Synthesis of L-(5-Chloro-2-pyridyl)glycine[1a]

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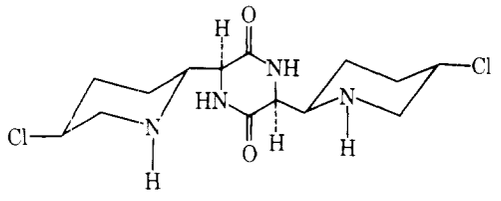
A practical synthesis of the new amino acid L-(5-chloro-2-pyridyl)glycine (**2**) was developed (Scheme I). The reaction sequence was initiated by condensing 2-chloro-5-nitropyridine with diethyl acetamidomalonate to afford diethyl (5-nitro-2-pyridyl)acetamidomalonate (**5**). Reduction of the 5-nitro group and application of the isoamyl nitrite–carbon tetrachloride reagent provided diethyl (5-chloro-2-pyridyl)acetamidomalonate (**8**). Selective base hydrolysis followed by enzymatic hydrolysis furnished amino acid **2**.

Recently, we reported a structural determination of the antitumor antibiotic known as 593A (**1**)[2] from *Streptomyces*



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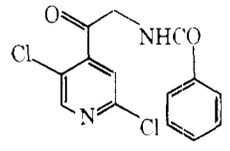

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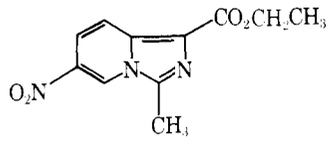

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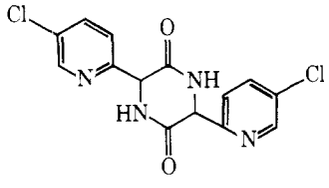

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griseoluteus. Because of considerable interest in 593A as a cancer chemotherapeutic drug,[2,3] we began to investigate possible synthetic approaches. The first objective was to develop a practical synthesis of the hitherto unknown amino acid L-(5-chloro-2-pyridyl)glycine (**2**, Scheme I).[4] For this purpose we evaluated two readily available 2-chloropyridines as potential starting material for condensation with diethyl acetamidomalonate to afford a 2-substituted pyridine system.[5] Suitable degradation of the diethyl acetamidomalonate moiety would then lead to amino acid **2**.[6]

The most expeditious route to amino acid **2** appeared to be via 2,5-dichloropyridine; however, the 2-chloro group could not be displaced by diethyl acetamidomalonate carbanion. In another attempt, a stronger nucleophile was generated from methyl hippurate using lithium diisopropylamine (LDA).[7] Upon reaction of the hippurate nucleophile with 2,5-dichloropyridine a number of products were obtained. The 4-substituted pyridine **3** was isolated in highest yield. Apparently the C-4 proton of 2,5-dichloropyridine was removed by the strong base and underwent Claisen condensation with the ester group of methyl hippurate.[8]

At this point the more reactive 2-chloro-5-nitropyridine was selected for further study. The condensation of diethyl acetamidomalonate carbanion with 2-chloro-5-nitropyridine proceeded smoothly in dimethylformamide, furnishing pyridine **4**. When 1,2-dimethoxyethane was used as solvent, formation of 1-(ethoxycarbonyl)-3-methyl-6-nitroimidazo[1,5-*a*]pyridine (**5**) became significant. In an effort to shorten the number of steps to amino acid **2**, the anion of benzylideneglycine ethyl ester was allowed to react with 2-chloro-5-nitropyridine.[9] However, the product was imidazole **6**.

Conversion of nitropyridine **4** to chloropyridine **8** was accomplished by way of diethyl (5-amino-2-pyridyl)acetamidomalonate (**7**). Reduction of 5-nitropyridine **4** to 5-aminopyridine **7** was achieved by catalytic hydrogenation using platinum on carbon. Synthesis of 5-chloropyridine **8** proved to be more difficult. Application of the classic Sandmeyer reaction[10] resulted in extensive decomposition. Thus, several nonacidic methods were evaluated. Reaction of aminopyridine **7** with cupric chloride nitrosyl (generated from cupric chloride and nitric oxide)[11] gave an unsatisfactory mixture. Although one product appeared to be the 5-chloropyridine **8**, the yield was quite poor. Next, isoamyl nitrite was allowed to react with aminopyridine **7** in the presence of carbon tetrachloride.[12] The result was a 42% yield of 5-chloropyridine **8**.

Attempted generation of DL-amino acid **2** from 5-chloropyridine **8** by acid hydrolysis gave 2-(aminomethyl)-5-chloropyridine (**10**). Variation of reaction conditions failed to eliminate the decarboxylation. Mild base hydrolysis[13] of 5-chloropyridine **8** followed by careful acidification of the reaction mixture at 5 °C provided DL-α-(acetylamino)-5-chloro-2-pyridineacetic acid (**9**). The aceturic acid **9** proved to be somewhat unstable and was characterized as the methyl ester **11**.[14] Acid hydrolysis of aceturic acid **9** led to (aminomethyl)pyridine **10**. The required L-amino acid **2** was readily prepared from aceturic acid **9** using hog renal acylase I.[15]

With the synthesis of L-amino acid **2** successfully completed, attention was focused on the feasibility of converting L-amino acid **2** to piperazinedione **13**. Because of the usual ready dimerization of amino acid esters to piperazinediones, initial experiments were directed at preparing an ester derivative of L-amino acid **2**. Esterification procedures attempted included thionyl chloride–methanol,[16] *p*-toluenesulfonic acid-methyl acetate,[17] perchloric acid–methyl acetate[18] (instantaneous decarboxylation occurred in this case), boron trifluoride–methanol,[19] diazomethane,[20] and trimethyl orthoformate.[21] Each set of reaction conditions proved unrewarding.

The esterification difficulties, particularly with diazomethane, were attributed to side reactions involving the primary amine. Thus, attempts were made to block the amino position of L-amino acid **2** with a *tert*-butoxycarbonyl[16] (Boc) group and by converting to a Schiff base derivative.[22] With the Boc approach, decarboxylation occurred during isolation (acidic) to yield Boc-amine **12**. Although the product was not fully characterized, decarboxylation also seemed to occur during Schiff base formation.[23]

Attempted dimerization of L-amino acid **2** by heating without solvent (under vacuum) at temperatures up to 250 °C cleanly produced the (aminomethyl)pyridine **10**. Use of a solvent, for example, ethylene glycol, resulted in extensive decomposition. Analogously, employment of dehydrating agents[24] to promote dimerization gave complex mixtures.

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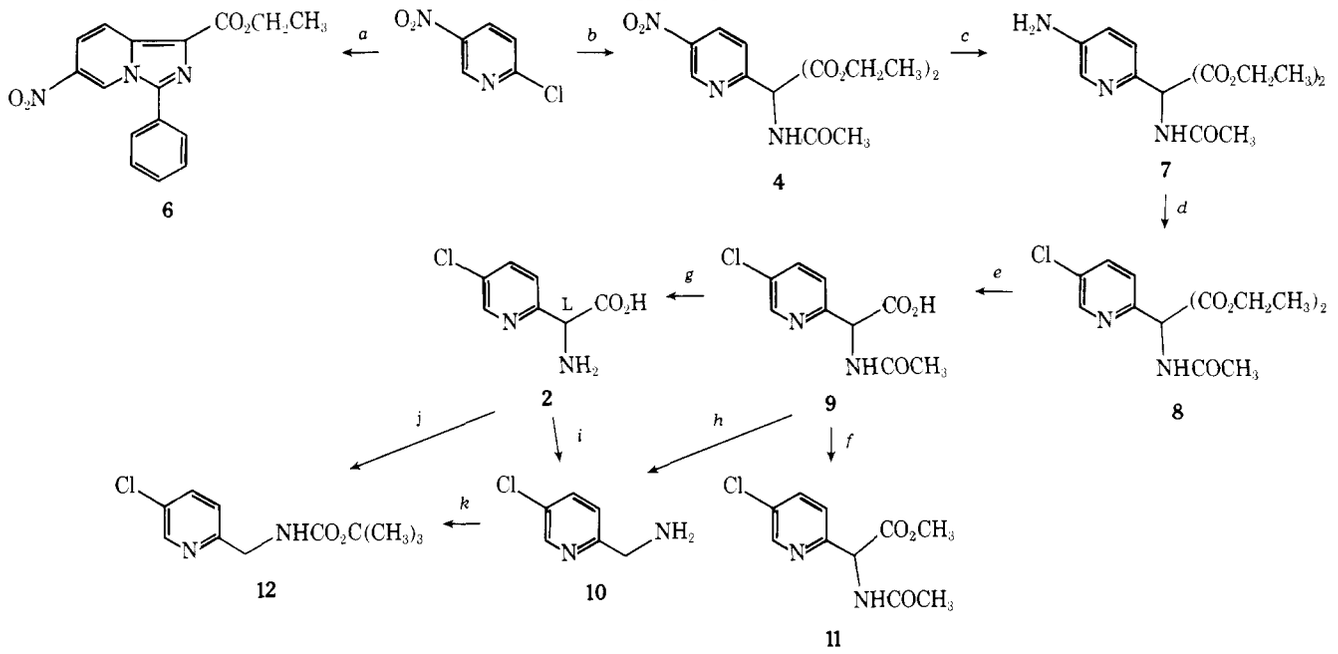
Scheme I



[a] $[(CH_3)_2CH)]_2NLi$, HMPA, THF, $EtO_2CCH_2N{=}CHC_6H_5$. [b] $CH_3CONHCH(CO_2CH_2CH_3)_2$, DMF, NaH. [c] Pd/C, CH_3OH. [d] $(CH_3)_2CHCH_2CH_2ONO$, CCl_4. [e] NaOH, H^+ at 5 °C. [f] CH_2N_2. [g] Hog renal acylase I. [h] HCl, Δ. [i] HCl and/or Δ. [j,k] $(CH_3)_3COCON_3$, NaOH, citric acid.

A unique reaction for dimerization of amino acids to piperazinediones reported by Rosenmund and Kaiser[25] involves synthesis of an *N*-carboxyanhydride derivative (NCA) of an amino acid followed by treatment with aziridine to afford the piperazinedione. Approaches to the NCA derivative of L-amino acid **2** using phosgene again resulted in extensive decomposition, and this route was abandoned. An alternative approach based on first reducing amino acid **2** to the corresponding piperidine derivative also proved to be impractical due to consistent hydrogenolysis of the 5-chloro substituent.

Precedence for the facile decarboxylation of L-amino acid **2** has been observed most notably with α-(2-pyridyl)glycine,[4] α-(4-pyridyl)glycine,[26] and DL-α-(2-thiazolyl)glycine.[27] Because of the instability of L-amino acid **2**, other routes to 593A (**1**) are currently being studied. However, amino acid **2** appears to be of further interest in respect to biological properties. The L-amino acid **2** has been found to significantly inhibit (ED_{50} = 3.8 μg/mL) growth of the P388 (murine lymphocytic leukemia) in vitro cell line.[28]

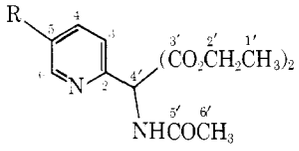
Table I summarizes the carbon-13 resonances for the pyridine derivatives synthesized in this study. Assignments were made using off-resonance proton-decoupled spectra, single-frequency decoupling experiments, and a survey of substituent effects in aromatic systems by Levy and Nelson.[29]

Experimental Section

Purchased reagents were obtained from J. T. Baker, Mallinckrodt, Inc., Aldrich Chemical, MC/B Manufacturing Chemists, Ventron, Div. of Thiokol (*n*-butyllithium), Fairfield Chemical (*N*-nitroso-*N*-methylurea), and Columbia Organic Chemicals (2,5-dichloropyridine). All solvents were redistilled prior to use, and ligroin refers to a fraction boiling at ~60 °C. Solvent extracts of aqueous solutions were dried over anhydrous sodium sulfate. Silica gel F-254 (0.25 mm) was used for thin-layer chromatography (TLC), and E. Merck silica gel (70–230 mesh) was used for column chromatography. TLC plates were visualized with UV light, ninhydrin spray, or bromocresol green spray.

The ^1H NMR spectra (obtained with a Varian Associates XL-100 and Bruker WH-90 instrument) were provided by Dr. J. Witschel, while one of us (M.T.E.) recorded the ^1H NMR spectra using a Varian Associates T-60. Tetramethylsilane (Me_4Si) was used as the internal standard. All ^{13}C NMR spectra were recorded by Dr. Witschel using a Bruker WH-90 instrument. Infrared spectra were recorded using a Perkin-Elmer 237B spectrophotometer and optical rotations employing an O.C. Rudolph Model 80 polarimeter. Low-resolution mass spectra (70 eV) were provided by Mr. E. Kelly using an Atlas

Table I. 2,5-Disubstituted Pyridine ^{13}C NMR Chemical Shifts[a]



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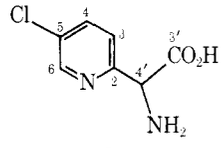

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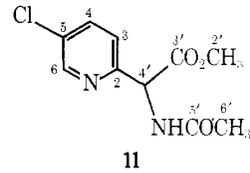

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compd no.	R	registry no.	1′	2′	3′	4′	5′	6′	2	3	4	5	6
4	NO_2	67938-67-4	13.88	63.30	165.96	70.25	169.34	22.76	161.31	126.10	131.63	143.89	143.56
7	NH_2	67938-68-5	13.95	62.52	167.39	69.41	169.24	22.95	143.82[b]	124.25[c]	122.27[c]	142.98[b]	135.08
8	Cl	67983-69-6	13.88	62.78	166.51	70.03	169.28	22.69	153.90	126.04	136.44	131.89	146.81
11		67983-70-9		52.86	170.06	56.96	170.06	22.85	153.09	124.38	136.96	131.92	148.34
2[d]		67983-71-0			171.79	58.98			151.66	126.36	138.76	133.10	149.10

[a] In parts per million relative to $(CH_3)_4Si$ in $CDCl_3$. [b] Assignments for C-2 and C-5 may be reversed. [c] Assigned by single-frequency proton decoupling. [d] Original data were relative to dioxane (D_2O) and were converted to the $(CH_3)_4Si$ scale using 67.40 ppm.

CH-4B spectrometer. A Kofler melting point apparatus was employed to determine melting points (uncorrected). Elemental analyses were determined by Spang Microanalytical Laboratory, Ann Arbor, Mich.

2,5-Dichloro-4-(1-oxo-2-benzamidoethyl)pyridine (3). A 250-mL three-neck flask was equipped with a mechanical stirrer, nitrogen source, and rubber injection system. Dry tetrahydrofuran (50 mL), dry diisopropylamine (5.6 mL, 4.0 g, 40 mmol), and dry *N,N,N',N'*-tetramethylethylenediamine (4.6 g, 40 mmol) were added; the solution was cooled to −78 °C, and *n*-butyllithium (2.4 M in heptane, 17.0 mL, 40 mmol) was introduced via the rubber septum. Before methyl hippurate (3.9 g, 20 mmol) in tetrahydrofuran (20 mL) was injected, the brown solution was stirred for 20 min. After stirring the bright yellow mixture for 1 h at −78 °C, a tetrahydrofuran (20 mL) solution of 2,5-dichloropyridine (3.0 g, 20 mmol) was added (through the injection port). As the mixture was allowed to warm to 25 °C, a deep red color appeared and water (100 mL) was slowly added. The organic layer was extracted with water (2 × 50 mL) and 1.2 M hydrochloric acid (75 mL). The combined aqueous extract was adjusted to pH 2–3 and extracted with ether (2 × 100 mL). Removal of solvent from the ether extract gave a brown oil. The oil (~2 g) was dry-loaded on silica gel (10 g) and chromatographed on a wet-packed ligroin–acetone, (4:1) silica gel (200 g) column. After elution with 1 L of the same solvent, a pale yellow oil was recovered in the next 150-mL fraction. Triturating the oil with ether caused crystallization (0.25 g, 3.6%, mp 115–116 °C). Recrystallization (twice) from chloroform–ether (−5 °C) afforded colorless needles of dichloropyridine **3**: mp 120.5–121.5 °C; IR (KBr) 3280, 1710, 1630, 1520, 1355, 1310, 1112, 878, 680 cm^{-1}; ^1H NMR ($CDCl_3$) δ 4.76 (d, 2 H, J = 6 Hz, CH_2), 7.22 (broad s, 1 H, NH, partially obscured by aromatic H, exchanges with D_2O), 7.36–7.54 (m, 3 H, phenyl H), 7.55 (s, 1 H, C-3 H), 7.74–7.91 (m, 2 H, phenyl H), 8.48 (s, 1 H, C-6 H); mass spectrum *m/e* (rel intensity) 312 (<1), 310 (<1), 308 (<1), 280 (<1), 278 (1), 275 (<1), 273 (1), 179 (5), 178 (2), 177 (28), 176 (4), 175 (36), 149 (6), 147 (9), 135 (5), 134 (30), 129 (7), 106 (19), 105 (100), 77 (29).

Anal. Calcd for $C_{14}H_{10}Cl_2N_2O_2$: C, 54.39; H, 3.27; Cl, 23.07; N, 9.05. Found: C, 54.35; H, 3.22; Cl, 23.07; N, 9.02.

Diethyl (5-Nitro-2-pyridyl)acetamidomalonate (4). To a stirred slurry of sodium hydride (50% oil dispersion, 1.5 g, 0.032 mol) in dimethylformamide (100 mL, distilled from CaO) was slowly added diethyl acetamidomalonate (6.9 g, 0.032 mol). After the initial reaction, the slurry was heated to 50 °C for 30 min and then 2-chloro-5-nitropyridine (5.0 g, 0.032 mol) in dimethylformamide (50 mL) was added. The mixture became dark brown during addition of the 2-chloro-5-nitropyridine. Heating at 50 °C was continued for 2 h. The cool solution was filtered and the solvent removed to give a brown oil. The oil was dry-loaded (on 20 g of silica gel) and chromatographed on a dry-packed silica gel column (250 g). The column was eluted with ligroin (1 L), ligroin-acetone (9:1, 1 L), and ligroin-acetone (7:3, until malonate **4** was isolated). Fractions containing nitropyridine **4** were combined and concentrated to an oil which was crystallized from ethyl ether–pentane to give nearly colorless crystals (5.5 g, 51%). Two additional recrystallizations provided an analytical sample: mp 82–83 °C; IR (KBr) 3370, 1740, 1680, 1605, 1355, 1260, 1200, 1030, 865 cm^{-1}; NMR (acetone-d_6) δ 1.26 (t, 6 H, J = 8 Hz, OCH_2CH_3), 2.04 (s, 3 H, $COCH_3$), 4.31 (q, 4 H, J = 8 Hz, OCH_2CH_3), 8.12 (broad s, 1 H, NHCO, exchanges with D_2O), 8.31 (d, 1 H, J = 9 Hz, C-3 H), 8.71 (dd, 1 H, J = 9 and 3 Hz, C-4 H), 9.25 (d, 1 H, J = 3 Hz, C-6 H); mass spectrum, *m/e* (rel intensity) 339 (2), 309 (<1), 294 (6), 268 (2), 267 (18), 266 (20), 250 (8), 249 (20), 226 (5), 225 (29), 224 (100), 208 (11), 204 (9), 199 (28), 178 (8), 171 (7), 152 (18), 150 (21), 106 (11), 104 (14), 59 (14).

Anal. Calcd for $C_{14}H_{17}N_3O_7$: C, 49.56; H, 5.05; N, 12.38. Found: C, 49.59; H, 5.00; N, 12.34.

1-(Ethoxycarbonyl)-3-methyl-6-nitroimidazo[1,5-*a*]pyridine (5). Sodium hydride (50% oil dispersion, 1.5 g, 0.032 mol) was slowly added to a mechanically stirred solution of diethyl acetamidomalonate (6.9 g, 0.032 mol) in dry 1,2-dimethoxyethane (80 mL). After heating the resulting slurry at 40 °C for 20 min, 2-chloro-5-nitropyridine (5.0 g, 0.032 mol) in 1,2-dimethoxyethane (30 mL) was introduced (1 min). The temperature of the reaction mixture was slowly (1 h) raised to reflux. The clear reaction mixture changed to orange and at reflux to deep red. After heating at reflux for 15 h, the dark red-brown mixture was cooled, diluted with an equal volume of chloroform, and filtered to remove a brown, water-soluble solid. Solvent removal led to a brown oil which was composed (by TLC, 7:3 ligroin–acetone) of at least five compounds, including 2-chloro-5-nitropyridine (12%) and diethyl acetamidomalonate. The oil was chromatographed (ligroin–acetone, 7:3) on a silica gel (250 g) column. Fractions (35 mL) 27–31 were combined and concentrated to a brown viscous residue. Treatment with activated charcoal (methanol) gave, after solvent removal, 2.03 g (25%) of imidazole **5** as a light brown oil.

Fractions 47–53 were combined and evaporated to dryness, furnishing bright orange needles. Four recrystallizations from acetone–ligroin yielded imidazole **5** as orange needles (1.63 g, 20%): mp 135.5–136.5 °C; IR (KBr) 1690, 1638, 1336, 1216, 1054, 1034, 880, 810, 737 cm^{-1}; NMR (acetone-d_6) δ 1.40 (t, 3 H, J = 8 Hz, OCH_2CH_3), 2.84 (s, 3 H, $-C(CH_3)=N$), 4.39 (q, 2 H, J = 8 Hz, OCH_2CH_3), 7.76 (dd, 1 H, J = 10 and 2 Hz, C-7 H), 8.17 (d, 1 H, J = 10 Hz, C-8 H), 9.29 (d, 1 H, J = 2 Hz, C-5 H); mass spectrum, *m/e* (rel intensity) 249 (100), 234 (1), 221 (25), 204 (35), 178 (9), 177 (42), 158 (13), 151 (17), 150 (45), 131 (7), 104 (18).

Anal. Calcd for $C_{11}H_{11}N_3O_4$: C, 53.01; H, 4.45; N, 16.86. Found: C, 53.14; H, 4.46; N, 16.80.

Repetition of the above procedure under completely anhydrous conditions led to a reversal in product yields. Immediately prior to use, DME was distilled from sodium hydride and diethyl acetamidomalonate and 2-chloro-5-nitropyridine were dried in vacuo over potassium hydroxide for 5 h. Product yields changed from 12% **4** and 20% **5** to 28% **4** and 7% **5**.

1-(Ethoxycarbonyl)-3-phenyl-6-nitroimidazo[1,5-*a*]pyridine (6). Into a cold (−78 °C) solution of dry tetrahydrofuran (500 mL), diisopropylamine (2.4 mL, 1.7 g, 17 mmol), and hexamethylphosphoramide (31 mL) under nitrogen contained in a 1-L flask (nitrogen) with a rubber injection septum was injected *n*-butyllithium (2.4 M in heptane, 7.2 mL, 17 mmol). The injection septum was replaced with an addition funnel containing the benzylidine derivative of glycine ethyl ester (3.2 g, 17 mmol) in tetrahydrofuran (20 mL). Slow addition (15 min) of the benzylidene to the colorless lithium diisopropylamide solution produced a dark red-brown mixture. Stirring was continued at −78 °C for 30 min, 2-chloro-5-nitropyridine (2.7 g, 0.017 mol) was added, and 2 h later the temperature was increased to 25 °C for 15 h. The brown suspension changed to a vivid purple upon warming and discolored upon addition of saturated ammonium chloride solution (600 mL). The two-phase system was extracted with ether (3 × 100 mL), and the combined extract was washed with saturated sodium chloride solution (300 mL) and concentrated to a dark brown oil (5.0 g). The oil was dry-loaded on silica gel (77 g) and placed on a dry-packed silica gel (600 g) column. The column was eluted with ligroin (1500 mL), ligroin-acetone (9:1, 1000 mL), and ligroin-acetone (4:1, 4000 mL). Fractions (50 mL) 26–45 were combined and concentrated to an orange solid. Recrystallization (acetone-ligroin) led to imidazo **6** as orange needles (406 mg, 8%): mp 176–177 °C; IR (KBr) 1716, 1334, 1187, 1084, 1043, 883, 790, 706 cm^{-1}; NMR ($CDCl_3$) δ 1.45 (t, 3 H, J = 8 Hz, OCH_2CH_3), 4.53 (q, 2 H, J = 8 Hz, OCH_2CH_3), 7.74–7.94 (m, 6 H, phenyl ring H and pyridine C-7 H), 8.36 (d, 1 H, J = 9 Hz, C-8 H), 9.32 (d, 1 H, J = 3 Hz, C-5 H); mass spectrum *m/e* (rel intensity) 312 (14), 311 (89), 284 (2), 283 (12), 267 (1), 266 (8), 265 (2), 240 (2), 239 (13), 193 (4), 192 (5), 191 (2), 106 (4), 105 (100), 77 (8).

Anal. Calcd for $C_{16}H_{13}N_3O_4$: C, 61.72; H, 4.22; N, 13.50. Found: C, 61.74; H, 4.17; N, 13.44.

Diethyl (5-Amino-2-pyridyl)acetamidomalonate (7). To a suspension of 5% Pd/C (200 mg) in methanol (100 mL) was added diethyl (5-nitro-2-pyridyl)acetamidomalonate (5.8 g, 17 mmol). The mixture, in a 200-mL flask equipped with a stirring bar, was cooled to 0 °C, and the flask was swept several times with hydrogen. After 7 h hydrogenation was essentially complete (1 L). The solution was filtered through Celite, and the filtrate was concentrated to furnish 4.9 g (92%) of aminopyridine **7** as an off-white solid (mp 154–155 °C). Recrystallization of the solid from acetone-ligroin furnished colorless rods: mp 156–157 °C; IR (KBr) 3415, 3300, 1735, 1710, 1640, 1350, 1189, 1115, 1000 cm^{-1}; NMR ($CDCl_3$) δ 1.22 (t, 6 H, J = 8 Hz, OCH_2CH_3), 2.05 (s, 3 H, $COCH_3$), 3.82 (broad s, 2 H, NH_2), 4.26 (q, 4 H, J = 8 Hz, OCH_2CH_3), 6.98 (dd, 1 H, J = 9 and 3 Hz, C-4 H), 7.60 (d, 2 H, J = 9 Hz, C-3 H and NHCO), 7.91 (d, 1 H, J = 3 Hz, C-6 H); mass spectrum *m/e* (rel intensity) 309 (5), 264 (1), 237 (4), 236 (27), 195 (8), 194 (100), 174 (2), 122 (11), 120 (21), 93 (8).

Anal. Calcd for $C_{14}H_{19}N_3O_5$: C, 54.36; H, 6.19; N, 13.58. Found: C, 54.32; H, 6.15; N, 13.56.

Diethyl (5-Chloro-2-pyridyl)acetamidomalonate 8. Procedure 1. To a 500-mL three-neck flask equipped with a reflux condenser and mechanical stirrer was added carbon tetrachloride (100 mL) and isoamyl nitrite (420 mg, 3.6 mmol). The resulting yellow solution was heated at reflux for 2 min before solid diethyl (5-amino-2-pyridyl)-acetamidomalonate (500 mg, 1.6 mmol) was introduced in several small portions (5-min period). Within 45 s, the reaction mixture containing undissolved amine turned dark yellow and finally to a dark orange-red (in 2 or 3 min). After a 2-h reflux period, the dark red solution was concentrated to a brown tarry residue (529 mg) composed of at least three compounds, as illustrated by TLC (ligroin–acetone,

7:3). The residue was dry-loaded on 8 g of silica gel and placed on a column of wet-packed (ligroin-acetone, 4:1) silica gel (100 g). Fractions (5 mL) 63–98 were combined and concentrated to yield an oily solid (299 mg). Crystallization of this material from ethyl ether-pentane afforded 215 mg (41%) of 5-chloropyridine **8** as colorless leaflets: mp 89–90 °C; IR (KBr) 3280, 1745, 1670, 1364, 1285, 1250, 1025, 1015, 860 cm^{-1}; NMR ($CDCl_3$) δ 1.23 (t, 6 H, J = 7 Hz, OCH_2CH_3), 2.06 (s, 3 H, $COCH_3$), 4.32 (q, 4 H, J = 7 Hz, OCH_2CH_3), 7.45 (broad s, 1 H, NHCO), 7.71 (dd, 1 H, J = 9 and 3 Hz, C-4 H), 8.00 (d, 1 H, J = 9 Hz, C-3 H), 8.46 (d, 1 H, J = 3 Hz, C-6 H); mass spectrum, *m/e* (rel intensity) 330 (<1), 328 (1), 285 (1), 283 (3), 257 (2), 255 (11), 240 (2), 238 (5), 215 (26), 213 (84), 200 (17), 199 (5), 143 (13), 142 (3), 141 (51), 140 (9), 139 (65), 114 (9), 113 (7), 112 (22), 43 (100).

Anal. Calcd for $C_{14}H_{17}ClN_2O_5$: C, 51.14; H, 5.22; Cl, 10.78; N, 8.52. Found: C, 51.27; H, 4.94; Cl, 10.85; N, 8.54.

Procedure 2. A suspension of diethyl (5-amino-2-pyridyl)acetamidomalonate (53 mg, 0.17 mmol) and cupric chloride (0.10 g, 0.63 mmol) in acetonitrile (40 mL) was placed in a sealed reaction flask (a 250-mL round-bottom flask with a 110 × 18 mm test tube sealed to the bottom and fitted with a removable gas bubbler that extended to the bottom of the test tube). The system was swept with nitrogen, and nitric oxide (AWECO Medical) was bubbled through the green slurry, turning it to a deep blue. After exposure to nitric oxide for 65 min, the system was again swept with nitrogen. With removal of excess nitric oxide the slurry became light green. Acetonitrile was evaporated and replaced by methanol (4 mL). The mixture was saturated with hydrogen sulfide and the solution filtered to remove a dark brown precipitate. Solvent removal provided a yellow oil, which was chromatographed (chloroform–methanol, 9:1) using a preparative thin-layer plate. A band at R_f 0.60–0.70 was isolated and extracted with chloroform-methanol (1:1). Concentration of the combined extracts furnished 5-chloropyridine **8** as a tan solid (10 mg, 19%).

DL-α-(Acetylamino)-5-chloro-2-pyridineacetic Acid (9). Diethyl (5-chloro-2-pyridyl)acetamidomalonate (2.36 g, 7.2 mmol) was dissolved in 95% ethanol (14 mL). To the stirred solution (25 °C) was added sodium hydroxide (7.67 mL of 3.74 N, 28.7 mmol). After 2 h, the mixture (containing a solid) was cooled to 5 °C and acidified to pH 2 with 5.88 N hydrochloric acid (~6 mL). As the first portion of hydrochloric acid was added, gas evolution occurred. At pH ~7 the solution was completely homogeneous, and colorless solid began to precipitate near pH 2. The mixture was allowed to stand at 5 °C for 20 min, and the solid was collected by filtration and washed with a small amount of cold ethanol, yielding 1.53 g (93%) of acid **9**: dp 107–109 °C; IR (KBr) 3235 (broad), 1732, 1615, 1543, 1255, 1230, 1180, 1019, 688 cm^{-1}; mass spectrum, *m/e* (rel intensity) 186 (6, $M^+ - 44$), 184 (24, $M^+ - 44$), 143 (36), 142 (9), 141 (100), 128 (4), 126 (9), 116 (2), 114 (15).

Methyl DL-α-(Acetylamino)-5-chloro-2-pyridineacetate (11). A suspension of α-(5-chloro-2-pyridyl)aceturic acid (0.536 g, 2.3 mmol) in 1,2-dimethoxyethane (30 mL) at 25 °C was treated with excess diazomethane[14] in 1,2-dimethoxyethane. After stirring for 5 min in the presence of excess diazomethane, the solution was concentrated to a brown oil. The oil (0.652 g) was filtered through silica gel (50 g, 4:1 ligroin–acetone), furnishing 0.568 g (quantitative) of a colorless oil that crystallized on standing. Recrystallization from ethyl ether–pentane afforded ester **11** as colorless plates: mp 94–95 °C; IR (KBr) 3407, 1744, 1653, 1532, 1232, 1183, 1025, 857, 793 cm^{-1}; NMR ($CDCl_3$) δ 2.08 (s, 3 H, $COCH_3$), 3.77 (s, 3 H, CO_2CH_3), 5.72 (d, 1 H, J = 8 Hz, CH), 7.15 (broad s, 1 H, NH), 7.47 (d, 1 H, J = 9 Hz, C-3 H), 7.70 (dd, 1 H, J = 9 and 3 Hz, C-4 H), 8.49 (d, J = 3 Hz, C-6 H); mass spectrum, *m/e* (rel intensity) 244 (1), 242 (2), 186 (1), 185 (20), 184 (5), 183 (58), 144 (2), 143 (31), 142 (7), 141 (100), 140 (2), 139 (9), 115 (1), 114 (3), 113 (4), 112 (2), 78 (6).

Anal. Calcd for $C_{10}H_{11}ClN_2O_3$: C, 49.50; H, 4.57; N, 11.55; Cl, 14.61. Found: C, 49.45; H, 4.60; N, 11.55; Cl, 14.58.

L-α-(5-Chloro-2-pyridyl)glycine (2). Dilute solutions of ammonium hydroxide and acetic acid (if needed) were used to adjust the pH of α-(5-chloro-2-pyridyl)aceturic acid (0.70 g, 3.1 mmol) suspended in water (40 mL) to 7.0. Additional water was added to the solution to bring the total volume to 95 mL. The solution was placed in a water bath (38 °C), and hog renal acylase I(Sigma Chemical Co., 10 mg) was introduced. After stirring for 22 h, a second portion of hog renal acylase I (10 mg) was added. One day later, 2 mL of 50% acetic acid was added and the resulting solution was filtered through Celite. Removal of solvent furnished a colorless solid. Trituration with methanol, cooling to −10 °C (for 2 h), and filtration afforded 0.22 g (86%) of L-amino acid **2** as a powder. Crystallization from water–methanol (~2:1) gave fine colorless needles: dp 146–147 °C; R_f 0.47 (butanol–acetic acid–water, 40:10:10); $[\alpha]^{25}_D$ +43.1° (*c* 1.200, water); IR (KBr) 3250–2700, 1760–1570, 1360, 1112, 1012, 914, 890, 782, 688 cm^{-1}; NMR (Me_2SO-d_6) δ 4.45 (s, 1 H, CH), 7.63 (d, 1 H, J = 9 Hz, C-3 H), 7.92 (dd, 1 H, J = 9 and 3 Hz, C-4 H), 8.54 (d, 1 H, J = 3 Hz, C-6 H); mass spectrum *m/e* (rel intensity) 144 (22, $M^+ - 44$), 143 (22), 142 (71, $M^+ - 44$), 141 (58), 116 (37), 115 (18), 114 (100), 113 (39).

Anal. Calcd for $C_7H_7ClN_2O_2$: C, 45.06; H, 3.78; Cl, 19.00; N, 15.01. Found: C, 45.12; H, 3.55; Cl, 19.07; N, 15.09.

2-(Aminomethyl)-5-chloropyridine (10). Procedure 1. A sample of DL-α-(5-chloro-2-pyridyl)aceturic acid (0.50 g, 2.20 mmol) was mixed with hydrochloric acid (2.9 M, 10 mL) and heated to 60 °C for 2 h. The suspended solid dissolved during the first 10 min of heating. The reaction mixture was maintained at 25 °C for 15 h and adjusted to pH 9 with 3.74 N sodium hydroxide (~7 mL). Extraction with chloroform (4 × 30 mL) and concentration of the extract provided amine **10** as a pale yellow liquid (0.21 g, 67%). Further purification was achieved using microdistillation at 42 °C (0.5 mm) followed by crystallization of the distillate from ethyl ether–pentane (at −11 °C) to give colorless feathery needles (decomposed on prolonged contact with air): mp 32.5–33 °C; IR (KBr) 1557, 1475, 1365, 1106, 1013, 906, 820, 805 cm^{-1}; NMR ($CDCl_3$) δ 1.87 (s, 2 H, NH_2), 3.96 (s, 2 H, CH_2), 7.24 (d, 1 H, J = 8 Hz, C-3 H), 7.66 (dd, 1 H, J = 8 and 3 Hz, C-4 H), 8.50 (d, 1 H, J = 3 Hz, C-6 H); mass spectrum *m/e* (rel intensity) 144 (23), 143 (23), 142 (70), 141 (56), 116 (42), 115 (20), 114 (100), 113 (40), 78 (38), 44 (72).

Procedure 2. A 10-mg (0.055-mmol) protion of L-α-(5-chloro-2-pyridyl)glycine was added to a micro test tube and placed under vacuum (0.4 mm). The test tube was plunged into a Wood's metal bath (preheated to 250 °C) to a depth of about 1 cm. Within 90 s the solid had vaporized and resolidified on a cooler area of the tube. TLC (ethyl acetate–benzene–ammonium hydroxide, 60:35:5) showed only pure amine **10**.

2-(*N*-*tert*-Butoxycarbonylaminomethyl)-5-chloropyridine (12). Procedure 1. To a solution of 2-(aminomethyl)-5-chloropyridine (554 mg, 3.9 mmol) in dioxane (5 mL) was added *tert*-butoxycarbonyl azide (0.57 g, 4.0 mmol). After stirring at 25 °C for 5 min, a solid began to precipitate that proved to be 2-(aminomethyl)-5-chloropyridine (**10**). An additional 5 mL of dioxane was added, and stirring at 25 °C was continued for 40 h. The solution was filtered and concentrated to a brown oil. The oil was dry-loaded on silica gel (2 g) and placed on a column containing 10 g of dry-packed silica gel. Elution with ligroin–acetone (7:3) gave a brown oil which solidified at −11 °C. The solid was sublimed at 60 °C (0.2 mm), affording 0.60 g (65%) of colorless product. Two recrystallizations from ethyl ether–pentane (at −11 °C) afforded Boc-amine **12** as colorless fine needles: mp 55–56 °C; IR (KBr) 3235, 1703, 1370, 1278, 1130, 1016, 925, 856 cm^{-1}; NMR ($CDCl_3$) δ 1.48 (s, 9 H, $C(CH_3)_3$), 4.43 (d, 2 H, J = 6 Hz, CH_2), 5.72 (broad s, 1 H, NH), 7.27 (d, 1 H, J = 9 Hz, C-3 H), 7.68 (dd, 1 H, J = 9 and 3 Hz, C-4 H), 8.54 (d, 1 H, J = 3 Hz, C-6 H); mass spectrum, *m/e* (rel intensity) 244 (<1), 242 (<1), 189 (28), 188 (24), 187 (92), 186 (59), 171 (15), 169 (47), 144 (7), 143 (27), 142 (24), 141 (47), 128 (12), 126 (43), 116 (9), 114 (34), 57 (100), 41 (59).

Anal. Calcd for $C_{11}H_{15}ClN_2O_2$: C, 54.43; H, 6.24; Cl, 14.61; N, 11.54. Found: C, 54.45; H, 6.16; Cl, 14.58; N, 11.52.

Procedure 2. A mixture of L-(5-chloro-2-pyridyl)glycine (50 mg, 0.27 mmol), sodium hydroxide (0.15 mL of a solution composed of 7.7 g of sodium hydroxide in 100 mL of water), and dioxane (0.30 mL) was placed in a micro test tube. To the filtered solution was added *tert*-butoxycarbonyl azide (40 mg, 28 mmol), water (~0.5 mL), and dioxane (~0.5 mL). After stirring the homogeneous reaction mixture for 24 h, an equal volume of water was added and the resulting solution was extracted with ether (3 × 1 mL). The aqueous layer was acidified to pH 3.5 with citric acid. During this process a gas (decarboxylation) was evolved. The aqueous layer was saturated with sodium chloride and extracted with ethyl acetate. The solvent extract was concentrated to yield Boc-amine **12** as a yellow liquid (26 mg).

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Registry No.—**3**, 67938-72-1; **5**, 67938-73-2; **6**, 67938-74-3; **9**, 67938-75-4; **10**, 67938-76-5; **12**, 67938-77-6; methyl hippurate, 1205-08-9; 2,5-dichloropyridine, 16110-09-1; diethyl acetamidomalonate, 1068-90-2; 2-chloro-5-nitropyridine, 4548-45-2; benzylideneglycine ethyl ester, 40682-54-0; isoamyl nitrite, 110-46-3; cupric chloride, 7447-39-4; nitric oxide, 10102-43-9; hog renal acylase I, 9012-37-7; *tert*-butoxycarbonyl azide, 1070-19-5.

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Halo Sugar Nucleosides. 6. Synthesis of Some 5′-Deoxy-5′-iodo and 4′,5′-Unsaturated Purine Nucleosides[1]

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Reactions of the 5′-hydroxyl groups of suitably substituted purine nucleosides with methyltriphenoxyphosphonium iodide (1) in nonpolar solvents such as tetrahydrofuran and dichloromethane give the corresponding 5′-deoxy-5′-iodo nucleosides in high yield. Previously, N^3,5′-cyclonucleosides were the major products of these reactions when DMF was used as solvent. Efficient syntheses of 9-(5-deoxy-β-D-*erythro*-pent-4-enofuranosyl)purine nucleosides are described via dehydrohalogenation of various 5′-deoxy-5′-iodoinosine, -guanosine, and -adenosine derivatives with either silver fluoride in pyridine or 1,5-diazabicyclo[4.3.0]non-5-ene in DMF or pyridine.

In previous papers in this series,[1,3] we have described the synthesis of various 5′-deoxy-4′,5′-unsaturated nucleosides by dehydrohalogenation of the corresponding 5′-deoxy-5′-iodo derivatives. These halogenated nucleosides[4] were obtained by reaction of the 5′-hydroxyl group of suitably substituted pyrimidine nucleosides with methyltriphenoxyphosphonium iodide[5] (1) in dimethylformamide. However, the reaction of 2′,3′-*O*-isopropylidene derivatives of purine nucleosides **3**, **10**, and **25a** with 1 gave mainly the corresponding N^3,5′-cyclonucleosides.[4] Previous work by Jahn[6] has shown that acylation of the 6-amino function in the adenosine series decreases the tendency toward formation of N^3,5′-cyclonucleosides via reduction of the electronegativity of N^3. Along these lines we have reported that reaction of N^6,N^6,$O^{2'}$,$O^{3'}$-tetrabenzoyladenosine with 1 leads to the corresponding 5′-deoxy-5′-iodonucleoside in high yield.[1]

While the above method has been of great synthetic value, the incorporation of *N*-acyl substituents is sometimes not desirable. In this paper, we describe an alternate method for minimizing the formation of N^3,5′-cyclonucleosides, thus permitting the synthesis, in good yield, of 5′-deoxy-5′-iodopurine nucleosides. The latter compounds can then be transformed into the corresponding 5′-deoxy-4′,5′-unsaturated derivatives by dehydrohalogenation using previously developed methods.

During the reaction of a purine nucleoside derivative with **1**, a common oxyphosphonium intermediate, **2**, is considered to be the precursor of both the desired 5′-deoxy-5′-iodo compound (via path b) and the undesired N^3,5′-cyclonucleoside (via path a). Rather than seek new methods to reduce the nucleophilicity of N^3 through heterocyclic derivatization, we have attempted to control the relative rates of paths a and b via changes in solvent and temperature.

It has been demonstrated that triphenylphosphine dihalides exist largely in a dissociated form [e.g., $(C_6H_5)_3P^+$–X X^-] in polar solvents and in a pentacovalent form [e.g., $(C_6H_5)_3PX_2$] in nonpolar media.[7] A similar situation probably exists with the related reagent **1**. It is therefore likely that in solvents such as dimethylformamide[8] electrostatic attraction between the 5′-oxygen of the nucleoside and the positive

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