

- ii Reference 35 shows Gla as di-O-methyl-Gla using a methylation procedure and a modified gradient. In addition, O-methyl Asp and O-methyl Glu are also described.
- iii Successive Edman cycles results in deglycosylation.
- iv Of three 5-Hydroxy-Lys contributors, this is the only one to indicate this peak.
- v The methyl lysines (mono-, di-, and tri-) have proven to be particularly problematic in establishing their elution position. Different contributors have shown them eluting in basically two places. The majority show them eluting in a wide area between alanine and DPTU and also after leucine. In some instances, a contributor will indicate both positions, and in others, only one of the two positions. In an effort to resolve this discrepancy, we obtained samples of the mono-, di- and tri-methyl lysines and ran them on a standard Applied Biosystems 477A sequencer simply by loading an aliquot into the reaction vessel and running a sequencer cycle. Both mono- and dimethyl lysine show both early and late peaks, while trimethyl lysine shows only the early peak. The elution order of the early peaks is mono- before di- before tri-, with a fairly limited range between Ala and Met. The late eluting peaks tend to co-elute just after leu (and nleu). So, what is the explanation? Without chemical proof we can only speculate, but we offer the following possibility. Mono- and dimethyl lysine are alkyl amines that may be capable of becoming protonated and assuming a positive charge. Trimethyl lysine is a quaternary amine that is always positively charged. As such, the charge should cause them to run relatively early in the chromatogram and, like His and Arg, their elution position will probably be very sensitive to ionic strength. Hence, varying ionic strength from different systems may explain the wide variance reported in the elution positions of the early peaks. The late peaks may be due to a portion of the mono- and dimethyl lysine side chains reacting with PITC in a manner similar to that of lysine, since they retain a free pair of electrons on the nitrogen that can participate in nucleophilic attack on the PITC. In our hands, this appears to be a major reaction for monomethyl lysine and a minor reaction for dimethyl lysine, but others have reported variable ratios. This variability may be cycle dependent. Trimethyl lysine does not possess an unbonded pair of electrons and thus would not be expected to react with PITC at this position. Hence, we do not see a late eluting peak.  
Again, it must be stressed that this is only a hypothesis and particular care must be taken in interpreting your results. However, the general behavior of the methylated lysines, whatever the reason, is well documented and should aid in their identification.
- vi Also reports minor peaks between Ser and Gln and at dehydroalanine.