Presence of an *N*-6 Acetate Group Shifts the Alkylation Site of the Ambident Nucleophile Sodium 1-*N*-Methylisoguanide

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Inclusion of an *N*-6 acetate into the ambident nucleophile, sodium 1-*N*-methylisoguanide, resulted in a shift in alkylation preference from *N*-9 to *N*-3. The preparation of the *N*-6 acetate of 1-*N*-methylisoguanine, 9-*N*-acetyl-1-*N*-methylisoguanine (**4**), and related acetate analogs are described and evidence presented for their structural determination. Analysis of long range ^{13}C-^{1}H coupling data facilitated the structural elucidation of the predominant alkylation products and provided evidence for the unusual *N*-7 hydrogen tautomeric form in one of them, 3-*N*-benzyl-6-*N*-acetyl-1-*N*-methylisoguanine (**8**).

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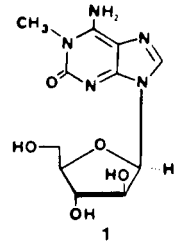
During the synthesis of a new arabinosyl nucleoside, ara-1-*N*-methylisoguanine or "ara-Doridosine (ara-D)" (**1**) [1,2], the preparation of a suitably protected form of 1-*N*-methylisoguanine for eventual coupling with 2,3,5-tri-*O*-benzyl-D-arabinofuranosyl chloride was deemed to be a major goal. The N-6 acetate derivative of 1-*N*-methylisoguanine was chosen as a likely candidate. Acetylation of 1-*N*-methylisoguanine with acetic anhydride in refluxing tetrahydrofuran led to crystalline diacetate **2** [1], and with acetic anhydride under reflux led to crystalline triacetate **3**. Methanolysis of **2** led to "monoacetate A" and stepwise methanolysis of **3** led to both a diacetate and a different monoacetate, "monoacetate B."

Determination of the position of the acetate groups on this framework so dominated by heteroatoms proved to be a relatively challenging problem. The acetates might conceivably be positioned on any one or more of the N-6, N-9, N-7, N-3 or O-2 heteroatoms of 1-*N*-methylisoguanine. A comparison of the cmr spectra of the monoacetates (Figure 1) indicated that with the exception of C-8, all of the resonances of monoacetate B were displaced to a significant degree downfield from those of monoacetate A. This suggested that monoacetate B might contain a tertiary amide as part of the ring system at N-9 (structure 4) or N-7 and monoacetate A a secondary amine isolated from the imidazolide ring at N-6 (structure 5). Amide resonance in structure **4** would place a positive charge on N-9 or N-7 in the ring system while in structure **5** such resonance could be offset by enolization [3,4]. Unfortunately, the C-8 cmr peak, potentially the most diagnostic in differentiating between structures **4** and **5** occurred at very similar chemical shifts in the two monoacetates A and B. However, further analysis of the long range ^{13}C-^{1}H coupling data to gain additional structural information proved rewarding. In both triacetate **3** and monoacetate B coupling between the C-8 hydrogen and an acetate carbonyl (J = 3 Hz) could be observed, demonstrating the presence of an acetate group on N-9 in both compounds as well as the identity of monoacetate B as **4**. No such coupling was observed in monoacetate A, which was therefore assigned structure **5**, the desired candidate. X-ray diffraction data on **2** and results of a mass spectral isotope fragmentation experiment on monoacetate A [1] confirmed this assignment. This left only the structure of the diacetate precursor of **4** unassigned.



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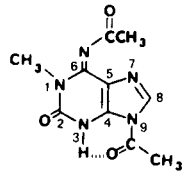

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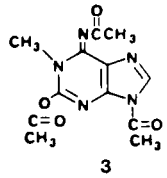

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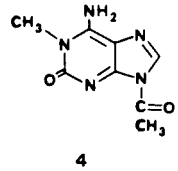

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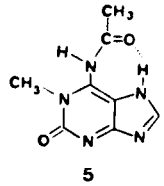

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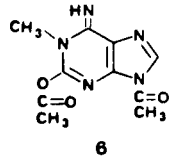

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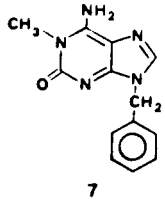

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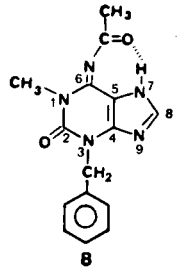

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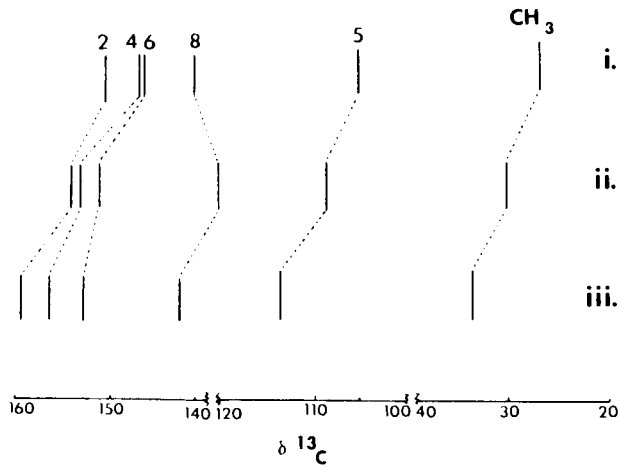

Figure 1. Comparison of ^{13}C resonances of i) 6-*N*-acetyl-1-*N*-methylisoguanine, **5** (monoacetate A), ii) 1-*N*-methylisoguanine, iii) 9-*N*-acetyl-1-*N*-methylisoguanine, **4** (monoacetate B). With the exception of the C-8 resonance, the resonances of **4** are shifted to a significant degree downfield of those of **5**.

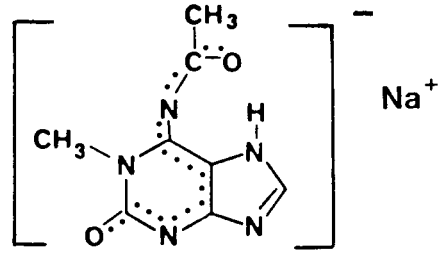

Figure 2. Resonance stability afforded to an anion of **5** centered at N-3.

The presence of an N-9 acetate in monoacetate **4** demonstrates that an N-9 acetate must also be present in its diacetate precursor. Consistent with this concept was the indication from the three-bond ^{13}C-^{1}H coupling data, between C-4 and C-5 and the C-8 hydrogen [5,6], that the imidazolide double bond resided at the N-7/C-8 location. Given the nonequivalence of this diacetate with **2**, only O-2 and N-3 remained as possible locations for the second acetate. Evidence in the ir spectrum for vinyl ester carbonyl stretching suggested **6** as the probable structure for this diacetate.

Alkylation Trials.

Recent literature reports [7-9] have described the alkylation patterns of a number of adenine salts with various alkyl chloride and ribofuranosyl chloride derivatives. We chose to determine the alkylation site preference of the sodium salts of *N*--6-acetyl-1-*N*-methylisoguanine (**5**) and 1-*N*-methylisoguanine to determine the effect of the N-6 acetyl group on this preference.

Condensation of sodium 1-*N*-methylisoguanide with benzyl chloride in dimethylsulfoxide led predominantly to a crystalline product with a parent ion of 255 in the mass spectrum, consistent with the presence of a single benzyl group. Analysis of the ^{13}C-^{1}H long range coupling data on this product facilitated the location of the benzyl group. Specifically, coupling between the benzyl methylene hydrogens and C-8 (J = 4 Hz) narrowed the possible attachment sites to N-7 and N-9. Long range coupling constants of 12 Hz between C-5 and the C-8 hydrogen and of 3 Hz between C-4 and the C-8 hydrogen pinpointed [5,6] the site of benzyl group attachment at N-9 as depicted in structure **7**.

In contrast, the analogous condensation with the sodium salt of **5** led to 3-*N*-benzyl-6-*N*-acetyl-1-*N*-methylisoguanine (**8**), trapped in the N-6 imino form, as the predominant product. A parent ion peak at 297 in the mass spectrum confirmed the presence of a single benzyl group. The absence of a fragment at m/z 240, *i.e.* loss of isocyanic acid, provided further evidence that **8** is trapped in an N-6 imino form. Long range ^{13}C-^{1}H coupling between C-2 (J = 9 Hz) and C-4 (J = 4 Hz) and the benzyl methylene hydrogens pinpointed the site of benzyl attachment at N-3. Further analysis of long range coupling between C-5 and C-4 and the C-8 hydrogen ($J_{4\text{-}8H}$ = 12 Hz) indicated [5,6] that the imidazolide double bond resided at the unusual N-9/-C-8 position, perhaps stabilized by a hydrogen bond between the N-6 acetate carbonyl and the N-7 hydrogen. A chemical shift of δ 181.0 for the N-6 acetate carbonyl resonance in the cmr spectrum of **8** is consistent with the proposed hydrogen bond. Such a N-7-hydrogen to N-6 acetate carbonyl hydrogen bond is also believed to exist in precursor **5** [1].

The observed shift in alkylation preference from N-9 to N-3 in sodium 1-*N*-methylisoguanide upon inclusion of an N-6 acetate is presumably due to the greater resonance stabilization afforded by the acetate to the N-3 anion (Figure 2).

EXPERIMENTAL

General.

Melting points were determined using a Thomas Hoover Unimelt and a Mel-Temp apparatus and are uncorrected. Proton nuclear magnetic resonance (pmr) spectra were recorded on either a Varian EM-390 or a Nicolet 200 spectrometer. Chemical shifts are reported as δ values in parts per million relative to internal tetramethylsilane. Carbon nuclear magnetic resonance (cmr) spectra were recorded on a JEOL JNM-PFT-100 spectrometer at 25 MHz. Chemical shifts are reported as δ values in parts per million relative to internal tetramethylsilane. Samples for pmr and cmr were dissolved in DMSO-d_6 unless otherwise stated. Mass spectra were obtained on a MM 70/70 HS mass spectrometer (V.G. Organic Ltd., Manchester, England). Electron Ionization (EI) spectra (70 eV) were obtained by use of standard direct introduction probe, at a source temperature of 190°. Accurate mass measurements, obtained with a VG 2035 data system, were on full scan spectra at 7000 resolution. Ultraviolet spectra were determined on a Hewlett Packard 3451A Diode Array spec-

trometer. A Beckman Model 3550 digital *p*H meter was utilized to monitor *p*H values and a Harrison Model 7924 Chromatotron instrument (Harrison Research Inc., Palo Alto, CA) with either a 1 or 2 mm silica gel circular plate was used for centrifugal circular preparative tlc. Analytical tlc was conducted on 2.5 × 10 cm precoated aluminum sheets: silica gel 60 F-254, layer thickness 0.2 mm, manufactured by E. Merck and Co., Darmstadt, Germany.

2-*O*-6,9-*N*-Triacetyl-1-*N*-methylisoguanine (**3**).

A suspension of 1-*N*-methylisoguanine (4.56 g, 27.6 mmoles) [1] in anhydrous acetic anhydride (65 ml), anhydrous tetrahydrofuran (65 ml) and anhydrous pyridine (2 ml) was refluxed for 24 hours. The brown liquid was filtered and the solvent removed *in vacuo* to give a syrup which was dissolved in tetrahydrofuran, treated with carbon black, and filtered. Removal of the solvent gave a residue which when crystallized from methylene chloride afforded crystalline **3** (6.6 g, 82%). Further recrystallization gave **3** as colorless needles, mp 134-136°; pmr (200 MHz): δ 2.24 (s, 6-N(CO)CH_3 and 9-N(CO)CH_3, 6H), 2.62 (s, 2-O(CO)CH_3, 3H), 3.39 (s, N-CH_3, 3H), 8.80 (s, H-8, 1H); pmr (deuteriochloroform, 200 MHz): δ 2.40 (s, 6-N(CO)CH_3 and 9-N(CO)CH_3, 6H), 2.99 (s, 2-O(CO)CH_3, 3H), 3.67 (s, N-CH_3, 3H), 8.72 (s, H-8, 1H); cmr: δ 20.9 (q, ^1J = 131 Hz, 2-O(CO)CH_3), 24.4 (q, ^1J = 130 Hz, 9-N(CO)CH_3), 24.9 (q, ^1J = 131 Hz, 6-N(CO)CH_3), 35.1 (q, ^1J = 142 Hz, N-CH_3), 125.1 (d, $^3J_{5\text{-}8H}$ = 13 Hz, C-5), 141.3 Hz (d, ^1J = 221 Hz, C-8), 153.1 (d, $^3J_{4\text{-}8H}$ = 4 Hz, C-4), 155.2 (C-6), 155.5 (q, ^3J = 3 Hz, C-2), 167.0 (q, ^2J = 7 Hz, 6-N(*C*O)CH$_3$), 168.0 (q, ^2J = 7 Hz, 2-O(*C*O)CH$_3$), 171.5 (dq, ^3J_d = 3 Hz, ^2J_q = 6 Hz; 9-N(*C*O)CH$_3$); ms: 291.0990 [Calcd. for $C_{12}H_{13}N_5O_4$: 291.0967] (M^+, 5), 249.0860 (M^+ -H_2C=C=O, 25), 207.0761 (249 - H_2C=C=O, 39), 165.0656 (207 - H_2C=C=O, 100), 148 (207 - CH_3N=C=O, retro Diels-Alder fragmentation, 15), 135 (10), 122 (6), 108 (165 - CH_3N=C=O, retro Diels-Alder fragmentation, 6), 43 ($CH_3C{\equiv}O^+$, 84); uv (95% ethanol): λ max nm 251 (ε 9100) and 300 (ε 8300); ir (potassium bromide): 1753 cm^{-1} (ester), 1695 cm^{-1} (amide); R_f (tetrahydrofuran, silica gel): 0.67.

Anal. Calcd. for $C_{12}H_{13}N_5O_4$: C, 49.48; H, 4.50; N, 24.04. Found: C, 49.32; H, 4.36; N, 24.31.

2-*O*-9-*N*-Diacetyl-1-*N*-methylisoguanine (**6**).

2-*O*-6,9-*N*-Triacetyl-1-*N*-methylisoguanine, **3** (2.52 g, 8.6 mmoles), was dissolved in methanol (20 ml) and chilled for 24 hours depositing crystals which were a 1:1 mixture of **3** and diacetate **6** (pmr and tlc). The mixture was separated by cctlc (ethyl acetate eluent, 2 mm plate) to yield 0.5 g of **6** (23%) as white crystalline plates, mp 131.8-133°; pmr (90 MHz): δ 2.20 (s, 9-N(CO)CH_3, 3H), 2.87 (s, 2-O(CO)CH_3, 3H), 3.40 (s, N-CH_3, 3H), 8.70 (s, H-8, 1H), 12.3 (br s, deuterium oxide exchangeable, 6=NH, 1H); cmr: δ 23.8 (q, ^1J = 129 Hz, 2-O(CO)CH_3), 24.8 (q, ^1J = 131 Hz, 9-N(CO)CH_3), 34.4 (q, ^1J = 140 Hz, N-CH_3), 122.0 (d, $^3J_{5\text{-}8H}$ = 12 Hz, C-5), 140.7 Hz (d, ^1J = 222 Hz, C-8), 154.4 (d, $^3J_{4\text{-}8H}$ = 5 Hz, C-4), 161.2 (C-6), 161.2 (C-2), 167.8 (q, ^2J = 7 Hz, 2-O(*C*O)CH$_3$), 170.6 (br m, 9-N(*C*O)CH$_3$); ms: 249.0870 [Calcd. for $C_{10}H_{11}N_5O_3$: 249.0862] (M^+, 18), 207.0764 (M^+ - H_2C=C=O, 33), 165.0651 (207 - H_2C=C=O, 100), 148 (14), 136 (13), 122 (5), 108 (165 - CH_3N=C=O, retro Diels-Alder fragmentation, 4), 43 ($CH_3N{\equiv}O^+$, 61); uv (dichloromethane): λ max nm 285 (ε 7100) and 316 (ε 2900); ir (potassium bromide): 1758 cm^{-1} (ester), 1672 cm^{-1} (tertiary amide); R_f (tetrahydrofuran, silica gel): 0.44.

Anal. Calcd. for $C_{10}H_{11}N_5O_3$: C, 48.19; H, 4.46; N, 28.10. Found: C, 48.12; H, 4.51; N, 28.26.

9-*N*-Acetyl-1-*N*-methylisoguanine (**4**).

2-*O*-9-*N*-Diacetyl-1-*N*-methylisoguanine, **6** (110 mg, 0.4 mmole), was dissolved in 20 ml of 50% aqueous methanol and chilled for 18 hours (5°), a precipitate was collected and reprecipitated from methanol to give **4** (25 mg, 30%) as a white powder, mp >280° dec; ^1H nmr (200 MHz): δ 2.20 (s, 9-N(CO)CH_3, 3H), 3.38 (s, N-CH_3, 3H), 3.40 (br s, *H*OD + -NH_2), 8.18 (s, H-8, 1H); cmr: δ 23.7 (q, ^1J = 129 Hz, 9-N(CO)CH_3), 34.4 (q, ^1J = 140 Hz, N-CH_3), 117 (br d, $^3J_{5\text{-}8H} \cong$ 11 Hz, C-5), 142.6 Hz (d, ^1J = 210 Hz, C-8), 152.9 (br s, C-6), 156.4 (d, $^3J_{4\text{-}8H}$ = 8 Hz, C-4), 159.4 (C-2), 170.8 (dq, ^2J_q = 6 Hz, ^3J_d = 3 Hz; 9-N(*C*O)CH$_3$); ms: 207.0749 [Calcd. for $C_8H_9N_5O_2$: 207.0756] (M^+, 20), 149.0533 (M^+ - CH_3, 23), 165.0651 (M^+ - $H_2C{=}C{=}O$, 100), 148.0409 (M^+ - CH_3N=C=O, retro Diels-Alder fragmentation, 20), 135.0306 (28), 108.0427 (165 - CH_3N=C=O, retro Diels-Alder fragmentation, 17), 43 ($CH_3C{\equiv}O^+$, 57); uv (95% ethanol): 246 nm (ε 10100) and 324 (ε 8100); ir (potassium bromide): 1710 cm^{-1} and 1695 nm (carbonyl); R_f (ethanol, silica gel): 0.38.

Anal. Calcd. for $C_8H_9N_5O_2$: C, 46.37; H, 4.38; N, 33.81. Found: C, 46.21; H, 4.40; N, 33.63.

Reaction of 1-*N*-Methylisoguanine With Acetic Anhydride.

A suspension of 1-*N*-methylisoguanine (500 mg, 3.0 mmoles) [1], in acetic anhydride (50 ml) was refluxed for 18 hours. Removal of the acetic anhydride at reduced pressure gave a dark syrup which was treated with decolorizing carbon in ethanol. The residue was chromatographed in portions by cctlc (100% ethanol eluent, silica gel, 2 mm plate) to give pure **4** (150 mg, 24%), **6** (50 mg, 7%) and **3** (62 mg, 7%). These were shown to be identical to authentic samples prepared *via* the alternate routes presented here (mp, pmr, cmr, uv and tlc).

9-*N*-Benzyl-1-*N*-methylisoguanine (**7**).

1-*N*-Methylisoguanine (787 mg, 4.8 mmoles) [1], was added to a solution of sodium methoxide (265 mg, 4.9 mmoles) in anhydrous methanol and refluxed for 1 hour. The solvent was removed *in vacuo* and the dry white powdery residue collected. A pmr spectrum confirmed the formation of the sodium salt, displaying singlet resonances at δ 3.36 (N-CH_3) and δ 7.42 (H-8). Benzyl chloride (1 ml, 8.8 mmoles) was added to a solution of this powdery residue in anhydrous dimethylsulfoxide (20 ml) and stirred for 18 hours. The reaction mixture was lyophilized to remove the dimethylsulfoxide and the residue crystallized from methanol to give **7** (523 mg, 43%), mp >300° dec; pmr (90 MHz): δ 3.36 (s, N-CH_3 3H), 5.11 (m, 9-NC$H_2C_6H_5$, 2H), 7.30 (br s, 9-NCH$_2C_6H_5$, 5H), 7.86 (s, H-8, 1H); cmr: δ 30.2 (q, ^1J = 140 Hz, N-CH_3), 45.2 (br t, ^1J = 140 Hz, 9-N$CH_2C_6H_5$), 108.3 (d, $^3J_{5\text{-}8H}$ = 12 Hz, C-5), 127.5 (br d, ^1J = 160 Hz, C-3' and C-4'), 128.6 (br d, ^1J = 161 Hz, C-2'), 137.4 (br s, C-1'), 139.0 (dt, ^1J_d = 212 Hz, ^3J_t = 4 Hz, C-8), 151.4 (d, ^3J = 3 Hz, C-4), 152.6 (C-6), 154.5 (C-2); ms: 255.1121 [Calcd. for $C_{13}H_{13}N_5O$: 255.1119] (M^+, 21), 239.0917 (M^+ - NH_2, 21), 164.0573 (M^+ - $CH_2C_6H_5$, 8), 107.0476 (6), 91.0556 ($CH_2C_6H_5^+$, 100); uv (95% ethanol): λ max nm 233 (ε 4800) and 297 (ε 12200); ir (potassium bromide): 1680 cm^{-1} (carbonyl); R_f (methanol/1-butanol/water [10/1/2], silica gel): 0.64.

Anal. Calcd. for $C_{13}H_{13}N_5O$: C, 61.17; H, 5.13; N, 27.43. Found: C, 61.23; H, 5.17; N, 27.25.

3-*N*-Benzyl-6-*N*-acetyl-1-*N*-methylisoguanine (**8**).

6-*N*-Acetyl-1-*N*-methylisoguanine, **7** (250 mg, 1.2 mmoles), was dissolved in a solution of sodium methoxide (66 mg, 1.2 mmoles) in anhydrous methanol (100 ml) and stirred for 1 hour. The solvent was removed *in vacuo* and the powdery residue dried. A pmr spectrum of its nucleoside salt revealed singlet peaks at δ 3.26 (N-CH_3) and 7.41 (H-8). The residue was dissolved in 30 ml of anhydrous dimethylsulfoxide to which benzyl chloride (140 μl, 1.2 mmoles) and 4 Å molecular sieves (100 mg) were added. After 2 hours the suspension was filtered and the filtrate lyophilized to a white powder which was crystallized from anhydrous methanol to yield **8** (110 mg, 30%), mp 250° dec; pmr (90 MHz): δ 2.21 (s, 6-NH(CO)CH_3, 3H), 3.38 (s, 3H, N-CH_3), 5.20 (m, 3-NC$H_2C_6H_5$, 2H), 7.29 (m, 3-NCH$_2C_6H_5$, 5H), 7.96 (s, H-8, 1H); cmr: δ 27.3 (q, ^1J = 127 Hz, 6-NH(CO)CH_3), 30.8 (q, ^1J = 142 Hz, N-CH_3), 46.3 (t, ^1J = 142 Hz, N-CH_3), 46.3 (t, ^1J = 142 Hz, 3-N$CH_2C_6H_5$), 104.5 (d, $^3J_{5\text{-}8H}$ = 6 Hz, C-5), 141.2 (dt, ^1J_d = 214 Hz, $^2J_t \cong$ 13 Hz, C-2'), 128.3 (d, ^1J = 160 Hz, C-3'), 139.0 (m, C-1'), 146.5 (br s, C-6), 147.4 (dt, $^3J_{4\text{-}8H}$ = 12 Hz, ^3J_t = 4 Hz, C-4), 151.4 (t, ^3J = 9 Hz, C-2), 181.0 (q, ^2J = 6 Hz, 6-NH(*C*O)CH$_3$); ms: 297.1230 [Calcd. for $C_{15}H_{15}N_5O_2$: 297.1224] (M^+, 6), 282.0994 (M^+ -CH_3, 33), 255.1092 (M^+-$H_2C{=}C{=}O$, 6), 239.0930 (20), 91.0541 ($CH_2C_6H_5^+$, 100), 43 ($CH_3C{\equiv}O^+$, 35); uv (95% ethanol): λ max nm 252 (ε 6600) and 302 (ε 11800); ir (potassium bromide): 1705 cm^{-1} and 1665 cm^{-1} (carbonyl); R_f (tetrahydrofuran, silica gel): 0.58.

Anal. Calcd. for $C_{15}H_{15}N_5O_2$: C, 60.60; H, 5.09; N, 23.55. Found: C, 60.74; H, 4.91; N, 23.29.

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