Carbohydrate Research, 180 (1988) 195–205
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands

SYNTHESIS OF METHYL 3-[3-(2-*O*-α-L-RHAMNOPYRANOSYL-α-L-RHAMNOPYRANOSYLOXY)DECANOYLOXY]DECANOATE, A RHAMNOLIPID FROM *Pseudomonas aeruginosa*

PIETER WESTERDUIN, PAUL E. DE HAAN, MICHEL J. DEES, AND JACQUES H. VAN BOOM*
Gorlaeus Laboratories, P. O. Box 9502, 2300 RA Leiden (The Netherlands)
(Received October 26th, 1987; accepted for publication, December 18th, 1987)

ABSTRACT

Condensation of (3*R*)-3-levulinoyloxydecanoic acid (**16**) with methyl (3*R*)-3-hydroxydecanoate (**13**) afforded, after selective delevulinoylation, methyl (3*R*)-3-[(3*R*)-3-hydroxydecanolyloxy]decanoate (**18**). Boron trifluoride etherate-promoted coupling of 3,4-di-*O*-benzyl-2-*O*-chloroacetyl-α-L-rhamnosyl fluoride (**11**) with **18** yielded exclusively an α-glycoside **19**, which, after removal of the chloroacetyl group, coupling with **11**, and removal of the protecting groups, afforded the title rhamnolipid **1**.

INTRODUCTION

Pseudomonas aeruginosa produces rhamnolipids which are mainly released extracellularly during the stationary growth phase or conditions that are growth-limiting[1,2]. These rhamnolipids are reported to be bactericidal, mycoplasmacidal, and antiviral[3], and play a possible role in the pathogenesis of *Pseudomonas* infections[2,4]. One of the rhamnolipids has been identified[5] as methyl 3-[3-(2-*O*-α-L-rhamnopyranosyl-α-L-rhamnopyranosyloxy)decanoyloxy]decanoate (**1**) and we now report a convenient synthesis of **1**, containing (3*R*)-3-hydroxydecanoic acid residues.

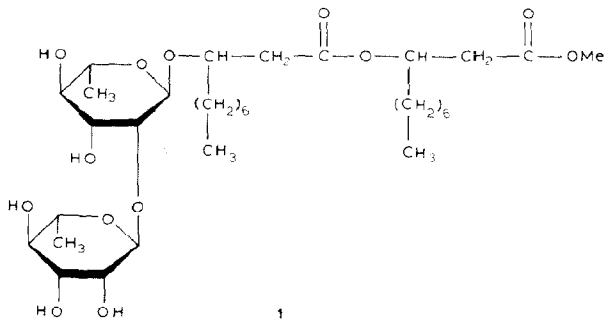
RESULTS AND DISCUSSION

The strategy adopted for the synthesis of **1** involved (*a*) the preparation of the glycosyl donor **11**, which allowed stereoselective formation of the required α-glycosidic bonds and selective deblocking of HO-2, (*b*) the preparation of the lipid aglycon **18**, and (*c*) the coupling of **11** to **18** and **20**.

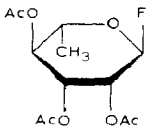
The ester function at position 2 in **11** was selected because it should promote the formation of the 1,2-*trans* glycosidic linkage[6]. An acetyl group, as used by Bundle and co-workers[7], is not suitable since the lipid moiety of **1** contains two ester functions which will react under the conditions necessary for *O*-deacetylation.

*Author for correspondence

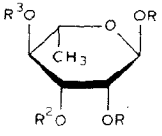
0008-6215/88/$03.50



1

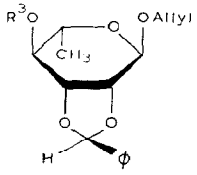


2



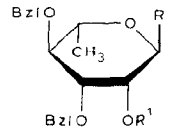
3 R = allyl, $R^1 = R^2 = R^3 = Ac$

4 R = allyl, $R^1 = R^2 = R^3 = H$



5 $R^3 = H$

6 $R^3 = Bzl$



7 R = O-allyl, $R^1 = H$

8 R = O-allyl, $R^1 = ClCH_2CO$

9 R = O-prop-1-enyl, $R^1 = ClCH_2CO$

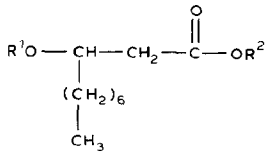
10 R = OAc, $R^1 = ClCH_2CO$

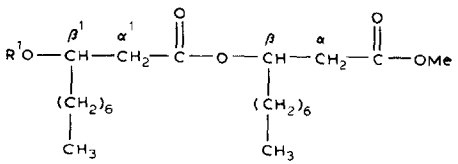
11 R = F, $R^1 = ClCH_2CO$

Therefore, the chloroacetyl group was selected since it can be removed under neutral conditions and, to our knowledge, does not decrease the reactivity of the glycosyl donor during coupling reactions[8].

Treatment of 2,3,4-tri-*O*-acetyl-α-L-rhamnosyl fluoride[9] (**2**) with allyl alcohol in the presence of boron trifluoride etherate[10] afforded the allyl glycoside **3**. Zemplén deacetylation of **3** followed by acid-catalysed reaction of the product **4** with benzaldehyde dimethyl acetal gave **5**. Synthesis of **4** by the Fischer procedure is more straightforward, but the route selected was used to determine whether a rhamnopyranosyl fluoride would suppress the formation of orthoester under Koenigs-Knorr-like conditions[11,12]. Benzylation of **5** afforded crystalline allyl 4-*O*-benzyl-*exo*-2,3-*O*-benzylidene-α-L-rhamnopyranoside (**6**, 72% from **2**). Hydrogenolysis of the *exo*-2,3-benzylidene acetal of **6** with $LiAlH_4$–$AlCl_3$ yielded the 3,4-di-*O*-benzyl derivative[13] **7**, which, with chloroacetic anhydride in the presence of sodium hydrogencarbonate[14], furnished the chloroacetate **8** (71% from **6**). Iridium(I)-catalysed isomerisation[15] of the allyl glycoside **8** afforded the prop-1-enyl glycoside **9**, hydro-

lysis[16] of which followed by treatment with diethylaminosulfur trifluoride (DAST)[17] gave the glycosyl fluoride **11** (64% of a 2:1 $\alpha\beta$-mixture). On the other hand, treatment of the 1-acetate **10** with hydrogen fluoride in pyridine[18] gave exclusively the α-rhamnosyl fluoride **11** (81% islolated yield).

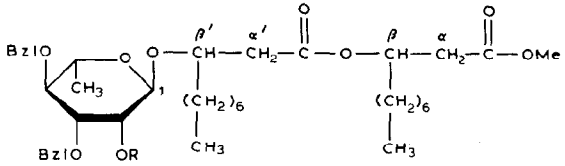




12 $R^1 = R^2 = H$
13 $R^1 = H, R^2 = Me$
14 $R^1 = H, R^2 = PhCOCH_2$
15 R^1 = levulinoyl, $R^2 = PhCOCH_2$
16 R^1 = levulinoyl, $R^2 = H$

17 R^1 = levulinoyl
18 $R^1 = H$

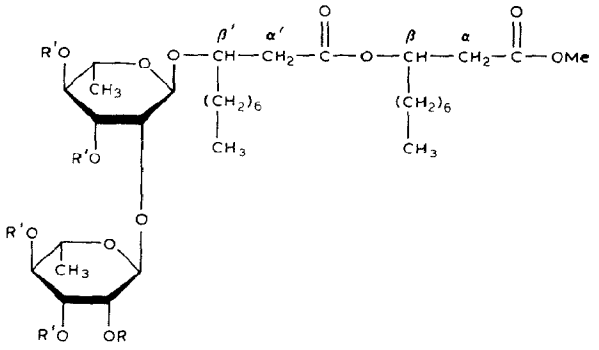
The lipid moiety **18** was obtained from optically pure (3*R*)-3-hydroxydecanoic acid[19] (**12**). Treatment of **12** with diazomethane afforded the methyl ester **13**. Reaction of **12** with phenylacetyl bromide in the presence of triethylamine yielded **14**, which was reacted[20] with levulinic (4-oxopentanoic) anhydride[21] in pyridine in the presence of 4-dimethylaminopyridine to give **15**. Removal of the phenylacetyl group from **15** with activated zinc dust[22] in acetic acid afforded **16**, which was condensed with **13** in the presence of di-isopropylcarbodi-imide and 4-dimethylaminopyridine[23] to give methyl (3*R*)-3-[(3*R*)-3-levulinoyloxydecanoyloxy]decanoate (**17**, 60% from **12**). Selective removal of the levulinoyl group from **17** with hydrazine in pyridine–acetic acid afforded the 3′-hydroxy compound **18**.



19 R = $ClCH_2CO$
20 R = H

Boron trifluoride etherate-promoted coupling of **18** with **11** gave the glycoside **19** (83% isolated yield). The exclusive formation of an α-glycosidic bond was confirmed by ^{13}C- and ^1H-n.m.r. spectroscopy. The $J_{C\text{-}1,H\text{-}1}$ value (170 Hz) for the anomeric carbon is characteristic[24] of an α-L-rhamnopyranoside. Selective removal of the chloroacetyl group from **19** with hydrazine dithiocarbonate[25] afforded 95% of

20, which was condensed with **11** as for **18** to give 80% of the rhamnolipid derivative **21**. Treatment of **21** with hydrazine dithiocarbonate removed the chloroacetyl group and hydrogenolysis (Pd/C) furnished **1** as a waxy solid, the identity and homogeneity of which were established by ^{13}C- and ^{1}H-n.m.r. spectroscopy. The following ^{13}C-resonances are particularly relevant: C-1 and C-1′ at 99.40 and 103.87 p.p.m., the low-field resonance at 80.13 p.p.m. which is characteristic[26–28] for C-2, and the resonances at 75.51, 74.02, and 73.67 p.p.m. which are assigned to C-4, C-β', and C-4′, respectively.



21 R = $ClCH_2CO$, R′ = Bzl

22 R = H, R′ = Bzl

EXPERIMENTAL

T.l.c. was performed on Silica Gel F 1500LS254 (Schleicher & Schüll), with detection by charring with sulfuric acid or by spraying with 1% potassium permanganate in aqueous 5% potassium carbonate for compounds containing an alkenic component. Column chromatography was carried out on Kieselgel 60 (Merck, 230–400 mesh). Evaporations were performed at 40° under reduced pressure. Optical rotations were measured at 20° with a Perkin–Elmer 241 polarimeter. N.m.r. spectra (internal Me_4Si) were recorded with a Jeol JNM-FX (^{13}C, 50.1 MHz, internal Me_4Si or MeOH) or Bruker WM-300 spectrometer equipped with an ASPECT-2000 computer (^{1}H, 300 MHz, internal Me_4Si).

Allyl 2,3,4-tri-O-acetyl-α-L-rhamnopyranoside (**3**). — To a stirred solution of **2**[9] (13.25 mmol) and allyl alcohol (20 mmol) in dichloromethane (50 mL) containing molecular sieves (4 Å, 15 g) was added, dropwise, boron trifluoride etherate (1.55 mL) at room temperature. The mixture was stirred for 20 min, diluted with dichloromethane (50 mL), filtered, washed with aqueous sodium hydrogenencarbonate (50 mL) and water (50 mL), dried ($MgSO_4$), and concentrated. Column chromatography (dichloromethane–acetone, 100:0→ 98:2) of the residue gave **3** as a colourless oil (4.28 g, 98%), $[\alpha]_D$ −53° (*c* 1.4 chloroform), R_F 0.42 (97:3 dichloro-

methane–acetone). N.m.r. data ($CDCl_3$): ^1H, δ 3.90 (m, 1 H, $J_{4,5}$ 9.8 Hz, H-5), 4.78 (d, 1 H, $J_{1,2}$ 1.6 Hz, H-1), 5.07 (dd, 1 H, $J_{3,4}$ 10 Hz, H-4), 5.32 (dd, 1 H, H-3), 5.88 (cm, 1 H, C*H*=CH_2); ^{13}C, δ 17.37 (C-6), 20.70, 20.79, 20.91 (3 $COCH_3$), 96.48 (C-1), 69.40, 117.36, 133.77 (allyl).

Allyl exo-*2,3*-O-*benzylidene*-α-L-*rhamnopyranoside* (**5**) *and the 4*-O-*benzyl derivative* (**6**). — To a solution of **3** (2.74 g) in dry methanol (20 mL) was added potassium *tert*-butoxide (15 mg), and the mixture was stirred for 30 min at room temperature, then neutralised with Dowex 50W (H^+) resin (100–200 mesh), filtered, and concentrated. A solution of the residue **4** in dry *N,N*-dimethylformamide (25 mL) was stirred with benzaldehyde dimethyl acetyl (2.25 mL) and toluene-*p*-sulfonic acid (10–20 mg) for 2 h, then neutralised with triethylamine, and concentrated. Column chromatography (3→5% acetone in dichloromethane) of the residue gave **5** (2.20 g, 91%). $[\alpha]_D$ −75° (*c* 1, chloroform). ^1H-N.m.r. data ($CDCl_3$); δ 1.355 (d, 3 H, $J_{5,6}$ 6.2 Hz, Me-5), 3.571 (dd, 1 H, $J_{3,4}$ 7.3, $J_{4,5}$ 9.3 Hz, H-4), 3.756 (m, 1 H, H-5), 4.157 (d, 1 H, $J_{2,3}$ 5.3 Hz, H-2), 4.416 (dd, 1 H, H-3), 5.062 (s, 1 H, H-1), 5.878 (m, 1 H, C*H*=CH_2), 6.124 (s, 1 H, C*H*Ph), 6.902–7.231 (m, 5 H, Ph).

Treatment of **5** (2.1 g, 7.19 mmol) with sodium hydride/benzyl bromide in *N,N*-dimethylformamide (20°, 3 h) gave a 90% yield of **6**, $[\alpha]_D$ −75° (*c* 1, chloroform), R_F 0.77 (95:5 dichloromethane–acetone). ^1H-N.m.r. data ($CDCl_3$): δ 1.334 (d, 3 H, $J_{5,6}$ 6.3 Hz, Me-5), 3.337 (dd, 1 H, $J_{3,4}$ 7.2, $J_{4,5}$ 9.8 Hz, H-4), 3.787 (m, 1 H, H-5), 4.166 (d, 1 H, $J_{2,3}$ 5.5 Hz, H-2), 4.626 (dd, 1 H, H-3), 4.691–4.981 (AB, CH_2Ph), 5.042 (s, 1 H, H-1), 5.886 (m, 1 H, C*H*=CH_2), 6.011 (s, 1 H, C*H*Ph), 7.38–7.26 (10 H, 2 Ph).

Allyl 3,4-di-O-*benzyl*-α-L-*rhamnopyranoside* (**7**). — To a solution of **6** (2.4 g, 6.28 mmol) in a mixture of dry ether and dichloromethane (30 mL, 1:1) was added lithium aluminium hydride (8 mmol), and the mixture was heated to reflux. A solution of aluminium chloride (8 mmol) in dry ether (15 mL) was added dropwise and stirring was continued under reflux for 30 min. The mixture was cooled, and methanol was added, followed by water until precipitation was complete. The mixture was filtered, diluted with dichloromethane (100 mL), washed with water (3 × 50 mL), dried ($MgSO_4$), and concentrated. Colomn chromatography (1 → 3% of acetone in dichlomethane) of the residue afforded **7** (1.90 g, 79%), $[\alpha]_D$ −35° (*c* 1, chloroform), R_F 0.45 (95:5 dichloromethane–acetone). N.m.r. data ($CDCl_3$): ^1H, δ 1.304 (d, 3 H, $J_{5,6}$ 6.2 Hz, Me-5), 2.505 (d, 1 H, OH), 3.426 (t, 1 H, $J_{3,4} = J_{4,5}$ = 9.3 Hz, H-4), 3.742 (m, 1 H, H-5), 3.852 (dd, 1 H, $J_{2,3}$ 3.4 Hz, H-3), 4.032 (m, 1 H, $J_{1,2}$ 1.7 Hz, H-2), 4.846 (d, 1 H, H-1), 7.27–7.33 (m, 10 H, 2 Ph); ^{13}C, δ 17.87 (C-6), 67.31, 67.80, 68.53, 71.69, 75.37, 79.77, 79.92 (C-2/5, CH_2Ph, CH_2–CH=), 98.08 (C-1), 113.91–138.41 (Ph), 117.36 (CH_2=), 133.77 (CH=).

Allyl 3,4-di-O-*benzyl-2*-O-*chloroacetyl*-α-L-*rhamnopyranoside* (**8**). — A suspension of **7** (4.7 mmol), chloroacetic anhydride (1.5 g), and sodium hydrogencarbonate (0.7 g) in *N,N*-dimethylformamide (50 mL) was stirred for 2 h at room temperature, then diluted with dichloromethane (75 mL), washed with aqueous 10%

sodium hydrogencarbonate (25 mL) and water (25 mL), dried ($MgSO_4$), and concentrated. Column chromatography (dichloromethane) of the residue gave **8** (1.94 g, 90%), $[\alpha]_D$ −41° (*c* 1, chloroform), R_F 0.52 (dichloromethane–acetone, 97:3). N.m.r. data ($CDCl_3$): ^1H, δ 1.328 (d, 3 H, $J_{5,6}$ 6.2 Hz, Me-5), 3.401 (t, 1 H, $J_{3,4} = J_{4,5}$ = 9.5 Hz, H-4), 3.786 (m, 1 H, H-5), 3.90–4.17 (m, 5 H, CH_2-CH=, H-3, CH_2Cl), 4.64 (AB, CH_2Ph), 4.70 (AB, CH_2Ph), 4.796 (d, 1 H, $J_{1,2}$ 1.8 Hz, H-1), 5.15-5.20 (m, 2 H, CH_2=CH), 5.443 (dd, 1 H, $J_{2,3}$ 3.3 Hz, H-2), 5.88 (m, 1 H, CH=CH_2, 7.25–7.35 (m, 10 H, 2 Ph); ^{13}C, δ 17.76 (C-6), 40.71 (CH_2Cl), 67.68 (C-5), 67.86 (CH_2-CH=), 70.66 (C-2), 71.77, 75.25 (2 CH_2Ph), 77.79 (C-3), 79.66 (C-4), 96.13 (C-1), 117.62 ($CH=CH_2$), 127.55–128.19, 137.62, 138.15 (Ph), 133.15 (-CH=CH_2), 166.62 (C=O).

Anal. Calc. for $C_{25}H_{29}ClO_6$: C, 65.1; H, 6.3. Found: C, 64.9; H, 6.3.

*Prop-1-enyl 3,4-di-*O*-benzyl-2-*O*-chloroacetyl-α-*L*-rhamnopyranoside* (**9**). — To a solution of **8** (4.23 mmol) in tetrahydrofuran (20 mL, freshly distilled from lithium aluminium hydride) was added 1,5-cyclo-octadienebis[methyldiphenylphosphine]iridium hexafluorophosphate (5 mg). The stirred solution was degassed, placed under hydrogen for 2 min, degassed, and placed under nitrogen. After 2 h, the solvent was evaporated and the catalyst was removed by short-column chromatography (dichloromethane), to give **9** (quantitative), R_F 0.56 (97:3 dichloromethane-acetone). N.m.r. data ($CDCl_3$): ^1H, δ 1.501 (dd, 3 H, =CH-CH_3), 5.105 (dq, *J* 12.3 and 6.2 Hz, =CH-CH_3), 6.186 (dq, *J* 12.3 and 1.3 Hz, OCH=); ^{13}C, δ 12.31, 104.82, 143.81 (3 C, prop-1-enyl).

*1-*O*-Acetyl-3,4-di-*O*-benzyl-2-*O*-chloroacetyl-*L*-rhamnopyranose* (**10**). — A solution of **9** (1.84 g) in aqueous 90% acetone (15 mL) was stirred for 2 h in the presence of mercury(II) oxide (4.28 mmol) and mercury(II) chloride (4.28 mmol). The mixture was filtered through Celite, diluted with dichloromethane (50 mL), washed with aqueous potassium iodide (4 × 25 mL) and water (25 mL), dried ($MgSO_4$), and concentrated. The residue was stirred with pyridine (20 mL) and Ac_2O (10 mL) for 16 h at 25°, and then concentrated *in vacuo*. Column chromatography (97:3 dichloromethane–acetone) of the residue gave **10** (1.61 g, 87%), R_F 0.48 (97:3 dichloromethane–acetone). ^{13}C-N.m.r. data ($CDCl_3$): δ 17.55 (C-6), 20.33 (CH_3), 40.33 (CH_2Cl), 69.15, 69.58 (C-2,5), 71.54 (CH_2Ph), 75.01 (CH_2Ph), 77.06 (C-3), 78.75 (C-4), 90.23 (C-1), 127.37–127.95, 137.15, 137.71 (Ph), 166.05, 167.93 (2 C=O).

*3,4-Di-*O*-benzyl-2-*O*-chloroacetyl-α-*L*-rhamnopyranosyl fluoride* (**11**). — A solution of 70% HF in pyridine (1.7 mL) at 0° was added to a solution of **10** (0.775 g, 1.68 mmol) in dichloromethane (2 mL). The mixture was kept for 2 h at 20°, then poured into ice-cold M potassium fluoride (25 mL), and extracted with dichloromethane (2 × 50 mL). The combined extracts were dried (Na_2SO_4) and concentrated. Column chromatography (1:1 ether–light petroleum) of the residue afforded **11** (0.667 g, 94%), $[\alpha]_D$ +3.8° (*c* 1, chloroform), R_F 0.63 (1:1 ether–light petroleum). N.m.r. data (C_6D_6): ^1H, δ 1.356 (d, 3 H, $J_{5,6}$ 6.2 Hz, Me-5), 3.69 (m, 3 H, H-4, CH_2Cl), 4.082 (dd, 1 H, $J_{2,3}$ 3.2, $J_{3,4}$ 9.3 Hz, H-3), 4.194 (m, 1 H, H-5), 5.531

(dd, 1 H, $J_{1,F}$ 49.1, $J_{1,2}$ 1.8 Hz, H-1), 5.701 (dd, 1 H, $J_{2,3}$ 3.2 Hz, H-2), 7.25–7.38 (m, 10 H, 2 Ph); ^{13}C ($CDCl_3$), δ 16.75 (C-6), 39.58 (CH_2Cl), 67.96 (d, $J_{2,F}$ 41.0 Hz, C-2), 69.29 (d, $J_{5,F}$ 2.9 Hz, C-5), 71.1, 74.40 (2 CH_2Ph), 75.96 (d, $J_{3,F}$ 2.2 Hz, C-3), 77.66 (C-4), 103.81 (d, $J_{1,F}$ 219.1 Hz, C-1), 126.77–127.41, 136.34, 136.96 (2 Ph), 165.43 ($COCH_2Cl$).

Anal. Calc. for $C_{22}H_{24}ClFO_5$: C, 62.5; H, 5.7. Found: C, 62.4; H, 5.7.

Methyl (3R)-3-hydroxydecanoate (**13**). — A M solution of diazomethane in dichloromethane (10.5 mL) was added dropwise to a solution of **12** (1.88 g, 10 mmol) in dichloromethane. After 5 min, acetic acid (0.5 mL) was added, the mixture was concentrated, and toluene was evaporated twice from the residue. Column chromatography (dichloromethane) of the residue gave **13** (1.96 g, 97%), $[\alpha]_D$ −20° (*c* 1, chloroform), R_F 0.64 (95:5 dichloromethane–methanol). ^{13}C-N.m.r. data ($CDCl_3$): δ 13.52 (CH_3), 22.19, 25.08, 28.82, 29.08, 31.39, 36.38 [-$(CH_2)_6$-], 41.23 (Cα), 51.10 (OCH_3), 67.54 (Cβ), 172.80 (CO).

Phenacyl (3R)-3-hydroxydecanoate (**14**). — To a solution of **12** (3 g, 15.9 mmol) and phenylacyl bromide (3.58 g, 18 mmol) in ethyl acetate (100 mL) was added, dropwise, triethylamine (5.5 mL). After stirring for 3.5 h, the mixture was filtered, extracted with water, dried ($MgSO_4$), and concentrated. Column chromatography (2→5% acetone in dichloromethane) of the residue gave **14**, R_F 0.46 (95:5 dichloromethane–methanol). ^{13}C-N.m.r. data ($CDCl_3$): δ 13.90 (CH_3), 22.54, 25.49, 29.14, 29.38, 31.68, 36.47 [-$(CH_2)_6$-], 42.02 (Cα), 65.90 (CH_2COPh), 68.33 (Cβ), 127.72, 128.80, 133.70, 134.06 (Ph), 171.84 (CO), 192.46 (COPh).

Phenacyl (3R)-3-levulinoyloxydecanoate (**15**). — A mixture of **14** (2.75 g, 9 mmol), levulinic (4-oxopentanoic) anhydride (20 mmol), and 4-dimethylaminopyridine (10 mg) in pyridine–1,4-dioxane (70 mL, 1:1) was stirred for 30 min at 20°. Water (2.5 mL) was added, the mixture was concentrated, and a solution of the residue in dichloromethane (75 mL) was washed with saturated aqueous sodium hydrogencarbonate (30 mL) and water (30 mL), dried ($MgSO_4$), and concentrated. Column chromatography (dichloromethane) of the residue yielded **15** (3.34 g, 9%), $[\alpha]_D$ +3.5° (*c* 1, chloroform), R_F 0.41 (25:1 toluene–acetone). ^{13}C-N.m.r. data ($CDCl_3$): δ 13.87 (CH_3), 22.58, 25.51, 29.16, 29.40, 31.69, 36.44 [-$(CH_2)_6$-], 27.71, 29.23, 37.49 (levulinoyl), 42.06 (Cα), 65.69 (CH_2COPh), 70.24 (Cβ), 127.71, 128.79, 133.69, 134.05 (Ph), 171.79 (CO), 174.70 (CO), 192.41 (COPh), 206.79 (CO).

(3R)-3-Levulinoyloxydecanoic acid (**16**). — A solution of **15** (3.15 g, 7.8 mmol) in acetic acid (50 mL) was stirred with activated zinc dust for 1.5 h at 20°, then filtered, diluted with dichloromethane (100 mL), washed with water (3 × 50 mL), dried ($MgSO_4$), and concentrated. Column chromatography (2→6% methanol in dichloromethane) of the residue afforded **16** (1.96 g, 88%), $[\alpha]_D$ −2.8° (*c* 1, chloroform), R_F 0.57 (96:4 dichloromethane–methanol). ^{13}C-N.m.r. data ($CDCl_3$): δ 13.64 (CH_3), 22.19, 24.62, 28.70, 28.85, 31.30, 33.46 [-$(CH_2)_6$-], 27.71, 29.23, 37.49 (levulinoyl), 38.40 (Cα), 70.25 (Cβ), 171.78 (CO), 174.70, 206.79 (2 CO levulinoyl).

Methyl (3R)-3-[(3R)-3-levulinoyloxydecanoyloxy]decanoate (**17**). — To a

solution of **13** (2.76 mmol) and **16** (2.51 mmol) in dichloromethane (10 mL) was added di-isopropylcarbodi-imide (2.60 mmol). The mixture was stirred for 5 min under nitrogen and a solution of 4-dimethylaminopyridine (1.25 mmol) in dichloromethane (2 mL) was added dropwise. The mixture was stirred for 6 h, diluted with chloromethane (50 mL), washed with aqueous sodium hydrogencarbonate and water, dried ($MgSO_4$), and concentrated. The residue was eluted from a column of Sephadex LH-20 with 2:1 dichloromethane–methanol to give **17** (1.00 g, 85%), $[\alpha]_D$ −0.7° (*c* 1, chloroform), R_F 0.60 (95:5 dichloromethane–acetone). ^{13}C-N.m.r. data ($CDCl_3$): δ 13.73 (2 CH_3), 22.28-33.61 [2 -$(CH_2)_6$-], 27.80, 29.41, 37.58 (levulinoyl), 38.52 (C′α), 38.87 (Cα), 51.33 (OCH_3), 70.40 (C′β), 70.49 (Cβ), 169.30, 170.35, 171.61 (3 OCO), 205.95 (CO).

Methyl (3R)-*3*-[(3R)-*3-hydroxydecanoyloxy]decanoate* (**18**). — To a solution of **17** (0.650 g, 1.38 mmol) in dichloromethane (10 mL) and pyridine (12 mL) was added 6:4:0.5 pyridine–acetic acid–hydrazine hydrate (11.3 mL), and the mixture was stirred for 10 min at 20°. Dichloromethane (150 mL) was added, and the mixture was washed with water (2 × 75 mL), aqueous sodium hydrogencarbonate (75 mL), and water, dried ($MgSO_4$), and concentrated. Column chromatography (2:1 light petroleum–ether) of the residue afforded **18** (0.44 g, 86%), $[\alpha]_D$ −9.7° (*c* 1, chloroform), R_F 0.43 (2:1 light petroleum–ether). ^{13}C-N.m.r. data ($CDCl_3$): δ 13.64, 13.84 (2 CH_3), 22.43–38.69 [2 -$(CH_2)_6$-], 38.78 (C′α), 41.76 (Cα), 51.63 (OCH_3), 68.07 (C′β), 70.58 (Cβ), 172.13, 173.28 (2 OCO).

Anal. Calc for $C_{21}H_{40}O_5$: C, 67.7; H, 10.8. Found: C, 67.9; H, 10.9.

Methyl (3R)-*3*-[(3R)-*3*-(*3,4-di*-O-*benzyl*-*2*-O-*chloroacetyl*-α-L-*rhamnopyranosyloxy)decanoyloxy]decanoate* (**19**). — To a stirred mixture of **11** (325 mg, 0.77 mmol), **18** (250 mg, 0.67 mmol), and powdered molecular sieves (4 Å, 250 mg) in dichloromethane (5 mL) was added boron trifluoride etherate (1 mmol) at 0°. The mixture was stirred for 30 min at 20°, then diluted with dichloromethane (30 mL), filtered, washed with aqueous sodium hydrogencarbonate (15 ml) and water (15 mL), dried ($MgSO_4$), and concentrated. The residue was eluted from a column of Sephadex LH-20 with 2:1 dichloromethane–methanol to give **19** (430 mg, 83%), $[\alpha]_D$ −17° (*c* 0.6, chloroform). N.m.r. data ($CDCl_3$): ^{1}H, δ 1.25–1.35 [m, 23 H, 2 -$(CH_2)_5$-, CH_3], 1.51–1.59 (m, 4 H, 2 CH_2CH), 2.40–2.55 (m, 4 H, 2 CH_2CO), 3.381 (t, 1 H, $J_{3,4} = J_{4,5}$ = 9.4 Hz, H-4), 3.612 (s, 3 H, OMe), 3.825 (m, 1 H, H-5), 3.92 (dd, 1 H, $J_{2,3}$ 3.2 Hz, H-3), 4.043 (m, 1 H, Hβ), 4.163 (s, 2 H, CH_2Cl), 4.61 (AB, 2 H, CH_2Ph), 4.73 (AB, 2 H, CH_2Ph), 4.862 (d, 1 H, $J_{1,2}$ 1.8 Hz, H-1), 5.190 (m, 1 H, Hβ), 5.336 (dd, 1 H, $J_{2,1}$ 1.8, $J_{2,3}$ 3.2 Hz, H-2), 7.22 – 7.32 (m, 10 H, 2 Ph); ^{13}C, δ 13.87 (2 CH_3), 17.55 (C-6), 22.40–33.61 [2 -$(CH_2)_6$-], 38.60 (C′α), 39.92 (Cα), 40.68 (CH_2Cl), 51.42 (OMe), 67.68 (C-5), 70.55 (Cβ), 70.98 (C-2), 71.69 (CH_2Ph), 74.63 (C′β), 75.01 (CH_2Ph), 77.58 (C-3), 79.51 (C-4), 96.04 (C-1), 127.34–128.10, 137.56, 138.17 (Ph), 166.58 ($COCH_2Cl$), 170.09, 170.41 (2 CO).

Anal. Calc. for $C_{43}H_{63}ClO_{10}$: C, 66.6; H, 8.2. Found: C, 66.9; H, 8.3.

Methyl (3R)-*3*-[(3R)-*3*-(*3,4-di*-O-*benzyl*-α-L-*rhamnopyranosyloxy)decanoyloxy]decanoate* (**20**). — To a solution of **19** (0.55 mmol) in lutidine (4 mL) and acetic

acid (1.35 mL) was added 4.4 mL of freshly prepared 0.375M hydrazine dithiocarbonate[25]. The mixture was stirred for 20 min at 20°, diluted with dichloromethane (50 mL), washed with water (2 × 25 mL), dried ($MgSO_4$), and concentrated. Column chromatography (98:2 dichloromethane–acetone) of the residue afforded **20** (364 mg, 95%), $[\alpha]_D$ −26° (*c* 1, chloroform), R_F 0.58 (97:3 dichloromethane–acetone). ^{13}C-N.m.r. data ($CDCl_3$): δ 13.96 (2 CH_3), 17.64 (C-6), 22.48–33.76 [2 $-(CH_2)_5-$], 38.75 (C′α), 40.15 (Cα), 51.54 (OCH_3), 67.56 (C-5), 68.76 (C-2), 70.56 (Cβ), 71.86 (CH_2Ph), 74.31 (C′β), 75.10 (CH_2Ph), 79.83 (C-3,4), 98.11 (C-1), 127.46–128.33, 137.95, 138.38 (Ph), 170.35, 170.62 (2 CO).

Methyl (3R)-*3*-{(3R)-*3*-[*3,4-di*-O-*benzyl*-*2*-O-(*3,4-di*-O-*benzyl*-*2*-O-*chloroacetyl*-α-L-*rhamnopyranosyl*)-α-L-*rhamnopyranosyloxy*]*decanoyloxy*}*decanoate* (**21**). — To a stirred mixture of **11** (0.75 mmol), **20** (0.50 mmol), and molecular sieves (4 Å) in dichloromethane (4 mL) was added BF_3Et_2O (0.80 mmol) at 0°. The mixture was stirred for 15 min at 20°, diluted with dichloromethane (30 mL), filtered, washed with aqueous sodium hydrogencarbonate (15 mL) and water (15 mL), dried, and concentrated. The residue was eluted from a column of Sephadex LH-20 with dichloromethane–methanol (2:1), to give **21** (440 mg, 80%), $[\alpha]_D$ −16° (*c* 1, chloroform), R_F 0.65 (2:1 light petroleum–ether). N.m.r. data ($CDCl_3$): ^{1}H, δ 0.88 (m, 6 H, 2 CH_3), 1.25–1.35 [m, 23 H, 2 $-(CH_2)_5-$, CH_3], 1.53–1.61 (m, 4 H, 2 CH_2CH), 2.30–2.9 (m, 4 H, 2 CH_2CO), 3.33–3.42 (m, 2 H, H-4,4′), 3.61 (s, 3 H, OMe), 3.723 (m, 1 H, $J_{4,5}$ 9.4, $J_{5,6}$ 6.2 Hz, H-5), 3.78–3.82 (m, 2 H, H-3,5), 3.901 (dd, 1 H, $J_{1,2}$ 2.0, $J_{2,3}$ 2.9 Hz, H-2), 3.97 (m, 1 H, H′β), 3.962 (dd, 1 H, $J_{2',3'}$ 3.3, $J_{3',4'}$ 9.3 Hz, H-3′), 4.106 (AB, 2 H, J_{AB} 16 Hz, CH_2Cl), 4.783 (d, 1 H, $J_{1,2}$ 2.0 Hz, H-1), 4.53–4.90 (m, 8 H, 4 CH_2Ph), 4.963 (d, 1 H, $J_{1',2'}$ 1.8 Hz, H-1′), 5.196 (m, 1 H, Hβ), 5.567 (dd, 1 H, H-2′), 7.21–7.34 (m, 20 H, 4 Ph); ^{13}C, δ 13.98 (2 CH_3), 17.78 (C-6,6′), 22.51–33.87 [2 $-(CH_2)_5-$], 38.78 (Cα), 40.21 (C′α), 40.82 (CH_2Cl), 67.62, 68.12 (C-5,5′), 70.63 (Cβ), 71.13 (C-2′), 71.86, 72.04 (2 CH_2Ph), 74.34 (C′β), 74.72 (C-2), 75.07, 75.22 (2 CH_2Ph), 77.71 (C-3′), 79.74, 79.83 (C-3,4,4′), 98.63 (C-1), 99.65 (C-1′), 127.28–128.22, 137.68, 138.20 (Ph), 166.35 ($COCH_2Cl$), 170.26, 170.59 (2 CO).

Anal. Calc. for $C_{63}H_{85}ClO_{14}$: C, 68.7; H, 7.8. Found: C, 68.6; H, 7.8.

Methyl (3R)-*3*-{(3R)-*3*-[*3,4-di*-O-*benzyl*-*2*-O-(*3,4-di*-O-*benzyl*-α-L-*rhamnopyranosyl*)-α-L-*rhamnopyranosyloxy*]*decanoyloxy*}*decanoate* (**22**). — A solution of **21** (385 mg, 0.35 mmol) was treated as described for the preparation of **20**, to give **22** (329 mg, 92%), $[\alpha]_D$ −32° (*c* 0.7, chloroform), R_F 0.45 (2:1 light petroleum–ether). ^{13}C-N.m.r. data ($CDCl_3$): δ 14.02 (2 CH_3), 17.78, 17.84 (C-6,6′), 22.54–33.78 [2 $-(CH_2)_5-$], 38.81 (C′α), 51.59 (OCH_3), 67.80 (C-5′), 68.18 (C-5), 68.62 (C-2′), 70.63 (Cβ), 72.06, 72.24 (2 CH_2Ph), 74.37 (C′β), 74.63 (C-2), 75.04, 75.28 (2 CH_2Ph), 79.48 (C-3′), 79.69 (C-4′), 79.92 (C-3), 80.21 (C-4), 98.14 (C-1), 100.59 (C-1′), 127.46–128.39, 138.29, 138.52 (Ph), 170.32, 170.64 (2 CO).

Methyl (3R)-*3*-[(3R)-*3*-(2-O-α-L-*rhamnopyranosyl*-α-L-*rhamnopyranosyloxy*)*decanoyloxy*]*decanoate* (**1**). — A solution of **22** (150 mg, 0.14 mmol) in *tert*-butyl alcohol (10 mL) containing acetic acid (0.5 mL) was hydrogenated in the presence of

10% Pd/C (125 mg) for 16 h, then filtered, and concentrated. The residue was eluted from a column of Sephadex LH-20 with 1:1 dichloromethane–methanol to give **1** as a waxy solid (65 mg, 70%), $[\alpha]_D$ −46° (*c* 1, methanol), R_F 0.45[5] (chloroform–methanol–water, 60:30:5). N.m.r. data (CD_3CN–D_2O, 2:1): ^1H, δ 0.82 (m, 6 H, 2 CH_3), 1.141, 1.152 (2 d, 6 H, $J_{5,6} = J_{5',6'} = 6.3$ Hz, 3 H-6, 3 H-6'), 1.21–1.37 [m, 20 H, 2 -$(CH_2)_5$-], 1.451 (m, 2 H, CH_2CH), 1.523 (m, 2 H, CH_2CH), 2.46–2.52 (m, 4 H, 2 CH_2CO), 3.261 (t, 1 H, $J_{3,4} = J_{4,5} = 9.5$ Hz, H-4), 3.278 (t, 1 H, $J_{3',4'} = J_{4',5'} = 9.5$ Hz, H-4), 9.51–3.61 (cm, 4 H, H-3,5,3',5'), 3.581 (s, 3 H, OMe), 3.687 (dd, 1 H, $J_{1',2'}$ 1.7, $J_{2',3'}$ 3.5 Hz, H-2'), 3.884 (dd, 1 H, $J_{1,2}$ 1.8, $J_{2,3}$ 3.4 Hz, H-2), 3.036 (m, 1 H, H'β), 4.783 (d, 1 H, $J_{1,2}$ 1.8 Hz, H-1), 4.821 (d, 1 H, $J_{1',2'}$ 1.7 Hz, H-1'), 5.113 (m, 1 H, Hβ); ^{13}C (CD_3OD), δ 14.31 (2 CH_3), 17.96 (C-6,6'), 23.42–34.78 [2 -$(CH_2)_5$-], 39.65 (Cα), 41.20 (C'α), 52.27 (OCH_3), 69.99 (C-5,5'), 71.65, 71.86, 71.95, 72.10 (C-2',3,3', Cβ), 73.67 (C-4'), 74.02 (C'β), 75.51 (C-4), 80.13 (C-2), 99.40 ($^1J_{C,H}$ 169 Hz, C-1), 103.87 ($^1J_{C,H}$ 170 Hz, C-1').

Anal. Calc. for $C_{33}H_{60}O_{13}$: C, 59.6; H, 9.1. Found: C, 59.5; H, 9.1.

REFERENCES

1 M. K. JOHNSON AND D. BOESE-MARAZZO, *Infect. Immunol.*, 29 (1980) 1028–1033.
2 C. SYLDATK, S. LANG, F. WAGNER, V. WRAY, AND L. WITTE, *Z. Naturforsch., Teil C*, 40 (1985) 51–60.
3 T. LEISINGER AND R. MARGRAFF, *Microbiol. Rev.*, 43 (1979) 422–442.
4 A. T. HASTIE, S. T. HINGLEY, M. L. HIGGINS, F. KUEPPERS, AND T. SHRYOCK, *Cell Motility Cytoskeleton*, 6 (1986) 502–509.
5 T. HIRAYAMA AND I. KATO, *FEBS Lett.*, 139 (1982) 81–85.
6 K. IGARASHI, *Adv. Carbohydr. Chem. Biochem.*, 34 (1977) 243–283; S. HANESSIAN AND J. BANOUB, *Carbohydr. Res.*, 53 (1977) c13–c16; B. HELFERICH AND K. WEIS, *Chem. Ber.*, 89 (1956) 314–321.
7 S. JOSEPHSON AND D. R. BUNDLE, *J. Chem. Soc., Perkin Trans. 1*, (1980) 297–301; B. M. PINTO, D. G. MOISETTE, AND D. R. BUNDLE, *ibid.*, (1987) 9–14.
8 H. PAULSEN, *Angew. Chem., Int. Ed. Engl.*, (1982) 155–194.
9 YA. V. VOZNY, I. S. KALICHEVA, AND A. A. GALOYAN, *Bioorg. Khim.*, 12 (1986) 521–526.
10 K. C. NICOLAOU, A. CHUCHOLOWSKI, R. E. DOLLE, AND J. L. RANDALL, *J. Chem. Soc., Chem. Commun.*, (1984) 1155–1156.
11 H. R. GOLDSCHMID AND A. S. PERLIN, *Can. J. Chem.*, 39 (1961) 2025–2029.
12 P. A. J. GORIN AND A. S. PERLIN, *Can. J. Chem.*, 37 (1959) 1930–1935.
13 A. LIPTÁK, P. FÜGEDI, AND P. NÁNÁSI, *Carbohydr. Res.*, 51 (1976) c19–c21.
14 G.J.F. CHITTENDEN AND H. REGELING, *Recl. Trav. Chim. Pays-Bas*, 106 (1987) 44–47.
15 J. J. OLTVOORT, C. A. A. VAN BOECKEL, J. H. DE KONING, AND J. H. VAN BOOM, *Synthesis*, (1981) 305–308.
16 R. GIGG AND C. D. WARREN, *J. Chem. Soc., C* (1968) 1903–1911.
17 G. H. POSNER AND S. R. HAINES, *Tetrahedron Lett.*, 26 (1985) 5–8.
18 M. HAYASHI, S. HASHIMOTO, AND R. NOYORI, *Chem. Lett.*, (1984) 1747–1750.
19 W. J. GOTTSTEIN AND L. C. CHENEY, *J. Org. Chem.*, 30 (1965) 2072–2073; M. DEMARY, G. PUZO, AND J. ASSELINEAU, *Nouv. J. Chim.*, 2 (1978) 373–378.
20 D. CHARON, R. CHABY, A. MALINVAUD, M. MONDAGNE, AND L. SZABÓ, *Biochemistry*, 24 (1985) 2736–2742.
21 H. J. KOENERS, J. VERHOEVEN, AND J. H. VAN BOOM, *Recl. Trav. Chim. Pays-Bas*, 100 (1981) 65–72.
22 J. H. VAN BOOM, P. M. J. BURGERS, G. VAN DER MAREL, C. H. M. VERDEGAAL, AND G. WILLE, *Nucl. Acids Res.*, 4 (1977) 1047–1063.
23 A. HASSNER AND V. ALEXANDER, *Tetrahedron Lett.*, 19 (1978) 4475–4478.

24 K. BOCK AND C. PEDERSEN, *J. Chem. Soc., Perkin Trans. 2,* (1974) 293–297. K. BOCK AND C. PEDERSEN, *Acta Chem. Scand., Ser B,* 29 (1975) 258–264.
25 C. A. A. VAN BOECKEL AND T. BEETZ, *Tetrahedron Lett.,* 24 (1983) 3775–3778.
26 R. KASAI, M. OKIHARA, J. ASAKAWA, K. MIZUTANI, AND O. TANAKA, *Tetrahedron,* 35 (1979) 1427–1432.
27 V. POZSGAY, P. NÁNÁSI, AND A. NESZEMÉLYI, *Carbohydr. Res.,* 90 (1981) 215–231.
28 J. H. BRADBURY AND G. A. JENKINS, *Carbohydr. Res.,* 126 (1984) 125–156.