Gas-Phase Conjugation to Arginine Residues in Polypeptide Ions via *N*-Hydroxysuccinimide Ester-based Reagent Ions

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Supplemental Information

Materials.

Methanol, acetic anhydride, and glacial acetic acid were purchased from Mallinckrodt (Phillipsburg, NJ). The peptide RARARAA was synthesized from CHI Scientific (Maynard, MA), the peptide ARAAARA was synthesized from NeoBioSci (Cambridge, MA), and the peptide YGGFLR was synthesized from CPC Scientific (San Jose, CA). The N-hydroxysuccinimide ester of 4-trimethylammonium butyrate (NHS-TMAB) reagent used to generate TMAB-YGGFLR in solution[1] was a generous donation from Prof. Fred Regnier. The *m*-maleimidobenzoyl-*N*-hydroxysulfosuccinimide ester (sulfo-MBS) was purchased from Soltec Ventures, Inc. (Beverly, MA). The crosslinker bis(sulfosuccinimidyl) suberate (BS^3) was purchased from PierceNet (Rockford, IL). The peptide acetylation procedure has been described previously,[2] with the propionylation procedure following in the same manner, using propionic anhydride rather than acetic anhydride.

Mass Spectrometry.

Ion/ion reactions were performed on a prototype version of a hybrid triple quadrupole/linear ion trap QTRAP mass spectrometer[3] (AB Sciex, Concord, ON, Canada), equipped with a home-

built, alternately pulsed nanoelectrospray ionization source.[4] Peptide solutions were prepared to concentrations of about 1ng/ml and were dissolved in 49.5:49.5:1 H_2O:MeOH:HOAc mixtures. Sulfo-NHS reagents were prepared with concentrations of about 100ng/ml and were dissolved in a 50:50 mixture of H_2O and MeOH. Analyte and reagent ions were sequentially mass-selected in Q1 and subsequently transferred to the collision cell (q2) for ion/ion reactions to be performed in mutual storage mode for varying periods of time. The reaction product ions were then transferred to Q3 where they were mass-selected and collisionally activated using ion trap collision-induced dissociation (CID). The ions were then mass analyzed using mass selective axial ejection (MSAE).[5]

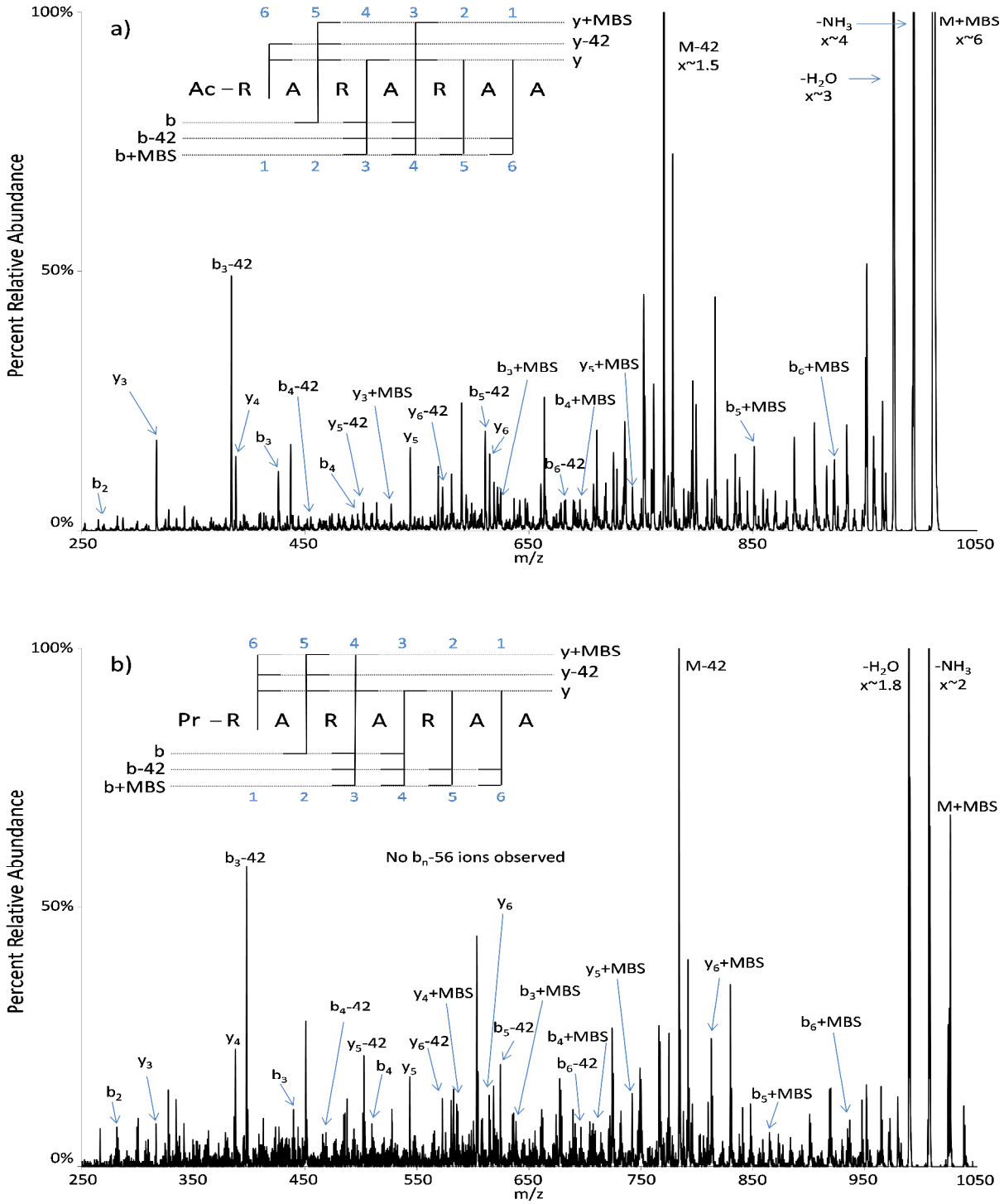

Figure S1 CID of the sulfo-NHS loss peak from a) [Ac-RARARAA+(sulfo-MBS-Na)+2H]$^+$, where the N-terminal acetylation has added 42Da to the nominal peptide mass. Loss of 42 is believed to be from the arginine side chains after reacting with MBS, as demonstrated with b) CID of the N-terminally propionylated complex, with near identical losses observed, indicating the loss of 42 does not come from the N-terminus.

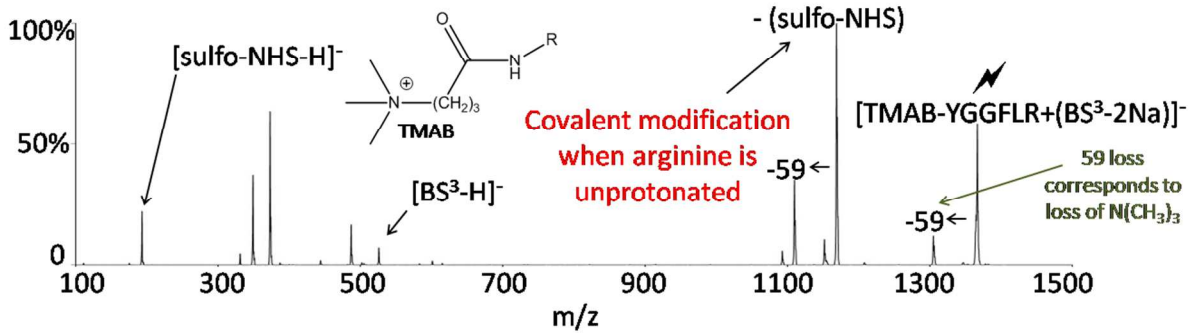

Figure S2 Product ion spectrum from collisional activation of the ion/ion complex [TMAB-YGGFLR+(BS^3-2Na)]$^-$ generated by the reaction of [TMAB-YGGFLR]$^+$ with (BS^3-2Na)$^{2-}$.

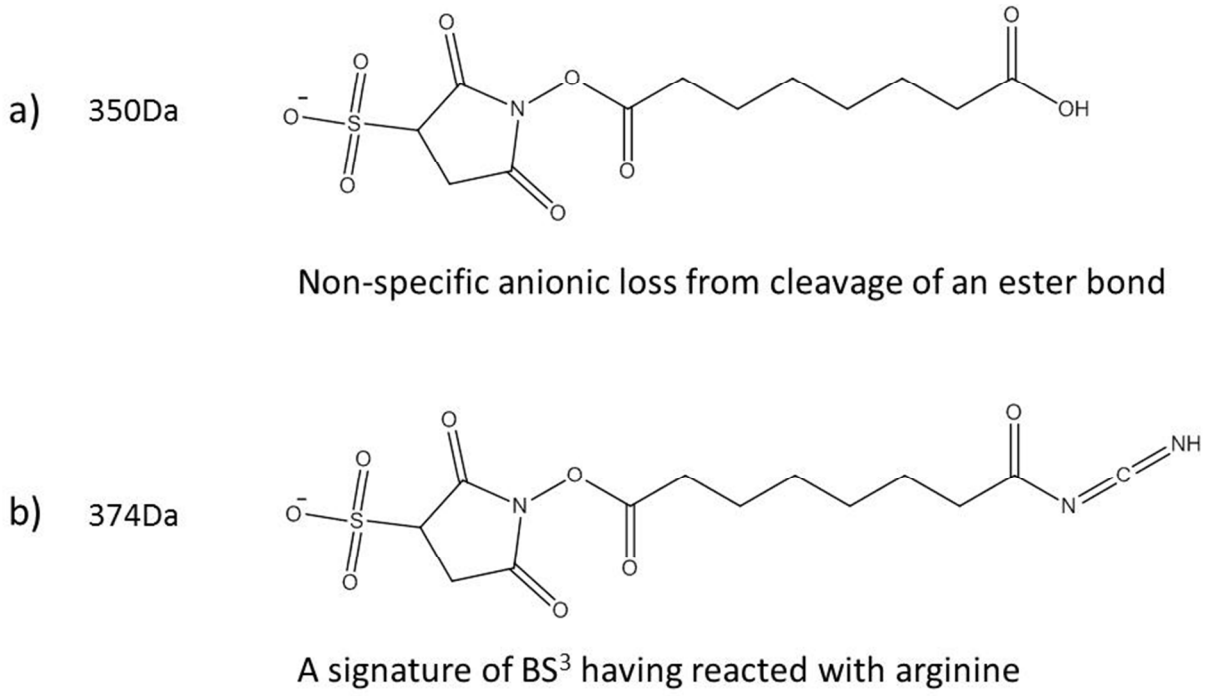

Figure S3 Anionic losses related to the BS^3 crosslinker with a) a cleavage at one of the ester bonds and b) NH=C=N- leaving from the arginine side chain, resulting in a loss of 42 from the peptide.

[1] Xia, Y.; Gunawardena, H.P.; Erickson, D.E.; McLuckey, S.A. *J. Am. Chem. Soc.*, **2007**, *129*, 12232-12243.
[2] Reid, G. E.; Simpson, R. J.; O'Hair, R. A. J. *J. Am. Soc. Mass Spectrom.* **1998**, *9*, 945-956
[3] Hager, J. W. *Rapid Commun. Mass Spectrom.* **2002**, *16*, 512-526.
[4] Liang, X.; Xia, Y.; McLuckey, S. A. *Anal. Chem.* **2006**, *78*, 3208-3212.
[5] Londry, F. A.; Hager, J. W. *J. Am. Soc. Mass Spectrom.* **2003**, *14*, 1130-1147.