Electronic Supplementary Material (ESI) for ChemComm.

Supporting Information

Palladium-Catalyst Stabilized in Chiral Environment of Monoclonal Antibody in Water

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Materials

Keyhole limpet hemocyanin (KLH), Bovine serum albumin (BSA) was purchased from Medical & Biological Laboratories Co. Ltd. Hypoxanthine thymidine (HT), hypoxanthine aminopterin thymidine (HAT) supplement, Cell culture medium (RPMI 1640) and alkaline phosphatase labeled anti-mouse IgG were obtained from Sigma Aldrich Co. BCA protein assay reagents A and B, Freund's complete adjuvant (FCA) were purchased from Thermo Scientific Co. Ltd. Female Balb/c mice were purchased from Japan SLC, Inc. Poly (ethylene glycol) (PEG; Mw 1,500, PEG 50% w/v in 75 mM HEPES) was obtained from Roche Applied Science. Myeloma cell (SP2/0-Ag14) was purchased from RIKEN Bio Resource Center. Fetal bovine serum (FBS) was obtained from MP Biomedicals, LLC. 2,6,10,14-Tetramethylpentadecane (pristane) was purchased from Funakoshi Co., Ltd. OBSA was purchased from Medical & Biological Laboratories Co. Ltd. Alkaline phosphatase labeled anti-mouse IgG were obtained from Sigma Aldrich Co. Other reagents and solvents were used without further purification. η^3-Allyl-1,5-cyclooctadiene palladium(II) perchlorate[1] and bis(1,5-cyclooctadiene) rhodium(I) perchlorate[2] were prepared according to previously described methods. 0.1 M Phosphate borate buffer (PBB pH 9.0) was prepared by dissolving potassium dihydrogenphosphate (KH_2PO_4; 2.38 g, 17.5 mmol) and sodium tetraborate decahydrate ($Na_2B_4O_7 \cdot 10H_2O$; 15.8 g, 41.4 mmol) in H_2O (1.00 L).

Measurements

The ^1H NMR and ^{13}C NMR spectra were recorded with a JEOL JNM-ECA 500 NMR spectrometer. The absorption spectra were measured with a JASCO V-650 and a Shimadzu UV-2500 PC spectrometer at room temperature. Absorbance at 405 nm for Enzyme-linked immunosorbent assay (ELISA) was measured by an iMark microplate absorbance reader (Bio-Rad).

The HPLC analyses were carried out using JASCO HPLC system equipped with a HPLC pump (PU-2080), an UV-Vis detector (UV-2075), a CD detector (CD-4095), and a CHIRALCEL OD-H (0.46 cm × 5 cm) (Daicel Chemical Industrials, Ltd.) attached CHIRALCEL OD-H Guard Column (0.46 cm × 1 cm) (0.46 cm × 5 cm) (Daicel Chemical Industrials, Ltd.). Condition for HPLC analyses is hexane/2-propanol (100/2), 40°C, 0.6 mL/min, UV and CD detection at 220 nm.

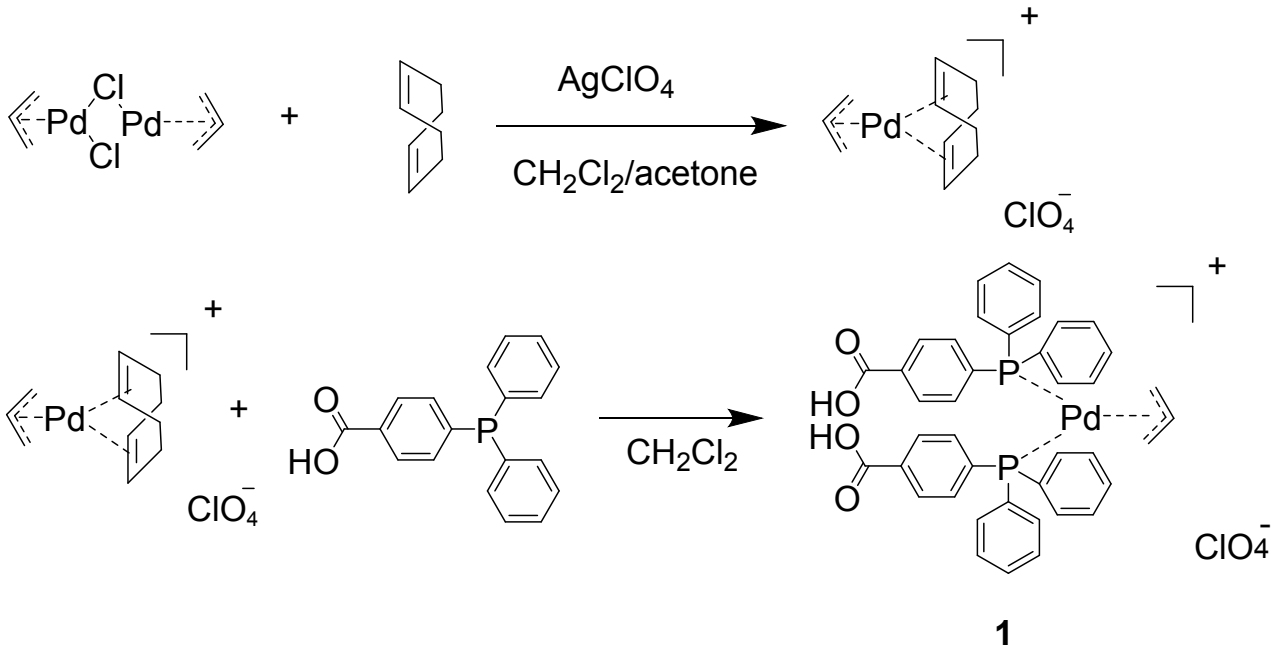
Enzyme-linked immunosorbent assay (ELISA)

Each well of 96 well microplates was coated with the compound-BSA solution (100 μL) and incubated at 4 °C for overnight. After the removal of the solutions, Blocking One (1:5 dilution) (180 μL) was added and the plates were incubated at 4 °C for overnight. After removal of the solutions, a drop of the culture supernatant was added to each well of the plates and the plates were incubated at 37 °C for 90 min. After removal of the solutions, the well was washed twice with 10 mM phosphate buffer saline (PBS) containing 20 mM NaN_3 and 10 mM of Tween 20 (washing buffer) (150 μL). Then alkaline phosphatase labeled anti-mouse IgG (1:1000 dilution) solution (100 μL) was added to each well and the plates were incubated at 37 °C for 90 min. After removal of the solutions, the well was washed with three times with washing buffer (150 μL). Then *p*-nitrophenyl phosphate sodium salt in substrate buffer (1.0 mg/mL) (150 μL) was added. The substrate buffer was prepared by dissolving 1 M di-ethanolamine, 3 mM sodium azide and 1 mM magnesium chloride in water and adjusting the pH to 9.8 by 10 hydrochloric acid solution. Absorbance at 405 nm derived from the product of the enzyme reaction was recorded.

Preparation of antigens

Preparation of η³-allyl-bis{4-(diphenylphosphino)benzoic acid} palladium(Ⅱ) perchlorate (1)

Scheme S1. Preparation of **1**.



1

4-(Diphenylphosphino)benzoic acid (0.13 g, 0.44 mmol) dissolved in CH_2Cl_2 (5.0 mL) was dropped into CH_2Cl_2 solution (5.0 mL) containing η³-allyl-1,5-cyclooctadiene palladium(II) perchlorate (75 mg, 0.21 mmol) and then the solution was stirred at room temperature for 5 h under an Ar atmosphere. The reaction solution was poured into excess diethyl ether (0.12 mL) and the suspension was centrifuged (4000 rpm, 3 min). The resultant precipitation was washed 3 times with diethyl ether and *n*-hexane, respectively, and dried in vacuo (Yield: 47%). ^{1}H NMR (500 MHz, CD_3CN, 25 °C): δ = 7.83 (m, 4H, -PC$_6$*H*$_4$COOH), 7.20–7.57 (m, 24H, *H* of Ph), 6.02 (m, 1H, C*H* of allyl), 4.10 (m, 2H, C*H*$_2$ of allyl), 3.52 (m, 2H, C*H*$_2$ of allyl). ^{13}C NMR (125 MHz, DMSO-d_6): δ = 166.5 136.8, 133.7, 133.0, 132.7, 131.4, 130.3, 129.3, 129.0, 124.2, 79.5, 27.5. ^{31}P NMR (500 MHz, CD_3CN, 25 °C): 23.4 MALDI-TOF-MS: Calcd. for ($C_{41}H_{35}O_8P_2ClPd$); 759; Found; 759 Elemental Anal: Calcd. for ($C_{41}H_{35}O_8P_2ClPd\cdot H_2O$); C, 56.18; H, 4.14; Found; C, 56.30; H, 4.04.

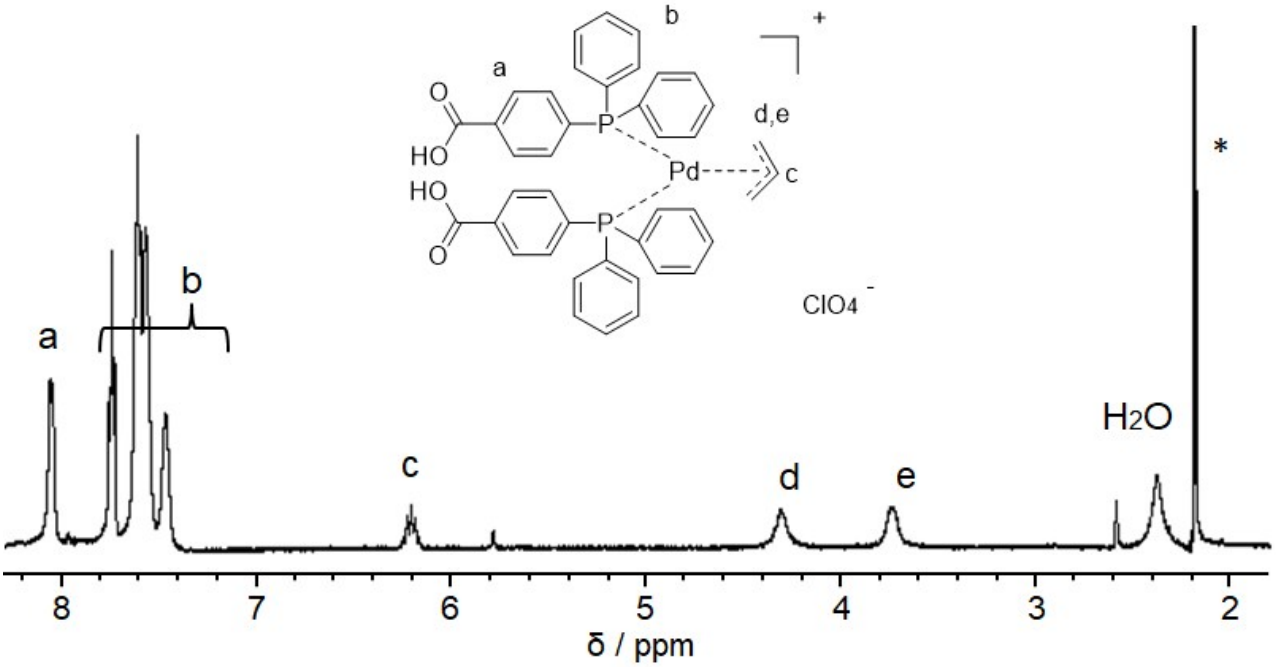


Figure S1. ^{1}H NMR spectrum of **1** in CD_3CN.

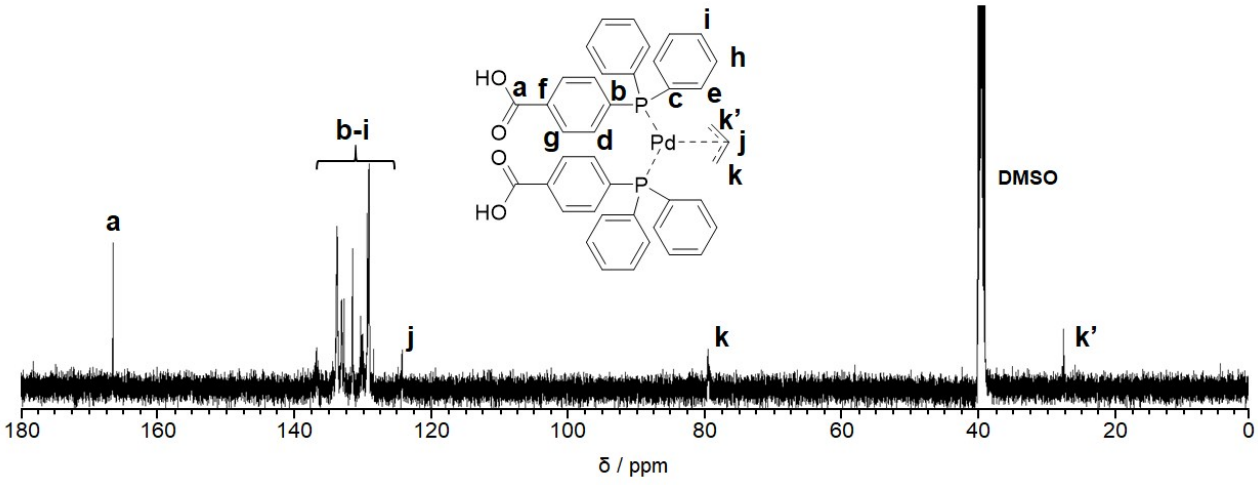


Figure S2. ^{13}C NMR spectrum of **1** in DMSO-d_6.

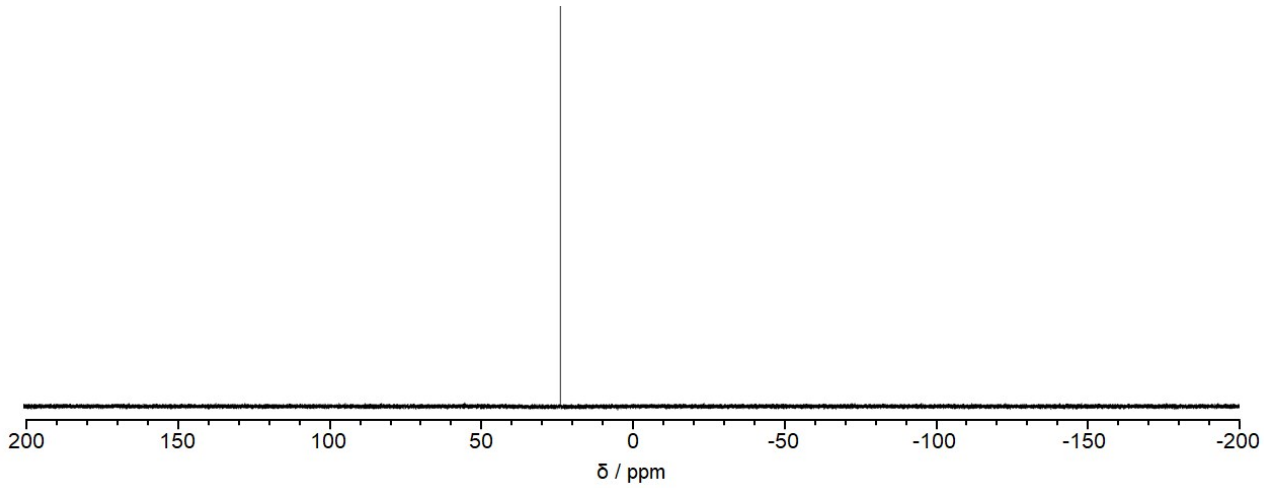
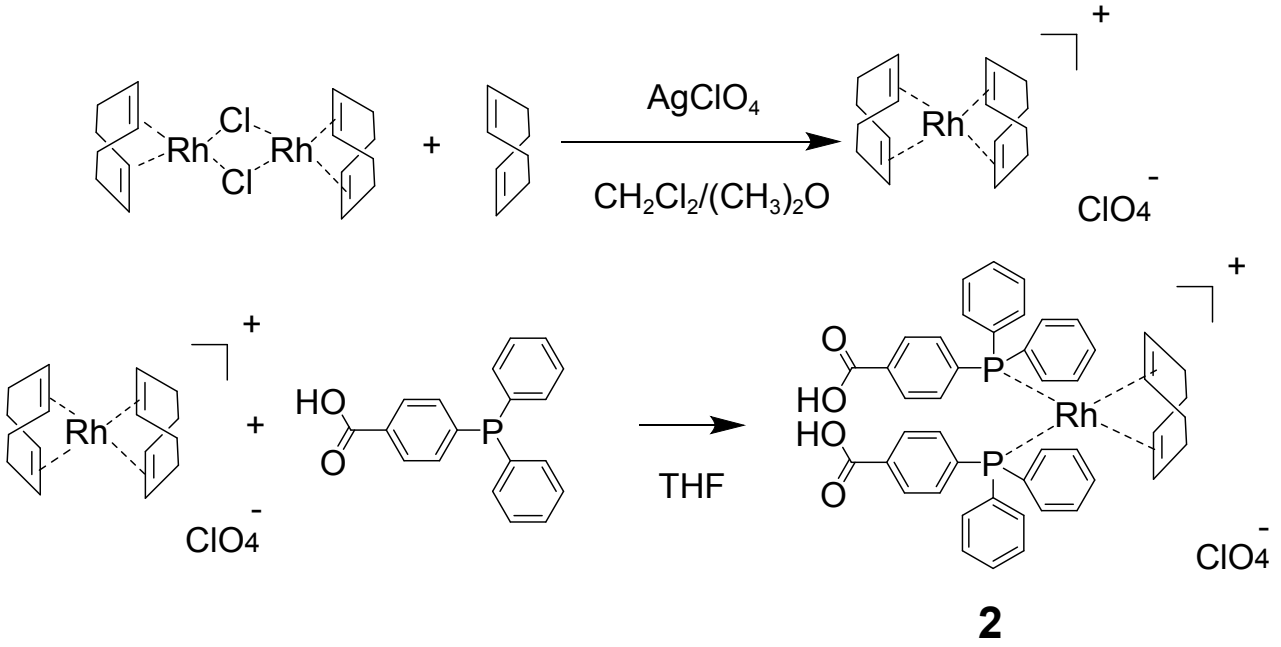


Figure S3. ^{31}P NMR spectrum of **1** in CD_3CN.

Preparation of [1,5-cyclooctadiene-bis{4-(diphenylphosphino)benzoic acid} rhodium(I) perchlorate] (2)

Scheme S2. Preparation of 2



2

4-(Diphenylphosphino)benzoic acid (0.16 g, 0.53 mmol) dissolved in THF (20 mL) was dropped into THF solution (10 mL) containing bis(1,5-cyclooctadiene)rhodium(I) perchlorate (0.10 g, 0.24 mmol) and the mixture was stirred for 1 h at r.t. under an Ar atmosphere. The reaction mixture was concentrated to ~5 mL and poured into diethylether (0.12 L). The suspension was centrifuged (4000 rpm, 5 min) and the resultant precipitation was washed 3 times with diethyl ether (5.0 mL). The product was dried in vacuo. (yield: 47%). ^{1}H NMR (500 MHz, CD_3CN, 25 °C): δ = 2.17 (m, b, 4H; CH_2 of 1,5-cyclooctadiene), 2.42-2.47 (m, b, 4H; CH_2 of 1,5-cyclooctadiene), 4.60 (s, b, 4H; C*H* of 1,5-cyclooctadiene), 7.45–7.60 (m, 24H, *H* of Ph), 8.01 (m, 4H, C_6H_4CO); ^{31}P NMR (500 MHz, CD_3CN, 25 °C): 28.2 (s); MALDI-TOF-MS for $C_{46}H_{42}O_4P_2Rh$: calcd 823; found 823.

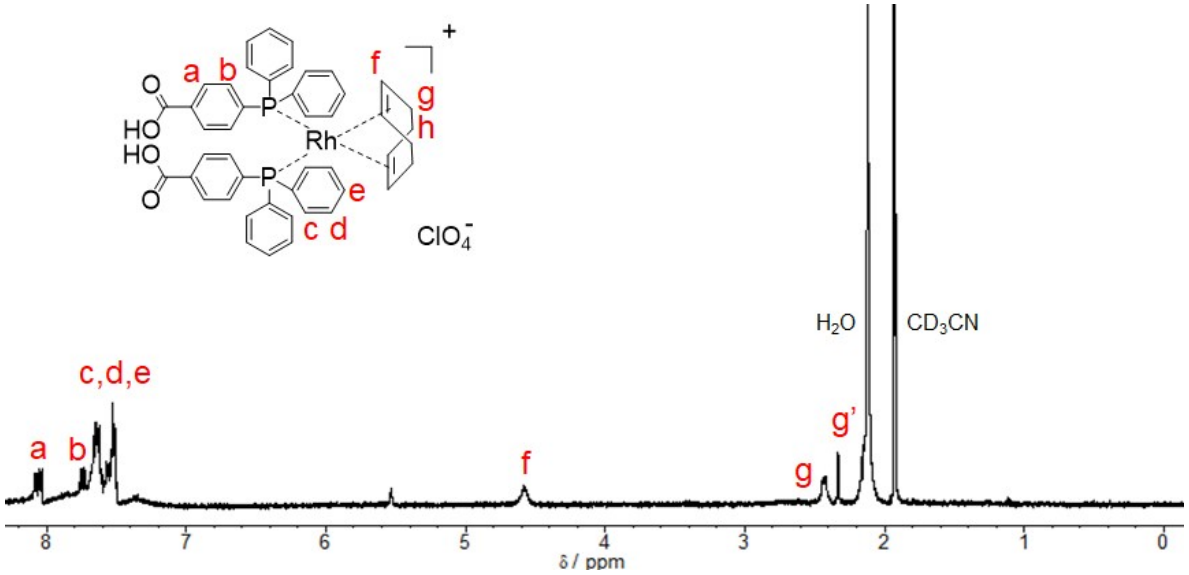


Figure S4. ^{1}H NMR spectrum of **2** in CD_3CN.

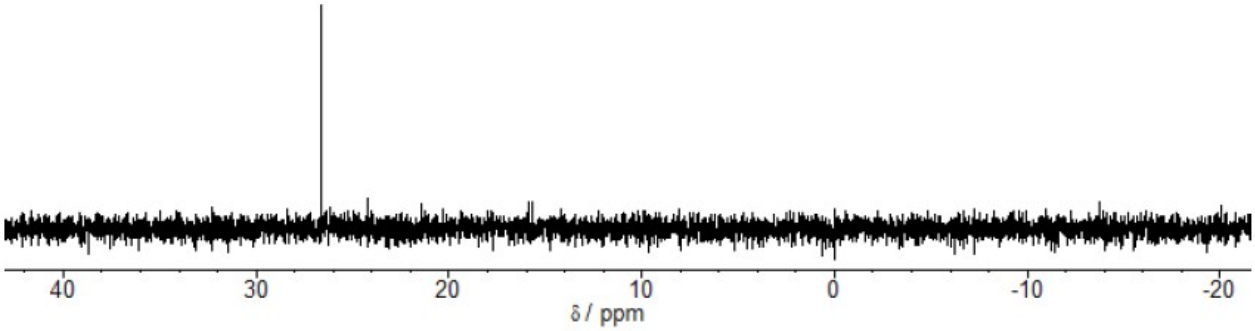
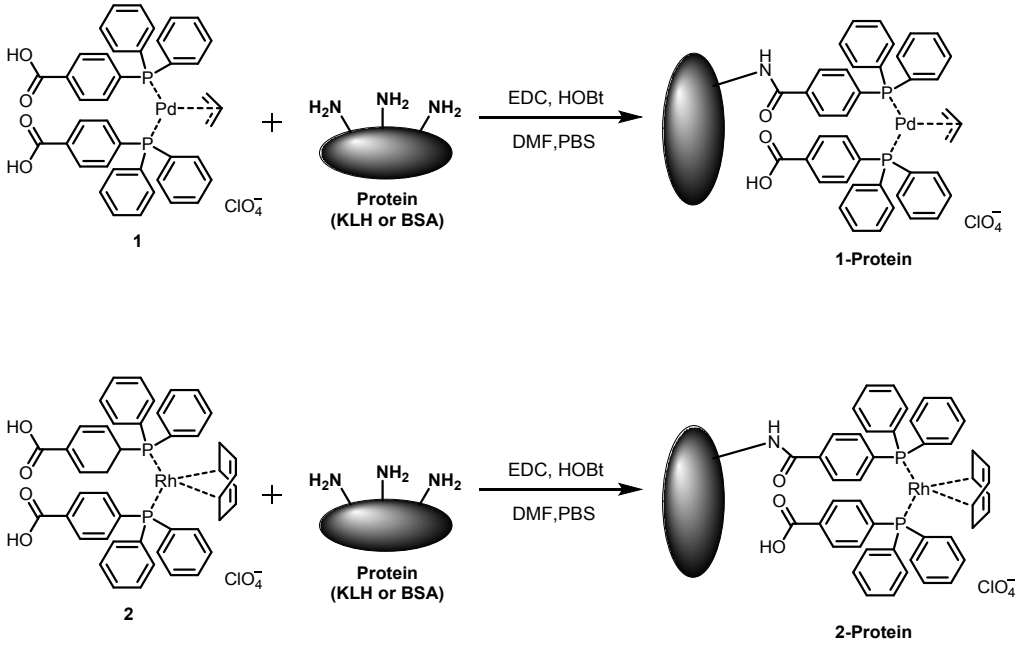


Figure S5. ^{31}P NMR spectrum of **2** in CD_3CN.

Preparation of the antigens for immunization and assay

Scheme S3. Preparation of the antigens for immunization and assay



The **1** or **2** (2.5 μmol) and carbonyldiimidazole (1.6 mg, 10 μmol) were dissolved in DMF (1.0 mL) and stirred for 2 hours at 0 °C. Subsequently this solution was added dropwise to a 0.1 M PBS (pH 7.0) including protein (KLH or BSA) (1.0 mg/mL) over a period of 1.5 hours and stirred for 2 hours at 0 °C and 6 hours at r.t. The resultant solution was purified using ultrafiltration membrane with 10 kDa molecular weight cutoff (Sartorius).

Quantification of metal complexes immobilized on proteins

Determination of protein concentrations

Protein concentration was determined by protein assay reagent (PIERCE). Albumin standard (0-1 mg/mL, Nacalai Tesque, Inc.) was used for calibration curve. Reagent A (1 mL) and Reagent B (20 mL) was mixed. The **1**- or **2**-protein solution or albumin standard (100 μL) was added to the mixed solution of reagent A and Reagent B (2 mL) and incubated at 37 °C for 30 min. The mixture was cooled to stop the color reaction and measured absorbance at 562 nm. Protein concentration was calculated from calibration curve.

Determination of the number of 1 or 2

The introduction amount of the **1**- or **2**- for KLH or BSA was determined by 2,4,6-trinitrobenzenesulfonic acid (TNBS) method.[3-5] We prepared solution A [0.1 M Na_2SO_3 aq (1.50 mL) + 0.1 M NaH_2PO_4 aq. (98.5 mL)] and TNBS solution [TNBS (10 mg) was dissolved in 0.2 M $Na_2B_4O_7$ (50.0 mL) + 0.2 M NaOH (50.0 mL)]. **1**- or **2**-KLH or BSA or glycine were dissolved in 0.1 M Phosphate Borate Buffer (PBB; pH 9.0) (10-100 μg/mL). Glycine solution was used for calibration curve. TNBS was dissolved in solution B (0.1 mg/mL). The sample solution (125 μL) or glycine solution were added to TNBS solution (125 μL). The mixture solution was stirred for 5 min at r.t., and quenched by solution A (1.00 mL). The absorption of the solution was measured at 420 nm. The number of the **1** and **2** was calculated from calibration curve.

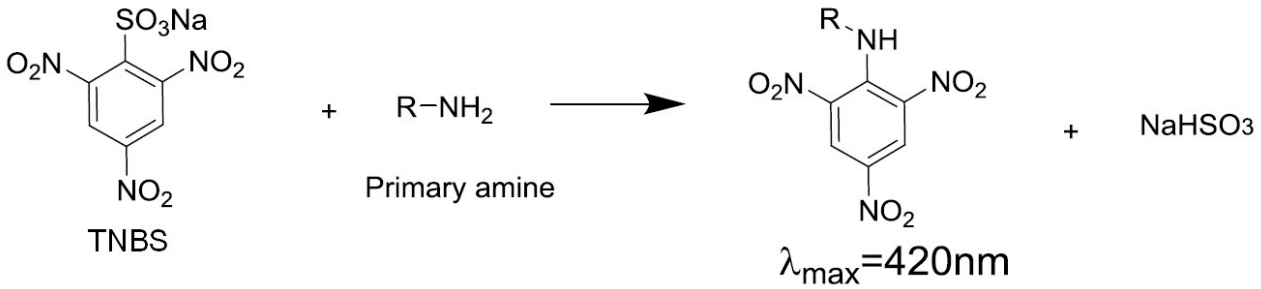


Table S1. The amount of the metal-complexes introduced into KLH or BSA

	The amount of metal complexes[a]
1-KLH	1000
1-BSA	7
2-KLH	600
2-BSA	8

[a] mol of metal-complexes/mol of proteins

Stability of 1 in water

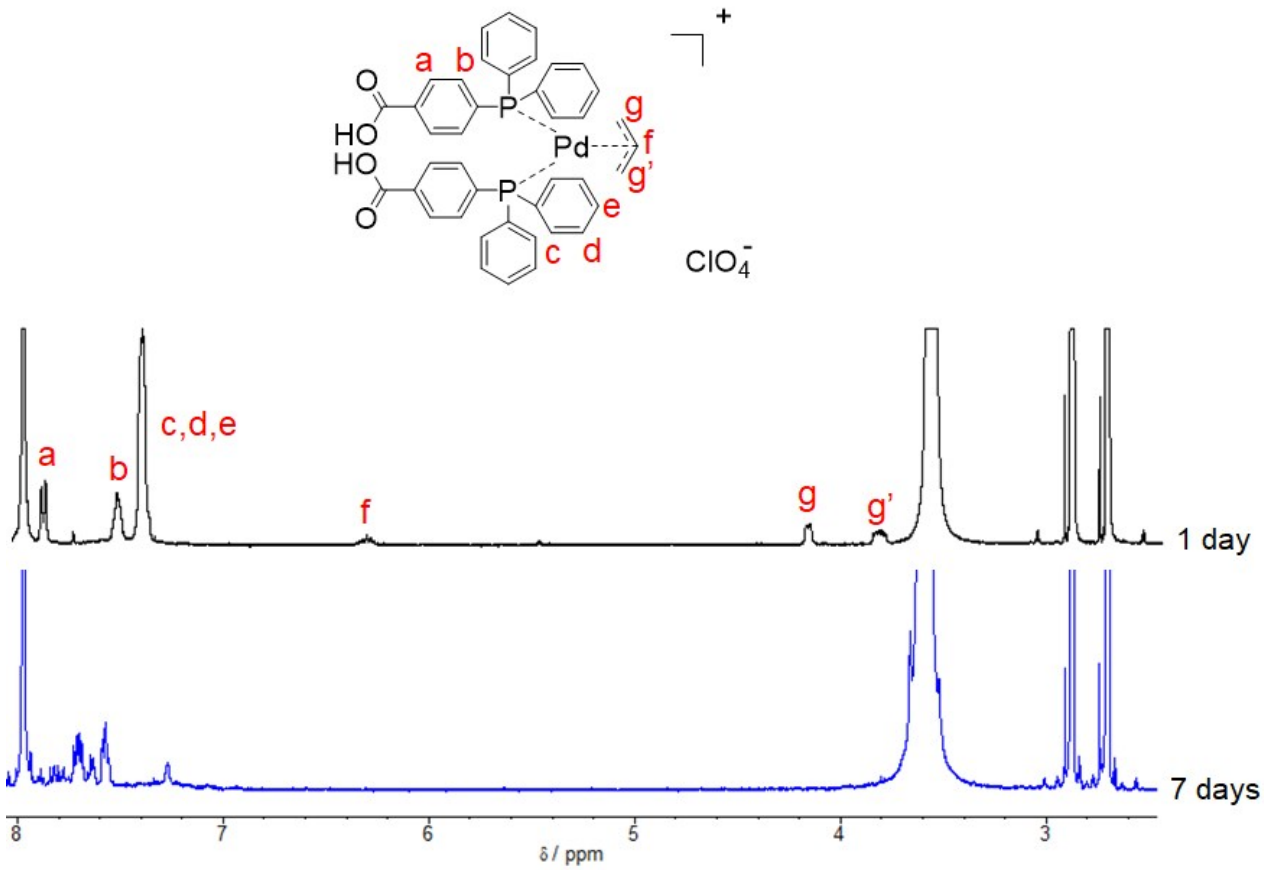


Figure S6. ^{1}H NMR spectra of **1** in DMF-d_7/D_2O (10/1) at 25 ºC.

Production of antibodies for 2

To confirm antibodies production, we performed ELISA measurement of the blood from immunized and non-immunized mice using **2**-BSA coated plate. In the case of immunized mouse, the absorbance of **2**-BSA was higher than that of BSA (Fig. S7). This result shows production of antibodies for **2**.

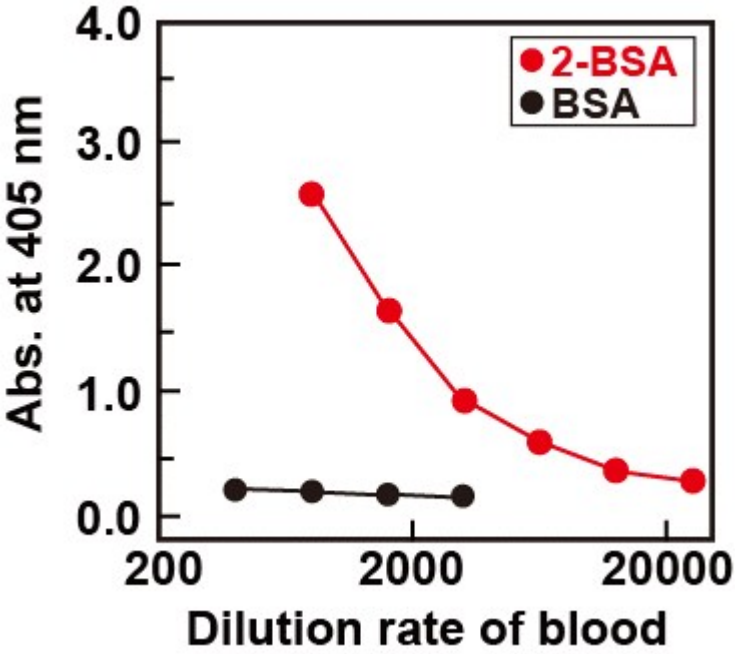


Figure S7. Results of ELISA for blood obtained from the mouse immunized with **2**-KLH response for the **2**-BSA (red) and BSA(black).

Preparation of monoclonal antibodies (mAbs)

All animal experiments are approved by Animal Experimentation Committee, Osaka University.

Procedure of immunization

The **1**- or **2**-KLH (0.3 mg/mL) was emulsified with equal amount of FCA. A group of Balb/c mice were immunized with the **1**- or **2**-KLH (100 μg) three times at interval of two weeks. At boost, these mice were immunized with the **1**- or **2**-KLH (80 μg) without the adjuvant.

Preparation of mAbs

Myeloma cells were cultured in 10% FBS RPMI medium. The spleen cells were removed from immunized mice three days after the boost and fused with myeloma cells using PEG. The cells were suspended in 2.5% FBS medium with HAT and plated in 96 well microplates. When the size of colony became observable, the antibodies produced by the cells were assayed by ELISA method (described later). The cells which produce antibodies specific for the **2** were cloned by limited dilution method in 10% FBS RPMI medium. The antibodies produced were assayed again and selected. The hybridomas selected were injected to pristane-primed mice. After 10 days, the ascites fluid was obtained. The ascites fluid was centrifuged and the supernatant was stored at -20 °C. mAbs were purified by affinity chromatography using HiTrap IgM Purification HP column (GE healthcare). The purity of mAbs was investigated by SDS-PAGE analysis. The subclass of mAb obtained was determined by IsoStrip (Roche).

Determination of the dissociation constants (K_d) of mAb and compounds

0.30 nM of mAbs dissolved in 0.1 M PBB (pH 9.0) (60 μL) and various concentration of the compound dissolved in 0.1 M PBB (pH 9.0) (10-7 to 10-3 M, 60 μL) were mixed on a BSA-coated plate. The mixed solutions were incubated at 4 °C overnight and added to a compound-BSA coated plate. The plates were incubated at 37 °C for 90 min under 5% CO_2. After removal of the solutions, the wells were washed twice with washing buffer (180 μL). Then alkaline phosphatase labeled anti-mouse IgG (1:1000 dilution, in 20 mM PBS, 100 μL) was added and the plates were incubated at 37 °C for 90 min. After removal of the solutions, the wells were washed three times with washing buffer (180 μL). Then p-nitrophenyl phosphate sodium salt in substrate buffer (1.0 mg/mL in substrate buffer, 150 μL) was added. Absorbance at 405 nm derived from the enzymatic hydrolysis product (p-nitrophenolate) by alkalinephosphate labeled to the secondary antibody was recorded and we determined the amount of the antibody binding to compound-BSA.

Dissociation constants were determined by Klotz plot[6] (Fig. S8 and S9):

$$A_0/(A_0 - A) = 1 + K_d \cdot (1/c)$$

where A_0 and A indicate absorbance at 405 nm in the absence or presence of competitive molecules, respectively. The character c shows the concentration of antigen. K_d represents the dissociation constant.

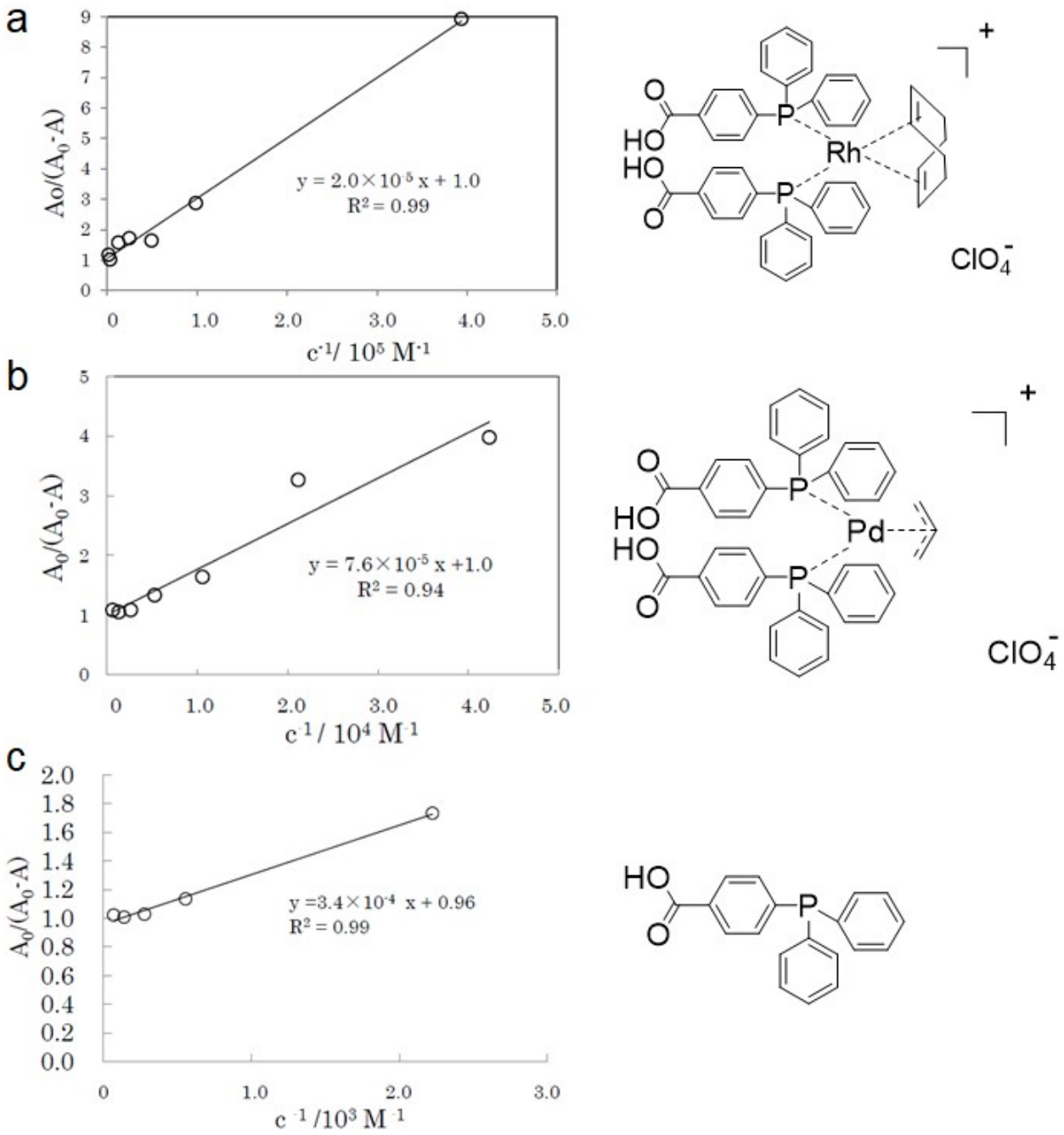


Figure S8. Klotz plots of mAb with (**a**) **2**, (**b**) **1**, or (**c**) **3**.

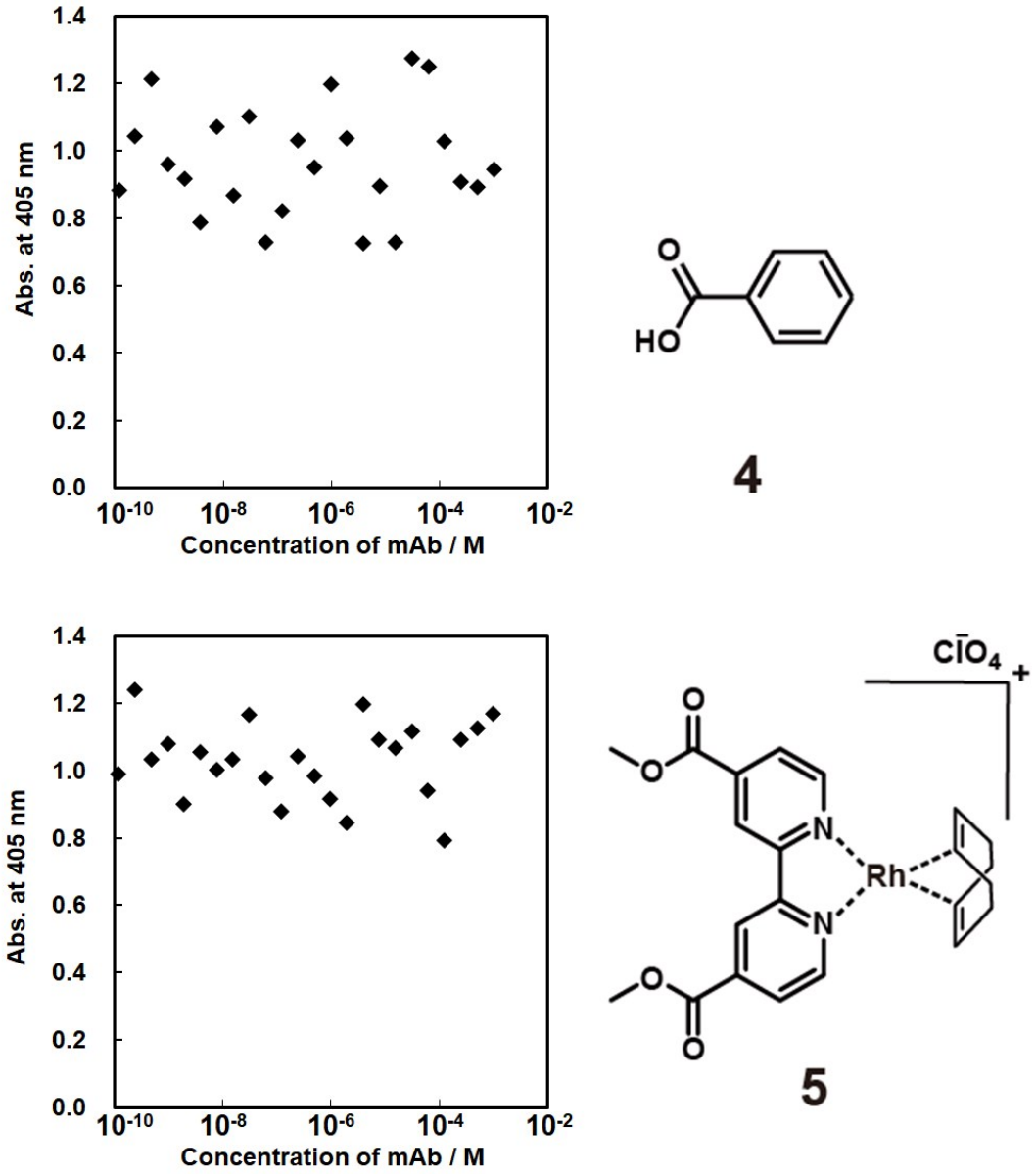
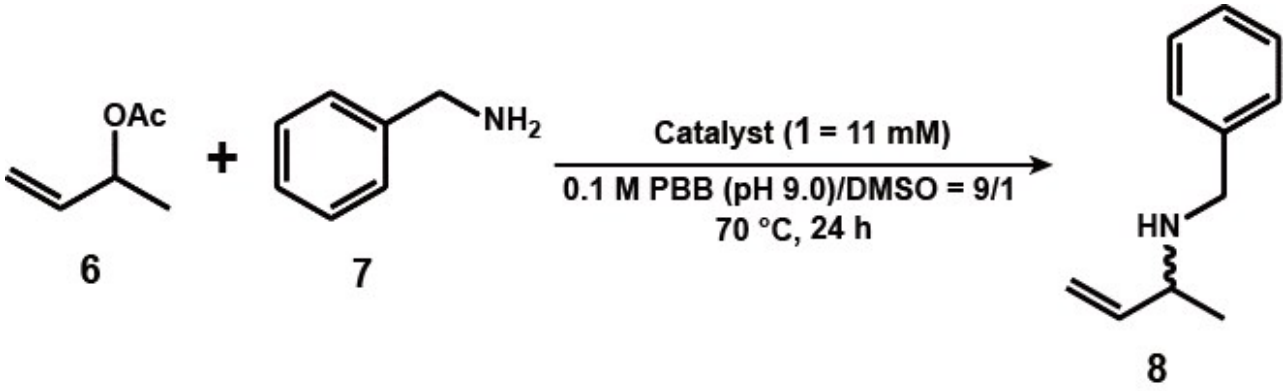


Figure S9. Competitive ELISA of mAb with (**a**) **4** or (b) **5**.

Synthesis of standard sample of *N*-benzylbut-3-en-2-amine (allylic amination product;8).

Scheme S4. Allylic amination reaction of **6** and **7**.



The reaction was carried out under an N_2 atmosphere. **1** (4.7 mg, 5.5 μmol) dissolved in DMSO (0.25 mL) was dropped into 0.1 M PBB (pH 9.0) (4.5 mL) and DMSO (0.25 mL) containing 3-buten-2-yl-acetate (**6**) (0.28 mL, 2.2 mmol) and benzylamine (**7**) (0.48 mL, 4.4 mmol). The mixture was stirred over night at 70 °C, and quenched by pouring water. The mixture was extracted with diethyl ether, the organic layer was dried over $MgSO_4$, and the organic layer was evaporated under reduced pressure. The residue was purified on a flash column chromatography (60 mg, 20% yield).

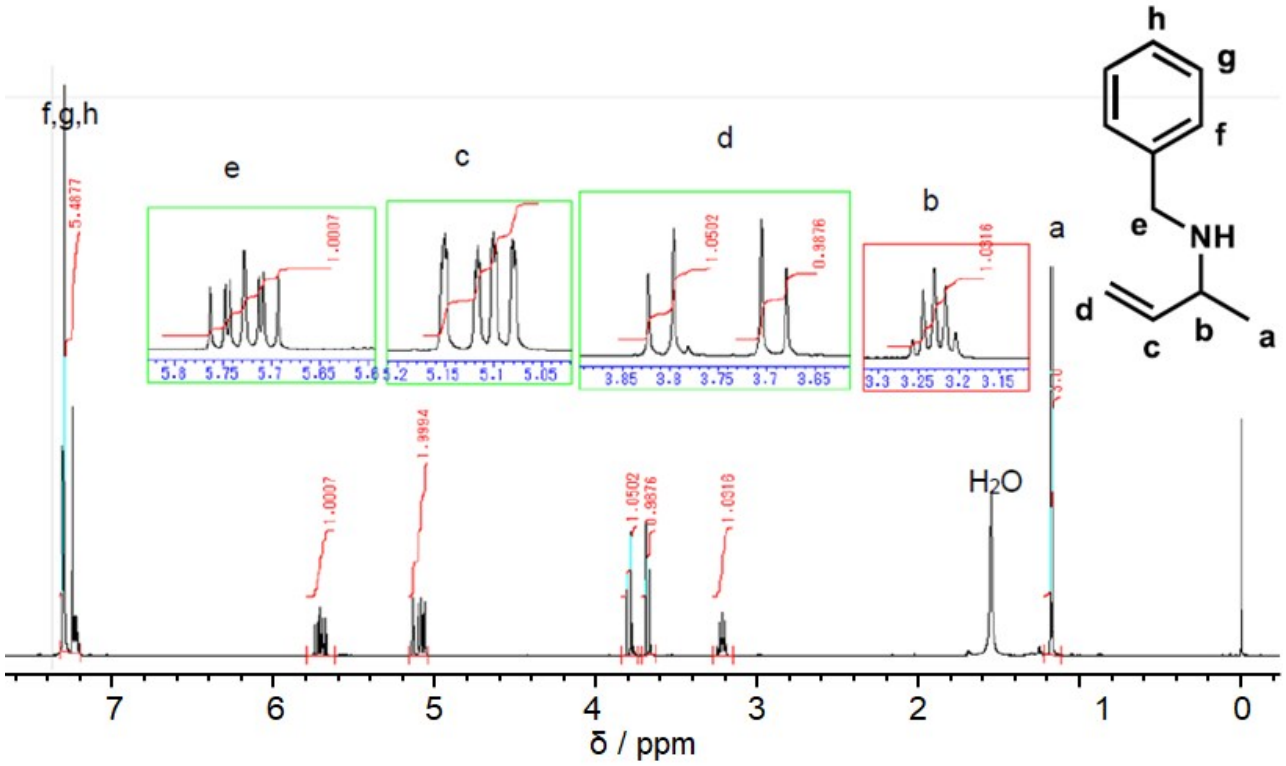


Figure S10. ^{1}H NMR spectrum of **8** in $CDCl_3$ at 25 ºC.

Determination of the product (8) concentration using internal standard (IS)

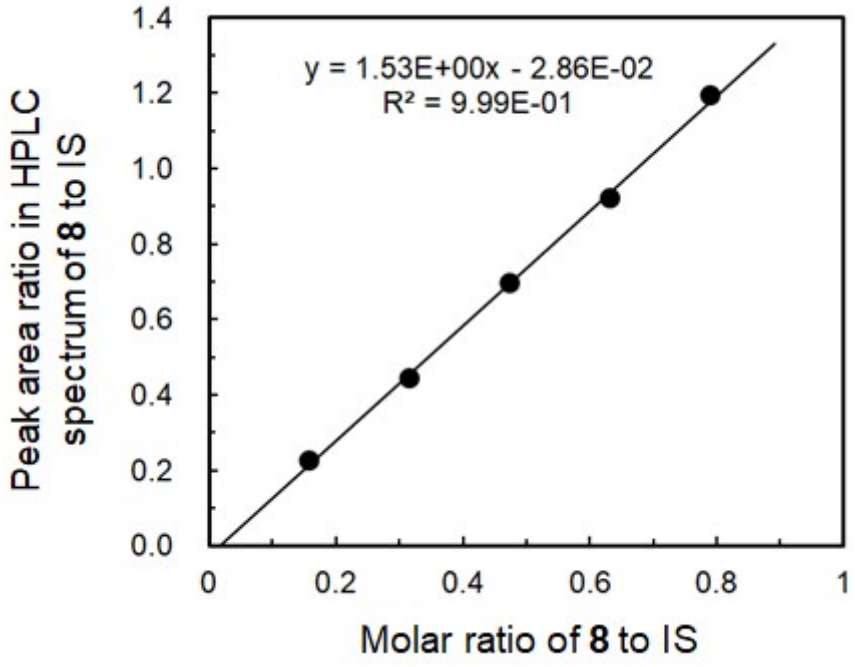


Figure S11. Calibration curve for **8**.

1-catalyzed allylic amination reaction in the absence and presence of mAb or BSA

In contrast to without mAb (Fig. S12a), the reaction catalysed by **1** with mAb was found to proceed with excellent (*R*)-enantioselectivity with a 98 ± 2% ee (Fig. S12b). This asymmetric allylic amination was not observed in the presence of BSA (ee < 2%) (Fig. S12c). These results indicate that the binding site of mAb functions as a reaction field for the asymmetric reaction.

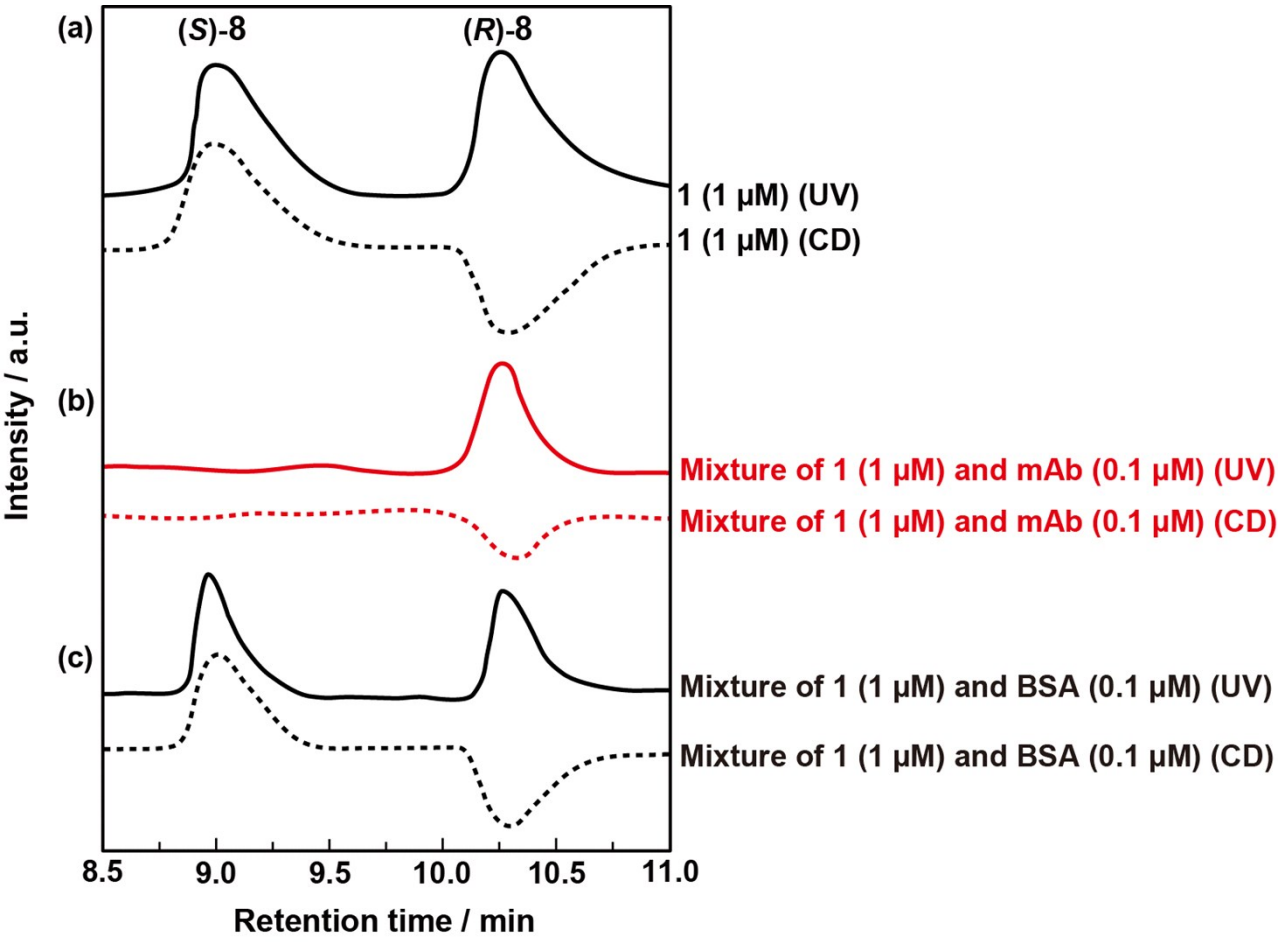


Figure S12. HPLC spectra (UV: solid line, CD: dash line) of **1**-catalyzed allylic amination reaction in the absence and presence of mAb or BSA. (a) **1** (1 μM), (**b**) mixture of **1** (1 μM) and mAb (0.1 μM), and (**c**) mixture of **1** (1 μM) and BSA (0.1 μM). The assignment of *S* and *R*-form was performed with reference to literature.[1]

Table S2. Turn over number (TON) of 1 in the absence and presence of mAb or BSA.

Entry	Catalyst	TON[a]
1	**1**	1900
2	**1**+mAb	300
3	**1**+BSA	550

[a]TON units (mol of product/mol of Pd complex)

References

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5. S. L. Snyder and P. Z. Sobocinski, *Anal. Biochem.*, 1975, **64**, 284.
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