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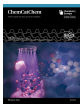

The Synthesis of Chiral γ-Lactones by Merging Decatungstate Photocatalysis with Biocatalysis

Fatma Feyza Özgen,[a] Alexandra Jorea,[b] Luca Capaldo,[b, c] Robert Kourist,[d] Davide Ravelli,*[b] and Sandy Schmidt*[a]

The implementation of light-driven catalytic processes in biocatalysis opens a golden window of opportunities. We hereby report the merging of photocatalytic C–C bond formation with enzymatic asymmetric reduction for the direct conversion of simple aldehydes and acrylates or unsaturated carboxylic acids into chiral γ-lactones. Tetrabutylammonium decatungstate (TBADT) is employed as the photocatalyst to trigger the hydroacylation of the starting olefins, yielding the corresponding keto esters/acids. Subsequently, an alcohol dehydrogenase converts the intermediate to the chiral alcohol, which undergoes lactonization to the desired γ-lactone. The photochemoenzymatic synthesis of aliphatic and aromatic γ-lactones was thereby achieved with up to >99% *ee* and >99% yield. This synthesis highlights the power of building molecular complexity by merging photocatalysis with biocatalysis to access high-value added chiral compounds from simple, cheap and largely available starting materials.

Introduction

Building molecular complexity from cheap commodity chemicals is a desirable feature in every synthetic endeavor.[1] An intriguing opportunity is offered by the combination of successive catalytic transformations, wherein each step occurs with a precise control over the regio-/chemo- and stereochemical outcome. This is particularly true whensoever these transformations are executed in a streamlined fashion, avoiding lengthy separation processes and purification procedures of the involved intermediates. This can limit waste formation and energy consumption, thus boosting the sustainability of the overall process in view of a transition from academia to an industrial setting.[2]

Recently, the combination of enzymes with chemocatalysts (e.g., metal-, organo-, and photocatalysts) has been subject of interest as a very attractive approach for implementing multistep synthetic processes.[3–6] In this context, the outstanding features of enzymes,[7] such as their broad reaction scope and exquisite selectivity and stereospecificity, make them particularly interesting tools for the preparation of complex and expensive, high-value added (chiral) molecules.[8–10]

γ-Lactones are important biologically active molecules and provide building blocks for various fine chemicals (Scheme 1a).[11–16] Moreover, they are highly relevant structures both in the food and cosmetic industries as flavors and fragrances.[24] For instance, γ-decalactone provides a characteristic aroma of peaches.[19,24]

Interestingly, the adoption of a biocatalytic route with the possibility to control the stereochemistry of the final product is particularly suited for constructing this coveted scaffold.[19–21,23,25–30] For this purpose, alcohol dehydrogenases (ADHs) are the elective tools, since these enzymes are capable of reversibly catalyzing the selective reduction of aldehydes and ketones, respectively, to primary and secondary alcohols[31] with high activity and enantioselectivity.[32] In fact, 1,4-keto esters and acids can be conveniently used as starting materials, and upon enzymatic reduction of the carbonyl to the secondary alcohol and acid-promoted lactonization, the desired chiral γ-lactones are obtained with high levels of enantioselectivity (Scheme 1b).[20,22,23,27]

Developing a versatile and robust methodology to form said 1,4-keto esters and acids would allow to harness ADHs' potential at its fullest extent, thus offering access to a virtually

[a] F. F. Özgen, Asst. Prof. S. Schmidt
Department of Chemical and Pharmaceutical Biology,
Groningen Research Institute of Pharmacy
Antonius Deusinglaan 1
9713 AV Groningen (The Netherlands)
E-mail: s.schmidt@rug.nl
Homepage: http://schmidt-researchgroup.com
[b] A. Jorea, Dr. L. Capaldo, Prof. D. Ravelli
PhotoGreen Lab,
Department of Chemistry,
University of Pavia
viale Taramelli 12
27100 Pavia (Italy)
E-mail: davide.ravelli@unipv.it
Homepage: http://www-2.unipv.it/photogreenlab/
[c] Dr. L. Capaldo
Present address: Flow Chemistry Group,
Van't Hoff Institute for Molecular Sciences (HIMS),
University of Amsterdam
Science Park 904
1098 XH Amsterdam (The Netherlands)
[d] Prof. R. Kourist
Institute of Molecular Biotechnology,
Graz University of Technology
Petersgasse 14/1
8010 Graz (Austria)

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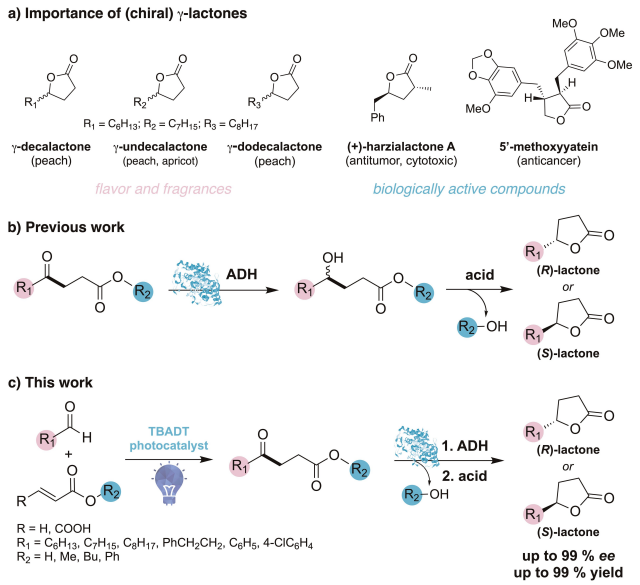


Scheme 1. a) Importance of γ-lactones as flavor and fragrance and biologically active compounds.[17–19] b) Literature-reported enzymatic conversion of 1,4-keto esters/acids into γ-lactones.[20–23] c) In this study, decatungstate photocatalyzed hydroacylation of simple olefins is combined with asymmetric reduction of 1,4-keto esters/acids catalyzed by ADH, yielding diverse enantiomerically pure (*R*)- and (*S*)-γ-lactones after acid-promoted lactonization.

unlimited number of structurally diverse chiral γ-lactones in a concise multi-step sequence. Thus, a straightforward entry to these building blocks is offered by the radical hydroacylation of α,β-unsaturated esters or acids with aldehydes occurring under photocatalytic conditions mediated by tetrabutylammonium decatungstate ($(n\text{Bu}_4\text{N})_4[\text{W}_{10}\text{O}_{32}]$), TBADT, Scheme 1c).[33,34] This polyoxometalate-based photocatalyst is able to cleave directly and with high chemoselectivity the C–H bond in a plethora of aliphatic fragments,[35–37] including the formyl C–H bond of both aliphatic[38] and aromatic aldehydes,[39] via a hydrogen atom transfer (HAT)[40,41] step upon near-ultraviolet (UV) light irradiation. Indeed, TBADT has previously been applied for the synthesis of various keto esters, which underwent a chemical reduction step with $NaBH_4$ to yield γ-lactones in flow, although with no control over the stereochemistry of the final products.[42]

Based on these preliminary data, we strived to develop a photochemoenzymatic approach enabling the direct conversion of cheap and largely available aldehydes and olefins to the desired γ-lactones with high enantioselectivity (Scheme 1c). Herein, we demonstrate the general feasibility of such a photochemoenzymatic γ-lactone synthesis together with the characterization of the crucial parameters determining the catalytic efficiencies of both the photocatalyst and the enzyme.

Results and Discussion

We initiated our investigations by examining a panel of aliphatic and aromatic aldehydes **1a–f**[38,39] and several electron-poor olefins **2a–c** (full list of respective substrates, intermediates and products can be found in the Supporting Information) in the presence of 2 mol% TBADT (Figure 1 and Scheme 2). The photocatalytic reactions were carried out in a home-made photoreactor equipped with 365 nm light-emitting diodes (LEDs, 24 W, Figures S1–2). When employing heptaldehyde **1a** (50 mM) and methyl acrylate **2a** (1.2 equiv.) dissolved in acetonitrile (MeCN) in a reaction that was irradiated for 24 hours, the desired ketone **3a** was obtained in decent yield (13.8 mM; Figure 1).

Interestingly, almost no remaining aldehyde **1a** could be detected anymore (< 1 mM). In addition, only trace amounts of heptanoic acid were detected after 24 h reaction. In contrast, when a mixture of acetone/water (Ac/H_2O 4:1 v/v) was used as solvent, around 4–5 mM **1a** remained after 24 hours of reaction. We thus continued to screen different reaction conditions that would allow for high activity of TBADT, while not impairing the activity of the enzyme in the subsequent step. MeCN, which has been reported to provide both good solubility and good reactivity of the photocatalyst,[33,34] typically led to higher product formation in most cases; however, in case of **3c**, an improved performance could be achieved in Ac/H_2O (Figure 1). Next, we screened several ADHs (*Ras*ADH from *Ralstonia* sp.

Sandy Schmidt is a tenure-track Assistant Professor (Rosalind Franklin Fellow) at the Groningen Research Institute of Pharmacy of the University of Groningen. She completed her Ph.D. in 2015 in the group of Prof. Uwe Bornscheuer at the University of Greifswald. After a research stay at Delft University of Technology as postdoctoral fellow within the group of Prof. Frank Hollmann, she was working as group leader at Graz University of Technology. Her research interests include the design and exploitation of novel biocatalysts and the development of photobiocatalytic approaches for applications in organic synthesis.

Davide Ravelli is currently Associate Professor at the Department of Chemistry of the University of Pavia, where he also obtained his Ph.D. in Chemistry in 2012 (Prof. A. Albini as the supervisor). His main research interests focus on the generation of radical intermediates through photocatalyzed hydrogen atom transfer reactions and their application in sustainable organic synthesis, particularly in C–C bond formations.

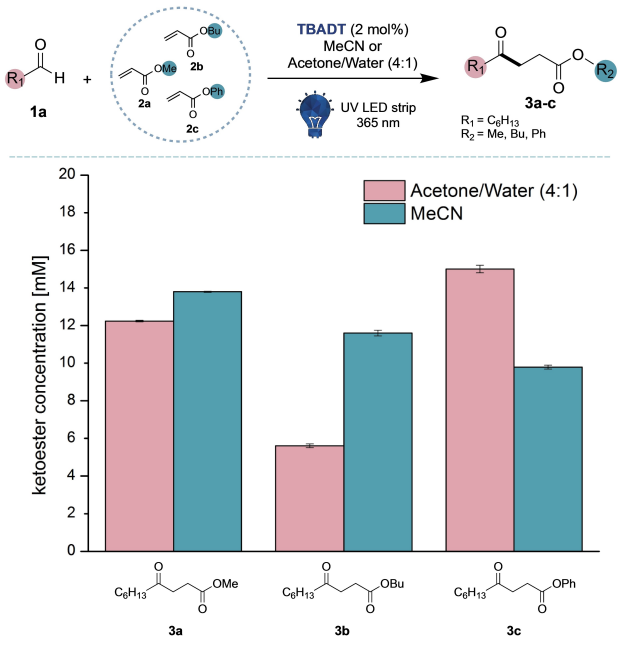


Figure 1. Photocatalytic synthesis of substituted keto esters **3a–c** with olefins **2a–c**. Reaction conditions: [aldehyde, **1a**] = 50 mM and [acrylates, **2a–c**] = 60 mM, 2 mol% TBADT, in 1 mL of MeCN or Ac/H_2O 4:1 v/v, irradiated with 365 nm UV LED strips for 24 hours. Concentrations have been determined via GC-FID with a calibration based on authentic standards.

DSM 6428,[43] *SY*ADH from *Sphingobium yanoikuyae* DSM 6900,[44] *LK*ADH from *Lactobacillus kefir*,[45] and the engineered variant *Sm*CR$_{m4}$ from *Serratia marcescens*[23]) for their activity against several keto esters **3a–c**. Glucose dehydrogenase was employed for cofactor regeneration for all ADHs. The highest activity was achieved for **3c**, while activity towards **3a–c** was observed for all investigated ADHs (Figure S4).

Due to the high reactivity of aldehydes with hard biological nucleophiles, e.g., primary nitrogen groups on lysine residues,[46] enzyme activity might be impaired in the presence of elevated aldehyde concentrations. Thus, we investigated the activity of ADHs in the presence of several aldehydes (Figure S5a–b) and acrylates (Figure S5c). Indeed, a decrease in enzyme activity has been observed, which was more detrimental for *SY*ADH at higher aldehyde concentrations (5 mM), while *Ras*ADH retained its activity. For acrylates, the effect on the enzyme was much more detrimental, and *Ras*ADH lost up to 85% of its activity.

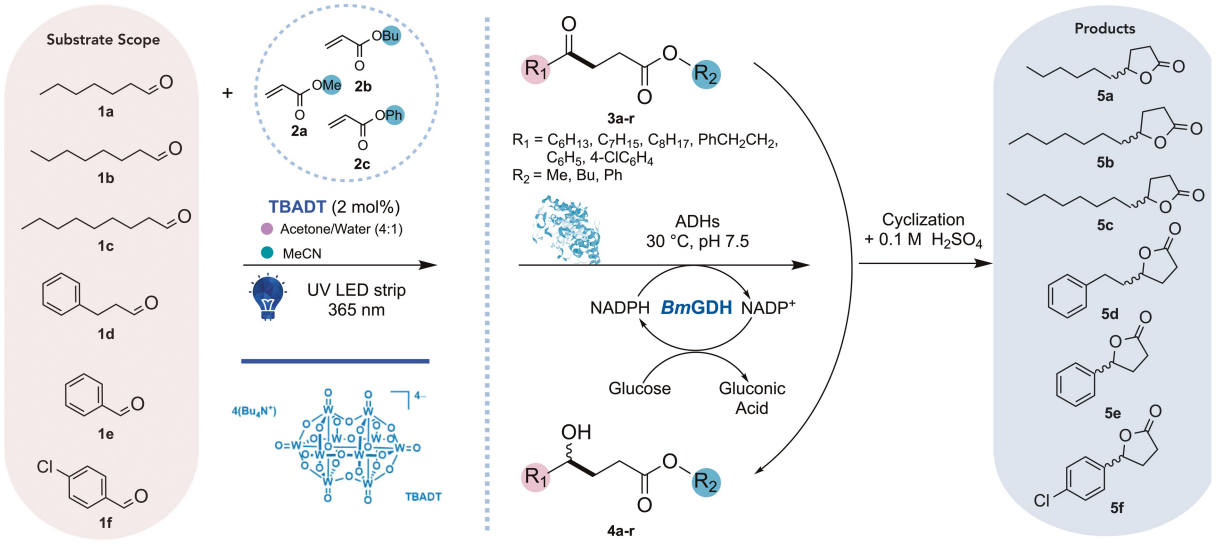
Next, we turned our attention to the combination of the photocatalytic and enzymatic reaction steps. Due to the observed enzyme deactivation in the presence of aldehydes/acrylates, the high amount of solvent, the undesired reduction of the starting aldehyde to the corresponding alcohol by the ADHs and the UV irradiation, we decided to perform the enzymatic reduction after the photocatalytic C–C bond-forming step was run to completion. In addition, the solvent applied might impair the activity of the enzyme in the subsequent reduction reaction, but could also influence the enantioselectivity as previously reported for several ADHs.[47]

Thus, the reactions starting from **1a–f** and **2a–c** were performed in both, MeCN or Ac/H_2O, in order to investigate the effect of the solvent on the efficiency and enantioselectivity of the ADH-catalyzed reaction (Schemes 2 and S1-6). For instance, substrate **3a** that resulted from the photocatalytic reaction between **1a** and **2a** was almost fully reduced by *Sm*CR$_{m4}$ to (***R***)-**4a** (95% yield, 96% *ee*) when Ac/H_2O was used, and after acidic work-up, readily cyclized to yield (***R***)-**5a** with an *ee* of 96% and 95% yield (Scheme 2).

Similarly, the same reaction was performed with all selected ADHs and acrylates **2b–2c** and in the presence of MeCN or Ac/H_2O (Scheme S1). The desired lactone **5a** was obtained with varying yields, while the reduction of the butyl ester **3b** was typically less efficient compared to the methyl ester **3a** and phenyl ester **3c**. Overall, the reduction of **3a** by *Sm*CR$_{m4}$ in Ac/H_2O and *SY*ADH in MeCN was showing similar results in view of yield, while the reduction of **3c** by *SY*ADH gave (***S***)-**5a** with the highest *ee* of >99%. Surprisingly, the reductions were typically more efficient when MeCN was used as solvent.

We continued our investigations by performing time course experiments to monitor how fast the enzymatic conversion proceeds (Figure S7a–c). The reactions typically run to completion within 6 hours, while the majority of product is already formed in the first hour. In the time-course experiments, the *ee* did not significantly change over time, but slightly decreased when increasing amounts of MeCN were used (Table S6).

Building on the results obtained, we became particularly interested in *Sm*CR$_{m4}$ which has been engineered toward the asymmetric reduction of various aliphatic and aromatic γ-keto esters/acids with an excellent *ee* of >99%.[23] Therefore, we expanded our substrate panel to further aliphatic **1b–d** and aromatic aldehydes **1e–f** (Scheme 2). Similar to the photochemoenzymatic reactions performed with aldehyde **1a** and acrylates **2a–c**, aliphatic lactones (***R***)-**5b** and (***R***)-**5c** were obtained with high yields (up to 99%) and *ee*'s (up to 98%) with *Sm*CR$_{m4}$, while reactions with *SY*ADH gave the corresponding lactones (***S***)-**5b** and (***S***)-**5c** with good *ee* (up to 93%). We continued to investigate the conversion of aromatic aldehydes **1e–f** and acrylates **2a–c** with our ADH panel. *Ras*ADH showed high yield for the aromatic lactone (***S***)-**5e** in MeCN (>99% yield), while in Ac/H_2O the yield was lower (84%), although a higher *ee* (99%) was observed. Similarly, all possible substrate combinations have been explored with our ADH panel (Schemes S1–6). Overall, depending on the chosen ADH and reaction conditions (MeCN or Ac/H_2O), the lactone yields and enantioselectivities obtained with *Ras*ADH and *LK*ADH typically lacked behind the performance of *Sm*CR$_{m4}$ and *SY*ADH. Nonetheless, both enantiomers of the respective lactones **5a–d** and **5f** were obtained with either *Sm*CR$_{m4}$, *LK*ADH or *SY*ADH with high yields of up to 99% (e.g., for (***S***)-**5a**) and good to excellent enantioselectivities (Scheme 2). The only exception is **5e**, for which only the (*S*)-enantiomer could be obtained with our chosen ADH panel. Overall, the photochemoenzymatic approach enabled the direct conversion of simple aldehydes **1a–f** and olefins **2a–c** into the respective keto esters **3a–r**, which are further converted to yield the desired γ-lactones **5a–f** after acid-promoted lactonization.



Enantioselectivity of Ketone Reduction

Aldehyde	Olefin	Lactone	Yield [%]	*ee* [%]	Enzyme
1a	2a	5a	95	96 (*R*)	*Sm*CR$_{m4}$
	2c		95	>99 (*S*)	*S*YADH
1b	2a	5b	60	98 (*R*)	*Sm*CR$_{m4}$
	2c		75	93 (*S*)	*S*YADH
1c	2a	5c	99	96 (*R*)	*Sm*CR$_{m4}$
	2b		81	89 (*S*)	*S*YADH
1d	2c	5d	10	88 (*S*)	*S*YADH
	2c		36	95 (*R*)	*LK*ADH
1e	2c	5e	84	99 (*S*)	*Ras*ADH
	2c		>99	97 (*S*)	*Ras*ADH
1f	2c	5f	56	>99 (*S*)	*Sm*CR$_{m4}$
	2c		61	99 (*R*)	*S*YADH

Scheme 2. The photochemoenzymatic synthesis of chiral γ-lactones starting from simple aldehydes and acrylates. The first step comprises the photocatalytic C–C bond formation catalyzed by TBADT under UV light irradiation (365 nm) to the corresponding keto esters, followed by an ADH-promoted asymmetric reduction to the corresponding hydroxy esters, which fully lactonize under acidic conditions to the desired γ-lactones. Photocatalytic reaction conditions: [aldehyde, **1a–f**] = 50 mM and [acrylates, **2a–c**] = 60 mM, 2 mol% TBADT, in 1 mL of either ●MeCN or ●Ac/H_2O (4:1 v/v), irradiated with 365 nm LEDs for 24–30 hours. Enzymatic reaction conditions: **3a–r** = [6.25% (v/v) from photocatalytic step], Glucose (50 mM), $NADP^+$ (0.5 mM), purified *E. coli*/*Bm*GDH (0.2–0.5 kU/mL), cell free extract of ADHs (100 mg/mL), in 0.5 mL buffer solution 50 mM KPi, pH 7.5 or 100 mM NaPi pH 7 (in the case of *Sm*CR$_{m4}$), 30 °C, 24 h. Analytical yields determined by GC-FID are reported.

While the conversions and enantioselectivities achieved with acrylates **2a–c** were encouraging, we became interested in replacing the acrylates with fumaric acid **2d**, offering the advantage of being less volatile and prone to undergo side reactions (e.g., oligomerization). Moreover, fumaric acid was recently shown to be an excellent substrate for the preparation of γ-keto acids under TBADT-photocatalyzed conditions in the presence of aldehydes, outperforming the performance offered by olefins substituted with a single electron-withdrawing group. Thus, the process initially led to a dicarboxylic acid adduct, which spontaneously underwent decarboxylation to afford the desired product (Figure 2).[48]

Accordingly, we first investigated whether the C–C bond formation between our aldehydes panel **1a–f** and **2d** under conditions compatible with the follow-up biocatalytic step can be efficiently catalyzed by TBADT (Figure 2). Similar to the reactions with the acrylates, we studied the photocatalytic synthesis in the presence of MeCN/H_2O (water was here required to ensure full solubility of the reaction components) or Ac/H_2O, starting from a higher (100 mM) substrate concentration.[48] Furthermore, while reactions with acrylates **2a–c** have been irradiated at 365 nm, it was more convenient to adopt 395 nm light irradiation to promote the desired acylation of **2d** (Table S7). Thus, the highest conversion was achieved for **1d** with **2d**, yielding 92 mM of **3v**. While in reactions with **2a–c** the acrylates had to be applied in excess due to the high volatility of these compounds, equimolar amounts of aldehydes **1a–f** and **2d** could be used and much better mass balances were consistently observed. Interestingly, the reaction time had to be significantly prolonged to 40 hours, because in case of **1d–f** remaining aldehyde (< 5–7 mM) was still detected.

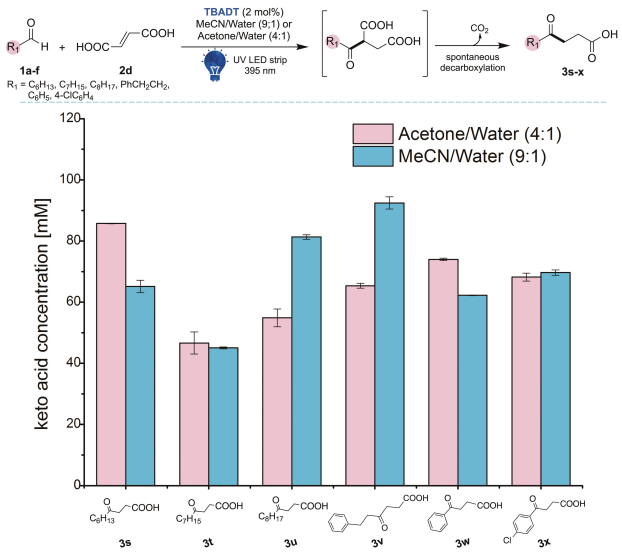
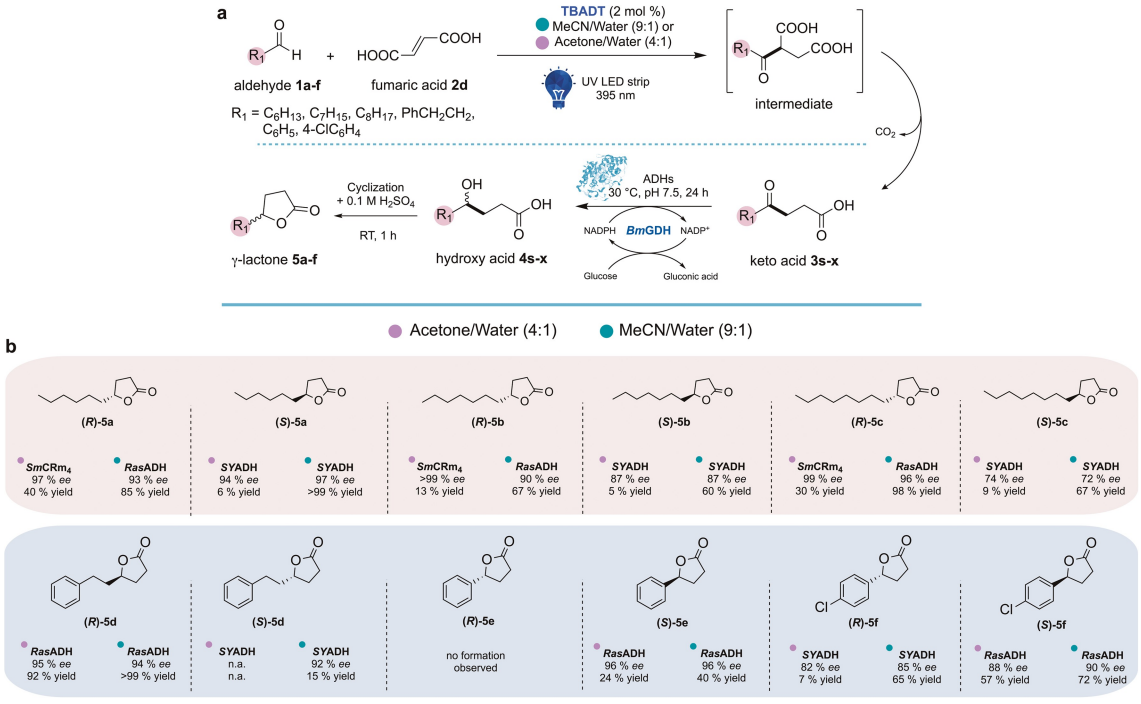


Figure 2. Photocatalytic synthesis of substituted keto acids with fumaric acid. Photocatalytic reaction conditions: [aldehyde, **1 a–f**] = 100 mM and [fumaric acid, **2 d**] = 100 mM, 2 mol% TBADT, in 1 mL of MeCN/H_2O 9:1 or Ac/H_2O 4:1 v/v, irradiated with 395 nm LED strips for 40 h.

Next, we combined the photocatalytic keto acid synthesis with the enzymatic reduction catalyzed by our ADH panel (Scheme 3). *Sm*CR$_{m4}$ showed comparable activity toward the medium-chain keto acids as for the respective keto esters (Figure S4). In particular, the selectivity for these substrates was high, and the corresponding (*R*)-lactones **5 a–c** could be obtained with an *ee* of up to >99% (Scheme 3 and S7). Similarly, the (*R*)-lactones **5 a–c** were obtained with *Ras*ADH, while yields were in general higher compared to reactions performed with *Sm*CR$_{m4}$, and the enantioselectivity was slightly lowered. In another example, *Ras*ADH produced up to 5.8 mM of the aromatic lactone **(*R*)-5 d** with excellent yield (>99%) and 94% *ee* (Scheme 3).

The corresponding (*S*)-lactones **5 a–c** were obtained with *SY*ADH; however, enantioselectivity was typically only moderate, except for **(*S*)-5 a** that was obtained with >99% yield and 97% *ee*, providing 4 mM of final product. As observed for the reactions with keto esters (Scheme 2), the solvent had a strong effect on the efficiency of the ADH-catalyzed reduction. The γ-lactones **5 d–f** were predominantly obtained by *Ras*ADH and *SY*ADH (Scheme 3 and S7), and reactions performed in MeCN/H_2O typically gave higher yields. In particular, **(*R*)-5 d**, **(*S*)-5 e** and **(*R*)-5 f** were obtained with good selectivities of up to 96% *ee*.

To further demonstrate the synthetic usefulness of this photochemoenzymatic approach, we performed semi-preparative scale synthesis of a few selected γ-lactones (**(*S*)-5 a**, **(*R*)-5 b**, **(*R*)-5 c**, **(*R*)-5 d**, **(*S*)-5 e**, **(*S*)-5 f**) at a volume of 12 mL. For the majority of products, good to excellent enantiocontrol (up to 99% *ee*) and analytical yields were obtained (72 to >99%). For instance, *Ras*ADH showed high catalytic activity in the synthesis



Scheme 3. a) The photochemoenzymatic synthesis of chiral γ-lactones starting from simple aldehydes and fumaric acid. The photocatalytic C–C bond formation catalyzed by TBADT under UV irradiation (395 nm) proceeds under spontaneous decarboxylation to the keto acid. b) Product panel accomplished from reactions with fumaric acid. Photocatalytic reaction conditions: [aldehyde, **1 a–f**] = 100 mM and [fumaric acid, **2 d**] = 100 mM, 2 mol% TBADT, in 1 mL of ●MeCN/H_2O 9:1 v/v or ●Ac/H_2O 4:1 v/v, irradiated with 395 nm LED strips for 40 hours. Enzymatic reaction conditions: intermediate **3 s–x** = [6.25% (v/v) from photocatalytic step], glucose (50 mM), $NADP^+$ (0.5 mM), purified *E. coli*/*Bm*GDH (0.2–0.5 kU/mL), cell free extract of ADHs (100 mg/mL), in 0.5 mL buffer solutions 50 mM KPi, pH 7.5 or 100 mM NaPi pH 7 (in the case of *Sm*CR$_{m4}$) at 30 °C, 24 h. Analytical yields determined by GC-FID are reported. n.a. not applicable.

of (*R*)-**5c**. After 24 hours, 98% **3u** was converted to afford (*R*)-**5c** in 85% yield of the isolated product (11 mg product) and with 96% *ee*. Similarly, (*S*)-**5e** was obtained with 53% isolated yield (4 mg) and 99% *ee* (Table S8 and Figures S14–19). While the conversions showed high catalytic activity of the respective ADHs, the obtained isolated yields were only between 39–85%. This can mainly be attributed to the loss of lactones during product isolation due to high volatility, although a combination of chromatography and liquid-liquid extraction methods was investigated.

Conclusion

In conclusion, a convenient methodology to enantioselectively produce γ-lactones from simple starting materials via photocatalytic C–C bond formation and enzymatic asymmetric reduction has been realized. While it facilitates the enantioselective synthesis of various aliphatic γ-lactones as highly important flavor and fragrance compounds, the product scope even comprises several aromatic γ-lactones, which are frequently used precursors for the preparation of pharmaceuticals. While this is the first report to combine decatungstate-catalyzed C–C bond formation with enzyme catalysis, we believe that the insights gained herein will inspire further advances in the use of photo- and biocatalysis for complex (chiral) molecule building.

Experimental Section

Gene cloning and expression of alcohol dehydrogenases

General information on strains and plasmids, and the details of gene cloning protocols can be found in the Supporting Information, "Sections Bacterial Strains, Plasmids and Primers". Site-directed mutagenesis was performed according to the Stratagene QuikChange™ protocol, using primers as listed in the Supporting Information. The presence of the desired mutations in all constructs was verified by sequencing.

Escherichia coli BL21(DE3) was used as expression strain for all ADHs. Plasmids containing the *adh* genes were isolated and transformed into *E. coli* BL21(DE3) by the heat shock method. Cells were routinely cultivated from a single fresh colony. *E. coli* BL21(DE3) cells carrying the *Ras*ADH, *SY*ADH, *LK*ADH, and *Sm*CR$_{m4}$ plasmids, respectively, were grown in 400 mL TB medium with the respective antibiotic (40 μg/mL kanamycin or 100 μg/mL ampicillin in case of *LK*ADH). For *Ras*ADH, the medium was further supplemented with 0.6 mM $CaCl_2$. The medium was inoculated with an overnight culture to give an OD_{600} of 0.05. Cells were grown in 2 L baffled shake flasks at 37 °C until an OD_{600} of 0.6–0.8 was reached and induced by the addition of isopropyl-β-D-thiogalactopyranoside (IPTG) to a final concentration of 1 mM for *LK*ADH, 0.5 mM for *SY*ADH and *Ras*ADH and 0.2 mM for *Sm*CR$_{m4}$. For *Sm*CR$_{m4}$, induced cultures were incubated for 24 hours at 16 °C before harvesting the cells by centrifugation (3,400×*g* for 15 min) at 4 °C and washing with sodium phosphate buffer (100 mM, pH 7). For all others, induced cultures were incubated for 20 hours at 20 °C before harvesting the cells by centrifugation (1,344×*g* for 15 min) at 4 °C and washing with potassium phosphate buffer (50 mM, pH 7.5). Cell pellets were centrifuged again with the same speed and resuspended in the same washing buffer to give a wet cell weight (WCW) of 300 g_{wcw}/L. Cell disruption was performed using ultrasonication with 70% duty cycle, out-put 7–8 sec for 2 min. Cell debris was separated from the crude extract by centrifugation (16,000×*g*, 45 min, 4 °C). The crude cell extracts were filtered (0.45 μm, Whatman®) and aliquoted to be stored at −20 °C.

Photochemoenzymatic synthesis of chiral γ-lactones

The photocatalytic reactions were performed in 2 mL glass vials. A mixture of aldehydes (50 mM, 1 equiv.), acrylates (60 mM, 1.2 equiv.), and a catalytic amount of TBADT (2 mol%, 1 mM) was dissolved in 1 mL acetonitrile or acetone/water (4:1 v/v). In case of fumaric acid reactions, a mixture of aldehydes (0.1 M, 1 equiv.) and fumaric acid (0.1 M, 1 equiv.) in the presence of a catalytic amount of TBADT (2 mol%, 2 mM) was dissolved in 1 mL either MeCN/H_2O (9:1 v/v) or Ac/H_2O (4:1 v/v). The resulting solutions were purged with nitrogen for 3 minutes, screw-capped and irradiated at 365 or 395 nm by using a home-made light-setup (24 W; see Supporting Information, "Section Photocatalytic Setup" and Figures S1,2 for further details). The light reactor was then placed into a shaking incubator and reactions were incubated at 24 °C and 220 rpm for 24–40 h. After the reaction was complete (aldehyde consumption was monitored via GC-FID), 6.25% of reaction solution containing the corresponding γ-keto esters or γ-keto acids was transferred to a new 2 mL glass vial. To this solution, the crude cell extract containing the overexpressed (*R*)- or (*S*)-selective ADHs (70–200 mg/mL), glucose (50 mM), $NADP^+$ (0.5 mM), and purified *Bm*GDH (0.2–0.5 kU/mL) were added. To reach the total reaction volume of 0.5 mL, 50 mM KPi buffer at pH 7.5 or 100 mM NaPi buffer at pH 7 in case of *Sm*CR$_{m4}$ was added. The mixture was then stirred in a thermomixer at 30 °C, 700 rpm under dark conditions. After 20–24 h, the reaction was terminated by adding 0.1 M sulfuric acid (95-97%), and the reaction samples (200 μL) were extracted with ethyl acetate (200 μL) and dried with a spatula tip of $MgSO_4$ after incubating at RT for 1 h. Analytical yields and enantioselectivity of lactones were directly determined by GC-FID. Details of columns and analytical methods, with chromatograms, can be found in the Supporting Information, "Section GC analytics".

Semi-preparative scale syntheses

In a 2 mL glass vial, a mixture of aldehyde (0.1 M, 0.1 mmol, 1 equiv.) and fumaric acid (0.1 M, 0.1 mmol, 1 equiv.) in the presence of a catalytic amount of TBADT (2 mol%, 2 mM) were dissolved in 1 mL MeCN/water (9:1 v/v). In case of acrylate reaction, aldehydes (0.1 M, 0.1 mmol, 1 equiv.), acrylates (0.12 M, 0.12 mmol, 1.2 equiv.) and a catalytic amount of TBADT (2 mol%, 2 mM) was dissolved in 1 mL Ac/water (4:1 v/v). The resulting solutions were purged with nitrogen for 3 minutes, screw-capped and irradiated at either 365 nm or 395 nm by using the home-made light-setup. The light reactor was placed into a shaking incubator and reactions were incubated at 24 °C and 220 rpm for 30–40 h. 6.25% of the reaction solution containing the corresponding γ-keto esters/acids was transferred to a new 20 mL glass vial. To this solution, the crude cell extract containing the overexpressed (*R*)- or (*S*)-selective ADHs (100 mg/mL), glucose (50 mM), $NADP^+$ (0.5 mM), and purified *Bm*GDH (0.2–0.5 kU/mL) were added. To reach the total reaction volume of 12 mL, 50 mM KPi buffer at pH 7.5 or 100 mM NaPi buffer at pH 7 in case of *Sm*CR$_{m4}$ was added. The reactions were placed in an incubation shaker at 30 °C, and were incubated at 180 rpm for 20–24 h under dark conditions. The reaction mixture was then extracted with DCM (3 times) after acidification at pH 2.0 using 0.5 M aqueous H_2SO_4. The lactonization reaction was carried out at RT and 180 rpm for 1 h. The organic layer was dried over anhydrous $MgSO_4$, followed by the addition of trifluoroacetic acid

(0.04 mL/g lactones) in 10 mL anhydrous DCM at room temperature, followed by stirring at room temperature for 4 h. The pH of the reaction mixture was maintained with saturated aqueous $NaHCO_3$ (10 mL) and subsequently extracted with DCM (3×10 mL). The organic phase was separated and dried over $MgSO_4$ and then concentrated in vacuum. The lactones were purified via column chromatography eluting with a mixture of ethyl acetate and pentane (1:4) to provide the pure γ-lactones. ^{1}H NMR (500 MHz) spectra were recorded in $CDCl_3$. Isolated yields were determined by calculating the amount of purified product obtained.

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Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Keywords: photobiocatalysis · C–C bond formation · decatungstate anion · asymmetric reduction · alcohol dehydrogenases

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