Concerning the Regioselectivity of Gas Phase Reactions of Glycine with Electrophiles. The Cases of the Dimethylchlorinium Ion and the Methoxymethyl Cation[1]

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The gas phase reactions of glycine with the dimethylchlorinium ion and the methoxymethyl cation in the CI source of a tandem mass spectrometer have been investigated. The mechanisms of these reactions were determined by analyzing the unimolecular fragmentation reactions of the product ions. $(CH_3)_2Cl^+$ undergoes a nonregioselective S_N2 reaction, with methylation occurring at both nitrogen and oxygen. This is consistent with the relative methyl cation affinities (MCAs) of CH_3Cl (MCA (CH_3Cl) = 62.0 kcal mol^{-1}) and those calculated via *ab initio* calculations (at the MP2/6-31G*//HF/6-31G* level) for the three nucleophilic sites in glycine: MCA (H_2N group) = 116.0 kcal mol^{-1}, MCA (C=O group) = 90.8 kcal mol^{-1}, and MCA (HO group) = 80.6 kcal mol^{-1}. In contrast, the ambident electrophile $CH_3OCH_2^+$ reacts almost exclusively via a regiospecific pathway involving addition at nitrogen followed by elimination of CH_3OH to form a $[M + CH]^+$ ion with the structure $CH_2{=}NHCH_2CO_2H^+$. The experimental results are consistent with the *ab initio* calculated (at the MP2/6-31G*//HF/6-31G* level) relative stabilities of isomeric $[M + CH]^+$ ions.

Introduction

It has been recognized for some time that mutagenic and carcinogenic chemicals tend to be reactive electrophiles capable of modifying biological macromolecules such as DNA and proteins.[2,3] Despite progress in determining the sites of modification of DNA[4] and proteins and developing assays for carcinogens,[5] a full understanding of the mechanisms of modification of biomolecules by reactive electrophiles at the molecular level remains elusive. To this end a number of groups have carried out theoretical studies of model systems using molecular orbital calculations.[6] Since these studies are of isolated species (i.e. in the absence of solvent molecules), the best test of their validity is to carry out experimental studies in the gas phase.

Although mass spectrometry of biomolecules is now a well established discipline, much of the work on the fundamental reactivity of gas phase biomolecules has focused on their Brønsted acid–base properties[7–9] or their complexation with metal cations.[10] Fewer studies have thoroughly investigated the gas phase reactions of bio-

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molecules with other classes of electrophiles. A notable exception is the pioneering work of Cooks et al., who showed that tandem mass spectrometric techniques could be used to establish the site(s) of methylation in gas phase methylation reactions between dimethylhalonium ions and a range of aromatic compounds and heterocyclic compounds including the nucleosides guanine, cytosine, thymine, and adenine.[11] Another interesting observation, which has not been thoroughly investigated, is that amino acids form product ions due to the addition of CH_3-CH_2^+ under methane chemical ionization conditions.[12] More recently Freeman et al. have made the intriguing proposition that gas phase ion–molecule reactions might be used to detect biologically reactive contaminants in the environment.[13] In their work, they note a correlation between the results of Ames tests of the relative mutagenicity of a series of allylic compounds with the order of gas phase reactivity of mass selected radical cations of pyridine (used to model nucleophilic bases of DNA) toward the same series of allylic reagents.[13] In a similar fashion, Gross and co-workers have modeled the mutagenicity of polycyclic aromatic hydrocarbons (PAHs) by studying the gas phase reactivity of their radical cations with nitrogen nucleophiles.[14]

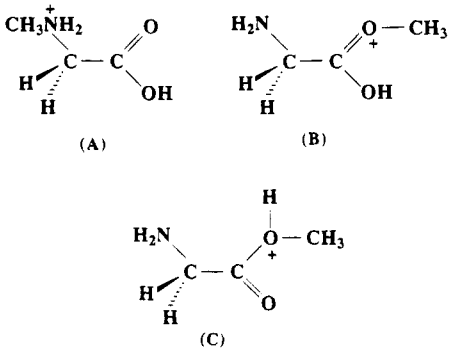
Our goal is to carry out studies on the intrinsic reactivity of biological molecules (such as amino acids, simple peptides, nucleosides, nucleotides, and related model compounds) by examining the fundamental aspects of their gas phase ion–molecule reactions with a wide range of electrophiles. This paper describes the gas phase reactions of glycine with the dimethylchlorinium ion $(CH_3)_2Cl^+$,[15] a methylating reagent (eq 1), and the methoxymethyl cation $CH_3OCH_2^+$, an ambident electrophile which can potentially react via either a S_N2 pathway (eq 2a) or an alternative pathway involving addition followed by elimination of CH_3OH (eq 2b).[16] We have

$$H_2NCH_2CO_2H + (CH_3)_2Cl^+ \longrightarrow [H_2NCH_2CO_2H + CH_3]^+ + CH_3Cl \quad (1)$$

$$H_2NCH_2CO_2H + CH_3OCH_2^+ \longrightarrow \begin{cases} [H_2NCH_2CO_2H + CH_3]^+ + CH_2O & (2a) \\ [H_2NCH_2CO_2H + CH]^+ + CH_3OH & (2b) \end{cases}$$

addressed the issue of the regioselectivity of electrophilic attack onto glycine by interrogating the structures of the $[M + CH_3]^+$ and $[M + CH]^+$ product ions formed in eqs 1 and 2 via tandem mass spectrometric techniques.[17] The key to this work is being able to relate the fragmentation reactions to the original structure of the ion. To gain a greater understanding of the mechanisms of these fragmentation reactions we have used isotopically labeled glycine ($H_2NCD_2CO_2H$ and $H_2{}^{15}NCH_2CO_2H$).

In theory there are three potential nucleophilic sites in glycine. For example, methylation of the nitrogen atom of the H_2N group yields structure **A**, while methylation at the oxygen atom of the C=O group and the oxygen atom of the OH group yields structures **B** and **C**, respectively. Another important issue involves the possible interconversion of the products **A**, **B**, and **C** via intramolecular methyl transfer.[18]



In the case of methylation, the thermodynamics is controlled by the relative methyl cation affinity (MCA) of the reactant and product Lewis bases. The gas phase MCA of a species X is defined in eq 3 and can either be (i) calculated from experimental heats of formations of ions available from other thermochemical measurements (such as proton affinity measurements),[19] (ii) experimentally measured from methyl cation transfer reactions,[20] or (iii) calculated using *ab initio* techniques.[20b] Using experimentally derived data, it is possible to predict that methylation at the nitrogen atom of the H_2N group of

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glycine (to give **A**) is exothermic by 48 kcal mol^{-1} and 31.7 kcal mol^{-1} for eqs 1 and 2a, respectively.[21] Unfortunately there is a dearth of thermochemical data on the heats of formation of other ions related to the gas phase reactions of electrophiles with glycine. Thus we have turned to *ab initio* methods to calculate the MCAs of the three nucleophilic sites of glycine, as well as the thermochemistry associated with the formation of the $[M + CH]^+$ ions (eq 2b).[22]

$$CH_3X^+ \xrightarrow{MCA\,(X)} CH_3^+ + X \quad (3)$$

$$MCA\,(X) = \Delta H^0_f(CH_3^+) + \Delta H^0_f(X) - \Delta H^0_f(CH_3X^+)$$

Computational Methods

Structures of ions and neutrals were optimized at the Hartree–Fock level using either the GAMESS[23] or GAUSSIAN 92[24] programs with the standard 6-31G* basis set.[25] All optimized structures were then subjected to frequency calculations with the same basis set, followed by a calculation of the correlated energy using the MP2(FC)/6-31G* level of theory (FC = frozen core). Energies are corrected for zero-point vibrations scaled by 0.9.[26] In each case, a set of possible rotamers was explored.[27] Transition states were located with the aid of analytical second-derivatives and are characterized by a single negative eigenvalue. In some cases, the intrinsic reaction coordinate (IRC)[28] was explored to insure that the transition state linked the proper minima. Complete structural details and lists of vibrational frequencies for each HF/6-31G* optimized structure and transition state can be found in the supplementary material.

Experimental Section

All experiments were performed on a Fisons/VG (Manchester, UK) Autospec-Q instrument of E1BE2qQ geometry (where E = electric sector, B = magnetic sector, q = RF only quadrupole and Q = quadrupole). MS/MS experiments were performed in the unimolecular MIKES mode, in which the ion of interest was mass selected using E1B, and the metastable fragments were determined by scanning E2. Methylated glycine ions, $[M + CH_3]^+$, were formed in the chemical ionization source using either CH_3Cl or $(CH_3)_2O$ as the CI gas. $[M + CH]^+$ ions of glycine were formed using $(CH_3)_2O$ as the CI gas. In each instance the CI plasma conditions were optimized for the ion of interest (i.e. $(CH_3)_2Cl^+$ or $CH_3OCH_2^+$) before introducing glycine through a heated direct insertion probe. Typical source conditions were source temperature = 250 °C; electron energy = 70 eV; emission current = 200 mA; source pressure was 6×10^{-5} mBarr, measured on the source ion gauge. All of the source CI mass spectra are listed in the supplementary material.

The major ions observed in the CH_3Cl CI plasma were CH_2Cl^+ (*m/z* 49/51), CH_3ClH^+ (*m/z* 51/53) and $(CH_3)_2Cl^+$ (*m/z* 65/67). In contrast, the ions CH_3^+ (*m/z* 15) and CH_3Cl^+ (*m/z* 50/52) were only minor components. The modes of formation of these ions have been described previously.[15e&h] The $(CH_3)_2O$ CI plasma yielded the following major ions: $CH_3OCH_2^+$ (*m/z* 45) and $(CH_3)_2OH^+$ (*m/z* 47) and to a lesser extent $(CH_3)_3O^+$ (*m/z* 61), $CH_3OCH_2^+(CH_3)_2O$ (*m/z* 91), and $((CH_3)_2O)_2H^+$ (*m/z* 93). The ions CH_3^+ (*m/z* 15); COH^+ (*m/z* 28), and CH_2OH^+ (*m/z* 31) were only minor components. Each of these ions have been observed previously under similar CI conditions.[16h]

All reagents were commercially available and were used without further purification. $H_2NCD_2CO_2H$ (98% D) and $H_2{}^{15}NCH_2CO_2H$ (99% ^{15}N) were both obtained from Cambridge Isotope Laboratories.

The immonium ions $CH_2{=}NHCH_2CO_2H^+$, $CH_2{=}{}^{15}NHCH_2CO_2H^+$, and $CH_2{=}NHCD_2CO_2H^+$ were formed by reacting the appropriate glycine isotopomer (≈1 mg) with 40 μL of a formaldehyde solution (37% in methanol) and 10 μL of formic acid (90%). An aliquot (1 μL) of the resultant reaction mixture was placed on a FAB probe tip and analyzed by FAB MS (glycerol matrix, cesium ion gun operating at 25 kV).

Results and Discussion

(A) *Ab Initio* Calculations on the Methylation of Glycine at Various Sites. In order to gain insights into the most likely site of attack of a methyl cation onto glycine, we have turned to *ab initio* calculations to calculate the structures and energies of glycine, CH_3^+ as well as the 3 different $[H_2NCH_2CO_2H + CH_3]^+$ isomers (A, B, and C) due to methylation of glycine at nitrogen, at the oxygen atom of the carbonyl group and at the oxygen atom of the hydroxyl group. The HF/6-31G* optimized structures of glycine and the 3 different $[H_2NCH_2CO_2H + CH_3]^+$ isomers are shown in Figure 1, while their relevant geometric parameters and vibrational frequencies are available in the supplementary material. The energies of glycine, CH_3^+ as well as the three different $[H_2NCH_2CO_2H + CH_3]^+$ isomers at various levels of theory are listed in Table 1.[27] Using the data in this table it is possible to calculate the MCA of the individual functional groups within the glycine molecule (at 0 K) via eq 4 where $E(CH_3^+)$ is the total

$$MCA(X) = E(CH_3^+) + E(X) - E(CH_3X^+) \quad (4)$$

energy of the methyl cation (corrected for zero point energy vibrations), $E(X)$ is the total energy of glycine (corrected for zero point energy vibrations), and $E(CH_3X^+)$ is the total energy of the methylated glycine species (corrected for zero point energy vibrations). The *ab initio* MCAs thus obtained are listed in Table 2.

Deakyne and Meot-Ner have shown that when using HF/6-31G* optimized geometries, the best agreement between experimental and *ab initio* MCAs are those calculated using MP2/6-31G* single point energies.[20b] In

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(27) An exhaustive search for all possible conformations of the each of the isomeric $[H_2NCH_2CO_2H + CH_3]^+$ ions is beyond the scope of this work. An approach aimed at identifying all possible gas phase conformations of the molecules alanine, serine, and cysteine via *ab initio* techniques has recently been described: Gronert, S.; O'Hair, R. A. J. *J. Am. Chem. Soc.* **1995**, *117*, 2071.

(28) Gonzales, C.; Schlegel, H. B. *J. Chem. Phys.* **1991**, *95*, 5853.

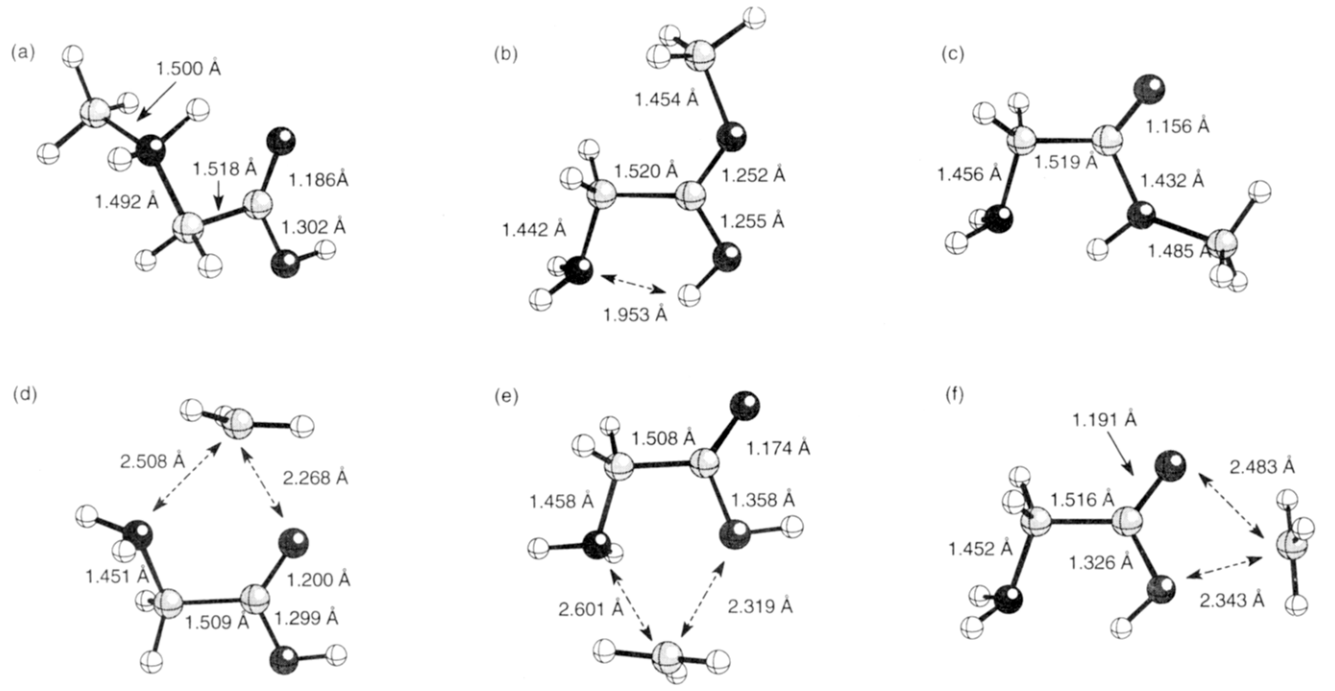


Figure 1. HF/6-31G* optimized structures of (a) N-methylated glycine; (b) CO-methylated glycine; (c) OH-methylated glycine; (d) transition state for CH_3^+ transfer between N and CO; (e) transition state for CH_3^+ transfer between N and OH; and (f) transition state for CH_3^+ transfer between HO and CO.

Table 1. *Ab Initio* Total Energies and Zero Point Energies of Species Related to the Methylation of Methylamine, Methanol, and Glycine

species	energies (Hartrees)[a]			relative energies (kcal/mol)[i]	
	HF/6-31G*	MP2(FC)/6-31G*	ZPE[b]	MP2	(HF)
CH_3^+	−39.23064	−39.32514	0.03034		
CH_3NH_2	−95.20983	−95.50583	0.06199		
$(CH_3)_2NH_2^+$	−134.61353	−135.03568	0.10422		
CH_3OH	−115.03542	−115.34493	0.04980		
$(CH_3)_2OH^+$	−154.38238	−154.81314	0.08972		
$H_2NCH_2CO_2H$	−282.83110	−283.59956	0.07800		
$CH_3NH_2CH_2CO_2H^+$ [c]	−322.22689	−323.12098	0.11982	0.0	(0.0)
$H_2NCH_2C(OCH_3)OH^+$ [d]	−322.19178	−323.07859	0.11755	25.2	(20.6)
$H_2NCH_2CO(HOCH_3)^+$ [e]	−322.16408	−323.06057	0.11576	35.4	(36.9)
TS1[f]	−322.12803	−323.00722	0.11385	67.6	(58.3)
TS2[g]	−322.10589	−322.98306	0.11300	82.3	(71.6)
TS3[h]	−322.11780	−322.99421	0.11225	74.8	(63.7)

[a] All calculations were carried out on the HF/6-31G* optimized geometries. [b] Scaled by 0.9. [c] See Figure 1a for geometry. [d] See Figure 1b for geometry. [e] See Figure 1c for geometry. [f] Transition state for CH_3^+ transfer from N to C=O. See Figure 1d for geometry. [g] Transition state for CH_3^+ transfer from N to HO. See Figure 1e for geometry. [h] Transition state for CH_3^+ transfer from HO to C=O. See Figure 1f for geometry. [i] Energies relative to N-methylated glycine. Zero point energies included. Hartree−Fock values given parenthetically.

Table 2. Calculated Methyl Cation Affinities (MCA) of Methylamine, Methanol, and the Various Nucleophilic Sites in Glycine

nucleophile	methyl cation affinity (MCA) in kcal mol^{-1} [a]		
	HF/6-31G*	MP2(FC)/6-31G*	expt[b]
CH_3NH_2	101.1	121.0	117.0[c]
CH_3OH	67.0	83.8	81.0[c]
nitrogen atom of NH_2 group[d]	96.4	116.0	110
oxygen atom of C=O group[e]	75.8	90.8	*g*
oxygen atom of OH group[f]	59.6	80.6	*g*

[a] All calculations were carried out with the energies given in Table 1 using eq 4 in the text. [b] Calculated from the experimental heats of formation (ref 19b) of N-protonated sarcosine (59 kcal mol^{-1}), CH_3^+ (262 kcal mol^{-1}) and glycine (−93 kcal mol^{-1}). [c] From ref 20c. [d] See Figure 1a for geometry. [e] See Figure 1b for geometry. [f] See Figure 1c for geometry. [g] Unknown.

order to gain further insight into the capabilities of this level of theory at reproducing experimentally derived MCAs, we have calculated the MCAs of methylamine and methanol, two species whose MCAs have recently undergone experimental reevaluation.[20c] There is reasonable agreement between the experimentally derived MCAs of CH_3NH_2 and CH_3OH and the MP2/6-31G* *ab initio* MCAs (Table 2). For glycine, the only experimental data available is for methylation at nitrogen, where there is reasonable agreement between the experimentally derived MCA (110 kcal mol^{-1}) and the MP2/6-31G* *ab initio* MCA (116.0 kcal mol^{-1}) (Table 2). After nitrogen, the *ab initio* calculations predict that the next favored site of methylation is the oxygen atom of the C=O group, with a MCA of 90.8 kcal mol^{-1} (at the MP2/6-31G* level, Table 2). The least favored site of methylation is predicted to be the oxygen atom of the OH group, with a MP2/6-31G* *ab initio* MCA of 80.6 kcal mol^{-1} (Table 2).

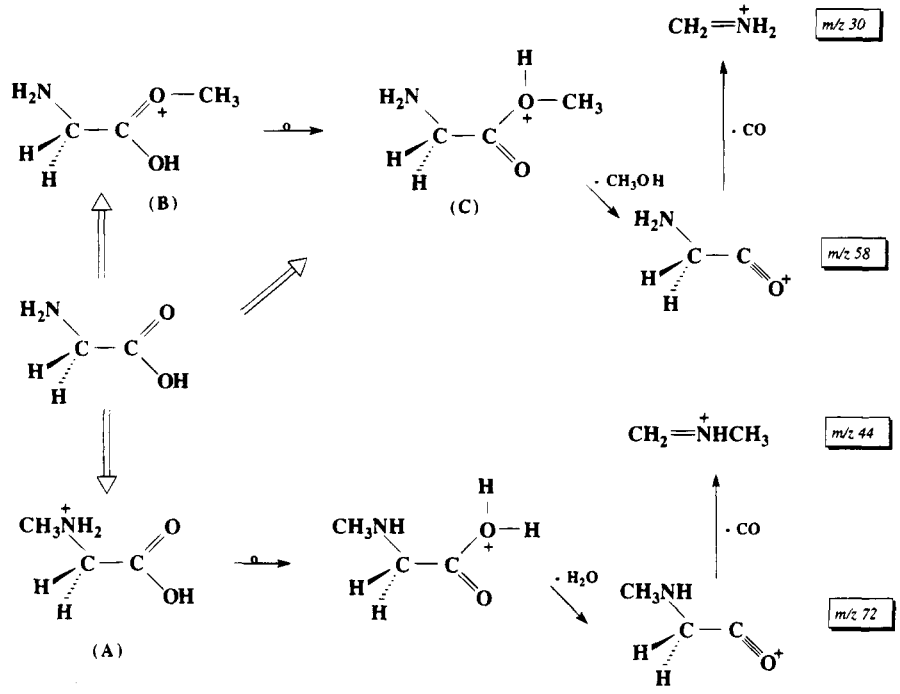
We next addressed the issue of intramolecular methyl transfer between **A**, **B** and **C** by determining the transition states to processes **A** → **B**, **A** → **C** and **B** → **C**. The transition state for each process is shown in Figure 1, while their relevant geometric parameters and vibrational frequencies are available in the supplementary material. It is clear from the geometries that the reactions are S_N1-like and that the transition states

Table 3. MS/MS Spectra of the $[M + CH_3]^+$ Ions of Glycine Formed in the CH_3Cl CI Plasma

precursor ion, *m/z*	unimolecular MIKE spectra [*m/z* (loss) abundance]
$[H_2NCH_2CO_2H + CH_3]^+$, 90	72 (H_2O) 1; 62 (CO) 77; 58 (CH_3OH) *, 44 (CH_2O_2) 100; 30 ($C_2H_4O_2$) 16; 28 ($C_2H_6O_2$) 4.
$[H_2{}^{15}NCH_2CO_2H + CH_3]^+$, 91	73 (H_2O) 7; 63 (CO) 38; 59 (CH_3OH) 1, 45 (CH_2O_2) 100; 31 ($C_2H_4O_2$) 16; 29 ($C_2H_6O_2$) 8.
$[H_2NCD_2CO_2H + CH_3]^+$, 92	74 (H_2O) *; 64 (CO) 60; 60 (CH_3OH) *, 46 (CH_2O_2) 100; 32 ($C_2H_4O_2$) 16; 30 ($C_2H_6O_2$) 6.

* Abundance of ion is less than 1% of the base peak.

Scheme 1



resemble ion–dipole complexes between glycine and the methyl cation. Intramolecular S_N2 reactions are not favorable in these systems because the cyclic transition states would be too small (four or five-membered rings) to support a trigonal-bipyramidal carbon with a linear X–C–Y linkage (X, Y = O or N).[18] As expected, the transition states are all very unstable with respect to the global minimum, **A** (from 67 to 82 kcal mol^{-1} less stable). The barriers from **C** to **B**, **B** to **A**, and **C** to **A** are 39.4, 42.5, and 46.9 kcal mol^{-1}, respectively; however, each of these transition states is ~15–30 kcal mol^{-1} *above* the energy of the separated reactants ($(CH_3)_2Cl^+$ and glycine). Therefore, once glycine is methylated under these conditions, rearrangements are highly unlikely.

(B) Experimental Studies of the Gas Phase Methylation of Glycine. On the basis of *ab initio* calculations described above, we decided to test the regioselectivity for methylation of glycine in the gas phase by comparing the reaction of glycine with a hard methylating reagent ($(CH_3)_2Cl^+$) versus a softer methylating reagent ($CH_3OCH_2^+$).[29] Abundant $[M + CH_3]^+$ ions of glycine were formed using CH_3Cl as the CI gas.[30] The unimolecular fragmentation reactions of these ions were studied in a MIKE experiment (Table 3). The base peak is due to the formation of the immonium ion $CH_2{=}NHCH_3^+$ via loss of the elements of CO and H_2O. The next largest peak is due to loss of CO. Another immonium ion ($CH_2{=}NH_2^+$), formed via the loss CO and CH_3OH, is the third largest peak in the spectrum. The losses of H_2O and CH_3OH and the formation of $[H_2,N,C]^+$ ions represent minor channels in comparison. The results from the isotopically labeled experiments using $H_2NCD_2CO_2H$, which allow the distinction between the methylene protons of glycine and the methyl protons derived from the methylating agent, indicate that the two immonium ions are formed from at least two different $[M + CH_3]^+$ precursor ions. The formation of $CD_2{=}NHCH_3^+$ is indicative of methylation on nitrogen (to yield structure **A**), while the formation of $CD_2{=}NH_2^+$ is indicative of methylation on oxygen (to yield either structure **B** and/or structure **C**. The mechanisms proposed for the formation of these immonium ions (Scheme 1) follow retro Koch reaction pathways[31] in which H_2O is lost from **A** and CH_3OH is lost from **C** to form the intermediate acylium ions, which then lose CO. Although Meot-Ner and Field have presented an alternative mechanism for the loss of the elements of $[CH_2O_2]$ from the $[M + H]^+$ ions of amino acids involving a concerted loss of formic acid,[32] we prefer the retro Koch reaction pathway, since this is entirely consistent with (i) the fact that the intermediate acylium ions are observed[33] and (ii) mechanisms previously proposed for the fragmentation reactions of simple amino acids and their esters.[12,34] To further substantiate this mechanism as well as ruling out the possibilities of intramolecular methyl transfer (which would isomerize structures **A**, **B** and **C**), we formed the $[M + H]^+$ ions of sarcosine ($CH_3NHCH_2CO_2H$)

(29) The possibilities of glycine undergoing methylation by other ions present in the CI plasmas cannot be excluded. We are currently building a flowing afterglow selected ion flow tube (FA-SIFT) to study the relative reactivities of each of these ions with a range of nucleophiles.

(30) Based upon the appropriate mass shifts using the various glycine isotopomers, the only other major ions observed in the CH_3Cl CI spectra were the $[M + H]^+$ ion of glycine, as well as the immonium ions $CH_2{=}NHCH_3^+$ and $CH_2{=}NH_2^+$.

(31) For gas phase studies of the retro Koch reaction of protonated carboxylic acids see: (a) Davidson, W. R.; Meza-Hojer, S.; Kebarle, P. *Can. J. Chem.* **1979**, *57*, 3205. (b) Davidson, W. R.; Lau, Y. K.; Kebarle, P. *Can. J. Chem.* **1978**, *56*, 1016. (c) Harrison, A. G. *Can. J. Chem.* **1979**, *57*, 3205.

(32) Meot-Ner, M.; Field, F. H. *J. Am. Chem. Soc.* **1973**, *95*, 7207.

and glycine methyl ester ($H_2NCH_2CO_2CH_3$).[35] Under unimolecular MIKE conditions, the $[M + H]^+$ ion of sarcosine loses H_2O, CO, as well as (CO + H_2O) while the $[M + H]^+$ ion of glycine methyl ester loses CH_3OH, CO, as well as (CO + CH_3OH). These results support our proposed mechanism (Scheme 1) and indicate that intramolecular methyl transfer is negligible under our experimental conditions.

In order to determine whether a softer methylating reagent would exhibit more selectivity, we studied the reaction of glycine with $CH_3OCH_2^+$. The major glycine CI ions formed under our source conditions were $[M + H]^+$ (base peak) and $[M + CH]^+$ (typically 50–65% of the base peak), while the yield of the $[M + CH_3]^+$ ion was less than 5% of the base peak. These results indicate that the methoxymethyl cation favors the addition/elimination pathway (eq 2b) over the S_N2 pathway (eq 2a), consistent with previous studies on the reactions of simple amines with $CH_3OCH_2^+$.[16a,c] For example the rate of reaction of H_3N with $CH_3OCH_2^+$ to form the addition/elimination product (eq 5b) is nearly 4 times faster than the S_N2 reaction (eq 5a), despite the S_N2 pathway being favored thermochemically.[16c] Both the $[M + CH]^+$ and $[M + CH_3]^+$ ions of glycine were subjected to MS/MS studies. The results on the structures of $[M + CH_3]^+$ ions are inconclusive since they gave such weak MIKE spectra.[36] In contrast, the $[M + CH]^+$ ions gave abundant fragment ions which were structurally diagnostic. The structures of the $[M + CH]^+$ are described in detail below.

$$H_3N + CH_3OCH_2^+ \longrightarrow \begin{cases} CH_3NH_3^+ + CH_2O & (\Delta H = -26 \text{ kcal mol}^{-1}) \quad (5a) \\ CH_2{=}NH_2^+ + CH_3OH & (\Delta H = -16 \text{ kcal mol}^{-1}) \quad (5b) \end{cases}$$

(C) *Ab Initio* and Experimental Studies on the Structures of the $[M + CH]^+$ Ions of Glycine. The key question to be addressed here is what is the structure of the $[M + CH]^+$ ion formed in reaction 2b and can we relate its structure to the original site of attack by $CH_3OCH_2^+$ onto glycine? As a reference point, it is worth summarizing some of the known gas phase ion–molecule reactions of the methoxymethyl cation with organic molecules.[16] The reactions of $CH_3OCH_2^+$ with amines (e.g. eq 5),[16a,c] and the oxygen nucleophiles $(CH_3)_2O$ (eq 6),[16b,i] $(CH_3)_2CO$ (eq 7)[16g,i] and CH_3CO_2H (eq 8)[16e] have all been examined in some detail. More importantly, the reactions of $CH_3OCH_2^+$ with compounds containing 2 nucleophilic sites (eq 9) were first investigated by Caserio and Kim[16d] and more recently by Eichmann and Brodbelt.[16f] The key points are that all primary and

$$(CH_3)_2O + CH_3OCH_2^+ \longrightarrow (CH_3)_3O^+ + CH_2O \quad (6)$$

$$(CH_3)_2C{=}O + CH_3OCH_2^+ \longrightarrow (CH_3)_2COCH_3^+ + CH_2O \quad (7)$$

$$CH_3CO_2H + CH_3OCH_2^+ \longrightarrow (CH_3CO_2CH_3 + H)^+ + CH_2O \quad (8)$$

$$X(CH_2)_nY + CH_3OCH_2^+ \longrightarrow (X(CH_2)_nY + CH)^+ + CH_3OH \quad (9)$$

secondary amines undergo addition followed by elimination of CH_3OH (c.f. eq 5b), while most oxygen nucleophiles undergo methylation instead (eqs 6–8). Bifunctional compounds of the type $X(CH_2)_nY$ (where n = 2–4 and X = CH_3O, CH_3NH, $(CH_3)_2N$, and CH_3S, and Y = HO, H_2N, and HS) exhibit special reactivity with the addition/elimination channel often dominating (eq 9). Caserio and Kim were the first to suggest that certain of the bifunctional $[M + CH]^+$ ions may gain extra stability by adopting either cyclic conformations or cyclic covalent structures.[16d] They were also the first to note that the loss of CH_2O from $[M + CH]^+$ ions where X = CH_3O or CH_3S and Y = HO is indicative of attachment of $CH_3OCH_2^+$ onto the HO site. Eichmann and Brodbelt studied the fragmentation reactions of the $[M + CH]^+$ ions derived from the reaction of $CH_3OCH_2^+$ with the amino alcohols $H_2NCH_2CH_2OH$, $CH_3NHCH_2CH_2OH$, and $(CH_3)_2NCH_2CH_2OH$. They note that the loss of CH_2O, indicative of attachment of $CH_3OCH_2^+$ onto the HO site, changes from being a minor channel for $H_2NCH_2CH_2OH$ to being the dominant channel for $(CH_3)_2NCH_2CH_2OH$.[16f] They also note that the fact that tertiary amines and ethers fail to give $[M + CH]^+$ ions on reaction with $CH_3OCH_2^+$ emphasizes the need for a H atom to be bonded to the heteroatom to which $CH_3OCH_2^+$ initially attaches.

Based upon the previous studies described above, possible scenarios for the reaction of glycine with the methoxymethyl cation to yield the observed $[M + CH]^+$ ions are outlined in Scheme 2. Attack by $CH_3OCH_2^+$ onto the nitrogen atom of the H_2N group would yield the energized adduct **D**, which could decompose via a four centered transition state to the immonium ion **G**. Such a pathway is consistent with the requirement that a H atom be attached to N as well as with *ab initio* calculations on the mechanism of reaction 5b.[16c] Methoxymethylation at the oxygen atom of the C=O group and the oxygen atom of the OH group would yield the adducts **E** and **F** respectively. **F** could lose CH_3OH to form **H** in a similar fashion to the formation of **G** from **D**. Although the formation of **H** from **E** does not meet the requirement of a H atom being present on the heteroatom to which $CH_3OCH_2^+$ is initially attached, a plausible six-centered transition state has been proposed in Scheme 2. We have also considered the possibilities that once the $[M + CH]^+$ ions are formed, they may undergo the cyclization reactions shown in Scheme 3. These species **I**–**K**, which are the various protonated forms of the parent ring structure 5-oxazolidinone, have received little attention. For example it is not known whether **I**–**K** are stable with respect to ring opening, as is the case for γ-butyrolactone.[37]

In order to gain insights into the energetics of the processes shown in Schemes 2 and 3, we have performed *ab initio* calculations on the species **G**–**K**. The results are summarized in Figure 2 and Table 4, while their relevant geometric parameters and vibrational frequencies are available in the supplementary material. The first noteworthy aspect, is that the *ab initio* results

(33) The acylium ions are never major ions in the MS/MS spectra of simple aliphatic amino acids. A simple explanation is that these acylium ions are not very stable species. For example *ab initio* calculations (J. A. R. Schmidt and R. A. J. O'Hair, unpublished results, 1994) at the MP2(FC)/6-31G*//HF/6-31G* level of theory indicate that the covalently bound form of the acylium ion $H_2NCH_2CO^+$ can pass over a small barrier (≈ 0.4 kcal mol^{-1}) to form a *more stable* (by about 16.8 kcal mol^{-1}) ion molecule complex between $H_2NCH_2^+$ and CO. Overall the fragmentation of the covalently bound form of the acylium ion $H_2NCH_2CO^+$ to produce $H_2NCH_2^+$ and CO is predicted to be exothermic (by about 11.7 kcal mol^{-1}).

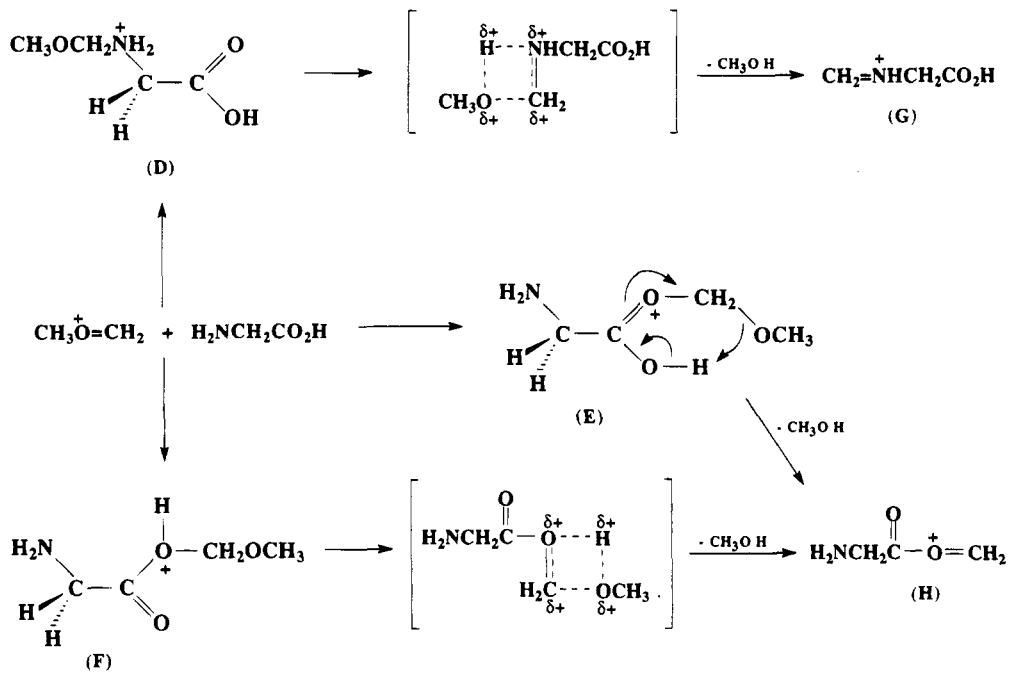
(34) For a selection of papers on the fragmentation reactions of protonated amino acids see: (a) Kulik, W.; Heerma, W. *Biomed. Mass Spectrom.* **1988**, *15*, 419. (b) Bouchonnet, S.; Denhez, J.-P.; Hoppilliard, Y.; Mauriac, C. *Anal. Chem.* **1992**, *64*, 753.

(35) The $[M + H]^+$ ions used as the standards were formed via FAB (cesium ion gun operating at 25 kV) by placing either sarcosine or glycine methyl ester hydrochloride salt on the FAB probe tip and using a glycerol matrix.

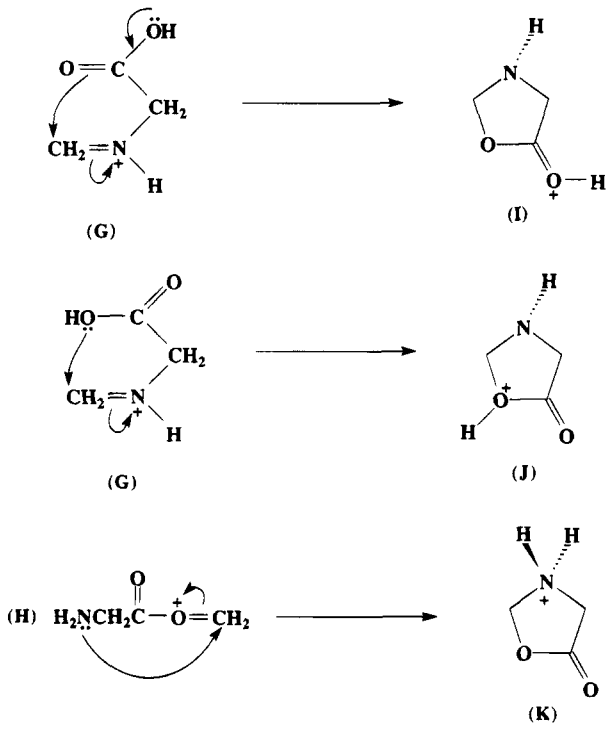
(36) This problem was further exacerbated by the fact that the $[M + CH_3]^+$ ions overlapped with background $(CH_3)_2O$ plasma ions of the same mass.

(37) Bordejé, M. C.; Mó, O.; Yáñez, M.; Herreros, M.; Abboud, J.-L. M. *J. Am. Chem. Soc.* **1993**, *115*, 7389.

Scheme 2



Scheme 3



predict that the formation of **G** from $CH_3OCH_2^+$ + glycine (scheme 2) is *exothermic* by 26.9 kcal mol^{-1} (at the MP2/6-31G* level).[38] All attempts to optimize **H** at the HF/6-31G* level of theory, led to the formation of an ion–molecule complex **H'**, formally between the acylium ion ($H_2NCH_2CO^+$) and formaldehyde.[39] Overall, the formation of **H'** from $CH_3OCH_2^+$ and glycine is *endothermic* by 10.0 kcal mol^{-1} (at the MP2/6-31G* level). Two of the cyclic structures are stable species, the formation of **I** from $CH_3OCH_2^+$ and glycine being *exothermic* by 7.4 kcal mol^{-1}, while the formation of **K** is *exothermic* by 17.9 kcal mol^{-1}. In contrast, all attempts to optimize the ring structure **J**, resulted in ring opening to form a slightly higher energy (+5.7 kcal mol^{-1}) conformer of **G**. These results clearly favor the formation of **G**.

From the labeling experiments (Table 5), it is possible to unambiguously assign the losses observed in the metastable spectra of the source formed $[M + CH]^+$ ions. These ions fragment via the losses of H_2O, CO, CO_2, and (H_2O + CO). The ion $[H_2,C,N]^+$ is also observed. How can we use these losses to determine the structure of the $[M + CH]^+$ ion? Firstly, we note the similarities in connectivity between the possible structures of the $[M + CH]^+$ ions **G** and **H** and those discussed in detail for the structures of $[M + CH_3]^+$ ions of glycine **A** and **C**. Using this analogy, we would expect **G** to lose H_2O and (H_2O + CO), and **H** to lose CH_2O and (CH_2O + CO). The results of the second approach, which involves independently synthesizing **G** and **H** and studying their MS/MS spectra, are described in detail below.

The immonium ion $CH_2{=}NHCH_2CO_2H^+$ (**G**) was independently synthesized by allowing glycine to react with formaldehyde in solution (under acid-catalyzed conditions) and then analyzing the reaction mixture by FAB MS/MS. The resultant metastable spectra of the various isotopomers are listed in Table 5. We propose the mechanisms outlined in Scheme 4 to explain the four major fragmentation reactions of the immonium ion **G**. Path A (Scheme 4) involves a concerted loss of CO. Similar types of mechanisms have been proposed to explain the EI fragmentation reactions of amino acid derivatives (eq 10) and trimethylsilyl esters of amino acids.[40] The loss of CO_2 is best explained via a six centered transition state involving a hydride transfer

(38) As a check for how well the MP2(FC)/6-31G*//HF/6-31G* level of theory can predict the energetics of these reactions, we have calculated the enthalpy of reaction 5b at the same level of theory. The *ab initio* value of −17.9 kcal mol^{-1} reproduces the experimental value[19b] of −16.0 kcal mol^{-1} quite well.

(39) In order to probe the potential energy surface connecting **H** and **H'**, we have carried out calculations by fixing the C–O bond and allowing all other variables to optimize under C_s symmetry at the MP2/6-31G*//HF/6-31G* and HF/6-31G*//HF/6-31G* levels of theory. Although there is a shoulder at the HF/6-31G* level, both levels indicate that **H** decomposes to **H'** without activation. The MP2 surface suggests a somewhat shorter separation (≈2 Å), but the potential energy surface is very shallow.

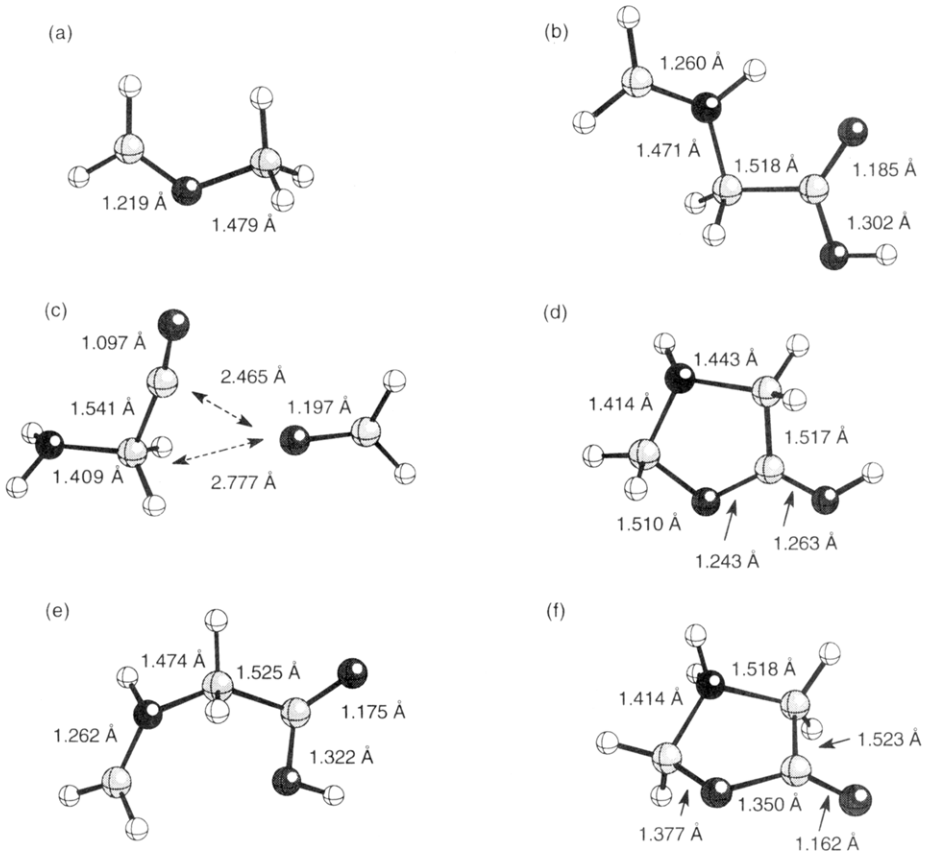


Figure 2. HF/6-31G* optimized structures of (a) $CH_3OCH_2^+$, (b) $CH_2{=}NHCH_2CO_2H^+$, (c) $H_2NCH_2CO^+{\cdots}O{=}CH_2$ ion−molecule complex, (d) CO-protonated 5-oxazolidinone, (e) ring-opened O-protonated 5-oxazolidinone, and (f) N-protonated 5-oxazolidinone.

Table 4. Energies Associated with the Formation of $[M + CH]^+$ Ions in the Reaction of $H_2NCH_2CO_2H$ and $CH_3OCH_2^+$ (Schemes 2 and 3)

species	energies (Hartrees)[a]		
	HF/6-31G*	MP2(FC)/6-31G*	ZPE[b]
$CH_3OCH_2^+$ [c]	−153.20403	−153.61873	0.06675
$CH_2{=}NHCH_2CO_2H^+$ [d]	−321.03995	−321.91669	0.09547
$H_2NCH_2CO^+{\cdots}O{=}CH_2$ [e]	−320.97972	−321.85134	0.08895
CO-protonated 5-oxazolidinone[f]	−321.01153	−321.88764	0.09745
$CH_2{=}NHCH_2CO_2H^+$ [g]	−321.03046	−321.90760	0.09544
N-protonated 5-oxazolidinone[h]	−321.02228	−321.90621	0.09916

[a] All calculations were carried out on the HF/6-31G* optimized geometries. [b] Scaled by 0.9. [c] See Figure 2a for geometry. [d] See Figure 2b for geometry. [e] See Figure 2c for geometry. [f] See Figure 2d for geometry. [g] Ring opened O-protonated 5-oxazolidinone. See Figure 2e for geometry. [h] See Figure 2f for geometry.

mechanism (Path B, Scheme 4), which is analogous to that proposed to explain the loss of CD_2O from CD_3OCH_2-$CH_2NH{=}CH_2^+$.[16d] Finally the loss of H_2O and (H_2O + CO) (Path C, Scheme 4) are similar to the retro Koch reactions described above for the $[M + CH_3]^+$ ions of glycine.

$$[XCH_2N(CH_3)CH(R)CO_2CH_3]^{\ddagger} \xrightarrow{-X} CH_2{=}\overset{+}{N}(CH_3)CH(R)CO_2CH_3$$

$$\downarrow -CO$$

$$CH_3OCH_2(CH_3)\overset{+}{N}{=}CH(R) \quad (10)$$

(X = CH_3O, $(CH_3)_3CS$, C_6H_5S; R = H, $CH_2CO_2CH_3$)

We also attempted to independently synthesize the ion **H** via electron impact on an ester derivative of glycine with a leaving group β to the ester oxygen (eq 11). The system studied, glycine β-phenethyl ester, failed to give an ion of *m/z* 88 with sufficient intensity for MS/MS studies.[41]

$$H_2NCH_2CO_2CH_2X \xrightarrow{EI} H_2NCH_2C(O)\overset{+}{O}{=}CH_2 + X \quad (11)$$

(X = $C_6H_5CH_2$)

On the basis of the fragmentation reactions of independently synthesized **G** together with the complete absence of losses of CH_2O and (CH_2O + CO), we conclude that the $[M + CH]^+$ ions sampled from the source reaction of $CH_3OCH_2^+$ and glycine only have structure **G**, with no contributions from structures **H** or **H′**.[42,43] Thus CH_3-OCH_2^+ undergoes a regiospecific attack at the nitrogen atom of glycine in the gas phase.

How do our gas phase results compare with solution data? Although the dimethylchlorinium ion[44] and the

(40) (a) Kostyanovsky, R. G.; Voznesensky, V. N.; Kadorkina, G. K.; El"natanov, Y. I. *Org. Mass Spectrom.* **1980**, *15*, 412. (b) Lawson, A. M.; Ramsden, D. B.; Raw, P. J.; Hoffenberg, R. *Biomed. Mass Spectrom.* **1974**, *1*, 374.

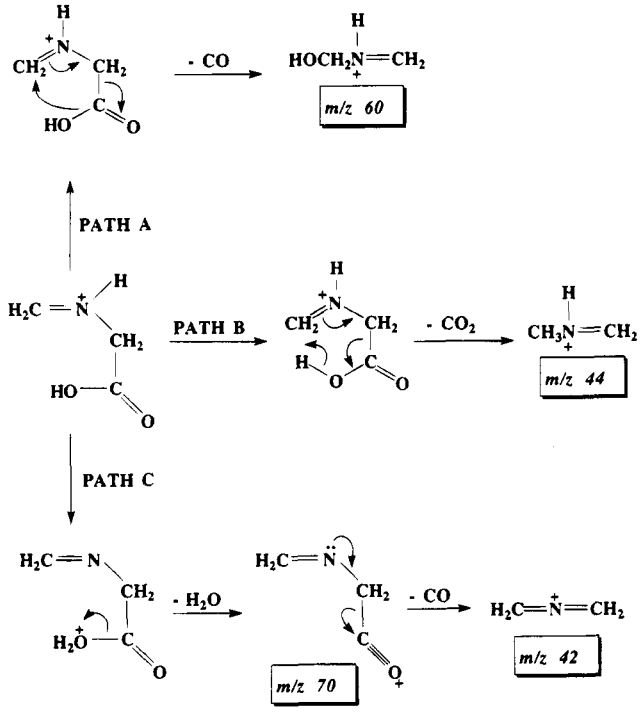
(41) Glycine β-phenethyl ester was synthesized according to a literature procedure: Taylor-Papadimitriou, J.; Yovanidis, C.; Paganou, A.; Zervas, L. *J. Chem. Soc. (C)* **1967**, 1830. The 70 eV mass spectrum of glycine β-phenethyl ester is listed in the supplementary material.

Table 5. MS/MS MIKE Spectra of the [M + CH]$^+$ Ions of Glycine

precursor ion, *m/z*	unimolecular MIKE spectra [*m/z* (loss) abundance]
$[H_2NCH_2CO_2H + CH]^+$, 88[a]	70 (H_2O) 26; 60 (CO) 100; 44 (CO_2) 34; 42 (CH_2O_2) 53; 28 ($C_2H_4O_2$) 16.
$[H_2{}^{15}NCH_2CO_2H + CH]^+$, 89[a]	71 (H_2O) 20; 61 (CO) 100; 45 (CO_2) 25; 43 (CH_2O_2) 30; 29 ($C_2H_4O_2$) 9.
$[H_2NCD_2CO_2H + CH]^+$, 90[a]	72 (H_2O) 14; 62 (CO) 100; 46 (CO_2) 14; 44 (CH_2O_2) 19; 28 ($C_2H_2D_2O_2$) 5.
$[CH_2{=}NHCH_2CO_2H]^+$, 88[b]	70 (H_2O) 38; 60 (CO) 100; 44 (CO_2) 6; 42 (CH_2O_2) 6; 28 ($C_2H_4O_2$) 1.
$[CH_2{=}{}^{15}NHCH_2CO_2H]^+$, 89[b]	71 (H_2O) 26; 61 (CO) 100; 45 (CO_2) 8; 43 (CH_2O_2) 9; 29 ($C_2H_4O_2$) 2.
$[CH_2{=}NHCD_2CO_2H]^+$, 90[b]	72 (H_2O) 25; 62 (CO) 100; 46 (CO_2) 4; 44 (CH_2O_2) 6; 28 ($C_2H_2D_2O_2$) 1.

[a] Formed in the gas phase $(CH_3)_2O$ CI plasma reaction between glycine and $CH_3OCH_2^+$. [b] FAB analysis of an aliquot of the acid-catalyzed condensed phase reaction of glycine and formaldehyde.

Scheme 4



methoxymethyl cation[45] have been generated in solution, their reactivity with simple amino acids does not seem to have been probed.[46] A further difficulty in comparing results of the two phases is that in aqueous solutions the zwitterion form of unprotected amino acids predominates. Of most relevance to this discussion is the work of Hughes et al., who studied the alkylation reactions of unprotected amino acids in nonaqueous solvents under a range of conditions.[47] They note that despite the zwitterionic form of the amino acid predominating in polar aprotic solvents, N-alkylation was competitive with O-alkylation. Furthermore the ratio of O-alkylation to N-alkylation was improved by moving to harder alkylating agents, or by "salting out" the zwitterion with LiBr. Thus, in a qualitative sense, the change in regioselectivity of attack by electrophiles onto an amino acid on moving from soft to hard alkylating agents is mirrored in both the gas and nonaqueous solution phases.

Conclusions

The lack of regioselectivity in the S_N2 reactions of $(CH_3)_2Cl^+$ with glycine and many other organic molecules[15] indicates that it is a hard Lewis acid in the gas phase. We predict that methyl cation donors with a MCA less than that of CH_3Cl should also display a lack of regioselectivity in their gas phase methylation reactions with biomolecules. A species which we expect to fall into this category is the methyldiazonium ion, an electrophile implicated in the condensed phase methylation of DNA. In contrast, the ambident electrophile $CH_3OCH_2^+$ exhibits regiospecific attack at nitrogen with subsequent loss of methanol to form the immonium ion $CH_2{=}NHCH_2{-}CO_2H^+$. The results presented here illustrate the importance of using isotopically labeled glycine in establishing the fragmentation mechanisms of the product ions. Studies are currently underway to determine (i) the reactivity of other amino acids and DNA model compounds with a range of electrophiles and (ii) kinetics of reactions between electrophilic species and neutral nucleophiles.

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Supplementary Material Available: Listing of source CI mass spectra, 70 eV EI mass spectrum of glycine β-phenethyl ester, and optimized geometries in the form of Cartesian coordinates and vibrational frequencies of all structures at the HF/6-31G* level (18 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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(42) We cannot totally exclude the possibility that the reason why we are not sampling ions of structure **H** or **H'** in our MIKE experiments is that these ions are so unstable that they *completely* decompose in the source prior to mass selection. Although very minor traces of the immonium ions $CH_2{=}NHCH_3^+$ and $CH_2{=}NH_2^+$ are observed in the CH_3Cl CI spectra (these underwent appropriate mass shifts using the various glycine isotopomers), their origins remain unclear. Further arguments against the *completely* source decomposing ions of structure **H** of **H'** stem from previous studies of metastable weakly bound ion–molecule complexes, which *have* been observed under similar experimental conditions: Heerma, W.; Kulik, W.; Burgers, P. C.; Terlouw, J. K. *Int. J. Mass Spectrom. Ion Proc.* **1988**, *84*, R1.

(43) We concur with a reviewer's suggestion that we cannot totally rule out the possibility that part of the [M + CH]$^+$ ions sampled from the source reaction of $CH_3OCH_2^+$ and glycine may have a cyclic structure such as **K**. Unfortunately MS/MS studies on protonated 5-oxazolidinone were not possible, since there is no reported literature synthesis of the parent 5-oxazolidinone.

(44) Olah, G. A. *Halonium Ions*; Wiley: New York, 1975.

(45) Olah, G. A., Svoboda, J. J. *Synthesis* **1973**, 52.

(46) Olah, G. A. private communication, 1994.

(47) Hughes, D. L.; Bergan, J. J.; Grabowski, E. J. J. *J. Org. Chem.* **1986**, *51*, 2579.