ethanol several times to give 0.388 g of a white solid. Recrystallization from ethyl acetate–hexane gave 0.293 g (66%) of analytically pure **28**: mp 135.5–136.5 °C; TLC-SG, ethyl acetate/cyclohexane (1:1), one spot with R_f = 0.35; UV (pH 1) λ_{max} 276 (ε 13 700), λ_{min} 246 (3500) nm; (pH 7) λ_{max} 274 (ε 14 400), λ_{min} 246 (3800) nm; (pH 13) λ_{max} 274 (ε 14 600), λ_{min} 246 (4000) nm; ^{1}H NMR (80 MHz, DMSO-d_6) δ 8.44 (s, 1 H, H-8), 7.19 (AB q, 4 H, Ar H), 5.38 (s, 2 H, CH_2), 3.40 (br s, 6 H, $N(CH_3)_2$), 3.34 (s, 3 H, SO_2CH_3), 2.26 (s, 3 H, CH_3); MS, *m/e* 345 (M^+), 316 ($M^+ - 29$), 266 ($M^+ - SO_2CH_3$), 240 ($M^+ - C_8H_9$), 105 ($C_8H_9^+$). Anal. ($C_{16}H_{19}N_5O_2S$) C, H, N.

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Synthesis and Cytostatic and Antiviral Activities of 1-β-D-Ribofuranosyl-5-alkylcytosine (5-Alkylcytidine) Cyclic 3′,5′-Monophosphates

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A series of 5-alkylcytidines and their 5′-monophosphates and cyclic 3′,5′-monophosphates have been synthesized and evaluated for antiviral and antitumor activity. The 5-alkyl cyclic nucleotides were not cytostatic (ID_{50} > 200 μg/mL) against leukemia L1210 cells and a deoxycytidine kinase-deficient subline thereof. Certain of the corresponding nucleosides and their 5′-monophosphates did show activity within the range of 35–162 μg/mL, as did the unsubstituted cytidine cyclic 3′,5′-monophosphate. No antiviral activity was found for any of the compounds at 400 μg/mL. A drug design rationale for utilization of 5-alkylcytidines based on their potential conversion to biologically active 5-alkyl-2′-deoxyuridines is not supported by these experimental findings.

Nucleoside cyclic 3′,5′-monophosphates have been of interest in recent years because of the implicated role of cAMP in the malignant transformation process.[1] Moreover, it has been suggested that cyclic 3′,5′-monophosphates could be the metabolites responsible for the antitumor and antiviral activities of certain nucleosides.[2] The potent in vivo antitumor and antiviral activities of cyclic nucleotides derived from araA,[3] araC,[4] and the 6-mercapto- and 6-(methylthio)-9-β-D-ribofuranosylpurines[5] have been known for some time, as has the antiviral capacity of the cyclic 3′,5′-monophosphate of ribavirin.[6] More recently we have examined a large number of cyclic 3′,5′-monophosphates derived from 5-alkyl-,[7] 5-halo-,[8] and 5-(trifluoromethyl)-2′-deoxyuridines,[8] 5-halocytidines,[9] and 5-halouridines.[10] Potent antiviral and cytostatic activities were found for a number of these compounds, although the cyclic diesters were normally 10–100 times less active than the corresponding nucleosides or 5′-monophosphates. However, significant cytostatic activity of the cyclic diesters against thymidine kinase deficient tumor cell lines was not found. A striking inhibitory effect against thymidine kinase deficient herpes simplex virus type 1 (HSV-1) was noted[11] for certain 5-substituted 2′-deoxyuridine cyclic 3′,5′-monophosphates as well as the corresponding nucleosides and nucleoside 5′-monophosphates which are known to inhibit thymidylate synthase. Active cyclic diesters probably are prodrug forms of the nucleoside or nucleoside monophosphate in most cases; however, studies of the potent antiviral activity of the cyclic phosphate diester of 2′-nordeoxyguanosine (DHPG) suggest a biological role of the cyclic compound itself.[12]

On the basis of the above and the well-known[13,14] striking

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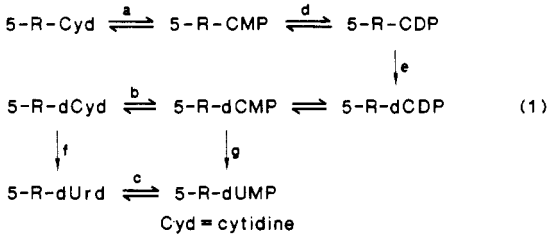
Table I. Synthetic Data for 5-Alkyl-CMP's, 1a–8a

no.	R	formula	analyses	phosphorylation conditions: 5-alkylcytidine	phosphorylation conditions: dissolved in $(EtO)_3PO$ at (°C)	phosphorylation conditions: react time, min	isolated yields, %	R_f[a] 1[b]	R_f[a] 2[b]
1a	CH_3	$C_{10}H_{22}N_5O_8P$	C, H, N, P	HCl	165	75	75	0.26	0.14
2a	CH_2CH_3	$C_{11}H_{24}N_5O_8P$	C, H, N, P	HCl	150–160	75	68	0.29	0.16
3a	$CH(CH_3)_2$	$C_{12}H_{26}N_5O_8P$	C, H, N, P	HCl	80–90	75	55	0.37	0.18
4a	$CH_2CH_2CH_3$	$C_{12}H_{26}N_5O_8P$	C, H, N, P	free base	110–120	75	69	0.41	0.19
5a	$CH_2(CH_2)_2CH_3$	$C_{13}H_{28}N_5O_8P$	C, H, N, P	HCl	70–80	60	85	0.46	0.24
6a	$CH_2(CH_2)_3CH_3$	$C_{14}H_{30}N_5O_8P$	C, H, N, P	HCl	30–40	75	78	0.46	0.26
7a	$CH_2(CH_2)_4CH_3$	$C_{15}H_{32}N_5O_8P$	C, H, N, P	free base	< 100	60	32	0.48	0.32
8a	$CH_2(CH_2)_6CH_3$	$C_{17}H_{36}N_5O_8P$	C, H, N, P	free base	130–140	75	42	0.51	0.34

[a] On silica gel TLC sheets. [b] Solvent system. See the Experimental Section.

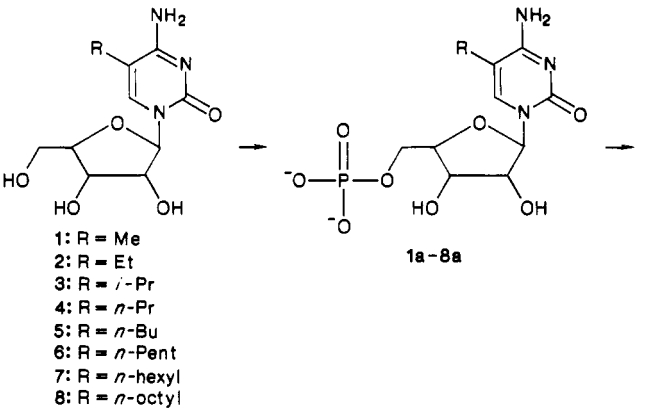
biological effects of substituent alteration at the 5-position of the pyrimidine ring of uridine, 2'-deoxyuridine, 2'-deoxycytidine, and uracil arabinoside and cytosine arabinoside, we undertook a study of the antiviral and cytostatic properties of a series of 5-alkylcytidines and their 5'- and cyclic 3',5'-monophosphates (5-R-cCMP's). Synthesis of these nucleosides was reported elsewhere.[15] If not biologically active in their own right, these analogues may potentially be converted to 2'-deoxycytidine and/or 2'-deoxyuridine monophosphates, certain of which may have antiviral and/or cytostatic potency[13,14] (eq 1). The



(1)

kinases necessary for steps a–d are well-known[16] as are the ribonucleotide reductase (step e),[16] deoxycytidine deaminase (step f),[16,17] and deoxycytidylate deaminase (step g),[16] although the virally encoded enzymes may not necessarily have the same activities as the cellular ones.

Scheme I



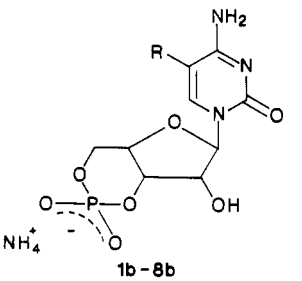


Table II. Synthetic Data for 5-Alkyl-cCMP's, 1b–8b

no.	R	formula	analyses	yields, %	R_f[a] 1[b]	R_f[a] 2[b]
1b	CH_3	$C_{10}H_{17}N_4O_7P$	C, H, N, P	26	0.35	0.66
2b	CH_2CH_3	$C_{11}H_{19}N_4O_7P$	C, H, N, P	35	0.42	0.70
3b	$CH(CH_3)_2$	$C_{12}H_{21}N_4O_7P$	C, H, N, P	64	0.46	0.70
4b	$CH_2CH_2CH_3$	$C_{12}H_{21}N_4O_7P$	C, H, N, P	33	0.47	0.72
5b	$CH_2(CH_2)_2CH_3$	$C_{13}H_{23}N_4O_7P$	C, H, N, P	60	0.48	0.75
6b	$CH_2(CH_2)_3CH_3$	$C_{14}H_{25}N_4O_7P$	C, H, N, P	50	0.50	0.77
7b	$CH_2(CH_2)_4CH_3$	$C_{15}H_{27}N_4O_7P$	C, H, N, P	46	0.51	0.79
8b	$CH_2(CH_2)_6CH_3$	$C_{17}H_{31}N_4O_7P$	C, H, N, P	44	0.56	0.80

[a] On silica gel TLC sheets. [b] Solvent system.

Results and Discussion

Chemistry. A series of 1-β-D-ribofuranosyl-5-alkylcytosine (5-alkylcytidine) 3',5'-cyclic phosphates (5-R-cCMP's), **1b–8b**, have been prepared (Scheme I, Table II). Conventional cyclization of the *N,N'*-dicyclohexyl-4-morpholinecarboxamidine salts of the corresponding 5'-mononucleotides (5-R-CMP's), **1a–8a**, with *N,N'*-dicyclohexylcarbodiimide (DCC) in refluxing pyridine, according to the procedure described for the preparation of ribonucleoside 3',5'-cyclic phosphates,[18] led to isolation (yield

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26–64%) of **1b–8b**. The sufficient solubility of the above salts of **1a–8a** in refluxing pyridine did not require them to be converted to more soluble derivatives[18] or the use of a modified reaction medium,[19] except for one case. A pyridine/DMF solvent mixture[19] was used when converting the above salt of **4a** to **4b**. Even in this case, no evidence was found (^{1}H, ^{13}C NMR) for the formation of the 2′,3′-cyclic phosphate, a potential side-product.[9,20]

The required 5-alkylcytidine 5′-monophosphate (5-R-CMP) precursors, **1a–8a**, were obtained (yield 32–85%) by the Yoshikawa method.[21] Limited room-temperature solubility of the 5-alkylcytidines (hydrochloride or free base) required that they be dissolved at 30–165 °C (Table I). At higher temperatures the nucleosides decomposed slowly; hence it was necessary that the dissolution of the nucleosides be carried out quickly. ^{1}H (Experimental Section) and ^{13}C NMR data (supplementary material) confirmed the structures of the 5-R-CMP's. No evidence for the formation of the 3′-monophosphate, a potential side product,[22] was found.

Each 5-R-CMP, **1a–8a**, and its corresponding 5-R-cCMP, **1b–8b**, was routinely isolated by DEAE-Sephadex A-25 anion-exchange column chromatography with a linear aqueous salt gradient. In a few cases, the 5′-mononucleotide was separated from the inorganic salts by repeated anion-exchange column chromatography. The structures of **1b–8b** were confirmed by ^{1}H (Experimental Section) and ^{13}C (supplementary materials) NMR spectroscopy as well as by mass spectrometry and quantitative elemental analysis (Table II). In the ^{1}H NMR spectra, the presence of sharp singlet signals in the range δ 5.75–6.05 (H1′) showed the β nature of the configuration at anomeric C1′.[23] For **1b–8b** a *singlet* at about δ 7.34–7.70 (H6) was observed, which is consistent with alkyl substitution at C5. The ^{13}C chemical shifts and J_{PC} values for **1b–8b** (Table IV) are typical of those found for other nucleoside cyclic 3′,5′-phosphates.[24] The chemical shift assignments for C3′ and C4′ follow the revisions made for similar diesters on the basis of single frequency decoupling experiments.[25] Trimethylsilylation of **1b–8b** prior to electron-impact mass spectrometry led to tri- and/or disilylation as evidenced by the observation of the corresponding molecular ion and M − 15 peaks (Experimental Section).

Protein Kinase Activity. Preliminary results showed **2b–8b** and cCMP itself to interact with protein kinase type I (rabbit skeletal muscle) with K_a (concentration for half-maximal activation) values ranging from 0.8 to 2.8 μM (K_a for cAMP, 30 nM). The presence of the pyrimidine base rather than a purine one evidently renders the cyclic nucleotides of the present study less effective activators of the kinase. Details of these experiments will be reported elsewhere.

Cytostatic Activity. None of the 5-R-cCMP's **1b–8b** was endowed with significant cytostatic activity against the murine leukemia L1210 cell line and its deoxycytidine kinase deficient L1210/araC subline (ID_{50} > 200 μg/mL). Their ID_{50} values for L1210/0 and L1210/araC[26] cell growth exceeded those of the reference compound, 5-fluoro-2′-deoxycytidine (FDC),[27] by a factor of at least 200 000 and 8000, respectively. The 5-R-cytosine, -cytidines, and -CMP's were also inactive against the proliferation of L1210/0 and L1210/araC cells at a concentration of 200 μg/mL, except for the following congeners: 5-*n*-octylcytosine (ID_{50} for L1210/0 cell growth: 190 μg/mL), 5-methylcytidine (ID_{50} for L1210/0 cell growth: 94 μg/mL), 5-isopropylcytidine (ID_{50} for L1210/0 and L1210/araC cell growth: 35 and 39 μg/mL, respectively), and 5-methyl-CMP (ID_{50} for L1210/0 cell growth: 162 μg/mL). The unsubstituted CMP and cCMP inhibited the growth of L1210/0 cells at an ID_{50} of 43 μg/mL; they were not inhibitory to L1210/araC cells at a concentration of 200 μg/mL. 5-*n*-Propyl- and 5-*n*-butylcytosine as well as 5-*n*-butylcytidine were not examined.

Antiviral Activity. None of the 5-R-cCMP's **1b–8b** exhibited an inhibitory effect on the replication of either herpes simplex virus type 1 (HSV-1, KOS; HSV-1, F; HSV-1, McIntyre), herpes simplex virus type 2 (HSV-2, G; HSV-2, 196; HSV-2, Lyons), vaccinia virus, or vesicular stomatitis virus at 400 μg/mL. Likewise, the 5-R-CMP's (**1a–8a**), 5-alkylcytidines, and 5-alkylcytosines were devoid of antiviral activity, irrespective of the nature of their 5-alkyl substituent. Neither cCMP nor CMP exhibited any antiviral effect.[28] Again, 5-*n*-propyl- and 5-*n*-butylcytosine as well as 5-*n*-butylcytidine were not examined. The reference compound was (*E*)-5-(2-bromovinyl)-2′-deoxyuridine.[29]

Conclusions

In earlier reports, several series of 5-alkyl-2′-deoxyuridines, 5-halo-2′-deoxyuridines, 5-halouridines, and their corresponding 5′-monophosphates and 3′,5′-cyclic monophosphates were described. Considerable cytostatic and antiviral potencies[7,8,10] were found in a number of instances. As a rule, the phosphorylated derivatives appeared to require extracellular hydrolysis to the nucleoside before functioning as cytostatic or antiviral agents, and proved generally less effective than the corresponding nucleosides. The phosphorylated 5-alkylcytidine derivatives described in this paper showed low levels of biological activity at best. Also, their corresponding nucleoside counterparts, except for 5-isopropylcytidine and 5-methylcytidine, which demonstrated mild potency as cytostatic agents, proved barely active. This implies that the drug design rationale based on the conversion of 5-R-CMP to 5-R-dUMP utilizing the intracellular deamination, (de)phosphorylation, and reduction steps of sequence (1), does not seem applicable under our experimental conditions. (A number of the 5-alkyl-2′-deoxyuridines[7] and some 5-alkyl-2′-deoxycytidines[30,31] are active cytostatic agents or antivirals.)

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These findings only pertain to the in vitro situation and do not exclude the possibility of conversion of 5-R-CMP to 5-R-dUMP in vivo. However, the present in vitro data do not point to the potential promise of 5-R-Cyd's, 5-R-CMP's, and 5-R-cCMP's as antiviral or cytostatic agents.

Experimental Section

Chromatography. Precoated TLC sheets (Kieselgel 60 F_{254}, 0.2 mm × 20 cm × 20 cm) from Merck (Darmstadt, FRG) were used. Solvent systems (v/v) for silica gel TLC were (1) isobutyric acid/25% ammonium hydroxide/water (66/1/33) and (2) 2-propanol/25% ammonium hydroxide/water (7/1/2) with added H_3BO_3. DEAE-Sephadex A-25 was purchased from Pharmacia Fine Chemicals.

Spectroscopy. Proton NMR spectra were recorded with a Varian XL-100 FT NMR system operating at 100.1 MHz. Carbon-13 spectra were acquired on a disk-augmented Varian XL-100/15 spectrometer operating at 25.2 MHz. TMS was used as internal reference in both cases. Mass spectral measurements were carried out on an AEI MS-902 double-focusing instrument with ionizing energy of 70 eV and an ion source temperature of 200 °C. All samples were introduced by the direct probe method. Compounds **1b–8b** were silylated with BSTFA in dry pyridine for 15 min at 150 °C before introduction. Only peaks diagnostic of molecular weight are given here.

Chemistry. Triethyl phosphate was vacuum distilled before use. Phosphoryl chloride, pyridine, and *N,N*-dimethylformamide were freshly distilled from P_2O_5.

General Procedure for the Synthesis of 5-Alkylcytidine 5'-Monophosphate Diammonium Salts, 1a–8a. The appropriate 5-alkylcytidine, 1–8, was quickly dissolved in triethyl phosphate at 30–165 °C (Table I). To a stirred solution of 5-alkylcytidine (1–4 mmol) in triethyl phosphate (2.5–10 mL) at 0 °C was added phosphoryl chloride (2–8 mmol). After 60–75 min at this temperature (see Table I), the reaction mixture was quenched with ice–water (ca. 20 mL). The pH of the solution was adjusted to 7 with 2 M sodium hydroxide (5–15 mL). This solution was applied to a DEAE-Sephadex A-25 (HCO_3^-) column (4.7 × 90 cm), which was washed with water (ca. 0.5–1 L) until no more UV absorbance (260 nm) was observed and then eluted (20 mL/5 min per fraction) with a linear aqueous salt gradient of equal volumes of water (2.5–3.0 L) and 1 M NH_4HCO_3 to give pure **1a–8a**. Unless indicated otherwise below, a single chromatography gave pure 5'-monophosphate. Relevant ^1H NMR data given for **1a–8a** and later **1b–8b** are those peaks most readily distinguishable and diagnostic of structure.

5-Methylcytidine 5'-Monophosphate Diammonium Salt (1a). 5-Methylcytidine hydrochloride (0.293 g, 1.00 mmol) was used as the starting compound. Salt **1a** (0.278 g, 0.75 mmol, 75%) was isolated by evaporation of fractions 173–191. Relevant ^1H NMR data (D_2O): δ 7.92 (s, 1 H, H6), 6.17 (s, 1 H, H1'), 2.17 (s, 3 H, CH_3).

5-Ethylcytidine 5'-Monophosphate Diammonium Salt (2a). 5-Ethylcytidine hydrochloride (1.23 g, 4.00 mmol) was used as starting material. Product **2a** was chromatographed twice and isolated (1.05 g, 2.73 mmol, 68%) on evaporation of fractions 196–205. Relevant ^1H NMR data (D_2O): δ 7.75 (s, 1 H, H6), 6.05 (d, $J_{H1'H2'} \simeq 4$ Hz, 1 H, H1'), 1.21 (d of t, 3 H, CH_3CH_2).

5-Isopropylcytidine 5'-Monophosphate Diammonium Salt (3a). 5-Isopropylcytidine hydrochloride (1.19 g, 3.70 mmol) gave salt **3a** (0.819 g, 2.05 mmol, 55%), which was isolated by evaporation of fractions 195–203. Relevant ^1H NMR data (D_2O): δ 7.68 (s, 1 H, H1'), 6.11 (d, $J_{H1'H2'} \simeq 4$ Hz, 1 H, H1'), 1.33 (d, 6 H, $(CH_3)_2CH$).

5-*n*-Propylcytidine 5'-Monophosphate Diammonium Salt (4a). 5-*n*-Propylcytidine (0.980 g, 3.44 mmol) yielded salt **4a** (0.952 g, 2.38 mmol, 69%), isolated by evaporation of fractions 171–183. Relevant ^1H NMR data (D_2O): δ 7.72 (s, 1 H, H6), 6.04 (d, $J_{H1'H2'} \simeq 4$ Hz, 1 H, H1'), 0.93 (t, 3 H, $CH_3CH_2CH_2$).

5-*n*-Butylcytidine 5'-Monophosphate Diammonium Salt (5a). 5-*n*-Butylcytidine hydrochloride (0.928 g, 2.76 mmol) gave compound **5a** (0.966 g, 2.34 mmol, 85%), isolated by evaporation of fractions 135–147. Relevant ^1H NMR data (D_2O): δ 7.80 (s, 1 H, H6), 6.15 (s, 1 H, H1'), 1.03 (t, 3 H, $CH_3(CH_2)_3$).

5-*n*-Pentylcytidine 5'-Monophosphate Diammonium Salt (6a). 5-*n*-Pentylcytidine hydrochloride (1.38 g, 3.94 mmol) yielded salt **6a** (1.31 g, 3.06 mmol, 78%), isolated by evaporation of fractions 177–186. Relevant ^1H NMR data (DMSO-d_6/$CDCl_3$): δ 7.62 (s, 1 H, H6), 5.87 (d, $J_{H1'H2'} \simeq 4$ Hz, 1 H, H1'), 0.89 (t, 3 H, $CH_3(CH_2)_4$).

5-*n*-Hexylcytidine 5'-Monophosphate Diammonium Salt (7a). Reactant 5-*n*-hexylcytidine (1.26 g, 3.85 mmol) yielded **7a**, which was chromatographed three times. Compound **7a** (0.540 g, 1.22 mmol, 32%) was isolated by evaporation of fractions 175–193. Relevant ^1H NMR data (D_2O): δ 7.80 (s, 1 H, H6), 6.07 (s, 1 H, H1'), 0.92 (br t, 3 H, $CH_3(CH_2)_5$).

5-*n*-Octylcytidine 5'-Monophosphate Diammonium Salt (8a). 5-*n*-Octylcytidine (0.995 g, 2.80 mmol) was used as starting material. Product **8a** was chromatographed twice. Salt **8a** (0.553 g, 1.18 mmol, 42%) was isolated by evaporation of fractions 190–200. Relevant ^1H NMR data (D_2O): δ 7.90 (s, 1 H, H6), 6.04 (s, 1 H, H1'), 0.86 (br t, 3 H, $CH_3(CH_2)_7$).

General Procedure for the Synthesis of 5-Alkylcytidine 3',5'-Cyclic Monophosphate Ammonium Salts, 1b–8b. The appropriate 5-alkylcytidine 5'-phosphate diammonium salt, **1a–8a** (1–2.8 mmol), was added to refluxing pyridine (100–280 mL). A clear solution resulted on addition of an equimolar amount of *N,N'*-dicyclohexyl-4-morpholinecarboxamidine. This solution, kept close to its reflux temperature, was added dropwise to a refluxing and vigorously stirred solution of dicyclohexylcarbodiimide (DCC, 2–5.6 mmol) in pyridine (100–280 mL) over a period of 1.5–2 h. After an additional 1.5–2 h reflux, the solution was evaporated to dryness and 100 mL each of ether and water was added. The insoluble dicyclohexylurea was filtered off. The aqueous phase was concentrated to a smaller volume (ca. 20 mL) and applied to a DEAE-Sephadex A-25 (HCO_3^-) column. The column was washed with water (ca. 0.5–1 L) and then eluted with a linear gradient of water and NH_4HCO_3 to give product **1b–8b**.

5-Methylcytidine 3',5'-Cyclic Monophosphate Ammonium Salt (1b). A 1.00 mmol (0.371 g) quantity of **1a** was used. Chromatographic conditions: column, 2.7 × 65 cm; gradient, water (2 L) and 0.75 M NH_4HCO_3 (2 L); fractions, 20 mL/10 min. Compound **1b** (0.086 g, 0.26 mmol, 26%) was isolated by evaporation of fractions 40–45. EI-MS, *m/e* (relative intensity): 463 (M^+ + 2 TMS (<25), 448 (M^+ + 2 TMS − 15 (100)). Relevant ^1H NMR data (D_2O): δ 7.56 (s, 1 H, H6), 6.00 (s, 1 H, H1'), 2.12 (s, 3 H, CH_3).

5-Ethylcytidine 3',5'-Cyclic Monophosphate Ammonium Salt (2b). A 1.51 mmol (0.581 g) amount of **2a** was used. Chromatographic conditions: column, 2.7 × 60 cm; gradient, water (1.5 L) and 0.75 M NH_4HCO_3 (1.5 L); fractions, 20 mL/10 min. Compound **2b** (0.184 g, 0.53 mmol, 35%) was isolated by evaporation of fractions 56–58. EI-MS, *m/e* (relative intensity): 477 (M^+ + 2 TMS (1)), 462 (M^+ + 2 TMS − 15 (12)), 549 (M^+ + 3 TMS (11)), 534 (M^+ + 3 TMS − 15 (100)). Relevant ^1H NMR data (D_2O): δ 7.55 (s, 1 H, H6), 5.98 (s, 1 H, H1'), 1.28 (t, 3 H, CH_3CH_2).

5-Isopropylcytidine 3',5'-Cyclic Monophosphate Ammonium Salt (3b). A 2.05-mmol (0.819-g) quantity of **3a** was used. Chromatographic conditions: **3b** was chromatographed twice; column, 4.7 × 90 cm; gradient, water (2.5 L) and 1 M NH_4HCO_3 (2.5 L); fractions, 20 mL/5 min. Salt **3b** (0.477 g, 1.31 mmol, 64%) was isolated by evaporation of fractions 131–143. EI-MS, *m/e* (relative intensity): 491 (M^+ + 2 TMS (2)), 476 (M^+ + 2 TMS − 15 (21)), 563 (M^+ + 3 TMS (9)), 548 (M^+ + 3 TMS − 15 (100)). Relevant ^1H NMR data (D_2O): δ 7.43 (s, 1 H, H6), 5.92 (s, 1 H, H1'), 1.30 (d, 6 H, $(CH_3)_2CH$).

5-*n*-Propylcytidine 3',5'-Cyclic Monophosphate Ammonium Salt (4b). A 2.13-mmol (0.850-g) amount of **4a** was used. Chromatographic conditions: column, 2.7 × 50 cm; gradient, water (1.5 L) and 0.75 M NH_4HCO_3 (1.5 L); fractions, 20 mL/10 min. Compound **4b** (0.255 g, 0.700 mmol, 33%) was isolated by evaporation of fractions 56–61. EI-MS, *m/e* (relative intensity): 491 (M^+ + 2 TMS (13)), 476 (M^+ + 2 TMS − 15 (100)). Relevant ^1H NMR data (D_2O): δ 7.38 (s, 1 H, H6), 5.83 (s, 1 H, H1'), 0.92 (t, 3 H, $CH_3CH_2CH_2$).

5-*n*-Butylcytidine 3',5'-Cyclic Monophosphate Ammonium Salt (5b). A 1.97-mmol (0.816-g) amount of **5a** was used. Chromatographic conditions: column, 4.7 × 90 cm; gradient, water

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(2.5 L) and 1 M NH_4HCO_3 (2.5 L); fraction, 20 mL/5 min. Salt **5b** (0.448 g, 1.18 mmol, 60%) was isolated by evaporation of fractions 89–101. EI-MS, *m/e* (relative intensity): 505 (M^+ + 2 TMS (10)), 490 (M^+ + 2 TMS − 15 (100)), 577 (M^+ + 3 TMS (5)), 562 (M^+ + 3 TMS − 15 (22)). Relevant ^1H NMR data (D_2O): δ 7.57 (s, 1 H, H6), 6.05 (s, 1 H, H1′), 1.11 (t, 3 H, $CH_3(CH_2)_3$).

5-*n*-Pentylcytidine 3′,5′-Cyclic Monophosphate Ammonium Salt (6b). A 2.80-mmol (1.20-g) quantity of **6a** was used. Chromatographic conditions: **6b** was chromatographed twice; column, 4.7 × 90 cm; gradient, water (2.5 L) and 1 M NH_4HCO_3 (2.5 L); fractions, 20 mL/5 min. Compound **6b** (0.550 g, 1.40 mmol, 50%) was isolated by evaporation of fractions 165–178. EI-MS, *m/e* (relative intensity): 519 (M^+ + 2 TMS (≈8)), 504 (M^+ + 2 TMS − 15 (100)). Relevant ^1H NMR data (D_2O): δ 7.42 (s, 1 H, H6), 5.90 (s, 1 H, H1′), 0.91 (t, 3 H, $CH_3(CH_2)_4$).

5-*n*-Hexylcytidine 3′,5′-Cyclic Monophosphate Ammonium Salt (7b). A 1.22-mmol (0.540-g) amount of **7a** was used. Chromatographic conditions: **7b** was chromatographed twice; column, 4.7 × 90 cm; gradient, water (2.5 L) and 1 M NH_4HCO_3 (2.5 L); fractions 20 mL/5 min. Salt **7b** (0.222 g, 0.546 mmol, 46%) was isolated by evaporation of fractions 210–227. EI-MS, *m/e* (relative intensity): 533 (M^+ + 2 TMS (7)), 518 (M^+ + 2 TMS − 15 (100)), 605 (M^+ + 3 TMS (2)), 590 (M^+ + 3 TMS − 15 (13)). Relevant ^1H NMR data (DMSO-d_6/$CDCl_3$): δ 7.34 (s, 1 H, H6), 5.75 (s, 1 H, H1′), 0.88 (br t, 3 H, $CH_3(CH_2)_5$).

5-*n*-Octylcytidine 3′,5′-Cyclic Monophosphate Ammonium Salt (8b). A 1.00-mmol (0.469-g) amount of **8a** was used. Chromatographic conditions: **8b** was chromatographed twice; column, 4.7 × 90 cm; gradient, water (2.5 L) and 1.5 M NH_4HCO_3 (2.5 L); fractions 20 mL/5 min. Compound **8b** (0.193 g, 0.444 mmol, 44%) was isolated by evaporation of fractions 207–225. EI-MS, *m/e* (relative intensity): 561 (M^+ + 2 TMS (≈10)), 546 (M^+ + 2 TMS − 15 (100)). Relevant ^1H NMR data (D_2O): δ 7.49 (s, 1 H, H6), 5.88 (s, 1 H, H1′), 0.91 (br t, 3 H, $CH_3(CH_2)_7$).

Cytostatic Assays. L1210/0 and L1210/araC cell lines were characterized as described.[27] The cytostatic assays were performed according to previously established procedures.[32]

Antiviral assays were performed as reported previously.[33] The origin and preparation of the virus stocks have also been documented in ref 31.

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Supplementary Material Available: Tables listing ^{13}C NMR data for **1a–8a** and **1b–8b** (2 pages). Ordering information is given on any current masthead page.

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Synthesis of Acylguanidine Analogues: Inhibitors of ADP-Induced Platelet Aggregation

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Routine screening of compounds for inhibition of ADP-induced platelet aggregation in vitro revealed that 1,1′-hexamethylenebis[3-cyclohexyl-3-[(cyclohexylimino)(4-morpholinyl)methyl]urea] (1) was active and represented the first example of a bis(acylguanidine) with possible antithrombotic activity. In order to develop a structure–activity relationship for this class of compounds, we synthesized a number of new bis(acylguanidines). These were tested in vitro, and several analogues were also active. Ex vivo testing revealed that compounds **22**, **41**, **58**, and **70–73** were orally active in rats or guinea pigs.

Researchers continue to emphasize the role of platelet-active drugs in the control of cardiovascular and cerebrovascular diseases.[1] These agents are believed to control or ameliorate the biochemical events that lead to thrombosis and vessel-wall damage. The common explanation for the series of events leading to thrombosis is fracture of the vessel's endothelium layer followed by platelet adherence to the exposed subendothelium. The adhering platelets then release other agents including thromboxane A_2 and adenosine diphosphate (ADP). These agents are responsible for the growth of platelet aggregates. At the same time, platelet membrane changes encourage the formation of thrombin. This induces further platelet aggregation and enmeshes the platelet mass in fibrin, stabilizing a thrombus.[2] In previous work on platelet drugs, we developed a compound that inhibits platelet aggregation induced by collagen.[3] Subsequently, we focused on an agent that would be a well-tolerated, orally active inhibitor of ADP-induced platelet aggregation. Others have also identified inhibitors of primary-wave ADP-induced platelet aggregation.[4]

During the course of our work, we discovered compound 1, which inhibits primary-wave ADP-induced platelet aggregation in human platelet-rich plasma (PRP), in vitro. This molecule presented an attractive lead as it possesses

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