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The Synthesis of Active-Site Directed Inhibitors of Some β-Glucan Hydrolases

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Abstract

The 2,3-epoxypropyl, 3,4-epoxybutyl and 4,5-epoxypentyl β-glycosides of D-glucose, cellobiose and laminaribiose have been prepared. As well, the 4,5-epoxypentyl β-glycosides of cellotriose, laminaritriose and two other trisaccharides have been synthesized. 3,4-Epoxybutyl β-cellobioside has also been prepared with a ^{14}C-label in the cellobiose residue.

Recently, we reported our results on inhibition experiments for some β-glucan hydrolases.[1] The potential inhibitors chosen were epoxyalkyl β-glycosides, based on the observed success of closely related compounds in the inhibition of some cellulases.[2] Here, we would like to present our synthetic efforts towards these glycosides.

The required sugars were D-glucose, cellobiose, laminaribiose, cellotriose, laminaritriose, 3-*O*-β-cellobiosyl-D-glucose and 4-*O*-β-laminaribiosyl-D-glucose, all in peracetylated form. Although D-glucose pentaacetate and cellobiose (4-*O*-β-D-glucopyranosyl-D-glucose) octaacetate are commercially available, laminaribiose (3-*O*-β-D-glucopyranosyl-D-glucose) is not,[3,4] and we decided to survey the methods available for the synthesis of this disaccharide. In our hands, condensation of the bromide (1) with the alcohol (2)[5] in the presence of silver triflate and tetramethylurea gave a poor yield (34%) of the desired laminaribioside (3), and longer reaction times produced increasing amounts of the diol (4). However, when the reaction was repeated with mercury(II) cyanide/mercury(II) bromide[6] in acetonitrile in the presence of molecular sieves, a 59% yield of the laminaribioside (3) was obtained. Also attempted were the couplings of β-D-glucose pentaacetate with the alcohol (2) and with the alcohol (5), both in the presence of trimethylsilyl triflate.[7] The former yielded the laminaribioside (3) in a disappointing 41% yield, while the latter appeared

[1] Høj, P. B., Rodriguez, E. B., Stick, R. V., and Stone, B. A., *J. Biol. Chem.*, 1989, **264**, 4939.
[2] Legler, G., and Bause, E., *Carbohydr. Res.*, 1973, **28**, 45.
[3] Barry, V. C., and McCormick, J. E., *Methods Carbohydr. Chem.*, 1962, **1**, 328.
[4] Whelan, W. J., *Methods Carbohydr. Chem.*, 1962, **1**, 330.
[5] Takeo, K., *Carbohydr. Res.*, 1979, **77**, 245.
[6] Paulsen, H., *Angew. Chem., Int. Ed. Engl.*, 1982, **21**, 155.
[7] Paulsen, H., and Paal, M., *Carbohydr. Res.*, 1984, **135**, 53.

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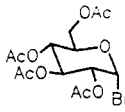
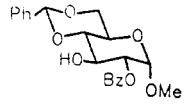
to give a mixture of α- and β-laminaribiose octaacetate, together with the silyl ether (6). Therefore, in light of these various synthetic observations, the laminaribioside (3) was prepared through the 'mercury(II)' route, and transformed easily into α-laminaribiose octaacetate.


(1)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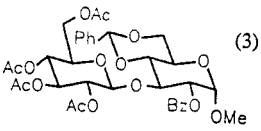
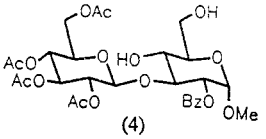

(3)

Bz = PhCO
Bn = $PhCH_2$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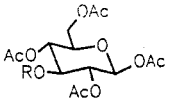
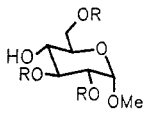
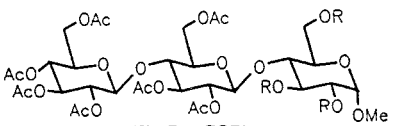
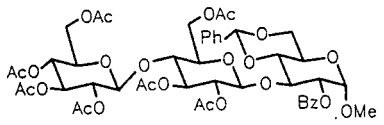
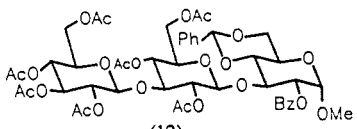

(5) R = H
(6) R = $SiMe_3$

For the synthesis of the four trisaccharides, the general approach utilized modified Koenigs–Knorr procedures whereby suitably protected monosaccharides were coupled with either hepta-*O*-acetyl-α-cellobiosyl bromide or hepta-*O*-acetyl-α-laminaribiosyl bromide. Thus, condensation of the cellobiosyl bromide with the alcohol (7) in the presence of silver triflate and molecular sieves, but without the addition of the usual tetramethylurea, yielded the glycoside (8) in 47% yield. When the alcohol (7) was replaced by the more reactive[8] alcohol (9),[9] the glycoside (10) was obtained in 74% yield. Condensation of the cellobiosyl bromide with the alcohol (2) in acetonitrile by using the 'mercury(II)' route yielded the glycoside (11) in reasonable yield. No improvement was observed by using silver triflate- and trimethylsilyl triflate-mediated procedures. The laminaritrioside (12) was similarly prepared by using the 'mercury(II)' route on the laminaribiosyl bromide and the alcohol (2). Applying the silver triflate/molecular sieves combination to the laminaribiosyl bromide and the alcohol (9) gave the glycoside (13) in good yield. Standard procedures converted the glycosides (8) and (11)–(13) into the corresponding peracetates.


(7) R = COPh
(9) R = CH_2Ph


(8) R = COPh
(10) R = CH_2Ph


(11)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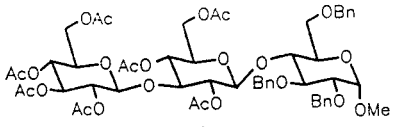
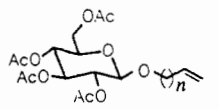

(13)

The stage was now set for the synthesis of the epoxyalkyl β-glycosides themselves, and each of the peracetylated sugars was converted into the corresponding glycosyl bromide by treatment with hydrogen bromide in glacial

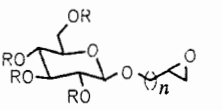
[8] Sinaÿ, P., *Pure Appl. Chem.*, 1978, **50**, 1437.
[9] Garegg, P. J., and Hultberg, H., *Carbohydr. Res.*, 1981, **93**, C10.

acetic acid. By using D-glucose as the model substrate, treatment of the bromide (1) with allyl alcohol in the presence of mercury(II) oxide/mercury(II) bromide[10] gave the prop-2-enyl β-D-glucoside (14), and subsequent epoxidation with *meta*-chloroperbenzoic acid and deacetylation with sodium methoxide in methanol gave 2,3-epoxypropyl β-D-glucopyranoside (15).[2] The synthesis of 3,4-epoxybutyl β-D-glucopyranoside (16) was uneventful and followed the general pathway, but synthesis of the pent-4-enyl β-D-glucopyranoside (17) was not successful when employing the mercury(II) promoters, possibly due to oxymercuration of the pent-4-en-1-ol. However, the use of silver carbonate as the promoter gave the β-D-glucoside (17) in moderate yield, and transformation into 4,5-epoxypentyl β-D-glucopyranoside (18) followed easily. Similar sequences then allowed the synthesis of the epoxyalkyl β-glycosides (19)–(21) and (22)–(24) from the cellobiosyl and laminaribiosyl bromides, respectively.

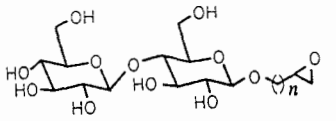
For the synthesis of the epoxyalkyl trisaccharide glycosides, early results from our own work[1] and others[2] had indicated that the 4,5-epoxypentyl glycoside would be the best inhibitor. Therefore, following the normal sequence, only the epoxypentyl glycosides (25)–(28) were prepared.



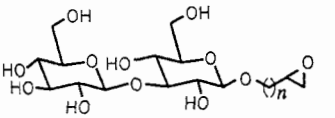
(14) $n=1$
(17) $n=3$



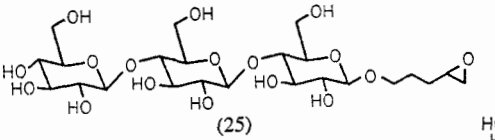
	R	n
(15)	H	1
(16)	H	2
(18)	H	3
(29)	Ac	1



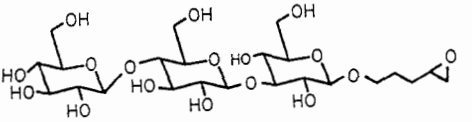
(19) $n=1$
(20) $n=2$
(21) $n=3$



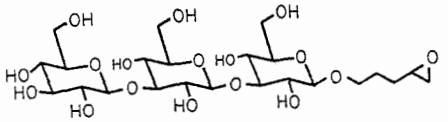
(22) $n=1$
(23) $n=2$
(24) $n=3$



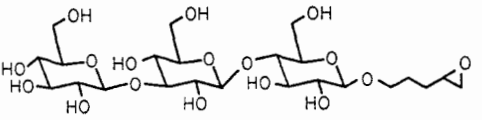
(25)



(26)



(27)

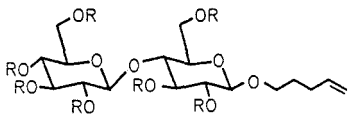


(28)

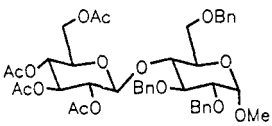
During the course of this work it became evident (^{13}C n.m.r.) that the epoxides produced from oxidation of the various alkenyl glycosides were mixtures (approximately 1 : 1) of diastereoisomers and, not surprisingly, inseparable by the normal chromatographic techniques. Also, the final step in the above syntheses, namely the deacetylation employing sodium methoxide in methanol followed by neutralization with an ion-exchange resin (H^+ form), naturally casts doubts on the integrity of the epoxide in the product. As well, the resulting epoxyalkyl β-glycosides are generally obtained as syrups

[10] Schroeder, L. R., and Green, J. W., *J. Chem. Soc. C*, 1966, 530.

or foams, and normally used without purification. Although some attention has been given to this problem of epoxide integrity and purity,[2,11] we decided to utilize ^{13}C n.m.r. spectroscopy to monitor the products of deacetylation. Thus, and typically throughout this work, compound (29) showed resonances for the epoxide carbons at δ 44·1, and 50·3 and 50·6, whereas the product of deacetylation, namely compound (15), showed corresponding resonances only at δ 45·1, and 51·4 and 51·7. As a further check, the pent-4-enyl cellobioside (30) was first deacetylated to give (31), and subsequent oxidation provided the 4,5-epoxypentyl cellobioside (21), identical to the material produced by the normal oxidation–deacetylation sequence.



(30) R = Ac
(31) R = H



(32)

Two other experiments were performed to test the stability of these epoxyalkyl glycosides under storage, and under the conditions of incubation with an enzyme. Thus, in the first experiment, an amount of 3,4-epoxybutyl β-cellobioside (20) was prepared and kept as the dry powder at 0°. No change was detected by ^{13}C n.m.r. spectroscopy over a period of eight weeks. In the second experiment, the 4,5-epoxypentyl glycoside (26) was incubated with *Rhizopus arrhizus* (1→3)-β-D-glucan-3(4)-glucano-hydrolase[1] and the mixture monitored by ^1H n.m.r. (300 MHz) spectroscopy by using Hore's pulse sequence,[12,13] which allowed the suppression of the water signal. After 18 h, there was no apparent decomposition of (26), as adjudged by the relative intensity of the epoxide protons to that of the internal sodium acetate buffer.

At this stage of our work, the early inhibition experiments had shown that 3,4-epoxybutyl β-cellobioside (20) was an excellent inhibitor of a variety of β-glucanases.[1] It therefore seemed appropriate to complete our synthetic work by preparing a radiolabelled version of (20) for use in mapping the active sites of the enzymes, and we chose to start with D-[U-^{14}C]glucose. Acetylation and treatment with hydrogen bromide provided (1), and condensation with unlabelled (9) provided the β-cellobioside (32). Standard transformations then gave hepta-*O*-acetyl-α-cellobiosyl bromide that was routinely transformed into the heptaacetate of labelled (20), in an overall yield of 12% from the D-glucose.

Experimental

'Normal workup' means extraction into an organic solvent, sequential washing of the organic extract with saturated aqueous sodium bicarbonate solution and water, drying ($MgSO_4$), and concentration on a rotary evaporator at water aspirator pressure and bath temperature of 45°. All solvents were purified by distillation and, where indicated, were dried by standard procedures. Petrol is a mixture of hydrocarbons boiling at 64–68°.

Melting points were determined on a Kofler hot stage apparatus and are uncorrected. Optical rotations were measured with a Perkin Elmer 141 polarimeter for solutions in chloroform (1·0 g per 100 ml) at room temperature, unless otherwise stated.

[11] Ross, W. C. J., *J. Chem. Soc.*, 1950, 2257.
[12] Hore, P. J., *J. Magn. Reson.*, 1983, **54**, 539.
[13] Hore, P. J., *J. Magn. Reson.*, 1983, **55**, 283.

^{1}H n.m.r. spectra were recorded with a Hitachi Perkin Elmer R-24B (60 MHz) spectrometer, a Bruker WP-80 (80 MHz) spectrometer, a Bruker HX-90 (90 MHz) spectrometer, or a Bruker AM 300 (300 MHz) spectrometer. Due to the complexity of the spectra, the assignment of chemical shifts was confined to the protons (and carbons, below) with resolved and identifiably useful signals. Chemical shifts are quoted relative to tetramethylsilane ($SiMe_4$) as internal standard (δ 0·0) for solutions in (D)chloroform, and to 2,2-dimethyl-2-silapentane-5-sulfonate (dss) for solutions in D_2O, unless otherwise stated.

^{13}C n.m.r. spectra were recorded with a Bruker WP-80 (20·1 MHz) spectrometer or a Bruker AM 300 (75·5 MHz) spectrometer. Chemical shifts are quoted relative to $SiMe_4$ (δ 0·0) or $CDCl_3$ (middle peak at δ 79·0) as internal standards for solutions in (D)chloroform, and to dss or MeOH (δ 49·0) for solutions in D_2O, unless otherwise stated.

'Flash chromatography' was performed on short columns (10–15 cm) of silica gel (Fluka, cat. No. 60739, <230 mesh) under positive pressures (30–40 psi). 'Rapid silica chromatography' refers to elution through silica gel (Fluka 60739) packed in a sintered glass funnel and application of vacuum to the collection flask. Thin-layer chromatography (t.l.c.) was performed on silica gel 60 F 254, pre-coated aluminium sheets (Merck Art. 5554); detection was effected by spraying with 10% sulfuric acid in ethanol, or 10% ammonium molybdate in 10% sulfuric acid in water, followed by heating.

'Sep-pak' refers to a pre-packed plug of silica gel in a polyethylene cartridge, used for rapid sample preparation (Waters Associates part No. 51900).

Mass spectra (in subsequent papers) were recorded on a Hewlett–Packard 5986 spectrometer. Microanalyses were performed by M.H.W. Laboratories, Phoenix, Arizona, U.S.A.

Methyl 2-O-Benzoyl-4,6-O-benzylidene-3-O-(tetra-O-acetyl-β-D-glucopyranosyl)-α-D-glucoside (3)

(i) To a solution of the alcohol (2)[14] (5·90 g, 15·3 mmol) in dry acetonitrile (50 ml) were added sequentially powdered molecular sieve 4A (10 g), mercury(II) bromide (6·92 g, 19·2 mmol), mercury(II) cyanide (4·85 g, 19·2 mmol) and a solution of the bromide (1) (9·45 g, 23·0 mmol) in dry acetonitrile (50 ml). The mixture was stirred (room temperature) with the rigorous exclusion of light and moisture and, after 2 h, t.l.c. (EtOAc/petrol, 3 : 2) revealed the absence of the bromide. The mixture was diluted with dichloromethane, filtered (Celite) and subjected to normal workup, including an aqueous potassium chloride wash. The syrupy residue was crystallized from ethanol and the mother liquor subjected to flash chromatography (EtOAc/petrol, 3 : 2) to yield the glycoside (3) (6·5 g, 59%), m.p. 187–189° (EtOH/$CHCl_3$) (lit.[5] 185–186°), $[\alpha]_D$ +34·8° (lit.[5] +35·3°).

(ii) To a solution of penta-*O*-acetyl-β-D-glucopyranose (878 mg, 2·2 mmol) and the alcohol (2) (965 mg, 2·5 mmol) in dry dichloromethane (13 ml) was added molecular sieve 4A (2·5 g). The mixture was stirred for 1 h under argon at room temperature, after which trimethylsilyl triflate (0·05 ml) was added. The mixture was stirred for another 6 h, neutralized with triethylamine, filtered (Celite) and then concentrated to a syrup from which the glycoside (3) (428 mg, 26%) crystallized upon the addition of ethanol. Flash chromatography (EtOAc/petrol, 1 : 2) of the mother liquor yielded additional product (238 mg, 15%).

Attempted Synthesis of Octa-O-acetyl-β-laminaribiose by Using the Alcohol (5)

Treatment of the alcohol (5)[15,16] (195 mg, 0·56 mmol) with penta-*O*-acetyl-β-D-glucopyranose (195 mg, 0·5 mmol) in the presence of $Me_3SiO_3SCF_3$ (0·04 ml) overnight, as described above, followed by flash chromatography (EtOAc/petrol, 1 : 1), gave a syrup (66 mg) that appeared [^{1}H n.m.r. (60 MHz)] to be the silyl ether (6). Another less mobile syrup (85 mg) was eluted that, according to the ^{1}H n.m.r. (60 MHz) spectrum, appeared to be a mixture of α- and β-laminaribiose octaacetates (δ 6·05, $J_{1,2}$ 3·5 Hz, H 1α; 5·50, $J_{1,2}$ 7·0 Hz, H 1β).

[14] Hönig, H., and Weidmann, H., *Carbohydr. Res.*, 1975, **39**, 374.
[15] Takeo, K., Nakaji, T., and Shinmitsu, K., *Carbohydr. Res.*, 1984, **133**, 275.
[16] Ogawa, T., and Nakabayashi, S., *Carbohydr. Res.*, 1981, **93**, C1.

Octa-O-acetyl-α-laminaribiose

A suspension of the glycoside (3) (870 mg, 1·2 mmol) in dry methanol (8 ml) was treated with methanolic sodium methoxide (2 ml of 0·1 M), the mixture stirred (room temperature) for 1 h, diluted with water, neutralized (Amberlite IR-120, H^+), filtered and the filtrate evaporated to give a white solid that was dissolved in 60% aqueous acetic acid (8 ml) and heated (100°) for 20 min. The solvents were evaporated and the last traces of water were removed by repeated codistillation with toluene to give a white solid that on conventional acetylation with 1 : 1 acetic anhydride/pyridine (8 ml) yielded another white solid. A solution of the solid in acetic anhydride (4 ml) was cooled in an ice-bath and 4% sulfuric acid in acetic anhydride (4 ml) added dropwise. The mixture was stirred (room temperature) for 4 h, then poured into ice-water that contained solid sodium bicarbonate and the product extracted (4×) with dichloromethane. The extract was washed with saturated aqueous sodium chloride solution, dried ($MgSO_4$), and concentrated until cloudy, then petrol was added until crystallization occurred. Filtration and drying afforded the octaacetate as white, fine needles (730 mg, 88%), m.p. 78–90° [lit.[5] 77–78° (EtOH)], $[\alpha]_D$ +18·0° (*c*, 2·44) (lit.[5] +20·5°).

Methyl O-(Tetra-O-acetyl-β-D-glucopyranosyl)-(1→4)-O-(tri-O-acetyl-β-D-glucopyranosyl)-(1→4)-tri-O-benzoyl-α-D-gluycopyranoside (8)

A solution of the cellobiosyl bromide (5·0 g, 7·2 mmol) in dry 1,2-dichloroethane (30 ml) was added dropwise over 30 min, with rigorous exclusion of light and moisture, to a stirred mixture (−30°) of the alcohol (7) (2·4 g, 4·7 mmol), silver triflate (2·2 g, 8·5 mmol) and powdered molecular sieve 4A (6 g) in dry 1,2-dichloroethane (20 ml). The resulting mixture was stirred (0°) for 1 h, allowed to warm to room temperature, and then stirred for another 5·5 h. Filtration (Celite) and normal workup, followed by flash chromatography (EtOAc/petrol, 1 : 1), afforded the *glycoside* (8) (2·5 g, 47%), m.p. 122–123° (EtOH), $[\alpha]_D$ +45·5° (Found: C, 57·6; H, 5·3. $C_{54}H_{60}O_{26}$ requires C, 57·6; H, 5·4%). ^{13}C n.m.r. (75·5 MHz, $CDCl_3$) δ 20·2–20·5, 7C, Me; 55·2, OMe; 75·5, 76·5, C4,4′; 96·5, C1; 99·9, 100·4, C1′,1″; 164·8–165·8, 3C, Ph**C**=O; 168·7–170·2, 7C, Me**C**=O.

Methyl O-(Tetra-O-acetyl-β-D-glucopyranosyl)-(1→4)-O-(tri-O-acetyl-β-D-glucopyranosyl)-(1→4)-tri-O-benzyl-α-D-glucopyranoside (10)

The cellobiosyl bromide and the alcohol (9), in the presence of silver triflate and molecular sieve, gave the *glycoside* (10) as an amorphous solid in 74% yield, $[\alpha]_D$ −4·6° (Found: C, 59·7; H, 6·2. $C_{54}H_{66}O_{23}$ requires C, 59·9; H, 6·1%). ^{13}C n.m.r. (75·5 MHz, $CDCl_3$) δ 20·4–20·6, 7C, Me; 55·2, OMe; 73·2, 73·6, 74·7, 3C, **C**H_2Ph; 78·6, 79·8, C4,4′; 98·2, C1; 99·7, 100·7, C1′, 1″; 168·9–170·3, 7C, C=O.

O-(Tetra-O-acetyl-β-D-glucopyranosyl)-(1→4)-O-(tri-O-acetyl-β-D-glucopyranosyl)-(1→4)-tetra-O-acetyl-α-D-glucopyranose

Sequential treatment of the glycoside (8) with sodium methoxide and acetic anhydride/sulfuric acid gave crystalline α-cellotriose undecaacetate in 76% yield, m.p. 217–221° (EtOH/diisopropyl ether) [lit.[17] 223–224° ($CHCl_3$/Et_2O); lit.[18] 221–222° (EtOH)], $[\alpha]_D$ +20·2° [lit.[17] +22·6° (*c*, 5); lit.[18] +23·0° (*c*, 4·4)].

Methyl O-(Tetra-O-acetyl-β-D-glucopyranosyl)-(1→4)-O-(tri-O-acetyl-β-D-glucopyranosyl)-(1→3)-2-O-benzoyl-4,6-O-benzylidene-α-D-glucoside (11)

The cellobiosyl bromide and the alcohol (2), in the presence of mercury(II) cyanide and mercury(II) bromide, gave the *glycoside* (11) as an amorphous solid in 50% yield, $[\alpha]_D$ +22·9° (Found: C, 56·0; H, 5·7. $C_{47}H_{56}O_{24}$ requires C, 56·2; H, 5·6%). ^{13}C n.m.r. (75·5 MHz, $CDCl_3$) δ 19·8–20·7, 7C, Me; 55·3, OMe; 76·2, C4′; 79·4, C3; 97·5, C1; 100·4, 100·7, C1′,1″; 101·3, Ph**C**H; 165·7, Ph**C**=O; 168·9–170·3, 7C, Me**C**=O.

[17] Dickey, E. E., and Wolfrom, M. L., *J. Am. Chem. Soc.*, 1949, **71**, 825.
[18] Takeo, K., Yasato, T., and Kuge, T., *Carbohydr. Res.*, 1981, **93**, 148.

O-(Tetra-O-acetyl-β-D-glucopyranosyl)-(1→4)-O-(tri-O-acetyl-β-D-glucopyranosyl)-(1→3)-tetra-O-acetyl-α-D-glucopyranose

Sequential treatment of the glycoside (11) with sodium methoxide, aqueous acetic acid, acetic anhydride/pyridine and acetic anhydride/sulfuric acid yielded the crystalline *peracetylated trisaccharide* in 61% yield, m.p. 119–120° ($EtOH/Et_2O$), $[\alpha]_D$ +8·2° (Found: C, 49·3; H, 5·8. $C_{40}H_{54}O_{27}$ requires C, 49·7; H, 5·6%). ^{13}C n.m.r. (75·5 MHz, $CDCl_3$) δ 20·2–20·7, 11C, Me; 75·9, 76·1, C3,4′; 89·0, C1; 100·6, C1′,1″; 168·5–170·6, 11C, C=O.

Methyl O-(Tetra-O-acetyl-β-D-glucopyranosyl)-(1→3)-O-(tri-O-acetyl-β-D-glucopyranosyl)-(1→3)-2-O-benzoyl-4,6-O-benzylidene-α-D-glucoside (12)

The laminaribiosyl bromide and the alcohol (2), in the presence of mercury(II) cyanide and mercury(II) bromide, gave the *glycoside* (12) as an amorphous solid in 39% yield, $[\alpha]_D$ +10·0° (Found: C, 56·4; H, 5·9. $C_{47}H_{56}O_{24}$ requires C, 56·2; H, 5·6%). ^{13}C n.m.r. (75·5 MHz, $SiMe_4$) δ 20·1–20·7, 7C, Me; 55·4, OMe; 78·7, 79·0, C3,3′; 97·6, C1; 100·7, C1′,1″; 101·2, Ph**C**H; 168·7, Ph**C**=O; 169·1–170·9, 7C, Me**C**=O.

O-(Tetra-O-acetyl-β-D-glucopyranosyl)-(1→3)-O-(tri-O-acetyl-β-D-glucopyranosyl)-(1→3)-tetra-O-acetyl-α-D-glucopyranose

The glycoside (12) was treated sequentially with sodium methoxide, aqueous acetic acid and sulfuric acid in acetic anhydride to yield *α-laminaritriose undecaacetate* as an amorphous solid in 53% yield, $[\alpha]_D$ −8·5° (Found: C, 49·7; H, 5·7. $C_{40}H_{54}O_{27}$ requires C, 49·7; H, 5·6%). ^{13}C n.m.r. (75·5 MHz, $SiMe_4$) δ 20·4–21·0, 11C, Me; 75·8, 78·9, C3,3′; 89·2, C1; 100·9, 101·0, C1′, 1″; 168·6–170·8, 11C, C=O.

Methyl O-(Tetra-O-acetyl-β-D-glucopyranosyl)-(1→3)-O-(tri-O-acetyl-β-D-glucopyranosyl)-(1→4)-tri-O-benzyl-α-D-glucopyranoside (13)

Condensation of the laminaribiosyl bromide with the alcohol (9) by utilizing silver triflate gave the *glycoside* (13) as an amorphous solid in 63% yield, $[\alpha]_D$ −5·7° (Found: C, 59·9; H, 6·1. $C_{54}H_{66}O_{23}$ requires C, 59·9; H, 6·1%). ^{13}C n.m.r. (75·5 MHz, $SiMe_4$) δ 20·3–20·9, 7C, Me; 55·4, OMe; 73·4, 73·8, 75·1, 3C, Ph**C**H_2; 78·9, 79·8, C3′,4; 98·4, C1; 100·0, 100·1, C1′,1″; 168·4–170·7, 7C, C=O.

O-(Tetra-O-acetyl-β-D-glucopyranosyl)-(1→3)-O-(tri-O-acetyl-β-D-glucopyranosyl)-(1→4)-tetra-O-acetyl-α-D-glucopyranose

A solution of the glycoside (13) (1·7 g) in acetic acid (17 ml) was hydrogenated (room temperature) in the presence of 10% Pd/C (1·1 g) under pressure (40 psi) overnight. The reaction mixture was filtered (Celite), the solids washed thoroughly with methanol and the filtrate concentrated. The residue was then treated with acetic anhydride/sulfuric acid to give the crystalline *peracetylated trisaccharide* (1·30 g, 85%), m.p. 140–142° (EtOH/diisopropyl ether), $[\alpha]_D$ +9·1° (Found: C, 49·6; H, 5·8. $C_{40}H_{54}O_{27}$ requires C, 49·7; H, 5·6%). ^{13}C n.m.r. (75·5 MHz, $SiMe_4$) δ 20·3–20·9, 11C, Me; 75·6, 78·6, C3′,4; 88·9, C1; 100·8, 100·9, C1′,1″; 168·5–170·5, 11C, C=O.

Prop-2-enyl Tetra-O-acetyl-β-D-glucopyranoside (14)

A suspension of yellow mercury(II) oxide (440 mg, 2·0 mmol), mercury(II) bromide (40 mg, 0·1 mmol) and anhydrous calcium sulfate (1·5 g) in dry allyl alcohol (7 ml) and dry dichloromethane (7 ml) was stirred with the exclusion of moisture for 30 min at room temperature, after which tetra-*O*-acetyl-α-D-glucopyranosyl bromide (1) (1·0 g, 2·4 mmol) was added. The mixture was stirred overnight and then filtered (Celite). Evaporation of the filtrate gave a viscous oil which was dissolved in a minimum amount of dichloromethane to precipitate the inorganic salts. Removal of the salts by filtration, evaporation of the filtrate and flash chromatography (EtOAc/petrol, 3 : 2) of the residue, followed by recrystallization,

gave prop-2-enyl tetra-*O*-acetyl-β-D-glucopyranoside as white needles (755 mg, 80%), m.p. 87–88° (MeOH) [lit.[2] 85° (EtOH); lit.[19] 82–84°], $[\alpha]_D$ −20·8° (lit.[2] −25·5°; lit.[19] −20°).

2,3-Epoxypropyl Tetra-O-acetyl-β-D-glucopyranoside (29)

The prop-2-enyl β-D-glucoside (14) (388 mg, 1·0 mmol) was dissolved in dichloromethane (20 ml), *m*-chloroperbenzoic acid (345 mg, 2·0 mmol) and 2,6-di-t-butyl-4-methylphenol (2·5 mg) added,[20] and the mixture heated gently at reflux until the test for double bonds [reaction mixture spotted on t.l.c. plate, developed in EtOAc/petrol (3 : 2) and sprayed with 0·5% $KMnO_4$ in 5% Na_2CO_3] was negative (4 h). The reaction mixture was then cooled (0°) and filtered. The filtrate was washed successively with 5% sodium bicarbonate solution, water and saturated sodium chloride solution and dried ($MgSO_4$). Concentration, followed by recrystallization of the residue, gave the epoxide (29) as white needles (257 mg, 64%), m.p. 115–117° (EtOH/Et_2O) [lit.[2] 106°; lit.[21] 115–117° (EtOH)], $[\alpha]_D$ −18·0° (*c*, 0·05) (lit.[21] −18·9°). ^1H n.m.r. (90 MHz) δ 1·99, 2·02, 2·06, 2·08, 4s, 12H, Me; 2·47–2·84, m, CH_2, epoxide; 3·02–3·23, m, CH, epoxide. ^{13}C n.m.r. (20·1 MHz, $SiMe_4$) δ 20·6, 4C, Me; 44·1, CH_2, epoxide; 50·3, 50·6, CH, epoxide; 62·0, C6; 68·6, C4; 69·3, 70·7, OCH_2; 71·4, 72·0, 72·9, C2,3,5; 100·6, 101·1, C1; 169·6, 170·3, 170·8, 4C, C=O.

2,3-Epoxypropyl β-D-Glucopyranoside (15)

A stirred suspension of the epoxide (29) (100 mg, 0·25 mmol) in dry methanol (2 ml) was treated with methanolic sodium methoxide (0·1 ml of 1 M). After complete dissolution of the epoxide, the solution was stirred for a further 30 min, then neutralized (Amberlite IRC-50, H^+), filtered and the filtrate concentrated to dryness to give the epoxide (15) as a syrup (79 mg) which was homogenous by t.l.c. (Me_2CO/MeOH, 2 : 1). ^1H n.m.r. (80 MHz, D_2O, dss) δ 2·75–3·12, m, CH_2, epoxide. ^{13}C n.m.r. (20·1 MHz, D_2O, MeOH) δ 45·1, CH_2, epoxide; 51·4, 51·7, CH, epoxide; 60·8, C6; 69·7, C4; 70·3, 70·5, OCH_2; 73·2, 75·8, 76·0, C2,3,5; 102·5, 102·8, C1.

But-3-enyl Tetra-O-acetyl-β-D-glucopyranoside

Yield 72%,* m.p. 77–78° (diisopropyl ether/petrol) [lit.[2] 70° (EtOH)], $[\alpha]_D$ −19·7° (lit.[2] −23·2°).

3,4-Epoxybutyl Tetra-O-acetyl-β-D-glucopyranoside

Yield 90%, m.p. 62–65° [lit.[2] 65° (EtOH/Et_2O)], $[\alpha]_D$ −12·5°. ^1H n.m.r. (60 MHz) δ 1·97, 2·05, 2s, 12H, Me; 2·30–2·63, m, CH_2, epoxide; 2·70–3·10, m, CH, epoxide; 4·45, d, $J_{1,2}$ 7·0 Hz, H1. ^{13}C n.m.r. (20·1 MHz, $SiMe_4$) δ 20·6, 4C, Me; 32·7, $OCH_2\mathbf{C}H_2$; 46·9, 47·2, CH_2, epoxide; 49·5, 49·6, CH, epoxide; 62·1, C6; 66·8, 67·0, $O\mathbf{C}H_2CH_2$; 68·6, C4; 71·4, 72·0, 73·0, C2,3,5; 100·9, 101·1, C1; 169·6, 170·4, 170·8, 4C, C=O.

3,4-Epoxybutyl β-D-Glucopyranoside (16)

Deacetylation of the epoxybutyl β-D-glucoside above (127 mg) gave the epoxide (16) as a syrup (97 mg). ^1H n.m.r. (80 MHz, D_2O, dss) δ 2·63–2·84, 2·88–3·03, 2m, CH_2, epoxide; 4·44, d, $J_{1,2}$ 7·4 Hz, H1. ^{13}C n.m.r. (20·1 MHz, D_2O, MeOH) δ 32·0, 32·1, $OCH_2\mathbf{C}H_2$; 48·1, CH_2, epoxide; 51·6, CH, epoxide; 61·0, C6; 67·2, 67·4, $O\mathbf{C}H_2CH_2$; 69·9, C4; 73·3, 75·9, 76·1, C2,3,5; 102·4, 102·6, C1.

* The value quoted here simply represents the percentage yield for the condensation of the unsaturated alcohol with the glycosyl bromide, in the presence of either mercury(II) salts or silver carbonate.

[19] Bessell, E. M., and Westwood, J. H., *Carbohydr. Res.*, 1972, **25**, 11.
[20] Kishi, Y., Aratani, M., Tanino, H., Fukuyama, T., and Goto, T., *J. Chem. Soc., Chem. Commun.*, 1972, 64.
[21] Barnett, J. E. G., and Ralph, A., *Carbohydr. Res.*, 1971, **17**, 231.

Pent-4-enyl Tetra-O-acetyl-β-D-glucopyranoside (17)

A suspension of silver carbonate (3·3 g, 12 mmol), iodine (a crystal) and anhydrous calcium sulfate (4 g) in pent-4-en-1-ol (3·0 ml, 30 mmol) and dry dichloromethane (20 ml) was stirred with the exclusion of light and moisture for 30 min at room temperature, after which the α-D-glucosyl bromide (1) (2·5 g, 6 mmol) was added. The mixture was stirred overnight, filtered (Celite), the filtrate evaporated to dryness and the residue subjected to flash chromatography (EtOAc/petrol, 3 : 2) to give the β-D-glucoside (17) as a white crystalline solid (1·6 g, 64%), m.p. 45–46° (diisopropyl ether) [lit.[2] 42° (EtOH)], $[\alpha]_D$ −19·4° (lit.[2] −22·5°).

4,5-Epoxypentyl Tetra-O-acetyl-β-D-glucopyranoside

Yield 96%, m.p. 55–58° (diisopropyl ether) [lit.[2] 55° (EtOH)], $[\alpha]_D$ −20·3°. ^{1}H n.m.r. (60 MHz) δ 1·97, 2·03, 2s, 12H, Me; 2·30–2·53, m, CH_2, epoxide; 2·53–3·20, m, CH, epoxide; 4·43, d, $J_{1,2}$ 7·0 Hz, H1. ^{13}C n.m.r. (75·5 MHz, $SiMe_4$) δ 20·58, 20·61, 20·7, 4C, Me; 25·8, 26·1, $OCH_2CH_2\mathbf{C}H_2$; 28·8, 28·9, $OCH_2\mathbf{C}H_2$; 46·88, 46·93, CH_2, epoxide; 51·78, 51·82, CH, epoxide; 62·0, C6; 68·4, C4; 69·3, 69·5, $O\mathbf{C}H_2CH_2$; 71·3, 71·7, 72·8, C2,3,5; 100·7, C1; 169·3, 169·4, 170·2, 170·6, 4C, C=O.

4,5-Epoxypentyl β-D-Glucopyranoside (18)

Deacetylation of the epoxypentyl β-D-glucoside (65 mg) gave the epoxide (18) as a syrup (46 mg). ^{1}H n.m.r. (300 MHz, D_2O, dss) δ 2·66–2·72, 2·89–2·95, 2m, CH_2, epoxide; 3·15–3·21, m, CH, epoxide; 4·46, d, $J_{1,2}$ 7·9 Hz, H1. ^{13}C n.m.r. (75·5 MHz, D_2O, MeOH) δ 25·5, $OCH_2CH_2\mathbf{C}H_2$; 28·3, $OCH_2\mathbf{C}H_2$; 48·4, CH_2, epoxide; 53·9, CH, epoxide; 60·9, C6; 69·8, C4; 70·0, $O\mathbf{C}H_2CH_2$; 73·3, 75·9, 76·1, C2,3,5; 102·3, C1.

Prop-2-enyl Hepta-O-acetyl-β-cellobioside

Yield 69%, m.p. 185–187° (MeOH) [lit.[2] 179° (EtOH); lit.[22] 186–188° (EtOH)], $[\alpha]_D$ −24·8° [lit.[2] −21·0°; lit.[22] −23° (*c*, 4·8)].

2,3-Epoxypropyl Hepta-O-acetyl-β-cellobioside

Yield 51%, m.p. 190–192° ($EtOH/Et_2O$) (lit.[2] 170°),* $[\alpha]_D$ −19·2°. ^{1}H n.m.r. (90 MHz) δ 1·97, 1·99, 2·02, 2·03, 2·07, 2·11, 6s, 21H, Me; 2·46–2·81, m, CH_2, epoxide; 3·03–3·21, m, CH, epoxide. ^{13}C n.m.r. (20·1 MHz, $SiMe_4$) δ 20·6, 20·8, 22·1, 7C, Me; 44·2, CH_2, epoxide; 50·3, 50·6, CH, epoxide; 69·6, 70·6, OCH_2; 78·8, C4; 100·5, 100·9, C1,1′; 169·2, 169·5, 169·8, 170·0, 170·4, 170·5, 170·6, 7C, C=O.

2,3-Epoxypropyl β-Cellobioside (19)

Deacetylation of the above epoxide (200 mg) gave the epoxide (19) (134 mg) as a foam, essentially pure by t.l.c. (Me_2CO/MeOH, 2 : 1) and ^{13}C n.m.r. (20·1 MHz) spectroscopic analysis. ^{1}H n.m.r. (80 MHz, D_2O, dss) δ 2·66–3·03, m, CH_2, epoxide. ^{13}C n.m.r. (20·1 MHz, D_2O, MeOH) δ 45·3, CH_2, epoxide; 51·7, 51·8, CH, epoxide; 70·6, OCH_2; 78·9, C4; 102·5, 102·8, C1,1′.

3,4-Epoxybutyl Hepta-O-acetyl-β-cellobioside

Yield 39% from cellobiose octaacetate, m.p. 192–194° [lit.[2] 178° ($EtOH/Et_2O$)], $[\alpha]_D$ −21·5°. ^{1}H n.m.r. (60 MHz) δ 2·00, 2·05, 2·10, 3s, 21H, Me; 2·30–3·15, m, 3H, epoxide. ^{13}C n.m.r.

* The difference in the melting point with that reported in the literature may be due to varying proportions of the diastereoisomers.

[22] Chernyak, A. Ya., Antonov, K. V., Kochetkov, N. K., Padyukov, L. N., and Tsvetkova, N. V., *Carbohydr. Res.*, 1985, **141**, 199.

(20·1 MHz, $SiMe_4$) δ 20·6, 20·8, 7C, Me; 32·7, $OCH_2\mathbf{C}H_2$; 46·8, 47·2, CH_2, epoxide; 49·5, CH, epoxide; 66·7, 66·9, $O\mathbf{C}H_2CH_2$; 76·6, C4; 100·7, 100·9, C1,1′; 169·2, 169·4, 169·7, 169·9, 170·4, 170·6, 7C, C=O.

3,4-Epoxybutyl β-Cellobioside (20)

Deacetylation of the above epoxide (150 mg) gave the epoxide (20) as a foam (87 mg). ^{1}H n.m.r. (80 MHz, D_2O, dss) δ 1·51–2·32, m, $OCH_2\mathbf{C}H_2$; 2·62–2·87, 2·87–3·12, 2m, CH_2, epoxide. ^{13}C n.m.r. (20·1 MHz, D_2O, MeOH) δ 31·9, 32·0, $OCH_2\mathbf{C}H_2$; 48·0, CH_2, epoxide; 51·6, CH, epoxide; 67·2, 67·4, $O\mathbf{C}H_2CH_2$; 78·9, C4; 102·2, 102·5, 102·7, C1,1′.

Pent-4-enyl Hepta-O-acetyl-β-cellobioside (30)

Yield 77%, m.p. 159–160° [lit.[2] 154° (EtOH)], $[\alpha]_D$ −25·4°.

4,5-Epoxypentyl Hepta-O-acetyl-β-cellobioside

Yield 68%, m.p. 160–162° [lit.[2] 154° (EtOH/Et_2O)], $[\alpha]_D$ −23·3°. ^{1}H n.m.r. (60 MHz) δ 1·40–1·80, m, $OCH_2C\mathbf{H}_2C\mathbf{H}_2$; 1·95, 2·05, 2·10, 3s, 21H, Me; 2·22–2·50, 2·60–3·17, 2m, 3H, epoxide. ^{13}C n.m.r. (20·1 MHz, $SiMe_4$) δ 20·6, 20·8, 7C, Me; 26·0, 26·1, $OCH_2CH_2\mathbf{C}H_2$; 28·9, $OCH_2\mathbf{C}H_2$; 47·0, CH_2, epoxide; 51·9, CH, epoxide; 69·5, $O\mathbf{C}H_2CH_2$; 76·6, C4; 100·8, 100·9, C1,1′; 169·2, 169·5, 169·7, 170·0, 170·4, 170·5, 170·6, 7C, C=O.

4,5-Epoxypentyl β-Cellobioside (21)

Deacetylation of the above epoxide (143 mg) gave a solid which on recrystallization afforded the epoxide (21) (65 mg, 77%), m.p. 172–174° (MeOH/diisopropyl ether) (lit.[2] 153°), $[\alpha]_D$ −19·1° (*c*, 0·43 in H_2O) (lit.[2] −26·8°). ^{1}H n.m.r. (300 MHz, D_2O, dss) δ 1·47–1·83, 2m, $OCH_2C\mathbf{H}_2C\mathbf{H}_2$; 2·64–2·69, 2·87–2·93, 2m, CH_2, epoxide; 3·11–3·19, m, CH, epoxide. ^{13}C n.m.r. (75·5 MHz, D_2O, MeOH) δ 25·6, $OCH_2CH_2\mathbf{C}H_2$; 28·4, $OCH_2\mathbf{C}H_2$; 48·5, CH_2, epoxide; 54·0, CH, epoxide; 70·1, $O\mathbf{C}H_2CH_2$; 78·9, C4; 102·3, 102·8, C1,1′.

Prop-2-enyl Hepta-O-acetyl-β-laminaribioside

Yield 68%, m.p. 145–146° (EtOH), $[\alpha]_D$ −42·8° (Found: C, 51·4; H, 6·1. $C_{29}H_{40}O_{18}$ requires C, 51·5; H, 6·0%). ^{1}H n.m.r. (60 MHz) δ 2·00, 2·05, 2s, 21H, Me; 5·40–6·20, m, =CH. ^{13}C n.m.r. (20·1 MHz, $SiMe_4$) δ 20·5, 20·7, 20·9, 7C, Me; 69·7, OCH_2; 79·1, C3; 99·7, 101·1, C1,1′; 117·5, $=CH_2$; 133·6, =CH; 169·1, 169·4, 169·6, 170·5, 170·6, 170·8, 7C, C=O.

2,3-Epoxypropyl Hepta-O-acetyl-β-laminaribioside

Yield 88%, m.p. 157–158° (EtOH/Et_2O), $[\alpha]_D$ −42·6° (Found: C, 50·2; H, 5·6. $C_{29}H_{40}O_{19}$ requires C, 50·3; H, 5·8%). ^{1}H n.m.r. (60 MHz) δ 1·95, 2·00, 2·10, 3s, 21H, Me; 2·37–2·87, 2·87–3·27, 2m, 3H, epoxide. ^{13}C n.m.r. (20·1 MHz, $SiMe_4$) δ 20·6, 20·8, 21·0, 7C, Me; 44·0, CH_2, epoxide; 50·3, 50·7, CH, epoxide; 78·7, C3; 100·6, 101·1, 101·2, C1,1′; 169·2, 169·4, 169·6, 170·5, 170·6, 170·9, 7C, C=O.

2,3-Epoxypropyl β-Laminaribioside (22)

Deacetylation of the above epoxide (62 mg) gave the epoxide (22) as a foam (35 mg). ^{1}H n.m.r. (300 MHz, D_2O, dss) δ 2·78–2·83, 2·92–2·98, 2m, CH_2, epoxide; 4·73, d, *J* 7·9 Hz, 1H, anomeric H. ^{13}C n.m.r. (75·5 MHz, D_2O, MeOH) δ 45·1, 45·2, CH_2, epoxide; 51·6, 51·8, CH, epoxide; 70·55, 70·63, OCH_2; 84·6, C3; 102·2, 102·9, C1,1′.

But-3-enyl Hepta-O-acetyl-β-laminaribioside

Yield 69%, m.p. 141–142° (EtOH), $[\alpha]_D$ −43·0° (Found: C, 52·2; H, 6·3. $C_{30}H_{42}O_{18}$ requires C, 52·2; H, 6·1%). ^{1}H n.m.r. (60 MHz) δ 1·98, 2·05, 2·10, 3s, 21H, Me; 5·33–6·10,

m, =CH. ^{13}C n.m.r. (20·1 MHz, $SiMe_4$) δ 20·6, 20·8, 20·9, 7C, Me; 33·9, $OCH_2\mathbf{C}H_2$; 69·1, $O\mathbf{C}H_2CH_2$; 79·1, C3; 100·9, 101·1, C1,1′; 116·8, $=CH_2$; 134·8, =CH; 169·0, 169·5, 169·6, 170·5, 170·6, 170·8, 7C, C=O.

3,4-Epoxybutyl Hepta-O-acetyl-β-laminaribioside

Yield 89%, m.p. 152–153° (EtOH/Et_2O), $[\alpha]_D$ −37·6° (Found: C, 50·8; H, 5·7. $C_{30}H_{42}O_{19}$ requires C, 51·0; H, 6·0%). ^{1}H n.m.r. (60 MHz) δ 1·95, 2·05, 2·10, 3s, 21H, Me; 2·30–2·55, 2·55–3·15, 2m, 3H, epoxide. ^{13}C n.m.r. (20·1 MHz, $SiMe_4$) δ 20·3, 20·6, 20·9, 7C, Me; 32·7, $OCH_2\mathbf{C}H_2$; 46·9, 47·4, CH_2, epoxide; 49·5, 49·7, CH, epoxide; 66·4, 66·6, $O\mathbf{C}H_2CH_2$; 79·0, C3; 101·1, C1,1′; 169·1, 169·4, 170·5, 170·6, 170·9, 7C, C=O.

3,4-Epoxybutyl β-Laminaribioside (23)

Deacetylation of the above epoxide (70 mg) gave 3,4-epoxybutyl β-laminaribioside (23) as a foam (46 mg). ^{1}H n.m.r. (300 MHz, D_2O, dss) δ 2·71–2·75, 2·91–2·96, 2m, CH_2, epoxide; 3·22–3·26, m, CH, epoxide; 4·51, 4·74, 2d, *J* 8·0, 7·9 Hz, H1,1′. ^{13}C n.m.r. (75·5 MHz, D_2O, MeOH) δ 32·0, 32·1, $OCH_2\mathbf{C}H_2$; 48·05, 48·08, CH_2, epoxide; 51·58, 51·63, CH, epoxide; 67·2, 67·5, $O\mathbf{C}H_2CH_2$; 84·56, 84·63, C3; 102·0, 102·3, 102·9, C1,1′.

Pent-4-enyl Hepta-O-acetyl-β-laminaribioside

Yield 86%, m.p. 145–146° (EtOH), $[\alpha]_D$ −47·2° (Found: C, 52·7; H, 6·4. $C_{31}H_{44}O_{18}$ requires C, 52·8; H, 6·3%). ^{1}H n.m.r. (60 MHz) δ 1·90, 1·95, 2·00, 3s, 21H, Me; 5·15–6·05, m, =CH. ^{13}C n.m.r. (20·1 MHz, $SiMe_4$) δ 20·4, 20·5, 20·9, 7C, Me; 28·6, 29·9, $OCH_2\mathbf{C}H_2\mathbf{C}H_2$; 69·0, $O\mathbf{C}H_2CH_2$; 79·1, C3; 100·9, 101·0, C1,1′; 115·2, $=CH_2$; 138·0, =CH; 169·0, 169·5, 170·4, 170·5, 170·9, 7C, C=O.

4,5-Epoxypentyl Hepta-O-acetyl-β-laminaribioside

81%, m.p. 127–129° (EtOH/diisopropyl ether), $[\alpha]_D$ −40·9° (Found: C, 51·7; H, 6·2. $C_{31}H_{44}O_{19}$ requires C, 51·7; H, 6·2%). ^{1}H n.m.r. (60 MHz) δ 1·95, 2·05, 2·10, 3s, 21H, Me; 2·30–2·55, 2·55–3·05, 2m, 3H, epoxide. ^{13}C n.m.r. (20·1 MHz, $SiMe_4$) δ 20·3, 20·5, 20·7, 20·9, 7C, Me; 25·8, 26·1, $OCH_2CH_2\mathbf{C}H_2$; 28·9, $OCH_2\mathbf{C}H_2$; 46·8, CH_2, epoxide; 51·8, CH, epoxide; 69·0, 69·2, $O\mathbf{C}H_2CH_2$; 79·1, C3; 100·8, 101·1, C1,1′; 169·0, 169·4, 170·4, 170·5, 170·8, 7C, C=O.

4,5-Epoxypentyl β-Laminaribioside (24)

Deacetylation of the above epoxide (65 mg) gave 4,5-epoxypentyl β-laminaribioside (24) as a foam (42 mg). ^{1}H n.m.r. (300 MHz, D_2O, dss) δ 1·49–1·85, 2m, $OCH_2C\mathbf{H}_2C\mathbf{H}_2$; 2·67–2·71, 2·89–2·95, 2m, CH_2, epoxide; 3·17–3·19, m, CH, epoxide; 4·49, 4·73, 2d, *J* 8·0, 7·9 Hz, H1,1′. ^{13}C n.m.r. (75·5 MHz, D_2O, MeOH) δ 25·4, $OCH_2CH_2\mathbf{C}H_2$; 28·3, $OCH_2\mathbf{C}H_2$; 48·4, CH_2, epoxide; 53·9, CH, epoxide; 70·0, $O\mathbf{C}H_2CH_2$; 84·7, C3; 102·1, 103·0, C1,1′.

Pent-4-enyl O-(Tetra-O-acetyl-β-D-glucopyranosyl)-(1→4)-O-(tri-O-acetyl-β-D-glucopyranosyl)-(1→4)-tri-O-acetyl-β-D-glucopyranoside

Yield 64%, m.p. 185–186° (EtOH), $[\alpha]_D$ −23·9° (Found: C, 52·2; H, 6·2. $C_{43}H_{60}O_{26}$ requires C, 52·0; H, 6·1%). ^{13}C n.m.r. (75·5 MHz, $SiMe_4$) δ 20·5–20·8, 10C, Me; 28·5, 29·8, $OCH_2CH_2\mathbf{C}H_2$; 69·2, $O\mathbf{C}H_2CH_2$; 76·1, 76·5, C4,4′; 115·1, $=CH_2$; 137·8, =CH; 169·1–170·5, 10C, C=O.

4,5-Epoxypentyl O-(Tetra-O-acetyl-β-D-glucopyranosyl)-(1→4)-O-(tri-O-acetyl-β-D-glucopyranosyl-(1→4)-tri-O-acetyl-β-D-glucopyranoside

Yield 78%, m.p. 181–182° (EtOH), $[\alpha]_D$ −23·6° (Found: C, 51·2; H, 6·0. $C_{43}H_{60}O_{27}$ requires C, 51·2; H, 6·0%). ^{1}H n.m.r. (300 MHz) δ 2·42–2·47, 2·71–2·75, 2m, CH_2, epoxide;

2·86–2·93, m, CH, epoxide. ^{13}C n.m.r. (75·5 MHz, $SiMe_4$) δ 20·5–20·9, 10C, Me; 25·8, 26·0, $OCH_2CH_2\mathbf{C}H_2$; 28·8, 28·9, $OCH_2\mathbf{C}H_2$; 46·9, CH_2, epoxide; 51·79, 51·82, CH, epoxide; 69·3, 69·4, $O\mathbf{C}H_2CH_2$; 76·1, 76·5, C4,4′; 100·5, 100·8, C1,1′1″; 169·1–170·5, 10C, C=O.

4,5-Epoxypentyl O-β-D-*Glucopyranosyl-(1→4)*-O-β-D-*glucopyranosyl-(1→4)*-β-D-*glucopyranoside (25)*

Deacetylation of the above epoxide (60 mg) gave the epoxypentyl β-cellotrioside as a foam (36 mg). ^{1}H n.m.r. (300 MHz, D_2O, dss) δ 2·67–2·71, 2·89–2·93, 2m, CH_2, epoxide; 3·09–3·21, m, CH, epoxide. ^{13}C n.m.r. (75·5 MHz, D_2O, MeOH) δ 25·4, $OCH_2CH_2\mathbf{C}H_2$; 28·3, $OCH_2\mathbf{C}H_2$; 48·4, CH_2, epoxide; 53·9, CH, epoxide; 70·7, $O\mathbf{C}H_2CH_2$; 78·5, 78·7, C4,4′; 102·2, 102·5, 102·7, C1,1′,1″.

Pent-4-enyl O-(*Tetra*-O-*acetyl*-β-D-*glucopyranosyl*)-*(1→4)*-O-(*tri*-O-*acetyl*-β-D-*glucopyranosyl*)-*(1→3)-tri*-O-*acetyl*-β-D-*glucopyranoside*

Yield 66%, m.p. 157–158° (EtOH), $[\alpha]_D$ −36·0° (Found: C, 52·2; H, 6·0. $C_{43}H_{60}O_{26}$ requires C, 52·0; H, 6·1%). ^{13}C n.m.r. (20·1 MHz, $SiMe_4$) δ 20·6–21·0, 10C, Me: 28·0, 28·7 $OCH_2\mathbf{C}H_2\mathbf{C}H_2$; 76·5, C4′; 79·3, C3; 100·9, C1,1′,1″; 115·2, $=CH_2$; 138·0, =CH; 169·0–170·9, 10C, C=O.

4,5-Epoxypentyl O-(*Tetra*-O-*acetyl*-β-D-*glucopyranosyl*)-*(1→4)*-O-(*tri*-O-*acetyl*-β-D-*glucopyranosyl*)-*(1→3)-tri*-O-*acetyl*-β-D-*glucopyranoside*

Yield 79%, m.p. 151–152° (EtOH), $[\alpha]_D$ −37·5° (Found: C, 51·4; H, 6·0. $C_{43}H_{60}O_{27}$ requires C, 51·2; H, 6·0%). ^{1}H n.m.r. (300 MHz) δ 1·98–2·13, 12s, 3OH, Me; 2·43–2·47, 2·73–2·76, 2m, CH_2, epoxide; 2·87–2·94, m, CH, epoxide; 4·36, 4·47, 4·55, 3d, J 8·2, 7·9, 8·1 Hz, H1,1′,1″. ^{13}C n.m.r. (75·5 MHz, $CDCl_3$) δ 20·2–20·8, 10C, Me; 25·8, 26·0, $OCH_2CH_2\mathbf{C}H_2$; 28·7, 28·8, $OCH_2\mathbf{C}H_2$; 46·75, 46·82, CH_2, epoxide; 51·7, 51·8, CH, epoxide; 68·9, 69·0, $O\mathbf{C}H_2CH_2$; 76·2, C4′; 79·1, C3; 100·6, 100·7, 100·9, C1,1′,1″; 168·8–170·6, 10C, C=O.

4,5-Epoxypentyl O-β-D-*Glucopyranosyl-(1→4)*-O-β-D-*glucopyranosyl-(1→3)*-β-D-*glucopyranoside (26)*

Deacetylation of the above epoxide (80 mg) gave the epoxide (26) as a foam (52 mg). ^{1}H n.m.r. (300 MHz, D_2O, dss) δ 2·65–2·69, 2·88–2·93, 2m, CH_2, epoxide; 3·11–3·18, m, CH, epoxide. ^{13}C n.m.r. (75·5 MHz, D_2O, MeOH) δ 25·3, $OCH_2CH_2\mathbf{C}H_2$; 28·1, $OCH_2\mathbf{C}H_2$; 48·3, CH_2, epoxide; 53·8, CH, epoxide; 69·9, $O\mathbf{C}H_2CH_2$; 78·6, C4′; 84·5, C3; 102·0, 102·6, C1,1′,1″.

Pent-4-enyl O-(*Tetra*-O-*acetyl*-β-D-*glucopyranosyl*)-*(1→3)*-O-(*tri*-O-*acetyl*-β-D-*glucopyranosyl*)-*(1→3)-tri*-O-*acetyl*-β-D-*glucopyranoside*

Yield 60%, $[\alpha]_D$ −41·2° (Found: C, 51·9; H, 5·9. $C_{43}H_{60}O_{26}$ requires C, 52·0; H, 6·1%). ^{13}C n.m.r. (75·5 MHz, $SiMe_4$) δ 20·4–21·1, 10C, Me; 28·6, 29·8, $OCH_2\mathbf{C}H_2\mathbf{C}H_2$; 68·9, $O\mathbf{C}H_2CH_2$; 78·3, 79·0, C3,3′; 100·7, 101·1, C1,1′,1″; 115·1, $=CH_2$; 137·9, =CH; 168·8–170·8, 10C, C=O.

4,5-Epoxypentyl O-(*Tetra*-O-*acetyl*-β-D-*glucopyranosyl*)-*(1→3)*-O-(*tri*-O-*acetyl*-β-D-*glucopyranosyl*-*(1→3)-tri*-O-*acetyl*-β-D-*glucopyranoside*

Yield 84%, $[\alpha]_D$ −49·1° (Found: C, 50·9; H, 6·1. $C_{43}H_{60}O_{27}$ requires C, 51·2; H, 6·0%). ^{1}H n.m.r. (300 MHz) δ 1·98–2·16, 12s, 30H, Me; 2·43–2·48, 2·74–2·77, 2m, CH_2, epoxide; 2·88–2·95, m, CH, epoxide; 4·37, 4·45, 4·51, 3d, J 8·1, 8·1, 8·1 Hz, H1,1′,1″. ^{13}C n.m.r. (75·5 MHz, $CDCl_3$) δ 20·2–20·9, 10C, Me; 25·7, 26·0, $OCH_2CH_2\mathbf{C}H_2$; 28·7, 28·8, $OCH_2\mathbf{C}H_2$; 46·7, 46·8, CH_2, epoxide; 51·7, CH, epoxide; 68·8, 69·0, $O\mathbf{C}H_2CH_2$; 78·2, 78·9, C3,3′; 100·5, 100·6, 100·9. C1,1′,1″; 168·7–170·6, 10C, C=O.

4,5-Epoxypentyl O-β-D-Glucopyranosyl-(1→3)-O-β-D-glucopyranosyl-(1→3)-β-D-glucopyranoside (27)

Deacetylation of the above epoxide (80 mg) gave the epoxypentyl β-laminaritrioside (27) as a foam (30 mg). ^{1}H n.m.r. (300 MHz, D_2O, dss) δ 2·66–2·71, 2·89–2·93, 2m, CH_2, epoxide; 3·11–3·19, m, CH, epoxide. ^{13}C n.m.r. (75·5 MHz, D_2O, MeOH) δ 25·5, $OCH_2CH_2\mathbf{C}H_2$; 28·3, $OCH_2\mathbf{C}H_2$; 48·4, CH_2, epoxide; 54·0, CH, epoxide; 70·1, $OCH_2\mathbf{C}H_2$; 84·4, 84·5, C3,3′; 102·1, 102·7, 103·0, C1,1′,1″.

Pent-4-enyl O-(Tetra-O-acetyl-β-D-glucopyranosyl)-(1→3)-O-(tri-O-acetyl-β-D-glucopyranosyl)-(1→4)-tri-O-acetyl-β-D-glucopyranoside

Yield 51%, m.p. 149–150° (EtOH), $[\alpha]_D$ −37·1° (Found: C, 52·2; H, 5·9. $C_{43}H_{60}O_{26}$ requires C, 52·0; H, 6·1%). ^{1}H n.m.r. (300 MHz) δ 1·98–2·14, 10s, 30H, Me; 4·32, 4·44, 4·54, 3d, *J* 8·0, 8·0, 8·0 Hz, H1,1′,1″; 5·69–5·83, m, =CH. ^{13}C n.m.r. (75·5 MHz, $SiMe_4$) δ 20·3–20·9, 10C, Me; 28·6, 29·8, $OCH_2\mathbf{C}H_2\mathbf{C}H_2$; 69·3, $O\mathbf{C}H_2CH_2$; 76·2, 78·6, C3′,4; 100·6, 100·8, C1,1′,1″; 115·1, $=CH_2$; 137·8, =CH; 168·4–170·6, 10C, C=O.

4,5-Epoxypentyl O-(Tetra-O-acetyl-β-D-glucopyranosyl)-(1→3)-O-(tri-O-acetyl-β-D-glucopyranosyl)-(1→4)-tri-O-acetyl-β-D-glucopyranoside

Yield 95%, m.p. 153–155° (EtOH), $[\alpha]_D$ −37·6° (Found: C, 51·2; H, 5·8. $C_{43}H_{60}O_{27}$ requires C, 51·2; H, 6·0%). ^{1}H n.m.r. (300 MHz) δ 1·98–2·14, 8s, 30H, Me; 2·43–2·48, 2·72–2·76, 2m, CH_2, epoxide; 2·88–2·95, m, CH, epoxide; 4·32, 4·46, 4·55, 3d, *J* 7·9, 7·8, 8·0 Hz, H1,1′,1″. ^{13}C n.m.r. (75·5 MHz, $SiMe_4$) δ 20·3–20·9, 10C, Me; 25·8, 26·0, $OCH_2CH_2\mathbf{C}H_2$; 28·82, 28·85, $OCH_2\mathbf{C}H_2$; 46·9, CH_2, epoxide; 51·77, 51·82, CH, epoxide; 69·37, 69·45, $O\mathbf{C}H_2CH_2$; 76·1, 78·6, C3′,4; 100·55, 100·65, 100·8, C1,1′,1″; 168·4–170·6, 10C, C=O.

4,5-Epoxypentyl O-β-D-Glucopyranosyl-(1→3)-O-β-D-glucopyranosyl-(1→4)-β-D-glucopyranoside (28)

Deacetylation of the above epoxide (80 mg) gave the deprotected epoxide (28) as a foam (50 mg). ^{1}H n.m.r. (300 MHz, D_2O, dss) δ 2·65–2·70, 2·88–2·93, 2m, CH_2, epoxide; 3·11–3·19, m, CH, epoxide; 4·46, 4·52, 4·73, 3d, *J* 8·0, 8·0, 7·8 Hz, H1,1′,1″. ^{13}C n.m.r. (75·5 MHz, D_2O, MeOH) δ 25·6, $OCH_2CH_2\mathbf{C}H$; 28·4, $OCH_2\mathbf{C}H_2$; 48·5, CH_2, epoxide; 54·1, CH, epoxide; 70·2, $O\mathbf{C}H_2CH_2$; 79·2, 84·6, C3′,4; 102·4, 102·6, 103·1, C1,1′,1″.

Methyl 2,3,6-Tri-O-benzoyl-α-D-glucopyranoside (7)

(i) To a stirred and ice-cooled solution of methyl 4,6-*O*-benzylidene-α-D-glucoside (1·41 g, 5·0 mmol) in dry dichloromethane (10 ml) containing pyridine (12 ml, 150 mmol) was added dropwise benzoyl chloride (1·75 ml, 15·0 mmol). The resulting mixture was stirred overnight at room temperature. Normal workup (CH_2Cl_2), including an aqueous acid wash (1 M HCl), followed by recrystallization, afforded the 2,3-dibenzoate as white needles (2·4 g, 98%), m.p. 158–159° (EtOH) (lit.[23] 148°).

(ii) A suspension of the dibenzoate (5·0 g) in 60% aqueous acetic acid (70 ml) was heated for 30 min on a steam bath. Evaporation of the solvents, followed by flash chromatography (EtOAc/petrol, 3 : 1) of the residue, afforded a yellowish glass identified [^{1}H n.m.r. (60 MHz)] as the 4,6-diol.[24]

(iii) (*a*) To an ice-cooled solution of the diol (402 mg, 1·0 mmol) in dry dichloromethane (10 ml) were added triethylamine (0·2 ml) and benzoyl chloride (0·15 ml, 1·1 mmol). The resulting mixture was stirred (0°) for 30 min, after which water was added to quench the reaction. Normal workup (CH_2Cl_2), followed by flash chromatography (EtOAc/petrol, 3 : 7), gave the alcohol (7) (399 mg, 79%), m.p. 127–128° (diisopropyl ether/petrol) (lit.[25] 127–129°).

[23] Ohle, H., and Spencker, K., *Ber. Dtsch. Chem. Ges.*, 1928, **61**, 2387.
[24] Mathers, D. S., and Robertson, G. J., *J. Chem. Soc.*, 1933, 1076.
[25] Ogawa, T., and Matsui, M., *Tetrahedron*, 1981, **37**, 2363.

(*b*) To a stirred mixture of the diol (402 mg, 1·0 mmol) and benzoyl cyanide[26] (131 mg, 1·0 mmol) in acetonitrile (10 ml) was added triethylamine (2 drops), resulting in the rapid dissolution of the diol. After 30 min, the reaction mixture was then concentrated, methanol added to the residue and the solvents evaporated. Flash chromatography (EtOAc/petrol, 3 : 7) of the residue gave the crystalline alcohol (7) (405 mg, 80%), m.p. 128–129°. ^{1}H n.m.r. (300 MHz) δ 3·45, s, OMe; 3·90, t, J 9·5 Hz, H4; 4·08–4·16, m, H5; 4·64, dd, J 2·2, 12·2 Hz, H6; 4·78, dd, J 4·7, 12·2 Hz, H6; 5·15, d, J 4·0 Hz, H1; 5·26, dd, J 3·7, 10·1 Hz, H2; 5·82, t, J 10·1 Hz, H3.

Epoxidation of Pent-4-enyl β-Cellobioside (31)

Deacetylation of the cellobioside (30) (845 mg) in the usual way gave a foam (491 mg) identified [^{1}H n.m.r. (60 MHz)] as pent-4-enyl β-cellobioside (31). A solution of this cellobioside (200 mg, 0·5 mmol) in methanol was treated with *m*-chloroperbenzoic acid (173 mg, 1·0 mmol) and stirred (room temperature) for 2 days. The solvent was evaporated and the residue taken up in water; residual solids were removed by filtration, the aqueous filtrate evaporated and the residue dried under vacuum to yield the epoxide (21) (206 mg) which contained some minor chemical impurities (^{13}C n.m.r.). Due to the polarity of the epoxide, no further purification was carried out. ^{1}H n.m.r. (80 MHz, D_2O, dss) δ 2·58–2·77, 2·77–3·00, 2m, CH_2, epoxide. ^{13}C n.m.r. (20·1 MHz, D_2O, MeOH) δ 25·2, 25·5, $OCH_2CH_2\mathbf{C}H_2$; 28·9, 29·5, $OCH_2\mathbf{C}H_2$; 48·4, CH_2, epoxide; 54·0, CH, epoxide; 69·7, C4′; 70·1, $O\mathbf{C}H_2CH_2$; 79·1, C4; 102·3, 102·8, C1,1′.

Methyl Tri-O-benzyl-4-O-(tetra-O-acetyl-β-D-glucopyranosyl)-α-D-glucopyranoside (32)

A mixture of D-glucose (180 mg, 1·0 mmol) and anhydrous sodium acetate (220 mg) in acetic anhydride (3 ml) was heated at reflux for 30 min. The reaction mixture was then poured into an ice-water mixture and subjected to normal workup (CH_2Cl_2) to give a solid identified [^{1}H n.m.r. (60 MHz)] as penta-*O*-acetyl-β-D-glucose (388 mg). The pentaacetate was dissolved in acetic acid (2 ml), 40% hydrogen bromide in acetic acid (1 ml) added and the mixture stirred (room temperature) for 2 h. Normal workup gave a syrup which was dissolved in dry 1,2-dichloroethane (5 ml) and added dropwise during 30 min, with the rigorous exclusion of light and moisture, to a stirred mixture (–30°) of the alcohol (9) (325 mg, 0·70 mmol), silver triflate (450 mg, 1·75 mmol) and powdered molecular sieve 4A (1 g) in dry 1,2-dichloroethane (5 ml). The mixture was allowed to attain 0° gradually, and then stirred at this temperature for 1 h. Filtration (Celite) and normal workup, followed by flash chromatography (EtOAc/petrol, 1 : 1), gave the disaccharide glycoside (32) (318 mg, 40%), m.p. 111–113° (diisopropyl ether), $[\alpha]_D$ +5·1° (Found: C, 63·2; H, 6·4. $C_{24}H_{50}O_{15}$ requires C, 63·5; H, 6·3%). ^{13}C n.m.r. (75·5 MHz, $SiMe_4$) δ 20·5, 20·6, 20·7, 4C, Me; 55·4, OMe; 73·5, 73·7, 75·2, 3C, $Ph\mathbf{C}H_2$; 79·9, C4; 98·4, 100·0, C1,1′; 169·0, 169·4, 170·2, 170·6, 4C, C=O.

3,4-Epoxybutyl Tetra-O-acetyl-β-cellobioside

A solution of the disaccharide glycoside (32) (258 mg, 0·32 mmol) in acetic acid (10 ml) was hydrogenated (room temperature) in the presence of 10% Pd/C (170 mg) under pressure (40 psi) overnight. The reaction mixture was filtered (Celite), the solids washed thoroughly with methanol and the filtrate concentrated. To the stirred and ice-cooled solution of the residue in acetic anhydride (5 ml) was added dropwise 4% sulfuric acid in acetic anhydride (5 ml). Stirring (room temperature) was continued for another 4 h, after which the reaction mixture was poured into ice-water containing solid sodium bicarbonate. Normal workup (EtOAc) afforded octa-*O*-acetyl-α-cellobiose (209 mg) [^{1}H n.m.r. (60 MHz)]. The octaacetate was dissolved in acetic acid and dichloromethane (3 ml of 1 : 1), 40% hydrogen bromide in acetic acid (2 ml) added and the mixture stirred at room temperature for 2 h. After the usual workup, a syrup was obtained. A solution of the syrup in dry dichloromethane (5 ml) was added to a mixture of but-3-en-1-ol (0·6 ml, 3·1 mmol), silver carbonate (255 mg,

[26] Koenig, K. E., and Weber, W. P., *Tetrahedron Lett.*, 1974, 2275.

0·92 mmol), a crystal of iodine, anhydrous calcium sulfate (750 mg) and powdered molecular sieve 4A (750 mg) in dry dichloromethane (5 ml) which had been stirred (room temperature) with the exclusion of light and moisture for 30 min. The mixture was stirred for 24 h and, after the usual workup followed by flash chromatography (EtOAc/petrol, 2 : 1), but-3-enyl hepta-*O*-acetyl-β-cellobioside (145 mg) was obtained. A solution of the cellobioside, *m*-chloroperbenzoic acid (80%, 90 mg, 0·42 mmol) and a few crystals of 2,6-di-t-butyl-4-methylphenol in dichloromethane was heated at reflux for 8 h. Normal workup, followed by flash chromatography (EtOAc/petrol, 2 : 1), gave 3,4-epoxybutyl hepta-*O*-acetyl-β-cellobioside, m.p. 191–193° (EtOH/Et_2O).

^{14}C-Labelled Epoxide

D-[U-^{14}C]Glucose (9·25 MBq, 250 μCi package), of molecular weight 189, specific activity 1·48 mCi/mg, and radioactive concentration 7·40 MBq/ml, was obtained from Amersham Australia Pty Ltd.

The sequence of reactions used for the preparation of the epoxybutyl cellobioside above was followed exactly. To begin with, the aqueous solution of radioactive D-glucose was freeze-dried and the residue diluted with cold D-glucose (180 mg). After the eight steps, the ^{14}C-labelled epoxide (93 mg, 13% overall yield) was obtained.

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