

Title (en)

LABELED PEPTIDES, PROTEINS AND ANTIBODIES AND PROCESSES AND INTERMEDIATES USEFUL FOR THEIR PREPARATION

Title (de)

MARKIERTE PEPTIDE, PROTEINE UND ANTIKÖRPER UND VERFAHREN UND ZWISCHENPRODUKTE ZUR DEREN HERSTELLUNG

Title (fr)

PEPTIDES, PROTEINES ET ANTICORPS ETIQUETES ET PROCESSUS ET PRODUITS INTERMEDIAIRES UTILES POUR LEUR PREPARATION

Publication

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Application

**EP 01954689 A 20010713**

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Abstract (en)

[origin: WO0208245A2] The invention provides peptide synthons having protected functional groups for attachment of desired moieties (e.g. functional molecules or probes). Also provided are peptide conjugates prepared from such synthons, and synthon and conjugate preparation methods including procedures for identifying optimum probe attachment sites. Biosensors are provided having functional molecules that can locate and bind to specific biomolecules within living cells. Biosensors can detect chemical and physiological changes in those biomolecules as living cells are moving, metabolizing and reacting to its environment. Methods are included for detecting GTP activation of a Pho GTP are protein using polypeptide biosensors. When the biosensor binds GTP-activated Rho GTPase protein, an environmentally sensitive dye emits a signal of a different lifetime, intensity or wavelength than when not bound. New fluorophores whose fluorescence responds to environmental changes are also provided that have improved detection and attachment properties, and that can be used in living cells, or in vitro.

[origin: WO0208245A2] A compound (I) which is a synthetic intermediate (a synthon) useful for preparing modified peptides, is new. A compound (I) which is a synthetic intermediate (a synthon) useful for preparing modified peptides. (I) Has the structure of formula (A). [Image] R1>hydrogen or amino protecting group; R2>hydrogen or carboxy protecting group; and R : an organic radical comprising one or more aminoxy groups. Independent claims are also included for the following: (1) a peptide (IIa) comprising a backbone and one or more aminoxy groups, provided the peptide is not glutathione and the peptide has at least one aminoxy group that is not part of a group NH2-O-CH2-C(=O)- positioned at the N-terminus of the peptide or that is not part of a group -C(=N)-O-CH2-C(=O)- that is in the backbone; (2) a peptide (IIb) comprising a backbone and one or more secondary aminoxy groups, provided the peptide having an aminoxy group that is not part of an oxime (C=N-O-) in the backbone; (3) a peptide conjugate (III) having the structure of formula (B); (4) identifying (IV) an optimal position for replacement of a functional molecule on a peptide having a peptide backbone and a known activity, by making a series of peptide conjugates, each peptide conjugate having the same amino acid sequence and the same functional molecule, where the functional molecule is linked at a different location along the backbone of every peptide conjugate in the series, and observing which functional molecule location does not substantially interfere with the known activity of the peptide; (5) a polypeptide biosensor (V) which comprises (III); (6) a polypeptide biosensor (VI) comprising a polypeptide capable of binding a GTP-activated Rho GTPase protein, where the polypeptide is operatively linked to a functional molecule; (7) a fusion protein (VII) comprising a biologically active Rho GTPase protein domain operatively linked to a fluorescent protein by (III), where the Rho GTPase protein domain is capable of binding GTP and forming an activated GTPase:GTP complex; (8) detecting binding of an antibody to an antigen, by reacting an antibody comprising (III) with an antigen or vice versa and detecting an antibody-antigen complex; (9) a fluorescent compound (VIII) of the formula (C); (10) a peptide biosensor comprising (VIII); (11) a protein, polypeptide, peptide, antibody or its fragment, or a nucleic acid attaching linked to (VIII); (12) attaching a biosensor to a cellular protein within a living cell, by providing the living cell with a biosensor capable of binding to a tag on the cellular protein, where the tag is a peptide segment that has been fused to the cellular protein expressed by the living cell; (13) a nucleic acid (IX) encoding the tag fused to above cellular protein; (14) a nucleic acid (X) encoding (VII); (15) an isolated vector comprising (IX); (16) an expression vector capable of expressing (VII) or the tag fused to the cellular protein as above; and (17) a cell comprising the above vector. [Image] R6>peptide or polypeptide; X : a direct bond or a linking group; R7>hydrogen, 1-6C alkyl, amino protecting group, or a radical comprising one or more aminoxy groups; Y : direct bond or a linking group; and D : a functional molecule. [Image] m : 1-3; n : 0-5; R8>, R11>, R12>CO, SO2, C=C(CN)2, S, O or C(CH3)2; R13>alkyl, branched alkyl or heterocyclic ring derivatized with charged groups to enhance water solubility and enhance photostability; and R9> and R10>alkyl chain derivatized with charged groups to enhance water solubility or with reactive groups for conjugation to other molecules.

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