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SYNTHESIS OF "9-DEAZAADENOSINE"; A NEW CYTOTOXIC C-NUCLEOSIDE ISOSTERE OF ADENOSINE[1]

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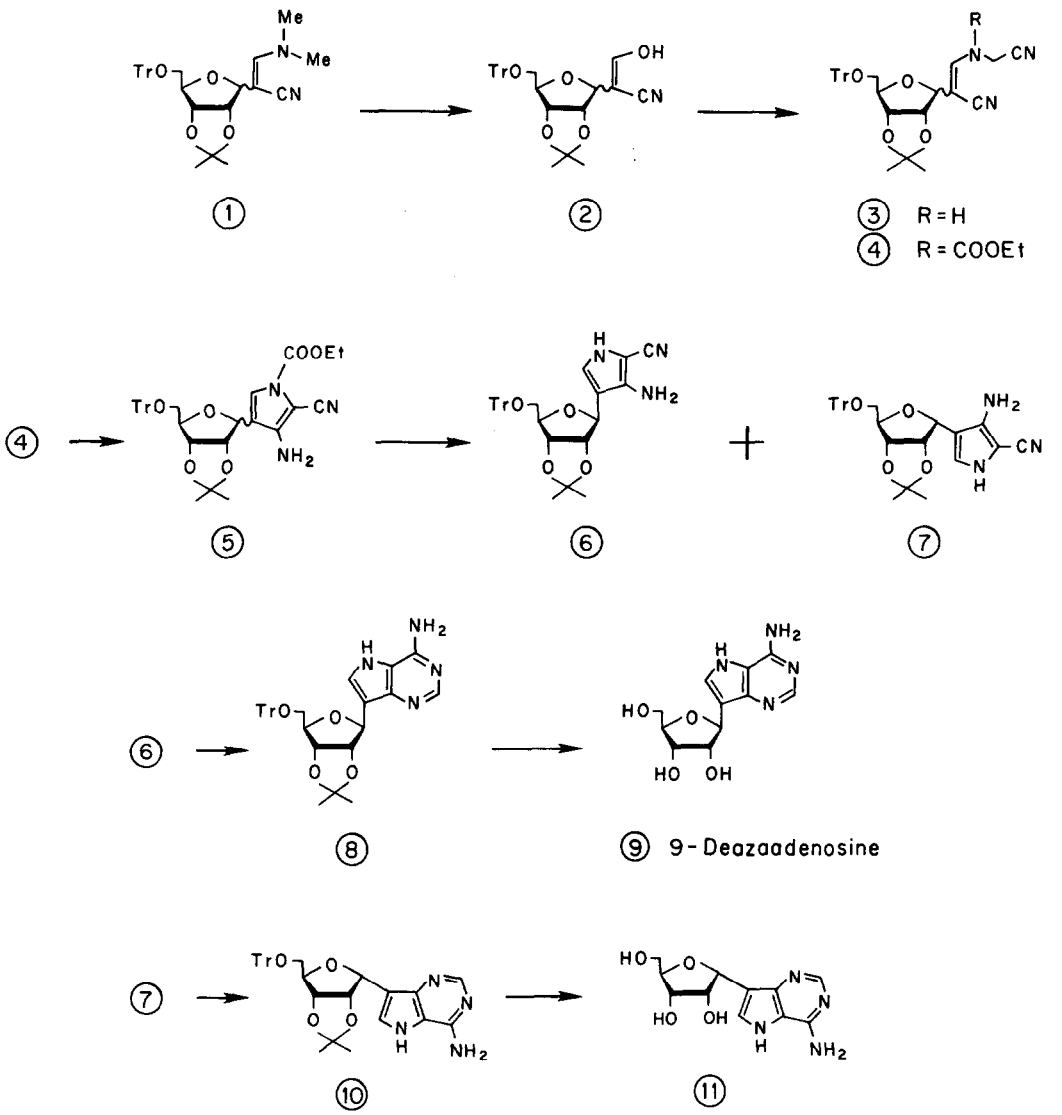
ABSTRACT: The synthesis of 7-(β-D-ribofuranosyl)-4-amino-5H-pyrrolo[3,2-d]pyrimidine (9-deazaadenosine) 9 is described. It involves base-catalyzed cyclization of N-carboethoxy-enamine 4 to give β- and α-ribosylated 3-amino-2-cyanopyrroles 6 and 7, respectively, followed by a one-step conversion to the desired pyrrolo[3,2-d]pyrimidine system.

The discovery that a nucleoside antibiotic, tubercidin (4-amino-7-(β-D-ribofuranosyl)-pyrrolo[2,3-d]pyrimidine or 7-deazaadenosine) is a powerful antibacterial, antifungal and cytotoxic analog of adenosine[2] spurred interest in the synthesis of several structurally related deazapurine nucleosides.[3] Of the four possible monodeazaadenosines, tubercidin,[4] 1-deaza-[5] and 3-deazaadenosine[6] have already been synthesized. We now wish to report the synthesis of the last remaining member of this series, viz., 9-deazaadenosine, a new synthetic pyrrolo[3,2-d]pyrimidine C-nucleoside of exceptional cytotoxicity to several lines of mouse and human leukemias (see below).

We have reported recently the synthesis of 7-(β-D-ribofuranosyl)-4-oxo-3H,5H - pyrrolo-[3,2-d]pyrimidine (9-deazainosine) by a two-step conversion of a 4-ribosylated 3-amino-2-carboalkoxy pyrrole.[7] Synthesis of this key intermediate relied upon ring-closure of an N-benzyl-β-enaminoacrylonitrile. While final removal of the N-benzyl blocking group could be readily achieved on a small scale by treatment with sodium naphthylide in tetrahydrofuran at 20°, [7,8] its removal by catalytic hydrogenolysis under milder conditions was not successful. The synthesis of 9-deazaadenosine described below utilizes instead a carboalkoxy group for protection of enamine 3 and should be of general applicability to the large-scale preparation of other structurally related 9-deazapurine C-nucleosides.

The intermediate 2-(D-ribofuranosyl)-2-formylacetonitrile 2,[7] readily obtained from 3-dimethylaminoacrylonitrile[9] 1 by mild acid hydrolysis, was converted to enamine 3 (81.5% from 1) by condensation with aminoacetonitrile hydrochloride (1.3 equiv.) in the presence of sodium acetate (1.5 equiv.) in aqueous methanol (25°, 20 hr). Our earlier studies[10] had indicated that blocking of the enamino-NH group of 3 was essential for effective ring-closure to the desired pyrrole. Such blocking was carried out by reaction of 3 with ethyl chloroformate (1.5 equiv.) and 1,5-diazabicyclo[4,3,0]nonene-5 (DBN, 2 equiv.) in methylene chloride

Figure 1


Me
N
Me
TrO
O
CN
OH
R
CN
③ R = H
④ R = COOEt
COOEt
NH2
⑤
⑥
⑦
⑧
⑨ 9-Deazaadenosine
HO
OH
⑩
⑪

(0°, 1 hr) to afford carbamate intermediate 4 as a mixture of epimers. Cyclization to pyrrole 5 could be carried out without isolation of this intermediate by treatment of the reaction mixture containing 4 with one additional equivalent of DBN (25°, 20 hr) to give N-carboethoxypyrrole 5 (84% overall yield from 3) as a mixture of α-β isomers.

Removal of the carboethoxy group at this stage was readily carried out by treatment of 5 with sodium carbonate (0.1 equiv.) in methanol (25°, 50 min) to give the desired unblocked β-, and α-3-amino-2-cyanopyrroles 6 (m.p. 170-172° from ethyl acetate-ethyl ether) and 7 (m.p. 176°, dec. from acetone-hexane), respectively (85% overall yield from 3, β/α ~5/2). These were separated by silica gel column chromatography[11] (toluene-ethyl acetate, 8:1) and identified by their NMR spectra.[12]

Although the α- and β-epimers of each of 4 and 5 can be readily separated and have been fully characterized,[12] it was found more convenient for large-scale preparative work to delete isolation of the desired β-isomer until completion of the decarboethoxylation step as we describe above. It is noteworthy that conversion of the isolated β-isomer of 4 to 5 and finally to 6 could be carried out without detectable epimerization at C-1'.

Treatment of 6 with formamidine acetate[13] (3 equiv.) in boiling ethanol (5 hr) afforded pyrrolopyrimidine 8 in 93.6% yield; H^1NMR ($CDCl_3$): δ 1.31, 1.51 (2s, 3H each, $C(CH_3)_2$), 3.37 (m, 2H, H-5', H-5"), 4.26 (m, 1H, H-4'), 4.77 (m, 1H, H-3'), 4.95 (m, 1H, H-2'), 5.22 (d, 1H, H-1', J = 4.3 Hz), 6.62 (bs, 2H, NH_2, exch. with D_2O), 7.17-7.39 (m, 16H, trityl and H-6), 7.70 (s, 1H, H-2). By an identical procedure, the corresponding α-isomer 7 was converted to 10; H^1NMR ($CDCl_3$): δ 1.28, 1.47 (2s, 3H each, $C(CH_3)_2$), 3.27 (m, 2H, H-5', H-5"), 4.36 (t, 1H, H-4'), 4.70-4.91 (m, 2H, H-2', H-3'), 5.49 (d, 1H, H-1', J = 3.1 Hz), 6.49 (bs, 2H, NH_2, exch. with D_2O), 7.21-7.50 (m, 16H, trityl and H-6), 8.16 (s, 1H, H-2).

Treatment of 8 with 12% HCl/MeOH (25°, 2 hr) afforded unblocked 9-deazaadenosine 9 which crystallized directly from the reaction mixture (75%). Recrystallization from ethanol afforded an analytically pure sample of the hydrochloride salt, m.p. 179-183°; H^1NMR (DMSO-d_6) δ 3.64 (d, 2H, H-5' and 5", J = 3, 1 Hz), 3.91-3.99 (m, 3H, H-2',3',4'), 4.88 (d, 1H, H-1', J = 6.4 Hz), 7.87 (d, 1H, H-6, J = 2.7 Hz, singlet with D_2O), 8.51 (s, 1H, H-2), 8.93 (bs, 2H, NH_2, exch. with D_2O), 12.16 (bs, 1H, NH, exch. with D_2O). Similarly, unblocking of the α-isomer 10 afforded the corresponding α-isomer of 9-deazaadenosine obtained as a hygroscopic solid; H^1NMR (DMSO-d6) δ 3.49-3.60 (m, 2H, H-5' and 5"), 4.06-4.21 (m, 3H, H-2',3',4'), 5.15 (d, 1H, H-1', J = 3.1 Hz), 7.81 (d, 1H, H-6, J = 2.7 Hz, singlet with D_2O), 8.47 (s, 1H, H-2), 9.02 (bs, 2H, NH_2, exch. with D_2O), 12.88 (bs, 1H, NH, exch. with D_2O).

The preliminary *in vitro* data[14] summarized in Table I indicate that 9-deazaadenosine has pronounced growth inhibitory activity against several mouse and human leukemic cell lines.

TABLE I

In Vitro Activity (ID_{50} in μg/ml) of 9-Deazaadenosine[14]

Mouse leukemia cell lines	P815	L5178Y	L1210	Human leukemia cell lines	HL60	K562	RPMI 8402
ID_{50}	0.001	0.002	0.0008	ID_{50}	0.0007	0.002	0.002

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14. The authors are indebted to Dr. Joseph H. Burchenal of this Institute for the communication of these results.
15. All new compounds were characterized by their NMR spectra and elemental analysis supported in some instances by mass spectroscopic data.

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