dissolved in 0.5 mL of formamide. The reaction mixture was dried and treated with silver nitrate according to the procedure described for **23**. The solution was stirred at 45 °C for 36 h with the exclusion of moisture. The rest of the workup was the same as the procedures described for adenosine 5'-phosphoric α-D-glucopyranosylphosphonic anhydride (**23**) except for the Dowex 2 (×8) column chromatography, in which the elution was performed with 0.5 M LiCl in 0.003 M HCl and then with 0.1 M LiCl. Compound **24** was eluted with the latter solvent: ^{1}H NMR (D_2O) δ 3.2 (t, 1 H, H_4 of galactose), 3.4–4.4 (11 H, galactose and ribose), 5.7 (d, 1 H, H_6 of uridine), 5.76 (d, 1 H, of ribose), 7.7 (d, 1 H, H_5 of uridine); ^{31}P NMR (34 MHz, pH 12) (proton decoupled) δ −8.9 (d, P of UMP, J_{P1P2} = 28 Hz), 9.4 (d, P of phosphonate). Anal. ($C_{15}H_{30}N_4O_{16}P_2$) C, H, N, P.

Galactosyltransferase Assay. Assays were performed in a total volume of 100 μL. The assay medium contained ovalbumin (Calbiochem, La Jolla, CA) (1 mg/mL), 0.1 M cacodylate buffer (Sigma Chemical Co., St. Louis, MO) (pH 7.0), 10 mM $MnCl_2$, and 2 mM AMP; inhibitor (20 or 50 μg/mL), 10 μL of varying concentrations of cold UDP-galactose and 1 μCi of UDP-[^{3}H]-galactose ([4,5-^{3}H]galactose, 40 Ci/mmol) (Dupont-NEN, Boston, MA), and 10 μL of cell-free L1210 leukemia ascites fluid as the enzyme source.[47] Tumor ascites fluid was obtained from DBA/2J mice (Jackson Laboratories, Bar Harbor, ME), implanted 5–7 days previously with 10^5 L1210 tumor cells. Cells were removed by centrifugation. The enzyme assay was incubated for 30 min at 37 °C and the reaction stopped with 1 mL of 1% phosphotungstic acid (PTA) in 0.5 N HCl. The precipitate was washed with 10% trichloroacetic acid (TCA) and once with ethanol/ether, 2:1. The precipitate was dissolved in 200 μL of 1 N NaOH and neutralized with 1 N HCl, and the solution was counted in 10 mL of scintillation fluid (Liquiscint 2, National Diagnostics, Somerville, NJ) by using a β-scintillation counter (efficiency 33% for tritium). All other chemicals were purchased from either Fisher Scientific, Rochester, NY, or Sigma. All assays were performed in duplicate. Enzyme kinetic calculations were performed by using a software program (Rosfit) prepared by Dr. William Greco.[48] Data were graphed as v vs. [S], v vs. [I], $1/v$ vs. $1/[S]$, and $1/v$ vs. [I] and the kinetic constants obtained from these plots.

Inhibition of Tumor Cell Growth in Vitro. Compounds were evaluated for their ability to inhibit growth of L1210 leukemia, human B-lymphoblastic leukemia (WI-L2) and human T-lymphoblastic leukemia (CEM) according to our previous procedures.[49]

In Vitro Antiviral Evaluations. Glycopyranosylphosphonates and their nucleotide sugar analogues were evaluated for their ability to inhibit virus induced cytopathic effect (CPE) produced by six viruses. Viruses used in this evaluation included parainfluenza virus type 3 (para 3) strain C243, herpes simplex virus type 2 (HSV-2) strain MS, adenovirus type 2, rhinovirus, influenza virus, and visna virus. Antiviral experiments were performed according to our previous procedures.[49]

Acknowledgment. We thank Edwin Banta and Marilyn Hillman for excellent technical assistance.

Supplementary Material Available: Anisotropic thermal parameters for non-hydrogen atoms of α-D-glucopyranosylphosphonate (Table 1S) and table of inhibition of galactosyltransferase activity by nucleotide sugar analogues (Table 4S) (2 pages); observed and calculated structure factors for α-D-glucopyranosylphosphonate (Table 2S) (5 pages). Ordering information is given on any current masthead page.

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Synthesis of Certain Nucleoside Methylenediphosphonate Sugars as Potential Inhibitors of Glycosyltransferases†

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The synthesis of α-D-glucopyranosyl 1-(methylenediphosphonate) (**11**), α-D-galactopyranosyl 1-(methylenediphosphonate) (**14**), and α-D-mannopyranosyl 1-(methylenediphosphonate) (**17**) has been accomplished. [(Diphenoxyphosphinyl)methyl]phosphonic acid (diphenyl-MDP) (**5**), synthesized by two different procedures, was fused with β-D-glucopyranose pentaacetate followed by catalytic hydrogenation to give 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl methylenediphosphonate (glucose-MDP) (**10**). The anomeric configuration of **10** was assigned on the basis of NMR spectral studies. Condensation of **10** with 2',3'-di-*O*-acetyladenosine was accomplished by using 1-(mesitylene-2-sulfonyl)-3-nitro-1,2,4-triazole (MSNT) as coupling agent, and removal of the blocking groups gave adenosine 5'-[(α-D-glucopyranosylhydroxyphosphinyl)methyl]phosphonate (**20**). Uridine 5'-[(α-D-galactopyranosylhydroxyphosphinyl)methyl]phosphonate (**23**) and guanosine 5'-[(α-D-mannopyranosylhydroxyphosphinyl)methyl]phosphonate (**26**) were similarly prepared. Using a specific glycoprotein galactosyltransferase (EC 2.4.1.38) assay, uridine 5'-[(α-D-galactopyranosylhydroxyphosphinyl)methyl]phosphonate (**23**) demonstrated competitive inhibition with an apparent K_i of 97 μM. The adenosine analogue did not inhibit the enzyme. None of the above compounds show any in vitro antitumor or antiviral activity. Such specific inhibitors of glycosyltransferases may serve as specific probes to study various glycosyltransferases that might be involved in the process of metastasis.

The synthesis of phosphonate analogues of natural products has been a subject of interest in recent years.[1,2] The synthesis of phosphonate analogues of nucleoside 5'-phosphates,[3,4] nucleoside 3'-phosphates,[5,6] and cyclic

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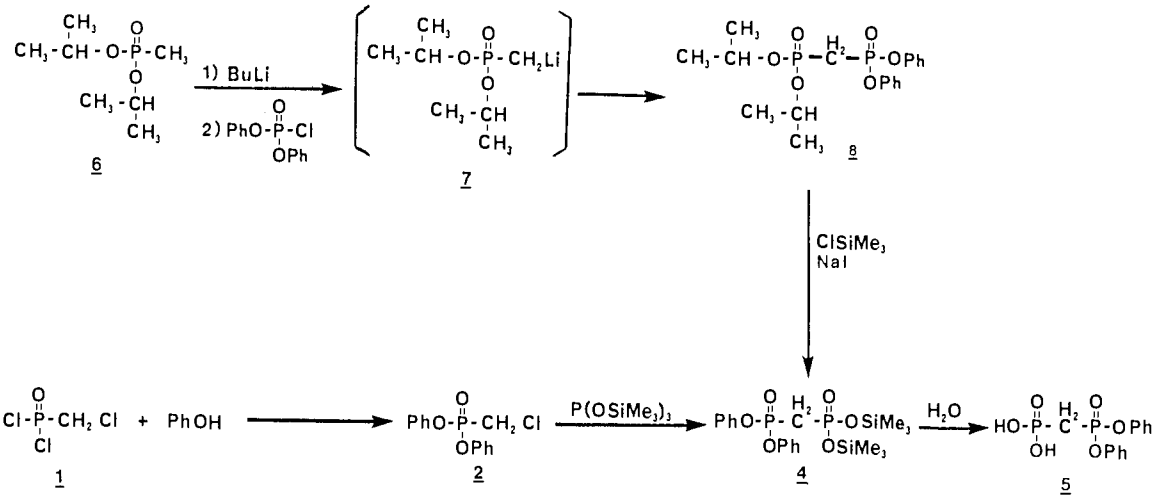
Scheme I



nucleotides[4] has been reported. The phosphonate analogues of both glucopyranosyl 6-phosphate[7] and glucopyranosyl 1-(methylenephosphate)[8,9] have been reported.

The major changes in the carbohydrate composition of transformed cells have been reviewed.[10] In general, glycopeptides derived from the surface of malignant cells are shifted toward a higher molecular weight over that of the normal cell. Potential inhibitors of specific glycosyltransferases responsible for addition to certain cell surface carbohydrates represent a new approach to the regulation of uncontrolled growth and metastasis. The biochemical rationale for a synthetic project to prepare potential glycosyltransferase inhibitors has been outlined in our preceding paper.

An appropriate nucleoside diphosphate sugar analogue[11] inhibitor of galactosyltransferase stable to enzymic hydrolysis of pyrophosphate linkage could exert selective inhibition at the surface of the tumor cell membrane. We here report the synthesis and biochemical evaluation of potential substrate inhibitors of glycosyltransferase in which the oxygen bridge between the two phosphorus atoms has been replaced by a methylene group.

Results and Discussion

The first goal of the present study was to prepare α-D-glucopyranosyl 1-(methylenediphosphonate). The synthesis of an α-D-glycopyranosyl 1-phosphate was first reported in 1949 by Krahl and Cori[12] and then by Posternak,[13] who coupled the silver salt of the phosphoric acid or diphenyl phosphate with 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide. In 1962 MacDonald[14] introduced a more conventient procedure by fusing dry phosphoric acid with β-D-glucopyranose pentaacetate at 50 °C. MacDonald's fusion procedure was not applicable when methylenediphosphonic acid was used. Consequently, [(diphenoxyphosphinyl)methyl]phosphonic acid was prepared as follows.

Diisopropyl methylphosphonate (**6**)[15] was treated with *n*-butyllithium and diphenyl chlorophosphate by the general procedure of Bartlett et al.[16] to give diisopropyl [(diphenoxyphosphinyl)methyl]phosphonate (**8**) (Scheme I). The isopropyl group was then replaced with trimethylsilyl by using chlorotrimethylsilane and sodium iodide[17] to yield bis(trimethylsilyl) [(diphenoxyphosphinyl)methyl]phosphonate (**4**), which was then hydrolyzed with water to give [(diphenoxyphosphinyl)-methyl]phosphonic acid (**5**).

Compound **5** was also synthesized through an alternative route. Chloromethanephosphonyl dichloride[18] (**1**) was reacted with phenol to give diphenyl(chloromethyl)-phosphonate (**2**) by the general procedure of McCall and McConnell[19] The alkyl halide of **2** was then reacted with tris(trimethylsilyl) phosphite[20] (**3**) to give bis(trimethylsilyl) [(diphenoxyphosphinyl)methyl]phosphonate (**4**) (Scheme I). Hydrolysis of **4** with water gave the selectively blocked [(diphenoxyphosphinyl)methyl]phosphonic acid (**5**). This procedure was superior to the previous procedure described above.

Compound **5**, which has a considerably lower melting point than methylenediphosphonic acid, was fused at 170 °C with β-D-glucopyranose pentaacetate. Under these conditions, 2,3,4,6-tetra-*O*-acetyl-α,D-glucopyranosyl 1-[[(diphenoxyphosphinyl)methyl]phosphonate] (**9**) (Scheme II) was obtained in over 50% yield. The coupling of methylenediphosphonate with β-D-glucopyranose pentaacetate at such a high temperature favors the formation of the α-anomer, which is thermodynamically controlled. The α configuration of this product was confirmed by ^{1}H NMR and ^{13}C NMR spectra (Table I). The ^{1}H NMR

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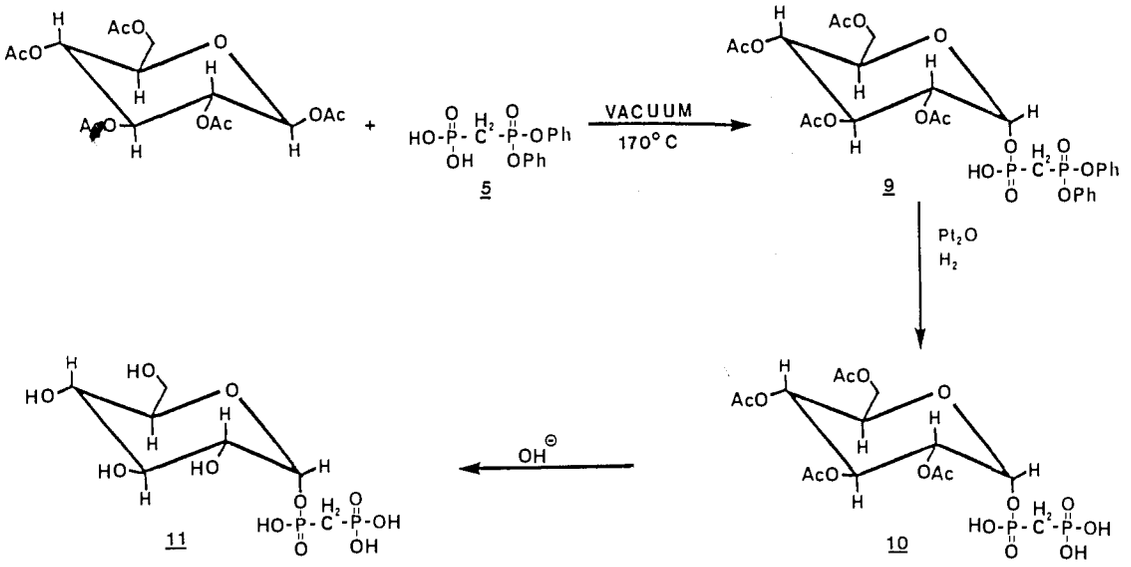
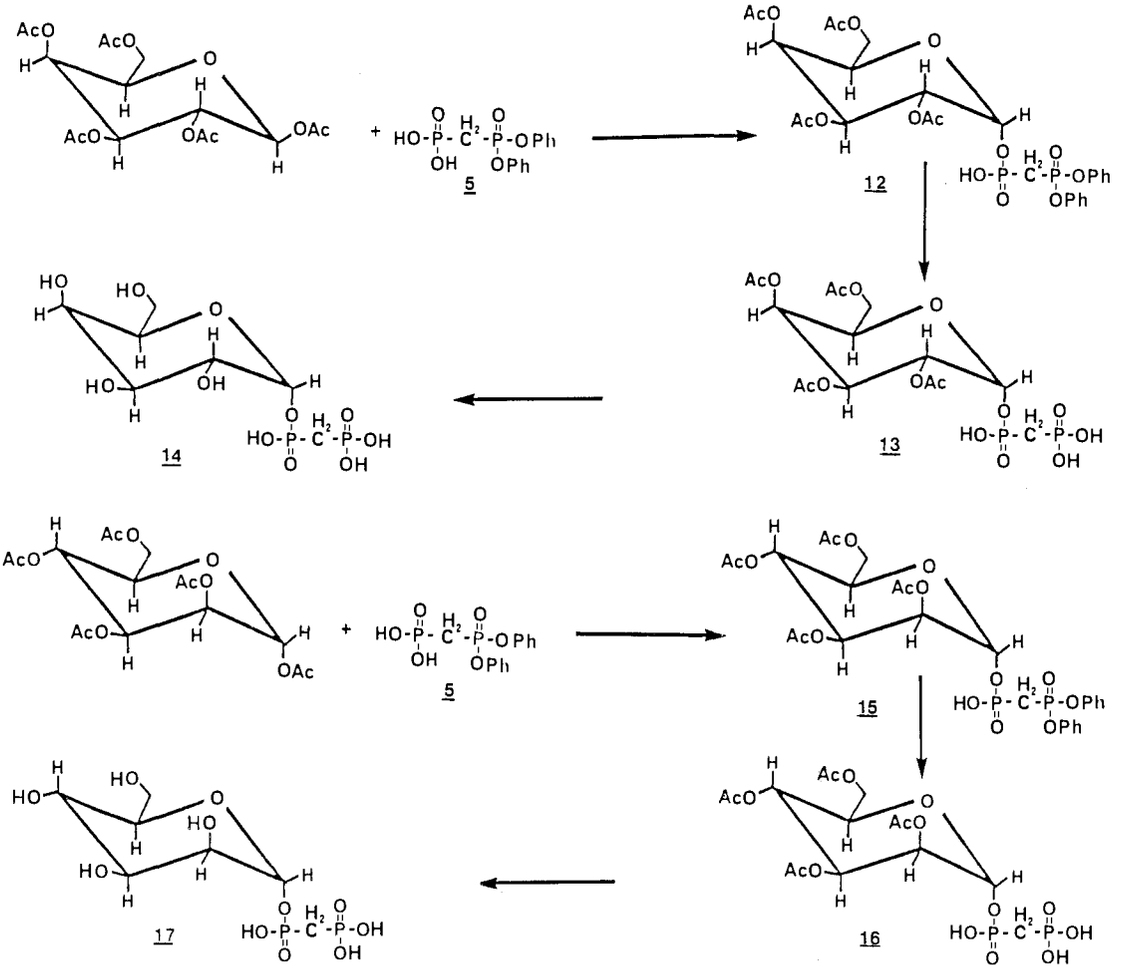
Scheme II



Scheme III



spectrum shows that the anomeric proton appears as a doublet of doublets at 6.05 ppm.

The phenyl groups were removed by catalytic hydrogenation using platinum oxide as catalyst[21] at 1000 psi to give 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl 1-7-methylenediphosphonate) (**10**). The ^{13}C NMR spectrum of compound **10** shows a doublet for C_1 at 93.9 ppm, which is characteristic of the α-anomer.[22]

Compound **10** was deacetylated by ammonium hydroxide solution to give α-D-glucopyranosyl 1-(methylenediphosphonate) (11) in quantitative yield.

The β-D-galactose pentaacetate (from Sigma) was treated with [(diphenoxyphosphinyl)methyl]phosphonic acid (**5**)

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Table I. Nuclear Magnetic Resonance (NMR) Data of Certain α-D-Glycopyranosyl 1-(Methylenediphosphonates)

	chemical shifts (ppm) and coupling constants (Hz)											
	^{1}H NMR			^{31}P NMR				^{13}C NMR				
				decoupled		proton coupled						
compound	H_1 (J_{H1P}, J_{H1H2})	H_2–H_6	PH_2P (J_{HP})	P_1 (J_{P1P2})	P_2	P_1 (J_{PCH2})	P_2 (J_{PH1})	C_1 (J_{C1P})	C_2 (J_{C2P})	C_3, C_4	C_5, C_6	PCH_2P (J_{CP1}, J_{CP2})
glucose-MDP, (**10**)	5.4 (dd), (3.18, 7.5)	3.3–4.2	2.1 (t) (20)	14.3 (d) (11)	23.9 (d)	14.3 (t of d) (18.31)	23.9 (m) (9.76)	93.9 (d) (7.65)	72.3 (d) (3.37)	69.67, 72.67	73.12, 60.75	29.4 (d of d) (116.5, 128.29)
galactose-MDP, (**13**)	5.42 (dd) (3.1, 7.45)	3.4–4.4	2.1 (t) (20)	14.2 (d) (11)	23.8 (d)	14.2 (t of d) (18.31)	23.8 (m) (8.54)	94.8 (d) (6.8)	70.3 (d) (6.3)	66.8, 70.8	71.5, 60.9	25.4 (d of d) (114, 126)
mannose-MDP (**16**)	5.3 (d) (8.2)	3.6–4.2	2.2 (t) (20)	14.1 (d) (8.54)	23.6 (d)	14.1 (t of d) (18.31)	23.6 (m) (8.54)	95.02 (d) (6.11)	70.81 (6.4)	66.9, 70.13	73.4, 60.92	24.5 (d of d) (114)

under identical reaction conditions as with β-D-glucose pentaacetate, to give 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl 1-[[(diphenoxyphosphinyl)methyl]phosponate] (**12**). Catalytic hydrogenation of **12** afforded 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl 1-(methylenediphosponate) (**13**) in 52% yield (Scheme III). The ^{1}H NMR spectrum of compound **12** shows a doublet of doublets at 6.05 ppm for H_1. On the other hand, the ^{13}C NMR spectrum of **13** shows a doublet at 94.8 ppm for C_1, which is characteristic of the α-anomer.[22] Treatment of compound **13** with ammonium hydroxide gave α-D-galactopyranosyl 1-(methylenediphosphonate) (**14**). In the case of mannose, the starting sugar was α-D-mannose pentaacetate. The reaction time in this case was longer, and after the removal of the phenyl groups, the yield of 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl 1-(methylenediphosphonate) (**16**) was 35% (Scheme III). The ^{1}H NMR spectrum of **16** shows a doublet for H_1, in which the coupling constant for H_1–P is 8.2 Hz and the coupling constant for H_1H_2 is 0 Hz, which is characteristic of the α-anomer of mannose.[23] The α configuration can also be established from the ^{13}C NMR spectrum of **16**, which shows a doublet for C_1 at 95.02 ppm.[22] Compound **16** was deacetylated to give α-D-mannopyranosyl 1-(methylenediphosphonate) (**17**).

The NMR data of compounds **10**, **13**, and **16** are summarized in Table I. The CH_2 of methylenediphosphonate appears as a doublet of doublets as it is coupled with the two neighboring phosphorus atoms. The coupling constant in this case is relatively large.

The ^{31}P NMR spectrum, when decoupled, shows a doublet in each case with a coupling constant of 11 Hz for **10** and **13** and 8.54 Hz for **16**. On the other hand, in the proton-coupled ^{31}P NMR spectrum, the phosphorus that is attached to the sugar (P_2) has a chemical shift of around 24 ppm and shows 7–9 clear peaks, which are in fact triplets of doublets of a doublet. The coupling constants are shown in Table I. The P_1 or the other phosphorus has a chemical shift of around 14 ppm, and when it is proton-coupled, it shows triplets of a doublet. The ^{31}P NMR of methylenediphosphonic acid appears at 18.7 ppm as a singlet, and when it is coupled with the protons, it shows a triplet with a coupling constant of 19 Hz.

The ^{13}C NMR spectra of **10**, **13**, and **16** clearly show a doublet signal at around 94–95 ppm, which is characteristic of a single anomer in each instance. The coupling constant between C_1 and phosphorus is 6.82 Hz for **10**, 6.8 Hz for **13**, and 6.11 Hz for **16**. C_2 of the sugar also coupled with phosphorus with a coupling constant of 8.54 Hz for **13**, 8.6 Hz for **10**, and 8.1 Hz for **16**. The CH_2 of methylenediphosphonate in each case appeared at 24.4–24.5 as a triplet with J_{CP} of 114–128 Hz.

The triethylammonium salt of **10** was coupled with 2′,3′-di-*O*-acetyladenosine (**18**) in dry pyridine. 1-(Mesitylene-2-sulfonyl)-3-nitro-1,2,4-triazole (MSNT) was used as coupling agent. This reagent has been used recently by Jones et al.[24] and Chattopadhyaya and Reese[25] with satisfactory results. The product was 2′,3′-di-*O*-acetyladenosine 5′-[(2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosylhydroxyphosphinyl)methyl]phosphonate (**19**), which was treated with 1 N ammonium hydroxide at room temperature to yield adenosine 5′-[(α-D-glucopyranosylhydroxyphosphinyl)methyl]phosphonate (**20**) in 48% yield (Scheme IV).

The triethylammonium salt of the 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl 1-(methylenediphosphonate) (**13**) was coupled with 2′,3′-di-*O*-acetyluridine (**21**),[26] and the product was 2′,3′-di-*O*-acetyluridine 5′-[(2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosylhydroxyphosphinyl)-methyl]phosphonate (**22**). Compound **22** was deacetylated with ammonium hydroxide to yield **23** in 38% overall yield (Scheme V).

N-(2-Methyl-1-oxopropyl)-2′,3′-dibenzoylguanosine (**24**) was synthesized according to the procedure of Ohtsuka et al.[27] Compound **24** was then reacted with the triethylammonium salt of 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl 1-(methylenediphosphonate) (**16**) in dry dimethylformamide, and MSNT was added as coupling reagent (Scheme VI). It should be mentioned that when pyridine was used as a solvent in this reaction no coupling took place. The product of the above reaction was *N*-(2-methyl-1-oxopropyl)-2′,3′-dibenzoylguanosine 5′-[(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosylhydroxyphosphinyl)-methyl]phosphonate (**25**), which was deblocked with methanolic ammonia to give guanosine 5′-[(α-D-mannopyranosylhydroxyphosphinyl)methyl]phosphonate (**26**) in 32% yield.

The galactosyltransferase inhibitory activities of compounds **20**, **23**, and **26** were evaluated. The adenosine methylenediphosphonate glucose analogue at concentrations up to 50 μg/mL was without significant inhibitory effect while concentrations of the uridine galactose de-

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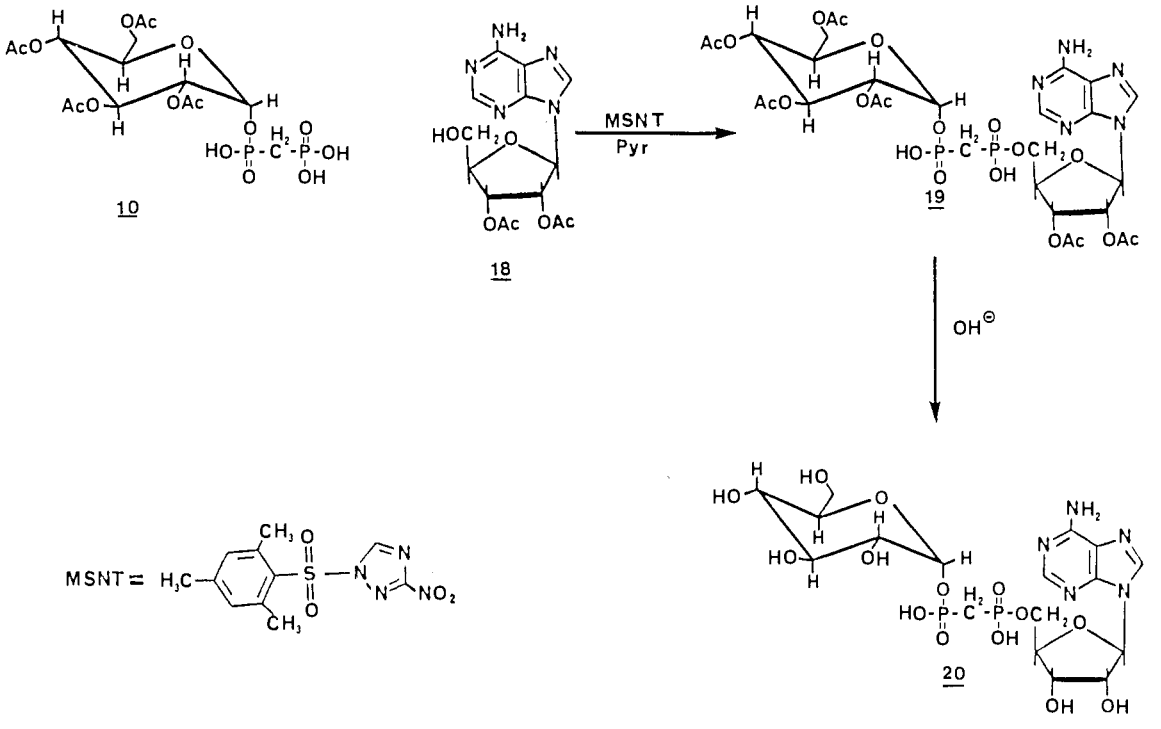
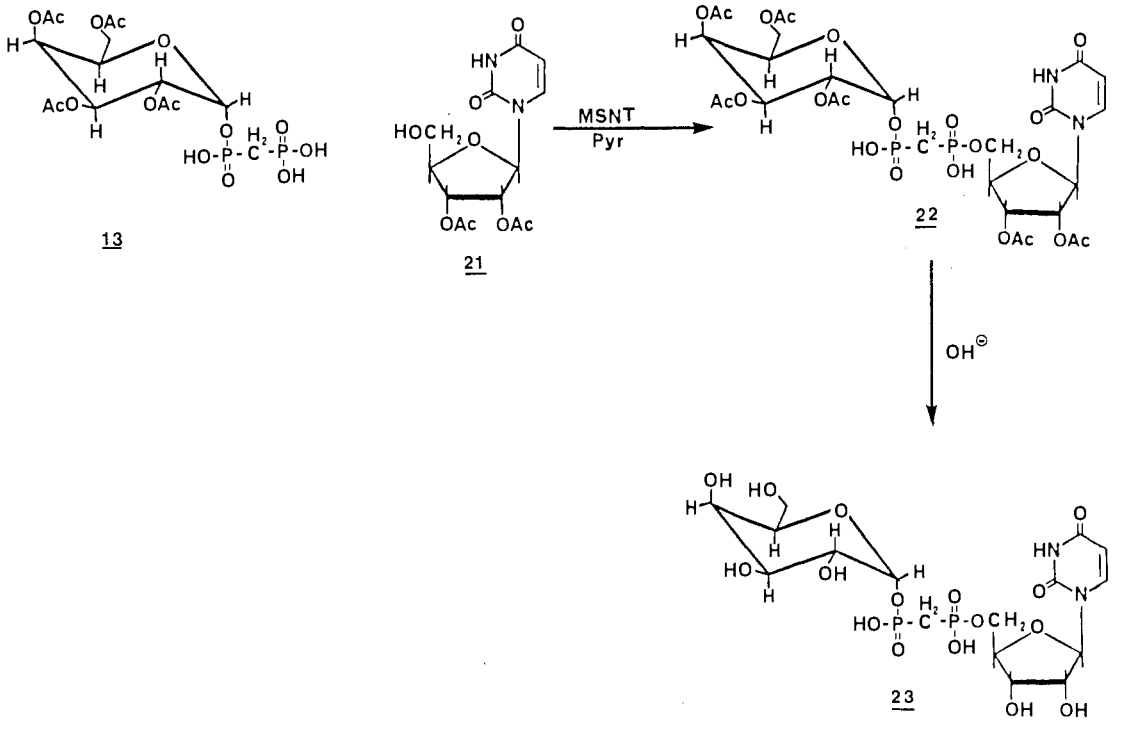
Scheme IV



10 18 19 20

Scheme V



13 21 22 23

rivative at 1 μg/mL reduced galactosyltransferase activity by over 40%. With variation of UDP-galactose concentrations from 1 to 80 μM and maintenance of compound **23** (inhibitor) concentrations at either 16.7 or 33.4 μM, a series of enzyme assays were performed. The double reciprocal plot (Figure 1) demonstrated competitive inhibition caused by the uridine methylenediphosphonate galactose. The apparent K_i calculated for compound **23** was 96.9 μM (Table II), which was less than that observed (165 μM) for the uridine 5′-phosphoric α-D-galactopyranosylphosphonic anhydride reported in our adjunct paper. Although it has been proposed that in the natural products of this type the bond between the sugar and the phosphate is being cleaved by the enzyme, inhibition of the enzyme by **23** might be due to another mechanism.

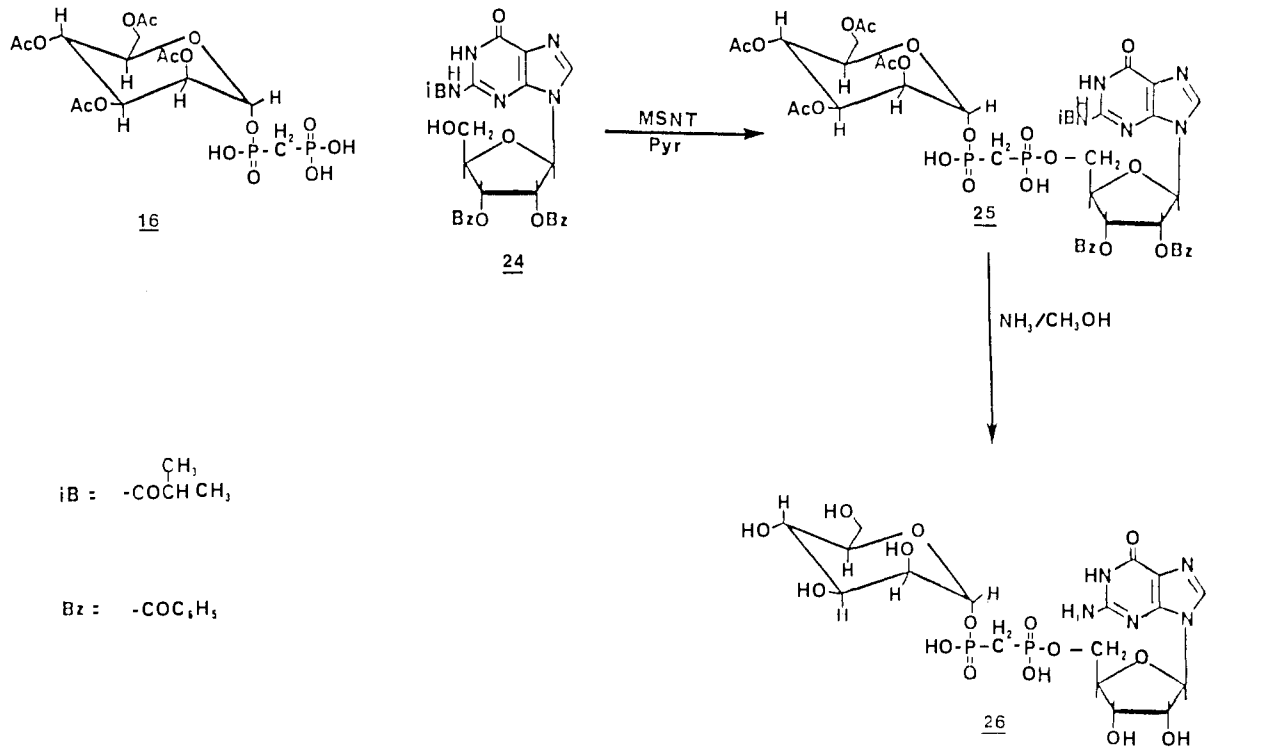
Table II.[a] Kinetic Constants for Galactosyltransferase

V_{max}	60.6 ± 2.7 pmol/0.5 h
K_m (UDP-galactose)	12.5 ± 2.5 μM
K_i (**23**)	96.9 ± 6.1 μM

[a] Assays were performed as described in the Experimental Section. All assays were performed in duplicate. Data are expressed as the mean ± SD. Enzyme kinetic parameters were calculated by using Rosfit computer software.

Antitumor Evaluations. The in vitro antitumor activities of α-D-glucopyranosyl 1-(methylenediphosphonate) (11), α-D-galactopyranosyl 1-(methylenediphosphonate) (14), and α-D-mannopyranosyl 1-(methylenedi-

Scheme VI



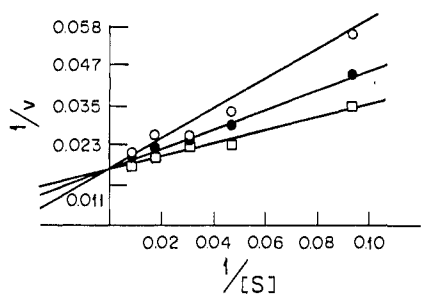


Figure 1. Double reciprocal plot for galactosyltransferase activity in the presence of uridine 5′-[(α-D-galactopyranosylhydroxyphosphinyl)methyl]phosphonate (○, 33 μM; ●, 17 μM; □, 0 μM). Enzyme activity was measured as described in the Experimental Section. UDP-galactose concentrations were varied from 1 to 80 μM.

phosphonate) (**17**) as well as their corresponding nucleotide sugar analogues of **20**, **23**, and **26** were measured against L1210 leukemia, human B-lymphoblastic leukemia (WW-L2), and human T-lymphoblastic leukemia (CEM). None of the above compounds show any antitumor activity in cell culture.

Antiviral Evaluations. The in vitro antiviral activities of compounds **11**, **14**, **17**, **20**, **23**, and **26** were measured against herpes simplex virus type 2 (HSV-2), parainfluenza virus type 3 (para 3), adenovirus type 2 (Adeno 2), rhinovirus (Picorna), influenza virus (Influenza A Chile), and visna viruses. These compounds did not show any significant activity against any viruses. Only compound **17** showed slight activity against rhinovirus.

Experimental Section

General Information. Ultraviolet spectra were recorded on a Cary Model 118 spectrophotometer. Infrared (IR) spectra were obtained on a Beckman Acculab 2 spectrophotometer. Melting points were taken on a Fisher-Johns plate melting point apparatus and are uncorrected. Nuclear magnetic resonance (NMR) spectra were taken on a JEOL FX 90Q spectrometer. The chemical shift values are expressed in δ values (parts per million) and referenced with tetramethylsilane (for ^1H NMR), phosporic acid (85%) (for ^{31}P NMR), and dioxane (for ^{13}C NMR). High-performance liquid chromatography (HPLC) studies were performed on a Beckman Model 110A gradient liquid chromatograph with a Hitachi Model 110-40 UV monitor and an Altex C-R1A recorder. A column of ODS-2 (C-AT reverse-phase silica gel) was used, and the elutions for HPLC were performed with both 5% acetonitrile in water and 0.05 M ammonium phosphate buffer at pH 6.5.

Thin-layer chromatograms (TLC) were run on silica gel 60 F-254 (EM Reagent) plates. EM silica gel (200–400 mesh) was used for flash column chromatography. Bio-Rad AG 2-X8 and Sigma DEAE-Sephadex A-25 were used for ion-exchange chromatography. The components were detected on TLC plates by UV light and with 10% H_2SO_4 in methanol spray followed by heating. The solvent systems used for TLC were as follows: (A) 2% MeOH in $CHCl_3$; (B) 5% MeOH in $CHCl_3$; (C) 15% MeOH in $CHCl_3$; (D) 0.1 N NH_4Cl, CH_3CN (3:7); (E) acetone, hexane (3:7).

Evaporations were carried out on a rotary evaporator (Büchi Rotavapor R110), under reduced pressure applied by a tap water aspirator with a bath temperature of 30–35°C. Freeze-drying was carried out with a Precision Vacuum pump Model DD 195.

β-D-Glucopyranose pentaacetate, β-D-galactopyranose pentaacetate, α-D-mannopyranose pentaacetate, 2′,3′-di-*O*-acetyladenosine, and 2′,3′-di-*O*-acetylguanosine were purchased from Sigma Chemical Company, St. Louis, MO. Chlorotrimethylsilane, diphenyl chlorophsphate, *n*-butyllithium, platinum(IV) oxide, and phosphorous trichloride were from Aldrich Chemical Co., Milwaukee, WI. Phosphorous acid and triethylamine were obtained from Fisher Scientific, Fair Lawn, NJ. 2′,3′-Di-*O*-acetyluridine was synthesized according to the procedure of Verheyden and Moffatt.[26]

Elemental analyses were performed by Galbraith Laboratories, Knoxville, TN., and by Schwarzkopf Microanalytical Laboratory, Woodside, NY.

Synthetic Procedures. Diphenyl (Chloromethyl)phosphonate (2). Chloromethanephosphonyl dichloride (**1**)[16] (177.4 g, 1 mol) and phenol (206 g, 2.1 mol) were mixed in a 1000-mL round-bottom flask equipped with a stirring bar and a reflux condenser through which ~60 °C H_2O passed. This mixture was heated to reflux in a silicon oil bath (200–210 °C). A white gas (HCl + phenol) escaped through the condenser. After about 3 h the refluxing ceased, and the heating was continued for another 5 h during which a slight vacuum (100 mm) was applied twice for about 10 min to remove the trace of HCl. The excess phenol was removed at 10-mm pressure and about 140–150 °C bath temperature. A hot (50 °C) condenser was used to prevent solidification of phenol inside the condenser channel. When the

fractionation was completed, the bath temperature rose to 220–230 °C and the pressure lowered to 0.15 mm during which a good rate of reflux started. The boiling point at this pressure was 145–147 °C, and only one fraction was obtained. The yield was 265 g (94%): TLC on silica gel with solvent B, single spot (R_f 0.7); d_4^{25} 1.30; ^{1}H NMR ($CDCl_3$) δ 3.8 (d, 24 H), 7.25 (m, 10 H). Anal. ($C_{13}H_{12}O_3ClP$) C, H, Cl, P.

[(Diphenoxyphosphinyl)methyl]phosphonic Acid (5). Method 1. In a 500-mL round-bottom flask, diphenyl (chloromethyl)phosphonate (**2**) (282 g, 1 mol) was mixed with tris(trimethylsilyl) phosphite (328 g, 1.1 mol) and heated to 220 °C with stirring. The flask was equipped with a 50-cm-long column with a distillation condenser at the top. The temperature was kept at 220–230 °C for 3 h while the chlorotrimethylsilane (byproduct) was distilling from the reaction mixture. The heating was continued for another $^1/_2$ h after which the reaction mixture was cooled to room temperature; 700 mL of H_2O and 1000 mL of 20% ammonium bicarbonate were added, and the mixture was extracted with 2 × 200 mL of chloroform. The bicarbonate solution was neutralized with 3 N HCl, and 300 mL of excess HCl was added. The acidic solution was immediately extracted with 3 × 500 mL of $CHCl_3$, and the chloroform extracts were combined and dried over anhydrous sodium sulfate. Compound **5** crystallized upon addition of 500 mL of petroleum ether (bp 30–60 °C) overnight at room temperature. The yield was 160 g (49%). The compound was recrystallized from chloroform: mp 150 °C; ^{1}H NMR (Me_2SO-d_6) δ 2.76 (t, 2 H, J_{HP} = 21 Hz), 7.1–7.5 (m, 10 H); ^{31}P NMR δ 16.93 (d, 1 P, $P(OH)_2$, J_{P1P2} = 8.54 Hz), 21.64 (d, 1 P, $P(OPh)_2$). Anal. ($C_{13}H_{14}O_6P_2$) C, H, P.

Method 2. Diisopropyl methylphosphonate (**6**) was synthesized by a modified procedure of Ford-Moore and Williams[15] as follows: Triisopropyl phosphite (1 mol) was mixed with 1.5 mol of methyl iodide and heated gently to 57 °C at which point a vigorous reaction started, and heating was discontinued until there was no more refluxing. The temperature was increased slowly to 100 °C and kept at 100 °C for about 1 h. The isopropyl iodide was distilled off at normal pressure, and then the product was distilled at 60 °C (1 mm). The yield was 95%: ^{1}H NMR ($CDCl_3$) δ 1.4 (d, 12 H), 1.42 (d, 3 H), 4.66 (m, 2 H).

Diisopropyl [(diphenoxyphosphinyl)methyl]phosphonate (**8**) was synthesized by a method similar to the procedure of Bartlett et al.,[16] who reported the synthesis of some methylenediphosphonate esters related to compound **8**.

In a 1-L three-neck flask, diisopropyl methylphosphonate (**6**) (180 g, 1.09 mol) was dissolved in 400 mL of dry tetrahydrofuran (THF), and the solution was cooled to −78 °C with an acetone/dry ice bath. To this cold solution was added 1.09 mol of *n*-butyllithium (404 mL of 2.7 M in hexane) dropwise during $^1/_2$ h such that the temperature was kept below −60 °C. Stirring was continued for an additional 15 min, and then 148 g of diphenyl chlorophosphate (0.54 mol) in 150 mL of THF was added dropwise over 1 h. Stirring was continued for another 15 min after which 202 mL of 2.7 M *n*-butyllithium (0.54 mol) was added dropwise. This sequence was repeated with 0.27 mol of diphenyl chlorophosphate, followed by 0.27 mol of *n*-butyllithium and 0.13 mol of diphenyl chlorophosphate. The reaction mixture was stirred for an additional $^1/_2$ h, poured into 1500 mL of saturated sodium bicarbonate, and extracted with 400 mL of ethyl acetate. The ethyl acetate solution was washed with water and dried with anhydrous sodium sulfate. The solvent was evaporated on a rotovap and then under vacuum at 0.05 Torr up to 110 °C. The yield of diisopropyl [(diphenoxyphosphinyl)methyl]phosphonate (**8**) was 329 g. Compound **8** was 96% pure on TLC. To obtain an analytically pure sample, 1 g of **8** was further purified on a 2.5 × 30 cm silica gel column (200–400 mesh) that was eluted with 2% methanol in chloroform: ^{1}H NMR ($CDCl_3$) δ 1.35 (d, 12 H), 2.75 (t, 2 H), 4.81 (m, 2 H), 7–7.4 (m, 10 H). Anal. ($C_{19}H_{26}O_6P_2$) C, H, P.

In a 1-L round-bottom flask, diisopropyl [(diphenoxyphosphinyl)methyl]phosphonate (**8**) (206 g, 0.5 mol) was added with stirring to a solution of sodium iodide (160 g, 1.08 mol) in 400 mL of dry acetonitrile. Then chlorotrimethylsilane (120 g, 1.12 mol) was added slowly with stirring over a period of 10 min. The reaction mixture was stirred at 50 °C for 3 h and then treated with 100 mL of water. The acetonitrile was removed at reduced pressure, and 400 mL of 4 M ammonium bicarbonate was added. The mixture was extracted with 2 × 200 mL of chloroform, and the water solution was neutralized with 4 N hydrochloric acid. An additional 50 mL of HCl was added and immediately extracted with 3 × 20 mL of chloroform. The chloroform solution was dried over anhydrous sodium sulfate and filtered. About 1 volume of petroleum ether was added, and the compound crystallized overnight. The yield was 110 g (34%). This compound was identical with the compound synthesized by method 1.

2,3,4,6-Tetra-*O*-acetyl-α-D-glucopyranosyl 1-[[(Diphenoxyphosphinyl)methyl]phosphonate] (9). A melt of [(diphenoxyphosphinyl)methyl]phosphonic acid (**5**) (10 g, 31 mmol) was prepared at 170 °C in a 100-mL round-bottom flask. At 0.05 Torr vacuum, it was stirred for 10 min. At the temperature of 170 °C, β-D-glucopyranose pentaacetate (7 g, 18 mmol) was added under argon gas, and immediately vacuum was applied and the reaction mixture was stirred. As soon as the color of the reaction mixture turned brown (ca. 7 min), the heat source was removed, and the reaction mixture was air-cooled under vacuum with stirring. When the temperature of the reaction mixture was around 60 °C, the crude product was dissolved in 100 mL of chloroform. The chloroform solution was then extracted with 100 mL of 20% ammonium bicarbonate solution to remove the unreacted acid (which can be recovered by acidification and extraction with chloroform, followed by crystallization as described in the synthesis of compound **5**). The chloroform solution was dried over anhydrous sodium sulfate and evaporated to dryness. The dark brown residue was dissolved in 50 mL of ethanol, and most of the color was removed by boiling the ethanolic solution with active charcoal. To the clear yellow solution was added 20 g of silica gel, and the mixture was dried at reduced pressure. This compound was then purified on a flash silica gel column (5 × 50 cm) that was eluted with 300 mL of $CHCl_3$, 1 L of 4% methanol in chloroform, and 2 L of 15% methanol in chloroform in turn. The last fraction contained 6.2 g of **9** (greater than 95% pure), which was dried under reduced pressure, redissolved in ethanol (50 mL), decolorized with charcoal, and dried. The yield of the title compound was 52.5%. For analytical purposes, a small fraction (0.5 g) was further purified on a preparative silica gel TLC plate that was developed with 15% methanol in chloroform. The compound was removed from the plate, dissolved in methanol, filtered through a glass fiber, and dried. The glassy solid melted at 188–190 °C with decomposition: ^{1}H NMR ($CDCl_3$) δ 2.73 (t, 2 H, PCH_2P, J_{PH} = 20 Hz), 6.10 (d of d, 1 H, H_1, J_{H1P} = 8.34 Hz, J_{H1H2} = 2.71 Hz) anomeric; ^{31}P NMR (decoupled spectrum) δ 9.52 (d, 1 P, J_{P1P2} = 4.88 Hz, P_2), 19.7 (d, 1 P, P_1); proton-coupled ^{31}P NMR δ 9.52 (d of t, J_{PCH2} = 18.3 Hz), 19.7 (m, J_{P1H1} = 9.76 Hz); ^{13}C NMR δ 91.77 (s, 1 C, $C_1\alpha$), 26.02 (t, 1 C, PCH_2P, J_{CP} = 108 Hz). Anal. ($C_{27}H_{32}O_{15}P_2$) C, H, P.

α-D-Glucopyranosyl 1-(Methylenediphosphonate) (11). To a solution of 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl 1-[[(diphenoxyphosphinyl)methyl]phosphonate] (**9**) (2 g, 3.0 mmol) in 20 mL of absolute ethanol was added 1 g of platinum oxide. The mixture was placed in a glass-lined high-pressure reactor. The reaction vessel was sealed and purged twice with 200 psi of hydrogen, and the content was agitated with hydrogen gas at 40 °C overnight at a pressure of 1000 psi. The progress of hydrogenation was followed by TLC on a silica gel F254 plate that was developed with solvent D. The products were poured into 10 mL of 1 M triethylamine in ethanol and dried at 30 °C under reduced pressure. The product, even though relatively pure, was applied to a 2.5 × 40 cm DEAE-Sephadex A-25 (bicarbonate form) column and eluted with 100 mL of water and then with a gradient of 0–0.4 M triethylammonium bicarbonate (1 L in each container). The UV monitor was used to distinguish any phenyl-containing product. The fraction that lacked UV absorption and charred (on silica gel) upon treatment with 10% H_2SO_4 in CH_3OH was collected and dried at 30 °C under reduced pressure. Coevaporation with absolute ethanol was performed three times. For analytical purposes, a fraction of compound **10** was absorbed on a bicarbonate form of a DEAE-Sephadex column. The ammonium salt of compound **10** was made by elution of **10** with ammonium bicarbonate gradient solution (0.1–0.3 M) from a DEAE-Sephadex column. The powder decomposed at 220 °C: ^{1}H NMR (D_2O) δ 5.52 (d of d, $H_1\alpha$), 2.1 (t, PCH_2P), the aromatic protons at 7–7.3 were eliminated, otherwise it has the same pattern as its precursor; ^{31}P NMR (decoupled) δ 14.3 (d, P_1, J_{P1P2} = 11 Hz), 23.8 (d, P_2);

proton-coupled ^{31}P NMR δ 14.3 (t of d, J_{PCH_2} = 18.31 Hz), 23.8 (m, J_{PH_1} = 9.76 Hz). Anal. ($C_{15}H_{33}H_3O_{15}P_2$) C, H, N, P.

Hydrolysis of compound **10** with 1 N ammonium hydroxide at room temperature for 1 h gave α-D-glucopyranosyl 1-(methylenediphosphonate) (**11**) in powder form and in quantitative yield. ^1H NMR and ^{13}C NMR spectral data (Table 2S) are included in supplementary material. Anal. ($C_7H_{25}N_3O_{11}P_2$) C, H, N, P.

2,3,4,6-Tetra-*O*-acetyl-α-D-galactopyranosyl 1-[[(Diphenoxyphosphinyl)methyl]phosphonate] (12). A melt of [(diphenoxyphosphinyl)methyl]phosphonic acid (**5**) (10 g, 31 mmol) was prepared at 170 °C under 0.05 Torr vacuum. β-D-Galactopyranose pentaacetate (7 g, 18 mmol) was added under argon gas, and the mixture was stirred at 170 °C under 0.05 Torr vacuum. As soon as the mixture turned brown (ca. 5 min), the heat source was removed and the reaction was air-cooled to about 60 °C while still under vacuum. Purification of compound **12** was conducted in a similar manner to that of 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl 1-[[(diphenoxyphosphinyl)methyl]phosphonate] (**9**). The yield was 5.5 g (46.6%). The pure compound melts at 187–190 °C with decomposition: ^1H NMR ($CDCl_3$) δ 2.73 (t, 2 H, J_{HP} = 20.02 Hz, PCH_2P), 6.06 (d of d, J_{H1P} = 8.58 Hz, J_{H1H2} = 2.71 Hz, $H_{1\alpha}$). Anal. ($C_{27}H_{32}O_{15}P_2$) C, H, P.

α-D-Galactopyranosyl 1-(Methylenediphosphonate) (14). To obtain the title compound, 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl 1-[[(diphenoxyphosphinyl)methyl]phosphonate] (**12**) (5.5 g, 8 mmol) was hydrogenated and purified in a similar manner to that of 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl 1-(methylenediphosphonate) (**10**), and 3.8 g of the triethylammonium salt of compound **13** was obtained as white powder. The compound decomposed at 210 °C: ^1H NMR (D_2O) δ 2.1 (t, PCH_2P, J_{PH} = 20 Hz), 5.42 (d of d, $H_1\alpha$), also other sugar and acetyl protons, no aromatic protons; ^{31}P NMR (decoupled) δ 14.2 (d, P_1, J_{P1P2} = 11 Hz), 23.8 (d, P_2); proton-coupled ^{31}P NMR δ 14.2 (t of d, J_{PCH_2} = 18.31 Hz), 23.8 (m, $J_{P2H1\alpha}$ = 8.54 Hz). Anal. ($C_{15}H_{33}H_3O_{15}P_2$) C, H, N, P.

Hydrolysis of compound **13** with 1 N ammonium hydroxide at room temperature gave α-D-galactopyranosyl 1-(methylenediphosphonate) (**14**). ^1H NMR and ^{13}C NMR data of compound **14** are included in supplementary material. Anal. ($C_7H_{25}N_3O_{11}P_2$) C, H, Nn, P.

2,3,4,6-Tetra-*O*-acetyl-α-D-mannopyranosyl 1-[[(Diphenoxyphosphinyl)methyl]phosphonate] (15). A melt of [(diphenoxyphosphinyl)methyl]phosphonic acid (**5**) (10 g, 31 mmol) was prepared at 170 °C and 0.05 Torr vacuum. α-D-Mannopyranose pentaacetate (7 g, 18 mmol) was added under argon gas, and the mixture was stirred at 175 °C under 0.05 Torr vacuum. As soon as the mixture turned brown (ca. 5 min), the heat source was removed and the product was treated in a similar manner to that of 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl 1-[[(diphenoxyphosphinyl)methyl]phosphonate] (**9**). The yield of compound **15** was 3.1 g (26.3%). The glassy solid **15** melts at 184–188 °C: ^1H NMR (D_2O) δ 2.73 (t, 2 H, J_{HP} = 20.60 Hz, PCH_2P), 5.88 (d, 1 H, J_{H1H2} = 8.34 Hz, $H_1\alpha$), also peaks corresponding to acetyl, phenyl, and sugar were present; ^{31}P NMR δ 8.16 (d, 1 P, J_{P1P2} = 4.89 Hz, P_2), 19.52 (d, 1 P, P_2); proton-coupled ^{31}P NMR 8.16 (m, P_2), 19.52 (t of d, J_{P1CH_2} = 19.95 Hz); ^{13}C NMR δ 27.66 (t, 1 C, J_{CP} = 109.2 Hz, PCH_2P), 92.2 (s, 1 C, $C_1\alpha$). Anal. ($C_{27}H_{32}O_{15}P_2$) C, H, P.

α-D-Mannopyranosyl 1-(Methylenediphosphonate) (17). To obtain the title compound, 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl 1-[[(diphenoxyphosphinyl)methyl]phosphonate] (**15**) (3.1 g, 4.6 mmol) was hydrogenated and purified according to the procedure described for the D-glucose analogue of **10** to yield 2.4 g of triethylammonium salt of compound **16**: ^1H NMR (D_2O) δ 2.19 (t, PCH_2P, J_{PH} = 20 Hz), 5.3 (d, $H_{1\alpha}$), also other sugar and acetyl protons and no aromatic protons; ^{31}P NMR (decoupled) δ 14.1 (d, P_1, J_{P1P2} = 8.54 Hz), 23.6 (d, P_2); proton-coupled ^{31}P NMR δ 14.1 (t of d, J_{P1CH_2} = 18.31 Hz), 23.6 (m, $J_{P2H1\alpha}$ = 8.54 Hz). Anal. ($C_{15}H_{33}N_3O_{15}P_2$) C, H, N, P.

Hydrolysis of compound **16** with 1 N ammonium hydroxide at room temperature gave α-D-mannopyranosyl 1-(methylenediphosphonate) (**17**). ^1H NMR and ^{13}C NMR data of compound **17** are included in supplementary material. Anal. ($C_7H_{25}N_3O_{11}P_2$) C, H, N, P.

Adenosine 5′-[(α-D-Glucopyranosylhydroxyphosphinyl)methyl]phosphonate (20). One millimole of triethylammonium salt of 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl 1-(methylenediphosphonate) (**10**) and 0.9 mmol of 2′,3′-di-*O*-acetyladenosine (**18**) were dissolved in 10 mL of dry pyridine and rendered dry by evaporation under reduced pressure. This procedure was repeated four times, the residue was dissolved in 10 mL of dry pyridine, 2 mmol of 1-(mesitylene-2-sulfonyl)-3-nitro-1,2,4-triazole (MSNT) was added, and the mixture was stirred at room temperature overnight. Pyridine was removed by evaporation, and then 50 mL of 1 N ammonium hydroxide was added to the residue. After the solution was stirred for 3 h at room temperature, the solvent was removed by evaporation. The residue was suspended in 50 mL of ethanol, mixed thoroughly filtered, and washed with ethanol. The precipitate was then dissolved in 20 mL of water and loaded on a DEAE-Sephadex (bicarbonate form) column (3 × 40 cm). The column was eluted with 500 mL of water and then with a gradient of 0.1–0.25 M ammonium bicarbonate (1 L in each flask). Compound **20** appeared toward the second half of the gradient and was collected and evaporated under reduced pressure. The product was then dissolved in 10 mL of water and freeze-dried for 48 h. The yield was 0.53 mmol (59%) as ammonium salt. The white powder of **20** decomposed before melting: ^1H NMR (D_2O) δ 2.1 (t, 2 H, PCH_2P), 3.2–4.5 (11 H, glucose and ribose protons), 5.4 (d of d, 1 H, $H_1\alpha$ of glucose), 5.95 (d, 1 H, $H_1\beta$ of ribose), 8.0 (1 H, aromatic), 8.3 (1 H, aromatic). Anal. ($C_{17}H_{33}N_7O_{14}P_2$) C, H, N, P.

Uridine 5′-[(α-D-Galactopyranosylhydroxyphosphinyl)methyl]phosphonate (23). One millimole of compound **13** was coupled with 0.9 mmol of 2′,3′-di-*O*-acetyluridine (**21**) by a similar procedure to the one that was described for compound **20** above. After DEAE-Sephadex column chromatography, the yield of compound **23** was 0.41 mmol (45.5%) as ammonium salt: ^1H NMR (D_2O) δ 2.1 (t, 2 H, PCH_2P), 3.5–4.4 (11 H, ribose and galactose protons), 5.5 (d of d, 1 H, $H_1\alpha$ of galactose), 5.8–5.9 (2 H, H_6 of uracil and H_1 of ribose), 6.9 (s, 1 H, H_3 of uracil), 7.85 (d, 1 H, H_5 of uracil). Anal. ($C_{16}H_{32}N_4O_{16}P_2 \cdot 2H_2O$) C, H, N, P.

Guanosine 5′-[(α-D-Mannopyranosylhydroxyphosphinyl)methyl]phosphonate (26). *N*-(2-Methyl-1-oxopropyl)-2′,3′-dibenzoylguanosine (**24**) was synthesized according to the procedures of Ohtsuka et al.[27]

Triethylammonium salt of 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl 1-(methylenediphosphonate) (**16**) (1 mmol) was dissolved in 10 mL of DMF, and 2 mmol of **24** was added. The solution was dried at 35 °C and at reduced pressure. This dry mixture was dissolved in 10 mL of dry DMF, 3 mmol of MSNT was added, and the reaction mixture was stirred at 45 °C for 36 h with the exclusion of moisture. DMF was removed by evaporation at reduced pressure, and the residue was dissolved in 20 mL of methanolic ammonia (previously saturated at 0 °C) in a pressure bottle and stirred at room temperature for 12 h. The solvent was removed by evaporation in vacuo. The residue was stirred in 20 mL of ethanol, filtered, and washed with 3 × 10 mL of ethanol. The precipitate was dissolved in water and filtered. The filtrate was purified on a DEAE-Sephadex column (3 × 30 cm) (bicarbonate form). The column was eluted with 500 mL of water and with a gradient of 0.1–0.25 M ammonium bicarbonate solution (1 L each). The title compound was eluted, pooled, and freeze-dried. The yield was 0.32 mmol (32%) of solid: ^1H NMR (D_2O) δ 2.06 (t, 2 H, PCH_2P, J_{PH} = 20 Hz), 3.3–4.4 (11 H, ribose and mannose), 5.35 (d, 1 H, anomeric proton of mannose), 5.75 (d, 1 H, anomeric proton of ribose), 7.9 (s, 1 H, aromatic). Anal. ($C_{17}H_{33}N_7O_{15}P_2$) C, H, N, P.

Galactosyltransferase Assay. Assays were performed in a total volume of 100 μL. The assay medium contained ovalbumin (Calbiochem, La Jolla, CA) (1 mg/mL), 0.1 M cacodylate buffer (Sigma Chemical Co., St. Louis, MO) (pH 7.0), 10 μM $MnCl_2$, and 2 mM AMP; inhibitor (20 or 50 μg/mL), 10 μL of varying concentrations of cold UDP-galactose and 1 μCi of UDP-[^{3}H]galactose ([4,5-^{3}H]galactose, 40 Ci/mmol) (Dupont-NEN, Boston, MA), and 10 μL of cell-free L1210 leukemia ascites fluid as the enzyme source.[28] Tumor ascites fluid was obtained from DBA/2J mice (Jackson Laboratories, Bar Harbor, ME), implanted 5–7 days

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previously with 10^5 L1210 tumor cells. Cells were removed by centrifugation. The enzyme assay was incubated for 30 min at 37 °C and the reaction stopped with 10% phosphotungstic acid (PTA) in 0.5 N HCl. The precipitate was washed with 10% trichloroacetic acid (TCA) and once with ethanol/ether, 2:1. The precipitate was dissolved in 200 μL of 1 N NaOH and neutralized with 1 N HCl, and the solution was counted in 10 mL of scintillation fluid (Liquiscint 2, National Diagnostics, Somerville, NJ) by using a β-scintillation counter (efficiency 33% for tritium). All other chemicals were purchased from either Fisher Scientific, or Sigma. All assays were performed in duplicate. The enzyme kinetic calculations were performed by using a software program by Dr. William Greco.[29] Data were graphed as δ vs. [S], v vs. [I], $1/v$ vs. $1/[S]$, and $1/v$ vs. [I] and the kinetic constants obtained from these plots.

Inhibition of Tumor Cell Growth in Vitro. Compounds were evaluated for their ability to inhibit growth of L1210 leukemia, human B-lymphoblastic leukemia (WI-L2), and human T-lymphoblastic leukemia (CEM) according to our previous procedure.[30]

In Vitro Antiviral Evaluations. Glycopyranosyl phosphonates and their nucleotide sugar analogues were evaluated for their ability to inhibit virus-induced cytopathic effect (CPE) produced by six viruses. Viruses used in this evaluation included parainfluenza virus type 3 (para 3) strain C243, herpes simplex virus type 2 (HSV-2) strain MS, adenovirus type 2, rhinovirus, influenza virus, and visna virus. Antiviral experiments were performed according to our previous procedures.[30]

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Supplementary Material Available: Analytical data for compounds **2**, **5**, **8–17**, **20**, **23**, and **26** (Table 15) and ^1H and ^{13}C NMR spectral data for glycopyranosyl methylenediphosphonates (Table 25) (3 pages). Ordering information is given on any current masthead page.

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Effects of Derivatives of Kahweol and Cafestol on the Activity of Glutathione S-Transferase in Mice

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The coffee constituents cafestol and kahweol are inducers of the activity of the detoxifying enzyme, glutathione S-transferase in laboratory animals. The two active functional groups, furan and glycol, on opposite ends of the diterpene nucleus of these two compounds have been modified. The resulting derivatives were evaluated for their ability to induce glutathione S-transferase activity in different tissues of the ICR/Ha mice. Derivatives of both cafestol and kahweol, which were the products of the modifications on the glycol function, retained much of the inducing properties of the parent compounds in the liver and mucosa of the small bowel. The effects of these compounds on the tissue acid-soluble sulfhydryl level, in general, were similar to those of the parent compounds. Some derivatives of kahweol, however, appeared to have lost their ability to induce increased levels of sulfhydryls in the liver. Catalytic hydrogenation of the furan moiety gave two products, the dihydro and tetrahydro derivatives. Both compounds and their corresponding acetates lost their effectiveness as inducers of glutathione S-transferase activity. In contrast, these compounds still retained their ability to induce increased levels of acid-soluble sulfhydryls in both the liver and the mucosa of the small bowel. These findings indicate that the furan moiety of cafestol and kahweol is vital to their biological activity as inducers of increased glutathione S-transferase activity.

Glutathione S-transferase (GST) is a major detoxifying enzyme system that catalyzes the binding of a variety of electrophiles, including the reactive forms of chemical carcinogens, to glutathione.[1,2] In most instances this conjugation results in rendering these substances to be less harmful and facilitates their excretion. Thus the enhancement of the activity of this enzyme system potentially could increase the capacity of the organism to withstand the neoplastic effects of chemical carcinogens. Since glutathione is a mandatory substrate in the conjugation of xenobiotics catalyzed by this enzyme system, the concentration of this compound in the tissues may be vital in the overall detoxifying process.

Green coffee beans fed in the diet were found to induce increased GST activity and tissue sulfhydryl (SH) level in the mucosa of the small intestine and in the liver of rats and mice. Using the induction of GST activity as an assay method, we followed the enzyme-inducing activity of the extracts of green coffee beans and isolated from the petroleum ether fraction two diterpene esters, kahweol palmitate and cafestol palmitate. These two natural products are potent inducers of the GST enzyme system and are good inducers of tissue SH level.[3]

The parent compounds of these two esters are similar diterpenes differing from each other by one double bond on the A ring of the diterpene nucleus.[4,5] Kahweol, IIa, which has the Δ^1 double bond, is a better inducer of the GST activity than cafestol. This difference in activity as a result of small change in the diterpene structure suggests the possibility of producing a more potent inducer by modifying the structure of the parent compound. Besides the double bond on kahweol (IIa) there are two functional groups on opposite ends of the diterpene nucleus of both cafestol (Ia) and kahweol (IIa). A furan ring is attached to the A ring at C3 and C4 while a glycol function is at C16 and C17. To determine the contribution of these functional groups to the inducing potential of increased GST activity, studies of structure activity relationships were

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