Experiment 1

医薬品リドカインの合成

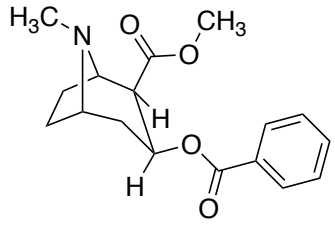
Synthesis of lidocaine as a synthetic drug

石原研究室担当

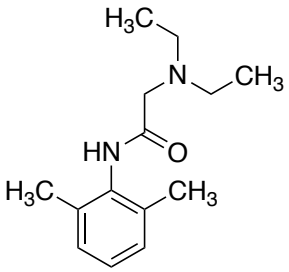
Ishihara Group

Introduction

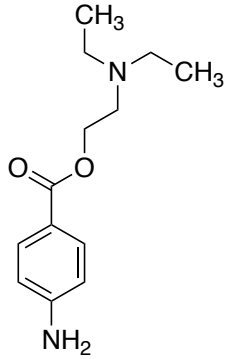
Local anesthetics (pain killers), like sulfa drugs, comprise a group of compounds having a key structural feature that imparts a specific pharmacological property. These are important and well-studied classes of synthetic drugs. Some common local anesthetics are shown below. Of these, only cocaine is a naturally occurring compound, and synthetic drugs are used to avoid the narcotic effects of the former. The suffix *caine* used so commonly with synthetic local anesthetics is derived from cocaine, which in turn is a combination of coca- + *-ine*, meaning a nitrogenous compound from coca plants.



Cocaine



Lidocaine (Xylocaine)



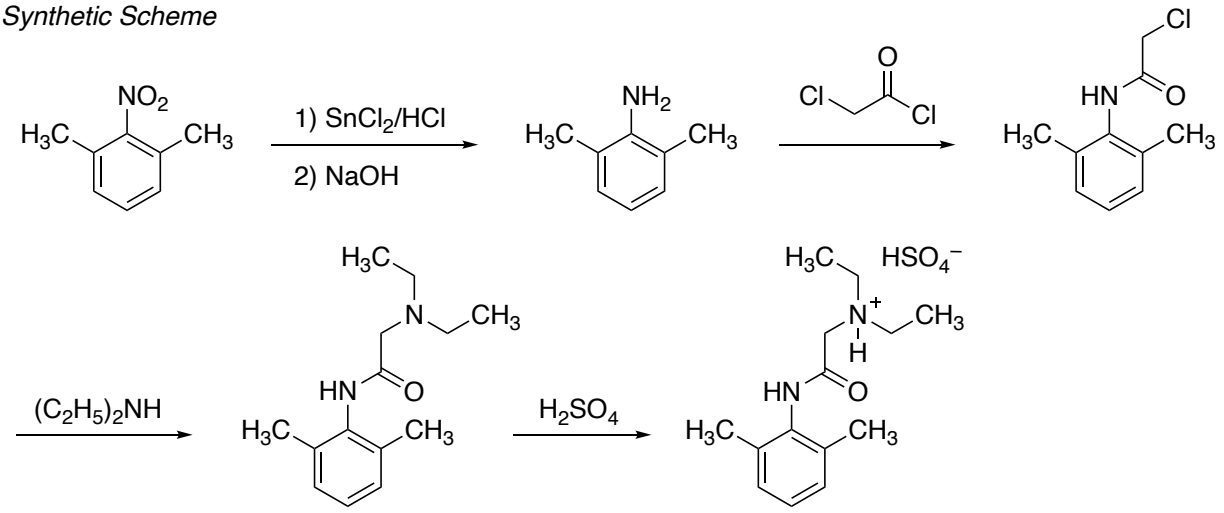
Procaine (Novocaine)

In most of the hundreds of local anesthetics that have been synthesized, two structural features are prominent: the compounds are benzoate esters or anilides and contain a dialkylamino group separated by 1 to 4 atoms from the carbonyl center, as indicated in the structure of cocaine. The dialkylamino group is a characteristic unit in the structures of many diverse medical agents such as antihistamines, antimalarial compounds, and tranquilizers.

In these experiments, the local anesthetics lidocaine will be synthesized and isolated in the form of its bisulfate salt. The hydrochloride is the salt generally used in medicine, but it is considerably more difficult to purify. Lidocaine (the generic name) is sold under various trade names, the most common of which is "*Xylocaine*". It is noted for its relatively high anesthetic activity when applied to the skin or injected into nerves, and it has low toxicity and incidence of side effects.

This synthetic sequence illustrates several important reactions. The reduction of an aromatic nitro compound is most accomplished with metals such as iron, zinc, or tin. Stannous chloride is more rapid and convenient because the reaction is homogeneous. The second and third steps in the synthesis illustrate the very large difference in reactivity of the two electrophilic centers in chloroacetyl chloride.

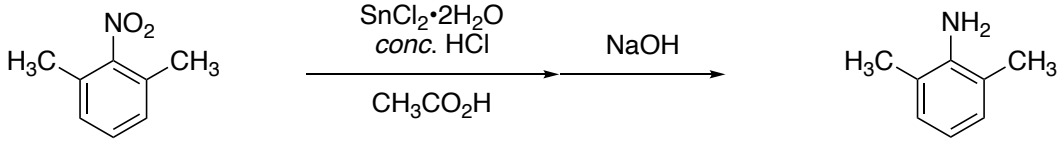
Synthetic Scheme



Experimental Procedures

The organic solvents used in this experiment are highly flammable. Since chloroacetyl chloride is a lachrymator, the experiment should be carried out in a well-ventilated hood.

1. Synthesis of 2,6-Dimethylaniline (2,6-Xylidine) by $SnCl_2$ Reduction

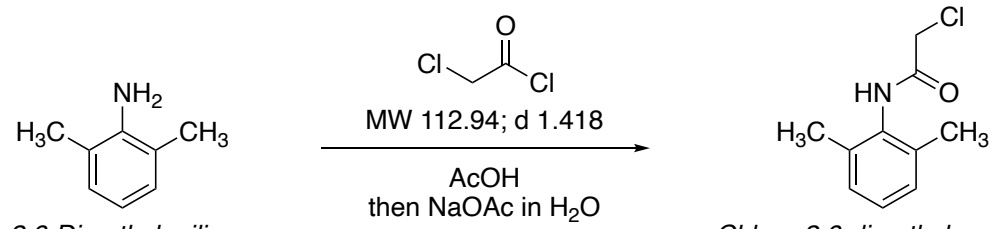


2,6-Dimethylnitrobenzene
MW 151.17; d 1.112
mp 14~16 °C, bp 225 °C/744 mm

2,6-Dimethylaniline
MW 121.18; d 0.984
mp 10~12 °C, bp 214 °C/739 mm

Dissolve 2.5 g of 2,6-dimethylnitrobenzene (2-nitro-*m*-xylene) in 25 mL of glacial acetic acid in a 100 mL Erlenmeyer flask. In a 100 mL Erlenmeyer flask, dissolve 10 g of $SnCl_2 \cdot 2H_2O$ in 20 mL of concentrated hydrochloric acid, heating on an oil bath if necessary. Add the $SnCl_2$ solution in one portion to the nitroxylene solution, swirl to mix, and let the mixture stand for 15 minutes. Cool the mixture and collect the crystalline salt in a Buchner-funnel. Transfer the moist crystals to an Erlenmeyer flask, add 12 mL of water, and make strongly basic by carefully adding 30% NaOH solution (20~25 mL required). After cooling, extract with 15 mL and 10 mL portions of diethyl ether, wash the ether extracts twice with 10 mL of water and once with 10 mL of brine, and dry over Na_2SO_4. Transfer the dried and filtered solution to a 100 mL round-bottom flask (measuring the weight of the empty flask beforehand will be useful in measuring the product weight), and complete evaporation. Weigh and calculate the percentage yield of 2,6-dimethylaniline.

2. Synthesis of α-Chloro-2,6-dimethylacetanilide (α-Chloroaceto-2,6-xylidide)

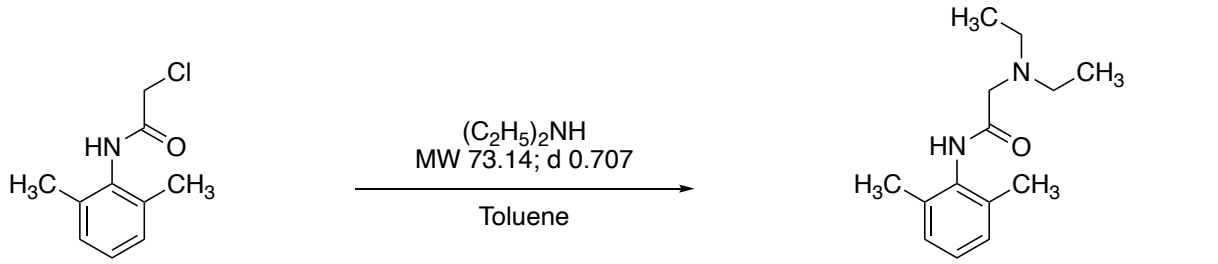


2,6-Dimethylaniline
MW 121.18; d 0.984

α-Chloro-2,6-dimethylacetanilide
MW 197.67; mp 145~146 °C

To a test tube, add the xylidine, glacial acetic acid (5 mL per 1 g of xylidine), and 1.85 g (1.3 mL) of chloroacetyl chloride, in that order. Warm the solution on an oil bath to 40 to 50 °C for 15~20 minutes. Remove the test tube from the oil bath. To a solution of 2.5 g of sodium acetate in 50 mL of water was added the reaction mixture. Cool the mixture and collect the product in a Buchner funnel. Rinse the solid in the funnel with water until the acetic acid odor is gone, and dry as much as possible by pressing and drawing air through the filter cake in the funnel. Transfer the product to a disk of filter paper and let it air-dry until the next laboratory period. Weigh and calculate the percentage yield of xylidide.

3. Synthesis of α-Dimethylamino-2,6-dimethylacetanilide (Lidocaine)



α-Chloro-2,6-dimethylacetanilide
MW 197.67; mp 145~146 °C

α-Diethylamino-2,6-dimethylacetanilide
MW 234.35; mp 68~69 °C

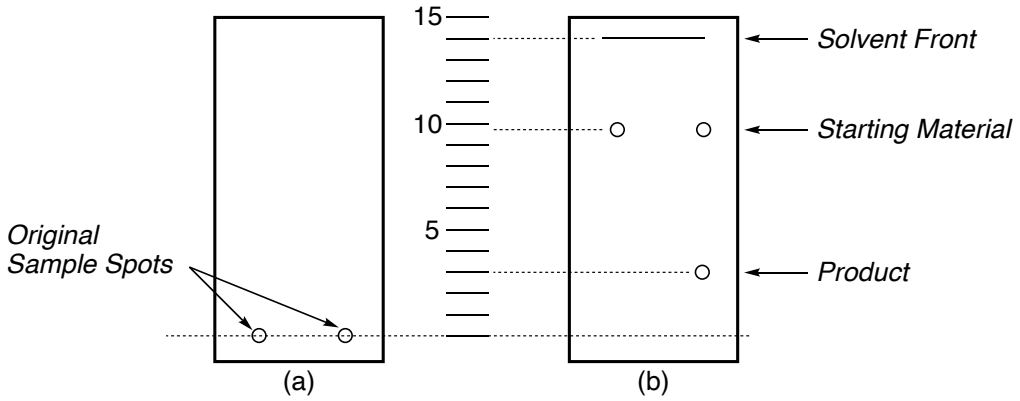
In a test tube, place the chloroacetoxylidide obtained from the preceding experiment and 5 mL of dry toluene; then add six moles of diethylamine per mole of xylidide (Save out a few mg of the starting material for TLC comparison). Warm the solution in an oil bath to 100 °C. The progress of the reaction can be monitored conveniently at 30 minutes intervals by TLC analysis (For details, see the next section). [Stop heating and, after boiling stops, take a sample with a fine capillary; stir the reaction mixture and resume the refluxing. Spot the solution and the starting material on a silica gel plate and develop with hexane–ethyl acetate]

After the starting material is gone (checked by TLC) or after 3 h, whichever comes first, cool the mixture, and filter out the crystals; rinse them with a small amount of pentane, air-dry, and weigh.

Transfer the filtrate to a separatory funnel, and extract with two 10 mL portions of 3 *M* HCl. Cool the acidic aqueous layer in an Erlenmeyer flask and add 30% aqueous NaOH carefully until the solution is strongly basic. Extract with 20 mL of pentane. Wash the pentane layer with six 5 mL portions of water, dry over Na_2SO_4, and concentrate in a 50 mL round-bottom flask to an oil. Weigh and calculate the percentage yield of lidocaine. Take the ^1H NMR spectrum (in $CDCl_3$ solvent) of your lidocaine sample.

Thin Layer Chromatography (TLC)

Silica gel TLC plates are available in the laboratory. Place a reaction solution (few μL) on the TLC plate about 0.5 cm from one edge and about 0.5 cm from the bottom, using a capillary tube to apply the spot. The spot should be 1~2 mm in diameter. Allow the spot to air-dry. Apply another spot of the starting material (few μL of 1~2% ether solution) on the plate at the same distance from the bottom as the first and leave about 0.5 cm between the spots. Again, allow the plate to air-dry.



$$R_f = \frac{\text{distance traveled by substance}}{\text{distance traveled by solvent}}$$

$$R_f \text{ (starting material)} = \frac{9.8 \text{ cm}}{14 \text{ cm}} = 0.70$$

$$R_f \text{ (product)} = \frac{3 \text{ cm}}{14 \text{ cm}} = 0.21$$

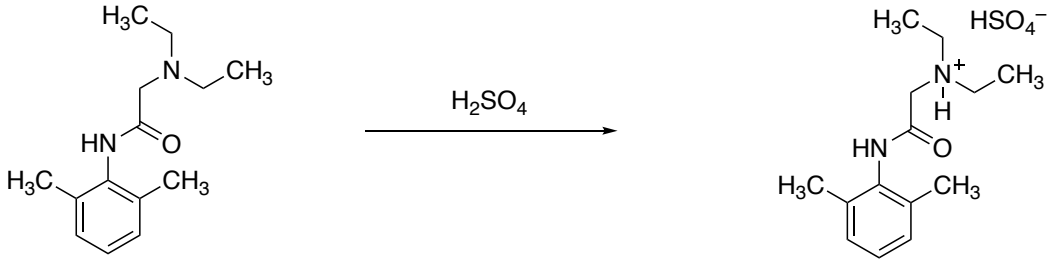
Figure 1. Thin-layer chromatogram: (a) original plate; (b) developed chromatogram.

Prepare a suitable solvent mixture (5~10 mL of hexane/ethyl acetate = 1:3) and place a 0.2~0.3 cm layer of the solution in the bottom of the developing chamber. Fold a piece of filter paper and place it into the developing chamber. Saturate a chamber with the vapors of the solvents by gentle

shaking. This inhibits the evaporation of solvents from the plate during the development of the chromatogram. The piece of filter paper aids in the maintenance of this saturated state.

Place the TLC plate in the chamber, being careful not to splash the solvent onto the plate. The spot must be above the solvent level. Cover the chamber with a lid. Allow the solvent to climb to within about 5 mm of the top of the plate and then remove the plate and allow it to air-dry. Spots are made visible using the UV lamp and a coloring agent.

4. Synthesis of Bisulfate Salt of α-Dimethylamino-2,6-dimethylacetanilide



α-Diethylamino-2,6-dimethylacetanilide
MW 234.35; mp 68~69 °C

α-Diethylamino-2,6-dimethylacetanilide bisulfate salt
MW 332.42; mp 210~212 °C

Dissolve the lidocaine in ethyl ether (10 mL per 1 g of lidocaine) and add 2 mL of 2.2 *M* sulfuric acid in ethanol per 1 g of lidocaine. Stir and scratch with a glass rod to mix and induce crystallization. Dilute the mixture with an equal volume of acetone to aid filtration and collect the salt in a small Buchner funnel. Rinse the solid on the funnel with a few mL of acetone and air dry and weigh the crude product. Recrystallize the salt by dissolving it in an equal weight of hot water and adding, *slowly*, 5 times this volume of acetone. Mix well and let stand to crystallize. Collect the crystals, rinsing with acetone, air dry, weigh, and determine the percentage yield of the product.

The filtrates (acidic water) should be neutralized with solid sodium carbonate and then discarded.

Questions and Discussions

(1) Elemental analysis of the crystalline salt isolated from the $SnCl_2$ reduction gave the following results: 33.4% C, 4.2% H, 36.8% Cl, 4.9% N, 20.6% Sn. Calculate its empirical formula and suggest a structure.

(2) Write a balanced equation for the $SnCl_2$ reduction. Compare your answer with the actual molar ratio of reactants used.

(3) What is the function of sodium acetate in the second step of the synthesis? Write a complete equation for the reaction.

(4) What is the compound that is crystallized from the refluxing toluene in the 3rd step? Write a balanced equation for the reaction.

References

(1) Ariens, E. J.; Simmonis, A. M.; van Rossum, J. M. Chemical and Physical Properties of Drugs with Local Anesthetic Action. In *Molecular Pharmacology*; Ariens, E. J. Ed.; Vol I; Academic Press, New York, 1964; pp 352–363.

(2) A. R. Patel, General Anesthetics. In *Medicinal Chemistry*, 3rd ed.; Burger, A. Ed.; Wiley-Interscience, New York, 1970; p 1314–1326.

(3) Takman, B. H.; Camougis, Q. Local Anesthetics. In *Medicinal Chemistry*, 3rd ed.; Burger, A. Ed.; Wiley-Interscience, New York, 1970; p 1607–1632.

(4) Buechi, J.; Perlia, X. The Design of Local Anesthetics. In *Drug Design*; Ariens, E. J. Ed.; Vol III; Academic Press, New York, 1964; pp 244–391.

(5) Gupta, S. P. *Chem. Rev.* **1991**, *91*, 1109.

(6) Josephson, P.; Nykvist, V.; Qasim, W.; Blomkvist, B.; Dinér, P. *J. Chem. Educ.* **2019**, *96*, 1389–1394.

Experiment 2

Synthesis of benzene ring via [2+2+2] cycloisomerization of alkynes with ruthenium catalyst

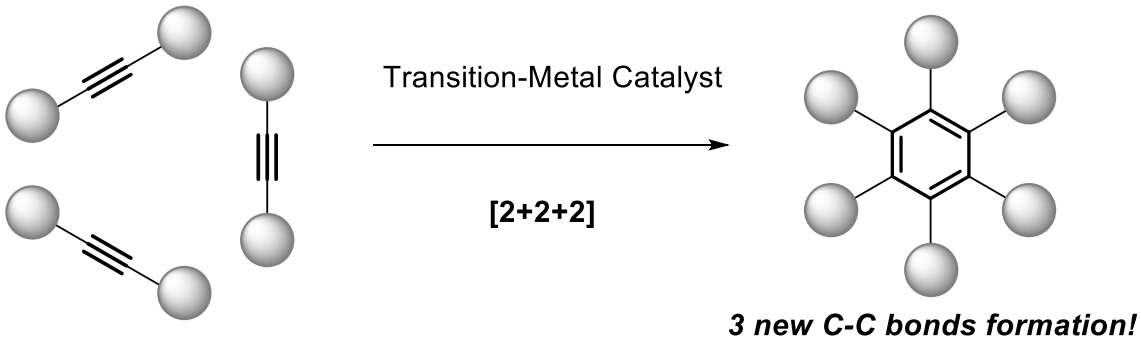
山本研究室担当

Yamamoto Group

Synthesis of benzene ring via [2+2+2] cycloisomerization of alkynes with ruthenium catalyst

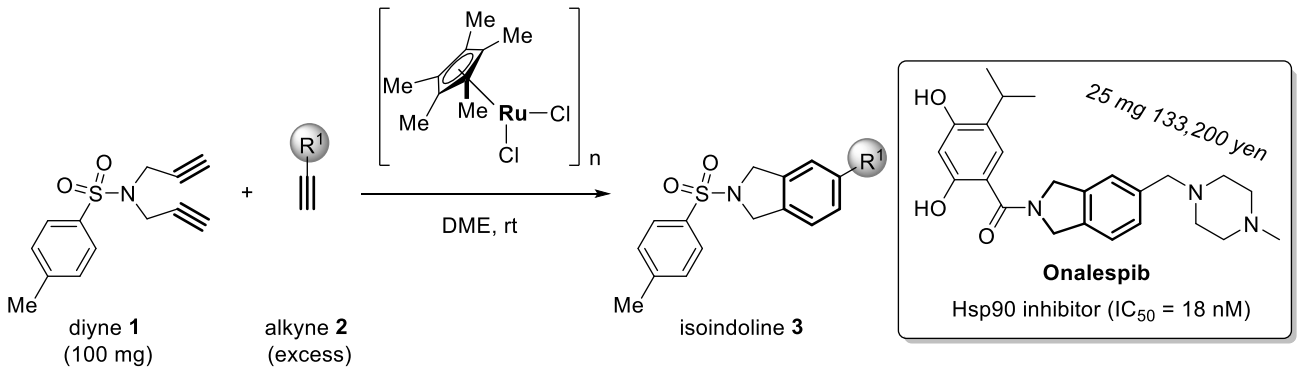
Introduction

Transition-metal catalysis has been used for many organic reactions such as Pd-catalyzed Suzuki-Miyaura cross coupling, Ru-catalyzed olefin (alkyne) metathesis, and Cu-catalyzed 1,4-addition to enones…, which are very useful for synthesizing drugs, agricultural chemicals, materials, and so on because new C-C bonds can be formed. Ru-catalyzed [2+2+2] cycloisomerization is one of the most useful C-C bond forming reactions, and it enables to access to the synthesis of a benzene ring from three alkynes in high atom economy (Scheme 1).



Scheme 1. Transition-metal-catalyzed [2+2+2] cycloisomerization of alkynes

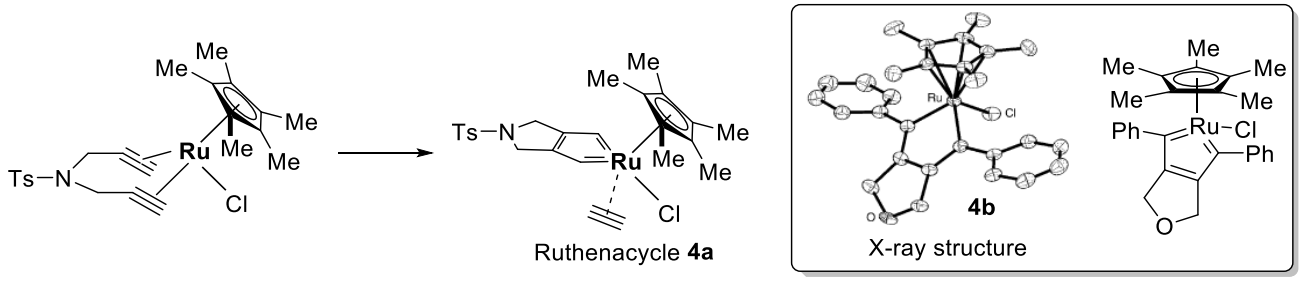
The intermolecular cycloaddition of diynes with alkynes, or the intramolecular cyclization of triynes have been developed as a promising and powerful tool to assemble polycyclic aromatic frameworks from simple acyclic precursors. For example, Onalespib, which is a selective inhibitor of Hsp90 and shows antitumor activity in mice bearing early stage HCT116 human colon carcinoma xenografts, has the isoindoline core. This structure would be easily and efficiently synthesized via intermolecular [2+2+2] cycloisomerization of diyne **1** with alkyne **2** (Surprisingly, the price of Onalespib is ¥133,200 per only 25 mg!!).



Scheme 2. Ru-catalyzed [2+2+2] cycloisomerization of diyne **1** with monoalkyne **2**

The reaction should proceed via ruthenacycle intermediate **4a** prepared from diyne **1** and the

ruthenium catalyst (Scheme 3). The ruthenacycle structure of **4b** was unambiguously confirmed by X-ray diffraction study. A ruthenacycle complex is a potential intermediate of the [2+2+2] cycloadditions of diynes and alkynes because the isolated **4b** was heated at 40 °C under the acetylene atmosphere for 5 days to give the expected cycloadduct.



Scheme 3. Ruthenacycle intermediate

In this experiment, you will synthesize an isoindoline derivative via [2+2+2] cycloisomerization of diyne **1** with one of alkynes **2a**–**c** (shown below) using the ruthenium catalyst $[Cp^*RuCl_2]_n$.

Alkynes

2a	**2b**	**2c**
Chemical Formula: C_8H_6 Molecular Weight: 102.14 density = 0.93 g/mL	Chemical Formula: C_3H_4O Molecular Weight: 56.06 density = 0.95 g/mL	Chemical Formula: C_4H_6O Molecular Weight: 70.09 density = 0.93 g/mL

References

1. Y. Yamamoto *et al. J. Am. Chem. Soc*. **2003**, *125*, 12143-12160.
2. J. Woodhead *et al. J. Med. Chem*. **2010**, *53*, 5956–5969.

Experimental procedure

Day 1

For the reaction using alkyne 2a

A solution of diyne **1** (100 mg, 0.40 mmol) in ethyl acetate (EtOAc, 2 mL) is added dropwise (***over 5 min***) to a solution of Ru catalyst (3 mg, 0.01 mmol) and alkyne **2a** (**0.2 mL**) in EtOAc (3 mL) at room temperature. The reaction is monitored by a TLC analysis (eluent: hexane–EtOAc = **4:1**). After the reaction is completed (the disappearance of the substrate spot on TLC will be observed within 20 minutes. *The product spot is very close to that of the substrate!*), the reaction mixture is directly purified by silica gel column chromatography (eluent: hexane–EtOAc = **4:1**). The collected fractions (30 mL each) are checked by TLC, and the fractions which contained the desired product is collected to a round-bottom flask and concentrated by using a rotary evaporator. The residue is suspended with EtOAc (ca. **3 mL**) and heated to 70 ºC until being clear solution, and hexane (ca. **10 mL**) is slowly added as a poor solvent. The crystals are collected by filtration, washed with ca. 20 mL of hexane, and then air-dried to afford the desired product.

For the reaction using alkyne 2b

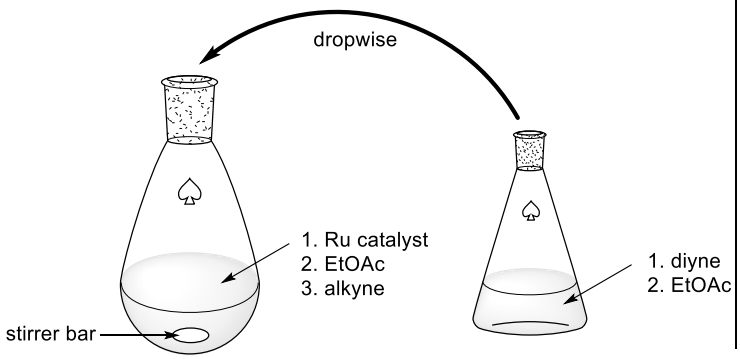
A solution of diyne **1** (100 mg, 0.40 mmol) in ethyl acetate (EtOAc, 2 mL) is added dropwise (***over 5 min***) to a solution of Ru catalyst (3 mg, 0.01 mmol) and alkyne **2b** (**0.1 mL**) in EtOAc (3 mL) at room temperature. The reaction is monitored by a TLC analysis (eluent: hexane–EtOAc = **1:2**). After the reaction is completed (the disappearance of the substrate spot on TLC will be observed within 20 minutes.), the reaction mixture is directly purified by silica gel column chromatography (eluent: hexane–EtOAc = **1:2**). The collected fractions (30 mL each) are checked by TLC, and the fractions which contained the desired product is collected to a round-bottom flask and concentrated by using a rotary evaporator. The residue is suspended with EtOH (ca. **4 mL**) and heated to 70 ºC until being clear solution, and water (ca. **15 mL**) is added as a poor solvent (a white solid will be precipitated). The crystals are collected by filtration, washed with ca. 20 mL of water, and then air-dried to afford the desired product.

For the reaction using alkyne 2c

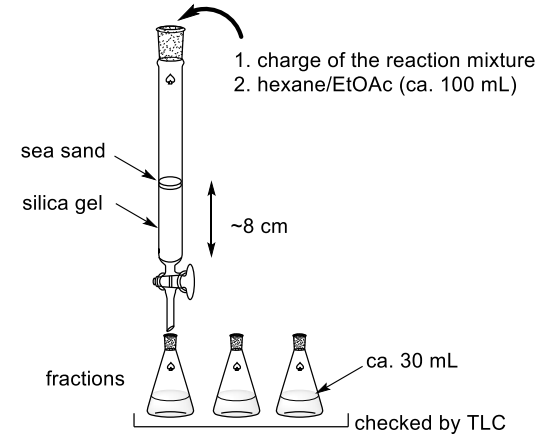
A solution of diyne **1** (100 mg, 0.40 mmol) in ethyl acetate (EtOAc, 2 mL) is added dropwise (***over 5 min***) to a solution of Ru catalyst (3 mg, 0.01 mmol) and alkyne **2c** (**0.15 mL**) in EtOAc (3 mL) at room temperature. The reaction is monitored by a TLC analysis (eluent: hexane–EtOAc = **1:2**). After the reaction is completed (the disappearance of the substrate spot on TLC will be observed within 20 minutes.), the reaction mixture is directly purified by silica gel column chromatography (eluent: hexane–EtOAc = **1:2**). The collected fractions (30 mL each) are checked by TLC, and the fractions which contained the desired product is collected to a round-bottom flask and concentrated by using a rotary evaporator. The residue is suspended with EtOAc (ca. **2 mL**) and heated to 70 ºC until being

clear solution, and hexane (ca. **10 mL**) is slowly added as a poor solvent (a white solid will be precipitated). The crystals are collected by filtration, washed with ca. 20 mL of hexane, and then air-dried to afford the desired product.

Schematic diagram of the reaction



Column chromatography



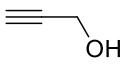
Day 2

The product is transferred to a 2 mL of vial using a medical packing paper. Do **NOT** discard your product because it should be checked by ^{1}H NMR to get the structural information.

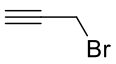
Questions

1. Describe the structure of alkyne **2** that you used and the estimated structure of your product and explain the reason.
2. Display the assignment of sp, sp^2, or sp^3 to all carbon atoms in your substrates (diyne **1** and alkyne **2**) and product.
3. Why is it necessary that an excess amount of alkyne **2** was used and diyne **1** was slowly added to the reaction mixture? Explain the reason. If one equivalent of alkyne **2** was used and/or diyne **1** was added to the reaction mixture immediately, what would be happened?
4. Propose a practical synthetic route for diyne **1** (*cf*. commercially available reagents that may be relevant to the synthesis are shown in the next page.).

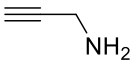
Commercially available reagents regarding question 4



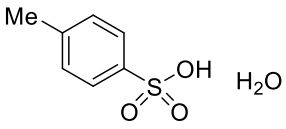
Propargyl alcohol
25 mL 2000 yen



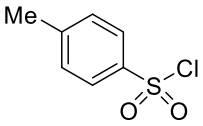
Propargyl Bromide
25 g 4000 yen



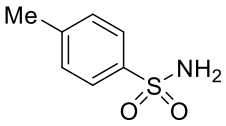
Propargyl amine
5 mL 8400 yen



p-Toluenesulfonic Acid Monohydrate
25 g 1600 yen



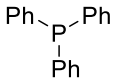
p-Toluenesulfonyl Chloride
25 g 2300 yen



p-Toluenesulfonamide
25 g 1700 yen

Ammonia (28% in Water)
500 mL 1600 yen

Phosphorus Tribromide
300 g 3300 yen

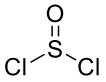


Triphenylphosphine
25 g 1800 yen

Bromine
500 g 5500 yen

Potassium Carbonate
500 g 1450 yen

Sodium Bicarbonate
500 g 1350 yen

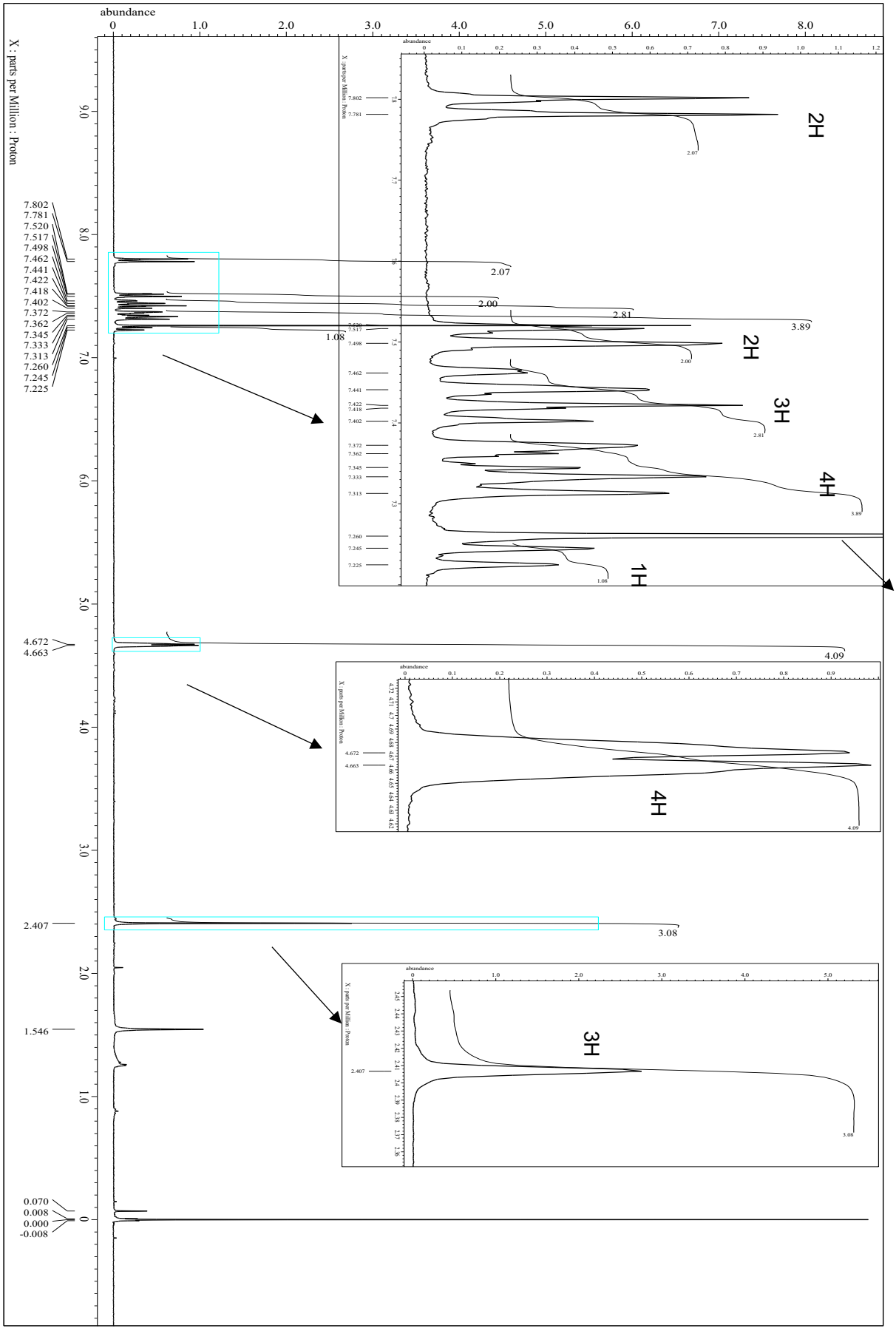


Thionyl Chloride
500 mL 3100 yen

<u>Instructions regarding your report</u>

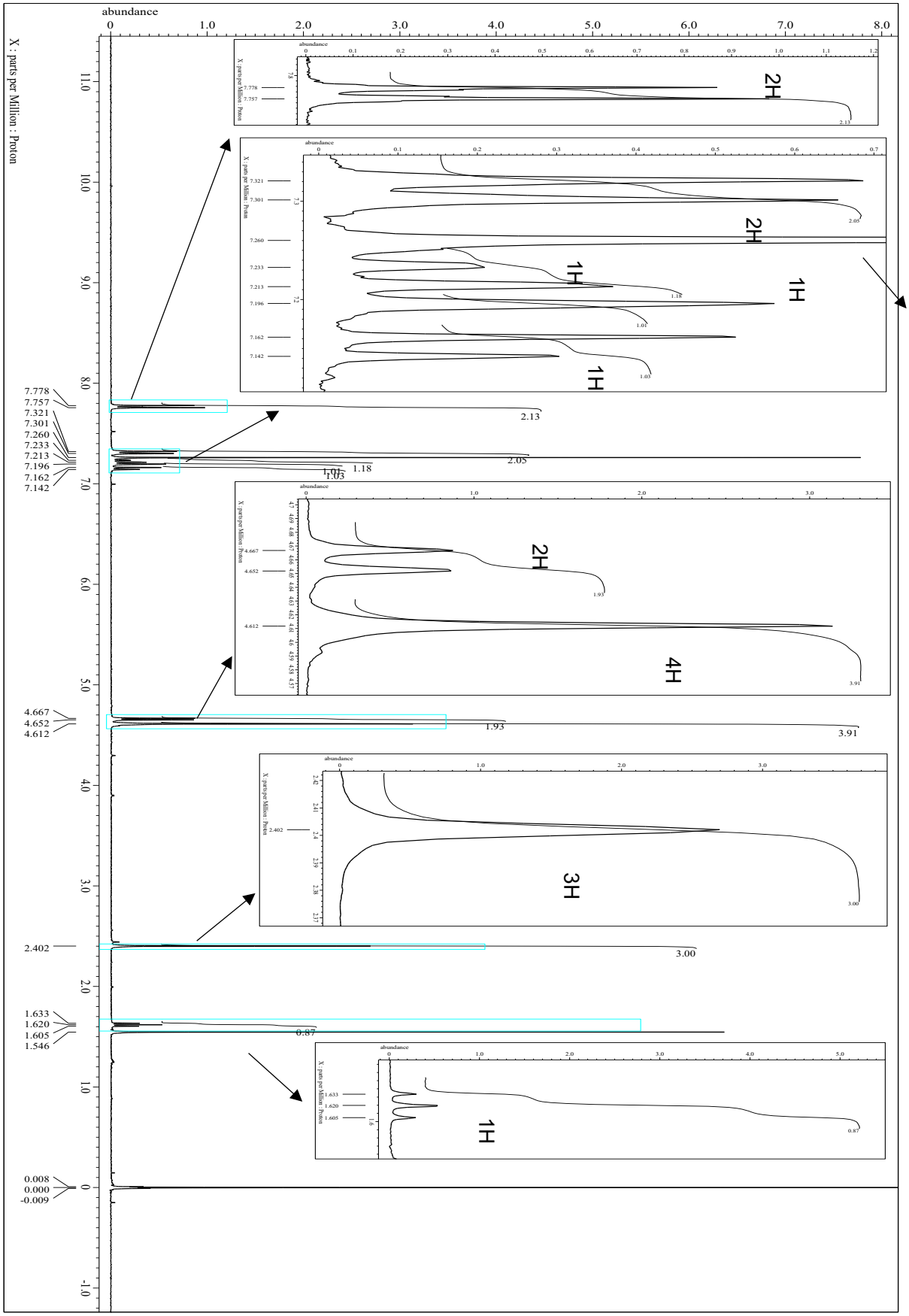
1. A4 size, within 2 pages (except for a copy of your experimental notebook and your answers for the questions).
2. The report should include the reaction scheme, information of the reagents you used, operation of the reaction, TLC information, results, and total discussion and consideration of the experiment. Reaction process and operation including the state of reaction should be described as a flowchart.
3. A copy of your experimental notebook should be attached to your report.
4. Your answers for the questions (A4 size, within 2 pages) should be attached to your report.

^{1}H NMR spectra



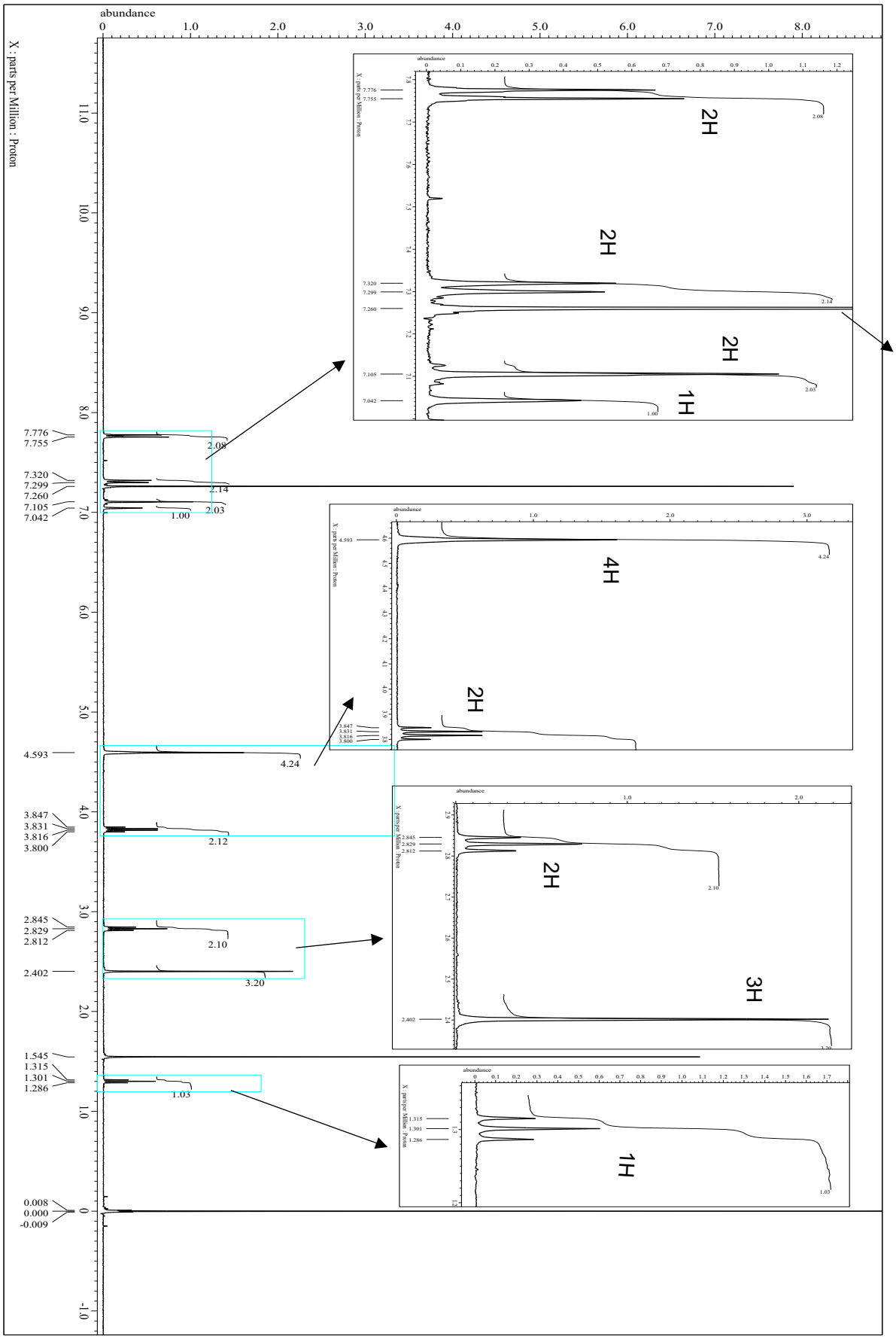
For alkyne **2b** adduct

solvent



For alkyne **2c** adduct

solvent



Experiment 3

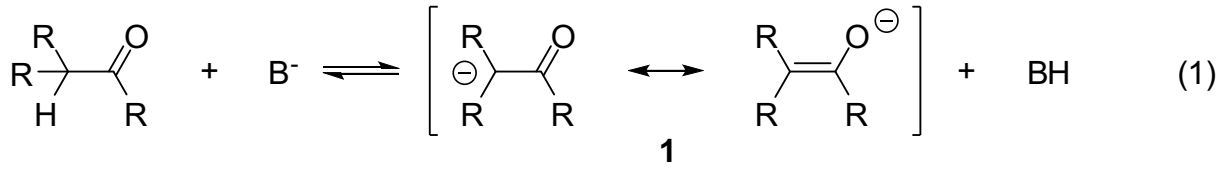
Carbon-Carbon Bond Formation with Enolate Anions

山下研究室担当

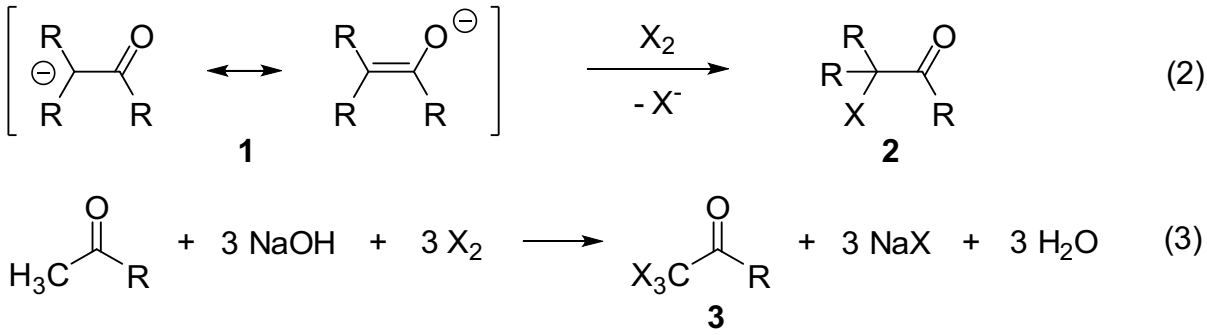
Yamashita Group

Carbon-Carbon Bond Formation with Enolate Anions

The nature of carbonyl groups affects their neighboring α- and β-carbon atoms. The partial positive charge on the carbonyl carbon makes the hydrogens on the α-carbons (the "α-hydrogens") acidic, and to produce an enolate anion in which the resulting carbanion is stabilized by resonance with the carbonyl π-electron system (**1**, eq. 1).

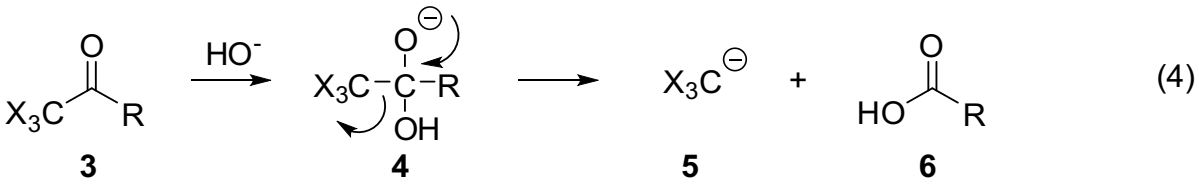


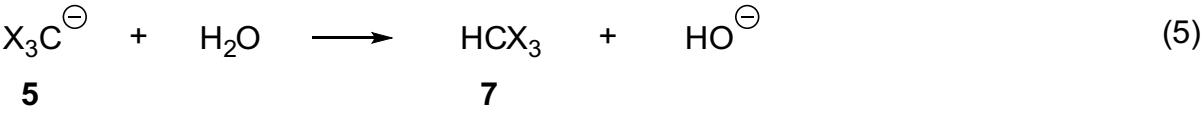
Haloform Reactions. When an aldehyde or ketone that has α-hydrogens is treated with a halogen in a basic medium, the enolate anion reacts rapidly with the halogen (eq. 2). In the case of a methyl ketone, all three of the α-hydrogens of the methyl group are replaced with halogen to give **3** (eq. 3, R = H or alkyl).

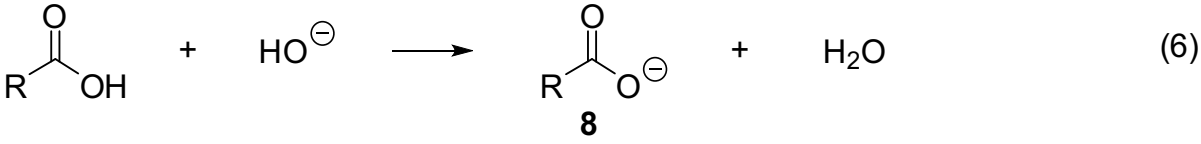


When the first α-hydrogen is replaced with halogen, the remaining hydrogens attached to this α-carbon atom become more acidic due to the inductive effect of the electronegative halogen atom, so that further substitution by halogen at this site occurs more rapidly than at the other α-carbon atom. The inductive effect of the three halogens makes the carbon atom of the carbonyl group particularly susceptible to nucleophilic addition of hydroxy anion. The intermediate adduct **4** readily undergoes C-C bond cleavage, as shown in eq. 4, and the fragments **5** and **6** are immediately converted to the products, a haloform **7** and a carboxylic acid salt **8** as shown in eq. **5** and **6**. (**7** and **8** may also be formed simply by transfer of a proton from **6** to **5**.) The overall equation for the reaction of a methyl

ketone or acetaldehyde (R = H) is given in eq. 7.

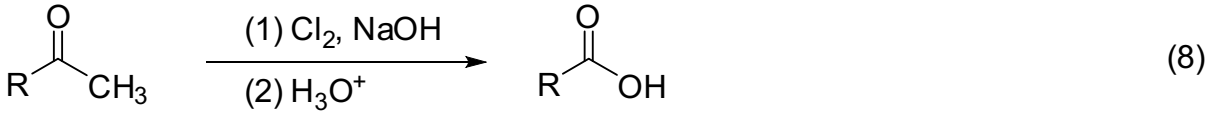






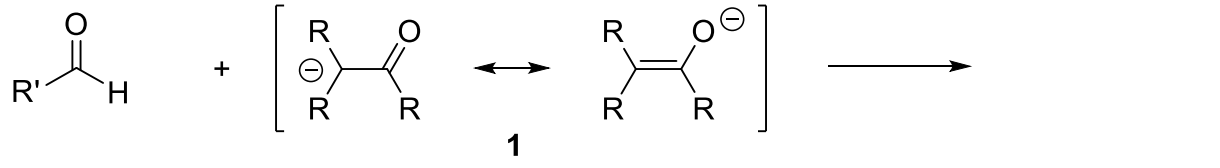
Although all carbonyl compounds having α-hydrogens undergoes halogenation at α-positions, only *methyl* ketones undergo the carbon-carbon cleavage, since three halogen atoms attached to the one carbon atom are required to weaken the bond. This fact is utilized in two important ways. (1) Since iodoform (CHI_3) is a highly insoluble crystalline yellow solid with a characteristic odor, its formation is used as a *qualitative test* for the structural moieties shown here (R = H, alkyl, or aryl). Note that *alcohols* shown below also give a positive result since halogens can partially oxidize them to the corresponding carbonyl compounds. (2) The conversion of a methyl ketone to a carboxylic acid

with one less carbon atom is often useful in synthesis. In this case, chlorine is the halogen of choice because of its cost and availability.

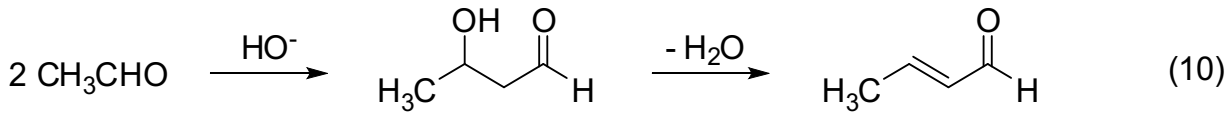


Aldol Additions and Condensations. Another important reaction of enolate anions is the addition to the carbonyl group. In this way, an anionic product **9** is produced, which stabilizes itself by abstracting a proton from the solvent (water or alcohol), as shown in eq. 9. The β-hydroxycarbonyl compound

of type **10** is called “aldol”, since it is both an aldehyde and an alcohol. The term “aldol addition” is also applied generally to the base-catalyzed self-addition of ketones as well as to aldehydes. The overall change for the reaction of acetaldehyde is given in eq. 10. Most β-hydroxy aldehydes and ketones undergo dehydration readily to α,β-unsaturated aldehydes and ketones. In this case, the overall reaction is referred to as “aldol condensation” since a molecule of water is eliminated from the adduct.

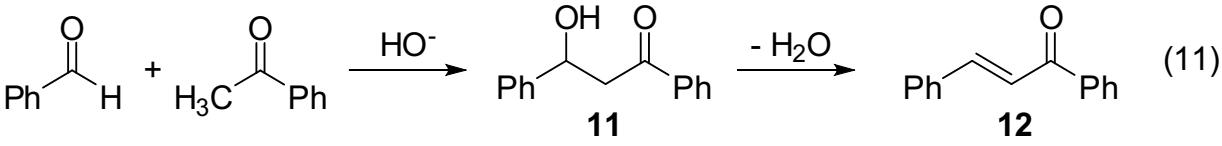


(9)



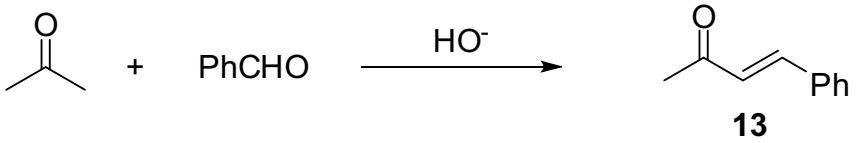
(10)

In general, ketones do not undergo self-addition as readily as aldehydes; in fact, special conditions must usually be employed to obtain good yield in such reactions. (Refer to a textbook for a discussion of the reaction of acetone, for example.) “Mixed (or cross) aldol condensation” between two different aldehydes or a pair of an aldehyde and a ketone is possible. Such mixed condensations are synthetically practical only between (1) an aldehyde bearing no α-hydrogens and hence only serving as an electrophile and (2) a ketone or aldehyde which can form enolate but does not easily undergo self-condensation. A good example is the reaction of benzaldehyde with acetophenone in the presence of dilute sodium hydroxide solution (eq. 11). The product **12** is called benzalacetophenone (1,3-diphenylpropen-1-one). Under the condition of the experiment, the dehydration of the aldol **11** is spontaneous.

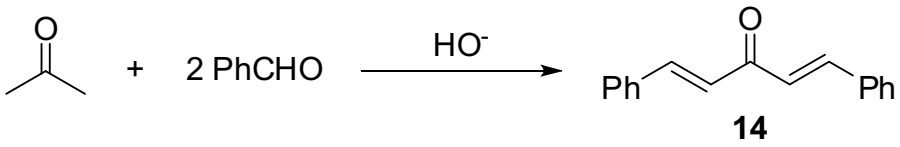


(11)

However, when acetone is used instead of acetophenone, both α-carbons are equally susceptible to aldol condensation under conventional aqueous basic conditions to give dibenzalacetone (1,5-diphenyl-1,4-pentadien-3-one **14**) in the presence of two equivalents of benzaldehyde. Slightly controlled manipulation is required for the selective formation of benzalacetone (4-phenyl-3-buten-2-one **13**).



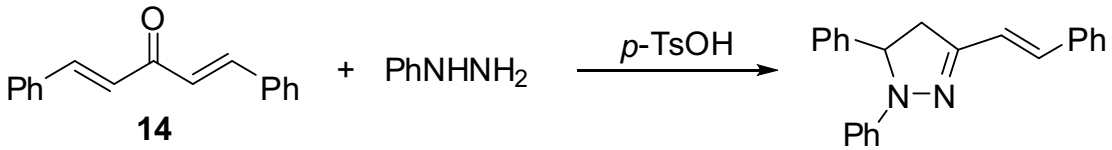
13 (12)



14 (13)

Reactions of Dibenzalacetone.

2,4-Dinitrophenylhydrazones and phenylhydrazones. 2,4-Dinitrophenylhydrazones and phenylhydrazones of aldehydes and ketones are prepared by an analogous method. However, the 2,4-dinitrophenylhydrazone derivatives are generally easier to prepare and purify than the phenylhydrazone derivatives. Therefore, they are usually the derivative of choice, especially for lower molecular weight carbonyl compounds. 2,4-Dinitrophenylhydrazone **14** is uniformly formed under conventional conditions. However, when phenylhydrazone is added instead of 2,4-dinitrophenylhydrazone, the resultant hydrazone reacts consecutively to give pyrazoline **15** in the presence of a catalytic amount of *para*-toluenesulfonic acid (eq. 14).



14 **15** (14)

Experimental Procedure

A. Preparation of dibenzalacetone 14 (Day 1)

In a 100-mL round bottom flask equipped with a stirring bar, sodium hydroxide (2.0 g) was dissolved in water (25 mL), and the solution was diluted with ethanol (20 mL). To the solution at room temperature with vigorous stirring, one half of a mixture of benzaldehyde (2.6 g) and acetone (0.73

g) was added. Within 5 minutes, a yellow cloud was observed which quickly turned into a flocculent precipitate. Then, to the mixture was added the rest of the mixed reagents. The container of the mixed reagents was rinsed with a small amount of ethanol and the ethanol solution was combined with the reaction mixture. The combined mixture was vigorously stirred for an additional 30 min, and the mush was filtered with suction on a Büchner funnel. The crude product was thoroughly washed with water and dried by pressing between filter paper three times. The yield was 2.57 g (88% of the theoretical amount) of a product which melts at 104-107 ºC.

The crude dibenzalacetone was recrystallized from hot ethyl acetate, using 2.5 mL of ethyl acetate per gram of the crude product. The recovery in this purification was about 55%; the purified product melts at 110-111 ºC. Its ^{1}H NMR spectrum is given in Figure 1.

B. Formation of 2,4-dinitrophenylhydrazone of dibenzalacetone (Day 2 and 3)

To a 50-mL Erlenmeyer flask was charged with 2,4-dinitrophenylhydrazine (0.19 g) and ethanol (15 mL). The mixture was stirred for 5‑10 min in a water bath at 80 ºC. To the mixture was added dibenzalacetone (0.24 g) dissolved in ethanol (5 mL), and *para*-toluenesulfonic acid (10 mg, as a solid). The container of dibenzalacetone was rinsed with a small amount of ethanol and the ethanol solution was combined with the reaction mixture. The combined mixture is heated in a water bath at 80 ^{o}C for 30‑60 min until a dark red solid appears. The reaction mixture is cooled to room temperature using another water bath. The formed crystals were collected by filtration and washed with 10 mL of ethanol. Then the dark red crystals are dissolved in a minimum amount of ethyl acetate at 80 ºC and then 4‑5 mL of hexane is added to the clear solution. The solution is kept for 1 night at ambient temperature to yield dark red prisms. After filtration, pure 2,4-dinitrophenylhydrazone of dibenzalacetone was obtained; yield 0.14 g; m.p. 173.5 – 175.0 ºC.

C. Reaction of Phenylhydrazine with Dibenzalacetone (Day 2)

To a 50-mL Erlenmeyer flask was charged with dibenzalacetone (0.33 g), *para*-toluenesulfonic acid (10 mg), phenylhydrazine (0.36 g), and ethanol (10 mL). The mixture was stirred at 80 ºC for 1 h and kept at ambient temperature for 12 h. A formed yellow precipitate was collected by filtration and washed with 10 mL of aqueous ethanol (70 vol. %) to give 0.10 g of a crude product. The yellow solid is dissolved in a minimum amount of ethanol at 80 ºC and then 3 mL of hexane is added into the clear solution. Cooling the solution in an ice-water bath yielded yellow microneedles of the pyrazoline; yield 0.04 g; m.p. 146.0 – 148.0 ºC. The ^{1}H NMR spectrum of the pyrazoline is shown in Figure 2.

Problems

1. Write a stepwise mechanism of the formation of benzalacetone.

2. Assign the ^{1}H NMR spectrum of 1,5-diphenyl-3-styrylpyrazoline (Fig. 2) formed from the reaction of dibenzalacetone with phenylhydrazine. Particular attention should be paid to explain the coupling pattern of signals around δ 2.9 – 5.4.

3. Write a stepwise mechanism of the formation of 1,5-diphenyl-3-styrylpyrazoline and explain the reason why products are different when dibenzalacetone was treated with phenylhydrazine and 2,4-dinitrophenylhydrazine.

Fig.1 ^{1}H NMR spectrum of dibenzalacetone (300MHz)

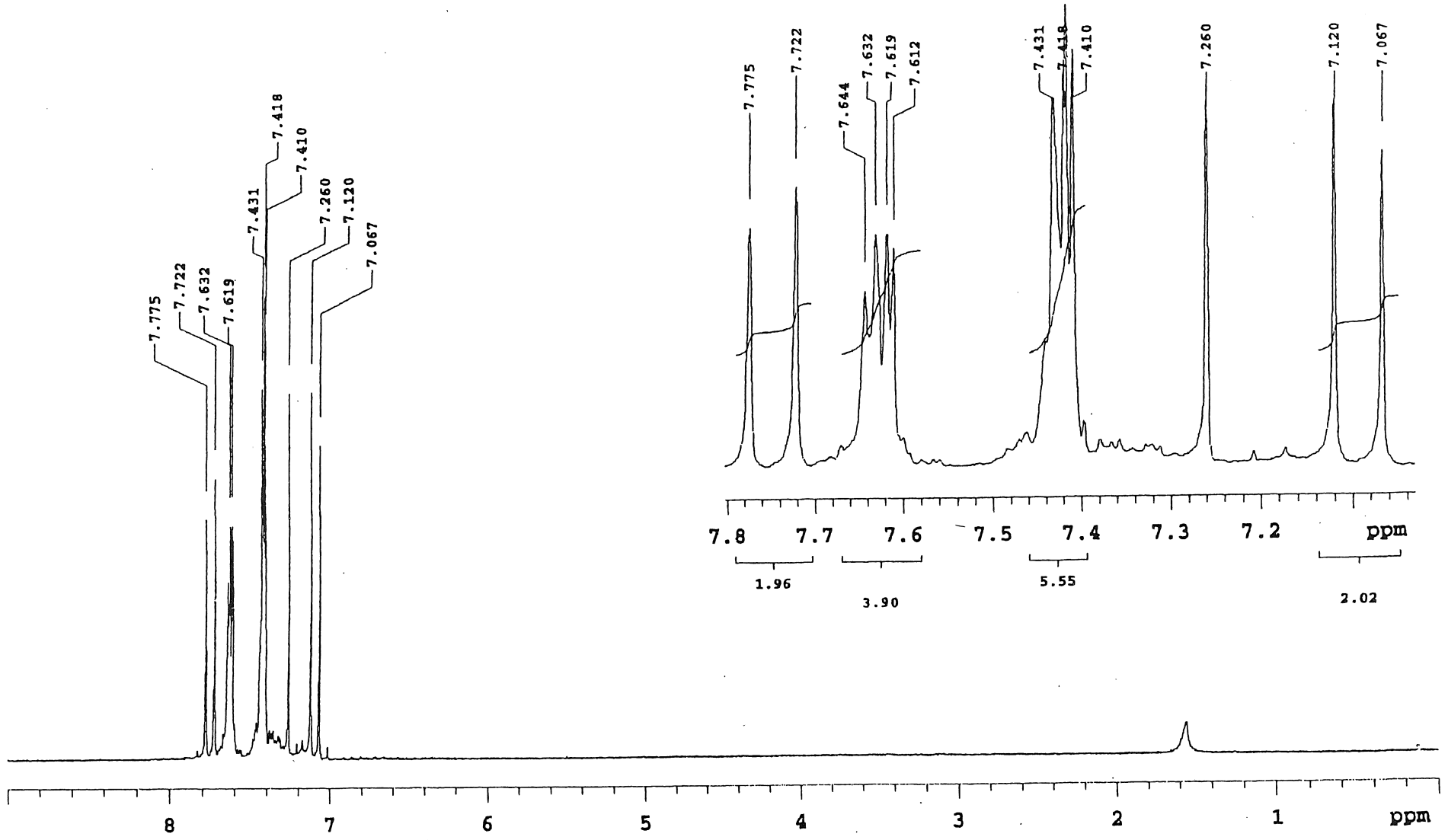
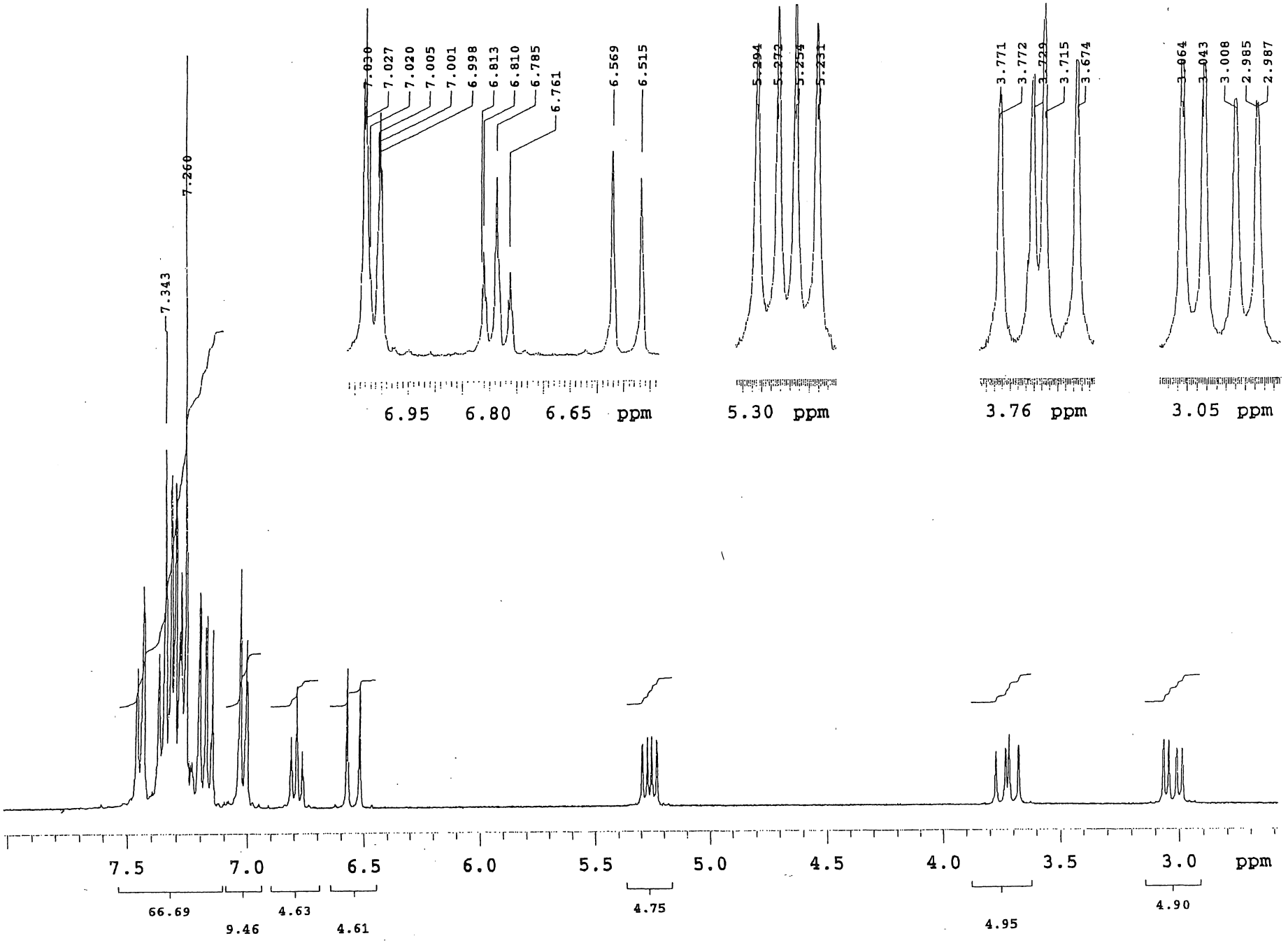


Fig.2. ^{1}H NMR spectrum of 1,5−diphenyl−3−styrylpyrazoline (300MHz)



Experiment 4

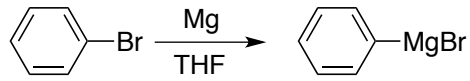
・Cross-Coupling Reaction with Grignard Reagents

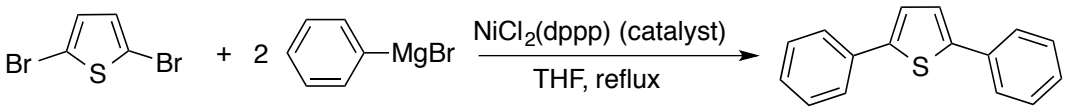
・Chemiluminescence with Luminol

忍久保研究室担当

Shinokubo Group

I. Nickel-Catalyzed Cross-Coupling Reaction with Grignard Reagents (Kumada–Tamao–Corriu Coupling) (2 days)





Transition metal-catalyzed cross-coupling reactions are useful tools in organic synthesis. In this section, we synthesize 2,5-diphenylthiophene by means of Kumada–Tamao–Corriu coupling with a Ni(II) catalyst and a Grignard reagent. Through this experiment, we learn how to treat moisture- and air-sensitive reactions as well as how to isolate a functional organic dye.

※ This reaction is moisture- and air-sensitive. The reaction must be conducted with using dry equipment and solvent under nitrogen atmosphere.

Table 1. Molecular weight of reagents

Compounds	***Mw***
magnesium	24.31
bromobenzene	157.01
2,5-dibromothiophene	241.93
1,3-bis(diphenylphosphino)propane nickel dichloride	542.04
2,5-diphenylthiophene	236.33

1. Preparation

Place a magnetic stirring bar and magnesium (6.6 mmol) in a two-neck round-bottom flask (100 mL) equipped with a reflux condenser, a three-way stopcock with a N_2-filled balloon, and a rubber septum (see Figure 1). Evacuate the flask and refill it with N_2 three times according to the following procedure (Figure 2).

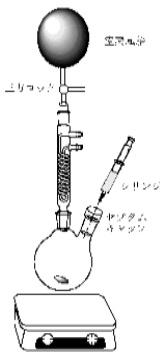


Figure 1.

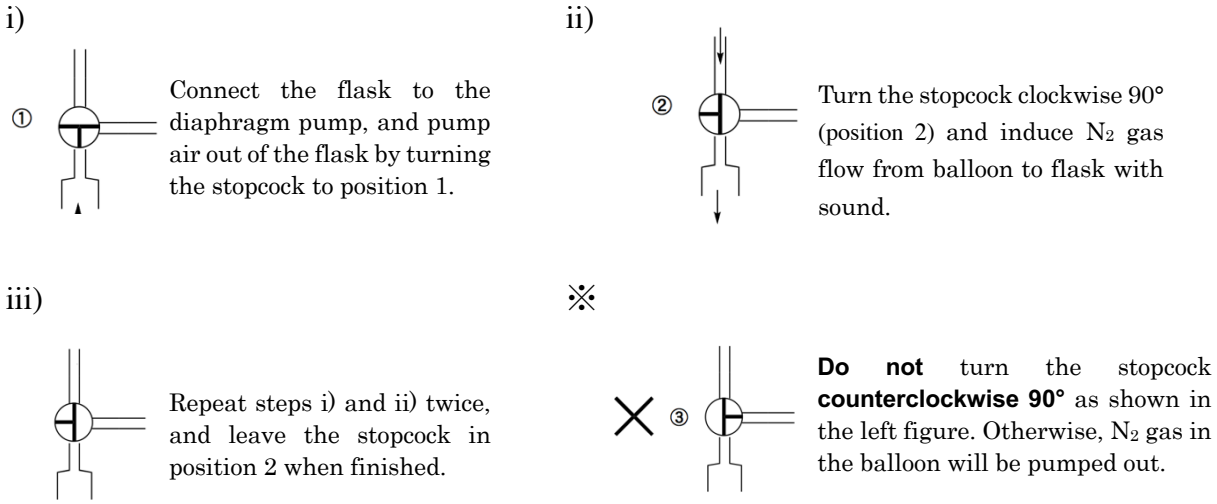


Figure 2. Instructions for a degassing process with a three-way stopcock

2. Generation of Grignard reagent

Draw a THF solution of bromobenzene (1.32 M, 5.0 mL) with a 12 mL syringe. To the round-bottom flask prepared in Section 1, add this bromobenzene solution (approximately 1 mL) dropwise through a rubber septum *via* a syringe. Stir the resulting mixture vigorously until the reaction begins. After the reaction starts, add the remaining solution of bromobenzene dropwise. (**Caution!** The reaction is exothermic. **DO NOT** add the solution in one portion.) After all the solution is added, stir the mixture for an additional 15 min at room temperature.

* Monitor the color change upon the generation of Grignard reagent carefully.

3. Nickel-catalyzed cross-coupling reaction

Draw the solution of Grignard reagent through a septum with a 12 mL syringe. (Keep the solution in the syringe still you use it again.) Add a THF solution of 2,5-dibromothiophene (0.75 M, 4.0 mL) to the round-bottom flask with another 12 mL syringe. Turn the three-way stopcock 45°, allowing nitrogen gas to flow out gently. Add 1,3-bis(diphenylphosphino)propane nickel(II) dichloride (0.033 mmol) by removing the septum. After that, the flask should be sealed with the septum as quickly as possible. Return the stopcock to the previous position. Add the stored Grignard solution dropwise through the septum *via* the syringe. Heat the reaction mixture to reflux for 30 min.

After stirring the reaction for 1 day at room temperature, quench it with $NH_4Cl_{(aq)}$ (10 mL). Add ethyl acetate (20 mL) to the mixture. Remove insoluble materials by suction filtration. Wash the insoluble materials with ethyl acetate (10 mL) and water (10 mL). Transfer the obtained solutions to a separatory funnel, and collect the organic layer. Wash the aqueous layer with ethyl acetate

(approximately 10 mL) twice. Combine the organic layers, and dry them over Na_2SO_4. Check the reaction progress by TLC. After checking TLC, add SiO_2 (about 4 g) to the solution. After swirling the solution for 1 min, remove Na_2SO_4 and SiO_2 by filtration. Put the filtrate in a 100-mL round-bottom flask. Remove the solvent under reduced pressure by using a rotary evaporator. Add MeOH (about 30 mL) to the residue. Conduct the suction filtration of the resulting suspension. Drying the filtrate provides pale yellow solids.

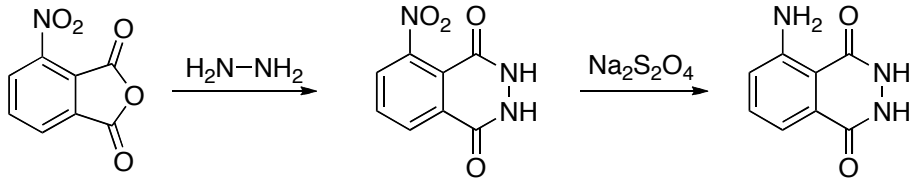
4. Luminescence of 2,5-diphenylthiophene

Observe the luminescence of 2,5-diphenylthiophene upon UV irradiation, both in solution and solid state.

Questions

1. Describe a reaction mechanism for the cross-coupling reaction in this experiment. List two examples of cross-coupling reactions using transition metal catalysts.
2. Show products provided by the reactions of an excess amount of phenylmagnesium bromide with the following three electrophiles, respectively: acetaldehyde, ethyl acetate, and acetone. These reactions will be quenched by diluted HCl aq. In addition, list these electrophiles in descending order of the reactivity.

II. Chemiluminescence of Luminol (1 day)



I **II** **III**

3-Aminophthalhydrazide (**III**) is known as luminol. It exhibits chemiluminescence upon oxidation. In this experiment, we will prepare compound **III** from 3-nitrophthalic anhydride (**I**) *via* 3-nitrophthalhydrazide (**II**). We will then observe the chemiluminescence upon treatment of **III** with hydrogen peroxide and potassium ferricyanate in a basic solution.

1. Synthesis of 3-Nitrophthalhydrazide (**II**)

Add 3-nitrophthalic anhydride (0.50 g) and acetic acid (2 mL) to a tube capped with an aluminum foil. Heat the resulting mixture at 120 °C. Stir it until the solid becomes completely dissolved. Cool the solution to room temperature. Add hydrazine monohydrate[*1] (0.14 mL) dropwise with shaking the tube vigorously in order to prevent the mixture from solidifying. (**Caution!** Wear gloves at the time of handling hydrazine monohydrate because it is harmful.) Again heat the resulting mixture at 120 °C and stir it for 10 min. White precipitates will be generated after cooling to room temperature. Vacuum filtration followed by washing with water and then a small amount of methanol produces a pale-yellow solid (0.5 g) (m.p. > 300°C decomp.). (The product is moderately soluble in methanol. One wash with methanol is enough.)

2. Synthesis of 3-Aminophthalhydrazide (**III**)

Place 3-nitrophthalhydrazide, synthesized in Section 1, into the tube and dissolve it in 10% (*w*/*w*) $NaOH_{(aq)}$ (2.5 mL). (When solid samples remain undissolved, crack them with a spatula and stir the mixture.) To the resulting dark red solution, add sodium hydrosulfite (1.5 g) and wash the walls with a small amount of water. Heat the solution for 5 min with stirring (watch the color change!!). After cooling to room temperature, add acetic acid (1.0 mL) and further cool the tube by using flowing water until the formation of yellow precipitates is complete. Filtration affords compound **III** as a pale-yellow solid (0.2 g) (m.p. = 316–320°C).

3. Chemiluminescence of luminol

Dissolve luminol (0.1–0.15 g) in 2% $NaOH_{(aq)}$ (10 mL). Then, dilute this solution (5 mL) using water (15 mL) (Solution A). Prepare another solution by mixing 10% aqueous potassium ferricyanate (2.5 mL), 10% $H_2O_{2(aq)}$ (2.5 mL), and water (10 mL) (Solution B). Pour solutions A and B into a 300-mL Erlenmeyer flask using a large funnel in the dark.

Questions

1. Explain why the color of the solution of **II** changes from colorless to dark red upon treatment with a NaOH solution.

2. Why does luminol display chemiluminescence? Explain and describe the emission mechanism.

Experiment 5

キラル相間移動触媒を利用したフェニルアラニンの不斉合成

Asymmetric Synthesis of Phenylalanine Using
Chiral Phase-Transfer Catalysts

および

(±)-シトロネラールの誘導化

Derivatization of (±)-Citronellal

大井研究室担当

Ooi Group

Molecules are groups of atoms covalently bound together that behave as a single unit. The placement of atoms relative to one another in a molecule can have a huge impact on the physical and biological properties and function of a molecule. Understanding and controlling this relationship is one of the key goals of organic chemistry. The purpose of this student experiment is to understand how the structure of molecules is important, and you will conduct two issues: one is “asymmetric synthesis of phenylalanine using chiral phase-transfer catalysts (For details, see chapter 1-3)” and another is “derivatization of (±)-citronellal (chapter 4)”. **Both two experiments will be started from the first day.**

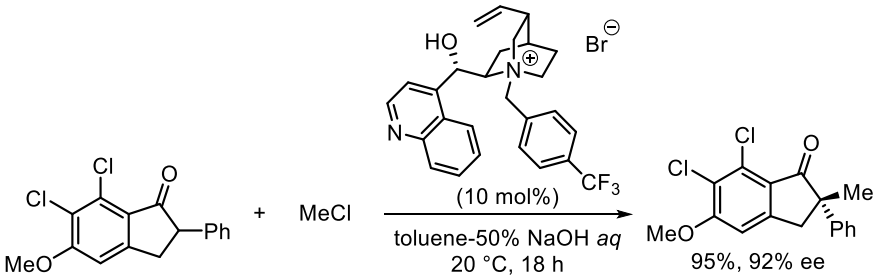
1. Asymmetric Phase-Transfer Catalysis

1.1 Introduction

Phase-transfer catalysis has long been recognized as a versatile methodology for organic synthesis in both industrial and academic laboratories, featuring its simple experimental operations, mild reaction conditions, inexpensive and environmentally benign reagents and solvents, and possibility to conduct large-scale preparations. In particular, during more than the last two decades, asymmetric phase-transfer catalysis based on the use of structurally well-defined chiral, nonracemic catalysts has become a topic of great scientific interest, and recent enormous efforts have resulted in notable achievements, making it feasible to perform various bond-formation reactions under mild phase-transfer-catalyzed conditions. An essential issue for optimal asymmetric catalysis is the rational design of catalysts for targeted reaction, which allows generation of a well-defined chiral ion pair that reacts with electrophiles in a highly efficient and stereoselective manner. This concept, together with the synthetic versatility of phase-transfer catalysis, provides a reliable and general strategy for the practical asymmetric synthesis of highly valuable organic compounds.

1.2 Cinchona Alkaloids-Derived Chiral Phase-Transfer Catalysts (PTCs) for Asymmetric Alkylation

Quaternary onium salts, represented by quaternary ammonium and phosphonium salts, have been routinely employed in synthetic organic chemistry and have played various important roles as stoichiometric reagents, reactive intermediates, ionic liquids, and catalysts. The use of this class of compounds, particularly chiral nonracemic ones, as catalysts gather significant attention because of their unique properties such as chemical stability, ease of handling, and the ability of directly controlling the reactivity of the anionic species. The first example of a successful asymmetric catalysis of a chiral quaternary onium salt was reported in 1984 by the Merck research group: a cinchona-alkaloid-derived ammonium salt was used as a PTC for the enantioselective alkylation of an enolate under biphasic conditions (Scheme 1).



Scheme 1.

1.3 Representative Mechanism of Phase-Transfer-Catalyzed Alkylations

The quaternary ammonium salt-catalyzed phase-transfer alkylations under basic conditions are supported by an interfacial mechanism as depicted in Figure 1. A metal carbanion [$M^+ \cdot C^-$] is generated by the influence of an inorganic base (MOH) at the interface of the organic and aqueous phases, followed by the extraction of the carbanion from the interfacial region into the organic phase by the action of an ammonium ion as a PTC. When the formation of a new ion pair [$Q^+ \cdot C^-$] in the organic phase, the initial anion of the quaternary ammonium salt (X^-) is simultaneously liberated into the aqueous phase in the form of [$M^+ \cdot X^-$]. A subsequent C–C bond formation of [$Q^+ \cdot C^-$] with an alkyl halide (RX) would afford a product with concomitant regeneration of the ammonium salt [$Q^+ \cdot X^-$] (Figure 1).

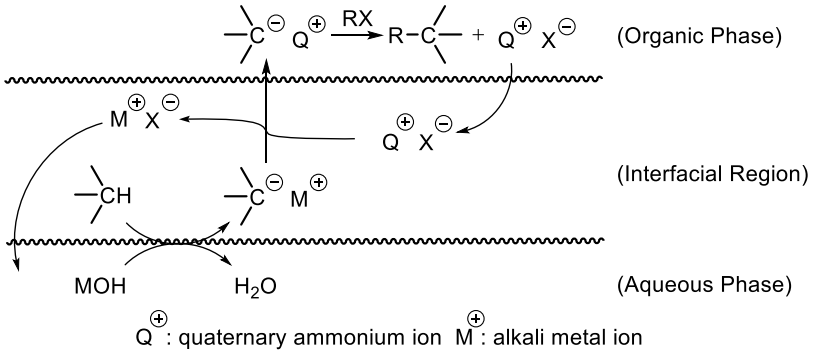
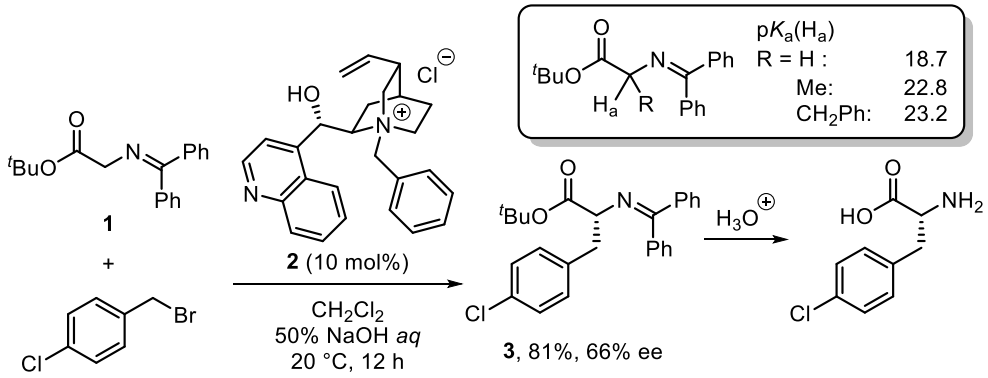


Figure 1. Proposed mechanism of the phase-transfer alkylation.

2. α-Amino Acid Synthesis via Monoalkylation of Glycine-Derived Schiff Bases

In 1989, O'Donnell and coworkers successfully utilized cinchona alkaloid-derived chiral quaternary ammonium salts for the asymmetric synthesis of α-amino acids using *tert*-butyl glycinate benzophenone Schiff base **1** as a key substrate. The asymmetric alkylation of **1** proceeded smoothly under mild phase-transfer conditions, with *N*-benzylcinchonium chloride **2** as a catalyst, to give the alkylation product in a good yield with a moderate enantioselectivity. The Schiff base **1** is an active methylene compound, in which both the carboxylate and the amino group of glycine are protected towards proton abstraction. In addition, the other important aspect of this reaction is the selective formation of the monoalkylated product **3** without concomitant formation of the undesired dialkylated product, as long as the benzophenone Schiff base is employed as a starting substrate, due to the considerable difference in acidity between **1** and **3**. This acidity-weakening

effect is also crucial for securing the configuration stability of the newly created α-stereogenic center under the reaction conditions (Scheme 2).



Scheme 2.

After the report by O'Donnell, research groups over the world have made major contributions to this area of chemistry. This combined effort has helped to establish the asymmetric alkylation by the use of PTCs as a powerful tool for not only natural and unnatural α-amino acids but also the construction of various biologically active natural products. Furthermore, major efforts towards this direction have recently resulted in notable achievements, whereby it has become feasible to perform a variety of enantioselective bond-forming reactions under mild phase-transfer conditions, and inspired the further design of cinchona alkaloid-derived PTCs and other forms of chiral PTCs.

References:

1. *Asymmetric Phase Transfer Catalysis*; Maruoka, K., Ed.; Wiley-VCH: Weinheim, Germany, 2008.

2.「有機分子触媒の新展開」柴崎正勝編, CMC Books, 2006.

3.「進化を続ける有機触媒―有機合成を革新する第三の触媒」丸岡啓二編, 化学同人, 2009.

4. Maruoka, K.; Ooi, T. *Chem. Rev.* **2003**, *103*, 3013-3028.

5. Ooi, T.; Maruoka, K. *Angew. Chem., Int. Ed.* **2007**, *46*, 4222-4266.

3. Experiment 1

****

"Preparation in Advance"

You will synthesize one catalyst molecule from cinchona alkaloid analogue (Core Unit A) and alkyl halide (Subunit B). Therefore, choose your favorite combination of one Core Unit A and Subunit B from the following list. During the course of your choice, you should consider the transition-state structure of the catalyst with glycinate Schiff base.

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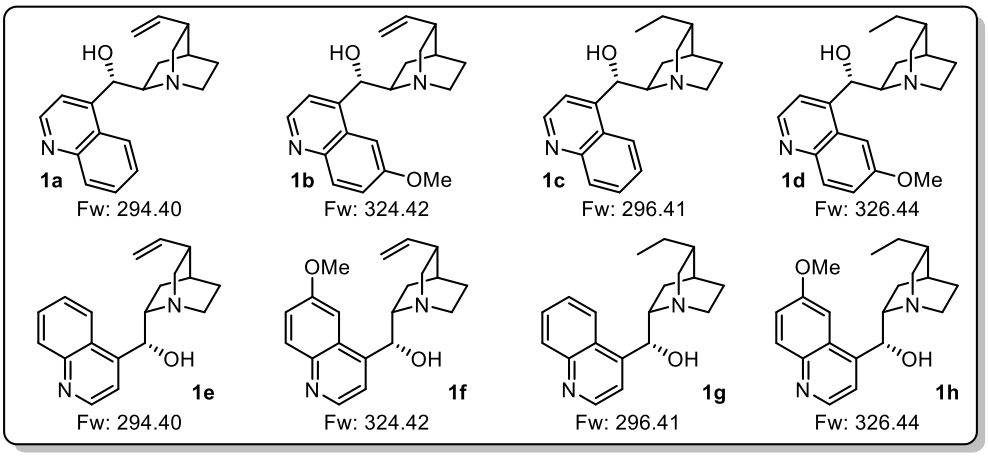
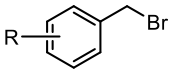
3.1 List of a Core Unit A and Subunit B

Core Unit A: Cinchona Alkaloids



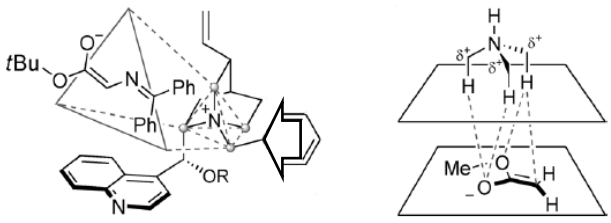
+

Subunit B: Alkyl Halides



Chose one unit from 6 candidates.

The structures differ between first and second halves.

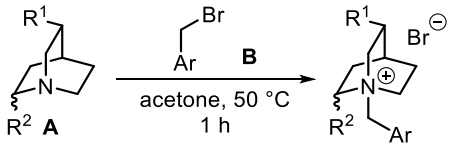


Hint! [Proposed structure in transition state]

Ref. Corey, E. J.; Xu, F.; Noe, M. C. *J. Am. Chem. Soc.* **1997**, *119*, 12414-12415.

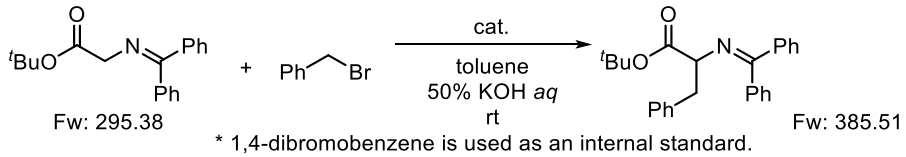
Figure 2.

3.2 Synthesis of Cinchona-Derived Chiral PTCs



Alkyl halide **B** (>0.2 mmol), and cinchona alkaloid **A** (0.2 mmol) that you chose are placed into a test tube. After addition of 2 mL of acetone at room temperature, the resulting suspension is stirred vigorously for 1 h at 50 °C. The reaction mixture is cooled to room temperature and diluted with 8 mL of hexane. After the stirring is kept for further several minutes, the precipitate is filtered and washed with hexane to collect the corresponding ammonium bromide as a powdery material.

3.3 Asymmetric Monoalkylation under the Phase-Transfer Conditions



* 1,4-dibromobenzene is used as an internal standard.

N-(Diphenylmethylene)glycine *tert*-butyl ester (0.29 g, 1.0 mmol), 1,4-dibromobenzene (0.24 g, 1.0 mmol), and the ammonium bromide (0.02-0.03 g) are dissolved in 6.0 mL of toluene. into the vial. Then, benzyl bromide solution (1.1 M in toluene, 1.1 mmol) and 2.0 mL of 50% aqueous KOH are successively introduced into the vial. The reaction mixture is vigorously stirred for about 20 h at room temperature. The reaction is quenched by addition of 4 mL of water and the aqueous phase is removed by use of a pipette.

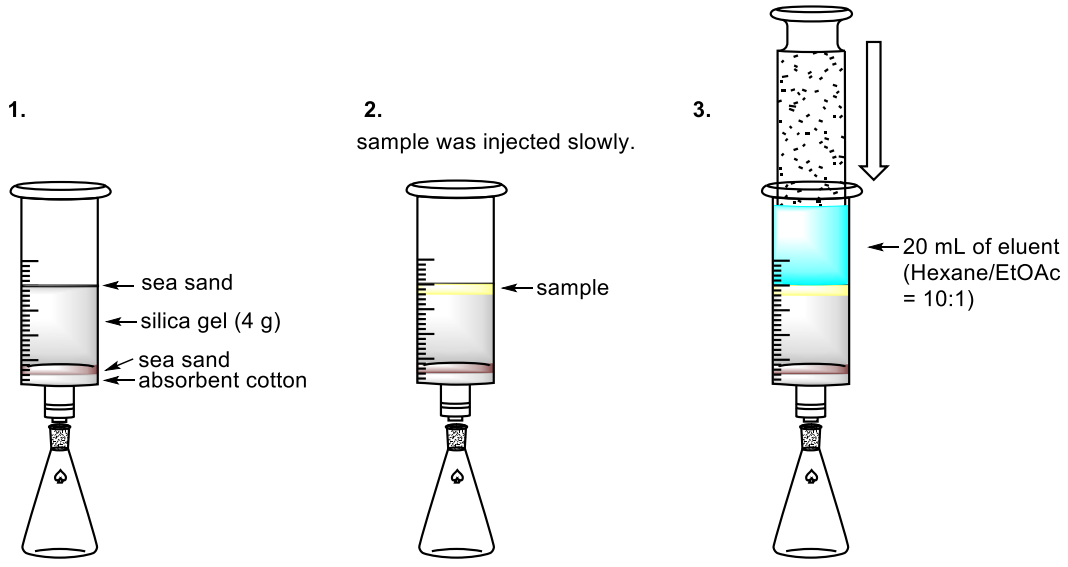


Figure 3.

The remaining organic phase is passed through a short path silica-gel column (eluent hexane/ethyl acetate = 10:1) as illustrated in Figure 3. A little amount of the collected solution is submitted for the determination of a yield and an enantiomeric excess of the desired product by chiral stationary phase HPLC analysis

(DAICEL CHIRALCEL OD-H, hexane:isopropanol = 10:1, Flow rate = 0.75 mL/min, retention time; 4.8 min for Internal Standard, 5.3 min for *R*-isomer, and 6.8 min for *S*-isomer).

***Calculation*:** SM: Schiff base, P: Product, IS: Internal Standard, RF: Response Factor

- Product Yield

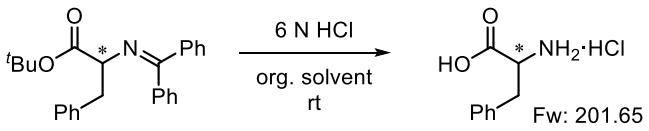
$$\text{mmol (P)} = \frac{\text{area (P}(R) + \text{P}(S)) \times \text{mmol (IS)}}{\text{area (IS)} \times \text{RF}} \qquad (\text{RF} = 3.544)$$

$$\%\ \text{yield (P)} = \frac{\text{mmol (P)}}{\text{mmol (SM)}} \times 100$$

- Enantiomeric excess

$$\%\ \text{ee (P)} = \frac{\text{area\% (P(major)} - \text{P(minor))}}{\text{area\% (P}(R) + \text{P}(S))} \times 100$$

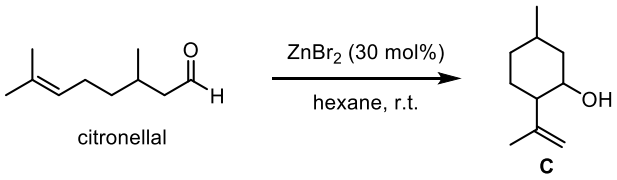
3.4 Preparation of Phenylalanine hydrochloride by Complete Deprotection of the Alkylated Product



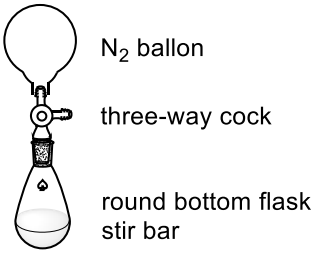
To the remaining solution of the Schiff base is added 4 mL of 6 N aqueous HCl solution, and the whole mixture is stirred vigorously for over 20 h. The mixture is diluted with 4 mL of H_2O and is stand for 5 min. Then, the aqueous phase is transferred into the 100 mL of round bottom flask and is concentrated in vacuo (30-50 hPa) with mild heating (50 ˚C) to give white solids, which are filtered by the aid of 10 mL of ethyl acetate and dried to give phenylalanine hydrochloride as a colorless solid (mp = <220 °C).

4. Experiment 2

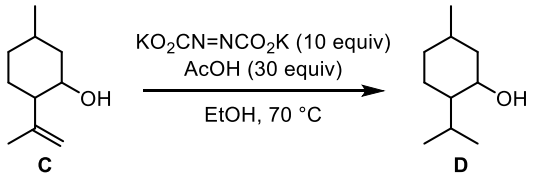
4.1 Ene reaction of (±)-citronellal



Zinc bromide (0.37 g, 1.65 mmol) is placed into a round bottom flask equipped with magnetic stir bar. After addition of 15 mL of hexane at room temperature, citronellal (1.0 mL, 5.5 mmol) is introduced (this operation should be conducted in a fume hood). Three-way cock and nitrogen balloon are then attached to a reaction flask, and the whole mixture is stirred at room temperature overnight. Filtration is then conducted with the aid of hexane and the resulting filtrate is evaporated to remove solvent. The crude residue is purified by silica-gel column chromatography [using Fuji silysia NH (1 g) as stationary phase and EtOAc/hexane = 1:10 (20 mL) as eluent] to give the compound **C** as a colorless oil.



4.2 Reduction of compound C



Compound **C** (0.15 mL, ~1 mmol) and potassium azodicarboxylate (1.9 g, 10 mmol) are placed in a test tube equipped with magnetic stir bar. After addition of 5 mL of EtOH, the suspension is heated at 70 °C (using dry aluminum block bath). A solution of acetic acid (1.2 mL, 20 mmol) in EtOH (5 mL) is then added dropwise to the reaction mixture. After stirring overnight, the reaction mixture is cooled to room temperature. The mixture is then diluted with saturated aqueous solution of $NaHCO_3$ (10 mL), and extracted with ethyl acetate (twice). Combined organic extracts are washed with brine, dried over Na_2SO_4, filtered, and evaporated to give the material including compound **D**.