OIST OKINAWA INSTITUTE OF SCIENCE AND TECHNOLOGY GRADUATE UNIVERSITY

Chemistry and Chemical Bioengineering Unit (Fujie Tanaka)

FY2019 Annual Report

Chemistry and Chemical Bioengineering Unit
Professor Dr. Fujie Tanaka, Professor

Abstract

The ability to design and synthesize organic molecules constitutes the foundation that underlies basic research as well as applied science. The ability is also essential for the development of pharmaceuticals and biofunctional molecules. This unit develops efficient, concise, and safe chemical transformation methods and strategies for constructing small molecules bearing functional groups and/or chiral centers that are relevant to the creation of biofunctional molecules. We design and create small organic molecule catalyst systems for designed chemical transformations, and we develop reaction strategies that use small organic molecules as enzyme-like catalysts. With the use of organic molecules as catalysts, we minimize the need for protection and deprotection steps that are usually required for the synthesis of functionalized molecules. When a reaction method does not affect functional groups that are not at the reaction site, the reaction method can be used for the synthesis of a series of molecules bearing various functional groups. This means that a series of molecules of interest may be synthesized using the same method in a short route, providing advantages for the synthesis of biofunctional candidate molecules. We also investigate the chemical bases of the reactions to understand the mechanisms of the catalysis and molecular interactions provided by organic molecules. By taking advantage of the use of features of our developing molecules, we also develop strategies and methods for conjugation of proteins and peptides with other molecules. The research undertaken by this unit advances the chemistry of catalysis and of molecular synthesis. The studies by this unit accelerate the creation of molecules used in biomedical research and contribute to the development of new therapeutics, therapeutic strategies, and diagnostic methods.

1. Staff

- Dr. Ravindra D. Aher
- Dr. Venkati Bethi
- Dr. Santosh Chavan
- Dr. Yuvraj Garg
- Dr. Yarkali Krishna
- Dr. Lingaiah Maram
- Dr. Prodip M. Roy
- Dr. Muhammad Sohail
- Kola Shilpa
- Santanu Mondal, Graduate Student

- Maira Pasha, Special Research Student
- Hoan Dihn, Graduate Student (Rotation)
- Vineet Marthur, Intern Student
- Honoka Watanabe, Intern Student
- Shiho Chinen, Research Unit Administrator

2. Activities and Findings

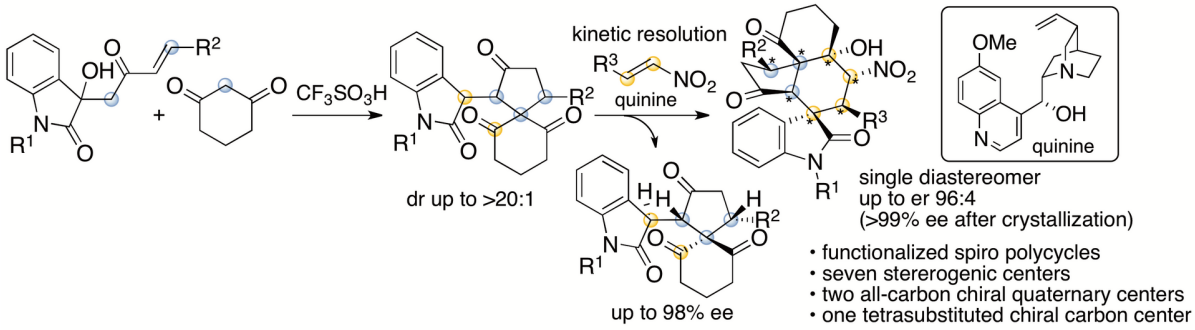
2.1. Development of new chemical transformation methods and synthesis of functionalized molecules

We have been developing small organic molecule catalysts (organocatalysts) and organocatalytic molecular transformation methods useful for the synthesis of functionalized molecules under mild conditions in short routes. We also investigate the chemical bases of the catalyses and the chemical transformations to understand the mechanisms of the catalysis and molecular interactions provided by organic molecules to further the creation of useful molecules.

Traditional synthetic methods often require high or very low temperatures and/or absolute conditions. In addition, functional groups on substrate molecules must be protected prior to reactions. That is, depending on functional groups present in target molecules to be synthesized, synthetic routes, including protection and deprotection steps, have to be designed for each molecule. To concisely synthesize functionalized molecules, chemical transformation methods that are not affected by functional groups presenting in starting materials are needed. It is a great advantage when the same reaction method can be used for the synthesis of a series of molecules bearing various functional groups without the need of product-specific protection and deprotection steps. In addition, it is desired that such reactions can be performed under safe, mild, and environmentally benign conditions. We address these points in our research as we design and develop catalysts and chemical transformation methods. By using organic molecules as catalysts, we concisely synthesize novel functionalized molecules including those that are often difficult to synthesize by traditional synthetic strategies. Our investigations into the chemical basis of the developed catalysts and chemical transformation methods further the understanding of the chemistry of organic molecules and their reactions.

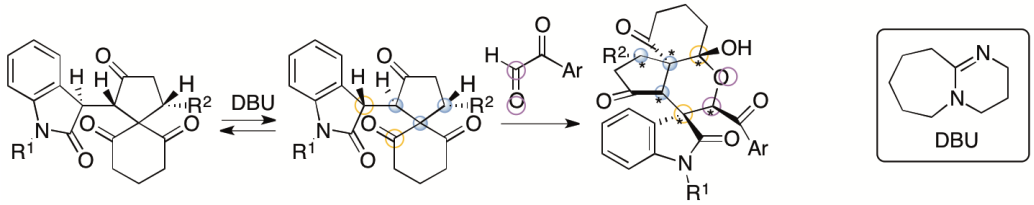
2.1.1. Construction of spirooxindole polycycles: formal (4+1) cycloaddition reactions, Michael-Henry cascade reactions, aldol-oxa-cyclization reactions, and dimerization reactions

Spirooxindole derivatives are found in bioactive natural products. Functionalized molecules with spirooxindole cores including those with polycyclic systems should be useful in drug discovery efforts. We have recently reported formal (4+1) cycloaddition and enantioselective Michael-Henry cascade reactions that provide spirooxindole polycycles via spiro[4,5]decane derivatives (Scheme 1) (Huang, Sohail, Taniguchi, Monde, and Tanaka, *Angew. Chem. Int. Ed.* 2017, 56, 5853). The reactions provided spirooxindole all-carbon polycyclic derivatives with seven stereogenic centers, including two all-carbon chiral quaternary centers and one tetrasubstituted chiral carbon center.



Scheme 1

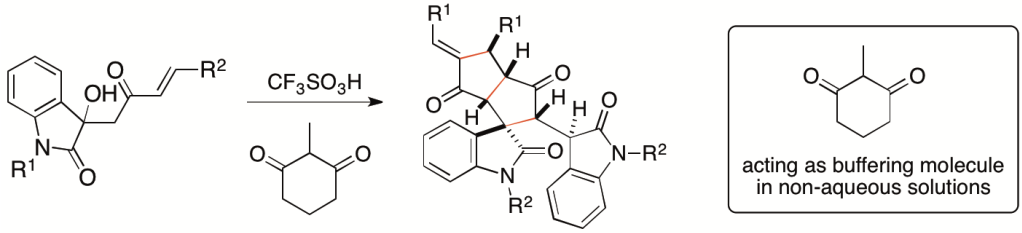
We have also developed annulation via aldol-oxa-cycliztion cascade reactions to afford spirooxindole pyran polycycles from the same spiro[4,5]decane derivatives (Scheme 2) (Sohail and Tanaka, *Communications Chemistry* 2020, 2, article number 73). With the use of arylglyoxal derivatives as reactants, the formation of C-C and C-O bonds was achieved. Whereas the all-carbon polycycles mentioned above had a *trans-cis* relationship for the generated 5-6-6 ring system, the pyran polycycles generated by this method had a *cis-cis* relationship for the 5-6-6 ring system. During the reaction, the spiro[4,5]decane derivative was isomerized to the diastereomer, and this was the key to afford the product. From the spiro[4,5]decane derivatives obtained by kinetic resolutions, highly enantiomerically enriched single diastereomers of the spirooxindole pyran polycycles were obtained.



Scheme 2

Usually, these complex, polycyclic molecules are synthesized by multi step routes. Our strategies allowed the access to the complex functionalized molecules including those as highly enantiomerically enriched forms in a short route.

We have also developed a dimerization reaction strategy to construct spirooxindole polycycles. The enone aldol derivatives used for the synthesis of the spiro[4,5]decane derivatives mentioned above were transformed to spirooxindile octahydropentalene derivatives under the conditions with the use 2-methyl-1,3-cyclohexanedione as a buffering molecule that works in non-aqueous solutions (Sohail an Tanaka, *Chem. Eur. J.* 2020, 26, 222) (Scheme 3). The buffering functions of the 1,3-cyclohexanedione derivatives are separately described in section 2.1.8 of this report.

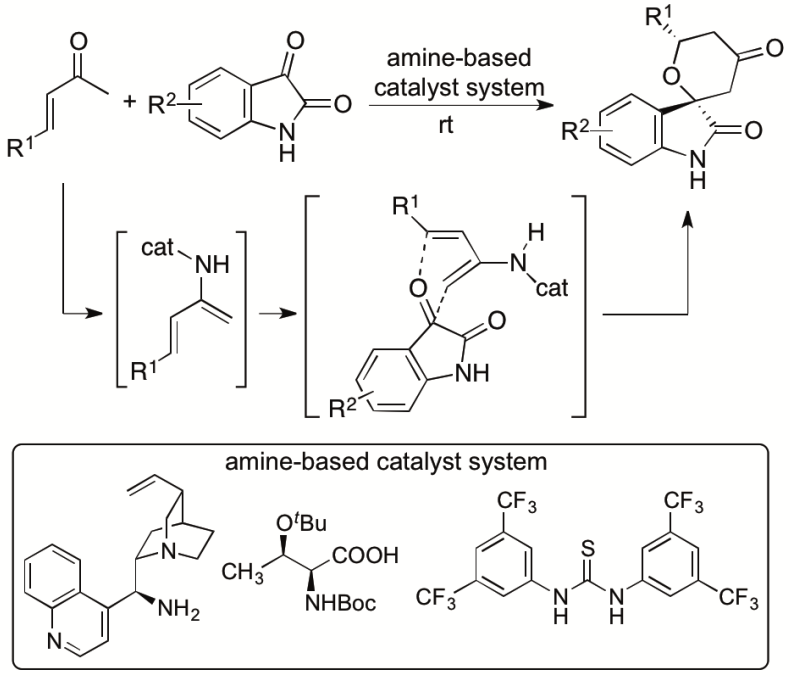


Scheme 3

We have been investigating the mechanisms of the reactions reading to the formation of the spiro[4,5]decane derivatives. We are also investigating to provide other polycyclic products and to expand the capabilities for the synthesis of functionalized molecules with polycyclic ring systems.

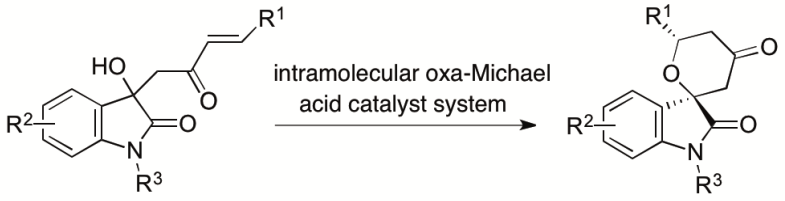
2.1.2. Construction of spirooxindole tetrahydropyrans: oxa-Diels-Alder reactions and oxa-Michael reactions

We previously reported catalytic asymmetric hetero-Diels-Alder reactions of enones with isatins (2,3-dioxyindoles or 2,3-dioxyindolins) that provide functionalized spirooxindole tetrahydropyranones with high diastereo- and enantioselectivities (Scheme 4) (Cui and Tanaka, *Chem. Eur. J.* 2013, 19, 6213). Novel amine-based catalyst systems composed of three-types of molecules (amine, acid, and thiourea) were developed to catalyze the reactions. The design and synthesis of single-molecule catalysts that provide all the required interactions for the catalysis and stereocontrol for designed reactions especially for new reactions are often difficult. We have demonstrated that the use of multicomponent catalyst systems provides a way to bypass the limitations in the access to efficient single component catalysts. We also elucidated the mechanism of the reactions and key factors for the high diastero- and enantioselectivities achieved by the three-component catalyst system (Cui, Chouthaiwale, Yin, and Tanaka, *Asian J. Org. Chem.* 2016, 5, 153).



Scheme 4

Whereas these hetero-Diels-Alder reactions provided the spirooxindole tetrahydropyrans with high diastereo- and enantioselectivities, the catalyst systems that we initially developed as described above were capable to afford only one type of the diastereomers as the major products. To synthesize the previously minor diastereomers as the main products, we have developed intramolecular oxa-Michael reactions of the aldols generated from the enones with isatins (Scheme 5) (Pasha, Sohail, and Tanaka, *Heterocycles* 2020, 101, 339). When enantiomerically enriched forms of aldols were used as the starting materials, the products retaining the enantiopurities of the starting materials were obtained.

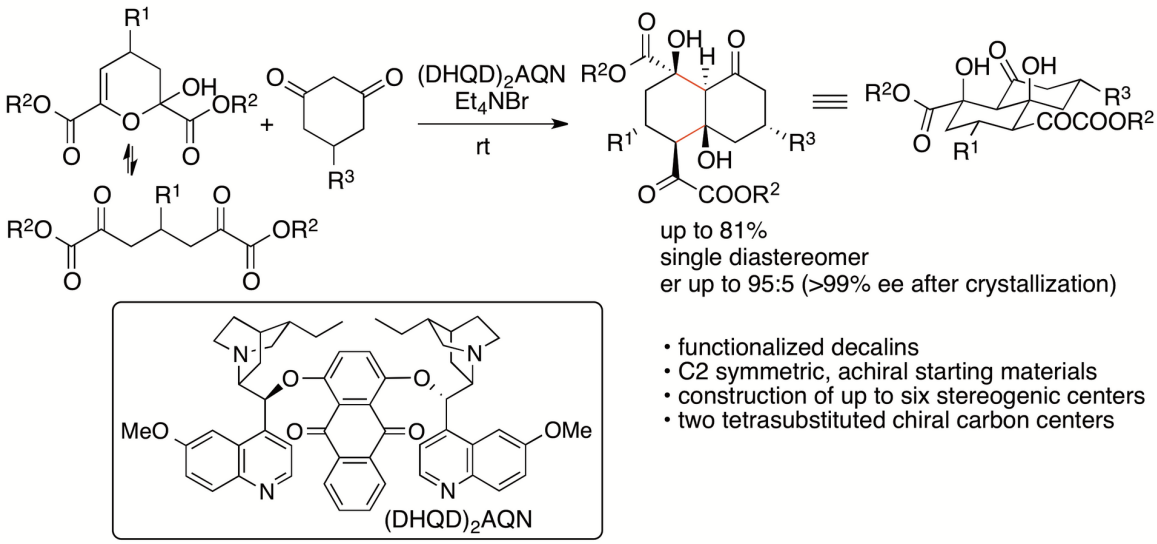


Scheme 5

We are also expanding the hetero-Diels-Alder reactions of enones beyond the use of the oxindole dienophiles to the use of various ketones and aldehydes as dienophiles. We have been studying and developing diastereo- and enantioselective hetero-Diels-Alder reactions and other strategies in order to allow the concise access to functionalized various tetrahydropyran derivatives with highly diastereo- and enantioselective manners.

2.1.3. Construction of highly functionalized decalin derivatives: aldol-aldol annulation reactions

We have recently developed catalytic enantioselective formal (4+2) cycloaddition reactions of dihydropyran derivatives with cyclohexane-1,3-diones that afford functionalized decalins through aldol-aldol annulation (Scheme 6) (Chouthaiwale, Aher, and Tanaka, *Angew. Chem. Int. Ed.* 2018, 57, 13298). The decalin ring system is found in diterpenes, diterpenoids, steroids, and other bioactive molecules, so the development of methods for the synthesis of functionalized decalin derivatives is of interest in drug discovery and related research. Our strategy enabled the construction of polyfunctionalized decalins bearing five to six chiral carbon centers with high diastereo- and enantioselectivities from achiral molecules in a single transformation that formed two C-C bonds.



Scheme 6

Key factors of the success of the reactions include the use of the dihydropyran derivatives as the starting materials, which are obtained from pyruvates and aldehydes (Chouthaiwale and Tanaka, *Chem. Commun.* 2014, 50, 14881; Chouthaiwale, Lapointe, and Tanaka, *Heterocycles* 2017, 95, 587). The starting material dihydropyran derivatives retain the α-ketoester group of pyruvates and are C2 symmetric molecules, allowing direct enantioselective transformations to provide the products in high yields (in contrast to that kinetic resolutions result in yielding the products in no higher than 50%).

We are currently investigating the detail mechanisms of the reactions. We are also investigating essential parts of the catalyst structure for the catalysis and the stereocontrol.

2.1.4. Reactions of pyruvates to synthesize various functionalized molecules

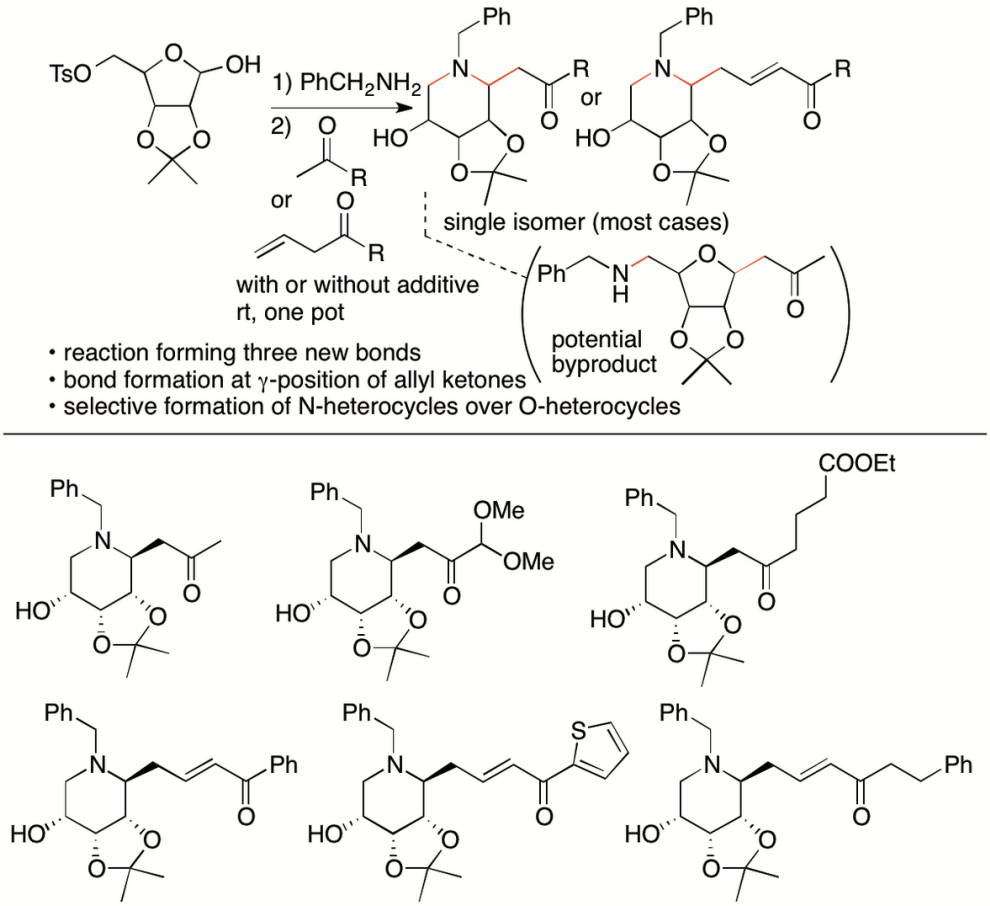
Pyruvates can act as nucleophiles and electrophiles and thus are expected to be useful synthons. However, the dual reactivities of pyruvates are difficult to control. We have recently developed concise cascade reactions of pyruvates to provide various functionalized dihydropyrans in one pot under mild conditions (Chouthaiwale and Tanaka, *Chem. Commun.* 2014, 50, 14881; Chouthaiwale, Lapointe, and Tanaka, Heterocycles 2017, 95, 587). We have demonstrated that the product dihydropyrans are readily transformed to various molecules including amino group-substituted and fluoro group-substituted dihydropyrans, cyclohexane-derived amino acids, dihydrodiazepines, and pyridines. As described in section 2.1.3., we have shown that the dihydropyrans are also used for the synthesis of functionalized decalin derivatives (Chouthaiwale, Aher, and Tanaka, *Angew. Chem. Int. Ed.* 2018, 57, 13298).

We are further developing transformation methods that use the dihydropyrans obtained from pyruvates and aldehydes to concisely provide enantiomerically enriched, polyfunctionalized molecules by taming the reactivities of pyruvate derivatives. We are also developing catalyst systems that enable the reactions of pyruvates to provide functionalized molecules.

2.1.5. Constructions of functionalized piperidine derivatives: reactions of imines and enamines generated from carbohydrate derivatives in situ

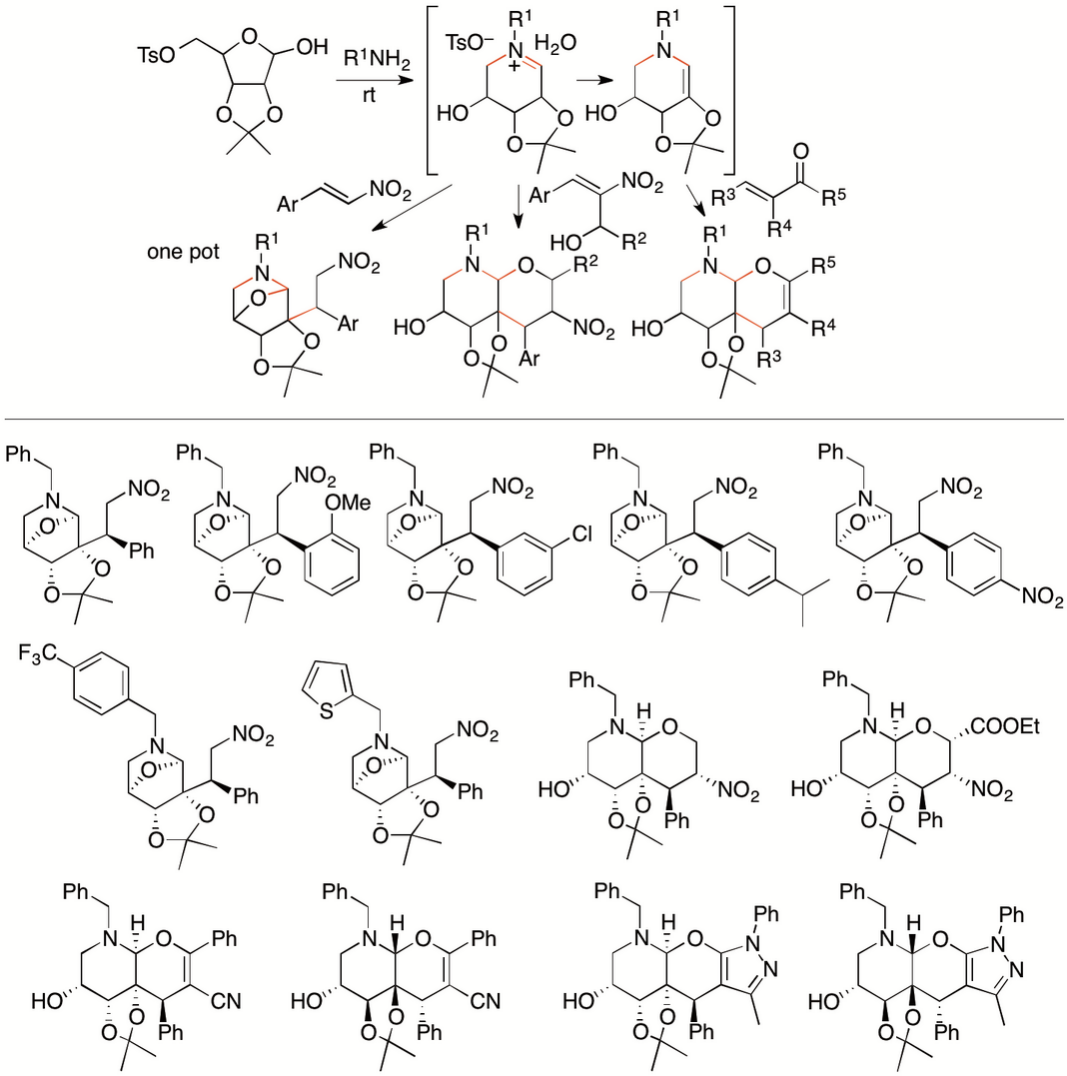
Polyoxy-functionalized piperidine derivatives are important as pharmaceuticals, probes, and their building blocks. Whereas various reaction methods for the synthesis of piperidine derivatives have been reported, most provide piperidines bearing only mono- and di-substitutions on the carbons of the piperidine rings. For the synthesis polyoxy-functionalized piperidines, strategies that are different from those used for the synthesis of simple piperidines are required. We have developed Mannich reactions of sugar derivatives with ketones that afford polyoxy-substituted piperidine derivatives bearing ketone groups (Scheme 7) (Maram and Tanaka, *Org. Lett.* 2019, 21, 1165). In our strategy, benzylamine used for the formation of the iminium ion in situ also activates the ketones via the formation of the enamines or the enolates.

The utility of this reaction method was further demonstrated by transformations of the products. For example, the piperidine derivatives obtained by this method were readily transformed to N-containing bicyclic derivatives, such as quinolizine derivatives.



Scheme 7

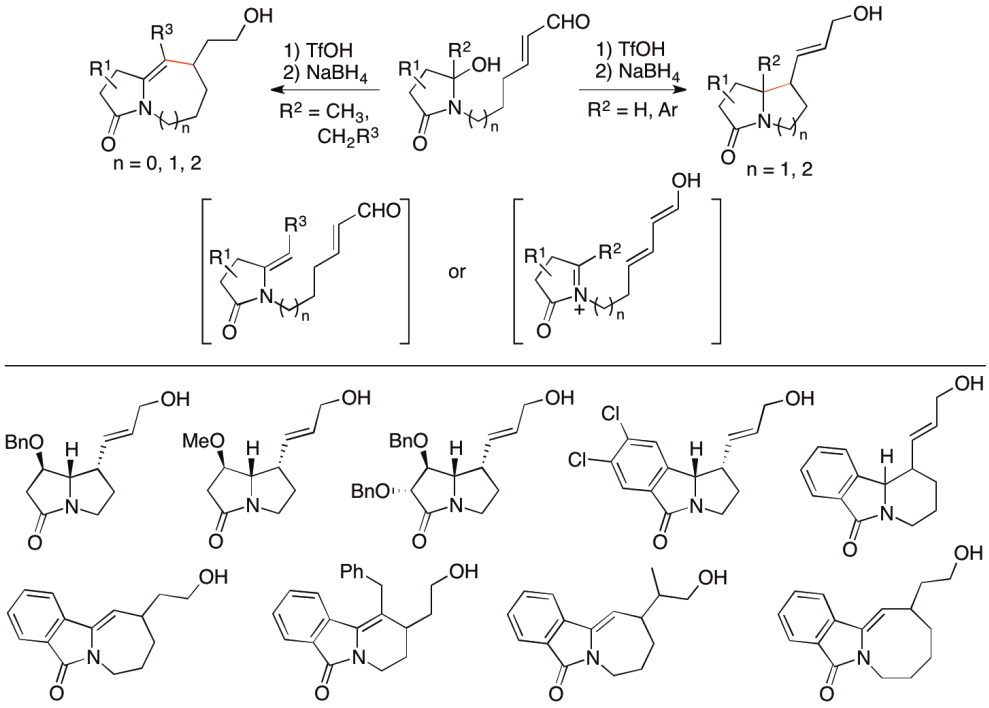
We have also demonstrated that iminium ions, which act as electrophiles, generated in situ from the sugar derivatives can be converted to enamines in situ, which act as nucleophiles (Scheme 8) (Maram and Tanaka, *Org. Lett.* 2020, 22, 2751). With this strategy, substituents were introduced at the 3-position or both 2- and 3-positions of the piperidines bearing polyoxy groups through Michael, Michael-annulation, oxa-Diels-Alder, or [4+2] cycloaddition reactions.



Scheme 8

2.1.6. Constructions of bicyclic N-heterocycles: Intramolecular Mannich and Michael annulation reactions

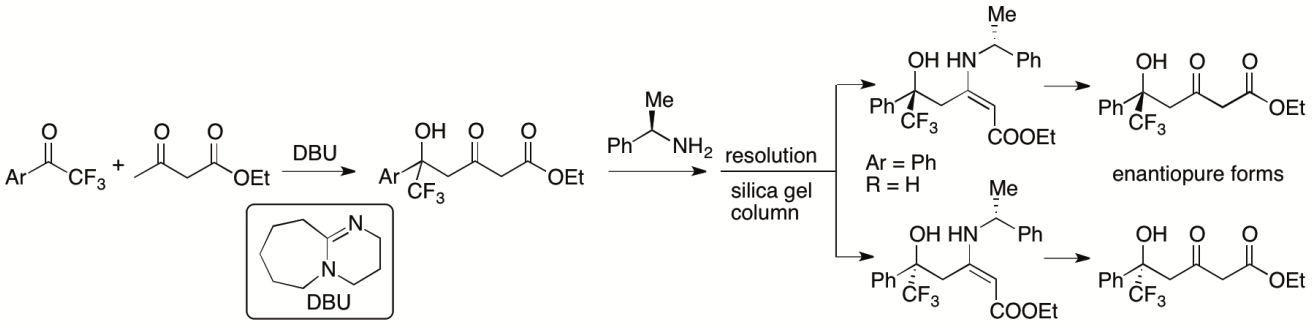
Bicyclic N-heterocycles bearing pyrrolidine rings or pyrrolidine-2-one moieties are found in bioactive natural products. Methods for the synthesis of these molecules are of interests in drug discovery efforts. We have developed a strategy that allow the synthesis of various pyrrolidinone-fused bicyclic N-heterocycles with 5- to 8-membered rings (Scheme 9) (Krishna, Y., Shilpa, K., and Tanaka, F. *Org. Lett.* 2019, 21, 8444.) From hydroxylactam enals as the starting materials, depending on the substituents, the C-C bond formation for the ring formation occurred through either intramolecular Mannich reactions of the N-acyliminium ion generated in situ or intramolecular Michael reactions of the enamide also generated in situ.



Scheme 9

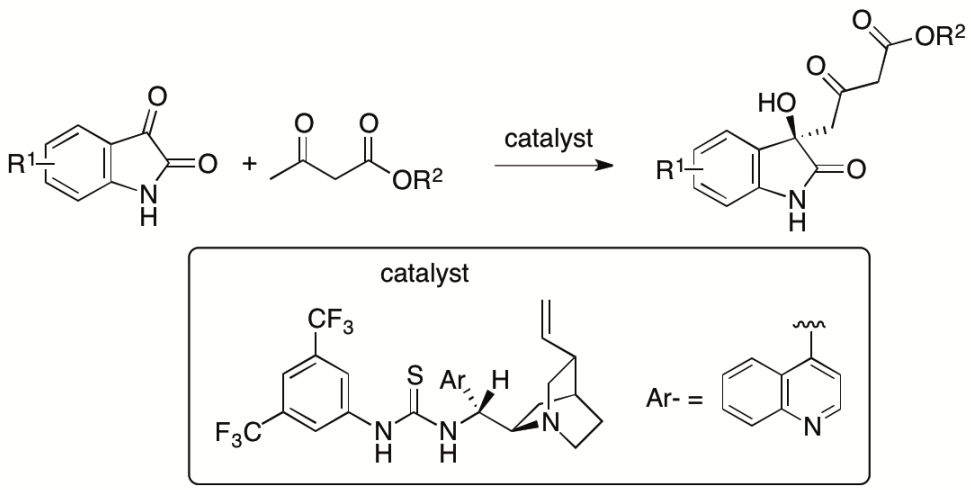
2.1.7. Construction of δ-hydroxy-β-ketoesters: γ-position selective aldol reactions of β-ketoesters

In most aldol and Mannich reactions of β-ketoesters in which the β-ketoesters are used as nucleophiles, the bond formation occurs at the α-position of the β-ketoesters. Previously, we developed γ-selective aldol reactions of β-ketoesters with aryl trifluoromethyl ketones catalyzed by 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) (Scheme 10) (Zhang and Tanaka, *Adv. Synth. Catal.* 2015, 357, 3458).



Scheme 10

Whereas resolutions of the γ-selective aldol products provided the enantiopure forms, enantioselective γ-selective aldol reactions of β-ketoesters are more desired than the resolution for the access to the enantiomerically enriched aldol products. In collaborating Prof. Dongxin Zhang, former PhD student of the Tanaka Unit, we have developed γ-selective aldol reactions of β-ketoesters with isatins (Scheme 11) (Zhang, Chen, Cai, Yin, Zhong, Man, Zhang, Bethi, and Tanaka, *Org. Lett.* 2020, 22, 6).

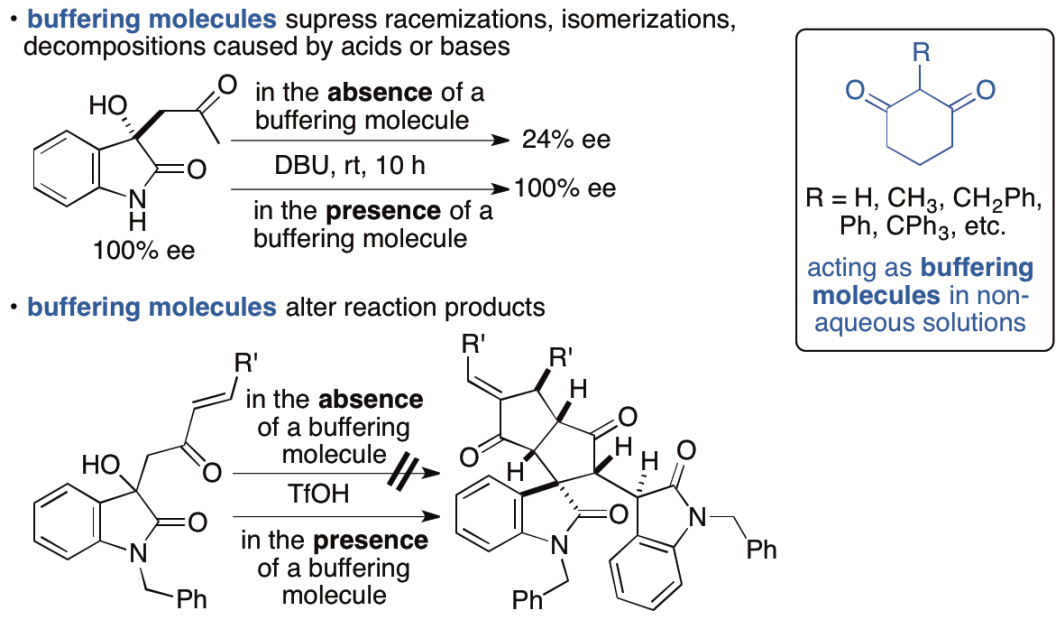


Scheme 11

We are also working to expand the γ-selective aldol reactions of β-ketoesters to those with other acceptor substrates (i.e., other than isatines). We are also developing enantioselective γ-selective Mannich and other reactions of β-ketoesters.

2.1.8. Control of chemical reactions using 1,3-cyclohexanedione derivatives as buffering molecules in non-aqueous solutions

Control of chemical reactions is necessary to obtain desired chemical transformation products and to prevent decompositions and isomerizations of molecules of interest. To control chemical reactions in aqueous solutions or to maintain conditions suitable for enzyme-catalyzed reactions and for storage of biological samples such as enzymes and antibodies, the use of buffers is a common practice. However, no molecules that have buffering functions in non-aqueous solutions to maintain conditions suitable for chemical reactions were commonly used. We have introduced the "buffering" concept into the events that occur in non-aqueous solutions and have demonstrated that 1,3-cyclohexanedione derivatives have buffering functions in non-aqueous solutions (Scheme 12) (Sohail and Tanaka, *Chem. Eur. J.* 2020, 26, 222). 1,3-Cyclohexanedione derivatives inhibited both acid-catalyzed and base-catalyzed isomerizations and decompositions in organic solvents. To suppress decompositions and isomerizations of the molecules by using the buffering molecules that we have disclosed, there is no need to consider whether the decomposition and/or isomerization is caused by a base or an acid or which type of base or acid is causing the decomposition, isomerization, and/or racemization. Additionally, simply adding the buffering molecule to reaction mixtures can completely alter the pathways of the reactions. No such simple operations to control chemical reactions were commonly demonstrated previously. The use of buffering molecules that work in organic solvents provides a strategy to control chemical reactions and expands the range of compounds that can be synthesized.



Scheme 12

2.2. Development of bioconjugation systems

Protein labeling methods are required for the synthesis of antibody-drug conjugates and other protein conjugates; these molecules are important as therapeutics and as detection devices for molecules of interest. Conjugation reactions are also needed to create multifunctional molecules. We are developing efficient protein labeling systems and molecules with desired reactivities that can be used for protein labeling reactions at targeted sites.

2.3. Search of biofunctional molecules

As described above, we have synthesized various functionalized molecules. In collaboration with researchers whose expertise is in biology and screening for biofunctional molecules, we have been searching new biofunctional molecules and drug leads. The collaborations include: Professor Dr. Hiroshi Tomoda, Kitasato University.

We have also worked to expand the chemical space of molecules that we can synthesize and search for biofunctionl molecules by combining organic synthesis with microbial transformations in collaboration with Professor Dr. Katsuhiro Ueda, University of the Ryukyus.

3. Publications

3.1 Journals

1. Sohail, M.; Tanaka F. Dynamic stereoselective annulation via aldol-oxacyclization cascade reaction to afford spirooxindole pyran polycycles. *Communications Chemistry* **2019**, *2*, article number 73, doi: 10.1038/s42004-019-0177-5.
2. Pasha, M.; Sohail, M.; Tanaka F. Intramolecular oxa-Michael reactions of aldols generated from enones and isatins to afford spirooxindole tetrahydropyrans. *Heterocycles* **2020**, *101*, 339-346, doi: 10.3987/COM-19-S(F)26.
3. Krishna, Y.; Shilpa, K.; Tanaka, F. Intramolecular Mannich and Michael annulation reactions of lactam derivatives bearing enals to afford bicyclic N-heterocycles. *Organic Letters* **2019**, *21*, 8444-8448, doi: 10.1021/acs.orglett.9b03210.
4. Sohail, M.; Tanaka F. Control of chemical reactions using molecules that buffer non-aqueous solutions. *Chemistry - A European Journal* **2020**, *26*, 222-229, doi: 10.1002/chem.201903552.
5. Zhang, D.; Chen, Y.; Cai, H.; Yin, L.; Zhong, J.; Man, J.; Zhang, Q.-F.; Bethi, V.; Tanaka F. Direct catalytic asymmetric synthesis of oxindole-derived δ-hydroxy-β-ketoesters by aldol reactions. *Organic Letters* **2020**, *22*, 6-10, doi: 10.1021/acs.orglett.9b03527.
6. Maram, L.; Tanaka, F. Switching electrophile intermediates to nucleophiles: Michael and oxa-Diels-Alder reactions to afford polyoxy-functionalized piperidine derivatives with tetrasubstituted carbon. *Organic Letters* **2020**, *22*, 2751-2755, doi: 10.1021/acs.orglett.0c00735.

3.2 Oral and Poster Presentations

1. Aher, R.; Chouthaiwale, P.; Tanaka, F. Organocatalytic enantioselective synthesis of functionalized decalins via desymmetrization of substituted dihydropyrans and 1,3-diketones, in the 254th ACS National Meeting, Orlando, Florida, 2019.03.31-2019.04.04. (oral, ORGN 523)
2. Aher, R.; Chouthaiwale, P.; Tanaka, F. Enantioselective synthesis of functionalized decalins via desymmetrization of substituted dihydropyrans and 1,3-diketones, in the 27th International Society of Heterocyclic Chemistry Congress, Kyoto, Japan, 2019.09.01-2019.09.06. (poster No. 2P-022s)
3. Sohail, M.; Tanaka, F. Dynamic stereoselective annulation to afford spirooxindole pyran polycycles, in the 27th International Society of Heterocyclic Chemistry Congress, Kyoto, Japan, 2019.09.01-2019.09.06. (flash presentation No. 2F-B-3 and poster No. 2P-025s)
4. Maram, L.; Tanaka, F. Synthesis of polyoxy-functionalized piperidines via Mannich and Micheal reactions of carbohydrate derivatives, in the 27th International Society of Heterocyclic Chemistry Congress, Kyoto, Japan, 2019.09.01-2019.09.06. (poster No. 3P-069s)
5. Garg, Y.; Tanaka, F. Enantioselective Mannich reactions of ketones catalyzed by 3-pyrrolidinecarboxylic acid in the presence of metal salts, in The 12th Symposium on Organocatalysis, Kyoto, 2019.12.04-2019.12.05 (poster No. P1)
6. Krishna, Y.; Tanaka, F. Intramolecular Mannich and Michael annulation reactions to synthesize bicyclic N-heterocycles, in The 140th Annual Meeting of the Pharmaceutical Society of Japan, Kyoto, Japan (2020), 2020.03.25-2020.03.28. (poster No. 26P-am013)
7. Sohail, M.; Tanaka, F. Control of chemical reactions using 1,3-cyclohexanedione derivatives as buffering molecules, in The 140th Annual Meeting of the Pharmaceutical Society of Japan, Kyoto, Japan (2020), 2020.03.25-2020.03.28. (poster No. 28Q-am073)
8. Bethi, V.; Tanaka, F. Organocatalytic γ-position-selective Mannich reactions of β-ketoesters, in The 140th Annual Meeting of the Pharmaceutical Society of Japan, Kyoto, Japan (2020), 2020.03.25-2020.03.28. (poster No. 28Q-am075)

4. Intellectual Property Rights and Other Specific Achievements

1. Tanaka, F.; Sohail, M. 1,3-CYCLOHEXANEDIONE DERIVATIVES AND 1,3-CYCLOPENTANEDIONE DERIVATIVES AS BUFFERING MOLECULES IN NON-AQUEOUS SOLUTIONS, United States provisional patent application 62/915,824, filed on 2019.10.16.

5. Meetings and Events

5.1 Seminars Hosted

- Date: August 28, 2019
- Venue: OIST campus
- Speaker: Professor Dr. Hideko Nagasawa, Gifu Pharmaceutical University, Japan
- Title: Development of antitumor drugs targeting tumor microenvironment: highly constrained bicyclic octadepsipeptids with potent antitumor activity and HIF-1 inhibitory activity

- Date: December 17, 2019
- Venue: OIST campus
- Speaker: Professor Dr. Kounosuke Oisaki, Graduate School of Pharmaceutical Sciences, The University of University, Japan
- Title: Chemoselective Protein Modification Using Organoradicals

- Date: February 6, 2020
- Venue: OIST campus
- Speaker: Professor Dr. Keiji Maruoka, Kyoto University, Japan
- Title: Design of High-Performance Organocatalysts for Asymmetric Catalysis and Radical Chemistry