

Application of *Safirinium* N-hydroxysuccinimide esters to derivatization of peptides for high-resolution mass spectrometry, tandem mass spectrometry, and fluorescent labeling of bacterial cells.

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Rok wydania

2020

Czasopismo

International Journal of
Molecular Sciences

Numer woluminu

21

Strony

9643/1-9643/27

DOI

10.3390/ijms21249643

Kolekcja

Naukowa

Język

Angielski

Streszczenie

Mass spectrometry methods are commonly used in the identification of peptides and biomarkers. Due to a relatively low abundance of proteins in biological samples, there is a need for the development of novel derivatization methods that would improve MS detection limits. Hence, novel fluorescent N-hydroxysuccinimide esters of dihydro-[1,2,4]triazolo[4,3-a]pyridin-2-ium carboxylates (*Safirinium* P dyes) have been synthesized. The obtained compounds, which incorporate quaternary ammonium salt moieties, easily react with aliphatic amine groups of peptides, both in solution and on the solid support; thus, they can be applied for derivatization as ionization enhancers. *Safirinium* tagging experiments with ubiquitin hydrolysate revealed that the sequence coverage level was high (ca. 80%), and intensities of signals were enhanced up to 8-fold, which proves the applicability of the proposed tags in the bottom-up approach. The obtained results confirmed that the novel compounds enable the detection of trace amounts of peptides, and fixed positive charge within the tags results in high ionization efficiency. Moreover, *Safirinium* NHS esters have been utilized as imaging agents for fluorescent labeling and the microscopic visualization of living cells such as *E. coli* Top10 bacterial strain.

Słowa kluczowe

fluorescence microscopy, fluorescent probe, H/D exchange, live cell imaging, ionization tag, mass spectrometry, protein analysis, peptide labeling, tandem mass spectrometry

Typ publikacji

Artykuł

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Adres publiczny

<http://dx.doi.org/10.3390/ijms21249643>

Strona internetowa wydawcy

<http://www.mdpi.com/journal/metals>

Plik został wygenerowany dnia 2026-04-23 04:08:35

Adres w repozytorium <https://old.chem.uni.wroc.pl/pl/repozytorium/Tv39som>.