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4-Aza-7,9-Dideazaadenosine, a New Cytotoxic Synthetic C–Nucleoside Analogue of Adenosine

Shirish A. Patil, Brian A. Otter and Robert S. Klein

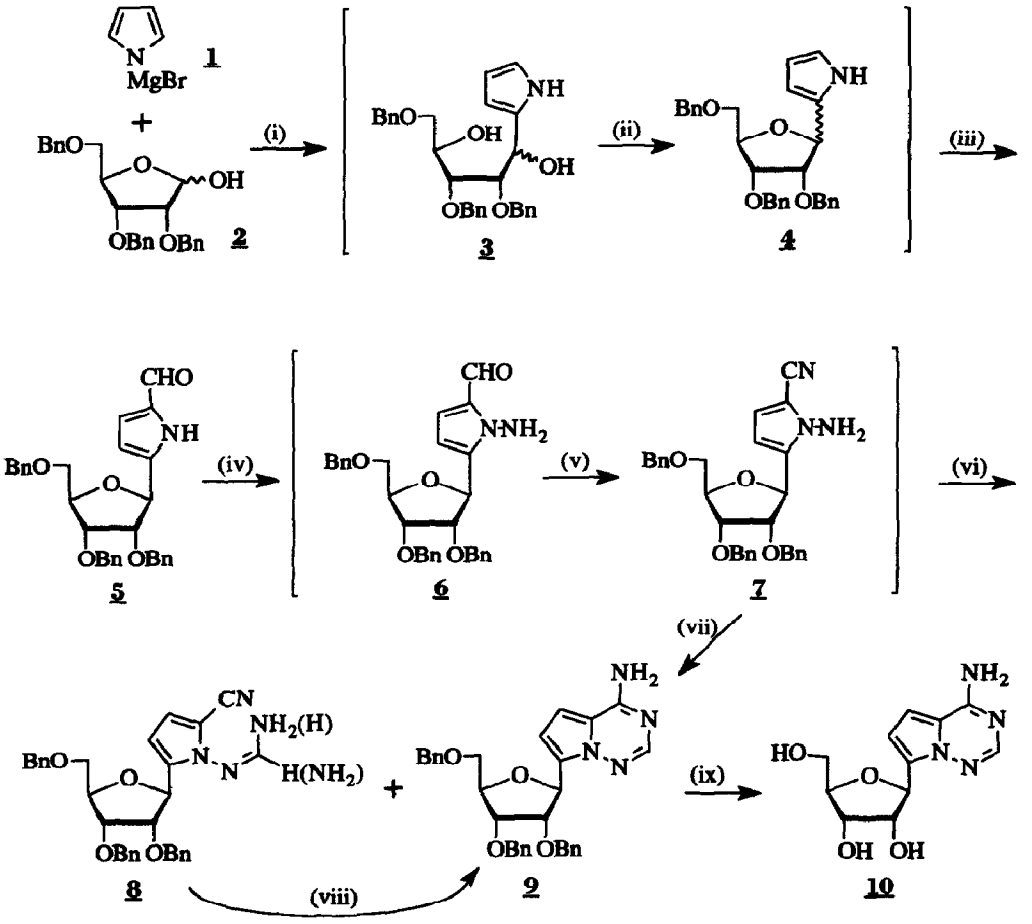
Albert Einstein College of Medicine Cancer Center and Medicinal Chemistry Laboratory, Department of Oncology, Montefiore Medical Center, 111 East 210th St., Bronx, NY 10467.

Abstract: The first synthesis of **10** , the pyrrolo[2,1-*f*][1,2,4]triazine C-nucleoside congener of adenosine is described. The key intermediate ribofuranosyl pyrrole **4** is obtained by the direct C-ribosylation of pyrrolemagnesiumbromide with 2,3,5-tri-*O*-benzyl ribose followed by an acid-catalyzed dehydration. Vilsmeier formylation of **4** followed by N-amination and CHO –> CN conversion affords N-amino nitrile intermediate **7** which can be cyclized with formamidine acetate to the blocked title compound **9**. Hydrogenolytic debenzylation completes the synthesis. *In vitro* growth inhibitory activities of **10** against leukemic cell lines (0.8 - 15 nM) are comparable to those of 9-deazaadenosine.

As part of our program directed towards the design and synthesis of novel purine-like C-nucleosides of potential biomedical interest, we recently initiated the investigation of a new class of analogues where the purine moiety is replaced by the pyrrolo[2,1-*f*][1,2,4]triazine system (also referred to herein as 4-aza-7,9-dideaza purine). Our interest in such analogues was prompted by, among other considerations, the considerable similarity between the computer-generated molecular electrostatic potential (MEP) maps of adenosine and of its pyrrolo[2,1-*f*][1,2,4]triazine congener when plotted in the plane of the base. As a preliminary to the investigation of this new class of C-nucleosides, we described recently [1] a simple synthetic approach to several 4-mono- and 2,4-difunctionalized pyrrolo[2,1-*f*][1,2,4]triazine derivatives which are also new analogues of the common nucleic acid purine bases. The developed method was expected to be amenable to the synthesis of the corresponding C-nucleosides and relies on the utilization of either hydroxylamine-*O*-sulfonic acid (HOSA) [2] or *O*-mesitylenesulfonylhydroxylamine (MSH) [3] to bring about both N-amination and conversion of CHO –> CN of appropriately substituted 2-pyrrole aldehydes. We wish to report herein its application to the synthesis of the adenosine analogue **10**.

We had envisaged the prerequisite intermediate for this synthesis to be a 5-ribosyl pyrrole-2 aldehyde such as **5** or, alternatively, its unformylated precursor **4**. Maeba et al. had previously reported the synthesis of the 2,3,5-tri-*O*-benzoyl- and 2,3-*O*-isopropylidene- derivatives of **5** as well as the fully unblocked ribofuranosyl-pyrrole 2-carboxaldehyde.[4] These authors also reported the synthesis of 2-(β- and α-D-ribofuranosyl) pyrrole.[5] Though all were deemed suitable for our purpose, they were obtained by fairly lengthy routes involving oxidation of a 2-ribosylated furan or a 5-ribosylated 2-furfuryl alcohol followed by treatment of the products with an appropriate monoamine. We opted for a shorter approach involving *direct ribosylation* of the pyrrole ring based on recent studies of the reaction between indolylbromomagnesium salts and protected furanoses which showed that solvent effects played a critical role in determining the regioselectivity of N- vs C-glycosylation, [6] with C-alkylation becoming almost exclusive with the use of CH_2Cl_2. Extension of these studies to the direct C-heteroarylation of acyclic and cyclic sugars with pyrrole [7] had further demonstrated the possibility for good regio- and stereoselective control.

Scheme 1



(i) CH_2Cl_2 / 0 °C; (ii) CF_3COOH; (iii) $POCl_3$-DMF / 0 °C; (iv) O-Mesitylenesulfonylhydroxylamine, NaH / THF / 0 °C; (v) HOSA, KOH / Dioxane-H_2O / 0 °C; (vi) Formamidine acetate, EtOH / Reflux; (vii) Formamidine acetate, DMA / 140 °C; (viii) K_2CO_3 / MeOH; (ix) 10% Pd/C, H_2, AcOH.

Reaction between **2** [8] and pyrrolemagnesium bromide [7,9] **1** (5 equiv.) in CH_2Cl_2 at 0 °C for 1 hr. followed by quenching with aqueous NH_4Cl afforded a mixture of products containing alditolylpyrrole **3**. Without isolation, **3** was subjected to an *in situ* acid-promoted dehydrative annulation to **4** by addition of CF_3COOH. Thin layer chromatography indicated that the reaction was complete after 1 hr. at room temperature. In initial experiments, partial chromatographic separation of the products indicated a mixture of both C- and N-

nucleosides, the complete characterization of which was rendered difficult by their slow degradation throughout the separation procedures. Since we expected the inductive effect of the carboxaldehyde function in intermediate **5** to reduce susceptibility of these pyrrole derivatives to oxidation and other degradative processes, no further attempts were made to isolate **4** in pure form at this stage. Instead, a direct Vilsmeier formylation of the aromatic nucleus was carried out by the dropwise addition of the crude product containing **4** to a solution of $POCl_3$ (1.8 equiv.) in DMF at 0 °C followed by quenching with sodium acetate. The major product, pyrrole aldehyde **5**, was isolated by flash chromatography on silica gel [ethyl acetate (8%-15%) in hexanes] (26 % yield from **2**). The ^1H NMR spectrum of the material obtained from the major fractions containing **5** indicated also a small amount of an isomeric product, presumably the α-anomer. A crystalline sample of **5** [10] was obtained by cooling concentrated elution fractions; mp 103-106 °C. X-Ray crystallographic techniques established its identity as the desired β–anomer and revealed a strong hydrogen bonding between N1-H and O-5'.

For the further elaboration of aldehyde **5** to the desired N-amino nitrile intermediate **7**, we found it advantageous to carry out first the N-amination of **5** with MSH followed by conversion of the aldehyde function of crude intermediate **6** to a nitrile with HOSA. Generation of the anion of **5** with NaH (2 equiv.) in THF and treatment with MSH [3a] (1.1 equiv.) at 0 °C for 45 minutes led to a complete conversion to **6**. Without extensive purification, the crude product (**6**, in dioxane-water, 3:2) was treated with HOSA (3.5 equiv.) for 2 hr at 0 °C and the resulting oxime -*O*-sulfonate intermediate was degraded *in situ* by the addition of a cold dioxane-water (1:1) solution of potassium hydroxide. Crude N-amino nitrile **7** was isolated by standard extractive procedures and used as such in the next step.

Conversion of **7** to the blocked adenosine analogue **9** by treatment with formamidine acetate (10 equivs.) in boiling ethanol (23hr) was moderately successful, affording the desired product **9** in 27 % yield together with a small amount of the N-formamidino intermediate **8** (7 % yield). These were readily separated by preparative TLC ($CHCl_3$-MeOH, 96:4). As expected from our earlier studies,[1] intermediate **8** could be readily converted to **9**[11] (58 %) by treatment with base (K_2CO_3) in MeOH for 4 hr at ambient temperature. The relatively modest yields obtained in the direct annulation of **7** -> **9** with formamidine acetate in boiling ethanol stand in sharp contrast with the much higher ones (93%) we had obtained in the conversion of a 4-ribosylated 3-amino-2-cyano pyrrole to the corresponding pyrrolo[3,2-*d*]pyrimidine congener of adenosine (a 9-deazaadenosine derivative) under similar conditions [12] and may reflect the much lower nucleophilicity of the N-NH_2 group in **7**. A better conversion of **7** -> **9** was ultimately achieved by treating **7** with formamidine acetate (10 equivs.) in DMA for 1.5 hr at 140 °C. Isolation of the product by flash chromatography on silica gel ($CHCl_3$-MeOH, 99.5:0.5) gave **9** in 45 % yield. The synthesis of 4-amino-7-(β-D-ribofuranosyl)pyrrolo[2,1-*f*][1,2,4]triazine (**10**) was completed by the hydrogenolytic debenzylation of **9** over Pd/C (10 %, Degussa type) in AcOH as solvent and with H_2 at atmospheric pressure for 21 hr to give **10** in 46 % yield, mp (water) 210-214 °C.[13,14]

The preliminary *in vitro* data summarized in Table 1 indicate that 4-aza-7,9-dideazaadenosine **10** has pronounced growth inhibitory activity against several mouse and human neoplastic cell lines comparable to that of 9-deazaadenosine.

Table 1

In Vitro Antitumor Activity (ID_{50} in μM) of **10** and of 9-Deazaadenosine			
	L1210-C2	S180	HL60-JG
10	0.0035	0.015	0.00082
9-Deazaadenosine	0.0014	0.014	0.00064

Such biological activities, presumably a direct consequence of the antimetabolic properties of **10**, support our initial premise that MEP may serve as a useful tool for the selection of potentially active structural analogues.

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10) For **5**: ^{1}H NMR ($CDCl_3$, 200 MHz) : δ 3.57 (dd, 1H, H-5", $J_{5",5'}$ = 10.5 Hz, $J_{5",4'}$ = 1.6 Hz), 3.84 (dd, 1H, H-5', $J_{5',4'}$ = 2.7 Hz), 3.92 (dd, 1H, H-2', $J_{2',3'}$ = 4.3 Hz; $J_{2',1'}$ = 2.6 Hz), 4.16 (dd, 1H, H-3', $J_{3',4'}$ = 7.3 Hz), 4.26 (dq, 1H, H-4'),4.43 and 4.32 (two 1H d, CH_2, J_{gem} = 11.6 Hz), 4.89 and 4.67 (two 1H d, CH_2, J_{gem} = 12.5 Hz), 4.65 (s, 2H, CH_2), 5.18 (d, 1H, H-1'), 6.03 (dd, 1H, H-4, $J_{4,NH}$ = 2.6 Hz, $J_{4,3}$ = 3.8 Hz), 6.86 (dd, 1H, H-3, $J_{3,NH}$ = 2.4 Hz), 7.14-7.48 (m, 15H, Phenyl), 9.38 (s, 1H, CHO), 10.99 (bs, 1H, NH).
11) For **9**: ^{1}H NMR ($CDCl_3$, 200 MHz) : δ 3.66 (dd, 1H, H-5", $J_{5",5'}$ = 10.8 Hz, $J_{5",4'}$ = 4.0 Hz), 3.80 (dd, 1H, H-5', $J_{5',4'}$ = 3.2 Hz), 4.13 (m, 1H, H-3'), 4.28 (m, 1H, H-2'), 4.33-4.87 (m, 7H, Benzyl - CH_2 and other sugar protons), 5.41 (br s, 2H, NH_2), 5.69 (d, 1H, H-1', $J_{1',2'}$ = 3.6Hz), 6.51 (d, 1H, H-6, $J_{6,5}$ = 4.4 Hz), 6.70 (d, 1H, H-5), 7.30 (m, 15H, Phenyl), 7.93 (s, 1H, H-2). In addition to the peaks for **9**, the ^{1}H NMR spectrum of material obtained by chromatography indicates very minor amounts of an isomeric product, presumably the α-anomer. The data reported here is for the β-anomer only.
12) Lim, M-I.; Klein, R. S. *Tetrahedron Lett.* **1981**, *22*, 25-28.
13) For **10**: ^{1}H NMR (methyl sulfoxide-d_6 + D_2O) : δ 3.43 (dd, 1H, H-5", $J_{5",5'}$ = 11.7 Hz, $J_{5",4'}$ = 4.9 Hz), 3.53 (dd, 1H, H-5', $J_{5',4'}$ = 3.8 Hz), 3.77 (m, 1H, H-4'), 3.93 (t, 1H, H-3', $J_{3',4'}$ = 4.7Hz), 4.21 (dd, 1H, H-2', $J_{2',3'}$ = 5.4Hz), 5.09 (d, 1H, H-1', $J_{1',2'}$ = 6.5 Hz), 6.66 (d, 1H, H-6, $J_{6,5}$ = 4.4 Hz), 6.83 (d, 1H, H-5), 7.80 (s, 1H, H-2). Prior to the addition of D_2O, the exchangeable proton resonances appear at δ 4.77 (t, 1H, 5'-OH, J = 5.6 Hz), 4.87 (d, 1H, 3'-OH, J = 4.8 Hz), 4.96 (d, 1H, 2'-OH, J = 6.4 Hz) and 7.69 (bs, 2H, NH_2). ^{13}C NMR (methyl sulfoxide-d_6) : δ 62.0 (C-5'), 71.2 (C-3'), 73.6 (C-2'), 75.3 (C-1'), 84.4 (C-4'), 100.6 (C-5), 109.6 (C-6), 114.8 (C-4a), 128.8 (C-7), 147.6 (C-2), 155.5 (C-4).
14) Satisfactory elemental analyses (C, H and N within 0.400 %) were obtained for isolated compounds **5**, **9** and **10**.

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