Supplementary Material (ESI) for Organic and Biomolecular Chemistry

Electronic Supplementary Information

Asymmetric hydrogenation with antibody-achiral rhodium complex

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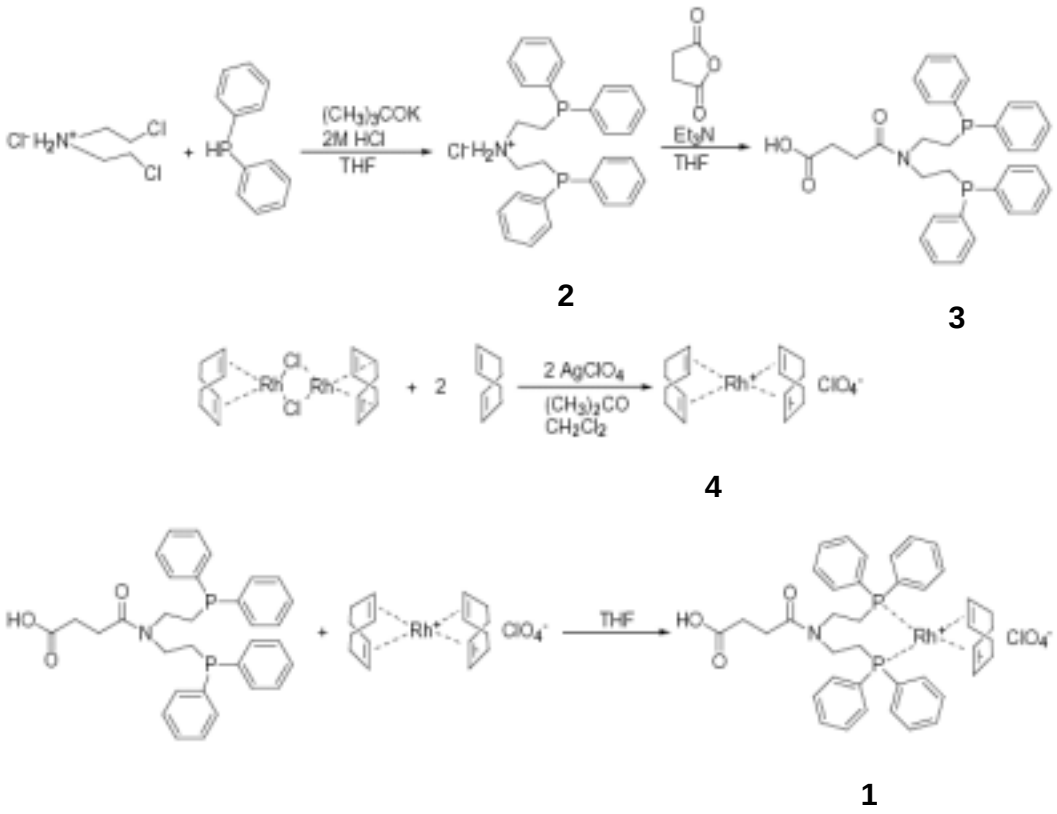
General Procedures. Diphenylphosphine, bis(2-chloroethylamine)chloride, chloro(1,5-cyclooctadiene)rhodium(I) dimer and 1,5-cychlooctadiene were purchased from Tokyo Kasei Kogyo Co., Ltd. Potassium *tert*-butxide, succinic anhydride and triethylamine were purchased from Nacalai Tesque, Inc. Silver perchlorate was purchased from Wako Pure Chemical Industries, Ltd. All solid chemicals were used without further purification, and all organic solvents and liquid chemicals were distilled and water was degassed before use.

The synthesis of Rh complex **1** was carried out under argon atmosphere using standard Schlenk techniques. ^{1}H and ^{31}P-NMR spectra were recorded on JEOL JMN Excaliber-400 spectrometer or JEOL Lambda-500 spectrometer. Chemical shifts are reported in ppm, the solvent residual peak was set as internal standard on ^{1}H-NMR and the peak of 9% trimethyl phosphate in $CDCl_3$ was set as external standard on ^{31}P-NMR. Fast atom bombardment mass spectra (FAB-MS) were recorded on a JEOL LMS-SX 102 spectrometer.

Synthesis of Hapten 1. Achiral Rh complex **1**, [(1,5-cyclooctadiene){bis(2-diphenylphosphinoethyl) succinamido}rhodium(I)]perchlorate, was synthesized as shown in Scheme S1.

Scheme S1. Synthesis of Hapten 1



Compounds **2** and **3** were synthesized according to the literatures.[1, 2] Bis(1,5-cyclooctadiene)rhodium(I) perchlorate (**4**) was prepared as follows. Chloro(1,5-cyclooctadiene) rhodium(I) dimer (429 mg, 0.87 mmol) and 1,5-cychlooctadiene (0.20 mL, 1.74 mmol) was dissolved in dichloromethane (15 mL). Silver perchlorate (465 mg, 2.24 mmol) dissolved in acetone (5 mL) was dropped into the solution and stirred for 30 min. The suspension was centrifuged (3500 rpm, 3 min), the supernatant was concentrated and poured into THF. The precipitate was washed twice with THF (10 mL) and further twice with diethyl ether (5 mL). The product was dried in vacuo. Yield: 63%. ^{1}H-NMR (500 MHz, $CDCl_3$, 30 ˚C): δ = 2.49 (d, *J* = 8.9 Hz, 4H; CH_2), 2.66 (d, *J* = 9.9 Hz, 4H; CH_2), 5.36 (s, 4H; CH). [1,5-Cyclooctadiene{bis(2-diphenylphosphinoethyl)succinamido}rhodium(I)] perchlorate (**1**) was synthesized by the reaction of **3** with **4**. Compound **3** (104 mg, 0.191 mmol) dissolved in THF (30 mL) was dropped into THF solution (10 mL) containing Rh complex **4** (123 mg, 0.294 mmol). After 1 hr stirring, the reaction mixture was concentrated to 15 mL and poured into diethyl ether (120 mL). The suspension was centrifuged (4000 rpm, 5 min) and the resulting precipitation was washed 3 times with diethyl

ether (5 mL). THF soluble part was collected and distilled under vacuum. The product was dried in vacuo. Yield 45%. ^{1}H-NMR (500 MHz, $CDCl_3$, 30 ˚C): δ = 2.06 (s, 4H; CH_2-P), 2.50-2.53 (m, 8H; CH_2, 1,5-COD), 2.60-2.61 (m, 4H; CH_2-N), 2.88-2.91 (m, H; CH_2-CO), 3.00-3.02 (m, H; CH_2-CO), 3.12 (s, 2H; CH_2-COOH), 5.30-539 (d, *J* = 22.9 Hz, 4H; CH, 1,5-COD), 7.39-7.43 (m, 4H; *p*-Ph), 7.45-7.51 (m, 8H; *m*-Ph), 7.53-7.60 (m, 8H; *o*-Ph); ^{31}P-NMR (202 MHz, CDCl3, 30 ˚C): 25.9 (d, $J_{P\text{-}Rh}$ = 149.1 Hz); FAB-MS for $C_{40}H_{45}NO_3P_2Rh$: calcd 752; found 752.

Preparation and Characterization of Antigens. Hapten **1** (5 mg) and carbonyldiimidazole (6 eq.) were dissolved in 2 mL of THF. Protein solutions (1 mg/mL, KLH, 1/3000 eq. or BSA, 1/5 eq.) dissolved in 0.1 M phosphate buffered saline (PBS, pH 7, 5 mL) were dropped into the hapten solution, respectively. The mixed solution was stirred for 2 hrs at 4 ˚C and a half of a day at r.t. The resulting protein-hapten conjugates were purified by using a Sephadex G-100. The concentrations of proteins were determined by BCA protein assay and Rh concentration in the hapten-carrier protein conjugate was determined with induction coupling plasma spectroscopy (λ_{max} = 343.489 nm). The conjugates were found to contain 946 mol of hapten / mol of KLH and 1 mol of hapten / mol of BSA, respectively.

Preparation, Purification, Characterization of Monoclonal Antibodies

Preparation of Antibodies. Balb/c mice were immunized with KLH-**1** in saline emulsified 1 : 1 in Freund's complete adjuvant 11 times at two weeks intervals. Three days after the final injection (boost), spleen cells were removed and used for the fusion experiments. Spleen cells from a mouse were fused with the SP 2/0 mouse myeloma cells.[3] Hybridomas secreting antibodies for the Rh complex were cloned twice by limiting dilution. The antibodies for the hapten were detected by enzyme-linked immunosorbent assay (ELISA). The tissue culture supernatants were added onto the ELISA plate coated with 0.3 mg /ml BSA-**1** and incubated 90 min. The amount of the antibody bound to the antigen was measured by using goat anti-mouse immunoglobulins (IgG, IgA, and IgM) labeled with alkaline phosphatase.

Purification of Antibodies. The class of monoclonal antibodies was determined by using a mouse monoclonal antibody isotyping kit (Amersham Biosciences). A 0.5 ml of pristane was injected to Balb/c mice 10 days prior to injection of hybridoma cells. The ascites fluid was harvested after 9-13 days and separated from cells by centrifugation. The monoclonal antibodies were purified with ImmunoPure IgM purification kit (Pierce). The purity of antibodies was checked by SDS-PAGE electrophoresis (Phast System, Pharmacia LKB, Uppsala, Sweden).

Determination of the Dissociation Constant (K_d) of the Complex between Antibody 1G8 and the Rh Complex by ELISA. The antibody solution (1.0×10^{-8}M, 60 μL) and the solutions of the Rh complex with various concentrations from 10^{-9} to 10^{-3} M (60 μL) were mixed on a BSA-coated plate. The mixed solutions were allowed to stand for 1 day at room temperature and then each

mixed solution (100μL) was transported to the ELISA plates precoated with BSA-**1** and BSA blocking buffer. Absorbance at 405 nm for the resulting enzymatic hydrolysis product (*p*-nitrophenolate) by alkalinephosphatase of the second antibody was recorded to determine the amount of the antibody that bound BSA-**1**. The K_d of the complex between antibody 1G8 and the Rh complex was determined by Klotz plot (Figure S1). Klotz equation is described as follows:

$$A_0 / (A_0 - A) = 1 + K_d / a_0$$

Where A_0 and A are absorbance at 405 nm in the absence and presence of the Rh complex, respectively, and a_0 is the concentration of the Rh complex. Direct coordination of the protein to the metal centre via displacement of the labile cyclooctadiene ligand is thought to be negligible from the papers on the hydrogenation of substrates catalyzed by Rh complexes in the presence of proteins reported by the other researchers.[4-6]

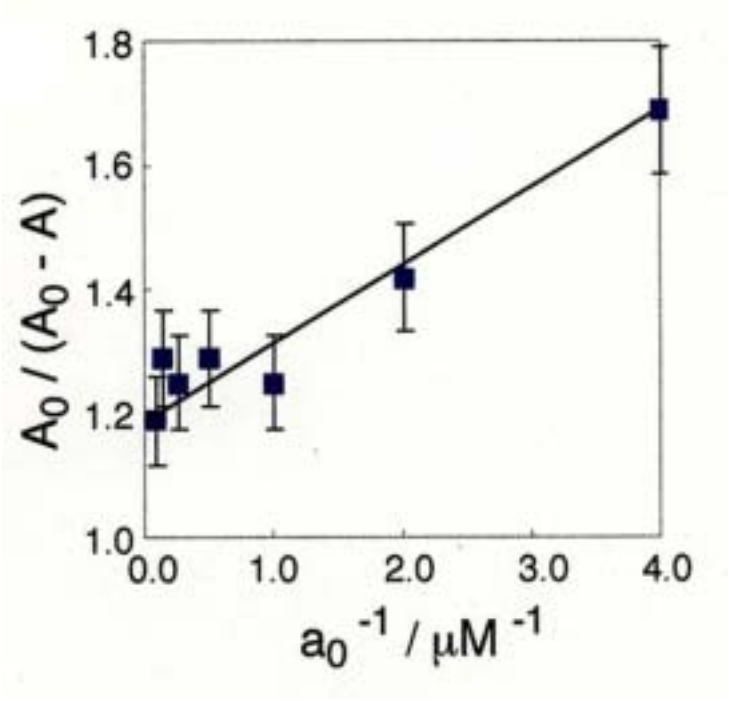


Figure S1. Binding of antibody 1G8 to achiral Rh complex **1** as shown by a Klotz plot.

Hydrogenation Procedures

Reaction Conditions of Hydrogenation of Substrates by the Rh Complex in the presence of Antibody 1B8. The mixture of Rh complex **1** (16μg, 21nmol) and monoclonal antibody 1G8 (4.0 mg, 4.2 nmol, [the antigen binding site] = 42 nmol) was dissolved in 5 mL of phosphate buffer (0.1 M, pH 7.0). The substrates (78 μmol) were added into Schlenk containing the aqueous solution of the complex between antibody 1G8 and Rh complex **1**. Argon was purged at first for 1 hr, and then

hydrogen was introduced to the solution through a needle. After stirring for 12 hrs at 37 ˚C, the conversion and the turnover frequency of the reaction product were determined by HPLC or GC. The ee values were also determined by chiral HPLC or GC. Detection conditions were described in the caption of Figure S2. The limit of detection of the GC and HPLC measurements was checked by using standard D/L-amino acid derivatives mixing with various concentrations. The experimental errors in GC and HPLC analyses were within 1% and 5%, respectively.

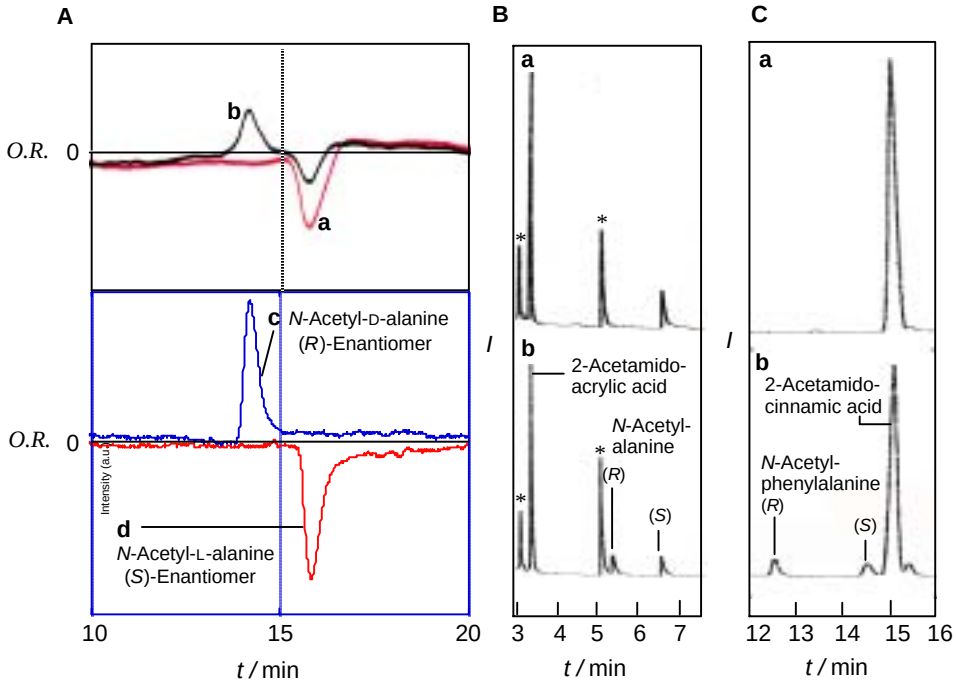


Figure S2. Hydrogenation of amino acid precursors (2-acetamidoacrylic acid and 2-acetamidocinnamic acid) to amino acid derivatives catalyzed by the Rh complex **1** in the presence and absence of antibody 1G8. (**A**) HPLC diagrams of the hydrogenation products of 2-acetamidoacrylic acid monitored by an optical rotation detector. **a**, The hydrogenation products catalyzed by the antibody-Rh complex; **b**, the products in the absence of the antibody; **c**, *N*-acetyl-D-alanine ((*R*)-enantiomer); **d**, *N*-acetyl-L-alanine ((*S*)-enantiomer). HPLC analysis was performed by using a Chiralcel AD-H column (Daicel Chemical Industries, Japan) at 25 ˚C, flow rate 0.5 mL min^{-1}, and hexane-2-propanol (9:1) as an eluent. Retention times (*t*) for (*R*)- and (S)- enantiomers were 14.2 min and 15.7 min, respectively. *N*-Acetyl-D-leucine: *t* (min) = 14.8, *N*-acetyl-L-leucine: *t* (min) = 17.7 (spectra not shown). (**B**) GC diagrams of the reaction products of 2-acetamidoacrylic acid catalyzed by the Rh complex with antibody 1G8 (**a**) and without antibodies (**b**). Peaks * show the reagent for methyl esterification and its derivative. (**C**) GC diagrams of the reaction products of 2-acetamidocinnamic acid catalyzed by the Rh complex with antibody 1G8 (**a**)

and without antibodies (**b**). The hydrogenation products and remaining substrates were converted *in situ* to their methyl esters using trimethylsulfonium hydroxide before FID-GC analysis on a 25 m x 0.25 mm fused silica of Chirasil-L-Val with helium as the mobile phase. *N*-Acetylalanine methyl ester: GC (100 ˚C): *t* (min) = 5.4 (*R*), 6.6 (*S*). Methyl ester of 2-acetamidoacrylic acid: (100 ˚C): *t* (min) = 3.5. *N*-Acetylphenylalanine methyl ester: GC (140 ˚C): *t* (min) = 12.7 (*R*), 14.6 (*S*). Methyl ester of 2-acetamidocinnamic acid: (140 ˚C): *t* (min) = 15.2.

References

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