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Staudinger Ligation: A Peptide from a Thioester and Azide

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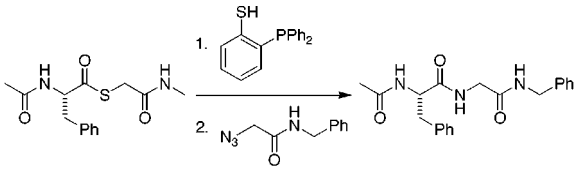
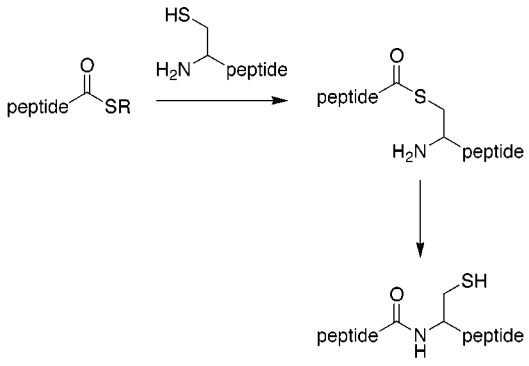
ABSTRACT



The technique of native chemical ligation enables the total chemical synthesis of proteins. This method is limited, however, by an absolute requirement for a cysteine residue at the ligation juncture. Here, this restriction is overcome with a new chemical ligation method in which a phosphinobenzenethiol is used to link a thioester and azide. The product is an amide with no residual atoms.

New methods are facilitating the total chemical synthesis of proteins.[1] In particular, Kent and others have developed an elegant means to stitch together two unprotected peptides in aqueous solution.[2] In this method, which is called "native chemical ligation", the thiolate of an *N*-terminal cysteine residue in one peptide attacks the carbon of a *C*-terminal thioester in another peptide to produce, ultimately, an amide bond between the two peptides (Scheme 1). Recently, Muir and others have expanded the utility of native chemical ligation by demonstrating that the thioester fragment can be produced readily with recombinant DNA (rDNA) techniques.[3]

Scheme 1



Though powerful, native chemical ligation has a serious limitation. The method has an absolute reliance on the formation of a peptide bond to a cysteine residue.[4] Creating

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(3) (a) Muir, T. W.; Sondhi, D.; Cole, P. A. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *9*, 6705−6710. (b) Evans, T. C., Jr.; Benner, J.; Xu, M.-Q. *Protein Sci.* **1998**, *7*, 2256−2264. (c) Ayers, B.; Blaschke, U. K.; Camarero, J. A.; Cotton, G. J.; Holford, M.; Muir, T. W. *Biopolymers* **2000**, *51*, 343−354. For reviews, see: (d) Holford, M.; Muir, T. W. *Structure* **1998**, *15*, 951−956. (e) Cotton, G. J.; Muir, T. W. *Chem. Biol.* **1999**, *6*, R247−R256. (f) Evans, T. C., Jr.; Xu, M.-Q. *Biopolymers* **2000**, *51*, 333−342.

(4) For a few specific ligation reactions, this restriction has been overcome by using the native conformation of the protein to guide the regioselective coupling of an activated ester and amine. For examples, see: (a) Homandberg, G. A.; Laskowski, M. *Biochemistry* **1979**, *18*, 586−592. (b) Wuttke, D. S.; Gray, H. B.; Fisher, S. L.; Imperiali, B. *J. Am. Chem. Soc.* **1993**, *113*, 8455−8456. (c) Vogel, K.; Chmielewski, J. *J. Am. Chem. Soc.* **1994**, *116*, 11163−11164. (d) Beligere G. S.; Dawson, P. E. *J. Am. Chem. Soc.* **1999**, *121*, 6332−6333.

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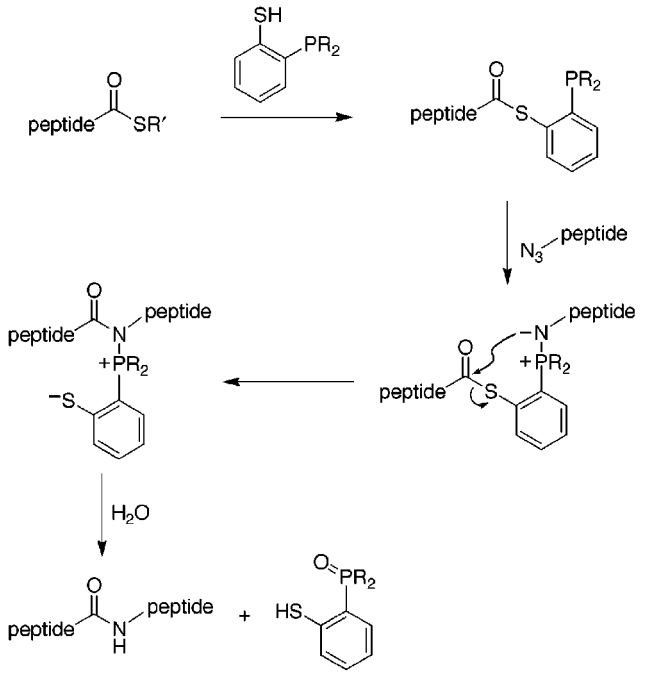
a linkage at a natural Xaa−Cys bond is not always possible, as cysteine comprises only 1.7% of the residues in globular proteins.[5] Installing an extra cysteine residue is often undesirable. Cysteine is by far the most reactive residue toward disulfide bonds, O_2(g), and other electrophiles.[6] In addition, the sulfhydryl group of cysteine can suffer β-elimination to form dehydroalanine, which can undergo further reaction.[7] Thus, the impact of native chemical ligation would be even greater were it not limited to creating an Xaa−Cys bond.

Offer and Dawson have described a means to remove the limitation inherent in native chemical ligation.[8] In their method, a peptide bond is formed from a thioester and an *o*-mercaptobenzylamine. Though effective, this method is engrammic, leaving *o*-mercaptobenzylamine in the ligation product.

Here, we describe a method for peptide ligation that eliminates the need for a cysteine residue and leaves no residual atoms in the peptide product. Our method is inspired by the Staudinger reaction.[9] In the Staudinger reaction, a phosphine is used to reduce an azide to an amine: $PR_3 + N_3R' + H_2O \rightarrow O{=}PR_3 + H_2NR' + N_2$(g). The intermediate in the reaction is an iminophosphorane ($R_3P^+-{}^-NR'$), which has a nucleophilic nitrogen. Vilarrasa and others have shown that this nitrogen can attack an acyl donor in an intermolecular or intramolecular reaction.[10] The final product, after hydrolysis of the amidophosphonium salt, is an amide. Saxon and Bertozzi have shown that the acyl group can originate from the phosphine itself and be transferred to the iminophosphorane nitrogen in an intramolecular reaction in water.[11] Their product is an amide containing a phosphine oxide.

To apply the Staudinger reaction to peptide synthesis, we use a phosphinothiol to unite a thioester and azide. A putative mechanism for this version of the "Staudinger ligation"[11] is shown in Scheme 2. The ligation begins by transthioesterification with the phosphinothiol. Coupling of the resulting phosphinothioester with a peptide azide leads to the formation of the reactive iminophosphorane. Attack of the iminophosphorane nitrogen on the thioester leads to an amidophosphonium salt. Hydrolysis of the amidophosphonium salt produces the desired amide and a phosphine oxide. Significantly, no atoms from the phosphinothiol remain in the amide product.[12]

Scheme 2



A critical aspect in effecting the Staudinger ligation of a thioester and azide is selecting an appropriate phosphinothiol. We chose an *o*-phosphinobenzenethiol ($R_2PC_6H_4$-*o*-SH) because it allows a six-membered ring to form in the transition state for acyl transfer. Moreover, $R_2PC_6H_4$-*o*-SH does not allow for the formation of an episulfide and a stable amidophosphine ($R_2PNR'C(O)R''$) by C−P bond cleavage in the amidophosphonium salt, as would simple alkanethiols such as $R_2PCH_2CH_2SH$. Further, thiophenol itself is known to effect the transthioesterification of thioesters during native chemical ligation.[13]

We demonstrated the efficacy of the Staudinger ligation by effecting the transformation shown in Scheme 3 (R = Bn). In this transformation, the peptide AcPheGlyNHBn (**5**) was synthesized from a phenylalanyl thioester (**1**) and a glycyl azide (**4**) by the action of *o*-(diphenylphosphino)-benzenethiol (**2**).[14] Thioester **3** was prepared in quantitative yield by the transthioesterification of thioester **1** with an excess of phosphinobenzenethiol **2** in DMF containing diisopropylethylamine (DIEA).[15] Excess thiol was removed by covalent immobilization to a Merrifield resin (chloromethylpolystyrene-divinylbenzene). Azide **4** (1 equiv) was

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(6) (a) Schneider, C. H.; de Weck, A. L. *Biochim. Biophys. Acta* **1965**, *168*, 27−35. (b) Raines, R. T. *Nat. Struct. Biol.* **1997**, *4*, 424−427.
(7) Friedman, M. *Adv. Exp. Med. Biol.* **1999**, *459*, 145−159.
(8) Offer, J.; Dawson, P. E. *Org. Lett.* **2000**, *2*, 23−26.
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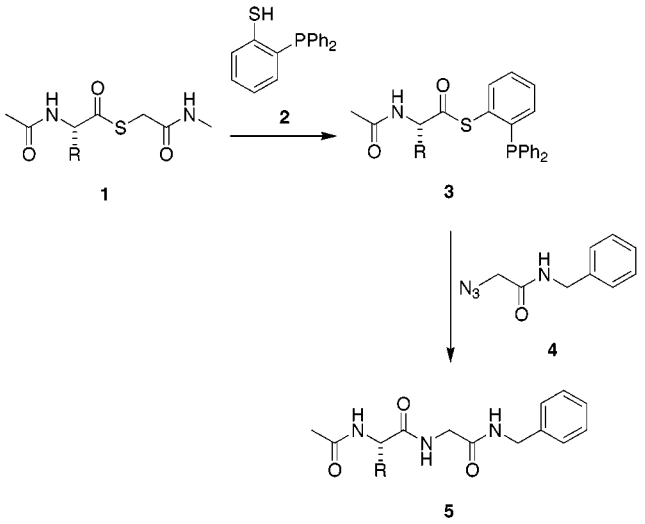
(10) For examples, see: (a) Bosch, I.; Romea, P.; Urpf, F.; Vilarrasa, J. *Tetrahedron Lett.* **1993**, *34*, 4671−4674. (b) Bosch I.; Urpi F.; Vilarrasa J. *J. Chem. Soc., Chem. Commun.* **1995**, 91−92. (c) Shalev, D. E.; Chiacchiera, S. M.; Radkowsky, A. E.; Kosower, E. M. *J. Org. Chem.* **1996**, *61*, 1689−1701. (d) Bosch, I.; Gonzalez, A.; Urpi, F.; Vilarrasa, J. *J. Org. Chem.* **1996**, *61*, 5638−5643. (e) Tang, Z.; Pelletier, J. C. *Tetrahedron Lett.* **1998**, *39*, 4773−4776. (f) Ariza X.; Urpi, F.; Viladomat, C.; Vilarrasa J. *Tetrahedron Lett.* **1998**, *39*, 9101−9102.
(11) Saxon, E.; Bertozzi, C. R. *Science* **2000**, 287, 2007−2010.
(12) Amide formation by intramolecular acyl transfer from an *N*-acyl imidazole to an iminophosphorane nitrogen is also traceless and effective (Bertozzi, C. R. Presented at the 218th National Meeting of the American Chemical Society, New Orleans, LA, August 1999; Paper ORGN 233).
(13) Dawson, P. E.; Churchill, M.; Ghadiri, M. R.; Kent, S. G. H. *J. Am. Chem. Soc.* **1997**, *119*, 4325−4329.
(14) *o*-(Diphenylphosphino)benzenethiol (**2**) was prepared by reaction of chlorodiphenylphosphine and ortholithiated thiophenol, as described by Block, E.; Ofori-Okai, G.; Zubieta, J. *J. Am. Chem. Soc.* **1989**, *111*, 2327−2329.
(15) Phosphines are remarkable catalysts of acyl transfer reactions (Vedejs, E.; Diver, S. T. *J. Am. Chem. Soc.* **1993**, *115*, 3358−3359). Hence, thioester **3** may result from the formation of an acylphosphonium salt ($Ph_2P^+(C_6H_4$-*o*-SH)C(O)R), followed by intramolecular *P*- to *S*-acyl migration.

Scheme 3



added to a solution of thioester **3** in unbuffered THF/H_2O (3:1), and the resulting solution was stirred at room temperature for 12 h. The reaction was then acidified by the addition of 2 N HCl, and solvents were removed under reduced pressure. Chromatography on silica gel gave purified amide **5** in 35% yield.[16,17] The Staudinger ligation was also effective for coupling azide **4** with the *o*-phosphinobenzenethioester of *N*-acetyl glycine (R = H in Scheme 3).

Amide **5** could be formed by a mechanism other than that in Scheme 2. Specifically, the amide product of the Staudinger ligation could, in theory, arise from the reduction of the azide followed by acyl transfer to the resulting amine. To test for this occurrence, we mixed thioester **3** and authentic GlyNHBn under conditions (reactant concentrations, solvent, temperature, and time) identical to those used to effect the ligation of thioester **3** and glycyl azide **4**. We saw no evidence for the formation of amide **5**. This result argues against the alternative mechanism.

Phosphinothiol **2** has the attributes necessary to effect the Staudinger ligation. Still, phosphinothiol **2** has low aqueous solubility and bestows a yield that may be too low for some applications. We anticipate, however, that these limitations can be overcome by structural optimization.

We note that an optimized version of the Staudinger ligation would expand the scope of protein synthesis. Staudinger ligation of a thioester fragment[18] and an azide fragment[19] would be orthogonal to native chemical ligation as well as other strategies[20] for the coupling of unprotected peptides. Scheme 4 depicts the simplest proteins that would

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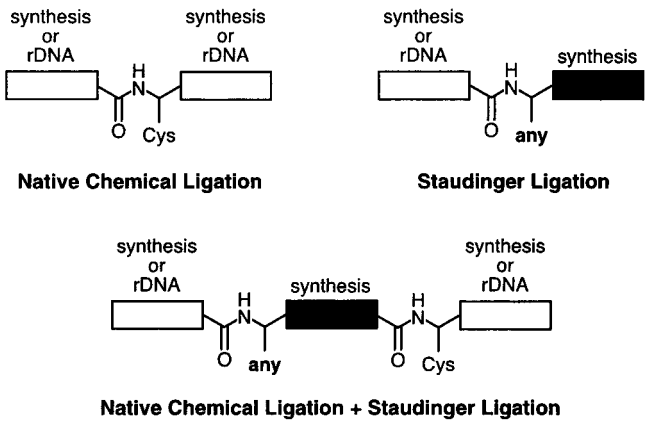

be accessible by native chemical ligation alone, the Staudinger ligation alone, and a sequential combination of the two methods. The use of thiol- or azide-protecting groups would extend the versatility of these methods even further.

Finally, we suggest that the Staudinger ligation could also be used with natural thioesters. For example, both the biosynthesis of polyketides and the nonribosomal biosynthesis of peptides proceed via the elaboration of thioester intermediates.[21] Interception of these intermediates with a phosphinothiol would allow for Staudinger ligation to an azide. Most significantly, ligation of a biosynthetic library of thioesters with a chemical library of azides could be a facile means to increase molecular diversity.

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Supporting Information Available: Procedures for the preparation of compounds **1** and **3**−**5** and related analytical data. This material is available free of charge via the Internet at http://pubs.acs.org.

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(16) The other major product was GlyNHBn, which can derive from the Staudinger reaction (ref 9).

(17) The effect of alternative solvent conditions on the coupling efficiency of thioester **3** and azide **4** was explored. The reaction was performed in THF/H_2O (3:1) buffered at pH 2, 4, 8, and 13.5. The reaction was also performed in methylene chloride or dimethyl formamide, followed by an acidic aqueous workup. None of these conditions improved the yield of product compared to that obtained in unbuffered THF/H_2O (3:1).

(18) A peptide or protein with a *C*-terminal thioester can be produced from Fmoc-based solid-phase synthesis (Ingenito, R.; Bianchi, E.; Fattori, D.; Pessi, A. *J. Am. Chem. Soc.* **1999**, *121*, 11369−11374. Shin, Y.; Winans, K. A.; Backes, B. J.; Kent, S. B. H.; Ellman, J. A.; Bertozzi, C. R. *J. Am. Chem. Soc.* **1999**, *121*, 11684−11689) or rDNA techniques (ref 3).

(19) A peptide with an *N*-terminal azide can be synthesized by reaction of the α-amino group of a protected peptide with $CF_3SO_2N_3$ (Zaloom, J.; Roberts, D. C. *J. Org. Chem.* **1981**, *46*, 5173−5176).

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