Reaction of 2-Acetamido-3,7-dihydropyrrolo[2,3-*d*]pyrimidin-4-one With Dimethylamine and Formaldehyde. Formation of Two Isomeric Mannich Bases

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2-Acetamido-3,7-dihydropyrrolo[2,3-*d*]pyrimidin-4-one reacts with dimethylamine and formaldehyde in glacial acetic acid to afford the two isomeric Mannich products, 2-acetamido-3,7-dihydro-5-dimethylaminomethylpyrrolo[2,3-*d*]pyrimidin-4-one, and 2-acetamido-3,7-dihydro-6-dimethylaminomethylpyrrolo[2,3-*d*]-pyrimidin-4-one, in a ratio of 3:1, respectively.

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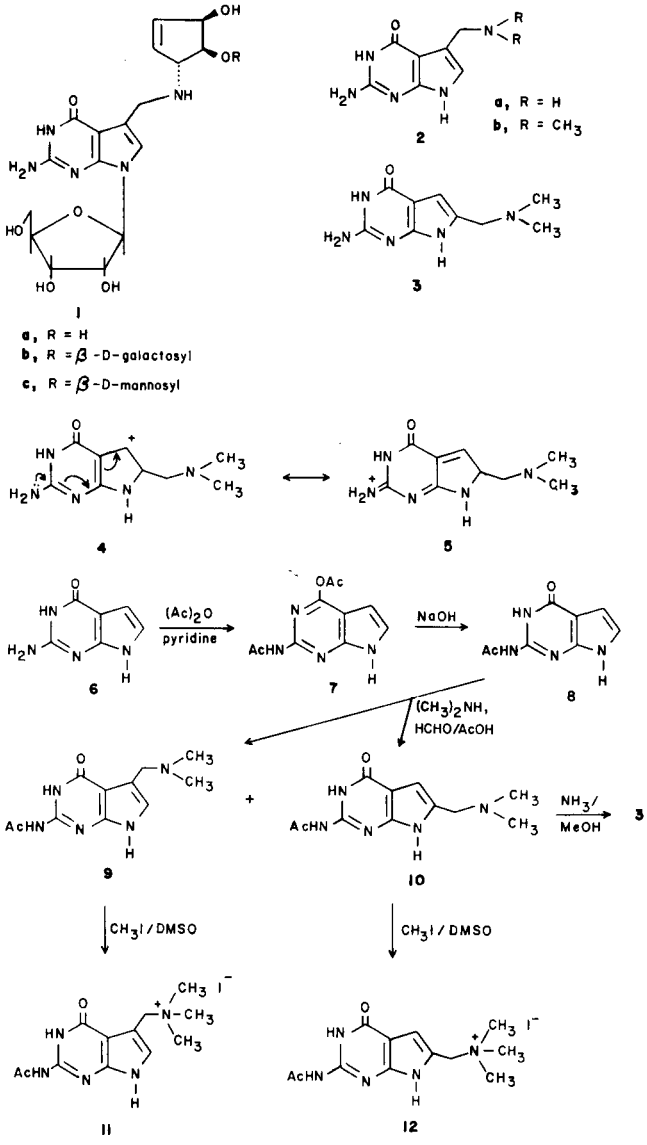
Recent interest has been focused on the pyrrolo[2,3-*d*]-pyrimidine ring system due to its presence in the structure of the hypermodified nucleosides Q (**1a**) [1] and Q* (**1b** and **1c**) [2], which have both been shown to be present in the initial position of the anticodon of $tRNA^{Tyr}$, $tRNA^{His}$, $tRNA^{Asn}$ and $tRNA^{Asp}$ from various organisms [3]. Several synthetic routes to 2-amino-5-aminomethyl-3,7-dihydropyrrolo[2,3-*d*]pyrimidin-4-one (pre Q base) (**2a**), the heterocyclic moiety in the nucleoside Q structure, have been attempted [4-6]. Of the strategies used, the only successful synthesis was that reported by Ohgi *et al.* [6], which involved a lengthy thirteen step synthesis from 4-methoxy-5-methylthio-3,7-dihydropyrrolo[2,3-*d*]pyrimidine. Of particular interest to us was the report by Seela and Lupke [5] of the reaction of 2-amino-3,7-dihydropyrrolo[2,3-*d*]pyrimidin-4-one with dimethylamine and formaldehyde. This reaction afforded not the expected Mannich product **2b**, but only the isomeric base **3** due to the electrophilic attack of the intermediate iminium ion $CH_2=^+N(CH_3)_2$ at C_6 rather than C_5, which is the usual position of attack in the formation of azaindolic Mannich bases of this type [7,8]. The formation of **3** has been attributed to the greater stability of the intermediate indolic carbocation resulting from attack at C_6, as a consequence of the electron releasing properties of the 2-amino group (see cannonical forms **4** and **5**). Only one other anomalous Mannich product has been reported, with the theophylline analog 1,3-dimethyl-3,7-dihydropyrrolo[2,3-*d*]pyrimidine-2,4-dione, which undergoes a Mannich reaction to afford exclusively the 6-substituted product [9].

Because of the facile nature of the above reaction, the facility for varying the secondary amine reactant, and the possibility that acylation of the 2-amino group of **6** may affect the position of attack of the electrophilic iminium species at the indole nucleus, we decided to investigate the reaction of 2-acetamido-3,7-dihydropyrrolo[2,3-*d*]pyrimidin-4-one with formaldehyde and dimethylamine.

Acetylation of **6** with pyridine and acetic anhydride, using a modification of a procedure described by Townsend [10], afforded the diacetyl derivative, **7** in 70% yield. Deacetylation of **7** was smoothly accomplished in 0.25 *N* sodium hydroxide, the monoacetyl derivative, **8**, being precipitated from solution in 87% yield. This method represents an improved procedure for the selective mono-



deacetylation of **7**. A glacial acetic acid solution of **8** was reacted with formaldehyde and dimethylamine at 70° for 5 hours in a stoppered flask. Evaporation of the solvent under reduced pressure, afforded a gummy residue whose ^{1}H-nmr spectrum showed two singlets at δ 6.63 and δ 6.10 in a ratio of 2:1 respectively which, upon thin layer chromatographic analysis (silica gel-methanol) afforded two spots of R_f 0.55 and 0.22. Column chromatography of the reaction product over silica gel using methanol as eluant afforded an initial band which, upon evaporation of solvent, gave a white solid, showing a single spot at R_f 0.22 by thin layer chromatographic analysis (silica gel-methanol) and singlets, at δ 6.10 (1H), δ 3.32 (2H) and δ 2.10 (9H) in its ^{1}H-nmr spectrum. Later methanol fractions gave a second band showing a single spot by tlc at R_f 0.55 (silica gel-methanol), which on pooling and evaporation of solvent afforded a white solid showing singlets at δ 6.63 (1H), δ 3.53 (2H) and δ 2.10 (9H) in its ^{1}H-nmr spectrum. From the ^{1}H-nmr data, the product with R_f 0.55 was tentatively assigned as **9**. The singlet at δ 6.63 was assigned to the C_6 proton, which resonates, as would be expected, downfield from the singlet for the C_5 proton in the isomeric structure **10**, this latter structure being assigned to the product with R_f 0.22. These assignments were confirmed by deacetylation of **10**, in methanolic ammonia solution, to the previously reported **3**. To our knowledge, this is the first reported observation of two isomeric Mannich base products being isolated from the same reaction mixture and suggests that electronic factors may play an important role in deciding the position of attack at the azaindole nucleus by the electrophilic iminium intermediate.

Both **9** and **10** could be converted into their corresponding trimethylammonium iodide salts **11** and **12** respectively, by reaction with methyl iodide in dimethylsulfoxide. In both cases, no indication of ring *N*-methylation was observed during the course of the reactions. Since it has been shown that facile displacement of the trimethylammonium group in compounds of this type can be carried out with a variety of nucleophiles [11-14] compound **11** should prove to be a useful intermediate for the synthesis of pre Q base analogs.

EXPERIMENTAL

The ^{1}H-nmr spectra were recorded on a Varian spectrometer using tetramethylsilane as internal reference. All melting points are uncorrected and were taken on a Reichert hot-stage microscope. Evaporations were carried out under reduced pressure on a rotary evaporator. Yields of solids refer to products obtained prior to recrystallization, unless otherwise stated, and are not maximized. Thin layer chromatographic analyses were carried out on Eastman Chromagram silica gel sheets containing fluorescent indicator and spots were visualized under ultra violet light at 254 nm.

2-Acetamido-4-acetoxy-3,7-dihydropyrrolo[2,3-*d*]pyrimidine (**7**).

A suspension of 2-amino-3,7-dihydropyrrolo[2,3-*d*]pyrimidin-4-one [15] (2.0 g, 13.3 mmoles) in pyridine (45 ml) and acetic anhydride (40 ml) was heated at reflux temperature for 5 hours. Water (80 ml) was then added to the cooled, dark solution which was allowed to stand at 4° for 1 hour. The resulting precipitate was filtered off, washed with chilled distilled water and then acetone, to afford 2.2 g (71%) of **7** as a light tan solid; mp 307-308° (from methanol); ^{1}H-nmr (perdeuterioacetic acid): δ 7.47 (d, 1H, H-6, J = 4 Hz), 6.63 (d, 1H, H-5, J = 4 Hz), 2.82 (s, 3H, $OCOCH_3$), 2.37 (s, 3H, $NCOCH_3$).

Anal. Calcd. for $C_{10}H_{10}N_4O_3$ (234.2): C, 51.38; H, 4.30; N, 23.92. Found: C, 51.14; H, 4.41; N, 24.17.

2-Acetamido-3,7-dihydropyrrolo[2,3-*d*]pyrimidin-4-one (**8**).

A mixture of **7** (1.0 g, 4.27 mmoles) in 0.25 *N* aqueous sodium hydroxide (18 ml, 4.5 mmoles) was stirred at room temperature for 0.5 hours. The resulting precipitate was filtered off, washed with chilled distilled water and then acetone to afford 0.65 g (79%) of **8** as a white solid; mp 342-343° dec (from methanol); ^{1}H-nmr (DMSO-d_6): δ 11.36 (bs, 3H, labile H's, deuterium oxide replaceable), 6.81 (d, 1H, H-6, J = 4 Hz), 6.30 (d, 1H, H-5, J = 4 Hz); 2.13 (s, 3H, CH_3).

Anal. Calcd. for $C_8H_8N_4O_2$ (192.1): C, 50.00; H, 4.19; N, 29.15. Found: C, 50.29; H, 4.31; N, 29.36.

Reaction of **8** with Dimethylamine and Formaldehyde. Formation of **9** and **10**.

Glacial acetic acid (6 ml) was added slowly to a 40% w/v aqueous solution of dimethylamine (3 ml, 26.7 mmoles) cooled in an ice-bath, at such a rate that the temperature did not rise above 5°. Aqueous formaldehyde (40%, w/v, 2.2 ml, 29.3 mmoles) was then added, followed by **8** (1.5 g, 7.8 mmoles) and the mixture heated at 70° in a stoppered flask for 5 hours. The resulting yellow solution was evaporated under reduced pressure to a gummy residue. Water (50 ml) was added to the residue and mixture evaporated to dryness. This procedure was repeated three more times, and then three times using methanol (50 ml) in place of water. Examination of the residue by thin layer chromatography on silica gel sheets using methanol as developing solvent showed two uv detectable spots at R_f 0.22 and 0.55. The ^{1}H-nmr examination of this residue showed two signals in the aromatic region resonating at δ 6.63 and δ 6.10, with integral ratio 3:1, respectively. The residue was dissolved in methanol (5 ml) and applied to the top of a silica gel-methanol column (Fisons, 35-65 mesh, 4 × 7 cm) and the column eluted with methanol. An initial band was collected which gave a single spot of R_f 0.22 by tlc on silica sheets. Evaporation of solvent afforded 0.35 g (18%) of **10** as a white powder; mp 248-251° dec (from methanol-diethyl ether); ^{1}H-nmr (DMSO-d_6): δ 11.33 (bs, 3H, CH_3CONH, OH and NH, deuterium oxide replaceable), 6.10 (s, 1H, H-5), 3.32 (s, 2H, CH_2-N), 2.10 (s, 9H, $N(CH_3)_2$ and CH_3CO).

Anal. Calcd. for $C_{11}H_{15}N_5O_2$ (249.3): C, 53.00; H, 6.07; N, 28.09. Found: C, 53.39; H, 6.24; N, 27.88.

Continued elution with methanol afforded a second band which gave a single spot at R_f 0.55 by tlc on silica sheets. Evaporation of solvent yield 0.72 g (37%) of **9** as a tan solid; mp 218-221° dec (from methanol-diethyl ether); ^{1}H-nmr (DMSO-d_6): δ 10.85 (bs, 3H, CH_3CONH, OH, NH, deuterium oxide replaceable), 6.63 (s, 1H, H-6), 3.53 (s, 2H, CH_2-N), 2.10 [s, 9H, $N(CH_3)_2$ and CH_3CO].

Anal. Calcd. for $C_{11}H_{15}N_5O_2$ (249.3): C, 53.00; H, 6.07; N, 28.09. Found: C, 53.31; H, 6.14; N, 27.92.

2-Amino-3,7-dihydro-6-dimethylaminomethylpyrrolo[2,3-*d*]pyrimidin-4-one (**3**).

A solution of **10** (0.1 g, 0.4 mmole) dissolved in methanol saturated with ammonia gas (20 ml) was stirred at ambient temperature for 16 hours. The mixture was concentrated to low volume on a rotary evaporator, chilled and the resulting precipitated solid was filtered off and washed with distilled water to give 55 mg (66%) of **3** as a white solid, which had identical melting point and spectral properties of those of an authentic sample prepared from **4** by the method of Seela and Lupke [5].

2-Acetamido-3,7-dihydro-5-trimethylammoniomethylpyrrolo[2,3-*d*]pyrimidin-4-one Iodide (**11**).

Methyl iodide (0.04 ml, 0.66 mmole) was added to a solution of **9** (0.15 g, 6.0 mmole) in DMSO (2 ml). The solution was stoppered and stirred at room temperature for 0.5 hours, and then poured into distilled water (30 ml). The aqueous solution was washed with chloroform (5 × 40 ml) and then lyophilized to give 0.16 g (68%) of **11** as a hygroscopic off-white powder; mp 180-183°; ^{1}H-nmr (DMSO-d_6): δ 11.60 (bs, 3H, labile H's, deuterium oxide replaceable), 7.28 (s, 1H, H-6), 4.58 (s, 2H, CH_2-N^+), 3.06 [s, 9H, $^+N(CH_3)_3$], 2.15 (s, 3H, CH_3CO).

Anal. Calcd. for $C_{12}H_{18}IN_5O_2 \cdot 2H_2O$ (427.2): C, 33.74; H, 5.19; N, 16.39. Found: C, 33.41; H, 5.2; N, 16.52.

2-Acetamido-3,7-dihydro-6-trimethylammoniomethylpyrrolo[2,3-*d*]pyrimidin-4-one Iodide (**12**).

A solution of **10** (0.1 g, 0.4 mmole) in DMSO (2 ml) was treated with methyl iodide (0.027 ml, 0.44 mmole) in the same manner as described for the preparation of **11**. Work up of the reaction mixture as before afforded 90 mg (58%) of **12** as a hygroscopic, tan powder; mp 205-207°; ^{1}H-nmr (DMSO-d_6): δ 11.73 (bs, 3H, labile H's, deuterium oxide replaceable), 6.70 (s, 1H, H-5), 4.54 (s, 2H, CH_2-N^+), 3.06 [s, 9H, $^+N(CH_3)_3$], 2.15 (s, 3H, CH_3CO).

Anal. Calcd. for $C_{12}H_{18}N_5O_2I \cdot \frac{1}{2}H_2O$ (418.3): C, 34.45; H, 5.08; N, 16.74. Found: C, 34.81; H, 5.20; N, 17.01.

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