Design, Synthesis, and Evaluation of the Multidrug Resistance-Reversing Activity of D-Glucose Mimetics of Hapalosin

Tam Q. Dinh,[†] Charles D. Smith,[§] Xiaohui Du,[†] and Robert W. Armstrong*,[†]

Department of Chemistry and Biochemistry, University of California, Los Angeles, California 90095, and Fox Chase Cancer Center, Philadelphia, Pennsylvania 19111

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When five substituents of hapalosin were placed on D-glucose, molecular modeling revealed that the substituents on mimetics **2** and **3** occupy similar spatial positions as the corresponding substituents on hapalosin. Mimetic **3** and all the glucopyranoside intermediates generated in its synthesis were assessed for their ability to reverse multidrug resistance (MDR) mediated by P-glycoprotein (P-gp) or the multidrug resistance-associated protein (MRP). None of the sugar compounds were as effective as hapalosin in inhibiting P-gp in cytotoxicity and drug accumulation assays using MCF-7/ADR cells. By contrast, four D-glucose compounds exhibited similar efficacy as hapalosin in antagonizing MRP in cytotoxicity assays with HL-60/ADR cells.

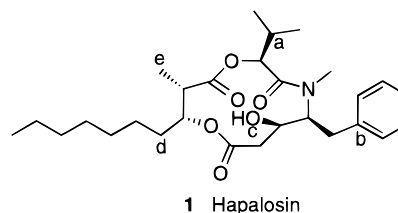
Introduction

Many cancer chemotherapy programs often provide temporary clinical improvement. Unfortunately, the emergence of less easily treated tumor cells frequently results in the ultimate failure of chemotherapy. Tumor cells which survive the initial therapeutic attack often recover with increased resistance to both the original therapeutic agent and other structurally unrelated drugs. This phenomenon is known as multiple drug resistance (MDR).[1]

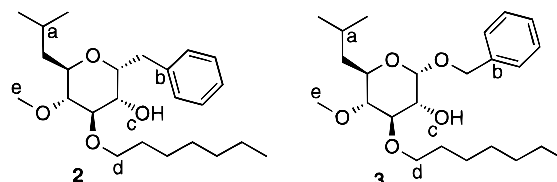
The most common mechanism for acquired MDR involves overexpression of drug transporters which act as ATP-dependent drug-efflux pumps, actively removing a broad spectrum of structurally diverse compounds. Enhanced efflux of these compounds reduces their intracellular accumulation and, hence, their cytotoxicity. The best-studied member of this family of ATPases is P-glycoprotein (P-gp), a transmembrane protein which extrudes many natural product drugs, including adriamycin, *Vinca* alkaloids, etoposide, gramicidin D, actinomycin D, and taxol. A related drug transporter that can confer drug resistance is the multidrug resistance-associated protein (MRP),[2] although its profiles of substrates and antagonists are not identical to those of P-gp.[3] However, virtually all currently described MDR-reversing agents inhibit the activity of both P-gp and MRP.

Since each of these drug transporters can be expressed in human tumor cells, a significant amount of effort has been directed toward the identification and development of compounds which inhibit the drug-efflux property of P-gp and/or MRP. A number of compounds have been shown to reverse MDR in vitro, usually by competing with cytotoxic drugs for binding to P-gp.[4] However, the majority of compounds shown to have anti-MDR activity are synthetic derivatives of a small number of chemical families, e.g., dihydropyridines, thioxanthenes, and phenothiazines. Importantly, none of these compounds have yet proven to be clinically useful because of their intrinsic toxicity or their adverse effects on the toxicity of accompanying anticancer drugs. It would clearly be advantageous to develop new MDR modulators with superior therapeutic profiles.

Hapalosin (**1**), a cyclic depsipeptide isolated from the cyanobacterium *Hapalosiphon welwitschii*, reverses MDR by inhibiting P-gp.[5] The relationship between the structure and anti-MDR activity of hapalosin[6] and its analogues has been the focus of our research. We have examined the non-*N*-Me analogue,[6d,7] analogues incorporating *trans*-4-hydroxy-L-proline,[7] and the triamide analogue of hapalosin.[8] The elegant work of Hirschmann et al. on nonpeptidal mimetics of the cyclic hexapeptide L-363,301 intrigued us.[9] They created peptidomimetics containing D-glucose as the scaffold which were agonists of the somatostatin receptor. Molecular modeling suggested that D-glucose might also be a good scaffold for mimetics of hapalosin (**2** and **3**). We have prepared a series of D-glucose mimetics of hapalosin and assessed their ability to antagonize P-gp and MRP.



1 Hapalosin



Results and Discussion

Design. The substituents of hapalosin (**1**) which might be important for its anti-MDR activity were taken as the isopropyl group (represented by atom a), the

[†] University of California.
[§] Fox Chase Cancer Center.

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Table 1. Distances (Å) between Atoms[a]

compound	a−b	a−c	a−d	a−e	b−c	b−d	b−e	c−d	c−e	d−e
hapalosin (**1**)	4.77	6.79	6.38	5.01	4.33	6.00	6.92	6.87	7.71	3.00
mimetic (**2**)	4.43	6.50	7.14	5.25	4.38	6.64	7.10	3.66	5.66	3.59
mimetic (**3**)	5.49	6.41	7.38	5.33	4.24	7.08	8.13	3.20	5.68	4.10

[a] For all three compounds, the *n*-heptyl group was replaced with a methyl group to simplify the computation.

benzyl group (atom b), the hydroxyl group (atom c), the *n*-heptyl group (atom d), and the α-methyl group (atom e). Maintaining the same clockwise order of the groups, the groups were placed at each of the five sites of functionalization on D-glucose in order to determine the optimum positioning of the substituents. Furthermore, ether or aliphatic linkages at C-1 and C-6 of D-glucose were examined.

Molecular modeling studies were performed with Macromodel (v4.5)[10] using AMBER* force field and GB/SA chloroform solvation[11] as previously described.[6d,8] To reduce the number of conformations found and computational time, the *n*-heptyl chain was replaced with a methyl group in hapalosin and the sugar compounds. For hapalosin (the major conformer), distance constraints between protons exhibiting NOESY cross-peaks were applied.[6d,8] The lowest-energy conformations of hapalosin and mimetics **2** and **3** are considered in this paper.

The D-glucose compounds which best resemble hapalosin are mimetics **2** and **3**. The only difference between the two mimetics is that **2** is a *C*-glycoside whereas **3** is an *O*-glycoside. Table 1 lists the distances between the substituents of the three compounds. The distances for the two mimetics are similar to those for hapalosin except for the c−d and c−e distances. In hapalosin, the hydroxyl group is substantially farther from the *n*-heptyl group and the methyl group than it is in the two sugar mimetics. Nearly all the distances between substituents for *C*-glycoside **2** are closer to those for hapalosin than the distances for *O*-glycoside **3**.

Hapalosin is superimposed on mimetics **2** and **3** in Figure 1. Figure 1a offers a top view and Figure 1b an angular view. The fit in the overlay of the five substituents is good, particularly for the *C*-Bn glycoside **2**. It is interesting that although the ring skeleton of hapalosin is clearly larger than the pyranose ring of the mimetics, the corresponding isopropyl, phenyl, *n*-heptyl (replaced by a methyl group to simplify computation), and methyl groups of the superimposed structures occupy similar spatial positions. Only the hydroxyl group of the two mimetics does not overlap well with that of hapalosin. The distances between the corresponding substituents of superimposed hapalosin and mimetics are presented in Table 2. On the basis of the molecular modeling results, D-glucose compounds **2** and **3** seem to mimic hapalosin well, with *C*-Bn **2** being the more analogous mimetic.

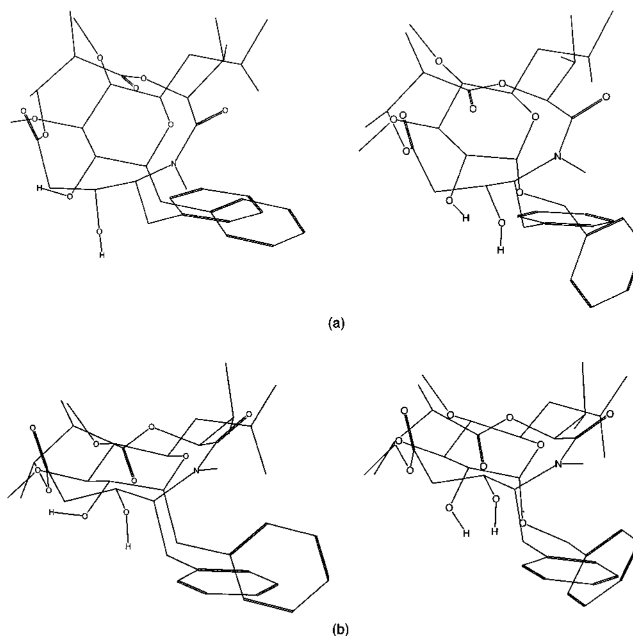


Figure 1. Superimposition of hapalosin and mimetics **2** and **3**: (a) top view with *C*-Bn **2** on the left and *O*-Bn **3** on the right; (b) angular view with **2** on the left and **3** on the right. The *n*-heptyl group of the three compounds was replaced with a methyl group to simplify the computation.

Table 2. Distances (Å) between Corresponding Atoms of Superimposed Hapalosin and Mimetic[a]

mimetic	a−a	b−b	c−c	d−d	e−e
2	0.57	1.04	2.19	1.47	0.32
3	0.64	1.59	2.77	1.69	0.32

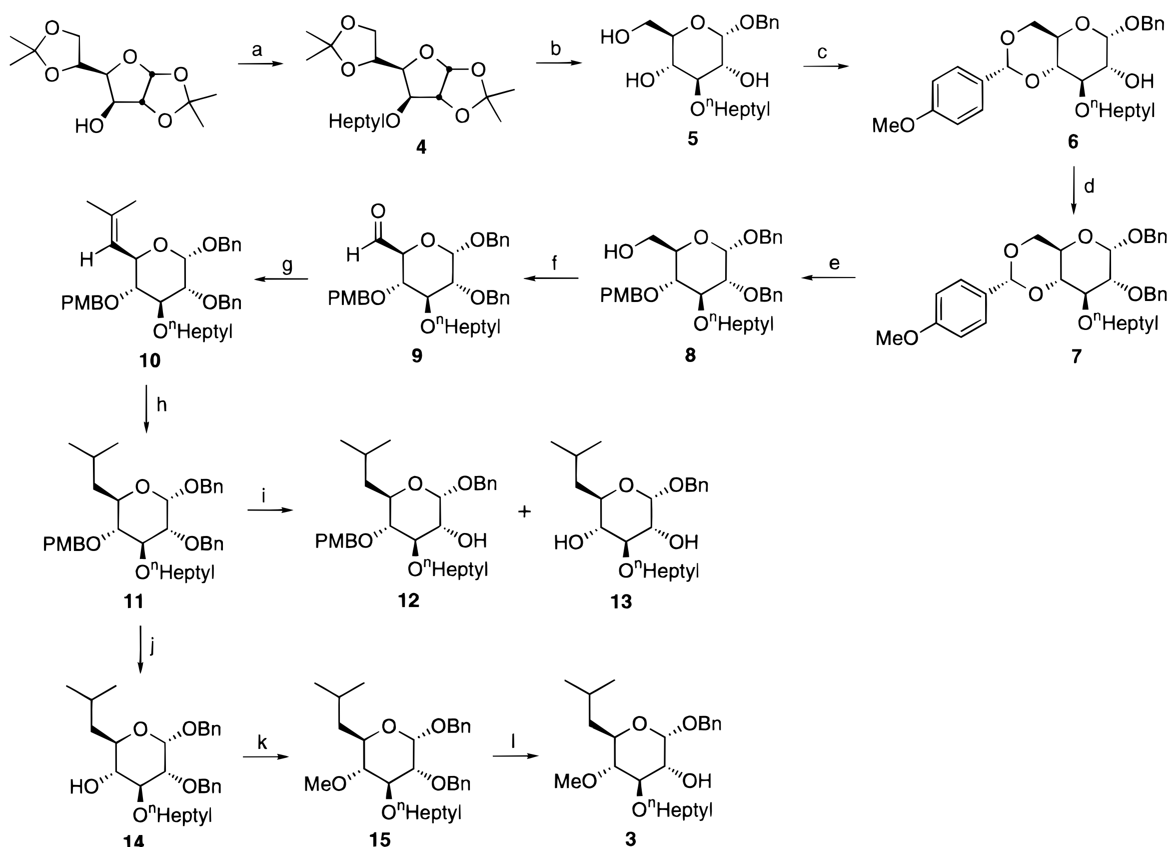
[a] For hapalosin and the mimetics, the *n*-heptyl group was replaced with a methyl group to simplify the computation.

Synthesis. Introduction of an α-*C*-Bn group at C-1 of several tetra-*O*-benzyl-D-glucopyranosides was attempted with BnTMS. It was hoped that C-glycosidation could be accomplished with BnTMS as it could routinely be with allylsilanes. However, reactions of the methyl glycoside with TMSOTf/BnTMS,[12] of the *p*-nitrobenzoyl glycoside with BF_3·OEt_2/BnTMS,[13] and of the glycosyl fluoride with BF_3·OEt_2/BnTMS[14] were unsuccessful. Electrophilic aromatic substitution of BnTMS, promoted by the positive charge being stabilized by a β C−Si bond, was not a problem in all three reactions. Considering that an α-*O*-Bn glycoside could be much more easily made than an α-*C*-Bn glycoside and that the *O*-Bn glycoside **3** also appeared to be a good mimetic of hapalosin, we pursued the synthesis of **3**.

The synthesis of mimetic **3** is illustrated in Scheme 1. It commenced with the alkylation of diacetone D-glucose[15] to produce *n*-heptyl ether **4**. The diacetonide was hydrolyzed back to the lactol form,[9] and the lactol was converted to the α-*O*-Bn glycoside **5**. The C-4 and C-6 hydroxyl groups were protected as the *p*-methoxybenzylidene acetal **6**. Benzylation of the hydroxyl group afforded the dibenzyl ether **7**. Regioselective cleavage of the *p*-methoxybenzylidene acetal was achieved with DIBAH in toluene.[16] The acetal did not react with DIBAH in CH_2Cl_2.

Transformation of activated derivatives of alcohol **8** to isobutyl **11** was attempted with ^{i}PrMgCl. However, the tosylate, the bromide,[17] and the triflate[18] derivatives did not react with ^{i}PrMgCl. Therefore, alcohol **8** was oxidized to aldehyde **9** which underwent a Wittig reaction.[19] Most of olefin **10** did not undergo diimide

Scheme 1[a]



[a] (a) *n*-Heptyl-I, NaH, DMF (100%); (b) (i) 60% aq AcOH, 90 °C, (ii) AcCl, BnOH, 80 °C (71% for 2 steps, $\alpha:\beta = 2.4:1$); (c) *p*-anisaldehyde, cat. PTSA, PhH, 110 °C, Dean–Stark (88%); (d) NaH, BnBr, DMF (100%); (e) DIBAH, PhMe, 0 °C; (f) Swern [O] (67% for 2 steps); (g) $^iPrPPh_3I$, ^nBuLi, THF, 0 °C (95%); (h) $PhS(O)_2NHNH_2$, aq NaOH, 2-MeOEtOH, reflux, 3 cycles (77%); (i) 10% Pd/C, H_2 (1 atm), EtOH, 25 min (29% **12**, 52% **13**); (j) DDQ, H_2O, CH_2Cl_2 (87%); (k) NaH, MeI, DMF (88%); (l) 10% Pd/C, H_2 (1 atm), EtOH, 25 min (77%).

reduction with $H_2NNH_2/CuSO_4/AcOH/NaIO_4$,[20] with $H_2NNH_2/CuSO_4/H_2O_2$,[21] or with $HONH_3HCl/NaOAc/EtOAc$.[22] Diimide reduction of olefin **10** was effected with benzenesulfonyl hydrazide and NaOH.[23]

Hydrogenolysis of compound **11** over 10% Pd/C in 25 min yielded 29% of monool **12** and 52% of diol **13**. In a shorter reaction time, more monool **12** could be formed than diol **13**. The two products did not contain a deprotected C-1 benzyl ether group since only their α-anomer was detected. During overhydrogenolysis of compounds **10**, **11**, and **15**, anomers resulting from deblocking of the C-1 benzyl ether group were observed. Hydrogenolysis of compound **11** over W-2 Raney nickel[24] was slow during which the formation of diols and the triol competed with that of monool **12**.

Completion of the synthesis of mimetic **3** involved DDQ deprotection of the PMB ether **11**. Methylation of alcohol **14** furnished dibenzyl ether **15** which was hydrogenolyzed to mimetic **3**. Only the α-anomer of the hydrogenolysis product was observed. Hydrolysis of compound **15** under acidic conditions generated anomers of the lactol which differed from the hydrogenolysis product of **15**.

Multidrug Resistance-Reversing Activity. The anti-MDR activities of synthetic hapalosin (**1**), mimetic **3**, compounds **5**–**15**, and verapamil (as a positive control) were evaluated. For P-gp-mediated MDR, cytotoxicity assays (Figure 2) were conducted with MCF-7/ADR cells[25] which overexpress P-gp but not MRP. Cells were exposed to one of eight doses of a compound alone (the PBS curves) or in the presence of 25 nM actinomycin D, which is transported by P-gp. In drug accumulation assays, MCF-7/ADR cells were treated with one of eight doses of a compound and then incubated with [^{3}H]vinblastine, which is also transported by P-gp. For MRP-mediated MDR, cytotoxicity assays (Figure 3) were conducted with HL-60/ADR cells[26] which express MRP but not P-gp. Cells were exposed to varying amounts of a compound alone (the PBS curves) or in the presence of 2 nM vincristine, an MRP-transported drug.

None of the sugar compounds reversed P-gp-mediated MDR as well as hapalosin and verapamil. Compounds **5**, **7**, **10**, **11**, and **15** exhibited very weak activity in cytotoxicity assays with MCF-7/ADR cells. The intrinsic cytotoxicity of aldehyde **9** was the greatest of all the compounds. The MDR-reversing profiles of compounds **3**, **6**, **8**, and **12**–**14** are presented in Figure 2. All of these compounds, particularly mimetic **3**, were at least significantly less potent than hapalosin and verapamil. In addition, all of the sugar compounds exerted very little effect on the accumulation of [^{3}H]vinblastine in MCF-7/ADR cells. Only diol **13** had a modest impact.

On the other hand, compounds **8** and **12**–**14** showed similar efficacy in reversing MRP-mediated MDR as hapalosin and verapamil (Figure 3). Mimetic **3** surprisingly displayed the weakest activity in Figure 3. Dibenzyl ether **7** has the same profile as alcohol **6**. The dramatic rise in the intrinsic cytotoxicity of hapalosin and compound **8** from 5 to 10 μg/mL is curious. As it

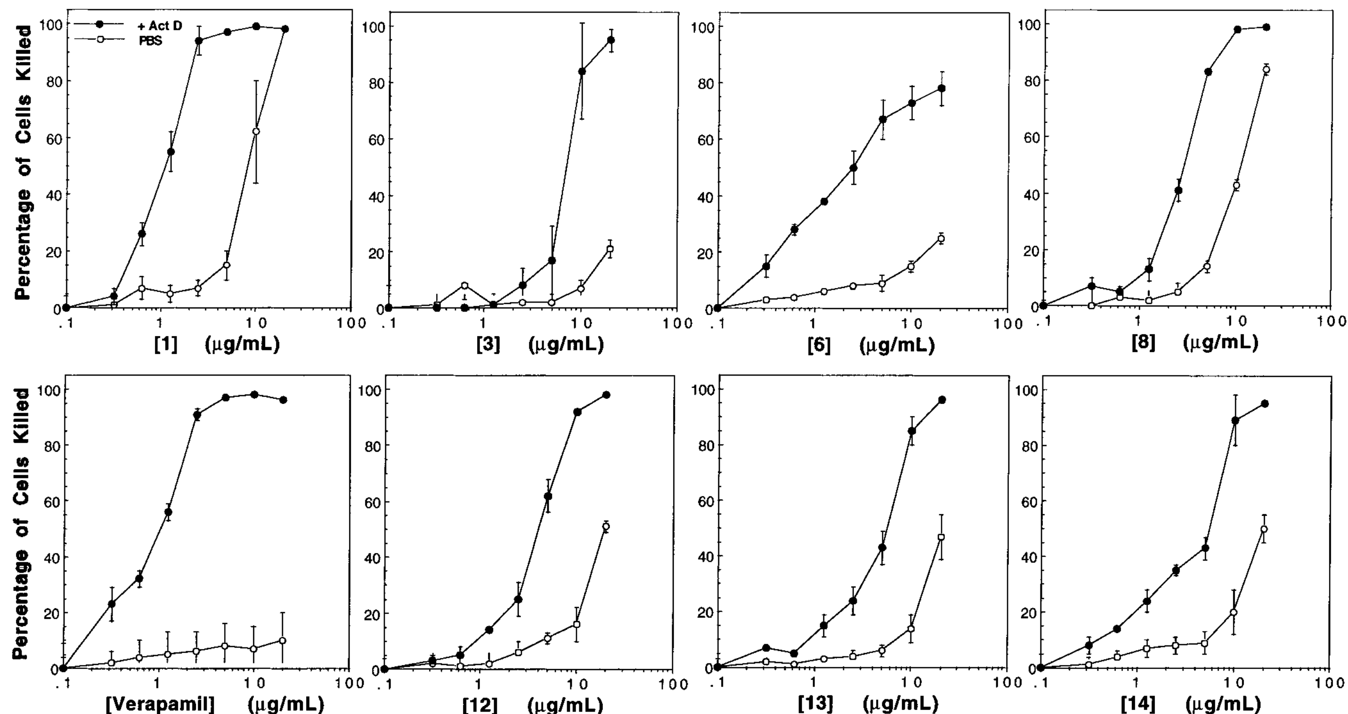


Figure 2. Reversal of P-gp-mediated MDR. MCF-7/ADR cells were incubated with the indicated concentrations of a compound in the presence of phosphate-buffered saline (as a control) (○) or 25 nM actinomycin D (●). Cell survival after 48 h was determined as indicated in the Experimental Section. Values represent the mean ± SD of triplicate samples.

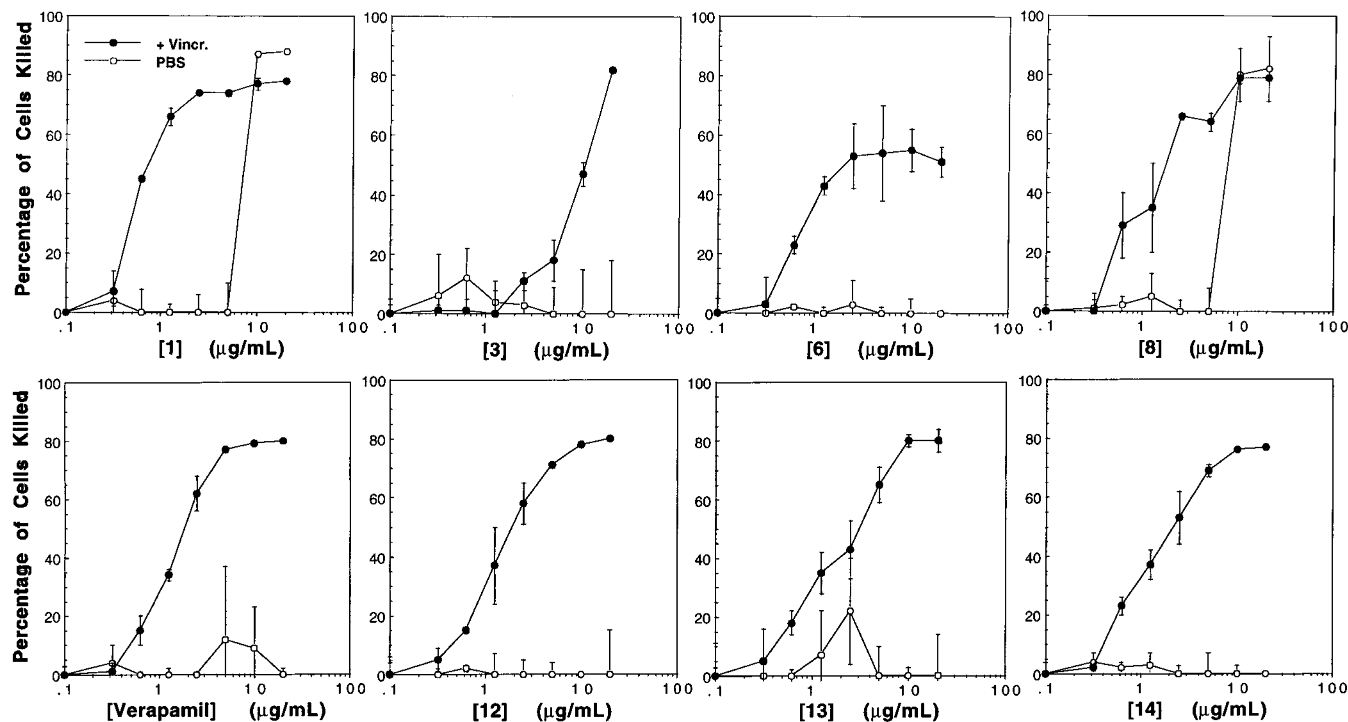


Figure 3. Reversal of MRP-mediated MDR. HL-60/ADR cells were incubated with the indicated concentrations of a compound in the presence of phosphate-buffered saline (as a control) (○) or 2 nM vincristine (●). Cell survival after 48 h was determined as indicated in the Experimental Section. Values represent the mean ± SD of triplicate samples.

was to MCF-7/ADR cells, aldehyde **9** was the most inherently cytotoxic to HL-60/ADR cells. Compounds **5**, **10**, **11**, and **15** were much less effective in potentiating the cytotoxicity of vincristine than compounds **12**–**14**.

The results of the in vitro cytotoxicity assays are summarized in Table 3. IC_{20} is the approximate concentration of a compound which inhibits cell proliferation by 20% in the absence of a cytotoxic drug and is usually considered the maximum dose appropriate for combination with a cytotoxic drug. EC_{50} is the approximate concentration of a compound which enhances the toxicity of actinomycin D or vincristine to the point where 50% of the cells are killed.

Table 3. Reversal of P-gp- and MRP-Mediated MDR

	MCF-7/ADR cells[a]		HL-60/ADR cells[b]	
compound	IC_{20}[c]	EC_{50}[d]	IC_{20}[c]	EC_{50}[d]
hapalosin	6	1	6	0.7
verapamil	>20	1	≫20	1.8
3	20	7	≫20	10
5	≫20	>20	≫20	10
6	12	2.5	≫20	2.5
7	≫20	≫20	≫20	2.5
8	6	3	6	1.7
9	5	4	1.8	1.5
10	≫20	≫20	≫20	>20
11	≫20	≫20	>20	>20
12	12	4	≫20	1.8
13	12	6	≫20	3.5
14	10	6	≫20	2.5
15	≫20	>20	≫20	18

[a] P-gp-mediated MDR assay using 25 nM actinomycin D. [b] MRP-mediated MDR assay using 2 nM vincristine. [c] The approximate concentration (μg/mL) of a compound which inhibits cell proliferation by 20% in the absence of the particular cytotoxic drug. [d] The approximate concentration (μg/mL) of a compound which results in 50% of the cells being killed in the presence of the particular cytotoxic drug.

Conclusion

None of the D-glucose compounds in Scheme 1 possess an effective capacity to reverse P-gp-mediated MDR. However, the ability of compounds **8** and **12**–**14** to antagonize MRP-mediated MDR is similar to that of hapalosin. In both types of cytotoxicity assays, the sugar compounds with no free hydroxyl group have much weaker activities than the compounds with a free hydroxyl group (except for triol **5**).

Mimetic **3** exhibits poor anti-MDR activity even though its five substituents seem to occupy similar spatial positions as the corresponding substituents of hapalosin. This may be due to a couple of reasons. First, the computed lowest-energy, distance-constrained conformation of the major conformer of hapalosin in chloroform may not be the bioactive conformation. Hapalosin is flexible and can adopt many other conformations, one or some of which being responsible for inhibition of drug transporters. By contrast, the D-glucose compounds are most likely constrained to the chair conformation in which four of the substituents are equatorial. Second, the amide–diester ring skeleton of hapalosin may be important in its interaction with and antagonism of P-gp and MRP.

Experimental Section

Cytotoxicity Assays. MCF-7 breast carcinoma cells and MCF-7/ADR cells, an MDR subline,[25] were obtained from the Division of Cancer Treatment of the National Cancer Institute and were grown in RPMI 1640 containing 10% fetal bovine serum and 50 μg/mL gentamycin sulfate.

To test for reversal of P-gp-mediated MDR, MCF-7/ADR cells were seeded in 96-well tissue culture dishes at approximately 15% confluency and were allowed to attach and recover for 24 h. The cells were then treated with varying concentrations of a compound alone or in the presence of 25 nM actinomycin D for 48 h according to previously described procedures.[27] The number of surviving cells was then determined by staining with sulforhodamine B.[28] The percentage of cells killed was calculated as the percentage decrease in sulforhodamine B binding compared with control cultures. Control cultures included equivalent amounts of ethanol (as the solvent control), which did not modulate the growth or drug sensitivity of these cells at doses utilized in these studies. Reversal of MDR is defined as the ability of a compound to potentiate the cytotoxicity of actinomycin D toward the MCF-7/ADR cells.

To test for reversal of MRP-mediated MDR, human promyelocytic leukemia HL-60/ADR cells[26] were treated with varying concentrations of a compound alone or in the presence of 2 nM vincristine for 48 h. The number of surviving cells was then determined using the CellTiter AQueous assay system from Promega. The percentage of cells killed was calculated as the percentage decrease in MTS tetrazolium metabolism compared with control cultures. Reversal of MDR is defined as the ability of a compound to potentiate the cytotoxicity of vincristine toward the HL-60/ADR cells.

[^{3}H]Drug Accumulation Assay. MCF-7/ADR cells were plated into 24-well tissue culture dishes and allowed to grow to 90% confluency. The cells were washed with phosphate-buffered saline (PBS) and then incubated in 0.5 mL of RPMI 1640 medium containing a compound and 20 nM [^{3}H]vinblastine sulfate (Amersham Corp.) for 60 min at 37 °C. The cultures were rapidly washed three times with ice-cold PBS. Intracellular [^{3}H]drug was solubilized with 0.3 mL of 1% sodium dodecyl sulfate in water and quantified by liquid scintillation counting.

General Procedures. ^{1}H and ^{13}C NMR spectra were obtained at room temperature with a Bruker ARX-400 or ARX-500 spectrometer. ^{1}H NMR chemical shifts are referenced to TMS (0.00 ppm) and those for ^{13}C NMR to $CDCl_3$ (77.0 ppm). IR spectra were recorded with a Nicolet 510P FT-IR spectrometer. Optical rotations were measured with a Perkin-Elmer 241MC polarimeter, and concentrations (*c*) are reported in g/mL. High-resolution mass spectroscopy (HRMS) was performed with a VG Autospec instrument for the EI and CI methods and a VG ZAB-SE instrument for the FAB method.

All water-sensitive reactions were conducted in oven- or flame-dried glassware under a nitrogen atmosphere. The starting materials were azeotroped two or three times with benzene before the reactions. Solvents were distilled immediately prior to use: CH_2Cl_2 from P_2O_5, PhMe from CaH_2, MeOH from magnesium metal, and THF from sodium metal/benzophenone ketyl. Anhydrous DMF was purchased from the Aldrich Chemical Co. and utilized without further purification. Most commercially available reagents were distilled before use. Thin-layer chromatography (TLC) was performed on silica gel-coated plates (Merck, Kieselgel 60 F_{254}, 0.25 mm thickness for analytical and 0.5 mm for preparative TLC) and visualized by UV light and/or *p*-anisaldehyde staining. After all aqueous extractions of crude reaction products, the combined organic layers were dried over $MgSO_4$ and concentrated in vacuo before further treatment.

3-*O*-Neoheptyl-1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose (4). To a solution of diacetone D-glucose (5.00 g, 19.2 mmol) and 1-iodoheptane (4.70 mL, 28.8 mmol) in DMF (50 mL) at 0 °C was added NaH (deoiled, 691 mg, 28.8 mmol) in three portions. After the mixture stirred at 0 °C for 15 min and then at 25 °C for 2 h, the reaction was quenched with water. Extraction in CH_2Cl_2 (3 × 100 mL) with water (100 mL) resulted in pure heptyl ether **4** (6.91 g, 100% yield) as a colorless oil.

Benzyl 3-*O*-Neoheptyl-α-D-glucopyranoside (5). A solution of diacetonide **4** (6.80 g, 19.0 mmol) in 60% aqueous acetic acid (35 mL) was stirred at 90 °C for 15 h. The solvent was removed in vacuo, and the crude product was azeotroped with benzene (3 × 15 mL). Addition of acetyl chloride (1.35 mL, 19.0 mmol) to a mixture of the crude lactol in benzyl alcohol (50 mL) quickly dissolved the sugar. After the solution was stirred at 80 °C for 15 h, BnOH was removed under high vacuum. Flash chromatography with silica gel (gradient from 10% to 90% EtOAc/hexanes) furnished α-*O*-benzyl glycoside **5** (3.51 g, 50% yield for two steps) and the β-anomer (1.47 g, 21% yield for two steps), both as white solids.

Benzyl 3-*O*-Neoheptyl-4,6-(*p*-methoxybenzylidene)-α-D-glucopyranoside (6). A solution of triol **5** (3.45 g, 9.36 mmol), *p*-anisaldehyde (2.30 mL, 18.7 mmol), and *p*-toluenesulfonic acid monohydrate (89 mg, 0.47 mmol) in benzene (60

mL) was refluxed at 110 °C for 15 h with a Dean–Stark trap attached. PTSA was quenched with triethylamine (7 drops from a Pasteur pipet), and benzene was removed in vacuo. Flash chromatography with silica gel (gradient to 40% EtOAc/hexanes) afforded *p*-methoxybenzylidene acetal **6** (4.02 g, 88% yield) as a white solid.

Benzyl 2-*O*-Benzyl-3-*O*-neoheptyl-4,6-(*p*-methoxybenzylidene)-α-D-glucopyranoside (7). To a solution of alcohol **6** (3.67 g, 7.54 mmol) and benzyl bromide (1.08 mL, 9.05 mmol) in DMF (35 mL) at 0 °C was added NaH (80% oil dispersion, 339 mg, 11.3 mmol) in two portions. The mixture was stirred at 0 °C for 10 min and then at 25 °C for 14 h. The reaction was quenched with water and the mixture extracted in $CHCl_3$ (2 × 100 mL) with water (70 mL). Flash chromatography with silica gel (gradient from 100% $CHCl_3$ to 0.6% EtOAc/$CHCl_3$) produced dibenzyl ether **7** (4.36 g, 100% yield) as a white solid.

Benzyl 2-*O*-Benzyl-3-*O*-neoheptyl-4-(*p*-methoxybenzyl)-α-D-glucopyranoside (8). DIBAH (4.60 mL, 4.60 mmol in hexanes) was added slowly to a solution of *p*-methoxybenzylidene acetal **7** (1.32 g, 2.29 mmol) in toluene (10 mL) at 0 °C (the solution solidified at −78 °C). After the mixture stirred at 0 °C for 1 h, the reaction was carefully quenched with cold MeOH (5 mL), and the mixture was stirred at 0 °C for a few minutes and extracted in $CHCl_3$ (2 × 50 mL) with 1 M HCl (50 mL) followed by water (30 mL). The crude product (1.33 g of white solid, 100% crude yield) contained mostly the C_4-OPMB, C_6-OH regioisomer **8**.

Benzyl 2-*O*-Benzyl-3-*O*-neoheptyl-4-*O*-(*p*-methoxybenzyl)-5-(dehydroxymethyl)-5-formyl-α-D-glucopyranoside (9). DMSO (839 μL, 11.8 mmol) was added dropwise to a solution of oxalyl chloride (516 μL, 5.91 mmol) in CH_2Cl_2 (16 mL) at −78 °C. The solution was stirred at −78 °C for 20 min, and then a solution of crude alcohol **8** (1.14 g, 1.97 mmol) in CH_2Cl_2 (23 mL) was added at −78 °C at a moderate rate. After the mixture stirred at −78 °C for 40 min, triethylamine (2.47 mL, 17.7 mmol) was added slowly at −78 °C and then the dry ice/acetone bath was removed. The mixture was stirred at 0 °C for 30 min, the reaction quenched with 0.1 M $NaHSO_4$, and the mixture extracted in CH_2Cl_2 (2 × 50 mL) with 0.1 M $NaHSO_4$ (2 × 40 mL). Flash chromatography with silica gel (gradient to 50% EtOAc/hexanes) gave aldehyde **9** (766 mg, 67% yield for two steps) as a colorless oil.

Benzyl 2-*O*-Benzyl-3-*O*-neoheptyl-4-*O*-(*p*-methoxybenzyl)-5-(dehydroxymethyl)-5-(2-methylprop-1-enyl)-α-D-glucopyranoside (10). To a suspension of isopropyltriphenylphosphonium iodide (heated at 100 °C under high vacuum overnight; 1.19 g, 2.75 mmol) in THF (25 mL) at 0 °C was added ⁿBuLi (1.31 mL, 2.54 mmol in pentane) dropwise. The dark-blood-red mixture was stirred at 0 °C for 5 min and then at 25 °C for 30 min. The mixture was recooled to 0 °C, a solution of aldehyde **9** (730 mg, 1.27 mmol) in THF (15 mL) was added slowly, and the pretty dark-red mixture was stirred at 0 °C for 3 h. After the reaction was quenched with water, much THF was removed in vacuo. Extraction in CH_2Cl_2 (2 × 40 mL) with brine (40 mL) and flash chromatography with silica gel (gradient to 14% EtOAc/hexanes) afforded olefin **10** (724 mg, 95% yield) as a colorless oil. *Note*: Do not expose the phosphonium reagent or the ylide to air since the ylide is easily quenched by air!

Benzyl 2-*O*-Benzyl-3-*O*-neoheptyl-4-(*p*-methoxybenzyl)-6-deoxy-6-isopropyl-α-D- glucopyranoside (11). To a solution of olefin **10** (280 mg, 0.465 mmol) and benzenesulfonyl hydrazide (1.60 g, 9.29 mmol) in 2-methoxyethanol (13 mL) was slowly added 3 M NaOH (4.65 mL, 14.0 mmol). The solution was refluxed for 15 h and then extracted in CH_2Cl_2 (2 × 40 mL) with brine (30 mL) followed by 0.1 M $NaHSO_4$ (2 × 30 mL). ^{1}H NMR of the crude product showed **10**:**11** = 2.3:1. After the crude product was submitted to a second cycle of diimide reduction with the same amount of reagents, the **11**:**10** ratio increased to 2.2:1. A third cycle of diimide reduction using the same amount of reagents resulted in **11**:**10** = 5.5:1. Flash chromatography with silica gel (gradient to 10% EtOAc/hexanes) provided isobutyl **11** (215 mg, 77% yield) as a colorless oil. *Note*: Reaction of olefin **10** with 60 equiv of benzenesulfonyl hydrazide and 70 equiv of NaOH for 3 days resulted in only **11**:**10** = 1:1.2.

Benzyl 3-*O*-Neoheptyl-4-*O*-(*p*-methoxybenzyl)-6-deoxy-6-isopropyl-α-D-glucopyranoside (12) and Benzyl 3-*O*-Neoheptyl-6-deoxy-6-isopropyl-α-D-glucopyranoside (13). Ten percent palladium on activated carbon (Degussa type, 15 mg) was added to a solution of **11** (41 mg, 0.068 mmol) in EtOH (1.3 mL), and the flask was purged with N_2 followed by H_2. After being stirred at 25 °C for 25 min under H_2 (1 atm), the mixture was filtered through a plug of Celite, and the plug was washed well with MeOH and EtOAc. Preparative TLC with silica gel (20% EtOAc/hexanes eluent) provided monool **12** (R_f = 0.43, 10 mg, 29% yield) and diol **13** (R_f = 0.37, 14 mg, 52% yield), both as colorless oils.

Benzyl 2-*O*-Benzyl-3-*O*-neoheptyl-6-deoxy-6-isopropyl-α-D-glucopyranoside (14). To a mixture of PMB ether **11** (139 mg, 0.230 mmol) and water (190 μL) in CH_2Cl_2 (3.4 mL) was added DDQ (111 mg, 0.491 mmol). The flask was sealed, and the mixture was stirred vigorously at 25 °C for 1.5 h. Extraction in CH_2Cl_2 (2 × 25 mL) with saturated $NaHCO_3$ (2 × 20 mL) and flash chromatography with silica gel (gradient to 12% EtOAc/hexanes) furnished alcohol **14** (97 mg, 87% yield) as a colorless oil.

Benzyl 2-*O*-Benzyl-3-*O*-neoheptyl-4-*O*-methyl-6-deoxy-6-isopropyl-α-D-glucopyranoside (15). To a solution of alcohol **14** (97 mg, 0.20 mmol) and MeI (62 μL, 1.0 mmol) in DMF (1.7 mL) at 0 °C was added NaH (deoiled, 17 mg, 0.70 mmol). The mixture was stirred at 0 °C for 5 min and then at 25 °C for 10 h. After the reaction was quenched with water, extraction in CH_2Cl_2 (2 × 20 mL) with water (15 mL) and preparative TLC with silica gel (8% EtOAc/hexanes eluent) generated methyl ether **15** (R_f = 0.42, 88 mg, 88% yield) as a colorless oil.

Benzyl 3-*O*-Neoheptyl-4-*O*-methyl-6-deoxy-6-isopropyl-α-D-glucopyranoside (3). A suspension of dibenzyl ether compound **15** (30 mg, 0.060 mmol) and 10% Pd/C (Degussa type, 10 mg) in EtOH (1.0 mL) was stirred at 25 °C for 25 min under H_2 (1 atm) and then filtered through a plug of Celite. (The longer the reaction transpires, the more diol anomers are formed!) Preparative TLC with silica gel (15% EtOAc/hexanes eluent) produced C-2 alcohol **3** (R_f = 0.41, 19 mg, 77% yield) as a colorless oil.

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Supporting Information Available: Complete characterization data of compounds **3**–**15**, including specific rotation, IR, HRMS, and ^{1}H and ^{13}C NMR (6 pages). Ordering information is given on any current masthead page.

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