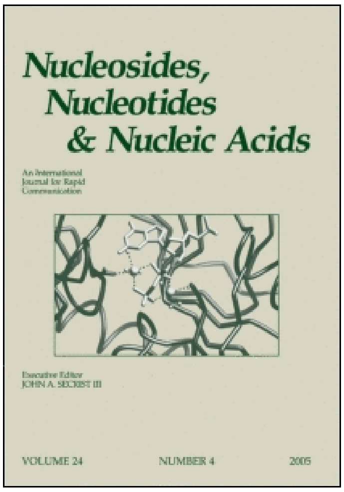

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The Synthesis of Novel Transition State Analogues Related to Imidazole and Purine Nucleotides Involved in the *De novo* Biosynthesis of Purines

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THE SYNTHESIS OF NOVEL TRANSITION STATE ANALOGUES RELATED TO IMIDAZOLE AND PURINE NUCLEOTIDES INVOLVED IN THE *de novo* BIOSYNTHESIS OF PURINES

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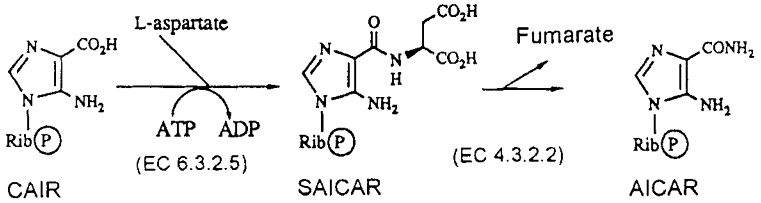
ABSTRACT

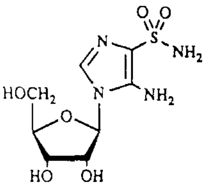
5-Amino-4-sulphonamidoimidazole ribofuranosyl nucleosides and a related imidazothiadiazine dioxide nucleoside have been synthesized as potential inhibitors of enzymes involved in the *de novo* biosynthesis of purine nucleotides.

The *de novo* biosynthesis of purine nucleotides is known to have an important rôle in rapidly proliferating cells; hence, inhibitors of this pathway, in such cells, have potential as anti-tumor agents[1].

Two steps, which are central in the pathway, involve the amination of 4-carboxy-5-aminoimidazole ribonucleotide (CAIR) with L-aspartate and ATP to give the N-succinylamide SAICAR followed by trans-elimination to give the carboxamide AICAR. The two enzymes involved are SAICAR-kinosynthetase (EC 6.3.2.5) and adenylosuccinate lyase (EC 4.3.2.2), respectively. The CAIR to SAICAR step, catalysed by EC 6.3.2.5., involves reaction of CAIR with ATP to give an acyl phosphate, which with L-aspartate would give a tetrahedral intermediate. Therefore,



we initiated a programme to synthesize the series of 5-amino-4-sulfonamidoimidazole nucleosides **1**, **2** and **3** as transition state analogues and potential enzyme inhibitors. In addition we synthesized an analogue of inosine namely, 5-(β-D-ribofuranosyl)imidazole[4,5-e]-1,2,4-thiadiazine-1,1-dioxode (**4**) as a potential inhibitor of the enzyme adenylosuccinate synthetase (EC 6.3.4.4) which catalyses the amination of inosinic acid with L-aspartate and GTP to give adenylosuccinate (ASA), the immediate precursor of AMP.



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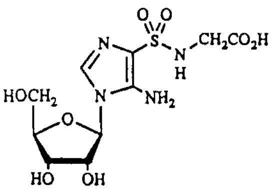

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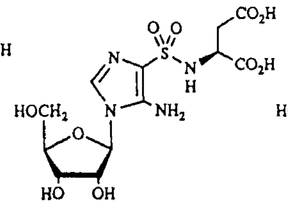

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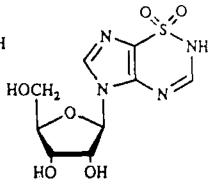


4

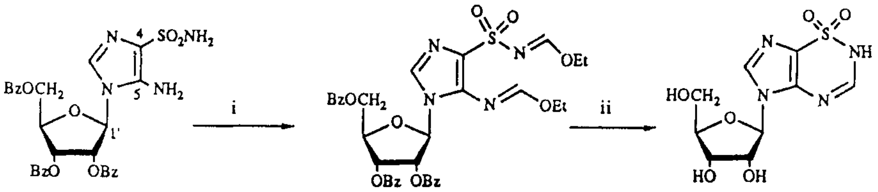
A comparison of thermodynamic and kinetic control was made for the condensation of silylated (using TMSCl, HMDS in xylene) 4(5)-nitro-5-(4)-sulphonamidoimidazole (**5**)[2] with 1-O-acetyl-2,3,5-tri-O-benzoyl-α-D-ribofuranose (**6**) in the presence of trimethylsilyl trifluoromethanesulfonate (TMSOTf) and MeCN. After 16 h, over which the reaction temperature was allowed to raise from 0°C to room temperature (thermodynamic control conditions), an anomeric mixture of 4-nitro-5-sulphonoamido-1-(2,3,5-tri-O-benzoyl-D-ribofuranosyl)imidazole (**7**) was obtained; the mixture was separated by silica gel column chromatography to yield 7α[3](39%)and. 7β(48%). In a parallel reaction which was stopped after 3 min (kinetic control conditions), no α-nucleosides were detected. Instead, the reaction gave a separable (by silica gel column chromatography) mixture of 7β(41%) and 5-nitro-4-sulphonamido-1-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)imidazole (**8**)(44%). Reduction of **8** with H_2, Pd/C in EtOAc gave the corresponding 5-amino-4-sulphonamide-1-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)imidazole, in high yield, which upon treatment with NH_3, MeOH gave 5-amino-4-sulphonamino-1-(β-D-ribofuranosyl)imidazole (**1**)(82%) which is a close analogue of AICAR.

Structural elucidation of the nucleosides obtained was based on NMR spectroscopy. All nucleosides obeyed the following empirical rules: (a) for an anomeric pair of nucleosides the signal for H-1′ of the α-anomer was at lower field and had a larger $J_{1',2'}$ than for the corresponding β-anomer[3,4] (b) for a regioisomeric pair, reduction of the nitro group to an amino group produced a greater upfield shift for H-1′ for the 5-nitro regioisomers than in the case of the corresponding 4-nitro isomers, (c) for a regiosomeric pair of aminosulfonamides, the C-4 and C-5 signals had larger separations in the 4-amino regioisomers (Δδ~50 ppm) than in the corresponding 5-amino isomers (Δδ~25 ppm). A similar correlation was observed in our earlier work with 4/5-aminoimidazole-5/4-carboxamide and carboxylate nucleosides.[5]

A similar synthetic strategy was followed for the synthesis of SAICAR analogues. Thus, ethyl N-[4(5)-nitroimidazole-5(4)-sulfonyl]glycinate (prepared in 56% yield by treatment of the sulfonyl chloride[2] with ethyl glycinate hydrochloride in Et_3N and DMF) was reacted with **6** under the kinetic control conditions to give, as expected, N-[5-nitro-1-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)imidazole-4-sulfonyl]glycine(**9**) and its corresponding 4-nitro regioisomer **10**(34%). Reduction of **9**(33%) with H_2, Pd/C in EtOAc gave the corresponding amino derivative which was deprotected using NaOMe (cat.), MeOH and then NaOHaq. to give **2**(84%).

Similarly, diethyl N-[4(5)-nitroimidazole-5(4)-sulphonyl]-L-aspartate (prepared from the sulphonyl chloride[2] and diethyl L-aspartate in DMF and Et_3N) was converted, under conditions of thermodynamic control, to N-[5-nitro-1-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)imidazole-4-sulphonyl]-L-aspartate (**11**)(29%) and its corresponding α-anomer **12**(38%), which were separated by silica gel column chromatography. The former, **11**, was reduced to the corresponding aminosulfonamide (96%) (δ_c 140.7 and 117.7, Δδ 23.0), which was debenzoylated and hydrolyzed to give **3**(31%).

Extension of this work to the synthesis of purine analogues was achieved by ring closure of **8** by reaction with triethylorthoformate. Interestingly, this reaction first gave the bis-(ethoxymethylene) derivative **13**(76%) which, on treatment with aqueous methanolic NaOH (pH10)[6], gave the desired inosine analogue **47** in 71% yield. The 7-(β-D-ribofuranosyl)-regioisomer of this nucleoside was previously synthesized by direct ribosylation of the bicyclic heterocycle.[6]



i, $HC(OEt)_3$, 120 °C, 4 h; ii, MeOH, NaOH aq to pH 8, 2 h, then adjust to pH 10, 2 h

Preliminary *in vitro* testing of the nucleosides **1**,**2**,**3** and **4** indicated that they were not cytotoxic against the IMC 15A cell line at concentrations below 10μg/mL.

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7. $[\alpha]_D$-60.0° (c.0.3, H_2O);λ_{max}(H_2O,pH 8) 228 and 273nm; δ_H (D_2O) 5.85(1H,d,*J* 6.40,H-1'), 7.63 (1H,s,H-6),7.96 (1H,s,H-3); δ_C[50 MHz, $(CD_3)_2SO$] 62.2 (C-5'),71.7, 74.8 (C-2', C-3'), 87.2 (C-4'), 90.2 (C-1'), 120.9 (C-7a), 134.3 (C-4a), 135.8 (C-6), 145.9 (C-3).