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Synthesis of the 2-Chloro Analogues of 3′-Deoxyadenosine, 2′,3′-Dideoxyadenosine, and 2′,3′-Didehydro-2′,3′-dideoxyadenosine as Potential Antiviral Agents

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2-Chloro-3′-deoxyadenosine (2-chlorocordycepin), 2-chloro-2′,3′-dideoxyadenosine (2-ClddAdo), and 2-chloro-2′,3′-didehydro-2′,3′-dideoxyadenosine (2-ClddeAdo) were synthesized from 2-chloroadenosine (2-ClAdo) as candidate antiretroviral agents on the basis that 2-chloro substitution would prevent enzymatic deamination and increase efficacy relative to 2′,3′-dideoxyadenosine (ddAdo). Reduction of 2-chloro-5′-(4,4′-dimethoxytrityl)-2′,3′-*O*-thiocarbonyladenosine with *n*-Bu_3SnH, followed by detritylation with AcOH, unexpectedly gave a mixture of 2-chlorocordycepin and 2-chloroadenine. Treatment of the crude *n*-Bu_3SnH reduction product with 1,1′-thiocarbonyldiimidazole, followed by another cycle of *n*-Bu_3SnH reduction and detritylation with silica gel afforded 2-ClddAdo and a byproduct identified as 2-chloro-2′,3′-*O*-methyleneadenosine. Treatment of 2-chloro-5′-*O*-(4,4′-dimethoxytrityl)-2′,3′-thiocarbonyladenosine with 1,3-dimethyl-2-phenyl-1,3,2-diazaphospholidine followed by silica gel detritylation afforded 2-ClddeAdo. 2-ClddAdo and 2-ClddeAdo were tested for activity against human immunodeficiency virus (HIV) in a cultured human $T4^+$ lymphocyte cell line. At a concentration of 100 μM, 2-ClddAdo inhibited reverse transcriptase (RT) production by 97%, while 2′,3′-dideoxyadenosine (ddAdo) gave >99% inhibition. In growth assays against uninfected $T4^+$ cells, however, 100 μM 2-ClddAdo gave 23% inhibition while 100 μM ddAdo was nontoxic. At a nontoxic concentration of 20 μM, RT production was 75% inhibited by ddAdo but only 43% inhibited by 2-ClddAdo. Thus, a 2-chloro substituent increased host cell toxicity but decreased antiretroviral activity. The unsaturated analogue 2-ClddeAdo was more cytotoxic than 2-ClddAdo, and antiviral effects could not be measured above 20 μM, where there was only 75% inhibition of RT production. Because of the decreased therapeutic index of 2-ClddeAdo relative to 2-ClddAdo and ddAdo, >90% inhibition of viral protein synthesis at a noncytotoxic concentration could not be achieved. In growth assays with cultured human T and B lymphocytes, 100 μM 2-chlorocordycepin gave 60–70% growth inhibition, while the IC_{50} against mouse fibroblasts was only 30 μM. The high cytotoxicity of 2-chlorocordycepin precluded consideration of this compound as an antiviral agent.

2′,3′-Dideoxyadenosine (ddAdo, **1**) and 2′,3′-didehydro-2′,3′-dideoxyadenosine (ddeAdo, **2**) have attracted attention as antiretroviral agents and especially as inhibitors of human immunodeficiency virus (HIV), the causative agent of acquired immune deficiency syndrome (AIDS).[1] The first report of the antiretroviral activity of **1** against HIV in cultured human $T4^+$ lymphocytes was by Mitsuya and Broder,[2] who achieved full protection from the cytopathic effects of the virus at concentrations of **1** that were completely nontoxic to host cells. Subsequent reports confirmed these results[3–6] and extended them to other retroviral models.[7–9] The 2′,3′-didehydro-2′,3′-dideoxy analogue **2** has likewise been reported to block HIV proliferation in human $T4^+$ lumphocytes,[3,6] but its therapeutic index is less favorable than that of **1**.

At the biochemical level, it has been demonstrated that the selective antiviral action of **1** is due to intracellular formation of the 5′-triphosphate, which competitively inhibits viral reverse transcriptase (RT) while leaving host cell DNA polymerase less affected.[4] The efficacy of **1** may vary as a function of cellular dATP pool sizes, as indicated by the finding that ribavirin, a potent inhibitor of IMP dehydrogenase and hence of de novo dATP synthesis, enhances the activity of **1** in HIV-infected T lymphocytes.[10] An important aspect of the biochemical pharmacology of **1** is a recently uncovered metabolic pathway whereby **1** is converted sequentially to 2′,3′-dideoxyinosine (ddIno)[11] and 2′,3′-dideoxyinosine 5′-monophosphate (ddIMP), the latter of which can be "salvaged" by the adenylosuccinate synthetase–lyase system.[12] The existence of this pathway may play an important role in the selective action of **1** in T cells, but it is not known to what extent it plays a role in non-T cells. The fact that HIV can infect not only T cells but also B cells,[13] monocyte macrophages,[13,14] and brain capillary endothelial cells[15] has been a cause of concern because these non-T cells can serve as sanctuaries for the virus. 2′,3′-Dideoxynucleosides, including **1**, have been found to be inactive against HIV in terminally differentiated, nondividing, primary human monocyte macrophages,[16] and it is possible that these compounds are

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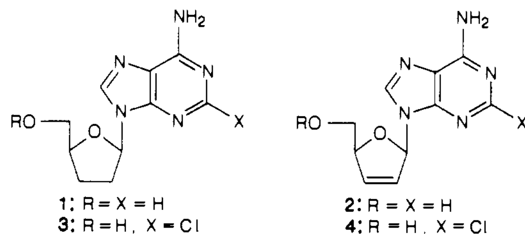
similarly inactive in other non-T cells that harbor HIV. The lack of activity of 1 in monocyte macrophages cannot be explained solely on the basis of low nucleoside kinase levels,[16] and may be due in part to an inability on the part of these cells to "salvage" ddIMP.

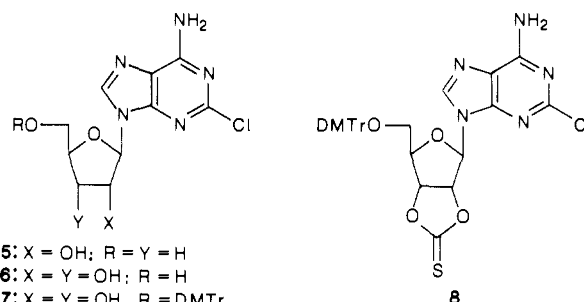
On the basis of these considerations we felt that deaminase-resistant analogues of 1 and 2 might be of interest as biochemical probes and potential therapeutic agents. Since adenosine and 2'-deoxyadenosine analogues with a 2-halogen substituent are well known for their stability to adenosine deaminase,[17,18] we undertook to prepare 2-chloro-2',3'-dideoxyadenosine (3) and 2-chloro-2',3'-didehydro-2',3'-dideoxyadenosine (4).[51] Haertle and co-



workers[19] independently obtained 3 and its 2-fluoro and 2-bromo analogues enzymatically from the corresponding purine bases, and compared their antiretroviral activity with that of 1. In this paper we report our chemical synthesis of 3 from 2-chloroadenosine, and also report the preparation of the unsaturated congener 4 and the cordycepin analogue 2-chloro-3'-deoxyadenosine (5), neither of which has been described until now. Our bioassay results confirm that 3 has high anti-HIV activity only at concentrations toxic to host cells. In addition, we find the therapeutic selectivity of 4 to be lower than that of 3, leading to the conclusion that 2-chloro substitution in both 1 and its 2',3'-dehydro analogue 2 is unfavorable for antiretroviral activity.

Chemistry

A number of synthetic routes have been described in the literature for the preparation of 1[20-31] as well as 2.[21,24,25,28,30] We chose to start with 2-chloroadenosine (6), which is commercially available and for which a convenient route starting from adenosine has been developed.[32] Protection of the 5'-position was accomplished by means of a 4,4'-dimethoxytrityl (DMTr) group.[33] Treatment of 6 with a slight excess of 4,4'-dimethoxytrityl chloride in dry pyridine afforded a 69% yield of the crystalline 5'-DMTr derivative 7, along with some unchanged starting material, which could be easily recovered by extraction from $CHCl_3$ into water. Condensation of 7 with 1,1'-thiocarbonyldiimidazole (TCDI)[21] in MeCN at room temperature was straightforward, giving the 2',3'-*O*-thiocarbonyl derivative 8 in 80% yield. However, reduction of 8 with *n*-Bu_3SnH in toluene at 110–120 °C in the presence of α,α'-azobisisobutyronitrile (AIBN)[34] was more complex. Analysis by TLC revealed two major spots, one of which was judged on the basis of its R_f value in comparison with 2'- and 3'-deoxyadenosine to be an unresolved mixture of the 2'- and 3'-deoxy derivatives 9 and 10. The second major spot had a higher R_f value, and although its structure was not immediately recognized it was identified subsequently as the 2',3'-*O*-methylene derivative 11, presumably arising from 8 by reductive desulfurization.[21] The yield of mixed isomers 9 and 10 after separation from 11 by column chromatography was 59%. Treatment of this mixture with 80% aqueous AcOH at 50 °C for 30 min was then carried out with the expectation of forming a mixture of 5 and 2-chloro-2'-deoxyadenosine. To our surprise, the only products isolated from this reaction proved to be 5 and *2-chloroadenine*, the latter of which presumably arose by selective acidolysis of the glycosidic bond in 2-chloro-2'-deoxyadenosine. The identity of 5 was established from the fact that its melting point differed substantially from that of the 2'-deoxy isomer, from its mass spectrum, which showed a strong (M + 1) peak at the expected value of 287, and from its ^1H NMR spectrum in CD_3OD solution, which showed the nonequivalent protons at the 3'-position as multiplets at δ 2.05 and 2.35, in good agreement with the report[25] that these protons in the spectrum of 3'-deoxyadenosine (cordycepin), in DMSO-d_6 solution, give multiplets at δ 1.88 and 2.30. The overall yield of 5 starting from 8 was 15%.



When the crude mixture of 9–11 from the *n*-Bu_3SnH reduction of 8 was condensed with TCDI in MeCN and then reduced again with *n*-Bu_3SnH and AIBN, some unchanged 11 was recovered, along with a major new product (12) with an R_f value higher than that of 5. Separation of the two compounds required repeated column chromatography, but ultimately yielded TLC-homogeneous samples. The ^1H NMR spectrum of the minor product contained singlets at δ 5.13 and 5.20 that integrated for one hydrogen each and were consistent with nonequivalent CH_2 hydrogens in a 2',3'-*O*-methylene structure, as in 11. The major product was assigned the 2',3'-dideoxy structure

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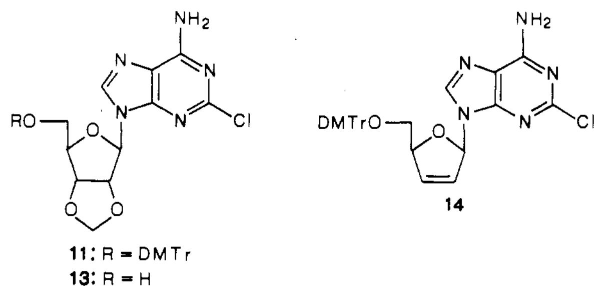
Table I. Effect of 2′,3′-Dideoxyadenosine (1) and 2-Chloro-2′,3′-dideoxyadenosine (3) on the Growth of Human T4$^+$ Lymphocytes (C8166) in Culture

compd	concn, μM	cell growth, % of control
1 (ddAdo)	10	116
	100	116
	500	90
	1000	91
3 (ClddAdo)	10	94
	100	77
	500	37
	1000	0

12. Removal of the 5′-protecting groups in 11 and 12 to obtain 3 and 13 was accomplished in reasonable yield by allowing the compounds to stand on silica gel columns[5] for 120 and 60 h, respectively. Approximately 30% of the unchanged 5′-DMTr compound was recovered in each case and could be recycled. The deprotected 2′,3′-dideoxy derivative 3 (65% yield) was identified on the basis of its elemental analysis, its mass spectrum which showed a strong (M + 1) peak at the expected value of 270, and its ^{1}H NMR spectrum, in CD_3OD solution, which showed the hydrogens at the 3′- and 2′-positions as multiplets at δ 2.15 and 2.45, respectively. The mass spectrum of the deprotected 2′,3′-*O*-methylene derivative 13 contained a strong (M + 1) peak at 314, which was consistent with its assigned structure and the formulation of its 5′-blocked precursor as 11. While the literature on 2′,3′-*O*-methylene derivatives of ribonucleosides is scanty, it is of interest that attempted desulfurization of 2′,3′-*O*-thiocarbonyladenosine with a sponge nickel catalyst has been reported to give, instead of the desired olefin, a low yield of 2′,3′-*O*-methyleneadenosine as the only identifiable product.[21]



Convenient access to the unsaturated analogue 4 was discovered in the reaction of 8 with 1,3-dimethyl-2-phenyl-1,3-diazaphospholidine, a reagent whose use in converting 1,2-diols to olefins via the thiocarbonate esters was first described by Corey and Hopkins.[35] A 60% yield of the 5′-protected olefin 14 was obtained after 24 h at room temperature in THF solution. Gentle detritylation on a silica gel column afforded 4 (69% yield) and the presence of the 2′,3′-ene structure was clearly confirmed by the ^{1}H NMR spectrum, which contained multiplets at δ 6.1 and 6.5 corresponding to two vinyl hydrogens and a multiplet δ 5.0 and 7.0 corresponding to the allylic $C_{4'}$ and $C_{1'}$ hydrogens, respectively. The chemical shifts for the latter hydrogens in the saturated analogue 3 occurred at δ 4.2 and 6.2. This appears to be the first example of the use of this facile and potentially general method to prepare 2′,3′-dehydro-2′,3′-dideoxyribonucleosides from the corresponding ribonucleosides. Previous attempts to convert the 2′,3′-*O*-thiocarbonyl derivative of a ribonucleoside to an olefin by other means, such as sponge nickel or trimethyl phosphite, were unsuccessful.[21]

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Table II. Effect of 2′,3′-Dideoxyadenosine (1), 2-Chloro-2′,3′-dideoxyadenosine (3), and 2-Chloro-2′,3′-didehydro-2′,3′-dideoxyadenosine (4) on Reverse Transcriptase (RT) Production by HIV-Infected Human T4$^+$ Lymphocytes (C8166)

compd	concn, μM	RT, % of control
1 (ddAdo)	2	25
	20	25
	100	<1
	200	<1
3 (ClddAdo)	2	100
	20	57
	100	3
	200	<1
4 (ClddeAdo)	2	53
	20	25
	100	ND[a]

[a] ND = not determined because of high toxicity to host cells.

Table III. Relative Effects of 2′,3′-Dideoxyadenosine (1) and 2-Chloro-2′,3′-dideoxyadenosine (3) on p24 Viral Core Protein Production by HIV-Infected Human T4$^+$ Lymphocytes (C8166)

concn, μM	p24, ng/mL		ratio (3:1)
	1	3	
20	28	16	0.57
100	1.8	2.2	1.2
200	0.2	0.2	1.0

Biological Activity

The ability of ddAdo (1) and 2-ClddAdo (3) to inhibit the growth of a line of immortalized human T4$^+$ lymphocytes (C8166 cells)[36] was compared in culture (Table I). While 1 showed only minimal toxicity at concentrations as high as 1 mM, the 2-chloro derivative 3 caused 23% inhibition of growth at 100 μM and 67% inhibition at 500 μM. The IC_{50} estimated from the growth curve was 360 μM. Thus, replacement of hydrogen by chlorine at the 2-position caused increased toxicity to noninfected cells. Compounds 1 and 3 were then compared for their ability to block viral RT production by HIV-infected C8166 cells (Table II). Incubation of the cells with 20 μM ddAdo, which was minimally toxic, led to a 75% decrease in level of RT in culture supernatants, whereas similar treatment with 3 led to a decrease of only 43%. Thus, at a concentration well tolerated by host cells, the 2-chloro derivative was less effective than the parent drug. At a concentration of 200 μM, virtually complete inhibition of RT production was achieved with both drugs, but as indicated above, this concentration was already somewhat toxic where 3 was concerned. Antiretroviral activity was also estimated on the basis of radioimmunoassays of p24, a major viral core protein encoded in the HIV genome,[37] in culture supernatants of infected cells (Table III). The amount of p24 after treatment of the cells with 1 or 3 over a range of concentrations from 20 to 200 μM was found to be similar within the limits of sensitivity of the assay. Expressed as a ratio, the activity of the two compounds differed by less than 2-fold.

The antiretroviral activity of 4 was also evaluated (Table II). At a concentration of 20 μM, there was 75% inhibition of RT production by HIV-infected C8166 lymphocytes. The activity of 4 at this concentration was therefore comparable to that of 1 and greater than that of 3. Unfortu-

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Table IV. Effect of 2-Chlorocordycepin (5) on the Growth of Human T Lymphocytes ($SupT_1$), Human B Lymphocytes (Raji), and Mouse Fibroblasts (SC-1) in Culture

	cell growth, % of control		
concn, μM	$SupT_1$	Raji	SC-1
1	100	98	82
10	81	69	53
100	73	43	2
500	13	8	ND[a]
1000	8	3	ND

[a] ND = not determined.

nately, however, 4 was also more toxic to cells and could not be tested at concentrations greater than 20 μM. Thus, antiviral activity appeared to be enhanced by the introduction of double bond in the sugar moiety but was accompanied by a larger increase in toxicity to host cells. The net outcome was a decrease in therapeutic selectivity.

The reason for the decreased therapeutic selectivity of 3 and 4 in comparison with 1 is not well understood. Compound 3 is known to be resistant to adenosine deaminase and to be converted readily to its 5′-triphosphate by CEM T-lympoblasts.[19] Comparable biochemical studies with 4 have not been done. However, the cytotoxicity of 4 to T cells is consistent with metabolism to a 5′-triphosphate which, like the 5′-triphosphate of 3, is a better inhibitor of the host cell's DNA polymerase(s) than is the 5′-triphosphate of 1. Another possibility that cannot be discounted at this time, on the other hand, is that 3 and 4 undergo partial cleavage to 2-chloroadenine, and that the latter is converted to 2-chloroadenosine 5′-phosphate by adenine phosphoribosyltransferase, which catalyzes a similar reaction with 2-fluoroadenine.[38]

The results of cytotoxicity assays performed with 2-chlorocordycepin (5) against three types of cells are shown in Table IV. Against the transformed human T lymphocyte cell line $SupT_1$,[36] the IC_{50} of 5 was estimated from the growth curve to be 30 μM, a value approximately 10-fold lower than that previously reported for CEM cells, which are likewise of T lineage. It thus appeared that a 2-chloro substituent enhances the cytotoxicity of cordycepin, probably by preventing enzymatic deamination, which is known to occur rapidly with this drug.[39-41] The ability of 2-halo substituents to block deamination in other adenine nucleosides is well established.[18] Interestingly, the toxicity of 5 appeared to be greater toward $SupT_1$ cells than Raji cells, which are of B rather than T lineage; the IC_{50} of 5 against Raji cells was determined from the growth curve to be 100 μM. Against murine fibroblasts (SC-1 cells),[42] whose growth was measured after 5 days of drug exposure, as compared with 2 days in the case of the lymphocytes lines, 5 was found to have an IC_{50} of 30 μM (Table IV).[43] Other workers using chick embryo fibroblasts and the same duration of drug treatment (5 days) have reported cordycepin to have an IC_{50} of 25–100 μM.[44] In another study,[45] it was observed that 2-fluorocordycepin, which is resistant to the action of adenosine deaminase, is 40 times more potent than cordycepin against human epithelial carcinoma cells (HEp-2) in culture. Biochemical evidence was provided to support the idea that the toxicity of 2-fluorocordycepin is due to cleavage to 2-fluoroadenine and subsequent conversion of the base to its very toxic ribonucleotide by adenine phosphoribosyltransferase.[46] Similar studies with 5 would be of interest in eludicating its mode of action in comparison with cordycepin and 2-fluorocordycepin.

Experimental Section

Ultraviolet spectra were obtained on a Cary Model 210 UV/vis spectrophotometer. ^1H NMR spectra of compounds 3–5 were obtained on a JEOL Model FX270 instrument with Me_4Si as the reference; spectra of other compounds were obtained on a Varian T60 instrument. Chemical shifts given in brackets indicate the presence of fractional amounts of solvents, e.g. MeOH and EtOH, in the test sample. Mass spectra were obtained on a Finnigan MAT-312 instrument. TLC was carried out on Whatman MK6F and Baker 250F silica gel plates containing a fluorescent indicator. Spots were visualized under 254-nm ultraviolet light or with the aid of I_2. Column chromatography was performed on Baker 3405 (60–200 mesh) or Baker "Flash" (40 mm) silica gel. Melting points were measured on a Fisher-Johns hot-stage apparatus and are not corrected. Solvents were routinely stored over 4A molecular sieves. Chemicals were purchased from Aldrich (Milwaukee, WI) and Sigma (St. Louis, MO). 1,3-Dimethyl-2-phenyl-1,3,2-diazaphospholidine was synthesized according by the method of Corey and Hopkins.[35]

2-Chloro-5′-*O*-(4,4′-dimethoxytrityl)adenosine (7). 4,4′-Dimethoxytrityl chloride (1.74 g, 5.15 mmol) was added to a solution of 6 (1.51 g, 5 mmol) in anhydrous pyridine (30 mL), and the mixture was stirred at room temperature for 24 h. The reaction was quenched by addition of MeOH (5 mL), and after evaporation to dryness under reduced pressure, the residue was taken up into $CHCl_3$ (250 mL) and the solution extracted repeatedly with H_2O. Evaporation of the aqueous layer yielded 0.35 g (23% recovery) of unchanged starting material (R_f 0.30; silica gel, 4:1 $CHCl_3$–MeOH). Drying of the organic layer (Na_2SO_4) and evaporation afforded crude 7 as a yellow gum (3.3 g), which was purified by chromatography on a silica gel column (40 × 2.5 cm) with $CHCl_3$ followed by 2% MeOH in $CHCl_3$ as eluents. Fractions containing pure 7 (R_f 0.77) were pooled and evaporated to a colorless foam (2.1 g, 69% yield, 86% based on consumed starting material): mp 189–191 °C; NMR ($CDCl_3$) δ 3.43 (m, 2 H, $C_{5'}$-H), 3.73 (s, 6H, OCH_3), 4.1–4.8 (br m, 3 H, $C_{2'}$-H, $C_{3'}$-H, $C_{4'}$-H), 6.0 (m, 1 H, $C_{1'}$-H), 6.8–7.5 (m, 13 H, aromatic), 8.13 (s, 1 H, C_8-H). Anal. ($C_{31}H_{30}ClN_5O_6{\cdot}CH_3OH$) C, H, Cl, N.

2-Chloro-5′-*O*-(4,4′-dimethoxytrityl)-2′,3′-*O*-thiocarbonyladenosine (8). TCDI (2.63 g, 16.5 mmol) was added to a solution of 7 (3.93 g, 6.5 mmol) in anhydrous MeCN (100 mL), and the mixture was stirred at room temperature overnight under an atmosphere of N_2. Solvent evaporation at 35 °C (bath temperature) left a yellow gum (7.7 g). The product was purified by chromatography on a silica gel column (40 × 2.5 cm), which was eluted slowly with $CHCl_3$. Fractions containing mainly the desired product (R_f 0.8; silica gel, 9:1 $CHCl_3$–MeOH) were pooled, and the solution was concentrated to a volume of 5–10 mL and added to cold hexane to obtain a solid (3.2 g, 80%): mp 235–246 °C; NMR ($CDCl_3$) δ 3.2 (m, 2 H, $C_{5'}$-H), 3.7 (s, 6 H, OCH_3), 4.8 (m, 1 H, $C_{2'}$-H), 5.7 (m, 1 H, $C_{3'}$-H), 5.8 (m, 1 H, $C_{1'}$-H), 6.2–7.4 (m, 13 H, aromatic), 7.8 (s, 1 H, C_8-H). Anal. Calcd ($C_{32}H_{28}ClN_5O_6S$) C, H, Cl, N, S.

2-Chloro-3′-deoxyadenosine (2-Chlorocordycepin, 5). To a solution of 8 (2.26 g, 3.5 mmol) in anhydrous toluene (100 mL) were added AIBN (0.060 g, 0.36 mmol) and n-Bu_3SnH (6.6 mL, 25.3 mmoL). The reaction mixture was purged with dry N_2 gas for 30 min, then heated at 110–120 °C for 3.5 h while still under N_2, and finally left to stand at room temperature overnight. Solvent evaporation under reduced pressure left an oil, which was applied onto a silica gel column (55 × 5 cm). The column was

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eluted first with 1% MeOH in $CHCl_3$ to obtain a faster moving fraction (R_f 0.7; silica gel, 9:1 $CHCl_3$–MeOH) consisting of the 5′-blocked derivative **11** along with some $(n\text{-}Bu_3Sn)_2$, and a slower moving fraction (R_f 0.5), which was assumed to consist of a mixture of the 2′-deoxy and 3′-deoxy derivatives **9** and **10**. Evaporation of the fractions containing **9** and **10** gave a colorless foam (1.2 g, 59% yield), mp 185–189 °C. A portion of the latter material (0.25 g, 0.43 mmol) was treated with 80% AcOH–H_2O, whereupon a clear solution was formed. The solution was kept at 50 °C for 30 min and evaporated to dryness under reduced pressure (40 °C bath temperature). Final traces of AcOH were removed by coevaporation with 50% MeOH–H_2O (3 × 10 mL), and the crude product was partitioned between $CHCl_3$ and H_2O with vigorous agitation. The aqueous layer, along with some undissolved colorless solid which remained suspended at the interface, was concentrated to dryness. The residue was triturated with hot EtOH (20 mL), undissolved *2-chloroadenine* (identical with an authentic sample) was filtered off, and the filtrate was concentrated to a small volume and placed in the refrigerator, whereupon **5** crystallized out. The crystals were collected, washed with cold EtOH, and dried in vacuo at 40 °C for 48 h: yield 0.036 g (28%); mp 251–253 °C; R_f 0.5 (silica gel, 20% MeOH–$CHCl_3$); UV λ_{max} 265 nm; NMR (CD_3OD) δ [1.2 (t, 0.3CH_3CH_2OH)], 2.05 (m, 1 H, $C_{3'}$-H_a), 2.35 (m, 1 H, $C_{3'}$-H_b), 3.60 (d, 1 H, $C_{5'}$-H_a), 3.70 (dd, 1 H, $C_{5'}$-H_b), [3.94 (q, 0.3CH_3CH_2OH)], 4.52 (m, 1 H, $C_{4'}$-H), 4.64 (q, 1 H, $C_{2'}$-H), 5.9 (d, 1 H, $C_{1'}$-H), 8.4 (s, 1 H, C_8-H); MS 287 (M + 1). Anal. ($C_{10}H_{12}ClN_5O_3 \cdot 0.3EtOH \cdot 0.25H_2O$) C, H, Cl, N.

2-Chloro-2′,3′-dideoxyadenosine (2-ClddAdo, 3). A crude mixture of compounds **9–11** from an experiment similar to the one described in the preceding section (1.56 g, ca. 5 mmol) was dissolved in MeCN (100 mL) and the solution was treated with TCDI (2.45 g, 12.4 mmol). After being stirred overnight at room temperature under N_2, the mixture was evaporated to dryness. The residue (4 g), whose TLC (silica gel, 9:1 $CHCl_3$–MeOH) still showed two spots of starting material along with two new spots, was redissolved in toluene (50 mL). AIBN (0.12 g, 0.72 mmol) was added to the solution, and the latter was purged with N_2 at room temperature for 30 min and treated with $n\text{-}Bu_3SnH$ (6.0 mL, 23.5 mmol). After being kept for 48 h at 120–130 °C under N_2, the reaction mixture, whose TLC now showed only two spots (R_f 0.88 and 0.83), was evaporated to dryness and the residue was applied onto a silica gel column (40 × 2.5 cm). The column was eluted first with 25% AcOH in hexane to remove $(n\text{-}Bu_3Sn)_2$ and then with 0.5% MeOH in $CHCl_3$, which still yielded a mixture of two products with R_f 0.88 and 0.83. The mixture was rechromatographed on a larger column (70 × 5 cm), which was eluted first with a large volume of $CHCl_3$ and then with 0.5% MeOH in $CHCl_3$ to give 0.45 g of pure **12** (R_f 0.88) and 0.58 g of still unresolved mixture. Further purification of this mixture by passage through a silica gel column (70 × 5 cm) two more times yielded another 0.17 g of pure **12** (total 0.62 g), 0.18 g of **11** (R_f 0.83), and 0.21 g of mixture. The NMR spectrum of **11** in $CDCl_3$ gave signals at δ 3.3 (t, 2 H, $C_{5'}$-H), 3.75 (s, 6 H, CH_3O), 4.45 (m, 1 H, $C_{4'}$-H), 5.05 (m, 1 H, $C_{2'}$-H), 5.13 and 5.20 (2 s, 2 H, OCH_2O), 6.1 (d, 1 H, $C_{1'}$-H), 6.6–7.2 (m, 13 H, aromatic), 7.9 (s, C_8-H). The spectrum was generally similar to that of 2-chloroadenosine except for the additional presence of the singlets at δ 5.13 and 5.20 consistent with diamagnetically nonequivalent methylenedioxy protons.

A portion of the 5′-blocked derivative **12** (125 mg, 0.218 mmol) was applied onto a silica gel colunn (25 × 2.5 cm) packed in $CHCl_3$. The column was washed with benzene to remove the $CHCl_3$ and allowed to stand at room temperature for 60 h, and the product was eluted with 5% MeOH in $CHCl_3$. A small amount (25 mg) of intact **12** eluted early and was set aside for recycling. Fractions containing the desired product (R_f 0.26; silica gel, 9:1 $CHCl_3$–MeOH) were pooled and evaporated, giving **3** as a white solid (39 mg, 65% yield, 83% based on consumed starting material): mp 238–240 °C; NMR (CD_3OD) δ 2.15 (m, 2 H, $C_{3'}$-H), 2.45 (m, 2 H, $C_{2'}$-H), [3.35 (s, CH_3OH)], 3.70 (m, 2 H, $C_{5'}$-H), 4.2 (m, 1 H, $C_{4'}$-H), 6.2 (m, 1 H, $C_{1'}$-H), 8.35 (s, 1 H, C_8-H); MS 270 (M + 1). Anal. ($C_{10}H_{12}ClN_5O_2 \cdot 0.2MeOH$) C, H, Cl, N.

A portion of the 5′-blocked derivative **11** (175 mg, 0.282 mmol) was applied onto a silica gel column (20 × 1 cm) packed in $CHCl_3$. After elution with benzene, the column was left to stand at room temperature for 120 h and then eluted with $CHCl_3$ followed by 5% MeOH in $CHCl_3$. A small amount (50 mg) of unchanged **11** eluted early and was recycled. Fractions containing pure product (R_f 0.38; silica gel, 9:1 $CHCl_3$–MeOH) were pooled and evaporated, giving **13** as a waxy white solid (42 mg, 47% yield, 67% based on consumed starting material): mp 228–230 °C; MS 314 (M + 1). The microanalytical sample was not dried, resulting in values consistent with the presence of fractional amounts of all three chromatographic solvents. Anal. ($C_{11}H_{12}ClN_5O_4 \cdot 0.2C_6H_6 \cdot 0.3CH_3OH \cdot 0.03CHCl_3$) C, H, Cl, N.

2-Chloro-2′,3′-didehydro-2′,3′-dideoxy-5′-*O*-(4,4′-dimethoxytrityl)adenosine (14). 1,3-Dimethyl-2-phenyl-1,3,2-diazaphospholidine (0.157 g, 0.81 mmol) was added to a solution of **8** (0.175 g, 0.27 mmol) in anhydrous THF (2 mL), and the reaction mixture was stirred at room temperature for 24 h and evaporated to dryness under reduced pressure. The crude product was chromatographed on a silica gel column (25 × 2 cm), which was eluted first with $CHCl_3$ (100 mL) and then with 0.5% MeOH in $CHCl_3$ (250 mL). Fractions containing the product (R_f 0.80; silica gel, 9:1 $CHCl_3$–MeOH) were poled and evaporated to a colorless foam (100 mg, 60% yield): mp 179–181 °C; NMR ($CDCl_3$) δ 3.4 (m, 2 H, $C_{5'}$-H), 3.8 (s, 6 H, OCH_3), 5.1 (m, 1 H, $C_{4'}$-H), 6.0 (m, 1 H, $C_{3'}$-H), 6.6 (m, 1 H, $C_{2'}$-H), 6.9 (m, 1 H, $C_{1'}$-H), 7.3 (m, 13 H, aromatic), 8.0 (s, 1 H, C_8-H). Anal. ($C_{31}H_{28}ClN_5O_4 \cdot 0.3CHCl_3$) C, H, Cl, N.

2-Chloro-2′,3′-didehydro-2′,3′-dideoxyadenosine (2-Cl-ddeAdo, 4). Compound 14 (275 mg, 0.454 mmol) was applied onto a silica gel column (25 × 1 cm) packed in $CHCl_3$. The column was washed with benzene to remove the $CHCl_3$, left to stand for 96 h, and finally eluted with 5% MeoH in $CHCl_3$. Some unreacted 14 (51 mg) was recovered in the early fractions. Later fractions containing the product (R_f 0.34, silica gel, 9:1 $CHCl_3$–MeOH) were pooled and evaporated to a white solid (84 mg, 69% yield, 80% based on consumed starting material): mp 200–205 °C dec; NMR ($CDCl_3$ + CD_3OD) δ 3.8 (d, 2 H, $C_{5'}$-H), [4.3 (s, CH_3OH)], 5.0 (m, 1 H, $C_{4'}$-H), 6.1 (m, 1 H, $C_{3'}$-H), 6.5 (d, 1 H, $C_{2'}$-H), 7.0 (m, 1 H, $C_{1'}$-H), 8.2 (s, 1 H, C_8-H). Anal. ($C_{10}H_{10}ClN_5O_2 \cdot 0.4MeOH$) C, H, Cl, N.

Virus Inhibition Assays. The C8166 human $T4^+$ lymphocytes were maintained in RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS), penicillin, and streptomycin. Experiments were conducted with either heat-inactivated (55 °C, 60 min) or non-heated FBS. Approximately 2×10^5 cells were seeded into each well of a 24-well microtiter plate. Drug was dissolved in culture medium and added to appropriate wells at various dilutions from a stock solution. Then, 2500 RT units[47] of cell-free virus (HXBc2 strain of HIV) was added to the cells, and the final volume of each well was adjusted to 1 mL with culture medium. Medium was replaced every 3 days with fresh drug-containing medium. On day 6, virus replication was assessed by radioimmunoprecipation of labeled cell lysates and supernatants,[48] by RT assay of cell supernatants,[49] or by p24 radioimmunoassay with a commercially available ELISA kit (Dupont).[50]

For the cytotoxicity assays againt the C6188, $SupT_1$, and Raji cells, uninfected cells were maintained in growth medium as described above, and various dilutions of drug were added. After 48 h, cells were stained with trypan blue were counted and the percentage of viable cells (based on dye exclusion) in the drug-treated cultures relative to controls was determined. Results shown in Tables I and IV are those for a typical experiment. Cytotoxicity in uninfected SC-1 fibroblasts was determined sim-

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ilarly, except that the cells were grown as monolayers in plastic dishes containing Dulbecco's modified Eagle's medium supplemented with 10% calf serum and antibiotics and were stained and counted after 5 days.[42]

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Registry No. 3, 114849-58-0; 4, 119530-56-2; 5, 115044-75-2; 6, 146-77-0; 7, 119530-57-3; 8, 119530-58-4; 9, 119530-59-5; 10, 119530-60-8; 11, 119530-61-9; 12, 119530-62-0; 13, 119530-63-1; 14, 119530-64-2.

Book Reviews

Imidazopyridines in Sleep Disorders: A Novel Experimental and Therapeutic Approach. L.E.R.S. Monograph Series, Volume 6. Edited by J. P. Sauvanet, S. Z. Langer, and P. L. Morselli. Raven, New York. 1988. xxv + 400 pp. 16 × 24 cm. ISBN 0-88167-377-3. $35.00.

The proceedings of a Laboratories d'Etudes et de Recherches Synthelabo (L.E.R.S.) Symposium held in Paris, France, October 22–24, 1986, are presented in this volume. The subject was the development of a sedative–hypnotic nonbenzodiazepine active at type-1 benzodiazepine receptors and known by its International Nonproprietary Name (INN) zolpidem [SL 80,0750-23N, *N*,*N*,6-trimethyl-2-(4-methylphenyl)imidazo[1,2-*a*]pyridine-3-acetamide hemitartrate]. Thirty-three major presentations are grouped into five main areas: Basic Sciences and Pre-Clinical Studies, Pre-Clinical and Pharmacokinetic Studies, Pharmacodynamics and Polysomnographic Studies in Healthy Volunteers, Polysomnographic and Short-Term Clinical Studies in Insomniacs, and Intermediate and Long-Term Studies in Insomniacs. Also included are 14 *Poster Abstracts* that contain data supporting the main presentations. Since this material was presented in a symposium that dealt with a specific pharmacologic agent, the authors discussed their subjects without full explanations of some aspects of this specialization, such as stages of sleep and criteria for measuring efficacy. However, the uninitiated can easily utilize references in order to develop full appreciation of this field. Background discussions include: the need for hypnotic–sedative drugs in sleep disorders, benzodiazepine receptors and ligands, and desirable profiles of sedatives. Readers specifically interested in this field and those desiring to enter it can learn how zolpidem was developed from synthesis and SAR studies to its mechanism of action, pharmacokinetics, safety, and subsequent clinical development.

The scientific content is of high quality. Presentations are error-free, and each is thoroughly referenced. The Subject Index is comprehensive. The volume should be a valuable reference book for libraries, pharmacologists, and medicinal chemists. The price is reasonable.

Burroughs Wellcome Co.
Research Triangle Park,
North Carolina 27709
Janet L. Rideout

Handbook of Research Laboratory Management. By Virginia P. White. ISI Press, Philadelphia, PA. 1988. xvi + 240 pp. 18 × 26 cm. ISBN 0-89495-065-7. $49.95.

The author of this volume begins from a premise with which most individuals engaged in scientific administration would agree: research management requires specialized skills and knowledge that can be difficult to identify and acquire. And it is also true that those who come to be involved in research direction and leadership normally have no specific training that would give them those skills. This handbook has been written to provide a source of such information.

Virginia P. White, who has been a director at several important laboratories, appropriately begins her book with a brief chapter highlighting the importance of both intellectual prowess and a sound management foundation to the success of a research enterprise. This is followed by chapters on personnel—scientific administrators, the administrative staff, and the research staff. The organization of a research institute and the communication of scientific results in conferences, seminars, and publications are then discussed. Lastly, the nuts and bolts of research are reviewed—library, patents, safety, human and animal subjects, buildings and equipment, and financial resources.

No one would argue that all of these topics are not highly relevant to the management of research. But to whom is the level of this presentation appropriate? The book abounds in banalities: "All research scientists go through fallow periods that usually prove to be temporary. The reason for this is not fully understood." "The formula for furthering creativity in a research-and-development organization given by S. J. (Sol) Buchsbaum, executive vice-president of AT&T Bell Laboratories, is 'Hire the best, give them the best, demand the best'.". "In some laboratories, small boards where humorous material may be posted by anyone at will provide lighthearted moments during a monotonous task, a grueling experiment, or just a bad day."

By contrast, the more sophisticated findings and weighty issues in the management of innovation—stimulating creativity, maximizing teamwork in multidisciplinary facilities, motivating productivity, interfacing with business groups, focusing research efforts, project management systems—are inadequately addressed or ignored.

This book will be useful to entry level nonscientist research administrators and project managers who have had little contact with a labortory environment. It should be of interest to students and postdoctorals seeking to learn about their future workplace. For these reasons it merits a place in academic and industrial science libraries. But it is too elementary to be of much help to senior managers of large research departments.

Immunopharmaceutics, Inc.
San Diego, California 92127
Manfred E. Wolff

The Basics of Technical Communicating. By B. Edward Cain. ACS Professional Reference Book. American Chemical Society, Washington, DC. 1988. xiii + 198 pp. 16 × 23.5 cm. ISBN 0-8412-1451-4. $29.95.

Teachers of scientific or technological subjects who have had to read compositions by students in college, graduate, or engineering schools have repeatedly encountered agonizing sentence structures, faulty word designations, illogical connections, and other serious flaws that interfere with easy reading and prevent concentration on the technical content of the text. Mature scientists themselves are not less to blame. Members of NIH, NSF, and DOD study sections who are supposed to judge the scientific value of grant applications are often stymied by excessive wordage, complex sentence structures, and lack of linguistic conciseness. Some engineering schools have installed courses in English that transcend the topics offered by a learned but literature-minded college faculty. Students in such courses in practical English learn