# Chapter 10

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

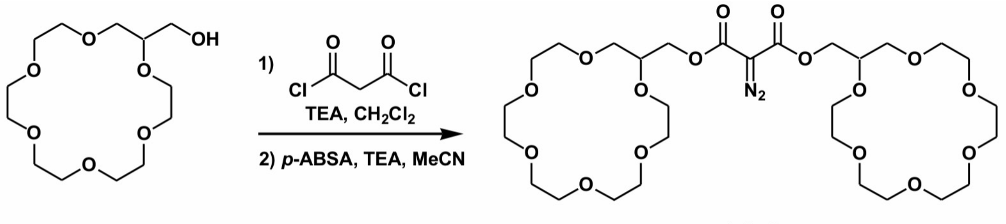
Published previously in: Julian, R. R.; May, J. A.; Stoltz, B. M.; Beauchamp, J. L. *Angew. Chem. Int. Ed.* **2003**, *42(9)*, 1012-1015.

## 10.1 Introduction

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 (ESI).[3] Attempts to effect intermolecular reactions between the cluster components are often frustrated by the lability of noncovalent complexes due to 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 which 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”.

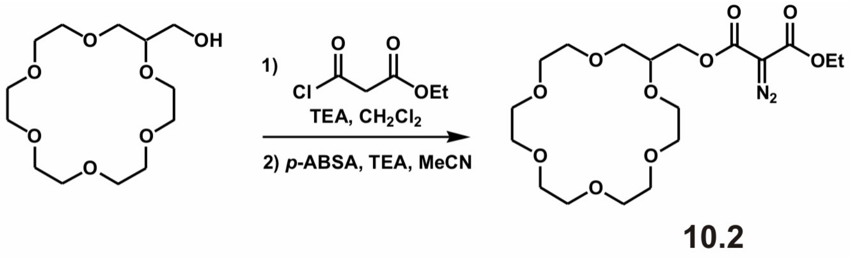
## 10.2 Experimental Section.

**General Information**: Due caution should always be used when handling diazo compounds. Reactions were performed in flame-dried glassware under a nitrogen atmosphere using freshly distilled solvents. All other reagents were used as received from commercial sources. Reaction temperatures were controlled by an IKAmag temperature modulator. $^1$H NMR spectra were recorded on a Varian Mercury 300 spectrometer (at 300 MHz) and are internally referenced to the chloroform peak (7.27 ppm) relative to $Me_4Si$. Data for $^1$H NMR spectra are reported as follows: chemical shift (δppm), multiplicity, coupling constant (Hz), and integration. IR spectra were recorded on a Perkin Elmer Paragon 1000 spectrometer and are reported in frequency of absorption ($cm^{-1}$). Preparatory reversed phase HPLC was performed on a Beckman HPLC with a Waters DeltaPak 25 x 100 mm, 100 µm C18 column equipped with a guard.



10.1

**Compound 10.1**: To a stirred, dry solution of 18-crown-6-methanol (50.0 µl, 0.16 mmol), dichloromethane (1.5 ml), and triethylamine (25 µl, 0.18 mmol) was added malonyl dichloride (9.0 µl, 0.09 mmol). The mixture was heated to reflux for eight hours, cooled, and then evaporated *in vacuo*. The residue was dissolved in acetonitrile (1.2 ml), and treated with triethylamine (220 µl, 1.58 mmol). To this solution was added *p*-acetamidobenzenesulfonyl azide (31.9 mg, 0.13 mmol), and the mixture was stirred for ten hours. The solvent was removed *in vacuo*, the residue dissolved in a minimal amount of dichloromethane (500 µl), and the undesired salts were precipitated out of solution with the addition of ether (5 ml). Filtration through celite and removal of solvent *in vacuo* yielded **10.1** (41.8 mg, 81% yield). A small sample (~15 mg) was chromatographed to analytical purity by HPLC, (0.1% (wt/v) TFA in water, 8.0 ml/min, 0.30% acetonitrile/min, 83-85 min). FTIR (thin film) 3429, 2918, 2143, 1743, 1691, 1595, 1454, 1356, 1251, 1108 cm$^{-1}$; $^1$H NMR (300 MHz, $CDCl_3$) δ 4.44 (dd, J = 3.85, 11.5 Hz, 1H), 4.26 (dd, J = 5.49, 11.0 Hz, 1H), 3.87-3.58 (m, 23H); MS *m/z* 683.3 ($H^+$).



**Compound 10.2**: To a stirred, dry solution of 18-crown-6-methanol (50.0 µl, 0.16 mmol), dichloromethane (1.5 ml), and triethylamine (33 µl, 0.24 mmol) was added ethyl malonyl chloride (28 µl, 0.22 mmol). The mixture was heated to reflux for eight hours, cooled, and then evaporated *in vacuo*. The residue was dissolved in acetonitrile (750 µl), and treated with triethylamine (30 µl, 0.22 mmol). To this solution was added *p*-acetamidobenzenesulfonyl azide (53.1 mg, 0.22 mmol), and the mixture was stirred for ten hours. The solvent was removed *in vacuo*, the residue dissolved in a minimal amount of dichloromethane (500 µl), and the undesired salts were precipitated out of solution with the addition of ether (5 ml). Filtration through celite and removal of solvent *in*

*vacuo* yielded **10.2** (59.8 mg, 87% yield). A small sample (~15 mg) was chromatographed to analytical purity by HPLC (0.1% (wt/v) TFA in water, 8.0 ml/min, 0.30% acetonitrile/min, 82-85 min). FTIR (thin film) 2879, 2142, 1755, 1689, 1457, 1326, 1102, 762; $^1$H NMR (300 MHz, $CDCl_3$) δ 4.45 (dd, J = 3.85, 12.1 Hz, 1H), 4.31 (q, J = 7.14 Hz, 2H), 4.27 (m, 1H), 3.85 (t, J = 4.95), 3.80 (s, broad, 1H), 3.67 (s, broad, 21H), 1.32 (t, j = 7.14 Hz, 3H); MS *m/z* 435.2 ($H^+$).

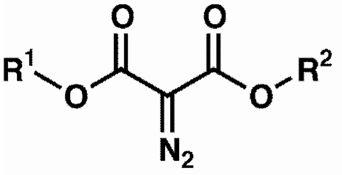
**Mass Spectrometry.** All spectra were obtained using a Finnigan LCQ ion trap quadrupole mass spectrometer without modification. Sample concentrations were typically kept in the ~10 to 100 μM range for all species of interest. All samples were electrosprayed in a mixture of 80:20 methanol/water. The appropriate host was added to the sample and electrosprayed with the guest in order to observe adducts. Semi-empirical calculations for Figure 10.1 were performed on HyperChem 5.1 Professional Suite using the PM3 parameter set.

**Theory.** Calculations to determine the singlet/triplet splittings were performed on structures fully optimized at the B3LYP/CCPVTZ(-F)$^+$ level. 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 of the best experimental or theoretical value.[8] Zero point energy corrections were not included. Reactions were modeled at the B3LYP/6-31G** level by minimizing structures containing both reactants, with several different starting geometries. Initial geometries included likely starting points for the most probable reaction mechanisms i.e. hydrogen abstraction, concerted insertion, and ylide formation.

The DFT calculations were carried out using Jaguar 4.1 (Schrödinger, Inc., Portland, Oregon).

## 10.3 Results and Discussion

We have synthesized and examined the chemistry of the prototypical molecular mousetraps **10.1** and **10.2**. 18-crown-6 (18C6) is a well-known host for protonated primary amines, both in solution and in the gas phase.[9] For example, we have recently shown that 18C6 selectively binds to lysine residues in small peptides.[3a] Mousetrap **10.1** is designed to bind molecules with either one or, preferentially, two protonated primary amines. Mousetrap **10.2**, with a single 18C6, binds to a single protonated primary amine. Structure **10.3** was used as a model compound in computations.



***10.1 $R^1$=$R^2$=$CH_2$18C6***
***10.2 $R^1$=Et, $R^2$=$CH_2$18C6***
***10.3 $R^1$=$R^2$=Me***

The interaction between **10.1** and doubly protonated 1,6-diaminohexane (DAH) is shown in Figure 10.1. This complex forms in solution and can be transferred intact to the gas phase by ESI, as seen in Figure 10.2a. The complex can be isolated and subjected to CAD as shown in Figure 10.2b. 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 10.2b provides evidence for covalent bond cleavage in preference to dissociation of the complex. The loss of $N_2$ from the diazo should yield the corresponding carbene (**:10.1**) as a highly reactive, short lived intermediate. This carbene can then react intermolecularly or intramolecularly.

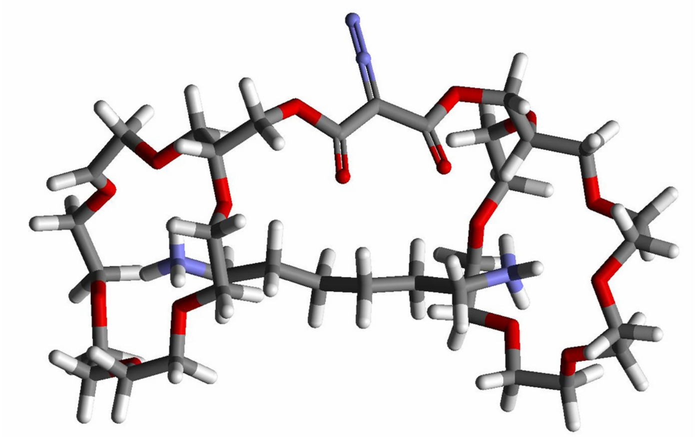


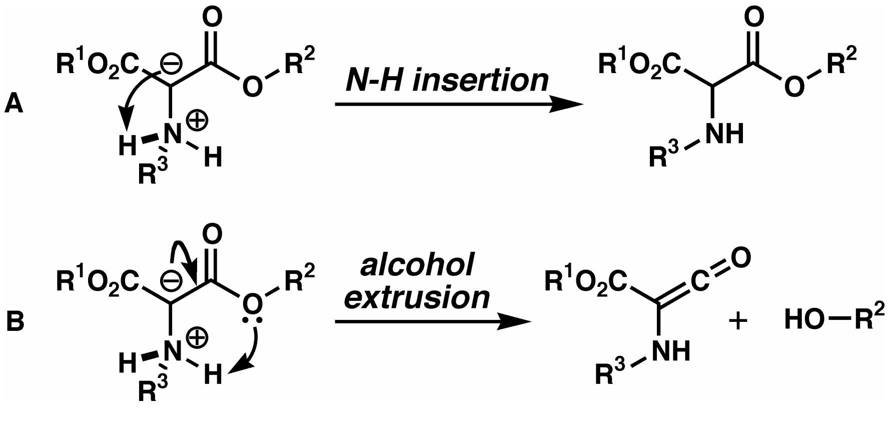
**Figure 10.1** One possible structure for the noncovalent adduct of **10.1** and doubly protonated 1,6-diaminohexane (DAH) in the gas phase, as determined by PM3 semi-empirical calculations.

The product from Figure 10.2b is subjected to further collisional activation in Figure 10.2c. 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 of the complex by C-H insertion of the carbene has occurred. 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.[10] It is also observed in Figure 10.2c that some of the DAH simply dissociates from the complex, suggesting an intramolecular process[11] is competitive in this case.

Singly charged DAH has a lower binding energy to **10.1** than the doubly charged species, yet Figure 10.2d illustrates that [:**10.1**+DAH+H]$^+$ is generated with high efficiency from the [**10.1**+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 hydroxymethyl

18C6. This additional loss is observed for all complexes of both **10.1** and **10.2** in which there is an unprotonated primary amine or alcohol available (in experiments with **10.2**, the loss of ethanol is also observed). DFT calculations at the at the B3LYP/CCPVTZ(-F)$^+$ level on **:10.3** yield a singlet ground state with a singlet/triplet splitting of 3±1 kcal/mol. This suggests that the singlet state is certainly accessible and perhaps favorable, which is in agreement with experimental results.[12] DFT calculations at the B3LYP/6-31G** level on **:10.3** and methylamine lead to the formation of an ammonium ylide without 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.[13] From this ammonium ylide two reaction pathways with minimal barriers are possible as shown in Scheme 10.1, and it should be pointed out that both lead to covalent attachment of the host/guest complex by intermolecular reactions. One leads to formal N-H insertion, and the other leads to the loss of an alcohol and the generation of a ketene.

**Scheme 10.1.**



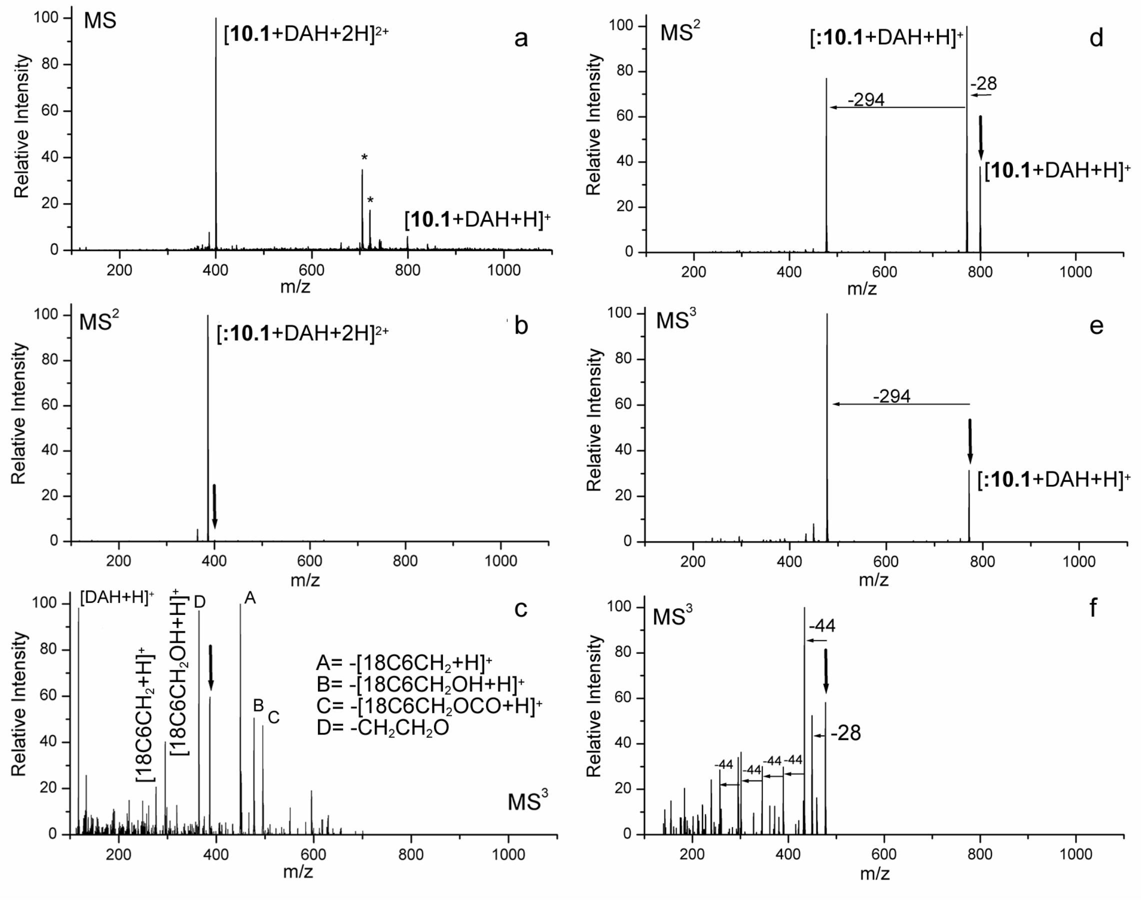


**Figure 10.2** (a) Mass spectrum of **10.1** with DAH. (b) CAD spectrum of [**10.1**+DAH+2H]$^{2+}$ which shows the almost exclusive loss of 28 Da, indicating the generation of carbene **:10.1**. (c) MS$^3$ on the [**:10.1**+DAH+2H]$^{2+}$ peak. Dissociation is accompanied by covalent bond cleavage, suggesting C-H insertion by **:10.1** and the formation of a new molecule. (d) CAD of [**10.1**+DAH+H]$^+$, leading entirely to intermolecular reaction products. (e) MS$^3$ on [**:10.1**+DAH+H]$^+$, resulting only in the loss of 294 Da. The absence of complex dissociation suggests covalent attachment. (f) MS$^3$ on [**:10.1**+DAH+H-294]$^+$. In the absence of both crowns, DAH is retained, confirming the covalent coupling of the complex. A bold downward arrow indicates the peak being subjected to CAD. * 1+alkali metal adduct peaks.

Further excitation of the isolated [**:10.1**+DAH+H]$^+$ peak following the loss of nitrogen yields exclusively the loss of 294 Da as shown in Figure 10.2e. The N-H insertion product shown in Scheme 10.1A has a proton on the secondary amine. Transfer of this proton to the ester can lead to the loss observed in Figure 10.2e by alcohol extrusion. It is also possible, though unlikely, that the ammonium ylide formed in Figure 10.2d could be sufficiently long-lived to yield this product directly.

Figure 10.2f offers several critical results. First, the loss of 28 Da is probably the loss of CO from the ketene product shown in Scheme 10.1B. Second, the fragment being subjected to further collisional activation in Figure 10.2f contains only a single remaining crown. The primary losses are multiple $CH_2CH_2O$ fragments from this remaining crown. The data reveals the sequential removal of nearly the entire remaining crown ether without the loss of the guest molecule. In the absence of both crowns, the retention of the guest can only be explained by a newly formed covalent bond.

## 10.4 Conclusion

These studies demonstrate that reagents which bind to specific functional groups in complex molecules can be derivatized to introduce the means to covalently couple them to the 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. More details of the chemistry and applications of these and related molecular mousetraps are described in Chapter 11.

## 10.5 References

---

[1] Peczuh, M. W.; Hamilton, A. D. *Chem. Rev.* **2000**, *100*, 2479-2494.

[2] Beer, P. D.; Gale, P. A.; Smith, D. K. *Supramolecular Chemistry*; Oxford University Press, New York, 1999.

[3] (a) Julian, R. R.; Beauchamp, J. L. *Int. J. Mass. Spectrom*. **2001**, *210*, 613-623; (b) Smith, R. D.; Bruce, J. E.; Wu Q. Y.; Lei, Q. P. *Chem. Soc. Rev*. **1997**, *26*, 191-202; (c) Veenstra, T. D. *Biophys. Chem*. **1999**, *79*, 63-79. (d) Loo, J. A. *Int. J. Mass Spectrom*. **2000**, *200*, 175-186.

[4] A neutral, two electron carbene is formed. Other studies have focused on carbene radical cations such as $CH_2^+$, see for example: Flammang, R.; Nguyen, M. T.; Bouschoux, G.; Gerbaux, P. *Int. J. Mass Spectrom*. **2002**, *202*, A8-A25.

[5] (a) Marzluff, E. M.; Beauchamp, J. L. in *Large Ions: Their Vaporization, Detection, and Structural Analysis*; Baer, T., Ng, C. Y., Powis, I.; Eds.; John Wiley & Sons Ltd.: New York, 1996; 115-143; (b) McLuckey, S. A. *J. Am. Soc. Mass Spectrom*. **1992**, *3*, 599-614; (c) Hayes, R. N.; Gross, M. L. *Methods Enzymol.* **1990**, *193*, 237-263.

[6] (a) Rice, F. O.; Glasebrook, A. L. *J. Am. Chem. Soc.* **1934**, *56*, 2381-2383; (b) Herzberg, G. *Proc. Roy. Soc. Lond. Ser. A*. **1961**, *262*, 291-317; (c) Moss, R. A.; Jones, M., Jr. Eds. *Carbenes, Vols. 1 and 2*; Wiley: New York, 1973, 1975; (d) Rynbrandt, J. D.; Rabinovitch, B. S. *J. Phys. Chem*. **1970**, *74*, 4175-4176; (e) Rynbrandt, J. D.; Rabinovitch, B. S. *J. Chem. Phys.* **1971**, *54*, 2275-2276; (f) Poutsma, J. C.; Nash, J. J.; Paulino, J. A.; Squires, R. R. *J. Am. Chem. Soc*. **1997**, *119*, 4686-4697; (g) Paulino, J. A.; Squires, R. R. *J. Am. Chem. Soc.* **1991**, *113*, 5573-5580; (h) Leopold, D. G.; Murray, K. K.; Miller, A. E. S.; Lineberger, W. C. *J. Chem. Phys*. **1985**, *83*, 4849-4865 and

---

references therein; (i) Bertani, R.; Michelin, R. A.; Mozzon, M.; Traldi, P.; Seraglia, R.; Busetto, L.; Cassani, M. C.; Tagleatesta, P.; D'Arcangelo, G. *Organometallics*, **1997**, *16*, 3229-3233.

[7] (a) Doyle, M. P.; McKervey, M. A.; Ye, T. *Modern Catalytic Methods for Organic Synthesis with Diazo Compounds*; Wiley-Interscience: New York, 1998; (b) Moody, C. J.; Whitham, G. H. *Reactive Intermediates*; Oxford University Press: New York, 1992; 26-50.

[8] Scott, A. P.; Platz, M. S.; Radom, L. *J. Am. Chem. Soc*. **2001**, *123*, 6069-6076.

[9] (a) Bradshaw, J. S.; Izatt, R. M.; Borkunov, A. V.; Zhu, C. Y.; Hathaway, J. K. *Comprehensive Supramolecular Chemistry, Vol. 1*; G. W. Gokel, Pergamon/Elsevier: Oxford, 1996; 35-95; (b) Maleknia, S.; Brodbelt, J. *J. Am. Chem. Soc.* **1993**, *115*, 2837-2843; (c) Dearden, D. V.; Dejsupa, C.; Liang, Y.; Bradshaw, J. S.; Izatt, R. M. *J. Am. Chem. Soc.* **1997**, *119*, 353-359; (d) Schalley, C. A. *Mass Spectrom. Rev*. **2001**, *20*, 253-309; (e) Loo, J. A. *Int. J. Mass Spectrom*. **2000**, *200*, 175-186.

[10] 18C6 locks protons onto primary amines, see ref 3a and Lee, S.-W.; Lee, H.-N.; Kim, H. S.; Beauchamp, J. L. *J. Am. Chem. Soc.* **1998**, *120*, 5800-5805.

[11] The likely competing reaction is Wolff rearrangement of the carbene. See, for example, (a) Likhotvorik, I.; Zhendong, Z.; Tae, E. L.; Tippmann, E.; Hill, B. T.; Platz, M. S. *J. Am. Chem. Soc*. **2001**, *123*, 6061-6068; (b) ref 8.

[12] Both results obtained the current work and in: Richardson, D. C.; Hendrick, M. E.; Jones, M. *J. Am. Chem. Soc*. **1971**, *93*, 3790-3791, where Wolff rearrangement is observed, which must proceed through the singlet state.

[13] Pliego, J. R.; Almeida, W. B. *J. Phys. Chem. A* **1999**, *103*, 3904-3909.