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(54) Title: ISOTOPE-CODED IONIZATION-ENHANCING REAGENTS (ICIER) FOR HIGH-THROUGHPUT PROTEIN IDENTIFICATION AND QUANTITATION USING MATRIX-ASSISTED LASER DESORPTION IONIZATION MASS SPECTROMETRY

(57) Abstract: Arginine-containing cysteine-modifying compounds useful for MALDI-MS analysis of proteins are provided. These compounds termed isotope-coded ionization enhancement reagents (ICIER) can provide ionization enhancement in MALDI-MS, relative quantitation, and additional database searching constraints at the same time without any extra sample manipulation. More specifically, ICIER increase the ionization efficiency of cysteine-containing peptides by attachment of a guanidino functional group. ICIER also increase the overall hydrophilicity of these peptides due the hydrophilic nature of ICIER and thus increase the percentage of recovery of these peptides during sample handling and processing such as in-gel digestion or liquid chromatography. Finally, a combination of both light and heavy ICIER provides an accurate way to obtain relative quantitation of proteins by MALDI-MIS and additional database searching constraints (number of cysteine residues in every single peptide peak) to increase the confidence of protein identification by peptide mass mapping.

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INTERNATIONAL SEARCH REPORT

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A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 G01N33/68 G01N33/58 C07C279/14 C07D207/456

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 G01N C07C C07D

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, BIOSIS, MEDLINE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	MCKENDRICK J E ET AL: "Rapid mass spectrometric determination of preferred irreversible proteinase inhibitors in combinatorial libraries" INTERNATIONAL JOURNAL OF MASS SPECTROMETRY, ELSEVIER SCIENCE PUBLISHERS, AMSTERDAM, NL, vol. 176, no. 1-2, 1 June 1998 (1998-06-01), pages 113-124, XP004128436 ISSN: 1387-3806 page 116, right-hand column --- -/--	14

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
28 October 2003	20/11/2003

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Vogt, T

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INTERNATIONAL SEARCH REPORT

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
Y	SECHI S & CHAIT BT: "Modification od Cysteine Residue by Alkylation. A Tool in Peptide Mapping and Protein Identification" ANALYTICAL CHEMISTRY, AMERICAN CHEMICAL SOCIETY. COLUMBUS, US, vol. 70, no. 24, 15 December 1998 (1998-12-15), pages 5150-5158, XP002192468 ISSN: 0003-2700 the whole document	1-20
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Y	KRAUSE E ET AL: "The dominance of arginine-containing peptides in MALDI-derived tryptic mass fingerprints of proteins." ANALYTICAL CHEMISTRY. UNITED STATES 1 OCT 1999, vol. 71, no. 19, 1 October 1999 (1999-10-01), pages 4160-4165, XP002259382 ISSN: 0003-2700 the whole document	1-20
P,X	BRANCIA FRANCESCO L ET AL: "Improved matrix-assisted laser desorption/ionization mass spectrometric analysis of tryptic hydrolysates of proteins following guanidination of lysine-containing peptides" RAPID COMMUNICATIONS IN MASS SPECTROMETRY, vol. 14, no. 21, 2000, pages 2070-2073, XP009018972 ISSN: 0951-4198 the whole document	1-20
E	WO 02 48717 A (GENETICS INST LLC) 20 June 2002 (2002-06-20) the whole document	1-20

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International Application No
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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	KRATZER R ET AL: "Suppression effects in enzymatic peptide ladder sequencing using ultraviolet - matrix assisted laser desorption/ionization - mass spectormetry." ELECTROPHORESIS. GERMANY AUG 1998, vol. 19, no. 11, August 1998 (1998-08), pages 1910-1919, XP009019035 ISSN: 0173-0835 Introduction, in particular p. 1911, 1. col. 1st paragraph the whole document	1-20

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INTERNATIONAL SEARCH REPORT

Information on patent family members

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