Carbohydrate Research, 62 (1978) 143–154

STUDIES ON ANTIVIRAL GLYCOSIDES. SYNTHESIS AND BIOLOGICAL EVALUATION OF VARIOUS PHENYL GLYCOSIDES

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(Received October 13th, 1976; accepted for publication in revised form, May 24th, 1977)

ABSTRACT

A variety of analogues and derivatives of phenyl glycosides were synthesized for examination of their biological activities and of the relationship between structure and antiviral activity. For antiviral activity, a 6-deoxy-6-halogeno-D-glucose residue was most suitable for the carbohydrate moiety and *p*-alkylphenyl groups for the aglycone moiety. Based on these results, *p*-(*sec*-butyl)phenyl 6-chloro-6-deoxy-β-D-glucopyranoside and *p*-(*sec*-butyl)phenyl 6-deoxy-6-iodo-β-D-glucopyranoside were prepared, and the former compound was found to be the most potent antiviral substance, in this series, against influenza and *Herpes simplex* virus. The anomeric configuration of phenyl glycosides did not contribute to the antiviral activity.

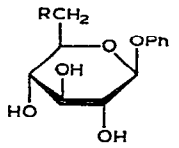
INTRODUCTION

A number of studies on biologically active sugar derivatives with immuno-adjuvant[1], antivirus[2], or interferon-inducing activities[3] have been reported. Among them, 2-deoxy-D-glucose (2-deoxy-D-*arabino*-hexose), known as an inhibitor of glycoprotein biosynthesis[4], was reported to have antiviral activity against enveloped viruses such as those of influenza[5], Newcastle disease[6], and *Herpes simplex* viruses[7]. In order to obtain more potent antiviral substances, many alkyl or aryl glycosides were synthesized, attention being given firstly to a comparison of the antiviral activity of deoxy or chlorodeoxy sugars with that of 2-deoxy-D-glucose, and secondly to the introduction into these structures of a lipophilic residue that might increase the interaction with viral envelopes. Thus, the effect of various substituents at C-6 of D-glucose and at the *para* position of the phenyl residue in phenyl β-D-glucopyranoside was investigated.

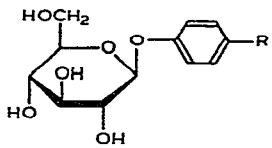
RESULTS AND DISCUSSION

Phenyl D-glucopyranoside and *p*-substituted phenyl D-glucopyranoside were prepared by fusion of phenol or *p*-substituted phenol with 1,2,3,4,6-penta-*O*-acetyl-D-glucose in the presence of catalysts, the α anomers being obtained with zinc chloride, and the β anomers with *p*-toluenesulfonic acid[8].

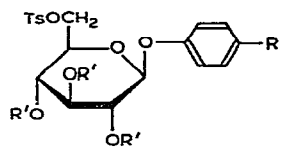
Reaction of the appropriate sugars with sulfuryl chloride in pyridine gave chlorodeoxy sugars, which were reduced to deoxy sugars with tributyltin hydride and 2,2′-azobis(isobutyronitrile), as previously reported[9]. On the other hand, phenyl 6-substituted-β-D-glucopyranosides were generally obtained *via* the intermediates phenyl 6-*O*-tosyl-β-D-glucopyranosides.



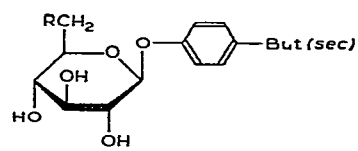
1 R = OH
2 R = H
3 R = F
4 R = Cl
5 R = Br
6 R = I
7 R = SH
8 R = N_3
9 R = $NH_2 \cdot HCl$
10 R = OCH_2CO_2Na



11 R = H
12 R = F
13 R = Cl
14 R = NO_2
15 R = NH_2
16 R = Me
17 R = Et
18 R = Pro
19 R = Isopro
20 R = *tert*-But
21 R = *sec*-But
22 R = *tert*-Amyl



23 R = sec-But, R′ = H
24 R = H, R′ = Ac
25 R = R′ = H



26 R = Cl
27 R = I

p-(*sec*-Butyl)phenyl 6-chloro-6-deoxy-β-D-glucopyranoside (**26**) was synthesized *via* two routes: direct chlorination[10] of the primary hydroxyl group of *p*-(*sec*-butyl)-phenyl β-D-glucopyranoside (**21**) and the other was a fusion reaction of *p*-(*sec*-butyl)-phenol with 1,2,3,4-tetra-*O*-acetyl-6-chloro-6-deoxy-D-glucopyranose[11]. *p*-(*sec*-Butyl)phenyl 6-deoxy-6-iodo-β-D-glucopyranoside (**27**) was synthesized by substitution, with sodium iodide, of the tosyl group of *p*-(*sec*-butyl)phenyl 6-*O*-tosyl-β-D-glucopyranoside (**23**). Physical properties of the synthesized compounds are presented in Tables I–VI.

These compounds were examined for cytotoxicity against HeLa cells, and for antiviral activity against influenza and *Herpes simplex* viruses in culture cells. As shown in Table I, phenyl 2-deoxy-D-*arabino*-hexopyranoside ("phenyl 2-deoxy-α-D-glucopyranoside") and phenyl 6-deoxy-β-D-glucopyranoside showed appreciable antiviral activity against influenza as well as *Herpes simplex* virus. However, phenyl 3-deoxy-β-D-*ribo*-hexopyranoside ("phenyl 3-deoxy-β-D-glucopyranoside") and phenyl 4-deoxy-β-D-*xylo*-hexopyranoside ("phenyl 4-deoxy-β-D-glucopyranoside") generally had no effect at concentrations lower than 2mM. The chlorodeoxy compounds, phenyl 6-chloro-6-deoxy-β-D-glucopyranoside (**4**) and phenyl 4,6-dichloro-4,6-dideoxy-β-D-galactopyranoside, showed about twelve times the antiviral activity of 2-deoxy-D-*arabino*-hexose. However, phenyl 4-chloro-4-deoxy-β-D-galactopyranoside and phenyl 3-chloro-3-deoxy-β-D-allopyranoside were inactive at the standardized

TABLE I

PHYSICAL PROPERTIES AND BIOLOGICAL ACTIVITIES OF THE SYNTHESIZED COMPOUNDS

Compound	*Formula*	*Anal.*						*M.p.*(°)	$[\alpha]_D^{25}$ (°)[a]	*Cytotoxicity*[b] ED_{50} (μg/ml)	ED_{90} (mM)[b] *Influenza*	ED_{50} (mM)[b] Herpes simplex
		Calc.			*Found*							
		C	*H*	*Cl*	*C*	*H*	*Cl*					
2-Deoxy-D-*arabino*-hexose											2.0	
Phenyl β-D-glucopyranoside	$C_{12}H_{16}O_6$	56.24	6.29		56.21	6.18		172–173	−62.0±1.0 (W, 1.002)	>1000	>2.0	
p-Chlorophenyl β-D-glucopyranoside	$C_{12}H_{15}ClO_6$	49.58	5.20	12.19	48.73	5.26	12.16	174–175	−71.4±1.1 (W, 1.011)	650	2.0	2.0
Phenyl 2-deoxy-α-D-*arabino*-hexopyranoside	$C_{12}H_{16}O_5$	59.99	6.71		60.00	6.73		142–145	+154.3±3.6 (P, 0.545)	>1000	2.0	2.0
p-Chlorophenyl 2-deoxy-α-D-*arabino*-hexopyranoside	$C_{12}H_{15}ClO_5$	52.47	5.50	12.91	52.57	5.48	12.84	206–208	+155.5±2.3 (P, 0.844)	240	1.0	
Phenyl 3-deoxy-β-D-*ribo*-hexopyranoside	$C_{12}H_{16}O_5$	59.99	6.71		59.89	6.76		181–183	−62.4±4.5 (P, 0.229)		>2.0	
Phenyl 4-deoxy-β-D-*xylo*-hexopyranoside	$C_{12}H_{16}O_5$	59.99	6.71		59.94	6.66		114–116	−76.4±2.4 (W, 0.484)		>2.0	
Phenyl 6-deoxy-β-D-glucopyranoside	$C_{12}H_{16}O_5$	59.99	6.71		59.50	6.61		160–161	−84.5±2.1 (W, 0.593)	1000	1.0	1.0
Phenyl 3-chloro-3-deoxy-β-D-allopyranoside	$C_{12}H_{15}ClO_5$	52.47	5.50	12.91	52.55	5.44	13.19	137–138	−81.1±1.2 (P, 1.003)		2.0	
p-Chlorophenyl 3-chloro-3-deoxy-β-D-allopyranoside	$C_{12}H_{14}Cl_2O_5$	46.62	4.56	22.94	46.72	4.61	22.97	140–141	−76.0±1.0 (P, 0.609)		0.5	
Phenyl 4-chloro-4-deoxy-β-D-galactopyranoside	$C_{12}H_{15}ClO_5$			12.91			12.87	189–190	−38.2±1.4 (W, 0.579)	1000	2.0	
Phenyl 6-chloro-6-deoxy-β-D-glucopyranoside	$C_{12}H_{15}ClO_5$	52.47	5.50	12.91	52.75	5.53	12.96	151–152	−67.9±2.0 (P, 0.536)	580	0.25	1.0
p-Chlorophenyl 6-chloro-6-deoxy-β-D-glucopyranoside	$C_{12}H_{14}Cl_2O_5$	46.62	4.56	22.94	46.43	4.56	23.07	154–155	−76.9±1.1 (P, 1.032)	150	0.125	0.5
Phenyl 4,6-dichloro-4,6-dideoxy-β-D-galacto-pyranoside	$C_{12}H_{14}Cl_2O_4$	49.17	4.81	24.19	48.93	4.86	24.48	220–221	−24.6±0.8 (P, 0.820)		0.25	
p-Chlorophenyl 4,6-dichloro-4,6-dideoxy-β-D-galactopyranoside	$C_{12}H_{13}Cl_3O_4$	44.00	4.00	32.47	43.76	4.00	32.26	241–242	−33.7±0.8 (P, 0.905)			

[a]In parentheses, solvent (W, water; P, pyridine) and concentration (*c*). [b]See text for methodology.

TABLE II

PHYSICAL PROPERTIES AND ANTIVIRAL ACTIVITY OF SOME 6-SUBSTITUTED PHENYL β-D-GLUCOPYRANOSIDES

Compound	*Substituent*	*Formula*	*Anal.*						*M.p.*(°)	$[\alpha]_D^{25}$ (°)[a]	ED_{90} (mM) *Influenza*
			Calc.			*Found*					
			C	*H*	*Others*	*C*	*H*	*Others*			
1[b]	OH										>2.0
2[b]	H										1.0
3	F	$C_{12}H_{15}FO_5$	55.81	5.85	7.36 (F)	55.76	6.14	7.43 (F)	145–147	−103.8 ± 3.2 (W, 0.445)	1.0
4[b]	Cl										0.25
5	Br	$C_{12}H_{15}BrO_5$	45.16	4.74	25.04 (Br)	45.23	4.75	25.12 (Br)	140–141 (dec.)	−76.6 ± 2.1 (P, 0.547)	0.2
6	I	$C_{12}H_{15}IO_5$	39.36	4.13	34.66 (I)	39.16	4.21	34.42 (I)	160–162	−91.7 ± 1.8 (P, 0.745)	0.2
7	SH	$C_{12}H_{16}O_5S$	52.93	5.92	11.77 (S)	53.04	5.81	11.67 (S)	149–152	−64.5 ± 5.7 (P, 0.183)	0.25
8	N_3	$C_{12}H_{21}N_3O_8$	53.07[c]	5.20	10.31 (N)	52.95[c]	5.28	10.26 (N)	Amorph.	−126.1 ± 3.2 (W, 0.514)	1.0
9	$NH_2 \cdot HCl$	$C_{12}H_{18}ClNO_5$	49.41	6.22	12.15 (Cl), 4.80 (N)	48.50	6.02	12.84 (Cl), 4.97 (N)	183 (dec.)	−49.6 ± 0.9 (W, 1.024)	>2.0
10	OCH_2CO_2Na	$C_{14}H_{18}NaO_8$							Amorph.		>2.0

[a]See footnote to Table I. [b]See data in Table I. [c]Compound 8 being amorphous, the per-*O*-acetyl derivative was analyzed.

TABLE III

PHYSICAL PROPERTIES AND BIOLOGICAL ACTIVITIES OF SOME *para*-SUBSTITUTED PHENYL β-D-GLUCOPYRANOSIDES

Compound	*Substituent*	*Formula*	*Anal.*						*M.p.*(°)	$[\alpha]_D^{25}$ (°)[a]	*Cytotoxicity* ED_{50} (μg/ml)	ED_{90}(mM) *Influenza*
			Calc.			*Found*						
			C	*H*	*Others*	*C*	*H*	*Others*				
1	H[d]											>2.0
11	OH[d]											>2.0
12	F	$C_{12}H_{15}FO_6$	54.30[c]	5.24	4.29 (F)	54.36[c]	5.30	4.23 (F)	Amorph.	−36.1 ± 1.5 (W, 0.511)		>2.0
13	Cl[d]										650	2.0
14	NO_2[e]	$C_{12}H_{15}NO_8$								−108.3 ± 2.7 (W, 0.540)		>2.0
15	NH_2[f]	$C_{12}H_{17}NO_6$	53.13	6.32	5.16 (N)	53.39	6.31	5.15 (N)	156–157	−41.0 ± 1.6 (P, 0.567)		>2.0
16	Me	$C_{13}H_{18}O_6$	57.77	6.71		57.79	6.72		178–179	−50.7 ± 1.6 (W, 0.562)	>1000	2.0
17	Et	$C_{14}H_{20}O_6$	59.15	7.09		58.93	7.03		164–165	−53.7 ± 3.2 (W, 0.296)	>1000	1.0
18	Propyl	$C_{15}H_{22}O_6$	60.39	7.43		60.23	7.44		143–144	−72.0 ± 1.1 (W, 1.041)	620	1.0
19	Isopropyl	$C_{15}H_{22}O_6$	60.39	7.43		60.51	7.52		161–163	−50.7 ± 1.1 (W, 0.817)		1.0
20	*tert*-Butyl	$C_{16}H_{24}O_6$	61.52	7.75		61.60	7.75		148–149	−61.6 ± 1.0 (W, 1.004)	480	0.5
21	*sec*-Butyl	$C_{16}H_{24}O_6$	61.52	7.75		61.09	7.78		131–134	−57.5 ± 1.0 (W, 1.023)	350	0.5
22	*tert*-Amyl	$C_{17}H_{26}O_6$	60.72[c]	6.93		60.83[c]	6.89		Amorph.	−51.9 ± 1.0 (P, 0.936)	380	

[a]See footnote to Table I. [b]See ref. 19. [c]Compounds **12** and **22** being amorphous, the per-*O*-acetyl derivatives were analyzed. [d]See data in Table I. [e]Purchased from Nakari Chemicals, Ltd., Karasuma-Nijōdōri, Nakakyo-ku, Kyoto 604, Japan. [f]Prepared by hydrogenation of *p*-nitrophenyl β-D-glucopyranoside.

TABLE IV

PHYSICAL PROPERTIES AND ANTIVIRAL ACTIVITY OF SOME ALKYL β-D-GLUCOPYRANOSIDES

Alkyl	*Formula*	*Anal.*				*M.p.* (°)	$[\alpha]_D^{25}$ (°)[a]	ED_{90} (mM) *Influenza*
		Calc.		*Found*				
		C	*H*	*C*	*H*			
Benzyl	$C_{13}H_{18}O_6$	57.97	6.71	57.50	6.86	120–121	−54.7 ± 1.0 (0.909)	>2.0
Cyclohexyl	$C_{12}H_{22}O_6$	54.95	8.45	55.01	8.40	128–130	−41.6 ± 1.3 (0.640)	>2.0
Cyclooctyl	$C_{14}H_{26}O_6$	57.91	9 03	57.44	9.07	127–128	−38.1 ± 0.8 (1.006)	2.0
Butyl[b]	$C_{10}H_{20}O_6$	50.83	8.53	50.14	8.81		−37.1 ± 0.7 (1.061)	>2.0
Amyl	$C_{11}H_{22}O_6$	52.78	8.86	52.62	8.58	90– 92	−35.3 ± 0.8 (1.001)	>2.0
Octyl	$C_{14}H_{28}O_6$	57.51	9.65	57.58	9.65	Amorph.	−28.3 ± 0.7 (1.027)	toxic

[a]In water, *c* in parentheses. [b]Highly hygroscopic.

TABLE V

INFLUENCE OF THE GLYCOSYL MOIETY OF PHENYL GLYCOPYRANOSIDES ON THE BIOLOGICAL ACTIVITY

Phenyl glycopyranoside	ED_{90} (mM) *Influenza*
α-D-Mannopyranoside[a]	>2.0
β-D-Galactopyranoside[a]	>2.0
β-D-Xylopyranoside[a]	2.0
β-L-Arabinopyranoside[a]	2.0
α-L-Rhamnopyranoside	0.5
α-L-Fucopyranoside[a]	1.0
2-Acetamido-2-deoxy-β-D-glucopyranoside[b]	>2.0
2-Acetamido-2,6-dideoxy-β-D-glucopyranoside[c]	>2.0
2-Acetamido-2,4-dideoxy-β-D-*xylo*-hexopyranoside[c]	>2.0
2-Acetamido-2,3-dideoxy-β-D-*ribo*-hexopyranoside[c]	>2.0

[a]Purchased from Nakarai Chemicals, Ltd. [b]See ref. 20. [c]See ref. 9.

dose of 2mM. Substitution at the *para* position of the phenyl residue with a chlorine atom enhanced antiviral activity. Comparison of the activities of *p*-chlorophenyl α-D-glucopyranoside and *p*-chlorophenyl β-D-glucopyranoside clearly showed that the anomeric configuration was unrelated to antiviral activity.

Removal of the phenyl residue from phenyl 6-chloro-6-deoxy-β-glucopyranoside (**4**) or phenyl 6-deoxy-β-D-glucopyranoside (**2**) resulted in loss of their antiviral activity, which means that the lipophilic property is necessary for interaction with viruses. Interestingly, the poliomyelitis virus, which has no envelope, was not affected by phenyl 6-chloro-6-deoxy-β-D-glucopyranoside (**4**). This suggests that interaction between the hydrophobic part of the phenyl glycosides and the virus envelope plays an important role in antiviral activity. Some of the compounds showed weak cytotoxicity against HeLa cells in tissue culture, substitution at the *para* position with chlorine causing more toxicity than the phenyl residue. Substitution at the C-6 position of the D-glucose moiety with a halogen atom enhanced the antiviral activity in the order F < Cl ⩽ Br ⩽ I, as shown in Table II. However, compounds with such substituents as carboxymethoxy or amino groups at C-6 of the D-glucose moiety in phenyl β-D-glucopyranoside did not show any antiviral activity at a standardized dosage of 2mM. The antiviral activity of *para*-substituted phenyl β-D-glucopyranosides tends to increase as the chain length of the substituent, alkyl groups become longer up to the butyl residue (see Table III). β-D-Glucopyranosides with various aglycons other than phenyl or *p*-substituted phenyl groups were prepared and examined for antiviral activity. However, none had any significant activity (see Table IV). Examination of the carbohydrate moiety in a series of phenyl glycopyranosides showed that sugars having no hydroxyl group at C-6 (L-rhamnose, L-fucose, etc.) showed considerable activity against influenza virus, as shown in Table V. These results seem to have some structure-activity relationship for the phenyl 6-deoxy- and 6-deoxy-6-halogeno-

TABLE VI

PHYSICAL PROPERTIES AND BIOLOGICAL ACTIVITIES OF *p*-(*sec*-BUTYL)PHENYL 6-DEOXY-6-HALOGENO-β-D-GLUCOPYRANOSIDES (**26** AND **27**)

Properties and activities	*Compound*	
	6-Chloro (**26**)	*6-Iodo* (**27**)
Formula	$C_{16}H_{23}ClO_5$	$C_{16}H_{23}IO_5 \cdot H_2O$
Anal.		
Calc. C	58.09	43.65
H	7.01	5.72
Cl	10.72	
I		28.82
Found C	57.74	44.01
H	6.96	5.64
Cl	10.66	
I		28.74
M p. (°)	148–149	172–174
$[\alpha]_D^{25}$	-63.7 ± 1.9	-86.1 ± 1.4
c (pyridine) (°)	(0.543)	(0.887)
Cytotoxicity, ED_{50} (g/ml)	60	
ED_{90} (mM) Influenza	0.03	0.125[a]
ED_{50} (mM) *Herpes simplex*	0.03	

[a]This compound was sparingly soluble in water, and the maximum solubility was 0.125mM.

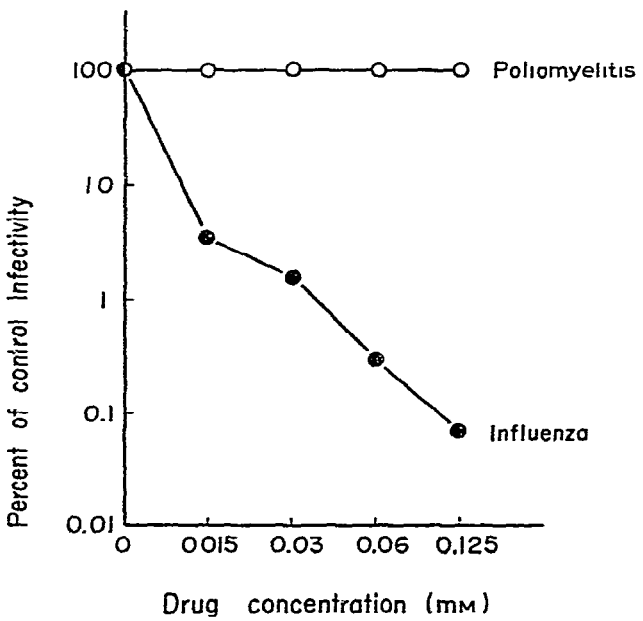


Fig. 1. Effect of *p*-(*sec*-butyl)phenyl 6-chloro-6-deoxy-β-D-glucopyranoside (**26**) on the infectivities of poliomyelitis and influenza viruses.

β-D-glucopyranosides reported in Table I. Phenyl 2-acetamido-2-deoxy-β-D-glucopyranoside and its analogues did not show any appreciable activity against viruses. Therefore, a hydrophobic substituent at C-6 of the hexose moiety seems necessary to increase antiviral activity.

Consideration of these data led us to prepare *p*-alkylphenyl 6-deoxy-6-halogeno-β-D-glucopyranosides as the most desirable substances in these series. Among them, *p*-(*sec*-butyl)phenyl 6- chloro-6-deoxy-β-D-glucopyranoside (**26**) markedly inhibited growth of influenza and *Herpes simplex* viruses in cell culture at a dosage of 0.03mM, which was about 70 times more active than 2-deoxy-D-*arabino*-hexose (2-deoxy-D-glucose) (see Table VI). Like phenyl 6-chloro-6-deoxy-β-D-glucopyranoside (**4**), *p*-(*sec*-butyl)phenyl 6-chloro-6-deoxy-β-D-glucopyranoside (**26**) was also inactive *vs.* poliomyelitis virus, which has no envelope, at the concentration of 0.125mM, whereas the infectivity of influenza virus was reduced by more than 3 $\log_{10}$ (see Fig. 1). This suggests that the antiviral activity of these glycosides is due to an interaction with the viral envelope. More-detailed studies on the use of different modes of administration, different dosage regiments, and mode of antiviral mechanism will be presented elsewhere.

EXPERIMENTAL

General. — All melting points are uncorrected. Optical rotations were determined at 25±2° with a Perkin–Elmer 141 polarimeter. Thin-layer chromatography (t.l.c.) was performed with Silica gel G (Merck). Silica gel column chromatography was performed on silicic acid (100-mesh Mallinckrodt) with 1:1 (v/v) toluene–ethyl acetate as the eluent.

Cytotoxicity. — Following the general procedure of Geran *et al.*[12], HeLa cells (10^5/ml) were incubated in Eagle's minimum essential medium (MEM) supplemented with 10% bovine serum for two days at 37°. The medium was removed and fresh medium containing serial dilutions of the compound to be tested was added to the cell cultures. After 48 h, the cells were dispersed with 0.02% trypsin and 0.04% Versene, and the number of cells was counted with a Model B Coulter counter. Values of ED_{50} were determined by plotting the percentage of inhibition *vs.* the concentration of the compound, and are summarized in Tables I–VI.

Antiviral activity. — Antiviral activity of each compound was determined by the virus-yield test for influenza virus and the plaque-reduction test for *Herpes simplex* virus. For the virus-yield test, monolayers of $LLCMK_2$ cells, which is an established cell line from rhesus monkey kidney grown for three days in Falcon plastic bottles (25 cm^2) with MEM (5 ml) supplemented with 10% bovine serum[13], were infected with influenza virus (Ao/WSN) at a multiplicity of infectivity of 2. After 2 h of virus-adsorption time, the infected cells were washed twice with Hanks' balance salt solution, and MEM (5 ml), supplemented with 0.2% skim milk (Difco) containing various concentration of each compound to be tested was added, and the cells were incubated for 24 h after virus infection at 37°. The cultures were stored

at −20°. For virus titration, the harvested cultures were frozen in dry ice–isopropyl alcohol solution and thawed in three cycles, and then centrifuged at 3000 r.p.m. for 15 min to remove the cell debris. The virus was titrated by plaque assay of $LLCMK_2$ cells. The drug concentration is expressed in terms of the mM concentration that gives a 10-fold reduction of virus yield of the control (ED_{90}). As presented in Tables I and VI, the antiviral activity of some compounds against *Herpex simplex* virus (HF strain) was examined by the plaque reduction test, as described previously[14]. The 50%-plaque-reduction concentration of the compound (ED_{50}) was expressed in terms of the mM concentration of the test compounds.

Reaction of sulfuryl chloride with sugars[15]. — A calculated amount of sulfuryl chloride was added dropwise to the sample dissolved in anhydrous pyridine under cooling with ice–water. The mixture was kept overnight at room temperature, extracted with chloroform several times, washed with water, and evaporated to dryness. The residue was dissolved in methanol and decolorized with Norit A, and then crystallized from methanol.

Reduction of chlorodeoxy sugars with tributyltin hydride. — Acetylated chlorodeoxy sugars were dissolved in anhydrous toluene and a calculated amount of tributyltin hydride (prepared by thermal decomposition of tributyltin formate[16]) and a trace of 2,2′-azobis(isobutyronitrile) were added to the solution. The reaction mixture was stirred for 30 min at 80°, and the solvent was removed by evaporation. The syrupy product was purified by being passed through a silica gel column with toluene–ethyl acetate as the eluent.

Preparation of alkyl β-D-glucopyranosides. — Alkyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosides were prepared by the method of Heidt and Purves[17]. Butyl, benzyl, and cyclohexyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosides were crystallized from ethanol, and the cyclooctyl derivative was crystallized from hexane. Amyl and octyl derivatives failed to crystallize. *O*-Deacetylation of these compounds was performed as described by Heidt and Purves[17]. Physical properties and antiviral activity are reported in Table IV.

*Phenyl 6-*O*-p-tolylsulfonyl-β-D-glucopyranoside* (**25**). — Phenyl β-D-glucopyranoside (**1** 1.0 g) was dissolved in anhydrous pyridine (20 ml), and then *p*-toluenesulfonyl chloride (0.9 g) was added under cooling in an ice–water bath. The solution was kept for 48 h at −20°, and poured into ice–water. The separated syrup was collected and washed with water several times, and dried *in vacuo* to give an amorphous powder (1.2 g).

*Phenyl 2,3,4-tri-*O*-acetyl-6-*S*-acetyl-6-deoxy-β-D-glucopyranoside.* — To a solution of phenyl 2,3,4-tri-*O*-acetyl-6-*O*-*p*-tolylsulfonyl-β-D-glucopyranoside (**24**, 300 mg) obtained by acetylation of the compound just described, in *N*,*N*-dimethylformamide (7.0 ml) was added potassium thioacetate (400 mg). The mixture was heated on a steam bath for 1 h. Water (10 ml) was added to the cooled solution, and the mixture was extracted with chloroform. The chloroform extract was extensively washed with water, dried (calcium chloride), and evaporated *in vacuo* to give a syrup. Recrystalli-

zation from methanol gave colorless crystals (105 mg), m.p. 95–96°, $[\alpha]_D^{25}$ −11.3 ± 1.1° (*c* 1.031, chloroform).

Anal. Calc. for $C_{20}H_{24}O_9S$: C, 54.54; H, 5.49; S, 7.28. Found: C, 54.36; H, 5.36; S, 7.38.

Phenyl 6-azido-6-deoxy-β-D-glucopyranoside (**8**). — To a solution of **25** (1.0 g) in acetone (25 ml) was added sodium azide (1.0 g in 5 ml of water). The mixture was boiled under reflux for 72 h, and the solvent evaporated *in vacuo*. The residue was deposited on a charcoal column (3 × 2 cm) and eluted stepwise with water (50 ml) and 60% aqueous ethanol (100 ml). The product obtained from the aqueous ethanol fraction gave a single spot on t.l.c., but failed to crystallize (yield 700 mg).

Phenyl 6-amino-6-deoxy-β-D-glucopyranoside hydrochloride (**9**). — A solution of **8** (500 mg) in ethanol (20 ml) was hydrogenated[18] with palladium-black at room temperature, while being gradually neutralized with dilute hydrochloric acid. The catalyst was removed by filtration, and the filtrate was concentrated *in vacuo* to give a syrup. Crystallization from ethanol gave colorless needles (222 mg), dec. 183°.

p-(sec-*Butyl*)*phenyl 6-chloro-6-deoxy-β-D-glucopyranoside* (**26**). — (*a*). 1,2,3,4-Tetra-*O*-acetyl-6-chloro-6-deoxy-D-glucose (11.5 g) was treated with *p*-(*sec*-butyl)-phenol (12.0 g) in the presence of *p*-toluenesulfonic acid. Recrystallization from ethanol gave *p*-(*sec*-butyl)phenyl 2,3,4-tri-*O*-acetyl-6-chloro-6-deoxy-β-D-glucopyranoside (4.1 g), m.p. 127–128°, $[\alpha]_D^{25}$ −18.9 ± 1.1° (*c*, chloroform).

Anal. Calc. for $C_{22}H_{29}ClO_8$: C, 57.83; H, 6.40; Cl, 7.76. Found: C, 57.94; H, 6.35; Cl, 7.90.

Deacetylation of this compound gave *p*-(*sec*-butyl)phenyl 6-chloro-6-deoxy-β-D-glucopyranoside (**26**).

(*b*). To a solution of *p*-(*sec*-butyl)phenyl β-D-glucopyranoside (**21**, 1.0 g) in *N*,*N*-dimethylformamide (15 ml) was added methanesulfonyl chloride (0.8 g). The mixture was stirred for 3 h at 65°, neutralized by adding M sodium hydroxide with cooling in an ice-water bath, and concentrated *in vacuo*. The residual syrup was dissolved in water and deposited on a charcoal column (6 × 1 cm) which was eluted successively with water (200 ml), 10% aqueous ethanol (100 ml), and 90% aqueous ethanol (300 ml). The fraction eluted with 90% ethanol was evaporated *in vacuo* and the residue was crystallized from toluene. Recrystallization from the same solvent gave colorless crystals (500 mg) having the same m.p. as the product described under (*a*).

p-(sec-*Butyl*)*phenyl 6-deoxy-6-iodo-β-D-glucopyranoside* (**27**). — *p-sec*-(Butyl)-phenyl β-D-glucopyranoside (**21**, 3.3 g) was dissolved in anhydrous pyridine (80 ml), and *p*-toluenesulfonyl chloride (2.7 g) was added. The mixture was kept in a refrigerator overnight, concentrated, and poured into 100 ml of ice–water. The precipitates were collected, washed several times with cold water, and dried *in vacuo* by codistillation with anhydrous toluene, to give an amorphous powder (3.5 g) that gave a single spot on t.l.c. This compound (3.4 g) was mixed with *N*,*N*,-dimethylformamide (50 ml) and sodium iodide (4.5 g), and the solution was stirred overnight at 70°. It was concentrated to a syrup which was deposited on a charcoal column (6 × 3 cm).

The column was eluted successively with water (100 ml) and ethanol (800 ml). The ethanol eluate was concentrated and the residue crystallized from toluene. Recrystallization from 1:1 (v/v) ethanol–water afforded colorless needles (2.2 g), m.p. 172–174°.

ACKNOWLEDGMENTS

The authors thank Prof. Y. Matsushima, Faculty of Science, Osaka University, Osaka, for his helpful discussions and gift of phenyl 2-acetamido-2,6-dideoxy-β-D-glucopyranoside and phenyl 3-deoxy-β-D-*ribo*-hexopyranoside, and Dr. O. Shiratori and Mr. S. Takase for the cytotoxicity tests.

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