DOWNLOAD PDF SITE-SPECIFIC MODIFICATION OF PROTEINS BY THE STAUDINGER-PHOSPHITE REACTION PAUL MAJKUT . [ET AL.]

Chapter 1 : Phosphorous Acids: Majkut, Paul - racedaydvl.com

Chemoselective reactions are important tools for the modification of peptides and proteins. Thereby the modification is desired to be site specific and bioorthogonal. Here we describe the site-specific modification of azido-proteins via a Staudinger-type phosphite ligation. The reaction was carried.

Protein modification in bacterial cell lysate In a recent communication we have presented an efficient transformation of the isolated azido - protein 6 by a Staudinger- phosphite reaction with 9 into phosphoramidate The importance of such a study was rationalized by the fact that biochemical studies are often executed in whole cell lysates, which are much easier to exploit experimentally in comparison to living cells or animals. In order to visualize the bioorthogonal conversion by the Staudinger- phosphite reaction, we first prepared radioactively labeled protein 6 by cell -free unnatural protein translation in E. Next to the full length protein Fig. After addition of phosphite 9 to the lysate, protein 6, but not the truncated one, underwent an efficient Staudinger- phosphite transformation as verified by a radiogram of SDS-PAGE. The band of the modified protein 13 was shifted upwards compared to 6, whereas no migration of the corresponding p-azidophenylalanine lacking protein occurred Fig. This observation confirms that the Staudinger- phosphite reaction can be successfully conducted in whole lysed cell systems. Coomassie stain; Lane 1. Protein PEGylation Covalent attachment of polyethylene glycol chains to peptides and proteins has gained much attention, especially in the field of medicinal chemistry. This unnatural protein modification was found to reduce immuno- and antigenicity of therapeutic polypeptides , while simultaneously increasing their hydrodynamic size and plasma half life times. It is important to notice that the PEGylation strategy presented here differs in two aspects from the other previously mentioned chemoselective PEGylation routes. Second, the photo-sensitive phosphites e. The precursor for 14 was obtained in a nucleophilic substitution reaction between mPEG tosylate and 4- hydroxymethyl methoxynitrophenol , in analogy to the preparation of its homologues utilized for the synthesis of 9 and Scheme 4 Preparation of reagents 14 and 15 for polypeptide PEGylation. The reactivities of phosphites 14 and 15 were first assessed in consumption studies with azido - phenylalanine. In a subsequent experiment 14 C-labeled proteins 6 and 8 that were isolated from translation mixtures by their C-terminal His 6 -Tag, were used. Truncated proteins that lack His 6 -Tag could not be separated from the full length proteins , due to a strong tendency of SecB to form tetramers. Although, these amber terminated proteins were present in the mixtures, they did not interfere with the chemoselective transformation. The proteins were incubated in phosphate buffer pH 8. Protein 6 could be efficiently modified with either phosphite 14 or 15 as verified by the SDS-PAGE , in which the bands of the modified proteins 16 and 17 were shifted upwards according to the molecular weight of the modification. The lower efficiency of the formation of phosphoramidate 17 could be explained on the basis of the partial hydrolytic instability of 15 in aqueous systems, in analogy to its homologue 4. Finally, we have investigated the efficiency of the light-induced removal of the PEG moiety from protein After the Staudinger- phosphite reaction, SecB was separated from modifier 14, and it was irradiated at nm to yield protein This removal of polymer conjugates could potentially be utilized for a modulation of polypeptide self-assembly. In analogy to the antiimmunogenic function of polyethylene glycol, which is based on the mechanical shielding of proteinous epitops by polymer strains, 61 one could envision that by the same principle PEG could shield aggregation prone stretches and thus attenuate or even inhibit the self-assembly of polypeptides. Currently, we are exploring the use of reagent 14 for a temporal control of the aggregation process in a peptide model system. Conclusions We have demonstrated that phosphites are useful reagents for aqueous Staudinger-based transformations, in particular for the attachment of PEG-chains to proteins. However, certain structural criteria need to be met in order for them to become efficient modifiers. We have shown that tris oligoethylene glycol and the corresponding 2-nitrobenzyl substituted phosphites are relatively stable in aqueous media at physiological pH and thus are able to efficiently modify azides when added portionwise to the reaction

medium. In addition to the sufficient stability a high solubility of the phosphites has to be ensured to guarantee efficient ligations in biologically relevant media. The decoration of phosphites with chemically inert solubilizing groups i. A previously reported reagent for chemical phosphorylation seems to be a good example of a phosphite possessing such optimized properties. As demonstrated in the present report, this reagent can also be used to efficiently modify azido - polypeptides in a complex E. Finally, we have demonstrated for the first time the decoration of a protein with branched oligoethylene glycol scaffolds as a result of Staudinger-phosphite reaction, and thus expanded the scope of this modification methodology. In summary, the Staudinger- phosphite reaction appears to be a powerful metal-free method for bio- conjugations. Current efforts in our laboratory are devoted to the preparation of other symmetrical and unsymmetrical phosphites that will be used for further functionalization of azido - proteins. All buffers were treated with Chelex Sigma before use.

Chapter 2 : - NLM Catalog Result

Verena BÃ¶hrsch, Remigiusz Serwa, Paul Majkut, Eberhard Krause and Christian P. R. Hackenberger, Site-specific functionalisation of proteins by a Staudinger-type reaction using unsymmetrical phosphites, Chemical Communications, /ba, 46, 18, (), ().

Chapter 3 : FMP Berlin: Publications

*Site-specific PEGylation of proteins by a Staudinger-phosphite reaction Remigiusz Serwa, a Paul Majkut, a Benjamin Horstmann, a Jean-Marie Swiecicki, a Michael Gerrits, b Eberhard Krause c and Christian P. R. Hackenberger * a.*

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Chapter 5 : 1, results in SearchWorks catalog

Site-Specific Modification of Proteins by the Staudinger-Phosphite Reaction January Â· Methods in molecular biology (Clifton, N.J.) Chemoselective reactions are important tools for the.

Chapter 6 : Publications Authored by Remigiusz Serwa | PubFacts

Site-specific modification of proteins by the staudinger-phosphite reaction. Site-specific functionalisation of proteins by a Staudinger-type reaction using unsymmetrical phosphites.

Chapter 7 : Publications Authored by Christian P R Hackenberger | PubFacts

Site-specific functionalization of proteins by bioorthogonal modification offers a convenient pathway to create, modify, and study biologically active biopolymers. In this paper the Staudinger reaction of aryl-phosphonites for the chemoselective functionalization of azido-peptides and proteins was probed.