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Purine Nucleosides XVII.

The Synthesis and Conformation of 6-Amino-9-β-D-ribopyranosylpurine and Related Derivatives Prepared via the Fusion Procedure (1)

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The preparation of 2,6-dichloro-9-(2',3',4'-tri-*O*-acetyl-β-D-ribopyranosyl)purine (I) has been accomplished utilizing the acid catalyzed fusion procedure. The displacement of the 6-chloro group, or the 2- and 6-chloro group has been studied. Several new 6-substituted-9-(β-D-ribopyranosyl)purines have been prepared by catalytic dehalogenation of the corresponding 2-chloropurine nucleosides. The conformation and configuration of these D-ribopyranosylpurines has been assigned with the assistance of proton magnetic resonance studies.

Interest in the nucleic acid field has produced numerous analogs of the naturally occurring 9-β-D-ribofuranosylpurines and has prompted the development of several diverse methods for their preparation (2,3) while purine ribonucleosides possessing ribose in the pyranose form have received very little attention. The previous methods utilized for the preparation of 9-D-ribopyranosylpurines include the classical procedure of Todd (4) and the halomercury procedure of Davoll (5). It has been recently shown (6,7) that a contamination of mercuric ions in a concentration of as low as 10^{-8} *M*, which can be introduced in the halomercury method, must be excluded in order to obtain a true evaluation of the biological activity of a nucleoside. We have now succeeded in preparing 9-D-ribopyranosylpurine nucleosides utilizing 1,2,3,4-tetra-*O*-acetyl-β-D-ribopyranose (8) for the first time in the acid catalyzed fusion procedure (9) which avoids the possibility of contamination by mercuric ions.

A mixture of 2,6-dichloropurine and 1,2,3,4-tetra-*O*-acetyl-β-D-ribopyranose was heated at 160° in the presence of a catalyst (dichloroacetic acid) *in vacuo* to furnish a 63% yield of 2,6-dichloro-9-(2',3',4'-tri-*O*-acetyl-β-D-ribopyranosyl)purine (I). The site of glycosidation was readily ascertained for I by a comparison of its ultraviolet absorption spectra with the ultraviolet absorption spectra of 2,6-dichloro-9-(2',3',5'-tri-*O*-acetyl-β-D-ribofuranosyl)purine (10), 2,6-dichloro-9-methylpurine (11) and 2,6-dichloro-7-methylpurine (11). The exclusion of *N*-1 and *N*-3 as possible sites of glycosidation can be made on the basis that methylation studies (11,12) of 6-chloropurine, 2,6-dichloropurine and 2-chloropurine produce only the 7- and 9-methyl isomers. Treatment of I with ethanolic ammonia at room temperature resulted in removal of the acetyl groups from the carbohydrate moiety with the concomitant nucleophilic displacement of the 6-chloro group to furnish 6-amino-2-chloro-9-(β-D-ribopyranosyl)purine (II). The catalytic removal of the 2-chloro group with palladium on carbon in the presence of hydrogen provided the adenosine analog 6-amino-9-(β-D-ribopyranosyl)purine (V). The ultraviolet absorption spectra of V was consistent with the ultraviolet absorption spectra of a 9-substituted adenine and provided substantiation of the above assignment of nucleophilic displacement. The pmr spectrum of V in DMSO-d_6 displayed a broad absorption peak (2 protons) centered at 7.2 δ which disappeared on the addition of deuterium oxide and was therefore assigned to the 6-amino group. The anomeric configuration of V was previously (5) assigned as *beta* on the basis of optical rotation. This assignment was presumably based on the optical rotations observed (4) for the α-($[\alpha]_D$ +94°) and β-($[\alpha]_D$ -34°) anomers of the 2-methylthio-tri-*O*-acetyl derivative of V. Other investigators have subsequently utilized the same procedure for the assignment of anomeric configuration for D-ribopyranosylpurines. It has been recently reported (13) that the unambiguous assignment of anomeric configuration for certain D-ribopyranosylpyrimidines has been accomplished utilizing nuclear magnetic resonance. Therefore, this procedure was utilized to provide an unambiguous assignment of anomeric configuration for the nucleosides prepared in the present investigation. The absorption peak in the pmr spectrum of V attributed to the anomeric proton occurred at 5.72 δ and possessed a coupling constant ($J_{1,2}$) of 9.0 cps. This large coupling constant can only be explained (13,14,15) by an axial-axial interaction or *trans* relationship between the protons residing at C-1' and C-2' and provided additional corroboration for the initial (5) anomeric assignment as ***beta***. In fact, of the four possible ideal chair conformations for D-ribopyranosylpurines it can be seen (Figure 1) that only the β-anomer possesses vicinal diaxial protons at C-1' and C-2'. Therefore, this unambiguously confers the *beta* assignment to all nucleo-

sides prepared in this investigation.

Conformational analysis was initiated on the assumption that these nucleosides would exist preponderantly in a specific conformation since the bulky purine group should assume an equatorial position. Therefore, a justification of either the C1 or 1C conformation would be all that is required since we were concerned specifically with the β-anomer. It was obvious from a visual inspection (Figure 1) of the two possible chair conformations that the nucleosides prepared in this investigation exist primarily in the C1 conformation since the coupling constants ($J_{1,2}$) observed approaches 10 cps. A conformational equilibrium would display a coupling constant ($J_{1,2}$) smaller than 10 cps since protons in an equatorial-equatorial juxtaposition, *e.g.*, the protons residing at C-1' and C-2' of a β-D-ribopyranosylpurine in a 1C chair conformation, would exhibit (16) a much smaller coupling constant ($J_{1,2}$). Justification for exclusion of the alternate chair conformation (1C) can also be made on the basis of several instability factors (17-19), *e.g.*, the bulky purine group residing in an axial position, 1,3 diaxial interactions between the hydroxyl groups at C-2' and C-4', etc.

Treatment of I with methylamine and dimethylamine effected removal of the blocking groups on the carbohydrate moiety and nucleophilic displacement of chlorine to yield 2-chloro-6-methylamino-9-(β-D-ribopyranosyl)purine (III) and 2-chloro-6-dimethylamino-9-(β-D-ribopyranosyl)purine (IV), respectively. Catalytic dehalogenation of IV furnished 6-dimethylamino-9-(β-D-ribopyranosyl)purine (VII) which possessed ultraviolet absorption spectra very similar to the corresponding ribofuranoside (20,21). The facile displacement of the chloro group from position 6 (*vide supra*) was conducted in the presence of an excess of the basic amine. It is of considerable interest that the chloro group in position 6 of I is so reactive that nucleophilic displacement has now been shown to occur without the concomitant

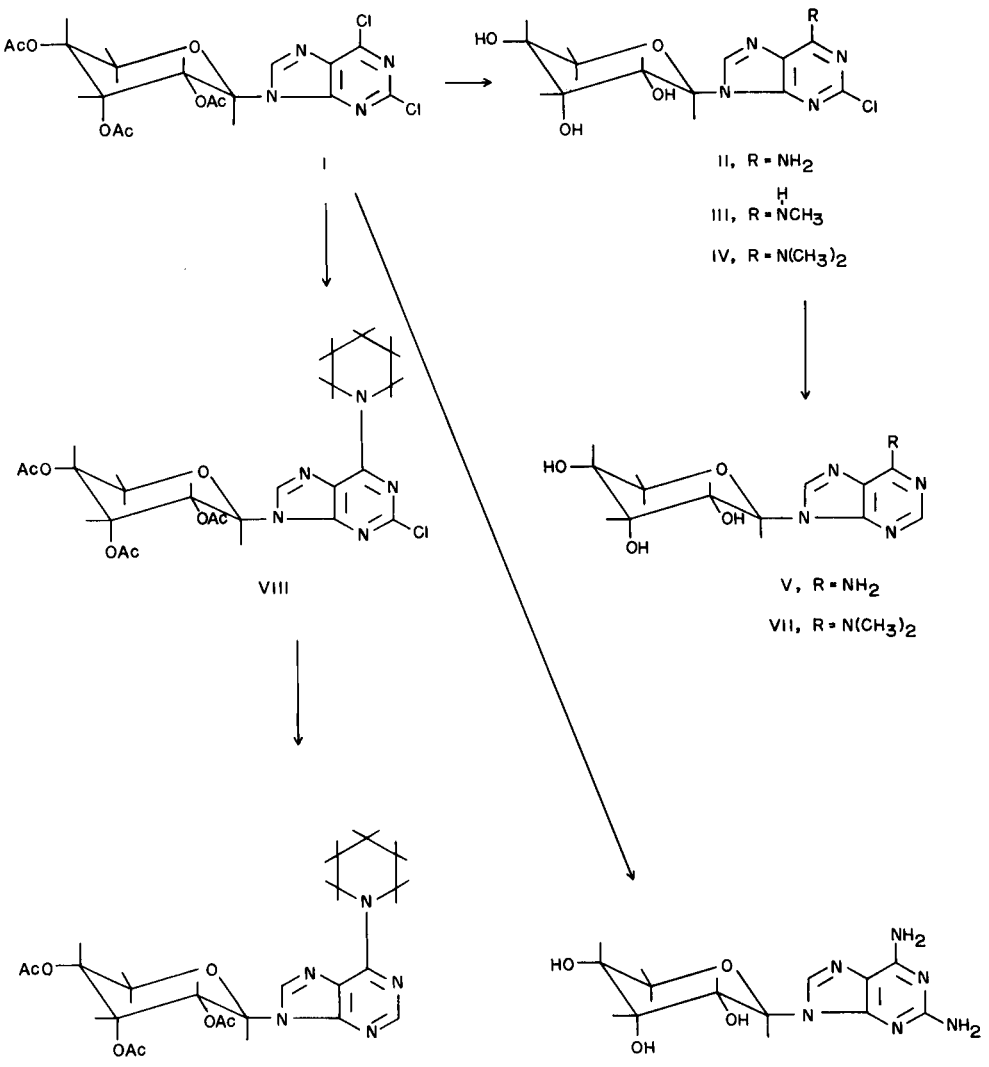
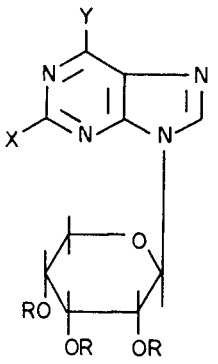


TABLE I

Ultraviolet Absorption Spectra (a) of Certain Ribopyranosylpurines.



Compound	R	X	Y	λ max (pH 1) mμ	ε	λ max (MeOH) mμ	ε	λ max (pH 11) mμ	ε
I	Ac	Cl	Cl	273.5	(8,000)	271	(7,400)	273	(8,700)
II	H	Cl	NH_2	263.5	(12,200)	273.5	(12,200)	263.5	(12,800)
III	H	Cl	$NHCH_3$	273	(11,000)	277.5	(11,800)	278	(13,100)
IV	H	Cl	$N(CH_3)_2$	277	(18,100)	276	(19,200)	276	(18,900)
VIII	Ac	Cl	NC_5H_{10}	280	(15,900)	277	(19,900)	280	(16,100)
V	H	H	NH_2	258.5	(14,100)	259.5	(14,700)	260	(15,100)
VII	H	H	$N(CH_3)_2$	266	(17,100)	274	(15,900)	274.5	(17,000)
IX	Ac	H	NC_5H_{10}	271	(11,900)	277.5	(14,200)	280	(12,600)
X	H	NH_2	NH_2	290	(10,200)	280	(9,600) (b)	278.5	(10,400)
				252	(12,600)	256.5	(9,900) (b)	256	(10,100)

(a) Determined on a Beckman DK-2 spectrophotometer. (b) Ethanol.

removal of the blocking groups. Treatment of I with approximately 1.5 equivalents of piperidine produced 2-chloro-6-(1-piperidyl)-9-(2',3',4'-tri-*O*-acetyl-β-D-ribopyranosyl)purine (VIII). The pmr spectra revealed the presence of three acetyl groups and further confirmation was provided by elemental analysis. Catalytic removal of the chloro group at position 2 furnished the acetylated nucleoside, 6-(1-piperidyl)-9-(2',3',4'-tri-*O*-acetyl-β-D-ribopyranosyl)purine (IX). Thus, it is apparent that by controlling the equivalents of amine utilized in the reaction it is possible to obtain either a deblocked or fully acetylated 2-chloro-6-substituted-9-D-ribopyranosylpurine. The chloro group in position 2 is relatively inert toward nucleophilic displacement especially since displacement occurs first at position 6 and the introduction of this basic group at position 6 contributes to the inertness of the 2-chloro group. Treatment of I however with ethanolic ammonia at 160° in a sealed vessel effects complete deblocking and replacement of both chloro groups to yield 2,6-diamino-9-(β-D-ribopyranosyl)purine (X). The ultraviolet absorption spectra of X was consistent with the assigned structure and the pmr spectra revealed a broad absorption peak (4 protons) at 6.8 δ and a doublet (1 proton, $J_{1,2}$ 9.0 cps) centered at 5.6 δ

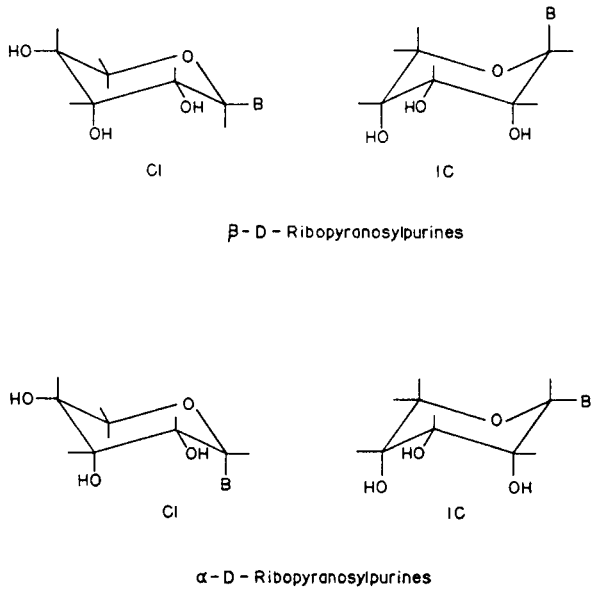


Figure 1. Ideal chair conformations for D-ribopyranosylpurines.

attributable to the anomeric proton.

It is of interest that I has demonstrated (22) sufficient activity in preliminary testing against Walker Carcinosarcoma 256 (intramuscular) to pass stage 1 of the sequential screen. The biological and chemotherapeutic properties of these compounds will be communicated at a later date and the utilization of 1,2,3,4-tetra-*O*-acetyl-β-D-ribopyranose to prepare additional 9-(β-D-ribopyranosyl)purines *via* the fusion procedure is under further investigation in this laboratory.

EXPERIMENTAL (23)

2,6-Dichloro-9-(2',3',4'-tri-*O*-acetyl-β-D-ribopyranosyl)purine (I).

A finely ground mixture of 2,6-dichloropurine (24) (14 g.) and 1,2,3,4-tetra-*O*-acetyl-D-ribopyranose (8) (23.8 g.) was placed in an oil bath which had been preheated to 160°. The mixture was allowed to form a clear melt, then 28 drops of dichloroacetic acid was added and the melt stirred for five (5) minutes while maintaining the same temperature at atmospheric pressure. A water aspirator vacuum was then applied and the melt was heated at 160° *in vacuo* until the color of the melt began to darken (approximately fifteen minutes). The reaction flask was removed from the oil bath and allowed to cool to room temperature. The melt was dissolved in ethyl acetate (140 ml.) and the ethyl acetate extracted with a cold (5°) aqueous saturated solution of sodium bicarbonate (3 x 50 ml.) followed by ice water (2 x 50 ml.). The ethyl acetate was dried with anhydrous sodium sulfate overnight and the sodium sulfate then removed by filtration. The filtrate was evaporated to dryness *in vacuo* and diethyl ether (100 ml.) was added to the residue and again evaporated to dryness *in vacuo* to afford a syrup. This syrup was dissolved in diethyl ether (140 ml.) and added dropwise to rapidly stirring *n*-pentane (560 ml.). The solid which separated from solution was collected by filtration and recrystallized from a diethyl ether-*n*-pentane mixture (1:4) to furnish 20 g. of chromatographically pure 2,6-dichloro-9-(2',3',4'-tri-*O*-acetyl-β-D-ribopyranosyl)purine (I), m.p. 80-82°.

Anal. Calcd. for $C_{16}H_{16}Cl_2N_4O_7$: C, 42.99; H, 3.58; N, 12.53. Found: C, 42.83; H, 3.72; N, 12.20.

6-Amino-2-chloro-9-(β-D-ribopyranosyl)purine (II).

2,6-Dichloro-9-(2',3',4'-tri-*O*-acetyl-β-D-ribopyranosyl)purine (I, 3.0 g.) was dissolved in 80 ml. of ethanolic ammonia (anhydrous ethanol previously saturated with ammonia at -10°). The reaction solution was placed in a pressure bottle, sealed and allowed to stand at room temperature for seven days. The solution was then evaporated to dryness *in vacuo* and the resulting residue was triturated with chloroform (5 x 50 ml.) at room temperature followed by trituration with boiling diethyl ether (5 x 50 ml.). The remaining solid was recrystallized from water to furnish 0.4 g. of chromatographically pure 6-amino-2-chloro-9-(β-D-ribopyranosyl)purine (II), m.p. 162-165°.

Anal. Calcd. for $C_{10}H_{12}ClN_5O_4 \cdot H_2O$: C, 37.7; H, 4.40; N, 21.95. Found: C, 37.83; H, 4.48; N, 21.61.

6-Amino-9-(β-D-ribopyranosyl)purine (V).

2-Chloro-6-amino-9-(β-D-ribopyranosyl)purine (II, 0.77 g.) was dissolved in anhydrous ethanol (50 ml.) and this solution added to 100 ml. of water containing 3.2 ml. of 1 *N* sodium hydroxide and 5% palladium on carbon (2.0 g.). The reaction mixture was placed on a Parr hydrogenator at 47 psi of hydrogen and shaken for six hours. The catalyst was removed by filtration and washed with boiling ethanol (300 ml.) and the combined filtrate and washings evaporated to dryness *in vacuo*. The resulting residue was crystallized from anhydrous methanol to furnish 0.23 g. of product, m.p. sintering at 230° and melting at 250-254°; $[\alpha]_D^{23}$ -32.4° (C=0.28%, H_2O) [reported (25), m.p. 254°; $[\alpha]_D^{26}$ -37° (C=0.6%, H_2O)].

Anal. Calcd. for $C_{10}H_{13}N_5O_4 \cdot H_2O$: C, 42.10; H, 5.26; N, 24.55. Found: C, 42.31; H, 5.48; N, 24.20.

2-Chloro-6-dimethylamino-9-(β-D-ribopyranosyl)purine (IV).

2,6-Dichloro-9-(2',3',4'-tri-*O*-acetyl-β-D-ribopyranosyl)purine (I, 1.0 g.) was dissolved in 25 ml. of anhydrous methanol containing 5 ml. of anhydrous dimethylamine. The solution was sealed in a pressure vessel and allowed to stand at room temperature for seven days. The solution was then evaporated to dryness *in vacuo* and the resulting residue recrystallized two times from anhydrous ethanol to furnish 0.23 g. of product, m.p. 242-245°.

Anal. Calcd. for $C_{12}H_{16}ClN_5O_4$: C, 43.80; H, 4.85; N, 21.30. Found: C, 43.61; H, 5.01; N, 21.40.

6-Dimethylamino-9-(β-D-ribopyranosyl)purine (VII).

2-Chloro-6-dimethylamino-9-(β-D-ribopyranosyl)purine (IV, 2.4 g.) was dissolved in anhydrous methanol (100 ml.) and this solution added to 50 ml. of water containing 5% palladium on carbon (2.4 g.). The reaction mixture was placed on a Parr hydrogenator at 47 psi of hydrogen and shaken for three hours. The catalyst was removed by filtration and washed with 300 ml. of hot (60°) methanol. The combined filtrate and washings were evaporated to dryness *in vacuo* and the resulting residue recrystallized from an ethanol-ethyl acetate mixture to furnish 1.08 g. of product, m.p. 201-202°. This solid was slurried with aqueous Dowex 1 (OH form) resin, filtered and the filtrate evaporated to dryness *in vacuo* and the resulting residue recrystallized from methanol to afford an analytical sample, m.p. 211-212°.

Anal. Calcd. for $C_{12}H_{17}N_5O_4$: C, 48.80; H, 5.80; N, 23.72. Found: C, 48.55; H, 5.79; N, 23.50.

2-Chloro-6-(1-piperidyl)-9-(2',3',4'-tri-*O*-acetyl-β-D-ribopyranosyl)-purine (VIII).

2,6-Dichloro-9-(2',3',4'-tri-*O*-acetyl-β-D-ribopyranosyl)purine (I, 4.47 g.) was dissolved in 30 ml. of anhydrous methanol containing 1.28 g. of piperidine at room temperature. This solution was heated at reflux temperature on a steam bath for one hour. The solution was then evaporated to dryness *in vacuo* at room temperature and the resulting residue triturated with diethyl ether (3 x 50 ml.) at room temperature. The resulting residue was crystallized from anhydrous ethanol to furnish 4.7 g. of a white crystalline product, m.p. 155-157°. A small sample was recrystallized from an ethanol-ethyl acetate mixture for analysis, m.p. 158-159°.

Anal. Calcd. for $C_{21}H_{26}ClN_5O_7$: C, 50.75; H, 5.28; N, 14.12. Found: C, 50.95; H, 5.58; N, 14.15.

6-(1-Piperidyl)-9-(2',3',4'-tri-*O*-acetyl-β-D-ribopyranosyl)purine (IX).

2-Chloro-6-(1-piperidyl)-9-(2',3',4'-tri-*O*-acetyl-β-D-ribopyranosyl)-purine (VIII, 1.4 g.) was dissolved in 40 ml. of hot (70°) ethanol and the solution then cooled to room temperature and 5% palladium on carbon (1.0 g.) was added. The reaction mixture was placed on a Parr hydrogenator at 45 psi of hydrogen and shaken for four hours. The catalyst was removed by filtration and washed with hot (70°) ethanol (3 x 5 ml.). The combined filtrate and washings were reduced to 1/5 of the initial volume and allowed to stand at 5° for 18 hours. The solid which had separated from solution was collected by filtration to furnish 0.4 g. of product, m.p. 245°. A small sample was recrystallized from a mixture of ethanol-water for analysis.

Anal. Calcd. for $C_{21}H_{27}N_5O_7 \cdot {}^1/_2H_2O$: C, 53.51; H, 6.00; N, 14.90. Found: C, 53.55; H, 5.78; N, 15.02.

2,6-Diamino-9-(β-D-ribopyranosyl)purine (X).

2,6-Dichloro-9-(2',3',4'-tri-*O*-acetyl-β-D-ribopyranosyl)purine (I, 3.0 g.) was dissolved in 150 ml. of ethanolic ammonia (ethanol saturated with ammonia at -10°). This solution was sealed in a pressure vessel and heated at 160° for six hours. The solvent was then removed *in vacuo* and the resulting residue dissolved in boiling water, treated with charcoal and the charcoal removed by filtration. The filtrate was allowed to stand at 5° for 18 hours and the solid collected by filtration to furnish 0.51 g. of product, m.p. 187-190°; $[\alpha]_D^{25}$ -22.6° (C=0.68%, H_2O) [reported (5), m.p. 188-190°; $[\alpha]_D^{23}$ -22° (C=0.68%, H_2O)].

Anal. Calcd. for $C_{10}H_{14}N_6O_4 \cdot H_2O$: C, 40.00; H, 5.34; N, 28.00. Found: C, 40.28; H, 5.50; N, 27.65.

2-Chloro-6-methylamino-9-(β-D-ribopyranosyl)purine (III).

2,6-Dichloro-9-(2',3',4'-tri-*O*-acetyl-β-ribopyranosyl)purine (I, 10.0 g.) was dissolved in 100 ml. of anhydrous methanol which had been previously saturated with gaseous methylamine at room temperature. The reaction vessel was covered and allowed to stir at room temperature for 18 hours. The solution was evaporated to dryness at room temperature *in vacuo* and to the resulting residue was added acetone (100 ml.) and again evaporated to dryness *in vacuo*. The resulting residue was crystallized from anhydrous methanol to furnish 1.8 g. of crystalline product. A small sample was recrystallized from anhydrous ethanol for analysis.

Anal. Calcd. for $C_{11}H_{14}ClN_5O_4$: C, 41.90; H, 4.45; N, 22.20. Found: C, 41.83; H, 4.78; N, 22.51.

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