

— Lecture Note —

Mitsunobu Reaction in My Chemistry: Lecture at VU Study Tour

Shinya HARUSAWA

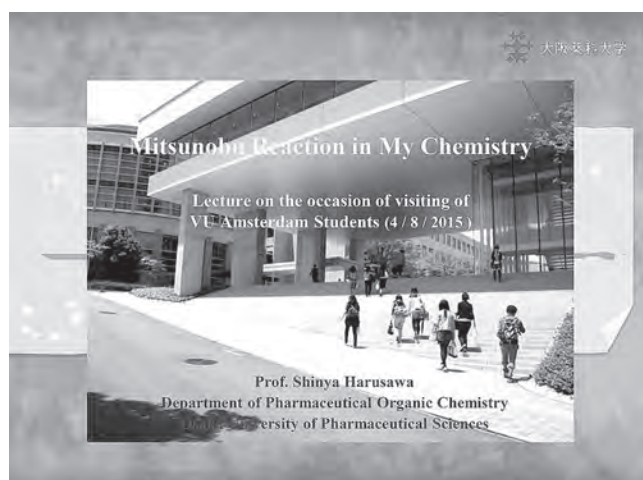
*Osaka University of Pharmaceutical Sciences
4-20-1 Nasahara, Takatsuki, Osaka, Japan 569-1094*

(Received November 18, 2015)

Abstract A total of 20 pharmaceutical sciences and chemistry students of VU University Amsterdam (VU), who are graduating bachelors or first-year master students, travelled to Osaka for the study tour Japan at the beginning of April (4/3-15, 2015). The tour was accompanied by Prof. Martine J. Smit and Dr. Maikel Wijtmans. On Wednesday April 8th, VU students visited Osaka University of Pharmaceutical Sciences (OUPS). I prepared a lot of programs for them as a head of the hosts. Maikel and I gave the lectures for VU and OUPS students at the opening. This is the note of my lecture entitled “Mitsunobu Reaction in My Chemistry”.

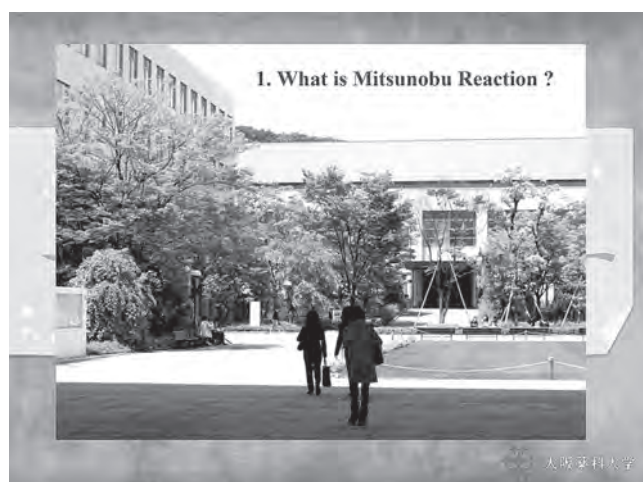
Key words — Mitsunobu Reaction; VU University Amsterdam; Study Tour Japan; Lecture Notes

1.



Today, I'd like to talk VU Amsterdam students on the subject Mitsunobu Reaction in My Chemistry. I often met Mitsunobu reaction at turning points in my chemistry so far.

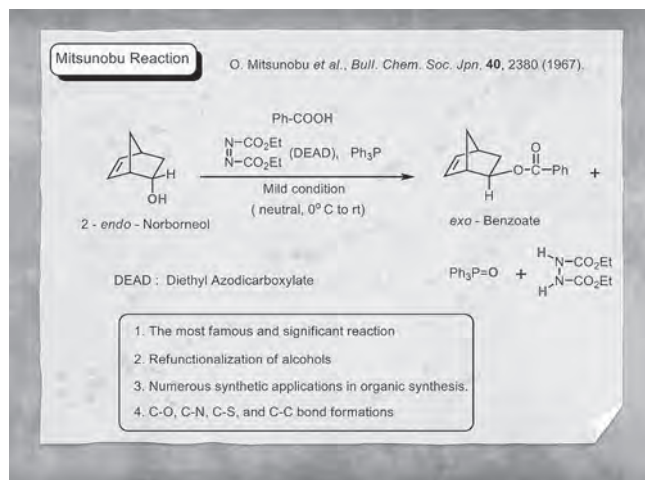
2.



First, what is Mitsunobu-reaction ?

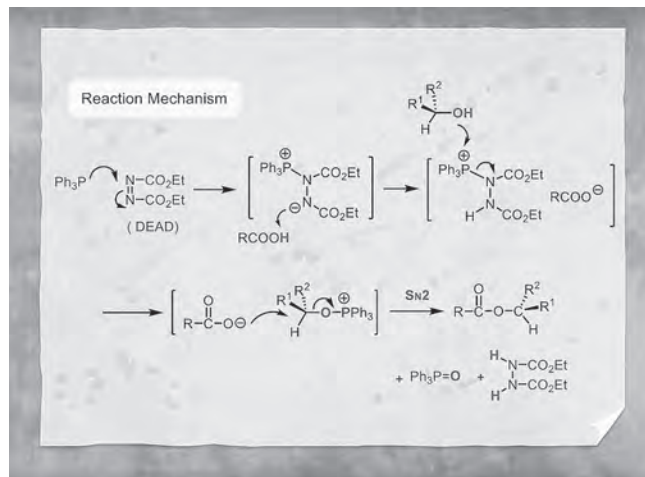
Have you already carried out the Mitsunobu reaction, or heard the reaction-name ?

3.



It is one of the reactions which have been widely used in organic chemistry. In 1967, professor Oyo Mitsunobu at Aoyamagakuin University in Tokyo described the reaction. It was the synthesis of esters from alcohols and carboxylic acids in the

4.



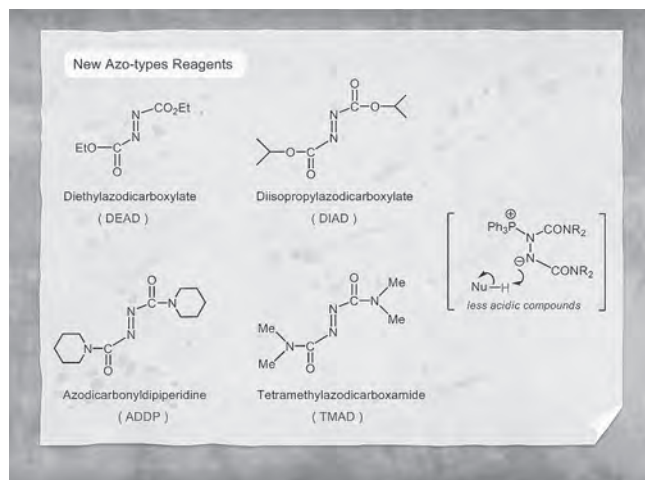
presence of diethyl azodicarboxylate (DEAD) and triphenylphosphine, with inversion of the configuration for asymmetric alcohols, together with triphenylphosphine oxide and hydrazine decarboxylate. Since then, this reaction has become the most famous and significant reaction for refunctionalization of alcohols, and found numerous synthetic applications in organic synthesis.

Apart from the C-O bond formation, Mitsunobu reaction gives C-N, C-S, and C-C bonds. The Mitsunobu reaction involves the reaction of an alcohol with an acidic nucleophile like a carboxylic acid in the presence of triphenylphosphine and DEAD to give reformed C-O, C-N, and C-S bonds. I will describe them right after this. The Mitsunobu reaction is a highly stereoselective process occurring in neutral condition and at 0 degree to room temperature.

The generally accepted mechanism is outlined as follows. Initially, the triphenylphosphine makes a nucleophilic attack on DEAD producing a betaine intermediate, which deprotonates from carboxylic acid. Then, the secondary alcohol attacks on the phosphonium cation to form the key oxyphosphonium ion. The attack of the carboxylate anion on the activated alcohol gives the ester product via Walden inversion of S_N2 reaction, together with triphenylphosphine oxide and hydrazine decarboxylate as by-products.

Viewing two reagents, triphenylphosphine is oxidized to an oxide, while DEAD is reduced to the hydrazine dicarboxylate. Wholly, H₂O was removed from the starting alcohol and carboxylic acid. Accordingly, Mitsunobu reaction is a condensation-dehydration reaction through the oxidation and reduction process.

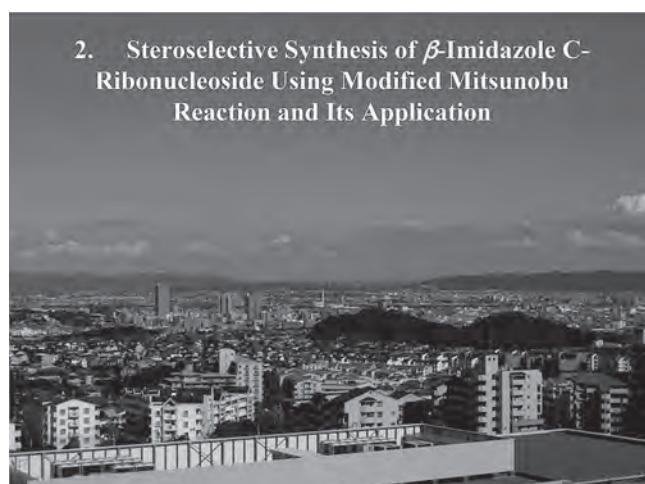
5.



Further, efficient and new azo-types reagents have been developed in particular by replacement of OEt group in DEAD by isopropyl or bulky NR₂ groups, as shown in this slide: DEAD, diisopropylazodicarboxylate (DIAD), azodicarbonyldipiperidine (ADDP), and tetramethylazodicarboxamide (TMAD).

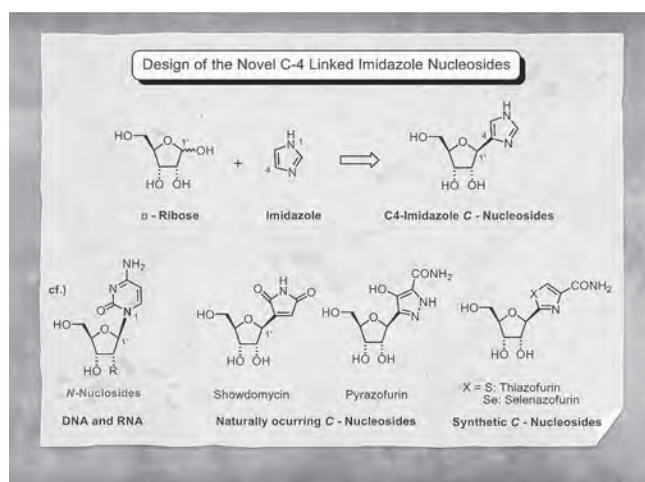
It is postulated that efficiency of the azo-amide compounds is due to enhanced basicity of the betaine, which expands versatility of the reaction with less acidic compounds.

6.



I used first this reaction in the study on stereoselective synthesis of β -imidazole C-nucleosides using a modified Mitsunobu reaction and its application.

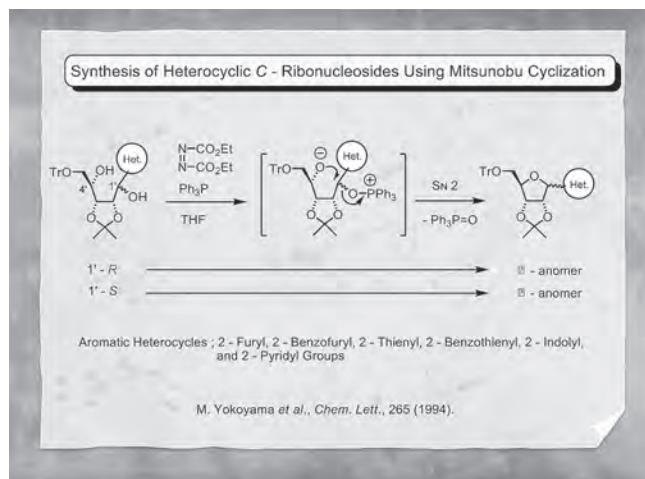
7.



C-Nucleosides are connected by the glycosidic linkage of C-C bond between a heterocycle and the C1' position of a ribose, in contrast to N-nucleosides containing N-1 linkage to the sugar in DNA and RNA. I'll show you some C-nucleosides. Showdomycin and pyrazofurin are known well as naturally occurring C-nucleosides, and thiazofurin and selenazofurin from synthetic studies.

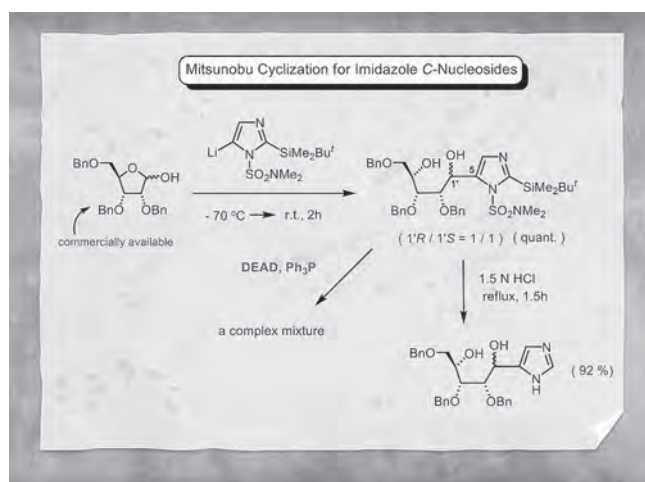
On the other hand, imidazoles are biologically important heterocyclic compounds and they are containing in a variety of useful therapeutic agents. Therefore, I directed my attention to synthesizing C4 linked C-nucleosides having the imidazole as the base moiety, starting from D-ribose and imidazole. The problem is how we make the imidazol C-nucleosides.

8.



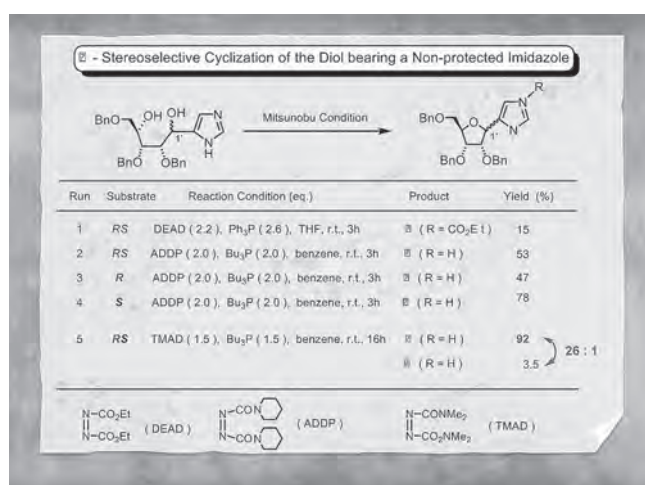
Yokoyama had reported synthesis of heterocyclic C-ribonucleosides having typical aromatic heterocycles in 1994, in which the cyclization of the corresponding diols proceeds through intramolecular S_N2 reaction under Mitsunobu conditions. Orientation of the glycosidic linkage is controlled by the C1' configuration of the substrate, that is, R isomer affords an α -anomer, downward-heterocycle moiety, and S isomer gives the other upward β -anomer which is a natural type.

9.



So, we first used the Mitsunobu cyclization for our synthetic study of imidazole C-nucleosides. Reaction of commercially available tribenzyl D-ribose with lithium salt of bis-protected imidazole gave an inseparable epimeric mixture of the corresponding 5-ribosylimidazole. This compound is a 1 to 1 mixture of R and S isomers at C1' position. When we carried out the cyclization of the mixture under standard Mitsunobu conditions (DEAD and triphenylphosphine), only a complex mixture was obtained. Meanwhile, hydrochloric acid hydrolysis of the diol afforded unsubstituted imidazole, so we investigated the Mitsunobu cyclization for the intact imidazole derivative.

10.



The standard Mitsunobu reaction of a R and S mixture gave a single crystalline compound with an ethoxycarbonyl group at N in the imidazole in only 15% yield. The stereochemistry of the C-1' in the product was determined unambiguously by X-ray analysis. Treatment of the mixture with the other reagent system ADDP-tributylphosphine afforded the β -anomer in modest yield. This result suggested the β -anomer to be produced from both R and S isomers. In fact, the cyclization of R isomer afforded β , and the S-isomer also brought about β -isomer. These three reactions were clear, but the isolated yields were variable owing to the difficulty in product isolation

from the hydrazone byproduct.

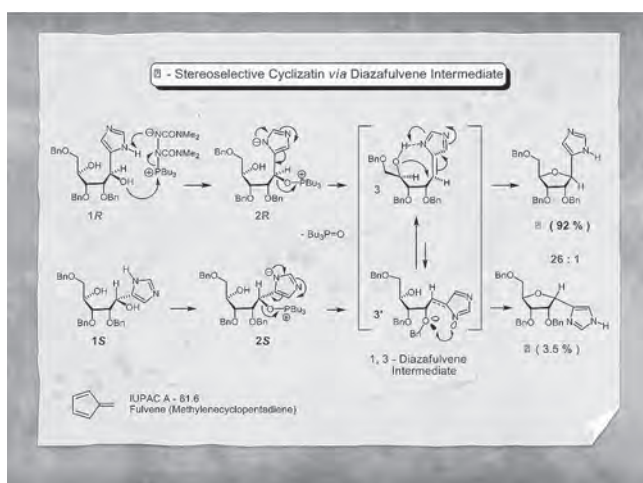
The problem was solved by a water-soluble TMAD. Treatment of the RS mixture with TMAD and tributylphosphine at room temperature in benzene produced the desired β -anomer in 92% yield, together with a small amount of α -anomer (only 3.5%). The ratio of β - and α -isomers was 26 to 1. Of particular interest, it is possible to synthesize the β -anomer without separation of R and S isomers, or without stereoselective synthesis of the R-isomers. It is in

contrast to the results of Yokoyama.

My question is why a combination of TMAD-tributylphosphine supplies exclusively the desirable β -anomer from both R and S-isomers. It is important for us to rationalize that.

The unsubstituted imidazole is indispensable for the exclusive formation of β -anomer. Because, Mitsunobu cyclization of diols bearing mono- or disubstituted imidazole proceeded via S_N2 process.

11.

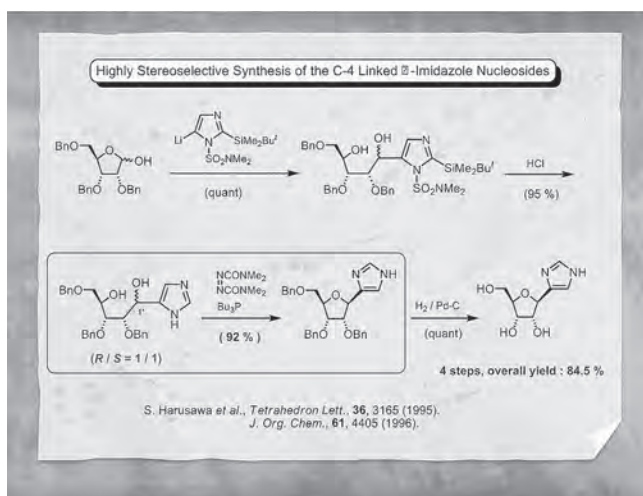


The reaction may be explained as in this Scheme. Reaction of TMAD-tributylphosphine adduct with R

isomer forms the zwitterion 2R. Elimination of stable tributylphosphine oxide indicated by arrows from 2R leads to diazafulvene 3. IUPAC uses fulvene as a traditional term for methylene-cyclopentadiene. The two nitrogen-containing moiety becomes diazafulvene. Spontaneous cyclization assisted by a hydrogen bond gives β -anomer. Alternatively, the S-isomer similarly leads to the active species 3', but it gives the β -anomer via rotamer 3 which is thermodynamically more stable. The remarkable stereoselectivity (26 to 1) may be facilitated by electron repulsion in the intermediate 3'.

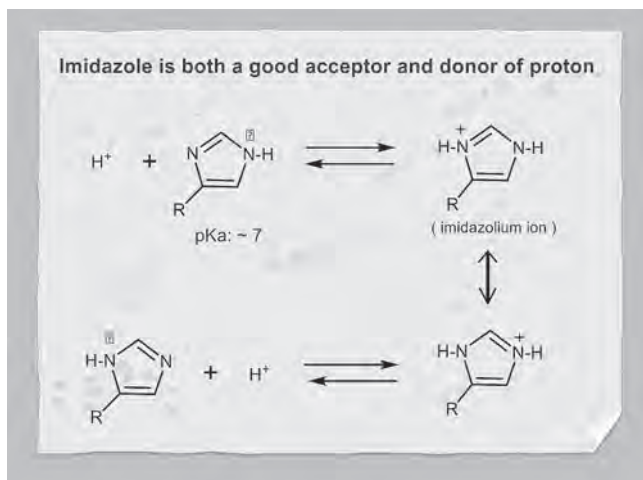
This mechanism is different from that of the standard Mitsunobu S_N2 reaction. Principal intermediate is the diazafulvene, so we call the reaction a modified Mitsunobu cyclization.

12.



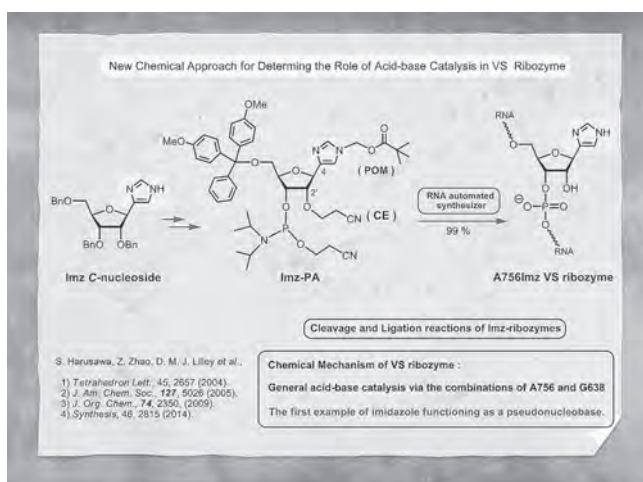
On the whole, we achieved highly stereoselective synthesis of β -imidazole C-nucleoside. The overall yield is 84.5% in four steps from tribenzyl D-ribose, addition, hydrolysis, the modified Mitsunobu cyclization and catalytic reduction. However, the compound and its derivatives did not show any antiviral and anticancer activities.

13.



Do you know imidazole with pKa 7 is used extensively as a building block or as a Brønsted acid and base catalyst in organic chemistry? This slide shows imidazole accepts a proton to form imidazolium ion which through a resonance also donates the proton. τ N-H Imidazole has a tautomer relationship with π N-H imidazole. Given that imidazole is both a good donor and acceptor, imidazole C-nucleosides may be applied to probe general acid and base catalysis of ribozymes in RNA chemistry.

14.

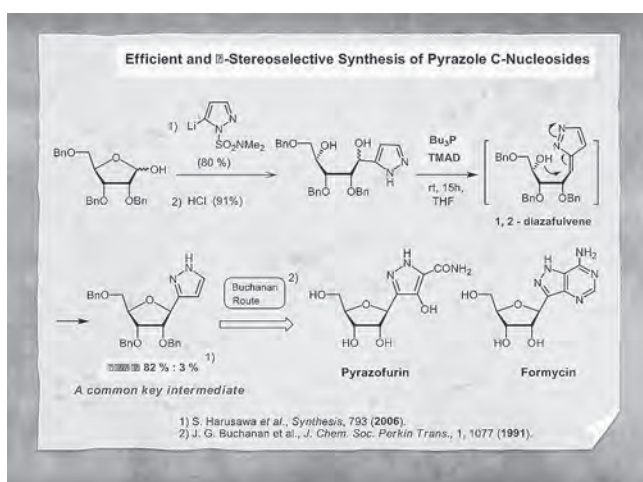


Imidazole-based compounds have not carried out important roles as chemical probes in nucleic acid chemistry, because to date there are few imidazole-intercalating agents into RNA. From this view point, we developed a new chemical approach for

determining the role of acid-base catalysis in ribozyme function, in which imidazole was inserted into A756 of VS ribozyme as a pseudo-base. In this study, we reported the efficient synthesis of C4-linked imidazole ribonucleoside phosphoramidites. During the synthesis of the phosphoramidites, pivaloyloxymethyl (POM) and cyanoethyl (CE) groups were used as a new protecting system for imidazole-nitrogen and C2'-hydroxy functions, respectively. The phosphoramidite was subjected to RNA automated synthesizer to provide Alanine (A) 756 imidazole-substituted VS ribozyme in 99% coupling yield.

Cleavage and ligation reactions of the imidazole-ribozymes indicated that the chemical mechanisms of VS ribozyme involve general-acid base catalysis via the combinations of A756 and G638. This is the first example of imidazole functioning as a pseudo-nucleobase.

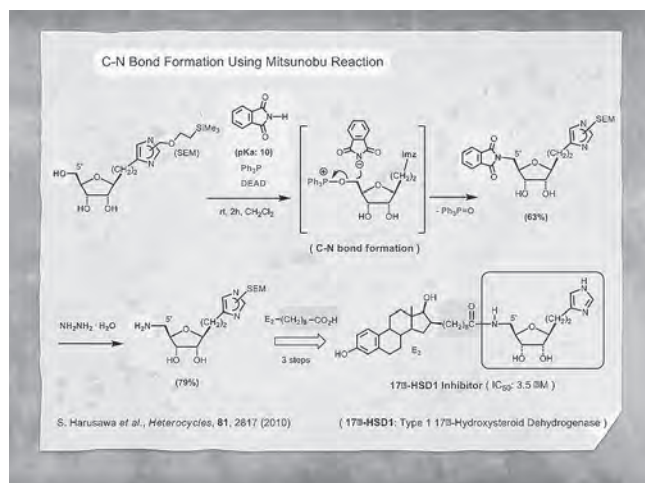
15.



Pyrazoles have neighboring two nitrogen-atoms in five membered ring. Taking account of the analogies between imidazoles and pyrazoles, the synthesis of pyrazole C-nucleosides is feasible by using the modified Mitsunobu cyclization. Just like imidazoles, ribofuranosylpyrazole were stereoselectively synthesized by cyclization of 1, 2-diazafulvene intermediate. The selectivity of β and α is 82% and 3% yields, respectively. This C-nucleoside is a common key intermediate in the route of formycin and pyrazofurin synthesis by Buchanan coworkers.

Therefore, the synthesis of pyrazole C-nucleosides can be seen as the formal synthesis of these compounds.

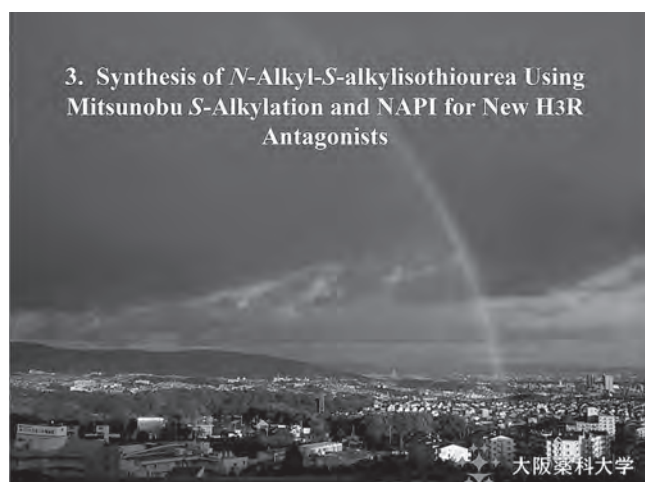
16.



Next, I would like to show a C-N bond formation using Mitsunobu reaction. It was used in the synthesis of estradiol-imidazole C₂-ribonucleoside hybrid compound which exhibits 17 β -HSD1 inhibitory effects

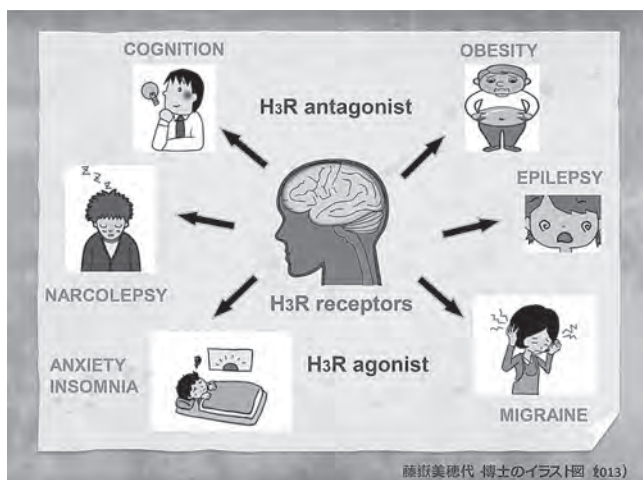
against type 1-17 β -hydroxysteroid dehydrogenase (17 β -HSD1). The enzyme functions the last step in the biosynthesis of estradiol from estrone. 17 β -HSD1 inhibitors are regarded as promising new agents against breast cancer. 5'-Hydroxy group of imidazole C₂-ribonucleoside was converted into a phthalimide by a standard Mitsunobu reaction using acidic phthalimide with pKa 10, triphenylphosphine, and DEAD. In the same way as the standard Mitsunobu S_N2 mechanism of Mitsunobu reaction, the activated hydroxyl group as oxyphosphonium ion is attacked by phthalimide anion to give a C5'-N bond. Deprotection with hydrazine hydrate provided 5'-amino intermediate. Accordingly, the original C-O bond was changed to a C-N bond. The following three steps contain an amide bond formation between the amine and the carboxylic acid derived from estradiol, gave the hybrid product.

17.



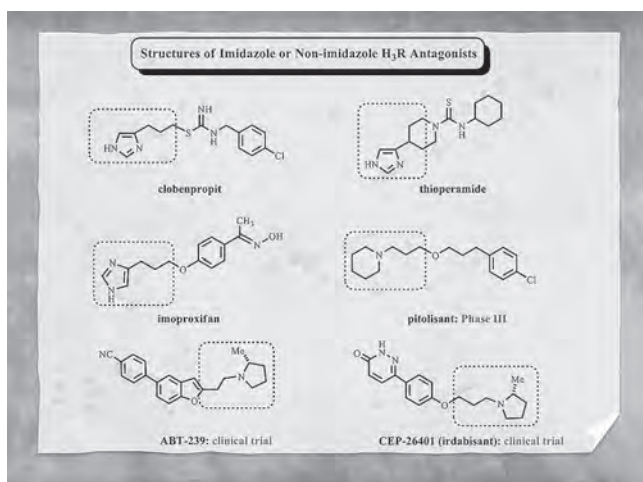
The third subject is my recent work: Synthesis of *N*-alkyl-*S*-alkylisothioureas using Mitsunobu *S*-alkylation and NPAI for new H₃R antagonists.

18.



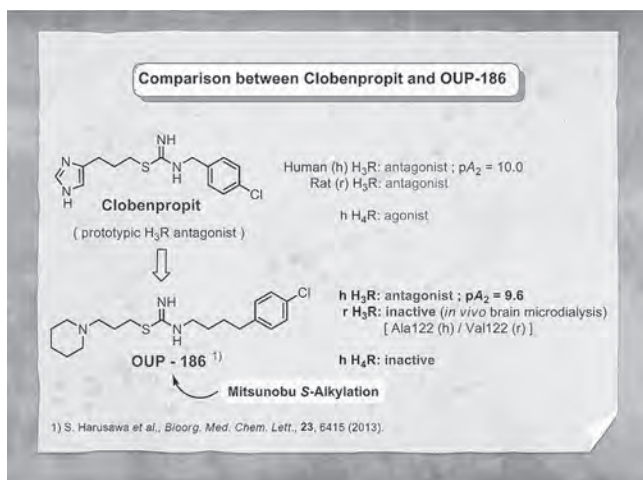
As Maikel has just talked in his lecture, H₃R antagonists increase histamine levels in brain. So, H₃R antagonists are now expected to be potential drugs such as narcolepsy, cognition such as Alzheimer's diseases, obesity, epilepsy, and so on. Meanwhile, as H₃R agonists lead to inhibition of histamine release, they are regarded as a target for anxiety, insomnia, and migraine.

19.



Typical structures of imidazole or non-imidazole H₃R antagonists are shown here. Clobenpropit was developed by Hendrik Timmerman at VU University Amsterdam (I met him once in Kyoto 10 years ago. But, he is one of my teachers, because I have learned a lot of things from his papers), and thioperamide by J. C. Schwarz in Paris. They have been widely used in pharmacology as potent prototype of H₃R antagonists. A few non-imidazole compounds have already reached clinical trials, including pitolisant, ABT-239, and irdabisant. They have much in common on the structure, that is, the spacer between the cyclic amines and central functional groups is set by three methylene-carbons, and the whole lengths are almost 15 to 16 Å (angstroms).

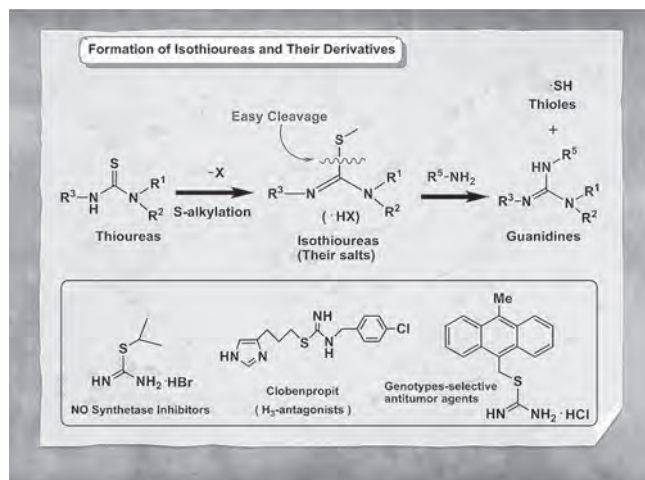
20.



We recently reported the synthesis of a novel H₃R antagonist, OUP-186 based on the S-alkyl-N-

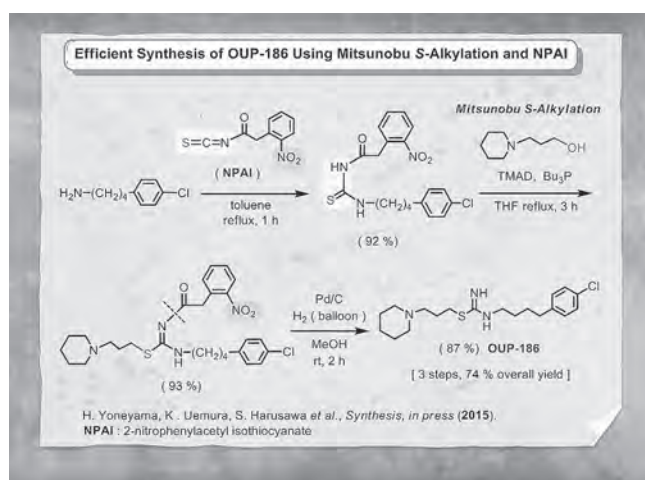
alkylisothiourea structure of clobenpropit. Clobenpropit exhibits potent antagonistic activities against human and rat H₃R, while it shows agonistic activity against human H₄R. OUP-186 with pA₂ = 9.6 was also found to exhibit potent and selective H₃R antagonistic activity against *in vitro* human H₃R, while being inactive against H₄R. Interestingly, it proved to be inactive for stimulating histamine release against rat H₃R in *in-vivo* rat brain microdialysis. The *in silico* docking studies revealed the species-selectivity of OUP-186 is caused by an Alanine 122/Valine 122 mutation between the antagonist-docking cavities in human and rat H₃Rs. Here, I would like to show the efficient synthesis of OUP-186 using Mitsunobu S-alkylation.

21.



Isothiourea functional group constitutes the main structural element in clobenpit, and the isothioureas have received considerable attention owing to interesting agrochemical and medicinal applications like NO synthetase inhibitors and genotype-selective antitumor agents as well as its application in organo- and transition-metal catalysis. The main synthetic route to isothioureas involves the reaction of thiourea with alkyl halides. The isothioureas are crucial intermediates in the synthesis of guanidines. As the S-alkyl moieties of isothioureas are good leaving groups, treatment of isothioureas with amines results in the generation of guanidines, removing thiols.

22.

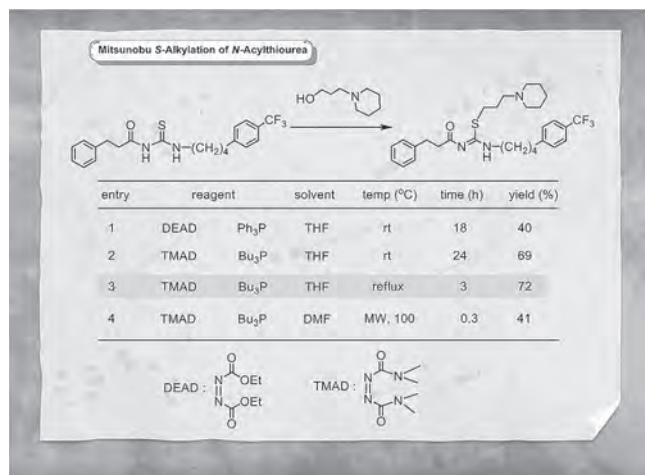


We could very recently developed an efficient synthesis of OUP-186 by using Mitsunobu S-alkylation

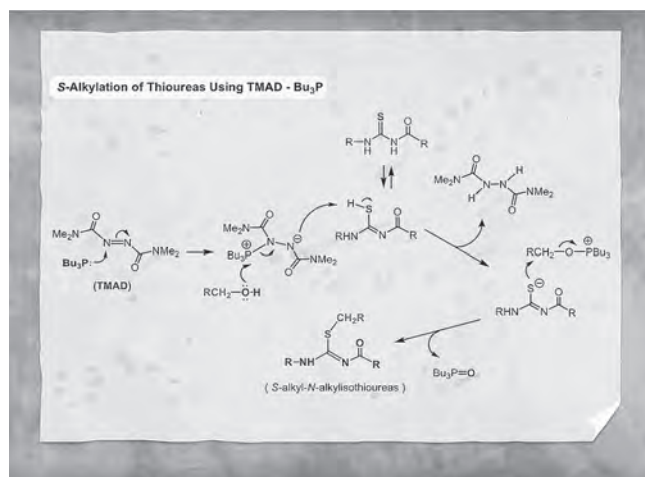
and 2-nitrophenyl-acetylisothiocyanate (NPAI). NPAI is a novel bifunctional SCN source bearing a nitro group. First, the starting phenylbutylamine attacks the central carbon of SCN moiety of NPAI, giving a thiourea intermediate.

Here, we used Mitsunobu S-alkylation. When the reaction of the thiourea with piperidinepropanol was carried out in the presence of TMAD and tributylphosphine in refluxing THF for 3 hours, the C-S bond formation proceeded smoothly to give an N-phenylacetyl-S-alkylisothiourea in 93% yield. We have to remove the unnecessary phenylacetyl-moiety to get the product. We got OUP-186 in 87% using only palladium-carbon and hydrogen. I will discuss the removal process later.

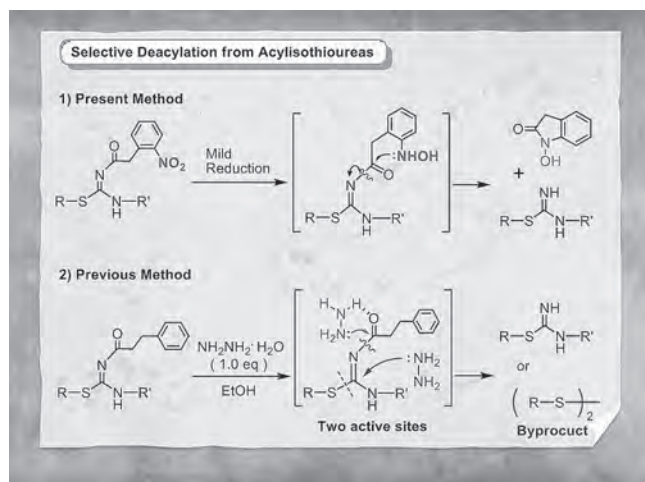
23.



We investigated the reaction condition of Mitsunobu S-alkylation. The standard reagent system, DEAD and triphenylphosphine was no good; THF, room temperature, and 18 hours gave only 40% yield. Meanwhile, the combination of TMAD and tributylphosphine improved the reaction, giving 69% yield of the product at room temperature for 24 hours. Further, when the reaction was refluxed in THF, the reaction time could be shorted to 3 hours.



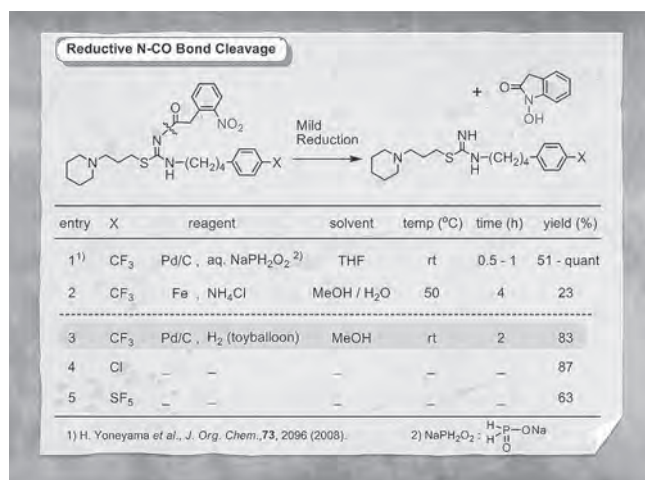
The mechanism of the S-alkylation is essentially the same as that of the Mitsunobu reaction mentioned previously. TMAD and tributylphosphine forms a betaine intermediate. Picking up a proton of the isothiurea which is brought about by tautomerization of an thiourea, and at the same time, attack of alcohol to the phosphine cation generates the oxyphosphonium ion together with hydrazine dicarboxamide. Successively, reaction of thiolate anion with the activated alcohol produce S-alkyl-N-alkylisothiurea and tributylphosphine oxide.



On the elimination of the unnecessary acyl moiety, we used two synthetic approaches. One approach involves direct cleavage of the N-CO bond by hydrazine. However, the deacylation led to a thiol which was oxidized to a disulfide with air, owing to the sensitive nature of C-S bonds. Therefore, we directed our interest to an alternative intramolecular amide cleavage using reductive cyclization of the nitro group.

I assume that mild reduction gave a hydroxylamine followed by subsequent cyclization with retention of the S-alkyl moiety, producing the desired isothiurea

and N-hydroxy-2-oxoindole.



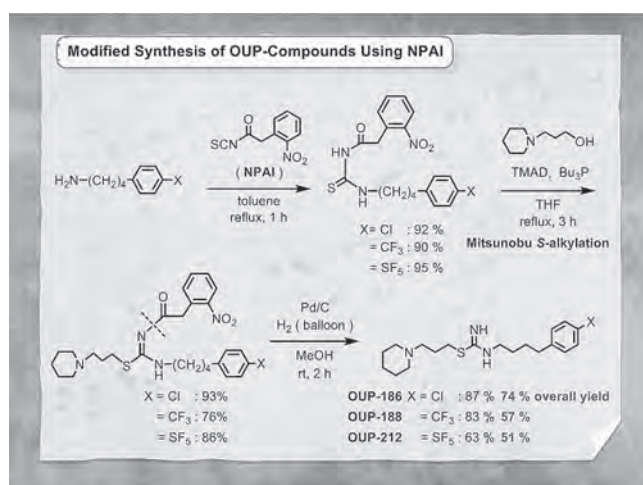
Next, we investigated reduction condition of the nitro group using a model compound bearing trifluoromethyl group. Among them, use of palladium carbon under hydrogen gave a 83% of isothiurea in methanol at room temperature for two hours. Chloro- and pentafluorosulfanyl compounds were also obtained in 87 and 63% yields, respectively.

27.



Experimental equipment is very simple, only flask with a balloon.

28.



Finally, I summarize the modified synthesis of OUP-compounds using NPAI. Starting from amines, thiourea formation using NPAI, Mitsunobu S-alkylation, and intramolecular amide cleavage by reduction of the nitro group.

Thank you for your kind attention.