Design of a Pd(0)-CalB CLEA Biohybrid Catalyst and its Application in a One-Pot Cascade Reaction

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Supporting Information Placeholder

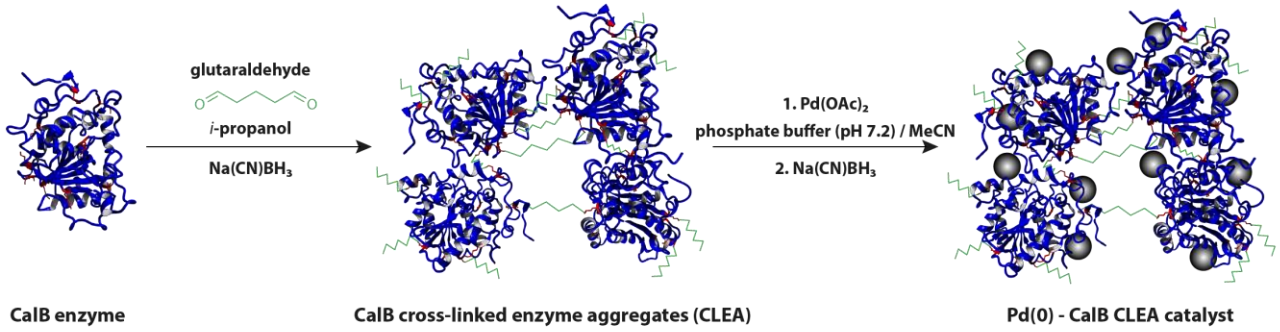
ABSTRACT: Herein, a rational design of a biohybrid catalyst is described, consisting of Pd nanoparticles and a cross-linked network of aggregated lipase B enzyme of *Candida antarctica* (CalB CLEA) functioning as an active support for the Pd nanoparticles. Both entities of the hybrid catalyst showed good catalytic activity. The applicability was demonstrated in a one-pot reaction where Pd-catalyzed cycloisomerization of 4-pentynoic acid afforded a lactone that serves as acyl donor in a subsequent selective enzymatic kinetic resolution of a set of *sec*-alcohols. The catalyst proved to be robust and could be recycled five times without significant loss of activity.

Keywords: kinetic resolution, biohybrid catalyst, Pd nanoparticles, CalB, cycloisomerization, one-pot cascade reaction

Over the past few decades, the field of catalysis has been greatly propelled by advances in material and nanotechnology research, which have enabled the development of a variety of efficient and robust heterogeneous catalysts for a plethora of organic transformations. Today, basically any type of catalytic species, ranging from metal nanoparticles[1] and organometallic complexes[2] to organocatalysts[3] and enzymes,[4] can be conveniently immobilized onto heterogeneous supports. Here, well-elaborated synthetic protocols that allow for precise tailoring of the morphology and physical properties of the support materials have played a crucial role in the progress. To date, a great number of solid supports can be prepared with tunable pore sizes and surface functionalization, which include for example carbon-based materials,[5] metal-organic frameworks,[6] metal oxides,[7] polymers[8] and silicas.[9] For catalytic applications, the support material should ideally show high stability over a wide range of reaction conditions to enable reuse. In addition, the support should possess a large internal surface area to allow for high loadings of the catalytic species and grant protection from mechanical grinding.

Another, and often overlooked aspect of the support materials is their intrinsic reactivity. Generally, inert support materials are chosen to ensure that no unwanted side reactions occur, as the latter may reduce the yield of the desired product that is produced by the immobilized catalytic species. However, an alternative and more interesting approach, is to design synergistic cascade reactions that make use of the reactivity of both the immobilized catalytic species and the surface functionality of the support. For example, bifunctional catalysts involving nanometal species immobilized on acidic or basic supports have been successfully applied in a number of different redox-condensation reaction sequences.[10] Heterogeneous materials containing site-isolated acid and base functionalities have also attracted considerable interest as they enable cascade reactions that are challenging to perform through homogeneous chemistry.[11] Unfortunately, the number of different reactions that can be achieved with these types of acid/base-type supports are rather limited, and as a result they have mainly been used for cascades involving condensation reaction steps.

A promising approach to expand the chemistry of bifunctional catalysts has been explored by the groups of Zare[12] and Palomo[13], where enzymes were converted to heterogeneous matrices upon treatment with metal salts. Interestingly, the enzymes within these bioinorganic hybrids were all found to retain their catalytic activity, and in the latter study the group of Palomo impressively managed to carry out a number of reactions where the reactivity of both the *Candida antartica* lipase B (CalB) and the entrapped Pd nanoparticles were utilized.[13] The metal salt-based enzyme heterogenization technique used in these studies is reminiscent of the cross-linked enzyme aggregate (CLEA) methodology, where insoluble and catalytically-competent aggregates of enzymes are generated by the use of cross-linking agents, such as glutaraldehyde.[14] However, unlike the more recent and more scarce number of methods employing metal salts for the cross-linking of enzym-



Scheme 1. Synthesis of Pd(0)-CalB CLEA heterogeneous catalyst.

es, the CLEA methodology with glutaraldehyde is well-established and has been successfully used to produce a wide range of heterogeneous enzyme materials in large quantities suitable for industrial-scale applications.[14c] A key advantage of cross-linking enzymes into aggregates compared to immobilizing them onto a support is that one avoids a reduction of the catalytic activity per mass unit of the catalytic material, which is often the case for supported biocatalysts where typically 90–99% of the mass is not catalytically productive.[14c] Moreover, as the enzyme itself constitutes the heterogeneous phase, the CLEA is by nature biodegradable and green. A possible drawback of the CLEA methodology compared to immobilization on a solid support is that enzyme stabilization is less profound. Moreover, there may be diffusion problems associated with CLEA.[15]

To improve further on the applicability and scalability of the synthesis of metal-enzyme composites, we envisioned to create a bifunctional hybrid catalyst by combining these two abovementioned strategies. The idea was to conduct glutaraldehyde-assisted cross-linking of the enzyme and make use of the formed heterogeneous matrix as a support for metal species. Our group has previously reported on the synthesis of a Pd-CalB hybrid catalyst,[16] in which Pd nanoparticles were first supported onto a siliceous mesocellular foam[17] and then CalB was subsequently immobilized onto the support using glutaraldehyde as linker.[18] In contrast to the previous protocol, the proposed CLEA-based method would provide more expedient access to a Pd-CalB hybrid catalyst although there would be less control of the Pd immobilization compared to the previous method. An advantage with the CLEA method is that it would not require the use of any support material. Herein, we report on the synthesis of a novel Pd(0)-CalB CLEA catalyst, and its application in a cycloisomerization/kinetic resolution reaction cascade that furnishes enantiomerically enriched 1-phenylethyl-4-oxopentaoate derivatives.

For the method of generating the CLEA from CalB, we chose to follow a protocol reported by Sheldon and coworkers (see the Supporting Information).[19] In our modified protocol, a commercial CalB solution was used as the enzyme source and the cross-linking was conducted with glutaraldehyde and $Na(CN)BH_3$ in isopropanol. The CalB CLEA was separated by centrifugation and subsequently washed with neutral phosphate buffer to remove excess reagents. In the next step, the CalB CLEA was re-suspended in a mixture of phosphate buffer (pH 7.2) and MeCN, and was treated with $Pd(OAc)_2$. After stirring for 16 h at room temperature, the Pd(II)-CalB CLEA was separated by centrifugation and the non-bound Pd(II)-species was washed away with phosphate buffer using centrifugation technique. To reduce the entrapped Pd(II)-species to Pd(0) nanoparticles, the Pd(II)-CalB CLEA was subjected to $Na(CN)BH_3$ in phosphate buffer for 30 min at room temperature (Scheme 1).[20]

Elemental analysis (ICP-OES) of the hybrid Pd(0)-CalB CLEA catalyst determined the palladium content to 4.7 wt%. The morphology of the Pd(0)-CalB CLEA catalyst was investigated and compared to the non-Pd-functionalized CalB CLEA using scanning electron microscopy (SEM) (Figure 1a,b). In comparison to the non-functionalized CalB CLEA, which has a well-defined spherical morphology (Figure 1A) (first reported by the group of Sheldon[19]), the Pd-functionalized CalB CLEA has lost the ordered spherical structure and adopted a relatively more amorphous morphology (Figure 1B). This observation suggests that the Pd nanoparticles are incorporated and bound within the CLEA matrix. Interestingly, this morphological change did not lead to any significant lowering of the enzymatic activity (see Table S1). To gain further support for the incorporation of the Pd nanoparticles within the CLEA matrix and visualize their structure, the catalyst was analyzed by high-angle annular dark-field scanning transmission electron microscopy (HAADF-STEM) (Figure 1C,D). The STEM images revealed a well-dispersed pattern of Pd nanoparticles with a particle size of 2–4 nm within the cross-linked enzyme structure (see Figure S1 in the Supporting Information).

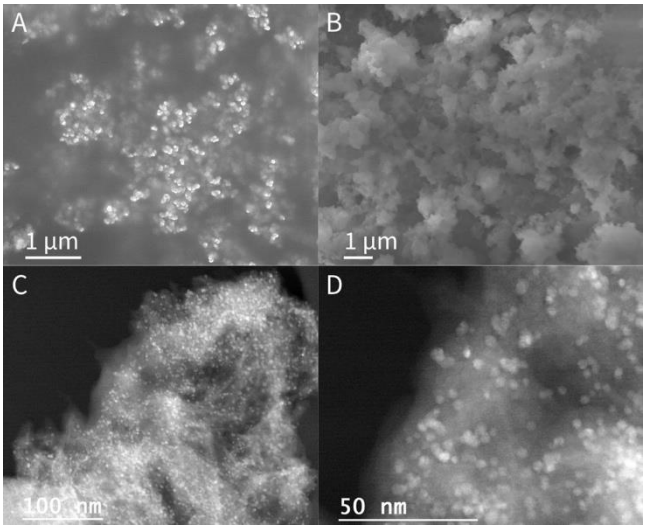
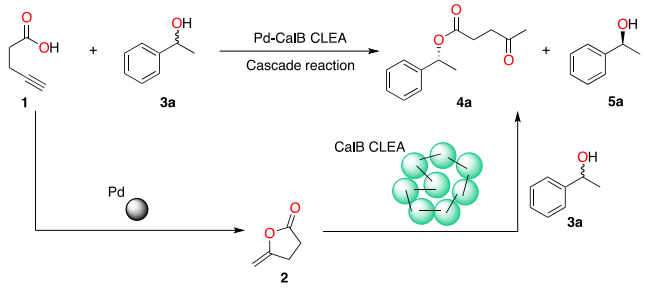


Figure 1. SEM images of the CalB CLEA catalyst (A) and the functionalized Pd(0)-CalB CLEA catalyst (B). HAADF-STEM images of the Pd(0)-CalB CLEA catalyst with 100 nm scale bar (C) and 50 nm scale bar (D).

To demonstrate the power of this new type of bifunctional CLEA catalyst, a one-pot, two-step cascade reaction was chosen as the model transformation for our catalytic studies (Scheme 2). In the first step of the cascade, 4-pentynoic acid (**1**) undergoes a Pd-catalyzed cycloisomerization to 5-methylenedihydrofuran-2(3*H*)-one (**2**), which in the next step can serve as an acyl donor for a

CalB-catalyzed kinetic resolution of 1-phenylethanol (**3a**) to produce (*R*)-1-phenylethyl 4-oxopentaoate (**4a**) as the final product. The cycloisomerization of various alkynoic acid derivatives has been extensively studied by our group, and we have demonstrated that there are several different heterogeneous Au and Pd nanocatalysts that can be used to efficiently catalyze this reaction.[21] Based on these previous results, we were confident that the Pd nanoparticles entrapped in the Pd(0)-CalB CLEA would be able to catalyze this reaction as well. The kinetic resolution (KR) of *sec*-alcohols with CalB is also a well-established transformation in the literature;[22] however, to the best of our knowledge there exist no previous examples where lactones have been used as acyl donors. In this respect, our proposed cascade holds great synthetic value as it allows for an unprecedented and expedient route to enantiomerically pure 1-phenylethyl 4-oxopentaoate derivatives through KR from simple starting materials. This strategy was inspired by work done by Akai and Kita,[23] in which acyl donors were used that enabled further functionalizations. In their study, unsaturated ethoxyvinyl esters were employed as acyl donors in the dynamic kinetic resolution (DKR) of 3-vinylcyclohex-2-en-1-ols, which allowed for subsequent intramolecular Diels-Alder reactions.[20]

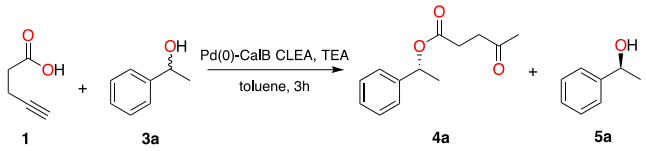


Scheme 2. The envisioned one-pot reaction catalyzed by Pd(0)-CalB CLEA catalyst.

To our delight, we found that the developed Pd(0)-CalB CLEA was a highly efficient and selective bifunctional catalyst for this cascade transformation, and we thus commenced investigating the impact of a number of parameters on the reaction yield (Table 1). In order to clearly assess the effects when changing the different reaction parameters, the reaction was sampled already after 3 h. In our previous studies of nanometal-catalyzed cycloisomerizations,[18] trimethylamine (TEA) was found to be the ideal choice of base, so instead of a comprehensive base screening, only variations in the TEA equivalents were explored. Toluene was selected as the solvent as it has been shown previously to exhibit excellent compatibility with lipases, allowing for both high enzyme activity and enantioselectivity.

First, the loading of the catalyst was investigated when running the reaction in 0.5 mL dry toluene with 0.1 equiv of TEA and 2 equiv of **1** with respect to **3a** at 60 °C. With 5 mg catalyst, a good conversion of 46 % was obtained (Table 1, entry 1), while a 2.5 mg catalyst loading only gave 31% conversion (Table 1, entry 2). The performance of the reaction could be further improved by increasing the concentration, and with 5 mg Pd(0)-CalB CLEA in 0.1 mL toluene 50% conversion was achieved (Table 1, entry 3), which is the theoretical maximum result for a KR reaction. To our delight, the conversion remained at respectable 47% with 2.5 mg catalyst under the same conditions (Table 1. entry 4). Next, we tried to lower the loading of **1**, and it was found that the reaction also worked excellently when using only 1 equiv of **1** (Table 1, entry 6), while the use of 0.5 equiv dramatically lowered the conversion (Table 1, entry 5). Interestingly, the loading of base could also be reduced with only a negligible loss in conversion (Table 1, entry 7), which is beneficial from a catalyst recycling perspective as TEA could potentially promote leaching of Pd from the catalyst. On the other hand, a temperature of 60 °C was found to be necessary for an efficient reaction and 40 °C and 50 °C led to markedly lower conversions (Table 1, entries 8 and 9). In conclusion, the best result was obtained by performing the reaction in dry toluene at 60 ºC with 1.0 equiv of 4-pentynoic acid (**1**), 0.75 equiv of Et_3N and 2.5 mg of Pd(0)-CalB CLEA for 3 h. Fortunately, this reaction setup proved to be robust and could be scaled up ten times (Table 1. entry 10). In all cases, the *ee* of the product **4** was found to be excellent (>99 %).

Table 1. Optimization of the reaction conditions.

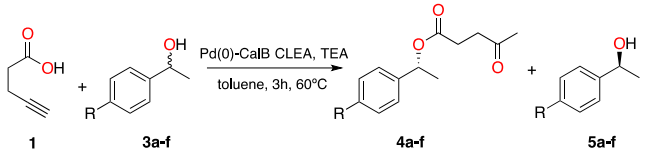


entry	1 [mmol]	3a [mmol]	Cat. [mg]	TEA [mmol]	T [°C]	conv. (%)
1[a]	0.2	0.1	5.0	0.1	60	46
2[a]	0.2	0.1	2.5	0.1	60	31
3[b]	0.2	0.1	5.0	0.1	60	50
4[b]	0.2	0.1	2.5	0.1	60	47
5[b]	0.05	0.1	2.5	0.1	60	29
6[b]	0.1	0.1	2.5	0.1	60	49
7[b]	0.1	0.1	2.5	0.075	60	48
8[b]	0.1	0.1	2.5	0.075	40	15
9[b]	0.1	0.1	2.5	0.075	50	41
10[c]	1.0	1.0	25	0.75	60	50

[a]Conditions: Pd(0)-CalB CLEA and substrate 1 were dispersed in 0.5 mL toluene ([b]0.1 mL toluene, [c]0.75 mL toluene), substrate **3a** and TEA were added and the reaction was sealed and stirred at the indicated temperatures. The conversion was determined by 1H-NMR with 1,3,5-trimethoxybenzene as internal standard. The *ee* was 99 % in every reaction as determined by chiral GC or HPLC.

The catalytic studies of the Pd(0)-CalB CLEA catalyst proceeded with a brief survey of different 1-phenylethanol derivatives bearing substituents of various electronic and steric properties (Table 2). 1-(4-Methylphenyl)ethanol (**3b**) gave comparable results to that of **3a**, affording 46% isolated yield after 3 h (Table 2, entry b). Slightly lower isolated yields were observed for the reaction sequence involving 1-(4-methoxyphenyl)ethanol (**3c**), which gave 42% yield of **4c** (Table 2, entry c). On the other hand, the Pd(0)-CalB CLEA did not seem to be negatively affected by substrates containing bulkier substituents as the reaction with 1-(naphthalene-2-yl)ethanol (**3d**) resulted in 45% isolated yield of **4d** (Table 2, entry d). The reactions with substrates containing electron-withdrawing groups, such as 1-(4-chlorophenyl)ethanol (**3e**) and 1-(4-nitrophenyl)ethanol (**3f**) were found to work satisfactory as well, giving 43% and 45% yield, respectively (Table 2, entries e and f). In the 1-(4-nitrophenyl)ethanol (**3f**) reaction the corresponding (*S*)-1-(4-nitrophenyl)ethan-1-ol (>99% ee) was isolated in 44 % yield. Also, for the reactions described in Table 2, the catalyst displayed excellent enantioselectivity and allowed the products to be obtained in >99% *ee* in all cases.

Table 2. Substrate scope of substituted 1-phenylethanol compounds.[a]



entry	R-	1 [mmol]	3a-f [mmol]	Cat. [mg]	isol. yield (%)	ee_P (%)
a	H	1.0	1.0	25	47	99
b	CH_3	1.0	1.0	25	46	99
c	OMe	1.0	1.0	25	42	99
d	naph.	1.0	1.0	25	45	99
e	Cl	1.0	1.0	25	43	99
f	NO_2	1.0	1.0	25	45	99

[a]Conditions: Pd(0)-CalB CLEA and substrate 1 were dispersed in 0.75 mL toluene, substrate 3a-f and TEA (0.75 equiv.) were added and the reaction was sealed and stirred at 60 °C. The *ee* was determined by chiral GC and HPLC.

To demonstrate the recyclability of the Pd(0)-CalB CLEA catalyst, the reaction with the model substrate **3a** was carried out over six repetitive cycles (Figure 2). After 3 h, the reaction mixture was separated and the catalyst was washed twice with 1 mL of dry toluene using centrifugation. To our delight, the conversion was found to be essentially the same over all cycles within the margins of error (40% and 45% in all runs). In all cases, perfect optical purity for the (*R*)-1-phenylethyl 4-oxopentanoate (**4a**) was achieved. It is gratifying that the amount of Pd in the reaction mixture was found to be less than 1 ppm, demonstrating the highly efficient trapping of the metal nanoparticles within the CLEA structure. Moreover, according to the SEM images the morphology of the recycled catalyst was identical to that of the fresh Pd(0)-CalB CLEA catalyst.

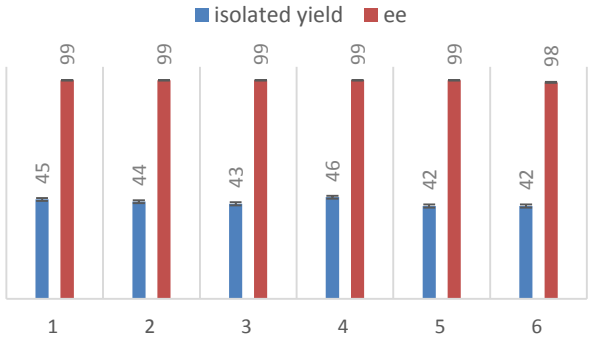


Figure 2. Recyclability of the Pd(0)-CalB CLEA catalyst.

In conclusion we have rationally designed a support-free enzyme-palladium hybrid catalyst. The capability of the Pd(0)-CalB CLEA catalyst was demonstrated by a one-pot reaction, where 4-pentynoic acid (**1**) was cyclized to 5-methylenedihydrofuran-2(3*H*)-one (**2**) and subsequently used as a lactone-based acyl donor with *Candida antarctica* lipase B to kinetically resolve differently substituted 1-phenylethanol compounds (**3a–f**) to (*R*)-1-phenylethyl 4-oxopentaoate derivatives (**4a–f**). The hybrid catalyst showed good recyclability with less than 1 ppm Pd leaching, demonstrating its high stability under the employed reaction conditions.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI:

Experimental procedures and compound characterization data for all new compounds (PDF)

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Notes

The authors declare no competing financial interests.

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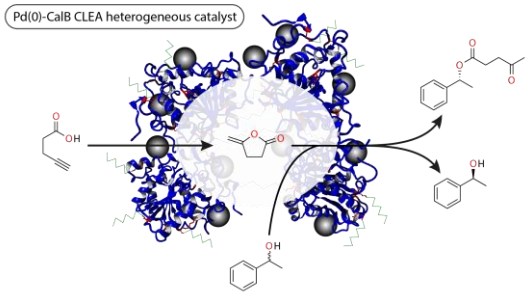
Pd(0)-CalB CLEA heterogeneous catalyst