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Reactive pseudo-oligosaccharides as potential irreversible inhibitors for sugar-binding proteins: synthesis of the diastereoisomers of (3,4,6/5)-3,4-epoxy-6-(β-D-galactopyranosylthio)-5-hydroxycyclohexene

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ABSTRACT

Reaction of (±)-(3/4,5,6)-4-bromo-5,6-epoxy-3-hydroxycyclohexene with 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-galactopyranose, followed by treatment of the products with base under phase-transfer conditions and then acetylation, gave the hexa-acetates of the title diastereoisomers one of which (**6b**) was isolated crystalline. The title diastereoisomer **7b**, obtained by *O*-deacetylation of **6b**, competitively inhibited *E. coli* β-D-galactosidase (K_i 85 μM). Although **7b** decomposed in buffer at pH 6.8 ($t_{\frac{1}{2}}$ ~3.3 h), it deactivated the enzyme irreversibly at a high initial rate.

INTRODUCTION

A vinyloxirane unit, attached to a monosaccharide, is not only useful for regio- and stereo-specific chemical reactions[1], but also for active-site-directed chemical modification of sugar-binding proteins[2]. The allylic carbon of the oxirane is susceptible to attack by all types of nucleophilic functional groups. In contrast to ordinary oxiranes, frequently used as irreversible inhibitors of sugar-binding proteins[3–5], vinyloxiranes do not need activation by protonation of the ring oxygen atom[1,2]. Unfortunately, when the vinyloxirane group is part of a sugar, the conformation of the latter may be altered to such an extent that the affinity for the binding site and the selectivity of the reaction are decreased considerably[2]. However, if binding does not depend solely on the monosaccharide moiety but also on a well-recognised part of a natural ligand, then binding to, and subsequent alkylation of, the protein may ensue. We now describe the synthesis of a pseudodisaccharide that mimics lactose. The compound (**7b**) comprises a β-D-galactopyranosyl moiety that has an affinity for a β-D-galactoside-binding site, and a reactive 3,4-epoxycyclohexene moiety.

RESULTS AND DISCUSSION

(±)-(3,4/5,6)-Diepoxycyclohexene* (**1**; only one enantiomer is depicted) is a versatile synthon for the preparation of pseudo-oligosaccharides. The enantiomeric

*Alternatively named 5,8-dioxabicyclo[5.1.0.$0^{2,4}$]oct-5-ene.

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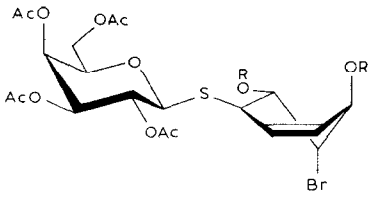
conduritol derivatives **1** resemble monosaccharides and can replace variously, the reducing, inner, and non-reducing units of an oligosaccharide[6]. Compound **1** can be prepared readily in several steps from *p*-benzoquinone[7,8] and, although it can be reacted directly with 1-thio sugars, it is more convenient to use (±)-(3/4,5,6)-4-bromo-5,6-epoxy-3-hydroxycyclohexene (**2**; only one enantiomer is depicted).

1

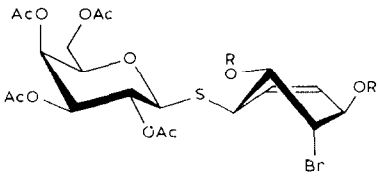
2

Thus, reaction of **2** with 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-galactopyranose in aqueous methanol at room temperature gave a mixture of the diastereoisomeric thioglycoside derivatives **3a** and **3b**, acetylation of which gave a mixture of the hexa-acetates **4a** and **4b** that was resolved by chromatography. Treatment of **3ab** with base under phase-transfer conditions gave a mixture of the vinyloxiranes **5a** and **5b**. Acetylation of **5ab** afforded a mixture of the penta-acetates **6a** and **6b** that could be resolved by chromatography and one, arbitrarily assigned as **6b**, was obtained crystalline. Zemplén *O*-deacetylation of **6b** gave the target pseudodisaccharide, (3,4,6/5)-3,4-epoxy-6-(β-D-galactopyranosylthio)-5-hydroxycyclohexene (**7b**) that was reasonably stable ($t_{\frac{1}{2}}$ ~3.3 h) in buffer at pH 6.8.

As expected, **7b** was a good competitive inhibitor of *E. coli* β-D-galactosidase (K_i



3a R = H
4a R = Ac



3b R = H
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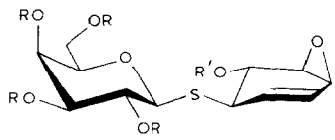
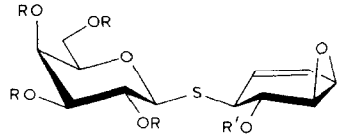


5a R = Ac, R′ = H
6a R = R′ = Ac
7a R = R′ = H



5b R = Ac, R′ = H
6b R = R′ = Ac
7b R = R′ = H

The compounds (**3**–**7**) of series **a** (higher R_F values) and **b** are diastereoisomers, the absolute configurations of which have not been established and are assigned arbitrarily.

85 μM) and also effected irreversible inhibition (Fig. 1). Initially, the latter effect was pronounced, although specificity studies[9] indicated little contact between the protein and the more remote parts of the aglycon. A low probability for alkylation by the oxirane group, together with the decomposition of the compound, may explain the decline in the irreversible deactivation. Additionally, the products of decomposition of **7b** in the aqueous buffer at pH 6.8 (1-thio-β-D-galactosides; K_i for decomposed **7b** is 265 μM) protected the enzyme, as did the added competitive inhibitor isopropyl 1-thio-β-D-galactopyranoside (IPTG) (Fig. 1).

Although, as expected, **1** and **2** had only weak affinities for the binding site of the enzyme (protection by IPTG was extremely effective; with equimolar concentrations, there was no loss of activity), they inhibited the enzyme irreversibly (Figs. 2 and 3).

Compounds **1** and **2** are too unspecific to be efficient active-site-directed irreversible inhibitors for sugar-binding proteins. Their high cytotoxicity is suggestive of indiscriminate alkylation[10], and a homing device such as an attached sugar moiety is necessary in order to make them useful for blocking sugar-binding proteins *in vivo*.

EXPERIMENTAL

General. — The m.p. is uncorrected and the optical rotations were measured with a Perkin–Elmer 141 polarimeter. All reactions were monitoried by t.l.c. on Silica Gel 60 F_{254} (Merck), using the solvents indicated. Flash-column chromatography[11] was performed on Silica Gel 60 (0.04–0.063 mm, Merck). ^{1}H-N.m.r. spectra were recorded for

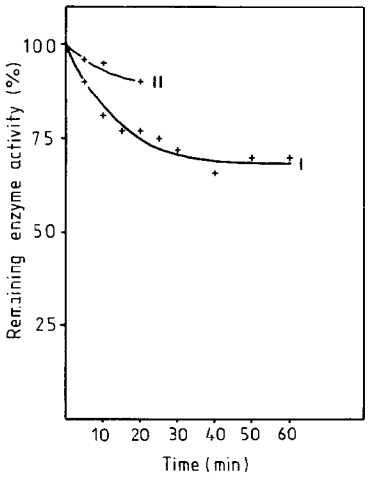


Fig. 1. Deactivation of β-D-galactosidase: I, 0.323M **7b**; II, 0.304M **7b** plus 0.83M isopropyl 1-thio-β-D-galactopyranoside.

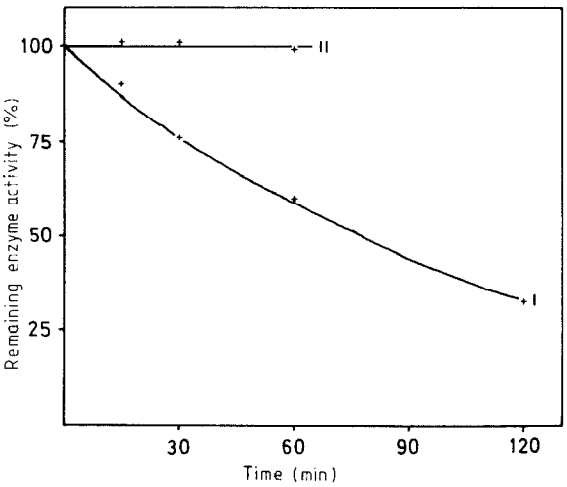


Fig. 2. Deactivation of β-D-galactosidase: I, 0.122M **1**; II, 0.142M **1** plus 0.289M isopropyl 1-thio-β-D-galactopyranoside.

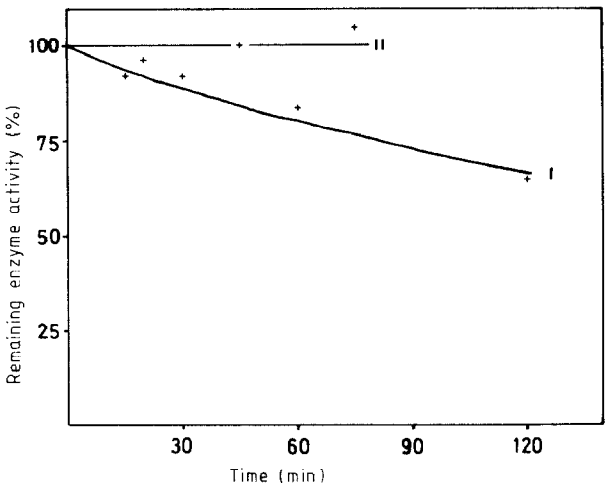


Fig. 3. Deactivation of β-D-galactosidase: I, 0.114M **2**; II, 0.129M **2** plus 0.289M isopropyl 1-thio-β-D-galactopyranoside.

solutions in $CDCl_3$ (internal Me_4Si) with a Bruker WM-250 or AM-400 spectrometer.

Enzymic reactions. — β-D-Galactosidase (β-D-galactoside galactohydrolase, EC 3.2.1.23) from *Escherichia coli* was purchased from Boehringer Mannheim, and isopropyl 1-thio-β-D-galactopyranoside (IPTG) from Sigma. Reactions were performed at 30° in 50mM sodium potassium phosphate buffer (pH 6.8) and mM magnesium chloride. The K_i value for **7b** was determined using an assay with 0.17–2.66mM *o*-nitrophenyl β-D-galactopyranoside (oNPGal), 0–0.4mM inhibitor, and 0.1 μg of β-D-galactosidase. In order to determine the K_i for the product(s) of decomposition, a solution of **7b** in the

buffer was kept at room temperature for 16 h. The assay was then carried out as described above and the concentrations were varied in the range 0–39.5mM. The half-life ($t_{\frac{1}{2}}$) of **7b** in the buffer was estimated by measuring the decrease in extinction at 260 nm. The kinetics of irreversible deactivation of β-D-galactosidase by **1**, **2**, and **7b** (the concentrations are noted in the legends to Figs. 1–3) were carried out severally with 0.313 mg of β-D-galactosidase dissolved in 1 mL of buffer. Aliquots, taken at intervals, were diluted immediately 1:200 with buffer, and 50 μL was assayed for residual enzyme activity using oNPGal (2.66mM) in buffer (2.5 mL).

TABLE I

^{1}H-N.m.r. Data

Proton	**3a**[a]	**3b**[a]	**4a**[b]	**4b**[b]
H-1	5.88 m	5.82 m	5.79 m	5.78 m
H-2	5.73 m	5.81 m	5.73 m	5.72 m
H-3	3.76 m	3.88 m	3.89 m	3.78 m
H-4	4.14 m	4.13 m	5.37 m	5.65 m
H-5	4.44 dd	4.41 dd	4.58 dd	4.65 dd
H-6	4.55 m	4.55 m	5.68 m	5.69 m
H-1′[c]	4.60 d	4.68 d	4.72 d	4.77 d
H-2′	5.28 t	5.22 t	5.28 t	5.20 t
H-3′	5.07 dd	5.09 dd	5.09 dd	5.08 dd
H-4′	5.42 dd	5.44 dd	5.43 dd	5.46 dd
H-5′	3.98 dt	4.01 dt	4.07 dt	3.98 dt
H-6′a	4.11 dd	4.13 dd	4.17 dd	4.10 dd
H-6′b	4.21 dd	4.17 dd	4.19 dd	4.21 dd
OAc	2.17 s	2.17 s	2.17 s	2.17 s
	2.10 s	2.09 s	2.16 s	2.15 s
	2.09 s	2.08 s	2.15 s	2.14 s
	2.00 s	2.00 s	2.10 s	2.06 s
			2.05 s	2.03 s
			2.00 s	2.00 s
$J_{1,2}$	10.2		9.8	9.8
$J_{1,3}$				1.3
$J_{1,6}$	3.3		3.8	3.9
$J_{2,3}$	3.3		2.5	2.5
$J_{2,4}$				1.2
$J_{2,6}$				1.2
$J_{3,4}$	5.7	5.9	3.0	3.0
$J_{4,5}$	2.7	2.6	2.5	2.5
$J_{5,6}$	5.2	5.4	8.5	9.0
$J_{1',2'}$	10.2	10.2	10.0	10.0
$J_{2',3'}$	10.2	10.2	10.0	10.0
$J_{3',4'}$	3.3	3.3	3.5	3.5
$J_{4',5'}$	1.2	1.2	1.2	1.2
$J_{5',6'a}$	6.8	6.3	6.3	6.8
$J_{5',6'b}$	5.7	6.9	6.9	5.7
$J_{6'a,6'b}$	11.0	10.9	10.9	11.0

[a] 250 MHz. [b] 400 MHz. [c] Primed numbers refer to the β-D-Gal*p* moiety.

(3,6/4,5)-4-Bromo-3,5-dihydroxy-6-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosylthio)cyclohexene (**3ab**). — To a solution of 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-galactopyranose[12,13] (10.2 g, 28 mmol) and **2** (4.5 g, 23 mmol) in methanol (12 mL) was added water (0.5 mL), and the mixture was stored for 2 days at room temperature, then concentrated to dryness *in vacuo*. Flash-column chromatography (chloroform–acetone, 6:1) of the residue gave syrupy, colourless **3ab** (12.9 g) in nearly quantitative yield, R_F 0.13. For the ^{1}H-n.m.r. data, see Table I.

Treatment of **3ab** (164 mg, 0.29 mmol) conventionally with pyridine–acetic anhydride (2:1, 0.5 mL), followed by concentration of the mixture *in vacuo* and repeated flash-column chromatography (ethyl acetate–cyclohexane, 1:3) of the residue (190 mg), gave the penta-acetates **4a** (R_F 0.18) and **4b** (R_F 0.13) (t.l.c.; ethyl acetate–cyclohexane, 1:2). For the ^{1}H-n.m.r. data, see Table I.

(3,4,6/5)-5-Acetoxy-3,4-epoxy-6-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosylthio)cyclohexene (**6ab**). — Powdered potassium hydroxide (1.6 g, 21 mmol) and tetraethylammonium chloride (70 mg, 0.4 mmol) were added to a solution of **3ab** (11.8 g, 21 mmol) in dichloromethane (200 mL). The mixture was stirred vigorously at room temperature for 5 h, then filtered, and concentrated to dryness *in vacuo*. Flash-column chromatography (ethyl acetate–cyclohexane, 2:1) of the residue gave **5ab** as a colourless syrup (6.6 g, 65%), the ^{1}H-n.m.r. spectrum of which was complex.

A mixture of **5ab** (6.4 g, 13.5 mmol) and triethylamine (6.25 g, 62 mmol) in dry dichloromethane (65 mL) was treated for 12 h at room temperature with acetic anhydride (4.2 g, 41 mmol), then concentrated to dryness *in vacuo*. Flash-column chromatography (ethyl acetate–cyclohexane, 1:1) of the residue gave a product that crystallised partly from ether. The recrystallised product **6b** (1.9 g, 27%) had m.p. 136°, $[\alpha]_D$ −220° (*c* 1, chloroform), R_F 0.19.

Anal. Calc. for $C_{22}H_{28}O_{12}S$: C, 51.16; H, 5.46; S, 6.21. Found: C, 51.22; H, 5.50; S, 6.13.

Flash-column chromatography (ethyl acetate–cyclohexane, 1:2) of the material in the mother liquor gave syrupy **6a** (1.8 g, 26%), $[\alpha]_D$ −157° (*c* 1, chloroform), R_F 0.23 (ethyl acetate–cyclohexane, 1:1).

The ^{1}H-n.m.r. data of **6a** and **6b** are given in Table II.

(3,4,6/5)-3,4-Epoxy-6-(β-D-galactopyranosylthio)-5-hydroxycyclohexene (**7b**). — Crystalline **6b** (98 mg, 0.19 mmol) was *O*-deacetylated conventionally by the Zemplén method (2 mL of solution, 1 h). The solution was deionised by elution from a column (1.5 × 4.0 cm) of silica gel with methanol. Concentration of the eluate yielded amorphous **7b** (58 mg, quantitative), R_F 0.33 (ethyl acetate–methanol–water, 7:2:1), re-acetylation of which gave **6b**.

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TABLE II

^{1}H-N.m.r. Data (400 MHz)

Proton	**6a**	**6b**	*Proton*	**6a**	**6b**
H-1	3.37 m	3.37 dt	$J_{1,2}$	4.0	4.2
H-2	6.13 dd	6.11 dd	$J_{1,3}$	1.2	1.8
H-3	5.91 m	5.92 dddd	$J_{1,6}$	4.0	4.2
H-4	3.61 m	3.57 m	$J_{2,3}$	9.9	9.9
H-5	5.66 m	5.76 m	$J_{3,4}$	6.0	6.0
H-6	3.61 m	3.60 m	$J_{4,5}$	2.0	2.0
H-1'[a]	4.73 d	4.55 d	$J_{1',2'}$	10.0	10.0
H-2'	5.26 t	5.34 t	$J_{2',3'}$	10.0	10.0
H-3'	5.08 dd	5.04 dd	$J_{3',4'}$	3.5	3.5
H-4'	5.44 dd	5.44 dd	$J_{4',5'}$	1.2	1.2
H-5'	3.99 dt	3.98 dt	$J_{5',6'a}$	6.5	6.5
H-6'a	4.12 dt	4.13 dt	$J_{5',6'b}$	7.2	7.0
H-6'b	4.19 dd	4.20 dd	$J_{6'a,6'b}$	10.5	11.2
OAc	2.13 s	2.16 s			
	2.08 s	2.08 s			
	2.07 s	2.07 s			
	2.05 s	2.05 s			
	2.00 s	2.00 s			

[a] Primed numbers refer to the β-D-Gal*p* moiety.

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