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Synthesis of 2-substituted 9-oxa-guanines {5-aminooxazolo[5,4-*d*]pyrimidin-7(6*H*)-ones} and 9-oxa-2-thio-xanthines {5-mercaptooxazolo[5,4-*d*]pyrimidin-7(6*H*)-ones}

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Full Research Paper

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Abstract

Oxazolo[5,4-*d*]pyrimidines can be considered as 9-oxa-purine analogs of naturally occurring nucleic acid bases. Interest in this ring system has increased due to recent reports of biologically active derivatives. In particular, 5-aminooxazolo[5,4-*d*]pyrimidine-7(6*H*)-ones (9-oxa-guanines) have been shown to inhibit ricin. The preparation of a series of 2-substituted 5-aminooxazolo[5,4-*d*]pyrimidin-7(6*H*)-ones and related 5-thio-oxazolo[5,4-*d*]pyrimidines is described, including analogs suitable for further elaboration employing "click" chemistry utilizing copper-catalyzed Huisgen 1,3-dipolar cycloadditions. Two of the compounds prepared were found to inhibit ricin with IC_{50} *ca.* 1–3 mM.

Introduction

Oxazolo[5,4-*d*]pyrimidines have been reported to possess a variety of biological activities including kinase inhibition [1,2], adenosine receptor antagonism [3] and tumor growth inhibition [4]. In particular, 5-aminooxazolo[5,4-*d*]pyrimidin-7(6*H*)-ones [9-oxa-guanines] and related heterocycles have been shown to inhibit the ability of ricin to inactivate ribosomes [5]. Given these reports, and the expectation of further applications based upon similarity to naturally occurring nucleic acid bases, this heterocyclic ring system continues to generate interest.

Approaches to the oxazolo[5,4-*d*]pyrimidine ring system generally involve either cyclodehydration of an 5-(acylamino)-4-hydroxypyrimidine [6-10] or elaboration of a 4-cyano- or 4-(alkoxycarbonyl)-5-aminooxazole [11-15] (Figure 1), with only isolated reports of alternative routes [16,17]. However, few publications have described the preparation of 5-aminooxazolo[5,4-*d*]pyrimidin-7(6*H*)-ones [5,8,14] or 5-mercaptooxazolo[5,4-*d*]pyrimidin-7(6*H*)-ones [9-oxa-2-thio-xanthines] [7,15], and in both cases, the conditions do not

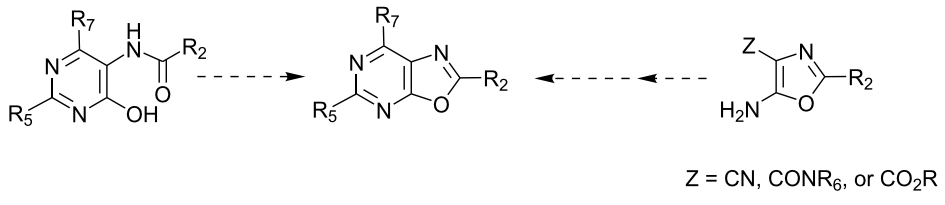

Figure 1: The two general synthetic approaches to oxazolo[5,4-*d*]pyrimidines.

appear to be amenable to the preparation of oxazolo[5,4-*d*]pyrimidines with variations at the 2-position, particularly those with additional functional groups or increased steric demand.

In pursuing 8-methyl-9-oxa-guanine [2-methyloxazolo[5,4-*d*]pyrimidin-7(6*H*)-one] as an initial lead in the design of inhibitors of the ribosome-inactivating protein ricin [5], we set out to prepare a series of 2-substituted 5-aminooxazolo[5,4-*d*]pyrimidin-7(6*H*)-ones and related 5-thio-oxazolo[5,4-*d*]pyrimidines. Here we report these studies, which have led to the preparation of analogs suitable for further elaboration, for example by "click" chemistry employing copper-catalyzed Huisgen 1,3-dipolar cycloadditions [18].

Results and Discussion

Our previous route [5] to 8-methyl-9-oxa-guanine (**2a**) involved the thermal cyclodehydration of 5-(acetylamino)-2-amino-4,6-dihydroxypyrimidine (**1a**) [19] (Figure 2). Unfortunately, for other 5-acylamino analogs of **1a**, this route failed to afford the required oxazolo[5,4-*d*]pyrimidines, presumably due to decomposition of the product at the temperatures required for cyclodehydration. Other cyclodehydration conditions ($POCl_3$, PPA) were also unsuccessful.

An alternative route was sought in which the oxazole ring was formed first, followed by elaboration of the pyrimidine ring. Our previous work had demonstrated that in the case of ethyl 5-amino-2-methyloxazole-4-carboxylate **3a** [20], direct annulation with chloroformamidine in DMSO at 120 °C or with 2-methylisothiourea sulfate neat or in ethylene glycol at 170 °C did not afford the oxazolo[5,4-*d*]pyrimidine **2** (Figure 2). Thus, a stepwise annulation [15] strategy was explored for the preparation of analogs of **2** bearing different 2-positions substituents. The required 5-aminooxazoles **3b,c** were prepared from the (acylamino)cyanoacetates **5b,c**, which in turn were derived from ethyl cyanoglyoxylate oxime (**4**) by reduction followed by acylation by isobutyric anhydride or azidoacetyl chloride (Figure 3). Reaction of the aminooxazoles **3b,c** with benzoylisothiocyanate afforded the thioureas **6b,c**. Direct cyclization of **6b,c** to oxazolo[5,4-*d*]pyrimidines **2b,c** by treatment with ammonia in methanol was unsuccessful. However, thioureas **6b,c** were converted to the desired 2-substituted the oxazolo[5,4-*d*]pyrimidines **2b,c** by stepwise methylation followed by cyclization in the presence of ammonia in methanol. Although the intermediate products after methylation were not purified, in the case of **6c** the ^{1}H NMR spectrum of the crude product following methylation retains the signals for the ethoxy group of **6c** and displays a new resonance for the *S*-methyl group at 2.0 ppm (see Supporting Information File 2), indicating that the intermediate is the *S*-methylisothiourea **A** (Figure 3). The mechanism involved in the conversion of intermediates such as **A** to **2** is not clear. It is unlikely that the 5-(methylthio)oxazolo[5,4-*d*]pyrimidine is an intermediate because treatment of 5-(methylthio)oxazolo[5,4-*d*]pyrimidine **8a** (see below) with ammonia in methanol fails to afford **2a**.

The thiourea **6b** and its analog **6a**, which was prepared from aminooxazole **3a** in 51% yield, were also subjected to cyclization in the presence of ethanolic KOH to afford the 5-mercaptooxazolo[5,4-*d*]pyrimidin-7(6*H*)-ones **7a,b** (Figure 4). Further elaboration of **7a** by alkylation with methyl iodide,

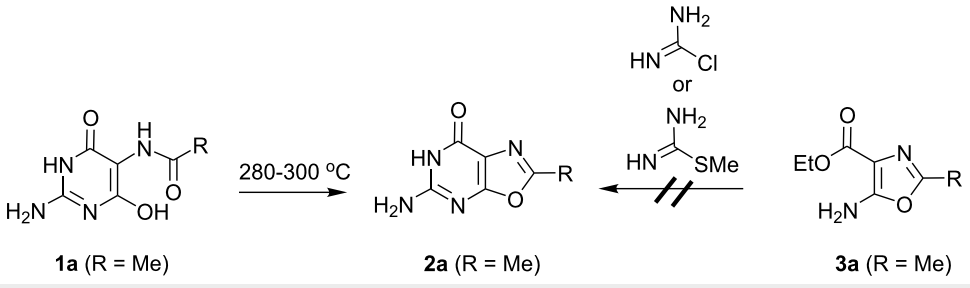

Figure 2: Thermal cyclodehydration route to 9-oxo-guanine.

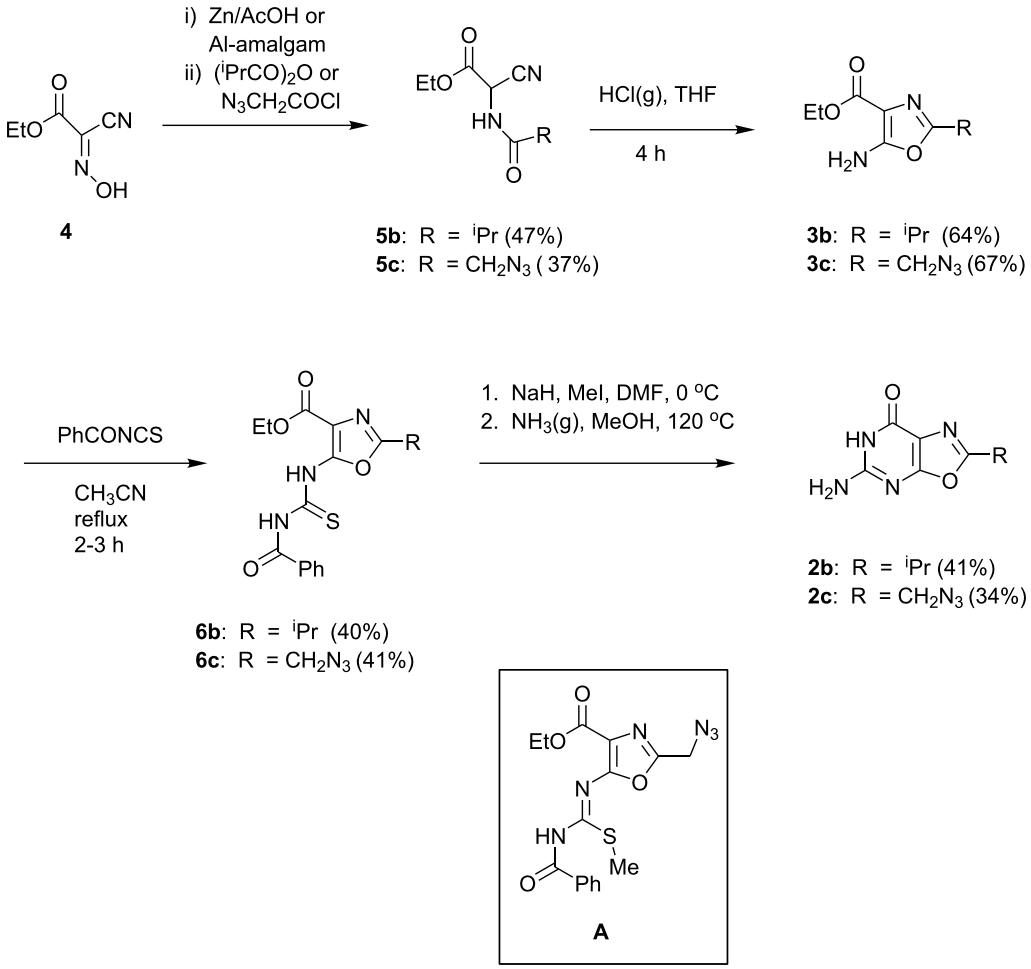

Figure 3: Preparation of 2-substituted 5-aminooxazolo[5,4-*d*]pyrimidin-7(6*H*)-ones.

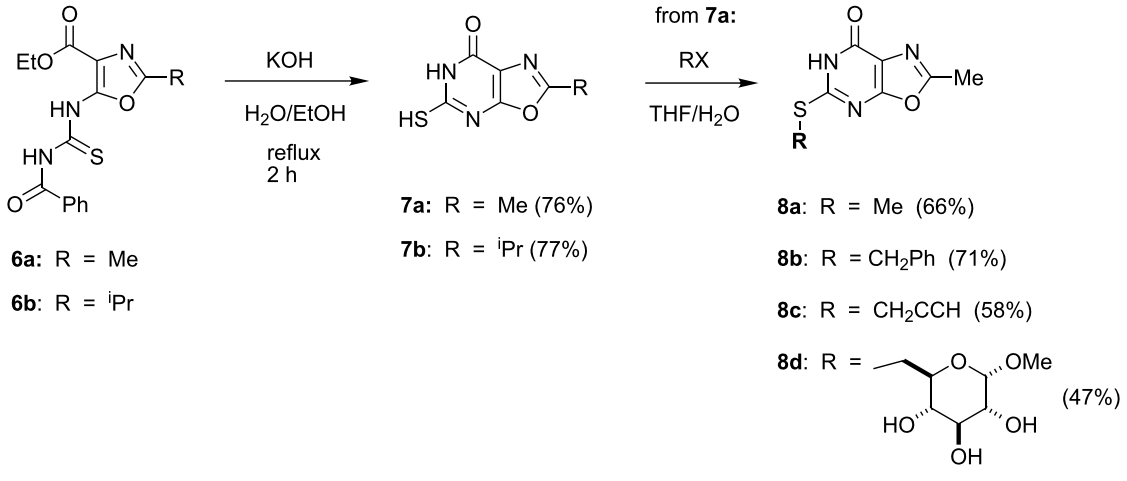

Figure 4: Preparation of 2-substituted 5-aminooxazolo[5,4-*d*]pyrimidin-7(6*H*)-ones and related thioethers.

benzyl bromide, propargyl bromide, or methyl 6-*O*-(tolylsulfonyl)-α-D-glucopyranoside [21] afforded the thioethers **8a**–**d**, respectively. The structural assignment of **8a**–**d** as thioethers rest principally on their ^{1}H and ^{13}C NMR spectra, which contain resonances more commensurate with groups attached to sulfur (e.g., for **8a**: 2.2 ppm in ^{1}H and 13 ppm in ^{13}C NMR, respectively) than to nitrogen, as would be the case for 4- or 3-alkylated 5-mercaptooxazolo[5,4-*d*]pyrimidine-7(6*H*)-ones.

The thioether **8c** bearing a propargyl substituent was subjected to "click" chemistry coupling with benzyl azide or 2-morpholinoethyl azide in the presence of catalytic $CuSO_4$ and sodium ascorbate to afford the triazoles **9** and **10**, respectively, in good yield (Figure 5). The copper-catalyzed Huisgen cycloaddition of terminal alkynes and alkyl azides favors formation of the 1,4-triazole regioisomers [22], and in the case of triazole **9**, this regiochemistry was confirmed by ^{1}H NOE spectra (see Supporting Information File 2).

Conclusion

In conclusion, routes to functionalized oxazolo[5,4-*d*]pyrimidines from 2-substituted 5-aminooxazole-4-carboxylic acid ethyl esters were developed, the key to which is the relatively mild conditions employed in the step-wise elaboration of the pyrimidine ring. Compounds **2b,c**, **7a,b**, **8a**–**d**, **9**, and **10** were evaluated for their ability to inhibit recombinant, catalytically active ricin A-chain (RTA) employing a modification of the previously reported assay [5]. Briefly, the synthesis of protein from endogenous globin mRNA by rabbit reticulocyte lysate was determined in the absence of RTA, in the presence of sufficient RTA to inhibit 90% of protein synthesis (*ca.* 10 pM), and in the presence of both RTA and increasing concentrations of the oxazolo[5,4-*d*]pyrimidines. The ability of these compounds to inhibit RTA and thereby rescue protein synthesis was determined. Compounds **2b** (IC_{50} = 2.8 mM) and **9** (IC_{50} = 1.6 mM), displayed some activity; whereas, none of the other compounds examined showed any significant RTA inhibitory activity.

Supporting Information

Supporting Information File 1

This file includes full experimental details for all new compounds.
[http://www.beilstein-journals.org/bjoc/content/supplementary/1860-5397-4-26-S1.doc]

Supporting Information File 2

Copies of ^{1}H and ^{13}C NMR spectra of compounds **2b**,**c**; **3a**–**c**; **5b**,**c**; **6a**–**c**; **A**; **7a**,**b**; **8a**–**d**; **9**; and **10**.
[http://www.beilstein-journals.org/bjoc/content/supplementary/1860-5397-4-26-S2.doc]

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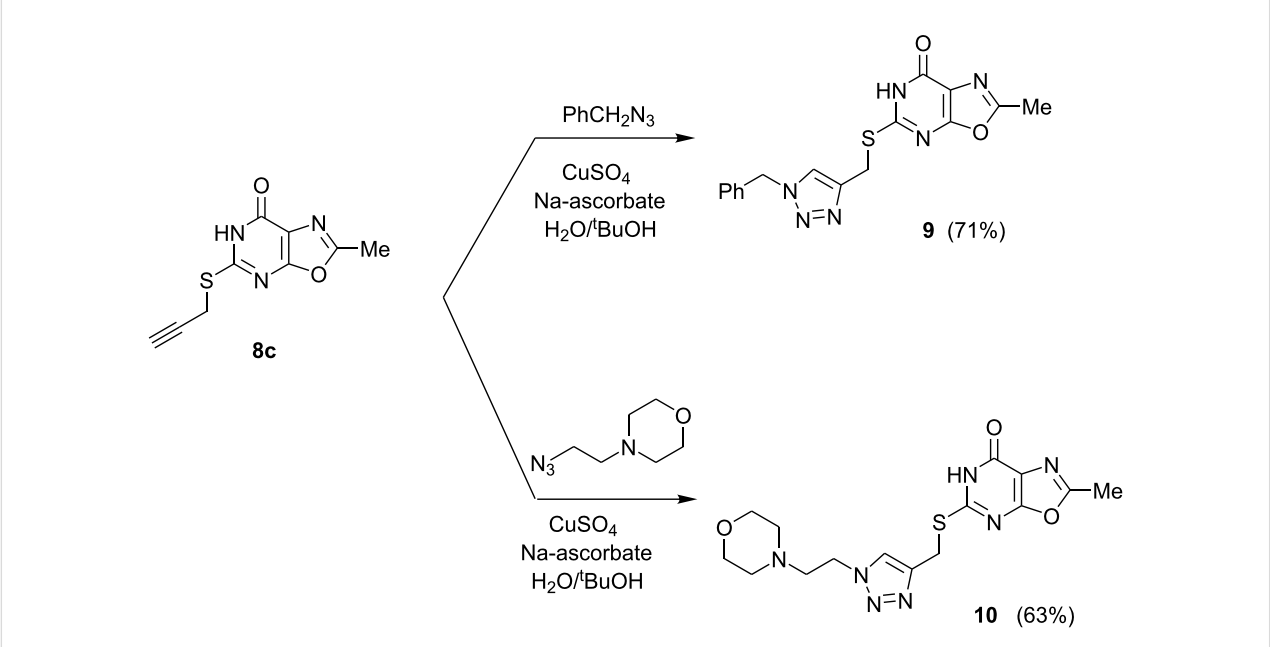

Figure 5: Click chemistry elaboration of a 5-(propargylthio)oxazolo[5,4-*d*]pyrimidine.

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