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COMMUNICATION

Palladium-Catalyst Stabilized in Chiral Environment of 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 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 of 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 a *non-direct* method.

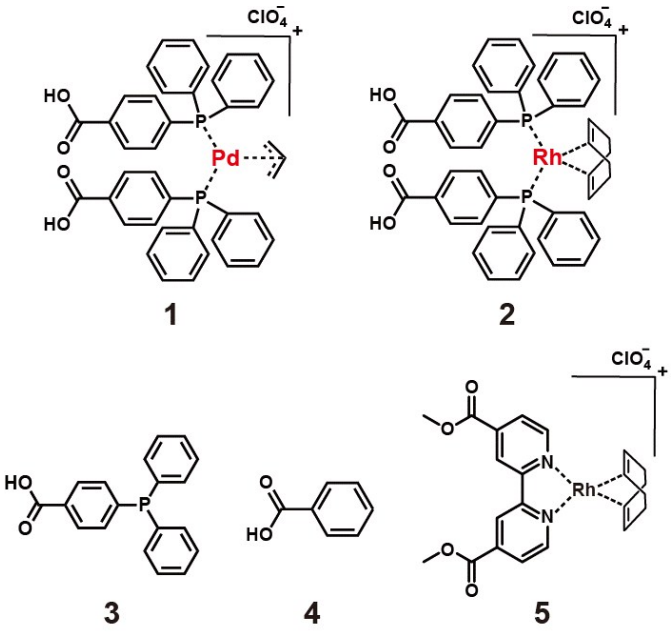

Fig. 1. Chemical structures of **1**–**5**.

One example is the utilization of avidin-biotin interactions during 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

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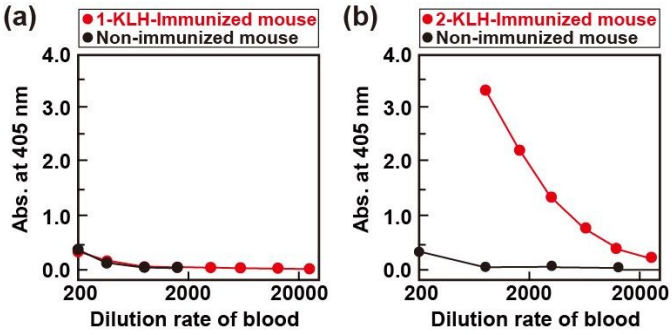


Fig. 2. Results of ELISA for blood obtained from the mouse immunized with transition-metal complex modified KLH (red) and non-immunized mouse (black) [(**a**) **1**-KLH and (**b**) **2**-KLH].

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 mAb has yet to be reported because typical Pd-complexes are unstable in water and degrade during immunization. Currently, there is only one report on complexation of a Pd-complex of a porphyrin.[32] This may be due to the difficulty preparing mAbs capable of recognizing Pd-complexes.

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

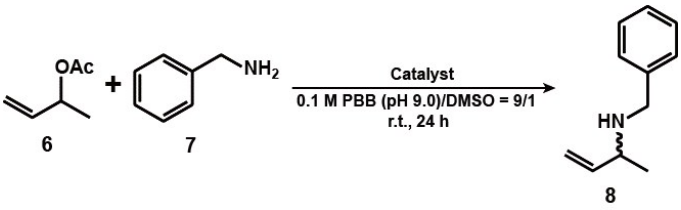
We chose a η^3-allyl palladium (II) complex as a late transition metal complex because they promote various coupling reactions such as allylic amination reaction.[33] To obtain mAbs capable of recognizing **1**, we prepared antigens in which **1** was modified to keyhole limpet hemocyanin (KLH) (**1**-KLH, Scheme S3 and Table S1) 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 interval. To investigate antibody 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 **1**-BSA coated plate were performed. However, there were no difference of the absorbance ascribable to the enzymatic reaction product in ELISA for the immunized and non-immunized mice, showing that no antibodies for **1** has been produced (Fig. 2a). This is most probably due to the low stability of Pd-complex in water. In fact, the proton nuclear magnetic resonance (^{1}H NMR) spectrum of **1** was changed by immersed in *N*,*N*-dimethylformamide-d_7/ deuterium oxide for 7 days, showing decomposition of **1**. (Fig. S6). The **1** was decomposed during immunization, which could not allow for creation of antibodies.

To overcome this problem, we employed cross-reactivity of antibody. Because Rh-complexes are stable in water, we immunized with **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 assay, respectively (**2**-KLH and **2**-BSA) (Scheme S3). The modification ratio was estimated by 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). Balb/c mice were immunized with **2**-KLH in saline emulsified 1:1 in Freund's complete adjuvant four times at two-week interval. To confirm antibodies production, we performed ELISA measurement of the blood from immunized and non-immunized mice using **2**-BSA coated plate. In contrast to **1**, the absorbance ascribable to the enzymatic reaction product in ELISA for immunized mouse was higher than that of non-immunized mouse (Fig. 2b). Moreover, in the case of immunized mouse, the absorbance of **2**-BSA was higher than that of BSA (Fig. S7). These results clearly show 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 HiTrap IgM Purification HP column (GE healthcare). The class of mAbs was determined with Iso Strip Mouse Monoclonal Antibody Isotyping Kit. As a result, the obtained mAb was Immunoglobulin M (IgM), and the light chain was kappa chain.

To quantitatively investigate the affinity of mAb to **1**, we determined the dissociation constant (K_d) of the complex between mAb and **1** or **2**. The K_d of the complexes between mAb and **1** or **2** were found to be 2.0 or 7.6×10^{-5} M, respectively (Figs. S8a and S8b), showing that the affinities of mAb to **1** and that of mAb to **2** were almost same. To understand this reason, the binding form of mAb to **2** was estimated using K_d of the complex between 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) (Figs. S8c and S9). This result indicates that the mAb recognizes the molecular elements of 3 in complex 2. The ligand structure of 2 and that of 1 was the same, thus the mAb can form complexes with **1**. We prepared for the first time the mAb for Pd-complex using the cross-reactivity of 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, 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 (Figure S10 and Schemes S4). Under this conditions, the binding site of mAb and **1** are equivalent. 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) and calculated from the peak area ratio of (*R*)- and (*S*)-enantiomers of **8** of HPLC spectra (Fig. S12). The yield and ee of **8** were decreased with

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, concentrations of **1** and the binding site of mAb are equivalent.

increasing of the concentration of **1** and mAb. These results suggest that the aggregation of IgM causes **1** to be 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). In contrast, interestingly, the reaction catalysed by **1** incorporated into the binding site of mAb was found to proceed with excellent (*R*)-enantioselectivity with a 98 ± 2% ee (Table 1, Entry 2 and and Fig. S12b). These result 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 and Fig. S12c). 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 Pd-catalyst without mAb. In contrast, the (*R*)-enantiomer (98 ± 2% ee) is obtained in the presence of the complex between mAb and Pd-catalyst.

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There are no conflicts to declare.

Notes and references

1. E. Negishi, Q. Hu, Z. H. Huang, M. X. Qian and G. W. Wang, *Aldrichim. Acta*, 2005, **38**, 71.
2. E. Negishi, G. W. Wang, H. H. Rao and Z. Q. Xu, *J. Org. Chem.*, 2010, **75**, 3151.
3. D. Haas, J. M. Hammann, R. Greiner and P. Knochel, *ACS Catal.*, 2016, **6**, 1540.
4. N. Miyaura and A. Suzuki, *Chem. Commun.*, 1979, 866.
5. N. Miyaura, K. Yamada and A. Suzuki, *Tetrahedron Lett.*, 1979, **20**, 3437.
6. N. Miyaura and A. Suzuki, *Chem. Rev.*, 1995, **95**, 2457.
7. T. Mizoroki, K. Mori and A. Ozaki, *Bull. Chem. Soc. Jpn.*, 1971, **44**, 581.
8. R. F. Heck and J. P. Nolley, *J. Org. Chem.*, 1972, **37**, 2320.
9. I. P. Beletskaya and A. V. Cheprakov, *Chem. Rev.*, 2000, **100**, 3009.
10. A. B. Dounay and L. E. Overman, *Chem. Rev.*, 2003, **103**, 2945.
11. F. Schwizer, Y. Okamoto, T. Heinisch, Y. F. Gu, M. M. Pellizzoni, V. Lebrun, R. Reuter, V. Kohler, J. C. Lewis and T. R. Ward, *Chem. Rev.*, 2018, **118**, 142.
12. S. Abe, J. Niemeyer, M. Abe, Y. Takezawa, T. Ueno, T. Hikage, G. Erker and Y. Watanabe, *J. Am. Chem. Soc.*, 2008, **130**, 10512.
13. M. Filice, O. Romero, A. Aires, J. M. Guisan, A. Rumbero and J. M. Palomo, *Adv. Synth. Catal.*, 2015, **357**, 2687.
14. J. Pierron, C. Malan, M. Creus, J. Gradinaru, I. Hafner, A. Ivanova, A. Sardo and T. R. Ward, *Angew. Chem. Int. Ed.*, 2008, **47**, 701.
15. A. Chatterjee, H. Mallin, J. Klehr, J. Vallapurackal, A. D. Finke, L. Vera, M. Marsh and T. R. Ward, *Chem. Sci.*, 2016, **7**, 673.
16. B. L. Iverson and R. A. Lerner, *Science*, 1989, **243**, 1184.
17. A. Harada, K. Okamoto, M. Kamachi, T. Honda and T. Miwatani, *Chem. Lett.*, 1990, **19**, 917.
18. A. G. Cochran and P. G. Schultz, *Science*, 1990, **249**, 781.
19. V. A. Roberts, B. L. Iverson, S. A. Iverson, S. J. Benkovic, R. A. Lerner, E. D. Getzoff and J. A. Tainer, *PNAS*, 1990, **87**, 6654.
20. R. A. Lerner, S. J. Benkovic and P. G. Schultz, *Science*, 1991, **252**, 659.
21. P. Ghosh, D. Shabat, S. Kumar, S. C. Sinha, F. Grynszpan, J. Li, L. Noodleman and E. Keinan, *Nature*, 1996, **382**, 339.
22. D. A. Blake, P. Chakrabarti, M. Khosraviani, F. M. Hatcher, C. M. Westhoff, P. Goebel, D. E. Wylie and R. C. Blake, *J. Biol. Chem.*, 1996, **271**, 27677.
23. A. Harada, H. Fukushima, K. Shiotsuki, H. Yamaguchi, F. Oka and M. Kamachi, *Inorg. Chem.*, 1997, **36**, 6099.
24. M. Khosraviani and R. C. Blake, *Bioconjugate Chem.*, 2000, **11**, 267.
25. K. M. Nicholas, P. Wentworth, C. W. Harwig, A. D. Wentworth, A. Shafton and K. D. Janda, *PNAS*, 2002, **99**, 2648.
26. R. Ricoux, H. Sauriat-Dorizon, E. Girgenti, D. Blanchard and J. P. Mahy, *J. Immunol. Methods*, 2002, **269**, 39.
27. H. Yamaguchi, K. Tsubouchi, K. Kawaguchi, E. Horita and A. Harada, *Chem. Eur. J.*, 2004, **10**, 6179.
28. R. C. Blake, A. R. Pavlov, M. Khosraviani, H. E. Ensley, G. E. Kiefer, H. Yu, X. Li and D. A. Blake, *Bioconjugate Chem.*, 2004, **15**, 1125.

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DOI: 10.1039/C9CC08756G

29. J. P. Mahy, J. D. Marechal and R. Ricoux, *Chem. Commun.*, 2015, **51**, 2476.
30. H. Yamaguchi, T. Hirano, H. Kiminami, D. Taura and A. Harada, *Org. Biomol. Chem.*, 2006, **4**, 3571.
31. T. Adachi, A. Harada and H. Yamaguchi, *Sci. Rep.*, 2019, **9**, 13551.
32. A. P. Savitsky, M. V. Demcheva, E. Y. Mantrova and G. V. Ponomarev, *FEBS Lett.*, 1994, **355**, 314.
33. M. Johannsen and K. A. Jorgensen, *Chem. Rev.*, 1998, **98**, 1689.
34. K. Satake, T. Okuyama, M. Ohashi and T. Shinoda, *J. Biochem.*, 1960, **47**, 654.
35. L. C. Mokrasch, *Anal. Biochem.*, 1967, **18**, 64.
36. S. L. Snyder and P. Z. Sobocinski, *Anal. Biochem.*, 1975, **64**, 284.