Efficient and directed peptide bond formation in the gas phase via ion/ion reactions

William M. McGee and Scott A. McLuckey[1]

Department of Chemistry, Purdue University, West Lafayette, IN 47907

Edited by Peter B. Armentrout, University of Utah, Salt Lake City, UT, and accepted by the Editorial Board December 18, 2013 (received for review September 24, 2013)

Amide linkages are among the most important chemical bonds in living systems, constituting the connections between amino acids in peptides and proteins. We demonstrate the controlled formation of amide bonds between amino acids or peptides in the gas phase using ion/ion reactions in a mass spectrometer. Individual amino acids or peptides can be prepared as reagents by (*i*) incorporating gas phase–labile protecting groups to silence otherwise reactive functional groups, such as the N terminus; (*ii*) converting the carboxyl groups to the active ester of *N*-hydroxysuccinimide; and (*iii*) incorporating a charge site. Protonation renders basic sites (nucleophiles) unreactive toward the *N*-hydroxysuccinimide ester reagents, resulting in sites with the greatest gas phase basicities being, in large part, unreactive. The N-terminal amines of most naturally occurring amino acids have lower gas phase basicities than the side chains of the basic amino acids (i.e., those of histidine, lysine, or arginine). Therefore, reagents may be directed to the N terminus of an existing "anchor" peptide to form an amide bond by protonating the anchor peptide's basic residues, while leaving the N-terminal amine unprotonated and therefore reactive. Reaction efficiencies of greater than 30% have been observed. We propose this method as a step toward the controlled synthesis of peptides in the gas phase.

peptide ligation | ion chemistry

The formation of amide bonds between amino acids holds widespread interest as it is relevant to the origin of life. Although the process by which these early peptides were formed remains an open question, laboratory-based approaches for peptide synthesis have been in place for over a century. The first controlled synthesis of diglycine via hydrolysis of diketopiperazine by Fisher in 1901 (1) marked the beginning of a long path of development of peptide synthesis approaches. Since that time, peptide synthesis has undergone several developments, beginning as solution phase reactions and eventually evolving in 1963 with Merrifield's introduction of solid resins in peptide synthesis (2), more commonly referred to as solid phase peptide synthesis (SPPS). Although this technique has undergone a dynamic maturation over the past few decades, it is still regarded as expensive and inelegant (3, 4). Furthermore, there are still several challenges that are not likely to be overcome without a significant change to the synthesis approach, beyond inherent challenges such as, for example, solubility of reagents. SPPS requires significant use of protecting and deprotecting agents, cleaving agents, and other solvents for washing, drying, and dissolving the newly formed peptides. This method quickly becomes a rather wasteful approach when considering the sacrifices made to produce just a single amide bond (3). Alternative synthesis approaches, such as chemical ligation (5, 6) and molecular machine-mediated peptide synthesis (7), offer more elegant approaches despite maintaining several of the previously mentioned requirements.

Amide bond formation is among the most widely executed reactions in organic chemistry with the most common approach involving the activation of a carboxyl group followed by nucleophilic attack of an unprotonated (and, therefore, nucleophilic) amine. The formation of amide bonds in the gas phase has been studied through computational work involving ion/molecule reactions (8) and has been demonstrated previously through uncatalyzed (9) and catalyzed reactions (10), photoexcitation reactions of proton-bound dimers (11, 12), ion/molecule reactions (13–15), and directed ion/ion reactions (16–18) using *N*-hydroxysulfosuccinimide (sulfoNHS) esters. Gas phase separation of isomeric species has also been achieved through ion mobility techniques (19), which could be used as a further purification method. Furthermore, methods for collecting peptides and proteins in the gas phase have been developed based on soft-landing techniques (20–22), which have even been extended to create microarrays of protein ions (23) from a mass spectrometer. By activating the carboxyl group of amino acids or peptides with NHS or sulfoNHS esters and protecting reactive functionalities, such as the amines, with gas phase–labile protecting groups, we generate a reagent that can be covalently attached to the N terminus of an existing unprotected peptide, which we call the "anchor" peptide. The directed N-terminal addition stems from both the preferential protonation of basic sites and the requirement of a nucleophilic attack on the carbonyl carbon of the sulfoNHS ester. Basic sites, such as arginine, lysine, or histidine, on the anchor peptide have higher gas phase basicities (24–30) than the N-terminal amines of the naturally occurring amino acids. Protonation of those sites is much more favorable than the N terminus, which renders them unreactive toward the activated carboxyl groups, while leaving the unprotonated N-terminal amine as the main reactive site. The reagent synthesis is demonstrated using a variety of amino acids with different reactive functionalities and is extended to small peptides such as di- and triglycine, which are then covalently added to the N terminus of an anchor peptide.

Here, we present a gas phase method that allows the directed formation of amide bonds between amino acids or short peptides with anchor peptides via ion/ion reactions in a mass

Significance

The generation of peptide bonds is of longstanding interest both from the standpoints of the origin of life and for synthesis. We describe here a general approach for forming peptide linkages in the gas phase via ion/ion reactions. Examples of the addition of a single amino acid to the N terminus of a peptide and the addition of a polypeptide to the N terminus of a peptide are demonstrated. This work constitutes a unique means for generating peptide linkages that are fast (<1 s), efficient (tens of percent), and flexible. Possible application areas include the generation of peptide libraries for the generation of tandem MS data and for the soft landing of peptide products for microarrays.

Author contributions: W.M.M. and S.A.M. designed research; W.M.M. performed research; and W.M.M. and S.A.M. wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission. P.B.A. is a guest editor invited by the Editorial Board.

[1]To whom correspondence should be addressed. E-mail: mcluckey@purdue.edu.

This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1317914111/-/DCSupplemental.

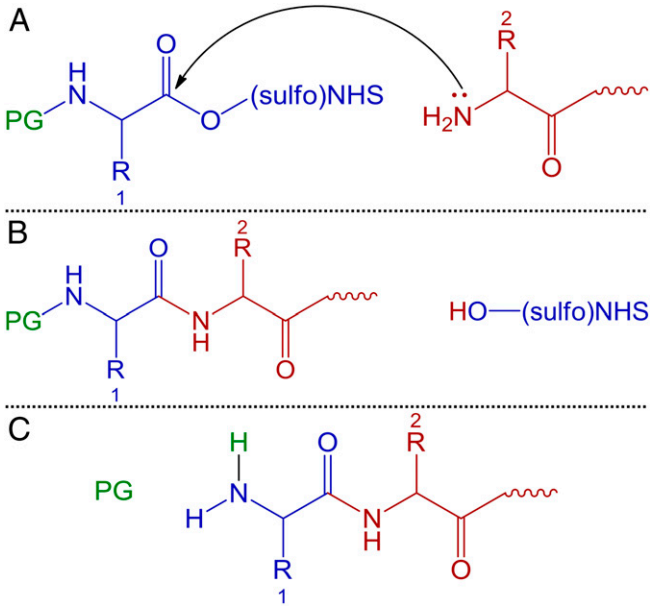


Fig. 1. Reaction process for covalent addition of a reagent amino acid or peptide to the anchor peptide. After forming a long-lived electrostatic complex between the protected sulfoNHS ester reagent, shown here as PG-R1-sulfoNHS (blue), where PG is the protecting group (green), and the anchor peptide, shown as the N-terminal R2 residue (red), (*A*) activation of the complex results in nucleophilic attack of an unprotonated amine of the anchor peptide on the carbonyl carbon of the sulfoNHS ester, resulting in (*B*) the formation of an amide bond and loss of a neutral sulfoNHS molecule, forming an extended and protected peptide. Subsequent activation of the new and protected peptide (*C*) results in loss of the PG with simultaneous transfer of a hydrogen from the PG to regenerate an amine, forming the new and unprotected peptide.

spectrometer. The method offers a rapid, efficient, and flexible approach for the generation of gaseous linear peptide ions and unique branched polypeptides. Furthermore, the absence of solvent impedes the formation of carbocations, which essentially eliminates the possibility of racemization.

Results and Discussion

Peptide Extension: A General Mechanism. Ion/ion reactions in a mass spectrometer are fast and highly exothermic as a result of, among other things, a strong Coulombic attraction (31–35). Competing pathways in these reactions mainly include proton transfer (PT), electron transfer (ET), and electrostatic complex formation, all of which are heavily dependent on the nature of the two reactants. If the anionic reagent has a low enough electron affinity to transfer an electron, then the reactivity between ET begins to compete with PT (36). However, if the reagent does not have a low electron affinity, then competition between PT and electrostatic complex formation is the main competing pathway to consider. In the case of sulfoNHS ester reagents, the electron affinities are high enough to prevent ET from being a competitive channel. Furthermore, if the reagent contains a sufficiently delocalized, or "sticky," charge-bearing site, then electrostatic complex formation tends to be the dominant pathway over PT. In the case of a multiply protonated anchor peptide and an anionic sulfoNHS ester reagent, the strong attraction between the oppositely charged ions results in the formation of a long-lived electrostatic complex. Upon activation of the electrostatic complex via energetic collisions, a nucleophilic attack by the unprotonated N-terminal amine of the anchor peptide on the carbonyl carbon of the sulfoNHS ester takes place within the complex (Fig. 1*A*), resulting in the formation of an amide bond at the N terminus of the anchor peptide with concomitant loss of the neutral sulfoNHS molecule (Fig. 1*B*). At this point, the anchor peptide has been extended; however, the protecting groups, labeled PG in Fig. 1, remain on reactive functionalities. A second activation step results in the loss of the protecting group(s), regenerating the initial functional group(s) (Fig. 1*C*). The protecting group *tert*-butyloxycarbonyl (boc) is used to protect amines, such as the N-terminal amine, the ε-amine of lysine, and the secondary amine of the imidazole ring of histidine. Several similar protecting groups have been shown to be labile in the gas phase such that, upon activation, these protecting groups are lost and the corresponding functional group is regenerated. A list of protecting groups investigated and the matching functional groups is provided in Table 1. Data demonstrating gas phase deprotection of several functional groups are provided in *SI Text* along with a table that summarizes the amino acids and di- and tripeptides that have been added to anchor peptides and the reagents used for their coupling.

Addition of a Tripeptide Reagent to an Anchor Peptide. Peptide extension is demonstrated here by adding the tripeptide GGG, where G is glycine, to the anchor peptide PKAAAKA, where P is proline, K is lysine, and A is alanine. Unless described as being N-terminally protected with a boc group or C-terminally activated with a sulfoNHS ester, the peptides are otherwise unmodified. We have shown previously that primary amines, such as the ε-amine of lysine and the N-terminal amines, are highly reactive toward sulfoNHS esters via ion/ion reactions (16–18). This is a demonstration of covalent modification of a secondary amine using sulfoNHS esters via gas phase ion/ion reactions. Here, proline was chosen to show that, despite the greater basicity of the secondary amine of proline compared with the primary amines of the remaining naturally occurring amino acids, protonation at the basic residue, lysine in this case, was still more favorable. Consequently, this results in covalent modification occurring at the N-terminal

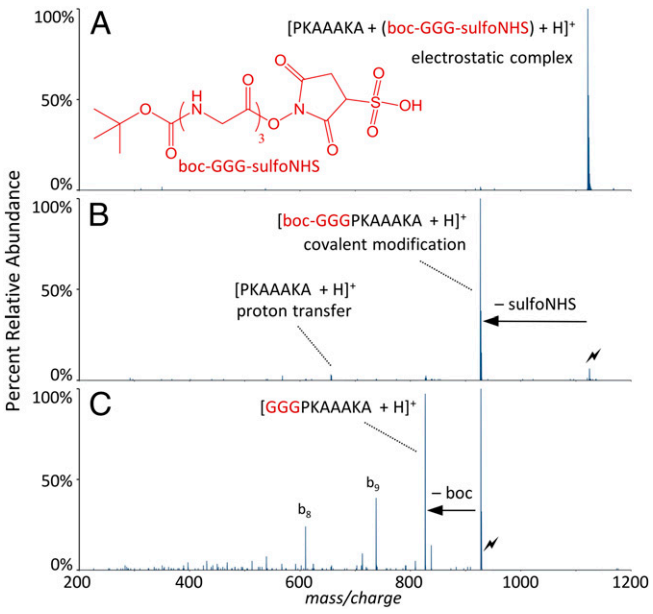
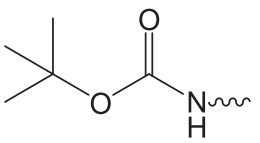
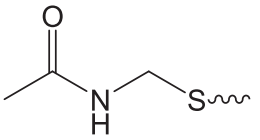
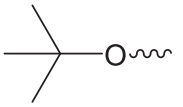
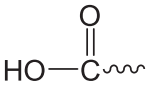
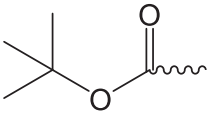
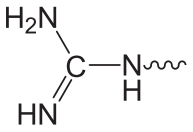
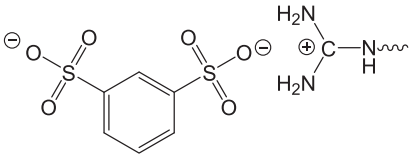


Fig. 2. Addition of the triglycine reagent [boc–GGG–sulfoNHS – H]$^-$ to the anchor peptide $[PKAAAKA + 2H]^{2+}$. Following the formation and isolation of (*A*) the electrostatic complex, (*B*) activation results in two competing channels: rupturing of the electrostatic interaction, observed as a PT, or covalent modification, observed as the loss of neutral sulfoNHS molecule. (*C*) Activation of the covalently modified and protected peptide, [boc–GGGPKAAAKA + H]$^+$, results in subsequent loss of the boc protecting group, forming [GGGPKAAAKA + H]$^+$. The lightning bolt represents the activated peak(s).

Table 1. Functional groups considered and the corresponding gas phase–labile protecting groups used

Functional group	Functional structure	Protecting group	Protected structure
Amine*	H_2N〰	*N*-boc	
Sulfhydryl	HS〰	Acetamidomethyl (S-acm)	
Hydroxy	HO〰	*Tert*-butyl ester (O-*t*Bu)	
Carboxylic Acid		*Tert*-butyl ester (O-*t*Bu)	
Guanidine**		1,3-benzenedisulfonate (BDSA)	

The single asterisk (*) indicates that primary or secondary amines can be protected with boc. The double asterisk (**) indicates that rather than a covalently bound protecting group, the guanidinium–sulfonate interaction is electrostatic.

proline residue. The gas phase ion/ion reaction between [boc–GGG–sulfoNHS – H]$^-$ and [PKAAAKA + 2H]$^{2+}$ initially forms a long-lived electrostatic complex [PKAAAKA + (boc–GGG–sulfoNHS) + H]$^+$ (Fig. 2*A*). Collision-induced dissociation (CID) of the electrostatic complex (Fig. 2*B*) can access two competing fragmentation pathways (31–37). One pathway leads to a net PT, as reflected in the detachment of the neutral boc–GGG–sulfoNHS ester, indicating no covalent reaction. The second pathway involves covalent modification, as reflected by the signature loss of a neutral sulfoNHS molecule (16–18). Isolation and monoisotopic activation of the covalently modified peptide [boc–GGGPKAAAKA + H]$^+$ (Fig. 2*C*) results mainly in the loss of the boc protecting group, generating the unprotected peptide [GGGPKAAAKA + H]$^+$. Additional fragmentation is observed by the presence of the b_8 and b_9 ions (Fig. 2*C*), which are produced from subsequent fragmentation of the unprotected peptide GGGPKAAAKA.

Comparison Between Gas Phase–Prepared and Solution-Prepared Peptides. Tandem mass spectrometry (MS/MS) can be performed on the peptide GGGPKAAAKA, prepared via ion/ion reactions to determine the sequence. To validate the identity of the newly formed peptide, its fragmentation spectrum was compared with that of a peptide of the same sequence prepared by NEOBioLab using SPPS. Fig. 3 compares the dissociation behavior of [GGGPKAAAKA + H]$^+$ prepared in the mass spectrometer (Fig. 3*A*) to the common SPPS method (Fig. 3*B*). The two spectra are very nearly identical; however, the presence of y-ions labeled with a solid diamond, in the gas phase–prepared peptide spectrum (Fig. 3*A*), indicates evidence for reaction of a lysine side chain as a minor reaction channel for the peptide reactant ions. This would occur if a proton was bound at the N-terminal amine, leaving the ε-amine of one of the lysine residues unprotonated and therefore reactive toward the reagent. Although it is a much less favorable pathway, linkages to the side chains of lysine residues can be observed. This side reaction has not been observed when histidine or arginine residues are used as the basic proton-bearing sites in the anchor peptide. The precursor ion used to produce the spectrum of Fig. 3*A* could contain a mixture of the desired peptide, GGGPKAAAKA, and the anchor peptide with a modification at either lysine. The similarities of both the peak locations on the mass scale and their relative abundances produced from fragmentation of the gas phase–prepared peptide (Fig. 3*A*) to those produced from fragmentation of the SPPS-prepared peptide (Fig. 3*B*) suggest that the two precursor ions are nearly identical. In this regard, it is likely that the mixture of isomers of Fig. 3*A* is comprised, in large part, of the desired peptide, GGGPKAAAKA, with any other isomeric products present at under 10%. In general, to achieve separation of isomers that may be produced from reaction at two or more sites, one could envisage the use of ion mobility MS as a purification method for the desired product.

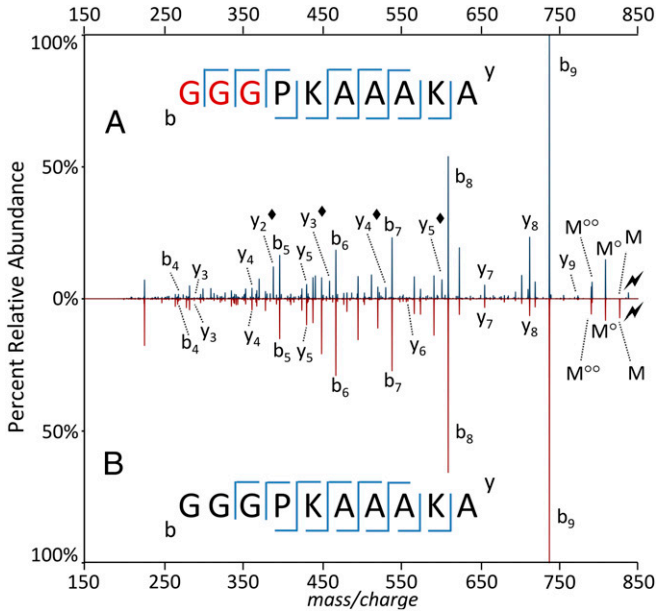


Fig. 3. Comparison of the CID spectra of M = [GGGPKAAAKA + H]$^+$ prepared (*A*) in the gas phase and (*B*) via SPPS. The lightning bolt represents the peak that is activated and water loss is labeled as a degree sign (°). The solid diamond represents fragments corresponding to a modification on a lysine.

Reaction Efficiency. The overall reaction efficiency, defined as the percentage of initial peptide reactant ions that are converted to product, is dependent on several factors, such as the extent to which the peptide reactant ion is allowed to react with the reagent (with sufficient reagent anion numbers and reaction time, 100% depletion of the peptide reactant is possible), the extent to which the ion/ion complex is dissociated (under optimal activation conditions, 100% efficiency is possible), the competition between PT and covalent reaction, and the deprotection efficiency. Fig. 4 illustrates the overall efficiency for the addition of diglycine to the anchor peptide using conditions tuned to give relatively high step-wise efficiencies. In this case, an overall efficiency of 31% was measured. This value is based on comparing the abundance of the precursor anchor peptide, [AKAAAKA + 2H]$^{2+}$, to the extended and deprotected peptide ion, [GGAKAAAKA + H]$^+$, generated using the gas phase process and is based on the sum of 20 scans for each spectrum. The various steps leading from the reactants to the final product are listed in Fig. 4, *Inset* and are analogous to those described in Fig. 2.

Conclusion

This process has applied common concepts of conjugation chemistry to gas phase ion/ion reactions performed in the mass spectrometer to create a method for rapid directed and efficient formation of amide bonds in peptides. A variety of amino acids and small peptides have been used to form amide bonds to the primary or secondary (proline) N-terminal amines of anchor peptides. Thus far, there has been no restriction to the amino acid(s) added to the anchor peptide or to the sequence of the anchor peptide, provided that certain criteria are met. The criteria for reaction are (*i*) the anchor peptide must bear n protons such that its absolute charge, |z|, is greater than that of the reagent ion, |z–1|; (*ii*) the number of basic sites, including the N-terminal amine, must be n+1 for n protons; (*iii*) the reagent ion must have the nucleophilic functional groups protected with a gas phase–labile protecting group (boc in this case); (*iv*) the carboxyl group must be activated with a reactive ester (NHS); and (*v*) the reagent must contain a charge-bearing site (sulfonate). This approach can be modified to create branched peptides by protecting (or protonating) nucleophilic sites on the anchor peptide such as the N terminus or by introducing sites with higher gas phase basicity, such as arginine residues, to favor protonation at those sites, keeping the less basic amines of lysine unprotonated and therefore free to react.

Materials and Methods

Boc–gly–gly–OH and boc–gly–gly–gly–OH were purchased from Bachem Americas, Inc. SulfoNHS and 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC) were purchased from PierceNet. Methanol (MeOH) and *N*,*N*-dimethylformamide (DMF) were purchased from Macron Chemicals. The peptides AKAAAKA, PKAAAKA, and GGGPKAAAKA were synthesized by NeoBioSci.

SulfoNHS Ester Reagent Preparation. Boc-protected amino acid and small peptides were dissolved in mixtures of MeOH and DMF. SulfoNHS esters were prepared by activating the carboxyl derivatives of the boc-protected amino acids or peptides using an equimolar amount of EDC in a mixture of DMF and H_2O. To this, equimolar amounts of sulfoNHS in H_2O were added, which resulted in the formation of an ester between the carboxyl group and the *N*-hydroxy of the succinimide, leaving the boc-protected sulfoNHS ester amino acid or peptide reagent. Final concentrations of sulfoNHS ester reagents were prepared to between 25 and 50 mM.

MS. All MS experiments were performed on QqQ hybrid triple quadrupole/linear ion trap (LIT) mass spectrometers (QTRAP 2000 and QTRAP 4000, AB Sciex), modified with home-built, alternately pulsed nanoelectrospray ionization sources (38). Analyte and reagent ions were sequentially mass-selected in the Q1 mass filter, and then subsequently transferred to the q2 reaction cell for mutual storage ion/ion reactions (39) for periods between 500 ms and 1,000 ms in length. Mutual storage reactions were performed by applying alternating current waveforms to the IQ2 and IQ3 lenses, while ions of both polarities were held in the q2 reaction cell. The long-lived electrostatic complex reaction product ions were then transferred to the Q3 LIT, where they were mass-selected and collisionally activated using ion trap CID by applying a low amplitude dipolar waveform for periods between 100 and 2,000 ms. For all subsequent fragmentation steps, additional quadrupolar radio frequency and direct current voltage isolations followed by ion-trap CID were performed. The ions were then mass-analyzed using mass-selective axial ejection (40).

Activation of the electrostatic complex between the reagent ion and the anchor peptide required the longest activation period with the lowest amplitude. By using a low-amplitude waveform to slowly heat the electrostatic complex, the covalent modification pathway often becomes more favorable than the PT pathway. Although this behavior is highly dependent on the nature of the ions involved in the electrostatic complex, all of the reagents investigated herein have reacted in a manner consistent with this observation.

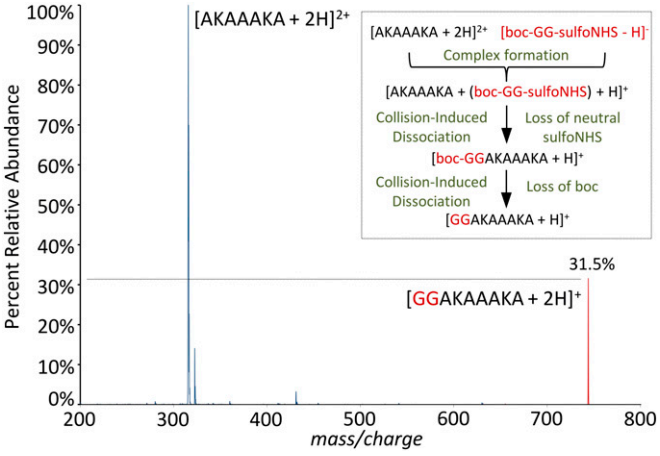


Fig. 4. Determining the overall efficiency of the addition of diglycine (GG) to the anchor peptide based on the ratio of the final product ion, [GGAKAAAKA + H]$^+$, shown in red on the right, to the precursor anchor peptide ion, [AKAAAKA + 2H]$^{2+}$, shown in blue on the left. The process by which GG was added is shown in the top right of the figure.

Following the formation of an amide bond between the protected reagent ion and the anchor peptide, subsequent activation steps may vary in the amplitude and time required to initiate gas phase deprotection of functional groups. In general, shorter activation periods with higher amplitude may be used to initiate gas phase deprotection; however, the higher the amplitude used, the more likely it becomes that cleavages occurring along the peptide backbone will be observed in competition with deprotection events. Therefore, extended activation periods with low amplitudes may also be used for deprotection to maximize the formation of the unprotected peptide.

ACKNOWLEDGMENTS. Dr. Ryan T. Hilger, Dr. Boone M. Prentice, and Dr. John R. Stutzman are acknowledged for helpful discussions. This work was supported by National Institutes of Health Grant GM 45372.

1. Fischer E, Fourneau E (1901) On the derivatives of glycocolls. *Ber Dtsch Chem Ges* 34(2):2868–2877.
2. Merrifield RB (1963) Solid phase peptide synthesis. I. The synthesis of a tetrapeptide. *J Am Chem Soc* 85(14):2149–2154.
3. Pattabiraman VR, Bode JW (2011) Rethinking amide bond synthesis. *Nature* 480(7378):471–479.
4. Coin I, Beyermann M, Bienert M (2007) Solid-phase peptide synthesis: From standard procedures to the synthesis of difficult sequences. *Nat Protoc* 2(12): 3247–3256.
5. Dawson PE, Muir TW, Clark-Lewis I, Kent SBH (1994) Synthesis of proteins by native chemical ligation. *Science* 266(5186):776–779.
6. Hackeng TM, Griffin JH, Dawson PE (1999) Protein synthesis by native chemical ligation: Expanded scope by using straightforward methodology. *Proc Natl Acad Sci USA* 96(18):10068–10073.
7. Lewandowski B, et al. (2013) Sequence-specific peptide synthesis by an artificial small-molecule machine. *Science* 339(6116):189–193.
8. Redondo P, Martínez H, Cimas A, Barrientos C, Largo A (2013) Computational study of peptide bond formation in the gas phase through ion-molecule reactions. *Phys Chem Chem Phys* 15(31):13005–13012.
9. Jensen JH, Baldridge KK, Gordon MS (1992) Uncatalyzed peptide bond formation in the gas phase. *J Phys Chem* 96(21):8340–8351.
10. Remko M, Rode BM (2001) Catalyzed peptide bond formation in the gas phase. *Phys Chem Chem Phys* 3(21):4667–4673.
11. Lee S, Valentine SJ, Reilly JP, Clemmer DE (2011) Controlled formation of peptide bonds in the gas phase. *J Am Chem Soc* 133(40):15834–15837.
12. Lee S, Julian RR, Valentine SJ, Reilly JP, Clemmer DE (2012) Biomolecular condensation via ultraviolet excitation in vacuo. *Int J Mass Spectrom* 316:6–11.
13. Wincel H, Fokkens RH, Nibbering NMM (2000) Peptide bond formation in gas-phase ion/molecule reactions of amino acids: A novel proposal for the synthesis of prebiotic oligopeptides. *Rapid Commun Mass Spectrom* 14(3):135–140.
14. O'Hair RAJ, Androutsopoulos NK (2000) Can transacylation reactions occur via $S_{(N)}2$ pathways in the gas phase? Insights via ion-molecule reactions of *N*-acylpyridinium ions and *ab initio* calculations. *Org Lett* 2(17):2567–2570.
15. O'Hair RAJ, Androutsopoulos NK, Reid GE (2000) Do amines react with protonated peptides in the gas phase via transacylation reactions to induce peptide bond cleavage? *Rapid Commun Mass Spectrom* 14(18):1707–1716.
16. Mentinova M, McLuckey SA (2010) Covalent modification of gaseous peptide ions with *N*-hydroxysuccinimide ester reagent ions. *J Am Chem Soc* 132(51):18248–18257.
17. Mentinova M, McLuckey SA (2011) Intra- and inter-molecular cross-linking of peptide ions in the gas phase: Reagents and conditions. *J Am Soc Mass Spectrom* 22(5): 912–921.
18. Mentinova M, Barefoot NZ, McLuckey SA (2012) Solution versus gas-phase modification of peptide cations with NHS-ester reagents. *J Am Soc Mass Spectrom* 23(2): 282–289.
19. Eiceman GA, Karpas Z (2005) *Ion Mobility Spectrometry* (CRC, Boca Raton, FL).
20. Laskin J, Futrell JH (2003) Energy transfer in collisions of peptide ions with surfaces. *J Chem Phys* 119(6):3413–3420.
21. Volný M, et al. (2007) Surface-enhanced Raman spectroscopy of soft-landed polyatomic ions and molecules. *Anal Chem* 79(12):4543–4551.
22. Laskin J, Wang P, Hadjar O (2008) Soft-landing of peptide ions onto self-assembled monolayer surfaces: An overview. *Phys Chem Chem Phys* 10(8):1079–1090.
23. Ouyang Z, et al. (2003) Preparing protein microarrays by soft-landing of mass-selected ions. *Science* 301(5638):1351–1354.
24. Gorman GS, Speir JP, Turner CA, Amster IJ (1992) Proton affinities of the 20 common α-amino acids. *J Am Chem Soc* 114(10):3986–3988.
25. Gorman GS, Amster IJ (1993) Gas-phase basicity measurements of dipeptides that contain valine. *J Am Chem Soc* 115(13):5729–5735.
26. Wu Z, Fenselau C (1994) Gas-phase basicities and proton affinities of lysine and histidine measured from the dissociation of proton-bound dimers. *Rapid Commun Mass Spectrom* 8(9):777–780.
27. Carr SR, Cassady CJ (1996) Gas-phase basicities of histidine and lysine and their selected di- and tripeptides. *J Am Soc Mass Spectrom* 7(12):1203–1210.
28. Ewing NP, Zhang X, Cassady CJ (1996) Determination of the gas-phase basicities of proline and its di- and tripeptides with glycine: The enhanced basicity of prolylproline. *J Mass Spectrom* 31(12):1345–1350.
29. Nold MJ, Cerda BA, Wesdemiotis C (1999) Proton affinities of the N- and C-terminal segments arising upon the dissociation of the amide bond in protonated peptides. *J Am Soc Mass Spectrom* 10(1):1–8.
30. Schroeder OE, et al. (2004) Proton affinity of lysine homologues from the extended kinetic method. *J Phys Chem A* 108(2):326–332.
31. McLuckey SA, Stephenson JL, Jr. (1998) Ion/ion chemistry of high-mass multiply charged ions. *Mass Spectrom Rev* 17(6):369–407.
32. Pitteri SJ, McLuckey SA (2005) Recent developments in the ion/ion chemistry of high-mass multiply charged ions. *Mass Spectrom Rev* 24(6):931–958.
33. McLuckey SA (2010) The emerging role of ion/ion reactions in biological mass spectrometry: Considerations for reagent ion selection. *Eur J Mass Spectrom (Chichester, Eng)* 16(3):429–436.
34. Prentice BM, McLuckey SA (2013) Gas-phase ion/ion reactions of peptides and proteins: Acid/base, redox, and covalent chemistries. *Chem Commun (Camb)* 49(10): 947–965.
35. Prentice BM, Stutzman JR, McLuckey SA (2013) Reagent cluster anions for multiple gas-phase covalent modifications of peptide and protein cations. *J Am Soc Mass Spectrom* 24(7):1045–1052.
36. Gunawardena HP, et al. (2005) Electron transfer versus proton transfer in gas-phase ion/ion reactions of polyprotonated peptides. *J Am Chem Soc* 127(36):12627–12639.
37. McGee WM, McLuckey SA (2013) Gas phase dissociation behavior of acyl-arginine peptides. *Int J Mass Spectrom* 354:181–187.
38. Liang X, Xia Y, McLuckey SA (2006) Alternately pulsed nanoelectrospray ionization/atmospheric pressure chemical ionization for ion/ion reactions in an electrodynamic ion trap. *Anal Chem* 78(9):3208–3212.
39. Xia Y, Wu J, McLuckey SA, Londry FA, Hager JW (2005) Mutual storage mode ion/ion reactions in a hybrid linear ion trap. *J Am Soc Mass Spectrom* 16(1):71–81.
40. Londry FA, Hager JW (2003) Mass selective axial ion ejection from a linear quadrupole ion trap. *J Am Soc Mass Spectrom* 14(10):1130–1147.