Chemistry of Bacterial Endotoxins. Part 3.[1] Reactions of Oxazolines Derived from 1,3,4,6-Tetra-*O*-acetyl-2-[(3*R*)-3-hydroxytetradecanamido]-β-D-glucopyranose

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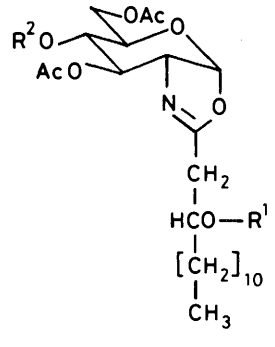
In acidic medium the oxazoline derived from 2-[(3RS)-3-acetoxytetradecanamido]-1,3,4,6-tetra-*O*-acetyl-2-deoxy-β-D-glucopyranose readily eliminates elements of acetic acid: it is transformed into an oxazoline that carries an olefinic substituent having the *E* configuration; this compound, when treated with an alcohol, yields the corresponding β-D-glucosaminide. No elimination takes place with the analogous 3-hydroxy-or 3-benzyloxy-tetradecanamidoglucosamine derivatives. (3*R*)-3-Benzyloxytetradecanoic acid was obtained in 60% yield upon treatment of methyl (3*R*)-3-hydroxytetradecanoate in benzene with benzyl bromide, silver oxide, and anhydrous calcium sulphate, followed by saponification. Under similar conditions 3,4,6-tri-*O*-acetyl-1,2-dideoxy-4',5'-dihydro-2-[(2*R*)-2-hydroxytridecyl]-α-D-glucopyranoso[2,1-*d*]oxazole was transformed into the corresponding (2*R*)-2-benzyloxyoxazoline.

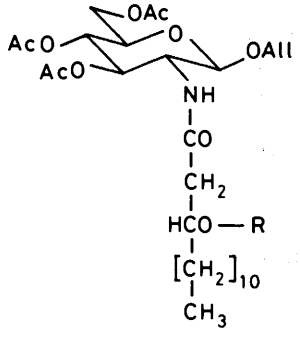
Because of their great potency and unique biological properties, endotoxins are widely used as reagents in immunological studies. It is thought that it is mainly the hydrophobic region ('Lipid A') which is responsible for the so-called 'endotoxic properties' (pyrogenicity, lethal toxicity, sensitizing action adjuvant, mitogenic effects, *etc.*) of these macromolecules. It has been established by Hase and Rietschel[2] that the disaccharide glucosaminyl-β1,6-glucosamine, phosphorylated at positions 1 and 4', and carrying (3*R*)-3-hydroxytetradecanoic acid residues in an amide linkage and various fatty acids in an ester linkage, was an essential constituent of the hydrophobic region of endotoxins of many gram-negative bacteria. This observation was confirmed and extended in a number of laboratories during the last few years,[3–5] and the phosphorylated disaccharide, and analogues of the basic structure, have been synthesized.[6–8] However, none of them appear to have the same biological potencies as the intact endotoxins or their isolated 'Lipid A' moieties.[9]

In Part 1 of this series an unsuccessful attempt, aimed at the synthesis of a phosphorylated glucosaminyl-β1,6-glucosamine by condensation of the oxazoline (**1**) with the diol (**10**) was described; it was then observed that in some instances the acetoxy group of the fatty acid was lost during the reaction. This reaction was examined further using the oxazoline (**2**), readily prepared from the easily accessible 2-[(3*RS*)-3-acetoxytetradecanamido]-1,3,4,6-tetra-*O*-acetyl-2-deoxy-β-D-glucopyranose (**11**). When the oxazoline (**2**)[10] was treated with allyl alcohol in the presence of toluene-*p*-sulphonic acid, two products, separable by t.l.c., were formed in about equal amounts. In the ^{1}H n.m.r. spectrum of the first one, which had the lower R_F value, four acetate groups could be detected: the compound was the expected allyl β-glycoside, (**5**), in which the acetoxy group of the fatty acid residue was conserved. In the ^{1}H n.m.r. spectrum of the other product, which was also a β-glycoside (δ_H 4.7, 1 H, d, $J_{1,2}$ 8.5 Hz), only three acetate groups appeared, but with a signal due to a clearly discernible double bond having the *E* configuration [δ_H 5.65, 1 H, d, $J_{2',3'}$ 16 Hz (1'-H) and δ_H 6.75, 1 H, dt, $J_{3',4'}$ 7 Hz (3'-H)]; as expected, the compound strongly absorbed u.v. light ($\lambda_{max.}$ 220 nm; ε 9 600). These results clearly established that the acetoxy group, originally present on C-3 of the fatty acid (C-3'), had been eliminated, and that the compound having the higher R_F value was allyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-(tetradec-2-enamido)-β-D-glucopyranoside (**13**).

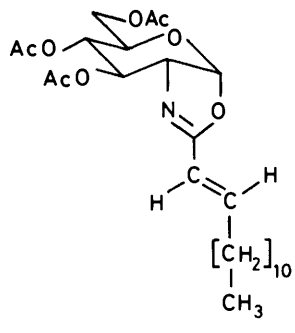
To answer the question as to whether elimination took place before, concurrently with, or after the condensation reaction, the oxazoline (**2**), dissolved in toluene, was treated, in the absence of any acceptor alcohol, with toluene-*p*-sulphonic (or acetic) acid: a single product, strongly absorbing u.v. light, was



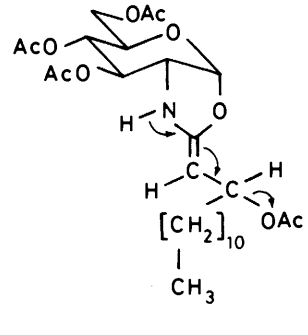
(**1**) R^1 = Ac, R^2 = P(OPh)$_2$
(**2**) R^1 = R^2 = Ac
(**3**) R^1 = H, R^2 = Ac
(**4**) R^1 = Bn, R^2 = Ac



(**5**) R = Ac
(**6**) R = H
(**7**) R = Bn

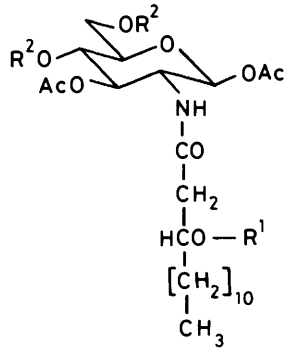


(**8**)

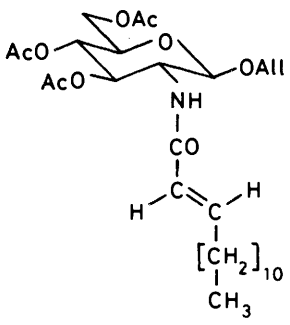


(**9**)

All = allyl, Bn = benzyl



(10) R^1 = Ac, R^2 = H
(11) R^1 = R^2 = Ac
(12) R^1 = H, R^2 = Ac



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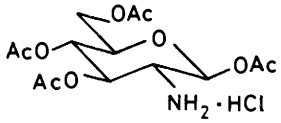
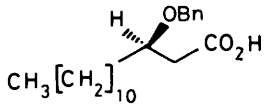


(14)



(15)

formed and isolated in 60% yield. Its ^{1}H n.m.r. spectrum and elemental analysis showed that it was the oxazoline (**8**) bearing an olefinic chain. As a corollary, when allowed to react with allyl alcohol, it gave a 60% yield of the allyl β-glycoside (**13**).

It was thus established that during the acid-catalysed condensation with alcohol, oxazolines derived from 2-acylamido-2-deoxyglucose derivatives, in which the acylamide residue is substituted in the β position by an acetoxy (or, presumably, by any other acyloxy) radical, may undergo two types of reaction: condensation to yield the expected β-glycoside, or elimination of the acyloxy residue to yield an oxazoline with an olefinic substituent; the latter will then react with the alcohol to form a β-glycoside bearing an olefinic acylamido group. The proportions of the products will depend on the reactivity of the reagents and reaction conditions. As it has been observed previously[11] that in simple oxazolines the hydrogen atom attached to C-1′ is labile, it is reasonable to suggest that elimination of the acyloxy group from the oxazoline (**2**) is initiated by protonation of the nitrogen atom: this leads to the isomeric structure (**9**) from which the olefinic compound is formed by elimination.

It is firmly established that the rate of an elimination reaction is, *inter alia*, a function of the nature of the leaving group, release of an acetyl group occurring much more rapidly than that of a hydroxy group. Accordingly, production of a β-glycoside from a 2-(3-hydroxytetradecanamido)-2-deoxyglucose derivative *via* the corresponding oxazoline was attempted. Thus, 1,3,4,6-tetra-*O*-acetyl-2-deoxy-[(3*R*)-3-hydroxytetradecanamido]-β-D-glucopyranose (**12**) – prepared by the dicyclohexylcarbodi-imide-promoted condensation of (3*R*)-3-hydroxytetradecanoic acid with 1,3,4,6-tetra-*O*-acetyl-2-amino-2-deoxy-β-D-glucopyranose – was transformed into the corresponding oxazoline (**3**)[10] which was then allowed to react with allyl alcohol in the presence of toluene-*p*-sulphonic acid. A greater than 60% yield of the expected glycoside (**6**) was isolated, and apparently no product bearing an olefinic fatty acid was formed concomitantly. It is noteworthy that self-condensation of the oxazoline (**3**) did not occur either, despite the presence of a free hydroxy group in the molecule.

It has been observed[4,12,13] that the hydroxy groups of 3-hydroxytetradecanoic acid units attached to the 1,6-linked glucosamine molecules present in the hydrophobic region of endotoxins are often esterified by another fatty acid. In order to safeguard the possibility of specifically introducing appropriate substituents in these positions during late stages of syntheses leading to natural products, the stability of a benzyl radical as temporary protecting group of 3-hydroxytetradecanoic acid in condensation reactions, *via* oxazolines, was evaluated.

When, to this end, attempts were made to prepare 3-benzyloxytetradecanoic acid by treating the methyl ester of 3-hydroxytetradecanoic acid with benzyl bromide in the presence of potassium hydroxide in toluene,[14] with barium oxide–barium hydroxide,[15] with sodium hydride,[16] or with silver oxide[17] in dimethylformamide, the reaction failed: in all cases (*E*)-tetradec-2-enoic acid or its methyl ester, and dodecanal – produced in variable amounts and proportions, the former by an elimination reaction, the latter by a retro-Reformatsky reaction – were the only identified products. It has been demonstrated that 4-hydroxy butan-2-one, when treated with benzyl bromide and silver oxide in benzene, gave a 40—50% yield of 4-benzyloxybutan-2-one;[18] when benzylation of methyl 3-hydroxytetradecanoate was carried out under these conditions, a less than 20% yield of the required benzyl ether was obtained, the rest of the fatty acid being recovered unchanged; large amounts of dibenzyl ether, and some benzyl alcohol, were formed simultaneously. It was considered that the dibenzyl ether may have been produced by reactions (1)—(4), the water

$$ROH + Ag_2O + BnBr \longrightarrow ROBn + AgBr + AgOH \quad (1)$$

$$2\,AgOH \longrightarrow Ag_2O + H_2O \quad (2)$$

$$BnBr + Ag_2O + H_2O \longrightarrow BnOH + AgBr + AgOH \quad (3)$$

$$BnOH + BnBr + Ag_2O \longrightarrow BnOBn + AgOH + AgBr \quad (4)$$

$$2\,BnBr + Ag_2O \xrightarrow{H_2O} BnOBn + 2\,AgBr \quad (5)$$

produced serving as the catalyst for the overall reaction (5). The validity of this hypothesis was strengthened when it was found that upon addition of a dehydrating agent (Drierite) to the reaction mixture, the amount of methyl 3-benzyloxytetradecanoate formed increased considerably to give, after saponification of the ester group, an overall yield of 60% of 3-benzyloxytetradecanoic acid. A synthesis of the same compound by another route was described by Kusumoto *et al.*[7]

Although this acid can be condensed in reasonable yield with 1,3,4,6-tetra-*O*-acetyl-2-amino-2-deoxy-β-D-glucopyranose to give the corresponding amide, it was considered that better yields would be obtained with the expensive (3*R*)-3-hydroxytetradecanoic acid, if either 1,3,4,6-tetra-*O*-acetyl-2-deoxy-2-[(3*R*)-3-hydroxytetradecanamido]-β-D-glucopyranose (**12**), or the oxazoline (**3**) derived therefrom, could be benzylated. While benzylation of the tetra-acetate (**12**) under the conditions just described failed, the labile-β-acetate being lost, the oxazoline (**3**) could be benzylated even in the absence of Drierite, presumably because of the enhanced nucleophilicity of the hydroxy group, to give a greater than 40% yield of the expected product. Treatment of this with allyl alcohol and toluene-*p*-sulphonic acid gave the benzylated β-glycoside in 63% yield. As no attempts were made to optimize reaction conditions, yields

could probably be improved for most of the reactions described.

Experimental

General methods are described in Parts 1 (ref. 8) and 2 (ref. 1).

1,3,4,6-*Tetra*-O-*acetyl*-2-*deoxy*-2-[(3R)-3-*hydroxytetradecanamido*]-β-D-*glucopyranose* (**12**).—A mixture, prepared by adding triethylamine (0.95 mol equiv.), dicyclohexylcarbodiimide (1 mol equiv.), and (3*R*)-3-hydroxytetradecanoic acid (1 mol equiv.) to a suspension of 1,3,4,6-tetra-*O*-acetyl-2-amino-2-deoxy-β-D-glucopyranose hydrochloride (**14**)[19] (1 mol equiv.) in anhydrous pyridine (15 ml g^{-1} of the salt), was stirred at room temperature for 48 h. The precipitate was filtered off, and the residue, recovered after removal of the solvent, was redissolved in (1:1) dichloromethane–ethyl acetate, and the solution was percolated through a column of silica gel (10 g g^{-1} of product). Fractions containing the amide (**12**) [t.l.c., (7:7:1) diethyl ether–toluene–methanol; R_F 0.6] were pooled, the solvent was removed, and the solid residue was crystallized from ethyl acetate (yield 68%), m.p. 136—138 °C; $[\alpha]_D^{20}$ +7.1° (*c* 1 in $CDCl_3$) {lit.,[10] $[\alpha]_D^{20}$ +6.7° (*c* 1 in $CHCl_3$)}.

2-[(3RS)-3-*Acetoxytetradecanamido*]-1,3,4,6-*tetra*-O-*acetyl*-2-*deoxy*-β-D-*glucopyranose* (**11**).—*N*-Acylation of the 2-amino-2-deoxyglucopyranose derivative (**14**) was carried out as described above, but using (3*RS*)-3-hydroxytetradecanoic acid. The filtered reaction mixture was evaporated, and acetic anhydride (1 ml g^{-1}) and sodium acetate (0.1 g g^{-1}) were added to the residue. The mixture was kept at 100 °C for 1 h, and the acetic anhydride was evaporated off, complete removal being achieved by repeated co-evaporation with toluene. The residue was then purified by column chromatography [(1:1) dichloromethane–ethyl acetate]: fractions containing the product (t.l.c. same solvent; R_F 0.75) were pooled, the solvent was evaporated off, and the solid residue was crystallized from ethanol–diethyl ether (yield 65%), m.p. 145—150 °C; $[\alpha]_D^{20}$ +4.3° (*c* 1 in $CHCl_3$) {lit.,[20] $[\alpha]_D^{20}$ +2.9° (*c* 1 in $CHCl_3$)}.

2′-[(2RS)-2-*Acetoxytridecyl*]-3,4,6-*tri*-O-*acetyl*-1,2-*dideoxy*-4′,5′-*dihydro*-α-D-*glucopyranoso*[2,1-d]*oxazole* (**2**).—Anhydrous iron(III) chloride (5 g) was added to a solution of the β-acetate (**11**) (5 g) in dry dichloromethane (100 ml). The mixture was saturated with dry nitrogen, the reaction vessel was closed, the mixture was stirred for 3 h at room temperature, and the solids were allowed to settle. The brown solution was decanted, washed with ice–water until colourless, dried, treated with charcoal, and evaporated to give the homogeneous [t.l.c., (7:7:1) diethyl ether–toluene–methanol; R_F 0.75] oxazoline (4.5 g, 85%) as an oil that could not be induced to crystallize. In its ^1H n.m.r. spectrum (90 MHz; $CDCl_3$) the following coupling constants were measured: $J_{1,2}$ 7.5 Hz; $J_{2,3}$ 3 Hz; $J_{3,4}$ 3 Hz; $J_{4,5}$ 9 Hz. For the analogous *N*-acetyl derivative Kiso *et. al.*[10] found $J_{1,2}$ 7.3 Hz; $J_{2,3}$ 2.6 Hz; $J_{3,4}$ 2.2 Hz; $J_{4,5}$ 9.2 Hz.

3,4,6-*Tri*-O-*acetyl*-1,2-*dideoxy*-4′,5′-*dihydro*-2′-[(E)-*tridec*-1-*enyl*]-α-D-*glucopyranoso*[2,1-d]*oxazole* (**8**).—Dry toluene-*p*-sulphonic acid (20 mg) was added to a solution of the oxazoline (**2**) (4.1 g) in anhydrous toluene (25 ml), and the mixture was kept at 50 °C overnight. Pyridine (10 μl) was added, and the solvent was removed. The residual material was purified by column chromatography [(3:1) ethyl acetate–dichloromethane]; fractions containing the *title compound* [t.l.c., (7:7:1) diethyl ether–toluene–methanol; R_F 0.8] were pooled, and the solvent was removed. The residual oily oxazoline (2.2 g, 60%) could not be induced to crystallize (Found: C, 63.0; H, 8.2; N, 3.0. $C_{26}H_{41}NO_8$ requires C, 63.0; H, 8.3; N, 2.8%); $[\alpha]_D^{20}$ +69° (*c* 1 in $CHCl_3$); $\lambda_{max.}$ 225 nm (ε 13 500); δ_H (90 MHz; $CDCl_3$) 0.5—2.2 (32 H, 3 × $COCH_3$, and $CH_3[CH_2]_{10}$), 3.6 (1 H, ddd, 5-H), 4.0—5.0 (4 H, m, 6-H_2, 2-H, and 4-H), 5.15 (1 H, t, $J_{3,4} = J_{2,3}$ = 2 Hz, 3-H), 5.9 (1 H, d, 1″-H), 6.0 (1 H, d, $J_{1,2}$ 6 Hz, 1-H), 6.8 (1 H, dt, $J_{2',3'}$ 15, $J_{3',4'}$ 6 Hz, 2″-H). In the ^{13}C n.m.r. spectrum the resonances appearing at δ_C 116.18, 146.53, and 164.35 p.p.m. were attributed to CH_2CH=C, CH=*C*HCO, and C=N, respectively.

Allyl 2-[(3RS)-3-*Acetoxytetradecanamido*]-3,4,6-*tri*-O-*acetyl*-2-*deoxy*-β-D-*glucopyranoside* (**5**) *from Oxazoline* (**2**).—Dry toluene-*p*-sulphonic acid (20 mg) and allyl alcohol (600 mg, 4 mol equiv.) were added to a solution of the oxazoline (**2**) (1.8 g) in a mixture (1:1) of dry nitromethane and toluene (30 ml), and the stirred mixture was kept at 50 °C overnight. Pyridine (10 μl) was added, and the solvents were removed. The residual material was submitted to column chromatography [(7:7:1) diethyl ether–toluene–methanol]; fractions containing material with R_F 0.6 (t.l.c., same solvent) were pooled, the solvent was removed, and the *glycoside* (**5**) (600 mg, 25%) was crystallized from ethyl acetate, m.p. 141—142 °C (Found: C, 60.4; H, 8.4; N, 2.4. $C_{31}H_{51}NO_{11}$ requires C, 60.7; H, 8.4; N, 2.3%); $[\alpha]_D^{20}$ −1.9° (*c* 1 in $CHCl_3$). In the ^1H n.m.r. spectrum (90 MHz; $CDCl_3$), the signal at δ_H 4.87 (1 H, d, $J_{1,2}$ 9 Hz, 1-H) confirmed the β-anomeric configuration.

Allyl 3,4,6-*Tri*-O-*acetyl*-2-*deoxy*-2-[(E)-*tetradec*-2-*enamido*]-β-D-*glucopyranoside* (**13**).—(a) *From the oxazoline* (**2**). The oxazoline (**2**) was condensed with allyl alcohol as described above for the synthesis of the glycoside (**5**). Fractions containing material eluted during column chromatography just before the glycoside (**5**) [t.l.c., (7:7:1) diethyl ether–toluene–methanol; R_F 0.67] were pooled, the solvent was removed, and the *title product* (600 mg, 25%) was recovered as a white solid, m.p. 119—120 °C (Found: C, 63.1; H, 8.4; N, 2.7. $C_{29}H_{47}NO_9$ requires C, 62.9; H, 8.50; N, 2.5%); $[\alpha]_D^{20}$ +8.4° (*c* 1 in $CHCl_3$); δ_H (90 MHz; $CDCl_3$) 4.7 (1 H, d, $J_{1,2}$ 7.5 Hz, 1-H), 5.65 (1 H, d, J_{vic} 15 Hz, OCC*H*=CHCH$_2$), and 6.75 (1 H, dt, OCCH=C*H*CH$_2$); the remainder of the spectrum was not interpreted.

(b) *From the oxazoline* (**8**). Allyl alcohol (0.6 ml, 4 mol equiv.) and toluene-*p*-sulphonic acid (20 mg) were added to a solution of the olefinic oxazoline (**8**) (1.8 g) in a mixture (1:1) of toluene and nitromethane (30 ml), and the stirred mixture was kept overnight at 50 °C. Pyridine (10 μl) was added, and the solvents were removed. The pure glycoside (**13**) (1.25 g, 65%) was obtained after column chromatography [(3:1) dichloromethane–ethyl acetate] of the residue. It had the same physical constants as the compound obtained by method (a).

3,4,6-*Tri*-O-*acetyl*-1,2-*dideoxy*-4′,5′-*dihydro*-2′-[(2R)-2-*hydroxytridecyl*]-α-D-*glucopyranoso*[2,1-d]*oxazole* (**3**).—Dry iron(III) chloride (5 g) was added to a solution of 1,3,4,6-tetra-*O*-acetyl-2-deoxy-2-[(3*R*)-3-hydroxytetradecanamido]-β-D-glucopyranose (**12**) (5 g) in dichloromethane (100 ml). The mixture was saturated with nitrogen, and the stirred mixture was kept in a closed vessel at room temperature for 3 h. Solids were filtered off, and the filtrate was washed with ice–water until completely colourless, dried (Na_2SO_4), treated with charcoal, filtered, and evaporated. The product (**3**) (4.3 g, 85%) could not be induced to crystallize. It appeared homogeneous upon t.l.c. [(7:7:1) diethyl ether–toluene–methanol], and had $[\alpha]_D^{20}$ +16° (*c* 1 in $CHCl_3$); δ_H (90 MHz; $CDCl_3$) 0.8—2.5 (34 H, m, 3 × $COCH_3$, $[CH_2]_{10}CH_3$, and 2′-CH_2), 3.65 (1 H, ddd, 5-H), 3.75—4.25 (3 H, m, 2-H and 6-H_2), 4.85 (1 H, m, 4-H), 5.25 (1 H, t, $J_{2,3} = J_{3,4}$ = 2 Hz, 3-H), and 5.85 (1 H, d, $J_{1,2}$ 7.5 Hz, 1-H). The material was used without undue delay.

Allyl 3,4,6-*Tri*-O-*acetyl*-2-*deoxy*-2-[(3R)-3-*hydroxytetradecanamido*]-β-D-*glucopyranoside* (**6**).—Allyl alcohol (3 ml, 4 mol equiv.) and toluene-*p*-sulphonic acid (100 mg) were added to a solution of the oxazoline (**3**) (6 g) in a mixture (1:1) of *nitro*methane and toluene (60 ml), and the mixture was kept at 50 °C overnight. Pyridine (50 μl) was added, the solvents were removed, and the residue was purified by column chromatography [(1:1) dichloromethane–ethyl acetate] to yield the crude product (4.2 g, 61%) as a white solid. Crystallization (ethyl acetate–hexane) afforded the pure *glycoside* (**6**) (3.2 g, 47%), m.p. 131—132 °C (Found: C, 61.2; H, 8.55; N, 2.7. $C_{29}H_{49}NO_{10}$ requires C, 60.9; H, 8.6; N, 2.45%); $[\alpha]_D^{20}$ −0.67° (*c* 1 in $CHCl_3$). In the ^1H n.m.r. spectrum (90 MHz; $CDCl_3$) the resonance of the anomeric proton appeared at δ_H 4.75, $J_{1,2}$ 9 Hz.

(3R)-3-*Benzyloxytetradecanoic Acid* (**15**).—Freshly prepared[21] silver oxide (3 g, 1 mol equiv.) and anhydrous calcium sulphate (12 g) were suspended in benzene (50 ml), and methyl (3R)-3-hydroxytetradecanoate (2.6 g, 0.8 mol equiv.) and benzyl bromide (2.4 ml, 1.8 mol equiv.) were added to the stirred mixture kept in the dark at room temperature. The mixture was stirred for 1 h, when more silver oxide (3 g), calcium sulphate (12 g), and benzyl bromide (2 ml) were added. After a further 2 h the addition was repeated, and the mixture was stirred for another 3 h. The mixture, diluted with dichloromethane, was then filtered and evaporated. The residual oil was then applied to a column (100 g) of silica gel and eluted with a mixture (6:1) of hexane–diethyl ether. Fractions containing the methyl ester of the title compound (t.l.c., R_F 0.6; same solvent) were pooled, and the solvent was evaporated. The residual oil (3.5 g) was dissolved in methanol (50 ml), the solution was heated to 80 °C, and aqueous potassium hydroxide [3 g KOH (1 mol equiv.), in 50 ml water] was added in portions. After 2.5 h the mixture was cooled to room temperature, diethyl ether (300 ml) and sulphuric acid (0.5M; 200 ml) were added and, after being thoroughly mixed, the organic layer was separated, washed with water until neutral, dried, and evaporated. The residual oil was submitted to column chromatography [(1:1) hexane–diethyl ether; t.l.c. (same solvent), R_F 0.4]. Appropriate fractions were pooled, and the solvent was removed, to afford the *title compound* as an oil (2.15 g, 60%) which solidified at −20 °C (Found: C, 75.4; H, 10.2 $C_{21}H_{43}O_3$ requires C, 75.4; H, 10.25%); $[\alpha]_D^{20}$ −4.2° (*c* 2 in $CHCl_3$); δ_H (90 MHz; $CDCl_3$) 1.23 (23 H, m, $[CH_2]_{10}CH_3$), 2.5 (2 H, dd, 2-H_2), 3.8 (1 H, q, 3-H), 4.5 (2 H, s, OCH_2Ph), 7.25 (5 H, s, Ph), and 8.95 (1 H, m, CO_2H).

3,4,6-*Tri*-O-*acetyl*-2′-[(2R)-2-*benzyloxytridecyl*]-1,2-*dideoxy*-5′,6′-*dihydro*-α-D-*glucopyranoso*[2,1-d]*oxazole* (**4**).—Benzyl bromide (3.75 ml, 3 mol equiv.) and freshly prepared[21] silver oxide (3 g, 1.33 mol equiv.) were added to a solution of the oxazoline (**3**) (5 g) in anhydrous benzene (50 ml). The stirred mixture, protected from light, was kept at 45 °C for 2 h, after which time silver oxide (1 g) and benzyl bromide (1 ml) were added and the additions were again repeated 2 h later. The reaction was then allowed to proceed for another 5 h at 45 °C. T.l.c. [(7:7:1) diethyl ether–toluene–methanol] then revealed the presence of three compounds having R_F 0.63 (major), 0.58 (benzyl alcohol), and 0.9 (dibenzyl ether), respectively. The mixture was diluted with dichloromethane and filtered. The solvent was removed from the filtrate at a bath temperature below 40 °C (critical!) and the residue was passed through a column of silica gel (100 g) with (4:1) dichloromethane–ethyl acetate as eluant. Fractions containing the major product contaminated with benzyl alcohol were pooled, and the solvent was removed below 40 °C. To the residue dissolved in pyridine (10 ml) and cooled to 0 °C was added acetic anhydride, and the mixture was kept in ice–water for 2 h. Solvents were evaporated below 40 °C, the last traces being removed by co-evaporation with toluene. The residue was submitted to column chromatography with (5:1) dichloromethane–ethyl acetate as eluant. Benzyl acetate was well separated from the *product* (**4**) which was obtained as an oil after removal of the solvent from the pooled fractions (2.53 g, 43%) (Found: C, 65.9; H, 8.3; N, 2.4. $C_{33}H_{49}NO_9$ requires C, 65.65; H, 8.2; N, 2.3%). The ^1H n.m.r. spectrum, in particular δ_H 5.2 (1 H, t, $J_{3,4} = J_{2,3}$ = 2 Hz, 3-H) and 5.9 (1 H, d, $J_{1,2}$ 7 Hz, 1-H), confirmed the proposed structure.

Allyl 3,4,6-*Tri*-O-*acetyl*-2-[(3R)-3-*benzyloxytetradecanamido*]-2-*deoxy*-β-D-*glucopyranoside* (**7**).—Toluene-*p*-sulphonic acid (80 mg) and allyl alcohol (2 ml, 4 mol equiv.) were added to a solution of the oxazoline (**4**) (4 g) in a mixture (1:1) of toluene and dichloromethane (50 ml). The stirred mixture was kept at 50 °C overnight. Pyridine (40 μl) was added, the solvents were removed, and the residue was submitted to column chromatography [(7:7:1) diethyl ether–toluene–methanol]. Fractions containing the glycoside (t.l.c., same solvent; R_F 0.58) were pooled, the solvent was removed, the residue was taken up in ethyl acetate, and the *glycoside* (**7**) (2.75 g, 63%) was precipitated as a solid by addition of hexane (Found: C, 65.3; H, 8.45; N, 2.1. $C_{36}H_{55}NO_{10}$ requires C, 65.3; H, 8.4; N, 2.1%); $[\alpha]_D^{25}$ −14.4° (*c* 1 in $CHCl_3$); δ_H (90 MHz; $CDCl_3$) *inter alia* 4.45 (2 H, s, CH_2Ph), 4.55 (1 H, d, $J_{1,2}$ 8 Hz, 1-H), 4.8—5.3 (4 H, m, 3-H, 4-H, and $CHCH_2$), 5.8 (1 H, m, $CH_2CH{=}CH_2$), 6.45 (1 H, d, *J* 9 Hz, NH), and 7.3 (5 H s, Ph).

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