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Antiproliferation and apoptosis induced by *C*-glycosides in human leukemia cancer cells

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Abstract—A large series of alkyl *C*-glycosides was synthesized from D-glucal or D-galactal. These compounds were screened against the human promyelocytic leukemia cell line (HL60), showing significant activity and apoptosis. Up to 13 *C*-glucopyranosides, but no *C*-galacto- or *C*-mannopyranosides, exhibited inhibitory concentrations (IC_{50} values) below 20 μM, five of them in the range 4–8 μM. Preliminary structure–activity relationships were established.

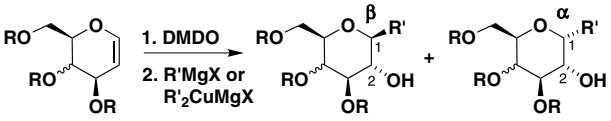
Cell surface glycoproteins are known to be active in numerous important disease states.[1] Their carbohydrate epitopes have been shown to play a central role in a variety of significant biological events, including inflammation, metastasis, immune response, and bacterial and viral infection. This has recently stimulated the development of effective therapeutic strategies based upon recognition of these cell surface carbohydrates. One approach lies in the replacement of the exocyclic oxygen with a carbon, to afford *C*-glycosidic analogs, due to their increased resistance to degradation by glycosidases. This has led to a wealth of synthetic approaches[2] and consequently biological data on *C*-glycosides have started to emerge.[3]

In addition, comparative biological studies have revealed that *C*-glycosides retain the biological properties of natural *O*-glycosides.[4]

Since glycosylarenes[5] (or *C*-aryl glycosides), 4-keto unsaturated *C*-glycosides,[6] *C*-glycoglycerolipids,[7] and unsaturated *C*-glycosides with an attached ring system bonded at C-4 and C-6 exhibit diverse biological activities,[8] including antitumor and antiviral action, the aim of the present study was to carry out the synthesis of easily obtained simple *C*-glycosides and check their cytotoxic activities.

Among the different methods for the preparation of *C*-glycosides,[2] the addition of carbon nucleophiles to activated glycal epoxides has been widely used.[9] Therefore, the *C*-glycoside derivatives under study were prepared in three steps: (i) *O*-alkylation of D-glucal or D-galactal; (ii) epoxidation using dimethyldioxirane in CH_2Cl_2, according to Danishefsky's protocol;[10] and (iii) epoxide opening with Grignard reagents or diorganocuprates (Scheme 1).[11]

Thus, addition of numerous Grignard reagents or diorganocuprates to the 1,2-anhydrosugar led to a mixture of α- and β-*C*-glycoside derivatives, the α/β ratio being variable and in most cases favoring the β-isomer. In some cases, further simple structural modifications were



Scheme 1. Synthesis of *C*-gluco- and *C*-galactopyranosides.

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performed by means of standard chemical transformations. The stereochemistry of the synthesized *C*-glycosides (Scheme 1) was established by analyzing the ^{1}H NMR $J_{1,2}$ value (β-configuration around 9.5 Hz, α-configuration around 5.8 Hz). Sometimes it was confirmed by means of the T-ROESY experiments, by observing the clear crosspeaks involving the *pseudo*-anomeric proton H1.

The cytotoxic activity of the whole series was measured as growth inhibition or decreased viability on the human promyelocytic leukemia cell line HL60. Table 1 shows the compounds with a cytotoxic effect, with their 50% inhibitory concentrations (IC_{50} values) below 50 μM. These data were determined by the MTT assay[12] and calculated from at least three independent experiments. Schemes 2 and 3 show the structures of those compounds exhibiting IC_{50} values below 20 μM and in the range 20–50 μM, respectively.

The *C*-glucopyranosides **1**–**13** inhibited growth below 20 μM. Compounds **3**, **4**, **5**, and **7** showed IC_{50} values around 8.5 μM, while compound **9**, the most active one, exhibited an IC_{50} of 4.1 μM (Scheme 2). A moderate or low activity was observed for compounds **14**–**23** (Scheme 3), whereas compounds **24**–**64**, having an IC_{50} higher than 50 μM, were not active.

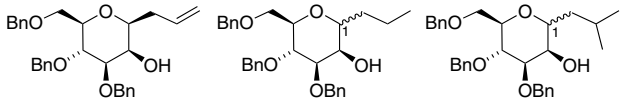
Some preliminary structure–activity relationships can be established. Thus, the present study reveals the importance of the underivatized hydroxyl group at C-2 for cytotoxic activity. All compounds having antiproliferative activity against HL60 cell line below 20 μM (Scheme 2), except compound **12**, possess an underivatized hydroxyl group at C-2 with an equatorial configuration. Compounds with an acetyl, benzyl, 2-deoxy, etc., group at position 2 showed a lowering or total loss of activity (Schemes 3 and 4).

Table 1. Effects of the *C*-glycosides on the growth of the HL-60 cell line

Compound	IC_{50}	SD
1	15.1	2.5
2	11.8	3.4
3	8.7	1.5
4	8.4	2.5
5	8.2	5.0
6	11.7	3.9
7	8.5	1.2
8	13.9	4.0
9	4.1	1.1
10	17.2	1.4
11	12.1	0.7
12	12.5	3.4
13	19.3	2.7
14	26.9	0.4
15	31.8	5.7
16	26.3	7.0
17	28.2	3.0
18	39.3	2.9
19	38.5	4.1
20	46.4	3.2
21	20.2	3.7
22	35.3	3.9
23	26.6	1.5
24–64	n.a.[a]	—

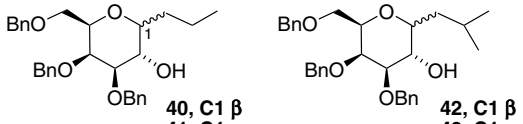
[a] n.a., not active.

Furthermore, the ketone **15**, obtained by oxidation of the alcohol **1** by treatment with dimethylsulfoxide/acetic anhydride, as well as the β-*C*-mannopyranoside **35**, derived from **15** by reduction with $NaBH_4$ in CH_2Cl_2/MeOH (1:1),[13] led to a lowering or total loss of activity. The mannopyranosides **36**–**39**, similarly obtained from their corresponding glucopyranosides (**6**–**8** and **17**) did not show activity either. Therefore, the equatorial disposition of the hydroxyl group at position 2, glucose configuration, seems to be crucial for the inhibitory process.



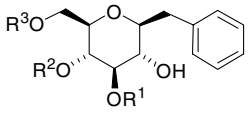
35 **36, C1 β** **37, C1 α** **38, C1 β** **39, C1 α**

To test the importance of the configuration at C-4, the galactopyranoside derivatives **40**–**43** were analyzed. Their lack of cytotoxic activity confirms the importance of having the glucose configuration, as in the *C*-mannopyranosides.



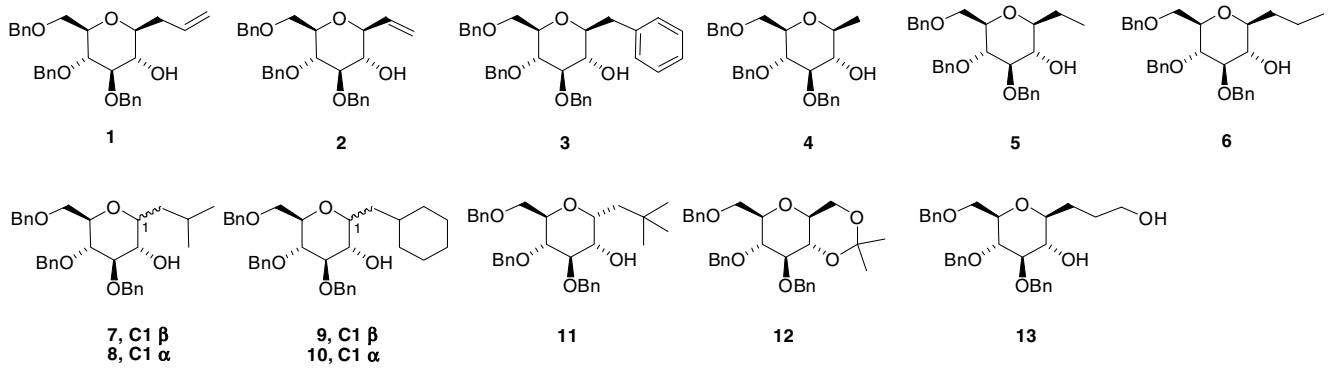
40, C1 β **41, C1 α** **42, C1 β** **43, C1 α**

To test the role of the benzyl groups linked to the hydroxyl groups at C-3, C-4, and C-6 in cytotoxic activity, all possible deprotected derivatives of the active compound **3** (compounds **21**–**23** and **44**–**47**) were synthesized. They were satisfactorily obtained from compound **3** by partial hydrogenolysis with H_2 and Pd–C as catalyst, when a 90% conversion was allowed.

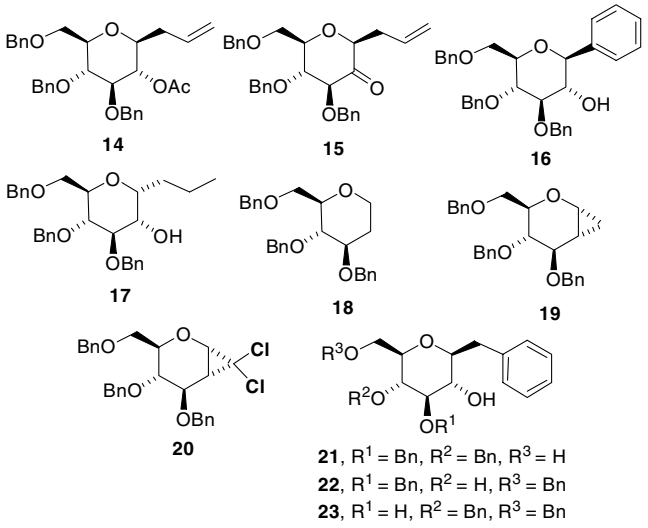


44, R^1 = Bn, R^2 = H, R^3 = H
45, R^1 = H, R^2 = Bn, R^3 = H
46, R^1 = H, R^2 = H, R^3 = Bn
47, R^1 = H, R^2 = H, R^3 = H

As seen in Table 1, only those compounds retaining two out of three *O*-benzyl groups (**21**–**23**) showed activity, somewhat lower than their parent compound **3**. On the basis of their IC_{50} values, the contribution to the inhibitory effect of **3** on the growth of HL60 was higher when an *O*-benzyl group was located at C-4 rather than at C-3, and at C-3 higher than C-6. Meanwhile, those having one *O*-benzyl group or none (**44**–**47**) showed no significant cytotoxic activity. This highlights the con-

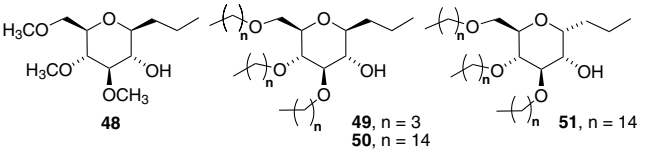


Scheme 2. Structures of *C*-glycosides with antiproliferative activity against HL60 cell line below 20 μM.



Scheme 3. Structures of *C*-glycosides with antiproliferative activity against HL60 cell line between 20 and 50 μM.

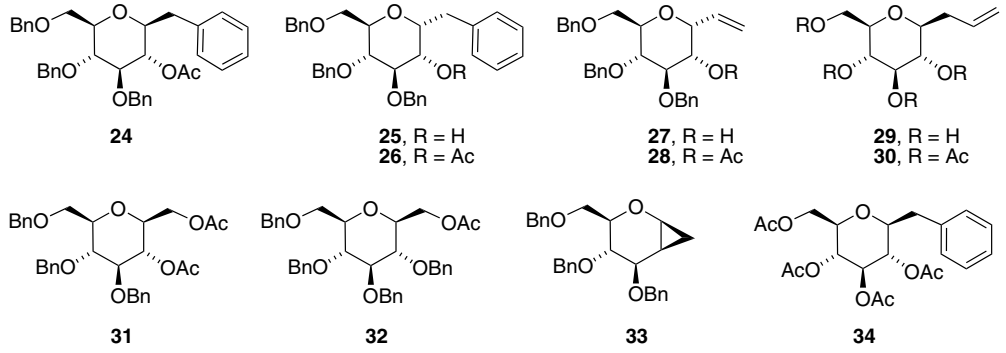
venience of having the three above-mentioned hydroxyl groups protected. Furthermore, when these aromatic groups are changed for methyl, *n*-butyl, or pentadecyl (**48**–**51**), no cytotoxic activity was observed. Consequently, the benzyl groups at C-3, C-4, and C-6, besides increasing cell permeability, may favor an aromatic interaction with the receptor, resulting in increased binding affinity.[14]



Regarding the *C*-aglycon, the data reveal a preference for the β equatorial configuration at C-1 to achieve a growth inhibiting effect. Most of the α axial epimers at C-1 showed no activity or a lower activity compared with their β stereoisomer, except for the neopentyl stereoisomers, where the α isomer is cytotoxic (**11**), and its β is not (**58**).

The relationships observed between the cytotoxicity and the length of the saturated chain of the *C*-aglycon are really striking. While the methyl, ethyl, and propyl 3,4,6-tri-*O*-benzyl β-*C*-glucopyranosides **4**–**6** exhibited cytotoxicity, the corresponding *n*-butyl (**56**) and *n*-pentyl (**57**) did not. Regarding their axial epimers at C-1, only the propyl α-*C*-glucopyranoside **17** showed some cytotoxic activity. Compounds having a branched aglycon, for example, **7**–**11**, also inhibited cell growth; the β stereoisomers **7** and **9** to a greater extent than their α isomers **8** and **10**. The neopentyl derivative **11** has the opposite behavior, as already mentioned.

In addition, an IC_{50} data comparison between *C*-glycosides with unsaturated aglycons and their saturated analogs was performed. Thus, compounds **1**, **2**, and **3**



Scheme 4. Structures of *C*-glycosides with no antiproliferative activity against HL60.

showed higher IC_{50} values than compounds **6**, **5**, and **9**, respectively, revealing more potent cytotoxic activity for *C*-glycosides with saturated aglycons (Scheme 5).

To test if these *C*-glucosides induce apoptosis in HL60 leukemia cells, some active compounds were analyzed. The cells were cultured in absence (C) or presence of 20 μM of the selected compounds for 24 h, then fixed with PFA, stained with DAPI, and nuclei were visualized using fluorescence microscopy (Fig. 1). The percentage of apoptotic cells was determined by quantitative fluorescence microscopy and the results of a representative experiment are shown. Each point represents average ± SE of duplicate determinations.

These data indicate that 3,4,6-tri-*O*-benzyl β-*C*-glucosides **1**, **5**, **7**, and **12** induce apoptosis in HL60 cells, the most potent being compound **12**.

In summary, a large series of *C*-glycosides have been synthesized and screened against the human promyelocytic leukemia cell line (HL60). Several *C*-glucopyranosides showed promising antiproliferative and apoptotic activity against HL60 cells. The preliminary structure–activity study revealed some structural features related with the potency of the cytotoxic activity. A β equatorial configuration at C-1, an underivatized equatorial hydroxyl group at C-2, and *O*-benzyl groups at C-3, C-4, and C-6 led to the highest antiproliferative activity. However, compound **12** having an acetonide group was the most apoptotic derivative.

Since compounds which regulate apoptosis and overcome apoptosis deficiency of cancer cells are of high medical significance, further chemical and biochemical studies are currently under way.

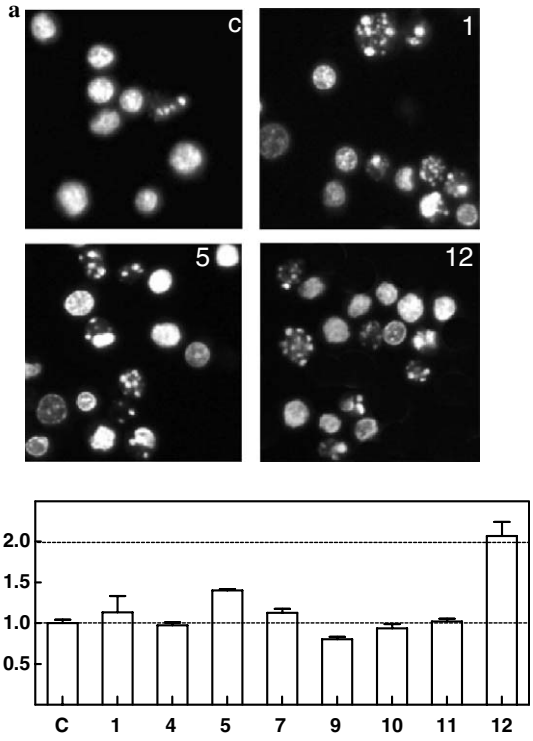
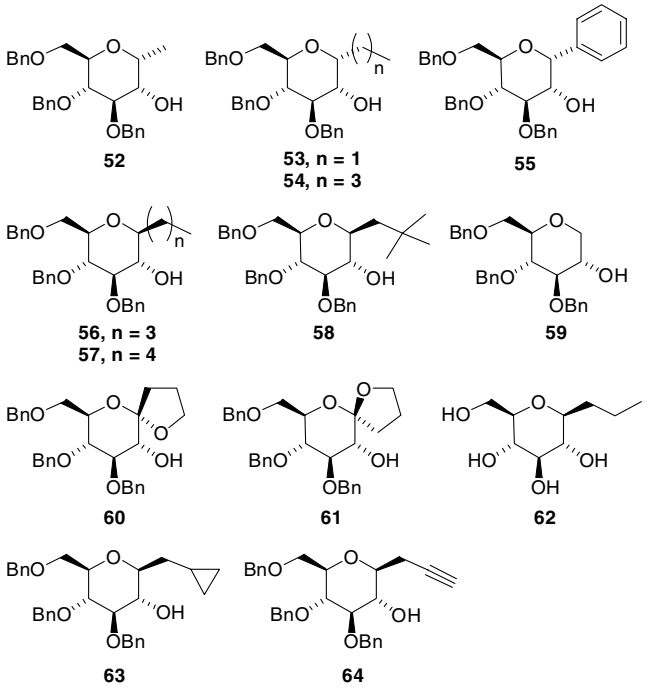


Figure 1. (a) Fluorescence microscopy of untreated (C) or treated (compounds **1**, **5**, and **12**) HL60 cells. (b) Percentage of apoptotic cells determined by quantitative fluorescence microscopy.



Scheme 5. Structures of *C*-glycosides without antiproliferative activity against HL60.

Acknowledgments

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