博士論文

Studies on Utilization of Unique Properties and Reactivities of Azulene Derivatives

（アズレン誘導体のユニークな物性と反応性の利用に関する研究）

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1. General Introduction

Azulene (AZ) (Fig. 1a) is a ten carbon, non-alternant, aromatic hydrocarbon, comprising a fused 5,7-bicyclic system. This compound has been attracted chemists due to its blue color from unique S_0-S_2 transition and a high dipole moment of 1.08 D. The latter character is attributable to one electron transfer from the seven-membered ring to the five-membered one resulting in 6π aromatization of each ring (Fig. 1b). As a consequence, the five membered ring of AZ has cyclopentadienyl-anion-like character and the seven membered ring is tropylium cation. Interestingly, this molecular skeleton is also found in nature as a sesquiterpene compound guaiazulene (GA, Fig. 1c)
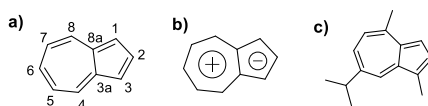

Figure 1. a) structure of AZ; b) charge separation of AZ c) structure of GA.

AZ and its derivatives have high reactivity toward various electrophiles, thanks to the aforementioned anionic character of the five-membered ring. Because HOMO of AZ has the large coefficient at the 1- and 3-positions, electrophilic aromatic substitution of AZ occurs at these sites. In addition, this compound also undergoes metal catalyzed C-H activation exclusively at 2-positon, which enables the synthesis of various 2-substituted azulenes. Due to highly polarized character of AZ, this compound also shows very high α-methyl-cation-stabilizing effect as shown in Scheme 1, and this property enlarges the chemical diversity of 1- and 3-substituted AZs. AZ and its derivatives have been applied in diverse area, including medicinal chemistry, solar cells, organic electronics, sensors. However, the versatile chemical behaviors of AZ in different reactions has not been fully explored yet. In this thesis, I utilized the diverse behaviors of AZ derivatives to study their unique properties in different reactions.
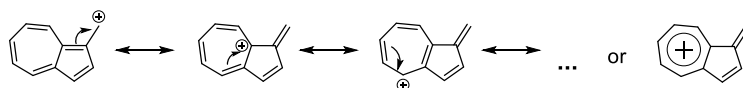
Scheme 1. Resonance structures of azulene-1-ylmethyl cation.

2. Development of peptide library screening method utilizing reactivity and color nature of azulene derivatives[1]

To date, this laboratory has focused on the development of selective peptide catalysts. For the purpose of finding catalytically active peptides, high-throughput screening of peptide library has been carried out (Figure 2), where the substrate and the resin-bound peptide forms an iminium ion, to which a dye-containing malonate attacks to resulting in the formation of colored beads. In that process, the nucleophile is malonate anion, and the peptides found in this screening worked well with other anionic nucleophiles such as nitromethane. However, they do not necessarily give good results with neutral nucleophiles such as indoles. This might be due to the difference in the interactions of the nucleophiles and peptides. Therefore, library screening with using neutral nucleophiles is desirable.
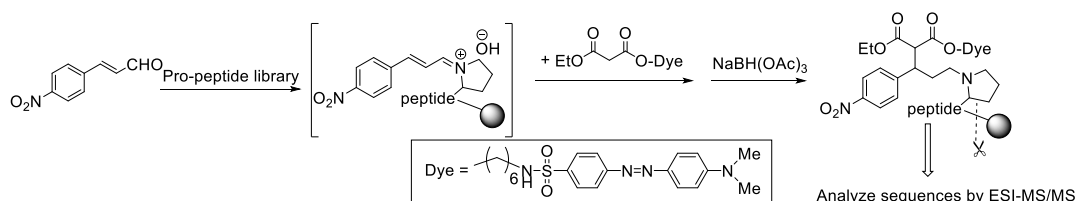
Figure 2. Procedure for the screening of a peptide library in previous studies.

In this context, I tried to utilize AZ, a neutral aromatic molecule, for such a purpose in order to avoid complexity due to 'double-nucleophilic' nature of AZ, I used 1-methylated natural product GA, instead. As expected, when GA and acrolein were mixed in the presence of peptide TFA salt, the reaction proceeded smoothly to give the corresponding Michael adduct. Then I changed the electrophile into 4-nitrocinnamaldehyde to find active and enantioselective peptide catalysts. However, in this case, the reaction gave 1,2-addition products (Scheme 2). Moreover, the 1,2-adduct further reacted with another GA molecule to give a bis-adduct. As the reaction conditions are only weakly acidic, the formation of such compounds should be due to very high ability of GA to stabilize α-methyl cation.
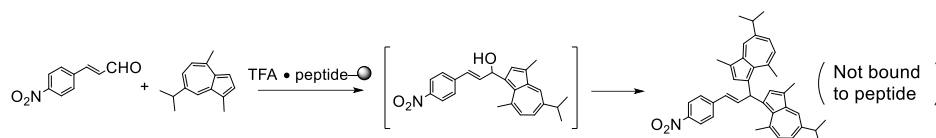
Scheme 2. The reaction of GA with 4-nitroaldehyde to give a bis-adduct.

Our group has previously developed helical-peptide-catalyzed asymmetric Michael addition of styryl boronates to 4-hydroxybut-2-enal[1]. In that reaction, the nucleophilic site was the carbon connected to the boron atom. I tried this reaction using azulene-2-ylboronic acid as a nucleophile. In this case, the desired 1.4-adduct was obtained. Here, I set out to explore brand new peptide sequences which does not rely on simple peptide secondary structures through a peptide library screening. Initially, the library consisting of N-terminal D-proline and the variable residues were constructed by a split-and-mix method (Figure 3).
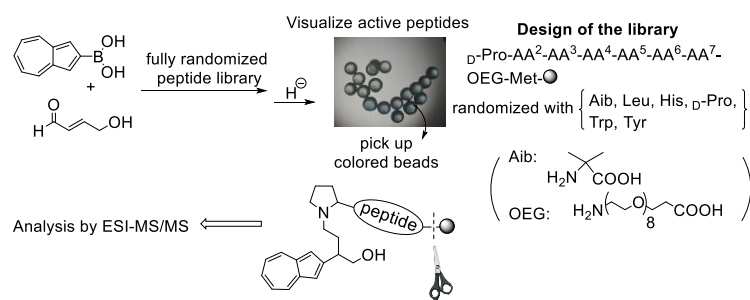
Figure 3. Procedure for the screening of a peptide library.5

Table 1. Detected Sequences.

No.	AA^2	AA^3	AA^4	AA^5	AA^6	AA^7	
1	Tyr	Trp	Tyr	His	Leu	Aib	
2	Tyr	Tyr	Tyr	Aib	Trp	His	
3	Tyr	Trp	Tyr	Leu	Trp	Leu	type I
4	Trp	Trp	Trp	Leu	Trp	D-Pro	
5	Trp	Trp	Trp	Aib	Trp	His	
6	Aib	Trp	Trp	Aib	Trp	His	
7	Aib	His	Trp	Trp	Trp	His	type II
8	Aib	Tyr	His	Tyr	Leu	Trp	
9	Tyr	Aib	Trp	Trp	Tyr	His	
10	Leu	Trp	Trp	Trp	Leu	D-Pro	

As expected, some beads turned blue which indicates that the peptide bound to those showed catalytic activity. Peptides were cleaved from the resin and analyzed by MS/MS. The detected sequences were as shown in Table 1: bulky aromatic amino acids were frequently detected at AA^2, AA^3 and AA^4 positions (type I) and Aib was frequently detected next to D-Pro (type II). Several sequences which contain these features were synthesized and subjected to evaluation of catalytic performance (Table 2).
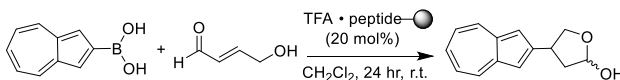
Table 2. Evaluation of detected sequences from library screening.

Entry	Peptide sequence	Conv. [%]$^{a)}$	ee [%]$^{b)}$
1	Pro-D-Pro-Aib-(Ala)$_5$ (**1**)$^{c)}$	71	-36$^{d)}$
2	D-Pro-Tyr-Trp-Tyr-His-Leu-Aib	87	7

3	D-Pro-Trp-Trp-Trp-Leu-Tyr-Leu	90	5
4	D-Pro-TyrTyr-Tyr-Aib-Trp-His	67	20
5	D-Pro-Aib-His-Trp-Trp-Trp-Tyr	90	5
6	D-Pro-Aib-Trp-Trp-Aib-Trp-His (**2**)	90	54

a) Determined by ^{1}H NMR. b) Determined by HPLC after reduction and esterification. c) This sequence was found in our previous study. d) The minus sign means opposite configuration.

Table 3. Optimization of peptide sequences.

| Entry	Sequence	Conv. [%][a]	ee [%][b]
1	D-Pro-Aib-Trp-Pya-Aib-Trp-His	78	48
2	D-Pro-Aib-Trp-Phe-Aib-Trp-His (**3**)	66	56
3	D-Pro-Aib-Trp-D-Pro-Aib-Trp-His	87	7
4	D-Pro-Aib-Trp-His-Aib-Trp-His	87	20
5	D-Pro-Phe-Trp-Phe-Aib-Trp-His	85	40

a) Determined by ^{1}H NMR; b) Determined by HPLC after reduction and esterification.

They all showed reasonable catalytic activity, among these, **2** showed the best enantioselectivity, which was allowed for further optimization (Table 3). After Alanine-scanning of this peptide, several positions in the sequence of **2** were modified. Although small difference in the enantioselecitivity was observed through optimization, **3** was found to be the best enantioselective catalyst for this reaction. To conclude, a new screening method to find brand new sequences of efficient peptide catalysts has been successfully developed.

3. Facile substitution reaction of guaiazulene-3-methanol derivatives with thiols and its application to labelling of thiol-containing biomolecules[2]

As shown in Scheme 2, the guaiazulene-3-methanol derivative (**4**) was proved to be highly reactive toward nucleophilic guaizulene, and this led my interest to its reaction with other nucleophiles (Scheme 3). As a result, it was found that a thiol readily reacted with **4** to give the substitution product. Other nucleophiles such as an alcohol, an amine, a carboxylic acid, and a sulfide did not give any substitution products. In case of the reaction with the carboxylic acid, a dehydrative bimolecular condensation of **4** occurred to give **5**.
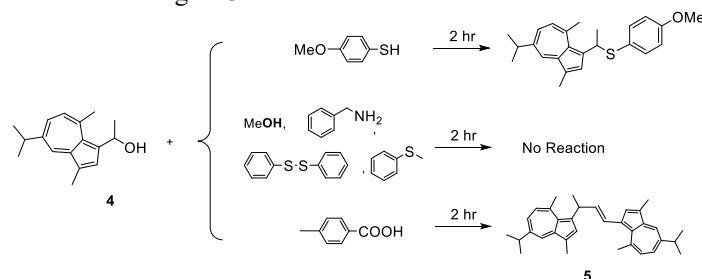
Scheme 3. Reactions of **4** with different types of nucleophiles.

This thiol preference for the substitution can be explained by both weakly acidic and nucleophilic nature of the thiol functional group. The hydroxyl group in **4** might be first protonated by thiol leading to spontaneous elimination of water molecule to form an azulenylmethyl cation, and the thiolate anion should attack to this intermediate. For the reaction with non-acidic functional groups, such as alcohol, amine and sulfide, the first dehydration may hardly occur. In contrast, carboxylic acid should enhance the formation of intermediate cation but not nucleophilic enough to participate the substitution reaction resulting in the formation of bimolecular condensation product **5**.

Besides the aromatic thiol, an aliphatic thiol (PhCH$_2$CH$_2$SH) also underwent spontaneous substitution although slower. As pKa of the former is lower than that of the latter, I thought efficient protonation might be the key of this reaction. Consequently, effect of an acid on the rate of this reaction was examined. As expected, in the presence of 0.3 eq. of acetic
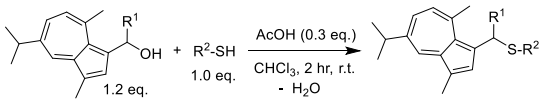
acid, the reaction was considerably accelerated. Generality of this reaction was screened with several kinds of guaiazulene-3-methanols and thiols (Table 4). For the electrophiles, no large effect of the substituent on the reactivity was observed. By contrast, when the nucleophilic thiol is sterically congested, the reaction was considerably slower.

Then I tried to apply this thiol-specific reaction to the labelling of biomolecules having thiol units. Physiological thiols, such as glutathione (GSH), cysteine (Cys) and homocysteine (Hcy), have been recognized to play important roles in maintaining biological systems through reversible oxidative dimerization to give a disulfide and back reduction to thiols, hence estimation of the amount of free thiol is important in biological studies. In

Table 4. Reaction of GA derived alcohol with thiols.

Entry	R^1	R^2	Yield [%]
1	Me	$4\text{-}MeOC_6H_4\text{-}$	90
2	Me	$Ph(CH_2)_2\text{-}$	80
3	iPr	$4\text{-}MeOC_6H_4\text{-}$	89
4	iPr	Ph	70
5	iPr	$2\text{-}CH_3C_6H_4$	84
6[a,b]	iPr	$4\text{-}NO_2C_6H_4\text{-}$	15
7	iPr	$Ph(CH_2)_2\text{-}$	82
8	iPr	$CH_3(CH_2)_5\text{-}$	90
9	iPr	cyclohexyl	81
10[b]	iPr	tBu	40
11	tBu	$4\text{-}MeOC_6H_4\text{-}$	85
12	Ph	$Ph(CH_2)_2\text{-}$	87

a) MeOH as a solvent. b) For 12 hr.

this context, kinds of thiol labelling agents have been developed[2]. Among them, colorimetric method is the most convenient but the known methods are based on conjugate addition of the thiols to maleimide derivatives which suffers from formation of 'false positive' by other nucleophilic functional groups in biomolecules. Use of the compound **4** is expected to avoid such unwanted side reactions.

By mixing **4** and Ac-Cys-OH, the reaction proceeded spontaneously and completed in 5 min to give the thiol-labelled product in 89% yield. This reaction was then extended to a labelling experiment of GSH and a peptide (Figure 4). The alcohol **4** was used as a labelling agent to achieve the naked-eye visualizations of biothiols on filter paper. When GSH was employed as a test molecule, semi-quantitative nature of this staining could be confirmed. In case insulin was used, it was found that the native insulin having three disulfide bonds and no free thiols did not turn blue, while its reduced product successfully get color upon treatment with **4** in ethanol.
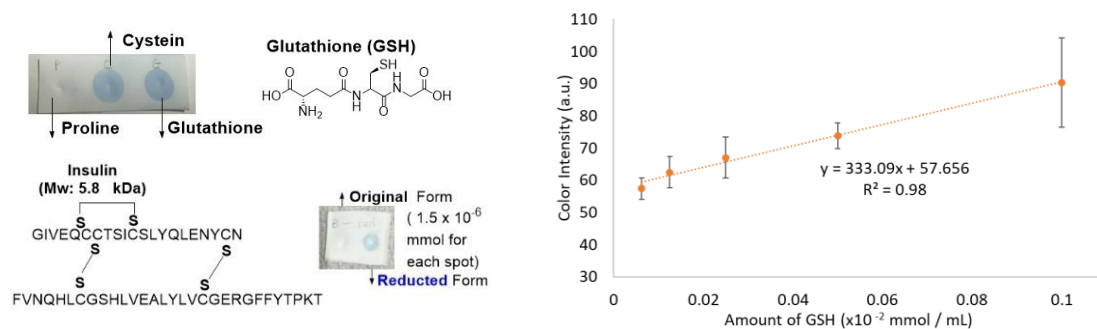

Figure 4. Staining of biothiols on filter paper (left);The relationship between loaded amount of GSH and color (right).

As described above, I succeeded to find a facile reaction of thiols with azulenylmethyl alcohols. From other viewpoint, this reaction is considered to be a protection of thiol group. Thiol group protection is also desirable in the biochemistry research field especially those related to proteins and peptides in which sometimes orthogonal protection of more than one thiol group is required. So I tried to develop removal of the S-bound azulenylmethyl group. After screening a number of conditions, I finally found that the deprotection can be realized by the treatment with tris(2-carboxyethyl)phosphine (TCEP) in MeOH. TCEP is well known reductive deprotecting agent for disulfide-protected thiols. However, in the

present case, the S-adduct is not a disulfide but a sulfide. This means that, the deprotection occurred through simple substitution of the thiol group. Use of azulenylmethyl-protected cysteine might be useful for the solution phase synthesis of peptides because the peptide containing such unit is blue and this will make the chromatographic separation of the product easier. A demonstrating experiment utilizing such an advantageous character of the labelled Cys was carried out as shown in Scheme 4. Orthogonally protected two Cys were connected to give a dipeptide. First deprotection with dithiothreitol (DTT) successfully underwent disulfide bod cleavage, and the subsequent treatment with TCEP gave fully protected dipeptide.
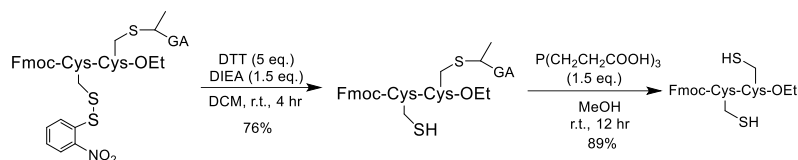
Scheme 4. Applications to selective deprotection of thiol groups on a peptide.

4 . Synthesis of calix[5]azulene with using guaiazulene as a leaving group[3]

During the studies mentioned in previous chapters, I had happened to find that the GA works as not only a nucleophile, but also as a leaving group. Treatment of bis(guaiazulen-3-yl)methane (**6**) with 4-methoxybenzenethiol in the presence of TFA resulted in the substitution of GA by the thiol (Scheme 5). In case this nature of GA is combined with bi-nucleophilic AZ, it is expected to form linear polymer containing AZ part in the main chain.
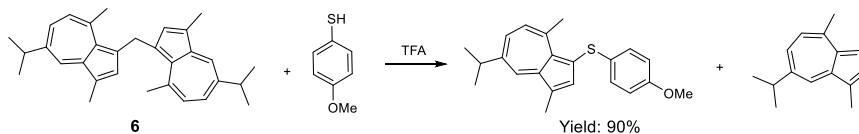

Scheme 5. Reaction of **6** with 4-methoxybenzenethiol to generate GA.

Due to intriguing character of AZ from the viewpoint of optical and electronic properties, polymers containing AZ part have been studied by several groups. Most of studies are highly conjugated rigid homo- or copolymer of AZs. On the other hand, AZ-pendent flexible methacrylate polymer has also been reported. A polymer with alternating 1,3-azulenediyl and methylene units should have an intermediate rigidity and is expected to show unique properties, but such a polymer has not been reported so far. I tried a polymerization using **6** and AZ in the presence of TFA. Although the starting **5** was completely consumed, high-molecular weight polymer could not be obtained. From the analysis of the product, it was supposed that 3 to 8-mer having GA in one terminus was mianly formed. Besides them, cyclic tetramer (calix[4]azulene) and pentamer (calix[5]azulene) were also observed in a 40:60 ratio.

Concerning the formation of these cyclic compounds, it has been reported starting from AZ and formaldehyde under acidic conditions[3]. In that report, they reported that the main products were calixazulenes in 80:20 ratio of tetramer / pentamer, from which only the former compound could be successfully isolated. That research was followed by other groups for utilization of calix[4]azulene as a host compounds for ammonium salts or as potential precursor for conjugated cyclic compound. In contrast, isolation of calix[5]azulene has not been realized to date. So I changed my goal to the preparation / isolation of calix[5]azulene. First, I had optimized the reaction conditions for the exclusive formation of calixazulenes. Such a result was obtained when I did the reaction in xylene in the presence of H_2SO_4. The resulting cyclic tetramer / pentater ratio was 24:76. After screening of several methods for seperation, I found that the calix[4]azulene and calix[5]azulene show considerably different solubility by repetitive washing with chloroform, and less soluble

calix[5]azulene was isolated.
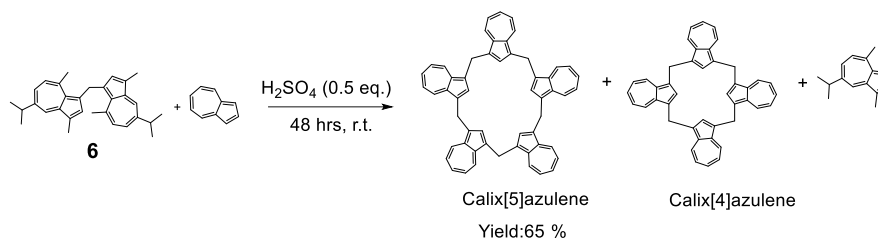
Scheme 6. Synthesis of calix[5]azulene from **6**.

The preferential formation of calix[5]azulene over calix[4]azulene in the reaction of **6** can be explained as follows (Figure 5): the reaction of AZ and paraformaldehyde gives a linear tetramer (Fig. 5 **a**). which adopts an easy-to-cyclize conformation, the corresponding tetramer from compound **6** has bulky GA moiety at the reaction center which prevents cyclization (Fig. 5 **b**). Instead, pentamer gave easy to cyclize structure (Fig. 5 **c**).
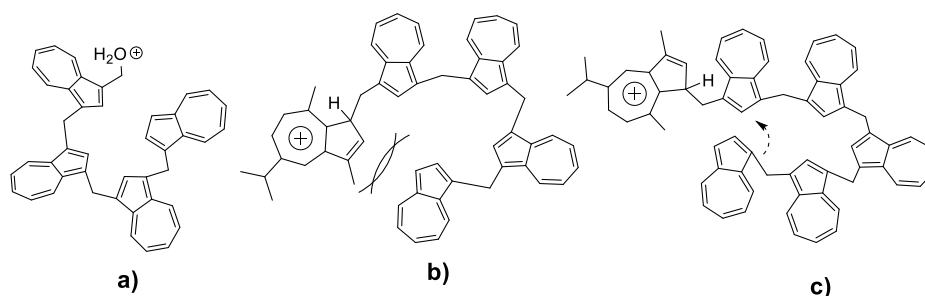

Figure 5. Proposed mechanism of formation of calixazulenes.

5. Conclusion

As AZ has its unique blue color and is highly polarized to show a high α-methyl-cation-stabilizing character. In this thesis, I utilized its unique properties on its five-membered ring to achieve its versatile behaviors in different chemical reactions. Firstly, azulene-2-ylbronic acid was successfully employed in peptide library screening based on its reactivity and color nature to find brand new sequences of efficient peptide catalysts. Secondly, guaiazulene-3-methanol derivatives were found to selectively and spontaneously react with thiols. This reactivity was successfully applied to lablelling of thiol-containing biomolecules. Thirdly, GA was utilized as a leaving group in the exclusive formation calix[5]azulene, which led successful isolation of calix[5]azulene from reaction mixtures. Although the chemistry of AZ is rich, its various behaviors has not been fully understood yet. Throughout this thesis, I am expecting broaden understanding of AZ chemistry and its applications in diverse area.

6. References

(1) K. Akagawa, M. Sugiyama, K. Kudo, *Org. Biomol. Chem.*, **2012**, *10*, 4839.
(2) X. Chen, Y. Zhou, X. Peng, J. Yoon, *Chem. Soc. Rev.*, **2010**, *39*, 2120.
(3) D. Al. Colby, T. D. Lash, *J. Org. Chem.*, **2002**, *67*, 1031.

7. Publications

[1] Y. Jin; K. Akagawa; K. Kudo; *In preparation*.
[2] Y. Jin; K. Akagawa; K. Kudo; *In preparation*.
[3] Y. Jin; K. Akagawa; K. Kudo; *In preparation*.

8. Related Publication

P. Cowper, Y. Jin, M. D. Turton, G. Kockik-Köhn, S. E. Lewis, *Angew. Chem. Int. Ed.*, **2016**, *55*, 2564.