Small molecule inhibitors of Nicotinamide N-Methyltransferase (NNMT)

Gao, Y.

Chapter 2

Bisubstrate inhibitors of nicotinamide *N*-methyltransferase (NNMT) with enhanced activity

Parts of this chapter have been published in:

Gao, Y.; van Haren, M. J.; Moret, E. E.; Rood, J. J. M.; Sartini, D.; Salvucci, A.; Emanuelli, M.; Craveur, P.; Babault, N.; Jin, J.; Martin, N. I .（2019）. Bisubstrate Inhibitors of Nicotinamide *N*-Methyltransferase (NNMT) with Enhanced Activity. *J. Med. Chem*. 62, 6597−6614.

Abstract

Nicotinamide N-methyltransferase (NNMT) catalyzes the methylation of nicotinamide to form *N*- methylnicotinamide. Overexpression of NNMT is associated with a variety of diseases, including a number of cancers and metabolic disorders, suggesting a role for NNMT as a potential therapeutic target. By structural modification of a lead NNMT inhibitor previously developed in our group, we prepared a diverse library of inhibitors to probe the different regions of the enzyme's active site. This investigation revealed that incorporation of a naphthalene moiety, intended to bind the hydrophobic nicotinamide binding pocket via π–π stacking interactions, significantly increases the activity of bisubstrate-like NNMT inhibitors (IC_{50}1.41 μM). These findings are further supported by isothermal titration calorimetry binding assays as well as modeling studies. The most active NNMT inhibitor identified in the present study demonstrated a dose-dependent inhibitory effect on the cell proliferation of the HSC-2 human oral cancer cell line.

1. Introduction

Nicotinamide *N*-methyltransferase (NNMT) is an important metabolic enzyme that catalyzes the transfer of a methyl group from the co-factor *S*-adenosyl-L-methionine (SAM) onto its various substrates, most notably nicotinamide (NA) and other pyridines, to form 1-methyl-nicotinamide (MNA) or the corresponding pyridinium ions.[1–3] The past decade has seen a renewed interest in the biological function of NNMT in a range of human diseases. While it was previously assumed that NNMT's primary roles were limited to nicotinamide metabolism and xenobiotic detoxification of endogenous metabolites, broader roles for NNMT in human health and disease are becoming clearer.[4] NNMT has been found to be overexpressed in a variety of diseases, including metabolic disorders[5–7], cardiovascular disease[8,9], cancer[10–14] and Parkinson's disease[15,16]. In general, overexpression of NNMT has been linked to disease progression in the aforementioned afflictions, with the exception of its role in Parkinson's disease where NNMT seems to be neuroprotective.[17,18] Collectively, NNMT appears to play a unique role in the regulation of post-translational modifications and signal transduction, making it an attractive and viable therapeutic target.

Despite growing interest, few small-molecule NNMT inhibitors have been described to date. Among these structures, the product of the enzymatic reaction, MNA, is a known inhibitor of NNMT and has generally been used in biochemical activity assays.[19] Recently, Cravatt and coworkers reported chloroacetamide-based covalent NNMT inhibitors that react with cysteine C165 in the SAM-binding pocket of the enzyme.[20] Notably, Sanofi researchers also recently reported a series of nicotinamide analogues that inhibit NNMT activity, leading to decreased MNA production, stabilization of insulin levels, glucose regulation, and weight loss in mouse models of metabolic disorders.[21,22] In another approach, the group of Watowich focused on the development of inhibitors based on NNMT's alternative substrate quinoline. Their compounds showed improvement of symptoms in diet-induced obese mice.[23] Previous work in our group has focused on bisubstrate inhibitors designed to mimic the transition state of the methylation reaction catalyzed by NNMT with compound **1** (Figure 1) showing activity on par with the known general methyltransferase inhibitor sinefungin.[24]

Designing bisubstrate analogues as inhibitors is an established and effective strategy that has been applied to a range of methyltransferase enzymes including catechol *O*-methyltransferase (COMT),[25-26] histone lysine methyltransferases,[27] arginine methyltransferases,[28–30] and more recently nicotinamide *N*-methyltransferase.[24,31] A recently published co-crystal structure of a bisubstrate inhibitor bound to NNMT (PDB ID: 6CHH) clearly delineates key interactions with residues in the enzyme active site, providing valuable information for further optimization of

improved bisubstrate-like inhibitors.[31] The work here described builds on our previous findings for "trivalent" inhibitor **1**, which is assumed to simultaneously bind in the adenosine, amino acid, and nicotinamide binding pockets of the NNMT active site. Based upon insights provided by recent NNMT crystal structures, we have designed new inhibitors wherein the nicotinamide moiety is replaced by other aromatic substituents accompanied by variation in the length of the linker connecting the amino acid moiety. Based on the high conservation of the residues in the adenosine binding pocket, no changes were made to the adenosine group. A schematic overview of the design strategy is been presented in Figure 1.

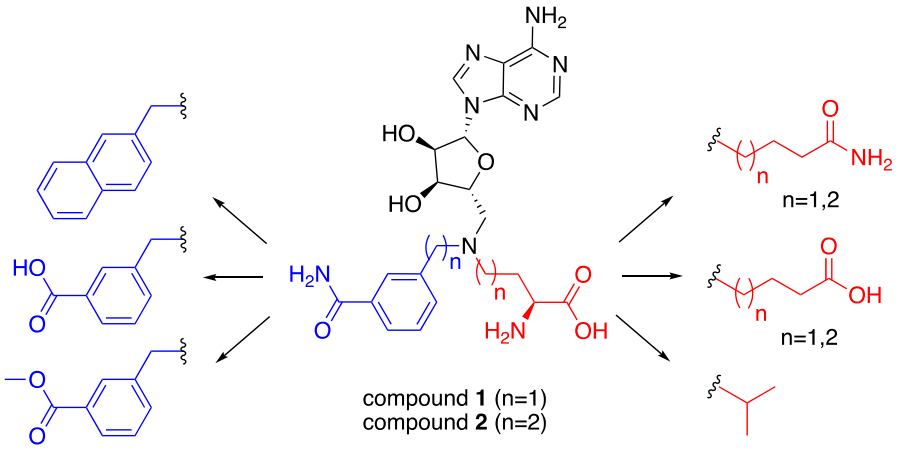


Figure 1. Schematic overview of the design strategy of the second generation of inhibitors based on trivalent bisubstrate compounds **1**[24] and **2**[31]

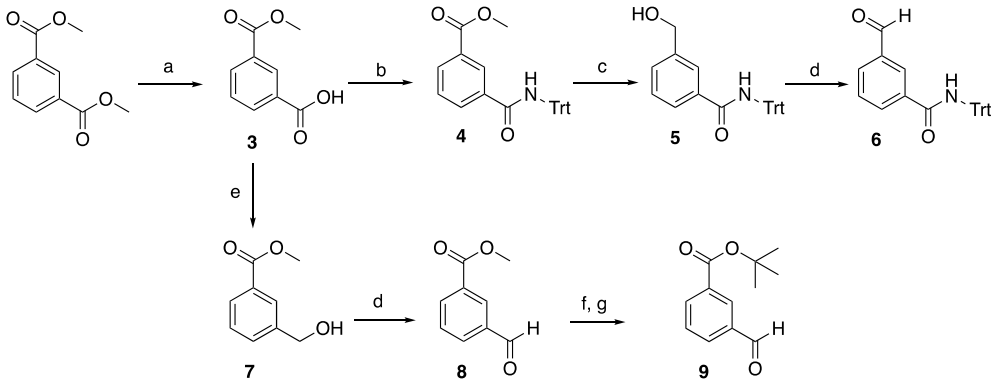
2. Results and discussion

Design: The ternary crystal structure of NNMT (PDB ID: 3ROD) reveals the interactions of nicotinamide and *S*-Adenosyl-L-homocysteine (SAH) with the active site residues.[32] The active site can be roughly divided into three binding regions for the adenosine group, the amino acid moiety, and the nicotinamide unit. The starting point was trivalent bisubstrate compound **1**, which was designed to bind all three binding regions. In order to find the optimal substitutions, a systematic approach was applied, where variations were made to the nicotinamide mimic on the one hand and the amino acid moiety on the other. The benzamide group, representing nicotinamide, was also replaced by methyl benzoate or benzoic acid moieties. Notably, the crystal structure of the NNMT–nicotinamide–SAH ternary complex reveals π-π stacking between tyrosine residue Y204 and the nicotinamide substrate.[32] We therefore also prepared an analogue bearing a naphthalene unit in the presumed nicotinamide position with the aim of introducing stronger π-π stacking with the tyrosine residues of the NNMT active site. We also explored variation of the amino acid moiety as part of our design strategy: in some analogues the amine of

the amino acid unit was omitted to reduce charge and in others the carboxylic acid replaced by the corresponding primary amide. In addition, variation in the length of the carbon chain linking the amino acid moiety was examined. Furthermore, inspired by the structure of histone methyltransferase DOTL1 inhibitor pinometostat,[33] we also investigated the incorporation of an isopropyl group to replace the amino acid moiety entirely.

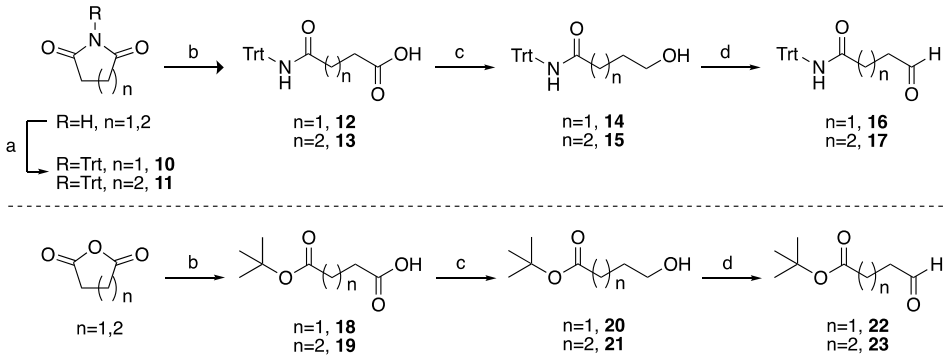
Synthesis: Key aldehyde intermediates (compounds **6**, **8**, **9**, **16**, **17**, **22**, **23**, **27**, **28**) required for the synthesis of the various bisubstrate analogues pursued were prepared from commercially available materials, in good overall yields, as summarized in Scheme 1-3. The trivalent inhibitors were then prepared via a convenient double reductive amination strategy starting from the commercially available 2'-3'-*O*-isopropylidene-6-aminomethyl-adenosine starting material and the corresponding aldehydes (Scheme 4 and 5).

The preparation of aromatic aldehydes **6**, **8**, and **9** began with the selective mono-deprotection of dimethyl isophthalate using sodium hydroxide (Scheme 1).[34] Monomethyl isophthalate (**3**) was subsequently transformed into trityl-protected amide **4** using tritylamine via its acid chloride intermediate and reduced by diisobutylaluminum hydride (DIBAL-H) to give alcohol **5**. The alcohol was oxidized to aldehyde **6** using pyridinium dichromate (PDC). For aldehydes **8** and **9**, the carboxylic acid of **3** was selectively reduced using a mixture of sodium borohydride and boron trifluoride diethyl etherate.[35] The resulting alcohol (**7**) was oxidized using PDC to yield the corresponding aldehyde (**8**). Following hydrolysis of the methyl ester in **8** and subsequent conversion to the *tert*-butyl ester, aldehyde **9** was obtained.[36]



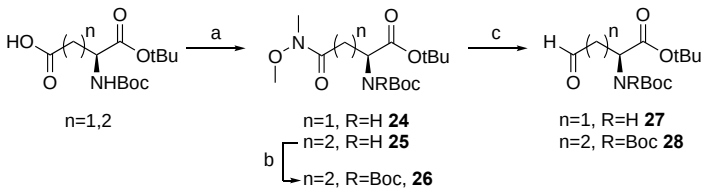
Scheme 1. Synthetic route for aldehydes **6**, **8** and **9**. Reagents and conditions: (a) NaOH, MeOH, rt, 16 h (95%); (b) i) $SOCl_2$, reflux, 2h, ii) tritylamine, CH_2Cl_2, 0°C-rt, 2 h (72%); (c) DIBAL-H, -78°C-rt, 2 h (85%); (d) PDC, CH_2Cl_2, rt, 2 h (53-64%); (e) $NaBH_4$, $BF_3{\cdot}Et_2O$, THF, 0°C-rt, 2 h (89%); (f) LiOH, THF/H_2O (2:1); (g) 2-tert-butyl-1,3-diisopropylisourea, CH_2Cl_2, tert-butanol (39% over 2 steps).

Aliphatic aldehydes **16** and **17** containing trityl-protected amide functionalities were prepared from succinimide and glutarimide respectively (Scheme 2). The cyclic amides were first trityl-protected and subsequently ring-opened using potassium hydroxide. Reduction to the corresponding alcohols and oxidization using PDC gave aldehydes **16** and **17**.[37,38] In analogous fashion, aldehydes **22** and **23**, both containing *tert*-buytl ester moieties, were prepared by ring opening of succinic or glutaric anhydride with *tert*-butyl alcohol to obtain mono-esters **18** and **19**.[39,40] The carboxylic acid functionalities were reduced to alcohols **20** and **21** and then oxidized using PDC to yield aldehydes **22** and **23**.



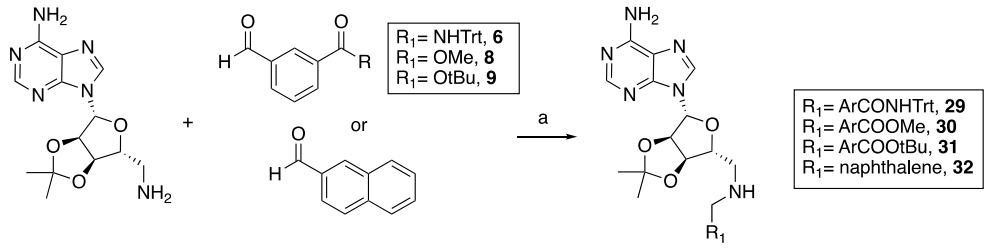
Scheme 2. Synthetic route for aldehydes **16**, **17**, **22** and **23**. Reagents and conditions: (a) TrtCl, CH_3CN, K_2CO_3, rt, 48 h (20-28%); (b) KOH, EtOH, reflux, overnight (37-93%) (c) $NaBH_4$, $BF_3{\cdot}Et_2O$, THF, 0°C-rt, 2 h (64-81%); (d) PDC, CH_2Cl_2, rt, 2 h (65-78%); (e) tert-butanol, DMAP, *N*-Hydroxysuccinimide, Et_3N, toluene, overnight (25-93%).

Aldehydes **27** and **28**, both containing protected amino acid functionalities, were prepared starting from the appropriately protected aspartic acid and glutamic acid building blocks (Scheme 3). Conversion of the side chain carboxylates to their corresponding Weinreb amides yielded intermediates **24** and **25**. Reduction of aspartate-derived **24** with DIBAL-H gave amino acid aldehyde **27** in high yield. For the preparation of aldehyde **28**, a similar route was followed with the addition of a second Boc-protection of intermediate **25** to avoid an intramolecular cyclization side reaction.[24,41]

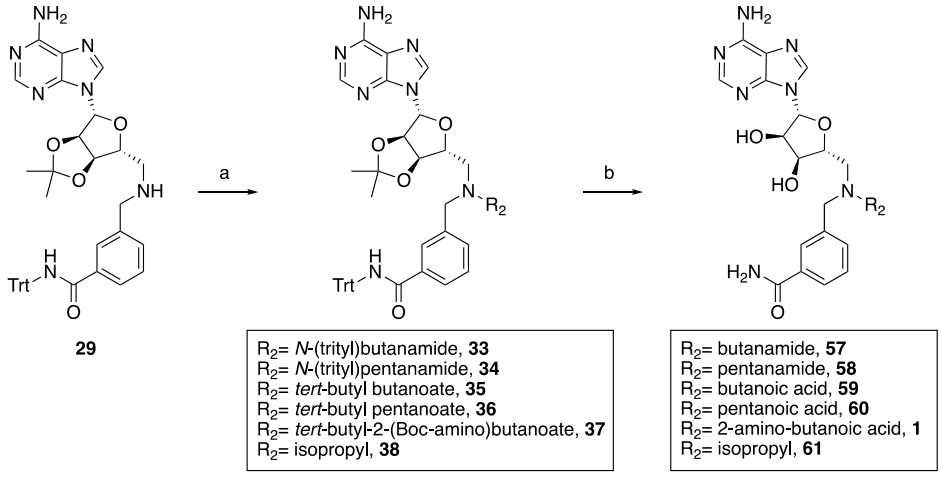


Scheme 3. Synthetic route for aldehydes **27** and **28**. Reagents and conditions: (a) $CH_3NHOCH_3{\cdot}HCl$, BOP, Et_3N, CH_2Cl_2, rt, 2 h (85-88%); (b) $(Boc)_2O$, Et_3N, DMAP, CH_2Cl_2 (94%); (c) DIBAL-H in hexanes (1 M), THF, -78°C, assumed quant.

With the necessary aldehyde building blocks in hand, assembly of the bisubstrate inhibitors was performed in each case starting from commercially available 2'-3'-*O*-isopropylidene-6-aminomethyl-adenosine (Scheme 4). Using a reliable reductive amination approach, aromatic aldehydes **6**, **8**, **9**, and commercially available 2-naphthaldehyde were each coupled to the protected adenosine species to yield intermediates **29-32.** These intermediates were next connected with aliphatic aldehydes **16**, **17**, **22**, **23**, **27, 28** or acetone via a second reductive amination step to give the corresponding protected tertiary amine intermediates **33-56 (Scheme 5)**. Global deprotection of the acid-labile protecting groups was carried out in CH_2Cl_2/TFA (1:1) with isopropylidene group cleavage facilitated by subsequent addition of water. The crude products were purified by preparative HPLC to yield bisubstrate analogues **1**, **57-61**.



Scheme 4. Synthesis of intermediate compounds **29-32**. Reagents and conditions: (a) $NaBH(OAc)_3$, AcOH, DCE, rt, overnight (50-74%).



Scheme 5. Representative scheme for the synthesis of the final compounds, shown for compounds **1** and **57-61**. The same procedure was used starting from aldehydes **30-32** to form intermediate compounds **39-56** and **80** and final compounds **62-79** and **81** as detailed in the experimental section. Reagents and conditions: (a) aldehyde, $NaBH(OAc)_3$, AcOH, DCE, rt, overnight (49-77%); (b) i) TFA, CH_2Cl_2, rt, 2h, ii) H_2O, rt, 30 min (47-73%).

Inhibition Studies: The bisubstrate analogues were next tested for their NNMT inhibitory activity using a method recently developed in our group.[2] This assay employs Ultra High Performance (UHP) Hydrophilic Liquid Interaction Chromatography (HILIC) coupled to Quadrupole Time-Of-Flight Mass Spectrometry (QTOF-MS) to rapidly and efficiently assess NNMT inhibition by analysis of the formation of MNA. The NNMT inhibition of all compounds was initially screened at a fixed concentration of 250 μM for all of the compounds. In cases where at least 50% inhibition was detected at this concentration full inhibition curves were measured in triplicate to determine the corresponding IC_{50} values. As reference compounds, we included the well-established and general methyltransferase inhibitors sinefungin and SAH. In addition, we also synthesized two recently described NNMT inhibitors, compound **2** and 6-(methylamino)-nicotinamide, following the procedures described in the corresponding publications.[21,31] The structures of these reference compounds are provided in Figure 2.

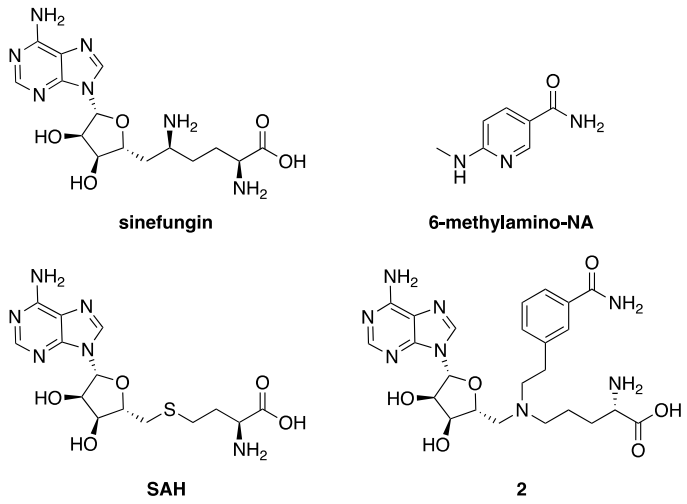
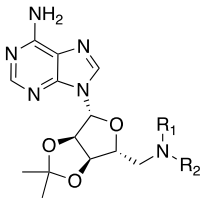
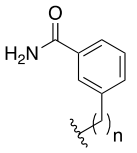
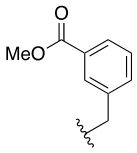
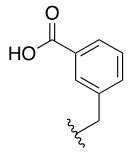
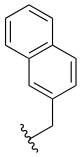
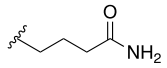
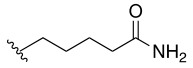
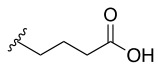
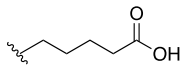
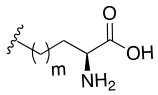


Figure 2. Chemical structures of the reference compounds used in NNMT inhibition studies

The results of the NNMT inhibition studies are summarized in Table 1 and clearly show that only minor adjustments to the functional groups found in the enzyme's natural substrates are tolerated. Among the compounds studied, the most potent inhibition was observed when the aliphatic moiety corresponded to the same length in the amino acid side chain as present in the methyl donor SAM. Notably, the preferred aromatic moiety was found to be the naphthalene group, an apparent confirmation of our hypothesis that increased π-π stacking can lead to enhanced binding in the nicotinamide pocket. The bisubstrate analogue containing both of these elements (compound **78**), displayed the highest inhibitory activity against NNMT with an IC_{50} of 1.41 μM. Interestingly, the amino acid and naphthyl moieties were also found to independently

enhance the activity of the other inhibitors prepared. In this way a suboptimal moiety at one position can be compensated – to an extent – by including either the SAM amino acid motif or the naphthalene unit at the other position. For example, bisubstrate analogues containing the benzamide, benzoic acid, or methyl benzoate groups only show inhibitory activity if they also contain the amino acid motif (compounds **1**, **2**, **66**, **72**) with IC_{50} values of 4.36-23.4 μM respectively. On the other hand, among the bisubstrate analogues lacking the amino acid motif, inclusion of the naphthalene moiety (compounds **74**-**79**) enhances NNMT inhibition albeit with moderate IC_{50} values in the range of 52.6-129.9 μM.

Table 1. Tabulated overview of the chemical structures and inhibition results of the final compounds and reference compounds

		IC_{50} values (μM)[a]			
Reference compounds		**Sinefungin**	**SAH**		**6-methylamino-nicotinamide**
IC_{50} (μM)		12.51 ± 2.11	35.30 ± 5.48		19.81 ± 2.50
		**R_1**			
					
R_2		**57** (n=1): >250	**62**: >250	**68**: >250	**74**: 111.50 ± 28.79
		**58** (n=1): >250	**63**: >250	**69**: >250	**75**: 52.62 ± 9.08
		**59** (n=1): >250	**64**: >250	**70**: >250	**76**: >250
		**60** (n=1): >250	**65**: >250	**71**: >250	**77**: >250
		**1** (m=1, n=1): 14.90 ± 2.07 **2** (m=2, n=2): 4.36 ± 0.27	**66** (m=1, n=1): 17.45 ± 2.65	**72** (m=1, n=1): 23.41 ± 4.86	**78** (m=1, n=1): 1.41 ± 0.16 **81** (m=2, n=2): >250
		**61** (n=1): >250	**67**: >250	**73**: >250	**79**: 129.90 ± 14.80

[a]Assays performed in triplicate on at least six different inhibitor concentrations. Standard errors of the mean reported.

Other notable findings were the results obtained with the reference compounds. The general methyltransferase inhibitors sinefungin and SAH showed inhibitory activities in line with those previously reported.[24] Interestingly, the 6-methylamino-NA compound recently described by Sanofi to be a submicromolar inhibitor,[21] gave an IC_{50} of 19.8 μM in our assay. The recently published bisubstrate analogue **2** exhibited good activity (IC_{50} 4.4 μM) on par with published values.[31] Given the potent inhibition measured for both compound **2** and **78**, we also prepared and tested compound **81**, an analogue of **78** bearing the same naphthyl moiety but with the amino acid motif containing an additional methylene unit as in **2**. Somewhat surprisingly, this linker elongation resulted in a complete loss of inhibitory activity (IC_{50}>250 μM).

To gain insight into the selectivity of compound **78**, we also tested its activity against representative members of both the arginine and lysine families of methyltransferases, PRMT1 and NSD2 respectively. In both cases compound **78** was tested at a concentration of 50 μM and showed no significant inhibition (>50% of the enzyme's activity remained).

ITC binding studies: To further evaluate the binding interactions of the most active bisubstrate analogues with NNMT, isothermal titration calorimetry (ITC) studies were performed, Compounds, **1**, **66**, **72** and **78**, all containing the amino acid moiety but with varying aromatic substituents, were investigated. As illustrated in Figure 3, the dissociation constants (K_d) measured for these compounds track very well with the IC_{50} values measured in the in vitro assay. Compounds **1** and **66** display similar binding to NNMT with K_d values of 36 μM and 25 μM respectively while compound **72** binds less tightly with a K_d of 124 μM. In good agreement with the results of the inhibition assay, the most active inhibitor, compound **78**, also displayed the highest binding affinity for NNMT with a K_d of 5.6 μM. As expected, the inhibitors were each found to bind the enzyme with a 1:1 stoichiometry.

Modeling studies: To further investigate the way in which the inhibitors bind within the NNMT active site modeling studies were performed. Working from the available crystal structure of NNMT protein bounded to nicotinamide and SAH (PDB ID: 3ROD)[32], compounds **1**, **2**, **78**, and **81** were modeled in the binding pocket. In an attempt to explain the significant difference in activity of **78** and **81** additional molecular dynamic simulations were also performed for compounds **1**, **2**, **78** and **81**. While these simulations suggest differences in the binding interaction of the compounds, the calculated binding energies for each are all very similar. In terms of their active site orientations, compounds **1**, **2**, **78** and **81** are all predicted to position their three branches roughly in the same regions of the active site, however their orientations and interactions are quite different.

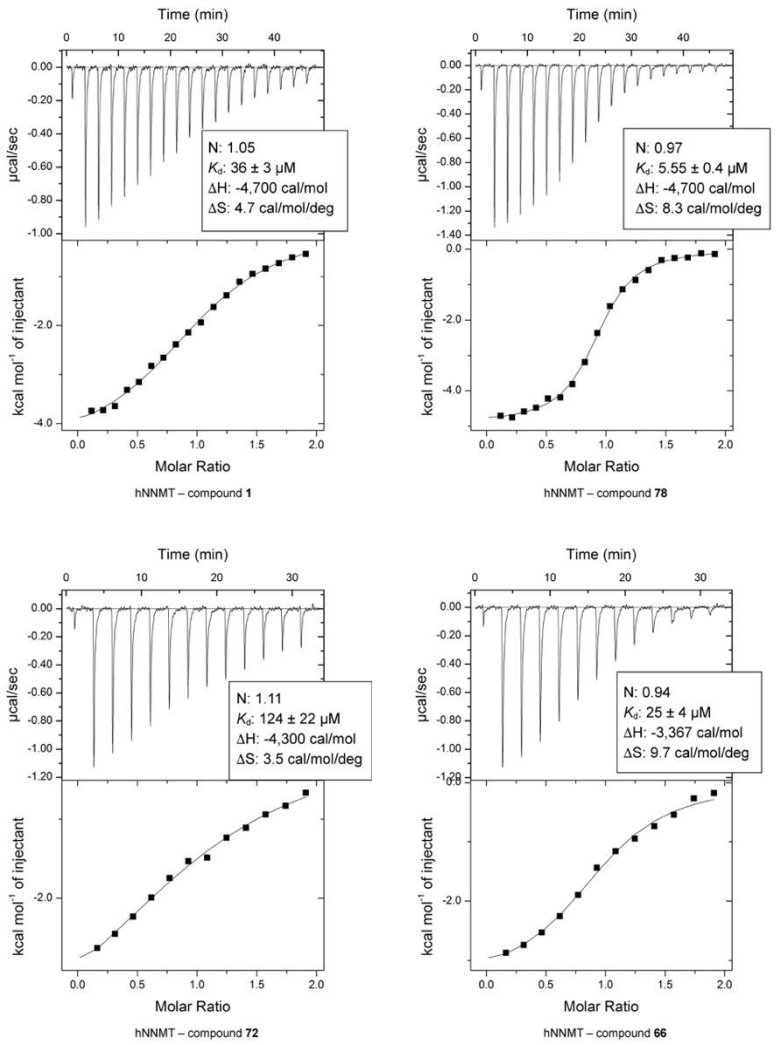


Figure 3. ITC isotherms and thermograms including thermodynamic binding parameters measured for compounds **1**, **66**, **72**, and **78** with hNNMT.

From the modeling data, two distinct features are apparent. First, when the chain linking the amino acid moiety is shorter (as in compounds **1** and **78**), the formation of an intramolecular hydrogen bond interaction was observed between the carboxylate of the amino acid moiety and the protonated tertiary amine (see Figure 4). This intramolecular interaction is highly stable for compound **78** and less stable for compound **1**. This additional interaction reduces the entropic energy of the ligand, thereby potentially stabilizing its binding, and re-orients the amino acid part in the pocket, preventing the polar interactions with neighboring residues (e.g. Y25, D61, Y69, and T163) observed when the chain is longer (as present in compounds **2** and **81**). This intramolecular hydrogen bond may explain the difference in activity observed between compounds **78** and **81**. The second distinct feature is the tyrosine rich environment around the naphthalene moiety of **78** compared to the nicotinamide unit of **1**. The orientation of the tyrosine

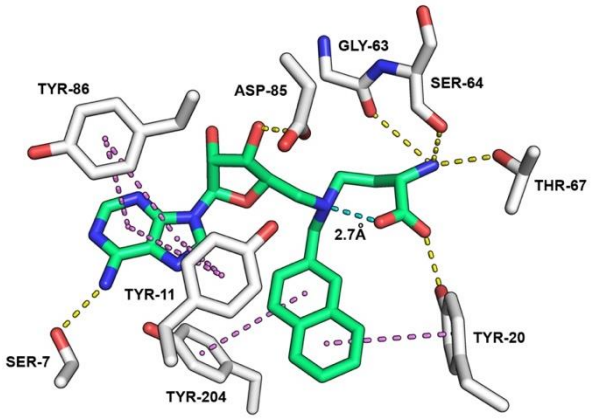


Figure 4. Modeling results for compound **78** in the NNMT active site (PDB ID: 3ROD). Molecular dynamics simulation indicates the presence of an intramolecular hydrogen bond (2.7Å, shown in cyan) specific to compound **78** (in green) that would be expected to reduce the entropic energy of the ligand and potentially stabilize binding to NNMT (in white). Proposed intermolecular hydrogen bond network (in yellow) and π-π stacking interactions with Tyr residues (in purple) stabilize compound **78** in the NNMT active site (hydrogens omitted for clarity).

residues surrounding this part of the molecule leads to π-π stacking interactions with the naphthalene and hint at an explanation for the strong inhibition and high affinity of compound **78** with NNMT protein (Figure 4).

Cell-based assays: To evaluate the cellular activity of the bisubstrate inhibitors, the compounds were tested for their effect on cell proliferation in the human oral cancer cell line HSC-2. We recently found that NNMT expression levels are high in this particular cell line and may contribute to its proliferation and tumorigenic capacity.[42] As shown in Figure 5, there were no significant differences in cell proliferation rate between HSC-2 cells treated with DMSO at 0.1% concentration and cells grown with culture medium only, at any time of each performed assay. Upon treatment with the NNMT inhibitors, cell proliferation was not significantly inhibited by compounds **1**, **2** and **81** (Figure 5). On the contrary, relative to the DMSO control, treatment with compound **78** led to a notable decrease in cell proliferation. In particular, cell proliferation was significantly ($p < 0.05$) inhibited by compound **78** at 10μM (20% reduction), 50 μM (21% reduction) and 100 μM (27% reduction) concentrations, 48 hours after treatment. Interestingly, at the longest 72-hour time-point taken, treatment with compound **78** lead to an even greater and significant ($p < 0.01$) decrease in cell proliferation (44% reduction), at the highest concentration (100 μM) (Figure 5).

We next investigated the effect of compound **78** on cellular NNMT activity by assessing its impact on MNA production in the same HSC-2 cell line. Cells were treated with 100 μM of **78**

and MNA levels determined after 0, 1, 2, and 3 days. Cells treated with compound 78 show a significant ($p < 0.01$) decrease in the levels of MNA (50% reduction) compared to controls after 48 hours. Interestingly, at 72 hours an increase in cellular MNA production was detected, however, the same effect was also observed in the DMSO control (but not in the untreated control) suggesting an effect attributable to longer-term DMSO exposure.

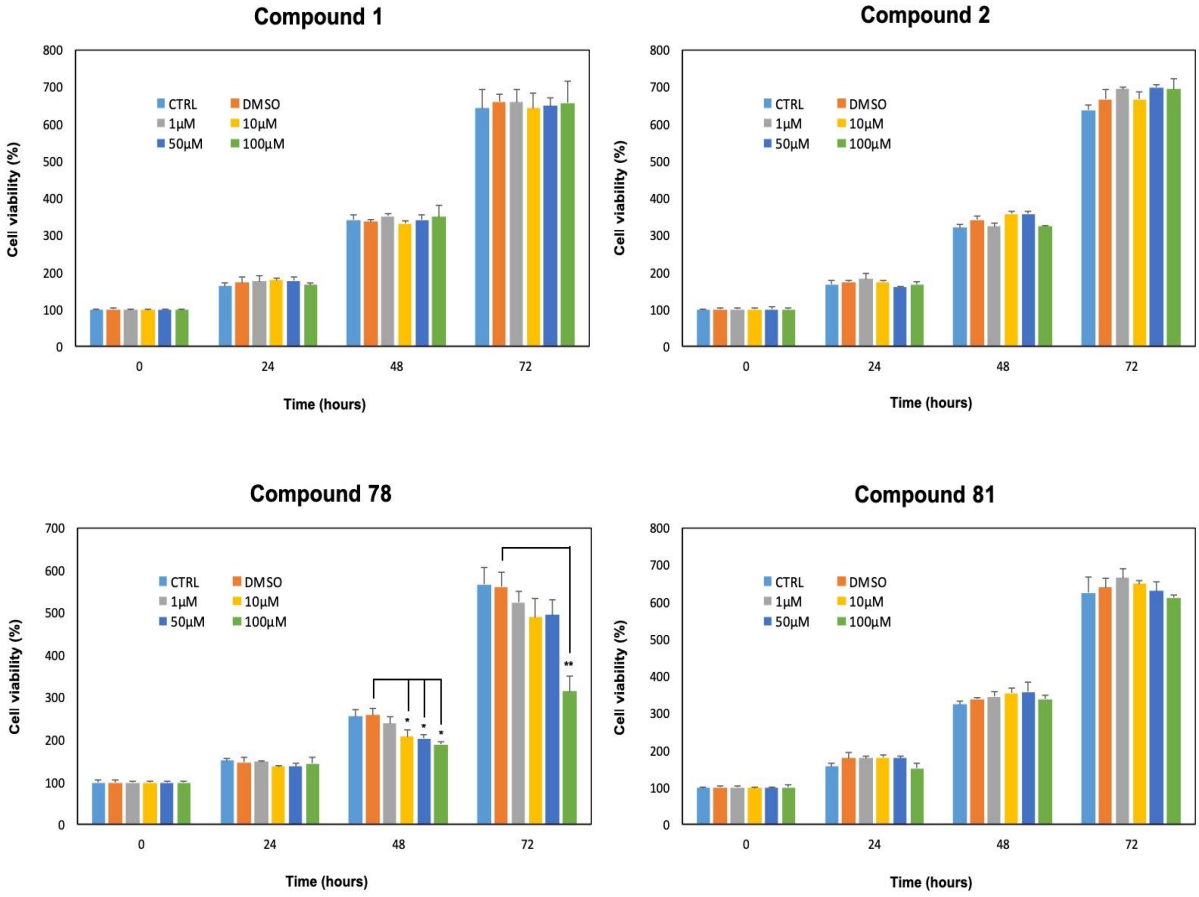


Figure 5. Results of the MTT cell viability assay on HSC-2 human oral cancer cells. Only compound **78** showed a significant effect on cell proliferation after 48 and 72 hours

3. Conclusion

Building from our earlier findings with first reported ternary bisubstrate NNMT inhibitor **1**,[24] we designed and prepared a focused library of novel inhibitors to provide new structure-activity insights. In doing so, various structural motifs were investigated for the ability to enhance inhibitor activity and binding within the NNMT active site. By probing the SAM and NA binding pockets with different spacers and functional groups, we found that the optimal ligands are the endogenous amino acid side-chain and the naphthalene moiety. Among the naphthalene-containing bisubstrate analogues prepared, compound **78** showed the most potent NNMT inhibition. In this way the activity of our initial NNMT inhibitor 1 (IC_{50} = 14.9 μM) was improved

10-fold with compound **78** displaying an IC_{50} value of 1.41 μM. Notably, using an assay designed to directly measure NNMT product formation, compound **78** was shown to be more potent than most other NNMT inhibitor reported to date. ITC-based binding studies provided additional insights in the affinity of the inhibitors for the enzyme with measured K_d value following a trend similar to that observed for the IC_{50} data obtained in the *in vitro* inhibition assays. From modeling studies, the improved activity of compound **78** can be rationalized by the apparent presence of an intramolecular hydrogen bonding interaction predisposing the compound to an active conformation with lower entropic cost. In addition, the modeling indicates that the naphthalene group in **78** is properly oriented so as to benefit from additional π-π stacking interactions with several tyrosine residues in the nicotinamide binding pocket of the enzyme. The cellular data obtained for compound **78** shows a significant inhibitory effect on cell proliferation in HSC-2 oral cancer cells. These promising results provide important new insights for the design and further optimization of potent NNMT inhibitors.

4. Experimental procedures

General Procedures: All reagents employed were of American Chemical Society (ACS) grade or finer and were used without further purification unless otherwise stated. For compound characterization, ^{1}H NMR spectra were recorded at 400 MHz with chemical shifts reported in parts per million (ppm) downfield relative to tetramethylsilane (TMS), H_2O (δ 4.79), $CHCl_3$ (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 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. High-resolution mass spectrometry (HRMS) analysis was performed using a Q-TOF instrument. Compounds **1**,[24] **2**,[31] **3**,[34] **7**,[34] **8**,[36] **9**,[37] **10**,[43] **12**,[38] **14**,[38] **16**,[44] **18**,[40] **19**,[40] **20**,[45] **21**,[46] **22**,[47] **23**,[40] **24**,[41] **25**,[41] **26**,[48] **27**,[41] **28**[48] were prepared as previously described and had NMR spectra and mass spectra consistent with the assigned structures. Purity was confirmed to be ≥ 95% by analytical RP-HPLC using a Phenomenex Kinetex C18 column (5 μm, 250 × 4.6 mm) eluted with a water–acetonitrile gradient moving from 0% to 100% CH_3CN (0.1% TFA) in 30 minutes. The compounds were purified via preparative HPLC using a Reprosil-Pur C18-AQ column (10 μm, 250 × 22 mm) eluted with a water–acetonitrile gradient moving from 0% to 50% CH_3CN (0.1% TFA) over 60 minutes at a flow rate of 12.0 ml min^{-1} with UV detection at 214 nm and 254 nm.

Methyl 3-(tritylcarbamoyl)benzoate (4). Mono-methylisophthalate **3** (0.98 g, 5.4 mmol) was refluxed in 10 mL $SOCl_2$ at 90 °C for about one hour (until the reaction mixture is a clear solution). The $SOCl_2$ was removed under reduced pressure and the acid chloride intermediate was redissolved in 15 mL dry CH_2Cl_2 and transferred to a cooled (ice-bath) solution of tritylamine (1.41 g, 5.4 mmol) and 2 mL triethylamine in 30 mL CH_2Cl_2. The reaction was stirred overnight under N_2 atmosphere, allowing the mixture to warm to room temperature. After the reaction was completed (monitored by TLC (petroleum ether / CH_2Cl_2= 1:1)), the reaction mixture was washed with water and brine and the organic phase dried over Na_2SO_4 and concentrated. The crude product was purified by column chromatography (petroleum ether / CH_2Cl_2= 2:1) to give compound **4** as a white powder (1.64 g, 72% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.45 (t, *J* = 1.6 Hz, 1H), 8.18 (m, 1H), 8.03 (m, 1H), 7.53 (t, *J* = 7.8 Hz, 1H), 7.41 – 7.26 (m, 15H), 3.94 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.3, 165.4, 144.5, 135.6, 132.5, 131.7, 130.6, 128.9, 128.7, 128.1, 128.1, 127.6, 127.2, 71.0, 52.4. HRMS (ESI): calculated for $C_{28}H_{23}NO_3$ $[M+Na]^+$ 444.1576, found 444.1581.

3-(Hydroxymethyl)-*N*-tritylbenzamide (5). Methyl 3-(tritylcarbamoyl)benzoate **4** (0.56 g, 1.33 mmol) was dissolved in dry CH_2Cl_2 (20 mL) under a N_2 atmosphere, the reaction solution was cooled down to -78 °C, and then diisobutylaluminum hydride (DIBAL-H) (5.5 mL, 1.0 M hexane solution) was added slowly. The reaction mixture was stirred at -78 °C for 2 hours. Saturated aq. NH_4Cl (50 mL) was added slowly to quench the reaction under -78 °C, followed by the addition of a saturated Rochelle salt solution (100 mL). The mixture was stirred at room temperature overnight, extracted with CH_2Cl_2 and the organic layers dried over Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by column chromatography (CH_2Cl_2/EtOAc = 9:1) to obtain **5** as a white powder (0.44 g, 85% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.92 (s, 1H), 7.78 (s, 1H), 7.75 – 7.71 (m, 1H), 7.47 (d, *J* = 7.8 Hz, 1H), 7.40 (t, *J* = 7.6 Hz, 1H), 7.36 – 7.18 (m, 15H), 5.26 (br, 1H), 4.54 (s, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 167.0, 145.3, 143.0, 135.5, 129.6, 128.9, 128.3, 127.9, 126.7, 126.5, 126.2, 79.6, 69.9, 69.9, 63.0. HRMS (ESI): calculated for $C_{27}H_{23}NO_2$ $[2M+Na]^+$ 809.3355, found 809.3359.

3-Formyl-*N*-tritylbenzamide (6). 3-(hydroxymethyl)-*N*-tritylbenzamide **5** (0.20 g, 0.51 mmol) and pyridinium dichromate (PDC) 0.23 g, 0.61 mmol) were placed in a 50 mL round bottom flask and 10 mL of dry CH_2Cl_2 was added under N_2 atmosphere at room temperature. The reaction was stirred till completion, as monitored by TLC (petroleum ether / CH_2Cl_2 = 5:1). The mixture was filtered and the organic layer was washed with brine, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The resulting crude product was purified by

column chromatography (petroleum ether / CH_2Cl_2 = 9:1) to obtain **6** as a white powder (0.13 g, yield 64%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.09 (s, 1H), 9.31 (s, 1H), 8.39 (s, 1H), 8.17 (d, *J* = 7.7 Hz, 1H), 8.06 (d, *J* = 7.7 Hz, 1H), 7.68 (t, *J* = 7.7 Hz, 1H), 7.41 – 7.17 (m, 15H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 191.5, 165.1, 144.4, 136.5, 136.2, 133.0, 132.5, 129.5, 128.6, 128.5, 128.1, 127.7, 127.3, 77.2, 71.1. HRMS (ESI): calculated for $C_{27}H_{21}NO_2$ [2M+Na]$^+$ 805.3042, found 805.3047.

***N*-(triphenylmethyl)glutarimide (11).** Glutarimide (2.8 g, 25 mmol), triphenylchloromethane (7.4 g, 25 mmol), and potassium carbonate (3.7 g, 25 mmol) were added to 100 mL acetonitrile and the mixture was stirred at room temperature overnight. Saturated aqueous $NaHCO_3$ (50 mL) was added and the mixture was extracted with EtOAc. The combined organic layers were dried with anhydrous Na_2SO_4 and the solvent was removed under reduced pressure. The crude product was purified by column chromatography (petroleum ether / EtOAc = 4:1) to obtain **11** as a white powder (1.8 g, yield 20%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.45 – 7.35 (m, 6H), 7.20 (t, *J* = 7.8 Hz, 6H), 7.08 (t, *J* = 7.3 Hz, 3H), 2.66 (t, *J* = 6.4 Hz, 4H), 2.01 (p, *J* = 6.5 Hz, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.4, 143.4, 128.5, 127.3, 125.9, 35.5, 16.7. HRMS (ESI): calculated for $C_{24}H_{21}NO_2$ [M+Na]$^+$ 378.1470, found 378.1493.

5-Oxo-5-(tritylamino)pentanoic acid (13). To 2.80 g of KOH dissolved in 50 ml of ethanol was added *N*-tritylglutarimide **11** (1.00 g, 2.8 mmol) and the mixture was refluxed for 48 hours. The mixture was then concentrated to dryness and redissolved in H_2O. Acidification of the basic solution with conc. HCl to pH=2 and filtration of the product gave compound **13** as a white powder (0.96 g, yield 91%). ^{1}H NMR (400 MHz, CD_3OD) δ 7.30 – 7.17 (m, 15H), 2.37 (t, *J* = 7.4 Hz, 2H), 2.25 (t, *J* = 7.4 Hz, 2H), 1.79-1.87 (m 2H). ^{13}C NMR (101 MHz, CD_3OD) δ 175.5, 173.3, 144.6, 128.6, 127.3, 127.2, 126.7, 126.3, 35.2, 32.6, 20.7. HRMS (ESI): calculated for $C_{24}H_{23}NO_3$ [M+Na]$^+$ 396.1576, found 396.1573.

5-Hydroxy-*N*-tritylpentanamide (15). To a solution of **13** (2.60 g, 6.96 mmol) in dry THF (60 mL) cooled to 0 °C was added $NaBH(OAc)_3$ (0.28 g, 7.3 mmol). The solution was stirred until evolution of H_2 stopped, and $BF_3.OEt_2$ (1.1 mL, 8.8 mmol) was added dropwise. The reaction was stirred at room temperature for 4 hours. The reaction was quenched by adding 50 mL H_2O at 0 °C. The mixture was extracted with EtOAc and the combined organic layers were washed with sat. aq. Na_2CO_3, brine and dried over Na_2SO_4. The crude product was purified by column chromatography (100% EtOAc) to give compound **15** as a white powder (1.60 g, 64% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.22 – 6.74 (m, 15H), 6.36 (br, 1H), 3.29 – 3.19 (br, 2H), 2.01 (t, *J* = 7.2 Hz, 2H), 1.46 – 1.36 (m, 2H), 1.24 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ

171.9, 144.7, 128.6, 127.9, 127.0, 62.0, 37.0, 32.0, 21.4. HRMS (ESI): calculated for $C_{24}H_{25}NO_2$ $[M+Na]^+$ 382.1783, found 382.1783.

5-Oxo-*N*-tritylpentanamide (17). 5-hydroxy-*N*-tritylpentanamide **15** (1.30 g, 3.6 mmol) and PDC (2.00 g, 5.4 mmol) were dissolved in 50 mL of dry CH_2Cl_2 and stirred for 2 hours under N_2 atmosphere at room temperature. The mixture was filtered and the organic layer was washed with brine, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by column chromatography (100% CH_2Cl_2) to give compound **17** as an off-white powder (0.84 g, 65% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.71 (s, 1H), 7.36 – 7.10 (m, 15H), 6.59 (s, 1H), 2.44 (t, *J* = 7.0 Hz, 2H), 2.32 (t, *J* = 7.2 Hz, 2H), 1.97 – 1.88 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 202.0, 170.8, 144.6, 128.6, 127.9, 127.0, 70.5, 42.9, 36.1, 17.9. HRMS (ESI): calculated for $C_{24}H_{23}NO_2$ $[M+Na]^+$ 380.1626, found 380.1629.

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)amino)methyl)-*N*-tritylbenzamide (29). 3-Formyl-*N*-tritylbenzamide **6** (1.22 g, 3.12 mmol), 2'-3'-*O*-isopropylidene-6-aminomethyl-adenosine (1.00 g, 3.43 mmol) and acetic acid (0.45 mL, 8 mmol) were dissolved in 1,2-dichloroethane (DCE, 50 mL) and stirred at room temperature under a N_2 atmosphere. After 3 hours, $NaBH(OAc)_3$ (1.09 g, 5.15 mmol) was added and the reaction mixture was stirred overnight at room temperature. The reaction was quenched by adding 1 N NaOH solution (50 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 CH_2Cl_2) to give compound 29 as a white powder (1.25 g, 59% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.89 (s, 1H), 8.34 (s, 1H), 8.06 (s, 1H), 7.79 (s, 1H), 7.71 (d, *J* = 7.7 Hz, 1H), 7.43 (d, *J* = 7.7 Hz, 1H), 7.39 – 7.24 (m, 15H), 7.20 (m, 3H), 6.09 (d, *J* = 3.1 Hz, 1H), 5.76 (s, 1H), 5.46 (M, 1H), 5.00 (m, 1H), 4.28 – 4.23 (m, 1H), 3.73 (s, 2H), 2.75 - 2.66 (m, 2H), 1.54 (s, 3H), 1.31 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 166.9, 156.5, 153.1, 149.3, 145.3, 140.4, 135.6, 128.9, 128.3, 127.9, 127.6, 126.7, 126.5, 119.7, 113.7, 89.7, 85.3, 83.1, 82.6, 69.9, 55.3, 53.0, 50.8, 27.5, 25.7. HRMS (ESI): calculated for $C_{40}H_{39}N_7O_4$ $[M+Na]^+$ 704.2961, found 704.2975.

Methyl-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)amino)benzoate (30). Following the procedure described for compound **29**, coupling methyl 3-formylbenzoate **8** (0.51 g, 3.12 mmol) and 2'-3'-*O*-isopropylidene-6-aminomethyl-adenosine (1.00 g, 3.43 mmol) afforded compound **30** as a white powder (0.92 g, 65% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.08 (s, 1H), 7.92 (s, 1H), 7.90 – 7.83 (m, 2H), 7.44 (d, *J* = 7.6 Hz, 1H), 7.32 (t, *J* = 7.6 Hz, 1H), 6.37 (d, *J* = 5.7 Hz, 2H), 5.95 (d, *J* = 3.1 Hz, 1H), 5.45 (m, 1H), 5.04 (m, 1H), 4.40 – 4.34 (m, 1H), 3.86 (s, 3H), 3.79 (s, 2H), 2.90-

2.83 (m, 2H), 1.58 (s, 3H), 1.35 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 167.1, 155.8, 155.8, 153.0, 149.2, 140.4, 140.4, 139.8, 132.6, 132.6, 130.1, 129.1, 129.1, 128.4, 128.4, 128.2, 120.2, 114.4, 91.0, 85.5, 83.2, 83.2, 82.2, 82.2, 53.3, 52.1, 52.1, 50.6, 27.3, 27.2, 25.4, 25.3. HRMS (ESI): calculated for $C_{22}H_{26}N_6O_5$ [M+H]$^+$ 455.2043, found 455.2050.

tert-Butyl-3-(((((3aR,4R,6R,6aR)-6-(6-amino-9H-purin-9-yl)-2,2-dimethyltetrahydrofuro[3,4-d][1,3]dioxol-4-yl)methyl)amino)methyl)benzoate (31). Following the procedure described for compound **29**, coupling *tert*-butyl 3-formylbenzoate **9** (0.64 g, 3.12 mmol) and 2'-3'-*O*-isopropylidene-6-aminomethyl-adenosine (1.00 g, 3.43 mmol) afforded compound **31** as a white powder (0.77 g, 50% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.11 (s, 1H), 7.89 (s, 1H), 7.86-7.83 (m, 2H), 7.43 (d, *J* = 7.7 Hz, 1H), 7.31 (t, *J* = 7.6 Hz, 1H), 6.36 – 6.27 (m, 2H), 5.96 (d, *J* = 3.3 Hz, 1H), 5.46 (m, 1H), 5.04 (m, 1H), 4.38 (m, 1H), 3.80 (s, 2H), 2.94-2.81 (m, 2H), 1.58 (s, 3H), 1.55 (s, 9H), 1.36 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 165.7, 155.8, 155.8, 153.0, 149.3, 140.2, 139.8, 132.0, 132.0, 129.0, 128.2, 128.1, 120.3, 114.5, 91.0, 85.4, 83.2, 82.2, 80.9, 53.4, 50.6, 28.1, 27.3, 25.4. HRMS (ESI): calculated for $C_{25}H_{32}N_6O_5$ [M+H]$^+$ 497.2512, found 497.2511.

9-((3a*R*,4*R*,6*R*,6a*R*)-2,2-dimethyl-6-(((naphthalen-2-ylmethyl)amino)methyl)tetrahydrofuro-[3,4-d][1,3]dioxol-4-yl)-9*H*-purin-6-amine (32). Following the procedure described for compound **29**, coupling 2-naphthaldehyde (0.49 g, 3.12 mmol) and 2'-3'-*O*-isopropylidene-6-aminomethyl-adenosine (1.00 g, 3.43 mmol) afforded compound **32** as a white powder (1.03 g, 74% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.11 (s, 1H), 7.88 (s, 1H), 7.78 (m, 3H), 7.70 (s, 1H), 7.48 – 7.38 (m, 3H), 6.05 (s, 2H), 5.99 (d, *J* = 3.3 Hz, 1H), 5.48 (m, 1H), 5.06 (m, 1H), 4.45 – 4.39 (m, 1H), 3.95 (s, 2H), 3.01 – 2.87 (m, 2H), 2.33 (br, 2H), 1.61 (s, 3H), 1.38 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 155.7, 153.0, 149.3, 139.9, 137.4, 133.3, 132.6, 128.0, 127.6 126.4, 126.4, 126.0, 125.5, 120.3, 114.5, 91.0, 85.6, 83.3, 82.3, 53.9, 50.7, 27.3, 25.4. HRMS (ESI): calculated for $C_{24}H_{26}N_6O_3$ [M+H]$^+$ 447.2145, found 447.2167.

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)(4-oxo-4-(tritylamino)butyl)amino)methyl)-*N*-tritylbenzamide (33). Oxo-*N*-tritylbutanamide **16** (62 mg, 0.18 mmol), compound **29** (100 mg, 0.15 mmol) and AcOH (1 drop) were dissolved in 1,2-dichloroethane (DCE, 10 mL) and stirred at room temperature under a N_2 atmosphere. After 3 hours, $NaBH_4$ (49 mg, 0.23 mmol) was added and the reaction mixture was stirred overnight at room temperature. 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 (10% MeOH in CH_2Cl_2) to give compound **33** as a white powder (83 mg, 55% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.15 (s, 1H), 7.69 (s, 1H), 7.67 (s, 1H), 7.53 (d, *J* = 7.1 Hz, 2H), 7.39 – 7.09 (m, 32H), 6.61 (s, 1H), 5.95 (d, *J* = 1.9 Hz, 1H), 5.65 (s, 2H), 5.36 (m, 1H), 4.89 (m, 1H), 4.40 – 4.34 (m, 1H), 3.56 (d, *J* = 3.4 Hz, 2H), 2.68 (d, *J* = 6.8 Hz, 2H), 2.46 (m, 2H), 2.26 (m, 2H), 1.81 – 1.69 (m, 2H), 1.52 (s, 3H), 1.30 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.5, 166.7, 155.4, 152.9, 149.0, 144.8, 144.7, 140.0, 139.9, 135.3, 131.5, 128.8, 128.7, 128.0, 127.9, 127.0, 126.9, 125.3, 114.1, 90.8, 85.7, 83.8, 83.4, 70.7, 70.4, 58.6, 56.0, 53.5, 34.9, 27.0, 25.3, 22.7. HRMS (ESI): calculated for $C_{63}H_{60}N_8O_5$ $[M+H]^+$ 1009.4765, found 1009.4765.

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)(5-oxo-5-(tritylamino)pentyl)amino)methyl)-*N*-tritylbenzamide (34). Following the procedure described for compound **33**, coupling compound **29** (100 mg, 0.15 mmol) with 5-oxo-*N*-tritylpentanamide **17** (64 mg, 0.18 mmol) afforded compound **34** as a white powder (88 mg, 57% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.16 (s, 1H), 7.67 (s, 2H), 7.57 (br, 1H), 7.52 (s, 1H), 7.41 – 7.13 (m, 32H), 6.62 (s, 1H), 5.96 (d, *J* = 1.8 Hz, 1H), 5.83 (br, 2H), 5.38 (m, 1H), 4.92 (m, 1H), 4.40 – 4.34 (m, 1H), 3.54 (s, 2H), 2.65 (d, *J* = 6.9 Hz, 2H), 2.46 – 2.38 (m, 2H), 2.13 (m, 2H), 1.56 (s, 3H), 1.42 – 1.33 (m, 2H), 1.30 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.6, 166.6, 155.5, 152.9, 149.1, 144.8, 144.8, 140.3, 140.0, 135.2, 131.6, 128.8, 128.7, 128.2, 128.0, 127.9, 127.5, 127.0, 127.0, 125.4, 114.1, 90.9, 85.9, 83.8, 83.4, 77.3, 70.7, 70.4, 58.6, 56.1, 53.9, 37.1, 27.1, 26.3, 25.4, 23.1. HRMS (ESI): calculated for $C_{64}H_{62}N_8O_5$ $[M+H]^+$ 1023.4921, found 1023.4918.

***tert*-Butyl 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)(3-(tritylcarbamoyl)benzyl)amino)butanoate (35).** Following the procedure described for compound **33**, coupling *tert*-butyl 4-oxobutanoate **22** (29 mg, 0.18 mmol) and compound **29** (100 mg, 0.15 mmol) afforded compound 35 as a white powder (61 mg, 49% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.16 (s, 1H), 7.70 (d, *J* = 5.8 Hz, 2H), 7.58 (d, *J* = 7.6 Hz, 2H), 7.38 – 7.15 (m, 17H), 5.97 (d, *J* = 2.0 Hz, 3H), 5.36 (m, 1H), 4.93 (m, 1H), 4.35 (m, 1H), 3.63 – 3.52 (m, 2H), 2.76 – 2.63 (m, 2H), 2.47 (t, *J* = 7.1 Hz, 2H), 2.23 – 2.12 (m, 2H), 1.75 – 1.65 (m, 2H), 1.55 (s, 3H), 1.36 (s, 9H), 1.29 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.7, 166.7, 155.5, 152.9, 149.0, 144.8, 144.8, 140.0, 139.9, 131.6, 128.8, 128.7, 128.0, 128.0, 127.0, 125.5, 120.1, 90.8, 85.8, 83.9, 83.4, 80.1, 70.7, 61.9, 58.8, 55.9, 53.4, 33.0, 32.4, 28.0, 27.1, 25.3, 22.4. HRMS (ESI): calculated for $C_{48}H_{53}N_7O_6$ $[M+H]^+$ 824.4136, found 824.4142.

***tert*-Butyl-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)(3-(tritylcarbamoyl)benzyl)amino)pentanoate (36).** Following the

procedure described for compound **33**, coupling *tert*-butyl 5-oxopentanoate **23** (31 mg, 0.18 mmol) and compound **29** (100 mg, 0.15 mmol) afforded compound **36** as a white powder (67 mg, 53% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.17 (s, 1H), 7.70 (d, *J* = 8.7 Hz, 2H), 7.58 (d, *J* = 7.8 Hz, 1H), 7.51 (s, 1H), 7.42 – 7.27 (m, 14H), 7.19 (t, *J* = 7.7 Hz, 1H), 5.98 (s, 1H), 5.72 (s, 2H), 5.38 (m, 1H), 4.94 (s, 1H), 4.40 – 4.33 (m, 1H), 3.57 (s, 2H), 2.70 – 2.65 (m, 2H), 2.47 (t, *J* = 7.0 Hz, 2H), 2.13 (t, *J* = 7.2 Hz, 2H), 1.57 (s, 3H), 1.52 – 1.45 (m, 2H), 1.41 (s, 9H), 1.32 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.9, 166.6, 155.4, 152.9, 149.1, 144.8, 140.2, 139.9, 135.2, 131.6, 128.7, 128.2, 128.0, 127.4, 127.0, 125.4, 120.2, 114.1, 90.8, 85.8, 83.9, 83.4, 80.0, 70.7, 58.7, 56.0, 54.0, 35.2, 28.1, 27.1, 26.3, 25.3, 22.7. HRMS (ESI): calculated for $C_{49}H_{55}N_7O_5$ $[M+H]^+$ 838.4292, found 838.4298.

***tert*-Butyl-(S)-4-((((3a*R*,4*R*,6*R*,6a*R*)-6-(6-amino-9H-purin-9-yl)-2,2-dimethyltetrahydrofuro[3,4-d][1,3]dioxol-4-yl)methyl)(3-(tritylcarbamoyl)benzyl)amino)-2-((*tert*-butoxycarbonyl)amino)butanoate (37).** Following the procedure described for compound **33**, coupling *tert*-butyl (*R*)-2-((*tert*-butoxycarbonyl)amino)-4-oxobutanoate **27** (49 mg, 0.18 mmol) and compound **29** (100 mg, 0.15 mmol) afforded compound **37** as a white powder (94 mg, 67% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.15 (s, 1H), 7.70 (m, 2H), 7.57 (d, *J* = 9.9 Hz, 2H), 7.41 – 7.14 (m, 15H), 5.98 (s, 1H), 5.59 (s, 2H), 5.37 (m, 2H), 4.91 (s, 1H), 4.36 (s, 1H), 4.17 (s, 1H), 3.62 (d, *J* = 13.8 Hz, 1H), 3.54 (d, *J* = 13.8 Hz, 1H), 2.76 – 2.48 (m, 4H), 1.99 (d, *J* = 6.2 Hz, 1H), 1.76 (br, 1H), 1.57 (s, 3H), 1.39 (m, 15H), 1.32 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.6, 166.6, 155.4, 152.9, 149.0, 144.8, 140.0, 139.5, 135.4, 131.6, 128.8, 128.0, 127.0, 125.5, 114.2, 90.7, 85.6, 83.9, 83.4, 81.7, 79.4, 70.7, 58.9, 58.2, 55.9, 52.7, 50.9, 50.6, 36.5, 29.7, 28.3, 28.2, 28.0, 27.9, 27.1, 25.3. HRMS (ESI): calculated for $C_{53}H_{61}N_8O_8$ $[M+H]^+$ 939.4749, found 939.4784.

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)(*iso*propyl)amino)methyl)-*N*-tritylbenzamide (38). Following the procedure described for compound **33**, coupling 5 mL dry acetone (large excess) and compound **29** (100 mg, 0.15 mmol) afforded compound **38** as a white powder (68 mg, 63% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.22 (s, 1H), 7.75 (s, 1H), 7.60 (d, *J* = 10.4 Hz, 2H), 7.49 – 7.40 (m, 2H), 7.33 – 7.21 (m, 15H), 5.91 (s, 1H), 5.34 (m, 2H), 4.92 – 4.87 (m, 1H), 4.23 (d, *J* = 3.2 Hz, 1H), 3.57 (s, 2H), 2.92 – 2.83 (m, 1H), 2.76 – 2.68 (m, 1H), 2.59 (m, 1H), 1.49 (s, 3H), 1.25 (s, 3H), 1.01 (d, *J* = 6.6 Hz, 3H), 0.90 (d, *J* = 6.6 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.7, 155.7, 152.9, 149.0, 144.8, 141.4, 139.9, 135.2, 131.4, 128.7, 128.3, 128.0, 127.1, 125.4, 120.2, 114.0, 90.9, 86.5, 83.7, 83.3, 70.7, 54.4, 51.8, 50.5, 27.1, 25.4, 18.7, 17.2. HRMS (ESI): calculated for $C_{43}H_{45}N_7O_4$ $[M+H]^+$ 724.3611, found 724.3618.

Methyl 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)(4-oxo-4-(tritylamino)butyl)amino)methyl)benzoate (39). Following the procedure described for compound **33**, coupling 4-oxo-N-tritylbutanamide **16** (62 mg, 0.18 mmol) and compound **30** (68 mg, 0.15 mmol) afforded compound **39** as a white powder (63 mg, 54% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.17 (s, 1H), 7.90 (s, 1H), 7.84 – 7.79 (m, 1H), 7.77 (s, 1H), 7.40 (d, *J* = 7.7 Hz, 1H), 7.31 – 7.08 (m, 17H), 6.60 (s, 1H), 5.97 (d, *J* = 2.2 Hz, 1H), 5.67 (s, 2H), 5.34 (m, 1H), 4.86 (m, 1H), 4.34 (m, 1H), 3.85 (s, 3H), 3.57 (m, 2H), 2.74 – 2.62 (m, 2H), 2.45 (t, *J* = 7.0 Hz, 2H), 2.31-2.16 (m, 2H), 1.75 (m, 2H), 1.53 (s, 3H), 1.32 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.5, 167.0, 155.4, 153.0, 149.1, 144.7, 139.9, 139.6, 133.3, 129.9, 129.7, 128.7, 128.2, 128.1, 127.9, 127.0, 126.9, 120.2, 114.2, 90.8, 85.4, 83.7, 83.4, 70.4, 58.5, 55.7, 53.6, 52.1, 34.9, 27.0, 25.2, 22.7. HRMS (ESI): calculated for $C_{45}H_{48}N_7O_6$ $[M+H]^+$ 782.3666, found 782.3666.

Methyl 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)(5-oxo-5-(tritylamino)pentyl)amino)methyl)benzoate (40). Following the procedure described for compound **33**, coupling 5-oxo-*N*-tritylpentanamide **17** (64 mg, 0.18 mmol) and compound **30** (68 mg, 0.15 mmol) afforded compound **40** as a white powder (67 mg, 53% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.17 (s, 1H), 7.89 (s, 1H), 7.83 (d, *J* = 7.8 Hz, 1H), 7.76 (s, 1H), 7.44 (d, *J* = 7.6 Hz, 1H), 7.26-7.17 (m, 14H), 6.69 (s, 1H), 6.35 (br, 2H), 5.99 (d, *J* = 1.8 Hz, 1H), 5.39 (m, 1H), 4.90 (m, 1H), 4.36 (m, 1H), 3.83 (s, 3H), 3.62 – 3.48 (m, 2H), 2.65 (m, 2H), 2.41 (t, *J* = 6.9 Hz, 2H), 2.14 (p, *J* = 8.2 Hz, 2H), 1.56 (s, 3H), 1.33 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.7, 167.1, 155.8, 152.9, 149.1, 144.8, 139.9, 139.8, 133.4, 129.9, 129.8, 128.7, 128.2, 128.2, 127.9, 126.9, 114.1, 90.8, 85.6, 83.7, 83.4, 70.4, 58.5, 55.8, 53.8, 52.1, 37.1, 27.1, 26.3, 25.3, 23.1. HRMS (ESI): calculated for $C_{46}H_{49}N_7O_6$ $[M+H]^+$ 796.3823, found 796.3814.

Methyl 3-(((((3a*R*,4*R*,6*R*,6a*R*)-6-(6-amino-9*H*-purin-9-yl)-2,2-dimethyltetrahydrofu ro-[3,4-d][1,3]dioxol-4-yl)methyl)(4-(*tert*-butoxy)-4-oxobutyl)amino)methyl)benzoate (41). Following the procedure described for compound **33**, coupling *tert*-butyl 4-oxobutanoate **22** (29 mg, 0.18 mmol) and compound **30** (68 mg, 0.15 mmol) afforded compound **41** as a white powder (65 mg, 73% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.13 (s, 1H), 7.85 (s, 1H), 7.80 (d, *J* = 8.1 Hz, 2H), 7.41 (d, *J* = 7.6 Hz, 1H), 7.23 (t, *J* = 7.6 Hz, 1H), 6.47 (s, 2H), 5.98 (d, *J* = 1.9 Hz, 1H), 5.33 (d, *J* = 6.4 Hz, 1H), 4.87 (m, 1H), 4.30 (m, 1H), 3.84 (s, 3H), 3.61-3.48(m, 2H), 2.75 – 2.69 (m, 2H), 2.43 (m, 2H), 2.16-2.10 (m, 2H), 1.53 (s, 3H), 1.39 – 1.30 (m, 15H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.8, 167.0, 155.8, 152.9, 148.9, 139.7, 139.6, 133.3, 129.9, 129.7, 128.2, 128.1, 120.0, 114.1, 114.1, 90.7, 85.4, 83.7, 83.3, 80.0, 61.6, 61.1, 58.6, 55.6, 53.4, 52.0,

32.9, 32.3, 28.0, 27.5, 27.0, 25.3, 22.3. HRMS (ESI): calculated for $C_{30}H_{40}N_6O_7$ $[M+H]^+$ 597.3037, found 597.3037.

Methyl 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)(5-(*tert*-butoxy)-5-oxopentyl)amino)methyl)benzoate (42). Following the procedure described for compound **33**, coupling *tert*-butyl 5-oxopentanoate **23** (31 mg, 0.18 mmol) and compound **30** (68 mg, 0.15 mmol) afforded compound **42** as a white powder (56 mg, 61% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.12 (s, 1H), 7.84 (s, 1H), 7.79 (d, *J* = 7.8 Hz, 2H), 7.39 (d, *J* = 7.5 Hz, 1H), 7.21 (t, *J* = 7.7 Hz, 1H), 6.52 (s, 2H), 5.97 (s, 1H), 5.36 – 5.30 (m, 1H), 4.86 (m, 1H), 4.33 – 4.26 (m, 1H), 3.82 (s, 3H), 3.58 (d, *J* = 13.8 Hz, 1H), 3.47 (d, *J* = 13.7 Hz, 1H), 2.69-2.56 (m, 2H), 2.43 – 2.35 (m, 2H), 2.07 (t, *J* = 6.8 Hz, 2H), 1.52 (s, 3H), 1.48 – 1.42 (m, 2H), 1.34 (s, 9H), 1.30 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.8, 167.0, 155.8, 152.9, 149.0, 139.7, 139.7, 133.3, 129.9, 129.7, 128.2, 128.1, 128.1, 120.1, 114.1, 90.8, 85.5, 83.6, 83.5, 83.3, 79.9, 58.6, 55.6, 53.9, 52.0, 35.2, 28.0, 27.1, 26.2, 25.3, 22.7. HRMS (ESI): calculated for $C_{31}H_{42}N_6O_7$ $[M+H]^+$ 611.3193, found 611.3182.

Methyl 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)-2-((*tert*-butoxycarbonyl)amino)-4-oxobutyl)amino)methyl)benzoate (43). Following the procedure described for compound **33**, coupling *tert*-butyl (*R*)-2-((*tert*-butoxycarbonyl)amino)-4-oxobutanoate **27** (49 mg, 0.18 mmol) and compound **30** (68 mg, 0.15 mmol) afforded compound **43** as a white powder (62 mg, 58% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.19 (s, 1H), 7.87 (d, *J* = 6.5 Hz, 2H), 7.82 (s, 1H), 7.48 (d, *J* = 7.7 Hz, 1H), 7.30 (t, *J* = 7.9 Hz, 1H), 6.01 (s, 1H), 5.73 (s, 2H), 5.38 (m, 2H), 4.89 (m, 1H), 4.35 (m, 1H), 4.20 – 4.11 (m, 1H), 3.90 (s, 3H), 3.71-3.52 (m, 2H), 2.78 (m, 1H), 2.65 (m, 2H), 2.51 (m, 1H), 1.96 (s, 2H), 1.76 (m, 1H), 1.59 (s, 3H), 1.40 (m, 18H), 1.37 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.6, 167.0, 155.4, 155.4, 153.0, 149.1, 139.9, 139.2, 133.4, 130.0, 129.8, 128.4, 128.3, 120.3, 114.3, 90.7, 85.3, 83.8, 83.3, 81.7, 79.4, 58.6, 55.7, 52.7, 52.1, 50.5, 29.5, 28.3, 27.9, 27.1, 25.3. HRMS (ESI): calculated for $C_{35}H_{49}N_7O_9$ $[M+H]^+$ 712.3670, found 712.3682.

Methyl 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)(*iso*propyl)amino)methyl)benzoate (44). Following the procedure described for compound **33**, coupling dry acetone (5 mL, large excess) and compound **30** (68 mg, 0.15 mmol) afforded compound **44** as a white powder (42 mg, 57% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.22 (s, 1H), 7.94 (s, 1H), 7.84 (d, *J* = 7.7 Hz, 1H), 7.77 (s, 1H), 7.52 (d, *J* = 7.6 Hz, 1H), 7.29 (t, *J* = 7.7 Hz, 1H), 5.96 (d, *J* = 2.4 Hz, 2H), 5.36 (dd, *J* = 6.4, 2.4 Hz, 1H), 4.87 (dd, *J* = 6.4, 3.0 Hz, 1H), 4.26 – 4.20 (m, 1H), 3.88 (s, 3H), 3.62 (d, *J* = 14.2 Hz, 1H), 3.54 (d, *J* =

14.2 Hz, 1H), 2.88 (p, *J* = 6.6 Hz, 1H), 2.73-2.59(m, 2H), 1.53 (s, 3H), 1.33 (s, 3H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 167.1, 155.6, 152.9, 149.2, 141.0, 139.9, 133.2, 129.9, 129.6, 128.1, 128.1, 120.2, 114.0, 91.0, 86.1, 83.5, 83.2, 60.3, 54.3, 52.0, 51.4, 50.3, 27.1, 25.3, 21.0, 18.9, 16.8, 14.2. HRMS (ESI): calculated for $C_{35}H_{32}N_6O_5$ $[M+H]^+$ 497.2512, found 497.2510.

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)(4-oxo-4-(tritylamino)butyl)amino)methyl)benzoate (45). Following the procedure described for compound **33**, coupling 4-oxo-*N*-tritylbutanamide **16** (62 mg, 0.18 mmol) and compound **31** (75 mg, 0.15 mmol) afforded compound **45** as a white powder (93 mg, 75% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.23 (s, 1H), 7.90 (s, 1H), 7.84 – 7.79 (m, 2H), 7.43 (d, *J* = 7.6 Hz, 1H), 7.31 – 7.16 (m, 17H), 6.68 (s, 1H), 6.07 (s, 2H), 6.01 (d, *J* = 2.2 Hz, 1H), 5.39 (m, 1H), 4.89 (m, 1H), 4.37 (m, 1H), 3.66 (d, *J* = 13.9 Hz, 1H), 3.55 (d, *J* = 13.9 Hz, 1H), 2.76 (m, 1H), 2.66 (m, 1H), 2.47 (t, *J* = 6.8 Hz, 2H), 2.28 (m, 2H), 1.77 (m, 2H), 1.59 (s, 9H), 1.56 (s, 3H), 1.35 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.6, 165.7, 155.6, 153.0, 149.1, 144.8, 139.7, 139.4, 132.8, 131.9, 129.6, 128.7, 128.1, 128.0, 127.9, 126.9, 120.2, 114.2, 90.8, 85.4, 83.6, 83.4, 80.9, 70.4, 58.6, 55.7, 53.6, 34.9, 28.2, 27.1, 25.3, 22.7. HRMS (ESI): calculated for $C_{49}H_{53}N_7O_6$ $[M+H]^+$ 824.4136, found 824.4123.

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)(5-oxo-5-(tritylamino)pentyl)amino)methyl)benzoate (46). Following the procedure described for compound **33**, coupling 5-oxo-*N*-tritylpentanamide **17** (64 mg, 0.18 mmol) and compound **31** (75 mg, 0.15 mmol) afforded compound **46** as a white powder (97 mg, 77% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.23 (d, *J* = 7.6 Hz, 1H), 7.90 (d, *J* = 9.6 Hz, 1H), 7.85 – 7.79 (m, 2H), 7.47 (d, *J* = 7.7 Hz, 1H), 7.32 – 7.15 (m, 16H), 6.71 (d, *J* = 8.4 Hz, 1H), 6.35 (d, *J* = 14.9 Hz, 2H), 6.03 (d, *J* = 2.1 Hz, 1H), 5.43 (m, 1H), 4.93 (m, 1H), 4.41-4.37 (m, 1H),, 3.65 (d, *J* = 13.8 Hz, 1H), 3.54 (d, *J* = 13.8 Hz, 1H), 2.75 -2.62 (m, 1H), 2.48 – 2.39 (m, 2H), 2.16 (t, *J* = 7.2 Hz, 2H), 1.59 (s, 12H), 1.45 – 1.37 (m, 2H), 1.36 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.7, 165.8, 155.8, 153.0, 149.1, 144.8, 144.8, 139.9, 139.6, 132.9, 131.8, 129.7, 128.7, 128.2, 128.1, 128.0, 128.0, 127.9, 126.9, 120.2, 114.1, 90.9, 90.8, 85.6, 83.7, 83.6, 83.4, 80.9, 70.4, 58.6, 58.6, 55.7, 53.8, 53.6, 37.1, 34.9, 28.2, 27.1, 27.1, 26.3, 25.3, 25.3, 23.1, 22.7. HRMS (ESI): calculated for $C_{49}H_{55}N_7O_6$ $[M+H]^+$ 838.4292, found 838.4314.

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)(4-(*tert*-butoxy)-4-oxobutyl)amino)methyl)benzoate (47). Following the procedure described for compound **33**, coupling *tert*-butyl 4-oxobutanoate **22** (29 mg, 0.18 mmol) and compound **31** (75 mg, 0.15 mmol) afforded compound **47** as a white powder (64 mg, 67% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.21 (s, 1H), 7.86 – 7.77 (m, 3H), 7.42 (d, *J*

= 7.6 Hz, 1H), 7.26 (d, *J* = 7.7 Hz, 1H), 6.15 (s, 2H), 5.99 (d, *J* = 2.2 Hz, 1H), 5.36 (m, 1H), 4.88 (m, 1H), 4.32 (m, 1H), 3.65 (d, *J* = 13.8 Hz, 1H), 3.50 (d, *J* = 13.8 Hz, 1H), 2.78-2.73 (m, 1H), 2.64-2.59 (m,1H), 2.42 (m, 2H), 2.22 – 2.09 (m, 2H), 1.55 (s, 12H), 1.36 (s, 9H), 1.33 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.8, 165.7, 155.7, 153.0, 149.1, 139.8, 139.5, 132.8, 131.8, 129.6, 128.1, 128.0, 120.2, 114.2, 90.8, 85.5, 83.7, 83.3, 80.8, 80.0, 58.7, 55.6, 53.4, 33.0, 28.2, 28.0, 27.1, 25.3, 22.4. HRMS (ESI): calculated for $C_{33}H_{46}N_6O_7$ $[M+H]^+$ 639.3506, found 639.3506.

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)(5-(*tert*-butoxy)-5-oxopentyl)amino)methyl)benzoate (48). Following the procedure described for compound **33**, coupling *tert*-butyl 5-oxopentanoate **23** (31 mg, 0.18 mmol) and compound **31** (75 mg, 0.15 mmol) afforded compound **48** as a white powder (72 mg, 73% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.20 (s, 1H), 7.84 – 7.77 (m, 3H), 7.42 (d, *J* = 7.6 Hz, 1H), 7.24 (t, *J* = 7.6 Hz, 1H), 6.19 (s, 2H), 5.99 (d, *J* = 2.2 Hz, 1H), 5.37 (m, 1H), 4.88 (m, 1H), 4.35 – 4.30 (m, 1H), 3.65 -3.48 1H), 2.71-2.59 (m, 1H), 2.46 – 2.38 (m, 2H), 2.10 (t, *J* = 7.1 Hz, 2H), 1.55 (s, 12H), 1.44 (m, 2H), 1.37 (s, 9H), 1.33 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.9, 165.7, 155.7, 153.0, 149.1, 139.7, 139.5, 132.9, 131.8, 129.6, 128.0, 128.0, 120.2, 114.1, 90.8, 85.5, 83.6, 83.3, 80.8, 79.9, 58.7, 55.6, 53.9, 35.2, 28.2, 28.1, 27.1, 26.2, 25.3, 22.7. HRMS (ESI): calculated for $C_{34}H_{48}N_6O_7$ $[M+H]^+$ 653.3663, found 653.3669.

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)benzoate (49). Following the procedure described for compound **33**, coupling *tert*-butyl (*R*)-2-((*tert*-butoxycarbonyl)amino)-4-oxobutanoate **27** (49 mg, 0.18 mmol) and compound **31** (75 mg, 0.15 mmol) afforded compound **49** as a white powder (85 mg, 75% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.18 (s, 1H), 7.79 (d, *J* = 6.7 Hz, 3H), 7.44 (s, 1H), 7.28 – 7.23 (m, 1H), 6.20 (s, 2H), 5.99 (s, 1H), 5.50 – 5.43 (m, 1H), 5.34 (d, *J* = 5.6 Hz, 1H), 4.86 (m, 1H), 4.31 (m, 1H), 4.15 – 4.07 (m, 1H), 3.67 (br, 1H), 3.47 (br, 1H), 2.76 (br, 2H), 2.59 (m, 2H), 2.44 (m, 2H), 1.93 (m, 1H), 1.73 (m, 1H), 1.54 (s, 12H), 1.35 (m, 21H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.6, 165.6, 155.7, 155.7, 155.3, 153.0, 149.1, 139.8, 139.0, 132.9, 131.8, 129.6, 128.2, 128.2, 120.2, 114.3, 90.6, 85.3, 83.7, 83.3, 81.6, 80.9, 79.3, 58.8, 55.7, 52.7, 50.5, 29.4, 28.3, 28.2, 27.9, 27.1, 25.4. HRMS (ESI): calculated for $C_{38}H_{55}N_7O_9$ $[M+H]^+$ 754.4140, found 754.4129.

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)(isopropyl)amino)methyl)benzoate (50). Following the procedure described for compound **33**, coupling 5 mL dry acetone (large excess) and compound **31** (75 mg,

0.15 mmol) afforded compound 50 as a white powder (85 mg, 79% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.26 (s, 1H), 7.90 (s, 1H), 7.80 (m, 2H), 7.51 (d, *J* = 7.6 Hz, 1H), 7.28 (t, *J* = 7.7 Hz, 1H), 5.97 (d, *J* = 2.4 Hz, 1H), 5.92 (s, 2H), 5.37 (m, 1H), 4.86 (m, 1H), 4.26 – 4.20 (m, 1H), 3.64 (br, 1H), 3.54 (br, 1H), 2.87 (m, 1H), 2.73-2.56 (br, 2H), 1.57 (s, 9H), 1.53 (s, 3H), 1.33 (s, 3H), 1.03 (d, *J* = 6.6 Hz, 3H), 0.89 (d, *J* = 6.5 Hz, 3H). ^{13}C NMR (101 MHz, CDCl3-*d*) δ 165.8, 155.5, 153.0, 149.2, 140.8, 139.9, 132.7, 131.8, 129.4, 128.0, 127.9, 120.2, 114.0, 91.0, 86.1, 83.4, 83.2, 80.8, 54.5, 51.3, 50.4, 28.2, 27.1, 25.3, 19.0, 16.7. HRMS (ESI): calculated for $C_{28}H_{38}N_6O_5$ $[M+H]^+$ 539.2982, found 539.2982.

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-2-ylmethyl)amino)-*N*-tritylbutanamide (51). Following the procedure described for compound **33**, coupling 4-oxo-*N*-tritylbutanamide **16** (62 mg, 0.18 mmol) and compound **32** (67 mg, 0.15 mmol) afforded compound **51** as a white powder (85 mg, 73% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.10 (s, 1H), 7.79 – 7.73 (m, 2H), 7.72 – 7.66 (m, 2H), 7.61 (s, 1H), 7.46 – 7.38 (m, 3H), 7.28 – 7.12 (m, 15H), 6.59 (s, 1H), 5.97 (d, *J* = 2.2 Hz, 1H), 5.81 (s, 2H), 5.30 (m, 1H), 4.83 (m, 1H), 4.38 (s, 1H), 3.77 (d, *J* = 13.7 Hz, 1H), 3.63 (d, *J* = 13.7 Hz, 1H), 2.78-2.64 (m, 2H), 2.51 (t, *J* = 6.9 Hz, 2H), 2.30 – 2.20 (m, 2H), 1.79 (m, 2H), 1.52 (s, 3H), 1.31 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.6, 155.5, 153.0, 144.8, 139.7, 136.6, 133.2, 132.7, 128.7, 127.9, 127.8, 127.6, 127.6, 127.4, 127.2, 126.9, 126.0, 125.6, 114.2, 90.8, 85.4, 83.7, 83.4, 70.4, 59.1, 55.8, 53.8, 35.0, 27.0, 25.3, 22.8. HRMS (ESI): calculated for $C_{47}H_{47}N_7O_4$ $[M+H]^+$ 774.3768, found 774.3769.

5-((((3a*R*,4*R*,6*R*,6a*R*)-6-(6-amino-9H-purin-9-yl)-2,2-dimethyltetrahydrofuro[3,4-d][1,3]dioxol-4-yl)methyl)(naphthalen-2-ylmethyl)amino)-*N*-tritylpentanamide (52). Following the procedure described for compound **33**, coupling 5-oxo-*N*-tritylpentanamide **17** (64 mg, 0.18 mmol) and compound **32** (67 mg, 0.15 mmol) afforded compound **52** as a white powder (85 mg, 64% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.12 (s, 1H), 7.84 – 7.55 (m, 5H), 7.42 (m, 3H), 7.28-7.16 (m, 14H), 6.57 (s, 1H), 5.98 (s, 1H), 5.76 (s, 2H), 5.35 (d, *J* = 6.3 Hz, 1H), 4.88 (d, *J* = 6.2 Hz, 1H), 4.39 (s, 1H), 3.75 (d, *J* = 13.6 Hz, 1H), 3.62 (d, *J* = 13.6 Hz, 1H), 2.77 – 2.62 (m, 2H), 2.52 – 2.37 (m, 2H), 2.16-2.09 (m, 2H), 1.56 (s, 3H), 1.45-1.38 (m, 2H), 1.32 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.7, 155.4, 152.9, 149.2, 144.8, 139.9, 136.8, 133.2, 132.7, 128.7, 128.4, 127.9, 127.8, 127.6, 127.4, 127.2, 127.0, 125.9, 125.5, 120.2, 114.1, 90.9, 85.7, 83.7, 83.5, 70.4, 59.1, 55.8, 53.8, 37.2, 27.1, 26.3, 25.3, 23.2. HRMS (ESI): calculated for $C_{48}H_{49}N_7O_4$ $[M+H]^+$ 788.3924, found 788.3932.

***tert*-Butyl-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-2-ylmethyl)amino)butanoate (53).** Following the

procedure described for compound **33**, coupling *tert*-butyl 4-oxobutanoate **22** (29 mg, 0.18 mmol) and compound **32** (67 mg, 0.15 mmol) afforded compound **53** as a white powder (67 mg, 76% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.12 (s, 1H), 7.82 (s, 1H), 7.78 – 7.60 (m, 4H), 7.48 – 7.35 (m, 3H), 6.74 (s, 2H), 6.02 (s, 1H), 5.34 (m, 1H), 4.89 (m, 1H), 4.38 (m, 1H), 3.79 (d, *J* = 13.6 Hz, 1H), 3.60 (d, *J* = 13.6 Hz, 1H), 2.80 (m, 1H), 2.65 (m, 1H), 2.57-2.44 (m, 2H), 2.29-2.13 (m, 2H), 1.75 (p, *J* = 7.3 Hz, 2H), 1.57 (s, 3H), 1.40 – 1.08 (m, 12H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.9, 156.0, 152.9, 152.9, 149.0, 139.6, 136.7, 133.2, 132.7, 127.8, 127.6, 127.3, 127.2, 125.9, 125.5, 120.1, 114.1, 90.8, 85.5, 83.7, 83.5, 80.0, 59.2, 55.7, 53.6, 33.0, 28.0, 27.5, 27.1, 25.4, 22.4. HRMS (ESI): calculated for $C_{32}H_{40}N_6O_5$ $[M+H]^+$ 589.3138, found 589.3143.

tert-Butyl-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)(naphthalen-2-ylmethyl)amino)pentanoate (54). Following the procedure described for compound **33**, coupling *tert*-butyl 5-oxopentanoate **23** (31 mg, 0.18 mmol) and compound **32** (67 mg, 0.15 mmol) afforded compound **54** as a white powder (62 mg, 69% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.11 (s, 1H), 7.80 (d, *J* = 24.4 Hz, 2H), 7.73 (d, *J* = 8.4 Hz, 2H), 7.64 (s, 1H), 7.47 – 7.38 (m, 3H), 6.42 (s, 2H), 6.02 (s, 1H), 5.34 (d, *J* = 6.3 Hz, 1H), 4.90 (m, 1H), 4.42 – 4.36 (m, 1H), 3.80 (d, *J* = 13.6 Hz, 1H), 3.62 (d, *J* = 13.6 Hz, 1H), 2.77 (m, 1H), 2.70 – 2.62 (m, 1H), 2.54 (s, 2H), 2.15 (t, *J* = 6.7 Hz, 2H), 1.58 (s, 3H), 1.48 (d, *J* = 9.8 Hz, 2H), 1.41 (s, 9H), 1.35 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.9, 155.8, 152.9, 149.1, 139.7, 136.8, 133.2, 132.7, 127.7, 127.6, 127.5, 127.3, 127.2, 125.9, 125.5, 120.2, 114.1, 90.9, 85.5, 83.7, 83.6, 83.4, 79.9, 59.1, 55.7, 54.1, 35.3, 28.1, 28.0, 27.1, 26.3, 25.3, 22.8. HRMS (ESI): calculated for $C_{33}H_{42}N_6O_5$ $[M+H]^+$ 603.3295, found 603.3311.

tert-Butyl-(*R*)-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-2-ylmethyl)amino)-2-((*tert*-butoxycarbonyl)amino)butanoate (55). Following the procedure described for compound **33**, coupling *tert*-butyl (*R*)-2-((*tert*-butoxycarbonyl)amino)-4-oxobutanoate **27** (49 mg, 0.18 mmol) and compound **32** (67 mg, 0.15 mmol) afforded compound **55** as a white powder (72 mg, 68% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.08 (s, 1H), 7.87 – 7.67 (m, 4H), 7.61 (s, 1H), 7.54-7.39 (m, 3H), 6.27 (d, *J* = 11.3 Hz, 2H), 6.00 (s, 1H), 5.71 – 5.61 (m, 1H), 5.30 (d, *J* = 5.1 Hz, 1H), 4.84 (m, 1H), 4.39 – 4.34 (m, 1H), 4.23 – 4.14 (m, 1H), 3.84 (d, *J* = 13.5 Hz, 1H), 3.59 (d, *J* = 13.5 Hz, 1H), 2.82 (br, 2H), 2.65 (br, 2H), 2.57-2.51 (m, 1H), 2.06 – 1.94 (m, 1H), 1.86 – 1.78 (m, 1H), 1.57 (s, 3H), 1.50-1.18 (m, 21H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.7, 155.4, 152.9, 149.0, 139.7, 136.2, 133.2, 132.7, 127.9, 127.6, 127.6, 127.5, 127.2, 127.1, 125.9, 125.6, 120.1, 114.3, 90.7, 85.3, 83.7, 83.4, 81.6, 79.3, 59.2, 55.7, 52.9, 50.8, 29.4, 28.3, 27.9, 27.9, 27.1, 25.4. HRMS (ESI): calculated for $C_{33}H_{42}N_6O_5$ $[M+H]^+$ 704.3772, found 704.3777.

9-((3a*R*,4*R*,6*R*,6a*R*)-6-((*iso*propyl(naphthalen-2-ylmethyl)amino)methyl)-2,2-dimethyltetrahydrofuro[3,4-d][1,3]dioxol-4-yl)-9*H*-purin-6-amine (56). Following the procedure described for compound **33**, coupling 5 mL dry acetone (large excess) and compound **32** (67 mg, 0.15 mmol) afforded compound **56** as a white powder (35 mg, 48% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.21 (s, 1H), 7.83 – 7.68 (m, 5H), 7.53 (m, 1H), 7.47 – 7.39 (m, 2H), 6.26 (s, 2H), 5.98 (d, *J* = 2.3 Hz, 1H), 5.32 (m, 1H), 4.85 (m, 1H), 4.32-4.27 (m, 1H), 3.79 (d, *J* = 13.9 Hz, 1H), 3.64 (d, *J* = 14.0 Hz, 1H), 3.00-2.93 (m, 1H), 2.78 (m 1H), 2.64 (m, 1H), 1.53 (s, 3H), 1.31 (s, 3H), 1.09 (d, *J* = 6.6 Hz, 3H), 0.95 (d, *J* = 6.6 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 155.7, 152.9, 149.1, 139.7, 138.0, 133.2, 132.7, 127.7, 127.6, 127.5, 127.2, 127.0, 125.9, 125.4, 120.2, 113.9, 91.1, 86.2, 83.5, 83.3, 77.3, 54.9, 51.4, 50.4, 27.0, 25.3, 19.2, 16.6. HRMS (ESI): calculated for $C_{27}H_{32}N_6O_3$ $[M+H]^+$ 489.2614, found 489.2611.

3-(((4-Amino-4-oxobutyl)(((2*R*,3*S*,4*R*,5*R*)-5-(6-amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl)amino)methyl)benzamide (57). To a solution of compound **33** (100 mg, 0.098 mmol) in 5 mL CH_2Cl_2 was added 5 mL TFA and the mixture was stirred at room temperature. After 2 hours, 2 mL H_2O was added and the mixture was stirred for 1 hour at room temperature. The mixture was concentrated and the crude product was purified by preparative HPLC affording compound **57** as a white powder. ^{1}H NMR (400 MHz, D_2O) δ 8.46 – 8.06 (m, 2H), 7.87 – 7.26 (m, 4H), 6.08 (br, 1H), 4.75 – 4.36 (m, 4H), 4.27 (br, 1H), 3.84 – 3.27 (m, 4H), 2.38 (br, 2H), 2.10 (br, 2H). ^{13}C NMR (101 MHz, D_2O) δ 177.5, 162.8, 162.5, 149.6, 143.8, 134.8, 134.1, 132.7, 129.6, 129.1, 128.3, 118.9, 117.6, 114.7, 90.4, 77.7, 73.6, 71.5, 57.9, 54.8, 31.8, 19.0. HRMS (ESI): calculated for $C_{22}H_{28}N_8O_5$ $[M+H]^+$ 485.2261, found 485.2265.

3-(((5-Amino-5-oxopentyl)(((2*R*,3*S*,4*R*,5*R*)-5-(6-amino-9*H*-purin-9-yl)-3,4-dihydroxy-tetrahydrofuran-2-yl)methyl)amino)methyl)benzamide (58). Following the procedure described for compound **57**, compound **34** (50 mg, 0.049 mmol) was deprotected to obtain compound **58** as a white powder (16 mg, 56% yield). ^{1}H NMR (400 MHz, D_2O) δ 8.43 – 8.12 (m, 2H), 7.84 – 7.26 (m, 4H), 6.08 (br, 1H), 4.65 – 4.21 (m, 5H), 3.63-3.48 (m, 2H), 3.34 (br, 2H), 2.35 (br, 2H), 1.84 (br, 2H), 1.58 (br, 2H). ^{13}C NMR (101 MHz, D_2O) δ 177.6, 170.6, 162.8, 149.6, 147.3, 143.8, 134.8, 129.7, 129.6, 129.4, 129.1, 128.3, 117.6, 114.7, 90.5, 77.8, 77.4, 71.6, 71.4, 57.8, 54.6, 32.6, 22.4, 21.0. HRMS (ESI): calculated for $C_{23}H_{30}N_8O_5$ $[M+H]^+$ 499.2417, found 499.2420.

4-((((2*R*,3*S*,4*R*,5*R*)-5-(6-amino-9H-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl)(3-carbamoylbenzyl)amino)butanoic acid (59). Following the procedure described for compound **57**, compound **35** (50 mg, 0.060 mmol) was deprotected to obtain compound **59** as a

white powder (21 mg, 60% yield). ^{1}H NMR (400 MHz, D_2O) δ 8.38 – 8.06 (m, 2H), 7.71 – 7.26 (m, 4H), 6.05 (br, 1H), 4.64 – 4.21 (m, 5H), 3.53 (br, 2H), 3.35 (s, 2H), 2.41 (br, 2H), 2.02 (br, 2H). ^{13}C NMR (101 MHz, D_2O) δ 176.4, 170.5, 149.6, 147.3, 143.8, 134.8, 132.7, 129.6, 129.5, 128.3, 117.5, 114.6, 90.4, 77.7, 73.5, 71.4, 57.8, 52.8, 38.6, 30.2, 18.4. HRMS (ESI): calculated for $C_{22}H_{28}N_7O_6$ $[M+H]^+$ 486.2101, found 486.2103.

5-((((2*R*,3*S*,4*R*,5*R*)-5-(6-amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl)(3-carbamoylbenzyl)amino)pentanoic acid (60). Following the procedure described for compound **57**, compound **36** (50 mg, 0.059 mmol) was deprotected to obtain compound **60** as a white powder (17 mg, 50% yield). ^{1}H NMR (400 MHz, D_2O) δ 8.29 (br, 2H), 7.84 – 7.58 (m, 3H), 7.46 (br, 1H), 6.13 (br, 1H), 4.70 – 4.33 (m, 5H), 3.66 (br, 2H), 3.48 – 3.31 (m, 2H), 2.42 (br, 2H), 1.88 (s, 2H), 1.65 (br, 2H). ^{13}C NMR (101 MHz, D_2O) δ 177.7, 170.8, 163.0, 150.5, 147.6, 145.1, 143.1, 134.5, 132.9, 129.7, 129.3, 128.4, 119.0, 117.7, 90.3, 77.7, 73.3, 32.8, 22.4, 21.0. HRMS (ESI): calculated for $C_{23}H_{30}N_7O_6$ $[M+H]^+$ 500.2258, found 500.2267.

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)methyl)benzamide (61). Following the procedure described for compound **57**, compound **38** (50 mg, 0.069 mmol) was deprotected to obtain compound **61** as a white powder (22 mg, 60% yield). ^{1}H NMR (400 MHz, Acetone-d_6) δ 8.48-8.39 (m, 2H), 8.26 (br, 1H), 7.94 (d, *J* = 7.8 Hz, 1H), 7.9-7.73 (m, 2H), 7.46 (m, 1H), 6.81 (br, 1H), 6.13 (d, *J* = 3.4 Hz, 1H), 4.74 (br, 2H), 4.65 (s, 1H), 4.53 (br, 1H), 4.46 (br, 1H), 3.93 – 3.69 (m, 3H), 3.31 (s, 1H), 1.49-1.45 (m, 6H). ^{13}C NMR (101 MHz, Acetone-d_6) δ 152.7, 148.4, 146.1, 142.5, 135.0, 134.0, 130.5, 130.2, 119.9, 90.6, 79.3, 73.5, 72.5, 55.6, 54.3, 54.0, 51.4, 16.6, 15.0. HRMS (ESI): calculated for $C_{21}H_{28}N_7O_4$ $[M+H]^+$ 442.2203, found 442.2203.

Methyl 3-(((4-amino-4-oxobutyl)(((2*R*,3*S*,4*R*,5*R*)-5-(6-amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl)amino)methyl)benzoate (62). Following the procedure described for compound **57**, compound **39** (50 mg, 0.064 mmol) was deprotected to obtain compound **62** as a white powder (20 mg, 53% yield). ^{1}H NMR (400 MHz, D_2O) δ 8.38 – 7.98 (m, 2H), 7.88 – 7.50 (m, 3H), 7.35 (br, 1H), 6.05 (br, 1H), 4.64 – 4.32 (m, 4H), 4.20 (br, 1H), 3.78 (s, 3H), 3.55 (br, 1H), 3.47 – 3.30 (m, 2H), 2.39 (br, 2H), 2.08 (br, 2H). ^{13}C NMR (101 MHz, D_2O) δ 177.5, 167.3, 149.5, 147.2, 143.7, 143.6, 135.8, 134.6, 131.3, 130.7, 129.9, 129.8, 129.4, 129.1, 118.8, 90.6, 77.8, 77.4, 73.8, 73.1, 71.7, 71.4, 57.6, 56.9, 55.2, 54.8, 53.6, 52.7, 31.8, 19.0. HRMS (ESI): calculated for $C_{23}H_{29}N_7O_6$ $[M+H]^+$ 500.2258, found 500.2265.

Methyl 3-(((5-amino-5-oxopentyl)(((2*R*,3*S*,4*R*,5*R*)-5-(6-amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl)amino)methyl)benzoate (63). Following the procedure described for compound **57**, compound **40** (50 mg, 0.063 mmol) was deprotected to obtain

compound **63** as a white powder (21 mg, 55% yield).^{1}H NMR (400 MHz, D_2O) δ 8.44 – 8.07 (m, 2H), 7.96 – 7.34 (m, 4H), 6.12 (br, 1H), 4.50 (br, 4H), 4.32 (s, 1H), 3.86 (s, 3H), 3.62 (br, 1H), 3.52 – 3.34 (m, 2H), 2.32 (br, 2H), 1.89 (br, 2H), 1.68 (br, 2H). ^{13}C NMR (101 MHz, D_2O) δ 178.7, 167.5, 162.6, 149.6, 143.8, 143.6, 135.9, 130.9, 130.0, 129.9, 129.5, 129.1, 117.6, 114.7, 111.8, 71.4, 52.7, 33.9, 22.4, 21.9. HRMS (ESI): calculated for $C_{24}H_{31}N_7O_6$ $[M+H]^+$ 514.2414, found 514.2415.

4-((((2*R*,3*S*,4*R*,5*R*)-5-(6-amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl)(3-(methoxycarbonyl)benzyl)amino)butanoic acid (64). Following the procedure described for compound **57**, compound **41** (50 mg, 0.084 mmol) was deprotected to obtain compound **64** as a white powder (24 mg, 49% yield). ^{1}H NMR (400 MHz, D_2O) δ 8.29 (s, 1H), 8.11 (s, 1H), 7.94 – 7.64 (m, 4H), 7.45 (t, *J* = 7.9 Hz, 1H), 6.09 (s, 1H), 4.62 (br, 4H), 4.49 (br, 1H), 3.88 (s, 3H), 3.67 (br, 2H), 3.55-3.41 (m, 6.9 Hz, 3H), 2.53 (t, *J* = 6.4 Hz, 2H), 2.18 – 2.09 (m, 2H). ^{13}C NMR (101 MHz, D_2O) δ 176.8, 167.6, 150.3, 147.4, 144.8, 143.3, 130.1, 129.8, 129.5, 129.2, 90.5, 77.7, 71.6, 57.7, 52.8, 30.6, 18.5. HRMS (ESI): calculated for $C_{23}H_{28}N_6O_7$ $[M+H]^+$ 501.2098, found 501.2097.

5-((((2*R*,3*S*,4*R*,5*R*)-5-(6-amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl)(3-(methoxycarbonyl)benzyl)amino)pentanoic acid (65). Following the procedure described for compound **57**, compound **42** (50 mg, 0.082 mmol) was deprotected to obtain compound **65** as a white powder (30 mg, 59% yield).^{1}H NMR (400 MHz, D_2O) δ 8.22 (br, 2H), 7.86 (br, 2H), 7.67 (br, 1H), 7.46 (br, 1H), 6.08 (br, 1H), 4.55 (br, 4H), 4.35 (br, 1H), 3.88 (s, 3H), 3.64 (br, 1H), 3.43 (br, 2H), 2.45 (br, 2H), 1.90 (br, 2H), 1.67 (br, 2H). ^{13}C NMR (101 MHz, D_2O) δ 178.1, 168.0, 150.3, 144.5, 143.8, 130.5, 130.2, 129.9, 129.6, 118.0, 115.1, 72.0, 53.1, 33.1, 23.6, 22.8, 21.4. HRMS (ESI): calculated for $C_{24}H_{30}N_6O_7$ $[M+H]^+$ 515.2254, found 515.2257.

(*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-(methoxycarbonyl)benzyl)amino)butanoic acid (66). Following the procedure described for compound **57**, compound **43** (50 mg, 0.070 mmol) was deprotected to obtain compound **66** as a white powder (26 mg, 60% yield).^{1}H NMR (400 MHz, D_2O) δ 8.24 (s, 1H), 8.01 (s, 1H), 7.72 (br, 1H), 7.64 (d, *J* = 7.8 Hz, 1H), 7.54 (d, *J* = 7.8 Hz, 1H), 7.31 (t, *J* = 7.8 Hz, 1H), 6.03 (s, 1H), 4.56 – 4.52 (m, 1H), 4.50 – 4.35 (m, 4H), 4.07 (m, 1H), 3.74 (s, 3H), 3.69 – 3.54 (m, 4H), 2.55-2.45 (m, 1H), 2.41-2.33 (m, 1H). ^{13}C NMR (101 MHz, D_2O) δ 170.8, 167.2, 162.7, 162.3, 149.5, 147.2, 143.6, 143.6, 131.0, 130.0, 129.5, 129.3, 129.1, 118.8, 117.6, 114.7, 90.8, 77.5, 73.5, 71.4, 53.9, 50.7, 24.4. HRMS (ESI): calculated for $C_{23}H_{29}N_7O_7$ $[M+H]^+$ 516.2207, found 516.2206.

Methyl 3-(((((2*R*,3*S*,4*R*,5*R*)-5-(6-amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofu ran-2-yl)methyl)(*iso*propyl)amino)methyl)benzoate (67). Following the procedure described for compound **57**, compound **44** (50 mg, 0.101 mmol) was deprotected to obtain compound **67** as a white powder (33 mg, 59% yield).^{1}H NMR (400 MHz, D_2O) δ 8.19 (d, *J* = 3.9 Hz, 1H), 7.99 – 7.54 (m, 4H), 7.29 (t, *J* = 7.6 Hz, 1H), 5.91 (s, 1H), 4.62 – 4.46 (m, 3H), 4.33 – 4.25 (m, 1H), 4.20 (br, 1H), 3.87 (s, 3H), 3.79-3.62 (m, 2H), 3.39 – 3.28 (m, 1H), 1.54 – 1.37 (m, 6H). ^{13}C NMR (101 MHz, D_2O) δ 167.6, 149.7, 147.3, 144.1, 143.3, 136.1, 131.3, 130.1, 129.7, 128.7, 118.7, 114.8, 90.6, 78.6, 73.6, 71.4, 58.7, 55.2, 52.7, 50.5, 16.2, 15.7. HRMS (ESI): calculated for $C_{22}H_{28}N_6O_5$ $[M+H]^+$ 457.2199, found 457.2196.

3-(((4-Amino-4-oxobutyl)(((2*R*,3*S*,4*R*,5*R*)-5-(6-amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl)amino)methyl)benzoic acid (68). Following the procedure described for compound **57**, compound **45** (50 mg, 0.061 mmol) was deprotected to obtain compound **68** as a white powder (15 mg, 42% yield). ^{1}H NMR (400 MHz, D_2O) δ 8.21 (br, 2H), 7.86 (br, 2H), 7.65 (d, *J* = 7.6 Hz, 1H), 7.43 (br, 1H), 6.08 (br, 1H), 4.70 – 4.24 (m, 5H), 3.63 (br, 1H), 3.52 – 3.38 (m, 2H), 2.48 (br, 2H), 2.12 (br, 2H). ^{13}C NMR (101 MHz, D_2O) δ 177.5, 149.8, 147.4, 144.0, 143.6, 131.2, 130.3, 129.9, 129.1, 117.7, 114.8, 90.5, 77.7, 71.5, 31.8, 19.1. HRMS (ESI): calculated for $C_{22}H_{26}N_7O_6$ $[M+H]^+$ 486.2101, found 486.2089.

3-(((5-Amino-5-oxopentyl)(((2*R*,3*S*,4*R*,5*R*)-5-(6-amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl)amino)methyl)benzoic acid (69). Following the procedure described for compound **57**, compound **46** (50 mg, 0.059 mmol) was deprotected to obtain compound **69** as a white powder (19 mg, 65 % yield). ^{1}H NMR (400 MHz, D_2O) δ 8.19 (br, 2H), 7.83(br, 2H), 7.63 (br, 1H), 7.40 (br, 1H), 6.03 (br, 1H), 4.58-4.41 (m, 4H), 4.31 (br, 1H), 3.63 (br, 1H), 3.42 (d, *J* = 7.8 Hz, 2H), 2.34 (br, 2H), 1.90 (br, 2H), 1.68 (br, 2H). ^{13}C NMR (101 MHz, D_2O) δ 178.7, 149.7, 143.9, 143.5, 130.3, 129.8, 129.8, 129.1, 120.6, 117.7, 114.8, 105.0, 77.9, 71.5, 33.9, 21.9. HRMS (ESI): calculated for $C_{23}H_{30}N_7O_6$ $[M+H]^+$ 500.2258, found 500.2253.

3-(((((2*R*,3*S*,4*R*,5*R*)-5-(6-amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl)(3-carboxypropyl)amino)methyl)benzoic acid (70). Following the procedure described for compound **57**, compound **47** (50 mg, 0.078 mmol) was deprotected to obtain compound **70** as a white powder (21 mg, 46% yield). ^{1}H NMR (400 MHz, D_2O) δ 8.27 (s, 1H), 8.14 (s, 1H), 7.85 (br, 2H), 7.64 (d, *J* = 7.7 Hz, 1H), 7.49 – 7.35 (m, 1H), 6.08 (br, 1H), 4.55 (br, 5H), 3.65 (br, 1H), 3.48-3,43 (m, 2H), 2.52 (br, 2H), 2.13 (br, 2H). ^{13}C NMR (101 MHz, D_2O) δ 176.6, 168.8, 149.8, 147.4, 144.0, 143.5, 131.4, 130.4, 129.7, 129.1, 117.7, 114.8, 90.5, 77.7, 71.6, 30.4, 22.1, 18.5. HRMS (ESI): calculated for $C_{22}H_{27}N_6O_7$ $[M+H]^+$ 487.1941, found 487.1945.

3-(((((2*R*,3*S*,4*R*,5*R*)-5-(6-amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl)(4-carboxybutyl)amino)methyl)benzoic acid (71). Following the procedure described for compound **57**, compound **48** (50 mg, 0.076 mmol) was deprotected to obtain compound **71** as a white powder (24 mg, 52% yield). ^{1}H NMR (400 MHz, D_2O) δ 8.20 (br, 2H), 7.83 (br, 2H), 7.63 (d, *J* = 7.5 Hz, 1H), 7.42 (br, 1H), 6.05 (br, 1H), 4.53 (br, 4H), 4.31 (br, 1H), 3.63 (br, 1H), 3.49 – 3.32 (m, 2H), 2.42 (br, 2H), 1.88 (br, 2H), 1.66 (br, 2H). ^{13}C NMR (101 MHz, D_2O) δ 177.7, 150.0, 144.3, 143.4, 131.4, 130.4, 130.1, 129.7, 129.1, 117.7, 114.8, 90.4, 77.7, 71.6, 32.7, 22.4, 21.0. HRMS (ESI): calculated for $C_{23}H_{29}N_6O_7$ $[M+H]^+$ 501.2098, found 501.2096.

3-((((*S*)-3-Amino-3-carboxypropyl)(((2*R*,3*S*,4*R*,5*R*)-5-(6-amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl)amino)methyl)benzoic acid (72). Following the procedure described for compound **57**, compound **49** (50 mg, 0.066 mmol) was deprotected to obtain compound **72** as a white powder (24 mg, 61% yield). ^{1}H NMR (400 MHz, D_2O) δ 8.23 (bs, 1H), 8.06 (s, 1H), 7.76 (s, 1H), 7.69 (d, *J* = 7.6 Hz, 1H), 7.56 (d, *J* = 7.8 Hz, 1H), 7.33 (t, *J* = 7.8 Hz, 1H), 6.06 – 5.98 (m, 1H), 4.59 – 4.34 (m, 5H), 4.06 (m, 1H), 3.72 – 3.51 (m, 4H), 2.55 – 2.4m (m, 1H), 2.40-2.32 (m, 1H). ^{13}C NMR (101 MHz, D_2O) δ 171.0, 168.4, 162.8, 162.4, 149.5, 147.3, 143.7, 143.5, 131.3, 130.3, 129.7, 129.5, 129.1, 118.9, 117.6, 114.7, 90.6, 77.5, 73.5, 71.5, 50.9, 24.4. HRMS (ESI): calculated for $C_{22}H_{27}N_7O_7$ $[M+H]^+$ 502.2050, found 502.2048.

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)methyl)benzoic acid (73). Following the procedure described for compound **57**, compound **50** (50 mg, 0.093 mmol) was deprotected to obtain compound **73** as a white powder (27 mg, 54% yield). ^{1}H NMR (400 MHz, D_2O) δ 8.21 (s, 1H), 8.16 (s, 1H), 7.90 (s, 1H), 7.58 (m, 2H), 7.27 (t, *J* = 7.3 Hz, 1H), 5.89 (s, 1H), 4.55 (t, *J* = 10.4 Hz, 2H), 4.31 – 4.15 (m, 2H), 3.98 – 3.90 (m, 1H), 3.78 (br, 1H), 3.32 (br, 1H), 1.50 (m, 6H). ^{13}C NMR (101 MHz, D_2O) δ 215.3, 168.7, 149.9, 147.3, 144.4, 143.1, 136.1, 131.7, 129.9, 128.7, 118.7, 117.7, 114.8, 89.7, 78.5, 73.5, 71.4, 58.8, 55.3, 51.7, 30.1, 16.2, 15.7. HRMS (ESI): calculated for $C_{21}H_{26}N_6O_5$ $[M+H]^+$ 443.2043, found 443.2040.

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)butanamide (74). Following the procedure described for compound **57**, compound **51** (50 mg, 0.064 mmol) was deprotected to obtain compound **74** as a white powder (22 mg, 58% yield). ^{1}H NMR (400 MHz, D_2O) δ 8.11 (br, 1H), 7.57 – 7.25 (m, 4H), 7.09 (d, *J* = 8.0 Hz, 1H), 5.89 (br, 1H), 4.35 (br, 4H), 3.94 – 3.63 (m, 1H), 3.49 – 3.22 (m, 3H), 2.44 (br, 2H), 2.13 – 1.82 (br, 2H). ^{13}C NMR (101 MHz, D_2O) δ 177.5, 148.4, 146.0, 143.3, 142.3, 132.1, 131.6, 129.5, 128.0, 127.7, 127.2, 127.0, 126.8, 126.7, 125.1, 118.0, 90.6,

77.8, 74.1, 71.2, 58.0, 55.9, 54.9, 32.0, 19.0. HRMS (ESI): calculated for $C_{25}H_{29}N_7O_4$ $[M+H]^+$ 492.2359, found 492.2363.

5-((((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)pentanamide (75). Following the procedure described for compound 57, compound **52** (50 mg, 0.063 mmol) was deprotected to obtain compound **75** as a white powder (23 mg, 60% yield). ^{1}H NMR (400 MHz, D_2O) δ 8.11 (s, 1H), 7.72 – 7.61 (m, 2H), 7.55 – 7.48 (m, 2H), 7.46 – 7.39 (m, 1H), 7.37 – 7.15 (m, 2H), 5.89 (br, 1H), 4.66 – 4.48 (m, 2H), 4.42 – 4.11 (m, 3H), 3.61 – 3.33 (m, 4H), 2.43 – 2.27 (m, 2H), 1.89 (d, *J* = 9.8 Hz, 2H), 1.77 – 1.61 (m, 2H). ^{13}C NMR (101 MHz, D_2O) δ 178.8, 148.6, 146.3, 143.8, 143.4, 142.7, 142.2, 132.4, 131.8, 129.9, 128.2, 127.8, 127.4, 126.8, 125.1, 90.7, 78.1, 74.2, 71.3, 58.6, 57.8, 55.8, 54.6, 34.0, 22.5, 22.0. HRMS (ESI): calculated for $C_{26}H_{31}N_7O_4$ $[M+H]^+$ 506.2516, found 506.2520.

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 (76). Following the procedure described for compound **57**, compound **53** (50 mg, 0.085 mmol) was deprotected to obtain compound **76** as a white powder (30 mg, 60% yield). ^{1}H NMR (400 MHz, D_2O) δ 8.14 (br, 1H), 7.75 – 7.23 (m, 8H), 5.95 (br, 1H), 4.62 – 4.54 (m, 1H), 4.43 – 4.26 (m, 4H), 3.69 – 3.53 (m, 2H), 3.45 (m, 2H), 2.54 (t, *J* = 6.5 Hz, 2H), 2.19 – 2.04 (m, 2H). ^{13}C NMR (101 MHz, D_2O) δ 179.8, 179.8, 152.0, 149.1, 146.3, 145.7, 134.9, 134.4, 132.5, 130.6, 129.9, 129.8, 129.3, 121.0, 120.2, 117.3, 93.3, 80.2, 74.2, 60.8, 33.6, 21.4. HRMS (ESI): calculated for $C_{23}H_{30}N_8O_5$ $[M+H]^+$ 493.2199, found 493.2199.

5-((((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)pentanoic acid (77). Following the procedure described for compound **57**, compound **54** (50 mg, 0.083 mmol) was deprotected to obtain compound **77** as a white powder (31 mg, 61% yield). ^{1}H NMR (400 MHz, D_2O) δ 8.27 (br, 2H), 7.86 – 7.55 (m, 5H), 7.52 – 7.47 (m, 1H), 7.40 (d, *J* = 7.7 Hz, 1H), 5.95 (s, 1H), 4.66-4.40 (m, 4H), 4.31 (br, 1H), 3.70 – 3.43 (m, 4H), 2.50 (br, 2H), 1.97 (br, 2H), 1.80 – 1.67 (br, 2H). ^{13}C NMR (101 MHz, D_2O) δ 178.0, 163.0, 162.7, 149.5, 146.6, 143.8, 143.1, 132.4, 131.9, 130.0, 128.0, 127.3, 127.2, 126.8, 117.7, 38.6, 32.9, 21.2. HRMS (ESI): calculated for $C_{26}H_{28}N_6O_5$ $[M+H]^+$ 507.2356, found 507.2355.

(*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 (78). Following the procedure described for compound **57**, compound **55** (50 mg, 0.071 mmol) was deprotected to obtain compound **78** as a white powder (28 mg, 65% yield). ^{1}H NMR (400 MHz,

D_2O) δ 8.03 (bs, 2H), 7.54 – 6.97 (m, 7H), 5.83 (bs, 1H), 4.55 – 4.25 (m, 3H), 4.24 – 4.16 (m, 1H), 4.16 – 4.09 (m, 1H), 3.65 (s, 2H), 3.49 (s, 1H), 2.46 (br, 2H). ^{13}C NMR (101 MHz, D_2O) δ 170.8, 148.3, 146.0, 143.3, 142.2, 132.0, 131.5, 129.7, 127.1, 126.9, 126.6, 126.3, 118.2, 117.6, 114.7, 90.9, 77.5, 73.8, 71.4, 50.7, 24.5. HRMS (ESI): calculated for $C_{25}H_{29}N_7O_5$ $[M+H]^+$ 508.2308, found 508.2309.

(2*R*,3*R*,4*S*,5*R*)-2-(6-amino-9*H*-purin-9-yl)-5-((isopropyl(naphthalen-2-ylmethyl) amino)methyl) tetrahydrofuran-3,4-diol (79). Following the procedure described for compound **57**, compound **56** (50 mg, 0.102 mmol) was deprotected to obtain compound **79** as a white powder (36 mg, 65% yield).^{1}H NMR (400 MHz, D_2O) δ 7.86 (s, 1H), 7.64 – 7.47 (m, 2H), 7.42 – 7.10 (m, 6H), 5.58 (s, 1H), 4.41 – 4.35 (m, 1H), 4.29 (br, 1H), 4.16 – 4.04 (m, 2H), 3.92 (br, 1H), 3.80-3.73(m, 1H), 3.58 (br, 1H), 3.24 (m, 1H), 1.35 (m, 6H). ^{13}C NMR (101 MHz, D_2O) δ 148.7, 146.3, 143.6, 143.0, 142.6, 132.2, 131.7, 130.4, 128.3, 127.5, 127.4, 118.0, 90.0, 77.5, 73.9, 71.9, 59.1, 51.7, 49.6, 17.4, 15.9. HRMS (ESI): calculated for $C_{24}H_{28}N_6O_3$ $[M+H]^+$ 449.2301, found 449.2299.

tert-Butyl (*S*)-4-((((3a*R*,4*R*,6*R*,6a*R*)-6-(6-amino-9*H*-purin-9-yl)-2,2-dimethyltetrahydro-furo-[3,4-d][1,3]dioxol-4-yl)methyl)(naphthalen-2-ylmethyl)amino)-2-bis(*tert*-butoxycarbonyl)amino)pentanoate (80). Following the procedure described for compound **33**, *tert*-butyl (*R*)-2-((*tert*-butoxycarbonyl)amino)-5-oxopentanoate **28** (312 mg, 0.80 mmol) and compound 32 (300 mg, 0.67mmol) to obtain compound **80** as a white powder (319 mg, 58% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.05 (s, 1H), 7.79 (s, 1H), 7.73 m, 1H), 7.68 (d, *J* = 8.6 Hz, 2H), 7.61 (s, 1H), 7.45 – 7.34 (m, 3H), 6.59 (s, 2H), 5.98 (d, *J* = 2.2 Hz, 1H), 5.27 (dd, *J* = 6.4, 2.2 Hz, 1H), 4.84 (dd, *J* = 6.4, 3.1 Hz, 1H), 4.69 (dd, *J* = 9.6, 5.2 Hz, 1H), 4.37 (m, 1H), 3.80 (br, 1H), 3.57 (br, 1H), 2.76 (m, 1H), 2.64 – 2.47 (m, 3H), 2.04 (m, 1H), 1.83 (m, 1H), 1.54 (s, 3H), 1.43 (s, 16H), 1.41 (s, 8H), 1.31 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 155.9, 152.9, 152.5, 139.5, 127.7, 127.5, 127.3, 127.2, 125.8, 125.5, 90.8, 85.4, 83.6, 83.41, 82.6, 81.0, 59.0, 55.6, 54.0, 28.0, 27.1, 27.0, 25.3, 23.8. HRMS (ESI): calculated for $C_{43}H_{59}N_7O_9$ $[M+H]^+$ 818.4453, found 818.4458.

(*S*)-2-Amino-5-((((2*R*,3*S*,4*R*,5*R*)-5-(6-amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl)(naphthalen-2-ylmethyl)amino)pentanoic acid (81). Following the procedure described for compound **57**, compound **80** (120 mg, 0.15 mmol) was deprotected to obtain compound **81** as a white powder (58 mg, 63% yield). ^{1}H NMR (600 MHz, D_2O) δ 8.14 (br, 1H), 7.69–6.93 (m, 8H), 5.93 (br, 1H), 4.59–4.43 (m, 2H), 4.27 (br, 2H), 4.15–3.73 (m, 2H), 3.47 (m, 4H), 2.16–1.90 (m, 4H). ^{13}C NMR (151 MHz, D_2O) δ 171.6, 162.9, 162.7, 148.6, 131.8 127.3, 127.1, 126.8, 119.2, 117.3, 115.3, 90.7, 78.0, 74.3, 71.3,

58.8, 52.3, 26.9, 19.4. HRMS (ESI): calculated for $C_{26}H_{31}N_7O_5$ $[M+H]^+$ 522.2465, found 522.2468.

Inhibition studies: Expression and purification of full-length wild type NNMT protein (NNMTwt) were performed as previously described.[32] The purity of the enzyme was confirmed using 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 per hour of incubation at 37 °C. The specific activity of the batch used in the inhibitory activity assays was 18,665 units per mg of protein at a protein concentration of 0.56 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 DTT). The compounds were dissolved in DMSO and diluted with water to concentrations ranging from 0.1 μM to 500 μM (DMSO was kept constant at 1.25% final concentration). The compounds were incubated with the enzyme for 10 minutes at 37 °C before initiating the reaction with a mixture of NA and AdoMet at their K_M values of 200 μM and 8.5 μM respectively. The formation of MNA was measured after 30 minutes at 37 °C. The reaction was quenched by addition of 15 μL sample to 70 μL acetonitrile containing 50 nM deutero-methylated nicotinamide as internal standard. The enzymatic activity assays were performed using UHP-HILIC-MS/MS as previously described with minor modifications.[24] The UHP-HILIC-MS/MS system consisted of a binary UHPLC system, consisting of two LC-30AD pumps, a SIL30-ACmp auto-sampler, a CTO-20AC column oven, and a DGU-20A5R degasser, (all from Shimadzu, 's-Hertogenbosch, The Netherlands). Isocratic elution was performed after 1 μL injections on a Waters Acquity BEH Amide HILIC column (3.0 x 100 mm, 1.7 μm particle size, Waters, Milford, USA), using water containing 300 μM formic acid and 550 μM NH_4OH (pH 9.2) at 40% v/v and acetonitrile at 60% v/v, with a run-time of 3 min. Calibration samples were prepared using 75 μL internal standard d_3-MNA at 50 nM in acetonitrile and 25 μL of an aqueous solution of reference standard MNA with concentrations ranging from 2500 nM to 1.221 nM. For detection, a Sciex QTRAP® 5500 triple quadrupole mass spectrometer, with Analyst 1.6.2, and MultiQuant 3.0.1 software (Sciex, Ontario, Canada) was used. Settings used for the ionization source were: curtain gas, 40 psi; collision gas, 'medium'; ionspray voltage, 5000 V; temperature, 600 °C; ion source gas 1, 60 psi; ion source gas 2, 80 psi. Dwell times were 10 msec, and entrance potential was set to 10 V; compound specific parameters can be found in Table 2. The whole eluate was transferred to the electrospray probe from 1.0 till 2.8 min using the MS diverter valve. Ratios of the sums of the MNA and d_3-MNA transitions were calculated and plotted versus concentration.

Table 2. Tuned MS/MS parameters for all quantified components.

Compound	Q1 (m/z)	Q3 (m/z)	DP	CE	CXP
MNA	137.101	94.0	136	27	12
		92.0	136	29	12
		78.0	136	35	10
MNA-d_3	140.128	97.1	121	29	12
		95.1	121	31	12
		78.0	121	35	10

The entrance potential was set at 10 V for all compounds, dwell-time was 10 msec. Q1: quadrupole 1, Q3: quadrupole 3, z: charge, DP: declustering potential, CE: collision energy, CXP: collision cell exit potential.

Isothermal Titration Calorimetry: Expression and purification of full-length wild type NNMT protein (NNMTwt) were performed as previously described.[31] Isothermal titration calorimetry (ITC) measurements were made at 25 °C on a MicroCal ITC200 Instrument (Malvern Instruments) with 2 μL injections. NNMTwt was diluted at 200 μM in ITC buffer (50 mM Tris (pH 8.0), 150 mM NaCl) supplemented with 4% DMSO. Compounds were dissolved in DMSO at 50 mM and diluted to 2 mM in ITC buffer with a final DMSO concentration of 4%. Binding constants were calculated by fitting the data using the ITC data analysis module in Origin 7.0 (OriginLab Corp.).

Modeling studies: Docking computations were performed using Autodock 4.2.[49] Compounds **1**, **2**, **78**, and **81** were docked into the catalytic pocket of the structure taken from PDB ID: 3ROD.[32] Four molecular dynamic simulations were performed with GROMACS 2018.2[50] using the AMBER03 force field[51]. Each structure was immersed in a cubic box using TIP3P water molecules[52] and neutralized with counter ions. A production step of 250 ns was carried out using the Parrinello-Rahman algorithm[53] for temperature and pressure control, with coupling constants of T=0.1 ps and P=2.0 ps, for compounds **1**, **2**, **81** and extended to 450 ns for compound **78**, in order to reach equilibrium of the system. Coordinates were saved every 200 ps and the protein/ligand binding energy was estimated using g_mmpbsa calculations[54,55] on the last 50 ns of each trajectory. The conformation of minimal energy in these 50 ns was extracted from the simulations and minimized in order to represent the interactions between the ligands and NNMT protein.

Enzyme assay for selectivity: Methyltransferase inhibition assays were performed as described[56] by using commercially available chemiluminescent assay kits for PRMT1 and NSD2

(purchased from BPS Bioscience). The enzymatic reactions were conducted in duplicate at room temperature for 1 h (PRMT1) or 2 h (NSD2) in substrate-coated well plates at a final reaction volume of 50 µL containing: the manufacturer's proprietary assay buffer, AdoMet (at a concentration of 5 times the respective Km value for each enzyme), the methyltransferase enzyme: PRMT1 (100 ng per reaction) and NSD2 (500 ng per reaction), and inhibitor **78**. Before addition of AdoMet, the enzyme was first incubated with the inhibitor for 15 min at room temperature. Positive controls were performed in the absence of inhibitor using water to keep the final volume consistent. Blanks and substrate controls were performed in the absence of the enzyme and AdoMet, respectively. Following the enzymatic reactions, 100 µL of primary antibody (recognizing the respective immobilized methylated product) was added to each well, and the plate was incubated at room temperature for an additional 1 h. Then, 100 µL of secondary horseradish peroxidase (HRP)-conjugated antibody was added to each well, and the plate was incubated at room temperature for additional 30 min. Finally, 100 µL of an HRP substrate mixture was added to the wells, and the luminescence was measured directly by using a standard microplate reader. The luminescence data were normalized with the positive controls defined as 100% activity and blank defined as 0%.

Cell culture and treatment with compounds: The HSC-2 human oral cancer cell line was 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. Compounds **1**, **2**, **78**, and **81** were tested for their inhibitory effect on cell proliferation of HSC-2 cells. Each compound was dissolved in DMSO at 100mM concentration. This stock solution was then diluted in culture medium to final concentration values ranging between 1 µM and 100 µM. 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 $1x10^3$ cells/well. Cells were allowed to attach overnight and then incubated with compounds at different final concentrations, or with DMSO only, for 24, 48 and 72 hours. All experiments were performed in triplicate.

MTT assay: 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. HSC-2 cell proliferation was evaluated by measuring the conversion of the

tetrazolium salt MTT to formazan crystals upon treatment with compounds 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 5 µl of MTT reagent (5mg/ml in PBS). The medium was removed and 200 µl 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 software (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 as statistically significant.

Quantitative measurements of MNA levels in cultured cells: The analysis was performed as previously described[57] with minor modifications. Cellular MNA levels were determined using the same UHP-HILIC-MS/MS employed for the inhibition studies as described above. To determine the effect of compound **78** on NNMT activity in the HSC-2 oral cancer cell line used cells were treated with **78** at 100 µM (final DMSO content 0.1%) and incubated for 24, 48, or 72 hours. The day prior to starting treatment, cells were seeded in 6-well plates, at a density of $3x10^4$ cells/well. Cells were allowed to attach overnight and were then incubated with compound **78**. All experiments were performed in duplicate. Following treatment, medium was removed, and adherent cells were trypsinized and harvested by centrifugation at 1,000xg for 3 min at 4°C. Supernatant was then discarded and cell pellets were stored at -80 °C until further use. The extraction of MNA from the cell pellets was performed as previously described.[58] Briefly, 100 µL acetonitrile containing 50 nM d_3-MNA (as internal control) was added to the cell pellets and the cells were lysed for 20 minutes at room temperature with mild shaking. 50µL of purified water was added, followed by mixing and the resulting cell debris centrifuged for 10 minutes at 5,000 rpm. 100 µL of the resulting supernatant was then transferred to a 96-well plate and analysed for MNA content.

References

1. Alston TA, Abeles RH. Substrate specificity of nicotinamide methyltransferase isolated from porcine liver. Arch Biochem Biophys. 1988;260(2):601-8.
2. van Haren MJ, Sastre Toraño J, Sartini D, Emanuelli M, Parsons RB, Martin NI. A Rapid and Efficient Assay for the Characterization of Substrates and Inhibitors of Nicotinamide N -Methyltransferase. Biochemistry. 2016;55(37):5307-15.
3. Thomas MG, Sartini D, Emanuelli M, van Haren MJ, Martin NI, Mountford DM, et al. Nicotinamide N-methyltransferase catalyses the N-methylation of the endogenous -carboline norharman: evidence for a novel detoxification pathway. Biochem J. 2016;473(19):3253-67.
4. Pissios P. Nicotinamide N -Methyltransferase: More Than a Vitamin B3 Clearance Enzyme. Trends Endocrinol Metab. 2017;28(5):340-53.
5. Jung J, Kim LJY, Wang X, Wu Q, Sanvoranart T, Hubert CG, et al. Nicotinamide metabolism regulates glioblastoma stem cell maintenance. JCI Insight. 2017;2(10):1-23.
6. Kraus D, Yang Q, Kong D, Banks AS, Zhang L, Rodgers JT, et al. Nicotinamide N-methyltransferase knockdown protects against diet-induced obesity. Nature. 2014;508(7495):258-62.
7. ten Klooster JP, Sotiriou A, Boeren S, Vaessen S, Vervoort J, Pieters R. Type 2 diabetes-related proteins derived from an in vitro model of inflamed fat tissue. Arch Biochem Biophys. 2018;644(February):81-92.
8. Khalil EM, Mackie BD, Mao Y. Methyltransferases: Key Regulators in Cardiovascular Development and Disease. Ann Vasc Med Res. 2016;3(2):1032-9.
9. Fedorowicz A, Mateuszuk Ł, Kopec G, Skórka T, Kutryb-Zając B, Zakrzewska A, et al. Activation of the nicotinamide N-methyltransferase (NNMT)-1-methylnicotinamide (MNA) pathway in pulmonary hypertension. Respir Res. 2016;17(1):108.
10. Ulanovskaya OA, Zuhl AM, Cravatt BF. NNMT promotes epigenetic remodeling in cancer by creating a metabolic methylation sink. Nat Chem Biol. 2013;9(5):300-6.
11. Palanichamy K, Kanji S, Gordon N, Thirumoorthy K, Jacob JR, Litzenberg KT, et al. NNMT Silencing Activates Tumor Suppressor PP2A, Inactivates Oncogenic STKs, and Inhibits Tumor Forming Ability. Clin Cancer Res. 2017;23(9):2325-34.
12. Zhang J, Wang Y, Li G, Yu H, Xie X. Down-Regulation of Nicotinamide N-methyltransferase Induces Apoptosis in Human Breast Cancer Cells via the Mitochondria-Mediated Pathway. Filleur S, ed. PLoS One. 2014;9(2):e89202.
13. Sartini D, Muzzonigro G, Milanese G, Pierella F, Rossi V, Emanuelli M. Identification of

Nicotinamide N-Methyltransferase as a Novel Tumor Marker for Renal Clear Cell Carcinoma. J Urol. 2006;176(5):2248-54.

14. Sartini D, Santarelli A, Rossi V, Goteri G, Rubini C, Ciavarella D, et al. Nicotinamide N-Methyltransferase Upregulation Inversely Correlates with Lymph Node Metastasis in Oral Squamous Cell Carcinoma. Mol Med. 2007;13(7-8):415-21.
15. Parsons RB, Smith SW, Waring RH, Williams AC, Ramsden DB. High expression of nicotinamide N-methyltransferase in patients with idiopathic Parkinson's disease. Neurosci Lett. 2003;342(1-2):13-6.
16. Parsons RB, Smith M-L, Williams AC, Waring RH, Ramsden DB. Expression of Nicotinamide N-Methyltransferase (E.C. 2.1.1.1) in the Parkinsonian Brain. J Neuropathol Exp Neurol. 2002;61(2):111-24.
17. Thomas MG, Saldanha M, Mistry RJ, Dexter DT, Ramsden DB, Parsons RB. Nicotinamide N-methyltransferase expression in SH-SY5Y neuroblastoma and N27 mesencephalic neurones induces changes in cell morphology via ephrin-B2 and Akt signalling. Cell Death Dis. 2013;4(6):e669-e669.
18. van Haren MJ, Thomas MG, Sartini D, Barlow DJ, Ramsden DB, Emanuelli M, et al. The kinetic analysis of the N -methylation of 4-phenylpyridine by nicotinamide N -methyltransferase: Evidence for a novel mechanism of substrate inhibition. Int J Biochem Cell Biol. 2018;98(September 2017):127-36.
19. Aksoy S, Szumlanski CL, Weinshilboum RM. Human liver nicotinamide N-methyltransferase. cDNA cloning, expression, and biochemical characterization. J Biol Chem. 1994;269(20):14835-40. http://www.ncbi.nlm.nih.gov/pubmed/8182091
20. Horning BD, Suciu RM, Ghadiri DA, Ulanovskaya OA, Matthews ML, Lum KM, et al. Chemical Proteomic Profiling of Human Methyltransferases. J Am Chem Soc. 2016;138(40):13335-43.
21. Ruf S, Hallur MS, Anchan NK, Swamy IN, Murugesan KR, Sarkar S, et al. Novel nicotinamide analog as inhibitor of nicotinamide N-methyltransferase. Bioorganic Med Chem Lett. 2018;28(5):922-5.
22. Kannt A, Rajagopal S, Kadnur SV, Suresh J, Bhamidipati RK, Swaminathan S, et al. A small molecule inhibitor of Nicotinamide N-methyltransferase for the treatment of metabolic disorders. Sci Rep. 2018;8(1):3660.
23. Neelakantan H, Wang HY, Vance V, Hommel JD, McHardy SF, Watowich SJ. Structure-Activity Relationship for Small Molecule Inhibitors of Nicotinamide N-Methyltransferase. J Med Chem. 2017;60(12):5015-28.

24. van Haren MJ, Taig R, Kuppens J, Sastre Toraño J, Moret EE, Parsons RB, et al. Inhibitors of nicotinamide N-methyltransferase designed to mimic the methylation reaction transition state. Org Biomol Chem. 2017;15(31):6656-67.
25. Lerner C, Masjost B, Ruf A, Gramlich V, Jakob-Roetne R, Zürcher G, et al. Bisubstrate inhibitors for the enzyme catechol-O-methyltransferase (COMT): influence of inhibitor preorganisation and linker length between the two substrate moieties on binding affinity. Org Biomol Chem. 2003;1(1):42-9.
26. Paulini R, Trindler C, Lerner C, Brändli L, Schweizer WB, Jakob-Roetne R, et al. Bisubstrate Inhibitors of CatecholO-Methyltransferase (COMT): the Crucial Role of the Ribose Structural Unit for Inhibitor Binding Affinity. ChemMedChem. 2006;1(3):340-57.
27. Mori S, Iwase K, Iwanami N, Tanaka Y, Kagechika H, Hirano T. Development of novel bisubstrate-type inhibitors of histone methyltransferase SET7/9. Bioorg Med Chem. 2010;18(23):8158-66.
28. Dowden J, Hong W, Parry R V, Pike RA, Ward SG. Toward the development of potent and selective bisubstrate inhibitors of protein arginine methyltransferases. Bioorg Med Chem Lett. 2010;20(7):2103-5.
29. Van Haren M, Van Ufford LQ, Moret EE, Martin NI. Synthesis and evaluation of protein arginine N-methyltransferase inhibitors designed to simultaneously occupy both substrate binding sites. Org Biomol Chem. 2015;13(2).
30. van Haren MJ, Marechal N, Troffer-Charlier N, Cianciulli A, Sbardella G, Cavarelli J, et al. Transition state mimics are valuable mechanistic probes for structural studies with the arginine methyltransferase CARM1. Proc Natl Acad Sci. 2017;114(14):3625-30.
31. Babault N, Allali-Hassani A, Li F, Fan J, Yue A, Ju K, et al. Discovery of Bisubstrate Inhibitors of Nicotinamide N -Methyltransferase (NNMT). J Med Chem. 2018;61(4):1541-51.
32. Peng Y, Sartini D, Pozzi V, Wilk D, Emanuelli M, Yee VC. Structural Basis of Substrate Recognition in Human Nicotinamide N -Methyltransferase. Biochemistry. 2011;50(36):7800-8.
33. Waters NJ. Preclinical Pharmacokinetics and Pharmacodynamics of Pinometostat (EPZ-5676), a First-in-Class, Small Molecule S-Adenosyl Methionine Competitive Inhibitor of DOT1L. Eur J Drug Metab Pharmacokinet. 2017;42(6):891-901.
34. af Gennäs GB, Talman V, Aitio O, Ekokoski E, Finel M, Tuominen RK, et al. Design, Synthesis, and Biological Activity of Isophthalic Acid Derivatives Targeted to the C1 Domain of Protein Kinase C. J Med Chem. 2009;52(13):3969-81.

35. Cho SD, Park YD, Kim JJ, Falck JR, Yoon YJ. Facile Reduction of Carboxylic Acids, Esters, Acid Chlorides, Amides and Nitriles to Alcohols or Amines Using NaBH 4 /BF 3 ·Et 2 O. Bull Korean Chem Soc. 2004;25(3):407-9.
36. Maruoka H, Muto T, Tanaka T, Imajo S, Tomimori Y, Fukuda Y, et al. Development of 6-benzyl substituted 4-aminocarbonyl-1,4-diazepane-2,5-diones as orally active human chymase inhibitors. Bioorg Med Chem Lett. 2007;17(12):3435-9.
37. Pola Chemical Industries Inc.; Yokoyama, Kouji; Kimura, Makoto; Tamai, Masashi; Saitoh, Yuko; Kato, Tomomi; Ikeda Y. Melanin Production Inhibitor. :US Patent 8,846,012, September 30, 2014.
38. Schreiber KC, Fernandez VP. The Lithium Aluminum Hydride Reduction of Some N-Substituted Succinimides 1. J Org Chem. 1961;26(6):1744-7.
39. Colombo R, Mingozzi M, Belvisi L, Arosio D, Piarulli U, Carenini N, et al. Synthesis and Biological Evaluation (in Vitro and in Vivo) of Cyclic Arginine–Glycine–Aspartate (RGD) Peptidomimetic–Paclitaxel Conjugates Targeting Integrin α V β 3. J Med Chem. 2012;55(23):10460-74.
40. Chi Y, English EP, Pomerantz WC, Horne WS, Joyce LA, Alexander LR, et al. Practical Synthesis of Enantiomerically Pure β 2 -Amino Acids via Proline-Catalyzed Diastereoselective Aminomethylation of Aldehydes. J Am Chem Soc. 2007;129(18):6050-5.
41. Wernic D, DiMaio J, Adams J. Enantiospecific synthesis of L-.alpha.-aminosuberic acid. Synthetic applications in preparation of atrial natriuretic factor analogs. J Org Chem. 1989;54(17):4224-8.
42. Seta R, Mascitti M, Campagna R, Sartini D, Fumarola S, Santarelli A, et al. Overexpression of nicotinamide N-methyltransferase in HSC-2 OSCC cell line: effect on apoptosis and cell proliferation. Clin Oral Investig. 2019;23(2):829-38.
43. Myung-Hwa Kim, Kwangwoo Chun, Jae-Won, ChoiBo-Young Joe, Sang-Woo Park, Kwang Hee Kim, Byung-Kyu Oh J-HC. Tricyclic derivatives or pharmaceutically acceptable salts thereof, their preparations and pharmaceutical compositions containing them. :US Patent 2007/0179143 A1, August 2, 2007.
44. Ewa B, Maciej W, Marcin S, Grzegorz D, Michał Z, Jan P, et al. The development of first Staphylococcus aureus SplB protease inhibitors: Phosphonic analogues of glutamine. Bioorg Med Chem Lett. 2012;22(17):5574-8.
45. Chen S, Zhao X, Chen J, Chen J, Kuznetsova L, Wong SS, et al. Mechanism-Based Tumor-Targeting Drug Delivery System. Validation of Efficient Vitamin Receptor-Mediated

Endocytosis and Drug Release. Bioconjug Chem. 2010;21(5):979-87.

46. Berk SC, Kreutzer KA, Buchwald SL. A catalytic method for the reduction of esters to alcohols. J Am Chem Soc. 1991;113(13):5093-5.
47. Liu F, Zha H-Y, Yao Z-J. Synthesis of a New Conformation-Constrained L-Tyrosine Analogue as a Potential Scaffold for SH2 Domain Ligands. J Org Chem. 2003;68(17):6679-84.
48. Floyd N, Vijayakrishnan B, Koeppe JR, Davis BG. Thiyl Glycosylate of Olefinic Proteins: S-Linked Glycoconjugate Synthesis. Angew Chemie Int Ed. 2009;48(42):7798-802.
49. Morris GM, Huey R, Lindstrom W, Sanner MF, Belew RK, Goodsell DS, et al. AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility. J Comput Chem. 2009;30(16):2785-91.
50. Abraham MJ, Murtola T, Schulz R, Páll S, Smith JC, Hess B, et al. GROMACS: High performance molecular simulations through multi-level parallelism from laptops to supercomputers. SoftwareX. 2015;1-2:19-25.
51. Ponder JW, Case DA. Force fields for protein simulations. Adv Protein Chem. 2003;66:27-85. http://www.ncbi.nlm.nih.gov/pubmed/14631816
52. Mahoney MW, Jorgensen WL. A five-site model for liquid water and the reproduction of the density anomaly by rigid, nonpolarizable potential functions. J Chem Phys. 2000;112(20):8910-22.
53. Parrinello M, Rahman A. Polymorphic transitions in single crystals: A new molecular dynamics method. J Appl Phys. 1981;52(12):7182-90.
54. Kumari R, Kumar R, Lynn A. g_mmpbsa —A GROMACS Tool for High-Throughput MM-PBSA Calculations. J Chem Inf Model. 2014;54(7):1951-62.
55. Baker NA, Sept D, Joseph S, Holst MJ, McCammon JA. Electrostatics of nanosystems: Application to microtubules and the ribosome. Proc Natl Acad Sci. 2001;98(18):10037-41.
56. Vedadi M, Barsyte-Lovejoy D, Liu F, Rival-Gervier S, Allali-Hassani A, Labrie V, et al. A chemical probe selectively inhibits G9a and GLP methyltransferase activity in cells. Nat Chem Biol. 2011;7(8):566-74.
57. Neelakantan H, Vance V, Wetzel MD, Wang HYL, McHardy SF, Finnerty CC, et al. Selective and membrane-permeable small molecule inhibitors of nicotinamide N-methyltransferase reverse high fat diet-induced obesity in mice. Biochem Pharmacol. 2018;147:141-52.
58. Policarpo RL, Decultot L, May E, Kuzmič P, Carlson S, Huang D, et al. High-Affinity Alkynyl Bisubstrate Inhibitors of Nicotinamide N -Methyltransferase (NNMT). J Med

Chem. 2019;62(21):9837-73.