A Facile Synthesis of 8-(β-D-Ribofuranosyl)-4-thioxo-3*H*-pyrazolo[1,5-*a*]-1,3,5-triazine and Its α-Anomer

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A versatile intermediate **2** for *C*-nucleoside synthesis was treated with thiosemicarbazide to obtain thiosemicarbazone **6**, which was then converted to 3-aminopyrazol-2*N*-thiocarboxamide derivatives **7** and **8** by the reaction of **6** and sodium ethoxide. 4-Thioxo-4*H*-pyrazolo[1,5-*a*]-1,3,5-triazine *C*-nucleosides **11** and **12** were obtained by the ring closure reaction of **7** and **8** with triethyl orthoformate. Brief treatment of **11** and **12** with 10% methanolic hydrogen chloride afforded *C*-nucleosides **4** and **13**, respectively, without anomerization. Identification of compounds **4** and **13** was made on the basis of ^{1}H nmr and uv spectra, as well as chemical conversion to known compounds with established configurations. Model compounds were also synthesized in order to confirm the heterocyclic moieties.

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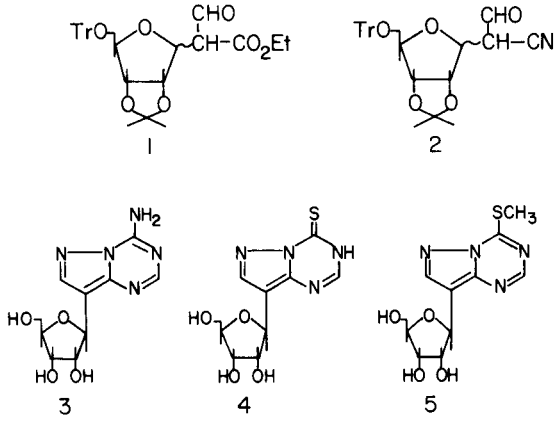
During our efforts to develop a general synthetic method for *C*-nucleosides related to the naturally occurring compounds, we have prepared two extremely useful intermediates **1** [1,2] and **2** [3,4] from which pyrimidine [1-3], 3-aminopyrazole [5] and 3-aminopyrazole-2*N*-carboxamide [4] *C*-nucleosides have been synthesized. The latter two *C*-nucleosides, have been utilized for the preparation of various "purine-like" *C*-nucleosides [5,6,10]. Furthermore, 2-formyl-2-(2,3-*O*-isopropylidene-5-*O*-trityl-D-ribofuranosyl)acetonitrile (**2**) proved to be a very versatile intermediate in the synthesis of thieno[3,2-*d*]pyrimidine [7] and pyrrolo[3,2-*d*]pyrimidine [8,9] *C*-nucleosides.

As a continuation of our efforts to utilize intermediate **2** in *C*-nucleoside synthesis, we report a facile synthesis of 8-(β-D-ribofuranosyl)-4-thioxo-3*H*-pyrazolo[1,5-*a*]-1,3,5-triazine (**4**) [10] and its α-isomer **13**, which is hereto unknown in the literature. Previously, Tam, *et al.* [10] reported the synthesis of **4** and its *S*-methyl derivative **5** from 4-amino-8-(β-D-ribofuranosyl)pyrazolo[1,5-*a*]-1,3,5-triazine (APTR) **3**. These compounds were found to be potent antileukemic agents *in vitro* [10,11]. Particularly, **5** exhibited extremely high antitumor activities against L1210 and P815 cell lines. This interesting biological activity warrants further synthetic studies of these compounds.

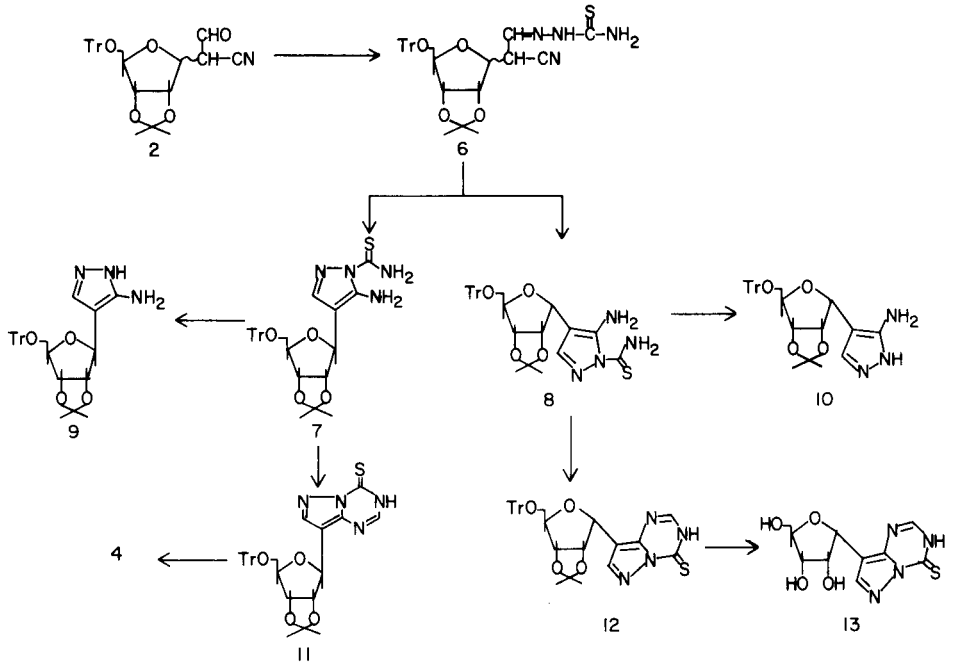
Figure 1



The aldehydoacetonitrile derivative [3,4] of ribose **2** was treated with an excess of thiosemicarbazide under acidic condition to obtain the thiosemicarbazone **6**. Unexpectedly, tlc mobilities of the product **6** and that of the starting material **2** were the same (Rf = 0.5 in chloroform-methanol = 20:1). Tlc indicated, however, that there was no sign of deblocking of the protecting groups of ribose during the reaction. Attempts to purify and separate the α and β-isomers failed because of a partial conversion to **7** and **8** on the silica gel column or on the preparative tlc plates. Thus without purification, the thiosemicarbazone **6** was treated with sodium ethoxide solution to obtain the α,β-mixture of 3-aminopyrazol-2*N*-thiocarboxamides **7** and **8**. Sodium hydroxide solution was also tried for the cyclization, however, it hydrolyzed significant amounts of **7** and **8** to 3-aminopyrazole derivatives **9** and **10**. The α (slower moving spot) and β (faster moving spot) mixture of 3-aminopyrazole-2*N*-thiocarboxamides were then cleanly separated into the individual anomer on a silica gel column using an ethyl acetate-hexane (1:4) mixture. The product ratio of the α/β isomer was 3:2 after the column separation.

The identification of these products **7** and **8** was based on nmr data as well as uv spectra. The configuration assignment of the α and β-isomer was mainly based on the chemical shifts of the anomeric protons, in which the β-anomer **7** appeared at higher field (δ 4.76) than that of α-anomer (δ 5.09). This method has been used successfully for *C*-nucleosides without exception [1-5]. Imbach's rule [12] was not applicable for an unambiguous assignment as the differences of chemical shifts between the methyl groups (Δδ) in isopropylidene moieties are 0.21 and 0.22

Scheme 1



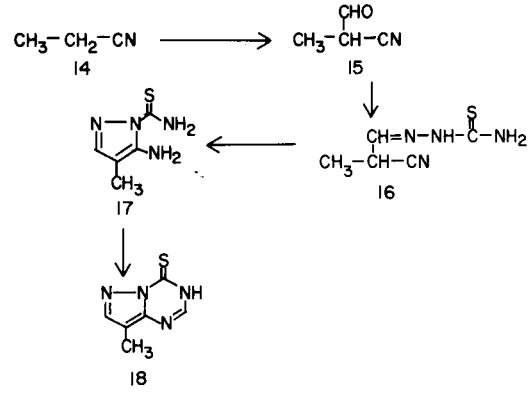
ppm for the α and β-anomer, respectively.

Additionally, in order to confirm the presence of the heterocyclic moieties in **7** and **8**, a model compound **17** was prepared in low yield by a method similar to that used for **7** and **8**. The structure of **17** was confirmed by cyclization to **18**, for which uv spectra could be compared to that of **4**. The uv spectra of **7** and **8** exhibited similar patterns at various *p*H's to that of the heterocycle **17**. Thus, 3-aminopyrazole-2*N*-thiocarboxamide moiety of **7** and **8** was unambiguously confirmed. Final identification of **7** and **8** came from the chemical conversion to **9** and **10**, for which their anomeric configurations have been previously established [5]. Brief treatment of the **8** (α) and **7** (β) anomer with sodium hydroxide solution gave **10** (α) and **9** (β) isomer, respectively, without anomerization.

Once the structure has been established, **7** and **8** were treated independently with triethyl orthoformate to yield **11** (50%) and **12** (64%) after silica gel column. An anomerization was not observed during the reaction. However, a partial decomposition of 3-aminopyrazole-2*N*-thiosemicarboxamides to 3-aminopyrazole *C*-nucleosides was observed at 90-95°. Since this reaction condition was not optimized, it is felt that careful control of the reaction condition may impove the yield significantly. The chemical shift data of the anomeric protons of **11** and **12** are in agreement with other anomeric pairs, in which β anomer appeared at higher field (δ 4.69) than that of α-anomer (δ 5.39). Imbach's rule [12] also was not applicable for the assignment.

The final compounds **4** and **13** were obtained in good yield from the brief treatment of **11** and **12** with 10% methanolic hydrogen chloride. It should be mentioned that under these conditions no anomerization was observed. Although compound **5** has been prepared from **3** [6], its α anomer **13** has not been reported.

Scheme 2



EXPERIMENTAL

Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. ^{1}H nmr spectra were recorded on a JEOL FX90Q or FX270 fourier transform spectrometer. Tetramethylsilane was the internal standard for organic solvents and sodium 3-(trimethylsilyl)-1-propane-1-sulfonate (DSS) was the internal standard for deuterium oxide; chemical shifts are reported in parts per million (δ), and signals are described as s (singlet), d (doublet), t (triplet), q (quartet), b broad), m (multiplet). Ultraviolet spectra were recorded on a Bausch and Lomb Spectronic 2000 spectrometer. Tlc was performed on Uniplates purchased from Analtech Co. or Pre-coated tlc sheets (Silica gel 60

(F-254) by EM Laboratories, Inc. Elemental analysis were performed by Atlantic Microlab, Inc., Atlanta, GA.

2-Formyl-2-(2,3-*O*-isopropylidene-5-*O*-trityl-D-ribofuranosyl)acetonitrile-thiosemicarbazone (**6**).

A mixture of aldehydoacetonitrile **2** (5.7 g, 11.5 mmoles) [3,4], thiosemicarbazide (1.5 g, 16.5 mmoles), acetic acid (2 ml), water (10 ml) and ethanol (35 ml) were heated at 55-60° for 30 minutes. The mixture was then evacuated *in vacuo* to a syrup. Water (50 ml) was added and cooled in an ice-water bath. The resulting solid was crushed, filtered to collect, and then dried (vacuum) overnight to collect 5.1 g (80%). Tlc indicated overlapping two spots (Rf = 0.5 in chloroform-methanol = 20:1) which could not be distinguishable from that of the starting material. Attempts to separate the anomeric mixture failed because of a partial conversion to **7** and **8** during the separation on a silica gel column or preparative tlc plates.

3-Amino-2*N*-thiocarbamoyl-4-(2,3-*O*-isopropylidene-5-*O*-trityl α- and β-D-Ribofuranosyl)pyrazole (**7** and **8**).

Compound **6** (5.1 g, 9.2 mmoles) was treated with 1*M* sodium ethoxide solution (2 ml) in ethanol (30 ml) at room temperature for 15 minutes. The solution was neutralized with acetic acid and then evaporated to a syrup at a temperature below 30°. Tlc showed two distinctive spots (Rf = 0.24 and 0.33) on a silica gel plate (ethyl acetate/hexane = 1:4). A part of the syrup (2.2 g) was separated on a silica gel column (4.5 × 32 cm) using the above solvent system, from which the fast moving compound **7** (β-anomer, 500 mg) was obtained; uv: λ max 273 nm (methanol), 273 (methanol + hydrochloric acid), 269 (methanol + sodium hydroxide); ^{1}H nmr (deuteriochloroform): δ 1.36 and 1.58 (s, 3, isopropylidene methyl), 3.35-3.45 (m, 3, H-2′, 5′ and 5″), 4.10-4.24 (m, 1, H-3′), 4.76 (m, 2, H-1′ and 2′), 6.70 (b, 2, NH_2), 7.20-7.65 (m, 16, trityl and H-5), 8.50 (b, 2, NH_2).

Anal. Calcd. for $C_{31}H_{32}N_4O_4S$: C, 66.91; H, 5.76; N, 10.07; S, 5.76. Found: C, 67.10; H, 5.94; N, 9.51 [13]; S, 5.42.

From the same column the slow moving isomer **8** (α, 760 mg) was obtained; uv: λ max 273 nm (methanol), 273 (methanol + hydrochloric acid), 268 (methanol + sodium hydroxide); ^{1}H nmr (deuteriochloroform): δ 1.34 and 1.55 (s, 3, isopropylidene methyl), 3.25-3.32 (m, 2, H-5′ and 5″), 4.27 (m, 1, H-4′), 4.81-4.83 (m, 2, H-2′ and 3′), 5.09 (d, 1, H-1′, J = 2.7 Hz), 6.81 (b, 2, NH_2), 7.26-7.44 (m, 16, trityl and H-5), 8.51 (b, 2, NH_2).

Anal. Calcd. for $C_{31}H_{32}N_4O_4S$: C, 66.91; H, 5.76; N, 10.07; S, 5.76. Found: C, 67.09; H, 5.69; N, 9.72; S, 5.74.

An unresolved mixture (400 mg) was also obtained from the column.

Hydrolysis of **7** to **9** and **8** to **10**.

A mixture of **7** (50 mg) and 2 *N* sodium hydroxide (1 drop) in methanol (0.5 ml) was heated at 40° for one minute. After neutralization with acetic acid, the hydrolytic product **9**, which was formed as a sole product, was compared with the authentic β-isomer of 4-(β-2,3-*O*-isopropylidene-5-*O*-tritylribofuranosyl)-3-aminopyrazole as well as with its anomeric mixture on tlc (chloroform/methanol = 10:1). It was confirmed from the experiment that the β-isomer **7** gave the β-isomer **9**.

Additional identification of **9** was substantiated from the comparison of ^{1}H nmr spectrum to that of the authentic sample prepared by the previous method [4,5]. Under similar experimental conditions **8** gave **10**. Thus, this confirmed the anomeric configuration of **7** and **8** unambiguously.

7-(β-D-2,3-*O*-Isopropylidene-5-*O*-tritylribofuranosyl)-4-thioxo-3*H*-pyrazolo[1,5-*a*]-1,3,5-triazine (**11**).

A mixture of **7** (400 mg, 0.72 mmole) and triethyl orthoformate (6 ml) was heated at 85-90° for 2 hours. The reaction mixture was evacuated *in vacuo* to a syrup, which was purified on a silica gel column (2.5 × 14 cm) using chloroform-methanol (20:1) as an eluent to yield 260 mg (50%) of **11** as a white foam; ^{1}H nmr (dimethylsulfoxide-d_6): δ 1.27 and 1.50 (s, 3, isopropylidene methyl), 3.25-3.33 (m, 2, H-5′ and 5″), 4.11-4.14 (m, 1, H-4′), 4.65-4.69 (m, 1, H-3), 5.02-5.05 (m, 2, H-1′ and H-2′), 7.25-7.35 (m, 15, trityl), 8.03 (s, 1, H-2), 8.27 (s, 1, H-1).

Anal. Calcd. for $C_{32}H_{30}N_4O_4S$: C, 67.84; H, 5.30; N, 9.89; S, 5.65. Found: C, 67.82; H, 5.84; N, 9.65; S, 5.62.

7-(α-D-2,3-*O*-Isopropylidene-5-*O*-tritylribofuranosyl)-4-thioxo-3*H*-pyrazolo[1,5-*a*]-1,3,5-triazine (**12**).

A mixture of **8** (900 mg, 1.62 mmoles) and triethyl orthoformate (6.5 ml) was heated at 85-90° for 6 hours. The reaction mixture was evacuated *in vacuo* to a syrup, which was separated on a silica gel column (5.5 × 12 cm) using chloroform-methanol (30:1) as an eluent to afford 460 mg (64%) of **12** as a white solid; ^{1}H nmr (dimethylsulfoxide-d_6): δ 1.23 and 1.46 (s, 3, isopropylidine methyl), 3.16-3.30 (m, 2, H-5′ and 5″), 4.15-4.22 (m, 1, H-4′), 4.70-4.76 (m, 2, H-2′ and 3′), 5.39 (d, 1, H-1′), 7.25-7.40 (m, 15, trityl), 8.09 (s, 1, H-2), 8.23 (s, H, H-7).

Anal. Calcd. for $C_{32}H_{30}N_4O_4S$: C, 67.84; H, 5.30; N, 9.89. S, 5.65. Found: C, 67.81; H, 5.50; N, 9.48; S, 5.49.

8-(β-D-Ribofuranosyl)-4-thioxo-3*H*-pyrazolo[1,5-*a*]-1,3,5-triazine (**4**).

A mixture of **11** (400 mg, 0.71 mmole) and 10% methanolic hydrogen chloride (4 ml) was stirred at room temperature for 10 minutes and the mixture was evacuated to dryness. Ether (10 ml) was added, triturated, and then decanted the solvent. This procedure was repeated three times in order to remove the by-product (triphenylmethyl alcohol). Absolute ethanol was then added to the remaining solid and the mixture was stirred for 10 minutes while cooling. The resulting suspension was filtered to yield 165 mg of amorphous powder. Another 60 mg was obtained from the filtrate to give a combined yield of 165 mg (83%), mp 205-207° dec (206-208° reported). The uv and ^{1}H nmr data of **4** were in agreement with the reported value [10].

8-(α-D-Ribofuranosyl)-4-thioxo-3*H*-pyrazolo[1,5-*a*]-1,3,5-triazine (**13**).

A mixture of **11** (430 mg, 0.76 mmole) and 10% methanolic hydrogen chloride (4 ml) was stirred at room temperature for 10 minutes. The solvent was then evaporated to near dryness, in which ether (10 ml) was added, triturated, and filtered to collect a solid. The resulting solid was again suspended in absolute ethanol (5 ml) and stirred for 10 minutes, and then filtered to collect and vacuum dried to afford 189 mg (88%) of amorphous powder, mp 209-210°; uv: λ max 275 nm (ε 8,890) and 321 (ε 9,790) (*p*H 7), 277 (ε 22,010) and 305 (ε 9,940) (*p*H 1); ^{1}H nmr (dimethylsulfoxide-d_6): δ 3.49-3.61 (m, 2, H-5′, 5″, $J_{5',5''}$ = 11.6 Hz), 3.95 (m, 1, H-4′, $J_{4',5''}$ = 4.7 Hz), 3.98 (m, 1, H-2′, $J_{2',3'}$ = 4.3 Hz), 4.13 (m, 1, H-3′, $J_{3',4'}$ = 7.8 Hz), 5.17 (d, 1, H-1′, $J_{1',2'}$ = 3.2 Hz), 8.00 (s, 1, H-2), 8.28 (s, 1, H-7).

Anal. Calcd. for $C_{10}H_{12}N_4O_4S$: C, 42.25; H, 4.23; N, 19.72; S, 11.26. Found: C, 41.97; H, 4.32; N, 19.56; S, 11.10.

3-Amino-4-methylaminopyrazole-2*N*-thiocarboxamide (**17**).

To a suspension of sodium hydroxide (11.6 g, 0.24 mole, 50% in oil) in dry ether (100 ml), a mixture of ethyl formate (40 ml, excess), propionitrile (13.2 g, 0.24 mole) and absolute ethanol (2 ml) in ether (30 ml) was added dropwise at such a rate that a brisk reaction sustained throughout the addition. After addition, which took about 30 minutes, the reaction mixture was stirred for 15 hours, during which the mixture solidifed. Ether and excess ethyl formate were evaporated *in vacuo* at below 30°, water (50 ml) was cautiously added to the resulting solid, and then the mixture was neutralized with acetic acid. To the resulting solution of aldehyde derivative **15**, thiosemicarbazide hydrochloride (23 g, 0.24 mole) was added, and the mixture was stirred for 15 hours. After stirring, 10% sodium hydroxide solution was added slowly to adjust the *p*H to 8. During this time, greenish crystals precipitated (12.5 g, 33%). Recrystallized from water gave cryme crystals, mp 128-129°; uv: λ max 271 and 239 (*p*H 7 and *p*H 1), 270 and 239 (*p*H 12); ^{1}H nmr (dimethylsulfoxide-d_6): δ 1.81 (s, 3, CH_3), 7.26 (s, 1, H-5), 9.05 (b, 1, NH_2), 9.45 (b, 1, NH_2).

Anal. Calcd. for $C_5H_8N_4S$: C, 38.46; H, 5.13; N, 35.90; S, 20.51. Found: C, 38.27; H, 5.13; N, 35.67; S, 20.55.

8-Methyl-4-thioxo-3*H*-pyrazolo[1,5-*a*]-1,3,5-triazine (**18**).

A mixture of **17** (400 mg, 2.5 mmoles) and triethyl orthoformate (10 ml)

was refluxed for two hours. During this time solid precipitated, which was filtered to yield 350 mg (83%) of white amorphous powder; mp 313-315°; uv: λ max 276 and 323 (*p*H 7-12), 279 and 305 (sh) (*p*H 1); ^{1}H nmr (dimethylsulfoxide-d_6): δ 2.16 (s, 3, CH_3), 7.96 (s, 1, H-2), 8.15 (s, 1, H-7).

Anal. Calcd. for $C_6H_6N_4S$: C, 43.37; H, 3.61; N, 33.73; S, 19.28. Found: C, 43.24; H, 3.61; N, 33.54; S, 19.09.

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