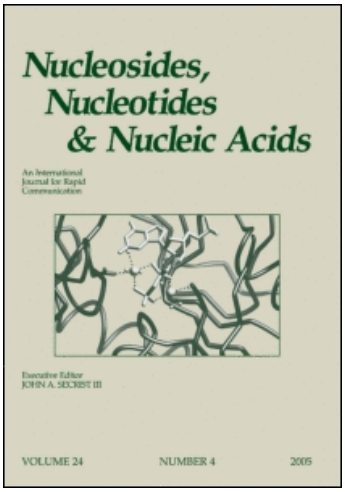

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Development of Methodologies for the Strategic Modification of Purim Ribonwxeoside System

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DEVELOPMENT OF METHODOLOGIES FOR THE STRATEGIC MODIFICATION OF PURINE RIBONUCLEOSIDE SYSTEMS

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Abstract:

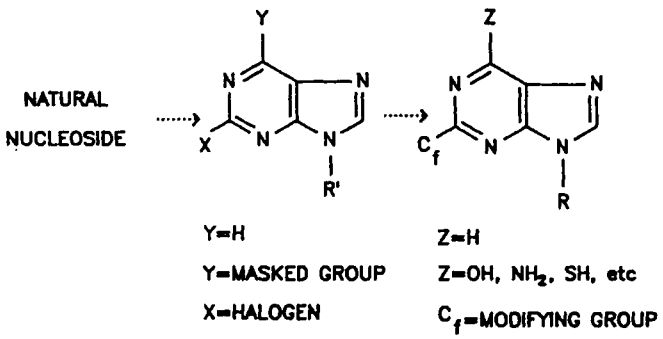
Development and application of new methodologies for the synthesis of novel functionalized analogues of nebularine, inosine, and adenosine of antiviral interest are described.

Synthesis of rare and previously inaccessible purine nucleosides has elicited considerable attention recently because of observations that some compounds belonging to this family have potent antiviral therapeutic activity as well as being of enzymological usefulness as biological probes for the study of key mammalian and viral-encoded enzymes.[1-10] In an ongoing program in our laboratory directed at the synthesis of new antiviral nucleosides, we have developed methods for the specific modification of both the base and the carbohydrate moieties of purine nucleosides.

Our research efforts in the area of base-modified purine nucleosides have focused attention on regiospecific functionalization involving the 2-position of nebularine, inosine, and adenosine.[11-13]. The natural nucleoside, nebularine [9-(β-D-ribofuranosyl)purine], is an antibiotic with strong competitive inhibitory properties for the enzyme, adenosine deaminase.[14] Additionally, it has been studied as an anticancer agent.[15]

Although analogues of nebularine, such as the 2-amino, 2-methyl-, and 2-trifluoromethyl- systems have shown some very interesting biological activities,[16-21] relatively few congeners of this interesting natural nucleoside involving functionalization at the 2-position are known.[11,22,23] While the synthesis of a number of C-2 substituted hypoxanthine nucleosides are known,[24-26] very few functionalized alkylated derivatives have been reported.[27] Functionalized C-2 alkylated inosine analogues are not only of potential interest as antiviral agents, but there is enzymological interest in these novel compounds as potential inhibitors of a key purine metabolizing enzyme, inosine monophosphate dehydrogenase.[28] However, limitations in synthetic methodology restrict accessibility to a variety of novel C-2 functionalized hypoxanthine nucleosides. The ring closure reaction of appropriately constructed imidazole nucleosides has provided the major approach to the synthesis of most 2-substituted inosines.[24,25] Other methods known for entry into this general class of compounds appear to be of more limited scope.[29] The aforementioned comments pertaining to limitations in synthetic methodology for C-2 functionalization, are also applicable to analogues of adenosine.[24-6,30] Such analogues and their deoxygenated congeners are of interest as potential anticancer and antiviral agents and they are also of enzymological interest with respect to purine metabolizing enzymes such as adenosine deaminase and nucleoside kinases.[2,9,31-39]

The general synthetic approach to the aforementioned 2-substituted nucleosides is shown in Scheme 1. The starting compound was a natural or readily available synthetic nucleoside which was converted in a series of steps to a key intermediate where both the carbohydrate and base moieties were protected (if needed). The 2-position was appropriately substituted with a halogen (commonly iodine). This key intermediate was converted through a key transformation to an immediate product which was either deprotected or elaborated further to the final target molecule. The major synthetic emphasis of this paper will be on the key step of the synthesis.

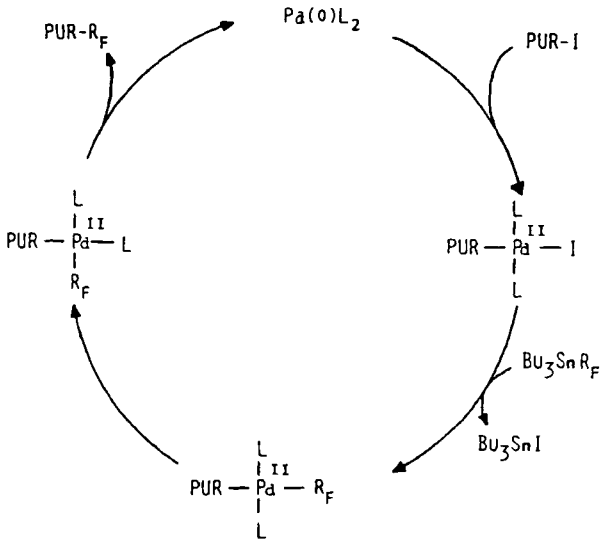


Scheme 1

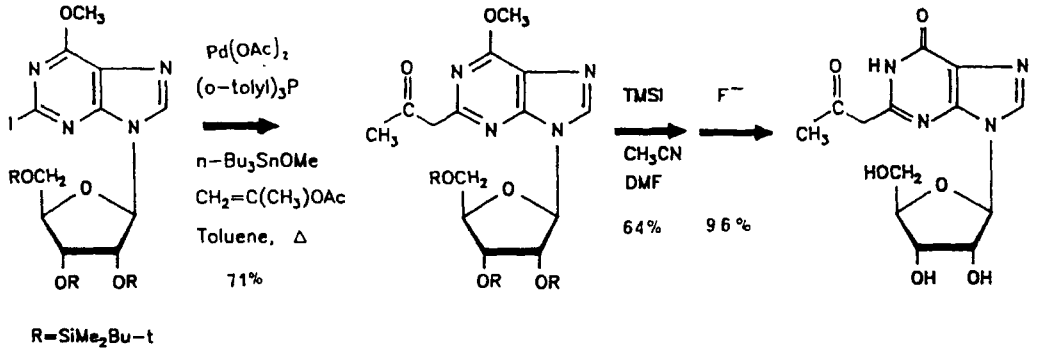
In previous work from our laboratory involving structural modification at the 6-position of the purine ring, reductive deaminations, deamination-halogenations, thermal and photochemical alkylthiolations, and photoinduced $S_{RN}1$ reactions were developed for the synthesis of specific target nucleosides. Unfortunately, the photochemical $S_{RN}1$ reaction,[40,41] one of the key functionalizing steps used in these syntheses, failed when applied to the 2-position. Various other approaches including the Eschenmoser sulfide contraction and Meerwein-type reactions were all unsuccessful.

We have now developed a general methodology for the regiospecific functionalization of the 2-position of purine nucleosides. This methodology involves a palladium-catalyzed cross-coupling reaction between an organostannane containing the desired synthon to be introduced and a 2-iodinated purine system.[12] A plausible mechanistic explanation of this reaction is summarized in Scheme 2.

The methodology can be illustrated with the synthesis of 2-acetonylinosine from protected 2-iodo-6-methoxypurine ribonucleoside as summarized in Scheme 3.[13] The final target nucleoside was purified by reversed-phase HPLC on Amberlite XAD-4 resin with ethanol/water as the eluting solvent and characterized by FAB HRMS, high-field multinuclear NMR data, and by UV and FTIR spectroscopy. It exhibits high <u>in vitro</u> antiviral



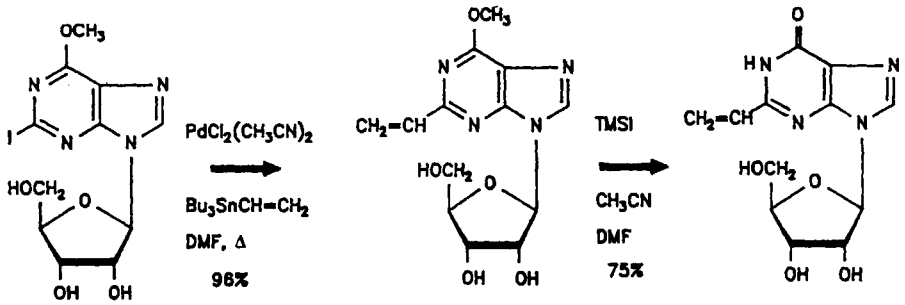
Scheme 2



Scheme 3

activity (Ti = MTC/ID_{50} = >1000) against the Sandfly Fever Virus (an RNA virus of the Phlebovirus family).

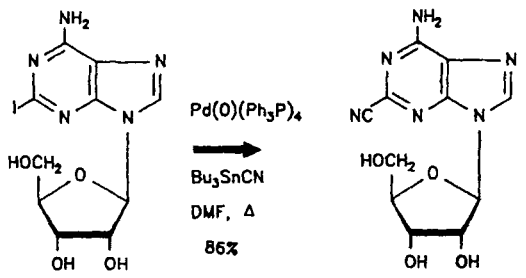
This palladium-catalyzed cross-coupling reaction with enol acetates appears to have considerable generality. In addition, these reactions can be extended to include other functionalized organostannanes. For example, 2-vinylinosine, a novel hypoxanthine nucleoside analogue which is



Scheme 4

potentially a precursor to a variety of new inosine compounds (*via* ozonolysis, hydroboration, osmylation, etc.), is also readily available using this methodology (Scheme 4). Allylation reactions are also possible. Interestingly, 2-vinylinosine exhibits low but broad-spectrum *in vitro* activity against a number of RNA viruses of the Flavi, Bunya, Pox, and Arena families.

The aforementioned and other reactions involving organostannanes can be extended to the nebularine series. The starting nucleoside for these syntheses was isoadenosine, synthesized by a photochemical reductive dehalogenation previously described by us.[11] Application to adenine nucleosides is also possible with 2-iodoadenosine as the tailored precursor.[42] A series of C-2 functionalized adenosine analogues have been synthesized using this and related organozinc coupling reactions. An example of the key transformation is illustrated in Scheme 5 for the direct synthesis of 2-cyanoadenosine (cf. reference 43). Interestingly, the series of C-2 functionalized adenosines synthesized are either very poor substrates (<< 1% compared to adenosine) or in most cases, totally resistant, to hydrolytic deamination by the ubiquitous enzyme, adenosine deaminase.

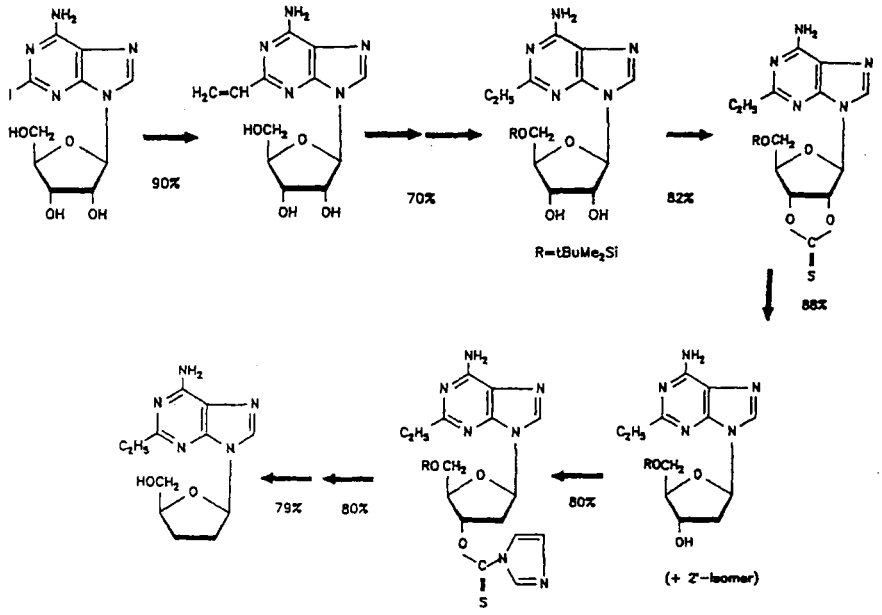


Scheme 5

In our work directed at novel analogues of the anti-AIDS compound, 2',3'-dideoxyadenosine[2,8], we have synthesized several congeners of this dideoxygenated that have greater stability than the parent compound with respect to deamination by adenosine deaminase and hydrolytic cleavage of the glycosidic bond. One of our approaches is shown in Scheme 6 with the synthesis of 2-ethyl-2',3'-dideoxyadenosine from 2-iodoadenosine.

Glycosidic bond stabilities were determined by differential UV spectroscopy.[44] Results for some representative cases are shown in Table 1. The analogues were resistant to deamination by adenosine deaminase.

Finally, it should be mentioned that the glycosidic bond conformation of purine nucleosides may play a critical role in determining their ability to be substrates for key purine metabolizing enzymes and also subsequently in their antiviral and related biological activities. We have previously reported that the preferred conformation of purine ribonucleosides in solution may be qualitatively determined through correlation of their high-field ^{13}C NMR data.[45] A key factor in this determination is the magnitude of the chemical shift difference between C-2' and C-3'. This correlation can be extended to dideoxypurine nucleosides as illustrated in Table 2. It appears that for the dideoxynucleosides in the preferred <u>syn</u> conformation the magnitude of the chemical shift difference between C-2'



Scheme 6

Table 1. Relative Rates of Hydrolysis of Dideoxyadenosines (25° C, pH 3)

2',3'-Dideoxyadenosine	100
2',3'-Dideoxynebularine	177
8-Methoxy-2',3'-dideoxyadenosine	61
2-Ethyl-2',3'-dideoxyadenosine	75

Table 2. Preferred Conformation of Purine Dideoxynucleosides Discerned from High-Field ^{13}C NMR Data

90.6 MHz Carbon Data (ppm)	ddAdo	ddNeb	2-Et ddAdo	8-OCH_3 ddAdo
C-2'	31.72	31.74	31.87	28.88
C-3'	25.62	25.36	26.00	26.96
(C2' - C3')	6.10	6.38	5.87	1.92

and C-3' is < 2 ppm whereas in the preferred anti conformation, this difference is > 5 ppm.

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