Synthesis of 7- and 9-β-D-Ribofuranosides of 3-Deaza-6-thioguanine and 3-Deaza-2,6-diaminopurine by a Novel Ring Closure of 4(5)-Cyano-5(4)-cyanomethylimidazole β-D-Ribofuranosides

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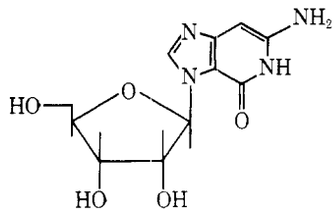
3-Deaza-7-β-D-ribofuranosyl-6-thioguanine [6-amino-3-β-D-ribofuranosylimidazo[4,5-*c*]pyridine-4(5*H*)-thione (**11**)] and 3-deaza-6-thioguanosine [6-amino-1-β-D-ribofuranosylimidazao[4,5-*c*]pyridine-4(5*H*)-thione (**16**)] were synthesized by the novel cyclization (and subsequent deblocking) of 5-cyano-4-cyanomethyl-1-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)imidazole (**6**) and 4-cyano-5-cyanomethyl-1-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)imidazole (**9**), respectively, with hydrogen sulfide and triethylamine. 3-Deaza-2,6-diamino-7-β-D-ribofuranosylpurine [4,6-diamino-3-β-D-ribofuranosylimidazo[4,5-*c*]pyridine (**18**)] and 3-deaza-2,6-diamino-9-β-D-ribofuranosylpurine [4,6-diamino-1-β-D-ribofuranosylimidazo[4,5-*c*]pyridine (**19**)] were synthesized directly by the novel cyclization of blocked imidazole nucleosides **6** and **9**, respectively, with ammonia. Blocked imidazole nucleosides **6** and **9** were prepared from the stannic chloride catalyzed condensation of 4(5)-cyano-5(4)-cyanomethyl-1-trimethylsilylimidazole (**4**) and 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose (**5**). 6-Amino-3-β-D-ribofuranosylimidazo[4,5-*c*]pyridine (**12**) and 6-amino-1-β-D-ribofuranosylimidazo[4,5-*c*]pyridine (**17**) were obtained from the Raney nickel desulfurization of imidazo[4,5-*c*]pyridine nucleosides **18** and **19**, respectively. Debromination and deblocking of 6-amino-4-bromo-1-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)imidazo[4,5-*c*]pyridine (**20**) (obtained by the cyclization of blocked imidazole nucleoside **9** with anhydrous hydrogen bromide) also led to imidazo[4,5-*c*]pyridine nucleoside **17**. The structures of the nucleosides were established by the use of proton NMR. Mechanistic implications of these cyclizations are discussed.

The synthesis[2] and antibacterial activity[3] of 3-deaza-7-β-D-ribofuranosylguanine [(6-amino-3-β-D-ribofuranosylimidazo[4,5-*c*]pyridin-4(5*H*)-one (**1**)] was recently described. Since it is likely that **1** acts as a prodrug of 3-deazaguanine (**2**),



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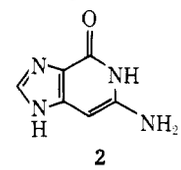

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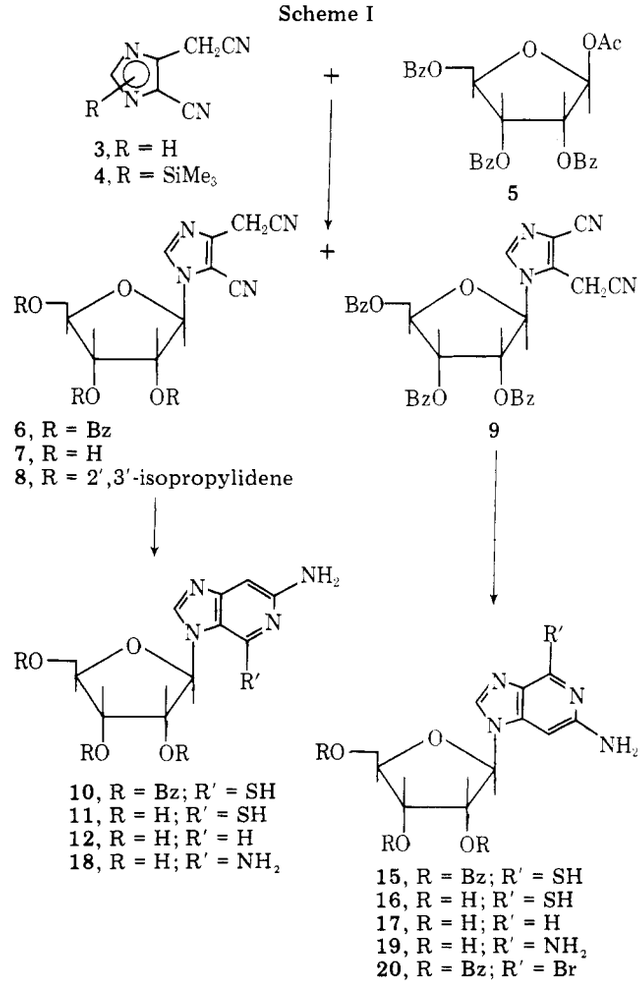
a potent guanine antimetabolite with anticancer,[4] antiviral,[5] and antibacterial activity,[6] the synthesis of the 3-deaza-7-β-D-ribofuranosyl modifications of 6-thioguanine and 2,6-diaminopurine, both known antimetabolites with anticancer activity,[7] was considered. Furthermore, the 9-β-D-ribofuranosyl derivative of 3-deaza-6-thioguanine and 3-deaza-2,6-diaminoguanine was considered an equally interesting synthetic goal.

Previous studies by Robins, Townsend, and their coworkers which led to the synthesis of certain 3-deazapurines,[8a,b] 3-deazaguanosine, and **1**[2] indicated that the ribosides of 4(5)-cyano-5(4)-cyanomethylimidazole (**3**) could be cyclized with various reagents directly into the desired 4,6-disubstituted imidazo[4,5-*c*]pyridine nucleosides.[9] Imidazole **3** has previously been cyclized with anhydrous hydrogen bromide to provide 6-amino-4-bromoimidazo[4,5-*c*]pyridine.[8b] This bicyclic intermediate, its 9-riboside,[12] and 4,6-dichloro-1-β-D-ribofuranosyl imidazol[4,5-*c*]pyridine[12b] do not lead to 4,6-disubstituted imidazo[4,5-*c*]pyridines and their ribosides due to lack of reactivity of the halogen atoms.[8b,12] Furthermore, in an attempt to circumvent the resistance to nucelophilic substitution of the chlorine atoms in imidazo[4,5-*c*]-pyridines, Kroon et al. recently prepared the fluoro analogue, 4,6-difluoroimidazo[4,5-*c*]pyridine for substitution studies.[14] Brief investigations of this intermediate revealed a reactivity similar to 4,6-dichloroimidazo[4,5-*c*]pyridine and, consequently, not a suitable intermediate to 4,6-disubstituted imidazo[4,5-*c*]pyridines. Thus, imidazole **3** was considered for ribosylation and subsequent cyclization procedures.

The stannic chloride catalyzed condensation of trimethylsilylated imidazoles with fully acylated ribofuranoses[15] has been found to be an excellent procedure for preparing the

Scheme I



3, R = H
4, R = $SiMe_3$

5

6, R = Bz
7, R = H
8, R = 2′,3′-isopropylidene

9

10, R = Bz; R′ = SH
11, R = H; R′ = SH
12, R = H; R′ = H
18, R = H; R′ = NH_2

15, R = Bz; R′ = SH
16, R = H; R′ = SH
17, R = H; R′ = H
19, R = H; R′ = NH_2
20, R = Bz; R′ = Br

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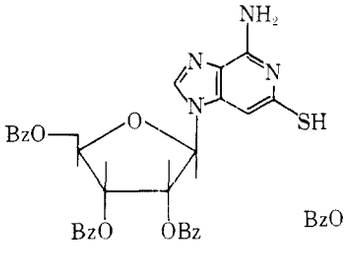
requisite imidazole nucleosides for conversion into 7- and 9-ribosides of 3-deazaguanine[2] and imidazo[4,5-*d*]pyridazine ribosides.[10] Thus, treatment of 4(5)-cyano-5(4)-cyanomethyl-1-trimethylsilyimidazole (**4**) in 1,2-dichloroethane with 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose (**5**) and 1.44 molar equiv of anhydrous stannic chloride provided two blocked imidazole nucleosides, 5-cyano-4-cyanomethyl-1-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)imidazole (**6**, 72%) and 4-cyano-5-cyanomethyl-1-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)imidazole (**9**, 18%), tentatively assigned the structures as shown in Scheme I. Reduction of the amount of anhydrous stannic chloride in this ribosylation procedure to 0.72 molar equiv significantly affected the ratio of positional isomers (50% of **6**, 25% of **9**). This corresponds to the stannic chloride catalyzed ribosylation of methyl 4(5)-cyanomethyl-1-trimethylsilyimidazole-5(4)-carboxylate,[2] although not to such a marked extent.[16]

Reaction of nucleoside **6** with ethanolic hydrogen sulfide and triethylamine at room temperature for 72 h followed by 0.45 h of reflux provided only one product in 94% yield. Proton NMR and UV absorption studies indicated a bicyclic product, and elemental analysis indicated only one sulfur atom. Of the numberous possibilities, only six reasonable structures are consistent with this data: tribenzoyl-blocked 7- and 9-ribosides of 3-deaza-6-thioguanine (the desired products), 3-deaza-2-mercaptoadenine (**13**), and 6-amino-4-iminoimidazo[4,5-*c*]thiine (**14**). Hydrogenolysis of the deblocked



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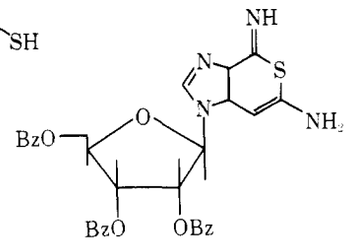

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thiated nucleoside with Raney nickel provided an aromatic, bicyclic nucleoside without a sulfur atom, as determined by proton NMR, UV absorption, and elemental analysis. The lack of an AX coupling system in the proton NMR spectrum confirmed the structure of the aglycones **10**, **11**, and **12**. Dethiation of **11** to afford **12** has also provided some indication as to the location of the ribofuranosyl moiety in nucleosides **6–12**, since a large upfield shift of the anomeric proton ($H_{1'}$) was observed for **12** (ΔH from **11** to **12** was 1.62 ppm). This results from removing the anisotropic effect of a thione group in close proximity to the anomeric proton ($H_{1'}$) in nucleoside **11**.[17] Therefore, 3-deazapurine nucleosides **11** and **12** are 7-ribosides and, hence, the position of the blocked ribofuranosyl moiety in imidazole nucleosides **6** and **9** are correct as shown in Scheme I. Furthermore, the dissimilarity of the UV absorption spectra of nucleosides **11** and **12** to 3-deaza-6-thioguanosine and 6-amino-1-β-D-ribofuranosylimidazo[4,5-*c*]pyridine, respectively, both recently prepared by May and Townsend,[12] provide conclusive proof that deazapurines **11** and **12** are not the 9-ribosides.

The β-configuration was expected for nucleosides **6–12** due to the propensity of the stannic chloride catalyzed ribosylations of silylated heterocycles to form exclusively β-anomers.[2,15] Proof that the nucleosides **6–8** and **10–12** were of the β-configuration was obtained by considering the difference in the chemical shift of the methyl groups of 5-cyano-4-cyanomethyl-1-(2,3-isopropylidene-β-D-ribofuranosyl)imidazole (**8**), obtained from deblocking nucleoside **6** and subsequent isopropylidenation of the resulting imidazole **7**. A difference of 0.19 ppm was found which is a reliable indicator for the β-configuration according to Imbach and workers.[18]

Treatment of nucleoside **9** with hydrogen sulfide and triethylamine at ambient temperature provided 6-amino-1-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)imidazo[4,5-*c*]pyridine-4(5*H*)-thione (**15**) in near quantitative yield. Deblocking of **15** with sodium methoxide in methanol provided 3-deaza-6-thioguanosine (**16**) in an excellent yield. The anomeric proton ($H_{1'}$) in **16** is considerably upfield compared to the isomeric compound 3-deaza-7-β-D-ribofuranosyl-6-thioguanine (**11**) (δ 5.63 compared to 7.47). This is due to the lack of a magnetic anisotropy effect of a closely positioned thione group as found in nucleoside **11**. Dethiation of nucleoside **16** with Raney nickel provided 6-amino-1-β-D-ribofuranosylimidazo[4,5-*c*]pyridine (**17**). The chemical shift of the anomeric proton ($H_{1'}$) in nucleoside **17** is, as expected, only slightly removed from the chemical shift of $H_{1'}$ in the isomeric dethiated nucleoside **12** and 3-deaza-6-thioguanosine (**16**) (δ 5.85 compared to 5.76 and 5.63, respectively). Nucleosides **16** and **17** are, respectively, spectroscopically identical to 3-deaza-6-thioguanosine and 6-amino-1-β-D-ribofuranosylimidazo[4,5-*c*]pyridine, which were prepared by May and Townsend's procedure.[12] Thus, as usual,[2,10,15] the stannic chloride catalyzed ribosylation of silylated heterocycles has provided an excellent yield of blocked nucleosides in the β-configuration.

4,6-Diamino-3-β-D-ribofuranosylimidazo[4,5-*c*]pyridine (**18**), another interesting modification of 3-deaza-7-β-D-ribofuranosylguanine (**1**), was obtained in 75% yield from treatment of blocked imidazole **6** with methanolic ammonia (130 °C) for 16 h. The corresponding isomer, 4,6-diamino-1-β-D-ribofuranosylimidazo[4,5-*c*]pyridine (**19**) was also obtained in excellent yield from imidazole **9** and methanolic ammonia at ambient temperature. Proof that nucleosides **18** and **19** were indeed the diaminobicyclic structures rather than a mono- or diamidine imidazole nucleoside was evident from elemental analysis; UV absorption, which exhibits the characteristic bathochromic shifts due to the annelation of the pyridine ring to the imidazole ring;[19] lack of a nitrile absorption band at 2220 cm^{-1}, which was present in nucleosides **6** and **9**; and proton NMR, which indicated an additional aromatic proton (C_7H) in comparison with **6** and **9**, the lack of a methylene group, and two aromatic amino groups.

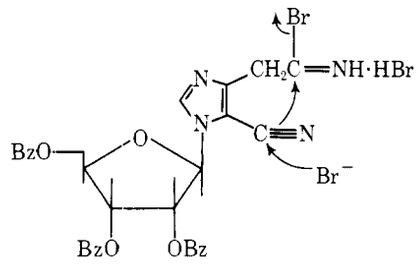
Attempts to cyclize nucleoside **6** with anhydrous hydrogen bromide in methylene chloride provided, according to thin-layer chromatography, unreacted starting material and several trace products. However, treatment of imidazole **9** under the same conditions provided a moderate yield of a slightly impure 6-amino-4-bromo-1-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)imidazo[4,5-*c*]pyridine (**20**). Structure proof of nucleoside **20** was obtained by subjecting it to deblocking and subsequent hydrogenolysis procedures to form **17**.

Acid-catalyzed intramolecular cyclizations between a cyanomethyl group and an adjacent cyano group located on a benzene,[20] pyridine,[21] or imidazole ring[8b] to form isoquinolines, pyrido[*c*]pyridines, or imidazo[4,5-*c*]pyridines, respectively, have been reported. Furthermore, Alhaique et al.,[22] in the most recent report concerning cyclizations of dinitriles, describes the synthesis of aminoalkoxynaphthyridines (pyrido[*c*]pyridines) via sodium alkoxides.

In the present work, the use of hydrogen sulfide or ammonia to affect this type of cylization does not appear to have been described in the literature.[23] Since our cyclizations with hydrogen sulfide and ammonia were carried out in a basic medium, just as Alhaique et al.'s, the same direction of cyclization would be expected. However, our basic cyclization with hydrogen sulfide proceeded in the "reverse" manner to provide 6-aminoimidazo[4,5-*c*]pyridine-4(5*H*)-thione ribosides **10** and **15** rather than the 4-aminoimidazo[4,5-*c*]pyridine-6(5*H*)-

thione ribosides (e.g., 13). As to speculations concerning a mechanism for these cyclizations, one might assume an attack of hydrogen sulfide–triethylamine or ammonia at the carbon atom of the aromatic nitrile first, followed by nucleophilic cyclization of the imino group and the cyanomethyl group, but the possibility of bis(thiocarboxamide) or bis(amidine) formation would complicate the problem.

Anhydrous hydrogen bromide cyclizations of aromatic nitriles with an adjacent cyanomethyl group have all proceeded in the same direction;[8b,20,21] that is, the nitrogen of the aromatic nitrile becomes the ring nitrogen of the pyridine moiety. Our cyclization of nucleoside 9 with anhydrous hydrogen bromide, although proceeding poorly, did afford the expected product. The greater reactivity of aliphatic nitriles to acid as compared to aromatic nitriles might account for this in that the aliphatic imino hydrogen bromide is preferentially formed (21 and 22) and then nucleophilic attack by the aromatic ni-



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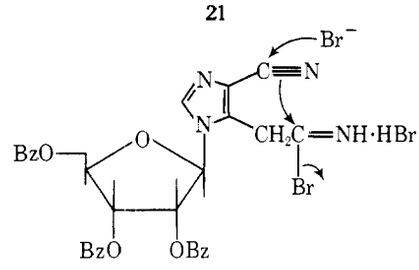

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trile takes place. This mechanism might also account for the failure of nucleoside 6 to react with hydrogen bromide, since the approach of the bromide for attack on the carbon atom of the aromatic nitrile 21 would encounter considerable steric hinderance as compared with the isomeric aliphatic imino hydrogen bromide 22. More strenuous conditions were required in all cyclizations described in this paper which led to the 7-ribosides as compared with the 9-ribosides. This was also evident in the cyclization of the ribosides of methyl 4-cyanomethylimidazole-5-carboxylate to 7- and 9-ribosides of 3-deazaguanine.[2] The tribenzoyl-blocked ribose and the cyanomethyl (or addition product thereof, such as imino hydrogen bromide) located on the nitrogen in the 1 position and the carbon atom in the 4 position of nucleoside 6, respectively, provide considerable steric hinderance to a nitrile or carbomethoxy group in the 5 position of the imidazole ring system according to space-filling models (CPK). We suspect that steric hinderance is the main reason for the reduced reactivity of imidazole nucleosides in cyclization leading to 7-ribosides of 3-deazapurines as compared to the corresponding isomer which lacks this steric hinderance and leads to 9-riboside of 3-deazapurines.

The biological evaluation of these modifications of 3-deaza-7-β-D-ribofuranosylguanine (1) and 3-deazaguanosine will be reported elsewhere.

Experimental Section

Melting points were determined with a Thomas-Hoover capillary melting point apparatus and are uncorrected. Specific rotations were measured in a 1-dm tube with a Perkin-Elmer Model 141 automatic digital readout polarimeter. Proton magnetic resonance (^{1}H NMR) spectra were obtained on a Varian A-60 spectrometer and a Perkin-Elmer R-20A spectrometer in Me_2SO-d_6 using DSS as an internal reference. Ultraviolet spectra were recorded on a Cary Model 15 spectrophotometer and infrared spectra on a Perkin-Elmer 257 spectrophotometer (KBr pellets). Elemental analyses were performed by Galbraith Laboratories, Inc., Knoxville, Tenn. Evaporations were carried out under reduced pressure with bath temperature below 40 °C unless otherwise noted. Detection of components on silica gel (ICN Life Sciences Group, Woelm F254) was by ultraviolet light and with anisaldehyde, methanol, sulfuric acid (1:10:100) spray followed by heating. ICN Life Sciences Group Woelm silica gel (0.063–0.2 mm) was used for column chromatography.

5-Cyano-4-cyanomethyl-1-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)imidazole (6) and 4-Cyano-5-cyanomethyl-1-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)imidazole (9). 4(5)-Cyano-5(4)-cyanomethylimidazole[8b] (3) (15.0 g, 113.6 mmol) was refluxed under anhydrous conditions for 8–24 h with hexamethyldisilazane (200 mL) and ammonium sulfate (250 mg). The excess hexamethyldisilazane was removed by distillation under reduced pressure providing the trimethylsilyl derivative as a tan oil. The oil was dissolved in dry 1,2-dichloroethane (500 mL). 1-*O*-Acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose (5) (57.25 g, 113.6 mmol) was added to the solution followed by direct addition of anhydrous stannic chloride (19 mL, 163.6 mmol) in one portion. TLC (silica gel, benzene–ethyl acetate, 4:1) of an ethanolized aliquot indicated almost complete conversion of the sugar and base to products after 15 min of stirring at ambient temperature. The reaction solution was stirred further for 5 h and then poured slowly into a vigorously stirred 5% sodium hydrogen carbonate solution (2 L). Chloroform (2 L) was added and stirring continued for 0.5 h. The mixture was filtered through Celite and the organic layer was removed. The aqueous layer was extracted with chloroform (300 mL). The combined, dried ($MgSO_4$) extracts were evaporated in vacuo (50 °C) to a light-beige foam (64.4 g, 98%). The foam was dissolved in benzene and placed on a column of silica gel (1800 g, packed in benzene). Elution with benzene–ethyl acetate (5:1) provided 9 (11.7 g of colorless foam, 18%) as the first isomer off the column. Recrystallization from methanol afforded 9 as colorless crystals: mp 145–146 °C; IR (KBr) 2220 (w) (C≡N) cm^{-1}; ^{1}H NMR (Me_2SO-d_6) δ 6.55 (d, 1, J = 4 Hz, $H_{1'}$), 8.51 (s, 1, C_2H).

Anal. Calcd for $C_{32}H_{24}N_4O_7$ (576.54): C, 66.66; H, 4.20; N, 9,72. Found: C, 66.55; H, 4.30; N, 9.67.

Further elution of the column with benzene–ethyl acetate (5:1) afforded 6 as a colorless foam (46.8 g, 72%): IR (KBr) 2220 (w) (C≡N) cm^{-1}; ^{1}H NMR (Me_2SO-d_6) δ 6.54 (d, 1, J - 4 Hz, $H_{1'}$), 8.50 (s, 1, C_2H).

Anal. Calcd for $C_{32}H_{24}N_4O_7$ (576.54): C, 66.66; H, 4.20; N, 9.72. Found: C, 66.45; H, 4.22; N, 9.60.

Utilizing the same conditions, except that the amount of anhydrous stannic chloride was decreased to 0.72 molar equiv (9.5 mL, 81.8 mmol), provided 9 and 6 in 25 and 50% yields, respectively.

5-Cyano-4-cyanomethyl-1-(2,3-isopropylidene-β-D-ribofuranosyl)imidazole (8). Nucleoside 6 (10 g, 17.4 mmol) and liquid ammonia (70 mL) were placed in a steel bomb (140 mL) and heated at 100 °C for 3 h. The ammonia was allowed to evaporate at room temperature, and the residue was subjected to a vacuum overnight to remove the last traces of ammonia. The brown residue was dissolved in methanol, absorbed on silica gel (40 g), and placed on a column of silica gel (300 g, packed in chloroform). Elution with chloroform–methanol (5:1) provided 5-cyano-4-cyanomethyl-1-β-D-ribofuranosylimidazole (7, 3.5 g, 76%) as a colorless foam. This foam was dissolved in a solution of dry acetone (50 mL), 2,2-dimethoxypropane (25 mL), and 70% perchloric acid (700 mg) and stirred at ambient temperature for 10 min. Saturated sodium carbonate solution (1 mL) was added, and the mixture was absorbed on silica gel (10 g) with the aid of methanol and placed on a column of silica gel (100 g, packed in chloroform). Elution with chloroform–methanol (10:1) provided the isopropylidene 8 as a colorless foam (3.2 g, 79%); ^{1}H NMR (Me_2SO-d_6) δ 1.39 (s, 3, CH_3), 1.58 (s, 3, CH_3), 4.20 (s, 2, C_4-CH_2), 5.99 (d, 1, J = 2 Hz, $H_{1'}$), 8.36 (s, 1, C_2H).

Anal. Calcd for $C_{14}H_{16}N_4O_4$ (304.3): C, 35.87; H, 5.30; N, 18.41. Found: C, 35.81; H, 5.28; N, 18.23.

6-Amino-3-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)imidazo[4,5-*c*]pyridine-4(5*H*)-thione (10). A mixture of 6 (7.0 g, 12.2 mmol), ethanol saturated at −10 °C with hydrogen sulfide (250 mL), and triethylamine (1.8 mmol) was kept in a steel bomb (300 mL) for 3 days at ambient temperature. TLC (silica gel, chloroform–methanol, 10:1) indicated two products in approximately equal amounts. The yellow suspension was refluxed for 0.45 h (complete dissolution obtained at start of reflux). This cleanly converts the higher R_f valued product into the lower R_f valued product. The solvents were removed

by evaporation in vacuo, and the yellow residue was dissolved in chloroform and placed on a column of silica gel (100 g, packed in chloroform). Elution with methylene chloride–methanol (10:1) provided pure **10** as a yellow foam (7.0 g, 94%). A sample of the foam was crystallized from ethyl ether to afford light yellow crystals: mp 223–224 °C dec (after drying at 100 °C for 5 h); ^{1}H NMR (Me_2SO-d_6) δ 6.08 (s, 2, NH_2), 6.17 (s, 1, C_7H), 8.73 (s, 1, C_2H), 12.11 (s, 1, NH).

Anal. Calcd for $C_{32}H_{26}N_4O_7S$ (610.66): C, 62.94; H, 4.29; N, 9.18; S, 5.25. Found: C, 63.02; H, 4.18; N, 9.01; S, 5.34.

6-Amino-3-β-D-ribofuranosylimidazo[4,5-*c*]pyridine-4(5*H*)-thione [3-Deaza-7-β-D-ribofuranosyl-6-thioguanine (11)]. Nucleoside **10** (2.5 g, 4.09 mmol) was dissolved in dry methanol (175 mL) containing sodium methoxide (from 5 mg of sodium) and kept at ambient temperature for 24 h. The yellow solution was refluxed 5 min, cooled, and treated with Amberlite IRC-50 (H^+) (10 mL). The resin was filtered and washed with hot ethanol. The filtrate was evaporated in vacuo in the presence of silica gel (5 g). The residue was slurried with chloroform and placed on a column of silica gel (45 g). Elution with chloroform–methanol (4:1) and evaporation of the product containing fractions provided the pure nucleoside as a yellow foam (1.05 g, 86%). Recrystallization from ethanol–water afforded yellow rosettes: mp grad dec >155 °C (after drying at 100 °C for 5 h); $[\alpha]^{25}_D$ +202° (*c* 1.0, DMF); UV λ_{max} (pH 1) 227 (ϵ 15 830), 255 (5000), 283 (6380), 378 nm (13 880); λ_{max} (pH 7) 231 (20 000), 264 (8610), 373 nm (13 880); λ_{max} (pH 11) 227 (17 500), 261 (6110), 343 nm (8330); ^{1}H NMR (Me_2SO_4-d_6) δ 5.96 (s, 2, NH_2), 6.10 (s, 1, C_1H), 7.48 (d, 2, J = 3.5, $H_{1'}$), 8.63 (s, 1, C_2H), 11.95 (s, 1, NH).

Anal. Calcd for $C_{11}H_{14}N_4O_4S$ (298.32): C, 44.29; H, 4.73; N, 18.78; S, 10.75. Found: C, 44.10; H, 4.91; N, 18.57; S, 10.59.

6-Amino-3-β-D-ribofuranosylimidazo[4,5-*c*]pyridine (12). A mixture of **11** (100 mg, 0.335 mmol), Raney nickel (ca. 500 mg), and ethanol (20 mL) was stirred and refluxed for 10 min and then filtered hot. The filtrate was concentrated in vacuo to a small volume and cooled overnight to afford **12** as colorless needles (63 mg, 70%): mp 226–227 °C dec (after drying at 100 °C for 2 h); UV λ_{max} (pH 1) 222 (ϵ 34 990), 224 (4700), 339 nm (6000); λ_{max} (H_2O) 215 (30 550), 253 (sh) (2870), 314 nm (3390); λ_{max} (pH 11) 221 (16 710), 253 (sh) (2870), 312 nm (3655); ^{1}H NMR (Me_2SO-d_6) δ 5.41 (s, 2, NH_2), 5.82 (d, 1, J = 6 Hz, $H_{1'}$), 6.69 (s, 1, C_7H), 8.40 (s, 1, C_2H or C_4H), 8.55 (s, 1, C_2H or C_4H).

Anal. Calcd for $C_{11}H_{14}N_4O_4$ (266.27): C, 49.62; H, 5.30; N, 21.04. Found: C, 49.53; H, 5.26; N, 21.34.

6-Amino-1-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)imidazo-[4,5-*c*]pyridine-4(5*H*)-thione (15). A mixture of nucleoside **9** (8.0 g, 13.88 mmol), ethanol saturated at 0 °C with hydrogen sulfide (250 mL), and triethylamine (2.5 g, 24.7 mmol) was kept in a steel bomb (300 mL) for 48 h at ambient temperature. The reaction solution was evaporated in vacuo to a yellow foam which was coevaporated with ethanol several times. TLC (silica gel, chloroform–methanol, 10:1) indicated one major product and several small products from partially deblocked material. This material was sufficiently pure for the next reaction.

6-Amino-1-β-D-ribofuranosylimidazo[4,5-*c*]pyridine-4-(5*H*)-thione [3-Deaza-6-thioguanosine (16)]. Nucleoside **15** (8.0 g, 13.0 mmol) was dissolved in dry methanol (200 mL) containing sodium methoxide (from 460 mg of sodium) and refluxed 15 min. The cooled solution was treated with Amberlite IRC-50 (H^+ (20 mL) and stirred for 0.5 h. The resin was filtered and washed with hot water. The combined filtrates were evaporated in vacuo to dryness and the resulting residue was triturated with methanol to afford quite pure **16** (3.5, 90%). Recrystallization from water provided large yellow chunks of **16**: mp 226–227 °C dec (after drying at 100 °C for 2 h) (lit. mp 185 °C dec as dihydrate);[10] $[\alpha]^{25}_D$ −55.7° (*c* 0.945, DMF); UV λ_{max} (pH 1) 223 (sh) (ϵ 16 870), 245 (sh) (6630), 291 (7230), 374 nm (16 870); λ_{max} (pH 7) 228 (17 770), 253 (6630), 283 (8430), 353 nm (18 370); λ_{max} (pH 11) 226 (15 360), 245 (sh) (9640), 283 (6630), 323 nm (13 550); ^{1}H NMR (Me_2SO-d_6) δ 5.62 (d, 1, J = 5 Hz, $H_{1'}$), 6.13 (s, 2, NH_2), 6.12 (s, 1, C_7H), 8.18 (s, 1, C_2H), 11.95 (s, 1, NH).

Anal. Calcd for $C_{11}H_{14}N_4O_4S$ (298.32): C, 44.29; H, 4.73; N, 18.78; S, 10.75. Found: C, 43.99; H, 4.84; N, 18.59; S, 10.44.

6-Amino-1-β-D-ribofuranosylimidazo[4,5-*c*]pyridine (17). A mixture of **16** (500 mg, 1.67 mmol), Raney nickel (ca. 2.5 g), ethanol (100 mL), and water (20 mL) was refluxed with stirring for 10 min and then filtered hot through celite. The filtrate was concentrated in vacuo to a small volumn and cooled overnight to provide **17** as beige needles (325 mg, 73%); mp 219–220 (after drying at 100 °C for 2 h) (lit. 221–223 °C dec);[10] $[\alpha]^{25}_D$ −44.2° (*c* 0.96, H_2O); UV λ_{max} (pH 1) 226 (ϵ 43 970), 254 (sh) (3921), 261 (4761), 268 (3921), 320 nm (3641); λ_{max} (pH 7) 218 (31 092), 256 (5042), 298 nm (3081); λ_{max} (pH 11) 222 (48 739), 255 (5602), 297 nm (3641); ^{1}H NMR (Me_2SO-d_6) δ 5.63 (s, 2, NH_2), 5.76 (d, 1, J = 6 Hz, $H_{1'}$), 5.62 (s, 1, C_7H), 8.26 (s, 1, C_2H or C_4H), 8.40 (s, 1, C_2H or C_4H).

Anal. Calcd for $C_{11}H_{14}N_4O_4$·0.5H_2O (275.27): C, 47.99; H, 5.49; N, 20.35. Found: C, 47.99; H, 5.35; N, 20.24.

4,6-Diamino-3-β-D-ribofuranosylimidazo[4,5-*c*]pyridine (18). A mixture of nucleoside **6** (5 g, 8.68 mmol), liquid ammonia (10 mL), and methanol (10 mL) was heated in a steel bomb (40 mL) at 125–135 °C for 16 h. The reaction solution was evaporated in vacuo and the residue triturated several times with ethyl ether–methanol (3:1). The residue was absorbed on silica gel (5 g) with the aid of methanol and placed on a column of silica gel (150 g, packed in chloroform). Elution with chloroform–methanol (1:1) provided benzamide and a small amount of pure 5-cyano-4-cyanomethyl-1-β-D-ribofuranosylimidazole (**7**) (0.26 g, 11%). Elution with chloroform–methanol (1:1) removed the desired nucleoside. Evaporation of the product containing fractions and recrystallization of the residue from methanol afforded **18** as beige crystals (1.44 g in 2 crops, 60%): mp 130–132°C dec (after drying at 100 °C for 2 h); UV λ_{max} (pH 1) 214 (ϵ 20 057), 273 (6857), 338 nm (6571); λ_{max} (pH 7) 218 (2286), 248 (4286), 318 (4857); λ_{max} (pH 11) 222 (15 428), 248 (4857), 313 nm (5143); ^{1}H NMR (Me_2SO-d_6) δ 5.90 (d, 2, J = 5 Hz, $H_{1'}$), 6.18 (s, 1, C_7H), 5.30–7.00 (br s, 2, NH_2), 7.20 (s, 2, NH_2), 8.58 (s, 1, C_2H).

Anal. Calcd for $C_{11}H_{15}N_5O_4$ (281.27): C, 46.97; H, 5.38; N, 24.90. Found: C, 47.22; H, 5.24; N, 24.99.

4,6-Diamino-1-β-D-ribofuranosylimidazo[4,5-*c*]pyridine (19). A mixture of nucleoside **9** (3.0 g, 5.2 mmol) and dry methanol saturated at 0 °C with ammonia (150 mL) was kept in a steel bomb at ambient temperature for 24 h and then evaporated in vacuo to dryness. The residue was triturated with ether, dissolved in methanol, absorbed on silica gel (10 g), and placed on a column of silica gel (60 g, packed in chloroform). Elution with chloroform–methanol (1:1) removed the product from the column. The product containing fractions were combined and concentrated in vacuo to a small volumn. Addition of ether until the cloud point was obtained and cooling provided **19** as colorless cyrstals (780 mg). An additional 430 mg of **19** was obtained in a second crop (1.21 g total, 83%): mp 210–212 °C dec (after drying at 100 °C, 3 h); $[\alpha]^{25}_D$ −41.8° (*c* 0.99, DMF); UV λ_{max} (pH 1) 217 (ϵ 29 539), 271 (11 382), 315 nm (8943); λ_{max} (pH 7) 217 (29 539), 272 (11 110), 295 nm (sh) (7046); λ_{max} (pH 14) 218 (18 690), 273 (10 570), 288 nm (sh) (8940); ^{1}H NMR (Me_2SO-d_6) δ 5.14 (br s, 2, NH_2), 5.65 (d, 1, J = 2 Hz, $H_{1'}$), 5.82 (s, 1, C_7H), 5.83 (s, 2, NH_2), 7.98 (s, 1, C_2H).

Anal. Calcd for $C_{11}H_{15}N_5O_4$ (281.27): C, 46.97; H, 5.38; N, 24.90. Found: C, 47.0; H, 5.29; N, 25.08.

6-Amino-4-bromo-1-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)imidazo[4,5-*c*]pyridine (20). A solution of nucleoside **9** (6.4 g, 11.1 mmol) and dry chloroform (300 mL) was cooled to −30 °C and saturated with anhydrous hydrogen bromide at −30 °C. The cooling bath was removed and the reaction solution stirred 6 h at ambient temperature. The residue, obtained from removal of the chloroform in vacuo at 20 °C, was dissolved in chloroform and washed with sodium hydrogen carbonate solution. TLC of the dried ($MGSO_4$) chloroform solution (silica gel, chloroform–methanol, 10:1) indicated one major spot which turns yellow with anisaldehyde–methanol–sulfuric acid spray (1:10:100) and then chars, on heating, some starting material and several other small spots. The solution was evaporated in vacuo to a small volumn and placed on a column of silical gel (250 g, packed in chloroform). Elution with chloroform–methanol (20:1) provided **20** as a slighlty impure light yellow foam (3.6 g, 49%) as determined by TLC. Structure proof of this material was obtained by deblocking with methanolic sodium methoxide and subsequent hydrogenolysis with palladium on charcoal to provide nucleoside **17**.

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Synthesis of a Cyclic Charge Transfer Labeled Analogue of the Luteinizing Hormone-Releasing Factor[1]

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The synthesis of

p-Glu-His-Trp-Glu-Tyr-D-Ala-Orn-Nva(Nic$^+$Cl$^-$)-Pro-Gly-NH_2

a cyclic analogue of the luteinizing hormone-releasing factor carrying a charge-transfer label, is described. The linear peptide *p*-Glu-His-Trp-Glu-Tyr-D-Ala-Orn-Orn(TFA)-Pro-Gly-NH_2 was synthesized by the solid-phase method in a 32% overall yield. The side-chain cyclization was carried out in pyridine at high dilution in 65% yield by using 30 equiv of dicyclohexylcarbodiimide and 2 equiv of *N*-hydroxysuccinimide as coupling reagents. Selectivity in the side-chain deprotection of the two ornithine residues was provided by using the benzyloxycarbonyl and the trifluoroacetyl protecting groups.

Introduction

We have undertaken a systematic investigation of the conformation of the luteinizing hormone-releasing factor by using charge-transfer labels[2,3] in order to visualize side chain–side chain interactions. In an attempt to obtain quantitative intramolecular charge-transfer effects, we have prepared a nicotinamidium-labeled cyclic analogue in which the folding of the peptide backbone at the central tetrapeptide sequence is forced by a covalent bond between the side chains of residues at positions 4 and 7. In this paper, we describe the synthesis of [*cyclo*(Glu^4,D-Ala^6,Orn^7),Nva^8(Nic$^+$)]LRF·Cl$^-$. The conformational studies of this and similarly labeled LRF analogues will be reported in a subsequent paper.[4]

Results and Discussion

A combination of the solid phase and the classical peptide synthesis methodologies has been applied to prepare the desired LRF analogues. Schemes I and II outline these syntheses. Selectivity in deprotection of the δ-amino side chains of the two ornithine residues in positions 7 and 8 was provided through the use of the benzyloxycarbonyl group and the trifluoroacetyl group, the latter of which is stable to the hydrogen fluoride treatment employed to cleave the peptide from the resin. This procedure results in a linear peptide in which the ornithine side chain in position 7 is deprotected in preparation for ring closure with the γ-carboxyl side chain of the glutamic acid in position 4. Selective N^{δ}-trifluoroacetylation of orni-

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