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Brief Article

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# Discovery of Leucyladenylate Sulfamates as Novel Leucyl-tRNA Synthetase (LRS)-targeted Mammalian Target of Rapamycin Complex 1 (mTORC1) Inhibitors

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**ABSTRACT:** Recent studies indicate that LRS may act as a leucine sensor for the mTORC1 pathway, potentially providing an alternative strategy to overcome rapamycin-resistance in cancer treatments. In this study, we developed leucyladenylate sulfamate derivatives as LRS-targeted mTORC1 inhibitors. Compound **18** selectively inhibited LRS-mediated mTORC1 activation and exerted specific cytotoxicity against colon cancer cells with a hyperactive mTORC1, suggesting that compound **18** may offer a novel treatment option for human colorectal cancer.

## ■ INTRODUCTION

Mammalian target of rapamycin (mTOR) is a multi-domain kinase that plays a central role in the regulation of cell growth, proliferation, metabolism and autophagy. mTOR consists of two structurally and functionally distinct protein complexes, mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2).[1] In particular, mTORC1 regulates protein synthesis by phosphorylating the two major substrates, S6 kinase 1 (S6K1) and the translational regulators eukaryotic translation initiation factor 4E (elF4E)-binding protein 1 (4E-BP1), both of which are known to play crucial roles in cell proliferation and tumorigenesis.[2-4] Therefore, much research effort has focused on the development of compounds that suppress the kinase activity of mTORC1 and act as anti-cancer agents.[5-7] The most studied compounds are rapamycin and its derivatives that directly bind near the mTOR kinase domain by forming a complex with the FK506-binding protein 12 (FKBP12); temsirolimus and everolimus have been approved by the FDA for the treatment of advanced renal cell carcinoma,[8, 9] and ridaforolimus showed a promising outcome in a recent clinical trial for advanced soft tissue and bone sarcoma.[10, 11] Unfortunately, the efficacy of these rapamycin analogs for the treatment of various cancer patients has been generally disappointing, mainly because these agents are cytostatic and only partially inhibit mTORC1 activity.[7, 12] Although rapamycin is a highly specific allosteric inhibitor of mTORC1, the mTORC1 pathway involves multiple regulatory mechanisms and complex feedback loops that have not yet been fully understood, thus resulting in an incomplete inhibition of kinase activity. Therefore, small molecules targeting other possible regulators may offer an alternative strategy for the suppression of mTORC1 activity, and have the potential to overcome rapamycin-resistance in cancer treatment.

mTORC1 activity is regulated by various environmental signals, such as the levels of nutrients, energy, and oxygen. Although the complete mechanism of how mTORC1 senses these signals still remains a mystery, the proteinogenic amino acid leucine is considered to be the master controller in amino acid-dependent mTORC1 signaling.[13, 14] More importantly, several recent studies have reported that leucyl-tRNA synthetase (LRS) may act as an intracellular leucine sensor by directly binding to RagD GTPase, one of the key mediators of the amino acid-dependent mTORC1 pathway.[15, 16] LRS is a member of the class I aminoacyl-tRNA synthetase (ARSs) family that catalyzes the ATP-dependent ligation of amino acids to cognate transfer RNA (tRNA) in protein biosynthesis. Because of their pivotal role in cell survival, ARSs have been effective targets for antibiotics to overcome bacterial resistance,[17-20] and also have been studied as anti-cancer agents.[21-23] In addition to its traditional role, LRS appears to participate in mTORC1 activation by acting as a GTPase-activating protein (GAP) for Rag GTPase in a leucine-dependent manner. Moreover, the leucine-induced activation of mTORC1 can be inhibited by leucine analogues, such as leucinol, without affecting the leucine charging ability of LRS, suggesting that LRS-targeted inhibitors can suppress mTORC1 activity.[15, 24, 25]

Previously, we have demonstrated that a leucine analog, (*S*)-4-isobutyloxazolidin-2-one, selectively inhibited downstream phosphorylation of mTORC1 by blocking the leucine-sensing ability of LRS.[26] Furthermore, this analog exhibited cytotoxicity against rapamycin-resistant colon cancer cells without affecting the catalytic activity of LRS, indicating that LRS has the potential to serve as a novel therapeutic target. In a contin-

uing effort to develop LRS-targeted mTORC1 inhibitors, we designed a new series of compounds based on the structure of aminoacyl adenylate **1**, an enzymatic reaction intermediate of ARSs (**Figure 1**). Aminoacyl adenylates have been extensively studied as a lead compound for ARS inhibitors.[27, 28] Specifically, replacing the hydrolytically unstable acylphosphate group with its isosteres, such as acylsulfamate, resulted in chemically stable and potent inhibitors with $IC_{50}$ values in the submicromolar to low micromolar range versus the corresponding ARSs.[29-31] Based on these previous findings, we synthesized a library of leucyladenylate sulfamates by modifying adenine (group 1), ribose (group 2) and leucine moieties (group 3) and evaluated their biological activity. When we tested these compounds by using immunoblots, we found that group 1 compounds with a lipophilic $R_2$ group generally showed good activity against mTORC1, while $R_2$ = I derivatives were most active. On the other hand, any modifications in the ribose moiety (group 2) lead to the loss of activity. Among the group 3 compounds, replacement of the α-amino group of leucine ($R_1$) with a hydroxyl group significantly enhanced inhibitory activity against mTORC1, while introduction of an additional hydroxyl group also resulted in good inhibitory effect. Among the more than 70 compounds that we tested, herein we report three representative compounds described in **Figure 1**. Each of these three compounds contains distinct structural features that are required for either LRS inhibition or LRS-mediated mTORC1 suppression. In this paper, we describe their syntheses, and inhibitory effects on the mTORC1 pathway together with their leucylation activity. We also evaluated cancer cell-specific cytotoxicity of these analogs in various types of cancer cells.

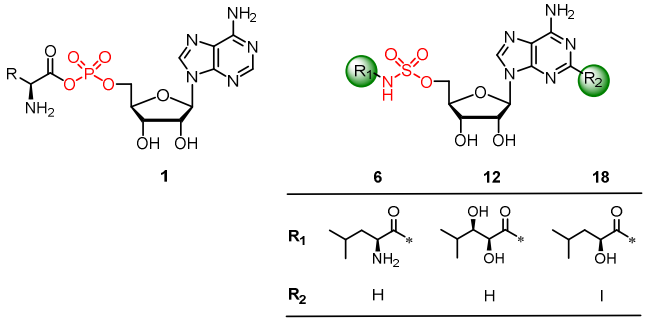


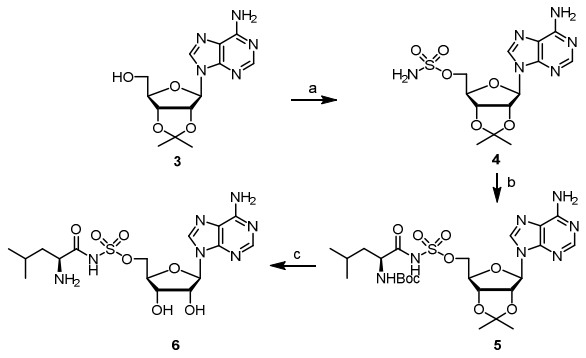

**Figure 1**. Amionacyl adenylate **1** and leucyladenylate sulfamates

## RESULTS AND DISCUSSION

**Chemistry.** Synthesis of leucyladenylate sulfamate **6** began with commercially available 2',3'-isopropylidene adenosine **3** as shown in **Scheme 1**. Compound **3** was reacted with sulfamoyl chloride prepared in a quantitative yield from chlorosulfonyl isocyanate according to previously described procedures.[32] Sulfamoylated adenosine **4** was then coupled with *N*-Boc-leucine to give the leucine adduct **5,** which was subsequently hydrolyzed to leucyladenylate sulfamate **6**.

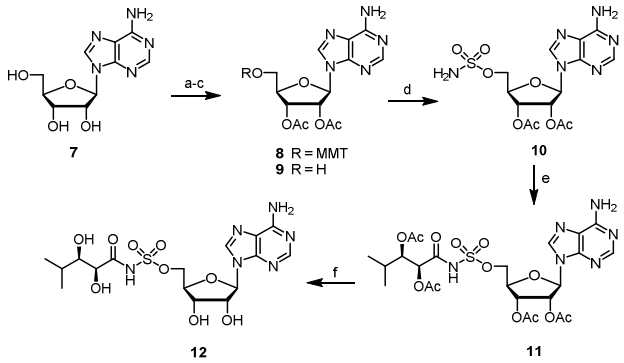
Synthesis of a *syn*-diol analog of leucyladenylate sulfamate **12** was carried out as illustrated in **Scheme 2**. 5'-*O*-Monomethoxytritylation (MMT) of compound **7** followed by acetylation of adenosine provided the fully protected adenosine **8**. Selective removal of MMT group and subsequent sulfamoylation produced the sulfamate intermediate **10**. (2*S*,3*R*)-dihydroxy-4-methylpentanoic acid (DMPA) protected by *O*-diacetyl was prepared from 4-methyl-2-pentenoic acid in 4 steps by Sharpless asymmetric dihydroxylation, and its configuration was confirmed by following previously described procedures.[33] Amide coupling between compound **10** and the protected chiral acid produced compound **11**, four acetyl groups of which were then removed in the presence of sodium methoxide to yield the final compound **12**.

**Scheme 1. Synthesis of compound 6**[a]



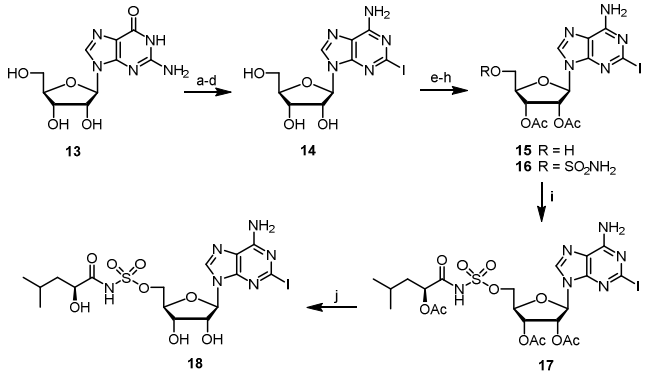

[a]*Reagents & conditions*: (a) i) NaH, THF, ii) $NH_2SO_2Cl$, THF; (b) *N*-Boc leucine, DCC, DMAP, $CH_2Cl_2$; (c) 80% aq. TFA.

**Scheme 2. Synthesis of compound 12** [a]




[a]*Reagents & conditions*: (a) MMTCl, pyridine, DMF; (b) $Ac_2O$, $NEt_3$, DMAP, $CH_3CN$; (c) 80% aq. AcOH; (d) i) NaH, THF, ii) $NH_2SO_2Cl$, THF; (e) chiral acid, DCC, DMAP, $CH_2Cl_2$; (f) NaOMe in MeOH.

**Scheme 3. Synthesis of compound 18** [a]




[a]*Reagents & conditions*: (a) $Ac_2O$, $NEt_3$, DMAP, $CH_3CN$; (b) $POCl_3$, $Et_4NCl$, $PhNMe_2$, $CH_3CN$; c) isoamyl nitrite, $I_2$, CuI, $CH_2I_2$, THF; (d) 7M $NH_3$ in MeOH; (e-j) the same conditions as (a-f) in scheme 2

Synthesis of a hydroxyl surrogate of leucyladenylate sulfamate **18** is shown in **Scheme 3**. 2-Iodoadenosine (**14**) was synthesized from guanosine (**13**) in 4 steps according to previ-

ously described procedures.[34] Compound **14** was converted to the corresponding 5'-*O*-sulfamoylated intermediate **16** by following the route described in **Scheme 2**. (2*S*)-hydroxyisocaproic acid (HICA, *L*-leucic acid) protected by *O*-acetyl was prepared by acetylation of commercially available *L*-leucic acid. A peptide coupling reaction between compound **16** and *O*-acetyl-*L*-leucic acid followed by deprotection of the acetyl group provided the final compound **18**.

**Biological Activity.** Because LRS catalyzes leucylation reaction with its cognate *t*RNA during protein synthesis, we first assessed the inhibitory effects of compounds **6**, **12** and **18** on the catalytic activity of LRS by performing aminoleucylation assays to determine the $IC_{50}$ values. Given that the previously reported acylsulfamate adenylates were found to be potent inhibitors of the corresponding ARSs, these compounds would be likely to exhibit inhibitory effects of LRS. As shown in **Figure 2**, compound **6** proved to be a potent inhibitor of LRS with an $IC_{50}$ value of 22.34 nM. However, compounds **12** and **18** inhibited LRS to a much lesser extent, showing 3- and 15-fold higher $IC_{50}$ values (70.04 nM and 337.1 nM, respectively) than compound **6**. Replacement of the α-amino group of leucine side chain with a hydroxyl group (**12**), and the introduction of a 2-iodo group to adenine (**18**) weakened the overall binding affinity toward the LRS active site. Moreover, considering fold-changes between compounds, we conclude that modification of the adenine moiety has a higher impact on the catalytic activity than the modification of the leucyl side chain.

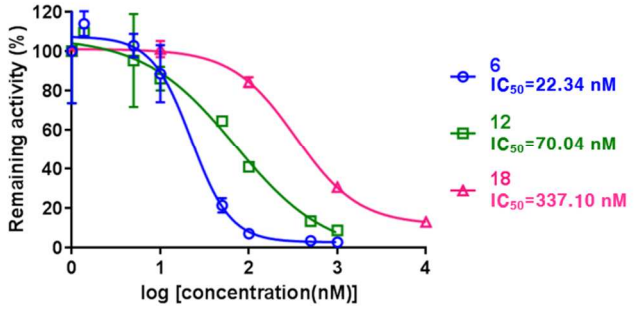



**Figure 2.** Inhibitory activities of compounds **6, 12** and **18** for catalytic leucylation.

Next, we determined the effects of compounds **6**, **12**, and **18** on leucine-induced mTORC1 activation using the immunoblotting method. In our previous study, pretreatment of leucinol analogs blocked leucine-induced phosphorylation of S6K, an mTORC1 substrate, by directly interacting with LRS[26]; because the newly designed compounds have additional adenosine moieties, we would expect to observe stronger inhibitory effects for S6K phosphorylation. We pretreated HEK293 cells with each compound at different concentrations as well as with rapamycin as a control at 100 nM, and then activated mTORC1 by treating the cells with leucine for 10 min. As shown in **Figure 3**, pretreatment of rapamycin blocked phosphorylation of S6K, whereas pretreatment of compound **6** did not affect S6K phosphorylation at all (**Figure 3A**). Notably, compounds **12** and **18** showed a dose-dependent inhibition of mTORC1 in HEK293 cells (**Figure 3B, 3C**) while compound **18** appeared to be more potent than compound **12**. Interestingly, these three sulfamates inhibited leucine-induced mTORC1 activation in the order of **18** > **12** > **6**, in contrast to the order of their inhibitory effects for the catalytic reaction (**6** > **12** > **18**). This apparently opposite trend suggests that LRS-mediated mTORC1 activation is independent of the leucylation activity of LRS on its cognate tRNA, which is again in agreement with previously reported observations.[15, 26] More interestingly, the side chain modifications of an α-hydroxyl group in the leucyl side chain and a 2-iodo group in the adenine, both of which adversely affected the catalytic activity of LRS, resulted in favorable effects for the mTORC1 inhibition

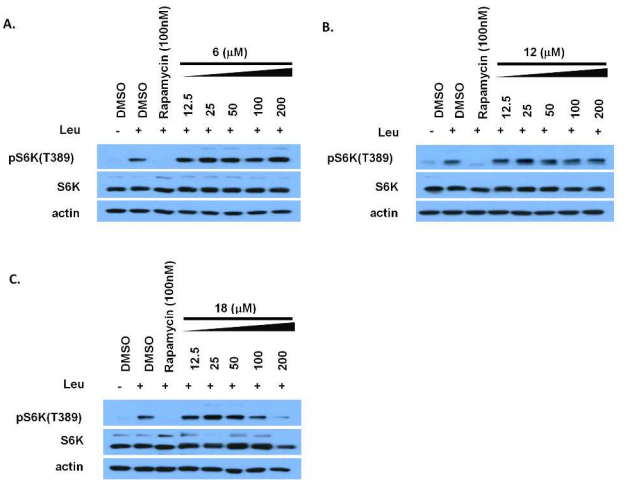



**Figure 3.** Dose-dependent inhibition of leucine-induced mTORC1 activation of compounds **6**, **12** and **18** in HEK293 cells

To further investigate the mechanisms of these sulfamates in LRS-mediated mTORC1 activation, we performed *in vitro* mTORC1 kinase assays using purified mTOR. As demonstrated in **Figure 4**, compounds **12** and **18** both did not inhibit the kinase activity of the purified mTOR at 200 μM, suggesting that these compounds are highly selective toward LRS, and did not act as an ATP-competitive inhibitor of the mTOR complex. We believe that these two sulfamates, **12** and **18**, inhibited the mTORC1 activity by selectively blocking the LRS-mediated mTORC1 activation pathway, rather than directly interacting with mTOR.

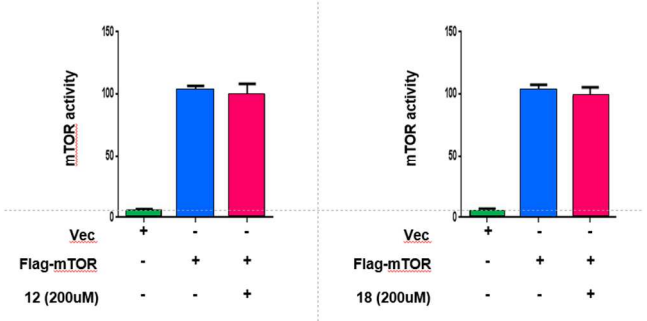



**Figure 4.** Effects of compounds **12** and **18** on the kinase activity of the purified mTOR.

Finally, to examine the anti-cancer activity of leucyladenylate sulfamates, we performed the sulforhodamine B (SRB) colorimetric assays for cytotoxicity.[35] We treated compounds **6**, **12**, and **18** with six different types of cancer cell lines together with etoposide as a positive control. To determine cancer cell selectivity, we also treated all four compounds with normal human lung epithelial cells (MRC-5), with the measured $IC_{50}$ values shown in **Table 1**. Compound **6**, the most

**Table 1. Relative cell growth inhibition of compounds 6, 12 and 18 for various cancer cell types and normal cells.** [a]

| $IC_{50}$ (μM) | A549 | HCT116 | K562 | MDA-MB-231 | SK-HEP-1 | SNU638 | MRC5 |
|---|---|---|---|---|---|---|---|
| **6** | 0.59 | 0.24 | 0.4 | 0.73 | 0.54 | 0.84 | 5.9 |
| **12** | 1.64 | 1.44 | 2.22 | 7.01 | 6.81 | 8.98 | >50 |
| **18** | 1.75 | 0.54 | 1.06 | 12.6 | 5.63 | 5.7 | >50 |
| Etoposide | 0.24 | 1.25 | 1.79 | 7.35 | 0.25 | 0.56 | 12.7 |

[a]A549: lung cancer cells, MDA-MB-231: breast cancer cells, SK-Hep-1: liver cancer cells, SNU638: stomach cancer cells, HCT116: colon cancer cells, K562: leukemia cells, MRC5: lung normal epithelial cell

potent LRS inhibitor, exhibited the greatest cytotoxicity that was even greater than that of etoposide in all types of cells, except A549 and SNU638. Given that LRS plays a crucial role in protein synthesis, it is not surprising that compound **6** is highly cytotoxic in both cancer cells and normal cells. In contrast, compounds **12** and **18** showed selective cytotoxicity against cancer cells. Specifically, compound **18** showed potent cytotoxicity against colon cancer cells (HCT116) and leukemia cells (K562) while exhibiting much less cytotoxicity against normal cells compared with compound **6** and etoposide. This result is particularly promising because several recent studies have reported that hyperactive mTORC1 is one of the distinctive features in human colorectal cancer.[36, 37] suggesting that compound **18** exerted colon cancer specific cytotoxicity by selective inhibition of mTORC1.

## ■ CONCLUSION

In this study, we developed leucyladenylate sulfamate derivatives that directly interact with LRS to inhibit the mTORC1 pathway. Compound **6** inhibited the catalytic activity of LRS but did not affect the leucine-induced mTORC1 activation, whereas compound **18** inhibited mTORC1 activation, while it also inhibited the catalytic activity of LRS to a much lesser degree compared to compounds **6** and **12**. Furthermore, both compounds **12** and **18** did not affect the kinase activity of the purified mTOR, indicating that the mTORC1-specific activity of these compounds arose from blocking the leucine-sensing ability of LRS rather than from interacting with mTOR directly. Cytotoxicity screening in various types of cancer cells and normal cells revealed that compound **6** showed the greatest cytotoxicity, probably due to a non-specific inhibition of LRS. Compounds **12** and **18** demonstrated cytotoxicity against all types of cancer cells but not against normal cells. Most notably, compound **18** exerted highly specific cytotoxicity against colon cancer cells that are known to have hyperactive mTORC1. We believe that compound **18** may serve as a useful tool to study the role of LRS in the mTORC1 pathway but may also offer a novel treatment option for human colorectal cancer.

## ■ EXPERIMENTAL SECTION

**General Methods.** All chemical reagents were commercially available. Melting points were determined on a Büchi Melting Point B-540 apparatus and are uncorrected. Silica gel column chromatography was performed on silica gel 60, 230-400 mesh, Merck. Nuclear magnetic resonance ($^1$H-NMR and $^{13}$C-NMR) spectra were recorded on JEOL JNM-LA 300 [300 MHz ($^1$H), 75 MHz ($^{13}$C)] and Bruker Avance 400 MHz FT-NMR [400 MHz ($^1$H), 100 MHz($^{13}$C)] spectrometers. Chemical shifts are reported in ppm units with $Me_4Si$ as a reference standard. Mass spectra were recorded on a VG Trio-2 GC-MS and 6460 Triple Quad LC/MS. All final compounds were purified to >95% purity, as determined by high-performance liquid chromatography (HPLC). HPLC was performed on an Agilent 1120 Compact LC (G4288A) instrument using an Agilent Eclipse Plus C18 column (4.6 x 250 mm, 5 $\mu$m) and a Daicel Chiralcel OD-H column (4.6 x 250 mm, 5 $\mu$m).

((3aR,4R,6R,6aR)-6-(6-amino-9H-purin-9-yl)-2,2-dimethyltetrahydrofuro[3,4-d][1,3]dioxol-4-yl)methyl sulfamate (4). Compound 4 was prepared by following the reported procedure.[32] Yield 69%, white solid; $^1$H-NMR (300MHz, $CD_3OD$) δ 8.27 (s, 1H, CH), 8.22 (s, 1H, CH), 6.24 (d, 1H, CH, *J* = 2.55 Hz), 5.42 (dd, 1H, CH, *J* = 6.24 Hz, 2.58 Hz), 5.13 (dd, 1H, CH, *J* = 6.21 Hz, 2.73 Hz), 4.51 (m, 1H, CH), 4.32 (dd, 1H, CH, *J* = 10.62 Hz, 4.59 Hz), 4.24 (dd, 1H, CH, *J* = 10.62 Hz, 5.31 Hz), 1.60 (s, 3H, $CH_3$), 1.39 (s, 3H, $CH_3$)

((3aR,4R,6R,6aR)-6-(6-amino-9H-purin-9-yl)-2,2-dimethyltetrahydrofuro[3,4-d][1,3]dioxol-4-yl)methyl ((tert-butoxy carbonyl)-L-leucyl)sulfamate (5). To a solution of compound 4 (0.54 mmol) in anhydrous MC (25 mL) was added *N*-Boc leucine (0.81 mmol) and DMAP (0.01 mmol) at 0$^o$C. 1M DCC in MC (0.81 mmol) was added dropwise, stirred for 2 h at room temperature. The solution was filtered on celite pad, washed with EtOAc (20 mL), and then concentrated. The filtrate was purified by column chromatography over silica gel (EtOAc:MeOH=10:1) to give the compound 5 (0.46 mmol). Yield 85%, white solid; $^1$H-NMR (300MHz, $CD_3OD$) δ 8.47 (s, 1H, CH), 8.21 (s, 1H, CH), 6.22 (d, 1H, CH, *J* = 3.30 Hz), 5.33 (dd, 1H, CH, *J* = 5.88 Hz, 3.48 Hz), 5.10 (d, 1H, CH, *J* = 5.67 Hz), 4.53 (m, 1H, CH), 4.23 (d, 2H, $CH_2$, *J* = 3.48 Hz), 4.06 (m, 1H, CH), 1.69 (m, 1H, CH), 1.60 (s, 3H, $CH_3$), 1.52 (m, 2H, $CH_2$), 1.43 (s, 9H, $CH_3$), 1.40 (s, 3H, $CH_3$), 0.93 (dd, 6H, $CH_3$, *J* = 6.60 Hz, 2.76 Hz)

((2R,3S,4R,5R)-5-(6-amino-9H-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl (L-leucyl) sulfamate trifluoroacetate salt (6). The compound 5 (0.38 mmol) was dissolved in 80% aquous TFA (2mL) and stirred for 2 h at room temperature. The reaction mixture was evaporated and washed with EtOAc (20 mL). Aqueous layer was concentrated under reduced pressure to give crude pale yellow solid. The solid was purified by ion-exchange resin (HP20SS) to give the compound 6 (0.16 mmol). Yield 42%, white solid; $^1$H-NMR (500MHz, $CD_3OD$) δ 8.49 (s, 1H, CH), 8.20 (s, 1H, CH), 6.07 (d, 1H, CH, *J* = 5.30 Hz), 4.62 (t, 1H, CH, *J* = 5.10 Hz), 4.39-4.29 (m, 4H, , 2 CH, $CH_2$), 3.61 (dd, 1H, CH, *J* = 8.50 Hz, 4.85 Hz), 1.78 (m, 2H, $CH_2$), 1.57 (m, 1H, CH), 0.95 (dd, 6H, $CH_3$, *J* = 15.95 Hz, 6.00 Hz); HRMS-FAB *m/z* $[M+H]^+$ $C_{16}H_{25}N_7O_7SH^+$ calcd 460.1536, Found: 460.4898.

(2R,3R,4R,5R)-2-(6-amino-9H-purin-9-yl)-5-(((4-methoxyphenyl) diphenylmethoxy)methyl)tetrahydrofuran-3,4-diyl diacetate (8). 5'-O-Monomethoxytrityl (MMT) protected adenosine (2.27 mmol) was prepared by following the reported procedure.[38] To a solution of 5'-O-MMT protected adenosine (2.05 mmol) in acetonitrile (50 mL) at 0$^o$C was added DMAP (0.21 mmol), TEA (6.15 mmol) and acetic anhydride (6.15 mmol). The mixture was stirred for 4hr at room temperature. Aqueous portion was extracted with EtOAc (50 mL). The organic phase was dried over $MgSO_4$ and concentrated, which was purified by column chromatography (EtOAc:MeOH = 40:1, v/v) to give compound 8 (1.6 mmol). Yield 78%, white solid; $^1$H-NMR (300MHz, $CDCl_3$) δ 8.01 (S, 1H, CH), 7.90 (S, 1H, CH), 7.42 (m, 4H, Ar), 7.32 (m, 4H, Ar), 7.25 (m, 4H, Ar), 6.18 (m, 4H, Ar), 6.26 (d, 1H, CH, *J* = 6.6 Hz), 6.07 (t, 1H, CH, *J* = 5.3 Hz), 5.67 (dd, 1H, CH,

*J* = 5.1 Hz, 3.1 Hz), 4.31 (m, 1H, CH), 3.78 (S, 3H, $CH_3$), 3.46 (m, 1H, CH), 2.10 (S, 3H, $CH_3$), 2.05 (s, 3H, $CH_3$)

(2R,3R,4R,5R)-2-(6-amino-9H-purin-9-yl)-5-(hydroxymethyl) tetrahydrofuran-3,4-diyl diacetate (9). 80% aquous AcOH (100 mL) was slowly added to compound 8 (1.52 mmol) and the reaction mixture was stirred for 12 h at room temperature. The mixture was evaporated, neutralized with $NaHCO_3$ and extrated with EtOAc (150 mL x 2). Organic layer was combined, dried over $MgSO_4$ and evaporated. The residue was purified by column chromatography (EtOAc:MeOH = 20:1, v/v) to give compound 9 (0.97 mmol). Yield 64%, white solid; $^1$H-NMR (300MHz, $CDCl_3$) δ 8.32 (s, 1H, CH), 7.85 (S, 1H, CH), 6.26 (S, 2H, $NH_2$), 6.03 (d, 2H, $CH_2$, *J* = 2.0 Hz), 5.70 (m, 1H, CH), 4.37 (s, 1H, CH), 3.99 (dd, 1H, CH, *J* = 13.0 Hz, 1.4 Hz), 3.86 (d, 1H, CH, *J* = 12.3 Hz), 2.18 (s, 3H, $CH_3$), 2.02 (s, 3H, $CH_3$)

(2R,3R,4R,5R)-2-(6-amino-9H-purin-9-yl)-5-((sulfamoyloxy) methyl)tetrahydrofuran-3,4-diyl diacetate (10). Compound 10 was prepared by following the procedure described for compound 4. Yield 93%, white solid; $^1$H-NMR (300MHz, $CDCl_3$) δ 8.34 (s, 1H, CH), 8.00 (s, 1H, CH), 6.16 (d, 1H, CH, *J* = 6.1 Hz), 5.91 (t, 1H, CH, *J* = 5.9 Hz), 5.72 (m, 1H, CH), 2.16 (s, 3H, $CH_3$), 2.07 (s, 3H, $CH_3$)

(2R,3R,4R,5R)-2-(6-amino-9H-purin-9-yl)-5-(((N-((2S,3R)-2,3-diacetoxy-4-methylpentanoyl)sulfamoyl)oxy)methyl)tetrahydrofuran-3,4-diyl diacetate (11). Compound 11 was prepared by following the procedure described for compound 5. Yield 79%, white solid; $^1$H-NMR (300MHz, $CD_3OD$) δ 8.47 (s, 1H, CH), 8.34 (s, 1H, CH), 6.29 (d, 1H, CH, *J* = 5.3 Hz), 5.85 (t, 1H, CH, *J* = 5.49 Hz), 5.68 (dd, 1H, CH, *J* = 5.67 Hz, 4.0 Hz), 5.14 (dd, 1H, CH, *J* = 8.8 Hz, 2.7 Hz), 5.07 (d, 1H, CH, *J* = 2.7 Hz), 4.52 (m, 2H, CH, NH), 3.68 (s, 1H, CH), 2.14 (s, 3H, $CH_3$), 2.03 (s, 3H, $CH_3$), 2.01 (s, 3H, $CH_3$), 2.04 (m, 1H, CH), 0.94 (d, 3H, $CH_3$, *J* = 2.5 Hz), 0.91 (d, 3H, $CH_3$, *J* = 2.4 Hz)

((2R,3S,4R,5R)-5-(6-amino-9H-purin-9-yl)-3,4-dihydroxytetra hydrofuran-2-yl)methyl ((2S,3R)-2,3-dihydroxy-4-methylpentanoyl) sulfamate (12). Compound 11 (0.056 mmol) was dissolved in 0.02 M sodium methoxide solution in methanol (3 mL) and stirred for 2 h at room temperature. DOWEX 50WX8 hydrogen form resin (10 mg) was added in portions, filtered and concentrated to afford compound 12 (0.03 mmol). Yield 56%, white solid; $^1$H-NMR (600MHz, $CD_3OD$) δ 8.52 (s, 1H, CH), 8.18 (s, 1H, CH), 6.08 (d, 1H, CH, *J* = 2.8 Hz), 4.64 (t, 1H, CH, *J* = 2.5 Hz), 4.40 (m, 1H, CH), 4.31 (m, 3H, CH), 4.05 (d, 1H, CH, *J* = 1.0 Hz), 3.51 (dd, 1H, CH, *J* = 4.4 Hz, 1.0 Hz), 1.86 (m, 1H, CH), 1.01 (d, 3H, $CH_3$, *J* = 3.2 Hz), 0.94 (d, 3H, $CH_3$, *J* = 3.2 Hz); HRMS-ESI *m/z* $[M+H]^+$ $C_{16}H_{24}N_6O_9SH^+$ calcd 477.1325, Found: 477.1395.

(2R,3R,4S,5R)-2-(6-amino-2-iodo-9H-purin-9-yl)-5-(hydroxyl methyl)tetrahydrofuran-3,4-diol (14). Compound 14 was prepared by following the reported procedure.[34] Yield 11% in 4 steps, yellow solid; $^1$H-NMR (300MHz, $CD_3OD$) δ 8.20 (s, 1H, CH), 5.89 (d, 1H, CH, *J* = 6.03 Hz), 4.66 (t, 1H, CH, *J* = 5.31 Hz), 4.30 (dd, 1H, CH, *J* = 5.10 Hz, 3.09 Hz), 4.15-4.12 (m, 1H, CH), 3.88 (dd, 1H, CH, *J* = 12.45 Hz, 2.73 Hz), 3.74 (dd, 1H, CH, *J* = 12.45 Hz, 2.94 Hz), 2.18 (s, 3H, $CH_3$), 2.14 (s, 3H, $CH_3$), 2.11 (s, 3H, $CH_3$)

(2R,3R,4R,5R)-2-(6-amino-2-iodo-9H-purin-9-yl)-5-(hydroxy methyl)tetrahydrofuran-3,4-diyl diacetate (15). Compound 15 was prepared by following the procedure described for compounds 8 and 9. Yield 67% in 3 steps, colorless oil; $^1$H-NMR (300MHz, $CDCl_3$) δ 7.77 (s, 1H, CH), 6.47 (br, 1H, OH), 5.97 (d, 1H, CH, *J* = 7.68 Hz), 5.91 (m, 1H, CH), 5.67 (dd, 1H, CH, *J* = 5.13 Hz, 1.29 Hz), 4.36 (d, 1H, CH, *J* = 1.08 Hz), 4.51 (dd, 1H, CH, *J* = 13.02 Hz, 1.47 Hz), 3.88 (d, 1H, CH, *J* = 11.73 Hz), 2.17 (s, 3H, $CH_3$), 2.11 (s, 3H, $CH_3$)

(2R,3R,4R,5R)-2-(6-amino-2-iodo-9H-purin-9-yl)-5-((sulfamoyl oxy)methyl)tetrahydrofuran-3,4-diyl diacetate (16). Compound 16 was prepared by following the procedure described for compound 10. Yield 86%, white solid; $^1$H-NMR (300MHz, $CDCl_3$) δ 7.93 (s, 1H, CH), 6.14 (d, 1H, CH, *J* = 5.49 Hz), 5.84 (br, 2H, $NH_2$), 5.78 (t, 1H, CH, *J* = 5.31 Hz), 5.71 (t, 1H, CH, *J* = 4.02 Hz), 4.53 (d, 2H, $CH_2$, *J* = 3.48 Hz), 4.47 (m, 1H, CH), 2.16 (s, 3H, $CH_3$), 2.09 (s, 3H, $CH_3$)

(2R,3R,4R,5R)-2-(((N-((S)-2-acetoxy-4-methylpentanoyl)sulfamoyl)oxy)methyl)-5-(6-amino-2-iodo-9H-purin-9-yl)tetrahydrofuran-3,4-diyl diacetate (17). Compound 17 (0.03 mmol) was prepared by following the procedure described for compound 11. Yield 81%, white solid; $^1$H-NMR (300MHz, $CDCl_3$) δ 8.31 (s, 1H, CH), 6.28 (d, 1H, CH, *J* = 6.69 Hz), 5.75 (m, 1H, CH), 5.54 (m, 1H, CH), 4.70 (m, 1H, CH), 4.42-4.34 (m, 2H, $CH_2$), 2.14 (s, 3H, $CH_3$), 1.98 (s, 6H, $CH_3$), 1.64 (m, 2H, $CH_2$), 1.50 (m, 1H. CH), 0.79(dd, 6H, $CH_3$, *J* = 13.17 Hz, 6.60 Hz)

((2R,3S,4R,5R)-5-(6-amino-2-iodo-9H-purin-9-yl)-3,4-dihydroxy tetrahydrofuran-2-yl)methyl ((S)-2-hydroxy-4-methylpentanoyl) sulfamate (18). Compound 18 was prepared by following the procedure described for compound 12. Yield 67%, white solid; $^1$H-NMR (500MHz, $CD_3OD$) δ 8.26 (s, 1H, CH), 5.98 (d, 1H, CH, *J* = 4.90 Hz), 4.55 (t, 1H, CH, *J* = 4.85 Hz), 4.50 (dd, 1H, CH, *J* = 11.20 Hz, 2.80 Hz), 4.44 (dd, 1H, CH, *J* = 11.2 Hz, 3.40 Hz), 4.36 (t, 1H, CH, *J* = 4.70 Hz), 4.28 (q, 1H, CH, *J* = 3.60 Hz), 4.03 (t, 1H, CH, *J* = 6.30 Hz), 1.83-1.79 (m, 1H, CH), 1.52-1.47 (m, 2H, $CH_2$), 0.90 (dd, 6H, $CH_3$, *J* = 6.60 Hz, 2.25 Hz); HRMS-ESI *m/z* $[M+H]^+$ $C_{16}H_{23}IN_6O_8SH^+$ calcd 587.0343, Found 587.0410.

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### Notes

The authors declare no competing financial interest.

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## ABBREVIATIONS

mTOR, mechanistic target of rapamycin; mTORC1, mTOR complex 1; LRS, leucyl-tRNA synthetase; S6K1, S6 kinase 1; MMT, monomethoxytrityl; DMF, dimethyl formamide; THF, tetrahydrofuran; MC, methylene chloride; DCC, dicyclohexylcarbodiimide; DMAP, dimethylaminopyridine;

## ASSOCIATED CONTENT

### Supporting Information

HPLC purities of all final compounds. Molecular formula strings. This material is available free of charge via the Internet at http://pubs.acs.org.

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