

Abschlussbericht für ein Max-Buchner Stipendium 2840

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Thema:

„Chemoselektive Fluoreszenzmarkierung von Proteinen durch eine Staudinger-Phosphit Reaktion“

1. Kurze Zusammenfassung der Förderung durch das Max-Buchner Stipendium

Innerhalb der zweijährigen Förderung ist es Frau Wilkening gelungen, einige sehr schöne methodische Entwicklungen der Staudinger-Phosphit Reaktionen zu entwickeln und in hochrangigen Journalen zu publizieren (siehe 2. Publikationen). Diese Reaktionen bildeten die Grundlagen für weitreichende Applikationen der chemoselektiven Staudinger-Phosphit Reaktion in biotechnologischen Anwendungen, u.a. in einer chemischen Phosphorylierungs-, PEGylierungs- und Biotinylierungsstrategie (siehe 3. Wissenschaftliche Zusammenfassung), die derzeit in Zusammenarbeit mit industriellen Partnern weiterentwickelt und kommerzialisiert werden (siehe 4. Industrielle Partner).

Zusammenfassend lässt sich damit festhalten, dass die Förderung durch die Max-Buchner Stiftung sehr erfolgreich war, und einen wichtigen Beitrag zur Etablierung der jungen Arbeitsgruppe von Prof. Christian Hackenberger an der FU Berlin geleistet hat.

2. Liste der aus diesem Projekt hervorgegangenen Publikationen:

a) Wissenschaftliche Journale (peer-review):

1. I. Wilkening, G. del Signore, W. Ahlbrecht, C. P. R. Hackenberger
Synthesis **2011**, DOI: 10.1055/s-0030-1260141
Lewis acid or alkyl halide promoted rearrangements of phosphor- and phosphinimidates to N,N-disubstituted phosphor- and phosphinamidates
(Feature Article)
2. I. Wilkening, G. del Signore, C. P. R. Hackenberger
Chem. Commun. **2011**, *47*, 349-351.
Synthesis of phosphoramidate peptides by Staudinger reactions of silylated phosphinic acids and esters
(Emerging Investigator Issue)

3. R. Serwa, P. Majkut, B. Horstman, J.-M. Swiecicki, M. Gerrits, E. Krause, C. P. R. Hackenberger
Chemical Science **2010**, *1*, 596-602.
Site-Specific PEGylation of Proteins by a Staudinger-Phosphite Reaction
4. V. Böhrsch, R. Serwa, P. Majkut, E. Krause, C. P. R. Hackenberger
Chem. Commun. **2010**, *46*, 3176 – 3178.
Site-specific Functionalisation of Proteins by a Staudinger-type reaction using unsymmetrical phosphites
5. R. Serwa, I. Wilkening, G. del Signore, M. Mühlberg, I. Claußnitzer, C. Weise, M. Gerrits, C. P. R. Hackenberger
Angew. Chem. **2009**, *121*, 8382-8387.
Angew. Chem. Int. Ed. **2009**, *47*, 8234-8239.
Chemoselective Staudinger-Phosphite Reaction of Azides for the Phosphorylation of Proteins
(highlighted in „Mimicking phosphorylation“ in *C&EN News*, issue August 3, 2009, p. 28)
6. I. Wilkening, G. del Signore, C. P. R. Hackenberger*
Chem. Comm. **2008**, 2932-2934.
Synthesis of N,N-disubstituted phosphoramidates via a Lewis-acid catalyzed phosphorimidate rearrangement

Anmerkung: Die Publikation 1.-4. sind diesem Bericht beigelegt. Publikationen 5. und 6. wurden bereits mit dem Zwischenbericht beigelegt. Im folgenden Bericht sind die jeweiligen Publikationen in der hier erfolgten (chronologischen) Aufzählung noch einmal kurz erwähnt.

b) Patent

C. P. R. Hackenberger, R. Serwa, G. del Signore, PCT/EP2010/052968
Mit einer Phosphoramidatgruppe modifizierte Makromoleküle und deren Verwendung

3. Kurze Darstellung der wissenschaftlichen Ergebnisse (auf Englisch)

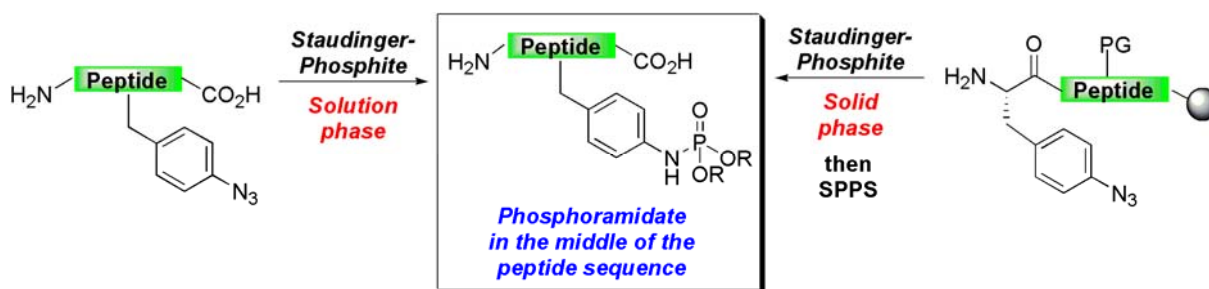
In the course of the investigation of a Lewis-acid catalyzed phosphorimidate-phosphoramidate rearrangement, which delivers *N,N*-disubstituted phosphoramidates as precursors of secondary amines in high yields and with a broad substrate scope, we discovered a quantitative hydrolysis of the phosphorimidate intermediates upon the addition of water.^[1] Alternatively, the Staudinger reaction of unprotected azido-peptides with silylated phosphinic acids and esters on the solid support allowed us to publish a straightforward acid-free entry to different phosphoramidate peptide esters or acids under mild conditions in high purity and yield.^[2]

Building upon these synthetic methodology developments, we engineered the *Staudinger-phosphite* reaction to convert azides into phosphoramidates either in solution or on the solid phase (Scheme 1).

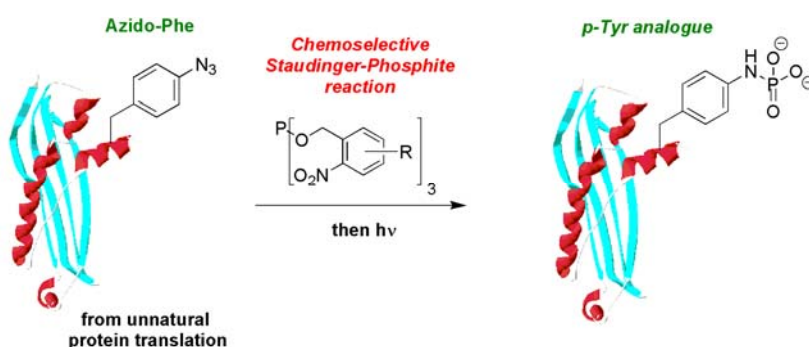
^[1] a) I. Wilkening, G. del Signore, C. P. R. Hackenberger, *ChemComm.* **2008**, 2932-2934; Publikation Nr. 6

b) I. Wilkening, G. del Signore, W. Ahlbrecht, C. P. R. Hackenberger, *Synthesis* **2011**, DOI: 10.1055/s-0030-1260141; Publikation Nr. 1

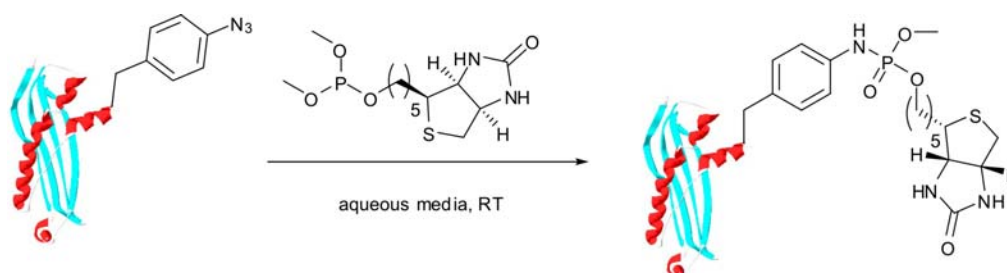
^[2] I. Wilkening, G. del Signore, C. P. R. Hackenberger, *Chem. Commun.* **2011**, *47*, 349-351. Publikation Nr. 2



In a first biological application of this reaction we developed a chemoselective phosphorylation of proteins, which allowed in combination with unnatural protein translation a site-specific incorporation of a charged phosphoramidate moiety into a protein (Scheme 2), which is recognized by a phosphor-Tyr specific antibody.^[3]



In a subsequent study, unsymmetrical phosphites were reacted in a Staudinger reaction with *p*-azido-phenylalanine, which can be applied for the site-specific biotinylation of proteins. Thereby, this procedure expands the scope of the chemoselective Staudinger-phosphite reaction of aryl azides with symmetrical phosphites to the corresponding phosphoramidates (Scheme 3).^[4] This study clearly points towards the main goal of the proposed Max-Buchner project, since a protein could be conjugated to a label by this metal-free chemical reaction.

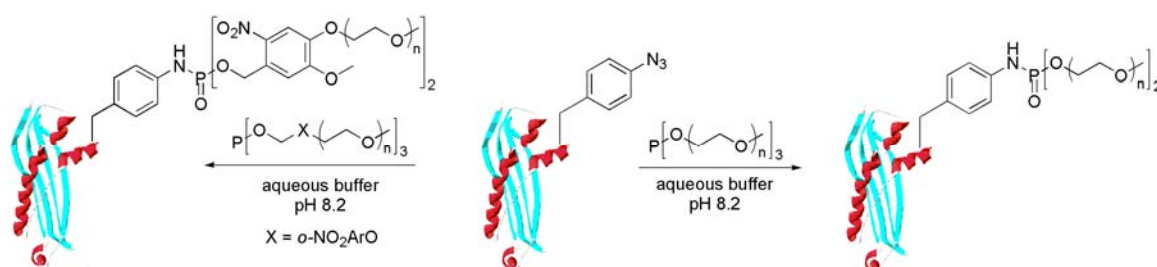


Finally, we demonstrated that the Staudinger-phosphite is an efficient transformation even in a highly crowded bio-environment, such as *E. coli* lysate. Consequently, we further employed this reaction for an efficient and metal-free PEGylation of a model azido-phenylalanine containing protein, which delivers a new class of branched oligoethylene

^[3] R. Serwa, I. Wilkening, G. del Signore, M. Mühlberg, I. Claußnitzer, C. Weise, M. Gerrits, C. P. R. Hackenberger, *Angew. Chem.Int. Ed.* **2009**, *48*, 8234-8239. [Publikation Nr. 5](#)

^[4] V. Böhrsch, R. Serwa, P. Majkut, E. Krause, C. P. R. Hackenberger, *Chem. Commun.* **2010**, *46*, 3176-3178. [Publikation 4](#)

glycol scaffolds (Scheme 4).^[5] This study points to a very attractive application of the Staudinger-phosphite reaction for the solubilization and stabilization of proteins, which is expected to be of particular interest for industry collaborations.



Scheme 4

4. Industrial collaborations and commercial use of the research findings

The identification and application of the chemoselective Staudinger-phosphite reaction as a method to chemically phosphorylate proteins was filed as a patent. Within this project we also have established a strong interaction with the small biotech company RiNA GmbH, in which a Ph.D. student is working in both laboratories (funded by FU Berlin sources), as well as with IRIS Biotech, who intends to commercialize our products.

^[5] R. Serwa, B. Horstman, P. Majkut, J.-M. Swiecicki, M. Gerrits, E. Krause, C. P. R. Hackenberger, *Chemical Science* **2010**, *1*, 596-602. [Publikation 3](#)