Secondary Metabolites by Chemical Screening, 39[‡]

Acyl α-L-Rhamnopyranosides, a Novel Family of Secondary Metabolites from *Streptomyces* sp.: Isolation and Biosynthesis

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In the course of our chemical screening program, the novel acyl α-L-rhamnopyranosides (**1–6**) were detected as metabolites from five different strains of Streptomycetes. The structures of all these compounds were elucidated by chemical and spectroscopic methods. The biosynthesis of **1** and **3** was established by feeding ^{13}C-labelled acetate, glycerol, and D-glucose to *Streptomyces griseoviridis* (strain Tü 3634), and resulted in a complete labelling pattern of the 2,4-dimethyl-3-furanylcarbonyl and benzoyl residues, as well as the rhamnose moiety. These results reveal biosynthetic pathways of general importance and give an insight into the generation of the hexose phosphates, from which deoxysugars are formed. The acyl rhamnosides are members of a novel family of microbial metabolites and are considered as rhamnoconjugates from Streptomycetes.

Introduction

Deoxyhexoses are typical building blocks of microbial secondary metabolites either as individual moieties or as components of oligosaccharides.[2,3] Usually they are linked to an aglycone as *O*-glycosides or to another sugar moiety and modulate the biological activity of a large number of antibiotics[4] e.g. anthracyclines, angucyclines, macrolides, enediynes, and the aureolic acid group.[5] Whilst the diversity of biological activity is tremendous and depends on the number and substituents present on the sugar, the mode of action is seldom known, e.g. interaction of sugars with specific DNA regions.[6,7] The *O*-glycosidic linkage is formed in the late biosynthesis by glycosyltransferases, which utilize deoxysugars activated at the anomeric C-atom and free aliphatic or phenolic hydroxy groups of the aglycone.[8] Using the well established chemical screening method for the detection of new secondary metabolites,[9,10] we have discovered a novel family of glycosides due to their remarkable yellow to green coloration on silica gel TLC plates when stained with anisaldehyde–sulfuric acid. This paper deals with the isolation and structure elucidation of six new acyl α-L-rhamnopyranosides (**1–6**) and related compounds (**7–9**) from different strains of Streptomycetes. Although not strictly glycosides, we use the term here for the acylated sugars described in this paper. Biosynthetic studies from feeding experiments with ^{13}C-labelled sodium acetate, glycerol, and D-glucose, respectively, gave interesting and unexpected results on the respective pathways and intermediates for the aglyca and the sugar assembly.

Results

Producing Organisms and Isolation

During an extensive screening program we found five strains, which produce varying ratios of an unusual type of glycoside with different co-metabolites. *Streptomyces griseoviridis* (strain Tü 3634), isolated from a soil sample from Nepal, produces compound **1** and **3** in an oatmeal medium, **2** after addition of ammonium hydrogenphosphate, **2** and **4** in a malt/yeast extract/glycerol medium and **6** in a malt/yeast extract medium. Strain FH-S 2087 produces **3**, **4**, and **5** in a glycerol/casein peptone medium, which yields only small amounts of compound **1** with strains Tü 3634, FH-S 1071 (*Streptomyces fragilis*), FH-S 1512, and FH-S 1298.[11] In addition different amounts of butanolides, e.g. acetomycin (**7**) and its derivatives **8** and **9** can be detected as by-products.[12–14] In the screening procedure, cultivations were run in 300-mL Erlenmeyer flasks containing 100 mL of the different media for 3–4 days at 28–30 °C. Using the isolation procedures described below,[15] we obtained crude extracts of the mycelium and the culture filtrate. Analysis by chromatography on silica gel TLC plates indicated that the glycosides were predominantly present in the culture fil-

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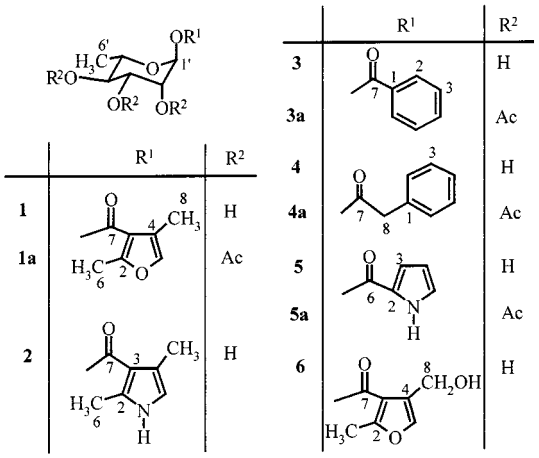
trate. Their *Rf*-values and color reactions with different staining reagents are given in Table 1.

Table 1. *Rf* values and discoloration of the natural acyl α-L-rhamnopyranosides **1–6** and some of their triacetates on silica gel TLC plates

Compound	A[a]	B	C	I	II
1	0.39	0.64	0.19	bright green	pink
1a	0.82	0.77	0.63	yellow	pink
2	0.21	0.62	0.11	bright yellow	red violet
3	0.31	0.65	0.18	yellow	–
3a	0.81	0.76	0.62	yellow	–
4	0.32	0.65	0.20	yellow	pink
4a	0.83	0.80	0.64	yellow	yellow
5	0.20	0.61	0.14	light brown	violet
5a	0.76	0.79	0.58	yellow	violet
6	0.09	0.75	0.15	yellow	–

[a] Solvent systems: (A) $CHCl_3$/MeOH = 9:1; (B) acetic acid/1-butyl alcohol/water = 1:4:5 (upper layer); (C) ethyl acetate/*n*-hexane (4:1). – Staining reagents: (I) anisaldehyde/sulfuric acid, (II) Ehrlich's reagent; after spraying the plates were heated at 120 °C for 5 min.

In order to isolate reasonable quantities of the glycosides, strains Tü 3634 and FH-S 2087 were cultivated in 1 L and 10 L fermentors, respectively, with different culture media. After harvesting, the culture filtrate was separated by filtration and applied to Amberlite XAD-2 or MCI-gel HP-20. Elution with methanol or a methanol/water gradient gave a crude extract, which was separated by column chromatography on Sephadex LH-20 (MeOH) and on silica gel (CH_2Cl_2/MeOH, 4:1) according to analysis by TLC (Table 1). Whilst **1**, **2**, **4**, and **6** could be obtained by this procedure, further purification on silica gel with acetone/hexane (2:1) or $CHCl_3$/MeOH (9:1) as eluents were necessary to obtain pure samples of **3** and **5**.[16] Typical fermentations (on 1-L and 10-L scale) followed by the purification procedure yielded 11 mg/L of acyl α-L-rhamnopyranoside **1**, 3−5 mg/L of **2**, 6 mg/L of **3**, 23 mg/L of **4**, 0.8 mg/L of **5** and 0.4 mg/L of **6**. All the glycosides are colorless solids.



Structure Elucidation

The pure acyl rhamnosides were spectroscopically characterized and, in some cases, derivatives were prepared by methanolysis and acetylation.

2,4-Dimethyl-3-furanylcarbonyl α-L-Rhamnopyranoside (1)

The molecular formula $C_{13}H_{18}O_7$ of **1** was established from the high resolution EI mass spectrum (M^+ = 286). The elemental analysis gave no reproducible values due to the inclusion of varying amounts of water and other solvents. The fragmentation peak at *m/z* = 140 originates from the loss of $C_6H_{10}O_4$, indicating a deoxy sugar moiety. UV maxima (MeOH) at 201 and 254 nm characterize the chromophore and the IR spectrum exhibits a strong band at 1710 (sh) indicating a conjugated ester.

The ^{1}H-NMR spectrum ($CDCl_3$) reveals 18 protons, three of which were exchangeable in [D_4]methanol. The presence of one methyl singlet (δ = 2.54), two methyl doublets [δ = 1.26 (*J* = 6.0 Hz), 2.15 (*J* = 1.3 Hz)], four aliphatic protons as multiplets (δ = 3.48–3.91), the anomeric proton as a doublet [δ = 6.16 (*J* = 1.9 Hz)] and an aromatic methine proton [δ = 7.18 (*J* = 1.3 Hz)] were observed. Acetylation gave the triacetate **1a**, thus indicating the presence of three hydroxyl groups. The sugar moiety of **1** was determined as rhamnose by comparing the chemical shifts with literature data and by an analysis of the coupling patterns of 1′-H (δ_H = 6.16) to 6′-H (δ_H = 1.26).[17] From the ^{13}C chemical shift (δ = 95.2) and the $^1J_{C-H}$ coupling constant (*J* = 172.5 Hz) at C-1′, an α-glycosidic linkage was deduced.[18] In order to assign the absolute configuration of the deoxyhexose, the glycoside was cleaved with methanol/HCl and gave the methyl rhamnopyranosides in both anomeric forms (α-anomer: $[\alpha]_D^{29}$ = −65, β-anomer: $[\alpha]_D^{29}$ = +86).[19] A comparison of the optical rotation values with those in the literature allowed the L-configuration to be assigned for the rhamnose moiety in **1** ($[\alpha]_D^{20}$ = −42). The α-configuration of L-deoxyhexoses is a characteristic structural feature in microbial secondary metabolites.

Thirteen signals were observed in the ^{13}C-NMR spectrum and APT experiment (CD_3OD), six of which confirmed the rhamnose moiety. The remaining seven signals originate from two methyl groups (δ_C = 14.7, 10.6), one ester carbonyl (δ_C = 164.0) and four furan ring carbons (δ_C = 162.4, 139.6 d, 122.2, 113.9), one of which carries a hydrogen atom (δ_H = 7.18). These structural elements form the aglycone, the structure of which was confirmed by 2D NMR methods and by comparison with synthetic 2,4-dimethylfuran-3-carboxylic acid (**11**). The synthesis of **11** was readily achieved by the dropwise addition of bromine to an aqueous suspension of isodehydracetic acid. Ether extraction followed by chromatography on silica gel (CH_2Cl_2/MeOH, 7:1) gave the pure aglycone **11** (95%).[20] A natural sample of **11** and synthetic **11** had identical properties (NMR and MS). The upfield shift of the anomeric carbon (C-1′, δ_C = 95.2) of **1** and the long-range correlation of the ester carbonyl C-7 at δ_C = 164.0 with the anomeric proton

1′-H at $\delta_H = 6.16$ in a COLOC-experiment account for an ester glycosidic linkage.[17] The appearance of only one set of NMR signals clearly indicates that **1** is a diastereomerically pure natural product. The structure of the new rhamnopyranoside was assigned as indicated in formula **1**.

2,4-Dimethyl-3-pyrrolylcarbonyl α-L-Rhamnopyranoside (2)

The analogue of **1,** containing nitrogen was produced (3 to 5 mg/L) in medium A enriched with ammonium hydrogenphosphate as nitrogen source or in medium C. The spectroscopic data of **2** are similar to those obtained for **1**. From HREI-mass spectral data (*m/z* = 285, M^+, $C_{13}H_{19}NO_6$), component **2** exhibits one O-atom less than **1** and an additional NH. In the IR spectrum the band for the ester carbonyl appears at 1683 cm^{-1}. A comparison of the ^{1}H-NMR and ^{13}C-NMR spectra (CD_3OD) revealed the same number of signals with different chemical shifts for the aglycone moiety only. The upfield shift of C-2 and C-5 of **2** indicates the exchange of oxygen by NH in the five-membered ring. Thus, structure **2** was deduced, which gave an insight into alternative routes in the biosynthesis of the aglycone starting from a common C_7 precursor.

Benzoyl α-L-Rhamnopyranoside (3)

Compound **3** was formed in culture broths of strain Tü 3634 and in smaller amounts from strain FH-S 2087. The DCI-MS of **3** shows the molecular ion at *m/z* = 286 ($M + NH_4^+$). The presence of a benzoyl group was indicated by characteristic UV absorption maxima in methanol and from the ester carbonyl IR absorption at 1726 cm^{-1}. The upfield region of the ^{1}H- and ^{13}C-NMR (CD_3OD) spectra were attributed to an ester linked rhamnose moiety, which was confirmed by facile conversion into the triacetate **3a**. Signals in the low field range of the NMR spectra indicate the aglycone to be a benzoyl residue. The optical rotation value ($[\alpha]_D^{20} = -52$) again supports the stereochemical assignment of a α-L-rhamnopyranoside. All these data confirm the structure as **3**.

Phenylacetyl α-L-Rhamnopyranoside (4)

Compound **4** was isolated from the culture broth of strains Tü 3634 and FH-S 2087 in amounts up to 23 mg/L. The results of the DCI-MS spectra (*m/z* = 300, $M + NH_4^+$) indicate one more methylene group than **3**. The IR spectrum reveals an ester absorption at 1745 cm^{-1}. In the ^{1}H- and ^{13}C-NMR spectra (CD_3OD) all the signals due to the rhamnose moiety are identical to those in **1–3**. This also suggests the α-L-configuration of the deoxysugar.[16,19] Again, this was confirmed by conversion into the triacetate **4a** and from isolation of the anomeric methyl rhamnosides obtained on methanolysis. Focussing on the aglycone: the methylene signals ($\delta_H = 3.68$, $\delta_C = 42.1$) and the presence of aromatic signals allow the identification of the benzyl group, which subsequently leads to structure **4**.

2-Pyrrolylcarbonyl α-L-Rhamnopyranoside (5)

Compound **5** was only isolated (0.8 mg/L) from the extracts of strain FH-S 2087. The DCI-MS [*m/z* = 258 ($M + H^+$), 275 ($M + NH_4^+$)] confirms the molecular weight in agreement with the molecular formula $C_{11}H_{15}NO_6$ as established from the spectral data and from the elemental analysis of the triacetate **5a**. The IR spectrum shows the characteristic ester carbonyl at 1696 cm^{-1}. In the upfield region of the ^{1}H-NMR spectrum (CD_3OD) all signals are again in agreement with those of an α-L-rhamnopyranosyl moiety. The optical rotation ($[\alpha]_D^{20} = -39$) of **5** again points to the α-L-configuration of the rhamnose in **5**. Three doublets of doublets in the lowfield region ($\delta_H = 6.21, 6.91, 7.01$, $J = 1.5, 2.5, 3.5$ Hz) correspond to a 2-pyrrolylcarbonyl aglycone in **5**, which was confirmed from the ^{13}C-NMR spectroscopic data. In addition to the four ^{13}C-NMR signals of the pyrrole nucleus ($\delta_C = 125.7, 122.7, 117.5, 111.0$), the ester carbon appears as a single signal at $\delta_C = 160.6$.

4-Hydroxymethyl-2-methyl-3-furanylcarbonyl α-L-Rhamnopyranoside (6)

Compound **6** was isolated (0.4 mg/L) from a culture of strain Tü 3634 in a 10 L airlift-loop fermentor with a high

Table 2. ^{13}C-NMR analysis of enriched **1** with stable isotope precursors; *as estimated by spin simulation

	1	Specific incorporation (J_{C-C} [Hz])[a]	J_{C-C} [Hz]	Specific incorporation	J_{C-C} [Hz]
Carbon	δ_c	[1-^{13}C]acetate	[1,2-$^{13}C_2$]acetate	[2-^{13}C]glycerol	[U-$^{13}C_3$]glycerol
2	162.4	21.5 (8.0)	52.3	1.8	52.7
3	113.9	–	86.4	–	86. 0
4	122.2	–	–	1.7	48.0, 72.0
5	139.9	–	–	–	72.0, 6.0
6	14.7	–	52.3	–	52.7
7	164.0	22.2 (8.0)	86.4	[0.9]	86.0
8	10.6	–	–	–	48.0, 6.0
1′	95.2	–	–	–	47, 3*
2′	71.4	–	–	1.6	47, 37,
3′	72.4	–	–	–	37, 3*
4′	73.4	–	–	8.0	40, 1
5′	72.7	–	–	–	40, 40
6′	18.1	–	–	–	40, 1

[a] Statistical coupling.

Table 3. ^{13}C-NMR analysis of **1** and **3** enriched with stable isotope precursors; *as estimated by spin simulation

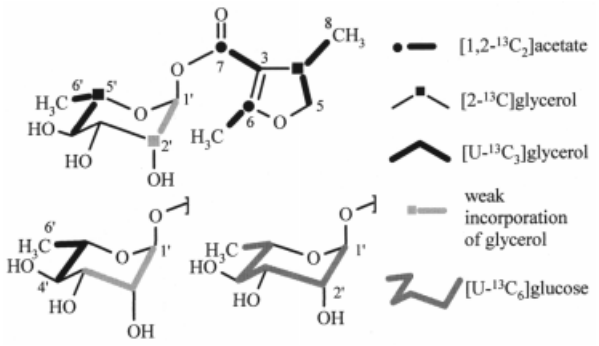
	3	Sugar moiety of **1**	Specific incorporation	J_{C-C} [Hz]	J_{C-C} [Hz]
Carbon	δ_c	δ_c	[2-^{13}C]glycerol	[U-$^{13}C_3$]glycerol	[U-$^{13}C_6$]glucose
1	130.8		3.44	75*, 64	–
2/6	130.7		–	56*, 64*, 2*	–
3/5	129.8		2.93	52, 56	–
4	134.8		–	52	–
7	166.0		–	75*, 2*	–
1′	96.0	95.2	–	47, 3*	47, 3*
2′	71.3	71.4	1.6	47, 37,	47, 37
3′	72.3	72.4	–	37, 3*	37*, 38*, 3*
4′	73.5	73.4	8.0	40, 1	38, 40, 1
5′	72.6	72.7	–	40, 40	40, 40
6′	18.1	18.1	–	40, 1	40, 1

aeration rate. The DCI-MS [m/z = 320 (M + NH_4^+), 302 (M^+)] confirms the molecular weight as $C_{13}H_{18}O_8$. Comparison with the ^{1}H-NMR spectrum (CD_3OD) of **1** showed that the methyl group of **1** (C-8) is replaced by a methylene group as indicated by a doublet (J = 1.0 Hz) at δ_H = 4.64. The ^{13}C-NMR spectrum of **6** exhibits all thirteen signals with the methylene group (δ_C = 57.1) in place of the methyl group of **1**. From all spectroscopic data, the metabolite clearly has structure **6** and is probably produced by the oxidation of **1**.

Biosynthetic Studies

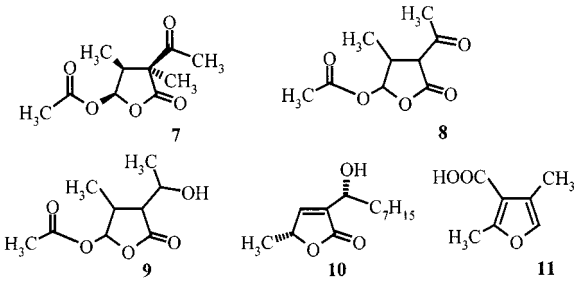
A working hypothesis for the biosynthesis of the novel acyl rhamnoside **1** envisaged a C_3+C_4 pathway for the aglycone (**11**), while L-rhamnose should be derived from the usual carbohydrate pathway.[4] We postulated that **11** is assembled from a triketide and a C_1 unit or a diketide and a C_3 unit (e.g. glycerol) as in acetomycin (**7**), A-factor, virginae butanolides or acaterin (**10**).[12,14,21] Consequently, we carried out feeding experiments with *Streptomyces griseoviridis* (strain Tü 3634) adding ^{13}C-labelled acetate, methionine, glycerol, and glucose, respectively, to the growing cultures.

Once the production of **1** had commenced (TLC analysis, 16 hours after inoculation), precursors were continuously added to the culture over a period of 10 hours. The cultures were harvested after 60 hours. Isolation by the above described procedure gave varying yields of **1** and, additionally,



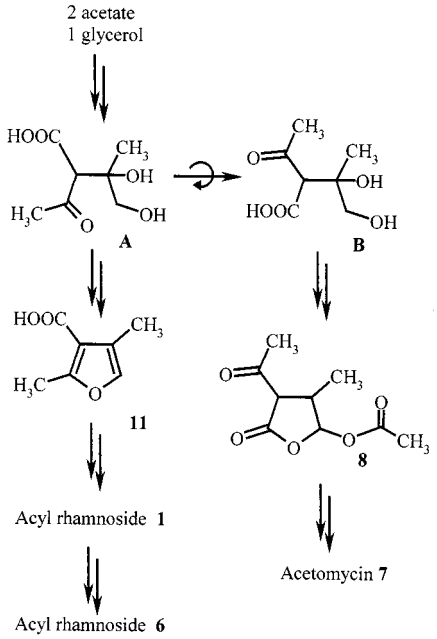
Scheme 1. Labelling pattern of 2,4-dimethyl-3-furanylcarbonyl α-L-rhamnopyranoside (**1**) derived from ^{13}C-labelled precursors

3. The results of ^{13}C-NMR spectral analysis are presented in Table 2 and Table 3 and in Scheme 1.



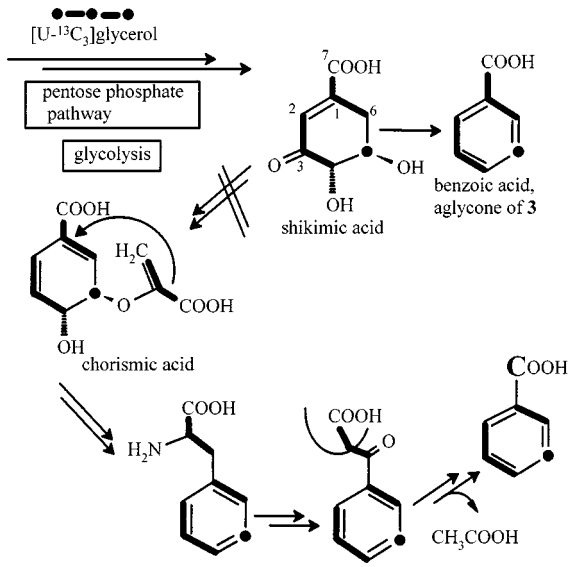
Feeding of sodium [1-^{13}C]acetate resulted in enhanced ^{13}C-NMR signals for C-2 and C-7 of the aglycone only. The ^{13}C-NMR spectrum of **1**, obtained from feeding of sodium [1,2-$^{13}C_2$]acetate, revealed two pairs of signals assigned by the coupling constants as C-6/C-2 and C-3/C-7. Thus, a diketide precursor was assumed for C-6/C-2/C-3/C-7. While feeding experiments with L-[methyl-^{13}C]methionine gave no enrichments whatsoever, experiments with [U-$^{13}C_3$]glycerol led to an intact incorporation at C-5/C-4/C-8 as indicated by the $^1J_{C-C}$ and $^3J_{C-C}$ coupling constants. Due to scrambling from glycerol into acetate, intact C_2-units were also detected for the diketide. Likewise [2-^{13}C]glycerol gave enrichments of C-2, C-7, and C-4 (Table 2). Thus, the assembly of the aglycone requires a diketide and a C_3-unit derived from glycerol. The C_7-intermediate (conformation A, Scheme 2) is converted into **11** by formation of a cyclic hemiacetal and subsequently into **1** in the predominant pathway. Remarkably, strains Tü 3634, FH-S 1071, and FH-S 1512 also produce butanolides of the acetomycin type, e.g. **8** and **9**, as minor components (see ref.[10–12]). For these, we propose the same C_7-intermediate (conformation B) which is converted by lactonization and further transformations (Scheme 2).

In the preceding feeding experiments with strain Tü 3634, no incorporation of labelled acetate, methionine and glucose was observed for the aglycone of **3**. Whereas [2-^{13}C]glycerol labelled C-1 and C-3/C-5 (chemical equivalent nuclei) and [U-$^{13}C_3$]glycerol labelled C-4/C-5/C-6 and C-2/C-1/C-7 (Table 3). These findings were in accord with the biosyn-



Scheme 2. Proposed biosynthetic pathways for acyl rhamnosides **1**, **6** and acetomycin **7** starting with a C_7 unit

thesis of **3** via a shikimate pathway from phosphoenolpyruvate and erythrose-4-phosphate.[22] Analysis of the extensive labelling pattern of **3**, obtained from the complex ^{13}C-NMR spectra with higher order couplings after feeding with [U-$^{13}C_3$]glycerol, was accompanied by spin simulations. Based on these results the biosynthetic pathway is assumed to start directly from shikimate and thus rules out a pathway via phenylalanine (Scheme 3).[23] A decisive fact here is, that instead of a strong singlet indicating a biosynthetic pathway via phenylalanine, a broad multiplet for C-7 3(δ = 166.0) is observed. Thus C-7 must be part of a higher



Scheme 3. Labelling pattern and proposed biosynthetic pathway to benzoic acid, the aglycone of **3**, ruling out a pathway via phenylalanine

order spin coupling system, which consequently makes quantitative determinations impossible as the eligible coupling partners C-1 (δ = 130.84) and C-2/C-6 (δ = 130.72) have similar chemical shifts. Therefore further evidence for an intact C-7/C-1/C-2-unit is not directly available by observing C-1 and C-2. Spin simulation experiments for C-7/C-1/C-2 gave a nearly corresponding coupling pattern for the C-7 signal, and from the spin simulations for C4/C-5/C-6, the signal patterns at δ = 130.2–131.2 were found to be comparable.[24] A strong doublet at δ = 134.8 (J = 52 Hz) accounts for C-4 as an outer carbon of a glycerol unit. Spin-spin coupling of the overlapping signals for C-3/C-5 (δ = 129.8, J = 52, 56 Hz) point to the incorporation of at least one intact C_3-unit. The incorporation pattern for the aglycone of **3** (Scheme 3) results from an examination of the coupling constants of 52 (C-4/C-5) and 56 Hz (C-5/C-6), the assumption of a singlet for C-3 and spin simulations, and is in accord with that of shikimate.[22] Feeding of unlabelled L-phenylalanine (3.3 mM, 7.3 mM) under the conditions of normal incorporation experiments did not raise the yields of **3**. Thus the biogenesis of an unsubstituted benzoyl moiety from a microbial source is described for the first time.[25]

Additionally, feeding experiments with ^{13}C-labelled glycerol and D-glucose led to interesting incorporation patterns in the rhamnose residues of **1** and **3**. Glucose feeding was followed by a decrease in the amount of products and **3** could be isolated in only small yield. Both C-2′ (weak) and C-5′ (strong) were unevenly enriched with [2-^{13}C]glycerol. [U-$^{13}C_3$]glycerol gave intact incorporation which led to variable intensities for the "top" half (C-1′/C-2′/C-3′, weak) and "bottom" half (C-4′/C-5′/C-6′, very strong). Moreover, an additional strong doublet within the C-2′-coupling pattern also indicated the incorporation of an intact C_2-unit (C-1′/C-2′). Feeding [U-$^{13}C_6$]glucose to strain Tü 3634 resulted in the ^{13}C-NMR spectra of a highly enriched rhamnose moiety in **3** which showed further additional complex coupling patterns as discussed above. This was especially the case for C-4′, which could be clearly analyzed as a doublet of doublets (J = 38, 40 Hz) thus indicating an intact incorporation of glucose (Scheme 1). It is well-known that deoxyhexoses e.g. rhamnose arise intact from glucose phosphate. Its labeling pattern reflects the labelling of the hexose phosphate pool, which is formed by different pathways: The "top" and the "bottom" C_3-unit are formed from triose phosphate pools and since these are not equilibrating, the addition of [U-$^{13}C_3$]glycerol via phosphoglyeraldehyde and dihydroxyacetone phosphate leads to uneven pulse-labelling as previously described in the literature.[26] The separate C_2-unit (C-1′/C-2′) derived from a clear coupling pattern of C-2′ (d, $^1J_{C\text{-}1/C\text{-}2}$ = 47 Hz) indicates, that the pentose phosphate pathway also feeds the hexose phosphate pool, since transketolases transfer C_2-units and thus labels the final product hexose. Although much is known about the biosynthesis of deoxysugars from glucose, detailed investigations of the hexoses as sources have been rare, and a separate C-1/C-2-coupling has been observed only in the case of polyketomycin.[27]

Discussion

The acyl rhamnopyranosides (**1–6**) represent a novel family of natural products produced by *Streptomyces* strains and were discovered by the chemical screening method. Glycosyltransferases use carboxylic acids as acceptor, the resulting ester linked rhamnose moiety is very uncommon in microbial secondary metabolites. The phenazine derivatives aestivophoenins and phenazoviridins are the only examples for acyl glycosides known so far.[17] In its biosynthesis, the aglycone of **1** is formed from a diketide and a glycerol unit (Scheme 1). In the presence of an increased amount of a nitrogen source the corresponding pyrrole derivative **2** is formed, presumably by a new pyrrole biosynthetic pathway.[28] Higher aeration during the fermentation led to **6** by a hydroxylation of **1**. The benzoyl residue of **3** is derived from the shikimate pathway by dehydration and reduction of the precursor. Investigations of the biosynthesis of the α-L-rhamnosyl moiety with [U-$^{13}C_3$]glycerol and [U-$^{13}C_6$]glucose revealed striking differences in the amount of incorporation in the "top" and "bottom" halves of the deoxyhexose and a characteristic labelling pattern which indicated the pentose phosphate pathway as one additional source for the hexose phosphate pool used for L-rhamnose.

One important result of the work presented here is the observation that different aglyca are combined with the α-L-rhamnopyranosyl residue in a regio- and stereoselective manner. The best interpretation is, that the rhamnosyltransferase is specific for L-rhamnosyl nucleotide phosphates but much less specific for carboxylic acids as the accepting aglycone. Experiments to study this observation are in progress.

Experimental Section

General: Melting points: Reichert hot-stage microscope (not corrected). – IR spectra: Perkin–Elmer 298 spectrometer. – UV spectra: Kontron Uvikon 860 spectrophotometer. – Optical rotation: Perkin–Elmer 241. – EI-MS: Varian 311 A, 70 eV, direct insert, high resolution with perfluorokerosine as standard. – DCI-MS: Finnigan MAT 95, 200 eV, reaction gas: NH_3. – ^{1}H- and ^{13}C-NMR spectra: Varian VXR 500, U300, Bruker AMX 300. Chemical shifts are expressed in δ values, TMS was used as internal standard. – TLC: silica gel 60 (Macherey–Nagel, SIL G/UV 254+366, 0.25 mm), Polygram (Macherey–Nagel, Alox N/UV254 0.20 mm); *Rf* values were determined on 20 × 20 cm plates; the evaluation length was 10 cm. – Column chromatography: silica gel < 0.08 mm (Macherey–Nagel), Sephadex LH-20 (Pharmacia). – Staining reagents: Anisaldehyde/sulfuric acid: 1.0 mL of anisaldehyde in 85 mL of methanol, 5 mL of conc. sulfuric acid and 10 mL of acetic acid; vanillin/sulfuric acid: 1 g of vanillin in 100 mL of sulfuric acid; Ehrlich's reagent: 1 g of 4-dimethylamino benzaldehyde, 25 mL conc. hydrochloric acid, 75 mL of methanol – Fermentation: Biostat E (10 L), Biostat M (1 L), Biostat U (50 L), all Braun-Diessel (Melsungen, Germany), Airlift-loop fermentor (10 L).

Nutrient Solutions: Medium A: oatmeal 20 g/L, 2.5 mL trace element solution, pH = 6.8 prior to sterilization; medium B: malt extract 10 g/L, yeast extract 4 g/L, glucose 4 g/L, pH = 7.0 prior to sterilization; medium C: malt 10 g/L, yeast extract 4 g/L, glycerol 20 g/L, $CaCO_3$ 20 mg/L, pH = 7.0 prior to sterilization; medium D: meat meal 20 g/L, malt extract 100 g/L, $CaCO_3$ 10 g/L, pH = 7.2 prior to sterilization; medium E: glycerol 30 g/L, casein peptone 2 g/L, K_2HPO_4 1 g/L, NaCl 1 g/L, $MgSO_4{\cdot}7H_2O$ 0.5 g/L, 5 mL trace element solution, pH = 7.3 prior to sterilization; trace element solution: $CaCl_2{\cdot}2H_2O$ 3 g/L, Fe^{III}-citrate 1 g/L, $MnSO_4$ 0.2 g/L, $ZnCl_2$ 0.1 g/L, $CuSO_4 \cdot 5\,H_2O$ 25 mg/L, $Na_2B_4O_7 \cdot 10\,H_2O$ 0.02 g/L, $CoCl_2$ 4 mg/L, $Na_2MoO_4 \cdot 2\,H_2O$ 0.01 g/L.

Fermentation: a) Strain Tü 3634 (*Streptomyces griseoviridis*) was maintained as a stock culture on agar slants containing medium C stored at 4 °C no longer than 14 d. A 1-cm^2 piece of agar from 7 d old cultures was used to inoculate 100 mL of medium A in 1000-mL Erlenmeyer flasks with flow spoilers. These cultures were incubated on a rotary shaker (250 rpm) at 28 °C for 48 h as standard conditions. In order to isolate **1** and **4** 100 mL of the culture broth were used to inoculate a stirred vessel (1 L working volume, medium A, 700 rpm, 28 °C, aeration 1.6 vvm). During the fermentation the pH decreased from 6.5 to 5.3 and after 12 h of fermentation the pH was maintained between 6.7 and 5.3 automatically. Acyl rhamnosides could be detected after 16 h and the culture was harvested after 67 h. For the isolation of **2**, strain Tü 3634 was cultivated in a 1-L fermentor under standard conditions (see above) for 3 d in flasks each containing 100 mL of medium C. For the isolation of **3**, medium A was used. Compound **6** was isolated from the culture filtrate of Tü 3634 in medium B (airlift-loop fermentor, 10 L, 28 °C, 72 h, aeration 4 vvm).

b) Strains FH-S 1071 (*Streptomyces fragilis*, DSM 4200), FH-S 1512 (DSM 4355), FH-S 2087, and FH-S 1298 (*Streptomyces* sp., DSM 4211) were maintained as stock cultures on agar slants containing medium B. A 72 h old submerged culture, cultivated on a rotary shaker, 180 rpm at 30 °C for 72 h, medium A or medium D, was used to inoculate (3%) a fermentor (10 L working volume, medium A or D, 200 rpm, 30 °C, 0.5 vvm). All cultures were harvested after 96 h.

Isolation and Purification: a) For **1** to **4** and **6** similar procedures were applied. The culture broths of strain Tü 3634 (*Streptomyces griseoviridis*) were separated from the mycelium by filtration. The mycelium was discarded. The light-red solutions obtained were passed through Amberlite XAD-2 columns and impurities washed out with deionized water and the metabolites then eluted with methanol. Evaporation yielded crude extracts (approx. 180 mg/L).

b) For the isolation of **1** and **3** strain Tü 3634 was cultivated in medium A. To obtain **6** cultivation was carried out in medium B. The crude extract obtained (180 mg/L) was chromatographed on Sephadex LH-20 (column: 100 × 2.5 cm, methanol) and the main fractions (detection by TLC) further purified on silica gel (column: 30 × 2.5 cm, CH_2Cl_2/MeOH 4:1) to yield 11 mg/L of pure **1**, 6 mg/L of **3** and 0.4 mg/L of **6**.

c) Rhamnoside **2** was isolated from the culture broth of strain Tü 3634, either obtained from medium C in 1-L flasks or medium A with additional 2.9 g (21.9 mM) ammonium hydrogenphosphate in a 1-L fermentor. The crude extract was chromatographed on Sephadex LH-20 (column: 100 × 2.5 cm, MeOH) and silica gel (column: 25 × 1.5 cm, CH_2Cl_2/MeOH, 4:1). Compound **2**, 5.3 mg/L (medium C) or 2.7 mg/L [medium A/$(NH_4)_2HPO_4$] was obtained along with **1** (8.0 mg/L) as white powders.

d) Rhamnoside **4** was isolated from the culture broth of strain Tü 3634 (medium C, 1-L fermentor). The crude extract was chromato-

graphed on Sephadex LH-20 (column: 100 × 2.5 cm, MeOH) and silica gel (column: 25 × 1.5 cm, CH_2Cl_2/MeOH 4:1). Compound **4** (23 mg/L) was obtained as a white powder. Other acyl rhamnosides were not detected.

e) Rhamnosides **3**, **4**, and **5** were obtained by separation of the culture filtrate of strain FH-S 2087 (cultivated in a 10-L fermentor, medium E) from the mycelium by filtration or centrifugation. The aqueous solution was applied to MCI-Gel HP-20, followed by elution with a 50–100% MeOH gradient in H_2O to yield 6 fractions of crude material. The isolation of the rhamnosides **3** and **5** from strain FH-S 2087 was accomplished by chromatography on silica gel (column: 40 × 5 cm, acetone/hexane 2:1, acetone/hexane/H_2O 2:1:1), on Sephadex LH-20 (column: 100 × 2.5 cm, MeOH) and on silica gel (column: 35 × 2.5 cm, $CHCl_3$/MeOH 9:1) to yield 1 mg/L of pure **3** and 0.8 mg/L of pure **5** as white powders. For the isolation of **4,** the main fraction of crude material was purified by chromatography on Sephadex LH-20 (column: 100 × 2.5 cm, MeOH) and on silica gel (column: 35 × 2.5 cm, CH_2Cl_2/MeOH 3:1) and yielded 3.1 mg/L of **4**.

All glycosides (**1–6**) are readily soluble in MeOH, ethyl acetate, DMSO, slightly soluble in $CHCl_3$ and water, and insoluble in *n*-hexane. They are unstable under acidic (pH < 4) or alkaline (pH > 9) conditions, as well as on heating above 40 °C.

2,4-Dimethyl-3-furanylcarbonyl α-L-Rhamnopyranoside (1): m.p. 87 °C (dec.). – $[\alpha]_D^{20}$ = −42 (*c* = 0.05 in MeOH). − IR (KBr): $\tilde{\nu}$ = 3400 cm^{-1}, 2980, 2930, 1710, 1610, 1560. − UV (MeOH): λ_{max} (log ε) = 201 (3.94), 254 (3.30). − ^{1}H NMR (500 MHz, CD_3OD): δ = 1.26 (d, *J* = 6.0 Hz, 3 H, 6′-H_3), 2.15 (d, *J* = 1.3 Hz, 3 H, 8-H_3), 2.54 (s, 3 H, 6-H_3), 3.48 (dd, *J* = 9.5, 9.5 Hz, 1 H, 4′-H), 3.65 (dq, *J* = 6.5, 9.5 Hz, 1 H, 5′-H), 3.77 (dd, *J* = 9.5, 3.5 Hz, 1 H, 3′-H), 3.91 (dd, *J* = 3.5, 1.9 Hz, 1 H, 2′-H), 6.16 (d, *J* = 1.9 Hz, 1 H, 1′-H), 7.18 (q, *J* = 1.3 Hz, 1 H, 5-H). − ^{13}C- NMR (125.7 MHz, CD_3OD): δ = 10.6 (q, C-8), 14.7 (q, C-6), 18.1 (q, C-6′), 71.4 (d, C-2′), 72.4 (d, C-3′), 72.7 (d, C-5′), 73.4 (d, C-4′), 95.2 (d, C-1′), 113.9 (s, C-3), 122.2 (s, C-4), 139.6 (d, C-5), 162.4 (s, C-2), 164.0 (s, C-7). − HREI-MS: calcd. for $C_{13}H_{18}O_7$: 286.1052, found: 286.1058. − EI MS: *m/z* (%) = 286 (9) [M^+], 140 (53) [$M^+ - C_6H_{10}O_4$], 123 (100) [$M^+ - C_6H_{11}O_5$]. − $C_{13}H_{18}O_7$ (286.3).

2,4-Dimethyl-3-pyrrolylcarbonyl α-L-Rhamnopyranoside (2): m.p. 90 °C (dec.). − $[\alpha]_D^{20}$ = −22 (*c* = 0.1 in MeOH). − IR (KBr): $\tilde{\nu}$ = 3406 cm^{-1}, 2924, 2363, 1683, 1582, 1441, 1383, 1259. − UV (MeOH): λ_{max} (log ε) = 205 (4.10), 221 (3.81), 233 (3.88), 252 (3.62), 262 (3.64). − ^{1}H NMR (500 MHz, CD_3OD): δ = 1.27 (d, *J* = 6.0 Hz, 3 H, 6′-H_3), 2.20 (d, *J* = 1.3 Hz, 3 H, 8-H_3), 2.44 (s, 3 H, 6-H_3), 3.48 (dd, *J* = 9.5, 9.5 Hz, 1 H, 4′-H), 3.70 (dq, *J* = 6.5, 9.5 Hz, 1 H, 5′-H), 3.81 (dd, *J* = 9.5, 3.5 Hz, 1 H, 3′-H), 3.89 (dd, *J* = 3.5, 1.9 Hz, 1 H, 2′-H), 6.13 (d, *J* = 1.9 Hz, 1 H, 1′-H), 6.35 (q, *J* = 1.3 Hz, 1 H, 5-H). − ^{13}C NMR (75.5 MHz, CD_3OD): δ = 13.5 (q, C-8), 14.3 (q, C-6), 18.1 (q, C-6′), 71.7 (d, C-2′), 72.3 (d, C-3′), 72.5 (d, C-5′), 73.5 (d, C-4′), 94.3 (d, C-1′), 110.1 (s, C-3), 116.1 (d, C-5), 122.1 (s, C-4), 138.6 (s, C-2), 165.9 (s, C-7). − EI-MS: *m/z* (%) = 285 (20) [M^+], 139 (100) [$M^+ - C_6H_{10}O_4$], 122 (98) [$C_7H_8NO^+$], 94 (10) [$C_6H_8N^+$]; DCI-MS: *m/z* (%) = 303 (30) [M + NH_4^+], 286 (100) [M + H^+]. − HREI-MS: found as calcd. for $C_{13}H_{19}NO_6$: 285.1212. – $C_{13}H_{19}NO_6$ (285.3).

Benzoyl α-L-Rhamnopyranoside (3): m.p. 145 °C (dec.). − $[\alpha]_D^{20}$ = −52 (*c* = 0.35 in MeOH). − IR (KBr): $\tilde{\nu}$ = 3384 cm^{-1}, 2979, 2916, 1726, 1601, 1451, 1320, 1271. − UV (MeOH): λ_{max} (log ε) = 201 (4.02), 209 (3.51), 230 (4.11), 258 (2.83), 274 (2.98). − ^{1}H NMR (500 MHz, CD_3OD): δ = 1.28 (d, *J* = 6.0 Hz, 3 H, 6′-H_3), 3.50 (dd, *J* = 9.5, 9.5 Hz, 1 H, 4′-H), 3.76 (dq, *J* = 6.5, 9.5 Hz, 1 H, 5′-H), 3.83 (dd, *J* = 9.5, 3.5 Hz, 1 H, 3′-H), 3.97 (dd, *J* = 3.5, 1.9 Hz, 1 H, 2′-H), 6.18 (d, *J* = 1.9 Hz, 1 H, 1′-H), 7.50 (dd, *J*= 7.5, 7.5, 2 H, 3-H, 5-H), 7.64 (tt, *J* = 7.5, 1.5 Hz, 1 H, 4-H), 8.03 (dd, *J* = 7.5, 1.5 Hz, 2 H, 2-H, 6-H). − ^{13}C NMR (125.7 MHz, CD_3OD): δ = 18.1 (q, C-6′), 71.2 (d, C-2′), 72.3 (d, C-3′), 72.6 (d, C-5′), 73.5 (d, C-4′), 95.9 (d, C-1′), 129.8 (d, C-3, C-5), 130.5 (s, C-1), 130. 7 (d, C-2, C-6), 134.8 (d, C-4), 166.0 (s, C-7). − DCI-MS: *m/z* (%) = 303 (100) [M + NH_4^+ + NH_3], 286 (33) [M + NH_4^+]. – $C_{13}H_{16}O_6$ (268.27).

Phenylacetyl α-L-Rhamnopyranoside (4): m.p. 85 °C (dec.). – $[\alpha]_D^{20}$ = –52 (*c* = 1.0 in MeOH). – IR (KBr): $\tilde{\nu}$ = 3400 cm^{-1}, 2980, 2930, 1745, 1500, 1455, 1250, 1130, 1060, 970. – UV (MeOH): λ_{max} (log ε) = 206 (3.48), 258 (2.58); (MeOH/HCl): λ_{max} (log ε) = 206 (3.78); (MeOH/NaOH): λ_{max} (log ε) = 206 (3.88), 258 (2.55). – ^{1}H NMR (500 MHz, CD_3OD): δ = 1.18 (d, *J* = 6.0 Hz, 3 H, 6′-H_3), 3.38 (dd, *J* = 9.5, 9.5 Hz, 1 H, 4′-H), 3.48 (dq, *J* = 6.5, 9.5 Hz, 1 H, 5′-H), 3.57 (dd, *J* = 9.5, 3.5 Hz, 1 H, 3′-H), 3.68 (s, 2 H, 8-H_2), 3.75 (dd, *J* = 3.5, 1.9 Hz, 1 H, 2′-H), 5.93 (d, *J* = 1.9 Hz, 1 H, 1′-H), 7.28 (m, 5 H, C_6H_5). – ^{13}C NMR (75.5 MHz, CD_3OD): δ = 17.9 (q, C-6′), 42.1 (t, C-8), 71.1 (d, C-2′), 72.0 (d, C-3′), 72.3 (d, C-5′), 73.3 (d, C-4′), 95.7 (d, C-1′), 128.2 (d, C-4), 129.6 (2 d, C-3, C-5), 130.3 (2 d, C-2, C-6), 135.3 (s, C-1), 171.4 (s, C-7). – DCI-MS: *m/z* (%) = 317 (35) [M + NH_4^+ + NH_3], 300 (100) [M + NH_4^+]. – $C_{14}H_{18}O_6$ (282.3).

2-Pyrrolylcarbonyl α-L-Rhamnopyranoside (5): m.p. 89 °C (dec.). – $[\alpha]_D^{20}$ = –39 (*c* = 0.56 in MeOH). – IR (KBr): $\tilde{\nu}$ = 3396 cm^{-1}, 2980, 2931, 2362, 1696, 1553, 1448, 1410, 1306. – UV (MeOH): λ_{max} (log ε) = 210 (3.37), 267 (4.23). – ^{1}H NMR (500 MHz, CD_3OD): δ = 1.26 (d, *J* = 6.0 Hz, 3 H, 6′-H_3), 3.47 (dd, *J* = 9.5, 9.5 Hz, 1 H, 4′-H), 3.74 (dq, *J* = 6.5, 9.5 Hz, 1 H, 5′-H), 3.83 (dd, *J* = 9.5, 3.5 Hz, 1 H, 3′-H), 3.90 (dd, *J* = 3.5, 2.0 Hz, 1 H, 2′-H), 6.09 (d, *J* = 2.0 Hz, 1 H, 1′-H), 6.21 (dd, *J* = 2.5, 3.5 Hz, 1 H, 4-H), 6.91 (dd, *J* = 1.5, 3.5 Hz, 1 H, 3-H), 7.01 (dd, *J* = 1.5, 2.5 Hz, 1 H, 5-H). – ^{13}C NMR (125.7 MHz, CD_3OD): δ =18.0 (q, C-6′), 71.4 (d, C-2′), 72.2 (d, C-3′), 72.3 (d, C-5′), 73.6 (d, C-4′), 95.1 (d, C-1′), 111.0 (d, C-4), 117.5 (d, C-3), 122.7 (s, C-2), 125.7 (d, C-5), 160.6 (s, C-6). – DCI-MS: *m/z* (%) = 258.3 (20) [M + H^+], 275 (100) [M + NH_4^+]. – $C_{11}H_{15}NO_6$ (257.24).

4-Hydroxymethyl-2-methyl-3-furanylcarbonyl α-L-Rhamnopyranoside (6): m.p. 119 °C. – $[\alpha]_D^{20}$ = –21 (*c* = 0.09 in MeOH). – IR (KBr): $\tilde{\nu}$ = 3425 cm^{-1}, 2926, 2363, 1632, 1383, 1062. – UV (MeOH): λ_{max} (log ε) = 201 (3.88), 248 (3.35). – ^{1}H NMR (500 MHz, CD_3OD): δ = 1.28 (d, *J* = 6.0 Hz, 3 H, 6′-H_3), 2.56 (s, 3 H, 6-H_3), 3.48 (dd, *J* = 9.5, 9.5 Hz, 1 H, 4′-H), 3.69 (dq, *J* = 6.5, 9.5 Hz, 1 H, 5′-H), 3.76 (dd, *J* = 9.5, 3.5 Hz, 1 H, 3′-H), 3.91 (dd, *J* = 3.5, 2.0 Hz, 1 H, 2′-H), 4.64 (d, *J* = 1.0 Hz, 2 H, 8-H_2), 6.15 (d, *J* = 2.0 Hz, 1 H, 1′-H), 7.37 (t, *J* = 1.0 Hz, 1 H, 5-H). – ^{13}C NMR (125.7 MHz, CD_3OD): δ =14.6 (q, C-6), 18.1 (q, C-6′), 57.1 (t, C-8), 71.3 (d, C-2′), 72.4 (d, C-3′), 72.8 (d, C-5′), 73.4 (d, C-4′), 95.6 (d, C-1′), 112.6 (s, C-3), 127.8 (s, C-4), 140.2 (d, C-5), 162.7 (s, C-2), 163.8 (s, C-7). – DCI-MS: *m/z* (%) = 320 (100) [M + NH_4^+], 302 (10) [M^+]. – $C_{13}H_{18}O_8$ (302.28).

Acetylation of 1, 3, 4, and 5: The respective rhamnoside (0.04 mmol) was dissolved in acetic anhydride (2 mL) at 0 °C. Pyridine (0.75 mL) was added, the solution stirred for 16 h at room temperature and the reaction stopped by hydrolysis with ice water (10 mL) for 2 h. The reaction product was extracted twice with ethyl acetate or chloroform, the organic phases dried with Na_2SO_4, and pyridine removed by evaporation with toluene in vacuo. Flash chromatography on silica gel (column: 20 × 1, $CHCl_3$/MeOH 98:2) yielded 14.8 mg (0.03 mmol, 75%) of **1a**, 18.8 mg (0.05 mmol, 90%) of **3a**,

6.0 mg (0.015 mmol, 30%) of **4a** and 21 mg (0.05 mmol, 100%) of **5a**.

2,4-Dimethyl-3-furanylcarbonyl 2,3,4-Tri-*O*-acetyl-α-L-rhamnopyranoside (1a): Colorless oil. – $[\alpha]_D^{20}$ = –65 (*c* = 0.2 in MeOH). – IR (KBr): $\tilde{\nu}$ = 3431 cm^{-1}, 2986, 1752, 1610, 1562. – UV (MeOH): λ_{max} (log ε) = 203 (4.02), 256 (3.54). – ^{1}H NMR (300 MHz, CD_3OD): δ = 1.20 (d, *J* = 6.0 Hz, 3 H, 6′-H_3), 1.97 (s, 3 H, CH_3CO), 2.06 (s, 3 H, CH_3CO), 2.17 (s, 3 H, CH_3CO), 2.18 (d, *J* = 1.5 Hz, 3 H, 8-H_3), 2.57 (s, 3 H, 6-H_3), 3.98 (dq, *J* = 6.0, 9.5 Hz, 1 H, 5′-H), 5.11 (dd, *J* = 9.5, 9.5 Hz, 1 H, 4′-H), 5.33 (m, 1 H, 2′-H), 5.35 (dd, *J* = 9.5, 3.5 Hz, 1 H, 3′-H), 6.18 (d, *J* = 2.0 Hz, 1 H, 1′-H), 7.20 (q, *J* = 1.5 Hz, 1 H, 7-H). – ^{13}C NMR (75.5 MHz, CD_3OD): δ = 10.6 (q, C-8), 14.8 (q, C-6), 17.9 (q, C-6′), 20.5 (q, CH_3CO), 20.6 (q, CH_3CO), 20.6 (q, CH_3CO), 70.1 (d, C-2′), 70.4 (d, C-5′), 70.6 (d, C-3′), 71.4 (d, C-4′), 91.7 (d, C-1′), 113.5 (s, C-3), 122.2 (s, C-4), 139.8 (d, C-5), 163.0 (s, C-2 or C-7), 163.1 (s, C-2 or C-7), 171.4 (s, CH_3CO), 171.5 (s, CH_3CO), 171.7 (s, CH_3CO). – EI-MS: *m/z* (%) = 412 (10) [M^+], 273 (50) [$M^+ - C_7H_7O_3$], 123.1 (100) [$C_7H_7O_2$]. – $C_{19}H_{24}O_{10}$ (412.4).

Benzoyl 2,3,4-Tri-*O*-acetyl-α-L-rhamnopyranoside (3a): Colorless oil. – $[\alpha]_D^{20}$ = –74 (*c* = 0.5 in MeOH). – IR (KBr): $\tilde{\nu}$ = 3450 cm^{-1}, 2980, 2920, 1750, 1600, 1580. – UV (MeOH): λ_{max} (log ε) = 202 (2.66), 228 (3.79), 273 (2.78). – ^{1}H NMR (500 MHz, CD_3OD): δ = 1.24 (d, *J* = 6.0 Hz, 3 H, 6′-H_3), 2.00 (s, 3 H, CH_3CO), 2.06 (s, 3 H, CH_3CO), 2.19 (s, 3 H, CH_3CO), 4.02 (dq, *J* = 6.0, 9.5 Hz, 1 H, 5′-H), 5.17 (dd, *J* = 9.5, 9.5 Hz, 1 H, 4′-H), 5.40 (dd, *J* = 3.5, 2.0 Hz, 1 H, 2′-H), 5.43 (dd, *J* = 9.5, 3.5 Hz, 1 H, 3′-H), 6.25 (d, *J* = 2.0 Hz, 1 H, 1′-H), 7.47 (dd, *J* = 7.5, 8.0 Hz, 2 H, 3-H, 5-H), 7.61 (tt, *J* = 1.5, 7.5 Hz, 1 H, 4-H), 8.08 (dd, *J* = 1.5, 8.0 Hz, 2 H, 2-H, 6-H). – ^{13}C NMR (75.5 MHz, CD_3OD): δ = 17.8 (q, C-6′), 20.6 (q, CH_3CO), 20.6 (q, CH_3CO), 20.6 (q, CH_3CO), 69.9 (d, C-2′), 70.3 (d, C-5′), 70.6 (d, C-3′), 71.5 (d, C-4′), 92.7 (d, C-1′), 129.9 (2 d, C-3, C-5), 130.2 (s, C-1), 130.9 (2 d, C-2, C-6), 135.1 (d, C-4), 165.2 (s, C-7), 171.4 (s, CH_3CO), 171.6 (s, CH_3CO), 171.7 (s, CH_3CO). – DCI-MS: *m/z* (%) = 412 (100) [M + NH_4^+]. – HREI-MS: calcd. for $C_{19}H_{22}O_9$: 394.1264, found: 394.1263. – $C_{19}H_{22}O_9$ (394.4).

Phenylacetyl 2,3,4-Tri-*O*-acetyl-α-L-rhamnopyranoside (4a): Colorless oil. – $[\alpha]_D^{20}$ = –35 (*c* = 1.15 in MeOH). – IR (KBr): $\tilde{\nu}$ = 2980 cm^{-1}, 2930, 1750, 1495, 1450. – ^{1}H NMR (500 MHz, CD_3OD): δ = 1.08 (d, *J* = 6 Hz, 3 H, 6′-H_3), 1.98 (s, 3 H, CH_3CO), 2.06 (s, 3 H, CH_3CO), 2.15 (s, 3 H, CH_3OH), 3.64 (dq, *J* = 6.0, 9.5 Hz, 1 H, 5′-H), 3.75 (d, *J* = 14.5 Hz, 1 H, 8-H), 3.79 (d, *J* = 14.5 Hz, 1 H, 8-H), 5.02 (dd, *J* = 9.5, 9.5 Hz, 1 H, 4′-H), 5.16 (dd, *J* = 3.5, 9.5 Hz, 1 H, 3′-H), 5.25 (dd, *J* = 2.0, 3.5 Hz, 1 H, 2′-H), 5.98 (d, *J* = 2.0 Hz, 1-H, 1′-H), 7.29 (m, 1 H, 4-H), 7.34 (m, 4 H, 2-H, 3-H, 5-H, 6-H). – ^{13}C NMR (75.5 MHz, CD_3OD): δ = 17.7 (q, C-6′), 20.5 (2q, 2 CH_3CO), 20.6 (q, CH_3CO), 41.2 (t, C-2), 69.8 (d, C-2′), 69.8 (d, C-5′), 70.4 (d, C-3′), 71.4 (d, C-4′), 92.2 (d, C-1′), 128.3 (d, C-4), 129.8 (2 d, C-3, C-5), 130.4 (2 d, C-2, C-6), 135.1 (s, C-1), 170.6 (s, C-7), 171.5 (s, CH_3CO), 171.6 (2s, 2 CH_3CO). – DCI-MS: *m/z* (%) = 426 (100) [M + NH_4^+]. – $C_{20}H_{24}O_9$ (408.4).

2-Pyrrolylcarbonyl 2,3,4-Tri-*O*-acetyl-α-L-rhamnopyranoside (5a): Light-yellow oil. – $[\alpha]_D^{20}$ = –89 (*c* = 0.2 in MeOH). – IR (KBr): $\tilde{\nu}$ = 3426 cm^{-1}, 2926, 1743, 1653, 1635, 1410. – UV (MeOH): λ_{max} (log ε) = 267 (4.20). – ^{1}H NMR (500 MHz, CD_3OD): δ = 1.20 (d, *J* = 6.0 Hz, 3 H, 6′-H_3), 1.99 (s, 3 H, $CDCl_3$), 2.06 (s, 3 H, CH_3CO), 2.17 (s, 3 H, CH_3CO), 4.00 (dq, *J* = 9.5, 6.0, 1 H, 5′-H), 5.14 (dd, *J* = 9.5, 9.5, 1 H, 4′-H), 5.36 (dd, *J* = 3.5, 2.0, 1 H, 2′-H), 5.39 (dd, *J* = 9.5, 3.5 Hz, 1 H, 3′-H), 6.17 (d, *J* = 2.0 Hz, 1 H, 1′-H), 6.29 (dd, *J* = 2.5, 3.8 Hz, 1 H, 4-H), 7.02 (m, 2 H, 3-H, 5-H). – ^{13}C NMR (125.7 MHz, CD_3OD): δ =17.8 (q, C-6′), 20.6 (q, CH_3CO), 20.6 (q, CH_3CO), 20.7 (q, CH_3CO), 69.9 (d, C-2′), 70.2 (d, C-5′), 70.6 (d, C-3′), 71.7 (d, C-4′), 91.8 (d, C-1′), 111.3 (d, C-4), 118.2 (d, C-3), 122.0 (s, C-2), 126.2 (d, C-5), 159.5 (s, C-6), 171.4 (s, CH_3CO), 171.6 (s, CH_3CO), 171.6 (s, CH_3CO). – DCI-MS: *m/z* (%) = 258.3 (20) [M + H^+], 275 (100) [M + NH_4^+]. – $C_{17}H_{21}NO_9$ (383.4): calcd. C 53.26, H 5.52; found C 53.49, 5.63.

Methanolysis of 1 and 4: a) Compound **1** (49 mg, 0.171 mmol) was stirred with MeOH/HCl (3 mL, 0.1 M) for 24 h at room temperature. Solvent and HCl were removed *in vacuo* and the residue chromatographed on silica gel (column 28 × 2.5, gradient $CHCl_3$/MeOH 95:5 to 8:1) to yield 14 mg (0.1 mmol, 58%) of aglycone **10**, 5 mg (0.03 mmol, 16%) of methyl α-L-rhamnopyranoside and 4 mg (0.02 mmol, 13%) of methyl β-L-rhamnopyranoside. – **b)** Compound **4** (40 mg, 0.14 mmol) was mixed with MeOH/HCl (2 mL, 0.1 M) and stirred for 16 h at room temperature. An identical isolation procedure yielded 13 mg (0.088 mmol, 62%) of phenyl acetic acid methyl ester, 9.0 mg (0.05 mmol, 72%) of methyl α-L-rhamnopyranoside and 6 mg (0.03 mmol, 48%) of methyl β-L-rhamnopyranoside.

Methyl α-L-Rhamnopyranoside: Colorless oil. – *Rf* = 0.20, $CHCl_3$/MeOH, 85:15. – $[\alpha]_D^{20}$ = –65 (*c* = 0.33 in MeOH). – ^{1}H NMR (500 MHz, CD_3OD): δ = 1.26 (d, *J* = 6.0 Hz, 3 H, 6-H_3), 3.33 (s, 1-OCH_3), 3.35 (dd, *J* = 9.5, 9.5, 1 H, 4-H), 3.52 (dq, *J* = 9.5, 6.0 Hz, 1 H, 5-H), 3.58 (dd, *J* = 9.5, 3.5 Hz, 1 H, 3-H), 3.77 (dd, *J* = 3.5, 2.0 Hz, 1 H, 2-H), 4.54 (d, *J* = 2.0 Hz, 1 H, 1-H). – DCI-MS: *m/z* (%) = 213 (24) [M + NH_4^+ + NH_3], 196 (100) [M + NH_4^+). – $C_7H_{14}O_5$ (178.2).

Methyl β-L-Rhamnopyranoside: Colorless oil. – *Rf* = 0.17 $CHCl_3$/MeOH, 85:15. – $[\alpha]_D^{20}$ = +85.8 (*c* = 0.33 in MeOH). – ^{1}H NMR (500 MHz, CD_3OD): δ = 1.31 (d, *J* = 6.0 Hz, 3 H, 6-H_3), 3.22 (dq, *J* = 9.5, 6.0 Hz, 1 H, 5-H), 3.32 (dd, *J* = 9.5, 9.5 Hz, 1 H, 4-H), 3.38 (dd, *J* = 9.5, 3.5 Hz, 1 H, 3-H), 3.49 (s, 3 H, 1-OCH_3), 3.84 (dd, *J* = 3.5, 2.0 Hz, 1 H, 2-H), 4.36 (d, *J* = 2.0 Hz, 1 H, 1-H). – DCI-MS: *m/z* (%) = 196 (100) [M + NH_4^+]. – $C_7H_{14}O_5$ (178.2).

2,4-Dimethylfuran-3-carboxylic Acid (11): White amorphous powder, m.p. 132 °C. – *Rf* = 0.54 ($CHCl_3$/MeOH 85:15). – IR (KBr): $\tilde{\nu}$ = 3400 cm^{-1}, 3000, 2620, 1670, 1605, 1560. – UV (MeOH): λ_{max} (log ε) = 202 (3.89), 243 (3.43). – ^{1}H NMR (300 MHz, $CDCl_3$/CD_3OD): δ = 2.16 (d, *J* = 1.3 Hz, 3 H, 8-H_3), 2.58 (s, 3 H, 6-H_3), 7.06 (d, *J* = 1.3, 1 H, 5-H). – ^{13}C NMR (50.3 MHz, $CDCl_3$): δ = 9.9 (t, C-8), 14.6 (t, C-6), 112.8 (s, C-3), 121.5 (s, C-4), 137.9 (d, C-5), 162.0 (s, C-2), 170.4 (s, C-7). – EI-MS: *m/z* (%) = 140 (100) [M^+], 122 (48) [$M^+ - H_2O$], 44 (100) [CO_2]. – HREI-MS: found as calcd. for $C_7H_8O_3$: 140.0473.

Phenylacetic Acid Methyl Ester: ^{1}H NMR (500 MHz, CD_3OD): δ =3.63 (s, 2 H, CH_2), 3.66 (s, 3 H, CH_3O), 7.27 (m, 5 H, C_6H_6). – EI-MS: *m/z* (%) = 150 (50) [M^+], 91 (100) (C_7H_7). – HREI-MS: calcd. for $C_9H_{10}O_2$: 150.0681, found: 150.0680).

Incorporation Experiments: Feedings were carried out in standard cultivations (1-L fermentor) by continuous addition of labelled precursors in aqueous solutions (7 mL/h) for 10 h by a low rate pump. Addition was started 16 h after inoculation. Precursors were dissolved in 100 mL of deionized water, adjusted to pH = 6.5 with 2 M citric acid and sterilized at 120 °C for 30 min. The following quantities of labelled compounds (all obtained from Campro Scientific or Cambridge Isotope Laboratories) were used: 9.1 mM of sodium [1-^{13}C]acetate (99.0% ^{13}C), 5.5 mM of sodium [1,2-$^{13}C_2$]acetate (99.0% ^{13}C), 0.7 mM of L-[methyl-^{13}C]methionine (99.0%

^{13}C), 5.4 mM of [2-^{13}C]glycerol (99.0% ^{13}C), 5.4 mM of [U-$^{13}C_3$]glycerol (99.0% ^{13}C) and 2.8 mM of [U-$^{13}C_6$]glucose. Labelled products were obtained in the following amounts: 7.5 mg **1**, 2.0 mg **3** ([1-^{13}C]acetate); 10.2 mg **1**, 2.0 mg **3** ([1,2-$^{13}C_2$]acetate); 11.6 mg **1**, 2.0 mg **3** ([2-^{13}C]glycerol); 5.0 mg **1**, 1.0 mg **3** ([U-$^{13}C_3$]glycerol); 1.0 mg **1**, 1.0 mg **3** ([U-$^{13}C_6$]glucose). Specific incorporations, summarized in Table 2 and Table 3, were calculated according Scott et al.[29]

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