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NUCLEOSIDES AND NUCLEOTIDES. 117. A TRANSITION-STATE ANALOGUE IN PURINE NUCLEOTIDE BIOSYNTHESIS: THE DESIGN AND SYNTHESIS OF AN IMIDAZO[4,5-*c*]AZEPINE NUCLEOSIDE[1]

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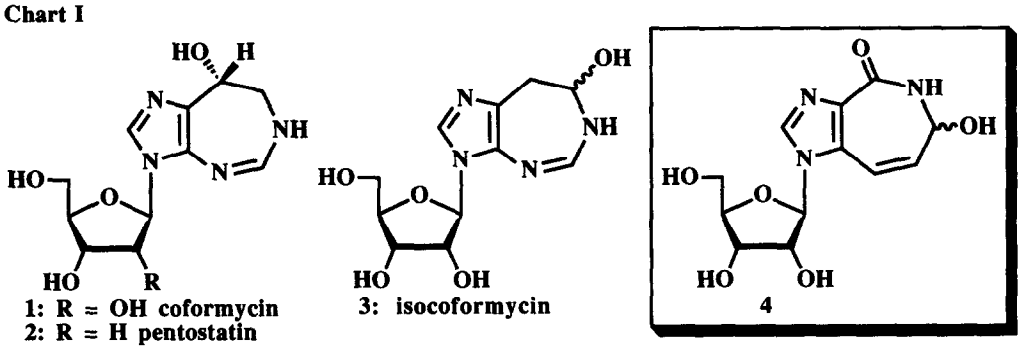
Key Words: nucleoside; imidazo[4,5-*c*]azepine nucleoside; transition-state analogue; GMP synthase, antileukemic activity

Abstract: The design, synthesis, and antileukemic activity of 6-hydroxy-1-β-D-ribofuranosyl-5,6-dihydroimidazo[4,5-*c*]azepin-4(1*H*)-one (**4**) are described.

In the research efforts into isolation and pharmacological evaluation of naturally occurring nucleoside antibiotics, discovery of coformycin (**1**)[2] and its 2'-deoxy analogue (**2**, pentostatin)[3] was one of the most epoch-making things not only for their biological and pharmacological properties but also their structural uniqueness. They have a 5:7-fused imidazo[4,5-*d*][1,3]diazepine ring system in their structures, which are regarded as ring-expanded purine nucleosides. Isocoformycin (**3**)[4] was also synthesized as its analogue during the synthesis of coformycin (Chart I). All compounds are exceedingly tight-binding inhibitors of adenosine deaminase (ADA).[5] The mechanism of ADA-catalyzed deamination of adenosine has been proposed to form a tetrahedral intermediate at C-6 of the purine,[6] which is structurally quite similar to their inhibitors. Therefore, these are called transition-state analogues. However, almost all the transition-state analogues so far reported[7] in the purine nucleotide biosynthesis are for catabolic pathways, but little is known for anabolic pathways.

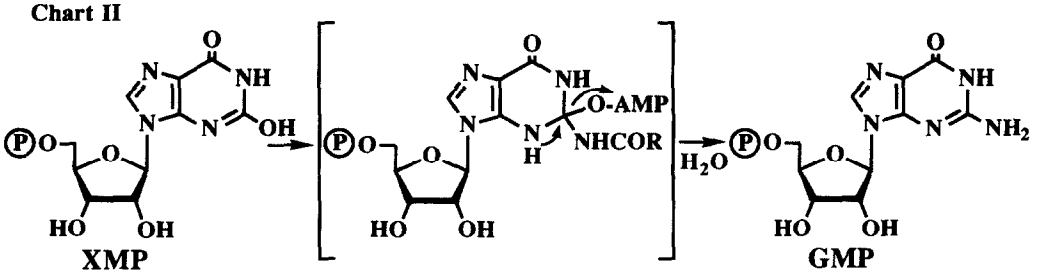
From these considerations, we thought about the metabolic pathways of IMP→XMP→GMP[8] in *de novo* purine nucleotide synthesis, and planned the design and synthesis of a transition-state inhibitor of GMP synthase, which catalyzes the conversion of XMP to GMP. GMP synthase as well as IMP dehydrogenase are key enzymes that regulate intracellular GMP levels. Lagerkvist reported a mechanism for GMP synthase,[9] in

Chart I



1: R = OH coformycin
2: R = H pentostatin
3: isocoformycin
4

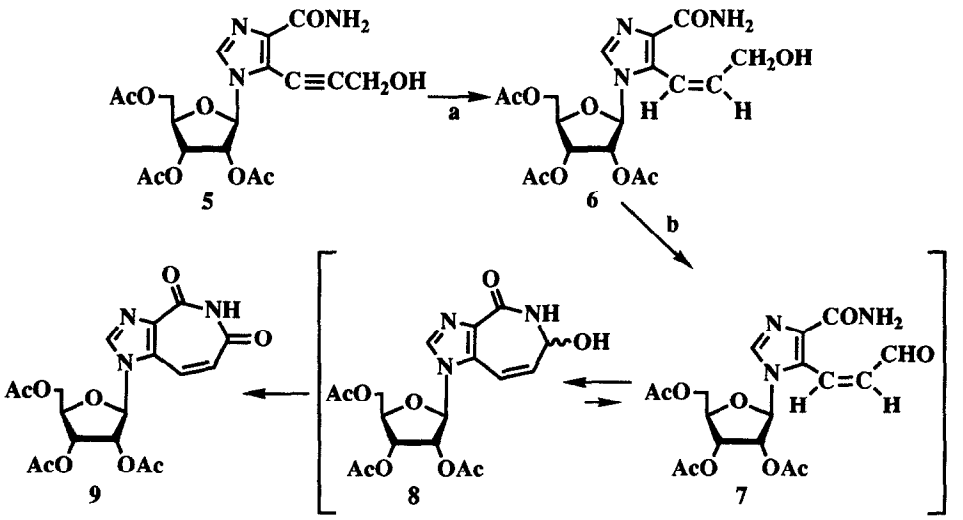
Chart II



which they suggested a tetrahedral intermediate as shown in Chart II. On the bases of this knowledge, we designed 6-hydroxy-1-β-D-ribofuranosyl-5,6-dihydroimidazo[4,5-*c*]azepin-4(1*H*)-one (**4**), a 5'-monophosphate of which could act as a transition-state inhibitor of GMP synthase (Chart I).

Initially, we attempted to synthesize **4** as shown in Scheme I. Since it is obvious that the target compound **8** is in an equilibrium with (*Z*)-formylvinyl derivative **7**, it could be easily derived from 3-hydroxypropyne derivative **5**.[10] Treatment of **5** with a palladium-on-carbon catalyst under H_2 atmosphere gave compound **6**[11] in 93% yield. Oxidation of **6** with barium manganate,[12] however, gave 1,5-dihydroimidazo[4,5-*c*]azepine-4,6-dione derivative **9**[13] in 67% yield but not the desired **7** and **8**. The formation of **9** suggested that oxidation of **6** gave **7** and spontaneous cyclization took place to afford **8**. Since **8** has also an allylic alcohol unit in the structure, it was further oxidized to give **9**. From these results, oxidation of the allylic alcohol in **6** should be done before intramolecular cyclization. Therefore, we introduced the (*E*)-3-hydroxypropenyl group at 5-position of the imidazole ring (Scheme II). Treatment of 5-iodo derivative **10**[10] with (*E*)-1-tributylstannylprop-1-en-3-ol (**11**)[14] in the presence of $(PhCN)_2PdCl_2$ gave (*E*)-5-(3-hydroxy-1-propenyl)-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)imidazole-4-carboxamide (**12**) in 68%

Scheme I[a]

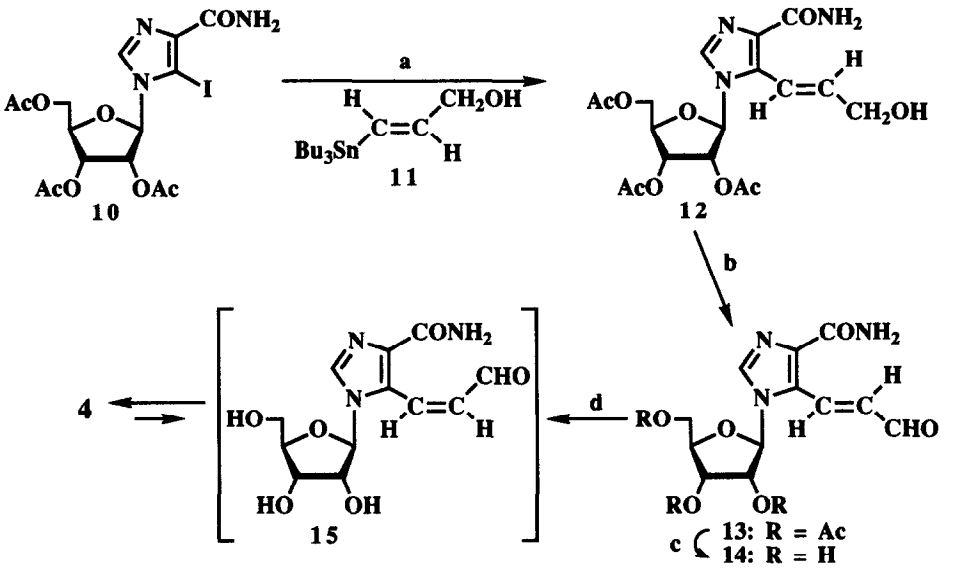


[a]a) H_2, Pd / C, EtOH; b) $BaMnO_4$ in CH_2Cl_2.

yield. Conversion of **12** to **13**[11] was achieved in 81% yield by using barium manganate. Compound **13** was treated briefly with sodium methoxide to give (*E*)-5-(2-formylvinyl)-1-β-D-ribofuranosylimidazole-4-carboxamide (**14**) in 76% yield. Photo-irradiation of **14** in methanol with a high pressure Hg lamp using a Pyrex filter gave a 1:1 mixture of the 6R and 6S diastereomeric alcohols **4**[15] in 56% yield after purification by preparative HPLC, and in which the (*Z*)-formylvinyl derivative **15** was not detected. Further, the formyl proton in **4** was not observed in its ^{1}H-NMR measurement. Therefore, the equilibrium between **4** and **15** is thought to lie far toward the cyclized form **4**.

Compound **4** showed moderate cytotoxicity (EC_{50} = 5.0 μg/ml) toward murine L1210 cells *in vitro*. Whether the cytotoxicity of **4** is due to the inhibition of GMP synthase or not is unclear in this study. The nucleoside should be converted into its 5'-monophosphate to be acting *via* the proposed mode of action. Since **4** itself could act as an alkylator of amines of proteins, it would be interesting to synthesize the 5'-monophosphate of **4** and test it for the ability to inhibit GMP synthase.

Scheme II[a]



[a]a) reagent **11**, $(PhCN)_2PdCl_2$ in CH_3CN, 100 °C; b) $BaMnO_4$ in CH_2Cl_2; c) 1 N NaOMe in MeOH, room temperature, 30 min; d) 100 W-high pressure Hg lamp in MeOH, 30 min.

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References and Notes

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8. The following abbreviations are used: IMP, inosine 5'-phosphate; XMP, xanthosine 5'-phosphate; GMP, guanosine 5'-phosphate.
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11. In ^{1}H-NMR spectra, the coupling constant of olefin protons of **6** was 11.7 Hz, which showed *Z*-geometry, while for **13**, it was 16.5 Hz, which showed *E*-geometry.
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13. **9**: MS *m/z* 421 (M^+); ^{1}H NMR ($CDCl_3$) 8.65 (br s, 1 H, NH), 8.10 (s, 1 H, H-2), 7.29 (d, 1 H, H-8, J = 12.5 Hz), 6.50 (d, 1 H, H-7, J = 12.5 Hz), 5.98 (d, 1 H, H-1', $J_{1',2'}$ = 5.1 Hz), 5.48 (dd, 1 H, H-2', $J_{2',1'}$ = 5.1, $J_{2',3'}$ = 5.5 Hz), 5.38 (dd, 1 H, H-3', $J_{3',2'}$ = 5.5, $J_{3',4'}$ = 4.8 Hz), 4.56~4.36 (m, 3 H, H-4', 5'a, b), 2.17, 2.16, 2.15 (each s, each 3 H, acetyl).
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15. **4**: FAB-MS *m/z* 298 (M^++1); UV λ_{max} (H_2O) 278 nm (ε 5600); UV λ_{max} (0.5 N NaOH) 268 nm (ε 5400); ^{1}H NMR (DMSO-d_6) 8.09 (s, 1 H, H-2), 7.99 (d, 0.5 H, NH of isomer A, J = 5.5 Hz), 7.91 (d, 0.5 H, NH of isomer B, J = 4.4 Hz), 6.76 (d, 1 H, H-8, J = 10.4 Hz), 6.31 (d, 0.5 H, 6-OH of isomer A, J = 5.5 Hz), 6.24~6.18 (m, 1.5 H, H-7, 6-OH of isomer B), 5.61 (d, 1 H, H-1', $J_{1',2'}$ = 5.5 Hz), 5.52 (br s, 1 H, 2'-OH), 5.20 (d, 1 H, 3'-OH, $J_{3'\text{-OH},3'}$ = 4.4 Hz), 5.08 (br s, 1 H, 5'-OH), 4.98~4.94 (m, 1 H, H-6), 4.22~4.18 (m, 1 H, H-2'), 4.06~4.05 (m, 1 H, H-3), 3.94~3.91 (m, 1 H, H-4'), 3.67~3.53 (m, 2 H, H-5'a, b); Anal. Calcd for $C_{12}H_{15}N_3O_6$: C, 48.48; H, 5.09; N, 14.14. Found: C, 48.36; H, 5.20; N, 13.85.