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Synthesis and photophysical characterization of 1- and 4-(purinyl) triazoles

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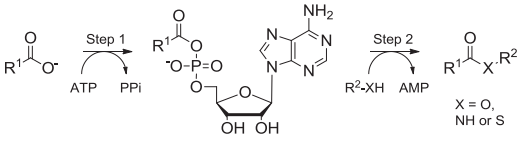
ABSTRACT

Fluorescent adenine mimetics are useful tools for studying DNA, RNA and enzyme functions. Herein we describe the synthesis of eight 1-(purinyl)triazoles and two 4-(purinyl)triazoles utilizing the 1,4-regioselective copper-catalyzed azide–alkyne cycloaddition (CuAAC) reaction as the key step. The fluorescence properties of five of the synthesized 1-(purinyl)triazoles are also presented. The five measured compounds displayed low quantum yields. The results, when compared to previously published data, suggest a high influence of the substitution pattern of the triazole on the fluorescence properties.

1. Introduction

Adenylate-forming enzymes are involved in a range of different essential biological processes, such as ribosomal and non-ribosomal peptide synthesis, fatty acid oxidation, and enzyme regulation.[1]

In the first step catalyzed by adenylate-forming enzymes, a carboxylate reacts with ATP to afford an acyl-adenylate intermediate (acyl-AMP) with concomitant release of pyrophosphate (PP_i) (Scheme 1, Step 1). In the second step the reactive intermediate reacts with a nucleophile to form the final product together with a release of AMP (Scheme 1, Step 2). Some of the adenylate-forming enzymes have been regarded as potential drug targets, such as aminoacyl-tRNA synthetases,[2] *Mycobacterium tuberculosis* pantothenate synthetase,[3,4] and aryl acid adenylating enzymes involved in siderophore biosynthesis in *M. tuberculosis*.[5,6] Since the acyl-AMP is assumed to bind tightly to the active site of adenylate-forming enzymes it is envisaged that non-reactive analogues of acyl-AMP could potentially serve as inhibitors of the enzymes. There are several examples in the literature where non-reactive



Scheme 1. The two-step adenylation process catalyzed by adenylate-forming enzymes, which results in the formation of thioesters, amides, and esters.

analogues of acyl-AMP have been synthesized and evaluated as inhibitors of specific adenylate-forming enzymes.[1,2,7–16] One such example is the use of sulfamoyl-adenylate analogues as inhibitors of aminoacyl-tRNA synthetases (Fig. 1, A).[2,16] We have previously reported the design and synthesis of several non-hydrolyzable sulfamoyl analogues of acyl-AMP, which have been used in structural studies of a number of tRNA synthetases.[17–21]

Fluorescence is a useful technique to study macromolecules, such as DNA and RNA. For this purpose artificial base analogues with intrinsic fluorescence are potentially useful tools.[22,23] We have previously reported the synthesis of 4-(adenosinyl)triazoles as fluorescent base analogues utilizing the 1,4-regioselective copper-catalyzed azide–alkyne cycloaddition (CuAAC).[24,25] The use of CuAAC facilitates the variation of substituents in the 1- and 4-positions of the 1,2,3-triazole through the use of a variety of azides/acetylenes. Small fluorescent compounds capable of detecting specific enzyme targets have been shown to be useful for studying enzymatic activation and regulation within the cell. Such

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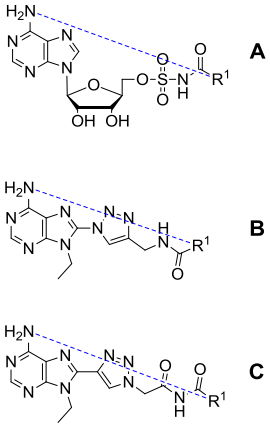


Fig. 1. A. Structures of sulfamoyloxy- and purinyltriazole-based (B and C) analogues of acyl-AMP. Blue dashed lines indicate similarities in the end groups of the compounds in their extended conformations.

tools have been used in enzyme function studies of kinases,[26] ATPase,[27] and glutathione transferase.[28]

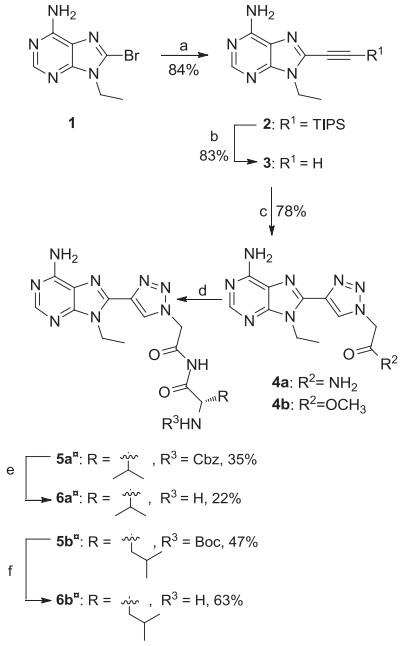
We hypothesized that purinyltriazoles (Fig. 1B and C) may fit into the adenylate-binding site of tRNA synthetases in a manner similar to the sulfamoyl analogues of acyl-AMP (Fig. 1A). Exchanging the ribose unit for a small lipophilic substituent would eliminate the need for the rather extensive protecting group strategies required for ribose-containing structures. These compounds could potentially be used as fluorescent probes to study adenylate-forming enzymes. In this paper we present the synthesis and photophysical characterization of a small series of 1- and 4-purinyltriazoles.

2. Results and discussion

2.1. Synthesis

The initial strategy to obtain aminoacyl bearing 4-purinyltriazoles utilized our previously described route to 8-(1*H*-1,2,3-triazole-4-yl) adenosine derivatives.[24] 8-Bromo-9-ethyladenine (**1**)[29] was synthesized in two steps from adenine. A Sonogashira cross-coupling was performed on **1** to introduce (triisopropylsilyl)acetylene in the 8-position using $Pd(PPh_3)_2Cl_2$ and CuI as catalysts with Amberlite IRA-67 as base (Scheme 2). The desired product **2** was obtained in 84% yield. The TIPS protecting group was removed with polymer-supported fluoride, which enabled work-up by filtration and compound **3** was isolated in 83% yield after purification. Initial attempts to synthesize **3** using (trimethylsilyl)acetylene resulted in in situ deprotection and low yields. The 1,2,3-triazole ring was synthesized using a copper-catalyzed [3+2]-cycloaddition between the alkyne **3** and an appropriate azide. Compound **4a** was isolated by precipitation from water in 78% yield and was used in the next step without further purification.

Initial attempts to prepare imides from **4a** using nitrophenyl activated esters and *n*-BuLi as base, were unsuccessful.[30] Changing the base to sodium hydride (NaH) resulted in acylation of **4a** using Cbz-L-valine 4-nitrophenyl ester or Boc-L-leucine 4-nitrophenyl ester affording **5a** and **5b** in 35% and 47% yield, respectively. The benzyl carbamate (Cbz) protecting group in **5a** was removed using a continuous-flow catalytic hydrogenation reactor (H-cube®) with Pd/C (10 wt % catalyst cartridge) and MeOH as solvent, affording **6a** in 22% yield after purification by preparative HPLC. The low isolated yield of **6a** can be partly attributed to the formation of the methyl ester **4b** (identified by NMR and LC/MS) by nucleophilic attack of MeOH on the imide functionality of **5a**. Compound **5a** could not be



Scheme 2. Synthesis of imide-based compounds. Reagents and conditions: (a) $Pd(PPh_3)_2Cl_2$ (5 mol %), CuI (20 mol %), Amberlite IRA-67 (5 equiv), ethynyltriisopropylsilane (3.3 equiv), THF, MW 120 °C, 30 min. (b) PS-fluoride (2.4–3.6 equiv, 2–3 mmol/g loading), THF, rt, N_2, 24 h. (c) 1. NaN_3 (1.2 equiv), 2-bromoacetamide (1.1 equiv), DMF, MW 80 °C, 20 min. 2. **3** (1.0 equiv), sodium ascorbate (0.6 equiv), CuI (0.2 equiv), *N*,*N*′-dimethylenediamine (0.3 equiv), MW 80 °C, 2 h. (d) **4a**, NaH (2.0 equiv), R^2-amino acid-ONp (1.1 equiv), THF, 0 °C 15 min then at rt, 3–5 h. (e) H_2/Pd/C (10% CatCart, 30×4 mm, H-cube®, 21 °C, 25 min, MeOH, flow rate: 1 ml/min). (f) 50% TFA in DCM, 1–1.5 h. ¤Unstable compounds.

purified by flash chromatography using an eluent containing MeOH, since the same side reaction occurred on the column. It was however not possible to identify a suitable replacement eluent system for this purification. The Boc-protecting group in **5b** was removed using TFA (50% in DCM), resulting in the isolation of **6b** in 63% yield after purification by preparative HPLC. Although, these compounds degraded within days when stored at −10 °C.

Due to the instability of **6a** and **6b** in the presence of nucleophiles like MeOH as well as on storage, it was decided to prepare compounds in which the imide was replaced by the more stable amide functionality. The existing route to the 4-(purinyl)triazoles involved a Sonogashira coupling on the 8-bromopurine derivative and subsequent desilylation followed by CuAAC with different azides to obtain the purinyltriazoles. Inverting the triazole would enable the cyclization on a range of more easily accessible alkynes. Aminoacyl substituted 1-(purinyl)triazoles would be accessible from a two-step azide formation/cyclization reaction with the alkynylamide of the amino acid and 8-bromo-9-alkyladenines, such as **1**.[29]

As in the case of the 4-(purinyl)triazoles, the synthesis of 1-(purinyl)triazoles started from 8-bromo-9-ethyladenine (**1**).[29] Propargylamides **7a**–**c** and β-alkynylamides **7d**,**e** were synthesized by amide coupling of the corresponding Boc-protected amino acids with propargylamine and 4-butynylamine, respectively (Table 1). Heating **1** and sodium azide at 90 °C for 22 h in DMF with the exclusion of light resulted in the formation of 8-azido-9-ethyladenine (observed by LC/MS). Existing protocols for similar transformations using DMSO failed to provide satisfactory conversions in our hands.[31,32]

Without isolation of the formed azide intermediate, copper(I) iodide (CuI), sodium ascorbate (NaAsc), *N*,*N*′-dimethylethylenediamine (DMEDA), and Cbz-valine-propargylamide were added to the reaction mixture and heated at 90 °C for 24 h. Although ^1H NMR