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Ab initio insights into amide bond cleavage reactions of formamide with substituted methyl cations XCH_2^+ (X = OH, F, and Cl)

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Abstract

The gas phase reactions of the substituted methyl cations, XCH_2^+ (X = F, HO, and Cl), with formamide were investigated using ab initio calculations. Addition of the carbocations at the nitrogen atom of the amide bond yields "N adducts" that are less stable than the "O adducts" arising from attack at the carbonyl oxygen atoms. The subsequent fragmentation reactions of these adducts were examined including: (1) fragmentation of the "O adduct" by elimination of HX via a transition structure involving 1, 6 H^+ transfer, resulting in the formation of $HC(OCH_2)NH^+$; (2) fragmentation of the "N adduct" by elimination of HX via a transition structure involving 1, 3 H^+ transfer resulting in the formation of $HC(O)NHCH_2^+$; (3) fragmentation of the "N adduct" via a transition structure involving 1, 3 $HC(O)^+$ transfer resulting in the formation of $HC(O)XCH_2NH_2^+$, which decomposes to yield HC(O)X and $CH_2NH_2^+$ (when X = OH and Cl); (4) fragmentation of the "N adduct" via extrusion of CO (when X = F and Cl). (Int J Mass Spectrom 195/196 (2000) 303–317) © 2000 Elsevier Science B.V.

Keywords: Amide bond cleavage; Substituted methyl cations; Ab initio calculations; Formamide

1. Introduction

There has been considerable recent interest in examining the gas phase ion–molecule reactions of biomolecules as a means of gaining structural information that is complementary to that obtained via other mass spectrometry based experiments (such as collision-induced dissociation (CID) of $[M + H]^+$ ions) [1]. To date, the most commonly examined ion–molecule reactions have been proton transfer reactions and hydrogen deuterium (H/D) exchange reactions of protonated peptides [2]. A few other types of reactions have been reported: McLuckey has examined the addition of HI molecules to protonated peptides and proteins [3], whereas Nibbering has shown that protonated peptides can react with $CH_3C(O)CH_2CH_2C(O)CH_3$ with explusion of two water molecules to give pyrroles via a Paal–Knorr type of reaction [4]. As part of our ongoing research into the gas phase electrophilic modification of biomolecules and model compounds [5,6], we became

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Part 20 of the series "Gas Phase Ion Chemistry of Biomolecules."

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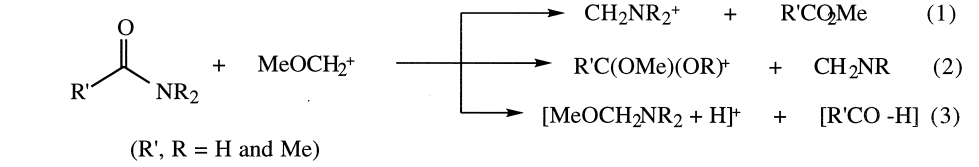
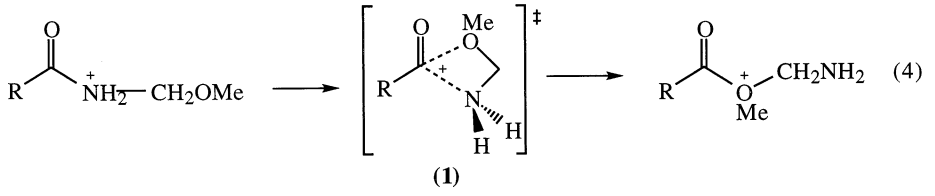
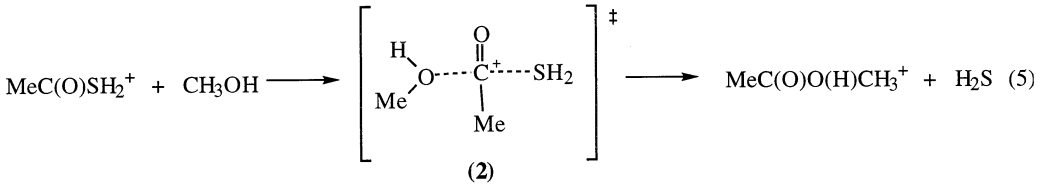
interested in designing reagents to sequence biomolecules via specific *gas phase* ion–molecule reactions. Thus for peptides, our aim is to develop site specific ion–molecule reactions to cleave the peptide bonds. During a recent flowing afterglow study on the rates of ion–molecule reactions of the methoxymethyl cation [7], we became encouraged by the rediscovery of a reaction [8] that leads to products arising from amide bond cleavage [Eqs. (1)–(3)].

In order to examine whether these reactions also occur for peptides, we then probed the behaviour of various peptides and model systems under chemical ionization—tandem mass spectrometry (CI/MS/MS) conditions [9]. We found that free peptides do not undergo peptide bond cleavage because of the high reactivity of the N-terminal amino group [10]. The gas phase reactivity of the N-terminal amino group can, however, be moderated via N acetylation, thereby yielding product ions in the MS/MS spectra of the $[M + CH_3OCH_2]^+$ ions that are due to peptide bond cleavage. A plausible mechanism for these amide bond cleavage reactions involves initial attack at the amide nitrogen followed by a 1,3 intramolecular acyl transfer reaction as shown in Eq. (4) [7,8]. Note that this corresponds to a nucleophilic acyl substitution where the nucleophile and the nucleofuge are parts of the same molecule and are thus forced to be on the same face of the migrating acyl cation. Such reactions are not without precedence: (1) the analogous gas phase intermolecular acyl transfer has been well documented [Eq. (5)] [11]; (2) related intramolecular acyl transfer reactions are believed to play an important role in organic chemistry [12a,b] and biology [12c] (although they may involve the formation of cyclic tetrahedral intermediates). Furthermore, these reactions are of fundamental interest from a mechanistic aspect, especially with regard to the nature of acylation [13], acid catalyzed H/D exchange in amides, amide isomerization, and amide hydrolysis [13]. In particular, are species such as (**1**) and (**2**) in Eqs. (4) and (5) transition structures on the reaction coordinate or are they intermediate ion–molecule complexes?



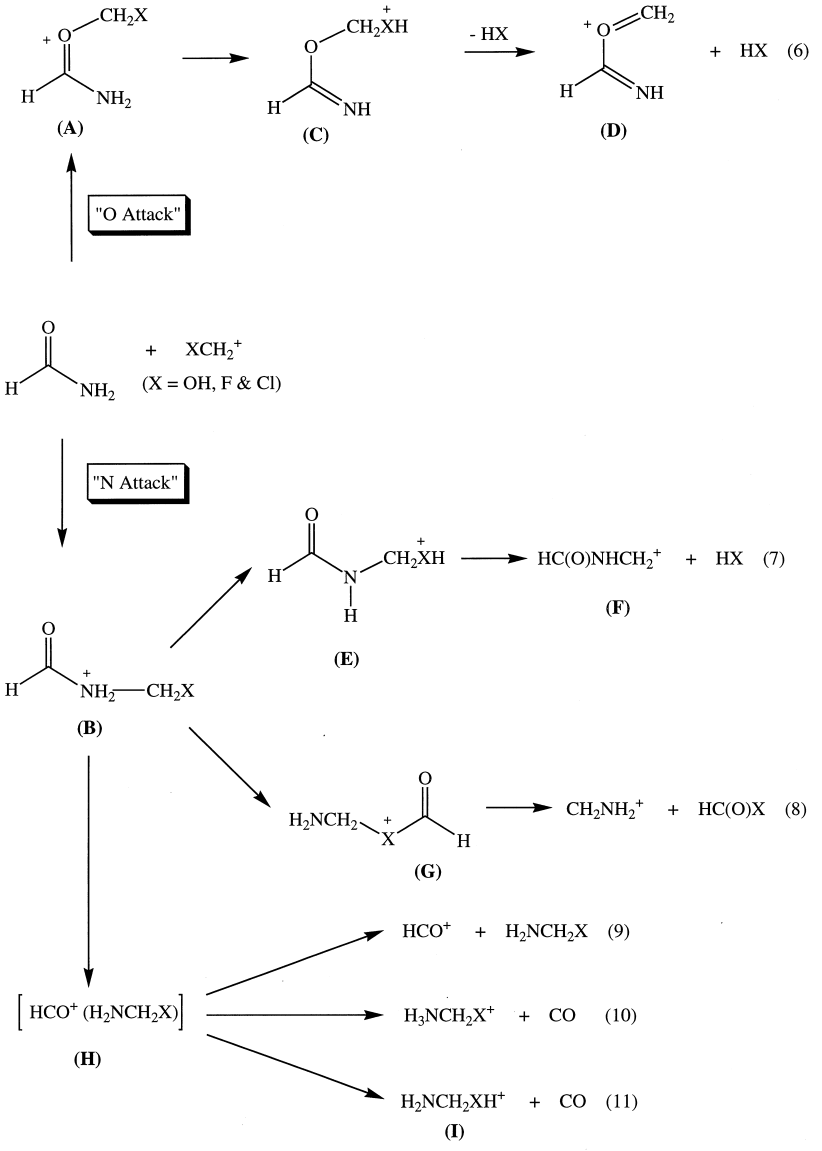




In order to gain further insights into the mechanisms of these reactions, especially in regard to evaluating the use of other reagent ions that might more efficiently cleave peptide bonds, we herein examine the modes of reactivity of formamide with various substituted methyl cations XCH_2^+ (X = F, HO, and Cl) [14]. The main types of reactions that we have examined are shown in Scheme 1 [15].

2. Computational methods

Structures of minima and transition structures were initially optimized at the Hartree–Fock level using the GAMESS programme with the standard 6-31G* basis set [16,17], and then reoptimized at the MP2(fc)/6-31G* level of theory using GAUSSIAN 94 [18]. Vibrational frequencies were calculated whenever geometry opti-



Scheme 1.

Table 1
Ab initio energies for the reactions of $H(C)ONH_2$ with XCH_2^+ at the MP2(fc)/6-31G* level of theory

	X = OH[b]		X = F[b]		X = Cl[b]	
Species[a]	MP2/6-31G*	ZPE[c]	MP2/6-31G*	ZPE[c]	MP2/6-31G*	ZPE[c]
$HC(O)NH_2$	−169.39446	0.04898	—	—	—	—
XCH_2^+	−114.44463	0.04382	−138.36897	0.02930	−498.39395	0.02720
O Adduct (**A1**)	−283.92538	0.10081	−307.90046	0.08762	−667.90355	0.08537
O Adduct (**A2**)	−283.92536	0.10142	−307.88593	0.08754	−667.90080	0.08596
O Adduct (**A3**)	—	—	−307.89827	0.08815	—	—
N Adduct (**B1**)	−283.90140	0.10067	−307.87555	0.08737	−667.88546	0.08546
N Adduct (**B2**)	−283.90025	0.10024	−307.87555	0.08737	−667.88484	0.08541
N Adduct (**B3**)	−283.90025	0.10025	—	—	—	—
TS [Eq. (6)]	—	—	−307.82388	0.08303	−667.82325	0.07910
(**C**) [Eq. (6)]	—	—	−307.82466	0.07903	−667.82527	0.07613
HX	−76.19685	0.02298	−100.18217	0.00993	−460.19236	0.00726
(**D**) [Eq. (6)]	−207.62225	0.06721	—	—	—	—
syn TS [Eq. (7)]	−283.85659	0.09491	−307.81206	0.08070	−667.81457	0.07770
syn (E) [Eq. (7)]	−283.89644	0.09512	−307.87240	0.08045	−667.87368	0.07732
syn (F) [Eq. (7)]	−207.67220	0.06887	—	—	—	—
anti TS [Eq. (7)]	−283.84926	0.09438	−307.80641	0.08034	−667.80729	0.07725
anti (E) [Eq. (7)]	−283.89434	0.09481	−307.87286	0.08024	−667.86762	0.07708
anti (F) [Eq. (7)]	−207.67031	0.06866	—	—	—	—
TS [Eq. (8)]	−283.87226	0.09637	—	—	−667.84383	0.07890
(**G**) [Eq. (8)]	−283.93460	0.09747	—	—	−667.89473	0.08130
HC(O)X	−189.24178	0.03705	−213.21953	0.02315	−573.21603	0.02110
$CH_2NH_2^+$	−94.65958	0.05844	—	—	—	—
TS [Eq. (12)]	—	—	−307.84972	0.08287	−667.84503	0.07662
IMC [Eq. (12)]	—	—	−307.89422	0.07797	−667.89061	0.07418
HCO^+	−113.25712	0.0181	—	—	—	—
H_2NCH_2X	−170.53228	0.07481	−194.52717	0.06136	−554.53609	0.05902
CO	−113.02122	0.00556	—	—	—	—
$H_3NCH_2X^+$	−170.89464	0.09057	−194.87032	0.07794	−554.88070	0.07569
$H_2NCH_2XH^+$	−170.88222	0.08465	−194.85949	0.07003	−554.86069	0.06704

[a] Refer to species as numbered in equations in Scheme 1 and in text.
[b] Energies are in Hartrees and are calculated using the MP2(fc)/6-31G* optimized geometries.
[c] Uncorrected. ZPE, zero-point vibration energy.

mizations were obtained to determine the nature of located stationary points. Energies were corrected for zero-point vibrations scaled by 0.9125 [19]. Complete structural details and lists of vibrational frequencies for each optimized structure are available from the authors.

3. Results and discussion

We have examined the reactants, intermediates, transition structures, and products associated with each of the reaction channels shown in Scheme 1, and they are discussed separately below. The energies for each species are given in Table 1.

3.1. "N adduct" versus "O adduct" formation

A key issue in the reactivity of the amide bond concerns the site of attack of the electrophile and how this controls subsequent fragmentation reactions. The reactions of formamide with the simplest electrophile, H^+, have been examined in detail using both experi-

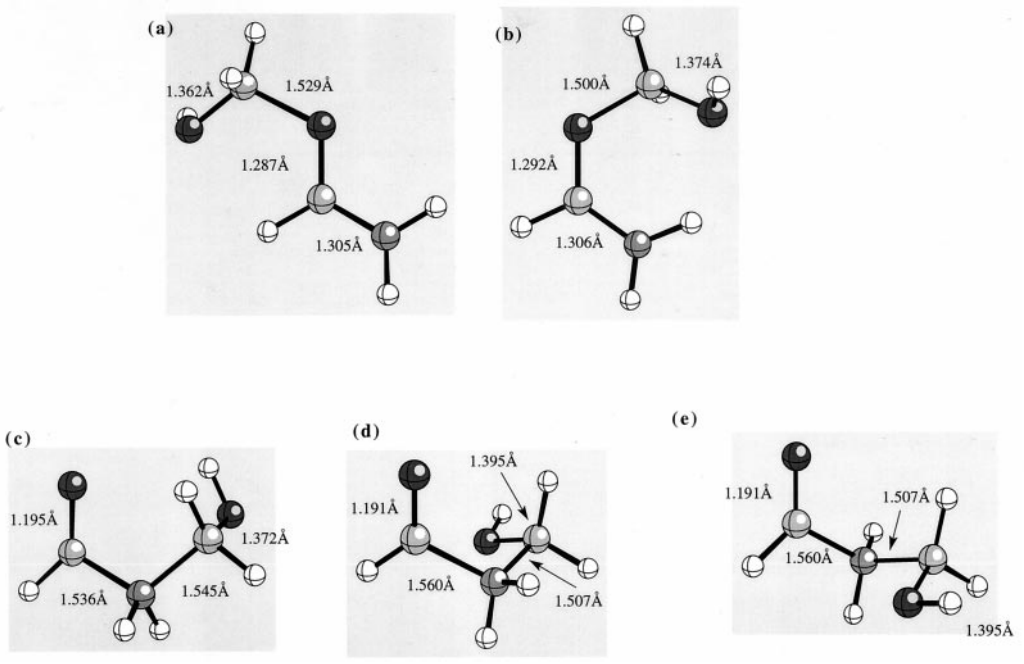


Fig. 1. MP2(FC)/6-31G* optimized structures of the O and N adducts formed in the reactions of formamide with $HOCH_2^+$: (a) O adduct (**A1**); (b) O adduct (**A2**); (c) N adduct (**B1**); (d) N adduct (**B2**); (e) N adduct (**B3**).

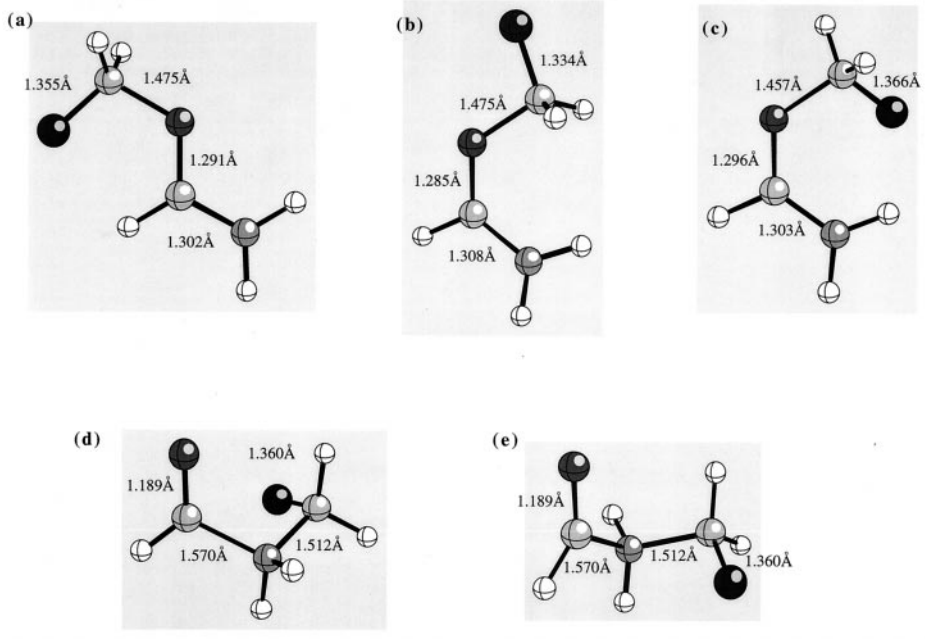


Fig. 2. MP2(FC)/6-31G* optimized structures of the O and N adducts formed in the reactions of formamide with FCH_2^+: (a) O adduct (**A1**); (b) O adduct (**A2**); (c) O adduct (**A3**); (d) N adduct (**B1**); (e) N adduct (**B2**).

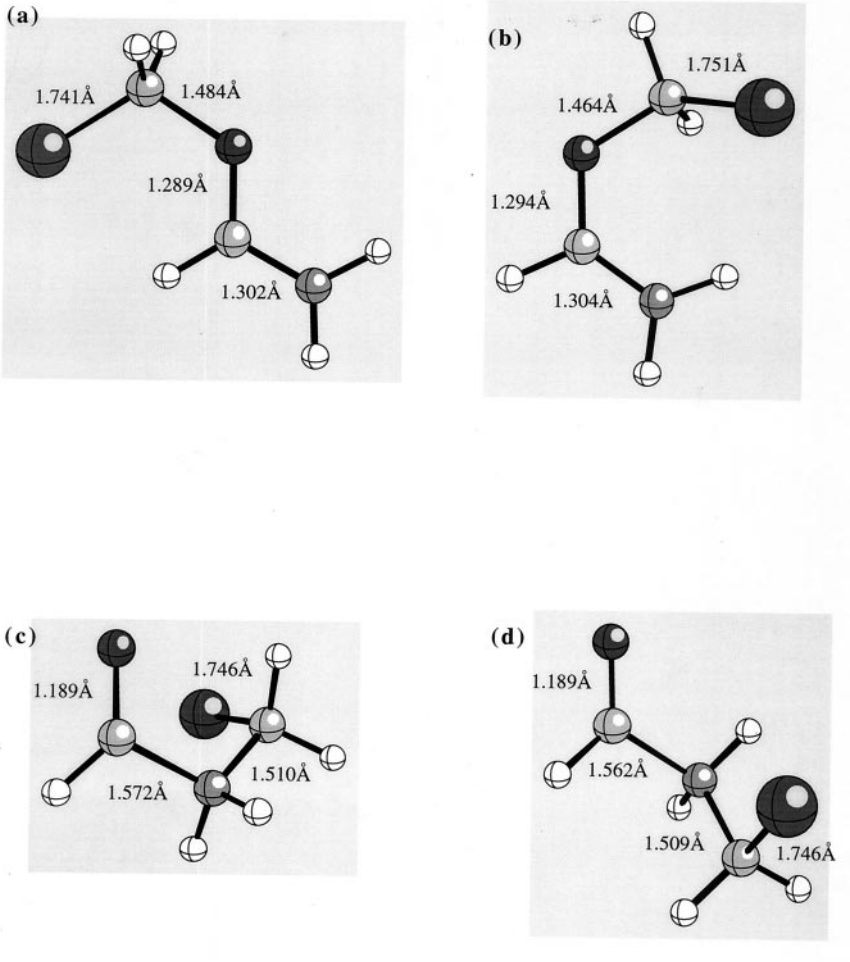


Fig. 3. MP2(FC)/6-31G* optimized structures of the O and N adducts formed in the reactions of formamide with $ClCH_2^+$: (a) O adduct (**A1**); (b) O adduct (**A2**); (c) N adduct (**B1**); (d) N adduct (**B2**).

mental and ab initio techniques [13a,20]. O protonation is favored over N protonation by 14.3 kcal mol^{-1} at the MP2/6-31G** level of theory. Furthermore, the ab initio calculations predict that the O-protonated isomer fragments via loss of water and ammonia, whereas the N-protonated isomer fragments via CO loss [20].

Our results allow us to compare the structures (Figs. 1–3) and energetics of attack (Table 2) by the carbocations XCH_2^+ (X = HO in Fig. 1; X = F in Fig. 2; X = Cl in Fig. 3) onto both the carbonyl oxygen [to yield structure (**A**) in Scheme 1] and the amino nitrogen [to yield structure (**B**) in Scheme 1]. Several different conformations were found for each type of adduct. Thus two O adducts (**A1**) and (**A2**) were found for each of the systems and are shown in Fig. 1(a) and (b) (X = HO); Fig. 2(a) and (b) (X = F); Fig. 3(a) and (b) (X = Cl). In all cases adduct (**A1**) is more stable than adduct (**A2**) (Table 2). For the N adducts, three adducts (**B1**), (**B2**), and (**B3**) were found for the $HOCH_2^+$ system [see Fig. 1(c), (d), and (e)], whereas only two adducts (**B1**) and (**B2**) were found for each of the other systems, as shown in Fig. 2(c) and (d) (X = F); Fig. 3(c) and (d) (X = Cl). The differences in energy (Table 2) between each of the N adducts are small (0.5 kcal mol^{-1} or less).

As would be intuitively expected, attack at oxygen

Table 2
Ab initio energies for reactions of XCH_2^+ with $HC(O)NH_2$ (X = HO, F, and Cl)

Reaction[b]	Species[b]	Energies kcal mol^{-1} [a]		
		X = HO	X = F	X = Cl
	"O Adduct"(**A1**)	−49.6	−80.6	−67.0
	"O Adduct" (**A2**)	−49.2	−71.6	−64.9
	"O Adduct" (**A3**)		−78.9	
	"N Adduct" (**B1**)	−34.6	−65.1	−55.6
	"N Adduct" (**B2**)	−34.1	−65.1	−55.2
	"N Adduct" (**B3**)	−34.1		
Eq. (6)	TS	[c]	−35.2	−20.2
	$HC(NH)OCH_2XH^+$	[c]	−38.0	−23.2
	$HC(NH)OCH_2^+ + HX$	+11.0	−26.4	−17.4
Eq. (7)	TS	−9.8	−29.1	−15.5
(syn)	$HC(O)NHCH_2XH^+$	−34.7	−67.1	−52.9
	$HC(O)NHCH_2^+ + HX$	−19.3	−56.8	−47.8
Eq. (7)	TS	−5.5	−25.8	−11.2
(anti)	$HC(O)NHCH_2XH^+$	−33.5	−67.5	−49.2
	$HC(O)NHCH_2^+ + HX$	−18.3	−55.7	−46.8
Eq. (8)	TS	−18.8	[d]	−33.2
	$H_2NCH_2XC(O)H^+$	−57.3	[d]	−63.8
	$H_2NCH_2^+ + HC(O)X$	−37.5	−70.7[d]	−52.8
Eq. (9)	$HCO^+ + H_2NCH_2X$	+31.2	−12.4	−2.5
Eq. (10)	$H_3NCH_2X^+ + CO$	−46.3	−77.4	−68.3
Eq. (11)	$H_2NCH_2XH^+ + CO$	−41.9	−75.1	−60.7
Eq. (12)	TS	[e]	−51.5	−35.3
	IMC	[e]	−82.2	−65.3
	$H_2NCH_2^+ + HX + CO$	[e]	−65.0	−56.0

[a] Relative to separated reactants at 0 kcal mol^{-1}; calculated using the MP2(fc)/6-31G* optimized geometries, include ZPE corrections.
[b] Refer to species as numbered in equations in Scheme 1 and throughout the text.
[c] Neither structure was located because this reaction is barrierless.
[d] Reaction does not occur. Instead CO extrusion reaction [Eq. (12)] occurs.
[e] Reaction does not occur.

results in O adducts (**A**) in which the C–O bond length has increased whereas the C–N bond length has shortened relative to those found in formamide. The reverse holds true for the N adducts (**B**), whereby the C–O bond length has decreased whereas the C–N bond length has increased. Furthermore, in all cases attack at the carbonyl oxygen is favored over the amino nitrogen by more that 10 kcal mol^{-1}.

Despite the O adduct (**A**) being thermodynamically favored over the N adduct (**B**), the kinetically important adduct in terms of fragmentation pathways will depend upon the relative barrier heights of the competing subsequent reactions [Eqs. (6)–(11) in Scheme 1]. Thus we have examined the various reaction coordinates involving both the O and N adducts, and these are discussed individually below.

3.2. Fragmentation of the "O adduct" by elimination of HX

A possible fragmentation of the O adduct (**A**) (other than back dissociation into reactants) involves

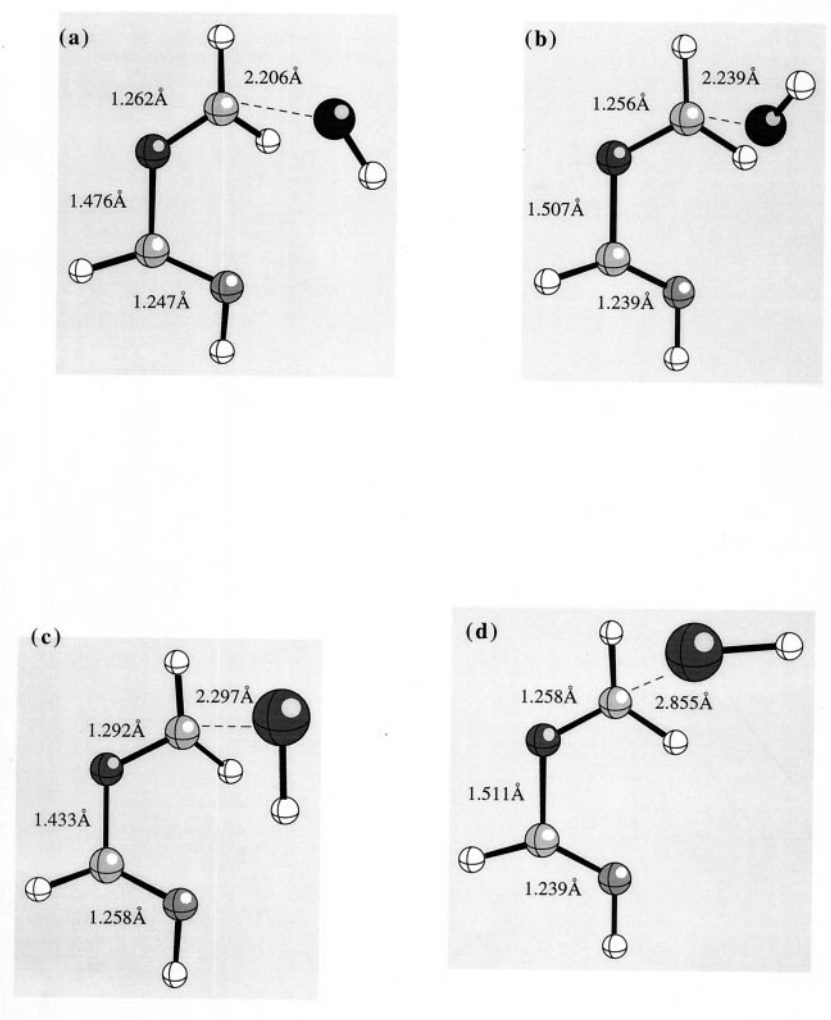


Fig. 4. MP2(FC)/6-31G* optimized structures of transition structure (**TS**) and intermediate (**C**) involved in Eq. (6): (a) (**TS**) where X = F; (b) (**C**) where X = F; (c) (**TS**) where X = Cl; (d) (**C**) where X = Cl.

1, 6 H^+ transfer to form intermediate (**C**), which can subsequently eliminate HX to form the ionic product (**D**), as outlined in Eq. (6) of Scheme 1. The overall reaction energetics (Table 2) for Eq. (6) are: X = HO + 11.0 kcal mol^{-1}; X = F − 26.4 kcal mol^{-1}; X = Cl − 17.4 kcal mol^{-1}. For the case of the hydroxymethyl cation, no transition structure or intermediate (**C**) were found [21]. In contrast, for the other cases (XCH_2^+, X = F, Cl), both the energy of the transition structure as well as the energy of the intermediate (**C**) associated with Eq. (6) lie below the energies of the separated reactants and thus these reactions should be thermodynamically viable. The structures of the transition structure involving the 1, 6 H^+ transfer as well as intermediate (**C**) are shown in Fig. 4 for the cases (XCH_2^+, X = F, Cl). Interestingly, the structures of the intermediates (**C**) are not covalent species (Scheme 1), but are rather ion–molecule complexes between the oxonium ions $HNCH(OCH_2)^+$ and HX [see Fig. 4(b) and (d)].

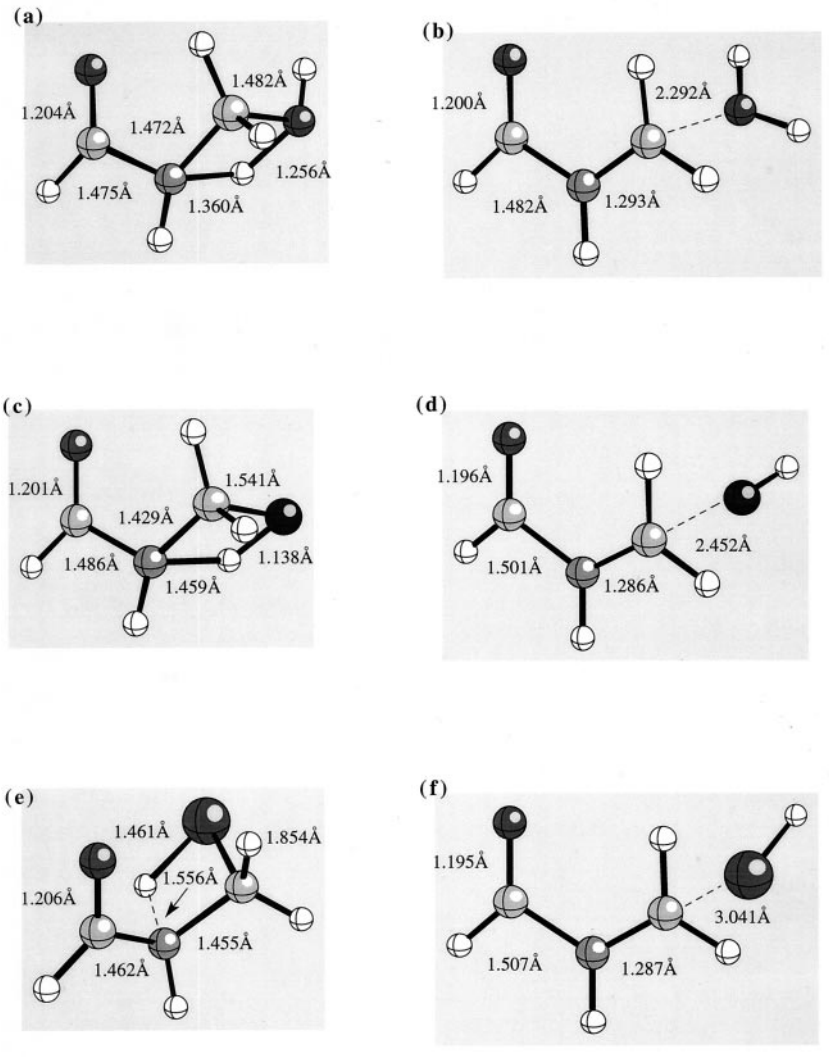


Fig. 5. MP2(FC)/6-31G* optimized structures of transition structure (**TS**) and intermediate (**E**) involved in the formation of *s*-cis $HC(O)NHCH_2^+$ (**F**) shown in Eq. (7): (a) (**TS**) where X = OH; (b) (**E**) where X = OH; (c) (**TS**) where X = F; (d) (**E**) where X = F; (e) (**TS**) where X = Cl; (f) (**E**) where X = Cl.

3.3. Fragmentation of the "N adduct" by elimination of HX

Fragmentation of the N adduct (**B**) can occur via a 1, 3 H^+ transfer to form intermediate (**E**), which ultimately eliminates HX to form the ionic product $HC(O)NHCH_2^+$ (**F**), as outlined in Eq. (7) of Scheme 1. Note that this $[M + CH]^+$ ion is isomeric to that shown in Eq. (6). Furthermore, the elimination of HX from the N adducts can proceed via two different transition structures (Figs. 5 and 6) to produce either the *s*-cis or *s*-trans $HC(O)NHCH_2^+$ product, with the former being the more stable species. The structures of the transition structures and intermediates (**E**) exhibit features similar to ab initio structures previously described for the formation of $[M + CH]^+$ ions in the reactions of saturated nucleophiles with substituted methyl cations [7,14]. In particular the structures

of the intermediates (**E**) are in fact not covalent species as depicted in Scheme 1, but are rather ion–molecule complexes between the immonium ions $HC(O)NHCH_2^+$ and HX [see Fig. 5(b), (d), and (f) and Fig. 6(b), (d), and (f)].

These reactions are exothermic in all instances and each of the transition structures lies below the energies of the separated reactants. It is interesting to compare the energetics (Table 2) for the elimination of HX from the O and N adducts to yield the isomeric product ions shown in Eqs. (6) and (7). In each case the O adduct is more stable than N adduct, but the stability of the final product from elimination of HX is the reverse (i.e. $HC(O)NHCH_2^+$ is more stable than $HC(NH)OCH_2^+$). However, the transition structure energies (relative to the energies of the separated reactants) are lower for the 1,6 H transfer than those for the 1,3 H transfer.

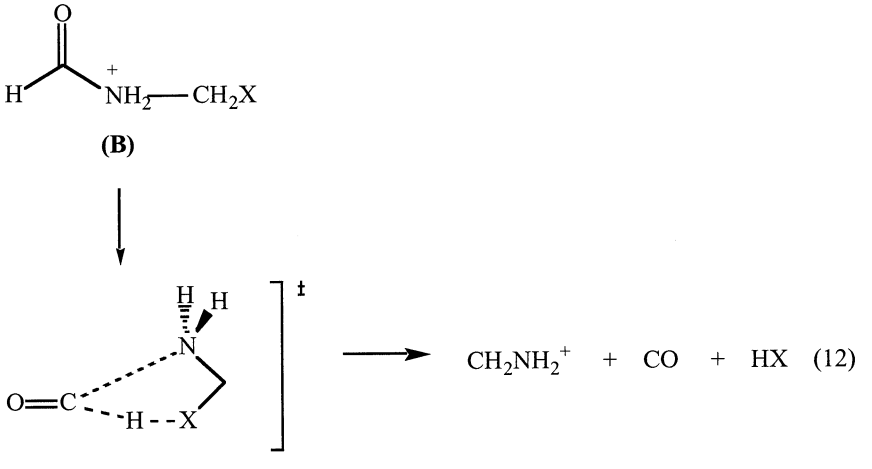
3.4. Fragmentation of the "N adduct" by "indirect" amide bond cleavage

An alternative fragmentation channel for the N adduct (**B**) involves a 1, 3 $HC(O)^+$ transfer resulting in the formation of $HC(O)XCH_2NH_2^+$ (**G**), which then decomposes to yield HC(O)X and $CH_2NH_2^+$, as shown in Eq. (8) of Scheme 1. Given that there have been no previous theoretical studies on this class of reactions, it is interesting to note the following results: (1) the final products from this amide bond cleavage [Eq. (8)] are the thermodynamically most favored from Eqs. (6)–(8); (2) this reaction proceeds via bona fide transition structures (Fig. 5), whose barriers for 1,3 $HC(O)^+$ transfer are lower than those associated with either 1,3 H^+ [Eq. (7)] or 1,6 H^+ transfer [Eq. (6)] in all cases. Thus we have termed this class of reaction an indirect amide bond cleavage as it proceeds via a transition structure rather than via an ion–molecule complex intermediate; (3) the structures of the intermediates (**G**) are not covalent species (Scheme 1), but are ion–molecule complexes between the immonium ions $CH_2NH_2^+$ and HC(O)X (see Fig. 7(b) and (e); (4) this reaction only appears to operate for the hydroxy and chloro substituted methyl cations, but not for FCH_2^+, which instead proceeds via an extrusion reaction (discussed further in Sec. 3.5 below).

An important consequence of these results is that the products from indirect amide bond cleavage [Eq. (8)] are both thermodynamically as well as kinetically favored over those of Eqs. (6) and (7).

3.5. Fragmentation of the "N adduct" via extrusion of CO

As noted above, the reaction of FCH_2^+ proceeds differently from the other substituted methyl cations. In this instance the final products are $H_2NCH_2^+$ + HF + CO [Eq. (12)] instead of $H_2NCH_2^+$ + HC(O)F, as seen in the transition structure and intermediate for this process [Fig. 8(a) and (b)]. Given this novel



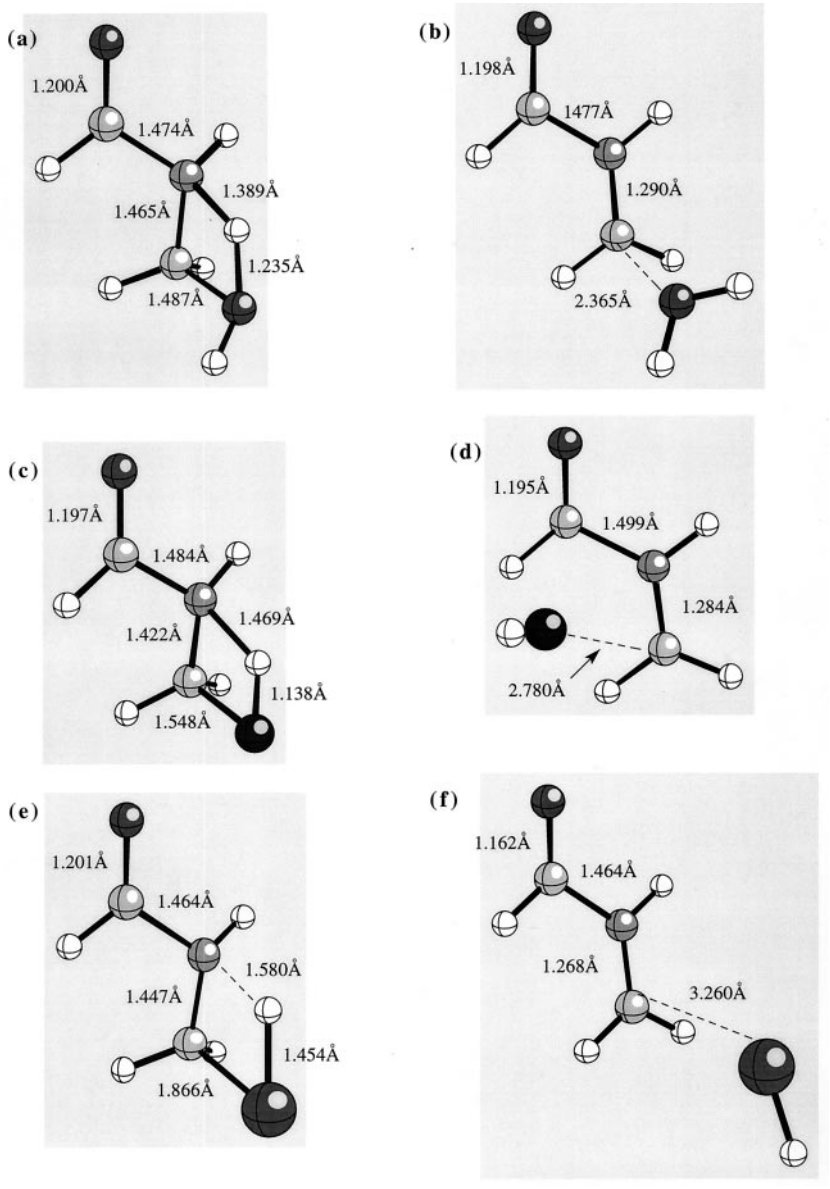


Fig. 6. MP2(FC)/6-31G* optimized structures of transition structure (**TS**) and intermediate (**E**) involved in the formation of *s*-trans $HC(O)NHCH_2^+$ (**F**) shown in Eq. (7): (a) (**TS**) where X = OH; (b) (**E**) where X = OH; (c) (**TS**) where X = F; (d) (**E**) where X = F; (e) (**TS**) where X = Cl; (f) (**E**) where X = Cl.

fragmentation pathway for the N adduct, we have also looked for this process in both other substituted methyl cations (XCH_2^+ where X = HO and Cl). When X = HO, this CO extrusion pathway does not operate, but a transition structure was found in the case of X = Cl [Fig. 8(c)].

3.6. Search for a "direct" amide bond cleavage fragmentation channel of the "N adduct"

The results from Sec. 3.5 above clearly reveal that a facile "indirect" amide bond cleavage channel can operate when X = OH and Cl via 1,3 HC(O) transfer

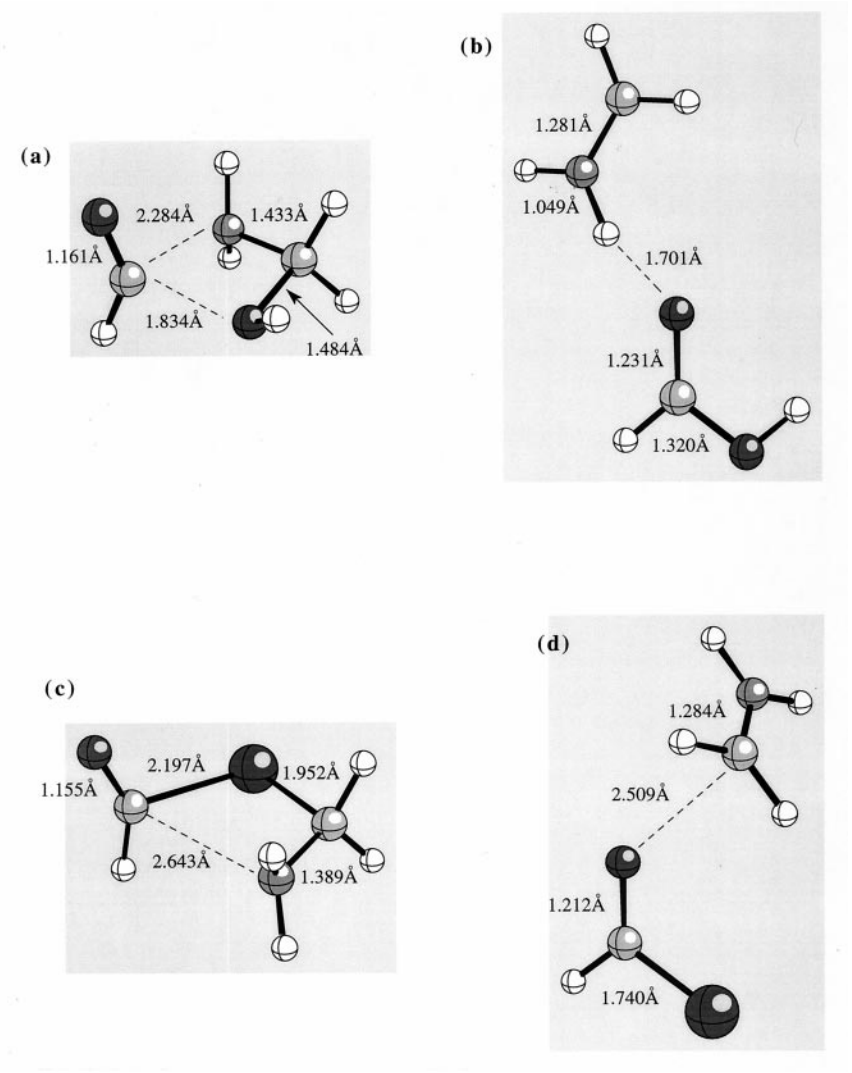


Fig. 7. MP2(FC)/6-31G* optimized structures of transition structure (**TS**) and intermediate (**G**) involved in the indirect bond cleavage reaction shown in Eq. (8): (a) (**TS**) where X = OH; (b) (**G**) where X = OH; (c) (**TS**) where X = Cl; (d) (**G**) where X = Cl.

[Eq. (8)]. We have also looked for the "direct" amide bond cleavage, in which the N adduct (**B**) directly fragments to yield an ion–molecule complex (**H**), that can then undergo three different types of reactions: loss of XCH_2NH_2 to yield $HC(O)^+$ [Eq. (9)]; proton transfer to the amino group of XCH_2NH_2 with concomitant loss of CO [Eq. (10)]; proton transfer to the heteroatom X of XCH_2NH_2 with concomitant loss of CO [Eq. (11)]. Of these channels, fragmentation to form the formyl cation is the least favored thermodynamically. Proton transfer to the amino group of XCH_2NH_2 is energetically most favored [Eq. (10)], with proton transfer to the other heteroatom X of XCH_2NH_2 being slightly less exothermic. In agreement with Uggerud [14], the structures (data not shown) of all the ionic products (**I**) due to X protonation [Eq. (11)] are not covalent, but are in fact ion–molecule complexes between the immonium ion $CH_2NH_2^+$ and HX. Finally, all attempts to find this ion–molecule complex (**H**) failed; in each case these

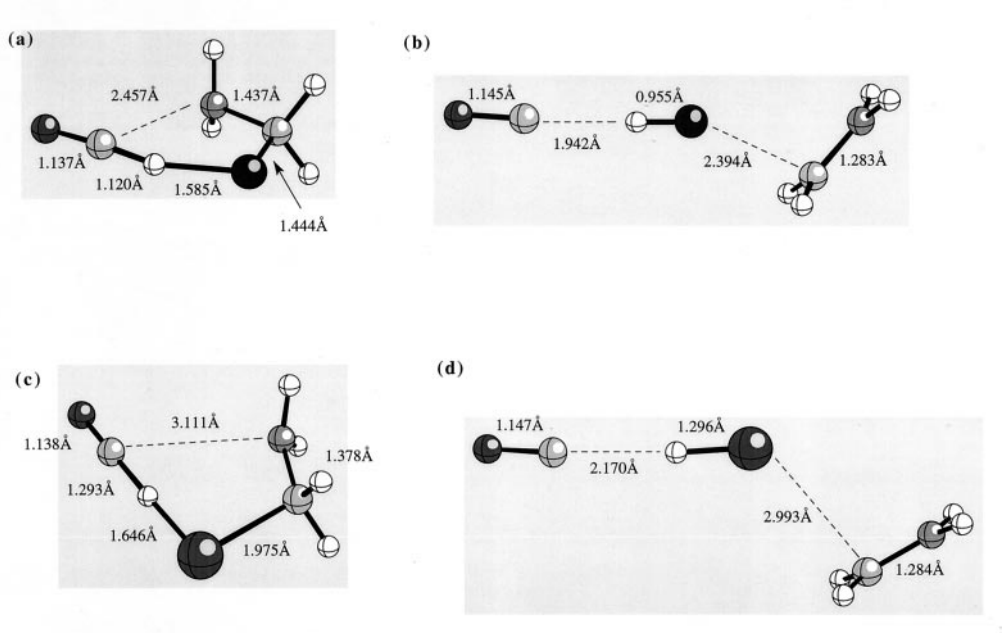


Fig. 8. MP2(FC)/6-31G* optimized structures of transition structure (**TS**) and intermediate (**G**) involved in the CO extrusion reaction shown in Eq. (12): (a) (**TS**) where X = F; (b) ion–molecule complex where X = F; (c) (**TS**) where X = Cl; (d) ion–molecule complex where X = Cl.

optimizations resulted in the formation of either (**B**) or (**G**).

4. Conclusions

These theoretical studies have revealed a number of different reaction channels for formamide reacting with the three substituted methyl cations XCH_2^+ (X = F, HO, and Cl). In all cases attack at the carbonyl oxygen atom to yield the O adduct (**A**) is thermodynamically favored over attack at the amide nitrogen atom to yield (**B**). The loss of HX from the O and N adducts yield isomeric $[M + CH]^+$ ions [Eq. (6) versus Eq. (7)] [22], but both these pathways are always thermodynamically and kinetically less favored than the amide bond cleavage reactions [Eqs. (8) and (12)]. Note that each of the substituted methyl cations exhibits different overall reactivity modes: (1) $HOCH_2^+$ reacts via a total of four pathways including Eqs. (7) (syn and anti), Eq. (8) (both the thermodynamically and kinetically favored product), and Eq. (12); (2) FCH_2^+ also reacts via a total of four pathways including Eq. (6), Eqs. (7) (syn and anti), and Eq. (12) (both the thermodynamically and kinetically favored product); (3) $ClCH_2^+$ exhibits the richest chemistry (Fig. 9), reacting via a total of five pathways including Eq. (6), Eq. (7) (syn and anti), Eq. (8), and Eq. (12) (both the thermodynamically and kinetically favored product). What do these theoretical results suggest in terms of sequencing strategies for peptides? Clearly for such reactions to be successfully applied, the peptide bond cleavage reaction(s) must be kinetically preferred over other reaction pathways. Whereas this is the case for all the substituted methyl cations studied here, the other "nonpeptide bond cleavage" reaction channels may become more competitive for FCH_2^+ and $ClCH_2^+$.

Experiments are currently underway to further examine the bimolecular reactions of peptides with a view to gaining structural information. In particular, the structures of: (1) neutral peptides will be probed through the use of ionic reagents (including substi-

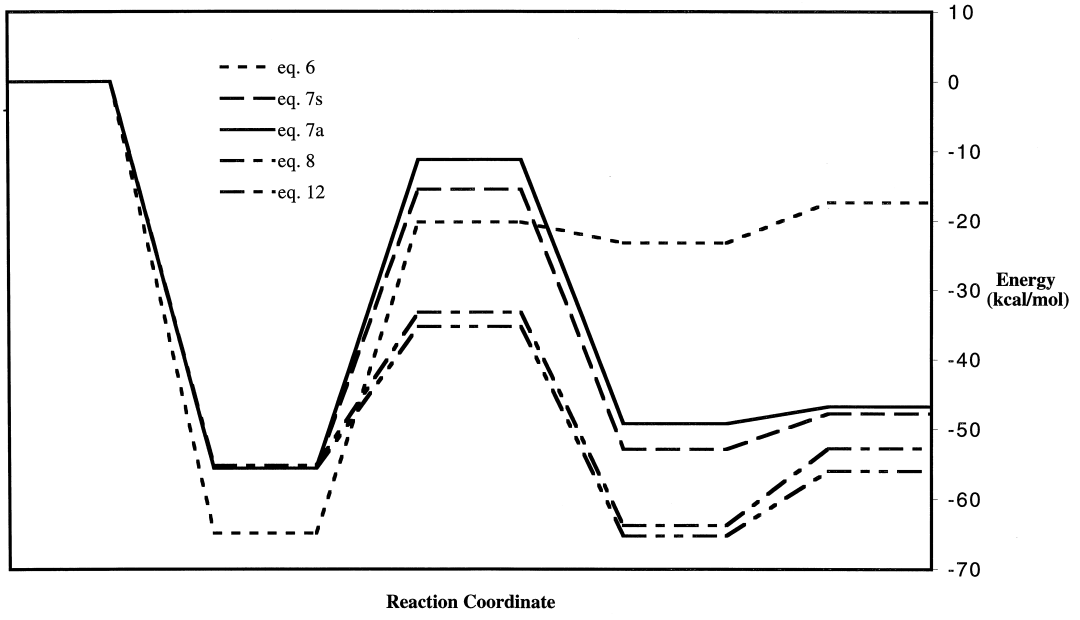


Fig. 9. Reaction coordinate diagrams showing the relative energies (at the MP2(FC)/6-31G* level of theory) of the reactants, intermediates, transition structures, intermediates,and separated products for Eqs. (6), (7) [syn (s) and anti (a)], 8, and 12 for $ClCH_2^+$ reacting with $HC(O)NH_2$.

tuted methyl cations XCH_2^+) [9]; (2) protonated and deprotonated peptides will be probed using neutral reagents [23].

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[21] An alternative explanation is that the reverse reaction involving addition of H_2O to the cation **(D)** is without barrier, also proceeding via a barrierless proton transfer to **(A)**.

[22] It remains to be seen whether these isomeric $[M + CH]^+$ ions can be experimentally distinguished via tandem mass spectrometry techniques; based on the fragmentation reactions of protonated formamide (see [19]), (**D**) may fragment via loss of formaldehyde whereas (**F**) could fragment via loss of CO. As a referee has pointed out, however, (**D**) and (**F**) may interconvert via intramolecular methylene transfer.

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