Heterocyclic Synthesis via a 1,3-Dicyclohexylcarbodiimide-Mediated Cyclodesulfurative Annulation Reaction. New Methodology for the Preparation of Guanosine and Guanosine-Type Nucleoside Analogues[1]

Michael P. Groziak, Ji-Wang Chern, and Leroy B. Townsend*

Department of Medicinal Chemistry, College of Pharmacy, and Department of Chemistry, University of Michigan, Ann Arbor, Michigan 48109-1065

Received July 15, 1985

Treatment of 5-amino-1-β-D-ribofuranosylimidazole-4-carboxamide (1, AICA-ribonucleoside) with methoxycarbonyl isothiocyanate followed by cyclodesulfurization of the resulting methoxycarbonylated thioureido derivative with 1,3-dicyclohexylcarbodiimide (DCC) has furnished 5-[3-(methoxycarbonyl)-1-ureido]-1-β-D-ribofuranosylimidazole-4-carbonitrile (6), not 2-[(methoxycarbonyl)amino]-9-β-D-ribofuranosylpurin-6-one (4). Using 1 labeled with ^{18}O in the carboxamide moiety, the conversion of 1 to 6 is shown to proceed with retention of the ^{18}O label. This finding has suggested the presence of a [1,3]oxazine intermediate in an intramolecular dehydration reaction mechanism. Under similar reaction conditions, methyl 5-amino-1-β-D-ribofuranosylimidazole-4-carboximidate (13) affords 6-methoxy-2-[(methoxycarbonyl)amino]-9-β-D-ribofuranosylpurine (14), which gives guanosine upon deprotection with iodotrimethylsilane. The use of this methoxycarbonyl isothiocyanate/DCC cyclodesulfurization method on heterocyclic *o*-amino carboximidate esters thus provides a highly efficient entry into the class of guanosine-type nucleoside analogues.

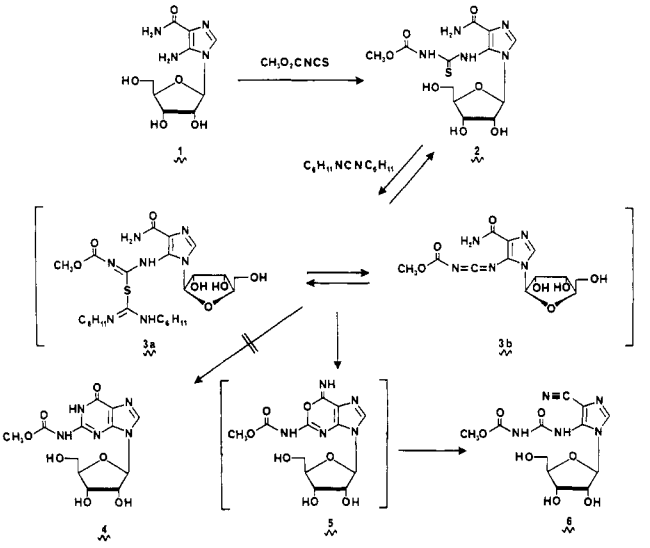
Alkoxycarbonyl isothiocyanates are highly reactive, versatile reagents that have been useful in the construction of a wide range of heterocyclic ring systems.[2] The reaction of an ortho-substituted heterocyclic amine with such a reagent, for example, affords a 3-(alkoxycarbonyl)-1-thioureido-substituted derivative, which, when subjected to cyclodesulfurative conditions, can be used to prepare annulated heterocyclic compounds. Recently, we have reported facile procedures for the synthesis of an alkyl 2-amino-6-(benzyloxy)purine-8-carbamate[3a] and an alkyl 6-amino-4-(benzylamino)-oxazolo[5,4-*d*]pyrimidine-2-carbamate[3b] using the mild method of cyclodesulfurization of 3-(alkoxycarbonyl)-1-thioureido derivatives with 1,3-dicyclohexylcarbodiimide (DCC).[4]

In order to explore the scope of this mild alkoxycarbonyl isothiocyanate/DCC annulation methodology, we have initiated additional studies using various heterocyclic precursors. Our initial goal was the synthesis of guanosine from 5-amino-1-β-D-ribofuranosylimidazole-4-carboxamide (1, AICA-ribonucleoside) under reaction conditions similar to those employed in our previous cyclizations.[3] The preparation of guanosine from AICA-riboside was first reported by Yamazaki and co-workers in 1967,[5a] and although the yield has subsequently been improved,[5b] we felt that the use of the alkoxycarbonyl isothiocyanate/DCC method might afford a milder and higher yielding alternate synthesis of guanosine and, consequently, of guanosine-type analogues.

Results and Discussion

AICA-riboside (1) was condensed with methoxycarbonyl isothiocyanate[2b,6] in DMF at room temperature to afford the desired 5-[3-(methoxycarbonyl)-1-thioureido]-1-β-D-ribofuranosylimidazole-4-carboxamide (2). On the basis of the results of similar cyclocondensations,[3,4] we expected that treatment of 2 with DCC would afford a reactive carbodiimide intermediate[3b] that would then ring close to give 2-[(methoxycarbonyl)amino]-9-β-D-ribofuranosylpurin-6-one [N^2-(methoxycarbonyl)guanosine (4)] (Scheme I). Guanosine could then be obtained by a simple saponification of 4.

Scheme I



In order to effect the cyclodesulfurization, compound 2 was treated with an excess of DCC at room temperature and the product mixture was purified by column chromatography to afford 1,3-dicyclohexylthiourea and a sole nucleoside product that initially appeared to be the desired N^2-methoxycarbonylated guanosine derivative 4, based upon the ^1H NMR spectral and elemental analysis data. We realized, however, that based upon these criteria alone the product could be not only 4 but also 1-(4-carbamoyl-1-β-D-ribofuranosylimidazol-5-yl)-3-(methoxycarbonyl)-carbodiimide (**3b**), 7-imino-5-[(methoxycarbonyl)-amino]-3-β-D-ribofuranosylimidazo[4,5-*d*][1,3]oxazine (5),

(1) A preliminary report of this research has been presented: Groziak, M. P.; Chern, J.-W.; Townsend, L. B. 10th International Congress of Heterocyclic Chemistry, Waterloo, Ontario, Canada 11–16 Aug 1985; P3–82 (Absts).

(2) For recent reviews of ethoxycarbonyl isothiocyanate, see: George B.; Papadopaulos, E. P. *J. Heterocycl. Chem.* **1983**, *20*, 1127. Esmail, R.; Kurger, F. *Synthesis* **1975**, 301.

(3) (a) Ram, S.; Wise, D. S.; Townsend, L. B. *Heterocycles* **1984**, *22*, 1789. (b) Chern, J.-W.; Wise, D. S.; Townsend, L. B. *J. Heterocycl. Chem.* **1984**, *21*, 1245.

(4) For the DCC-mediated cyclodesulfurization of *o*-(3-alkyl/aryl-1-thioureido)anilines, -phenols, and -thiophenols, see: Omar, A. M. M. E.; Habib, N. S.; Aboulwafa, O. M. *Synthesis* **1977**, 864.

(5) (a) Yamazaki, A.; Kumashiro, I.; Takenishi, T. *J. Org. Chem.* **1967**, *32*, 1825. (b) For a review of cyclization reactions of AICA-ribonucleoside, see: Yamazaki, A.; Okutsu, M. *J. Heterocycl. Chem.* **1978**, *15*, 353.

(6) (a) Lanon, R. W. *J. Heterocycl. Chem.* **1968**, *5*, 837. (b) Gensler, W. J.; Chan, S.; Ball, D. B. *J. Org. Chem.* **1981**, *46*, 3407.

0022-3263/86/1951-1065$01.50/0

Table I. Theoretical and Experimental Mass Spectral Data for 1, 9, 11a, 11b, 12a, and 12b

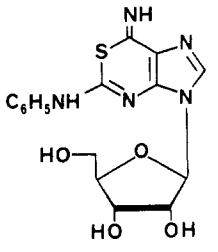
compd[a]	parent ion, m/e	M	M + 1	M + 2	M + 3	M + 4	% ^{18}O[b]
1 (n = 2)	258 (M^+)	100.00	19.46	1.60			
1 (theor)[c]		100.00	11.91	1.64			
9 (n = 3)		19.59	3.36	100.00	18.19	1.11	84 ± 4
11a (n = 2)	557 (M^+)	100.00	29.45	16.09			
11a (theor)		100.00	41.37	19.48			
11b (n = 4)		26.36	5.92	100.00	38.44	6.28	78 ± 9
12a (n = 2)	629 (M^+)	100.00	46.10	28.52			
12a (theor)		100.00	49.91	26.59			
12b (n = 2)		28.31	13.92	100.00	45.75	19.69	77 ± 8

[a] The number in parentheses is the number of individual spectra used to obtain the averaged values. [b] See the Experimental Section for details. [c] The theoretical values were calculated by the methods described in ref 20.

or 4-cyano-5-[3-(methoxycarbonyl)-1-ureido]-1-β-D-ribofuranosylimidazole (**6**). The UV and ^{13}C NMR spectral data for this product were not consistent with those expected for an N-2-acylated guanosine derivative. It was of considerable interest that the infrared spectrum of the product showed a strong, sharp absorption at 2230 cm^{-1}, which supports the presence of a cyano group in the molecule. Therefore, on the basis of ^1H NMR, ^{13}C NMR, IR, UV spectral, and elemental analysis data, we have assigned the structure of this unexpected product as the 4-cyanoimidazole nucleoside **6**.

Since **1** was found to be inert to DCC under the same reaction conditions, the dehydration of the 4-carboxamide moiety of **2** to afford the 4-cyanoimidazole nucleoside **6** must be the result of an initial reaction of the 3-(methoxycarbonyl)-1-thioureido substituent with the carbodiimide reagent. As shown in Scheme I, the formation of **6** from **2** most probably involves the initial reaction of the 5-acylthiourea substituent with DCC to form the intermediate **3a**, which may afford the carbodiimide intermediate **3b** upon loss of 1,3-dicyclohexylthiourea.[3] Ring cyclization by an intramolecular attack of the 4-carboxamide oxygen atom on the sp^2-hybridized carbon atom of the isothiourea moiety of **3a** and loss of 1,3-dicyclohexylthiourea or by a similar intramolecular attack on the sp-hybridized carbon atom of the carbodiimide moiety of **3b** then occurs to form the [1,3]oxazine intermediate **5**. The ring opening of **5** by a proton transfer then affords the product **6**.

The implication of the [1,3]oxazine intermediate **5** in the reaction sequence is of interest in that a similar [1,3]-thiazine analogue (**7**) has been reported to be stable to



7

isolation under neutral conditions but affords the thioguanosine analogue upon treatment with aqueous hydroxide,[7a] presumably via a Dimroth-type rearrangement. The presence of the strongly electron-withdrawing methoxycarbonyl group in **5** may account for its instability relative to **7**. This group would be expected to weaken the NH bond of the imino group of **5** through the tautomer that possesses two exocyclic carbon–nitrogen double bonds; the imino NH bond of **7** would not experience this effect. Of related interest is the stable nucleoside antibiotic oxanosine, the [1,3]oxazine derivative of guanosine.[7b]

There are several possible reasons why the oxygen atom and not the nitrogen atom of the carboxamide moiety of **3** acts as the nucleophile in this specific ring-closure reaction. Since we have found that **6** could also be obtained from **2** by treatment with methoxycarbonyl isothiocyanate followed by treatment with the mildly acidic DCC–pentafluorophenol complex,[8] the possibility of excess reagent acting as a base to remove a proton from the carboxamide nitrogen atom of **3** is unlikely. A more reasonable explanation is that the "hard" end of the neutral nucleophilic carboxamide group (the oxygen atom) reacts with the "hard" electrophilic group at the 5-position of the imidazole ring of **3**.[9] In a related study, Hegarty and Bruice found that the nature of the alkoxy leaving group ability in various 2-ureidobenzoic acid esters determined whether these compounds underwent cyclization under neutral conditions via nitrogen atom attack to afford 2,4-(1*H*,3*H*)-quinazolinedione or via oxygen atom attack to afford 2-amino-3,4-benzo-6-oxo-1,3-oxazine.[10]

Interestingly, other workers have reported no dehydration of the 4-carboxamide substituent in AICA-ribonucleoside analogues in which an acylcarbodiimide substituent is apparently generated from an *S*-methyl isothioureido substituent at the C-5 position of the imidazole ring.[5b,11] Instead, these workers report the isolation of a cyclonucleoside in which the 2′-hydroxyl group has added in a Michael fashion to the acylcarbodiimide substituent. Although attempts to form a similar cyclonucleoside from **6** by treatment with a large excess of DCC resulted only in the recovery of starting material, we had to consider the possibility of such a cyclonucleoside as an intermediate in the conversion of **2** to **6**.

In order to provide evidence for the putative intramolecular oxygen atom transfer in the conversion of **2** to **6**, and hence for the [1,3]oxazine intermediate **5**, we undertook a mass spectral study of this reaction. The hydrolysis of nitriles to carboxamides with alkaline hydrogen peroxide is known to proceed through a peroxycarboximidic acid intermediate;[12] thus, the use of ^{18}O-labeled hydrogen peroxide in this procedure should afford ^{18}O-labeled carboxamides. Using a published procedure,[13] we prepared

(7) (a) Omura, K.; Marumoto, R.; Furukawa, Y. *Chem. Pharm. Bull.* **1981**, *29*, 1970. (b) Yagisawa, N.; Takita, T.; Umezawa, H. *Tetrahedron Lett.* **1983**, *24*, 931.

(8) Kovacs, J.; Kisfaludy, L.; Ceprim, M. Q. *J. Am. Chem. Soc.* **1967**, *89*, 183.

(9) For a review of hard and soft acid/base theory, see: Ho, T.-L. *Chem. Rev.* **1975**, *75*, and references contained therein.

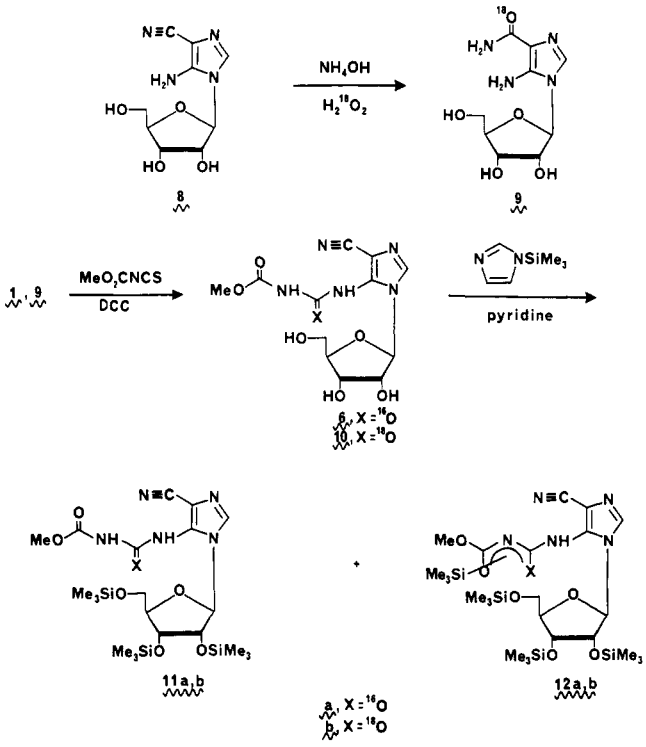
(10) (a) Hegarty, A. F.; Bruice, T. C. *J. Am. Chem. Soc.* **1970**, 92, 6575. (b) Bruice, T. C.; Hegarty, A. F. *Proc. Natl. Acad. Sci. U.S.A.* **1970**, *65*, 805.

(11) (a) Okutsu, M.; Yamazaki, A. *Nucleic Acids Res.* **1976**, *3*, 237. (b) Yamazaki, A. Okutsu, M.; Yamada, Y. *Nucleic Acids Res.* **1976**, *29*, 1870.

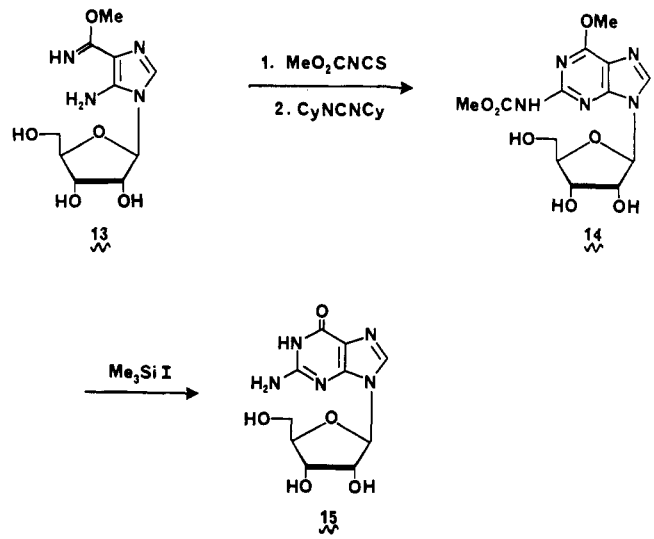
(12) (a) Wiberg, K. *J. Am. Chem. Soc.* **1953**, *75*, 3961. (b) Payne, G. B.; Deming, P. H.; Williams, P. H. *J. Org. Chem.* **1974**, *39*, 2667.

(13) (a) Sawaki, Y.; Foote, C. S. *J. Am. Chem. Soc.* **1979**, *101*, 6292. (b) Rosenthal, I. *J. Labelled Compd. Radiopharm.* **1976**, *12*, 317.

Scheme II



Scheme III



^{18}O-labeled hydrogen peroxide from ^{18}O-labeled oxygen gas by autooxidation of benzhydrol in the presence of potassium *tert*-butoxide and hydrolyzed 5-amino-1-β-D-ribofuranosylimidazole-4-carbonitrile (**8**, AICN-ribonucleoside)[14] with concentrated ammonium hydroxide in the presence of this ^{18}O-labeled hydrogen peroxide (Scheme II). The ^{18}O-labeled AICA-ribonucleoside **9** thus obtained was analyzed by electron impact–mass spectrometry and was found to possess 84% ^{18}O atom content (Table I). Compound **9** was then treated with methoxycarbonyl isothiocyanate followed by DCC to obtain a 4-cyanoimidazole nucleoside product. The product obtained from **9** was heated with an excess of (trimethylsilyl)-imidazole in anhydrous pyridine, and the reaction mixture was analyzed by gas chromatography–mass spectrometry. Two sets of spectral lines in the mass spectrum were observed that, on the basis of the molecular weights, were determined to be due to the tris(trimethylsilyl) derivative **11** and the tetrakis(trimethylsilyl) derivative **12**. Analysis of the mass spectra of **11** and **12**, derived from the ^{18}O-labeled precursor **9**, indicated that all of the ^{18}O label had been retained within experimental error, during the methoxycarbonyl isothiocyanate/DCC reaction and thus that the product of this reaction was **10**, the ^{18}O-labeled derivative of **6**. The results of the mass spectral study are in agreement with an intramolecular oxygen atom transfer in the mechanism of the reaction **2** → **6** and thus provide indirect evidence for the postulated [1,3]oxazine intermediate **5**.

The formation of nucleoside **6** from the reaction of **2** and DCC is very closely related to the dehydration observed when asparagine and N-substituted maleamic acids are treated with DCC.[15] In the case of asparagine, the dehydration was found to proceed via the internal acylation of the amide oxygen atom, followed by a ring opening of the oxazine intermediate to afford a nitrile product. This mechanism is essentially identical with the one proposed by us for the conversion of **2** to **6**.

The use of an imidate ester derivative of **1** as starting material would provide for the exclusivity of nitrogen atom attack in the cyclodesulfurization reaction and thus would circumvent the DCC-mediated dehydration reaction observed with **1**. Indeed, when methyl 5-amino-1-β-D-ribofuranosylimidazole-4-carboximidate (**13**)[16] was treated with methoxycarbonyl isothiocyanate followed by DCC, the desired 6-methoxy-2-[(methoxycarbonyl)amino]-9-β-D-ribofuranosylpurine (**14**) was obtained in good yield (Scheme III). Compound **14** was then smoothly deprotected with iodotrimethylsilane[17] to afford guanosine.

The use of this alkoxycarbonyl isothiocyanate/DCC cyclodesulfurative ring closure methodology on a heterocyclic *o*-amino amide results in an intramolecular dehydration reaction, most probably proceeding through a [1,3]-oxazine intermediate, and affords an *o*-[3-(alkoxycarbonyl)-1-ureido] carbonitrile as product. This methodology, when applied to a heterocyclic *o*-amino carboximidate ester, however, smoothly effects of pyrimidine ring annulation reaction and affords an N^2-(alkoxycarbonyl)-O^6-alkylguanine derivative that is readily transformed into the guanine analogue. This mild and highly efficient ring-closure methodology is therefore ideally suited to the formation of guanosine-type nucleoside analogues from appropriate *o*-amino carboximidate ester precursors.

Experimental Section

General Methods. Melting points were obtained on a Thomas-Hoover Unimelt apparatus and are uncorrected. All rotary evaporations were conducted at less than 50 °C, using either a water aspirator or vacuum pump. Infrared spectra were recorded on a Perkin-Elmer Model 281 spectrophotometer; ^1H and ^{13}C nuclear magnetic resonance spectra were recorded on a Bruker Model WM-360SY spectrometer, using Me_2SO-d_6 as solvent and Me_2SO-d_5 as internal standard. UV spectra were recorded on a Hewlett-Packard Model 8450a UV/vis spectrophotometer with a Model 9875A cartridge tape unit and a Model 7245A printer plotter. Mass spectra were obtained on a Finnigan Model 4021 GC/MS/DC spectrometer (70 eV), and gas chromatography was

(14) AICN-ribonucleoside was prepared according to the method described in: *Chem. Abstr.* **1969**, *71*, P12561a; Jpn. Patent 69 05 225.

(15) (a) Ressler, C.; Ratzkin, H. *J. Org. Chem.* **1961**, *26* 3356. (b) Kashelikar, D. V.; Ressler, C. *J. Org. Chem.* **1964**, *86*, 2467. (c) Paul, R.; Kende, A. S. *J. Am. Chem. Soc.* **1964**, *86*, 4162.

(16) Srivastava, P. C.; Ivanovics, G. A.; Rousseau, R. J.; Robins, R. K. *J. Org. Chem.* **1975**, *40*, 2920.

(17) (a) Silverman, R. B.; Radak, R. E.; Hacker, N. P. *J. Org. Chem.* **1979**, *44*, 4970. (b) Matsuda, A.; Satoh, K.; Miyasaka, T.; Ueda, T. *Chem. Pharm. Bull.* **1984**, *32*, 2048.

performed on a 6 ft by 2 mm i.d. glass column (5% SE-30, 250 °C) by Jim Houdak at the University of Michigan. Analtech thin-layer silica gel chromatography plates were used for TLC analysis; Merck silica gel 60 from Anspec, Ann Arbor, MI, was used for column chromatography. Merck preparative TLC plates (silica gel, 20 × 20 cm, 0.5 mm thick) were also obtained from Anspec.

^{18}O-labeled oxygen gas (99%) was purchased from Stohler Isotope Chemicals. Methyl chloroformate, potassium thiocyanate, 1,3-dicyclohexylcarbodiimide (DCC), iodotrimethylsilane, and potassium *tert*-butoxide were purchased from the Aldrich Chemical Co. Tri-Sil Z [*N*-(trimethylsilyl)imidazole in anhydrous pyridine] is a trademark of the Pierce Chemical Co. AICA-ribonucleoside was purchased from United States Biochemical Corp.

Anhydrous benzene was distilled from sodium benzophenone ketyl under nitrogen; *N*,*N*-dimethylformamide (DMF) was distilled from CaH_2 under nitrogen and stored over 4-Å molecular sieves. Absolute methanol was distilled from $Mg(OCH_3)_2$ and stored under nitrogen. Benzhydrol was obtained from the University of Michigan Chemistry Department and was recrystallized from low-boiling petroleum ether before use.

5-[3-(Methoxycarbonyl)-1-ureido]-1-β-D-ribofuranosylimidazole-4-carbonitrilie (6). Freshly distilled methoxycarbonyl isothiocyanate[2b,6] (351 mg, 3.0 mmol) was added to a solution of AICA-ribonucleoside (516 mg, 2.0 mmol) in 4 mL of anhydrous DMF under nitrogen, and the solution was stirred at room temperature for 2.5 h. The reaction mixture was then treated with DCC (619 mg, 3.0 mmol) and stirred at room temperature for 6 h. The reaction mixture was rotary evaporated to dryness in vacuo and the residue dissolved in 20 mL of warm absolute ethanol. The solution was rotary evaporated onto 2 g of silica gel, which was then loaded onto a column of silica gel (30 g, 1.8 × 22 cm) slurry-packed in 5% ethanol/ethyl acetate. Elution with this same solvent system afforded 346 mg (72%) of 1,3-dicyclohexylthiourea: mp 180–181 °C (from CH_3CN, lit.[18] mp 176–178 °C); IR spectrum, identical with that of an authentic sample. Further elution of the column with 10% ethanol/ethyl acetate afforded 476 mg (70%) of **6**: mp 176.5–177.5 °C; ^1H NMR (Me_2SO-d_6) δ 10.80 (s, 1 H, NH), 9.70 (s, 1 H, NH), 8.14 (s, 1 H, H-2), 5.58 (b, 1 H, OH), 5.46 (d, J = 4.2 Hz, 1 H-1′), 5.22 (d, 1 H, OH)), 5.09 (s, 1 H, OH), 4.17 (b, 1 H, H-2′), 4.04 (m, 1 H, H-3′), 3.90 (m, 1 H, H-4′), 3.74 (s, 3 H, OCH_3), 3.63–3.53 (m, 1 H, H-5′); ^{13}C NMR ($MeSO_2$-d_6) δ 154.5, 150.8, 135.0, 133.8, 114.4, 108.4, 88.6, 85.4, 75.0, 69.6, 60.5, 52.9; IR (KBr) 1600, 1700–1730, 2230, 2850, 2930, 3260, 3320 cm^{-1}; UV λ_{max} nm ($\epsilon \times 10^4$) (pH 1) 228 (9.2), (CH_3OH) 227 (9.0), (pH 11) 272 (1.0). Anal. Calcd for $C_{12}H_{15}N_5O_7$: C, 42.23; H, 4.43; N, 20.52. Found: C, 41.97; N, 4.49; N, 20.38.

The use of water as the solvent in the above procedure furnished **6**. A substitution of the DCC–pentafluorophenol complex[7] for DCC in this procedure also gave **6**; however, both of these latter procedures furnished a lower yield of **6**.

5-Amino-1-β-D-ribofuranosylimidazole-4-carbonitrile (8, AICN-ribonucleoside). This compound was prepared according to a literature procedure.[14] **8**: IR (KBr) 1580, 1670, 2200, 3100–3500 cm^{-1}.

^{18}O-Labeled Hydrogen Peroxide.[13a] A three-necked round-bottom flask, flame-dried under nitrogen, was charged with potassium *tert*-butoxide (833 mg, 7.5 mmol), evacuated with a water aspirator, and then charged with ^{18}O-labeled oxygen gas (99%) by displacement with anhydrous benzene. A solution of benzhydrol (1.38 g, 7.5 mmol) in 20 mL of anhydrous benzene was then added from a nonpressure equalizing addition funnel. As the ^{18}O-labeled oxygen gas was consumed, this solution was drawn into the reaction vessel. As soon as all of the benzhydrol solution had been added, the stopcock to the addition funnel was closed, and the remainder of the ^{18}O-labeled oxygen gas was transfered by displacement with anhydrous benzene. The solution was stirred for 1 h at room temperature, and then the $H_2^{18}O_2$ was extracted into 3.75 mL of a 2 N aqueous HCl solution.

5-Amino-1-β-D-ribofuranosylimidazole-4-[^{18}O]carboxamide (9). A solution of **8** (240 mg, 1.0 mmol) in 1 mL of concentrated NH_4OH was treated dropwise with the aqueous solution of ^{18}O-labeled hydrogen peroxide (vide supra). The reaction mixture was stirred at room temperature overnight and then rotary evaporated to dryness. Column chromatography (40 g of silica gel, 1.8 × 28 cm, 20% methanol/chloroform as eluent) of the residue afforded 227 mg (88%) of **9**. Recrystallization of this residue from methanol gave white crystals: mp 216–219 °C (lit.[19] mp 215–216 °C); IR spectrum, identical with that of an authentic, unlabeled sample.

5-[3-(Methoxycarbonyl)-1-[^{18}O]ureido]-1-β-D-ribofuranosylimidazole-4-carbonitrile (10). Freshly distilled methoxycarbonyl isothiocyanate[2b,6] (4.4 mL, 0.045 mmol) was added to a solution of **9** (11 mg, .043 mmol) in 0.2 mL of anhydrous DMF under nitrogen and the resultant mixture stirred at room temperature for 2 h. The solution was then treated with DCC (10.3 mg, 0.050 mmol) and stirred at room temperature overnight. The reaction mixture was applied directly to a preparative TLC plate (20 × 20 cm, 0.5-mm thickness) and eluted with 20% methanol/chloroform to afford 12 mg (82%) of **10** as a pale yellow oil; IR spectrum was identical to that obtained for **6**.

Tris(trimethylsilyl) and Tetrakis(trimethylsilyl) Derivatives of 6 and 10 (11, 12a and 11, 12b, Respectively). A mixture of **6** or **10** (5–6 mg) in 0.5 mL of Tri-Sil Z (1.5 mg/mL) was heated in a sealed vial at 90 ± 5 °C for 1 h and then allowed to cool to room temperature. This solution was injected directly for a GC/MS determination.

Mass Spectral Analysis. According to the method of Sawaki and Foote,[13a] the percentage of ^{18}O atom content was determined from the average of the five-line normalized spectra by (1) determining the value of M + 2 attributable to the M + 2 peak of unlabeled constituent (using the theoretical M + 2 value[17] and actual M value observed), (2) subtracting this value from the observed M + 2 value, and (3) using the relative ratio of (M + 2 corrected)/[(M + 2 corrected) + (M observed)].

This method is justified by the fact that the observed M + 2 values for **1**, **10**, and **11** are very close to the calculated values[20] (see Table I). A representative calculation (for **9**) is given: M + 2 corrected = M + 2 observed-[(M observed)(M + 2 theoretical %)]; % ^{18}O content = [(M + 2 corrected)(100%)]/[(M observed) + (M + 2 corrected)]. Thus, M + 2 corrected for **9** = 100 − [(19.59)(0.0164)] = 99.68 and % ^{18}O content for **9** = [(99.68)(100%)]/(19.59 + 99.68) = 84%.

6-Methoxy-2-[(methoxycarbonyl)amino]-9-β-D-ribofuranosylpurine (14). A suspension of methyl 5-amino-1-β-D-ribofuranosylimidazole-4-carboximidate[15] (100 mg, 1.5 mmol) in 4.5 mL of anhydrous DMF under nitrogen was treated with methoxycarbonyl isothiocyanate[2b,6] (193 mg, 1.67 mmol). The reaction mixture was stirred under nitrogen overnight, treated with DCC (412 mg, 2.0 mmol), and again stirred overnight (16 h). The reaction mixture was then rotary evaporated onto 2 g of silica gel and applied to the top of a column (1.8 × 2.5 cm) wet packed with 35 g of silica gel using 20% methanol/chloroform as eluent. Elution with this same solvent system afforded 320 mg (60%) of **14** as a pale yellow solid, which was recrystallized from methanol to afford pure **14**: mp 222–224 °C dec; ^1H NMR (Me_2SO-d_6) δ 10.34 (s, 1 H, exchanges with D_2O, NH), 8.43 (s, 1 H, H-8), 5.88 (d, J = 6.0 Hz, 1 H, H-1′), 5.48 (d, exchanges with D_2O, 1 H, OH), 5.18 (d, exchanges with D_2O, 1 H, OH), 4.94 (t, exchanges with D_2O, 1 H, 5′-OH), 4.61 (m, 1 H, H-2′), 4.18 (m, 1 H, H-3′), 4.07 (s, 3, 6-OCH_3), 3.91 (m, 1 H, H-4′), 3.67 (s, 3 H, CH_3O_2Ce, 3.63 and 3.55 (m, 2 H, 5′-CH_2); IR (KBr) 1605, 1740, 3260, 3330, 3390 cm^{-1}; UV λ_{max} nm ($\epsilon \times 10^4$) (pH 1) 270 (1.1), (CH_3OH) 267 (1.4), (pH 11) 266 (1.2). Anal. Calcd for $C_{13}H_{17}N_5O_7$: C, 43.95; H, 4.82; N, 19.71. Found: C, 44.18; N, 5.03; N, 19.73. CI-mass spectrum (ammonia), m/z: 356 ($M + H^+$), 283, 238, 224, 105, 88.

2-Amino-9-β-D-ribofuranosylpurin-6-one (Guanosine, 15). Iodotrimethylsilane (1 mL) was added to a suspension of 6-methoxy-2-[(methoxycarbonyl)amino]-9-β-D-ribofuranosylpurine (**14**; 13 mg) in 5 mL of anhydrous acetonitrile. The mixture was stirred at room temperature. The reaction mixture was completely converted into a single product, but this was not the desired guanosine (as determined by TLC). After 24 h at room tem-

(18) Alliger, G.; Smith, G. E. P.; Carr, E. L.; Stevens, H. P. *J. Org. Chem.* **1949**, *14*, 962.

(19) Panzica, R. P.; Townsend, L. B. *J. Org. Chem.* **1971**, *36*, 1594.

(20) McLafferty, F. W. "Interpretation of Mass Spectra"; University Science Books: Mill Valley, CA, 1980.

perature, the mixture was heated to reflux in an oil bath. The mixture was then evaporated to dryness in vacuo (water pump) at 70 °C, with anhydrous acetonitrile (5 mL) and iodotrimethylsilane (0.5 mL) then being added to the residue. The mixture was heated to reflux in an oil bath, and after 1 h, a complete conversion into guanosine had been effected as determined by comparing the reaction mixture with authentic guanosine by TLC.

Acknowledgment. This investigation was supported by Grant No. CA-28381 awarded by the National Cancer Institute, by DHEW, and in part by the UNDP/World Bank/WHO Special Program for Research and Training in Tropical Diseases and the Scientific Working Group on Filariasis (I.D. 840398). We also thank Deanna VanSickle for assistance in the preparation of this paper and acknowledge the NIH Biomedical Research Support (Grant RR01437) for funds toward the purchase of an IBMWP-270SY NMR spectrometer.

Registry No. 1, 2627-69-2; **6**, 99268-01-6; **8**, 23192-64-5; **9**, 99268-02-7; **10**, 99268-03-8; **11a**, 99268-04-9; **11b**, 99268-05-0; **12a**, 99268-22-1; **12b**, 99268-23-2; **13**, 56086-84-1; **14**, 99268-06-1; **15**, 118-00-3; $^{18}O_2$, 32767-18-3.

Highly Selective Total Synthesis of Enantiomerically Pure (–)-Anisomycin

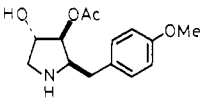
Hideo Iida, Naoki Yamazaki, and Chihiro Kibayashi*

Tokyo College of Pharmacy, Horinouchi, Hachioji, Tokyo 192-03, Japan

Received October 2, 1985

A chiral total synthesis of optically pure (–)-anisomycin (**1**) has been achieved. The method consists of the virtually complete regio- and stereocontrolled reactions involving no separation of isomers through the entire sequence. The scheme starts with the readily available 4-*O*-benzyl-2,3-*O*-bis(methoxymethyl)-L-threose (**5**) from diethyl L-tartrate as the chiral building block and involves highly selective α-chelation-controlled addition of hydride using $Zn(BH_4)_2$ to (3*R*,4*S*)-5-(benzyloxy)-3,4-bis[(methoxymethyl)oxy]-1-(4-methoxyphenyl)pentan-2-one (**9**) yielding the *lyxo* alcohol **8b** and stereospecific cyclization of (2*S*,3*R*,4*S*)-1-azido-2,3-bis[(methoxymethyl)-oxy]-5-(4-methoxyphenyl)-4-[(methylsulfonyl)oxy]pentane (**11**) to the (2*R*,3*S*,4*S*)-anisylpyrrolidine **12** via intramolecular S_N2 displacement. At the final critical stage, the selective introduction of the acetyl group into *N*-(benzyloxycarbonyl)deacetylanisomycin (**14**) is achieved with complete regiochemical control via the reaction sequence involving protection by the *tert*-butyldimethylsilyl group followed by acetylation–deprotection.

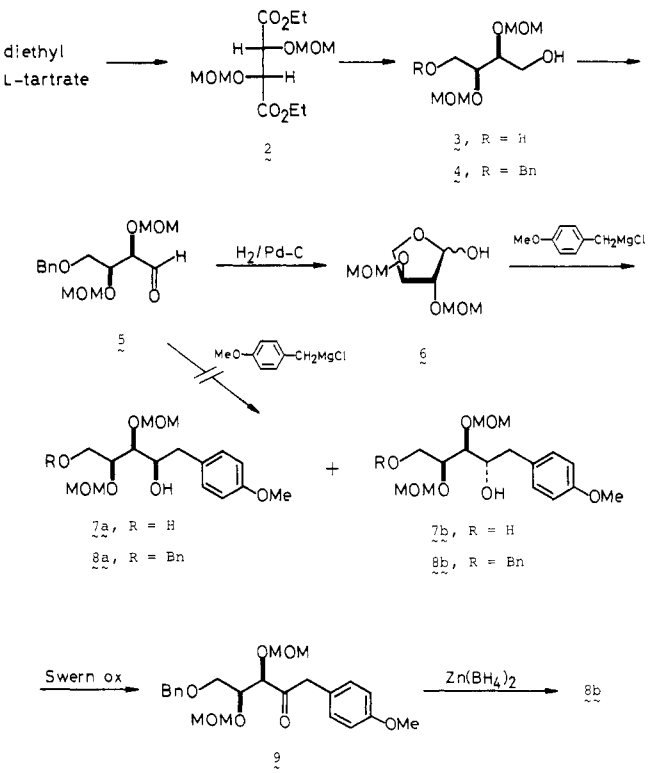
Anisomycin (**1**) is an antibiotic that has been isolated from fermentation broth filtrates of various species of *Streptomyces*.[1] The structure and relative stereochem-



istry of anisomycin were first studied chemically[2] and then determined by X-ray crystallographic analysis[3] to be **1**. The absolute stereochemistry was established as 2*R*,3*S*,4*S* by chemical correlation studies.[4] Anisomycin possesses strong and selective activities against pathogenic protozoa and fungi and has been used successfully clinically in the treatment of amebic dysentery and trichomonas vaginitis.[5] It has been shown to block ribosomal peptide synthesis.[5]

Anisomycin has attracted considerable synthetic interest, and two total syntheses of the racemic form[6] and four chiral syntheses[7] have been reported. Of the chiral syntheses two early ones starting from tartaric acid[7a] and diethyl tartrate[7b] were nonstereoselective and resulted in very low overall yields. Alternative elegant approaches by Moffatt et al.[7c] and Buchanan et al.[7d] have used carbohydrates as chiral templates. A problem encountered in the synthesis of anisomycin has been the chemo-, regio-, and stereoselective introduction of the acetyl group in the 3-position of the pyrrolidine ring. To resolve this problem,

Scheme I



(1) Sobin, B. A.; Tanner, F. W., Jr. *J. Am. Chem. Soc.* **1954**, *76*, 4053.
(2) Beereboom, J. J.; Butler, K.; Pennington, F. C.; Solomons, I. A. *J. Org. Chem.* **1965**, *30*, 2334.
(3) Schaefer, J. P.; Wheatley, P. J. *J. Org. Chem.* **1968**, *33*, 166.
(4) Wong, C. M. *Can. J. Chem.* **1968**, *46*, 1101.
(5) Korzybski, T.; Kowszyk-Gindifer, Z.; Kurytowicz, W. "Antibiotics"; American Society of Microbiology: Washington, DC, 1978; Vol. 1, pp 343–346.
(6) (a) Oida, S.; Ohki, E. *Chem. Pharm. Bull.* **1969**, *17*, 1405. (b) Schumacher, D. P.; Hall, S. S. *J. Am. Chem. Soc.* **1982**, *104*, 6076.
(7) (a) Wong, C. M.; Buccini, J.; Chang, I.; Te Raa, J.; Schwenk, R. *Can. J. Chem.* **1969**, *47*, 2421. (b) Felner, I.; Schenker, K. *Helv. Chim. Acta* **1970**, *53*, 754. (c) Verheyden, J. P. H.; Richardson, A. C.; Bhatt, R. S.; Grant, B. D.; Fitch, W. L.; Moffatt, J. G. *Pure Appl. Chem.* **1978**, *50*, 1363. (d) Buchanan, J. G.; MacLean, K. A.; Paulsen, H.; Wightman, R. H. *J. Chem. Soc., Chem. Commun.* **1983**, *486*.

0022-3263/86/1951-1069$01.50/0