

Switching between Enantiomers by Combining Chromoselective Photocatalysis and Biocatalysis

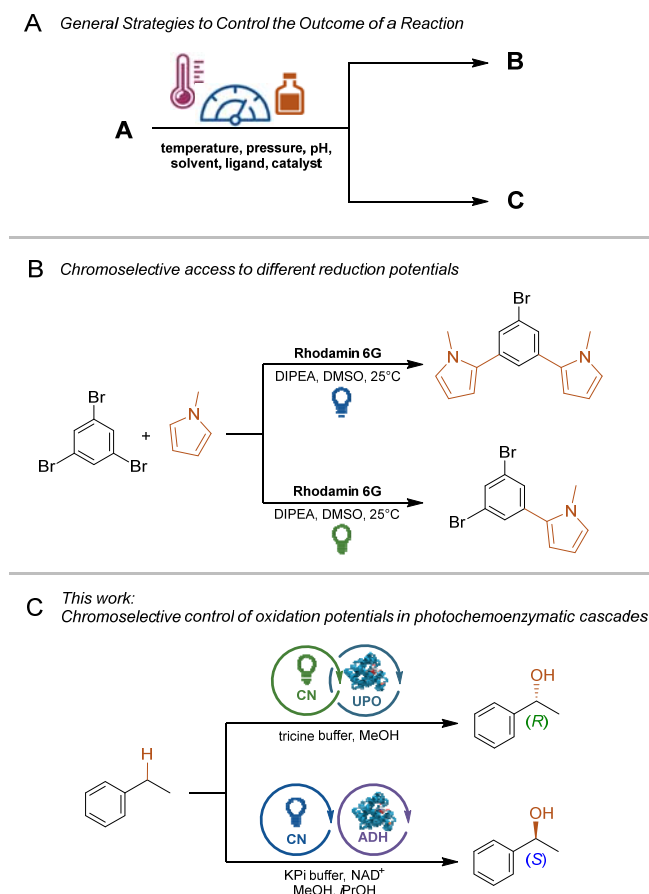
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Abstract: Controlling the selectivity of a chemical reaction with external stimuli is common in thermal processes, but rare in visible-light photocatalysis. Here we show that the redox potential of a carbon nitride photocatalyst (CN-OA-m) can be tuned by changing the irradiation wavelength to generate electron holes with different oxidation potentials. This tuning was the key to realizing photo-chemo-enzymatic cascades that give either the (*S*)- or the (*R*)-enantiomer of phenylethanol. In combination with an unspecific peroxygenase from *Agrocyste aegerita*, green light irradiation of CN-OA-m led to the enantioselective hydroxylation of ethylbenzene to (*R*)-1-phenylethanol (99% *ee*). In contrast, blue light irradiation triggered the photocatalytic oxidation of ethylbenzene to acetophenone, which in turn was enantioselectively reduced with an alcohol dehydrogenase from *Rhodococcus ruber* to form (*S*)-1-phenylethanol (93% *ee*).

Many parameters influence the selectivity of a chemical reaction.^[1] For instance, catalytic reactions can be controlled by varying the catalyst/coordinated ligands, directing groups^[2] or by tuning external parameters (Scheme 1, A).^[1a, 3] The selectivity of photochemical reactions varies with different wavelengths,^[4] but examples that use this for visible-light photocatalysis are rare.^[5]

In one example, selective control between either a one- or two-fold substitution of 1,3,5-tribromobenzene with *N*-methylpyrrole using Rhodamin 6G (Rh-6G) as photocatalyst was demonstrated (Scheme 1, B).^[5a] This selectivity switch is explained by the chromoselective generation of two photocatalytic species that differ in their reduction potential. Green light irradiation results in a common photoredox cycle and the expected mono-substituted product. In case of blue light, the Rh-6G radical anion, which is formed after quenching of Rh-6G* with a sacrificial electron donor, can absorb a second photon, resulting in the highly reducing Rh-6G^{•-} species that enables the formation of the di-substituted product.^[5a]

Here we show that electron holes with different oxidation potentials can be generated using a heterogeneous carbon nitride (CN) catalyst by changing the incident photon energy. The combination of this strategy with biocatalysis allowed us to selectively produce the (*S*)- or (*R*)-enantiomer of a chiral alcohol in photo-chemo-enzymatic reaction sequences (Scheme 1C).



Scheme 1. A) General approaches to control of the outcome of a chemical reaction; B) Chromoselective control in photocatalytic C-H-arylations^[5a]; C) This study: Chromoselective control of the stereochemical outcome of photo-chemo-enzymatic reactions.

We recently realized that the choice of the wavelength is crucial for high selectivities in metallophotocatalytic cross couplings using a heterogeneous carbon nitride material, which is made from urea and oxamide in molten salt (CN-OA-m).^[5b, 5c, 6] While this can be rationalized by a purely kinetic effect, there is also evidence that a wavelength-controlled generation of excited species with different oxidation potentials could be responsible for this phenomenon. CN-OA-m has a strong absorption up to ~460 nm and a comparably weaker absorption band up to ~700 nm, which were ascribed as the π - π^* and n - π^* electron transitions, respectively (Figure 1, A).^[7] The selective induction of the n - π^* electron transition using long wavelengths (525 nm) should result in electron holes with a lower oxidation potential compared to irradiation using blue light (440 nm). The choice of the wavelength should not affect the reduction potential of the electron that is promoted into the valence band. Although such a behavior was previously suggested,^[7] there is, to the best of our knowledge, no report that applies this concept for controlling the selectivity of chemical reactions.

We hypothesised that such a strategy would allow us to induce a photocatalytic reaction of a substrate with green light selectively in the presence of a second compound that is only photo-oxidized when shorter wavelengths are used. The photocatalytic aerobic oxidation of benzylic sp^3 C-H bonds, which is feasible with other members of the carbon nitride family and blue light irradiation,^[8] served as a model reaction for our initial studies. In a series of experiments, we were indeed able to show that only blue light results in the desired carbonyl products and no reaction occurs at longer wavelengths (Figure 1, B).

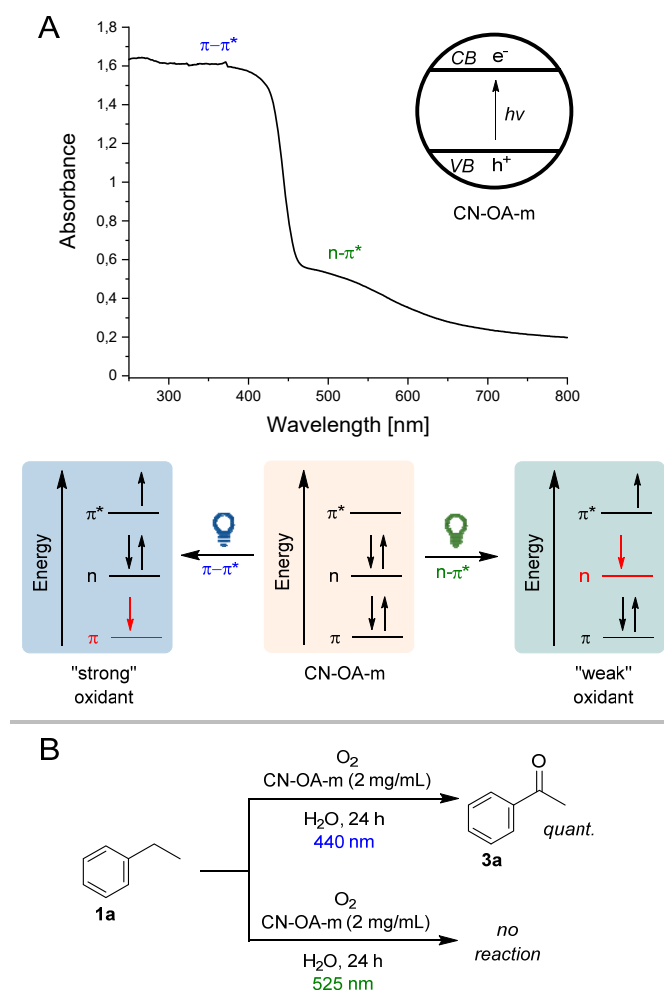


Figure 1. Chromoselective generation of excited CN-OA-m species with different oxidation potentials. A) Switching between $\pi\text{-}\pi^*$ and $n\text{-}\pi^*$ electron transitions using different wavelengths. B) The oxidation of ethylbenzene **1a** to acetophenone **3a** is only possible using blue light.

Carbon nitrides are used to catalyse the formation of O_2 and H_2 via water oxidation^[9] and the production of hydrogen peroxide from oxygen and alcohols, which requires the reduction of O_2 .^[10] Hydrogen peroxide can then be used to fuel the enantioselective biocatalytic hydroxylation of ethylbenzene derivatives by the unspecific peroxygenase^[11] (UPO) from *A. aegerita*^[12] (*AaeUPO*).^[13]

We hypothesized that a chromoselective activation of CN-OA-m with green light enables the selective formation of H_2O_2 in the presence of ethylbenzene (**1**) and the *AaeUPO*, which in turn catalyses the asymmetric hydroxylation of **1** (Figure 2). Performing the reaction in tricine buffer using 528 nm LEDs indeed resulted in a high selectivity towards (*R*)-1-phenylethanol formation [(*R*)-**2a**, up to 3.8 mM, 98% *ee*] with low amounts (3%) of acetophenone (**3a**). When the same reaction was carried out using shorter wavelengths, **3a** became the main product, thus supporting our hypothesis. Ketone (**3a**) formation was also the preferred reaction in the presence of blue light in phosphate buffer.

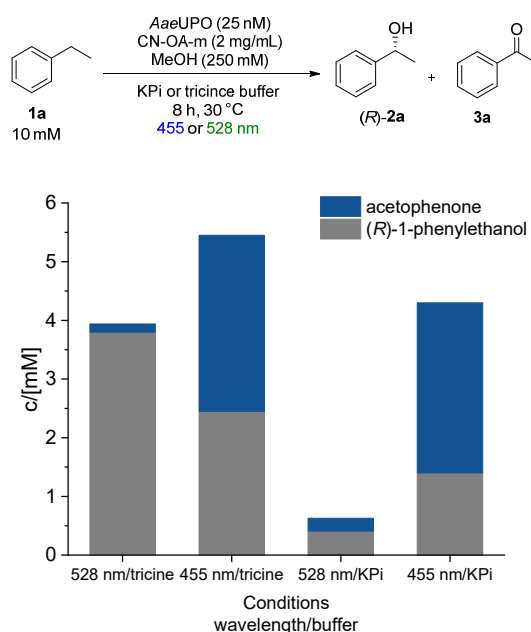
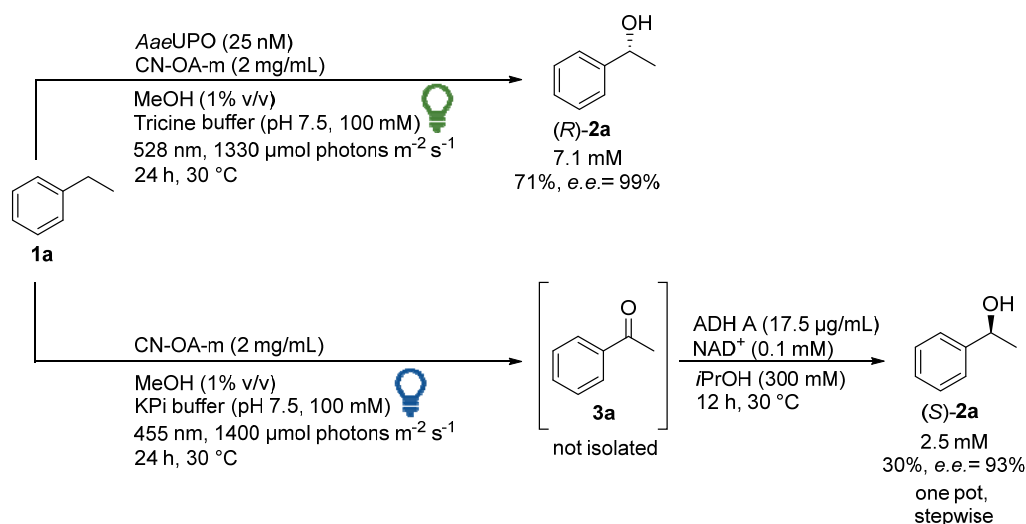


Figure 2. Influence of different wavelengths and buffers on the photoenzymatic hydroxylation of ethylbenzene; reaction conditions: *AaeUPO* (25 nM), ethylbenzene (10 mM), CN-OA-m (2 mg mL⁻¹), MeOH (250 mM), KPi (100 mM, pH 7.5) or tricine (100 mM, pH 7.5), 455 nm (1440 μmol photons m⁻² s⁻¹) or 528 nm (1330 μmol photons m⁻² s⁻¹), 30 °C, 8 h.

It was previously shown that UPOs are deactivated in the presence of blue light, a photocatalyst and O₂ due to the generation of reactive oxygen species (ROS) that harm the enzyme.^[14] Consequently, one might expect that green light might be less harmful to the UPO and lead to higher conversions in comparison to blue light. To investigate this aspect, UPO and CN-OA-m were incubated for one hour in the presence of oxygen and green or blue light, before **1a** was added (Figure S46). The mixture incubated at longer wavelengths indeed led to a higher conversion for the asymmetric hydroxylation after addition of **1a**.

The milder conditions subsequently allowed an extension of the substrate scope for *AaeUPO* (Scheme 2). Nine additional substrates were converted with high stereoselectivity (>98% *e.e.*) to the corresponding alcohols with concentrations of 1.0–6.0 mM. None of these ethylbenzene derivatives has been transformed with *AaeUPO* using an *in situ* H₂O₂ generation system before.

Ethylbenzenes bearing a methyl-substituent in the *ortho*- or *meta*-position were hydroxylated with 99% regioselectivity at the ethyl group to give the desired chiral alcohols (*R*)-**2b-c**. This ability to distinguish between a methyl and an ethyl group has not been reported before. Acetophenone substituted with ethyl in the *para*-position (**1i**) allowed to access a bi-functionalised chiral hydroxyketone **2i**, which is otherwise difficult to make. The same is true for **2j**.



Scheme 3. Light-driven enantioselective oxyfunctionalizations of **1a** by using chromoselective CN-OA-m and AaeUPO or ADH-A.

To the best of our knowledge this is the first example in which it was possible to utilize the same photocatalyst to either oxidize an organic substrate or to provide *in situ* formed H_2O_2 without photocatalytic oxidation of the substrate, all controlled only by the choice of the wavelength.

In summary, we showed that electron holes with different oxidation potentials can be generated using a carbon nitride material by simply changing the photon energy. In the presence of blue light this enables the oxidation of ethylbenzene to acetophenone in an aqueous solution. Using green light the organic substrate does not react and only H_2O_2 is formed. This was the key for designing chromoselective photo-chemo-enzymatic cascade reactions. Selective hydrogen peroxide generation enabled the hydroxylation of ethylbenzene to give (*R*)-1-phenylethanol (*R*)-**2a** using an UPO, whereas the photocatalytic oxidation to acetophenone was coupled with an enantioselective reduction to (*S*)-1-phenylethanol (*S*)-**2a** by an ADH. Additionally, low energy photons (green light) increased the stability of UPO compared to blue light, which permitted the expansion of the substrate scope of this enzyme. Controlling the outcome of a photocatalytic reaction merely through the choice of wavelength employed presents exciting new options in reaction design and could be an important new tool for controlling reactivity and stereoselection in organic synthesis.

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References

- [1] a) T. Gaich, E. Winterfeldt, *Directed Selectivity in Organic Synthesis: A Practical Guide*, 1st ed., Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim, **2014**; b) G. Statham, *Synthese* **2017**, 194, 4815–4838.
- [2] a) I. Fleming, A. Barbero, D. Walter, *Chem. Rev.* **1997**, 97, 2063–2192; b) C. Sambigao, D. Schönbauer, R. Blicke, T. Dao-Huy, G. Pototschnig, P. Schaaf, T. Wiesinger, M. F. Zia, J. Wencel-Delord, T. Besset, B. U. W. Maes, M. Schnürch, *Chem. Soc. Rev.* **2018**, 47, 6603–6743; c) G. Rousseau, B. Breit, *Angew. Chem. Int. Ed.* **2011**, 50, 2450–2494; *Angew. Chem.* **2011**, 123, 2498–2543; d) Z. Huang, G. Dong, *Acc. Chem. Res.* **2017**, 50, 465–471.
- [3] J. Jurczak, *Physica B+C* **1986**, 139–140, 709–716.
- [4] S. Protti, D. Ravelli, M. Fagnoni, *Photochem. Photobiol. Sci.* **2019**, 18, 2094–2101.
- [5] a) I. Ghosh, B. König, *Angew. Chem. Int. Ed.* **2016**, 55, 7676–7679; *Angew. Chem.* **2016**, 128, 7806–7810; b) S. Gisbertz, S. Reischauer, B. Pieber, *Nat. Catal.* **2020**, 3, 611–620; c) C. Cavedon, E. T. Sletten, A. Madani, O. Niemeyer, P. H. Seeberger, B. Pieber, **2020**; d) A. M. Martínez-

- Gualda, R. Cano, L. Marzo, R. Pérez-Ruiz, J. Luis-Barrera, R. Mas-Ballesté, A. Fraile, V. A. de la Peña O'Shea, J. Alemán, *Nat. Commun.* **2019**, *10*, 2634.
- [6] B. Pieber, J. A. Malik, C. Cavedon, S. Gisbertz, A. Savateev, D. Cruz, T. Heil, G. Zhang, P. H. Seeberger, *Angew. Chem. Int. Ed.* **2019**, *58*, 9575-9580; *Angew. Chem.* **2019**, *131*, 9676-9681.
- [7] G. Zhang, G. Li, Z.-A. Lan, L. Lin, A. Savateev, T. Heil, S. Zafeiratos, X. Wang, M. Antonietti, *Angew. Chem. Int. Ed.* **2017**, *56*, 13445-13449; *Angew. Chem.* **2017**, *129*, 13630-13634.
- [8] a) P. Geng, Y. Tang, G. Pan, W. Wang, J. Hu, Y. Cai, *Green Chem.* **2019**, *21*, 6116-6122; b) W. Zhang, A. Bariotaki, I. Smonou, F. Hollmann, *Green Chem.* **2017**, *19*, 2096-2100.
- [9] W. J. Ong, L. L. Tan, Y. H. Ng, S. T. Yong, S. P. Chai, *Chem. Rev.* **2016**, *116*, 7159-7329.
- [10] a) Y. Shiraishi, S. Kanazawa, Y. Kofuji, H. Sakamoto, S. Ichikawa, S. Tanaka, T. Hirai, *Angew. Chem. Int. Ed.* **2014**, *53*, 13454-13459; *Angew. Chem.* **2014**, *126*, 13672-13677; b) Y. Shiraishi, S. Kanazawa, Y. Sugano, D. Tsukamoto, H. Sakamoto, S. Ichikawa, T. Hirai, *ACS Catal.* **2014**, *4*, 774-780; c) Z. Wei, M. Liu, Z. Zhang, W. Yao, H. Tan, Y. Zhu, *Energy Environ. Sci.* **2018**, *11*, 2581-2589; d) X. Zeng, Y. Liu, Y. Kang, Q. Li, Y. Xia, Y. Zhu, H. Hou, M. H. Uddin, T. R. Gengenbach, D. Xia, C. Sun, D. T. McCarthy, A. Deletic, J. Yu, X. Zhang, *ACS Catal.* **2020**, *10*, 3697-3706.
- [11] a) Y. Wang, D. Lan, R. Durrani, F. Hollmann, *Curr. Opin. Chem. Biol.* **2017**, *37*, 1-9; b) M. Faiza, S. Huang, D. Lan, Y. Wang, *BMC Evol. Biol.* **2019**, *19*, 76; c) M. Hofrichter, R. Ullrich, *Curr. Opin. Chem. Biol.* **2014**, *19*, 116-125; d) M. Hobisch, D. Holtmann, P. Gomez de Santos, M. Alcalde, F. Hollmann, S. Kara, *Biotechnol. Adv.* **2020**, 107615.
- [12] a) P. Molina-Espeja, E. Garcia-Ruiz, D. Gonzalez-Perez, R. Ullrich, M. Hofrichter, M. Alcalde, *Appl. Environ. Microbiol.* **2014**, *80*, 3496-3507; b) P. Molina-Espeja, S. Ma, D. M. Mate, R. Ludwig, M. Alcalde, *Enzyme Microb. Technol.* **2015**, *73-74*, 29-33.
- [13] a) W. Zhang, B. O. Burek, E. Fernandez-Fueyo, M. Alcalde, J. Z. Bloh, F. Hollmann, *Angew. Chem. Int. Ed.* **2017**, *56*, 15451-15455; *Angew. Chem.* **2017**, *129*, 15654-15658; b) L. Schermund, V. Jurkaš, F. F. Özgen, G. D. Barone, H. C. Büchenschütz, C. K. Winkler, S. Schmidt, R. Kourist, W. Kroutil, *ACS Catal.* **2019**, *9*, 4115-4144; c) B. O. Burek, S. Bormann, F. Hollmann, J. Z. Bloh, D. Holtmann, *Green Chem.* **2019**, *21*, 3232-3249; d) S. J. P. Willot, E. Fernández-Fueyo, F. Tieves, M. Pesic, M. Alcalde, I. W. C. E. Arends, C. B. Park, F. Hollmann, *ACS Catal.* **2019**, *9*, 890-894; e) F. Tieves, S. J.-P. Willot, M. M. C. H. van Schie, M. C. R. Rauch, S. H. H. Younes, W. Zhang, J. Dong, P. Gomez de Santos, J. M. Robbins, B. Bommarius, M. Alcalde, A. S. Bommarius, F. Hollmann, *Angew. Chem. Int. Ed.* **2019**, *58*, 7873-7877; *Angew. Chem.* **2019**, *131*, 7955-7959; f) Y. Ni, E. Fernández-Fueyo, A. G. Baraibar, R. Ullrich, M. Hofrichter, H. Yanase, M. Alcalde, W. J. H. van Berkel, F. Hollmann, *Angew. Chem. Int. Ed.* **2016**, *55*, 798-801; *Angew. Chem.* **2016**, *128*, 809-812; g) D. S. Choi, Y. Ni, E. Fernández-Fueyo, M. Lee, F. Hollmann, C. B. Park, *ACS Catal.* **2017**, *7*, 1563-1567; h) E. Churakova, M. Kluge, R. Ullrich, I. Arends, M. Hofrichter, F. Hollmann, *Angew. Chem. Int. Ed.* **2011**, *50*, 10716-10719; *Angew. Chem.* **2011**, *123*, 10904-10907; i) M. Hobisch, M. M. C. H. van Schie, J. Kim, K. R. Andersen, M. Alcalde, R. Kourist, C. B. Park, F. Hollmann, S. Kara, *ChemCatChem* **2020**, *12*, 4009-4013; j) J. Yoon, J. Kim, F. Tieves, W. Zhang, M. Alcalde, F. Hollmann, C. B. Park, *ACS Catal.* **2020**, 5236-5242; k) W. Zhang, E. Fernandez-Fueyo, Y. Ni, M. van Schie, J. Gacs, R. Renirie, R. Wever, F. G. Mutti, D. Rother, M. Alcalde, F. Hollmann, *Nat. Catal.* **2018**, *1*, 55-62.
- [14] a) S. Bormann, A. Gomez Baraibar, Y. Ni, D. Holtmann, F. Hollmann, *Catal. Sci. Technol.* **2015**, *5*, 2038-2052; b) B. O. Burek, S. R. d. Boer, F. Tieves, W. Zhang, M. van Schie, S. Bormann, M. Alcalde, D. Holtmann, F. Hollmann, D. W. Bahnemann, J. Z. Bloh, *ChemCatChem* **2019**, *11*, 3093-3100.
- [15] a) H. E. Bonfield, J. D. Williams, W. X. Ooi, S. G. Leach, W. J. Kerr, L. J. Edwards, *ChemPhotoChem* **2018**, *2*, 938-944; b) H. E. Bonfield, K. Mercer, A. Diaz-Rodriguez, G. C. Cook, B. S. J. McKay, P. Slade, G. M. Taylor, W. X. Ooi, J. D. Williams, J. P. M. Roberts, J. A. Murphy, L. Schermund, W. Kroutil, T. Mielke, J. Cartwright, G. Grogan, L. J. Edwards, *ChemPhotoChem* **2020**, *4*, 45-51.
- [16] K. Edegger, C. C. Gruber, T. M. Poessl, S. R. Wallner, I. Lavandera, K. Faber, F. Niehaus, J. Eck, R. Oehrlin, A. Hafner, W. Kroutil, *Chem. Commun.* **2006**, 2402-2404.
- [17] W. Stampfer, B. Kosjek, C. Moitz, W. Kroutil, K. Faber, *Angew. Chem. Int. Ed.* **2002**, *41*, 1014-1017.
- [18] M. M. C. H. van Schie, W. Zhang, F. Tieves, D. S. Choi, C. B. Park, B. O. Burek, J. Z. Bloh, I. W. C. E. Arends, C. E. Paul, M. Alcalde, F. Hollmann, *ACS Catal.* **2019**, *9*, 7409-7417.