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An efficient chemo-enzymatic approach towards variably functionalized benzotropolones

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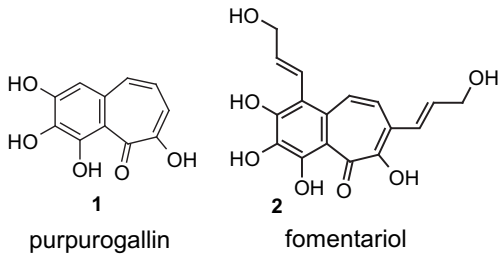
ABSTRACT

An efficient three-step synthesis for benzotropolones via three catalytic steps is presented. Pyrogallol phenones are formed in the first step starting from pyrogallol, which is acylated by proton-catalysis. Catalytic hydrogenation of the phenones yields the corresponding alkylated pyrogallyl dervatives. In the final enzyme-catalyzed step the pyrogallol derivatives are annulated to form the benzotropolone cores. An alternative pathway via the Pechmann reaction is also presented. The combination of the three catalytic steps gives access to a wide range of benzotropolone congeners.

1. Introduction

The protection of human skin from environmental stress like oxygen radicals and UV irradiation is an ongoing research area in the personal care and cosmetics industry. To comply with the changing consumer trends we looked into the preparation of novel naturally derived compounds like the polyphenols. Polyphenols with a benzotropolone core (see Scheme 1) are conveniently found in fruits and fermented tea leaves, e.g., theaflavins, and exhibit a wide range of beneficial biological effects.[1–3] Besides their anti-inflammatory activities they exhibit a high anti-oxidative potential and a strong UV-absorbing power.[4]



Scheme 1. Natural benzotropolone structures.

However, benzotropolones are only found as a mixture of a number of various isomers in their natural sources mostly attached to carrier compounds like e.g., coumarinolignans, ferulic acid, quinic acid or polyhydroxy cinnamic acid.[2,4,18] In addition, the total amount found in any source is limited and quite low, e.g., 0.5–5% of dry weight. So large scale recovery and accumulation of those compounds from natural sources is tedious and accompanied by the production of large amounts of waste by-products. A more environmentally benign way is the specific synthesis of a defined compound, ideally combining both chemical and enzymatic catalysis.[5]

In order to meet the stringent requirements for cosmetic ingredients our synthetic concept has been pegged to four key features:

(a) use starting materials from renewable resources
(b) use only benign chemical reactions
(c) use biotransformations preferentially
(d) prepare a 'modified' biomolecule.

The selected target structures (Scheme 1) are derived from the naturally occurring, however unstable, fomentariol **2** isolated from the tree sponge *Fomes fomentarius*[6] or purpurogallin **1**, which represents the underivatized core structure.

A number of naturally occurring derivatives are known, which differ in their degree of hydroxylation on the aromatic ring system and alkylation of the hydroxyl groups. The chemical synthesis of some non-natural benzotropolones with only limited structural variability has been disclosed.[7] However, a large excesses of an *ortho*-chinone as one precursor had to be used to obtain acceptable yields with pyrogallol as the second reaction partner. The chinones were separately prepared in the presence of excess heavy metal oxides starting from the corresponding phenols.

More appropriate to our requirements would be an in situ generation of the chinone structures via a biocatalytic method with atmospheric oxygen as the preferred oxidant followed by the

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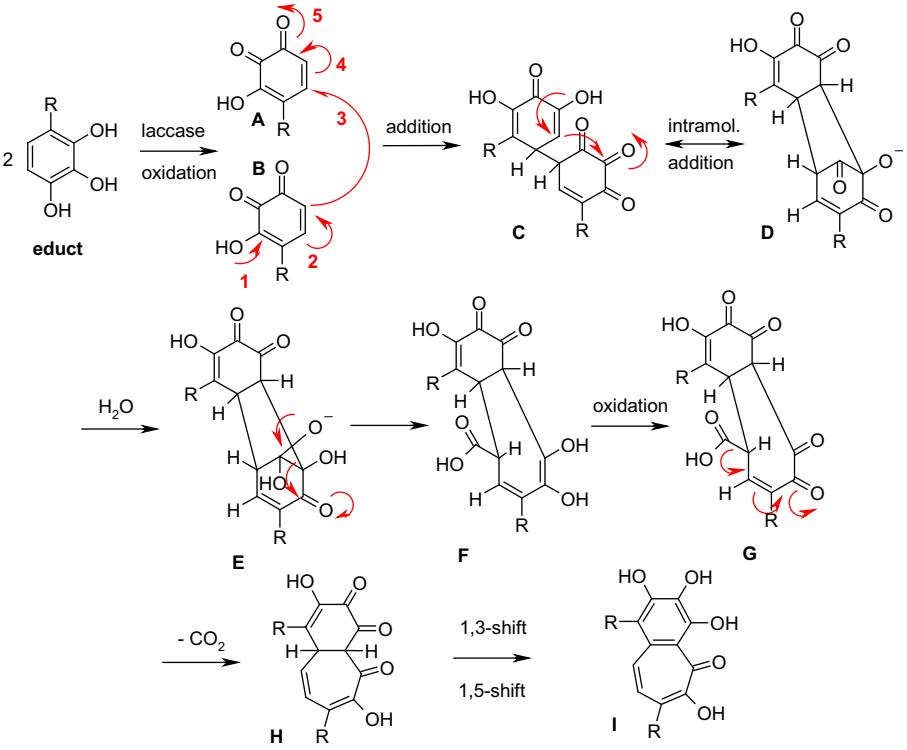
desired annulation to the benzotropolone system in one pot. Fungal laccases (benzene:oxygen oxido reductase EC 1.10.3.2) are a class of extracellular enzymes, which use molecular oxygen as an oxidative reagent.[8,9] The enzymes possess a particularly high redox potential[10] and can be used in mixtures of aqueous buffers and organic solvents.

The mechanism for the oxidative formation of the benzotropolone core has been elucidated recently regarding studies with natural catechins (confer also Scheme 2).[3] Here the intermediate *ortho*-chinones **A** and **B** are generated in situ via laccase-catalyzed oxidation. Subsequently, both compounds dimerize to form the chinone structure **C**. We could isolate this structure and characterize it by NMR and MS when R is a tertiary butyl moiety! In that case the reaction stops at this point. When R is less sterically demanding a second addition takes place to give the postulated intermediate **D**. Addition of water forms a transient carboxylate **F**, which is oxidized to the dione **G** subsequently. Release of carbon dioxide gives compound **H**, which aromatizes via two hydrogen shifts to form the final benzotropolone structure **I**.

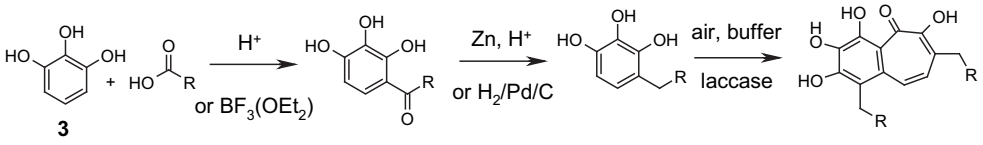
2. Results and discussion

Our target was to deliver both lipophilic and hydrophilic benzotropolone ingredients being applicable to various cosmetic formulations via a general protocol. Surprisingly, we found that the laccases accepted a very wide range of quite unusual compounds as substrates and converted them to the desired benzotropolone cores (compare Schemes 3 and 4). In that respect this class of enzymes luckily showed an equally broad substrate promiscuity in vitro as has been observed with glycosyl-tranferases, previously,[11] which makes both of them versatile synthetic reagents.

A close inspection of fomentariol (Scheme 1) reveals that it is derived from only one precursor. So we tackled two routes to prepare appropriately stable phenol precursors (Scheme 3). In a first approach the three-step synthesis starts with the naturally occurring pyrogallol **3**, which is treated with a series of aliphatic carboxylic acids or acid anhydrides in the presence of a proton source to form the phenones **4**. Unfortunately, these phenones could not be reacted with laccases up to now! So the ketone moiety

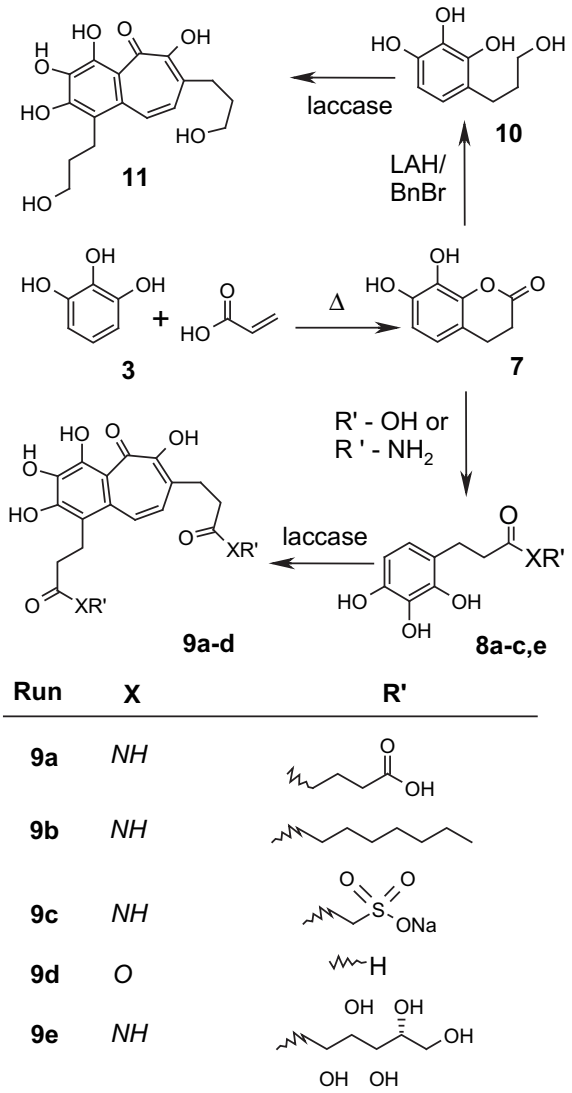


Scheme 2. Proposed mechanism of benzotropolone formation.



R = CH_3	**4a**	comm.	**5a**	95 %	**6a**	66 %
R = C_2H_5	**4b**	51 %	**5b**	91 %	**6b**	37 %
R = C_3H_7	**4c**	53 %	**5c**	96 %	**6c**	88 %
R = $C_{11}H_{23}$	**4d**	38 %	**5d**	65 %	**6d**	32 %
R = $CH(C_2H_5)C_4H_9$	**4e**	56 %	**5e**	44 %	**6e**	------
R = C_2H_4COOEt(H)	**4f**	20 %	**5f**	49 %	**6f**	51 %

Scheme 3. Synthesis of phenol precursors.



Run	X	R'
9a	*NH*	
9b	*NH*	
9c	*NH*	
9d	*O*	
9e	*NH*	

Scheme 4. Synthesis of functional benzotroplones.

is reduced using Clemmensen conditions[12] or catalytic hydrogenation to yield the intermediates **5**. Subsequently, compounds **5** are incubated with catalytic amounts of laccase (*Trametes versicolor*) in an open beaker and oxidized to the corresponding benzotropolones **6**. Protocols have been optimized for lead compound **6c** only.

Although the highly lipophilic compound **5d** is also accepted by the enzyme to form **6d**, the α-branched **5e** is not converted to the expected **6e**, instead an uncyclized product previously postulated[3] as a reaction intermediate has been isolated (corresponding to compound **C** in Scheme 2). During our optimization processes we found that the wasteful Clemmensen reduction with zinc metal as reductant could be replaced by a catalytic hydrogenation[13] more efficiently, **5c**. Succinic anhydride is condensed to pyrogallol **3** in the presence of an excess boron trifluoro etherate to give a mixture of the corresponding ethyl ester and the free acid **4f**, which gives the free acid **5f** after reduction. Surprisingly, also this polar acid is converted in the same manner as the lipophilic derivatives **5** to the desired diacid **6f** in acceptable yields. The sodium salt of this acidic benzotropolone exhibits the desired good water solubility.

The above sequence gives good access only to lipophilic unfunctionalized benzotropolone congeners. The harsh Friedel–Crafts conditions are not compatible with more sensitive functional groups. So we looked into a more versatile route towards hydrophilic benzotropolones, which is achieved via the Pechman reaction[14,15] (Scheme 4).

In the presence of a strongly acidic ion exchange resin pyrogallol **3** can be reacted with acrylic acid to form the lactone **7**. Reduction of the lactone with lithium aluminium hydride in the presence of excess benzyl bromide gives the alcohol **10**, which is incubated with laccases to provide the fomentariol congener **11**. The lactone **7** can be opened easily with oxygen or nitrogen nucleophiles to yield a series of esters or amides **8**. That way additional functionalities can be incorporated into the core system. The series comprises lipophilic amides like **8a** and **8b** and highly polar or charged compounds like the glucamide **8e** or the taurine amide **8c**. Compounds **8** are subsequently treated with laccase. Surprisingly, all of them were converted to the corresponding benzotropolone derivatives **9a**–**e** despite their quite unusual substitution patterns. The derivatives **9** could be isolated in 30–57% yield although no mediator had been added, so all the compounds had to approach the catalytic centre of the enzyme and be recognized as a substrate. Interestingly, the highly polar glucamin **8e**, e.g., is converted to **9e** in 57%. The charged hydrophilic compound **9c**, however, could not be purified satisfactorily from the aqueous incubation mixture. The ^{13}C NMR spectra of the crude product, however, revealed the presence of the tropolone core. The signal at about 181 ppm is characteristic of the tropolone carbon C(=O)-atom.

Regarding the strong oxidative power of the laccases one could imagine that further oxidative conversion of the electron-rich benzotropolone system might take place. Indeed this is observed with benzotropolone congeners, which are well soluble in the incubation medium, resulting in intractable brown materials. The ultimate success for the presented benzotropolone synthesis stems from the insolubilities of the desired final structures. On the contrary, when taking into consideration the proposed mechanism for the enzymatic benzotropolone formation,[3] which runs via several unstable intermediates (according the pathway depicted in Scheme 2),[3] these intermediates should be well solubilized until the formation of the final benzotropolone structures.

With appropriately substituted precursors **8** we did indeed isolate some of those intermediates, accidentally, when their low solubilities made them drop out of the incubation medium. So only a well balanced solvent system consisting of an appropriate buffer solution and an adjusted amount of organic solvent guarantees the isolation of the desired benzotropolones in high yields. Nonetheless, yields could be optimized well up to 90% as is exemplified by the synthesis our lead compound **6c**.

The benzotropolone derivatives have been screened in several personal care applications as UV-protectants and radical scavengers.[16]

3. Conclusion

In summary we could show that commercial laccases are very versatile synthetic tools for the preparation of benzotropolone structures. They exhibit a useful broad substrate promiscuity in vitro and convert both lipophilic and hydrophilic pyrogallol precursors in one step to the corresponding symmetrical benzotropolone systems. Based on the intricate reaction pathway towards these structures a diligent screening of the reaction conditions is mandatory for each derivative, however rewarding, in order to optimize the over-all yields for a competitive commercialization of the desired benzotropolones.

By that way we obtained a series of lipophilic and hydrophilic benzotropolone derivatives in an efficient three-step sequence starting from *proton-catalyzed* Friedel–Crafts acylation of

pyrogallol, followed by a *palladium-catalyzed* reduction of the resulting pyrogallol phenones and a *laccase-catalyzed* benzannulation towards the final benzotropolones with atmospheric oxygen as benign oxidant.

The Pechmann-adduct obtained from pyrogallol and acrylic acid gives access to an even wider range of symmetrical starting materials bearing more sensitive functionalities e.g., carbohydrate derivatives. Replacing acrylic acid by propiolic acid or malic acid should give access to the natural daphnetin,[17] respectively. Via this precursor congeners of the natural fomentariol should be accessible easily. Currently, this route is explored in our labs further.

The presented preparative route could also successfully be applied to the synthesis of a series of 'unsymmetrical' benzotropolone congeners starting with e.g., pyrogallol and properly derivatized *ortho*-diphenols. However, the reaction conditions have to be adjusted more strictly in these cases as the desired cross-coupling products become contaminated with the two possible 'homo-coupled' side products among many others.[18] Results concerning these investigations will be presented in due course elsewhere.

4. Experimental

4.1. General

Chemicals used were reagent grade and were used as supplied except where noted. Solvents were either dried by standard protocols or used as purchased. Commercial laccase (*T. versicolor*, ca. 26 U/mg; FLUKA) was dissolved in 0.05 M phospate buffer (pH=4.8). Analytical thin-layer-chromatography (TLC) was performed using silica gel 60 F_{254} glass plates (MERCK); compound spots were visualized by UV light (254 nm) and by staining with a solution containing *p*-anisaldehyde in concentrated sulfuric acid in ethanol. Flash column chromatography was performed on silica gel 60 (0.040–0.063 mesh, MERCK).

^{1}H NMR and ^{13}C NMR spectra were recorded on a BRUKER dpx 300 spectrometer. ^{1}H NMR and ^{13}C NMR shifts are referenced to the solvent line (given in ppm) unless otherwise stated. ESI-HRMS were measured on an LTQ-FT machine (Thermo Fischer), and substances dissolved in acetonitrile/water mixtures.

4.2. Preparation of phenones 4

*4.2.1. Compound **4b**.* Pyrogallol **3** (50.0 g, 396 mmol), propionic acid (44.0 g, 594 mmol) and strongly acidic ion exchanger AMBERLYST 15 (30.0 g) are heated to 120 °C for 24 h. After cooling down to room temperature the resultant mixture is taken up in ethyl acetate and filtered. The organic phase is thoroughly extracted with water and brine. After drying over magnesium sulfate the solvent is evaporated and the residue chromatographed (eluent: hexane/ethyl acetate 7:3) to give 37.0 g (51%) of phenone **4b** as a whitish solid, sufficiently pure for the ensuing step. Yields are unoptimized!

^{1}H NMR ($CDCl_3$): 1.23 (3H, t, *J*=7.3 Hz), 2.95 (2H, q, *J*=7.3 Hz), 5.62 (OH), 5.96 (OH), 6.53 (1H, d, *J*=9.1 Hz), 7.29 (1H, d, *J*=9.1 Hz), 12.9 (OH). ^{13}C NMR ($CD_3OD/CDCl_3$): 12.4, 35.0, 111.3, 117.0, 126.1, 135.9, 155.1, 155.4, 210.1.

4.2.2. Compound 4c. According to the above protocol for **4b**, compound **4c** is obtained from pyrogallol and butyric acid in 52% yield.

^{1}H NMR ($CDCl_3$): 1.00 (3H, t, *J*=7.3 Hz), 1.75 (2H, sext., *J*=7.3 Hz), 2.87 (2H, t, *J*=7.3 Hz), 5.85 (br, 2×OH), 6.48 (1H, d, *J*=9.1 Hz), 7.26 (1H, d, *J*=9.1 Hz), 12.9 (OH). ^{13}C NMR (CD_3OD): 13.2, 18.5, 39.6, 107.2, 113.3, 122.4, 132.4, 151.9, 152.2, 205.9.

*4.2.3. Compound **4d**.* According to the above protocol for **4b**, compound **4d** is obtained from pyrogallol and laurinic acid in 38% yield.

^{1}H NMR ($CDCl_3$): 0.84 (3H, t, *J*=7.6 Hz), 1.22 (16H, multiplet), 1.67 (2H, quint., *J*=7.6 Hz), 2.84 (2H, t, *J*=7.6 Hz), 5.00 (br, 3×OH), 6.40 (1H, d, *J*=8.5 Hz), 7.21 (1H, d, *J*=8.5 Hz). ^{13}C NMR ($CD_3OD/CDCl_3$): 18.1, 24.9, 26.9, 29.4, 33.5, 33.6, 33.7, 33.8, 36.1, 42.1, 111.5, 117.3, 126.4, 136.1, 155.6, 155.9, 209.9.

*4.2.4. Compound **4e**.* According to the above protocol for **4b**, compound **4e** is obtained from pyrogallol **3** and 2-ethyl-hexanoic acid in 56% yield.

^{1}H NMR ($CDCl_3$): 0.84 (3H, t, *J*=7.1 Hz), 0.87 (3H, t, *J*=7.3 Hz), 1.25 (4H, multiplet), 1.55 (2H, multiplet), 1.75 (2H, multiplet), 3.26 (1H, multiplet), 6.61 (1H, d, *J*=9.1 Hz), 6.58 (br, 2×OH), 7.26 (1H, d, *J*=9.1 Hz), 13.8 (OH). ^{13}C NMR ($CDCl_3$): 12.2, 14.3, 23.0, 26.3, 30.1, 32.5, 47.2, 107.5, 114.4, 122.9, 131.5, 150.3, 151.5, 210.2.

*4.2.5. Compound **4f**.* Pyrogallol **3** (20.0 g, 159 mmol) and succinic anhydride (16.0 g, 160 mmol) are dissolved in 130 ml dry dioxane and cooled to −30 °C. Boron trifluoro etherate (60 ml) are added to this mixture with vigorous stirring. The mixture is slowly warmed up to room temperature over 24 h and subsequently quenched with 40 ml of methanol and evaporated at 40 °C to leave a brown mass, which is purified over a silica gel column (eluent: dichloromethane/methanol 10:0.5). The resulting waxy mass can be further purified by crystallization (hexane/ethyl acetate mixtures) to give 7.6 g (20%) of the desired methyl ester **4f**. The free acid is generated in situ during the strongly acidic conditions of the Clemmensen reduction (see **5f**).

^{1}H NMR (CD_3OD): 2.71 (2H, t, *J*=7.3 Hz), 3.29 (2H, t, *J*=7.3 Hz), 3.70 (3H, s), 6.46 (1H, d, *J*=9.1 Hz), 7.37 (1H, d, *J*=9.1 Hz). ^{13}C NMR (CD_3OD): 28.7, 33.6, 52.3, 108.6, 114.3, 123.2, 133.6, 153.1, 153.3, 175.2, 204.5.

4.3. Preparation of alkylated pyrogallols 5

*4.3.1. Compound **5a**.* 2,3,4-Trihydroxy acetophenone (16.3 g, 97 mmol) and zinc powder (10.0 g, 153 mmol) are added to 45 ml of water and heated to about 50 °C. The slurry is vigorously stirred and treated with 48 ml concentrated hydrogen chloride and subsequently heated to reflux for 1.5–2 h until all starting material has disappeared. After cooling down, the mixture is filtered over Celite and taken up in ethyl acetate. The organic phase is then separated, washed with brine and dried over magnesium sulfate. Filtration and removal of solvent leaves compound **5a** as a white amorphous solid, 12.2 g (89%).

Alternatively, compound **5a** can be obtained via a catalytic hydrogenation. The tri-hydroxyphenone (5.0 g, 297 mmol) is dissolved in methanol water (50 ml:2.5 ml) containing 0.25 g *ortho*-phosphoric acid and 0.5 g of palladium on charcoal (10 wt %). The mixture is heated in an autoclave under hydrogen pressure (7 bar) until complete consumption of the phenone, usually 24–30 h to give 4.4 g (95%) of compound **5a** after filtration over Celite.

^{1}H NMR (CD_3OD): 1.13 (3H, t, *J*=7.6 Hz), 2.50 (2H, q, *J*=7.6 Hz), 6.26 (1H, d, *J*=8.3 Hz), 6.40 (1H, d, *J*=8.3 Hz). ^{13}C NMR (CD_3OD): 14.1, 22.8, 106.5, 118.7, 122.7, 131.8, 143.6, 143.7.

*4.3.2. Compound **5b**.* According to the above protocol for **5a**, compound **5b** is obtained from pyrogallol phenone **4b** in 91% yield as a white solid.

^{1}H NMR (CD_3OD): 0.81 (3H, t, *J*=7.6 Hz), 1.45 (2H, sext., *J*=7.6 Hz), 2.37 (2H, t, *J*=7.6 Hz), 6.15 (1H, d, *J*=8.1 Hz), 6.28 (1H, d, *J*=8.1 Hz). ^{13}C NMR (CD_3OD): 14.3, 24.5, 32.9, 107.7, 120.9, 122.2, 134.1, 144.9, 145.1.

*4.3.3. Compound **5c**.* According to the above protocol for the hydrogenation of **5a**, compound **5c** is obtained from pyrogallol phenone **4c** in 96% yield as a white solid.

^{1}H NMR (CD_3OD): 0.82 (3H, t, J=7.5 Hz), 1.27 (2H, sext., J=7.5 Hz), 1.42 (2H, quint., J=7.5 Hz), 2.40 (2H, t, J=7.5 Hz), 6.15 (1H, d, J=8.4 Hz), 6.29 (1H, d, J=8.4 Hz). ^{13}C NMR (CD_3OD): 14.5, 23.6, 30.5, 33.7, 107.7, 120.8, 122.4, 134.1, 144.9, 145.0.

*4.3.4. Compound **5d**.* According to the above protocol for **5a**, compound **5d** is obtained from pyrogallol phenone **4d** in 65% yield as a waxy solid.

^{1}H NMR (CD_3OD): 0.87 (3H, t, J=7.5 Hz), 1.26 (18H, multiplet), 1.53 (2H, quint., J=7.5 Hz), 2.49 (2H, t, J=7.5 Hz), 6.28 (1H, d, J=8.2 Hz), 6.40 (1H, d, J=8.2 Hz). ^{13}C NMR (CD_3OD): 13.9, 22.8, 29.6, 29.7, 29.8 (2C), 29.9 (2C), 30.0 (2C), 30.4, 32.1, 106.8, 119.9, 121.5, 132.7, 143.5, 143.7.

*4.3.5. Compound **5e**.* According to the above protocol for **5a**, compound **5e** is obtained from pyrogallol phenone **4e** in 44% yield as a syrup after silica gel chromatography (eluent: hexane/ethyl acetate 1:1).

^{1}H NMR ($CDCl_3$): 0.86 (6H, t, J=7.3 Hz), 1.27 (7H, multiplet), 1.56 (2H, multiplet), 2.45 (2H, d, J=7.1 Hz), 6.39 (1H, d, J=8.2 Hz), 6.50 (1H, d, J=8.2 Hz). ^{13}C NMR ($CDCl_3$): 11.2, 14.5, 23.4, 26.0, 29.2, 32.9, 34.2, 40.1, 107.4, 121.1, 121.7, 131.8, 141.7, 142.8.

*4.3.6. Compound **5f**.* According to the above protocol for **5a**, the free acid **5f** is obtained from pyrogallol phenone **4f**, which is predissolved in dioxane/water, in 49% yield as a syrup after silica gel chromatography (eluent: dichloromethane/methanol 10:1).

^{1}H NMR (CD_3OD): 1.15 (2H, quint., J=7.2 Hz), 2.28 (2H, t, J=7.2 Hz), 2.57 (2H, t, J=7.2 Hz), 6.28 (1H, d, J=8.0 Hz), 6.40 (1H, d, J=8.0 Hz). ^{13}C NMR (CD_3OD): 26.6, 30.1, 34.3, 107.7, 121.0 (2×C), 131.8, 145.3 (2×C), 176.3.

4.4. Preparation of benzotropolone derivatives 6

*4.4.1. Compound **6a**.* Pyrogallol derivative **5a** (0.300 g as obtained) is dissolved in 20 ml of the indicated buffer solution containing 7.0 mg of laccase and are incubated in an open beaker with vigorous stirring at room temperature until all starting material is consumed (usually 20–24 h). The resulting orange precipitate is filtered off, washed with water and lyophilized from dioxane to give 0.170 g (66%) of benzotropolone **6a**.

The reaction can be sped up significantly by increasing the amount of enzyme. Clear incubation mixtures with the more lipophilic starting materials are obtained by admixing appropriate amounts of acetone or dioxane (up to 50% v/v).

^{1}H NMR ($CDCl_3$): 1.15 (3H, t, J=7.6 Hz), 1.21 (3H, t, J=7.6 Hz), 1.55 (OH), 2.75 (2H, q, J=7.6 Hz), 2.98 (2H, q, J=7.6 Hz), 6.14 (2×OH), 6.79 (1H, d, J=12.6 Hz), 7.53 (1H, d, J=12.6 Hz), 8.80 (OH). ^{13}C NMR ($CDCl_3$): 13.7, 14.6, 19.6, 28.1, 116.3, 120.6, 128.4, 128.6, 131.2, 132.4, 134.3, 147.1, 148.6, 152.1, 181.6. ESI-HRMS m/z 275.09236 $[M-H]^-$ (calcd for $C_{15}H_{15}O_5$: 275.09250).

*4.4.2. Compound **6b**.* According to the above protocol for **6a**, compound **6b** is obtained from pyrogallol derivative **5b** (dissolved in buffer/acetone 4:1) in 37% yield as an orange solid.

^{1}H NMR (CD_3OD): 0.88 (3H, t, J=7.6 Hz), 0.92 (3H, t, J=7.6 Hz), 1.46 (2H, sext., J=7.5 Hz), 1.59 (2H, sext., J=7.5 Hz), 2.62 (2H, t, J=7.6 Hz), 2.86 (2H, q, J=7.6 Hz), 6.64 (1H, d, J=12.3 Hz), 7.45 (1H, d, J=12.3 Hz). ^{13}C NMR (CD_3OD): 14.5, 23.4, 24.2, 28.9, 37.3, 116.9, 120.4, 128.9, 129.1, 131.9, 132.7, 135.2, 150.5, 150.8, 153.2, 183.0. ESI-HRMS m/z 303.12369 $[M-H]^-$ (calcd for $C_{17}H_{19}O_5$: 303.12380).

*4.4.3. Compound **6c**.* According to the above protocol for **6a**, compound **6c** is obtained from pyrogallol derivative **5c** (dissolved in buffer/acetone 7:3) in 88% yield as an orange solid.

^{1}H NMR ($CDCl_3$): 0.84 (6H, 2×t, J=7.6 Hz), 1.61–1.68 (8H, multiplet), 2.73 (2H, t, J=7.6 Hz), 2.93 (2H, t, J=7.6 Hz), 6.19 (2×OH), 6.76 (1H, d, J=12.3 Hz), 7.53 (1H, d, J=12.3 Hz), 8.78 (OH). ^{13}C NMR ($CDCl_3$): 14.4 (2×C), 23.2, 23.3, 26.0, 31.6, 32.5, 34.6, 116.3, 119.3, 128.3, 129.0, 131.4, 132.3, 133.2, 147.4, 148.6, 152.3, 181.6. ESI-HRMS m/z 331.15497 $[M-H]^-$ (calcd for $C_{19}H_{23}O_5$: 331.15510).

*4.4.4. Compound **6d**.* According to the above protocol for **6a**, compound **6d** is obtained from pyrogallol derivative **5d** (dissolved in buffer/acetone 2.5:1) in 32% yield as an orange syrup after extraction of the incubation mixture with ethyl acetate and silica gel chromatography (eluent: hexane/ethyl acetate 1:1) of the crude product.

^{1}H NMR ($CDCl_3$): 0.88 (6H, 2×t, J=7.6 Hz), 1.40–1.70 (40H, multiplet), 2.79 (2H, t, J=7.6 Hz), 2.95 (2H, t, J=7.6 Hz), 6.30 (OH), 6.40 (OH), 6.81 (1H, d, J=12.3 Hz), 7.58 (1H, d, J=12.3 Hz), 8.78 (OH). ^{13}C NMR ($CDCl_3$): 14.4 (2×C), 23.2, 23.3, 26.0, 31.6, 32.5, 34.6, 116.5, 119.7, 128.1, 129.0, 131.3, 132.4, 133.1, 147.2, 148.5, 153.1, 181.4. ESI-HRMS m/z 555.40530 $[M-H]^-$ (calcd for $C_{35}H_{55}O_5$: 555.40550).

*4.4.5. Compound **6f**.* According to the above protocol for **6a**, compound **6f** is obtained from pyrogallol derivative **5f** (dissolved in buffer:acetone 12:1) in 51% yield as an orange solid.

^{1}H NMR (CD_3OD): 1.78 (2H, quint., J=7.3 Hz), 1.88 (2H, quint., J=7.3 Hz), 2.20 (4H, t, J=7.3 Hz), 2.71 (2H, t, J=7.3 Hz), 2.97 (2H, t, J=7.3 Hz), 6.78 (1H, d, J=12.3 Hz), 7.60 (1H, d, J=12.3 Hz). ^{13}C NMR (CD_3OD): 25.2, 25.3, 26.1, 33.8, 36.3, 37.4, 115.5, 118.2, 127.6, 128.0, 130.6, 131.1, 134.2, 148.9, 150.1, 151.9, 175.8, 181.4. ESI-HRMS m/z 391.10325 $[M-H]^-$ (calcd for $C_{19}H_{19}O_9$: 391.10346).

4.5. Compounds from the Pechman-route: 7–11

*4.5.1. Compound **7**.* Pyrogallol **3** (50.0 g, 397 mmol) is mixed with 32.6 ml acrylic acid and 15 g strongly acidic ion exchange resin AMBERLYST 15 in 250 ml toluene and refluxed in a Dean–Stark trap until the theoretical amount of water has been liberated. The mixture is filtered while hot and then cooled down. The resulting solid is repeatedly washed with diethyl ether to yield 27.0 g (38%) of lactone **7** as a solid, which is pure enough for the ensuing transformations.

^{1}H NMR (DMSO-d_6): 2.73 (2H, dd, J=5.7, 12.3 Hz), 2.85 (2H, dd, J=5.7, 12.3 Hz), 6.51 (1H, s), 8.85 (OH), 9.13 (OH). ^{13}C NMR (DMSO-d_6): 22.7, 29.1, 110.8, 114.5, 116.9, 119.2, 132.2, 140.7, 145.4, 168.5.

*4.5.2. Compound **8a**.* 4-Amino butyric acid methyl ester hydrochloride salt (3.75 g, 24.4 mmol) and lactone **7** (4.00 g, 22.2 mmol) are dissolved in a mixture of 15 ml dimethylformamide and 6.1 ml of a solution of 4 N sodium hydroxyde with vigorous stirring. The mixture is stirred until complete consumption of the starting materials is indicated by TLC. The mixture is extracted with ethyl acetate, washed with water and brine and dried over magnesium sulfate. Evaporation of a the solvent leaves a **8a** as a syrupy residue in 41% (2.7 g) yield.

^{1}H NMR (DMSO-d_6): 1.62 (2H, quint., J=7.6 Hz), 2.27 (4H, m), 2.62 (2H, t, J=7.6 Hz), 3.04 (2H, q, J=7.6 Hz), 3.57 (3H, s), 6.18 (1H, d, J=8.1 Hz), 6.31 (1H, d, J=8.1 Hz), 7.84 (NH), 8.13 (OH), 8.27 (OH), 8.74 (OH). ^{13}C NMR (DMSO-d_6): 24.5, 25.4, 30.67, 36.0, 37.9, 51.2, 106.3, 118.8, 119.2, 132.9, 143.40, 144.2, 172.6, 173.1.

*4.5.3. Compound **8b**.* The lactone **7** (2.50 g, 13.8 mmol) and 2.3 ml of octylamine (13.8 mmol) are dissolved in 10 ml dimethylformamide at room temperature and are stirred for 5 h. The mixture is subsequently extracted with ethyl acetate, washed with water and

dried over magnesium sulfate. Evaporation leaves a crude syrup, which is taken up in diethyl ether and extracted with 4 N hydrogenchloride solution. Evaporation of the organic phase gives 2.1 g (50%) of the amide **8b** as a colourless syrupy mass.

^{1}H NMR ($CDCl_3$): 0.80 (3H, t, J=6.9 Hz), 1.13–1.23 (10H, m), 1.35 (2H, quint., J=7.2 Hz), 2.44 (2H, t, J=8.4 Hz), 2.73 (2H, t, J=8.4 Hz), 3.42 (2H, q, J=7.6 Hz), 5.70 (NH, t, J=7.0), 6.39 (2H, s). ^{13}C NMR (DMSO-d_6): 14.1, 22.6, 24.3, 26.8, 29.1, 29.2, 29.3, 31.8, 37.4, 40.1, 107.6, 120.3, 120.4, 133.2, 142.5, 142.9, 174.8.

*4.5.4. Compound **8c**.* Commercial food additive taurin (3.27 g, 26.2 mmol) is dissolved at room temperature in dioxan/water (25 ml:12.5 ml) and 6.53 ml of 4 N sodiumhydroxide solution. Lactone **7** (5.0 g, 26.6 mmol) is then added portionwise to this mixture at 0 °C and stirred for 1 h. The mixture is then lyophilized and the resulting white powder extensively washed with acetone. The taurin sodium salt adduct **8c** is obtained as a very hygroscopic material in 98% yield (8.4 g) after a final lyophilization from water.

^{1}H NMR (D_2O): 1.90 (3H, t, J=6.6 Hz), 2.20 (2H, t, J=6.6), 2.41 (2H, t, J=6.9 Hz), 2.93 (2H, t, J=6.9 Hz), 5.89 (2H, 2×s). ^{13}C NMR (DMSO-d_6): 25.4, 35.2, 36.6, 52.4, 106.8, 118.1, 118.4, 133.9, 141.9, 143.4, 172.7.

*4.5.5. Compound **8e**.* The lactone **7** (2.20 g, 12 mmol) is added to a solution of glucamine (2.20 g, 12 mmol) in 15 ml of dimethylformamide at room temperature and stirred for 24 h. The clear orange solution is then dropped into 100 ml of dichloromethane and the resulting precipitate recovered by centrifugation and washed subsequently with dichloromethane and diethyl ether. The glucamine adduct **8e** is obtained in 98% (4.20 g).

^{1}H NMR (D_2O): 2.542 (2H, t, J=7.2 Hz), 2.82 (2H, t, J=7.92 Hz), 3.16 (1H, dd, J=7.5 Hz, 13.8 Hz), 3.36 (1H, dd, J=4.5 Hz, 13.8 Hz), 3.62 (3H, m), 3.72 (3H, m), 6.46 (1H, d, J=8.4 Hz), 6.58 (1H, d, J=8.4 Hz). ^{13}C NMR (D_2O): 25.5, 36.6, 47.7, 62.6, 70.0, 71.0, 71.1, 71.4, 107.9, 120.2, 120.8, 132.8, 143.4, 143.8, 176.3.

*4.5.6. Compound **9a**.* According to the above protocol for **6a**, compound **9a** is obtained from pyrogallol derivative **8a** (1.00 g, 3.3 mmol). After incubation for 24 h in buffer/acetone (20 ml:10 ml) the resulting residue is filtered off, washed with water and lyophilized from dioxane to give **9a** as an orange solid in 30% (0.28 g) yield.

^{1}H NMR (CD_3OD): 1.58 (4H, 2×t, J=7.6 Hz), 2.18 (4H, 2×t, J=7.6 Hz), 2.43 (2H, t, J=6.6 Hz), 2.55 (2H, t, J=6.6 Hz), 2.92 (2H, t, J=7.8 Hz), 3.13 (4H, m), 3.21 (2H, t, J=7.8 Hz), 6.65 (1H, d, J=12.1 Hz), 7.44 (1H, d, J=12.1 Hz), 7.85 (2H, br, NH). ^{13}C NMR (CD_3OD): 14.4, 25.6, 32.0, 36.4, 31.6, 37.2, 39.7, 52.1, 117.0, 119.1, 128.8, 129.1, 131.9, 132.3, 136.0, 150.9, 151.1, 153.6, 173.8, 174.0, 174.2, 174.5, 182.8. ESI-HRMS m/z 585.35442 $[M-H]^-$ (calcd for $C_{33}H_{49}O_5N_2$: 585.35453).

*4.5.7. Compound **9b**.* According to the above protocol for **6a**, compound **9b** is obtained from pyrogallol derivative **8b** (1.00 g, 3.2 mmol). After incubation for 24 h in buffer/acetone (25 ml:25 ml) a sticky orange residue is filtered off, washed with water and lyophilized from dioxane. The resulting residue is chromatographed over silica gel (eluent: dichloromethane/methanol 10:1) to give 0.81 g (45%) of amide **9b**.

^{1}H NMR (CD_3OD): 0.80 (6H, t, J=6.9 Hz), 1.10–1.25 (20H, m), 1.35 (4H, quint., J=7.2 Hz), 2.23 (2H, t, J=7.5 Hz), 2.45 (2H, t, J=7.5 Hz), 2.93 (2H, t, J=7.5 Hz), 3.04 (4H, t, J=7.6 Hz), 3.19 (2H, t, J=7.6), 6.78 (1H, d, J=12.6 Hz), 7.50 (1H, d, J=12.6 Hz). ^{13}C NMR (DMSO-d_6): 14.1, 23.7, 27.0, 27.2, 30.4, 30.5, 31.9, 36.0, 37.0, 37.9, 40.6, 117.1, 118.7, 128.9, 129.1, 130.8, 132.0, 136.1, 151.0, 151.1, 153.6, 174.9, 175.4, 182.9. ESI-HRMS m/z 585.35442 $[M-H]^-$ (calcd for $C_{33}H_{49}O_5N_2$: 585.35453).

*4.5.8. Compound **9d**.* The lactone **7** (1.00 g, 5.5 mmol) is directly incubated according **6a** in buffer/acetone (20 ml:4 ml). The resulting orange solid is filtered off, washed with water and lyophilized from dioxane to yield 0.32 g (32%) of benzotropolone **9d**.

^{1}H NMR (DMSO-d_6): 2.36 (2H, t, J=6.3 Hz), 2.55 (2H, t, J=6.3 Hz), 2.93 (2H, t, J=6.0 Hz), 3.17 (2H, t, J=6.0 Hz), 3.38 (2OH), 6.90 (1H, d, J=12.1 Hz), 7.56 (1H, d, J=12.1 Hz), 9.61 (OH), 9.81 (OH), 10.22 (OH). ^{13}C NMR (DMSO-d_6): 21.5, 29.6, 32.6, 33.7, 115.3, 117.1, 127.3, 127.9, 129.7, 129.8, 134.1, 149.8, 149.9, 152.0, 173.7, 173.8, 181.3. ESI-HRMS m/z 363.07203 $[M-H]^-$ (calcd for $C_{17}H_{15}O_9$: 363.07216).

*4.5.9. Compound **9e**.* According to the above protocol for **6a**, compound **9e** is obtained from pyrogallol derivative **8e** (0.56 g, 1.5 mmol). After incubation for 2 d in buffer a sticky orange residue is filtered off, washed with water and lyophilized from dioxane to give 0.30 g (57%) of benzotropolone glucamide **9b**.

^{1}H NMR (DMSO-d_6): 2.30 (2H, t, J=6.0 Hz), 2.42 (2H, t, J=6.0 Hz), 2.92 (2H, t, J=6.0 Hz), 3.06 (2H, m), 3.27 (2H, t, J=6.0 Hz), 3.36–3.75 (28H, m), 6.86 (1H, d, J=9.3 Hz), 7.57 (1H, d, J=9.3 Hz), 7.84 (1H, br t, NH), 7.93 (1H, br t, NH). ^{13}C NMR (DMSO-d_6): 22.2, 29.8, 34.4, 35.3, 42.0, 42.1, 63.3, 69.5, 71.4, 71.7, 72.0, 115.2, 117.7, 127.2, 127.5, 129.7, 130.6, 134.3, 149.6, 149.9, 151.8, 171.6, 172.1, 181.2. ESI-HRMS m/z 689.24027 $[M-H]^-$ (calcd for $C_{29}H_{41}O_{17}N_2$: 689.23997).

*4.5.10. Compound **10**.* In an argon atmosphere lithium aluminium hydride (0.59 g, 15.5 mmol) suspended in 5 ml of dry tetrahydrofuran containing benzyl bromide (2.60 g, 15.2 mmol) is stirred for 15 min at room temperature. Thereafter the flask is cooled to 0 °C and lactone **7** (1.00 g, 5.6 mmol) dissolved in 10 ml tetrahydrofuran is dropped in slowly whereby a white precipitate forms. After 0.5 h the mixture is quenched by successively adding 2 N hydrogenchloride solution and ethylacetate. The organic phase is then separated and extracted with water, hydrogenchloride solution and dried over magnesium sulfate. Evaporation of the solvent leaves 0.80 g (78%) of compound **10** as an oily residue, which is used as obtained.

^{1}H NMR (DMSO-d_6): 1.62 (2H, quint., J=6.0 Hz), 2.43 (2H, t, J=6.0 Hz), 3.39 (OH and 2H, t, J=6.0 Hz), 6.20 (1H, d, J=6.3 Hz), 6.32 (1H, d, J=6.3 Hz), 8.02 (OH), 8.12 (OH), 8.74 (OH). ^{13}C NMR (DMSO-d_6): 25.9, 33.1, 59.7, 106.2, 118.9, 119.6, 132.8, 143.9, 144.1, 170.8.

*4.5.11. Compound **11**.* According to the above protocol for **6a**, compound **11** is obtained from pyrogallol derivative **10** (0.70 g, 3.8 mmol). After incubation for 24 h in buffer/acetone (20 ml:25 ml) an orange residue is filtered off, washed with water and lyophilized from dioxane to give 0.36 g (56%) of benzotropolone **11**.

^{1}H NMR (CD_3OD): 1.68 (2H, quint., J=6.0 Hz), 1.77 (2H, quint., J=6.0 Hz), 2.71 (2H, t, J=6.0 Hz), 2.94 (2H, t, J=6.0 Hz), 3.51 (4H, t, J=6.0 Hz), 6.66 (1H, d, J=12.3 Hz), 7.49 (1H, d, J=12.3 Hz). ^{13}C NMR (CD_3OD): 23.2, 31.8, 33.0, 33.7, 62.7, 62.8, 117.0, 119.8, 129.0, 129.1, 131.8, 132.3, 135.3, 150.6, 150.8, 153.3, 182.9. ESI-HRMS m/z 335.11329 $[M-H]^-$ (calcd for $C_{17}H_{19}O_7$: 335.11363).

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References and notes

1. Haslam, E. *Phytochemistry* **2003**, *64*, 61–73.
2. Sang, S.; Tian, S.; Meng, X.; Stark, R. E.; Rosen, R. T.; Yang, C. S.; Ho, C.-T. *Tetrahedron Lett.* **2002**, *43*, 7129–7133.
3. Matsuo, Y.; Tanaka, T.; Kuono, I. *Tetrahedron* **2006**, *62*, 4774–4783.
4. Ho, C. -T.; Ghai, G.; Sang S.; Jhoo, J. -W.; Huang, M. -T.; Rosen, T. R.; Dushenkov, S. U.S. Patent 0,049,284, 2005.

5. Armmitage, B. A. *Annu. Rep. Prog. Chem., Sect. B* **2001**, *97*, 483–513.
6. Arpin, N.; Fauvre-Bovin, J.; Steglich, W. *Phytochemistry* **1974**, *13*, 1949–1952.
7. Horner, L.; Dürckheimer, W. German Patent 1091114, 1960.
8. Leonowicz, A.; Cho, N.-S.; Lutarek, J.; Wilkolazka, A.; Wojtas-Wasilewska, M.; Matszewska, A.; Hofrichter, M.; Wesenberg, D.; Rogalski, J. *J. Basic Microbiol.* **2001**, *41*, 185–227.
9. Riva, S. *Trends Biotechnol.* **2006**, *25*, 209–226.
10. Burton, S. G. *Trends Biotechnol.* **2003**, *21*, 543–549.
11. Oehrlein, R. *Top. Curr. Chem.* **1999**, *200*, 227–254.
12. Clemmensen, E. *Chem. Ber.* **1914**, 51–63.
13. Borzatta, V.; Brancaleoni, D.; Battistini, C. European Patent 1048664, 2000.
14. Hoefnagel, A. J.; Gunnewegh, E. A.; Downing, R. S.; van Bekkum, H. *J. Chem. Soc., Chem. Commun.* **1995**, 225–226.
15. Laufer, M. C.; Hausmann, H.; Hölderich, W. F. *J. Catal.* **2003**, *218*, 315–320.
16. WO Patent submitted, 2008.
17. Molyneux, R.; Jurd, L. *Aust. J. Chem.* **1974**, *27*, 2697–2702.
18. Bhardwaj, S.; Mishra, A. K.; Kaushik, N. K.; Khan, M. A. *Trends Appl. Sci. Res.* **2006**, *1*, 115–122.