University of Groningen

Merging Whole-cell Biosynthesis of Styrene and Transition-metal Catalyzed Derivatization Reactions

Maaskant, Ruben V.; Chordia, Shreyans; Roelfes, Gerard

Published in:
ChemCatChem

DOI:
10.1002/cctc.202001896

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Publication date:
2021

Link to publication in University of Groningen/UMCG research database

Citation for published version (APA):
Maaskant, R. V., Chordia, S., & Roelfes, G. (2021). Merging Whole-cell Biosynthesis of Styrene and Transition-metal Catalyzed Derivatization Reactions. *ChemCatChem*, *13*(6), 1607-1613. https://doi.org/10.1002/cctc.202001896

Copyright
Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

The publication may also be distributed here under the terms of Article 25fa of the Dutch Copyright Act, indicated by the "Taverne" license. More information can be found on the University of Groningen website: https://www.rug.nl/library/open-access/self-archiving-pure/taverne-amendment.

Take-down policy
If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): http://www.rug.nl/research/portal. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.

Merging Whole-cell Biosynthesis of Styrene and Transition-metal Catalyzed Derivatization Reactions

Ruben V. Maaskant,[a] Shreyans Chordia,[a] and Gerard Roelfes*[a]

The approach of combining enzymatic and transition-metal catalysis has been focused almost exclusively on using purified, isolated enzymes. The use of whole-cell biocatalysis, instead of isolated enzymes, with transition-metal catalysis, however, has been investigated only sparsely to date. Herein we present the development of two transition-metal catalyzed reactions used to derivatize styrene obtained from whole-cell biosynthesis. Using a biocompatible ruthenium cross-metathesis catalyst up to 1.5 mM stilbene could be obtained in the presence of *E. coli*, which simultaneously produced styrene. Using palladium catalysts and arylboronic acids, titers of up to 1 mM of several stilbene derivatives were obtained. These two transition-metal catalyzed reactions are valuable additions to the toolbox of combined whole-cell biocatalysis and transition-metal catalysis, offering the possibility to supplement biosynthetic pathways with the chemical versatility of abiological transition-metal catalysis.

Introduction

The ever increasing understanding of biosynthetic pathways and the associated enzymes, enable the creation of new biosynthetic routes to obtain non-natural molecules via metabolic engineering of microorganisms.[1–5] Whereas biosynthesis is highly versatile and can be used to synthesize a wide range of interesting molecules, it uses a relatively limited number of reaction classes to do so, at least in comparison to organic synthesis. Hence, combining biosynthesis with transition-metal catalysis may dramatically expand the synthetic scope of biosynthesis.[6] Combining biosynthesis and transition-metal catalysis was first applied in the field of dynamic kinetic resolutions using isolated lipases and dirhodium(II) or palladium (II) catalysts.[7–11] Over the last decade this approach has been expanded to include a wide variety of other reaction classes.[12] Compatible transition-metal and enzyme catalyzed reactions now include palladium catalyzed cross-couplings,[13–16] copper catalyzed Sonogashira cross-couplings,[17] ruthenium catalyzed metathesis reactions,[18–20] ruthenium and iridium catalyzed hydride transfer reactions,[21–23] gold and palladium catalyzed cycloisomerizations,[24] enzymatic hydrogenations,[14,22,24] P450-catalyzed epoxidations[19,20] and hydroxylations,[17] and dehalogenations.[21,25]

Being able to use cell free extracts or whole microbial cells for biocatalysis would be attractive because it negates the need to purify enzymes, and in addition, the use of whole microbial cells would enable the cell to regulate the use and regeneration of any required co-factors.[26,27] However, a challenge is the potential mutual incompatibility of transition-metal catalysts and cells or their extracts.[28–32] These challenges and potential approaches to mitigate these were illustrated in recent reports on the use of decarboxylases in cell free extracts with ruthenium cross metathesis reactions.[33,34]

Kourist and co-workers found that the cross metathesis of alkenols, generated in situ by enzymatic decarboxylation of fatty acids, gave low conversions to the product, which they attributed to the incompatibility of cell free extracts with the metathesis catalyst.[33] By performing the reaction in a sequential one-pot mode, adding the metathesis catalyst after decarboxylation, higher conversions could be achieved.

Schwaneberg and co-workers investigated the cross metathesis reaction on styrene derivatives obtained by decarboxylation of cinnamic acid derivatives, where they found that no decarboxylation or cross metathesis occurred when performing the reaction in concurrent mode.[34] The activity of the decarboxylase was restored by performing the reaction in sequential mode, adding the metathesis catalyst after one hour. Anticipating deactivation of the metathesis catalyst by cell lysate contents (e.g. glutathione), a cross metathesis catalyst was embedded in a protein scaffold, shielding the metal center from inhibition. By employing this protected, biohybrid catalyst in sequential mode, activity of both reactions could be restored and the stilbene derivatives were obtained with good yields. These approaches to avoid incompatibilities, together with the development in the last decade of a range of metal catalysts for the in vivo activation of profluorophores or prodrugs in mammalian cells, indicate that the challenges and potential incompatibilities associated with using whole microbial cells with transition-metal catalysts can be overcome.[35–47]

To date, only a few reports exist in which whole-cell biocatalysis is combined with transition-metal catalysis for synthetic purposes. Goss and co-workers reported a bromina-

[a] *R. V. Maaskant, S. Chordia, Prof. G. Roelfes*
Stratingh Institute for Chemistry
University of Groningen
Nijenborgh 4, 9747 AG Groningen (The Netherlands)
E-mail: j.g.roelfes@rug.nl

Supporting information for this article is available on the WWW under https://doi.org/10.1002/cctc.202001896

© 2021 The Authors. ChemCatChem published by Wiley-VCH GmbH. This is an open access article under the terms of the Creative Commons Attribution Non-Commercial License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited and is not used for commercial purposes.

tion of tryptophan using a halogenase expressed by an *E. coli* RG-1500 strain, followed by incorporation of the obtained bromotryptophan into the antibiotic pacidamycin by engineered Streptomyces coelicolor. The bromotryptophan in the pacidamycin molecule was subsequently arylated via a palladium catalyzed Suzuki coupling.[48]

Ward and co-workers reported the creation of chemoenzymatic cascades using *E. coli*, containing up to six- or eight enzymatic conversions, to obtain cycloalkenes from oleic acid, using a transition-metal catalyzed ring-closing metathesis reaction as the final step in the cascades.[49] Integration of an upstream hydrolase enabled the use of olive oil for the production of oleic acid, and consequently the cycloalkenes.

Balskus and co-workers reported a palladium catalyzed hydrogenation reaction of a range of alkenes, using hydrogen generated in the same pot by an *E. coli* strain and, in two successive studies showed that styrene, produced from glucose by an engineered *E. coli* NST74 strain, was efficiently derivatized using a cyclopropanation reaction catalyzed by iron porphyrins and iron phthalocyanines.[50–52] By adding a designer surfactant, TPGS-750-M, the styrene was sequestered in the formed micelles and its toxic effect on the *E. coli* cells was decreased. This allowed for an increase of the cyclopropane product titer.

We envisioned to extend the scope of transition-metal catalyzed derivatization reactions of styrene, obtained from biosynthesis by *E. coli*, by developing biocompatible versions of transition-metal catalyzed reactions. Herein we present the development of a biocompatible cross-metathesis reaction and palladium-catalyzed cross-coupling reactions on styrene produced by *E. coli*.

Results and Discussion

Preparation of a styrene producing *E. coli* strain

An *E. coli* strain was engineered to express the proteins PAL2 and FDC1, which have been shown to enable the biosynthesis of styrene from L-phenylalanine.[53] PAL2 catalyzes the ammonia lysis of L-phenylalanine to form cinnamic acid, which is subsequently decarboxylated by FDC1 to form styrene. In a first step, the genes encoding for the proteins PAL2 and FDC1 were inserted into a pTrcHis plasmid. This plasmid was subsequently used to transform chemically competent *E. coli* C43(DE3), producing styrene upon supplementation of L-phenylalanine. creating a cell line (hereafter called *E. coli* C43PF) capable of expressing proteins PAL2 and FDC1 and, consequently, *E. coli* C43PF was grown in minimal media MM1 to an OD600 of 0.6-0.8, at which point protein expression was induced. At the same time, 10 mM L-phenylalanine was supplemented (in solid form) to the culture. It was found that over 2 mM styrene was obtained in MM1. This could be increased to over 6 mM by the addition of 2.5 w/v% of the surfactant TPGS-750-M (Figure 1, Supporting Information SI1), which is close to the toxicity limit for *E. coli*.[52,54]

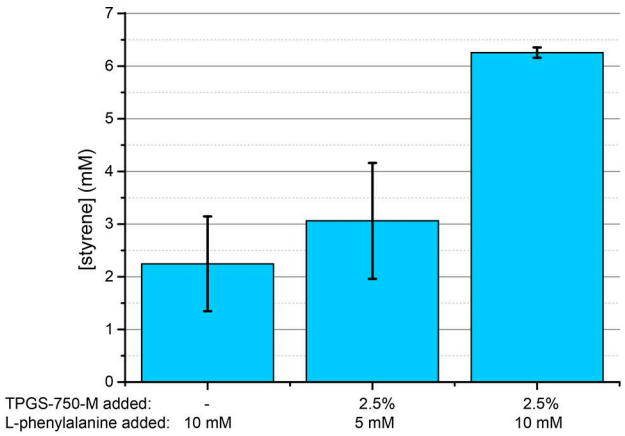


Figure 1. The production of styrene by *E. coli* C43PF from supplemented L-phenylalanine, as determined by GC-FID with 2-methylanisole as internal standard. Conditions: 50 mL cultures grown in minimal media MM1 in 300 mL brown glass erlenmeyers, 40 h incubation. Results are the average of two experiments.

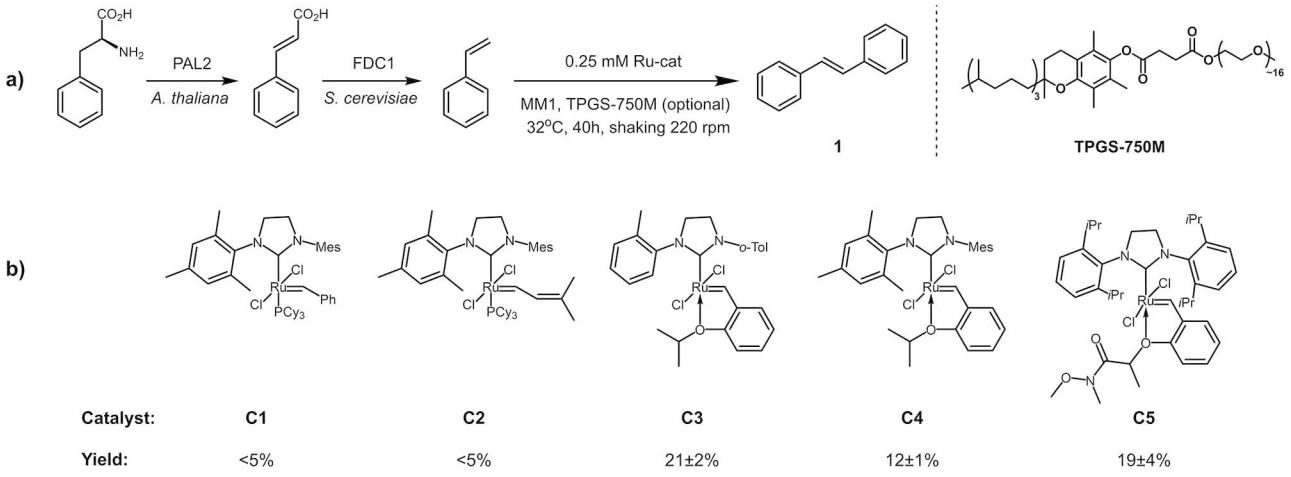
Development of the olefin cross-metathesis reaction

Drawing inspiration from the work of Lipshutz and co-workers on transition-metal catalysis in aqueous TPGS-750-M solutions, it was envisioned to developed a cross-metathesis reaction of styrene to form stilbene (Scheme 1a).[54] Olefin cross-metathesis is a powerful tool to couple two alkene groups which has no equivalent reaction in nature.[55] Additionally, in this case the reaction only requires the supplementation of a catalyst as the addition of a coupling partner other than styrene is not necessary. Five commercially available second generation Grubbs-type metathesis catalysts were screened under relevant conditions, without *E. coli* cells present, in sterile minimal media MM1 with 2.5% TPGS-750-M and 2.5 mM supplemented styrene (Scheme 1b). After shaking at 32 °C for 24 hours, only trace amounts of stilbene were obtained using catalysts **C1** and **C2**, which both contain phosphine ligands, which may be due to degradation of the catalysts in an aqueous environment.[56,57] Catalysts **C3** to **C5**, possessing a chelating ortho-alkoxybenzylidene ligand, were able to form stilbene in 12 to 21% yield. Grubbs C571 catalyst (**C3**), a slightly less sterically hindered version of Grubbs-Hoveyda 2 catalyst **C4**, gave the highest yield of 21% and was selected for trials in combination with the bacterial styrene production.

Olefin cross metathesis in presence of styrene producing *E. coli*

The engineered *E. coli* C43PF was grown in MM1 to an OD600 of 0.6–0.8 and subsequently protein expression was induced with ITPG, while simultaneously adding 0.25 mM catalyst, solid TPGS-750-M (final concentration 2.5%) and L-phenylalanine (final concentration 10 mM). After 40 hours, using 0.25 mM **C3** with *E. coli* C43PF, a styrene titer of up to 5 mM was obtained. This shows that the cross metathesis catalyst used is compatible

© 2021 The Authors. ChemCatChem published by Wiley-VCH GmbH



Scheme 1. a) Ruthenium-catalyzed cross-metathesis of styrene obtained from biosynthesis by *E. coli*. The surfactant TPGS-750 M was added to increase styrene titers in the cell cultures. b) Screening of the cross-metathesis of styrene under relevant conditions. Reactions conditions: 0.25 mM ruthenium catalyst, 2.5 mM styrene, shaking at 220 rpm at 32 °C for 24 hours, 10 mL 2.5 w/v% TPGS-750 M in MM1 in 50 mL brown glass erlenmeyers. Results are the average of two experiments as determined by ^{1}H NMR using 1,3,5-trimethoxybenzene as internal standard.

with *E. coli* and protein expression. Unfortunately, no stilbene was detected (Figure 2, Supporting Information SI2). Without surfactant present, styrene titers were approximately three fold lower, albeit that a small quantity ($\leq$0.2 mM) of stilbene was detected in the mixture.

In addition, two new cross metathesis catalysts, **C6** and **C7**, were tested in combination with TPGS-750-M. **C6** is the cationic and water-soluble Aquamet catalyst, which was expected to be located outside the micelle in the bulk aqueous phase. A high styrene titer was obtained, albeit that no stilbene was formed (Figure 2, Supporting Information SI2). Curiously, styrene was not obtained in a reaction with **C6** in the absence of surfactant.

C7 is an immobilized Zhan II catalyst,[58,59] which gave rise to a high styrene titer but, as expected, no stilbene in the presence of surfactant. From a screening of different concentrations of surfactant it was found that the addition of 0.25% TPGS-750-M resulted in the formation of 1 mM stilbene while allowing for a significantly higher styrene titer of over 4.5 mM. In reactions with **C7** without surfactant it was observed that styrene titers were again approximately three fold lower. However, up to 1.5 mM stilbene was obtained, which is equivalent to the consumption of 3 mM styrene (Figure 2, Supporting Information SI2). This is more than the 2 mM styrene measured at any time point, showing that styrene was being produced while the cross-metathesis reaction took place. The 1.5 mM stilbene titer obtained corresponds to a yield of ~30% based on phenylalanine added and ~12 turnovers of the catalyst.

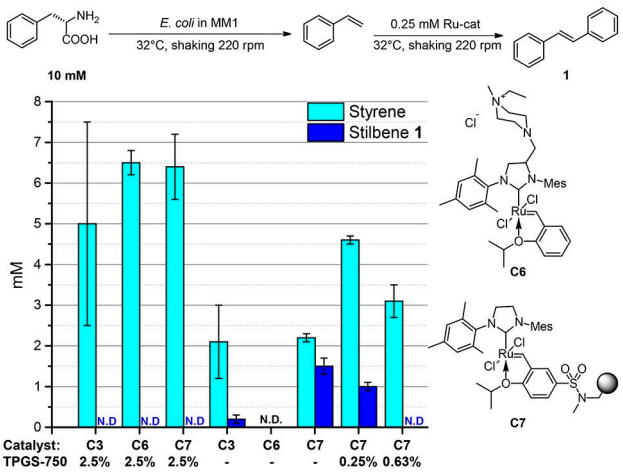


Figure 2. Cross-metathesis reaction of styrene produced by *E. coli* C43PF. L-phenylalanine, 2.5% TPGS-750-M and the catalysts were added upon induction with IPTG. Reactions performed on 50 mL scale in 300 mL brown glass erlenmeyers, 40 h incubation. Results are the average of two experiments as determined by GC-FID with 2-methylanisole as internal standard.

It was observed that the reaction mixture became more viscous and slimy over time, which could indicate slime formation due to environmental stress on the *E. coli* cells, as was described for the combined styrene production/cyclopropanation.[52] However, styrene titers remained constant over the duration of the experiment (40 hours) while stilbene titers increased (Supporting Information SI2). This illustrates that the efficiency of the styrene biosynthesis was not affected significantly by the presence of the transition-metal catalyst. It is notable that immobilized **C7** afforded a significantly higher stilbene titer than **C3**. This might be due to the relatively hydrophobic resin- and PEG-linked support, which may sequester some styrene itself. Alternatively, it might be that the support protects the metathesis catalyst from inhibition by potential poisons emanating from the cells.

Development of a palladium catalyzed cross-coupling reaction under relevant conditions

The positive results obtained in the olefin cross metathesis reaction, encouraged the development of another transition-metal catalyzed reaction which could be combined with styrene

© 2021 The Authors. ChemCatChem published by Wiley-VCH GmbH

biosynthesis. We aimed for a palladium-catalyzed cross coupling of styrene with boronic acids, which could allow the formation of asymmetric stilbenes. An initial screening was performed on the cross coupling of 5 mM styrene and 10 mM phenylboronic acid with 0.25 mM palladium catalyst under relevant conditions in sterile MM1, without *E. coli* cells present. 2.5% TPGS-750-M was added as most palladium catalysts were expected to fully dissolve in the surfactant solution and as higher styrene titers could be obtained in the presence of surfactant. After shaking at 32 °C for 24 hours, it was observed that 6 out of 28 tested palladium catalysts were able to catalyze the formation of stilbene, obtaining titers ranging from 0.4 mM to 1 mM (Figure 3, Supporting Information SI3). The other 22 tested palladium catalysts gave no reaction or only trace amounts (≤ 0.2 mM) of stilbene (Supporting Information SI3).

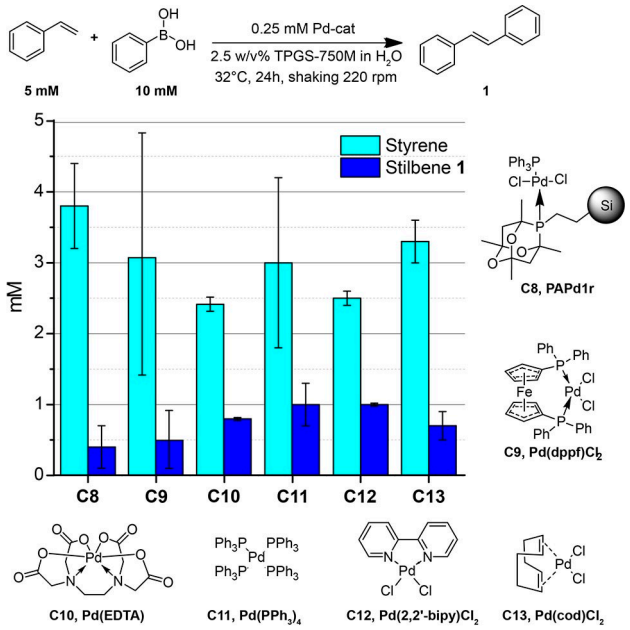


Figure 3. Palladium-catalyzed cross-coupling of phenylboronic acid and styrene in MM1 containing 2.5% TPGS-750-M, in absence of *E. coli*. Reactions performed on 10 mL scale in 50 mL brown glass erlenmeyers, 24 h reaction time. Results are the average of two experiments as determined by GC-FID with 2-methylanisole as internal standard.

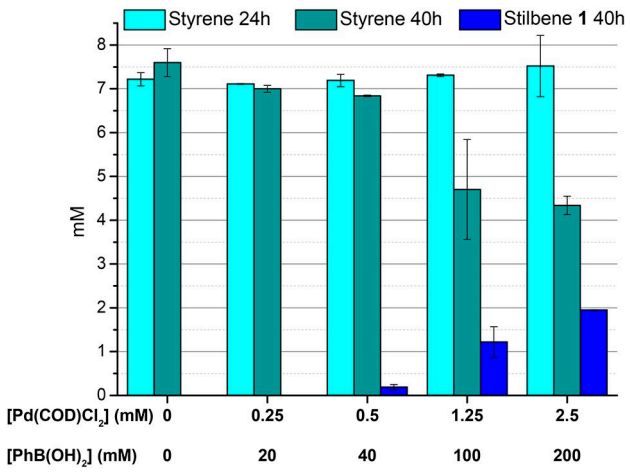


Figure 4. Palladium-catalyzed cross-coupling of phenylboronic acid and styrene produced by *E. coli* C43PF. L-phenylalanine and 2.5% TPGS-750-M were added upon induction with IPTG. Reactions performed on 50 mL scale in 300 mL brown glass erlenmeyers. Results are the average of two experiments after 40 h reaction time as determined by GC-FID with 2-methylanisole as internal standard.

Palladium catalyzed cross-coupling reaction in presence of styrene producing *E. coli*

Three catalysts, palladium(0)-tetrakis(triphenylphosphine) (**C11**), 2,2′-bipyridylpalladium dichloride (**C12**) and dichloro(1,5-cyclooctadiene)palladium(II) (**C13**), were selected for further screening with *E. coli* C43PF. 0.25 mM catalyst, 10 mM

L-phenylalanine, 20 mM phenylboronic acid and 2.5% TPGS-750-M were added to *E. coli* cultures in MM1, as soon as OD600 values of 0.6–0.8 were obtained and protein expression was induced by the addition of IPTG. However, neither styrene nor stilbene was detected after 40 hours of incubation (Figure 4, Supporting Information SI4), suggesting poisoning of the microorganism by the palladium catalysts. Therefore, in this case we opted for a sequential process: the palladium catalysts and phenylboronic acid were added after 24 hours of incubation, instead of directly upon induction, giving *E. coli* C43PF the time needed to produce styrene. Styrene was produced in titers up to 5.5 mM after 24 hours, after which the palladium and boronic acid were added (Figure 4, Supporting Information SI5). After another 16 hours of incubation with the palladium and boronic acid (40 hours total), again no stilbene was obtained. Increasing the concentrations of phenylboronic acid and palladium catalyst ten-fold to 200 mM and 2.5 mM respectively, resulted in the formation of almost 2 mM of styrene and 2.6 mM of stilbene with **C13**. Upon prolonging the reaction times, the stilbene titers still increased further, albeit at a lower rate, to approximately 3 mM as the remaining styrene reacted (Supporting Information SI5). This corresponds to a yield of ~30% based on phenylalanine added, albeit that the number of turnovers of the Pd catalyst is relatively low, i.e. 2–3. After establishing the optimal conditions (Figure 5, Supporting Information SI6), 1.25 mM **C13** was used to catalyze the cross-coupling of styrene with 100 mM of various substituted arylboronic acids (Scheme 2). After 40 hours of incubation, a cross-coupling with 2,4-difluorophenylboronic acid resulted in the formation of trace amounts of 2,4-difluoro-trans-stilbene 2. Gratifyingly, 4-methyl-trans-stilbene **3** and 4-methoxy-trans-stilbene **4** were obtained from their corresponding arylboronic acids in 1 mM concentrations. A reaction with 4-acetylphenylboronic acid gave the corresponding stilbene 5 in 0.7 mM concentration. While stilbene **5** was obtained in a good titer, more importantly the acetyl group present in stilbene **5** offers a functional handle for further derivatization, for example by an enzymatic transamination or a ketone reduction.[60–64]

© 2021 The Authors. ChemCatChem published by Wiley-VCH GmbH

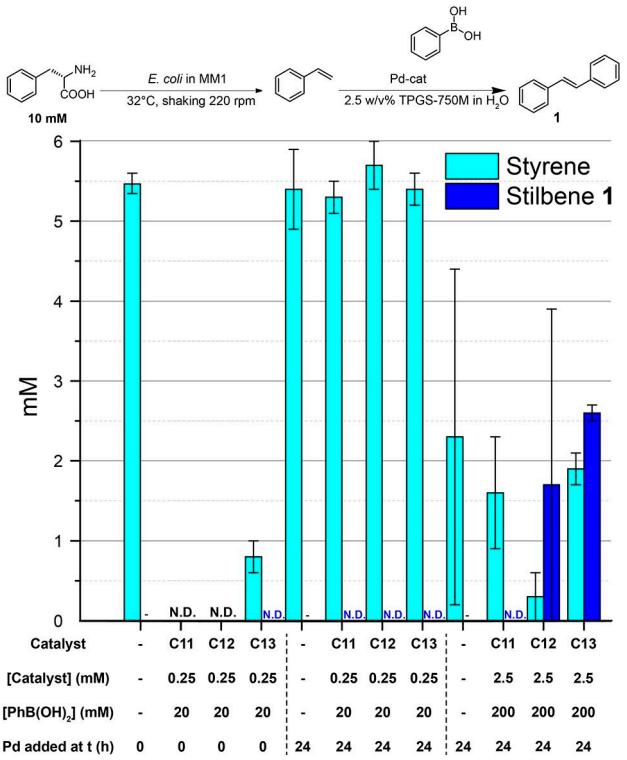
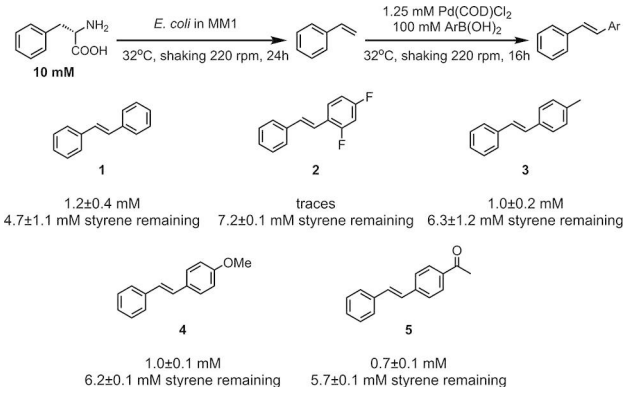


Figure 5. Optimization of the palladium-catalyzed cross-coupling of phenylboronic acid and styrene produced by *E. coli* C43PF. L-phenylalanine, 2.5% TPGS-750-M, phenylboronic acid and the catalyst were added upon induction with IPTG. Reactions performed on 50 mL scale in 300 mL brown glass erlenmeyers. Shown results are after 24 h incubation (prior to the addition of catalyst and boronic acid) and 40 h incubation. Results are the average of two experiments as determined by GC-FID with 2-methylanisole as internal standard.



Scheme 2. Substrate scope of the cross-coupling of styrene, generated in situ from 10 mM L-phenylalanine, with arylboronic acids. Reactions performed on 50 mL scale in 300 mL brown glass erlenmeyers. Palladium catalyst and arylboronic acids were added after 24 h incubation. Results are the average of two experiments after 40 h incubation as determined by GC-FID using 2-methylanisole as internal standard.

Conclusion

In conclusion, here we have demonstrated that whole cell biosynthesis of styrene can be coupled directly to a follow-up abiological ruthenium catalyzed cross metathesis reaction, using an immobilized ruthenium catalyst. This represents the first demonstration of homocoupling of two styrene molecules produced by whole-cell biocatalysis and a metathesis catalyst in a concurrent reaction, showing that metathesis catalysts and enzymes can be fully mutually compatible. In addition, we reported the development of a cross coupling reaction for the derivatization of styrene obtained by biosynthesis. Albeit not compatible with the styrene biosynthesis, the palladium catalyzed cross-coupling enables the conversion of whole cell biocatalytically produced styrene into a range of stilbenes. These two transition-metal catalyzed reactions are powerful additions to the toolbox of combined whole-cell biocatalysis and transition-metal catalysis, offering the possibility to supplement biosynthetic pathways with abiological transition metal catalyzed reactions.

Experimental Section

Representative procedure for the production of styrene by *E. coli* C43PF

A −80 °C glycerol stock of *E. coli* C43PF was streaked onto a LB agar plate with 100 μg/mL ampicillin, which was incubated overnight at 37 °C. A single colony was picked from the plate and used to inoculate 10 mL LB with 10 μL ampicillin stock solution. The culture was incubated overnight at 37 °C and 135 rpm. To autoclaved 300 mL amber glass Erlenmeyers with NS29 grind was added 49 mL MM1, 50 μL ampicillin stock solution and 1 mL of the saturated overnight culture. The flasks were closed with a cotton plug & aluminium foil, after which the cultures were grown at 32 °C and 220 rpm to an OD600 of 0.6–0.8. Upon reaching the correct OD600 value, 10 μL filter-sterilized 1 M IPTG solution (0.2 mM final concentration), solid L-phenylalanine (82.6 mg, 10 mM final concentration) and optionally solid TPGS-750-M were added to the flask, after which it was closed with a sterile glass stopper. Cultures were incubated at 32 °C and 220 rpm for at least 48 hours. Samples for the quantification of metabolites were taken after 16, 24, 40 and 48 h post-induction. An 800 μL aliquot of the culture was added to a 2 mL Eppendorf cup. 720 μL benzene was added, followed by 80 μL 10 mM 2-methylanisole in benzene (1 mM final concentration in organic phase). The mixture was vortexed for 20 minutes and subsequently centrifuged for 10 minutes at 13000 rpm. The organic layer was filtered through a 0.2 μm syringe-filter and analyzed by GC-FID.

Representative procedure for the cross-metathesis of styrene produced by *E. coli* C43PF

A −80 °C glycerol stock of *E. coli* C43PF was streaked onto a LB agar plate with 100 μg/mL ampicillin, which was incubated overnight at 37 °C. A single colony was picked from the plate and used to inoculate 10 mL LB with 10 μL ampicillin stock solution. The culture was incubated overnight at 37 °C and 135 rpm. To autoclaved 300 mL amber glass Erlenmeyers with NS29 grind was added 49 mL MM1, 50 μL ampicillin stock solution and 1 mL of the saturated overnight culture. The flasks were closed with a cotton plug & aluminium foil, after which the cultures were grown at 32 °C and 220 rpm to an OD600 of 0.6–0.8. Upon reaching the correct OD600 value, 10 μL filter-sterilized 1 M IPTG solution (0.2 mM final concentration), solid L-phenylalanine (82.6 mg, 10 mM final concentration), 0.25 mM metathesis catalyst and optionally solid TPGS-750-

© 2021 The Authors. ChemCatChem published by Wiley-VCH GmbH

M were added to the flask, after which it was closed with a sterile glass stopper. Cultures were incubated at 32 °C and 220 rpm for at least 48 hours. Samples for the quantification of metabolites were taken after 16, 24, 40 and 48 h post-induction. An 800 μL aliquot of the culture was added to a 2 mL Eppendorf cup. 720 μL benzene was added, followed by 80 μL 10 mM 2-methylanisole in benzene (1 mM final concentration in organic phase). The mixture was vortexed for 20 minutes and subsequently centrifuged for 10 minutes at 13000 rpm. The organic layer was filtered through a 0.2 μm syringe-filter and analyzed by GC-FID.

Representative procedure for the cross-coupling of arylboronic acids and styrene produced by *E. coli* C43PF

A −80 °C glycerol stock of *E. coli* C43PF was streaked onto a LB agar plate with 100 μg/mL ampicillin, which was incubated overnight at 37 °C. A single colony was picked from the plate and used to inoculate 10 mL LB with 10 μL ampicillin stock solution. The culture was incubated overnight at 37 °C and 135 rpm. To autoclaved 300 mL amber glass Erlenmeyers with NS29 grind was added 49 mL MM1, 50 μL ampicillin stock solution and 1 mL of the saturated overnight culture. The flasks were closed with a cotton plug & aluminium foil, after which the cultures were grown at 32 °C and 220 rpm to an OD600 of 0.6–0.8. Upon reaching the correct OD600 value, 10 μL filter-sterilized 1 M IPTG solution (0.2 mM final concentration), solid L-phenylalanine (82.6 mg, 10 mM final concentration) and optionally solid TPGS-750-M were added to the flask, after which it was closed with a sterile glass stopper. Cultures were incubated at 32 °C and 220 rpm for 24 h. After 24 h, the styrene concentration was determined by GC (sample preparation described below) and 1.25 mM palladium catalyst and 100 mM arylboronic acid were added to the flask, sealing it again with a glass stopper. Samples for the quantification of metabolites were taken after 40 and 48 h post-induction. An 800 μL aliquot of the culture was added to a 2 mL Eppendorf cup. 720 μL benzene was added, followed by 80 μL 10 mM 2-methylanisole in benzene (1 mM final concentration in organic phase). The mixture was vortexed for 20 minutes and subsequently centrifuged for 10 minutes at 13000 rpm. The organic layer was filtered through a 0.2 μm syringe-filter and analyzed by GC-FID.

Acknowledgements

Financial support from the Netherlands Ministry of Education, Culture and Science (Gravitation program no. 024.001.0035) and the Netherlands Organisation for Scientific Research (NWO, Vici grant 724.013.003) is gratefully acknowledged.

Conflict of Interest

The authors declare no conflict of interest.

Keywords: Biosynthesis · whole-cell biocatalysis · styrene · cross-metathesis · palladium · cross-coupling · transition-metal catalysis

[1] K. Watanabe, *Biosci. Biotechnol. Biochem.* **2008**, *72*, 2491–2506.
[2] Y. Luo, B.-Z. Li, D. Liu, L. Zhang, Y. Chen, B. Jia, B.-X. Zeng, H. Zhao, Y.-J. Yuan, *Chem. Soc. Rev.* **2015**, *44*, 5265–5290.
[3] W. Zhang, Y. Tang, *J. Med. Chem.* **2008**, *51*, 2629–2633.
[4] L. B. Pickens, Y. Tang, Y.-H. Chooi, *Annu. Rev. Chem. Biomol. Eng.* **2011**, *2*, 211–236.
[5] S. M. Pearsall, C. N. Rowley, A. Berry, *ChemCatChem* **2015**, *7*, 3078–3093.
[6] B. M. Trost, *Transit. Met. Org. Synth.*, John Wiley & Sons, Ltd **2008**, pp. 2–14.
[7] P. M. Dinh, J. A. Howarth, A. R. Hudnott, J. M. J. Williams, W. Harris, *Tetrahedron Lett.* **1996**, *37*, 7623–7626.
[8] A. L. E. Larsson, B. A. Persson, J.-E. Bäckvall, *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 1211–1212.
[9] J. Deska, C. del Pozo Ochoa, J.-E. Bäckvall, *Chem. Eur. J.* **2010**, *16*, 4447–4451.
[10] M. T. Reetz, K. Schimossek, *Chimia* **1996**, *50*, 668–669.
[11] O. Pàmies, A. H. Éll, J. S. M. Samec, N. Hermanns, J.-E. Bäckvall, *Tetrahedron Lett.* **2002**, *43*, 4699–4702.
[12] J. H. Schrittwieser, S. Velikogne, M. Hall, W. Kroutil, *Chem. Rev.* **2018**, *118*, 270–348.
[13] E. Burda, W. Hummel, H. Gröger, *Angew. Chem. Int. Ed.* **2008**, *47*, 9551–9554; *Angew. Chem.* **2008**, *120*, 9693–9696.
[14] A. Boffi, S. Cacchi, P. Ceci, R. Cirilli, G. Fabrizi, A. Prastaro, S. Niembro, A. Shafir, A. Vallribera, *ChemCatChem* **2011**, *3*, 347–353.
[15] S. T. Ahmed, F. Parmeggiani, N. J. Weise, S. L. Flitsch, N. J. Turner, *ACS Catal.* **2015**, *5*, 5410–5413.
[16] M. Cortes-Clerget, N. Akporji, J. Zhou, F. Gao, P. Guo, M. Parmentier, F. Gallou, J.-Y. Berthon, B. H. Lipshutz, *Nat. Commun.* **2019**, *10*, 2169.
[17] M. A. S. Mertens, F. Thomas, M. Nöth, J. Moegling, I. El-Awaad, D. F. Sauer, G. V. Dhoke, W. Xu, A. Pich, S. Herres-Pawlis, U. Schwaneberg, *Eur. J. Org. Chem.* **2019**, *2019*, 6341–6346.
[18] K. Tenbrink, M. Seßler, J. Schatz, H. Gröger, *Adv. Synth. Catal.* **2011**, *353*, 2363–2367.
[19] C. A. Denard, H. Huang, M. J. Bartlett, L. Lu, Y. Tan, H. Zhao, J. F. Hartwig, *Angew. Chem. Int. Ed.* **2014**, *53*, 465–469; *Angew. Chem.* **2014**, *126*, 475–479.
[20] C. A. Denard, M. J. Bartlett, Y. Wang, L. Lu, J. F. Hartwig, H. Zhao, *ACS Catal.* **2015**, *5*, 3817–3822.
[21] R. M. Haak, F. Berthiol, T. Jerphagnon, A. J. A. Gayet, C. Tarabiono, C. P. Postema, V. Ritleng, M. Pfeffer, D. B. Janssen, A. J. Minnaard, B. L. Feringa, J. G. de Vries, *J. Am. Chem. Soc.* **2008**, *130*, 13508–13509.
[22] F. G. Mutti, A. Orthaber, J. H. Schrittwieser, J. G. de Vries, R. Pietschnig, W. Kroutil, *Chem. Commun.* **2010**, *46*, 8046–8048.
[23] N. Ríos-Lombardía, C. Vidal, M. Cocina, F. Morís, J. García-Álvarez, J. González-Sabín, *Chem. Commun.* **2015**, *51*, 10937–10940.
[24] M. J. Rodríguez-Álvarez, N. Ríos-Lombardía, S. Schumacher, D. Pérez-Iglesias, F. Morís, V. Cadierno, J. García-Álvarez, J. González-Sabín, *ACS Catal.* **2017**, *7*, 7753–7759.
[25] S. T. Ahmed, F. Parmeggiani, N. J. Weise, S. L. Flitsch, N. J. Turner, *Org. Lett.* **2016**, *18*, 5468–5471.
[26] H. Zhao, W. A. van der Donk, *Curr. Opin. Biotechnol.* **2003**, *14*, 583–589.
[27] S. P. France, L. J. Hepworth, N. J. Turner, S. L. Flitsch, *ACS Catal.* **2017**, *7*, 710–724.
[28] S. Hippeli, E. F. Elstner, *FEBS Lett.* **1999**, *443*, 1–7.
[29] K. Helbig, C. Bleuel, G. J. Krauss, D. H. Nies, *J. Bacteriol.* **2008**, *190*, 5431–5438.
[30] K. W. Becker, E. P. Skaar, *FEMS Microbiol. Rev.* **2014**, *38*, 1235–1249.
[31] Y. M. Wilson, M. Dürrenberger, E. S. Nogueira, T. R. Ward, *J. Am. Chem. Soc.* **2014**, *136*, 8928–8932.
[32] P. Chandrangsu, C. Rensing, J. D. Helmann, *Nat. Rev. Microbiol.* **2017**, *15*, 338–350.
[33] S. Bojarra, D. Reichert, M. Grote, Á. G. Baraibar, A. Dennig, B. Nidetzky, C. Mügge, R. Kourist, *ChemCatChem* **2018**, *10*, 1192–1201.
[34] M. A. S. Mertens, D. F. Sauer, U. Markel, J. Schiffels, J. Okuda, U. Schwaneberg, *Catal. Sci. Technol.* **2019**, *9*, 5572–5576.
[35] P. K. Sasmal, C. N. Streu, E. Meggers, *Chem. Commun.* **2013**, *49*, 1581–1587.
[36] S. V. Chankeshwara, E. Indrigo, M. Bradley, *Curr. Opin. Chem. Biol.* **2014**, *21*, 128–135.
[37] J. T. Weiss, J. C. Dawson, C. Fraser, W. Rybski, C. Torres-Sánchez, M. Bradley, E. E. Patton, N. O. Carragher, A. Unciti-Broceta, *J. Med. Chem.* **2014**, *57*, 5395–5404.
[38] G. Y. Tonga, Y. Jeong, B. Duncan, T. Mizuhara, R. Mout, R. Das, S. T. Kim, Y.-C. Yeh, B. Yan, S. Hou, V. M. Rotello, *Nat. Chem.* **2015**, *7*, 597–603.
[39] M. Tomás-Gamasa, M. Martínez-Calvo, J. R. Couceiro, J. L. Mascareñas, *Nat. Commun.* **2016**, *7*, 12538.
[40] F. Wang, Y. Zhang, Z. Du, J. Ren, X. Qu, *Nat. Commun.* **2018**, *9*, 1209.

© 2021 The Authors. ChemCatChem published by Wiley-VCH GmbH

[41] S. Learte-Aymamí, C. Vidal, A. Gutiérrez-González, J. L. Mascareñas, *Angew. Chem. Int. Ed.* **2020**, *59*, 9149–9154.
[42] R. Martínez, C. Carrillo-Carrión, P. Destito, A. Alvarez, M. Tomás-Gamasa, B. Pelaz, F. Lopez, J. L. Mascareñas, P. del Pino, *Cell Rep. Phys. Sci.* **2020**, *1*, DOI 10.1016/j.xcrp.2020.100076.
[43] J. Miguel-Ávila, M. Tomás-Gamasa, J. L. Mascareñas, *Angew. Chem. Int. Ed.* **2020**, *59*, 17628–17633.
[44] M. Martínez-Calvo, J. L. Mascareñas, *Coord. Chem. Rev.* **2018**, *359*, 57–79.
[45] M. O. N. van de L'Isle, M. C. Ortega-Liebana, A. Unciti-Broceta, *Curr. Opin. Chem. Biol.* **2021**, *61*, 32–42.
[46] J. L. Mascarenas, P. Destito, C. Vidal, F. López, *Chem. Eur. J.* **2021**, DOI: 10.1002/chem.202003927.
[47] C. Vidal, M. Tomás-Gamasa, P. Destito, F. López, J. L. Mascareñas, *Nat. Commun.* **2018**, *9*, 1913.
[48] S. V. Sharma, X. Tong, C. Pubill-Ulldemolins, C. Cartmell, E. J. A. Bogosyan, E. J. Rackham, E. Marelli, R. B. Hamed, R. J. M. Goss, *Nat. Commun.* **2017**, *8*, 229.
[49] S. Wu, Y. Zhou, D. Gerngross, M. Jeschek, T. R. Ward, *Nat. Commun.* **2019**, *10*, 5060.
[50] G. Sirasani, L. Tong, E. P. Balskus, *Angew. Chem. Int. Ed.* **2014**, *53*, 7785–7788; *Angew. Chem.* **2014**, *126*, 7919–7922.
[51] S. Wallace, E. P. Balskus, *Angew. Chem. Int. Ed.* **2015**, *54*, 7106–7109; *Angew. Chem.* **2015**, *127*, 7212–7215.
[52] S. Wallace, E. P. Balskus, *Angew. Chem. Int. Ed.* **2016**, *55*, 6023–6027; *Angew. Chem.* **2016**, *128*, 6127–6131.
[53] R. McKenna, D. R. Nielsen, *Metab. Eng.* **2011**, *13*, 544–554.
[54] B. H. Lipshutz, S. Ghorai, A. R. Abela, R. Moser, T. Nishikata, C. Duplais, A. Krasovskiy, R. D. Gaston, R. C. Gadwood, *J. Org. Chem.* **2011**, *76*, 4379–4391.
[55] B. H. Lipshutz, S. Ghorai, *Olefin Metathesis* (Ed.: K. Grela), John Wiley & Sons, Inc. **2014**, pp. 515–521.
[56] A. G. Santos, G. A. Bailey, E. Nicolau dos Santos, D. E. Fogg, *ACS Catal.* **2017**, DOI 10.1021/acscatal.6b03557.
[57] W. L. McClennan, S. A. Rufh, J. A. M. Lummiss, D. E. Fogg, *J. Am. Chem. Soc.* **2016**, *138*, 14668–14677.
[58] Z.-Y. Zhan, *Ruthenium Complex Ligand, Ruthenium Complex, Carried Ruthenium Complex Catalyst and the Preparing Methods and the Use Thereof* **2007**, WO2007003135 A1.
[59] Z.-Y. Zhan, *Recyclable Ruthenium Catalysts for Metathesis Reactions* **2007**, US20070043180 A1.
[60] K. Nakamura, R. Yamanaka, T. Matsuda, T. Harada, *Tetrahedron: Asymmetry* **2003**, *14*, 2659–2681.
[61] S. A. Kelly, S. Pohle, S. Wharry, S. Mix, C. C. R. Allen, T. S. Moody, B. F. Gilmore, *Chem. Rev.* **2018**, *118*, 349–367.
[62] M. Fuchs, J. E. Farnberger, W. Kroutil, *Eur. J. Org. Chem.* **2015**, *2015*, 6965–6982.
[63] J. E. Dander, M. Giroud, S. Racine, E. R. Darzi, O. Alvizo, D. Entwistle, N. K. Garg, *Commun. Chem.* **2019**, *2*, 1–9.
[64] Y.-G. Zheng, H.-H. Yin, D.-F. Yu, X. Chen, X.-L. Tang, X.-J. Zhang, Y.-P. Xue, Y.-J. Wang, Z.-Q. Liu, *Appl. Microbiol. Biotechnol.* **2017**, *101*, 987–1001.

Manuscript received: November 25, 2020
Revised manuscript received: December 22, 2020
Accepted manuscript online: January 11, 2021
Version of record online: February 2, 2021

© 2021 The Authors. ChemCatChem published by Wiley-VCH GmbH