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PURINE 2',3'-ACYCLONUCLEOSIDES: IMPROVED SYNTHESIS AND ANTIPARASITIC ACTIVITY

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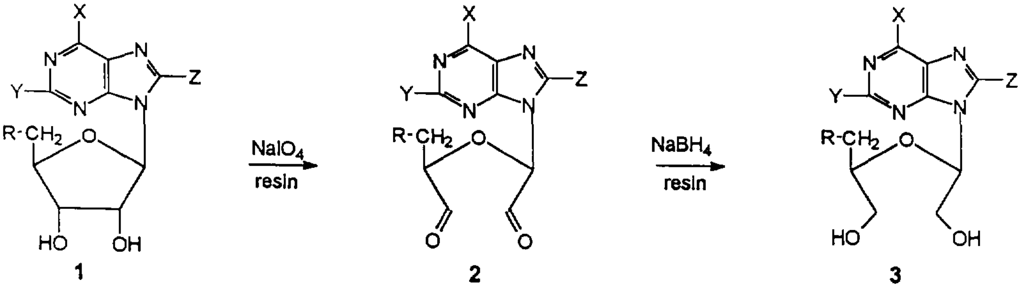
Abstract: Synthesis of purine 2',3'-acyclonucleosides has been improved by using methanol as solvent for sequential oxidation/reduction reactions on periodate- and borohydride-bound resins. Several O-acetylated derivatives of acyclic analogs prepared by this procedure have significant antitrypanosomal activity *in vitro*.

Introduction:

Acyclic ribonucleoside analogs have received attention as potential antimicrobial agents.[1] Many of these analogs can be processed by purine and/or pyrimidine pathway enzymes that are less discriminating in microorganisms than in mammalian cells. 9-(2-Hydroxyethoxymethyl)guanine (acyclovir) is a widely used antiherpetic drug[2] whose success has stimulated the search for other biologically active acyclic nucleosides.[3] Acyclic modifications of the ribose ring have resulted in structures of varying complexity[1] whose synthesis can be especially challenging if chiral features are retained. 2',3'-Acyclonucleosides are among the simplest of these acyclic structures and are of interest as potential therapeutic agents as well as synthetic intermediates.

Procedures for synthesizing 2',3'-acyclonucleosides (e.g.[4,5,6,7,8,9,10,11]) are fundamentally similar: a form of periodate is used to oxidize a ribonucleoside to an acyclic 2',3'-dialdehyde; a form of borohydride is next used to reduce the dialdehyde to the acyclic 2',3'-diol. Despite the straightforward application of oxidation-reduction agents, difficulties in workup and/or isolation of products have required the use of various ion exchange resins, cellulose and/or charcoal for separation and/or purification of products. A simplified procedure was reported by Bessodes and Antonakis[12] who used a mixed bed of periodate and borohydride supporting resins to form the desired acyclic ribonucleosides in a single flask. In this manner, the product could be easily recovered by filtration of the reaction mixture followed by evaporation of the aqueous filtrate. When we applied this method to prepare 2',3'-acyclo-5'-deoxy-5'-chloroadenosine, we unexpectedly obtained an epimerized product. We determined by ^{1}H-NMR spectroscopy that epimerization had occurred at C-1 during oxidation of the ribonucleoside to the 2'-3'-dialdehyde. In an effort to eliminate undesired formation of epimerized product, we explored the use of nonaqueous solvents to carry out this heterogeneous reaction. We subsequently found that concomitant oxidation/reduction in the presence of the mixed bed resins could be carried out cleanly in methanol, giving high yields of unepimerized product. We have prepared a series of analogs (see Table 1) according to the synthetic route that follows and have found this to be an improved general method to access purine 2',3'-acyclonucleosides.



Our interest in acyclic purine nucleosides relates to their potential as antiparasitic agents. Protozoan parasites do not synthesize purines *de novo* and rely on salvage of preformed purines from their host.[13] As a result, agents that interfere with purine salvage in these parasites provide a potential for chemotherapeutic selectivity. In addition, our discovery that 5'-alkyl-substituted analogs of 5'-deoxy-5'-(methylthio)adenosine (MTA) are trypanocidal[14] led us to consider acyclic analogs of MTA as a possible means to enhance their activity. We have examined a series of purine 2',3'-acyclonucleosides for antiparasitic activity *in vitro* against *Trypanosoma brucei*, *Trypanosoma cruzi* and *Leishmania donovani*.

Chemistry:

Preparation of periodate and borohydride resins.

A. Periodate resin. To a suspension of sodium periodate (233 g) in water (3500 ml) was added Amberlyst A27 ion-exchange resin (200 g; Aldrich catalogue # 21643-7) in a 4 L beaker. The mixture was stirred (using a mechanical stirrer with paddle) for 4 hours, filtered, washed thoroughly with water and rinsed with methanol. The resin was dried overnight in a vacuum oven with a vacuum pump at 55°C and then stored at 4°C.

B. Borohydride resin. Amberlyst A27 ion-exchange resin (200 g) was added to an aqueous solution (3500 ml) of sodium borohydride (66 g) and then prepared as described above.

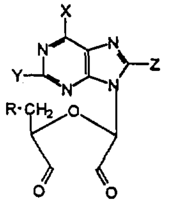
General procedure for preparation of purine 2',3'-acyclonucleosides. To a solution (or suspension) of purine nucleoside (1 mM) in methanol (15-25 ml) was added 1 g each of periodate and borohydride resins. The mixture was stirred (RT) until the reaction was complete (4-24 hr) as determined by thin layer chromatography: in all cases, the intermediate dialdehyde had a higher R_f and the 2',3'-acyclonucleoside, a lower R_f than the starting ribonucleoside. The resin beds were filtered and washed with methanol. The methanol filtrate was evaporated under reduced pressure to obtain the desired purine 2',3'-acyclonucleoside.

These highly polar 2',3'-acyclonucleosides can be purified by converting them to their O-acetylated derivatives [acetic anhydride/triethylamine/4-dimethylaminopyridine (DMAP)/acetonitrile] which are readily chromatographed on silica gel columns or preparative plates. The O-acetylated derivatives (**4a-4h, 4j**) were also assayed for antiparasitic activity. Unexpectedly, several of these acetylated derivatives (i.e. **4a**, **4g** and **4j**) were much more active against *T. b. brucei* than the corresponding unacetylated acyclic analogs.

Biology: The *in vitro* antiparasitic activity of the prepared acyclic nucleoside analogs was examined against 1) the LAB 110 EATRO isolate of *Trypanosoma brucei brucei*; 2) *Trypanosoma cruzi* (strain MHOM/BR/OO/Y) amastigote-infected macrophages and; 3) *Leishmania donovani* (strain MHOM/ET/67/L82) amastigote-infected macrophages. Compounds were tested against *T. b. brucei* and *L. donovani* according to our published procedures.[15,16] The method for testing compounds against *T. cruzi* is described below.

Assay for activity against *T. cruzi*. *T. cruzi* trypomastigotes, derived from the overlay of infected L6 myoblast cultures, were used to infect mouse peritoneal macrophages at a parasite:macrophage ratio of 5:1. Infected macrophage cultures were incubated with test compounds and nifurtimox as a standard drug in RPMI 1640 medium plus 10% foetal calf serum at 37°C in 5%CO_2/air. After 3 days of exposure to drug, cultures were fixed and stained and a proportion of amastigote-infected macrophages assessed by microscopy in drug treated and untreated cultures. Each drug concentration was tested in quadruplicate.

TABLE 1. Structures and Preparative Yields of Purine 2',3'-Acyclonucleosides



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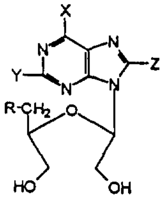

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Product (No.)	X	Y	Z	R	Yield(%)
Adenosine Analogs					
2',3'-acycloadenosine (3a)	NH_2	H	H	OH	57
2',3'-acyclo-2-aminoadenosine (3b)	NH_2	NH_2	H	OH	91
2',3'-acyclo-2-fluoroadenosine (3c)	F	NH_2	H	OH	25*
2',3'-acyclo-5'-deoxy-5'-chloroadenosine (3d)	NH_2	H	H	Cl	85
2',3'-acyclo-5'-deoxyadenosine (3e)	NH_2	H	H	H	96
2',3'-acyclo-5'-deoxy-5'-(ethylthio)adenosine (3f)	NH_2	H	H	CH_3CH_2S	28
2',3',-acyclo-5'-deoxy-5'-(hydroxyethylthio)adenosine (3g)	NH_2	H	H	$HOCH_2CH_2S$	52**
2',3'-acyclo-5'-deoxy-5'-(methylthio)adenosine (3h)	NH_2	H	H	CH_3	29
2',3'-acyclo-5'-trityladenosine-2',3'-dialdehyde (2i)	NH_2	H	H	O-trityl	100
Inosine Analogs					
2',3'-acycloinosine (3j)***	OH	H	H	OH	30
2',3'-acyclo-5'-deoxy-5'-chloroinosine (3k)***	OH	H	H	Cl	25

*yield of **4c**, prepared from **4b** using $HBF_4/NaNO_2$.[17] **3g prepared from **3d** in 3 steps (overall yield of 52%):1) **3d** was treated with mercaptoethanol/aqueous NaOH according to our described procedure;[18] 2) unpurified product (99%) was O-acetylated to give **4g** (recrystallized from ethyl acetate, 66% yield from **3d**); 3) **4g** was treated with ammonia/methanol at 0°C to give **3g** (80% yield from **4g**). ***Low yields reflect high affinity of inosine analogs for resin beds.

Results and Discussion:

A group of purine 2',3'-acyclonucleosides and their O-acetylated derivatives were prepared by an improved synthetic method. Compounds **3a, 3d, 3e, 3f, 3g, 3j** and **4a, 4b, 4c, 4d, 4e, 4f, 4g, 4j** were screened for activity *in vitro* against 3 related protozoan parasites, *T. b. brucei, T. cruzi and L. donovani* (Compounds **4a, 4b, 4c, 4d, 4e, 4f, 4g** and **4j** are the fully O-acetylated derivatives of compounds **3a, 3b, 3c, 3d, 3e, 3f, 3g** and **3j**, respectively). None of the compounds showed activity against *L. donovani.* Several compounds were moderately active at 30 μM against *T. cruzi* (i.e., growth inhibition by **3d, 3e, 4b, 4c, 4d** and **4e** ranged between 20-40% as compared to untreated cells). It is interesting that **3d, 4d, 3e** and **4e** are among the compounds to show moderate activity. This may be related to the absence of a 5'-hydroxy-substituent in these analogs, thereby precluding phosphorylation at this position.

Significant antiparasitic activity against *T. b. brucei* was seen for the O-acetylated derivatives of 2',3'-acycloadenosine, 2',3'-acyclo-5'-deoxy-5'-(hydroxyethylthio)adenosine and 2',3'-acycloinosine which had respective IC_{50} values of 7, 5.3 and 5.4 μM. The corresponding unacetylated analogs, **3a, 3g** and **3j** were considered inactive, with IC_{50} values ≥100 μM. This finding leads us to propose that the fully O-acetylated derivatives of **3a, 3g** and **3j**, i.e., **4a, 4g** and **4j**, are lipophilic prodrugs with an enhanced ability to penetrate African trypanosomes

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