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Allylic Selenosulfide Rearrangement: A Method for Chemical Ligation to Cysteine and Other Thiols

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The development of methods for the chemical ligation of small molecules to peptides and proteins, for conjoining two proteins, and for the attachment of oligosaccharides to peptides and proteins is a current frontier in chemical biology.[1] One established ligation method involves the formation of mixed disulfide linkages,[2] various modifications of which have recently been applied to the synthesis of glycopeptides and proteins.[3] The disulfide ligation is especially attractive in conjunction with native peptide ligation; however, the ease of disulfide exchange renders the method impermanent.[4] We report preliminary results on the development of a new method of chemical ligation based on the facile formation of mixed selenosulfides, which are then rendered permanent by a sigmatropic rearrangement with loss of selenium.

Allylic disulfides are in equilibrium with allylic thiosulfoxides, by virtue of a 2,3-sigmatropic rearrangement.[5] The equilibrium very strongly favors the allylic disulfide and is only revealed when the reaction is driven in the forward direction, either by a further rearrangement or by the addition of a thiophilic reagent, typically a phosphine. At 60 °C in benzene, rate constants for the rearrangement with transfer of sulfur to triphenyl phosphine were found to range from 0.7 to 190 $\times$ 10^{-4} s^{-1}, depending on the substituent pattern.[5a] Allylic diselenides, on the other hand, undergo the analogous rearrangement at room temperature in a matter of hours.[6]

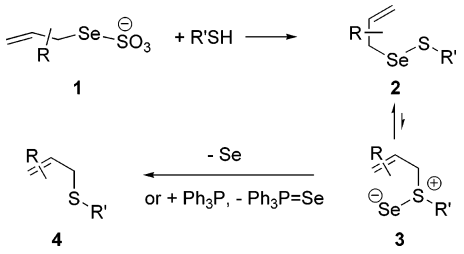
We hypothesized that *Se*-allyl selenosulfides **2** would undergo a similar 2,3-sigmatropic rearrangement at room temperature and that the weakness of the Se−S bond in the selenosulfoxide product **3** would result in the facile loss of selenium, thereby driving the reaction even in the absence of a phosphine. We further hypothesized that the requisite *Se*-allylic selenosulfides could be readily accessed at room temperature from simple *Se*-allyl seleno Bunte salts **1**, as was known for *Se*-alkyl seleno Bunte salts,[7,8] and that the combination of these two processes would provide a convenient new chemical ligation process (Scheme 1).

Reaction of K_2SO_3 with Se powder and then allyl bromide gave potassium *Se*-allyl selenosulfate **5** as an orange solid.[8b,9] Reaction of this *Se*-Bunte salt with primary aliphatic thiols in methanol at room temperature resulted in transfer of the allyl group to sulfur, with loss of selenium (Scheme 2), establishing the validity of the initial hypothesis.

We next examined the attachment of allyl and substituted allyl groups to cysteine derivatives (Table 1). These reactions were conducted in two stages, with formation of the selenosulfide in methanol and, following filtration on silica gel, triphenylphosphine-promoted deselenylative rearrangement in $CDCl_3$, all at room temperature. The 2,3-sigmatropic nature of the rearrangement step is unmasked with the formation of the *S*-linalyl cysteine **14** starting from the *Se*-geranyl *Se*-Bunte salt **13**.[10]

Functionalization of small cysteine-containing peptides was explored with Boc-(α-OMe)-γ-L-Glu-L-Cys-Gly-OMe, **15**, and with Boc-L-Cys-L-Ala-L-Trp-OMe **16** using a selection of allylic *Se*-Bunte salts (Table 2). These reactions were conducted in methanol

Scheme 1. Chemical Ligation via *Se*-Allyl Selenosulfides



Scheme 2 . Allylation of Simple Thiols

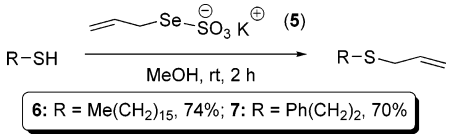
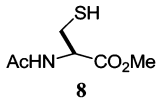
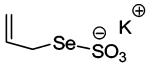
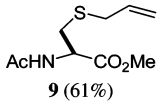
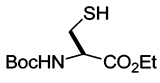
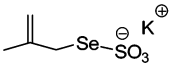
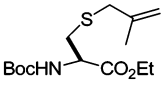
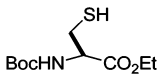
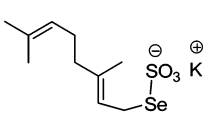
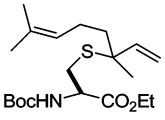


Table 1. Application to Cysteine Derivatives[a,b]

Thiol	*Se*-Bunte Salt	Product (Yield)
 **8**	 **5**	 **9** (61%)
 **10**	 **11**	 **12** (62%)
 **10**	 **13**	 **14** (46%)

[a] With *Se*-Bunte salt **13**, the first step was conducted in a methanol/CH_2Cl_2 mixture. [b] Compound **14** was formed as an approximately 1:1 unassigned mixture of diastereomers.

at room temperature with filtration of the excess Bunte salt before addition of the phosphine. It is noteworthy that this new cysteine functionalization method is compatible with the indole in tryptophan and enables the incorporation of the geranyl and farnesyl chains as the transposed linalyl and nerolidyl groups. We have also demonstrated application to a carbohydrate-based thiol **24** (Scheme 3). This rearrangement, which proceeded spontaneously over a period of several days at room temperature or in a matter of hours on addition of triphenyl phosphine, took place with complete retention of the anomeric stereochemistry.

In summary, we describe a new, permanent chemical ligation method. The method has potential for the attachment of lipids to protein-based thiols, an area of considerable current interest.[11] Applications in combinatorial chemistry and for the attachment of a wide variety of groups, including poly(ethylene glycol)[12] and

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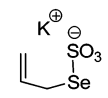
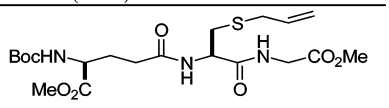
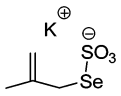
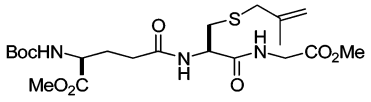
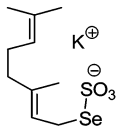
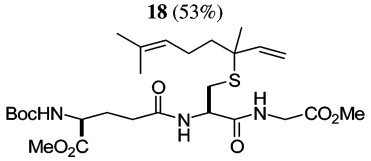
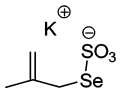
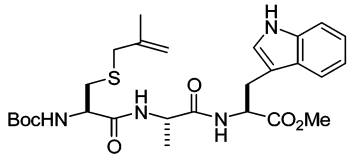
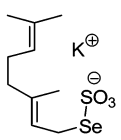
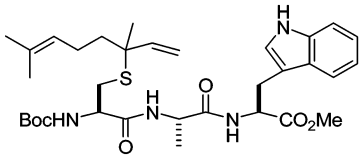
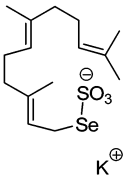
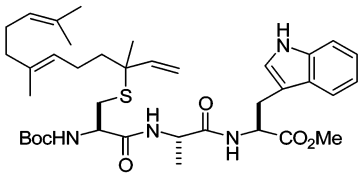
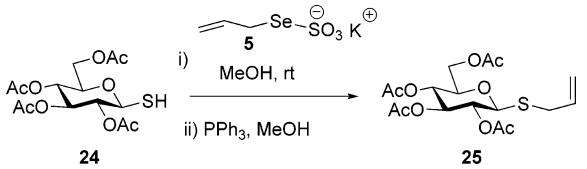
Table 2. Application to Cysteine-Containing Tripeptides[a]

Peptide	*Se*-Bunte Salt	Product (Yield)
15	 **5**	 **17** (58%)
15	 **11**	 **18** (53%)
15	 **13**	 **19** (43%)
16	 **11**	 **20** (60%)
16	 **13**	 **21** (51%)
16	 **22**	 **23** (47%)

[a] Compounds **19**, **21**, and **23** were formed as an approximately 1:1 unassigned mixture of diastereomers.

Scheme 3. Allylation of an Anomeric Thiol



fluorous chains[13] and oligosaccharide units to thiols[3,14] of biological interest, are currently under development.

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

References

(1) (a) Kochendoerfer, G. G.; Kent, S. B. H. *Curr. Opin. Chem. Biol.* **1999**, *3*, 665. (b) Dawson, P. E.; Kent, S. B. H. *Annu. Rev. Biochem.* **2000**, *69*, 923. (c) Köhn, M.; Breinbauer, R. *Angew. Chem., Int. Ed.* **2004**, *43*, 3106. (d) van Swieten, P. F.; Leeuwenburgh, M. F.; Kessler, B. M.; Overkleeft, H. S. *Org. Biomol. Chem.* **2005**, *3*, 20. (e) Peri, F.; Nicotra, F. *Chem. Commun.* **2004**, 623. (f) Breinbauer, R.; Köhn, M. *ChemBioChem* **2003**, *4*, 1147. (g) Kubler-Kielb, J.; Pozsgay, V. *J. Org. Chem.* **2005**, *70*, 6987. (h) Langenhan, J. M.; Thorson, J. S. *Curr. Org. Synth.* **2005**, *2*, 59. (i) He, Y.; Hinklin, R. J.; Chang, J.; Kiessling, L. *Org. Lett.* **2004**, *6*, 4479. (j) Grandjean, C.; Boutonnier, A.; Guerreiro, C.; Fournier, J.-M.; Mulard, L. A. *J. Org. Chem.* **2005**, *70*, 7123. (k) Lin, H.; Walsh, C. T. *J. Am. Chem. Soc.* **2004**, *126*, 13998. (l) Liu, H.; Wang, L.; Brock, A.; Wong, C.-H.; Schultz, P. G. *J. Am. Chem. Soc.* **2003**, *125*, 1702. (m) Manetsch, R.; Krasinski, A.; Radic, Z.; Raushel, J.; Taylor, P.; Sharpless, K. B.; Kolb, H. C. *J. Am. Chem. Soc.* **2004**, *126*, 12809. (n) Krasinski, A.; Radic, Z.; Manetsch, R.; Raushel, J.; Taylor, P.; Sharpless, K. B.; Kolb, H. C. *J. Am. Chem. Soc.* **2005**, *127*, 6686. (o) Kolb, H. C.; Finn, M. G.; Sharpless, K. B. *Angew. Chem., Int. Ed.* **2001**, *40*, 2004. (p) Davis, B. G. *Chem. Rev.* **2002**, *102*, 579. (q) 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. (r) Macmillan, D.; Bertozzi, C. R. *Angew. Chem., Int. Ed.* **2004**, *43*, 1355. (s) MacMillan, D. W. C.; Bill, R. M.; Sage, K. A.; Fern, D.; Flitsch, S. L. *Chem. Biol.* **2001**, *8*, 133.

(2) (a) Lundblad, R. L. *Chemical Reagents for Protein Modification*, 3rd ed.; CRC Press: Boca Raton, FL, 2005. (b) Kenyon, G. L.; Bruice, T. W. *Methods Enzymol.* **1977**, *47*, 407. (c) Wynn, R.; Richards, F. M. *Methods Enzymol.* **1995**, *251*, 351. (d) Faulstich, H.; Heintz, D. *Methods Enzymol.* **1995**, *251*, 357.

(3) (a) Gamblin, D. P.; Garnier, P.; van Kasteren, S.; Oldham, N. J.; Fairbanks, A. J.; Davis, B. G. *Angew. Chem., Int. Ed.* **2004**, *43*, 828. (b) Gamblin, D. P.; Garnier, S. J.; Ward, N. J.; Oldham, A. J.; Fairbanks, A. J.; Davis, B. G. *Org. Biomol. Chem.* **2003**, *1*, 3642. (c) Macindoe, W. M.; van Oijen, A. H.; Boons, G.-J. *Chem. Commun.* **1998**, 847.

(4) As illustrated by applications in dynamic combinatorial libraries. (a) Otto, S.; Furlan, R. L. E.; Sanders, J. K. M. *J. Am. Chem. Soc.* **2000**, *122*, 12063. (b) Otto, S.; Furlan, R. L. E.; Sanders, J. K. M. *Science* **2002**, *297*, 590. (c) Brisig, B.; Sanders, J. K. M.; Otto, S. *Angew. Chem., Int. Ed.* **2003**, *42*, 1270. (d) Leclaire, J.; Vial, L.; Otto, S.; Sanders, J. K. M. *Chem. Commun.* **2005**, 1959.

(5) (a) Höfle, G.; Baldwin, J. E. *J. Am. Chem. Soc.* **1971**, *93*, 6307. (b) Baechler, R. D.; Hummel, J. P.; Mislow, K. *J. Am. Chem. Soc.* **1973**, *95*, 4442. (c) Block, E.; Iyer, R.; Grisoni, S.; Saha, C.; Belman, S.; Lossing, F. P. *J. Am. Chem. Soc.* **1988**, *110*, 7813. (d) Kutney, G. W.; Turnbull, K. *Chem. Rev.* **1982**, *82*, 333.

(6) Sharpless, K. B.; Lauer, K. B. *J. Org. Chem.* **1972**, *37*, 3973.

(7) Synthesis of selenosulfides by reaction of selenols with disulfides was discounted due to the complex synthesis and instability of allylic selenols. Riague, E. H.; Guillemin, J.-C. *Organometallics* **2002**, *21*, 68.

(8) (a) Patarasakulchai, N.; Southwell-Keely, P. T. *Phosphorus Sulfur* **1985**, *22*, 277. (b) Klayman, D. L.; Günther, W. H. H., Eds.; *Organic Selenium Compounds: Their Chemistry and Biology*; Wiley-Interscience: New York, 1973.

(9) (a) Price, T. S.; Jones, L. M. *J. Chem. Soc.* **1909**, *95*, 1729. (b) Günther, W. H. H.; Mautner, H. G. *J. Med. Chem.* **1964**, *7*, 229.

(10) Attempted acceleration of the rearrangement with more nucleophilic phosphines resulted in a changed mechanism: nucleophilic attack on the selenosulfide with loss of the sigmatropic rearrangement component. Presumably, this occurs analogously to loss of sulfur from disulfides with hexaalkylphosphoramines. Hexamethylphosphoramine also caused racemization of the cysteine moiety as evidenced by incorporation of deuterium at the α-position when the reaction was conducted in CD_3OD. Harpp, D. N.; Gleason, J. G. *J. Am. Chem. Soc.* **1971**, *93*, 2437.

(11) (a) Schelhaas, M.; Nägele, E.; Kuder, N.; Bader, B.; Kuhlmann, J.; Wittinghofer, A.; Waldmann, H. *Chem.−Eur. J.* **1999**, *5*, 1239. (b) Schelhaas, M.; Glomsda, S.; Hänsler, M.; Jakubke, H.-D.; Waldmann, H. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 106. (c) Brown, M. J.; Milano, P. D.; Lever, D. C.; Epstein, W. W.; Poulter, C. D. *J. Am. Chem. Soc.* **1991**, *113*, 3176. (d) Yang, C.-C.; Marlowe, C. K.; Kania, R. *J. Am. Chem. Soc.* **1991**, *113*, 3177. (e) Pachamuthu, K.; Zhu, X.; Schmidt, R. R. *J. Org. Chem.* **2005**, *70*, 3720.

(12) (a) Veronese, F. M.; Pasut, G. *Drug Discovery Today* **2005**, *10*, 1451. (b) Doherty, D. H.; Rosendahl, M. S.; Smith, D. J.; Hughes, J. M.; Chlipala, E. A.; Cox, G. N. *Bioconjugate Chem.* **2005**, *16*, 1291. (c) Manjula, B. N.; Tsai, A.; Upadhya, R.; Perumalsamy, K.; Smith, P. K.; Malavalli, A.; Vanderegriff, K.; Winslow, R. M.; Intaglietta, M.; Prabhakaran, M.; Friedman, J. M.; Acharya, A. S. *Bioconjugate Chem.* **2003**, *14*, 464.

(13) Brittain, S. M.; Ficarro, S. B.; Brock, A.; Peters, E. C. *Nat. Biotechnol.* **2005**, *23*, 463.

(14) (a) Ohnishi, Y.; Ichikawa, M.; Ichikawa, Y. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 1289. (b) Jobron, L.; Hummel, G. *Org. Lett.* **2000**, *2*, 2265. (c) Cohen, S. B.; Halcomb, R. L. *Org. Lett.* **2001**, *3*, 405. (d) Knapp, S.; Myers, D. S. *J. Org. Chem.* **2002**, *67*, 2995. (e) Zhu, X. M.; Pachamuthu, K.; Schmidt, R. R. *Org. Lett.* **2004**, *6*, 1083. (f) Zhu, X. M.; Schmidt, R. R. *Chem.−Eur. J.* **2004**, *10*, 875. (g) Thayer, D. A.; Yu, H. N.; Galan, M. C.; Wong, C.-H. *Angew. Chem., Int. Ed.* **2005**, *44*, 4596.

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