Synthetic Methods

DOI: 10.1002/anie.201206019

A Photocatalyst/Enzyme Couple That Uses Solar Energy in the Asymmetric Reduction of Acetophenones**

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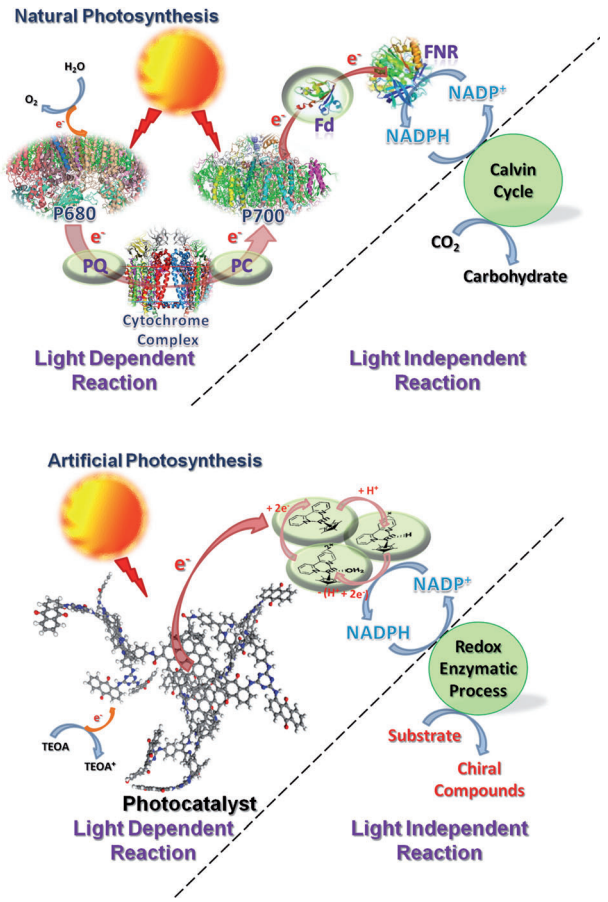
Synthesis of fine chemicals and fuel materials through the utilization of solar energy emerges as one of the most important scientific challenges in recent years as it is becoming more and more significant from the viewpoint of green chemistry.[1] In green plants, chlorophyll absorbs solar energy in the visible region and high-energy electrons are generated. These photoexcited electrons are transferred and absorbed by the photosynthetic reaction center and results in the formation of the reduced form of the nicotinamide cofactors NADPH (Scheme 1). NADPH serves as an energy source in the Calvin cycle for the production of carbohydrates from CO_2 through a series of sequential redox enzymatic reactions, and is subsequently regenerated through photosynthesis for use in another cycle. Encouraged and fascinated by the orchestration between photocatalysis and biocatalysis in natural photosynthesis, scientists have been engaged in devising a corresponding photocatalyst/biocatalyst systems as an artificial analogue (Scheme 1). In recent years artificial photosynthesis has been identified as one of the most potentially useful and environmentally benign methods for solar energy conversion.[2] Biocatalytic processes have gained increasing significance in recent years because of the unique capabilities of redox enzymes to catalyze complex organic reactions under mild reaction conditions where conventional ones fail.[3] However, in spite of their high potential, they are not used a lot because of the high costs of enzyme-specific cofactors (NADH/NADPH), which are required in stoichiometric quantities for successful application, and is a disadvantage for the industrialization of many promising enzymatic processes.[4] To overcome these drawbacks, in situ cofactor regeneration from their oxidized counterpart appears to be crucial and so far a plethora of such methods have been developed in last few decades. For example, there are substrate-coupled,[3a] enzyme-coupled,[3a,5] electrochemical approaches[5,6] etc. However all these have drawbacks.[4a,6] As a result, the necessity for an efficient cofactor regeneration method still exists and keeps scientists engaged in exploring new methods.[3a,4b,5] Of late, photochemical methods for cofactor regeneration has gained increasing significance as it provides a way of harnessing clean and abundant solar energy.[7–9] In earlier work, we reported $W_2Fe_4Ta_2O_{17}$ as a photocatalyst for a photocatalyst/enzyme-coupled system.[8] Recently we developed a graphene-based photocatalyst, composed of chemically converted graphene coupled with a multianthraquinone-substituted porphyrin (CCGCMAQSP),[9] and used it in a photocatalyst/enzyme-coupled, artificial photosynthesis system wherein visible-light mediated the regeneration of NADH and photoenzymatic synthesis of formic acid proceeded from CO_2.[9]



Scheme 1. Illustrative comparison of natural photosynthesis (above) and a photocatalyst/enzyme-coupled artificial photosynthesis system (bottom). Light-mediated NADPH regeneration by an electron-transport system with subsequent cofactor dependent redox enzymatic synthesis of final products is the essential feature in both the systems. Fd = ferredoxin, FNR = ferredoxin $NADP^+$-reductase, PC = plastocyanin, PQ = plastoquinone, TEOA = triethanolamine.

In continuation of this research, we envisaged an analogous system for chiral synthesis of fine chemicals using a photocatalyst (Scheme 1) and the regenerated cofactor with

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[**] This work was supported by the KRICT 2020 project program.
Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/anie.201206019.

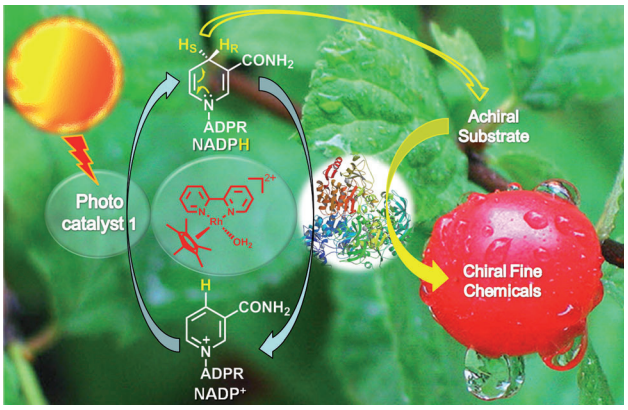


Figure 1. Solar energy in asymmetric artificial photosynthesis of chiral compounds from an achiral substrate. ADPR = adenosine diphosphate ribose.

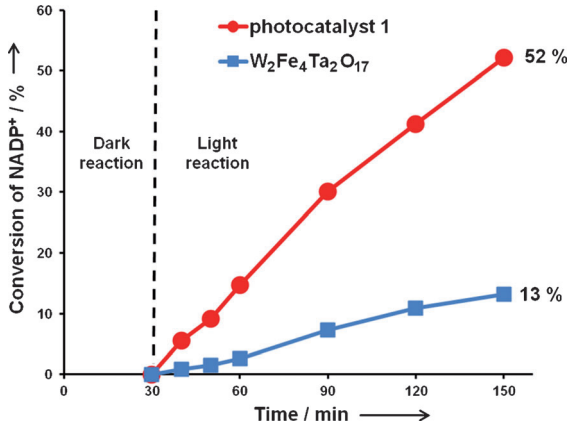


Figure 2. Regeneration of NADPH from $NADP^+$ under dark and light conditions by photocatalyst **1** and $W_2Fe_4Ta_2O_{17}$. Keeping 0.5 h in the dark, the reactor was illuminated with visible light ($\lambda > 420$ nm).

an achiral substrate in the presence of a suitable enzyme. Figure 1 depicts such an asymmetric, artificial photosynthetic system based on photocatalysis with the photocatalyst **1** (CCGCMAQSP;[9] see Figure S1 in the Supporting Information) and biocatalysis using an enzyme. Thus, we describe herein the use of **1** for the regeneration of NADPH in the presence of visible light and its subsequent use in the asymmetric enzymatic reduction of substituted acetophenones to give the pharmaceutically useful chiral 1-phenylethanol derivatives[10,11] (Figure 1). Since, energetically almost half of the total solar light available on Earth falls within the visible range,[12] the present work demonstrates a useful way to employ solar energy and is of great importance from the perspective of green chemistry.

We employed a commercially available alcohol dehydrogenase from *Lactobacillus kefir* (LKADH),[13] which is known to be an NADPH-dependent reductase and necessitates an efficient and inexpensive means of NADPH regeneration. Using our previously reported method,[9] the system consisted of the photocatalyst **1**, pentamethylcyclopentadienyl rhodium bipyridine, $[Cp^*Rh(bpy)(H_2O)]^{2+}$ ($\mathbf{M_{ox}}$; $Cp^* = \eta^5\text{-}C_5Me_5$, bpy = 2,2-bipyridyl) as an electron mediator and a hydride-transfer catalyst,[14] along with commercially available $NADP^+$ in an aqueous buffer solution at pH 7. The reaction was carried out in a quartz reactor using visible-light irradiation (with a $\lambda = 420$ nm cut-off-filter) under argon. Although no regeneration of NADPH from its oxidized form, $NADP^+$, was observed in the absence of visible light (0.5 h), formation of NADPH was detected upon irradiation ($\lambda >$ 420 nm) of the system. The concentration of NADPH was measured spectrophotometrically by recording the absorbance at $\lambda = 340$ nm. For the optimized set of parameters (for experimental details, see the Supporting Information), rapid NADPH formation took place and resulted in more than 50% conversion of $NADP^+$ within 2 hours (Figure 2).

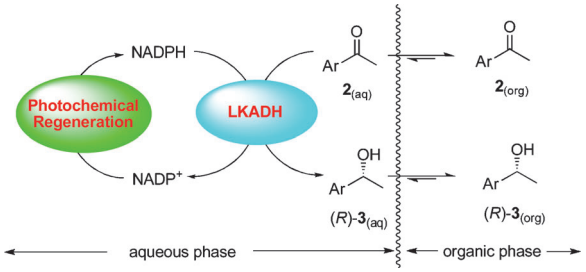
This observation establishes that **1** performs its role of cofactor regeneration. Moreover, **1** showed superior photocatalytic activity over the previously developed $W_2Fe_4Ta_2O_{17}$,[8] which under similar experimental conditions resulted a mere 13% conversion of $NADP^+$ (Figure 2).

After successfully devising a visible-light-driven photoregeneration system, our next task was to exploit the regenerated cofactor NADPH for the stereoselective reduction of inexpensive prochiral ketones (**2a–e**) to their corresponding chiral alcohols, which are used extensively for the industrial synthesis of a variety of pharmaceutical and agrochemical intermediates, active pharmaceuticals, and other bioactive compounds.[3,10,11] For this purpose **2a** was chosen as the model substrate for carrying out asymmetric reduction employing the cofactor regeneration process in conjunction with the enzyme LKADH. However, the reaction did not produce satisfactory results as only a negligible conversion of **2a** was observed. It was indeed not surprising because of the poor solubility of the hydrophobic ketone substrate in aqueous media. One solution was to introduce an organic cosolvent to improve the ketone solubility, however, this would be at the expense of enzyme deactivation to different extents depending upon enzyme and solvent used.[15,16] So we sought a reaction medium that would minimize the effect of the solvent on the function of the enzyme.[15,16] For this purpose, known organic solvents were employed (as 20% v/v) and it was observed that polar solvents like DMF, DMSO, and THF failed to produce satisfactory results as only about 14–17% conversion was achieved after 50 hours (see the Supporting Information). Comparatively better results were obtained using nonpolar solvents like *n*-hexane and *n*-heptane,[13,15] where approximately 40–42% conversion was observed with an enantioselectivity of greater than 99% in favor of (*R*)-1-phenylethanol [(*R*)-**3a**]. With a prolonged reaction time, the reaction rate gradually diminished. Thus *n*-heptane emerged as the solvent of choice for the reaction.

The effect of the concentrations of heptane, $NADP^+$, and LKADH on the outcome of the reaction needed to be assessed to optimize the reaction. In a systematic study, one of these three concentrations was varied while keeping the other two constant. The optimized reaction conditions, thus obtained, were: $NADP^+$ at 0.4 mM and LKADH at 1 unit (per 20 mM conc. of substrate **2a**) in 20% (v/v) *n*-heptane/water (for experimental details, see the Supporting Informa-

tion). Under these reaction conditions, the reduction of **2a** proceeded, albeit slowly, and its best conversion was 42% after 50 hours with exclusive formation of (*R*)-**3a** (>99% *ee*). Efforts to improve the conversion of the starting ketone additionally were not successful.

The underlying principles for such biphasic reaction media are depicted in Scheme 2 and shows the equilibrium at the interface between the aqueous and organic phases for

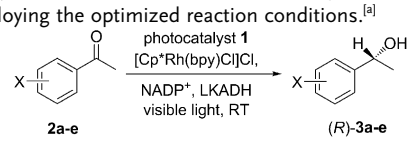


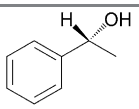
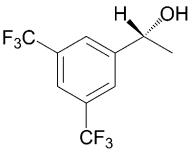
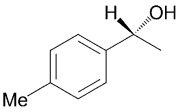
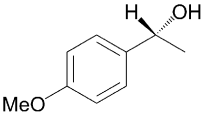
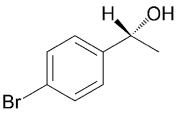
Scheme 2. Plausible rationale behind enzymatic reduction of **2** in biphasic media.

the substrates [$\mathbf{2}_{(aq)}$ versus $\mathbf{2}_{(org)}$] and products [(*R*)-$\mathbf{3}_{(aq)}$ versus [(*R*)-$\mathbf{3}_{(org)}$].[15] Once reduction starts and alcohols are generated, they cross the interface and move to organic phase thereby facilitating more of the ketone to move towards aqueous phase to undergo enzymatic reduction multiple cycles.

Having proved the applicability of the new photosynthesis system, we then focused upon the asymmetric reduction of a few representative acetophenone derivatives using the optimized reaction conditions (see the Supporting Information). As with **2a**, enzymatic reduction of the ketones **2b**–**e** furnished exclusively the *R*-configured alcohols (>99% *ee* except **2b** with ca. 82% *ee*; Table 1). From these results it appears that inductive effects of the substituents present in the aromatic ring of aryl ketones does not have a significant impact upon the overall outcome. Stereoselective reduction of ketones under enzymatic conditions has been studied extensively over the years,[11,13,15,16] however, in all cases the associated cofactors are regenerated by employing either substrate-[11a,13] or enzyme-coupled[11b,15,16] methods as well as others. To the best of our knowledge, the present asymmetric reduction of acetophenone derivatives exemplifies the first of its kind, wherein regeneration of the cofactor is achieved by means of a visible-light-mediated reaction. Moreover, it proceeds in the absence of either a co-substrate or a second enzyme, and instead utilizes a cheap and renewable energy source (i.e., solar energy), thus reducing the overall cost of the method. The chiral products and derivatives thereof can serve as key intermediates for the synthesis of a myriad of drugs,[10,11] and (*R*)-**3b** is of particular interest in the pharmaceutical industry as it is incorporated into the orally active Human neurokinin-1 (NK1) receptor antagonist (EMEND, Aprepitant) which is an anti-emetic for cancer chemotherapy.[11] In spite of the excellent stereoselectivity in the products, and environmental and economical benefits of the method, it is limited by incomplete consumption of the

Table 1: Enzymatic reduction with different acetophenone derivatives (**2a–e**) employing the optimized reaction conditions.[a]

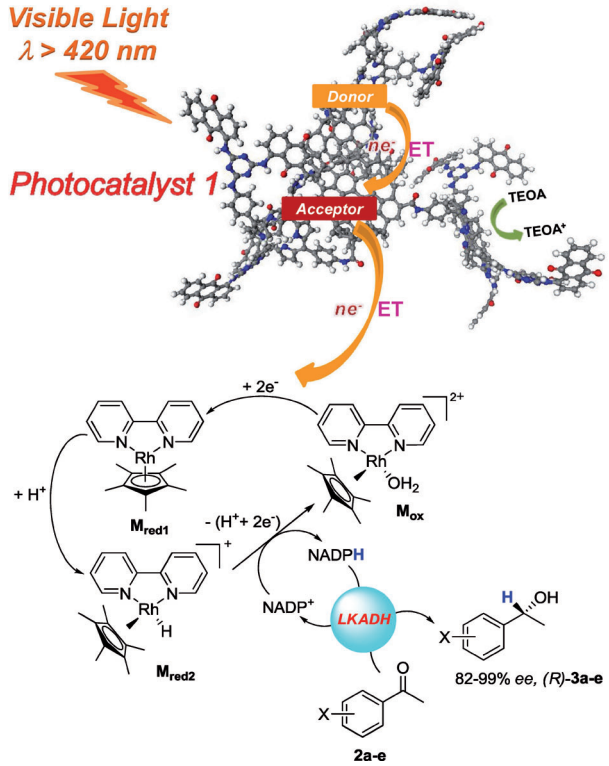


Entry	Product		Conv.[b] [%]	*ee*[c] [%]
1		(*R*)-**3a**	42	>99
2		(*R*)-**3b**	34	ca. 82
3		(*R*)-**3c**	26	>99
4		(*R*)-**3d**	20	>99
5		(*R*)-**3e**	25	>99

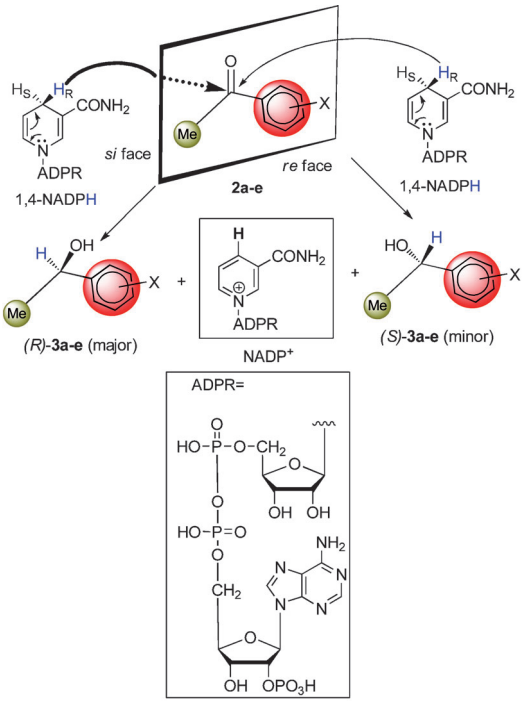
[a] Total reaction mixture of 3 mL comprises 2.4 mL sodium phosphate (NaH_2PO_4-Na_2HPO_4) buffer (pH ≈ 7.0, 0.1 M), 0.6 mL *n*-heptane, 20–40 mM **2a–e**, 1 mM $NADP^+$, and 1–2 U LKADH (for experimental details, see the Supporting Information). [b] Conversions of ketones **2a–e** were determined on the basis of GC analysis. [c] The enantiomeric excesses of the product alcohols **3a–e** were measured by GC analysis using a chiral stationary phase.

starting ketone (20–42%) and longer reaction times which reduce its practical utility. Although confirmation is needed, these limitations could be attributed to the time-dependent decrease of enzymatic activity of LKADH towards the acetophenones **2a–e**, given the present reaction conditions. Nevertheless, the present photoenzymatic method for asymmetric synthesis seems to be promising and has the potential for future applications because of its environmental advantage.

A plausible mechanism for the overall reaction is depicted in Scheme 3. Upon absorption of visible light by **1**, electronic excitation takes place, thus leading to the transport of photoexcited electrons from the triazine-linked porphyrin anthraquinone triads to the graphene part of the photocatalyst by the interconnecting amide linkage and then final transfer of two such electrons to the organometallic rhodium complex $\mathbf{M}_{ox}$. The reduced species $\mathbf{M}_{red1}$ is thus formed, and subsequent proton abstraction from aqueous solution gives $\mathbf{M}_{red2}$, which loses two electrons and one proton to generate NADPH and the initial $\mathbf{M}_{ox}$. Thus, the rhodium-based organometallic compound **M** acts as an efficient electron shuttle between **1** and the oxidized cofactor $NADP^+$. The in situ generated reactive species $\mathbf{M}_{red2}$ ($[Cp^*Rh(bpy)(H)]^+$) is actually responsible for regiospecific reduction of natural



Scheme 3. Plausible mechanism of asymmetric reduction of acetophenones **2a–e** by the LKADH/1,4-NADPH system under visible light. ET = electron transfer.



Scheme 4. Stereochemical mechanism of hydride transfer from 1,4-NADPH to the carbonyl carbon atom of substrates **2a–e** bearing small (Me) and large (Ar) groups.

NADP$^+$ to the enzymatically active 1,4-NADPH,[14] which is subsequently used in the next step for the enzymatic asymmetric reduction of prochiral ketones (Scheme 4). At this stage, the active site of LKADH, present in the reaction medium, in combination with the regenerated cofactor 1,4-NADPH, presumably binds with the carbonyl group of the substrate to facilitate preferential transfer of the *pro*-R hydride from the cofactor to the *si* face of the carbonyl group,[3b,13] thereby producing (*R*)-**3a–e** as major products (ca. 82–99%). NADP$^+$, sequentially dissociates from the enzyme, then follows the same photocatalytic cycle, thereby resulting in the photoregeneration of 1,4-NADPH and subsequent enzymatic asymmetric reduction of the ketones. In contrast, TEOA acts as a sacrificial agent as it gets oxidized in the presence of light and supplies electrons to **1** during each photocatalytic cycle (Scheme 3). Such a synchronized coupling between these two catalytic cycles guarantees sustainable photoregeneration of the cofactor in active form and hence the redox enzymatic production of chiral 1-phenylethanols.

In summary, we demonstrated a bioinspired photoenzymatic system for the synthesis of chiral compounds by incorporating nicotinamide cofactor photoregeneration with biocatalysis. In the photocatalyst/enzyme-coupled artificial photosynthesis process, efficient regeneration of cofactors NADH/NADPH is a crucial step to trigger enzymatic reactions. Although several methods are reported for cofactor regeneration, the photochemical regeneration process gains additional impetus because of the ready availability of clean solar energy. We have described the visible-light-mediated regeneration of NADPH employing a graphene-based photocatalyst and successful utilization in the asymmetric enzymatic reduction of acetophenone derivatives to produce pharmaceutically useful chiral 1-phenylethanols. The outcome of the reaction depended on the concentrations of solvent, NADP$^+$ and enzyme used, and optimum results were obtained using an enzyme-compatible biphasic reaction medium. The reaction represents the first example of its kind where chirality is incorporated into an achiral ketone through the enzyme-supported transfer of hydride from a cofactor, which is regenerated photocatalytically. This work lays the foundation for future studies on asymmetric, photoenzymatic reaction systems and heralds a new paradigm in artificial photosynthesis. The present approach to using solar energy is a nice application of photochemical regeneration of the nicotinamide cofactor to induce asymmetry in an achiral molecule, and holds promise for applications in the synthesis of chiral fine chemicals.

Received: July 27, 2012
Published online: October 12, 2012

Keywords: alcohols · enzyme catalysis · photosynthesis · reduction · synthetic methods

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