mixture was stirred for 5 h at room temperature, and the solvent was removed in vacuo to give a solid, which was washed with a mixture of ethyl acetate and ether (1:1) to afford 1.95 g (88.6%) of **7**: mp 150–151 °C; recrystallization from MeOH/ethyl acetate gave mp 151 °C; NMR (Me_2SO) δ 8.34 (d, 1 H, J = 1.9 and 8.9 Hz), 7.30 (s, 1 H), 7.26 (s, 1 H), 3.30 (s, 3 H). Anal. ($C_{11}H_{10}O_2S$) C, H, S.

(6,7-Dihydroxy-2-tetralinyl)dimethylsulfonium Bromide (6). A solution of BBr_3 (1.0 mL, 1 M solution in CH_2Cl_2) was added to a solution of **18** (180 mg) in CH_2Cl_2 (5 mL) cooled in a dry ice–acetone bath under nitrogen. After stirring for 30 min, the reaction mixture was warmed to room temperature, and then stirring was continued for 15 h at room temperature. Anhydrous MeOH (2 mL) was added to the resulting mixture with cooling in a dry ice bath, followed by stirring for 3 h at room temperature. Removing the solvent gave a residue, which solidified. The residue was washed with ethyl acetate to yield 218 mg of white solids (dec pt 125–127 °C). Recrystallization from MeOH–ethyl acetate afforded 166 mg (72%) of colorless crystals: dec pt 126–127 °C; NMR (D_2O) δ 6.70 (s, 2 H), 3.85–3.65 (m, 1 H), 3.30–2.60 (m, 4 H), 2.95 (s, 3 H), 2.93 (s, 3 H), 2.50–1.85 (m, 2 H). Anal. ($C_{12}H_{17}SO_2Br$) C, H, S.

Preparation of Brain Slices. Male Swiss Webster mice (Harlan labs) were used for all experiments. After the mice were decapitated, the brains were removed, and the striatal tissue rostral to the anterior commissures were dissected.[20] The tissue was cut into 0.5 × 0.5 mm sections with a McIlwain tissue chopper and dispersed into a Krebs Ringer bicarbonate medium. The medium contained (mM) NaCl (118), KCl (4.8), $CaCl_2$ (1.3), $MgSO_4$ (1.2), $NaHCO_3$ (25), KH_2PO_4 (1.2), ascorbic acid (0.6), disodium EDTA (0.03), glucose (11). It was bubbled with a 95% O_2–5% CO_2 mixture and adjusted to pH 7.2 with NaOH. The slices were incubated for 20 min with [^{3}H]choline at a final concentration of 0.1 M. This low concentration of [^{3}H]choline favors the selective uptake of choline into cholinergic neurons through a high-affinity uptake system.[21] After the slices were rinsed, they were transferred to a superfusion system[22] and superfused with medium at 37 °C at a constant rate of 0.5 m/min. Fractions were collected every 5 min, starting at 40 min after the onset of superfusion. Transmitter release was induced by superfusion with medium containing 12.5 M K^+ for 5 min starting at 60 (S_1) and 90 (S_2) min after the onset of the superfusion. Drugs were added to the medium 15 min before S_2. The slices were superfused for a total of 110 min. At the end of the superfusion, the radioactivity remaining in the tissue was extracted by homogenizing the tissue in 0.4 N perchloric acid. The radioactivity in the superfusate samples and tissue extracts was determined by liquid scintillation counting.

The outflow of tritium into the superfusion medium during each 5-min interval is expressed as a fraction of the total tritium content of the tissue at the beginning of the interval (fractional release). This was calculated by correcting the tissue content of each fraction for the radioactivity lost to the medium. The K^+-induced increase in release (denoted S_2 and S_1) is the sum of the fractional release obtained following the addition of the high K^+ medium above the baseline of spontaneous outflow (Sp). The latter is the fractional release of tritium obtained during the 5-min interval prior to the addition of the high K^+ medium. The effects of dopaminergic agonists on K^+-induced release were evaluated by determining the ratio S_2/S_1 for control- and drug-treated slices. The effect of drugs on the spontaneous release of tritium was evaluated by determining the fractional release of radioactivity during the 5-min intervals preceding S_2 and S_1, respectively, and was expressed as the ratio (Sp_2/Sp_1).

The tritium released by the high K^+ medium was not chemically characterized in these experiments, since several previous studies have demonstrated that radioactive acetylcholine formed from radiolabeled choline can be released from brain slices by K^+-depolarization.[23-28] In these studies, physostigmine was added to the medium to inhibit the metabolism of acetylcholine. However, the inhibition of acetylcholine metabolism can cause high extracellular levels of acetylcholine, which has been shown to inhibit the depolarization-induced release of acetylcholine by the process of feedback inhibition.[29,30] In the present study, physostigmine was omitted from the medium. Under our conditions, the K^+-induced release of tritium is completely dependent on the presence of calcium ions in the superfusion medium.

Registry No. **5**, 89017-40-3; **6**, 89017-41-4; **7**, 89017-42-5; **8**, 56221-32-0; (*R*)-**9**, 89017-43-6; (*S*)-**9**, 89017-44-7; **10**, 89017-45-8; **11**, 5333-34-6; **12**, 13575-74-1; **13**, 13575-75-2; **15**, 35491-96-4; **16**, 89017-46-9; **17**, 33212-93-0; **18**, 89017-47-0; Ach, 51-84-3; veratrol, 91-16-7; tetralol, 529-33-9; succinic anhydride, 108-30-5.

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Potential Inhibitors of Nucleotide Biosynthesis. 2. Halomethyl Ketone Derivatives of Pyrimidine Nucleosides[1]

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Several halomethyl ketone derivatives of pyrimidine nucleosides have been prepared for evaluation as cytotoxic agents. The first series are 1-(8-halo-2,5,6,8-tetradeoxy-β-D-*erythro*-oct-7-ulofuranosyl)thymines (**7–9**), whereas the second type are halo derivatives of acetophenone (**12–14** and **16**). These compounds are cytotoxic, and one (**13**) showed activity against the P388 leukemia in vivo.

A detailed rationale for the preparation of nucleosides containing a chemically reactive function attached through a spacer to C-5′ that may act as active-site-directed, irreversible inhibitors of enzymes that metabolize the corresponding nucleotides has been presented.[3] As a part of this program, we prepared diazo ketone derivatives of thymidine and uridine,[4] since such a functional group should react under in vivo conditions with protonated guanidine groups that act as binding points for the phosphate moieties for the corresponding nucleotides.[5] An

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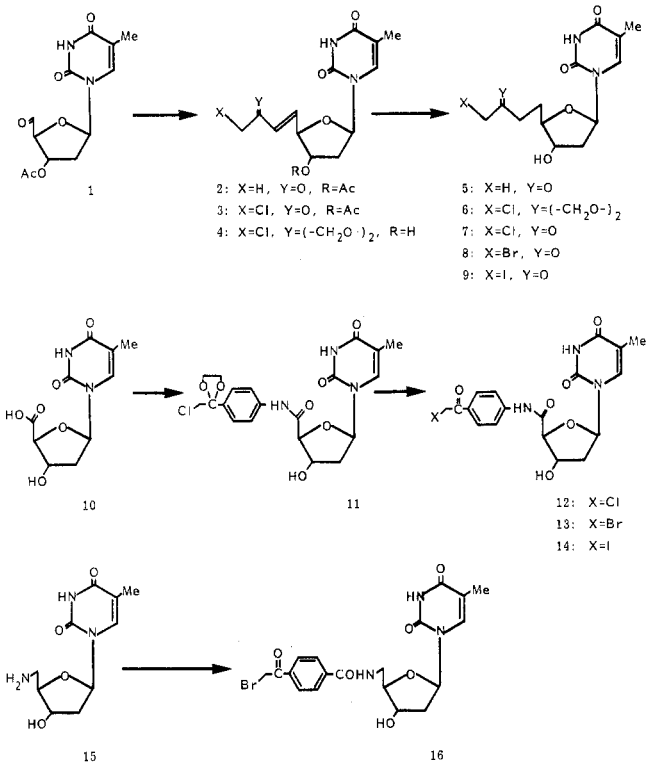
Scheme I



effort was made to properly position the diazomethyl ketone group by extending the carbon chain attached to C-4 of the furanose ring of these nucleosides, but they were unexpectedly unreactive, which might account for their lack of biological activity, since closely related compounds containing more reactive functions are both cytotoxic in vitro and active against the P388 leukemia in vivo.[6] These results led us to the preparation of a series of the more reactive halomethyl ketone nucleosides, which is the subject of this paper.

Previously, we obtained nucleosides containing a chloromethyl ketone group as byproducts from the preparation of the diazomethyl ketones from the acid chlorides,[4] but these compounds were obtained in low yields and were unstable as isolated. Rather than attempt to maximize the yields of the chloro ketones from this procedure, we elected to devise other approaches to this type of structure. The route selected begins with an intermediate used in the preparation of the diazomethyl ketone nucleosides—1-(3-*O*-acetyl-2-deoxy-β-D-*erythro*-pento-1,5-dialdo-1,4-furanosyl)thymine (**1**), which was first allowed to react with triphenylacetonylphosphorane as a model to give 1-(3-*O*-acetyl-2,5,6,8-tetradeoxy-β-D-*erythro*-oct-5-en-7-ulofuranosyl)thymine (**2**). Catalytic reduction of the double bond of **2**, followed by deacetylation, gave 1-(2,5,6,8-tetradeoxy-β-D-*erythro*-oct-7-ulofuranosyl)thymine (**5**). In the same way, reaction of **1** generated in situ with triphenylchloroacetonylphosphorane[7] gave **3**, which was purified by TLC. The instability of **3**, however, defeated efforts to further purify **3** or study its reactions with azide or hydroxide ions; these attempts resulted in decomposition, and no products could be isolated. Therefore, it was characterized by MS and ^{1}H NMR and then converted by a standard procedure to its stable dioxolane derivative.

Table I. Chemical Reactivity

no.	$T_{1/2}$,[a] h
7	>52 (26% dec)
8	1.8
9	4
12	>52 (14% dec)
13	4
14	13
16	1

[a] $T_{1/2}$ = chemical half life in water at room temperature as judged by HPLC.[3]

The resulting deactivation of the chlorine allowed recrystallization from methanol to give an analytical sample, a procedure that resulted in removal of the acetyl group to produce 1-[8-chloro-2,5,6,8-tetradeoxy-7-(ethylenedioxy)-β-D-*erythro*-oct-5-enofuranosyl]thymine (**4**). The use of aldehyde **1** prepared from its 1,2-dianilinoethane derivative[2] resulted in a simpler workup requiring no chromatographic purification, which, perhaps as a result, gave a higher yield of **4** (51 vs. 34%). Compound **4** was reduced with hydrogen and 30% palladium on charcoal catalyst, after which the deactivating dioxolane function of **6** was hydrolyzed with dilute sulfuric acid to the ketone **7**. Exchange of the chlorine of **7** for bromine and iodine to give **8** and **9** was readily accomplished by heating with the corresponding sodium halides in acetone. The conversion of the aldehyde **1** to the bromo analogue of **3** (in 25% yield) was also accomplished by its reaction with triphenylbromoacetonylphosphorane, but in view of the ease of the exchange reactions, this route to **8** was not pursued further.

For the second type of halomethyl ketone nucleoside, derivatives of acetophenone, the ribofuranuronic acid **10** was prepared by the oxidation of thymidine according to a literature procedure.[8] Since attempts to cause 1,2-dideoxy-1-(3,4-dihydro-5-methyl-2,4-dioxo-1(2*H*)-pyrimidinyl)-β-D-ribofuranuronic acid (**10**) to react with 4-amino-α-chloroacetophenone to give **12** were unsuccessful due to reaction of the amino ketone with itself (MS), its dioxolane was prepared by a modification of the literature procedure,[9] which in our hands failed to produce the product. Reaction of the dioxolane with the ribofuranuronic acid **10** via the acid azide prepared in situ by means of diphenylphosphoryl azide was poor; the yields ranged between 20 and 35%. Hydrolysis of the dioxolane **11** with dilute acid gave the desired *N*-[4-(chloroacetyl)phenyl]-1,2-dideoxy-1-[3,4-dihydro-5-methyl-2,4-dioxo-1(2*H*)-pyrimidinyl]-β-D-*erythro*-pentofuranuronamide (**12**). Although preliminary results indicated that the bromo compound **13** can be made in a similar manner, **13** and **14** were readily prepared by the halide exchange reaction described above for **8** and **9**.

A third type of halomethyl ketone nucleoside, 5′-[[4-(bromoacetyl)benzoyl]amino]-5′-deoxythymidine (**16**), was prepared by the reaction of in situ generated 4-(bromoacetyl)benzoyl bromide with 5′-amino-5′-deoxythymidine (**15**)[10] in *N*,*N*-dimethylacetamide. Confirmation of the acylation of the 5′-amino group was obtained by a negative ninhydrin reaction (TLC).

The half-lives of these compounds were determined[3] as a measure of their relative chemical reactivities (Table I). The chloro compounds were unexpectedly stable, relative to the bromo compounds, which proved to be somewhat

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Table II. Biological Activity

	cytotoxicity I_{50},[a] μM			P388 in vivo	
no.	H.Ep.-2	L1210	H/L ratio	dose,[b] mg/kg	% ILS[c]
5					
7	25	66	0.4	50	10
8	55	110	0.5	12.5	16
9	17	20	0.8	25	16
12	30	28	1.1	100	14
13	12	6	2	200	38
14	29	8	3.6	25	5
16	>40	>70		50	15

[a] I_{50} is the concentration that produces 50% inhibition of proliferation of L1210 cells in suspension culture in 48 h or 50% inhibition of cloning of H.Ep.-2 cells over a 12-day period relative to growth in the controls. [b] Single injection on days 1-5. [c] Percent increase in life span relative to untreated controls.

more reactive than the iodo compounds. The aliphatic ketones were significantly more reactive than the acetophenone derivatives, but none of these differences is reflected in the cytotoxicities to H.Ep.-2[11] and L1210[12] cells in culture, nor is the reversal in I_{50} ratios for the two cell lines between the aliphatic (0.4–0.8) and aromatic (1.1–3.6) ketones easy to explain. Of these compounds, only one, **13**, showed activity against the P388 leukemia in vivo.[13] This compound and its relatives (**12** and **14**) all inhibit both RNA and DNA, but not protein, synthesis at 25 μM.[14] The aliphatic chloro ketone **7**, however, showed no effect on macromolecular synthesis at 30 μM.

Experimental Section

All evaporations were carried out in vacuo with a rotary evaporator. Analytical samples were normally dried in vacuo over P_2O_5 at room temperature for 16 h. Analtech precoated (250 μm) silica gel G(F) plates were used for TLC analyses; the spots were detected by irradiation with a Mineralight and by charring after spraying with saturated $(NH_4)_2SO_4$. Compounds containing the halomethyl ketone function were also detected with (*p*-nitrobenzyl)pyridine. All analytical samples were essentially TLC homogeneous. Melting points were determined with a Mel-Temp apparatus and are not corrected. The UV absorption spectra were determined in 0.1 N HCl (pH 1), pH 7 buffer, and 0.1 N NaOH (pH 13) with a Cary 17 spectrophotometer: the maxima are reported in nanometers (10^{-3} ε). The NMR spectra were determined with a Varian XL-100-15 spectrometer in Me_2SO-d_6 (unless otherwise specified) with tetramethylsilane as an internal reference: chemical shifts (δ) quoted in the case of multiplets are measured from the appproximate center. The mass spectral data were obtained with a Varian MAT 311A mass spectrometer in the electron-impact (EI), field-desorption (FD), or fast-atom-bombardment (FAB) mode.

1-(3-*O*-Acetyl-2,5,6,8-tetradeoxy-β-D-*erythro*-oct-5-en-7-ulofuranosyl)thymine (2). To a solution of 284 mg (1.0 mmol) of 3′-*O*-acetylthymidine in 4 mL of dimethyl sulfoxide was added 825 mg (4.0 mmol) of dicyclohexylcarbodiimide, followed by 40 μL (0.5 mmol) of dichloroacetic acid. After stirring for 20 h at ambient temperature, the reaction mixture was treated with 40 μL of pyridine and 318 mg (1.0 mmol) of triphenylacetonylphosphorane, stirred for 20 h, and then evaporated to dryness in vacuo. The residue was purified by preparative TLC on Brinkmann silica gel (2 mm) plates developed in 95:5 chloroform–methanol. A glass was obtained: yield 315 mg (98%); MS (EI), *m/z* 197 (sugar$^+$), 322 (M^+); ^{1}H NMR (Me_2SO-d_6) δ 1.81 (s, CH_3), 2.09 (s, CH_3 of acetyl), 2.28 (s, 3 $H_{8'}$), 2.30 (m, 2 $H_{2'}$), 4.6 br m, $H_{4'}$), 5.23 (q, $H_{3'}$), 6.2 (m, $H_{6'}$), 6.28 (m, $H_{1'}$), 6.95 (q, $H_{5'}$), 7.59 (d, H_6) (spectrum indicates the trans isomer).

1-(8-Chloro-2,5,6,8-tetradeoxy-β-D-*erythro*-oct-5-enofuranosyl)thymine (3). To a solution of 284 mg (1.0 mmol) of 3′-*O*-acetylthymidine in 4 mL of dimethyl sulfoxide was added 825 mg (4.0 mmol) of dicyclohexylcarbodiimide, followed by 40 μL (0.5 mmol) of dichloroacetic acid. After stirring for 20 h at ambient temperature, the reaction mixture was treated with 40 μL of pyridine and 353 mg (1.0 mmol) of triphenylchloroacetonylphosphorane, stirred for 3 h, and then evaporated to dryness in vacuo. The residue was purified by preparative TLC on Brinkmann silica gel (2 mm) plates with chloroform–methanol (95:5) as the developing solvent. A yellow semisolid was obtained: yield 340 mg (95%); MS/(EI), *m/z* 231 (sugar$^+$), 356 (M^+); ^{1}H NMR ($CDCl_3$) δ 1.95 (m, CH_3), 2.13 (s, CH_3 of acetyl), 2.35 (m, 2 $H_{2'}$), 4.22 (s, 2 $H_{8'}$), 4.6 (m, $H_{4'}$), 5.17 (m, $H_{3'}$), 6.35 (m, $H_{1'}$), 6.7 (m, $H_{6'}$), 7.1 (m, $H_{5'}$), 7.2 (m, H_6) (spectrum indicates the trans isomer).

1-[8-Chloro-2,5,6,8-tetradeoxy-7-(ethylenedioxy)-β-D-*erythro*-oct-5-enofuranosyl]thymine (4). A mixture of 320 mg (0.9 mmol) of 1-(8-chloro-2,5,6,8-tetradeoxy-β-D-*erythro*-oct-5-enofuranosyl)thymine (**3**) and 25 mg of *p*-toluenesulfonic acid in 25 mL of ethylene glycol and 50 mL of benzene was refluxed for 24 h using a Dean–Stark trap. It was then evaporated to dryness in vacuo at 70 °C. A chloroform solution of the residue was purified by preparative TLC plates of Brinkmann (2 mm) silica gel developed in 95:5 chloroform–methanol. The product band was extracted with methanol. Evaporation gave a white crystalline solid: yield 112 mg (34%).

The analytical sample was obtained by recrystallization of a small portion from ethanol: mp 160–165 °C dec; UV λ_{max} ($\epsilon \times 10^{-3}$) at pH 1, 266 nm (9.77); at pH 7, 266 (9.64); at pH 13, 266 (7.27); MS (EI), *m/z* 358 (M^+); ^{1}H NMR (Me_2SO-d_6) δ 1.8 (s, CH_3), 2.07 (m, 2 $H_{2'}$), 3.3–3.5 (m, H_2O, CH_3OH), 3.72 (s, $H_{8'}$), 3.95 (m, ethylene protons), 4.2 (m, $H_{3'}$, $H_{4'}$), 5.43 (m, $O_{3'}$ H), 5.72 (m, $H_{6'}$), 6.10 (m, $H_{5'}$), 6.2 (m, $H_{1'}$), 7.38 (m, H_6), 11.3 (br m, H_3). Anal. ($C_{15}H_{19}N_2O_6Cl$·0.65MeOH) C, H, N.

B. A solution of 3.37 g (7.0 mmol) of the *N,N′*-diphenylethylenediamine derivative of 1-(3-*O*-acetyl-2-deoxy-β-D-*erythro*-pento-1,5-dialdo-1,4-furanosyl)thymine in 160 mL of a 1:1 solution of water–tetrahydrofuran was stirred for 20 h with 10 g of Dowex 50 (W-X 8) ion-exchange resin (50–100 mesh) (hydrogen form) and then filtered, and the filtrate was evaporated in vacuo to remove the tetrahydrofuran. The resulting precipitate was removed by filtration, and the filtrate was lyophilized.

A solution of the residue in 100 mL of acetonitrile containing 2.47 g (7.0 mmol) of triphenylchloroacetonylphosphorane was kept at ambient temperature for 20 h and then filtered, and the filtrate was evaporated to dryness in vacuo. A solution of the residue in 200 mL of benzene and 100 mL of ethylene glycol containing 100 mg of *p*-toluenesulfonic acid was stirred and refluxed with a Dean–Stark trap for 20 h and then evaporated to dryness in vacuo. A solution of the residue in 50 mL of methanol was treated with 7 mL of 1 N methanolic sodium methoxide, kept for 1 h at ambient temperature, then stirred with Amberlite IR (120) ion-exchange resin (30–50 mesh) (hydrogen form), and filtered, and the filtrate was evaporated to dryness in vacuo. The residue was recrystallized from ethanol as a white solid: yield 1.41 g (51%); MS (EI), *m/z* 235 (sugar$^+$), 360 (M^+).

1-(2,5,6,8-Tetradeoxy-β-D-*erythro*-oct-7-ulofuranosyl)thymine (5). A solution of 315 mg (0.98 mmol) of 1-(3-*O*-acetyl-2,5,6,8-tetradeoxy-β-D-*erythro*-oct-5-en-7-ulofuranosyl)thymine (**2**) in 20 mL of glacial acetic acid was hydrogenated for 2 h at ambient temperature and atmospheric pressure in the presence of 100 mg of 30% palladium on carbon catalyst. The catalyst was removed by filtration. Mass spectral examination of the solution indicated complete reaction. Evaporation of the solution gave 277 mg of a white glass. A solution of the glass in 10 mL of methanol was treated with 1 mL of 1 N methanolic sodium methoxide. After $^1/_2$ h at ambient temperature, the solution was stirred with Amberlite IR (120) ion-exchange resin (hydrogen form), and filtered, and the filtrate was concentrated to 2 mL and streaked across four Brinkmann preparative silica

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gel plates. After the plates were developed in 9:1 chloroform–methanol, the product band was extracted with methanol. Evaporation of the methanol solution gave a solid: yield 57 mg (24%); mp 151–154 °C.

The analytical sample was obtained by recrystallization from methanol: mp 154–155 °C; UV λ_{max} ($\epsilon \times 10^{-3}$), at pH 1, 267 nm (9.84); at pH 7, 266 (9.89); at pH 13, 267 (7.48); MS (EI), m/z 157 (sugar$^+$), 282 (M^+); ^{1}H NMR (Me_2SO-d_6) δ 1.80 (s, CH_3), 1.6–2.0 (m, 2 $H_{5'}$), 2.1 (M, 2 $H_{2'}$), 2.65 (m, 2 $H_{6'}$), 2.8 (s, 3 $H_{8'}$), 3.6 (m, $H_{4'}$), 4.05 (m, $H_{3'}$), 6.11 (t, $H_{1'}$), 7.37 (m, H_6). Anal. ($C_{13}H_{18}N_2O_5$) C, H, N.

1-[8-Chloro-2,5,6,8-tetradeoxy-7-(ethylenedioxy)-β-D-*erythro*-octofuranosyl]thymine (6). A solution of 663 mg (1.85 mmol) of 1-(8-chloro-2,5,6,8-tetradeoxy-7-(ethylenedioxy)-β-D-*erythro*-oct-5-enofuranosyl]thymine (**4**) in 500 mL of ethanol was hydrogenated for 20 h at ambient temperature and atmospheric pressure in the presence of 333 mg of 30% palladium on charcoal catalyst. The catalyst was removed by filtration. Mass spectral examination of an aliquot indicated incomplete reaction. The reduction was repeated wtih 100 mg of catalyst. Filtration and evaporation of the solution gave a white crystalline solid: yield 640 mg (96%); MS (EI), m/z 235 (sugar$^+$), 360 (M^+).

1-(8-Chloro-2,5,6,8-tetradeoxy-β-D-*erythro*-oct-7-ulofuranosyl)thymine (7). A solution of 892 mg (2.47 mmol) of 1-[8-chloro-2,5,6,8-tetradeoxy-7-(ethylenedioxy)-β-D-*erythro*-octofuranosyl]thymine (**6**) in 28 mL of 1 N sulfuric acid was refluxed for 15 min and cooled. The product precipitated as a crystalline solid and was collected by filtration: yield 248 mg; mp 166–167 °C. The filtrate was neutralized with solid barium carbonate and filtered. Evaporation of the filtrate gave a solid that was recrystallized from methanol: yield 235 mg; mp 165–167 °C. Purification of the methanol filtrate by preparative TLC on Brinkmann (2 mm) silica gel plates developed in 95:5 chloroform–methanol gave a third crop of 75 mg; total yield 71%.

The analytical sample was obtained from a previous reaction by recrystallization from ethanol: mp 166–167 °C; UV λ_{max} ($\epsilon \times 10^{-3}$) at pH 1, 267 nm (9.83); at pH 7, 268 (9.77); at pH 13, 267 (7.45); MS (EI), m/z 191 (sugar$^+$), 316 (M^+); ^{1}H NMR (Me_2SO-d_6) δ 1.80 (s, CH_3), 1.5–1.9 (m, 2 $H_{5'}$), 2.16 (m, 2 $H_{2'}$), 2.64 (m, 2 $H_{6'}$), 4.4 (m, $H_{3'}$), 4.50 (s, 2 $H_{8'}$), 5.5 (d, $O_{3'}$ H), 6.11 (t, $H_{1'}$), 7.3 (m, H_6), 11.26 (s, NH). Anal. ($C_{13}H_{17}ClN_2O_5$) C, H, N.

1-(8-Bromo-2,5,6,8-tetradeoxy-β-D-*erythro*-oct-7-ulofuranosyl)thymine (8). A solution of 100 mg (0.32 mmol) of 1-(8-chloro-2,5,6,8-tetradeoxy-β-D-*erythro*-oct-7-ulofuranosyl)-thymine (**7**) in 250 mL of anhydrous acetone containing a suspension of 1.00 g (9.70 mmol) of anhydrous pulverized sodium bromide was refluxed and stirred for 3 h. Examination by HPLC [reverse-phase Spherisorb no. 279, acetonitrile–water (9:1)] showed 14% starting compound. The solution was filtered, treated with an additional 1 g of sodium bromide, refluxed, stirred for 20 h and then for 24 h at ambient temperature, filtered again, and then evaporated to dryness in vacuo. The residue crystallized upon addition of water. The solid was collected by filtration and washed water: yield 64 mg (56%); mp 162–163 °C dec. HPLC showed less than 1.5% starting compound.

The analytical sample was obtained by recrystallization from acetone; mp 165–166 °C dec; UV λ_{max} ($\epsilon \times 10^{-3}$) at pH 1 and 7, 267 nm (9.63); at pH 13, 267 (7.27); MS (EI), m/z 235 (sugar$^+$), 360 (M^+); ^{1}H NMR (Me_2SO-d_6) δ 1.80 (s, CH_3), 1.6–2.0 (m, 2 $H_{5'}$), 2.1 (m, 2 $H_{2'}$), 2.69 (2 $H_{6'}$), 3.6 (m, $H_{4'}$), 4.05 (m, $H_{3'}$), 4.34 (s, 2 $H_{8'}$), 6.11 (t, $H_{1'}$), 7.37 (m, H_6), 11.24 (s, NH). Anal. ($C_{13}H_{17}BrN_2O_5$) C, H, N.

1-(8-Iodo-2,5,6,8-tetradeoxy-β-D-*erythro*-oct-7-ulofuranosyl)thymine (9). A solution of 100 mg (0.32 mmol) of 1-(8-chloro-2,5,6,8-tetradeoxy-β-D-*erythro*-oct-7-ulofuranosyl)-thymine (**7**) and 144 mg (0.96 mmol) of anhydrous sodium iodide in 150 mL of anhydrous acetone was stirred for 30 min at ambient temperature. Examination by HPLC [reverse-phase Spherisorb no. 279, acetonitrile–water (9:1)] showed complete conversion. After filtration, the solution was evaporated to dryness in vacuo. The addition of water to the residue gave a crystalline solid that was collected by filtration: yield 106 mg (81%); mp 160-161 °C dec; UV λ_{max} ($\epsilon \times 10^{-3}$) at pH 1, 267 nm (9.80); at pH 7, 267 (9.59); at pH 13, 267 (7.27); MS (FAB), m/z 283 (sugar$^+$), 409 [$(M + 1)^+$]; ^{1}H NMR (Me_2SO-d_6) δ 1.81 (CH_3), 1.7–2.0 (m, 2 $H_{5'}$), 2.15 (m, 2 $H_{2'}$), 2.78 (t, 2 $H_{6'}$), 3.6 (m, $H_{4'}$), 4.04 (m, $H_{3'}$), 4.07 (s, 2 $H_{8'}$), 6.13 (t, $H_{1'}$), 7.39 (s, H_6), 11.26 (s, NH). Anal. ($C_{13}H_{17}IN_2O_5$) C, H, N.

***N*-[4-[2-(Chloromethyl)-1,3-dioxolan-2-yl]phenyl]-1,2-dideoxy-1-[3,4-dihydro-5-methyl-2,4-dioxo-1(2*H*)-pyrimidinyl]-β-D-*erythro*-pentofuranuronamide (11).** To a cold (−10 °C) solution of 1.28 g (5.00 mmol) of 1,2-dideoxy-1-[3,4-dihydro-5-methyl-2,4-dioxo-1(2*H*)-pyrimidinyl]-β-D-ribofuranuronic acid (**10**) and 1.28 g (6.00 mmol) of 2-(*p*-aminophenyl)-2-(chloromethyl)-1,3-dioxolane in 50 mL of dimethylformamide was added 1.82 mL (12.0 mmol) of triethylamine, followed by 1.35 mL (6.00 mmol) of diphenylphosphoryl azide. The solution was stirred for 20 h at ambient temperature and evaporated to dryness in vacuo. A methanol solution of the residue was placed on a dry silica gel column (Woelm), and the column was developed in 9:1 chloroform–methanol. Extraction of the product band with methanol gave 1.32 g of a syrup. The syrup was purified by preparative TLC on Brinkmann (2 mm) silica gel plates developed in 9:1 chloroform–methanol. Methanol extraction gave a solid: yield 782 mg (34%); MS (FD), m/z 451 (M^+); ^{1}H NMR (Me_2SO-d_6) δ 1.79 (s, CH_3), 2.2 (m, 2 $H_{2'}$), 3.39 (br s, H_2O and OH), 3.86 (m, $H_{8'}$), 3.98 (m, ethylene protons), 4.5 (s, $H_{3'}$, $H_{4'}$), 6.37 (t, $H_{1'}$), 7.55 (q, phenyl protons), 8.15 (s, H_6), 11.48 (s, phenyl NH).

***N*-[4-(Chloroacetyl)phenyl]-1,2-dideoxy-1-[3,4-dihydro-5-methyl-2,4-dioxo-1(2*H*)-pyrimidinyl]-β-D-*erythro*-pentofuranuronamide (12).** A solution of 737 mg (1.62 mmol) of *N*-[4-[2-(chloromethyl)-1,3-dioxolan-2-yl]phenyl]-1,2-dideoxy-1-(3,4-dihydro-5-methyl-2,4-dioxo-1(2*H*)-pyrimidinyl)-β-D-*erythro*-pentofuranuronamide (**11**) in 32 mL of a 1:1 solution of 0.1 N hydrochloric acid and ethanol was refluxed for 90 min. Cooling produced a crystalline product that was collected by filtration: yield 552 mg (84%); mp 212–214 °C.

The analytical sample was obtained in another reaction: mp 216–218 °C; UV λ_{max} ($\epsilon \times 10^{-3}$) at pH 1 and 7, 267 nm (23.5); at pH 13, 275 (18.2); MS (FD), m/z 407 (M^+); ^{1}H NMR (Me_2SO-d_6) δ 1.80 (s, 5-CH_3), 2.27 (m, br, 2 $H_{2'}$), 3.33 (s, H_2O), 4.4 (br, $H_{4'}$), 4.5 (m, $H_{3'}$), 5.15 (s, $ClCH_2$), 5.77 (d, $O_{3'}$ H), 6.39 (m, $H_{1'}$), 7.9 (m, phenyl protons), 8.05 (s, H_6), 10.6 (s, NHCO), 11.4 (s, 3 NH of pyrimidine). Anal. ($C_{18}H_{18}ClN_3O_6 \cdot 0.9H_2O$) C, H, N.

***N*-[4-(Bromoacetyl)phenyl]-1,2-dideoxy-1-[3,4-dihydro-5-methyl-2,4-dioxo-1(2*H*)-pyrimidinyl]-β-D-*erythro*-pentofuranuronamide (13).** A solution of 100 mg (0.25 mmol) of *N*-[4-(chloroacetyl)phenyl]-1,2-dideoxy-1-[3,4-dihydro-5-methyl-2,4-dioxo-1(2*H*)-pyrimidinyl]-β-D-*erythro*-pentofuranuronamide (**12**) in 300 mL of anhydrous acetone containing a suspension of 900 mg (8.75 mmol) of anhydrous pulverized sodium bromide was stirred for 20 h at ambient temperature. Examination by HPLC [reverse-phase Spherisorb no. 279, acetonitrile–water (9:1)] showed 10% starting compound. The solution was filtered, recharged with 900 mg of sodium bromide, stirred for 48 h at ambient temperature, again filtered, evaporated to about 30 mL in vacuo, refiltered, and concentrated to about 10 mL by a stream of nitrogen. The addition of about 30 mL of water to this solution caused precipitation of a crystalline solid. It was collected by filtration and washed with water: yield 83 mg (73%); mp 186–187 °C dec; HPLC showed no starting compound; UV λ_{max} ($\epsilon \times 10^{-3}$) at pH 1, 276 nm (20.4); at pH 7, 276 (20.8), at pH 13, 273 (16.7); MS (FAB), m/z 326 (sugar$^+$), 452 [$(M + 1)^+$]; ^{1}H NMR (Me_2SO-d_6) δ 1.79 (s, 5-CH_3), 2.25 (m, br, 2 $H_{2'}$), 3.2–3.6 (m, H_2O), 4.25 (d, $H_{3'}$, $H_{4'}$), 4.86 (s, $BrCH_2$), 6.38 (t, $H_{1'}$), 7.88 (m, phenyl protons), 8.06 (d, H_6), 10.6 (s, NHCO), 11.4 (s, 3 NH of pyrimidine). Anal. ($C_{18}H_{18}BrN_3O_6 \cdot 0.9H_2O$) C, H, N.

***N*-[4-(Iodoacetyl)phenyl]-1,2-dideoxy-1-[3,4-dihydro-5-methyl-2,4-dioxo-1(2*H*)-pyrimidinyl]-β-D-*erythro*-pentofuranuronamide (14).** A solution of 408 mg (1.00 mmol) of *N*-[4-(chloroacetyl)phenyl]-1,2-dideoxy-1-[3,4-dihydro-5-methyl-2,4-dioxo-1(2*H*)-pyrimidinyl]-β-D-*erythro*-pentofuranuronamide (**12**) and 450 mg (3.00 mmol) of anhydrous sodium iodide in 200 mL of anhydrous acetone was stirred for 2 h at ambient temperature and filtered, and the filtrate was evaporated to dryness in vacuo. The residue was recrystallized from acetone–water: yield 427 mg (82%); mp 193–195 °C dec; HPLC [reverse-phase Spherisorb no. 279, acetonitrile–water (9:1)] showed no starting compound.

The analytical sample was obtained from a similar reaction: mp 195–197 °C dec; UV λ_{max} ($\epsilon \times 10^{-3}$) at pH 1, 275 nm (19.1), 297 (sh) (17.4); at pH 7, 275 (19.5), 295 (sh) (17.9); at pH 13, 276

(19.0), 296 (sh) (15.8); MS (EI), m/z 499 (M^+); ^1H NMR (Me_2SO-d_6) δ 1.79 (s, 5-CH_3), 2.25 (m, 2 $H_{2'}$), 3.0–4.0 (br, H_2O), 4.25 (d, $H_{3'}$, $H_{4'}$, ICH_2), 6.38 (t, $H_{1'}$), 7.9 (m, phenyl protons), 8.06 (s, H_6), 10.6 (s, NHCO), 11.4 (s, 3 NH of pyrimidine). Anal. ($C_{18}H_{18}IN_3O_6 \cdot H_2O$) C, H, N.

5'-[[4-(Bromoacetyl)benzoyl]amino]-5'-deoxythymidine (16). A suspension of 4-(bromoacetyl)benzoic acid[15] (300 mg, 1.23 mmol) in benzene (5 mL) and $SOBr_2$ (2 mL) was refluxed under N_2 for 1.75 h to give a clear solution, which was evaporated to dryness in vacuo and evaporated with toluene (2 × 4 mL) to remove traces of $SOBr_2$. A solution of the acyl bromide in DMAC (5 mL) was added dropwise over 20 min to an ice-cooled, stirred mixture of 5'-amino-5'-deoxythymidine[10] (**15**; 282 mg, 1.17 mmol); anhydrous, finely powdered NaBr (2.00 g, 19.4 mmol); and *N,N*-diisopropylethylamine (220 μL, 1.23 mmol) in DMAC (15 mL). The suspension was stirred for 30 min at 25 °C, filtered, and evaporated to dryness under high vacuum. The gummy residue was triturated with Et_2O (3 × 10 mL), followed by H_2O (2 × 10 mL). A solution of the dried solid in a minimum of hot MeOH was applied to four Brinkmann silica gel plates (20 cm × 20 cm × 2 mm) and developed with $CHCl_3$–MeOH (9:1). The product band was extracted with EtOH (300 mL), and the extract was evaporated to dryness. The residue was washed with Et_2O and then H_2O and dried in vacuo (P_2O_5): yield 142 mg (26%); mp 210 °C dec (Kofler-Heizbank); TLC $CHCl_3$–MeOH (9:1); UV λ_{max} ($\epsilon \times 10^{-3}$) at pH 1, 259 nm (23.6); at pH 7, 259 (24.0). Anal. ($C_{19}H_{20}BrN_3O_6$) C, H, N.

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Registry No. **1**, 5983-15-3; **2**, 89299-46-7; **3**, 89299-47-8; **4**, 89254-77-3; **5**, 80647-12-7; **6**, 80647-13-8; **7**, 80647-14-9; **8**, 89196-50-9; **9**, 89196-51-0; **10**, 3544-99-8; **11**, 80647-05-8; **12**, 80647-04-7; **13**, 89196-52-1; **14**, 89196-53-2; **15**, 25152-20-9; **16**, 89196-54-3; 3'-*O*-acetylthymidine, 21090-30-2; triphenylacetonylphosphorane, 89196-55-4; triphenylchloroacetonylphosphorane, 89196-56-5; 2-(*p*-aminophenyl)-2-(chloromethyl)-1,3-dioxolane, 2705-89-7; 4-(bromoacetyl)benzoic acid, 20099-90-5; 4-(bromoacetyl)benzoyl bromide, 89196-57-6.

Amnesia-Reversal Activity of a Series of *N*-[(Disubstituted-amino)alkyl]-2-oxo-1-pyrrolidineacetamides, Including Pramiracetam†

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A series of *N*-[(dialkylamino)alkyl]-2-oxo-1-pyrrolidineacetamides was synthesized. The title compounds reversed electroconvulsive shock (ECS) induced amnesia in mice when administered subsequent to the ECS treatment and were inactive in a general observational test for central nervous system (CNS) activity. Active compounds exhibited an inverted U-shaped dose–response curve. Among the compounds with the broadest dose–response curve, as well as the most potent, were those with the *N*-[2-[bis(1-methylethyl)amino]ethyl] or 2,6-dimethylpiperidinoethyl residues as amide substituent. The *N*-(dialkylamino) substituent markedly enhances amnesia-reversal activity, with ethylene providing the optimal chain length. *N*-[2-[Bis(1-methylethyl)amino]ethyl]-2-oxo-1-pyrrolidineacetamide *N*-(dialkylamino) substituent was selected for preclinical toxicological evaluation, assigned the investigational number CI-879 and the U.S. adopted name (USAN) pramiracetam. Pramiracetam demonstrated a wide margin of safety in animals and was well tolerated in normal human volunteers. It has shown encouraging activity in an open label trial in patients with primary degenerative dementia (PDD or senile dementia of the Alzheimer's type).

Congnitive dysfunctions occur in persons of all ages. They may result from disease, accidents, injury, developmental defects, or aging. Recent increases in average longevity and the accompanying rise in the number of elderly persons, however, have made the development of new treatments for age-related cognitive impairments a particularly urgent goal of medical science. An agent that reverses or ameliorates cognitive impairments, especially in aged patients, would be of enormous medical, social, economic, and scientific importance.[1,2]

Cognitive impairments in the elderly were long attributed to atherosclerotic disruption of cerebral blood flow. Recent evidence indicates, however, that as few as 10% of senile patients have such circulatory disorders.[3,4] The majority suffer from senile dementia of the Alzheimer's type (SDAT). The primary symptoms of SDAT are as follows: (1) forgetfulness, poor memory, and memory loss; (2) confusion and disorientation in space and/or time; (3) poor attention or distractability; (4) affective disturbances (agitation, depression, apathy, and lethargy).[5]

Drugs reported to treat the cognitive impairments of the elderly have shown only limited benefits and therefore are not widely accepted.[6,7] Many useful reviews covering drug effects upon cognition and neurotransmitters involved in cognition have been written.[8-12]

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