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Synthesis of 4-Amino-1-(ß-D-ribofuranosyl)pyrrolo[2,3-*d*]pyridazine; An Entry into a Novel Series of Adenosine Analogs

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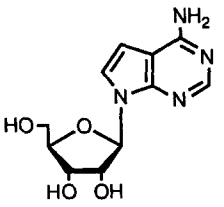
Summary: The synthesis of a novel adenosine analog, 4-amino-1-(ß-D-ribofuranosyl)pyrrolo[2,3-*d*]pyridazine, has been accomplished using pyrrole nucleoside precursors.

Analogs of the naturally occurring nucleoside adenosine have proven to be important leads for therapeutically useful agents. Sugar modified analogs such as 6-amino-9-(ß-D-arabinofuranosyl)purine[1] (ara A) have been used clinically for the treatment of certain viral disorders and another adenosine analog, 2',3'-dideoxyadenosine, has recently undergone phase I clinical trials for the treatment of HIV.[2] Modifications of adenosine in the heterocyclic base moiety have also afforded biologically active molecules.[3] The naturally occurring pyrrolo[2,3-*d*]pyrimidine nucleoside tubercidin (**1**), which contains a carbon atom in place of the 7-nitrogen atom, possesses some significant antineoplastic[4] and antiviral properties.[5] Several other biologically active pyrrolo[2,3-*d*]pyrimidine adenosine analogs, have been either synthesized or isolated from naturally occurring sources. While the chemical and biological effects of exchanging a carbon atom for the N-7 atom of the adenine base moiety have been well studied, the effects of an additional interchange involving the N-3 and C-2



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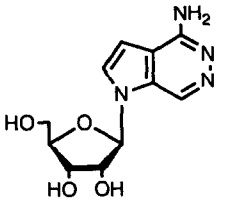

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atoms of adenosine remained to be investigated. This prompted us to initiate a synthesis of 4-amino-1-(ß-D-ribofuranosyl)pyrrolo[2,3-*d*]pyridazine (**2**), a structural isomer of the nucleoside antibiotic tubercidin (**1**), in order to investigate the effects which these chemical changes would have on the biological activity.

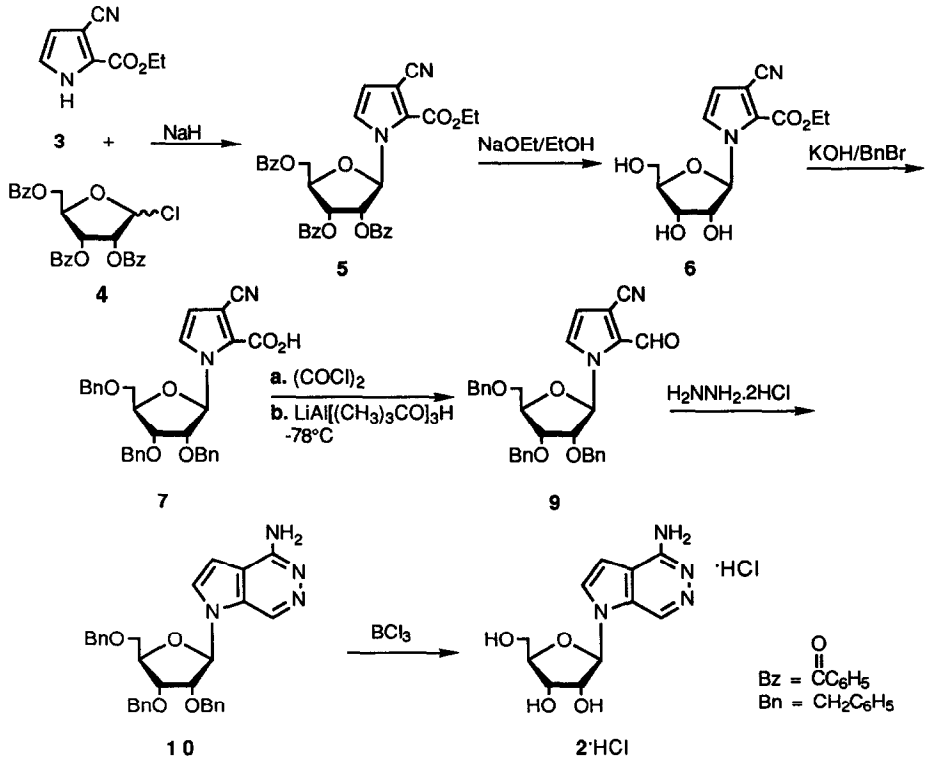
A literature search revealed that a number of syntheses which generate the pyrrolo[2,3-*d*]pyridazine heterocyclic nucleus had been reported,[6] however, there were no reports involving the synthesis of pyrrolo-

[2,3-*d*]pyridazine nucleosides. The ring annulation of a 2,3-disubstituted pyrrole precursor, as described by Bisagni[7] and coworkers, appeared to be the most relevant to our study. Using an appropriately substituted pyrrole with a sugar moiety at position 1 would allow us a facile entry into the pyrrolo[2,3-*d*]pyridazine nucleoside system. This approach was especially appealing since this would eliminate the problem of regioselectivity which is usually associated with a direct glycosylation of a bicyclic heterocycle. In addition, a convenient method for the stereoselective glycosylation of pyrroles with a number of different 1-halo sugars has been reported.[8,9] Thus, if the synthetic strategy was successful it would provide a route for the regioselective introduction of other sugars to this heterocyclic system.

We chose to use ethyl 3-cyanopyrrole-2-carboxylate (**3**)[10] as our starting material because of the favorable juxtaposition of substituents and the different chemical reactivity of these substituents which would allow us to accomplish the requisite manipulations. The sodium salt glycosylation of **3** with 1-chloro-2,3,5-tri-O-benzoyl-D-ribose (**4**)[11] in acetonitrile provided a 95% yield of a single anomer of ethyl 3-cyano-1-(2,3,5-tri-O-benzoyl-ß-D-ribofuranosyl)pyrrole-2-carboxylate (**5**) as judged by ^{1}H NMR (Scheme 1). In contrast to a previous report,[9] we did not observe any product which would have resulted from an attack of the pyrrole anion on the 2'-carbonyl moiety of the benzoyl protected sugar. In order to prevent unwanted side reactions, due to deblocking during the modification of the pyrrole substituents, the base labile benzoyl blocking groups were replaced by the more stable benzyl blocking groups. In order to effect this interchange, the benzoyl blocking groups of **5** were first removed with sodium ethoxide in ethanol to provide **6** in 77% yield. The sugar hydroxyl groups of ethyl 3-cyano-1-(ß-D-ribofuranosyl)pyrrole-2-carboxylate (**6**) were then benzylated with potassium hydroxide/benzyl bromide with a simultaneous saponification of the ester moiety of the pyrrole to provide 3-cyano-1-(2,3,5-tri-O-benzyl-ß-D-ribofuranosyl)pyrrole-2-carboxylic acid (**7**, 70%). A peak at 2235 cm^{-1} in the IR spectrum confirmed that the nitrile moiety of **7** was still present.

In order to reduce the carboxylic acid function to an aldehyde function, a selective procedure that would not affect the nitrile function was required. Lithium tri-*tert*-butoxyaluminohydride[12] has been used to selectively reduce an acyl chloride to an aldehyde moiety, without further reduction to the alcohol, while nitrile moieties are resistant to this hydride reagent.[13] This procedure was applied to our problem by first converting **7** to 3-cyano-1-(2,3,5-tri-O-benzyl-ß-D-ribofuranosyl)pyrrole-2-carboxylic acid chloride (**8**) by treatment with oxalyl chloride. Compound **8** was used, without further isolation, in the reaction with lithium tri-*tert*-butoxyaluminohydride in diglyme at -78°C to afford 3-cyano-(2,3,5-tri-O-benzyl-ß-D-ribofuranosyl)pyrrole-2-carboxaldehyde (**9**). For analytical purposes, a sample of **9** was purified by column chromatography and its ^{1}H NMR spectrum revealed a

peak at 9.84 ppm corresponding to the aldehydic proton. The IR spectrum of **9** contained a peak at 2230 cm^{-1}, confirming the presence of the nitrile moiety. It was also at this stage that the anomeric configuration of the



pyrrole nucleoside was determined. In the ^1H NMR spectrum of **9**, the peak assigned to the anomeric proton appeared as a singlet at 6.61 ppm. A coupling constant of <1 Hz is possible only for a trans relationship of the H-1' and H-2' in the ribofuranosyl ring[14] which established the ß configuration for nucleoside **9**.

Annulation of crude **9** with hydrazine dihydrochloride in ethanol at reflux proceeded smoothly to provide, after column chromatography, 4-amino-1-(2,3,5-tri-O-benzyl-ß-D-ribofuranosyl)pyrrolo[2,3-*d*]pyridazine (**10**) in a 33% overall yield from **7**. Evidence that ring closure had occurred was confirmed by the loss of the absorbance due to the nitrile in the IR spectrum, and by the molecular ion at 536 in the EI mass spectrum of **10**. The benzyl groups of **10** were removed with BCl_3 to provide the target compound **2**, isolated as its HCl salt (59% yield).[15] For **2**, a complete lack of cytotoxicity was observed at 100 µM in the L1210 assay, (tubercidin, complete inhibition of growth rate at 0.043 µM) and also no activity against human cytomegalovirus at 100 µM.

In summary, we have designed a synthetic route that provides an entry into a novel class of adenosine analogs. The application of this route for the synthesis of other adenosine analogs is under investigation.

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15) ^{1}H NMR (DMSO-d_6): δ 14.45 (br s, 1H), 9.08 (s, 1H, H-7), 8.77 (br s, 2H, -NH_2), 8.04 (d, 1H, pyrrole H), 7.27 (d, 1H, pyrrole H), 6.04 (d, 1H, H-1'), 5.95-5.28 (m, 3H, OH's), 4.16-4.02 (m, 3H, H-2', H-3', H-4'), 3.65 (m, 2H, H-5'); UV λ_{max} nm (ε x 10^4); (pH 7) 267 (0.69), 285 (0.74); (pH 1) 266 (0.54), 286 (0.62); (pH 11) 261 (0.52), 294 (0.76); IR (KBr pellet) υ (cm^{-1}) 3600-2400, 3430.5, 3198.4, 2931.2, 1658.6, 1384.4, 1103.1, 1053.9, 976.6, 618.0; mp 231.5-233°C; Anal. (C, H, N).