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# EFFECT OF 1-EPOXYALKYL-1-DEOXYNOJIRIMYCINS ON EXOGLUCOSIDASES

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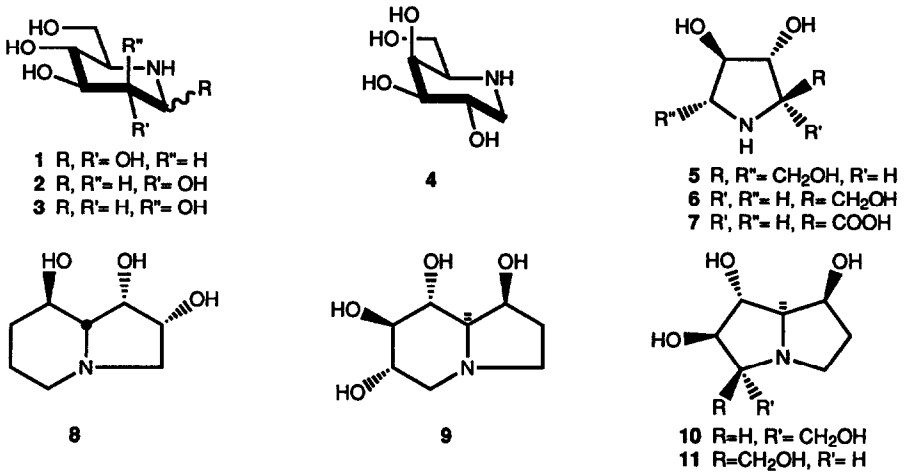
**Abstract:** The total synthesis of two nojirimycin derivatives **13** and **33** has been achieved. These substances act as inactivators or inhibitors of α- and β-glucosidases.

In the hierarchy of enzymes that process carbohydrates, those which split glycoside bonds to release mono or oligosaccharides are among the most important. Glycosidases are ubiquitous in nature and are essential for the normal growth and development of all organisms. They are involved in a variety of important cellular functions including the breakdown of carbohydrate foodstuffs,[1] the processing of eucaryotic glycoproteins[2] and the catabolism of polysaccharides and glycoconjugates.[3]

Over the last several years, efforts to design and synthesize competitive inhibitors and irreversible inactivators of glycosidases have surged, especially because such compounds promise to be useful tools for probing the details and intricacies of catalytic mechanism. Interest has in part been stimulated by the discovery that plants produce several unusual nitrogen-containing natural products with structures and shapes very reminiscent of monosaccharides. Several of these novel alkaloids are potent inhibitors of the glycosidases whose substrates they most closely resemble.[4] Among them are (a) polyhydroxylated piperidines like nojirimycin **1**,[5] 1-deoxynojirimycin (1-dNM) **2**,[5] 1-deoxymannonojirimycin **3**[6] and the galactose analog **4**,[7] (b) polyhydroxylated pyrrolidines such as **5**,[8] **6**[9] and **7**,[10] (c) the indolizidine alkaloids swainsonine **8**[11] and castanospermine **9**[12] and (d) the pyrrolizidine alkaloids australine[13] **10** and 1-epiaustraline[14] **11** (Scheme 1). Synthetic efforts in recent years have culminated in the preparation of many of these alkaloids, along with unnatural stereoisomers and analogs too numerous to mention here.

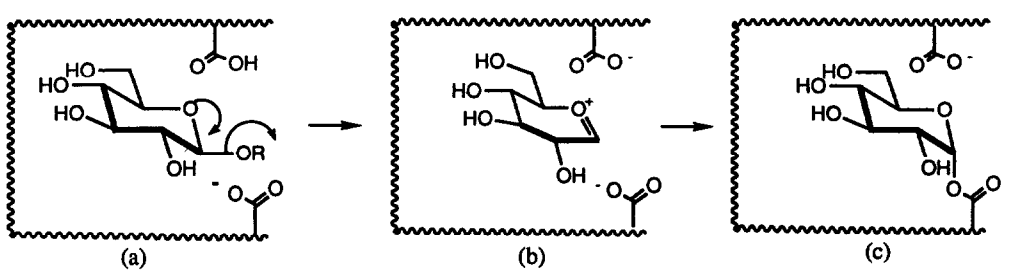
Many enzyme-specific glycosidase inhibitors already demonstrate promising therapeutic applications. For example, inhibitors of intestinal α-glucosidases are effective in maintaining serum glucose control for patients suffering from diabetes mellitus.[15] Swainsonine exhibits potent immunomodulatory activity and can boost levels of natural killer cells.[16] Castanospermine **9**, 1-deoxynojirimycin **2** and several derivatives thereof can all reduce the ability of the human immunodeficiency virus (HIV) to infect cell cultures, at least in part because of changes to gp120, a highly glycosylated surface glycoprotein which is critical for infectivity.[17]

**SCHEME 1**




Specific inhibitors have also been used to probe the mechanisms of carbohydrate-splitting enzymes.[18] For instance, Dinur *et al.* have labelled the active site of human β-glucosidase using conduritol B epoxide[19] and Withers *et al.* have studied the inactivation of 11 different enzymes using 2-deoxy-2-fluoro-D-glycosyl fluorides.[20] Together with our own work to develop aziridinylsugars as irreversible inactivators[21] and amidine analogues of monosaccharides as transition state mimics,[22] these studies strongly implicate the presence of one or more active site carboxylic acid residues in most glycoside-splitting enzymes. The generally accepted catalytic mechanism which emerges (Figure 1)[23] for enzymes that hydrolyze with retention of configuration involves *(a)* protonation of the glycosidic oxygen and fragmentation with departure of the aglycone ROH producing *(b)* a transient, point-charge-stabilized oxocarbonium ion which subsequently collapses to *(c)* a glycosyl-enzyme intermediate that eventually undergoes hydrolysis at the active site.

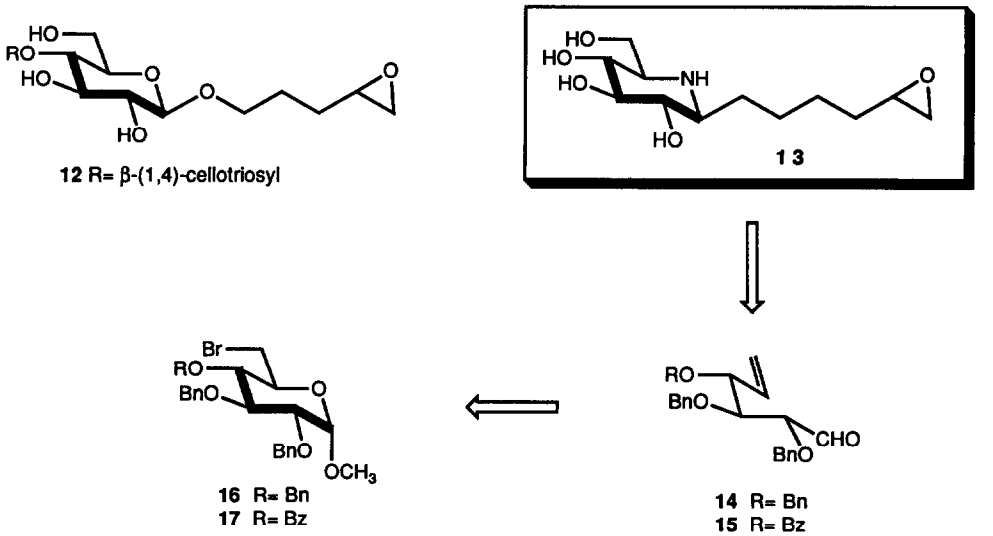
**FIGURE 1**



In recent work we have attempted to extend the use of azasugars to map the active site domains of endoglycosidases. These more complex enzymes (belonging to the chitinase, cellulase and endohexosaminidase families) are insensitive to monosaccharide-sized inhibitors and usually cleave internal glycoside bonds within oligosaccharide domains. We have shown in the case of endocellulases that by substituting a deoxynojirimycin unit for one glucopyranose residue, potent competitive inhibitors are produced. Such structures might potentially be used to deliver an irreversible alkylating agent to the active site environment.[24] We were therefore attracted to the work of Legler and Bause who almost thirty years ago demonstrated that oligo-β-D-glucosides like **12** (Scheme 2) having a terminal epoxide function in the aglycone were able to inactivate cellulases from several microbial sources.[25] Although these epoxyalkylglucosides were bound no more tightly than substrate (i.e. millimolar $K_M$'s), Legler and Bause reported that enzyme inactivation reached its optimum with epoxypentyl aglycone groups. It seemed reasonable to expect that the binding of (and subsequent inactivation by) such substrate analogues might be appreciably improved by incorporating an epoxyalkyl chain of the same molecular dimension within the basic framework of a 1-dNM residue. Thus was conceived the possibility of linking an (ω-epoxyhexyl)piperidine like **13** (in appropriately protected form) to a cellobiosyl or cellotriosyl unit. Such an approach to endoglycosidase inactivators would hinge upon a practical synthetic route to 1-alkylated-1-dNM derivatives.

The retrosynthetic analysis shown in Scheme 2 was attractive for several reasons. First, nucleophilic addition to the known aldehyde **14**[26] or its congener **15** and replacement of the nascent hydroxyl by an amine would permit subsequent elaboration of the heterocyclic ring through intramolecular aminomercuration, a technique we have exploited with considerable success in constructing highly functionalized nitrogen heterocycles.[21,24] It should moreover be noted that the differentiation of protecting groups in aldehyde **15**, readily achievable by reductive ring opening of the corresponding 6-bromo-D-glucopyranoside **17**,[27] would be particularly well-suited later in the synthesis for constructing oligosaccharide-based inhibitor frameworks. We now report the successful conversion of **14** to 1-β-(ω-epoxyhexyl)-1-dNM **13** as well as the corresponding α-substituted epimer **33**. Inhibition and inactivation studies on several glucosidases are also described which set the stage for further experiments with oligosaccharide inactivators based on **13**.

**SCHEME 2**

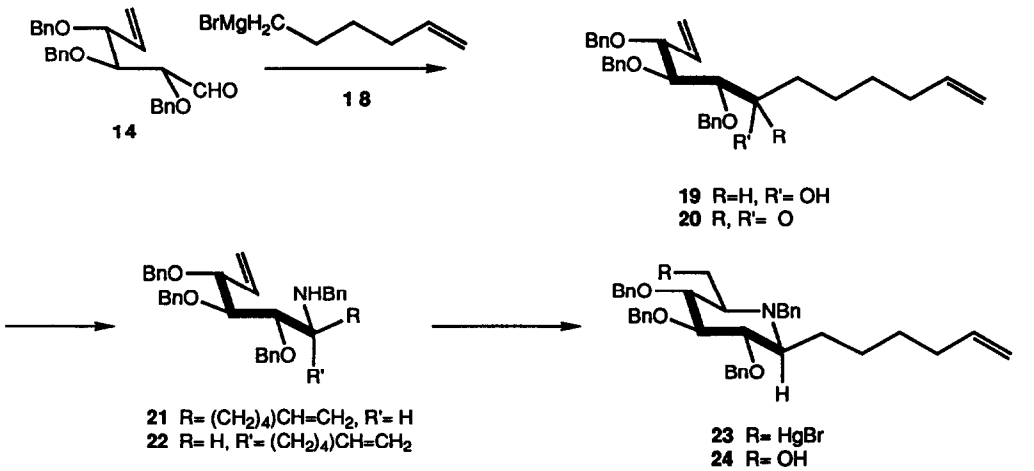



Upon modification of the procedure of Vasella and Bernet,[26] reductive ring opening of methyl 6-bromo-6-deoxy-2,3,4-tri-O-benzyl-α-D-glucopyranoside **16** using activated zinc in combination with $CeCl_3 \cdot 7H_2O$ afforded multigram quantities of aldehyde **14** (81%) as shown in Scheme 3. At this juncture several approaches were explored for the stereoselective introduction of an hexenyl chain along with the requisite amine functionality prior to the ring-closing aminomercuration step. Chelation-controlled Grignard addition to the corresponding imine of **14** appeared promising, however attempts to make the N-benzylimine of **14** were plagued by epimerization of this very sensitive aldehyde. Instead we resorted to nucleophilic addition of hexenyl magnesium bromide **18** to **14**, which gave a mixture of diastereoisomeric secondary alcohols **19** in 91% yield. Since elimination reactions predominated when either stereoisomer of **19** underwent Mitsunobu or other $S_N2$ replacements of the hydroxyl with nitrogen nucleophiles (azide, phthalimide, benzylamine), the alcohol mixture was oxidized under Swern conditions (DMSO-oxalyl chloride) to afford a single ketodiene **20** (92%).

Reductive amination of **20** ($NaBH_3CN$, added $ZnBr_2$) afforded a 3:1 ratio of the desired N-benzylamine **21** and its epimer **22**. Epimerization of **20** during the Borch reaction also gave rise to substantial amounts of undesired D-manno product isomers (15-25%) which could be separated by flash chromatography. With pure **21** in hand, cyclization in the presence of $Hg(OCOCF_3)_2$-THF (with ligand exchange during workup) afforded a

1:1 mixture of axial and equatorial organomercurials. Reductive oxygenation of the desired isomer **23** introduced the requisite C6-hydroxyl group, from which pure **24** was obtained by chromatography.

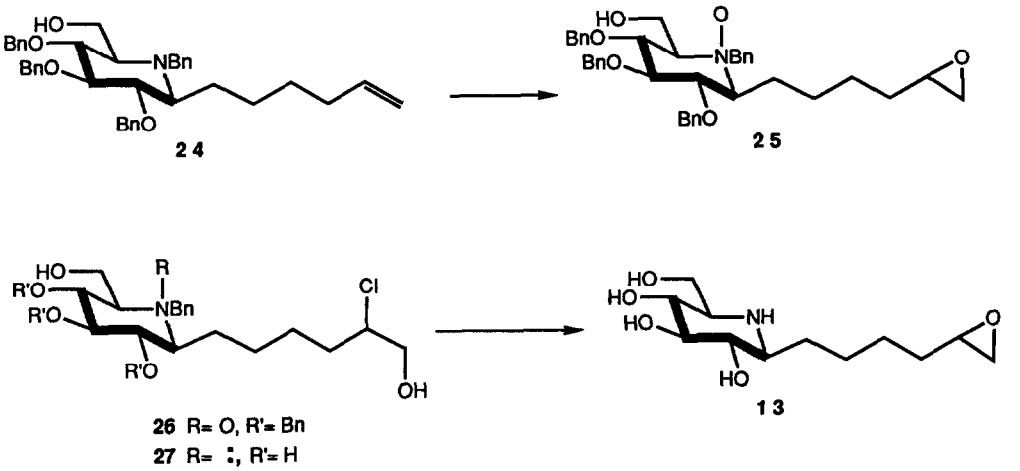
**SCHEME 3**




With the correct carbon framework assembled, it remained to install the reactive epoxide group on the alkyl sidechain and deprotect the amine and alcohol functions. Epoxidation of the terminal alkene in **24** with peracids was nonstereoselective, and was always accompanied by tertiary amine oxidation. In fact 1 equiv of *m*-chloroperoxybenzoic acid in $CH_2Cl_2$ selectively oxidized the N-benzylamine group in **24**. With 2.2 equiv of oxidant, **24** could cleanly be converted to epoxy N-oxide **25** (Scheme 4). Since catalytic hydrogenolysis of the N-oxide and benzyl groups also reduced the epoxide to a secondary alcohol, we opted first to protect the oxirane ring as its chlorohydrin. Brief treatment of **25** with anhydrous HCl in $CH_2Cl_2$ gave **26** which upon reduction ($H_2$, Pd) led to deprotected chlorohydrin **27** as its hydrochloride salt in quantitative yield. Interestingly, no hydrogenolysis of the C-Cl bond was detected. The epoxide was then reformed from **27** in KOH-$CH_3OH$ to produce the target inhibitor **13**.

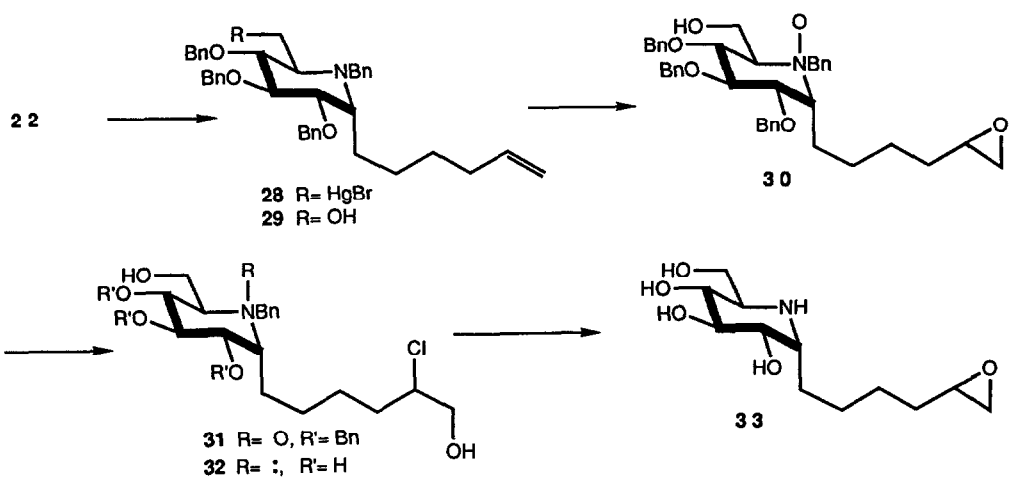
In principle, halohydrin-forming reagents might have been employed directly on alkene **24** to save an additional step. However the different regiochemical outcome of HO-X additions would have produced a primary halide, thus raising the potential risk of lytic cleavage during the final deprotection.

**SCHEME 4**




For purposes of evaluating structure-activity relationships, the axially substituted epoxyalkyl-1-dNM derivative **33** (Scheme 5) was likewise prepared from aminoalkene **22** (Scheme 3) as shown below. In the case of **22**, cyclization with mercuric trifluoroacetate was powerfully controlled by the incipient axial hexenyl sidechai so that only the desired equatorial organomercurial **28** was formed in 75% yield. After reductive oxygenation, alcohol **29** was then oxidized to epoxy N-oxide **30** and transformed according to the strategy just discussed via N-oxides **30** and **31** to chlorohydrin **32** and thence to the target **33** in good overall yield.

**SCHEME 5**



With ample supplies of **13** and **33** in hand, the biological activity of both 1-epoxyalkyl-1-deoxynojirimycins was assayed. At 1 m*M* concentration, β-substituted isomer **13** was found to exhibit classic time-dependent inactivation of both almond emulsin β–glucosidase (β-glu) as well as yeast α-glucosidase (α-glu), although its effect on the latter, rather unstable enzyme was an order of magnitude weaker. Jackbean α-mannosidase, bovine β-galactosidase and coffee α-galactosidase were not affected in any time-dependent fashion. Detailed kinetic studies of *p*-nitrophenyl β-D-glucopyranoside hydrolysis by almond β-glu at different concentrations of **13** revealed time-dependent first-order inactivation of the enzyme. Not surprisingly, inactivation was also pH-dependent over the range from 5 to 7, reaching its maximum at pH 5.6, as might be expected for a covalent modification step involving acid-catalyzed nucleophilic opening of the epoxide. All subsequent studies were conducted at pH 5.6. A plot of $1/k_{obs}$ vs 1/[I] (Figure 2) gave a dissociation constant for the non-covalent enzyme-**13** complex [$K_I$ = 0.4 m*M*] as well as the first-order rate constant with which the complex was converted into inactivated enzyme [$k_{inact} = 2.6 \times 10^{-2}$ min$^{-1}$]. To ascertain whether inactivation was truly active site-directed, the effect of **13** was measured in the presence of varying concentrations of the well-known competitive inhibitor 1-dNM **2** (Figure 3). In fact, **2** protected β-glu against irreversible inactivation by **13**.

In similar assays of the activity of **13** against α-glu the corresponding kinetic parameters were also obtained [$K_I$ = 5.2 m*M*; $k_{inact}$ = 0.16 min$^{-1}$]. Clearly the β-substituted epoxyalkyl structure was more weakly bound to (and a much less effective inactivator of) an α-glycoside processing enzyme.

**FIGURE 2**
**Analysis of Time-Dependent Kinetics Between Almond ß-Glucosidase and 13**

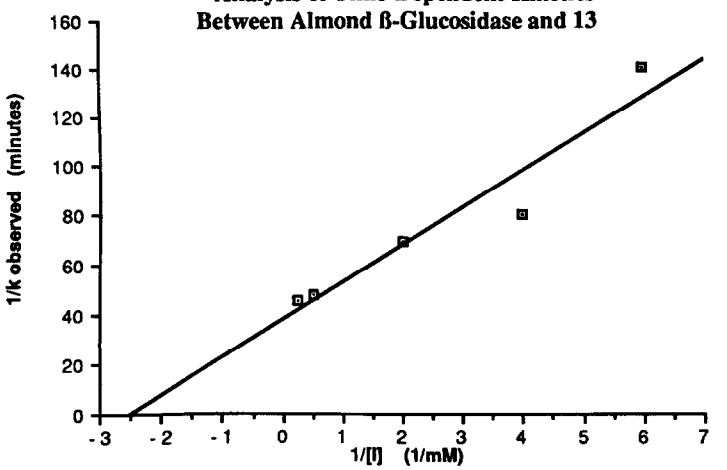
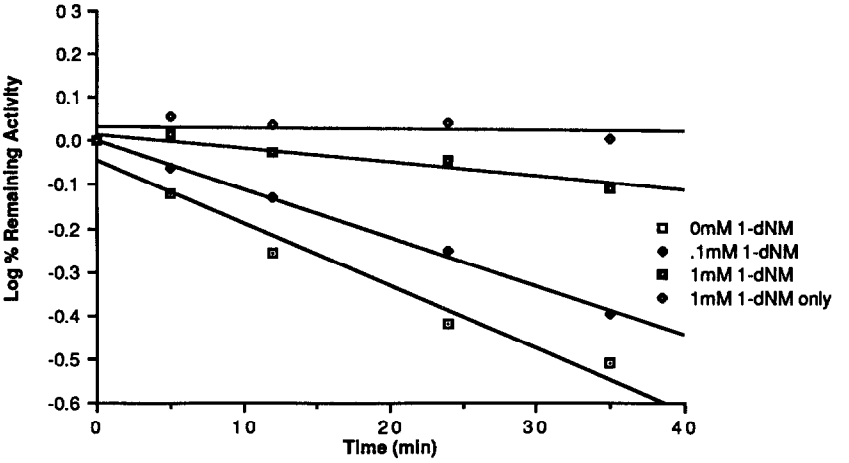


**FIGURE 3**
**Protection of ß-Glu Against Inactivation by 13 Using 1-dNM**




Preliminary screening of α-substituted isomer **33** against several readily available glycosidases indicated no effect on jackbean α-mannosidase, green coffee α-galactosidase or β-glu but quite promising activity against α-amyloglucosidase from *Aspergillus niger*. No time-dependent behavior was noted, however **33** proved to be a rather potent competitive inhibitor of amyloglucosidase. Under steady-state conditions at pH 5.0 (*p*-nitrophenyl-α-D-glucopyranoside as substrate, $K_M$=3-4 m*M*) analysis of the inhibition by **33** using Lineweaver-Burk (Figure 4) and Dixon (Figure 5) plots indicated that $K_I = 27 \pm 5$ μ*M*.

**FIGURE 4**
**Lineweaver-Burk Slopes vs [I] for 33 Against Alpha-Amyloglucosidase**

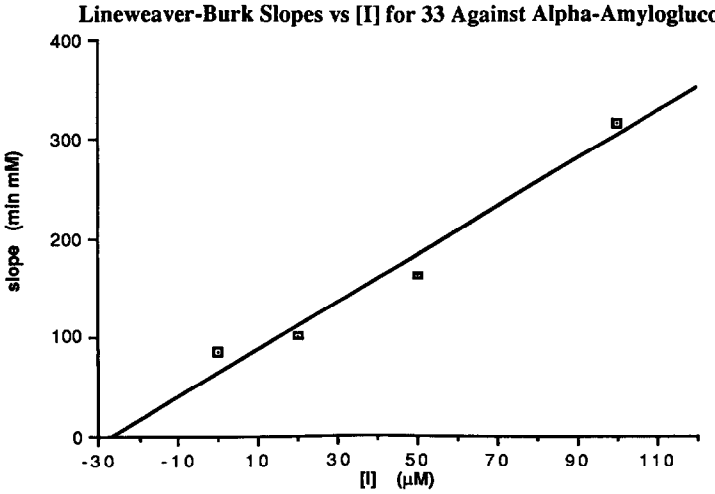
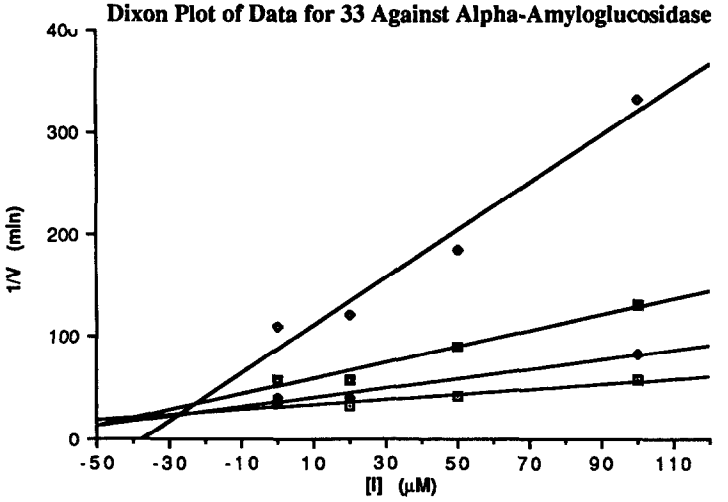


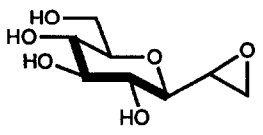
**FIGURE 5**
**Dixon Plot of Data for 33 Against Alpha-Amyloglucosidase**



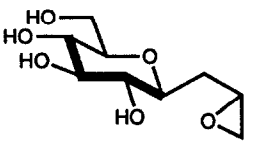

Apparently the 6-carbon chain's axial disposition did not interfere with binding, but directed the electrophilic epoxide functionality away from any critical active site residues that might have otherwise have been modified.

In conclusion, 1-(ω-epoxyalkyl)-1-deoxynojirimycins **13** and **33** have been synthesized and their effect on a variety of glycosidases has been examined. Compound **13** bound moderately well to almond β-glucosidase, its equatorially disposed alkyl chain causing an as-yet uncharacterized covalent modification of the enzyme. One useful parameter for quantitatively comparing **13** with other known glucosidase inactivators (shown in Scheme 6) is the apparent second-order rate constant for the association of free enzyme with inhibitor ($k_{inact}/K_M$) which reflects how fast inactivation occurs relative to dissociation of the enzyme-**I** complex. In the case of **13**, $k_{inact}/K_M$ = 65 $min^{-1}M^{-1}$ for almond β-glucosidase. This represents about $10^3$ more potent and specific inactivation than was observed with 1'R or 1'S-β-D-glucopyranosylepoxyethanes **34** and 2'R or 2'S-β-D-glucopyranosyl-2',3'-epoxypropanes **35** [$k_{inact}/K_I$ = 0.04-0.1 $min^{-1}M^{-1}$],[28] and about twentyfold better activity than that reported for the corresponding glycosylmethyl-(*p*-nitrophenyl)triazene **36** [$k_{inact}/K_I$ = 3.8 $min^{-1}M^{-1}$].[29] However **13** was a somewhat less efficient inactivator of β-glu than structures like conduritol B epoxide **37** and bromoconduritol B epoxide **38** [$k_{inact}/K_I$ = 76 and 420 $min^{-1}M^{-1}$, respectively] whose epoxide groups are strategically located where the pyranosyl ring would bind.[30]

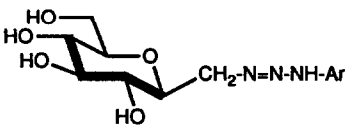
**SCHEME 6**



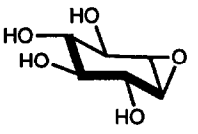
**34**



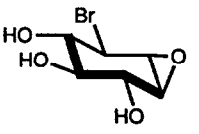
**35**




**36**



**37**




**38**

Axially substituted stereoisomer **33** showed much higher binding affinity for α-amyloglucosidase ($K_I$ = 27 μ*M*), but failed to inactivate the enzyme. To the extent further generalization was possible, our data also seemed to indicate that both α- and β-glucosidases were able to recognize and discriminate the 'anomeric' configuration of epoxyalkyl stereoisomers **13** and **33**. We thus conclude that the exercise of linking an (ω-epoxyhexyl)-piperidine like **13** (in appropriately protected form) to a cellobiosyl or cellotriosyl unit might indeed constitute a viable approach to endoglycosidase inactivators.

## EXPERIMENTAL SECTION

*General*

General experimental procedures were recently described.[31] Activated zinc refers to zinc dust that was heated at 90°C in glacial acetic acid with a catalytic amount of silver acetate, filtered, rinsed with methanol and ether, and dried *in vacuo*. The enzymes α-glucosidase (yeast), α-amyloglucosidase (*Aspergillus niger*), β-glucosidase (almonds), α-galactosidase (green coffee beans), β-galactosidase (bovine liver), and α-mannosidase (jackbean) were purchased from Sigma Chemical Company. Unless otherwise stated, all other commercially available reagents were used as received. Substrates used in the biological assays were *p*-nitrophenyl α-(or β)-D-glucopyranosides. Assays were done at 37°C, in phosphate-citrate buffers. Three sets of data were collected and averaged for each enzyme. The enzyme, buffer, and inhibitor were incubated for five min, then substrate was added. The reaction was quenched with a pH 10 glycine buffer after 15 min. Absorbance readings were taken on a Hewlett-Packard UV Spectrophotometer at 400 nm using distilled deionized water as a control.

1-Epoxyhexyl-1-deoxynojirimycin **13** -- Chlorohydrin **27** (15.4 mg, 0.046 mmol) was dissolved in $CH_3OH$ (10 mL). Five percent methanolic KOH (160 mL, 0.14 mmol) was added and the mixture stirred at rt for 18.5 h. The reaction was neutralized (pH 7) with 1% trifluoroacetic acid in $CH_3OH$ and evaporated to dryness. The residue was dissolved in $H_2O$ (7 mL) to which was added 2 drops of NaOH (2.5 *M*). Activated charcoal was added, the flask swirled for 2 min, then filtered through Celite. The charcoal was rinsed first with $H_2O$ (5 mL) then with 1:1 EtOH:$H_2O$ (30 mL). The $H_2O$ rinse was discarded and the EtOH:$H_2O$ fractions evaporated to afford **13** (11.3 mg, 94 %) as an amorphous solid: $R_f$ = 0.36 (7:3:1 $CH_2Cl_2$:$CH_3OH$:$NH_4OH$); $[\alpha]_D$ = -6.3° (c = 0.31, $H_2O$); $^1$H-NMR ($D_2O$): δ 3.74 (dd, J = 3.0, 11.5 Hz, 1 of $CH_2OH$, 1 H), 3.41 (dd, J = 7.3, 11.5 Hz, 1 of $CH_2OH$, 1 H), 3.17 (t, J = 9.1 Hz, $H_4$, 1 H), 3.06 (t, J = 9.6 Hz, $H_3$, 1 H), 2.98 (m, $H_5$', 1 H), 2.95 (t, J = 9.4 Hz, $H_5$, 1 H), 2.77 (t, J = 4.3 Hz, $H_{6a}$', 1 H), 2.53 (t, J = 3.7 Hz, $H_{6b}$', 1 H), 2.48 (ddd, J = 3.1, 7.4, 10.1 Hz, $H_2$, 1 H), 2.38 (m, $H_6$, 1 H), 1.69 (m, $H_{1a}$', 1 H), 1.51 (m, $H_{1b}$', 1 H), 1.50-1.15 (m, $H_2$', $H_3$', $H_4$', 6 H); $^{13}$C-NMR ($D_2O$): d 68.9, 65.3, 62.3, 52.4, 50.8, 49.1, 44.8, 38.9, 21.9, 21.5, 15.9, 15.2; IR (film): 3580-3020, 2920, 2870, 1675, 1370 cm$^{-1}$; HRMS: Calc. for $C_{12}H_{24}NO_5$: 262.1654; Found 262.1652

(2R,3S,4R)-2,3,4-Tribenzyloxyhex-5-enal **14** -- Acid treated zinc (10.5 g, 160 mmol), $CeCl_3 \cdot 7H_2O$ (2.98 g, 8.0 mmol), and bromopyranoside **16** (2.1 g, 3.98 mmol) were suspended in $CH_3OH$ (200 mL). After refluxing 3 h and cooling for 1 h the mixture was filtered to remove excess zinc. The residue was rinsed with $CH_3OH$ (3 x 50 mL) and the combined filtrates evaporated to dryness in vacuo. The resulting residue was dissolved in acetone (170 mL) containing *p*-toluenesulfonic acid (210 mg, 1.10 mmol). The solution was stirred at rt for 1.5 h then stored in the freezer overnight. After evaporating to dryness the crude product was dissolve in a mixture of 1.2 *M* HCl (150 mL) and $CH_2Cl_2$ (75 mL). The $CH_2Cl_2$ was removed and the aqueous extracted with $CH_2Cl_2$ (3 x 75 mL). Organics were combined, dried, filtered, evaporated to dryness and chromatographed through silica ($CH_2Cl_2$) to afford **14** (1.34 g, 81%) which was identical with a previously reported sample.[26]

(3R,4S,5S)-3,4,5-Tribenzyloxydodeca-1,11-dien-6-ol **19** -- Ground (mortar and pestle), flame-dried magnesium filings (97 mg, 3.99 mmol) were suspended in ether (4 ml). 6-Bromo-1-hexene (627 mL, 4.68 mmol) was added slowly by syringe. After 15 min aldehyde **14** (1.34 g, 3.22 mmol) was added slowly as a solution in ether (4 mL) and a vigorous reaction ensued. After 4 h at rt the reaction mixture was poured over a mixture of ice (8 mL) and 1 *M* $H_2SO_4$ (8 mL), the organic layer separated and the aqueous phase extracted with ether (3 x 20 mL). The combined organic layers were dried, filter and concentrated to afford **19** (91%) as a diastereomeric mixture: $R_f$ = 0.23 ($CH_2Cl_2$); $^1$H-NMR ($CDCl_3$): δ 7.31-7.24 (m, aromatics, 15 H), 6.02-5.85 (m, $H_2$, 1 H), 5.85-5.70 (m, $H_{11}$, 1 H), 5.29 (m, $H_1$, 2 H), 4.94 (m, $H_{12}$, 2 H), 4.85-4.30 (m, 3 x $OCH_2Ph$, 6 H), 4.17 (bt, J = 5.2 Hz, 1/2 $H_3$, 1/2 H), 4.01 (dd, J = 3.5, 7.4 Hz, 1/2 $H_3$, 1/2 H), 3.80-3.35 (m, $H_4$, $H_5$, $H_6$, 3 H), 2.84 (d, J = 6 Hz, 1/2 OH, 1/2 H), 2.28 (d, J = 8.4 Hz, 1/2 OH, 1/2 H), 2.00 (m, $H_{10}$, 2 H), 1.47-1.25 (m, $H_7$, $H_8$, $H_9$, 6 H); IR (film): 3600-3360, 3060, 3030, 2920, 2860, 1500, 1455, 1390, 1350, 1205 $cm^{-1}$; CIMS: m/e (relative intensity) 502 (M+2, 27), 501 (M+1, 21), 107 (100).

(3R,4S,5R)-3,4,5-Tribenzyloxydodeca-1,11-dien-6-one **20** -- To a -65°C solution of oxalyl chloride (285 mL, 3.34 mmol) in $CH_2Cl_2$ (7mL) was added DMSO (480 mL, 6.76 mmol) in $CH_2Cl_2$ (1.5 mL). The mixture was allowed to react for 5 min, then a $CH_2Cl_2$ (3 mL) solution of alcohol **19** (1.47 g, 2.94 mmol) was slowly added. After 15 min triethylamine (2 mL) was added. The mixture was stirred at -65°C for 5 min then warmed to rt and $H_2O$ (18 mL) was added. The two phase mixture was stirred for 10 min then separated. The aqueous layer was extracted with $CH_2Cl_2$ (3 x 25 mL). The combined organics were dried, filtered, evaporated to dryness and chromatographed ($CH_2Cl_2$) to afford ketone **20** (1.34 g, 92%): $R_f$ = 0.45 ($CH_2Cl_2$); $[\alpha]_D$ = 5.7° (c = 0.95, $CH_2Cl_2$); $^1$H-NMR ($CDCl_3$): δ 7.22-7.13 (m, aromatics, 15 H), 5.76-5.62 (m, $H_2$, $H_{11}$, 2 H), 5.22-5.14 (m, $H_1$, 2 H), 4.91-4.81 (m, $H_{12}$, 2 H), 4.73, 4.43 (AB q, J = 11.3 Hz, $OCH_2Ph$, 2 H), 4.50, 4.31 (AB q, J = 11.5 Hz, $OCH_2Ph$, 2 H), 4.48, 4.28 (AB q, J = 11.4 Hz, $OCH_2Ph$, 2 H), 4.08 (t, J = 7.2 Hz, $H_3$, 1 H), 3.85 (d, J = 3.5 Hz, $H_5$, 1 H), 3.77 (dd, J = 3.0, 6.7 Hz, $H_4$, 1 H), 2.45 (dt, J = 7.3, 18.2 Hz, $H_7$, 1 H), 2.30 (dt, J = 7.5, 18.2 Hz, $H_{7'}$, 1 H), 1.86 (m, $H_{10}$, 2 H), 1.30 (m, $H_8$, 2 H), 1.16 (m, $H_9$, 2 H); $^{13}$C-NMR ($CDCl_3$): δ 211.5, 138.5, 138.2, 138.1, 137.2, 134.8, 128.3, 128.1, 128.1, 127.8, 127.5, 127.4, 119.3, 114.3, 84.4, 82.6, 81.3, 74.9, 73.5, 70.8, 39.7, 33.4, 28.3, 22.3; IR (film): 3060, 3030, 3000, 2975, 2920, 2860, 1715, 1640, 1495, 1450, 1395, 1350, 1330, 1305 $cm^{-1}$; CIMS: m/e (relative intensity) 500 (M+2, 9), 499 (M+1, 9), 91 (100).

(3R,4S,5S,6S)-6-Amino-(N-benzyl)-3,4,5-tribenzyloxydodeca-1,11-diene **21** and (3R,4S,5S,6R)-6-Amino-(N-benzyl)-3,4,5-tribenzyloxydodeca-1,11-diene **22** -- A solution containing ketone **20** (890 mg, 1.78 mmol), benzylamine (3.5 mL, 32.2 mmol), zinc bromide (400 mg, 1.78 mmol), and $NaBH_3CN$ (3.5 mL of a 1 *M* THF solution, 3.5 mmol) in 19:1 1-propanol:$H_2O$ (30 mL) was heated to reflux for 3 days. Upon cooling the mixture was filtered, evaporated to dryness, and dissolved in a mixture of 1.2 *M* HCl (25 mL), $CHCl_3$ (17 mL), and $CH_3OH$ (38 mL). The resulting solution was stirred for 45 min then basified with 2.5 *M* NaOH (15 mL). Water (20 mL) was addded and the reaction mixture extracted with $CH_2Cl_2$ (4 x 50 mL). The organics were combined, dried, filtered, and concentrated to a viscous yellow liquid which was run through a plug of silica (diameter = 2 cm, height = 3 cm) eluting with 1:1 hexane:EtOAc (75 mL). After concentrating to near-dryness the eluant was chromatographed (10:1 $CH_2Cl_2$:$CH_3CN$) to separate higher-running ($R_f$ > 0.30) compounds from benzylamine. The higher-running fractions were then chromatographed through ammonia-deactivated silica ($CHCl_3$). The silica was deactivated by placing in a chamber containing $NH_4OH$-soaked sand for 7 min just prior to packing the column. Mixed fractions were rechromatographed twice to afford two components, impure **22** (349 mg, 33%), and pure **21** (471 mg, 45%). The first component was rechromatographed through untreated silica (40:1 to 2:1 $CH_2Cl_2$:$CH_3CN$) to afford **22** (163 mg, 16%). For **21**: $R_f$ = 0.66 ($CHCl_3$, $NH_3$-deactivated TLC plates); $[\alpha]_D$ = -25° (c = 0.48, $CH_2Cl_2$); $^1$H-NMR ($CDCl_3$): δ 7.50-7.00 (m, aromatics, 20 H), 5.88 (ddd, J = 7.4, 10.6, 17.7 Hz, $H_2$, 1 H), 5.75 (ddt, J = 17.0, 10.3, 6.8 Hz, $H_{11}$, 1 H), 5.25-5.19 (m, $H_1$, 1 H), 4.95 (d, J = 17.2 Hz, $H_{12}$, 1 H), 4.91 (d, J = 10.4 Hz, $H_{12'}$, 1 H), 4.74, 4.67 (AB q, J = 11.4 Hz, $OCH_2Ph$, 2 H), 4.74, 4.62 (AB q, J = 11.7 Hz, $OCH_2Ph$, 2 H), 4.56, 4.28 (AB q, J = 11.8 Hz, $OCH_2Ph$, 2 H), 3.92 (bdd, $H_3$, 1 H), 3.83

(bdd, J = 6.1 Hz, $H_5$, 1 H), 3.67 (bdd, J = 5.9 Hz, $H_4$, 1 H), 3.59, 3.52 (AB q, J = 13.4 Hz, $NCH_2Ph$, 2 H), 2.64 (m, $H_6$, 1 H), 1.97 (dt, J = 7.1, 6.9 Hz, $H_{10}$, 2 H), 1.50-1.15 (m, $H_7$, $H_8$, $H_9$, 6 H); $^{13}$C-NMR ($CDCl_3$): δ 139.0, 135.8, 128.2, 128.1, 127.8, 127.5, 127.4, 127.3, 126.6, 118.2, 114.1, 82.7, 80.9, 79.6, 74.7, 74.0, 70.0, 58.0, 51.6, 33.7, 30.4, 29.1, 25.9; IR (film): 3300, 3050, 3010, 2950, 2850, 1950, 1875, 1820, 1730, 1650, 1610, 1590, 1500, 1450, 1400, 1350, 1200 cm$^{-1}$; CIMS: m/e (relative intensity) 593 (30), 592(80), 591 (M+2, 100), 590 (M+1, 60). For **22**: $R_f$ = 0.55 ($CHCl_3$, $NH_3$-deactivated TLC plates); $[\alpha]_D$ = 15° (c = 0.6, $CH_2Cl_2$); $^1$H-NMR ($CDCl_3$): δ 7.23-7.16 (m, aromatic, 20 H), 5.88 (ddd, J = 7.7, 10.5, 17.7 Hz, $H_2$, 1 H), 5.69 (ddt, J = 17.0, 10.1, 7.0 Hz, $H_{11}$, 1 H), 5.18 (d, J = 8.8 Hz, $H_1$, 1 H), 5.14 (d, J = 16.7 Hz, $H_1$', 1H), 4.91 (d, J = 17.0 Hz, $H_{12}$, 1 H), 4.86 (d, J = 9.9 Hz, $H_{12}$', 1 H), 4.79, 4.44 (AB q, J = 11.4 Hz, $OCH_2Ph$, 2 H), 4.68, 4.58 (AB q, J = 11.2 Hz, $OCH_2Ph$, 2 H), 4.48, 4.01 (AB q, J = 11.9 Hz, $OCH_2Ph$, 2 H), 3.88 (dd, J= 3.0, 7.6 Hz, $H_4$, 1 H), 3.75, 3.38 (AB q, J = 13.0 Hz, $NCH_2Ph$, 2 H), 3.71 (dd, J = 2.1, 7.7 Hz, $H_5$, 1 H), 3.63 (dd, J = 2.8, 7.4 Hz, $H_3$, 1 H), 2.22 (m, $H_6$, 1 H), 1.90 (dt, J = 6.9, 6.9 Hz, $H_{10}$, 2 H), 1.57-1.04 (m, $H_7$, $H_8$, $H_9$, 6 H); $^{13}$C-NMR ($CDCl_3$): δ 139.5, 139.1, 136.6, 128.5, 128.3, 128.2, 128.2, 127.8, 127.5, 127.3, 126.8, 117.9, 114.3, 104.5, 83.4, 80.9, 80.4, 75.2, 74.9, 70.4, 57.1, 51.2, 33.7, 30.6, 29.4, 26.0; IR (film): 3050, 3010, 2930, 2850, 1650, 1500, 1450, 1400, 1360, 1210, 1130 cm$^{-1}$; CIMS: m/e (relative intensity) 593 (33), 592 (83), 591 (M+2, 100), 590 (M+1, 56).

(N-Benzyl)-2-bromomercuriomethyl-3(R),4(R),5(S)-tribenzyloxy-6(S)-(5-hexenyl)-piperidine **23** -- Aminodiene **21** (68.3 mg, 0.116 mmol) in THF (2 mL) was treated with $Hg(OTFA)_2$ (49.5 mg, 0.116 mmol). After 6.5 hr the reaction was treated with saturated $NaHCO_3$ (2 mL), stirred for 10 min and treated with saturated KBr (2 mL). The two phase mixture was stirred vigorously for 45 min, then extracted with ether (3 x 5 mL). The combined organics were dried, filtered, evaporated and chromatographed (9:1 hexane:EtOAc) to afford **23** (71.3 mg, 73%) as a 1:1 mixture of diastereomers: $R_f$ = 0.23 (9:1 hexane:EtOAc); $^1$H-NMR ($CDCl_3$): δ 7.29-7.06 (m, aromatics, 20 H), 5.70 (m, $H_5$', 1 H), 4.97-4.44 (m, 3 x $OCH_2Ph$, $H_6$', 8 H), 3.81-3.23 (m, $NCH_2Ph$, $H_3$, $H_4$, $H_5$, 5 H), 3.02 (m, $H_2$, 1 H), 2.89 (m, $H_{6ax}$, 1/2 H), 2.60 (dt, J = 4.8, 8.8 Hz, $H_{6eq}$, 1/2 H), 2.11-1.80 (m, $CH_2HgBr$, $H_4$', $H_1$', 5 H), 1.65-0.75 (m, $H_1$', $H_2$', $H_3$', 5 H); IR (film): 3050, 3020, 2910, 2850, 1640, 1490, 1450, 1350, 1200 cm$^{-1}$; CIMS: m/e (relative intensity) 632 [$M^+$-HgBr + $(CH_3)_2CH(+)$, 17], 91 (100).

(N-Benzyl)-2(R)-hydroxymethyl-3(R),4(R),5(S)-tribenzyloxy-6(S)-(5-hexenyl)-piperidine **24** -- $NaBH_4$ (60 mg, 1.59 mmol) was dissolved in DMF (4 mL). The resulting solution was cooled to -15°C and oxygen vigorously bubbled in by means of a ceramic bubbler. The mixture **23** (667 mg, 0.77 mmol) was introduced dropwise as a solution in DMF (11 mL) over a 1.5 h period. The oxygen flow was continued for 30 min at 0°C and 1 h at rt after the addition was complete. The excess $NaBH_4$ was quenched with a solution of 0.6 *M* HCl (20 mL) in methanol (40 mL). After 45 min 2.5 *M* NaOH (15 mL) was added and the mixture extracted with $CH_2Cl_2$ (4 x 75 mL). The combined organics were dried, filtered, evaporated *in vacuo* and chromatographed (80:1 to 40:1 $CH_2Cl_2$:$CH_3CN$) to afford two components: the desired alcohol **24** (117 mg, 25%) and its axial diastereomer **29** (171 mg, 37%): $R_f$ = 0.35 (40:1 $CH_2Cl_2$:$CH_3CN$); $[\alpha]_D$ = -3.5° (c = 0.13, $CH_2Cl_2$); $^1$H-NMR ($CDCl_3$): δ 7.54-7.10 (m, aromatics, 20 H), 5.67 (ddt, J = 17.1, 10.5, 6.5 Hz, $H_5$', 1 H), 4.93-4.87 (m, $H_6$', 2 H), 4.84, 4,65 (AB q, J = 11 Hz, $OCH_2Ph$, 2 H), 4.78, 4.58 (AB q, J = 11.2 Hz, $OCH_2Ph$, 2 H), 4.74 (s, $OCH_2Ph$, 2 H), 3.82-3.56 (m, $CH_2OH$, $NCH_2Ph$, $H_3$, $H_4$, 6 H), 3.49 (t, J = 6.6 Hz, $H_5$, 1 H), 2.80 (m, $H_2$, $H_6$, 2 H), 1.87 (m, $H_4$', 2 H), 1.63-1.40 (m, $H_1$', 2 H), 1.23-1.17 (m, $H_2$', $H_3$', 4 H); $^{13}$C-NMR ($CDCl_3$): δ 138.9, 128.7, 128.5, 128.5, 128.4, 128.0, 128.0, 127.9, 127.9, 127.8, 127.7, 127.7, 127.2, 114.4, 105.3, 86.4, 80.5, 78.1, 74.8, 73.9, 73.7, 63.8, 61.5, 59.4, 54.9, 33.6, 31.5, 28.9, 25.6; IR (film): 3550-3330, 3050, 3000, 2910, 2850, 1640, 1585, 1480, 1445, 1375, 1340, 1310, 1290, 1250 cm$^{-1}$; CIMS: m/e (relative intensity) 608 (43), 607 (M+2, 100), 606 (M+1, 98).

(N-Benzyl)-2(R)-hydroxymethyl-3(R),4(R),5(S)-tribenzyloxy-6(S)-(5,6-epoxyhexyl)-piperidine-N-oxide **25** -- A $CH_2Cl_2$ (4.2 mL) solution of hydroxypiperidine **24** (107 mg, 0.177 mmol) at 0°C was treated with *m*-chloroperoxybenzoic acid (108 mg, 0.63 mmol). After 15 min at 0°C the mixture was allowed to stir at rt overnight. Saturated $NaHCO_3$ (8 mL) was added and the mixture stirred vigorously for 20 min, then extracted with $CH_2Cl_2$ (5 x 12 ml). The combined organics were dried, filtered, concentrated and chromatographed (10:5:1 $CH_2Cl_2$:$CH_3CN$:$CH_3OH$) to afford **25** (75.4 mg, 67%): $R_f$ = 0.23 (10:5:1 $CH_2Cl_2$:$CH_3CN$:$CH_3OH$); $^1$H-NMR ($CDCl_3$): δ 7.4-7.2 (m, aromatic, 20 H), 5.1-4.6 (m, 3 x $OCH_2Ph$, 1/2 x $NCH_2Ph$, $CH_2OH$, $H_3$, 10 H), 4.24-4.15 (m, 1/2 $NCH_2Ph$, $H_5$, 2 H), 3.44 (t, J = 9.5 Hz, $H_4$, 1 H), 3.44 (m, $H_2$, $H_6$, 2 H), 2.85 (m, $H_6$', 1 H), 2.71 (m, $H_5$', 1 H), 2.42 (td, J = 5.0, 2.7 Hz, $H_4$', 2 H), 2.41 (m, $H_6$', 1 H), 1.91 (m, $H_1$', 2 H), 1.48 (m, $H_2$', $H_3$', 4 H); IR (film): 3600-3100, 3050, 3020, 2920, 2865, 1725, 1650, 1620, 1600, 1575, 1500, 1450, 1395, 1360, 1260 cm$^{-1}$; CIMS: m/e (relative intensity) 638 (M+1, 2), 91 (100).

2(R)-Hydroxymethyl-3(R),4(R),5(S)-hydroxy-6(S)-(5-chloro-6-hydroxyhexyl)-piperidine **27** -- Freshly prepared HCl-saturated $CH_2Cl_2$ (3 mL) was added to a solution of epoxide **25** (52.8 mg, 0.082 mmol) in $CH_2Cl_2$ at 0°C. The solution was maintained at 0°C for 15 min then evaporated to dryness. The residue was dissolved in ethanol (10 mL) which contained 1.2 *M* methanolic HCl (600 mL). Catalyst (10% Pd/C, ~60 mg) was added and the reaction maintained under $H_2$ (1 atmosphere) for 8 h. The catalyst was filtered, rinsed with 1:1 EtOH:$H_2O$ (15 mL) and the combined filtrates evaporated to dryness. The residue was demercurated using aqueous $H_2S$ followed by elemental sulfur. After lyophilization, the HCl salt of chlorohydrin **27** (27.5 mg 100%) was obtained as an amorphous, hygroscopic solid: $R_f$ = 0.29 (7:3:1 $CH_2Cl_2$:$CH_3OH$:$NH_4OH$); $[\alpha]_D$ = -4.4° (c = 0.95, $H_2O$); $^1$H-NMR ($D_2O$): δ 3.8-3.3 (bm, $H_3$, $H_4$, $H_5$, $H_{5'}$, $H_{6'}$, $CH_2OH$, 8 H), 3.03 (bm, $H_2$, $H_6$, 2 H), 1.83, 1.55 (bm, $H_{1'}$, 2 H), 1.4-1.3 (bm, $H_{2'}$, $H_{3'}$, $H_{4'}$, 6 H); $^{13}$C-NMR ($D_2O$): δ 66.9, 62.0, 61.6, 58.1, 56.6, 50.8, 49.7, 48.2, 40.2, 23.6, 19.8, 15.4; IR (film): 3650-2650, 2920, 2850, 1630, 1430, 1350, 1275, 1150-1000 $cm^{-1}$; CIMS: m/e (relative intensity) 300 ($^{37}$Cl: M+1, 15), 298 ($^{35}$Cl: M+1, 45), 69 (100).

Alkenyl Mercuric Bromide **28** --To a solution of aminodiene **22** (11.5 mg, 0.0195 mmol) in THF (400 μL) was added mercuric trifluoroacetate (13.3 mg, 0.0312 mmol) and the solution stirred for 1.75 h. Then saturated $NaHCO_3$ (0.4 mL) was added and the solution stirred vigorously for 10 min, during which time a white precipitate appeared. A saturated KBr solution (0.4 mL) was added to this suspension and stirred for 2 h. The organic and aqueous layers were separated, and the aqueous layer was extracted with diethyl ether (3 x 3 mL). The organics were combined and dried over $MgSO_4$, then filtered through Celite. The filtrate was concentrated under vacuum, then chromatographed (7% EtOAc in pentane) and concentrated under vacuum again to yield alkenyl mercuric bromide **28** (12.8 mg, 75%): $R_f$= 0.24 (7:93 EtOAc:pentane); $[\alpha]_D$=32° (c=0.75, $CH_2Cl_2$); $^1$H-NMR ($CDCl_3$) δ 7.28-7.12 (m, aromatics, 20 H), 5.72 (ddt, J= 6.6, 10.2, 17.0 Hz, $H_{5'}$), 4.97-4.78 (m, 4 H), 4.72, 4.59 (AB q, J=10.7 Hz, $OCH_2Ph$, 2 H), 4.51, 4.45 (AB q, J=11.6 Hz, $OCH_2Ph$, 2 H), 3.80, 3.55 (AB q, J=14.2 Hz, $NCH_2Ph$, 2 H) 3.83-3.77 (m, $H_5$,), 3.70 (t, J=8.2 Hz, $H_4$), 3.28 (t, J=9.8 Hz, $H_3$), 3.22 (m, $H_2$), 2.90 (dt, J= 4.8, 9.0 Hz, $H_6$), 2.21 (dd, J=5.3, 12.1 Hz, one $CH_2HgBr$ H), 2.01-1.89 (m, one $CH_2HgBr$, two $H_{4'}$, 3 H), 1.58-1.16 (m, $H_{1'}$, $H_{2'}$, $H_{3'}$, 6 H); IR (film) 3070, 3030, 2950, 2880, 1625, 1500, 1450, 1360, 1200, 1175, 1040, 1030, 1000 $cm^{-1}$; CIMS (isobutane) m/e 590 (M+1-HgBr, 21%).

Alcohol **29** -- To a -15°C solution of $NaBH_4$ (4.2 mg, 0.111 mmol) in DMF (120 μL) was added a solution of alkenyl mercuric bromide **28** (12.7 mg, 0.0146 mmol) in DMF (600 μL) while maintaining a vigorous stream of $O_2$ through the reaction mixture. The mixture was then warmed to rt and allowed to stir for 1 h. The $O_2$ was then shut off and a solution (3.4 mL) of HCl, $H_2O$, and $CH_3OH$ (1:1:4) was added to the flask. After 45 min of vigorous stirring, NaOH (10%, 0.85 mL) was added and the reaction was stirred for another 10 min. The reaction mixture was then extracted with ether (4 x 5 mL), the extracts were combined, dried over $MgSO_4$, filtered and concentrated under vacuum. The resulting residue was chromatographed (160:1 $CH_2Cl_2$:$CH_3CN$) and concentrated *in vacuo* to afford alcohol **29** (2.1 mg, 53%): $R_f$= 0.67 (40:1 $CH_2Cl_2$:$CH_3CN$); $[\alpha]_D$=25° (c=0.325, $CH_2Cl_2$); $^1$H-NMR ($CDCl_3$) δ 7.35-7.16 (m, aromatics, 20 H), 5.74 (ddt, J= 6.4, 10.4, 17.2 Hz, $H_{5'}$, 1 H), 4.99-4.87 (m, 4 H), 4.79 (1/2 AB q, J= 10.8 Hz, OCH2Ph, 1 H), 4.62-4.50 (m, 3 H), 3.87-3.78 (m, 4 H), 3.69 (dd, J= 7.5, 11.0 Hz, 1 H), 3.65-3.58 (m, 2 H), 3.06 (ddd, J= 4.5, 7.4, 10.0 Hz, $H_2$), 2.93 (dt, J= 8.5, 5.0 Hz, $H_6$), 1.98 (dt, J= 6.7, 7.2 Hz, $H_{4'}$), 1.59 (m, $H_{1'}$), 1.36 (m, $H_{2'}$), 1.26-1.05 (m, 3 H); IR (film) 3600-3280, 3050, 3000, 2910, 2830, 2390, 2350, 1625, 1600, 1500, 1450, 1400, 1360, 1340, 1310, 1270, 1200, 1075, 1030 $cm^{-1}$; CIMS (isobutane): m/e 608 (100%), 607 (M+1, 97%).

Epoxide **30** --To a 0°C solution of alcohol **29** (8.5 mg, 0.014 mmol) in $CH_2Cl_2$ (distilled, 300 μL) was added solid *m*-chloroperoxybenzoic acid (8.2 mg, 0.0475 mmol). The solution was stirred under argon at 0°C for 15 min, then warmed to rt and capped. The mixture was stirred overnight. Saturated $NaHCO_3$ (0.68 mL) was added and the mixture was stirred vigorously for 20 min. The organic and aqueous layers were separated, and the aqueous layer was extracted with $CH_2Cl_2$ (5 x 1 mL). The organic extracts were combined, dried over $Na_2SO_4$, filtered through $MgSO_4$ and concentrated *in vacuo*. After chromatography (6:3:0.3 $CH_2Cl_2$:$CH_3CN$:$CH_3OH$) and concentration, epoxide **30** (4.8 mg, 54%) was obtained: $R_f$= 0.44 (6:3:0.3 $CH_2Cl_2$:$CH_3CN$:$CH_3OH$); $[\alpha]_D$=30° (c=0.65, $CH_2Cl_2$); $^1$H-NMR ($CDCl_3$) δ 7.65-7.0 (m, aromatics, 20 H), 4.9-4.0 (bm, 12 H), 3.9-3.7 (bm, $H_4$, $H_6$), 3.0-2.9 (bm, $H_2$, one $H_{6'}$, 2 H), 2.71 (bm, one $H_{6'}$), 2.61 (bm, $H_{5'}$), 2.32 (bm, two $H_{4'}$), 1.72 (bm, one $H_{1'}$), 1.5-1.2 (bm, one $H_{1'}$, two $H_{2'}$, two $H_{3'}$, 5 H); CIMS (methane): m/e 639 (M+1, 1%), 624 (M+1-$CH_3$, 23%).

Deprotected Chlorohydrin **32** -- A solution of epoxide **30** (4.8 mg, 7.5 x $10^{-3}$ mmol) in distilled $CH_2Cl_2$ (2 mL) was cooled to 0°C under argon. To it was added 8 drops of HCl-$CH_2Cl_2$, generated by adding conc. $H_2SO_4$ (1 mL) to dry NaCl (1 g) and passing the resulting gas through Drierite into a flask containing $CH_2Cl_2$ (2 mL). The reaction mixture was allowed to stir for 5 min, then concentrated *in vacuo* to yield chlorohydrin **31** (5.0 mg,

98%), $R_f$= 0.49 (6:3:0.3 $CH_2Cl_2$:$CH_3CN$:$CH_3OH$). To a solution of **31** (4.8 mg, 7.1 x $10^{-3}$ mmol) in ethanol was added an HCl solution (3 drops, 9:1 $CH_3OH$:conc. HCl), and 10% Pd/C catalyst (15 mg). The mixture was placed under $H_2$ at rt for 1.5 h, then filtered through Celite and rinsed with 10 mL of 1:1 absolute ethanol:water. The filtrate was concentrated *in vacuo*, then lyophilized to afford deprotected chlorohydrin **32** (2.33 mg, 98%): $R_f$: 0.21 (7:3:1 $CH_2Cl_2$:$CH_3CN$:$CH_3OH$); $^1$H-NMR ($D_2O$) δ 3.85-3.65 (m, -$CH_2O$-, $H_{5'}$, $H_4$, 4 H), 3.65-3.35 (m, $H_3$, $H_5$, $H_6$, two $H_{6'}$, 5 H), 3.21 (m, $H_2$), 1.78 (bm, $H_{1'}$, 2 H), 1.6-1.2 ($H_{2'}$, $H_{3'}$, $H_{4'}$, 6 H).

Azasugar **33** -- Traces of mercury were first removed from chlorohydrin **32** (4.5 mg) by bubbling $H_2S$ into a solution of **32** in water (5 mL) for 5 min. Elemental sulfur was then added and the suspension allowed to stir for 40 h. The reaction mixture was filtered through Celite, rinsed with water, then lyophilized to afford crude **32**. To the sample of demercurated chlorohydrin **32** (5.1 mg) dissolved in methanol (4 mL) was added a KOH/$CH_3OH$ solution (170 μL, 5:95 KOH:$CH_3OH$). The resulting mixture was stirred under argon at rt for 36 h. To it was added 1:99 $CF_3CO_2H$:$CH_3OH$ (1.2 mL) to bring the solution to pH 7. The solution was then concentrated *in vacuo*. The resulting residue was dissolved in water (3 mL) and extracted with $CH_2Cl_2$ (3 x 1.5 mL). The aqueous layer was separated, $CH_3OH$ (4.5 mL) was added to make a homogeneous solution, and the solution was concentrated *in vacuo* to yield **33** (3.5 mg, 98%): $R_f$=0.26 (7:3:1 $CH_2Cl_2$:$CH_3CN$:$CH_3OH$); $[\alpha]_D$=26° (c=0.36, water); $^1$H-NMR ($D_2O$) δ 3.71 (dd, J= 3.0, 11.3 Hz, 1 H), 3.49 (dd, J= 5.2, 9.3 Hz, $H_5$, 1 H), 3.41-3.35 (m, $H_4$, one -$CH_2O$-, 2 H), 3.02 (t, J= 9.2 Hz, $H_3$), 2.98-2.92 (m, $H_{5'}$, $H_6$), 2.76 (t, J= 3.8 Hz, $H_{6'}$, 1 H), 2.61 (m, $H_2$), 1.37-1.15 (m, $H_{1'}$, $H_{2'}$, 2 $H_{3'}$, 2 $H_{4'}$, 8 H).

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