Biocatalytic Friedel–Crafts Acylation and Fries Reaction

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# Table of Contents

1 General Information .................................................................................................................. S1
   Materials .................................................................................................................................. S1
   Methods .................................................................................................................................... S1
2 Plasmid Construction .............................................................................................................. S2
3 DNA- & Protein Sequences ..................................................................................................... S3
   \( Pb\)ATaseWT .................................................................................................................... S3
   \( Pp\)ATaseWT .................................................................................................................... S4
   \( Pp\)ATaseCH .................................................................................................................. S5
4 Biocatalyst Preparation & Protein Expression ....................................................................... S7
   Shake flask cultivation of the \( Pseudomonas\) wildtypes ...................................................... S7
   Shake flask cultivation and expression of the recombinant ATases ........................................ S7
   Purification of \( Pp\)ATaseCH ............................................................................................. S8
5 Activity Assay ........................................................................................................................ S9
6 Biotransformations ................................................................................................................ S10
   General procedure for the Friedel-Crafts bioacetylation using different acetyl donors .... S10
   General procedure for the Friedel-Crafts biopropanoylation of \( 1b\) using different acyl donors ................................................................. S10
   General procedure for the enzymatic Fries rearrangement-like reaction of \( 4b\) into \( 2b\) .... S10
   Time studies using \( Pp\)ATaseCH with different donors ......................................................... S11
   Inter- versus intramolecular reaction .................................................................................... S10
7 Analytical Methods ................................................................................................................. S13
   Determination of conversion ................................................................................................. S13
8 Spectroscopic Data ................................................................................................................ S16
9 NMR- & GC-MS-Spectra ....................................................................................................... S20
10 References ............................................................................................................................ S38
1 General Information

Materials
All starting materials were obtained from Sigma-Aldrich, Alfa Aesar or TCI-Chemicals and used as received unless stated otherwise. Commercially available resorcinol diacetate (5b) was purified by flash chromatography prior to applications. 1,3-Diacetyl-2,4,6-trihydroxy benzene (DAPG, 3a), 1,3-dipropanoyl-2,4,6-trihydroxy benzene (DPPG) and resorcinol monoacetate (4b) were chemically synthesized as previously described.\textsuperscript{1} Pseudomonas protegens DSM 19095 and Pseudomonas brassicacearum DSM 13227 were obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ). Pseudomonas fluorescens Pf-5 was obtained from the American Type Culture Collection (ATCC BAA-477). gBlocks® gene fragments, primers for polymerase-chain reactions (PCR) were purchased from IDT and Eurofins MWG Operon. The expression vector pASK-IBA3plus was purchased from IBA©-Solutions for Lifeciences. Restriction enzymes and the PureLink® Genomic DNA Minikit were obtained from ThermoFisher Scientific. Chemically competent \textit{E. coli} DH5α and \textit{E. coli} BL21 (DE3) cells and the Gibson Assembly® master mix were purchased from New England Biolabs. Size-exclusion chromatography (SEC) columns and PD-10 desalting columns were purchased from GE Healthcare Life Sciences.

Methods
TLC was carried out with pre-coated aluminum sheets (TLC Silica gel 60 F\textsubscript{254}, Merck) with detection by UV (254 nm) and/or by staining with cinnamaldehyde/HCl solution [abs. EtOH (72.2 vol%), conc. HCl (3.6 vol%), \textit{trans}-cinnamaldehyde (3.6 vol%)]. GC-MS spectra were recorded with an Agilent 7890A GC-system, equipped with an Agilent 5975C mass selective detector and a HP-5 MS column (30 m × 0.25 mm × 0.25 µm). Analysis was performed according to the following parameters: injector 250 °C, constant flow 0.7 mL; temperature program: 100 °C (hold 0.5 min) 100 °C to 300 °C (10 °C min\textsuperscript{-1}), 300 °C (hold 2 min). Helium was used as carrier gas and CH\textsubscript{2}Cl\textsubscript{2} or EtOAc were used as solvent. \textsuperscript{1}H- and \textsuperscript{13}C-NMR spectra were recorded at 20 °C on a 300 Bruker NMR unit; chemical shifts are given in ppm relative to Me\textsubscript{4}Si (\textsuperscript{1}H: Me\textsubscript{4}Si = 0.0 ppm) or relative to the resonance of the solvent (\textsuperscript{1}H: acetone-\textit{d}\textsubscript{6} = 2.05 ppm; \textsuperscript{13}C: acetone-\textit{d}\textsubscript{6} = 29.84 & 206.6 ppm).
2 Plasmid Construction

All primer sequences, plasmids and bacterial strains used in this study are collated in Table S1. To construct the recombinant PpATaseWT and PbATaseWT, the genomic DNA of the respective Pseudomonas wild-type served as template to amplify the ATase-encoding operon phlACB by PCR. The PCR products were digested (KpnI/BamHI), gel-purified and ligated into the digested target vector pASK-Iba3plus. The resulting expression vectors carry the wild-type-derived ATase encoding genes phlACB under the control of the P_Tet promoter. To construct the recombinant PpATaseCH, the ATase encoding open-reading frames phlA, phlC and phlB of P. protegens were codon-optimized by manually matching the codon-frequency of the Pseudomonas wild-type with E. coli. For this purpose, codon-usage tables for Escherichia coli B and Pseudomonas fluorescens were obtained from the Kazusa-database (http://www.kazusa.or.jp/codon/). Ribosomal binding sites suitable for E. coli were introduced upstream of each start codon of every individual phl gene. The optimized phl genes were obtained as gene fragments (gBlocks©) and assembled together with the double-digested pASKIBA3plus backbone (EcoRI/HindIII) by Gibson cloning (Gibson Assembly® master mix) and subsequent overlap extension-PCR (OE-PCR). The resulting expression vector carries the E. coli codon-optimized ATase encoding genes phlACB under the control of the P_Tet promoter.

Table S1. Strains, plasmids and primers used in this study. Mutagenized codons are shown in bold, restriction sites are underlined. Ribosomal binding sites are shown in lowercase letters.

| Bacterial Strains | Origin (Strain ID) | GenBankID (phlACB gene locus/comments) |
|-------------------|-------------------|-------------------------------------|
| Pseudomonas brassicaeearum DSMZ (DSM 13227) | LT629713.1 (bp: 1051432-1054193) / phlACB from P. brassicaeearum DSM13227 | |
| Pseudomonas protegens DSMZ (DSM 19095) | CP003190.1 (bp: 6560049-6562816) / other designation: CHA0 | |
| Pseudomonas fluorescens Pf5 ATCC (ATCC BAA-477) | CP000076.1 (bp: 6766435-6769202) | |

| Plasmids | Origin (GenBankID) | Description/Comments |
|----------|-------------------|---------------------|
| pASKIBA3plus | IBA-Lifescience this study | Pro+, Amp', ColE1, C-terminal StrepTag |
| pEG330 | (KY173354) | Wild-type-derived phlACB genes of P. brassicaeearum DSM13227, isolated from genomic DNA by PCR; PCR primers: PpWT-Fow/Rev. |
| pEG331 | (CP003190.1) | Wild-type-derived phlACB genes of P. protegens DSM19095, isolated from genomic DNA by PCR; PCR primers: PpWT-Fow/Rev. |
| pEG332 | (KY173355) | Codon-optimized gene fragments phlA, phlC and phlB based on phlACB from P. protegens DSM19095, assembled by Gibson cloning and overlap-extension PCR. PCR primers: OE1-AATaseCH-Fow/Rev. |

| Primers | Origin | Sequence (5'→3') |
|----------|-------|-----------------|
| PpWT-Fow | Eurofins | ATATAGGTACCATGAACGTGAAAAAGATAGGTATTG |
| PpWT-Rev | Eurofins | ATATAGGTACCATGAACGTGAAAAAGATAGGTATTG |
| PbWT-Fow | Eurofins | ATATAGGTACCATGAACGTGAAAAAGATAGGTATTG |
| PbWT-Rev | Eurofins | ATATAGGTACCATGAACGTGAAAAAGATAGGTATTG |
| OE1AATaseCH-Fow | IDT | ATATAGGTACCATGAACGTGAAAAAGATAGGTATTG |
| OE2AATaseCH-Fow | IDT | ATATAGGTACCATGAACGTGAAAAAGATAGGTATTG |
| OE3AATaseCH -Rev | IDT | ATATAGGTACCATGAACGTGAAAAAGATAGGTATTG |
| OE4AATaseCH -Rev | IDT | ATATAGGTACCATGAACGTGAAAAAGATAGGTATTG |
3 DNA- & Protein Sequences

Nucleotide sequences used in this study have been deposited at NCBI.

PbATaseWT (wild-type nucleotide *phlACB* gene sequence, *P. brassicacearum* DSM 13227, Genbank accession no.: KY173354)

PpATaseWT (wild-type nucleotide *phlACB* gene sequence, *P. protegens* DSM 19095, Genbank accession no.: CP003190.1)

PpATaseCH (codon-optimized *phlACB* gene sequence, *P. protegens* DSM 19095, Genbank accession no.: KY173355)

**PbATaseWT**

**DNA-sequence** - (start- and stop-codon of the ORFs are highlighted)

```
5’ ATGAAATTAAGTGGAAATTGAGCTATTGAGCTACGGGCGGGCCATCCGGCTCGTCAGGTGGAGACGACGTGATCC
AGGTGGATTTATGGGAACTTTTGGGGAGCTATGGGCCTCGTCGCTACGGGATTTTTCTGCCCTGAGG
ATGATATGTCGACTGGGATTTTGCTGAGGCGGGGGGCGCGACGGTCTGGCCTATGGCGCAGATGGGCTGG
AGTACGGGTTGATCTTATTGATTTGCTGACGCTCTCTTGTTGCTGAGATGTCGACTGTGGGGGATTTGGGG
AGTATAGTCGACTGGGTTTATGCTGGTATCTTGGTATAGTCGACTGGGTTTATGCTGGTATCTTGGTATAG
GTCGACTGGGTTTATGCTGGTATCTTGGTATAGTCGACTGGGTTTATGCTGGTATCTTGGTATAGTCGAC
GGGTTTATGCTGGTATCTTGGTATAGTCGACTGGGTTTATGCTGGTATCTTGGTATAGTCGACTGGGTTT
ATGCTGGTATCTTGGTATAGTCGACTGGGTTTATGCTGGTATCTTGGTATAGTCGACTGGGTTTATGCTG
GGTATCTTGGTATAGTCGACTGGGTTTATGCTGGTATCTTGGTATAGTCGACTGGGTTTATGCTGGTATAG
TCGACTGGGTTTATGCTGGTATCTTGGTATAGTCGACTGGGTTTATGCTGGTATCTTGGTATAGTCGACT
GGGTTTATGCTGGTATCTTGGTATAGTCGACTGGGTTTATGCTGGTATCTTGGTATAGTCGACTGGGTTT
ATGCTGGTATCTTGGTATAGTCGACTGGGTTTATGCTGGTATCTTGGTATAGTCGACTGGGTTTATGCTG
GGTATCTTGGTATAGTCGACTGGGTTTATGCTGGTATCTTGGTATAGTCGACTGGGTTTATGCTGGTATAG
TCGACTGGGTTTATGCTGGTATCTTGGTATAGTCGACTGGGTTTATGCTGGTATCTTGGTATAGTCGACT
GGGTTTATGCTGGTATCTTGGTATAGTCGACTGGGTTTATGCTGGTATCTTGGTATAGTCGACTGGGTTT
ATGCTGGTATCTTGGTATAGTCGACTGGGTTTATGCTGGTATCTTGGTATAGTCGACTGGGTTTATGCTG
GGTATCTTGGTATAGTCGACTGGGTTTATGCTGGTATCTTGGTATAGTCGACTGGGTTTATGCTGGTATAG
TCGACTGGGTTTATGCTGGTATCTTGGTATAGTCGACTGGGTTTATGCTGGTATCTTGGTATAGTCGACT
GGGTTTATGCTGGTATCTTGGTATAGTCGACTGGGTTTATGCTGGTATCTTGGTATAGTCGACTGGGTTT
ATGCTGGTATCTTGGTATAGTCGACTGGGTTTATGCTGGTATCTTGGTATAGTCGACTGGGTTTATGCTG
GGTATCTTGGTATAGTCGACTGGGTTTATGCTGGTATCTTGGTATAGTCGACTGGGTTTATGCTGGTATAG
TCGACTGGGTTTATGCTGGTATCTTGGTATAGTCGACTGGGTTTATGCTGGTATCTTGGTATAGTCGACT
GGGTTTATGCTGGTATCTTGGTATAGTCGACTGGGTTTATGCTGGTATCTTGGTATAGTCGACTGGGTTT
ATGCTGGTATCTTGGTATAGTCGACTGGGTTTATGCTGGTATCTTGGTATAGTCGACTGGGTTTATGCTG
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TCGACTGGGTTTATGCTGGTATCTTGGTATAGTCGACTGGGTTTATGCTGGTATCTTGGTATAGTCGACT
GGGTTTATGCTGGTATCTTGGTATAGTCGACTGGGTTTATGCTGGTATCTTGGTATAGTCGACTGGGTTT
ATGCTGGTATCTTGGTATAGTCGACTGGGTTTATGCTGGTATCTTGGTATAGTCGACTGGGTTTATGCTG
GGTATCTTGGTATAGTCGACTGGGTTTATGCTGGTATCTTGGTATAGTCGACTGGGTTTATGCTGGTATAG
TCGACTGGGTTTATGCTGGTATCTTGGTATAGTCGACTGGGTTTATGCTGGTATCTTGGTATAGTCGACT
GGGTTTATGCTGGTATAGTCGACTGGGTTTATGCTGGTATCTTGGTATAGTCGACTGGGTTTATGCTGGTAT
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**Protein sequence**

### PhlA:

MNKYGIVSYGAGIPVFCKLKDVIQVWKNTDLNVKQLVGEAADVLPDGQELTVGADINRNATPGDLTESEYGAAALNLGTENIAHFADSFSCAADVADNPQGDYIRSGMGDSKNSQEGDREELQGASKLMAKIHQAOGDFYQNFLNLSYPSLIGKHLGTTQAIEFGYIAQSGVGDAAGAPSLGLGVLNLQARPGERILVSYVGFGAGSDAIALTVDTAEAYQKTNVLPRSLLEDKYYVDGTISIKYEFKYLPDYALTAYL

### PhlC:

MSARRVAIVSAAYTPKPGSSRVRQTFKEMIVESAYQALNAIKMHPRELQAVAYGYHGEGISEYGGLGPTISDALGISPAPTFMTSTANNCTSSSVFQMAHMVGASGYEDVLCGPAFEKTMHDHINAYEYGSTSECEYDFLIGISHTDAPALATAEYEKYFGGAEADVLATFGQMRIYAHNTFTATRYGVPISLEAKSSEACSGMSLWAYESEGCALIYVAEHLAHRYTPVFVIRGCAITYGVSHYFGTYHRYNTPLQFQGFKPGMAVSNMAACAEIAYKAKTAGTAKTDIAVQYDRLGAGLQMSMAGCGPQGADVFVLLEGIALDGQLPLTDGNNIGRHRGACGDLGIHELITFRQLRGESNNQVKGARIGVSQNLGGAHYHVNLSVHNL

### PhlB:

PpATaseWT

**DNA-sequence - (start- and stop-codon of the ORFs are highlighted)**

```
5' ATGAACGTGAAAAAGATAGGTATTGTCAGCTATGGCGCGGGTATTCCGGTATGCCGCCTGAAAGTCCAGGAAGTGATCAGTGAAAAAAACGCCACCTCAAGCTCTTGGAGAAACACCTCGGCGTCAAGGAAGAGCGCTTGGC
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AAACCGGATGAAGATGTCATCACCCTCGGGGTGCTGGCGGCGCAACGGGCCCTGGATAAAGTCCCCGGTCATCAGATCGATGGCCAAGCTGCACACCAGCCCCGCCACTATGACTATGTGGTGTTCCAGCAAAACCTGGTGTCCACGCCCTACTCCCTGGCCAAGCACCTGGGCTTCAACCCCAAGCAGGTGGAACCGGGCATCTATGCCGGCAACGTTGGCGATGGCGGATCGGCCAGCCCGCTGCTCGGCCTGATCAACGTACTGGACCAGGCACGCCCGGGGCAGAAAATTCTTTTGGTGTCCTACGGTTTCGGCGCCGGCAGCGACGCCATCGCCCTGACCGTCACCGACGCCATCGAGCAGTACCAGAAGCACAACAAGCTCCTGGCCTATCAGTACATGCTCGTACACTCGGCAAGATAGCTGGCCGCCATTTCCGACGCCCTGGGCATCAGCCCGGCGCCGACCTTCATGAGCACCCGAACCTGCACCAGCAGCTCGGTGTCGTTCCAGATGGGCCACCAGATGGTGCCGGGGAGTACGACATCGTCCTGTGCGGCGGCTTCGAGAAGATGACCGATCACTTCAACTACGCCGAATAATCGACGGGATTACGATGGCCGCCATGGGCTATGGCGAAACCGTGCCGCGCATCATGGCCATGGTGCGCCTGGACGACGGCCCTGGTGATCGCTTCGGAAATCGTCGATGTGTGCGATCAGCAACAGCTGAAAGTCGGTGCGCCGGTGCGCATGGTGATCCGCAAGCATGTGCGCGAAAGCAACCTGGCCTGGCAATACGCTTATAAGTTCGTACTCGATATATAA-3'
```
Protein sequence
PhlA:
MNVKIGIVSYGAFVCPRLKVEQVENWKTNDLKVLEENLGVTQAVLPQPDDVITLGVLAQQRALDKVFGHQI
EALYLGCTCFNYDSRASAIIILEMGLGYDAVCDVFQPKGSSTALICQIALVASMGTGSAALIAGDTRRNAT
FGLDTEYAGAAMALLISSQDVIAEFDADCSFADVIRIINRFQGDYIRSMGLGDSKNS1GLEDQTRAAEGL
MAKLLHPSADYDVFFQVNLYSTVSYSLAKHLGPNFKQVFEGPIYAGNYVGAGDASPLLGLIVNLQARFQKILLV
SYGFAGASDAIALTVDIAEIQYQKHNKPLRELLESKIYVHDGTSIKEYFKLRYADILTAYL

PhlC:
MCARRVAIVSAAYTPKPGSSRVRQTFKEMIVESAYKALKDAKMHPREIQAVAYGYHGEGISEYGLGLPTISDALG
ISPAFTFMSTANCTSSSVSFQMGHMQVSAEYDVILCGGEFKETMDHMNAYIGSSTECEYDFGLISHTDAPAF
ATAEYQFXQFGAGREADVFALTFRQRMIYASQNTFTATYRGQPISELVLEKSNFAAGGSAWGAEAGCAIYVAHEL
AHYTKDFFVKFVRGAYGTVSYGFTFHRNPTLHFLGFDFKVMNASVINSACAEYKAYAGTANDVQYVYDL
LGACGLIQMSEGIGKQAGDFVFLEGIALDGGPLNLDGGNSRGRHSGCDGLIHTELFRQLRGEESDNQVKG
RIGVSNLGGYAHAAHNSVLD

PhlB:
MSMYFQ1HRMTTASMELREWHRGGKRYLEGGSQCECNE1EFFPRRTTVGACNSLSKVYPCARSKIGEVMAPAEN
PILAAMGYGETVPVRIMAVLRDGLVIAEISEIVDVCDQQQLKVGAIVPVRMVHJRPHRESNLAWQYAYKFLID

PpATaseCH
DNA-sequence - (start- and stop-codon of the ORFs are highlighted; RBS in lowercase letters)
5’ aaggagatatacatatg
ATG AATGTGAAGAAAATAGGTATCGTTAGCTACGGCGCCGGTATCCCGGTATGTCG
CCTGAAAGTCTAGGAAGTATTAAAGTTTGGAGAAGATAACGGATTCGAACCTCGATCGTGGCGGCTACACGCCGAAGCCAGGAAGTTCACGAGTACGGCAGACGTTTAAAGAAATGATTGTTGAGTCTGCGTATAAAGCACTCAAAGATGCGAAAATGCATCCACGGGAAATTCCAGGCGGTGGCGTACGGTTACCATGGTGAAGGCATCTCGGAATACGGCGGTCTGGGCCCGACCATCTCTGATGCGCTGGGCATTAGCCCGCCCCGACCTTTATGAGCACCGCGAATTGCACCAGCAGCTCGGTGTCGTTTCAGATGGGCATCAGATGGTGGCCTCGGGGGAGTATGATATTGTTCTGTGCGGCGGTTTTGAGAAAATGACCGACCATTTTAATATGCGGAATATATTGGCTCGAGCACTGAATGTGAATATGACTACTTTCTCGGCATCTCTCATACCGACGCGTTTGGCGCTGGCGACCGCGGAGTATTTTTCAGAAATTTGGCTACGCGGGTCGCGAGGCGGATGTACTGGCGACCTTTGGCGCGGCAGATGCGCATTTATGCACAGAATACCCCAACCGCGACCCGTTACGGCCAGCCGATCCCATCGCTGGAAGTGTTGAAAAATAGCGAAGCGTGCGGCTCGATGCTGGCGTGCGGGCGAAGCGAGTGGCTGCGCCATTCTGGTGGCGGAA
CATCTGGCGCATAAATATACCGACAAGCCGGTGTTTGTACGCGGTTGCGCGTACACCGGGGTTTCTCATTACTTT
GGTACGCGCTTCCATAATCCGACCCTGCACCATCCGGGCCTGCCAAAAGACGTGGGCATGGCCGTCTCGGCGAAT
TCTATTGCGTGTGCCGAGATTGCGTATAAAAAGGCGGGGATTACCGCGAAAGATATTGATGTGGCGCAGGTTTAT
GATCTGCTCGGCGCAGGGCTGATTCAGATGGGAATCTATGGGCATTTGTGGCAAAGGCCAGGCGGGCGATTTTGTG
CTCGAAGGCGGTATCGCGCTGGACGGCCAGCTGCCGCTCAACACCGATGGCGGTAATATTGGCCGCGGCCACGCG
TCTGGCTGCGATGGCATTCTGCATATTACCGAGCTGTTTCGGCAGCTGAGAGGTGAATCGGATAATCAGGTTAAA
GGCGCGCGCATTGGCGTGTCGCAGAATCTTGGCGGTTATGCCGCGCATAATTCCGTGATCGTTCTCTCGAATGAT
TTGAATTTAAATCACATGCTCGGCTCAGTCGCCTGCGGATGCATGGTCTGGATGTGCCGAGGTCTGCGTCGGTGCA
GACTCAGTCGACGAGATCT

5’ aaggagatatacatatg
ATG AATGTGAAGAAAATAGGTATCGTTAGCTACGGCGCCGGTATCCCGGTATGTCG
CCTGAAAGTCTAGGAAGTATTAAAGTTTGGAGAAGATAACGGATTCGAACCTCGATCGTGGCGGCTACACGCCGAAGCCAGGAAGTTCACGAGTACGGCAGACGTTTAAAGAAATGATTGTTGAGTCTGCGTATAAAGCACTCAAAGATGCGAAAATGCATCCACGGGAAATTCCAGGCGGTGGCGTACGGTTACCATGGTGAAGGCATCTCGGAATACGGCGGTCTGGGCCCGACCATCTCTGATGCGCTGGGCATTAGCCCGCCCCGACCTTTATGAGCACCGCGAATTGCACCAGCAGCTCGGTGTCGTTTCAGATGGGCATCAGATGGTGGCCTCGGGGGAGTATGATATTGTTCTGTGCGGCGGTTTTGAGAAAATGACCGACCATTTTAATATGCGGAATATATTGGCTCGAGCACTGAATGTGAATATGACTACTTTCTCGGCATCTCTCATACCGACGCGTTTGGCGCTGGCGACCGCGGAGTATTTTTCAGAAATTTGGCTACGCGGGTCGCGAGGCGGATGTACTGGCGACCTTTGGCGCGGCAGATGCGCATTTATGCACAGAATACCCCAACCGCGACCCGTTACGGCCAGCCGATCCCATCGCTGGAAGTGTTGAAAAATAGCGAAGCGTGCGGCTCGATGCTGGCGTGCGGGCGAAGCGAGTGGCTGCGCCATTCTGGTGGCGGAA
CATCTGGCGCATAAATATACCGACAAGCCGGTGTTTGTACGCGGTTGCGCGTACACCGGGGTTTCTCATTACTTT
GGTACGCGCTTCCATAATCCGACCCTGCACCATCCGGGCCTGCCAAAAGACGTGGGCATGGCCGTCTCGGCGAAT
TCTATTGCGTGTGCCGAGATTGCGTATAAAAAGGCGGGGATTACCGCGAAAGATATTGATGTGGCGCAGGTTTAT
GATCTGCTCGGCGCAGGGCTGATTCAGATGGGAATCTATGGGCATTTGTGGCAAAGGCCAGGCGGGCGATTTTGTG
CTCGAAGGCGGTATCGCGCTGGACGGCCAGCTGCCGCTCAACACCGATGGCGGTAATATTGGCCGCGGCCACGCG
TCTGGCTGCGATGGCATTCTGCATATTACCGAGCTGTTTCGGCAGCTGAGAGGTGAATCGGATAATCAGGTTAAA
GGCGCGCGCATTGGCGTGTCGCAGAATCTTGGCGGTTATGCCGCGCATAATTCCGTGATCGTTCTCTCGAATGAT
TTGAATTTAAATCACATGCTCGGCTCAGTCGCCTGCGGATGCATGGTCTGGATGTGCCGAGGTCTGCGTCGGTGCA
GACTCAGTCGACGAGATCT
GGCATTATGGCCATGGGATGTGATGGGCTGGATGATGGTGTCGGAAATTGTTGACGTGTGCGACCAGCAA
CAGCTGAAGGTTGGTGCGCCGGTGCGCATGGTGATTCGCAAACACGTGCGCGAAAGCAATCTGGCGTGGCAATAT
GCTTACAAATTGTACTTGACATA

Protein sequence
PhlA:
MNVKKIGIVSYGAGIPVCRLKVQEVINVWKNTDRLVEENLVTERAVLQPDENVTLGVLAQAQRALDKVPGHQI
EALYLGTCNPYDSRASASIIILEMLGSYGDAYCADVQFAGKSQTSALQICQALVASGTMGSAALAIQADTNNTA
PGDLTESYAGAGAAALLIGSQQDVIAGAFSADSFCAADVADNRIPQGDRYIRSGMGLGSDKNSIGLEDQTRRAEGL
MAKLHTPADYDYVVFQQNLSTPYSLAKLGFFNPQVEFIYAGNVGDAASPLGLGLINVLDQARPGKQILLV
SYGFAGGDSAIALTVDIAIEQYQKHNKPLRELLESKIYVGDTSIKYEFKYLRAVLYALTAYL

PhlC:
MCARRVAIVSAAYTPFGLSSRVRQFKEVIVESAYKALKDAKMKFREIQAVAYGYHSEGISEYGGLGPTISDALG
ISPAPTFMSTANCTSSSVSFMGQVMASGEYDIVILCGGSFGKEFMTHFYAEYIGSSECEYDFGLISHTDAFAL
ATAEQFQKFQAGREADVATFGRQMRITYAQTFTATRYGQPIPSLEVLKNEACGSMHLWGEASGCAILVAEHL
AHKYTDKPVVFRCAYTVSVHYFGRTRFHNPIIHLHHFGLFKDGMAVSAANSIAAEIAYKAGITAKDIQVLYL
LGAGLQMESMGCGKQAGDFVLEGIGIALDGQLPLNTDGNNIGRASHGCDGILHITELFRQLRESDNQVRGA
RIGVSNLGGYAHNSIVLNS

PhlB:
MSMYPEQIHRMTTASMLREREWREHGGKRLEGCGGECEEINEIFPPRTVCACNSLSVKPRRCRASGKIEVMAPAEN
PILAAMGGETVPRIMAMVRDGLVIAEIVDQCDQQQLKVGPVRMVIRKHVRESNLAWSQAYKFVLDI
4 Biocatalyst Preparation & Protein Expression

Shake flask cultivation of the *Pseudomonas* wildtypes
For the cultivation of the *Pseudomonas* wildtype strains, *P. protegens* and *P. brassicacearum*, a pre-culture (100 mL) containing M1-media (peptone 5 g L⁻¹, meat-extract 3 g L⁻¹) was inoculated with a glycerol stock (250 µL). In case of *P. fluorescens* Pf-5, tryptone/soya broth (30 g L⁻¹, pH 7.3) was used instead. The pre-culture was grown at 28 °C (*P. protegens*) or 30 °C (*P. brassicacearum & P. fluorescens* Pf-5) for 3 days and shaken with 120 rpm. The main culture (3 × 330 mL) in baffled shake flasks (1 L) was inoculated with the pre-culture (10 mL) and incubation was continued for another 3 days under the given conditions. The cells were harvested by centrifugation (10 min, 8,000 rpm) and washed with potassium phosphate buffer (50 mM, pH 7.5) prior to lyophilization.

Shake flask cultivation and expression of the recombinant ATases
The expression plasmids (pEG330-*Ph*ATaseWT, pEG331-*Pp*ATaseWT or pEG332-*Pp*ATaseCH) were introduced into chemically competent *E. coli* BL21 (DE3) host cells. A single colony, picked from an agar-plate (LB/ampicillin, 100 µg mL⁻¹), was used for inoculation of an overnight-culture (10 mL, LB/Amp, 100 µg mL⁻¹) which was grown at 37 °C and 135 rpm for 15 h. The main culture (1 L) containing LB/Amp (100 µg mL⁻¹) was then inoculated with the overnight culture (10 mL) and shaken in a non-baffled flask (5 L) at 37 °C and 140 rpm until the OD₆₀₀ reached 0.7. The cells were induced with anhydrotetracycline (AHTC, 200 µg L⁻¹) and protein expression was continued for 21 h at 30 °C (*Pp*ATaseWT or *Pp*ATaseCH) or 6 h at 25 °C (*Ph*ATaseWT). The cells were harvested by centrifugation (15 min, 8,000 rpm), washed with potassium phosphate buffer (50 mM, pH 7.5) and resuspended again in the same buffer (7 mL buffer to 1 g wet cells). The suspension was disrupted by ultrasonication (40 % amplitude, 8 min, pulse 1 sec, pause 4 sec). After centrifugation (30 min, 14,000 rpm), the cell-free extract was analyzed by SDS-PAGE (Figure S1), shock-frozen in liquid nitrogen and stored at -20 °C until direct use for biotransformations. Initial rates and protein concentrations were measured to determine the batch activity (*vide infra*).

![Figure S1. SDS-PAGE analysis of the cell-free *E. coli* extract containing the recombinant *Pp*ATaseWT, *Ph*ATaseWT or *Pp*ATaseCH. Empty *E. coli* BL21 (DE3) host cells served as negative control (C). The ATase encoding genes *phlA*, *phlC* and *phlB* of all ATases were overexpressed in soluble form.](image)
Purification of *Pp*ATaseCH

Purification of the *Pp*ATaseCH was achieved by size-exclusion chromatography with a Superdex 200 16/600 HiLoad-column. The column was initially washed with water, followed by conditioning (potassium phosphate buffer, 50 mM, pH 7.5, 100 mM NaCl). The cell-free extract (3.5 mL ≡ 0.5 g wet cells) was filtered (0.45 μm) prior to loading onto the column. The *Pp*ATaseCH eluted after ~94 min with a flow-rate of 0.75 mL min⁻¹ and the size of the protein (~91 kDa) was determined by comparison to a GelFiltration standard (BioRad). The purity of the *Pp*ATaseCH-fractions was estimated by SDS-PAGE (Figure S2). All enzyme-containing fractions (20 × 500 μL) were combined and concentrated to approximately 2.5 mL with a Vivaspin column (MWCO 30,000). NaCl was removed by filtration through a PD-10-desalting column (final buffer = potassium phosphate buffer, 50 mM, pH 7.5) and the enzyme solution was concentrated again. Initial rates and protein concentrations were measured to determine the batch activity (*vide infra*).

In total, 7.53 mg of purified enzyme (5.45 U mg⁻¹) were obtained from 0.5 g wet cells.

**Figure S2.** SDS-PAGE analysis of the *Pp*ATaseCH after purification via size-exclusion chromatography. Cell-free extract (input), flow-through (FT) and purified fractions of *Pp*ATaseCH (elution).
5 Activity Assay

ATase-batch activities were measured on a Thermo Scientific Genesys 10 UV Scanning UV/Vis spectrophotometer according to a modified procedure from literature.[2] When following the disproportionation of 2a into 3a and 1a spectrophotometrically, an increase of absorption is recorded due to the formation of 3a (ε = 205.6 M⁻¹ cm⁻¹, λ = 395 nm). One unit of activity was defined as the amount of ATase that catalyzed the formation of 1 µmol of 3a per minute under the following conditions: potassium phosphate buffer (800 µL, 50 mM, pH 7.5) and 2a (2.0 µmol, 100 µL of a 20 mM stock solution prepared in DMSO) were added to a cuvette and preheated to 35 °C. The reaction (1 mL total volume, 10 vol% DMSO) was started by the addition of the enzyme-containing cell-free extract (100 µL = 14.3 mg wet cells). The reaction was followed for 3 minutes. All reactions were performed as a duplicate. As a negative control, a sample with cell-free extract of E. coli without plasmid (empty host cells) was run. The protein concentration (Bradford) was measured [(ε = 0.083 mL mg⁻¹ cm⁻¹, λ = 595 nm)] and specific activities were determined as units per mg cell-free extract or as units per mg purified protein (Table S2).

Table S2. Typical ATase batch activities. The protein concentration was determined (Bradford).

| ATase       | Specific activity (U mg⁻¹ cell free extr.)[a] |
|-------------|---------------------------------------------|
| PpATaseWT   | 0.38±0.02                                   |
| PpATaseCH   | 1.93±0.07                                   |
| PpATaseCH   | 5.45±0.05[b]                               |
| PhATaseWT   | 0.16±0.002                                  |

[a] purified PpATaseCH.
6 Biotransformations

General procedure for the Friedel-Crafts bioacetylation using different acetyl donors
Acceptor 1b-j (10 mM final concentration in the reaction mixture) was dissolved in potassium phosphate buffer (50 mM, pH 7.5) and preheated to 35 °C for 10 minutes. Cell-free extract of the recombinant ATase (2.5 U, 4.7 µM) was subsequently added to the preheated mixture. The bioacetylation was started by addition of the donor at the following final concentrations: DAPG, (15 mM), N-AcIm (250 mM, added from a 1 M stock solution prepared in the reaction buffer), IPEA (100 mM) or vinyl acetate (100 mM). DAPG and p-cyano phenyl acetate were dissolved in DMSO (100 µL) to improve its solubility in buffer (10 vol% DMSO final concentration). Reactions with IPEA and vinyl acetate optionally contained imidazole (100 mM, added from a 1 M stock solution prepared in the reaction buffer), which was added prior to the donor causing the pH to increase to 8.30. To ensure proper suspension of the donor in the mixture, the vessel was manually shaken thoroughly right after starting the reaction. The reaction mixture (1 mL total volume) was horizontally shaken for 18 h at 35 °C and 750 rpm in an orbital shaker. Except for reactions with acceptor 1f which were quenched with MeOH (1 mL), all reactions were aborted by addition of acetonitrile (1 mL). The precipitated protein was removed by centrifugation (10 min, 14,000 rpm) and the supernatant (900 µL) was transferred to an eppendorf tube and left standing for another 40 minutes. Any residual precipitated protein was once again removed by centrifugation and the supernatant was directly subjected to HPLC for determination of conversions. Each reaction was performed as a duplicate. As a control, reactions without enzyme were performed.

General procedure for the Friedel-Crafts biopropanoylation of 1b using different acyl donors
Acceptor 1b (10 mM final concentration) was dissolved in potassium phosphate buffer (50 mM, pH 7.5) and preheated to 35 °C for 10 minutes. Cell-free extract of the recombinant ATase (2.5 U, 4.7 µM) was subsequently added to the preheated mixture. The bioacylation was started by addition of DPPG, MPPG (15 mM) or vinyl propionate (100 mM). DPPG or MPPG were dissolved in DMSO (100 µL) to improve their solubility in buffer (10 vol% DMSO final concentration). Reactions with vinyl propionate contained imidazole (100 mM, added from a 1 M stock solution prepared in the reaction buffer). The reaction (total volume 1 mL) was carried out and worked up as described above. The formation of the C-propanoylated product 6b was analyzed by HPLC. Each reaction was performed as a duplicate. As control, reactions without enzyme were performed.

General procedure for the enzymatic Fries rearrangement-like reaction of 4b into 2b
Potassium phosphate buffer (50 mM, pH 7.5) was preheated to 35 °C for 10 minutes prior to the addition of PpATaseCH (2.5 U, 4.7 µM). The rearrangement reaction was started by the addition of O-acetyl ester 4b (10 mM final concentration) which was dissolved in DMSO (100 µL). The reaction (total volume 1 mL, 10 vol% DMSO) was carried out and worked up as described above (vide supra). Each reaction was performed as a duplicate. As negative control, reactions without enzyme were performed.
Modification for preparative scale biotransformation: O-Acetyl ester 4b (76 mg, 0.5 mmol, 10 mM final concentration) was dissolved in potassium phosphate buffer (50 mM, pH 7.5) in a baffled shaking flask at 35 °C for 5 min. The cell-free extract containing the PpATaseCH (83 U, 1.6 µM) was thawed at 21 °C and preheated to 35 °C for 5 min. The enzyme solution was subsequently added to the mixture to start the rearrangement reaction (total volume 50 mL) which was carried out and worked up as described above (vide supra).

Time studies using PpATaseCH with different donors
The PpATaseCH catalyzed formation of 2b was investigated over time using either IPEA (Figure S3) or DAPG (Figure S4) as acetyl donor. In case of IPEA, the enzymatic reaction was accompanied by the spontaneous formation of monoester 4b, whereas no O-acetylation was observed with DAPG. The reaction was carried out and worked up as described in Section 6 (Biotransformations, vide supra). Each reaction was performed as a duplicate. As negative control, reactions without enzyme were performed.

Figure S3. Time course of the PpATaseCH catalyzed Friedel-Crafts bioacetylation of 1b (10 mM) with IPEA (15 mM). The relative amounts of product 2b and substrate 1b (orange) were determined by HPLC according to standard curves with authentic samples. Symbols represent the mean of two (n = 2) experiments.
Figure S4. Time course of the PpATaseCH catalyzed Friedel-Crafts bioacetylation of 1b (10 mM) with DAPG (15 mM). See Methods for representative procedures. The relative amounts of product 2b and substrate 1b (orange) were determined by HPLC according to standard curves with authentic samples. Symbols represent the mean of two (n = 2) experiments.

Inter- versus intramolecular reaction
To address the question, whether in the biocatalytic Fries reaction the acetyl moiety is transferred intra-molecularly or inter-molecularly, the following experiment was performed: to detect possible crossover transfers the rearrangement of monoester 4b catalyzed by PpATaseCH was performed in the presence of 4-hexylresorcinol (1h) (Scheme S1). Besides a small amount of rearrangement product 2b (6%) and hydrolysis leading to the formation 1b (42%), C-acetylated product 2h (42%) was identified as the crossover product. This suggests that in the enzymatic Fries reaction is actually an intermolecular reaction.

Scheme S1. Crossover experiment with resorcinol monoacetate (4b) and 4-hexylresorcinol (1h).
7 Analytical Methods

Determination of conversion

The conversions were measured at 25 °C by HPLC using a Shimadzu-Prominence liquid chromatograph, equipped with a SPD-M20A diode array detector and an achiral C18 column (Phenomenex Luna C18(2) 100A (0.46 cm × 25 cm, 5 µm particle size). Gradient or isocratic elution was performed to separate the compounds. Reaction products were quantified at 280 nm (or at 230 nm, only if diester 5b was detected in the reaction mixture) from the peak areas on the basis of standard curves with authentic samples.

Gradient elution, method A (reactions of 1a-e and 1i-j):
Eluent: H₂O/MeCN (+ TFA, 0.1 vol%). 0-15% MeCN (0-5 min), 15-60% MeCN (5-22 min), 60-100% MeCN (22-25 min), 100-0% MeCN (25-30 min), flow rate = 1 mL min⁻¹, detection wavelength = 280 nm, sample vol. = 2 µL.

Gradient elution, method B (reactions of 1g-h):
Eluent: H₂O/MeCN (+ TFA, 0.1 vol%). 0-60% MeCN (0-2 min), 60-100% MeCN (2-8 min), 100% MeCN (8-20 min), 100-0% MeCN (20-25 min), flow rate = 1 mL min⁻¹, detection wavelength = 280 nm, sample vol. = 2 µL.

Isocratic elution, method C (reactions of 1f):
Eluent: H₂O/MeOH (40:60, + TFA 0.1 vol%) for 30 min, flow rate = 0.7 mL min⁻¹, detection wavelength = 280 nm, sample vol. = 2 µL.

Retention times (min):
2a: 16.4 min, 3a: 23.9 min
1b: 11.9 min, 2b: 18.2 min, 4b: 16.6 min, 5b: 20.6 min, 6b: 15.6 min
1c: 16.94 min, 2c: 21.6 min
1d: 15.5 min, 2d: 20.9 min
1e: 17.9 min, 2e: 22.8 min
1f: 10.1 min, 2f: 22.7 min
1g: 8.5 min, 2g: 8.9 min
1h: 8.7 min, 2h: 10.1 min
1i: 13.6 min, 2i: 17.9 min
1j: 12.6 min, 2j: 20.6 min
MPPG: 14.2 min, DPPG: 26.9 min

Sample chromatograms are shown on the following pages:
Figure S5. HPLC-chromatogram showing the bioacetylation of 1b into 2b using IPEA/imidazole as donor system, after 24 h (crude reaction mixture).

Figure S6. HPLC-chromatogram showing the crude reaction mixture of the enzymatic Fries-rearrangement of 4b into 2b. Residual 4b hydrolyzed to 1b after the given reaction time of 24 h.

Figure S7. HPLC-chromatogram showing the bioacetylation of 1c into 2c using IPEA/imidazole as donor system, after 24 h (crude reaction mixture).

Figure S8. HPLC-chromatogram showing the bioacetylation of 1d into 2d using IPEA/imidazole as donor system, after 24 h (crude reaction mixture). In addition, O-acetyl product 4d was detected.

Figure S9. HPLC-chromatogram showing the bioacetylation of 1e into 2e using IPEA/imidazole as donor system, after 24 h (crude reaction mixture).
Figure S10. HPLC-chromatogram showing the bioacetylation of 1f into 2f using IPEA/imidazole as donor system, after 24 h (crude reaction mixture).

Figure S11. HPLC-chromatogram showing the bioacetylation of 1g into 2g using IPEA/imidazole as donor system, after 24 h (crude reaction mixture).

Figure S12. HPLC-chromatogram showing the bioacetylation of 1h into 2h using IPEA/imidazole as donor system, after 24 h (crude reaction mixture).

Figure S13. HPLC-chromatogram showing the bioacetylation of 1h into 2h using DAPG (3a) as donor, after 24 h (crude reaction mixture). In addition, MAPG (2a) was detected.

Figure S14. HPLC-chromatogram showing the bioacetylation of 1j into 2j using IPEA/imidazole as donor system, after 24 h (crude reaction mixture).
8 Spectroscopic Data

All reaction products were prepared according to the general procedure described in the Experimental Section of the main text or according to the procedure for enzymatic Fries-rearrangements (vide supra). NMR- & GC-MS-spectra are shown on the following pages.

1-(2,4-Dihydroxyphenyl)ethan-1-one (2b)

Resorcinol (1b, 100 mg, 0.91 mmol) was used to afford pure 2b as colorless solid (103 mg, 0.68 mmol, 68%) after purification by flash chromatography (CH₂Cl₂/EtOAc, 85:15). R_F = 0.7 (CH₂Cl₂/EtOAc, 85:15), m.p. 120-125 °C (lit: 146-148 °C[3]). ¹H- and ¹³C-NMR-data are in accordance with literature.[⁴] ¹H-NMR (300 MHz, acetone-d₆): δ [ppm] = 2.56 (s, 3 H, 1b-H), 6.33 (ds, J = 2.3 Hz, 1 H, 3-H), 6.45 (dd, J = 8.7 Hz, 2.3 Hz, 1 H, 5-H), 7.78 (d, J = 8.7 Hz, 1 H, 6-H), 9.43 (s, 1 H, Ar-OH), 12.75 (s, 1 H, Ar-OH). ¹³C-NMR (75 MHz, acetone-d₆): δC [ppm] = 25.36 (C-1b), 102.6, 107.9, 113.4, 133.4, 164.6, 165.2 (6 × arom. C), 202.8 (C-1a). GC-MS (EI⁺, 70 eV): m/z (%) = 152 [M⁺] (39), 137 [C₇H₅O₃⁺] (100), 109 [C₆H₅O₂⁺] (3).

1-(2,4-Dihydroxyphenyl)ethan-1-one (2b) - obtained via enzymatic Fries-rearrangement

Resorcinol monoacetate (4b, 76 mg, 0.5 mmol) was used to afford pure 2b as colorless solid (35 mg, 0.23 mmol, 46%) without further purification. R_F = 0.6 (CH₂Cl₂/EtOAc, 85:15), m.p. 117-118 °C (lit: 146-148 °C[3]). ¹H-NMR and MS-data are in accordance with literature.[⁴] ¹H-NMR (300 MHz, acetone-d₆): δ [ppm] = 2.56 (s, 3 H, 1b-H), 6.33 (ds, J = 2.3 Hz, 1 H, 3-H), 6.45 (dd, J = 8.7 Hz, 2.3 Hz, 1 H, 5-H), 7.78 (d, J = 8.7 Hz, 1 H, 6-H), 9.47 (s, 1 H, Ar-OH), 12.76 (s, 1 H, Ar-OH). ¹³C-NMR (75 MHz, acetone-d₆): δC [ppm] = 25.36 (C-1b), 102.6, 107.8, 133.5 (3 × CH-arom.), 113.4, 164.7, 165.2 (3 × arom. C₉), 202.8 (C-1a). GC-MS (EI⁺, 70 eV): m/z (%) = 152 [M⁺] (41), 137 [C₇H₅O₃⁺] (100), 109 [C₆H₅O₂⁺] (4).

1-(5-Chloro-2,4-dihydroxyphenyl)ethan-1-one (2c)

4-Chlororesorcinol 1c (146 mg, 1.01 mmol) was used to afford pure 2c as colorless solid (179 mg, 0.96 mmol, 95%) after purification by flash chromatography (CH₂Cl₂/EtOAc, 90:10). R_F = 0.8 (CH₂Cl₂/EtOAc, 90:10), m.p. 150-155 °C (lit: 165-167 °C[⁵]). ¹H-NMR and MS-data are in accordance with literature.[⁵] ¹H-NMR (300 MHz, acetone-d₆): δ [ppm] = 2.62 (s, 3 H, 1b-H), 6.51 (s, 1 H, 3-H), 7.91 (s, 1 H, 6-H), 9.97 (s, 1 H, Ar-OH), 12.59
(s, 1 H, Ar-OH). $^{13}$C-NMR (75 MHz, acetone-$d_6$): $\delta$ [ppm] = 25.57 (C-1b), 103.8, 111.5, 114.0, 132.6, 159.7, 163.4 (6 × arom. C), 202.7 (C-1a). GC-MS (EI$^+$, 70 eV): $m/z$ (%) = 186 [M$^+$] (36), 170 [C$_7$H$_3$ClO$_3^+$] (100).

1-(2,4-Dihydroxy-5-methylphenyl)ethan-1-one (2d)

4-Methylresorcinol 1d (124 mg, 1.00 mmol) was used to afford crude 2d (64 mg, 39%). Two by-products were not successfully separated from 2d by flash chromatography (overlying spots on TLC). GC-MS- and $^1$H-NMR-analysis of crude 2d led to the indication that the by-products resulted from the spontaneous $O$-acetylation of 1d into the corresponding monoesters 4d, respectively. Purification of 2d was achieved by saponification which was performed as followed: Crude 2d (64 mg) was treated in aq. NaOH (3 M, 20 mL) at 60 °C for 2 h. Acidification (conc. HCl, to pH 1.0), extraction (CH$_2$Cl$_2$) and flash chromatography eventually rendered pure 2d as a colorless solid (26.5 mg, 0.16 mmol, 16%). R$_f$ = 0.7 (CH$_2$Cl$_2$/EtOAc, 85:15), m.p. 132-136 °C (lit: 167-168 °C$^{[6]}$). $^1$H- and $^{13}$C-NMR-data and MS-data are in accordance with literature.$^{[6]}$ $^1$H-NMR (300 MHz, acetone-$d_6$): $\delta$ [ppm] = 2.00 (s, 3 H, 5a-H), 2.39 (s, 3 H, 1b-H), 6.20 (s, 1 H, 3-H), 7.50 (s, 1 H, 6-H), 9.29 (s, 1 H, ArOH), 12.47 (s, 1 H, ArOH). $^{13}$C-NMR (75 MHz, acetone-$d_6$): $\delta$ [ppm] = 14.52 (C-5a), 25.38 (C-1b), 102.0, 113.0, 116.4, 133.2, 162.8, 163.5 (6 × arom. C), 202.7 (C-1a). GC-MS (EI$^+$, 70 eV): $m/z$ (%) = 166 [M$^+$] (39), 151 [C$_8$H$_7$O$_3^+$] (100).

1-(5-Ethyl-2,4-dihydroxyphenyl)ethan-1-one (2e)

4-Ethylresorcinol 1d (138 mg, 1.00 mmol) was used to afford pure 2e as colourless solid (148 mg, 0.82 mmol, 82%) after purification by flash chromatography (CH$_2$Cl$_2$/EtOAc, 90:10). R$_f$ = 0.7 (CH$_2$Cl$_2$/EtOAc, 90:10), m.p. 120-122 °C (lit: 116 °C$^{[7]}$). $^1$H-NMR and MS-data are in accordance with literature.$^{[5]}$ $^1$H-NMR (300 MHz, acetone-$d_6$): $\delta$ [ppm] = 1.19 (t, $J$ = 7.5 Hz, 3 H, 5b-H), 2.59 (m, 5 H), 6.35 (s, 1 H, 3-H), 7.65 (s, 1H, 6-H), 9.42 (s, 1 H, Ar-OH), 12.63 (s, 1 H, Ar-OH). $^{13}$C-NMR (75 MHz, acetone-$d_6$): $\delta$ [ppm] = 13.80 (C-5b), 22.38 (C-5a), 25.41 (C-1b), 102.3, 113.2, 122.8, 132.0, 162.5, 162.4. (6 × arom. C), 202.8 (C-1a). GC-MS (EI$^+$, 70 eV): $m/z$ (%) = 180 [M$^+$] (32), 165 [C$_9$H$_7$O$_3^+$] (100).
1-(2,4-Dihydroxy-5-isopropylphenyl)ethan-1-one (2f)

4-Isopropylresorcinol 1f (152 mg, 1.00 mmol) was used to afford pure 2f as colorless solid (158 mg, 0.81 mmol, 81%) after purification by flash chromatography (CH$_2$Cl$_2$/EtOAc, 90:10). R$_f$ = 0.6 (CH$_2$Cl$_2$/EtOAc, 90:10), m.p. 92 °C (lit: 142-144 °C [6]). $^1$H-NMR and MS-data are in accordance with literature. $^5$ $^1$H-NMR (300 MHz, acetone-$d_6$): δ [ppm] = 1.24 (d, $J$ = 6.9 Hz, 6 H, 5b-H), 2.57 (s, 3 H, 1b-H), 3.22 (sept, $J$ = 6.9 Hz, 1 H, 5a-H), 6.36 (s, 1 H, 3-H), 7.65 (s, 1 H, 6-H), 9.46 (s, 1 H, Ar-OH), 12.62 (s, 1 H, Ar-OH). $^{13}$C-NMR (75 MHz, acetone-$d_6$): δC [ppm] = 13.45 (C-5b), 25.42 (C-1b), 26.52 (C-5a), 102.4, 113.2, 127.1, 129.2, 162.1, 163.1. (6 × arom. C), 202.7 (C-1a). GC-MS (EI$,^+$, 70 eV): $m/z$ (%) = 194 [M$^+$] (25), 179 [C$_{10}$H$_{11}$O$_3$]$^+$ (100).

1-(5-Butyl-2,4-dihydroxyphenyl)ethan-1-one (2g)

4-Butylresorcinol 1g (166 mg, 1.00 mmol) was used to afford pure 2g as colourless solid (167 mg, 0.80 mmol, 80%) after purification by flash chromatography (CH$_2$Cl$_2$/EtOAc, 90:10). R$_f$ = 0.7 (CH$_2$Cl$_2$/EtOAc, 90:10), m.p. 60-70 °C (lit: 88 °C [6]). $^1$H-NMR and MS-data are in accordance with literature. $^{10}$ $^1$H-NMR (300 MHz, acetone-$d_6$): δ [ppm] = 0.94 (t, $J$ = 7.3 Hz, 3 H, 5d-H), 1.38 (sext, $J$ = 7.3 Hz, 2 H, 5c-H), 1.59 (mc, 2 H), 2.58 (mc, 5 H), 6.35 (s, 1 H, 3-H), 7.65 (s, 1 H, 6-H), 9.42 (s, 1 H, Ar-OH), 12.63 (s, 1 H, Ar-OH). $^{13}$C-NMR (75 MHz, acetone-$d_6$): δC [ppm] = 13.37 (C-5d), 22.29 (C-5c), 25.41 (C-1b), 32.07 (C-5b, C-5a), 102.3, 113.1, 121.3, 132.7, 162.6, 163.4. (6 × arom. C), 202.8 (C-1a). GC-MS (EI$,^+$, 70 eV): $m/z$ (%) = 208 [M$^+$] (24), 193 [C$_{11}$H$_{13}$O$_3$]$^+$ (12), 165 [C$_9$H$_7$O$_3$]$^+$ (100).

1-(2,4-Dihydroxy-5-hexylphenyl)ethan-1-one (2h)

4-Hexylresorcinol 1h (194 mg, 1.00 mmol) was used to afford pure 2h as colourless solid (123 mg, 0.52 mmol, 52%) after purification by flash chromatography (CH$_2$Cl$_2$/EtOAc, 95:05). R$_f$ = 0.5 (CH$_2$Cl$_2$/EtOAc, 95:05), m.p. 60-63 °C (lit: 87 °C [6]). $^1$H-NMR and MS-data are in accordance with literature. $^{10}$ $^1$H-NMR (300 MHz, acetone-$d_6$): δ
[ppm] = 0.89 (m, 3 H), 1.34 (m, 6 H), 1.60 (m, 2 H), 2.58 (m, 5 H), 6.35 (s, 1 H, 3-H), 7.65 (s, 1 H, 6-H), 9.41 (s, 1 H, Ar-OH), 12.63 (s, 1 H, Ar-OH). 13C-NMR (75 MHz, acetone-\textit{d}6): \( \delta \)C [ppm] = 13.45 (C-5f), 22.41 (C-5e), 25.38 (C-1b), 29.26 (C-5c), 29.81 (C-5a), 31.58 (C-5b, C-5d), 102.2, 113.1, 121.4, 132.7, 162.5, 163.4. (6 × arom. C), 202.7 (C-1a). GC-MS (EI+, 70 eV): \( m/z \) (%) = 236 [M\textsuperscript{+}] (17), 221 [C\textsubscript{13}H\textsubscript{17}O\textsubscript{3}\textsuperscript{+}] (7), 165 [C\textsubscript{9}H\textsubscript{9}O\textsubscript{3}\textsuperscript{+}] (100).

1-(2,4-Dihydroxy-6-methoxyphenyl)ethan-1-one (2j)

5-Methoxyresorcinol 1j (140 mg, 1.00 mmol) was used to afford pure 2j as orange solid (18 mg, 0.10 mmol, 10%) after purification by flash chromatography (CH\textsubscript{2}Cl\textsubscript{2}/EtOAc, 90:10). \( R_F = 0.5 \) (CH\textsubscript{2}Cl\textsubscript{2}/EtOAc 90:10), m.p. 160-165 °C (lit: 204-205 °C\textsuperscript{[11]}). 1H-NMR-data is in accordance with literature.\textsuperscript{[12]} 1H-NMR (300 MHz, acetone-\textit{d}6): \( \delta \) [ppm] = 2.41 (s, 3 H, 1b-H), 3.77 (s, 3 H, 6b-H), 5.81 (d, \( J = 2.2 \) Hz, 1 H, 3-H), 5.89 (d, \( J = 2.2 \) Hz, 1 H, 5-H). 13C-NMR (75 MHz, acetone-\textit{d}6): \( \delta \)C [ppm] = 32.04 (C-1b), 55.21 (C-6b), 90.85, 95.73 (2 × CH-arom.), 105.1, 163.8, 164.8, 167.3 (4 × arom. C\textsubscript{\textit{q}}), 202.6 (C-1a). GC-MS (EI+, 70 eV): \( m/z \) (%) = 182 [M\textsuperscript{+}] (33), 167 [C\textsubscript{9}H\textsubscript{10}O\textsubscript{3}\textsuperscript{+}] (100).
Figure S15. $^1$H-NMR of 1-(2,4-dihydroxyphenyl)ethan-1-one (2b).

Figure S16. $^{13}$C-NMR of 1-(2,4-dihydroxyphenyl)ethan-1-one (2b).
Figure S17. Mass spectrum of 1-(2,4-dihydroxyphenyl)ethan-1-one (2b).
Figure S18. $^1$H-NMR of 1-(2,4-dihydroxyphenyl)ethan-1-one (2b) originating from the enzymatic rearrangement reaction of monoester 4b.

Figure S19. $^{13}$C-NMR of 1-(2,4-dihydroxyphenyl)ethan-1-one (2b) originating from the enzymatic rearrangement reaction of monoester 4b.
Figure S20. Mass spectrum of 1-(2,4-dihydroxyphenyl)ethan-1-one (2b) originating from the enzymatic rearrangement reaction of monoester 4b.
Figure S21. $^1$H-NMR of 1-(5-chloro-2,4-dihydroxyphenyl)ethan-1-one (2c).

Figure S22. $^{13}$C-NMR of 1-(5-chloro-2,4-dihydroxyphenyl)ethan-1-one (2c).
Figure S23. Mass spectrum of 1-(5-chloro-2,4-dihydroxyphenyl)ethan-1-one (2c).
Figure S24. $^1$H-NMR of 1-(2,4-dihydroxy-5-methylphenyl)ethan-1-one (2d).

Figure S25. $^{13}$C-NMR of 1-(2,4-dihydroxy-5-methylphenyl)ethan-1-one (2d).
Figure S26. GC-chromatograms of crude (a) and pure (b) 1-(2,4-dihydroxy-5-methylphenyl)ethan-1-one (2d). Mass spectrum of 4d (c) and pure 2d (d) after saponification.
Figure S27. $^1$H-NMR of 1-(5-ethyl-2,4-dihydroxyphenyl)ethan-1-one (2e).

Figure S28. $^{13}$C-NMR of 1-(5-ethyl-2,4-dihydroxyphenyl)ethan-1-one (2e).
**Figure S29.** Mass spectrum of 1-(5-ethyl-2,4-dihydroxyphenyl)ethan-1-one (2e).
Figure S30. $^1$H-NMR of 1-(2,4-dihydroxy-5-isopropylphenyl)ethan-1-one (2f).

Figure S31. $^{13}$C-NMR of 1-(2,4-dihydroxy-5-isopropylphenyl)ethan-1-one (2f).
Figure S32. Mass spectrum of 1-(2,4-dihydroxy-5-isopropylphenyl)ethan-1-one (2f).
Figure S33. $^1$H-NMR of 1-(5-butyl-2,4-dihydroxyphenyl)ethan-1-one (2g).

Figure S34. $^{13}$C-NMR of 1-(5-butyl-2,4-dihydroxyphenyl)ethan-1-one (2g)
Figure S35. Mass spectrum of 1-(5-butyl-2,4-dihydroxyphenyl)ethan-1-one (2g).
Figure S36. $^1$H-NMR of 1-(2,4-dihydroxy-5-hexylphenyl)ethan-1-one (2h).

Figure S37. $^{13}$C-NMR of 1-(2,4-dihydroxy-5-hexylphenyl)ethan-1-one (2h).
Figure S38. Mass spectrum of 1-(2,4-dihydroxy-5-hexylphenyl)ethan-1-one (2h).
Figure S39. $^1$H-NMR of 1-(2,4-dihydroxy-6-methoxyphenyl)ethan-1-one (2j).

Figure S40. $^{13}$C-NMR of 1-(2,4-dihydroxy-6-methoxyphenyl)ethan-1-one (2j).
Figure S41. Mass spectrum of 1-(2,4-dihydroxy-6-methoxyphenyl)ethan-1-one (2j).
10 References

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