A New and Efficient Synthesis of Guanosine†

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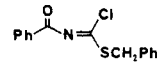
New methodology for the preparation of guanosine-type nucleoside analogues from *o*-amino carbamyl nucleoside precursors has been developed and is demonstrated by the three-step, high-yield synthesis of guanosine (**16**) from 5-amino-1-(β-D-ribofuranosyl)imidazole-4-carboxamide (**1**, AICA-riboside). Treatment of 1-(alkoxycarbonyl)-3-(arylmethyl)thioureas **8** with phosgene in the presence of triethylamine affords the highly electrophilic 1-(alkoxycarbonyl)-3-(arylmethyl)carbodiimide reagents **9** in high yield. These reagents are shown to condense with AICA-riboside readily at room temperature to afford *N*-acyl-*N'*-(arylmethyl)guanidino-substituted imidazole nucleoside derivatives. One of these derivatives, 5-(*N*-benzyl-*N'*-(ethoxycarbonyl)guanidino)-1-(β-D-ribofuranosyl)imidazole-4-carboxamide (**11a**), is smoothly debenzylated with cyclohexene in the presence of Pd(0) to afford 5-(*N*-(ethoxycarbonyl)guanidino)-1-(β-D-ribofuranosyl)imidazole-4-carboxamide (**14**). Prolonged heating of **14** in ethanol at reflux affords *N*-2-(ethoxycarbonyl)guanosine (**15**) in high yield. The ethoxycarbonyl protecting group of **15** is removed with concentrated NH_4OH/pyridine to afford guanosine. This new methodology is much more efficient than those previously reported and should find application for the preparation of a wide variety of guanosine-type nucleoside analogues.

Several naturally occurring guanosine-type nucleoside analogues have demonstrated significant biological and chemotherapeutic activity. This has prompted a continuing interest in developing more efficient synthetic routes for the preparation of guanosine and guanosine-type analogues by annulation (formation of the pyrimidine ring) from a readily available precursor.

Since its appearance, the ring-closure methodology reported by Yamazaki and co-workers in 1967[1a] for the formation of guanosine (**5**, R = β-D-ribofuranosyl) from 5-amino-1-(β-D-ribofuranosyl)imidazole-4-carboxamide (**1**, R = β-D-ribofuranosyl, AICA-riboside) has served as the principal method by which guanosine analogues are obtained from *o*-amino carbamyl nucleoside precursors. According to this method (Scheme I),[1,2] the 2',3'-*O*-isopropylidene-protected AICA-riboside is treated with benzoyl isothiocyanate to afford a 3-benzoyl-1-thioureido derivative (**2**) which is *S*-methylated to give the corresponding *S*-methyl isothioureido adduct **3**. Compound **3** is then subjected to aminolysis under high temperature and pressure to give, presumably, the benzoylated guanidino derivative **4**, which affords 2',3'-*O*-isopropylideneguanosine upon ring closure and concomitant debenzoylation in aqueous alkali. This multistep method has been employed in the preparation of a number of guanosine-type nucleoside analogues but has suffered the drawback of proceeding in a low overall yield, typically in the 15–20% range.[3,4] This prompted us to initiate research designed to provide a new methodology for the preparation of guanosine-type nucleoside analogues in a high-yielding, short synthesis, directly from *o*-amino carbamyl nucleoside precursors. We now report the successful development of just such a methodology.

A careful examination of the Yamazaki ring-closure synthesis revealed that several steps could be circumvented by the use of a specific new electrophilic reagent. Acton and Ryan have recently reported the preparation and use of *S*-benzyl-*N*-benzoylcarbonochloridimidothioate[5] (**6**) in



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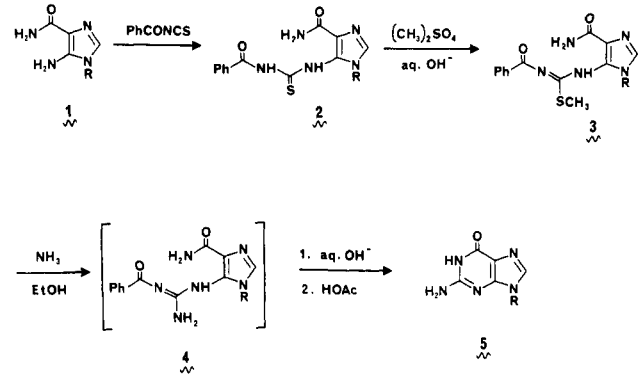
Scheme I. Standard Ring Closure Method for the Preparation of Guanosine-Type Nucleoside Analogues (R = 2',3'-*O*-Isopropylidene-β-D-ribofuranosyl)[1a]



the synthesis of the thioguanosine analogue in the pyrazolo[4,3-*d*]pyrimidine series.[6] This reagent reacts with heterocyclic *o*-amino thiocarboxamides directly to afford *S*-benzyl isothiourea thiocarbamyl derivatives of **3** (Scheme I), thus circumventing the first two steps in the Yamazaki synthesis.

Our approach was to develop a new electrophilic reagent which could be used to circumvent the first *three* steps in the Yamazaki ring-closure synthesis. Specifically, we sought an electrophilic reagent which would react with unprotected AICA-riboside directly to generate an *N*-acyl-*N'*-alkylguanidine moiety at the 5-carbon atom position of the imidazole ring and allow for the subsequent selective removal of the acyl and alkyl protecting groups. As the 1-(alkoxycarbonyl)-3-(arylmethyl)carbodiimides appeared well suited for this purpose, we focused our at-

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(1) (a) Yamazaki, A.; Kumashiro, I.; Takenishi, T. *J. Org. Chem.* **1967**, *32*, 1825. (b) Kumashiro, I.; Yamazaki, A.; Meguro, T.; Takanishi, T.; Tsunoda, T. *Biotechnol. Bioeng*, **1968**, *10*, 303. (c) Okutsu, M.; Yamazaki, A. *Nucleic Acids Res.* **1976**, *3*, 237. (d) Yamazaki, A.; Okutsu, M. *Nucleic Acids Res.* **1976**, *3*, 251.

(2) For a review on cyclization reactions of AICA-riboside, see: Yamazaki, A.; Okutsu, M. *J. Heterocycl. Chem.* **1978**, *15*, 353.

(3) (a) Lewis, A. F.; Townsend, L. B. *J. Am. Chem. Soc.* **1982**, *104*, 1073. (b) Taylor, E. C.; Fletcher, S. R. *J. Org. Chem.* **1984**, *49*, 3226.

(4) Babin, F.; Dinh, T. H.; Igolen, J. *J. Heterocycl. Chem.* **1983**, *20*, 1169.

(5) Acton, E. M.; Ryan, K. J. *Nucleic Acids, Symp. Ser.* **1981**, *9*, 243.

(6) Acton, E. M.; Ryan, K. J. *J. Org. Chem.* **1984**, *49*, 528.

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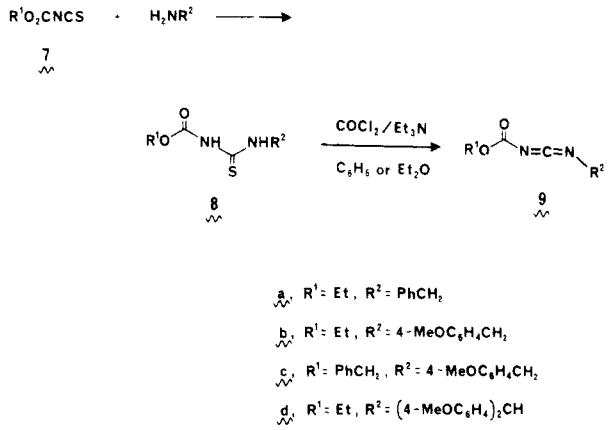
Scheme II. Preparation of 1-Acyl-3-(arylmethyl)carbodiimide Reagents



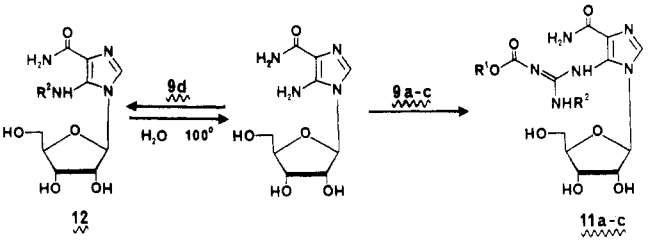
tention on the preparation of this type of reagent.[7]

Results and Discussion

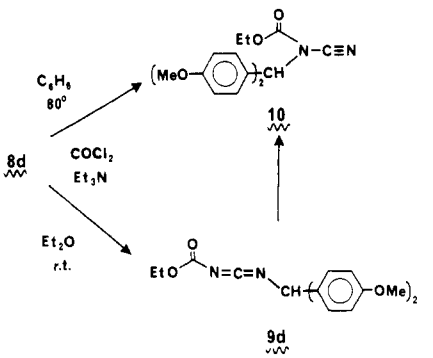
(Alkoxycarbonyl)carbodiimides have been prepared by the photochemical addition of nitrenes to isonitriles[8] and by the desulfurization of thioureas with diethyl azodicarboxylate or mercuribenzamide.[9] Base-promoted ring cleavage of oxadiazolium tetrafluoroborate salts has been shown to afford acylcarbodiimides as well.[10]

Goerdeler and co-workers have prepared 1-carbamyl-, 1-thiocarbamyl-, and 1-imidoyl-3-alkylcarbodiimides by the treatment of the corresponding thioureas with cyanuric chloride in the presence of triethylamine.[11] Other investigators have reported the preparation of 1-cyclohexyl-3-(*p*-toluenesulfonyl)carbodiimide[12] and 1-alkyl-3-(ethoxycarbonyl)carbodiimides[13] by a similar reaction of appropriate thioureas with phosgene. We prepared a series of 1-(alkoxycarbonyl)-3-(arylmethyl)thioureas (**8a–d**) by the condensation of appropriate alkoxycarbonyl isothiocyanates[14] with substituted benzylamines (Scheme II). It is interesting to note that in the preparation of the benzyloxycarbonyl isothiocyanate reagent **7c**,[15] from benzyloxycarbonyl chloride and potassium thiocyanate, the use of dry acetonitrile as solvent was found to afford the decarboxylation product, benzyl thiocyanate. The use of dry ethyl acetate as solvent, however, afforded the desired **7c** with no evidence of a decarboxylation reaction being observed. Apparently, the nucleophilicity of the thiocyanate anion is sufficiently enhanced in the more polar acetonitrile solvent to allow for nucleophilic attack at the benzylic carbon atom. (Allyloxy)carbonyl chloride has been found to undergo a similar decarboxylation reaction upon treatment with KSCN in acetonitrile.[16]

Scheme III. Condensation Reactions of AICA-riboside with 9 (R^1 and R^2 as in Scheme II)



Treatment of thioureas **8a–d** with phosgene in the presence of triethylamine in benzene at reflux afforded the desired 1-(alkoxycarbonyl)-3-(arylmethyl)carbodiimide reagents **9a–c** in excellent yields with one exception. Under these conditions, 1-(4,4′-dimethoxybenzhydryl)-3-(ethoxycarbonyl)thiourea (**8d**) afforded a rearranged product, 1-(4,4′-dimethoxybenzhydryl)-1-(ethoxycarbonyl)cyanamide (**10**), identified by ^{1}H NMR and IR



spectral data. However, under milder conditions, treatment with phosgene in the presence of triethylamine in diethyl ether at room temperature, **8d** afforded the desired carbodiimide reagent **9d**.

The infrared spectra of the 1-(alkoxycarbonyl)-3-(arylmethyl)carbodiimides **9a–d** showed a very strong, broad absorption in the 2160–2180-cm^{-1} region, indicative of the acylated carbodiimide moiety.[13] In contrast, the infrared spectrum of the cyanamide product **10** showed a strong, sharp absorption at 2240 cm^{-1}. These reagents (**9**) were found to decompose slowly at room temperature, probably via a polymerization reaction,[13] as indicated by the infrared spectrum obtained after 6 h, 12 h, and 24 h, and therefore were used immediately upon isolation. However, it was subsequently found they could be stored at 0 °C for several days without appreciable decomposition (determined by IR).

As expected, the acylated carbodiimide reagents **9a–d** were highly electrophilic[17] and were readily condensed with AICA-riboside at room temperature to afford the corresponding *N*-acyl-*N*′-alkylguanidino-substituted imidazole nucleosides **11a-c** (Scheme III) in good yields. Unexpectedly, **9d** reacted with AICA-riboside to afford 5-((4,4′-dimethoxybenzhydryl)amino)-1-(β-D-ribofuranosyl)imidazole-4-carboxamide (**12**), identified by ^{1}H NMR, IR, and UV spectral data and by the fact that it readily reverts to AICA-riboside upon treatment with hot methanol or water. Nucleoside **12** was also obtained by the treatment of AICA-riboside with a mixture of thiourea **8d** and 1,3-dicyclohexylcarbodiimide, conditions which generate **9d** in situ.[18] Transfer of the labile 4,4′-dimeth-

(7) Under aqueous acidic conditions, arylamines are reported to condense with (methoxycarbonyl)cyanamide to afford methoxycarbonylated arylguanidines: Gotz, N.; Zeeh, B. *Synthesis* **1970**, 3931.

(8) Kozlowska-Gramsz, E.; Descotes, G. *Tetrahedron Lett.* **1982**, *23*, 1585.

(9) Mitsunobu, O.; Tomari, M.; Morimoto, H.; Sato, T.; Sudo, M. *Bull. Chem. Soc. Jpn.* **1972**, *45*, 3607.

(10) Zimmerman, D. M.; Olofson, R. A. *Tetrahedron Lett.* **1970**, 3453.

(11) (a) Goerdeler, J.; Raddatz, S. *Chem. Ber.* **1980**, *113*, 1095. (b) Goerdeler, J.; Losch, R. *Ibid.* **1980**, *113*, 79. (c) Goerdeler, J.; Lohmann, H. *Ibid.* **1977**, *110*, 2996. (d) Goerdeler, J.; Lohmann, H.; Losch, R.; Raddatz, S. *Tetrahedron Lett.* **1971**, 2765.

(12) Gupta, R. K.; Stammer, C. H. *J. Org. Chem.* **1968**, *33*, 4368.

(13) (a) Neidlein, R.; Heukelbach, E. *Arch. Pharm.* **1966**, *299*, 709. (b) Neidlein, R.; Heukelbach, E. *Tetrahedron Lett.* **1965**, 149.

(14) (a) Esmail, R.; Kurzer, R. *Synthesis* **1975**, *5*, 301. (b) Gensler, W. J.; Chan, S.; Ball, D. B. *J. Org. Chem.* **1961**, *46*, 3407.

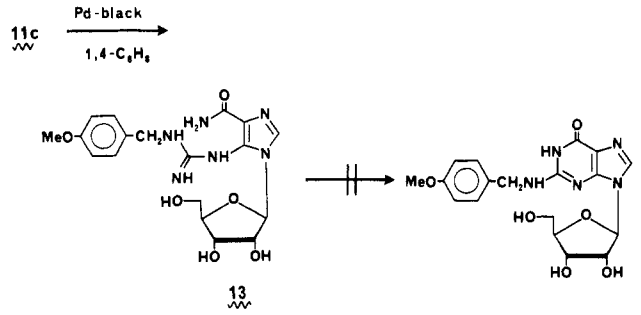
(15) Teruhisa, N.; Keisuke, K.; Kimpei, K. U.S. Patent 4 020 095 to Nippon Soda Co., Ltd.; *Chem. Abstr.* **1977**, *87*, P134749f.

(16) Takamizawa, A.; Hirai, K.; Matusi, K. *Bull Chem. Soc. Jpn.* **1963**, *36*, 1214.

(17) For reviews on carbodiimide chemistry, see: (a) Williams, A.; Ibrahim, I. T. *Chem. Rev.* **1981**, *81*, 589. (b) Kurzer, F.; Douraghi-Zadeh, K. *Chem. Rev.* **1967**, *67*, 107.

oxybenzhydryl group of **9d** to the amino group of AICA-riboside thus was a more facile reaction than the desired carbodiimide condensation, under the conditions employed.

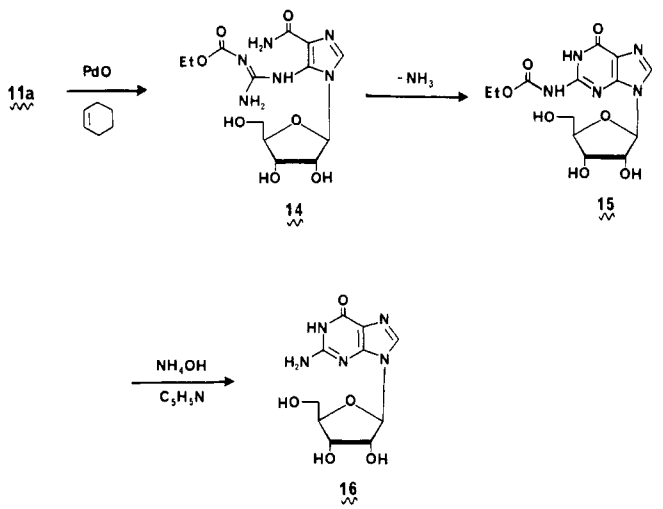
Removal of one of the guanidino protecting groups from nucleosides **11** would afford an *N*-acyl- or *N*-benzyl-guanidino-substituted imidazole nucleoside which might undergo a cyclization reaction to afford an N-2-substituted guanosine derivative. As the presence of the unprotected 5′-hydroxyl group precluded an oxidative removal of the protecting groups, we directed our attention to the hydrogenolytic removal of the benzyl groups. Catalytic transfer hydrogenolysis has been employed successfully in nucleoside synthesis;[19] indeed when **11c** was treated with 1,4-cyclohexadiene[20] in the presence of palladium black, generated in situ from palladium(II) acetate,[21] a smooth debenzylative decarboxylation reaction occurred to afford 5-[3-(4′-methoxybenzyl)-1-guanidino]-1-(β-D-ribofuranosyl)imidazole-4-carboxamide (**13**) isolated in a 57% yield after chromatographic separation. Compound **13** was



identified by its ^{1}H NMR, IR, and UV spectral data. Unfortunately, all attempts (base catalysis or neutral conditions) to promote a ring-closure reaction of **13** to the *N*-2-(4′-methoxybenzyl)guanosine derivative were unsuccessful. This may be interpreted to imply that the presence of an electron-withdrawing (acidifying) group on the guanidino moiety is necessary for a facile cyclization[22] to occur.

We then turned our attention to the debenzylation of **11a** or **11b**. Both of these compounds were inert to the conditions used (vide supra) to prepare nucleoside **13**. Moreover, these compounds were inert to catalytic hydrogenation (Pd/C, 1 or 34 atm H_2 in ethanol), even when freshly prepared and scrupulously washed palladium black[23] was employed as a catalyst. Catalytic transfer hydrogenolysis with methanolic formic acid[24] also failed to effect a debenzylation reaction. As palladium hydroxide on carbon (Pearlman's catalyst)[25] is known to be useful in the removal of certain *N*-benzyl groups, we subjected nucleoside **11a** to the debenzylation conditions of Hanessian et al. (cyclohexene, $Pd(OH)_2/C$ in refluxing ethanol).[26]

Scheme IV. Synthesis of Guanosine



This furnished a 20% yield of the desired debenzylated product **14**, identified by its ^{1}H NMR, IR, UV, and FAB–mass spectral properties (Scheme IV).

We suspected that the low yield might be due to the absorption of the product onto the carbon catalyst support, and therefore palladium(II) oxide was substituted for Pearlman's catalyst in this procedure. Interestingly, the debenzylation product **14** was isolated (20% purified yield) from the reaction mixture after 8 h at reflux, while the desired N-2-ethoxycarbonylated guanosine derivative **15** was isolated (92% yield) from the reaction mixture after 48 h at reflux (Scheme IV). This result is interpreted to indicate that nucleoside **14** is a direct precursor to **15** under the reaction conditions. Nucleoside **15**, identified by its ^{1}H NMR, IR, UV, and FAB–mass spectral data, was then subjected to the conditions of Letsinger and Miller for the deprotection of *N*-6-((isobutyloxy)carbonyl)deoxycytidine derivatives (concentrated NH_4OH/pyridine).[27] After 48 h at 45 °C, a removal of the ethoxycarbonyl protecting group from the N-2-position of **15** was complete, as judged by TLC analysis; guanosine (**16**) was isolated in a 95% yield (Scheme IV). The guanosine thus obtained was identical with an authentic sample by melting point, infrared spectral, ultraviolet spectral (pH 1, H_2O, and pH 11), and TLC R_f value data; fast-atom bombardment mass spectral analysis showed the molecular ion peak at m/z of 284 (MH^+) amu.

Conclusions

We have developed a new, efficient three-step synthesis of guanosine-type nucleoside analogues from *o*-amino carbamyl nucleoside precursors utilizing a 1-acyl-3-benzylcarbodiimide reagent. The methodology reported herein should find application to the preparation of a wide variety of guanosine-type nucleoside analogues, not only from *o*-amino carbamyl nucleoside precursors but also from *o*-amino thiocarbamyl and possibly *o*-amino carboxylic ester nucleoside precursors as well.

Experimental Section

General Methods. Melting points were determined on a Thomas-Hoover Unimelt apparatus and are uncorrected. Rotary evaporations were conducted at less than 50 °C, using a water aspirator or a vacuum pump. ^{1}H nuclear magnetic resonance spectra were recorded at 60, 200, 270, or 360 MHz, using either deuteriochloroform as solvent and tetramethylsilane as internal standard or dimethyl-d_6 sulfoxide as solvent and dimethyl-d_5

(18) 1,3-Disubstituted thioureas are known to undergo an equilibrium reaction with 1,3-dicyclohexylcarbodiimide: Wragg, R. T. *Tetrahedron Lett.* **1970**, 3931.
(19) See, for example: Watkins, B. E.; Kiely, J. S.; Rappoport, H. *J. Am. Chem. Soc.* **1982**, *104*, 5702.
(20) Felix, A. M.; Heimer, E. P.; Lambros, T. J.; Tzougraki, C.; Meienhofer, J. *J. Org. Chem.* **1978**, *43*, 4194.
(21) Khan, S. A.; Sivanandaiah, K. M. *Synthesis* **1978**, 750.
(22) The exact mechanism of the successful cyclization reaction described in this report is unclear at present; the nucleophilic amino group involved in this reaction remains to be identified with an appropriate ^{15}N-label mass spectral study.
(23) Greenstein, J. P.; Winitz, M. "Chemistry of Amino Acids"; John Wiley and Sons, Inc.: New York, 1961; Vol. 2, p 1233.
(24) AlAmin, B.; Anatharamaiah, G. M.; Royer, G. P.; Means, G. E. *J. Org. Chem.* **1979**, *44*, 3442.
(25) Pearlman, W. M. *Tetrahedron Lett.* **1967**, 1663.
(26) Hanessian, S.; Liek, T. J.; Vanasse, B. *Synthesis* **1981**, 396.
(27) Letsinger, R. L.; Miller, P. S. *J. Am. Chem. Soc.* **1969**, *91*, 3356.

sulfoxide as internal standard. H_a and H_b denote the different carboxamide proton resonances observed in the ^1H NMR spectrum of compounds which undergo intramolecular hydrogen bonding. ^{13}C nuclear magnetic resonance spectra were recorded at 90.4 MHz. Fast-atom bombardment–mass spectra were obtained in the Mass Spectrometry Laboratory, School of Chemical Sciences, University of Illinois, supported in part by a grant from the National Institute of General Medical Sciences (GM 27029).

Benzene was distilled from sodium benzophenone ketyl under nitrogen prior to use. Acetonitrile and ethyl acetate were distilled from P_2O_5 under nitrogen. *N,N*-Dimethylformamide, *N,N*-dimethylacetamide, and triethylamine were distilled from CaH_2 under nitrogen and were stored over 4-Å molecular sieves.

Potassium isothiocyanate, benzyl chloroformate, ethoxycarbonyl isothiocyanate, 4-methoxybenzylamine, 1,3-dicyclohexylcarbodiimide, 4,4′-dimethoxybenzhydrol, and 1,4-cyclohexadiene were purchased from the Aldrich Chemical Co., Milwaukee, WI. Benzylamine was obtained from the Medicinal Chemistry stockroom, University of Michigan, and was distilled prior to use. Phosgene (12.5% in benzene) was a product of Matheson, Coleman and Bell. Palladium(II) acetate trimer was purchased from the AESAR group, Johnson Matthey, Inc. Palladium oxide was a product of Engelhard Industries, Inc.

Caution! Phosgene is a severe poison and should be handled in a well-ventilated hood at all times. Consult Merck Index 1976, 9, 7146 for warning which describes test strips to be used whenever phosgene is handled.

4,4′-Dimethoxybenzhydrylamine[28] mp 60–61 °C (lit. mp 58–59 °C,[28b] 61–62 °C[28c]).

Benzyloxycarbonyl Isothiocyanate (7c). A suspension of finely ground potassium isothiocyanate (2.9 g, 40 mmol) in 40 mL of anhydrous ethyl acetate under nitrogen was cooled to 0 °C and treated dropwise with benzyl chloroformate (5.7 mL, 40 mmol). The reaction mixture was allowed to warm to room temperature slowly and then stirred under nitrogen overnight. The reaction mixture was filtered through Celite, rotary evaporated, and the product was purified by Kugelrohr distillation to afford 3.94 g (51%) of benzyloxycarbonyl isothiocyanate as a pale yellow solid: bp 95–105 °C (2 mmHg); mp ~20 °C; ^1H NMR ($CDCl_3$) δ 7.36 (m, 5 H, C_6H_5), 4.12 (s, 2 H, CH_2); IR (neat) 1745, 1970, 3020 cm^{-1}.

Anal. Calcd for $C_9H_7NO_2S$: C, 55.95; N, 3.65; N, 7.25; S, 16.59. Found: C, 55.82; H, 3.74; N, 7.40; S, 16.31.

Substitution of acetonitrile for ethyl acetate in the above procedure afforded, after distillation, a 59% yield of benzyl thiocyanate: mp 41–43 °C, bp 114–118 °C (2 mmHg).

1-Acyl-3-alkylthioureas. The preparation of 1-benzyl-3-(ethoxycarbonyl)thiourea (**8a**) is representative. A solution of benzylamine (4.36 g, 40 mmol) in 200 mL of anhydrous diethyl ether under nitrogen was treated with ethoxycarbonyl isothiocyanate (4.7 mL, 40 mmol) dropwise to avoid excessive boiling. The reaction mixture was stirred for 30 min at room temperature, and the product was collected by suction filtration and washed with hexane to afford 8.85 g (93%) of **8a**, which was recrystallized from diethyl ether/*n*-hexane: mp 105–107 °C; ^1H NMR (270 MHz, $CDCl_3$) δ 9.95 (s, exchanges with D_2O, 1 H, CONH), 8.09 (s, exchanges, 1 H, CSNH), 7.35 (m, 5 H, C_6H_5), 4.87 (d, J = 5.4 Hz, 2 H, $ArCH_2$), 4.21 (q, J = 7.1 Hz, 2 H, CH_2O), 1.30 (t, J = 7.1 Hz, 3 H, CH_3); IR (KBr) 1545, 1727, 3190, 3250 cm^{-1}.

Anal. Calcd for $C_{11}H_{14}N_2O_2S$: C, 55.44; H, 5.92; N, 11.76. Found: C, 55.58; H, 6.01; N, 11.66.

1-(Ethoxycarbonyl)-3-(4′-methoxybenzyl)thiourea (8b): 82% yield; mp 91–93 °C; ^1H NMR (270 MHz, $CDCl_3$) δ 9.86 (s, exchanges, 1 H, CONH), 8.07 (s, exchanges, 1 H, CSNH), 7.28 (d, J = 8.6 Hz, 2 H, Ar H), 6.88 (d, J = 8.6 Hz, 2 H, Ar H), 4.78 (d, J = 5.2 Hz, collapses to a singlet upon D_2O addition, 2 H, $ArCH_2$), 4.20 (q, J = 7.2 Hz, 2 H, CH_2O), 3.80 (s, 3 H, $ArOCH_3$), 1.29 (t, J = 7.2 Hz, 3 H, CH_3); IR (KBr) 1555, 1725, 3170, 3240 cm^{-1}.

Anal. Calcd for $C_{12}H_{16}N_2O_3S$: C, 53.71; H, 6.01; N, 10.44. Found: C, 53.81; H, 5.95; N, 10.51.

1-(Benzyloxycarbonyl)-3-(4′-methoxybenzyl)thiourea (8c): 85% yield; mp 114–115.5 °C; ^1H NMR (270 MHz, $CDCl_3$) δ 9.81 (s, exchanges, 1 H, CONH), 8.07 (s, exchanges, 1 H, CSNH), 7.36 (m, 5 H, C_6H_5), 7.27 (d, J = 8.8 Hz, 2 H, Ar H), 6.88 (d, J = 8.8 Hz, 2 H, Ar H), 5.15 (s, 2 H, CH_2O), 4.77 (d, J = 5.2 Hz, collapses to a singlet upon D_2O addition, 2 H, $ArCH_2$), 3.80 (s, 3 H, $ArOCH_3$); IR (KBr) 1535, 1550, 1725, 3205 cm^{-1}.

Anal. Calcd for $C_{17}H_{18}N_2O_3S$: C, 61.80; H, 5.49; N, 8.48; S, 9.70. Found: C, 61.63; H, 5.57; N, 8.47; S, 9.44.

1-(4,4′-Dimethoxybenzhydryl)-3-(ethoxycarbonyl)thiourea (8d): 92% yield; mp 121.5–122.5 °C; ^1H NMR (270 MHz, $CDCl_3$) δ 11.18 (s, exchanges, 1 H, CONH), 10.62 (d, exchanges, 1 H, CSNH), 7.16 (d, J = 8.7 Hz, 4 H, Ar H), 6.92 (d, J = 8.7 Hz, 4 H, ArH), 6.53 (d, J = 8.0 Hz, collapses to a singlet upon D_2O addition, 1 H, benzhydryl CH), 4.16 (q, J = 7.0 Hz, 2 H, CH_2O), 3.73 (s, 6 H, $ArOCH_3$), 1.22 (t, J = 7.0 Hz, 3 H, CH_3); IR (KBr) 1540, 1712, 3200, 3260 cm^{-1}.

Anal. Calcd for $C_{19}H_{22}N_2O_4S$: C, 60.94; H, 5.92; N, 7.48; S, 8.56. Found: C, 61.03; H, 6.05; N, 7.52; S, 8.63.

1-Benzyl-3-(ethoxycarbonyl)carbodiimide (9a). A solution of **8a** (7.16 g, 30 mmol) in 80 mL of anhydrous benzene under nitrogen was treated with dry triethylamine (10.5 mL, 75 mmol) and then dropwise with phosgene (28.5 mL of a 12.5% solution in anhydrous benzene, 33 mmol). The resulting thick mixture was heated at reflux under nitrogen for 1 h, allowed to cool to room temperature, and then rotary evaporated to dryness in a hood. The residue was treated with 200 mL of anhydrous diethyl ether and filtered through Celite, and the Celite washed with an additional 100 mL of anhydrous diethyl ether. The combined ether solutions were rotary evaporated in vacuo, and the resulting yellowish oil was pumped free of residual benzene at room temperature to afford 6.1 g (quantitative) of **9a** as a pale yellow oil: ^1H NMR (270 MHz, $CDCl_3$) δ 7.36 (m, 5 H, C_6H_5), 4.71 (s, 2 H, $ArCH_2$), 4.17 (q, J = 7 Hz, 2 H, CH_2O), 1.28 (t, J = 7 Hz, 3 H,CH_3); IR (neat) 1720, 2180, 2985 cm^{-1}. Kugelrohr distillation of this material afforded 2.45 g (40%) of analytically pure **9a** (bp 75–85 °C (0.025 mmHg)). The infrared spectrum was identical with that of undistilled material. As distillation resulted in loss of product due to decomposition, freshly prepared undistilled **9a** was employed in subsequent reactions.

Anal. Calcd for $C_{11}H_{12}N_2O_2$: C, 64.69; H, 5.92; N, 13.72. Found: C, 64.45; H, 5.97; N, 13.91.

1-(Ethoxycarbonyl)-3-(4′-methoxybenzyl)carbodiimide (9b). Subsitution of **8b** for **8a** in the above procedure afforded an 94% yield of undistilled **9b** as an analytically pure yellow oil; ^1H NMR (270 MHz, $CDCl_3$) δ 7.26 (d, J = 8.7 Hz, 2 H, $^1/_2$ C_6H_4), 6.89 (d, J = 8.7 Hz, 2 H, $^1/_2$ C_6H_4), 4.65 (s, 1 H, $ArCH_2$), 4.15 (q, J = 7.2 Hz, 2 H, CH_2O), 3.79 (s, 3 H, $ArOCH_3$), 1.27 (t, J = 7.2 Hz, 3 H, CH_3); IR (neat) 1720, 2175, 2980 cm^{-1}.

Anal. Calcd for $C_{12}H_{14}N_2O_3$: C, 61.53; H, 6.02; N, 11.96. Found: C, 61.39; H, 6.12; N, 11.70.

1-(Benzyloxycarbonyl)-3-(4′-methoxybenzyl)carbodiimide (9c). Substitution of **8c** for **8a** in the procedure for the preparation of **9a** afforded a quantitative yield of undistilled **9c** as an analytically pure pale yellow oil: ^1H NMR (270 MHz, $CDCl_3$) δ 7.33 (m, 5 H, C_6H_5), 7.20 (d, J = 8.7 Hz, 2 H, Ar H), 6.93 (d, J = 8.7 Hz, 2 H, Ar H), 5.14 (s, 2 H, CH_2), 4.65 (s, 2 H, CH_2), 3.80 (s, 3 H, $ArOCH_3$); IR (neat) 1720, 2180, 2960 cm^{-1}.

Anal. Calcd for $C_{17}H_{16}N_2O_3$: C, 68.91; H, 5.44; N, 9.45. Found: C, 69.10; H, 5.50; N, 9.47.

1-(4,4′-Dimethoxybenzhydryl)-3-(ethoxycarbonyl)cyanamide (10). Substitution of **8d** for **8a** in the above procedure afforded, after purification by column chromatography (silica gel, $CHCl_3$ as eluent), a 54% yield of **10** as a thick colorless oil: ^1H NMR (270 MHz, $CDCl_3$) δ 7.21 (d, J = 8.7 Hz, 4 H, Ar H), 6.98 (d, J = 8.7 Hz, 4 H, Ar H), 6.29 (s, 1 H, benzhydryl CH), 4.27 (q, J = 7.2 Hz, 2 H, CH_2), 3.77 (s, 6 H, $ArOCH_3$), 1.23 (t, J = 7.2 Hz, 3 H, CH_3); IR (neat) 1755, 2240 (sharp), 2960 cm^{-1}.

1-(4,4′-Dimethoxybenzhydryl)-3-(ethoxycarbonyl)carbodiimide (9d). A solution of **8d** (1.87 g, 5.0 mmol) in 15 mL of anhydrous diethyl ether under nitrogen was cooled to 0 °C and treated dropwise with triethylamine (1.75 mL, 12.5 mmol) and then dropwise with phosgene (4.75 mL of a 12.5% solution in benzene, 5.5 mmol). The reaction mixture was stirred at room temperature for 1.5 h and then worked up as in the preparation of **9a** to afford 1.7 g (quantitative) of **9d** as a thick pale yellow oil: IR (neat) 1735, 1760, 2160 (br), 2960 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 7.24 (d, J = 8.7 Hz, 4 H, Ar H), 6.89 (d, J = 8.7 Hz, 4

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H, Ar H), 6.28 (s, 1 H, benzhydryl CH), 4.28 (q, J = 7.2 Hz, 2 H, CH_2), 3.73 (s, 6 H, $ArOCH_3$), 1.27 (t, J = 7.2 Hz, 3 H, CH_3). The use of any heat or prolonged reaction times in this procedure resulted in the partial isomerization of **9d** to **10**, as detected by the presence of a sharp absorption at 2240 cm^{-1} in the infrared spectrum of the product.

5-(3-Benzyl-3′-(ethoxycarbonyl)-1-guanidino)-1-(β-D-ribofuranosyl)imidazole-4-carboxamide (11a). A solution of 5-amino-1-(β-D-ribofuranosyl)imidazole-4-carboxamide (387 mg, 1.5 mmol) in 7 mL of anhydrous *N,N*-dimethylacetamide under nitrogen was treated dropwise with **9a** (552 mg, 2.3 mmol) and stirred at room temperature overnight. The reaction mixture was rotary evaporated to dryness in vacuo, and the residue was dissolved in 7 mL of methanol and rotary evaporated onto 1 g of silica gel. The silica gel was loaded onto the top of a column wet-packed with 80 g of silica gel using 20% methanol/chloroform as eluent, and the column was eluted with the same solvent system to afford 434 mg (64%) of **11a** as a pale yellow foam. Recrystallization from water afforded pure **11a** as a white powder: mp 118–121 °C; ^1H NMR (360 MHz, Me_2SO-d_6) δ 10.62 (s, exchanges, 1 H, guanidino H-1), 7.94 (t, J = 5.7 Hz, 1 H, guanidino H-3), 7.77 (s, 1 H, H-2), 7.43–7.23 (m, 5 H, C_6H_5), 7.34 (s, exchanges, 1 H, $CONH_a$), 7.19 (s, exchanges, 1 H, $CONH_b$), 5.58 (d, J = 4.4 Hz, 1 H, H-1′), 5.28 (d, J = 5.4 Hz, exchanges, 1 H, 2′-OH), 5.05 (d, J = 5.3 Hz, exchanges, 1 H, 3′-OH), 4.99 (t, J = 5.3 Hz, 1 H, 5′-OH), 4.54 (d of d, J = 5.7, 20 Hz, 2 H, $ArCH_2$), 4.14 (m, 1 H, H-2′), 4.05 (m, 3 H, H-3′ and CO_2CH_2), 3.80 (m, 1 H, H-4′), 3.66–3.49 (m, 2 H, 5′-CH_2), 1.17 (t, J = 7.1 Hz, 3 H, CH_3); IR (KBr) 1655, 1730, 2925, 3200–3500 cm^{-1}; UV λ_{max} nm (ε × 10^3) (pH 1) 250 (8.0), (CH_3OH) 278 (8.1), (pH 11) 268 (8.0); FAB–mass spectrum, m/z 463 (MH^+).

Anal. Calcd for $C_{19}H_{26}N_6O_7$: C, 50.66; H, 5.82; N, 18.66. Found: C, 50.46; H, 5.69; H, 18.76.

5-[3-(Ethoxycarbonyl)-3′-(4′-methoxybenzyl)-1-guanidino]-1-(β-D-ribofuranosyl)imidazole-4-carboxamide (11b). Substitution of **9b** for **9a** in the above procedure afforded a 64% yield of **11b** as a pale yellow foam. Recrystallization from water afforded pure **11b**: mp 116–118 °C; ^1H NMR (360 MHz, Me_2SO-d_6) δ 10.61 (s, exchanges with 1 H, guanidino H-1), 7.78 (s, 1 H, H-2), 7.38 (s, exchanges, 2 H, $CONH_a$), 7.35 (d, J = 8.6 Hz, 2 H, Ar H), 7.19 (s, exchanges, 1 H, 3′-OH), 5.01 (t, J = 5.3 Hz, exchanges, 1 H, 5-OH), 4.46 (d of d, J = 5.6, 15.5 Hz, 2 H, $ArCH_2$), 4.15 (m, 1 H, H-2′), 4.05 (m, 3 H, H-3′ and CO_2CH_2), 3.82 (m, 1 H, H-4′), 3.73 (s, 3 H, $ArOCH_3$), 3.67–3.49 (m, 2 H, 5′-CH_2), 1.17 (t, J = 7.1 Hz, 3 H, CH_3); IR (KBr) 1665, 1720, 2940, 3200–3500 cm^{-1}; ^{13}C NMR (90.4 MHz, Me_2SO-d_6) δ 166.7, 158.2, 153.5, 146.6, 139.3, 130.6, and 118.5 (quaternary), 130.9, 128.9, 113.6, 86.7, 84.1, 74.5, 69.5, and 30.5 (tertiary), 61.0, 60.7, and 44.0 (secondary), 54.9 and 14.4 (primary); UV λ_{max} nm (ε × 10^4) (pH 1) 250 (1.0), (CH_3OH) 276 (1.1), (pH 11) 273 (1.0); CI–mass spectrum (ammonia), m/z 481, 403, 361, 198, 181.

Anal. Calcd for $C_{20}H_{28}N_6O_8$: C, 50.00; H, 5.87; N, 17.49. Found: C, 49.89; H, 6.01; N, 17.61.

5-[3-(Benzyloxycarbonyl)-3′-(4′-methoxybenzyl)-1-guanidino]-1-(β-D-ribofuranosyl)imidazole-4-carboxamide (11c). Subsitution of **9c** for **9a** in the above procedure afforded a 54% yield of **11c** as a pale yellow foam. Recrystallization from water afforded pure **11c**: mp 141–144 °C; ^1H NMR (270 MHz, Me_2SO-d_6) δ 10.82 (s, exchanges, 1 H, guanidino H-1), 7.88 (t, J = 5.6 Hz, exchanges, 1 H, guanidino H-3), 7.78 (s, 1 H, H-2), 7.40–7.30 (m, 8 H, C_6H_5 and $^1/_2$ C_6H_4 and $CONH_a$ (exchanges)), 7.18 (s, exchanges, 1 H, $CONH_b$), 6.87 (d, J = 8.7 Hz, 2 H, $^1/_2$ C_6H_4), 5.61 (d, J = 4.4 Hz, 1 H, H-1′), 5.28 (d, J = 5.3 Hz, exchanges, 1 H, 2′-OH), 5.09 (s, 2 H, $ArCH_2O$), 5.06 (d, J = 5.3 Hz, exchanges, 1 H, 3′-OH), 4.99 (t, J = 5.2 Hz, exchanges, 1 H, 5′-OH), 4.48 (d of d, J = 5.8, 22.7 Hz, 2 H, $ArCH_2N$), 4.16 (m, 1 H, H-2′), 4.05 (m, 1 H, H-3′), 3.82 (m, 1 H, H-4′), 3.73 (s, 1 H, $ArOCH_3$), 3.70–3.54 (m, 2 H, 5′-CH_2); IR (KBr), 1655, 1730, 2920, 3200–3500 cm^{-1}; UV λ_{max} nm (ε × 10^4) (pH 1) 250 (1.0), (CH_3OH) 276 (1.1), (pH 11) 273 (1.0); CI–mass spectrum (ammonia), m/z 481, 361, 198, 181, 138, 121.

Anal. Calcd for $C_{26}H_{30}N_6O_8$: C, 56.31; H, 5.45; N, 15.15. Found: C, 56.13; H, 5.45; N, 14.97.

5-[(4,4′-Dimethoxybenzhydryl)amino]-1-(β-D-ribofuranosyl)imidazole-4-carboxamide (12). A mixture of 5-amino-1-(β-D-ribofuranosyl)imidazole-4-carboxamide (1.29 g, 5 mmol), **8d** (2.25 g, 6 mmol), and 1,3-dicyclohexylcarbodiimide (2.06 g, 10 mmol) in 10 mL of anhydrous *N,N*-dimethylacetamide under nitrogen was heated at 100 ± 5 °C for 3 h. The orange reaction mixture was allowed to cool to room temperature and then rotary evaporated in vacuo at 45 °C to afford a gum. The residue was dissolved in 5 mL of methanol at room temperature and absorbed onto 3 g of silica gel keeping the temperature below 30 °C. Column chromatography (300 g silica gel, 15% methanol/chloroform as eluent) afforded 1.10 g (43%) of **12** as a pale yellow foam, which reverted to starting material upon attempted recrystallization from hot water. Preparative thin-layer chromatography (20 cm × 20 cm × 0.5 mm silica gel plate, 20% methanol/chloroform as eluent) afforded pure **12**: ^1H NMR (270 MHz, Me_2SO-d_6) δ 7.57 (s, 1 H, H-2), 7.25 (d, J = 8.6 Hz, 2 H, $^1/_2$ C_6H_4), 7.20 (J = 8.6 Hz, 2 H, $^1/_2$ C_6H_4), 7.06 (s, exchanges, 1 H, $CONH_a$), 6.91 (s, exchanges, 1 H, $CONH_b$), 6.86 (d, J = 8.6 Hz, 4 H, $^1/_2$ C_6H_4 and $^1/_2$ C_6H_4), 6.69 (d, J = 11.3 Hz, exchanges, 1 H, 2′-OH), 5.25 (d, J = 5.2 Hz, exchanges, 1 H, 3′-OH), 5.04 (t, J = 5.1 Hz, exchanges, 1 H, 5′-OH), 4.25 (m, 1 H, H-2′), 4.04 (m, 1 H, H-3′), 3.91 (m, 1 H, H-4′), 3.71 (s, 6 H, $ArOCH_3$), 3.67–3.60 (m, 2 H, 5′-CH_2); IR (KBr) 1510, 1580, 1640, 1640, 2925, 3100–3500 cm^{-1}; UV λ_{max} nm (ε × 10^4) (pH 1) 270 (1.7), (CH_3OH) 273 (1.6), (pH 11) 272 (1.6).

By TLC analysis, treatment of AICA-riboside with **9d** in anhydrous *N,N*-dimethylacetamide at room temperature also afforded **12**, which reverted to starting material upon treatment with boiling methanol.

5-[3-(4-Methoxybenzyl)-1-guanidino]-1-(β-D-ribofuranosyl)imidazole-4-carboxamide (13). A mixture of **11c** (555 mg, 1.0 mmol) and palladium(II) acetate trimer (674 mg, 3.0 mmol Pd) in 5 mL of anhydrous *N,N*-dimethylformamide under nitrogen was warmed to 80 ± 5 °C and treated dropwise with 1,4-cyclohexadiene (1.0 mL). The black reaction mixture was stirred at 80 ± 5 °C for 4 h; an additional 1.0 mL of 1.4-cyclohexadiene was added after 2 h. The reaction mixture was allowed to cool to room temperature and diluted to 15 mL with anhydrous DMF, and the palladium black was separated by filtration and washed with 5 mL of anhydrous DMF. The combined filtrate and washing was rotary evaporated in vacuo at 50 °C. The residue was purified by column chromatography (80 g silica gel, 50% methanol/chloroform as eluent) to afford 240 mg (57%) of **13** as a pale yellow foam: ^1H NMR (270 MHz, Me_2SO-d_6) δ 7.65 (s, 1 H, H-2), 7.31 (d, J = 8.6 Hz, 2 H, $^1/_2$ C_6H_4), 7.01 (s, exchanges, 2 H, $CONH_a$), 6.89 (d, J = 8.6 Hz, 2 H, $^1/_2$ C_6H_4), 6.73 (s, exchanges, 1 H, $CONH_b$), 6.42 (t, J = 5 Hz, exchanges, 1 H, benzyl NH), 5.65 (s, exchanges, 2 H, guanidino NH_2), 5.57 (d, J = 4.5 Hz, 1 H, H-1′), 5.26 (m, exchanges, 1 H, 2′-OH), 5.07 (m, exchanges, 1 H, 3′-OH), 4.97 (m, exchanges, 1 H, 5′-OH), 4.34 (m, 2 H, $ArCH_2$), 4.15 (m, 1 H, H-2′), 4.04 (m, 1 H, H-3′), 3.79 (m, 1 H, H-4′), 3.73 (s, 3 H, $ArOCH_3$), 3.65–3.45 (m, 2 H, 5′-CH_2); IR (KBr) 1510, 1540, 1640, 1920, 3100–3500 cm^{-1}; UV λ_{max} nm (ε × 10^3) (pH 1) 264 (sh) (2.5) (CH_3OH) 264 (7.3), (pH 11) 263 (6.8).

1*H*-2-((Ethoxycarbonyl)amino)-9-(β-D-ribofuranosyl)-purin-6-one (*N*-2-(Ethoxycarbonyl)guanosine, 15). A solution of **11a** (333 mg, 0.72 mmol) in 15 mL of absolute ethanol was treated with 5 mL of cyclohexene and 50 mg of palladium(II) oxide, and the reaction mixture was heated at reflux under nitrogen for 48 h. An additional 50 mg of Pd(0) was added after 2, 4, and 6 h. The reaction mixture was allowed to cool to room temperature, filtered through Celite, and rotary evaporated to dryness in vacuo to afford 235 mg (92%) of **15** as a pale yellow foam. Column chromatography (100 g of silica gel, 15% methanol/chloroform as eluent) of this material afforded 187 mg (73%) of pure **15** as a colorless powder: mp 165–175 °C; ^1H NMR (200 MHz, Me_2SO-d_6) δ 11.40 (s, exchanges, 1 H, NH), 8.20 (s, 1 H, H-2), 5.77 (d, J = 5 Hz, 1 H, H-1′), 5.53 (m, exchanges, 1 H, OH), 5.23 (m, exchanges, 1 H, OH), 5.07 (t, J = 6 Hz, exchanges, 1 H, 5′-OH), 4.45 (m, 1 H, H-2′), 4.22 (q, J = 7 Hz, 2 H, CO_2CH_2), 4.14 (m, 1 H, H-3′), 3.90 (m, 1 H, H-4′), 3.59 (m, 2 H, H-5′), 1.26 (t, J = 7 Hz, 3 H, CH_3); IR (KBr) 1569, 1613, 1687, 2943, 3216 cm^{-1}; UV λ_{max} nm (ε × 10^4) (pH 1) 257 (1.3), (CH_3OH) 256 (1.1), (pH 11) 263 (1.2); FAB–mass spectrum, m/z 356 (MH^+), 378 (MNa^+).

When the reaction mixture from above was allowed to cool to room temperature after only 8 h at reflux and the product purified by preparative TLC (20 cm × 20 × 0.25 mm silica gel plate, 30% methanol/chloroform as eluent), the intermediate 5-(3-(ethoxycarbonyl)-1-guanidino)-1-(β-D-ribofuranosyl)imidazole-4-carbox-

amide (14) was obtained (20% yield) as a colorless powder: mp 94–96 °C (gas evolution above 110 °C); ^{1}H NMR (200 MHz, Me_2SO-d_6) δ 10.22 (s, exchanges, 1 H, guanidino NH), 7.77 (s, 1 H, H-2), 7.5–6.7 (b, exchanges, 2 H, guanidino NH_2), 7.12 (s, exchanges, 1 H, $CONH_a$), 6.90 (s, exchanges, 1 H, $CONH_b$), 5.49 (d, J = 4.5 Hz, 1 H, H-1′), 5.30 (m, exchanges, 1 H, OH), 5.05 (m, exchanges, 1 H, OH), 5.00 (m, exchanges, 1 H, OH), 4.20–4.00 (m, 4 H, H-2′, H-3′, and CO_2CH_2), 3.81 (m, 1 H, H-4′), 3.70–3.40 (m, 2 H, 5′-CH_2), 1.22 (t, J = 7.1 Hz, 3 H, CH_3); IR (KBr) 1653, 1701, 1734, 2964, 3417 cm^{-1}; UV λ_{max} nm (ε × 10^4) (pH 1) 237 (sh) (1.0), (CH_3OH) 263 (1.0); (pH 11) 261 (0.9); FAB–mass spectrum, m/z 373 (MH^+), 395 (MNa^+).

1*H*-2-Amino-9-(β-D-ribofuranosyl)purin-6-one (Guanosine, 16). A solution of **15** (25 mg, 0.07 mmol) in 1.5 mL of concentrated NH_4OH and 0.5 mL of pyridine was allowed to stand at 45 ± 1 °C in a sealed flask for 48 h. The reaction mixture was rotary evaporated to dryness in vacuo, and the residue was dissolved in 5 mL of water and again rotary evaporated. The residue was pumped dry at 50 °C in vacuo overnight to afford 19 mg (95%) of **16** as a white powder: mp 248–251 °C dec (lit. mp 235 °C[29]). A mixture melting point with authentic guanosine showed no depression. The infrared and ultraviolet (pH 1, H_2O, and pH 11) spectra were identical with those obtained from an authentic sample; TLC analysis (solvent system I[30]) showed the product to be homogeneous, and identical in R_f value (0.12) with authentic guanosine. FAB–mass spectrum, m/z 284 (MH^+).

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Registry No. **7** (R^1 = Et), 16182-04-0; **7** (R^1 = $PhCH_2$), 63220-36-0; **8a**, 54035-70-0; **8b**, 100313-31-3; **8c**, 100313-32-4; **8d**, 100313-33-5; **9a**, 100313-34-6; **9b**, 100313-35-7; **9c**, 100313-36-8; **9d**, 100313-37-9; **10**, 100313-38-0; **11a**, 100313-39-1; **11b**, 100334-11-0; **11c**, 100313-40-4; **12**, 100313-41-5; **13**, 100313-42-6; **14**, 100313-44-8; **15**, 100313-43-7; **16**, 118-00-3; H_2NCH_2Ph, 100-46-9; $H_2NCH_2C_6H$-*p*-OMe, 2393-23-9; $H_2NCH(C_6H_4$-*p*-OMe)2, 19293-62-0; ethyl chloroformate, 541-41-3; benzyl chloroformate, 501-53-1; benzyl thiocyanate, 3012-37-1; 5-amino-1-(β-D-ribofuranosyl)imidazole-4-carboxamide, 2627-69-2.

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Synthesis of Azotomycin[1]

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Total synthesis of the *Streptomyces ambofaciens* anticancer constituents (−)-azotomycin (1) from γ-OBzl-*N*-Boc-L-Glu (3) has been accomplished in nine steps. Selective protection with *N*-*tert*-butoxycarbonyl and *N*-trifluoroacetyl for the amino groups and benzyl or methyl esters for carboxyl groups, combined with the mixed carbonic anhydride peptide bond forming procedure, comprised the general strategy. Synthesis of bis diazo ketone 9 from dicarboxylic acid **8a** proved challenging and required development of precise experimental conditions for treating the diacid chloride intermediate with diazomethane. The (−)-azotomycin synthesis presents a useful alternative to the original fermentation–isolation route.

The biosynthetic virtuosity of *Streptomyces ambofaciens* began to be revealed over 30 years ago with discovery[2] of the spiramycin antibiotics (against gram-positive bacteria and rickettsia) of the erythromycin–carbomycin group. By 1960–1962 Rao had isolated and characterized, from the same microorganism, the anticancer constituents azotomycin (1)[3] and DON (2).[4] Evaluation of azotomycin against experimental neoplasms began in 1963, and Duvall[5a] reported consistent activity against a wide variety of animal tumors (predominantly murine leukemias). By 1968 azotomycin had exhibited excellent antineoplastic activity against the mouse sarcoma 180, carcinoma–755, L1210 lymphocytic leukemia, and the rat Walker 256 carcinoma.[6,7] In this period Brockman and co-workers[8] reported that azotomycin (1) was activated by in vivo conversion to DON (2). That route was further substantiated by a 1971 study.[9] And this was consistent with a more recent report[10] that azotomycin and DON have nearly identical antitumor activity against murine tumor systems such as the L1210 and P388 lymphocytic leukemias, C-26 and C-38 colon tumors, and $CD8F_1$ mammary carcinoma.

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