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(±)-6-Acetamido-1,2-anhydro-6-deoxy-*myo*-inositol: a tight-binding inhibitor and pseudosubstrate for *N*-acetyl-β-glucosaminidases

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ABSTRACT

A five-step procedure is described for the synthesis of the title compound (*N*-acetylconduramine B *trans*-epoxide, **14**) from tetra-*O*-acetylconduritol B [(±)-(1,3/2,4)-1,2,3,4-tetra-*O*-acetyl-5-cyclohexene-1,2,3,4-tetrol]. Inhibition studies with *N*-acetyl-β-glucosaminidases from bovine kidney, jack beans, and *Helix pomatia* gave K_i values for **14** of 0.50–1.6 μM, i.e., 500–8000-fold lower than the K_i for 2-acetamido-2-deoxy-D-glucose. The K_i values for *N*-acetylconduramine B [(±)-(1,3/2,4)-4-acetamido-5-cyclohexene-1,2,3-triol] and its *cis*-epoxide [(±)-1-acetamido-2,3-anhydro-2-deoxy-*myo*-inositol] were several orders of magnitude larger than for **14**. In contrast to the interaction of other glycosidases with anhydro-inositols of appropriate configuration, there was no covalent, irreversible inhibition. Instead, the first two enzymes catalysed a transformation of **14** into a compound (presumably the oxazoline) which underwent spontaneous hydrolysis at pH ≤ 5. No inhibition was observed with the *N*-acetyl-β-glucosaminidase from *Aspergillus niger*.

INTRODUCTION

Conduritol epoxides (1,2-anhydro-inositols) with the appropriate configurations are mechanism-based active-site-directed inhibitors of numerous glycosidases[1,2]. Their specificity rests on non-covalent interactions of the glycon-binding site with the hydroxyl groups of the inhibitor combined with catalytic features of the active site, which normally effect hydrolysis of bound substrate molecules. These features are (*a*) an acidic group AH, which can transfer a proton to the glycosidic oxygen atom of the substrate or the oxirane ring of the inhibitor; and (*b*) a carboxylate group which stabilises a (partial) positive charge in the first transition state of substrate hydrolysis or which forms an ester bond with the activated oxirane, thus giving a covalent enzyme–inhibitor complex (**1** → **2** in Scheme 1).

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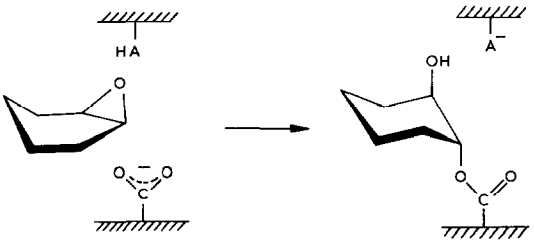


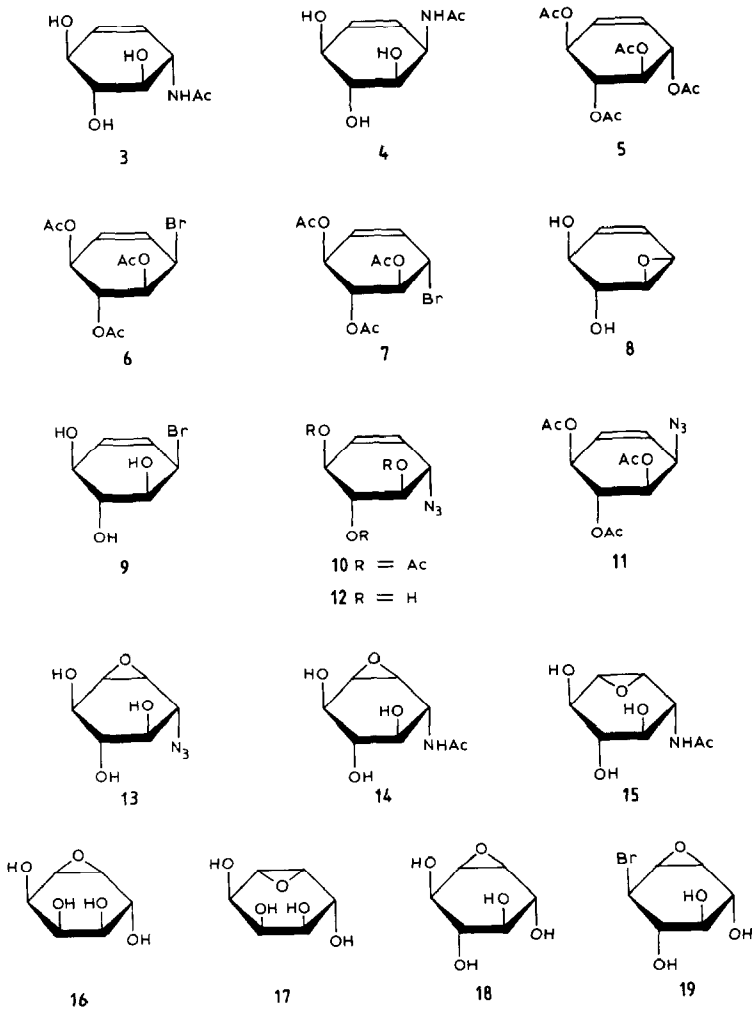
Scheme 1. Reaction of glycon-related epoxides with β-glycoside hydrolases (hydroxyl groups omitted).

We have extended this principle to *N*-acetyl-β-glucosaminidases by the synthesis of (±)-6-acetamido-1,2-anhydro-6-deoxy-*myo*-inositol (*N*-acetylconduramine B *trans*-epoxide, **14**). Exploratory experiments with epoxides prepared from a mixture of *N*-acetylconduramines B (**3**) and F (**4**) [data not reported] showed a weak immediate, but no time-dependent, inhibition of *N*-acetyl-β-glucosaminidases from jack beans and bovine epididymis. Presumably epoxidation occurred exclusively *cis* to the acetamido group[3]. If *N*-acetyl-β-glucosaminidases act by a mechanism similar to that of β-glucosidases[1,2], then the oxirane should be *trans* to the acetamido group in the potential inhibitor. In order to overcome the orientational effects of the neighbouring acetamido group, the precursor azide (**12**) was epoxidised and the product (**13**) was used in the synthesis of the title compound (**14**).

RESULTS AND DISCUSSION

The reaction of tetra-*O*-acetylconduritol B (**5**) with hydrogen bromide in acetic acid gave exclusively the all-*trans* product (±)-(1,3/2,4)-1,2,3-tri-*O*-acetyl-4-bromo-5-cyclohexene-1,2,3-triol (**7**). With acetonitrile as solvent, a 3:2 mixture of the desired (1,3,4/2)-isomer **6** and **7** was obtained together with one (in acetic acid) or two (in acetonitrile or water) 1,4-dibromo derivatives. The reaction of **5** in acetonitrile is similar to that in hydrobromic acid[4]. The results may be rationalised by an S_N1 reaction in polar solvents. The complete retention of configuration in the reaction of **5** in acetic acid is probably due to the formation of an intermediate cyclic acetoxonium ion.

Treatment of the mixture of **6** and **7** with ethanolic sodium ethoxide converted **7** into the unstable[4] epoxide **8** and deacetylated **6** (→ **9**). Treatment of the mixture of **8** and **9** with tetrabutylammonium azide and acetylation of the product gave the azide triacetate **10**. Treatment of **7** with tetrabutylammonium azide in acetonitrile effected azide displacement with inversion of configuration to give the azide triacetate **11**. Whereas epoxidation of *N*-acetylconduramine B (**3**), obtained from **12**, gave exclusively the *cis* isomer **15**, the *trans*-epoxide **13** was the only product when **12**, obtained by deacetylation of **10**, was epoxidised under the same conditions. The outcome of the epoxidation of **3** and **12** show that the directing effect of



the acetamido group greatly exceeds that of the hydroxyl group. Reduction of the azide group in **13** with triphenylphosphine and *N*-acetylation of the resulting amide gave the title epoxide **14**.

All of the compounds synthesised are racemic; the enantiomers with closest resemblance to 2-acetamido-2-deoxy-D-glucose are shown in the formulae.

The stability of the epoxide **14** was tested by measuring the inhibitory potency against *N*-acetyl-β-glucosaminidases from jack beans and bovine kidney (see below) under different conditions of storage. From the decrease of $1/K_i$, the half-life in water was calculated to be 20 h at 20–22° and 50 h at 4°. In 50 mM citrate/phosphate buffer (pH 4.5), $1/K_i$ decreased with k_{hydr} 0.0067 min^{-1} ($t^{1/2}$ 105 min), but the decrease was $\leqslant 5\%$ after 90 min at pH 3.5 and 6.0. The

TABLE I

Relative rates of reaction of epoxides with thiosulfate [a]

Epoxide	Time (s)	Relative rate
Cyclohexene oxide	18 ± 0.5	100
14	730 ± 10	2.4
15	350 ± 5	5.2
Conduritol B epoxide (**18**)	255 ± 5	7.0
Bromoconduritol B epoxide (**19**)	73 ± 3	24.5
Conduritol C *cis*-epoxide[21] (**16**)	330 ± 5	5.5
Conduritol C *trans*-epoxide[21] (**17**)	930 ± 20	1.9

[a] Time required to give the same colour with 0.05% phenolphthalein in 0.5 M thiosulfate as seen with 0.09 mM NaOH. The epoxide concentration was 25 mM.

occurrence of a maximum of instability indicates a participation of both the acid and base of the buffer in the decomposition process.

The reactivity of **14** with nucleophiles in the absence of acid catalysis was tested by the thiosulfate–phenolphthalein method[5] and the results are shown in Table I.

The differences in the rates of reaction of **14** and **15** and the conduritol C *cis*-(**16**) and *trans*-epoxide (**17**) are probably caused by steric effects. In the slower reacting isomers, the bulky acetamido group in **14** and the axial hydroxyl group in **17** are located on the side where attack of the thiosulfate ion occurs. The instability of **14** near pH 4.5, which is in marked contrast to the other conduritol epoxides, is not due to exceptional reactivity with nucleophiles, but decomposition might occur via an intramolecular reaction as proposed below (Scheme 2).

Enzymic studies.—The addition of an active-site-directed, covalent inhibitor to a solution that contains enzyme and substrate should cause the rate of formation of the product to decrease with time. The initial rate v_i will be given by $v_i = v_0/(1 + S/K_m + I/K_i)$ where v_0 is the rate in the absence of inhibitor and K_i the dissociation constant of an initial non-covalent enzyme–inhibitor complex. If the inhibitor is stable under the conditions of the assay and is in large excess over the enzyme, the rate of formation of the product will *decrease* exponentially to zero in a first-order process governed by the rate constant for the conversion of the non-covalent into a covalent enzyme–inhibitor complex[1]. The K_i for glycon-related conduritol epoxides was of the same order of magnitude as K_i for the corresponding hexose except for the mammalian lysosomal β-glucosidase where K_i (epoxide) is 1500-fold lower than K_i (glucose)[6,7].

However, the results of the inhibition experiments with **14** differed markedly from those expected from the above kinetic scheme in two respects, namely (*a*) the dissociation constants K_i (Table II), based on v_i measured directly after mixing (≤ 5 s), were several 1000-fold lower than those for 2-acetamido-2-deoxy-D-glucose and (*b*) the rate of substrate hydrolysis in the presence of **14** *increased* with time (Fig. 1).

The data in Table II indicate that specific interactions with the oxirane ring of **14** are important for tight inhibition. Its absence, as in **3**, or its opposite orienta-

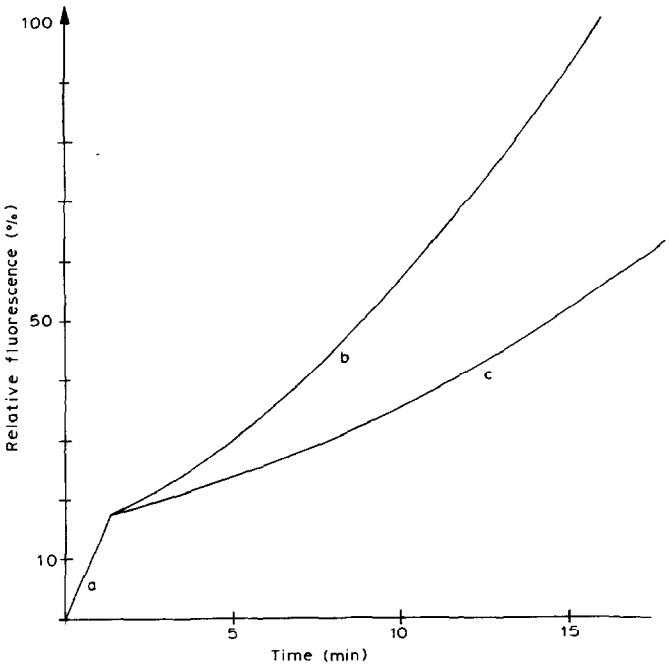


Fig. 1. Hydrolysis of 0.25 mM 4-methylumbelliferyl 2-acetamido-2-deoxy-β-D-glucopyranoside by *N*-acetyl-β-glucosaminidase A from bovine kidney in the absence (a) and presence of 5 (b) and 10 μM (c) **14** at pH 4.25 and 25°: 100% fluorescence corresponds to 4% of hydrolysis.

tion, as in **15**, decreased the affinity by several orders of magnitude. The lack of inhibition with the enzyme from *Aspergillus niger* was not unexpected as this enzyme probably has an unusual active site. The enzyme differs from the other *N*-acetyl-β-glucosaminidases in being inhibited[8] ⩾ 1000-fold less by derivatives of 2-acetamido-2-deoxy-D-glucose with a basic nitrogen function at C-1. Nothing unusual was seen in the concentration dependence of the inhibition: Dixon plots over a 10-fold concentration range of **14** (not shown) were linear and the K_i values calculated from the slopes of Lineweaver–Burk plots[9] (Fig. 2) were identical for different concentrations of **14**. A partially non-competitive inhibition (ordinate intersections above $1/V_{max}$) was also seen with other strong inhibitors[8] and became purely competitive at pH ⩾ 5.5. The pH dependence of the inhibition of hexosaminidase A from bovine kidney was maximal at pH 5.4 with half-maximal inhibition at pH 3.8 and 6.6 (data not shown).

The following model is proposed to account for the unexpected properties of **14**. The high affinity for the *N*-acetyl-β-glucosaminidases from bovine kidney, jack beans, and *Helix pomatia* is due to a strong hydrogen bond with the epoxide oxygen. In addition, the interactions with the acetamido and hydroxyl groups are more favourable than with 2-acetamido-2-deoxy-D-glucose, as seen from the 12- and 90-fold lower K_i values for **3** relative to those of 2-acetamido-2-deoxy-D-glu-

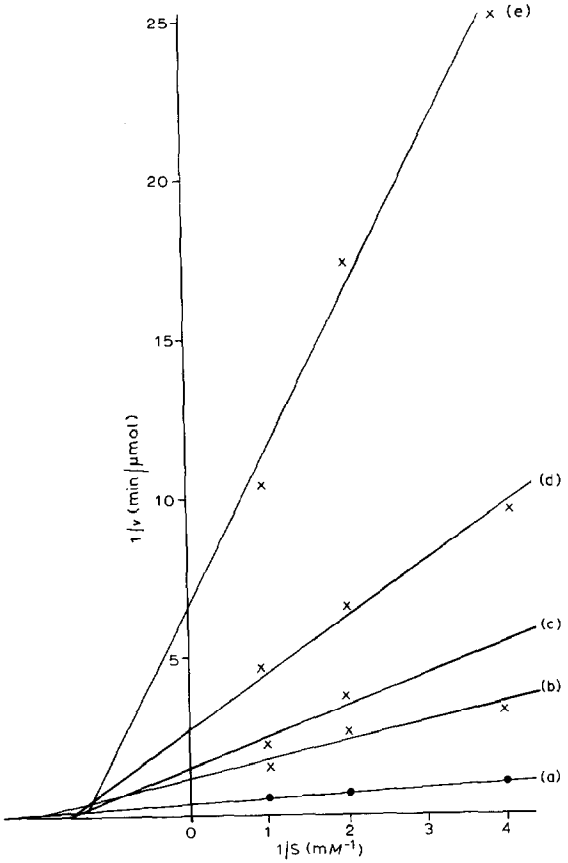


Fig. 2. Lineweaver–Burk plot of the hydrolysis of 4-methylumbelliferyl 2-acetamido-2-deoxy-β-D-glucopyranoside by *N*-acetyl-β-glucosaminidase from jack beans in the absence (a) and presence of 2.5 (b), 5.0 (c), 10 (d), and 25 μM (e) **14** at pH 4.5 and 25°. Reciprocal rates measured immediately after the addition of **14** (see Fig. 1) were plotted. The K_i values, calculated from the slopes in the presence and absence of **14**, were 0.82 (b), 0.85 (c), 0.89 (d), and 0.76 μM (e).

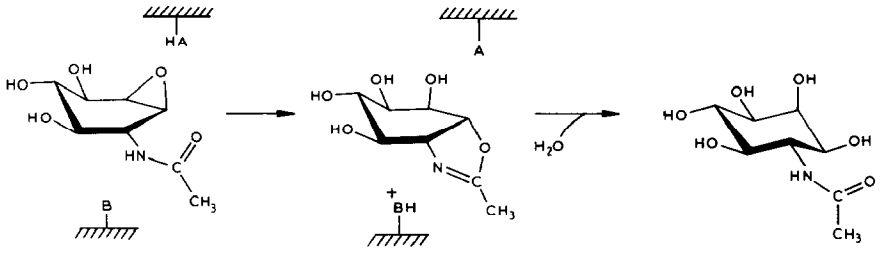
cose. In this respect, the enzyme–epoxide interaction resembles that of the lysosomal β-glucosidase from human placenta[6] and bovine spleen[7] with conduritol B epoxide and its 6-bromo-6-deoxy derivative, respectively, for which K_i of the

TABLE II

Inhibition [a] of *N*-acetyl-β-glucosaminidases

Enzyme source	Substrate			
	3	**14**	**15**	GlcNAc
Bovine kidney	45	0.39	1000	4000
Jack beans	330	0.83	1.100	4000
Helix pomatia	nd [b]	1.5	nd	800
Aspergillus niger	nd	> 1000	nd	6000

[a] K_i values in μM. [b] No inhibition detected.



Scheme 2. Proposed reaction of **14** with *N*-acetyl-β-glucosaminidases from bovine kidney and jack beans.

non-covalent complex is 1500-fold[6] and 200 000-fold[7] lower, respectively, than the K_i for D-glucose. However, the enzymes studied here differ from these β-glucosidases in that the latter form a covalent bond with the epoxide (Scheme 1).

Since the regain of activity of the enzymes from bovine kidney and jack beans was first order with a rate constant $k = 0.09 \pm 0.01$ min^{-1} that was independent of the inhibitor concentration and unaffected by the presence of substrate, it must be due to an enzyme-independent process. On the other hand, the regain was much larger than the rate of spontaneous decomposition (0.0067 min^{-1}). We propose that these two enzymes catalyse a rapid transformation of **14** into a compound of similar inhibitory potency, which undergoes an acid-catalysed decomposition into a non-inhibiting species because the regain of activity is only seen at pH $\leqslant 5$. Such a compound could be the oxazoline **20** which would undergo hydrolysis to the *N*-acetylinosamine **21** (Scheme 2). Thus, **14** can be classified as a pseudosubstrate[2] for these two *N*-acetyl-β-glucosaminidases. The lack of a similar regain of activity with the enzyme from *Helix pomatia* could point to an active site where a strong hydrogen bond with the oxirane is formed but transformation of **14** into **21** does not occur. A somewhat different pseudosubstrate-like interaction of conduritol epoxides and glycoside hydrolases was observed with β-glucosidases from *Aspergillus wentii*[10] and sweet almonds[11] where part of the inhibitor did not react as in **1** → **2** but underwent enzyme-catalysed hydrolysis or ring opening by halide ions present in the assay medium.

If the active site of an enzyme has evolved towards a structure which stabilises the transition state and/or a reactive intermediate, its affinity for structural analogues of these intermediates is much larger than for the substrate or product[12]. The postulated strong inhibition by the oxazoline **20** would then imply that the oxazoline derived from 2-acetamido-2-deoxyglucose, or its protonated counterpart, is on the reaction pathway of substrate hydrolysis. Acetamido-group participation with formation of an oxazoline has been postulated[13] from pH–rate studies of the non-enzymic hydrolysis of (1 → 4)-linked 2-acetamido-2-deoxy-β-D-glucose oligosaccharides and by lysozyme[14]. On the other hand, anchimeric assistance by the acetamido group was considered unimportant for lysozyme catalysis by Raftery and Rand-Meir[15] from a comparison of the rates of cleavage with substrates where the

acetamido group had been replaced by a hydroxyl group. Preliminary experiments showed the crude oxazoline prepared by deacetylation of 2-methyl-(1,4,6-tri-*O*-acetyl-1,2-dideoxy-α-D-glucopyrano[2,1-*d*]oxazoline to be an ~10-fold better inhibitor than 2-acetamido-2-deoxy-D-glucose for *N*-acetyl-β-glucosaminidase from bovine kidney.

Another model which could explain the strong inhibition by **14** and the observed reactivation is based on the rapid formation of a covalent enzyme–inhibitor complex which is then hydrolysed to the enzyme and a weakly inhibiting product. This possibility cannot be ruled out completely but appears unlikely because the inhibition was established within the time of mixing (< 5 s) even with an inhibitor concentration of 2.5 μM and a substrate concentration of > 2 K_m. Under these conditions, the rate constant for the approach to the inhibition is governed by $k_i(max)/K_i$ (cf. inhibition by glycals[2]). The failure to observe a lag phase in the approach to the inhibition (Fig. 1) requires that $k_i(max)/K_i$ should be $> 10^7$ M^{-1} min^{-1}, which is > 2000-fold larger than $k_i(max)/K_i$ for the fastest epoxide/glycosidase system reported so far[2].

EXPERIMENTAL

General.—Melting points were determined with a hot-stage microscope and are uncorrected. ^1H NMR spectra were recorded with Bruker AC 80 and AM 300 spectrometers, and mass spectra with a Finnigan MAT 4510 spectrometer. TLC was performed on Silica Gel 60 F_{254} (Merck), using *A*, light petroleum (bp 30–50°)–EtOAc (7:3); and *B*, $CHCl_3$–MeOH (3:1); and detection by charring with 30% H_2SO_4 in acetic acid and with 0.5% $KMnO_4$ in aq 5% Na_2CO_3. Column chromatography (MPLC) was performed on silica gel (Matrex LC 60 Å; 20–45μ, Amicon).

Bromination of (±)-tetra-O-acetylconduritol B (**5**).—Crystalline acetonitrile/hydrogen bromide adduct (16 g, prepared by saturating acetonitrile with gaseous hydrogen bromide at 0°) was added to a solution of **5**[16] (4.08 g) in acetonitrile (10 mL). The mixture was kept in a closed vessel at 55° for 48 h until ~80% of **5** had reacted [TLC (solvent *A*); R_F values: **5** 0.40, **6** 0.56, **7** 0.60, 1,4-dibromide(s) 0.87]. Work-up involved the addition of ice (30 g) and extraction with ether. The extract was washed with satd aq $NaHCO_3$ and concentrated. Column (3.5 × 40 cm) chromatography (light petroleum–EtOAc, 9:1 → 7:3) of the residue gave a mixture (0.6 g) of 1,4-dibromides, a mixture (2.9 g) of **6** and **7**, and **5** (0.7 g).

Rechromatography (light petroleum–EtOAc, 9:2) of the middle fraction gave (±)-(1,3,4/2)-1,2,3-tri-*O*-acetyl-4-bromo-5-cyclohexene-1,2,3-triol (**6**), isolated as a colourless syrup. ^1H NMR data (80 MHz, $CDCl_3$): δ 2.04, 2.05, and 2.10 (3 s, 3 H each, 3 Ac), 4.8–5.0 (m, 2 H, H-3,4), 5.5–5.7 (m, 3 H, H-1,2,5), 6.1 (m, 1 H, H-6).

(±)-(1,3/2,4)-1,2,3-Tri-O-acetyl-4-bromo-5-cyclohexene-1,2,3-triol (**7**).—A crystalline compound isolated from the first fractions of the mixture of **6** plus **7** after rechromatography (see above) was identified (^1H NMR, mixture mp) by comparison with **7**, prepared as described[16].

Tetrabutylammonium azide.—A solution of the salt in CH_2Cl_2 was prepared as described[17]. The azide concentration was determined by concentration of an aliquot (100 μL) in vacuo, and dissolution of the residue in water to give a concentration of 2–10 μmol N_3^-/mL. An aliquot (100 μL) of this solution was added to 0.5 M aq $FeCl_3$ (0.4 mL) and water (4 mL), and the A_{460} measured after 15 min. The azide content was determined from a standard curve prepared with NaN_3, the slope of which corresponded to E_{460} 2450 M^{-1} cm^{-1}. The yield of tetrabutylammonium azide was 60–70% and the solutions could be stored at −18° for several weeks; decomposition became noticeable after a few days at room temperature.

(±)-(1,3 / 2,4)-1,2,3-Tri-O-acetyl-4-azido-5-cyclohexene-1,2,3-triol (**10**).—To a solution of a mixture (340 mg, 1 mmol) of **6** and **7** in anhyd EtOH (15 mL) was added ethanolic 0.4 M NaOEt dropwise until the solution (which became brown after a few min) remained alkaline for ~ 10 min (pH 9, indicator paper). Tetrabutylammonium azide solution (1.5 mL) was added and the pH was adjusted to ~ 5 with *p*-toluenesulfonic acid. The solution was stored for at least 6 h at room temperature, then concentrated, and the residue was acetylated with acetic anhydride–pyridine. Work-up (addition of ice, extraction with ether, and MPLC as described for **6** and **7**) gave **10**, R_F 0.60 (solvent *A*) in a yield of 65–80% which decreased to ~ 30% when the reaction was scaled up to 5 mmol. Isolated by-products were **11** (5–10%), R_F 0.54, and (±)-(1,3,4/2)-1,2,3-tri-*O*-acetyl-4-*O*-ethyl-5-cyclohexene-1,2,3-triol (≤ 5%), R_F 0.68.

Compound **10** had mp 123–124° (from EtOAc–light petroleum); ν_{max}^{KBr} 2090 cm^{-1} (N_3). ^1H NMR data (300 MHz, $CDCl_3$): δ 2.00, 2.02, and 2.06 (3 s, 3 H each, 3 Ac), 4.17 (m, 1 H, H-4), 5.22 (m, 1 H, H-1), 5.25 (m, 1 H, H-2), 5.53 (m, 1 H, H-3), 5.70 (quasi s, 2 H, H-5,6). EI-mass spectrum: *m/z* 43 (100%, Ac^+), 255 [7%, $M - N_3)^+$]; other prominent peaks at *m/z* 212 (3%), 183 (12%), 170 (20%), 152 (4%), 128 (6%), 118 (10%).

Anal. Calcd for $C_{12}H_{15}N_3O_6$ (297.2): C, 48.49; H, 5.08; N, 14.13. Found: C, 48.10; H, 5.18; N, 13.90.

(±)-(1,3,4 / 2)-1,2,3-Tri-O-acetyl-4-azido-5-cyclohexene-1,2,3-triol (**11**).—A solution of **7** (1.68 g, 5 mmol) in acetonitrile (5 mL) was treated with a solution of tetrabutylammonium azide (10 mmol) for 30 min at room temperature. The solution was diluted with ether (300 mL), washed four times with water, and concentrated to dryness. Crystallisation of the residue from EtOAc–light petroleum gave **11** (1.26 g, 85%), mp 82–83°. ^1H NMR data (300 MHz, $CDCl_3$): δ 2.00, 2.01, and 2.07 (3 s, 3 H each, 3 Ac), 4.32 (m, 1 H, H-4), 5.08 (m, 1 H, H-1), 5.40 (m, 1 H, H-2), 5.43 (m, 1 H, H-3), 5.75 (m, 1 H, H-5), 5.83 (m, 1 H, H-6).

Anal. Calcd for $C_{12}H_{15}N_3O_6$ (297.2): C, 48.49; H, 5.08; N, 14.13. Found: C, 48.00; H, 5.21; N, 13.60.

1,2-Anhydro-6-azido-6-deoxy-myo-*inositol* (**13**).—Compound **10** (1.2 g) was deacetylated with a catalytic amount of NaOMe in MeOH (30 mL). After 30 min, the solution was neutralised with Dowex 50 (H^+) resin and concentrated to

dryness to give (±)-(1,3/2,4)-4-azido-5-cyclohexene-1,2,3-triol (**12**, 0.68 g), mp 84–85°, R_F 0.70 (solvent *B*); ν_{max}^{KBr} 2100 cm^{-1} (N_3). ^1H NMR data (80 MHz, pyridine-d_5): 4.10–4.68 (m, 4 H, H-1,2,3,4), 5.54–6.28 (m, 2 H, H-5,6), 6.44 (3 H, b s, OH).

A solution of **12** (0.65 g) in acetic acid (30 mL) and $CHCl_3$ (15 mL) was treated with *m*-chloroperoxybenzoic acid (2.5 g) for 48 h at room temperature. The solution was concentrated in vacuo to a few mL, diluted with water (50 mL), filtered, and extracted with ether until A_{280} became < 0.1. The aqueous phase was concentrated and then lyophilised to give **13** (0.49 g, 70%), mp 130–132° (from EtOH–ether), R_F 0.61 (solvent *B*). ^1H NMR data (80 MHz, pyridine-d_5): δ 3.23 [quasi d, 1 H, H-1(2)], 3.64 [m, 1 H, H-2(1)], 3.79–4.40 (m, 4 H, H-3,6).

Anal. Calcd for $C_6H_9N_3O_4$ (187.2): C, 38.49; H, 4.85; N, 22.44. Found: C, 39.20; H, 5.00; N, 21.90.

(±)-6-Acetamido-1,2-anhydro-6-deoxy-myo-*inositol* (**14**).—Triphenylphosphine (0.8 g, 3 mmol) was reacted at room temperature with **13** (0.28 g, 1.5 mmol) in anhyd tetrahydrofuran (15 mL) for 2.5 h. TLC (solvent *B*) then showed that **13** had been transformed into a compound with R_F 0.02 (ninhydrin positive). Water (20 μL) and acetic anhydride (0.2 mL) were added and crystallisation, induced by the addition of ether to faint turbidity, was completed by cooling to −18° and adding successive portions of ether, to give **14** (0.18 g, 58%), mp 120–122°. NMR data (pyridine-d_5): ^1H (300 MHz), δ 2.08 (s, 3 H, Ac); 3.52 (quasi d, 1 H, H-1), 3.69 (m, 1 H, H-2), 4.13 (m, 1 H, H-5), 4.21 (m, 1 H, H-4), 4.41 (m, 1 H, H-3), 4.72 (m, 1 H, H-6), 5.07, 6.92, 7.29 (s, OH), 9.21 (d, 1 H, NH); ^{13}C (75 MHz), δ 23.07 (CH_3), 54.15 (C-6), 56.65 (C-1), 58.28 (C-2), 72.96 (C-3), 73.47 (C-4), 73.92 (C-5), 170.71 (C=O).

Anal. Calcd for $C_8H_{13}NO_5$ (203.1): C, 47.29; H, 6.40; N, 6.90. Found: C, 46.82; H, 6.65; N, 7.45.

(±)-N-Acetylconduramine B [(1,3 / 2,4)-4-acetamido-5-cyclohexene-1,2,3-triol, **3***].*—Compound **12** (150 mg) was reduced with triphenylphosphine and the product was acetylated, as described for **13**, to give **3** (100 mg, 59%), mp 204–205°. ^1H NMR data (80 MHz, pyridine-d_5): δ 2.03 (s, 3 H Ac), 4.18, 4.65, 5.22 (m, 4 H, H-1/4), 5.89 (m, 2 H, H-5/6), 4.8 (s, 1 H, OH), 6.7 (s, 2 H, OH), 8.77 (d, 1 H, NH).

Anal. Calcd for $C_8H_{13}NO_4$ (187.2): C, 51.33; H, 7.00; N, 7.49; Found: C, 50.50; H, 7.15; N, 7.29.

(±)-1-Acetamido-2,3-anhydro-1-deoxy-myo-*inositol* (**15**).—Epoxidation of **3** (80 mg), as described for **12**, gave **15** (48 mg, 52%), mp 190° (dec), R_F 0.42 (solvent *B*).

Relative reaction rates of epoxides with thiosulfate.—Equal volumes of 50 mM epoxide in water and M sodium thiosulfate in aq 50% MeOH containing 0.1% of phenolphthalein were mixed, and the times noted for the development of a pink colour corresponding to an arbitrary standard in which the epoxide solution was replaced by 0.18 mM NaOH.

Enzymic studies.—The activities of *N*-acetyl-β-glucosaminidases were determined fluorimetrically[18] with 4-methylumbelliferyl 2-acetamido-2-deoxy-β-D-gluco-

pyranoside at 25° in 50 mM sodium citrate (pH 4.5), unless stated otherwise. The time of mixing was < 5 s. Values for K_i were calculated from the slopes of Lineweaver–Burk plots in the absence and presence of inhibitor[9]. The kinetics for the regain of activity were evaluated from plots of $\ln[S_0 - S_i(t)/S_i(0)]$ vs. time where S_0 is the slope in the absence, and $S_i(0)$ in the presence, of **14** immediately after its addition.

The enzymes were from the following sources. *N*-Acetyl-β-glucosaminidase from Jack beans (*Convallaria ensiformis*) was a commercial preparation (Sigma A 2264) and was used without further purification (K_m 0.42 mM). Hexosaminidase A was purified from freshly frozen bovine kidney by affinity chromatography[19] (K_m 0.65 mM). Commercial β-glucuronidase (Sigma G 7017) from the digestive juice of *Helix* was found to contain two, possibly three, *N*-acetyl-β-glucosaminidases. The isozyme (Hex A) with highest affinity for DEAE-Cellulose was purified by affinity chromatography[20] (K_m 0.010 mM at pH 5). The *N*-acetyl-β-glucosaminidase from *Aspergillus niger* was a commercial preparation (Sigma A 7053) and was used without further purification (K_m 0.1 mM).

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