Staudinger Ligation

DOI: 10.1002/anie.200801514

# Chemoselective Peptide Cyclization by Traceless Staudinger Ligation**

*Rolf Kleineweischede and Christian P. R. Hackenberger**

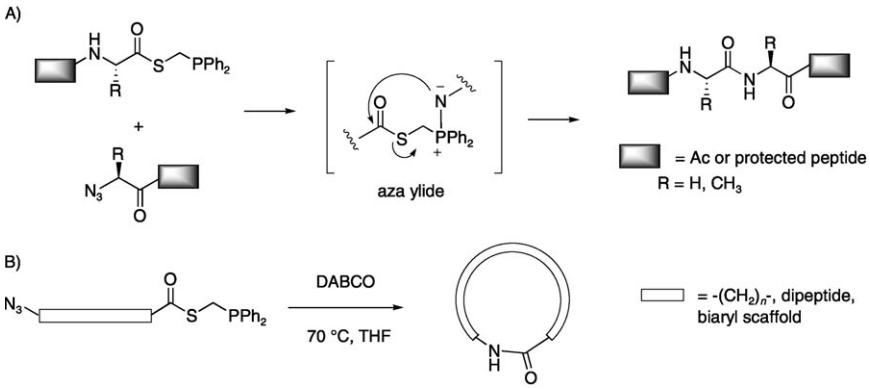
*Dedicated to Professor Michael Bienert on the occasion of his 65th birthday*

Complex cellular function is controlled by a network of protein–protein interactions which are often individually regulated by the post- or cotranslational attachment of specific functionalities to protein side chains, a process commonly referred to as protein modification.[1,2] To probe the specific impact of a particular modification on the protein's biological function, efforts have been focused on the acquisition of homogeneous protein material containing natural protein modifications, including glycosylation and phosphorylation, for comparative studies with their unmodified analogues.[2] The most effective semisynthetic strategy in protein synthesis employs the expressed protein ligation (EPL).[2a] In this chemoselective process an unprotected synthetic peptide fragment containing the particular modification of interest is ligated with an unmodified expressed protein by native chemical ligation (NCL) at a Cys junction.[3]

In contrast to impressive studies in which the biological and structural function of various protein modifications have been validated by this approach, only a few investigations have addressed the functional consequence of protein cyclization.[4] The protein head-to-tail cyclization represents an interesting posttranslational modification process, which occurs almost exclusively on ribosomally expressed proteins in bacterial organisms and less frequently in mammals and plants.[5] Most importantly, cyclic proteins display promising biological activity (especially antibacterial) and remarkable stability, thus making them ideal candidates for detailed investigations concerning the influence of the cyclization event on the structural stability and biological activity.[6]

Common synthetic strategies towards cyclic polypeptides either employ the cyclization of a peptide with protected side chains using activation reagents in solution or on the solid support,[7] or an intramolecular chemoselective amide bond formation by NCL.[8] Whereas the latter approach can directly deliver unprotected Cys-containing cyclic polypeptides and thereby utilize the advantages of structure-assisted intramolecular ring closure,[9] non-Cys-containing peptides have been generated by additional chemical transformations on the cyclization product including Cys desulfurization[10] and auxiliary-based methodologies.[11]

To develop a direct synthetic strategy for non-Cys-containing head-to-tail cyclic polypeptides, which would allow access to important members of the posttranslationally modified cyclic proteins like the 70-membered Bacteriocin AS48,[12] we decided to employ the traceless Staudinger ligation[13] for the conjunction of the peptide termini in small peptide model systems. The traceless Staudinger ligation, which was first described by the groups of Raines and Bertozzi,[14a,b] originates from the Staudinger reaction[15] of an azide with a phosphine yielding an iminophosphorane, which reacts intramolecularly as the aza ylide with an internal electrophile, in this case a thioester, to yield a native amide bond (Scheme 1 A). Consequently, the traceless Staudinger ligation, in contrast to NCL, does not require a Cys residue at the ligation site. Since its discovery in 2000, the traceless Staudinger ligation has been at the focus of extensive methodology studies,[14] including a recent description of a water-soluble variant,[16] detailed mechanistic investiga-

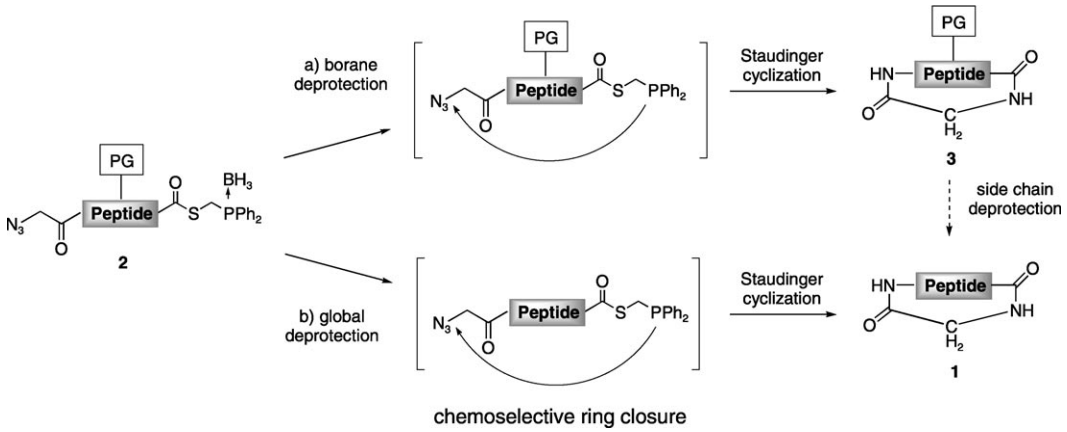



***Scheme 1.*** A) Traceless Staudinger ligation.[14] B) Base-induced Staudinger cyclization.[22] DABCO = 1,4-diazabicyclo[2.2.2]octane.

[*] R. Kleineweischede, Dr. C. P. R. Hackenberger
Institut für Chemie und Biochemie
Freie Universität Berlin
Takustrasse 3, 14195 Berlin (Germany)
Fax: (+49) 30-838-52551
E-mail: hackenbe@chemie.fu-berlin.de

[**] The authors acknowledge financial support from the German Science Foundation (DFG) within the Emmy-Noether program (HA 4468/2-1) and the SFB 765 "Multivalency" as well as from the Fonds der Chemischen Industrie (FCI). We thank Ilona Papp for preliminary experimental contributions.

Supporting information for this article is available on the WWW http://dx.doi.org/10.1002/anie.200801514.



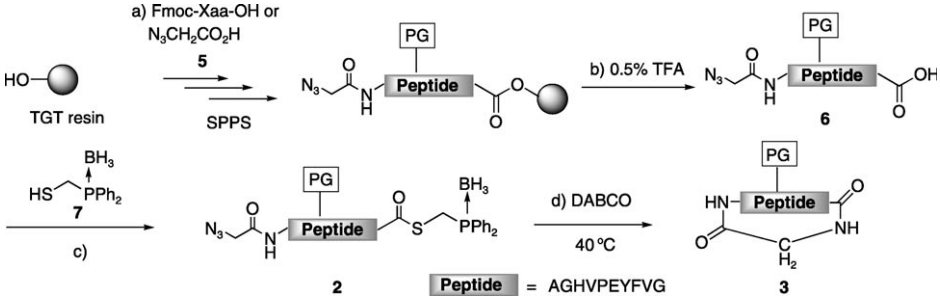

***Scheme 2.*** Synthetic strategy for the Staudinger cyclization of peptides a) with and b) without the presence of side chain protecting groups.

tions,[17] and applications, including site-specific immobilization of peptides on surfaces[18] and the ligation of protected (glyco-)peptide fragments.[19] However, the chemoselectivity of the traceless Staudinger ligation has only rarely been addressed,[18a,19c,20] in contrast to the "bioorthogonal" Staudinger ligation, which was developed by Bertozzi et al. for the chemoselective functionalization of metabolically engineered azidoglycans in cellular environment.[21] Along those lines, the "traceless" Staudinger ligation has yet not been applied in the ligation of unprotected peptide fragments.

In our strategy for the synthesis of cyclic peptides we planned to use a borane-protected phosphinothiol to install the reactive moieties for the Staudinger ligation—azide and phosphine—in the same molecule (Scheme 1B).[20,22] After borane removal under basic conditions[23] an intramolecular version of the traceless Staudinger ligation can occur, which was previously demonstrated for the synthesis of lactams and biaryl-containing natural product analogues. By employing the concept of an induced traceless Staudinger ligation, we envisioned two possible synthetic strategies towards cyclic peptides **1**, both starting from the protected bifunctional azidopeptide phosphinothioester **2**. In the first route the cyclization of **2** is initiated by borane cleavage in the presence of side-chain-protecting groups to deliver the protected cyclic peptide **3**, and thus a final deprotection is required (Scheme 2a). The second route utilizes a chemoselective amide bond ring closure in a globally deprotected peptide for direct access to the cyclized peptide **1** without additional chemical transformations or deprotections (Scheme 2b).

The previously employed conditions used for the borane deprotection of a phosphinothioester (70 °C) rendered the generally applicability in polypeptide ligations difficult. However, deprotection of the borane-protected acetylphosphinothiol **4**, which can be synthesized by published protocols, was complete at 40 °C with DABCO.[20,24] For an intramolecular application in peptide cyclization, we focused on the solid-phase peptide synthesis (SPPS) of a bifunctional azidopeptide phosphinothioester **2**. Residues 1–11 of Microcin J25 (GAGHVPEYFVG) were chosen as the model sequence, which resembles the terminal circular loop of this microbial peptide.[26] Standard 9-fluorenylmethoxycarbonyl(Fmoc)-based SPPS using azidoglycine **5** in the final coupling step on an acid-sensitive trityl resin (TGT resin) and cleavage with 0.5% TFA in $CH_2Cl_2$ containing 2.5% TIS delivered the C-terminal-deprotected peptide **6** (Scheme 3). This peptide was transformed into **2** by activation with DIC/DMAP in $CH_2Cl_2$. HPLC and HRMS (ESI-TOF) analysis (see Figure 1A and the Supporting Information) demonstrated full conversion to **2**, even when only 1.1 equivalents of phosphinothiol **7**, which was obtained by deprotection of **4**,[24] was used. The base-induced Staudinger cyclization of **2** under optimized conditions was performed at 7 mM concentration; the cyclized peptide **3** was obtained in very good overall conversion with only minimal amounts of oxidized side product (see the Supporting Information).

In addition to this head-to-tail cyclization of a protected peptide sequence by a base-induced traceless Staudinger




***Scheme 3.*** Staudinger cyclization of peptides by base-induced borane cleavage : SPPS synthesis of the bifunctional azidopeptide phosphinothioester **2**. Reagents and conditions: a) 1. Fmoc-Xaa-OH or **5**, HBTU/HOBt, DIPEA, NMP; 2. 20% piperidine, NMP; b) 0.5% TFA, 2.5% TIS, $CH_2Cl_2$, 2 h; c) **7** (1.5 equiv), DIC (3 equiv), DMAP (cat.), $CH_2Cl_2$, 8 h; d) DABCO (3 equiv), DMF, 40 °C 4 h then 25 °C, 36 h. HBTU = 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate, HOBt = *N*-hydroxybenzotriazole, DIPEA = diisopropylethylamine, NMP = *N*-methyl-2-pyrrolidone, TFA = trifluoroacetic acid, TIS = triisopropylsilane, DIC = diisopropylcarbodiimide.

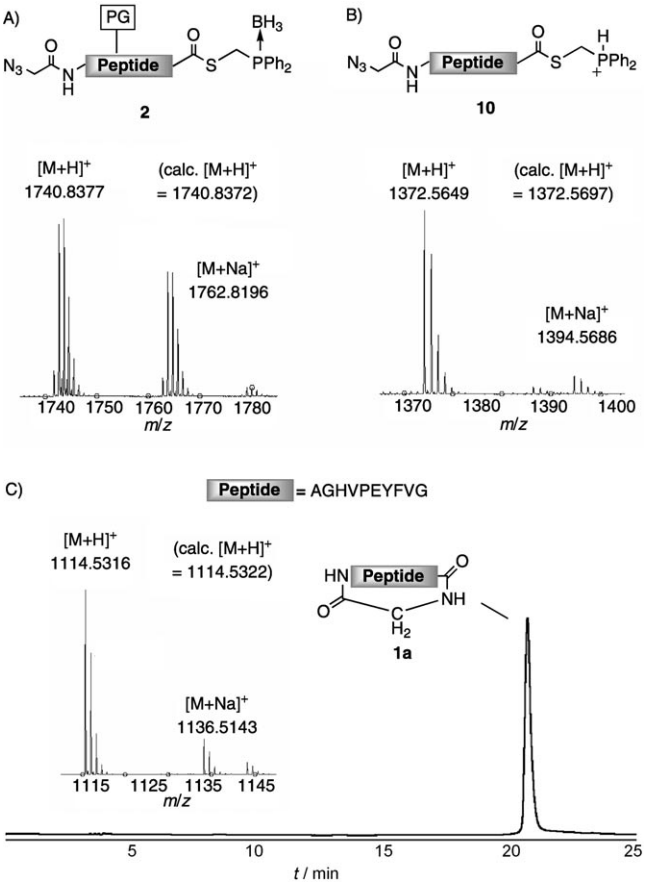
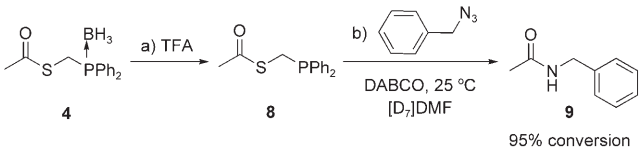



***Figure 1.*** HRMS (ESI-TOF) analysis of the bifunctional azidopeptide thioester **2** (A) and of the deprotected azido-peptide-phosphonium-thioester **10**. (B). C) HRMS (ESI-TOF) analysis and HPLC profile of purified cyclic peptide **1a**.

cyclization, we decided to further develop a system that made use of the chemoselective features of the Staudinger reaction (Scheme 2b). For such a strategy, deprotection of the peptide side chains as well as the borane removal is needed such that additional deprotection of the cyclized product is not required. We therefore decided to switch to less frequently used acidic conditions for the removal of borane protecting group.[27]

In model studies treatment of acetyl-protected phosphinothiol **4** with neat TFA and subsequent removal of the volatiles under high vacuum yielded the free phosphine **8** (Scheme 4). Detailed $^{1}H$, $^{31}P$, and $^{11}B$ NMR investigations confirmed complete borane cleavage and demonstrated the full conversion of the tetracoordinate borane into a less reactive three-valent borate species (see the Supporting Information).[28] A test reaction for the Staudinger ligation, in this case with benzyl azide[25] to yield *N*-benzylacetamide (**9**), was performed. Optimized conditions delivered an excellent conversion of up to 95% when DABCO or DIPEA was added to the reaction mixture to scavenge residual amounts of TFA (see the Supporting Information) and when short deprotection times (1 h) were maintained to prevent thioester cleavage resulting from extended exposure to TFA. The addition of a base was pivotal for high conversion

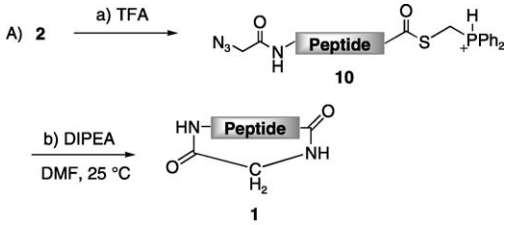



***Scheme 4.*** Model studies for the intermolecular traceless Staudinger ligation following borane removal under acidic conditions. Reagents and conditions: a) TFA, 1 h, then TFA removal under high vacuum, 1 h; b) benzylazide (1 equiv), DABCO (2 equiv), [$D_7$]DMF, 25 °C.

rates for the Staudinger ligation as residual TFA would lead to protonation of the iminophosphorane, which would potentially enhance the electrophilicity of phosphorus and favor the Staudinger reduction to the amine.

The use of acidic conditions for the deprotection has several benefits over the base-induced version: The deprotection can be performed at room temperature, the reactive borane species is converted into a chemically less reactive borate compound, and bases milder than DABCO can be used during the reaction sequence. However, and most importantly for peptide Staudinger ligations, TFA-mediated deprotection of an SPPS-derived azidopeptide phosphinothioester **2** leads to the simultaneous removal of all the peptide side chain protecting groups, which would allow an amide bond formation in the presence of unprotected side chains.

The latter advantage was demonstrated on the azidopeptide phosphinothioester **2** (Scheme 5B), which was treated with a solution containing 97.5% TFA and 2.5% TIS to yield the deprotected azidopeptide phosphoniumthioester **10**. Peptide **10**, which was characterized by HPLC and HRMS (ESI-ToF) analysis (Figure 1B and the Supporting Information),




B)

| Cyclic peptide sequence | yield[a] |
|---|---|
| GAGHVPEYFVG **1a** | 36% |
| GGIVPQFYSAG **1b** | 31% |
| GIGTPISFYGG **1c** | 20% |

***Scheme 5.*** Staudinger cyclization of peptides following borane removal under acidic conditions. A) Deprotection of the bifunctional azidopeptide phosphinothioester **2** by treatment with TFA and subsequent Staudinger cyclization. B) Sequences of cyclic peptides **1a–c** and yields of isolated product after HPLC purification. Reagents and conditions: a) 97.5% TFA, 2.5% TIS, 1 h, then precipitation from dry ethyl ether; b) DIPEA (20 equiv), DMF, 12 h. For details concerning the HPLC and HRMS analysis see the Supporting Information. [a] Yield of isolated product after HPLC purification (over three steps, see text and the Supporting Information).

did not show any tendency to undergo the Staudinger cyclization without the addition of a base. However, it was difficult to purify peptide **10** by HPLC as undesired partial oxidation of the phosphine was observed. We therefore decided to directly initiate the Staudinger cyclization after deprotection with TFA. After precipitation from dry, cold ethyl ether, peptide **10** was diluted to a concentration of 7 mM in dry DMF, and DIPEA was added to initiate the Staudinger cyclization. Analysis of the crude HPLC profile revealed that the main product of the ligation corresponds to the fully deprotected cyclic peptide **1a** as verified by HRMS analysis (see the Supporting Information). Further LC-MS analysis revealed the oxidized phosphinothioester peptide with an intact azide at the N terminus as a minor peptidic side product. Importantly, no by-products formed by intermolecular ligation were observed. Finally, the cyclic peptide **1a** (cyclo-GAGHVEPYFVG) was purified by preparative HPLC and characterized by HRMS (Figure 1C). As no intermediate HPLC purification between individual steps in the synthesis was performed, an overall yield of 36% was determined for the whole three-step synthetic scheme, including the thioester synthesis, global deprotection, and final cyclization.

This protocol was furthermore applied to the cyclization of two other 11-residue peptides: Peptide **1b** contained an additional hydroxy functionality and one side chain amide (sequence of **1b**: GGIVPQFYSAG). The overall conversion and side products were similar to those in the synthesis of **1a**, and the yield of isolated **1b** was 31% (Scheme 5B). Finally, peptide **1c** containing the remaining residues of the Microcin J25 sequence (GIGTPISFYGG, peptide **1c**) was obtained in 20% yield.

In summary, we have developed a method for the synthesis of cyclic peptides employing two strategies based on induced Staudinger ligation. Our approach offers an efficient route to protected and unprotected peptide phosphinothioesters starting with an acid-labile trityl resin. Particularly noteworthy is the fact that borane removal under acidic conditions allows a chemoselective peptide ligation in the presence of functional peptide side chains, therefore broadening the general scope of the Staudinger ligation in (poly-) peptide ligations as no Cys residues are needed for the amide bond formation. Along those lines, this strategy has the advantage that a concluding deprotection step with the final peptide product is not necessary. Although not all amino acid functionalities have been probed, this study demonstrates the potential of the traceless Staudinger ligation as a chemoselective amide bond forming reaction in the synthesis of naturally occurring peptide substrates.

The cyclization in the presence of unprotected side chains could take advantage of a structural preorganization of the termini, thus favoring an intra- over an intermolecular amide bond formation.[9] Studies concerning this structural influence in combination with peptide concentration on the cyclization yield, the development of chemoselective intermolecular peptide Staudinger ligations, and the cyclization of larger polypeptide sequences in aqueous solvents are currently underway in our laboratory.

## Experimental Section

Synthesis of the protected bifunctional azidopeptide phosphinothioester **2**: The azidopeptide was synthesized on an ABI 433a peptide synthesizer using standard Fmoc-based SPPS conditions (Fast-moc protocol with HBTU/HOBt activation). As the solid support, TGT resin (Novabiochem) was used with the first amino acid (Gly) already attached to the resin. The peptide was cleaved from the resin with 0.5% TFA in $CH_2Cl_2$ (including 2.5% TIS) for 2 h. The resin was filtered off and washed with $CH_2Cl_2$. The filtrate and the washing solution were combined, and the solvent was removed under high vacuum. The C-terminally deprotected peptide was dissolved in dry $CH_2Cl_2$ (5 mL/0.033 mmol). After the addition of 3 equiv DIC and catalytic amounts of DMAP, 1.5 equiv of borane-protected diphenylphosphinomethanethiol (**7**) was added and the reaction mixture was stirred for 12 h. The conversion to the corresponding phosphinothioester **2** was determined by HPLC and HRMS analysis (see the Supporting Information).

Deprotection of **2** with TFA and subsequent Staudinger cyclization: The crude reaction mixture containing the protected phopshinothioester **2** was treated with a solution containing 97.5% TFA and 2.5% TIS (0.5 mL/0.033 mmol) for 1 h. The globally deprotected thioester **10** was precipitated from 10 mL dry ethyl ether, characterized by HPLC and HRMS methods (see the Supporting Information), and redissolved in dry DMF (5 mL/0.033 mmol).

For the cyclization 20 equiv DIPEA was added to the reaction mixture and the reaction mixture was stirred for 12 h. Cyclic peptide **1a** was purified by preparative HPLC. HPLC and HRMS analysis were carried out to confirm the identity of the final products and the peptide intermediates.

For HPLC and HRMS analysis of peptides **1a–c**, **2**, **3**, and **10** see Figure 1 and the Supporting Information. For the NMR anaylsis concerning the TFA deprotection of **4** to give **8** as well as references regarding synthetic procedures, see the Supporting Information.

Received: March 31, 2008
Published online: July 4, 2008

**Keywords:** chemoselectivity · cyclization · peptides · protecting groups · protein design

[1] a) C. T. Walsh in *Posttranslational Modifications of Proteins*, Roberts and Company Publishers, Colorado, **2003**; b) B. G. Davis, *Science* **2004**, *303*, 480–482; c) F. Wold, *Annu. Rev. Biochem.* **1981**, *50*, 783–814.

[2] a) T. W. Muir, *Annu. Rev. Biochem.* **2003**, *72*, 249–289; b) J.-P. Pellois, T. W. Muir, *Curr. Opin. Chem. Biol.* **2006**, *10*, 487–491; c) D. Rauh, H. Waldmann, *Angew. Chem.* **2007**, *119*, 840–844; *Angew. Chem. Int. Ed.* **2007**, *46*, 826–829; d) C. P. R. Hackenberger, D. Schwarzer, *Angew. Chem.* DOI: 10.1002/ange.200801313; *Angew. Chem. Int. Ed.*, DOI: 10.1002/anie.200801313.

[3] P. E. Dawson, T. W. Muir, J. Clark-Lewis, S. B. H. Kent, *Science* **1994**, *266*, 776–779. For pioneering studies see: T. Wieland, E. Bockelmann, L. Bauer, H. U. Lang, H. Lau, *Justus Liebigs Ann. Chem.* **1953**, *583*, 129.

[4] For reviews on the structural and functional impact of cyclic peptides, see: a) J. S. Davies in *Cyclic Polymers*, 2nd ed. (Ed.: J. A. Semlyen), Kluwer Academic Publishers, Dordrecht, **2000**, pp. 85–124; b) H. Kessler, *Angew. Chem.* **1982**, *94*, 703–703; *Angew. Chem. Int. Ed. Engl.* **1982**, *21*, 512–689; c) P. Wipf, *Chem. Rev.* **1995**, *95*, 2115–2134.

[5] M. Trabi, D. J. Craik, *Trends Biochem. Sci.* **2002**, *27*, 132–138.

[6] For structural and folding studies on circular variants of src SH3 domains see: a) J. A. Camarero, D. Fushman, S. Sato, I. Giriat, D. Cowburn, D. P. Raleigh, T. W. Muir, *J. Mol. Biol.* **2001**, *308*,

1045 – 1062; b) V. P. Grantcharova, D. Baker, *J. Mol. Biol.* **2001**, *306*, 555 – 563.
[7] a) M. Chorev, E. Roubini, R. L. McKec, S. W. Gibbons, M. E. Goldman, M. P. Caulfield, M. Rosenblatt, *Biochemistry* **1991**, *30*, 5968 – 5974; b) M. Bodanszky in *Principles of Peptide Synthesis*, Springer, Berlin, **1984**; c) N. Seewald, H.-D. Jakubke in *Peptides: Chemistry and Biology*, Wiley-VCH, Weinheim, **2002**, pp. 311 – 319.
[8] a) J. A. Camarero, T. W. Muir, *Chem. Commun.* **1997**, 1369 – 1370; b) L. Zhang, J. P. Tam, *J. Am. Chem. Soc.* **1997**, *119*, 2363 – 2370; c) R. Quaderer, D. Hilvert, *Chem. Commun.* **2002**, 2620 – 2621.
[9] a) J. A. Camarero, J. Pavel, T. W. Muir, *Angew. Chem.* **1998**, *110*, 361 – 364; *Angew. Chem. Int. Ed.* **1998**, *37*, 347 – 349; b) G. S. Beligere, P. E. Dawson, *J. Am. Chem. Soc.* **1999**, *121*, 6332 – 6333; c) J. Blankenstein, J. Zhu, *Eur. J. Org. Chem.* **2005**, 1949 – 1964.
[10] a) L. Z. Yan, P. E. Dawson, *J. Am. Chem. Soc.* **2001**, *123*, 526 – 533; b) Q. Wan, S. J. Danishefsky, *Angew. Chem.* **2007**, *119*, 9408 – 9412; *Angew. Chem. Int. Ed.* **2007**, *46*, 9248 – 9252.
[11] Y. Shao, W. Lu, S. B. H. Kent, *Tetrahedron Lett.* **1998**, *39*, 3911 – 3914.
[12] a) M. Maqueda, A. Galvez, M. M. Bueno, M. J. Sanchez-Barrena, C. Gonzalez, A. Albert, M. Rico, E. Valdivia, *Curr. Protein Pept. Sci.* **2004**, *5*, 399 – 416; b) D. J. Craik, N. L. Daly, I. Saska, M. Trabi, K. J. Rosengren, *J. Bacteriol.* **2003**, *185*, 4011 – 4021.
[13] M. Köhn, R. Breinbauer, *Angew. Chem.* **2004**, *116*, 3168 – 3178; *Angew. Chem. Int. Ed.* **2004**, *43*, 3106 – 3116.
[14] a) B. L. Nilsson, L. L. Kiessling, R. T. Raines, *Org. Lett.* **2000**, *2*, 1939 – 1941; b) E. Saxon, J. I. Armstrong, C. R. Bertozzi, *Org. Lett.* **2000**, *2*, 2141 – 2143; c) B. L. Nilsson, L. L. Kiesling, R. T. Raines, *Org. Lett.* **2001**, *3*, 9 – 12; d) M. B. Soellner, A. Tam, R. T. Raines, *J. Org. Chem.* **2006**, *71*, 9824 – 9830.
[15] J. Meyer, H. Staudinger, *Helv. Chim. Acta* **1919**, *2*, 635 – 646.
[16] A. Tam, M. B. Soellner, R. T. Raines, *J. Am. Chem. Soc.* **2007**, *129*, 11421 – 11430.
[17] a) M. B. Soellner, B. L. Nilsson, R. T. Raines, *J. Am. Chem. Soc.* **2006**, *128*, 8820 – 8828; b) A. Tam, M. B. Soellner, R. T. Raines, *Org. Biomol. Chem.* **2008**, *6*, 1173 – 1175.
[18] a) M. B. Soellner, K. A. Dickson, B. L. Nilsson, R. T. Raines, *J. Am. Chem. Soc.* **2003**, *125*, 11790 – 11791; b) M. Köhn, R. Wacker, C. Peters, H. Schroeder, L. Soulere, R. Breinbauer, C. M. Niemeyer, H. Waldmann, *Angew. Chem.* **2003**, *115*, 6010 – 6014; *Angew. Chem. Int. Ed.* **2003**, *42*, 5830 – 5834; c) A. Watzke, M. Gutierrez-Rodriguez, M. Köhn, R. Wacker, H. Schroeder, R. Breinbauer, J. Kuhlmann, K. Alexandrov, C. M. Niemeyer, R. S. Goody, H. Waldmann, *Bioorg. Med. Chem.* **2006**, *14*, 6288 – 6306; d) A. Watzke, M. Köhn, M. Gutierrez-Rodriguez, R. Wacker, H. Schroeder, R. Breinbauer, J. Kuhlmann, K. Alexandrov, C. M. Niemeyer, R. S. Goody, H. Waldmann, *Angew. Chem.* **2006**, *118*, 1436 – 1440; *Angew. Chem. Int. Ed.* **2006**, *45*, 1408 – 1412.
[19] a) B. L. Nilsson, R. J. Hondal, M. B. Soellner, R. T. Raines, *J. Am. Chem. Soc.* **2003**, *125*, 5268 – 5269; b) L. Liu, Z.-Y. Hong, C.-H. Wong, *ChemBioChem* **2006**, *7*, 429 – 432; c) R. Merkx, D. T. S. Rijkers, J. Kemmink, R. M. J. Liskamp, *Tetrahedron Lett.* **2003**, *44*, 4515 – 4518.
[20] For the conjugation of unprotected sugar derivatives to protein carriers by the traceless Staudinger ligation see: C. Grandjean, A. Boutonnier, C. Guerreiro, J.-M. Fournier, L. A. Mulard, *J. Org. Chem.* **2005**, *70*, 7123 – 7132.
[21] a) E. Saxon, C. R. Bertozzi, *Science* **2000**, *287*, 2007 – 2010; b) N. J. Agard, J. M. Baskin, J. A. Prescher, A. Lo, C. R. Bertozzi, *Chem. Biol.* **2006**, *13*, 644 – 648. For a review see: J. A. Prescher, C. R. Bertozzi, *Nat. Chem. Biol.* **2005**, *1*, 13 – 21.
[22] a) O. David, W. J. N. Meester, H. Bieräugel, H. Hiemstra, J. H. van Maarseveen, *Angew. Chem.* **2003**, *115*, 4509 – 4511; *Angew. Chem. Int. Ed.* **2003**, *42*, 4373 – 4375; b) G. Masson, T. den Hartog, H. E. Schoemaker, H. Hiemstra, J. H. van Maarseveen, *Synlett* **2006**, 865 – 868.
[23] a) B. Carboni, L. Monnier, *Tetrahedron* **1999**, *55*, 1197 – 1248; b) T. Imamoto, T. Oshiki, T. Onazawaj, T. Kusmoto, K. Sato, *J. Am. Chem. Soc.* **1990**, *112*, 5244 – 5252.
[24] M. B. Soellner, B. L. Nilsson, R. T. Raines, *J. Org. Chem.* **2002**, *67*, 4993 – 4996.
[25] Although the azides reported here were apparently stable, we strongly recommend caution and the use of appropriate protection during the handling of azides, especially azides of low molecular weight, and during heating and/or concentrating steps. See also: S. Bräse, C. Gil, K. Knepper, V. Zimmermann, *Angew. Chem.* **2005**, *117*, 5320 – 5374; *Angew. Chem. Int. Ed.* **2005**, *44*, 5188 – 5240.
[26] M. J. Bayro, J. Mukhopadhyay, G. V. T. Swapna, J. Y. Huang, L.-C. Ma, E. Sineva, P. E. Dawson, G. T. Montelione, R. H. Ebright, *J. Am. Chem. Soc.* **2003**, *125*, 12382 – 12383.
[27] P. Shapland, E. Vedejs, *J. Org. Chem.* **2004**, *69*, 4094 – 4100.
[28] H. Nöth, B. Wrackmeyer in *NMR Spectroscopy of Boron Compounds*, Springer, Berlin, **1978**.