An Efficient Synthesis of (*R*)-3-{(*R*)-3-[2-*O*-(α-L-Rhamnopyranosyl)-α-L-rhamnopyranosyl]oxydecanoyl}oxydecanoic Acid, a Rhamnolipid from *Pseudomonas Aeruginosa*

Howard I. Duynstee, Michiel J. van Vliet, Gijsbert A. van der Marel, and Jacques H. van Boom*

Leiden Institute of Chemistry, Gorlaeus Laboratories, University of Leiden,
P. O. Box 9502, NL-2300 RA Leiden, The Netherlands
Fax: (internat.) +31(0)71/5274307

Received October 27, 1997

Keywords: *Pseudomonas aeruginosa* / Rhamnolipid / One-pot two-step glycosylation / Chemoselectivity / Esterification / Carbohydrates

N-iodosuccinimide/triflic acid mediated one-pot two-step glycosylation of ethyl 2,3,4-tri-*O*-benzyl-1-thio-α-L-rhamnopyranoside (**8**) with phenyl 3,4-*O*-(2,3-dimethoxybutane-2,3-diyl)-1-thio-α-L-rhamnopyranoside (**10b**) and phenacyl (*R*)-3-hydroxydecanoate (**13**) gave rhamnolipid **17**. The latter was transformed in five steps into the title compound **2**. Esterification of **2** with diazomethane resulted into the corresponding methyl ester derivative **1**.

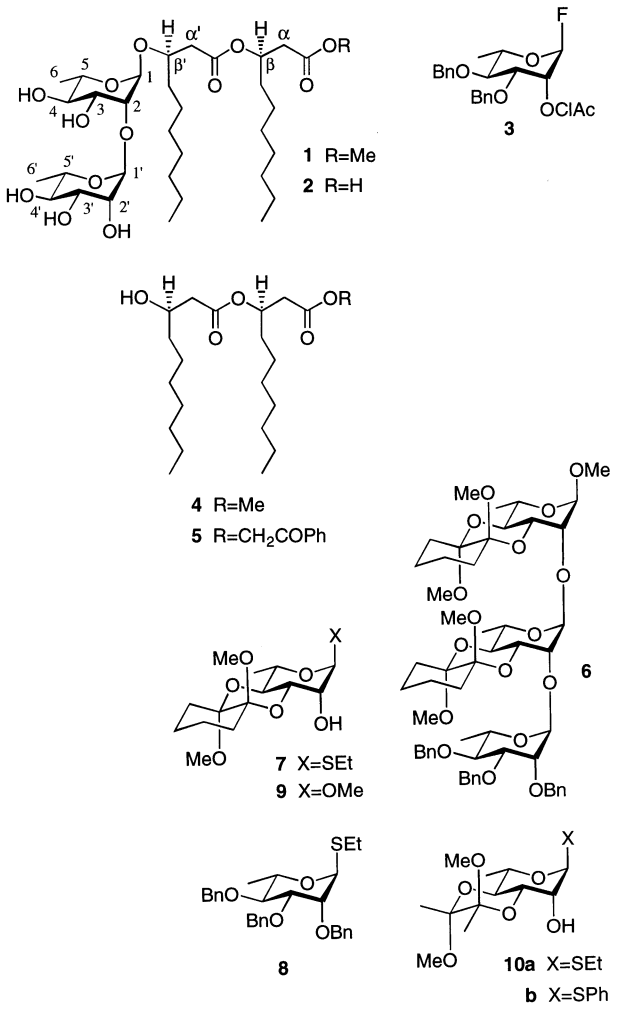
Introduction

It is well established[1] that the rhamnolipids methyl (*R*)-3-{(*R*)-3-[2-*O*-(α-L-rhamnopyranosyl)-α-L-rhamnopyranosyl]xydecanoyl}oxydecanoate (**1**) and the corresponding acid **2** are both produced by *Pseudomonas aeruginosa*. They exhibit interesting biological properties such as antibacterial, mycoplasmacidal, and antiviral activities[2]. In addition, compound **2** may also function as an immunomodulator of autoimmune diseases[3].

Several years ago we reported[4] the synthesis of rhamnolipid **1** from the building units 3,4-di-*O*-benzyl-2-*O*-chloroacetyl-α-L-rhamnosylpyranosyl fluoride (**3**) and methyl (*R*)-3-[(*R*)-3-hydroxydecanoyl]oxydecanoate (**4**). It is evident that the biologically interesting rhamnolipid **2** would be accessible in a similar fashion from **3** and phenacyl (*R*)-3-[(*R*)-3-hydroxydecanoyl]oxydecanoate (**5**). However, the synthesis of the target molecule **2** on a multigram scale is rather time-consuming due to the multi-step preparation of rhamnopyranosyl donor **3**.

Interestingly, Ley et al. revealed[5] that the fully protected methyl α-(1→2)-L-trirhamnoside **6** could be assembled stereospecifically in a one-pot two-step process by a sequential condensation of HO-2 and C-1 in the cyclohexane-1,2-diacetal (CDA) protected ethyl 1-thio-α-L-rhamnopyranoside (**7**) with ethyl 2,3,4-tri-*O*-benzyl-1-thio-α-L-rhamnopyranoside (**8**) and methyl 3,4-*O*-(1,2-dimethoxycyclohexane-1,2-diyl)-α-L-rhamnopyranoside (**9**). Later on, Frost et al. showed[6] that the butane-2,3-diacetal (BDA) group is an inexpensive and efficient alternative for the CDA group in the selective protection of vicinal diequatorial diols.

We here describe a convenient and straightforward synthetic route to rhamnolipid **2** starting from **8**, ethyl 3,4-*O*-(2,3-dimethoxybutane-2,3-diyl)-1-thio-α-L-rhamnopyranoside (**10a**) and phenacyl (*R*)-3-hydroxydecanoate (**13**).

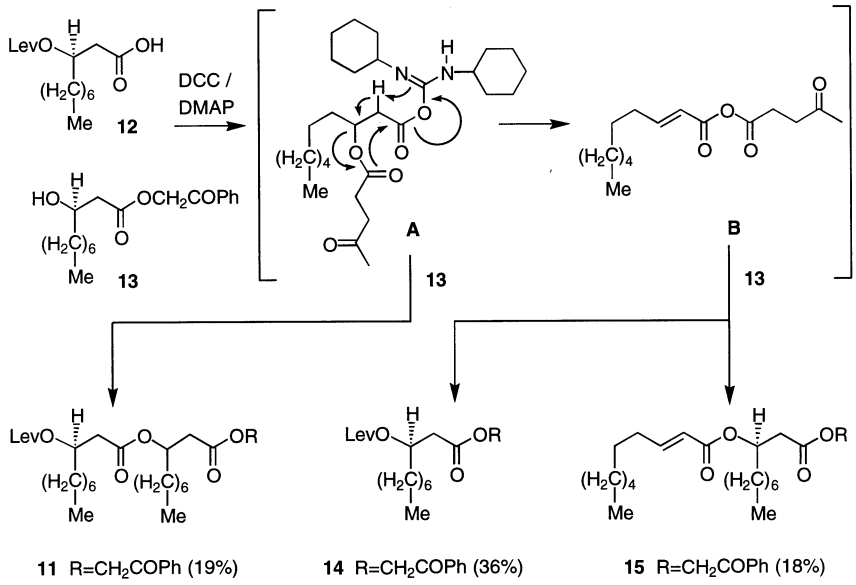


  1434−193X/98/0202−0303 $ 17.50+.50/0

Results and Discussion

It was expected[4] that removal of the 4-oxopentanoate (levulinate) ester from the fully protected di-lipid derivative **11**, prepared by esterification of (*R*)-3-levulinoyloxydecanoic acid (**12**)[4] with **13**[4], would afford the required lipid building unit **5**. However, esterification of **12** with **13** under the agency of either dicyclohexylcarbodiimide (DCC) or diisopropylcarbodiimide (DIC) in the presence of 4-(dimethylamino)pyridine (DMAP) led to the isolation of **11** in an unsatisfactory yield of 19%. Moreover, it was also found that the condensation was accompanied by the formation of the unwanted products **14**[4] (36%) and **15** (18%).

Scheme 1

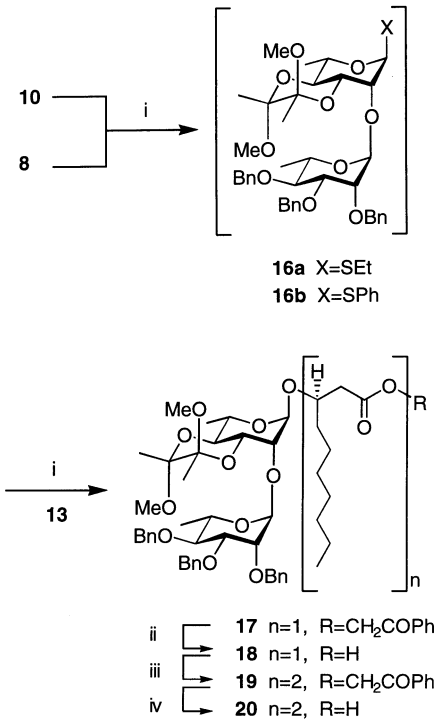


The low yield of **11**, as well as the occurrence of the latter side products may be explained by the migratory aptitude of the 3-levulinoyl function in the acylurea adduct **A** generated in the reaction of **12** with DCC or DIC. Thus, migration of the levulinoyl group in **A**, instead of esterification of **13** with **A**, will result in the mixed anhydride **B** which in turn reacts with **13** to give **14** and **15**. On the basis of the above result, it was decided to employ phenacyl (*R*)-3-hydroxydecanoate (**13**) for the introduction of the α-(1-3)-linked (*R*)-3-[(*R*)-3-hydroxydecanoyl] oxydecanoic acid moiety via a two-step process comprising glycosylation and esterification. In order to achieve this goal most effectively, we adopted the one-pot two-step protocol of Ley for the assemblage of the fully protected phenacyl (*R*)-3-[2-*O*-(α-L-rhamnopyranosyl)-α-L-rhamnopyranosyl]oxydecanoate (**17**, see Scheme 2). To this end, ethyl 3,4-*O*-(2,3-dimethoxybutane-2,3-diyl)-1-thio-α-L-rhamnopyranoside (**10a**), prepared according to Frost, was glycosylated in the presence of *N*-iodosuccinimide (NIS) and catalytic triflic acid (TfOH) with the known[7] ethyl 2,3,4-tri-*O*-benzyl-1-thio-α-L-rhamnopyranoside (**8**).

Analysis of the glycosylation by TLC showed the presence of **16a** as the main product. After addition of the phenacyl protected decanoate derivative **13**, followed by an additional amount of the same promotor NIS cat. TfOH, the fully protected rhamnolipid **17** was isolated in an overall yield of 55%. In this respect, it is of interest to note that a similar yield was obtained using the cyclohexane-1,2-diacetal, instead of the butane-2,3-diacetal, protected ethyl 1-thio-α-L-rhamnopyranoside **7** as the building unit in the same one-pot approach. These results indicate that the optimal level of chemoselectivity of the iodonium-ion mediated condensation of donor **8** with acceptor **10a** or **7** is not fully met. It occurred to us, that the chemoselectivity of the first step [i.e., formation of the α-(1′→2) linkage] could be enhanced by using the relatively more "disarmed"[8] phenyl 3,4-*O*-BDA-1-thio-α-L-rhamnopyranoside (**10b**) as acceptor. Indeed, the fully protected rhamnolipid **17** was obtained in an overall yield of 67% in the NIS cat. TfOH assisted one-pot two-step condensation of equimolar amounts of the three compounds **8**, **10b** and **13**. Apart from this, it was the more gratifying to establish that **17** was isolated in an excellent overall yield of 75% by executing the same one-pot two-step condensation with a slight excess of compound **8** (1.2 equiv.) and **13** (1.3 equiv.).

Next, attention was directed to the introduction of second (*R*)-3-hydroxydecanoic acid moiety. Thus, the phenacyl group in **17** was removed under the agency of activated zinc, and the resulting free acid derivative **18** was condensed with **13** in the presence of DCC/DMAP to give the fully protected target compound **19** in an overall yield of 70%.

Scheme 2. Reagents and conditions: i) a. **10b** (1 equiv.), **8** (1.2 equiv.), 1,2-dichloroethane/diethyl ether (1:1, v/v), NIS cat. TfOH (75%). b. **13** (1.3 equiv.), NIS cat. TfOH (75%). − ii) Zn in AcOH (1 h, 96%). − iii) **13** (1.2 equiv.), DCC (1.1 equiv.) and DMAP (0.6 equiv.) in CH_2Cl_2 (18 h, 73%). − iv) Zn in AcOH (1 h, 96%)



Complete deprotection of **19** proceeded smoothly by the following three-step process. Dephenacylation with activated zinc dust, followed by acid hydrolysis of the diacetal group in **20**, and subsequent debenzylation gave, after purification, homogeneous **2** in a yield of 76% over the three steps. Diazomethylation of **2** proceeded quantitatively to give the corresponding methyl ester **1**. The physical and spectral data of which were identical with those of an authentic sample[4].

Conclusion

The results presented in this paper clearly show that the one-pot synthesis of key intermediate **17** is a high yielding and stereoselective process. The latter possibility opens the way to a convenient multigram synthesis of the naturally occurring biological interesting rhamnolipids **1**−**2**.

Experimental Section

General Methods and Materials: Dichloromethane, diethyl ether and toluene were dried by distillation from P_2O_5 (5 g l^{-1}) and stored over molecular sieves (4 Å, Acros). 1,2-Dichloroethane (p.a., Rathburn) and tetrahydrofuran (p.a., Acros) were stored over molecular sieves (4 Å, Acros) and used without further purification. Acetic acid (p.a., Baker) and chloroform (p.a., Baker) were used as received. Trimethyl orthoformiat was refluxed for 2 h in the presence of CaH_2 (5 g l^{-1}), subsequently distilled. Solvents used for column chromatography were of technical grade and distilled before use. Trifluoromethanesulfonic acid, 2,3-butanedione, *N,N*-diisopropylcarbodiimide, *N,N*-dicyclohexylcarbodiimide, 4-(dimethylamino)pyridine, *N*-iodosuccinimide were purchased from Acros. 1-Methyl-3-nitro-1-nitrosoguanidine was purchased from Aldrich. 1-Methyl-3-nitro-1-nitrosoguanidine diazomethane generation apparatus was purchased from Aldrich. − Reactions were monitored by TLC analysis using Schleicher-and-Schüll DC Fertigfolien (F 1500 LS 254). − Compounds were visualised by UV light and by spraying with 20% sulphuric acid in methanol followed by charring at 140°C. − Column chromatography was performed on silica gel 60, 230−400 mesh (Merck). − Optical rotations were measured with a Propol polarimeter. − NMR spectra were recorded with a Jeol JNM-FX-200 (^1H and ^{13}C at 200 and 50.1 MHz, respectively), and a Bruker WM-300 spectrometer equipped with an Aspect 2000 computer (^1H and ^{13}C at 300 and 75 MHz, respectively). Chemical shift are given in ppm (δ) relative to tetramethylsilane as an internal standard. − Mass spectra were recorded with a Finnigan MAT TSQ-70 equipped with a custom-made Electrospray Interface (ESI).

Phenacyl (R)-3-[(R)-3-Levulinoyloxydecanoyl]oxydecanonate (**11**), *Phenacyl (R)-3-Levulinoyloxydecanate* (**14**) *and Phenacyl (R)-3-[(E)-2-Decenoyl]oxydecanate* (**15**): To a solution of **12**[4] (310 mg, 1.08 mmol) and **13**[4] (368 mg, 1.2 mmol) in CH_2Cl_2 (5 ml) was added *N,N*-diisopropylcarbodiimide and 4-(dimethylamino)pyridine (67 mg, 0.55 mmol). TLC-analysis showed, after stirring for 18 h, the formation of three products. The reaction mixture was diluted with CH_2Cl_2 (40 ml) washed with 1 M $NaHCO_3$ (20 ml) and H_2O, dried ($MgSO_4$) and concentrated. The mixture was purified by silca gel column chromatography (light petroleum/ethyl acetate, 9:1 → 6:4, v/v). Concentration of the appropriate fractions afforded **11**, known **14**[4] and **15** in 19%, 36% and 18% yield, respectively. **11**: $[\alpha]_D = -1.1$ (c = 1.0, $CHCl_3$). − R_f = 0.3 (light petroleum/ethyl acetate, 8:2). − ^1H NMR ($CDCl_3$): δ = 0.88 (m, 6 H, 2 × CH_3), 1.27 [m, 20 H, 2 × -$(CH_2)_5$-], 1.59 (m, 4 H, 2 × $CH_2\gamma$), 2.15 (s, 3 H, CH_3 Lev), 2.57 (m, 4 H, CH_2 Lev, $CH_2\alpha'$), 2.74 (m, 4 H, CH_2 Lev, $CH_2\alpha$), 5.23 (t, 1 H, CHβ′), 5.30 (t, 1 H, CHβ), 5.35 (s, 2 H, C*H*$_2$COPh), 7.48 (m, 2 H, CH_2CO*Ph*), 7.60 (m, 1 H, CH_2CO*Ph*), 7.92 (d, 2 H, CH_2CO*Ph*). − $^{13}C\{^1H\}$ NMR ($CDCl_3$): δ = 13.9 (CH_3), 22.5, 25.0, 29.0, 29.2, 31.6, 33.8 [2 × -$(CH_2)_6$-], 28.0 (CH_2 Lev), 29.6 (CH_3 Lev), 37.8 (CH_2 Lev), 38.6 (Cα), 39.0 (Cα′), 66.0 (*C*H2COPh), 70.7 (Cβ, Cβ′), 127.6−133.7 (CH_2CO*Ph*), 169.6 (C=O Lev), 171.8 (C=O), 192.4 (CH_2*C*OPh), 206.4 (C=O Lev). **14**: $[\alpha]_D = -2.8$ (c = 1.0, $CHCl_3$). − R_f = 0.2 (light petroleum/ethyl acetate, 8:2). − ^1H NMR ($CDCl_3$): δ = 0.86 (t, 3 H, CH_3), 1.27 [m, 10 H, -$(CH_2)_5$-], 1.55 (2 H, m, $CH_2\gamma$), 2.18 (s, 3 H, CH_3 Lev), 2.57 (t, 2 H, CH_2 Lev), 2.74 (m, 4 H, CH_2 Lev, $CH_2\alpha$), 5.30 (t, 1 H, CHβ), 5.35 (s, 2 H, C*H*$_2$COPh), 7.48 (m, 2 H, CH_2CO*Ph*), 7.60 (m, 1 H, CH_2CO*Ph*), 7.92 (d, 2 H, CH_2CO*Ph*). − $^{13}C\{^1H\}$ NMR ($CDCl_3$): δ = 13.9 (CH_3), 22.4, 24.9, 28.9, 29.1, 31.5, 33.8 [-$(CH_2)_6$-], 28.0 (CH_2 Lev), 28.8 (CH_3 Lev), 37.8 (CH_2 Lev), 38.6 (Cα), 65.9 (*C*H$_2$COPh), 70.5 (Cβ), 127.6−133.7 (CH_2CO*Ph*), 169.7 (C=O Lev), 171.9 (C=O), 192.4 (CH_2*C*OPh), 206.4 (C=O Lev). **15**: $[\alpha]_D = -1.9$ (c = 1.0, $CHCl_3$). − R_f = 0.8 (light petroleum/ethyl acetate, 8:2). − ^1H NMR ($CDCl_3$): δ = 0.88 (m, 6 H, 2 × CH_3), 1.27 [m, 20 H, 2 × -$(CH_2)_5$-], 1.59 (m, 2 H, $CH_2\gamma$), 2.21 (m, 2 H, $CH_2\gamma'$), 2.79 (m, 2 H, $CH_2\alpha$), 5.34 (m, 3 H, CHβ, C*H*$_2$COPh), 5.81 (d, 1 H, Hα′, $J_{\alpha',\beta'}$ = 17.4 Hz), 5.81 (m, 1 H, Hβ′), 7.48 (m, 2 H, CH_2CO*Ph*), 7.60 (m, 1 H, CH_2CO*Ph*), 7.92 (d, 2 H, CH_2CO*Ph*). − $^{13}C\{^1H\}$ NMR ($CDCl_3$): δ = 14.0 (CH_3), 22.5, 25.0, 27.9, 28.9, 29.0, 29.2, 31.6. 32.3, 33.9 [2 × -$(CH_2)_6$-], 38.8 (Cα), 66.0 (*C*H$_2$COPh), 70.1 (Cβ), 120.9 (Cα=*C*β), 127.6−133.7 (CH_2CO*Ph*), 149.8 (*C*α=Cβ), 169.6 (C=O Lev), 171.8 (C=O), 192.4 (CH_2*C*OPh), 206.4 (C=O Lev).

Ethyl 3,4-O-(2,3-Dimethoxybutane-2,3-diyl)-1-thio-α-L-rhamnopyranoside (**10a**): To a solution of ethyl 1-thio-α-L-rhamnopyrano-

side (3.34 g, 16.2 mmol), trimethylorthoformiaat (5.2 ml, 48 mmol) and 2,3-butanedione (1.6 ml, 18 mmol) in methanol (20 ml) was added camphersulfonic acid (200 mg). The mixture was heated under reflux for 18 h. The cooled reaction mixture was then neutralised with triethylamine and concentrated under reduced pressure. Purification by chromatography (light petroleum/Et_2O, 2:1 → 1:1, v/v) provided **10a** in 75% yield (5.22 g). R_f = 0.3 (light petroleum/Et_2O, 1:1, v/v). − ^{1}H NMR ($CDCl_3$): δ = 1.21−1.36 (m, 12 H, CH_3 SEt, 2 × CH_3 BDA, H-6), 2.60 (m, 2 H, CH_2 SEt), 3.23 (s, 3 H, OMe BDA), 3.26 (s, 3 H, OMe BDA), 3.75 (t, 1 H, H-4, $J_{3,4}$ = 9.8 Hz), 3.91 (dd, 1 H, H-3, $J_{2,3}$ = 3.0 Hz), 4.02 (br s, 1 H, H-2), 4.14 (m, 1 H, H-5), 5.24 (s, 1 H, H-1). − ^{13}C{^{1}H} NMR ($CDCl_3$): δ = 14.7 (C-6), 16.3 (CH_3 SEt), 17.3, 17.5 (2 × CH_3 BDA), 24.9 (CH_2 SEt), 47.3, 47.7 (2 × OMe BDA), 66.6, 68.4, 68.6, 71.0 (C-2, C-3, C-4, C-5), 84.1 (C-1), 99.5, 99.9 (2 × C_q BDA).

Phenyl 3,4-O-(2,3-Dimethoxybutane-2,3-diyl)-1-thio-α-L-rhamnopyranoside (**10b**): Phenyl 1-thio-α-L-rhamnopyranoside was converted in DBA-proteced **10b** as described above for the synthesis of **10a**. Yield 74%. − R_f = 0.85 (light petroleum/Et_2O, 3:2, v/v). − ^{1}H NMR ($CDCl_3$): δ = 1.16−1.30 (m, 6 H, 2 × CH_3 BDA), 1.32 (d, 3 H, H-6, $J_{5,6}$ = 5.0 Hz), 3.25 (s, 3 H, OMe BDA), 3.31 (s, 3 H, OMe BDA), 3.78 (t, 1 H, H-4, $J_{3,4}$ = 9.9 Hz), 3.98 (dd, 1 H, H-3, $J_{2,3}$ = 2.9 Hz), 4.18 (br s, 1 H, H-2), 4.26 (m, 1 H, H-5), 5.50 (s, 1 H, H-1), 7.30 (m, 3 H Ph), 7.45 (m, 2 H Ph). − ^{13}C{^{1}H} NMR ($CDCl_3$): δ = 15.9 (C-6), 16.9, 17.2 (2 × CH_3 BDA), 46.9, 47.3 (2 × OMe BDA), 67.1, 67.9, 68.5, 70.5 (C-2, C-3, C-4, C-5), 87.4 (C-1), 99.2, 99.6 (2 × C_q BDA), 130.5, 128.3, 130.5 (CH Ph), 133.8 (C_q Ph).

Phenacyl (R)-3-[2-O-(2′,3′,4′-Tri-O-benzyl-α-L-rhamnopyranosyl)-3,4-O-(2,3-dimethoxybutane-2,3-diyl)-α-L-rhamnopyranosyl]oxydecanoate (**17**): Ethyl 2,3,4-tri-*O*-benzyl-1-thio-α-L-rhamnopyranoside (**8**) (5.74 g, 12.0 mmol) and phenyl 3,4-*O*-(2,3-dimethoxybutane-2,3-diyl)-1-thio-α-L-rhamnopyranoside (**10b**) (3.70 g, 10.0 mmol) were dissolved in 1,2-dichloroethane ether (1:1,v/v, 100 ml). Powdered molecular sieves (4 Å, 5g) were added and the mixture were kept for 15 min. at 0°C. *N*-iodosuccinimide (2.70 g, 12.0 mmol) and triflic acid (108 µl, 1.2 mmol) was added. After stirring for 5 min, to the reaction mixture was added a solution of phenacyl (*R*)-3-hydroxydecanoate **13** (3.98 g, 13.0 mmol) in 1:1 1,2-dichloroethane ether (v/v, 30 ml). A second portion of *N*-iodosuccinimide (2.70 g, 12.0 mmol) and triflic acid (108 µl, 1.2 mmol) was added and stirring was continued for 10 min. The reacion mixture was filtered and the filtrate was diluted with CH_2Cl_2, washed with 1 M $Na_2S_2O_3$, 1 M $NaHCO_3$, H_2O, dried ($MgSO_4$) and concentrated. Purification of the residual oil on silica gel (100:0 → 95:5, toluene/ethyl acetate, v/v) furnished compound **17**. Yield 7.38 g (75%). − $[\alpha]_D$ = −83.2 (*c* = 1.0, $CHCl_3$). − R_f = 0.35 (light petroleum/Et_2O, 1:1, v/v). − ^{1}H NMR ($CDCl_3$): δ = 0.86 (t, 3 H, CH_3), 1.13−1.43 [m, 22 H, -$(CH_2)_5$-, 2 × BDA ,H-6, H-6′], 1.62 (br d, 2 H, $CH_2\gamma$), 2.73 (m, 2 H, CHα), 3.21 (s, 3 H, OMe BDA), 3.25 (s, 3 H, OMe BDA), 3.61 (m, 2 H, H-4, H-4′), 3.74 (m, 2 H, H-5, H-5′), 3.95 (m, 4 H, H-2, H-2′, H-3, H-3′), 4.15 (m, 1 H, CHγ), 4.50 (AB, 2 H, CH_2 Bn), 4.64 (AB, 2 H, CH_2 Bn), 4.81 (AB, 2 H, CH_2 Bn), 4.78 (s, 1 H, H-1′), 5.35 (s, 1 H, H-1), 5.37 (s, 2 H, CH_2COPh), 7.20−7.38 (m, 15H Bn), 7.46 (dd, 3 H, CH_2CO*Ph*), 7.92 (d, 2 H, CH_2CO*Ph*). − ^{13}C{^{1}H} NMR ($CDCl_3$): δ = 13.8 (CH_3), 16.4 (C-6), 17.5, 17.7, 17.8 (2 × CH_3 BDA, C-6′), 22.3, 24.4, 28.8, 29.2, 31.5, 33.1 [$(CH_2)_6$], 39.8 (Cα), 47.3, 47.6 (2 × OMe BDA), 65.5 (*C*H_2COPh), 71.3, 71.6, 72.4, (3 × CH_2 Bn), 67.0, 67.9, 68.4, 68.5, 73.6, 73.8, 74.4, 78.8, 80.2 (C-2,2′, C-3,3′, C-4,4′, C-5,5′, Cβ), 97.9, 98.2 (C-1,1′), 99.2, 99.3 (2 × C_q BDA), 127.2−133.4 (CH arom.), 133.9 (C_q, CH_2CO*Ph*), 138.1, 138.2, 138.3 (3 × C_q Bn), 170.2 (C=O), 191.6 (C=O, CH_2*C*OPh).

(R)-3-[2-O-(2′,3′,4′-Tri-O-benzyl-α-L-rhamnopyranosyl)-3,4-O-(2,3-dimethoxy-butane-2,3-diyl)-α-L-rhamnopyranosyl]oxydecanoic Acid (**18**): A solution of **17** (5.84 g, 5.94 mmol) in acetic acid (60 ml) was stirred with activated zinc dust for 1.5 h at 20°C, filtered and concentated. Traces of acetic acid were removed by evaporation with toluene. The product was chromatographed on silica gel (light petroleum/ether, 4:1 → 1:2, v/v) to give pure **18** in 96% yield (4.92 g). − $[\alpha]_D$ = −74.6 (*c* = 1.0, $CHCl_3$). − R_f = 0.35 (light petroleum/Et_2O, 1:1, v/v). − ^{1}H NMR ($CDCl_3$): δ = 0.86 (t, 3 H, CH_3), 1.15−1.47 (m, 22 H, -$(CH_2)_5$-, 2 × CH_3 BDA, H-6, H-6′), 1.65 (br d, 2 H, $CH_2\gamma$), 2.55 (m, 2 H, CH″), 3.20 (s, 3 H, OMe BDA), 3.24 (s, 3 H, OMe BDA), 3.58 (m, 2 H, H-4, H-4′), 3.70 (m, 2 H, H-5, H-5′), 3.95 (m, 4 H, H-2, H-2′, H-3, H-3′), 4.06 (m, 1 H, CHγ), 4.48 (AB, 2 H, CH_2 Bn), 4.73 (AB, 2 H, CH_2 Bn), 4.79 (AB, 2 H, CH_2 Bn), 4.80 (s, 1 H, H-1′), 5.35 (s, 1 H, H-1), 7.20−7.38 (m, 15H Bn). − ^{13}C{^{1}H} NMR ($CDCl_3$): δ = 14.0 (CH_3), 16.4 (C-6), 17.7, 17.8, 18.0 (2 × CH_3 BDA, C-6′), 22.5, 24.7, 29.1, 29.4, 31.6, 33.4 [-$(CH_2)_6$-], 40.0 (Cα), 47.6, 47.8 (2 × OMe BDA), 71.6, 71.9, 75.1, (3 × CH_2 Bn), 67.2, 68.2, 68.5, 68.7, 73.8, 74.1, 74.7, 79.1, 80.4 (C-2,2′, C-3,3′, C-4,4′, C-5,5′, Cβ), 98.0, 98.4 (C-1,1′), 99.4, 99.5 (2 × C_q BDA), 128.2−127.4 (CH arom.), 138.3, 138.5, (3 × C_q Bn), 176.0 (C=O).

Phenacyl (R)-3-{(R)-3-[2-O-(2′,3′,4′-Tri-O-benzyl-α-L-rhamnopyranosyl)-3,4-O-(2,3-dimethoxybutane-2,3-diyl)-α-L-rhamnopyranosyl]oxydecanoyl}oxydecanonate (**19**): To a solution of acid **18** (8.80 g, 10.2 mmol) and phenacyl (*R*)-3-hydroxydecanoate **13** (3.43 g, 11.16 mmol) in CH_2Cl_2 (120 ml) was added *N*,*N*-dicyclohexylcarbodiimide (2.52 g, 12.0 mmol) and 4-(dimethylamino)pyridine (744 mg, 5.0 mmol). After stirring for 18 h the reaction mixture was diluted with diethyl ether (400 ml) and the dicyclohexylurea was removed by filtration. The filtrate was washed with water (100 ml), dried ($MgSO_4$) and concentrated. Purification was preformed by column chromatography (light petroleum/ether, 4:1 → 1:2, v/v) to afford **19** in 73% yield (8.56 g, 7.4 mmol). $[\alpha]_D$ = −50.1 (*c* = 1.0, $CHCl_3$). − R_f = 0.8 (light petroleum/Et_2O, 1:1, v/v). − ^{1}H NMR ($CDCl_3$): δ = 0.87 (t, 6 H, 2 × CH_3), 1.15−1.38 (m, 32 H, 2 × -$(CH_2)_5$-, 2 × CH_3 BDA ,H-6, H-6′), 1.53 (br d, 2 H, $CH_2\gamma$), 1.62 (br d, 2 H, $CH_2\gamma'$), 2.79 (m, 4 H, CHα, $CH_2\alpha'$), 3.20 (s, 3 H, OMe BDA), 3.24 (s, 3 H, OMe BDA), 3.57 (m, 2 H, H-4, H-4′), 3.78 (m, 2 H, H-5, H-5′), 3.96 (m, 4 H, H-2, H-2′, H-3, H-3′), 4.06 (m, 2 H, CH(, CHγ′), 4.50 (AB, 2 H, CH_2 Bn), 4.68 (AB, 2 H, CH_2 Bn), 4.78 (AB, 2 H, CH_2 Bn), 4.79 (s, 1 H, H-1), 5.27 (d, 2 H, CH_2COPh), 5.37 (s, 1 H, H-1′), 7.20−7.35 (m, 15H Bn), 7.43 (dd, 3 H, CH_2CO*Ph*), 7.91 (d, 2 H, CH_2CO*Ph*). − ^{13}C{^{1}H} NMR ($CDCl_3$): δ = 13.9 (2 × CH_3), 16.4 (C-6), 17.6, 17.8, 18.0 (2 × CH_3 BDA, C-6′), 22.6, 24.7, 25.0, 29.0, 29.1, 29.5, 31.6, 33.6 [2 × -$(CH_2)_6$-], 38.6, 40.4 (Cα,α′), 47.5, 47.8 (2 × OMe BDA), 71.5, 71.9, 75.0, (3 × CH_2 Bn), 67.1, 68.1, 68.5, 68.7, 70.5, 73.7, 74.6, 79.0, 80.4 (C-2,2′, C-3,3′, C-4,4′, C-5,5′, Cβ,β′), 65.9 (CH_2COPh), 97.9, 99.1 (C-1,1′), 99.3, 99.4 (2 × C_q BDA), 127.2−133.6 (CH, arom.), 133.8 (C_q, CH_2CO*Ph*) 138.3, 138.5, (3 × C_q Bn), 169.6, 176.0 (2 × C=O), 191.5 (C=O, CH_2*C*OPh).

(R)-3-{(R)-3-[2-O-(2′,3′,4′-Tri-O-benzyl-α-L-rhamnopyranosyl)-3,4-O-(2,3-dimethoxybutane-2,3-diyl)-α-L-rhamnopyranosyl]oxydecanoyl}oxydecanoic Acid (**20**): Compound **20** was prepared in an indentical way as described for the synthesis of acid **18**. Purification was preformed by column chromatography (light petroleum/ether, 4:1 → 1:1, v/v) to afford **20** in 96% yield. − $[\alpha]_D$ = −74.0 (*c* = 1.0, $CHCl_3$). − R_f = 0.35 (light petroleum/Et_2O, 1:1, v/v). − ^{13}C{^{1}H} NMR ($CDCl_3$): δ = 13.9 (2 × CH_3), 16.3 (C-6), 17.6, 17.9, 18.0 (2 × CH_3 BDA, C-6′), 22.5, 24.6, 28.9, 29.1, 29.3, 29.5, 31.6, 32.5, 34.1 [2 × -$(CH_2)_6$ -], 38.6, 39.5 (Cα,α′), 47.6, 47.8 (2 × OMe BDA), 71.6, 71.9, 75.0, (3 × CH_2 Bn), 66.6, 68.1, 68.7,

70.8, 72.3, 73.8, 74.6, 79.0, 80.4 (C-2,2′, C-3,3′, C-4,4′, C-5,5′, Cβ,β′), 96.6, 98.1 (C-1,1′), 99.2, 100.0 (2 × C_q BDA), 126.8−128.1 (CH arom.), 138.3, 138.5 (3 × C_q Bn), 171.0, 171.9 (2 × C=O).

(R)-3-{(R)-3-[2-O-(2′,3′,4′-Tri-O-benzyl-α-L-rhamnopyranosyl)-α-L-rhamnopyranosyl]oxydecanoyl}oxy-(R)-decanoic Acid: Compound **20** (6.32 g, 6.12 mmol) was dissolved in 4:1 acetic acid-water (v/v, 80 ml) and stirred for 2 h at 100°C, then concentrated, and toluene (3 × 75 ml) was evaporated from the residue. Purification of the remaining oil on silica gel (toluene/ethyl acetate/AcOH, 100:0/0.5 → 75:25:0.5, v/v/v) afforded the title compound (4.88 g, 87%). − $[\alpha]_D$ = −53.6 (*c* = 1.0, $CHCl_3$). − R_f = 0.28 (toluene/ethyl acetate/AcOH, 85:15:0.5, v/v/v). − ^{1}H NMR ($CDCl_3$): δ = 0.88 (t, 6 H, 2 × CH_3), 1.10−1.30 (m, 26 H, 2 × -$(CH_2)_5$-, H-6, H-6′), 1.48 (br s, 2 H, $CH_2\gamma$), 1.52 (br s, 2 H, $CH_2\gamma'$), 2.15 (m, 2 H, $CH_2\alpha$), 2.32 (d, 2 H, $CH_2\alpha'$), 3.26 (t, 1 H, H-4′), 3.62−3.68 (m, 3 H, H-4, H-2′, H-5′), 3.67−3.88 (m, 3 H, H-5, H-3, H-3′), 4.04 (s, 1 H, H-2) 4.31 (br s, 2 H, CHβ), 4.62 (AB, 2 H Bn), 4.70 (AB, 2 H Bn), 4.83 (AB, 2 H Bn), 4.91 (d, 1 H, H-1), 4.97 (m, 1 H, H-1′), 5.46 (br s, 1 H, CHβ′), 7.23−7.44 (15H Bn). − ^{13}C{^{1}H} NMR ($CDCl_3$): δ = 14.0 (2 × CH_3), 17.5, 17.9 (C-6,6′) 22.5, 24.1, 25.0, 29.0, 29.1, 29.3, 29.7, 31.2, 31.7, 34.4 [2 × -$(CH_2)_6$-], 38.7, 39.6 (Cα,α′), 67.2, 68.7, 69.7, 70.4, 70.6, 74.3, 75.8, 79.6, 80.1, 80.4 (C-2,2′, C-3,3′, C-4,4′, C-5,5′, Cβ,β′), 72.1, 72.5, 75.2 (3 × CH_2 Bn) 93.2, 100.9 (C-1,1′), 127.0−127.9 (CH arom.), 138.4, 138.5, 138.8 (3 × C_q Bn), 171.3, 173.6 (2 × C=O).

(R)-3-{(R)-3-[2-O-(α-L-rhamnopyranosyl)-α-L-rhamnopyranosyl]oxydecanoyl}oxydecanoic Acid (**2**): Palladium on carbon (10%) was added to a solution of (*R*)-3-{(*R*)-3-[2-*O*-(2′,3′,4′-tri-*O*-benzyl-α-L-rhamnopyranosyl)-α-L-rhamnopyranosyl]oxydecanoyl}oxy-(*R*)-decanoic acid (4.36 g, 4.76 mmol) in a mixture of 2-propanol/water/acetic acid (20:1:1, v/v/v, 50 ml). The mixture was vigorous stirred for 48 h under hydrogen atmosphere. The reaction mixture was filtered and the filtrate was concentrated. The residual oil was applied onto a column of silica gel and elution effected with CH_2Cl_2/MeOH/AcOH (100:0:0.5 → 85:15:0.5, v/v/v) to give pure **2** (2.84 g, 92%), as a white solid. − $[\alpha]_D$ = −44.6 (*c* = 1.0, $CHCl_3$). − R_f = 0.31 (CH_2Cl_2/MeOH/AcOH, 85:15:0.5, v/v/v). − ^{1}H NMR ($CDCl_3$): δ = 0.89 (t, 6 H, 2 × CH_3), 1.22−1.30 [m, 26 H, 2 × -$(CH_2)_5$-, H-6, H-6′], 1.54 (br s, 2 H, $CH_2\gamma$), 1.62 (br s, 2 H, $CH_2\gamma'$), 2.54 (m, 4 H, $CH_2\alpha$,$CH_2\alpha'$), 3.38 (t, 1 H, H-4, H-4′ $J_{3,4}$.$J_{3'4'}$ = 9.5 Hz), 3.67 (m, 5 H, H-2, H-3, H-3′, H-5, H-5′), 3.97 (dd, 1 H, H-2′), 4.05 (m, 2 H, CHβ), 4.83 (d, 1 H, H-1′), 4.92 (m, 1 H, H-1), 5.25 (m, 1 H, CHβ′). − ^{13}C{^{1}H} NMR (MeOD): δ = 14.47 (2 × CH_3), 17.95, 17.97 (C-6,6′), 23.48, 25.56, 25.98, 30.07, 30.09, 30.20, 30.54, 32.69, 32.74, 33.94, 34.87 [2 × -$(CH_2)_6$-], 39.76, 40.95 (Cα,α′), 69.74, 69.95, 71.61, 71.69, 71.95, 72.05, 73.55, 74.04, 74.73, 80.16 (C-2,2′, C-3,3′, C-4,4′, C-5,5′, Cβ,β′) 98.56, 103.83 (C-1,1′), 172.0, 174.0 (2 × C=O). − MS (ESI); *m/z*: 673 $[M + Na]^+$.

Methyl (R)-3-{(R)-3-[2-O-(α-L-Rhamnopyranosyl)-α-L-rhamnopyranosyl]oxydecanoyl}oxydecanoate (**1**): A yellow solution of diazomethane in tetrahydrofuran was prepared using a 1-methyl-3-nitro-1-nitrosoguanidine diazomethane generation apparatus. The diazomethane solution was transferred by pipette into a solution of carboxylic acid **2** (239 mg, 0.19 mmol). The excess of diazomethane was quenched by dropwise addition of AcOH and the solution was concentrated under reduced pressure to afford rhamnolipid (**1**). The physical and spectral data of compound **1**[4] is in every aspect identical with an authentic sample prepared earlier by us.

[1] [1a] M. K. Johnson, D. Boese-Marazzo, *Infect. Immunol.* **1980**, *29*, 1028−1033. − [1b] C. Syldatk, S. Lang, F. Wagner, V. Wray, L.Witte, *Z. Naturforsch., Teil C* **1985**, *40*, 51−60. − [1c] T. Hirayama, I. Kato, *FEBS Lett.* **1982**, *139*, 81−85. − [1d] F. G. Jarvis, M. J. Johnson, *J. Am. Chem. Soc.* **1949**, *71*, 4124−4126.
[2] T. Leisinger, R. Margraff, *Microbiol. Rev.* **1979**, *43*, 422−442.
[3] P. Goran, P. Visnja, *Chem. Abstr.* **1996**, *124*, 106658.
[4] P. Westerduin, P.E. de Haan, M.J. Dees, J. H. van Boom, *Carbohydr. Res.* **1988**, *180*, 195−205.
[5] S. V. Ley, H. W. M. Priepke, *Angew. Chem. Int. Ed. Engl.* **1994**, *33*, 2292−2294.
[6] [6a] J. L. Montchamp, F. Tian, M. E. Hart, J. W. Frost, *J. Org. Chem.* **1996**, *61*, 2897−3899. − [6b] N. L. Douglas, S. V. Ley, H. M. I. Osborn, D. R. Owen, H. W. M. Priepke, S. L. Warriner, *Synlett* **1996**, 793−794.
[7] G. H. Veeneman, L. J. F. Gomes, J. H. van Boom, *Tetrahedron* **1989**, *45*, 7433−7448.
[8] H. M. Zuurmond, S. C. van der Laan, G. A. van der Marel, J. H. van Boom, *Carbohydr. Res.* **1991**, *215*, C1-C3.

[97327]