Communication

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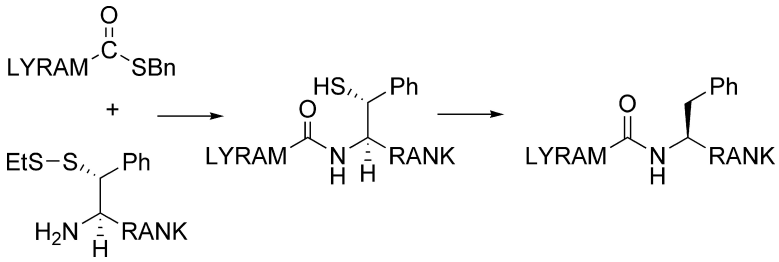

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Native Chemical Ligation at Phenylalanine

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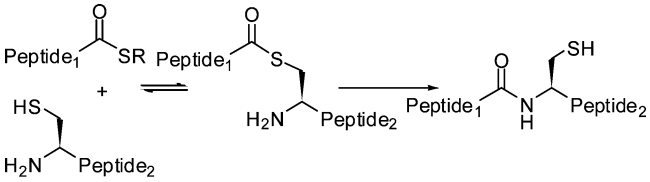
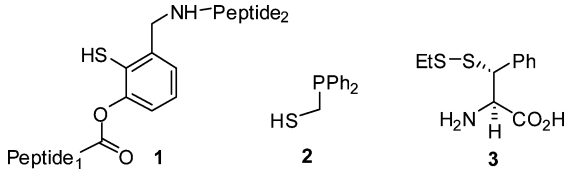
Native chemical ligation (NCL), a technique introduced by Kent and co-workers for the synthesis of peptides and proteins, relies on the combination of a N-terminal cysteine residue with a C-terminal thioester to form a peptide bond.[1] The method has two steps: a trans-thioesterification, followed by a rapid intramolecular S- to N-shift (Scheme 1). The power of this technique, or its biochemical equivalent, expressed protein ligation,[2] and the modification dubbed kinetically controlled ligation,[3] has enabled the synthesis of many moderate sized proteins[4] and glycoproteins,[5] culminating in the assembly of a 203 amino acid (AA) HIV protease covalent dimer.[6]

Scheme 1. General NCL Mechanism

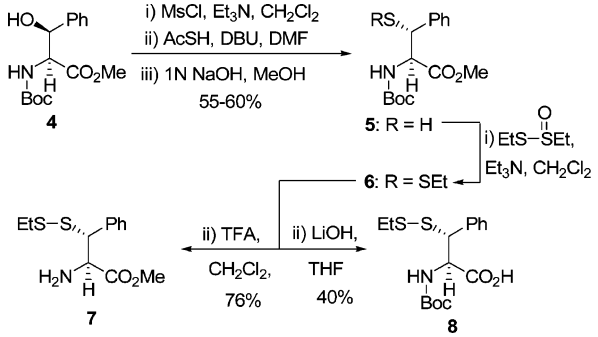


Extension of NCL beyond cysteine may be accomplished most directly by post-ligation desulfurization of the pivotal cysteine residue, affording ligation at alanine.[7,8] Alternatively, a variety of auxiliary thiols have been introduced for attachment to the N-terminal AA and traceless cleavage after ligation (e.g., **1**),[9] but none has complete generality.[10] The phosphine **2** bridges the gap with the Staudinger ligation but requires the synthesis of α-azido acids for use in peptide and protein synthesis.[11] Like Dawson,[7] we reasoned that NCL could be extended to further AAs if (i) a convenient synthesis of the *β*-mercapto derivatives was available, (ii) the trans-thioesterification step is not seriously affected by additional steric hindrance, and (iii) the final desulfurization can be conducted selectively. We report that the *β*-mercaptophenylalanine derivative **3** meets these conditions, as demonstrated through the synthesis of two complex decapeptides.

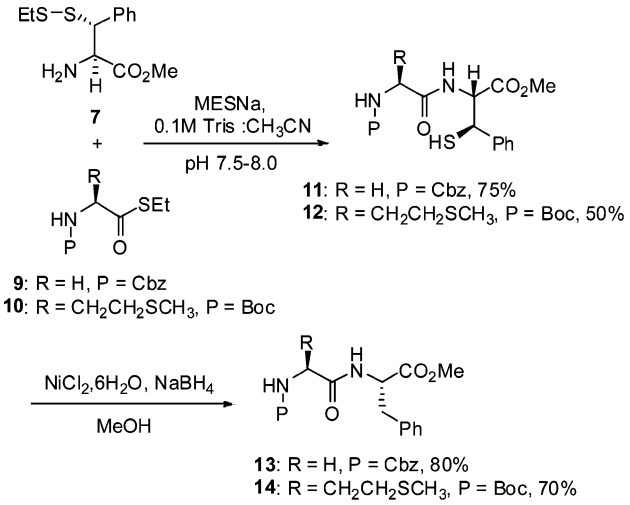


We focused on phenylalanine as direct, selective functionalization at the *β*-position was known and because we anticipated facile reductive cleavage of the benzylic C−S bond would ensure compatibility with other sulfur containing AAs.[12] Adapting Easton's bromination protocol,[13] we devised a convenient synthesis of the *threo*-*β*-hydroxy-L-phenylalanine derivative **4** from L-phenylalanine[14] and converted it to the *erythro*-thiol **5** by standard methods (Scheme 2). To avoid oxidative dimerization, the thiol group was protected by reaction with *S*-ethyl ethanethiosulfinate,[15] giving the disulfide **6**. Treatment with trifluoroacetic acid then afforded amino ester **7**, whereas saponification gave the acid **8** (Scheme 2).

Scheme 2. Precursor Synthesis



Scheme 3. Model Ligations



The *N*-Cbz glycine and *N*-Boc-L-methionine thioesters **9** and **10** were obtained under standard carbodiimide conditions and were ligated with the *β*-mercaptophenylalanine derivative **7** in the presence of sodium 2-mercaptoethanesulfonate (MESNa) in a mixture of acetonitrile and Tris buffer at pH 7.5−8.0. In this manner, the glycine-based dipeptide **11** and the methionine analogue **12** were obtained in 75 and 50% yields, respectively, reflecting the slower ligation at the more hindered thioester (Scheme 3). Blank experiments with L-phenylalanine methyl ester in place of **7** established the critical role of the thiol in these couplings.

Desulfurization of **11** with nickel boride,[16] obtained in situ by sodium borohydride reduction of nickel chloride, finally gave *N*-Cbz-Gly-L-Phe-OMe **13** in 80% yield. The cognate reduction of **12** afforded *N*-Boc-Met-L-Phe-OMe **14** in 70% isolated yield, thereby demonstrating the preferential cleavage of the benzylic C−S bond in the presence of methionine (Scheme 3). A comparable competition experiment (Supporting Information) established selec-

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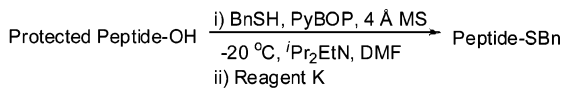
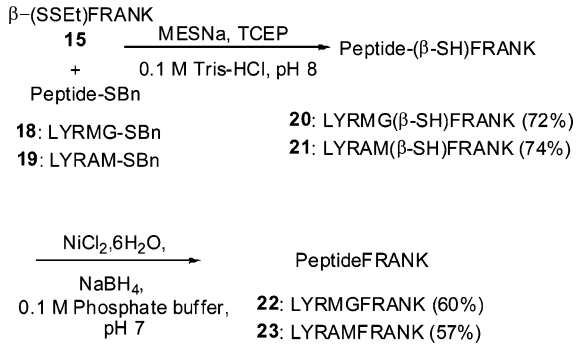
Scheme 4. Peptide Thioester Synthesis



16: Boc-LY(O^{t}Bu)R(Pbf)MG-OH
17: Boc-LY(O^{t}Bu)R(Pbf)AM-OH
18: LYRMG-SBn (78%)
19: LYRAM-SBn (71%)

Scheme 5. Decapeptide Synthesis



tive desulfurization in the presence of Cys(Acm) (*S*-acetamidomethyl cysteine).[17]

With proof of principle established, we turned to the synthesis of more challenging peptides. To probe the limits of the system, we selected two LYRMXXRANK sequences,[18] with their densely packed array of the more reactive AA side chains, which we modeled on Dawson's NCL probes.[19] The *N*-Boc acid **8** was employed as the final AA in the Fmoc-based SPPS of a pentapeptide containing the RANK residues. Release from the Wang resin and removal of the acid-sensitive protecting groups with reagent K afforded the β-(SSEt)-FRANK peptide **15**, thereby demonstrating compatibility with the standard Fmoc-SPPS conditions.

Two further pentapeptides Boc-LY(O^{t}Bu)R(Pbf)MG and Boc-LY(O^{t}Bu)R(Pbf)AM, **16** and **17**, were prepared by Fmoc-SPPS on chlorotrityl resin[20] and, after cleavage from the support with acetic acid were converted to their *S*-benzyl thioesters under the non-racemizing Kajihara conditions with activation by PyBOP.[21] Removal of the protecting groups and purification by RP-HPLC provided the pentapeptides **18** and **19** (Scheme 4). With **19**, the use of the Kajihara conditions was critical to the success of the enterprise as HATU-mediated thioesterification resulted in racemization of the methionine residue.

Ligation of both thioesters **18** and **19** to the β-(SSEt)-FRANK peptide **15** was achieved in the presence of excess sodium 2-mercaptoethanesulfonate and tris(2-carboxyethyl)phosphine hydrochloride (TCEP-HCl) in 0.1 M Tris buffer at pH 8 over 24 h at room temperature. After purification by RP-HPLC, hydrogenolytic desulfurization of both products was performed uneventfully in pH 7 phosphate buffer with nickel boride, resulting in the formation of the target peptides LYRMGFRANK and LYRAMFRANK in good overall yield (Scheme 5).

In conclusion, we have demonstrated that NCL can be extended to N-terminal phenylalanine, through the use of the readily accessible β-(SSEt)-Phe derivatives **7** and **8**. NCL conducted in this manner is not limited to coupling with C-terminal glycine thioesters, provided that appropriate conditions are employed to avoid racemization in the synthesis of the thioester. Furthermore, the final desulfurization step is compatible with the presence of methionine and ACM-protected cysteine. As we have described facile syntheses of *N*-Boc-*threo*-β-hydroxy-L-histidine methyl ester, and the corresponding tyrosine and tryptophan derivatives by the analogous route to that employed here for *N*-Boc-*threo*-β-hydroxy-L-phenylalanine methyl ester,[14] we anticipate that this chemistry will be readily extended to NCL at histidine, tyrosine, and tryptophan.

Acknowledgment. We thank the University of Illinois at Chicago for the Moriarty Fellowship (A.B.), the Protein Research Laboratory at UIC for SPPS, and Professor Yasuhiro Kajihara, Yokohama City University, for insightful discussions.

Note Added after ASAP Publication. After this work was published ASAP on July 21, 2007, we became aware that the concept of native chemical ligation at phenylalanine had been described previously: (a) Tchertchian, S.; Opligger, F.; Paolini, M.; Manganiello, S.; Raimondi, S.; Depresle, B; Dafflon, N.; Gaertner, H.; Botti, P. In *Understanding Biology Using Peptides*, Proceedings of the 19th American Peptide Symposium, San Diego, June 18−23, 2005; Blondelle, S. E., Ed.; Springer: New York, 2006; p 61. (b) Botti, P.; Tchertchian, S. WO/2006/133962. We apologize for this inadvertent oversight. This note was added on July 26, 2007.

Supporting Information Available: Full experimental details and characterization data for all compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) (a) Dawson, P. E.; Muir, T. W.; Clark-Lewis, I.; Kent, S. B. H. *Science* **1994**, *266*, 776. (b) Dawson, P. E.; Kent, S. B. H. *Annu. Rev. Biochem.* **2000**, *69*, 923. (c) Yeo, D. S. Y.; Srinivasan, R.; Chen, G. Y.; Yao, S. Q. *Chem.—Eur. J.* **2004**, *10*, 4664. (d) Macmillan, D. *Angew. Chem., Int. Ed.* **2006**, *45*, 7668.

(2) Muir, T. W.; Sondhi, D.; Cole, P. A. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 6705.

(3) Bang, D.; Pentelute, B. L.; Kent, S. B. H. *Angew. Chem., Int. Ed.* **2006**, *45*, 3985.

(4) (a) Bang, D.; Kent, S. B. H. *Angew. Chem., Int. Ed.* **2004**, *43*, 2534. (b) Bang, D.; Makhatadze, G. I.; Tereshko, V.; Kossiakoff, A. A.; Kent, S. B. *Angew. Chem., Int. Ed.* **2005**, *44*, 3852.

(5) (a) Brik, A.; Ficht, S.; Yang, Y.-Y.; Bennett, C. S.; Wong, C.-H. *J. Am. Chem. Soc.* **2006**, *128*, 15026. (b) Brik, A.; Yang, Y.-Y.; Ficht, S.; Wong, C.-H. *J. Am. Chem. Soc.* **2006**, *128*, 5626. (c) 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. (d) Dudkin, V. Y.; Miller, J. S.; Danishefsky, S. J. *J. Am. Chem. Soc.* **2004**, *126*, 736. (e) Miller, J. S.; Dudkin, V. Y.; Lyon, G. J.; Muir, T. W.; Danishefsky, S. J. *Angew. Chem., Int. Ed.* **2003**, *42*, 431.

(6) Torbeev, V. Y.; Kent, S. B. H. *Angew. Chem., Int. Ed.* **2007**, *46*, 1667.

(7) Yan, L. Z.; Dawson, P. E. *J. Am. Chem. Soc.* **2001**, *123*, 526.

(8) Selenocysteine has been employed to facilitate selective removal of the chalcogenide: Quaderer, R.; Hilvert, D. *Chem. Commun.* **2002**, 2620.

(9) Chen, G.; Warren, J. D.; Chen, J.; Wu, B.; Wan, Q.; Danishefsky, S. J. *J. Am. Chem. Soc.* **2006**, *128*, 7460.

(10) (a) Coltart, D. M. *Tetrahedron* **2000**, *56*, 3449. (b) Offer, J.; Boddy, C. N. C.; Dawson, P. E. *J. Am. Chem. Soc.* **2002**, *124*, 4642. (c) Clive, D. L. J.; Hisaindee, S.; Coltart, D. M. *J. Org. Chem.* **2003**, *68*, 9247. (d) Wu, B.; Chen, J. H.; Warren, J. D.; Chen, G.; Hua, Z. H.; Danishefsky, S. J. *Angew. Chem., Int. Ed.* **2006**, *45*, 4116.

(11) (a) Saxon, E.; Armstrong, J. I.; Bertozzi, C. R. *Org. Lett.* **2000**, *2*, 2141. (b) Nilsson, B. L.; Kiessling, L. L.; Raines, R. T. *Org. Lett.* **2001**, *3*, 9. (c) Nilsson, B. L.; Kiessling, L. L.; Raines, R. T. *Org. Lett.* **2000**, *2*, 1939. (d) Soellner, M. B.; Nilsson, B. L.; Raines, R. T. *J. Am. Chem. Soc.* **2006**, *128*, 8820.

(12) The expectation of selectivity is based on the weaker benzylic C−S bond (~60.4 kcal·mol^{-1}) compared with typical primary alkyl C−S bonds (73−74 kcal·mol^{-1}): Luo, Y.-R. *Bond Dissociation Energies in Organic Compounds*; CRC Press: Boca Raton, FL, 2003.

(13) Easton, C. J.; Hutton, C. A.; Roselt, P. D.; Tiekink, E. R. T. *Tetrahedron* **1994**, *50*, 7327.

(14) Crich, D.; Banerjee, A. *J. Org. Chem.* **2006**, *71*, 7106.

(15) (a) Hogg, D. R. In *Comprehensive Organic Chemistry*; Barton, D., Ollis, W. D., Eds.; Pergamon Press: Oxford, 1979; Vol. 3, p 261. (b) Furukawa, N.; Morishita, T.; Akasaka, T.; Oae, S. *J. Chem. Soc., Perkin Trans. 2* **1980**, 432.

(16) Back, T. G.; Baron, D. L.; Yang, K. *J. Org. Chem.* **1993**, *58*, 2407.

(17) For protection of cysteine against desulfurization with the Acm group, see: Pentelutte, B. L.; Kent, S. B. H. *Org. Lett.* **2007**, *9*, 687 and ref 8.

(18) L: leu, Y: tyr, R: arg, M: met, X: variable amino acid, A: ala, N: asn, K: lys, G: gly (except G all have the L-configuration).

(19) Hackeng, T. M.; Griffin, J. H.; Dawson, P. E. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 10068.

(20) Barlos, K.; Chatzi, O.; Gatos, D.; Stavropoulos, G. *Int. J. Peptide Protein Res.* **1991**, *37*, 513.

(21) (a) Kajihara, Y.; Yoshihara, A.; Hirano, K.; Yamamoto, N. *Carbohydr. Res.* **2006**, *341*, 1333. (b) Hogenauer, T. J.; Wang, Q.; Sanki, A. K.; Gammon, A. J.; Chu, C. H. L.; Kaneshiro, C. M.; Kajihara, Y.; Michael, K. *Org. Biomol. Chem.* **2007**, *5*, 759.

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