# The Synthesis of 3-(β-D-Ribofuranosyl)imidazo[4,5-c]pyridazines

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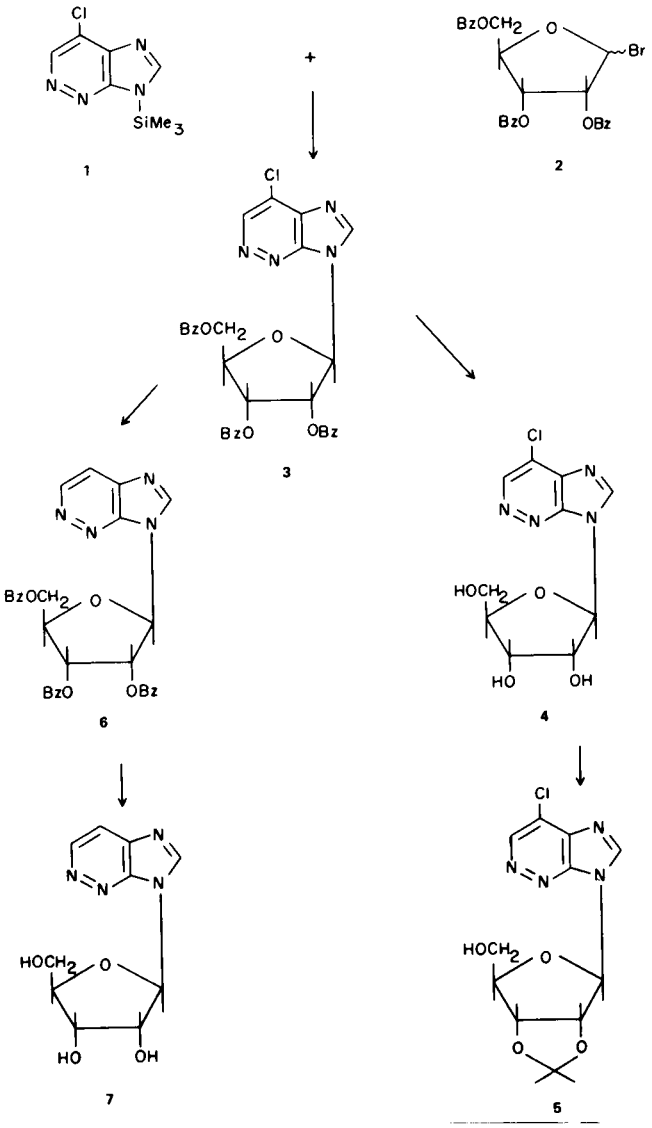
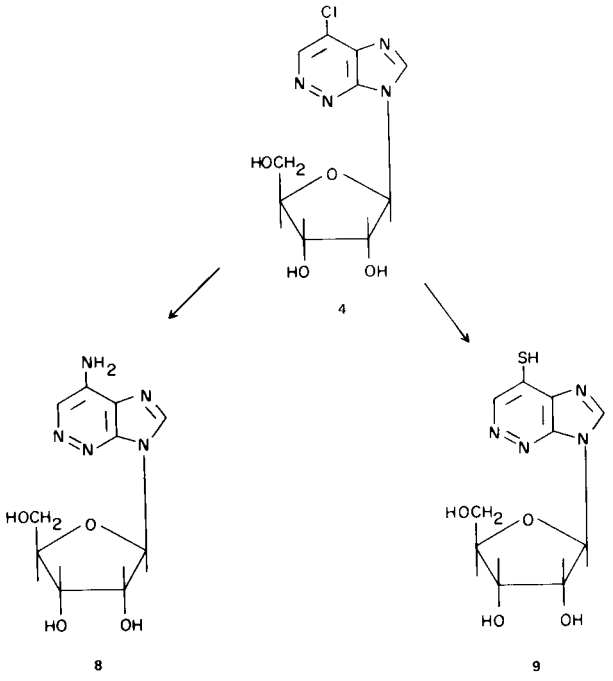
7-Chloro-3-(β-D-2,3,5-tri-*O*-benzoylribofuranosyl)imidazo[4,5-c]pyridazine (**3**), obtained from the condensation of 7-chloro-3-trimethylsilylimidazo[4,5-c]pyridazine (**1**) with 2,3,5-tri-*O*-benzoyl-D-ribofuranosyl bromide (**2**), served as the percursor of 7-chloro- (**4**), 7-amino- (**8**), and 7-mercapto-3-(β-D-ribofuranosyl)imidazo[4,5-c]pyridazine (**9**). 3-(β-D-ribofuranosyl)imidazo[4,5-c]pyridazine (**7**) was obtained from 3-(β-D-2,3,5-tri-*O*-benzoylribofuranosyl)imidazo-[4,5-c]pyridazine (**6**). The site of ribosidation is based upon uv spectral comparisons with model methyl compounds. The assignment of the anomeric configuration is derived from pmr spectral data.

The only nucleosides of imidazopyridazines previously reported are those of the imidazo[4,5-*d*]pyridazine system (1). 4-Amino-1-(β-D-ribofuranosyl)imidazo[4,5-*d*]pyridazine has been shown to be cytotoxic and to exhibit feedback inhibition of "*de novo*" purine biosynthesis (2). This work was undertaken to synthesize nucleosides in the imidazo[4,5-c]pyridazine ring system as possible purine antimetabolites.

7-Chloro-3-trimethylsilylimidazo[4,5-c]pyridazine (**1**) was prepared by allowing 7-chloroimidazo[4,5-c]pyridazine (3) to react with hexamethyldisilazane (HMDS) (4). 7-Chloro-3-(β-D-2,3,5-tri-*O*-benzoylribofuranosyl)imidazo-[4,5-c]pyridazine (**3**) was obtained in 60% yield by allowing **1** to condense with 2,3,5-tri-*O*-benzoyl-D-ribofuranosyl bromide (**2**) (5). Compound **3** was catalytically dechlorinated with hydrogen in the presence of 5% palladium on charcoal giving 3-(β-D-2,3,5-tri-*O*-benzoylribofuranosyl)imidazo[4,5-c]pyridazine (**6**) in 82% yield. The deblocked, unsubstituted riboside, 3-(β-D-ribofuranosyl)imidazo[4,5-c]pyridazine (**7**) was obtained in 74% yield by allowing **6** to stand in methanolic ammonia at room temperature for five days. Compound **3** was also deblocked directly to give 7-chloro-3-(β-D-ribofuranosyl)-imidazo[4,5-c]pyridazine (**4**) in 70% yield by the same deblocking procedure. The adenosine analog, 7-amino-3-(β-D-ribofuranosyl)imidazo[4,5-c]pyridazine (**8**), was obtained in 20% yield by treatment of **4** with ethanolic ammonia under pressure at elevated temperatures. Likewise the 6-mercaptopurine riboside analog, 7-mercapto-3-(β-D-ribofuranosyl)imidazo[4,5-c]pyridazine (**9**), was prepared in 52% yield by the action of methanolic sodium hydrosulfide solution on **4** under pressure at elevated temperatures.

That ribosylation took place on position 3 of the imidazo[4,5-c]pyridazine ring system is shown by the





almost identical uv spectrum of 3-(β-D-ribofuranosyl)-imidazo[4,5-c]pyridazine (**7**) and 3-methylimidazo[4,5-c]-pyridazine (6) and further substantiated by the nearly identical uv spectrum of 7-chloro-3-(β-D-ribofuranosyl)-imidazo[4,5-c]pyridazine (**4**) and 7-chloro-3-methylimidazo[4,5-c]pyridazine (7). These data are summarized in Table I.

TABLE I

Uv Spectral Data

| Compound | λ max (nm) | Ref. |
|---|---|---|
| 3-β-D-Ribofuranosylimidazo[4,5-c]-pyridazine | 251<br>281 | |
| 3-Methylimidazo[4,5-c]pyridazine | 254<br>286 | 6 |
| 1-Methylimidazo[4,5-c]pyridazine | 270 | 6 |
| 7-Chloro-3-β-D-ribofuranosylimidazo-[4,5-c]pyridazine | 259<br>287 | |
| 7-Chloro-3-methylimidazo[4,5-c]-pyridazine | 261<br>290 | 7 |

The assignment of the β-configuration to these nucleosides could not be based solely on the coupling constants of their anomeric protons, which ranged from ($J_{1',2'}$) 4.5 Hz to 6.0 Hz (8), nor on the "*trans*" rule (9) since exceptions to this rule have been reported (10). In an effort to diminish the coupling constant to within acceptable limits (11), 7-chloro-3-(2',3'-*O*-isopropylidene-β-D-ribofuranosyl)imidazo[4,5-c]pyridazine (**5**) was prepared and shown to have a coupling constant of 2.0 Hz which is indicative of the β-D-configuration. While the assignment of the β-configuration is not unequivocal, the pmr spectrum of **5** also shows the difference in chemical shift between the two methyl groups of the isopropylidene variety to be 0.22 Hz, a difference characteristic of the β-configuration (12,13).

## EXPERIMENTAL

Melting points were recorded on a Thomas-Hoover capillary melting point apparatus and are uncorrected. The pmr spectra were recorded on a Varian A-60A instrument in DMSO-$d_6$ and compared with TMS as an internal standard. The uv spectra were recorded on a Cary model 15 spectrophotometer. The optical rotations were recorded on a Perkin-Elmer Model 141 automatic digital readout polarimeter and were performed by ARB TechLab, University of Utah, Salt Lake City, Utah. All evaporations were carried out under reduced pressure in a rotary evaporator unless otherwise noted. Column chromatography was accomplished using J. T. Baker 3405 silicagel. Tlc was accomplished using Eastman Chromagram sheets "6060 Silica Gel with Fluorescent Indicator". Microanalyses were performed by the Heterocyclic Chemical Corporation, Harrisonville, Missouri 64701.

7-Chloro-3-trimethylsilylimidazo[4,5-c]pyridazine (**1**).

A mixture of dry 7-chloroimidazo[4,5-c]pyridazine (3) (1.24 g., 8 mmoles), 80 ml. of hexamethyldisilazane (HMDS) and 15 mg. of ammonium sulfate was refluxed with stirring and the exclusion of moisture for 28 hours. The solids dissolved within 30 minutes. The excess HMDS was removed under reduced pressure leaving the yellow-brown crystalline silyl derivative which was not further purified.

7-Chloro-3-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)imidazo[4,5-c]-pyridazine (**3**).

Mercuric cyanide (4.04 g., 16 mmoles) was suspended in 250 ml. of dry benzene and azeotroped until 50 ml. of benzene was collected in a Dean-Stark tube. To this mercuric cyanide suspension was added the entire quantity of the trimethylsilyl derivative (**1**) as a dry benzene suspension followed by a dry benzene solution of 2,3,5-tri-*O*-benzoyl-D-ribofuranosyl bromide (**2**) (14) [freshly prepared from 4.09 g. of 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-D-ribofuranose (15)]. The mixture was refluxed with stirring under anhydrous conditions for 90 minutes. Evaporation of the solvent left a thick green syrup which was dissolved in dichloromethane and filtered through a Celite pad. The filtrate was washed with three 45 ml. portions of 30% potassium iodide solution. The organic layer was then washed with saturated sodium bicarbonate solution, water and allowed to dry over sodium sulfate. After filtration and solvent evaporation, the resulting green foam was dissolved in benzene and chromatographed on a 3 x 40 cm silica gel column. The column was eluted with approximately 700 ml. of benzene-ethyl acetate (18:1 v/v; 1ℓ) before the nucleoside material was collected (Rf 0.46). The product was obtained after solvent evaporation as a yellow-brown foam (2.87 g., 60% yield). An analytical sample was prepared by dissolving a portion of the foam in benzene (Norite) followed by evaporation of the solution. Subsequent crystallizations (2x) from aqueous ethanol gave the pure product, m.p. 88° preceeded by shrinking; pmr: δ 7.00 (1H, d, $J_{1',2'}$ = 4 Hz, 1'*H*); δ 9.28 (1H, s, 6-*H*); δ 9.50 (1H, s, 3-*H*); (pmr indicated that water was introduced

during recrystallization).

*Anal.* Calcd. for $C_{31}H_{23}ClN_4O_7 \cdot H_2O$: C, 60.3; H, 3.9; N, 9.4. Found: C, 60.0; H, 4.1; N, 9.0.

7-Chloro-3-(β-D-ribofuranosyl)imidazo[4,5-c]pyridazine (**4**).

Two g. (3.3 mmoles) of 7-chloro-3-(2,3,6-tri-*O*-benzoyl-β-D-ribofuranosyl)imidazo[4,5-c]pyridazine (**3**) (16) was suspended in 100 ml. of methanolic ammonia (saturated at -10°) in a sealed pressure bottle and allowed to stand at room temperature for for five days (after a few hours, solution was complete). The resulting solution was evaporated to a brown residue which was dissolved in ethanol and allowed to stand overnight at 5°. The resulting crystalline product was separated by filtration and recrystallized (Norite) from ethanol to give 663 mg. (70% yield) of the deblocked product. An analytical sample was prepared by an additional recrystallization from ethanol to give colorless crystals, m.p. 156-158° dec.; pmr: δ 6.43 (1H, d, $J_{1',2'}$ = 4.5 Hz, 1'-*H*); δ 9.37 (1H, s, 6-*H*); δ 9.47 (1H, s, 3-*H*); uv λ max (ethanol): 259 nm (ε, 6,440) and 286 nm (ε, 5,565); $[\alpha]_D^{27}$ -44.0 (*c* 1.033, water).

*Anal.* Calcd. for $C_{10}H_{11}ClN_4O_4$: C, 41.9; H, 3.9; N, 19.5. Found: C, 41.9; H, 3.9; N, 19.4.

7-Chloro-3-(2',3'-*O*-isopropylidene-β-D-ribofuranosyl)imidazo[4,5-c]pyridazine (**5**).

A solution of 230 mg. (0.8 mmole) of 7-chloro-3-(β-D-ribofuranosyl)imidazo[4,5-c]pyridazine, 100 ml. of dry acetone, and 0.8 ml. of 2,2-dimethoxypropane was cooled to 0° and 0.3 ml. of 70% perchloric acid was added dropwise. The solution was allowed to rise to room temperature as it was stirred under anhydrous conditions for 45 minutes, then evaporated to one half the original volume. Ten ml. of saturated sodium carbonate solution was then added and the evaporation completed. To the white residue was added 10 ml. of distilled water and the mixture filtered. The white solid, 220 mg. (67% yield), was almost pure, m.p. 158-159° dec. One recrystallization of a portion of **5** from ethanol supplied an analytical sample m.p. 158° dec.; pmr: δ 6.5 (1H, d, $J_{1',2'}$ = 2 Hz, 1'-*H*); δ 9.20 (1H, s, 6-*H*); δ 9.40 (1H, s, 3-*H*); $CH_3$ groups of isopropylidene δ 1.41 and 1.63; (pmr spectrum confirms the presence of water); uv λ max (ethanol): 258 nm (ε, 7,320) and 285 nm (ε, 6,285).

*Anal.* Calcd. for $C_{13}H_{15}ClN_4O_4 \cdot \frac{1}{2}H_2O$: C, 46.5; H, 4.8; N, 16.7. Found: C, 46.5; H, 5.1; N, 16.8.

3-(2,3,5-Tri-*O*-benzoyl-β-D-ribofuranosyl)imidazo[4,5-c]pyridazine (**6**).

7-Chloro-3-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)imidazo[4,5-c]pyridazine (**3**), (599 mg., 1 mmole) and 172 mg. (2.1 mmoles) of sodium acetate were dissolved in 50 ml. of absolute ethanol by gentle warming. To this solution was added 400 mg. of 5% palladium on charcoal and the mixture was hydrogenated at atmospheric pressure and room temperature for 16 hours. The hydrogenated reaction mixture was filtered and the solvent removed giving a yellow syrup which was transferred to a separatory funnel with 50 ml. of water. The resulting suspension was extracted three times with 15 ml. portions of benzene, the benzene extracts combined, dried over sodium sulfate, concentrated and chromatographed on a 1.7 x 40 cm silica gel column. Elution was accomplished with 50 ml. of benzene followed by 200 ml. of benzene-ethyl acetate (4:1 v/v). The analytical sample (Rf value 0.28) was obtained by evaporation of the eluate (yield 82%), m.p. 73-76° preceeded by shrinking; pmr: δ 7.01 (1H, d, $J_{1',2'}$ = 4 Hz, 1'-*H*); δ 9.18 (1H, s, 6-*H*); peaks representing adjacent protons 3 and 4 are partially covered by the protons of the benzoyl groups but two peaks at δ 9.40 and δ 9.30 are apparent, J = 6 Hz, 3-*H*.

*Anal.* Calcd. for $C_{31}H_{24}N_4O_7$: C, 66.0; H, 4.3; N, 9.9. Found: C, 66.0; H, 4.7; N, 9.7.

3-(β-D-Ribofuranosyl)imidazo[4,5-c]pyridazine (**7**).

3-(2,3,5-Tri-*O*-benzoyl-β-D-ribofuranosyl)imidazo[4,5-c]pyridazine (**6**) (600 mg., 1.06 mmoles) was suspended in 50 ml. of methanolic ammonia (saturated at -10°) in a sealed pressure bottle and allowed to stand at room temperature for five days. (After a few hours, solution was complete.) The resulting solution was evaporated to a syrup to which was added 1-2 ml. of absolute ethanol and placed on a 1.7 x 40 cm silica gel column. The column was eluted with absolute ethanol and the deblocked nucleoside was collected after 70 ml. of ethanol had passed through the column. The Rf value in ethanol was 0.56. The analytical sample was recrystallized from ethyl acetate, yield 74%, m.p. 157-159°; pmr: δ 6.41 (1H, d, $J_{1',2'}$ = 6 Hz, 1'-*H*); δ 9.20 (1H, s, 6-*H*); δ 8.22 (1H, d, $J_{4,3}$ = 6 Hz, 4-*H*); δ 9.35 (1H, d $J_{3,4}$ = 6 Hz, 3-*H*); uv λ max (ethanol): 253 nm (ε, 6,045) and 280 (ε, 4,590); $[\alpha]_D^{27}$ -47.0 (*c*, 0.943, water).

*Anal.* Calcd. for $C_{10}H_{12}N_4O_4$: C, 47.6; H, 4.8; N, 22.2. Found: C, 47.3; H, 4.8; N, 22.0.

7-Amino-3-(β-D-ribofuranosyl)imidazo[4,5-c]pyridazine (**8**).

7-Chloro-3-(β-D-ribofuranosyl)imidazo[4,5-c]pyridazine (**4**) (287 mg., 1 mmole) was added to 100 ml. of absolute ethanolic ammonia (saturated at -10°) in the glass liner (150 ml. capacity) of a 1 ℓ. Paar stainless steel pressure vessel. The reaction mixture was rocked in the autoclave while heating between 180-200° for 6 hours (17). The solvent was evaporated and the resulting syrup dissolved in 1-2 ml. of hot acetone-methanol (6:1 v/v) and placed on a 1.7 x 40 cm silica gel column. The column was eluted with additional acetone-methanol and the product collected after about 70 ml. had been eluted. The product (53.5 mg., 20% yield) was isolated and the analytical sample was prepared by recrystallization from ethanol, m.p. 228-234°; pmr: δ 6.17 (1H, d, $J_{1',2'}$ = 6 Hz, 1-*H*); δ 7.03 (2H, s, $NH_2$ protons); δ 8.70 (2H, s, 6-*H* and 3-*H* superimposed); uv λ max (ethanol): 259 nm (ε, 10,295) and 305 nm (ε, 16,470); $[\alpha]_D^{27}$ -72.6 (*c* 0.894, water).

*Anal.* Calcd. for $C_{10}H_{13}N_5O_4$: C, 44.9; H, 4.9; N, 26.2. Found: C, 45.0; H, 5.0; N, 26.4.

7-Mercapto-3-(β-D-ribofuranosyl)imidazo[4,5-c]pyridazine (**9**).

To a suspension of 287 mg. (1 mmole) of 7-chloro-3-(β-D-ribofuranosyl)imidazo[4,5-c]pyridazine (**4**) in 8 ml. of anhydrous methanol in a pressure bottle was added 2 ml. of a 1*N* methanolic sodium hydrosulfide solution. This reaction mixture was heated at 140° for 1 hour, during which time a yellow product precipitated. The reaction mixture was filtered and additional product was obtained by evaporating the filtrate to dryness, dissolving the resulting residue in 95% ethanol and acidifying with acetic acid to precipitate an additional amount of **9**. A total of 224 mg. of crude product was recrystallized from methanol to give 140 mg. (52% yield) of **9**, m.p. 182-183° dec.; pmr: δ 6.05 (1H, d, $J_{1',2'}$ = 6 Hz, 1'-*H*); δ 8.75 (1H, s, 6-*H*); δ 8.92 (1H, s, 3-*H*); SH proton covered by sugar protons (pmr spectrum confirms the presence of water); uv λ max (ethanol): 272 nm (ε, 2,805), 306 nm (ε, 5,875) and 390 nm (ε, 9,240).

*Anal.* (18) Calcd. for $C_{10}H_{12}N_4O_4S \cdot H_2O$: C, 39.7; H, 4.7; N, 18.5. Found: C, 39.7; H, 4.5; N, 18.3.

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(16) During recrystallization compound **3** tends to form a syrup rather than a crystalline solid therefore in this procedure it was used without recrystallization.

(17) In another experiment the amino compound **8** crystallized from the original reaction mother liquor and was separated by filtration and recrystallized. The filtrate was evaporated and chromatographed as described.

(18) In response to the inquiry of one reviewer, the presence of sulfur was confirmed by the sodium fusion test for sulfur.