

## Parallel Solution-Phase Synthesis of an Adenosine Antibiotic Analog Library

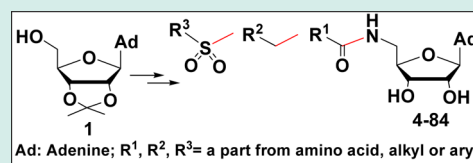
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## Supporting Information

**ABSTRACT:** A library of eighty one adenosine antibiotic analogs was prepared under the Pilot Scale Library Program of the NIH Roadmap initiative from 5'-amino-5'-deoxy-2',3'-O-isopropylidene-adenosine **3**. Diverse aldehyde, sulfonyl chloride and carboxylic acid reactant sets were condensed to **3**, in solution-phase fashion, leading after acid-mediated hydrolysis to the targeted compounds in good yields and high purity. No marked antituberculosis or anticancer activity was noted on preliminary cellular testing, but these nucleoside analogs should be useful candidates for other types of biological activity.

**KEYWORDS:** 5'-amino, carboxamide and sulfonyl-adenosine antibiotic analogs



## INTRODUCTION

Historically, nucleoside chemistry has primarily focused on low throughput production and screening of analogs for anticancer, antifungal, and antiviral antimetabolite activities.<sup>1–8</sup> Beyond the traditional nucleosides with an available 5'-hydroxyl that can enter nucleoside metabolic pathways, there are numerous examples of relatively simple to complex nucleoside antibiotics that exhibit diverse alternative mechanisms of action.<sup>9,10</sup> Natural nucleoside antibiotics, for example, demonstrate potent activities, such as protein synthesis inhibition, glycosyltransferase inhibition, and methyltransferase inhibition, among others.<sup>9–13</sup> More recently, there has been a trend to develop approaches for the synthesis and screening of this class of compounds, which do not exhibit typical antimetabolite activities based on nucleoside phosphorylation and incorporation into nucleoside metabolic pathways.<sup>14–18</sup> In many respects, the nucleoside scaffolds offer an opportunity for numerous diverse and directionally oriented substitutions to probe for activity against diverse protein binding sites. Furthermore, many of these basic scaffolds are not well represented in commercial chemical space, including the Molecular Library Small Molecule Repository (MLSMR) [see PubChem Substance at <http://www.ncbi.nlm.nih.gov/pcsubstance>]. Hence, we have designed and prepared nucleoside antibiotic-like small molecule libraries under the Pilot Scale Library Program of the NIH Roadmap Initiative to probe specific or general biological activities. We report herein the initial phase of this project which is the synthesis of a library of eighty one 5'-amido-, amino-, and sulfonylamido-adenosine analogs derived from 5'-amino-5'-deoxy-2',3'-O-isopropylidene-adenosine **3** (Scheme 1) using parallel solution phase chemistry.

Both **3** or its analog 5'-amino-5'-deoxy-adenosine are suitable precursors for the synthesis of a variety of biologically active nucleoside analogs<sup>19–36</sup> dating back to the first report describing the synthesis of 5'-amino-5'-deoxy-5'-N-aminoacyl peptide derivatives of guanosine, and adenosine and their effects on

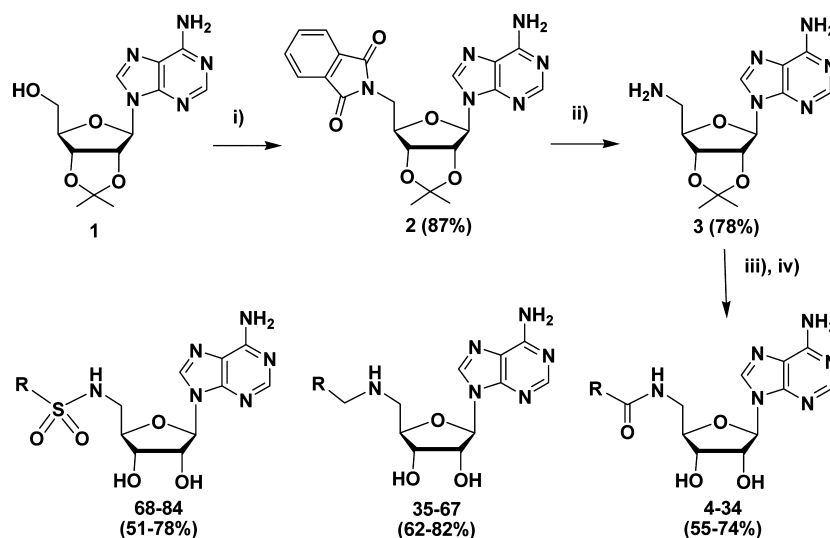
cell-free protein synthesis.<sup>37</sup> Hence, the 5'-amino group of the nucleoside ribose moiety was chosen as a site of diversification through robust peptidyl chemistry in order to prepare the target library of 5'-amido derivatives **4–34** (Scheme 1). The resulting nucleoside peptide library was expected to show reasonable stability to dissolution, storage and screening as evidenced for similar aminoacyl functions found in various nucleoside antibiotics, such as puromycin, gougerotin, ampicillin, and blasticidin S, all known inhibitors of protein synthesis. Thus, **3** was synthesized in two steps (Scheme 1) starting from 2',3'-O-isopropylidene adenosine **1**, which was reacted with triphenylphosphine, 1,3-dihydro-1,3-dioxo-2H-isoindole and diisopropyl azodicarboxylate to give 5'-deoxy-5'-(1,3-dihydro-1,3-dioxo-2H-isoindol-2-yl)-2',3'-O-isopropylidene adenosine **2**. Hydrazinolysis of **2** with hydrazine monohydrate in ethanol gave **3** in 78% yield according to the previously reported method.<sup>19</sup>

To achieve our targeted 5'-amido compounds **4–34** (Figure 1), we explored several methods for peptide coupling of **3** to diverse and readily available carboxylic acid-substituted or *N*-*tert*-butyloxycarbonyl (Boc) amino acid derivatives using 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU) and *N,N'*-dicyclohexylcarbodiimide (DCC). HATU [(2-(7-Aza-1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate] has been reported to rapidly provide the peptide linkage in high yield and with little or no racemization. In our hands, the best results were obtained with HATU (1 equiv) and *N,N*-diisopropylethylamine (DIEA, 1.5 equiv) in acetonitrile for 30–180 min. The utility of HATU allowed the facile preparation of the title compounds with a variety substituents and ready adaptation to a parallel format using a Radleys 12-place carousel reaction station on a 0.5 mmol scale. Subsequent acid-mediated deprotection of the acetone

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Scheme 1<sup>a</sup>

<sup>a</sup>Reagents and conditions: (i) phthalimide, PPh<sub>3</sub>, DIAD; (ii) H<sub>2</sub>NNH<sub>2</sub>, EtOH; (iii) R = a part from N-Boc amino acid, HATU, DIEA, CH<sub>3</sub>CN; R = a part from an aldehyde, MeOH, molecular sieves, 0–40 °C, NaBH<sub>4</sub>; R = a part from sulfonyl chloride, DMF, CsCO<sub>3</sub>; (iv) 50% formic acid, 70 °C.

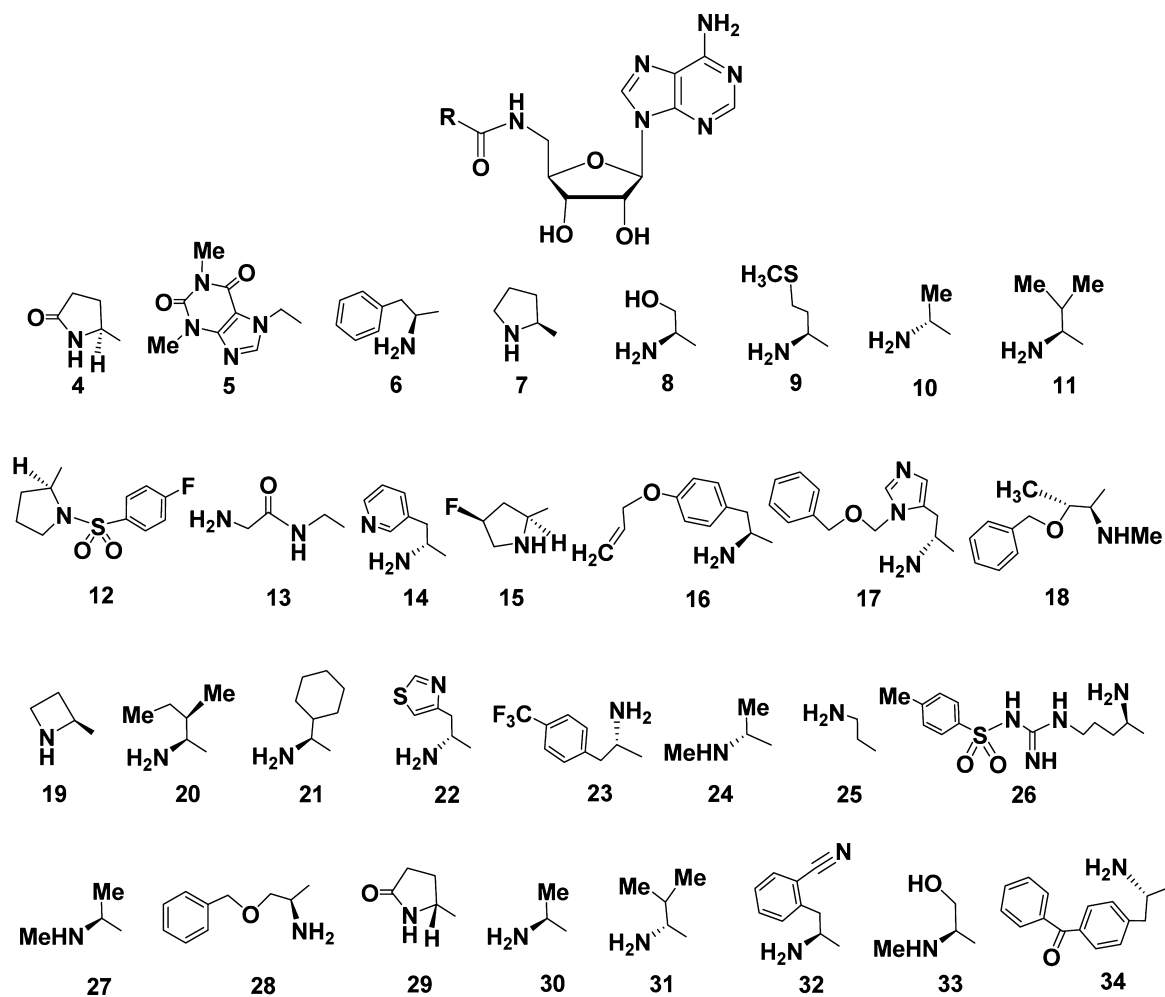


Figure 1. Structures of compounds 4–34.

as well as the Boc protecting group using 50% formic acid, again in a parallel format, furnished the desired compounds 4–34 in quantitative yields and high purity.

Next, we prepared a diverse small library of 5'-N-alkyl-5'-deoxy-adenosine derivatives 35–67 (Figure 2) based upon the important biological activity and stability exhibited by

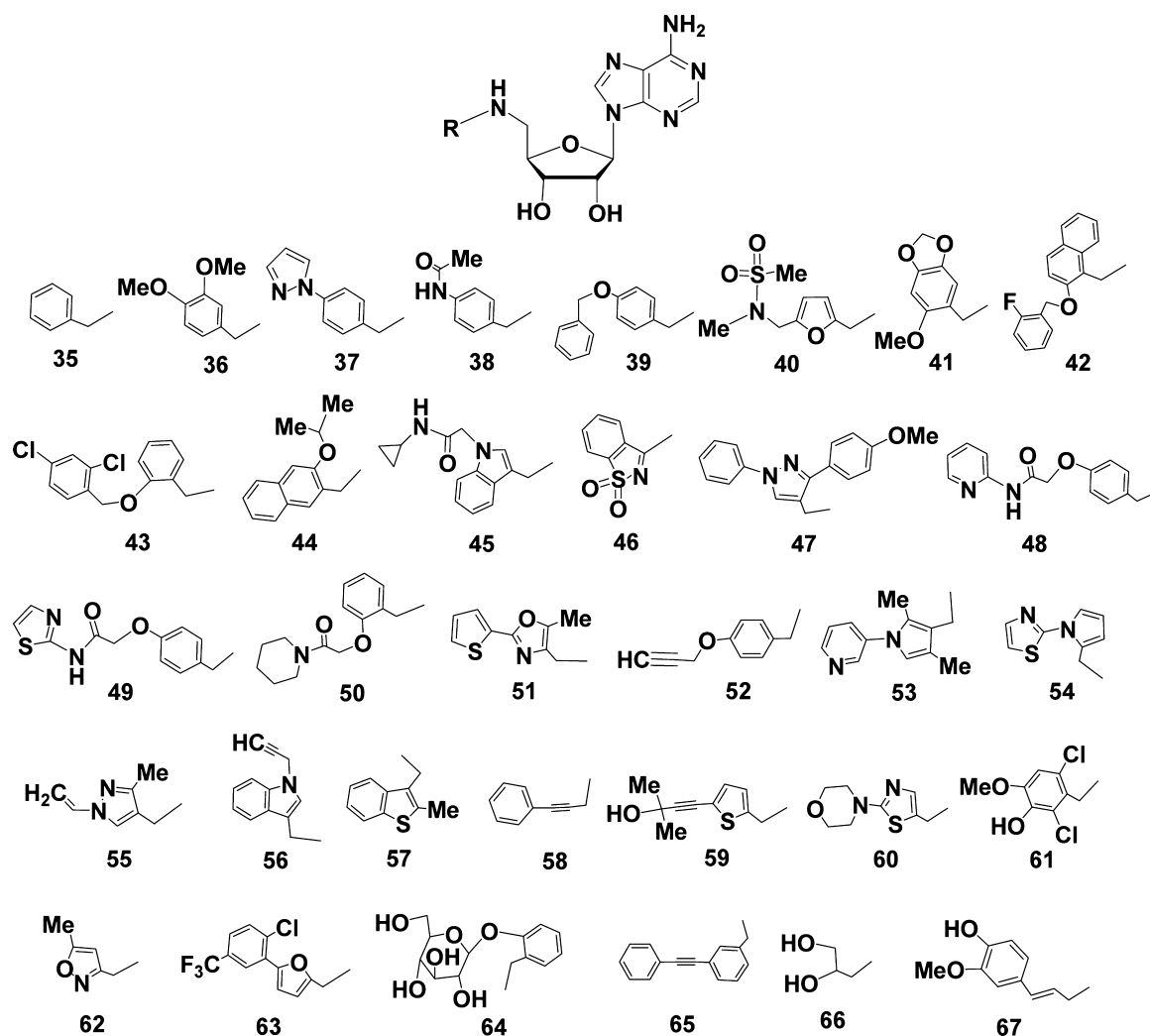


Figure 2. Structures of compounds 35–67.

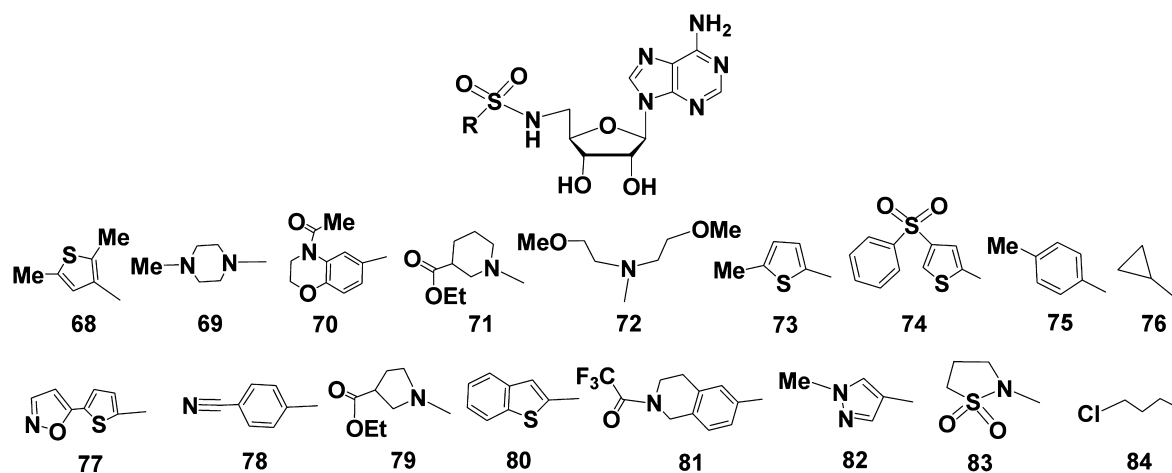


Figure 3. Structures of compounds 68–84.

known natural analogues. Reductive amination is an efficient method that is readily adaptable to parallel format and, hence, we adapted this reaction to couple compound 3 with thirty three commercially available aldehydes. Successful couplings were achieved in methanol in the presence of molecular sieves to efficiently drive intermediate imine formation. It must be

noted that the use of molecular sieves was crucial in terms of yield improvement and reaction time. The reaction was also readily adapted to a parallel format on a Radleys 12-place carousel reaction station at room temperature, although occasionally reactions were facilitated with less soluble aldehydes by warming for the first ten minutes at 40 °C.

The resulting aldimines were carefully treated in situ with solid sodium borohydride for one-half hour and the reaction was then preadsorbed and dried on silica gel without further workup followed by flash chromatography purification. Similarly, the hydrolysis of the isopropylidene blocking groups of the resulting intermediates using 50% formic acid was successful leading to the desired targets 35–67 in quantitative yields.

The synthesis of sulfonylamide adenosine analogs 68–84 (Figure 3) was achieved by reacting intermediate 3 with seventeen commercially available sulfonyl chlorides. The reactions were carried out in *N,N*-dimethylformamide (DMF) at room temperature using cesium carbonate as base followed by hydrolysis of the isopropylidene blocking groups as described above.

## BIOLOGICAL EVALUATION

The described analogs were tested in vitro for their effectiveness against *Mycobacterium tuberculosis* (*Mtb*).<sup>38</sup> None of the compounds showed appreciable activity at compound concentrations lower than 100  $\mu\text{M}$ . Only compound 42 exhibited slight inhibition with an  $\text{IC}_{50}$  of 43  $\mu\text{M}$ . Also, they were screened in vitro against three human tumor cell lines

(HT29 colon, PC3 prostate, and MDA-MB-231 breast). Only compounds 39, 42, and 43 showed toxicity at less than 50  $\mu\text{M}$  in these cells as shown in Table 1 (details of these biological studies are provided in Supporting Information).

All the prepared analogs have been submitted (20 mg) in the Molecular Libraries Small Molecule Repository (MLSMR) to be screened against a wide range of biological assays (see [www.ncbi.nlm.nih.gov/pcsubstance](http://www.ncbi.nlm.nih.gov/pcsubstance) search term Robert Reynolds). Certain analogs (Figure 4) exhibited interesting activities in these primary screens. For example, adenosine peptides 9 and 21 were found to be malaria HSP40-mediated yeast toxicity inhibitors; 16 was identified as an inhibitor of G-Protein coupled receptor kinase-2 (GRK2) (protein target = beta-adrenergic receptor kinase 1); 39 was identified as an activator of methionine sulfoxide reductase A (MsrA); compound 48 was found to be inhibitor of prion protein 5' UTR mRNA; 58 and 63 were identified as inhibitors of the histone demethylase GASC-1 (gene amplified in squamous cell carcinoma-1) and vitamin D receptor (VDR) respectively; sulfonylamide adenosine compound 71 inhibited the interaction of LANA 1–23 peptide with purified nucleosomes (containing H2A/H2B histone dimers) while the derivative 74 was found to inhibit scavenger receptor class B using DiI-HDL.

Table 1. Effect of Prepared Analogs on Cell Growth

compound	cancer screen		
	HT29 ( $\text{CC}_{50}$ ) <sup>a</sup>	PC3 ( $\text{CC}_{50}$ ) <sup>a</sup>	MDA ( $\text{CC}_{50}$ ) <sup>a</sup>
4–38, 40–41, and 44–84	>50	>50	>50
39	14	45	31
42	9	24	>50
43	12	>50	>50

<sup>a</sup> $\text{CC}_{50}$  = Concentrations of compound required for 50% growth inhibition of cancer cells.

## CONCLUSION

In conclusion, a small library of eighty one adenosine antibiotic-like analogs derived from 5'-amino-5'-deoxy-2',3'-*O*-isopropylideneadenosine was prepared in good yields and high purity using a parallel solution phase format. No marked anti-tuberculosis nor anticancer activity was witnessed and all the prepared analogs have been submitted for screening in the Molecular Libraries Probe Production Centers Network (MLPCN), preliminary screening via the MLPCN has

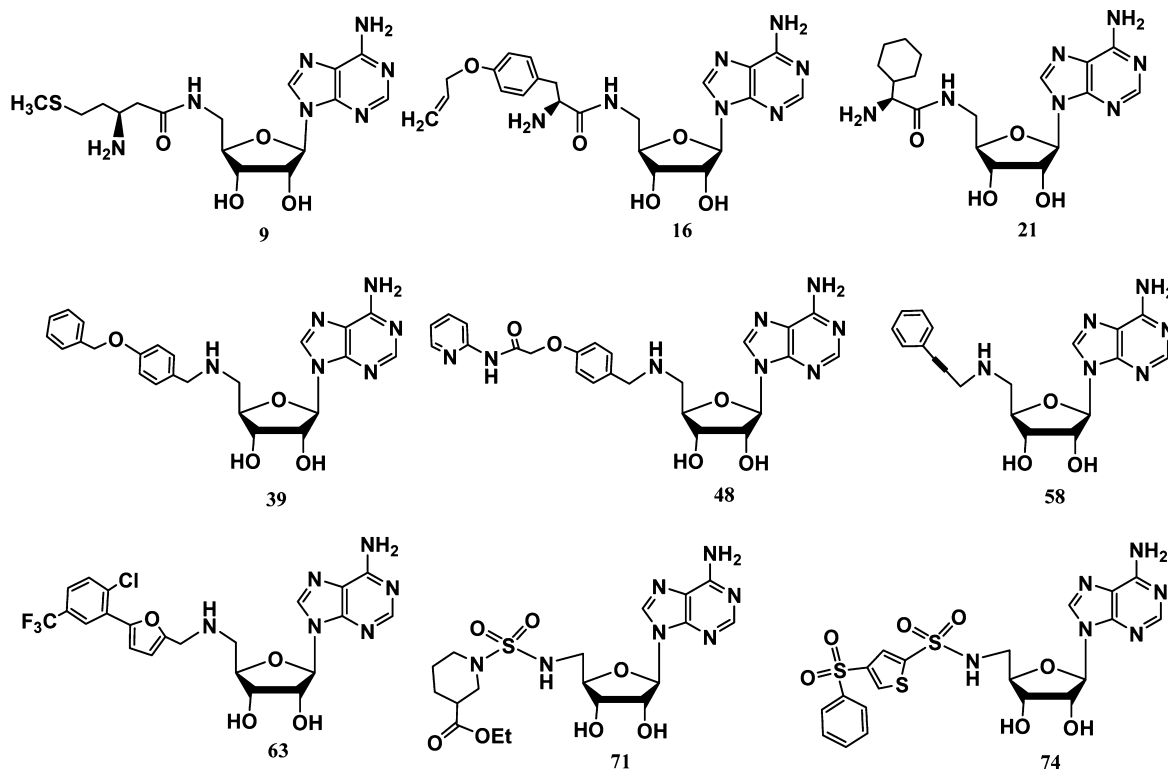


Figure 4. Some active adenosine analogues.

indicated a variety of interesting activities although full evaluation of the libraries is still under way (see <http://www.ncbi.nlm.nih.gov/pcsubstance> search term Robert Reynolds).

## ■ ASSOCIATED CONTENT

### Supporting Information

Additional material as described in the text. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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### Notes

The authors declare no competing financial interest.

## ■ ACKNOWLEDGMENTS

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