SYNTHESIS OF 3-AMINO-2-CARBAMIMIDOYLPYRAZOLE C-NUCLEOSIDES AND ITS CYCLIZATION TO 4-AMINOPYRAZOLO[1,5-a]-1,3,5-TRIAZINE C-NUCLEOSIDES

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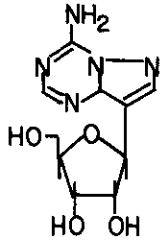
Abstract - 4-Amino-8-(β-D-2,3-O-isopropylidene-5-O-trityl-ribofuranosyl)pyrazolo[1,5-a]-1,3,5-triazine and its α-isomer were synthesized via 3-amino-2N-carbamimidoyl-4-(α and β-D-2',3'-O-isopropylidene-5'-O-tritylribofuranosyl)pyrazoles.

Success in C-nucleoside synthesis heavily depends on the availability of the appropriate preformed carbohydrate intermediates. In this matter we have been fortunate to develop two extremely versatile intermediates for a general synthesis of C-nucleosides.[1-5] From these intermediates we have synthesized pyrimidine,[1-3] 3-aminopyrazole,[4] and 3-aminopyrazole-2-carboxamide[5] C-nucleosides. One of the intermediates, 2-formyl-2-(2,3-O-isopropylidene-5-O-trityl-D-ribofuranosyl)acetonitrile[3,5] (2) was also found to be a very useful intermediate for the preparation of pyrrolo[3,2-d]pyrimidine[6,7] and thieno[3,2-d]pyrimidine[8] C-nucleosides. This report deals with a new synthesis of 4-amino-8-(β-D-ribofuranosyl)pyrazolo[1,5-a]-1,3,5-triazine (1),[9] which was shown to have good antileukemic activity in preliminary cell culture study.[10] Intermediate 2 was condensed with an excess of aminoguanidine nitrate in 2 N nitric acid to give an equal mixture of 4 and 5 via aminosemicarbazone derivative 3, which could not be isolated under the condition. It should be noted that there was no deblocking of the



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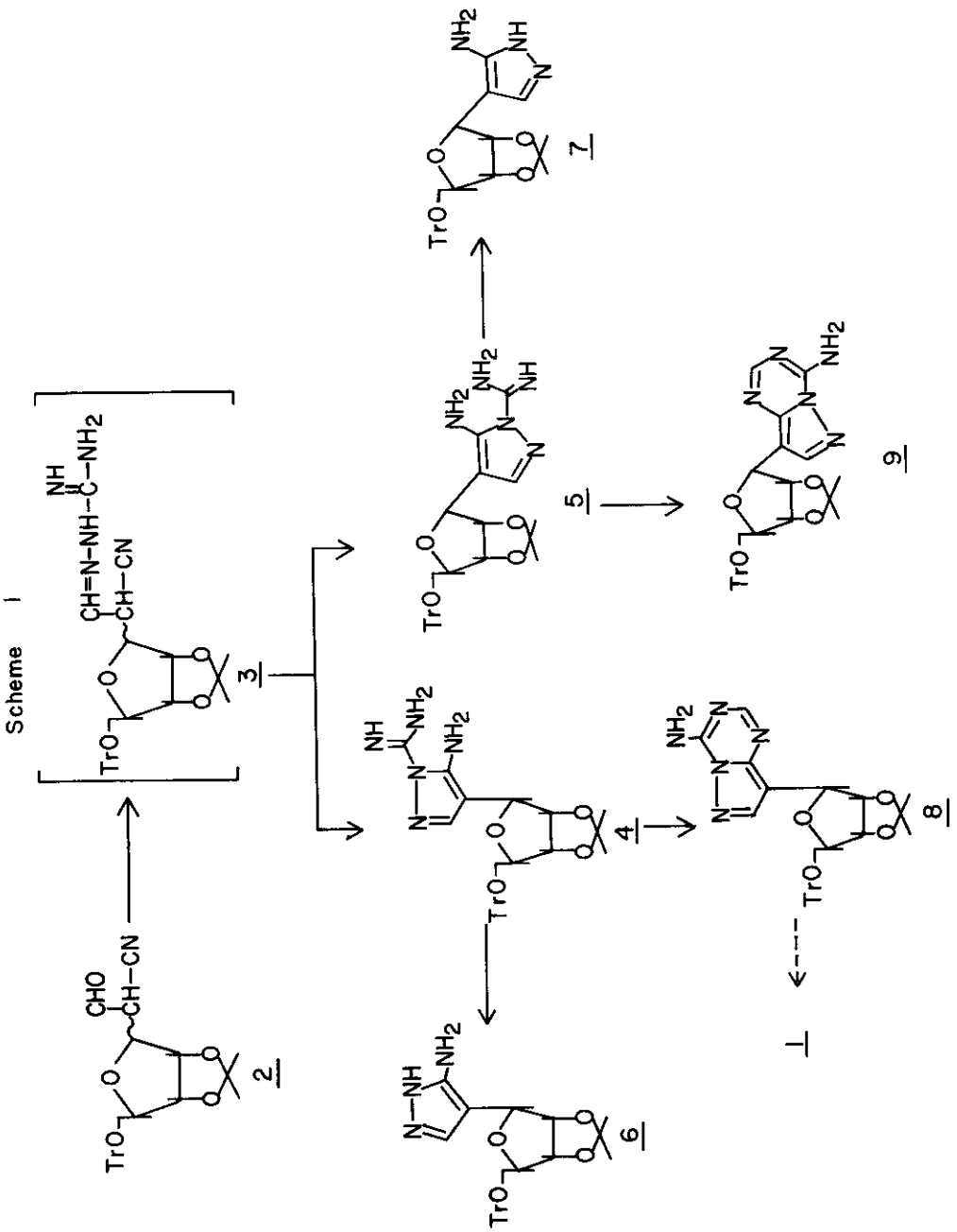
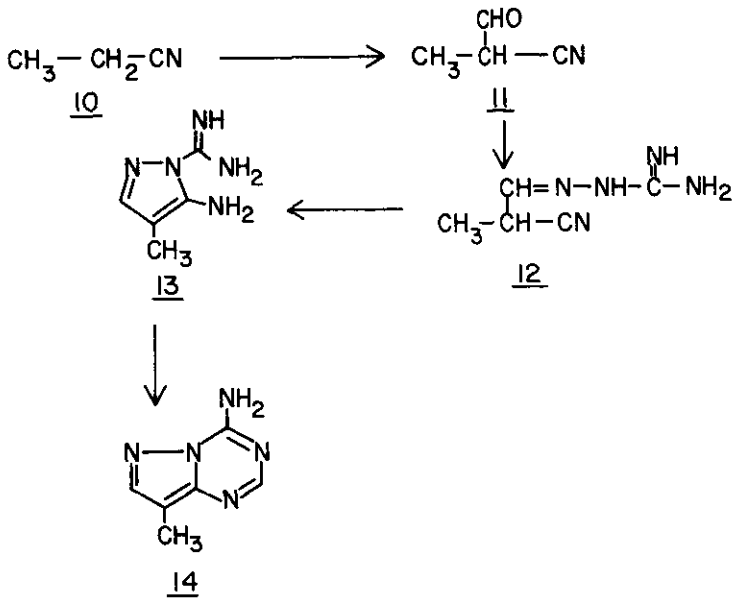

Scheme I
2
3
4
5
6
7
8
9
1
TrO
CHO
CH=N-NH-C-NH2
NH
CH-CN
NH2
NH
N

Scheme 2



ribose protecting groups during the reaction. The anomeric mixture (4 and 5) was separated on a silica gel column using ethyl acetate-petroleum ether (4:1).

The identification of 4 and 5 has been made on the basis of ^{1}H nmr as well as uv spectra. The chemical shift of anomeric proton of isomer 4 appeared at higher field (δ 4.76; H-1' is overlapping with H-2' & H-3') than that of β isomer 5 (δ 5.08). This method has been successfully utilized in the assignment of anomeric configuration of C-nucleosides[1-5] as well as that of N-nucleosides.[11] Imbach's rule[12] does not provide an unambiguous assignment as the differences of chemical shifts ($\Delta\delta$) between the methyl groups in isopropylidine moieties are 0.21 and 0.22 ppm for the α and β anomer, respectively. Additional confirmation of the configuration was based on the conversion of 4 and 5 to 6 and 7, for which their configurations have been established previously.[4]

In order to confirm the presence of a heterocyclic moiety in 4 and 5, a model compound 13 was prepared by a procedure similar to that reported previously.[5] However, uv absorption spectra of the 3-amino-2-carbamimidoyl moiety of 4 and 5 overlap with that of the trityl group. Thus a direct comparison of uv spectra between the C-nucleosides (3 and 4) and the heterocycle 13 was

not feasible. Because of this interference, identification of the aglycons of 4 and 5 was made indirectly. The final cyclization to 8 and 9 was accomplished in good yield by the treatment of 4 and 5 with triethyl orthoformate. No anomerization was observed during the reaction. This also gave the final structural proof for 4 and 5. Deblocking of the ribose protecting groups to give 1 and its isomer has been previously reported.[9] The structural identification of 13 was made on the basis of its cyclization to 14 with triethyl orthoformate, which exhibited similar uv spectral patterns to those of 8 and 9 (or 1) at various pH's.

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 FX 90Q Fourier transform spectrometer (90 MHz). Tetramethylsilane was the internal standard for organic solvents and sodium 3-trimethylsilyl)-1-propane-1-sulfonate (DSS) was the internal standard for D_2O; 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 & 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 analyses were performed by Atlantic Microlab, Inc., Atlanta, GA.

3-Amino-2-carbamimidoyl-4-(α and β-D-2,3-O-isopropylidene-5-O-tritylribofuranosyl)pyrazole (4 and 5)

Aminoguanidine nitrate (2.74 g, 0.02 mol) in 2 N nitric acid (12 ml) was added to an ethanolic solution (25 ml) of 2 (4.97 g, 0.01 mol) and the mixture was stirred at room temperature for 20 min. After neutralization, ethanol was evaporated in vacuo at low temperature(30°C). Water (30 ml) was added and the resulting precipitates were collected by filtration and air-dried overnight to give a solid (4.5 g, 84%). TLC (chloroform-methanol = 20:1) indicated two major spots (Rf = 0.45 and 0.50) which were separated on a silica gel column using a mixture of ethyl acetate-petroleum ether (4:1) to give the fast moving β-isomer 4 (1.11 g); ^{1}H nmr ($CDCl_3$): δ 1.35 and 1.57 (s, 3, isopropylidene methyl), 3.35 - 3.45 (m, 2, H-5' and H-5''), 4.12 - 4.18 (m, 1, H-4'), 4.70 - 4.76 (m, 3, H-1', 2' and 3'), 7.25 - 7.38 (m, 16, trityl and H-5).

Anal. Calcd. for $C_{31}H_{33}N_5O_4$: C, 69.02; H, 6.12; N, 12.99. Found: C, 68.84; H, 6.34; N, 12.64.

From the same column the slow moving α-isomer 5 (1.25 g) was obtained; ^{1}H nmr ($CDCl_3$): δ 1.33 and 1.54 (s, 1, isopropylidene methyl), 3.26-3.33 (m, 2, H-5' and H-5"), 4.27 (m, 1, H-4'), 4.79 (m, 2, H-2' and 3'), 5.08 (d, 1, H-1', $J_{1',2'}$ = 2.0 Hz), 7.26 - 7.68 (m, 16, trityl and H-5). Anal. Calcd. for $C_{31}H_{33}N_5O_4$: C, 69.02; H, 6.12; N, 12.99. Found: C, 69.28; H, 6.34; N, 12.62.

4-Amino-8-(β-D-2,3-O-isopropylidene-5-O-tritylribofuranosyl)pyrazolo[1,5-a]-1,3,5-triazine (8)

A mixture of 4 (539 mg, 0.001 mole) and triethyl orthoformate (10 ml) was heated at 95-100°C for 2.5 h. TLC indicated that all the starting material was converted to 8. Excess triethyl orthoformate was evaporated in vacuo to yield a syrup, which was purified on a silica gel column using chloroform-methanol (30:1) to give 8 (465 mg, 85%). ^{1}H nmr data, in comparison to those reported[9] values, confirmed the compound.

4-Amino-8-(α-D-2,3-O-isopropylidene-5-O-tritylribofuranosyl)pyrazolo[1,5-a]-1,3,5-triazine (9)

A mixture of 5 (539 mg, 0.001 mol) and triethyl orthoformate (10 ml) was heated at 95-100°C for 2 h. After heating, excess reagent was evaporated in vacuo to yield a syrup, which was chromatographed on a short silica gel column using chloroform-methanol (30:1) as eluent to obtain 9 (489 mg, 80%). ^{1}H nmr data confirmed the structure.[9]

Conversion of 3-Amino-2-carbamimidoyl C-Nucleosides (4 and 5) to 3-Aminopyrazole C-Nucleosides (6 and 7)

A mixture of 4 (60 mg) and 2 N aqueous NaOH (2 drops) in methanol (0.5 ml) was refluxed for 1 min. After neutralization with acetic acid, the product was isolated on a preparative TLC plate using a mixture of chloroform-methanol (10:1) as an eluent to obtain 6 (27 mg), which was compared with the authentic β-isomer of 3-aminopyrazole C-nucleosides. Thus, ^{1}H nmr and the TLC data indicated that the compound 6 was identical to an authentic sample prepared by the previously reported method.[4] A similar experiment with 5 also gave 7 as a sole product. No anomerization was observed during the reaction.

3-Amino-2-carbamimidoyl-4-methylpyrazole (13)

To a suspension of sodium hydride (8.0 g, 0.17 mol, 50% in oil) in dry ether (350 ml) was added dropwise a mixture of propionitrile (9.0 g, 0.16 mole), ethyl formate (20 ml), ether (50 ml), and absolute ethanol (3 ml). After addition, the mixture was stirred overnight. Excess ethyl for-

mate and ether were evaporated at low temperature (< 30°C). To the remaining grey solid, an ice-water was cautiously added, the solution was neutralized with acetic acid, and then aminoguanidine nitrate (13.7 g, 0.1 mol) was added. The reaction mixture was stirred at room temperature for 15 h and then the pH of the solution was adjusted to 9-10 with 50% aqueous NaOH. The solution was then immediately readjusted to pH 7 with acetic acid, during which a solid precipitated. Crystallization from methanol, decolorized with charcoal at room temperature (30°C), afforded white crystals (1.1 g), mp 179-180°C; uv: λ max 261 nm (pH 1-7), 262 (pH 12); ^{1}H nmr (DMSO-d_6): δ 1.89 (s, 1, CH_3), 7.6 (s, 1, H-5), 8.5 (b, 5, exchangeable with D_2O).

Anal. Calcd. for $C_5H_9N_5$: C, 43.17; H, 6.47; N, 50.36. Found: C, 43.25; H, 6.44; N, 50.55.

4-Amino-8-methylpyrazolo[1,5-a]-1,3,5-triazine (14)

A mixture of 13 (350 mg, 2.5 mmol) and triethyl orthoformate (5 ml) was refluxed for 1.5 h and cooled to room temperature. The resulting precipitates were collected and recrystallized from water to give white crystals, mp 239-240°C; uv: λ max 276 nm (pH 7-12), 257 (pH 1), shoulder 292; ^{1}H nmr (DMSO-d_6): δ 2.16 (s, 1, CH_3), 7.99 (s, 2, H-2 and H-7), 8.36 (b, 1, NH_2).

Anal. Calcd. for $C_6H_7N_5$: C, 48.32; H, 4.70; N, 46.98. Found: C, 48.13; H, 4.72; N, 46.73.

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