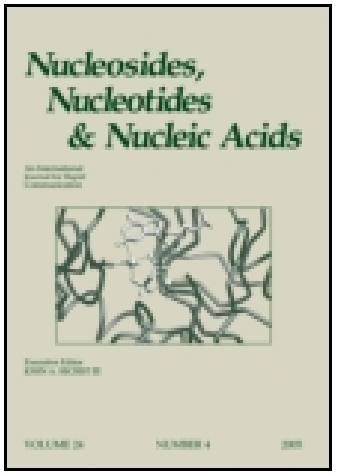

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NOVEL CARBOXALDEHYDE MEDIATED SYNTHETIC PATHWAY FOR 5′-AMINO ADENOSINE ANALOGUES

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□ *Modifications of the 5′-position of adenosine have been prepared via a novel 5′-carboxaldehyde synthon. The described methodology should prove useful for making related compounds in which amine-derived moieties off the 5′-position of adenosine (or related nucleoside congeners) can be easily incorporated via reductive amination, especially for the incorporation of aromatic amines.*

Keywords Synthetic methodology; reductive amination; modified nucleosides; enzyme inhibition

INTRODUCTION

Nucleoside derivatives are highly sought after as therapeutic agents for the treatment of various disease states, ranging from antibiotics and antivirals to treat infections[1,2] to DNA methyltransferases in cancer.[3] Diverse biological molecules are amenable to targeting via a nucleoside scaffold such as protein kinases,[4] histone methyltransferases,[5–7] reverse transcriptase,[2] poly(ADP-ribose) polymerase (PARP),[8] and many others. In conjunction with ongoing projects in our laboratory to develop small molecule inhibitors against cancer targets, we needed to establish a synthetic strategy for introducing different amino derivatives at the 5′-position of adenosine. Current synthetic strategies for 5′-modified adenosine analogues typically proceed via replacement of the 5′-hydroxyl with a chlorine leaving group and subsequent displacement with a thiol or amine. Activation via Mitsunobu reaction with phthalimide followed by hydrazine treatment to give the primary amine has also been utilized.[6,9,10] Within these strategies, subsequent homologation of installed amines is then carried out by alkylation or reductive amination

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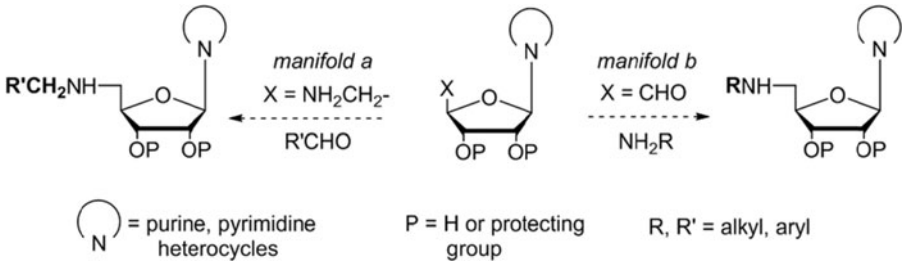


FIGURE 1 Strategies for synthesizing 5′-deoxy-5′-amino derivatives of adenosine via reductive amination.

methodologies. The latter is generically exemplified by *manifold a* (Figure 1), with many examples of the installation of $R'CH_2$ groups (R' = aryl) given in a recent paper.[11] Due to the mild conditions of reductive amination, we chose this methodology to access our target amines designed as bioisosteres of S-adenosylmethionine (SAM; **1**), shown in Figure 2. While it would be possible to access **4** and **5** by *manifold a*, we were interested in accessing all target compounds from a single carboxaldehyde intermediate as shown in *manifold b*. Examples of nucleoside 5′amine installation by *manifold b* are sparse, and *indeed an exhaustive literature search uncovered no examples of reductive amination of a nucleoside aldehyde with an aromatic amine*, required for target compounds **2** and **3**. Thus, we devised a synthetic strategy utilizing a convergent approach

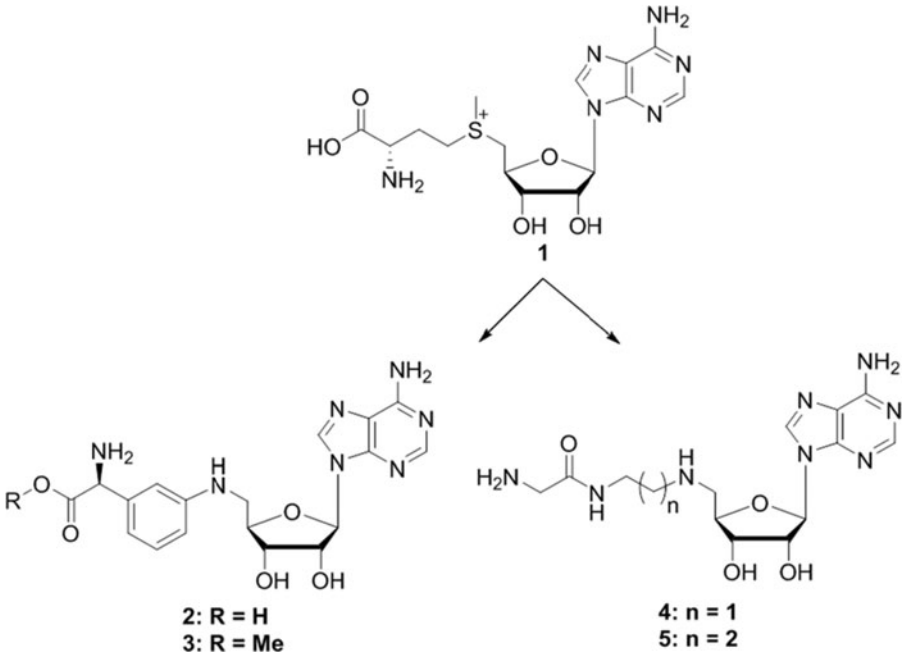
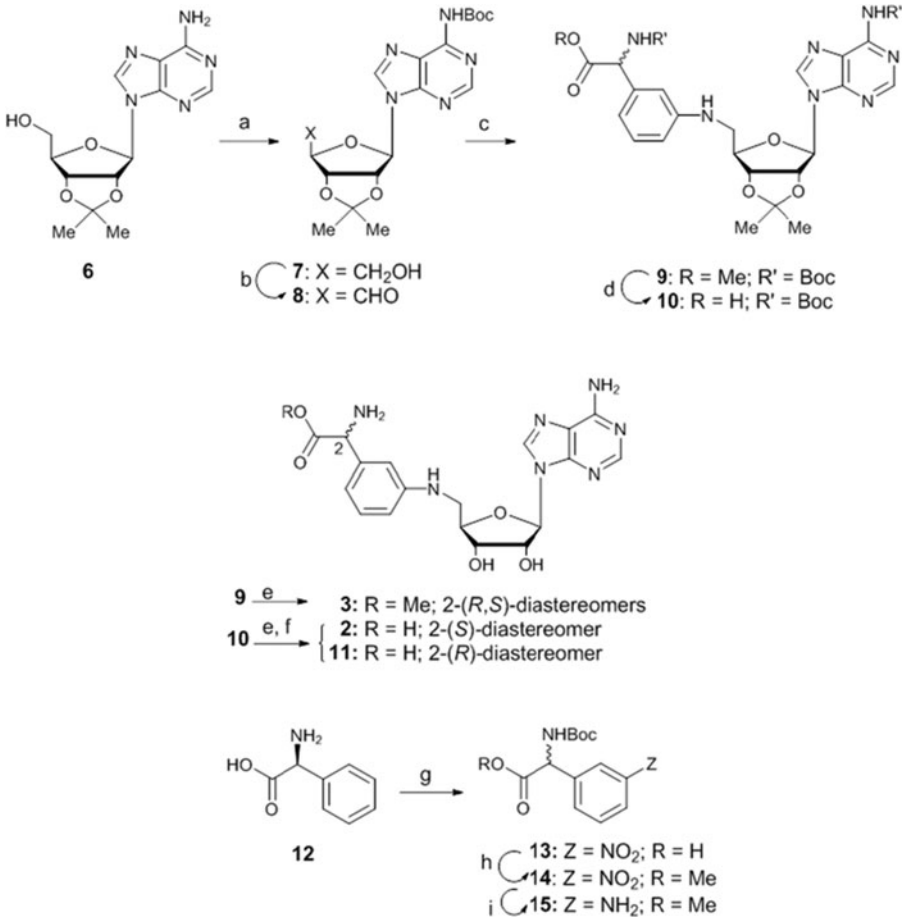


FIGURE 2 Target model compounds of 5′-adenosine modifications.



SCHEME 1 Reagents and conditions: (a) 1. HMDS, DMAP, TMSOTf; 2. Boc_2O, THF; 3. 5:1 MeOH: TEA (100%, 3 steps); (b) Dess-Martin periodinane, DCM, 0°C - rt (99%); (c) **15**, DCE, $NaBH(OAc)_3$ (26%); (d) LiOH, MeOH (39%); (e) aq. TFA (35% for **9**); (f) hplc purification; (g) 1. Fuming HNO_3, conc. H_2SO_4; 2. Boc_2O, $NaHCO_3$, 1,4-dioxane/H_2O [14]; (h) MeI, K_2CO_3, THF (65%); (i) H_2, 10% Pd/C, MeOH (87%).

in which a protected adenosine 5′-carboxaldehyde (**8**, Scheme 1) would be condensed with a requisite amine side chain via a reductive amination, providing a relatively novel and efficient method to access 5′-deoxy-5′-amino derivatives of adenosine.

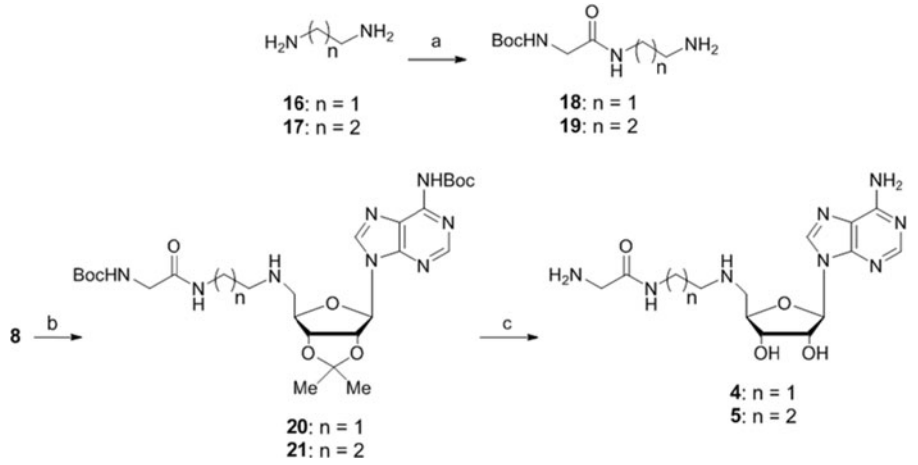
SYNTHESIS

Based on the use of S-adenosylmethionine (SAM; **1**) as a methyl donor by many methyltransferase enzymes, we sought to synthesize several model compounds **2–5** using our reductive amination strategy to provide bioisosteres

of the homocysteine tail of SAM (Figure 2). This required the synthesis of the novel adenosine synthon **8** as shown in Scheme 1. Accordingly, hexamethyldisilazane (HMDS) persilylation of 2′,3′-(isopropylidene)adenosine **(6)** followed by treatment with di-*t*-butyl dicarbonate was carried out by modification of a procedure used to introduce N^6-Boc functionality onto nucleosides.[12] The literature procedure mixes reactants using milling in a flask with Pyrex glass beads. However, we found this was not necessary and simply conducted our reaction with conventional magnetic stirring. This gave an intermediate N^6,N^6-bis(*tert*-butoxycarbonyl)-5′-*O*-(trimethylsilyl)adenosine, which was not isolated but was reacted directly with methanol:triethylamine (5:1) to simultaneously cleave the TMS ether and one of the N^6-Boc groups. This provided **7** in quantitative yield with the overall sequence representing a much superior method to make this reported compound.[13] Subsequent conversion of **7** to the 5′-carboxaldehyde **8** was then carried out by oxidation with Dess-Martin periodinane. This intermediate was then used for synthesis of designed compounds. For target compound **2** the side chain was derived from (*S*)-(+)-phenylglycine **12** in a series of standard reactions. Nitration followed by α-amine Boc protection was carried out by a literature procedure to provide compound **13**.[14] Methyl ester formation gave **14**, which was then hydrogenated to amine **15**. Reductive amination of aldehyde **8** with **15** then provided compound **9** in modest overall yield.

HPLC analysis of this product showed a ~1:1 mixture of diastereomers, indicating that racemization had taken place in the earlier nitration step of building up the side chain as previously documented.[15] Hydrolysis of the methyl ester with LiOH gave **10**, which was subjected to aqueous TFA hydrolyis to simultaneously remove the Boc and isopropylidene protecting groups. Upon HPLC purification, two diastereomers, **2** and **11**, were separated (Supplementary Figure 1). Alternatively, **9** was also subjected to TFA deprotection to provide methyl ester **3** as a mixture of diastereomers.

For compound **5**, the same general synthetic approach was utilized (Scheme 2). Compound **19** was prepared via amidation of 1,3-propanediamine (**17**) with *N*-Boc-glycine methyl ester using the method described by Morandeau et al.[16] Coupling of **19** with aldehyde **8** was performed under the same reductive amination conditions shown above providing the Boc and isopropylidene protected compound **21**. Subsequent global hydrolysis of the protecting groups provided **5** in good overall yield. The same sequence of steps was repeated on ethylenediamine (**16**) to make compound **4** with the truncated linker. All compounds reported in this paper were rigorously purified and their structural assignments are supported by mass spectrometry (ESI and HRMS target compounds) and ^{1}H NMR spectroscopy (Experimental and Supplementary Figures 2–16).



SCHEME 2 Reagents and conditions: (a) *N*-(Boc)-glycine methyl ester, MeOH, 0°C – rt (50–52% yield); (b) **18** or **19**, $NaBH(OAc)_3$, DCE (43–48% yield); (c) aq. TFA (54–56% yield).

Evaluation of *in vitro* DOT1L Inhibition

As several SAM analogues with 5′-adenosine modifications have been demonstrated to potently inhibit the histone methyltransferase Disruptor of Telomeric silencing 1 Like (DOT1L),[5,7,17] we tested the ability of the synthesized compounds to inhibit DOT1L histone methyltransferase activity *in vitro*. Briefly, the assay utilized ^{3}H-methyl-S-adenosylmethionine (^{3}H-SAM) as the source of radioisotopically labelled methyl donor. In the presence of DOT1L the ^{3}H-methyl group is transferred to the substrate histone H3 lysine 79 (H3K79) in a milieu of extracted core histones. The dose dependent curves together with the IC_{50} values of synthesized and characterized compounds are presented in Figure 3. S-adenosylhomocysteine (SAH) was used as a positive control and showed inhibition of the methyltransferase activity of DOT1L with IC_{50} of 1.6 μM, consistent with previously reported results. Compound **2** showed the most potent inhibition of DOT1L histone methyltransferase activity in comparison with other synthesized nucleoside analogues with an IC_{50} value of 220 μM. However, it was significantly less active in comparison to SAH. Interestingly compound **11**, the diastereomer of **2**, showed five-fold lower potency with IC_{50} of 1,055 μM. Based on the *in vitro* histone methyltransferase activity, compound **2** was assigned the natural 2-(*S*)-stereochemistry in the side chain since it showed higher potency, while compound **11** was assigned as the 2-(*R*)-diastereomer. Compound **3**, which is a diastereomeric mixture of the methyl ester of **2**/**11**, showed less potency ($IC_{50} > 1{,}500$ μM).

These findings are consistent with the reported DOT1L crystal structure[18] which demonstrates that the co-factor SAM forms a key hydrogen bond with the protein through the carboxylic acid moiety. Replacing the

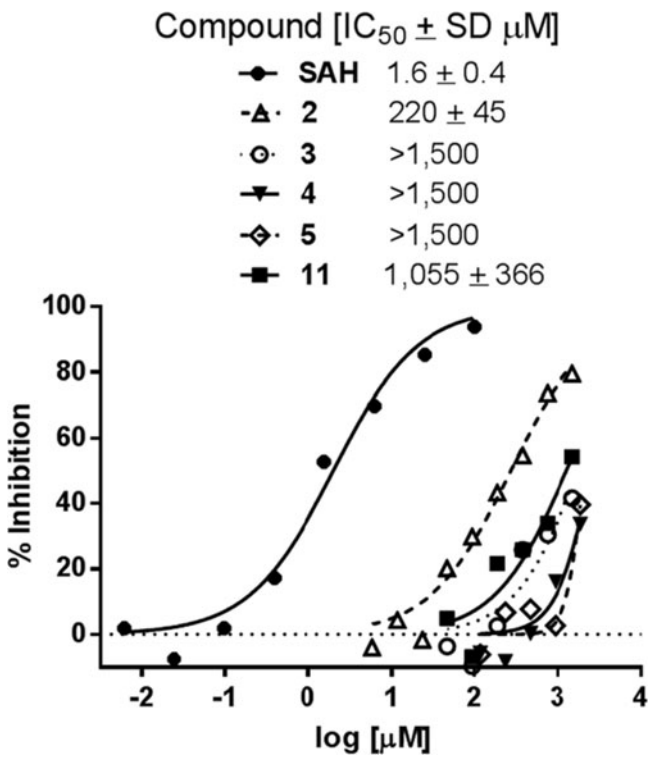


FIGURE 3 DOT1L inhibition by designed compounds. IC_{50} values of synthesized inhibitors assessed by *in vitro* radiometric DOT1L histone methyltransferase (HMTase) assay.

amino acid tail of SAM with an α-amino acetamide bioisostere resulted in a detrimental effect on the activity of compounds **4** and **5** with IC_{50} values > 1,500 μM. In addition, the different length of the linker in these two compounds seemed to have no effect on their potency. We believe that much of the difference in potency for these compounds compared with SAH can be attributed to disruption of the hydrogen bond network formed between SAM and DOT1L. In addition, a structure activity relationship (SAR) study of SAM analogues published while our work was in progress confirmed that slight alterations of the structure of SAM can dramatically affect the ability of small molecules to inhibit DOT1L.[9]

CONCLUSIONS

Herein we present a novel synthetic methodology utilizing a 5′-carboxaldehyde adenosine intermediate that can undergo reductive amination with representative amines to provide 5′-deoxy-5′-amino derivatives of adenosine. This strategy was applied to the synthesis of five model SAM analogues, which were tested for *in vitro* inhibition of the histone methyltransferase DOT1L. The newly synthesized SAM analogues demonstrate that

a rigid linker between adenosine and the amino acid tail portion of SAM is more favorable than a flexible α-amino acetamide bioisoteric replacement of the amino acid moiety for inhibition of DOT1L. Our synthetic strategy will be useful for making related compounds in which moieties off the 5′-position of adenosine (or related nucleoside congeners) can be easily constructed via reductive amination of a precursor 5′-carboxaldehyde. Thus, the utility clearly extends beyond SAM analogues, as adenosine is a useful scaffold for the development of numerous potential classes of inhibitors.

EXPERIMENTAL

General Chemistry Procedures

All starting materials were obtained from commercial suppliers and were used without further purification. ^{1}H NMR spectra were recorded on a Varian 400 instrument. Chemical shift values are recorded in δ units (ppm). Mass spectra were recorded on a Micromass TofSpec-2E Matrix-Assisted, Laser-Desorption, Time-of-Flight Mass Spectrometer in positive ESI mode unless otherwise noted. High resolution mass spectrometry (HRMS) analysis was performed on an Agilent Q-TOF system. Analytical HPLC was run on a reverse-phase column (Restek Ultra C18, 5 μm, 150 × 4.6 mm column; flow rate of 1 mL/min using a gradient of 40–80% acetonitrile in water over 20 min). Thin-layer chromatography (TLC) was performed on silica gel GHLF plates (250 microns) purchased from Analtech. Column chromatography was carried out in the flash mode utilizing silica gel. Extraction solutions were dried over $MgSO_4$ prior to concentration.

***tert*-Butyl (9-((3a*R*,4*R*,6*R*,6a*R*)-6-(hydroxymethyl)-2,2-dimethyltetrahydrofuro[3,4-*d*][1,3]dioxol-4-yl)-9*H*-purin-6-yl)carbamate (7)**.[13] Using a modification of a literature procedure,[12] a suspension of **6** (1.54 g, 5 mmol), hexamethyldisilazane (HMDS; 2 mL), and 4-dimethylaminopyridine (DMAP; 120 mg) was magnetically stirred together at 25°C in an oven-dried flask. To this was added drop-wise trimethylsilyl trifluoromethanesulfonate (TMSOTf; 20 μL) and the resultant suspension was heated at 75°C for 2 h. The mixture was concentrated to oil that was dissolved in 3 mL of dry THF with gentle warming. The solution was ice-cooled and treated with 3.27 g (15 mmol) of di-*t*-butyl dicarbonate and an additional 7 ml of THF, and allowed to stir at 25°C for 4 h. The solution was concentrated and then treated with 18 mL of 5:1(v/v) methanol: triethylamine. Following vigorous evolution of CO_2, the solution was stirred at 55°C for 16 h and then concentrated to an oil that was distributed between dichloromethane and water. The dichloromethane phase was dried and concentrated to an oil that was purified by flash chromatography, eluting with a 1:1 mixture of hexane and ethyl acetate. Combined product fractions were concentrated to an oil that was pumped *in vacuo* at 60°C to leave **7** (2.53 g; 100% yield)

as a white foam: NMR and mass spec data were identical to those previously reported.[13]

***tert*-Butyl (9-((3a*R*,4*R*,6*S*,6a*S*)-6-formyl-2,2-dimethyltetrahydrofuro[3,4-*d*][1,3]dioxol-4-yl)-9*H*-purin-6-yl)carbamate (8).** To an ice-cooled stirred suspension of Dess-Martin periodinane (674 mg, 1.6 mmol) in dichloromethane (7.5 mL) was added **7** (589 mg, 1.45 mmol) and the resultant mixture was allowed to warm to room temperature over 3 h. The mixture was concentrated to a white powder that was suspended in ethyl acetate, and the insoluble Dess-Martin reagent was removed by filtration. The filtrate was concentrated and additional residual amounts of Dess-Martin reagent were removed by the same process. The filtrate was concentrated to leave fairly pure **8** (583 mg, 99% crude yield) as a solid, which was suitable for use in the next reaction: ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.25 (s, 1H), 8.54 (s, 1H), 7.96 (s,1H), 6.17 (s, 1H), 5.49 (d, $J = 6.1$ Hz, 1H), 5.28 (d, $J = 6.1$ Hz, 1H), 4.62 (s, 1H), 1.54 (s, 3H), 1.50 (s, 9H), 1.34 (s, 3H); MS (ESI): *m/z* 438.1 $(M+Na)^+$.

Methyl (*S*)-2-((*tert*-butoxycarbonyl)amino)-2-(3-((((3a*R*,4*R*,6*R*,6a*R*)-6-(6-((*tert*-butoxycarbonyl)amino)-9*H*-purin-9-yl) - 2,2-dimethyltetrahydrofuro[3,4-*d*][1,3]dioxol-4-yl)methyl)amino)phenyl)acetate and *R*-diastereomer (9). To an ice-cold solution of **8** (370 mg, 0.91 mmol) in 1,2-dichloroethane (DCE; 2.5 mL) was added a solution of **15** (280 mg, 1 mmol) in DCE (2.5 mL) followed by sodium triacetoxyborohydride (STAB; 270 mg, 1.3 mmol). The reaction mixture was stirred overnight at room temperature and quenched with saturated aq. $NaHCO_3$. The mixture was diluted with water and extracted with ethyl acetate (3x). The combined extracts were dried and concentrated to an oil that was purified by flash chromatography, eluting with hexanes: ethyl acetate (2:1) to give **9** (157 mg, 26% yield) as a ~1:1 ratio of diastereomers by HPLC: ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.84, 8.67 (s each, 0.57H & 0.43H, H-8 of each diastereomer), 8.03 (br s, 1H), 7.97, 7.94 (s each, 0.49 & 0.51H, H-2 of each diastereomer), 7.10 (q, $J =$ 8.1 Hz, 1H), 6.65 (t, $J = 9.0$ Hz, 1H), 6.61–6.51 (m, 2H), 6.03, 5.96 (s each, 0.44H & 0.56H, anomeric H of each diastereomer), 5.54–5.09 (m, 4H), 4.60–4.50 (m,1H), 3.67 (s, 3H), 3.53–3.45 (m, 2H), 1.61 (s, 3H), 1.55 (s, 9H), 1.42 (s, 9H), 1.36 (s, 3H); MS (ESI): *m/z* 670.2 $(M+1)^+$, 692.2 $(M+Na)^+$.

(*S*)-2-((*tert*-Butoxycarbonyl)amino) - 2 -(3-((((3a*R*,4*R*,6*R*,6a*R*)-6-(6-((*tert*-butoxycarbonyl)amino)-9*H*-purin-9-yl)-2,2-dimethyltetrahydrofuro[3,4-*d*][1,3]dioxol-4-yl)methyl)amino)phenyl)acetic acid and (*R*)-diastereomer (10). To a stirred solution of **9** (160 mg, 0.24 mmol) in methanol (1 mL) at 0°C was added LiOH (11 mg, 0.26 mmol). Cooling was removed and reaction mixture was stirred at room temperature overnight. TLC showed incomplete conversion with a new spot of R_f ~0.4 (2:1 ethyl acetate: methanol). Additional LiOH (11 mg) was added and stirring was continued for 4 h. The mixture was concentrated and the residue was distributed between 5% aq. acetic acid added and ethyl acetate. The combined ethyl

acetate extracts were dried and concentrated to oil that was purified by flash chromatography, eluting with ethyl acetate: methanol (9:1). Product fractions were combined and concentrated to give **10** (61 mg, 39% yield): ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.66 (s, 1H), 7.98 (s, 1H), 7.11–6.97 (m, 1H), 6.71–6.45 (m, 3H), 6.03 (d, J = 4.4 Hz, 1H), 5.91–5.84 (m, 1H), 5.50–4.99 (m, 3H), 4.57 (m, 1H), 3.40–3.20 (m, 2H), 1.56 (s, 3H), 1.52 (s, 9H), 1.39 (s, 3H), 1.37 (s, 9H); MS (ESI): *m/z* 656.2 $(M+1)^+$.

Methyl (*S*)-2-amino-2-(3-((((2*R*,3*S*,4*R*,5*R*)-5-(6-amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl)amino)phenyl)acetate and *R*-diastereomer (3). To a solution of **9** (173.5 mg, 0.26 mmol) in ice-cold water (3 mL) was added trifluoroacetic acid (TFA; 3 mL) at 0°C. The mixture was stirred at room temperature overnight, concentrated and then diluted with aq. $NaHCO_3$. The mixture was extracted with ethyl acetate (3x), and the combined extracts were dried and concentrated to a residue that precipitated solids upon addition of ethyl acetate. The solids were collected and washed well with hexanes. The filtrate was concentrated and processed in a similar fashion to provide a second crop that was combined with the first to give **3** (39 mg, 35% yield): ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.23 (s, 1H), 7.86 (s, 1H), 7.12 (t, J = 7.7 Hz, 1H), 6.64 (d, J = 7.5 Hz, 1H), 6.56 (d, J = 10.7 Hz, 1H), 6.40 (d, J = 7.0 Hz, 1H), 6.30 (s, 1H), 6.00 (s, 1H), 5.51 (d, J = 5.9 Hz, 1H), 5.40 (d, J = 7.0 Hz, 1H), 5.23 (d, J = 5.4 Hz, 1H), 4.60 (m, 1H), 3.68 (s, 3H), 3.60 - 3.35 (m, 2H); MS (ESI): *m/z* 430.9 $(M+1)^+$, 452.1 $(M+Na)^+$.

(*S*)-2-Amino-2-(3-((((2*R*,3*S*,4*R*,5*R*)-5-(6-amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl)amino)phenyl)acetic acid (2) and (*R*)-diastereomer (11). To a solution of **10** (61 mg, 0.09 mmol) in dichloromethane (0.4 mL) at 0°C was added water (0.1 mL) and TFA (0.5 mL). The mixture was stirred at room temperature overnight and concentrated to a residue that was dissolved in H_2O: methanol (95:5). The solution was then purified by reverse-phase HPLC (Restek Ultra C18, 5 μm, 150 × 21.2 mm column; flow rate of 10 mL/min using a gradient of 10–30% acetonitrile in water over 25 min) followed by concentration and lyophilization of fractions corresponding to each isomer provided purified diastereomers. Diastereomer **11**: ^{1}H NMR (400 MHz, DMSO-d_6 + D_2O): δ 8.34 (s, 1H), 8.12 (s, 1H), 7.10 (t, J = 7.8 Hz, 1H), 6.70–6.59 (m, 3H), 5.88 (d, J = 7.4 Hz, 1H), 4.99 (d, J = 11.0 Hz, 1H), 4.73 (s, 1H), 4.65–4.57 (m, 1H), 4.23–4.17 (m, 1H), 3.26–3.20 (m, 2H); MS (ESI): *m/z* 416.1682 $(M+1)^+$; Diastereomer **2**: ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.58 (br s, 2H; exchanges with D_2O), 8.38 (s, 1H), 8.24 (s,1H), 7.69 (br s, 2H; exchanges with D_2O), 7.12 (t, J = 7.8 Hz, 1H), 6.68–6.61 (m, 3H), 5.87 (d, J = 6.0 Hz,1H), 5.47 (br s, 1H; exchanges with D_2O), 5.27(br s, 1H; exchanges with D_2O), 4.88 (d, J = 18.0 Hz,1H; collapses to s with D_2O wash), 4.77–4.68 (m, 1H), 4.14 (t, J = 4.3 Hz, 1H), 4.07 (d, J = 3.5 Hz, 1H), 3.32 (d, J = 7.2 Hz, 2H; peak revealed with D_2O wash); MS (ESI): *m/z* 416.1685 $(M+1)^+$.

Methyl (*S*)-2-((*tert*-butoxycarbonyl)amino)-2-(3-nitrophenyl)acetate (14).[19] A stirred solution of partially racemized (*S*)-2-((*tert*-butoxycarbonyl)amino)-2-(3-nitrophenyl)acetic acid[14] (**13**; 600 mg, 2.03 mmol), iodomethane (140 μL, 2.2 mmol), anhydrous potassium carbonate (415 mg, 3 mmol) and THF (10 mL) was heated at 60°C for 18 h. The mixture was filtered and the filtrate was diluted with water and extracted with dichloromethane (3x). The combined organic extracts were dried and concentrated to an oil that was purified by flash chromatography, eluting with hexanes: ethyl acetate (6:1). Product fractions were combined and concentrated to leave **14** (409 mg, 65% yield): (400 MHz, $CDCl_3$): δ 8.23 (t, $J = 2.0$ Hz, 1H), 8.19–8.15 (m, 1H), 7.72 (d, $J = 7.7$ Hz, 1H), 7.57–7.49 (m, 1H), 5.78 (s, 1H), 5.42 (d, $J = 6.7$ Hz, 1H), 3.73 (s, 3H), 1.51 (s, 9H).

Methyl (*S*)-2-(3-aminophenyl)-2-((*tert*-butoxycarbonyl)amino)acetate (15).[19] A mixture of **14** (720 mg, 2.3 mmol), 10% Pd/C (catalytic) and methanol (35 mL) was hydrogenated at 50 psi at room temperature overnight. The mixture was filtered over Celite® and the filtrate concentrated to leave **15** (566 mg, 87% yield) as a viscous yellow oil: ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.10 (t, $J = 7.8$ Hz, 1H), 6.70 (dt, $J = 7.7$, 1.2 Hz, 1H), 6.64 (t, $J = 2.0$ Hz, 1H), 6.62–6.59 (m, 1H), 5.46 (d, $J = 7.5$ Hz, 1H), 5.18 (d, $J = 7.5$ Hz, 1H), 3.69 (s, 3H), 1.41 (s, 9H).

***tert*-Butyl (2-((2-aminoethyl)amino)-2-oxoethyl)carbamate (18).** To ethylenediamine (**16**; 13.5 mL, 200 mmol) at 0°C was added drop-wise a solution of *N*-Boc-glycine methyl ester (948 mg, 5 mmol) in methanol (20 mL). The reaction mixture was stirred at room temperature for 6 h and concentrated to an oil that was partitioned between water and dichloromethane. The combined organic extracts were dried and concentrated to an oil that was purified by flash chromatography eluting with ethyl acetate: methanol: triethylamine (50:50:1). Product fractions were concentrated to give **18** (544 mg, 50% yield) as an oil: ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.58 (br s, 1H), 5.23 (br s, 1H), 3.77 (d, $J = 5.8$ Hz, 2H), 3.31 (m, 2H), 2.83 (br s, 2H), 1.44 (s, 9H), 1.26 (s, 2H); MS (ESI): *m/z* 218.2 $(M+1)^+$.

***tert*-Butyl (2-((3-aminopropyl)amino)-2-oxoethyl)carbamate (19).** Reaction of 1,3-propanediamine (**17**; 16.8 ml, 200 mmol), as described for the synthesis of **18**, gave **19** (600 mg, 52% yield): ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.10 (br s, 1H), 5.29 (br s, 1H), 3.74 (d, $J = 5.8$ Hz, 2H), 3.35 (q, $J = 6.2$ Hz, 2H), 2.77 (t, $J = 6.3$ Hz, 2H), 1.75–1.60 (m, 4H), 1.42 (s, 9H); MS (ESI): *m/z* 232.1 $(M+1)^+$, 254.1 $(M+Na)^+$.

***tert*-Butyl (9-((3a*R*,4*R*,6*R*,6a*R*)-6-(11,11-dimethyl-6,9-dioxo-10-oxa-2,5,8-triazadodecyl)-2,2-dimethyltetrahydrofuro[3,4-*d*][1,3]dioxol-4-yl)-9*H*-purin-6-yl)carbamate (20)**. To a solution of **8** (203 mg, 0.5 mmol) in DCE (2 mL) at 0°C was added **18** (120 mg, 0.55 mmol) dissolved in DCE (1 mL), followed by sodium triacetoxyborohydride (148 mg, 0.7 mmol). The reaction mixture was stirred overnight at room temperature, quenched

with saturated aq. $NaHCO_3$, and distributed between dichloromethane and water. The combined organic extracts were dried and concentrated to an oil that was purified by flash chromatography eluting with dichloromethane: methanol (19:1). Product fractions were combined and concentrated to give **20** (130 mg, 43% yield): ^{1}H NMR (400 MHz, $CDCl_3$ + D_2O): δ 8.72 (s, 1H), 8.13 (s, 1H), 6.07 (s, 1H), 5.48–5.41 (m, 1H), 4.99 (s, 1H), 4.35 (s, 1H), 3.77 (d, J = 5.3 Hz, 2H), 3.34–3.21 (m, 2H), 2.92–2.60 (m, 4H), 1.61 (s, 3H), 1.56 (s, 9H), 1.42 (s, 9H), 1.39 (s, 3H).

***tert*-Butyl (9-((3aR,4R,6R,6aR)-6-(12,12-dimethyl-7,10-dioxo-11-oxa-2,6,-9-triazatridecyl)-2,2-dimethyltetrahydrofuro[3,4-d][1,3]dioxol-4-yl)-9*H* -purin-6-yl)carbamate (21)**. Reaction of **19** (127 mg, 0.55 mmol) with **8** followed by purification, as described for the synthesis of **20**, gave **21** (153 mg, 48% yield): ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.70 (s, 1H), 8.10 (s, 1H), 7.15 (d, J = 6.5 Hz, 1H), 6.09 (d, J = 2.5 Hz, 1H), 5.47–5.36 (m, 2H), 5.07 (dd, J = 6.4, 3.4 Hz, 1H), 4.50–4.47 (m, 1H), 3.68 (d, J = 5.8 Hz, 2H), 3.30 (d, J = 6.2 Hz, 2H), 3.20–3.08 (m, 2H), 2.81–2.71 (m, 2H), 1.80–1.70 (m, 2H), 1.60 (s, 3H), 1.55 (s, 9H), 1.42 (s, 9H), 1.37 (s, 3H).

2-Amino-*N*-(2-((((2*R*,3*S*,4*R*,5*R*)-5-(6-amino-9*H*-purin-9-yl) -3,4-dihydroxytetrahydrofuran-2-yl)methyl)amino)ethyl)acetamide (4). To a solution of **20** (130 mg, 0.21 mmol) in dichloromethane (0.8 mL) at 0°C was added water (0.2 mL) and TFA (1.1 mL). The mixture was stirred at room temperature overnight and concentrated to a residue that was dissolved in methanol: water (5:95) and purified by preparative reverse-phase HPLC (Restek Ultra C18, 5 μm, 150 × 21.2 mm column; flow rate of 10 mL/min using an isocratic elution of 2% acetonitrile in water for 10 min) followed by concentration and lyophilization of fractions yielding **4** (44 mg, 56% yield): ^{1}H NMR (400 MHz, DMSO-d_6 + D_2O): δ 8.47 (s, 1H), 8.31 (s, 1H), 5.97 (d, J = 5.4 Hz, 1H), 4.65 (t, J = 5.0 Hz, 1H), 4.23–4.19 (m, 2H), 3.51 (s, 2H), 3.46–3.30 (m, 4H), 3.08–2.99 (m, 2H); MS (ESI): m/z 367.1841 $(M+1)^+$.

2-Amino-*N*-(3-((((2*R*,3*S*,4*R*,5*R*)-5-(6-amino-9*H*-purin-9-yl) -3,4-dihydroxytetrahydrofuran-2-yl)methyl)amino)propyl)acetamide (5). To a solution of **21** (185 mg, 0.3 mmol) in dichloromethane (1.2 mL) at 0°C was added water (0.3 mL) and TFA (1.5 mL). The mixture was stirred at room temperature overnight and concentrated to a residue that was dissolved in methanol: water (5:95) and purified by preparative reverse-phase HPLC (Restek Ultra C18, 5 μm, 150 × 21.2 mm column; flow rate of 10 mL/min using an isocratic elution of 2% acetonitrile in water for 10 min) followed by concentration and lyophilization of fractions yielding **5** (62 mg, 54% yield): ^{1}H NMR (400 MHz, DMSO-d_6 + D_2O): δ 8.42 (s, 1H), 8.31 (s, 1H), 5.95 (d, J = 5.3 Hz, 1H), 4.63 (t, J = 5.0 Hz, 1H), 4.25–4.15 (m, 2H), 3.50 (s, 2H), 3.36 (dd, J = 13.2, 9.6 Hz, 1H), 3.24 (dd, J = 13.4, 2.8 Hz, 1H), 3.12 (t, J = 6.8 Hz, 2H), 2.90 (t, J = 7.7 Hz, 2H), 1.79–1.64 (m, 2H); MS (ESI): m/z 381.2000 $(M+1)^+$.

In vitro DOT1L Histone Methyltransferase Assay

Enzymatic activity of DOT1L was assessed by incubating 125 nM of recombinantly expressed and nickel affinity purified GST-DOT1L catalytic domain (1–420), with N-terminal 6x His-tag, in the presence of 125 nM (0.28 μCi) ^{3}H-S-adenosylmethionine (NET155250UC; Perkin Elmer), and 1 μg of calf thymus extracted core histones (H9250; Sigma-Aldrich) in histone methyltransferase (HMTase) buffer (20 mM Tris pH 7.9, 4 mM EDTA, 1 mM DTT, 0.01% Triton X-100) at a final volume of 26.5 μL. For determination of inhibitor potency, compounds were added to the reaction mixture prior to initiation of the HMTase reaction with ^{3}H-SAM. The HMTase reaction was allowed to proceed for 1 hr at room temperature and was stopped by transferring 5 μL of reaction mixture to P81 filter paper. After drying, filter papers were washed three times with 50 mM $NaHCO_3$ pH 9.0. Filter papers were then dried and radioactivity measured in vials with 10 mL liquid scintillation fluid on a Tri-Carb 2800 TR liquid scintillation counter (Perkin Elmer).

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