Novel α-L-Rhamnopyranosides from a Single Strain of *Streptomyces* by Supplement-Induced Biosynthetic Steps[‡]

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Dedicated to Prof. Lutz F. Tietze on the occasion of his 60th birthday

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Various acyl and phenyl α-L-rhamnopyranosides were produced by *Streptomyces griseoviridis* (strain Tü 3634) in the presence of different supplements. Some of the added aromatic compounds underwent structural transformations prior to rhamnosylation (**1**, **3**, **5**, **8**, **9**). Especially remarkable was the formation of an indole (**9**) when 3,5-diaminobenzoic acid was added. Other precursors were not accepted by the strain, but did induce or enhance the biosynthesis of aromatic carboxylic acids from the shikimate pathway. These acids were then transformed into the acyl α-L-rhamnopyranosides **10**–**13**. This can be seen as a new strategy of pathway engineering. With derivatives **4** and **14**–**17** we gained further insight into the substrate specificity of the rhamnosyltransferase, in particular concerning the competition for rhamnosylation between a carboxylic acid and a phenol or aliphatic hydroxyl group. All isolated metabolites were detected by chemical screening and characterized by spectroscopic methods. The biosynthetic abilities of the strain result in a remarkable structural diversity within the family of α-L-rhamnopyranosides. The strain exemplifies the one strain/many compounds (OSMAC) approach.

Introduction

1-*O*-Acyl α-L-rhamnopyranosides, in which an aromatic or heteroaromatic carboxylic acid is ester-linked to the anomeric position of the sugar moiety, have been identified as microbial secondary metabolites from Streptomycetes.[1,2,3] In plants similar acyl glucosides derived from substituted aromatic acids, i.e. gallic acid[4] or sinapinic acid,[5] are quite common. These glucosides can be considered to be activated conjugates with similar reactivity to thioesters and have been reported to mediate acyl group transfer.[6] The function of the bacterial acyl rhamnosides, though, remains unknown. Some of them demonstrate inhibitory effects on 3α-hydroxysteroid dehydrogenase (3α-HDS),[3] a useful target in the search for anti-inflammatory and antiphlogistic agents. Recently, we reported the extension of this novel family of compounds by adding carboxylic acids as artificial precursors to growing cultures of *Streptomyces griseoviridis* (strain Tü 3634).[7] This precursor-directed biosynthesis (PDB) demonstrates the broad substrate specificity of the glycosyltransferase involved. To date more than twenty 1-*O*-acyl α-L-rhamnopyranosides derived from different acids have been described. Some of the added carboxylic acids did not result in the expected acyl rhamnosides. Instead, new metabolites were isolated and identified, which in some cases were derivatives of the added precursors (**2**, **6**, **7**) and were rhamnosylated to yield either phenyl (**1**, **3**, **5**) or acyl rhamnosides (**8**, **9**). Chemical screening[8] was found to be an efficient method for the detection of these new derivatives, which were formed as a result of transformation steps combined with the high rhamnosylation activity of the strain. Other carboxylic acids were neither accepted for rhamnosylation nor structurally transformed, but, surprisingly, led to the isolation of rhamnosides **10**–**15** whose aglycons are not derived from the added chemicals. Their formation was induced or enhanced by the added acid, probably via hitherto silent biosynthetic pathways. In this publication we describe the feeding experiments and the isolation and characterization of the compounds produced by our strain. Finally, the results are discussed in the context of the one strain/many compounds (OSMAC) approach[9] as an easy procedure to enhance the chemical diversity within different families of microbial secondary metabolites.

[‡] Studies of Precursor-Directed Biosynthesis with Streptomyces, 4. Part 3: Ref.[1]

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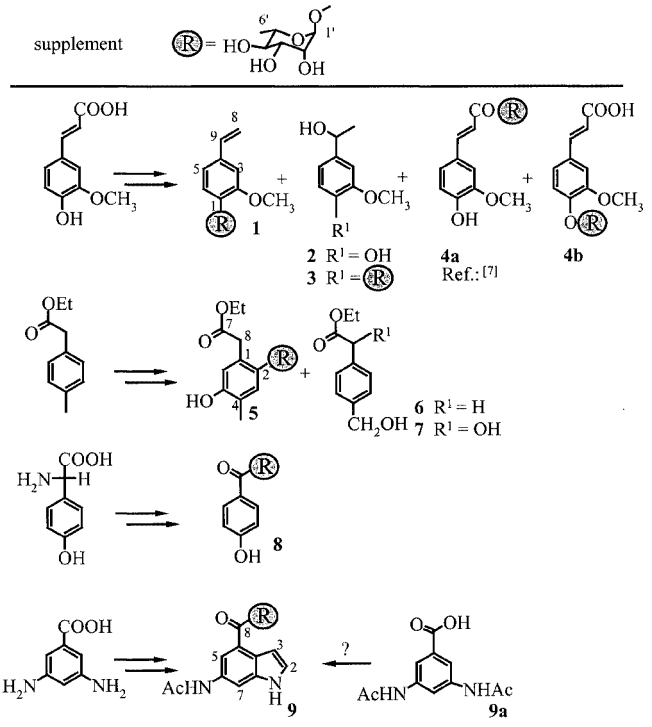
Results

Transformation Steps

The precursor-directed biosynthesis (PDB) was carried out by continuous addition of the compounds to growing

cultures of *Streptomyces griseoviridis* (strain Tü 3634) under standard conditions (final concentration of 7.3 mM, over 10 h).[7] All products were isolated by standard procedures.[1] The culture filtrate was separated by filtration and applied to Amberlite XAD-2. Extraction with methanol gave the crude extracts. The metabolites could be detected by TLC analysis due to their intense coloration when treated with anisaldehyde stain. Chromatography on Sephadex LH-20 (MeOH) and silica gel (CH_2Cl_2/MeOH, 4:1 or 7:1) afforded the described products. With the exception of ferulic acid, there was no evidence for the formation of either the expected acyl α-L-rhamnopyranosides or the metabolites normally produced by the strain. Instead, a surprising variety of altered and mostly rhamnosylated derivatives was observed, suggesting the potential for biotransformations using this strain (Scheme 1).



Scheme 1. α-L-Rhamnopyranosides resulting from structural transformations

Ferulic acid yielded the expected 1-*O*-feroyl α-L-rhamnopyranoside **4a**, (46 mg/L)[7] and four further transformed derivatives **1**−**4b** (4.1−52 mg/L), detected by their dark violet coloration on TLC plates with anisaldehyde stain. The DCI MS of the pleasant smelling **1** showed the molecular ion peak at m/z = 314 [M + NH_4^+]. The ^{1}H NMR spectroscopic data indicated the presence of an *O*-glycosidically linked rhamnose ($\delta_{1'\text{-H}}$ = 5.35 ppm) as previously identified in α-L-rhamnosides.[7] A vinyl group (δ_H = 5.15, 5.68, 6.67 ppm) was found by comparing the ^{1}H NMR spectrum of **1** with that of **4a**. A notable feature is the lack of carbonyl signals in the ^{13}C NMR spectrum indicating the decarboxylation of the precursor. All these assignments, along with an HMQC experiment, fit structure **1**, whose aglycon is 4-vinylguaiacol, a compound which has already been isolated from different strains by enzymatic decarboxylation of ferulic acid.[10] The EI mass spectrum of **2** reveals a strong [M^+] peak at m/z = 168. The ^{1}H NMR spectrum does not show signals for a rhamnose moiety, but three aromatic protons, one methoxy group (δ_H = 3.82 ppm) and methyl protons (δ_H = 1.40 ppm) adjacent to a methine group (δ_H = 4.73 ppm, q, J = 6.5 Hz) were observed. Analysis of the ^{13}C NMR and HMQC spectra and comparison with the spectra of **1** shows the presence of a 1-hydroxyethyl side chain (δ_C = 70.8, 25.5 ppm) as a new feature, thus confirming the structure of **2** as 4-(1-hydroxyethyl)-2-methoxyphenol.[11] The DCI mass spectrum of **3** displays the [M + NH_4^+] peak at m/z = 332. The ^{1}H NMR spectrum of **3** reveals the clear coupling patterns of an *O*-glycosidically linked rhamnopyranoside moiety ($\delta_{1'\text{-H}}$ = 5.32 ppm), as in **1**, and chemical shifts for the aglycon that are almost identical to those in **2**. Compound **3** shows UV maxima (MeOH) similar to **2** but not the bathochromic shift in alkaline solution indicative of a phenolic hydroxy group, as this now bears the rhamnosyl moiety. The rhamnosylated ferulic acid derivative **4b** (15.3 mg/L) demonstrates the *O*-rhamnosylation activity, and **1** and **3** give an indication of the metabolic potential of strain Tü 3634, as shown by the production of phenyl glycosides.

In a further experiment ethyl *para*-tolylacetate was added. In this case, no free hydroxyl group is available for rhamnosylation and the *para*-position is blocked by the methyl group. The isolated compounds (4.0−6.6 mg/L) exhibit green (**5**) and yellow (**6**, **7**) coloration with anisaldehyde stain on TLC plates. The DCI MS of **5** displays an intense peak with an unexpectedly high mass at m/z = 374 [M + NH_4^+]. The ^{1}H NMR spectrum reveals the spin system of an *O*-glycosidically rhamnose moiety ($\delta_{1'\text{-H}}$ = 5.22 ppm), along with signals for the ethyl and methylene group of the side chain already present in the precursor, and two singlets for aromatic protons (δ_H = 6.58 and 6.89 ppm). A COSY spectrum established the substitution pattern of **5**, with the proton resonating at δ_H = 6.58 ppm adjacent to the ester side chain and that at δ_H = 6.89 ppm in the *ortho*-position relative to the methyl group. A comparison of the ^{13}C NMR spectrum of **5** with that of the precursor revealed strong low-field shifts ($\delta_{C\text{-}2}$ = 149.1, $\delta_{C\text{-}5}$ = 151.1 ppm) that are indicative of hydroxyl substituents. The assignment was completed from HSQC and HMBC spectra and comparison with similar data in the literature (Table 1).[12] The oxygenase activity as a characteristic of strain Tü 3634 is not limited to an oxygenation of an aromatic ring as in **5** but is also effective on aliphatic carbons (**6**, **7**). The biotransformed derivative **6** bearing a hydroxymethyl group ($\delta_{9\text{-H}}$ = 4.58 ppm) instead of the 4-methyl shows all data identical with the literature.[13] Furthermore, **7** has a hydroxylated methine group ($\delta_{8\text{-H}}$ = 5.15 ppm) in the side chain in addition to a hydroxymethyl group.

Addition of 4-hydroxyphenylglycine, which is active against ischemic heart disease and is a substructural element of natural products such as vancomycin,[14] led to the isolation of the known 4-hydroxybenzoyl α-L-rhamnopyranoside **8** (10 mg/L), whose data are identical with those given in the literature.[7] We assume that transamination and oxidative decarboxylation precede rhamnosylation, al-

Table 1. Selected HMBC correlations of **5** and **9**

	5		9	
Position	δ_H (ppm)	δ_C (ppm)	δ_H (ppm)	δ_C (ppm)
2	–	–	7.39	103.4 (C-3, ^1J), 126.0 (C-3a, ^3J), 138.7 (C-7a, ^3J)
3	6.89	16.3 (C-9, ^3J), 123.2 (C-1, ^3J), 149.1 (C-2, ^2J), 151.1 (C-5, ^3J)	6.97	128.6 (C-2, ^2J), 138.7 (C-7a, ^3J)
5	–	–	7.84	
6	6.58	37.0 (C-8, ^3J), 125.4 (C-4, ^3J), 149.1 (C-2, ^3J), 151.1 (C-5, ^2J)	–	–
7		–	8.19	117.6 (C-5, ^3J), 126.0 (C-3a, ^3J)
8	3.45, 3.52	118.3 (C-6, ^3J), 123.2 (C-1, ^2J), 149.1 (C-2, ^3J), 173.9 (C-7, ^2J)	–	–
9	2.14	16.3 (C-9, ^1J), 118.4 (C-3, ^3J), 125.4 (C-4, ^2J), 151.1 (C-5, ^3J)	–	–
10	4.13	14.5 (C-11, ^2J), 173.9 (C-7, ^3J)	2.16	23.7 (C-10, ^1J), 172.0 (^2J)
11	1.23	62.0 (C-10, ^2J),	–	–
1′	5.22	149.1 (C-2, ^3J)	–	–

though a de novo synthesis from the shikimate pathway cannot be ruled out.

Addition of 3,5-diaminobenzoic acid under standard conditions yielded 6-acetamidoindole-4-carbonyl α-L-rhamnopyranoside **9** (4.4 mg/L) and acyl rhamnoside **10** (6.1 mg/L) rather than the expected acyl rhamnoside. The molecular formula of **9**, $C_{17}H_{20}N_2O_7$, was deduced from a DCI MS spectrum (m/z = 382 [M + NH_4^+]) and NMR spectroscopic data. UV maxima (MeOH) at 252 and 344 nm indicate the presence of an indole ring. The ^{1}H NMR spectrum of **9** shows clearly separated signals of an acyl α-L-rhamnose moiety ($\delta_{1'\text{-H}}$ = 6.12 ppm) as well as one additional methyl group and four aromatic protons. The connectivities of the latter were revealed by an HMQC experiment. The signals at δ_H = 2.16 and δ_C = 23.7/172.0 ppm were attributed to an *N*-acetyl group. Analysis of the HMBC spectrum supported the assignments and the novel structure **9** (Table 1). Formation of **9** might involve an enzymatic biotransformation process including bis-*N*-acetylation of 3,5-diaminobenzoic acid and indole ring closure (Scheme 1). However, addition of **9a** to strain Tü 3634 did not yield rhamnoside **9**.

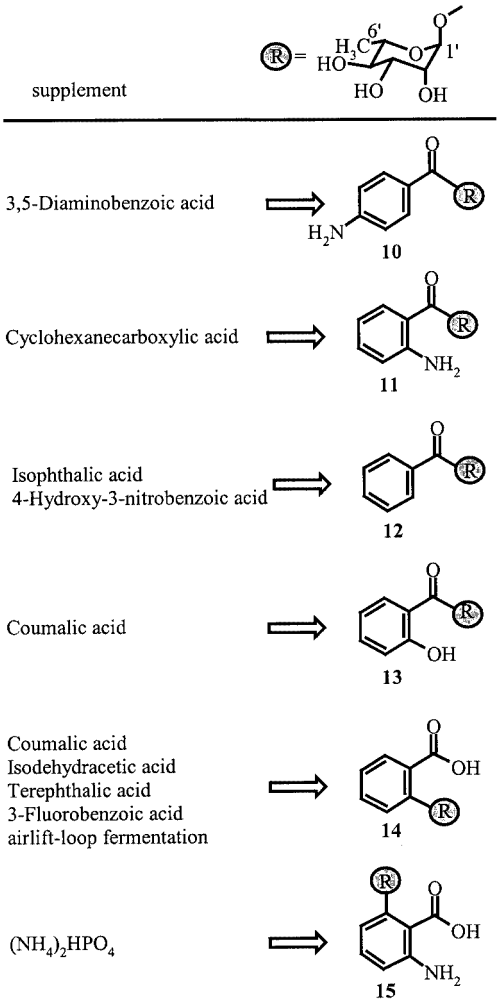
Induction of Metabolic Variability

The PDB experiment with 3,5-diaminobenzoic acid resulted in the formation of both **9** and **10**, the latter of which has already been isolated after the addition of 4-aminobenzoic acid.[7] It is unlikely that the aglycon of **10** results from biotransformation of the added acid , so we assume that it was biosynthesized de novo by strain Tü 3634 under the influence of the additive. Similar observations were made with *Streptomyces parvulus* (strain Tü 64): when 3,5-diaminobenzoic acid was added to the cultures a metabolite derived from 4-aminobenzoic acid — a common precursor for tetrahydrofolate in both plants and microorganisms — was formed with a yield five times greater than that of the expected manumycin analog.[15]

Similar unexpected metabolic shifts were detected in the following experiments: Addition of cyclohexanecarboxylic acid led to the isolation of 2-aminobenzoyl α-L-rhamnopyranoside (**11**; Scheme 2) (3.5 mg/L) as a single product, which shows identical spectroscopic data to the acyl rhamnoside formed when anthranilic acid is added directly.[7] Higher yields (up to 8 mg/L) of benzoyl α-L-rhamnopyranoside (**12**), a minor component of the natural secondary metabolite pattern of *Streptomyces griseoviridis*,[1] were produced by adding isophthalic or 4-hydroxy-3-nitrobenzoic acid. No formation of the respective acyl rhamnosides was observed. This is remarkable given the novel biosynthetic pathway to the benzoyl moiety found in this strain.[1] Furthermore the rhamnopyranosides **13** and **14**, known from feeding experiments with salicylic acid[7] and not found in the natural metabolite pattern of strain Tü 3634 so far, were isolated in yields of up to 11 mg/L from cultures supplemented with coumalic, terephthalic, isodehydracetic, or 3-fluorobenzoic acid. The increase of oxygen supplementation of the culture by using an airlift-loop fermentor without any chemical additives also resulted in the formation of **14** (3.0 mg/L). Increasing the nitrogen source by addition of $(NH_4)_2HPO_4$ led to the formation of rhamnopyranoside **15** (9 mg/L). This compound gives a characteristic molecular ion peak in the DCI mass spectrum (m/z = 299 (100) [M^+]). The ^{1}H NMR spectrum ([D_6]DMSO) shows signals for an *O*-glycosidically linked rhamnoside moiety ($\delta_{1'\text{-H}}$ = 5.25 ppm) as well as three signals for aromatic protons (δ_H = 6.45, 7.02, 7.24 ppm) with an ABC-coupling pattern and one for an amino group (δ_H = 6.18 ppm). These data are in agreement with the ^{13}C NMR signals for an aromatic ring, one carbonyl and a rhamnosyl residue, and led to the structural assignment of the novel metabolite **15**.

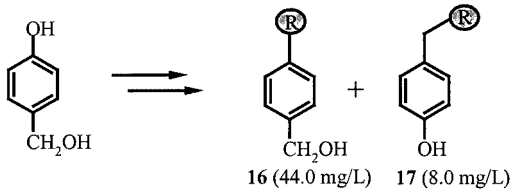
Discussion

Various acyl and phenyl α-L-rhamnopyranosides **1**, **3**–**5**, **8**–**15** are produced by *Streptomyces griseoviridis* (strain Tü



Scheme 2. α-L-Rhamnopyranosides resulting from additive-induced metabolic pathways

3634) when different compounds are added to the culture medium. With the exception of **12** these metabolites are not produced by the strain under normal conditions (oatmeal medium, 28 °C, 1.6 vvm);[1] seven of them are described here for the first time. Interestingly the aromatic carboxylic acids were not transformed into the expected acyl rhamnopyranosides, but instead induced unexpected metabolic steps. One rationale for these results is that the added compounds are transformed either by previously unobserved enzymatic activity in the strain or by chemical reactions in the culture broth. Cell-free experiments to further differentiate an enzymatic or chemical transformation during the fermentation process have not yet been carried out. In some cases the intermediates were accepted as substrates for the highly active rhamnosyltransferase, resulting in rhamnopyranosides **1**, **3**, **5**, **8**, **9**. The low substrate specificity of the enzyme leads to a wide variety of rhamnosylated aromatic compounds. These were easily detected and isolated from the culture filtrate due to their striking coloration with anisaldehyde stain on TLC plates and their moderate polarity. Apart from the previously mentioned transformations the examples show that phenyl rhamnosides (**1**, **3**, **4b**) can be produced as well as the acyl aromatic compounds, the given aliphatic hydroxy groups (**2**, **6**, **7**) were not successfully rhamnosylated. To confirm this observation we investigated the differentiation between phenolic and aliphatic hydroxy groups by addition of 4-hydroxybenzyl alcohol under standard conditions.[7] Each of the hydroxyl groups was rhamnosylated (**16**, **17**) although in different amounts (Scheme 3). Considering the addition of salicylic acid,[7] we can state now that aromatic acids are preferred as substrates, followed by phenols. Aliphatic hydroxy groups show the lowest substrate specificity and are only accepted if the concentration is high enough.



Scheme 3. α-L-Rhamnopyranosides resulting from addition of 4-hydroxybenzyl alcohol

Analysis of the aglycons produced by transformation reveals that strain Tü 3634 facilitates decarboxylation (**1**, **8**), hydroxylation (**2**, **3**, **5**−**7**) and a novel indole cyclization (**9**) in the presence of suitable precursors. The latter was totally unexpected, as indoles are normally generated via the shikimate pathway. The substitution pattern of **9** is indicative of a new biosynthetic pathway, which is currently under investigation.

During the feeding experiments to study the substrate specificity of the rhamnosyltransferase we observed the formation of new metabolites from the shikimate pathway (**10**−**15**). This effect can also be observed by enhancing the nitrogen or oxygen source. Compounds **14** and **15** were both isolated as a result of these experiments (Scheme 2), and also by supplementation of the respective precursor.[7] We can now state that feeding of certain chemicals leads to altered branchings or different activity levels in the shikimate pathway. How the respective enzyme activities are regulated at the molecular level remains open.

The investigation of the metabolic activity of strain Tü 3634 by using PDB has opened up the access to greater structural diversity within the family of rhamnopyranosides. The extended biosynthetic potential of a single strain is a variation of our OSMAC (one strain/many compounds) approach whereby the chemical diversity of natural products is enhanced by using the natural biosynthetic potential of the producing microorganism.[16]

Experimental Section

General Remarks: See ref.[1]

Nutrient Solutions: Medium A: Oatmeal 20 g/L, trace element solution 2.5 mL/L, 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. **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, $CuSO_4 \cdot 5H_2O$ 25 mg/L, $Na_2B_4O_7 \cdot 10H_2O$ 20 mg/L, $CoCl_2$ 4 mg/L, $Na_2MoO_4 \cdot 2H_2O$ 10 mg/L.

Fermentation and PDB: Strain Tü 3634 (*Streptomyces griseoviridis*) was maintained as a stock culture on agar slants (medium B) and cultivated in medium A under standard conditions in a stirred vessel (1 L working volume, 700 rpm, 28 °C, aeration 1.6 vvm, 72 h) as described previously.[1,7] Isolation and purification of **1**−**17** were carried out according to established protocols from the separated culture filtrate by chromatography on Sephadex LH-20 (column: 100 × 2.5 cm, MeOH) and silica gel (column: 25 × 1.5 cm, CH_2Cl_2/MeOH, 4:1 or 7:1).[7] All compounds (**1**−**17**) are readily soluble in MeOH, ethyl acetate, DMSO and slightly soluble in $CHCl_3$ and water. Under acidic (pH < 4) or alkaline (pH > 9) conditions the rhamnosides are unstable. They are destroyed by heating above 40 °C. Spectroscopic and other experimental data of **4a**, **8**, **10**−**14** were identical to those already published.[1,7] Feeding experiments (1-L fermentor) were carried out under standardized conditions whereby an additive was added continuously over 10 h to a final concentration of 7.3 mM by a low rate pump (7 mL/h), starting 16 h after inoculation. $(NH_4)_2HPO_4$ (2.90 g, 21.9 mM) was added to yield **15**. Precursors were dissolved in 70−80 mL of deionized water, adjusted to pH = 6.5 with 0.1 M NaOH and sterilized at 120 °C for 30 min. Compound **14** was isolated from the culture filtrate of strain Tü 3634 in medium A (airlift-loop fermentor, 10 L, 28 °C, 72 h, aeration 4 vvm). The molecular formulas of the metabolites of the novel acyl rhamnoside family were established by high resolution mass spectrometry.[1] Spectroscopic data led to the structures unambiguously, so elemental analysis data were not determined. Yields of products are given in the experimental data. Characterization of **4a, 8** and of **10**−**14** is given in previous papers.[1,7]

1-*O*-(2-Methoxy-4-vinylphenyl) α-L-Rhamnopyranoside (1): Addition of ferulic acid (1.42 g). Yield: 4.1 mg/L. M.p. 180−190 °C. $[\alpha]_D^{20}$ = −98 (*c* = 0.05 in MeOH). IR (KBr): $\tilde{\nu}$ = 3422 cm^{-1}, 1628, 1510. UV (MeOH): λ_{max} (log ε) = 212 nm (4.17), 255 (3.92). ^1H NMR (500 MHz, CD_3OD): δ = 1.22 (d, *J* = 6.0 Hz, 3 H, 6′-H_3), 3.45 (dd, *J* = 9.5, 9.5 Hz, 1 H, 4′-H), 3.79 (dd, *J* = 9.5, 6.0 Hz, 1 H, 5′-H), 3.84 (s, 3 H, 7-H_3), 3.88 (dd, *J* = 9.5, 3.5 Hz, 1 H, 3′-H), 4.06 (dd, *J* = 3.5, 2.0 Hz, 1 H, 2′-H), 5.15 (dd, *J* = 11.0, 1.0 Hz, 1 H, 8-H_a), 5.35 (d, *J* = 2.0 Hz, 1 H, 1′-H), 5.68 (dd, *J* = 17.0, 1.0 Hz, 1 H, 8-H_b), 6.67 (dd, *J* = 17.0, 11.0 Hz, 1 H, 9-H), 6.94 (dd, *J* = 8.0, 2.0 Hz, 1 H, 5-H), 7.05 (d, *J* = 8.0 Hz, 1 H, 6-H), 7.08 (d, *J* = 2.0 Hz, 1 H, 3-H) ppm. ^{13}C NMR (125.7 MHz, CD_3OD): δ = 18.0 (q, C-6′), 56.4 (q, C-7), 70.8 (d, C-5′), 72.2 (d, C-3′), 72.4 (d, C-2′), 73.8 (d, C-4′), 101.3 (d, C-1′), 111.1 (d, C-3), 112.9 (t, C-8), 119.4 (d, C-6), 120.2 (d, C-5), 134.7 (s, C-4), 137.7 (d, C-9), 146.7 (s, C-1), 152.0 (s, C-2) ppm. DCI-MS: *m*/*z* (%) = 314 (10) [M + NH_4^+], 164 (80) [$C_6H_{11}O_4$ + NH_3], 151 (100) [C_9H_9O + NH_4^+]. $C_{15}H_{20}O_6$ (296.32).

4-(1-Hydroxyethyl)-2-methoxyphenol (2): Addition of ferulic acid (1.42 g). Yield: 52.0 mg/L. M.p. 93 °C. $[\alpha]_D^{20}$ = +2.5 (*c* = 0.2 in MeOH). IR (KBr): $\tilde{\nu}$ = 3443 cm^{-1}, 3144, 2968, 1602, 1526. UV (MeOH): λ_{max} (log ε) = 206 nm (4.06), 227 (3.79), 279 (3.43). UV (MeOH/NaOH): λ_{max} (log ε) = 212 (4.10), 246 (3.76), 286 (3.44). ^1H NMR (500 MHz, CD_3OD): δ = 1.40 (d, *J* = 6.5 Hz, 3 H, 8-H_3), 3.82 (s, 3 H, 7-H_3), 4.73 (q, *J* = 6.5 Hz, 1 H, 9-H), 6.73 (d, *J* = 8.0 Hz, 1 H, 6-H), 6.77 (dd, *J* = 8.0, 2.0 Hz, 1 H, 5-H), 6.95 (d, *J* = 2.0 Hz, 1 H, 3-H) ppm. ^{13}C NMR (125.7 MHz, CD_3OD): δ =25.5 (q, C-8), 56.4 (q, C-7), 70.8 (d, C-9), 110.3 (d, C-3), 115.9 (d, C-6), 119.2 (d, C-5), 139.2 (s, C-4); 146.7 (s, C-1 or C-2), 148.9 (s, C-1 or C-2). EI-MS (70 eV): *m*/*z* (%) = 168 (75) [M^+], 153 (100) [M^+ − CH_3], 125 (55) [M^+ − C_2H_3O]. $C_9H_{12}O_3$ (168.19).

1-*O*-[4-(1-Hydroxyethyl)-2-methoxyphenyl] α-L-Rhamnopyranoside (3): Addition of ferulic acid (1.42 g). Yield: 10.0 mg/L. M.p. 71 °C. $[\alpha]_D^{20}$ = −98 (*c* = 0.1 in MeOH). IR (KBr): $\tilde{\nu}$ = 3408 cm^{-1}, 2971, 2931, 1595, 1513. UV (MeOH): λ_{max} (log ε) = 204 nm (sh) (4.36), 275 (3.43). UV (MeOH/NaOH): λ_{max} (log ε) = 206 nm (sh) (4.15), 275 (3.36). ^1H NMR (500 MHz, CD_3OD): δ = 1.21 (d, *J* = 6.2 Hz, 3 H, 6′-H_3), 1.42 (d, *J* = 6.5 Hz, 3 H, 8-H_3), 3.44 (dd, *J* = 9.5, 9.5 Hz, 1 H, 4′-H), 3.83 (concealed, 5′-H), 3.84 (s, 3 H, 7-H_3), 3.87 (dd, *J* = 9.5, 3.5 Hz, 1 H, 3′-H), 4.06 (dd, *J* = 3.5, 2.0 Hz, 1 H, 2′-H), 4.78 (q, *J* = 6.5 Hz, 1 H, 9-H), 5.31 (d, *J* = 2.0 Hz, 1 H, 1′-H), 6.87 (dd, *J* = 8.5, 1.5 Hz, 1 H, 5-H), 7.03 (d, *J* = 1.5 Hz, 1 H, 3-H), 7.04 (d, *J* = 8.5 Hz, 1 H, 6-H) ppm. ^{13}C NMR (125.7 MHz, CD_3OD): δ = 17.9 (q, C-6′), 25.6 (q, C-8), 56.4 (q, C-7), 70.6 (d, C-9), 70.8 (d, C-5′), 72.1 (d, C-2′), 72.3 (d, C-3′), 73.9 (d, C-4′), 101.5 (d, C-1′), 111.2 (d, C-3), 118.9 (d, C-5), 119.7 (d, C-6), 143.2/145.9/152.0 (s, C-1, C-2 or C-4). DCI-MS: *m*/*z* (%) = 332 (199) [M + NH_4^+], 297 (68) [M^+ − OH], 181 (15) [$C_6H_{11}O_5$ + NH_4^+]. $C_{15}H_{22}O_7$ (314.34).

1-*O*-(2-Methoxy-4-vinylcarboxyphenyl) α-L-Rhamnopyranoside (4b): Addition of ferulic acid (1.42 g). Yield: 15.3 mg/L. M.p. 128−130 °C. $[\alpha]_D^{20}$ = −92 (*c* = 0.05 in MeOH). IR (KBr): $\tilde{\nu}$ = 3425 cm^{-1}, 2930, 1686, 1636, 1509. UV (MeOH): λ_{max} (log ε) = 213 nm (4.17), 284 (4.12), 310 (4.08). UV (MeOH/HCl): λ_{max} (log ε) = 214 nm (4.10), 230 (4.04), 289 (4.11), 316 (4.12). UV (MeOH/NaOH): λ_{max} (log ε) = 214 nm (4.19), 279 (4.07), 303 (4.01), 342 (3.65). ^1H NMR (500 MHz, CD_3OD): δ = 1.22 (d, *J* = 6.0 Hz, 3 H, 6′-H_3), 3.36 (dd, *J* = 9.5, 9.5 Hz, 1 H, 4′-H), 3.73 (dd, *J* = 9.5, 6.0 Hz, 1 H, 5′-H), 3.86 (s, 3 H, 7-H_3), 3.88 (dd, *J* = 9.5, 3.5 Hz, 1 H, 3′-H), 4.06 (dd, *J* = 3.5, 2.0 Hz, 1 H, 2′-H), 5.42 (d, *J* = 2.0 Hz, 1 H, 1′-H), 6.40 (d, *J* = 16.0 Hz, 1 H, 9-H), 7.12 (dd, *J* = 8.0, 2.0 Hz, 1 H, 5-H), 7.14 (d, *J* = 8.0 Hz, 1 H, 6-H), 7.21 (d, *J* = 2.0 Hz, 1 H, 3-H), 7.56 (d, *J* = 16.0 Hz, 1 H, 10-H). ^{13}C NMR (125.7 MHz, CD_3OD): δ = 18.0 (q, C-6′), 56.5 (q, C-7), 70.9 (d, C-4′), 72.0 (d, C-5′), 72.2 (d, C-3′), 73.8 (d, C-2′), 100.9 (d, C-1′), 112.4, 118.8, 122.8, (d, C-3, C-5, C-6), 123.7 (d, C-9), 131.4 (s, C-4), 144.7 (d, C-10), 148.6, 152.3 (s, C-1, C-2), 146.7 (s, C-1), 172.0 (s, C-8). (−)-ESI-MS: *m*/*z* (%) = 339.6 (80) [M]$^-$; (+)-ESI-MS: *m*/*z* (%) = 363.6 (20) [M + Na]$^+$, 385.5 (65) [M − H + 2Na]$^+$. $C_{16}H_{20}O_8$ (340.33).

Ethyl (5-Hydroxy-4-methyl-2-α-L-rhamnopyranosylphenyl)acetate (5): Addition of ethyl *para*-tolylacetate (2.19 g), glycerol (0.5 g) and L-rhamnose (0.5 g). Yield: 4.0 mg/L. M.p. oil. $[\alpha]_D^{20}$ = −18 (*c* = 0.28 in MeOH). IR (KBr): $\tilde{\nu}$ = 3410 cm^{-1}, 2927, 1723, 1631, 1518. UV (MeOH): λ_{max} (log ε) = 203 nm (3.99), 288 (3.17). ^1H NMR (500 MHz, CD_3OD): δ =1.23 (t, *J* = 7.0 Hz, 3 H, 11-H_3), 1.24 (d, *J* = 6.5 Hz, 3 H, 6′-H_3), 2.14 (s, 3 H, 9-H_3), 3.42 (dd, *J* = 9.5, 9.5 Hz, 1 H, 4′-H), 3.45 (d, *J* = 16.0 Hz, 1 H, 8-H_a), 3.52 (d, *J* = 16.0 Hz, 1 H, 8-H_b), 3.64 (dd, *J* = 9.5, 6.5 Hz, 1 H, 5′-H), 3.78 (dd, *J* = 9.5, 3.5 Hz, 1 H, 3′-H), 3.93 (dd, *J* = 3.5, 2.0 Hz, 1 H, 2′-H), 4.13 (q, *J* = 7.0 Hz, 2 H, 10-H_2), 5.22 (d, *J* = 2.0 Hz, 1 H, 1′-H), 6.58 (s, 1 H, 6-H), 6.89 (s, 1 H, 3-H) ppm. ^{13}C NMR (125.7 MHz, CD_3OD): δ = 14.5 (q, C-11), 16.3 (q, C-9), 18.0 (q, C-6′), 37.0 (t, C-8), 62.0 (t, C-10), 70.5 (d, C-5′), 72.2 (d, C-2′), 72.4 (d, C-3′), 73.9 (d, C-4′), 100.7 (d, C-1′), 118.3 (d, C-6), 118.4 (d, C-3), 123.2 (s, C-1), 125.4 (s, C-4), 149.1(s, C-2), 151.1 (s, C-5), 173.9 (s, C-7). DCI-MS: *m*/*z* (%) = 374 (100) [M + NH_4^+]. $C_{17}H_{24}O_8$ (356.37).

Ethyl 4-Hydroxymethylphenylacetate (6): Addition of ethyl *para*-tolylacetate (2.19 g), glycerol (0.5 g) and L-rhamnose (0.5 g). Yield: 6.6 mg/L. ^1H NMR (500 MHz, CD_3OD): δ = 1.20 (t, *J* = 7.2 Hz, 3 H, OCH_2CH_3), 3.60 (s, 2 H, 8-H_2), 4.12 (q, *J* = 7.2 Hz, 2 H, OCH_2CH_3), 4.58 (s, 2 H, 9-H_2), 7.33 (dm, *J* = 8.5 Hz, 2-H, 6-H or 3-H, 5-H), 7.41 (dm, *J* = 8.5 Hz, 2-H, 6-H or 3-H, 5-H). $C_{11}H_{14}O_3$ (194.23).

Ethyl 2-Hydroxy-(4-hydroxymethylphenyl)acetate (7): Addition of ethyl *para*-tolylacetate (2.19 g), glycerol (0.5 g) and L-rhamnose (0.5 g). Yield: 5.5 mg/L. M.p. oil. $[\alpha]_D^{20} = +35$ ($c = 0.21$ in MeOH). IR (KBr): $\tilde{\nu} = 3406\ cm^{-1}$, 3282, 1734, 1635, 1512. UV (MeOH): λ_{max} (log ε) = 201 nm (4.04). UV (MeOH/NaOH): λ_{max} (log ε) = 221 nm (3.79). ^1H NMR (500 MHz, CD_3OD): δ = 1.17 (t, J = 7.2 Hz, 3 H, OCH_2CH_3), 4.12 (q, J = 7.2 Hz, 2 H, OCH_2CH_3), 4.59 (s, 2 H, 9-H_2), 5.15 (s, 1 H, 8-H), 7.34 (dm, J = 8.5 Hz, 2 H, 2-H, 6-H or 3-H, 5-H), 7.41 (dm, J = 8.5 Hz, 2-H, 6-H or 3-H, 5-H) ppm. ^{13}C NMR (125.7 MHz, CD_3OD): δ = 14.35 (s, OCH_2CH_3), 62.3 (t, OCH_2CH_3 or C-9), 64.8 (t, OCH_2CH_3 or C-9), 74.2 (s, C-8), 127.8 (d, C-2, C-6 or C-3, C-5), 128.1 (d, C-2, C-6 or C-3, C-5), 139.5 (s, C-1 or C-4), 142. 95 (s, C-1 or C-4), 174.5 (s, C-7). EI-MS (70 eV): *m/z* (%) = 210 (15) [M^+], 137(100) [$C_8H_9O_2$]. $C_{11}H_{14}O_4$ (210.23).

6-Acetamidoindole-4-carbonyl α-L-Rhamnopyranoside (9): Addition of 3,5-diaminobenzoic acid (2.22 g). Yield: 4.4 mg/L. M.p. 154 °C. $[\alpha]_D^{20} = -11$ ($c = 0.12$ in MeOH). IR (KBr): $\tilde{\nu}$ = 3381 cm^{-1}, 2927, 1710, 1588, 1554. UV (MeOH): λ_{max} (log ε) = 252 (4.18), 313 (3.54), 344 (3.60). ^1H NMR (500 MHz, CD_3OD): δ = 1.30 (d, J = 6.5 Hz, 3 H, 6′-H_3), 2.16 (s, 3 H, 10-H_3), 3.54 (dd, J = 9.5, 9.5 Hz, 1 H, 4′-H), 3.83 (dd, J = 9.5, 6.5 Hz, 1 H, 5′-H), 3.93 (dd, J = 9.5, 3.5 Hz, 1 H, 3′-H), 4.01 (dd, J = 3.5, 2.0 Hz, 1 H, 2′-H), 6.25 (d, J = 2.0 Hz, 1 H, 1′-H), 6.97 (dd, J = 3.0, 1.0 Hz, 1 H, 3-H), 7.39 (d, J = 3.0 Hz, 1 H, 2-H), 7.84 (d, J = 2.3 Hz, 1 H, 5-H), 8.19 (dd, J = 2.3, 1.0 Hz, 1 H, 7-H) ppm. ^{13}C NMR (125.7 MHz, CD_3OD): δ =18.1 (q, C-6′), 23.7 (q, C-10), 71.5 (d, C-2′), 72.5 (d, C-3′), 72.7 (d, C-5′), 73.6 (d, C-4′), 95.9 (d, C-1′), 103.4 (d, C-3), 110.1 (d, C-7), 117.6 (d, C-5), 121.1 (s, C-4), 126.0 (s, C-3a), 128.6 (d, C-2), 133.5 (s, C-6), 138.7 (s, C-7a), 165.5 (s, C-8), 172.0 (s, C-9). DCI-MS: *m/z* (%) = 382 (100) [M + NH_4^+], 290 (20) [$M^+ - C_2H_4NO_2$]. $C_{17}H_{20}N_2O_7$ (364.36).

2-Amino-6-*O*-(α-L-rhamnopyranosyl)benzoic Acid (15): Addition of $(NH_4)_2HPO_4$ (2.89 g). Yield: 8.7 mg/L. M.p. 89 °C. $[\alpha]_D^{20} = -34$ (c = 0.1 in MeOH). IR (KBr): $\tilde{\nu}$ = 3426 cm^{-1}, 1663, 1613, 1555, 1472, 1384. UV (MeOH): λ_{max} (log ε) = 215 nm (4.18), 246 (sh), 330 (3.41). ^1H NMR (500 MHz, [D_6]DMSO): δ = 1.12 (d, J = 6.2 Hz, 3 H, 6′-H_3), 3.28 (n.d., 4′-H), 3.50 (dd, J = 9.5, 6.5 Hz, 1 H, 5′-H), 3.73 (ddm, J = 9.5, 3.5 Hz, 1 H, 3′-H), 3.93 (m, 1 H, 2′-H), 4.56 (d, J = 6.0 Hz, 1 H, 4′-OH), 4.78 (d, J = 5.5 Hz, 1 H, 3′-OH), 4.97 (d, J = 4.0 Hz, 1 H, 2′-OH), 5.25 (d, J = 1.8 Hz, 1 H, 1′-H), 6.18 (s, 2 H, 6-NH_2), 6.45 (dd, J = 8.0, 8.0 Hz, 1 H, 4-H), 7.02 (dd, J = 8.0, 1.1 Hz, 1 H, 3-H or 5-H), 7.24 (dd, J = 8.0, 1.1 Hz, 1 H, 3-H or 5-H), 7.70 (s, 1 H, COOH) ppm. ^{13}C NMR (125.7 MHz, CD_3OD): δ =18.0 (q, C-6′), 70.8 (d, C-5′), 72.1 (d, C-2′), 72.3 (d, C-3′), 72.9 (d, C-4′), 100.4 (d, C-1′), 116.3 (d, C-3), 116.4 (s, C-1), 118.3 (d, C-5), 123.1 (d, C-4), 141.6 (s, C-6), 145.7 (s, C-2), 174.3 (s, C-7). EI-MS (70 eV): *m/z* (%) = 299 (10) [M^+], 152 (100) [$C_7H_6NO_3$], 135 (94). DCI-MS: *m/z* (%) = 299 (100) [M^+], 188 (58), 171 (69). $C_{13}H_{17}NO_7$ (299.28).

1-*O*-(4-Hydroxymethylphenyl) α-L-Rhamnopyranoside (16): Addition of 4-hydroxybenzyl alcohol (0.92 g). Yield: 44.0 mg/L. M.p. 142 °C. $[\alpha]_D^{20} = -31$ (c = 0.2 in MeOH). IR (KBr): $\tilde{\nu}$ =3422 cm^{-1}, 2925, 1612, 1511. UV (MeOH): λ_{max} (log ε) = 223 nm (3.97), 270 (2.63). ^1H NMR (500 MHz, CD_3OD): δ = 1.20 (d, J = 6.5 Hz, 3 H, 6′-H_3), 3.45 (dd, J = 9.5, 9.5 Hz, 1 H, 4′-H), 3.62 (dq, J = 9.5, 6.5 Hz, 1 H, 5′-H), 3.84 (dd, J = 9.5, 3.5 Hz, 1 H, 3′-H), 3.99 (dd, J = 3.5, 2.0 Hz, 1 H, 2′-H), 4.53 (s, 2 H, 7-H_2), 5.41 (d, J = 2.0 Hz, 1 H, 1′-H), 7.03 (dd, J = 8.5, 2.0 Hz, 2 H, 3-H, 5-H), 7.26 (dd, J = 8.5, 2.0 Hz, 2 H, 2-H, 6-H) ppm. ^{13}C NMR (125.7 MHz, CD_3OD): δ = 18.0 (q, C-6′), 64.7 (t, C-7), 70.5 (d, C-5′), 72.0 (d, C-2′), 72.1 (d, C-3′), 73.7 (d, C-4′), 99.7 (d, C-1′), 117.3 (d, C-3, C-5), 129.5 (d, C-2, C-6), 136.3 (s, C-4), 157.0 (s, C-1). DCI-MS: *m/z* (%) =305 (100) [M + NH_3 + NH_4^+], 288 (10) [M^+ + NH_4^+]. $C_{13}H_{18}O_6$ (270.28).

1-*O*-(4-Hydroxybenzyl) α-L-Rhamnopyranoside (17): Addition of 4-hydroxybenzyl alcohol (0.92 g). Yield: 8.0 mg/L. M.p. 100 °C. $[\alpha]_D^{20} = -112$ (c = 3.1 in MeOH). IR (KBr): $\tilde{\nu}$ =3422 cm^{-1}, 2925, 1613, 1507. UV (MeOH): λ_{max} (log ε) = 226 nm (3.50), 272 (2.57). ^1H NMR (500 MHz, CD_3OD): δ = 1.21 (d, J = 6.5 Hz, 3 H, 6′-H_3), 3.44 (dd, J = 9.5, 9.5 Hz, 1 H, 4′-H), 3.64 (dq, J = 9.5, 6.5 Hz, 1 H, 5′-H), 3.82 (dd, J = 9.5, 3.5 Hz, 1 H, 3′-H), 3.84 (s, 2 H, 7-H_2), 3.97 (dd, J = 3.5, 2.0 Hz, 1 H, 2′-H), 4.53 (s, 2 H, 9-H_2), 5.36 (d, J = 2.0 Hz, 1 H, 1′-H), 6.96 (dd, J = 8.5, 1.5 Hz, 2 H, 2-H, 6-H), 7.09 (dd, J = 8.5, 1.5 Hz, 2 H, 3-H, 5-H) ppm. ^{13}C NMR (125.7 MHz, CD_3OD): δ = 18.0 (q, C-6′), 41.1 (t, C-7), 70.5 (d, C-5′), 72.1 (d, C-2′), 72.2 (d, C-3′), 73.8 (d, C-4′), 99.9 (d, C-1′), 117.5 (d, C-2, C-6), 130.8 (d, C-3, C-5), 136.7 (s, C-4), 156.1 (s, C-1). $C_{13}H_{18}O_6$ (270.28).

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[1] S. Grond, H.-J. Langer, P. Henne, I. Sattler, R. Thiericke, S. Grabley, H. Zähner, A. Zeeck, *Eur. J. Org. Chem.* **2000**, 929−937.
[2] T. Kunigami, K. Shin-Ya, K. Furihata, Y. Hayakawa, H. Seto, *J. Antibiot.* **1998**, *51*, 880−882.
[3] J.-F. Hu, D. Wunderlich, I. Sattler, A. Härtl, I. Papastavrou, S. Grond, S. Grabley, X.-Z. Feng, R. Thiericke, *J. Antibiot.* **2000**, *53*, 944−953.
[4] G. Gross, *Biosynthesis of Hydrolyzable Tannins*, in *Comprehensive Natural Product Chemistry, Volume 3* (Eds.: D. Barton, K. Nakanishi, O. Meth-Cohn), Elsevier, Amsterdam, **1999**, 799−826.
[5] C. Milkowski, A. Baumert, D. Strack, *Planta* **2000**, *211*, 883−886.
[6] H.-P. Mock, D. Strack, *Phytochemistry* **1993**, *32*, 575−579.
[7] S. Grond, I. Papastavrou, A. Zeeck, *Eur. J. Org. Chem.* **2000**, 1875−1881.
[8] S. Grabley, R. Thiericke, A. Zeeck, in *Drug Discovery from Nature* (Eds.: S. Grabley, R. Thiericke), Springer, Heidelberg, **1999**, 124−148.
[9] J. Fuchser, A. Zeeck, *Liebigs Ann./Recueil* **1997**, 87−95; H.-J. Schiewe, A. Zeeck, *J. Antibiot.* **1999**, *52*, 635−642; R. Höfs, M. Walker, A. Zeeck, *Angew. Chem. Int. Ed.* **2000**, *39*, 3258−3261.
[10] B. Karmakar, R. M. Vohra, H. Nandanwar, P. Sharma, K. G. Gupta, R. C. Sobti, *J. Biotechnol.* **2000**, *80*, 195−202; I.-Y. Lee, T. G. Volm, J. P. N. Rosazza, *Enzyme Microb. Technol.* **1998**, *23*, 261−266.
[11] A. Castellan, S. Grelier, L. Kessab, A. Nourmamode, Y. Hannachi, *J. Chem. Soc., Perkin Trans.* 2 **1996**, 1131−1138.
[12] H. Inouye, K. Inoue, *Phytochem.* **1985**, *24*, 1857−1859; U. F. Castillo, A.L. Wilkins, D. R. Lauren, D. E. McNaughton, B. L. Smith, *J. Nat. Prod.* **1995**, *58*, 1889−1891.
[13] G. Biagi, O. Livi, V. Scartoni, E. Verugi, *Farmaco, Ed. Sci.* **1988**, *43* 597−611.
[14] D. H. Williams, B. Bardsley, *Angew. Chem. Int. Ed.* **1999**, *38*, 1172−1193.
[15] C. Puder, Diploma Thesis, Göttingen **1994**.
[16] H. B. Bode, B. Bethe, R. Höfs, A. Zeeck, *ChemBioChem.* **2002**, in press.

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