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THE SYNTHESIS AND CYTOTOXIC ACTIVITY OF SOME HALOACETAMIDOALKYL β-D-XYLOPYRANOSIDES

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ABSTRACT

2-Hydroxyethyl 2,3,4-tri-*O*-acetyl-β-D-xylopyranoside was prepared from 2,3,4-tri-*O*-acetyl-α-D-xylopyranosyl chloride by the action of 1,2-ethanediol and mercuric acetate. Subsequent mesylation and azide displacement gave 2-azidoethyl 2,3,4-tri-*O*-acetyl-β-D-xylopyranoside, which was hydrogenated over palladium-on-charcoal and the amine acylated with various haloacetyl halides, to afford 2-(haloacetamido)ethyl 2,3,4-tri-*O*-acetyl-β-D-xylopyranosides. Deprotection to obtain the free sugars was carried out with 5mM ethanolic sodium ethoxide. 2-(Chloroacetamido)ethyl 2,3,4-tri-*O*-acetyl-β-D-xylopyranoside was further modified by sequential azide displacement, hydrogenation, and subsequent acylation with various haloacetyl halides to afford 2-[(haloacetamido)acetylamino]ethyl 2,3,4-tri-*O*-acetyl-β-D-xylopyranosides, which were also deprotected to give the corresponding free sugars. The effects of these haloacetamido analogs on the growth of the melanoma cells in tissue culture was evaluated.

INTRODUCTION

The chemistry and structure of glycosaminoglycuronoglycans have been recently reviewed[1]. These macromolecules are important components of mammalian cells[2], acting in the maintenance and regulation of cellular functions, as well as providing intercellular support[3]. Their occurrence in both animal[4] and human[5] tumor tissue is of particular interest, since recent findings suggest that glycosaminoglycuronoglycans may be important in the metastatic process[6–8]. Furthermore, glycosaminoglycuronoglycans have also been reported to have growth-promoting effects on neoplastic cells[9]. Proteoglycans consist of protein and glycosaminoglycuronoglycans chain covalently bound. The biosynthesis of the linkage region connecting the glycosaminoglycuronoglycans to the protein core appears to be identical for chondroitin 4-sulfate[10,11], chondroitin 6-sulfate[12], dermatan sulfate[13], and heparan sulfate[14]. This process involves the stepwise transfer of various monosaccharide units from uridine nucleotide sugars *via* specific glycosyltransferases, the initial step being the formation of an *O*-β-D-xylopyranosyl-L-serine linkage[15]. Some β-D-xylose

derivatives have been demonstrated to function as initiators in the biosynthesis of glycosaminoglycuronoglycan chains in the presence of inhibitors of protein synthesis with both particulate enzyme systems[16] and a variety of neoplastic cell lines in culture[17,18]. For example, Robinson *et al.*[19] showed with a particulate enzyme system that a variety of alkyl β-D-xylopyranosides, such as butyl β-D-xylopyranoside, produced a marked stimulation of glucosaminoglycuronoglycan synthesis. These observations imply that some β-D-xylopyranoside derivatives are capable of acting as acceptors for D-galactosyltransferase I, the enzyme that catalyzes the transfer of D-galactose from UDP-D-galactose to the D-xylosylserine protein core.

Since it is well documented that haloacetamido derivatives act as active-site-directed irreversible enzyme inhibitors[20,21], a series of haloacetamidoalkyl β-D-xylopyranosides with the potential to function as inhibitors of the formation of glycosaminoglycuronoglycans have been synthesized. This work is part of a program directed towards a study of the cellular synthesis and release of glycosaminoglycuronoglycans in relation to tumor-cell growth and metastasis, and describes the synthesis and growth-inhibitory activity in culture of some of the compounds prepared.

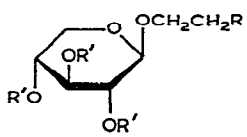
RESULTS AND DISCUSSION

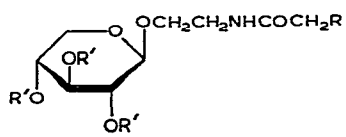
Synthesis of the desired haloacetamido glycosides was based on the initial preparation of an alkyl β-D-xylopyranoside containing a terminally reactive functional group. Thus, 2-hydroxyethyl 2,3,4-tri-*O*-acetyl-β-D-xylopyranoside (**1**) was prepared, by a slight modification of the procedure of Lindberg[22], from 2,3,4-tri-*O*-acetyl-α-D-xylopyranosyl chloride[23] in 80% yield. The structure of **1** was supported by its 270-MHz ^{1}H-n.m.r. spectrum, which showed the H-1 resonance as a doublet ($J_{1,2}$ 7.0 Hz) indicative of the β-D configuration. Furthermore, the $J_{4,5e}$ and $J_{5e,5a}$ coupling constants (5.1 and −11.8 Hz, respectively) indicate that the $^4C_1$ conformation preponderates[24]. Conversion of **1** into the corresponding 2-mesyloxyethyl 2,3,4-tri-*O*-acetyl-β-D-xylopyranoside (**2**) was achieved in 76% yield. Further treatment of **2** with sodium azide in *N*,*N*-dimethylformamide gave, in 88% yield, the crystalline azide **3** which was subsequently hydrogenated in ethanol solution to 2-aminoethyl 2,3,4-tri-*O*-acetyl-β-D-xylopyranoside (**4**), obtained in crystalline form in 80% yield.

Synthesis of the desired haloacetamido analogs was achieved by the procedure of Chang and Kyi[25], which involved treatment of **4** in the presence of chloroform and sodium hydrogencarbonate with the appropriate haloacetyl halides. The chloroacetamido **5**, bromoacetamido **7**, and dichloroacetamido **9** derivatives were all obtained as crystalline materials in yields of 86, 83, and 74%, respectively. The structures of **5**, **7**, and **9** were supported by their n.m.r. spectra which showed singlets due to the halo-methylene and -methine protons as expected. Subsequent deprotection with 5mM ethanolic sodium ethoxide gave in good yield **6**, **8**, and **10**, respectively, as crystalline materials.

Since the reactive portions of an enzyme are not limited to those amino acid residues involved in the catalytic center, but extend over a larger part of the molecule[26],

we were also interested in preparing haloacetamido derivatives linked to the D-xylose component by a longer chain. We took advantage of the reactive chloromethylene group of **5** and, by nucleophilic displacement with azide anion, introduced a group suitable for subsequent modification. Compounds containing two amido groups may be expected to interact more strongly with binding sites of the enzyme. Accordingly, treatment of **5** in *N,N*-dimethylformamide with sodium azide afforded the azidoacetamido **11** as a crystalline material in 79% yield. Subsequent hydrogenation afforded 2-(aminoacetamido)ethyl 2,3,4-tri-*O*-acetyl-β-D-xylopyranoside (**12**) as a crystalline material in 83% yield. Synthesis of the derivatives, performed as indicated previously, gave crystalline chloroacetamido **13**, bromoacetamido **15**, and dichloroacetamido **17**, in yields of 89, 57, and 66%, respectively; the structure of these compounds was supported by their 270-MHz ^{1}H-n.m.r. spectra. Base-catalyzed hydrolysis gave the chloroacetamido **14** as a crystalline product in 83% yield, and the corresponding bromoacetamido **16** and dichloroacetamido **18** as syrupy materials in 80 and 76% yields, respectively.





1 R = OH, R′ = Ac
2 R = OMs, R′ = Ac
3 R = N_3, R′ = Ac
4 R = NH_2, R′ = Ac
5 R = $NHCOCH_2Cl$, R′ = Ac
6 R = $NHCOCH_2Cl$, R′ = H
7 R = $NHCOCH_2Br$, R′ = Ac
8 R = $NHCOCH_2Br$, R′ = H
9 R = $NHCOCHCl_2$, R′ = Ac
10 R = $NHCOCHCl_2$, R′ = H

11 R = N_3, R′ = Ac
12 R = NH_2, R′ = Ac
13 R = $NHCOCH_2Cl$, R′ = Ac
14 R = $NHCOCH_2Cl$, R′ = H
15 R = $NHCOCH_2Br$, R′ = Ac
16 R = $NHCOCH_2Br$, R′ = H
17 R = $NHCOCHCl_2$, R′ = Ac
18 R = $NHCOCHCl_2$, R′ = H

The growth-inhibitory activity of some of the derivatives prepared was evaluated against B16 melanoma cells in culture. This cell line was selected as it is known to produce glycosaminoglycuronoglycans[27] and shows metastatic properties *in vivo* that can be measured[28]. The free sugars **6**, **8**, and **10**, each tested up to a concentration of 100 μM, did not produce any inhibition of cell proliferation (Table I). In contrast, the peracetylated derivatives **5** and **7** exhibited significant growth-inhibitory properties, with ID_{50}* values of 60 and 7 μM, respectively. The dichloro derivatives **9** and **17** produced little or no inhibition of cellular growth at concentrations up to 100 μM. While the bromoacetamido derivative **15** was less active than its shorter chain counterpart **7**, the chloroacetamido derivative **13** produced a level of inhibition similar to that

*The ID_{50} is the concentration necessary to inhibit cell replication by 50%.

TABLE I

EFFECT OF HALOACETAMIDOALKYL β-D-XYLOPYRANOSIDES ON THE GROWTH OF B16 MELANOMA CELLS IN CULTURE

Compound	*Concentration (μM)*[a]	*Inhibition (%)*
6	100	0
8	100	0
10	100	0
5[b]	60	50
7[b]	7	50
9	100	20
13[b]	70	50
15	20	25
17	100	0

[a]All compounds were tested up to a maximum concentration of either 100 or 20μM. [b]The 50%-inhibitory level (ID_{50}) was determined graphically.

of the shorter chain derivative **5**. Since the acetylated methanesulfonate **2** and azido **3** derivatives did not produce inhibition of cell growth up to a concentration of 100μM, the inhibitory activities of **5** and **7** cannot be attributed solely to the presence of the acetyl groups. The acetyl groups, however, were necessary for cytotoxicity, as the respective free sugars were inactive at similar concentrations. The greater inhibitory activity of the peracetates **5** and **7** compared to their corresponding free sugars suggests that differences in transport properties may be involved.

EXPERIMENTAL

General methods. — All evaporations were performed under reduced pressure. Melting points were determined with a Thomas–Hoover capillary melting point apparatus and are uncorrected. *N*,*N*-Dimethylformamide was dried over calcium hydride and then distilled under diminished pressure. Column chromatography was performed on Silica gel (Merck 7734, 70–235 mesh, E. Merck A.G., Darmstadt, Germany). All reactions were monitored by t.l.c. on Silica gel G (Merck). Petroleum ether refers to a fraction having b.p. 35–60°. Elemental analyses were performed and optical rotations determined by Baron Consulting Company, Orange, Connecticut, U.S.A.

*2-Hydroxyethyl 2,3,4-tri-*O*-acetyl-β-*D*-xylopyranoside* (**1**). — To a mixture of 2,3,4-tri-*O*-acetyl-α-D-xylopyranosyl chloride (1 g) and mercuric acetate (0.5 g) was added 1,2-ethanediol (10 mL). The solution was heated to 45° for 0.5 h when t.l.c. (2:1, v/v, petroleum ether–ethyl acetate) indicated the reaction to be complete. The solution diluted with water (20 mL) was extracted with chloroform (3 × 20 mL). The chloroform layer was then re-extracted with water (2 × 20 mL) and, after evaporation, the product dissolved in ethyl acetate was allowed to percolate through

a column of Chelex-100 (Na^+) chelating resin, to remove the excess of mercuric ions. The solution was then evaporated, and the product was chromatographed on silica gel with 2:1 (v/v) ethyl acetate–petroleum ether as eluent to give **1** (0.74 g, 80%) as a crystalline product from ethyl acetate–petroleum ether, m.p. 88–89°, $[\alpha]_D^{23}$ −29° (*c* 1 chloroform); n.m.r. ($CDCl_3$): δ 5.20 (t, 1 H, $J_{3,4}$ 8.8 Hz, H-3), 4.96 (m, 2 H, $J_{2,3}$ 8.8, $J_{4,5e}$ 5.1, $J_{4,5a}$ 9.2 Hz, H-2, -4), 4.52 (d, 1 H, $J_{1,2}$ 7.0 Hz, H-1), 4.15 (dd, 1 H, $J_{5e,5a}$ −11.8 Hz, H-5e), 3.80 (m, 5 H, -CH_2CH_2-OH), 3.38 (dd, H-5a), and 2.07, 2.05, 2.04 (OAc).

Anal. Calc. for $C_{13}H_{20}O_9$: C, 48.75; H, 6.25. Found: C, 48.78; H, 5.98.

2-Mesyloxyethyl 2,3,4-tri-O-acetyl-β-D-xylopyranoside (**2**). — To a solution of **1** (4.9 g) in pyridine (40 mL) cooled to −10° was added methanesulfonyl chloride (2.1 g). After maintaining the reaction temperature at 0° for 16 h, t.l.c. (2:1, v/v, ethyl acetate–petroleum ether) indicated completion of the reaction, and the solution diluted with chloroform (200 mL) was extracted with water (200 mL). The chloroform layer was then evaporated together with water to remove the residual pyridine. The product crystallized from ethanol, affording **2** (4.66 g, 76%) as a fine, white crystalline material, m.p. 105–106°, $[\alpha]_D^{23}$ −30° (*c* 1, chloroform); n.m.r. ($CDCl_3$): δ 5.18 (t, 1 H, $J_{3,4}$ 8.8 Hz, H-3), 4.94 (m, 2 H, $J_{2,3}$ 8.8, $J_{4,5e}$ 5.1, $J_{4,5a}$ 9.8 Hz, H-2, -4), 4.54 (d, 1 H, $J_{1,2}$ 7.0 Hz, H-1), 4.36 (m, 2 H, -CH_2CH_2OMs), 4.14 (dd, 1 H, $J_{5e,5a}$ −11.8 Hz, H-5e), 4.05 and 3.80 (m, 1 H, -CH_2CH_2OMs), 3.39 (dd, H-5a), 3.04 (OMs), and 2.08, 2.06, 2.05 (OAc).

Anal. Calc. for $C_{14}H_{22}O_{11}S$: C, 42.21; H, 5.53; S, 8.04. Found: C, 42.50; H, 5.64; S, 7.67.

2-Azidoethyl 2,3,4-tri-O-acetyl-β-D-xylopyranoside (**3**). — To a solution of **2** (1 g) in *N,N*-dimethylformamide (5 mL) was added sodium azide (0.5 g). The reaction mixture was heated to 65° for 1.5 h, whereupon t.l.c. (3:1, v/v, petroleum ether–ethyl acetate) showed one product. After being cooled, the solution was diluted with ethyl acetate (20 mL) and extracted with water (2 × 20 mL). The combined aqueous layers were then re-extracted with ethyl acetate (20 mL), and the combined organic phases were dried ($MgSO_4$) and evaporated to give a crystalline product. Recrystallization from ethyl acetate–petroleum ether afforded **3** (0.76 g, 88%), m.p. 102.5–104°, $[\alpha]_D^{23}$ −116° (*c* 1, chloroform); n.m.r. ($CDCl_3$): δ 5.17 (t, 1 H, $J_{3,4}$ 8.4 Hz, H-3), 4.95 (m, 2 H, $J_{2,3}$ 8.4, $J_{4,5e}$ 5.1, $J_{4,5a}$ 8.8 Hz, H-2, -4), 4.57 (d, 1 H, $J_{1,2}$ 6.6 Hz, H-1), 4.15 (dd, 1 H, $J_{5e,5a}$ −11.8 Hz, H-5e), 3.99 and 3.67 (m, 1 H, -$CH_2CH_2N_3$), 3.40 (dd, H-5a), 3.39 (m, 2 H, -$CH_2CH_2N_3$), and 2.07, 2.06, 2.04 (OAc).

Anal. Calc. for $C_{13}H_{19}N_3O_8$: C, 45.22; H, 5.51; N, 12.17. Found: C, 45.48; H, 5.61; N, 12.43.

2-Aminoethyl 2,3,4-tri-O-acetyl-β-D-xylopyranoside (**4**). — A solution of **3** (4 g) in ethanol (120 mL) was hydrogenated in the presence of 10% palladium-on-charcoal at a pressure of 2 atm for 16 h, whereupon t.l.c. (4:1, v/v, ethyl acetate–methanol) indicated a single product. The reaction mixture was filtered, and the filtrate evaporated to a syrupy product, which spontaneously crystallized to afford **4** (2.96 g, 80%). This was recrystallized from ethanol–petroleum ether, m.p. 83–85°,

$[\alpha]_D^{23}$ −62° (*c* 1, chloroform); n.m.r. ($CDCl_3$): δ 5.18 (t, 1 H, $J_{3,4}$ 8.8 Hz, H-3), 4.95 (m, 2 H, $J_{2,3}$ 8.8, $J_{4,5e}$ 5.1, $J_{4,5a}$ 9.2 Hz, H-2, -4), 4.51 (d, 1 H, $J_{1,2}$ 7.0 Hz, H-1), 4.13 (dd, 1 H, $J_{5e,5a}$ −11.8 Hz, H-5e), 3.84 and 3.55 (m, 1 H, -$CH_2$$CH_2NH_2$), 3.37 (dd, H-5a), 2.87 (m, 2 H, -$CH_2CH_2NH_2$), 2.07, 2.05, 2.04 (OAc), and 1.74 (broad NH_2 signal, which is exchanged by D_2O).

Anal. Calc. for $C_{13}H_{21}NO_8$: C, 48.90; H, 6.58; N, 4.39. Found: C, 49.17; H, 6.34; N, 4.15.

*Acylation reactions of 2-aminoethyl 2,3,4-tri-*O*-acetyl-β-*D*-xylopyranoside* (**4**). — A solution of **4** in chloroform, cooled to 0–5° in the presence of sodium hydrogencarbonate, was treated with an excess of acylating reagent (chloroacetyl chloride, bromoacetyl bromide, and dichloroacetyl chloride). The reaction mixture was stirred at room temperature until t.l.c. (2:1, v/v, ethyl acetate–petroleum ether) indicated the reaction to be complete. The reaction mixture was then evaporated together with silica gel to form a powder, which was applied to a column of silica gel and eluted with 2:1 (v/v) ethyl acetate–petroleum ether.

*2-(Chloroacetamido)ethyl 2,3,4-tri-*O*-acetyl-β-*D*-xylopyranoside* (**5**). Yield 86%, m.p. 123–125°, $[\alpha]_D^{23}$ −65° (*c* 1, chloroform); n.m.r. ($CDCl_3$): δ 7.05 (broad NH signal), 5.19 (t, 1 H, $J_{3,4}$ 8.8 Hz, H-3), 4.96 (m, 2 H, $J_{2,3}$ 8.8, $J_{4,5e}$ 5.1, $J_{4,5a}$ 9.2 Hz, H-2, -4), 4.50 (d, 1 H, $J_{1,2}$ 7.3 Hz, H-1), 4.13 (dd, 1 H, $J_{5e,5a}$ −11.8 Hz, H-5e), 4.06 (s, 2 H, -CH_2Cl), 3.85 and 3.72 (m, 1 H, -CH_2CH_2NH-), 3.52 (m, 2 H, -CH_2CH_2NH-), 3.37 (dd, H-5a), and 2.07, 2.05, 2.04 (OAc).

Anal. Calc. for $C_{15}H_{22}ClNO_9$: C, 45.51; H, 5.56; Cl, 8.98; N, 3.54. Found: C, 45.32; H, 5.76; Cl, 9.16; N, 3.61.

*2-(Bromoacetamido)ethyl 2,3,4-tri-*O*-acetyl-β-*D*-xylopyranoside* (**7**). Yield 83%, m.p. 118–119°, $[\alpha]_D^{23}$ −55° (*c* 1, chloroform); n.m.r. ($CDCl_3$): δ 6.93 (broad NH signal), 5.19 (t, 1 H, $J_{3,4}$ 8.8 Hz, H-3), 4.96 (m, 2 H, $J_{2,3}$ 8.8, $J_{4,5e}$ 5.1, $J_{4,5a}$ 9.2 Hz, H-2, -4), 4.50 (d, 1 H, $J_{1,2}$ 7.3 Hz, H-1), 4.14 (dd, 1 H, $J_{5e,5a}$ −11.7 Hz, H-5e), 3.89 (s, 2 H, -CH_2Br), 3.85 and 3.72 (m, 1 H, -CH_2CH_2NH-), 3.49 (m, 2 H, -CH_2CH_2NH-), 3.37 (dd, H-5a), and 2.08, 2.06, 2.05 (OAc).

Anal. Calc. for $C_{15}H_{22}BrNO_9$: C, 40.92; H, 5.00; Br, 18.16; N, 3.18. Found: C, 41.16; H, 5.20; Br, 17.86; N, 3.26.

*2-(Dichloroacetamido)ethyl 2,3,4-tri-*O*-acetyl-β-*D*-xylopyranoside* (**9**). Yield 74%, m.p. 146–148°, $[\alpha]_D^{23}$ −43° (*c* 1, chloroform); n.m.r. ($CDCl_3$): δ 7.03 (broad NH signal), 5.93 (s, 1 H, -$CHCl_2$), 5.19 (t, 1 H, $J_{3,4}$ 8.8 Hz, H-3), 4.96 (m, 2 H, $J_{2,3}$ 8.8, $J_{4,5e}$ 5.1, $J_{4,5a}$ 8.8 Hz, H-2, -4), 4.50 (d, 1 H, $J_{1,2}$ 7.0 Hz, H-1), 4.14 (dd, 1 H, $J_{5e,5a}$ −11.8 Hz, H-5e), 3.81 (m, 2 H, -CH_2CH_2NH-), 3.53 (m, 2 H, -CH_2CH_2-NH-), 3.38 (dd, H-5a), and 2.07, 2.05, 2.04 (OAc).

Anal. Calc. for $C_{15}H_{21}Cl_2NO_9$: C, 41.86; H, 4.88; Cl, 16.51; N, 3.26. Found: C, 42.20; H, 5.11; Cl, 16.84; N, 3.00.

Base-catalyzed hydrolysis of **5**, **7**, *and* **9**. — Compounds in 5mM ethanolic sodium ethoxide were kept at room temperature for 16 h, whereupon t.l.c. (9:1, v/v, ethyl acetate–methanol) indicated completion of hydrolysis. The solutions were neutralized with AG 50W-X8 cation-exchange resin and, after filtration and evapora-

tion, the products were eluted from a column of silica gel with 9:1 (v/v) ethyl acetate–methanol:

2-(Chloroacetamido)ethyl β-D-xylopyranoside (**6**). Yield 88%, m.p. 101–103°, $[\alpha]_D^{23}$ −21° (*c* 1, methanol).

Anal. Calc. for $C_9H_{16}ClNO_6$: C, 40.07; H, 5.94; Cl, 13.17; N, 5.20. Found: C, 40.23; H, 5.73; Cl, 12.93; N, 4.94.

2-(Bromoacetamido)ethyl β-D-xylopyranoside (**8**). Yield 84%, m.p. 95–97°, $[\alpha]_D^{23}$ −0.4° (*c* 1, methanol).

Anal. Calc. for $C_9H_{16}BrNO_6$: C, 34.41; H, 5.10; Br, 25.45; N, 4.46. Found: C, 34.72; H, 5.22; Br, 25.09; N, 4.39.

2-(Dichloroacetamido)ethyl β-D-xylopyranoside (**10**). Yield 79%, m.p. 125–126.5°, $[\alpha]_D^{23}$ −44° (*c* 1, methanol).

Anal. Calc. for $C_9H_{15}Cl_2NO_6$: C, 35.53; H, 4.93; Cl, 23.36; N, 4.61. Found: C, 35.89; H, 5.06; Cl, 23.03; N, 4.41.

2-(Azidoacetamido)ethyl 2,3,4-tri-O-acetyl-β-D-xylopyranoside (**11**). — To a solution of **5** (7 g) in *N*,*N*-dimethylformamide (40 mL) was added sodium azide (3 g). The reaction mixture, heated to 90° for 1 h, was then cooled and processed as described for the preparation of **3**. The impure product obtained was eluted from a column of silica gel with 2:1 (v/v) ethyl acetate–petroleum ether to afford **11** (5.64 g, 79%), which crystallized from ethyl acetate–petroleum ether, m.p. 80–82°, $[\alpha]_D^{23}$ −45° (*c* 1, chloroform); n.m.r. ($CDCl_3$): δ 6.76 (broad NH signal), 5.20 (t, 1 H, $J_{3,4}$ 8.8 Hz, H-3), 4.96 (m, 2 H, $J_{2,3}$ 8.8, $J_{4,5e}$ 5.1, $J_{4,5a}$ 9.2 Hz, H-2, -4), 4.49 (d, 1 H, $J_{1,2}$ 7.0 Hz, H-1), 4.13 (dd, 1 H, $J_{5e,5a}$ −11.8 Hz, H-5e), 3.99 (s, 2 H, $-CH_2N_3$), 3.84 and 3.70 (m, 1 H, $-CH_2CH_2NH-$), 3.49 (m, 2 H, $-CH_2CH_2NH-$), 3.37 (dd, H-5a), and 2.08, 2.05 (9 H, OAc).

Anal. Calc. for $C_{15}H_{22}N_4O_9$: C, 44.78; H, 5.47; N, 13.93. Found: C, 44.42; H, 5.39; N, 13.59.

2-(Aminoacetamido)ethyl 2,3,4-tri-O-acetyl-β-D-xylopyranoside (**12**). — A solution of **11** (0.5 g) in ethanol (10 mL) was hydrogenated in the presence of 10% palladium-on-charcoal and processed as described for the preparation of **4**. The product was crystallized from ethanol–petroleum ether to afford **12** (0.39 g, 83%), m.p. 111–113°, $[\alpha]_D^{23}$ −42° (*c* 1, chloroform).

Anal. Calc. for $C_{15}H_{24}N_2O_9$: C, 47.87; H, 6.38; N, 7.45. Found: C, 48.04; H, 6.59; N, 7.39.

Acylation reactions of 2-(aminoacetamido)ethyl 2,3,4-tri-O-acetyl-β-D-xylopyranoside (**12**). — Compound **12** was acylated with chloroacetyl chloride, bromoacetyl bromide, and dichloroacetyl chloride according to the procedure outlined for the preparation of **5**, **7** and **9**:

2-[(Chloroacetylamino)acetamido]ethyl 2,3,4-tri-O-acetyl-β-D-xylopyranoside (**13**). Yield 89%, m.p. 124–126°, $[\alpha]_D^{23}$ −43° (*c* 1, chloroform); n.m.r. ($CDCl_3$): δ 7.26 (broad NH signal), 6.32 (broad NH signal), 5.20 (t, 1 H, $J_{3,4}$ 8.8 Hz, H-3), 4.93 (m, 2 H, $J_{2,3}$ 8.8, $J_{4,5e}$ 5.1, $J_{4,5a}$ 8.8 Hz, H-2, -4), 4.48 (d, 1 H, $J_{1,2}$ 7.0 Hz, H-1), 4.27 (dd, 1 H, $J_{5e,5a}$ −11.8 Hz, H-5e), 4.11 (s, 2 H, $-CH_2Cl$), 3.97 (d, 2 H, $-COCH_2-$

NHCO-), 3.82 and 3.68 (m, 1 H, -$CH_2$$CH_2$NH-), 3.49 (m, 2 H, -$CH_2$$CH_2$NH-), 3.37 (dd, H-5a), and 2.08, 2.05 (9 H, OAc).

Anal. Calc. for $C_{17}H_{25}ClN_2O_{10}$: C, 45.08; H, 5.53; Cl, 7.85; N, 6.19. Found: C, 45.47; H, 5.27; Cl, 8.17; N, 5.89.

*2-[(Bromoacetylamino)acetamido]ethyl 2,3,4-tri-*O*-acetyl-β-*D*-xylopyranoside* (**15**). Yield 57%, m.p. 142–144°, $[\alpha]_D^{23}$ −84° (*c* 1, chloroform); n.m.r. ($CDCl_3$): δ 7.16 (broad NH signal), 6.27 (broad NH signal), 5.20 (t, 1 H, $J_{3,4}$ 8.8 Hz, H-3), 4.94 (m, 2 H, $J_{2,3}$ 8.8, $J_{4,5e}$ 5.1, $J_{4,5a}$ 9.2 Hz, H-2, -4), 4.48 (d, 1 H, $J_{1,2}$ 6.6 Hz, H-1), 4.13 (dd, 1 H, $J_{5e,5a}$ −11.8 Hz, H-5e), 3.95 (d, 2 H, -COCH_2NHCO-), 3.93 (s, 2 H, -CH_2Br), 3.82 and 3.68 (m, 1 H, -$CH_2$$CH_2$NH-), 3.49 (m, 2 H, -$CH_2$$CH_2$NH-), 3.37 (dd, H-5a), and 2.08, 2.05, 2.04 (OAc).

Anal. Calc. for $C_{17}H_{25}BrN_2O_{10}$: C, 41.06; H, 5.03; Br, 16.08; N, 5.64. Found: C, 41.30; H, 5.27; Br, 15.89; N, 5.47.

*2-[(Dichloroacetylamino)acetamido]ethyl 2,3,4-tri-*O*-acetyl-β-*D*-xylopyranoside* (**17**). Yield 66%, m.p. 111–113°, $[\alpha]_D^{23}$ −15° (*c* 1, chloroform); n.m.r. ($CDCl_3$): δ 7.34 (broad NH signal), 6.29 (broad NH signal), 6.00 (s, 1 H, -$CHCl_2$), 5.20 (t, 1 H, $J_{3,4}$ 8.8 Hz, H-3), 4.93 (m, 2 H, $J_{2,3}$ 8.8, $J_{4,5e}$ 5.1, $J_{4,5a}$ 9.2 Hz, H-2, -4), 4.48 (d, 1 H, $J_{1,2}$ 7.0 Hz, H-1), 4.13 (dd, 1 H, $J_{5e,5a}$ −11.8 Hz, H-5e), 3.98 (d, 2 H, -COCH_2NHCO-), 3.83 and 3.69 (m, 1 H, -$CH_2$$CH_2$NH-), 3.50 (m, 2 H, -$CH_2$$CH_2$-NH-), 3.37 (dd, H-5a), and 2.08, 2.05 (9 H, OAc).

Anal. Calc. for $C_{17}H_{24}Cl_2N_2O_{10}$: C, 41.89; H, 4.93; Cl, 14.58; N, 5.75. Found: C, 42.03; H, 5.11; Cl, 14.96; N, 5.47.

Base-catalyzed hydrolysis of **13**, **15**, *and* **17**. — Hydrolysis was performed as described for the preparation of **6**, **8**, and **10**:

*2-[(Chloroacetylamino)acetamido]ethyl β-*D*-xylopyranoside* (**14**). Yield 83%, m.p. 115–116.5°, $[\alpha]_D^{23}$ −27° (*c* 1, methanol).

Anal. Calc. for $C_{11}H_{19}ClN_2O_7$: C, 40.43; H, 5.82; Cl, 10.87; N, 8.58. Found: C, 40.19; H, 6.04; Cl, 10.55; N, 8.43.

*2-[(Bromoacetylamino)acetamido]ethyl β-*D*-xylopyranoside* (**16**). Yield 80%, $[\alpha]_D^{23}$ −24° (*c* 1, methanol).

Anal. Calc. for $C_{11}H_{19}BrN_2O_7 \cdot 4H_2O$: C, 29.80; H, 6.10; Br, 18.04; N, 6.32. Found: C, 30.04; H, 5.71; Br, 18.27; N, 6.76.

*2-[(Dichloroacetylamino)acetamido]ethyl β-*D*-xylopyranoside* (**18**). Yield 76%, $[\alpha]_D^{23}$ −25° (*c* 1, methanol).

Anal. Calc. for $C_{11}H_{18}Cl_2N_2O_7 \cdot H_2O$: C, 34.83; H, 5.28; Cl, 18.73; N, 7.39. Found: C, 34.77; H, 5.21; Cl, 19.01; N, 7.48.

Assay of cytotoxic activity in cell culture. — B16/F10 melanoma cells were grown at 37° as a monolayer culture in Ham's F-10 nutrient mixture containing 12.5% of horse serum and 2.5% of fetal bovine serum. Exponentially growing melanoma cells (8×10^4 cells) were transferred to 25-cm^2 plastic culture-flasks and were incubated for 48 h. The medium was then removed and fresh medium with or without test substance was added. After the cells had been incubated for another 48 h, the medium was withdrawn and the cells attached to the flask were removed by a

0.25-h exposure to 2mM sodium ethylenediaminetetraacetate in a phosphate-buffered 150mM sodium chloride solution. The total cell number in each of 3 flasks was then determined with a Model ZBI Coulter counter.

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