ESI-MS Detection of Ionic Intermediates in Phosphine-Mediated Reactions

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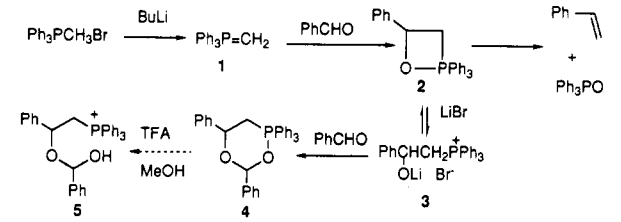
Abstract: This article describes the application of electrospray ionization mass spectrometry (ESI-MS) to the detection of transient intermediates in the Wittig, Mitsunobu, and Staudinger reactions directly from solution. Ionic intermediates in these reactions could be detected directly and zwitterionic intermediates indirectly upon quenching reaction aliquots with acid. For each reaction, intermediates were identified that correlate with those proposed on the basis of ^{31}P NMR analysis. Furthermore, ESI-MS spectra were uncomplicated by fragmentation of molecular ions and showed unambiguous single peaks for each species present and, consequently, interpretation of spectra was straightforward. The progress of reactions could be monitored over time by following the gradual appearance and disappearance of peaks corresponding to transient intermediates.

Electrospray ionization mass spectrometry (ESI-MS) has recently emerged as a powerful technique for the measurement of molecular masses of involatile and fragile compounds that would normally suffer fragmentation under most ionizing conditions necessary for mass measurement.[1] In particular, ESI-MS has attracted considerable attention for its capacity to accurately measure molecular masses of biopolymers such as proteins and nucleic acids.[2] An observation that has received less attention has been that ESI-MS can also be conveniently used to detect smaller organic ions in solution, giving unambiguous molecular ion signals with no fragmentation.[3] ESI-MS also exhibits exceptional sensitivity, giving clean signals at submicromolar sample concentrations. We felt that these qualities of ESI-MS might allow for detection of ionic intermediates in organic reactions, providing a mechanistic probe for the elucidation of transient intermediates. To test this hypothesis, we chose to examine three well-studied phosphine-mediated reactions in which ionic intermediates have been implicated and where a wealth of ^{31}P NMR data are available to corroborate our findings. We found that removal of reaction aliquots followed by dilution and ESI-MS analysis led to remarkably simple spectra wherein the expected ionic intermediates in all cases could be detected either by observation of the cations of ion pairs or by observation of molecular ions arising from protonation of zwitterionic intermediates.

Wittig Reaction. The mechanism of the Wittig reaction has been subjected to close scrutiny.[4] Scheme I illustrates the currently favored mechanism of the Wittig reaction using a well-studied system in which the reagents are methylene ylide **1** and benzaldehyde. In the past, the primary focus of controversy with respect to the mechanism of the Wittig reaction has been whether or not the phosphorus ylide and the carbonyl substrates combine to form oxaphosphetanes **2** directly, or if betaines **3** are formed first. It is now generally accepted that oxaphosphetanes are formed first and that these, in the presence of lithium halide salts, may open reversibly to betaine intermediates. This conclusion follows primarily from the work of Vedejs, who characterized intermediates in the Wittig reaction at low temperature by ^{31}P and ^{1}H NMR techniques under standard and salt free conditions.[5] Included in that work was a study of the reaction shown in Scheme I. We chose to examine this same reaction by ESI-MS since our detection of intermediates could be verified by comparison to the NMR data available in the literature. In a typical procedure,

Scheme I



treatment of methyltriphenylphosphonium bromide with *n*-butyllithium (Scheme I) leads to the formation of the ylide **1**. An aliquot quenched with acid at this stage showed an ESI-MS spectrum (Figure 1a) consisting of only a single peak for the protonated ylide (m/z = 277). The addition of benzaldehyde, according to the protocol of Vedejs et al., generates oxaphosphetane **2** which, due to the LiBr generated in situ, opens to the bentaine–LiBr adduct **3**.[5] An aliquot of a reaction mixture was quenched with 1% TFA/methanol after 5 min at −78 °C. A clean ESI-MS spectrum resulted, showing three major signals (Figure 1b). The peak at 383 corresponds to the hydroxyphosphonium cation of **3**. The peak at 489 has been assigned as phosphonium hemiacetal **5** derived from **4**, which opens upon quenching with 1% TFA/methanol. The presence of an intermediate such as **4** has not been previously observed in the Wittig reaction, although Vedejs noted the presence of extraneous ^{31}P NMR signals in the pentavalent phosphorus region in some Wittig reactions, particularly in those where lithium halides were present.[5] Until now, no structure accounting for these signals has been proposed. The peak at 563 is a complex consisting of two triphenylphosphine oxide molecules and one lithium cation.[6] The progress of the Wittig reaction can be monitored to completion. After 20 h at room temperature, ESI-MS shows only the two peaks at 489 and 563 in a 1:10 ratio. The fact that the peak at 489 remains suggests that the formation of side product **4** is irreversible.

To confirm our peak assignments we carried out the same experiment using *m*-tolualdehyde and obtained a spectrum (Figure 2) in which the signals at 383 and 489 had been replaced by new peaks at 397 and 517, a change in mass of 14 and 28 amu, respectively. Furthermore, when a threefold excess of tolualdehyde was used, the relative intensity of the peak at 517 increased, as expected if the intermediate betaine is being trapped with excess unreacted aldehyde to form an adduct such as **4**.

This represents the first use of mass spectrometry to detect molecular ions of intermediates (or their quenched derivatives) in the Wittig reaction. ESI-MS cannot contribute to resolving the controversy relating to betaine versus oxaphosphetane intermediates that previously were only characterized by ^{31}P and ^{1}H

(1) For a recent review see: Smith, R. D.; Loo, J. A.; Edmonds, C. G.; Barinaga, C. J.; Udseth, H. R. *Anal. Chem.* **1990**, *62*, 882–899.

(2) Fenn, J. B.; Mann, M.; Meng, C. K.; Wong, S. F.; Whitehouse, C. M. *Science* **1989**, *246*, 64–71.

(3) Yamashita, M.; Fenn, J. B. *J. Phys. Chem.* **1984**, *88*, 4451–4459.

(4) For recent reviews see: (a) McEwen, W. E.; Beaver, B. D.; Cooney, J. V. *Phosphorus Sulfur* **1985**, *25*, 255. (b) Maryannoff, B. E.; Reitz, A. B. *Chem. Rev.* **1989**, *89*, 863. Also see: Vedejs, E.; Marth, C. F. *J. Am. Chem. Soc.* **1990**, *112*, 3905–3909.

(5) Vedejs, E.; Meier, G. P.; Snoble, K. A. J. *J. Am. Chem. Soc.* **1981**, *103*, 2823–2831.

(6) Interestingly, we have observed that the 2 to 1 ratio of phosphine oxide to lithium cation is maintained over a range of concentrations from 0.001% to 1% of LiBr/methanol.

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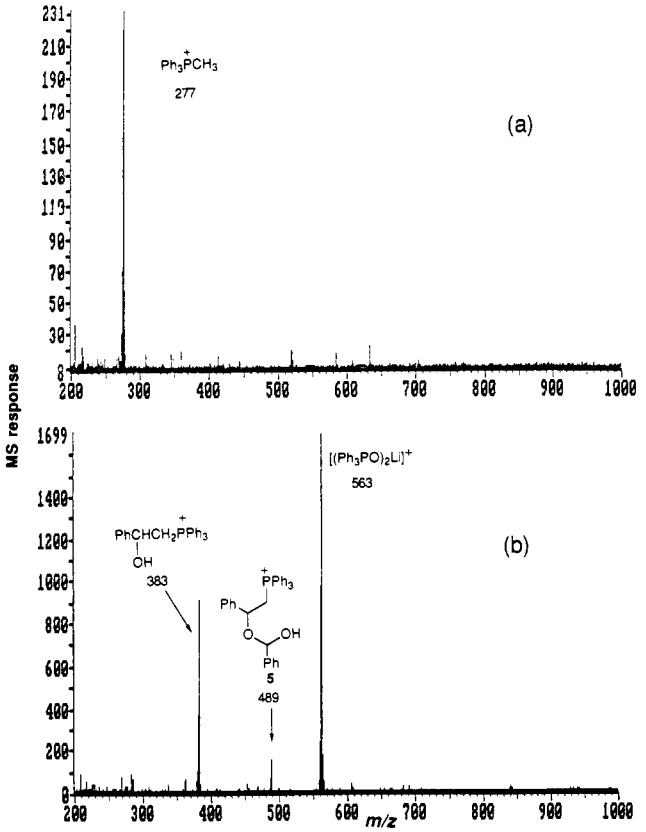


Figure 1. Spectra of the Wittig reaction mixture in 1% TFA/MeOH: (a) after formation of ylide **1**; (b) 5 min after addition of benzaldehyde.

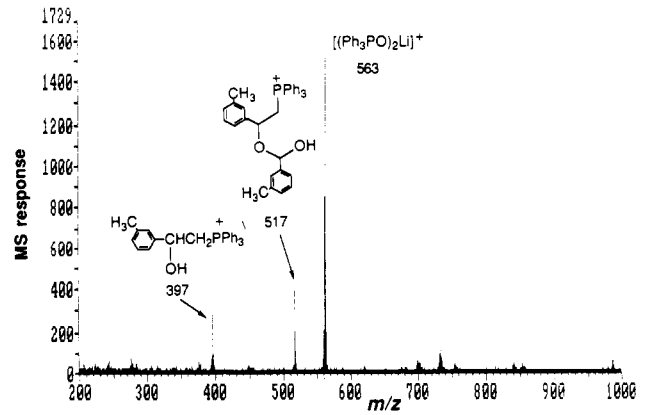


Figure 2. Spectrum of the Wittig reaction with a threefold excess of *m*-tolualdehyde after 15 min at −78 °C in 1% TFA/MeOH.

NMR. In addition, a new side product in the Wittig reaction is now proposed to have the structure **4**.

Mitsunobu Reaction. The mechanism of the Mitsunobu reaction has similarly been a subject of controversy over the past decade.[7] The structure of the activated alcohol species has been the focus of mechanistic debate. Mitsunobu originally proposed that the active alcohol species was an alkoxyphosphonium salt; however, it is now commonly believed that the alkoxyphosphonium salt **9** is in equilibrium with a dialkoxyphosphorane intermediate **8**. This mechanism is depicted in Scheme II with triphenylphosphine (TPP), diethyl azodicarboxylate (DEAD), benzoic acid, and two different alcohols, neopentyl alcohol and diacetone-*d*-glucose, as the reagents. We investigated the Mitsunobu reaction by ESI-MS using both of these alcohols since their reaction intermediates have already been characterized by ^{31}P NMR.[8] Treatment of betaine **6**, formed in the usual fashion[8] from TPP and DEAD, with 1 equiv

(7) For a recent review see: Hughes, D. L. *Organic Reactions*, Paquette, L. A., Ed.; John Wiley & Sons, Inc.: New York, 1992.
(8) Camp, D.; Jenkins, I. D. *J. Org. Chem.* **1989**, *54*, 3045–3049.

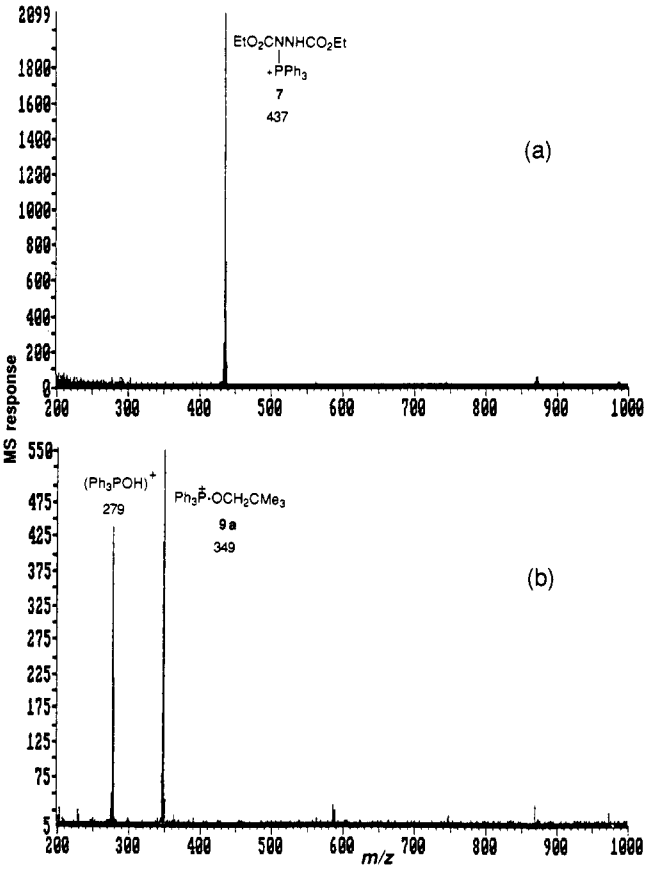


Figure 3. Spectra of the Mitsunobu reaction mixture, in CH_3CN, after adding 1 equiv of benzoic acid to (a) betaine **6** or (b) dialkoxyphosphorane **8a**.

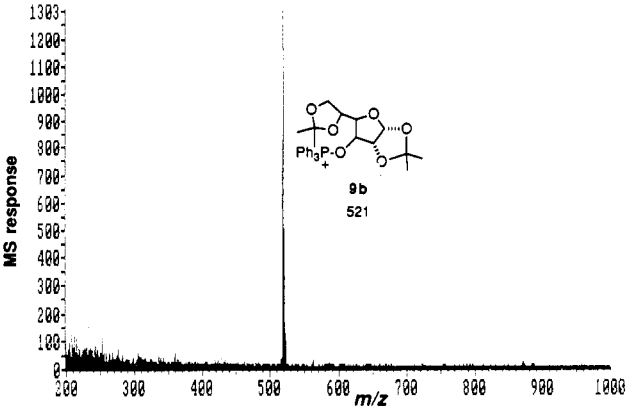
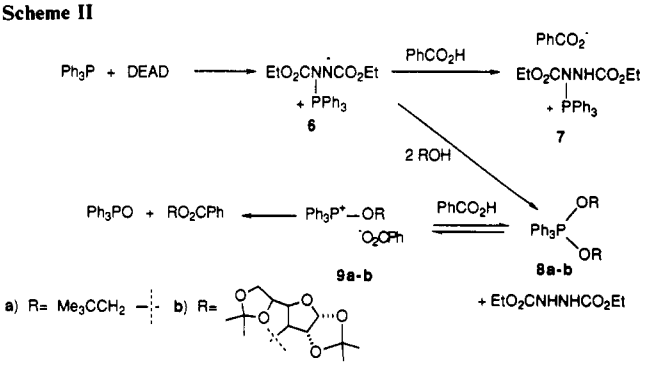


Figure 4. Reaction mixture 5 min after adding benzoic acid to dialkoxyphosphorane **8b** in CH_3CN.

Scheme II



of benzoic acid, followed by dilution of an aliquot in acetonitrile,[9] showed a single peak by ESI-MS with a mass of 437, corre-

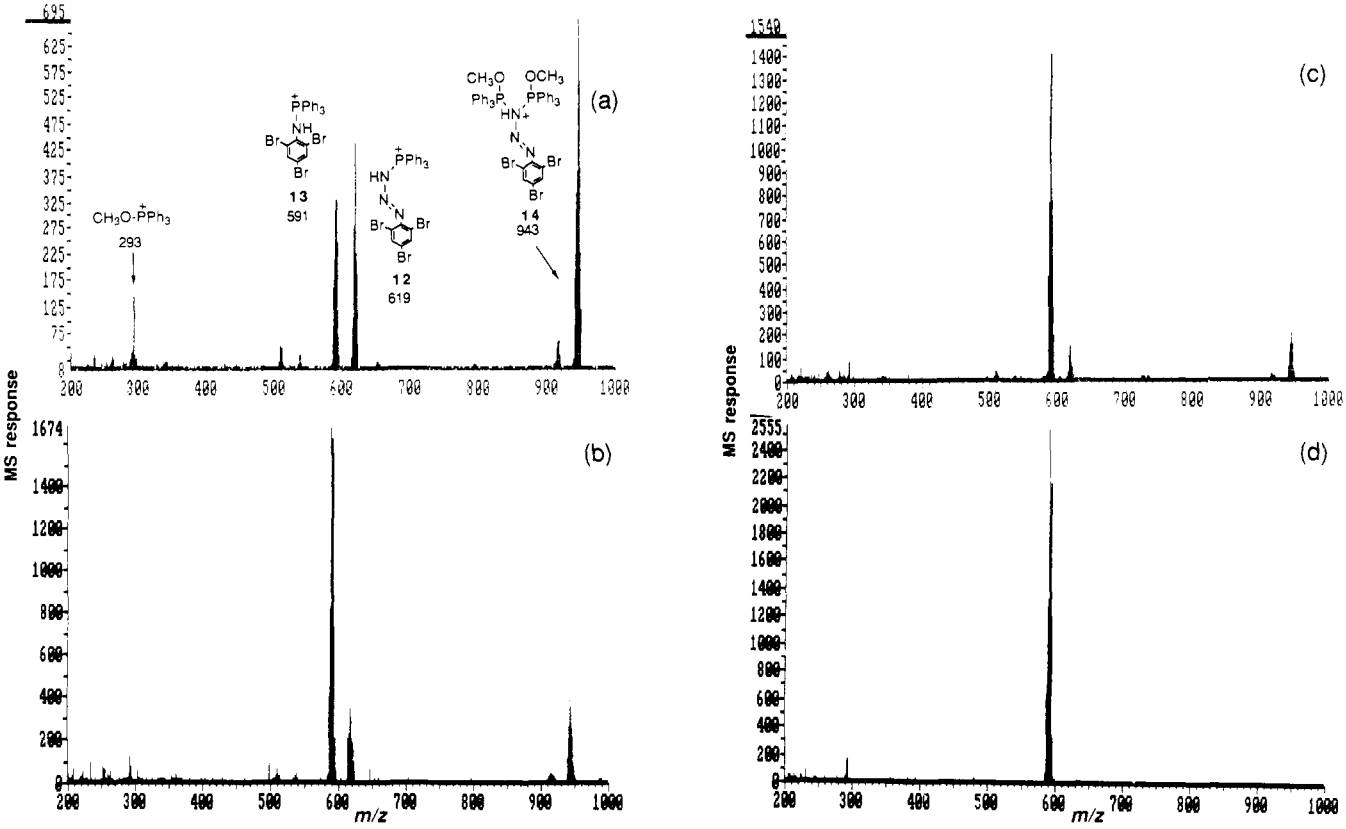


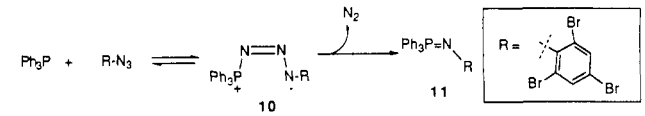
Figure 5. Spectra of the Staudinger reaction mixture in 1% TFA/MeOH: (a) after 2 min at 0 °C; (b) after 15 min at 0 °C; (c) after 30 min at 0 ° %C; (d) after 1 h at room temperature.

sponding to the expected protonated betaine **7** (Figure 3a). Subsequent treatment of betaine **6** was 2 equiv of neopentyl alcohol, followed by addition of 1 equiv of benzoic acid, led to the spectrum shown in Figure 3b. The signal at 279 corresponds to protonated triphenylphosphine oxide, while the signal at 349 corresponds to the alkoxyphosphonium cation of **9a**. When the same sequence was repeated using the secondary alcohol diacetone-*d*-glucose in place of neopentyl alcohol, the spectrum (Figure 4) showed the expected alkoxyphosphonium cation of **9b** at 521.

Although ESI-MS cannot be used to detect dialkoxyphosphoranes directly, we have found that all of the remaining intermediates shown in Scheme II can be detected either directly when they have cationic components or indirectly after quenching with acid to form protonated derivatives. In his examination of these same reactions, Jenkins relied primarily on ^{31}P NMR to identify the reaction intermediates.[8] Analysis of Mitsunobu reaction mixtures by ^{1}H and ^{13}C NMR using neopentyl alcohol was not very successful. The major problems with ^{1}H and ^{13}C NMR spectrometry are the difficulty of characterizing individual intermediates in a mixture of components and of finding an appropriate NMR solvent in which to carry out the reactions. ESI-MS, therefore, appears to provide a useful complementary technique for more complete characterization of Mitsunobu reaction intermediates.

Staudinger Reaction. The Staudinger reaction (Scheme III) proceeds by a two-step process involving reversible electrophilic attack of an azide onto a phosphine to generate phosphazide **10**, which decomposes with extrusion of nitrogen gas to give phosphinimine **11**. The mechanism of the Staudinger reaction has been well-studied and the phosphazide intermediates as well as the phosphinimine products (which are most often hydrolyzed to give the corresponding amines) have been characterized by ^{31}P, ^{15}N, and ^{13}C NMR.[10] In some cases, stable phosphazide intermediates

Scheme III



have even been isolated and their chemical composition verified by microanalysis.[11] The known basicity of both phosphinimines and phosphazides[12] allows for their protonation, making them amenable to detection by ESI-MS. We chose to examine the known Staudinger reaction between TPP and 2,4,6-tribromophenyl azide[11] by ESI-MS. Treatment of TPP with 2,4,6-tribromophenyl azide at 0 °C[13] followed by quenching after 2 min gave an ESI-MS spectrum (Figure 5a) showing two expected signals: one for the protonated phosphazide **12** at 619 and the other for the protonated phosphinimine **13** at 591. It has been suggested that protonation of phosphazides occurs at the nitrogen adjacent to phosphorus.[12] In addition, an unexpected peak at 943 was observed whose intensity during the course of the reaction roughly parallels that of **12**. The structure of this ion has tentatively been assigned as **14**[14] and seems to be a product formed on quenching of reaction aliquots and not a Staudinger reaction intermediate. ESI-MS can also be conveniently used to monitor the reaction with time, as shown in parts b–d of Figure 5, where reaction mixture aliquots were analyzed after 15 and 30 min at 0 °C and 1 h at room temperature, respectively. As the reaction progresses, a gradual

(9) In this case the solvent choice is crucial, since an aliquot quenched in methanol at −78 °C showed a single peak at 293 corresponding to the methoxytriphenylphosphonium cation.

(10) For a recent review see: Gololobov, Y. G.; Kasukhin, L. F. *Tetrahedron* **1992**, *48*(*8*), 1353–1406.

(11) Gololobov, Y. G.; Onys'ko, P. P.; Proklina, N. V.; Prokopenko, V. P. *Zh. Obshch. Khim.* **1984**, *54*(*2*), 325–333.

(12) Prokopenko, V. P.; Proklina, N. V.; Onys'ko, P. P. *Zh. Obshch. Khim.* **1984**, *54*, 812.

(13) We felt that our chances of detecting the phosphazide would be enhanced at 0 °C since extrusion of nitrogen proceeds rapidly at room temperature, with the reaction going to completion in only 1 h.

(14) The ion at 943 is not always observed, e.g., in 1% TFA/ethanol the peak is absent. Ion **14** might form from reaction of the methoxytriphenylphosphonium cation (note ion at 293 and cf. ref 9) with the azide.

disappearance of the peaks at 619 and 943 with a concomitant increase in the peak at 591 corresponding to product is observed. The minor peak at 293 is always present and appears to correspond to the methoxytriphenylphosphonium cation formed on quenching in 1% TFA/MeOH.

In order to further verify our peak assignments, we carried out Staudinger reactions with three other phosphines: diphenyl(*p*-tolyl)phosphine, diphenylmethylphosphine, and diphenyl(3-methyl-2-butyl)phosphine. In each case, the peaks for the proposed intermediates were appropriately shifted according to the difference in mass of the phosphine (Figures 6–8, supplementary material). For example, in Figure 6 the ESI-MS spectrum of a quenched aliquot (1% TFA/MeOH) of the Staudinger reaction between diphenyl(*p*-tolyl)phosphine and 2,4,6-tribromophenyl azide after 10 min of reaction of 0 °C is shown. The protonated phosphinimine and phosphazide now appear at 605 and 633, respectively, corresponding to an increase in mass of 14 amu in each case. The anomalous peak observed previously which arises on quenching with 1% TFA/MeOH has shifted by 28 amu, supporting the proposition that 2 equiv of the phosphine are incorporated in this adduct.

The Staudinger reaction intermediates can therefore be detected using ESI-MS. Again, the masses of quenched intermediates match what would be anticipated on the basis of structures derived from literature ^{31}P NMR characterizations. ESI-MS can also be used to monitor the Staudinger reaction with time. Although in the Wittig and Mitsunobu reactions essentially all the peaks could be attributed to known or suspected reaction intermediates, in the Staudinger reaction one major peak seemed to arise as a result of the quenching process.

Conclusions. We have shown that ESI-MS can be used to detect ionic intermediates in phosphine-mediated reactions, directly from solution, with no apparent fragmentation. The results agree well with literature identification of reaction intermediates in all cases, indicating that ESI-MS, when used in conjunction with the standard ^{31}P NMR analyses, can contribute to a more confident characterization of proposed reaction intermediates. Furthermore, a side product not previously reported in the Wittig reaction has also been detected and is proposed to have acetal structure **4**. In addition, ESI-MS may conveniently be used to monitor the progress of reactions.

Experimental Section

ESI-MS spectra were obtained on a Vestec Model M-200 single quadrupole mass spectrometer with a 2000-amu mass range.[15] Data collection was carried out with a Teknivent data system. Sample solutions were injected at 4 μL/min with the following instrument settings: needle voltage, 2.0–2.6 kV; ES chamber temperature, 45–60 °C; nozzle voltage, 200 V; block temperature, 245–250 °C; lens temperature, 120 °C; repeller voltage, 20 V; ES chamber pressure, 1.4–1.5 × 10^{-1} Torr. Samples were scanned for ≤5 min at 7.6–13.3 s/scan. Signal-to-noise ratio was improved by averaging the scans of the first 1–2 min. The sample concentrations reported below were experimentally found to give high intensity and high signal-to-noise ratio.

Most chemical reagents were purchased from Aldrich Chemical Co. THF and diethyl ether were dried by distillation from Na–benzophenone under nitrogen prior to use. HPLC grade methanol, ethanol, and acetonitrile were used for ESI-MS injections.

The Wittig Reaction (Figures 1a,b and 2). The experimental procedures for these reactions were similar to those reported by Vedejs and co-workers:[5] A suspension of 1.23 g (3.5 mmol) of $CH_3Ph_3P^+Br^-$ in dry THF (28 mL) is cooled to −78 °C under nitrogen. *n*-Butyllithium in hexane (1.61 mL of 2.5 M solution, 4.03 mmol) is added dropwise to the stirred suspension. The canary yellow color of ylide appears at once. A 25-μL sample withdrawn after 15 min is quenched with 1 mL of 1% TFA/MeOH to give the spectrum shown in Figure 1a. After 2 h at −78 °C, benzaldehyde or *m*-tolualdehyde (4.03 mmol) is added dropwise. The progress of the reaction is monitored by taking 25–50-μL aliquots, at different times, and quenching them with 1 mL of 1% TFA/methanol precooled to 78 °C and analyzing by ESI-MS within 5 min (cf. Figure 1b or 2).

The Mitsunobu Reaction (Figures 3a,b and 4). Procedures 1 and 2 are based on the work of I. D. Jenkins and co-workers.[8]

(1) Preparation of protonated betaine 9: Triphenylphosphine (262 mg, 1 mmol) in 6 mL of THF is stirred under nitrogen at −0 °C. Diethyl azodicarboxylate (157 mL, 1 mmol) is added. After 15 min, benzoic acid (122 mg, 1 mmol) is added and 5 min later a 50-μL aliquot is dissolved in 1 mL of acetonitrile and analyzed by ESI-MS.

(2) Preparation of alkoxyphosphoranes 11: Triphenylphosphine (262 mg, 1 mmol) in 6 mL of THF is stirred under nitrogen at 0 °C. Diethyl azodicarboxylate (157 mL 1 mmol) is added. After 15 min 2 equiv of the corresponding alcohol are added. The reaction mixture is brought to room temperature. After 15 min it is cooled to 0 °C again and benzoic acid (122 mg, 1 mmol) is added and 5 min later a 50 μL aliquot is dissolved in 1 mL of acetonitrile and analyzed by ESI-MS.

The Staudinger Reaction (Figures 5a–d and 6). 2,4,6-Tribromophenyl azide was prepared by the procedure of Y. G. Gololobov and co-workers.[11] Staudinger reactions with 2,4,6-tribromophenyl azide were also carried out in a similar fashion to that described by Gololobov. In a typical procedure, a solution of 2,4,6-tribromophenyl azide (27 mg, 7.59 mmol) in 1 mL of anhydrous THF or diethyl ether under nitrogen is cooled to 0 °C. An equimolar amount of triphenylphosphine (or other phosphine) is added. The progress of the reaction is monitored by taking 25–50 μL aliquots, at different times, and quenching them with 1 mL of 1% TFA/methanol precooled to −78 °C and analyzing by ESI-MS within 5 min.

Acknowledgment. We thank Mr. Yunhui Wu for advise and assistance with the ESI-MS experiments. In addition, J.P. thanks NYU for a Kramer Fellowship and A.P. thanks NYU for a Sokol fellowship.

Supplementary Material Available: Figures 6–8, MS spectra of the Staudinger reactions (3 pages). Ordering information is given on any current masthead page.

(15) Allen, M. H.; Vestal, M. L. *J. Am. Soc. Mass Spectrom.* **1992**, *3*, 15.