A Comparison of Two Methods for the Preparation of 3-Deazapurine Ribonucleosides

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The reaction of the trimethylsilyl derivative of 4,6-dichloroimidazo[4,5-*c*]pyridine with 2,3,5-tri-*O*-benzoyl-D-ribofuranosyl bromide gave four nucleosides—the α- and β-anomers of the 1-isomer and the α- and β-anomers of the 3-isomer (3.9:2.7:1.5:1). In contrast, the fusion reaction of 4,6-dichloroimidazo[4,5-*c*]pyridine with 1,2,3,5-tetra-*O*-acetyl-β-D-ribofuranose gave a high yield of the 1-β-isomer, which was converted to the known 3-deazaadenosine (4-amino-1-β-D-ribofuranosylimidazo[4,5-*c*]pyridine).

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Current interest in the inhibition of enzymatic transmethylations by 3-deazaadenosyl-L-homocysteine (I) (1) prompted a study of methods for the preparation of 3-deazaadenosine (XI) for its conversion to I.

Recently a general synthesis of 4-substituted 1-(β-D-ribofuranosyl)imidazo[4,5-*c*]pyridines (3-deazapurines) involving the reaction of the trimethylsilyl derivative of 4,6-dichloroimidazo[4,5-*c*]pyridine (II) with 2,3,5-tri-*O*-benzoyl-D-ribofuranosyl bromide (IV) followed by removal of the benzoyl protecting groups and appropriate manipulation of the chloro groups to give 3-deazaadenosine (XI) and related nucleosides was delineated (2). The initial reaction of this sequence is described as affording a mixture of the protected 1- and 3-ribosides (β-VI and β-IX), which was separated by fractional crystallization and column chromatography. In our hands, the reaction of II and IV produced not two but four nucleosides, which could not be induced to crystallize but which could be resolved by means of HPLC on a microporasil column (3). The ultraviolet spectra of the four nucleosides, determined during chromatography by stop-flow, showed that the two compounds present in lesser amounts were the 3-substituted 4,6-dichloroimidazo[4,5-*c*]pyridines, whereas the major products were 1-substituted derivatives. These nucleosides were separated by means of silica gel chromatography and their identity firmly established by uv and pmr spectrometry (Table I) as 4,6-dichloro-1-(2,3,5-tri-*O*-benzoyl-α- and β-D-ribofuranosyl)imidazo[4,5-*c*]pyridine (α- and β-VI) and 4,6-dichloro-3-(2,3,5-tri-*O*-benzoyl-α- and β-D-ribofuranosyl)imidazo[4,5-*c*]pyridine (α- and β-IX). The point of attachment of the sugar moiety was assigned from the uv spectra in accordance with the data of May and Townsend (2). The anomeric pairs were assigned by the chemical shifts of the anomeric protons, since the signals from α-anomers always appear downfield from those of the corresponding β-anomers (4). In the case of both isomeric pairs, the β to α ratio is about 1.5 to 1 as determined by integration of the HPLC peaks. This ratio is in good agreement with that (1.4 to 1 for the

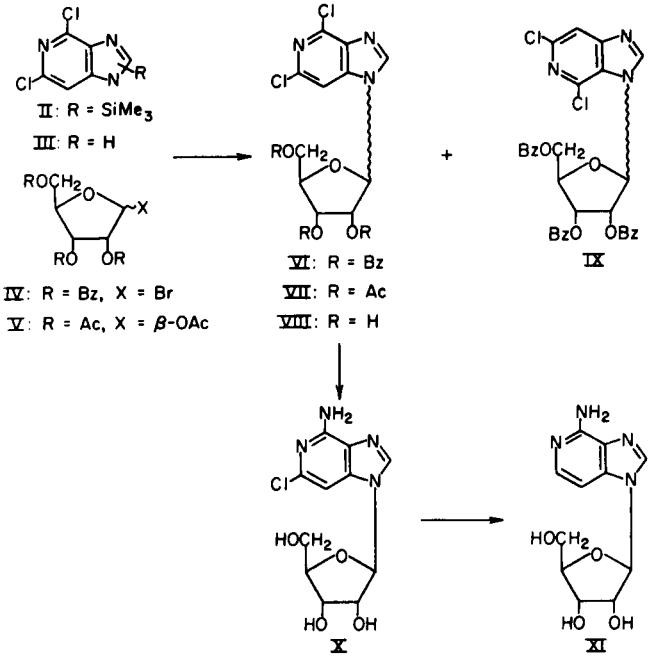

Table I

Pmr and Uv Data

Compound	Chemical Shifts, δ in ppm							Uv Maxima, nm (a)
	$2H_5'$	H_4'	H_3'	H_2'	H_1'	$J_{1'2'}$ (Hz)	H_2	
α-VI	4.75 (*m*)	5.5 (*m*)	6.00 (*m*)	6.23 (*t*)	7.00 (*d*)	5	8.92 (*s*)	259 (b), 274, 281 (b)
β-VI	4.7-5.1 (*m*)		5.95-6.25 (*m*)		6.80 (*d*)	5	8.93 (*s*)	259 (b), 274, 281 (b)
α-IX	4.78 (*m*)	5.60 (*m*)	6.04 (*m*)	6.39 (*t*)	7.4 (c)		9.23 (*s*)	277 (b), 283, 296 (b)
β-IX	4.7-5.1 (*m*)		6.11 (*t*)	6.30 (*m*)	7.12 (*d*)	4	9.12 (*s*)	277 (b), 283, 296 (b)

(a) In chloroform. (b) Shoulder. (c) Masked by phenyl absorption; located by INDOR. (s) Singlet. (d) Doublet. (t) Triplet. (m) Multiplet.

9-isomers and 1.5 to 1 for the 7-isomers) obtained by integration of the C-2 proton peaks in the pmr spectrum of the unresolved reaction mixture. By calculating the HPLC peak areas at the wavelength of the maxima of each isomer pair (VI at 273 nm and IX at 284 nm) and multiplying the peak areas obtained for IX by the ε max ratio (9.8:6), the ratio of 1- and 3-isomers (2.6 to 1) was also determined and confirmed again by pmr (3 to 1). Thus, the reaction mixture is comprised of 43% β-VI, 30% α-VI, 16% β-IX, and 11% α-IX.

In view of the complexity of the trimethylsilyl reaction and the necessity of separating anomers and isomers by chromatography, the fusion reaction, which had been used previously for the preparation of 4-chloro-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)imidazo[4,5-*c*]pyridine (5), was carried out with the dichloro compound III and 1,2,3,5-tetra-*O*-acetyl-β-D-ribofuranose (V). This reaction, in contrast to the trimethylsilyl procedure, gave essentially one nucleoside in high yield with only traces (tlc) of other nucleosidic material. The appearance of all of the methyl signals of the *O*-acetyl groups below 2 ppm is good evidence that this nucleoside has the β- (or trans) configuration with respect to the heterocycle and the 2'-acetoxy group (4). Treatment of this nucleoside (β-VII) with ethanolic ammonia in a bomb at 140° for 89 hours removed the protecting groups and displaced the 4-chloro group in one step to give 4-amino-6-chloro-1-β-D-ribofuranosylimidazo[4,5-*c*]pyridine (X) identical to authentic material (uv, pmr, tlc) (6). Shorter reaction times gave a mixture of VIII and X. Reductive dechlorination of X as previously described (2) gave 4-amino-1-β-D-ribofuranosyl-[4,5-*c*]pyridine (3-deazaadenosine, XI) in good yield. Characterization of this material showed it to be essentially identical with previously described samples (2,7,8) as to m.p., uv spectrum, and optical rotation. Its pmr spectrum, however, is at variance with that previously reported (2).

EXPERIMENTAL

All evaporations were carried out *in vacuo* with a rotary evaporator. Analytical samples were normally dried *in vacuo* over phosphorus pentoxide at room temperature for 16 hours. Analtech precoated (250 μm) silica gel G(F) plates were used for tlc analyses; the spots were detected by irradiation with a Mineralight and by charring after spraying with saturated ammonium sulfate. Compounds containing amino groups were also detected with ninhydrin spray. All analytical samples were essentially tlc homogeneous. Melting points were determined with a Mel-Temp apparatus and are not corrected. The uv absorption spectra were determined in 0.1 *N* hydrochloric acid (*p*H 1), *p*H 7 buffer, and 0.1 *N* sodium hydroxide (*p*H 13) with a Cary 17 spectrophotometer: the maxima are reported in nm ($\epsilon \times 10^{-3}$). The nmr spectra were determined with a Varian XL-100-15 spectrometer in deuteriodimethylsulfoxide with tetramethylsilane as an internal reference: chemical shifts (δ in ppm) quoted in the case of multiplets are measured from the approximate center. The high-pressure liquid chromatographic analysis was carried out with a Waters Associates ALC-242 chromatograph with an M-6000 pump and equipped with a μ Porasil column (¼" by 30 cm) using chloroform (1% ethanol) as the solvent.

1-and 3-(2,3,5-Tri-*O*-benzoyl-D-ribofuranosyl)-4,6-dichloroimidazo-[4,5-*c*]pyridine (α- and β-VI, α- and β-IX).

4,6-Dichloroimidazo[4,5-*c*]pyridine (5.1 g., 27.1 mmoles) was converted to its tetramethylsilyl derivative (II), which was allowed to react with 2,3,5-tri-*O*-benzoyl-D-ribofuranosyl bromide [IV, prepared from 15.2 g. (30 mmoles) of 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose] according to the published procedure (2). The syrup (18.6 g.), which failed to crystallize, was characterized by HPLC and pmr spectrometry before a sample of the mixture was resolved by chromatography on silica gel. Isomerically pure samples of three nucleosides were obtained, but the fourth, the 1-β compound, was not obtained completely free of the 1-α compound. These nucleosides were characterized by HPLC and by pmr and uv spectrometry (see Table I). The mixture proved to be 3.9 parts β-VI, 2.7 parts α-VI, 1.5 parts β-IX, and 1 part α-IX.

1-(2,3,5-Tri-*O*-acetyl-β-D-ribofuranosyl)-4,6-dichloroimidazo[4,5-*c*]pyridine (VII).

An intimate mixture of 4,6-dichloroimidazo[4,5-*c*]pyridine (III, 25.0 g., 133 mmoles), 1,2,3,5-tetra-*O*-acetyl-β-D-ribofuranose (V, 84.6 g., 266 mmoles), and *p*-toluenesulfonic acid (250 mg.) was heated at 160° with stirring *in vacuo* (25 mm) for 5-10 minutes until bubbling ceased and the melt solidified. The resulting solid was heated with chloroform (1.5 l.) with stirring and the suspension filtered hot; yield of white solid, 44.6 g. (75%). The chloroform solution was washed twice with bicarbonate solution (150 ml.) and then dried over magnesium sulfate. Evaporation and ether trituration gave additional white solid, 7.8 g.; total yield, 88% of a homogeneous solid (tlc). A small sample was recrystallized from chloroform-methanol (1:1) for analysis (80%

recovery); m.p. 239-242°; uv: *p*H 1, 7–259 (6.6), 275 (5.5); *p*H 13–260 (6.0), 276 (5.8); pmr: 2.05, 2.1, and 2.15 (3s, CH_3 of acetyl), 4.4 (m, H_4' and $2H_5'$), 5.5 (m, H_2' and H_3'), 6.4 (d, H_1', $J_{1'2'}$, 5 Hz), 8.03 (s, H_7), 8.8 (s, H_2).

Anal. Calcd. for $C_{17}H_{17}Cl_2N_3O_7$: C, 45.65; H, 3.88; N, 9.18. Found: C, 45.75; H, 3.84; N, 9.42.

4-Amino-6-chloro-1-β-D-ribofuranosylimidazo[4,5-c]pyridine (X).

1-(2,3,5-Tri-*O*-acetyl-β-D-ribofuranosyl)-4,6-dichloroimidazo-[4,5-c]pyridine (VII, 52.4 g., 117.5 mmoles) in ethanolic ammonia (750 ml., saturated at -30°) was heated in a bomb at 140° for 89 hours before the solution was evaporated to dryness *in vacuo*. A solution of the residue in 1.5 l. of water was washed with chloroform (3 x 200 ml.), treated with charcoal, filtered, and concentrated to 1.2 l. and refrigerated. A tan solid (homogeneous, tlc) deposited, 23.2 g. (66%). A small sample was recrystallized from water (charcoal), m.p. 223-225° (loses water of hydration at 115°); uv: *p*H 1–266 (11.3), 287 (9.2); methanol–272 (13.0); *p*H 7, 13–271 (12.7); pmr: 3.65 (m, $2H_5'$), 4.0 (m, H_4'), 4.1 (m, H_3'), 4.3 (m, H_2'), 5.15 (m, 3'- and 5'-OH), 5.47 (2'-OH), 5.76 (d, H_1', $J_{1'2'}$ 6.0 Hz), 6.67 (s, NH_2), 7.03 (s, H_7), 8.3 (s, H_2).

4-Amino-1-β-D-ribofuranosylimidazo[4,5-c]pyridine (3-Deazaadenosine, XI).

To a solution of 4-amino-6-chloro-1-β-D-ribofuranosylimidazo-[4,5-c]pyridine (X, 11.3 g., 37.7 mmoles) in water (1.1 l.) was added 1 *N* sodium hydroxide (37.7 ml.) and 30% palladium on charcoal catalyst (2.5 g.). The mixture was hydrogenated at 20 psi for 20 hours. A second reduction (12.4 g. of X) was combined with the first and filtered. The catalyst was washed with boiling water (400 ml.), and the combined filtrates concentrated to 600 ml. and chilled. A white solid deposited, yield 13.3 g. A second crop (4 g.) was obtained from the filtrate. Recrystallization of these crops gave a total yield of 15.2 g. (72%) of a homogeneous solid (tlc), m.p. 229-231°; $[\alpha]_D^{26}$ -45.8 ± 0.8 (c 0.6 in water); uv: *p*H 1, 7–262 (10.8), *p*H 13–265 (10.7); pmr: 3.6 (m, water + $2H_5'$), 4.0 (m, H_4'), 4.1 (m, H_3'), 4.3 (m, H_2'), 5.1 and 5.2 broad (3'- and 5'-OH), 5.5 broad (2'-OH), 5.8 (d, H_1', $J_{1'2'}$ 6.1 Hz), 6.2 (s, NH_2), 6.9 (d, H_7, $J_{6,7}$ 6 Hz), 7.7 (d, H_6, $J_{6,7}$ 6 Hz), 8.3 (s, H_2).

Anal. Calcd. for $C_{11}H_{14}N_4O_4$: C, 49.6; H, 5.30; N, 21.0. Found: C, 49.9; H, 5.43; N, 20.7.

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