Applications of Electrospray Ionization Mass Spectrometry to Neutral Organic Molecules Including Fullerenes

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The use of electrospray ionization mass spectrometry (ESI/MS) for the detection of neutral organic molecules becomes possible by their derivation with specific ESI/MS tagging reagents that have either proton or metal ion binding sites. We used the neutral crown ether group in several reagents to attach a metal binding site to substrate molecules. Application of this method to steroids, amino acids, vitamin D, fatty acids, and fullerenes is described. Besides characterization, tagged molecules can be used for studying organic reactions by ESI/MS. This work demonstrates that ESI/MS provides a unique window on fullerene solution chemistry. ESI/MS is not only an excellent tool for the analysis of biopolymers but is also useful for studying the organic chemistry of small neutral molecules. *(J Am Soc Mass Spectrom 1993, 4, 596–603)*

The use of electrospray ionization mass spectrometry (ESI/MS) for analysis of biopolymers, such as peptides, proteins, and oligonucleotides, continues to expand [1]. For detection, ESI/MS requires the presence of cationic groups, such as the ammonium salts of proteins in the positive ion mode, or anionic groups, such as the phosphates of nucleic acids in the negative ion mode. Neutral molecules have not generally been well studied by ESI/MS for several reasons. First, small neutral molecules are often volatile and may readily be determined by using gas chromatography mass spectrometry. Even so, neutrals are often derivatized to make them more thermally stable and volatile. Second, neutral molecules do not appear directly in ESI/MS because they are not charged. Nevertheless, there are significant advantages to the use of ESI/MS because this technique monitors to a great extent solution chemistry, where most chemical reactions take place. Many studies have appeared describing the use of mass spectrometry techniques, such as fast-atom bombardment (FAB) or laser desorption (LD) for quantitation and solution studies (for use of FAB for solution studies, see ref 2a–e; for use of liquid secondary ion mass spectrometry for solution studies, see ref 2f; for use of LD for solution studies, see ref 2g,h; for use of reagents for tagging molecules for mass spectrometry, see ref 2i–l). In addition, the use of ESI/MS to study solution chemistry has also been previously reported [3].

Organic chemistry often involves conversion of one neutral molecule to another. Although ionic intermediates are sometimes involved, more often they are not. We have examined applications of ESI/MS to organic chemistry [4]. This report surveys additional applications of ESI/MS to neutral organic molecules and describes our recent efforts to exploit specific tagging reagents to make neutral molecules more readily charged for the ESI/MS process. In addition, we discuss our strategy to prepare "ESI-active" tagged substrate molecules so that ESI/MS can be used as a tool in the study of chemical transformations in solution.

Obviously, neutral molecules must acquire a charge to be detected by mass analyzers during ESI/MS. There are four reactions that accomplish this:

$$X: + H^+ \rightarrow X - H^+ \quad (1)$$

$$X - H \rightarrow X^- + H^+ \quad (2)$$

$$\begin{cases} X = Y \leftrightarrow X^+ - Y^- + M^+ \rightarrow X^+ - YM \\ X = Y \leftrightarrow X^+ - Y^- + Z^- \rightarrow XZ - Y^- \end{cases} \quad (3)$$

or

$$X + Y \rightarrow X^{+\cdot}Y^{-\cdot} \quad (4)$$

The first two involve acid/base chemistry (i.e., protonation of a basic residue or deprotonation of an acidic one). The third involves protonation or ion attachment to a zwitterionic molecule, such as an ylide or *N*-oxide. In the fourth, formation of a charge transfer complex can also make measurement possible by detection of a radical cation or radical anion [5].

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Experimental

General

All ESI/MS spectra were obtained with a Vestec model 200 ESI/MS quadrupole instrument (Vestec Corp., Houston, TX) with a 2000-u range [6]. ESI spectra of pure compounds were obtained in the solvent indicated approximately 15–30 min after sample preparation by electrospraying the sample solution at 3–5 μL/min using the following instrument settings: needle voltage 2.0–2.6 kV; electrospray chamber temperature 50–65 °C; nozzle voltage 200 V; block temperature 220–250 °C; lens temperature 110–120 °C; repeller voltage 20 V.

Sample Preparation

Reagent **6** was purchased from Molecular Probes, Inc. (Eugene, OR), and reagent **5** was prepared as previously described [7]. The Diels-Alder reactions of **5** or **6** with vitamin D were carried out in refluxing $CHCl_3$. Reaction mixtures were diluted with NaOAc in methanol for ESI/MS studies.

Preparation of reagent **9** as well as the crown ether fulleroid **10** has been reported elsewhere [4h]. Derivatization of carboxylic acids with reagent **9** was performed in benzene at room temperature for 3 min. The nearly instant disappearance of purple from reagent **9** indicated the completion of the reaction. The solution was then mixed with an equal volume of KOAc methanol solution and used for ESI/MS analysis (see Figure 3).

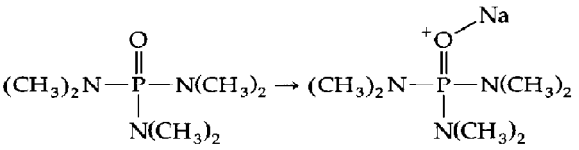
Reagent **11** was prepared by oxidation of 4-formylbenzo-18-crown-6 [4h] to the acid, with $KMnO_4$ in water at 80 °C for 2 h followed by reaction with excess $SOCl_2$. Derivatization of cholesterol was carried out in benzene at room temperature for 15–30 min. The reaction mixture was then diluted with KOAc methanol solution and used for ESI/MS measurement (see Figure 4).

Reagent **12** was prepared according to the procedure for phenyl isothiocynate (PITC) [8] from 4-amino-benzo-15-crown-5 (Parish Chemical Co., Odem, UT). Tagging of amino acids was carried out in the usual way. For example, L-alanine was dissolved in coupling buffer (CH_3CN/pyridine/ triethylamine/ H_2O, 10:5:2:3), and reagent **12** was added. The mixture was heated at 50 °C for 10 min. The volatiles were removed, and NaOAc water/methanol (1:1) solution was added to the residue. The resulting solution was used directly for ESI/MS analysis (see Figure 5).

ESI mass spectra of the reaction mixtures of **10** with vitamin D, α-methyl benzyl azide, *i*-butyl amine, bromine, and diborane (see Figures 6–8) were obtained by dilution of an aliquot with the solvents listed in the figure legends.

Results and Discussion

The ionic signals initially reported by Dole and coworkers [9] in their pioneering work on ESI/MS involved Na^+ attachment to neutral polyethers. Such interactions are common and indeed are often observed in other forms of soft ionization. We can detect hexamethylphosphoric triamide (HMPA) (**1**) (a common reagent in organic synthesis) by ESI/MS as its Na^+ adduct (eq 1). HMPA is used in synthesis



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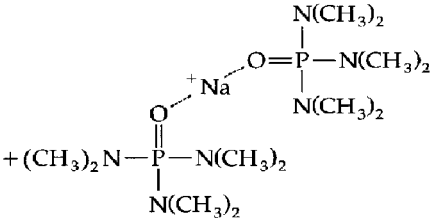
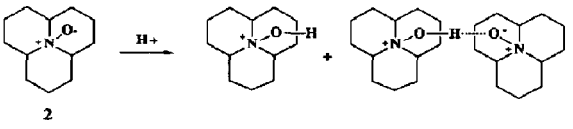


for cation coordination, and in its electrospray spectrum both $[M + Na]^+$ and $[2M + Na]^+$ are observed. Li^+ was found to be efficient for the observation of the neutral compound triphenylphosphine oxide ($Ph_3P{=}O$) [4g]. Even for certain types of alkaloids, such as colchicine, $[M + Na]^+$ was observed [4b]. For molecules with charge separation, such as *N*-oxide (**2**), simple protonation of the negative center will also change the molecule from neutral to positively charged (eq 2). The ESI spectrum of **2** shows both $[M + H]^+$ and a hydrogen-bonded dimer (Figure 1).



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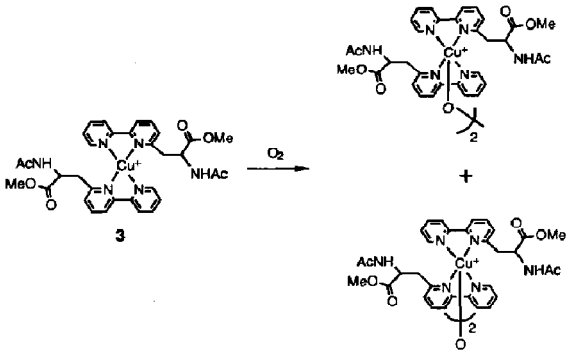
(2)

One of the most useful methods for detection of neutral molecules involves metal cation binding to specific ligands. The first direct study of ESI/MS with neutral ligands attached to metal ions was reported by Chait and co-workers [10]. We followed their lead in developing ESI/MS as a tool for determination of copper/peptide complexes [4a]. During the course of our study, an interesting spectrum was obtained from an air-oxidized sample of complex **3**. The spectrum was consistent with the formation of oxo-bridged

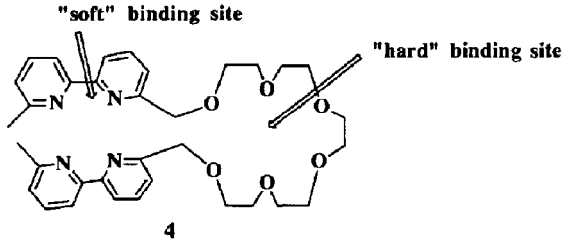
dimers $[Cu + O - O - Cu]^{2+}$ and $[Cu - O - Cu]^{2+}$ (eq 3) (Figure 2). These types of complexes are



(3)

known as models for metalloenzymes, such as hemocyanin [11], although they have not previously observed by mass spectrometry. Other studies of peptide–metal ion interactions have been reported [12], but the results from Figure 2 suggest that ESI/MS also has the potential to be used for studying oxygen transport proteins. Besides copper complexes, we reported examination of cationic nickel complexes and their redox chemistry by ESI/MS [4c, e].

We recently synthesized new molecules with both hard and soft metal binding sites and carried out several careful studies of their Cu^+ and K^+ complexes by ESI/MS. For example, compound **4** may form complexes with either alkali metal ions ("hard" site) or transition metal ions ("soft" site) [4i].



We examined the ion specificity of complexation of metals to polyether ligands by ESI/MS. For example, the complexation of a mixture of metal salts with 18-crown-6 allows direct determination of relative binding in solution [3].

We found that not only does 18-crown-6 provide an excellent binding site for Na^+ or K^+, but that neutral derivatives containing this group are readily prepared and purified, are stable, and may be used as reagents or ESI/MS tags for the study of organic reactions.

ESI Tagging Reagents

The first example of an ESI/MS tag was reported by Lam et al. [13]. Chemical modification of functional groups by the introduction of chargeable groups was proposed to make it possible to detect compounds that are not normally amenable to ESI/MS. Lam showed that conversion of a mercaptan to a pyridyl thio-ether introduced a basic site that was detectable by ESI/MS. More recently, a quaternary ammonium analog of the peptide sequencing reagent PITC was reported [8].

We began a program for design and synthesis of ESI tagging reagents about a year ago [4d] and proposed the following criteria for a good tagging reagent. First, derivatization should be easy, fast, and highly selective. Second, the tagged adduct must have high sensitivity for detection by ESI/MS. Finally, the tagged molecules must be stable under ESI/MS conditions. Although systematic design of tagging reagents for every type of neutral molecule is still in its infancy, we

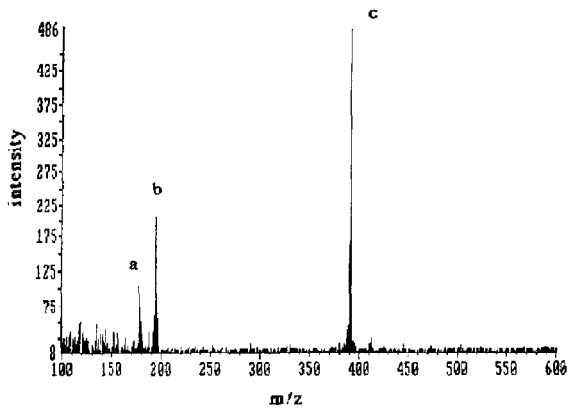


Figure 1. ESI mass spectrum of compound **2** (1×10^{-4} M) in 0.1% trifluoroacetic acid/CH_3OH: a, $[M + H - H_2O]^+$ (m/z 178); b, $[M + H]^+$ (m/z 196); c, $[2M + H]^+$, hydrogen-bonded dimer (m/z 391).

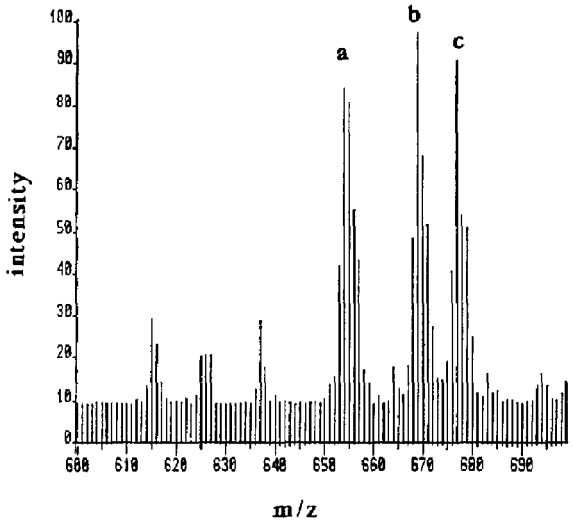
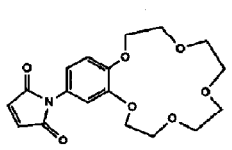


Figure 2. ESI mass spectrum of air-oxidized products of complex **3** ($\sim 10^{-6}$ M) in CH_3OH: a, unknown (m/z 654); b, $[Cu—O—Cu]^{2+}$ (m/z 669); c, $[Cu - O - O - Cu]^{2+}$ (m/z 677).

prepared several types of reagents that may be used for diverse functionalities. The crown ether (metal binding site) and tertiary amine (basic site) are two major structural features in our design. The first, reagent **5**, is a dienophile for introducing a metal binding site for ESI/MS detection of vitamin D. For



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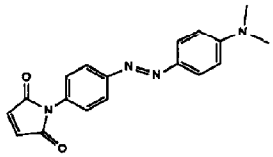

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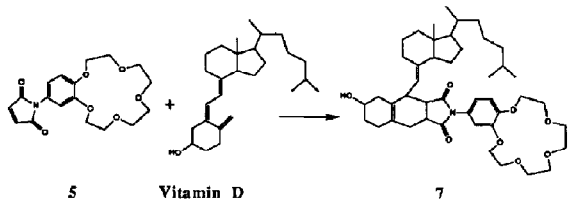
example, reagent **5** reacts with vitamin D_3 to produce adduct **7** (eq 4) [4d, 14]. In a similar manner, the com-



5 Vitamin D 7

(4)

mercially available compound **6** also undergoes a Diels-Alder reaction with vitamin D. Our studies showed that the derivatives from both reagents **5** and **6** had similar sensitivities and that only approximately 1 pmol of compound **7** is required for a spectrum. Besides a Diels-Alder reaction with dienes, the maleimide reagent **5** can also be used as an alkylating agent for tagging peptides having the —SH group [15].

Derivatization of Fullerenes

One of the most exciting recent developments in organic chemistry has been the discovery of a new form of carbon (besides diamond and graphite) called Buckminsterfullerene [C_{60} (**8**)] [16, 17]. In a process first reported in 1991, "soot" may be produced, containing up to 10% C_{60}, accompanied by its relative C_{70}. C_{60} is now commercially available. One of the interesting features of the molecule, besides its unique soccer ball structure, is that C_{60} is the only form of carbon that is a molecule! Another is that it is a rather reactive molecule; because of symmetry, there are six reactive double bonds. Unfortunately, for aspiring C_{60} chemists, the compound is not detectable by proton nuclear magnetic resonance, so that mass spectrometry is one of the few tools for structural analysis. Other major problems are that C_{60} is not very soluble, and many of its derivatives are very fragile and do not yield molecular ions, even by LD or FAB mass spectrometry [18].

Because there is a need for new structure identification tools in this area, we recently developed ESI/MS derivatization reagents for fullerenes such as C_{60} and

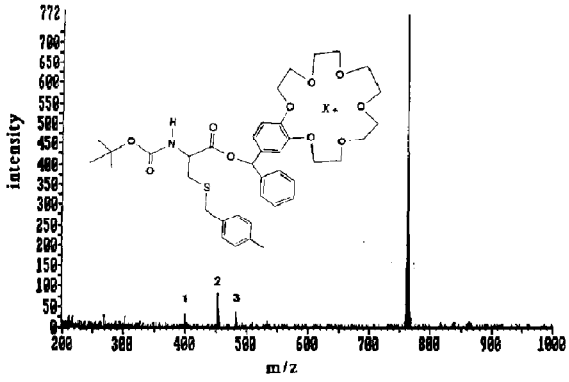
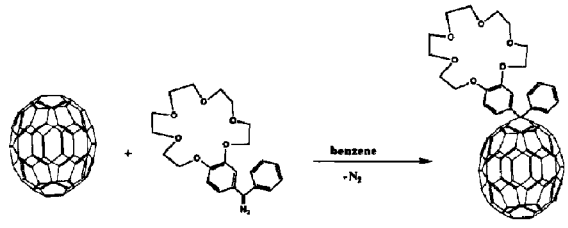


Figure 3. ESI mass spectrum of ester generated in situ from Boc-Cys(4-CH_3—Bn)—OH (10^{-3} M) and reagent **9** (10^{-4} M) in 1:1 C_6H_6/CH_3OH containing KOAc (m/z 764). Peaks 1, 2, and 3 are derived from reagent **9**.

C_{70}. We are able to tag quantitatively these molecules by using diazomethane reagent **9** (eq 5). The resulting



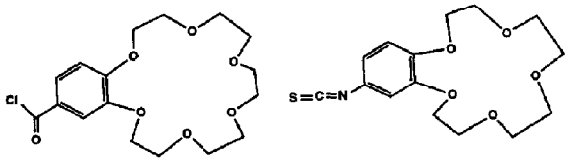
8 9 10

(5)

derivative **10** gives very strong signals in ESI/MS. Determination of C_{60}/C_{70} ratios directly from soot is possible by using this technique [4h].

Derivatization of Other Molecules

In addition, although carboxylic acids are detectable by negative ion ESI/MS, an alternative method involves their derivatization using reagent **9**. For example, an *N*-protected amino acid (Figure 3) can be detected in this manner in the positive mode. For oleic acid (spectrum not shown), only one peak at m/z 721 is seen in the spectrum of the derivatized material. Our own experience has been that positive ion ESI/MS is easier and more sensitive and reliable than the negative ion mode. For molecules having hydroxyl groups, acid chloride reagent **11** was used to form the



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esters that have the potential to be charged. Figure 4 shows that cholesterol may be detected in this manner. Finally, for specific peptide and amino acid detection

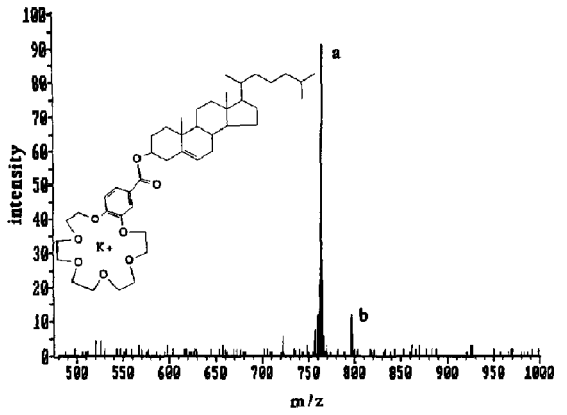


Figure 4. ESI mass spectrum of ester derived from reaction of cholesterol with reagent **11** (10^{-4} M) in C_6H_6/CH_3OH (1:1) containing KOAc: a, $[M + K]^+$ (m/z 763); b, $[M + K]^+ + CH_3OH$ (m/z 795).

systems, ESI/MS and the well-known PITC chemistry were combined. We prepared the crown-PITC reagent **12**. Reaction of alanine with reagent **12** gives a derivative that may be detected as its Na^+ adduct by ESI/MS (Figure 5).

Surveying Chemical Reactions by ESI / MS

A study of the catalytic cycle of the drug ebselen (**13**) by using its ESI-tagged crown ether analog (**14**) was recently reported [19]. We now describe how tagged derivatives may be used to follow other organic reactions in solution.

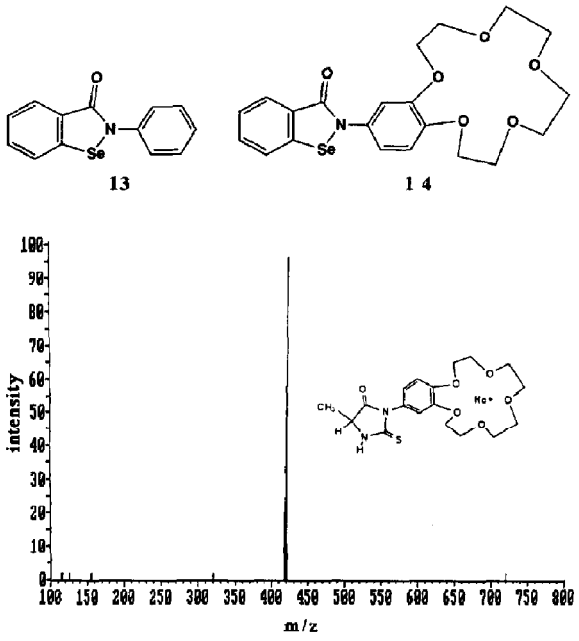
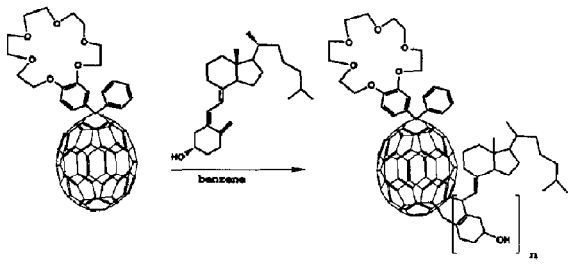


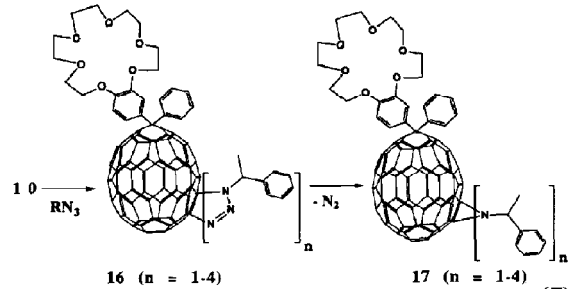
Figure 5. ESI mass spectrum of PITC derivative of L-alanine (10^{-5} M) in H_2O/CH_3OH (1:1) containing NaOAc (m/z 420).

We may use ESI/MS to survey reactions of the Buckminsterfullerene molecule C_{60} (**8**). We prepared the tagged C_{60} molecule that we call C_{61} fulleroid (**10**) (eq 5) in quantity and used this derivative to examine its reactions in solution using ESI/MS. It has been reported that C_{61} fulleroids have chemical properties nearly identical to C_{60} [17g]. We can therefore reliably use **10** to screen for chemical reactions of C_{60} itself [4h]. For example, the Diels-Alder reaction of C_{61} fulleroid with vitamin D_3 could be monitored by ESI/MS (eq 6) (Figure 6a–c). The relative reactivity of the vitamin D diene was followed to establish that the reaction is not reversible and to observe directly the reaction of one **15** ($n = 1$) and then two **15** ($n = 2$) vitamin D molecules with C_{61} fulleroid **10** (the position of attachment of vitamin D is not known).



(6)

With C_{61} fulleroid **10**, we detected many reactions that have been previously reported for C_{60} as well as some new ones. The reaction of C_{60} with amines [17b], bromine [20], and borane [21] are readily studied (Figure 7a–c). The reaction may be followed in solution and the product distribution directly observed. For example, we carried out the reaction of C_{61} fulleroid **10** with 1,3-dipoles, such as α-methylbenzyl azide (eq 7).



(7)

Several pathways for the reaction may be observed as a function of time and temperature. Initially, α-methylbenzyl azide adds one, two, three, and four times (Figure 8a) without expulsion of nitrogen to yield **16** ($n = 1$–4) (the position of attachment of azides is not known). At longer times (Figure 8b), loss of nitrogen may be observed **17** ($n = 1$). On heating at higher temperatures, the multiple adducts lose one or more moles of N_2 to produce the expected distribution of aziridine adducts.

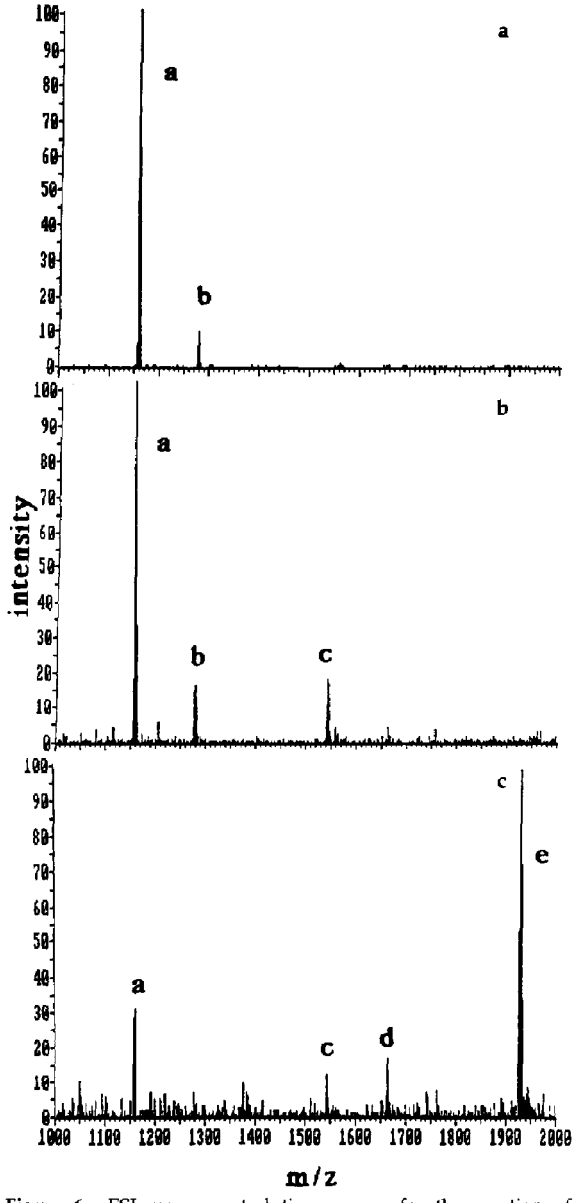


Figure 6. ESI mass spectral time course for the reaction of C_{60}/C_{70} mixture (9:1 ratio of compound **10** and its C_{70} analog, referred to below as $C_{61}X$ or $C_{71}X$, respectively) with vitamin D in C_6H_6. Aliquots were removed and diluted to 10^{-4}–10^{-6} M in C_6H_6/CH_3OH (1:1) containing KOAc. Peak assignments: a, $C_{61}X$ (m/z 1159); b, $C_{71}X$ (m/z 1279); c, $C_{61}X$–mono-vitamin D adduct (m/z 1543); d, $C_{71}X$–mono-vitamin D adduct (m/z 1663); e, $C_{61}X$–bis-vitamin D adduct (m/z 1927). (a) Start of reaction; (b) stirring 20 h at room temperature; (c) refluxing 4 h (benzene).

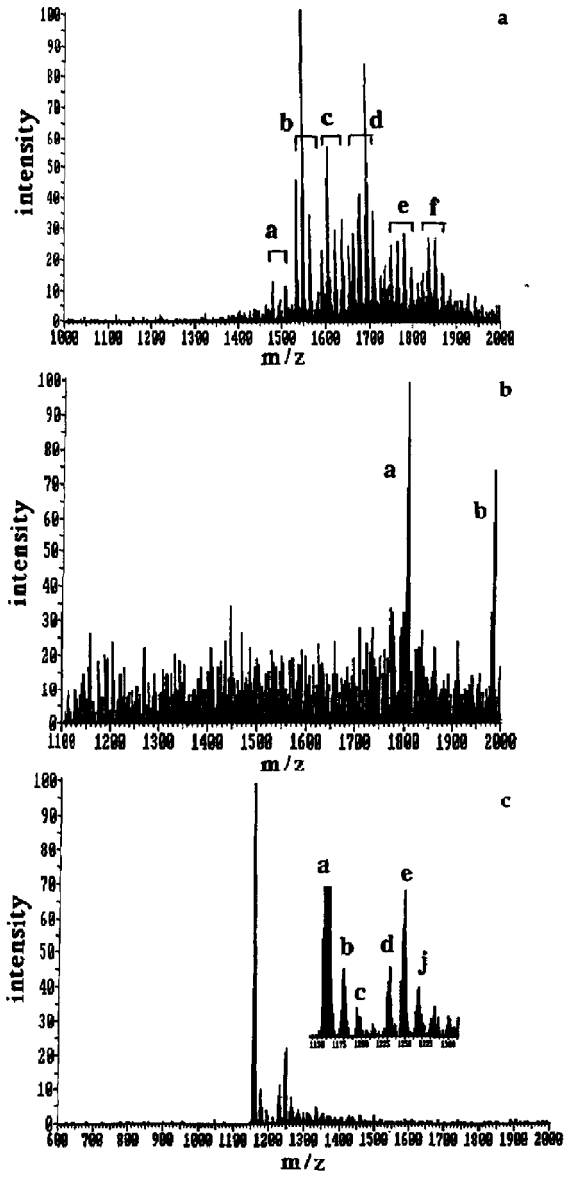


Figure 7. ESI mass spectra for $C_{61}X$ fulleroid **10** reaction products (10^{-4}–10^{-6} M) in C_6H_6/CH_3OH (1:1) containing KOAc (M = compound **10**). (a) Amination (neat *i*-butyl amine, 4 h; X = isobutyl amine; n = 0–5): a, $MO_nX_4K^+$; b, $MO_nX_5K^+$; c, $MO_nX_6K^+$; d, $MO_nX_7K^+$; e, $MO_nX_8K^+$; f, $MO_nX_9K^+$. (b) Bromination (neat Br_2, 25 °C, 2 h): a, $[MBr_8K]^+$ (centered at m/z 1805); b, $[MBr_{10}(CH_3OH)K]^+$ (centered at m/z 1985). (c) Hydroboration (excess BH_3-OEt_2 in $C_6H_5CH_3$, 25 °C, 2 h): a, $[MK]^+$ (m/z 1159); b, unknown (m/z 1180); c, unknown (m/z 1195); d, $[MO_2(BH_3)_5K]^+$ (m/z 1233); e, $[MO_3(BH_3)_3K]^+$ (m/z 1249); f, $[MO_4(BH_3)_3K]^+$ (m/z 1265).

Conclusions

ESI/MS is not only an excellent tool for the analysis of biopolymers but is also a very useful technique for characterization of small neutral organic molecules. We have shown that the rational design of tagging reagents can eliminate the ESI/MS limitation with regard to type of analyte. By using suitable strategies, applications of ESI/MS to organic chemistry, organic reaction mechanisms, and drug metabolism may be greatly improved.

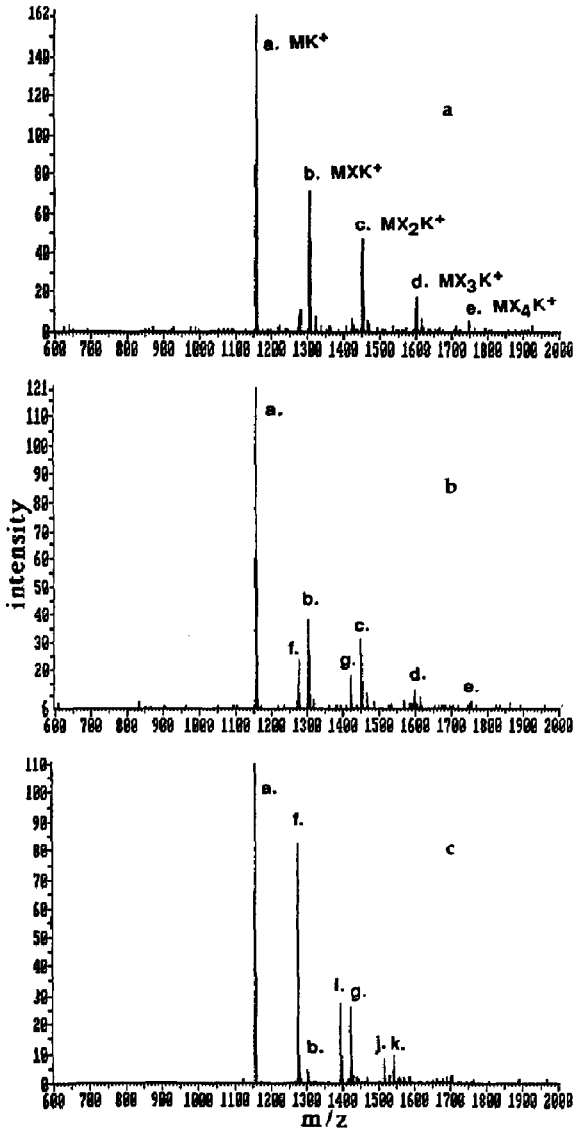


Figure 8. ESI mass spectral time course for the 1,3-dipolar addition of α-methylbenzyl azide to C_{61} fulleroid **10** in C_6H_6 for **a** and **b** and in $C_6H_5CH_3$ for **c**. Aliquots were diluted in C_6H_6/CH_3OH (2:1) containing KOAc (sample concentrations 10^{-4}–10^{-6} M); M = C_{61} fulleroid **10**; X = α-methylbenzyl azide. (a) 22 h, 65 °C: a, $[MK]^+$ (m/z 1159); b, $[MXK]^+$ (m/z 1306); c, $[MX_2K]^+$ (m/z 1453); d, $[MX_3K]^+$ (m/z 1600); e, $[MX_4K]^+$ (m/z 1747). (b) 30 h, 65 °C: f, $[(MX - N_2)K]^+$ (m/z 1278); g, $[(MX_2 - N_2)K]^+$ (m/z 1425); h, $[(MX_3 - N_2)K]^+$ (m/z 1572). (c) 20 h, 100 °C: i, $[(MX_2 - 2N_2)K]^+$ (m/z 1397); j, $[(MX_3 - 3N_2)K]^+$ (m/z 1514); k, $[(MX_3 - 2N_2)K]^+$ (m/z 1542).

References

1. (a) Fenn, J. B.; Mann, M.; Meng, C. K.; Wong, S. F.; Whitehouse, C. M. *Science* **1989**, *246*, 64; (b) Fenn, J. B.; Mann, M.; Meng, C. K.; Wong, S. F.; Whitehouse, C. M. *Mass Spectrom. Rev.* **1990**, *9*, 37; (c) Smith, R. D.; Loo, J. A.; Edmonds, C. G.; Barinage, C. J.; Udseth, H. R. *Anal. Chem.* **1990**, *62*, 882; (d) Beavis, R. C.; Chait, B. T. *Proc. Natl. Acad. Sci. USA* **1990**, *87*, 6873; (e) McEwen, C. N.; Larsen, B. S. *J. Am. Soc. Mass Spectrom.* **1991**, *2*, 205; (f) Dass, C.; Kusmierz, J. J.; Desiderio, D. M.; Jaruis, S. A.; Green, B. N. *J. Am. Soc. Mass Spectrom.* **1991**, 2, 149; (g) Guevremont, R.; Siu, K. W. M.; Le Blanc, J. C. Y.; Berman, S. S. *J. Am. Soc. Mass Spectrom.* **1992**, *3*, 216; (h) Chait, B. T.; Kent, S. B. H. *Science*, **1992**, *257*, 1885; (i) Loo, J. A.; Loo, R. R. O.; Light, K. J.; Edmonds, C. G.; Smith, R. D. *Anal. Chem.* **1992**, *64*, 81.
2. (a) Beckner, C.; Caprioli, R. *Biomed. Environ. Mass Spectrom.* **1984**, *11*, 60; (b) Caprioli, R. *Anal. Chem.* **1983**, *55*, 2387; (c) Johnstone, R. A. W.; Rose, M. E. *J. Chem. Soc., Chem. Commun.* **1983**, 1268; (d) Ikarashi, Y.; Maruyama, Y. *J. Chromatogr.* **1991**, *587*, 306; (e) Newton, R. P.; Walton, T. J.; Brenton, A. C.; Kingston, E. E.; Harris, F. M. *Rapid Commun. Mass Spectrom.* **1989**, *3*(6), 178–182; (f) Brown, S. M.; Busch, K. L. *Anal. Chim. Acta* **1989**, *218*, 231; (g) Lustig, D. A.; Lubman, D. M. *Rev. Sci. Instrum.* **1991** 62(4), 957–962; (h) Hahn, J. H.; Zenobi, R.; Zare, R. N. *J. Am. Chem. Soc.* **1987**, *109*, 2842; (i) Teesch, L. M.; Adams, J. *Org. Mass Spectrom.* **1992**, *27*, 931; (j) Brown, S. M.; Busch, K. L. *Anal. Chim. Acta* **1989**, *218*, 231; (k) DiDonato, G. C.; Busch, K. L. *Biomed. Environ. Mass Spectrom.* **1985**, *12*, 364; (l) Guarini, A.; Guglielmetti, G.; Andriollo, Nunzio; Vincenti, M. *Anal. Chem.* **1992**, *64*, 204.
3. Cheng, Z. L.; Siu, K. W. M.; Guevremont, R.; Berman, S. S. *J. Am. Soc. Mass Spectrom.* **1992**, *3*, 281.
4. (a) Wilson, S. R.; Wu, Y. *J. Org. Chem.* **1992**, *57*, 6941; (b) Wilson, S. R.; Perez, J.; Wu, Y. *Nat. Prod. Lett.* **1992**, *1*, 103; (c) Wilson, S. R.; Wu, Y. *Proceedings of the 40th ASMS Conference on Mass Spectrometry and Allied Topics*; Washington, DC, 1992; p 594; (d) Wilson, S. R.; Tulchinsky, M. L.; Wu, Y. *Proceedings of the 40th ASMS Conference on Mass Spectrometry and Allied Topics*; Washington, DC, 1992; p 1641; (e) Wilson, S. R.; Wu, Y. *Organometallics*, **1993**, *12*, 1478; (f) Perez, J. Ph.D. thesis, New York University, 1992; (g) Wilson, S. R.; Perez, J.; Pasternak, A. *J. Am. Chem. Soc.*, **1993**, *115*, 1994; (h) Wilson, S. R.; Wu, Y., *J. Chem. Soc. Chem. Commun.*, in press; (i) Wilson, S. R.; Wu, Y., *J. Am. Chem. Soc.*, in press.
5. Van Berkel, G. J.; McLuckey, S. A.; Glish, G. L. *Anal. Chem.* **1992**, *64*, 1586.
6. Allen, M. H.; Vestal, M. L. *J. Am. Soc. Mass Spectrom.* **1992**, *3*, 18.
7. Wilson, S. R.; Tulchinski, M.; Wu, Y. *Bioorg. Med. Chem. Lett.*, in press.
8. Aebersold, R.; Bures, E. J.; Namchuk, M.; Goghari, M. H.; Shushan, B.; Covey, T. C. *Protein Sci.* **1992**, *1*, 494.
9. (a) Dole, M.; Mach, L. L.; Hines, R. L.; Mobley, R. C.; Ferguson, L. P.; Alice, M. B. *J. Chem. Phys.* **1968**, *49*, 2240; (b) Mach, L. L.; Kralik, P.; Rheude, A.; Dole, M. *J. Chem. Phys.* **1970**, *52*, 4977.
10. Katta, V.; Chowdhury, S. K.; Chait, B. T. *J. Am. Chem. Soc.* **1990**, *112*, 5348.
11. Simmons, M. G.; Wilson, L. J. *J. Chem. Soc., Chem. Commun.* **1978**, 634.
12. Hutchens, T. W.; Allen, M. H. *Rapid Commun. Mass Spectrom.* **1992**, *6*, 469.
13. Lam, Z.; Reinhold, B. B.; Reinhold, V. N. *Proceedings of the 39th ASMS Conference on Mass Spectrometry and Allied Topics*; Nashville, TN, May 19–24, 1991; p 282.
14. Wilson, S. R.; Lu, Q.; Tulchinski, M.; Wu, Y. *J. Chem. Soc., Chem. Commun.*, in press.
15. Wilson, S. R.; Tulchinski, M., unpublished results.
16. Koshland, D. *Science* **1991**, *254*, 1705.
17. (a) Braun, T. *Angew. Chem. Int. Ed. Engl.* **1992**, *31*, 588; (b) Wudl, F.; Hirsch, A.; Khemani, K. C.; Suzuki, T.; Allemand, P.-M.; Koch, A.; Eckert, H.; Srdanov, G.; Webb, H. M. In *Fullerenes*; ACS Symposium Series; American Chemical Soci-

ety: Washington, DC, 1992; (c) Hirsch, A.; Khemani, K. C.; Suzuki, T.; Allermand, P.-M.; Kolch, A.; Srdanov, G.; Wudl, F. In *Large Carbon Clusters*; ACS Symposium Series; American Chemical Society: Washington, DC, 1991; (d) Howard, J. B.; McKinon, J. T.; Makarovsky, Y.; Lafleur, A. L.; Johnson, E. *Nature* **1991**, *352*, 139–141; (e) Coustel, N.; Bernier, P.; Aznar, R.; Zahad, A.; Lamber, L.-M.; Lyard, P. *J. Chem., Soc., Chem. Commun.* **1992**, 1402; (f) Scrievens, W. A.; Bedworth, P. V.; Tour, J. M.; *J. Am. Chem. Soc.* **1992**, *114*, 7917; (g) Khemani, K. C.; Prato, M.; Wudl, F. *J. Org. Chem.* **1992**, *57*, 3254; (h) Schwarz, H. *Angew. Chem. Int. Ed. Engl.* **1992**, *31*, 293; (i) Wudl, F. *Acc. Chem. Res.* **1992**, *25*, 157; (j) Hoke, S. H., II; Molstad, J.; Dilettato, D.; Jay, M. J.; Carlson, D.; Kahr, B.; Cooks, R. G. *J. Org. Chem.* **1992**, *57*, 5069.

18. McElvany, S. W.; Ross, M. M. *J. Am. Soc. Mass. Spectrom.* **1992**, *3*, 268.
19. Wilson, S. R.; Tulchinski, M., submitted.
20. Olah, G. A.; Bucsi, J.; Lambert, C.; Aniszfeld, R.; Truverdi, N. J.; Sensharman, D. K.; Prakash, G. K. S. *J. Am. Chem. Soc.* **1991**, *112*, 9385.
21. Cahill, P. A.; Henderson, C. C. *MRS Meeting Abstracts*, Boston, MA, Dec. 1992.