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A palladium-catalyst stabilized in the chiral environment of a monoclonal antibody in water†

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We report the first preparation of a monoclonal antibody (mAb) that can immobilize a palladium (Pd)-complex. The allylic amination reaction using a supramolecular catalyst consisting of the Pd-complex and mAb selectively gives the (*R*)-enantiomer product with an enantiomeric excess (ee) of 98 ± 2%. This is in sharp contrast to the reaction catalyzed by a conventional Pd-catalyst (ee < 2%).

Palladium (Pd)-complexes have attracted much attention because they promote various cross-coupling reactions such as Negishi coupling,[1–3] Suzuki–Miyaura cross-coupling,[4–6] and the Mizoroki–Heck reaction.[7–10] Recent efforts have focused on controlling the synthesis products and increasing the catalytic activity due to the importance of chirality for living systems. For structural control of a product, both the first coordination sphere, which is formed by metal ions and ligands, and the second coordination sphere must be appropriately constructed. Synthesizing a ligand with the desired structure can realize the desired first coordination sphere. However, the second coordination sphere is composed by multiple non-covalent interactions such as hydrogen bonding, hydrophobic effects, and electro-static interactions. Hence, it is difficult to produce the desired second coordination sphere with a low molecular weight system.

To this end, hybrid catalysts with various combinations of Pd-complexes and biomolecules,[11] such as ferritin,[12] lipase,[13] and biotin-streptavidin,[14,15] have been developed to obtain structure-controlled products. A Pd-complex anchored in a biomolecule provides asymmetric environmental fields in the second coordination sphere. In this case, the Pd-complex is incorporated into biomacromolecules by employing a non-direct method. One example is the utilization of avidin–biotin interactions during the complex formation of avidin with a biotinylated Pd-complex.[14,15] Because the space originally possessed by the biomacromolecules is used as the second coordination sphere of the Pd-complex, it is not suitable for the Pd-complex. If biomacromolecules can directly recognize Pd-complexes, complexation of the Pd-complex and biomacromolecules can construct an appropriate second coordination sphere, realizing a novel platform for a hybrid catalyst of the Pd-complex and biomacromolecules.

Our work has focused on monoclonal antibodies (mAbs) because the binding site of mAbs can be tailored to the antigen specifications.[16–29] This intriguing feature allows asymmetric environmental fields to be constructed as the second coordination sphere for transition metal complexes, enabling a stereospecific reaction.[30,31] However, to the best of our knowledge, a hybrid catalyst consisting of a Pd-complex and a mAb is yet to be reported because typical Pd-complexes are unstable in water and degrade during immunization. Currently, there is only one report on the complexation of a Pd-complex of a porphyrin.[32] This may be due to the difficulty in preparing mAbs capable of recognizing Pd-complexes.

In this study, we describe our efforts to create a supramolecular catalyst using a mAb to immobilize an unstable Pd-complex (**1**) (Fig. 1 and Fig. S1–S3, Scheme S1, ESI†) for the allylic amination reaction. Because **1** degrades during immunization, a mAb capable of recognizing **1** is prepared by the cross-reactivity of mAb binding to the rhodium (Rh)-complex (**2**) (Fig. 1 and Fig. S4, S5, Scheme S2, ESI†) with the same ligand as **1**. A supramolecular catalyst consisting of **1** and mAb catalyzes the allylic amination reaction, which selectively affords an (*R*)-enantiomer product.

We chose a η^3-allyl palladium(II) complex as a late transition metal complex because it promotes various coupling reactions such as allylic amination reactions.[33] To obtain mAbs capable of recognizing **1**, we prepared antigens in which **1** was modified to keyhole limpet hemocyanin (KLH) (**1**-KLH, Scheme S3 and Table S1, ESI†) for immunization and immunized **1**-KLH in saline emulsified 1 : 1 in Freund's complete adjuvant for Balb/c mice four times at two-week intervals. To investigate antibody

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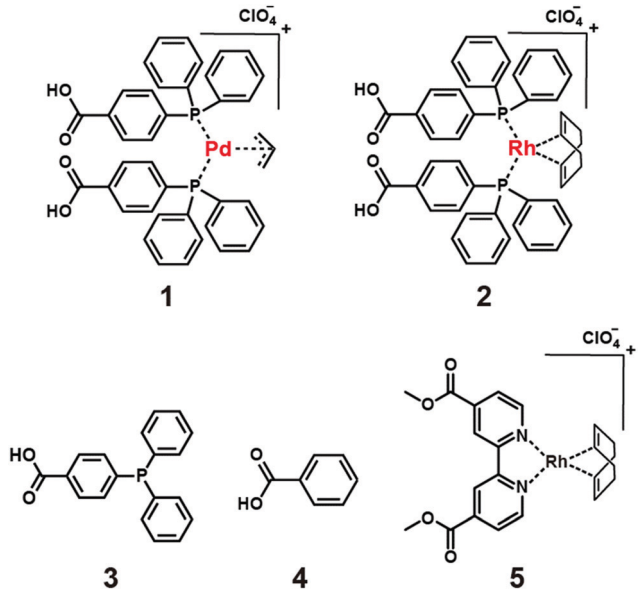


Fig. 1 Chemical structures of **1**–**5**.

production, **1** was introduced into bovine serum albumin (BSA) to obtain antigens for assays (**1**-BSA), and then enzyme-linked immunosorbent assay (ELISA) measurements of the blood from immunized and non-immunized mice using a **1**-BSA coated plate were performed. However, there were no differences in the absorbance ascribable to the enzymatic reaction product in ELISA for the immunized and non-immunized mice, showing that no antibodies for **1** have been produced (Fig. 2a). This is most probably due to the low stability of the Pd-complex in water. In fact, the proton nuclear magnetic resonance (^{1}H NMR) spectrum of **1** was changed by immersing it in *N*,*N*-dimethylformamide-d_7/deuterium oxide for 7 days, showing decomposition of **1**. (Fig. S6, ESI†). The **1** was decomposed during immunization, which could not allow for creation of antibodies.

To overcome this problem, we employed cross-reactivity of antibodies. Because Rh-complexes are stable in water, we immunized **2** with the same ligand as **1**. The **2** was prepared from the chloro(1,5-cyclooctadiene)rhodium(I) dimer. The **2** was introduced to the proteins, such as KLH and BSA, in 0.1 M PBS buffer (pH 7.0) to obtain the antigens for immunization and

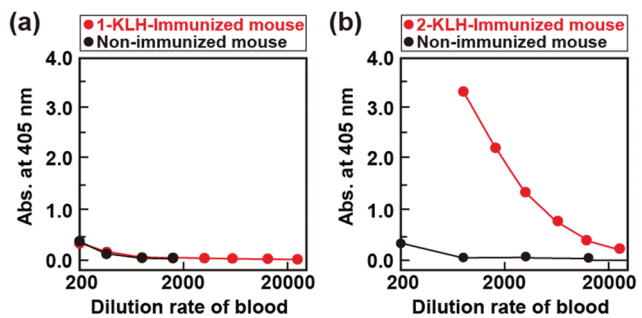


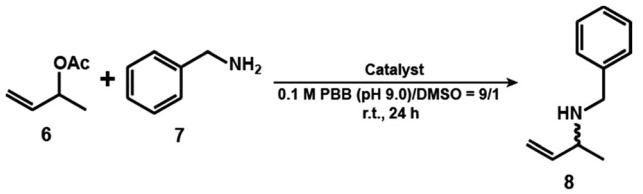
Fig. 2 Results of ELISA for the blood obtained from the mouse immunized with transition-metal complex modified KLH (red) and the non-immunized mouse (black) [(a) **1**-KLH and (b) **2**-KLH].

assays, respectively, (**2**-KLH and **2**-BSA) (Scheme S3, ESI†). The modification ratio was estimated by using the 2,4,6-trinitrobenzenesulfonic acid (TNBS) method,[34–36] and over six hundred or eight of **2** were introduced into one KLH or BSA, respectively (Table S1, ESI†). Balb/c mice were immunized with **2**-KLH in saline emulsified 1 : 1 in Freund's complete adjuvant four times at two-week intervals. To confirm antibody production, we performed ELISA measurement of the blood from immunized and non-immunized mice using a **2**-BSA coated plate. In contrast to **1**, the absorbance ascribable to the enzymatic reaction product in ELISA for an immunized mouse was higher than that of an non-immunized mouse (Fig. 2b). Moreover, in the case of an immunized mouse, the absorbance of **2**-BSA was higher than that of BSA (Fig. S7, ESI†). These results clearly show the preparation of antibodies for **2**. The hybridomas secreting antibodies specific to **2** were cloned twice by the limiting dilution method and then the obtained mAbs were purified from the ascites fluid by affinity chromatography using a HiTrap IgM Purification HP column (GE healthcare). The class of mAbs was determined using the Iso Strip Mouse Monoclonal Antibody Isotyping Kit. As a result, the obtained mAb was immunoglobulin M (IgM), and the light chain was the kappa chain.

To quantitatively investigate the affinity of the mAb to **1**, we determined the dissociation constant (K_d) of the complex between the mAb and **1** or **2**. The K_d values of the complexes between the mAb and **1** or **2** were found to be 2.0 or 7.6 $\times$ 10^{-5} M, respectively (Fig. S8a and b, ESI†), showing that the affinities of mAb to **1** and that of mAb to **2** were almost the same. To understand this reason, the binding form of mAb to **2** was estimated using K_d of the complex between the mAb and the ligand of **2** (**3**), benzoic acid (**4**), or the Rh-complex (**5**) whose structure is different from **2** (Fig. 1). The mAb showed no affinity to **4** and **5** ($>1.5 \times 10^{-2}$ and $>1.0 \times 10^{-3}$ M, respectively), whereas the mAb showed affinity to **3** (3.4×10^{-4} M) (Fig. S8c and S9, ESI†). This result indicates that the mAb recognizes the molecular elements of **3** in complex **2**. The ligand structure of **2** and that of **1** were the same, and thus the mAb can form complexes with **1**. We prepared for the first time the mAb for a Pd-complex using the cross-reactivity of the mAb.

A reaction in a nanoconfined geometry often gives products whose symmetries differ from those obtained in a solution reaction. We carried out an allylic amination reaction using **1** in the absence and presence of the mAb (Table 1). Firstly, the reaction concentrations of **1** and mAb were optimized. 3-Buten-2-yl acetate (**6**) (10 mM) and benzyl amine (**7**) (20 mM) were reacted with **1** (1 μM) and the mAb (0.1 μM, binding site: 1 μM) in a mixed solution of 0.1 M phosphate borate buffer (PBB; pH 9.0) and DMSO (9 : 1) at r.t. for 24 h (Fig. S10 and Scheme S4, ESI†). Under these conditions, the binding site of the mAb and **1** is the same. The yield and ee of the product (**8**) were determined by comparing the area ratio of **8** and allylbenzene as an internal standard in HPLC analysis (Fig. S11, ESI†) and calculated from the peak area ratio of (*R*)- and (*S*)-enantiomers of **8** of HPLC spectra (Fig. S12, ESI†). The yield and ee of **8** decreased with increasing concentration of **1** and mAb. This result suggests that the aggregation of IgM causes **1** to be

Table 1 Results of the 1-catalyzed allylic amination reaction



Entry	Catalyst	Conc. of Pd (μM)/mAb (μM)	Yield of **8**[a] (%)	ee[b] (%)
1	**1**	1/0	38	<2
2	**1** + mAb	1/0.1[c]	6	98 ± 2
3	**1** + BSA	1/0.1	11	<2

[a] Calculated from the peak area ratio of HPLC spectra using allylbenzene as an internal standard. [b] Calculated from the peak area ratio of (*R*)- and (*S*)-enantiomers of **8** of HPLC spectra. [c] Since the mAb is IgM with 10 binding sites, the concentrations of **1** and the binding site of mAb are equivalent.

adsorbed at locations other than where the product structure is controlled. The optimum reaction concentration of **1** and mAb was found to be 1 and 0.1 μM, respectively. The yield of **8** in the presence of the mAb (6%) was lower than that in the absence of the mAbs (38%) (Table 1, entries 1 and 2). Owing to the complex formation between **1** and mAb, it is difficult for the substrates such as **6** and **7** to access **1**. The decline in the yield of **8** was also observed in the presence of BSA (11%) (Table 1, entry 3), suggesting that the presence of protein reduces the catalytic activity of **1**. In the case of the absence of mAb, **1** gave racemic **8** (ee < 2%) (Table 1, entry 1 and Fig. S12a, ESI†). In contrast, interestingly, the reaction catalysed by **1** incorporated into the binding site of the mAb was found to proceed with excellent (*R*)-enantioselectivity with a 98 ± 2% ee (Table 1, entry 2 and Fig. S12b, ESI†). These results indicate that no catalytic reaction occurred outside the binding sites. Since the catalytic activity of free **1** is reduced by the presence of protein, the catalytic activity of free **1** would be negligible in the mAb system. This asymmetric allylic amination was not observed in the presence of BSA (ee < 2%) (Table 1, entry 3 and Fig. S12c, ESI†). These results indicate that the binding site of mAb functions as a reaction field for the asymmetric reaction.

In summary, we have demonstrated that Pd-complex catalysed allyl amination in the presence of mAb is a useful synthetic strategy to control the resultant product asymmetry. The substrate is converted to a racemic product by a Pd-catalyst without a mAb. In contrast, the (*R*)-enantiomer (98 ± 2% ee) is obtained in the presence of the complex between the mAb and the Pd-catalyst.

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Conflicts of interest

There are no conflicts to declare.

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