Synthesis of Glycopyranosylphosphonate Analogues of Certain Natural Nucleoside Diphosphate Sugars as Potential Inhibitors of Glycosyltransferases†

Morteza M. Vaghefi,*‡ Ralph J. Bernacki,§ N. Kent Dalley,∥ Bruce E. Wilson,∥ and Roland K. Robins‡

Cancer Research Center, Department of Chemistry, Brigham Young University, Provo, Utah 84602, Grace Cancer Drug Center, Department of Experimental Therapeutics, Roswell Park Memorial Institute, Buffalo, New York 14263, and Nucleic Acid Research Institute, Costa Mesa, California 92626. *Received March 25, 1987*

The synthesis of α-D-glucopyranosyl-, α-D-galactopyranosyl-, and α-D-mannopyranosylphosphonate is described. Condensation of tris(trimethylsilyl) phosphite with 2,3,4,6-tetrakis-*O*-(phenylmethyl)-1-*O*-acetyl-α-D-glucopyranose generated 2,3,4,6-tetrakis-*O*-(phenylmethyl)-α-D-glucopyranosylphosphonic acetic anhydride (**13**). The benzyl blocking groups were removed by catalytic hydrogenation, and the anhydride bond was cleaved by alkaline hydrolysis to obtain α-D-glucopyranosylphosphonate (**15**). α-D-Galactopyranosylphosphonate (**17**) and α-D-mannopyranosylphosphonate (**19**) were also similarly synthesized. The anomeric configuration of **15** was assigned by single-crystal X-ray analysis, and the structural assignments of **17** and **19** were made on the basis of comparative NMR spectral studies. Compound **15** was then coupled with adenosine 5′-phosphoric di-*n*-butylphosphinothioic anhydride in dry pyridine to give adenosine 5′-phosphoric α-D-glucopyranosylphosphonic anhydride (**23**). Similarly, uridine 5′-phosphoric α-D-galactopyranosylphosphonic anhydride (**24**) and guanosine 5′-phosphoric α-D-mannopyranosylphosphonic anhydride (**25**) were synthesized from **17** and **19**, respectively. With ovalbumin as an acceptor for [^{3}H]galactose, provided by UDP-[^{3}H]galactose, only uridine 5′-phosphoric α-D-galactopyranosylphosphonic anhydride (**24**) was shown to inhibit glycoprotein β-D-galactosyltransferase (EC 2.4.1.38), with an apparent K_i equal to 165 μM. Even though these ionic compounds hardly penetrate the cell membrane, preliminary in vitro antitumor screening shows that compounds **23** and **25** are slightly active against human B-lymphoblastic leukemia and human T-lymphoblastic leukemia. None of these compounds show any antiviral activity.

Transformation of normal cells to tumor cells is accompanied by an increase in the glycosylation of endogenous surface glycoproteins.[1-3] Such transformation modifies the membrane to allow the glycosyltransferase enzymes greater access to the outer cellular surface.[4]

Certain metastatic tumor cells can also be distinguished from their nonmetastatic counterparts on the basis of altered characteristics in the cell surface glycoconjugates. Metastasis is intimately associated with the biochemical properties of plasma membranes. Bosmann and coworkers[5] have shown that high levels of surface glycosyltransferases may contribute to cell movement within the circulation by adhering to normal circulating cell surfaces such as platelets and thus cause metastasis. An increase in glycosyltransferase in metastasizing tumors and serum has also been confirmed by several other studies.[3,6-10]

The importance of adhesion in metastasis is indicated by the fact that the more metastatic variants of B16 melanoma cells show increased sialyltransferase[11] and greater rates of adhesion to homotypic and heterotypic cell monolayers[12-14] or cell aggregates.[15] This suggests that the ability to consolidate adhesion is an important if not crucial early step in metastasis.

The involvement of cell surface glycosyltransferases in the process of cell adhesion in metastatic tumor cells has been postulated to be crucial.[16] Either these enzymes may be directly involved in adhesion of metastatic cells or their abundance at the surface of metastatic cells may produce a special class of glycoprotein or glucosaminoglycan, which acts as a receptor site.[17,18] However, inhibition of these enzymes has profound effects on the process of adhesion and prevention of metastasis. There is growing evidence in support of the concept that inhibitors of protein glycosylation possess certain antitumor properties.[19-25]

In the process of the biochemical synthesis of glycoproteins, both the ester linkage between sugar and phosphate and the anhydride linkage of the pyrophosphate are potential targets for enzymatic cleavage. Replacement of

† This work was supported in part by Research Grant 1-R01-CA-31783 and CA-13038 from the National Cancer Institute, USPHS.

‡ Nucleic Acid Research Institute, 3300 Hyland Aveneue, Costa Mesa, CA 92626.

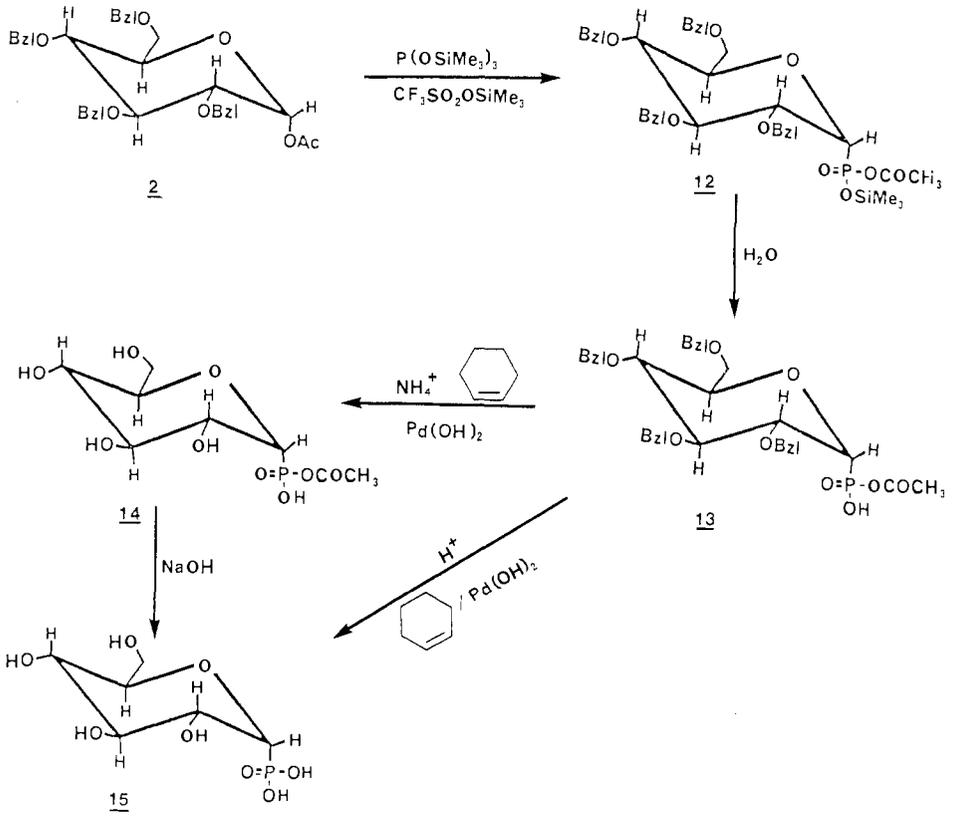
§ Roswell Park Memorial Institute.

∥ Brigham Young University.

(1) Bosmann, H. B.; Lockwood, T.; Morgan, H. R. *Exp. Cell Res.* **1974**, *83*, 15.
(2) Patt, L. M.; Grimes, W. J. *J. Biol. Chem.* **1974**, *249*, 4157.
(3) Den, H.; Schultz, A. M.; Basu, M.; Roseman, S. *J. Biol. Chem.* **1971**, *246*, 2721.
(4) Roth, S.; White, D. *Proc. Natl. Acad. Sci. U.S.A.* **1972**, *69*, 485.
(5) Bosmann, H. B.; Bieber, G. F.; Brown, A. G.; Case, K. R.; Gersten, C. M.; Kimmer, T. W.; Lione, A. *Nature (London)* **1973**, *246*, 487.
(6) Bernacki, R. J.; Kim, U. *Science (Washington, D.C.)* **1977**, *195*, 577.
(7) Charlson, A. J.; Reid, B. L. *J. Carbohydr. Nucleosides, Nucleotides* **1978**, *5*, 381 and 434.
(8) Roos, E.; Dingemans, K. P. *Biochim. Biophys. Acta* **1979**, *560*, 135.
(9) Bosmann, H. B. *Biochem. Biophys. Res. Commun.* **1972**, *48*, 523.
(10) Chatterjee, S. K. *Eur. J. Cancer* **1979**, *15*, 1351.
(11) Dobrossy, L.; Pavelic, Z. P.; Bernacki, R. J. *Cancer Res.* **1981**, *41*, 2262.
(12) Fidler, I. J.; Gersten, D. M.; Budmen, M. B. *Cancer Res.* **1976**, *36*, 3160.
(13) Fidler, I. J. *Nature (London), New Biol.* **1973**, *242*, 148.
(14) Winkelhake, J. L.; Nicolson, G. L. *J. Natl. Cancer Inst. (U.S.)* **1976**, *56*, 285.
(15) Raz, A.; Bucana, C.; McLellan, W.; Fidler, I. J. *Nature (London)* **1980**, *284*, 363.
(16) Weiser, M. W.; Podolsky, D. K. In *Cell Surface Carbohydrate Chemistry*; Harmon, R. E., Ed.; Academic: New York, 1978; pp 67–82.
(17) Rollins, B. J.; Cathcart, M. K.; Culp, L. A. In *The Glycoconjugates*; Horowitz, M. I., Ed.; Academic: New York, 1982; Vol. III, pp 289–329.
(18) Nicolson, G. L.; Irimura, T.; Nakajima, M.; Estrada, J. *Cancer Invasion and Metastasis: Biologic and Therapeutic Aspects*; Nicolson, G. L., Milar, L., Eds.; Raven: New York, 1984.
(19) Knowles, R. W.; Person, S. *J. Virol.* **1976**, *18*, 644.
(20) Buchsel, R.; Hassels-Vischer, B.; Tauber, R.; Reutter, W. *Eur. J. Biochem.* **1980**, *111*, 445.
(21) Schmidt, M. J. F.; Schwartz, R. T.; Ludwig, H. *J. Virol.* **1976**, *18*, 819.
(22) Bessell, E. M.; Courtenay, V. D.; Foster, A. B.; Jones, J.; Westwood, J. H. *Eur. J. Cancer* **1973**, *9*, 463.

0022-2623/87/1830-1383$01.50/0

Scheme I



the O–P or C–O linkage with a more stable C–P bond should provide nucleoside diphosphate sugar analogues that are more resistant to enzymatic degradation and therefore have greater potential to selectively inhibit glycoprotein synthesis. These nucleotides could act as substrate analogues for the various glycosyltransferases covalently bound to the outer surface of the plasma membrane. Recently, Kijima-Suda and co-workers[26] reported inhibition of lung metastasis and prolongation of survival in rat by a sialic acid–nucleoside conjugate. This compound, which is an inhibitor of sialyltransferase, was found to interfere with cell membrane glycosylation and to reduce the metastatic potential of the tumor cell.

In this and the following manuscripts, we report the synthesis and biological activity of certain analogues of naturally occurring nucleoside diphosphate sugars such as uridine diphosphate galactose (UDPGal), adenosine diphosphate glucose (ADPG), and guanosine diphosphate mannose (GDPMan). These analogues, which do not readily penetrate cellular membranes, can selectively inhibit glycosyltransferases at the surface of the tumor cellular plasma membrane.

Results and Discussion

The present work describes the synthesis of adenosine 5′-phosphoric α-D-glucopyranosylphosphonic anhydride (**23**), guanosine 5′-phosphoric α-D-mannopyranosylphosphonic anhydride (**25**), and uridine 5′-phosphoric α-D-galactopyranosylphosphonic anhydride (**24**). In order to prepare these nucleotide sugar analogues, the α-D-glycopyranosylphosphonate of each sugar was first prepared and then coupled with the corresponding nucleoside 5′-monophosphate.

Methyl 2,3,4,6-tetrakis-*O*-(phenylmethyl)-α-D-glucopyranoside[27] (**1**) was dissolved in acetic acid and treated with acetic anhydride and concentrated sulfuric acid to give 2,3,4,6-tetrakis-*O*-(phenylmethyl)-1-*O*-acetyl-α-D-glucopyranose (**2**) in over 85% yield. Similar treatment of methyl 2,3,4,6-tetrakis-*O*-(phenylmethyl)-α-D-mannopyranoside[28] (**5**) with acetic anhydride and sulfuric acid gave 2,3,4,6-tetrakis-*O*-(phenylmethyl)-1-*O*-acetyl-α-D-mannopyranose (**6**).

When the above procedure was employed for methyl 2,3,4,6-tetrakis-*O*-(phenylmethyl)-α-D-galactopyranoside (**3**), a mixture of α and β 2,3,4,6-tetrakis-*O*-(phenylmethyl)-1-*O*-acetyl-D-galactopyranose was obtained. The α-anomer of **4** was synthesized by acetylation of 2,3,4,6-tetrakis-*O*-(phenylmethyl)-α-D-galactopyranose according to the procedure of Austin et al.[29]

Tris(trimethylsilyl) phosphite (**7**)[30-32] was fused with 2,3,4,6-tetrakis-*O*-(phenylmethyl)-1-*O*-acetyl-α-D-glucopyranose (**2**) in the presence of trimethylsilyl trifluoromethanesulfonate (triflate catalyst) (Scheme I) to give trimethylsilyl[2,3,4,6-tetrakis-*O*-(phenylmethyl)-α-D-

(23) Schmidt, M. J. F.; Biely, P.; Krotka, Z.; Schartz, R. T. *Eur. J. Biochem.* **1978**, *87*, 55.
(24) Bernacki, R. J.; Korytnyk, W. In *Glycoconjugates*; Horowitz, M. I., Ed.; Academic: New York, 1982, Vol. IV, pp 245–263.
(25) Morin, M. J.; Bernacki, R. J. *Cancer Res.* **1983**, *43*, 1669.
(26) Kijima-Suda, I.; Miyamoto, Y.; Toyoshima, S.; Itoh, M.; Osawa, T. *Cancer Res.* **1986**, *46*, 858.
(27) Glaudemans, C. P. J.; Fletcher, H. G., Jr. *Methods in Carbohydrate Chemistry*; Whistler, R. L., BeMiller, J. N., Eds.; Academic: New York, 1972; Vol. 6, p 363.
(28) Koto, S.; Morishima, N.; Miyata, Y.; Zen, S. *Bull. Chem. Soc. Jpn.* **1976**, *49*, 2639.
(29) Austin, P. W.; Hardy, F. E.; Buchanan, J. G.; Baddiley, J. *J. Chem. Soc.* **1965**, 1419.
(30) Voronkov, M. S.; Skorik, Y. I. *Zh. Obshch. Khim.* **1965**, *35*, 106; *Chem. Abstr.* **1965**, *62*, 13175.
(31) Orlov, N. F.; Kaufman, B. L.; Sukhi, L.; Slesar, L. N.; Sudakova, E. V. *Khim. Prakt. Primen. Kremniiorg. Soedin., Tr. Soveshch., 1966* **1968**, 111; *Chem. Abstr.* **1970**, *72*, 21738.
(32) Sekine, M.; Okimoto, K.; Tamada, K.; Hata, T. *J. Org. Chem.* **1981**, *46*, 2097.

Scheme II

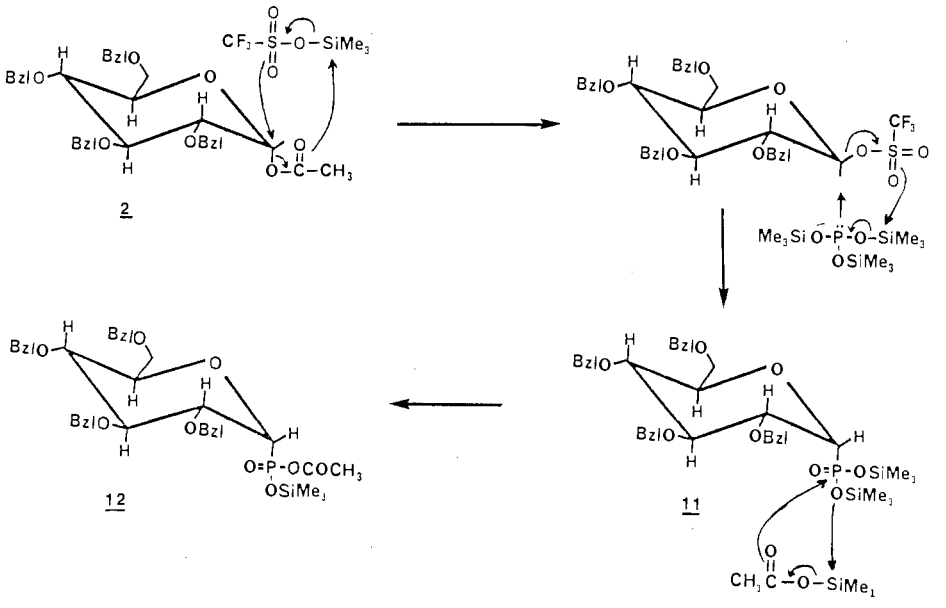


Table I.[a] Atom Coordinates ($\times 10^3$) and Temperature Factors ($\times 10^3$) for Atoms of α-D-Glucopyranosylphosphonate

atom	*x*	*y*	*z*	*U*
C1	6200 (4)	405 (3)	8186 (3)	27 (1)*[b]
C2	6024 (4)	-770 (3)	9042 (3)	32 (1)*
O2	4560 (4)	-624 (3)	9678 (2)	44 (1)*
HO(2)	4089 (4)	-1104 (32)	9538 (28)	22 (9)
C3	7475 (4)	-839 (3)	9873 (3)	31 (1)*
O3	7353 (4)	-2076 (3)	10550 (2)	41 (1)*
HO(3)	7483 (60)	-1962 (45)	11263 (39)	50 (12)
C4	9095 (4)	-818 (3)	9239 (3)	29 (1)*
O4	10374 (4)	-740 (3)	10072 (2)	45 (1)*
HO(4)	11006 (79)	-1518 (79)	9881 (48)	112 (21)
C5	9192 (4)	388 (3)	8396 (3)	29 (1)*
C6	10685 (5)	362 (4)	7640 (4)	43 (1)*
O6	10753 (4)	-817 (4)	6936 (2)	58 (1)*
HO(6)	10579 (225)	-500 (115)	6129 (46)	289 (45)
O	7787 (3)	407 (2)	7629 (2)	31 (1)*
P	5752 (1)	2073 (1)	8824 (1)	29 (1)*
OP(1)	6484 (4)	3062 (3)	7908 (3)	39 (1)*
HOP(1)	7241 (67)	3362 (50)	8023 (45)	38 (14)
OP(2)	3879 (3)	2251 (3)	8642 (3)	45 (1)*
HOP(2)	3208 (75)	2336 (48)	9241 (41)	60 (14)
OP(3)	6373 (3)	2233 (2)	10042 (2)	37 (1)*

[a] Esd values are in parentheses. Calculated positions for hydrogen atoms are omitted. [b] (*) Equivalent isotropic *U* defined as one-third of the trace of the orthogonalized U_{ij} tensor.

glucopyranosyl]phosphonic acetic anhydride (**12**) instead of the expected product bis(trimethylsilyl) glucopyranosylphosphonate (**11**). Aqueous hydrolysis of the silyl group yielded 2,3,4,6-tetrakis-*O*-(phenylmethyl)-α-D-glucopyranosylphosphonic acetic anhydride (**13**). Compound **13** contained four benzyl groups and one acetyl group according to the ^{1}H NMR spectrum. Scheme II shows a probable reaction mechanism involving a double S_N2 displacement, which results in the retention of configuration. Bis(trimethylsilyl) ether was detected as a byproduct in the generation of the anhydride bond.

Catalytic hydrogenation of the free acid of compound **13** for the removal of benzyl groups[33] resulted in the simultaneous hydrolysis of the acetic anhydride moiety and the formation of α-D-glucopyranosylphosphonate (**15**), whereas the catalytic hydrogenation of the ammonium salt of **13** gave α-D-glucopyranosylphosphonic acetic anhydride (**14**). The NMR spectrum of **14** showed the presence of an acetyl group, which was hydrolyzed with 2 N ammonium hydroxide to **15** (Scheme I).

Table II. Bond Lengths and Bond Angles of α-D-Glucopyranosylphosphonate (**15**)

1	2	3	length 1-2, Å	angle 1-2-3, deg
C2	C1	O	1.527 (2)	110.9 (3)
C2	C1	P	*a*	114.9 (2)
O	C1	P	1.441 (5)	110.4 (2)
C1	C2	O2	*a*	108.6 (3)
C1	C2	C3	*a*	111.6 (3)
O2	C2	C3	1.417 (5)	110.4 (3)
*H*O2	O2	C2	0.643 (34)	108.2 (29)
C2	C3	O3	1.505 (5)	108.5 (3)
C2	C3	C4	*a*	112.5 (3)
O3	C3	C4	1.455 (4)	109.0 (3)
*H*O3	O3	C3	0.824 (44)	113.2 (31)
C3	C4	O4	1.505 (5)	109.3 (3)
C3	C4	C5	*a*	110.7 (3)
O4	C4	C5	1.411 (5)	109.6 (3)
*H*O4	O4	C4	0.956 (74)	101.7 (36)
C4	C5	C6	1.538 (4)	112.8 (3)
O	C5	C4	1.440 (5)	110.2 (3)
C6	C5	O	1.490 (6)	107.6 (3)
C5	C6	O6	*a*	111.8 (3)
C6	O6	*H*O6	1.421 (6)	104.8 (67)
*H*O6	O6		0.979 (72)	
C1	O	C5	*a*	116.8 (2)
C1	P	*O*P1	1.848 (4)	103.3 (2)
C1	P	*O*P2	*a*	104.2 (2)
*O*P1	P	*O*P2	1.551 (3)	102.6 (2)
C1	P	*O*P3	*a*	113.3 (2)
*O*P3	P	*O*P1	1.481 (3)	115.2 (2)
*O*P2	P	*O*P3	1.553 (3)	116.7 (2)
*H*OP1	*O*P1	P	0.699 (52)	119.1 (42)
*H*OP2	*O*P2	P	0.877 (54)	121.5 (36)
O2	C2	C3	*a*	110.4 (3)

[a] Value listed earlier in the table.

A computer drawing of the results of the single-crystal X-ray analysis of compound **15** is shown in Figure 1. The phosphorus atom is in the axial position of the plane of the sugar, establishing the α-anomeric structure. The positional and thermal parameters (Table I) and the bond lengths and bond angles (Table II) are shown. The C–C and C–O bond length values are in the normal range. The C_1–P bond length is 1.848 (4) Å, which is similar to other C–P bonds found in organophosphorus compounds.[34] The

(33) Hanessian, S.; Liak, T. J.; Vanasse, B. *Synthesis* 1981, 396.

Table III.[a] Intermolecular Hydrogen Bond Data of α-D-Glucopyranosylphosphonate (15)

D	H	A	H···A, Å	D···A, Å	D–H···A, deg	translation of A
O2	HO2	O3	2.30 (3)	2.955 (5)	168 (4)	$-0.5 + x, -0.5 - y, 2 - z$
O3	HO3	O	2.20 (5)	2.889 (4)	141 (4)	$1.5 - x, -y, 0.5 + z$
O4	HO6	O3	1.85 (8)	2.799 (5)	175 (7)	$0.5 + x, -0.5 - y, 2 - z$
O6	HO4	O2	2.00 (8)	2.947 (5)	164 (6)[b]	$1.5 - x, -y, -0.5 + z$
OP1	HOP1	O6	1.83 (4)	2.523 (5)	171 (4)	$2 - x, 0.5 + y, 1.5 - z$
OP2	HOP2	OP3	1.76 (6)	2.585 (4)	156 (5)	$-0.5 + x, 0.5 - y, 2 - z$

[a] Esd values are in parentheses. [b] The esd value is estimated as the positional parameters of HO6 were not refined.

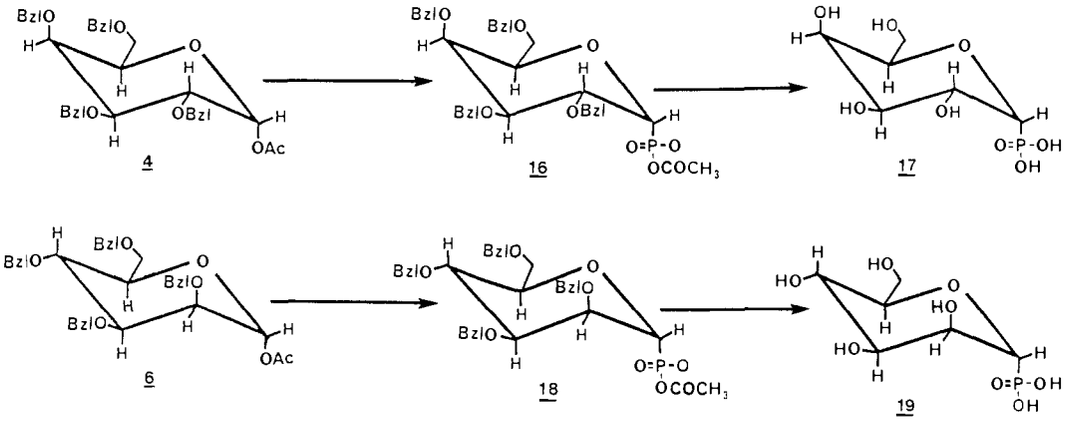
Table IV. Nuclear Magnetic Resonance (NMR) Data of Certain α-D-Glycopyranosylphosphonates

	^{1}H NMR											
no.	H_1[a]	J[b]	H_2	J	H_3	J	H_4	J	H_5	J	H_6	J
15	3.74 dd	10.2, 6.4	3.53 dd	9.2, 6.4	3.65 t	9.2	3.09 t	9.2	3.99 ddd	9.2, 6.1, 2.0	3.43–3.56 m	6.1, 2.0
17α	3.79 dd	9.8, 6.4	3.73 dd	9.1, 6.4	3.81 dd	9.1, 4.6	3.36 t	4.6	4.03 ddd	6.3, 4.6, 2.1	3.40–3.55 m	6.3, 2.1
19	3.50 d	15.2	3.24 t	9.6	3.52 dd	9.6, 6.8	2.99 t	6.8	3.80 br s		3.24–3.34 m	

	^{13}C NMR										^{31}P NMR (proton coupled)	
no.	C_1	J	C_2	J	C_3	J	C_4	J	C_5	J	C_6	P	J
15	73.19 d	148.1	70.07 s		73.34 s		69.45 s		76.66 **d**	1.74	60.80 s	15.51 dd	19.5, 11.0
17α	72.22 d	149.4	67.16 s		69.84 s		68.10 s		76.21 d	1.81	60.57 s	16.50 dd	21.0, 11.0
17β	75.65 d	161.9	67.58 s		74.15 **d**	16.3	69.23 s		80.42 **d**	14.5	61.67 s		
19	75.01 d	144.6	68.74 d	2.94	73.79 d	4.3	66.60 s		81.80 **d**	15.3	61.18 s	15.14 d	11.0

[a] Chemical shifts are in ppm. [b] Coupling constants are in hertz.

Scheme III



single-crystal X-ray of D-glucopyranosylphosphonate shows that the phosphonate moiety is not completely perpendicular to the plane of the pyranose ring and the bulkiness forces the P–C bond to a wider angle.

The α-D-glucopyranosyl ring is in the chair configuration. There are no intramolecular hydrogen bonds, but there is an extensive intermolecular hydrogen bonding network involving all of the hydrogen atoms in the molecule that are bonded to oxygen atoms. The hydrogen bond data is included in Table III. The anisotropic thermal parameters (Table 1S) and observed and calculated structure factors (Table 2S) are also included as supplementary material.

The NMR data for compound **15** are summarized in Table IV. The anomeric proton is shielded and is upfield at 3.99 ppm and appears as a doublet of doublets split by P and C_1H. The chemical shift in the ^{13}C NMR spectrum shows that C_1 is shielded and shifted upfield to 73.2 ppm and is coupled with phosphorus. Reports concerning the vicinal coupling of ^{13}C and ^{31}P indicate a Karplus-type variation[35] in which the coupling constant of *C–C–C–P* is related to the dihedral angle of the two atoms, which has

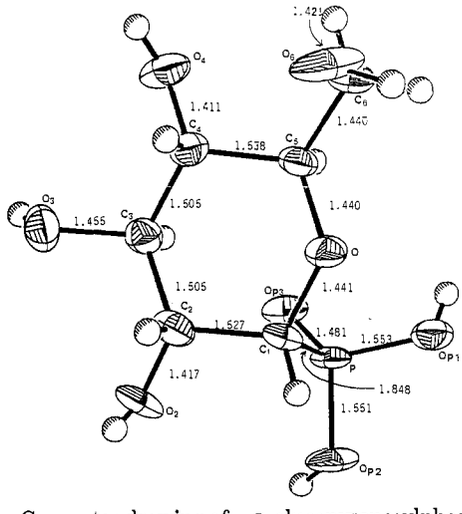


Figure 1. Computer drawing of α-D-glucopyranosylphosphonate (**15**) from single-crystal X-ray analysis with bond length (Å).

a maximum coupling constant at 0° and 180° and a minimum coupling constant at 60°. Coupling of P and C_3 of compound **15** was not resolvable due to the 60° angle, and the coupling constant of P and C_5 was 1.74 Hz, which agrees with the X-ray structure. The vicinal ^{31}P–C–C–^{1}H

(34) *International Tables for X-ray Crystallography*. Macgillary, C. H,, Rieck, G. D., Eds.; Kynoch: Birmingham, England, 1968; Vol. III.

(35) Buchanan, G. W.; Bowen, J. H. *Can. J. Chem.* **1977**, *55*, 604.

coupling constant also follows Karplus-type variation.[36] The coupling of ^{31}P–HC_2 (J = 19.5 Hz) agrees with the α-configuration.

When 2,3,4,6-tetrakis-*O*-(phenylmethyl)-1-*O*-acetyl-α-D-galactopyranose (**4**) was treated with **7**, by a procedure identical with the one described for 2,3,4,6-tetrakis-*O*-(phenylmethyl)-α-D-glucopyranosylphosphonic acetic anhydride (**13**), 2,3,4,6-tetrakis-*O*-(phenylmethyl)-α-D-galactopyranosylphosphonic acetic anhydride (**16**) was obtained (Scheme III). Catalytic hydrogenation of the free acid of **16** yielded the desired α-D-galactopyranosylphosphonate (**17**). A similar reaction with a 3:1 mixture of the α- and β-anomer of 4 gave a 3:1 mixture of the α- and β-anomer of **17**.[37]

The stereochemical structure and configuration of **17** are assigned from proton, carbon, and phosphorus NMR spectral data, which are summarized and tabulated (Table IV). The anomeric proton appeared at 4.03 ppm as a doublet of doublets similar to those of the glucose analogue. In addition, the proton-coupled ^{31}P NMR spectrum shows a doublet of doublets, split by C_1H (J = 11 Hz) and C_2H (J = 21.0 Hz), which are similar to those of the glucose analogue of **15**, which is in agreement with the α-conformation. The coupling constant between P and C_2H depends on the dihedral angle between the two atoms.[36] In compounds **15** and **17** the dihedral angle between P and C_2H is about 160–170°, which results in a relatively large coupling constant (J = 21.0 Hz). This suggests that the phosphorus on C_1 and the proton on C_2 are trans to each other and establishes the α-anomeric structure. The ^{13}C NMR spectrum of compound **17** shows a doublet at 72.22 ppm for C_1 with a coupling constant of 150.6 Hz, which is also characteristic of the α-anomeric configuration.[38] The ^{13}C NMR spectrum of the β-anomer of **17** (Table IV) shows a doublet at 75.01 ppm for C_1 with a coupling constant of 163.5 Hz. The vicinal coupling of P with C_3 and C_5 of compound **17** is similar to those of compound **15**. A relatively large coupling constant between C_3 and P (J = 16.3 Hz) as well as C_5 and P (J = 14.5 Hz) was observed in the β-anomer of **17**, which is due to the 180° dihedral angle.

In the next part, 2,3,4,6-tetrakis-*O*-(phenylmethyl)-1-*O*-acetyl-α-D-mannopyranose (**6**) was treated with compound **7** in the same manner as described for **13** to provide 2,3,4,6-tetrakis-*O*-(phenylmethyl)-α-D-mannopyranosylphosphonic acetic anhydride (**18**). Catalytic hydrogenation of **18** followed by ammonium hydroxide treatment of the product yielded α-D-mannopyranosylphosphonate (**19**) in 62% yield (Scheme III).

The ^{13}C NMR spectrum of **19** shows the presence of one anomer. The signal for C_6 appears at 61.18 ppm, and the C_1 signal displays a doublet at 75.01 ppm as it is coupled with phosphorus with a coupling constant of 144.6 Hz, which is smaller than the 149.9 Hz reported for the β-D-mannopyranosylphosphonate.[36] The ^{31}P NMR spectrum shows a signal at 15.14 ppm, which, when it is coupled with C_1H, shows only a doublet with a coupling constant of 11.0 Hz. The phosphorus does not show any resolvable coupling with C_2H because of the orientation of *P* and *H*, which are axial and equatorial, respectively, and the vicinal angle between P and C_2H is about 60°, with P and C_2H being cis to each other. Since the proton at C_2 is equatorial, the phosphorus at C_1 must be axial, which proves the α-configuration. The geminal coupling of P and C_2 (J = 2.94 Hz) and the vicinal coupling of P and C_3 (J = 4.3 Hz) are in agreement with reported values.[38]

Meuwly and Vasella[38] have reported the formation of the 1,2-cis configuration of phosphonates as major products of the reaction of compounds **2** and **6** with triethyl phosphite. In our study, when compounds **2**, **4**, and **6** were treated with tris(trimethylsilyl) phosphite, retention of configuration was observed in all cases. In another study, a mixture of the α- and β-anomer of **4** with tris(trimethylsilyl) phosphite gave a mixture of the α- and β-anomer of **17** in the same ratio as that of the starting material. These observations support the double S_N2 displacement mechanism as described above.

Adenosine 5′-monophosphate was coupled with di-*n*-butylphosphinothioyl bromide according to the procedure of Furusawa and co-workers[39] to give adenosine 5′-phosphoric di-*n*-butylphosphinothioic anhydride (**20**), which was then treated with α-D-glucopyranosylphosphonate (**15**) in dry pyridine and in the presence of silver nitrate to give adenosine 5′-phosphoric α-D-glucopyranosylphosphonic anhydride (**23**) in 51% yield (Scheme IV).

Uridine 5′-phosphate was similarly coupled with α-D-galactopyranosylphosphonate (**17**) to give uridine 5′-phosphoric α-D-galactopyranosylphosphonic anhydride (**24**) in 53% yield (Scheme IV).

Guanosine 5′-phosphate was similarly coupled with α-D-mannopyranosylphosphonate (**19**) to give guanosine 5′-phosphoric-α-D-mannopyranosylphosphonic anhydride (**25**) in 42% yield.

Table V.[a] Kinetic Constants for Galactosyltransferase

V_{max}	74.0 pmol/0.5 h
K_m (UDP-galactose)	13.7 ± 2.5 μM
K_i (**24**)	165 ± 58 μM
K_i (UTP)	128 ± 50 μM

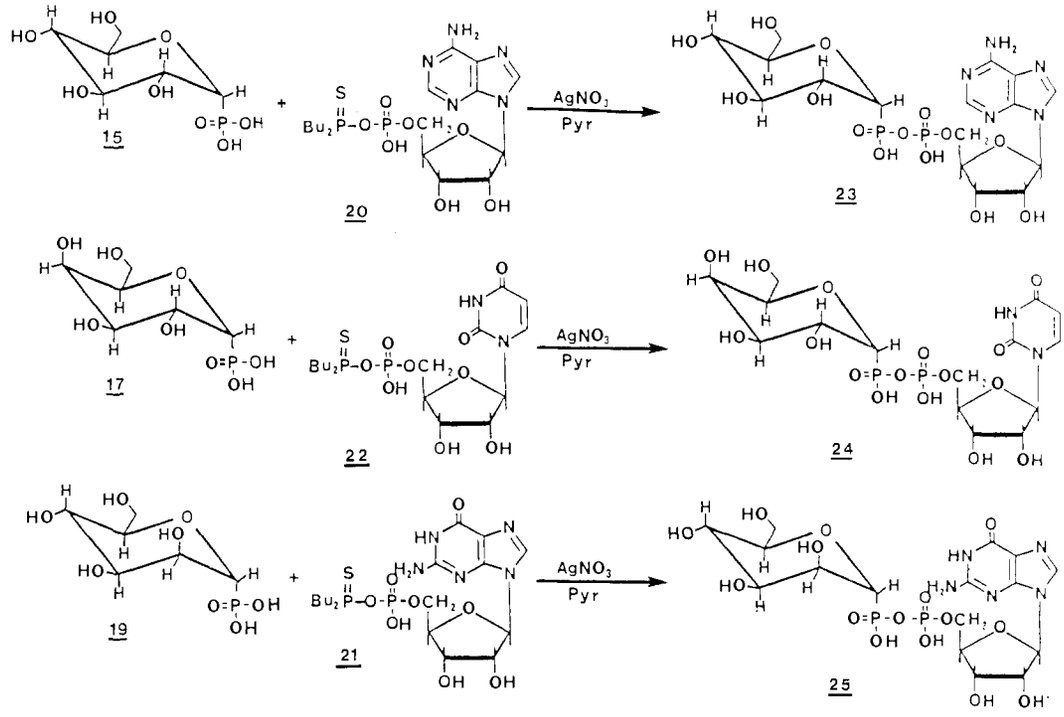
[a] Assays were performed as described in the Experimental Section. All assays were performed in duplicate. (Data are expressed as the mean ± SD.) Enzyme kinetic parameters were calculated by using a Rosfit software program (Greco et al., 1982). Data were graphed as v vs. [S], v vs. [I], $1/v$ vs. $1/[S]$, and $1/v$ vs. [I]. Inhibition by compound **24** and UTP best fit the model for competitive inhibition.

Specific Glycosyltransferase Inhibition Studies

Compounds **23–25** were evaluated as inhibitors of galactosyltransferase activity secreted by L1210 leukemia cells grown in mouse peritoneal ascites. With inhibitor concentrations of 20 and 50 μg/mL the nucleotide sugar analogue exhibiting the greatest inhibitory activity of galactosyltransferase activity was the UDP-galactose analogue, uridine 5′-phosphoric α-D-galactopyranosylphosphonic anhydride. At 20 μg/mL (35 μM) enzyme activity was decreased by 50% while a concentratin of 50 μg/mL (89 μM) resulted in 58% inhibition. Varying UDP-galactose concentrations from 1 to 80 μM, while maintaining inhibitor concentrations at either 35 or 89 μM **24**, we obtained a series of enzyme kinetic curves, including a double reciprocal plot (Figure 2), which resulted in an estimation of various enzyme kinetic constants for galactosyltransferase (Table V). The apparent K_m for UDP-galactose was 13.7 μM with a V_{max} of 74.0 pmol/0.5 h. The apparent K_i of **24** was 165 μM and was similar to the (128 μM) apparent K_i for the known competitive inhibitor UTP. The type of enzyme inhibition caused by **24** (Figure 2A) was competitive in nature and weak in relation to the apparent K_m for UDP-galactose.

(36) Neeser, J. R.; Tronchet, J. M. J.; Charollias, E. J. *Can. J. Chem.* **1983**, *61*, 2112.
(37) Unpublished results.
(38) Meuwly, R.; Vasella, A. *Helv. Chim. Acta* **1986**, *69*, 25.
(39) Furusawa, K.; Sekine, M.; Hata, T. *J. Chem. Soc., Perkin Trans. 1* **1976**, 1711.

Scheme IV



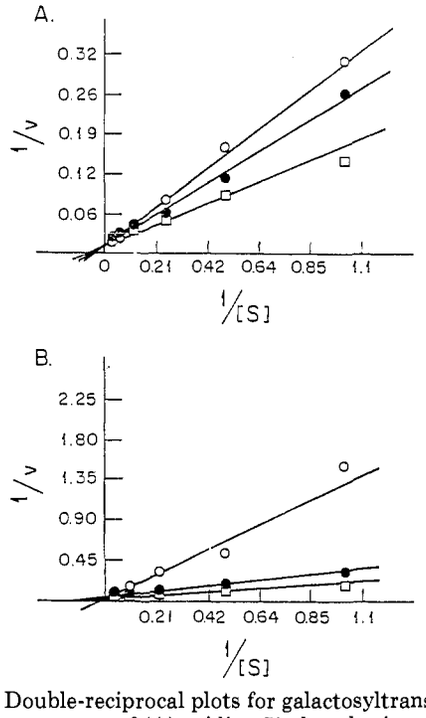


Figure 2. Double-reciprocal plots for galactosyltransferase activity in the presence of (A) uridine 5′-phosphoric α-D-galactopyranosylphosphonic anhydride (**24**) (□, 0 μM; ●, 35 μM; 0, 89 μM) and (B) UTP (□, 0 mM; ●, 0.1 mM; ○, 1.0 mM). Enzyme activity was measured as described in the Experimental Section. UDP-galactose concentrations were varied from 1 to 80 μM.

Tunicamycin, a naturally occurring antibiotic analogue of uridine 5′-diphosphate *N*-acetylglucosamine, is a very potent inhibitor of N-linked glycoprotein biosynthesis.[40] The transfer of *N*-acetylglucosamine phosphate from UDP-*N*-acetylglucosamine to lipid is blocked at nM concentration of tunicamycin while hydrolysis of tunicamycin, generating tunicaminyl uracil, results in a far less active inhibitor (Morin and Bernacki, unpublished results). The inhibitory activity for **24** is similar in magnitude (μM level) to tunicaminyl uracil.

The inhibition of galactosyltransferase activity caused by **24** also was similar in magnitude to the inhibition of sialyltransferase induced by *trans*-*N*-(acetylcyclohexyl)-5′-cytidylic acid hydrochloride, a synthetic CMP-sialic acid analogue.[41,42] At 100 μM *trans*-*N*-(acetylcyclohexyl)-5′-cytidylic acid hydrochloride a 25% decrease in sialyltransferase activity was noted. Additionally, 5′-fluoro-CMP and CMP were found to be competitive inhibitors of sialyltransferase, with apparent K_i's of 70 μM and 50 μM, respectively. The degree of galactosyltransferase inhibition caused by tunicaminyl uracil, *trans*-*N*-(acetylcyclohexyl)-5′-cytidylic acid hydrochloride, 5′-fluoro-CMP, and CMP is similar in magnitude to that noted for uridine 5′-phosphoric-α-D-galactopyranosylphosphonic anhydride (**24**).

Recently, Osawa and co-workers[43] reported the inhibition of sialyltransferase by 5-fluoro-2′,3′-*O*-isopropylidene-5′-*O*-(4-*N*-acetyl-2,4-dideoxy-3,6,7,8-tetra-*O*-acetyl-1-(methoxycarbonyl)-D-glycero-α-D-galacto-pyranosyl)uridine, a uridine–sialic acid complex. The inhibition of sialyltransferase by this compound has resulted in a significant prevention of lung metastasis in the mouse.[26]

The nucleoside 5′-phosphoric hexopyranosylphosphonic anhydrides were synthesized as potential inhibitors of specific glycosyltransferases. Uridine 5′-phosphoric α-D-galactopyranosylphosphoric anhydride has demonstrated specific, competitive inhibition of a glycoprotein glycosyltransferase. The inhibition of metastasis with these analogues has not been tested, but the abundance of sialyltransferase and galactosyltransferase at the surface of metastatic cells suggests that a potent inhibitor of these enzymes could efficiently prevent metastasis without

(40) Lehle, L.; Tanner, W. *FEBS Lett.* **1976**, *71*, 167.

(41) Klohs, W. D.; Bernacki, R. J.; Korytnyk, W. *Cancer Res.* **1979**, *39*, 1231.

(42) Korytnyk, W.; Angelino, N.; Klohs, W.; Bernacki, R. J. *Eur. J. Med. Chem.* **1980**, *15*, 77.

(43) Kijimi-Suda, I.; Toyoshima, S.; Itoh, M.; Furuhata, K.; Ogura, H.; Osawa, T. *Chem. Pharm. Bull.* **1985**, *33*, 730.

penetrating the cell membrane.

Antitumor Evaluations

α-D-Glucopyranosylphosphonate (**15**), α-D-galactopyranosylphosphonate (**17**), and α-D-mannopyranosylphosphonate (**19**) as well as their corresponding nucleotide sugar analogues **23**, **24**, and **25** were respectively evaluated for antitumor activity in vitro against L1210 leukemia, human B-lymphoblastic leukemia (WI-L2), and human T-lymphoblastic leukemia (CEM) cells. Compounds **23** and **25** showed slight cytotoxicity against WI-L2 and CEM but were inactive against L1210. Compounds **15**, **17**, **19**, and **24** showed no cytotoxic activity against any of those cell lines. The low cytotoxicity of these compounds is likely due partially to their ionic strength, which prevents the molecules from penetrating the cell membrane. Further in vivo studies are necessary to evaluate the antimetastatic activity of these compounds.

Antiviral Evaluations

The in vitro antiviral activity of α-D-glucopyranosylphosphonate (**15**), α-D-galactopyranosylphosphonate (**17**), and α-D-mannopyranosylphosphonate (**19**) and their corresponding nucleotide sugar analogues **23**, **24**, and **25** was 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 **15** showed slight activity against rhinovirus.

All compounds tested were nontoxic to confluent Vero cells at concentrations as high as 5000 μM, as assessed by microscopic observation.

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 recorded on an IBM NR300 FTNMR spectrometer (^1H (300 MHz), ^{13}C (75 MHz), ^{31}P (121 MHz)) or on a JEOL FX 90Q spectrometer (^1H (90 MHz), ^{13}C (22.5 MHz), ^{31}P (36 MHz)). The chemical shift values are expressed in δ values (parts per million). Compounds were normally dissolved in D_2O and referenced with HDO (for ^1H NMR), with external 85% phosphoric acid (for ^{31}P NMR), and with external dioxane (for ^{13}C NMR) unless otherwise mentioned. ^{31}P NMR were recorded at pH 12. 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 (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); and (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 temperture of 30–35 °C.

Methyl-α-D-glucopyranoside, methyl-α-D-galactopyranoside, and methyl-α-D-mannopyranoside were purchased from Sigma Chemical Company, St. Louis, MO. Adenosine 5′-monophosphate, guanosine 5′-monophosphate, and uridine 5′-monophosphate were obtained from Sigma.

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

Synthetic Procedures. 2,3,4,6-Tetrakis-*O*-(phenylmethyl)-1-*O*-acetyl-α-D-glucopyranose (2). Methyl 2,3,4,6-tetrakis-*O*-(phenylmethyl)-α-D-glucopyranoside (**1**) was synthesized according to the procedure of Glaudemans and Fletcher.[27]

The title compound, which was reported by Lemieux and coworkers,[44] was synthesized by an even more convenient procedure, as follows. The syrup of **1** (135 g, 0.28 mol) was dissolved in 450 mL of glacial acetic acid, and 100 mL of acetic anhydride was added. The mixture was cooled to 0 °C, and 20 mL of concentrated sulfuric acid was added dropwise during a half-hour with vigorous stirring. The reaction was allowed to continue at room temperature until all of the compound **1** had disappeared (about 2 h). The TLC of the mixture at this point showed one major spot (R_f 0.37) and a trace of a minor spot (R_f 0.63), which did not char upon treatment with H_2SO_4 spray. The reaction mixture was poured with vigorous mixing into 500 g of ice and extracted with 3 × 300 mL of chloroform. The combined chloroform solution was then washed with 3 × 300 mL of cold water and 2 × 200 mL of saturated sodium bicarbonate followed by 100 mL of water. The chloroform solution was dried with anhydrous sodium sulfate, and the chloroform was removed at reduced pressure. The resulting syrup was over 90% pure and was used without further purification. The yield was 88%. For analytical purposes a sample of 2 g was purified on a flash column of silica gel (230–400 mesh) (3 × 50 cm) and was eluted first with 1000 mL of cyclohexane and then with 1000 mL of cyclohexane/acetone (9:1). Compound **2** was obtained as a viscous syrup by evaporation of the solvent of the second fraction: ^1H NMR ($CDCl_3$, Me_4Si as reference) δ 2.10 (s, 3 H, CH_3), 3.5–5.1 (14 H, sugar and CH_2 of benzyl), 6.35 (d, 1 H, $H_1\alpha$), 7.3 (20 H, aromatic). Anal. ($C_{36}H_{38}O_{16}$) C, H.

2,3,4,6-Tetrakis-*O*-(phenylmethyl)-1-*O*-acetyl-α-D-mannopyranose (6).[36] Methyl 2,3,4,6-tetrakis-*O*-(phenylmethyl)-α-D-mannopyranoside (**5**) was synthesized according to the procedures of Koto and co-workers.[28] Compound **5** (134 g, 0.28 mol) was acetylated at C_1 by the same proceudre as described for the glucose analogue **2** above. The yield of compound **6** was 91%: ^1H NMR ($CDCl_3$) δ 2.02 (s, 3 H, CH_3), 3.7–5.0 (14 H, sugar and CH_2 of benzyl), 6.17 (1 H, H_1), 7.3 (20 H, aromatic). Anal. ($C_{36}H_{38}O_{16}$) C, H.

α-D-Glucopyranosylphosphonic Acetic Anhydride (14). In a 50-mL flask, 2,3,4,6-tetrakis-*O*-(phenylmethyl)-1-*O*-acetyl-α-D-glucopyranose (**2**) (10 g, 19.3 mmol) was mixed with tris(trimethylsilyl) phosphite (**7**) (15 g, 50 mmol) and heated to 80 °C. The reaction was catalyzed by 0.5 mL of trimethylsilyl triflate and stirred at 80 °C for 1/2 h. After removal of the excess **7** at 0.05 Torr, the clear solution was cooled to room temperature, and 30 mL of 20% ammonium bicarbonate solution was added. The mixture was shaken with 50 mL of ether, which resulted in a three-phase mixture. At the bottom of the three phases, 2,3,4,6-tetrakis-*O*-(phenylmethyl)-α-D-glucopyranosylphosphonic acetic anhydride (**13**) appeared as a yellow syrup, which was separated and dried under reduced pressure: ^1H NMR ($CDCl_3$) δ 1.8 (3 H), 3.3–4.9 (15 H, CH_2 of benzyl and sugar), 7.16 (20 H, aromatic). The dry compound was dissolved in 120 mL of ethanol, and 80 mL of cyclohexane was added. After the addition of 3 g of palladium hydroxide on carbon (20%), the reaction mixture was refluxed overnight during which the oil bath temperature did not exceed 100 °C (fresh cyclohexene was added if necessary). The reaction mixture was then cooled to room temperature. The precipitate along with the catalyst was filtered and washed with ethanol. The precipitate was heated with 60 mL of water on a steam bath, filtered to remove catalyst, and washed with water. The filtrate was concentrated at reduced pressure. α-D-Glucopyranosylphosphonic acetic anhydride (**14**) was crystallized from ethanol/water (2:1): ^1H NMR (D_2O) δ 1.95 (3 H, CH_3), 3.1–4.2 (7 H); ^{13}C NMR (Me_2SO-d_6) δ 20.5 (s, CH_3), 63.3 (s, C_6), 69.8 (s), 70.8 (s), 73.5 (d, C_1, J_{CP} = 145 Hz), 73.6 (s), 73.9 (s), 174.3 (s, C=O). Anal. ($C_8H_{18}NO_9P$) C, H, N. P.

α-D-Glucopyranosylphosphonate (15). The reaction procedure for the synthesis of **13** above was repeated, and after the removal of excess tris(trimethylsilyl) phosphite, 30 mL of water was added and the mixture was shaken vigorously. The water

(44) Lemieux, R. U.; Hendriks, K. B.; Stick, R. V.; James, K. *J. Am. Chem. Soc.* **1975**, *97*, 4056.

was removed, the wet residue was dissolved in 40 mL of 95% ethanol, 60 mL of cyclohexene was added, and the reaction mixture was refluxed in the presence of 1.5 g of palladium hydroxide on carbon overnight. The boiling reaction mixture was filtered, and compound **15** crystallized from the filtrate upon cooling. Compound **15** was redissolved in 5 mL of water and recrystallized by the addition of 80 mL of hot ethanol. The yield of compound **15** was 3.95 g (78%). Hydrolysis of compound **14** with 2 N ammonium hydroxide also resulted in the synthesis of compound **15**. For analytical purposes 0.5 g of the compound was further purified on a DEAE-Sephadex column (3 × 40 cm), which was eluted with a gradient of 0–0.4 M ammonium bicarbonate (1 L each). The α-D-glucopyranosylphosphonate fraction was evaporated to dryness at reduced pressure, redissolved in 10 mL of water, and freeze-dried for 48 h. The residue was dissolved in 5 mL of water and converted to free acid with Dowex 50 H^+ resin: ^1H NMR of free acid (D_2O, 300 MHz) δ 3.99 (dd, J = 10.2, 6.4 Hz, $H_1\alpha$), 3.53 (dd, J = 9.2, 6.4 Hz, H_2), 3.65 (t, J = 9.2 Hz, H_3), 3.09 (t, J = 9.2 Hz, H_4), 3.99 (ddd, J = 9.2, 6.1, 2.0 Hz, H_5), 3.43–3.65 (m, J = 6.1, 2.0 Hz, H_6); ^{31}P NMR (D_2O, pH 12, 120 MHz) (proton decoupled) δ 15.51 (s); proton-coupled ^{31}P NMR δ 15.53-(dd, J_{PH2} = 19.53 Hz, J_{PH1} = 11.0 Hz); ^{13}C NMR of free acid (proton decoupled) (D_2O, 75 MHz) δ 60.80 (s, C_6), 69.45 (s, C_4), 70.07 (s, C_2), 73.19 (d, C_1, J_{C1P} = 148.1 Hz), 73.35 (s, C_3), 76.66 (s, C_5). Anal. ($C_6H_{13}O_8P$) C, H, P.

X-ray Crystallography of α-D-Glucopyranosylphosphonate. The free acid of compound **15** above was made by passing 2 g of the compound in 20 mL of water through a Dowex 50 H^+ column. The solution was concentrated to about 3 mL, 15 mL of hot absolute ethanol was added, and a clear solution was achieved by heating. Clear crystals formed upon storing at room temperature for 48 h.

A suitable crystal of approximately equal dimensions (0.35 × 0.35 × 0.40 mm) was mounted on a Nicolet automated R3 diffractometer using graphite monochromated Cu radiation. Crystal and experimental data are summarized in Table II. The lattice parameters were obtained by using a least-squares technique of 24 centered reflections. $12° < 2\theta < 26°$. A total of 794 reflections were measured by using a θ–2θ scan procedure to a $\sin\theta/\lambda$ limit 0.55. These data were merged to 754 unique reflections, 11 being considered unobserved as $I < 2\sigma$ (I). The structure was solved by using direct methods and refined to R = 0.037 and R_w = 0.055 by using a blocked cascading least-squares procedure. The weights were based on counting statistics with $w = \sigma^2(F) + 0.002F^2$. All non-hydrogen atoms were refined anisotropically. Positions for hydrogen atoms bonded to oxygen atoms were obtained from difference maps, and these atoms were refined isotropically. Positions for the hydrogen atoms bonded to carbon atoms were calculated on the basis of geometrical conditions with the C–H distance of 0.96 Å. Each of these atoms was allowed to ride on the neighboring carbon atom and the temperature factor of the atom fixed at 1.2 times the U_{eq} of the adjacent atom. Atomic scattering factors were obtained from the *International Tables for X-ray Crystallography*.[45] The programs used in the structure solution are contained in the SHELXTL program package.[46]

α-D-Galactopyranosylphosphonate (17). Compound **17** was synthesized by treatment of 2,3,4,6-tetrakis-*O*-(phenylmethyl)-1-*O*-acetyl-α-D-galactopyranose (**4**)[29] (10 g, 19.3 mmol) with tris(trimethylsilyl) phosphite (**7**) as described for the synthesis of α-D-glucopyranosylphosphonate (**15**). The yield of the free acid of compound **17** was 3.5 g (70%). Final purification was performed on a DEAE-Sephadex column as described for compound **15**: ^1H NMR of free acid (D_2O, 300 MHz) δ 3.79 (dd, 1 H, J = 9.8, 6.4 Hz, $C_1\alpha H$), 3.73 (dd, J = 9.1, 6.4 Hz, H_2), 3.81 (dd, J = 9.1, 4.6 Hz, H_3), 3.36 (t, J = 4.6 Hz, H_4), 4.03 (m, J = 6.3, 4.6, 2.1 Hz, H_5), 3.40–3.55 (m, J = 6.3, 2.1 Hz, H_6); ^{31}P NMR (D_2O, pH 12, 120 MHz) (proton decoupled) δ 16.67 (s); proton-coupled ^{31}P NMR δ 16.5 (dd, J_{PH2} = 21 Hz, J_{PH1} = 11 Hz); ^{13}C NMR of free acid (D_2O, 75 MHz) δ 60.57 (s, C_6), 67.16 (s, C_4), 68.10 (s, C_2), 69.84 (s, C_3), 72.22 (d, C_1, J_{C1P} = 149.4 Hz), 76.22 (s, C_5). Anal. ($C_6H_{13}O_8P$) C, H, P.

α-D-Mannopyranosylphosphonate (19). Compound **19** was synthesized by treatment of 2,3,4,6-tetrakis-*O*-(phenylmethyl)-1-*O*-acetyl-α-D-mannopyranose (**6**) with tris(trimethylsilyl) phosphite (**7**) as described for the synthesis of α-D-glucopyranosylphosphonate (**15**). The yield of compound **19** was 3.2 g (64%). Final purification for analytical purposes was performed by DEAE-Sephadex column chromatography: ^1H NMR of free acid (D_2O, 300 MHz) δ 3.50 (d, J = 15.2 Hz, H_1), 3.24 (dd, J = 9.6 Hz, H_2), 3.52 (dd, J = 9.6, 6.8 Hz, H_3), 2.99 (t, J = 6.8 Hz, H_4), 3.80 (br s, H_5), 3.24–3.34 (m, H_6); ^{31}P NMR (D_2O, pH 12, 120 MHz) (proton decoupled) δ 15.05; ^{31}P NMR (proton coupled) δ 15.05 (d, J_{PH1} = 11 Hz); ^{13}C NMR of free acid (D_2O, 75 MHz) δ 61.18 (s, C_6), 66.6 (C_4), 68.75 (d, J_{PC} = 2.9 Hz), 73.79 (d, J_{PC} = 4.3 Hz), 81.8 (d, J_{PC} = 15.4 Hz), 75.01 (d, C_1, J_{C1P} = 144.6 Hz). Anal. ($C_6H_{13}O_8P$) C, H, P.

Adenosine 5′-Phosphoric α-D-Glucopyranosylphosphonic Anhydride (23). Adenosine 5′-phosphoric di-*n*-butylphosphinothioic anhydride (**20**) was synthesized according to the procedures of Furusawa and co-workers.[39] Tri-*n*-butylammonium salt of α-D-glucopyranosylphosphonate (**15**) (1 mmol) was dissolved in 0.5 mL of formamide, and **20** (2 mmol) was added. Dry pyridine was added and evaporated under reduced pressure. This process was repeated four times for complete removal of moisture. To the residue were added 10 mL of dry pyridine and then silver nitrate (8 mmol), and the reaction mixture was stirred at room temperature for 36 h. To the reaction mixture was added 20 mL of water, and the silver was precipitated by bubbling in hydrogen sulfide and filtered. The solvent was then removed by evaporation under reduced pressure. Ammonium hydroxide (20 mL, 1 N) was added to the residue and evaporated. The residue was dissolved in 30 mL of water and purified on a Dowex 2 (×8) (200–400 mesh) column (3 × 50 cm) (Cl^- form) and eluted successively with 500 mL of water, 0.01 M LiCl in 0.003 M HCl (2 L), and 0.06 M LiCl in 0.003 M HCl (2 L). The last elution contained compound **23**, which was neutralized with 1 N HCl and evaporated to about 5 mL. To the residue was added 100 mL of absolute ethanol, and the precipitate was separated by centrifugation and washed twice with ethanol. The yield was 295 mg (51%).

In order to obtain an analytically pure sample as the ammonium salt, compound **23** was further purified on a DEAE-Sephadex (bicarbonate form) column (2.5 × 30 cm), which was eluted with 200 mL of water and then a gradient of 0.1–0.3 M ammonium bicarbonate (1 L each). The compound was then freeze-dried to a constant weight: ^1H NMR (D_2O) δ 3.09 (t, H_4 of glucose), 3.5–4.5 (11 H, glucose and ribose), 5.92 (d, 1 H, H_1 of ribose, J_{H1H2} = 5.2 Hz), 8.0 (s, 1 H, aromatic), 8.3 (s, 1 H, Aromatic); ^{31}P NMR (34 MHz, pH 12) (proton decoupled) δ 6.24 (d, 1 P, P of AMP, J_{P1P2} = 28 Hz), 16.12 (d, P of phosphonate); proton-coupled ^{31}P NMR δ 6.24 (t of d, $J_{PH5'}$ = 4.89 Hz), 16.12 (ddd, J_{PH2} = 14.6 Hz, J_{PH1} = 10.99 Hz). Anal. ($C_{16}H_{31}N_7O_{15}P_2 \cdot 1.5H_2O$) C, H, N, P.

Guanosine 5′-Phosphoric α-D-Mannopyranosylphosphonic Anhydride (25). Guanosine 5′-phosphoric di-*n*-butylphosphinothioic anhydride (**21**) was synthesized by the procedures of Furusawa and co-workers.[39] Tri-*n*-butylammonium salt of α-D-mannopyranosylphosphonate (**19**) (1 mmol) was dissolved in 1.5 mL of formamide, and **21** (2 mmol) was added. The mixture was rendered dry and treated with silver nitrate, and the reaction mixture was then worked up by the same procedure as described for **23**. The elution from a Dowex 2 (×8) column, however, required 0.03 M LiCl in 0.003 M HCl (2 L) and 0.1 M LiCl in 0.003 M HCl (2 L). The yield was 42%: ^1H NMR (D_2O) δ 3.2 (1 H, H_4 of mannose), 3.5–4.5 (11 H, mannose and ribose), 5.82 (d, 1 H, H_1 of ribose), 7.98 (1 H, aromatic); ^{31}P NMR (34 MHz, pH 12) (proton decoupled) δ −8.9 (d, P of GMP, J_{P1P2} = 28 Hz), 7.75 (d, P of phosphonate); ^{31}P NMR (proton coupled) δ −8.9 (br d), 7.75 (dd, J_{PH1} = 15.87 Hz). Anal. ($C_{16}H_{31}N_7O_{15}P_2 \cdot 2.5H_2O$) C, H, N, P.

Uridine 5′-Phosphoric α-D-Galactopyranosylphosphonic Anhydride (24). Uridine 5′-phosphoric di-*n*-butylphosphinothioic anhydride (**22**) was synthesized according to the procedures of Furusawa and co-workers.[39] Tributylammonium salt of α-D-galactopyranosylphosphonate (**17**) (1 mmol) and **22** (2 mmol) were

(45) *International Tables for X-ray Crystallography*; Ibers, J. A., Hamilton, W. C., Eds.; Kynoch: Birmingham, England, 1984; Vol. 4, p 99.

(46) Sheldrick, G. M. *An Integrated System for Solving, Refining and Displaying Crystal Structures from Diffraction Data*, Shelxtl, 4th Revision; University of Gottinger: Federal Republic of Germany, 1983.

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.

(47) Bosmann, H. B.; Spataro, A. C.; Myer, M. W.; Bernacki, R. J.; Hillman, M. J.; Caputi, S. E. *Res. Commun. Chem. Pathol. Pharmacol.* **1975**, *12*, 499.

(48) Greco, W. R.; Priore, R. L.; Sharma, M.; Korytnyk, W. *Comput. Biomed. Res.* **1982**, *15*, 39.

(49) Vaghefi, M. M.; McKernan, P. A.; Robins, R. K. *J. Med. Chem.* **1986**, *29*, 1389.

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

Morteza M. Vaghefi,*‡ Ralph J. Bernacki,§ William J. Hennen,∥ and Roland K. Robins‡

Cancer Research Center, Department of Chemistry, Brigham Young University, Provo, Utah 84602, Grace Cancer Drug Center, Department of Experimental Therapeutics, Roswell Park Memorial Institute, Buffalo, New York 14263, and Nucleic Acid Research Institute, Costa Mesa, California 92626. *Received September 18, 1986*

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

† This work was supported in part by Research Grant 1-R01-CA-31783 and CA-13038 from the National Cancer Institute, USPHS.

‡ Nucleic Acid Research Institute, 3300 Hyland Ave, Costa Mesa, California 92626.

§ Roswell Park Memorial Institute.

∥ Brigham Young University.

(1) Engel, R. *Chem. Rev.* **1977**, *77*, 349.

(2) Hilderbrand, R. L.; Curley-Josef, J.; Lubansky, H. J.; Henderson, T. O. In *Topics in Phosphorous Chemistry*; Grayson, M., Griffin, E. J., Eds.; Wiley: New York, 1983; Vol. 2.

(3) Holy, A.; Rosenberg, I. *Nucleic Acids Symp. Ser.* **1981**, *No. 9*, 145.

(4) Jones, G. H.; Moffat, J. G. *J. Am. Chem. Soc.* **1968**, *90*, 5337.

0022-2623/87/1830-1391$01.50/0