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

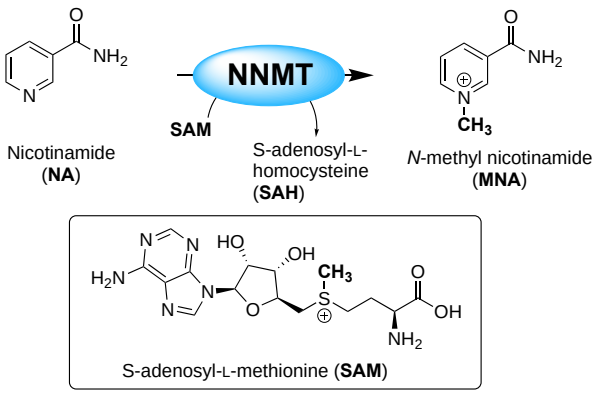


Figure 1. Methylation of nicotinamide (NA) by NNMT using *S*-adenosyl-L-methionine (SAM) as the methyl donor forming *N*-methyl nicotinamide (MNA) and *S*-adenosyl-L-homocysteine (SAH).

field (Figure 2) [21–24] Notably, the potency of bisubstrate NNMT inhibitors has improved from our first reported compounds with IC_{50} values in the micromolar range[19,20,25] to those more recently described by the groups of Shair and Huang with IC_{50} 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 IC_{50} 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 cell-based studies to assess investigate effects on the viability of several cancer cell lines.

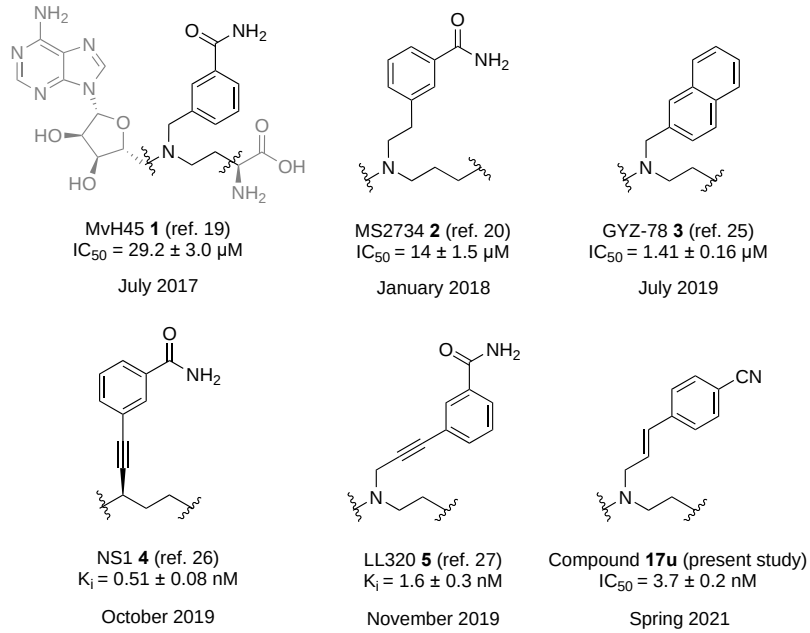


Figure 2. Chemical structures, inhibition data, and publication dates of bisubstrate inhibitors of NNMT.

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 substrate[30] 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

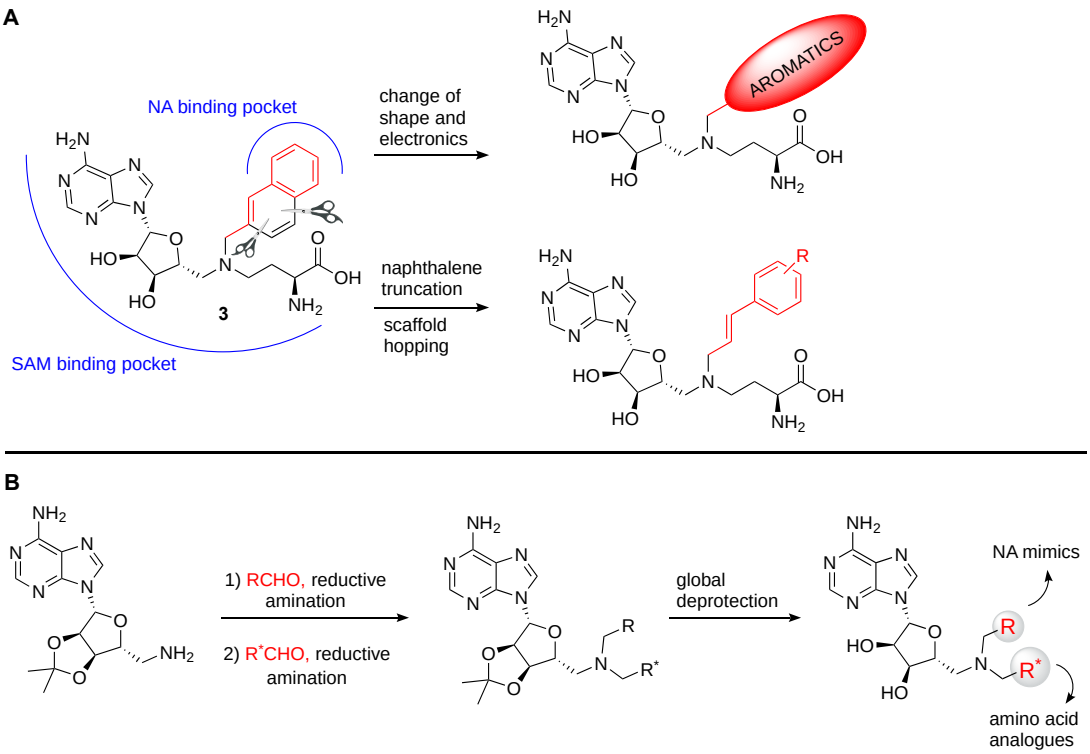
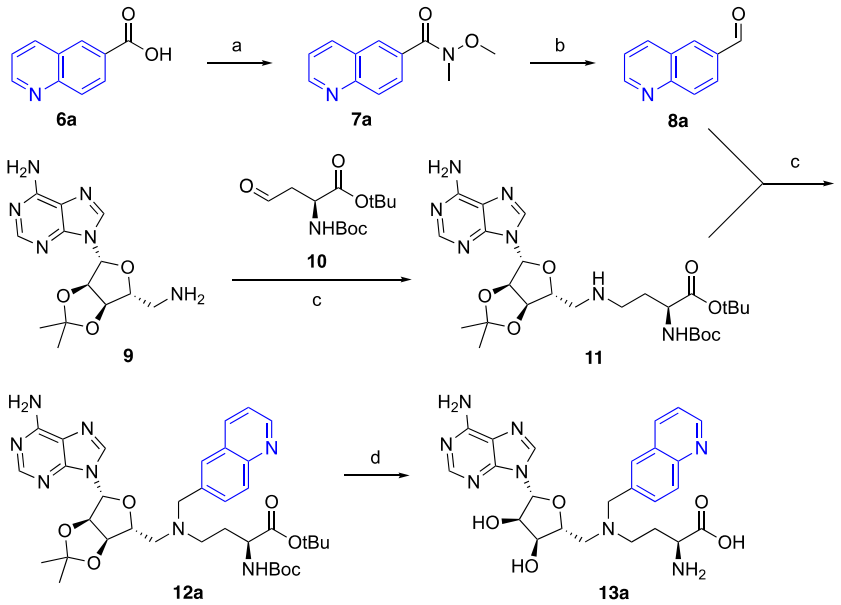


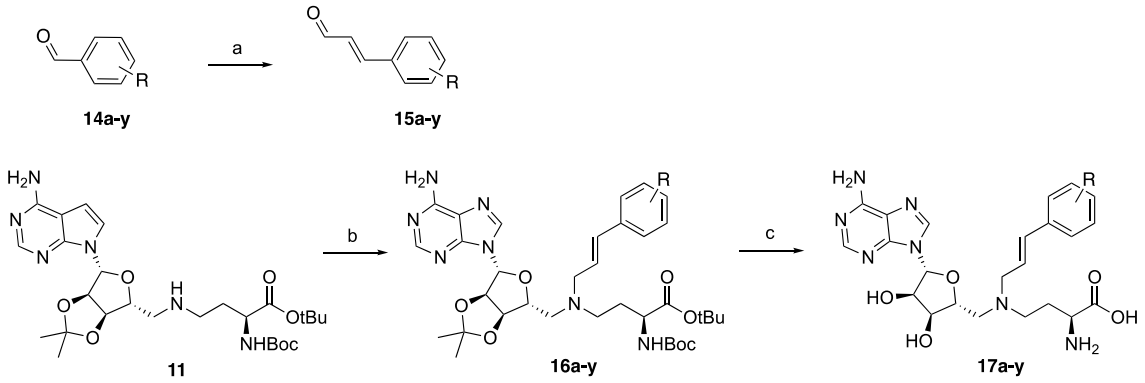
Figure 3. (A) Strategy for the modification and optimization of inhibitor **3** through introduction of a variety of aromatics and the truncation of the naphthalene moiety resulting in the introduction of the alkenyl linker; **(B)** General synthetic route for the preparation of NNMT inhibitors, based 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-l** 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/CH_2Cl_2, with isopropylidene group cleavage facilitated by the addition of water (Scheme 1 and 2).

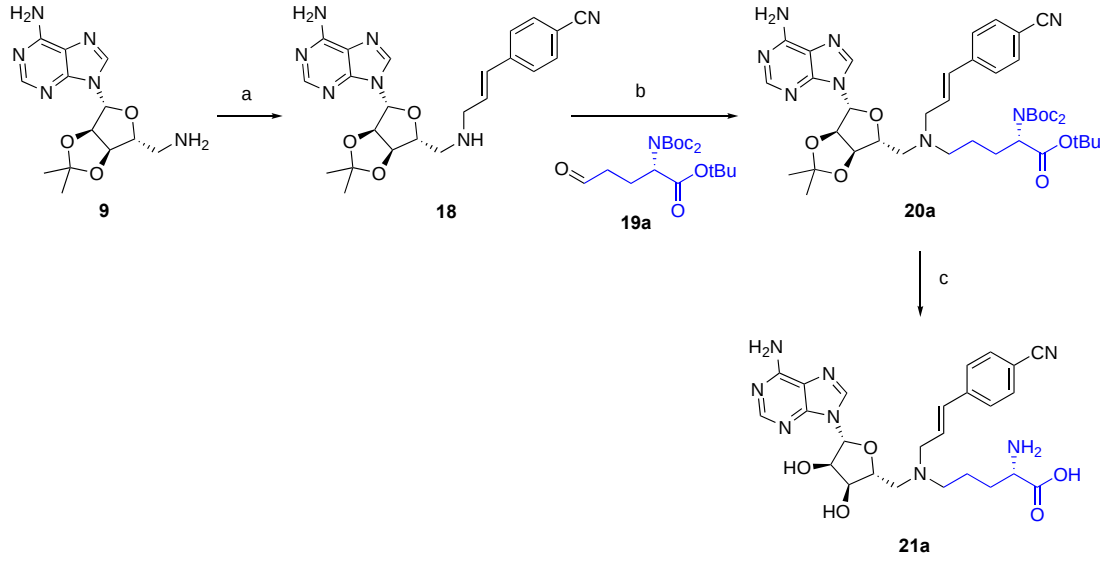


Scheme 1. Representative synthetic scheme for the preparation of bicyclic aromatic compounds **13a-l**, shown for quinoline-containing compound **13a**.[a] The variable group for compounds **6b-l**, **7b-l**, **8b-l**, **12b-l** and **13b-l** is indicated in blue. Reagents and conditions: (a) CH_3NHOCH_3·HCl, BOP, Et_3N, CH_2Cl_2, 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, CH_2Cl_2, H_2O, rt, 2 h. (86%).



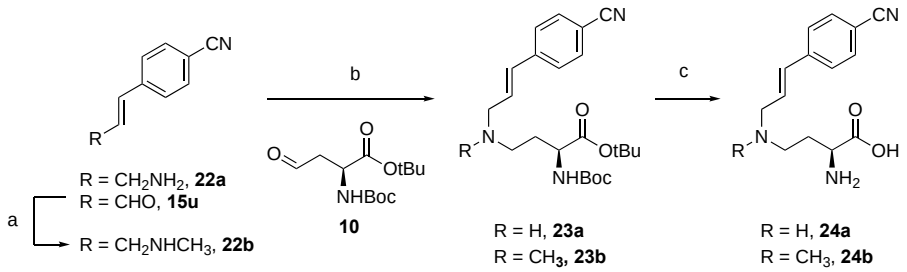
Scheme 2. Representative synthetic scheme for the preparation of substituted cinnamaldehydes **15a-y** and resulting alkenyl linked aromatic compounds **17a-y**. Reagents and conditions: (a) PPh_3=CHCHO, toluene, 80°C, overnight (45-77%); (b) aldehyde **15a-y**, $NaBH(OAc)_3$, AcOH, DCE, rt, overnight (43-81%); (c) TFA, CH_2Cl_2, H_2O, 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, CH_2Cl_2, H_2O, 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, CH_2Cl_2, H_2O, 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 μM. 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 (IC_{50}) 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 IC_{50} 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 IC_{50} values of

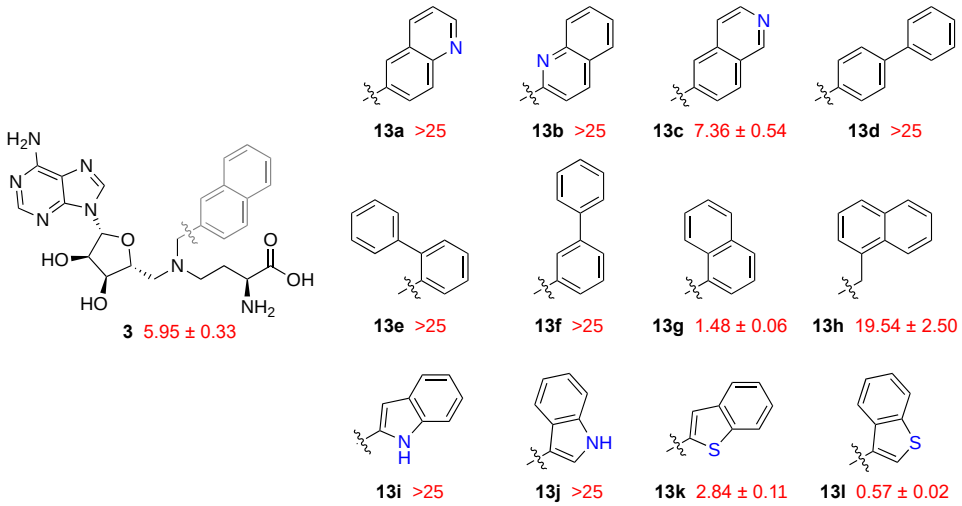


Figure 4. Structure–activity relationship (SAR) studies of bisubstrate NNMT inhibitors **13a-l** carrying bicyclic (hetero)aromatic side-chains to replace the naphthalene group of compound **3**. IC_{50} values (μM) and s.e.m. are shown in red.

compounds **13a** and **13b** were above the 25 μM threshold, with only compound **13c** showing moderate inhibition (IC_{50} = 7.36 μM). Although the incorporation of an α-naphthalene led to good inhibition (**13g**, IC_{50} = 1.48 μM), the addition of an extra carbon to the linker portion abrogated it (**13h**, IC_{50} = 19.54 μM) and switching to biphenyl resulted in a considerable drop in potency (**13d-f**, IC_{50} > 25 μM). A similar trend was observed with the introduction of an indole moiety, with inhibitors **13i** and **13j** failing to display IC_{50} values below 25 μM. 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 IC_{50} value of 0.57 μM (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** (IC_{50} = 1.16 μM) showed better

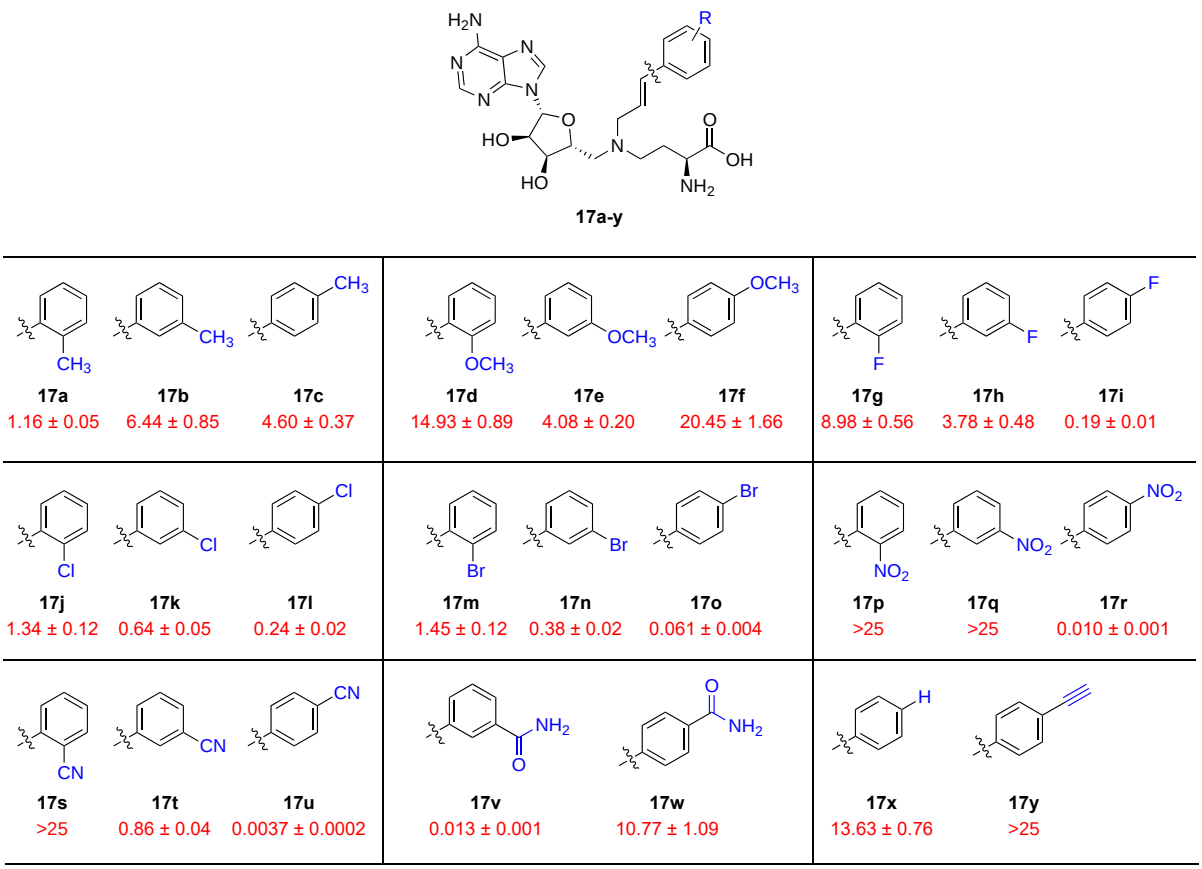


Figure 5. SAR studies of bisubstrate NNMT inhibitors **17a-y** carrying alkenyl linked substituted aromatics. IC_{50} values (μM) and s.e.m. are shown in red and the substitutions are highlighted in blue

activity than the corresponding *meta* (**17b**, IC_{50} = 4.60 μM) and *para* (**17c**, IC_{50} = 6.44 μM) analogues. Methoxy-substituted compounds **17d-f** all showed a somewhat lower potency (IC_{50} ≥ 4 μM). 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*-substitution. In the case of fluorinated ligands **17g-i**, the activity increased with an IC_{50} value of 8.98 μM for *ortho*-F, to 3.78 μM for *meta*-F and the most potent activity observed for the *para*-F substituted compound displaying an IC_{50} value of 0.19 μM. The introduction of a chlorine atom in the same styrene scaffold resulted in a similar trend in NNMT inhibitory activity. In this instance, the IC_{50} values for the *ortho*-Cl and *meta*-Cl compounds were 1.34 μM and 0.64 μM, respectively (**17j** and **17k**, Figure 5), while *para*-analogue **17l** (IC_{50} = 0.24 μM) 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**, IC_{50} = 1.45 and 0.38 μM, respectively), but positively impacted NNMT inhibition in the case of the *para*-Br compound **17o**, which displayed

nanomolar activity (IC_{50} = 0.061 μM, Figure 5). Even more striking was the case of nitro-substituted compounds **17p-r**: while the *para*-nitro-substituted analogue was found to be a highly potent inhibitor (**17r**, IC_{50} = 0.010 μM), both *ortho*-nitro and *meta*-nitro compounds failed to show any appreciable activity (**17p** and **17q**, IC_{50} >25 μM). 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 μM, *meta*-cyano analogue **17t** displayed good inhibition with an IC_{50} of 0.86 μM 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 IC_{50} value (IC_{50} = 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 μM) while the *meta*-amide proved to be an active NNMT inhibitor (IC_{50} = 0.013 μM). 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 μM). 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 μM. 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 **17o**, **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

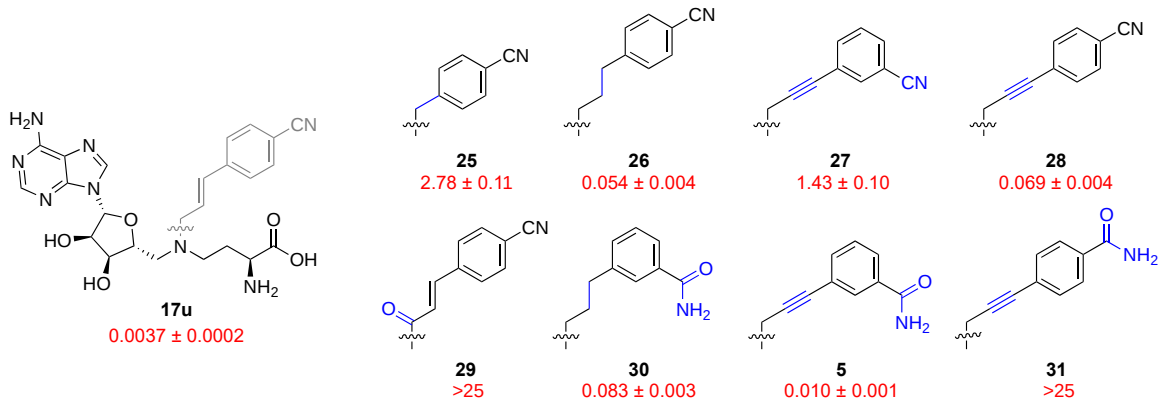


Figure 6. SAR studies of bisubstrate NNMT inhibitors **5** and **25**-**31** carrying different linkers. IC_{50} values (μM) and s.e.m. are shown in red. Changes introduced relative to the lead inhibitor **17u** are indicated in blue.

activity against NNMT (IC_{50} = 2.78 μM and > 25 μM respectively). When the carbon-carbon double bond of inhibitor **17u** was reduced to a saturated three carbon linker, the IC_{50} value increased more than 10-fold (**26**, IC_{50} = 0.054 μM), but the resulting compound still showed high potency. A similar outcome was observed when a propargyl spacer was introduced (**28**, IC_{50} = 0.069 μM).

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 (IC_{50} >25 μM) relative to the *meta* compound (**5**[27]) which was measured to have an IC_{50} value of 0.010 μM 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 (IC_{50} = 1.43 μM) than the *para* isomer **28** (IC_{50} = 0.069 μM, 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 (IC_{50} = 0.083 μM).

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 IC_{50} 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 μM (10 times its K_M value) in the biochemical assay, resulted in a 2- to 4-fold increase in IC_{50}, confirming the trend observed under standard assay conditions. In addition, the apparent K_i values were calculated using Morrison's equation for tight binding inhibitors[32] 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 μM or at 10 times its K_M value (85 μM)

	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 IC_{50} 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 μM). 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 μM). Amino amide analogue **21b** showed a strong decrease in potency (1.90 μM), which was further diminished upon removal of the primary amine (**21c** and **21d**, IC_{50} >25 μM). Replacement of the amino acid moiety with a pyridinone mimic[33] (**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 μM). 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 μM).

NNMT Inhibitor Binding Studies

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

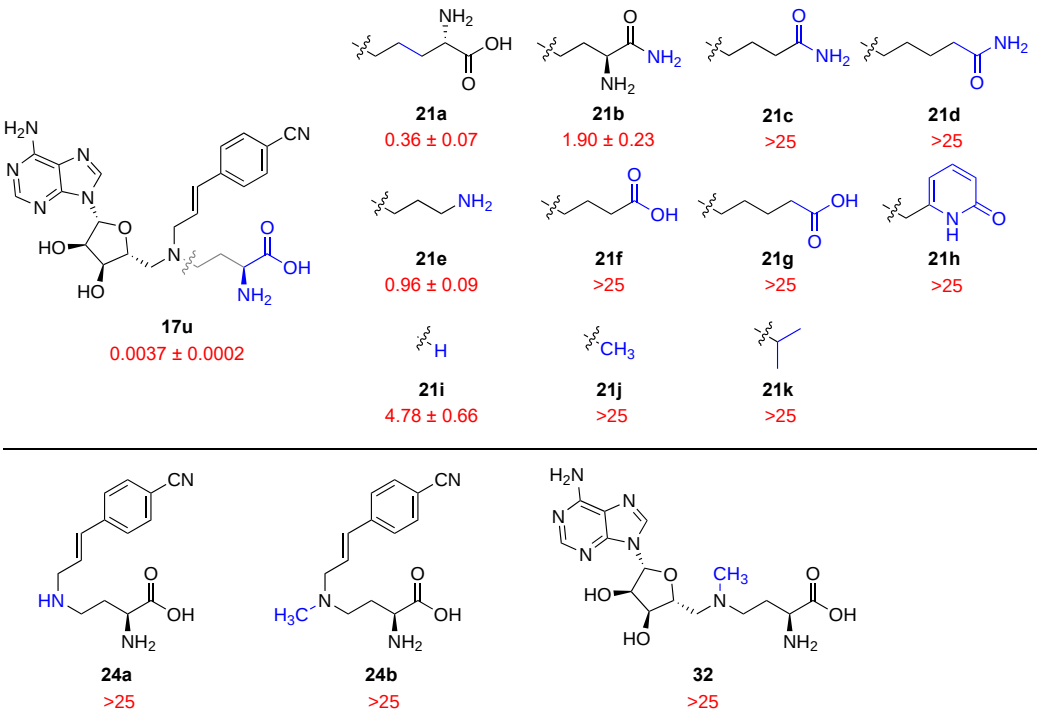


Figure 7. SAR studies of bisubstrate NNMT inhibitors **21a-k** bearing different amino acid substitutions and compounds **24a**, **24b** and **32** lacking either the adenosine unit or the nicotinamide mimicking aromatic sidechain. IC_{50} values (μM) and s.e.m. are shown in red. Changes introduced relative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

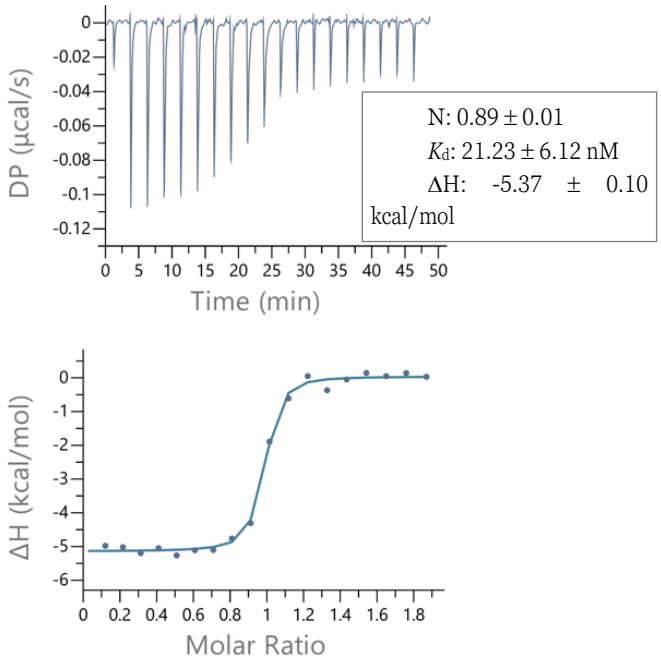


Figure 8. ITC thermogram of compound **17u** including the thermodynamic binding parameters obtained from three independent titration experiments with human wild-type NNMT.

NNMT Inhibitor Modeling

To learn more about the driving force of the *ortho-meta-para* effect observed for the electron-withdrawing (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**.

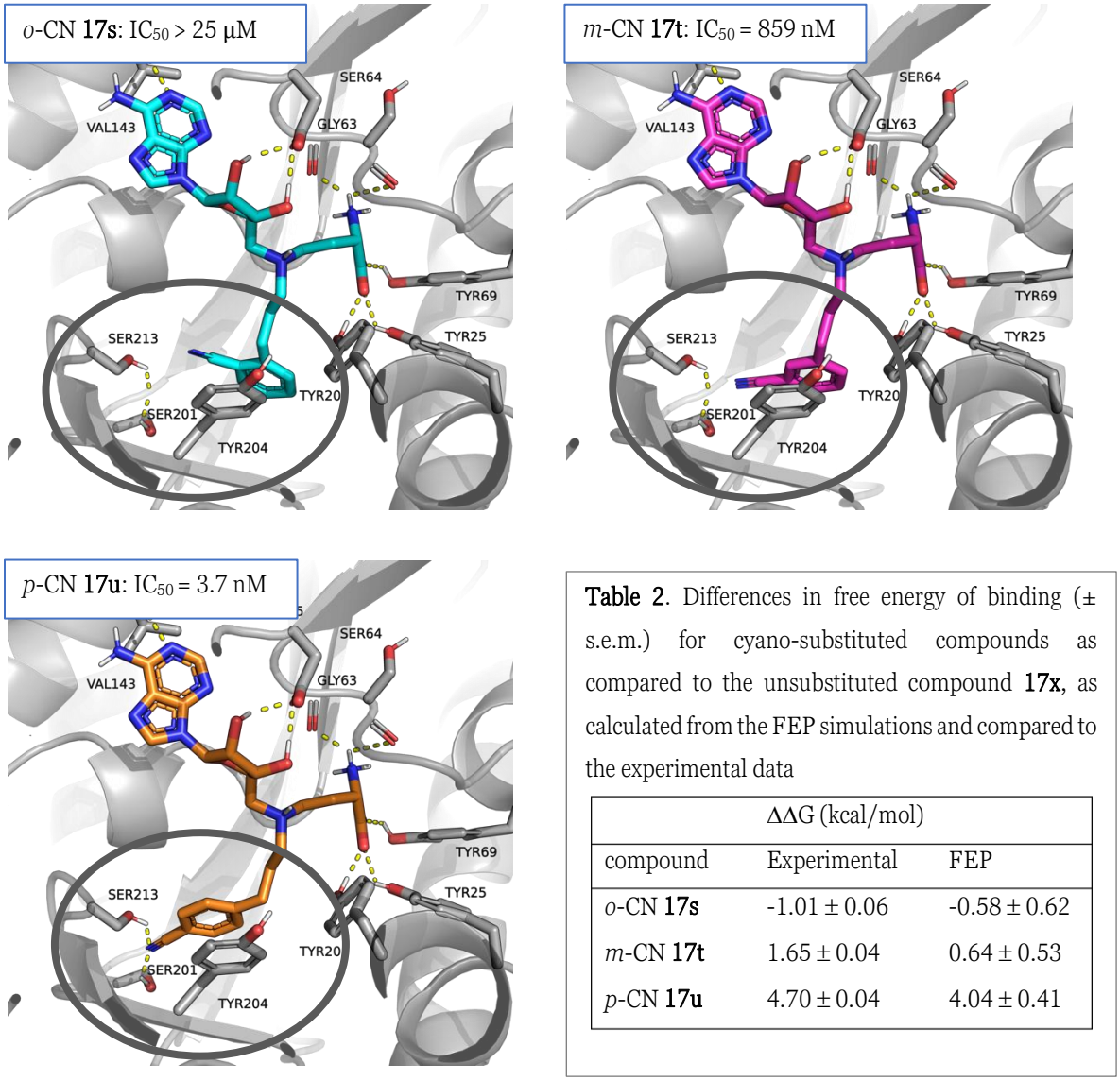


Table 2. Differences in free energy of binding (± s.e.m.) for cyano-substituted compounds as compared to the unsubstituted compound **17x**, as calculated from the FEP simulations and compared to the experimental data

	ΔΔG (kcal/mol)	
compound	Experimental	FEP
o-CN **17s**	-1.01 ± 0.06	-0.58 ± 0.62
m-CN **17t**	1.65 ± 0.04	0.64 ± 0.53
p-CN **17u**	4.70 ± 0.04	4.04 ± 0.41

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 (ΔΔG) 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 μM. Against PRMT5 and DOT1L, **17u** exhibited more than 50% inhibition at 10 μM, but this activity was abolished at 1 μM. The highest percentage inhibition was observed against lysine methyltransferase SMYD-2, with 19% and 39% activity remaining at the concentrations of 10 μM and 1 μM, 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 100μM. 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 and the poor potency in the cellular 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 IC_{50} 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 μM 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, ^{1}H NMR spectra were recorded at 400, 500 or 600 MHz with chemical shifts reported in parts per million downfield relative to H_2O (δ 4.79), CH_3OH (δ 3.31), $CHCl_3$ (δ 7.26), or DMSO (δ 2.50). ^{1}H 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. ^{13}C NMR spectra were recorded at 101, 126 or 151 MHz with chemical shifts reported relative to $CDCl_3$ (δ 77.16), methanol (δ 49.00), or DMSO (δ 39.52). The ^{13}C NMR spectra of the compounds recorded in D_2O 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] **30**[27] and **32**[38] were prepared as previously described and had NMR spectra and mass spectra consistent with the assigned structures. Purity was confirmed to be ≥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, 10 μm) 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, 1.8 μm) 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(1*H*,1*H*,3*H*-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)-2,2-dimethyltetrahydrofuro[3,4-d][1,3]dioxol-4-yl)methyl)amino)-2-((*tert*-butoxycarbonyl)amino)butanoate (11).** 9-((3a*R*,4*R*,6*R*,6a*R*)-6-(aminomethyl)-2,2-dimethyltetrahydrofuro[3,4-d][1,3]dioxol-4-yl)-9*H*-purin-6-amine **9** (7.3 g, 24 mmol), *tert*-butyl (*S*)-2-((*ter*t-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 round-bottom flask (RBF) and the mixture was stirred at room temperature under N_2 atmosphere overnight. The reaction was quenched by adding 1 N NaOH (20 mL), and the product was extracted with CH_2Cl_2. The combined organic layers were washed with brine and dried over Na_2SO_4. 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). ^{1}H NMR (400 MHz, $CDCl_3$) 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). ^{13}C NMR (101 MHz, $CDCl_3$) 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 $C_{26}H_{42}N_7O_7$ $[M+H]^+$ 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)-2,2-dimethyltetrahydrofuro[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-6-carbaldehyde **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 N_2 atmosphere overnight. The reaction was quenched by adding 1 N NaOH (10 mL), and the product was extracted with CH_2Cl_2. The combined organic layers were washed with brine and dried over Na_2SO_4. 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). ^{1}H NMR (400 MHz, $CDCl_3$) 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 – 2.48 (m, 1H), 1.96 (br, 1H), 1.77 (br, 1H), 1.51 (s, 3H), 1.33 – 1.27 (br m, 21H). ^{13}C NMR (101 MHz, $CDCl_3$) 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 $C_{36}H_{49}N_8O_7$ $[M+H]^+$ 705.3724, found 705.3728.

tert-butyl (*S*)-4-((((3aR,4R,6R,6aR)-6-(6-amino-9H-purin-9-yl)-2,2-dimethyltetrahydrofuro[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-((((3a*R*,4*R*,6R,6a*R*)-6-(6-amino-9*H*-purin-9-yl)-2,2-dimethyltetrahydrofuro[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). ^{1}H NMR (400 MHz, $CDCl_3$)

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). ^{13}C NMR (101 MHz, $CDCl_3$) 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 $C_{36}H_{49}N_8O_7$ $[M+H]^+$ 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). ^{1}H NMR (400 MHz, $CDCl_3$) 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). ^{13}C NMR (101 MHz, $CDCl_3$) 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 $C_{39}H_{52}N_7O_7$ $[M+H]^+$ 730.3928, found 730.3956.

tert-butyl (2S)-4-(([1,1'-biphenyl]-2-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 (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). ^{1}H NMR (400 MHz, $CDCl_3$) 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). ^{13}C NMR (101 MHz, $CDCl_3$) 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 $C_{39}H_{51}N_7O_7Na$ $[M+ Na]^+$ 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). ^{1}H NMR (400 MHz, $CDCl_3$) 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). ^{13}C NMR (101 MHz, $CDCl_3$) 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 $C_{39}H_{52}N_7O_7$ $[M+H]^+$ 730.3928, found 730.3938.

tert-butyl (2*S*)-4-((((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)(naphthalen-1-ylmethyl)amino)-2-((tert-butoxycarbonyl)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). ^{1}H NMR (600 MHz, $CDCl_3$) 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). ^{13}C NMR (151 MHz, $CDCl_3$) 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 $C_{37}H_{50}N_7O_7$ $[M+H]^+$ 704.3772, found 704.3775.

tert-butyl (2*S*)-4-((((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)(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). ^{1}H NMR (400 MHz, $CDCl_3$) 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). ^{13}C NMR (101 MHz, $CDCl_3$) 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 $C_{38}H_{52}N_7O_7$ $[M+H]^+$ 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). ^{1}H NMR (600 MHz, $CDCl_3$) 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). ^{13}C NMR (151 MHz, $CDCl_3$) 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 $C_{35}H_{49}N_8O_7$ $[M+H]^+$ 693.3724, found 693.3732.

tert-butyl 3-(((((3a*R*,4*R*,6*R*,6a*R*)-6-(6-amino-9*H*-purin-9-yl)-2,2-dimethyltetrahydrofuro[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). ^{1}H NMR (600 MHz, $CDCl_3$) 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).^{13}C NMR (151 MHz, $CDCl_3$) 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 $C_{40}H_{57}N_8O_9$ $[M+H]^+$ 793.4249, found 793.4256.

tert-butyl (2*S*)-4-((((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)(benzo[*b*]thiophen-2-ylmethyl)amino)-2-((*tert*-butoxycarbonyl)amino) butanoate (12k). Following the procedure described for compound **12a**, coupling compound **11** (112 mg, 0.20 mmol) with benzo[*b*]thiophene-2-carbaldehyde **8k** (39 mg, 0.24 mmol) afforded compound **12k** as a white powder (89 mg, 63% yield). ^{1}H NMR (400 MHz, $CDCl_3$) 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). ^{13}C NMR (101 MHz, $CDCl_3$) 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 $C_{35}H_{48}N_7O_7S$ $[M+H]^+$ 710.3336, found 710.3348.

tert-butyl (2*S*)-4-((((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)(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). ^{1}H NMR (400 MHz, $CDCl_3$) 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). ^{13}C NMR (101 MHz, $CDCl_3$) 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 $C_{35}H_{48}N_7O_7S$ $[M+H]^+$ 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 CH_2Cl_2 was added a mixture of 9 mL TFA and 1 mL H_2O, 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). ^{1}H NMR (400 MHz, D_2O) 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). ^{13}C NMR (101 MHz, D_2O) 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 $C_{24}H_{29}N_8O_5$ $[M+H]^+$ 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). ^{1}H NMR (400 MHz, D_2O) 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). ^{13}C NMR (101 MHz, D_2O) 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 $C_{24}H_{29}N_8O_5$ $[M+H]^+$ 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). ^{1}H NMR (400 MHz, D_2O) 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). ^{13}C NMR (101 MHz, D_2O) 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 $C_{24}H_{29}N_8O_5$ $[M+H]^+$ 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)-3,4-dihydroxy-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). ^{1}H NMR (400 MHz, D_2O) 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). ^{13}C NMR (101 MHz, D_2O) δ 171.1, 163.0, 162.6, 162.2, 143.6, 140.2, 137.8, 131.1,

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 $C_{27}H_{33}N_7O_5$ $[M+H]^+$ 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)-3,4-dihydroxy-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). ^{1}H NMR (400 MHz, D_2O) 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). ^{13}C NMR (101 MHz, D_2O) 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 $C_{27}H_{33}N_7O_5$ $[M+H]^+$ 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)-3,4-dihydroxy-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). ^{1}H NMR (400 MHz, D_2O) 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). ^{13}C NMR (101 MHz, D_2O) 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 $C_{27}H_{33}N_7O_5$ $[M+H]^+$ 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). ^{1}H NMR (400 MHz, D_2O) 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). ^{13}C NMR (101 MHz, D_2O) 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 $C_{25}H_{30}N_7O_5$ $[M+H]^+$ 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). ^{1}H NMR (400 MHz, CD_3OD) 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). ^{13}C NMR (101 MHz, CD_3OD) 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 $C_{26}H_{32}N_7O_5$ $[M+H]^+$ 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-dihydroxy-tetrahydrofuran-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). ^{1}H NMR (400 MHz, D_2O) 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). ^{13}C NMR (101 MHz, D_2O) 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 $C_{23}H_{29}N_8O_5$ $[M+H]^+$ 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-dihydroxy-tetrahydrofuran-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). ^{1}H NMR (500 MHz, CD_3OD) 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). ^{13}C NMR (126 MHz, CD_3OD) 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 $C_{23}H_{29}N_8O_5$ $[M+H]^+$ 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). ^{1}H NMR (400 MHz, D_2O) 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). ^{13}C NMR (101 MHz, D_2O) 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 $C_{23}H_{28}N_7O_5S$ $[M+H]^+$ 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). ^{1}H NMR (400 MHz, CD_3OD) 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). ^{13}C NMR (101 MHz, CD_3OD) 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 $C_{23}H_{28}N_7O_5S$ $[M+H]^+$ 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 N_2 for overnight and concentrated to dryness under vacuum. The crude product was purified by flash chromatography on silica gel (0–90% CH_2Cl_2 in petroleum ether) to give compound **15y** (1.2 g, 73%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) 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). ^{13}C NMR (101 MHz, $CDCl_3$) δ 193.5, 151.6, 132.9, 132.6, 128.3, 126.1, 104.3, 97.6. HRMS (ESI): calculated for $C_{14}H_{17}OSi$ $[M+H]^+$ 229.3740, found 229.3744.

***tert*-butyl (2*S*)-4-((((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)((*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) ^{1}H NMR (400 MHz, $CDCl_3$) 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). ^{13}C NMR (101 MHz, $CDCl_3$) 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 $C_{36}H_{52}N_7O_7$ $[M+H]^+$ 694.3928, found 694.3935.

tert-butyl (2*S*)-4-((((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)((*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). ^{1}H NMR (400 MHz, $CDCl_3$) 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). ^{13}C NMR (101 MHz, $CDCl_3$) 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 $C_{36}H_{52}N_7O_7$ $[M+H]^+$ 694.3928, found 694.3938.

tert-butyl (2*S*)-4-((((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)((*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). ^{1}H NMR (400 MHz, $CDCl_3$) 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). ^{13}C NMR (101 MHz, $CDCl_3$) 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 $C_{36}H_{52}N_7O_7$ $[M+H]^+$ 694.3928, found 694.3940.

tert-butyl (2S)-4-((((3a*R*,3a*R*,4*R*,6R,6a*R*,6a*R*)-6-(6-amino-9*H*-purin-9-yl)-2,2-dimethyltetra-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-(2-methoxyphenyl)acrylaldehyde **15d** (39 mg, 0.24 mmol) afforded compound **16d** as a white powder (75 mg, 53% yield). ^{1}H NMR (400 MHz, $CDCl_3$) 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

(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). ^{13}C NMR (101 MHz, $CDCl_3$) 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 $C_{36}H_{52}N_7O_8$ $[M+H]^+$ 710.3877, found 710.3882.

tert-butyl (2S)-4-((((3a*R*,3a*R*,4*R*,6R,6a*R*,6a*R*)-6-(6-amino-9*H*-purin-9-yl)-2,2-dimethyltetra-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-(2-methoxyphenyl)acrylaldehyde **15e** (39 mg, 0.24 mmol) afforded compound **16e** as a white powder (82 mg, 58% yield). ^{1}H NMR (400 MHz, $CDCl_3$) 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). ^{13}C NMR (101 MHz, $CDCl_3$) 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 $C_{36}H_{52}N_7O_8$ $[M+H]^+$ 710.3877, found 710.3885.

tert-butyl (2*S*)-4-((((3a*R*,3a*R*,4*R*,6R,6a*R*,6a*R*)-6-(6-amino-9*H*-purin-9-yl)-2,2-dimethyltetra-hydrofuro[3,4-d][1,3]dioxol-4-yl)methyl)((*E*)-3-(4-methoxyphenyl)/allyl)amino)-2-((*tert*-butoxy carbonyl)amino)butanoate (16f). Following the procedure described for compound **12a**, coupling compound **11** (112 mg, 0.20 mmol) with (*E*)-3-(4-methoxyphenyl)acrylaldehyde **15f** (39 mg, 0.24 mmol) afforded compound **16f** as a white powder (86 mg, 61% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.29 (s, 1H), 7.96 (s, 1H), 7.26 (d, *J* = 8.7 Hz, 2H), 6.84 (d, *J* = 8.8 Hz, 2H), 6.45 – 6.21 (m, 3H), 6.09 – 6.04 (m, 2H), 5.72 (d, *J* = 8.2 Hz, 1H), 5.47(d, *J* = 8.1 Hz, 1H), 5.01 – 4.99 (m, 1H), 4.41 – 4.40 (br d, *J* = 8.2 Hz, 1H), 4.25 – 4.20 (m, 1H), 3.81 (s, 3H), 3.36 – 3.30 (m, 1H), 3.25 – 3.17 (m, 1H), 2.87 – 2.82 (m, 1H), 2.71 – 2.53 (m, 3H), 2.03 – 1.96 (m, 1H), 1.86 – 1.75 (m, 1H), 1.63 (s, 3H), 1.47 – 1.41 (br m, 21H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.8, 159.1, 155.8, 155.6, 153.1, 149.3, 140.1, 132.5, 129.7, 127.5, 124.9, 120.3, 114.5, 114.0, 90.9, 85.5, 83.95, 83.4, 81.7, 57.1, 55.8, 55.3, 52.9, 29.5, 28.4, 28.0, 27.2, 25.5. HRMS (ESI): calculated for $C_{36}H_{52}N_7O_8$ $[M+H]^+$ 710.3877, found 710.3887.

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

((*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). ^{1}H NMR (600 MHz, $CDCl_3$) 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). ^{13}C NMR (151 MHz, $CDCl_3$) 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 $C_{35}H_{49}FN_7O_7$ $[M+H]^+$ 698.3678, found 698.3690.

tert-butyl (2*S*)-4-((((3a*R*,3a*R*,4*R*,6R,6a*R*,6a*R*)-6-(6-amino-9*H*-purin-9-yl)-2,2-dimethyltetra-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). ^{1}H NMR (600 MHz, $CDCl_3$) 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), 2.70 – 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). ^{13}C NMR (151 MHz, $CDCl_3$) 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 $C_{35}H_{49}FN_7O_7$ $[M+H]^+$ 698.3678, found 698.3682.

tert-butyl (2*S*)-4-((((3a*R*,3a*R*,4*R*,6R,6a*R*,6a*R*)-6-(6-amino-9*H*-purin-9-yl)-2,2-dimethyltetra-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 **12a**, 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). ^{1}H NMR (400 MHz, $CDCl_3$) 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

(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). ^{13}C NMR (101 MHz, $CDCl_3$) 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 $C_{35}H_{49}FN_7O_7$ $[M+H]^+$ 698.3678, found 698.3694.

tert-butyl (2*S*)-4-((((3a*R*,3a*R*,4*R*,6R,6a*R*,6a*R*)-6-(6-amino-9*H*-purin-9-yl)-2,2-dimethyltetra-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). ^{1}H NMR (400 MHz, $CDCl_3$) 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). ^{13}C NMR (101 MHz, $CDCl_3$) 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 $C_{35}H_{49}ClN_7O_7$ $[M+H]^+$ 714.3382, found 714.3389.

tert-butyl (2*S*)-4-((((3a*R*,3a*R*,4*R*,6R,6a*R*,6a*R*)-6-(6-amino-9*H*-purin-9-yl)-2,2-dimethyltetra-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). ^{1}H NMR (400 MHz, $CDCl_3$) 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). ^{13}C NMR (101 MHz, $CDCl_3$) 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 $C_{35}H_{49}ClN_7O_7$ $[M+H]^+$ 714.3382, found 714.3408.

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

((*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). ^{1}H NMR (400 MHz, $CDCl_3$) 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). ^{13}C NMR (101 MHz, $CDCl_3$) 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 $C_{35}H_{49}ClN_7O_7$ $[M+H]^+$ 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)-2,2-dimethyltetrahydrofuro[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). ^{1}H NMR (400 MHz, $CDCl_3$) 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). ^{13}C NMR (101 MHz, $CDCl_3$) 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 $C_{35}H_{49}BrN_7O_7$ $[M+H]^+$ 758.2877 found 758.2882.

tert-butyl (2*S*)-4-((((3a*R*,3a*R*,4*R*,6R,6a*R*,6a*R*)-6-(6-amino-9*H*-purin-9-yl)-2,2-dimethyltetra-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 **12a**, 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). ^{1}H NMR (600 MHz $CDCl_3$) 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). ^{13}C NMR (151 MHz, $CDCl_3$) 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 $C_{35}H_{49}BrN_7O_7$ $[M+H]^+$ 758.2877 found 758.2881.

tert-butyl (2S)-4-((((3a*R*,3a*R*,4*R*,6R,6a*R*,6a*R*)-6-(6-amino-9*H*-purin-9-yl)-2,2-dimethyltetra-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). ^{1}H NMR (400 MHz, $CDCl_3$) 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). ^{13}C NMR (101 MHz, $CDCl_3$) 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 $C_{35}H_{49}BrN_7O_7$ $[M+H]^+$ 758.2877, found 758.2895.

tert-butyl (2*S*)-4-((((3a*R*,3a*R*,4*R*,6R,6a*R*,6a*R*)-6-(6-amino-9*H*-purin-9-yl)-2,2-dimethyltetra-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). ^{1}H NMR (400 MHz, $CDCl_3$) 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). ^{13}C NMR (101 MHz, $CDCl_3$) 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 $C_{35}H_{49}N_8O_9$ $[M+H]^+$ 725.3633, found 725.3632.

tert-butyl (2*S*)-4-((((3a*R*,3a*R*,4*R*,6R,6a*R*,6a*R*)-6-(6-amino-9*H*-purin-9-yl)-2,2-dimethyltetra-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, $CDCl_3$) 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). ^{13}C NMR (151 MHz, $CDCl_3$) 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 $C_{35}H_{49}N_8O_9$ $[M+H]^+$ 725.3633, found 725.3634.

***tert*-butyl (2*S*)-4-((((3a*R*,3a*R*,4*R*,6R,6a*R*,6a*R*)-6-(6-amino-9*H*-purin-9-yl)-2,2-dimethyltetra-hydrofuro[3,4-d][1,3]dioxol-4-yl)methyl)((*E*)-3-(4-nitrophenyl)allyl)amino)-2-((*tert*-butoxy carbonyl)amino)butanoate (16r).** Following the procedure described for compound **12a**, coupling compound **11** (112 mg, 0.20 mmol) with (*E*)-3-(4-nitrophenyl)acrylaldehyde **15r** (42 mg, 0.24 mmol) afforded compound **16r** as a white powder (74 mg, 51% yield). ^1H NMR (600 MHz, $CDCl_3$) 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). ^{13}C NMR (151 MHz, $CDCl_3$) 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 $C_{35}H_{49}N_8O_9$ $[M+H]^+$ 725.3633, found 725.3639.

***tert*-butyl (2S)-4-((((3a*R*,3a*R*,4*R*,6R,6a*R*,6a*R*)-6-(6-amino-9*H*-purin-9-yl)-2,2-dimethyltetra-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, $CDCl_3$) 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). ^{13}C NMR (151 MHz, $CDCl_3$) 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 $C_{36}H_{49}N_8O_7$ $[M+H]^+$ 705.3724, found 705.3734.

tert-butyl (2*S*)-4-((((3a*R*,3a*R*,4*R*,6R,6a*R*,6a*R*)-6-(6-amino-9*H*-purin-9-yl)-2,2-dimethyltetra-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). ^{1}H NMR (400 MHz, $CDCl_3$) 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). ^{13}C NMR (151 MHz, $CDCl_3$) 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 $C_{36}H_{49}N_8O_7$ $[M+H]^+$ 705.3724, found 705.3732.

tert-butyl (2*S*)-4-((((3a*R*,3a*R*,4*R*,6R,6a*R*,6a*R*)-6-(6-amino-9*H*-purin-9-yl)-2,2-dimethyltetra-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). ^{1}H NMR (400 MHz, $CDCl_3$) 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). ^{13}C NMR (151 MHz, $CDCl_3$) 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 $C_{36}H_{49}N_8O_7$ $[M+H]^+$ 705.3724, found 705.3738.

tert-butyl (2S)-4-((((3a*R*,3a*R*,4*R*,6R,6a*R*,6a*R*)-6-(6-amino-9*H*-purin-9-yl)-2,2-dimethyltetra-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 H_2O_2 (30 % w/w) in H_2O (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 Na_2SO_4. 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). ^{1}H NMR (400 MHz, $CDCl_3$) 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). ^{13}C NMR (101 MHz, $CDCl_3$) 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 $C_{36}H_{51}N_8O_8$ $[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)-2,2-dimethyltetrahydrofuro[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). ^{1}H NMR (400 MHz, $CDCl_3$) 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). ^{13}C NMR (101 MHz, $CDCl_3$) 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 $C_{36}H_{51}N_8O_8$ $[M+H]^+$ 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)-2,2-dimethyltetrahydrofuro[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). ^{1}H NMR (400 MHz, $CDCl_3$) 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). ^{13}C NMR (101 MHz, $CDCl_3$) 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 $C_{35}H_{50}N_7O_7$ $[M+H]^+$ 680.3772, found 680.3780.

tert-butyl (2S)-4-((((3a*R*,3a*R*,4*R*,6R,6a*R*,6a*R*)-6-(6-amino-9*H*-purin-9-yl)-2,2-dimethyltetra-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). ^{1}H NMR (400 MHz, $CDCl_3$) 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). ^{13}C NMR (101 MHz, $CDCl_3$) 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 $C_{40}H_{58}N_7O_7Si$ $[M+H]^+$ 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). ^{1}H NMR (400 MHz, CD_3OD) 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). ^{13}C NMR (101 MHz, CD_3OD) 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 $C_{24}H_{32}N_7O_5$ $[M+H]^+$ 498.2465, found 498.2572.

(*S*)-2-amino-4-((((2R,3S,4R,5R)-5-(6-amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-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). ^{1}H NMR (400 MHz, CD_3OD) 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). ^{13}C NMR (101 MHz, CD_3OD) 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 $C_{24}H_{32}N_7O_5$ $[M+H]^+$ 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). ^{1}H NMR (400 MHz, D_2O) 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). ^{13}C NMR (101 MHz, D_2O) 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 $C_{24}H_{32}N_7O_5$ $[M+H]^+$ 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). ^{1}H NMR (400 MHz, CD_3OD) δ 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). ^{13}C NMR (101 MHz, CD_3OD) 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 $C_{24}H_{32}N_7O_6$ $[M+H]^+$ 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). ^{1}H NMR (400 MHz, CD_3OD) 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). ^{13}C NMR (101 MHz, CD_3OD) 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 $C_{24}H_{32}N_7O_6$ $[M+H]^+$ 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). ^{1}H NMR (400 MHz, D_2O) 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). ^{13}C NMR (101 MHz, D_2O) 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 $C_{24}H_{32}N_7O_6$ $[M+H]^+$ 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). ^{1}H NMR (400 MHz, D_2O) 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). ^{13}C NMR (101 MHz, D_2O) 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 $C_{23}H_{29}FN_7O_5$ $[M+H]^+$ 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). ^{1}H NMR (400 MHz, D_2O) 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). ^{13}C NMR (101 MHz, D_2O) 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 $C_{23}H_{29}FN_7O_5$ $[M+H]^+$ 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). ^{1}H NMR (400 MHz, D_2O) 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). ^{13}C NMR (101 MHz, D_2O) 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 $C_{23}H_{29}FN_7O_5$ $[M+H]^+$ 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). ^{1}H NMR (400 MHz, D_2O) 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). ^{13}C NMR (101 MHz, D_2O) 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 $C_{23}H_{29}ClN_7O_5$ $[M+H]^+$ 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). ^{1}H NMR (400 MHz, CD_3OD) 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). ^{13}C NMR (101 MHz, CD_3OD) 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 $C_{23}H_{29}ClN_7O_5$ $[M+H]^+$ 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). ^{1}H NMR (400 MHz, D_2O) 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). ^{13}C NMR (101 MHz, D_2O) 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 $C_{23}H_{29}ClN_7O_5$ $[M+H]^+$ 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). ^{1}H NMR (400 MHz, D_2O) 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). ^{13}C NMR (101 MHz, D_2O) 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 $C_{23}H_{29}BrN_7O_5$ $[M+H]^+$ 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). ^{1}H NMR (400 MHz, D_2O) 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). ^{13}C NMR (101 MHz, D_2O) 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 $C_{23}H_{29}BrN_7O_5$ $[M+H]^+$ 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).^{1}H NMR (400 MHz, D_2O) 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). ^{13}C NMR (101 MHz, D_2O) 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 $C_{23}H_{29}BrN_7O_5$ $[M+H]^+$ 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). ^{1}H NMR (400 MHz, D_2O) 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). ^{13}C NMR (101 MHz, D_2O) 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 $C_{23}H_{29}N_8O_7$ $[M+H]^+$ 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). ^{1}H NMR (500 MHz, CD_3OD) δ 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). ^{13}C NMR (126 MHz, CD_3OD) δ 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 $C_{23}H_{29}N_8O_7$ $[M+H]^+$ 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). ^{1}H NMR (500 MHz, CD_3OD) 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). ^{13}C NMR (126 MHz, CD_3OD) 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 $C_{23}H_{29}N_8O_7$ $[M+H]^+$ 529.2159, found 529.2178.

(*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-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). ^{1}H NMR (400 MHz, D_2O) 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). ^{13}C NMR (101 MHz, D_2O) 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 $C_{24}H_{29}N_8O_5$ $[M+H]^+$ 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). ^{1}H NMR (400 MHz, D_2O) 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). ^{13}C NMR (101 MHz, D_2O) 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 $C_{24}H_{29}N_8O_5$ $[M+H]^+$ 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). ^{1}H NMR (400 MHz, D_2O) 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). ^{13}C NMR (101 MHz, D_2O) 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 $C_{24}H_{29}N_8O_5$ $[M+H]^+$ 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). ^{1}H NMR (400 MHz, CD_3OD) 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). ^{13}C NMR (101

MHz, CD_3OD) 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 $C_{24}H_{31}N_8O_6$ $[M+H]^+$ 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, CD_3OD) 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). ^{13}C NMR (101 MHz, CD_3OD) 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 $C_{24}H_{31}N_8O_6$ $[M+H]^+$ 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, CD_3OD) 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). ^{13}C NMR (126 MHz, CD_3OD) 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 $C_{23}H_{30}N_7O_5$ $[M+H]^+$ 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, CD_3OD) 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). ^{13}C NMR (126 MHz, CD_3OD) 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 $C_{25}H_{30}N_7O_5$ $[M+H]^+$ 508.2308, found 508.2315.

4-((*E*)-3-((((3a*R*,3a*R*,4*R*,6R,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-((3a*R*,4*R*,6*R*,6a*R*)-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). ^{1}H NMR (400 MHz, $CDCl_3$) 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). ^{13}C NMR (101 MHz, $CDCl_3$) 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 $C_{23}H_{26}N_7O_3$ $[M+H]^+$448.2097, found 448.2106.

***tert*-butyl (*S*)-5-((((3 3a*R*,3a*R*,4*R*,6R,6a*R*,6a*R*)-6-(6-amino-9*H*-purin-9-yl)-2,2-dimethyltetrahydrofuro[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(*tert*-butoxycarbonyl)amino)-5-oxopentanoate **19a** (82 mg, 0.24 mmol) afforded protected intermediate **20a** as a white powder (113 mg, 69% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 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). ^{13}C NMR (101 MHz, $CDCl_3$) 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 $C_{42}H_{59}N_8O_9$ $[M+H]^+$ 819.4405, found 819.4410.

methyl (*S*)-4-((((3a*R*,3a*R*,4*R*,6R,6a*R*,6a*R*)-6-(6-amino-9*H*-purin-9-yl)-2,2-dimethyltetrahydro-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). ^{1}H NMR (400 MHz, $CDCl_3$) 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),

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). ^{13}C NMR (101 MHz, $CDCl_3$) 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 $C_{33}H_{43}N_8O_7$ $[M+H]^+$ 663.3255, found 663.3258.

4-((((3a*R*,3a*R*,4*R*,6R,6a*R*,6a*R*)-6-(6-amino-9*H*-purin-9-yl)-2,2-dimethyltetrahydrofuro[3,4-d][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). ^{1}H NMR (400 MHz, $CDCl_3$) 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). ^{13}C NMR (101 MHz, $CDCl_3$) 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 $C_{46}H_{47}N_8O_4$ $[M+H]^+$ 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[3,4-d][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). ^{1}H NMR (500 MHz, $CDCl_3$) 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). ^{13}C NMR (126 MHz, $CDCl_3$) 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 $C_{47}H_{49}N_8O_4$ $[M+H]^+$ 789.3877, found 789.3886.

***tert*-butyl (3-((((3a*R*,4*R*,6*R*,6a*R*)-6-(6-amino-9*H*-purin-9-yl)-2,2-dimethyltetrahydrofuro[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). ^{1}H NMR (500 MHz, $CDCl_3$) 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). ^{13}C NMR (126 MHz, $CDCl_3$) 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 $C_{31}H_{41}N_8O_5$ $[M+H]^+$ 605.3200, found 605.3211.

tert-butyl 4-((((3a*R*,3a*R*,4*R*,6R,6a*R*,6a*R*)-6-(6-amino-9*H*-purin-9-yl)-2,2-dimethyltetrahydro-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). ^{1}H NMR (400 MHz, $CDCl_3$) 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). ^{13}C NMR (101 MHz, $CDCl_3$) 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 $C_{31}H_{40}N_7O_5$ $[M+H]^+$ 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)-2,2-dimethyltetrahydro-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). ^{1}H NMR (500 MHz, $CDCl_3$) 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). ^{13}C NMR (126 MHz, $CDCl_3$) 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 $C_{32}H_{42}N_7O_5$ $[M+H]^+$ 604.3247, found 604.3255.

4-((*E*)-3-((((3a*R*,3a*R*,4*R*,6R,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-1-yl)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). ^{1}H NMR (400 MHz, $CDCl_3$) δ 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). ^{13}C NMR (101 MHz, $CDCl_3$) δ 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 $C_{33}H_{39}N_8O_4$ $[M+H]^+$ 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-amine[39] (64 mg, 0.20 mmol) with **15u** (34 mg, 0.22 mmol) afforded protected intermediate **20j** as a yellow powder (66 mg, 72% yield). ^{1}H 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). ^{13}C 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 $C_{24}H_{28}N_7O_3$ $[M+H]^+$ 462.2254, found 462.2259.

4-((*E*)-3-((((3a*R*,3a*R*,4*R*,6R,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). ^{1}H NMR (400 MHz, $CDCl_3$) 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). ^{13}C NMR (101 MHz, $CDCl_3$) δ 155.8, 153.0, 149.2, 141.6, 140.2,

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 $C_{26}H_{32}N_7O_3$ $[M+H]^+$ 490.2567, found 490.2573.

(*S*)-2-amino-5-((((*2R,3S,4R,5R*)-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). ^{1}H NMR (400 MHz, CD_3OD) 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). ^{13}C NMR (101 MHz, CD_3OD) 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 $C_{25}H_{32}N_8O_5$ $[M+H]^+$ 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). ^{1}H NMR (400 MHz, CD_3OD) 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). ^{13}C NMR (101 MHz, CD_3OD) 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 $C_{24}H_{40}N_9O_4$ $[M+H]^+$ 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-2-yl)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). ^{1}H NMR (400 MHz, CD_3OD) 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). ^{13}C NMR (101 MHz, CD_3OD) 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 $C_{24}H_{29}N_8O_4$ $[M+H]^+$ 493.2312, found 493.2320.

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)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). ^{1}H NMR (400 MHz, CD_3OD) 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). ^{13}C NMR (101 MHz, CD_3OD) 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 $C_{25}H_{31}N_8O_4$ $[M+H]^+$ 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). ^{1}H NMR (500 MHz, CD_3OD) δ 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). ^{13}C NMR (126 MHz, CD_3OD) δ 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 $C_{23}H_{29}N_8O_3$ $[M+H]^+$ 465.2363, found 465.2372.

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 (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). ^{1}H NMR (CD_3OD) 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). ^{13}C NMR (101 MHz, CD_3OD) 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 $C_{24}H_{28}N_7O_5$ $[M+H]^+$ 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-2-yl)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). ^{1}H NMR (400 MHz, CD_3OD) 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). ^{13}C NMR (101 MHz, CD_3OD) 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 $C_{25}H_{30}N_7O_5$ $[M+H]^+$ 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-2-yl)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). ^{1}H NMR (400 MHz, CD_3OD) 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). ^{13}C NMR (101 MHz, CD_3OD) 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 $C_{26}H_{27}N_8O_4$ $[M+H]^+$ 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-2-yl)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). ^{1}H NMR (500 MHz, CD_3OD) 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). ^{13}C NMR (126 MHz, CD_3OD) 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 $C_{20}H_{22}N_7O_3$ $[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-2-yl)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). ^{1}H NMR (400 MHz, CD_3OD) 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). ^{13}C NMR (101 MHz, CD_3OD) 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 $C_{21}H_{24}N_7O_3$ $[M+H]^+$ 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). ^{1}H NMR (500 MHz, CD_3OD) 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). ^{13}C NMR (101 MHz, CD_3OD) 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 $C_{23}H_{28}N_7O_3$ $[M+H]^+$ 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 CH_2Cl_2. The combined organic layers were washed with brine and dried over Na_2SO_4. 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). ^{1}H NMR (400 MHz, CD_3OD) 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). ^{13}C NMR (101 MHz, CD_3OD) δ 140.1, 136.5, 132.4, 127.3, 122.2, 118.1, 111.7, 50.0, 31.5. HRMS (ESI): calculated for $C_{11}H_{13}N_2$ $[M+H]^+$ 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).^{1}H NMR (400 MHz, $CDCl_3$) 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). ^{13}C NMR (101 MHz, $CDCl_3$) 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 $C_{23}H_{34}N_3O_4$ $[M+H]^+$ 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).^{1}H NMR (400 MHz, $CDCl_3$) 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).^{13}C NMR (101 MHz, $CDCl_3$) 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 $C_{24}H_{35}N_3O_4$ $[M+H]^+$ 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). ^{1}H NMR (400 MHz, CD_3OD) 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). ^{13}C NMR (101 MHz, CD_3OD) 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 $C_{14}H_{18}N_3O_2$ $[M+H]^+$ 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). ^{1}H NMR (400 MHz, CD_3OD 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). ^{13}C NMR (101 MHz, CD_3OD) 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 $C_{15}H_{20}N_3O_2$ $[M+H]^+$ 274.1556, found 274.1561.

***tert*-butyl (2*S*)-4-((((3 3a*R*,3a*R*,4*R*,6R,6a*R*,6a*R*)-6-(6-amino-9*H*-purin-9-yl)-2,2-dimethyltetra-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). ^{1}H NMR (500 MHz, $CDCl_3$) 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). ^{13}C NMR (126 MHz, $CDCl_3$) 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 $C_{34}H_{47}N_8O_7$ $[M+H]^+$ 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). ^{1}H NMR (500 MHz, CD_3OD) 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). ^{13}C NMR (126 MHz, CD_3OD) 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 $C_{24}H_{27}N_8O_5$ $[M+H]^+$ 483.2104, found 483.2115.

***tert*-butyl (2*S*)-4-((((3a*R*,3a*R*,4*R*,6R,6a*R*,6a*R*)-6-(6-amino-9*H*-purin-9-yl)-2,2-dimethyltetra-hydrofuro[3,4-d][1,3]dioxol-4-yl)methyl)(3-(4-cyanophenyl)propyl)amino)-2-((*tert*-butoxy carbonyl)amino)butanoate (26a).** Following the procedure described for compound **12a**, coupling compound **11** (112 mg, 0.20 mmol) with 4-(3-oxopropyl)benzonitrile (38 mg, 0.24 mmol) afforded protected intermediate compound **26a** as a white powder (80 mg, 57% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.30 (s, 1H), 7.92 (s, 1H), 7.54 (d, *J* = 8.1 Hz, 2H), 7.22 (d, *J* = 8.2 Hz, 2H), 6.24 (br s, 2H), 6.07 (d, *J* = 1.7 Hz, 1H), 5.76 (d, *J* = 8.0 Hz, 1H), 5.52 (d, *J* = 6.0 Hz, 1H), 5.02 (s, 1H), 4.32 – 4.28 (m, 1H), 4.20 – 4.14 (m, 2H), 3.01 – 2.73 (m, 2H), 2.67 – 2.57 (m, 4H), 2.50 – 2.34 (m, 3H), 2.16 – 1.88 (m, 2H), 1.75 – 1.64 (m, 3H), 1.62 (s, 3H), 1.48 – 1.38 (br m, 21H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.9, 153.1, 149.2, 148.0, 140.3, 132.2, 129.2, 120.4, 119.2, 114.4, 109.6, 90.9, 85.7, 83.9, 83.4, 81.7, 79.5, 60.4, 54.0, 53.7, 53.0, 52.6, 50.9, 42.1, 33.6, 30.8, 29.3, 28.4, 28.2, 27.2, 25.5, 20.0, 14.3. HRMS (ESI): calculated for $C_{36}H_{51}N_8O_7$ $[M+H]^+$ 707.3881, found 707.3882.

(*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). ^{1}H NMR (400 MHz, CD_3OD) 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). ^{13}C NMR (101 MHz, CD_3OD) 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 $C_{24}H_{31}N_8O_5$ $[M+H]^+$ 511.2417, found 511.2425.

tert-butyl (2*S*)-4-((((3a*R*,3a*R*,4*R*,6R,6a*R*,6a*R*)-6-(6-amino-9*H*-purin-9-yl)-2,2-dimethyltetra-hydrofuro[3,4-d][1,3]dioxol-4-yl)methyl)(3-(3-cyanophenyl)prop-2-yn-1-yl)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-1-yl)benzonitrile (37 mg, 0.24 mmol) afforded protected intermediate compound **27a** as a white powder (90 mg, 64% yield). ^{1}H NMR (400 MHz, $CDCl_3$) 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). ^{13}C NMR (101 MHz, $CDCl_3$) 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 $C_{36}H_{47}N_8O_7$ $[M+H]^+$ 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). ^{1}H NMR (500 MHz, CD_3OD) 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). ^{13}C NMR (126 MHz, CD_3OD) 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 $C_{24}H_{27}N_8O_5$ $[M+H]^+$ 507.2104, found 507.2108.

tert-butyl (2S)-4-((((3a*R*,3a*R*,4*R*,6R,6a*R*,6a*R*)-6-(6-amino-9*H*-purin-9-yl)-2,2-dimethyltetra-hydrofuro[3,4-d][1,3]dioxol-4-yl)methyl)(3-(4-cyanophenyl)prop-2-yn-1-yl)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-1-yl)benzonitrile (37 mg, 0.24 mmol) afforded protected intermediate compound **28a** as a white powder (104 mg, 74% yield). ^{1}H NMR (500 MHz, $CDCl_3$) 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). ^{13}C NMR (126 MHz, $CDCl_3$) 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 $C_{36}H_{47}N_8O_7$ $[M+H]^+$ 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). ^{1}H NMR (500 MHz, CD_3OD) 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). ^{13}C NMR (126 MHz, CD_3OD) 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 $C_{24}H_{27}N_8O_5$ $[M+H]^+$ 507.2104, found 507.2113.

***tert*-butyl (2*S*)-4-((*E*)-*N*-(((3a*R*,3a*R*,4*R*,6R,6a*R*,6a*R*)-6-(6-amino-9*H*-purin-9-yl)-2,2-dimethyl-tetrahydrofuro[3,4-d][1,3]dioxol-4-yl)methyl)-3-(4-cyanophenyl)acrylamido)-2-((tert-butoxy carbonyl)amino)butanoate (29a)** To a stirred solution of (*E*)-3-(4-cyanophenyl)acrylic acid (35 mg, 0.20 mmol) in CH_2Cl_2 (10 mL) under N_2 atmosphere were added BOP (97 mg, 0.22 mmol), compound **11** (112mg, 0.20 mmol) and Et_3N (0.1 mL) sequentially. The resulting reaction mixture was then stirred for 16 hours at room temperature. After washing with 5% $KHSO_4$ (2 × 80 mL), 5% $NaHCO_3$ (2 × 80 mL), and H_2O (80 mL), the organic phase was dried over Na_2SO_4. 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). ^{1}H NMR (400 MHz, $CDCl_3$) 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) ^{13}C NMR (101 MHz, $CDCl_3$) δ 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 $C_{36}H_{47}N_8O_8$ $[M+H]^+$ 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)-3,4-dihydroxytetrahydro-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). ^{1}H NMR (400 MHz, CD_3OD) 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). ^{13}C NMR (101 MHz, CD_3OD) 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 $C_{24}H_{27}N_8O_6$ $[M+H]^+$ 523.2054, found 523.2061.

tert-butyl (2*S*)-4-((((3a*R*,3a*R*,4*R*,6R,6a*R*,6a*R*)-6-(6-amino-9*H*-purin-9-yl)-2,2-dimethyltetra-hydrofuro[3,4-d][1,3]dioxol-4-yl)methyl)(3-(4-carbamoylphenyl)prop-2-yn-1-yl)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). ^{1}H NMR (400 MHz, $CDCl_3$) 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). ^{13}C NMR (101 MHz, $CDCl_3$) 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 $C_{36}H_{51}N_8O_8$ $[M+H]^+$ 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). ^{1}H NMR (400 MHz, CD_3OD) 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). ^{13}C NMR (101 MHz, CD_3OD) 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 $C_{24}H_{28}N_8O_6$ $[M+H]^+$ 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 μM (DMSO was kept constant at 1.25% final concentration). The compounds were screened for activity at fixed concentrations of 25 and 5 μM. When at least 50% inhibition was observed at 25 μM, full IC_{50} 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 μL of the sample to 70 μL of acetonitrile containing 50 nM deuteromethylated nicotinamide (d_3-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 × 100 mm, 1.7 μm particle size, Waters, Milford), using water containing 300 mM formic acid and 550 mM NH_4OH (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 d_3-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 IC_{50} 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 NaH_2PO_4, 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 μM. Compound **17u** was diluted to a final concentration of 114 μM in 20 mM Tris HCl, pH 7.0 with the addition of enzyme buffer to avoid any buffer mismatch during titration. Compound **17u** (114μM) was titrated into hNNMT (11.4 μM). The titrations are conducted at 25 °C under constant stirring at 750 rpm. Each binding

experiment consisted of an initial injection of 0.4 μL followed by 18 separate injections of 2.0 μL into the sample cell of 200 μL. The time between each injection is 150 seconds, the measurements are performed with the reference power set at 10 μcal 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 described[29] 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 μM and no inhibition was observed at the concentrations tested. The phenylethanolamine *N*-methyltransferase (PNMT) assay was developed using the Promega MTase-Glo™ Methyltransferase Assay (purchased from Promega Corporation, US). Compound **17u** was tested at concentrations of 1 and 10 μM and less than 50% inhibition was observed at the concentrations tested. Full details of the PNMT assay are provided below. All other methyltransferase assays are performed as previously described.[25]

PNMT selectivity assay: The phenylethanolamine N-methyltransferase (PNMT) assay was developed using the Promega MTase-Glo™ 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 μM for SAM and 5.9 μM for (+)-norepinephrine. The final conditions of the assay were set at 125 nM PNMT, 5 μM SAM, 10 μM (+)-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 μL. Inhibitors (2 μL) were pre-incubated with the enzyme in the presence of substrate (4 μL) for 10 minutes before the methyltransferase reaction was initiated through addition of cofactor SAM (4 μL). After 45 minutes, the MTase Glo detection solution (10 μL) 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 μM 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 IC_{50} 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 IC_{50} value was determined by the concentration resulting in a half- maximal percent activity at 21.34 ± 1.28 μM.

Modelling: The structure of NNMT was taken from PDB entry 6PVE[27] 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) protocol[41] 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/Å^2, 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/Å^2 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_server[46] 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/Å^2 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 IC_{50} values for each ligand using equation 1:

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

where R = 1.987x10^{-3} kcal/mol/K, and T = 298K.

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% CO_2 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)-2,5-diphenyl 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 ± 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® BioCoat™ 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 µM (2% DMSO (v/v)). The bottom plate (donor) was filled with 300 µL of diluted sample solution, while the top plate (acceptor, containing the synthetic phospholipid membrane) was filled with 200 µL 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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