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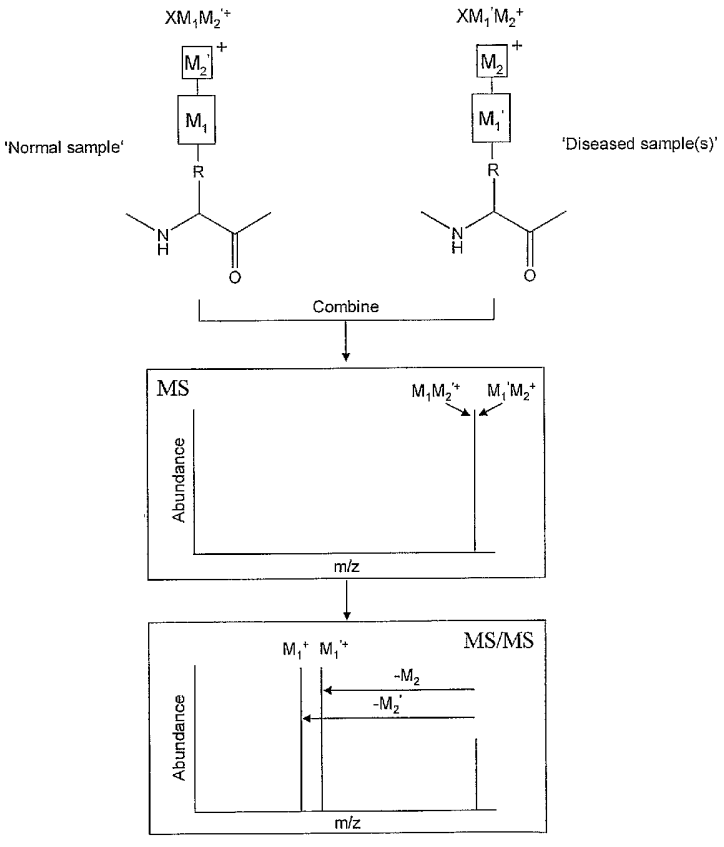
(54) Title: MODULAR ISOTOPE LABELLED MASS SPECTROMETRY REAGENTS AND METHODS FOR QUANTITATION OF AMINO ACIDS, PEPTIDES AND PROTEINS



(57) Abstract: This invention provides tandem mass spectrometry methods that can be employed in proteome analysis. The present invention also relates to 'modular' stable isotope labelled fixed charge containing compounds that are useful as mass spectrometry (MS) reagents for multiplex analyses. It is also concerned with methods for the quantitation of amino acids, peptides and proteins using tandem mass spectrometry techniques.

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MODULAR ISOTOPE LABELLED MASS SPECTROMETRY REAGENTS AND METHODS FOR QUANTITATION OF AMINO ACIDS, PEPTIDES AND PROTEINS

Field of the Invention

This invention provides tandem mass spectrometry methods that can be employed in proteome analysis. The present invention also relates to 'modular' stable isotope labelled fixed charge containing compounds that are useful as mass spectrometry (MS) reagents. It is also concerned with methods for the quantitation of amino acids, peptides and proteins using tandem mass spectrometry techniques.

Background of the Invention

The goal of proteomics may be broadly defined as (i) the systematic identification of all the gene products i.e., proteins, expressed by a particular cell or tissue type at a given time, (ii) quantitative analysis of the differences in protein abundances observed between two different states of a biological system i.e., such as that encountered between a normal and diseased cell or tissue, (iii) identification and characterization of co- and post-translational protein modifications, and (iv) identification and characterization of the protein complexes and specific protein-protein interactions, involved in the regulation of cellular behaviour [Blackstock, W.P. and Weir, M.P. *Trends Biotechnol.* **1999**, *17*, 121-127.; Mann, M., Hendrickson, R.C., Pandey, A. *Annu. Rev. Biochem.* **2001**, *70*, 437-473.]. It is widely anticipated that one of the major outcomes of proteomics research will be determination of the hitherto unknown functional role of the thousands of genes identified from recent genome sequencing initiatives, ultimately enabling a more complete understanding of the processes that control cellular biochemistry, as well as allowing the development of novel therapeutic agents targeted toward specific biomarkers of disease.

Substantial progress in the field has been achieved in recent years, primarily via developments in the application of mass spectrometry (MS) and associated sample handling methodologies for protein and peptide purification, separation and analysis, along with sophisticated bioinformatic tools for rapid protein identification and characterization via database interrogation of MS derived data [Aebersold, R. and Goodlett, D.R. *Chem. Rev.* **2001**, *101*, 269-295.]. However, there are a number of issues that currently limit the ability of these approaches to translate the 'promise' of proteomics into 'practice'. Foremost among

these is the analytical challenge presented by the enormous dynamic range and mixture complexity associated with the proteome, which is in a constant state of spatial and temporal flux, and which is further exacerbated by the diversity of transcriptional, translation and post translational protein modifications associated with protein expression throughout the cell cycle. Furthermore, most conventional methods for protein identification and characterization are based on MS analysis of individual peptides obtained following proteolytic digestion of an individual protein of protein mixture of interest, thereby further increasing the mixture complexity problem by several orders of magnitude.

Peptide mixture complexity presents a particular problem for the quantitative analysis of protein abundances. To date, the majority of methods for protein quantitation have employed either *in vivo* [Oda, Y.; Huang, K.; Cross, F. R.; Cowburn, D.; Chait, B. T. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 6591-6596.; Pasa-Tolic, L; Jensen, P. K.; Anderson, G. A.; Lipton, M. S.; Peden, K. K.; Martinovic, S; Tolic, N; Bruce, J. E.; Smith, R. D. *J. Am. Chem. Soc.* **1999**, *121*, 7949-7950.; Ong, Shao-En., Blagoev, B., Kratchmarova, I., Bach-Kristensen, D., Steen, H., Pandey, A. and Mann, M. *Mol. Cell. Proteomics.* **2002**, *1*, 376-386.] or *in vitro* [Gygi, S.P., Rist, B., Gerber, S.A., Turecek, F., Gelb, M.H. and Aebersold, R. *Nat. Biotechnol.* **1999**, *17*, 994-999.; Adamczyk, N.; Gebler, J. C.; Wu, J. *Rapid. Commun. Mass Spectrom.* **1999**, *13*, 1813-1817.; Mirgorodskaya, O.A., Kozmin, Y.P., Titov, M.I., Korner, R., Sonksen, C.P. and Roepstorff, P. *Rapid Commun. Mass Spectrom.* **2000**, *14*, 1226-1232.; Yao; X.; Freas, A.; Ramirez, J.; Demirev, P. A.; Fenselau, C. *Anal. Chem.* **2001**, *73*, 2836-2842.; Goodlett, D.R., Keller, A., Watts, J.D., Newitt, R., Yi, E.C., Purvine, S., Eng, J., von Haller, P., Aebersold, R. and Kolker, E. *Rapid Commun. Mass Spectrom.* **2001**, *15*, 1214-1221.; Sechi, S. *Rapid Commun. Mass. Spectrom.* **2002**, *16*, 1416-1424.; Gehanne, S.; Cecconi, D.; Carboni, L.; Righetti, P. G.; Domenici. E.; Hamdan, M. *Rapid Commun. Mass Spectrom.* **2002**, *16*, 1692-1698.; Chakroborty, A.; Regnier, F. E. *J. Chrom. A* **2002**, *949*, 173-184.; Qiu, Y.; Sousa, E. A.; Hewick, R. M.; Wang, J. H. *Anal. Chem.* **2002**, *74*, 4969-4979.; Zhou, H.; Ranish, J. A.; Watts, J. D.; Aebersold, R. *Nat. Biotechnol.* **2002**, *20*, 512-515; Ren, D.; Penner, N. A.; Slentz, B. E.; Mirzaei, H.; Regnier, F. E. *J. Proteome. Res.* **2003**, *2*, 321-329.; Hansen, K. C.; Schmitt-Ulms, G.; Chalkley, R.; Hirsch, J.; Baldwin, M. A.; Burlingame, A. L. *Mol. Cell Proteomics* **2003**, *2*, 299-314.; Shen, M.; Guo, L.; Wallace, A.; Fitzner, J.; Eisenman, J.; Jacobson, E.; Johnson, R.S. *Mol. Cell*

Proteomics, **2003**, *2*, 315-324.; Kuyama, H.; Watanabe, M.; Toda, C.; Ando, E.; Tanaka, K.; Nishimura, O. *Rapid Commun. Mass Spectrom.* **2003**, 17, 1642-1650.] labelling using naturally abundant or isotopically enriched derivatives, to introduce a differential mass 'tag' between two different samples of interest (e.g. a normal verus diseased cell or tissue state). By comparing the relative abundances of the peptide ions obtained from an isotopically enriched sample with those from a sample prepared using naturally abundant isotopes, quantitation of differences in protein abundance between the two samples may be obtained. These methods have also been applied to the quantitative analysis of phosphorylation status within post translationally modified proteins [Weckwerth, W., Willmitzer, L. and Fiehn, O. *Rapid Commun. Mass Spectrom.* **2000**, *14*, 1677-1681.; Goshe M.B., Conrads, T.P., Panisko, E.A., Angell, N.H., Veenstra, T.D. and Smith, R.D. *Anal Chem.* **2001**, *73*, 2578-2586.; Goshe, M.B.,Veenstra, T.D., Panisko, E.A., Conrads, T.P., Angell, N.H. and Smith, R.D. *Anal. Chem*, **2002**, *74*, 607-616.; Adamczyk, M; Gebler, J.C. and Wu, J. *Rapid Commun. Mass Spectrom.* **2002**, *16*, 999-1001.; Ficarro, S.; Chertihin, O.; Westbrook, V. E.; White, F.; Jayes, F.;Kalab, P.; Marto, J. A.; Shananowitz, J.; Herr, J. C.; Hunt, D. F.;Visconti, P. E. *J. Biol. Chem.* **2003**, *278*, 11579-11589.] by β–elimination of phosphoserine and phosphothreonine residues under strongly alkaline conditions to yield dehydroalanine or dehydroaminobutyric acid residues, respectively followed by Michael addition of naturally abundant or isotopically enriched nucleophiles [Meyer, H., Hoffman-Posorske, E., Korte, H. and Heilmeyer, L.J. *FEBS. Lett.* **1986**, *204*, 61-66.; Molloy, M.P. and Andrews, P.C. *Anal. Chem.* **2001**, 73, 5387-5394.; Li, W., Boykins, R.A., Backlund, P.S., Wang, G. and Chen, H-C. *Anal. Chem.* **2002** *74*, 5701-5710.; Steen, H. and Mann, M. *J. Am. Soc. Mass Spectrom.* **2002**, *13*, 996-1003.; Adamczyk, M., Gebler, J.C. and Wu, J. *Rapid Commun Mass Spectrom.* **2001**, *15*, 1481-1488.; Oda, Y., Nagasu, T. and Chait, B.T. *Nature Biotechnol.* **2001**, *19*, 379-382.]. For a recent review of quantitative phosphoproteome analysis see [Mann, M., Ong, S-E., Gronborg, M., Steen, H., Jensen, O.N. and Pandey, A. *Trends Biotechnol.* **2002**, 20, 261-268.].

A range of both 'targeted' and 'global' labelling strategies for protein quantitation have been described [reviewed in Julka, S. and Regnier, F. *J. Prot. Res.* **2004**, *3*, 350 – 363.]. The majority of these methods however, have been based on a common approach for identification of the differential mass "signature" between the two samples i.e., by mass analysis of their intact peptide precursor ions. Thus,

limitations are encountered when; (i) when one or both of the differentially labelled ions of interest are present at low levels (for example, approaching or below the limit of detection of the mass spectrometer) [Krutchinsky, A.N. and Chait, B.T. *J. Am. Soc. Mass Spectrom.* **2002**, *13*, 129-134.], (ii) the m/z values of low abundance differentially labelled peptide ions overlap with other higher abundance components present in the mixture, (iii) separation of the differential labelled "heavy" and "light" peptides occurs during chromatographic fractionation of the peptide mixture [Zhang, R.; Sioma, C.; Wang, S.; Regnier, F. E. *Anal. Chem.* **2001**, *73*, 5142-5149.; Zhang, R.; Sioma, C.; Thompson, R. A.; Xiong, L.; Regnier, F. E. *Anal. Chem.* **2002**, *74*, 3662-3669.] or (iv) the mass spectrometer lacks sufficient resolution to adequately resolve the two labelled components, thereby precluding their detection. A more recent report has described an alternative approach for peptide quantitation, based on measurement of the relative abundances of low mass isotopically encoded product ions formed by MS/MS from derivatized peptides, in an attempt to overcome many of these issues [Thompson, A., Schaefer, J., Kuhn, K., Kiene, S., Schwarz, J., Schmidt, G., Johnstone, R., Neumann, T. and Hamon, C. *Anal. Chem.* **2003**, *75*, 1895-1904.]. However, a limitation of this MS/MS based approach is that the desired fragmentation pathway giving rise to the product ion of interest is typically only one of many dissociation channels, thereby "diluting" the spectrum and limiting sensitivity, as well as potentially complicating its subsequent interpretation.

The reason for the desired fragmentation pathway often not yielding a dominant product ion may be rationalised by taking into consideration the generally accepted mechanisms and other factors (such as peptide ion charge state and amino acid composition) known to be responsible for the observed fragmentation reactions of protonated peptide ions. For example, the derivatization reagent employed in the strategy mentioned above contains a cleavage 'enhancement' group consisting of a proline residue to promote fragmentation within the label during MS/MS, as well as a guanidino containing 'sensitization' group to promote formation of a characteristic protonated low mass product ion following the fragmentation reaction. Under low-energy collisional activation conditions, the cleavage of most amide bonds within a protonated peptide ion, as well as many fragmentation reactions resulting in the loss of small molecules such as NH_3 or H_2O (e.g. from certain amino acid side chains), are generally thought to require localization of an ionizing proton at the cleavage site, and that fragmentation then proceeds via neighbouring group participation mechanisms

involving 'charge-directed' nucleophilic attack from an adjacent functional group at the cleavage site [Dongre, A.R., Jones, J.L., Somogyi, A. and Wysocki, V.H. *J. Am. Chem. Soc.* **1996,** *118*, 8365-8374.; O'Hair, R.A.J. *J. Mass Spectrom.* **2000**, *35*, 1377-1381.; Schlosser, A. and Lehmann, W.D *J. Mass Spectrom.* **2000**, *35*, 1382-1390.; Wysocki, V.H., Tsaprailis, G., Smith, L.L. and Breci, L.A. *J. Mass Spectrom.* **2000**, *35*, 1399-1406.]. The formation of product ions resulting from 'enhanced' cleavage at the N-terminal side of proline residues has been consistently noted in the literature, presumably due to the higher local proton affinity of the proline imide bond compared to a conventional amide bond. However, the high proton affinity of the guanidino side chains of arginine residues may act to strongly 'sequester' ionizing protons, thereby limiting their ability to be transferred along the peptide backbone to initiate cleavage by 'charge-directed' pathways. Indeed, a recent report has found that significantly enhanced N-terminal proline cleavage is only observed under conditions where the number of ionizing protons is greater than the number of arginine residues (in the case of the derivatization reagent described above, this would also include the guanidino 'sensitization' group) contained within the peptide ion [Kapp, E.A., Schütz, F., Reid, G.E., Eddes, J.S., Moritz, R.L., O'Hair, R.A.J., Speed, T.P. and Simpson, R.J. *Anal. Chem.* **2003**, *75*, 6251-6264.]. Note that under 'non-mobile' conditions, when the number of ionizing protons is less than or equal to the number of arginine residues within the peptide ion, 'charge-remote' mechanisms may become energetically more favourable, resulting in 'enhanced' preferential cleavage at sites such as the C-terminal side of aspartic acid residues or the side chains of methionine sulfoxide residues [Kapp, E.A., Schütz, F., Reid, G.E., Eddes, J.S., Moritz, R.L., O'Hair, R.A.J., Speed, T.P. and Simpson, R.J. *Anal. Chem.* **2003**, *75*, 6251-6264.; Reid, G.E., Roberts, K.D., Kapp, E.A. and Simpson, R.J. *J. Prot. Res.* **2004**, *3*, 751-759.]. Hence, the formation of abundant low mass product ions, characteristic of derivatized peptides in the approach mentioned above, is only expected for a sub-set of the total peptide ions (and their charge states) presented to the mass spectrometer for dissociation.

MS/MS methods have also been extensively employed previously for the identification of peptides containing selected structural features via formation of diagnostic "signature" product ions (e.g., amino acid immonium ions [Wilm M., Neubauer G. and Mann M. *Anal. Chem.* **1996**, *68*, 527-533.], product ions resulting from the side chain fragmentation of phosphopeptides [Carr S.A., Huddleston M.J.,

and Annan R.S. *Anal. Biochem.* **1996**, *239*, 180-192.; Schlosser A., Pipkorn R., Bossemeyer D., and Lehmann W.D. *Anal. Chem.* **2001**, *73*, 170-176. Steen H., Kuster B., Fernandez M., Pandey A., and Mann M. *Anal. Chem.* **2001**, *73*, 1440-1448.; Weckwerth, W., Willmitzer, L. and Fiehn, O. *Rapid Commun. Mass Spectrom.* **2000**, *14*, 1677-1681.; Molloy, M.P. and Andrews, P.C. *Anal. Chem.* **2001**, 73, 5387-5394.; Li, W., Boykins, R.A., Backlund, P.S., Wang, G. and Chen, H-C. *Anal. Chem.* **2002** *74*, 5701-5710. Steen, H. and Mann, M. *J. Am. Soc. Mass Spectrom.* **2002**, *13*, 996-1003.] or characteristic product ions from cross linked peptides [Back, J.W., Hartog, A.F., Dekker, H.L., Muijsers, A.O., de Koning, L.J. and de Jong, L. *J. Am. Soc. Mass Spectrom.* **2001**, 12, 222-227.]), via neutral loss and/or precursor ion scan modes of analysis [Schwartz, J.C., Wade, A.P., Enke, C.G. and Cooks, R.G. *Anal. Chem.* **1990**, *62*, 1809-1818.]. These methods have been demonstrated to yield greater specificity and increase sensitivity by 1-2 orders of magnitude over conventional MS based detection methods, due to the reduction in chemical noise associated with the formation of product ions at different m/z values to that of the mass selected precursor ion [Wilm M., Neubauer G. and Mann M. *Anal. Chem.* **1996**, *68*, 527-533]. However, similar issues to those mentioned above for the 'tandem mass tag' approach limit the general applicability of these methods.

Recently, an MS/MS based approach for selective protein identification and quantitation was described in WO 2004046731, the disclosure of which is incorporated herein by reference. In contrast to all of the MS and MS/MS based derivatization strategies outlined above, this approach is based on the formation of 'fixed-charge' derivatives on the side-chains of selected amino acids, or on the side-chains of selected amino acid residues contained within a protein or peptide. These side-chain fixed-charge derivatives are designed to direct the dissociation of the amino acid, peptide or protein ion containing the fixed charge toward exclusive formation of a single product ion that is characteristic of fragmentation occurring at the site of the fixed-charge, thereby allowing its selective identification by neutral loss scan mode MS/MS methods. By incorporation of 'light' and 'heavy' isotopically encoded labels into the fixed-charge derivatives, these methods have also been extended to the quantitative analysis of differential protein expression. These fixed-charge derivatization methods thereby offer the potential for significantly improved sensitivity and selectivity for the identification and quantitation of amino acids, peptides or proteins containing certain structural features, without need for prior

resolution or otherwise enrichment from a complex mixture prior to analysis, compared to existing methods.

However, a significant limitation of this fixed charge derivatization approach for quantitative protein analysis, is that two separate MS/MS scans must be acquired in order to determine abundance ratios, i.e., one MS/MS scan for 'light' labelled peptide ions, followed by an MS/MS scan for 'heavy' labelled peptide ions. Therefore, during capillary RP-HPLC peptide separation and neutral loss MS/MS analysis, if the concentration of the 'heavy' labelled peptide ion significantly changes in the time taken to acquire an MS/MS scan for the 'light' labelled peptide ion (or visa versa), errors in quantitation may result.

Here, we describe novel 'modular' isotopically labelled fixed charge derivatization reagents, and tandem mass spectrometry methods to enable the 'multiplexed' quantitative analysis of amino acids, peptides and proteins in a single MS/MS experiment.

Summary of the Invention

Described herein are what are believed to be novel fixed-charge derivatization reagents. The reagents may be selective for reaction with the N-terminal amino groups of amino acids, peptides and proteins, or with amino containing side chains of amino acids such as lysine, or with guanidine containing side chains of amino acids such as arginine or homoarginine, or with thiol containing side chains of amino acids such as cysteine or homocysteine, or with indole containing side chains of amino acids such as tryptophan, or with dehydroalanine or dehydroamino-2-butyric acid amino acids formed by β-elimination from O-linked phosphorylated or glycosylated serine or threonine, or with peptides or proteins comprising at least one residue of such amino acids. These novel reagents have potential application for the high throughput, sensitive and selective quantitation of these compounds when present in complex mixtures.

Accordingly, in one aspect, the present invention provides compounds of formula $XM_1'M_2^+$, and $XM_1'M_2'^+$ or salts thereof, wherein:

X is a reactive group specific to a functional group contained within an amino acid, peptide or protein, or peptide or protein containing at least one of such amino acid;

M_1' is a linker group between X and M_2^+ or $M_2'^+$, and is isotopically encoded by incorporation of one or more of ^2H, ^{13}C, ^{15}N or ^{18}O;

M_2^+ is selected from the group consisting of a tertiary alkyl sulfonium ion, a quaternary alkyl ammonium ion or a quaternary alkyl phosphonium ion; and

$M_2'^+$ is selected from the group consisting of a tertiary alkyl sulfonium ion, a quaternary alkyl ammonium ion or a quaternary alkyl phosphonium ion, and is isotopically encoded by incorporation of one or more of ^2H, ^{13}C, ^{15}N or ^{18}O.

The M_1' group may be a branched alkyl optionally interrupted or substituted with an alkyl, aryl, substituted alkyl, substituted aryl, amino, amide, acid, ester or thioester.

In a further aspect, the present invention provides a method for the quantitative analysis of amino acids, peptides or proteins, the method comprising subjecting:

a first amino acid, peptide or protein having a fixed-charge, introduced as a result of derivatization with a compound of formula $XM_1M_2^+$, wherein X and M_2^+ are as defined above, and M_1 is a linker group between X and M_2^+; and

a second amino acid, peptide or protein having a fixed-charge, introduced as a result of derivatization with a compound of formula $XM_1'M_2^+$, wherein X, M_1' and M_2^+ are as defined above,

to dissociation to form product ions that are characteristic of fragmentation occurring at the fixed-charge site.

The M_1 group may be a branched alkyl optionally interrupted or substituted with an alkyl, aryl, substituted alkyl, substituted aryl, amino, amide, acid, ester or thioester.

In another aspect, the present invention provides a method for the quantitative analysis of amino acids, peptides or proteins, the method comprising subjecting:

a first amino acid, peptide or protein having a fixed-charge, introduced as a result of derivatization with a compound of formula $XM_1M_2'^+$, wherein X, M_1 and $M_2'^+$ are as defined above, and

a second amino acid, peptide or protein having a fixed-charge, introduced as a result of derivatization with a compound of formula $XM_1'M_2^+$ or $XM_1'M_2'^+$, wherein X, M_1', M_2^+, and $M_2'^+$ are as defined above,

to dissociation to form product ions that are characteristic of fragmentation occurring at the fixed-charge site.

In another aspect, the present invention provides a reagent kit for quantitative analysis of amino acids, peptides or proteins by tandem mass spectrometry, comprising a container containing a compound of formula $XM_1'M_2^+$ or $XM_1'M_2'^+$ of the present invention.

In another aspect, the present invention provides a reagent kit for quantitative analysis of amino acids, peptides or proteins by tandem mass spectrometry, comprising a container containing the compounds of formula $XM_1M_2^+$ and $XM_1'M_2^+$ of the present invention.

In another aspect, the present invention provides a reagent kit for quantitative analysis of amino acids, peptides or proteins by tandem mass spectrometry, comprising a container containing the compounds of formula $XM_1M_2'^+$ and $XM_1'M_2'^+$.

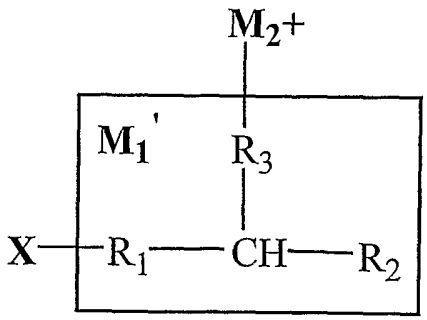
In another aspect, the present invention provides a reagent kit for quantitative analysis of amino acids, peptides or proteins by tandem mass spectrometry, comprising a container containing the compounds of formula $XM_1M_2'^+$, $XM_1'M_2'^+$ and $XM_1'M_2^+$.

In another aspect, the present invention also extends to compounds consisting of amino acids, peptides or proteins, that have been derivatized with a compound of formula $XM_1'M_2^+$ or $XM_1'M_2'^+$ as defined above.

In another aspect, the present invention provides a reagent kit comprising a container containing compounds consisting of amino acids, peptides or proteins that have been derivatized with a compound of formula $XM_1'M_2^+$ or $XM_1'M_2'^+$ as defined above.

Detailed Description of the Invention

In one particular embodiment, the present invention provides a compound of formula $XM_1'M_2^+$, or a salt thereof,



$XM_1'M_2^+$

wherein,

X is a thiol reactive group selected from the group consisting of a halide, a disulfide exchange group or a vinyl group;

M_1' is $-R_1CH(R_3)R_2$, where R_1 is selected from $-(CH_2)_n-$, -Y- or $-(CH_2)_nY-$; R_2 is selected from $-(CH_2)_nH$, $-C_6H_5$, $-CH_2C_6H_5$, $-NH_2$, -YH, $-Y(CH_2)_nH$, $-YC_6H_5$, $-YCH_2C_6H_5$; and R_3 is $-(CH_2)_n-$, and is isotopically encoded by incorporation of one or more of ^2H, ^{13}C, ^{15}N or ^{18}O;

n is from 1 to 3 inclusive;

Y is selected from CONH, NHCO and COO; and

M_2^+ is attached to the R_3 group of M_1' and is selected from the group consisting of a tertiary alkyl sulfonium ion, $-S^+CH_3R''$, where R'' is selected from $-CH_2COC_6H_5$, $-CH_2COC_6H_5CH_3$, and $-CH_2COC_6H_5CH_2CH_3$, or a quaternary alkyl ammonium ion $-N^+(R''')_3$, or a quaternary alkyl phosphonium ion $-P^+(R''')_3$, where R'''_3 is $-(CH_2)_nH$ (where n = 1 to 3), $-C_6H_5$, $-CH_2C_6H_5$.

The halide is preferably -Cl, -Br, or - I. The disulfide exchange group may be selected from -S-S-R′ where R′ is $-C_6H_5$, 3-carboxyl-4-nitrophenyl, 2,4-dinitrophenyl, 4-nitrophenyl, 2-nitrophenyl, 2-pyridyl, 5-nitropyridyl, 3-nitropyridyl, methanesulfonyl. Where X is a vinyl group it may be $-CH=CH_2$.

In a preferred embodiment, the present invention provides a compound of formula $XM_1'M_2^+$, or a salt thereof, wherein,

M_1' is $-R_1CH(R_3)R_2$, where R_1 is $-(CH_2)_n-$; R_2 is -YH, $-Y(CH_2)_nH$, $-YC_6H_5$, $-YCH_2C_6H_5$; and R_3 is $-(CH_2)_n-$, and is isotopically encoded by incorporation of one or more of ^2H, ^{13}C, ^{15}N or ^{18}O;

n is from 1 to 3 inclusive;

Y is CONH; and

X and M_2^+ are as defined above.

In a further preferred embodiment, the present invention provides a compound of formula $XM_1'M_2^+$, or a salt thereof, wherein,

M_1' is $-R_1CH(R_3)R_2$, where R_1 is -Y- or $-(CH_2)_nY-$; R_2 is $-(CH_2)_nH$, $-NH_2$, $-Y(CH_2)_nH$, $-YC_6H_5$, $-YCH_2C_6H_5$; and R_3 is $-(CH_2)_n-$, and is isotopically encoded by incorporation of one or more of ^2H, ^{13}C, ^{15}N or ^{18}O;

n is from 1 to 3 inclusive;

Y is CONH; and

X and M_2^+ are as defined above.

In a further preferred embodiment, the present invention provides a compound of formula $XM_1'M_2^+$, or a salt thereof, wherein,

M_1' is $-R_1CH(R_3)R_2$, where R_1 is $-(CH_2)_n-$; R_2 is -YH, $-Y(CH_2)_nH$, $-YC_6H_5$, $-YCH_2C_6H_5$; and R_3 is $-(CH_2)_n-$, and is isotopically encoded by incorporation of one or more of ^2H, ^{13}C, ^{15}N or ^{18}O;

n is from 1 to 3 inclusive;

Y is NHCO; and

X and M_2^+ are as defined above.

In a further preferred embodiment, the present invention provides a compound of formula $XM_1'M_2^+$, or a salt thereof, wherein,

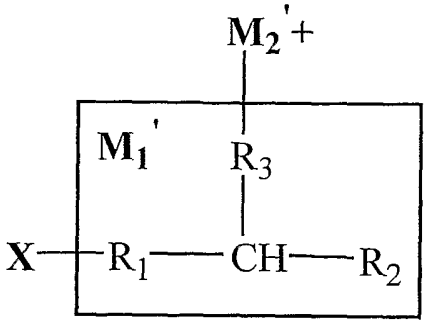
M_1' is $-R_1CH(R_3)R_2$, where R_1 is -Y- or $-(CH_2)_nY-$; R_2 is $-(CH_2)_nH$, $-NH_2$, $-Y(CH_2)_nH$, $-YC_6H_5$, $-YCH_2C_6H_5$; and R_3 is $-(CH_2)_n-$, and is isotopically encoded by incorporation of one or more of ^2H, ^{13}C, ^{15}N or ^{18}O;

n is from 1 to 3 inclusive;

Y is NHCO; and

X and M_2^+ are as defined above.

In another particular embodiment, the present invention provides an isotopically labelled compound of formula $XM_1'M_2'^+$, or a salt thereof,



$XM_1'M_2'^+$

wherein,

X is a thiol reactive group selected from the group consisting of a halide, a disulfide exchange group or a vinyl group;

M_1' is $-R_1CH(R_3)R_2$, where R_1 is selected from $-(CH_2)_n-$, -Y- or $-(CH_2)_nY-$; R_2 is selected from $-(CH_2)_nH$, $-C_6H_5$, $-CH_2C_6H_5$, $-NH_2$, -YH, $-Y(CH_2)_nH$, $-YC_6H_5$, $-YCH_2C_6H_5$; and R_3 is $-(CH_2)_n-$, and is isotopically encoded by incorporation of one or more of ^2H, ^{13}C, ^{15}N or ^{18}O;

n is from 1 to 3 inclusive;

Y is selected from CONH, NHCO and COO; and

$M_2'^+$ is attached to the R_3 group of M_1' and is selected from the group consisting of a tertiary alkyl sulfonium ion, $-S^+CH_3R''$, where R'' is selected from $-CH_2COC_6H_5$, $-CH_2COC_6H_5CH_3$, and $-CH_2COC_6H_5CH_2CH_3$, or a quaternary alkyl ammonium ion $-N^+(R''')_3$, or a quaternary alkyl phosphonium ion $-P^+(R''')_3$, where R'''_3 is $-(CH_2)_nH$ (where n = 1 to 3), $-C_6H_5$, $-CH_2C_6H_5$ and is isotopically encoded by incorporation of one or more of ^2H, ^{13}C, ^{15}N or ^{18}O.

The halide is preferably -Cl, -Br, or - I. The disulfide exchange group may be selected from -S-S-R′ where R′ is $-C_6H_5$, 3-carboxyl-4-nitrophenyl, 2,4-dinitrophenyl, 4-nitrophenyl, 2-nitrophenyl, 2-pyridyl, 5-nitropyridyl, 3-nitropyridyl, methanesulfonyl. Where X is a vinyl group it may be $-CH=CH_2$.

In a preferred embodiment, the present invention provides a compound of formula $XM_1'M_2'^+$, or a salt thereof, wherein,

M_1' is $-R_1CH(R_3)R_2$, where R_1 is $-(CH_2)_n-$; R_2 is -YH, $-Y(CH_2)_nH$, $-YC_6H_5$, $-YCH_2C_6H_5$; and R_3 is $-(CH_2)_n-$, and is isotopically encoded by incorporation of one or more of ^2H, ^{13}C, ^{15}N or ^{18}O;

n is from 1 to 3 inclusive;

Y is CONH; and

X and $M_2'^+$ are as defined above.

In a further preferred embodiment, the present invention provides a compound of formula $XM_1'M_2'^+$, or a salt thereof, wherein,

M_1' is $-R_1CH(R_3)R_2$, where R_1 is -Y- or $-(CH_2)_nY-$; R_2 is $-(CH_2)_nH$, $-NH_2$, $-Y(CH_2)_nH$, $-YC_6H_5$, $-YCH_2C_6H_5$; and R_3 is $-(CH_2)_n-$, and is isotopically encoded by incorporation of one or more of ^2H, ^{13}C, ^{15}N or ^{18}O;

n is from 1 to 3 inclusive;

Y is CONH; and

X and $M_2'^+$ are as defined above.

In a further preferred embodiment, the present invention provides a compound of formula $XM_1'M_2'^+$, or a salt thereof, wherein,

M_1' is $-R_1CH(R_3)R_2$, where R_1 is $-(CH_2)_n-$; R_2 is -YH, $-Y(CH_2)_nH$, $-YC_6H_5$, $-YCH_2C_6H_5$; and R_3 is $-(CH_2)_n-$, and is isotopically encoded by incorporation of one or more of ^2H, ^{13}C, ^{15}N or ^{18}O;

n is from 1 to 3 inclusive;

Y is NHCO; and

X and $M_2'^+$ are as defined above.

In a further preferred embodiment, the present invention provides a compound of formula $XM_1'M_2'^+$, or a salt thereof, wherein,

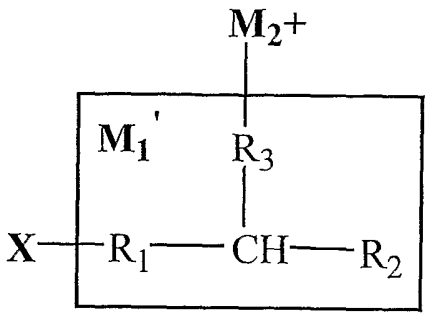
M_1' is $-R_1CH(R_3)R_2$, where R_1 is -Y- or $-(CH_2)_nY-$; R_2 is $-(CH_2)_nH$, $-NH_2$, $-Y(CH_2)_nH$, $-YC_6H_5$, $-YCH_2C_6H_5$; and R_3 is $-(CH_2)_n-$, and is isotopically encoded by incorporation of one or more of ^2H, ^{13}C, ^{15}N or ^{18}O;

n is from 1 to 3 inclusive;

Y is NHCO; and

X and $M_2'^+$ are as defined above

In another particular embodiment, the present invention provides a compound of formula $XM_1'M_2^+$, or a salt thereof,



$XM_1'M_2^+$

wherein,

X is an amino reactive group selected from the group consisting of an acid anhydride, an active ester, an acid halide, a sulfonylhalide, a substituted O-methyl isourea, an isocyanate or an isothiocyanate,

M_1' is $-R_1CH(R_3)R_2$, where R_1 is selected from $-(CH_2)_n-$, -Y- or $-(CH_2)_nY-$; R_2 is selected from $-(CH_2)_nH$, $-C_6H_5$, $-CH_2C_6H_5$, $-NH_2$, -YH, $-Y(CH_2)_nH$, $-YC_6H_5$, $-YCH_2C_6H_5$; and R_3 is $-(CH_2)_n-$, and is isotopically encoded by incorporation of one or more of ^2H, ^{13}C, ^{15}N or ^{18}O;

n is from 1 to 3 inclusive;

Y is selected from CONH, NHCO, COO and COS; and

M_2^+ is attached to the R_3 group of M_1 and is selected from the group consisting of a tertiary alkyl sulfonium ion, $-S^+CH_3R''$, where R'' is selected from $-CH_2COC_6H_5$, $-CH_2COC_6H_5CH_3$, and $-CH_2COC_6H_5CH_2CH_3$, or a quaternary alkyl ammonium ion $-N^+(R''')_3$, or a quaternary alkyl phosphonium ion $-P^+(R''')_3$, where R'''_3 is $-(CH_2)_nH$ (where n = 1 to 3), $-C_6H_5$, $-CH_2C_6H_5$.

In a preferred embodiment, the present invention provides a compound of formula $XM_1'M_2^+$, or a salt thereof, wherein,

M_1' is $-R_1CH(R_3)R_2$, where R_1 is $-(CH_2)_n-$; R_2 is -YH, $-Y(CH_2)_nH$, $-YC_6H_5$, $-YCH_2C_6H_5$; and R_3 is $-(CH_2)_n-$, and is isotopically encoded by incorporation of one or more of ^2H, ^{13}C, ^{15}N or ^{18}O;

n is from 1 to 3 inclusive;

Y is CONH; and

X and M_2^+ are as defined above.

In a further preferred embodiment, the present invention provides a compound of formula $XM_1'M_2^+$, or a salt thereof, wherein,

M_1' is $-R_1CH(R_3)R_2$, where R_1 is -Y- or $-(CH_2)_nY-$; R_2 is $-(CH_2)_nH$, $-NH_2$, $-Y(CH_2)_nH$, $-YC_6H_5$, $-YCH_2C_6H_5$; and R_3 is $-(CH_2)_n-$, and is isotopically encoded by incorporation of one or more of ^2H, ^{13}C, ^{15}N or ^{18}O;

n is from 1 to 3 inclusive;

Y is CONH; and

X and M_2^+ are as defined above.

In a further preferred embodiment, the present invention provides a compound of formula $XM_1'M_2^+$, or a salt thereof, wherein,

M_1' is $-R_1CH(R_3)R_2$, where R_1 is $-(CH_2)_n-$; R_2 is -YH, $-Y(CH_2)_nH$, $-YC_6H_5$, $-YCH_2C_6H_5$; and R_3 is $-(CH_2)_n-$, and is isotopically encoded by incorporation of one or more of ^2H, ^{13}C, ^{15}N or ^{18}O;

n is from 1 to 3 inclusive;

Y is NHCO; and

X and M_2^+ are as defined above.

In a further preferred embodiment, the present invention provides a compound of formula $XM_1'M_2^+$, or a salt thereof, wherein,

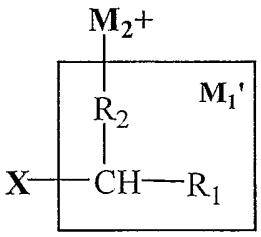
M_1' is $-R_1CH(R_3)R_2$, where R_1 is -Y- or $-(CH_2)_nY-$; R_2 is $-(CH_2)_nH$, $-NH_2$, $-Y(CH_2)_nH$, $-YC_6H_5$, $-YCH_2C_6H_5$; and R_3 is $-(CH_2)_n-$, and is isotopically encoded by incorporation of one or more of ^2H, ^{13}C, ^{15}N or ^{18}O;

n is from 1 to 3 inclusive;

Y is NHCO; and

X and M_2^+ are as defined above.

In another particular embodiment, the present invention provides a compound of formula $XM_1'M_2^+$, or a salt thereof,



$XM_1'M_2^+$

wherein,

X is an amino reactive group selected from the group consisting of an acid anhydride, an active ester, an acid halide, a sulfonylhalide, a substituted O-methyl isourea, an isocyanate or an isothiocyanate,

M_1' is $-CH(R_2)R_1$, where R_1 is selected from $-(CH_2)_nH$, $-C_6H_5$, $-CH_2C_6H_5$, $-NH_2$, -YH, $-Y(CH_2)_nH$, $-YC_6H_5$, $-YCH_2C_6H_5$; and R_2 is $-(CH_2)_n-$, and is isotopically encoded by incorporation of one or more of ^2H, ^{13}C, ^{15}N or ^{18}O;

n is from 1 to 3 inclusive;

Y is selected from CONH, NHCO, COO and COS; and

M_2^+ is attached to the R_2 group of M_1 and is selected from the group consisting of a tertiary alkyl sulfonium ion, $-S^+CH_3R''$, where R'' is selected from $-CH_2COC_6H_5$, $-CH_2COC_6H_5CH_3$, and $-CH_2COC_6H_5CH_2CH_3$, or a quaternary alkyl ammonium ion $-N^+(R''')_3$, or a quaternary alkyl phosphonium ion $-P^+(R''')_3$, where R'''_3 is $-(CH_2)_nH$ (where n = 1 to 3), $-C_6H_5$, $-CH_2C_6H_5$; and

In a preferred embodiment, the present invention provides a compound of formula $XM_1'M_2^+$, or a salt thereof, wherein,

M_1' is $-CH(R_2)R_1$, where R_1 is $-(CH_2)_nH$, $-C_6H_5$, $-CH_2C_6H_5$, $-NH_2$, -YH, $-Y(CH_2)_nH$, $-YC_6H_5$, $-YCH_2C_6H_5$; and R_2 is $-(CH_2)_n-$, and is isotopically encoded by incorporation of one or more of ^2H, ^{13}C, ^{15}N or ^{18}O;

n is from 1 to 3 inclusive;

Y is CONH; and

X and M_2^+ are as defined above.

In a further preferred embodiment, the present invention provides a compound of formula $XM_1'M_2^+$, or a salt thereof, wherein,

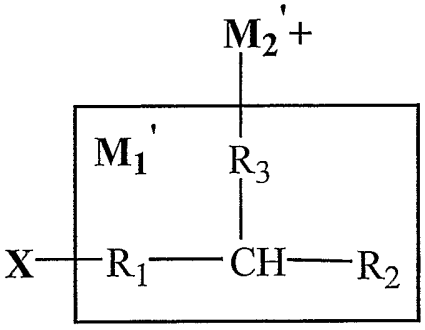
M_1' is $-CH(R_2)R_1$, where R_1 is $-(CH_2)_nH$, $-C_6H_5$, $-CH_2C_6H_5$, $-NH_2$, $-YH$, $-Y(CH_2)_nH$, $-YC_6H_5$, $-YCH_2C_6H_5$; and R_2 is $-(CH_2)_n-$, and is isotopically encoded by incorporation of one or more of ^2H, ^{13}C, ^{15}N or ^{18}O;

n is from 1 to 3 inclusive;

Y is NHCO; and

X and M_2^+ are as defined above.

In another particular embodiment, the present invention provides a compound of formula $XM_1'M_2'^+$, or a salt thereof,



$XM_1'M_2'^+$

wherein,

X is an amino reactive group selected from the group consisting of an acid anhydride, an active ester, an acid halide, a sulfonylhalide, a substituted O-methyl isourea, an isocyanate or an isothiocyanate,

M_1' is $-R_1CH(R_3)R_2$, where R_1 is selected from $-(CH_2)_n-$, -Y- or $-(CH_2)_nY-$; R_2 is selected from $-(CH_2)_nH$, $-C_6H_5$, $-CH_2C_6H_5$, $-NH_2$, $-YH$, $-Y(CH_2)_nH$, $-YC_6H_5$, $-YCH_2C_6H_5$; and R_3 is $-(CH_2)_n-$, and is isotopically encoded by incorporation of one or more of ^2H, ^{13}C, ^{15}N or ^{18}O;

n is from 1 to 3 inclusive;

Y is selected from CONH, NHCO, COO and COS; and

$M_2'^+$ is attached to the R_3 group of M_1 and is selected from the group consisting of a tertiary alkyl sulfonium ion, $-S^+CH_3R''$, where R'' is selected from $-CH_2COC_6H_5$, $-CH_2COC_6H_5CH_3$, and $-CH_2COC_6H_5CH_2CH_3$, or a quaternary alkyl ammonium ion $-N^+(R''')_3$, or a quaternary alkyl phosphonium ion $-P^+(R''')_3$, where R'''_3

is $-(CH_2)_nH$ (where n = 1 to 3), $-C_6H_5$, $-CH_2C_6H_5$; and is isotopically encoded by incorporation of one or more of ^2H, ^{13}C, ^{15}N or ^{18}O.

In a preferred embodiment, the present invention provides a compound of formula $XM_1'M_2'^+$, or a salt thereof, wherein,

M_1' is $-R_1CH(R_3)R_2$, where R_1 is $-(CH_2)_n-$; R_2 is -YH, $-Y(CH_2)_nH$, $-YC_6H_5$, $-YCH_2C_6H_5$; and R_3 is $-(CH_2)_n-$, and is isotopically encoded by incorporation of one or more of ^2H, ^{13}C, ^{15}N or ^{18}O;

n is from 1 to 3 inclusive;

Y is CONH; and

X and $M_2'^+$ are as defined above.

In a further preferred embodiment, the present invention provides a compound of formula $XM_1'M_2'^+$, or a salt thereof, wherein,

M_1' is $-R_1CH(R_3)R_2$, where R_1 is -Y- or $-(CH_2)_nY-$; R_2 is $-(CH_2)_nH$, $-NH_2$, $-Y(CH_2)_nH$, $-YC_6H_5$, $-YCH_2C_6H_5$; and R_3 is $-(CH_2)_n-$, and is isotopically encoded by incorporation of one or more of ^2H, ^{13}C, ^{15}N or ^{18}O;

n is from 1 to 3 inclusive;

Y is CONH; and

X and $M_2'^+$ are as defined above.

In a further preferred embodiment, the present invention provides a compound of formula $XM_1'M_2'^+$, or a salt thereof, wherein,

M_1' is $-R_1CH(R_3)R_2$, where R_1 is $-(CH_2)_n-$; R_2 is -YH, $-Y(CH_2)_nH$, $-YC_6H_5$, $-YCH_2C_6H_5$; and R_3 is $-(CH_2)_n-$, and is isotopically encoded by incorporation of one or more of ^2H, ^{13}C, ^{15}N or ^{18}O;

n is from 1 to 3 inclusive;

Y is NHCO; and

X and $M_2'^+$ are as defined above.

In a further preferred embodiment, the present invention provides a compound of formula $XM_1'M_2'^+$, or a salt thereof, wherein,

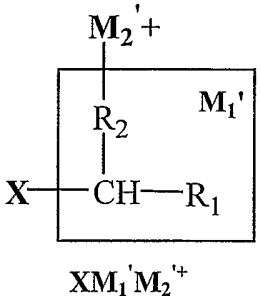
M_1 is $-R_1CH(R_3)R_2$, where R_1 is -Y- or $-(CH_2)_nY-$; R_2 is $-(CH_2)_nH$, $-NH_2$, $-Y(CH_2)_nH$, $-YC_6H_5$, $-YCH_2C_6H_5$; and R_3 is $-(CH_2)_n-$, and is isotopically encoded by incorporation of one or more of ^2H, ^{13}C, ^{15}N or ^{18}O;

n is from 1 to 3 inclusive;

Y is NHCO; and

X and $M_2^{'+}$ are as defined above.

In another particular embodiment, the present invention provides a compound of formula $XM_1'M_2^+$, or a salt thereof,



$XM_1'M_2^{'+}$

wherein,

X is an amino reactive group selected from the group consisting of an acid anhydride, an active ester, an acid halide, a sulfonylhalide, a substituted O-methyl isourea, an isocyanate or an isothiocyanate;

M_1' is $-CH(R_2)R_1$, where R_1 is selected from $-(CH_2)_nH$, $-C_6H_5$, $-CH_2C_6H_5$, $-NH_2$, -YH, $-Y(CH_2)_nH$, $-YC_6H_5$, $-YCH_2C_6H_5$; and R_2 is $-(CH_2)_n-$, and is isotopically encoded by incorporation of one or more of ^2H, ^{13}C, ^{15}N or ^{18}O;

n is from 1 to 3 inclusive;

Y is selected from CONH, NHCO, COO and COS; and

$M_2^{'+}$ is attached to the R_2 group of M_1 and is selected from the group consisting of a tertiary alkyl sulfonium ion, $-S^+CH_3R''$, where R'' is selected from $-CH_2COC_6H_5$, $-CH_2COC_6H_5CH_3$, and $-CH_2COC_6H_5CH_2CH_3$, or a quaternary alkyl ammonium ion $-N^+(R''')_3$, or a quaternary alkyl phosphonium ion $-P^+(R''')_3$, where R'''_3 is $-(CH_2)_nH$ (where n = 1 to 3), $-C_6H_5$, $-CH_2C_6H_5$; and is isotopically encoded by incorporation of one or more of ^2H, ^{13}C, ^{15}N or ^{18}O.

In a preferred embodiment, the present invention provides a compound of formula $XM_1'M_2'^+$, or a salt thereof, wherein,

M_1' is $-CH(R_2)R_1$, where R_1 is $-(CH_2)_nH$, $-C_6H_5$, $-CH_2C_6H_5$, $-NH_2$, -YH, $-Y(CH_2)_nH$, $-YC_6H_5$, $-YCH_2C_6H_5$; and R_2 is $-(CH_2)_n-$, and is isotopically encoded by incorporation of one or more of ^2H, ^{13}C, ^{15}N or ^{18}O;

n is from 1 to 3 inclusive;

Y is CONH; and

X and $M_2'^+$ are as defined above.

In a further preferred embodiment, the present invention provides a compound of formula $XM_1'M_2'^+$, or a salt thereof, wherein,

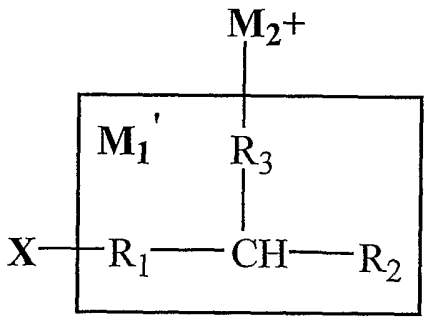
M_1' is $-CH(R_2)R_1$, where R_1 is $-(CH_2)_nH$, $-C_6H_5$, $-CH_2C_6H_5$, $-NH_2$, -YH, $-Y(CH_2)_nH$, $-YC_6H_5$, $-YCH_2C_6H_5$; and R_2 is $-(CH_2)_n-$, and is isotopically encoded by incorporation of one or more of ^2H, ^{13}C, ^{15}N or ^{18}O;

n is from 1 to 3 inclusive;

Y is NHCO; and

X and $M_2'^+$ are as defined above.

In another particular embodiment, the present invention provides a compound of formula $XM_1'M_2^+$, or a salt thereof,



$XM_1'M_2^+$

wherein,

X is a guanidino specific reactive group selected from the group consisting of a substituted 2,3-butanedione, a substituted 2,4-pentanedione, a substituted glyoxal, or a substituted phenylglyoxal;

M_1' is $-R_1CH(R_3)R_2$, where R_1 is selected from $-(CH_2)_n-$, -Y- or $-(CH_2)_nY-$; R_2 is selected from $-(CH_2)_nH$, $-C_6H_5$, $-CH_2C_6H_5$, $-NH_2$, -YH, $-Y(CH_2)_nH$, $-YC_6H_5$, $-YCH_2C_6H_5$; and R_3 is $-(CH_2)_n-$, and is isotopically encoded by incorporation of one or more of ^2H, ^{13}C, ^{15}N or ^{18}O;

n is from 1 to 3 inclusive;

Y is selected from CONH, NHCO, COO and COS; and

M_2^+ is attached to the R_3 group of M_1 and is selected from the group consisting of a tertiary alkyl sulfonium ion, $-S^+CH_3R''$, where R'' is selected from $-CH_2COC_6H_5$, $-CH_2COC_6H_5CH_3$, and $-CH_2COC_6H_5CH_2CH_3$, or a quaternary alkyl ammonium ion $-N^+(R''')_3$, or a quaternary alkyl phosphonium ion $-P^+(R''')_3$, where R'''_3 is $-(CH_2)_nH$ (where n = 1 to 3), $-C_6H_5$, $-CH_2C_6H_5$.

In a preferred embodiment, the present invention provides a compound of formula $XM_1'M_2^+$, or a salt thereof, wherein,

M_1' is $-R_1CH(R_3)R_2$, where R_1 is $-(CH_2)_n-$; R_2 is -YH, $-Y(CH_2)_nH$, $-YC_6H_5$, $-YCH_2C_6H_5$; and R_3 is $-(CH_2)_n-$, and is isotopically encoded by incorporation of one or more of ^2H, ^{13}C, ^{15}N or ^{18}O;

n is from 1 to 3 inclusive;

Y is CONH; and

X and M_2^+ are as defined above.

In a further preferred embodiment, the present invention provides a compound of formula $XM_1'M_2^+$, or a salt thereof, wherein,

M_1' is $-R_1CH(R_3)R_2$, where R_1 is -Y- or $-(CH_2)_nY-$; R_2 is $-(CH_2)_nH$, $-NH_2$, $-Y(CH_2)_nH$, $-YC_6H_5$, $-YCH_2C_6H_5$; and R_3 is $-(CH_2)_n-$, and is isotopically encoded by incorporation of one or more of ^2H, ^{13}C, ^{15}N or ^{18}O;

n is from 1 to 3 inclusive;

Y is CONH; and

X and M_2^+ are as defined above.

In a further preferred embodiment, the present invention provides a compound of formula $XM_1'M_2^+$, or a salt thereof, wherein,

M_1' is $-R_1CH(R_3)R_2$, where R_1 is $-(CH_2)_n-$; R_2 is -YH, $-Y(CH_2)_nH$, $-YC_6H_5$, $-YCH_2C_6H_5$; and R_3 is $-(CH_2)_n-$, and is isotopically encoded by incorporation of one or more of ^2H, ^{13}C, ^{15}N or ^{18}O;

n is from 1 to 3 inclusive;

Y is NHCO; and

X and M_2^+ are as defined above.

In a further preferred embodiment, the present invention provides a compound of formula $XM_1'M_2^+$, or a salt thereof, wherein,

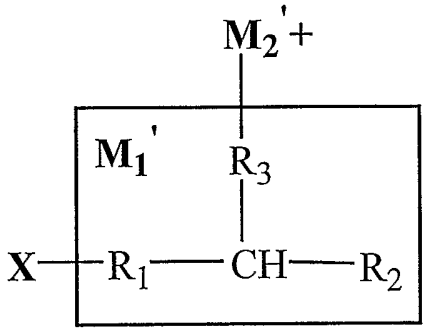
M_1' is $-R_1CH(R_3)R_2$, where R_1 is -Y- or $-(CH_2)_nY-$; R_2 is $-(CH_2)_nH$, $-NH_2$, $-Y(CH_2)_nH$, $-YC_6H_5$, $-YCH_2C_6H_5$; and R_3 is $-(CH_2)_n-$, and is isotopically encoded by incorporation of one or more of ^2H, ^{13}C, ^{15}N or ^{18}O;

n is from 1 to 3 inclusive;

Y is NHCO; and

X and M_2^+ are as defined above.

In another particular embodiment, the present invention provides a compound of formula $XM_1'M_2'^+$, or a salt thereof,



$XM_1'M_2'^+$

wherein,

X is a guanidino specific reactive group selected from the group consisting of a substituted 2,3-butanedione, a substituted 2,4-pentanedione, a substituted glyoxal, or a substituted phenylglyoxal;

M_1 is $-R_1CH(R_3)R_2$, where R_1 is selected from $-(CH_2)_n-$, Y- or $-(CH_2)_nY-$; R_2 is selected from $-(CH_2)_nH$, $-C_6H_5$, $-CH_2C_6H_5$, $-NH_2$, -YH, $-Y(CH_2)_nH$, $-YC_6H_5$, $-YCH_2C_6H_5$; and R_3 is $-(CH_2)_n-$, and is isotopically encoded by incorporation of one or more of ^2H, ^{13}C, ^{15}N or ^{18}O;

n is from 1 to 3 inclusive;

Y is selected from CONH, NHCO, COO and COS; and

$M_2^{'+}$ is attached to the R_3 group of M_1 and is selected from the group consisting of a tertiary alkyl sulfonium ion, $-S^+CH_3R''$, where R'' is selected from $-CH_2COC_6H_5$, $-CH_2COC_6H_5CH_3$, and $-CH_2COC_6H_5CH_2CH_3$, or a quaternary alkyl ammonium ion $-N^+(R''')_3$, or a quaternary alkyl phosphonium ion $-P^+(R''')_3$, where R'''_3 is $-(CH_2)_nH$ (where n = 1 to 3),
$-C_6H_5$, $-CH_2C_6H_5$; and is isotopically encoded by incorporation of one or more of ^2H, ^{13}C, ^{15}N or ^{18}O.

In a preferred embodiment, the present invention provides a compound of formula $XM_1'M_2^{'+}$, or a salt thereof, wherein,

M_1' is $-R_1CH(R_3)R_2$, where R_1 is $-(CH_2)_n-$; R_2 is -YH, $-Y(CH_2)_nH$, $-YC_6H_5$, $-YCH_2C_6H_5$; and R_3 is $-(CH_2)_n-$, and is isotopically encoded by incorporation of one or more of ^2H, ^{13}C, ^{15}N or ^{18}O;

n is from 1 to 3 inclusive;

Y is CONH; and

X and $M_2^{'+}$ are as defined above.

In a further preferred embodiment, the present invention provides a compound of formula $XM_1'M_2^{'+}$, or a salt thereof, wherein,

M_1' is $-R_1CH(R_3)R_2$, where R_1 is -Y- or $-(CH_2)_nY-$; R_2 is $-(CH_2)_nH$, $-NH_2$, $-Y(CH_2)_nH$, $-YC_6H_5$, $-YCH_2C_6H_5$; and R_3 is $-(CH_2)_n-$, and is isotopically encoded by incorporation of one or more of ^2H, ^{13}C, ^{15}N or ^{18}O;

n is from 1 to 3 inclusive;

Y is CONH; and

X and $M_2^{'+}$ are as defined above.

In a further preferred embodiment, the present invention provides a compound of formula $XM_1'M_2'^+$, or a salt thereof, wherein,

M_1' is $-R_1CH(R_3)R_2$, where R_1 is $-(CH_2)_n-$; R_2 is -YH, $-Y(CH_2)_nH$, $-YC_6H_5$, $-YCH_2C_6H_5$; and R_3 is $-(CH_2)_n-$, and is isotopically encoded by incorporation of one or more of ^2H, ^{13}C, ^{15}N or ^{18}O;

n is from 1 to 3 inclusive;

Y is NHCO; and

X and $M_2'^+$ are as defined above.

In a further preferred embodiment, the present invention provides a compound of formula $XM_1'M_2'^+$, or a salt thereof, wherein,

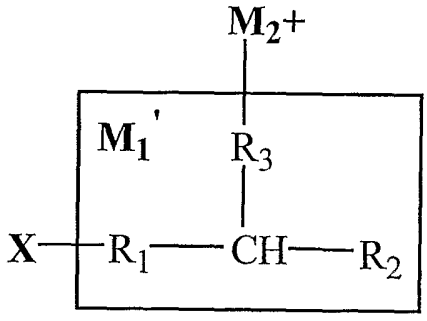
M_1' is $-R_1CH(R_3)R_2$, where R_1 is -Y- or $-(CH_2)_nY-$; R_2 is $-(CH_2)_nH$, $-NH_2$, $-Y(CH_2)_nH$, $-YC_6H_5$, $-YCH_2C_6H_5$; and R_3 is $-(CH_2)_n-$, and is isotopically encoded by incorporation of one or more of ^2H, ^{13}C, ^{15}N or ^{18}O;

n is from 1 to 3 inclusive;

Y is NHCO; and

X and $M_2'^+$ are as defined above.

In another particular embodiment, the present invention provides a compound of formula $XM_1'M_2^+$, or a salt thereof, for specific reaction with the C2-indole position of the side chain of tryptophan or tryptophan containing proteins or peptides,



$XM_1'M_2^+$

wherein,

X is a reactive group specific to the C2-indole position of the side chain of tryptophan selected from a halide or a dimethyl sulfonium ion, wherein the halide is preferably

-Cl, -Br, or – I, and wherein the dimethyl sulfonium ion is preferably $-S(CH_3)_2^+$;

M_1' is $-R_1CH(R_3)R_2$, where R_1 is selected from a -2-hydroxy-5-nitrobenzyl-, or -(2-hydroxy-5-nitrobenzyl)-4-Y- group; R_2 is selected from $-(CH_2)_nH$, $-C_6H_5$, $-CH_2C_6H_5$, $-NH_2$, -YH, $-Y(CH_2)_nH$, $-YC_6H_5$, $-YCH_2C_6H_5$, and R_3 is $-(CH_2)_n-$, and is isotopically encoded by incorporation of one or more of ^2H, ^{13}C, ^{15}N or ^{18}O;

n is from 1 to 3 inclusive;

Y is selected from CONH, NHCO, COO or COS; and

M_2^+ is attached to the R_3 group of M_1 and is selected from the group consisting of a tertiary alkyl sulfonium ion, $-S^+CH_3R''$, where R'' is selected from $-CH_2COC_6H_5$, $-CH_2COC_6H_5CH_3$, and $-CH_2COC_6H_5CH_2CH_3$, or a quaternary alkyl ammonium ion $-N^+(R''')_3$, or a quaternary alkyl phosphonium ion $-P^+(R''')_3$, where R'''_3 is $-(CH_2)_nH$ (where n = 1 to 3), $-C_6H_5$, $-CH_2C_6H_5$.

In a preferred embodiment, the present invention provides a compound of formula $XM_1'M_2^+$, or a salt thereof, wherein,

M_1' is $-R_1CH(R_3)R_2$, where R_1 is -2-hydroxy-5-nitrobenzyl-; R_2 is -YH, $-Y(CH_2)_nH$, $-YC_6H_5$, $-YCH_2C_6H_5$; and R_3 is $-(CH_2)_n-$, and is isotopically encoded by incorporation of one or more of ^2H, ^{13}C, ^{15}N or ^{18}O;

n is from 1 to 3 inclusive;

Y is CONH; and

X and M_2^+ are as defined above.

In a further preferred embodiment, the present invention provides a compound of formula $XM_1'M_2^+$, or a salt thereof, wherein,

M_1' is $-R_1CH(R_3)R_2$, where R_1 is -(2-hydroxy-5-nitrobenzyl)-4-Y-; R_2 is $-(CH_2)_nH$, $-C_6H_5$, $-CH_2C_6H_5$, $-NH_2$, -YH, $-Y(CH_2)_nH$, $-YC_6H_5$, $-YCH_2C_6H_5$; and R_3 is $-(CH_2)_n-$, and is isotopically encoded by incorporation of one or more of ^2H, ^{13}C, ^{15}N or ^{18}O;

n is from 1 to 3 inclusive;

Y is CONH; and

X and M_2^+ are as defined above.

In a further preferred embodiment, the present invention provides a compound of formula $XM_1'M_2^+$, or a salt thereof, wherein,

M_1' is $-R_1CH(R_3)R_2$, where R_1 is -2-hydroxy-5-nitrobenzyl-; R_2 is -YH, -$Y(CH_2)_nH$, -YC_6H_5, -$YCH_2C_6H_5$; and R_3 is -$(CH_2)_n$-, and is isotopically encoded by incorporation of one or more of ^2H, ^{13}C, ^{15}N or ^{18}O;

n is from 1 to 3 inclusive;

Y is NHCO; and

X and M_2^+ are as defined above.

In a further preferred embodiment, the present invention provides a compound of formula $XM_1'M_2^+$, or a salt thereof, wherein,

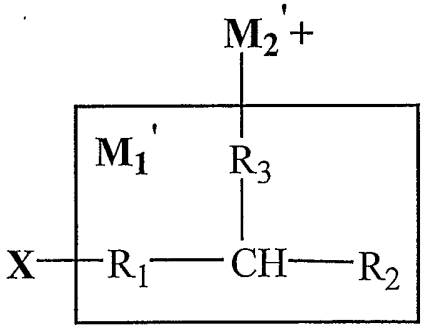
M_1' is $-R_1CH(R_3)R_2$, where R_1 is -(2-hydroxy-5-nitrobenzyl)-4-Y-; R_2 is -$(CH_2)_nH$, -C_6H_5, -$CH_2C_6H_5$, -NH_2, -YH, -$Y(CH_2)_nH$, -YC_6H_5, -$YCH_2C_6H_5$; and R_3 is -$(CH_2)_n$-, and is isotopically encoded by incorporation of one or more of ^2H, ^{13}C, ^{15}N or ^{18}O;

n is from 1 to 3 inclusive;

Y is NHCO; and

X and M_2^+ are as defined above.

In another particular embodiment, the present invention provides a compound of formula $XM_1'M_2'^+$, or a salt thereof, for specific reaction with the C2-indole position of the side chain of tryptophan or tryptophan containing proteins or peptides,



$XM_1'M_2'^+$

wherein,

X is a reactive group specific to the C2-indole position of the side chain of tryptophan selected from a halide or a dimethyl sulfonium ion, wherein the halide is preferably -Cl, -Br, or – I, and wherein the dimethyl sulfonium ion is preferably -$S(CH_3)_2^+$.

M_1' is $-R_1CH(R_3)R_2$, where R_1 is selected from a -2-hydroxy-5-nitrobenzyl-, or -(2-hydroxy-5-nitrobenzyl)-4-Y- group; R_2 is selected from $-(CH_2)_nH$, $-C_6H_5$, $-CH_2C_6H_5$, $-NH_2$,

-YH, $-Y(CH_2)_nH$, $-YC_6H_5$, $-YCH_2C_6H_5$, and R_3 is $-(CH_2)_n-$, and is isotopically encoded by incorporation of one or more of ^2H, ^{13}C, ^{15}N or ^{18}O;

n is from 1 to 3 inclusive;

Y is selected from CONH, NHCO, COO or COS; and

$M_2'^+$ is attached to the R_3 group of M_1 and is selected from the group consisting of a tertiary alkyl sulfonium ion, $-S^+CH_3R''$, where R'' is selected from -$CH_2COC_6H_5$, $-CH_2COC_6H_5CH_3$, and $-CH_2COC_6H_5CH_2CH_3$, or a quaternary alkyl ammonium ion $-N^+(R''')_3$, or a quaternary alkyl phosphonium ion $-P^+(R''')_3$, where R'''_3 is $-(CH_2)_nH$ (where n = 1 to 3),

$-C_6H_5$, $-CH_2C_6H_5$; and is isotopically encoded by incorporation of one or more of ^2H, ^{13}C, ^{15}N or ^{18}O.

In a preferred embodiment, the present invention provides a compound of formula $XM_1'M_2'^+$, or a salt thereof, wherein,

M_1' is $-R_1CH(R_3)R_2$, where R_1 is -2-hydroxy-5-nitrobenzyl-; R_2 is -YH, $-Y(CH_2)_nH$, $-YC_6H_5$, $-YCH_2C_6H_5$; and R_3 is $-(CH_2)_n-$, and is isotopically encoded by incorporation of one or more of ^2H, ^{13}C, ^{15}N or ^{18}O;

n is from 1 to 3 inclusive;

Y is CONH; and

X and $M_2'^+$ are as defined above.

In a further preferred embodiment, the present invention provides a compound of formula $XM_1'M_2'^+$, or a salt thereof, wherein,

M_1' is $-R_1CH(R_3)R_2$, where R_1 is -(2-hydroxy-5-nitrobenzyl)-4-Y-; R_2 is $-(CH_2)_nH$,

$-C_6H_5$, $-CH_2C_6H_5$, $-NH_2$, -YH, $-Y(CH_2)_nH$, $-YC_6H_5$, $-YCH_2C_6H_5$; and R_3 is $-(CH_2)_n-$, and is isotopically encoded by incorporation of one or more of ^2H, ^{13}C, ^{15}N or ^{18}O;

n is from 1 to 3 inclusive;

Y is CONH; and

X and $M_2^{'+}$ are as defined above.

In a further preferred embodiment, the present invention provides a compound of formula $XM_1'M_2^{'+}$, or a salt thereof, wherein,

M_1' is $-R_1CH(R_3)R_2$, where R_1 is -2-hydroxy-5-nitrobenzyl-; R_2 is -YH, $-Y(CH_2)_nH$, $-YC_6H_5$, $-YCH_2C_6H_5$; and R_3 is $-(CH_2)_n-$, and is isotopically encoded by incorporation of one or more of ^2H, ^{13}C, ^{15}N or ^{18}O;

n is from 1 to 3 inclusive;

Y is NHCO; and

X and $M_2^{'+}$ are as defined above.

In a further preferred embodiment, the present invention provides a compound of formula $XM_1'M_2^{'+}$, or a salt thereof, wherein,

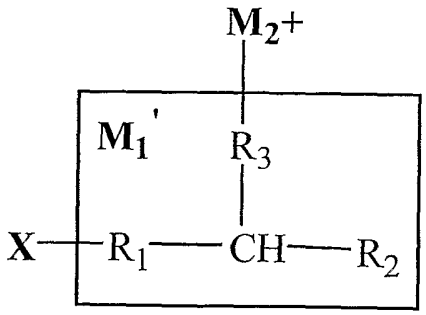
M_1' is $-R_1CH(R_3)R_2$, where R_1 is -(2-hydroxy-5-nitrobenzyl)-4-Y-; R_2 is $-(CH_2)_nH$, $-C_6H_5$, $-CH_2C_6H_5$, $-NH_2$, -YH, $-Y(CH_2)_nH$, $-YC_6H_5$, $-YCH_2C_6H_5$; and R_3 is $-(CH_2)_n-$, and is isotopically encoded by incorporation of one or more of ^2H, ^{13}C, ^{15}N or ^{18}O;

n is from 1 to 3 inclusive;

Y is NHCO; and

X and $M_2^{'+}$ are as defined above.

In another particular embodiment, the present invention provides a compound of formula $XM_1'M_2^+$, or a salt thereof, for specific reaction with the C2-indole position of the side chain of tryptophan or tryptophan containing proteins or peptides,



$XM_1'M_2^+$

wherein,

X is a reactive group specific to the C2-indole position of the side chain of tryptophan selected from a sulfenylhalide, wherein the sulfenylhalide is preferably -SCl, -SBr, or – SI;

M_1' is $-R_1CH(R_3)R_2$, where R_1 is selected from a -2-nitrophenyl- or -(2-nitrophenyl)-4-Y- group; R_2 is selected from $-(CH_2)_nH$, $-C_6H_5$, $-CH_2C_6H_5$, $-NH_2$, -YH, $-Y(CH_2)_nH$, $-YC_6H_5$, $-YCH_2C_6H_5$, and R_3 is $-(CH_2)_n-$, and is isotopically encoded by incorporation of one or more of ^2H, ^{13}C, ^{15}N or ^{18}O;

n is from 1 to 3 inclusive;

Y is selected from CONH, NHCO, COO or COS; and

M_2^+ is attached to the R_3 group of M_1 and is selected from the group consisting of a tertiary alkyl sulfonium ion, $-S^+CH_3R''$, where R'' is selected from $-CH_2COC_6H_5$, $-CH_2COC_6H_5CH_3$, and $-CH_2COC_6H_5CH_2CH_3$, or a quaternary alkyl ammonium ion $-N^+(R''')_3$, or a quaternary alkyl phosphonium ion $-P^+(R''')_3$, where R'''_3 is $-(CH_2)_nH$ (where n = 1 to 3), $-C_6H_5$, $-CH_2C_6H_5$.

In a preferred embodiment, the present invention provides a compound of formula $XM_1'M_2^+$, or a salt thereof, wherein,

M_1' is $-R_1CH(R_3)R_2$, where R_1 is -2-nitrophenyl-; R_2 is -YH, $-Y(CH_2)_nH$, $-YC_6H_5$, $-YCH_2C_6H_5$; and R_3 is $-(CH_2)_n-$. and is isotopically encoded by incorporation of one or more of ^2H, ^{13}C, ^{15}N or ^{18}O;

n is from 1 to 3 inclusive;

Y is CONH; and

X and M_2^+ are as defined above.

In a further preferred embodiment, the present invention provides a compound of formula $XM_1'M_2^+$, or a salt thereof, wherein,

M_1' is $-R_1CH(R_3)R_2$, where R_1 is -(2-nitrophenyl)-4-Y-; R_2 is $-(CH_2)_nH$, $-C_6H_5$, $-CH_2C_6H_5$, $-NH_2$, -YH, $-Y(CH_2)_nH$, $-YC_6H_5$, $-YCH_2C_6H_5$; and R_3 is $-(CH_2)_n-$, and is isotopically encoded by incorporation of one or more of ^2H, ^{13}C, ^{15}N or ^{18}O;

n is from 1 to 3 inclusive;

Y is CONH; and

X and M_2^+ are as defined above.

In a further preferred embodiment, the present invention provides a compound of formula $XM_1'M_2^+$, or a salt thereof, wherein,

M_1' is $-R_1CH(R_3)R_2$, where R_1 is -2-nitrophenyl-; R_2 is -YH, $-Y(CH_2)_nH$, $-YC_6H_5$, $-YCH_2C_6H_5$; and R_3 is $-(CH_2)_n-$, and is isotopically encoded by incorporation of one or more of ^2H, ^{13}C, ^{15}N or ^{18}O;

n is from 1 to 3 inclusive;

Y is NHCO; and

X and M_2^+ are as defined above.

In a further preferred embodiment, the present invention provides a compound of formula $XM_1'M_2^+$, or a salt thereof, wherein,

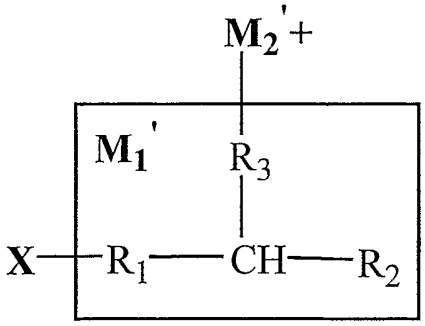
M_1' is $-R_1CH(R_3)R_2$, where R_1 is -(2-nitrophenyl)-4-Y-; R_2 is $-(CH_2)_nH$, $-C_6H_5$, $-CH_2C_6H_5$, $-NH_2$, -YH, $-Y(CH_2)_nH$, $-YC_6H_5$, $-YCH_2C_6H_5$; and R_3 is $-(CH_2)_n-$, and is isotopically encoded by incorporation of one or more of ^2H, ^{13}C, ^{15}N or ^{18}O;

n is from 1 to 3 inclusive;

Y is NHCO; and

X and M_2^+ are as defined above.

In another particular embodiment, the present invention provides a compound of formula $XM_1'M_2^+$, or a salt thereof, for specific reaction with the C2-indole position of the side chain of tryptophan or tryptophan containing proteins or peptides,



$XM_1'M_2'^+$

wherein,

X is a reactive group specific to the C2-indole position of the side chain of tryptophan selected from a sulfenylhalide, wherein the sulfenylhalide is preferably -SCl, -SBr, or – SI;

M_1' is $-R_1CH(R_3)R_2$, where R_1 is selected from a -2-nitrophenyl- or -(2-nitrophenyl)-4-Y- group; R_2 is selected from $-(CH_2)_nH$, $-C_6H_5$, $-CH_2C_6H_5$, $-NH_2$, -YH, $-Y(CH_2)_nH$, $-YC_6H_5$, $-YCH_2C_6H_5$, and R_3 is $-(CH_2)_n-$, and is isotopically encoded by incorporation of one or more of ^2H, ^{13}C, ^{15}N or ^{18}O;

n is from 1 to 3 inclusive;

Y is selected from CONH, NHCO, COO or COS; and

$M_2'^+$ is attached to the R_3 group of M_1 and is selected from the group consisting of a tertiary alkyl sulfonium ion, $-S^+CH_3R''$, where R'' is selected from $-CH_2COC_6H_5$, $-CH_2COC_6H_5CH_3$, and $-CH_2COC_6H_5CH_2CH_3$, or a quaternary alkyl ammonium ion $-N^+(R''')_3$, or a quaternary alkyl phosphonium ion $-P^+(R''')_3$, where R'''_3 is $-(CH_2)_nH$ (where n = 1 to 3), $-C_6H_5$, $-CH_2C_6H_5$; and is isotopically encoded by incorporation of one or more of ^2H, ^{13}C, ^{15}N or ^{18}O.

In a preferred embodiment, the present invention provides a compound of formula $XM_1'M_2'^+$, or a salt thereof, wherein,

M_1' is $-R_1CH(R_3)R_2$, where R_1 is -2-nitrophenyl-; R_2 is -YH, $-Y(CH_2)_nH$, $-YC_6H_5$, $-YCH_2C_6H_5$; and R_3 is $-(CH_2)_n-$. and is isotopically encoded by incorporation of one or more of ^2H, ^{13}C, ^{15}N or ^{18}O;

n is from 1 to 3 inclusive;

Y is CONH; and

X and $M_2^{'+}$ are as defined above.

In a further preferred embodiment, the present invention provides a compound of formula $XM_1'M_2^{'+}$, or a salt thereof, wherein,

M_1' is $-R_1CH(R_3)R_2$, where R_1 is -(2-nitrophenyl)-4-Y-; R_2 is $-(CH_2)_nH$, $-C_6H_5$, $-CH_2C_6H_5$, $-NH_2$, -YH, $-Y(CH_2)_nH$, $-YC_6H_5$, $-YCH_2C_6H_5$; and R_3 is $-(CH_2)_n-$, and is isotopically encoded by incorporation of one or more of ^2H, ^{13}C, ^{15}N or ^{18}O;

n is from 1 to 3 inclusive;

Y is CONH; and

X and $M_2^{'+}$ are as defined above.

In a further preferred embodiment, the present invention provides a compound of formula $XM_1'M_2^{'+}$, or a salt thereof, wherein,

M_1' is $-R_1CH(R_3)R_2$, where R_1 is -2-nitrophenyl-; R_2 is -YH, $-Y(CH_2)_nH$, $-YC_6H_5$, $-YCH_2C_6H_5$; and R_3 is $-(CH_2)_n-$, and is isotopically encoded by incorporation of one or more of ^2H, ^{13}C, ^{15}N or ^{18}O;

n is from 1 to 3 inclusive;

Y is NHCO; and

X and $M_2^{'+}$ are as defined above.

In a further preferred embodiment, the present invention provides a compound of formula $XM_1'M_2^{'+}$, or a salt thereof, wherein,

M_1' is $-R_1CH(R_3)R_2$, where R_1 is -(2-nitrophenyl)-4-Y-; R_2 is $-(CH_2)_nH$, $-C_6H_5$, $-CH_2C_6H_5$, $-NH_2$, -YH, $-Y(CH_2)_nH$, $-YC_6H_5$, $-YCH_2C_6H_5$; and R_3 is $-(CH_2)_n-$, and is isotopically encoded by incorporation of one or more of ^2H, ^{13}C, ^{15}N or ^{18}O;

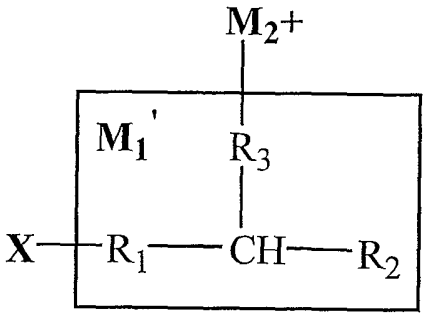
n is from 1 to 3 inclusive;

Y is NHCO; and

X and $M_2^{'+}$ are as defined above.

In another particular embodiment, the present invention provides a compound of formula $XM_1'M_2^+$, or a salt thereof, for specific reaction with dehydroalanine or

dehydroamino-2-butyric acid formed by β-elimination from O-linked phosphorylated or glycosylated serine or threonine, or dehydroalanine or dehydroamino-2-butyric acid residues formed by β-elimination from O-linked phosphorylated or glycosylated serine or threonine containing proteins or peptides respectively,



$XM_1'M_2^+$

wherein,

X is a thiol, wherein the thiol is preferably –SH;

M_1' is $-R_1CH(R_3)R_2$, where R_1 is selected from $-(CH_2)_n-$, -Y- or $-(CH_2)_nY-$; R_2 is selected from $-(CH_2)_nH$, $-C_6H_5$, $-CH_2C_6H_5$, $-NH_2$, -YH, $-Y(CH_2)_nH$, $-YC_6H_5$, $-YCH_2C_6H_5$, and R_3 is $-(CH_2)_n-$, and is isotopically encoded by incorporation of one or more of ^2H, ^{13}C, ^{15}N or ^{18}O;

n is from 1 to 3 inclusive;

Y is selected from CONH, NHCO or COO; and

M_2^+ is attached to the R_3 group of M_1 and is selected from the group consisting of a tertiary alkyl sulfonium ion, $-S^+CH_3R''$, where R'' is selected from $-CH_2COC_6H_5$, $-CH_2COC_6H_5CH_3$, and $-CH_2COC_6H_5CH_2CH_3$, or a quaternary alkyl ammonium ion $-N^+(R''')_3$, or a quaternary alkyl phosphonium ion $-P^+(R''')_3$, where R'''_3 is $-(CH_2)_nH$ (where n = 1 to 3), $-C_6H_5$, $-CH_2C_6H_5$.

In a preferred embodiment, the present invention provides a compound of formula $XM_1'M_2^+$, or a salt thereof, wherein,

M_1' is $-R_1CH(R_3)R_2$, where R_1 is $-(CH_2)_n-$; R_2 is -YH, $-Y(CH_2)_nH$, $-YC_6H_5$, $-YCH_2C_6H_5$; and R_3 is $-(CH_2)_n-$, and is isotopically encoded by incorporation of one or more of ^2H, ^{13}C, ^{15}N or ^{18}O;

n is from 1 to 3 inclusive;

Y is CONH; and

X and M_2^+ are as defined above.

In a further preferred embodiment, the present invention provides a compound of formula $XM_1'M_2^+$, or a salt thereof, wherein,

M_1' is $-R_1CH(R_3)R_2$, where R_1 is -Y- or $-(CH_2)_nY-$; R_2 is $-(CH_2)_nH$, $-C_6H_5$, $-CH_2C_6H_5$, $-NH_2$, -YH, $-Y(CH_2)_nH$, $-YC_6H_5$, $-YCH_2C_6H_5$; and R_3 is $-(CH_2)_n-$, and is isotopically encoded by incorporation of one or more of ^2H, ^{13}C, ^{15}N or ^{18}O;

n is from 1 to 3 inclusive;

Y is CONH; and

X and M_2^+ are as defined above.

In a further preferred embodiment, the present invention provides a compound of formula $XM_1'M_2^+$, or a salt thereof, wherein,

M_1' is $-R_1CH(R_3)R_2$, where R_1 is $-(CH_2)_n-$; R_2 is -YH, $-Y(CH_2)_nH$, $-YC_6H_5$, $-YCH_2C_6H_5$; and R_3 is $-(CH_2)_n-$, and is isotopically encoded by incorporation of one or more of ^2H, ^{13}C, ^{15}N or ^{18}O;

n is from 1 to 3 inclusive;

Y is NHCO; and

X and M_2^+ are as defined above.

In a further preferred embodiment, the present invention provides a compound of formula $XM_1'M_2^+$, or a salt thereof, wherein,

M_1' is $-R_1CH(R_3)R_2$, where R_1 is -Y- or $-(CH_2)_nY-$; R_2 is $-(CH_2)_nH$, $-C_6H_5$, $-CH_2C_6H_5$, $-NH_2$, -YH, $-Y(CH_2)_nH$, $-YC_6H_5$, $-YCH_2C_6H_5$; and R_3 is $-(CH_2)_n-$, and is isotopically encoded by incorporation of one or more of ^2H, ^{13}C, ^{15}N or ^{18}O;

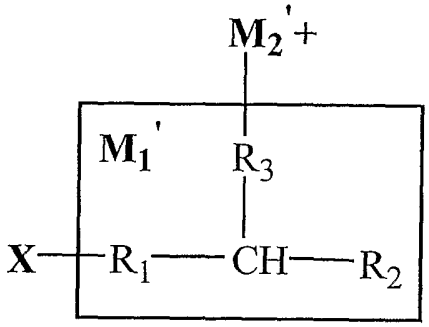
n is from 1 to 3 inclusive;

Y is NHCO; and

X and M_2^+ are as defined above.

In another particular embodiment, the present invention provides a compound of formula $XM_1'M_2'^+$, or a salt thereof, for specific reaction with dehydroalanine or dehydroamino-2-butyric acid formed by β-elimination from O-linked phosphorylated

or glycosylated serine or threonine, or dehydroalanine or dehydroamino-2-butyric acid residues formed by β-elimination from O-linked phosphorylated or glycosylated serine or threonine containing proteins or peptides respectively,



$XM_1'M_2'^+$

wherein,

X is a thiol, wherein the thiol is preferably –SH;

M_1' is $-R_1CH(R_3)R_2$, where R_1 is selected from $-(CH_2)_n-$, -Y- or $-(CH_2)_nY-$; R_2 is selected from $-(CH_2)_nH$, $-C_6H_5$, $-CH_2C_6H_5$, $-NH_2$, -YH, $-Y(CH_2)_nH$, $-YC_6H_5$, $-YCH_2C_6H_5$, and R_3 is $-(CH_2)_n-$, and is isotopically encoded by incorporation of one or more of ^2H, ^{13}C, ^{15}N or ^{18}O;

n is from 1 to 3 inclusive;

Y is selected from CONH, NHCO or COO; and

$M_2'^+$ is attached to the R_3 group of M_1 and is selected from the group consisting of a tertiary alkyl sulfonium ion, $-S^+CH_3R''$, where R'' is selected from $-CH_2COC_6H_5$, $-CH_2COC_6H_5CH_3$, and $-CH_2COC_6H_5CH_2CH_3$, or a quaternary alkyl ammonium ion $-N^+(R''')_3$, or a quaternary alkyl phosphonium ion $-P^+(R''')_3$, where R'''_3 is $-(CH_2)_nH$ (where n = 1 to 3), $-C_6H_5$, $-CH_2C_6H_5$; and is isotopically encoded by incorporation of one or more of ^2H, ^{13}C, ^{15}N or ^{18}O.

In a preferred embodiment, the present invention provides a compound of formula $XM_1'M_2^+$, or a salt thereof, wherein,

M_1' is $-R_1CH(R_3)R_2$, where R_1 is $-(CH_2)_n-$; R_2 is -YH, $-Y(CH_2)_nH$, $-YC_6H_5$, $-YCH_2C_6H_5$; and R_3 is $-(CH_2)_n-$, and is isotopically encoded by incorporation of one or more of ^2H, ^{13}C, ^{15}N or ^{18}O;

n is from 1 to 3 inclusive;

Y is CONH; and

X and $M_2'^+$ are as defined above.

In a further preferred embodiment, the present invention provides a compound of formula $XM_1'M_2'^+$, or a salt thereof, wherein,

M_1' is $-R_1CH(R_3)R_2$, where R_1 is -Y- or $-(CH_2)_nY-$; R_2 is $-(CH_2)_nH$, $-C_6H_5$, $-CH_2C_6H_5$, $-NH_2$, -YH, $-Y(CH_2)_nH$, $-YC_6H_5$, $-YCH_2C_6H_5$; and R_3 is $-(CH_2)_n-$, and is isotopically encoded by incorporation of one or more of ^2H, ^{13}C, ^{15}N or ^{18}O;

n is from 1 to 3 inclusive;

Y is CONH; and

X and $M_2'^+$ are as defined above.

In a further preferred embodiment, the present invention provides a compound of formula $XM_1'M_2'^+$, or a salt thereof, wherein,

M_1' is $-R_1CH(R_3)R_2$, where R_1 is $-(CH_2)_n-$; R_2 is -YH, $-Y(CH_2)_nH$, $-YC_6H_5$, $-YCH_2C_6H_5$; and R_3 is $-(CH_2)_n-$, and is isotopically encoded by incorporation of one or more of ^2H, ^{13}C, ^{15}N or ^{18}O;

n is from 1 to 3 inclusive;

Y is NHCO; and

X and $M_2'^+$ are as defined above.

In a further preferred embodiment, the present invention provides a compound of formula $XM_1'M_2'^+$, or a salt thereof, wherein,

M_1' is $-R_1CH(R_3)R_2$, where R_1 is -Y- or $-(CH_2)_nY-$; R_2 is $-(CH_2)_nH$, $-C_6H_5$, $-CH_2C_6H_5$, $-NH_2$, -YH, $-Y(CH_2)_nH$, $-YC_6H_5$, $-YCH_2C_6H_5$; and R_3 is $-(CH_2)_n-$, and is isotopically encoded by incorporation of one or more of ^2H, ^{13}C, ^{15}N or ^{18}O;

n is from 1 to 3 inclusive;

Y is NHCO; and

X and $M_2'^+$ are as defined above.

In another particular embodiment, the present invention provides a method for the quantitative analysis of amino acids, peptides or proteins, the method comprising subjecting

a first amino acid, peptide or protein having a fixed-charge, introduced as a result of derivatization with a compound of formula $XM_1M_2^+$, wherein X and M_2^+ are as defined above, and M_1 is a linker group having the same structure as M_1' below, but containing only naturally abundant isotopes; and

a second amino acid, peptide or protein having a fixed-charge, introduced as a result of derivatization with a compound of formula $XM_1'M_2^+$, wherein X, M_1' and M_2^+ are as defined above,

to dissociation to form product ions that are characteristic of fragmentation occurring at the fixed-charge site by the loss of M_2.

In another particular embodiment, the present invention provides a method for the quantitative analysis of amino acids, peptides or proteins, the method comprising subjecting

a first amino acid, peptide or protein having a fixed-charge, introduced as a result of derivatization with a compound of formula $XM_1M_2'^+$, wherein X and $M_2'^+$ are as defined above, and M_1 is a linker group having the same structure as M_1' below, but containing only naturally abundant isotopes; and

a second amino acid, peptide or protein having a fixed-charge, introduced as a result of derivatization with a compound of formula $XM_1'M_2^+$ or $XM_1'M_2'^+$, wherein X, M_1', M_2^+, and $M_2'^+$ are as defined above,

to dissociation to form product ions that are characteristic of fragmentation occurring at the fixed-charge site by the loss of M_2 or M_2'.

The compounds of the present invention are suitable for use in the tandem mass spectrometry methods for quantitative analysis described below. In this embodiment, the reagents of the invention are employed for the fixed-charge derivatization of an amino acid, peptide or protein, to enable their quantitation via selective and directed fragmentation during MS/MS dissociation.

Accordingly, in a further aspect, the present invention provides a method for quantitative analysis of amino acids, peptides or proteins, the method comprising:

(1) introducing a mixture of amino acids, peptides or proteins containing at least one selected amino acid, peptide or protein, or peptide or protein comprising at least one residue of the selected amino acid, derivatized to contain a fixed-charge

using compounds of formula $XM_1M_2^+$, $XM_1'M_2^+$, $XM_1M_2'^+$ and $XM_1'M_2'^+$, or salts thereof, as described above;

(2) passing the mixture of amino acids, peptides or proteins containing at least one derivatized amino acid or derivatized amino acid residue containing peptide or protein, through a first mass resolving spectrometer to select precursor protein or peptide ions having a first mass-to-charge ratio;

(3) subjecting the precursor ions of the first mass-to-charge ratio to dissociation to form a product ion having a second mass-to-charge ratio that is characteristic of the loss of M_2 or M_2' at the site of the fixed-charge; and

(4) detecting the product ions having the second mass-to-charge ratio.

The method of analysis may be used for the identification and/or quantitation of amino acids, peptides or proteins.

Preferably the amino acid, peptide or protein contains an N-terminal amino group, a cysteine, a homocysteine, a lysine, an arginine, a homoarginine, a tryptophan, a dehydroalanine or a dehydroamino-2-butyric acid.

The method of the latter aspect of the invention may include the preceding step of derivatizing the amino acid, peptide or protein with a compound of formula $XM_1M_2^+$, $XM_1'M_2^+$, $XM_1M_2'^+$ and $XM_1'M_2'^+$, or salts thereof.

Preferably, the method of the invention described above comprises the further step of: (5) determining the identity of the peptide or protein.

Step (5) may be performed by first repeating steps (1), (2), and (3) and then subjecting the product ion having the second characteristic mass-to-charge ratio formed by loss from the precursor to a further stage of dissociation to form a series of product ions having a range of mass to charge ratios, for the purpose of determining the amino acid sequence of the peptide or protein and subsequently, the identity of the protein of origin.

Alternatively, step (5) may be carried out by use of high resolution mass analyzers to obtain an "accurate mass tag" (i.e., a mass accuracy of, for example, approximately 1-5 ppm) on the product ion detected in step (4). This, coupled with database searching, may be employed for subsequent identification of those peptides found to contain a fixed-charge derivative. Previously, in cases where an "accurate mass tag "has been obtained for a precursor ion, and the presence of a particular amino acid is known (for example the presence of a cysteine residue), the specificity of database searching algorithms can be improved such that unambiguous

identification of the protein from which the peptide is derived has been achieved from this information alone.

The amino acid, peptide or protein ion may be dissociated by any suitable dissociation method including, but not limited to, collisions with an inert gas (known as collision-induced dissociation (CID or collisionally-activated dissociation (CAD); (ii) collisions with a surface (known as surface-induced dissociation or SID); (iii) interaction with photons (e. g. via a laser) resulting in photodissociation; (iv) thermal/black body infrared radiative dissociation (BIRD), (v) interaction with an electron beam, resulting in electron-induced dissociation for singly charged cations (EID), electron-capture dissociation (ECD) for multiply charged cations, or combinations thereof, or (vi) by electron transfer dissociation (ETD).

Analysis of the amino acid, peptide or protein ion may be performed by tandem mass spectrometry. The tandem mass spectrometer may be equipped with electrospray ionization (ESI) or matrix assisted laser desorption ionization (MALDI) interfaces to transfer the protein or peptide ion from solution into the gas-phase.

The methods of the invention in certain embodiments may also include one or more steps of protein extraction, protein separation, reduction and alkylation of cysteine disulfides and/or protein digestion.

The present invention also extends to a reagent kit for quantitative analysis of amino acids, peptides or proteins comprising a compound of the present invention. The kit may also include instructions for use of the compounds of the invention in the quantitative analysis of amino acids, peptides or proteins by mass spectrometry.

The reagent kit further may also contain one or more containers containing: cysteine disulfide reducing agents, alkylating agents, proteases or chemical cleavage agents, and/or solvents. The cysteine disulfide reducing agents preferably include dithiothreitol (DTT), mercaptoethanol, tris-carboxyethyl phosphine (TCEP),and/or tributylphosphine (TBP). The cysteine alkylating agents preferably include alkylhalides (e.g. iodoacetic acid, iodoacetamide), vinylpyridine or acrylamide. The proteases or chemical cleavage agents preferably include trypsin, Endoproteinase Lys-C, EndoproteinaseA. sp-N, Endoproteinase Glu-C, pepsin, papain,thermolysin, cyanogen bromide, hydroxylamine hydrochloride,2- [2'-nitrophenylsulfenyl]-3-methyl-3'-bromoindole (BNPS- skatole), iodosobenzoic acid, pentafluoropropionic acidand/or dilute hydrochloric acid. The solvents preferably include urea, guanidine hydrochloride, acetonitrile, methanol and/or water.

The invention in another aspect provides methods for providing an internal standard in a mass spectrometer method comprising adding to a sample a predetermined quantity of an isotopically encoded fixed charge derivatized amino acid, peptide or protein described above.

The present invention will now be described with reference to particular embodiments, however, the method of the present invention is not limited to these particular embodiments.

Brief Description of the Drawings

Figure 1. Schematic representation of the use of the reagents of the present invention for the 'multiplexed' quantitation of protein abundances observed between different samples ***in a single neutral loss or precursor ion scan mode MS/MS*** experiment.

Figure 2. Schematic representation of the use of the reagents of the present invention for the 'multiplexed' quantitation of protein abundances observed between different samples ***in a single product ion scan mode MS/MS*** experiment.

Non-Limiting Embodiments of the Invention

Definitions

Unless the context indicates otherwise, all technical and scientific terms used herein generally have the same meaning as commonly understood in the art to which the present invention belongs.

"**Fixed-charge**", as used herein, includes any charge localised to a specific heteroatom contained within a specific heteroatom contained within the derivatization reagent, by the attachment of any moiety.

"**Fixed-charge derivatization**", as used here, means the introduction of a fixed-charge as defined above.

"**Protein**", as used herein, means any protein, including, but not limited to peptides, enzymes, glycoproteins, hormones, receptors, antigens, antibodies, growth factors, etc., without limitation. Proteins may be endogenous, or produced from other proteins by chemical or proteolytic cleavage. Preferred proteins include those comprised of at least 15-20 amino acid residues.

"**Peptide**" as used herein includes any substance comprising two or more amino acids and includes di-, tri-, oligo and polypeptides etc according to the number of amino acids linked by amide (s) bonds. Peptides may be endogenous, or produced from other peptides or proteins by chemical or proteolytic cleavage. Preferred peptides include those comprised of up to 15-20 amino acid residues.

When the amino acids are α-amino acids, either the L-optical isomer or the D-optical isomer can be used. The L-isomers are generally preferred. For a general review, see, [Spatola, A. F., in Chemistry and Biochemistry of amino acids, peptides and proteins. 1983, B. Weinstein,eds., Marcel Dekker, New York, p. 267.]

The term "**salt thereof**" includes any suitable counter ion. Non-limiting examples of counter ions are halide ions, for example, chloride, bromide, iodide.

Abbreviations

CID Collision Induced Dissociation

ESI Electrospray Ionization

MALDI Matrix Assisted Laser Desorption Ionization

MS Mass spectrometry

MS/MS Tandem mass spectrometry

MS^n Multistage tandem mass spectrometry, where $n > 2$

The one-letter amino acid abbreviations used herein are well known to those skilled in the art (see, for example, Stryer, Lubert, Biochemistry, 1975, page 17).

Use of the compounds of the Invention

In one embodiment, the reagents of the present invention may be used for the 'multiplexed' quantitation of protein abundances observed between different samples ***in a single neutral loss or precursor ion scan mode MS/MS*** experiment, using the 'modular' fixed charge stable isotope labelling approach described below (shown in Figure 1 for reaction with alkylation reagents $XM_1M_2^+$ and $XM_1'M_2^+$). Here, derivatization of a 'normal' sample is carried out using an alkylation reagent $XM_1M_2^+$, where both the M_1 and M_2 'modules' contain only naturally abundant isotopes. Simultaneously, derivatization of multiple 'diseased' samples is carried out using isotopically distinct labelled alkylation reagents $XM_1'M_2^+$, where the M_1'

'module' contains an increasing number of isotopically enriched labels (for example ^{2}H, ^{13}C, ^{15}N or ^{18}O), preferably giving an increase of up to twelve mass units in increments of one, two, three, four or six mass units, compared to the M_1 module containing only naturally abundant isotopes, and where the M_2^+ module contains only naturally abundant isotopes. The samples are then combined and subjected to tandem mass spectrometry. Quantitative analysis of the relative peptide concentrations between the 'normal' and 'diseased' samples may then be achieved in a single neutral loss or precursor ion scan mode MS/MS experiment by measurement of the abundances of the isotopically distinct M_1 and M_1' containing product ions formed by the neutral loss of M_2, or by measurement of the abundances of the M_2 product ion formed by charged loss of M_2, respectively, via directed fragmentation occurring at the bonds between the M_1 and M_2^+ modules.

In another embodiment, the reagents of the present invention may be used for the 'multiplexed' quantitation of protein abundances observed between different samples ***in a single product ion scan mode MS/MS*** experiment, using the 'modular' fixed charge stable isotope labelling approach described below (shown in Figure 2 for reaction with alkylation reagents $XM_1M_2'^+$ and $XM_1'M_2^+$). Here, derivatization of a first 'normal' sample is carried out using an isotopically distinct labelled alkylation reagent $XM_1M_2'^+$, where the M_1 module contains only naturally abundant isotopes and where the $M_2'^+$ 'module' is isotopically enriched (for example with ^{2}H, ^{13}C, ^{15}N or ^{18}O), preferably giving an increase of up to twelve mass units compared to an M_2^+ module containing only naturally abundant isotopes. Simultaneously, derivatization of multiple 'diseased' samples may be carried out using (i) the isotopically distinct labelled alkylation reagent $XM_1'M_2^+$, where the M_1' 'module' is isotopically enriched (for example with ^{2}H, ^{13}C, ^{15}N or ^{18}O), preferably giving an increase of up to twelve mass units compared to an M_1 module containing only naturally abundant isotopes while the M_2^+ module contains only naturally abundant isotopes, and (ii) the isotopically distinct labelled alkylation reagents $XM_1'M_2'^+$, where the M_1' 'modules' contain an increasing number of isotopically enriched labels (in increments of one, two, three or four mass units, thereby allowing multiplexed analysis of 12, 6, 4 or 3 'diseased' samples', respectively) compared to that used in the 'normal' sample, while the $M_2'^+$ 'modules' contain an equally decreasing number of isotopically enriched labels (for example $^{2}H_n$, $^{13}C_n$, $^{15}N_n$ or $^{18}O_n$) compared to that used in the 'normal' sample. The masses of each of the alkylation reagents employed for

labelling both 'normal' and 'diseased' samples are then identical, such that the mass difference between 'normal' and 'diseased' derivatized samples is zero. The samples are then combined and subjected to tandem mass spectrometry. Quantitative analysis of the relative peptide concentrations between the 'normal' and 'diseased' samples may then be achieved in a single product ion scan mode MS/MS experiment by measurement of the abundances of the isotopically distinct M_1 and M_1' containing product ions formed by neutral loss of M_2' or M_2, or by measurement of the abundances of the isotopically distinct M_2' and M_2 product ions formed by charged loss of M_2' and M_2, respectively, via directed fragmentation occurring at the bond between the M_1 and M_2^+ modules.

It will be appreciated by persons skilled in the art that numerous variations and/or modifications may be made to the invention as shown in the specific embodiments without departing from the spirit or scope of the invention as broadly described. The present embodiments are, therefore, to be considered in all respects as illustrative and not restrictive.

Furthermore, throughout this specification the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated element, integer or step, or group of elements, integers or steps, but not the exclusion of any other element, integer or step, or group of elements, integers or steps.

Moreover, any discussion of documents, acts, materials, devices, articles or the like which has been included in the present specification is solely for the purpose of providing a context for the present invention. It is not to be taken as an admission that any or all of these matters form part of the prior art base or were common general knowledge in the field relevant to the present invention as it existed before the priority date of each claim of this application.

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims.

All references, including patent documents, disclosed herein are incorporated by reference in their entirety.

CLAIMS

1. A compound of formula $XM_1'M_2^+$ or $XM_1'M_2'^+$, or salts thereof, wherein:

X is a reactive group specific to a functional group contained within an amino acid, peptide or protein, or peptide or protein containing at least one of such amino acid;

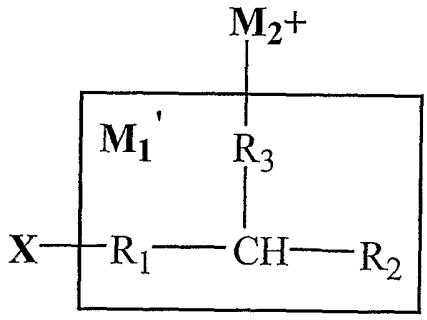
M_1' is a linker group between X and M_2^+ or $M_2'^+$, and is isotopically encoded by incorporation of one or more of ^2H, ^{13}C, ^{15}N or ^{18}O;

M_2^+ is selected from the group consisting of a tertiary alkyl sulfonium ion, a quaternary alkyl ammonium ion or a quaternary alkyl phosphonium ion; and

$M_2'^+$ is selected from the group consisting of a tertiary alkyl sulfonium ion, a quaternary alkyl ammonium ion or a quaternary alkyl phosphonium ion, and is isotopically encoded by incorporation of one or more of ^2H, ^{13}C, ^{15}N or ^{18}O.

2. The compound of claim 1, wherein the M_1' group is a branched alkyl, optionally interrupted or substituted with an alkyl, aryl, substituted alkyl, substituted aryl, amino, amide, acid, ester or thioester.

3. The compound of claim 1 of formula $XM_1'M_2^+$, or a salt thereof,



$XM_1'M_2^+$

wherein,

X is a thiol reactive group selected from the group consisting of a halide, a disulfide exchange group or a vinyl group;

M_1' is $-R_1CH(R_3)R_2$, where R_1 is selected from $-(CH_2)_n-$, -Y- and $-(CH_2)_nY-$; R_2 is selected from $-(CH_2)_nH$, $-C_6H_5$, $-CH_2C_6H_5$, $-NH_2$, -YH, $-Y(CH_2)_nH$, $-YC_6H_5$, and

$-YCH_2C_6H_5$; and R_3 is $-(CH_2)_n-$, and is isotopically encoded by incorporation of one or more of ^2H, ^{13}C, ^{15}N or ^{18}O;

n is from 1 to 3 inclusive;

Y is selected from CONH, NHCO and COO; and

M_2^+ is attached to the R_3 group of M_1' and is selected from the group consisting of a tertiary alkyl sulfonium ion, $-S^+CH_3R''$, where R'' is selected from $-CH_2COC_6H_5$, $-CH_2COC_6H_5CH_3$, and $-CH_2COC_6H_5CH_2CH_3$; and a quaternary alkyl ammonium ion, $-N^+(R''')_3$, or a quaternary alkyl phosphonium ion, $-P^+(R''')_3$, where R'''_3 is $-(CH_2)_nH$ (where n = 1 to 3); $-C_6H_5$; or $-CH_2C_6H_5$.

4. The compound of claim 3, wherein the halide is -Cl, -Br, or - I.

5. The compound of claim 3, wherein the disulfide exchange group is -S-S-R', wherein R' is selected from the group consisting of: $-C_6H_5$, 3-carboxyl-4-nitrophenyl, 2,4-dinitrophenyl, 4-nitrophenyl, 2-nitrophenyl, 2-pyridyl, 5-nitropyridyl, 3-nitropyridyl, and methanesulfonyl.

6. The compound of claim 3, wherein the vinyl group is $-CH=CH_2$.

7. The compound of claim 3 wherein,

M_1' is $-R_1CH(R_3)R_2$, where R_1 is $-(CH_2)_n-$; R_2 is -YH, $-Y(CH_2)_nH$, $-YC_6H_5$, or $-YCH_2C_6H_5$; and R_3 is $-(CH_2)_n-$, and is isotopically encoded by incorporation of one or more of ^2H, ^{13}C, ^{15}N or ^{18}O;

n is from 1 to 3 inclusive;

Y is CONH; and

X and M_2^+ are as defined above.

8. The compound of claim 3 wherein,

M_1' is $-R_1CH(R_3)R_2$, where R_1 is -Y- or $-(CH_2)_nY-$; R_2 is $-(CH_2)_nH$, $-NH_2$, $-Y(CH_2)_nH$,

$-YC_6H_5$, or $-YCH_2C_6H_5$; and R_3 is $-(CH_2)_n-$, and is isotopically encoded by incorporation of one or more of ^2H, ^{13}C, ^{15}N or ^{18}O;

n is from 1 to 3 inclusive;

Y is CONH; and

X and M_2' are as defined above.

9. The compound of claim 3 wherein,

M_1' is $-R_1CH(R_3)R_2$, where R_1 is $-(CH_2)_n-$; R_2 is -YH, $-Y(CH_2)_nH$, $-YC_6H_5$, or $-YCH_2C_6H_5$; and R_3 is $-(CH_2)_n-$, and is isotopically encoded by incorporation of one or more of ^2H, ^{13}C, ^{15}N or ^{18}O;

n is from 1 to 3 inclusive;

Y is NHCO; and

X and M_2^+ are as defined above.

10. The compound of claim 3 wherein,

M_1' is $-R_1CH(R_3)R_2$, where R_1 is -Y- or $-(CH_2)_nY-$; R_2 is $-(CH_2)_nH$, $-NH_2$, $-Y(CH_2)_nH$,

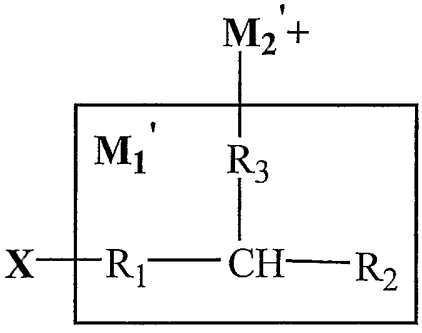
$-YC_6H_5$, or $-YCH_2C_6H_5$; and R_3 is $-(CH_2)_n-$, and is isotopically encoded by incorporation of one or more of ^2H, ^{13}C, ^{15}N or ^{18}O;

n is from 1 to 3 inclusive;

Y is NHCO; and

X and M_2^+ are as defined above.

11. The compound of claim 1 of formula $XM_1'M_2'^+$, or a salt thereof,



$XM_1'M_2'^+$

wherein,

X is a thiol reactive group selected from the group consisting of a halide, a disulfide exchange group and a vinyl group;

M_1' is $-R_1CH(R_3)R_2$, where R_1 is selected from $-(CH_2)_n-$, -Y- and $-(CH_2)_nY-$; R_2 is selected from $-(CH_2)_nH$, $-C_6H_5$, $-CH_2C_6H_5$, $-NH_2$, -YH, $-Y(CH_2)_nH$, $-YC_6H_5$, and $-YCH_2C_6H_5$; and R_3 is $-(CH_2)_n-$, and is isotopically encoded by incorporation of one or more of ^2H, ^{13}C, ^{15}N or ^{18}O;

n is from 1 to 3 inclusive;

Y is selected from CONH, NHCO and COO; and

$M_2'^+$ is attached to the R_3 group of M_1' and is selected from the group consisting of a tertiary alkyl sulfonium ion, $-S^+CH_3R''$, where R'' is selected from $-CH_2COC_6H_5$, $-CH_2COC_6H_5CH_3$, and $-CH_2COC_6H_5CH_2CH_3$; and a quaternary alkyl ammonium ion, $-N^+(R''')_3$, or a quaternary alkyl phosphonium ion, $-P^+(R''')_3$, where R'''_3 is $-(CH_2)_nH$ (where n = 1 to 3), $-C_6H_5$, or $-CH_2C_6H_5$ and is isotopically encoded by incorporation of one or more of ^2H, ^{13}C, ^{15}N or ^{18}O;

12. The compound of claim 11, wherein the halide is -Cl, -Br, or - I.

13. The compound of claim 11, wherein the disulfide exchange group is selected from -S-S-R′, where R′ is $-C_6H_5$, 3-carboxyl-4-nitrophenyl, 2,4-dinitrophenyl, 4-nitrophenyl, 2-nitrophenyl, 2-pyridyl, 5-nitropyridyl, 3-nitropyridyl, and methanesulfonyl.

14. The compound of claim 11, wherein the vinyl group is $-CH=CH_2$.

15. The compound of claim 11 wherein,

M_1' is $-R_1CH(R_3)R_2$, where R_1 is $-(CH_2)_n-$; R_2 is -YH, $-Y(CH_2)_nH$, $-YC_6H_5$, or $-YCH_2C_6H_5$; and R_3 is $-(CH_2)_n-$, and is isotopically encoded by incorporation of one or more of ^2H, ^{13}C, ^{15}N or ^{18}O;

n is from 1 to 3 inclusive;

Y is CONH; and

X and $M_2'^+$ are as defined above.

16. The compound of claim 11 wherein,

M_1 is $-R_1CH(R_3)R_2$, where R_1 is -Y- or $-(CH_2)_nY-$; R_2 is $-(CH_2)_nH$, $-NH_2$, $-Y(CH_2)_nH$, $-YC_6H_5$, or $-YCH_2C_6H_5$; and R_3 is $-(CH_2)_n-$, and is isotopically encoded by incorporation of one or more of ^2H, ^{13}C, ^{15}N or ^{18}O;

n is from 1 to 3 inclusive;

Y is CONH; and

X and $M_2^{'+}$ are as defined above.

17. The compound of claim 11 wherein,

M_1' is $-R_1CH(R_3)R_2$, where R_1 is $-(CH_2)_n-$; R_2 is -YH, $-Y(CH_2)_nH$, $-YC_6H_5$, or $-YCH_2C_6H_5$; and R_3 is $-(CH_2)_n-$, and is isotopically encoded by incorporation of one or more of ^2H, ^{13}C, ^{15}N or ^{18}O;

n is from 1 to 3 inclusive;

Y is NHCO; and

X and $M_2^{'+}$ are as defined above.

18. The compound of claim 11 wherein,

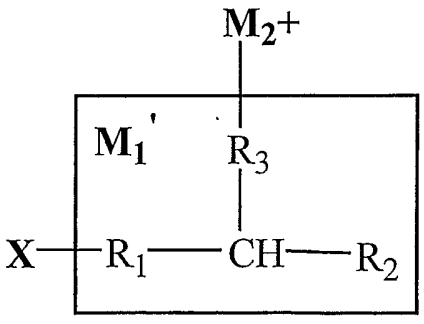
M_1' is $-R_1CH(R_3)R_2$, where R_1 is -Y- or $-(CH_2)_nY-$; R_2 is $-(CH_2)_nH$, $-NH_2$, $-Y(CH_2)_nH$, $-YC_6H_5$, or $-YCH_2C_6H_5$; and R_3 is $-(CH_2)_n-$, and is isotopically encoded by incorporation of one or more of ^2H, ^{13}C, ^{15}N or ^{18}O;

n is from 1 to 3 inclusive;

Y is NHCO; and

X and $M_2^{'+}$ are as defined above

19. The compound of claim 1 of formula $XM_1'M_2^+$, or a salt thereof,



$XM_1'M_2^+$

wherein,

X is an amino reactive group selected from the group consisting of an acid anhydride, an active ester, an acid halide, a sulfonylhalide, a substituted O-methyl isourea, an isocyanate and an isothiocyanate;

M_1' is $-R_1CH(R_3)R_2$, where R_1 is selected from $-(CH_2)_n-$, -Y- and $-(CH_2)_nY-$; R_2 is selected from $-(CH_2)_nH$, $-C_6H_5$, $-CH_2C_6H_5$, $-NH_2$, -YH, $-Y(CH_2)_nH$, $-YC_6H_5$, and $-YCH_2C_6H_5$; and R_3 is $-(CH_2)_n-$, and is isotopically encoded by incorporation of one or more of ^2H, ^{13}C, ^{15}N or ^{18}O;

n is from 1 to 3 inclusive;

Y is selected from CONH, NHCO, COO and COS; and

M_2^+ is attached to the R_3 group of M_1 and is selected from the group consisting of a tertiary alkyl sulfonium ion, $-S^+CH_3R''$, where R'' is selected from $-CH_2COC_6H_5$, $-CH_2COC_6H_5CH_3$, and $-CH_2COC_6H_5CH_2CH_3$; and a quaternary alkyl ammonium ion, $-N^+(R''')_3$ or a quaternary alkyl phosphonium ion, $-P^+(R''')_3$, where R'''_3 is $-(CH_2)_nH$ (where n = 1 to 3), $-C_6H_5$, or $-CH_2C_6H_5$.

20. The compound of claim 19 wherein,

M_1' is $-R_1CH(R_3)R_2$, where R_1 is $-(CH_2)_n-$; R_2 is -YH, $-Y(CH_2)_nH$, $-YC_6H_5$, or $-YCH_2C_6H_5$; and R_3 is $-(CH_2)_n-$, and is isotopically encoded by incorporation of one or more of ^2H, ^{13}C, ^{15}N or ^{18}O;

n is from 1 to 3 inclusive;

Y is CONH; and

X and M_2^+ are as defined above.

21. The compound of claim 19 wherein,

M_1' is $-R_1CH(R_3)R_2$, where R_1 is -Y- or $-(CH_2)_nY-$; R_2 is $-(CH_2)_nH$, $-NH_2$, $-Y(CH_2)_nH$, $-YC_6H_5$, or $-YCH_2C_6H_5$; and R_3 is $-(CH_2)_n-$, and is isotopically encoded by incorporation of one or more of ^2H, ^{13}C, ^{15}N or ^{18}O;

n is from 1 to 3 inclusive;

Y is CONH; and

X and M_2^+ are as defined above.

22. The compound of claim 19 wherein,

M_1 is $-R_1CH(R_3)R_2$, where R_1 is $-(CH_2)_n-$; R_2 is -YH, $-Y(CH_2)_nH$, $-YC_6H_5$, or $-YCH_2C_6H_5$; and R_3 is $-(CH_2)_n-$, and is isotopically encoded by incorporation of one or more of ^2H, ^{13}C, ^{15}N or ^{18}O;

n is from 1 to 3 inclusive;

Y is NHCO; and

X and M_2^+ are as defined above.

23. The compound of claim 19 wherein,

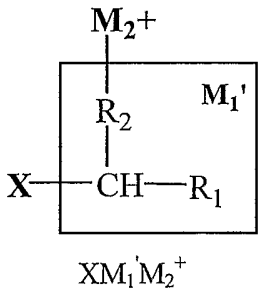
M_1' is $-R_1CH(R_3)R_2$, where R_1 is -Y- or $-(CH_2)_nY-$; R_2 is $-(CH_2)_nH$, $-NH_2$, $-Y(CH_2)_nH$, $-YC_6H_5$, or $-YCH_2C_6H_5$; and R_3 is $-(CH_2)_n-$, and is isotopically encoded by incorporation of one or more of ^2H, ^{13}C, ^{15}N or ^{18}O;

n is from 1 to 3 inclusive;

Y is NHCO; and

X and M_2^+ are as defined above.

24. A compound of claim 1 of formula $XM_1'M_2^+$, or a salt thereof,



$XM_1'M_2^+$

wherein,

X is an amino reactive group selected from the group consisting of an acid anhydride, an active ester, an acid halide, a sulfonylhalide, a substituted O-methyl isourea, an isocyanate and an isothiocyanate;

M_1' is $-CH(R_2)R_1$, where R_1 is selected from $-(CH_2)_nH$, $-C_6H_5$, $-CH_2C_6H_5$, $-NH_2$, -YH,

$-Y(CH_2)_nH$, $-YC_6H_5$, and $-YCH_2C_6H_5$; and R_2 is $-(CH_2)_n-$, and is isotopically encoded by incorporation of one or more of ^2H, ^{13}C, ^{15}N or ^{18}O;

n is from 1 to 3 inclusive;

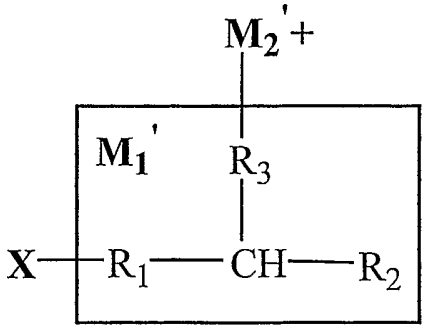
Y is selected from CONH, NHCO, COO and COS; and

M_2^- is attached to the R_2 group of M_1' and is selected from the group consisting of a tertiary alkyl sulfonium ion, $-S^+CH_3R''$, where R'' is selected from $-CH_2COC_6H_5$, $-CH_2COC_6H_5CH_3$, and $-CH_2COC_6H_5CH_2CH_3$; and a quaternary alkyl ammonium ion, $-N^+(R''')_3$, or a quaternary alkyl phosphonium ion, $-P^+(R''')_3$, where R'''_3 is $-(CH_2)_nH$ (where n = 1 to 3);
$-C_6H_5$, or $-CH_2C_6H_5$.

25. The compound of claim 24 wherein,
M_1' is $-CH(R_2)R_1$, where R_1 is $-(CH_2)_nH$, $-C_6H_5$, $-CH_2C_6H_5$, $-NH_2$, -YH, $-Y(CH_2)_nH$,
$-YC_6H_5$, or $-YCH_2C_6H_5$; and R_2 is $-(CH_2)_n-$, and is isotopically encoded by incorporation of one or more of ^2H, ^{13}C, ^{15}N or ^{18}O;
n is from 1 to 3 inclusive;
Y is CONH; and
X and M_2^+ are as defined above.

26. The compound of claim 24 wherein,
M_1' is $-CH(R_2)R_1$, where R_1 is $-(CH_2)_nH$, $-C_6H_5$, $-CH_2C_6H_5$, $-NH_2$, -YH, $-Y(CH_2)_nH$,
$-YC_6H_5$, or $-YCH_2C_6H_5$; and R_2 is $-(CH_2)_n-$, and is isotopically encoded by incorporation of one or more of ^2H, ^{13}C, ^{15}N or ^{18}O;
n is from 1 to 3 inclusive;
Y is NHCO; and
X and M_2^+ are as defined above.

27. A compound of claim 1 of formula $XM_1'M_2'^+$, or a salt thereof,



$XM_1'M_2'^+$

wherein,

X is an amino reactive group selected from the group consisting of an acid anhydride, an active ester, an acid halide, a sulfonylhalide, a substituted O-methyl isourea, an isocyanate and an isothiocyanate;

M_1' is $-R_1CH(R_3)R_2$, where R_1 is selected from $-(CH_2)_n-$, -Y- and $-(CH_2)_nY-$; R_2 is selected from $-(CH_2)_nH$, $-C_6H_5$, $-CH_2C_6H_5$, $-NH_2$, -YH, $-Y(CH_2)_nH$, $-YC_6H_5$, and $-YCH_2C_6H_5$; and R_3 is $-(CH_2)_n-$, and is isotopically encoded by incorporation of one or more of ^2H, ^{13}C, ^{15}N or ^{18}O;

n is from 1 to 3 inclusive;

Y is selected from CONH, NHCO, COO and COS; and

$M_2'^+$ is attached to the R_3 group of M_1' and is selected from the group consisting of a tertiary alkyl sulfonium ion, $-S^+CH_3R''$, where R'' is selected from $-CH_2COC_6H_5$, $-CH_2COC_6H_5CH_3$, and $-CH_2COC_6H_5CH_2CH_3$; and a quaternary alkyl ammonium ion, $-N^+(R''')_3$, or a quaternary alkyl phosphonium ion, $-P^+(R''')_3$, where R'''_3 is $-(CH_2)_nH$ (where n = 1 to 3), $-C_6H_5$, or $-CH_2C_6H_5$; and is isotopically encoded by incorporation of one or more of ^2H, ^{13}C, ^{15}N or ^{18}O.

28. The compound of claim 27 wherein,

M_1' is $-R_1CH(R_3)R_2$, where R_1 is $-(CH_2)_n-$; R_2 is -YH, $-Y(CH_2)_nH$, $-YC_6H_5$, or $-YCH_2C_6H_5$; and R_3 is $-(CH_2)_n-$, and is isotopically encoded by incorporation of one or more of ^2H, ^{13}C, ^{15}N or ^{18}O;

n is from 1 to 3 inclusive;

Y is CONH; and

X and $M_2'^+$ are as defined above.

29. The compound of claim 27 wherein,

M_1' is $-R_1CH(R_3)R_2$, where R_1 is -Y- or $-(CH_2)_nY-$; R_2 is $-(CH_2)_nH$, $-NH_2$, $-Y(CH_2)_nH$, $-YC_6H_5$, or $-YCH_2C_6H_5$; and R_3 is $-(CH_2)_n-$, and is isotopically encoded by incorporation of one or more of ^2H, ^{13}C, ^{15}N or ^{18}O;

n is from 1 to 3 inclusive;

Y is CONH; and

X and M_2' are as defined above.

30. The compound of claim 27 wherein,

M_1' is $-R_1CH(R_3)R_2$, where R_1 is $-(CH_2)_n-$; R_2 is -YH, $-Y(CH_2)_nH$, $-YC_6H_5$, or $-YCH_2C_6H_5$; and R_3 is $-(CH_2)_n-$, and is isotopically encoded by incorporation of one or more of ^2H, ^{13}C, ^{15}N or ^{18}O;

n is from 1 to 3 inclusive;

Y is NHCO; and

X and $M_2'^+$ are as defined above.

31. The compound of claim 27 wherein,

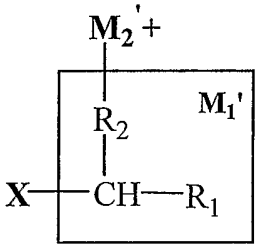
M_1' is $-R_1CH(R_3)R_2$, where R_1 is -Y- or $-(CH_2)_nY-$; R_2 is $-(CH_2)_nH$, $-NH_2$, $-Y(CH_2)_nH$, $-YC_6H_5$, or $-YCH_2C_6H_5$; and R_3 is $-(CH_2)_n-$, and is isotopically encoded by incorporation of one or more of ^2H, ^{13}C, ^{15}N or ^{18}O;

n is from 1 to 3 inclusive;

Y is NHCO; and

X and $M_2'^+$ are as defined above.

32. A compound of claim 1 of formula $XM_1'M_2^+$, or a salt thereof,



$XM_1'M_2'^+$

wherein,

X is an amino reactive group selected from the group consisting of an acid anhydride, an active ester, an acid halide, a sulfonylhalide, a substituted O-methyl isourea, an isocyanate and an isothiocyanate;

M_1' is $-CH(R_2)R_1$, where R_1 is selected from $-(CH_2)_nH$, $-C_6H_5$, $-CH_2C_6H_5$, $-NH_2$, -YH,

$-Y(CH_2)_nH$, $-YC_6H_5$, and $-YCH_2C_6H_5$; and R_2 is $-(CH_2)_n-$, and is isotopically encoded by incorporation of one or more of ^2H, ^{13}C, ^{15}N or ^{18}O;

n is from 1 to 3 inclusive;

Y is selected from CONH, NHCO, COO and COS; and

$M_2'^+$ is attached to the R_2 group of M_1' and is selected from the group consisting of a tertiary alkyl sulfonium ion, $-S^+CH_3R''$, where R'' is selected from $-CH_2COC_6H_5$, $-CH_2COC_6H_5CH_3$, and $-CH_2COC_6H_5CH_2CH_3$; and a quaternary alkyl ammonium ion, $-N^+(R''')_3$, or a quaternary alkyl phosphonium ion, $-P^+(R''')_3$, where R'''_3 is $-(CH_2)_nH$ (where n = 1 to 3); $-C_6H_5$, or $-CH_2C_6H_5$; and is isotopically encoded by incorporation of one or more of ^2H, ^{13}C, ^{15}N or ^{18}O.

33. The compound of claim 32 wherein,

M_1' is $-CH(R_2)R_1$, where R_1 is $-(CH_2)_nH$, $-C_6H_5$, $-CH_2C_6H_5$, $-NH_2$, -YH, $-Y(CH_2)_nH$, $-YC_6H_5$, or $-YCH_2C_6H_5$; and R_2 is $-(CH_2)_n-$, and is isotopically encoded by incorporation of one or more of ^2H, ^{13}C, ^{15}N or ^{18}O;

n is from 1 to 3 inclusive;

Y is CONH; and

X and $M_2'^+$ are as defined above.

34. The compound of claim 32 wherein,

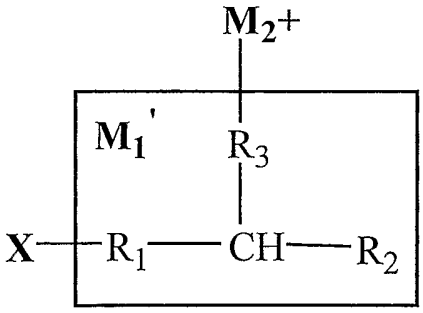
M_1' is $-CH(R_2)R_1$, where R_1 is $-(CH_2)_nH$, $-C_6H_5$, $-CH_2C_6H_5$, $-NH_2$, -YH, $-Y(CH_2)_nH$, $-YC_6H_5$, or $-YCH_2C_6H_5$; and R_2 is $-(CH_2)_n-$, and is isotopically encoded by incorporation of one or more of ^2H, ^{13}C, ^{15}N or ^{18}O;

n is from 1 to 3 inclusive;

Y is NHCO; and

X and $M_2'^+$ are as defined above.

35. A compound of claim 1 of formula $XM_1'M_2^+$, or a salt thereof,



$XM_1'M_2^+$

wherein,

X is a guanidino specific reactive group selected from the group consisting of a substituted 2,3-butanedione, a substituted 2,4-pentanedione, a substituted glyoxal, and a substituted phenylglyoxal;

M_1' is $-R_1CH(R_3)R_2$, where R_1 is selected from $-(CH_2)_n-$, -Y- and $-(CH_2)_nY-$; R_2 is selected from $-(CH_2)_nH$, $-C_6H_5$, $-CH_2C_6H_5$, $-NH_2$, -YH, $-Y(CH_2)_nH$, $-YC_6H_5$, and $-YCH_2C_6H_5$; and R_3 is $-(CH_2)_n-$, and is isotopically encoded by incorporation of one or more of ^2H, ^{13}C, ^{15}N or ^{18}O;

n is from 1 to 3 inclusive;

Y is selected from CONH, NHCO, COO and COS; and

M_2^+ is attached to the R_3 group of M_1' and is selected from the group consisting of a tertiary alkyl sulfonium ion, $-S^+CH_3R''$, where R'' is selected from $-CH_2COC_6H_5$, $-CH_2COC_6H_5CH_3$, and $-CH_2COC_6H_5CH_2CH_3$; and a quaternary alkyl ammonium ion, $-N^+(R''')_3$, or a quaternary alkyl phosphonium ion, $-P^+(R''')_3$, where R'''_3 is $-(CH_2)_nH$ (where n = 1 to 3), $-C_6H_5$, or $-CH_2C_6H_5$.

36. The compound of claim 35 wherein,

M_1' is $-R_1CH(R_3)R_2$, where R_1 is $-(CH_2)_n-$; R_2 is -YH, $-Y(CH_2)_nH$, $-YC_6H_5$, or $-YCH_2C_6H_5$; and R_3 is $-(CH_2)_n-$, and is isotopically encoded by incorporation of one or more of ^2H, ^{13}C, ^{15}N or ^{18}O;

n is from 1 to 3 inclusive;

Y is CONH; and

X and M_2^+ are as defined above.

37. The compound of claim 35 wherein,

M_1' is $-R_1CH(R_3)R_2$, where R_1 is -Y- or $-(CH_2)_nY-$; R_2 is $-(CH_2)_nH$, $-NH_2$, $-Y(CH_2)_nH$, $-YC_6H_5$, or $-YCH_2C_6H_5$; and R_3 is $-(CH_2)_n-$, and is isotopically encoded by incorporation of one or more of ^2H, ^{13}C, ^{15}N or ^{18}O;

n is from 1 to 3 inclusive;

Y is CONH; and

X and M_2^+ are as defined above.

38. The compound of claim 35 wherein,

M_1' is $-R_1CH(R_3)R_2$, where R_1 is $-(CH_2)_n-$; R_2 is -YH, $-Y(CH_2)_nH$, $-YC_6H_5$, or $-YCH_2C_6H_5$; and R_3 is $-(CH_2)_n-$, and is isotopically encoded by incorporation of one or more of ^2H, ^{13}C, ^{15}N or ^{18}O;

n is from 1 to 3 inclusive;

Y is NHCO; and

X and M_2^+ are as defined above.

39. The compound of claim 35 wherein,

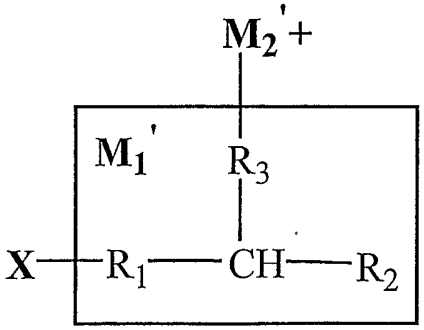
M_1' is $-R_1CH(R_3)R_2$, where R_1 is -Y- or $-(CH_2)_nY-$; R_2 is $-(CH_2)_nH$, $-NH_2$, $-Y(CH_2)_nH$, $-YC_6H_5$, or $-YCH_2C_6H_5$; and R_3 is $-(CH_2)_n-$, and is isotopically encoded by incorporation of one or more of ^2H, ^{13}C, ^{15}N or ^{18}O;

n is from 1 to 3 inclusive;

Y is NHCO; and

X and M_2^+ are as defined above.

40. A compound of claim 1 of formula $XM_1'M_2'^+$, or a salt thereof,



$XM_1'M_2'^+$

wherein,

X is a guanidino specific reactive group selected from the group consisting of a substituted 2,3-butanedione, a substituted 2,4-pentanedione, a substituted glyoxal, and a substituted phenylglyoxal;

M_1' is $-R_1CH(R_3)R_2$, where R_1 is selected from $-(CH_2)_n-$, Y- and $-(CH_2)_nY-$; R_2 is selected from $-(CH_2)_nH$, $-C_6H_5$, $-CH_2C_6H_5$, $-NH_2$, -YH, $-Y(CH_2)_nH$, $-YC_6H_5$, and $-YCH_2C_6H_5$; and R_3 is $-(CH_2)_n-$, and is isotopically encoded by incorporation of one or more of ^2H, ^{13}C, ^{15}N or ^{18}O;

n is from 1 to 3 inclusive;

Y is selected from CONH, NHCO, COO and COS; and

$M_2'^+$ is attached to the R_3 group of M_1 and is selected from the group consisting of a tertiary alkyl sulfonium ion, $-S^+CH_3R''$, where R'' is selected from $-CH_2COC_6H_5$, $-CH_2COC_6H_5CH_3$, and $-CH_2COC_6H_5CH_2CH_3$; and a quaternary alkyl ammonium ion, $-N^+(R''')_3$, or a quaternary alkyl phosphonium ion, $-P^+(R''')_3$, where R'''_3 is $-(CH_2)_nH$ (where n = 1 to 3), $-C_6H_5$, or $-CH_2C_6H_5$; and is isotopically encoded by incorporation of one or more of ^2H, ^{13}C, ^{15}N or ^{18}O.

41. The compound of claim 40 wherein,

M_1' is $-R_1CH(R_3)R_2$, where R_1 is $-(CH_2)_n-$; R_2 is -YH, $-Y(CH_2)_nH$, $-YC_6H_5$, or $-YCH_2C_6H_5$; and R_3 is $-(CH_2)_n-$, and is isotopically encoded by incorporation of one or more of ^2H, ^{13}C, ^{15}N or ^{18}O;

n is from 1 to 3 inclusive;

Y is CONH; and

X and $M_2'^+$ are as defined above.

42. The compound of claim 40 wherein,

M_1' is $-R_1CH(R_3)R_2$, where R_1 is -Y- or $-(CH_2)_nY-$; R_2 is $-(CH_2)_nH$, $-NH_2$, $-Y(CH_2)_nH$, $-YC_6H_5$, or $-YCH_2C_6H_5$; and R_3 is $-(CH_2)_n-$, and is isotopically encoded by incorporation of one or more of ^2H, ^{13}C, ^{15}N or ^{18}O;

n is from 1 to 3 inclusive;

Y is CONH; and

X and $M_2'^+$ are as defined above.

43. The compound of claim 40 wherein,

M_1' is $-R_1CH(R_3)R_2$, where R_1 is $-(CH_2)_n-$; R_2 is -YH, $-Y(CH_2)_nH$, $-YC_6H_5$, or $-YCH_2C_6H_5$; and R_3 is $-(CH_2)_n-$, and is isotopically encoded by incorporation of one or more of ^2H, ^{13}C, ^{15}N or ^{18}O;

n is from 1 to 3 inclusive;

Y is NHCO; and

X and $M_2^{'+}$ are as defined above.

44. The compound of claim 40 wherein,

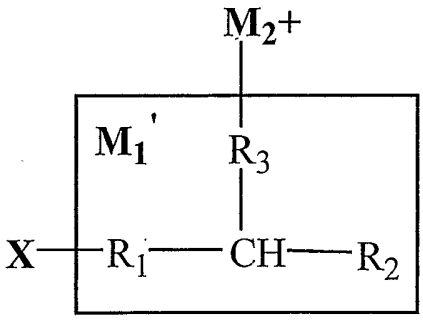
M_1' is $-R_1CH(R_3)R_2$, where R_1 is -Y- or $-(CH_2)_nY-$; R_2 is $-(CH_2)_nH$, $-NH_2$, $-Y(CH_2)_nH$, $-YC_6H_5$, or $-YCH_2C_6H_5$; and R_3 is $-(CH_2)_n-$, and is isotopically encoded by incorporation of one or more of ^2H, ^{13}C, ^{15}N or ^{18}O;

n is from 1 to 3 inclusive;

Y is NHCO; and

X and $M_2^{'+}$ are as defined above.

45. A compound of claim 1 of formula $XM_1'M_2^+$, or a salt thereof, for specific reaction with the C2-indole position of the side chain of tryptophan or tryptophan containing proteins or peptides,



$XM_1'M_2^+$

wherein,

X is a reactive group specific to the C2-indole position of the side chain of tryptophan selected from a halide and a dimethyl sulfonium ion;

M_1 is $-R_1CH(R_3)R_2$, where R_1 is selected from a -2-hydroxy-5-nitrobenzyl-, and a -(2-hydroxy-5-nitrobenzyl)-4-Y- group; R_2 is selected from $-(CH_2)_nH$, $-C_6H_5$, $-CH_2C_6H_5$, $-NH_2$, -YH, $-Y(CH_2)_nH$, $-YC_6H_5$, and $-YCH_2C_6H_5$, and R_3 is $-(CH_2)_n-$, and is isotopically encoded by incorporation of one or more of ^2H, ^{13}C, ^{15}N or ^{18}O;

n is from 1 to 3 inclusive;

Y is selected from CONH, NHCO, COO and COS; and

M_2^+ is attached to the R_3 group of M_1' and is selected from the group consisting of a tertiary alkyl sulfonium ion, $-S^+CH_3R''$, where R'' is selected from $-CH_2COC_6H_5$,

$-CH_2COC_6H_5CH_3$, and $-CH_2COC_6H_5CH_2CH_3$; and a quaternary alkyl ammonium ion, $-N^+(R''')_3$, or a quaternary alkyl phosphonium ion, $-P^+(R''')_3$, where R'''_3 is $-(CH_2)_nH$ (where n = 1 to 3),

$-C_6H_5$, or $-CH_2C_6H_5$.

46. The compound of claim 45 wherein the halide is -Cl, -Br, or - I.

47. The compound of claim 45 wherein the dimethyl sulfonium ion is $-S(CH_3)_2^+$.

48. The compound of claim 45 wherein,

M_1' is $-R_1CH(R_3)R_2$, where R_1 is -2-hydroxy-5-nitrobenzyl-; R_2 is -YH, $-Y(CH_2)_nH$,

$-YC_6H_5$, or $-YCH_2C_6H_5$; and R_3 is $-(CH_2)_n-$, and is isotopically encoded by incorporation of one or more of ^2H, ^{13}C, ^{15}N or ^{18}O;

n is from 1 to 3 inclusive;

Y is CONH; and

X and M_2^+ are as defined above.

49. The compound of claim 45 wherein,

M_1' is $-R_1CH(R_3)R_2$, where R_1 is -(2-hydroxy-5-nitrobenzyl)-4-Y-; R_2 is $-(CH_2)_nH$,

$-C_6H_5$, $-CH_2C_6H_5$, $-NH_2$, -YH, $-Y(CH_2)_nH$, $-YC_6H_5$, or $-YCH_2C_6H_5$; and R_3 is $-(CH_2)_n-$, and is isotopically encoded by incorporation of one or more of ^2H, ^{13}C, ^{15}N or ^{18}O;

n is from 1 to 3 inclusive;

Y is CONH; and

X and M_2^+ are as defined above.

50. The compound of claim 45 wherein,

M_1' is $-R_1CH(R_3)R_2$, where R_1 is -2-hydroxy-5-nitrobenzyl-; R_2 is -YH, -$Y(CH_2)_nH$,

$-YC_6H_5$, or $-YCH_2C_6H_5$; and R_3 is $-(CH_2)_n-$, and is isotopically encoded by incorporation of one or more of ^2H, ^{13}C, ^{15}N or ^{18}O;

n is from 1 to 3 inclusive;

Y is NHCO; and

X and M_2^+ are as defined above.

51. The compound of claim 45 wherein,

M_1' is $-R_1CH(R_3)R_2$, where R_1 is -(2-hydroxy-5-nitrobenzyl)-4-Y-; R_2 is -$(CH_2)_nH$,

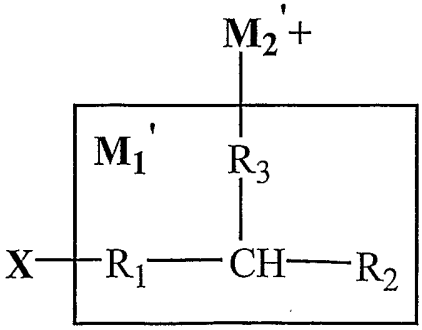
$-C_6H_5$, $-CH_2C_6H_5$, $-NH_2$, -YH, $-Y(CH_2)_nH$, $-YC_6H_5$, or $-YCH_2C_6H_5$; and R_3 is -$(CH_2)_n-$, and is isotopically encoded by incorporation of one or more of ^2H, ^{13}C, ^{15}N or ^{18}O;

n is from 1 to 3 inclusive;

Y is NHCO; and

X and M_2^+ are as defined above.

52. A compound of claim 1 of formula $XM_1'M_2'^+$, or a salt thereof, for specific reaction with the C2-indole position of the side chain of tryptophan or tryptophan containing proteins or peptides,



$XM_1'M_2'^+$

wherein,

X is a reactive group specific to the C2-indole position of the side chain of tryptophan selected from a halide or a dimethyl sulfonium ion;

M_1' is $-R_1CH(R_3)R_2$, where R_1 is selected from a -2-hydroxy-5-nitrobenzyl- and -(2-hydroxy-5-nitrobenzyl)-4-Y- group; R_2 is selected from $-(CH_2)_nH$, $-C_6H_5$, $-CH_2C_6H_5$, $-NH_2$,

-YH, $-Y(CH_2)_nH$, $-YC_6H_5$, and $-YCH_2C_6H_5$, and R_3 is $-(CH_2)_n-$, and is isotopically encoded by incorporation of one or more of ^2H, ^{13}C, ^{15}N or ^{18}O;

n is from 1 to 3 inclusive;

Y is selected from CONH, NHCO, COO and COS; and

$M_2'^+$ is attached to the R_3 group of M_1' and is selected from the group consisting of a tertiary alkyl sulfonium ion, $-S^+CH_3R''$, where R'' is selected from $-CH_2COC_6H_5$, $-CH_2COC_6H_5CH_3$, and $-CH_2COC_6H_5CH_2CH_3$, and a quaternary alkyl ammonium ion, $-N^+(R''')_3$, or a quaternary alkyl phosphonium ion $-P^+(R''')_3$, where R'''_3 is $-(CH_2)_nH$ (where n = 1 to 3), $-C_6H_5$, or $-CH_2C_6H_5$; and is isotopically encoded by incorporation of one or more of ^2H, ^{13}C, ^{15}N or ^{18}O.

53. The compound of claim 52 wherein the halide is -Cl, -Br, or - I.

54. The compound of claim 52 wherein the dimethyl sulfonium ion is $-S(CH_3)_2^+$.

55. The compound of claim 52 wherein,

M_1' is $-R_1CH(R_3)R_2$, where R_1 is -2-hydroxy-5-nitrobenzyl-; R_2 is -YH, $-Y(CH_2)_nH$, $-YC_6H_5$, or $-YCH_2C_6H_5$; and R_3 is $-(CH_2)_n-$, and is isotopically encoded by incorporation of one or more of ^2H, ^{13}C, ^{15}N or ^{18}O;

n is from 1 to 3 inclusive;

Y is CONH; and

X and $M_2'^+$ are as defined above.

56. The compound of claim 52 wherein,

M_1' is $-R_1CH(R_3)R_2$, where R_1 is -(2-hydroxy-5-nitrobenzyl)-4-Y-; R_2 is $-(CH_2)_nH$,

$-C_6H_5$, $-CH_2C_6H_5$, $-NH_2$, -YH, $-Y(CH_2)_nH$, $-YC_6H_5$, or $-YCH_2C_6H_5$; and R_3 is $-(CH_2)_n-$, and is isotopically encoded by incorporation of one or more of ^2H, ^{13}C, ^{15}N or ^{18}O;

n is from 1 to 3 inclusive;

Y is CONH; and

X and $M_2^{'+}$ are as defined above.

57. The compound of claim 52 wherein,

M_1' is $-R_1CH(R_3)R_2$, where R_1 is -2-hydroxy-5-nitrobenzyl-; R_2 is -YH, $-Y(CH_2)_nH$, $-YC_6H_5$, or $-YCH_2C_6H_5$; and R_3 is $-(CH_2)_n-$, and is isotopically encoded by incorporation of one or more of ^2H, ^{13}C, ^{15}N or ^{18}O;

n is from 1 to 3 inclusive;

Y is NHCO; and

X and $M_2^{'+}$ are as defined above.

58. The compound of claim 52 wherein,

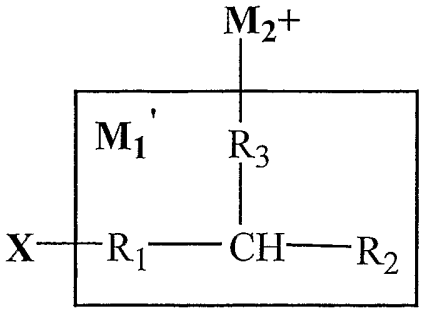
M_1' is $-R_1CH(R_3)R_2$, where R_1 is -(2-hydroxy-5-nitrobenzyl)-4-Y-; R_2 is $-(CH_2)_nH$, $-C_6H_5$, $-CH_2C_6H_5$, $-NH_2$, -YH, $-Y(CH_2)_nH$, $-YC_6H_5$, or $-YCH_2C_6H_5$; and R_3 is $-(CH_2)_n-$, and is isotopically encoded by incorporation of one or more of ^2H, ^{13}C, ^{15}N or ^{18}O;

n is from 1 to 3 inclusive;

Y is NHCO; and

X and $M_2^{'+}$ are as defined above.

59. A compound of claim 1 of formula $XM_1'M_2^+$, or a salt thereof, for specific reaction with the C2-indole position of the side chain of tryptophan or tryptophan containing proteins or peptides,



$XM_1'M_2^+$

wherein,

X is a reactive group specific to the C2-indole position of the side chain of tryptophan selected from a sulfenylhalide.

M_1' is $-R_1CH(R_3)R_2$, where R_1 is selected from a -2-nitrophenyl- and a -(2-nitrophenyl)-4-Y- group; R_2 is selected from $-(CH_2)_nH$, $-C_6H_5$, $-CH_2C_6H_5$, $-NH_2$, -YH, $-Y(CH_2)_nH$, $-YC_6H_5$, and $-YCH_2C_6H_5$; and R_3 is $-(CH_2)_n-$, and is isotopically encoded by incorporation of one or more of ^2H, ^{13}C, ^{15}N or ^{18}O;

n is from 1 to 3 inclusive;

Y is selected from CONH, NHCO, COO or COS; and

M_2^+ is attached to the R_3 group of M_1' and is selected from the group consisting of a tertiary alkyl sulfonium ion, $-S^+CH_3R''$, where R'' is selected from -$CH_2COC_6H_5$,

$-CH_2COC_6H_5CH_3$, and $-CH_2COC_6H_5CH_2CH_3$; and a quaternary alkyl ammonium ion, $-N^+(R''')_3$, or a quaternary alkyl phosphonium ion, $-P^+(R''')_3$, where R'''_3 is $-(CH_2)_nH$ (where n = 1 to 3),

$-C_6H_5$, or $-CH_2C_6H_5$.

60. The compound of claim 59 wherein, the sulfenylhalide is -SCl, -SBr, or - SI.

61. The compound of claim 59 wherein,

M_1' is $-R_1CH(R_3)R_2$, where R_1 is -2-nitrophenyl-; R_2 is -YH, $-Y(CH_2)_nH$, -YC_6H_5, or

$-YCH_2C_6H_5$; and R_3 is $-(CH_2)_n-$ and is isotopically encoded by incorporation of one or more of ^2H, ^{13}C, ^{15}N or ^{18}O;

n is from 1 to 3 inclusive;

Y is CONH; and

X and M_2^+ are as defined above.

62. The compound of claim 59 wherein,

M_1' is $-R_1CH(R_3)R_2$, where R_1 is -(2-nitrophenyl)-4-Y-; R_2 is $-(CH_2)_nH$, $-C_6H_5$, $-CH_2C_6H_5$, $-NH_2$, -YH, $-Y(CH_2)_nH$, $-YC_6H_5$, or $-YCH_2C_6H_5$; and R_3 is $-(CH_2)_n-$, and is isotopically encoded by incorporation of one or more of ^2H, ^{13}C, ^{15}N or ^{18}O;

n is from 1 to 3 inclusive;

Y is CONH; and

X and M_2^+ are as defined above.

63. The compound of claim 59 wherein,

M_1' is $-R_1CH(R_3)R_2$, where R_1 is -2-nitrophenyl-; R_2 is -YH, $-Y(CH_2)_nH$, $-YC_6H_5$, or $-YCH_2C_6H_5$; and R_3 is $-(CH_2)_n-$, and is isotopically encoded by incorporation of one or more of ^2H, ^{13}C, ^{15}N or ^{18}O;

n is from 1 to 3 inclusive;

Y is NHCO; and

X and M_2^+ are as defined above.

64. The compound of claim 59 wherein,

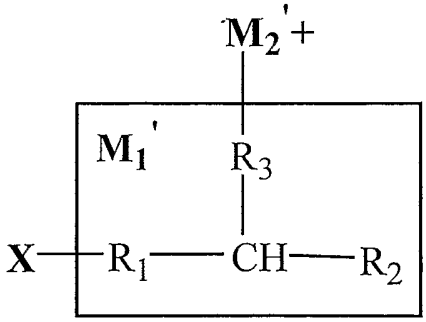
M_1' is $-R_1CH(R_3)R_2$, where R_1 is -(2-nitrophenyl)-4-Y-; R_2 is $-(CH_2)_nH$, $-C_6H_5$, $-CH_2C_6H_5$, $-NH_2$, -YH, $-Y(CH_2)_nH$, $-YC_6H_5$, or $-YCH_2C_6H_5$; and R_3 is $-(CH_2)_n-$, and is isotopically encoded by incorporation of one or more of ^2H, ^{13}C, ^{15}N or ^{18}O;

n is from 1 to 3 inclusive;

Y is NHCO; and

X and M_2^+ are as defined above.

65. A compound of claim 1 of formula $XM_1'M_2'^+$, or a salt thereof, for specific reaction with the C2-indole position of the side chain of tryptophan or tryptophan containing proteins or peptides,



$XM_1'M_2'^+$

wherein,

X is a reactive group specific to the C2-indole position of the side chain of tryptophan selected from a sulfenylhalide;

M_1' is $-R_1CH(R_3)R_2$, where R_1 is selected from a -2-nitrophenyl- and a -(2-nitrophenyl)-4-Y- group; R_2 is selected from $-(CH_2)_nH$, $-C_6H_5$, $-CH_2C_6H_5$, $-NH_2$, -YH, $-Y(CH_2)_nH$, $-YC_6H_5$, and $-YCH_2C_6H_5$; and R_3 is $-(CH_2)_n-$, and is isotopically encoded by incorporation of one or more of ^2H, ^{13}C, ^{15}N or ^{18}O;

n is from 1 to 3 inclusive;

Y is selected from CONH, NHCO, COO or COS; and

$M_2'^+$ is attached to the R_3 group of M_1' and is selected from the group consisting of a tertiary alkyl sulfonium ion, $-S^+CH_3R''$, where R'' is selected from $-CH_2COC_6H_5$, $-CH_2COC_6H_5CH_3$, and $-CH_2COC_6H_5CH_2CH_3$; and a quaternary alkyl ammonium ion, $-N^+(R''')_3$, or a quaternary alkyl phosphonium ion, $-P^+(R''')_3$, where R'''_3 is $-(CH_2)_nH$ (where n = 1 to 3),

$-C_6H_5$, or $-CH_2C_6H_5$; and is isotopically encoded by incorporation of one or more of ^2H, ^{13}C, ^{15}N or ^{18}O.

66. The compound of claim 65 wherein the sulfenylhalide is -SCl, -SBr, or - SI.

67. The compound of claim 65 wherein,

M_1' is $-R_1CH(R_3)R_2$, where R_1 is -2-nitrophenyl-; R_2 is -YH, $-Y(CH_2)_nH$, $-YC_6H_5$, or

$-YCH_2C_6H_5$; and R_3 is $-(CH_2)_n-$ and is isotopically encoded by incorporation of one or more of ^2H, ^{13}C, ^{15}N or ^{18}O;

n is from 1 to 3 inclusive;

Y is CONH; and

X and $M_2^{'+}$ are as defined above.

68. The compound of claim 65 wherein,

M_1' is $-R_1CH(R_3)R_2$, where R_1 is -(2-nitrophenyl)-4-Y-; R_2 is $-(CH_2)_nH$, $-C_6H_5$, $-CH_2C_6H_5$, $-NH_2$, -YH, $-Y(CH_2)_nH$, $-YC_6H_5$, or $-YCH_2C_6H_5$; and R_3 is $-(CH_2)_n-$, and is isotopically encoded by incorporation of one or more of ^2H, ^{13}C, ^{15}N or ^{18}O;

n is from 1 to 3 inclusive;

Y is CONH; and

X and $M_2^{'+}$ are as defined above.

69. The compound of claim 65 wherein,

M_1' is $-R_1CH(R_3)R_2$, where R_1 is -2-nitrophenyl-; R_2 is -YH, $-Y(CH_2)_nH$, $-YC_6H_5$, or $-YCH_2C_6H_5$; and R_3 is $-(CH_2)_n-$, and is isotopically encoded by incorporation of one or more of ^2H, ^{13}C, ^{15}N or ^{18}O;

n is from 1 to 3 inclusive;

Y is NHCO; and

X and $M_2^{'+}$ are as defined above.

70. The compound of claim 65 wherein,

M_1' is $-R_1CH(R_3)R_2$, where R_1 is -(2-nitrophenyl)-4-Y-; R_2 is $-(CH_2)_nH$, $-C_6H_5$, $-CH_2C_6H_5$, $-NH_2$, -YH, $-Y(CH_2)_nH$, $-YC_6H_5$, or $-YCH_2C_6H_5$; and R_3 is $-(CH_2)_n-$, and is isotopically encoded by incorporation of one or more of ^2H, ^{13}C, ^{15}N or ^{18}O;

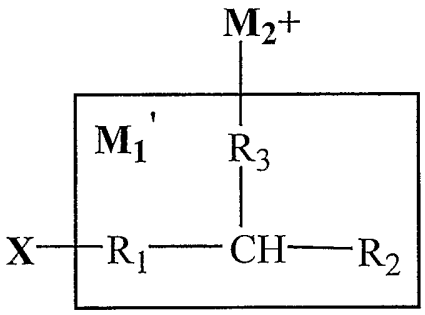
n is from 1 to 3 inclusive;

Y is NHCO; and

X and $M_2^{'+}$ are as defined above.

71. A compound of claim 1 of formula $XM_1'M_2^+$, or a salt thereof, for specific reaction with dehydroalanine or dehydroamino-2-butyric acid formed by β-elimination from O-linked phosphorylated or glycosylated serine or threonine, or dehydroalanine or dehydroamino-2-butyric acid residues formed by β-elimination

from O-linked phosphorylated or glycosylated serine or threonine containing proteins or peptides respectively,



$XM_1'M_2^+$

wherein,

X is a thiol;

M_1' is $-R_1CH(R_3)R_2$, where R_1 is selected from $-(CH_2)_n-$, -Y- and $-(CH_2)_nY-$; R_2 is selected from $-(CH_2)_nH$, $-C_6H_5$, $-CH_2C_6H_5$, $-NH_2$, -YH, $-Y(CH_2)_nH$, $-YC_6H_5$, and $-YCH_2C_6H_5$; and R_3 is $-(CH_2)_n-$, and is isotopically encoded by incorporation of one or more of ^2H, ^{13}C, ^{15}N or ^{18}O;

n is from 1 to 3 inclusive;

Y is selected from CONH, NHCO and COO; and

M_2^+ is attached to the R_3 group of M_1' and is selected from the group consisting of a tertiary alkyl sulfonium ion, $-S^+CH_3R''$, where R'' is selected from $-CH_2COC_6H_5$, $-CH_2COC_6H_5CH_3$, and $-CH_2COC_6H_5CH_2CH_3$; and a quaternary alkyl ammonium ion, $-N^+(R''')_3$, or a quaternary alkyl phosphonium ion, $-P^+(R''')_3$, where R'''_3 is $-(CH_2)_nH$ (where n = 1 to 3), $-C_6H_5$, or $-CH_2C_6H_5$.

72. The compound of claim 71 wherein the thiol is -SH.

73. The compound of claim 71 wherein,

M_1' is $-R_1CH(R_3)R_2$, where R_1 is $-(CH_2)_n-$; R_2 is -YH, $-Y(CH_2)_nH$, $-YC_6H_5$, or $-YCH_2C_6H_5$; and R_3 is $-(CH_2)_n-$, and is isotopically encoded by incorporation of one or more of ^2H, ^{13}C, ^{15}N or ^{18}O;

n is from 1 to 3 inclusive;

Y is CONH; and

X and M_2^+ are as defined above.

74. The compound of claim 71 wherein,

M_1' is $-R_1CH(R_3)R_2$, where R_1 is -Y- or $-(CH_2)_nY-$; R_2 is $-(CH_2)_nH$, $-C_6H_5$, $-CH_2C_6H_5$,

$-NH_2$, -YH, $-Y(CH_2)_nH$, $-YC_6H_5$, or $-YCH_2C_6H_5$; and R_3 is $-(CH_2)_n-$, and is isotopically encoded by incorporation of one or more of ^2H, ^{13}C, ^{15}N or ^{18}O;

n is from 1 to 3 inclusive;

Y is CONH; and

X and M_2^+ are as defined above.

75. The compound of claim 71 wherein,

M_1' is $-R_1CH(R_3)R_2$, where R_1 is $-(CH_2)_n-$; R_2 is -YH, $-Y(CH_2)_nH$, $-YC_6H_5$, or $-YCH_2C_6H_5$; and R_3 is $-(CH_2)_n-$, and is isotopically encoded by incorporation of one or more of ^2H, ^{13}C, ^{15}N or ^{18}O;

n is from 1 to 3 inclusive;

Y is NHCO; and

X and M_2^+ are as defined above.

76. The compound of claim 71 wherein,

M_1' is $-R_1CH(R_3)R_2$, where R_1 is -Y- or $-(CH_2)_nY-$; R_2 is $-(CH_2)_nH$, $-C_6H_5$, $-CH_2C_6H_5$,

$-NH_2$, -YH, $-Y(CH_2)_nH$, $-YC_6H_5$, or $-YCH_2C_6H_5$; and R_3 is $-(CH_2)_n-$, and is isotopically encoded by incorporation of one or more of ^2H, ^{13}C, ^{15}N or ^{18}O;

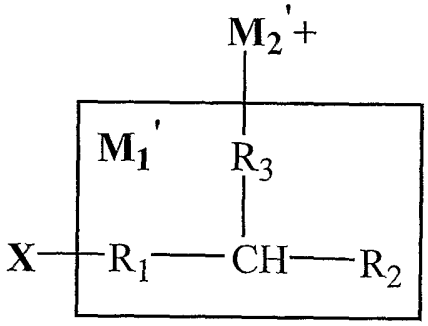
n is from 1 to 3 inclusive;

Y is NHCO; and

X and M_2^+ are as defined above.

77. A compound of claim 1 of formula $XM_1'M_2'^+$, or a salt thereof, for specific reaction with dehydroalanine or dehydroamino-2-butyric acid formed by β-elimination from O-linked phosphorylated or glycosylated serine or threonine, or dehydroalanine or dehydroamino-2-butyric acid residues formed by β-elimination

from O-linked phosphorylated or glycosylated serine or threonine containing proteins or peptides respectively,



$XM_1'M_2'^+$

wherein,

X is a thiol;

M_1' is $-R_1CH(R_3)R_2$, where R_1 is selected from $-(CH_2)_n-$, -Y- and $-(CH_2)_nY-$; R_2 is selected from $-(CH_2)_nH$, $-C_6H_5$, $-CH_2C_6H_5$, $-NH_2$, -YH, $-Y(CH_2)_nH$, $-YC_6H_5$, and $-YCH_2C_6H_5$; and R_3 is $-(CH_2)_n-$, and is isotopically encoded by incorporation of one or more of ^2H, ^{13}C, ^{15}N or ^{18}O;

n is from 1 to 3 inclusive;

Y is selected from CONH, NHCO and COO;

$M_2'^+$ is attached to the R_3 group of M_1' and is selected from the group consisting of a tertiary alkyl sulfonium ion, $-S^+CH_3R''$, where R'' is selected from $-CH_2COC_6H_5$, $-CH_2COC_6H_5CH_3$, and $-CH_2COC_6H_5CH_2CH_3$; and a quaternary alkyl ammonium ion, $-N^+(R''')_3$, or a quaternary alkyl phosphonium ion, $-P^+(R''')_3$, where R'''_3 is $-(CH_2)_nH$ (where n = 1 to 3), $-C_6H_5$, or $-CH_2C_6H_5$; and is isotopically encoded by incorporation of one or more of ^2H, ^{13}C, ^{15}N or ^{18}O.

78. The compound of claim 77 wherein the thiol is -SH.

79. The compound of claim 77 wherein,

M_1' is $-R_1CH(R_3)R_2$, where R_1 is $-(CH_2)_n-$; R_2 is -YH, $-Y(CH_2)_nH$, $-YC_6H_5$, or

$-YCH_2C_6H_5$; and R_3 is $-(CH_2)_n-$, and is isotopically encoded by incorporation of one or more of ^2H, ^{13}C, ^{15}N or ^{18}O;

n is from 1 to 3 inclusive;

Y is CONH; and

X and $M_2^{'+}$ are as defined above.

80. The compound of claim 77 wherein,

M_1' is $-R_1CH(R_3)R_2$, where R_1 is -Y- or $-(CH_2)_nY-$; R_2 is $-(CH_2)_nH$, $-C_6H_5$, $-CH_2C_6H_5$, $-NH_2$, -YH, $-Y(CH_2)_nH$, $-YC_6H_5$, or $-YCH_2C_6H_5$; and R_3 is $-(CH_2)_n-$, and is isotopically encoded by incorporation of one or more of ^2H, ^{13}C, ^{15}N or ^{18}O;

n is from 1 to 3 inclusive;

Y is CONH; and

X and $M_2^{'+}$ are as defined above.

81. The compound of claim 77 wherein,

M_1' is $-R_1CH(R_3)R_2$, where R_1 is $-(CH_2)_n-$; R_2 is -YH, $-Y(CH_2)_nH$, $-YC_6H_5$, or $-YCH_2C_6H_5$; and R_3 is $-(CH_2)_n-$, and is isotopically encoded by incorporation of one or more of ^2H, ^{13}C, ^{15}N or ^{18}O;

n is from 1 to 3 inclusive;

Y is NHCO; and

X and $M_2^{'+}$ are as defined above.

82. The compound of claim 77 wherein,

M_1' is $-R_1CH(R_3)R_2$, where R_1 is -Y- or $-(CH_2)_nY-$; R_2 is $-(CH_2)_nH$, $-C_6H_5$, $-CH_2C_6H_5$, $-NH_2$, -YH, $-Y(CH_2)_nH$, $-YC_6H_5$, or $-YCH_2C_6H_5$; and R_3 is $-(CH_2)_n-$, and is isotopically encoded by incorporation of one or more of ^2H, ^{13}C, ^{15}N or ^{18}O;

n is from 1 to 3 inclusive;

Y is NHCO; and

X and $M_2^{'+}$ are as defined above.

83. A compound consisting of amino acids, peptides or proteins that have been derivatized with a compound of any of claims 1-82.

84. A method for the quantitative analysis of amino acids, peptides or proteins, the method comprising subjecting:

a first amino acid, peptide or protein having a fixed-charge, introduced as a result of derivatization with a compound of formula $XM_1M_2^+$, wherein X and M_2^+ are as defined in claims 1-82, and M_1 is a linker group having the same structure as M_1' below, but containing only naturally abundant isotopes; and

a second amino acid, peptide or protein having a fixed-charge, introduced as a result of derivatization with a compound of formula $XM_1'M_2^+$, wherein X, M_1' and M_2^+ are as defined in claims 1-82, wherein M_1' is isotopically encoded by incorporation of one or more of ^2H, ^{13}C, ^{15}N or ^{18}O;

to dissociation to form product ions that are characteristic of fragmentation occurring at the fixed-charge site.

85. A method for the quantitative analysis of amino acids, peptides or proteins, the method comprising subjecting:

a first amino acid, peptide or protein having a fixed-charge, introduced as a result of derivatization with a compound of formula $XM_1M_2'^+$, wherein X and $M_2'^+$ are as defined in claims 1-82, and M_1 is a linker group having the same structure as M_1' below, but containing only naturally abundant isotopes; and

a second amino acid, peptide or protein having a fixed-charge, introduced as a result of derivatization with a compound of formula $XM_1'M_2^+$ or $XM_1'M_2'^+$, wherein X, M_1', M_2^+, and $M_2'^+$ are as defined in claims 1-82, wherein M_1' is isotopically encoded by incorporation of one or more of ^2H, ^{13}C, ^{15}N or ^{18}O;

to dissociation to form product ions that are characteristic of fragmentation occurring at the fixed-charge site.

86. The method of claim 84 or claim 85, wherein the M_1 group is a branched alkyl optionally interrupted or substituted with an alkyl, aryl, substituted alkyl, substituted aryl, amino, amide, acid, ester or thioester.

87. A method for quantitative analysis of amino acids, peptides or proteins, the method comprising:

(1) introducing a mixture of amino acids, peptides or proteins containing at least one selected amino acid, peptide or protein, or peptide or protein comprising at least one residue of the selected amino acid, derivatized to contain a fixed-charge using one or more compounds of formula $XM_1M_2^+$, $XM_1'M_2^+$, $XM_1M_2'^+$ and $XM_1'M_2'^+$, or salts thereof, as described in claims 1-82;

(2) passing the mixture of amino acids, peptides or proteins containing at least one derivatized amino acid or derivatized amino acid residue containing peptide or protein, through a first mass resolving spectrometer to select precursor protein or peptide ions having a first mass-to-charge ratio;

(3) subjecting the precursor ions of the first mass-to-charge ratio to dissociation to form a product ion having a second mass-to-charge ratio that is characteristic of the loss of M_2 or M_2' at the site of the fixed-charge; and

(4) detecting the product ions having the second mass-to-charge ratio.

88. The method of claim 87, wherein the method of analysis used for the identification and/or quantitation of amino acids, peptides or proteins.

89. The method of claim 87, wherein the amino acid, peptide or protein contains an N-terminal amino group, a cysteine, a homocysteine, a lysine, an arginine, a homoarginine, a tryptophan, a dehydroalanine or a dehydroamino-2-butyric acid.

90. The method of claim 87, further comprising a preceding step of derivatizing the amino acid, peptide or protein with the one or more compounds of formula $XM_1M_2^+$, $XM_1'M_2^+$, $XM_1M_2'^+$ and $XM_1'M_2'^+$, or salts thereof.

91. The method of claim 87, further comprising a further step of: (5) determining the identity of the peptide or protein.

92. The method of claim 91, wherein step (5) is performed by first repeating steps (1), (2), and (3) and then subjecting the product ion having the second characteristic mass-to-charge ratio formed by loss from the precursor to a further stage of dissociation to form a series of product ions having a range of mass to charge ratios, for the purpose of determining the amino acid sequence of the peptide or protein and subsequently, the identity of the protein of origin.

93. The method of claim 91, wherein step (5) is performed by use of high resolution mass analyzers to obtain an accurate mass tag on the product ion detected in step (4).

94. The method of claim C7, wherein the accurate mass tag is a mass accuracy of approximately 1-5 ppm.

95. The method of claim 91, further comprising database searching to identify those peptides found to contain a fixed-charge derivative.

96. The method of any of claims 87-95, wherein the amino acid, peptide or protein ion is dissociated by a dissociation method selected from the group consisting of: (i) collisions with an inert gas (collision-induced dissociation (CID) or collisionally-activated dissociation (CAD)); (ii) collisions with a surface (surface-induced dissociation or SID); (iii) interaction with photons (e. g. via a laser) resulting in photodissociation; (iv) thermal/black body infrared radiative dissociation (BIRD), (v) interaction with an electron beam, resulting in electron-induced dissociation for singly charged cations (EID), electron-capture dissociation (ECD) for multiply charged cations, or combinations thereof, and (vi) by electron transfer dissociation (ETD).

97. The method of any of claims 87-96, wherein analysis of the amino acid, peptide or protein ion is performed by tandem mass spectrometry.

98. The method of claim 97, wherein the tandem mass spectrometer is equipped with electrospray ionization (ESI) or matrix assisted laser desorption ionization (MALDI) interfaces to transfer the protein or peptide ion from solution into the gas-phase.

99. The method of any of claims 87-98, further comprising one or more steps of protein extraction, protein separation, reduction and alkylation of cysteine disulfides and/or protein digestion.

100. A reagent kit for quantitative analysis of amino acids, peptides or proteins by tandem mass spectrometry, comprising

a container containing a compound of formula $XM_1'M_2^+$ or $XM_1'M_2'^+$, wherein

X is a reactive group specific to a functional group contained within an amino acid, peptide or protein, or peptide or protein containing at least one of such amino acid;

M_1' is a linker group between X and M_2^+ or $M_2'^+$, and is isotopically encoded by incorporation of one or more of ^2H, ^{13}C, ^{15}N or ^{18}O;

M_2^+ is selected from the group consisting of a tertiary alkyl sulfonium ion, a quaternary alkyl ammonium ion or a quaternary alkyl phosphonium ion; and

$M_2'^+$ is selected from the group consisting of a tertiary alkyl sulfonium ion, a quaternary alkyl ammonium ion or a quaternary alkyl phosphonium ion, and is isotopically encoded by incorporation of one or more of ^2H, ^{13}C, ^{15}N or ^{18}O.

101. A reagent kit for quantitative analysis of amino acids, peptides or proteins by tandem mass spectrometry, comprising

a container containing compounds of formula $XM_1M_2^+$ and $XM_1'M_2^+$, wherein

X is a reactive group specific to a functional group contained within an amino acid, peptide or protein, or peptide or protein containing at least one of such amino acid;

M_1 is a linker group between X and M_2^+ or $M_2'^+$;

M_1' is a linker group between X and M_2^+ or $M_2'^+$, and is isotopically encoded by incorporation of one or more of ^2H, ^{13}C, ^{15}N or ^{18}O;

M_2^+ is selected from the group consisting of a tertiary alkyl sulfonium ion, a quaternary alkyl ammonium ion or a quaternary alkyl phosphonium ion; and

$M_2'^+$ is selected from the group consisting of a tertiary alkyl sulfonium ion, a quaternary alkyl ammonium ion or a quaternary alkyl phosphonium ion, and is isotopically encoded by incorporation of one or more of ^2H, ^{13}C, ^{15}N or ^{18}O.

102. A reagent kit for quantitative analysis of amino acids, peptides or proteins by tandem mass spectrometry, comprising

a container containing the compounds of formula $XM_1M_2'^+$ and $XM_1'M_2'^+$, wherein

X is a reactive group specific to a functional group contained within an amino acid, peptide or protein, or peptide or protein containing at least one of such amino acid;

M_1 is a linker group between X and M_2^+ or $M_2'^+$;

M_1' is a linker group between X and M_2^+ or $M_2'^+$, and is isotopically encoded by incorporation of one or more of ^2H, ^{13}C, ^{15}N or ^{18}O;

$M_2'^+$ is selected from the group consisting of a tertiary alkyl sulfonium ion, a quaternary alkyl ammonium ion or a quaternary alkyl phosphonium ion, and is isotopically encoded by incorporation of one or more of ^2H, ^{13}C, ^{15}N or ^{18}O.

103. A reagent kit for quantitative analysis of amino acids, peptides or proteins by tandem mass spectrometry, comprising

a container containing the compounds of formula $XM_1M_2'^+$, $XM_1'M_2'^+$ and $XM_1'M_2^+$, wherein

X is a reactive group specific to a functional group contained within an amino acid, peptide or protein, or peptide or protein containing at least one of such amino acid;

M_1 is a linker group between X and M_2^+ or $M_2'^+$;

M_1' is a linker group between X and M_2^+ or $M_2'^+$, and is isotopically encoded by incorporation of one or more of ^2H, ^{13}C, ^{15}N or ^{18}O;

M_2^+ is selected from the group consisting of a tertiary alkyl sulfonium ion, a quaternary alkyl ammonium ion or a quaternary alkyl phosphonium ion;

$M_2'^+$ is selected from the group consisting of a tertiary alkyl sulfonium ion, a quaternary alkyl ammonium ion or a quaternary alkyl phosphonium ion, and is isotopically encoded by incorporation of one or more of ^2H, ^{13}C, ^{15}N or ^{18}O.

104. A reagent kit comprising

a container containing compounds consisting of amino acids, peptides or proteins that have been derivatized with a compound of formula $XM_1'M_2^+$ or $XM_1'M_2'^+$, wherein

X is a reactive group specific to a functional group contained within an amino acid, peptide or protein, or peptide or protein containing at least one of such amino acid;

M_1' is a linker group between X and M_2^+ or $M_2'^+$, and is isotopically encoded by incorporation of one or more of ^2H, ^{13}C, ^{15}N or ^{18}O;

M_2^+ is selected from the group consisting of a tertiary alkyl sulfonium ion, a quaternary alkyl ammonium ion or a quaternary alkyl phosphonium ion;

$M_2^{'+}$ is selected from the group consisting of a tertiary alkyl sulfonium ion, a quaternary alkyl ammonium ion or a quaternary alkyl phosphonium ion, and is isotopically encoded by incorporation of one or more of ^{2}H, ^{13}C, ^{15}N or ^{18}O.

105. The reagent kit for quantitative analysis of amino acids, peptides or proteins of any of claims 100-104, further comprising instructions for use of the compounds in the quantitative analysis of amino acids, peptides or proteins by mass spectrometry.

106. The reagent kit of any of claims 100-105, further comprising one or more containers containing: one or more cysteine disulfide reducing agents, one or more alkylating agents, one or more proteases or chemical cleavage agents, and/or one or more solvents.

107. The reagent kit of claim 106, wherein the one or more cysteine disulfide reducing agents are selected from the group consisting of dithiothreitol (DTT), mercaptoethanol, tris-carboxyethyl phosphine (TCEP) and tributylphosphine (TBP).

108. The reagent kit of claim 106, wherein the one or more cysteine alkylating agents are selected from the group consisting of alkylhalides, vinylpyridine and acrylamide.

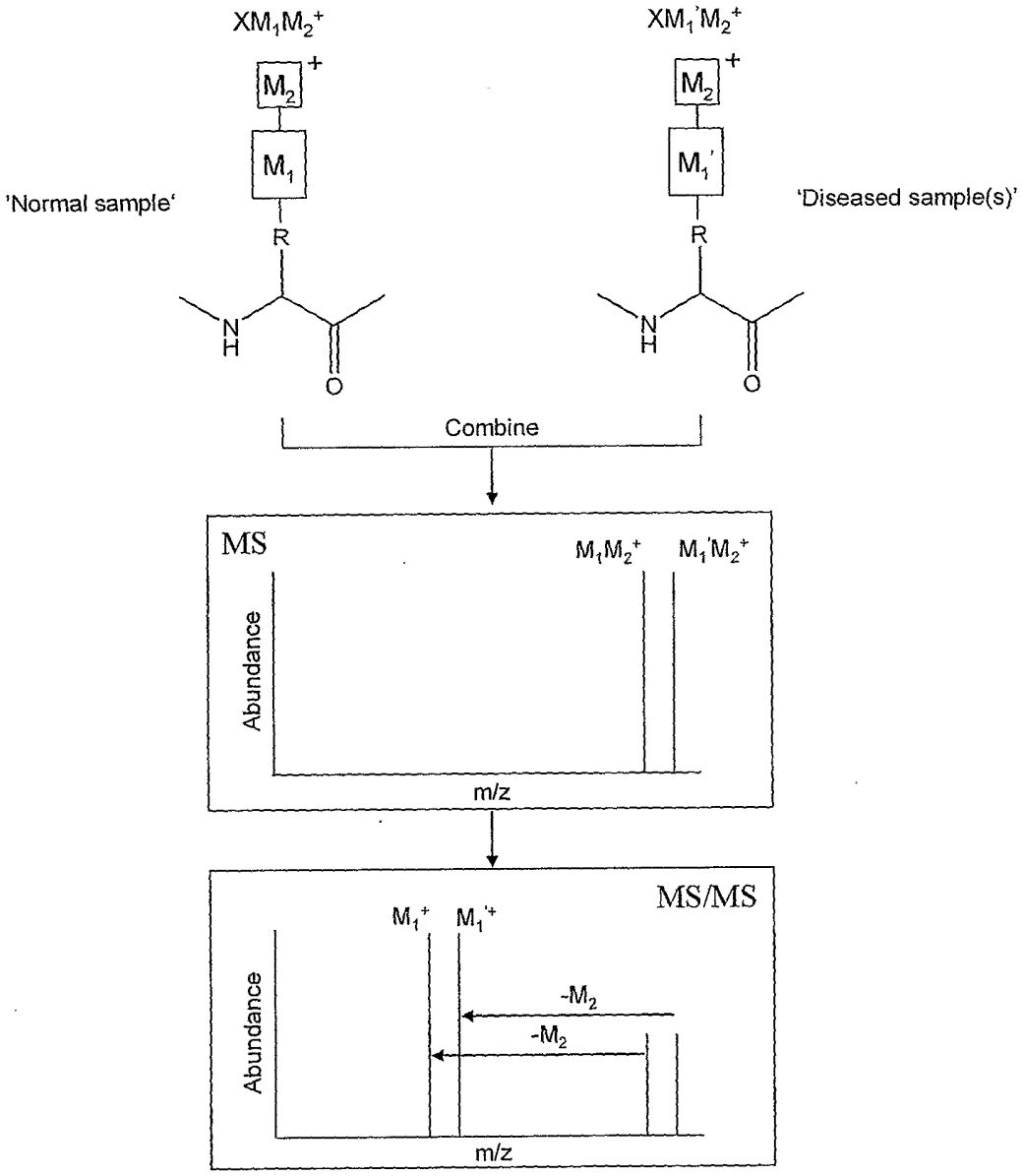
109. The reagent kit of claim 108, wherein the alkylhalides are selected from iodoacetic acid and iodoacetamide.

110. The reagent kit of claim 106, wherein the one or more proteases or chemical cleavage agents are selected from the group consisting of trypsin, Endoproteinase Lys-C, Endoproteinase Asp-N, Endoproteinase Glu-C, pepsin, papain, thermolysin, cyanogen bromide, hydroxylamine hydrochloride, 2-[2'-nitrophenylsulfenyl]-3-methyl-3'-bromoindole (BNPS-skatole), iodosobenzoic acid, pentafluoropropionic acid and dilute hydrochloric acid.

111. The reagent kit of claim 106, wherein the one or more solvents are selected from the group consisting of urea, guanidine hydrochloride, acetonitrile, methanol and water.

112. A method for providing an internal standard in a mass spectrometer method comprising

adding to a sample a predetermined quantity of an isotopically encoded fixed charge derivatized amino acid, peptide or protein as claimed in claim 83.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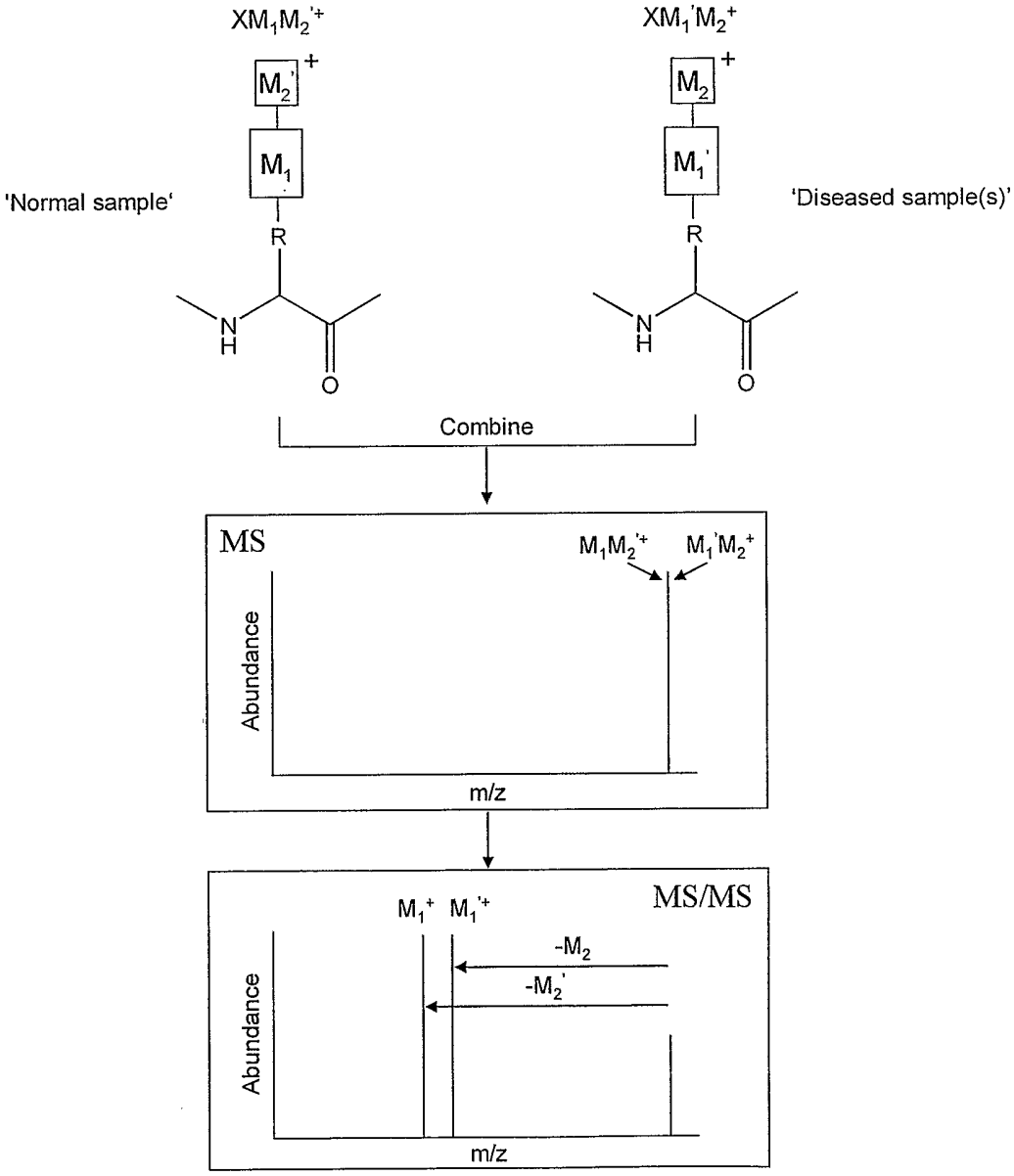
M_1
R
N
H
O
'Diseased sample(s)'
$XM_1'M_2^+$
M_2
M_1'
R
N
H
O
Combine
MS
$M_1M_2^+$
$M_1'M_2^+$
Abundance
m/z
MS/MS
M_1^+
$M_1'^+$
$-M_2$
$-M_2$
Abundance
m/z

Figure 1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M2'
+
M1
R
N
H
O
'Normal sample'
XM1'M2+
M2
+
M1'
R
N
H
O
'Diseased sample(s)'
Combine
MS
M1M2'+
M1'M2+
Abundance
m/z
MS/MS
M1+
M1'+
-M2
-M2'
Abundance
m/z

Figure 2