Synthesis, Intramolecular Hydrogen Bonding, and Biochemical Studies of Clitocine, a Naturally Occurring Exocyclic Amino Nucleoside

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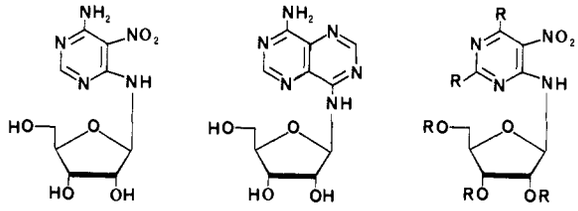
The total synthesis of clitocine [6-amino-5-nitro-4-(β-D-ribofuranosylamino)pyrimidine] (1), a nucleoside recently isolated from the mushroom *Clitocybe inversa*, has been accomplished. Glycosylation of 4,6-diamino-5-nitropyrimidine (4) with 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-D-ribofuranose afforded the protected nucleoside 6-amino-5-nitro-4-[(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)amino]pyrimidine (5) in good yield exclusively as the β-anomer. Deprotection of 5 with NaOMe/MeOH gave 1 as an 11.5:1 mixture of the β- and α-anomers, respectively. Recrystallization from MeOH, followed by chromatography, afforded 1 containing less than 1% of its α-anomer. X-ray crystal data revealed a planar aglycon moiety in clitocine with each oxygen atom of the nitro group intramolecularly hydrogen bonded to the hydrogen atoms of the two adjacent amino functions. Clitocine inhibited L1210 cells in vitro with an ID_{50} of 3×10^{-8} M. Clitocine was also found to be a substrate and inhibitor of adenosine kinase with a K_i value of 3×10^{-6} M.

The isolation and characterization of a new nucleoside, clitocine [6-amino-5-nitro-4-(β-D-ribofuranosylamino)pyrimidine] (1), from the mushroom *Clitocybe inversa* was recently reported by Kubo et al.[3] These workers also reported that this compound exhibited strong insecticidal activity against the pink bollworm *Pectinophora gossypiella*. As part of a continuing program of the synthesis of nucleosides and derivatives thereof that may have potential antiparasitic, antitumor, and antiviral activity, the total synthesis of clitocine was investigated.



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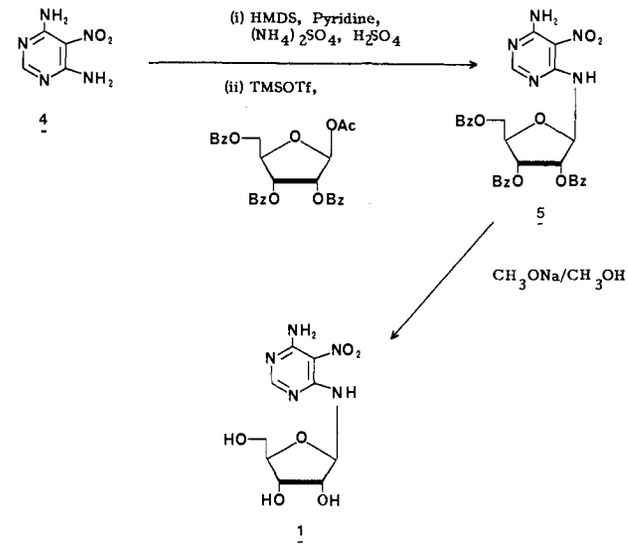
Robins et al.[4] reported the first synthesis of the exocyclic amino nucleoside, 4-amino-8-(β-D-ribofuranosylamino)pyrimido[5,4-*d*]pyrimidine (2), via a rearrangement of 6-cyano-9-β-D-ribofuranosylpurine. Townsend and Robins[5] reported the synthesis of 2-amino-6-hydroxy-5-(*N*-formyl-*N*-methylamino)-4-(D-ribofuranosylamino)pyrimidine by ring cleavage of 7-methylguanosine with aqueous ammonia. Niedballa and Vorbrüggen[6] isolated N^4-(pentaacetyl-β-D-glucopyranosyl)-2-thio-5-azacytosine as a side product during the reaction of 2-thio-5-azacytosine with pentaacetyl-β-D-glucopyranose, while Pfleiderer and coworkers[7] obtained a mixture of α- and β-anomers upon glycosylation of substituted 4-amino-5-nitropyrimidines with riboses during their synthesis of aminoglycosides with the general structure 3.

Results and Discussion

Preliminary studies of the synthesis of clitocine were approached by the displacement of the 4-chloro group in 4,6-dichloro-5-nitropyrimidine with 2,3-*O*-isopropylidene-β-D-ribofuranosylamine.[8] After chromatography, the major product isolated was identified as 6-chloro-5-nitro-4-[(2′,3′-*O*-isopropylidene-β-D-ribofuranosyl)amino]pyrimidine, on the basis of 300-MHz proton NMR and UV data. Treatment of the blocked nucleoside with methanolic ammonia followed by deprotection with 80% acetic acid at 80 °C gave (1) in low yields as a mixture of α- and β-anomers. ^{1}H NMR and UV spectral data of the β-anomer were consistent with those reported by Kubo et al.[3] The low yields coupled with the problems of anomeric purity of the final product obtained by this method prompted the investigation of the synthesis of 1 by alternate routes. The synthesis of clitocine was accomplished as per Scheme I.

Scheme I[a]



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[a] HMDS, hexamethyldisilazane; TMSOTf, trimethylsilyl trifluoromethanesulfonate.

4,6-Diamino-5-nitropyrimidine[9] (4) was silylated according to the procedure of Vorbrüggen et al.,[10] with slight

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(2) Present address: Microprobe Corporation, Bothell, WA 98021.
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modifications. A mixture of hexamethyldisilazane, pyridine, ammonium sulfate, and **4** in the presence of catalytic amounts of sulfuric acid was refluxed for 24 h. The silylated heterocycle was then directly glycosylated with 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-D-ribofuranose in acetonitrile with trimethylsilyl trifluoromethanesulfonate as the Lewis acid catalyst to give 6-amino-5-nitro-4-[(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)amino]pyrimidine (**5**) as a foam in 72% yield after chromatography. No significant amount of the α-anomer was isolated. It was observed that in the absence of sulfuric acid the silylation failed to occur, and unreacted **4** was recovered from the glycosylation mixture. The anomeric purity of **5** was confirmed by the single set of absorptions for the anomeric proton in the ^{1}H NMR spectrum at δ 6.36. This appears to be the first example of the direct glycosylation of an aminopyrimidine under acid-catalyzed conditions to give the exocyclic amino nucleoside exclusively as its β-anomer.

Treatment of **5** with a saturated solution of ammonia in methanol resulted in a colorless solid, the ^{1}H NMR spectrum of which was consistent with that reported[3] for compound **1**, with two exceptions. In addition to the absorptions reported[3] for **1**, a doublet of doublets at δ 6.02 (J = 4.7 and 8.4 Hz) and a doublet at δ 9.95 (J = 8.4 Hz) were observed. We attribute these absorptions to the presence of the α-anomer of **1** and assign these to the C_1'H and NH protons, respectively. Based on integration of the anomeric proton absorptions, the β/α ratio was 2.8. No improvement in this ratio was observed when **5** was treated with saturated ethanolic ammonia. Thus, this method of deblocking was accompanied by epimerization to give a mixture of α- and β-anomers of compound **1**.

In an effort to avoid anomerization apparently occurring during the deprotection step, the use of sodium methoxide in this step was investigated. Treatment of **5** with 0.2 molar equiv of NaOMe in methanol and dioxane (0–8 °C, 17 h) afforded an excellent yield of **1** containing less than 10% of the α-anomer, based on proton NMR spectral data. Further purification by fractional recrystallization from methanol (β-anomer more soluble) followed by chromatography resulted in compound **1** as the pure β-anomer.

While the ^{1}H and ^{13}C NMR spectra for **1** obtained as described above were identical with those reported,[3] slight differences in the UV and IR spectra[11,12] were observed. In particular, the reported UV absorptions for **1** were at 255 nm (ε 7950) and 300 nm (ε 2190) while our sample of **1** lacked the absorption at 255 nm and instead exhibited a peak at 215 (ε 55 627) in addition to one at 332 nm (ε 12 925).[11] The observed melting point for **1** was 230–231 °C as compared to the reported[3] melting point of 228–230 °C for this compound. The purity was further confirmed by the use of analytical HPLC and combustion analysis for elemental composition.

X-ray Crystallographic Studies. In order to confirm the chemical structure as well as β-configuration of **1**, an X-ray crystallographic analysis was undertaken. Crystallization from ethanol yielded crystals suitable for X-ray analysis. An ORTEP[13] drawing of **1** is shown in Figure 1. The hydrogen bond of the 4-amino hydrogen, HN4, to O5A of the nitro group postulated by Kubo et al.[3] is confirmed in the solid state. The HN4···O5 distance is 1.90 (3) Å. In addition, HN61 of the 6-amino group is hydrogen bonded to O5B of the nitro group [d(HN61···O5B) = 2.00 (4) Å]. The entire aglycon (pyrimidine) moiety resembles a planar tricyclic ring system as a result of this intramolecular hydrogen bonding. Details of the X-ray crystallographic analysis will be published elsewhere.[14]

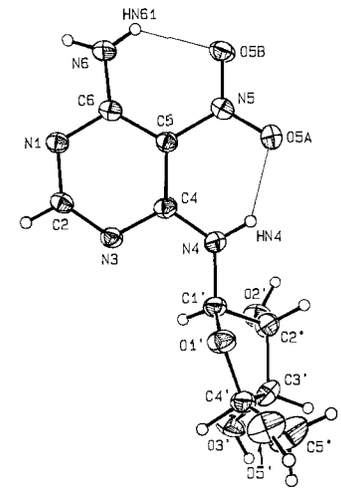

Figure 1. Thermal ellipsoid plot illustrating atom labeling and intramolecular hydrogen bonding. The ellipsoids are drawn at the 50% probability level.

NMR Spectral Data. Kubo et al.[3] postulated the existence of hydrogen bonding between the 4-N*H* and an oxygen atom of the 5-nitro group of clitocine. This was based on the position of the N*H* signal in the proton NMR in DMSO solution at low field, δ 9.3. The corresponding signal in the NMR of the nucleoside **2** appears at δ 8.4.[4] The room-temperature proton NMR of protected nucleoside 6-amino-5-nitro-4-[(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)amino]pyrimidine (**5**) in $CDCl_3$ showed a doublet at δ 9.6 for the 4-N*H*. The two 6-NH_2 protons resonated as broad singlets at δ 8.5 and 6.3, indicating that they are in different environments, presumably due to H bonding of one proton with the oxygen atom of the nitro group. Upon the sample being cooled to 0 °C, the 4-N*H* signal was shifted downfield to δ 9.7, and the two 6-NH_2 protons resonated at δ 8.5 and 6.7, with the proton at δ 8.5 now as a sharp doublet. At 60 °C, the 4-N*H* resonated at δ 9.4, while the two 6-NH_2 protons coalesced and now resonated with the aromatic protons from δ 7.2 to 7.6. The signal for the anomeric proton appeared at δ 6.4 at all three temperatures. The proton NMR for the deprotected nucleoside **1** in DMSO-d_6 at room temperature showed the signal for the 4-N*H* as a doublet at δ 9.3, while the 6-NH_2 protons resonated as a singlet at δ 8.5. At 90 °C, the doublet at δ 9.3 collapsed into a broad singlet at δ 9.1, and the 6-NH_2 protons were shifted upfield to δ 8.2. The aromatic proton singlet at δ 8.0 showed no change in chemical shift at the two temperatures. While the X-ray analysis confirmed the existence of intramolecular hydrogen bonding between the 4- and 6-amino hydrogens and the oxygen atoms of the 5-nitro group of clitocine in the crystalline state, these NMR data are indicative of the existence of such hydrogen bonding in solution as well.

Biochemical Studies. In a prior investigation of adenosine kinase activity with 3-β-D-ribofuranosyl-7-thia-1,2,3-triazolo[5,4-*d*]pyrimidine,[15] an exocyclic amino-

(11) These absorptions are very similar to those of 2,4-diamino-5-nitropyrimidine itself.

(12) See Figure 2 of ref 3. The IR spectrum of our sample lacks any absorptions in the region 1800–2700 cm^{-1}.

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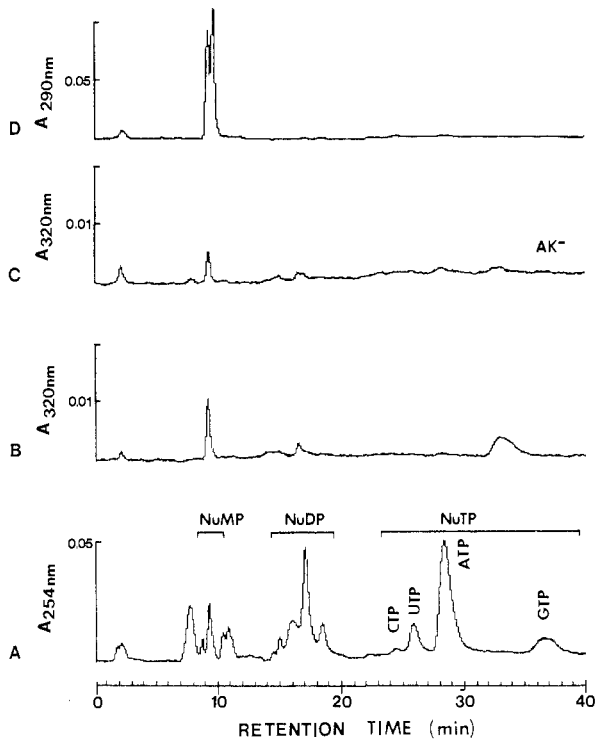

Figure 2. Analytical SAX–HPLC chromatograms of acid-soluble extracts from WI-L2 cells. WI-L2 cells or adenosine kinase deficient (AK⁻) WI-L2 cells were treated with **1** (40 μM) or **2** (10 μM) for 4 h. Each chromatogram represents extract from 4 × 10^6 cells/200 μL injection. Ultraviolet absorbance (A) was monitored in each case at the indicated wavelength. (A) Untreated control showing retention times of nucleoside mono- (NuMP), di- (NuDP), and triphosphates (NuTP), (B) WI-L2 cells treated with clitocine, (C) AKase-deficient WI-L2 cells treated with clitocine, (D) WI-L2 cells treated with **2**.

nucleoside, 4-(β-D-ribofuranosylamino)-1,2,3-thiadiazolo-[5,4-*d*]pyrimidine, similar in structure to **1** and **2** was suggested to be a substrate for adenosine kinase purified 430-fold from H.Ep.2 cells. It was therefore of interest to explore the role of adenosine kinase (AKase, E.C. 2.7.1.20) in the metabolism and biological activity of clitocine.

Clitocine (**1**) inhibits the growth of human B lymphoblast derived WI-L2 cells (ID_{50} = 0.03 μM). The intracellular nucleotide pools of drug-treated WI-L2 cells were examined by SAX–HPLC (Figure 2) and the mono-, di-, and triphosphates of clitocine were detected, indicating efficient phosphorylation of **1** after transport into the cell. 4-Amino-8-(β-D-ribofuranosylamino)pyrimido[5,4-*d*]pyrimidine, **2**, is also phosphorylated under similar conditions. In this latter case, however, only the 5′-monophosphate of **2** can be detected. The growth of AKase-deficient WI-L2 cells is not inhibited by **2**.

The route of phosphorylation of clitocine was investigated with use of a WI-L2 cell line deficient in adenosine kinase. SAX–HPLC analysis of AKase-deficient cell extracts showed two peaks identified as clitocine and 6-amino-5-nitro-4-(β-D-ribofuranosylamino)pyrimidine 5′-monophosphate (clitocine 5′-monophosphate). The peak representing the nucleoside 5′-monophosphate was reduced in area as compared with the same peak from AKase-sufficient cells, and no di- or triphosphates were detected. These results indicate that AKase is involved in the phosphorylation of **1**; however, either the mutation that causes deficiency of AKase activity is not sufficient to completely prevent the phosphorylation of clitocine or clitocine may be a substrate for another kinase activity. To distinguish between these possibilities, WI-L2 lysates were prepared, and **1** was tested for inhibition of conversion of various ^{14}C-labeled nucleosides to their respective 5′-monophosphate derivatives. Compound **1** did not inhibit the conversion of [^{14}C]uridine or [^{14}C]-2′-deoxycytidine to their respective nucleoside 5′-monophosphates at inhibitor concentrations up to 400 μM. However, **1** substantially inhibited the conversion of [^{14}C]adenosine to [^{14}C]AMP.

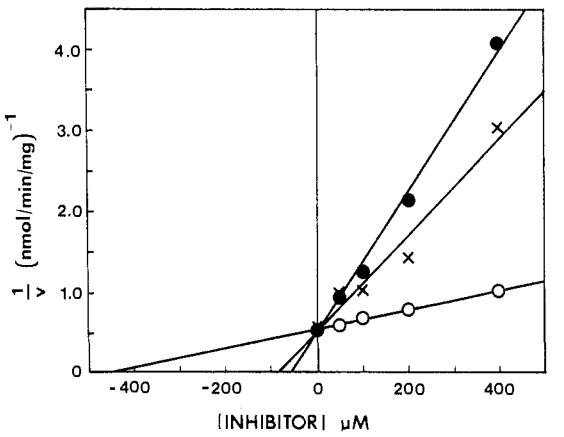

Figure 3. Comparative activity of inhibitors of adenosine kinase. The inhibition of conversion of adenosine (50 μM, 59 Ci/mol) to AMP by clitocine (●), unlabeled adenosine (X) or 5′-deoxyadenosine (○) is shown by plot of $1/v$ vs inhibitor concentration. Reaction velocity is in units of nmol/min per mg of protein. Each point represents the average of duplicate measurements.

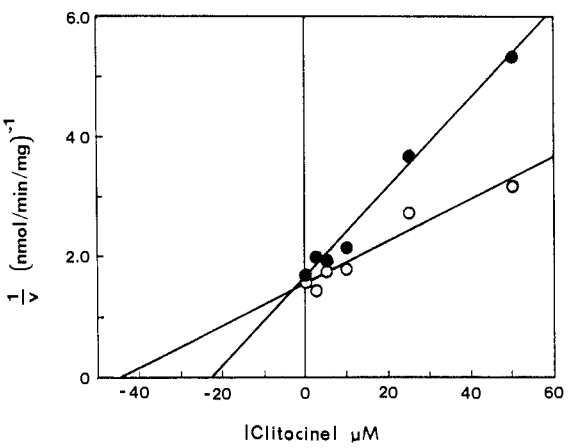

Figure 4. Determination of K_i value of clitocine for adenosine kinase from WI-L2 cells. Adenosine at concentrations of 5 (●) and 10 μM (○) was tested against graded concentrations of clitocine. Reciprocal reaction velocity is plotted against clitocine concentration. Reaction velocity is in units of nmol/min per mg of protein. Each point represents the average of duplicate measurements.

The degree of inhibition of AKase by clitocine was estimated by comparison with unlabeled adenosine and with 5′-deoxyadenosine a reported inhibitor of rabbit liver AKase (K_i value of 2.5 μM).[16] The relative inhibition is shown (Figure 3) by the reciprocal reaction velocity ($1/v$) as a function of inhibitor concentration. Unlabeled adenosine competed with [^{14}C]adenosine to a greater extent than did 5′-deoxyadenosine. This is demonstrated by the higher relative slope value for adenosine (1.00 vs 0.24 for 5′-deoxyadenosine). In the same experiment, **1** (slope, 1.8) was found to be 8-fold more potent an inhibitor than

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5′-deoxyadenosine and 2-fold more potent than unlabeled adenosine.

The K_i value for 1 with adenosine kinase from undialyzed WI-L2 lysate was investigated in a separate experiment utilizing adenosine as substrate at concentrations of 5 and 10 μM. Graded concentrations from 0 to 50 μM clitocine were tested (Figure 4). A K_i value of 3 μM was determined.

The inhibition of adenosine kinase by clitocine strongly suggests that adenosine kinase is responsible for the phosphorylation of clitocine in WI-L2 cells. We conclude that 1 is a structural adenosine analogue not only in the solid state, but also in aqueous media. This structural similarity allows the molecule to be a potent competitor for the adenosine kinase substrate binding site. Other enzymes that recognize clitocine and higher 5′-phosphate derivatives of clitocine as substrate or inhibitor are currently being investigated as part of a more complete study of the mechanism of action of clitocine.

Clitocine was tested (for experimental details, see ref 17) for its inhibitory effects in vitro on the growth of L1210 murine lymphocytic leukemia, WI-L2 human B lymphoblastic leukemia, and CCRF-CEM human T lymphoblastic leukemia in cell culture. It showed inhibitory effects on all these cell lines with an ID_{50} of 3.0×10^{-8} M. Similarly, clitocine was tested (for experimental details, see ref 18) against parainfluenza type 3, measles, vaccinia, and herpes simplex type 2 viruses in cell culture but was found to lack any significant antiviral activity.

Experimental Section

Chemistry. NMR data were obtained on an IBM NR-300 spectrometer in $(CD_3)_2SO$ solvent with use of the residual proton as internal reference. IR spectra were recorded with a Perkin-Elmer Model 1420 spectrophotometer and UV data with a Beckman DU-50 instrument. Melting points were obtained in open capillaries with a Haake-Buchler apparatus and are uncorrected. Combustion analyses were performed by Robertson Laboratories, Florham Park, NJ.

Biochemistry Materials. [5-^{3}H]-2′-Deoxycytidine, [5,6-^{3}H]uridine, and [8-^{14}C]adenosine were obtained from ICN Biomedicals. DE-81 ion-exchange filters and the Partisil 10 strong cation exchange (SAX) high-pressure liquid chromatography column were from Whatman Ltd.

Cell Lines and Nucleotide Pools. The WI-L2 and adenosine kinase deficient WI-L2 cell lines, preparation of nucleotide pools, and SAX–HPLC analytical methods have been previously described.[19] Cells were cultured in RPMI-1640 medium (Irvine Scientific) supplemented with 10% dialyzed fetal bovine serum (Flow Laboratories), 20 mM Hepes, pH 7.5, and 2 mM glutamine.

Cell Lysates. Log-phase cultures of WI-L2 cells (1×10^6 cells/mL) were harvested by centrifugation, cooled to 4 °C, and washed twice with Hanks buffer (Flow Laboratories). The washed cell pellet was resuspended to 5×10^7 cells/mL in buffer composed of 0.5 mM EDTA and 10 mM Tris-Cl, pH 7.4, and lysed by three cycles of freezing in liquid nitrogen and thawing at 37 °C. The suspension was centrifuged for 60 min at 100000*g*, and the supernatant fraction was stored at −70 °C.

Enzyme Assays. Kinase activities and inhibition studies were accomplished by the filter-binding assay method.[20] Adenosine kinase activity and comparative inhibition studies were done at 37 °C with an assay mixture containing 4 mM ATP, 1.5 mM $MgCl_2$, 50 μM [8-^{14}C]adenosine (59 Ci/mol), and 100 mM Tris-maleate, pH 5.5. Inhibitor was present at concentrations indicated in the figures. To prevent the enzymatic breakdown of adenosine, *erythro*-9-(2-hydroxy-3-nonyl)adenine (EHNA) was added to the cell-free lysate to give a 5 μM final assay concentration. Assays were started by the addition of protein. For K_i determinations, adenosine (59 Ci/mol) was used at concentrations of 5 and 10 μM. The amount of enzyme, time of assay, and sampling volume was adjusted to give acceptable conversion of [8-^{14}C]adenosine to [8-^{14}C]AMP. Each DE-81 filter was spotted with a constant sampling volume and immersed in distilled water (4 L) to terminate the reaction. Filters were washed three times with water and once with 95% ethanol (100 mL). Dry filters were placed in scintillation vials and radioactivity was determined in 10 mL of toluene-based scintillation cocktail.[21] Uridine kinase[22] was assayed in a solution containing 10 mM ATP, 11 mM $MgCl_2$, 40 μM [5,6-^{3}H]uridine (250 Ci/mol), and 100 mM Tris-Cl, pH 7.4. Sampling and filter processing was done as described for the adenosine kinase assay. 2′-Deoxycytidine kinase[20] was assayed in a solution containing 10 mM ATP, 12 mM $MgCl_2$, 12.5 mM dithiothreitol, and 25 μM [5-^{3}H]-2′-deoxycytidine (1 Ci/mmol). Sampling and filter processing was done as described for the adenosine kinase assay.

6-Amino-5-nitro-4-[(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)amino]pyrimidine (5). To a suspension of 4,6-diamino-5-nitropyrimidine (4) (2.5 g, 16.1 mmol) in HMDS (75 mL) were added H_2SO_4 (0.5 mL), pyridine (12 mL), and ammonium sulfate (0.075 g). The mixture was refluxed under a dry argon atmosphere for 16 h, and the resulting clear solution was cooled to room temperature. The excess HMDS was evaporated in vacuo, and the solid residue was dissolved in acetonitrile (125 mL). To this solution were added 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-D-ribofuranose (9.7 g, 19.2 mmol) and trimethylsilyl trifluoromethanesulfonate (5.0 mL, 26.7 mmol). The solution was stirred under a dry argon atmosphere for 18 h. Solvent was evaporated in vacuo, and the residual foam was dissolved in CH_2Cl_2 (150 mL). The organic phase was washed with saturated $NaHCO_3$ and dried over anhydrous $MgSO_4$. Evaporation of the solvent and chromatography (silica gel, with 5% acetone in CH_2Cl_2 as eluent) gave **5** (7.0 g, 72%) as a pale yellow foam, which softened at 106 °C: ^{1}H NMR δ 4.64 (m, 3 H), 5.96 (AB of ABX, 2 H, C_5′H), 6.36 (dd, 1 H, J = 4.8 and 8.2 Hz, C_1′H), 7.4–8.0 (m, 16 H, aromatic and C_2H), 8.62 (br s, 2 H, NH_2), and 9.63 (d, 1 H, J = 8.2 Hz, NH). Anal. ($C_{30}H_{25}N_5O_9$) C, H, N.

6-Amino-5-nitro-4-(β-D-ribofuranosylamino)pyrimidine (1). Compound **5** (5.7 g, 9.5 mmol) was dissolved in a mixture of methanol (225 mL, previously stored over 3-Å molecular sieves) and dioxane (45 mL). The solution was cooled to 0 °C, and freshly prepared methanolic solution of sodium methoxide (1.9 mL of a 1 M solution) was added. The reaction mixture was kept at 8 °C under a dry argon atmosphere for 17 h. The resulting clear solution was acidified by stirring with sufficient Dowex (H^+) to pH 6, filtered, and evaporated. The residue was triturated with ether (three times), after which a colorless solid material was obtained in quantitative yield as an 11.5:1 mixture of the β- and α-anomers of 1 as determined by ^{1}H NMR analysis. Recrystallization of this material from methanol by repeated concentrations of the mother liquor afforded 636 mg of 1, which was more than 98% of the β-anomer. A portion (570 mg) of this material was dissolved into methanol, adsorbed onto silica gel, and chromatographed (silica gel, with 20% methanol in CH_2Cl_2 as eluent) to give 427 mg of 1 as ≥99% β-anomer. This was then crystallized from ethanol to yield crystals suitable for X-ray crystallography: mp 230–231 °C (lit.[3] mp 228–230 °C); UV λ_{max} (EtOH) 332 (ε 12925), 215 nm (ε 55627); IR (KBr) 3425, 1590, 1515, 1245 cm^{-1}; ^{1}H NMR δ 3.41 (m, 1 H, C_5′H), 3.53 (m, 1 H, C_5′H), 3.78 (m, 1 H, C_4′H), 3.92 (m, 1 H, C_2′H), 4.06 (m, 1 H, C_3′H), 4.96 (d, 1 H, J = 5.9 Hz, C_3′OH), 5.10 (dd, 1 H, J = 5 Hz, C_5′OH), 5.23 (d, 1 H, J = 5.3 Hz, C_2′OH), 5.78 (dd, 1 H, J = 3.5 and 7.7 Hz, C_1′H), 8.00 (s, 1 H, C_2H), 8.58 (br s, 2 H, NH_2), and 9.29 (d, 1 H, J =

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7.7 Hz, NH); ^{13}C NMR δ 60.3, 69.9, 74.9, 83.4, 86.1, 111.8, 155.8, 158.6, and 159.3. Anal. ($C_9H_{13}N_5O_6$) C, H, N.

Acknowledgment. We thank Edwin B. Banta and Gina M. Whalin for expert assistance in obtaining the NMR and biochemical data, respectively.

Registry No. 1, 105798-74-1; 4, 2164-84-3; 4 (silylated), 112220-30-1; 5, 112220-31-2; AKase, 9027-72-9; 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose, 6974-32-9.

Synthesis and Biological Properties of Actinomycin D Chromophoric Analogues Substituted at Carbon 7 with Aziridine and Cyclopropyl Functions

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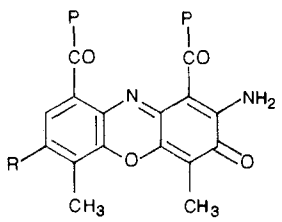
The growing importance of functionalized tricyclic rings, e.g., cyclopropyl and aziridine, in numerous organic biomolecules led us to develop syntheses of novel actinomycin D (AMD) analogues substituted with aziridine and cyclopropyl functions. Reaction of 7-hydroxyactinomycin D with 1-aziridineethyl iodide and bromomethylcyclopropane afforded the desired 7-[2-(1-aziridinyl)ethoxy] and cyclopropylmethoxy analogues, respectively. Calf thymus DNA binding of these analogues was comparable to that of AMD as examined by UV-vis difference spectral measurements, CD techniques, and relaxation of supercoiled closed circular SV40 DNA, indicating an intercalative mode of binding to the DNA duplex. Thermal denaturation of DNA experiments employing higher temperatures than room temperature exhibit a thermal lability of the DNA analogue complexes, suggestive of a probable covalent bond formation with DNA bases. The analogues were found to be $^1/_4$-$^1/_{40}$ as cytotoxic to human lymphoblastic CCRF-CEM leukemia and B_{16} melanoma cells in vitro as AMD, with ID_{50} values in the nanomolar concentration range.

Actinomycin D (AMD, **1b**, Chart I) is a clinically useful chemotherapeutic agent in the treatment of a limited number of tumors.[1,2] It binds to double-helical DNA of the cell by intercalation of its chromophore between guanosine–cytosine sequences with the peptide portion lying in the minor groove of the DNA helix as confirmed by spectroscopic and hydrodynamic studies of this complex.[3-5] This drug–DNA interaction is believed responsible for its selective inhibition of DNA-primed RNA synthesis.[6,7]

Actinomycin D is also known to cause chromosomal damage and appears to require active cell processes for this to occur. To explain this, an alternative mechanism involving enzymatic reduction of the quinone imine structure of AMD to a free-radical intermediate has been proposed.[8,9] These free radicals are presumed to be the critical activated form of the antibiotic to cause intracellular DNA damage and cell death.

In an earlier paper we reported the synthesis of 7-(2,3-epoxypropoxy)actinomycin D (**3b**), which appeared to retain the DNA binding property and to enhance the in vivo tumor-inhibiting activity of AMD.[10] In addition, other functionalized aziridines, namely AZQ and mitomycin C, are reported to act as tumor-inhibiting alkylating agents.[11] In light of these observations, we prepared the corresponding aziridine analogue (**4b**). During attempted preparation of the aziridine (**4b**) by an alternate route, we isolated the linear 2-aminopropoxy compound (**6b**).[12]

Chart I[a]



1. R = H
2. R = OH
3. R = $OCH_2CH(O)CH_2$
4. R = $OCH_2CH(NH)CH_2$
5. R = $OCH_2CH(CH_2)CH_2$
6. R = $OCH_2CH(NH_2)CH_3$
7. R = $OCH_2CH_2N(CH_2)CH_2$
8. R = $OCH_2CH_2NHCH_2CH_2OH$

[a] **a**. P = $N(C_2H_5)_2$. **b**. P = Thr-D-Val-Pro-Sar-MeVal–O

Although calf thymus DNA binding of actinomycin D analogues **4b** and **6b** was comparable to AMD, these were comparatively less cytotoxic to human lymphoblastic CCRF-CEM leukemia and B_{16} cells in vitro. As an extension of this work, we now report our results on cyclopropylmethoxy-substituted derivative (**5b**). We surmised that a cyclopropane ring containing a highly strained covalent bond would be prone to cleavage to an ion-pair intermediate, which would release strain.[13] Ring cleavage of cyclopropane rings by nucleophiles has long been known.[14] The cyclopropane ring could cleave to singlet diradical transition and zwitterion transition state in ep-

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