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Assessment of Chemoselective Neoglycosylation Methods Using Chlorambucil as a Model

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To systematically assess the impact of glycosylation and the corresponding chemoselective linker upon the anticancer activity/selectivity of the drug chlorambucil, herein we report the synthesis and anticancer activities of a 63-member library of chlorambucil-based neoglycosides. A comparison of *N*-alkoxyamine-, *N*-acylhydrazine-, and *N*-hydroxyamine-based chemoselective glycosylation of chlorambucil revealed sugar- and linker-dependent partitioning among open- and closed-ring neoglycosides and corresponding sugar-dependent variant biological activity. Cumulatively, this study represents the first neoglycorandomization of a synthetic drug and expands our understanding of the impact of sugar structure upon product distribution/equilibria in the context of *N*-alkoxyamino-, *N*-hydroxyamino-, and *N*-acylhydrazine-based chemoselective glycosylation. This study also revealed several analogues with increased in vitro anticancer activity, most notably D-threoside **60** (NSC 748747), which displayed much broader tumor specificity and notably increased potency over the parent drug.

Introduction

The sugars attached to pharmaceutically important natural products dictate the pharmacokinetics and/or pharmacodynamics of the selected agent.[1−3] Yet studies designed to systematically understand and/or exploit the attachment of carbohydrates in drug discovery remain limited by the availability of practical synthetic and/or biosynthetic tools.[4−8] Neoglycosylation takes advantage of a chemoselective reaction between free reducing sugars and *N*-methoxyamino-substituted acceptors.[9−19] This reaction has enabled the process of "neoglycorandomization" wherein alkoxyamine-appended natural product-based drugs are differentially glycosylated with a wide array of natural and unnatural reducing sugars.[4−8,20−24] Neoglycorandomization has led to the discovery of cardenolide neoglycosides with enhanced in vitro and in vivo anticancer activity and lower in vivo toxicity,[20,23] colchicine neoglycosides with a novel anticancer mechanism and lower in vivo toxicity,[21] vancomycin neoglycosides that displayed improved in vitro potency against clinical isolates of vancomycin-resistant *Enterococci*,[22] and betulinic acid neoglycosides improved for either in vitro anticancer or antiviral potency.[24] While these examples clearly highlight the potential impact of differential glycosylation in drug lead discovery, this work has not addressed the impact of the chemoselective glycosylation "handles"[17] employed.

To assess the impact of alternative chemoselective glycosylation methods in the context of neoglycorandomization, we selected the synthetic anticancer agent chlorambucil (Figure 1, **1**) as a model. First synthesized over 5 decades ago,[25] chlorambucil remains a current treatment for chronic lymphocytic leukemia (CLL[a])[26−30] and has served as the basis for newer generation analogues such as the recently approved bendamustine (**3**).[31] A nitrogen mustard, **1** leads to guanine alkylation and DNA cross-linking and ultimately prohibits DNA replication and transcription.[32,33] The primary cellular uptake mechanism of **1** is passive diffusion,[34] and like many cytotoxics, the lack of nitrogen mustard tumor-specificity contributes to serious side effects.[35,36] Thus, improvements have focused upon (i) the development of tumor-activated prodrugs, exemplified by the hypoxia-activated *N*-oxide PX-478 (Figure 1, **2**) currently in phase I[37] or (ii) modifications to engage tumor-specific transport, exemplified by the β-D-glucosyl analogue of ifosfamide (glufosfamide, Figure 1, **4**) which is actively transported into tumor cells by the sodium/D-glucose cotransporter SGLT3 (SAAT1).[38] While the specific glycosylation of **1** has presented analogues that display slight improvements in a perceived therapeutic index (slightly improved in vitro potency and subtle reductions of in vivo peripheral toxicity), the analogues synthesized to date have been restricted to the use of D-gluco- or D-galacto-based sugars.[39−46]

To systematically assess the impact of glycosylation and the corresponding chemoselective linker upon the anticancer activity/selectivity of **1**, herein we report the synthesis and anticancer activities of a 54-member library of **1** neoglycosides. Several analogues with increased in vitro anticancer activity were identified, most notably D-threoside **60** (NSC 748747) which displayed much broader tumor specificity and increases in potency of up to 15-fold compared to **1**, representing the most active chlorambucil glycoside reported to date. Representative sugars identified as hits in the context of

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[a] Abbreviations: $BH_3 \cdot Et_3N$, borane−triethylamine complex; CLL, chronic lymphocytic leukemia; CNS, central nervous system; DIC, *N*,*N*′-diisopropylcarbodiimide; DMAP, 4-(*N*′,*N*′-dimethylamino)pyridine; EDAC, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide; GI_{50}, growth inhibitory concentration for 50% of the cell population under study; GLUT1, glucose transporter 1; LAH, lithium aluminum hydride; NMM, *N*-methylmorpholine; SAAT1, sodium/amino acid transporter 1; SGLT3, sodium/glucose transporter 3; SPE, solid phase extraction; THF, tetrahydrofuran.

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N-methoxyamino-substituted **1** were subsequently conjugated via *N*-acylhydrazine- and *N*-hydroxyamine-based strategies and the products characterized and evaluated for anticancer activity. Analysis of these second generation neoglycoside analogues revealed sugar-dependent partitioning among open- and closed-ring neoglycosides and corresponding sugar-dependent variant biological activity. Cumulatively, this study represents the first neoglycorandomization of a synthetic drug and expands our understanding of the impact of sugar structure upon product distribution/equilibria in the context of *N*-alkoxyamino-, *N*-hydroxyamino-, and *N*-acylhydrazine-based chemoselective glycosylation.

Results and Discussion

Chlorambucil *N*-Alkoxyamino-Based Neoglycorandomization (Scheme 1). Chlorambucil was converted to the Weinreb amide **6** using the EDAC coupling agent in excellent yield (97%) and then selectively reduced to the corresponding aldehyde with LAH. Compound **7** was subsequently condensed with methoxyamine·HCl in the presence of organic base, providing a mixture of *E*- and *Z*-oximes (**8**). Reduction of the carbon−nitrogen double bond was accomplished with $BH_3 \cdot Et_3N$ in a manner similar to that for previously described neoaglycons.[20,21,23] This convenient strategy yielded neoaglycon **9** in four steps with an overall yield of 37% and required only a single chromatographic purification. While standard neoglycosylation conditions (3/1 DMF/HOAc)[9−22] provided a slightly greater yield (68%) of neo-D-riboside **53** in a pilot reaction, the use of MeOH with a small molar excess of HOAc (i.e., 1.5 equiv) as an acidic proton source was sufficient for the reaction to proceed in an equivalent amount of time (54% yield, Table S1 in Supporting Information) and also simplified subsequent solvent evacuation. By use of these optimized conditions (90 μM **9**, 2 equiv of sugar, 40 °C, 1.5 equiv of HOAc in MeOH), a 54-member library of neoglycosides (**10**−**63**; see Figure S1 in Supporting Information) was synthesized with an average isolated yield of 63% wherein high-throughput solid phase extraction provided an average purity of 92.9% (see Table S3 in Supporting Information). Product yields paralleled the overall reactivity trend: tetroses (79 ± 10%) > deoxy sugars (69 ± 6%) > pentoses (62 ± 7%) > hexoses (56 ± 5%), likely reflecting the general reducing sugar solubility in the selected solvent system. Unlike prior neoglycorandomized libraries,[9−24] a preference for β-anomer formation of chlorambucil neoglycosides was generally less predominate. Rather, a strong 1,2-trans relationship was typically observed (Table S2 in Supporting Information).

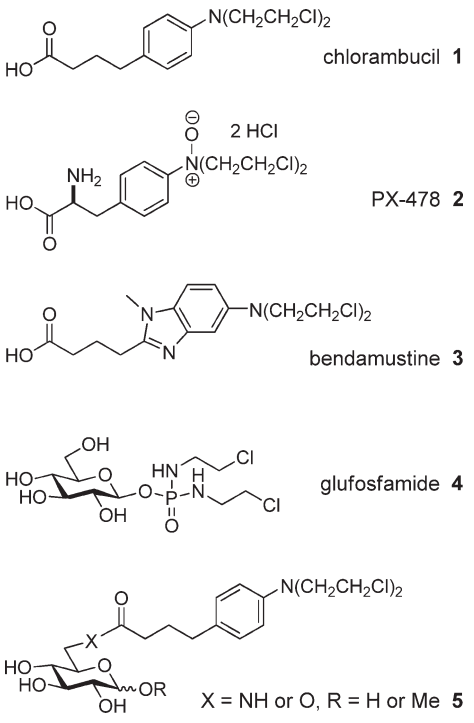
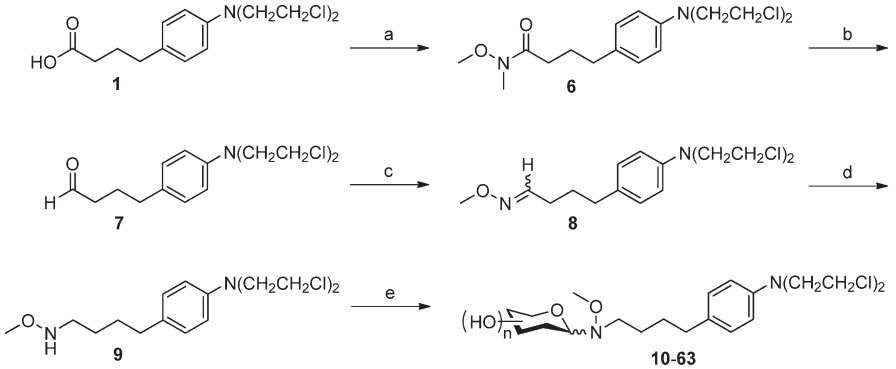


Figure 1. Chlorambucil (**1**) and other nitrogen mustard compounds including *N*-oxide **2** (PX-478), FDA-approved bendamustine (**3**), and glycosylated variants glufosfamide (**4**) and **5**. The latter was found to inhibit the brain/erythrocyte D-glucose GLUT1 transporter and thereby decrease glucose uptake (ref 42).

Anticancer Activity of Chlorambucil *N*-Alkoxyamino-Based Neoglycosides. The antiproliferative activity (i.e., GI_{50}) of neoglycosides **10**−**63** was evaluated using a 10-member panel of human carcinomas from various lung, colorectal, liver, breast, prostate, CNS, and ovarian cell lines with **1** and neoaglycon **9** as comparators (see Figure 2). On the basis of this study, aglycon **9** displayed slightly better GI_{50} values (1.1−4.4 μM) compared to the parent drug **1** (5.0−11.0 μM) and the vast majority of the library members had an average GI_{50} also comparable to or lower than chlorambucil (see Table S5 in Supporting Information). Out of the 54 analogues, 19 possessed GI_{50} values in the high nanomolar range in at least one cell line, six of which displayed GI_{50} values in the high nanomolar range in three or more lines. Of this latter set, two neoglycosides (D-glucuronolactonide **39** and D-threoside **60**;

Scheme 1. Synthesis of the *N*-Alkoxyamino-Based Chlorambucil Library[a]



[a] (a) MeON(H)Me, NMM, EDAC, 0 °C (97%); (b) LAH, THF, 0 °C (70%); (c) $MeONH_3Cl$, Et_3N, EtOH (84%); (d) $BH_3 \cdot Et_3N$, HCl, EtOH, 0 °C (65%); (e) reducing sugar, MeOH, HOAc (1.5 equiv), 40 °C (63% av).

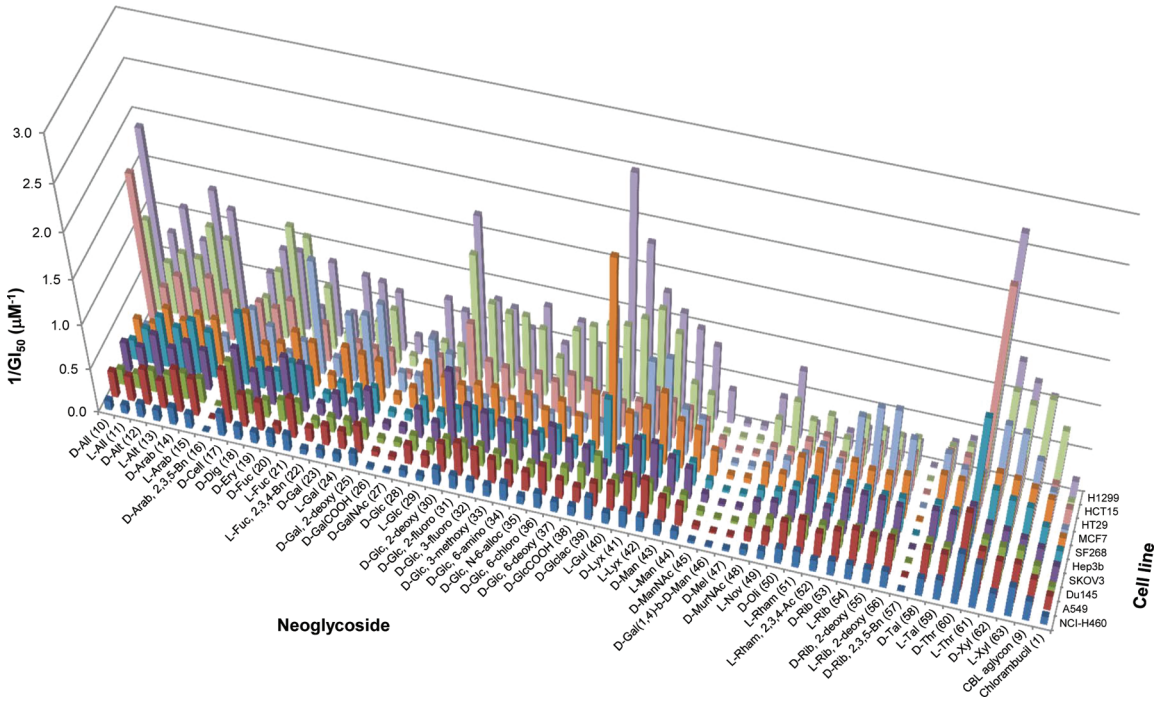


Figure 2. Summary of GI_{50} data from the high-throughput growth inhibition assay of **10**−**63** (reciprocal values displayed). Comparisons were performed against the aglycon (**9**) and chlorambucil (**1**). GI_{50} data and error values are provided in Supporting Information Table 5. Representative cancer cell lines tested include NCI-H460 (lung), A549 (lung), Du145 (prostate), SKOV3 (ovary), Hep3b (liver), SF268 (brain), MCF7 (breast), HT29 (colorectal), HCT15 (colorectal), H1299 (lung).

see Figure 3) were identified as the two most potent neoglycosides. In comparison to the parent **1**, **39** displayed a 6-fold improvement in average growth inhibition across the cell panel, with the most sensitive cell line being SF268 glioblastoma (12-fold improved). Likewise, D-threoside **60** presented an average elevated potency of 8-fold over **1**, with 12-, 13-, and 15-fold improved activities toward HT29 (colorectal), H1299 (lung), and HCT15 (colorectal) cancers, respectively, over the parent drug.

The activity assessment described above revealed the following general structure−activity relationships. First, sugars that favor furanosyl-derived neoglycosides (e.g., threosides **60** and **61**, glucuronolactonide **39**, arabinosides **14** and **15**, lyxosides **41** and **42**, and xylosides **62** and **63**) led to the greatest improvements in anticancer activity. Second, relative configuration within this furanoside-derived neoglycoside series influenced potency. Specifically, those tetroses or pentoses with a 2,3-*trans* dihydroxy orientation [e.g., D-threoside **60** (2*S*,3*R*), L-threoside **61** (2*R*,3*S*), and **39** (D-2*S*,3*R*)] were generally more potent than those with a corresponding 2,3-*cis* configuration [e.g., D-erythroside **19** (2*R*,3*R*), D-riboside **53** (2*R*,3*R*), and L-riboside **54** (2*S*,3*S*)], while D- and L-saccharide enantiomers of this furanoside-derived group were found to have similar values. Third, among neohexosides, a 3*R* hydroxyl group (i.e., axial in the chair conformation) enhances selectivity toward lung (H1299) and colon (HCT-15) cancer cell lines (e.g., allosides **10** and **11**, altrosides **12** and **13**, and L-guloside **40**). Finally, most neoglycosides derived from sugars known to be GLUT substrates and to mediate GLUT-dependent uptake of conjugates (e.g., D-glucoside **28** or 3-methoxy-D-glucoside **33**)[47−50] were not among the most active and/or selective hits identified. Additionally, comparison of the anomeric composition of either the most active neoglycosides or the library as a whole to the inhibitory data does not reveal that a distinct correlation exists between anomers and activity. To illustrate using the

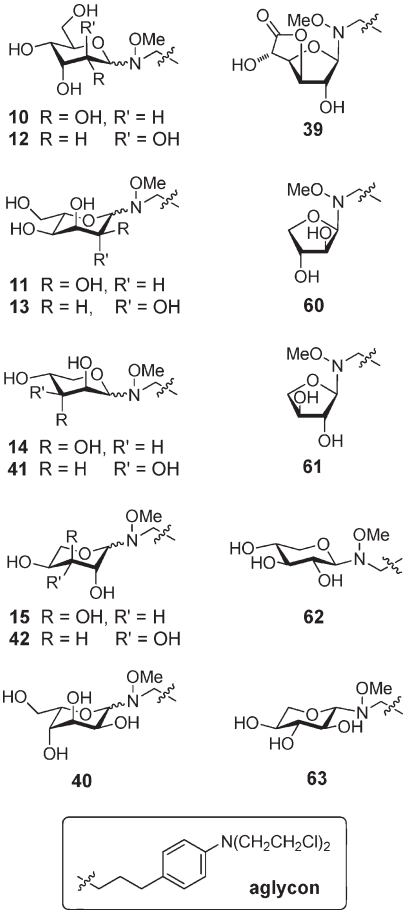
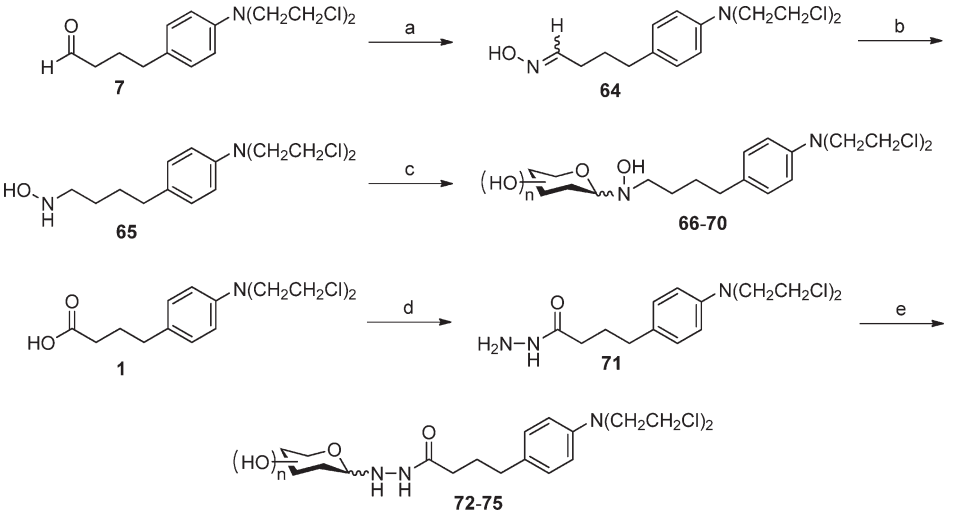


Figure 3. Structures of the most antiproliferative chlorambucil *N*-alkoxyamino-based neoglycosides against a 10-member carcinoma panel.

Scheme 2. Synthesis of the *N*-Acylhydrazine- and *N*-Hydroxyamino-Based Chlorambucil Libraries[a]



[a] (a) $HONH_3Cl$, Et_3N, EtOH (98%); (b) $BH_3 \cdot Et_3N$, HCl, EtOH, 0 °C (65%); (c) reducing sugar, MeOH, HOAc (1.5 equiv), 40 °C (47% av); (d) (i) *N*-hydroxysuccinimide, DIC, THF, 40 °C, (ii) NH_2NH_2, pyridine, DMAP, 40 °C (85%); (e) reducing sugar, MeOH, HOAc (1.5 equiv), 40 °C (62% av).

antiproliferative neo-D-pentosides (the two with the most extreme anomeric biases), D-arabinoside **14** (α/β 19/1) and D-xyloside **62** (β only) have similar GI_{50} values in 9 of the 10 cell lines, not providing any distinction between the two anomers and overall impact on growth inhibition.

Chlorambucil *N*-Hydroxyamino-Based Neoglycosylation (Scheme 2). Given the clear impact of chlorambucil neoglycosylation upon anticancer activity, we subsequently set out to examine the specific contribution of the neoglycoside handle. Similar to alkoxyamino-based chemoselective neoglycosylation,[9−24] previous studies have revealed hydroxyamines[51−53] and hydrazides[54−59] to also provide the corresponding closed-ring glycosides. Additionally, we envisioned that use of a hydroxyamino handle may allow for additional modification at the hydroxyl group, providing a facile means for further diversification.[23] To create the two modified aglycons, only slight changes in the synthesis were required. In a fashion similar to the procedure described in Scheme 1, aldehyde **7** was combined with hydroxyamine HCl to form a mixture of *E*- and *Z*-oximes (**64**) that were reduced with $BH_3 \cdot Et_3N$ complex. As with the synthesis described in Scheme 1, only aglycon **65** required column chromatography for purification in the four-step process from **1** (Scheme 2, 44% overall yield). Hydrazide formation (**71**) was achieved by reacting the *N*-hydroxysuccinimidyl ester of **1** with hydrazine in the presence of DMAP in pyridine. By use of the previously described neoglycosylation conditions (see Scheme 1), the focused hydroxyamine (**66**−**70**) and hydrazide (**72**−**75**) neoglycosyl sets were synthesized using sugars identified as hits from the alkoxyamino-based series **10**−**63**, specifically D-glucuronolactone and D-threose, which had superior performance throughout the panel; D-xylose, the most active of the pentoses; and D-fucose, which displayed the most pronounced selectivity toward one tumor line (HCT-15 colorectal).

NMR analysis of the **66**−**70** neoglycosides revealed that the desired closed-ring α- and β-anomeric forms were usually in equilibrium with an open-chain imine isomer. Evidence for this was based upon the observed ^{1}H NMR chemical shift of the H1 (doublets) and H2 (doublets of doublets) protons at 7.2−7.5 and 5.1−5.0 ppm, respectively, indicating the presence of an iminyl double bond. Such nitrone formation is

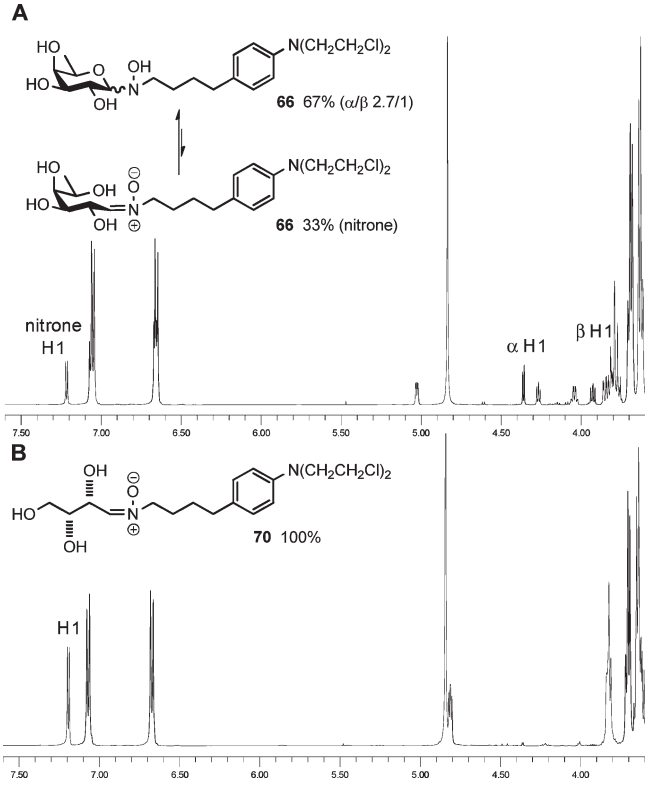


Figure 4. ^{1}H NMR spectra of *N*-hydroxyaminochlorambucil glycosides: (A) equilibrium between the cyclic neoglycoside and acyclic nitrone of D-fucoside **66**; (B) nitrone form of the chlorambucil D-threoside **70**. Both spectra were obtained at 500 MHz in CD_3OD.

well-precedented for the condensation of aldehydes and monosubstituted hydroxyamines.[60] However, in the context of glycoside formation, only a handful of such glycosides have been reported, comprising protected saccharides and small *N*-alkyl-*N*-hydroxyamines.[51−53] From the current study, the nature of the sugar influences the thermodynamic equilibrium and thereby product distribution. Specifically, the D-fucoside-derived **66** predominately adopted the closed-ring isomer (67%), D-glucuronolactone (**67**) and D-ribose (**68**) favored the open-chain nitrone (71% and 50%, respectively),

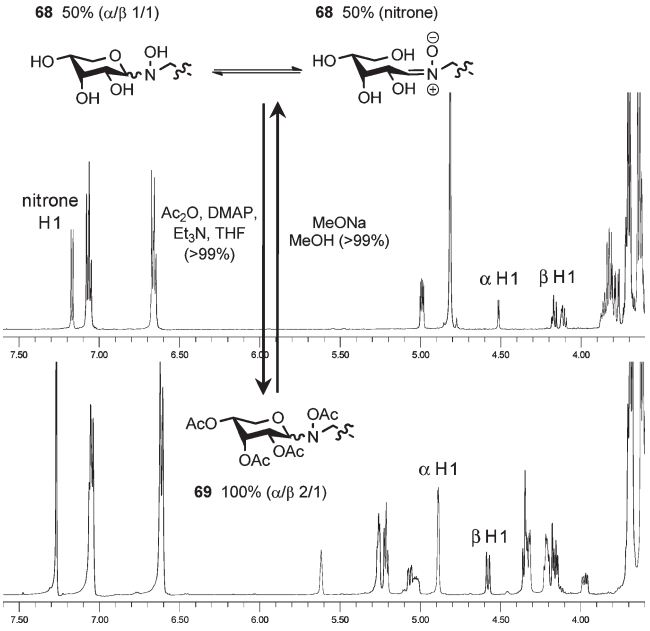


Figure 5. Interconversion of *N*-hydroxyaminochlorambucil-D-riboside between the cyclic neoglycoside and acyclic nitrone **68** and peracetylated analogue **69**. Both spectra were obtained at 500 MHz with **68** in CD_3OD and **69** in $CDCl_3$.

while D-threose (**70**) led solely to nitrone (Figure 4, Table S3 in Supporting Information). These equilibria could be shifted upon nitrone modification. For example, peracetylation of neoriboside **68** using acetic anhydride and DMAP in THF promoted ring closure based upon ^{1}H NMR (Figure 5) and ESI-MS analysis. Deacetylation of the peracetate **69** in base reestablished the mixture of isomers, demonstrating that changing the electronics of the nitrone oxygen will promote ring-opening and closure, as will the type of ligated sugar. Interestingly, the anomeric ratio also changed, moving from a 1/1 ratio as the nitrone to 2/1 as the tetraacetate, contrasting from the 1/3 α/β ratio of methoxyamine D-riboside (**53**).

Chlorambucil *N*-Acylhydrazine-Based Neoglycosylation (Scheme 2). A conformational study of sugar acetylhydrazides by Bendiak indicated that natural hexose and pentose (e.g., D-Glc, D-Gal, D-Xyl, etc.) analogues formed pyranosides, as evidenced by ^{1}H NMR.[54] Similar findings were reported for mono- and oligosaccharide chemoselective ligations with biotinyl,[55] long-chain acyl,[56] peptidyl,[57] and adipyl hydrazides.[59] In contrast, the current chlorambucil study revealed a trend that mirrored the trend observed for the **66**−**70** subset. Specifically, D-fucoside **72** was formed only as the cyclic compound (67% yield), D-glucuronolactonide **73** and D-xyloside **75** were a mixture of the open and closed conformers (46% and 47%, respectively), and D-threoside **74** adopted the open-chain imine (86%). These results indicate that closure of the glycoside may be dependent not only on the type of sugar but possibly on the nature of the aglycon as well. It was also notable that only the β-anomer of the closed rings was observed while the hydrazylimines were isolated as mixtures of *E*- and *Z*-isomers (Figure 6).

Anticancer Activity of Chlorambucil *N*-Hydroxyamino- and *N*-Acylhydrazine-Based Neoglycosides. Neoglycosides **66**−**70**, **72**−**75**, and their respective aglycons **65** and **71** were assayed for antiproliferative activity using the same cell lines as for **10**−**63**. In general, the alternative handle glycosides did not perform as

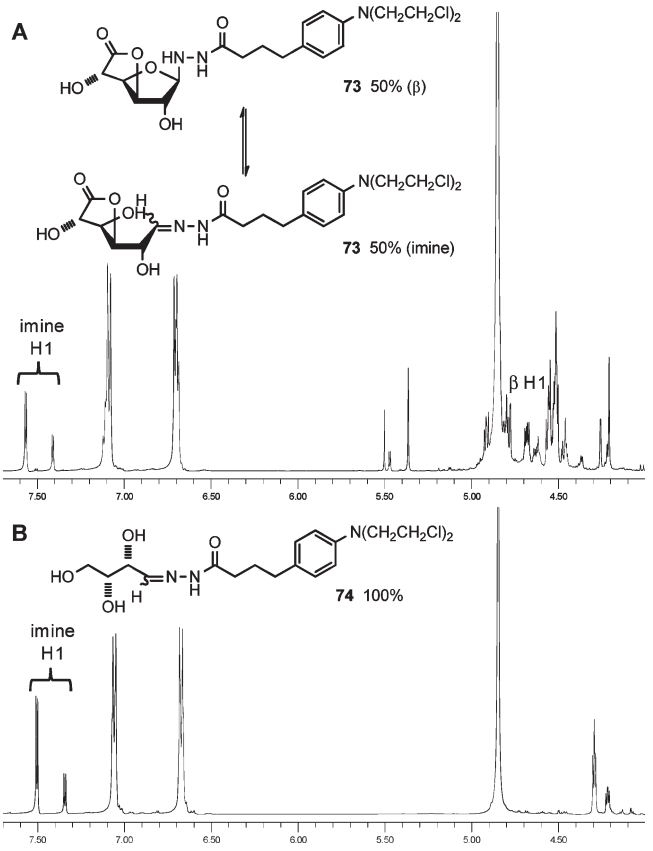


Figure 6. ^{1}H NMR spectra of *N*-hydrazidochlorambucil glycosides: (A) equilibrium between the cyclic neoglycoside and acyclic imine of D-glucurono-6,3-lactonide **73**; (B) nitrone form of the chlorambucil D-threoside **74**. Both spectra were obtained at 500 MHz in CD_3OD.

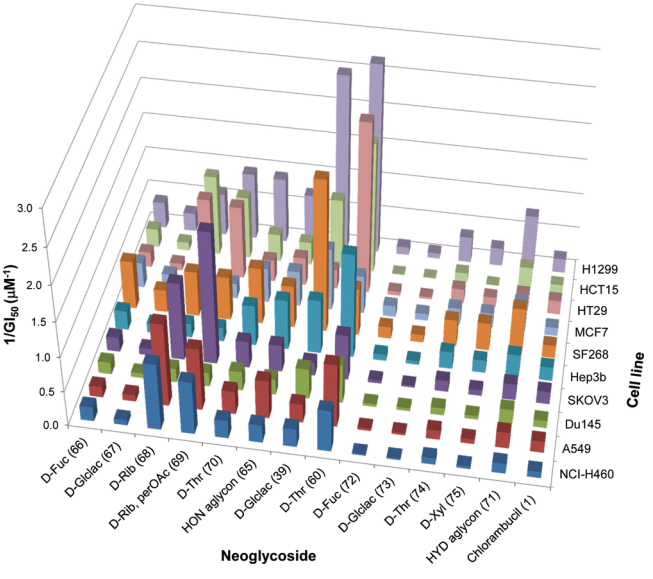


Figure 7. Summary of GI_{50} data from the high-throughput growth inhibition assay of **66**−**70** hydroxyamines and **72**−**75** hydrazides (reciprocal values displayed). Comparisons were performed against methoxyamines **39** and **60**, aglycons **65** and **71**, and chlorambucil (**1**). GI_{50} data and error values are provided in Supporting Information Table 5. Representative cancer cell lines tested include NCI-H460 (lung), A549 (lung), Du145 (prostate), SKOV3 (ovary), Hep3b (liver), SF268 (brain), MCF7 (breast), HT29 (colorectal), HCT15 (colorectal), H1299 (lung).

well as their methoxyamine analogues with the most notable difference being between the **66**−**70** and **72**−**75** analogues. While some of the hydroxyamine compounds produced comparable inhibitory responses to the methoxyamine group, **72**−**75** had universally diminished anticancer properties over **10**−**63** with average GI_{50} values 5- to 13-fold greater across the 10-member panel. **66**−**70** and **72**−**75** were also found to have GI_{50} values up to 6-fold larger than the corresponding aglycons, though aglycons **65** and **71** were of similar activity to **9** (Table S5 in Supporting Information).

Two exceptions to this overall trend, D-riboside **68** and its peracetylated variant **69**, were 2- to 5-fold more active than the alkoxyamino-based **53** in 6 of the 10 cell lines (0.53−1.3 μM vs 1.8−5.9 μM, respectively). **68** and **69** are also notable as being among the most active (i.e., mid- to high-nanomolar range) neoglycosides against the SKOV3 ovarian cancer line (see Figure 7). These data suggest the type of neoglycoside handle employed greatly influences desired activity. When taken into account that aglycons **9**, **65**, and **71** are of similar potency, the variation in activity between the methoxyamino-, hydroxyamino-, and acylhydrazine-based neoglycosides implicates the nature of the glycosidic bond to be the most significant contributor wherein a prevalence of the acyclic nitrone or imine conjugate led to a reduction in potency.

Conclusions

This study revealed a facile four-step process, which could be conducted on gram scale in less than 2 h of reaction time and required only a single chromatographic separation to modify the drug chlorambucil (**1**) for chemoselective glycosylation. While prior syntheses of **1** glycoconjugates focused upon the use of typical metabolic sugars designed to enhance sugar-mediated uptake and led to modest overall improvements compared to **1**,[39,40] the current study revealed that anticancer potency optimization was best accomplished via conjugation with novel nonmetabolic sugars, culminating in the discovery of D-threoside **60** as the most active chlorambucil glycoside reported to date. The discovery of **60** opens the door to a series of new questions relating to the precise mechanism(s) of improvement, including among the many possibilities: (i) modulation of uptake (via novel targeting of known transporters and/or even raising the possibility of new sugar transport/receptor mediated-processes); (ii) intracellular stabilization of the alkylating reagent (basically extending the intracellular $T_{1/2}$); (iii) enhancement of the productive agent−DNA interactions (e.g., DNA affinity and/or specificity); and/or even (iv) alternative targeting of the active species (e.g., RNA, proteins, and/or membrane targets). While the specific mechanisms remain to be elucidated, it is important to note that precedent does exists for **1** glucosylation to alter the mechanism of cellular uptake.[34,42]

In addition to the lead discovery aspect of this project, it is also important to note that the corresponding in-depth product distribution analysis among *N*-alkoxyamino-, *N*-acylhydrazine-, and *N*-hydroxyamino-based neoglycosylation reactions revealed sugar-dependent partitioning among open- and closed-ring neoglycosides in the last two cases. Thus, this cumulative study also sheds new light (and a potential note of caution) on an underappreciated chemical variability of glycoconjugates generated via the *N*-acylhydrazine- and *N*-hydroxyamine-based chemoselective glycosylation methods more commonly applied in glycobiology.[18]

Experimental Section

Materials and General Methods. Mass spectrometric data were obtained on either a Waters (Milford, MA) LCT time-of-flight spectrometer for electrospray ionization (ESI) or a Varian ProMALDI (Palo Alto, CA) Fourier transform ion cyclotron resonance mass spectrometer (FTICR) equipped with a 7.0 T actively shielded superconducting magnet and a Nd:YAG laser. NMR spectra were obtained on a Varian ^{Unity}Inova 500 MHz instrument (Palo Alto, CA) using 99.8% $CDCl_3$ with 0.05% v/v TMS or 99.8% CD_3OD in ampules. ^1H and ^{13}C chemical shifts were referenced to TMS (for $CDCl_3$) or nondeuterated solvent (for CD_3OD). Multiplicities are indicated by s (singlet), d (doublet), t (triplet), q (quartet), qui (quintet), m (multiplet), and br (broad). Chemical shift assignments for anomeric mixtures, where possible, are noted as α or β with the atom responsible for the shift. ^1H NMR characterization was supplemented with gCOSY for all neoglycoside library members as well as ^{13}C and gHSQC for pilot reactions and alternative handle compounds. Tetrahydrofuran was dried using a column of activated alumina. All other solvents were used as provided by the supplier. Reagents were obtained from Aldrich or Sigma and were used as received. Flash chromatography was performed using 40−63 μm particle size silica gel. Thin layer chromatography was performed on aluminum-backed, 254 nm UV-active plates with a silica gel particle size of 60 μm. Library purity was assessed by reverse phase HPLC using a Varian (Walnut Creek, CA) ProStar unit with a Phenomenex (Torrance, CA) Luna C18 4.6 mm × 250 mm column running a H_2O/MeCN 90/10 to 10/90 gradient over 13 m, followed by a 5 m isocratic flow, at a rate of 1.0 mL/m, A_{254} detection. The purity of the neoglycosides and aglycons was assessed to be greater than 95%, unless specified otherwise (see Table S4 in Supporting Information).

***N*,*O*-Dimethyl-4-(4-*N*′,*N*′-bis(2-chloroethyl)amino)phenylbutanamide (6).** Chlorambucil (**1**, 516 mg, 1.70 mmol) was dissolved in CH_2Cl_2 (15 mL) and cooled to 0 °C before adding *N*,*O*-dimethylhydroxylamine HCl (181 mg, 1.86 mmol) and *N*-methylmorpholine (200 μL, 1.82 mmol). The coupling agent EDAC (347 mg, 1.81 mmol) was then added to the reaction slowly over 5 min to ensure dissolution. After 60 min, the reaction was quenched with 5% HCl (20 mL) and the acidic layer extracted with CH_2Cl_2 (20 mL). The organic layers were combined, washed with saturated aqueous $NaHCO_3$ (20 mL), and the basic layer was extracted with CH_2Cl_2 (20 mL). After the organic layers were combined, washed with brine (20 mL), and dried with Na_2SO_4, solvent removal yielded a colorless oil (571 mg, 97%, R_f = 0.23, EtOAc/Hex 1:2) that was used without further purification. ^1H NMR ($CDCl_3$, 500 MHz) δ 7.09 (d, J = 8.8 Hz, 2 H), 6.62 (d, J = 8.8 Hz, 2 H), 3.71−3.65 (m, 4 H), 3.64 (s, 3 H), 3.62−3.59 (m, 4 H), 3.17 (s, 3 H), 2.58 (t, J = 7.7 Hz, 2 H), 2.44 (t, J = 7.0 Hz, 2 H), 1.95−1.88 (m, 2 H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 144.37, 131.17, 129.80, 112.31, 61.30, 53.74, 40.69, 34.33, 32.29, 31.35, 26.44; HRMS (ESI) m/z for $C_{16}H_{25}Cl_2N_2O_2$ ($[M + H]^+$) 347.1295, calcd 347.1288.

4-(4-*N*′,*N*′-Bis(2-chloroethyl)amino)phenylbutanal (7). Weinreb amide **6** (571 mg, 1.64 mmol) was dissolved in anhydrous THF (8 mL) under Ar and cooled to 0 °C. A suspension of lithium aluminum hydride in anhydrous THF (1.0 M, 1 mL) was added in one aliquot. After 5 min, the reaction was quenched with saturated aqueous $KHSO_4$ (5 mL) followed by deionized water (5 mL). Extraction of the aldehyde was performed with Et_2O (2 × 15 mL), and the organic layer was dried with Na_2SO_4. Solvent evaporation provided a yellowish oil (334 mg, 70%, R_f = 0.65, EtOAc/Hex 1:2). ^1H NMR ($CDCl_3$, 500 MHz) δ 9.80 (s, 1 H), 7.13 (d, J = 8.8 Hz, 2 H), 6.70 (d, J = 8.8 Hz, 2 H), 3.77−3.74 (m, 4 H), 3.69−3.66 (m, 4 H), 2.63 (t, J = 7.6 Hz, 2 H), 2.49 (td, J = 7.3, 1.5 Hz, 2 H), 2.00−1.94 (m, 2 H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 202.42, 144.46, 130.37, 129.69, 112.23, 53.56, 43.16, 40.60, 33.91, 23.93; HRMS (MALDI) m/z for $C_{14}H_{20}Cl_2NO$ ($[M + H]^+$) 288.090 91, calcd 288.091 65.

***N*-Methoxy-4-(4-*N*′,*N*′-bis(2-chloroethyl)amino)phenylbutanimine (8).** Aldehyde **7** (334 mg, 1.16 mmol) was dissolved in absolute EtOH (10 mL) followed by the addition of $MeONH_2 \cdot HCl$ (247 mg, 2.95 mmol) and Et_3N (400 μL, 2.87 mmol). After 35 min, the solvent was removed in vacuo, providing the crude product as a white crystalline solid. The material was suspended in EtOAc/Hex 1:7 (50 mL) and filtered through a silica gel plug, which was flushed with solvent (400 mL). The purified imine was provided as a colorless oil containing a mixture of *E*- and *Z*-isomers (308 mg, 84%, R_{fA} = 0.51, R_{fB} = 0.43, EtOAc/Hex 1:7). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.36 (t, *J* = 6.2 Hz, 1 H), 7.05 (d, *J* = 8.8 Hz, 2 H), 6.61 (d, *J* = 8.8 Hz, 2 H), 3.80 (s, 3 H), 3.69−3.65 (m, 4 H), 3.61−3.57 (m, 4 H), 2.55 (t, *J* = 7.7 Hz, 2 H), 2.18 (q, *J* = 7.7 Hz, 2 H), 1.75 (qui, *J* = 7.7 Hz, 2 H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 150.58, 144.38, 130.87, 129.74, 112.28, 61.27, 53.67, 40.61, 34.14, 29.03, 28.72; HRMS (ESI) *m*/*z* for $C_{15}H_{23}Cl_2N_2O$ ($[M + H]^+$) 317.1187, calcd 317.1182.

***N*-Methoxy-4-(4-*N*′,*N*′-bis(2-chloroethyl)amino)phenylbutylamine (9).** Imine **8** (308 mg, 0.971 mmol) was dissolved in absolute EtOH (15 mL), cooled to 0 °C, and the reducing agent $BH_3 \cdot Et_3N$ (710 μL, 4.83 mmol) was added in one aliquot. Concentrated HCl/EtOH 1:1 (800 μL) was then dripped in slowly over 5 min. The reaction was quenched with saturated aqueous $NaHCO_3$ (5 mL) 5 min after the acid solution was completely added, resulting in a white slurry. The organic solvent was removed under reduced pressure. Then more $NaHCO_3$ (5 mL) solution was added. The aqueous material was extracted with CH_2Cl_2 (3 × 20 mL) and dried with Na_2SO_4. The crude oil was purified by flash chromatography (SiO_2, EtOAc/Hex 1:2), providing the aglycon as a colorless oil (200 mg, 65%, R_f = 0.37, EtOAc/Hex 1:2). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.05 (d, *J* = 8.6 Hz, 2 H), 6.60 (d, *J* = 8.7 Hz, 2 H), 5.50 (s br, 1 H), 3.69−3.65 (m, 4 H), 3.61−3.57 (m, 4 H), 3.50 (s, 3 H), 2.91 (t, *J* = 7.1 Hz, 2 H), 2.53 (t, *J* = 7.5 Hz, 2 H), 1.61−1.58 (m, 2 H), 1.55−1.49 (m, 2 H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 144.20, 131.63, 129.62, 112.22, 61.80, 53.65, 51.80, 40.61, 34.66, 29.32, 26.98; HRMS (ESI) *m*/*z* for $C_{15}H_{25}Cl_2N_2O$ ($[M + H]^+$) 319.1334, calcd 319.1339.

***N*-Hydroxy-4-(4-*N*′,*N*′-bis(2-chloroethyl)amino)phenylbutanimine (64).** Aldehyde **7** (442 mg, 1.53 mmol) was dissolved in absolute EtOH (10 mL) followed by the addition of $HONH_2 \cdot HCl$ (269 mg, 3.87 mmol) and Et_3N (550 μL, 3.95 mmol). After 25 min, the solvent was removed in vacuo, providing the crude product as a white flaky solid. The material was purified by column chromatography (SiO_2, EtOAc/Hex 1:2), which yielded the imine as a colorless mixture of *E*- and *Z*-isomers (455 mg, 98%, R_{fA} = 0.53, R_{fB} = 0.45, EtOAc/Hex 1:2). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.29 (t, *J* = 6.3 Hz, 1 H), 7.06 (d, *J* = 8.8 Hz, 2 H), 6.61 (d, *J* = 8.8 Hz, 2 H), 3.68−3.65 (m, 4 H), 3.61−3.58 (m, 4 H), 2.55 (t, *J* = 7.7 Hz, 2 H), 2.15 (q, *J* = 7.7 Hz, 2 H), 1.75 (qui, *J* = 7.7 Hz, 2 H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 147.98, 144.45, 131.01, 129.69, 112.22, 53.76, 40.59, 33.23, 29.10, 28.73; HRMS (ESI) *m*/*z* for $C_{14}H_{21}Cl_2N_2O$ ($[M + H]^+$) 303.1019, calcd 303.1025.

***N*-Hydroxy-4-(4-*N*′,*N*′-bis(2-chloroethyl)amino)phenylbutylamine (65).** Imine **64** (455 mg, 1.50 mmol) was dissolved in absolute EtOH (10 mL), cooled to 0 °C, and the reducing agent $BH_3 \cdot Et_3N$ (1.1 mL, 7.5 mmol) was added in one aliquot. Concentrated HCl/EtOH 1:1 (1.26 μL) was then dripped in slowly over 5 min. The reaction was quenched with saturated aqueous $NaHCO_3$ (5 mL) 5 min after the acid solution was completely added, resulting in a white slurry. The organic solvent was removed under reduced pressure. Then more $NaHCO_3$ (5 mL) solution was added. The aqueous material was extracted with CH_2Cl_2 (4 × 25 mL) and dried with Na_2SO_4. The crude oil was purified by column chromatography (SiO_2, $MeOH/CH_2Cl_2$ 2:98 to 5:95), providing the aglycon as a colorless oil (302 mg, 66%, R_f = 0.36, $MeOH/CH_2Cl_2$ 5:95). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.05 (d, *J* = 8.7 Hz, 2 H), 6.61 (d, *J* = 8.7 Hz, 2 H), 5.23 (s br, 2 H), 3.70−3.64 (m, 4 H), 3.63−3.57 (m, 4 H), 3.40 (s, 2 H), 2.58−2.51 (m, 2 H), 1.96−1.84 (m, 2 H), 1.67−1.55 (m, 2 H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 144.60, 131.08, 129.84, 112.47, 53.81, 50.43, 40.88, 34.58, 29.00, 27.16; HRMS (ESI) *m*/*z* for $C_{14}H_{23}Cl_2N_2O$ ($[M + H]^+$) 305.1175, calcd 305.1182.

4-(4-*N*′,*N*′-Bis(2-chloroethyl)amino)phenylbutanoic Hydrazide (71). Chlorambucil (**1**, 319 mg, 1.05 mmol) was dissolved in THF (10 mL), along with *N*-hydroxysuccinimide (132 mg, 1.15 mmol) and DIC (180 μL, 1.16 mmol). The mixture was warmed to 40 °C and stirred for 5.5 h. Hydrazine (40 μL, 1.27 mmol), pyridine (10 μL), and DMAP (5 mg, 0.04 mmol) were then introduced, and the reaction proceeded for another 20 min. The solvent was removed in vacuo, yielding a yellowish solid, which was suspended in $MeOH/CH_2Cl_2$ 3:97 and purified by column chromatography (SiO_2, $MeOH/CH_2Cl_2$ 3:97). The hydrazide product was collected as an opaque oil that solidified at −20 °C to a white amorphous solid (285 mg, 85% R_f = 0.39, $MeOH/CH_2Cl_2$ 5:95). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.31 (s br, 1 H), 7.05 (d, *J* = 8.7 Hz, 2 H), 6.61 (d, *J* = 8.7 Hz, 2 H), 3.90 (s br, 2 H), 3.71−3.66 (m, 4 H), 3.63−3.58 (m, 4 H), 2.54 (t, *J* = 7.6 Hz, 2 H), 2.17−2.13 (m, 2 H), 1.95−1.89 (m, 2 H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 173.80, 144.46, 130.47, 129.72, 112.26, 53.62, 40.66, 40.88, 34.10, 33.77, 27.19; HRMS (ESI) *m*/*z* for $C_{14}H_{22}Cl_2N_3O$ ($[M + H]^+$) 318.1140, calcd 318.1134.

General Procedure for Neoglycoside Library Synthesis, Purification, and Characterization. Aglycon **9**, **65**, or **71** (typically 0.12−0.16 mmol) was added to 1 dram vials along with stir fleas and dissolved in MeOH such that the aglycon was at 90−100 mM. Glacial acetic acid (1.5 equiv) was introduced, reducing sugars (2 equiv) were added, the vials capped, and the vessels placed on a heating block/stir plate to react at 40 °C for 3−48 h. The vial caps were removed, and the solvent was evaporated by a Speedvac apparatus (55 °C, 3 h). Crude neoglycosides were suspended in CH_2Cl_2 (200 μL) using a vortex mixer and then loaded onto 2000 mg silica gel solid phase extraction (SPE) columns (Alltech, Deerfield, IL) that were prewashed with $MeOH/CH_2Cl_2$ 2/98. The SPEs were eluted using a vacuum manifold, collecting fractions with a volume of approximately 2 mL. After the initial two fractions were obtained, eluting any unreacted aglycon or relatively nonpolar material, the following step gradients were used: $MeOH/CH_2Cl_2$ 5/95 for tetroses, pentoses, and substituted hexoses; $MeOH/CH_2Cl_2$ 10/90 for hexoses; $MeOH/CHCl_3$ 15/85 for disaccharides; $MeOH/CHCl_3$ 20/80 for glycuronosides. For polyprotected saccharides, a gradient of EtOAc/Hex 1/6 to 1/5 was used. Typically, all neoglycoside was eluted by the 10th or 11th fraction, leaving unreacted sugar on the column. The fractions containing pure product were identified by TLC using UV light (254 nm) and *p*-anisaldehyde stain, then combined and dried. Library members were characterized by ^{1}H and gCOSY NMR as well as either high-resolution ESI or MALDI mass spectrometry (see Tables S2 and S3 in Supporting Information). Anomeric ratios were obtained by comparison of anomeric proton integration (see Tables S2 and S3 in Supporting Information).

***N*-Methoxy-4-(4-*N*′,*N*′-bis(2-chloroethyl)amino)phenylbutyl-amino-D-riboside (53).** Aglycon **9** (40 mg, 0.13 mmol) was placed into a 1 dram vial, dissolved in MeOH (1.34 mL), and mixed with glacial acetic acid (10.7 μL). After addition of D-ribose (57 mg, 0.38 mmol), the mixture was capped, warmed to 40 °C, and allowed to stir for 3 h. Solvent was subsequently removed in vacuo and the resulting crude solid suspended in $MeOH/CH_2Cl_2$ 2:98 (250 μL) by vortex mixer. The mixture was purified by SPE (SiO_2, $MeOH/CH_2Cl_2$ 2:98 to 5:95), providing the white solid neoglycoside as a mixture of anomers (31 mg, 54%, R_f = 0.34, $MeOH/CH_2Cl_2$ 5:95; α/β 1:3). ^{1}H NMR (CD_3OD, 500 MHz) δ 7.08 (d, *J* = 8.7 Hz, 2 H), 6.68 (d, *J* = 8.7 Hz, 2 H), 4.56 (d, *J* = 3.4 Hz, 0.25 H, α-H1′), 4.27 (d, *J* = 8.7 Hz, 0.75 H, β-H1′), 4.15 (dd, *J* = 5.4, 3.5 Hz, 0.25 H, α-H2′), 4.12 (s br, 0.75 H, β-H3′), 3.99 (t, *J* = 5.6 Hz, 0.25 H, α-H3′), 3.90−3.86 (m, 0.25, α-H4′), 3.74−3.68 (m, 5 H, α-$H5_A$′ + β-$H5_A$′), 3.68−3.60 (m, 6.5 H, α-$H5_B$′ + β-H2′ + β-H4′ + β-$H5_B$′), 3.58 (s, 3 H), 3.05−2.98 (m, 0.75 H, β-$H2_A$), 2.97−2.92 (m, 0.25 H, α-$H2_A$), 2.86−2.77 (m, 1 H, $H2_B$), 2.56 (t, *J* = 7.2 Hz, 2 H), 1.71−1.56 (m, 4 H); ^{13}C NMR (CD_3OD, 125 MHz) δ 145.86, 132.73, 130.66, 113.61, 100.76 (α-C1′), 91.14 (β-C1′), 84.50 (α-C4′), 73.16 (α-C2′), 72.52 (α-C3′), 72.46 (β-C3′), 68.94 (β-C2′), 68.65 (β-C4′), 65.94

(β-C5′), 64.21 (α-C5′), 62.82, 54.76 (α-C2), 54.68, 54.05 (β-C2), 41.82, 35.76, 30.70, 28.12; HRMS (ESI) *m*/*z* for $C_{20}H_{32}Cl_2N_2NaO_5$ ([M + Na]$^+$) 473.1581, calcd 473.1587.

***N*-Methoxy-4-(4-*N*′,*N*′-bis(2-chloroethyl)amino)phenylbutylamino-L-riboside (54).** By use of the same procedure as **53**, **9** (52 mg, 0.16 mmol) combined with L-ribose (48 mg, 0.32 mmol), yielded the anomeric mixture as a white solid (40 mg, 54%, R_f = 0.34, MeOH/CH_2Cl_2 5:95; α/β 1:3). ^{1}H NMR (CD_3OD, 500 MHz) δ 7.05 (d, *J* = 8.6 Hz, 2 H), 6.67 (d, *J* = 8.6 Hz, 2 H), 4.52 (d, *J* = 3.4 Hz, 0.25 H, α-H1′), 4.25 (d, *J* = 8.7 Hz, 0.75 H, β-H1′), 4.11 (dd, *J* = 5.4, 3.5 Hz, 0.25 H, α-H2′), 4.08 (s br, 0.75 H, β-H3′), 3.95 (t, *J* = 5.5 Hz, 0.25 H, α-H3′), 3.87−3.83 (m, 0.25, α-H4′), 3.71−3.67 (m, 5 H, α-$H5_A$′ + β-$H5_A$′), 3.65−3.61 (m, 5.75 H, α-$H5_B$′ + β-H4′ + β-$H5_B$′), 3.60−3.59 (m, 0.75 H, β-H2′), 3.55 (s, 3 H), 3.00−2.95 (m, 0.75 H, β-$H2_A$), 2.94−2.89 (m, 0.25 H, α-$H2_A$), 2.81−2.76 (m, 1 H, $H2_B$), 2.53 (t, *J* = 7.5 Hz, 2 H), 1.66−1.55 (m, 4 H); ^{13}C NMR (CD_3OD, 125 MHz) δ 145.96, 132.83, 130.69, 113.70, 100.85 (α-C1′), 91.20 (β-C1′), 84.59 (α-C4′), 73.24 (α-C2′), 72.61 (α-C3′), 72.56 (β-C3′), 69.02 (β-C2′), 68.74 (β-C4′), 66.00 (β-C5′), 64.28 (α-C5′), 62.81, 54.78 (α-C2), 54.76, 54.07 (β-C2), 41.86, 35.80, 30.75, 28.15; HRMS (ESI) *m*/*z* for $C_{20}H_{32}Cl_2N_2NaO_5$ ([M + Na]$^+$) 473.1579, calcd 473.1581.

***N*-Hydroxy-4-(4-*N*′,*N*′-bis(2-chloroethyl)amino)phenylbutylamino-D-fucoside (66).** By use of aglycon **65** (63 mg, 0.21 mmol), the mixture of compounds was yielded as a white solid (54 mg, 58%, R_f = 0.11, MeOH/CH_2Cl_2 5:95, α/β/nitrone 2.7:1:1.8). ^{1}H NMR (CD_3OD, 500 MHz) δ 7.25 (d, *J* = 5.8 Hz, 0.33 H, nitrone-H1′), 7.10−7.07 (m, 2 H), 6.70−6.67 (m, 2 H), 5.06 (dd, *J* = 5.8, 2.1 Hz, 0.33 H, nitrone-H2′), 4.40 (d, *J* = 4.9 Hz, 0.18 H, α-H1′), 4.30 (dd, *J* = 5.8, 4.9 Hz, 0.18 H, α-H2′), 4.08 (dd, *J* = 6.6, 1.8 Hz, 0.33 H, nitrone-H5′), 3.96 (dd, *J* = 7.5, 5.8 Hz, 0.18 H, α-H3′), 3.89 (dd, *J* = 8.7, 2.1 Hz, 0.33 H, nitrone-H3′), 3.86−3.79 (m, 1.33 H, nitrone-H2 + α-H5′ + β-H1′), 3.73−3.70 (m, 4.49 H, β-H2′), 3.67−3.63 (m, 4.18 H, α-H4′), 3.61−3.58 (m, 0.98 H, β-H4′ + β-H5′), 3.53−3.50 (m, 0.49 H, β-H3′), 3.48 (dd, *J* = 8.7, 1.8 Hz, 0.33 H, nitrone-H4′), 3.11−3.06 (m, 0.49 H, β-$H2_A$), 3.05−3.01 (m, 0.18 H, α-$H2_A$), 2.79−2.75 (m, 0.49 H, β-$H2_B$), 2.74−2.68 (m, 0.18 H, α-$H2_B$), 2.60−2.54 (m, 2 H), 1.91−1.87 (m, 0.66 H, nitrone-H3), 1.68−1.63 (m, 3.34 H), 1.30−1.26 (m, 3 H, H6′); ^{13}C NMR (CD_3OD, 125 MHz) δ 145.54 (nitrone-C1′), 144.64, 131.68, 129.45, 112.41, 99.46 (α-C1′), 94.89 (β-C1′), 86.70 (β-C2′), 78.52 (α-C2′), 77.32 (α-C3′), 74.99 (β-C3′), 73.68 (nitrone-C4′), 72.48 (β-C5′), 72.36 (β-C4′), 71.32 (nitrone-C3′), 67.76 (α-C5′), 67.38 (α-C4′), 67.26 (nitrone-C2′), 66.21 (nitrone-C5′), 64.69 (nitrone-C2), 54.87 (β-C2), 53.72 (α-C2), 53.49, 40.64, 34.59, 29.36, 28.16 (α/β-C3), 26.97 (nitrone-C3), 18.95 (α/β-C6′), 18.66 (nitrone-C6′); HRMS (MALDI) *m*/*z* for $C_{20}H_{32}Cl_2N_2NaO_5$ ([M + Na]$^+$) 473.159 11, calcd 473.158 05.

***N*-Hydroxy-4-(4-*N*′,*N*′-bis(2-chloroethyl)amino)phenylbutylamino-D-glucurono-6,3-lactonide (67).** By use of aglycon **65** (58 mg, 0.19 mmol), the mixture of compounds was yielded as a colorless syrup (49 mg, 56%, R_f = 0.28, MeOH/CH_2Cl_2 5:95, α/β/nitrone 0:1:2.5). ^{1}H NMR (CD_3OD, 500 MHz) δ 7.25 (d, *J* = 6.3 Hz, 0.71 H, nitrone-H1′), 7.10 (d, *J* = 8.6 Hz, 2 H), 6.70 (d, *J* = 8.6 Hz, 2 H), 5.11−5.08 (m, 0.71 H, nitrone-H2′), 4.85−4.83 (m, 0.29 H, β-H4′), 4.79 (d, *J* = 4.4 Hz, 0.29 H, β-H3′), 4.57 (d, *J* = 2.3 Hz, 0.29 H, β-H1′), 4.54 (d, *J* = 4.6 Hz, 0.71 H, nitrone-H5′), 4.49 (d, *J* = 2.0 Hz, 0.29 H, β-H5′), 4.47 (d, *J* = 2.8 Hz, 0.71 H, nitrone-H3′), 4.46 (d, *J* = 2.3 Hz, 0.29 H, β-H2′), 4.33 (dd, *J* = 4.6, 2.8 Hz, 0.71 H, nitrone-H4′), 3.91 (q, *J* = 7.0 Hz, 1.42 H, nitrone-H2), 3.74−3.71 (m, 4 H), 3.67−3.65 (m, 4 H), 2.99−2.96 (m, 0.29 H, β-$H2_A$), 2.69−2.64 (m, 0.29 H, β-$H2_B$), 2.59 (t, *J* = 7.5 Hz, 1.42 H, nitrone-H5), 2.69−2.64 (dd, *J* = 7.2, 6.7 Hz, 0.58 H, β-H5), 1.94−1.87 (m, 1.42 H, nitrone-H3), 1.68−1.62 (m, 2.58 H, β-H3 + H4); ^{13}C NMR (CD_3OD, 125 MHz) δ 176.39 (C6′), 144.87, 141.51 (nitrone-C1′), 130.76, 129.48, 112.45, 104.94 (β-C1′), 85.74 (β-C3′), 80.64 (nitrone-C3′), 77.57 (β-C4′), 77.24 (β-C2′), 70.90 (nitrone-C5′), 69.97 (nitrone-C4′), 69.65 (β-C5′), 66.13 (nitrone-C2′), 65.02 (nitrone-C2), 55.41 (β-C2), 53.42, 40.66, 34.55 (β-C5), 33.99 (nitrone-C5), 29.15 (β-C3), 28.17, 26.79 (nitrone-C3); HRMS (MALDI) *m*/*z* for $C_{20}H_{28}Cl_2N_2NaO_6$ ([M + Na]$^+$) 485.122 54, calcd 485.121 66.

***N*-Hydroxy-4-(4-*N*′,*N*′-bis(2-chloroethyl)amino)phenylbutylamino-D-riboside (68).** By use of aglycon **65** (50 mg, 0.16 mmol), the product mixture was yielded as a white solid that was visualized as a single spot by TLC (18 mg, 25%, R_f = 0.39 MeOH/CH_2Cl_2 10:90, α/β/nitrone 1:1:2). ^{1}H NMR (CD_3OD, 500 MHz) δ 7.17 (d, *J* = 6.3 Hz, 0.5 H, nitrone-H1′), 7.07 (d, *J* = 8.7 Hz, 2 H), 6.66 (d, *J* = 8.7 Hz, 2 H), 4.99 (dd, *J* = 6.3, 3.5 Hz, 0.5 H, nitrone-H2′), 4.51 (d, *J* = 2.9 Hz, 0.25 H, α-H1′), 4.18−4.17 (m, 0.25 H, α-H2′), 4.16 (d, *J* = 8.7 Hz, 0.25 H, β-H1′), 4.12−4.09 (m, 0.5 H, α/β-H4′ + β-H2′), 3.89−3.86 (m, 0.5 H, α-H3′ + α/β-$H5_A$′), 3.86−3.85 (m, 1 H, nitrone-H2), 3.84−3.81 (m, 0.25 H, α/β-$H5_A$′), 3.78 (dd, *J* = 11.5, 3.5 Hz, 0.5 H, nitrone-H3′), 3.74−3.67 (m, 5 H, nitrone-$H5_A$′ + α/β-H4′ + α/β-$H5_B$′), 3.65−3.58 (m, 5 H, nitrone-$H5_B$′ + α/β-$H5_B$′ + β-H3′), 3.55−3.51 (m, 0.5 H, nitrone-H4′), 3.08−3.02 (m, 0.25 H, α-$H2_A$), 2.98−2.93 (m, 0.25 H, α-$H2_B$), 2.74−2.67 (m, 0.5 H, β-H2), 2.57 (t, *J* = 7.4 Hz, 2 H), 1.92−1.86 (m, 2 H), 1.68−1.60 (m, 2 H); ^{13}C NMR (CD_3OD, 125 MHz) δ 144.79, 144.63 (nitrone-C1′), 130.80, 129.52, 112.45, 100.86 (α-C1′), 91.12 (β-C1′), 83.43 (α-C3′), 74.19 (nitrone-C3′), 72.98 (α-C2′), 72.31 (nitrone-C4′), 70.79 (β-C2′), 70.71 (α/β-C4′), 68.16 (nitrone-C2′), 67.88 (β-C3′), 67.55 (α/β-C4′), 64.85 (α/β-C5′), 64.82, 63.58 (nitrone-C5′), 62.59 (α/β-C5′), 53.54, 40.57, 34.09, 28.22, 26.78; HRMS (ESI) *m*/*z* for $C_{19}H_{30}Cl_2N_2NaO_5$ ([M + Na]$^+$) 459.1441, calcd 459.1424.

***N*-Acetoxy-4-(4-*N*′,*N*′-bis(2-chloroethyl)amino)phenylbutylamino-D-riboside Peracetate (69).** The D-riboside mixture designated **68** (23 mg, 0.053 mmol) was peracetylated by dissolving in THF (5 mL) and adding acetic anhydride (0.5 mL, 5 mmol), DMAP (1 mg, 0.008 mmol), and Et_3N (0.5 mL, 4 mmol). After 20 min, the solvent was removed in vacuo and the residue was purified by SPE chromatograpy (SiO_2, EtOAc/Hex 2:3). The product was collected as a mixture of anomers of a colorless oil (32 mg, >99%, $R_{f\beta}$ = 0.38 $R_{f\alpha}$ = 0.32, EtOAc/Hex 2:3, α/β = 2:1). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.06−7.04 (m, 2 H), 6.61 (d, *J* = 8.6 Hz, 2 H), 5.62 (s, 0.33 H, β-H3′), 5.27−5.25 (m, 0.67 H, α-H2′), 5.21 (dd, *J* = 6.1, 5.4 Hz, 0.67 H, α-H3′), 5.07 (dd, *J* = 9.2, 2.6 Hz, 0.33 H, β-H2′), 5.04−5.01 (m, 0.33 H, β-H4′), 4.89 (d, *J* = 2.9 Hz, 0.67 H, α-H1′), 4.58 (d, *J* = 9.2 Hz, 0.33 H, β-H1′), 4.33−4.31 (m, 0.67 H, α-$H5_A$′), 4.23−4.20 (m, 0.67 H, α-H4′), 4.16 (dd, *J* = 11.7, 5.4 Hz, 0.67 H, α-$H5_B$′), 3.97 (dd, *J* = 10.9, 5.4 Hz, 0.33 H, β-$H5_A$′), 3.71−3.67 (m, 4.33 H, β-$H5_B$′), 3.63−3.60 (m, 4 H), 3.20−3.15 (m, 0.33 H, β-$H2_A$), 3.11−3.06 (m, 0.67 H, α-$H2_A$), 2.99−2.91 (m, 1 H, α-$H2_B$ + β-$H2_B$), 2.57−2.47 (m, 2 H), 2.11 (s, 3 H), 2.10 (s, 3 H), 2.09 (s, 3 H), 2.05 (s, 3 H), 1.67−1.46 (m, 4 H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 170.72, 169.76, 169.51, 169.49, 144.35, 131.49, 129.74, 112.36, 96.56 (α-C1′), 88.60 (β-C1′), 77.85 (α-C4′), 71.99 (α-C2′), 70.58 (α-C3′), 68.93 (β-C3′), 66.47 (β-C4′), 65.72 (β-C2′), 63.55 (α-C5′), 63.21 (β-C5′), 54.06, 53.79, 40.71, 34.55, 29.20, 26.53, 20.93, 20.88, 20.68, 20.65; HRMS (ESI) *m*/*z* for $C_{27}H_{38}Cl_2N_2NaO_9$ ([M + Na]$^+$) 627.1859, calcd 627.1847.

***N*-Hydroxy-4-(4-*N*′,*N*′-bis(2-chloroethyl)amino)phenylbutylamino-D-threoside Nitrone (70).** By use of aglycon **65** (35 mg, 0.11 mmol), the neoglycoside nitrone was yielded as a colorless syrup (23 mg, 49%, R_f = 0.11, MeOH/CH_2Cl_2 5:95). ^{1}H NMR (CD_3OD, 500 MHz) δ 7.22 (d, *J* = 5.8 Hz, 1 H, H1′), 7.10 (d, *J* = 8.6 Hz, 2 H), 6.70 (d, *J* = 8.6 Hz, 2 H), 4.84 (dd, *J* = 5.8, 4.2 Hz, 1 H, H2′), 3.87−3.84 (m, 3 H, H3′ + H4′), 3.75−3.72 (m, 4 H), 3.68−3.61 (m, 6 H), 2.59 (t, *J* = 7.4 Hz, 2 H), 1.93−1.87 (m, 2 H), 1.69−1.63 (m, 2 H); ^{13}C NMR (CD_3OD, 125 MHz) δ 146.11, 146.01 (C1′), 132.10, 130.72, 113.71, 73.93 (C3′), 68.77 (C2′), 65.95 (C4′), 64.09, 54.70, 41.85, 35.30, 29.41, 27.93; HRMS (MALDI) *m*/*z* for $C_{18}H_{28}Cl_2N_2NaO_4$ ([M + Na]$^+$) 429.131 88, calcd 429.131 83.

4(4-*N*′,*N*′-Bis(2-chloroethyl)amino)phenylbutylhydrazido-β-D-fucoside (72). By use of aglycon **71** (47 mg, 0.15 mmol), the

product was yielded as a colorless syrup (46 mg, 67%, R_f = 0.28 MeOH/CH_2Cl_2 10:90). ^{1}H NMR (CD_3OD, 500 MHz) δ 7.09 (d, J = 8.6 Hz, 2 H), 6.70 (d, J = 8.6 Hz, 2 H), 3.86 (d, J = 8.7 Hz, 1 H, H1′), 3.74−3.72 (m, 4 H), 3.68−3.65 (m, 4 H), 3.64−3.62 (m, 2 H, H3′ + H5′), 3.54 (dd, J = 9.5, 3.2 Hz, 1 H, H4′), 3.48 (d, J = 8.7 Hz, 1 H, H2′), 2.56 (t, J = 7.5 Hz, 2 H), 2.21 (t, J = 7.5 Hz, 2 H), 1.90 (qui, J = 7.5 Hz, 2 H), 1.28 (d, J = 6.4 Hz 3 H); ^{13}C NMR (CD_3OD, 125 MHz) δ 174.72, 144.86, 130.58, 129.53, 112.44, 91.30 (C1′), 74.29 (C4′), 72.12 (C3′), 72.03 (C5′), 68.38 (C2′), 53.45, 40.61, 34.02, 33.28, 27.65, 24.17, 15.93 (C6′); HRMS (MALDI) m/z for $C_{20}H_{31}Cl_2N_3NaO_5$ ([M + Na]$^+$) 486.152 33, calcd 486.153 30.

4-(4-*N*′,*N*′-Bis(2-chloroethyl)amino)phenylbutylhydrazido-D-glucurono-6,3-lactonide (73). By use of aglycon **71** (61 mg, 0.19 mmol), the mixture of compounds was yielded as a colorless syrup (42 mg, 44%, R_{fA} = 0.55, R_{fB} = 0.47, MeOH/CH_2Cl_2 10:90, α/β/imine = 0:1:1). ^{1}H NMR (CD_3OD, 500 MHz) δ 7.57 (d, J = 4.3 Hz, 0.35 H, imine A-H1′), 7.41 (d, J = 4.1 Hz, 0.15 H, imine B−H1′), 7.09 (d, J = 8.6 Hz, 2 H), 6.70 (d, J = 8.6 Hz, 2 H), 4.91 (dd, J = 6.4, 4.7 Hz, 0.5 H, β-H4′), 4.85−4.79 (m, 0.5 H, β-H3′), 4.78 (d, J = 1.4 Hz, 0.5 H, β-H1′), 4.68 (dd, J = 7.6, 4.3 Hz, 0.35 H, imine A-H2′), 4.63 (dd, J = 7.8, 4.1 Hz, 0.15 H, imine B-H2′), 4.57−4.54 (m, 1 H, imine-H4′/H5′ + β-H2′), 4.53−4.50 (m, 1.35 H, imine A-H3′ + imine-H4′/H5′ + β-H5′), 4.46−4.44 (m, 0.15 H, imine B-H3′), 3.75−3.71 (m, 4 H), 3.68−3.65 (m, 4 H), 2.71−2.54 (m, 2 H), 2.27 (t, J = 7.5 Hz, 1 H), 2.20−2.15 (m, 1 H), 1.96−1.87 (m, 2 H); ^{13}C NMR (CD_3OD, 125 MHz) δ 176.71 (imine-C6′), 176.08 (β-C6′), 171.83, 148.17 (imine A-C1′), 144.93, 144.85 (imine B-C1′), 130.37, 129.50, 112.43, 99.79 (β-C1′), 84.14 (β-C3′), 81.62 (imine B-C3′), 81.53 (imine A-C3′), 77.83 (β-C4′), 70.96 (imine B-C4′/C5′), 70.88 (β-C2′), 70.11 (imine A-C4′/C5′), 69.97 (imine B-C4′/C5′), 69.84 (imine A-C4′/C5′), 69.49 (β-C5′), 69.20 (imine A-C2′), 69.59 (imine B-C2′), 53.40, 40.62, 33.99, 33.58 (β-C2), 33.47 (imine-C2), 27.36; HRMS (MALDI) m/z for $C_{20}H_{27}Cl_2N_3NaO_6$ ([M + Na]$^+$) 498.116 31, calcd 498.116 91.

4-(4-*N*′,*N*′-Bis(2-chloroethyl)amino)phenylbutylhydrazido-D-threosideimine (74). By use of aglycon **71** (36 mg, 0.11 mmol), the neoglycoside imine was yielded as a colorless syrup (41 mg, 85%, R_f = 0.19, MeOH/CH_2Cl_2 10:90). ^{1}H NMR (CD_3OD, 500 MHz) δ 7.53 (d, J = 4.9 Hz, 0.6 H, imine A-H1′), 7.37 (d, J = 5.5 Hz, 0.4 H, imine B-H1′), 7.08 (d, J = 8.6 Hz, 2 H), 6.70 (d, J = 8.6 Hz, 2 H), 4.32 (t, J = 4.4 Hz, 0.6 H, imine A-H2′), 4.25 (m, 0.4 H, imine B-H2′), 3.78−3.70 (m, 5 H, imine-H3′), 3.69−3.60 (m, 6 H, imine-H4′), 2.65 (t, J = 7.5 Hz, 0.8 H, imine B-H2), 2.58 (t, J = 7.5 Hz, 1.2 H, imine A-H2), 2.26 (t, J = 7.5 Hz, 1.2 H, imine A-H4), 1.98−1.90 (m, 2.8 H, imine-H3 + imine B-H3); ^{13}C NMR (CD_3OD, 125 MHz) δ 171.67, 151.04 (imine A-C1′), 147.62 (imine B-C1′), 144.92, 130.40, 129.49, 112.44, 73.65 (imine B-C3′), 73.46 (imine A-C3′), 71.49 (imine B-C2′), 71.40 (imine A-C2′), 62.60 (imine-C4′), 53.42, 40.59, 34.19 (imine B-C2), 34.00 (imine A-C2), 33.56 (imine A-C4), 31.76 (imine B-C4), 27.41 (imine A-C3), 26.82 (imine B-C3); HRMS (MALDI) m/z for $C_{18}H_{27}Cl_2N_3NaO_4$ ([M + Na]$^+$) 442.127 21, calcd 442.127 08.

4-(4-*N*′,*N*′-Bis(2-chloroethyl)amino)phenylbutylhydrazido-D-xyloside (75). By use of aglycon **71** (60 mg, 0.19 mmol), the mixture of compounds was yielded as a colorless syrup (40 mg, 45%, R_{fA} = 0.36, R_{fB} = 0.29, MeOH/CH_2Cl_2 10:90, α/β/imine = 0:2.3:1). ^{1}H NMR (CD_3OD, 500 MHz) δ 7.54 (d, J = 5.0 Hz, 0.15 H, imine A-H1′), 7.38 (d, J = 5.5 Hz, 0.15 H, imine B-H1′), 7.09 (d, J = 8.6 Hz, 2 H), 6.70 (d, J = 8.6 Hz, 2 H), 4.45 (d, J = 3.8 Hz, 0.15 H, imine A-H2′), 4.40 (t, J = 4.9 Hz, 0.15 H, imine B-H2′), 3.89 (dd, J = 11.3, 5.4 Hz, 0.7 H, β-H5$_A$′), 3.85 (d, J = 8.7 Hz, 0.7 H, β-H1′), 3.75−3.72 (m, 4.3 H, imine B-H3′ + imine A/B-H4′), 3.68−3.65 (m, 4.75 H, imine A/B-H4′ + imine-H5′), 3.59 (dd, J = 7.8, 4.0 Hz, 0.15 H, imine A-H3′), 3.52−3.45 (m, 0.7 H, β-H4′), 3.39−3.36 (m, 0.7 H, β-H3′), 3.23−3.16 (m, 1.4 H, β-H2′ + β-H5$_B$′), 2.56 (t, J = 7.5 Hz, 2 H), 2.27−2.24 (m, 0.6 H, imine-H2), 2.21−2.17 (m, 1.4 H, β-H2), 1.96−1.87 (m, 2 H); ^{13}C NMR (CD_3OD, 125 MHz) δ 174.67, 144.87, 130.53, 129.49, 112.43, 91.78 (β-C1′), 87.51 (imine A-C2′), 77.17 (β-C3′), 72.44 (imine A/B-C4′), 72.43 (imine B-C3′), 71.57 (imine A/B-C4′), 72.00 (imine B-C2′), 71.34 (β-C2′), 70.92 (imine A-C3′), 70.05 (β-C4′), 67.28 (β-C5′), 62.95 (imine-C5′), 53.43, 40.60, 33.99, 33.22, 27.67; HRMS (MALDI) m/z for $C_{19}H_{29}Cl_2N_3NaO_5$ ([M + Na]$^+$) 472.137 12, calcd 472.137 65.

Cell Proliferation Assays. Testing was performed by the Keck-UWCCC Small Molecule Screening Facility (Madison, WI). General carcinoma cell line maintenance, compound handling, and assay protocols have been previously reported.[20] Briefly, cells were plated in 50 μL volumes at a density of 500 cells per well in 384-well clear-bottom tissue culture plates. Serial dilutions of 30 mM DMSO compound stock solutions were accomplished in 96-well plates using a BioTek Precision XS liquid handler (Winooski, VT) to a concentration 100× greater than that of the most dilute assay. Final dilutions were performed in a 384-well plate in quadruplicate using a Beckman-Coulter Biomek FX liquid handler with a 384-channel pipetting head (Fullerton, CA) and were stored at −20 °C when not in use. Compounds were then added to the culture plates by the Biomek FX handler and were incubated at 37 °C for 7 days in an atmosphere containing 5% CO_2. The calcein AM reagent (acetoxymethyl ester, 10 μM) and ETHD-1 (100 μM, 30 μL total) were added, the treated cells incubated for 30 m at 37 °C, and plates read for fluorescent emission using a Tecan Safire2 microplate reader (Duram, NC) at the appropriate wavelengths. CellTiter-Glo reagent (15 μL; Promega Corp., Madison, WI) was added, and the plates were incubated for 10 m at room temperature with gentle agitation to lyse the cells. Each plate was re-examined for luminescence to verify inhibition. GI_{50} values for cytotoxicity were determined using XLfit 4.2 as previously reported.[20] For compound **60**, the most active neoglycoside, the NCI60 human tumor cancer cell line screen was performed by the Developmental Therapeutics Program of the National Cancer Institute (Rockville, MD) as previously described.[61,62]

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Supporting Information Available: Experimental procedures and characterization data for aglycons **9**, **65**, and **71**; characterization, structures, and growth inhibition data for the neoglycoside library; NCI60 anticancer screen data for **60**; and NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Křen, V.; Řezanka, T. Sweet antibiotics. The role of glycosidic residues in antibiotic and antitumor activity and their randomization. *FEMS Microbiol. Rev.* **2008**, *32*, 858–889.
(2) Thorson, J. S.; Hosted, T. J., Jr.; Jiang, J.; Biggins, J. B.; Ahlert, J. Nature's carbohydrate chemists: the enzymatic glycosylation of bioactive bacterial metabolites. *Curr. Org. Chem.* **2001**, *5*, 139–167.
(3) Weymouth-Wilson, A. C. The role of carbohydrates in biologically active natural products. *Nat. Prod. Rep.* **1997**, *14*, 99–110.
(4) Salas, J. A.; Méndez, C. Engineering the glycosylation of natural products in actinomycetes. *Trends Microbiol.* **2007**, *15*, 119–232.
(5) Thibodeaux, C. J.; Melançon, C. E.; Liu, H.-W. Unusual sugar biosynthesis and natural product glycodiversification. *Nature* **2007**, *446*, 1008–1016.
(6) Blanchard, S.; Thorson, J. S. Enzymatic tools for engineering natural product glycosylation. *Curr. Opin. Chem. Biol.* **2006**, *10*, 263–271.
(7) Griffith, B. R.; Langenhan, J. M.; Thorson, J. S. "Sweetening" natural products via glycorandomization. *Curr. Opin. Biotechnol.* **2005**, *16*, 622–630.

(8) Langenhan, J. M.; Griffith, B. R.; Thorson, J. S. Neoglycorandomization and chemoenzymatic glycorandomization: two complementary tools for natural product diversification. *J. Nat. Prod.* **2005**, *68*, 1696–1711.

(9) Peri, F.; Dumy, P.; Mutter, M. Chemo- and stereoselective glycosylation of hydroxylamino derivatives: a versatile approach to glycoconjugates. *Tetrahedron* **1998**, *54*, 12269–12278.

(10) Peri, F.; Deutman, A.; La Ferla, B.; Nicotra, F. Solution and solid-phase chemoselective synthesis of (1-6)-amino(methoxy) di- and trisaccharide analogs. *Chem. Commun.* **2002**, 1504–1505.

(11) Carrasco, M. R.; Nguyen, M. J.; Burnell, D. R.; MacLaren, M. D.; Hengel, S. M. Synthesis of neoglycopeptides by chemoselective reaction of carbohydrates with peptides containing a novel *N*′-methyl-aminooxy amino acid. *Tetrahedron Lett.* **2002**, *43*, 5727–5729.

(12) Carrasco, M. R.; Brown, R. T. A versatile set of aminooxy amino acids for the synthesis of neoglycopeptides. *J. Org. Chem.* **2003**, *68*, 8853–8858.

(13) Carrasco, M. R.; Brown, R. T.; Serafimova, I. M.; Silva, O. Synthesis of *N*-Fmoc-*O*-(*N*′-Boc-*N*′-methyl)-aminohomoserine, an amino acid for the facile preparation of neoglycopeptides. *J. Org. Chem.* **2003**, *68*, 195–197.

(14) Peri, F. Extending chemoselective ligation to sugar chemistry: convergent assembly of bioactive neoglycoconjugates. *Mini-Rev. Med. Chem.* **2003**, *3*, 651–658.

(15) Peri, F.; Jiménez-Barbero, J.; García-Aparicio, V.; Tvaroška, I.; Nicotra, F. Synthesis and conformational analysis of novel N(OCH_3)-linked disaccharide analogues. *Chem.—Eur. J.* **2004**, *10*, 1433–1444.

(16) Peri, F.; Nicotra, F. Chemoselective ligation in glycochemistry. *Chem. Commun.* **2004**, 623–627.

(17) Langenhan, J. M.; Thorson, J. S. Recent carbohydrate-based chemoselective ligation applications. *Curr. Org. Synth.* **2005**, *2*, 59–81.

(18) Carrasco, M. R.; Brown, R. T.; Doan, V. H.; Kandel, S. M.; Lee, F. C. 2-(*N*-Fmoc)-3-(*N*-Boc-*N*-methoxy)-diaminopropanoic acid, an amino acid for the synthesis of mimics of *O*-linked glycopeptides. *Biopolymers* **2006**, *84*, 414–420.

(19) Nicotra, F.; Cipolla, L.; Peri, F.; La Ferla, B.; Redaelli, C. Chemoselective neoglycosylation. *Adv. Carbohydr. Chem. Biochem.* **2007**, *61*, 353–398.

(20) Langenhan, J. M.; Peters, N. R.; Guzei, I. A.; Hoffman, M. A.; Thorson, J. S. Enhancing the anticancer properties of cardiac glycosides by neoglycorandomization. *Proc. Nat. Acad. Sci. U.S.A.* **2005**, *102*, 12305–12310.

(21) Ahmed, A.; Peters, N. R.; Fitzgerald, M. K.; Watson, J. A., Jr.; Hoffmann, F. M.; Thorson, J. S. Colchicine glycorandomization influences cytotoxicity and mechanism of action. *J. Am. Chem. Soc.* **2006**, *128*, 14224–14225.

(22) Griffith, B. R.; Krepel, C.; Fu., X.; Blanchard, S.; Ahmed, A.; Edmiston, C. E.; Thorson, J. S. Model for antibiotic optimization via neoglycosylation: synthesis of liponeoglycopeptides active against VRE. *J. Am. Chem. Soc.* **2007**, *129*, 8150–8155.

(23) Langenhan, J. M.; Engle, J. M.; Slevin, L. K.; Fay, L. R.; Lucker, R. W.; Smith, K. R.; Endo, M. M. Modifying the glycosidic linkage in digitoxin analogs provides selective cytotoxins. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 670–673.

(24) Goff, R. D.; Thorson, J. S. Enhancing the divergent activities of betulinic acid via neoglycosylation. *Org. Lett.* **2009**, *11*, 461–464.

(25) Everett, J. L.; Roberts, J. J.; Ross, W. C. J. Aryl-2-haloalkylamines. XII. Some carboxylic derivatives of *N*,*N*-bis(2-chloroethyl)aniline. *J. Chem. Soc.* **1953**, 2386–2392.

(26) Jaksic, B.; Brugiatelli, M.; Krc, I.; Losonczi, H.; Holowiecki, J.; Planinc-Peraica, A.; Kusec, R.; Morabito, F.; Iacopino, P.; Lutz, D. High dose chlorambucil versus Binet's modified cyclophosphamide, doxorubicin, vincristine, and prednisone regimen in the treatment of patients with advanced B-cell chronic lymphocytic leukemia. Results of an international multicenter randomized trial. *Cancer* **1997**, *79*, 2107–2114.

(27) Robak, T.; Kasznicki, M. Alkylating agents and nucleoside analogues in the treatment of B cell chronic lymphocytic leukemia. *Leukemia* **2002**, *16*, 1015–1027.

(28) Nicolle, A.; Proctor, S. J.; Summerfield, G. P. High dose chlorambucil in the treatment of lymphoid malignancies. *Leuk. Lymphoma* **2004**, *45*, 271–275.

(29) Robak, T. Recent progress in the management of chronic lymphocytic leukemia. *Cancer Treat. Rev.* **2007**, *33*, 710–728.

(30) Robak, T.; Jamroziak, K.; Robak, P. Current and emerging treatments for chronic lymphocytic leukemia. *Drugs* **2009**, *69*, 2415–2449.

(31) Kahl, B. S.; Bartlett, N. L.; Leonard, J. P.; Chen, L.; Ganjoo, K.; Williams, M. E.; Czuczman, M. S.; Robinson, K. S.; Joyce, R.; van der Jagt, R. H.; Cheson, B. D. Bendamustine is effective therapy in patients with rituximab-refractory, indolent B-cell non-hodgkin lymphoma: results from a multicenter study. *Cancer* **2010**, *116*, 106–114.

(32) Millard, J. T.; Raucher, S.; Hopkins, P. B. Mechlorethamine cross-links deoxyguanosine residues at 5′-GNC sequences in duplex DNA fragments. *J. Am. Chem. Soc.* **1990**, *112*, 2459–2460.

(33) Denny, W. A. DNA minor groove alkylating agents. *Curr. Med. Chem.* **2001**, *8*, 533–544.

(34) Bank, B. B.; Kanganis, D.; Liebes, L. F.; Silber, R. Chlorambucil pharmacokinetics and DNA binding in chronic lymphocytic leukemia lymphocytes. *Cancer Res.* **1989**, *49*, 554–559.

(35) Dorr, R. T.; Fritz, W. L. *Cancer Chemotherapy Handbook*; Elsevier Science: New York, 1980.

(36) Sanderson, B. J. S.; Johnson, K. J.; Henner, W. D. Induction of mutant lymphocytes in cyclophosphamide- and chlorambucil-treated patients. *Mutagenesis* **2001**, *16*, 197–202.

(37) Palayoor, S. T.; Mitchell, J. B.; Cerna, D.; Degraff, W. John-Aryankalayil, M.; Coleman, C. N. PX-478, an inhibitor of hypoxia-inducible factor-1α, enhances radiosensitivity of prostate carcinoma cells. *Int. J. Cancer* **2008**, *123*, 2430–2437.

(38) Veyhl, M.; Wagner, K.; Volk, C.; Gorboulev, V.; Baumgarten, K.; Weber, W.-M.; Schaper, M.; Bertam, B.; Wiessler, M.; Koepsell, H. Transport of the new chemotherapeutic agent β-D-glucosylisophosphoramide mustard (D-19575) into tumor cells is mediated by the Na^+-D-glucose cotransporter SAAT1. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 2914–2919.

(39) (a) Schein, P. S.; Green, D.; Hammer, C. F.; McPherson, E.; Talebian, A. 6-Bis-(2-chloroethyl)amino-6-deoxy-D-galactopyranose hydrochloride: synthesis, chemical characterization, murine P388 antitumor activity, and bone marrow toxicity. *J. Pharm. Sci.* **1989**, *78*, 918–921.

(40) Atassi, G.; Dumont, P.; Gosse, C.; Fournier, J. P.; Gouyette, A.; Roger, P. Characterization of the antitumor activity against solid tumors of a new nitrosoureido sugar: Cy 233. *Cancer Chemother. Pharmacol.* **1989**, *25*, 205–209.

(41) Sosnovsky, G.; Rao, N. U. M. In the search for new anticancer drugs. XXIII: exploration of a predictive design for anticancer drugs of carbohydrates containing *N*-nitrosochloroethylamino, *N*-nitrosomethyl, and *N*-nitrosoaminoxyl components. *J. Pharm. Sci.* **1991**, *80*, 693–699.

(42) Halmos, T.; Santarromana, M.; Antonakis, K.; Scherman, D. Synthesis of glucose-chlorambucil derivatives and their recognition by the human GLUT1 glucose transporter. *Eur. J. Pharmacol.* **1996**, *318*, 477–484.

(43) Iglesias-Guerra, F.; Candela, J. I.; Bautista, J.; Alcudia, F.; Vega-Perez, J. M. Potential anticancer drugs, part 2. Alkylating agents from sugars. Alkyl hexopyranoside derivatives as carrier systems for chlorambucil. *Carbohydr. Res.* **1999**, *316*, 71–84.

(44) Vega-Perez, J. M.; Candela, J. I.; Blanco, E.; Iglesias-Guerra, F. Potential anticancer drugs. Part 3. Alkylating agents from sugars. Stereoselective synthesis of 2,3-diaminoglucoses from 2-nitroalkenes, as intermediates in the synthesis of carriers of chlorambucil. *Tetrahedron* **1999**, *55*, 9641–9650.

(45) Iglesias-Guerra, F.; Candela, J. I.; Blanco, E.; Alcudia, F.; Vega-Perez, J. M. Alkylating agents from sugars: synthesis of chlorambucil derivatives carried by chiral glycosyl glycerols derived from D-glucosamine. *Chirality* **2002**, *14*, 199–203.

(46) Reux, B.; Weber, V.; Galmier, M.-J.; Borel, M.; Madesclaire, M.; Madelmont, J.-C.; Debiton, E.; Coudert, P. Synthesis and cytotoxic properties of new fluorodeoxyglucose-coupled chlorambucil derivatives. *Bioorg. Med. Chem.* **2008**, *16*, 5004–5020.

(47) Thorens, B.; Mueckler, M. Glucose transporters in the 21st century. *Am. J. Physiol.: Endocrinol. Metab.* **2010**, *298*, 141–145.

(48) Zhao, F.-Q.; Keating, A. F. Functional properties and genomics of glucose transporters. *Curr. Genomics* **2007**, *8*, 113–128.

(49) Macheda, M. L.; Rogers, S.; Best, J. D. Molecular and cellular regulation of glucose transporter (GLUT) proteins in cancer. *J. Cell. Physiol.* **2005**, *202*, 654–662.

(50) Joost, H.-G.; Thorens, B. The extended GLUT-family of sugar/polyol transport facilitators: nomenclature, sequence characteristics, and potential function of its novel members (review). *Mol. Membr. Biol.* **2001**, *18*, 247–256.

(51) Merino, P.; Franco, S.; Merchan, F. L.; Tejero, T. A facile synthesis of glycosyl hydroxylamines. *Synth. Commun.* **1997**, *27*, 3529–3537.

(52) Dondoni, A.; Giovannini, P. P.; Perrone, D. New synthesis of pyrrolidine homoazasugars via aminohomologation of furanoses and their use for the stereoselective synthesis of aza-*C*-disaccharides. *J. Org. Chem.* **2002**, *67*, 7203–7214.

(53) Cicchi, S.; Marradi, M.; Corsi, M.; Faggi, C.; Goti, A. Chiral nitrones from lactols, 1. Preparation of *N*-glycosylhydroxylamines

and their oxidation to nitrones for the enantioselective synthesis of isoxazolidines. *Eur. J. Org. Chem.* **2003**, 4152–4160.
(54) Bendiak, B. Preparation, conformation, and mild hydrolysis of 1-glycosyl-2-acetylhydrazines of the hexoses, pentoses, 2-acetamido-2-deoxyhexoses, and fucose. *Carbohydr. Res.* **1997**, *304*, 85–90.
(55) Leteux, C.; Childs, R. A.; Chai, W.; Stoll, M. S.; Kogelberg, H.; Feizi, T. Biotinyl-L-3-(2-naphthyl)-alanine hydrazide derivatives of *N*-glycans: versatile solid-phase probes for carbohydrate-recognition studies. *Glycobiology* **1998**, *8*, 227–236.
(56) Auge, J.; Lubin-Germain, N. Glycosylhydrazides, a new class of sugar surfactants. Preparation and amphiphilic properties of 1-glycosyl-2-acylhydrazines. *J. Carbohydr. Chem.* **2000**, *19*, 379–392.
(57) Peluso, S.; Ufret, M. L.; O'Reilly, M. K.; Imperiali, B. Neoglycopeptides as inhibitors of oligosaccharyl transferase: insight into negotiating product inhibition. *Chem. Biol.* **2002**, *9*, 1323–1328.
(58) Guillaumie, F.; Thomas, O. R. T.; Jensen, K. J. Immobilization of pectin fragments on solid supports: novel coupling by thiazolidine formation. *Bioconjugate Chem.* **2002**, *13*, 285–294.
(59) Flinn, N. S.; Quibell, M.; Monk, T. P.; Ramjee, M. K.; Urch, C. J. A single-step method for the production of sugar hydrazides: intermediates for the chemoselective preparation of glycoconjugates. *Bioconjugate Chem.* **2005**, *16*, 722–728.
(60) Grigor'ev, I. A. Nitrones: Novel Strategies in Synthesis. In *Nitrile Oxides, Nitrones, and Nitronates in Organic Synthesis*, 2nd ed.; Feuer, H., Ed.; John Wiley & Sons, Inc.: Hoboken, NJ, 2008; pp 129−434.
(61) Developmental Therapeutics Program NCI/NIH Home Page. http://dtp.nci.nih.gov/branches/btb/ivclsp.html (accessed May 4, 2010).
(62) Shoemaker, R. H. The NCI60 human tumour cell line anticancer drug screen. *Nat. Rev. Cancer* **2006**, *6*, 813–823.