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A New Class of C-Nucleoside Analogues. 1-(S)-aryl-1,4-dideoxy-1,4-imino-D-ribitols, Transition State Analogue Inhibitors of Nucleoside Hydrolase

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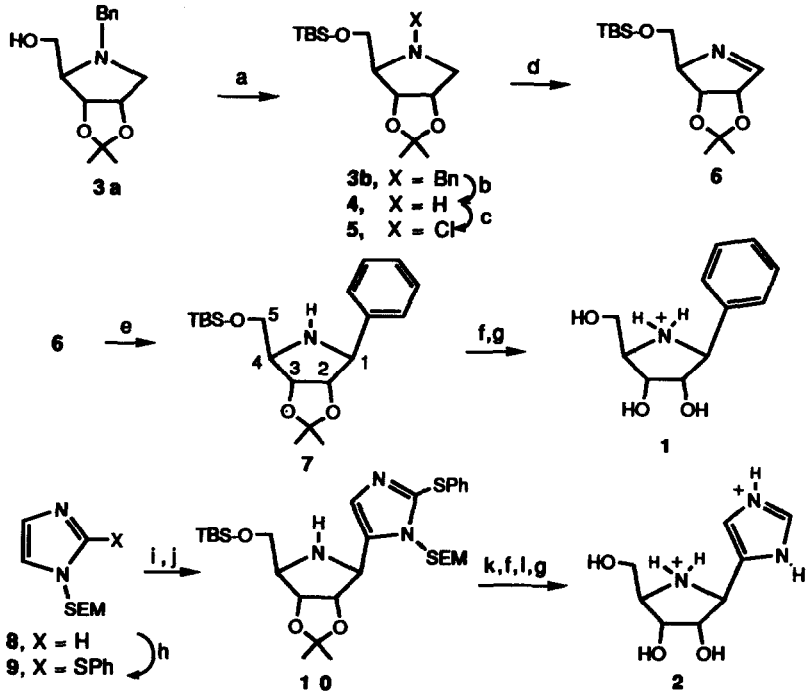
Abstract: The first members of a new class of N-glycohydrolase transition state analogue inhibitors, 1,4-dideoxy-1,4-imino-1-(S)-phenyl-D-ribitol, 1, and 1,4-dideoxy-1,4-imino-1-(S)-(4-imidazolyl)-D-ribitol, 2, have been synthesized. These compounds represent a new type of C-nucleoside analogue in which the endocyclic ribosyl ring oxygen has been replaced with nitrogen. The key synthetic step involves reaction of a protected ribosylimine with a metallated aryl species. The compounds are transition state analogues for enzymes which catalyze C-N glycosidic bond hydrolyses of nucleosides and have transition states with oxocarbenium ion character. Compounds 1 and 2 are potent competitive inhibitors with dissociation constants of 0.03 and 2.5 μM, for nucleoside hydrolase from the trypanosome *Crithidia fasciculata*. The highly effective inhibition observed demonstrates the importance of including both charge and aglycon mimicry in a glycosylase transition state analogue and differentiates it from simpler analogues.

N-glycohydrolases are a large group of enzymes which function by catalyzing the hydrolysis of the C-N glycosidic linkage for their respective substrates. This class includes nucleoside hydrolases, NAD glycohydrolases, ADP-ribosyl hydrolases, DNA glycosylases, and RNA glycosylases, which are known or implicated in pyrimidine and purine base salvage, regulation of the NAD pool, cellular regulation, DNA repair, and the action of plant toxins such as ricin, respectively.[1] Mechanistic study of these enzymes and characterization of their associated biology has lagged behind the characterization of O-glycohydrolases and would be facilitated by development of a general route to transition state analogue inhibitors. A common mechanistic theme for N-glycohydrolases is development of positive charge at the anomeric center in the transition state.[2] As for some O-glycohydrolases,[3] cationic transition states can be mimicked via exchange of nitrogen for the endocyclic ribosyl ring oxygen in the inhibitor. Previously synthesized glycosylase inhibitors have focused on the sugar oxocarbenium moiety as the first order target of transition state mimicry. However, transition states for glycosylase reactions have to varying degrees, involvement of a departing aglycon. Our mechanistic characterization and transition state analysis[1d,2a,4a] of the purine salvage enzyme nucleoside hydrolase, lead to a transition state model for inosine hydrolysis in which the ribosyl residue has substantial positive charge, yet is experiencing interaction with the departing hypoxanthine ring. Compounds 1 and 2, (Scheme 1) utilize *both* charge and aglycon mimicry and are analogues of this transition state.[4] These compounds represent entry into a new class of C-nucleoside analogue[5-8] and demonstrate that simultaneous charge and aglycon mimicry in a glycosylase transition state analogue can provide better inhibition than simple analogues which only mimic the saccharide residue.

We sought a convergent approach to **1** and **2** which used C-C bond formation between the iminoribitol and aglycon moieties as the key step. One way of achieving this construction is by addition of an organometallic aglycon species to an imine,[8] formally derived from 4-amino-4-deoxy-D-ribose. Imines may be synthesized under relatively mild conditions from N-halo amines by base-catalyzed elimination.[9] We anticipated that N-



Scheme 1

(a) t-butyl dimethylsilyl chloride, Et_3N, CH_2Cl_2. (b) 10% Pd-C, H_2(30 psi), EtOH. (c) N-chlorosuccinimide, pentane, 30 min. (d) Lithium tetramethyl piperidide, THF, -78 °C, 30 min. (e) 2 eq. PhMgBr, Et_2O, -78 °C, 30 min. (f) 50% aq. TFA, RT 12-16 h. (g) Dowex 50X8-200 (H^+ form) chromatography, load pH 7, wash H_2O, elute 5-10% aq NH_4OH., concentrate, then 1N aq HCl. (h) n-BuLi, THF, -78 °C, 1 h, then PhSSPh, -78 °C, 2 h, then RT, 1 h. (i) n-BuLi, THF, -78 °C, 45 min. (j) **6**, THF, -78 °C, 1 h. (k) Raney nickel, EtOH, 70 °C, 30 min (l) 2N aq HCl, 60-70° C, 20 h.

halogenation of a protected pyrrolidine having the ribose hydroxylation pattern, followed by dehydrohalogenation under kinetic control would regioselectively provide the desired imine. The requisite pyrrolidine **4** (Scheme 1) contains the required ribose hydroxylation pattern and can be conveniently prepared in multigram quantities from the known compound **3a** by the method of Fleet.[10] Conversion of **3a** to **4** proceeded in 92% overall yield by protection of the 5-hydroxyl as its TBS ether, followed by removal of the N-benzyl protecting group by catalytic hydrogenation.[11]

Reaction of **4** with 1.2 equivalents of N-chlorosuccinimide in pentane[12] afforded clean conversion to the N-chloro **5**, as evidenced by TLC and NMR analysis of the crude product. Chloroamine **5** was subjected to the following two-step one-pot procedure because of the significantly lower yields obtained when the intermediate

imine **6** was subject to aqueous workup and/or silica chromatography. After chlorination, **5** was immediately treated with lithium tetramethylpiperidide (THF, -78 °C, 15-30 min).[13] The reaction mixture was concentrated, and treated with PhMgBr (2 eq., Et_2O, -78 °C, then RT,30 min). After aqueous workup and chromatography (silica, 10:1 Hexane/EtOAc) **7** was obtained in 64% yield, from **4**.[14] The 1-(S) stereochemistry for **7** was established by NMR analysis. The observed complementary nOe's between H1 and H4, and the phenyl group and H-2 could only arise from the (S) epimer. In a separate reaction, imine **6** was purified by silica chromatography (7:1 Hexane/EtOAc) after aqueous workup. The regiochemistry for imine formation was established by observation of a doublet (1H, J=1.0) at 8.3 ppm which was assigned to the CH hydrogen of the imine double bond; the undesired imine lacks this hydrogen. Deprotection of **7** (50% aq. TFA) provided **1** in 76% yield as the HCl salt. Compound **2** was prepared by a route analogous to that used for **1**, via addition of lithiated[15] imidazole **9** (n-BuLi, THF -78°) to **6** (THF, -78° C), providing **10** in 26% yield. Imidazole **9** was synthesized in 84% yield from previously reported **8**[16,17] (n-BuLi, -78 °C; PhSSPh). As for **7**, the 1-S-configuration obtained for **10** was established by difference nOe experiments. Compound **10** was deprotected by sequential treatment with Raney nickel, 50% aq. TFA, and 2 N HCl, to provide inhibitor **2**·HCl in 57% yield from **10**. Both protonated and unprotonated forms of **1** and **2** are stable in aqueous solution. Compounds **1** and **2** show pKa's of 6.5 and 5.2, 8.7(imidazole, pyrrolidine).[4b]

Compound **1** was a competitive inhibitor of nucleoside hydrolase with a K_i of 0.17 μM and it showed a slow onset tighter binding phase, with an estimated equilibrium dissociation constant of 0.03 μM.[4b] Compound **2** was also a competitive inhibitor, with an inhibition constant of 2.5 μM.[4b] Both bind tighter than substrate inosine, with a K_d of 380 μM.[1d] The importance of the aglycon functionality[18] in **1** and **2** is underscored by the observation[4b] that the simple iminoribitol derived from complete deprotection of **3a**[10] which lacks aglycon functionality has an inhibition constant of 10 μM. Compounds such as **1** and **2** may be useful inhibitors of other N-glycohydrolases which proceed via transition states related to that of nucleoside hydrolase. Other applications may include affinity chromatography of N-glycohydrolases, and incorporation into RNA and DNA to effect charge and/or covalent manipulation of the sugar-phosphate backbone. Since the 5' TBS ether protecting group may be selectively removed with fluoride, synthesis of novel 5'-phosphorylated analogues of **1** and **2** is under investigation. Nucleic acid bases have not been employed in coupling reactions with imine **6**, as the present deprotection scheme would likely be incompatible with the labile product aminal. The strategy employed in this synthesis for construction of the 1-substituted imino-ribitols is currently being employed for synthesis of other new C-nucleoside and C-nucleotide analogues.

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