search grant AA00279 from the National Institute on Alcohol Abuse and Alcoholism, U.S. Public Health Service.

Registry No. **1d**, 5398-10-7; **3c**, 1457-39-2; **3d**, 5702-91-0; **3e**, 635-51-8; **4c**, 24766-10-7; **4d**, 18755-30-1; **4e**, 34861-81-9; **5b**, 2938-98-9; **5c**, 80676-17-1; **5d**, 18755-31-2; **5e**, 6837-05-4; **6b**, 4740-00-5; **6c**, 1551-24-2; **6d**, 1613-50-9; **6e**, 16766-62-4; **7a**, 1600-44-8; **7b**, 93111-02-5; **7c**, 93111-03-6; **7d**, 29711-02-2; **7e**, 93134-22-6; diethyl *n*-butylmalonate, 133-08-4; ethyl chloroacetate, 105-39-5; ethyl 3,3-dicarbethoxyheptanoate, 67610-46-2; 2-butyl-1,4-diiodobutane, 93111-04-7; alcohol dehydrogenase, 9031-72-5; ethanol, 64-17-5.

Uridine 5′-Diphosphate Glucose Analogues. Inhibitors of Protein Glycosylation That Show Antiviral Activity

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A series of analogues of uridine 5′-diphosphate glucose and uridine 5′-diphosphate glucosamine have been synthesized by reaction of 2,3,4,6-tetra-*O*-benzyl-, 2,3,4,6-tetra-*O*-benzoyl-, 2,3,4,6-tetra-*O*-acetyl-, and 2,3,4,6-tetra-*O*-palmitoyl-α-D-glucopyranose and 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranose with chlorosulfonyl isocyanate and 2′,3′-*O*-isopropylideneuridine. Isopropylidene and acetyl groups of the resulting 5′-*O*-[[[[(α-D-glucopyranosyl)oxy]carbonyl]amino]sulfonyl]-2′,3′-*O*-isopropylideneuridine derivatives were removed by reaction with a TFA/water (5:1) mixture and methanolic ammonia, respectively. The 5′-*O*-[[[[(2″,3″,4″,6″-tetra-*O*-benzyl- and 2″,3″,4″,6″-tetra-*O*-benzoyl-α-D-glucopyranosyl)oxy]carbonyl]amino]sulfonyl]-2′,3′-*O*-isopropylideneuridine (**13** and **19**) and the corresponding deisopropylidenated derivatives showed antiviral activity as determined by the inhibition of the cytopathic effect induced by HSV-1 replication and by the plaque assay method. Compound **13** inhibited glycosylation of proteins in HSV-1 infected HeLa cells.

Nucleoside diphosphate sugars are intermediates that donate glycosyl residues in the biosynthesis of polysaccharides, glycolipids, glycoproteins, and some components of the bacterial cell wall. Compounds that interfere with protein glycosylation show a variety of biological effects,[1,2] such as inhibition of the replication of enveloped animal viruses.[3-6] For instance, 2-deoxy-D-glucose is a glycosylation inhibitor having antiviral activity,[4-9] which is transformed to UDP-2dGlc (**3**) and GDP-2dGlc (**5**), and as such interferes with the biosynthesis of the dolichol-linked oligosaccharide precursor of the *N*-glycosylated glycoproteins.[10] These interferences are mainly due to the replacement of glucose and mannose by 2-deoxyglucose, i.e., the replacement of UDP-Glc (**1**) and GDP-Man (**4**) by UDP-2dGlc (**3**) and GDP-2dGlc (**5**) (Chart I). The nucleoside antibiotic tunicamycin[11] (**6**) and the related Streptomycete antibiotic streptovirudin[12] are effective protein glycosylation inhibitors that block the enzymes reversibly, translocating phosphoryl-*N*-acetyl-D-glucosamine from UDP-GlcNAc (**2**) to polyprenol phosphate.[2] However, the use of tunicamycin as an antiviral agent is hampered by its high toxicity. The metabolites of 2-deoxyglucose, GDP-2dGlc (**5**), and UDP-2dGlc (**3**), tunicamycin, and streptovirudin are structurally related to the natural nucleoside diphosphate sugars in that all of them have a sugar residue (glucose, *N*-acetylglucosamine 2-deoxyglucose, mannose) linked to the nucleoside moiety (uridine, guanosine) by a five-atom bridge. We hypothesized that this is an essential structural requirement for these compounds to act as substrates or inhibitors of glycosyltransferases and therefore we designed, synthesized, and tested as protein glycosylation inhibitors and as antiviral agents a series of analogues of uridine diphosphate glucose and of uridine diphosphate *N*-acetylglucosamine in which the diphosphate bridge has been replaced by an isosteric $-OCONHSO_2O-$ residue.

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Reaction of 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranose (**7**) with chlorosulfonyl isocyanate (**8**) in methylene chloride at low temperature and with exclusion of moisture afforded the unstable [[[(chlorosulfonyl)amino]carbonyl]oxy]glucose **9**, which, by in situ reaction with 2′,3′-*O*-isopropylideneuridine gave 5′-*O*-[[[[(2″,3″,4″,6″-tetra-*O*-benzyl-α-D-glucopyranosyl)oxy]carbonyl]amino]sulfonyl]-2′,3′-*O*-isopropylideneuridine (**13**) in 40% yield (Scheme I). Compound **9** was formed by reaction of the isocyanate group, more reactive toward nucleophiles than the chlorosulfonyl group of **8**,[13] with the glucose anomeric hydroxyl group. This was demonstrated by the obtention of the 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl carbamate **10** in 67% yield when the crude reaction mixture of **7** and **8** was left in contact with ambient moisture. Also in support of the assigned structure to **9**, and thus the nature of the indicated five-atom bridge as glucosyl–$OCONHSO_2O$–uridine and not the inverse one glucosyl–OSO_2NHCOO–uridine,

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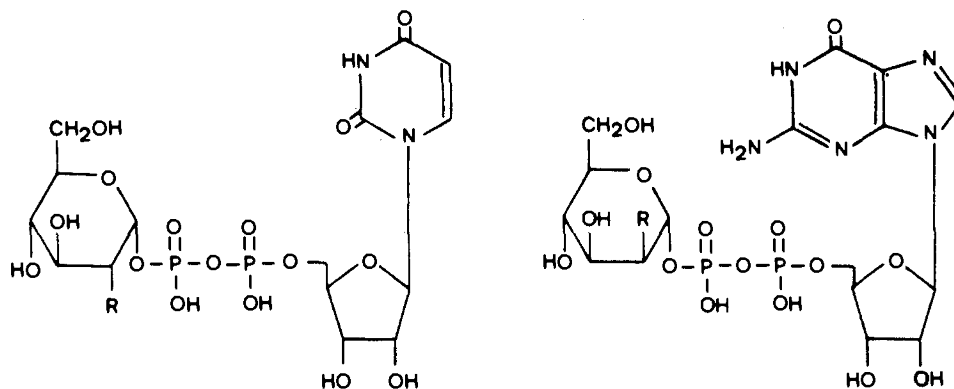
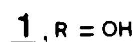
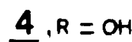
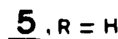
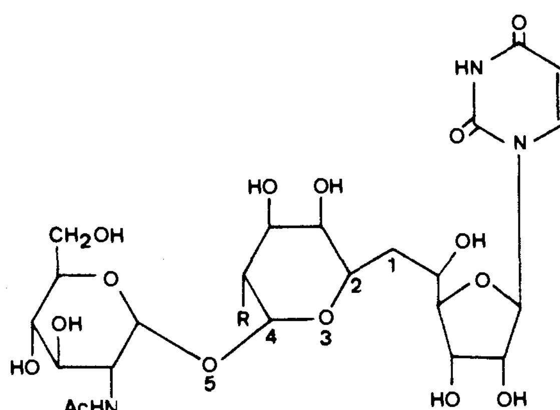
Chart I

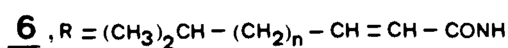








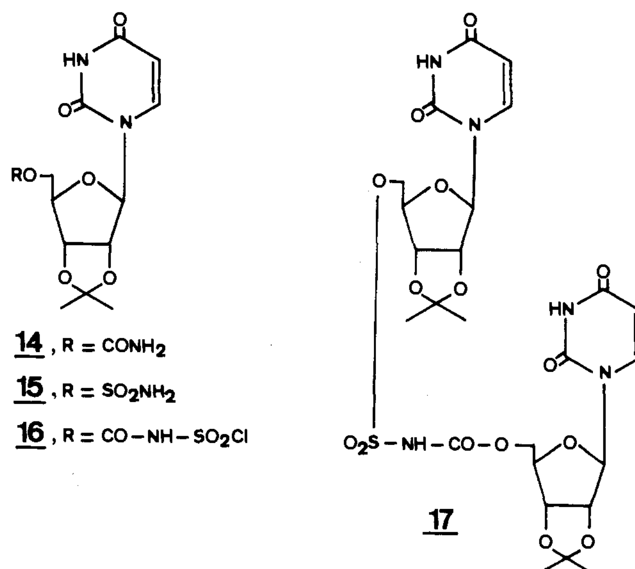


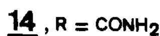


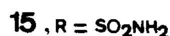
was, on one hand, the obtention of the (sulfamidocarbonyl)glucoside **12**, when the above crude reaction mixture of **7** and **8** was treated with a solution of ammonia in methylene chloride, and on the other, the fact that the reaction of compound **16**, obtained by in situ treatment of 2′,3′-*O*-isopropylideneuridine with **8**, did not afford the corresponding condensation product, an analogue of **13**, by reaction with **7**.

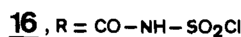
The structures of **10**, **12**, and **13** were confirmed by analytical and spectroscopic data. The α anomeric configuration of the tetrabenzylglucopyranosyl moiety was demonstrated by the appearance in the corresponding ^{1}H NMR spectra for **10** and **12** (90 MHz) and **13** (300 MHz) of doublets at δ 6.13 ($J_{1,2}$ = 3.5 Hz), 6.18 ($J_{1,2}$ = 3.5 Hz), and 6.21 ($J_{1'',2''}$ = 3.0 Hz), respectively. The attachment of the [[(glucosyloxy)carbonyl]amino]sulfonyl residue to the 5′-O-position of the uridine in **13** was demonstrated by the presence in its ^{1}H NMR spectrum of a singlet at δ 11.34, assigned to the uridine 3-NH proton, which disappeared by treatment with D_2O, as compared to a similar singlet at δ 11.43 for the same proton of **11** and by the downfield shift for the bands of the uridine moiety H-4′ and H-5′ protons, which appeared at δ 4.26 and 4.05, respectively, as compared to the same protons of 2′,3′-*O*-isopropylideneuridine, which appeared at δ 4.06 and 3.56, respectively.

Chart II



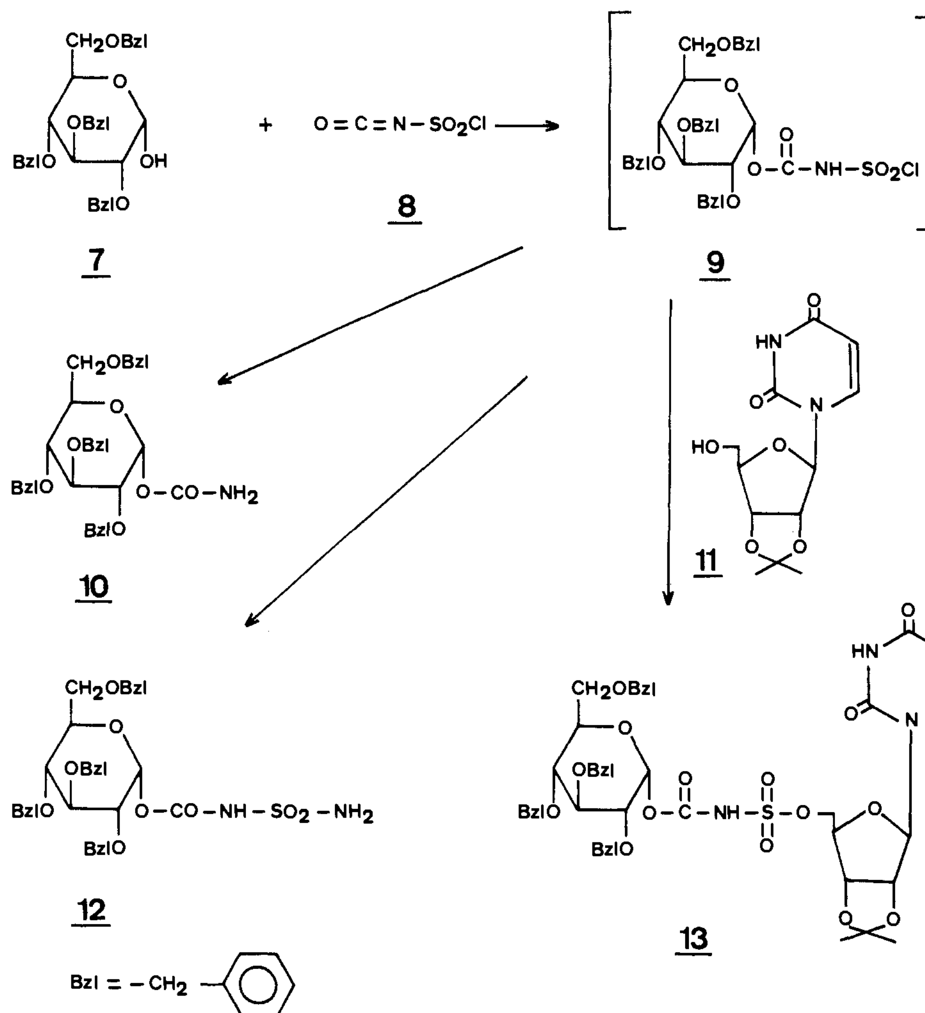






Small amounts of the 5′-*O*-carbamoyl **14** and 5′-*O*-sulfamoyl-2′,3′-*O*-isopropylideneuridine **15** were usually obtained as byproducts of the above one-pot reaction of

Scheme I



7, 8, and 11 (Chart II). The formation of 14 is similar to that of 10, i.e., reaction of 11 with unreacted chlorosulfonyl isocyanate to give intermediate 16, which in the presence of ambient moisture decomposes to afford carbamate 14.[13] This same compound 14 has also been obtained in 40% yield by reaction of 11 with chlorosulfonyl isocyanate, which confirms the above suggested formation pathway of 14. This is a ready, one-step procedure for the obtention of 5′-*O*-carbamoyluridine, an alternative to the methods previously described by this[14] and other laboratories.[15] The formation of the nonisolated intermediate 16 is evidenced by the obtention of traces of the bisuridine derivative 17, identified by ^{1}H NMR and mass spectra, and by reaction of 16 with a second molecule of 2′,3′-*O*-isopropylideneuridine. Compound 15 should be formed by reaction of sulfamoyl chloride, generated by reaction of chlorosulfonyl isocyanate with moisture,[16] with 11. This same procedure has been previously used to prepare several 5′-*O*-sulfamoyl nucleosides.[17,18] The structure of 15 was determined, as in the case of 13, by the $\Delta\delta$ = 0.14 and 0.64 ppm downfield shifts for the bands of the H-4′ and H-5′ protons, respectively, of 15 as compared to those of 11.

Reaction of other glucose or glucosamine derivatives, such as, 2,3,4,6-tetra-*O*-benzoyl-α-D-glucopyranose, 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranose, 2,3,4,6-tetra-*O*-palmitoyl-α-D-glucopyranose, and 2-acetamido-2-deoxy-3,4,6-tri-*O*-acetyl-α-D-glucopyranose with chlorosulfonyl isocyanate and 2′,3′-*O*-isopropylideneuridine as described before afforded analogues of UDP-glucose 19, 21, and 22 and the analogue of UDP-glucosamine 24 (Chart III). The structures of these compounds were determined from their 90- or 300-MHz ^{1}H NMR spectra in a way similar to that of 13. The α-anomeric configurations of the hexose moieties were determined from the $J_{1'',2''}$ = 3–4.2 Hz coupling constant values. In the case of the 2-acetamido-2-deoxy-3,4,6-tri-*O*-acetyl-α-D-glucopyranose, the reaction of 8 with the anomeric 1-OH group and not with the 2-AcNH group was demonstrated by the presence of the characteristic 2-NH doublet at δ 8.11 ($J_{2'',NH}$ = 9.1 Hz) and the downfield shift of the anomeric H-1 proton from δ 5.00 in the starting tetraacetylglucosamine to δ 5.72 in 24. The attachment of the [[(hexosyloxy)carbonyl]amino]sulfonyl residue to the 5′-O-position of uridine was similarly demonstrated by the presence in the ^{1}H NMR spectra for 19, 21, and 24 of singlets at δ 11.00–11.43 assigned to the 3-NH uracil proton and the downfield chemical shift of the H-4′ and H-5′ ribose residue protons as compared to those of 11.

Removal of the 2,3-*O*-isopropylidene protecting groups from uridine moieties of compounds 13, 19, and 24 was

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(17) Shuman, D. A.; Robins, M. J.; Robins, R. K. *J. Am. Chem. Soc.* **1970**, *92*, 3434.
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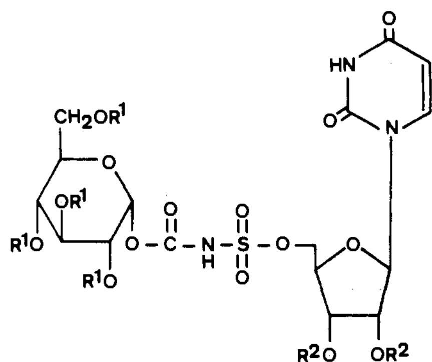
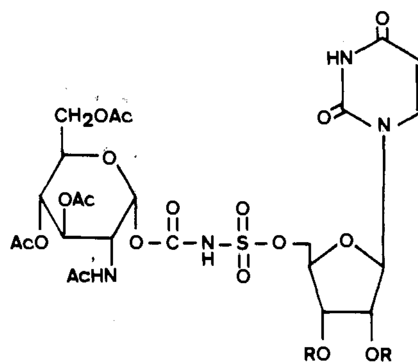
Chart III





18, $R^1 = CH_2C_6H_5$; $R^2 = H$

19, $R^1 = COC_6H_5$; $R^2 = C(CH_3)_2$

20, $R^1 = COC_6H_5$; $R^2 = H$

21, $R^1 = COCH_3$; $R^2 = C(CH_3)_2$

22, $R^1 = COC_{15}H_{31}$; $R^2 = C(CH_3)_2$

23, $R^1 = H$; $R^2 = C(CH_3)_2$

24, $R = C(CH_3)_2$

25, $R = H$

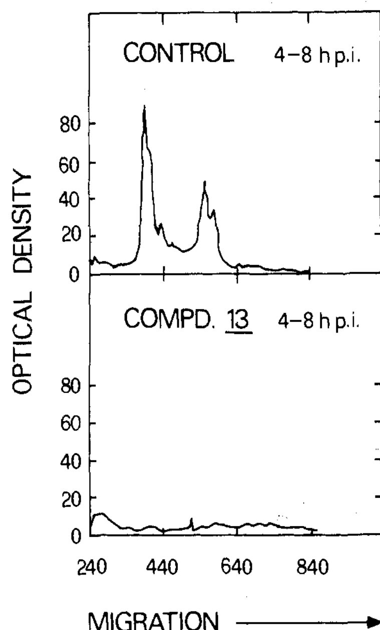


Figure 1. Densitometric scans of autoradiograms of [^{3}H]-glucosamine labeled HSV-1 glycoproteins synthesized in the absence (upper panel) and the presence (lower panel) of 100 μM compound 13.

achieved in 55–60% yield by treatment with a trifluoroacetic acid/water mixture to give compounds **18**, **20**, and **25**, respectively.

Removal of the glucose acyl protecting groups of **21** by treatment with methanolic ammonia afforded **23** in 54% yield. This low yield is due to partial cleavage of the five-atom bridge between glucose and uridine.

Biological Activity. Compound **13** was tested for its ability to inhibit protein glycosylation. For this purpose HeLa cell monolayers were mock-infected or infected with herpes simplex virus type 1 (HSV-1) strain KOS, at a multiplicity of infection (moi) of 10 pfu/cell in the presence of different concentrations of compound **13**. The glycosylation of proteins was examined from 4 to 8 h postinfection by incubating the cells with [^{3}H]glucosamine and analyzing the radioactive glycoproteins by polyacrylamide gel electrophoresis, followed by autoradiography of the dried gel.[19] Figure 1 represents the densitometric scans of the autoradiograms obtained in the absence (upper panel) and in the presence (lower panel) of 100 μM of compound **13**, which shows that this compound at the indicated concentration completely blocks glycosylation of HSV-1 proteins. At the same concentration (100 μM) of compound **13**, an inhibition of 73% was observed on the glycosylation of uninfected control cell proteins. Almost no effect of compound **13** was observed on the synthesis of proteins at 100 μM after 48 h. In fact, 6% inhibition was produced as measured by [^{35}S]methionine incorporation and subsequent separation of the labeled proteins in polyacrylamide gels.

The antiherpes activity of these new UDP-glucose analogues was first analyzed by their protection of the cytopathic effect induced by HSV-1 replication. Two well-known glycosylation inhibitors[2] (2-deoxy-D-glucose and tunicamycin) and three well-established antiviral

Table I. In Vitro Antiherpes Activity and Toxicity of UDP-Glucose Analogues

compd	CPE_{50},[a] μM	Tox_{50},[b] μM
10	>350	>350
13	85	230
14	>660	>660
15	740	740
18	90	360
19	30	220
20	75	>220
21	>540	540
22	230	230
23	>350	>350
24	>270	>270
25	>575	>575
2-deoxy-D-glucose	5000	5000
tunicamycin (**6**)	1	1
amantadine	200	500
acycloguanosine[c]	60	10000
BVdU[d]	1	400

[a] CPE_{50} represents the concentration of compound (μM) that protects by 50% the cytopathic effect induced by HSV-1. [b] Tox_{50} represents the concentration of compound (μM) that induces 50% cell toxicity. [c] Acycloguanosine is 9-[(2-hydroxyethoxy)methyl]guanine. [d] BVdU is (*E*)-5-(2-bromovinyl)-2′-deoxyuridine.

(19) Muñoz, A.; Carrasco, L. *J. Gen. Virol.* 1984, *65*, 377.

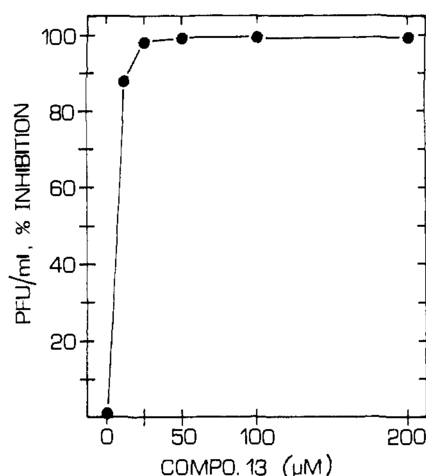


Figure 2. Effect of compound 13 on the formation of plaque-forming units.

compounds[3-6] (amantadine, acycloguanosine, and BVdU) have also been tested for antiviral activity under the same experimental conditions, and the data are given in Table I. This table shows that compounds **13**, **18**, **19**, and **20** have an antiviral effect as measured by this test. They also show a moderate specificity, when protection of the infected cell monolayers and toxicity to culture cells are compared. Neither tunicamylcin nor 2-deoxy-D-glucose showed any selectivity. To test further the antiherpes action of compound **13**, the production of new infectious HSV-1 was measured by the plaque assay method. Figure 2 shows that 12 μM of compound **13** reduced by 88% the production of new infectious HSV-1 and that at 100 μM the reduction was 99.6%. This latter value correlates well with the observed inhibition of protein glycosylation by 100 μM of **13**. The effect of compound **13** on cell multiplication was also examined. It was found that after 48-h incubation, 50 μM of **13** did not reduce at all the rate of HeLa cell growth, while a concentration of 100 μM reduced it by 15%. The same concentrations of **13** reduced by 98% and 99.6%, respectively, the production of infectious HSV-1.

The higher antiviral effect of compounds **13**, **18**, **19**, and **20** can be related to a favorable partition coefficient lipid/water, due to the presence of four benzyl or benzoyl hydroxyl protecting groups. The absence of protecting groups on the glucose hydroxyls, compound **23**, or the presence of protecting groups having very low (acetyl groups of **21**, **24**, and **25**) or very high (palmitoyl groups of **22**) lipophilic character gave compounds devoid of antiviral effect. Tunicamycin also has a fatty acid residue that may play a similar role. The antiviral effect of these UDP-hexose analogues only appeared when the three parts of the molecule, i.e., hexose, diphosphate-like five-atom bridge, and uridine, were present. Compounds **10** and **15**, which are fragments of molecule **13**, did not show any antiviral effect.

As regards to the antiviral spectrum of compound **13**, it was active against other enveloped viruses, such as vesicular stomatitis virus and Semliki Forest virus, and it had no effect on the growth of naked viruses such as poliovirus and encephalomyocarditis virus.[20] These results are in agreement with the idea that compound **13** is an inhibitor of protein glycosylation.

Since protein synthesis is not inhibited by compound **13**, glycosylation of viral proteins is inhibited to a greater extent than that of cellular proteins, this compound has no effect on cell multiplication while reducing the production of new infectious HSV-1, and the fact that **13** is active against enveloped viruses and inactive against naked viruses, it can be concluded that the present compound shows a selectivity in its mode of action not shown by the above-mentioned glycosylation inhibitors (2-deoxy-D-glucose and tunicamycin) and that the inhibition of viral replication is mediated by a blockade of the glycosylation of viral proteins.

Experimental Section

Melting points were determined on a Kofler hot-stage apparatus and are uncorrected. ^{1}H NMR spectra were recorded with a Varian XL-300 (300 MHz) and a Varian EM-390 spectrometer using Me_4Si as internal standard. UV absorption spectra were taken with a Perkin-Elmer 402 spectrophotometer. Optical rotations were determined with a Perkin-Elmer 141 polarimeter. Mass spectra were measured with a Hitachi Perkin-Elmer RMV-GMG spectrophotometer. Analytical TLC was performed on aluminum sheets coated with a 0.2-mm layer of silica gel 60 F_{254} purchased from Merck and preparative TLC on glass plates coated with a 2-mm layer of silica gel PF 254 (Merck).

Reaction of 2,3,4,6-Tetra-*O*-benzyl-α-D-glucopyranose (7) with Chlorosulfonyl Isocyanate (8) and 2′,3′-*O*-Isopropylideneuridine (11). A solution of 7 (3.24 g, 6 mmol) in dry methylene chloride (30 mL) cooled at −20 to −15 °C was treated in the absence of humidity with chlorosulfonyl isocyanate (0.52 mL, 6 mmol). The mixture was stirred at −20 to −15 °C until 7 disappeared and then a solution of 11 (1.13 g, 4 mmol) in dry acetonitrile (75 mL) containing dry pyridine (0.48 mL, 6 mmol) was added. The mixture was stirred at room temperature for 20 h and evaporated under reduced pressure, and the residue was chromatographed with $CHCl_3$/acetone (1:1) to afford one major and four minor bands, which were removed, and the compounds were extracted with EtOAc/MeOH (1:1).

1-*O*-Carbamoyl-2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranose (10). From the fastest running band 0.35 g (10%) of 10 was obtained: mp 96–97 °C (from MeOH–hexane); ^{1}H NMR [$(CD_3)_2SO$] δ 5.11 (br s, 2 H, NH_2, D_2O exchangeable), 6.23 (d, 1 H, H-1, $J_{1,2}$ = 3 Hz). Anal. ($C_{35}H_{37}NO_7$) C, H, N.

Compound 10 was also obtained as follows. To a solution of 7 (1.08 g, 2 mmol) in dry methylene chloride (20 mL) cooled at −20 to −15 °C was added 8 (0.18 mL, 2 mmol), and the resulting mixture was stirred at room temperature for 24 h. Then the reaction was concentrated under reduced pressure and the residue purified by preparative TLC with $CHCl_3$/acetone (10:1) to afford 10 (0.78 g, 67%), identical in all respects with that obtained before.

5′-*O*-[[[[(2″,3″,4″,6″-Tetra-*O*-benzyl-α-D-glucopyranosyl)oxy]carbonyl]amino]sulfonyl]-2′,3′-*O*-isopropylideneuridine (13). The major, fourth fastest running band gave 13 (1.48 g, 40%) as a white foam: ^{1}H NMR [$(CD_3)_2SO$, 300 MHz] δ 1.26 and 1.46 (2 s, 6 H, isopropylidene), 3.39–3.82 (m, 6 H, H-2″, H-3″, H-4″, H-5″, H-6″), 4.08 (m, 2 H, H-5′), 4.25 (m, 1 H, H-4′), 4.40–4.92 (m, 10 H, H-2′, H-3′, $CH_2C_6H_5$), 4.82 (dd, 1 H, H-3′, $J_{3',4'}$ = 2.2 Hz), 4.90 (dd, 1 H, H-2′, $J_{2',3'}$ = 6.5 Hz), 5.63 (d, 1 H, H-5, $J_{5,6}$ = 8.1 Hz), 5.87 (d, 1 H, H-1′, $J_{1',2'}$ = 2.5 Hz), 6.11 (d, 1 H, H-1″, $J_{1'',2''}$ = 3.5 Hz), 7.77 (d, 1 H, H-6), 11.50 (br s, 1 H, NH-3, D_2O exchangeable); UV λ_{max} (MeOH) 260 (ε 8150), 212 nm (18600). Anal. ($C_{47}H_{51}N_3O_{15}S$) C, H, N, S.

5′-*O*-Carbamoyl-2′,3′-*O*-isopropylideneuridine (14). The second fastest running band gave 0.078 g (6%) of 14: mp 184–186 °C (lit.[12] mp 178–179 °C); ^{1}H NMR [$(CD_3)_2SO$] δ 1.30 and 1.48 (2 s, 6 H, isopropylidene), 4.12 (m, 3 H, H-4′, H-5′), 4.73 (dd, 1 H, H-3′), 5.02 (dd, 1 H, H-2′), 5.62 (d, 1 H, H-5, $J_{5,6}$ = 8 Hz), 5.80 (d, 1 H, H-1′, $J_{1',2'}$ = 2.5 Hz), 6.53 (br s, 2 H, NH_2, D_2O exchangeable), 7.68 (d, 1 H, H-6), 11.30 (br s, 1 H, NH-3, D_2O exchangeable). Anal. ($C_{13}H_{17}N_3O_7$) C, H, N.

Compound 14 was also obtained as follows. To a mixture of 11 (0.57 g, 2 mmol) in dry acetonitrile (50 mL) and triethylamine (0.25 mL) cooled at −20 to −15 °C was added chlorosulfonyl isocyanate (0.2 mL, 2.2 mmol). The reaction was stirred at room temperature for 24 h and worked up as before. After preparative TLC using $CHCl_3$/MeOH (14:1) and crystallization from acetone–hexane, compound 14 (0.27 g, 40%) was obtained.

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5′-*O*-Sulfamoyl-2′,3′-*O*-isopropylideneuridine (15). The third fastest running band yielded 0.1 g (7%) of 15 as a white foam: ^{1}H NMR [$(CD_3)_2SO$] δ 1.30 and 1.50 (2 s, 6 H, isopropylidene), 4.20 (m, 3 H, H-4′, H-5′), 5.62 (d, 1 H, H-5, $J_{5,6}$ = 8 Hz), 5.82 (d, 1 H, H-1′, $J_{1',2'}$ = 2.5 Hz), 7.67 (d, 1 H, H-6). Anal. ($C_{12}H_{17}N_3O_8S$) C, H, S; N: calcd, 11.57; found, 10.27.

From the slowest running band, traces of compound 17 were obtained: ^{1}H NMR [$(CD_3)_2SO$] δ 1.30 and 1.50 (2 s, 12 H, isopropylidene), 4.03–4.30 (m, 6 H, H-4′, H-5′), 4.75 (dd, 1 H, H-3′, $J_{2',3'}$ = 6.5 Hz, $J_{3',4'}$ = 3.5 Hz), 4.78 (dd, 1 H, H-3′), 4.92 (dd, 2 H, H-2′), 5.58 (d, 1 H, H-5, $J_{5,6}$ = 8.5 Hz), 5.60 (d, 1 H, H-5), 5.83 (d, 2 H, H-1′, $J_{1',2'}$ = 2.5 Hz), 7.72 (d, 1 H, H-6), 7.77 (d, 1 H, H-6); MS, *m/e* 363, (*i*-Pr-Ur-5′-O-SO_2NH_2, 2%), 347 (*i*-Pr-Ur-5′-O-SO_2, 2%), 326 (*i*-Pr-Ur-5′-OCONH, 2%).

1-*O*-[[(Aminosulfonyl)amino]carbonyl]-2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranose (12). To a solution of 7 (1.62 g, 3 mmol) in dry methylene chloride (25 mL) cooled at −20 to −15 °C was added chlorosulfonyl isocyanate (0.26 mL, 3 mmol). After the solution was stirred for 1 h, a saturated solution of dry ammonia in methylene chloride (10 mL) was added and the resulting mixture was stirred at room temperature for 3 h. Then the solution was evaporated to dryness and the residue chromatographed with $CHCl_3$/MeOH (20:1) to give 12 (1 g, 53%) as a white foam: ^{1}H NMR [$(CD_3)_2SO$] δ 3.30 (br s, 2 H, NH_2, D_2O exchangeable), 6.18 (d, 1 H, H-1, $J_{1,2}$ = 3 Hz), 6.28 (br s, 1 H, NH, D_2O exchangeable). Anal. ($C_{35}H_{38}N_2O_9S$) C, H, N, S.

5′-*O*-[[[[(2″,3″,4″,6″-Tetra-*O*-benzyl-α-D-glucopyranosyl)oxy]carbonyl]amino]sulfonyl]uridine (18). A suspension of 13 (0.93 g, 1 mmol) in a mixture of TFA/water (5:1) (4 mL) was stirred at room temperature for 1 h. The solvent was evaporated in vacuo (bath temperature below 30 °C), and the residue was coevaporated three times with absolute EtOH and chromatographed with $CHCl_3$/MeOH (7:1) as eluent to give 18 (0.52 g, 58%) as a white foam: ^{1}H NMR [$(CD_3)_2SO$] δ 4.33–4.88 (m, 8 H, $CH_2C_6H_5$) 5.18 (br s, 1 H, OH, D_2O exchangeable), 5.35 (br s, 1 H, OH, D_2O exchangeable), 5.60 (d, 1 H, H-5, $J_{5,6}$ = 8.5 Hz), 5.82 (d, 1 H, H-1′, $J_{1',2'}$ = 3.5 Hz), 6.08 (d, 1 H, H-1″, $J_{1'',2''}$ = 3 Hz), 7.83 (d, 1 H, H-6), 11.23 (br s, 1 H, NH-3, D_2O exchangeable). Anal. ($C_{44}H_{47}N_3O_{15}S$) H, N, S; C: calcd, 59.39; found, 58.20.

5′-*O*-[[[[(2″,3″,4″,6″-Tetra-*O*-benzoyl-α-D-glucopyranosyl)oxy]carbonyl]amino]sulfonyl]-2′,3′-*O*-isopropylideneuridine (19). Following the procedure described for 13, a solution of 2,3,4,6-tetra-*O*-benzoyl-α-D-glucopyranose (0.596 g, 1 mmol) in acetonitrile (10 mL) reacted with 8 (0.09 mL, 1 mmol) and 11 (0.284 g, 1 mmol) in acetonitrile (50 mL) containing pyridine (0.08 mL, 1 mmol) to afford, after preparative TLC purification using EtOAc/MeOH (8:1) as solvent, compound 19 (0.27 g, 27%): mp 166–167 °C (from methanol/hexane); ^{1}H NMR [$(CD_3)_2SO$] δ 1.26 and 1.46 (2 s, 6 H, isopropylidene), 4.0–4.3 (m, 3 H, H-4′, H-5′), 5.60 (d, 1 H, H-5), $J_{5,6}$ = 8.0 Hz), 5.83 (d, 1 H, H-1′, $J_{1',2'}$ = 1.5 Hz) 6.35 (d, 1 H, H-1″, $J_{1'',2''}$ = 4.0 Hz), 11.15 (br s, 1 H, NH-3); UV λ_{max} (MeOH) 263 (ε 11 000), 232 nm (32 500). Anal. ($C_{47}H_{43}N_3O_{19}S$) C, H, N, S.

5′-*O*-[[[[(2″,3″,4″,6″-Tetra-*O*-benzoyl-α-D-glucopyranosyl)oxy]carbonyl]amino]sulfonyl]uridine (20). Compound 19 (0.985 g, 1 mmol) reacted with TFA/water as described for the obtention of 18 to give, after preparative TLC with EtOAc/MeOH (8:1), compound 20 (0.52 g, 55%): mp 173–174 °C (from methanol/ethyl ether); ^{1}H NMR [$(CD_3)_2SO$] δ 4.00 (m, 5 H, H-2′, H-3′, H-4′, H-5′), 5.60 (d, 1 H, H-5, $J_{5,6}$ = 8.5 Hz), 5.80 (d, 1 H, H-1′, $J_{1',2'}$ = 5 Hz), 6.36 (d, 1 H, H-1″, $J_{1'',2''}$ = 4 Hz); UV λ_{max} (MeOH) 263 (ε 10 650), 230 nm (35 750). Anal. ($C_{44}H_{39}N_3O_{19}S$) C, H, N, S.

5′-*O*-[[[[(2″,3″,4″,6″-Tetra-*O*-acetyl-α-D-glucopyranosyl)oxy]carbonyl]amino]sulfonyl]-2′,3′-*O*-isopropylideneuridine (21). 2,3,4,6-Tetra-*O*-acetyl-α-D-glucopyranose (0.348 g, 1 mmol) reacted with 8 and 11 as described for 19 to give 21 (0.18 g, 24%): mp 172–174 °C (from EtOAc/hexane); ^{1}H NMR [$(CD_3)_2SO$] δ 1.27 and 1.47 (2 s, 6 H, isopropylidene), 1.95, 1.97 (2 s, 12 H, AcO), 4.00–4.30 (m, 6 H, H-4′, H-5′, H-5″, H-6″), 5.57 (d, 1 H, H-5, $J_{5,6}$ = 8.5 Hz), 5.84 (d, 1 H, H-1′, $J_{1',2'}$ = 2 Hz), 5.97 (d, 1 H, H-1″, $J_{1'',2''}$ = 4 Hz), 7.73 (d, 1 H, H-6), 11.0 (br s, 1 H, NH-3); UV λ_{max} (MeOH) 258 (ε 8100), 209 nm (7200). Anal. ($C_{27}H_{35}N_3O_{19}S$) C, H, N, S.

5′-*O*-[[[[(2″,3″,4″,6″-Tetra-*O*-palmitoyl-α-D-glucopyranosyl)oxy]carbonyl]amino]sulfonyl]-2′,3′-*O*-isopropylideneuridine (22). 2,3,4,6-Tetra-*O*-palmitoyl-α-D-glucopyranose (1.132 g, 1 mmol) reacted with 8 and 11 as described for 19 to give 22 (0.47 g, 31%) as a white foam chromatographically homogeneous: ^{1}H NMR [$(CD_3)_2SO$] δ 0.85 (m, 12 H, $CH_3(CH_2)_{13}CH_2CO$), 1.23–1.49 (m, 11 OH, $CH_3(CH_2)_{13}CH_2CO$, isopropylidene), 2.03–2.28 (m, 8 H, $CH_3(CH_2)_{13}CH_2CO$), 5.64 (d, 1 H, H-5, $J_{5,6}$ = 7.9 Hz), 5.82 (m, 2 H, H-1′, H-1″), 7.70 (d, 1 H, H-6); UV λ_{max} ($CHCl_3$) 258 nm (ε 10 550). Anal. ($C_{83}H_{147}N_3O_{19}S$) C, H, N, S.

5′-*O*-[[[[(α-D-Glucopyranosyl)oxy]carbonyl]amino]sulfonyl]-2′,3′-*O*-isopropylideneuridine (23). A solution of 21 (0.737 g, 1 mmol), in saturated methanolic ammonia (50 mL) was stirred at room temperature for 2 h. Then the solvents were removed under reduced pressure, and the residue was chromatographed with EtOAc/MeOH (6:1) as eluent to give 23 (0.307 g, 54%) as a chromatographically homogeneous hygroscopic syrup: ^{1}H NMR [$(CD_3)_2SO$] δ 1.27 and 1.48 (2 s, 6 H, isopropylidene), 4.05 (m, 2 H, H-5′), 4.22 (m, 1 H, H-4′), 5.60 (d, 1 H, H-5, $J_{5,6}$ = 8.5 Hz), 5.72 (d, 1 H, H-1″, $J_{1'',2''}$ = 4.5 Hz), 5.85 (d, 1 H, H-1′, $J_{1',2'}$ = 3 Hz), 7.75 (d, 1 H, H-6); UV λ_{max} (MeOH) 260 nm (ε 5100); MS, *m/e* 551 ($M^+ - 18$, 7), 458 ($M^+ - 111$, 3), 362 (3), 303 (3), 295 (2), 279 (2), 267 (15), 251 (5), 221 (4), 207 (8), 191 (6), 179 (2), 176 (6).

5′-*O*-[[[[(2″-Acetamido-3″,4″,6″-tri-*O*-acetyl-2″-deoxy-α-D-glucopyranosyl)oxy]carbonyl]amino]sulfonyl]-2′,3′-*O*-isopropylideneuridine (24). Reaction of 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranose (2.42 g, 7 mmol) in dry acetonitrile (50 mL) with 8 (0.6 mL, 7 mmol) and a solution of 11 (1.99 g, 7 mmol) in dry acetonitrile (120 mL) and dry pyridine (0.56 mL) and workup as described above for the synthesis of 13 afforded, after preparative TLC with EtOAc/MeOH (10:1), compound 24 (1.82 g, 43%) as an amorphous solid chromatographically homogeneous: ^{1}H NMR [$(CD_3)_2SO$, 300 MHz] δ 1.28 and 1.48 (2 s, 6 H, isopropylidene), 1.79 (s, 3 H, AcNH), 1.92, 1.98, 2.00 (3 s, 9 H, AcO), 3.93–4.28 (m, 6 H, H-4′, H-5′, H-5″, H-6″), 4.85 (dd, 1 H, H-3′, $J_{2',3'}$ = 3 Hz, $J_{3',4'}$ = 6.1 Hz), 4.93 (dd, 1 H, H-2″), 4.98 and 5.20 (2 t, 2 H, H-3″, H-4″, *J* = 10 Hz), 5.61 (d, 1 H, H-5, $J_{5,6}$ = 8.0 Hz), 5.72 (d, 1 H, H-1″, $J_{1'',2''}$ = 3.4 Hz), 5.85 (d, 1 H, H-1′, $J_{1',2'}$ = 2.5 Hz), 7.75 (d, 1 H, H-6), 8.11 (d, 1 H, AcNH, $J_{NH,2''}$: 9.1 Hz), 11.43 (br s, 1 H, NH-3, D_2O exchangeable); UV λ_{max} (MeOH) 260 (ε 7250), 211 nm (6050); MS, *m/e* 736 (M^+). Anal. ($C_{27}H_{36}N_4O_{18}S$) C, H, N, S.

5′-*O*-[[[[(2″-Acetamido-3″,4″,6″-tri-*O*-acetyl-2″-deoxy-α-D-glucopyranosyl)oxy]carbonyl]amino]sulfonyl]uridine (25). Compound 24 (0.736 g, 1 mmol) reacted with TFA/water as described for the obtention of 18 and 20 to give 25 (0.39 g, 56%) as a white, chromatographically homogeneous foam: ^{1}H NMR [$(CD_3)_2SO$] δ 1.78 (s, 3 H, AcNH), 1.90, 1.96, 1.98 (3 s, 9 H, AcO), 3.40–4.20 (m, 9 H, H-2′, H-3′, H-4′, H-5′, H-2″, H-5″, H-6″), 4.90 and 5.19 (2 t, 2 H, H-3″, H-4″, *J* = 9.5 Hz), 5.65 (d, 1 H, H-5, $J_{5,6}$ = 8 Hz), 5.75 (d, 1 H, H-1″, $J_{1'',2''}$ = 3 Hz), 5.82 (d, 1 H, H-1′, $J_{1',2'}$ = 5 Hz), 7.74 (d, 1 H, H-6), 7.96 (d, 1 H, NH, $J_{NH-2''}$ = 8 Hz). Anal. ($C_{24}H_{32}N_4O_{18}S$) C, H, N, S.

Cells and Viruses. HeLa cells were grown in Dulbecco's modified Eagle's medium, DMEM (Gibco), supplemented with 10% new born calf serum (Gibco). Herpes simplex virus type 1 (HSV-1) strain KOS was grown and titrated in african green monkey kidney cells (vero cells).

Estimation of the Cytopathic Effect. Monolayers of HeLa cells were infected with HSV-1 at a multiplicity (moi) of 0.5 pfu (plaque forming unit) per cell, in the presence of several concentrations of the compound to be tested. After 48-h incubation at 37 °C, the cell monolayer was examined under a phase-contrast microscope and the cytopathic effect was recorded. Toxicity was also estimated under the microscope in uninfected cells.

[^{3}H]Glucosamine Labeling and Polyacrylamide Gel Electrophoresis. HeLa cell monolayers were infected with HSV-1 (moi, 10 pfu/cell) in presence or absence of 100 μM of compound 13. After 4 h at 37 °C, a 4-h pulse was given with 10 μCi/mL of [^{3}H]glucosamine (35 Ci/mmol, The Radiochemical Centre) in glucoseless medium. Then the medium was removed and the cell monolayer was washed once with 1 mL of saline phosphate buffer, and the cells were dissolved in 0.1 mL of sample buffer [62.5 mM Tris-HCl (pH 6.8), 2% sodium dodecyl sulfate,

0.1 M dithiothreitol, 17% glycerol]. Each sample was sonicated to reduce viscosity and heated to 90 °C for 5 min. Samples (7 μL each) were analyzed by polyacrylamide gel electrophoresis, using 15% acrylmide gels. The gels were run overnight at 30 V, and fluorography was carried out with 2,5-diphenyloxazole–dimethyl sulfoxide (20% wt/wt). The dried gels were exposed with use of XS-5 X-ray films (Kodak). Densitometric profiles of the films were performed in an Optronics P 1700 microdensitometer.

Assay of Infections Units. HeLa cells were infected with HSV-1 at a multiplicity of 0.5 pfu/cell in the presence of several concentrations of compound 13. Forty-eight-hour postinfection cells were collected and lysed by sonication with a MSE sonicator. Serial dilutions were made in saline phosphate buffer containing 1% calf serum. Vero cell monolayers were infected with 0.5 mL of each virus dilution. After 1 h at 37 °C, the cells were overloaded with DMEM containing 0.7% agar and 1% calf serum and incubated at 37 °C. After 4 days the medium was removed and 0.5 mL of 5% trichloroacetic acid was added over the monolayer, and plaques were counted.

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Metabolism of Phencyclidine. The Role of the Carbinolamine Intermediate in the Formation of Lactam and Amino Acid Metabolites of Nitrogen Heterocycles

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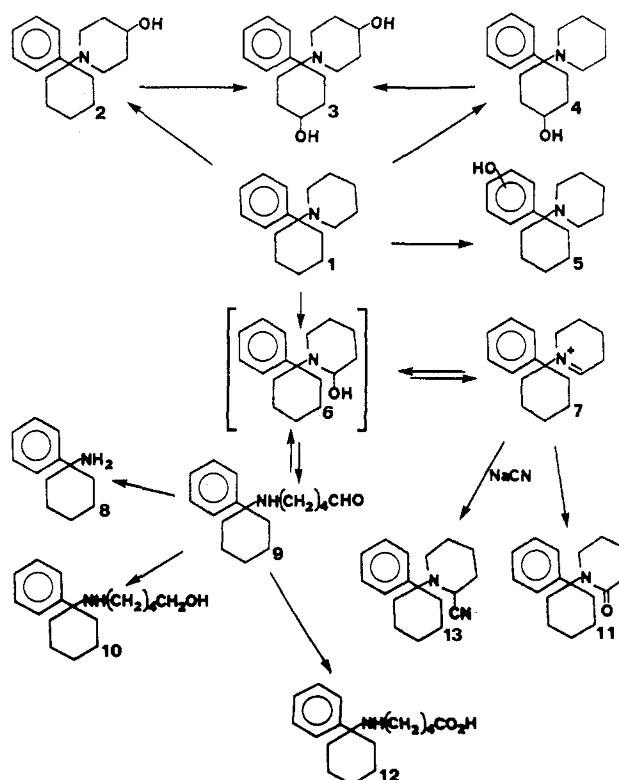
The transformation of phencyclidine in a mouse liver microsome preparation to several oxidative metabolites was studied. With use of GLC and HPLC methods with internal standards, phencyclidine and six metabolites were quantitated and the amino acid 12, resulting from the α-oxidation of the piperidine ring, was produced in 10–50 times greater amounts than the other metabolites. While most piperidines and pyrrolidines produce an amino acid and a corresponding lactam, it was found that phencyclidine was not converted to the lactam 11.

The metabolism of phencyclidine (1) has been of considerable interest because some of the delayed-onset effects or biphasic effects of the drug have been attributed to metabolites (of unknown structure) that may have a pharmacological profile very distinct from that of phencyclidine itself. In man, acute intoxication is characterized by hypotension, but the blood pressure may rise above normal 2 or 3 days later.[1] A schizophreniform condition appears in a small number of the subjects and the psychosis may persist for several weeks.[2–5]

Previous studies with several species including man have shown that phencyclidine is metabolized to small quantities of *cis*- and *trans*-4-phenyl-4-piperidinocyclohexanol (4)[6,7] and 1-(1-phenylcyclohexyl)-4-hydroxypiperidine (2),[6] and the structures of these metabolites were verified by comparisons to synthetic standards. GC–mass spectral data have been utilized to tentatively identify the dihydroxy metabolite 3 and the phenol 5.[8]

The major metabolic pathway for phencyclidine appears to be through oxidation of the α-carbon of the piperidine ring. The major metabolite of phencyclidine was first detected in dogs and found to be 5-[*N*-(1-phenylcyclohexyl)amino]pentanoic acid (12).[9] This amino acid metabolite has also been detected in the urine of humans,[10] as well. Other metabolites arising from the α-oxidation route that have been detected are the amine[8] (8) and the amino alcohol[11] (10) metabolites.



Most of the studies in the literature on the metabolism of piperidines and pyrrolidines have shown that this class of compounds undergo α-oxidation leading to the formation of a lactam and the corresponding amino acid. For

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