Nanoscale Molecular Cavities for Stabilization of Highly Reactive Species

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Abstract: Several kinds of bowl-type molecules were designed for regulation of the reactivities of the functional groups in their cavities, especially for kinetic stabilization of highly reactive species which have been difficult of access by conventional methods. The "peripheral steric protection" by these molecular cavities effectively prevents the bimolecular decomposition of the reactive species such as dimerization or self-condensation, and enabled the isolation of various species including a sulfenic acid, a selenenic acid, an S-nitrosothiol, and a Se-nitrososelenol, which have been known as important but elusive intermediates in biological reactions. Utilization of the molecular bowls as the ligands for transition metal complexes is also described.

1. Introduction

Regulation of the reactivity of functional groups by the appropriate design of the reaction environment is one of the most important topics in organic chemistry. In the active site of enzymes, the reactive species which are very unstable in the artificial system are sometimes stabilized and involved in the enzymatic reactions as functional intermediates. Recently, reactive species generated by oxidation or nitrosation of cysteine and selenocysteine residues have been attracting increasing attention in view of their roles in redox regulation and signaling in the biological systems. Typical examples of such species are sulfenic acids (RSOH) and selenenic acids (RSeOH). The sulfenic acid is a particularly reactive and versatile reversibly oxidized form of cysteine (Scheme 1), which has important roles as a catalytic center in enzymes and as a sensor of oxidative and nitrosative stress in enzymes and transcriptional regulators. The selenenic acid has been well recognized as the intermediate in the catalytic cycle of glutathione peroxidase (GPx) generated by oxidation of selenocysteine with peroxides (Scheme 1). Although these species have also been known as important intermediates in the reactions of organosulfur and organoselenium compounds, much of our knowledge of their chemistry has been derived in quite an indirect and speculative fashion owing to their high instability; they are known to undergo rapid self-condensation leading to the corresponding thiosulfinates or selenoseleninates (Scheme 2). Especially, selenenic acids are so unstable that there had been no example of isolation of a selenenic acid in pure form until our recent reports. For elucidation of the role of these reactive species in biological systems, it is considered to be necessary to synthesize stable "reference compounds" with which their properties can be experimentally elucidated in a direct manner, and a new methodology to stabilize these important but elusive species has long been awaited. In this article, we report the design and application of bowl-shaped molecular cavities for the stabilization of highly reactive species which have been difficult of access by conventional methods.

2. Design of Molecular Bowls for Stabilization of Reactive Species

For the stabilization of reactive species which undergo facile bimolecular decomposition such as dimerization and self-condensation, steric protection due to bulky substituents has been effectively employed. A wide variety of steric protection groups have been developed and successfully applied to the syntheses of various species. The representative bulky aromatic substituents and the examples of stabilization of reactive species are shown in Figure 1. However, steric protection of chalcogen-containing reactive species such as sulfenic acids and selenenic acids is rather difficult in comparison with reactive species containing other group elements. For example, the bimolecular decomposition of a selenenic acid cannot be prevented even with a 2,4,6-tri-tert-butylphenyl group (denoted as Mes* hereafter), one of the most effective steric protecting groups; a selenenic acid bearing a Mes* group undergoes disproportionation to the corresponding diselenide and seleninic acid via self-condensation reaction (Scheme 3). This is mainly because only one substituent can be introduced to such chalcogen-containing species unlike 1-3, which can carry multiple steric protection groups.
In principle, if the steric bulkiness of the ortho-substituents is further increased from tert-butyl groups, the bimolecular process will be hindered. However, too severe steric shielding of the central functionality would lead to difficulties in the functional group transformation for generation of the target species as well as in elucidation of the reactivity of the obtained species towards other molecules (Figure 2). Furthermore, the ortho-substituents in close vicinity of the reactive functionality tend to cause undesired side reaction with it as shown in Scheme 4.10

The stabilization of the reactive species in the active site of enzymes provides the clue to overcome these limitations of the steric protection. The reactive functionality in the active site is usually incorporated in the cavity or the cleft and isolated from other reactive groups in the same and/or different subunit by the topology and the higher-order structure of proteins. These functionalities are inert towards each other, but they can readily react with the substrates. For construction of such an environment, we designed bowl-shaped molecules schematically shown in Figure 3. In these molecules, the functional groups X and Y cannot approach each other because of the steric repulsion of the peripheral moieties, whereas they can react with other reagents because there is a relatively large space around them. The functionalities X and Y can be either of the same kind or of different kinds. Such mode of stabilization shown in Figure 3 can be referred to as “peripheral steric protection” in contrast with the conventional “proximal steric protection” shown in Figure 2. We have designed several kinds of molecular bowls as...
shown in Figure 4. In 1987, Luning et al. proposed the concept of “concave reagents”; that is, cavity-shaped molecules with an inwardly-directed functionality embedded in the concave position.11 Because these molecules are designed to increase the selectivity of reactions by making use of the concave shielding of the functionality, their sizes are generally not so large. For our purposes, however, large and shallow molecular bowls are desirable, and the compounds shown in Figure 4 have been designed so that they have a diameter of 1.3 or 1.4 nm.12

3. Bowl-type Bimacro cyclic Cyclophane

As the first bowl-type molecule, we designed the bimacro cyclic cyclophane Ar1X (Figure 5) by making a faithful translation of the schematic drawing in Figure 3 to a molecule.13 This molecule consists of an aryl benzyl ether framework because formation of this type of linkage is convenient for construction of a large cyclophane skeleton. The crystallographic analysis of Ar1Br (4) bearing this framework indicates that 4 has a shallow bowl-shaped cavity with a diameter of ca. 1.4 nm as expected (Figure 6).13 Sulfenic acid 6 was successfully synthesized by pyrolysis of butyl sulfoxide 5, which was derivatized from 4, and isolated as stable crystals (Scheme 5). The thermal stability of 6 was unprecedentedly high; even after heating at 90 °C for 12 h in toluene-d8, only slight decomposition was observed. On the other hand, 6 readily reacted with methyl propiolate and 1-butanethiol to produce 7 and 8, respectively. These results validate the concept of the molecular bowls described in the previous section. Furthermore, this cyclophane framework can be easily modified to give various derivatives such as the water-soluble molecular bowl Ar2X (Figure 5).14 It is well-known that hydrophobicity around the catalytic functionality in the active site of enzymes is one origin of the unique features of enzymatic reactions. The environment of the cavity of Ar2X was investigated by using the chromophore covalently fixed in the cavity. The micropolarity around the central functionality of 9 in water was estimated to be 0.70 in the E8 scale, which corresponds to the polarity of ethanol. Although the bowl-shaped cyclophane, Ar1X, proved to be useful as the molecular bowl, there are some problems associated with this framework. Including the CH2O linkages with considerable flexibility, the framework of Ar1X is not rigid enough to maintain the bowl shape in any conformation of the molecule, and its ability to prevent dimerization of reactive species is somewhat decreased. For example, the iodine oxidation of thiol 10 gives disulfide 11, although the reaction is so slow that it takes several hours (Scheme 6). The aryl benzyl ether linkages are also subject to degradation upon treatment with reactive agents such as alkylithiums, which were used for lithiation of bromide 4. Furthermore, the bicyclic framework of Ar1X requires multi-step synthesis and is not suitable for large-scale preparation.


For the design of more readily accessible and efficient bowl-type molecules, a calix[6]arene framework seems to be an attractive molecular platform.15 Calix[6]arenes can be prepared on a large scale in good yields, unlike other cyclophanes of this size. A wide variety of functionalization on their upper and lower rims has been extensively investigated. However, the functional groups on the calixarene macrocycle intrinsically tend to diverge away from the cavity. For the construction of a bowl-type molecule by incorporation of an inwardly-directed functional group into the cavity of calixarenes, we designed m-xylylene-bridged calix[6]arenes represented by general formula 12.16 Luning et al. have also reported the bridged calix[6]arenes bearing a framework similar to that of 12 independently from us.17 The framework of 12 can be easily constructed by building a bridge with a substituted m-xylylen unit over the distal positions of the parent calix[6]arene followed by capping the four remaining hydroxy
units with alkyl groups. For example, the bridging reaction to produce tert-butyl sulfide 13 proceeded in a high yield of 92%, and the methylation of 13 afforded 14 in 82% yield (Scheme 7).

The synthesis of a stable sulfenic acid was attained also by using this bridged calix[6]arene framework. Thermolysis of tert-butyl sulfoxide 15 prepared by oxidation of 14 in toluene afforded sulfenic acid 16 almost quantitatively (Scheme 8). X-ray crystallographic analysis of 16 revealed that the SOH functionality is surrounded by the calix[6]arene macrocycle adopting the 1,2,3-alternate conformation (Figure 8). The reaction of 16 with methyl propiolate afforded the adduct 17, although the reaction was much slower than that of 6 bearing the bicyclic cyclophane framework. This is probably because the vicinity of the SOH group of 16 is sterically more congested than that of 6.

When calix[6]arenes are used as a molecular platform, it is often problematic that their conformational flexibility is very large. In the case of the bridged calix[6]arene 12, however, several conformationally frozen isomers can be obtained by arylmethylation of the four hydroxy groups at the lower rim (Figure 9). Usually, the cone isomer (a) and the 1,2,3-alternate isomer (b) are obtained as the major isomers. In some cases, the partial cone isomer (c) is formed as a minor product. When the functionality X is a sterically demanding group such as butylseleno and phenylethynyl groups, the formation of the inverted cone isomer (a') instead of the cone isomer of (a) is kinetically preferred.

Because the steric protection endowed by the bridged calix[6]arene framework 12 is considered to be more effective than that of the aforementioned bicyclic cyclophane framework, the stabilization of a selenenic acid was then investigated. Selenenic acid 19a fixed in the cone conformation was synthesized by oxidation of butyl selenide 18a with the inverted cone conformation followed by thermolysis of the resulting selenoxide and isolated as stable crystals (Scheme 9). During the formation of 19a, the conformational change from the inverted cone (a') to the normal cone (a) took place with the flipping of the central bridging unit. The structure of 19a was established by X-ray crystallographic analysis (Figure 10), representing the first example of X-ray analysis of a selenenic acid. The SeOH functionality of 19a is deeply buried in the cavity of the calix[6]arene macrocycle with the cone conformation, and its self-condensation seems almost impossible. Selenenic acid 19a showed high stability both in the crystalline state and in solution. Even after heating at 120 °C for 5 h in CDCl3/CDCl3, no decomposition was observed. Considering the reported fact that even 2,4,6-tri-tert-butylbenzeneselenenic acid disproportinates completely within 2 h in 4% D2O/CD3CN at 25 °C, such sta-
bility of 19a is remarkable. Selenenic acid 19b fixed in the 1,2,3-alternate conformation was synthesized by a similar method (Scheme 9). This conformational isomer showed comparable stability to the cone isomer 19a, whereas the spectral properties and reactivities of 19b are somewhat different from those of 19a. For example, the OH absorption bands of 19a and 19b suggest that there are intramolecular hydrogen bonding interactions in 19b while no significant interaction of such kind exists in 19a. While the cone isomer 19a reacted with an excess amount of 1-butanethiol at room temperature to give the corresponding selenenyl sulfide, the 1,2,3-alternate isomer 19b underwent no reaction even at 50 °C (Scheme 10). These results demonstrate that the properties and reactivities of the endohedral functionality can be regulated by the conformation of the calix[6]arene framework. It was also found that the conformational isomers of a water-soluble derivative of the bridged calix[6]arene show different aggregating properties. The critical micelle concentrations of the cone and 1,2,3-alternate isomers of water-soluble calix[6]arene 20 are quite different from each other, which can be explained in terms of their monomer structures with contrasting arrangements of the charged groups (Figure 11).

5. Molecular Bowl Based on an All-Carbon Framework

The bridged calix[6]arene framework described above is a versatile molecular platform because several conformational isomers are available as the scaffolds and various functionalization can be done at their upper and lower rims to endow them special properties such as water-solubility. For investigation of the intrinsic properties of reactive species, however, the presence of the oxygen atoms in the framework is inconvenient because they cause electronic perturbation on the species. A molecular bowl based on a more inert and simpler framework without heteroatoms is desired for the kinetic stabilization of reactive species in general. In the course of the aforementioned studies on the bimacrocyclic cyclophane and the bridged calix[6]arene systems, it occurred to us that the essential condition for prevention of dimerization of reactive species is to surround the functional group from all sides and from a distance in any conformation of the molecule. Inspection of CPK models suggested that a simpler, acyclic molecule with large conformational flexibility could function as a molecular bowl if it meets these conditions. As such a molecular bowl with high accessibility, we designed the acyclic molecule shown in Figure 12 with an all-carbon framework, which is denoted as BmtX hereafter. Because BmtX has two rigid m-terphenyl units in the positions which correspond to the bridgeheads of bicyclic molecules, the central functionality X is effectively surrounded in any conformation of the molecule. Bromide 23 was readily prepared by a cross-coupling reaction between the m-terphenyl unit 21 and tribromide 22 in good yield. Functional group transformation can be carried out via lithiation of 23 without significant side reactions. The inert framework of the Bmt group is considered to be suitable for the elucidation of the intrinsic properties of reactive species. Stable sulfenic and selenenic acids bearing the Bmt group were synthesized, and their structures and various reactivities were investigated.

Scheme 9

\[
\begin{align*}
\text{ArSeBu}^+ & \quad 1) \text{mCPBA}, 0^\circ \text{C} & \quad \text{ArSeOH} \\
18a: \text{Ar} = \text{Ar}_{w}^+ & \quad 2) \text{toluene}, 80^\circ \text{C}, 2 \text{~h} & \quad 19a: \text{Ar} = \text{Ar}_{w}^+ (74\%) \\
18b: \text{Ar} = \text{Ar}_{h}^+ & \quad 19b: \text{Ar} = \text{Ar}_{h}^+ (44\%) 
\end{align*}
\]

Scheme 10

\[
\begin{align*}
\text{Ar}_{w}^+\text{SeOH} & \quad 10 \text{eq Bu}^+\text{SH} & \quad \text{Ar}_{w}^+\text{SeSBu}^+ (70\%) \\
19a & \quad \text{CDCl}_3, 25^\circ \text{C}, 4 \text{~h} & \quad \text{Ar}_{h}^+\text{SeOH} \\
\text{Ar}_{h}^+\text{SeOH} & \quad 10 \text{eq Bu}^+\text{SH} & \quad \text{Ar}_{h}^+\text{SeSBu}^+ \\
19b & \quad \text{CDCl}_3, 50^\circ \text{C}, 12 \text{~h} & \quad \text{Ar}_{h}^+\text{SeSBu}^+
\end{align*}
\]

Figure 10. ORTEP drawing of 19a (30% probability).

Figure 11. Water-soluble bridged calix[6]arenes.

Figure 12. Molecular bowl based on an all-carbon framework.

Scheme 11

\[
\begin{align*}
\text{MgBr}_2 \text{CuCl}_2 & \quad \text{THF} & \quad 22 \rightarrow \text{BmtBr} (78\%) \\
\text{21} & \quad \text{21} & \quad \text{21}
\end{align*}
\]

Sulfenic acids are generally assumed to be transient intermediates in the oxidation of thiols, both to disulfides and to sulfenic acids, and the redox process between a thiol and a...
sulfenic acid plays an important role in some biological reactions (Scheme 12). In such reaction schemes, however, sulfenic acids have always appeared in parentheses so far, and the evidence for these processes has been entirely circumstantial owing to their instability. The reaction processes in Scheme 12 were investigated using the compounds with the Bmt group.

Scheme 12

\[
\text{RSH} \xrightarrow{[O]} \text{RSOH} \xrightarrow{[O]} \text{RSO}_2\text{H}
\]

Oxidation of a thiol is the most straightforward and simplest way for the synthesis of a sulfenic acid. However, even the observation of an intermediary sulfenic acid in the direct oxidation of a thiol has never been reported. The reaction of thiol 24 with iodosobenzene, a mild oxidant which usually converts thiols to disulfides, afforded sulfenic acid 25, which was isolated as stable crystals (Scheme 13). The peripheral steric bulk of the Bmt group is considered to prevent the initially formed sulfenic acid 25 from reacting with a second molecule of thiol 24 during the oxidation, as well as to suppress the self-condensation of 25. On the other hand, sulfenic acid 25 was reactive enough to be reduced to thiol 24 by treatment with a relatively bulky reductant, triphenylphosphine. Sulfenic acid 25 was oxidized to sulfinic acid 27 by oxaziridine 26. The reactions of 25 with 1-butanol and thioephene produced the corresponding disulfides 28a and 28b, respectively. Sulfenic acid 25 was reduced to thiol 24 also by dithiothreitol via the intermediary disulfide 28c. Thus, by taking advantage of the Bmt group, all the processes in Scheme 12 have been demonstrated conclusively. The reaction of 25 with benzylamine afforded sulfenamide 29, indicating that a sulfenic acid exhibits electrophilic reactivity even under basic conditions.

Scheme 13

The structure of 25 was established by X-ray crystallographic analysis (Figure 13). The central SOH functionality is surrounded by two rigid m-terphenyl units like the brim of a bowl. Concerning the structure of a sulfenic acid, there has been controversy as to whether it takes the sulfenyl form (Figure 14, (A)) or sulfoxide form (Figure 14, (B)). Although there have been some examples of the X-ray analysis of sulfenic acids, all of those compounds have nitrogen, sulfur, or oxygen atoms in the vicinity of the SOH group and their S-O bond lengths fall between 1.58-1.63 Å. The S-O bond length of sulfenic acid 25 with the all-carbon framework is 1.675(5) Å, which is distinctly longer than that of sulfoxides [1.44-1.59 Å]. These results suggest that an arenesulfenic acid without perturbation by heteroatoms takes the sulfenyl form rather than the sulfoxide form. The properties and reactivities of sulfenic acid 25 were cited in the biochemical review as the reference data for a sulfenic acid.

Figure 13. ORTEP drawing of 25 (30% probability).

Figure 14. Two possible structures of a sulfenic acid.

The synthesis of a selenenic acid bearing a Bmt group was then investigated. In the catalytic cycle of GPx, a selenenic acid intermediate is considered to be generated by oxidation of a selenol with peroxides (Scheme 1). This process, which detoxifies the peroxides, has also been postulated to be included in the reactions of many synthetic GPx mimics. However, even trapping of an intermediary selenenic acid during oxidation of a selenol has not been reported yet. When selenol 30 bearing a Bmt group was treated with an equimolar amount of hydrogen peroxide, selenenic acid 31 was formed as the main product and isolated as stable pale yellow crystals in good yield of 77% (Scheme 14). X-ray crystallographic analysis established the structure of 31, which has the features similar to those of sulfenic acid 25. In 77Se NMR (CDCl3), 31 showed a signal at δ 1079. Selenenic acid 31 was further oxidized to seleninic acid 32 by mCPBA while treatment with triphenylphosphine reduced 31 to selenol 30. The selenenic acid form in the catalytic cycle of GPx is reduced to the selenol form via the selenenyl sulfide form by the reaction with two molecules of glutathione. Selenenic acid 31 reacted with various thiols to produce the corresponding selenenyl sulfides such as 33a-c. However, reduction of selenenyl sulfides 33a-c to selenol 30 by treatment of a thiol was found to be difficult. Treatment of 33a-c with an excess amount of thiols in the presence of triethylamine resulted in the thiol exchange on the selenium atom almost exclusively with the formation of only a trace amount of selenol 30. These results suggest that the equilibrium lies to the selenenyl sulfide and the thiol rather than the selenol and...
the disulfide (Scheme 15). In fact, when the mixture of selenol 30 and dibutyl disulfide was allowed to stand in the presence of triethylamine, selenol 30 disappeared completely and selenenyl sulfide 33a was obtained. For enhancement of the selenol formation, 1,4-dithiols were employed, which affords thermodynamically favorable cyclic disulfides upon oxidation. Reaction of selenenic acid 31 with an excess of 1,4-butanedithiol or DTT afforded the corresponding selenenyl sulfides 33d and 33e (Scheme 16). When 33d and 33e were treated with triethylamine, elimination of the cyclic disulfides took place and selenol 30 was obtained in good yields. Thus, the experimental demonstration of three processes included in the catalytic cycle of GPx has been achieved for the first time by taking advantage of the Bmt group. From the results obtained here using the selenium compounds with an inert, all-carbon framework, it is suggested that the high efficiency of the catalytic function of the selenoenzyme is at least partially due to the participation of the ancillary functional groups around the selenium center, which enhances the nucleophilic attack of the thiol at the sulfur atom of the selenenyl sulfide form, not at the selenium atom to result in the thiol exchange.

Selenenic acids can be regarded as a selenium analogue of hydroperoxides (ROOH) and their properties as oxidizing agents are intriguing in terms of comparison with hydroperoxides. While the mechanism of the reduction of hydroperoxides by trivalent phosphorus compounds has been elucidated in detail,25 no such mechanistic study has been performed on their heavier analogues such as selenenic acids. There are two possible mechanisms for the reduction of hydroperoxides and their heavier analogues. One mechanism involves the initial attack of the phosphorus on the hydroxyl oxygen atom to form the intermediate (I), and the other involves the phosphonium hydroxide intermediate (II) (Scheme 17). For the reaction of hydroperoxides, the experimental support for Route A was provided by a tracer study using H218O.25 In sharp contrast, the initial step of the reaction of selenenic acid 31 with triphenylphosphine was found to involve the attack of the phosphine on the selenium atom of 31 (Route B); the reaction in the presence of H218O afforded triphenylphosphine oxide containing 18O in the exchange rate of 87% (Scheme 18).26 Similarly, a high exchange rate of 77% was observed in the reaction of sulfenic acid 25 with triphenylphosphine, indicating that the reduction of 25 also proceeds via Route B. It is notable that the nucleophilic attack of the relatively bulky phosphine occurs at the sulfur and selenium atoms, even though they are incorporated in the cavity of the Bmt group and are less accessible than the hydroxylic oxygen on steric grounds. These results also corroborate the highly electrophilic character of the sulfur and selenium atoms of sulfenic and selenenic acids.

The Bmt group with an all-carbon framework can be applied to stabilization of reactive species containing an aluminium atom. It is known that organotrihydroaluminates (RAH3−) undergo facile ligand redistribution reaction described in Scheme 19, and very little is known about their reactivity as reducing agents in contrast to organotrihydrobo-
rates. The reaction between BmtLi and AlH₃NMe₃ in DME gave trihydroaluminate 34 (Scheme 20), whose monomeric structure was established by X-ray analysis.²⁷ It was found that 34 shows high reactivity as a reducing agent toward unsaturated compounds such as benzophenone, phenyl benzoate, and benzonitrile. In the reaction with benzophenone, 34 can reduce a 3 equimolar amount of substrate to benzhydrol.

Scheme 19

\[
2[\text{AlH}_2\text{Li}] \rightarrow [\text{AlH}_4]^+ + [\text{AlH}_2\text{Li}]^-
\]

Scheme 20

\[
\text{BmtBr} + \text{BuLi}, \text{AlH}_3\text{NMe}_3 \rightarrow \text{BmtAlH}_2\text{Li(DME)}_2 \rightarrow 34 \text{ (80%)}
\]

6. m-Terphenyl-based Dendrimer-type Molecular Cavities

6.1 Design, Synthesis, and Characteristics of Novel Dendrimer-type Molecular Cavities

When reactive species are prevented from undergoing intermolecular decomposition reactions such as dimerization and self-condensation, they tend to react intramolecularly with the substituents nearby. It is well known that the ortho-tert-butyl group of a Mes⁺ group often causes a side reaction with reactive species as shown in Scheme 4. Such kind of intramolecular reaction involving the ortho-alkyl group is one of the most difficult problems faced by the steric protection using bulky aromatic substituents. As described in the previous sections, the Bmt group effectively suppresses the intermolecular decomposition of reactive species, and it is relatively free from the intramolecular side reaction because it protects the central functionality from peripheral positions. In some special cases, however, the methylene groups at the ortho-positions of a Bmt group are involved in the reaction. For example, heating of N-thiosulfinylaniline 35 for a prolonged time resulted in intramolecular cyclization to afford 2,1-benzisothiazole 36 (Scheme 21).²⁸ Similar cyclization reactions were also reported for N-thiosulfinylanilines 37²⁹ and 38.³⁰ Recently, m-terphenyl-2'-yl groups represented by the general formula 39 have been widely utilized as excellent steric protection groups which are not liable to undergo such intramolecular reactions. Even in 39, however, the alkyl groups at 2,2'',6,6''-positions are considerably close to the central functionality and sometimes involved in its reaction as shown in Scheme 22.

Miller et al. reported the synthesis of 1,3,5-phenylene-based dendrimer 40.³³ It occurred to us that the framework of such a dendrimer can be used for stabilization of reactive species because there is an inert and relatively large space in the core position. Based on such an idea, we designed a novel dendrimer 41, where the m-terphenyl framework extends to form a large molecular cleft with no alkyl group around the central functionality. In this dendrimer, there is a large rotational freedom of the biaryl bonds whereas the absence of any sp³ carbon atoms in the skeleton makes the flexibility of the whole molecule very low. In any conformation of the molecule, the central functionality X is embedded in the molecular cleft and its dimerization is expected to be more difficult than the corresponding compound bearing a Bmt group. As the minimum generation model of 41, we designed a novel aromatic compound shown in Figure 16, which is denoted as BpqX hereafter,³⁴ and applied it to stabi-
lization of various reactive species. The CPK model examination indicates that the peripheral isopropyl groups of BpqX cannot be close to the central functionality X because its skeleton consists solely of rigid biaryl units.

The framework of BpqX can be readily constructed by repetition of Hart’s method for the m-terphenyl synthesis (Scheme 23). Functionalization of the X position can be done by quenching the second reaction with an appropriate electrophile or via lithiation of iodide 42.

As expected, N-thiosulfinylaniline 43 with this dendrimer-type substituent showed remarkable thermal stability in comparison with 35 bearing a Bmt group. No decomposition of 43 was observed after heating in benzene-d6 at 80 °C for 11 days and even after subsequent heating at 100 °C for 7 days in a sealed tube (Scheme 24). Compound 43 is much more stable than compound 38, which has so far been known as the most stable N-thiosulfinylaniline; 38 was reported to decompose to the corresponding benzisothiazole after 1 week upon heating at 80 °C in benzene.40 These results clearly demonstrate that the Bpq group is not only effective for prevention of the bimolecular decomposition of the reactive species but also provides a very inert reaction environment to the species.

6.2 Stabilization of an S-Nitrosothiol

S-Nitrosothiols (RSNO) have been attracting increasing attention in view of their role as potential biocatalysts and reagents for the storage and transport of nitric oxide (NO).45 Because of their inherent instability, however, only limited physical and structural data have been accumulated. As for aliphatic S-nitrosothiols, there have been several compounds isolated and structurally characterized so far.46 By contrast, aromatic S-nitrosothiols are much less stable than aliphatic derivatives and there has been no example of the isolation of an aromatic S-nitrosothiol. Usually, such compounds accumulate only transiently and rapidly decompose to the corresponding disulfide and NO. For elucidation of the properties of an aromatic S-nitrosothiol, its stabilization by use of a Bpq group was examined.

The reaction of thiol 44 bearing a Bpq group with ethyl nitrite afforded S-nitrosothiol 45, which was isolated as brownish-green crystals (Scheme 25).17 The conformation of the C-S-N-O linkage of S-nitrosothiols has been attracting interest in association with the properties of this functional group. X-ray crystallographic analysis established that the C-S-N-O-linkage adopts the syn conformation, which is in agreement with the theoretical calculation. S-Nitrosothiol 45 showed much higher thermal stability than hitherto known for S-nitrosothiols. It was found that, even after heating in benzene-d6 at 80 °C for 60 h, 38% of 45 remained unchanged (Scheme 26). The rest of 45 was converted to the dibenzoithiophene derivative 46 and thiol 44. In this reaction, formation of the symmetrical disulfide 47 was not detected. Considering the reported fact that half-life times of ArSNO (Ar = phenyl, p-methoxyphenyl, p-nitrophenyl, 3,5-di-tert-butyl-4-hydroxyphenyl) are 7-14 min in dichloromethane at room temperature,40 the stability of 45 is remarkable. The mechanism of thermolysis of S-nitrosothiols in hydrocarbon solvents is usually considered to involve the bimolecular reaction of an initially formed thiyl radical with the second molecule of S-nitrosothiol. The present results suggest that the Bpq group effectively suppressed the reaction of thiyl radical 48 with the second molecule of S-nitrosothiol 45, which enabled the very slow reaction to produce 46 and 44 to take place. Okazaki et al. reported the synthesis of a stable S-nitrosothiol bearing a Bmt group.49 BmtSNO (48) also showed high thermal stability, but it was found that 48 was
converted to the corresponding disulfide, BmtSSBmt, by heating in refluxing benzene for 75 h. Comparison of the stability of BpqSNO (45) and BmtSNO (48) indicates that the Bpq group is more effective in preventing the bimolecular decomposition of reactive species. In spite of such high thermal stability, S-nitrosothiol 45 undergoes some reactions as shown in Scheme 27, including the reaction to produce the first stable aromatic thionitrate 49.37

6.3 Application to the Synthesis of the First Stable Se-Nitrososelenol

While S-nitrosation of cysteine residues to produce S-nitrosothiols is one of the most important NO-mediated modifications of proteins, it has recently been suggested that the interactions of NO (or NO-derived species) with the SeH groups of selenoproteins are also involved in NO-mediated cellular functions.40 For example, GPx is inactivated by treatment of an S-nitrosothiol as well as by endogenous NO, presumably through Se-nitrosation of the selenocysteine residue.40d However, essentially no chemical information about Se-nitrososelenols (RSeNO) has been available to date, despite their potential physiological importance. For identification of Se-nitrosated species in proteins and elucidation of the mechanism of NO-mediated modification of selenoproteins, reference data on a Se-nitrososelenol are indispensable. Very recently, du Mont et al. reported that even a Se-nitrososelenol carrying the extremely bulky (Me3Si)3C group can be observed only at -78 °C, above which temperature the compound decomposes forming NO.41 In the studies on S-nitrosothiols, it was indicated that the Bpq group is the most efficient in suppressing the dimerization of reactive species among the bowl-type substituents.37 This group was then applied to the synthesis of a stable Se-nitrososelenol. Treatment of selenol 52 with ethyl nitrite led to the quantitative formation of Se-nitrososelenol 53, which was isolated as reddish-purple crystals, representing the first example of a stable Se-nitrososelenol (Scheme 28).42 The structure of 53 was established by X-ray crystallographic analysis (Figure 17). The structural parameters are in good agreement with the calculated values for a model Se-nitrososelenol, PhSeNO. Se-Nitrososelenol 53 showed characteristic spectral properties. In the 77Se NMR spectrum of 53 (CDCl3), a signal was observed at δ 2229, about 2100 ppm lower field than that of selenol 52, suggesting the strong magnetic deshielding effect of the NO moiety. The UV-vis spectrum (CHCl3) of 53 shows the n-π* absorption maximum at 485 nm (ε 150), which is shifted bathochromically by about 140 nm compared to the value for BpqSNO (45) (345 nm). In the IR spectrum of 53, the N-O stretching band was observed at 1563 cm⁻¹, which is slightly higher than that of BpqSNO (45) (1548 cm⁻¹). These spectral data are expected to help Se-nitrosated species to be identified in various situations.

The inactivation of GPx by an S-nitrosothiol has been proposed to proceed by a two-step reaction (Scheme 29).1c,40c The first step is Se-nitrosation of a selenocysteine moiety, which can be reversed by addition of a reducing agent such as dithiothreitol (DTT). The second reaction involves the formation of an interbridge selenenyl sulfide and cannot be reversed by a reducing agent. When selenol 52 was treated with S-nitrosoglutathione (GSNO), one of the important NO carriers in the biological system, the formation of Se-nitrososelenol 53 was observed by UV-vis spectroscopy with clear isobestic points (Scheme 30).42 The reaction of 53 with 1-butanethiol afforded selenenyl sulfide 54 quantitatively. While Se-nitrososelenol 53 was reduced to 52 by treatment of DTT in the presence of triethyamine, selenenyl sulfide 54 did not react with DTT under the same conditions. These results coincide with the proposed mechanism for the NO-mediated GPx inactivation shown in Scheme 29, and strongly suggest the possible involvement of Se-nitrosation of selenoproteins by NO-derived species in redox regulation of cellular functions.

For comparison, the synthesis and stability of a Se-nitrososelenol bearing a Bmt group were examined. When selenol 30 was treated with tert-butyl nitrite, quantitative formation of Se-nitrososelenol 55 was observed (Scheme 31).43 In the 77Se NMR spectrum (CDCl3), 55 showed a signal at δ 2125, an extremely low-field similar to 53. Although 55 was relatively stable in solution, 55 was gradually converted to diselenide 56 at room temperature, and it was difficult to isolate 55 as a pure specimen. These results demonstrate the readiness of the bimolecular decomposition of a Se-nitrososelenol and the great efficiency of the Bpq group for stabilization of reactive species.

Figure 17. ORTEP drawing of 53 (50% probability).
7. Molecular Bowls Based on Triarylmethyl-type Frameworks

While all the aforementioned bowl-type substituents are aromatic steric protection groups, development of aliphatic substituents with comparable efficiency would promote wider application of the molecular bowls. For this purpose, we designed triarylmethane derivative shown in Figure 18 (denoted as TrmX hereafter), where three m-terphenyl groups are connected to an sp³ carbon atom at their 5'-positions so that it would form a dendrimer-type structure. The molecular models of Me₃CSH, Ph₃CSH, and TrmSH are shown in Figure 19. The functionality X of TrmX is incorporated in the shallow molecular cavity and is surrounded by the peripheral 2,6-dimethylphenyl groups so that its dimerization or self-condensation would be effectively prevented. On the other hand, steric congestion in the vicinity of X is not increased as much in comparison with Ph₃CX because there is no ortho-substituent on the aromatic rings connected to the central carbon atom. Thiol 58 was readily prepared by the procedure shown in Scheme 32. The reaction of 58 with tert-butylnitrite afforded S-nitrosothiol 59, which was further oxidized by N₂O₄ to thionitrate 60. Both 59 and 60 were isolated as stable crystals and their structures were established by X-ray analysis. The crystallographic analysis of 60 (Figure 20) is the first example for a thionitrate. S-Nitrosothiol 59 showed much higher stability than that of Ph₃CSNO. Thermolysis of 59 in benzene-d₆ at 80 °C proceeded about 30 times slower than that of Ph₃CSNO, and the main product was trisulfide 61, not the corresponding disulfide, TrmSSTrm (Scheme 33). These results indicate that the lifetime of S-nitrosothiol 59 was elongated by inhibition of the disulfide formation due to the steric repulsion between the bowl-type substituents, and that the prolonged heating caused another mode of reaction involving the C-S bond cleavage to produce trisulfide 61 and carbinol 57.

![Figure 18. Triarylmethyl-type molecular bowl.](image)

![Scheme 32](image)

![Scheme 33](image)

![Figure 20. ORTEP drawing of 60 (30% probability).](image)

In the framework of the Trm group, the central carbon can be replaced by other elements to produce a variety of analogues bearing the same structural characteristics. As the silicon and germanium analogues of TrmX, the bowl-shaped silane and germane derivatives shown in Figure 21 (denoted as TRMS-X and TRMG-X, respectively, hereafter) were designed. A bowl-shaped silanol, TRMS-OH (63), and germanol, TRMG-OH (64), were synthesized, and X-ray analysis revealed that both 63 and 64 are monomeric in the crystalline state without any OH⋯O hydrogen bonding. The crystal structure of 63 is shown in Figure 22. Silanol 63 was found to undergo no self-condensation reaction even under the conditions where Ph₃SiOH affords the corresponding disiloxane, Ph₃SiOSiPh₃, while 63 can readily react with trimethylsilyl generated in situ to produce the correspond-
ing condensation product 65 (Scheme 34). Germanol 64 was also found to be extremely resistant to self-condensation.

8. Design and Application of Bowl-type Ligands

The concept of the peripheral steric protection of reactive species shown in Figure 3 can also be applied to the formation of low-coordinate metal complexes otherwise difficult of access. When the bowl-type molecules are utilized as the ligands, the number of the ligands introduced to the metal center can be regulated effectively by the steric repulsion among the peripheral moieties of the ligands. On the other hand, a relatively large space remains around the metal center for the reaction with substrates. As the bowl-type ligands, we synthesized triarylphosphines 66 and 67 (denoted as TRMP and TRIP, respectively, hereafter)\(^{47}\) bearing a framework similar to those of TrmX, TRMS-X, and TRMG-X. The synthesis of TRMP (66) was also reported by Tatsumi et al. independently.\(^{48}\) The cone angles of TRMP (66) and TRIP (67) were estimated to be as large as 174° and 206°, respectively, based on their crystal structures (Figure 24).\(^{47}\) On the other hand, the steric congestion in the vicinity of the phosphorus center of 66 and 67 is less severe than that of other bulky phosphines such as Mes3P because of the absence of any substituent in the ortho-positions of phosphorus. The reactions of tertiary phosphines with palladium(II) halides usually produce the mononuclear complex \([\text{PdX}_2(\text{PR}_3)_2]\) or dinuclear complex \([\text{PdX}_2(\text{PR}_3)_2]\) depending on the steric bulkiness of the phosphine and the reaction conditions. However, there has been no report of a phosphine ligand bulky enough to form the trinuclear complex \([\text{PdX}_2(\text{PR}_3)_2]\). Tatsumi et al. reported that, even in the case of TRMP (66), the reaction with a half equimolar amount of PdCl\(_2\) afforded the corresponding mononuclear complex, \([\text{PdCl}_2(\text{TRMP})_2]\).\(^{46b}\) In contrast, treatment of TRIP (67) with PdCl\(_2\) produced the trinuclear palladium(II) complex 68 as a single product even when an excess amount of phosphine 67 was employed (Scheme 35).\(^{47c,d}\) X-ray crystallography established the structure of 68, where the trimer of PdCl\(_2\) is terminated by two TRIP ligands. This is the first example of a structurally characterized PdCl\(_2\) trimer complex. The formation of the trinuclear complex 68 in the presence of an excess of TRIP (67) indicates that only one molecule of 67 can coordinate to one palladium atom

\[\text{PdCl}_2 \rightarrow \text{TRIP (67): } R = \text{Me} \quad \text{TRIP (67): } R = \text{i-Pr} \]

\[\text{Scheme 34}\]

\[\text{Scheme 35}\]

\[\text{Figure 21. Bowl-shaped silane and germaine derivatives.}\]

\[\text{Figure 22. ORTEP drawing of 63 (50% probability).}\]

\[\text{Figure 23. Bowl-shaped triarylphosphines.}\]

\[\text{Figure 24. ORTEP drawing of 67 (50% probability).}\]

\[\text{Figure 25. ORTEP drawing of 68 (50% probability).}\]
because the phosphorus center of 67 is embedded in its deep bowl-shaped cavity (Figure 24). Recently, Tsuji et al. utilized TRMP (66) for efficient rhodium-catalyzed hydrosilylation of ketones. These bowl-shaped phosphines are expected to work as potent ligands in various catalytic reactions.

Metal complexes of N-heterocyclic carbenes (NHC) have been recognized as an important class of compounds for coordination chemistry and catalysis. As the NHC analogue of the Bpq group, a novel bowl-type ligand 69 (denoted as ITmt hereafter) was designed. Crystals of the two-coordinate palladium(0) complex 70 bearing 69 directly and rapidly fixed from air not only O2 but also CO2, the concentration of which is 0.035%, to produce the corresponding peroxocarbonate complex 72 (Scheme 36). This is the first example of the solid-state fixation of both O2 and CO2 from air to a transition metal complex. The present reaction consists of dioxygenation of the palladium(0) complex 70 to the palladium(II) peroxo complex 71 and the subsequent CO2 insertion to produce the peroxocarbonate complex 72. Under the same conditions, complex 74 bearing IMes (73) reacts with O2, but the subsequent reaction with CO2 does not proceed. The comparison of the crystal structures of peroxo complexes 71 and 75 suggested that the unique reactivity of solid 71 toward air arises from the structural features of the bowl-shaped carbene ligand 69, where there is no alkyl group around the coordinating site.

Figure 26. Bowl-type N-heterocyclic carbene.

Scheme 36

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Product</th>
</tr>
</thead>
<tbody>
<tr>
<td>ITmt + Pd</td>
<td>ITmt-Pd</td>
</tr>
<tr>
<td>ITmt</td>
<td>solid state, r.t., 3 h</td>
</tr>
<tr>
<td>IMes (73)</td>
<td></td>
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<tr>
<td>IMes (73)</td>
<td></td>
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<td>IMes (73)</td>
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<td>IMes (73)</td>
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9. Conclusion

Various types of bowl-type steric protection groups have been developed so that an appropriate microenvironment can be constructed according to the characteristics of the target species to be stabilized as well as the intended use of the obtained species. The concept of the “peripheral steric protection” is expected to be useful in various aspects of the reaction control other than stabilization of reactive species, as indicated by the application of bowl-type ligands to the synthesis of novel transition metal complexes.

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