Article

Not peer-reviewed version

Synthesis of New DltA Inhibitors and Their Application as Adjuvant Antibiotics to Re-Sensitize Methicillin Resistant Staphylococcus aureus

David Leparfait , Alexandre Mahé , Xiao Feng , Delphine Coupri , Fabien Le Cavelier , Nicolas Verneuil , Emmanuel Pfund * , Aurélie Budin-Verneuil , Thierry Lequeux *

Posted Date: 22 May 2025

doi: 10.20944/preprints202505.1714.v1

Keywords: Nucleoside analogs; DltA inhibitor; antibiotic resistance

 doi:10.20944/preprints202505.1714.v1

Article

Synthesis of New DltA Inhibitors and Their Application as Adjuvant Antibiotics to Re-Sensitize Methicillin Resistant *Staphylococcus aureus*

David Leparfait [1], Alexandre Mahé [2], Xiao Feng [1], Delphine Coupri [2], Fabien Le Cavelier [1], Nicolas Verneuil [2], Emmanuel Pfund [1,*], Aurélie Budin-Verneuil [2] and Thierry Lequeux [1,*]

[1] ENSICAEN, Université de Caen Normandie, Univ Rouen Normandie, INSA Rouen Normandie, CNRS, Institut CARMeN UMR 6064, 14050 Caen, France

[2] CBSA UR 4312, Université de Caen Normandie, F-14000 Caen, Normandie, France

* Correspondence: emmanuel.pfund@ensicaen.fr (E.P.); thierry.lequeux@ensicaen.fr (T.L.)

Abstract: The synthesis of new acyclic and cyclic series of D-Ala-AMP analogues was reported. Chemical modifications were introduced on the carbohydrate, the sulfamate linker and/or the amino-acid *N*-terminal moiety in order to increase *in vivo* stability and cell permeability. These new compounds were evaluated *in vitro* as DltA inhibitors and also *in vivo* as adjuvant antibiotics to re-sensitize methicillin resistant Staphylococcus aureus. Indeed, we showed that compounds **8**, **9**, **18**, **27**, **28**, **35** and **36**, containing modifications onto the carbohydrate or the linker, had moderate to excellent IC_{50} values. We also showed that **18** and **27** were able to efficiently re-sensitize MRSA to imipenem. Quantification of D-alanyl esters confirmed that these two compounds reduced the level of bacterial cell wall D-alanyl residues by 50% and 80%.

Keywords: nucleoside analogs; DltA inhibitor; antibiotic resistance

1. Introduction

Antibiotics represent one of the most significant medical discoveries of the 20th century, revolutionizing the treatment of bacterial infections and saving countless lives worldwide for several decades [1]. Antibacterial agents were initially designed to either kill bacteria or inhibit their growth by counteracting essential biological functions such as cell wall synthesis, DNA replication and protein synthesis. However, these modes of action exert a selective pressure that modifies the human microbiome and induces antimicrobial resistances (AMR) [2]. As a result, inappropriate utilization and overuse of antibiotics in the past, have accelerated the emergence of antibiotic-resistant bacterial strains, rendering previously effective treatments obsolete, and posing grave threats to public health [3]. This phenomenon was further amplified with the COVID-19 pandemic where, in addition to the increased use of antibacterial cleaning products [4], approximatively 75% of patients were treated with antibiotics although bacterial co-infection was estimated at 8.6% [5]. In 2019, 4.95 million deaths worldwide were associated with bacterial resistance to antibiotics, including 1.27 million directly attributable to AMR, positioning it as a major global burden of diseases ahead of HIV and malaria [6]. The development of new therapeutic approaches involving active compounds towards new targets remains an important challenge and is an emergency requirement for human care. In this field, adjuvant antibiotics have emerged and represent a promising way to fight AMR infections [7]. These molecules are designed to improve antibiotic actions or re-sensitize pathogens to drugs from which they have become resistant, by targeting the antibiotic resistance mechanism. D-alanylation of teichoic acids serves as an effective strategy for Gram-positive bacteria to develop antibioresistance [8]. This process, catalyzed by five proteins (DltXABCD) encoded by the *dlt* operon [9], transfers a D-alanine residue onto the cell wall, altering its net charge and permeability, thereby reducing susceptibility to cationic antimicrobial peptides and glycopeptides such as vancomycin (Figure 1) [10].

 doi:10.20944/preprints202505.1714.v1

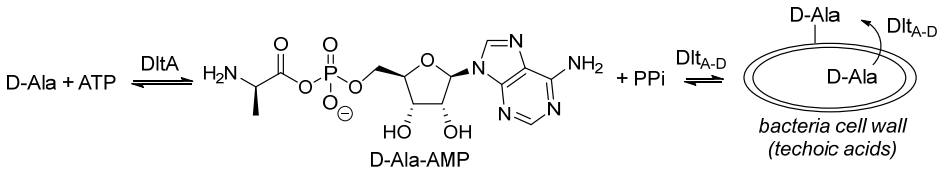


Figure 1. D-alanylation of cell wall teichoic acids.

Indeed, nucleoside **1**, designed as a D-Ala-AMP analog, was reported as the sole DltA inhibitor (Ki = 0.2 mM) and found to be efficient as an adjuvant to inhibit the growth of *Bacillus subtilis*, a non-pathogenic bacterium, in the presence of vancomycin [11]. We recently showed that compound **1** was also able to counteract the antibiotic resistance of methicillin resistant *Staphylococcus aureus* (MRSA) and β-lactam resistant *enterococci* including vancomycin resistant strains when co-administered with the antibiotics [12]. However, high concentrations (500 mM to 1 mM) of inhibitor **1** were needed suggesting that nucleoside **1** may suffer from a low metabolic stability and/or a weak cellular uptake. As a result, the design of new DltA inhibitors with improved stability and cell permeability still deserves to be developed for clinical use. In this context, synthesis of stable analogues of D-Ala-AMP, in which D-Alanine is attached to the nucleoside by a non-scissile bond in order to prevent its transfer and block the D-alanylation process, is of particular interest. In this paper, we report the synthesis, molecular docking and biological studies of new series of nucleosides derived from inhibitor **1** through chemical modifications of the carbohydrate, the sulfamate linker and/or the amino-acid *N*-terminal moiety (Figure 2).

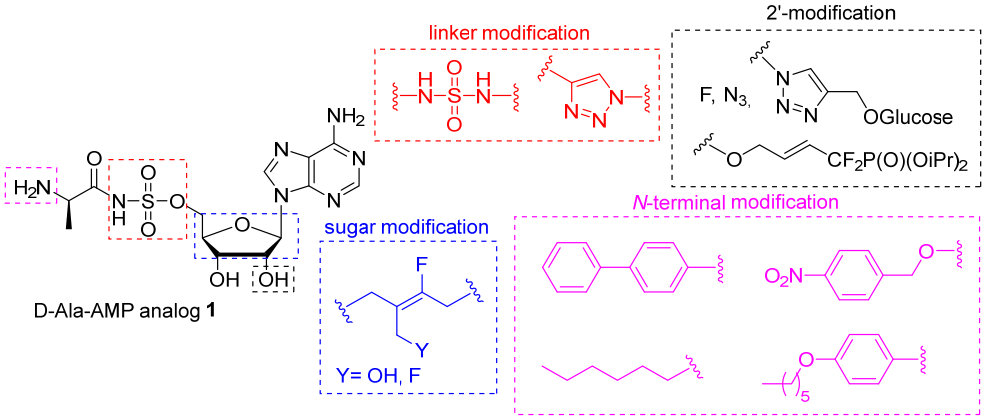


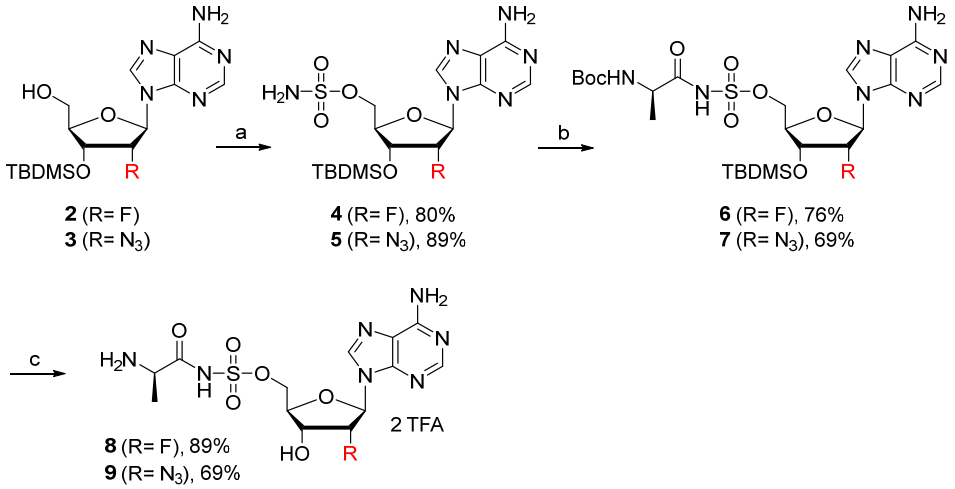
Figure 2. Chemical modifications envisaged for D-Ala-AMP.

2. Results and Discussion

2.1. Chemistry

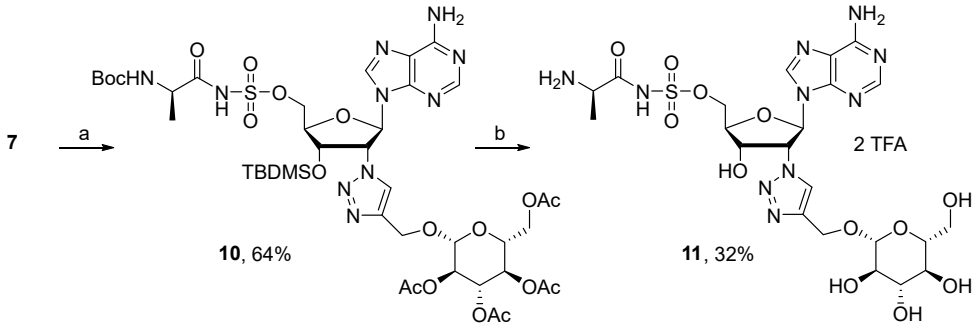
The introduction of electron-withdrawing groups onto the 2′-position of nucleosides is well known to increase the *N*-glycosidic bond stability. This chemical modification was first considered. Indeed, 2′-fluoro and 2′-azido *N*-acylsulfamate adenosine **8**-**9** were prepared according to the procedure reported by Herdewijn (Scheme 1) [13]. Known compounds **2** and **3** were reacted with NH_2SO_2Cl affording the corresponding *N*-sulfamoyl derivatives **4**-**5** in 80% and 89% yield, respectively. The desired nucleosides **8** and **9** were finally obtained in two-steps involving a coupling reaction with Boc-D-Ala-OSu followed by amine and alcohol deprotection.

 doi:10.20944/preprints202505.1714.v1



Scheme 1. Reagents and conditions: (a) i) NH_2SO_2Cl, DMA, MeCN, 20 °C, 2 h. ii) Et_3N, MeOH, 0 °C, 10 min. (b) Boc-D-Ala-OSu, DBU, DMF, 20 °C, 48 h for **6** and 16 h for **7**. (c) TFA, H_2O, CH_2Cl_2, 20 °C, 5 h for **8** and 12 h for **9**.

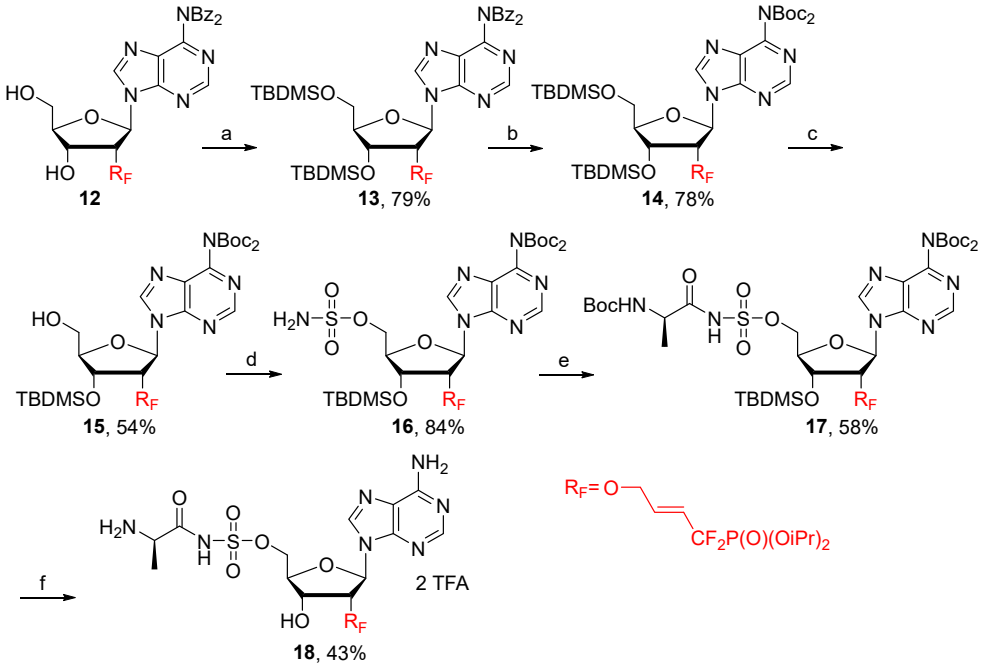
To facilitate cellular uptake using specific channels such as GLUT proteins [14], incorporation of a glucose moiety onto the 2′-position through a triazolyl linker was next envisaged (Scheme 2). D-glucose was introduced *via* a 1,3-dipolar cycloaddition reaction between **7** and 1-*O*-propargyl-2,3,4,-tetra-*O*-acetyl-D-glucopyranose. After 24 h stirring at 20 °C in *t*-BuOH/H_2O in the presence of $CuSO_4$ and sodium ascorbate, triazole **10** was isolated in 64% yield. Finally, additional treatment with TFA, sodium methoxide and $Et_3N.3HF$ led to the formation of the desired glucosyl-derived adenosine **11** in 32% yield over 3 steps.



Scheme 2. Reagents and conditions: (a) 1-*O*-propargyl-2,3,4,-tetra-*O*-acetyl-D-glucopyranose, $CuSO_4$, NaAsc, *t*-BuOH, H_2O, 20 °C, 24 h. (b) i) TFA, H_2O, 20 °C, 12 h. ii) MeONa, MeOH, 20 °C, 1 h. iii) $Et_3N.3HF$, THF, 20 °C, 12 h.

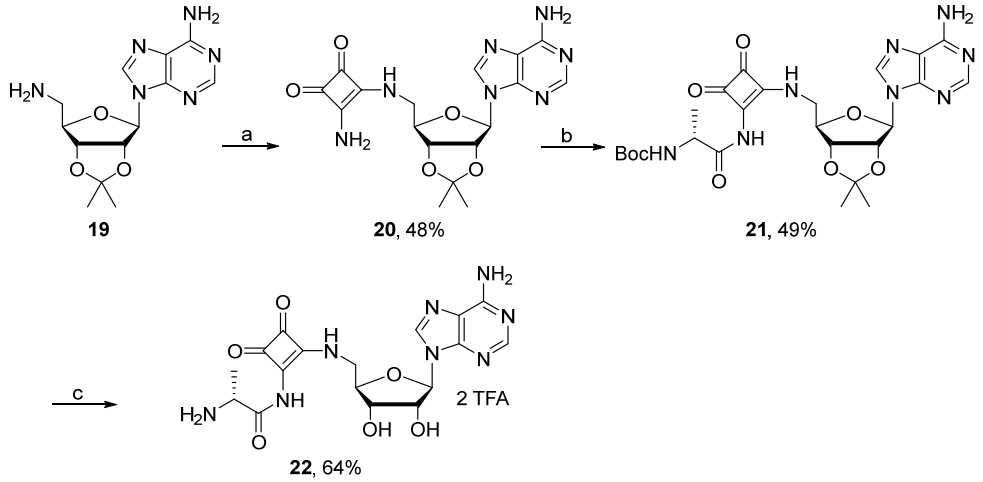
The last 2′-modification realized was the introduction of a difluorophosphonylated allylic ether moiety that has already been used by our group to improve the metabolic stability and lipophilicity of homouridylates [15]. The synthesis of the corresponding fluorinated *N*-acylsulfamate adenosine **18** was accomplished in 6 steps starting from **12** [16] (Scheme 3). Nucleoside **13**, easily obtained from **12**, was first treated with methylamine followed by an excess of Boc_2O to furnish **14** in 78% yield. After selective 5′-deprotection occurring in TFA/H_2O, the corresponding alcohol **15** was sulfamoylated with NH_2SO_2Cl and then engaged in a coupling reaction with Boc-D-Ala-OSu in the presence of DBU to afford **17** that was converted into the desired nucleoside **18** after a final deprotection conducted with TFA.

 doi:10.20944/preprints202505.1714.v1



Scheme 3. Reagents and conditions: (a) TBDMSCl, DMAP, Imidazole, DMF, 20 °C, 24 h. (b) i) $MeNH_2$, EtOH, 20 °C, 30 min. ii) $(Boc)_2O$, Et_3N, DMAP, DMF, 20 °C, 12 h. (c) AcOH, H_2O, THF, 20 °C, 64 h. (d) i) NH_2SO_2Cl, DMAc, MeCN, 20 °C, 2 h. ii) NEt_3, MeOH, 0 °C, 10 min. (e) Boc-D-Ala-OSu, DBU, DMF, 20 °C, 24 h. (f) TFA, H_2O, CH_2Cl_2, 20 °C, 24 h.

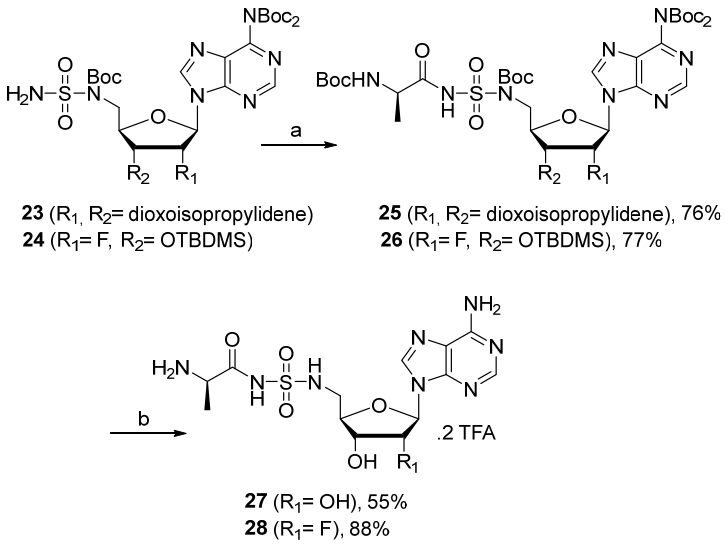
It was noticed that *N*-acylsulfamate adenosines could easily decompose into their corresponding cyclonucleosides *via* a nucleophilic substitution between the adenine N^3-atom and the 5′-sulfamoyl group [17]. To avoid this competitive reaction, the bridge modification was investigated. Substitution of the acylsulfamate linkage by a stable isosteric acylphosphate mimic such as acylsquaramide was first envisaged (Scheme 4). Indeed, squaramide **20**, easily obtained from **19** under standard conditions [18], was treated with activated alanylester in the presence of DBU. After 4 h stirring at 60 °C in DMF, nucleoside **21** was isolated in 49% yield. Additional treatment with TFA produced the ammonium salt **22** in good yield.



Scheme 4. Reagents and conditions: (a) i) Dimethyl squarate, MeOH, 20 °C, 4 h. ii) NH_3, MeOH, 20 °C, 8 h. (b) Boc-D-Ala-OSu, DBU, DMF, 60 °C, 4 h. (c) TFA, H_2O, CH_2Cl_2, 20 °C, 5 h.

 doi:10.20944/preprints202505.1714.v1

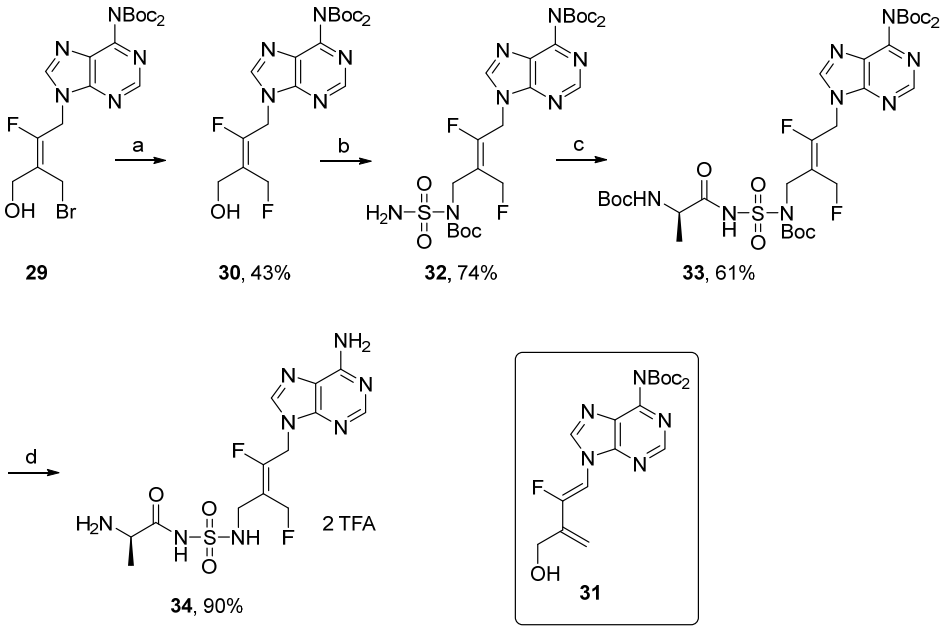
Substitution of the sulfamate oxygen with a nitrogen leading to the more stable corresponding acylsulfamide derivatives [19], was also considered (Scheme 5). Compound **23** was reacted with Boc-D-Ala-OSu in the presence of DBU to produce, after 16 h stirring in DMSO, nucleoside **25** in 76% yield. Under the same conditions, the reaction with **24** was slower and reached completion after 48 h. In this case, 2′-fluoroadenosine **26** was isolated in 77% yield. *N*-acylsulfamide adenosine **27** and 2′-fluoro *N*-acylsulfamide adenosine **28** were finally obtained after Boc and TBDMS deprotection with TFA.



Scheme 5. Reagents and conditions: (a) Boc-D-Ala-OSu, DBU, DMF, 20 °C, 16 h for **25** and 48 h for **26**. (b) TFA, H_2O, CH_2Cl_2, 20 °C, 5 h for **27** and 10 h for **28**.

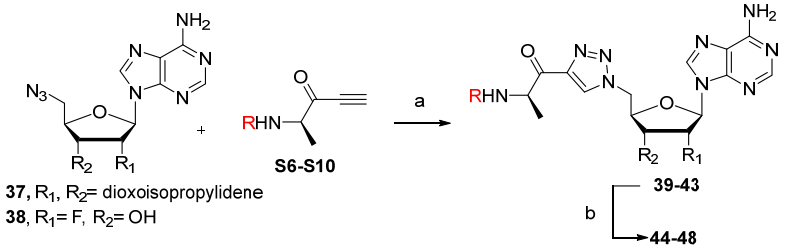
Agrofoglio showed that acyclonucleosides containing a transbutenyl motif were recognized by kinase as nucleoside mimic and we recently reported the fluorinated transbutenyl moiety can be used as stable nucleoside sugar surrogates [20], synthesis of *N*-acylsulfamides **34** was investigated to access to a mimic of fluorinated nucleoside **8** (Scheme 6). The synthesis started from bromo-alcohol **29**, easily obtained by oxetane ring-opening reaction. This later was first treated with cesium fluoride to give the corresponding acyclonucleoside **30** in 17% yield. In this case, a competitive elimination reaction occurred leading to diene **31** as major product. To overcome this limitation, *tert*-butanol was used as solvent in order to balance the basic character of the fluoride ion and emphasize its nucleophilicity [21]. Under these conditions, ^{19}F NMR analysis of the crude mixture revealed the presence of 92% of **30** and 8% of **31**, affording pure **30** in 43% yield. It is worthy of note that partial degradation of **30** was observed during purification. Compound **30** was next reacted with *tert*-butylsulfamoylcarbamate in the presence of PPh_3 and DIAD. After 16 h stirring at room temperature in THF, the corresponding fluoroalkene **32** was isolated in 74% yield. The desired acyclonucleoside **34** was finally obtained in two-steps involving a coupling reaction with Boc-D-Ala-OSu followed by a deprotection.

 doi:10.20944/preprints202505.1714.v1



Scheme 6. Reagents and conditions: (a) CsF, *t*BuOH, 70 °C, 4 h. (b) NH_2SO_2NHBoc, PPh_3, DIAD, THF, 20 °C, 16 h. (c) Boc-D-Ala-OSu, DBU, DMF, 20 °C, 16 h. (d) TFA, H_2O, CH_2Cl_2, 20 °C, 5h.

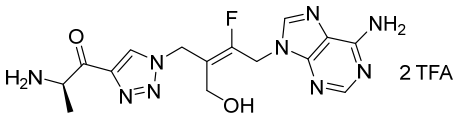
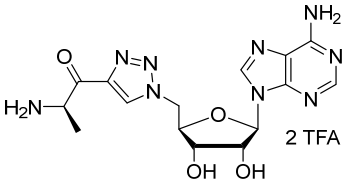
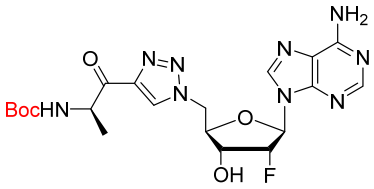
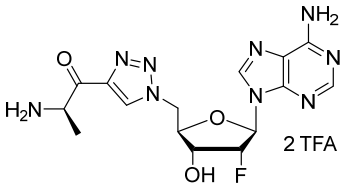
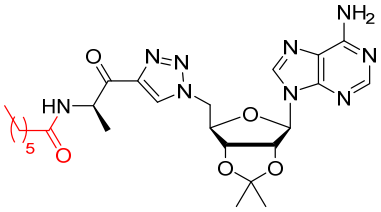
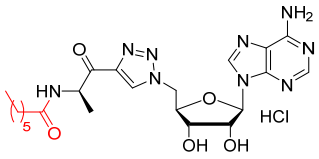
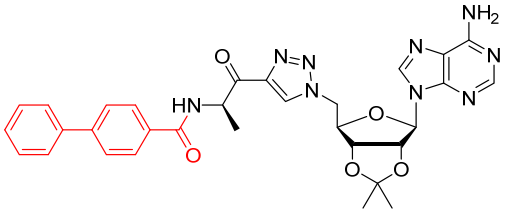
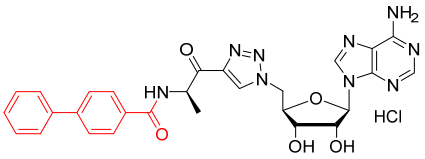
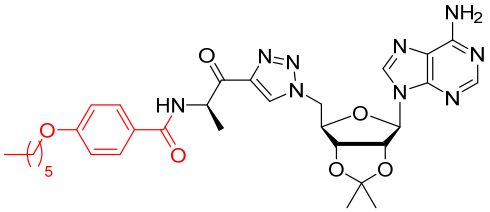
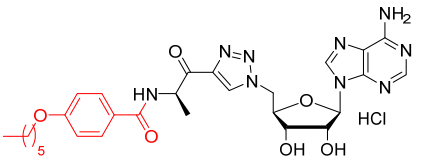
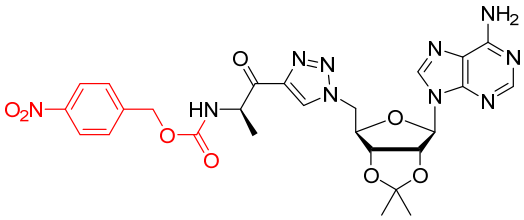
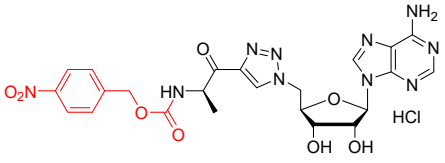
We showed the introduction of a triazole ring to link D-alanine to adenosine or its fluorinated transbutenyl mimic was not detrimental for the inhibitory activity *in vitro* towards DltA enzyme [20]. Indeed, nucleosides **35** and **36** were previously prepared (Scheme 7, Table 1, entries 1 and 2) and exhibited IC_{50} values in the same order of magnitude to those obtained from **1** [20]. To extend this series, analogs **44**-**48** was prepared (Table 1). Indeed, azide **38** was reacted with 4-*N*-Boc-amino-pent-1-yn-2-one in the presence of $CuSO_4$ and sodium ascorbate. After 24 h stirring at 20 °C in *t*-BuOH/H_2O, triazole **39** was isolated in 68% yield. Additional treatment with TFA afforded **44** in 88% yield (Table 1, entry 2). We envisaged to functionalize the amino group of the triazolyl moiety with various lipophilic acyl derivatives, that are also known to improve cell permeability in several bacteria [22]. To rapidly access these compounds, 1,3-dipolar cycloaddition reactions between azide **37** and keto-alkynes containing either a biphenyl, heptyl, hexyloxyphenyl or a 4-nitrobenzyl chain were chosen as key step (Table 1, entries 3-6 and *see* SI). Under the same previous conditions, triazoles **39**-**43** were obtained and then treated with HCl or TFA to produce the corresponding deprotected nucleosides **44**-**48** in 76-83% yield.



Scheme 7. Reagents and conditions: (a) $CuSO_4.5H_2O$, Sodium Ascorbate, *t*-BuOH/H_2O, 40 °C, 24 h. (b) TFA, H_2O, CH_2Cl_2, 20 °C, 5 h for **44** or HCl_{aq} (3 M), MeOH, 20 °C, 20 h for **45**-**48**.

 doi:10.20944/preprints202505.1714.v1

Table 1

Entry	Products	
1		
	35 [20]	**36** [20]
2		
	39, 68%	**44**, 88%
3		
	40, 79%	**45**, 89%
4		
	41, 77%	**46**, 83%
5		
	42, 81%	**47**, 76%
6		
	43, 64%	**48**, 79%

2.2. Molecular Docking and Biological Studies

Nucleoside **1** has already been reported to effectively inhibit the growth of *Bacillus subtilis* in the presence of vancomycin by acting as DltA inhibitor (K*i* = 0.2 mM) disrupting the D-alanylation process [11]. To validate the various proposed chemical modifications, *in silico* binding affinity of each molecule was measured towards *B. Subtilis* DltA (PDB 3FCC) and compared to the one obtained with **1**. Docking scores listed in Table 2 are the average of nine different minimized energy states. Functionalization of the 2′-position did not disturb the binding affinity which even seemed to

 doi:10.20944/preprints202505.1714.v1

increase with the size of the functional group. In fact, 2'-F and 2'-azido derivatives **8** (-8.2 kcal/mol) and **9** (-8.5 kcal/mol) showed binding affinities similar to **1** (-8.2 kcal/mol) while the introduction of bulky chains led to docking scores of -8.8 kcal/mol for **18** and -9.7 kcal/mol for **11** (Table 2, entries 1-5). Substitution of the sulfamate linkage by either a squaramide, a sulfamide or a triazole resulted in similar or slightly increased binding affinities with docking scores of -8.2 kcal/mol for **27**, -8.7 kcal/mol for **22** and -8.9 kcal/mol for **36** (Table 2, entries 6, 7 and 11). *N*-terminal moiety functionalization of triazole **36** improved affinity resulting in binding energies ranging from -8.9 kcal/mol to -10.5 kcal/mol (Table 2, entries 13-16). When the nucleosidic sugar pocket is replaced by the fluorinated transbutenyl moiety, the binding affinity of **34** (-7.7 kcal/mol) and **35** (-8.1 kcal/mol) was still closed to **1** (Table 2, entries 1, 9 and 10). To ensure these docking results could be extended to *E. faecalis*, *S. aureus* and *S. epidermidis*, we compared the 3D-structures of DltA from these four bacteria (Figure 3). Structural superposition indicated a RMSD ranging from 2.25 Å (480 aa aligned) to 0.790 Å (387 aa aligned) (*B. subtilis vs. S. epidermidis*), from 4.13 Å (476 aa aligned) to 0.859 Å (368 aa aligned) (*B. subtilis vs. E. faecalis*), and from 3.45 Å (479 aa aligned) to 0.787 Å (377 aa aligned) (*B. subtilis vs. S. aureus*). This suggested that the overall structure was well conserved. Regarding the ATP and D-alanine pockets, all the structures were perfectly homologous and exhibited the same spatial distribution. These results of structural homologies supported the idea that the proposed chemical modifications should not disturb the molecular recognition of the different inhibitors by DltA from *E. faecalis*, *S. aureus* and *S. epidermidis*.

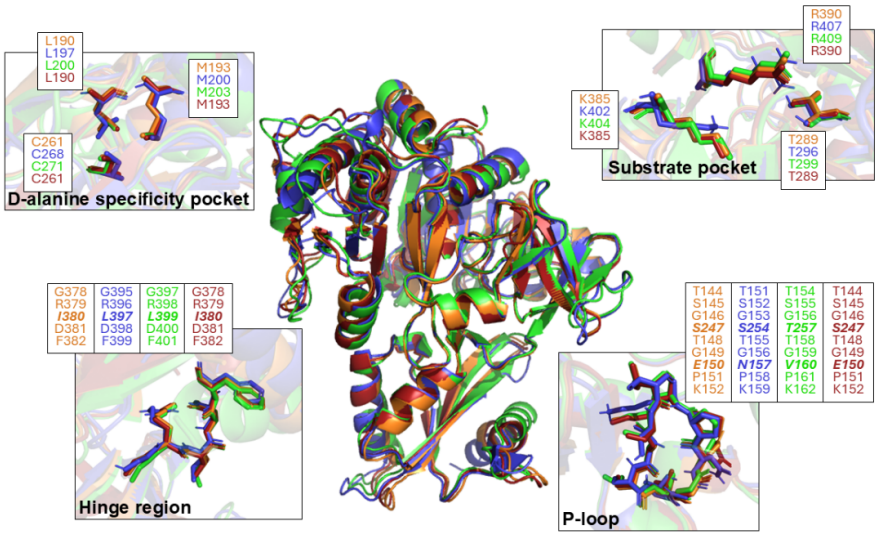


Figure 3. Structural superposition of DltA from *B. subtilis* MR168 (blue), *S. epidermidis* ATCC 35984 (orange), *S. aureus* Mu50 (dark red), and *E. faecalis* V583 (green). The structure of DltA from *S. epidermidis*, *S. aureus*, and *E. faecalis* were predicted using AlphaFold Server [23]. The structure of DltA from *B. subtilis* was previously determined by Yonus [24]. The aa that constitute each region are indicated in the boxes. Residues in bold italic indicate the aa differing between the four structures.

 doi:10.20944/preprints202505.1714.v1

Table 2

Entry	Inhibitor	Docking Score (kcal/mol)	IC_{50} (μM)	MIC of IPM[c] (μg/mL)	Reduction of D-Ala ester Residues (%)
1	**1**	-8.2	2.3 (0.4)[a], 2.4 (0.04)[b]	0.25	86.7 (10.5)
2	**8**	-8.2	2.6 (0.5)[a]	16	0
3	**9**	-8.5	3.8 (1.1)[a]	8	74.9 (9.4)
4	**11**	-9.7	39.5 (0.9)[b]	32	0
5	**18**	-8.8	7.6 (0.9)[b]	0.25	51.5 (14.2)
6	**22**	-8.7	28.4 (3.7)[a]	64	11.5 (1.3)
7	**27**	-8.2	1.8 (0.2)[a], 2.0 (0.3)[b]	1	80.9 (18.4)
8	**28**	-8.3	3.2 (0.8)[a]	32	0
9	**34**	-7.7	>100[b]	32	2
10	**35**	-8.1	7.4 (1.3)[a]	16	0
11	**36**	-8.9	4.5 (2.6)[a]	8	0
12	**44**	-9.0	14.7 (6.7)[a]	32	8.6 (1.1)
13	**45**	-8.9	22.2 (3.5)[b]	16	22.2 (5.0)
14	**46**	-10.5	12.4 (2.7)[b]	8	16.0 (4.2)
15	**47**	-9.3	16 (2.4)[b]	16	12.5 (14.0)
16	**48**	-10.0	21.9 (2.6)[b]	16	0

[a] DltA from *E. faecalis*; [b] Dlt from *S. epidermidis*, [c] Experiments performed with *S. aureus*.

All new compounds were then evaluated *in vitro* for enzyme inhibition against recombinant DltA from *E. faecalis* or *S. epidermis*. Half-maximal inhibitory concentrations (IC_{50}) were then determined by measuring the amount of PPi-released at different inhibitor concentrations (Table 2). Our results showed that the IC_{50} values of compound **1** against DltA from *E. faecalis* and *S. epidermidis* were similar, with values of 2.3 mM and 2.4 mM, respectively (Table 2, entry 1). The adenosine 2'-hydroxyl did not seem to be involved in the enzymatic recognition since its substitution by a fluorine atom leading to compound **8** showed the same activity as **1** (IC_{50} = 2.4 mM) with an IC_{50} value of 2.6 mM (Table 2, entries 1 and 2). We noticed the DltA enzyme tolerated the presence of various modification on 2'-position but the activity was inversely related to the size of the introduced group. Indeed, compounds **9** (IC_{50} = 3.8 mM) and **18** (IC_{50} = 7.6 mM), containing either an azide or a difluorophosphonylated allylic ether, were respectively 1.6 and 3 times less active than **1** while the activity of the glucose derivative **11** (IC_{50} = 39.5 mM) dropped 16-fold (Table 2, entries 1, 3-5). Substitution of the sulfamate linkage by a squaramide drastically decreased the enzyme activity with an IC_{50} value of 28.4 mM for **22** (Table 2, entry 6), despite a good predicted binding affinity (-8.7 Kcal/mol). Compounds **27** (IC_{50} = 1.8 mM) and **28** (IC_{50} = 3.2 mM), both containing the more acidic sulfamide linker instead of the squaramide, were as efficient as **1** (Table 2, entries 7 and 8). Surprisingly, when this linkage was introduced onto the fluorinated transbutenyl derivative **34**, a complete loss of the activity was observed (Table 2, entries 9). This result contrasted with our previous reported work in which triazolyl transbutenyl derivative **35** (IC_{50} = 7.5 mM) was found to inhibit DltA in the same order of magnitude as its corresponding nucleosidic analogue **36** (Table 2, entries 10 and 11) [22]. In this triazolyl series, the introduction of a fluorine atom onto 2'-position did not improve the enzymatic activity (Table 2, entries 11 and 12). Instead, fluorinated compound **44** (IC_{50} = 14.7 mM) was 3 times less active than non-fluorinated derivative **36** (IC_{50} = 4.5 mM). The presence of the amino group at the 5'-end seemed to be necessary since **45**-**48** were 3 to 5 times less active than the amine-free derivative **36** (Table 2, entries 11, 13-16).

All these compounds were also tested *in vivo* against MRSA clinical isolates. None of them had a significant effect on bacterial growth at concentrations up to 1 mM, thus excluding that they can act as antibiotics. The minimum inhibitory concentrations (MIC) of imipenem (IPM) were next determined in the absence or presence of 1 mM of the different DltA inhibitors to evaluate their potential to re-sensitize MRSA to this β-lactam (Table 2). In the absence of inhibitor, the MIC value of

 doi:10.20944/preprints202505.1714.v1

IPM against the tested MRSA strain was 32 µg/mL, which is well above the clinical breakpoint of resistance of 2 µg/mL. Among the best *in vitro* Dlt inhibitors with IC_{50} values ranging from 1.8 to 7.6 mM, only **18** (MIC = 0.25 mg/mL) and **27** (MIC = 1 mg/mL) acted as efficient adjuvants of IPM with MICs comparable with those previously reported with **1** (MIC = 0.25 µg/mL) (Table 2, entries 1, 5 and 7) [12], while compounds **8**, **9**, **28**, **35** and **36** were 32 to 128 times less active than **1** *in vivo* emphasizing their potential poor cell-permeability or metabolic instability (Table 2, entries 2, 3, 8, 10 and 11). Compounds with moderate and weak inhibitory activity such as **11**, **22**, **34**, **44**, **45**, **47** and **48** did not re-sensitize the bacteria to IPM since MIC values, ranging from 16 to 64 mg/mL, are above the breakpoint (Table 2, entries 4, 6, 9, 12, 13, 15 and 16). However, we observed that **46** (MIC = 8 mg/mL) slightly restored IPM antimicrobial activity even though this compound was weakly active towards DltA *in vitro* (Table 2, entry 14). This result seems to indicate that **46** was able to slightly potentiate IPM against MRSA, probably by targeting an enzyme other than DltA. To clarify this hypothesis, quantification of D-alanyl ester of the MRSA cell wall was performed as previously described in the presence of inhibitors (Table 2) [25]. At 1 mM, **36** (MIC = 8 mg/mL) did not affect D-alanylation while **46** (MIC = 8 mg/mL) and **9** (MIC = 8 mg/mL) reduced the level of bacteria cell wall D-alanyl ester residues by 16 % and 74.9%, respectively (Table 2, entries 3, 11 and 14). This clearly supports that IPM antimicrobial activity observed with the presence of **36** was not related to D-alanylation inhibition in contrast with **46** and **9** which had an effect on this pathway. In addition to **9** (reduction of 74.9%), molecules exhibiting the best *in vivo* activities targeted the D-alanylation process to re-sensitize MRSA to IPM. Indeed, a reduction of 51.5% and 80.9% in D-alanyl residues was observed in the presence of compounds **18** and **27** which were as efficient as **1** (reduction of 86.7%) (Table 2, entries 1, 5 and 7). These new series represent a promising structure entry not reported yet for the inhibition of DltA and the re-sensitization of bacteria toward known antibiotics.

3. Materials and Methods

3.1. General Information and Materials

Unless otherwise specified, all reagents were obtained from commercial suppliers and were used without purification. For anhydrous conditions, the glassware was flamed under a continuous nitrogen flow and cooled to room temperature before performing the experiment. Anhydrous solvents (THF, MeCN, Toluene, Et_2O and CH_2Cl_2) were obtained with PURE SOLV, Innovative Technology Solvent Purification System by passing the degassed solvents (N_2) through a column of activated alumina. Anhydrous Pyridine and DMF were distilled from CaH_2. Flash column chromatography was performed on silica gel (40-63 µm) and thin layer chromatography plates were revealed by UV light and/or $KMnO_4$ solution. ^{1}H NMR, ^{13}C NMR, ^{31}P NMR and ^{19}F NMR spectra were recorded on 500 or 600 MHz apparatus in deuterated solvent. All NMR spectra were calibrated by the residual peaks of the deuterated solvent according to Hugo E. Gottlieb's values [26]. ^{19}F and ^{31}P NMR spectral lines are with respect to the internal references $CFCl_3$ and H_3PO_4 respectively. All data are reported in the following order: chemical shifts (δ) in parts per million (ppm), multiplicity (s: singlet, d: doublet, t: triplet, q: quadruplet, m: multiplet, *br* s: broad signal), coupling constants (*J*) in Hertz (Hz), and number of protons. All proton and carbon assignments were realized by NMR methods involving DEPT-135, HSQC, HMBC and COSY experiments. Mass spectra and high resolution mass spectra (HRMS) were recorded on a Q-TOF (Quadrupole time-of-flight) micro instrument with an electrospray source in the ESI mode.

3.2. General Procedure

General procedure A for the sulfamoylation: (Method A) To a solution of alcohol (1.0 equiv.) in DMF (0.5 M) were added triethylamine (1.1 equiv.) and sulfamoyl choride (2.4 equiv.) at 0 °C. The reaction mixture was stirred at 0 °C for 2 h and overnight at 20 °C. The mixture was diluted with EtOAc then washed three times with H_2O and once with brine. The organic layer was dried over $MgSO_4$ and concentrated under reduced pressure to give the desired product. (Method B) To a

 doi:10.20944/preprints202505.1714.v1

solution of alcohol (1.0 equiv.) in DMAc (0.6 M) was added a cooled solution of sulfamoyl chloride (2.4 equiv.) in MeCN (1.9 M) at 0 °C. The mixture was stirred for 2 h at 20 °C then triethylamine (4.0 equiv.) with MeOH was added at 0 °C. The mixture was stirred for 10 min and concentrated under reduced pressure. The residue was taken up with EtOAc and the organic layer was washed twice with an aqueous solution of $NaHCO_3$ (1 M) and brine. The organic layer was dried over $MgSO_4$, filtered and concentrated under reduced pressure to give the desired product.

General procedure B for the amino acid introduction: To a solution of sulfamoyl derivative (1.0 equiv.) and activated alanine (1.0 equiv.) in DMF (0.1 M) was added DBU (1.5 equiv.) at 20 °C. The mixture was stirred for 16 h then the solvent was evaporated under reduced pressure. The crude product was purified by column chromatography to give the desired product.

General procedure C for the nucleoside deprotection: (Method A) To a solution of protected nucleoside analogue (1.0 equiv.) in MeOH (0.1 M) was added a solution of aqueous HCl (3 M). The reaction mixture was stirred at 20 °C for 20 h then the solvent was removed under reduced pressure. The residue was taken up with a small amount of MeOH and precipitated by addition of Et_2O. The desired product was isolated after filtration. (Method B): To a solution of protected nucleoside analogue (1.0 equiv.) in CH_2Cl_2/H_2O (5:1) was added TFA and the reaction mixture was stirred for 5 h at 20 °C. The solvent was removed under reduced pressure. The residue was taken up with a small amount of MeOH and precipitated by addition of Et_2O. The desired product was isolated after filtration.

General procedure D for the sulfamide introduction by Mitsunobu reaction: To a solution of alcohol (1.0 equiv.) in THF (0.05 M) were added *tert*-butyl sulfamoylcarbamate (3.0 equiv.) and triphenylphosphine (1.5 equiv.). Then, diisopropyl azodicarboxylate (1.05 equiv.) was added dropwise. The mixture was stirred overnight at 20 °C and concentrated under reduced pressure. The crude mixture was purified by column chromatography to afford the desired product.

General procedure E for the copper catalyzed azide alkyne cycloaddition: To a solution of alkyl azide (1.0 equiv.) in *t*-BuOH/H_2O (0.2 M, 1:1) were added alkyne (1.1 equiv.) followed by sodium ascorbate (10 mol %), and $CuSO_4.5H_2O$ (5 mol%) at 20 °C. The mixture was stirred for 24 h at 40 °C then the solvent was removed under reduced pressure. The residue was taken up with CH_2Cl_2/H_2O (1:1) and the aqueous layer was extracted three times with CH_2Cl_2. The combined organic layers were washed with brine, dried over $MgSO_4$, filtered then concentrated under reduced pressure. The crude was purified by column chromatography to give the desired product.

General procedure F for the alcohol protection by silyl ether: To a solution of adenosine derivative (1.0 equiv.) in DMF (0.2 M) were added Imidazole (6.6 equiv.) and DMAP (0.1 equiv.) at 20 °C. TBDMSCl (3.3 equiv.) was then added by portion at 0 °C. The reaction mixture was stirred for 24 h at 20 °C then quenched with an aqueous solution of NH_4Cl. The aqueous layer was extracted three times with EtOAc and the combined organic layers were washed with H_2O and brine. The organic layer was dried over $MgSO_4$, filtered and concentrated under reduced pressure. The crude product was purified by column chromatography to give the desired product.

General procedure G for the selective deprotection of silyl ether: (Method A) To a solution of protected nucleoside (1.0 equiv.) in H_2O and THF (1:1) was added glacial acetic acid (3 v/v) at 0 °C. The mixture was stirred for 2 days at 20 °C then quenched with a cooled aqueous solution of HCl (1 M). The aqueous layer was extracted three times with EtOAc and the combined organic layers were washed once with brine. The organic layer was dried over $MgSO_4$, filtered and concentrated under reduced pressure. The crude product was purified by column chromatography to give the desired product. (Method B) To a solution of protected nucleoside (1.0 equiv.) in THF (4 v/v) was added an aqueous solution of TFA (1:1) at 0 °C. The mixture was stirred for 2 h at 0 °C then quenched with a cooled aqueous solution of saturated $NaHCO_3$. The aqueous layer was extracted three times with EtOAc and the combined organic layers were washed once with H_2O and brine. The organic layer was dried over $MgSO_4$, filtered and concentrated under reduced pressure. The crude product was purified by column chromatography to give the desired product.

 doi:10.20944/preprints202505.1714.v1

3.3. Synthesis

*((2R,3R,4R,5R)-5-(6-amino-9H-purin-9-yl)-3-((tert-butyldimethylsilyl)oxy)-4-fluorotetrahydrofuran-2-yl)methyl sulfamate (**4**).* The general procedure A (Method B) was followed with compound **2** (590 mg, 1.54 mmol), prepared as previously described [13], in DMAc (2.4 mL), sulfamoyl chloride (426 mg, 3.69 mmol) in MeCN (1.9 mL). The crude product was purified by column chromatography (Eluent CH_2Cl_2/MeOH 94:6) to give compound **4** (570 mg, 1.23 mmol, 80%) as a white solid. ^{1}H NMR (CD_3CN, 500 MHz): δ 0.00 (s, 3H), 0.04 (s, 3H), 0.84 (s, 9H), 3.87 (dd, *J* = 12.2 Hz, *J* = 3.4 Hz, 1H), 4.03 (dd, *J* = 12.2 Hz, *J* = 2.3 Hz, 1H), 4.30–4.33 (m, 1H), 5.51 (ddd, *J* = 18.5 Hz, *J* = 7.2 Hz, *J* = 4.6 Hz, 1H), 5.76 (ddd, *J* = 51.9 Hz, *J* = 4.6 Hz, *J* = 1.8 Hz, 1H), 6.05 (br s, 4H), 6.26 (dd, *J* = 18.3 Hz, *J* = 1.8 Hz, 1H), 8.11 (s, 1H), 8.24 (s, 1H). ^{19}F NMR (CD_3CN, 470 MHz): δ –202.0 (dt, *J* = 51.9 Hz, *J* = 18.5 Hz). ^{13}C NMR (CD_3CN, 150 MHz): δ –5.4, –5.3, 19.0, 26.2, 61.8, 74.8 (d, *J* = 14.8 Hz), 82.0, 87.9 (d, *J* = 33.6 Hz), 92.4 (d, *J* = 189.9 Hz), 120.7, 140.5, 150.3, 154.1, 157.0. HRMS-ESI (m/z) calcd for $C_{16}H_{28}FN_6O_5SiS$ $[M+H]^+$ 463.1595, found 463.1602. NMR spectrum is identical to that previously reported [13].

*((2R,3S,4S,5R)-5-(6-amino-9H-purin-9-yl)-4-azido-3-((tert-butyldimethylsilyl)oxy)tetrahydrofuran-2-yl)methyl sulfamate (**5**).* The general procedure A (Method B) was followed with compound **3** (250 mg, 0.61 mmol), prepared as previously described [27], in DMAc (1 mL), sulfamoyl chloride (170 mg, 1.47 mmol) in MeCN (0.75 mL) to give compound **5** (265 mg, 0.54 mmol, 89%) as white solid. The compound was used in the next step without further purification. ^{1}H NMR (MeOD, 500 MHz): δ 0.23 (s, 3H), 0.24 (s, 3H), 1.00 (s, 9H), 4.28–4.32 (m, 2H), 4.40–4.43 (m, 1H), 4.72–4.74 (m, 1H), 4.87–4.89 (m, 1H), 6.17 (d, *J* = 6.1 Hz, 1H), 8.23 (s, 1H), 8.30 (s, 1H). ^{13}C NMR (MeOD, 125 MHz): δ –4.8, –4.6, 21.3, 26.3, 66.1, 68.8, 74.1, 84.6, 87.6, 120.6, 141.1, 150.6, 154.1, 157.5. HRMS-ESI (m/z) calcd for $C_{16}H_{28}N_9O_5SSi$ $[M+H]^+$ 486.1703, found 486.1710.

*((2R,3R,4R,5R)-5-(6-amino-9H-purin-9-yl)-3-((tert-butyldimethylsilyl)oxy)-4-fluorotetrahydrofuran-2-yl)methyl ((tert-butoxycarbonyl)-D-alanyl)sulfamate (**6**).* The general procedure B was followed with compound **4** (375 mg, 0.81 mmol), Boc-D-Ala-OSu (232 mg, 0.81 mmol) and DBU (0.18 mL, 1.22 mmol) in DMF (10.8 mL). The crude was purified by column chromatography (Eluent CH_2Cl_2/MeOH 94:6 to 9:1) to give compound **6** (392 mg, 0.61 mmol, 76%) as a white foam. ^{1}H NMR (DMSO-d_6, 600 MHz): δ –0.02 (s, 3H), 0.00 (s, 3H), 0.82 (s, 9H), 1.17 (d, *J* = 7.1 Hz, 3H), 1.33 (s, 9H), 3.75–3.83 (m, 2H), 3.91–3.95 (m, 1H), 4.16–4.20 (m, 1H), 4.37–4.46 (m, 1H), 5.66 (d, *J* = 52.1 Hz, 1H), 6.27 (dd, *J* = 17.3 Hz, *J* = 2.3 Hz, 1H), 6.46 (br s, 1H), 7.40 (br s, 2H), 8.14 (s, 1H), 8.27 (s, 1H). ^{19}F NMR (DMSO-d_6, 565 MHz): δ –201.5 (m). ^{13}C NMR (DMSO-d_6, 150 MHz): δ –5.6, –5.5, 18.1, 18.5, 25.8, 28.2, 51.3, 62.2, 74.0, 77.8, 81.5, 85.7 (d, *J* = 32.2 Hz), 91.2 (d, *J* = 190.2 Hz), 119.0, 139.1, 148.8, 152.7, 154.8, 156.1, 176.1. HRMS-ESI (m/z) calcd for $C_{24}H_{41}FN_7O_8SiS$ $[M+H]^+$ 634.2491, found 634.2489.

*((2R,3S,4S,5R)-5-(6-amino-9H-purin-9-yl)-4-azido-3-((tert-butyldimethylsilyl)oxy)tetrahydrofuran-2-yl)methyl ((tert-butoxycarbonyl)-D-alanyl)sulfamate (**7**).* The general procedure B was followed with compound **5** (438 mg, 0.90 mmol), Boc-D-Ala-OSu (258 g, 0.90 mmol) and DBU (0.20 mL, 1.35 mmol) in DMF (12 mL). The crude was purified by column chromatography (Eluent EtOAc with 1% AcOH) to give compound **7** (408 mg, 0.62 mmol, 69%) as a white foam. ^{1}H NMR (CD_3CN, 600 MHz): δ 0.18 (s, 3H), 0.19 (s, 3H), 0.96 (s, 9H), 1.27 (d, *J* = 7.1 Hz, 3H), 1.37 (s, 9H), 4.00–4.02 (m, 1H), 4.30–4.32 (m, 1H), 4.44 (dd, *J* = 11.2 Hz, *J* = 3.7 Hz, 1H), 4.55–4.60 (m, 2H), 4.78–4.79 (m, 1H), 5.82 (br s, 1H), 6.09 (d, *J* = 7.1 Hz, 1H), 8.34 (s, 1H), 8.38 (s, 1H). ^{13}C NMR (CD_3CN, 150 MHz): δ –4.7, –4.6, 18.6, 19.6, 26.1, 28.6, 53.7, 66.5, 68.1, 73.8, 79.7, 84.5, 86.5, 120.2, 141.1, 150.6, 154.1, 156.5, 156.7, 171.7. HRMS-ESI (m/z) calcd for $C_{24}H_{41}N_{10}O_8SSi$ $[M+H]^+$ 657.2599, found 657.2601.

*((2R,3R,4R,5R)-5-(6-amino-9H-purin-9-yl)-4-fluoro-3-hydroxytetrahydrofuran-2-yl)methyl (D-alanyl)sulfamate salt (**8**).* The general procedure C (Method B) was followed with compound **6** (498 mg, 0.78 mmol), TFA (9.7 mL) and CH_2Cl_2/H_2O (8/2 mL) to give compound **8** (450 mg, 0.69 mmol, 89%) as a white solid (2 TFA salt). ^{1}H NMR (D_2O, 500 MHz): δ 1.52 (d, *J* = 7.1 Hz, 3H), 3.81 (dd, *J* = 13.1 Hz, *J* = 3.7 Hz, 1H), 3.89 (q, *J* = 7.1 Hz, 1H), 3.98 (dd, *J* = 13.1 Hz, *J* = 2.3 Hz, 1H), 4.42–4.46 (m, 1H), 5.51 (ddd, *J* = 17.2 Hz, *J* = 7.6 Hz, *J* = 4.8 Hz, 1H), 5.76 (ddd, *J* = 51.1 Hz, *J* = 4.8 Hz, *J* = 2.0 Hz, 1H), 6.51 (dd, *J* = 18.4 Hz, *J* = 2.0 Hz, 1H), 8.45 (s, 1H), 8.51 (s, 1H). ^{19}F NMR (D_2O, 470 MHz): δ –200.8 (dt, *J* = 51.1 Hz, *J* = 17.6 Hz). ^{13}C NMR (D_2O, 150 MHz): δ 51.3, 59.2, 73.8 (d, *J* = 14.9 Hz), 81.5, 87.3 (d, *J* = 34.8 Hz),

 doi:10.20944/preprints202505.1714.v1

91.4 (d, *J* = 191.9 Hz), 116.2 (q, *J* = 291.4 Hz), 117.2, 119.0, 143.3, 144.6, 147.9, 150.0, 162.9 (q, *J* = 35.3 Hz, TFA), 176.4. HPLC purity: (96.6%, t_R = 4.48 min). HRMS-ESI (m/z) calcd for $C_{13}H_{18}FN_7O_6S$ $[M+H]^+$ 420.1102, found 420.1102.

*((2R,3S,4S,5R)-5-(6-amino-9H-purin-9-yl)-4-azido-3-hydroxytetrahydrofuran-2-yl)methyl (D-alanyl)sulfamate salt (**9**)*. To a solution of compound **7** (165 mg, 0.25 mmol, 1 equiv.) in H_2O (2.5 mL) was added TFA (10 mL) at 20 °C. The mixture was stirred for 12 h and concentrated under reduce pressure. The residue was taken up with a small amount of MeOH. By addition of Et_2O, a precipitate appeared which was isolated by filtration to give compound **9** (91 mg, 0.13 mmol, 54%) as a white solid (2 TFA salt). ^{1}H NMR (DMSO-d_6, 500 MHz): δ 1.29 (d, *J* = 7.1 Hz, 3H), 3.48–3.50 (m, 1H), 4.07 (dd, *J* = 11.1 Hz, *J* = 4.3 Hz, 1H), 4.14–4.17 (m, 1H), 4.19 (dd, *J* = 11.1 Hz, *J* = 3.7 Hz, 1H), 4.57–4.59 (m, 1H), 4.67–4.69 (m, 1H), 6.06 (d, *J* = 6.1 Hz, 1H), 6.17 (d, *J* = 5.4 Hz, 1H), 7.35 (br s, 1H), 7.81 (br s, 2H), 8.16 (s, 1H), 8.39 (s, 1H). ^{13}C NMR (DMSO-d_6, 150 MHz): δ 17.2, 50.8, 64.2, 67.2, 71.5, 83.0, 84.8, 118.9, 139.3, 149.3, 152.9, 156.1, 173.4. HPLC purity: (94.9 %, t_R = 4.59 min). HRMS-ESI (m/z) calcd for $C_{13}H_{19}N_{10}O_6S$ $[M+H]^+$ 443.1210, found 443.1208.

*(2R,3R,4S,5R,6R)-2-(acetoxymethyl)-6-((1-((2R,3R,4S,5R)-2-(6-amino-9H-purin-9-yl)-5-(((N-((tert-butoxycarbonyl)-D-alanyl)sulfamoyl)oxy)methyl)-4-((tert-butyldimethylsilyl)oxy)tetrahydrofuran-3-yl)-1H-1,2,3-triazol-4-yl)methoxy)tetrahydro-2H-pyran-3,4,5-triyl triacetate (**10**)*. The general procedure E was followed with compound **7** (269 mg, 0.41 mmol), glucosyl-*O*-propargyl ether (175 mg, 0.45 mmol), prepared as previously described [28], sodium ascorbate (8 mg, 0.04 mmol) and $CuSO_4.5H_2O$ (5 mg, 0.02 mmol) in *t*-BuOH/H_2O (4 mL). The crude was purified by column chromatography (Eluent EtOAc/MeOH 9:1) to give compound **10** (276 mg, 0.26 mmol, 64%) as a white solid. ^{1}H NMR (CD_3CN, 600 MHz): δ –0.18 (s, 3H), 0.01 (s, 3H), 0.73 (s, 9H), 1.23 (d, *J* = 7.4 Hz, 3H), 1.33 (s, 9H), 1.85 (s, 3H), 1.92 (s, 3H), 1.97 (s, 3H), 2.02 (s, 3H), 3.82 (ddd, *J* = 10.1 Hz, *J* = 4.7 Hz, *J* = 2.5 Hz, 1H), 3.98 (br s, 1H), 4.10 (dd, *J* = 12.4 Hz, *J* = 2.5 Hz, 1H), 4.25 (dd, *J* = 12.4 Hz, *J* = 4.7 Hz, 1H), 4.28 (dd, *J* = 10.4 Hz, *J* = 3.6 Hz, 1H), 4.38–4.39 (m, 1H), 4.44–4.46 (m, 1H), 4.68 (d, *J* = 12.4 Hz, 1H), 4.71 (d, *J* = 8.1 Hz, 1H), 4.81 (d, *J* = 12.4 Hz, 1H), 4.85–4.88 (m, 2H), 5.02 (t, *J* = 9.7 Hz, 1H), 5.18 (t, *J* = 9.7 Hz, 1H), 5.78 (br s, 1H), 5.96 (br s, 1H), 6.28 (br s, 2H), 6.80 (d, *J* = 5.6 Hz, 1H), 7.94 (s, 1H), 8.19 (s, 1H), 8.39 (br s, 1H). ^{13}C NMR (CD_3CN, 150 MHz): δ –4.9, –4.8, 18.3, 19.7, 20.8, 20.9 (2C), 21.0, 25.9, 28.6, 53.7, 62.7, 62.9, 66.2, 68.2, 69.2, 71.9, 72.5, 72.8, 73.4, 79.7, 85.1, 86.3, 100.3, 120.3, 126.0, 141.0, 144.3, 150.6, 154.1, 156.4, 156.8, 170.2, 170.5, 170.9, 171.4, 182.0. HRMS-ESI (m/z) calcd for $C_{41}H_{63}N_{10}O_{18}SSi$ $[M+H]^+$ 1043.3812, found 1043.3835.

*((2R,3S,4R,5R)-5-(6-amino-9H-purin-9-yl)-3-hydroxy-4-(4-((((2R,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-2-yl)oxy)methyl)-1H-1,2,3-triazol-1-yl)tetrahydrofuran-2-yl)methyl (D-alanyl)sulfamate salt (**11**)*. A solution of compound **10** (70 mg, 0.06 mmol, 1 equiv.) in H_2O (2 mL) and TFA (8 mL) was stirred for 12 h at 20 °C. Volatiles were removed under reduced pressure and the residue was dissolved in MeOH (1 mL) and a solution of MeONa (5.4 M) in MeOH (0.05 mL, 0.3 mmol, 5 equiv.) was added at 20 °C. The mixture was stirred for 1 h and quenched with an aqueous solution of TFA (10%) then concentrated under reduced pressure. The residue was dissolved in THF (1 mL) and a solution of 37% triethylamine trihydrofluoride (0.1 mL, 0.24 mmol, 4 equiv.) was added at 20 °C. The mixture was stirred for 12 h and volatiles were removed under reduced pressure. The crude was purified by HPLC RP-18 (MeCN/H_2O, linear gradient) to give compound **11** (17 mg, 0.26 mmol, 32%) as a white solid (2 TFA salt). ^{1}H NMR (D_2O, 600 MHz): δ 1.48 (d, *J* = 7.2 Hz, 3H), 3.24–3.26 (m, 1H), 3.33–3.37 (m, 1H), 3.41–3.45 (m, 2H), 3.66 (dd, *J* = 12.7 Hz, *J* = 6.3 Hz, 1H), 3.85–3.87 (m, 1H), 3.89–3.93 (m, 1H), 4.46–4.48 (m, 1H), 4.51–4.57 (m, 2H), 4.62–4.64 (m, 1H), 4.88 (d, *J* = 12.7 Hz, 1H), 4.99 (d, *J* = 12.7 Hz, 1H), 5.05 (t, *J* = 5.6 Hz, 1H), 5.99 (t, *J* = 5.6 Hz, 1H), 6.99 (d, *J* = 4.9 Hz, 1H), 8.26 (s, 1H), 8.39 (s, 1H), 8.60 (s, 1H). ^{13}C NMR (D_2O, 150 MHz): δ 16.3, 51.0, 60.6, 61.7, 65.8, 68.3, 69.5, 69.8, 72.9, 75.6, 75.9, 83.2, 86.3, 101.4, 116.2 (q, *J* = 291.1 Hz), 119.2, 126.6, 142.7, 143.7, 144.6, 148.1, 149.9, 162.9 (q, *J* = 35.8 Hz), 175.4. HPLC purity: (88.5 %, t_R = 2.42 min). HRMS-ESI (m/z) calcd for $C_{22}H_{33}N_{10}O_{12}S$ $[M+H]^+$ 661.2000, found 661.2016.

*diisopropyl ((E)-4-(((2R,3R,4R,5R)-2-(6-(N-benzoylbenzamido)-9H-purin-9-yl)-4-((tert-butyldimethylsilyl)oxy)-5-(((tert-butyldimethylsilyl)oxy)methyl)tetrahydrofuran-3-yl)oxy)-1,1-difluorobut-2-en-1-yl)phosphonate (**13**)*. The general procedure F was followed with compound **12** (320 mg, 0.44

 doi:10.20944/preprints202505.1714.v1

mmol), prepared as previously described [16], TBDMSCl (218 mg, 1.45 mmol), imidazole (196 mg, 2.89 mmol) and DMAP (4.9 mg, 0.04 mmol) in DMF (5 mL). The crude product was purified by column chromatography (Eluent Pentane/EtOAc 7:3) to give compound **13** (333 mg, 0.35 mmol, 79%) as a white foam. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 0.06 (s, 3H), 0.08 (s, 3H), 0.11 (s, 6H), 0.90 (s, 9H), 0.92 (s, 9H), 1.33 (t, *J* = 6.2 Hz, 6H), 1.35 (dd, *J* = 6.2 Hz, *J* = 1.7 Hz, 6H), 3.78 (dd, *J* = 11.7 Hz, *J* = 3.1 Hz, 1H), 3.98 (dd, *J* = 11.7 Hz, *J* = 3.4 Hz, 1H), 4.13 (dt, *J* = 5.7 Hz, *J* = 3.1 Hz, 1H), 4.22–4.31 (m, 2H), 4.36 (dd, *J* = 5.7 Hz, *J* = 3.6 Hz, 1H), 4.53–4.55 (m, 1H), 4.79–4.86 (m, 2H), 5.95–6.03 (m, 1H), 6.18 (d, *J* = 3.6 Hz, 1H), 6.26–6.33 (m, 1H), 7.33–7.36 (m, 4H), 7.46–7.49 (m, 2H), 7.84–7.86 (m, 4H), 8.37 (s, 1H), 8.65 (s, 1H). ^{19}F NMR ($CDCl_3$, 565 MHz): δ –109.5 (ddd, *J* = 298.3 Hz, *J* = 113.2 Hz, *J* = 12.4 Hz), –110.3 (ddd, *J* = 298.3 Hz, *J* = 113.2 Hz, *J* = 12.4 Hz). ^{31}P NMR ($CDCl_3$, 243 MHz): δ 4.3 (t, *J* = 113.2 Hz). ^{13}C NMR ($CDCl_3$, 150 MHz): δ –5.3, –5.2, –4.7, –4.5, 18.2, 18.6, 23.8 and 23.9 (d, *J* = 4.1 Hz), 24.3 (d, *J* = 3.4 Hz, 2C), 25.8, 26.2, 61.8, 69.3, 69.8, 73.9 (d, *J* = 7.1 Hz, 2C), 82.1, 85.1, 87.3, 116.8 (dt, *J* = 258.9 Hz, *J* = 220.7 Hz), 122.7 (dt, *J* = 22.3 Hz, *J* = 13.4 Hz), 128.3, 128.8 (4C), 129.6 (4C), 133.1 (2C), 134.2 (2C), 134.4 (dt, *J* = 9.8 Hz, *J* = 5.6 Hz), 143.7, 151.9, 152.3, 152.7, 172.4. HRMS-ESI (m/z) calcd for $C_{46}H_{67}F_2N_5O_9PSi_2$ $[M+H]^+$ 958.4183, found 958.4179.

*tert-butyl (tert-butoxycarbonyl)(9-((2R,3R,4R,5R)-4-((tert-butyldimethylsilyl)oxy)-5-(((tert-butyldimethylsilyl)oxy)methyl)-3-(((E)-4-(diisopropoxyphosphoryl)-4,4-difluorobut-2-en-1-yl)oxy)tetrahydrofuran-2-yl)-9H-purin-6-yl)carbamate (**14**).* To a solution of compound **13** (300 mg, 0.44 mmol, 1 equiv.) in EtOH (3.5 mL) was added CH_3NH_2 (2.1 mL, 33% in EtOH) at 20 °C. The mixture was stirred for 1 h and followed by TLC. When the conversion is quantitative the solvent was removed under reduced pressure. It was dissolved in DMF (4 mL) and triethylamine (0.17 mL, 1.20 mmol, 3 equiv.) and DMAP (5 mg, 0.04 mmol, 0.5 equiv.) were added at 20 °C. The solution was cooled and $(Boc)_2O$ (0.27 mL, 1.20 mmol, 3 equiv.) was added dropwise at 0 °C. The reaction mixture was stirred for 12 h and quenched with an aqueous solution of HCl (1 M). The aqueous layer was extracted three times with EtOAc. The combined organic layers were washed with brine, dried over $MgSO_4$, filtered and concentrated under reduced pressure. The crude product was purified by column chromatography (Eluent Pentane/EtOAc 7:3) to give compound **14** (326 mg, 0.34 mmol, 78%) as a waxy oil. ^{1}H NMR ($CDCl_3$, 600 MHz): δ 0.07 (s, 3H), 0.08 (s, 3H), 0.12 (s, 3H), 0.13 (s, 3H), 0.91 (s, 9H), 0.94 (s, 9H), 1.33 (dd, *J* = 6.4 Hz, *J* = 4.3 Hz, 6H), 1.36 (dd, *J* = 6.4 Hz, *J* = 1.9 Hz, 6H), 1.45 (s, 18H), 3.81 (dd, *J* = 11.6 Hz, *J* = 2.6 Hz, 1H), 4.05 (dd, *J* = 11.6 Hz, *J* = 3.4 Hz, 1H), 4.16 (dt, *J* = 6.3 Hz, *J* = 2.6 Hz, 1H), 4.24 (dd, *J* = 4.7 Hz, *J* = 3.1 Hz, 1H), 4.26–4.30 (m, 1H), 4.36–4.40 (m, 1H), 4.52 (dd, *J* = 6.3 Hz, *J* = 4.7 Hz, 1H), 4.80–4.86 (m, 2H), 5.98–6.06 (m, 1H), 6.22 (d, *J* = 3.1 Hz, 1H), 6.29–6.33 (m, 1H), 8.54 (s, 1H), 8.84 (s, 1H). ^{19}F NMR ($CDCl_3$, 565 MHz): δ –109.6 (ddd, *J* = 298.4 Hz, *J* = 112.4 Hz, *J* = 12.2 Hz), –110.2 (ddd, *J* = 298.4 Hz, *J* = 112.4 Hz, *J* = 12.2 Hz). ^{31}P NMR ($CDCl_3$, 243 MHz): δ 4.3 (t, *J* = 112.4 Hz). ^{13}C NMR ($CDCl_3$, 150 MHz): δ –5.3, –5.2, –4.8, –4.5, 18.2, 18.6, 23.8 and 23.9 (d, *J* = 4.3 Hz), 24.3 (d, *J* = 3.4 Hz, 2C), 25.8, 26.2, 27.9, 61.5, 69.3, 69.4, 73.9 (d, *J* = 7.1 Hz, 2C), 82.5, 84.0, 84.7, 87.3, 116.7 (dt, *J* = 259.1 Hz, *J* = 220.8 Hz), 122.7 (dt, *J* = 21.3 Hz, *J* = 13.4 Hz), 129.4, 134.4 (dt, *J* = 9.8 Hz, *J* = 5.3 Hz), 143.4, 150.4, 150.6, 152.2, 152.6. HRMS-ESI (m/z) calcd for $C_{42}H_{75}F_2N_5O_{11}PSi_2$ $[M+H]^+$ 950.4707, found 950.4697.

*tert-butyl (tert-butoxycarbonyl)(9-((2R,3R,4R,5R)-4-((tert-butyldimethylsilyl)oxy)-3-(((E)-4-(diisopropoxyphosphoryl)-4,4-difluorobut-2-en-1-yl)oxy)-5-(hydroxymethyl)tetrahydrofuran-2-yl)-9H-purin-6-yl)carbamate (**15**).* The general procedure G (method A) was followed with compound **14** (666 mg, 0.70 mmol) in H_2O (1.2 mL), THF (1.2 mL) and AcOH (3.75 mL). The crude product was purified by column chromatography (Eluent Cyclohexane/EtOAc 6:4 to 4:6) to give compound **15** (315 mg, 0.38 mmol, 54%) as a colorless oil. ^{1}H NMR ($CDCl_3$, 600 MHz): δ 0.12 (s, 6H), 0.93 (s, 9H), 1.31 (dd, *J* = 6.2 Hz, *J* = 2.6 Hz, 6H), 1.33 (d, *J* = 6.2 Hz, 6H), 1.46 (s, 18H), 3.73 (dd, *J* = 13.1 Hz, *J* = 1.4 Hz, 1H), 3.86–3.89 (m, 1H), 3.97 (dd, *J* = 13.1 Hz, *J* = 1.8 Hz, 1H), 4.08–4.11 (m, 1H), 4.21–4.23 (m, 1H), 4.57–4.58 (m, 1H), 4.72 (dd, *J* = 7.6 Hz, *J* = 4.5 Hz, 1H), 4.77–4.83 (m, 2H), 5.83–5.90 (m, 1H), 6.01 (d, *J* = 7.6 Hz, 1H), 6.10–6.15 (m, 1H), 8.22 (s, 1H), 8.82 (s, 1H). ^{19}F NMR ($CDCl_3$, 470 MHz): δ –109.7 (ddd, *J* = 315.6 Hz, *J* = 113.2 Hz, *J* = 12.3 Hz), –110.6 (ddd, *J* = 315.6 Hz, *J* = 113.2 Hz, *J* = 12.3 Hz). ^{31}P NMR ($CDCl_3$, 202 MHz): δ 4.2 (t, *J* = 113.2 Hz). ^{13}C NMR ($CDCl_3$, 150 MHz): δ –4.6, –4.5, 18.2, 23.8 and 23.9 (d, *J* = 2.3 Hz), 24.2 and 24.3 (d, *J* = 1.4 Hz), 25.8, 27.9, 62.9, 69.1, 71.6, 73.9 and 74.0 (d, *J* = 3.7 Hz), 80.9, 84.3, 89.5, 89.6, 116.6 (dt, *J* = 258.6 Hz, *J* = 220.6 Hz),

 doi:10.20944/preprints202505.1714.v1

122.7 (dt, *J* = 21.2 Hz, *J* = 13.4 Hz), 130.4, 134.0 (dt, *J* = 10.1 Hz, *J* = 5.8 Hz), 144.9, 150.5, 151.4, 151.6, 152.0. HRMS-ESI (m/z) calcd for $C_{36}H_{61}F_2N_5O_{11}PSi$ $[M+H]^+$ 836.3843, found 836.3840.

*((2R,3R,4R,5R)-5-(6-(bis(tert-butoxycarbonyl)amino)-9H-purin-9-yl)-3-((tert-butyldimethylsilyl)oxy)-4-(((E)-4-(diisopropoxyphosphoryl)-4,4-difluorobut-2-en-1-yl)oxy)tetrahydrofuran-2-yl)methyl sulfamate (**16**)*. The general procedure A (Method B) was followed with compound **15** (100 mg, 0.12 mmol) in DMAc (0.6 mL), sulfamoyl chloride (33 mg, 0.29 mmol) in MeCN (0.5 mL) to give compound **16** (105 mg, 0.10 mmol, 84%) with DMAc (13% w/w) as a colorless oil. This compound was used in the next step without further purification to avoid its degradation. ^{1}H NMR ($CDCl_3$, 600 MHz): δ 0.12 (s, 6H), 0.91 (s, 9H), 1.33 (dd, *J* = 6.2 Hz, *J* = 2.4 Hz, 6H), 1.36 (d, *J* = 6.2 Hz, 6H), 1.45 (s, 18H), 4.20–4.27 (m, 2H), 4.30–4.33 (m, 2H), 4.47–4.49 (m, 1H), 4.53–4.55 (m, 1H), 4.58–4.60 (m, 1H), 4.80–4.87 (m, 2H), 5.88 (br, s, 2H), 5.93–5.96 (m, 1H), 6.20 (d, *J* = 4.2 Hz, 1H), 6.20–6.24 (m, 1H), 8.44 (s, 1H), 8.85 (s, 1H). ^{19}F NMR ($CDCl_3$, 565 MHz): δ –109.7 (ddd, *J* = 334.1 Hz, *J* = 114.2 Hz, *J* = 12.8 Hz, 1F), –110.5 (ddd, *J* = 334.1 Hz, *J* = 114.2 Hz, *J* = 12.8 Hz, 1F). ^{31}P NMR ($CDCl_3$, 243 MHz): δ 4.0 (t, *J* = 114.2 Hz). ^{13}C NMR ($CDCl_3$, 150 MHz): δ –4.8, –4.6, 18.1, 23.8 and 23.9 (d, *J* = 4.7 Hz), 24.2 and 24.3 (d, *J* = 2.3 Hz), 25.8, 27.9, 67.9, 69.3, 70.5, 74.3 and 74.4 (d, *J* = 7.2 Hz), 80.6, 82.7, 84.3, 87.5, 116.5 (dt, *J* = 258.4 Hz, *J* = 220.7 Hz), 123.3 (dt, *J* = 21.3 Hz, *J* = 13.7 Hz), 128.9, 134.4 (dt, *J* = 9.8 Hz, *J* = 5.7 Hz), 143.8, 150.4, 150.6, 152.4, 152.7. HRMS-ESI (m/z) calcd for $C_{36}H_{62}F_2N_6O_{13}SiPS$ $[M+H]^+$ 915.3571, found 915.3564.

*((2R,3R,4R,5R)-5-(6-(bis(tert-butoxycarbonyl)amino)-9H-purin-9-yl)-3-((tert-butyldimethylsilyl)oxy)-4-(((E)-4-(diisopropoxyphosphoryl)-4,4-difluorobut-2-en-1-yl)oxy)tetrahydrofuran-2-yl)methyl ((tert-butoxycarbonyl)-D-alanyl)sulfamate (**17**)*. The general procedure B was followed with compound **16** (285 mg, 0.31 mmol), Boc-D-Ala-OSu (89 mg, 0.31 mmol) and DBU (0.07 mL, 0.46 mmol) in DMF (4.4 mL). The crude was purified by column chromatography (Eluent EtOAc/CycloHex 9:1) to give compound **17** (195 mg, 0.18 mmol, 58%) as a waxy oil. ^{1}H NMR (Acetone-d_6, 500 MHz): δ 0.19 (s, 3H), 0.21 (s, 3H), 0.97 (s, 9H), 1.29 (dd, *J* = 6.3 Hz, *J* = 1.8 Hz, 6H), 1.31 (d, *J* = 6.4 Hz, 3H), 1.33 (d, *J* = 6.2 Hz, 6H), 1.39 (s, 9H), 1.44 (s, 18H), 4.01–4.06 (m, 1H), 4.28–4.38 (m, 4H), 4.41–4.46 (m, 1H), 4.75–4.83 (m, 4H), 5.96–6.04 (m, 2H), 6.26–6.31 (m, 1H), 6.39–6.41 (m, 1H), 8.77 (s, 1H), 8.83 (s, 1H). ^{19}F NMR (Acetone-d_6, 470 MHz): δ –109.8 (ddd, *J* = 291.6 Hz, *J* = 113.4 Hz, *J* = 12.9 Hz), –110.5 (ddd, *J* = 291.6 Hz, *J* = 113.4 Hz, *J* = 12.9 Hz). ^{31}P NMR (Acetone-d_6, 202 MHz): δ 3.7 (t, *J* = 111.6 Hz). ^{13}C NMR (Acetone-d_6, 150 MHz): δ –4.5, –4.4, 18.6, 19.9, 23.9 and 24.0 (d, *J* = 3.1 Hz), 24.3 (d, $^3J_{CP}$ = 3.3 Hz, 2C), 26.2, 27.9, 28.7, 53.2, 68.9, 69.6, 71.9, 74.3 and 74.4 (d, *J* = 6.4 Hz), 79.0, 82.2, 84.0, 84.3, 87.4, 117.7 (dt, *J* = 257.6 Hz, *J* = 222.3 Hz), 122.7 (dt, *J* = 22.1 Hz, *J* = 13.5 Hz), 129.7, 135.8 (dt, *J* = 10.3 Hz, *J* = 5.6 Hz), 145.1, 151.0, 151.2, 152.7, 153.9, 155.9, 181.0. HRMS-ESI (m/z) calcd for $C_{44}H_{75}F_2N_7O_{16}PSSi$ $[M+H]^+$ 1086.4466, found 1086.4462.

*((2R,3R,4R,5R)-5-(6-amino-9H-purin-9-yl)-4-(((E)-4-(diisopropoxyphosphoryl)-4,4-difluorobut-2-en-1-yl)oxy)-3-hydroxytetrahydrofuran-2-yl)methyl (D-alanyl)sulfamate salt (**18**)*. The general procedure C (Method B) was followed with compound **17** (317 mg, 0.29 mmol), TFA (3.6 mL) and CH_2Cl_2/H_2O (3:0.7 mL). The crude was purified by HPLC RP-18 (MeCN/H_2O, linear gradient) to give compound **18** (112 mg, 0.12 mmol, 43%) as a white solid (2 TFA salt). ^{1}H NMR (MeOD, 600 MHz): δ 1.31 (d, *J* = 6.1 Hz, 6H), 1.34 (dd, *J* = 6.1 Hz, *J* = 1.8 Hz, 6H), 1.52 (d, *J* = 7.1 Hz, 3H), 3.87 (q, *J* = 7.1 Hz, 1H), 4.32–4.36 (m, 2H), 4.43–4.47 (m, 2H), 4.48–4.53 (m, 2H), 4.75–4.80 (m, 2H), 5.91–5.99 (m, 1H), 6.27 (d, *J* = 4.1 Hz, 1H), 6.29–6.33 (m, 1H), 8.42 (s, 1H), 8.62 (s, 1H). ^{19}F NMR (MeOD, 470 MHz): δ –111.5 (m), –111.2 (m). ^{31}P NMR (MeOD, 202 MHz): δ 4.2 (t, *J* = 114.6 Hz). ^{13}C NMR (MeOD, 150 MHz): δ 17.3, 24.0 (d, *J* = 4.9 Hz, 2C), 24.3 and 24.4 (d, *J* = 1.2 Hz), 52.0, 70.2, 70.5, 72.2, 75.9 (d, *J* = 7.3 Hz, 2C), 83.7, 85.0, 88.8, 117.8 (dt, *J* = 252.8 Hz, *J* = 225.1 Hz), 120.4, 122.6 (dt, *J* = 21.9 Hz, *J* = 13.8 Hz), 136.9 (dt, *J* = 9.8 Hz, *J* = 5.6 Hz), 143.4, 146.3, 149.8, 152.4, 174.1. HPLC purity: (77.6%, t_R = 10.53 min). HRMS-ESI (m/z) calcd for $C_{23}H_{37}F_2N_7O_{10}PS$ $[M+H]^+$ 672.2028, found 672.2036.

*2´,3´-O-isopropylidene-5´-N-(3-amino-cyclobut-3-ene-1,2-dione)aminodeoxyadenosine (**20**)*. To a solution of **19** (0.92 g, 3 mmol, 1 equiv.), prepared as previously described [29], in MeOH (20 mL) was added dimethyl squarate (0.89 g, 6.26 mmol, 2 equiv.) at 20 °C. The mixture was stirred for 4 h and concentrated under reduced pressure. The residue was dissolved in MeOH (70 mL) and a solution of NH_3 (7 N) in MeOH (4.87 mL) was added at 20 °C. The mixture was stirred for 16 h and the reaction mixture was filtered. The solid was washed with pentane to give compound **20** (0.58 g, 1.45 mmol,

 doi:10.20944/preprints202505.1714.v1

48%) as a white foam. The product was used in the next step without further purification. ^{1}H NMR (DMSO-d$_6$, 500 MHz): δ 1.32 (s, 3H), 1.54 (s, 3H), 3.70–3.76 (m, 1H), 3.94 (br s, 1H), 4.24–4.27 (m, 1H), 5.00–5.02 (m, 1H), 5.43 (s, 1H), 6.20 (s, 1H), 7.37 (br s, 2H), 7.44 (br s, 1H), 8.19 (s, 1H), 8.33 (s, 1H). ^{13}C NMR (DMSO-d$_6$, 150 MHz): δ 25.7, 27.5, 27.3, 45.6, 81.6, 83.6, 89.2, 114.2, 119.6, 140.4, 149.2, 153.4, 156.6, 170.1, 183.8.

*2´,3´-O-isopropylidene-5´-N-(N-tert-butoxycarbonyl-D-alanyl]-(3amino-cyclobut-3-ene-1,2-dione))aminodeoxy adenosine (**21**).* The general procedure B was followed with compound **20** (200 mg, 0.5 mmol), Boc-D-Ala-OSu (180 mg, 0.63 mmol) and DBU (0.19ml, 1.2mmol) in DMF (5 mL) at 60 °C for 4h. The crude mixture was purified by column chromatography (Eluent EtOAc/MeOH 9:1) to give compound **21** (140 mg, 0.244 mmol, 49%) as a white solid. ^{1}H NMR (DMSO-d$_6$, 500 MHz): δ 1.19–1.20 (m, 5H), 1.31 (s, 3H), 1.36 (s, 18H), 1.53 (s, 3H), 3.69–3.74 (m, 1H), 3.81–3.87 (m, 2H), 4.23–4.27 (m, 1H), 5.01–5.03 (m, 1H), 5.42 (s, 1H), 6.18 (s, 1H), 6.87 (br s, 1H), 7.36 (s, 1H), 7.85 (br s, 1H), 8.17 (s, 1H), 8.33 (s, 1H). ^{13}C NMR (DMSO-d$_6$, 150 MHz): δ 18.1, 25.7, 27.5, 28.6, 45.5, 49.7, 78.2, 81.7, 83.2, 83.6, 89.2, 113.8, 114.2, 119.6, 140.3, 149.3, 153.4, 155.5, 156.6, 170.2, 175.5, 183.7. HRMS-ESI(m/z) calcd for $C_{25}H_{33}N_8O_8$ [M+H]$^+$ 573.2421 found 573.2428.

*5´-N-(N-D-alanyl]-(3-amino-cyclobut-3-ene-1,2-dione))aminodeoxy adenosine salt (**22**).* The general procedure C (Method B) was followed with compound **21** (210 mg, 0.37 mmol), TFA (5 mL) and CH_2Cl_2/H_2O (4/1 mL). The crude mixture was purified by HPLC RP-C18 (MeCN/H_2O, linear gradient) to give compound **22** (100.8 mg, 0.153 mmol, 64%) as a white solid (2 TFA salt). ^{1}H NMR (DMSO-d$_6$, 500 MHz): δ 1.38-1.43 (m, 3H), 3.86-3.94 (m, 1H), 4.05-4.13 (m, 3H),4.18-4.23 (m, 1H), 4.61-4.65 (m, 1H), 5.93 (d, *J* = 5.5 Hz, 1H), 7.80 (br s, 1H), 8.10 (br s, 2H), 8.26 (br s, 2H), 8.28 (s, 1H), 8.43 (s, 1H), 11.98 (s, 1H). ^{13}C NMR (DMSO-d$_6$, 150 MHz): δ 16.6, 45.8, 48.5, 70.8, 73.1, 83.2,87.7, 119.1, 140.7, 148.9, 150.1, 154.1, 169.3, 172.1, 172.8, 181.4, 189.3. HPLC purity: (97.1%, t_R = 3.10 min). HRMS-ESI (m/z) calcd for $C_{17}H_{21}N_8O_6$ [M+H]$^+$ 433.1584, found 433.1585.

*tert-butyl (tert-butoxycarbonyl)(9-((3aR,4R,6R,6aR)-6-(((tert-butoxycarbonyl)(N-((tert-butoxycarbonyl)-D-alanyl)sulfamoyl)amino)methyl)-2,2-dimethyltetrahydrofuro[3,4-d][1,3]dioxol-4-yl)-9H-purin-6-yl)carbamate (**25**).* The general procedure B was followed with compound **23** (1.2 g, 1.76 mmol), prepared as previously described [30], Boc-D-Ala-OSu (640 mg, 2.23 mmol) and DBU (0.6 mL, 4.22 mmol) in DMF (25 mL). The crude was purified by flash chromatography (Eluent CH_2Cl_2/MeOH 9:1) to give compound **25** (1.1 g, 1.33 mmol, 76%) as a white foam. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 1.32 (d, *J* = 7.4 Hz, 3H), 1.39 (s, 3H), 1.43 (s, 18H), 1.44 (s, 9H), 1.62 (s, 3H), 4.04–4.15 (m, 3H), 4.20 (dd, *J* = 15.4 Hz, *J* = 5.9 Hz, 1H), 4.53–4.58 (m, 1H), 4.83–4.95 (m, 1H), 5.13–5.17 (m, 1H), 5.38–5.41 (m, 1H), 6.18 (d, *J* = 2.1 Hz, 1H), 8.21 (s, 1H), 8.87 (s, 1H). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 16.4, 25.6, 27.4, 27.9, 28.0, 28.4, 50.3, 82.4, 83.9, 84.5, 85.3, 85.4, 90.7, 114.9, 129.6, 144.0, 150.5, 150.7, 152.4, 152.7, 171.7. HRMS-ESI (m/z) calcd for $C_{31}H_{48}N_8O_{12}S$ [M+H]$^+$ 757.3191, found 757.3193.

*tert-butyl (tert-butoxycarbonyl)(9-((2R,3R,4R,5R)-5-(((tert-butoxycarbonyl)(N-((tert-butoxycarbonyl)-D-alanyl)sulfamoyl)amino)methyl)-4-((tert-butyldimethylsilyl)oxy)-3-fluorotetrahydrofuran-2-yl)-9H-purin-6-yl)carbamate (**26**).* The general procedure B was followed with compound **24** (185 mg, 0.24 mmol), prepared as previously described [30], Boc-D-Ala-OSu (70 mg, 0.24 mmol) and DBU (55 µL, 0.36 mmol) in DMF (3.3 mL). The crude was purified by column chromatography (Eluent Pentane/EtOAc 1:1) to give compound **26** (173 mg, 0.18 mmol, 77%) as a white foam. ^{1}H NMR (CD_3CN, 600 MHz): δ 0.16 (s, 3H), 0.19 (s, 3H), 0.95 (s, 9H), 1.19 (d, *J* = 7.2 Hz, 3H), 1.25 (s, 9H), 1.40 (s, 18H), 1.41 (s, 9H), 3.97–4.03 (m, 2H), 4.14–4.17 (m, 1H), 4.34–4.35 (m, 1H), 4.80–4.83 (m, 1H), 5.53–5.63 (m, 1H), 5.77 (br s, 1H), 6.30 (dd, *J* = 17.5 Hz, *J* = 2.4 Hz, 1H), 8.44 (s, 1H), 8.81 (s, 1H). ^{19}F NMR (CD_3CN, 470 MHz): δ –205.6 (m). ^{13}C NMR (CD_3CN, 150 MHz): δ –4.7, –4.5, 18.2, 18.7, 26.1, 27.9, 28.1, 28.7, 50.4, 53.3, 73.0 (d, *J* = 15.9 Hz), 80.9, 83.1, 85.0, 85.9, 88.3 (d, *J* = 32.9 Hz), 93.3 (d, *J* = 190.8 Hz), 130.1, 146.0, 151.0, 151.5, 152.8, 153.6, 155.6, 157.2, 179.5. HRMS-ESI (m/z) calcd for $C_{39}H_{66}FN_8O_{13}SiS$ [M+H]$^+$ 933.4223, found 933.4211.

*(R)-2-amino-N-(N-(((2R,3S,4R,5R)-5-(6-amino-9H-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl)sulfamoyl)propenamide salt (**27**).* The general procedure C (Method B) was followed with compound **25** (528 mg, 0.62 mmol), TFA (7.5 mL) and CH_2Cl_2/H_2O (6/1.5 mL). The crude was purified by HPLC RP-C18 (MeCN/H_2O, linear gradient) to give compound **27** (219 mg, 0.34 mmol, 55%) as a

 doi:10.20944/preprints202505.1714.v1

white solid (2 TFA salt). ^{1}H NMR (D_2O, 500 MHz): δ 1.52 (d, *J* = 7.2 Hz, 3H), 3.37–3.38 (m, 2H), 3.97 (q, *J* = 7.2 Hz, 1H), 4.31–4.34 (m, 1H), 4.41–4.44 (m, 1H), 4.80–4.83 (m, 1H), 6.04 (d, *J* = 5.8 Hz, 1H), 8.37 (s, 1H), 8.39 (s, 1H). ^{13}C NMR (D_2O, 125 MHz): δ 16.4, 44.3, 50.4, 71.0, 73.5, 83.7, 89.1, 119.3, 142.8, 147.1, 148.2, 151.7, 173.0. HRMS-ESI (m/z) calcd for $C_{13}H_{20}N_8O_6S$ $[M+H]^+$ 417.1305, found 417.1304.

*(R)-2-amino-N-(N-(((2R,3R,4R,5R)-5-(6-amino-9H-purin-9-yl)-4-fluoro-3-hydroxytetrahydrofuran-2-yl)methyl)sulfamoyl)propenamide salt (**28**).* The general procedure C (Method B) was followed with compound **26** (173 mg, 0.18 mmol), TFA (2.3 mL) and CH_2Cl_2/H_2O (2/0.5 mL) to give compound **28** (105 mg, 0.16 mmol, 88%) as a white solid (2 TFA salt). ^{1}H NMR (D_2O, 500 MHz): δ 1.47 (d, *J* = 7.2 Hz, 3H), 3.33 (dd, *J* = 14.3 Hz, *J* = 4.5 Hz, 1H), 3.42 (dd, *J* = 14.3 Hz, *J* = 2.8 Hz, 1H), 3.85 (q, *J* = 7.2 Hz, 1H), 4.28–4.32 (m, 1H), 4.70 (ddd, *J* = 18.3 Hz, *J* = 7.2 Hz, *J* = 4.7 Hz, 1H), 5.50 (ddd, *J* = 52.1 Hz, *J* = 4.7 Hz, *J* = 2.4 Hz, 1H), 6.34 (dd, *J* = 17.3 Hz, *J* = 2.4 Hz, 1H), 8.28 (s, 1H), 8.32 (s, 1H). ^{19}F NMR (D_2O, 470 MHz): δ –203.9 (dt, *J* = 52.1 Hz, *J* = 17.3 Hz, 1F). ^{13}C NMR (D_2O, 125 MHz): 16.6, 43.4, 51.0, 69.5 (d, *J* = 17.1 Hz), 81.6, 87.1 (d, *J* = 33.9 Hz), 93.0 (d, *J* = 187.9 Hz), 119.1, 141.3, 148.3, 150.6, 154.1, 175.3. HPLC purity: (98.3%, t_R = 2.08 min). HRMS-ESI (m/z) calcd for $C_{13}H_{19}FN_8O_5S$ $[M+H]^+$ 419.1261, found 419.1264.

*tert-butyl (E)-(tert-butoxycarbonyl)(9-(2,4-difluoro-3-(hydroxymethyl)but-2-en-1-yl)-9H-purin-6-yl)carbamate (**30**).* A solution of compound **29** (247 mg, 0.48 mmol, 1 equiv.), prepared as previously described [20], in *t*-BuOH (5 mL) was added CsF (111 mg, 0.96 mmol, 2.0 equiv.). The reaction mixture was stirred at 70 °C for 4 h and the solvent was removed under reduced pressure. The residue was taken up with EtOAc/H_2O and the aqueous layers was extracted three times with EtOAc. The combined organic layers were washed with brine, dried over $MgSO_4$, filtered and concentrated under reduced pressure. The crude product was purified by column chromatography (Eluent Pentane/EtOAc 1:1 to 6:4) to give compound **30** (93 mg, 0.21 mmol, 43%) as a colorless oil. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 1.44 (s, 18H), 2.33 (br s, 1H), 4.34 (s, 2H), 5.14 (dd, *J* = 21.7 Hz, *J* = 1.8 Hz, 2H), 5.35 (dd, *J* = 47.8 Hz, *J* = 2.2 Hz, 2H), 8.14 (s, 1H), 8.85 (s, 1H). ^{19}F NMR ($CDCl_3$, 470 MHz): δ –107.4 (t, *J* = 21.7 Hz), –212.1 (t, *J* = 47.8 Hz). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 27.9, 41.0 (d, *J* = 29.6 Hz), 56.3 (dd, *J* = 8.7 Hz, *J* = 1.2 Hz), 78.6 (dd, *J* = 166.7 Hz, *J* = 10.1 Hz), 84.1, 118.8 (dd, *J* = 15.5 Hz, *J* = 13.4 Hz), 128.5, 144.5, 150.5, 150.6, 152.5, 153.2, 153.7 (dd, *J* = 269.6 Hz, *J* = 10.8 Hz). HRMS-ESI (m/z) calcd for $C_{20}H_{28}F_2N_5O_5$ $[M+H]^+$ 456.2059, found 456.2054.

*tert-butyl (E)-(tert-butoxycarbonyl)(9-(4-((tert-butoxycarbonyl)(sulfamoyl)amino)-2-fluoro-3-(fluoromethyl)but-2-en-1-yl)-9H-purin-6-yl)carbamate (**32**).* The general procedure D was followed with compound **30** (120 mg, 0.26 mmol), *tert*-butyl sulfamoylcarbamate (155 mg, 0.78 mmol), triphenylphosphine (102 mg, 0.39 mmol) and diisopropyl azodicarboxylate (0.05 mL, 0.27 mmol) in THF dry (5.2 mL). The crude product was purified by column chromatography (EtOAc/Pentane 1:2) to give compound **32** (123 mg, 0.19 mmol, 74%) as a white foam. ^{1}H NMR ($CDCl_3$, 600 MHz): δ 1.44 (s, 9H), 1.46 (s, 18H), 4.57 (br s, 2H), 5.16 (d, *J* = 21.1 Hz, 2H), 5.33 (d, *J* = 47.8 Hz, 2H), 5.40 (br s, 2H), 8.15 (s, 1H), 8.85 (s, 1H). ^{19}F NMR ($CDCl_3$, 565 MHz): δ –103.1 (t, *J* = 21.1 Hz), –208.1 (t, *J* = 47.8 Hz). ^{13}C NMR ($CDCl_3$, 150 MHz): δ 27.9, 28.1, 41.1 (d, *J* = 30.1 Hz), 43.1 (d, *J* = 8.4 Hz), 79.6 (dd, *J* = 165.9 Hz, *J* = 10.4 Hz), 84.1, 85.3, 114.9 (dd, *J* = 13.9 Hz, *J* = 12.1 Hz), 128.6, 144.4, 150.6, 150.7, 151.9, 152.6, 153.1, 155.6 (dd, *J* = 263.6 Hz, *J* = 11.7 Hz). HRMS-ESI (m/z) calcd for $C_{25}H_{38}F_2N_7O_8S$ $[M+H]^+$ 634,2471 found 634.2474.

*tert-butyl (E)-(tert-butoxycarbonyl)(9-(4-((N-((tert-butoxycarbonyl)-D-alanyl)sulfamoyl)amino)-2-fluoro-3-(fluoromethyl)but-2-en-1-yl)-9H-purin-6-yl)carbamate (**33**).* The general procedure B was followed with compound **32** (130 mg, 0.20 mmol), Boc-D-Ala-OSu (59 mg, 0.20 mmol) and DBU (46 µL, 0.31 mmol) in DMF (2.6 mL). The crude was purified by column chromatography (Eluent EtOAc/Pentane 1:1 to 1:0) to give compound **33** (100 mg, 0.12 mmol, 61%) as a white foam. ^{1}H NMR ($CDCl_3$, 600 MHz): δ 1.31 (d, *J* = 6.9 Hz, 3H), 1.40 (s, 9H), 1.44 (s, 18H), 1.45 (s, 18H), 4.69 (br s, 2H), 5.17 (d, *J* = 21.3 Hz, 2H), 5.26 (d, *J* = 47.8 Hz, 2H), 8.18 (s, 1H), 8.86 (s, 1H). ^{19}F NMR ($CDCl_3$, 565 MHz): δ –103.9–103.6 (m), –209.6 (t, *J* = 47.8 Hz). ^{13}C NMR ($CDCl_3$, 150 MHz): δ 18.1, 27.8, 27.9, 28.4, 41.1 (d, *J* = 29.4 Hz), 44.1, 52.1, 78.4 (dd, *J* = 166.4 Hz, *J* = 9.6 Hz), 80.1, 84.1, 84.4, 116.2 (m), 128.4, 144.8, 150.2, 150.6, 152.3, 152.7, 153.3, 154.6 (dd, *J* = 262.3 Hz, *J* = 10.9 Hz), 156.0, 177.7. HRMS-ESI (m/z) calcd for $C_{33}H_{51}F_2N_8O_{11}S$ $[M+H]^+$ 805.3366, found 805.3391.

 doi:10.20944/preprints202505.1714.v1

*(R,E)-2-amino-N-(N-(4-(6-amino-9H-purin-9-yl)-3-fluoro-2-(fluoromethyl)but-2-en-1-yl)sulfamoyl)propenamide salt (**34**)*. The general procedure C (Method B) was followed with compound **33** (100 mg, 0.12 mmol), TFA (1.4 mL) and CH_2Cl_2/H_2O (1.2/0.3 mL) to give compound **34** (76 mg, 0.11 mmol, 90%) as a white solid (2 TFA salt). ^{1}H NMR (MeOD, 600 MHz): δ 1.52 (d, *J* = 7.1 Hz, 3H), 3.88 (br s, 2H), 3.92 (q, *J* = 7.1 Hz), 5.28 (dd, *J* = 20.6 Hz, *J* = 1.6 Hz, 2H), 5.33 (dd, *J* = 47.6 Hz, *J* = 1.8 Hz, 2H), 8.24 (s, 1H), 8.32 (s, 1H). ^{19}F NMR ($CDCl_3$, 565 MHz): δ -105.5 (m), -211.3 (m).^{13}C NMR (MeOD, 150 MHz): δ 17.1, 39.0 (d, *J* = 9.1 Hz), 41.8 (d, *J* = 28.8 Hz), 50.5, 79.3 (dd, *J* = 163.1 Hz, *J* = 9.2 Hz), 116.6 (dd, *J* = 15.1 Hz, *J* = 12.6 Hz), 118.1 (q, *J* = 292.1 Hz), 119.7, 143.8, 149.8, 150.6, 154.6, 156.5 (dd, *J* = 261.7 Hz, *J* = 10.8 Hz), 163.4 (q, *J* = 32.4 Hz), 170.1. HPLC purity: (95.8%, t_R = 3.23 min). HRMS-ESI (m/z) calcd for $C_{13}H_{19}N_8O_3F_2S$ $[M+H]^+$ 405.1269, found 405.1271.

*tert-butyl ((R)-1-(1-(((2R,3R,4R,5R)-5-(6-amino-9H-purin-9-yl)-4-fluoro-3-hydroxytetrahydrofuran-2-yl)methyl)-1H-1,2,3-triazol-4-yl)-1-oxopropan-2-yl)carbamate (**39**)*. The general procedure E was followed with compound **38** (410 mg, 1.39 mmol), prepared as previously described [31], alkyne **S6** (295 mg, 1.53 mmol), sodium ascorbate (28 mg, 0.14 mmol) and $CuSO_4.5H_2O$ (17 mg, 0.07 mmol) in *t*-BuOH/H_2O (9 mL). The crude was purified by column chromatography (Eluent EtOAc/MeOH 94:6) to give compound **39** (470 g, 0.94 mmol, 68%) as a white solid. ^{1}H NMR (DMSO-d_6, 500 MHz): δ 1.26 (d, *J* = 7.4 Hz, 3H), 1.32 (s, 9H), 4.34 (dt, *J* = 7.4 Hz, *J* = 3.2 Hz, 1H), 4.69–4.77 (m, 1H), 4.82 (dd, *J* = 14.8 Hz, *J* = 7.4 Hz, 1H), 4.88 (dd, *J* = 14.8 Hz, *J* = 2.8 Hz, 1H), 4.89–4.95 (m, 1H), 5.50 (ddd, *J* = 52.6 Hz, *J* = 4.5 Hz, *J* = 1.3 Hz, 1H), 6.08 (d, *J* = 6.4 Hz, 1H), 6.24 (dd, *J* = 20.6 Hz, *J* = 1.3 Hz, 1H), 7.24 (d, *J* = 6.8 Hz, 1H), 7.33 (br s, 2H), 8.10 (s, 1H), 8.23 (s, 1H), 8.60 (s, 1H). ^{19}F NMR (DMSO-d_6, 470 MHz): δ –201.2 (dt, *J* = 52.6 Hz, *J* = 20.6 Hz). ^{13}C NMR (DMSO-d_6, 125 MHz): δ 16.6, 28.1, 51.0, 52.3, 69.7 (d, *J* = 16.3 Hz), 78.0, 80.0, 86.5 (d, *J* = 34.8 Hz), 93.0 (d, *J* = 186.5 Hz), 119.2, 128.7, 140.0, 144.6, 148.6, 152.7, 155.2, 156.1, 193.4. HRMS-ESI (m/z) calcd for $C_{20}H_{27}FN_9O_5$ $[M+H]^+$ 492.2119, found 492.2123.

*N-((R)-1-(1-(((3aR,4R,6R,6aR)-6-(6-amino-9H-purin-9-yl)-2,2-dimethyltetrahydrofuro[3,4-d][1,3]dioxol-4-yl)methyl)-1H-1,2,3-triazol-4-yl)-1-oxopropan-2-yl)heptanamide (**40**)*. The general procedure E was followed with compound **37** (400 mg, 1.20 mmol), prepared as previously described [32], alkyne **S7** (277 mg, 1.32 mmol), sodium ascorbate (24 mg, 0.12 mmol) and $CuSO_4.5H_2O$ (15 mg, 0.06 mmol) in *t*-BuOH/H_2O (8 mL). The crude was purified by column chromatography (Eluent EtOAc/MeOH 1:0 to 98:2) to give compound **40** (511 mg, 0.95 mmol, 79%) as a brown foam. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 0.80 (t, *J* = 7.0 Hz, 3H), 1.19–1.25 (m, 6H), 1.35 (s, 3H), 1.44 (d, *J* = 7.6 Hz, 3H), 1.56 (s, 3H), 2.18 (t, *J* = 7.0 Hz, 2H), 4.56–4.61 (m, 1H), 4.84–4.86 (m, 2H), 5.18–5.20 (m, 1H), 5.38–5.39 (m, 1H), 5.44–5.52 (m, 1H), 6.07 (s, 1H), 6.61 (s, 1H), 6.67–6.72 (m, 1H), 7.86 (s, 1H), 8.03 (s, 1H), 8.25 (s, 1H). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 14.1, 18.8, 22.5, 25.4, 25.6, 27.2, 28.9, 31.6, 36.6, 52.0, 81.8, 84.1, 84.2, 85.3, 90.7, 115.1, 120.4, 128.3, 140.4, 145.3, 148.8, 152.9, 155.9, 172.9, 193.4. HRMS-ESI (m/z) calcd for $C_{25}H_{36}N_9O_5$ $[M+H]^+$ 542.2839, found 542.2841.

*N-((R)-1-(1-(((3aR,4R,6R,6aR)-6-(6-amino-9H-purin-9-yl)-2,2-dimethyltetrahydrofuro[3,4-d][1,3]dioxol-4-yl)methyl)-1H-1,2,3-triazol-4-yl)-1-oxopropan-2-yl)-[1,1'-biphenyl]-4-carboxamide (**41**)*. The general procedure E was followed with compound **37** (400 mg, 1.20 mmol), prepared as previously described [32], alkyne **S8** (367 mg, 1.32 mmol), sodium ascorbate (24 mg, 0.12 mmol) and $CuSO_4.5H_2O$ (15 mg, 0.06 mmol) in *t*-BuOH/H_2O (8 mL). The crude was purified by column chromatography (Eluent EtOAc/MeOH 1:0 to 99:1) to give compound **41** (561 mg, 0.92 mmol, 77%) as a brown foam. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 1.33 (s, 3H), 1.55 (s, 3H), 1.59–1.62 (m, 3H), 4.56–4.61 (m, 1H), 4.80–4.88 (m, 1H), 5.17–5.19 (m, 1H), 5.36–5.37 (m, 1H), 5.66–5.73 (m, 1H), 6.06 (br s, 1H), 6.62 (br s, 2H), 7.31–7.34 (m, 1H), 7.38–7.41 (m, 2H), 7.50–7.58 (m, 5H), 7.85–7.88 (m, 3H), 8.04–8.05 (m, 1H), 8.27 (br s, 1H). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 18.9, 25.4, 27.1, 29.7, 51.9, 52.7, 81.7, 84.1, 85.3, 90.6, 115.1, 120.4, 127.1 (2C), 127.2 (2C), 127.7 (2C), 128.4, 128.9 (2C), 132.8, 140.0, 140.2, 144.2, 145.2, 148.8, 153.3, 156.1, 166.6, 193.2. HRMS-ESI (m/z) calcd for $C_{31}H_{32}N_9O_5$ $[M+H]^+$ 610.2526, found 610.2531.

*N-((R)-1-(1-(((3aR,4R,6R,6aR)-6-(6-amino-9H-purin-9-yl)-2,2-dimethyltetrahydrofuro[3,4-d][1,3]dioxol-4-yl)methyl)-1H-1,2,3-triazol-4-yl)-1-oxopropan-2-yl)-4-(hexyloxy)benzamide (**42**)*. The general procedure E was followed with compound **37** (400 mg, 1.20 mmol), prepared as previously described [32], alkyne **S9** (398 mg, 1.32 mmol), sodium ascorbate (24 mg, 0.12 mmol) and $CuSO_4.5H_2O$

 doi:10.20944/preprints202505.1714.v1

(15 mg, 0.06 mmol) in *t*BuOH/H_2O (8 mL). The crude was purified by column chromatography (Eluent EtOAc/MeOH 99:1) to give compound **42** (617 mg, 0.97 mmol, 81%) as a brown foam. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 0.87–0.90 (m, 3H), 1.30–1.36 (m, 4H), 1.40 (s, 3H), 1.41–1.45 (m, 2H), 1.58–1.61 (m, 6H), 1.72–1.79 (m, 2H), 3.92–3.97 (m, 2H), 4.59–4.61 (m, 1H), 4.84–4.91 (m, 2H), 5.13–5.20 (m, 1H), 5.33–5.39 (m, 1H), 5.64–5.70 (m, 1H), 6.06 (br s, 1H), 6.82–6.88 (m, 2H), 7.75–7.77 (m, 2H), 7.88 (s, 1H), 8.04 (s, 1H), 8.28 (s, 1H). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 14.1, 19.1, 22.7, 25.4, 25.8, 27.2, 29.2, 31.6, 52.1, 52.7, 68.3, 81.8, 84.2, 85.4, 90.7, 114.2 (2C), 115.2, 120.5, 126.2, 128.4, 129.0 (2C), 129.7, 140.6, 145.4, 149.2, 152.6, 162.0, 166.4, 193.4. HRMS-ESI (m/z) calcd for $C_{31}H_{40}N_9O_6$ $[M+H]^+$ 634.3102, found 634.3104.

*4-nitrobenzyl ((R)-1-(1-(((3aR,4R,6R,6aR)-6-(6-amino-9H-purin-9-yl)-2,2-dimethyltetrahydrofuro[3,4-d][1,3]dioxol-4-yl)methyl)-1H-1,2,3-triazol-4-yl)-1-oxopropan-2-yl)carbamate (**43**).* The general procedure E was followed with compound **37** (400 mg, 1.20 mmol), prepared as previously described [32], compound **S10** (365 mg, 1.32 mmol), sodium ascorbate (24 mg, 0.12 mmol) and $CuSO_4.5H_2O$ (15 mg, 0.06 mmol) in *t*-BuOH/H_2O (8 mL). The crude was purified by column chromatography (Eluent EtOAc/MeOH 1:0 to 99:1) to give compound **43** (470 mg, 0.77 mmol, 64%) as a brown foam. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 1.37 (s, 3H), 1.53 (t, *J* = 7.6 Hz, 3H), 1.58 (s, 3H), 4.58–4.61 (m, 1H), 4.87–4.88 (m, 2H), 5.13–5.22 (m, 3H), 5.32–5.35 (m, 1H), 5.40–5.42 (m, 1H), 6.04–6.07 (m, 1H), 6.35 (br s, 2H), 7.46–7.47 (m, 2H), 7.88 (s, 1H), 7.97 (s, 1H), 8.11–8.16 (m, 1H), 8.29 (s, 1H). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 19.1, 25.4, 27.2, 29.8, 52.1, 53.7, 65.3, 81.9, 84.2, 85.4, 90.8, 115.2, 123.8 (2C), 128.1 (2C), 128.2, 140.6, 144.1, 145.2, 147.6, 152.9, 155.3, 155.8, 193.1. HRMS-ESI (m/z) calcd for $C_{26}H_{29}N_{10}O_8$ $[M+H]^+$ 609.2170, found 609.2173.

*(R)-2-amino-1-(1-(((2R,3R,4R,5R)-5-(6-amino-9H-purin-9-yl)-4-fluoro-3-hydroxytetrahydrofuran-2-yl)methyl)-1H-1,2,3-triazol-4-yl)propan-1-one salt (**44**).* The general procedure C (Method B) with compound **39** (380 mg, 0.77 mmol), TFA (9.5 mL) and CH_2Cl_2/H_2O (8.7/1.8 mL) to give compound **44** (470 mg, 0.68 mmol, 88%) as a white solid (2 TFA). ^{1}H NMR (D_2O, 500 MHz): δ 1.65 (d, *J* = 7.2 Hz, 3H), 4.58–4.61 (m, 1H), 4.73 (ddd, *J* = 22.7 Hz, *J* = 9.2 Hz, *J* = 4.4 Hz, 1H), 4.93 (q, *J* = 7.2 Hz, 1H), 5.00 (d, *J* = 3.9 Hz, 2H), 5.58 (dd, *J* = 51.6 Hz, *J* = 4.8 Hz, 1H), 6.42 (d, *J* = 20.3 Hz, 1H), 8.23 (s, 1H), 8.30 (s, 1H), 8.55 (s, 1H). ^{19}F NMR (D_2O, 470 MHz): δ –201.5 (ddd, *J* = 51.6 Hz, *J* = 22.7 Hz, *J* = 20.3 Hz, 1F). ^{13}C NMR (D_2O, 150 MHz): δ 16.2, 50.1, 52.6, 69.3 (d, *J* = 16.9 Hz), 79.6, 87.3 (d, *J* = 36.1 Hz), 92.8 (d, *J* = 186.1 Hz), 115.3, 118.7, 130.5, 142.8, 145.6, 147.7, 150.7, 189.7. HPLC purity: (98.5%, t_R = 10.24 min). HRMS-ESI (m/z) calcd for $C_{15}H_{19}FN_9O_3$ $[M+H]^+$ 392.1595, found 392.1594.

*N-((R)-1-(1-(((2R,3S,4R,5R)-5-(6-amino-9H-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl)-1H-1,2,3-triazol-4-yl)-1-oxopropan-2-yl)heptanamide salt (**45**).* The general procedure C (Method A) was followed with compound **40** (250 mg, 0.46 mmol), HCl aq (1 mL, 3 M) and MeOH (2.5 mL). The crude was precipitated in MeOH/Et_2O to give compound **45** (219 mg, 0.41 mmol, 89%) as a white solid (HCl salt). ^{1}H NMR (DMSO-d_6, 500 MHz): δ 0.83 (t, *J* = 7.1 Hz, 3H), 1.18–1.24 (m, 6H), 1.27–1.29 (m, 3H), 1.41–1.44 (m, 2H), 2.06–2.10 (m, 2H), 4.27–4.29 (m, 1H), 4.37–4.40 (m, 1H), 4.62–4.64 (m, 1H), 4.85–4.91 (m, 2H), 5.11–5.16 (m, 1H), 5.98 (d, *J* = 5.2 Hz, 1H), 8.27 (br s, 1H), 8.54 (s, 1H), 8.74 (s, 1H), 8.77 (s, 1H), 9.07 (br s, 1H), 9.78 (br s, 1H). ^{13}C NMR (DMSO-d_6, 125 MHz): δ 14.0, 16.7, 22.0, 25.2, 28.3, 31.1, 34.8, 50.9, 51.7, 70.8, 73.4, 82.5, 88.3, 118.9, 128.9, 142.8, 144.7, 144.9, 148.2, 150.3, 172.1, 193.0. HPLC purity: (93.6 %, t_R = 10.83 min). HRMS-ESI (m/z) calcd for $C_{22}H_{32}N_9O_5$ $[M+H]^+$ 502.2526, found 502.2527.

*N-((R)-1-(1-(((2R,3S,4R,5R)-5-(6-amino-9H-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl)-1H-1,2,3-triazol-4-yl)-1-oxopropan-2-yl)-[1,1'-biphenyl]-4-carboxamide salt (**46**).* The general procedure C (Method A) was followed with compound **41** (250 mg, 0.41 mmol), HCl aq (1 mL, 3 M) and MeOH (2.5 mL). The crude was precipitated in MeOH/Et_2O to give compound **46** (205 mg, 0.34 mmol, 83%) as a white solid (HCl salt). ^{1}H NMR (DMSO-d_6, 500 MHz): δ 1.46–1.48 (m, 3H), 4.29–4.31 (m, 1H), 4.39–4.43 (m, 1H), 4.63 (dt, *J* = 7.8 Hz, *J* = 5.1 Hz, 1H), 5.87–5.94 (m, 2H), 5.36–5.42 (m, 1H), 5.99–6.01 (m, 1H), 7.39–7.42 (m, 1H), 7.47–7.51 (m, 2H), 7.72–7.78 (m, 4H), 7.97–8.00 (m, 2H), 8.53 (d, *J* = 1.6 Hz, 1H), 8.75 (d, *J* = 4.8 Hz, 1H), 8.79 (d, *J* = 9.8 Hz, 1H), 8.90 (d, *J* = 6.2 Hz, 1H), 8.97 (br s, 1H), 9.67 (br s, 1H). ^{13}C NMR (DMSO-d_6, 125 MHz): δ 16.4, 51.7, 52.0, 70.8, 73.4, 82.5, 88.3, 118.9, 126.4, (2C), 126.9 (2C), 128.1, 128.3 (2C), 128.8, 129.1 (2C), 132.5, 139.1, 142.7, 142.9, 144.8, 145.3, 148.3, 150.5, 165.9, 192.7. HPLC purity: (98.8 %, t_R = 12.32 min). HRMS-ESI (m/z) calcd for $C_{28}H_{28}N_9O_5$ $[M+H]^+$ 570.2213, found 570.2214.

 doi:10.20944/preprints202505.1714.v1

*N-((R)-1-(1-(((2R,3S,4R,5R)-5-(6-amino-9H-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl)-1H-1,2,3-triazol-4-yl)-1-oxopropan-2-yl)-4-(hexyloxy)benzamide salt (**47**)*. The general procedure C (Method A) was followed with compound **42** (491 mg, 0.77 mmol), HCl aq (1.9 mL, 3 M) and MeOH (4.7 mL). The crude was precipitated in MeOH/Et_2O to give compound **47** (370 mg, 0.58 mmol, 76%) as a white solid (HCl salt). ^{1}H NMR (DMSO-d_6, 500 MHz): δ 0.87 (t, *J* = 7.0 Hz, 3H), 1.28–1.31 (m, 4H), 1.39–1.44 (m, 5H), 1.68–1.73 (m, 2H), 4.01 (t, *J* = 6.6 Hz, 2H), 4.28–4.30 (m, 1H), 4.38–4.42 (m, 1H), 4.61–4.64 (m, 1H), 4.88–4.91 (m, 1H), 5.31–5.38 (m, 1H), 5.98–6.00 (m, 1H), 6.95–6.98 (m, 2H), 7.84–7.86 (m, 2H), 8.53 (s, 1H), 8.65–8.66 (m, 1H), 8.74–8.75 (m, 1H), 8.76–8.78 (m, 1H), 8.96 (br s, 1H), 9.66 (br s, 1H). ^{13}C NMR (DMSO-d_6, 125 MHz): δ 13.9, 16.4, 22.1, 25.2, 28.6, 31.0, 51.6, 51.8, 67.7, 70.8, 73.3, 82.4, 88.3, 113.8 (2C), 118.9, 125.7, 128.8, 129.4 (2C), 142.7, 144.8, 145.3, 148.3, 150.1, 161.2, 165.7, 193.1. HPLC purity: (96.5 %, t_R = 14.41 min). HRMS-ESI (m/z) calcd for $C_{28}H_{36}N_9O_6$ $[M+H]^+$ 594.2789, found 594.2797.

*4-nitrobenzyl ((R)-1-(1-(((2R,3S,4R,5R)-5-(6-amino-9H-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl)-1H-1,2,3-triazol-4-yl)-1-oxopropan-2-yl)carbamate salt (**48**)*. The general procedure C (Method A) was followed with compound **43** (250 mg, 0.41 mmol), HCl aq (1 mL, 3 M) and MeOH (2.5 mL). The crude was precipitated in MeOH/Et_2O to give compound **48** (195 mg, 0.32 mmol, 79%) as a white solid (HCl salt). ^{1}H NMR (DMSO-d_6, 500 MHz): δ 1.34 (t, *J* = 7.4 Hz, 3H), 4.27–4.29 (m, 1H), 4.38–4.41 (m, 1H), 4.62–4.64 (m, 1H), 4.89–4.91 (m, 2H), 5.02–5.07 (m, 1H), 5.15 (s, 2H), 5.98 (d, *J* = 4.9 Hz, 1H), 7.59 (d, *J* = 8.1 Hz, 2H), 7.94 (d, *J* = 7.4 Hz, 1H), 8.22 (d, *J* = 8.1 Hz, 2H), 8.52 (s, 1H), 8.75 (s, 1H), 8.77 (s, 1H), 9.01 (br s, 1H), 9.69 (br s, 1H). ^{13}C NMR (DMSO-d_6, 125 MHz): δ 16.8, 51.6, 52.8, 64.2, 70.8, 73.4, 82.4, 88.3, 118.8, 123.5 (2C), 128.1 (2C), 128.9, 142.7, 144.5, 145.0, 145.1, 146.9, 148.2, 150.4, 155.5, 193.0. HPLC purity: (98.3 %, t_R = 10.74 min). HRMS-ESI (m/z) calcd for $C_{23}H_{25}N_{10}O_8$ $[M+H]^+$ 569.1857, found 569.1860.

3.4. Determination of the Binding Affinity (Molecular Docking)

The molecular docking was performed by AutoDock Vina software which uses non deterministic Docking Algorithm with 8 maximum exhaustiveness to determine the binding affinity for each ligand. Molecular docking was simulated with crystal structure of *Bacillus cereus* D-alanyl carrier protein ligase,[33] from Protein Data Bank (PDB ID: 3FFC) using Research Collaboratory for Structural Bioinformatics. Crystallization cofactors such as ATP, Magnesium as well as traces of residual water from the 3FCC structure were removed using PyMol software. Kollman and Gasteiger charges as well as the polar hydrogens were added respectively to the receptor and the ligands using the AutoDockTools software to prepare molecules in pdbqt format. The active site was defined by default through the ligand co crystallized in the 3FCC protein and the grid box was established with a dimension (Å) of X: 19.8903, Y: 20.2516 and Z: 24.5359 and for center X: 18.9110, Y: 9.0989 and Z: 13.3228. Docking scores listed are the average of nine different minimized energy states.

3.5. Determination of the Half-Maximal Inhibitory Concentration (IC_{50})

The DltA-catalyzed ATP-hydrolysis reaction was monitored *in vitro via* PPi release (DltA +D-alanine +ATP → DltA-DAla-AMP +PPi) using a coupled-enzyme assay. The purified DltA was pretreated with DltC purifiedfrom *E. coli* to clear D-Ala-AMP already present in the enzyme preparation after expression in *E. coli*. Holo-DltC is the natural protein partner of DltA, and DltC purified from *E. coli* BL21 (DE3) contained 51% of this active form of DltC as determined by HPLC analysis. DltA was pretreated with DltC (ratio 1:1.2), incubated 30 min at 37°C and 30 min at 4°C. Three successive washes were realized on Amicon Ultra-2 Centrifugal Filter Unit – Ultracel 30 membrane 30 kDa MWCO (Merck, Darmstadt, Germany) with elution buffer EB at 4°C. The components of the coupled enzyme assay included: 1.5 µM pre-treated DltA, 20 µM of ATP, and 14 µM of D-alanine. The half-maximal inhibitory concentration (IC_{50}) of potential DltA inhibitors was determined by the addition of different concentrations of the synthesized molecules. PPi release was determined using the PiPer – Pyrophosphate assay kit (Invitrogen, Waltham, MA, USA) according to the recommendation of the supplier. Reactions were carried out in 96-well Chimney Style, non-binding Microplates with µClear Film Bottom (Greiner Bio-one, Frickenhausen, Germany). Plates were incubated at 37°C for 5 h and read in FlexStation 3 Multi-Mode plate reader (Molecular Devices,

 doi:10.20944/preprints202505.1714.v1

Silicon Valley, CA, USA), with λ excitation of 530 nm and λ emission of 590 nm. Experiments were conducted in sextuplicate, and the IC_{50} was determined by linear regression of the curve.

3.6. MIC Determination

Colonies from BHI agar plates were suspended in saline solution (0.9% NaCl) and adjusted to an OD_{600} of 0.5. For growth kinetics determination, 96-Well microplates (Starlab, France) containing 200 µL of fresh BHI medium were inoculated with bacterial suspensions to an OD_{600} of 0.02 with a wide range of DltA inhibitor (from 0 to 2 mM). Plates were incubated at 37°C with shaking (orbital amplitude of 3 mm) in a microplate reader (InfiniteM Nano, Tecan). OD_{600nm} was measured every 10 min for 24 h. Determination of MIC/MBC of antibiotics and bacterial survival assays were performed as previously described using BHI medium [Ref]. The DltA inhibitor was diluted in pure water and added to the growth medium to a final concentration of 0, 0.1, 0.25, 0.5, 0.75, or 1 mM.

3.7. Quantification of D-alanyl Ester in the Bacterial Cell Wall

Ester-linked D-alanines were quantified as described previously [25], with the following modifications. Cultures were grown in 5 mL of BHI supplemented with DltA inhibitor to a final concentration of 0.05, 0.1, 0.25, 0.5, 0.75 or 1 mM for 16 h at 37 °C under shaking (120 rpm). Briefly, the quantification is based on the oxidation of a chromogen by H_2O_2 generated when free D-alanines are oxidized by a D-amino acid oxidase. Three independent experiments with duplicate samples were performed.

4. Conclusions

In this paper, new series of D-Ala-AMP analogues were synthesized and tested as adjuvants to re-sensitive MRSA to IPM. Modification of the 2'-position of adenosine was first envisaged to increase cell permeability and/or metabolic stability. Indeed, a fluorine atom, an azido group, a difluorophosphonylated allylic ether and a glucose moiety were successfully introduced. Squaramide, sulfamide and triazole derivatives were also prepared as phosphate surrogate as well as fluorinated transbutenyl acyclonucleosides as sugar mimic. Functionalization of the *N*-terminal moiety to facilitate bacterial penetration was also realized to produce a series of *N*-acylated derivatives. Binding affinity of each molecule was measured towards *B. Subtilis* DltA (PDB 3FCC) revealing docking scores closed to reference molecule **1**. Each new compound was tested *in vitro* as DltA inhibitor. Indeed, we observed **8**, **9**, **18**, **27**, **28**, **35** and **36**, containing modifications onto the carbohydrate or the linker, had IC_{50} values in the same order of magnitude as **1**. We also showed that **18** and **27** were able to re-sensitize MRSA to imipenem as efficiently as **1**. Quantification of D-alanyl esters confirmed that these two compounds reduced the level of bacterial cell wall D-alanyl residues by 50% and 80%. These new series represent a promising structure entry not reported yet for DltA inhibition and re-sensitization of bacteria towards known antibiotics. Further investigations will be realized to evaluate these new DltA inhibitors in MRSA-infected larvae of *Galleria mellonella* and will be reported in due course.

Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org, Experimental procedure for the preparation of **31**, **S1**-**S10**, ^{1}H, ^{13}C, ^{31}P and ^{19}F NMR spectra of compounds **5**-**9**, **10**, **11**, **13**-**18**, **20**-**22**, **25**-**28**, **32**-**34**, **39**-**48**, **S2**-**S5**, **S7**-**S10**, HPLC chromatogram for final products **8**, **9**, **11**, **18**, **22**, **27**, **28**, **34**, **44**-**48**.

Author Contributions: Conceptualization, E.P. and T.L.; methodology, D.L., X.F., A.M., D.C.; software, D.L. and A.M.; validation, E.P., T.L., N.V. and A.B.-V.; formal analysis, F.L.C.; investigation, D.L., X.F., A.M., D.C.; data curation, E.P., T.L., N.V. and A.B.-V.; writing—original draft preparation, E.P. and T.L.; writing—review and editing, E.P., T.L., N.V. and A.B.-V.; supervision, E.P., T.L., N.V. and A.B.-V.; project administration, E.P., T.L., N.V. and A.B.-V.; funding acquisition, E.P., T.L., N.V. and A.B.-V. All authors have read and agreed to the published version of the manuscript.

 doi:10.20944/preprints202505.1714.v1

Funding: This research was funded by the Agence National de la Recherche the DALATAR project (ANR-19-CE18-0008) and the excellence laboratory LabEx SYNORG (ANR-11-LABX-0029), the Conseil Régional de Normandie and ERDF funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data are contained within the article and Supplementary Materials.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. (a) Cook, M.A.; Wright, G.D. The Past, Present, and Future of Antibiotics. *Sci. Transl. Med.* **2022**, *14*, eabo7793, doi:10.1126/scitranslmed.abo7793. (b) Lima, L.M.; Silva, B.N.M.D.; Barbosa, G.; Barreiro, E.J. β-Lactam Antibiotics: An Overview from a Medicinal Chemistry Perspective. *European Journal of Medicinal Chemistry* **2020**, *208*, 112829, doi:10.1016/j.ejmech.2020.112829.
2. (a) Bhardwaj, S.; Mehra, P.; Dhanjal, D.S.; Sharma, P.; Sharma, V.; Singh, R.; Nepovimova, E.; Chopra, C.; Kuča, K. Antibiotics and Antibiotic Resistance- Flipsides of the Same Coin. *CPD* **2022**, *28*, 2312–2329, doi:10.2174/1381612828666220608120238. (b) Patangia, D.V.; Anthony Ryan, C.; Dempsey, E.; Paul Ross, R.; Stanton, C. Impact of Antibiotics on the Human Microbiome and Consequences for Host Health. *MicrobiologyOpen* **2022**, *11*, e1260, doi:10.1002/mbo3.1260. (c) Baran, A.; Kwiatkowska, A.; Potocki, L. Antibiotics and Bacterial Resistance—A Short Story of an Endless Arms Race. *IJMS* **2023**, **24**, 5777, doi:10.3390/ijms24065777.
3. (a) Giurazza, R.; Mazza, M.C.; Andini, R.; Sansone, P.; Pace, M.C.; Durante-Mangoni, E. Emerging Treatment Options for Multi-Drug-Resistant Bacterial Infections. *Life* **2021**, *11*, 519, doi:10.3390/life11060519. (b) Avershina, E.; Shapovalova, V.; Shipulin, G. Fighting Antibiotic Resistance in Hospital-Acquired Infections: Current State and Emerging Technologies in Disease Prevention, Diagnostics and Therapy. *Front. Microbiol.* **2021**, *12*, 707330, doi:10.3389/fmicb.2021.707330.
4. Mahoney, A.R.; Safaee, M.M.; Wuest, W.M.; Furst, A.L. The Silent Pandemic: Emergent Antibiotic Resistances Following the Global Response to SARS-CoV-2. *iScience* **2021**, *24*, 102304, doi:10.1016/j.isci.2021.102304.
5. Langford, B.J.; So, M.; Raybardhan, S.; Leung, V.; Soucy, J.P.R.; Westwood, D.; Daneman, N.; MacFadden, D.R.; Antibiotic prescribing in patients with COVID-19: rapid review and meta-analysis. *Clin Microbiol Infect*. **2021**, 27, 520-531. doi:10.1016/j.cmi.2020.12.018.
6. Murray, C.J.L.; Ikuta, K.S.; Sharara, F.; Swetschinski, L.; Robles Aguilar, G.; Gray, A.; Han, C.; Bisignano, C.; Rao, P.; Wool, E.; et al. Global Burden of Bacterial Antimicrobial Resistance in 2019: A Systematic Analysis. *The Lancet* **2022**, *399*, 629–655, doi:10.1016/S0140-6736(21)02724-0.
7. Kumar, V.; Yasmeen, N.; Pandey, A.; Ahmad Chaudhary, A.; Alawam, A.S.; Ahmad Rudayni, H.; Islam, A.; Lakhawat, S.S.; Sharma, P.K.; Shahid, M. Antibiotic Adjuvants: Synergistic Tool to Combat Multi-Drug Resistant Pathogens. Front. *Cell. Infect. Microbiol.* **2023**, *13*, 1293633, doi:10.3389/fcimb.2023.1293633.
8. Saar-Dover, R.; Bitler, A.; Nezer, R.; Shmuel-Galia, L.; Firon, A.; Shimoni, E.; Trieu-Cuot, P.; Shai, Y. D-Alanylation of Lipoteichoic Acids Confers Resistance to Cationic Peptides in Group B Streptococcus by Increasing the Cell Wall Density. *PLoS Pathog.* **2012**, *8*, e1002891, doi:10.1371/journal.ppat.1002891.
9. (a) Neuhaus, F.C.; Baddiley, J. A Continuum of Anionic Charge: Structures and Functions of d-Alanyl-Teichoic Acids in Gram-Positive Bacteria. *Microbiol. Mol. Biol. Rev.* **2003**, *67*, 686–723, doi:10.1128/MMBR.67.4.686-723.2003. (b) Schultz, B.J.; Snow, E.D.; Walker, S. Mechanism of D-Alanine Transfer to Teichoic Acids Shows How Bacteria Acylate Cell Envelope Polymers. *Nat. Microbiol.* **2023**, *8*, 1318–1329, doi:10.1038/s41564-023-01411-0.
10. Percy, M.G.; Gründling, A. Lipoteichoic Acid Synthesis and Function in Gram-Positive Bacteria. *Annu. Rev. Microbiol.* **2014**, *68*, 81–100, doi:10.1146/annurev-micro-091213-112949.
11. May, J.J.; Finking, R.; Wiegeshoff, F.; Weber, T.T.; Bandur, N.; Koert, U.; Marahiel, M.A. Inhibition of the D-alanine: D-alanyl Carrier Protein Ligase from Bacillus Subtilis Increases the

 doi:10.20944/preprints202505.1714.v1

Bacterium's Susceptibility to Antibiotics That Target the Cell Wall. *The FEBS Journal* **2005**, *272*, 2993–3003, doi:10.1111/j.1742-4658.2005.04700.x.

12. (a) Coupri, D.; Budin-Verneuil, A.; Hartke, A.; Benachour, A.; Léger, L.; Lequeux, T.; Pfund, E.; Verneuil, N. Genetic and Pharmacological Inactivation of D-Alanylation of Teichoic Acids Sensitizes Pathogenic Enterococci to β-Lactams. *Journal of Antimicrobial Chemotherapy* **2019**, *74*, 3162–3169, doi:10.1093/jac/dkz322. (b) Coupri, D.; Verneuil, N.; Hartke, A.; Liebaut, A.; Lequeux, T.; Pfund, E.; Budin-Verneuil, A. Inhibition of d-Alanylation of Teichoic Acids Overcomes Resistance of Methicillin-Resistant Staphylococcus Aureus. *Journal of Antimicrobial Chemotherapy* **2021**, *76*, 2778–2786, doi:10.1093/jac/dkab287. (c) Mahé, A.; Verneuil, N.; Coupri, D.; Hartke, A.; Cattoir, V.; Rincé, I.; Gueulle, S.; Feng, X.; Lequeux, T.; Pfund, E.; Budin-Verneuil, A, D-alanylation of lipoteichoic acids inhibitor provides anti-virulence and anti-resistance effects against methicillin-resistant *Staphylococcus epidermidis*. *Antimicrob Agents Chemother.* **2025**, 69:e01822-24, doi:10.1128/aac.01822-24.
13. Van De Vijver, P.; Ostrowski, T.; Sproat, B.; Goebels, J.; Rutgeerts, O.; Van Aerschot, A.; Waer, M.; Herdewijn, P. Aminoacyl-tRNA Synthetase Inhibitors as Potent and Synergistic Immunosuppressants. *J. Med. Chem.* **2008**, *51*, 3020–3029, doi:10.1021/jm8000746.
14. Thorens, B.; Mueckler, M. Glucose Transporters in the 21st Century. *American Journal of Physiology-Endocrinology and Metabolism* **2010**, *298*, E141–E145, doi:10.1152/ajpendo.00712.2009.
15. Pfund, E.; Dupouy, C.; Rouanet, S.; Legay, R.; Lebargy, C.; Vasseur, J.-J.; Lequeux, T. Difluorophosphonylated Allylic Ether Moiety as a 2′-Modification of RNA-Type Molecules: Synthesis, Thermal, and Metabolic Studies. *Org. Lett.* **2019**, *21*, 4803–4807, doi:10.1021/acs.orglett.9b01689.
16. Ceuninck, A.; Lequeux, T.; Pfund, E. Expanding the Scope of Group Transfer Radical Reaction: Toward the Synthesis of Fluorinated Nucleoside Analogues Incorporating Difluorophosphonylated Allylic Ether Moieties. *J. Org. Chem.* **2024**, *89*, 8099–8110, doi:10.1021/acs.joc.4c00763.
17. (a) Duckworth, B.P.; Geders, T.W.; Tiwari, D.; Boshoff, H.I.; Sibbald, P.A.; Barry, C.E.; Schnappinger, D.; Finzel, B.C.; Aldrich, C.C. Bisubstrate Adenylation Inhibitors of Biotin Protein Ligase from Mycobacterium Tuberculosis. *Chemistry & Biology* **2011**, *18*, 1432–1441, doi:10.1016/j.chembiol.2011.08.013. (b) Shuman, D.A.; Robins, R.K.; Robins, M.J. The Synthesis of Adenine 5'-O-Sulfamoyl Nucleosides Related to Nucleocidin. *J. Am. Chem. Soc.* **1969**, *9*, 3391–4000.
18. Evans, C.E.; Si, Y.; Matarlo, J.S.; Yin, Y.; French, J.B.; Tonge, P.J.; Tan, D.S. Structure-Based Design, Synthesis, and Biological Evaluation of Non-Acyl Sulfamate Inhibitors of the Adenylate-Forming Enzyme MenE. *Biochemistry* **2019**, *58*, 1918–1930, doi:10.1021/acs.biochem.9b00003.
19. Somu, R.V.; Boshoff, H.; Qiao, C.; Bennett, E.M.; Barry, C.E.; Aldrich, C.C. Rationally Designed Nucleoside Antibiotics That Inhibit Siderophore Biosynthesis of Mycobacterium tuberculosis. *J. Med. Chem.* **2006**, *49*, 31–34, doi:10.1021/jm051060o.
20. (a) Topalis, D.; Pradère, U.; Roy, V.; Caillat, C.; Azzouzi, A.; Broggi, J.; Snoeck, R.; Andrei, G.; Lin, J.; Eriksson, S.; Alexandre, J. A. C.; El-Amri, C.; Deville-Bonne, D.; Meyer, P.; Balzarini, J.; Agrofoglio, L. A. Novel Antiviral C5-Substituted Pyrimidine Acyclic Nucleoside Phosphonates Selected as Human Thymidylate Kinase Substrates. *J. Med. Chem.* **2011**, *54*, 222–232, doi:0.1021/jm1011462. (b) Leparfait, D.; Xiao, F.; Coupri, D.; Gueulle, S.; Desriac, F.; Budin-Verneuil, A.; Verneuil, N.; Hartke, A.; Pfund, E.; Lequeux, T. Oxetane Ring-Opening Reaction: A Key Step for the Preparation of Substituted (Fluoro)Alkenyl as Acyclonucleoside Mimics. *Eur. J. Org. Chem.* **2023**, *26*, e202300258, doi:10.1002/ejoc.202300258.
21. (a) Kim, D.W.; Ahn, D.-S.; Oh, Y.-H.; Lee, S.; Kil, H.S.; Oh, S.J.; Lee, S.J.; Kim, J.S.; Ryu, J.S.; Moon, D.H.; et al. A New Class of SN2 Reactions Catalyzed by Protic Solvents: Facile Fluorination for Isotopic Labeling of Diagnostic Molecules. *J. Am. Chem. Soc.* **2006**, *128*, 16394–16397, doi:10.1021/ja0646895. (b) Lee, J.-W.; Oliveira, M.T.; Jang, H.B.; Lee, S.; Chi, D.Y.; Kim, D.W.; Song, C.E. Hydrogen-Bond Promoted Nucleophilic Fluorination: Concept, Mechanism and Applications in Positron Emission Tomography. *Chem. Soc. Rev.* **2016**, *45*, 4638–4650, doi:10.1039/C6CS00286B.

 doi:10.20944/preprints202505.1714.v1

22. Davis, T.D.; Gerry, C.J.; Tan, D.S. General Platform for Systematic Quantitative Evaluation of Small-Molecule Permeability in Bacteria. *ACS Chem. Biol.* **2014**, *9*, 2535–2544, doi:10.1021/cb5003015.
23. Abramson, J.; Alder, J.; Dunger, J.; Evans, R.; Green, T.; Pritzel, A.; Ronneberger, O.; Willmore, L.; Ballard, A.J.; Bambrick, J.; Bodenstein, S.W.; Evans, D.A.; Hung, C.C.; O'Neil, M.; Reiman, D.; Tunyasuvunakool, K.; Wu, Z.; Zemgulyte, A.; Arvaniti, E.; Beattie, C.; Bertolli, O.; Bridgland, A.; Cherepanov, A.; Congreve, M.; Cowen-Rivers, A.I.; Cowie, A.; Figurnov, M.; Fuchs, F.B.; Gladman, H.; Jain, R.; Khan, Y.A.; Low, C.M.R.; Perlin, K.; Potapenko, A.; Savy, P.; Singh, S.;Stecula, A.; Thillaisundaram, A.; Tong, C.; Yakneen, S.; Zhong, E.D.; Zielinski, M.; Zidek, A.; Bapst, V.; Kohli, P.; Jaderberg, M.; Hassabis, D.; Jumper, J.M. Accurate structure prediction of biomolecular interactions with AlphaFold 3. *Nature* **2024**, *630*, 493-500, doi: 10.1038/s41586-024-07487-w.
24. Yonus, H.; Neumann, P.; Zimmermann, S.; May, J.J.; Marahiel, M.A., Stubbs, M.T. Crystal structure of DltA. Implications for the reaction mechanism of non-ribosomal peptide synthetase adenylation domains. *J. Biol. Chem.* **2008**, *283*, 32484-32491, doi: 10.1074/jbc.M800557200.
25. McBride, S.M.; Sonenshein, A.L. The Dlt Operon Confers Resistance to Cationic Antimicrobial Peptides in Clostridium Difficile. *Microbiology* **2011**, *157*, 1457–1465, doi:10.1099/mic.0.045997-0.
26. Gottlieb, H.E.; Kotlyar, V.; Nudelman, A. NMR Chemical Shifts of Common Laboratory Solvents as Trace Impurities. *J. Org. Chem.* **1997**, *62*, 7512–7515, doi:10.1021/jo971176v.
27. Lee, Seung-Jae; Hwang, Ki-Jun. A Convenient and Versatile Synthesis of 2′ (and 3′)-Amino (and Azido)-2′ (and 3′)-Deoxyadenosine as Diverse Synthetic Precursors of Cyclic Adenosine Diphosphate Ribose (cADPR). *Bulletin of the Korean Chemical Society.* **2004**, *25*, 243–248, doi:10.5012/BKCS.2004.25.2.243.
28. Morotti, A. L. M.; Lang, K. L.; Carvalho, I.; Schenkel, E. P.; Bernardes, L. S. C. Semi-Synthesis of New Glycosidic Triazole Derivatives of Dihydrocucurbitacin B. *Tetrahedron Letters* **2015**, *56* (2), 303–307, doi:10.1016/j.tetlet.2014.11.049.
29. Moreau, C.; Kirchberger, T.; Swarbrick, J. M.; Bartlett, S. J.; Fliegert, R.; Yorgan, T.; Bauche, A.; Harneit, A.; Guse, A. H.; Potter, B. V. L. Structure–Activity Relationship of Adenosine 5′-Diphosphoribose at the Transient Receptor Potential Melastatin 2 (TRPM2) Channel: Rational Design of Antagonists. *J. Med. Chem.* **2013**, *56* (24), 10079–10102, doi:10.1021/jm401497a.
30. Bockman, M. R.; Kalinda, A. S.; Petrelli, R.; De La Mora-Rey, T.; Tiwari, D.; Liu, F.; Dawadi, S.; Nandakumar, M.; Rhee, K. Y.; Schnappinger, D.; Finzel, B. C.; Aldrich, C. C. *J. Med. Chem.* **2015**, *58*, 7349–7369 doi:10.1021/acs.jmedchem.5b00719.
31. Dawadi, S.; Kawamura, S.; Rubenstein, A.; Remmel, R.; Aldrich, C. C. Synthesis and Pharmacological Evaluation of Nucleoside Prodrugs Designed to Target Siderophore Biosynthesis in Mycobacterium Tuberculosis. *Bioorganic & Medicinal Chemistry* **2016**, *24* (6), 1314–1321, doi:10.1016/j.bmc.2016.02.002.
32. Liu, F.; Austin, D. J. A General Synthesis of 5′-Azido-5′-Deoxy-2′,3′- *O* -Isopropylidene Nucleosides. *J. Org. Chem.* **2001**, *66* (25), 8643–8645, doi:10.1021/jo015800m.
33. Osman, K. T.; Du, L.; He, Y.; Luo, Y. Crystal Structure of Bacillus Cereus D-Alanyl Carrier Protein Ligase (DltA) in Complex with ATP. *Journal of Molecular Biology.* **2009**, *388* (2), 345–355, doi:10.1016/j.jmb.2009.03.040.