Synthesis of 3- and 4-(β-D-Ribofuranosyl)-*s*-Triazolo[2,3-*a*]pyrimid-7-one, Isomers of Inosine Containing a Bridgehead Nitrogen Atom (1)

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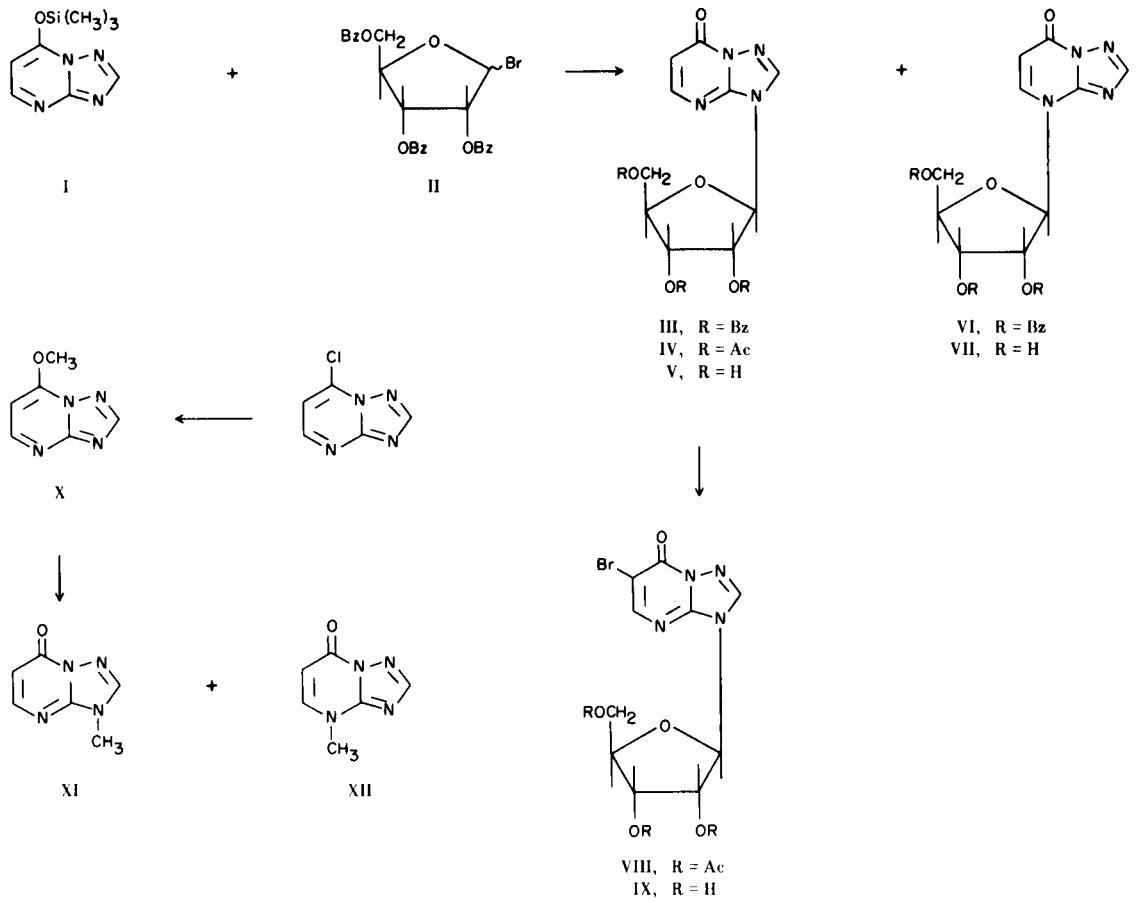
The first synthesis of a purine nucleoside analog containing a bridgehead nitrogen atom is here reported. The direct glycosylation of the trimethylsilyl derivative of *s*-triazolo[2,3-*a*]pyrimid-7-one has been shown to give 3-(β-D-ribofuranosyl)-*s*-triazolo[2,3-*a*]pyrimid-7-one (V) and 4-(β-D-ribofuranosyl)-*s*-triazolo[2,3-*a*]pyrimid-7-one (VII). The nucleoside V may be considered a close analog of inosine in which the nitrogen N_1 and C_5 of inosine have been interchanged. Bromination of the tri-*O*-acetyl derivative IV gave, after deblocking, 6-bromo-3-(β-D-ribofurnaosyl)-*s*-triazolo[2,3-*a*]pyrimid-7-one (IX). Structural assignments of the nucleosides were made on the basis of comparison of the ultraviolet absorption spectral characteristics with 3-methyl-*s*-triazolo-[2,3-*a*]pyrimid-7-one (XI) and 4-methyl-*s*-triazolo[2,3-*a*]pyrimid-7-one (XII) prepared by a standard procedure from 7-methoxy-*s*-triazolo[2,3-*a*]pyrimidine (X).

In view of a general program of nucleoside syntheses we wish to report the first synthesis of a purine nucleoside analog possessing a bridgehead nitrogen atom, (Structure V). This structure is of particular interest since it may be regarded as inosine but with C_5 and N_1 interchanged. The parent heterocycle, *s*-triazolo[2,3-*a*]pyrimid-7-one, is a close analog of 5-azahypoxanthine, a derivative of *s*-triazolo[2,3-*a*]-*s*-triazine. Taylor and Hendess (2) have viewed derivatives of the *s*-triazolo[2,3-*a*]-*s*-triazine ring system as analogs of the purines without an NH function in the five membered ring and as a consequence they proposed that "conversion to a normal riboside is impossible". In addition these authors speculated that biological activity might arise from the fact and that ribosidation might not take place *in vivo*. However, it now seems quite evident from the present work that the presence of an "NH" or pyrrole type nitrogen atom is not a requisite for glycosidation (or alkylation) in a condensed pyrimidine system. Further interest in structure V is found in that the usual hydrogen present at N_1 in inosine is absent. Such hydrogen bonding of the Watson, Crick type as found for instance in poly I:C should be drastically effected. This could shed considerable light on the relationship of hydrogen bonding and biological activity. For example this would be of interest in interferon production with polyinosinic acid and related polymers when combined with polycytidylic acid, to make double stranded copolymers (3). In the present study triazolo[2,3-*a*]pyrimid-7-one (4) was converted into its trimethylsilyl derivative (I) using hexamethyldisilazane and this derivative was allowed to react with 2,3,5-tri-*O*-benzoyl-D-ribofuranosyl bromide in acetonitrile according to the general procedure previously reported (5-8). After the usual workup followed by column chromatography on alumina two syrupy homogeneous blocked nucleosides III and VI were isolated. Removal of the blocking groups from III and VI gave the crystalline nucleosides 3-(β-D-ribofuranosyl)-*s*-triazolo[2,3-*a*]pyrimid-7-one (V) and 4-(β-D-ribofuranosyl)-*s*-triazolo[2,3-*a*]pyrimid-7-one (VII) in over all yields from II of 45% and 20% respectively.

The assignment of the position of attachment of the sugar was next considered. Makisumi and Kano (9) heated 5-methyl-7-methoxy-*s*-triazolo[2,3-*a*]pyrimidine which rearranged to yield two isomers, namely 3,5-dimethyl-*s*-triazolo[2,3-*a*]pyrimid-7-one and 4,5-dimethyl-*s*-triazolo[2,3-*a*]pyrimid-7-one. The structures of these isomers were unequivocally proved by these authors syntheses involving ring closures of requisite triazole precursors. In our hands a similar rearrangement of 7-methoxy-*s*-triazolo[2,3-*a*]-pyrimidine (X) again furnished two isomers namely 3-methyl-*s*-triazolo[2,3-*a*]pyrimid-7-one (XI) and 4 methyl-*s*-triazolo[2,3-*a*]pyrimid-7-one (XII) whose structures could



be unequivocally assigned by comparison of the uv spectral data with that of the corresponding 5-methyl derivatives obtained by Makisumi and Kano (9). 7-Methoxy-*s*-triazolo[2,3-*a*]pyrimidine (X) was readily prepared by treatment of 7-chloro-*s*-triazolo[2,3-*a*]pyrimidine (4) with sodium methoxide. 3-Methyl-*s*-triazolo[2,3-*a*]pyrimid-7-one (XI), m.p. 260-262°, exhibited λ max (*p*H 1) 245 mμ (ε, 6,000) 285 mμ (ε, 13,000), sh 294 mμ (ε, 9,000) and 4-methyl-*s*-triazolo[2,3-*a*]pyrimid-7-one (XII), m.p. 242-244°, exhibited λ max (*p*H 1) 245 mμ (ε, 5,900) 280 mμ (ε, 13,000). Comparison of the details of the uv absorption curves provided definitive diagnostic differences. 3-(β-D-Ribofuranosyl)-*s*-triazolo[2,3-*a*]pyrimid-7-one (V) exhibits λ max (*p*H 1) 243 mμ (ε, 6,000), 285 mμ (ε, 12,600) shoulder at 294 mμ (ε, 8,800). These data and similar uv data in other solvents and at various *p*H established the assignment of the β-D-ribofuranosyl moiety to position 3 since these curves were virtually identical to those of 3-methyl-*s*-triazolo[2,3-*a*]pyrimid-7-one (XI). Similarly 4-(β-D-ribofuranosyl)-*s*-triazolo[2,3-*a*]pyrimid-7-one (VII) exhibits λ max (*p*H 1) 244 mμ (ε, 5,900) 282 mμ (ε, 13,100) and exhibited no shoulder as for V. It is of interest that in the case of XII and VII no shifts in λ max was noted at different *p*H or in ethanol. In contrast the λ max of the major absorption peak of both XI and V exhibited a bathochromic shift to 289 mμ in absolute ethanol. The attachment of the β-D-ribofuranosyl moiety to position 1 is ruled out since alkylation at this position would be expected to give a bathochromic shift of near 10 mμ due to the favored conjugation and similarity of 7 substitution noted in the purine series. The proton magnetic resonance spectrum of V in deuterium oxide showed the signal for the proton at C_2 as a singlet at δ 9.50 ppm and that for the proton at C_5 as a doublet (J = 8 cps) at δ 8.10 ppm. In the case of VII the relative positions of these signals were reversed, i.e. the signal for the C_2 proton (in deuterium oxide) was at δ 8.62 ppm (singlet) and that for the C_5 proton was at δ 8.78 ppm (doublet, J = 8 cps). This effect is due to the presence of the electron withdrawing β-D-ribofuranosyl moiety which deshields the proton attached to the adjacent aromatic carbon atom. This has previously been noted particularly in 3-β-D-ribofurnaosyl purines (10). This data is confirmatory evidence of the assigned site of glycosidation. Bromination of 3-(β-D-ribofuranosyl)-*s*-triazolo[2,3-*a*]pyrimid-7-one (V) was next studied. Acetylation of V gave a crystalline acetate which was successfully

brominated with *N*-bromosuccinimide in chloroform to give crystalline 3-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-6-bromo-*s*-triazolo[2,3-*a*]pyrimid-7-one (VIII). Removal of the blocking groups gave crystalline 6-bromo-3-(β-D-ribofuranosyl)-*s*-triazolo[2,3-*a*]pyrimid-7-one (IX). The absence of the far upfield proton H_6 is ample evidence that bromination has occurred at position 6. This is not unexpected since Makisuma and Kano (4) have reported similar bromination of *s*-triazolo[2,3-*a*]pyrimid-7-one at position 6.

EXPERIMENTAL

Nucleosides V and VII derived from Triazolo[2,3-*a*]pyrimid-7-one.

To the trimethylsilyl derivative of *s*-triazolo[2,3-*a*]pyrimid-7-one (4) (prepared from 5.0 g. of the heterocycle) was added 2,3,5-tri-*O*-benzoyl-D-ribofuranosyl bromide (from 15 g. of 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose) in dry acetonitrile (100 ml.). The sealed solution was stirred at room temperature for 15 days. The mixture was evaporated to dryness and absolute ethanol was added to the residue. The mixture was evaporated to dryness and the residue dissolved in chloroform. This chloroform solution was washed with ice cold sodium carbonate solution (XI) and dried with magnesium sulfate. Solvent removal left a syrup which was dissolved in benzene and applied to a column (5.0 x 4.3 cm) of alumina (Merck) prepacked in benzene. Two hundred milliliter fractions were collected and the fractionation monitered by tlc on alumina Hf 254 with chloroform-ethyl acetate (1:) as developer. Benzene was the eluting solvent until fraction 5. Benzene-ethyl acetate 9:1 was used from fractions 6-15. Benzene-ethyl acetate (4:1) was used from fractions 16-33. Benzene-ethyl acetate (1:) was used from fraction 34. Fractions 17-25 were pooled and evaporated to yield 4.32 of homogenous syrupy 4-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-*s*-triazolo[2,3-*a*]pyrimid-7-one (VI). Similarly fractions 34-51 yielded 7.28 g. of homogeneous syrupy 3-[2,3,5-tri-*O*-benzoyl-1-(β-D-ribofuranosyl)]-*s*-triazolo[2,3-*a*]pyrimid-7-one (III).

Compounds VI and III were dissolved separately in ammonia saturated (at 0°) methanol (100 ml.) and left at room temperature for 3 days. The solution of VI slowly deposited white needles. Evaporation to smaller volume gave 1.06 g. (20%) white crystals of m.p. 216-221° dec. Recrystallization from water-ethanol gave pure 4-(β-D-ribofuranosyl)-*s*-triazolo[2,3-*a*]pyrimid-7-one (VII), m.p. 221-223° dec.; $[\alpha]_D^{30}$ -10.9° (c, 1, dimethylformamide); ν max (potassium bromide) 1690 cm^{-1} (C=O of heterocycle); λ max (pH 1) (4, 11) 282 mμ (ε, 13,100), pmr (DMSO-d_6) δ 6.14 (d, 1, $J_{1',2'}$ 4.7 cps, 1'-H), 6.21 (d, 1, $J_{6,5}$ 8.0 cps, 6-H), 8.62 (s, 1, 2-H), 8.78 (d, 1, $J_{5,6}$ 8.0 cps, 5-H).

Anal. Calcd. for $C_{10}H_{12}N_4O_5$: C, 44.78; H, 4.51; N, 20.89. Found: C, 44.55; H, 4.37; N, 20.77.

The solution of VI was evaporated to dryness and the residue was mixed with water and chloroform. The aqueous layer was extracted (X3) with chloroform and finally evaporated to small volume. Evaporation with absolute ethanol produced white crystals, yield 2.40 g. (45%), m.p. 237-241° dec. (sinters 175.177°). Recrystallization from water-ethanol gave pure 3-(β-D-ribofuranosyl)-*s*-triazolo[2,3-*a*]pyrimid-7-one (V), m.p. 243-248° dec.; $[\alpha]_D^{30}$ -39.3° (c, 1, water); ν max (potassium bromide) 1680, 1720 mμ (C=O of heterocycle); λ max (pH 1) (4, 11, 14) 285 mμ (ε, 12,600) sh 294 mμ (ε, 8,800), pmr (deuterium oxide) δ 5.94 (d, 1, $J_{1',2'}$ 4.0 cps, 1'-H), 6.22 (d, 1, $J_{6,5}$ 6.5 cps, 6-H), 8.10 (d, 1, $J_{5,6}$ 8.0 cps, 5-H), 9.50 (s, 1, 2-H).

Anal. Calcd. for $C_{10}H_{12}N_4O_5$: C, 44.78; H, 4.51; N, 20.89. Found: C, 44.59; H, 4.51; N, 20.82.

7-Methoxy-*s*-triazolo[2,3-*a*]pyrimidine.

7-Chloro-*s*-triazolo[2,3-*a*]pyrimidine (4) (3.09 g.) was added portionwise with magnetic stirring to a solution of sodium (0.46 g.) in anhydrous methanol (80 ml.) which had been cooled in ice. The solution was protected from moisture and stirred at room temperature for 2 hours. The mixture was evaporated at 30° to dryness. The residue was extracted with chloroform and the extract was washed with water three times. The dried (magnesium sulfate) solution was evaporated to smaller volume whereupon crystallization occurred. Hexane was added and the solution was cooled. The yield of white crystals was 1.73 g. (57%), m.p. 164-165°. Recrystallization from chloroform-hexane gave pure product having m.p. 164-165° dec.

Anal. Calcd. for $C_6H_6N_4O$: C, 48.00; H, 4.03; N, 37.32. Found: C, 47.31; H, 3.88; N, 37.84.

This compound was homogeneous by tlc on alumina Hf_{254} with ethyl acetate-methanol (9:1) as developer. No carbonyl absorption was present in the ir (potassium bromide) spectrum.

Isomerization of 7-Methoxy-*s*-triazolo[2,3-*a*]pyrimidine (X).

7-Methoxy-*s*-triazolo[2,3-*a*]pyrimidine (X) (2.3 g.) was heated at 175-180° for 30 minutes according to the procedure of Makisuma and Kano (9). The solidified melt was finely ground and extracted with hot chloroform (300 ml.). The cooled solution was applied to a column 47 x 2.8 cm of alumina prepacked in chloroform. Two hundred milliliter fractions were collected from 1-4 and from 21-33. The remaining fractions were of 100 ml. volume. At fraction 5 the eluting solvent was changed from chloroform to chloroform-ethyl acetate (9:1). At fraction 16 the solvent was changed to ethylacetate. Fractions 5-13 were evaporated to dryness to give a crystalline residue XII, yield 1.16 (50%), m.p. 237-239°. Fractions 16-33 were evaporated to dryness to give 0.27 g. (12%) of a crystalline residue XI m.p. 258-260°.

4-Methyl-*s*-triazolo[2,3-*a*]pyrimid-7-one, was recrystallized from chloroform-ether to give pure product, m.p. 242-244°; ν max (potassium bromide) 1715 cm^{-1} λ max (pH 1, 11) 245 mμ (ε, 5,900), 280 mμ (ε, 13,000); pmr (DMSO-d_6) δ 3.8 (s, 3, CH_3) 6.05 (d, 1, $J_{5,6}$ 1.5 cps 6-H) 8.15 (d, 1, $J_{5,6}$ 1.5 cps 5-H) 8.3 (s, 1, 2-H).

Anal. Calcd. for $C_6H_6N_4O$: C, 48.00; H, 4.03; N, 37.32. Found: C, 47.76; H, 4.06; N, 37.57.

This compound was homogeneous by tlc on alumina Hf_{254} with ethyl acetate-methanol (9:1) as a developer.

Recrystallization of 3-methyl-*s*-triazolo[2,3-*a*]pyrimid-7-one (XI) from chloroform-ether gave pure product m.p. 260-262°; ν max (potassium bromide) 1670 cm^{-1} (C=O); λ max (pH 1, 11) 245 mμ (ε, 6,000) 285 mμ (ε, 13,000), sh 294 mμ (ε, 9,000); pmr (DMSO-d_6) δ 3.7 (s, 3 CH_3) 6.1 (d, 1, $J_{5,6}$ 1 cps 6-H) 8.05 (d, 1, $J_{5,6}$ 1 cps 5-H) 8.91 (s, 1, 2-H).

Anal. Calcd. for $C_6H_6N_4O$: C, 48.00; H, 4.03; N, 37.32. Found: C, 48.05; H, 4.06; N, 37.04.

This compound was homogeneous by tlc on alumna Hf_{254} with acetate-methanol (9:1) as developer.

3-(2,3,5-Tri-*O*-acetyl-β-D-ribofuranosyl)-*s*-triazolo[2,3-*a*]pyrimid-7-one (IV).

3-(β-D-Ribofuranosyl)-*s*-triazolo[2,3-*a*]pyrimid-7-one (V) (1.50 g.) was stirred in a sealed mixture of acetic anhydride (15 ml.) and

pyridine (15 ml.) overnight. The solution was left a further day and then poured onto ice. The solution was extracted with chloroform. The chloroform extract was washed successively with water, 2*N* hydrochloric acid, water, and saturated aqueous sodium bicarbonate and water. The dried (magnesium sulfate) solution was evaporated to a syrup which was crystallized from ethyl acetate-hexane to give 2.12 g. (96%) of white crystals m.p. 168-169°. Recrystallization of this product from the same solvent raised the m.p. to 169-170°; ν max (potassium bromide) 1690 cm^{-1} (C=O heterocycle), 1745 (OAc); pmr (deuteriochloroform) δ 2.07 (s, 6, OAc), 2.10 (s, 3, OAc), 4.26-4.26-4.62 (m, 3, 5'-C*H*$_2$OAc (s) at 4.40 overlapped by 4'-H), 5.46-5.69 (m, 1, 3'-H), 5.89 (t, 1, $J_{2',1'}$ 4.7 cps, 2'-H), 6.11 (d, 1, $J_{1',2'}$ 4.7 cps, 1'-H), 6.21 (d, 1, $J_{6,5}$ 5.5 cps, 6-H), 7.90 (d, 1, $J_{5,6}$ 5.5 cps, 5-H), 8.60 (s, 1, 2-H).

Anal. Calcd. for $C_{16}H_{18}N_4O_8$: C, 48.73; H, 4.60; N, 14.21. Found: C, 48.48; H, 4.61; N, 14.21.

3-(2,3,5-Tri-*O*-acetyl-β-D-ribofurnaosyl)-6-bromo-*s*-triazolo[2,3-*a*]-pyrimid-7-one (VIII).

A mixture of 3-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-s-triazolo-[2,3-*a*]pyrimid-7-one (0.50 g.) and *N*-bromosuccinimide (0.27 g.) was heated in chloroform (15 ml.) under reflux for 2 hours. The precipitate of succinimide was filtered and the filtrate and washing were washed with water (X3). The dried (magnesium sulfate) solution was evaporated to a syrup which was crystallized from chloroform-ether to give 0.44 g. of white needles, m.p. 161-163°. Recrystallization from chloroform-ether gave pure material having m.p. 163-164°; ν max (potassium bromide) 1745 cm^{-1} (OAc) and 1690 (C=O of heterocycle); pmr (deuteriochloroform) δ 2.11 (s) and 2.12 (s, 9, OAc), 4.28-4.63 (m, 3, 5'-C*H*$_2$OH (s) centered at 4.43 overlapped by 4'-H), 5.46-5.73 (m, 1, 3'-H), 5.91 (t, 1, $J_{2',1'}$ 5.0 cps, 3'-H), 6.20 (d, 1, $J_{1',2'}$ 5.0 cps 1'-H) 8.52 (s, 1, 2-H), 9.04 (s, 1, 5-H).

Anal. Calcd. for $C_{16}H_{17}N_4O_8Br$: C, 40.60; H, 3.62; N, 11.84. Found: C, 39.70; H, 3.91; N, 11.54.

This compound was homogeneous as judged by tlc on SilicAR 7GF with ethyl acetate as developer.

6-Bromo-3-(β-D-ribofuranosyl)-*s*-triazolo[2,3-*a*]pyrimid-7-one (IX).

3-(2,3,5-Tri-*O*-acetyl-β-D-ribofuranosyl)-6-bromo-*s*-triazolo-[2,3-*a*]pyrimid-7-one (0.93 g.) was dissolved in ammonia saturated (at 0°) methanol (50 ml.) and the sealed solution left overnight at room temperature. The solution was filtered and evaporated to a syrup. The residue was coevaporated with absolute ethanol and the resulting syrup was crystallized from ethanol-2-propanol, yield 0.50 g., m.p. 170-175° dec. Recrystallization from water-2-propanol gave pure product m.p. gradually decomposed above 180°; $[\alpha]_D^{29}$ -15.9° (c, 1.00 water); ν max (potassium bromide) 1690 cm^{-1} (C=O); λ max (*p*H 1) 298 mμ (ε, 12,300), sh 309 mμ (ε, 7,650); λ min (*p*H 1) 266 mμ (ε, 3,100); λ max (*p*H 11) 297 mμ (ε, 12,300), sh 309 mμ (ε, 7,650); λ min (*p*H 11) 266 mμ (ε, 4,500); pmr (DMSO-d_6) δ 5.86 (d, 1, $J_{1',2'}$ 3.0 cps, 1'-H), 8.76 (s, 1, 2-H), 9.60 (s, 1, 5-H).

Anal. Calcd. for $C_{10}H_{11}N_4O_5Br$: C, 34.60; H, 3.20; N, 16.14. Found: C, 34.42; H, 3.06; N, 15.90.

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