Reactions in Host–Guest Complexes

Molecular Mousetraps: Gas-Phase Studies of the Covalent Coupling of Noncovalent Complexes Initiated by Reactive Carbenes Formed by Controlled Activation of Diazo Precursors**

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Molecular recognition is a powerful technique that can be used to generate noncovalently bound host–guest complexes for a variety of purposes.[1,2] These noncovalent complexes are easily transferred to the gas phase by electrospray ionization

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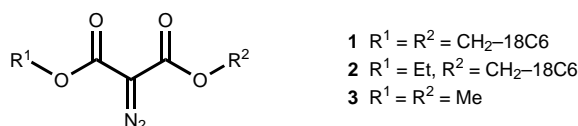E-mail: stoltz@caltech.edu

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(ESI).[3] Attempts to effect intermolecular reactions between the cluster components are often frustrated by the lability of noncovalent complexes, which results from the relatively weak interactions that hold them together. In the present work, we have successfully initiated intermolecular reactions in noncovalent clusters. First, a strongly bound host–guest complex is formed in solution and transferred to the gas phase by ESI. Second, a diazo group that has been incorporated into the host is efficiently and easily converted into a highly reactive carbene[4] by low-energy collision-activated dissociation (CAD).[5] This carbene[6,7] then reacts in an intermolecular fashion, covalently binding the host–guest complex. These reagents are herein referred to as "molecular mousetraps".

We have synthesized and examined the chemistry of the prototypical molecular mousetraps **1** and **2**. 18-Crown-6 (18C6) is a well-known host for protonated primary amines, both in solution and in the gas phase.[8] For example, we have recently shown that 18C6 selectively binds to lysine residues in small peptides.[3a] Mousetrap **1** is designed to bind molecules with either one or, preferentially, two protonated primary amines. Mousetrap **2**, with a single 18C6 unit, binds to a single protonated primary amine. Structure **3** was used as a model compound in computations.

$$R^1\diagdown O \underset{N_2}{\overset{\displaystyle O \quad\quad O}{\diagdown\diagup}} O \diagup R^2$$

1 $R^1 = R^2 = CH_2–18C6$
2 $R^1 = Et, R^2 = CH_2–18C6$
3 $R^1 = R^2 = Me$

A possible interaction between **1** and doubly protonated 1,6-diaminohexane (DAH) is shown in Figure 1. This complex forms in solution, and can be transferred intact to the gas phase by ESI, as seen in Figure 2a. The complex can be isolated and subjected to CAD, as shown in Figure 2b. The sole product results from a neutral loss of 28 Da, which is interpreted to be the loss of N_2 from the diazo group. Significantly, the result shown in Figure 2b provides evidence
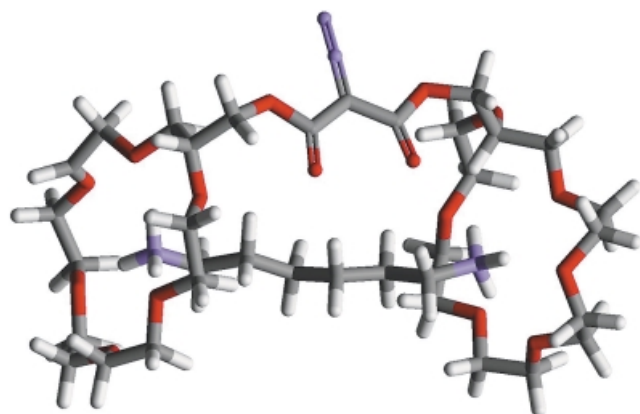
for covalent-bond cleavage in preference to dissociation of the complex. The loss of N_2 from the diazo group should yield the corresponding carbene (:**1**) as a highly reactive, short-lived intermediate. This carbene can then undergo either intermolecular or intramolecular reaction.

The product described in Figure 2b is subjected to further collisional activation, as shown in Figure 2c. The majority of the product-ion intensity results from covalent-bond cleavage with loss of a crown, or part of a crown and retention of DAH. The fragmentation of the host without the accompanying loss of the guest provides evidence that an intermolecular reaction involving covalent coupling between the host and guest occurs by C–H insertion of the carbene. For the doubly protonated DAH, the complexation of the protonated primary amines by the crown ethers reduces the likelihood of an N–H insertion reaction by the carbene.[9] It is also observed in Figure 2c that some of the DAH appears to dissociate from the complex, which suggests that an intramolecular process[10] is competitive in this case.

Singly charged DAH has a lower binding energy to **1** than the doubly charged species, yet Figure 2d illustrates that [:**1**+DAH+H]+ is generated with high efficiency from the [**1**+DAH+H]+ complex. The loss of nitrogen is accompanied by an additional loss of 294 Da, which can be accounted for by the loss of 18-crown-6 methanol. This additional loss is observed for all complexes of both **1** and **2** in which there is an unprotonated primary amine or alcohol available (in experiments with **2**, the loss of ethanol is also observed). DFT calculations at the B3LYP/CCPVTZ(-F)+ level on :**3** describe a singlet ground state, with a singlet/triplet splitting of 3 ± 1 kcal mol⁻¹. This result suggests that the singlet state is certainly accessible and perhaps favorable, which is in agreement with experimental results.[11] DFT calculations at the B3LYP/6-31G** level on :**3** and methylamine lead to the formation of an ammonium ylide without a barrier. The ammonium ylide is a local minimum on the potential energy surface, and previous reports have suggested that all carbenes will initially react with amines by the formation of an intermediate ylide.[12] From this ammonium ylide two reaction pathways with minimal barriers are possible, as shown in Scheme 1, and it should be pointed out that both pathways lead to covalent attachment of the host–guest complex through intermolecular reactions; one route leads to formal N–H insertion, and the other leads to the loss of an alcohol group and the generation of a ketene.
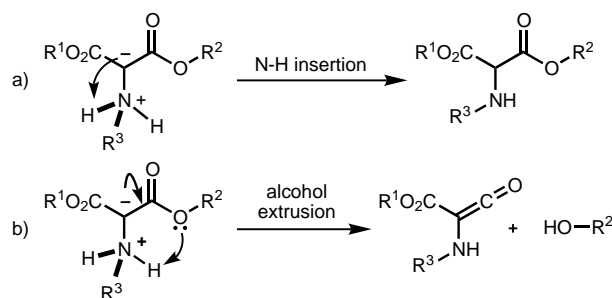
Figure 1. One possible structure for the noncovalent adduct of **1** and doubly protonated 1,6-diaminohexane (DAH) in the gas phase, as determined by PM3 semiempirical calculations.
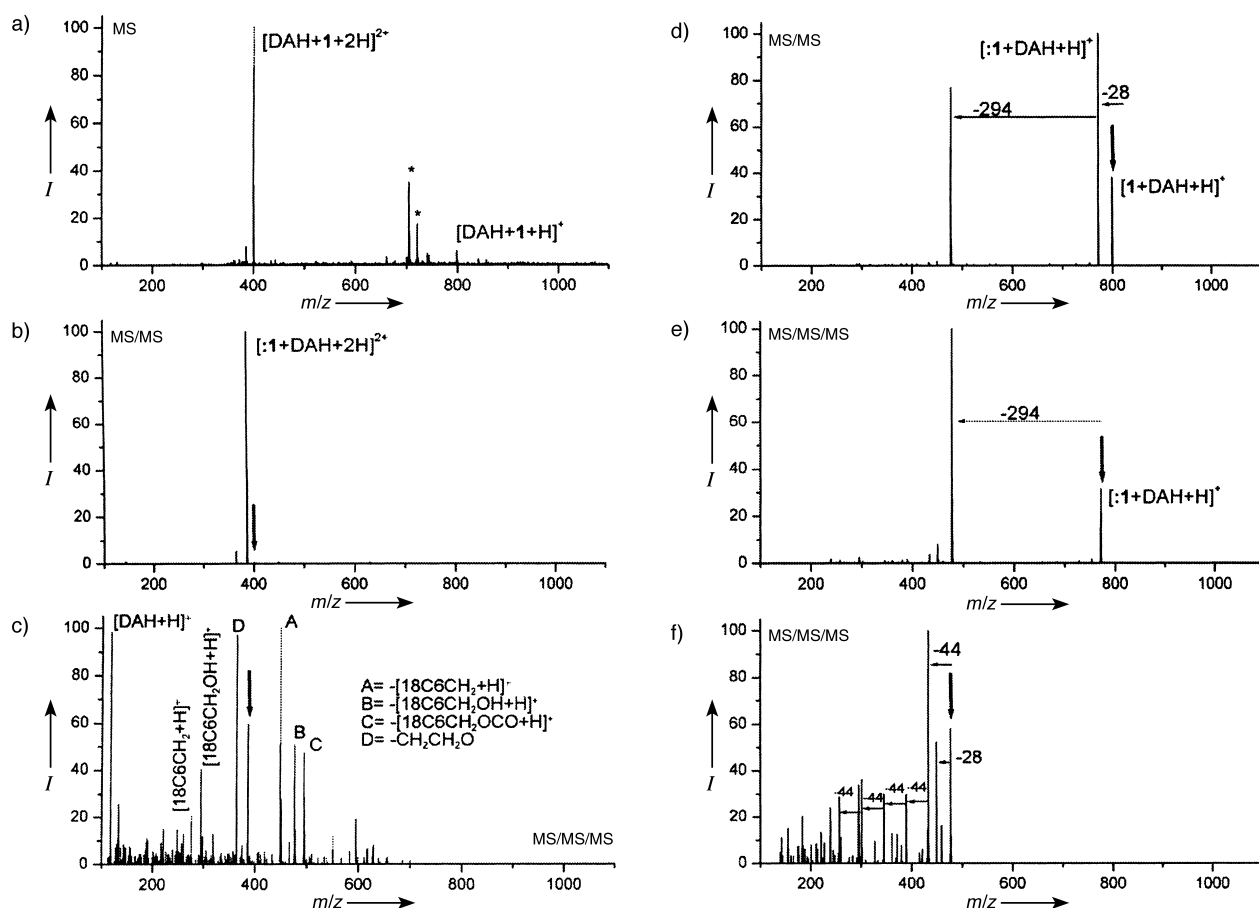
Scheme 1.

Figure 2. a) Mass spectrum of **1** with DAH; b) CAD spectrum of [1+DAH+2H]²⁺ which shows the almost exclusive loss of 28 Da and indicates the generation of carbene **:1**; c) MS/MS/MS analysis of the [:1+DAH+2H]²⁺ peak. Dissociation is accompanied by covalent bond cleavage, which suggests C−H insertion by **:1** and the formation of a new molecule; d) CAD of [1+DAH+H]⁺, which leads entirely to intermolecular reaction products; e) MS/MS/MS analysis of [:1+DAH+H]⁺, which only results in the loss of 294 Da. The absence of complex dissociation suggests covalent attachment; f) MS/MS/MS analysis of [:1+DAH+H-294]⁺. In the absence of both crowns DAH is retained, which confirms the covalent coupling of the complex. A bold downward arrow indicates the peak being subjected to CAD. *I* = relative intensity, * = 1 + alkali-metal adduct peaks.

Further excitation of the isolated [:1+DAH+H]⁺ species, after the loss of nitrogen, exclusively leads to the loss of 294 Da, as shown in Figure 2e. The N−H insertion product shown in Scheme 1a is protonated at the secondary amine. Transfer of this proton to the ester can lead to the loss observed in Figure 2e by alcohol extrusion. It is also possible, though unlikely, that the ammonium ylide, represented by the data in Figure 2d, could be sufficiently long-lived to yield this product directly.

Figure 2f offers several critical results. First, the loss of 28 Da is probably a result of the loss of CO from the ketene product shown in Scheme 1b. Second, the fragment being subjected to further collisional activation in Figure 2f contains only a single remaining crown. The primary losses are multiple {CH₂CH₂O} fragments from this remaining crown. The data reveals the sequential removal of almost the entire remaining crown ether without the loss of the guest molecule. In the absence of both crowns, retention of the guest can only be explained by a newly formed covalent bond.

These studies demonstrate that reagents that bind to specific functional groups in complex molecules can be derivatized to introduce the means to covalently couple them to target molecules with appropriate methods of activation. We have combined 18C6, which binds strongly to protonated primary amines,[3a] with a diazo precursor to a reactive carbene to form a potent "molecular mousetrap" that can be used to target lysines in peptides or proteins. Full details of the chemistry and applications of these and related molecular mousetraps will be described in further publications.

Experimental Section

All spectra were obtained using a Finnigan LCQ ion-trap quadrupole mass spectrometer without modification. Sample concentrations were typically kept in the 10 to 100 μM range for all species of interest. All samples were electrosprayed in an 80:20 mixture of methanol/water. The appropriate host was added to the sample and electrosprayed with the guest in order to observe adduct formation. Full characterization and synthetic details for hosts **1** and **2** are available in the Supporting Information. Semiempirical calculations for Figure 1 were performed on HyperChem 5.1 Professional Suite using the PM3 parameter set.

Calculations to determine the singlet/triplet splittings were performed on structures fully optimized at the B3LYP/CCPV-TZ(-F)[+] level of theory. Comparison of this methodology with previous computational and experimental results for the following carbenes: CH_2, HCCl, HCF, CCl_2, CF_2, and HCCHO yielded results within (on average) ± 0.6 kcal mol^{-1} of the best experimental or theoretical value.[13] Zero-point energy corrections were not included. Reactions were modeled at the B3LYP/6-31G** level of theory by minimizing structures containing both reactants, with several different starting geometries. Initial geometries included likely starting points for the most probable reaction mechanisms, that is, hydrogen abstraction, concerted insertion, and ylide formation. The DFT calculations were carried out using Jaguar 4.1 (Schrödinger, Inc., Portland, Oregon).

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