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Short Note

(*S*)-*N*-(*N*-(((2*R*,3*S*,4*R*,5*R*)-5-(6-Amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl)sulfamoyl)-5-oxopyrrolidine-2-carboxamide

Ravil N. Khaybullin, Xiao Liang and Xin Qi *

Department of Medicinal Chemistry, College of Pharmacy, University of Florida, Gainesville, FL 32610, USA; E-Mails: ravilx84@gmail.com (R.N.K.); xiao.liang@ufl.edu (X.L.)

* Author to whom correspondence should be addressed; E-Mail: xqi@cop.ufl.edu; Tel.: +1-352-294-5581; Fax: +1-352-392-9455.

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Abstract: Aminoacyl sulfamides represent a family of high-affinity inhibitors of the specific aminoacyl tRNA synthetase activity. In this paper we describe the synthesis of a novel sulfamide adenosine derivative **4** bearing pyroglutamyl fragment (pGlu-SA).

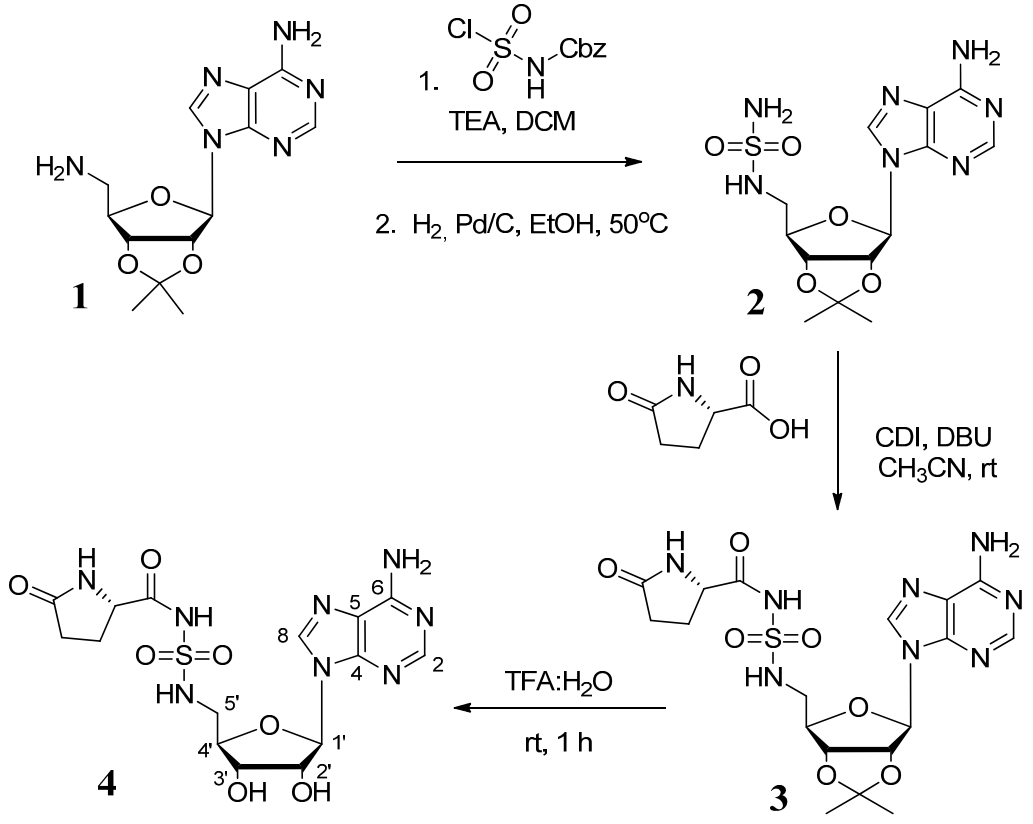
Keywords: aminoacyl sulfamide; tRNA synthetase inhibitor; pGlu-SA

The aminoacyl tRNA synthetase enzymes (aaRSs) are a large family of synthetases that play a fundamental role in protein biosynthesis processes [1]. All aaRSs use ATP to generate an activated form of the cognate amino acid—a mixed carbon-phosphorus anhydride termed aminoacyl adenylate (aaAMP), which is a key intermediate in protein biosynthesis. Because of their critical roles, the mimics of natural aaAMPs can serve as important aminoacyl tRNA synthetase inhibitors and consequently may possess significant biological activities such as anticancer or antiviral. Therefore, efficient synthetic strategies for obtaining these classes of compounds may facilitate research in these fields. One of the synthetic methods towards the aaAMP derivatives involves replacement of the high-energy mixed anhydride acylphosphate bond with the more stable and non-hydrolyzable bioisosteres [2]. For example, alkylphosphate [3], ester [4,5], hydroxamate [5], N-hydroxysulfamide [6], sulfamide [6,7], β-ketophosphonate [8], sulfamate [6,9], and sulfonamide [10] linkers have been used for that purpose.

Aminoacyl sulfamides are a class of stable structural mimics of aaAMPs and generally have submicromolar Ki values for their corresponding synthetases [11,12]. Although recently several types of synthetic derivatives of aminoacyl adenylates have been reported, novel aminoacyl sulfamides with improved therapeutics properties against various diseases hold great promise in drug development.

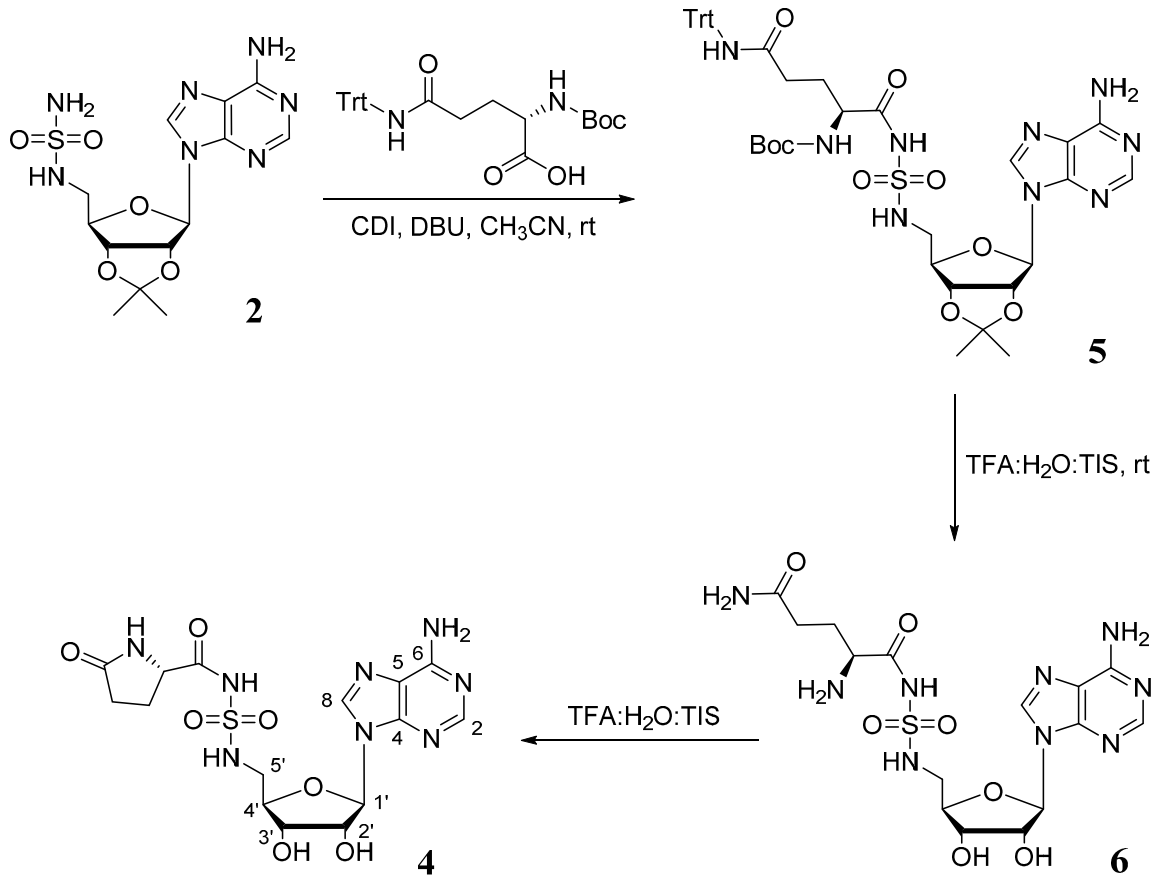
Recently we evaluated the potency of series of aminoacyl sulfamides for inhibition of protein translation and showed that these compounds significantly inhibited the activity of cognate aminoacyl tRNA synthetases [13]. Furthermore, in our current on-going efforts of anticancer and antiviral drug discovery, the series of aminoacyl sulfamide adenosine derivatives have been designed and synthesized. Herein we describe a novel sulfamide adenosine derivative **4** bearing pyroglutamyl fragment at the side chain position (Scheme 1). To the best of our knowledge, this is the first example of sulfamide adenosine derivatives with pyroglutamyl fragment.

The novel sulfamide **4** pGlu-SA was prepared as shown in Scheme 1 through the direct synthetic route. Briefly, the amine **1**, obtained following known procedures [14,15], was treated with N-carbobenzyloxysulfamoyl chloride and triethylamine in dichloromethane at ambient temperature. Cbz-protected sulfamide was then deprotected by hydrogenation using a 10% Pd/C catalyst in EtOH to afford 5′-deoxy-2′,3′-*O*-isopropylideneadenosine-5′-*N*-sulfamide **2**. The coupling reaction between intermediate **2** and L-pyroglutamic acid was carried out by using CDI activation of carboxylic group under basic condition. The resulting lactam adenosine conjugate **3** was purified by column chromatography. The acetonide group was further deprotected by treatment with aqueous TFA solution for 1 h. The pure target lactam **4** as a white solid was obtained after trituration with diethyl ether.



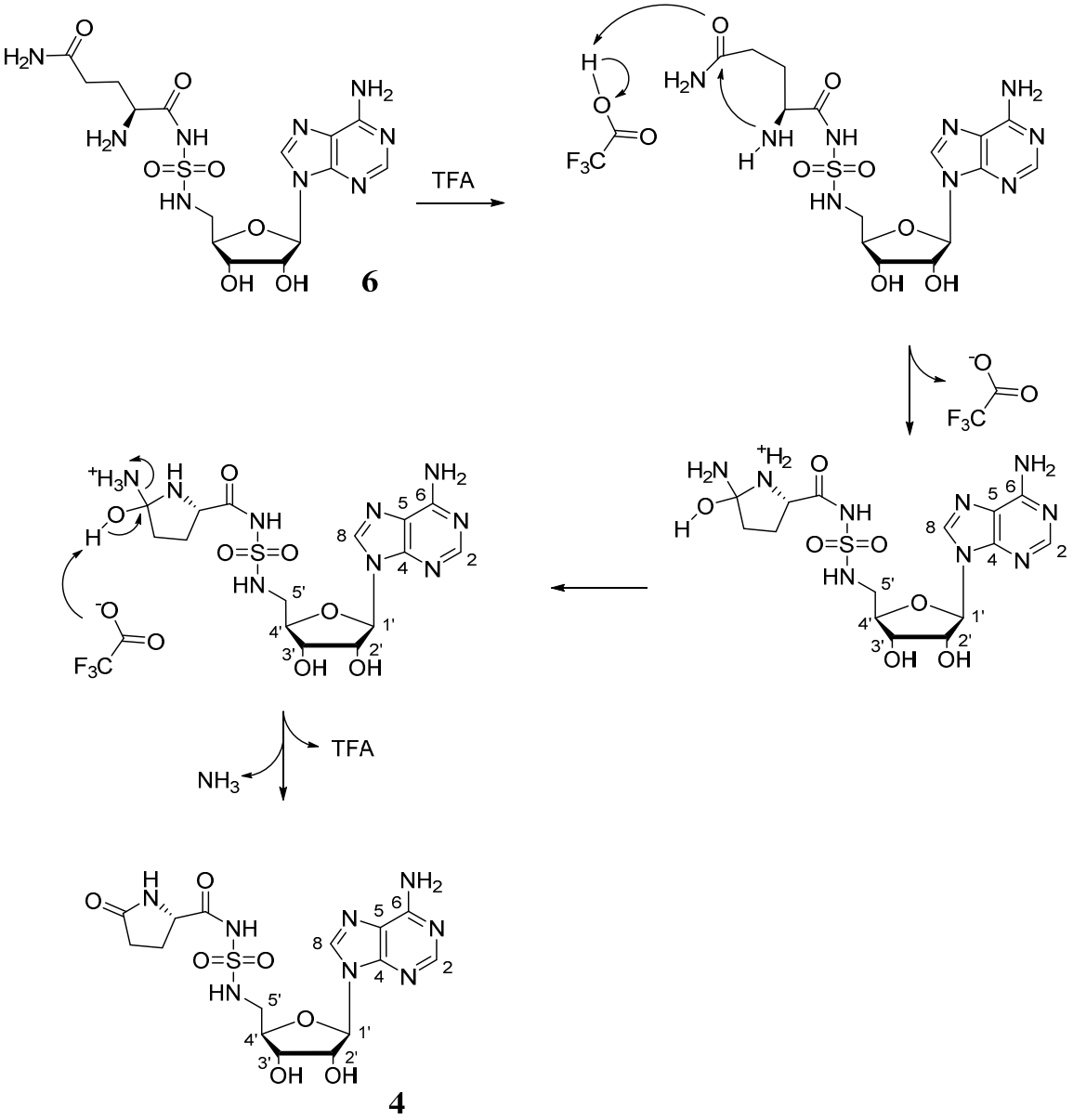
Scheme 1. Direct Synthetic Route of the pGlu-SA **4**.

Interestingly, during the process to synthesize glutamine sulfamide **6**, intramolecular cyclization product pGlu-SA **4** also was obtained (Scheme 2). The coupling reaction between intermediate **2** and Boc-L-Gln(Trt)-OH (Chem-Impex International, Wood Dale, IL, USA) was carried out by using CDI activation of carboxylic group under basic condition. The resulting conjugate **5** was further deprotected by treatment with aqueous TFA solution to take off Boc-, Trt- and acetonide groups simultaneously. Under this acidic condition, we observed the formation of pGlu-SA **4**. The mechanism involved in this process was proposed here that intramolecular cyclization to form pGlu-SA **4** from known glutamine sulfamide (Glu-SA) **6** was favorable under prolonged reaction time as well as increased temperature (Scheme 3).



Scheme 2. Indirect Synthetic Route of the pGlu-SA **4**.

In the current study, we showed that pGlu-SA can be synthesized efficiently from direct synthetic route. Furthermore, this novel sulfamide demonstrated potent inhibition to a panel of cancer cell lines with low micromolar IC_{50} using CellTiter-Glo luminescence viability assays (data not included [16]). Taken together, our discovery will lead to development of promising and potent anti-cancer therapeutics.



Scheme 3. Mechanism of pGlu-SA **4** formation from Glu-SA **6**.

Experimental Section

General Procedures

^{1}H and ^{13}C-NMR spectra were recorded on an Oxford Activated Shield NMR instrument (Varian, Inc., Palo Alto, CA, USA) operating at 400 MHz for ^{1}H, and 100 MHz for ^{13}C using D_2O as the solvent. Melting points were determined on a Fisher-Johns melting point apparatus and were uncorrected. All chemicals were purchased from Sigma Aldrich (St. Louis, MO, USA) or Acros (Pittsburgh, PA, USA) and were used as received, unless stated otherwise. High resolution mass spectra was acquired on a Thermo Scientific Q Exactive Plus Hybrid Quadrupole-Orbitrap mass spectrometer (Waltham, MA, USA) with electro spray ionization (ESI) mode. Reactions were carried out in oven-dried glassware under nitrogen atmosphere, unless otherwise noted. Analytical TLC was performed on E. Merck silica gel 60 F254 plates and visualized by UV and phosphomolybdic acid

(PMA) staining. Flash column chromatography was performed on E. Merck silica gel 60 (40–63 mm). Yields refer to chromatographically and spectroscopically pure compounds.

Direct Synthesis of pGlu-SA **4**

To a stirred solution of L-pyroglutamic acid (44 mg, 0.34 mmol) in dry acetonitrile/DMF mixture, the CDI (63 mg, 0.39 mmol) was added and the resulting mixture was stirred for an additional 30 min at room temperature. The above solution with activated L-pyroglutamic acid was added via syringe to a stirred suspension of sulfamide **2** (100 mg, 0.26 mmol) and DBU (55 mg, 0.36 mmol) in dry acetonitrile under nitrogen atmosphere. The reaction mixture was stirred at room temperature overnight and concentrated under reduced pressure. The pure compound **3** (obtained after column chromatography (silica gel, DCM/methanol 100:5; v/v)) was then treated with TFA:H_2O (5:2) mixture for 1 h at room temperature. The reaction mixture was concentrated under reduced pressure and residue was treated with Et_2O to afford 72 mg (61%) of pure lactam **4** as a white solid. This compound is readily soluble in water.

White solid. MP 170 °C (dec.)

^{1}H-NMR (D_2O, 400 MHz, ppm): δ 8.44 (s, 1H), 8.43 (s, 1H), 6.07 (d, J = 5.6 Hz, 1H), 4.83–4.75 (m, 1H), 4.49–4.40 (m, 1H), 4.39–4.22 (m, 2H), 3.44 (qd, J = 13.9, 4.0 Hz, 2H), 2.67–2.46 (m, 1H), 2.37 (t, J = 8.0 Hz, 2H), 2.19–2.00 (m, 1H).

^{13}C-NMR (D_2O, 100 MHz, ppm): δ 181.9, 173.5, 150.3, 147.8, 145.0, 143.3, 119.3, 89.3, 83.2, 73.3, 70.7, 56.9, 44.0, 28.9, 25.0.

HRMS (ESI): calcd. for $C_{15}H_{21}N_8O_7S$ $[M + H]^+$ 457.1254, found 457.1236.

Indirect Synthesis of pGlu-SA **4**

To a stirred solution of Boc-L-Gln(Trt)-OH (166 mg, 0.34 mmol) in dry acetonitrile, the CDI (63 mg, 0.39 mmol) was added and resulting yellow mixture was stirred additional 30 min at room temperature. The above solution with activated glutamine was added via syringe to a stirred suspension of sulfamide **2** (100 mg, 0.26 mmol) and DBU (55 mg, 0.36 mmol) in dry acetonitrile under nitrogen atmosphere. The reaction mixture was stirred at room temperature overnight and concentrated under reduced pressure. The pure compound **5** (187 mg, 0.20 mmol, 84% yield) obtained after column chromatography (silica gel, DCM/methanol 100:5; v/v) was then treated with TFA:H_2O (5:2) in presence of triisopropylsilane (5%) for 24 h at room temperature. The reaction mixture was concentrated under reduced pressure and the resulting residue was triturated with ether afforded white solid as crude products. The final separation and purification of target pGlu-SA **4** was performed by preparative HPLC (218TP510, 10 mm × 250 mm, Grace Corporate, Columbia, MD, USA), using a linear gradient of 0%–1% acetonitrile in 0.1% trifluoroacetic acid. After lyophilization the 23 mg (25% yield from **5**) of white solid lactam **4** was obtained.

Supplementary Materials

^{1}H-NMR (Figure S1), ^{13}C-NMR (Figure S2), and HRMS spectra (Figure S3) for titled compound pGlu-SA **4** are available in the supporting information.

Acknowledgments

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Author Contributions

R.K. carried out the synthetic work, characterized the sulfamide derivatives, and participated the manuscript preparation. X.L. performed part of the synthetic work and participated the manuscript preparation. X.Q. designed and performed the experiment, planned research, interpreted the results, and wrote the manuscript. All authors read and approved the final manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

References

1. Ibba, M.; Soll, D. Aminoacyl-tRNA synthesis. *Annu. Rev. Biochem.* **2000**, *69*, 617–650.
2. Kim, S.; Lee, S.W.; Choi, E.C.; Choi, S.Y. Aminoacyl-tRNA synthetases and their inhibitors as a novel family of antibiotics. *Appl. Microbiol. Biotechnol.* **2003**, *61*, 278–288.
3. Forrest, A.K.; Jarvest, R.L.; Mensah, L.M.; O'Hanlon, P.J.; Pope, A.J.; Sheppard, R.J. Aminoalkyl adenylate and aminoacyl sulfamate intermediate analogues differing greatly in affinity for their cognate staphylococcus aureus aminoacyl tRNA synthetases. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 1871–1874.
4. Lee, J.; Kang, S.U.; Kim, S.Y.; Kim, S.E.; Job, Y.J.; Kim, S. Vanilloid and isovanilloid analogues as inhibitors of methionyl-tRNA and isoleucyl-tRNA synthetases. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 965–968.
5. Lee, J.; Kang, S.U.; Kim, S.Y.; Kim, S.E.; Kang, M.K.; Jo, Y.J.; Kim, S. Ester and hydroxamate analogues of methionyl and isoleucyl adenylates as inhibitors of methionyl-tRNA and isoleucyl-tRNA synthetases. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 961–964.
6. Lee, J.; Kim, S.E.; Lee, J.Y.; Kim, S.Y.; Kang, S.U.; Seo, S.H.; Chun, M.W.; Kang, T.; Choi, S.Y.; Kim, H.O. *N*-Alkoxysulfamide, *N*-hydroxysulfamide, and sulfamate analogues of methionyl and isoleucyl adenylates as inhibitors of methionyl-tRNA and isoleucyl-tRNA synthetases. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 1087–1092.
7. Brown, P.; Richardson, C.M.; Mensah, L.M.; O'Hanlon, P.J.; Osborne, N.F.; Pope, A.J.; Walker, G. Molecular recognition of tyrosinyl adenylate analogues by prokaryotic tyrosyl tRNA synthetases. *Bioorg. Med. Chem.* **1999**, *7*, 2473–2485.

8. Balg, C.; Blais, S.P.; Bernier, S.; Huot, J.L.; Couture, M.; Lapointe, J.; Chenevert, R. Synthesis of beta-ketophosphonate analogs of glutamyl and glutaminyl adenylate, and selective inhibition of the corresponding bacterial aminoacyl-tRNA synthetases. *Bioorg. Med. Chem.* **2007**, *15*, 295–304.
9. Brown, M.J.; Mensah, L.M.; Doyle, M.L.; Broom, N.J.; Osbourne, N.; Forrest, A.K.; Richardson, C.M.; O'Hanlon, P.J.; Pope, A.J. Rational design of femtomolar inhibitors of isoleucyl tRNA synthetase from a binding model for pseudomonic acid-A. *Biochemistry* **2000**, *39*, 6003–6011.
10. Gadakh, B.; Smaers, S.; Rozenski, J.; Froeyen, M.; Van Aerschot, A. 5′-(N-aminoacyl)-sulfonamido-5′-deoxyadenosine: Attempts for a stable alternative for aminoacyl-sulfamoyl adenosines as aaRS inhibitors. *Eur. J. Med. Chem.* **2015**, *93*, 227–236.
11. Guo, M.; Schimmel, P. Structural analyses clarify the complex control of mistranslation by tRNA synthetases. *Curr. Opin. Struct. Biol.* **2012**, *22*, 119–126.
12. Tao, J.; Schimmel, P. Inhibitors of aminoacyl-tRNA synthetases as novel anti-infectives. *Expert Opin. Investig. Drugs* **2000**, *9*, 1767–1775.
13. Liang, X.; Khaybullin, R.; Fu, J.; Goncalves, K.; Xia, A.; Qi, X. Genetic code expansion using aminoacylated orthogonal tRNAs in conjunction with aminoacyl sulfamides. *Int. J. Recent Technol. Eng.* **2015**, *3*, 78–82.
14. Hill, J.M.; Kluge, A.F. Aminoacyl Sulfamides for the Treatment of Hyperproliferative Disorders. U.S. Patent 5824657, 20 October 1998.
15. Kolb, M.; Danzin, C.; Barth, J.; Claverie, N. Synthesis and biochemical properties of chemically stable product analogues of the reaction catalyzed by *S*-adenosyl-L-methionine decarboxylase. *J. Med. Chem.* **1982**, *25*, 550–556.
16. Qi, X.; Khaybullin, R.N.; Liang, X. Sulfamide Adenosine Derivatives and Uses Thereof. U.S. Patent Provisional Application No.: 62/174,362, 2015.