3, 3.9 Hz, 3H), 6.6 – 6.64 (br d, *J* = 12.0 Hz, 1H), 6.27 (s, 2H), 6.13 – 6.03 (m, 2H), 5.73 (d, *J* = 8.1 Hz, 1H), 5.48 (d, *J* = 5.1 Hz, 1H), 5.05 – 4.96 (m, 1H), 4.43 – 4.39 (m, 1H), 4.25 – 4.21 (m, 1H), 3.42 – 3.33 (m, 1H), 3.31 – 3.23 (m, 1H), 2.89 – 2.84 (m, 1H), 2.72 – 2.55 (m, 3H), 2.30 (s, 3H), 2.07 – 1.91 (m, 1H), 1.86 – 1.74 (m, 1H), 1.63 (s, 3H), 1.44 – 1.41 (br m, 21H). ¹³C NMR (101 MHz, CDCl₃) δ 171.9, 155.8, 153.1, 149.3, 140.0, 136.0, 135.2, 130.9, 127.4, 126.1, 125.7, 120.4, 114.5, 90.8, 85.5, 83.9, 83.4, 81.7, 57.2, 55.9, 52.9, 50.6, 29.5, 28.4, 28.0, 27.2, 25.5, 19.9. HRMS (ESI): calculated for C₃₆H₅₂N₇O₇ [M+H]⁺ 694.3928, found 694.3935.

tert-butyl (2*S*)-4-((((3*aR*,4*R*,6*R*,6*aR*)-6-(6-amino-9*H*-purin-9-yl)-2,2dimethyltetrahydrofuro[3,4-d][1,3]dioxol-4-yl)methyl)((*E*)-3-(m-tolyl)allyl)amino)-2-((*tert*butoxycarbonyl)amino)butanoate (16b). Following the procedure described for compound 12a, coupling compound 11 (112 mg, 0.20 mmol) with (*E*)-3-(*m*-tolyl)acrylaldehyde 15b (35 mg, 0.24 mmol) afforded compound 16b as a white powder (104 mg, 75% yield). ¹H NMR (400 MHz, CDCl₃) δ 8.26 (s, 1H), 7.92 (s, 1H), 7.18 – 7.09 (m, 3H), 7.01 (d, *J* = 7.3 Hz, 1H), 6.40 – 6.36 (br d, *J* = 16.0 Hz, 1H), 6.20 – 6.05 (m, 4H), 5.66 (d, *J* = 7.9 Hz, 1H), 5.44 (d, *J* = 6.1 Hz, 1H), 4.96 (d, *J* = 5.8 Hz, 1H), 4.38 (s, 1H), 4.24 – 4.08 (m, 1H), 3.39 – 3.14 (m, 2H), 2.84 – 2.79 (m, 1H), 2.71 – 2.50 (m, 3H), 2.31 (s, 3H), 2.00 – 1.93 (m, 1H), 1.82 – 1.73 (m 1H), 1.60 (s, 3H), 1.41 – 1.38 (br m, 21H). ¹³C NMR (101 MHz, CDCl₃) δ 171.8, 155.8, 153.1, 149.3, 141.0, 140.0, 135.1, 128.5, 126.1, 123.5, 120.4, 114.5, 90.8, 85.4, 83.3, 57.0, 55.9, 52.9, 50.6, 29.5, 28.4, 28.0, 27.2, 25.5. 21.4. HRMS (ESI): calculated for C₃₆H₅₂N₇O₇ [M+H]⁺ 694.3928, found 694.3938.

tert-butyl (2*S*)-4-(((((3*aR*,4*R*,6*R*,6*aR*)-6-(6-amino-9*H*-purin-9-yl)-2,2dimethyltetrahydrofuro[3,4-d][1,3]dioxol-4-yl)methyl)((*E*)-3-(p-tolyl)allyl)amino)-2-((*tert*butoxycarbonyl)amino)butanoate (16c). Following the procedure described for compound 12a, coupling compound 11 (112 mg, 0.20 mmol) with (*E*)-3-(*p*-tolyl)acrylaldehyde 15c (35 mg, 0.24 mmol) afforded compound 16c as a white powder (109 mg, 79% yield). ¹H NMR (400 MHz, CDCl₃) δ 8.26 (s, 1H), 7.92 (s, 1H), 7.19 (d, *J* = 8.1 Hz, 2H), 7.07 (d, *J* = 8.0 Hz, 2H), 6.39 – 6.35 (br *J* = 16.1 Hz, 1H), 6.25 – 5.98 (m, 4H), 5.66 (d, *J* = 8.1 Hz, 1H), 5.43 (d, *J* = 6.1 Hz, 1H), 4.96 (d, *J* = 6.1 Hz, 1H), 4.36 (br s, 1H), 4.21 – 4.17 (m, 1H), 3.33 – 3.16 (m, 2H), 2.84 – 2.79 (m, 1H), 2.67 – 2.53 (m, 3H), 2.30 (s, 3H), 1.98 – 1.93 (m, 1H), 1.84 – 1.71 (m, 1H), 1.60 (s, 3H), 1.44 – 1.37 (br m, 21H). ¹³C NMR (101 MHz, CDCl₃) δ 171.8, 155.8, 153.1, 149.3, 140.03, 136.8, 133.0, 128.6, 127.5, 126.3, 120.4, 114.5, 90.8, 85.5, 83.9, 83.4, 81.7, 57.0, 55.9, 52.9, 28.4, 28.0, 27.2, 25.5. HRMS (ESI): calculated for C₃₆H₅₂N₇O₇ [M+H]⁺ 694.3928, found 694.3940.

tert-butyl (2S)-4-(((((3a*R*,3a*R*,4*R*,6*R*,6a*R*,6a*R*)-6-(6-amino-9*H*-purin-9-yl)-2,2dimethyltetra-hydrofuro[3,4-d][1,3]dioxol-4-yl)methyl)((*E*)-3-(2-methoxyphenyl)allyl)amino)-2-((*tert*-butoxy carbonyl)amino)butanoate (16d). Following the procedure described for compound 12a, coupling compound 11 (112 mg, 0.20 mmol) with (*E*)-3-(2methoxyphenyl)acrylaldehyde 15d (39 mg, 0.24 mmol) afforded compound 16d as a white powder (75 mg, 53% yield). ¹H NMR (400 MHz, CDCl₃) δ 8.27 (s, 1H), 7.95 (s, 1H), 7.38 (d, *J* = 7.6 Hz, 1H), 7.24 – 7.17 (m, 1H), 6.93 – 6.75 (m, 3H), 6.33 (br s, 2H), 6.25 – 6.14 (m, 1H), 6.07 (d, *J* = 2.2 Hz, 1H), 5.74 (d, *J* = 8.2 Hz, 1H), 5.44 (d, *J* = 6.5 Hz, 1H), 5.06 – 4.93 (m, 1H), 4.43 – 4.39

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(m, 1H), 4.26 - 4.16 (m, 1H), 3.83 (s, 3H), 3.39 - 3.22 (m, 2H), 2.88 - 2.83 (m, 1H), 2.75 - 2.50 (m, 3H), 2.03 - 1.98 (m, 1H), 1.85 - 1.78 (m, 1H), 1.62 (s, 3H), 1.42 - 1.40 (br m, 21H). ¹³C NMR (101 MHz, CDCl₃) δ 171.3, 156.5, 155.8, 155.1, 153.6, 149.9, 141.4, 130.3, 126.8, 125.9, 120.7, 119.7, 114.5, 111.3, 90.8, 85.8, 84.0, 82.7, 81.7, 79.4, 57.4, 55.9, 55.4, 52.9, 49.9, 29.4, 28.4, 27.2, 25.5. HRMS (ESI): calculated for C₃₆H₅₂N₇O₈ [M+H]+ 710.3877, found 710.3882.

tert-butyl (2S)-4-((((3aR,3aR,4R,6R,6aR,6aR)-6-(6-amino-9*H*-purin-9-yl)-2,2dimethyltetra-hydrofuro[3,4-d][1,3]dioxol-4-yl)methyl)((*E*)-3-(3-methoxyphenyl)allyl)amino)-2-((*tert*-butoxy carbonyl)amino)butanoate (16e). Following the procedure described for compound 12a, coupling compound 11 (112 mg, 0.20 mmol) with (*E*)-3-(2methoxyphenyl)acrylaldehyde 15e (39 mg, 0.24 mmol) afforded compound 16e as a white powder (82 mg, 58% yield). ¹H NMR (400 MHz, CDCl₃) δ 8.30 (s, 1H), 7.96 (s, 1H), 7.23 (t, *J* = 7.9 Hz, 1H), 6.97 – 6.88 (m, 2H), 6.80 (dd, *J* = 8.2, 2.4 Hz, 1H), 6.44 – 6.40 (br d, *J* = 16.0 Hz, 1H), 6.28 – 6.17 (m, 1H), 6.09 – 6.03 (br d, *J* = 24.0 Hz, 3H), 5.66 – 5.48 (br m, 2H), 5.05 – 4.97 (m, 1H), 4.48 – 4.36 (m, 1H), 4.23 (d, *J* = 4.7 Hz, 1H), 3.83 (s, 3H), 3.43 – 3.18 (m, 2H), 2.88 – 2.83 (m, 1H), 2.75 – 2.53 (m, 3H), 2.00 – 1.97 (m, 1H), 1.88 – 1.73 (m, 1H), 1.64 (s, 3H), 1.45 – 1.42 (br m, 21H). ¹³C NMR (101 MHz, CDCl₃) δ 171.8, 159.8, 155.7, 151.9, 149.3, 140.1, 138.3, 133.5, 130.0, 127.6, 120.4, 119.9, 114.5, 113.3, 110.8, 93.1, 89.5, 81.7, 83.4, 81.72, 57.0, 55.9, 55.3, 50.6, 28.4, 28.0, 27.2, 25.5. HRMS (ESI): calculated for C₃₆H₅₂N₇O₈ [M+H]+710.3877, found 710.3885.

 $tert-butyl \qquad (2S)-4-((((3aR,3aR,4R,6R,6aR,6aR)-6-(6-amino-9H-purin-9-yl)-2,2-dimethyltetra-hydrofuro[3,4-d][1,3]dioxol-4-yl)methyl)((E)-3-(2-fluorophenyl)allyl)amino)-2-dimethyltetra-hydrofuro[3,4-d][1,3]dioxol-4-yl)methyl)((E)-3-(2-fluorophenyl)allyl)amino)-2-dimethyltetra-hydrofuro[3,4-d][1,3]dioxol-4-yl)methyl)((E)-3-(2-fluorophenyl)allyl)amino)-2-dimethyltetra-hydrofuro[3,4-d][1,3]dioxol-4-yl)methyl)((E)-3-(2-fluorophenyl)allyl)amino)-2-dimethyltetra-hydrofuro[3,4-d][1,3]dioxol-4-yl)methyl)((E)-3-(2-fluorophenyl)allyl)amino)-2-dimethyltetra-hydrofuro[3,4-d][1,3]dioxol-4-yl)methyl)((E)-3-(2-fluorophenyl)allyl)amino)-2-dimethyltetra-hydrofuro[3,4-d][1,3]dioxol-4-yl)methyl)((E)-3-(2-fluorophenyl)allyl)amino)-2-dimethyltetra-hydrofuro[3,4-d][1,3]dioxol-4-yl)methyl)(E)-3-(2-fluorophenyl)allyl)amino)-2-dimethyltetra-hydrofuro[3,4-d][1,3]dioxol-4-yl)methyl)(E)-3-(2-fluorophenyl)allyl)amino)-2-dimethyltetra-hydrofuro[3,4-d][1,3]dioxol-4-yl)methyl)(E)-3-(2-fluorophenyl)allyl)amino)-2-dimethyltetra-hydrofuro[3,4-d][1,3]dioxol-4-yl)methyl(E)-3-(2-fluorophenyl)allyl)amino)-2-dimethyltetra-hydrofuro[3,4-d][1,3]dioxol-4-yl)methyl(E)-3-(2-fluorophenyl)allyl)amino)-2-dimethyltetra-hydrofuro[3,4-d][1,3]dioxol-4-yl)methyl(E)-3-(2-fluorophenyl)allyl)amino)-2-dimethyltetra-hydrofuro[3,4-d][1,3]dioxol-4-yl)methyl(E)-3-(2-fluorophenyl)allyl)amino)-2-dimethyltetra-hydrofuro[3,4-d][1,3]dioxol-4-yl)methyl(E)-3-(2-fluorophenyl)allyl)amino)-2-dimethyltetra-hydrofuro[3,4-d][1,3]dioxol-4-yl)methyl(E)-3-(2-fluorophenyl)allyl)amino)-2-dimethyltetra-hydrofuro[3,4-d][1,3]dioxol-4-glamethyltetra-hydrofuro[3,4-d][1,3]dioxol-4-glamethyltetra-hydrofuro[3,4-d][1,3]dioxol-4-glamethyltetra-hydrofuro[3,4-d][1,3]dioxol-4-glamethyltetra-hydrofuro[3,4-d][1,3]dioxol-4-glamethyltetra-hydrofuro[3,4-d][1,3]dioxol-4-glamethyltetra-hydrofuro[3,4-d][1,3]dioxol-4-glamethyltetra-hydrofuro[3,4-d][1,3]dioxol-4-glamethyltetra-hydrofuro[3,4-d][1,3]dioxol-4-glamethyltetra-hydrofuro[3,4-d][1,3]dioxol-4-glamethyltetra-hydrofuro[3,4-d][1,3]dioxol-4-glamethyltetra-h$

(*tert*-butoxy carbonyl)amino)butanoate (16g). Following the procedure described for compound 12a, coupling compound 11 (112 mg, 0.20 mmol) with (*E*)-3-(2-fluorophenyl)acrylaldehyde 15g (36 mg, 0.24 mmol) afforded compound 16g as a white powder (96 mg, 69% yield). ¹H NMR (600 MHz, CDCl₃) δ 8.26 (s, 1H), 7.91 (s, 1H), 7.37 (t, *J* = 7.1 Hz, 1H), 7.19 – 7.15 (m, 1H), 7.04 (t, *J* = 7.9 Hz, 1H), 7.01 – 6.97 (m, 1H), 6.58 (m, 1H), 6.30 – 6.21 (m, 1H), 6.04 (s, 1H), 5.90 (s, 2H), 5.58 (d, *J* = 8.0 Hz, 1H), 5.44 (d, *J* = 5.4 Hz, 1H), 5.01 – 4.92 (m, 1H), 4.38 (s, 1H), 4.22 – 4.15 (m, 1H), 3.35 (d, *J* = 6.2 Hz, 1H), 3.29 – 3.19 (m, 1H), 2.86 – 2.80 (m, 1H), 2.71 – 2.51 (m, 3H), 1.99 – 1.96 (m, 1H), 1.82 – 1.72 (m, 1H), 1.60 (s, 3H), 1.40 – 1.38 (br m, 21H). ¹³C NMR (151 MHz, CDCl₃) δ 171.5, 168.8, 159.4, 156.6, 152.5, 147.4, 145.2, 141.6, 137.1, 127.2, 123.5, 121.5, 119.8, 116.2, 112.4, 91.7, 85.9, 83.3, 81.7, 79.9, 57.1, 54.8, 52.4, 51.8, 49.6, 28.1, 26.1, 24.4. HRMS (ESI): calculated for C₃₅H₄₉FN₇O₇ [M+H]⁺ 698.3678, found 698.3690.

tert-butyl (2S)-4-(((((3aR,3aR,4R,6R,6aR,6aR)-6-(6-amino-9H-purin-9-yl)-2,2dimethyltetra-hydrofuro[3,4-d][1,3]dioxol-4-yl)methyl)((E)-3-(3-fluorophenyl)allyl)amino)-2-((tert-butoxy carbonyl)amino)butanoate (16h). Following the procedure described for compound 12a, coupling compound 11 (112 mg, 0.20 mmol) with (*E*)-3-(3-fluorophenyl)acrylaldehyde 15h (36 mg, 0.24 mmol) afforded compound **16h** as a white powder (93 mg, 67% yield). ¹H NMR (600 MHz, CDCl₃) δ 8.26 (s, 1H), 7.91 (s, 1H), 7.25 – 7.18 (m, 1H), 7.03 (d, J = 7.7 Hz, 1H), 7.01 – 6.98 (m, 1H), 6.91 - 6.87 (m 1H), 6.36 (d, J = 8.1 Hz, 1H), 6.20 - 6.15 (m, 1H), 6.04 (s, 1H),5.91 (s, 2H), 5.57 (d, J = 8.0 Hz, 1H), 5.45 (d, J = 5.5 Hz, 1H), 4.97 (d, J = 5.7 Hz, 1H), 4.42 -4.34 (m, 1H), 4.19 (d, J = 4.9 Hz, 1H), 3.32 - 3.28 (m, 1H), 3.23 - 3.19 (m, 1H), 2.82 - 2.79 (m, 1H), 2.82 - 2.79 (m, 1H), 2.82 - 2.79 (m, 1H), 3.23 - 3.19 (m, 1H), 3.23 (m,1H), 2.70 - 2.50 (m, 3H), 2.03 - 1.91 (m, 1H), 1.79 - 1.75 (m, 1H), 1.60 (s, 3H), 1.42 - 1.38 (br m, 21H). ¹³C NMR (151 MHz, CDCl₃) δ 170.70, 162.85, 161.22, 154.58, 154.49, 152.06, 148.19, 139.03, 138.13, 130.67, 128.94, 128.88, 127.12, 121.17, 119.29, 113.45, 113.28, 113.14, 111.76, 111.61, 89.75, 84.52, 82.87, 82.26, 80.69, 55.83, 54.96, 51.81, 49.61, 28.54, 27.33, 26.95, 26.14, 24.42. HRMS (ESI): calculated for C35H49FN7O7 [M+H]+ 698.3678, found 698.3682.

tert-butyl (2*S*)-4-(((((3*aR*,3*aR*,4*R*,6*R*,6*aR*,6*aR*)-6-(6-amino-9*H*-purin-9-yl)-2,2dimethyltetra-hydrofuro[3,4-d][1,3]dioxol-4-yl)methyl)((*E*)-3-(4-fluorophenyl)allyl)amino)-2-((*tert*-butoxy carbonyl)amino)butanoate (16i). Following the procedure described for compound 12*a*, coupling compound 11 (112 mg, 0.20 mmol) with (*E*)-3-(4-fluorophenyl)acrylaldehyde 15i (36 mg, 0.24 mmol) afforded compound 16i as a white powder (86 mg, 62% yield). ¹H NMR (400 MHz, CDCl₃) δ 8.21 (s, 1H), 7.89 (s, 1H), 7.21 – 7.16 (m, 2H), 6.90 (t, *J* = 8.6 Hz, 2H), 6.51 (s, 2H), 6.32 – 6.29 (br d, *J* = 16.1 Hz, 1H), 6.08 – 5.99 (m, 2H), 5.74 (d, *J* = 8.1 Hz, 1H), 5.42 (d, *J* = 7.9 Hz, 1H), 4.96 (d, *J* = 3.5 Hz, 1H), 4.36 – 4.32 (m, 1H), 4.22–4.14 (m, 1H), 3.27 – 3.22

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(m, 1H), 3.18 - 3.12 (m, 1H), 2.80 - 2.75 (m, 1H), 2.66 - 2.57 (m, 2H), 2.54 - 2.46 (m, 1H), 1.99 - 1.88 (m, 1H), 1.78 - 1.69 (m, 1H), 1.56 (s, 3H), 1.36 - 1.34 (br m, 21H). ¹³C NMR (101 MHz, CDCl₃) δ 171.8, 163.3, 160.9, 155.9, 155.5, 153.0, 149.1, 139.9, 132.9, 131.5, 127.7, 126.2, 120.2, 115.4, 115.2, 114.3, 90.7, 85.5, 83.8, 83.2, 81.7, 79.3, 56.9, 55.8, 52.8, 50.5, 29.4, 28.3, 27.9, 27.1, 25.4. HRMS (ESI): calculated for C₃₅H₄₉FN₇O₇ [M+H]⁺ 698.3678, found 698.3694.

tert-butyl (2*S*)-4-(((((3*a*,3*a*,4*k*,6*R*,6*aR*,6*aR*)-6-(6-amino-9*H*-purin-9-yl)-2,2dimethyltetra-hydrofuro[3,4-d][1,3]dioxol-4-yl)methyl)((*E*)-3-(2-chlorophenyl)allyl)amino)-2-((*tert*-butoxy carbonyl)amino)butanoate (16j). Following the procedure described for compound 12a, coupling compound 11 (112 mg, 0.20 mmol) with (*E*)-3-(2-chlorophenyl)acrylaldehyde 15j (40 mg, 0.24 mmol) afforded compound 16j as a white powder (84 mg, 59% yield). ¹H NMR (400 MHz, CDCl₃) δ 8.25 (s, 1H), 7.91 (s, 1H), 7.44 – 7.41 (m, 1H), 7.29 (dd, *J* = 7.5, 1.7 Hz, 1H), 7.17 – 7.09 (m, 2H), 6.80 (d, *J* = 15.9 Hz, 1H), 6.22 – 6.10 (m, 3H), 6.04 (s, 1H), 5.65 (d, *J* = 8.0 Hz, 1H), 5.44 (d, *J* = 5.6 Hz, 1H), 4.98 (d, *J* = 9.5 Hz, 1H), 4.41 – 4.33 (m, 1H), 4.23 – 4.16 (m, 1H), 3.38 – 3.30 (m, 1H), 3.28 – 3.20 (m, 1H), 2.86 – 2.81 (m, 1H), 2.62 (br s, 2H), 2.56 (d, *J* = 12.9 Hz, 1H), 12.01 – 1.92 (m, 1H), 1.79 – 1.75 (m, 1H), 1.59 (s, 3H), 1.39 – 1.37 (br m, 21H). ¹³C NMR (101 MHz, CDCl₃) δ 174.1, 158.8, 158.6, 156.1, 152.2, 135.8, 132.6, 132.0, 131.5, 129.9, 129.8, 123.4, 117.5, 93.8, 88.5, 86.9, 86.3, 82.4, 59.0, 32.6, 31.4, 31.0, 30.2, 28.5. HRMS (ESI): calculated for C₃₅H₄₉ClN₇O₇ [M+H]+714.3382, found 714.3389.

tert-butyl (2*S*)-4-((((3*aR*,3*aR*,4*R*,6*R*,6*aR*,6*aR*)-6-(6-amino-9*H*-purin-9-yl)-2,2dimethyltetra-hydrofuro[3,4-d][1,3]dioxol-4-yl)methyl)((*E*)-3-(3-chlorophenyl)allyl)amino)-2-((*tert*-butoxy carbonyl)amino)butanoate (16k). Following the procedure described for compound 12a, coupling compound 11 (112 mg, 0.20 mmol) with (*E*)-3-(3-chlorophenyl)acrylaldehyde 15k (40 mg, 0.24 mmol) afforded compound 16k as a white powder (79 mg, 65% yield). ¹H NMR (400 MHz, CDCl₃) δ 8.22 (s, 1H), 7.88 (s, 1H), 7.17 – 7.09 (m, 3H), 6.32 – 6.28 (br d, *J* = 16.0 Hz, 1H), 6.16 (d, *J* = 5.8 Hz, 3H), 6.01 (s, 1H), 5.62 (d, *J* = 7.9 Hz, 1H), 5.42 (d, *J* = 5.6 Hz, 1H), 4.98 – 4.91 (m, 1H), 4.37 – 4.30 (m, 1H), 4.18 (s, 1H), 3.29 – 3.24 (m, 1H), 3.19 – 3.14 (m, 1H), 2.81 – 2.76 (m, 1H), 2.66 – 2.60 (m, 2H), 2.53 – 2.47 (m, 1H), 1.99 – 1.88 (m, 1H), 1.79 – 1.67 (m, 1H), 1.56 (s, 3H), 1.36 (d, *J* = 6.8, 21H). ¹³C NMR (101 MHz, CDCl₃) δ 174.0, 158.9, 158.7, 157.0, 150.5, 143.2, 141.2, 137.2, 135.1, 132.9, 131.5, 130.5, 129.4, 127.7, 124.1, 118.9, 95.0, 88.7, 87.6, 86.5, 84.8, 84.3, 60.7, 59.1, 53.8, 32.7, 31.6, 31.1, 28.6. HRMS (ESI): calculated for C₃₅H₄₉ClN₇O₇ [M+H]⁺714.3382, found 714.3408.

 $tert-butyl \qquad (2S)-4-((((3aR,3aR,4R,6R,6aR,6aR)-6-(6-amino-9H-purin-9-yl)-2,2-dimethyltetra-hydrofuro[3,4-d][1,3]dioxol-4-yl)methyl)((E)-3-(4-chlorophenyl)allyl)amino)-2-dimethyltetra-hydrofuro[3,4-d][1,3]dioxol-4-yl)methyl)((E)-3-(4-chlorophenyl)allyl)amino)-2-dimethyltetra-hydrofuro[3,4-d][1,3]dioxol-4-yl)methyl)((E)-3-(4-chlorophenyl)allyl)amino)-2-dimethyltetra-hydrofuro[3,4-d][1,3]dioxol-4-yl)methyl)((E)-3-(4-chlorophenyl)allyl)amino)-2-dimethyltetra-hydrofuro[3,4-d][1,3]dioxol-4-yl)methyl)((E)-3-(4-chlorophenyl)allyl)amino)-2-dimethyltetra-hydrofuro[3,4-d][1,3]dioxol-4-yl)methyl)((E)-3-(4-chlorophenyl)allyl)amino)-2-dimethyltetra-hydrofuro[3,4-d][1,3]dioxol-4-yl)methyl)((E)-3-(4-chlorophenyl)allyl)amino)-2-dimethyltetra-hydrofuro[3,4-d][1,3]dioxol-4-yl)methyl)((E)-3-(4-chlorophenyl)allyl)amino)-2-dimethyltetra-hydrofuro[3,4-d][1,3]dioxol-4-yl)methyl)((E)-3-(4-chlorophenyl)allyl)amino)-2-dimethyltetra-hydrofuro[3,4-d][1,3]dioxol-4-yl]methyltetra-$

(*tert*-butoxy carbonyl)amino)butanoate (16l). Following the procedure described for compound 12a, coupling compound 11 (112 mg, 0.20 mmol) with (*E*)-3-(4-chlorophenyl)acrylaldehyde 15l (40 mg, 0.24 mmol) afforded compound 16l as a white powder (79 mg, 56% yield). ¹H NMR (400 MHz, CDCl₃) δ 8.22 (s, 1H), 7.89 (s, 1H), 7.19 – 7.13 (m, 4H), 6.43 (s, 2H), 6.31 – 6.28 (br , *J* = 16.0 Hz, 1H), 6.12 – 6.05 (m, 1H), 6.02 (d, *J* = 4.1 Hz, 1H), 5.70 (d, *J* = 8.1 Hz, 1H), 5.42 (d, *J* = 5.9 Hz, 1H), 4.97 – 4.94(m, 1H), 4.37 – 4.32 (m, 1H), 4.22 – 4.14 (m, 1H), 3.28 – 3.22 (m, 1H), 3.19 – 3.13 (m, 1H), 2.80 – 2.76 (m, 1H), 2.68 – 2.58 (m, 2H), 2.54 – 2.47 (m, 1H), 2.00 – 1.89 (m, 1H), 1.75 (d, *J* = 9.4 Hz, 1H), 1.57 (s, 3H), 1.37 – 1.35 (br m, 21H). ¹³C NMR (101 MHz, CDCl₃) δ 171.7, 155.8, 155.5, 152.9, 149.1, 139.9, 135.2, 132.9, 131.5, 128.6, 127.4, 120.2, 114.3, 90.7, 85.5, 83.8, 83.3, 81.6, 79.4, 56.9, 55.9, 52.8, 50.5, 29.5, 28.3, 27.1, 25.4. HRMS (ESI): calculated for C₃₅H₄₉ClN₇O₇ [M+H]⁺714.3382, found 714.3403.

tert-butyl (*S*)-4-((((3a*R*,4*R*,6*R*,6a*R*)-6-(6-amino-9*H*-purin-9-yl)-2,2dimethyltetrahydrofuro[3,4-d][1,3]dioxol-4-yl)methyl)((*E*)-3-(2-bromophenyl)allyl)amino)-2-((*tert*-butoxycarbonyl)amino) butanoate (16m). Following the procedure described for compound 12a, coupling compound 11 (112 mg, 0.20 mmol) with (*E*)-3-(2-bromophenyl)acrylaldehyde 15m (51 mg, 0.24 mmol) afforded compound 16m as a white powder (80 mg, 53% yield). ¹H NMR (400 MHz, CDCl₃) δ 8.33 – 8.22 (m, 1H), 7.93 (s, 1H), 7.50 (dd, *J* = 7.9, 3.9 Hz, 1H), 7.47 – 7.38 (m, 1H), 7.28 (t, *J* = 4.4 Hz, 1H), 7.22 (d, *J* = 7.3 Hz, 1H), 7.06 (d, *J* = 7.4 Hz, 1H), 6.77 (d, *J* = 15.2 Hz, 1H), 6.19 (s, 2H), 6.16 – 6.01 (m, 2H), 5.67 (s, 1H), 5.46 (s, 1H), 5.01 (s, 1H), 4.40 (s, 1H), 4.22 (s, 1H), 3.32 (br d, *J* = 22.6 Hz, 2H), 2.84 (s, 1H), 2.63 (br d, *J* = 42.6 Hz, 3H), 1.98 (s, 1H), 1.79 (s, 1H), 1.61 (d, *J* = 3.8 Hz, 3H), 1.42 (d, *J* = 2.1 Hz, 21H). ¹³C NMR (101 MHz, CDCl₃) δ 171.7, 155.6, 155.5, 152.9, 149.0, 139.9, 136.3, 133.9, 132.3, 128.1, 128.0, 126.3, 124.0, 120.2, 90.9, 85.5, 83.8, 83.5, 81.7, 59.2, 56.0, 53.5, 52., 50.9, 29.5, 28.4, 27.9, 27.1, 25.4. HRMS (ESI): calculated for C₃₅H₄₉BrN₇O₇ [M+H]⁺758.2877 found 758.2882.

tert-butyl (2*S*)-4-(((((3*aR*,3*aR*,4*R*,6*R*,6*aR*,6*aR*)-6-(6-amino-9*H*-purin-9-yl)-2,2dimethyltetra-hydrofuro[3,4-d][1,3]dioxol-4-yl)methyl)((*E*)-3-(3-bromophenyl)allyl)amino)-2-((*tert*-butoxy carbonyl)amino)butanoate (16n). Following the procedure described for compound 12*a*, coupling compound 11 (112 mg, 0.20 mmol) with (*E*)-3-(3-bromophenyl)acrylaldehyde 15n (51 mg, 0.24 mmol) afforded compound 16n as a white powder (94 mg, 62% yield). ¹H NMR (600 MHz CDCl₃) δ 8.25 (s, 1H), 7.90 (s, 1H), 7.43 (s, 1H), 7.30 (d, J = 7.8 Hz, 1H), 7.18 – 7.10 (m, 2H), 6.33 – 6.30 (br d, *J* = 12.0 Hz, 1H), 6.19 – 6.14 (m, 1H), 6.05 (d, *J* = 8.1 Hz, 2H), 5.61 (d, *J* = 7.7 Hz, 1H), 5.44 (d, *J* = 5.3 Hz, 1H), 4.98 (s, 1H), 4.36 (s, 1H), 4.19 (s, 1H), 3.31 – 3.18 (m, 2H), 2.82 – 2.78 (m, 1H), 2.70 – 2.50 (m, 3H), 1.96(br d, *J* = 4.0 Hz, 1H), 1.78 (br d, *J* = 4.1 Hz, 1H), 1.59 (s, 3H), 1.39 (d, *J* = 10.7 Hz, 21H). ¹³C NMR (151 MHz, CDCl₃) δ 170.2, 156.2, 153.1, 147.5, 138.5, 131.3, 130.3, 130.0, 129.1, 122.7, 124.5, 122.7, 90.7, 85.2, 84.6, 83.9, 83.3, 81.3, 79.5, 56.0, 52.8, 49.9, 29.58, 29.6, 28.4, 27.2, 24.4. HRMS (ESI): calculated for C₃₅H₄₉BrN₇O₇ [M+H]+758.2877 found 758.2881.

tert-butyl (2S)-4-((((3aR,3aR,4R,6R,6aR,6aR)-6-(6-amino-9*H*-purin-9-yl)-2,2dimethyltetra-hydrofuro[3,4-d][1,3]dioxol-4-yl)methyl)(*(E*)-3-(4-bromophenyl)allyl)amino)-2-((*tert*-butoxy carbonyl)amino)butanoate (16o). Following the procedure described for compound 12a, coupling compound 11 (112 mg, 0.20 mmol) with (*E*)-3-(4-bromophenyl)acrylaldehyde 15o (51 mg, 0.24 mmol) afforded compound 16o as a white powder (122 mg, 81% yield). ¹H NMR (400 MHz, CDCl₃) δ 8.22 (s, 1H), 7.89 (s, 1H), 7.33 (d, *J* = 8.4 Hz, 2H), 7.08 (d, *J* = 8.4 Hz, 2H), 6.41 (s, 2H), 6.26 (s, 1H), 6.14 – 6.07 (m, 1H), 6.03 (d, J = 1.6 Hz, 1H), 5.70 (d, *J* = 8.1 Hz, 1H), 5.43 (d, *J* = 5.8 Hz, 1H), 4.96 (dd, *J* = 6.3 Hz, 3.6 Hz, 1H), 4.37 – 4.31 (m, 1H), 4.22 – 4.14 (m, 1H), 3.27 – 3.22 (m, 1H), 3.18 – 3.13 (m, 1H), 2.80 – 2.75 (m, 1H), 2.69 – 2.57 (m, 2H), 2.54 – 2.47 (m, 1H), 1.99 – 1.88 (m, 1H), 1.79 – 1.69 (m, 1H), 1.57 (s, 3H), 1.37 – 1.35 (br d, *J* = 8.3 Hz, 21H). ¹³C NMR (101 MHz, CDCl₃) δ 171.7, 155.8, 155.5, 153.0, 149.1, 139.9, 135.7, 131.5, 127.7, 127.5, 121.1, 120.2, 114.3, 90.7, 85.5, 83.8, 83.3, 81.6, 79.4, 56.89 55.9, 52.80 50.5, 29.5, 28.3, 27.9, 27.1, 25.4. HRMS (ESI): calculated for C₃₅H₄₉BrN₇O₇ [M+H]⁺ 758.2877, found 758.2895.

tert-butyl (2*S*)-4-((((3*a*,3*a*,4*k*,6*R*,6*a*,6*a*,6*a*)-6-(6-amino-9*H*-purin-9-yl)-2,2dimethyltetra-hydrofuro[3,4-d][1,3]dioxol-4-yl)methyl)((E)-3-(2-nitrophenyl)allyl)amino)-2-((*tert*-butoxy carbonyl)amino)butanoate (16p). Following the procedure described for compound 12a, coupling compound 11 (112 mg, 0.20 mmol) with (*E*)-3-(2-nitrophenyl)acrylaldehyde 15p (42 mg, 0.24 mmol) afforded compound 16p as a white powder (69 mg, 47% yield). ¹H NMR (400 MHz, CDCl₃) δ 8.21 (s, 1H), 7.91 (s, 1H), 7.82 (d, *J* = 8.1 Hz, 1H), 7.44 (d, *J* = 4.1 Hz, 2H), 7.29 (dd, *J* = 8.3, 4.2 Hz, 1H), 6.85 – 6.81 (br d, *J* = 16.1 Hz, 1H), 6.46 (s, 2H), 6.15 – 6.06 (m, 1H), 6.03 (d, *J* = 2.0 Hz, 1H), 5.73 (d, *J* = 8.1 Hz, 1H), 5.41 (d, *J* = 5.7 Hz, 1H), 4.96 (dd, *J* = 6.4, 3.6 Hz, 1H), 4.37 – 4.31 (m, 1H), 4.21 – 4.14 (m, 1H), 3.33 – 3.28 (m, 1H), 3.24 – 3.19 (m, 1H), 2.83 – 2.78 (m, 1H), 2.72 – 2.60 (m, 2H), 2.56 – 2.50 (m, 1H), 1.98 – 1.91 (m, 1H), 1.79 – 1.68 (m, 1H), 1.55 (s, 3H), 1.42 – 1.29 (br m, 21H). ¹³C NMR (101 MHz, CDCl₃) δ 171.7, 155.8, 155.5, 153.0, 149.1, 139.9, 135.7, 131.5, 127.7, 121.1, 120.2, 114.3, 90.7, 85.5, 83.8, 83.3, 81.6, 79.4, 56.9, 55.9, 53.4, 52.8, 50.6, 29.5, 28.3, 27.1, 25.4. HRMS (ESI): calculated for C₃₅H₄₉N₈O9 [M+H]+725.3633, found 725.3632.

tert-butyl (2*S*)-4-(((((3*aR*,3*aR*,4*R*,6*R*,6*aR*,6*aR*)-6-(6-amino-9*H*-purin-9-yl)-2,2dimethyltetra-hydrofuro[3,4-d][1,3]dioxol-4-yl)methyl)((*E*)-3-(3-nitrophenyl)allyl)amino)-2-((*tert*-butoxy carbonyl)amino)butanoate (16q). Following the procedure described for compound **12a**, coupling compound **11** (112 mg, 0.20 mmol) with (*E*)-3-(3-nitrophenyl)acrylaldehyde **15q** (42 mg, 0.24 mmol) afforded compound **16q** as a white powder (63 mg, 43% yield). 1H NMR (400 MHz, CDCl₃) δ 8.21 (s, 1H), 7.91 (s, 1H), 7.82 (d, *J* = 8.1 Hz, 1H), 7.44 (d, *J* = 4.1 Hz, 2H), 7.29 (dd, *J* = 8.3, 4.2 Hz, 1H), 6.85 – 6.81 (br d, *J* = 15.7 Hz, 1H), 6.46 (s, 2H), 6.15 – 6.06 (m, 1H), 6.03 (d, *J* = 2.0 Hz, 1H), 5.73 (d, *J* = 8.1 Hz, 1H), 5.41 (d, *J* = 5.7 Hz, 1H), 4.96 (dd, *J* = 6.4, 3.6 Hz, 1H), 4.37 – 4.31 (m, 1H), 4.21 – 4.14 (m, 1H), 3.33 – 3.19 (m, 2H), 2.83 – 2.78 (m, 1H), 2.72 – 2.60 (m, 2H), 2.56 – 2.50 (m, 1H), 1.98 – 1.93 (m, 1H), 1.79 – 1.68 (m, 1H), 1.55 (s, 3H), 1.42 – 1.29 (m, 21H). ¹³C NMR (151 MHz, CDCl₃) δ 170.7, 154.8, 154.4, 152.0, 148.6, 148.1, 138.7, 134.6, 129.3, 123.7, 123.4, 121.5, 119.2, 119.1, 114.1, 113.3, 89.73, 84.2, 82.6, 82.3, 80.6, 54.6, 52.4, 51.7, 49.7, 49.0, 28.5, 27.3, 27.2, 26.9, 26.1, 24.2. HRMS (ESI): calculated for C₃₅H₄₉N₈O₉ [M+H]⁺ 725.3633, found 725.3634.

tert-butyl (2*S*)-4-(((((3*a*,3*a*,4*k*,6*R*,6*a*,*R*,6*a*,*R*,6*a*,*G*,*a*,*G*,*B*)-6-(*6*-amino-9*H*-purin-9-yl)-2,2dimethyltetra-hydrofuro[3,4-d][1,3]dioxol-4-yl)methyl)((*E*)-3-(4-nitrophenyl)allyl)amino)-2-((*tert*-butoxy carbonyl)amino)butanoate (16*r*). Following the procedure described for compound 12*a*, coupling compound 11 (112 mg, 0.20 mmol) with (*E*)-3-(4-nitrophenyl)acrylaldehyde 15*r* (42 mg, 0.24 mmol) afforded compound 16*r* as a white powder (74 mg, 51% yield). ¹H NMR (600 MHz, CDCl₃) δ 8.24 (s, 1H), 8.10 (d, *J* = 7.7 Hz, 2H), 7.93 (s, 1H), 7.34 (d, *J* = 7.9 Hz, 2H), 6.45 – 6.43 (br d, *J* = 15.9 Hz, 1H), 6.36 – 6.31 (m, 1H), 6.24 (s, 2H), 6.08 (s, 1H), 5.62 (d, *J* = 7.7 Hz, 1H), 5.46 (d, *J* = 5.0 Hz, 1H), 5.02 (s, 1H), 4.40 (s, 1H), 4.24 (s, 1H), 3.37 – 3.23 (m, 2H), 2.87 – 2.81 (m, 1H), 2.78 (br d, *J* = 19.4 Hz, 1H), 2.66 (s, 1H), 2.61 – 2.57 (m, 1H), 2.03 – 1.99 (br d, *J* = 20.7 Hz, 1H), 1.84 – 1.73 (m, 1H), 1.61 (s, 3H), 1.42 – 1.40 (br d, *J* = 15.3 Hz, 21H). ¹³C NMR (151 MHz, CDCl₃) δ 171.6, 155.7, 153.0, 149.1, 146.7, 143.2, 140.1, 126.6, 123.9, 120.3, 114.43, 90.7, 85.7, 84.0, 83.3, 81.8, 79.5, 56.9, 56.1, 52.8, 50.8, 29.7, 28.4, 28.0, 27.2, 25.4. HRMS (ESI): calculated for C₃₅H₄₉N₈O₉ [M+H]+725.3633, found 725.3639.

tert-butyl (2S)-4-(((((3a*R*,3a*R*,4*R*,6*R*,6a*R*,6a*R*)-6-(6-amino-9*H*-purin-9-yl)-2,2dimethyltetra-hydrofuro[3,4-d][1,3]dioxol-4-yl)methyl)((*E*)-3-(2-cyanophenyl)allyl)amino)-2-((*tert*-butoxy carbonyl)amino)butanoate (16s). Following the procedure described for compound 12a, coupling compound 11 (112 mg, 0.20 mmol) with (*E*)-2-(3-oxoprop-1-en-1-yl)benzonitrile 15s (58 mg, 0.24 mmol) afforded compound 16s as a white powder (82 mg, 68% yield). ¹H NMR (600 MHz, CDCl₃) δ 8.26 (s, 1H), 7.97 (s, 1H), 7.58 (d, *J* = 7.7 Hz, 1H), 7.54 (d, *J* = 8.0 Hz, 1H), 7.49 (t, *J* = 7.6 Hz, 1H), 7.28 (t, *J* = 7.5 Hz, 1H), 6.78 (br d, *J* = 11.6 Hz, 1H), 6.54 (s, 2H), 6.43 – 6.34 (m, 1H), 6.09 (s, 1H), 5.78 (d, *J* = 8.2 Hz, 1H), 5.47 (d, *J* = 5.6 Hz, 1H), 5.03 (dd, *J* = 6.2, 3.6 Hz, 1H), 4.42 – 4.39 m, 1H), 4.26 – 4.23 (m, 1H), 3.40 – 3.37 (m, 1H), 3.32 – 3.28 (m, 1H), 2.89 – 2.85 (m, 1H), 2.77 – 2.73 (m, 1H), 2.70 – 2.66 (m, 1H), 2.62 – 2.56 (m, 1H), 2.06 – 1.93 (m, 1H), 1.85 – 1.73 (m, 1H), 1.62 (s, 3H), 1.49 – 1.34 (br m, 21H). ¹³C NMR (151 MHz, CDCl₃) δ 171.8, 155.9, 155.5, 153.0, 149.1, 132.7, 128.2, 127.5, 125.6, 120.2, 117.9, 114.4, 110.7, 90.6, 85.4, 83.9, 83.2, 81.6, 57.0, 56.0, 53.5, 52.8, 50.8, 29.6, 28.3, 27.9, 27.2, 25.4. HRMS (ESI): calculated for C₃₆H₄₉N₈O₇ [M+H]⁺ 705.3724, found 705.3734.

tert-butyl (2*S*)-4-((((3a*R*,3a*R*,4*R*,6*R*,6a*R*,6a*R*)-6-(6-amino-9*H*-purin-9-yl)-2,2dimethyltetra-hydrofuro[3,4-d][1,3]dioxol-4-yl)methyl)((*E*)-3-(3-cyanophenyl)allyl)amino)-2-((*tert*-butoxy carbonyl)amino)butanoate (16t). Following the procedure described for compound 12a, coupling compound 11 (112 mg, 0.20 mmol) with (*E*)-3-(3-oxoprop-1-en-1-yl)benzonitrile 15t (58 mg, 0.24 mmol) afforded compound 16t as a white powder (69 mg, 49% yield). ¹H NMR (400 MHz, CDCl₃) δ 8.21 (s, 1H), 7.90 (s, 1H), 7.51 (s, 1H), 7.49 – 7.41 (m, 2H), 7.33 (t, *J* = 7.7 Hz, 1H), 6.33 (d, *J* = 16.0 Hz, 1H), 6.27 – 6.15 (m, 3H), 6.04 (d, *J* = 1.9 Hz, 1H), 5.62 (d, *J* = 8.0 Hz, 1H), 5.43 (d, *J* = 5.9 Hz, 1H), 4.97 (dd, *J* = 6.3, 3.6 Hz, 1H), 4.39 – 4.32 (m, 1H), 4.23 – 4.14 (m, 1H), 3.31 – 3.18 (m, 2H), 2.82 – 2.77 (m, 1H), 2.72 – 2.58 (m, 2H), 2.56 – 2.49 (m, 1H), 2.02 – 1.90 (m, 1H), 1.77 – 1.70 (br, 1H), 1.58 (s, 3H), 1.40 – 1.36 (br m, 21H). ¹³C NMR (151 MHz, CDCl₃) δ 171.7, 155.8, 155.5, 153.0, 149.1, 140.0, 138., 130.7, 130.3, 129.7, 129.3, 120.3, 118.8, 114.4, 112.7, 90.7, 85.6, 83.9, 83.3, 81.7, 79.5, 56.8, 56.0, 52.8, 50.7, 29.6, 28.3, 27.2, 25.4. HRMS (ESI): calculated for C₃₆H₄₉N₈O₇ [M+H]+ 705.3724, found 705.3732.

tert-butyl (2*S*)-4-((((3a*R*,3a*R*,4*R*,6*R*,6a*R*,6a*R*)-6-(6-amino-9*H*-purin-9-yl)-2,2dimethyltetra-hydrofuro[3,4-d][1,3]dioxol-4-yl)methyl)((*E*)-3-(4-cyanophenyl)allyl)amino)-2-((*tert*-butoxy carbonyl)amino)butanoate (16u). Following the procedure described for compound 12a, coupling compound 11 (112 mg, 0.20 mmol) with (*E*)-4-(3-oxoprop-1-en-1-yl)benzonitrile 15u (58 mg, 0.24 mmol) afforded compound 16u as a white powder (93 mg, 66% yield). ¹H NMR (400 MHz, CDCl₃) δ 8.23 (s, 1H), 7.90 (s, 1H), 7.53 (d, *J* = 8.4 Hz, 2H), 7.30 (d, *J* = 8.3 Hz, 2H), 6.40 - 6.36 (br d, *J* = 16.0, 1H), 6.33 - 6.22 (m, 1H), 6.08 - 5.91 (m, 3H), 5.53 (d, *J* = 8.0 Hz, 1H), 5.44 (d, *J* = 6.0 Hz, 1H), 5.03 - 4.94 (m, 1H), 4.42 - 4.32 (m, 1H), 4.20 (d, *J* = 4.9 Hz, 1H), 3.36 - 3.20 (m, 2H), 2.84 - 2.79 (m, 1H), 2.72 (d, *J* = 5.2 Hz, 1H), 2.68 - 2.59 (m, 1H), 2.58 -2.50 (m, 1H), 2.03 - 1.91 (m, 1H), 1.75 (d, *J* = 9.6 Hz, 1H), 1.59 (s, 3H), 1.42 - 1.37 (br m, 21H). ¹³C NMR (151 MHz, CDCl₃) δ 171.6, 155.6, 153.0, 149.1, 140.1, 132.3, 126.7, 120.3, 119.0, 114.5, 110.6, 90.7, 85.7, 83.9, 81.8, 79.5, 56.9, 56.1, 53.4, 52.8, 50.7, 29.1, 28.3, 27.2, 25.5. HRMS (ESI): calculated for C₃₆H₄₉N₈O₇ [M+H]⁺705.3724, found 705.3738.

tert-butyl (2S)-4-(((((3a*R*,3a*R*,4*R*,6*R*,6a*R*,6a*R*)-6-(6-amino-9*H*-purin-9-yl)-2,2dimethyltetra-hydrofuro[3,4-d][1,3]dioxol-4-yl)methyl)((*E*)-3-(3-carbamoylphenyl)allyl)amino)-2-((*tert*-butoxy carbonyl)amino)butanoate (16v). To a solution of compound 16t (0.21 mmol, 150 mg) in DMSO (10 mL) was added KOH (0.25 mmol, 14 mg). The mixture was cooled to 0 °C and treated with H₂O₂ (30 % w/w) in H₂O (0.5 mL). The reaction mixture was warmed to room temperature and stirred for 3 hours at room temperature. The reaction was diluted with water and extracted with EtOAc (3x). The combined organic layers were dried over Na₂SO₄. The solvent was evaporated, and the crude product was purified by column chromatography (5% MeOH in EtOAc) to give compound **16v** as a white powder (127 mg, 82% yield). ¹H NMR (400 MHz, CDCl₃) δ 8.17 (s, 1H), 7.94 (s, 1H), 7.78 (s, 1H), 7.70 (d, *J* = 7.4 Hz, 1H), 7.37 – 7.24 (m, 3H), 7.03 (s, 1H), 6.64 (s, 2H), 6.32 (d, *J* = 15.8 Hz, 1H), 6.23 – 6.12 (m, 1H), 6.06 (d, *J* = 1.5 Hz, 1H), 5.89 (s, 1H), 5.43 (d, *J* = 6.2 Hz, 1H), 4.97 (dd, *J* = 6.2, 3.4 Hz, 1H), 4.39 – 4.35 (m, 1H), 4.22 – 4.17 (m, 1H), 3.26 – 3.13 (m, 2H), 2.78 – 2.55 (m, 4H), 1.97 (dd, *J* = 13.5, 6.0 Hz, 1H), 1.81 – 1.70 (m, 1H), 1.58 (s, 3H), 1.40 – 1.36 (br m, 21H). ¹³C NMR (101 MHz, CDCl₃) δ 172.0, 170.1, 156.0, 155.7, 153.0, 149.0, 137.1, 134.0, 128.7, 128.0, 126.6, 120.1, 114.3, 90.7, 85.8, 84.0, 83.4, 81.8, 79.5, 57.0, 50.6, 45.9, 29.7, 28.4, 28.0, 27.2, 25.5. HRMS (ESI): calculated for C₃₆H₅₁N₈O₈ [M+H]+723.3830, found 723.3838.

tert-butyl (*S*)-4-((((3a*R*,4*R*,6*R*,6a*R*)-6-(6-amino-9*H*-purin-9-yl)-2,2dimethyltetrahydrofuro[3,4-*d*][1,3]dioxol-4-yl)methyl)((*E*)-3-(4-carbamoylphenyl)allyl)amino)-2-((*tert*-butoxycarbonyl) amino)butanoate (16w). Following the procedure described for compound 16v, compound 16u was oxidized to afford compound 16w as a white powder (118 mg, 77% yield). ¹H NMR (400 MHz, CDCl₃) δ 8.09 (s, 1H), 7.92 (s, 1H), 7.75 (d, *J* = 8.0 Hz, 2H), 7.20 (d, *J* = 8.3 Hz, 2H), 6.72 (s, 2H), 6.34 – 6.30 (br d, *J* = 16 Hz, 1H), 6.20 – 6.10 (m, 1H), 6.04 (d, *J* = 1.6 Hz, 1H), 5.79 (d, *J* = 8.0 Hz, 1H), 5.41 (d, *J* = 6.2 Hz, 1H), 4.98 (dd, *J* = 6.1, 3.7 Hz, 1H), 4.38 – 4.34 (m, 1H), 4.21 – 4.17 (m, 1H), 3.22 (d, *J* = 5.6 Hz, 2H), 3.07 – 3.02 (br m, 1H), 2.80 – 2.67 (m, 2H), 2.61 – 2.55 (m, 2H), 1.58 (s, 3H), 1.44 – 1.36 (br m, 21H). ¹³C NMR (101 MHz, CDCl₃) δ 171.9, 169.9, 156.0, 155.6, 152.9, 149.0, 140.2, 132.3, 131.7, 127.9, 126.2, 90.7, 85.7, 84.0, 83.3, 81.8, 79.5, 56.9, 55.8, 52.9, 45.9, 30.3, 29.7, 28.4, 28.0, 27.2, 25.5. HRMS (ESI): calculated for C₃₆H₅₁N₈O₈ [M+H]+723.3830, found 723.3832.

tert-butyl (2*S*)-4-(((((3a*R*,4*R*,6*R*,6a*R*)-6-(6-amino-9*H*-purin-9-yl)-2,2dimethyltetrahydrofuro[3,4-d][1,3]dioxol-4-yl)methyl)(cinnamyl)amino)-2-((*tert*-

butoxycarbonyl)amino)butanoate (16x). Following the procedure described for compound **12a**, coupling compound **11** (112 mg, 0.20 mmol) with cinnamaldehyde **15x** (32 mg, 0.24 mmol) afforded compound **16x** as a white powder (110 mg, 50% yield). ¹H NMR (400 MHz, CDCl₃) δ 8.29 (s, 1H), 7.96 (s, 1H), 7.34 – 7.21 (m, 5H), 7.28 (s, 1H), 6.46 – 6.42 (br d, *J* = 16.0 Hz, 1H), 6.31 – 6.16 (m, 3H), 6.08 (d, *J* = 1.7 Hz, 1H), 5.71 (d, *J* = 8.1 Hz, 1H), 5.48 (d, *J* = 5.1 Hz, 1H), 5.02 – 5.00 (m, 1H), 4.50 – 4.35 (m, 1H), 4.22 (d, *J* = 7.4 Hz, 1H), 3.38 – 3.22 (m, 2H), 2.88 – 2.83 (m, 1H), 2.77 – 2.51 (m, 3H), 2.06 – 1.92 (m, 1H), 1.84 – 1.79 (m, 1H), 1.64 (s, 3H), 1.44

- 1.42 (br m, 21H). ¹³C NMR (101 MHz, CDCl₃) δ 171.8, 155.8, 153.1, 149.3, 140.0, 137.3, 134.1, 133.0, 129.3, 126.3, 125.3, 120.3, 114,5, 90.8, 85.5, 83.9, 83.4, 52.9, 50.6, 29.5, 28.41, 28.0, 27.2, 25.5, 21.2. HRMS (ESI): calculated for C₃₅H₅₀N₇O₇ [M+H]⁺ 680.3772, found 680.3780.

tert-butyl (2S)-4-(((((3a*R*,3a*R*,4*R*,6*R*,6a*R*,6a*R*)-6-(6-amino-9*H*-purin-9-yl)-2,2dimethyltetra-hydrofuro[3,4-d][1,3]dioxol-4-yl)methyl)((*E*)-3-(4-

((trimethylsilyl)ethynyl)phenyl)allyl)amino)-2-((*tert*-butoxycarbonyl)amino)butanoate (16y). Following the procedure described for compound **12a**, coupling compound **11** (112 mg, 0.20 mmol) with (*E*)-3-(4-((trimethylsilyl)ethynyl)phenyl)acrylaldehyde **15y** (55 mg, 0.24 mmol) afforded compound **16y** as a white powder (98 mg, 63% yield). ¹H NMR (400 MHz, CDCl₃) δ 8.27 (s, 1H), 7.93 (s, 1H), 7.38 (d, *J* = 8.4 Hz, 2H), 7.22 (d, *J* = 8.4 Hz, 2H), 6.40 – 6.36 (br d, *J* = 16.0, 1H), 6.25 – 6.15 (m, 1H), 6.19 – 6.06 (m, 2H), 5.68 – 5.66 (br d, *J* = 8.2 Hz, 1H), 5.47 – 5.45 (br d, *J* = 8.6 Hz, 1H), 5.04 – 4.94 (m, 1H), 4.39 (d, *J* = 4.9 Hz, 1H), 4.24 – 4.19 (m, 1H), 3.35 – 3.30 (br m, 1H), 3.25 – 3.19 (br m, 1H), 2.86 – 2.81 (m, 1H), 2.74 – 2.47 (m, 4H), 2.01 – 1.96 (br, 1H), 1.81 – 1.78 (br, 1H), 1.61 (s, 3H), 1.42 – 1.39 (br m, 21H), 0.25 (s, 9H). ¹³C NMR (101 MHz, CDCl₃) δ 171.8, 155.6, 153.1, 149.2, 140.1, 137.0, 132.2, 126.1, 122.2, 120.3, 114.5, 105.2, 90.8, 85.5, 83.9, 83.3, 79.5, 57.0, 56.0, 52.9, 50.7, 29.5, 28.0, 27.2, 25.5. HRMS (ESI): calculated for C₄₀H₅₈NrOrSi [M+H]+776.4167, found 776.4172.

(*S*)-2-amino-4-((((2*R*,3*S*,4*R*,5*R*)-5-(6-amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl)((*E*)-3-(o-tolyl)allyl)amino)butanoic acid (17a). Following the procedure described for compound 13a, compound 16a (50 mg, 0.072 mmol) was deprotected and purified, affording compound 17a as a white powder (31 mg, 71% yield). ¹H NMR (400 MHz, CD₃OD) δ 8.50 (s, 1H), 8.21 (s, 1H), 7.38 (d, *J* = 7.5 Hz, 1H), 7.23 – 7.11 (m, 3H), 6.99 – 6.5 (br d, *J* = 16.0 Hz, 1H), 6.29 – 6.23 (m, 2H), 4.69 (t, *J* = 4.2 Hz, 1H), 4.59 (d, *J* = 6.3 Hz, 2H), 4.19 – 4.08 (m, 3H), 3.91 – 3.85 (m, 1H), 3.75 – 3.55 (m, 3H), 2.59 – 2.49 (m, 1H), 2.38 – 2.32 (m, 1H), 2.20 (s, 3H). ¹³C NMR (101 MHz, CD₃OD) δ 170.53, 151.1, 147.9, 144.7, 143.2, 138.8, 135.8, 134.1, 130.1, 128.7, 126.0, 125.4, 119.63, 116.9, 91.0, 78.9. 735.5, 72.3, 54.39, 51.2, 51.0, 25.02, 18.3. HRMS (ESI): calculated for C₂₄H₃₂N₇O₅ [M+H]⁺ 498.2465, found 498.2572.

(*S*)-2-amino-4-((((2R,3S,4R,5R)-5-(6-amino-9*H*-purin-9-yl)-3,4dihydroxytetrahydrofuran-2-yl)methyl)((*E*)-3-(m-tolyl)allyl)amino)butanoic acid (17b). Following the procedure described for compound 13a, compound 16b (50 mg, 0.072 mmol) was deprotected and purified, affording compound 17b as a white powder (32 mg, 73% yield). ¹H NMR (400 MHz, CD₃OD) δ 8.48 (s, 1H), 8.23 (s, 1H), 7.21 (t, *J* = 7.5 Hz, 1H), 7.11 (dd, *J* = 18.9, 8.4 Hz, 3H), 6.69 (d, *J* = 15.8 Hz, 1H), 6.24 (dt, *J* = 15.3, 7.3 Hz, 1H), 6.17 (d, *J* = 3.6 Hz, 1H), 4.66 (t, *J* = 4.1 Hz, 1H), 4.56 (d, *J* = 6.8 Hz, 2H), 4.10 (dd, *J* = 8.3, 5.0 Hz, 3H), 3.88 (dd, *J* = 13.9, 10.1 Hz, 1H), 3.70 – 3.52 (m, 3H), 2.55 – 2.46 (m, 1H), 2.33 (s, 3H), 2.31 – 2.27 (m, 1H). ¹³C NMR (101 MHz, CD₃OD) δ 170.3, 151.3, 148.0, 145.0, 143.0, 141.0, 138.3, 135.1, 129.5, 127.0, 123.6, 119.8, 115.3, 91.1, 78.9, 72.3, 54.4, 51.2, 50.9.5.0. 20.0. HRMS (ESI): calculated for C_{24H32}N₇O₅ [M+H]⁺ 498.2465, found 498.2574.

(*S*)-2-amino-4-((((2*R*,3*S*,4*R*,5*R*)-5-(6-amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl)((*E*)-3-(p-tolyl)allyl)amino)butanoic acid (17c). Following the procedure described for compound 13a, compound 16c (50 mg, 0.072 mmol) was deprotected and purified, affording compound 17c as a white powder (31 mg, 70% yield). ¹H NMR (400 MHz, D₂O) δ 8.47 (s, 1H), 8.22 (s, 1H), 7.16 – 7.09 (m, 4H), 6.69 – 6.65 (br d, *J* = 16.0 Hz, 1H), 6.21 – 6.13 (m, 2H), 4.65 (t, *J* = 4.0 Hz, 1H), 4.54 (d, *J* = 6.2 Hz, 2H), 4.10 – 4.06 (m, 3H), 3.88 – 3.82 (m, 1H), 3.71 – 3.49 (m, 3H), 2.57 – 2.43 (m, 1H), 2.32 – 2.27 (m, 4H). ¹³C NMR (101 MHz, D₂O) δ 168.7, 163.1, 162.8, 152.7, 149.4, 146.3, 144.5, 142.3, 140.5, 133.73, 127.8, 121.1, 119.4, 116.5, 115.7, 92.4, 80.4, 75.0, 73.7, 55.8, 52.3, 26.40, 21.4. HRMS (ESI): calculated for C₂₄H₃₂N₇O₅ [M+H]+ 498.2465, found 498.2570.

(*S*)-2-amino-4-((((2*R*,3*S*,4*R*,5*R*)-5-(6-amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl)((*E*)-3-(2-methoxyphenyl)allyl)amino)butanoic acid (17d). Following the procedure described for compound 13a, compound 16d (50 mg, 0.070 mmol) was deprotected and purified, affording compound 17d as a white powder (32 mg, 72% yield). ¹H NMR (400 MHz, CD₃OD) 8 8.46 (s, 1H), 8.16 (s, 1H), 7.33 – 7.24 (m, 2H), 6.96 – 6.85 (m, 3H), 6.27 – 6.21 (m, 1H), 6.15 (d, *J* = 3.4 Hz, 1H), 4.62 (dd, *J* = 4.8, 3.5 Hz, 1H), 4.59 – 4.50 (m, 2H), 4.15 – 4.02 (m, 3H), 3.90 (dd, *J* = 13.9, 9.7 Hz, 1H), 3.77 (s, 3H), 3.69 – 3.51 (m, 3H), 2.55 – 2.45 (m, 1H), 2.36 – 2.28 (m, 1H). ¹³C NMR (101 MHz, CD₃OD) δ 170.3, 157.0, 151.0, 147.9, 144.4, 143.2, 136.0, 130.1, 127.0, 123.56, 119.8, 115.8, 110.8, 91.2, 79.0, 73.6, 72.3, 55.9, 54.6, 54.2, 50.8, 25.0. HRMS (ESI): calculated for C₂₄H₃₂N₇O₆ [M+H]⁺ 514.2414, found 514.2422.

(*S*)-2-amino-4-((((2*R*,3*S*,4*R*,5*R*)-5-(6-amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl)((*E*)-3-(3-methoxyphenyl)allyl)amino)butanoic acid (17e). Following the procedure described for compound 13a, compound 16e (50 mg, 0.070 mmol) was deprotected and purified, affording compound 17e as a white powder (34 mg, 77% yield). ¹H NMR (400 MHz, CD₃OD) δ 8.46 (s, 1H), 8.21 (s, 1H), 7.21 (t, *J* = 7.9 Hz, 1H), 6.89 – 6.83 (m, 2H), 6.77 (s, 1H), 6.68 – 6.64 (br d, *J* = 16.0 Hz, 1H), 6.27 – 6.19 (m, 1H), 6.15 (d, *J* = 3.5 Hz, 1H), 4.66 – 4.61 (m, 1H), 4.56 – 4.52 (m, 2H), 4.12 – 4.02 (m, 3H), 3.89 – 3.83 (m, 1H), 3.78 (s, 3H), 3.69 – 3.50 (m, 3H), 2.54 – 2.44 (m, 1H), 2.34 – 2.27 (m, 1H). ¹³C NMR (101 MHz, CD₃OD) δ 170.3, 160.0, 151.2, 148.0, 144.8, 143.1, 140.8, 136.5, 129.5, 119.8, 118.8, 91.1, 78.9, 73.6, 72.3, 54.4, 50.9, 25.0. HRMS (ESI): calculated for C₂₄H₃₂N₇O₆ [M+H]⁺ 514.2414, found 514.2419.

(*S*)-2-amino-4-((((2*R*,3*S*,4*R*,5*R*)-5-(6-amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl)((*E*)-3-(4-methoxyphenyl)allyl)amino)butanoic acid (17f). Following the procedure described for compound 13a, compound 16f (50 mg, 0.070 mmol) was deprotected and purified, affording compound 17f as a white powder (35 mg, 80% yield). ¹H NMR (400 MHz, D₂O) δ 8.47 (s, 1H), 8.24 (s, 1H), 7.21 (d, *J* = 8.5 Hz, 2H), 6.84 (d, *J* = 8.7 Hz, 2H), 6.65 (d, *J* = 15.7 Hz, 1H), 6.15 (d, *J* = 3.6 Hz, 1H), 6.07 – 6.03 (m, 1H), 4.66 (t, *J* = 4.0 Hz, 1H), 4.54 (d, *J* = 6.4 Hz, 2H), 4.12 – 4.01 (m, 3H), 3.88 – 3.82 (m, 4H), 3.70 – 3.47 (m, 3H), 2.54 – 2.44 (m, 1H), 2.34 – 2.26 (m, 1H). ¹³C NMR (101 MHz, D₂O) δ 169.0, 161.9, 152.7, 149.44, 146.3, 144.5, 142.0, 129.3, 129.1, 121.1, 115.2, 114.1, 92.4, 80.4, 75.0, 73.7, 55.8, 52.5, 52.1, 26.4. HRMS (ESI): calculated for C₂₄H₃₂N₇O₆ [M+H]⁺ 514.2414, found 514.2425.

(*S*)-2-amino-4-((((2*R*,3*S*,4*R*,5*R*)-5-(6-amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl)((*E*)-3-(2-fluorophenyl)allyl)amino)butanoic acid (17g). Following the procedure described for compound 13a, compound 16g (50 mg, 0.072 mmol) was deprotected and purified, affording compound 17g as a white powder (31 mg, 68% yield). ¹H NMR (400 MHz, D₂O) δ 8.30 (s, 1H), 8.04 (s, 1H), 7.56 – 7.52 (m, 1H), 7.38 (d, *J* = 5.4 Hz, 2H), 7.21 (s, 1H), 6.85 – 6.56 (m, 1H), 6.10 – 6.05 (m, 1H), 6.02 – 5.93 (m, 1H), 4.70 (dd, *J* = 7.1, 5.6 Hz, 1H), 4.57 (dd, *J* = 5.5, 2.4 Hz, 1H), 4.43 (s, 1H), 4.11 – 4.06 (m, 2H), 3.96 (s, 1H), 3.86 – 3.76 (m, 1H), 3.66 – 3.51 (m, 3H), 2.55 – 2.41 (m, 1H), 2.36 – 2.30 (m, 1H). ¹³C NMR (101 MHz, D₂O) δ 171.7, 163.2, 162.9, 162.5, 162.2, 149.0, 147.1, 144.0, 133.2, 130.9, 129.9, 128.7, 120.6, 118.7, 117.7, 114.8, 111.9, 91.1, 72.9, 72.3, 50.9, 25.0. HRMS (ESI): calculated for C₂₃H₂₉FN₇O₅ [M+H]+ 502.2214, found 502.2215.

(*S*)-2-amino-4-((((2*R*,3*S*,4*R*,5*R*)-5-(6-amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl)((*E*)-3-(3-fluorophenyl)allyl)amino)butanoic acid (17h). Following the procedure described for compound 13a, compound 16h (50 mg, 0.072 mmol) was deprotected and purified, affording compound 17h as a white powder (30 mg, 67% yield). ¹H NMR (400 MHz, D₂O) δ 8.30 (s, 1H), 8.04 (s, 1H), 7.20 – 7.15 (m, 1H), 6.96 (t, *J* = 8.4 Hz, 1H), 6.75 (d, *J* = 7.6 Hz, 1H), 6.68 (d, *J* = 9.4 Hz, 1H), 6.30 (s, 1H), 6.08 (s, 1H), 6.00 – 5.92 (m, 1H), 4.67 (d, *J* = 6.1 Hz, 1H), 4.54 – 4.48 (m, 1H), 4.39 (s, 1H), 4.07 – 3.76 (m, 4H), 3.46 – 3.45 (m, 3H), 2.42 – 2.36 (m, 1H), 2.30 – 2.15 (m, 1H). ¹³C NMR (101 MHz, D₂O) δ 172.1, 149.3, 147.2, 144.1, 143.6, 137.0, 130.5, 122.3, 119.2, 115.8, 115.6, 112.42, 112.19, 91.4, 72.0, 51.8. HRMS (ESI): calculated for C₂₃H₂₉FN₇O₅ [M+H]⁺ 502.2214, found 502.2218. (*S*)-2-amino-4-((((2*R*,3*S*,4*R*,5*R*)-5-(6-amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl)((*E*)-3-(4-fluorophenyl)allyl)amino)butanoic acid (17i). Following the procedure described for compound 13a, compound 16i (50 mg, 0.072 mmol) was deprotected and purified, affording compound 17i as a white powder (34 mg, 76% yield). ¹H NMR (400 MHz, D₂O) δ 8.32 (s, 1H), 8.02 (s, 1H), 6.98 – 6.81 (m, 4H), 6.28 (br s, 1H), 6.09 (s, 1H), 5.89 – 5.82 (m, 1H), 4.70 (dd, *J* = 6.9, 5.5 Hz, 1H), 4.55 – 4.53 (m, 1H), 4.43 (br s, 1H), 4.11 – 3.79 (m, 4H), 3.65 – 3.47 (m, 3H), 2.52 – 2.38 (m, 1H), 2.31 (s, 1H). ¹³C NMR (101 MHz, D₂O) δ 171.5, 163.8, 163.0, 161.3, 149.2, 147.1, 144.0, 143.5, 138.8, 131.0, 128.0, 127.9, 115.6, 115.3, 114.0, 112.0, 91.3, 73.6, 51.2, 24.9. HRMS (ESI): calculated for C₂₃H₂₉FN₇O₅ [M+H]+ 502.2214, found 502.2216.

(*S*)-2-amino-4-((((2*R*,3*S*,4*R*,5*R*)-5-(6-amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl)((*E*)-3-(2-chlorophenyl)allyl)amino)butanoic acid (17j). Following the procedure described for compound 13a, compound 16j (50 mg, 0.070 mmol) was deprotected and purified, affording compound 17j as a white powder (28 mg, 63% yield). ¹H NMR (400 MHz, D₂O) δ 8.31 (d, *J* = 10.5 Hz, 1H), 8.00 (br d, *J* = 12.0 Hz, 1H), 7.46 – 6.98 (m, 4H), 6.58 – 6.38 (br d, *J* = 80.0 Hz, 1H), 6.09 (d, *J* = 10.8 Hz, 1H), 6.00 – 5.88 (m, 1H), 4.74 – 4.68 (m, 1H), 4.50 (dd, *J* = 5.4, 2.2 Hz, 1H), 4.43 (s, 1H), 4.15 – 3.81 (m, 4H), 3.67 – 3.48 (m, 3H), 2.47 (s, 1H), 2.36 – 2.30 (m, 1H). ¹³C NMR (101 MHz, D₂O) δ 171.1, 163.3, 163.0, 162.6, 149.0, 147.0, 144.1, 143.4 138.5, 132.3, 130.3, 129.5, 127.3, 126.33, 125.4, 120.6, 119.1, 117.7, 114.9, 112.0, 91.4, 73.5, 72.0, 55.7, 50.9, 25.0. HRMS (ESI): calculated for C₂₃H₂₉ClN₇O₅ [M+H]+ 518.1919, found 518.1922.

(*S*)-2-amino-4-((((2*R*,3*S*,4*R*,5*R*)-5-(6-amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl)((*E*)-3-(3-chlorophenyl)allyl)amino)butanoic acid (17k). Following the procedure described for compound 13a, compound 16k (50 mg, 0.070 mmol) was deprotected and purified, affording compound 17k as a white powder (28 mg, 63% yield). ¹H NMR (400 MHz, CD₃OD) δ 8.48 (s, 1H), 8.27 (s, 1H), 7.34 – 7.23 (m, 4H), 6.73 (br d, *J* = 16.0 Hz, 1H), 6.37 – 6.29 (m, 1H), 6.16 (d, *J* = 3.8 Hz, 1H), 4.68 – 4.64 (m, 1H), 4.58 – 4.49 (m, 2H), 4.08 (dd, *J* = 8.3, 4.5 Hz, 3H), 3.86 – 3.80 (m, 1H), 3.63 – 3.53 (m, 3H), 2.54 – 2.44 (m, 1H), 2.33 – 2.25 (m, 1H). ¹³C NMR (101 MHz, CD₃OD) δ 170.5, 162.0, 161.2, 151.3, 148.1, 145.0, 143.0, 139.2, 137.3, 134.4, 130.0, 128.6, 126.2, 125.0, 119.6, 118.0, 117.6, 115.1, 54.66, 78.9, 73.6, 72.2, 54.7, 51.2, 51.0, 25.0, 22.9. HRMS (ESI): calculated for C₂₃H₂₉ClN₇O₅ [M+H]+ 518.1919, found 518.1928.

(*S*)-2-amino-4-((((2*R*,3*S*,4*R*,5*R*)-5-(6-amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl)((*E*)-3-(4-chlorophenyl)allyl)amino)butanoic acid (17l). Following the procedure described for compound 13a, compound 16l (50 mg, 0.070 mmol) was deprotected and purified, affording compound 17l as a white powder (30 mg, 69% yield). ¹H NMR (400 MHz, D₂O) δ 8.33 (s, 1H), 8.05 (d, *J* = 8.2 Hz, 1H), 7.14 (d, *J* = 7.8 Hz, 2H), 6.87 (d, *J* = 8.3 Hz, 2H), 6.27 (br s, 1H), 6.10 (s, 1H), 5.97 – 5.89 (m, 1H), 4.72 (dd, *J* = 6.9, 5.6 Hz, 1H), 4.56 – 4.38 (m, 2H), 4.10 – 3.79 (m, 4H), 3.64 – 3.50 (m, 3H), 2.53 – 2.39 (m, 1H), 2.37 – 2.24 (m, 1H). ¹³C NMR (101 MHz, D₂O) δ 171.8, 163.0, 162.7, 149.1, 147.1, 144.0, 1435, 138.6, 134.0, 133.2, 128.6, 127.4, 119.0, 117.8, 114.9, 91.4, 73.6, 72.0, 25.0. HRMS (ESI): calculated for C₂₃H₂₉ClN₇O₅ [M+H]⁺ 518.1919, found 518.1925.

(*S*)-2-amino-4-((((2*R*,3*S*,4*R*,5*R*)-5-(6-amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl)((*E*)-3-(2-bromophenyl)allyl)amino)butanoic acid (17m). Following the procedure described for compound 13a, compound 16m (50 mg, 0.066 mmol) was deprotected and purified, affording compound 17m as a white powder (26 mg, 59% yield). ¹H NMR (400 MHz, D₂O) δ 8.21 (s, 1H), 7.86 (s, 1H), 7.29 (d, *J* = 9.3 Hz, 1H), 7.03 (d, *J* = 7.2 Hz, 3H), 6.42 (s, 1H), 5.99 (s, 1H), 5.84 - 5.77 (m, 1H), 4.65 - 4.60 (m, 1H), 4.42 (dd, *J* = 5.4, 2.2 Hz, 1H), 4.34 (s, 1H), 4.02 (dd, *J* = 8.4, 4.9 Hz, 1H), 3.84 - 3.74 (m, 2H), 3.57 - 3.41 (m, 3H), 2.38 (br s, 1H), 2.29 - 2.20 (m, 1H). ¹³C NMR (101 MHz, D₂O) δ 171.1,163.3, 163.0, 162.2, 149.0, 146.9, 144.1, 143.4, 132.7, 130.5, 127.9, 126.5, 122.7, 120.6, 119.1, 117.7, 114.8, 111.9, 91.5, 73.5,72.0, 55.5, 50.8, 251. HRMS (ESI): calculated for C₂₃H₂₉BrN₇O₅ [M+H]⁺ 562.1414, found 562.1427.

(*S*)-2-amino-4-((((2*R*,3*S*,4*R*,5*R*)-5-(6-amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl)((*E*)-3-(3-bromophenyl)allyl)amino)butanoic acid (17n). Following the procedure described for compound 13a, compound 16n (50 mg, 0.066 mmol) was deprotected and purified, affording compound 17n as a white powder (28 mg, 64% yield). ¹H NMR (400 MHz, D₂O) δ 8.33 (s, 1H), 8.05 (s, 1H), 7.38 – 7.35 (m, 1H), 7.09 (t, *J* = 7.8 Hz, 1H), 7.00 (s, 1H), 6.95 (d, *J* = 7.8 Hz, 1H), 6.10 (s, 2H), 5.99 – 5.92 (m, 1H), 4.75 – 4.69 (m, 1H), 4.52 – 4.43 (m, 2H), 4.09 – 3.91 (m, 4H), 3.66 – 3.51 (m, 3H), 2.53 – 2.45 (m, 1H), 2.36– 2.30 (m, 1H). ¹³C NMR (101 MHz, D₂O) δ 171.3, 163.4, 162.7, 162.3, 149.0, 147.0, 144.0, 143.4, 138.4, 136.7, 131.6, 130.4, 128.5, 124.9, 122.2, 120.7, 119.1, 117.8, 114.9, 91.5, 73.7, 71.90, 51.1, 25.0. HRMS (ESI): calculated for C₂₃H₂₉BrN₇O₅ [M+H]⁺ 562.1414, found 562.1425.

(*S*)-2-amino-4-((((2*R*,3*S*,4*R*,5*R*)-5-(6-amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl)((*E*)-3-(4-bromophenyl)allyl)amino)butanoic acid (17o). Following the procedure described for compound 13a, compound 16o (50 mg, 0.066 mmol) was deprotected and purified, affording compound 17o as a white powder (33 mg, 75% yield).¹H NMR (400 MHz, D₂O) δ 8.33 (s, 1H), 7.93 (s, 1H), 7.21 – 7.07 (m, 2H), 6.65 (d, *J* = 8.3 Hz, 2H), 6.00 (s, 2H), 5.86 – 5.78 (m, 1H), 4.62 (d, *J* = 6.3 Hz, 1H), 4.42 – 4.33 (m, 2H), 4.04 – 3.79 (m, 4H), 3.66 – 3.41 (m, 3H), 2.41 – 2.21 (m, 2H). ¹³C NMR (101 MHz, D₂O) δ 171.3, 163.0, 162.6, 162.3 149.0, 147.0, 143.9, 143.4, 138.7, 135.5, 131.5, 127.6, 122.3, 120.7, 119.0, 117.8, 114.9, 91.4, 73.7, 71.9, 51.1, 25.0. HRMS (ESI): calculated for C_{23H29}BrN₇O₅ [M+H]⁺ 562.1414, found 562.1421. (*S*)-2-amino-4-((((2*R*,3*S*,4*R*,5*R*)-5-(6-amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl)((*E*)-3-(2-nitrophenyl)allyl)amino)butanoic acid (17p). Following the procedure described for compound 13a, compound 16p (50 mg, 0.069 mmol) was deprotected and purified, affording compound 17p as a white powder (18 mg, 43% yield). ¹H NMR (400 MHz, D₂O) δ 8.29 (s, 1H), 8.06 (s, 1H), 7.83 – 7.78 (m, 1H), 7.47 (s, 1H), 7.41 (d, *J* = 9.2 Hz, 1H), 7.27 (s, 1H), 6.72 (d, *J* = 13.6 Hz, 1H), 6.07 (s, 1H), 6.00 – 5.93 (m, 1H), 4.67 – 4.59 (m, 2H), 4.49 – 4.40 (m, 1H), 4.13 – 3.91 (m, 3H), 3.80 – 3.73 (m, 1H), 3.68 – 3.49 (m, 3H), 2.50 – 2.43(s, 1H), 2.37 – 2.29 (m, 1H). ¹³C NMR (101 MHz, D₂O) δ 170.8, 149.2, 147.3, 146.4, 143.9, 143.8, 136.1, 134.1,130.1, 129.8, 124.6, 117.7, 114.8, 111.9, 91.0, 73.2, 71.9, 67.9, 66.5, 50.6, 24.8, 17.9. HRMS (ESI): calculated for C₂₃H₂₉N₈O₇ [M+H]+529.2159, found 529.2166.

(*S*)-2-amino-4-((((2*R*,3*S*,4*R*,5*R*)-5-(6-amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl)((*E*)-3-(3-nitrophenyl)allyl)amino)butanoic acid (17q). Following the procedure described for compound 13a, compound 16q (50 mg, 0.069 mmol) was deprotected and purified, affording compound 17q as a white powder (20 mg, 45% yield). ¹H NMR (500 MHz, CD₃OD) & 8.51 (s, 1H), 8.27 (s, 1H), 7.45 (d, *J* = 8.4 Hz, 2H), 7.24 (d, *J* = 8.1 Hz, 2H), 5.02 (s, 2H), 4.69 (t, *J* = 4.5 Hz, 1H), 4.08 (h, *J* = 7.7 Hz, 3H), 3.85 – 3.80 (m, 1H), 3.68 (6.10 (d, *J* = 8.1 Hz, 1H), 3.64 – 3.55 (m, 2H), 2.54 – 2.46 (m, 1H), 2.32 – 2.24 (m, 1H). ¹³C NMR (126 MHz, CD₃OD) & 171.1, 161.9, 161.8, 161.6, 151.4, 148.2, 139.5, 134.3, 131.6, 128.3, 122.6, 119.5, 116.7. 90.5, 79.1, 73.6, 72.2, 54.7, 51.6, 51.2, 29.8, 25.0. HRMS (ESI): calculated for C₂₃H₂₉N₈O₇ [M+H]+ 529.2159, found 529.2162.

(*S*)-2-amino-4-((((2*R*,3*S*,4*R*,5*R*)-5-(6-amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl)((*E*/*Z*)-3-(4-nitrophenyl)allyl)amino)butanoic acid (17r, mixture of isomers). Following the procedure described for compound 13a, compound 16r (50 mg, 0.069 mmol) was deprotected and purified, affording compound 17r as a pink powder (mixture of E- and Z-isomers, 23 mg, 51% yield). ¹H NMR (500 MHz, CD₃OD) δ 8.48 (s, 1H), 8.38 (d, *J* = 6.2 Hz, 1H), 8.30 (s, 1H), 8.20 – 8.12 (m, 3H), 7.54 (s, 2H), 7.42 (d, *J* = 7.1 Hz, 1H), 7.01 – 6.85 (br m, 2H), 6.56 – 6.51 (m, 1H), 6.17 (d, *J* = 3.6 Hz, 1H), 6.12 – 5.99 (m, 1H), 4.70 – 4.66 (m, 1H), 4.61 – 4.53 (m, 3H), 4.42 – 4.35 (m, 1H), 4.30 (d, *J* = 6.3 Hz, 1H), 4.21 – 4.04 (m, 4H), 3.90 – 3.82 (m, 1H), 3.79 – 3.50 (m, 5H), 2.56 – 2.19 (m, 3H). (E/Z mixture). ¹³C NMR (126 MHz, CD₃OD) δ 171.1, 153.9, 148.4, 147.71, 141.6, 137.7, 134.5, 129.5, 123.6, 123.3, 121.6, 90.8, 79.3, 73.4, 73.3, 72.4, 72.1, 54.8,52.0, 25.0. HRMS (ESI): calculated for C₂₃H₂₉N₈O₇ [M+H]+ 529.2159, found 529.2178.

(S)-2-amino-4-((((2R,3S,4R,5R)-5-(6-amino-9H-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl)((E)-3-(2-cyanophenyl)allyl)amino)butanoic acid (17s). Following the procedure described for compound **13a**, compound **16s** (50 mg, 0.071 mmol) was deprotected and purified, affording compound **17s** as a white powder (34 mg, 78% yield). ¹H NMR (400 MHz, D₂O) δ 8.33 (s, 1H), 8.03 (s, 1H), 7.52 – 7.42 (m, 2H), 7.33 (t, *J* = 7.2 Hz, 2H), 6.44 (s, 1H), 6.22 – 6.15 (m, 1H), 6.11 (d, *J* = 2.3 Hz, 1H), 4.70 – 4.65 (m, 1H), 4.45 (t *J* = 7.8, 1H), 4.14 – 4.04 (m, 3H), 3.86 (d, *J* = 10.2 Hz, 1H), 3.67 – 3.51 (m, 3H), 2.53 – 2.43 (m, 1H), 2.37 – 2.29 (m, 1H). ¹³C NMR (101 MHz, D₂O) δ 171.0, 163.2, 162.9, 162.5, 162.2, 149.1, 147.1, 144.2, 143.7, 137.3, 135.2, 133.6, 133.0, 129.4, 125.6, 120.6, 119.0, 117.7, 117.3, 114.8, 109.1, 91.4, 73.4, 72.0, 55.4, 50.8, 24.9. HRMS (ESI): calculated for C₂₄H₂₉N₈O₅ [M+H]+ 509.2261, found 509.2271.

(*S*)-2-amino-4-((((2*R*,3*S*,4*R*,5*R*)-5-(6-amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl)((*E*)-3-(3-cyanophenyl)allyl)amino)butanoic acid (17t). Following the procedure described for compound 13a, compound 16t (50 mg, 0.071 mmol) was deprotected and purified, affording compound 17t as a white powder (34 mg, 77% yield). ¹H NMR (400 MHz, D₂O) δ 8.36 (s, 1H), 8.10 (s, 1H), 7.60 – 7.58 (m, 1H), 7.39 – 7.29 (m, 3H), 6.40 (br s, 1H), 6.13 – 6.05 (m, 2H), 4.71 (dd, *J* = 7.1, 5.5 Hz, 1H), 4.56 – 4.51 (m, 1H), 4.45 (s, 1H), 4.11 – 3.86 (m, 4H), 3.68 – 3.52 (m, 3H), 2.53 – 2.44 (m, 1H), 2.37 – 2.31 (m, 1H). ¹³C NMR (101 MHz, D₂O) δ 171.1, 149.2, 147.2, 144.1, 143.6, 137.8, 135.7, 132.3, 130.8, 129.7, 119.1, 118.9, 118.5, 117.7, 114.8, 111.5, 91.4, 71.8, 50.9, 24.9. HRMS (ESI): calculated for C₂₄H₂₉N₈O₅ [M+H]+ 509.2261, found 509.2264.

(*S*)-2-amino-4-((((2*R*,3*S*,4*R*,5*R*)-5-(6-amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl)((*E*)-3-(4-cyanophenyl)allyl)amino)butanoic acid (17u). Following the procedure described for compound 13a, compound 16u (50 mg, 0.071 mmol) was deprotected and purified, affording compound 17u as a white powder (35 mg, 80% yield). ¹H NMR (400 MHz, D₂O) δ 8.38 (s, 1H), 8.14 (s, 1H), 7.59 (d, *J* = 8.0 Hz, 2H), 7.16 (d, *J* = 8.1 Hz, 2H), 6.47 (d, *J* = 8.1, 1H), 6.23 – 6.13 (m, 2H), 4.75 – 4.72 (m, 1H), 4.58 – 4.56 (m, 1H), 4.47 (br s, 1H), 4.09 – 4.01 (m, 4H), 3.67 – 3.54 (m, 3H), 2.52 – 2.42 (m, 1H), 2.35 – 2.29 (m, 1H). ¹³C NMR (101 MHz, D₂O) δ 171.8, 149.3, 147.2, 144.1, 143.6, 138.1, 132.63, 126.6, 119.2, 114.9, 110.8, 91.4, 73.6, 71.9, 51.6, 20.5. HRMS (ESI): calculated for C₂₄H₂₉N₈O₅ [M+H]⁺ 509.2261, found 509.2266.

(*S*)-2-amino-4-((((2*R*,3*S*,4*R*,5*R*)-5-(6-amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl)((*E*)-3-(3-carbamoylphenyl)allyl)amino)butanoic acid (17v). Following the procedure described for compound 13a, compound 16v (50 mg, 0.069 mmol) was deprotected and purified, affording compound 17v as a white powder (34 mg, 77% yield). ¹H NMR (400 MHz, CD₃OD) δ 8.46 (s, 1H), 8.26 (s, 1H), 7.91 (s, 1H), 7.82 (d, *J* = 7.6 Hz, 1H), 7.53 – 7.41 (m, 2H), 6.16 (d, *J* = 3.8 Hz, 1H), 4.72 – 4.68 (m, 1H), 4.60 – 4.49 (m, 2H), 4.17 – 4.02 (m, 3H), 3.86 – 3.80 (m, 1H), 3.71 – 3.52 (m, 3H), 2.51 – 2.45 (m, 1H), 2.30 – 2.20 (m, 1H). ¹³C NMR (101

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MHz, CD₃OD) δ 170.7, 148.7, 144.6, 139.7, 137.0, 134.8, 129.8, 128.7, 127.5, 126.2, 118.0, 90.7, 79.5, 73.4, 71.9, 53.5, 52.4, 50.8, 25.0. HRMS (ESI): calculated for C₂₄H₃₁N₈O₆ [M+H]⁺ 527.2367, found 527.2378.

(*S*)-2-amino-4-((((2*R*,3*S*,4*R*,5*R*)-5-(6-amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl)((*E*)-3-(4-carbamoylphenyl)allyl)amino)butanoic acid (17w). Following the procedure described for compound 13a, compound 16w (50 mg, 0.069 mmol) was deprotected and purified, affording compound 17w as a white powder (34 mg, 77% yield). ¹H NMR (400 MHz, CD₃OD) δ 8.45 (s, 1H), 8.26 (s, 1H), 7.85 (d, *J* = 8.4 Hz, 2H), 7.41 (s, 2H), 6.81 (br d, *J* = 15.8 Hz, 1H), 6.45 – 6.37 (m, 1H), 6.16 (s, 1H), 4.73 – 4.68 (m, 1H), 4.55 (dd, *J* = 5.6, 2.8 Hz, 2H), 4.16 – 4.01 (m, 3H), 3.88 – 3.79 (m, 1H), 3.69 – 3.57 (m, 4H), 2.51 – 2.45 (m, 1H), 2.29 – 2.20 (m, 1H). ¹³C NMR (101 MHz, CD₃OD) δ 171.9, 148.2, 138.5, 129.0, 126.5, 120.9, 118.4, 51.40, 90.9, 79.0, 72.9, 72.3, 51.7, 51.4, 25.0. HRMS (ESI): calculated for C₂₄H₃₁N₈O₆ [M+H]+ 527.2367, found 527.2373.

(*S*)-2-amino-4-((((2*R*,3*S*,4*R*,5*R*)-5-(6-amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl)(cinnamyl)amino)butanoic acid (17x). Following the procedure described for compound 13a, compound 16x (50 mg, 0.074 mmol) was deprotected and purified, affording compound 17x as a white powder (35 mg, 79% yield). ¹H NMR (500 MHz, CD₃OD) δ 8.49 (s, 1H), 8.24 (s, 1H), 7.31 – 7.26 (s, 5H), 6.74 – 6.71 (br d, *J* = 12.0, 1H), 6.29 – 6.23 (m, 1H), 6.18 (d, *J* = 3.8 Hz, 1H), 4.70 – 4.68 (m, 1H), 4.60 – 4.56 (m, 2H), 4.14 – 4.10 (m, 3H), 3.90 – 3.85 (br m, 1H), 3.76 – 3.52 (m, 3H), 2.57 – 2.51 (m, 1H), 2.41 – 2.34 (m, 1H). ¹³C NMR (126 MHz, CD₃OD) δ 169.5, 162.0, 161.7, 161.4, 149.7, 151.0, 148.0, 140.9, 135.1, 128.8, 128.5, 125.7, 120.0, 119.8, 117.7, 115.5, 115.4, 91.1, 79.4, 73.6, 72.3, 55.5, 54.5, 50.9, 50.6, 25.0. HRMS (ESI): calculated for C₂₃H₃₀N₇O₅ [M+H]⁺ 484.2308, found 484.2311.

(*S*)-2-amino-4-((((2*R*,3*S*,4*R*,5*R*)-5-(6-amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl)((*E*)-3-(4-ethynylphenyl)allyl)amino)butanoic acid (17y). Following the procedure described for compound 13a, compound 16y (50 mg, 0.064 mmol) was deprotected and purified, affording compound 17y as a white powder (8 mg, 21% yield). ¹H NMR (500 MHz, CD₃OD) δ 8.41 (s, 1H), 8.28 (s, 1H), 7.43 (d, *J* = 8.3 Hz, 2H), 7.30 (d, *J* = 8.3 Hz, 2H), 6.77 – 6.73 (br d, *J* = 12.0, 1H), 6.37 – 6.25 (m, 1H), 6.14 (d, *J* = 3.5 Hz, 1H), 4.70 – 4.66 (m, 1H), 4.53 (dd, *J* = 5.5, 2.5 Hz, 2H), 4.14 – 3.94 (m, 3H), 3.82 – 3.77 (m, 1H), 3.66 – 3.63 (br d, *J* = 16.0, 1H), 3.61 (s, 1H), 3.60 – 3.47 (m, 2H), 2.48 – 2.40 (m, 1H), 2.24 – 2.16 (m, 1H). ¹³C NMR (126 MHz, CD₃OD) δ 148.6, 139.6, 135.6, 132.0, 126.5, 91.1, 79.1, 78.9, 73.4, 71.7, 54.6, 51.5, 25.2. HRMS (ESI): calculated for C₂₅H₃₀NrO5 [M+H]⁺ 508.2308, found 508.2315.

4-((*E*)-3-((((3a*R*,3a*R*,4*R*,6*R*,6a*R*,6a*R*)-6-(6-amino-9*H*-purin-9-yl)-2,2-

dimethyltetrahydrofuro[3,4-d][1,3]dioxol-4-yl)methyl)amino)prop-1-en-1-yl)benzonitrile (18). Following the procedure described for compound 12a, coupling 9-((3aR,4*R*,6*R*,6*aR*)-6-(aminomethyl)-2,2-dimethyltetrahydrofuro[3,4-d][1,3]dioxol-4-yl)-9*H*-purin-6-amine **9** (67 mg, 0.22 mmol) with (*E*)-4-(3-oxoprop-1-en-1-yl)benzonitrile 15u (31 mg, 0.20 mmol) afforded compound 18 as a yellow powder (49 mg, 55% yield). ¹H NMR (400 MHz, CDCl₃) δ 8.26 (s, 1H), 7.92 (s, 1H), 7.59 (d, *J* = 8.4 Hz, 2H), 7.41 (d, *J* = 8.3 Hz, 2H), 6.51 (s, 1H), 6.41 – 6.36 (m, 1H), 6.15 (s, 2H), 6.01 (d, *J* = 3.3 Hz, 1H), 5.52 – 5.47 (m, 1H), 5.10 (dd, *J* = 6.4, 3.3 Hz, 1H), 4.45 – 4.41 (m, 1H), 3.46 (t, *J* = 5.5 Hz, 2H), 3.06 – 2.93 (m, 2H), 2.61 (s, 3H), 1.64 (s, 3H), 1.41 (s, 3H). ¹³C NMR (101 MHz, CDCl₃) δ 155.7, 153.1, 149.3, 141.5, 140.1, 132.5, 129.8, 126.8, 114.8, 110.6, 91.1, 85.5, 83.3, 82.3, 51.6, 50.9, 27.4, 25.5. HRMS (ESI): calculated for C₂₃H₂₆N₇O₃ [M+H]+448.2097, found 448.2106.

tert-butyl (*S*)-5-((((3 3aR,3aR,4R,6R,6aR,6aR)-6-(6-amino-9H-purin-9-yl)-2,2dimethyltetrahydrofuro[3,4-d][1,3]dioxol-4-yl)methyl)((*E*)-3-(4-cyanophenyl)allyl)amino)-2-(bis(tert-butoxycarbonyl)amino)pentanoate (20a). Following the procedure described for compound 12a, coupling compound 18 (112 mg, 0.20 mmol) with tert-butyl (S)-2-(bis(tertbutoxycarbonyl)amino)-5-oxopentanoate 19a (82 mg, 0.24 mmol) afforded protected intermediate **20a** as a white powder (113 mg, 69% yield). ¹H NMR (400 MHz, CDCl₃) & 8.23 (s, 1H), 7.92 (s, 1H), 7.51 (d, *J* = 8.3 Hz, 2H), 7.28 (d, *J* = 8.2 Hz, 2H), 6.44 – 6.21 (m, 4H), 6.04 (d, *J* = 1.9 Hz, 1H), 5.44 (dd, *J* = 6.4, 1.9 Hz, 1H), 4.97 (dd, *J* = 6.4, 3.6 Hz, 1H), 4.71 (dd, *J* = 9.6, 5.2 Hz, 1H), 4.38 - 4.34 (m, 1H), 3.26 (d, J = 6.0 Hz, 2H), 2.79 - 2.69 (m, 2H), 2.56 - 2.51 (m, 2H), 2.07 -2.00 (m, 1H), 1.91 – 1.75 (m, 1H), 1.59 (s, 3H), 1.44 (br d, J = 21.0, 27H), 1.37 (s, 3H). ¹³C NMR (101 MHz, CDCl₃) δ 169.8, 155.8, 153.0, 152.6, 149.2, 141.4, 140.0, 132.4, 131.7, 130.6, 126.6, 119.1, 114.4, 110.4, 107.0, 90.8, 85.7, 83.9, 83.3, 82.8, 81.2, 77.5, 77.2, 76.9, 58.6, 54.3, 28.1, 27.2, 26.9, 25.5, 23.9. HRMS (ESI): calculated for C42H59N8O9 [M+H]+ 819.4405, found 819.4410.

methyl (S)-4-(((((3aR,3aR,4R,6R,6aR,6aR)-6-(6-amino-9H-purin-9-yl)-2,2dimethyltetrahydro-furo[3,4-d][1,3]dioxol-4-yl)methyl)((E)-3-(4-cyanophenyl)allyl)amino)-2-((*tert*-butoxycarbonyl) amino)butanoate (20b). Following the procedure described for compound 12a, coupling compound 18 (112 mg, 0.20 mmol) with methyl (S)-2-((*tert*butoxycarbonyl)amino)-4-oxobutanoate 19b (82 mg, 0.24 mmol) afforded protected intermediate 20b as a white powder (97 mg, 73% yield). ¹H NMR (400 MHz, CDCl₃) δ 8.21 (s, 1H), 7.94 (s, 1H), 7.49 (d, *J* = 8.3 Hz, 2H), 7.27 (d, *J* = 8.3 Hz, 2H), 6.60 (s, 2H), 6.41 – 6.25 (m, 1H), 6.26 – 6.19 (m, 1H), 6.06 (s, 1H), 5.94 (d, *J* = 8.1 Hz, 1H), 5.45 (d, *J* = 6.2 Hz, 1H), 5.03 – 4.95 (m, 1H),

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4.41 – 4.30 (m, 2H), 3.64 (s, 3H), 3.23 (d, J = 6.0 Hz, 2H), 2.79 – 2.69 (m, 2H), 2.58 – 2.50 (m, 2H), 2.07 – 2.00 (m, 1H), 1.86 – 1.79 (m, 1H), 1.58 (s, 3H), 1.41 – 1.33 (br m, 12H). ¹³C NMR (101 MHz, CDCl₃) δ 173.3, 155.9, 155.6, 155.3, 153.0, 149.0, 141.3, 140.1, 132.3, 131.0, 126.7, 120.2, 119.0, 114.4, 110.5, 90.7, 85.7, 83.9, 83.3, 56.6, 56.1, 53.6, 52.2, 50.6, 44.8, 29.2, 28.4, 27.2, 25.4. HRMS (ESI): calculated for C₃₃H₄₃N₈O7 [M+H]⁺ 663.3255, found 663.3258.

4-((((3a*R*,3a*R*,4*R*,6*R*,6a*R*,6a*R*)-6-(6-amino-9*H*-purin-9-yl)-2,2-dimethyltetrahydrofuro[3,4d][1,3]dioxol-4-yl)methyl)((*E*)-3-(4-cyanophenyl)allyl)amino)-*N*-tritylbutanamide (20c). Following the procedure described for compound 12a, coupling compound 18 (112 mg, 0.20 mmol) with 4-oxo-*N*-tritylbutanamide 19c (82 mg, 0.24 mmol) afforded protected intermediate 20c as a white powder (79 mg, 51% yield). ¹H NMR (400 MHz, CDCl₃) δ 8.27 (s, 1H), 7.90 (s, 1H), 7.54 (d, *J* = 6.6 Hz, 2H), 7.33 – 7.19 (m, 20H), 6.72 (br s, 1H), 6.43 – 6.39 (br d, *J* = 16.0 Hz, 1H), 6.32 – 6.23 (m, 1H), 6.04 (d, *J* = 2.1 Hz, 1H), 5.82 (s, 2H), 5.47 (dd, *J* = 6.4, 2.1 Hz, 1H), 4.99 (dd, *J* = 6.4, 3.5 Hz, 1H), 4.42 – 4.38 (m, 1H), 3.28 (t, *J* = 6.4 Hz, 2H), 2.77 (d, *J* = 6.7 Hz, 2H), 2.57 – 2.51 (m, 2H), 2.35 – 2.25 (m, 2H), 1.87 – 1.73 (m, 2H), 1.59 (s, 3H), 1.38 (s, 3H). ¹³C NMR (101 MHz, CDCl₃) δ 171.7, 154.9, 153.6, 149.2, 144.0, 140.8, 132.4, 128.7, 128.0, 127.0, 126.7, 120.8, 119.1, 114.4, 110.5, 90.9, 86.7, 83.9, 82.7, 69.1, 56.6, 56.0, 54.5, 35.6, 26.5, 25.4, 22.6. HRMS (ESI): calculated for C₄₆H₄₇N₈O₄ [M+H]⁺775.3720, found 775.3733.

5-((((3a*R*,4*R*,6*R*,6a*R*)-6-(6-amino-9*H*-purin-9-yl)-2,2-dimethyltetrahydrofuro[3,4d][1,3]dioxol-4-yl)methyl)((*E*)-3-(4-cyanophenyl)allyl)amino)-*N*-tritylpentanamide (20d). Following the procedure described for compound 12a, coupling compound 18 (112 mg, 0.20 mmol) with 5-oxo-*N*-tritylpentanamide 19d (86 mg, 0.24 mmol) afforded protected intermediate 20d as a white powder (88 mg, 56% yield). ¹H NMR (500 MHz, CDCl₃) δ 8.15 (s, 1H), 7.97 (s, 1H), 7.52 (d, *J* = 8.4 Hz, 2H), 7.29 – 7.27 (m, 7H), 7.25 – 7.18 (m, 11H), 7.07 (s, 2H), 6.75 (s, 1H), 6.05 (d, *J* = 2.0 Hz, 1H), 5.40 (dd, *J* = 6.4, 2.0 Hz, 1H), 4.97 (dd, *J* = 6.4, 3.5 Hz, 1H), 4.45 – 4.41 (m, 1H), 3.30 (d, *J* = 6.5 Hz, 2H), 2.78 (d, *J* = 6.6 Hz, 2H), 2.54 (dd, *J* = 10.8, 4.0 Hz, 2H), 2.28 – 2.25 (m, 2H), 1.60 (s, 3H), 1.46 – 1.41 (m, 2H), 1.37 (s, 3H). ¹³C NMR (126 MHz, CDCl₃) δ 175.9, 171.9, 155.8, 152.3, 148.9, 144.8, 141.2, 132.5, 131.5, 130.6, 128.8, 127.1, 126.8, 119.5, 119.1, 114.6, 110.7, 91.1, 86.1, 84.4, 83.3, 70.5, 60.5, 56.4, 55.8, 54.0, 37.2, 26.1, 23.2, 21.5. HRMS (ESI): calculated for C₄₇H₄₉N₈O₄ [M+H]⁺789.3877, found 789.3886.

tert-butyl (3-((((3a*R*,4*R*,6*R*,6a*R*)-6-(6-amino-9*H*-purin-9-yl)-2,2dimethyltetrahydrofuro[3,4-d][1,3]dioxol-4-yl)methyl)((*E*)-3-(4-

cyanophenyl)allyl)amino)propyl)carbamate (20e). Following the procedure described for compound 12a, coupling compound 18 (112 mg, 0.20 mmol) with *tert*-butyl (3-oxopropyl)carbamate 19e (41 mg, 0.24 mmol) afforded protected intermediate 20e as a white

powder (88 mg, 73% yield). ¹H NMR (500 MHz, CDCl₃) δ 8.09 (s, 1H), 7.97 (s, 1H), 7.54 (d, *J* = 8.4 Hz, 1H), 7.30 (d, *J* = 8.4 Hz, 2H), 6.98 (s, 2H), 6.40 (d, *J* = 15.9 Hz, 1H), 6.32 – 6.22 (m, 1H), 6.06 (s, 1H), 5.41 (d, *J* = 7.8 Hz, 1H), 4.40 – 4.37 (m, 1H), 3.26 (d, *J* = 6.5 Hz, 2H), 3.17 – 3.13 (m, 1H), 2.75 (d, *J* = 4.7 Hz, 1H), 2.56 (s, 1H), 1.42 (d, *J* = 8.6 Hz, 12H), 1.38 (s, 3H). ¹³C NMR (126 MHz, CDCl₃) δ 175.8, 154.8, 152.4, 149.7, 141.2, 132.6, 126.8, 119.6, 119.0, 114.6, 112.1, 90.9, 86.4, 84.1, 57.0, 51.2, 39.2, 28.5, 27.2, 26.5, 25.4, 22.0. HRMS (ESI): calculated for C₃₁H₄₁N₈O₅ [M+H]⁺ 605.3200, found 605.3211.

tert-butyl 4-((((3a*R*,3a*R*,4*R*,6*R*,6a*R*)-6-(6-amino-9*H*-purin-9-yl)-2,2dimethyltetrahydro-furo[3,4-d][1,3]dioxol-4-yl)methyl)((*E*)-3-(4-

cyanophenyl)allyl)amino)butanoate (20f). Following the procedure described for compound **12a**, coupling compound **18** (89 mg, 0.20 mmol) with *tert*-butyl 4-oxobutanoate **19f** (35 mg, 0.22 mmol) to afford protected intermediate **20f** as a white powder (84 mg, 71% yield). ¹H NMR (400 MHz, CDCl₃) δ 8.27 (s, 1H), 7.93 (s, 1H), 7.55 (d, *J* = 8.4 Hz, 2H), 7.32 (d, *J* = 8.4 Hz, 2H), 6.43 (d, *J* = 15.9 Hz, 1H), 6.33 – 6.26 (m, 1H), 6.10 (s, 2H), 6.07 (d, *J* = 2.1 Hz, 1H), 5.47 (dd, *J* = 6.5, 2.1 Hz, 1H), 5.01 (dd, *J* = 6.5, 3.6 Hz, 1H), 4.40 – 4.36 (m, 1H), 3.30 (d, *J* = 6.5 Hz, 2H), 2.83 – 2.73 (m, 2H), 2.54 (t, *J* = 7.3 Hz, 2H), 2.26 – 2.23 (m, 2H), 1.79 – 1.69 (m, 2H), 1.61 (s, 3H), 1.43 – 1.38 (br m, 12H). ¹³C NMR (101 MHz, CDCl₃) δ 173.7, 157.1, 153.0, 150.2, 142.2, 141.7, 140.1, 138.9, 136.8, 134.2, 130.7, 127.0, 120.9, 119.0, 114.4, 111.2, 90.8, 85.8, 84.6, 83.2, 79.5, 56.8, 56.0, 52.4, 33.1, 28.7, 27.2, 25.4, 22.4. HRMS (ESI): calculated for C₃₁H₄₀N₇O₅ [M+H]⁺ 590.3091, found 590.3097.

tert-butyl 5-((((3a*R*,3a*R*,4*R*,6R,6a*R*,6a*R*)-6-(6-amino-9*H*-purin-9-yl)-2,2dimethyltetrahydro-furo[3,4-d][1,3]dioxol-4-yl)methyl)((*E*)-3-(4-

cyanophenyl)allyl)amino)pentanoate (20g). Following the procedure described for compound **12a**, coupling compound **18** (112 mg, 0.20 mmol) with *tert*-butyl 5-oxopentanoate **19g** (41 mg, 0.24 mmol) afforded protected intermediate **20g** as a white powder (82 mg, 68% yield). ¹H NMR (500 MHz, CDCl₃) δ 8.23 (s, 1H), 7.92 (s, 1H), 7.53 (d, *J* = 6.7 Hz, 2H), 7.29 (d, *J* = 8.4 Hz, 2H), 6.40 (d, *J* = 16.0 Hz, 1H), 6.31– 6.25 (m, 1H), 6.20 (s, 2H), 6.05 (d, *J* = 2.1 Hz, 1H), 5.45 (dd, *J* = 6.4, 2.1 Hz, 1H), 4.99 (dd, *J* = 6.4, 3.6 Hz, 1H), 4.39 – 4.34 (m, 1H), 3.69 – 3.59 (m, 3H), 3.28 (d, *J* = 6.8 Hz, 2H), 2.75 (d, *J* = 6.7 Hz, 2H), 2.53 – 2.45 (m, 3H), 2.18 (t, *J* = 7.3 Hz, 2H), 1.76 – 1.68 (m, 1H), 1.65 – 1.48 (m, 8H), 1.47 – 1.43 (m, 2H), 1.41 (s, 9H), 1.37 (s, 3H). ¹³C NMR (126 MHz, CDCl₃) δ 173.0, 155.7, 153.1, 149.2, 141.4, 132.4, 126.7, 120.3, 119.1, 114.4, 111.3, 90.9, 85.8, 84.0, 83.3, 80.2, 62.5, 62.0, 56.9, 56.0, 54.4, 35.3, 32.4, 28.2, 27.2, 25.5, 23.5. HRMS (ESI): calculated for C₃₂H₄₂N₇O₅ [M+H]⁺ 604.3247, found 604.3255.

4-((*E***)-3-((((3a***R***,3a***R***,4***R***,6***R***,6a***R***,6a***R***)-6-(6-amino-9***H***-purin-9-yl)-2,2-dimethyltetrahydrofuro[3,4-d][1,3]dioxol-4-yl)methyl)((6-(***tert***-butoxy)pyridin-2-yl)methyl)amino)prop-1-en-1yl)benzonitrile (20h). Following the procedure described for compound 12a, coupling compound 18 (112 mg, 0.20 mmol) with 6-(***tert***-butoxy)picolinaldehyde 19h (43 mg, 0.24 mmol) afforded protected intermediate 20h as a white powder (73 mg, 60% yield). ¹H NMR (400 MHz, CDCl₃) 8 8.15 (s, 1H), 7.95 (s, 1H), 7.54 (d,** *J* **= 6.6 Hz, 2H), 7.45 – 7.41 (m, 1H), 7.30 (d,** *J* **= 6.5 Hz, 2H), 6.88 (d,** *J* **= 7.4 Hz, 1H), 6.59 (d,** *J* **= 6.8 Hz, 2H), 6.52 (dd,** *J* **= 8.2, 0.8 Hz, 1H), 6.44 (d,** *J* **= 16.0 Hz, 1H), 6.37 – 6.29 (m, 1H), 6.08 (d,** *J* **= 2.1 Hz, 1H), 5.42 (dd,** *J* **= 6.4, 2.1 Hz, 1H), 4.97 (dd,** *J* **= 6.4, 3.5 Hz, 1H), 4.50 – 4.56 (m, 1H), 3.75 (s, 2H), 3.46 – 3.32 (m, 2H), 2.90 (d,** *J* **= 6.6 Hz, 2H), 1.60 (s, 3H), 1.56 (s, 9H), 1.39 (s, 3H). ¹³C NMR (101 MHz, CDCl₃) 8 163.3, 156.0, 153.0, 149.2, 141.5, 139.9, 132.4, 131.8, 130.7, 126.6, 120.3, 119.1, 115.4, 114.3, 111.5, 110.5, 90.8, 85.8, 84.0, 83.2, 79.4, 60.2, 56.9, 56.0, 28.8, 27.2, 25.5. HRMS (ESI): calculated for C₃₃H₃₉N₈O₄ [M+H]⁺ 611.3094, found 611.3102.**

4-((*E***)-3-((((3a***R***,3a***R***,4***R***,6***R***,6a***R***,6a***R***)-6-(6-amino-9***H***-purin-9-yl)-2,2-dimethyltetrahydrofuro[3,4-d][1,3]dioxol-4-yl)methyl)amino)prop-1-en-1-yl)benzonitrile (20j). Following the procedure described for compound 12a, coupling 9-((3a***R***,4***R***,6***R***,6a***R***)-2,2-dimethyl-6-((methylamino)methyl)tetrahydrofuro[3,4-d][1,3]dioxol-4-yl)-9***H***-purin-6-amine³⁹ (64 mg, 0.20 mmol) with 15u (34 mg, 0.22 mmol) afforded protected intermediate 20j as a yellow powder (66 mg, 72% yield). ¹H NMR (400 MHz, CDCl3) δ 8.20 (s, 1H), 7.97 (s, 1H), 7.52 (d,** *J* **= 8.1 Hz, 2H), 7.28 (d,** *J* **= 8.5 Hz, 2H), 6.57 (s, 2H), 6.42 (d,** *J* **= 16.0 Hz, 1H), 6.34 – 6.23 (m, 1H), 6.08 (s, 1H), 5.45 (d,** *J* **= 7.9 Hz, 1H), 4.98 (dd,** *J* **= 6.3, 3.7 Hz, 1H), 4.48 – 4.35 (m, 1H), 3.28 – 3.11 (m, 2H), 2.80 – 2.74 (m, 1H), 2.65 – 2.60 (br m, 1H), 2.33 (s, 3H), 1.61 (s, 3H), 1.38 (s, 3H). ¹³C NMR (101 MHz, CDCl3) δ 155.8, 152.9, 149.1, 140.0, 132.4, 131.0, 126.6, 120.2, 119.1, 114.5, 110.6, 90.8, 85.2, 84.1, 83.2, 60.4, 59.0, 42.8, 29.7, 27.2, 25.4. HRMS (ESI): calculated for C_{24H28}NrO₃ [M+H]+ 462.2254, found 462.2259.**

4-((*E*)-3-((((3a*R*,3a*R*,4*R*,6*R*,6a*R*,6a*R*)-6-(6-amino-9*H*-purin-9-yl)-2,2-dimethyltetrahydrofuro[3,4-d][1,3]dioxol-4-yl)methyl)(isopropyl)amino)prop-1-en-1-yl)benzonitrile (20k). Following the procedure described for compound 12a, coupling compound 18 (112 mg, 0.20 mmol) with 2 mL dry acetone afforded protected intermediate 20k as a white powder (47 mg, 48% yield). ¹H NMR (400 MHz, CDCl₃) δ 8.29 (s, 1H), 7.92 (s, 1H), 7.54 (d, *J* = 8.4 Hz, 2H), 7.33 (d, *J* = 8.3 Hz, 2H), 6.49 – 6.38 (m, 3H), 6.37 – 6.29 (m, 1H), 6.06 (d, *J* = 2.0 Hz, 1H), 5.49 (dd, *J* = 6.4, 2.0 Hz, 1H), 5.04 (dd, *J* = 6.4, 3.5 Hz, 1H), 4.35 – 4.31 (m, 1H), 3.35 – 3.22 (m, 2H), 3.04 – 2.97 (m, 1H), 2.83 – 2.78 (m, 1H), 2.69 – 2.60 (m, 1H), 1.58 (s, 3H), 1.40 (s, 3H), 1.05 (d, *J* = 6.6 Hz, 3H), 0.93 (d, *J* = 6.6 Hz, 3H). ¹³C NMR (101 MHz, CDCl₃) δ 155.8, 153.0, 149.2, 141.6, 140.2,

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133.7, 132.4, 129.6, 126.6, 120.3, 119.1, 114.3, 110.3, 90.9, 86.6, 83.9, 83.1, 53.8, 51.7, 27.2, 25.5, 18.6, 17.9. HRMS (ESI): calculated for C₂₆H₃₂N₇O₃ [M+H]⁺ 490.2567, found 490.2573.

(*S*)-2-amino-5-((((2*R*,3*S*,4*R*,5*R*)-5-(6-amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl)((*E*)-3-(4-cyanophenyl)allyl)amino)pentanoic acid (21a). Following the procedure described for compound 13a, compound 20a (50 mg, 0.061 mmol) was deprotected and purified, affording compound 21a as a white powder (24 mg, 63% yield). ¹H NMR (400 MHz, CD₃OD) δ 8.47 (s, 1H), 8.33 (s, 1H), 7.68 (d, *J* = 8.3 Hz, 2H), 7.48 (d, *J* = 8.2 Hz, 2H), 6.82 – 6.78 (br d, *J* = 15.8 Hz, 1H), 6.51 – 6.43 (m, 1H), 6.18 (d, *J* = 3.2 Hz, 1H), 4.71 – 4.65 (m, 1H), 4.62 – 4.51 (m, 2H), 4.19 – 4.00 (m, 3H), 3.89 – 3.84 (m, 1H), 3.69 (d, *J* = 8.9 Hz, 1H), 3.47 – 3.37 (m, 2H), 2.13 – 1.91 (m, 4H). ¹³C NMR (101 MHz, CD₃OD) δ 170.0, 151.3, 148.1, 145.0, 143.1, 139.7, 138.5, 132.3, 127.2, 120.0, 119.7, 118.1, 118.0, 115.1, 111.9, 91.3, 73.5, 72.2, 55.3, 52.8, 27.1, 20.0. HRMS (ESI): calculated for C₂₅H₃₂N₈O₅ [M+H]+ 523.2417, found 523.2423.

(*S*)-2-amino-4-((((2*R*,3*S*,4*R*,5*R*)-5-(6-amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl)((*E*)-3-(4-cyanophenyl)allyl)amino)butanamide (21b). Compound 20b (50 mg, 0.076 mmol) was added to ammonia in MeOH (33% w/w, 5 mL) in a sealed tube and the mixture was stirred overnight at room temperature. The solvent was evaporated and the crude intermediate was deprotected and purified following the procedure described for compound **13a** affording compound **21b** as a white powder (33 mg, 71% yield, two steps). ¹H NMR (400 MHz, CD₃OD) δ 8.44 (s, 1H), 8.32 (s, 1H), 7.70 (d, *J* = 8.4 Hz, 2H), 7.50 (d, *J* = 8.4 Hz, 2H), 6.85 – 6.81 (br d, *J* = 15.8 Hz, 1H), 6.50 – 6.41 (m, 1H), 6.15 (d, *J* = 3.3 Hz, 1H), 4.68 (dd, *J* = 5.0, 3.4 Hz, 1H), 4.58 – 4.48 (m, 2H), 4.15 – 4.03 (m, 3H), 3.86 – 3.80 (m, 1H), 3.67 – 3.64 (br d, *J* = 9.0 Hz, 1H), 3.51 – 3.40 (m, 2H), 2.47 – 2.34 (m, 2H). ¹³C NMR (101 MHz, CD₃OD) δ 169.4, 163.1, 161.0, 152.6, 148.1, 140.1, 138.3, 132.7, 119.8, 118.0, 111.8, 91.1, 78.6, 73.4, 72.2, 55.4, 54.8, 49.3, 26.8. HRMS (ESI): calculated for C₂₄H₄₀N₉O₄ [M+H]⁺ 508.2421, found 508.2427.

4-((((2*R*,3*S*,4*R*,5*R*)-5-(6-amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2yl)methyl)((*E*)-3-(4-cyanophenyl)allyl)amino)butanamide (21c). Following the procedure described for compound 13a, compound 20c (50 mg, 0.065 mmol) was deprotected and purified, affording compound 21c as a white powder (19 mg, 59% yield). ¹H NMR (400 MHz, CD₃OD) δ 8.46 (s, 1H), 7.70 (d, *J* = 8.1 Hz, 2H), 7.53 (s, 2H), 6.88 – 6.84 (br d, *J* =9.0 Hz, 1H), 6.54 – 6.40 (m, 1H), 4.75 (br s, 1H), 4.57 (d, *J* = 6.1 Hz, 2H), 4.12 (dd, *J* = 7.4, 3.7 Hz, 2H), 3.88 – 3.82 (m, 1H), 3.68 – 3.64 (br d, *J* = 16.0 Hz, 1H), 3.37(s, 1H), 2.46 (t, *J* = 6.5 Hz, 2H), 2.10 – 2.04 (m, 2H). ¹³C NMR (101 MHz, CD₃OD) δ 174.8, 151.7, 149.1, 141.3, 137.9, 132.8, 127.2, 120.1, 117.0, 110.2, 91.0, 73.8, 71.1, 55.1, 30.1, 19.3. HRMS (ESI): calculated for C₂₄H₂₉N₈O₄ [M+H]+ 493.2312, found 493.2320.

5-((((2R,3S,4R,5R)-5-(6-amino-9H-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-

yl)methyl)((*E***)-3-(4-cyanophenyl)allyl)amino)pentanamide (21d).** Following the procedure described for compound **13a**, compound **20d** (50 mg, 0.063 mmol) was deprotected and purified, affording compound **21d** as a white powder (22 mg, 57% yield). ¹H NMR (400 MHz, CD₃OD) δ 8.44 (s, 1H), 8.32 (s, 1H), 7.71 (d, *J* = 7.9 Hz, 2H), 7.51 (s, 2H), 6.87 (s, 1H), 6.52 (s, 1H), 6.16 (d, *J* = 3.5 Hz, 1H), 4.70 (s, 1H), 4.60 – 4.48 (m, 2H), 4.11 (d, *J* = 7.2 Hz, 2H), 3.87 – 3.81 (m, 1H), 3.66 (br d, *J* = 15.8 Hz, 1H), 2.31 (t, *J* = 7.0 Hz, 2H), 1.89 – 1.77 (m, 2H), 1.72 – 1.64 (m, 2H). ¹³C NMR (101 MHz, CD₃OD) δ 175.5, 140.8, 138.6, 132.3, 127.2, 118.0, 112.7, 91.1, 73.4, 72.3, 54.4, 31.4, 23.1, 21.8. HRMS (ESI): calculated for C₂₅H₃₁N₈O₄ [M+H]⁺ 507.2468, found 507.2479.

3-((*E***)-3-((((2***R***,3***S***,4***R***,5***R***)-5-(6-amino-9***H***-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl) (3-aminopropyl)amino)prop-1-en-1-yl)benzonitrile (21e). Following the procedure described for compound 13a, compound 20e (50 mg, 0.083 mmol) was deprotected and purified, affording compound 21e as a white powder (41 mg, 72% yield). ¹H NMR (500 MHz, CD₃OD) \delta 8.48 (s, 1H), 8.33 (s, 1H), 7.64 (d,** *J* **= 8.4 Hz, 2H), 7.47 (s, 2H), 6.18 (d,** *J* **= 3.3 Hz, 1H), 4.68 (d,** *J* **= 3.3 Hz, 1H), 4.60 – 4.55 (m, 2H), 4.14 (d,** *J* **= 7.3 Hz, 2H), 3.90 – 3.83 (m, 1H), 3.73 – 3.70 (br d,** *J* **= 9.0 Hz, 1H), 3.08 (t,** *J* **= 7.5 Hz, 2H), 2.26 – 2.20 (m, 2H). ¹³C NMR (126 MHz, CD₃OD) \delta 151.1, 148.1, 139.7, 138.6, 132.3, 127.2, 120.0, 119.8, 118.2, 111.8, 91.3, 73.6, 72.3, 55.4, 54.6, 50.6, 36.51, 48.6, 36.5, 22.2. HRMS (ESI): calculated for C₂₃H₂₉N₈O₃ [M+H]+ 465.2363, found 465.2372.**

4-(((((2R,3S,4R,5R)-5-(6-amino-9H-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-

yl)methyl)((*E*)-3-(4-cyanophenyl)allyl)amino)butanoic acid (21f). Following the procedure described for compound 13a, compound 20f (50 mg, 0.085 mmol) was deprotected and purified, affording compound 21f as a white powder (37 mg, 71% yield). ¹H NMR (CD₃OD) δ 8.44 (s, 1H), 8.34 (s, 1H), 6.87 (d, *J* = 15.8 Hz, 1H), 6.51 – 6.43 (m, 1H), 6.16 (d, *J* = 3.7 Hz, 1H), 4.74 (t, *J* = 4.1 Hz, 1H), 4.55 (d, *J* = 6.9 Hz, 2H), 4.13 (d, *J* = 7.4 Hz, 2H), 3.37 (dd, *J* = 9.5, 6.9 Hz, 2H), 2.47 (t, *J* = 6.8 Hz, 2H), 2.11 – 2.02 (m, 2H). ¹³C NMR (101 MHz, CD₃OD) δ 178.8, 151.1, 147.5, 139.7, 139.2, 132.3, 127.3, 120.5, 118.1, 112.3, 95.4, 78.3, 73.4, 72.3, 57.9, 52.9, 32.6, 22.2. HRMS (ESI): calculated for C₂₄H₂₈N₇O₅ [M+H]⁺ 494.2152, found 494.2160.

5-((((2*R*,3*S*,4*R*,5*R*)-5-(6-amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2yl)methyl)((*E*)-3-(4-cyanophenyl)allyl)amino)pentanoic acid (21g). Following the procedure described for compound 13a, compound 20g (50 mg, 0.083 mmol) was deprotected and purified, affording compound 21g as a white powder (39 mg, 75% yield). ¹H NMR (400 MHz, CD₃OD) δ 8.45 (s, 1H), 8.33 (s, 1H), 7.71 (d, *J* = 8.0 Hz, 2H), 7.52 (br s, 2H), 6.85 (br d, *J* = 15.7 Hz, 1H), 6.48 - 6.41 (m, 1H), 6.17 (d, J = 4.3 Hz, 1H), 4.59 - 4.48 (m, 2H), 4.12 (d, J = 7.3 Hz, 2H), 3.86 - 3.81 (br m, 1H), 3.68 - 3.65 (br d, J = 12.2 Hz, 1H), 2.38 (t, J = 7.0 Hz, 2H), 1.86 (t, J = 7.8 Hz, 1H), 1.70 - 1.63 (m, 2H). ¹³C NMR (101 MHz, CD₃OD) δ 171.7, 139.1, 138.6, 131.7, 119.9, 118.0, 111.9, 91.1, 73.4, 72.2, 55.1, 53.0, 31.7, 23.0, 20.7. HRMS (ESI): calculated for C₂₅H₃₀N₇O₅ [M+H]⁺ 508.2308, found 508.2317.

4-((*E*)-3-((((2*R*,3*S*,4*R*,5*R*)-5-(6-amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2yl)methyl) ((6-oxo-1,6-dihydropyridin-2-yl)methyl)amino)prop-1-en-1-yl)benzonitrile (21h). Following the procedure described for compound 13a, compound 20h (50 mg, 0.082 mmol) was deprotected and purified, affording compound 21h as a white powder (25 mg, 59% yield). ¹H NMR (400 MHz, CD₃OD) δ 8.43 (s, 1H), 8.26 (s, 1H), 7.64 (d, *J* = 8.4 Hz, 2H), 7.57 (dd, *J* = 8.8, 7.0 Hz, 1H), 7.46 (d, *J* = 8.4 Hz, 2H), 6.79 – 6.69 (m, 2H), 6.57 – 6.43 (m, 2H), 6.14 (d, *J* = 3.1 Hz, 1H), 4.63 (dd, *J* = 4.9, 3.1 Hz, 1H), 4.58 – 4.49 (m, 2H), 4.35 (s, 2H), 4.13 – 4.00 (m, 2H), 3.76 – 3.57 (m, 2H). ¹³C NMR (101 MHz, CD₃OD) δ 164.1, 150.9, 148.0, 144.3, 143.3, 141.4, 137.3, 132.3, 127.1, 122.2, 119.8, 118.2, 117.8, 115.8, 114.9, 112.3, 111.5, 91.2, 79.4, 73.7, 72.3, 56.1, 55.4, 55.1. HRMS (ESI):) calculated for C₂₆H₂₇N₈O₄ [M+H]⁺ 515.2155, found 515.2164.

4-((*E***)-3-((((2***R***,3***S***,4***R***,5***R***)-5-(6-amino-9***H***-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2yl)methyl) amino)prop-1-en-1-yl)benzonitrile (21i). Following the procedure described for compound 13a**, compound **18** (50 mg, 0.11 mmol) was deprotected and purified, affording compound **21i** as a white powder (30 mg, 52% yield). ¹H NMR (500 MHz, CD₃OD) δ 8.49 (s, 1H), 8.38 (s, 1H), 7.69 (d, *J* = 8.4 Hz, 2H), 7.58 (d, *J* = 8.4 Hz, 2H), 6.86 (d, *J* = 15.9 Hz, 1H), 6.49 – 6.40 (m, 1H), 6.15 (d, *J* = 4.6 Hz, 1H), 4.83 (d, *J* = 4.9 Hz, 2H), 4.50 (t, *J* = 5.1 Hz, 1H), 4.47 – 4.43 (m, 1H), 3.95 (d, *J* = 7.2 Hz, 2H), 3.66 – 3.61 (br m, 1H), 3.56 – 3.53 (br m, 1H). ¹³C NMR (126 MHz, CD₃OD) δ 161.3, 150.8, 148.3, 140.3, 136.7, 132.5, 128.3, 122.2, 119.7, 118.6, 108.9, 90.6, 80.3, 73.7, 71.9, 50.7. HRMS (ESI): calculated for C₂₀H₂₂N₇O₃ [M+H]+408.1784, found 408.1792.

4-((*E*)-3-((((2*R*,3*S*,4*R*,5*R*)-5-(6-amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2yl)methyl) (methyl)amino)prop-1-en-1-yl)benzonitrile (21j). Following the procedure described for compound 13a, compound 20j (50 mg, 0.11 mmol) was deprotected and purified, affording compound 21j as a white powder (37 mg, 64% yield). ¹H NMR (400 MHz, CD₃OD) δ 8.46 (s, 1H), 8.37 (s, 1H), 7.73 (d, *J* = 8.3 Hz, 2H), 7.59 (d, *J* = 8.0 Hz, 2H), 6.94 – 6.90 (br d, *J* =16.0, 1H), 6.53 – 6.45 (m, 1H), 6.16 (d, *J* = 4.2 Hz, 1H), 4.81 – 4.76 (m, 1H), 4.59 – 4.46 (m, 2H), 4.09 (d, *J* = 5.0 Hz, 2H), 3.84 (br t, *J* = 9.0 Hz, 1H), 3.63 – 3.61 (br d, *J* = 8.0 Hz, 1H), 3.00 (s, 3H). ¹³C NMR (101 MHz, CD₃OD) δ 151.82, 148.9, 139.7, 138.7, 132.3, 127.8, 118.05, 111.1, 89.5, 78.7, 73.3, 72.2, 56.9. HRMS (ESI): calculated for C₂₁H₂₄N₇O₃ [M+H]⁺ 422.1941, found 422.1945.

4-((*E*)-3-(((((2*R*,3*S*,4*R*,5*R*)-5-(6-amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-

yl)methyl) (isopropyl)amino)prop-1-en-1-yl)benzonitrile (21k). Following the procedure described for compound 13a, compound 20k (50 mg, 0.10 mmol) was deprotected and purified, affording compound 21k as a white powder (32 mg, 69% yield). ¹H NMR (500 MHz, CD₃OD) δ 8.34 (s, 1H), 8.28 (s, 1H), 7.62 (d, *J* = 8.2 Hz, 2H), 7.44 (d, *J* = 6.9 Hz, 2H), 6.82 (d, *J* = 8.9 Hz, 1H), 6.44 – 6.38 (m, 1H), 6.12 – 6.10 (m, 1H), 4.64 (dd, *J* = 5.3, 3.2 Hz, 1H), 4.58 – 4.53 (m, 2H), 4.47 – 4.43 (m, 1H), 4.07 (d, *J* = 8.1 Hz, 2H), 3.89 – 3.81 (m, 1H), 3.69 (d, *J* = 4.2 Hz, 2H), 1.44 (d, *J* = 6.6 Hz, 3H), 1.41 (d, *J* = 6.6 Hz, 3H). ¹³C NMR (101 MHz, CD₃OD) δ 161.9, 161.6, 161.2, 160.8, 142.9, 139.8, 132.3, 119.7, 118.1, 115.1, 111.7, 91.3, 73.7, 72.1, 51.7. HRMS (ESI): calculated for C_{23H28N7O3} [M+H]⁺ 450.2254, found 450.2262.

(*E*)-4-(3-(methylamino)prop-1-en-1-yl)benzonitrile (22b). Aldehyde 15u (157 mg, 1.0 mmol), 5 mL methylamine in MeOH (33% w/w), NaBH(OAc)₃ (57 mg, 1.5 mmol) and AcOH (one drop) were added to DCE (10 mL) in a sealed tube and the mixture was stirred at room temperature overnight. The reaction was quenched by adding 1 N NaOH (10 mL), and the product was extracted with CH₂Cl₂. The combined organic layers were washed with brine and dried over Na₂SO₄. The solvent was evaporated, and the crude product was purified by column chromatography (20% MeOH in EtOAc) to give compound **22b** as a white powder (72 mg, 42% yield). ¹H NMR (400 MHz, CD₃OD) δ 7.76 (d, *J* = 8.6 Hz, 2H), 7.68 (d, *J* = 8.7 Hz, 2H), 6.97 – 6.93 (br d, *J* = 12.0 Hz, 1H), 6.52 – 6.45 (m, 1H), 3.85 (dd, *J* = 7.1, 1.3 Hz, 2H), 2.77 (s, 3H). ¹³C NMR (101 MHz, CD₃OD) δ 140.1, 136.5, 132.4, 127.3, 122.2, 118.1, 111.7, 50.0, 31.5. HRMS (ESI): calculated for C₁₁H₁₃N₂ [M+H]⁺ 173.1079, found 173.1084.

tert-butyl

(S,E)-2-((tert-butoxycarbonyl)amino)-4-((3-(4-

cyanophenyl)allyl)amino)butanoate (23a). Following the procedure described for compound **12a**, coupling (*E*)-4-(3-aminoprop-1-en-1-yl)benzonitrile **22a** (35 mg, 0.22 mmol) with *tert*-butyl (*S*)-2-((*tert*-butoxycarbonyl)amino)-4-oxobutanoate **10** (55 mg, 0.20 mmol) afforded compound **23a** as a white powder (40 mg, 48% yield).¹H NMR (400 MHz, CDCl₃) δ 7.57 (d, *J* = 8.3 Hz, 2H), 7.43 (d, *J* = 8.3 Hz, 2H), 6.56 – 6.52 (br d, *J* = 16.0 Hz , 1H), 6.45 – 6.35 (m, 1H), 5.57 (d, *J* = 8.1 Hz, 1H), 4.30 – 4.18 (m, 1H), 3.48 – 3.40 (m, 2H), 2.76 – 2.67 (m, 2H), 2.04 – 1.92 (m, 2H), 1.81 – 1.75 (m, 1H), 1.45 – 1.42 (m, 18H). ¹³C NMR (101 MHz, CDCl₃) δ 171.9, 155.7, 141.7, 132.4, 129.5, 126.8, 119.0, 110.5, 81.9, 79.6, 52.6, 51.5, 45.4, 32.9, 28.4. HRMS (ESI): calculated for C_{23H34}N₃O₄ [M+H]+ 416.2549, found 416.2563.

tert-butyl (*S*,*E*)-2-((*tert*-butoxycarbonyl)amino)-4-((3-(4-cyanophenyl)allyl)(methyl)amino) butanoate (23b). Following the procedure described for compound 12a, coupling (*E*)-4-(3-(methylamino)prop-1-en-1-yl)benzonitrile 22b (34 mg, 0.20 mmol) with *tert*-butyl (*S*)-2-((*tert*- butoxycarbonyl)amino)-4-oxobutanoate **10** (66 mg, 0.24 mmol) afforded compound **23b** as a white powder (66 mg, 77% yield).¹H NMR (400 MHz, CDCl₃) δ 7.59 (d, *J* = 8.3 Hz, 2H), 7.46 (d, *J* = 8.2 Hz, 2H), 6.53 (d, *J* = 16.0 Hz, 1H), 6.46 – 6.34 (m, 1H), 5.83 (d, *J* = 7.9 Hz, 1H), 4.23 (d, *J* = 6.4 Hz, 1H), 3.26 – 3.21 (m, 1H), 3.14 – 3.09 (m, 1H), 2.58 – 2.49 (m, 1H), 2.44 – 2.38 (m, 1H), 2.26 (s, 3H), 2.07 – 1.97 (m, 1H), 1.87 – 1.79 (m, 1H), 1.46 – 1.42 (br m, 18H).¹³C NMR (101 MHz, CDCl₃) δ 171.7, 156.9, 141.5, 132.4, 131.8, 130.8, 126.9, 119.0, 110.6, 81.7, 79.5, 60.2, 53.6, 53.3, 42.3, 28.4, 28.0. HRMS (ESI): calculated for C₂₄H₃₅N₃O₄ [M+H]⁺ 430.2706, found 430.2715.

(*S,E*)-2-amino-4-((3-(4-cyanophenyl)allyl)amino)butanoic acid (24a). Following the procedure described for compound 13a, compound 23a (20 mg, 0.048 mmol) was deprotected and purified, affording compound 24a as a white powder (14 mg, 76% yield). ¹H NMR (400 MHz, CD₃OD) δ 7.75 (d, *J* = 8.4 Hz, 2H), 7.68 (d, *J* = 8.4 Hz, 2H), 6.96 (d, *J* = 15.9 Hz, 1H), 6.54 – 6.47 (m, 1H), 4.10 (dd, *J* = 8.1, 5.3 Hz, 1H), 3.91 (d, *J* = 8.3 Hz, 2H), 3.43 – 3.35 (m, 1H), 2.45 – 2.21 (m, 2H). ¹³C NMR (101 MHz, CD₃OD) δ 169.4, 144.8, 136.5, 131.3, 127.8, 126.2, 122.5, 118.0, 114.1, 111.7, 50.7, 48.7, 42.8, 28.7. HRMS (ESI): calculated for C₁₄H₁₈N₃O₂ [M+H]+ 260.1399, found 260.1408.

(*S,E*)-2-amino-4-((3-(4-cyanophenyl)allyl)(methyl)amino)butanoic acid (24b). Following the procedure described for compound 13a, compound 23b (13 mg, 0.046 mmol) was deprotected and purified, affording compound 24b as a white powder (9 mg, 72% yield). ¹H NMR (400 MHz, CD₃OD δ 7.76 (d, *J* = 8.6 Hz, 2H), 7.71 (d, *J* = 8.5 Hz, 2H), 7.04 – 7.00 (br d, *J* = 15.8 Hz, 1H), 6.62 – 6.51 (m, 1H), 4.13 (dd, *J* = 8.0, 5.3 Hz, 1H), 4.06 (d, *J* = 7.3 Hz, 2H), 3.53 – 3.30 (br m, 2H), 2.96 (s, 3H), 2.53 – 2.42 (m, 1H), 2.41 – 2.30 (m, 1H). ¹³C NMR (101 MHz, CD₃OD) δ 171.7, 143.8, 140.9, 138.7, 132.3, 127.5, 120.3, 117.0, 115.1, 110.6, 54.9, 53.0, 50.8, 39.3, 23.8. HRMS (ESI): calculated for C₁₅H₂₀N₃O₂ [M+H]⁺ 274.1556, found 274.1561.

tert-butyl (2*S*)-4-((((3 3a*R*,3a*R*,4*R*,6*R*,6a*R*)-6-(6-amino-9*H*-purin-9-yl)-2,2dimethyltetra-hydrofuro[3,4-d][1,3]dioxol-4-yl)methyl)(4-cyanobenzyl)amino)-2-((*tert*butoxycarbonyl) amino)butanoate (25a). Following the procedure described for compound 12a, coupling compound 11 (112 mg, 0.20 mmol) with 4-formylbenzonitrile (31 mg, 0.24 mmol) afforded protected intermediate 25a as a white powder (79 mg, 50% yield). ¹H NMR (500 MHz, CDCl₃) δ 8.14 (s, 1H), 7.86 (s, 1H), 7.46 (d, *J* = 8.3 Hz, 2H), 7.32 (d, *J* = 8.2 Hz, 2H), 6.42 (s, 2H), 6.03 (s, 1H), 5.43 – 5.37 (m, 2H), 4.93 (dd, *J* = 6.5, 3.6 Hz, 1H), 4.38 – 4.33 (m, 1H), 4.21 – 4,17 (m, 1H), 3.69 – 3.53 (m, 2H), 2.82 – 2.67 (m, 2H), 2.65 – 2.48 (m, 2H), 2.00 – 1.96 (br m, 1H), 1.77 – 1.71 (br m, 1H), 1.59 (s, 3H), 1.43 – 1.37 (br m, 21H). ¹³C NMR (126 MHz, CDCl₃) δ 175.4, 171.7, 155.7, 148.9, 132.1, 129.3, 120.1, 119.0, 115.8, 109.5, 90.8, 85.7, 84.0, 83.5, 59.4, 52.7, 50.73, 29.8, 28.4, 27.2, 25.5. HRMS (ESI): calculated for C₃₄H₄₇N₈O₇ [M+H]⁺ 679.3568, found 679.3571.

(*S*)-2-amino-4-((((2*R*,3*S*,4*R*,5*R*)-5-(6-amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl)(4-cyanobenzyl)amino)butanoic acid (25). Following the procedure described for compound 13a, compound 25a (50 mg, 0.074 mmol) was deprotected and purified, affording compound 25 as a white powder (33 mg, 75% yield). ¹H NMR (500 MHz, CD₃OD) δ 8.40 (s, 1H), 8.34 (s, 1H), 7.69 (d, *J* = 8.5 Hz, 2H), 7.64 (d, *J* = 8.6 Hz, 2H), 6.11 (d, *J* = 3.2 Hz, 1H), 4.60 (dd, *J* = 5.3, 3.3 Hz, 1H), 4.48 – 4.40 (m, 3H), 4.33 (br d, *J* = 13.7 Hz, 1H), 3.98 (dd, *J* = 7.9, 5.1 Hz, 1H), 3.53 – 3.37 (m, 4H), 2.39 – 2.33 (m, 1H), 2.22 – 2.18 (m, 1H). ¹³C NMR (126 MHz, CD₃OD) δ 172.2, 161.2, 151.5, 147.8, 132.4, 128.0, 119.6, 118.3, 112.8, 90.8, 79.4, 73.1, 70.9, 57.3, 55.1, 51.7, 51.0, 39.1, 25.5. HRMS (ESI): calculated for C₂₄H₂₇N₈O₅ [M+H]⁺ 483.2104, found 483.2115.

(*S*)-2-amino-4-((((2*R*,3*S*,4*R*,5*R*)-5-(6-amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl)(3-(4-cyanophenyl)propyl)amino)butanoic acid (26). Following the procedure described for compound 13a, compound 26a (50 mg, 0.071 mmol) was deprotected and purified, affording compound 26 as a white powder (35 mg, 79% yield). ¹H NMR (400 MHz, CD₃OD) δ 8.47 (s, 1H), 8.38 (s, 1H), 7.61 (d, *J* = 8.3 Hz, 2H), 7.33 (d, *J* = 8.2 Hz, 2H), 6.12 (d, *J* = 3.9 Hz, 1H), 4.70 (t, *J* = 4.3 Hz, 1H), 4.50 – 4.43 (m, 2H), 4.08 (dd, *J* = 8.2, 4.7 Hz, 1H), 3.84 – 3.78 (m, 1H), 3.71 – 3.67 (br d, *J* = 16.0 Hz, 1H), 3.64 – 3.47 (m, 2H), 2.81 – 2.68 (m, 2H), 2.47 – 2.38 (m, 1H), 2.30 – 2.22(m, 1H), 2.07 (h, *J* = 7.4 Hz, 2H). ¹³C NMR (101 MHz, CD₃OD) δ 170.2, 161.9, 161.5, 161.2, 160.8, 151.6, 148.3, 146.1, 145.4, 142.8, 132.1, 129.1, 119.6, 118.4, 115.1, 109.9, 90.6, 78.7, 73.3, 72.2, 54.9, 51.1, 31.9, 24.7, 24.3. HRMS (ESI): calculated for C₂₄H₃₁N₈O₅ [M+H]⁺ 511.2417, found 511.2425.

tert-butyl (2*S*)-4-(((((3*aR*,3*aR*,4*R*,6*R*,6*aR*,6*aR*)-6-(6-amino-9*H*-purin-9-yl)-2,2dimethyltetra-hydrofuro[3,4-d][1,3]dioxol-4-yl)methyl)(3-(3-cyanophenyl)prop-2-yn-1yl)amino)-2-((*tert*-butoxycarbonyl)amino)butanoate (27a). Following the procedure described for compound 12a, coupling compound 11 (112 mg, 0.20 mmol) with 3-(3-oxoprop-1-yn-1yl)benzonitrile (37 mg, 0.24 mmol) afforded protected intermediate compound 27a as a white powder (90 mg, 64% yield). ¹H NMR (400 MHz, CDCl₃) δ 8.31 (s, 1H), 7.99 (s, 1H), 7.63 (s, 1H), 7.55 (dd, *J* = 7.7, 2.1 Hz, 2H), 7.39 (t, *J* = 7.8 Hz, 1H), 6.34 (d, *J* = 11.2 Hz, 2H), 6.09 (d, *J* = 2.3 Hz, 1H), 5.64 – 5.49 (br m, 2H), 5.08 – 5.00 (m 1H), 4.42 – 4.23 (m, 2H), 3.66 (s, 2H), 2.91 – 2.86 (m, 1H), 2.81 – 2.73 (br m, 1H), 2.65 (t, *J* = 6.9 Hz, 2H), 2.02 – 1.96 (m, 1H), 1.87 – 1.80 (m, 1H), 1.62 (s, 3H), 1.50 – 1.35 (br m, 21H). ¹³C NMR (101 MHz, CDCl₃) δ 175.9, 171.7, 155.8, 155.5, 153.0, 149.2, 140.1, 135.83, 135.8, 135.1, 131.4, 129.2, 124.5, 120.2, 118.1, 114.5, 112.8, 86.8, 85.7, 83.9, 83.4, 55.6, 52.67, 50.6, 43.5, 29.7, 28.4, 28.0, 27.2, 25.5. HRMS (ESI): calculated for C₃₆H₄₇N₈O7 [M+H]⁺703.3568, found 703.3582.

(*S*)-2-amino-4-((((2*R*,3*S*,4*R*,5*R*)-5-(6-amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl)(3-(3-cyanophenyl)prop-2-yn-1-yl)amino)butanoic acid (27). Following the procedure described for compound 13a, compound 27a (50 mg, 0.074 mmol) was deprotected and purified, affording compound 27 as a white powder (33 mg, 73% yield). ¹H NMR (500 MHz, CD₃OD) δ 8.48 (s, 1H), 8.38 (s, 1H), 7.83 (t, *J* = 1.3 Hz, 1H), 7.78 – 7.75 (m, 1H), 7.74 – 7.72 (m, 1H), 7.59 – 7.55 (m, 1H), 6.14 (d, *J* = 4.1 Hz, 1H), 4.74 – 4.71 (m, 1H), 4.50 – 4.46 (m, 1H), 4.44 (t, *J* = 5.4 Hz, 1H), 4.23 (s, 2H), 4.13 (t, *J* = 6.4 Hz, 1H), 3.60 – 3.49 (m, 2H), 3.40 (t, *J* = 6.9 Hz, 2H), 2.42 – 2.35 (m, 1H), 2.24 – 2.17 (m, 1H). ¹³C NMR (126 MHz, CD₃OD) δ 170.3, 161.0, 160.8, 151.2, 148.4, 135.8, 134.9, 132.4, 129.6, 123.1, 119.6, 117.4, 115.2, 112.8, 90.3, 86.1, 81.44, 80.2, 73.7, 72.2, 55.9, 51.7, 51.1, 42.7, 25.6. HRMS (ESI): calculated for C₂₄H₂₇N₈O₅ [M+H]⁺ 507.2104, found 507.2108.

tert-butyl (2S)-4-(((((3a*R*,3a*R*,4*R*,6*R*,6a*R*,6a*R*)-6-(6-amino-9*H*-purin-9-yl)-2,2dimethyltetra-hydrofuro[3,4-d][1,3]dioxol-4-yl)methyl)(3-(4-cyanophenyl)prop-2-yn-1yl)amino)-2-((*tert*-butoxycarbonyl)amino)butanoate (28a). Following the procedure described for compound 12a, coupling compound 11 (112 mg, 0.20 mmol) with 4-(3-oxoprop-1-yn-1yl)benzonitrile (37 mg, 0.24 mmol) afforded protected intermediate compound 28a as a white powder (104 mg, 74% yield). ¹H NMR (500 MHz, CDCl₃) δ 8.32 (s, 1H), 7.96 (s, 1H), 7.56 (s, 2H), 7.40 (d, *J* = 8.4 Hz, 2H), 6.09 (d, *J* = 9.8 Hz, 3H), 5.57 (d, *J* = 8.2 Hz, 1H), 5.50 (d, *J* = 8.7 Hz, 1H), 5.09 – 4.99 (m, 1H), 4.43 – 4.36 (m, 1H), 4.25 – 4.23 (m, 1H), 3.66 (d, *J* = 2.9 Hz, 2H), 2.90 – 2.75 (m, 2H), 2.64 (t, J = 6.9 Hz, 2H), 2.01 – 1.97 (m, 1H), 1.85 – 1.80 (m, 1H), 1.61 (s, 3H), 1.45 – 1.38 (br m, 21H). ¹³C NMR (126 MHz, CDCl₃) δ 171.7, 154.5, 153.1, 150.6, 132.3, 132.0, 129.0, 120.9, 118.5, 113.9, 110.2, 90.9, 89.0, 85.8, 83.2, 81.9, 80.2, 55.2, 50.6, 42.1, 30.1, 28.4, 28.1, 27.2, 25.5. HRMS (ESI): calculated for C₃₆H₄₇N₈O₇ [M+H]⁺ 703.3568, found 703.3577.

(*S*)-2-amino-4-((((2*R*,3*S*,4*R*,5*R*)-5-(6-amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl)(3-(4-cyanophenyl)prop-2-yn-1-yl)amino)butanoic acid (28). Following the procedure described for compound 13a, compound 28a (50 mg, 0.074 mmol) was deprotected and purified, affording compound 28 as a white powder (36 mg, 81% yield). ¹H NMR (500 MHz, CD₃OD) δ 8.48 (s, 1H), 8.37 (s, 1H), 7.73 (d, *J* = 8.6 Hz, 2H), 7.59 (d, *J* = 8.6 Hz, 2H), 6.13 (d, *J* = 4.1 Hz, 1H), 4.74 – 4.71 (m, 1H), 4.48 – 4.41 (m, 2H), 4.19 (s, 2H), 4.12 (t, *J* = 6.4 Hz, 1H), 3.53 – 3.42 (m, 2H), 3.35 (d, *J* = 6.5 Hz, 2H), 2.39 – 2.32 (m, 1H), 2.20 – 2.13 (m, 1H). ¹³C NMR (126 MHz, CD₃OD) δ 170.5, 161.0, 151.50, 148.4, 132.1, 126.6, 117.8, 115.3, 112.2, 90.2, 86.2, 84.2, 73.7, 72.2, 55.9, 52.2, 51.1, 42.7, 25.8. HRMS (ESI): calculated for C₂₄H₂₇N₈O₅ [M+H]+ 507.2104, found 507.2113.

tert-butyl (2S)-4-((E)-N-(((3aR,3aR,4R,6R,6aR,6aR)-6-(6-amino-9H-purin-9-yl)-2,2dimethyl-tetrahydrofuro[3,4-d][1,3]dioxol-4-yl)methyl)-3-(4-cyanophenyl)acrylamido)-2-((tertbutoxy carbonyl)amino)butanoate (29a) To a stirred solution of (E)-3-(4-cyanophenyl)acrylic acid (35 mg, 0.20 mmol) in CH₂Cl₂ (10 mL) under N₂ atmosphere were added BOP (97 mg, 0.22 mmol), compound 11 (112mg, 0.20 mmol) and Et₃N (0.1 mL) sequentially. The resulting reaction mixture was then stirred for 16 hours at room temperature. After washing with 5% KHSO₄ (2 × 80 mL), 5% NaHCO₃ (2×80 mL), and H₂O (80 mL), the organic phase was dried over Na₂SO₄. The solvent was evaporated, and the crude product was purified by column chromatography (5% MeOH in EtOAc) to give protected intermediate compound **29a** as a white powder (83 mg, 58% yield). ¹H NMR (400 MHz, CDCl₃) δ 8.32 (d, J = 8.1 Hz, 1H), 7.93 (d, J = 43.7 Hz, 1H), 7.73 – 7.59 (m, 2H), 7.52 – 7.36 (m, 2H), 7.08 – 6.87 (m, 2H), 6.76 (s, 1H), 6.70 – 6.47 (m, 1H), 6.07 (d, J = 9.4 Hz, 1H), 5.63 – 5.47 (br m, 1H), 5.27 (d, J = 6.4 Hz, 1H), 5.17 – 5.12 (m, 1H), 4.27 – 3.80 (m, 3H), 3.70 - 3.66 (br d, J = 16.0 Hz, 1H), 3.60 - 3.13 (m, 2H), 2.11 (s, 1H), 1.61 (d, J = 10.2 Hz, 3H), 1.46 – 1.36 (br m, 21H) ¹³C NMR (101 MHz, CDCl₃) & 175.7, 141.4, 166.2, 155.0, 153.1, 140.2, 139.5, 132.5, 128.5, 127.6, 124.8, 121.5, 118.7, 90.6, 89.9, 84.7, 81.8, 52.2, 50.5, 43.4, 28.4, 28.0, 25.5. HRMS (ESI): calculated for C₃₆H₄₇N₈O₈ [M+H]⁺719.3517, found 719.3524.

(*S*)-2-amino-4-((*E*)-*N*-(((2*R*,3*S*,4*R*,5*R*)-5-(6-amino-9*H*-purin-9-yl)-3,4dihydroxytetrahydro-furan-2-yl)methyl)-3-(4-cyanophenyl)acrylamido)butanoic acid (29). Following the procedure described for compound **13a**, compound **29a** (50 mg, 0.070 mmol) was deprotected and purified, affording compound **29** as a white powder (18 mg, 49% yield). ¹H NMR (400 MHz, CD₃OD) δ 8.40 (s, 1H), 8.32 (s, 1H), 7.68 (d, *J* = 8.4 Hz, 2H), 7.46 (d, *J* = 6.7 Hz, 2H), 7.43 – 7.39 (br d, *J* = 16.0, 1H), 7.18 (br s, 1H), 6.09 – 6.04 (m, 1H), 4.69 – 4.63 (m, 2H), 4.39 – 4.25 (m, 1H), 4.07 – 4.01 (m, 2H), 3.97 (dd, *J* = 7.7, 5.4 Hz, 1H), 3.89 – 3.82 (m, 1H), 3.74 – 3.63 (m, 1H), 2.40 – 2.35 (m, 1H), 2.27 – 2.20(m, 1H). ¹³C NMR (101 MHz, CD₃OD) δ 170.0, 168.4, 162.5, 151.6, 148.3, 140.1, 139.3, 133.2, 127.9, 121.2, 117.96, 112.52, 90.6, 82.5, 72.5, 70.9, 50.2, 42.6, 27.9. HRMS (ESI): calculated for C₂₄H₂₇N₈O₆ [M+H]⁺ 523.2054, found 523.2061.

tert-butyl (2*S*)-4-((((3*aR*,3*aR*,4*R*,6*R*,6*aR*,6*aR*)-6-(6-amino-9*H*-purin-9-yl)-2,2dimethyltetra-hydrofuro[3,4-d][1,3]dioxol-4-yl)methyl)(3-(4-carbamoylphenyl)prop-2-yn-1yl)amino)-2-((*tert*-butoxycarbonyl)amino)butanoate (31a). Following the procedure described for compound 16v, compound 28a was oxidized to afford protected intermediate compound 31a as a white powder (109 mg, 82% yield). ¹H NMR (400 MHz, CDCl₃) δ 8.31 (s, 1H), 7.99 (s, 1H), 7.76 (d, *J* = 8.3 Hz, 2H), 7.38 (d, *J* = 8.2 Hz, 2H), 6.47 (s, 2H), 6.18 (s, 2H), 6.10 (s, 1H), 5.62 (d, *J* = 8.0 Hz, 1H), 5.51 (d, *J* = 5.7 Hz, 1H), 5.12 – 5.03 (m, 1H), 4.43 (s, 1H), 4.32 – 4.21 (m, 1H), 3.67 (s, 2H), 2.92 – 2.80 (m, 2H), 2.69 – 2.63 (m, 2H), 1.99 (d, *J* = 5.5 Hz, 1H), 1.87 – 1.82 (br m, 1H), 1.64 (s, 3H), 1.44 – 1.42 (br d, *J* = 8.0 Hz, 21H). ¹³C NMR (101 MHz, CDCl₃) δ 171.8, 169.0, 155.6, 153.0, 149.2, 140.2, 132.7, 131.8, 127.4, 120.3, 114.5, 90.9, 86.8, 86.0, 83.0, 83.3, 55.7, 52.8, 50.7, 28.4, 28.0, 27.2, 25.50. HRMS (ESI): calculated for C₃₆H₅₁N₈O₈ [M+H]+ 723.3830, found 723.3841

(*S*)-2-amino-4-((((2*R*,3*S*,4*R*,5*R*)-5-(6-amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl)(3-(4-carbamoylphenyl)prop-2-yn-1-yl)amino)butanoic acid (31). Following the procedure described for compound 13a, compound 31a (50 mg, 0.074 mmol) was deprotected and purified, affording compound 31 as a white powder (34 mg, 80% yield). ¹H NMR (400 MHz, CD₃OD) δ 8.59 (s, 1H), 8.32 (s, 1H), 7.87 (d, *J* = 8.5 Hz, 2H), 7.53 (d, *J* = 8.4 Hz, 2H), 6.20 (d, *J* = 4.8 Hz, 1H), 4.79 (t, *J* = 4.8 Hz, 1H), 4.61 (dd, *J* = 7.9, 4.6 Hz, 1H), 4.50 (t, *J* = 4.9 Hz, 1H), 4.44 (s, 2H), 4.20 (dd, *J* = 8.1, 4.7 Hz, 1H), 3.85 – 3.68 (m, 2H), 3.67 – 3.53 (m, 2H), 2.57 – 2.48 (m, 1H), 2.35 – 2.22 (m, 1H). ¹³C NMR (101 MHz, CD₃OD) δ 171.4, 169.9, 162.2, 161.8, 161.5, 161.1, 151.2, 148.3, 144.9, 148.3, 144.9, 143.0, 134.2, 127.5, 124.6, 121.0, 119.2, 118.1, 115.2, 112.3, 89.9, 88.4, 73.7, 72.2, 55.7, 52.1, 51.8, 42.3, 25.3. HRMS (ESI): calculated for C₂₄H₂₈N₈O₆ [M+H]⁺ 525.2210, found 525.2223.

Enzymatic activity assay: Expression and purification of full-length wild-type NNMT protein (NNMTwt) were performed as previously described.³⁰ The purity of the enzyme was confirmed

using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) with Coomassie blue staining, and NNMT identity was confirmed using SDS-PAGE and Western blotting. Catalytic activity of the recombinant protein was evaluated with 1 unit of enzyme activity representing the formation of 1 nmol of MNA/h of incubation. The specific activity of the batch used in the inhibitory activity assays was 18060 units/mg of protein at a protein concentration of 0.98 mg/mL. NNMT was used at a final concentration of 100 nM diluted in assay buffer (50 mM Tris buffer (pH 8.4) and 1 mM dithiothreitol final concentrations). The compounds were dissolved in DMSO and diluted with water to concentrations ranging from 1 nM to 500 μ M (DMSO was kept constant at 1.25% final concentration). The compounds were screened for activity at fixed concentrations of 25 and 5 μ M. When at least 50% inhibition was observed at 25 μ M, full IC₅₀ curves were generated. The compounds were incubated with the enzyme for 10 minutes at room temperature before initiating the reaction with a mixture of NA and SAM at their K_M concentrations of 400 µM and 8.5 µM, respectively. The formation of MNA was measured after 30 minutes at room temperature. The reaction was quenched by addition of 30 μ L of the sample to 70 μ L of acetonitrile containing 50 nM deuteromethylated nicotinamide (d₃-MNA) as internal standard. Sample analysis was performed using Multiple Reaction Monitoring (MRM) on an LC-MS system as previously described with minor modifications.²⁰ The LC-MS system consisted of a Shimadzu 8040 triple quadrupole mass spectrometer (ESI ionization). Isocratic elution was performed after 5 µL injections on a Waters Acquity BEH Amide HILIC column $(3.0 \times 100 \text{ mm}, 1.7 \mu\text{m} \text{ particle size}, \text{Waters, Milford})$, using water containing 300 mM formic acid and 550 mM NH₄OH (pH 9.2) at 40% v/v and acetonitrile at 60% v/v, with a runtime of 1.7 min. Calibration samples were prepared using 70 µL of internal standard d₃-MNA at 50 nM in acetonitrile and 30 µL of an aqueous solution of reference standard MNA with concentrations ranging from 1 to 1024 nM. All compounds exhibiting an IC₅₀ value below 500 nM were considered tight binding inhibitors and were retested using an enzyme concentration of 10 nM and a reaction time of 45 minutes.

Isothermal Titration Calorimetry: All binding experiments are performed using a MicroCal PEAQ-ITC Automated microcalorimeter (Malvern). The samples are equilibrated to 20°C prior to the measurement. The hNNMT enzyme (8.4 mg/mL in 50mM NaH₂PO₄, pH 8, 300mM NaCl, 200mM imidazole, 0.5mM DTT, 1mM PMSF, 20% glycerol) was diluted with 20 mM Tris HCl, pH 7.0 to reach a final concentration of 11.4 μ M. Compound **17u** was diluted to a final concentration of 11.4 μ M. Compound **17u** was diluted to a final any buffer mismatch during titration. Compound **17u** (114 μ M) was titrated into hNNMT (11.4 μ M). The titrations are conducted at 25 °C under constant stirring at 750 rpm. Each binding

experiment consisted of an initial injection of 0.4 μ L followed by 18 separate injections of 2.0 μ L into the sample cell of 200 μ L. The time between each injection is 150 seconds, the measurements are performed with the reference power set at 10 μ cal s⁻¹ and the feedback mode set at 'high'. The calorimetric data obtained is analyzed using MicroCal PEAQ-ITC Analysis Software Version 1.20. ITC data fitting is made based on the "One set of sites" fitting model of the software. The best fit is defined by chi-square minimization. All thermodynamic parameters and thermograms are reported based on the measurements of three independent experiments.

Enzyme assays for selectivity: The PRMT4/CARM1 methyltransferase inhibition assay was performed as previously described²⁹ by using a commercially available chemiluminescent assay kit for PRMT4/CARM1 (purchased from BPS Bioscience). Compound **17u** was tested at concentrations of 3.7, 11.1, 33.3 and 100 μ M and no inhibition was observed at the concentrations tested. The phenylethanolamine *N*-methyltransferase (PNMT) assay was developed using the Promega MTase-GloTM Methyltransferase Assay (purchased from Promega Corporation, US). Compound **17u** was tested at concentrations of 1 and 10 μ M and less than 50% inhibition was observed at the concentration assay are performed as previously described.²⁵

PNMT selectivity assay: The phenylethanolamine N-methyltransferase (PNMT) assay was developed using the Promega MTase-GloTM Methyltransferase Assay (Promega Corporation, US, #V7601). In the coupled luminescence-based assay, the enzymatic product SAH is converted into ADP and subsequently into light.¹ Human recombinant PNMT was purchased from ProSpec-Tany TechnoGene Ltd, Israel (#ENZ-457). After establishing the concentration of enzyme to use in the assay, the K_M values for cofactor SAM and substrate (+)-norepinephrine were determined. The measured K_M values were 2.6 μ M for SAM and 5.9 μ M for (+)-norepinephrine. The final conditions of the assay were set at 125 nM PNMT, 5 μ M SAM, 10 μ M (+)-norepinephrine and a reaction time of 45 minutes. The reactions were performed in half area, flat bottom, white 96 well plates (Greiner Bio-One #675074) with a final volume of the reaction mixture of 10 μ L. Inhibitors (2 μ L) were pre-incubated with the enzyme in the presence of substrate (4 μ L) for 10 minutes before the methyltransferase reaction was initiated through addition of cofactor SAM (4 μ L). After 45 minutes, the MTase Glo detection solution (10 μ L) was added and incubated for 60 minutes followed by analysis of the luminescent signal in a plate reader. Compound **17u** was tested at 3.7, 11.1, 33.3 and 100 μ M in duplicate.

The luminescence data were analysed using GraphPad Prism (version 8.4.3). The luminescence of the positive control (Lp) in each dataset was defined as 100% activity. This value was included in the IC₅₀ graphs at a concentration of 1.5 log values below the lowest concentration tested. The luminescence data of the negative controls (Ln) in each dataset were subtracted from the obtained luminescence data. The percent activity in the presence of each inhibitor was calculated according to the following equation: % activity = (L - Ln)/(Lp - Ln), where L = the luminescence in the presence of the compound, Ln = the luminescence in the absence of the enzyme, and Lp = the luminescence in the absence of the inhibitor. The percent activity values were plotted as a function of inhibitor concentrations and fitted using non-linear regression analysis of the Sigmoidal dose–response curve generated using the equation Y=100/(1+10^((LogIC50-X)*HillSlope)))). The IC₅₀ value was determined by the concentration resulting in a half- maximal percent activity at 21.34 ± 1.28 μ M.

Modelling: The structure of NNMT was taken from PDB entry 6PVE²⁷ and subsequently prepared using the Protein Preparation Wizard in Maestro (Schrodinger, version 2020-3). Compounds were aligned to the co-crystallized ligand using flexible ligand alignment in Maestro, based on their chemical similarity. The generated protein-ligand complexes were used as starting point for molecular dynamics (MD) simulations performed in the software package Q.40 This software is tailored for different types of free energy calculations under spherical boundary conditions, and in particular we used the QligFEP utility as a free energy perturbation (FEP) protocol⁴¹ for the generation of all input files and subsequent analysis. A 25 Å radius sphere was solvated, based on the center of geometry of the ligand. Protein atoms in the boundary of the sphere (22-25 Å outer shell) had a positional restraint of 20 kcal/mol/Å², while solvent atoms were subject to polarization and radial restrains using the surface constrained all-atom solvent (SCAAS)^{42,43} model to mimic the properties of bulk water at the sphere surface. Atoms lying outside the simulation sphere are tightly constrained (200 kcal/mol/Å² force constant) and excluded from the calculation of non-bonded interactions. Long range electrostatics interactions beyond a 10 Å cut off were treated with the local reaction field method,⁴³ except for the atoms undergoing the FEP transformation where no cut-off was applied. Solvent bonds and angles were constrained using the SHAKE algorithm.⁴⁴ All titratable residues outside the sphere were neutralized and histidine protonation states were assigned by the Protein Preparation Wizard. The OPLS-AA/M force field ⁴⁵ was adopted for protein and solvent (TIP3P model) parameters, while compatible OPLS2005 ligand parameters were generated using the ffld_server⁴⁶ and translated to Q format using QligFEP. The simulation sphere was warmed up from 0.1 to 298 K, during a first equilibration period of 0.61

nanoseconds, where an initial restraint of 25 kcal/mol/Å² imposed on all heavy atoms was slowly released for all complexes. Thereafter the system was subject to ten parallel replicates of unrestrained MD, starting in all cases with a 0.25 nanosecond unbiased equilibration period using randomized initial velocities. Thereafter the FEP protocol follows for every investigated ligand pair, which consists of 101 FEP λ -windows, where the coupling parameter λ is unevenly distributed using a sigmoidal function, each window sampled for 10 ps. In order to close a thermodynamic cycle and calculate relative binding free energies, for each ligand pair an analogous FEP transformation is run in parallel in a sphere of water. In these water simulations, the same parameters apply (i.e., sphere size, simulation time, etc.), and the relative binding free energy difference was estimated by solving the thermodynamic cycle utilizing the Bennett acceptance ratio (BAR).⁴⁷ The corresponding experimental values were extracted from the herein reported IC₅₀ values for each ligand using equation 1:

$$\Delta \Delta G_{exp} = -RT \ln \left(\frac{IC50_{17s-\nu}}{IC50_{17x}} \right) \qquad \text{Equation (1)}$$

where $R = 1.987 \times 10^{-3} \text{ kcal/mol/K}$, and T = 298 K.

Cell culture and treatment with compounds: HSC-2 human oral cancer cell line, T24 human bladder cancer cell line and A549 human lung cancer line were purchased from the American Type Culture Collection (ATCC, Rockville, MD, USA), and cultured in DMEM/F12 medium, supplemented with 10% fetal bovine serum and 50 µg/ml gentamicin, at 37 °C in a humidified 5% CO₂ incubator. Compound 17u was dissolved in DMSO at 100mM concentration. This stock solution was then diluted in culture medium to final concentration values ranging between 1µM and 100µM. For each sample, DMSO was kept constant at 0.1% final concentration. The day before starting treatment, cells were seeded in 96-well plates, at a density of $2x10^3$ cells/well. Cells were allowed to attach overnight and then incubated with compound 17u at different final concentrations, or with DMSO only, for 24, 48 and 72 hours. All experiments were performed in triplicate. Cell proliferation was determined using a colorimetric assay with 3-(4,5-dimethylthiazol-2-yl)-2,5diphenyl tetrazolium bromide (MTT). The MTT assay measures the conversion of MTT to insoluble formazan by dehydrogenase enzymes of the intact mitochondria of living cells. Cell proliferation was evaluated by measuring the conversion of the tetrazolium salt MTT to formazan crystals upon treatment with compound 17u or DMSO only for 24, 48 and 72 hours. Briefly, cells were incubated for 2 hours at 37 °C with 100 µl fresh culture medium containing 5µl of MTT

reagent (5 mg/ml in PBS). The medium was removed and 200µl isopropanol were added. The amount of formazan crystals formed correlated directly with the number of viable cells. The reaction product was quantified by measuring the absorbance at 540nm using an ELISA plate reader. Experiments were repeated three times. Results were expressed as percentage of the control (control equals 100% and corresponds to the absorbance value of each sample at time zero) and presented as mean values \pm standard deviation of three independent experiments performed in triplicate. Data were analysed using GraphPad Prism (GraphPad Software, San Diego, CA). Significant differences between groups were determined using the one-way analysis of variance (ANOVA). A p-value <0.05 was considered statistically significant.

Parallel Artificial Membrane Permeability Assay

The PAMPA assay was carried out with a Corning® BioCoatTM Pre-coated PAMPA Plate System (cat. 353015). The stock solutions were prepared at 10 mM concentration in DMSO and diluted with PBS to achieve a final sample concentration of 200 μ M (2% DMSO (v/v)). The bottom plate (donor) was filled with 300 μ L of diluted sample solution, while the top plate (acceptor, containing the synthetic phospholipid membrane) was filled with 200 μ L of PBS. The acceptor plate was then placed on the donor plate and the system incubated for 5 h at 25 °C. The plate sandwich was separated, and the concentrations of samples in both the donor and acceptor compartments were evaluated by means of UV spectrometry using a Tecan plate reader set at 280 nM.

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