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Selenocysteine-mediated backbone cyclization of unprotected peptides followed by alkylation, oxidative elimination or reduction of the selenol

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An unprotected 16 residue peptide containing a C-terminal thioester and an N-terminal selenocysteine residue efficiently cyclizes in the presence of thiophenol; subsequent reduction, elimination or alkylation of the selenol yields modified cyclic peptides with alanine, dehydroalanine or a non-natural amino acid at the site of ligation.

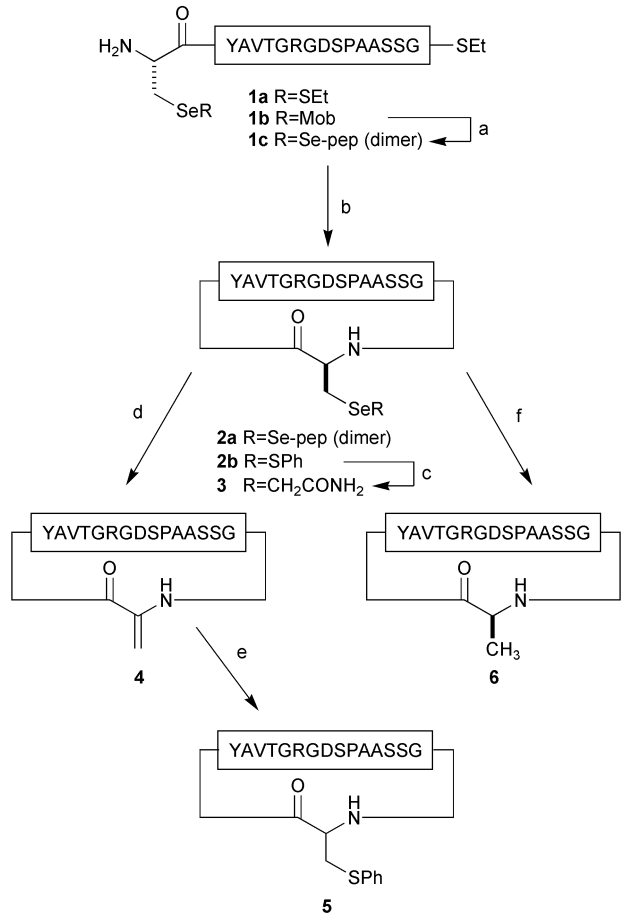
Cyclic peptides exhibit valuable activity as drugs and hormones[1] and considerable effort has been invested in developing efficient methods for their synthesis.[2] Chemoselective strategies for ligation[3] are particularly attractive in this regard because they allow unprotected peptides to be cyclized under mild conditions in aqueous buffer.[4,5] Such cyclizations tend to occur in preference to oligomerization even at relatively high peptide concentrations;[6] they can also be initiated directly from a solid support.[7] The synthesis of a circular protein domain[8] and cyclization of peptides containing up to 6 cysteines through successive ring expansion (the so-called 'thia-zip' reaction)[9] illustrate the broad utility of these methods. Here we report an extension of this approach that exploits the ability of selenocysteine to mediate chemical ligation.[10]

Under reducing conditions, peptides containing an N-terminal selenocysteine have been shown to react with peptides containing a C-terminal thioester to form a selenoester intermediate that spontaneously rearranges to give the more stable amide bond. This strategy has been used to prepare a variety of selenopeptides and selenoproteins.[10] The ease with which selenols can be subsequently modified provides considerable flexibility with respect to the formal choice of ligation site.

The molecule chosen to illustrate the utility of this method for preparation of cyclic peptides (**1**, Scheme 1) is based on a model system previously described by Muir[5] for cysteine-mediated cyclizations. It consists of 16 residues and contains a C-terminal ethyl thioester, an N-terminal selenocysteine in place of cysteine, and an RGD motif that is involved in binding to the integrin class of cell adhesion receptors.

The linear peptide was synthesized by conventional Fmoc-based solid-phase peptide synthesis on a PAM resin preloaded with glycine. The peptide chain was assembled on a 0.1 mmol scale in stepwise fashion up to Tyr2 on an automated synthesizer using standard HBTU/HOBt/NMP activation protocols[11] for Fmoc-chemistry. Sec1 was introduced in the last step as an Fmoc-Sec(Mob)-OPfp ester to minimize problems with racemization and β-elimination.[12] The peptide was cleaved from the resin at room temperature using an aluminium-thiolate reagent, prepared from trimethyl aluminium (0.2 M) and ethanethiol (0.6 M),[13] in CH_2Cl_2 under an inert atmosphere for 5 h to give the thioester directly. Following removal of the side chain protecting groups with a cleavage cocktail containing TFA and scavengers (TFA/H_2O/EtSH/PhOH/triisopropylsilane 87.5:4:4:2:0.5; 4 °C for 75 min) and trituration with cold diethyl ether, the crude product was dissolved in a mixture of water and acetonitrile and lyophilized. The product was purified by preparative RP-HPLC and characterized by electrospray ionization mass spectrometry (ESI-MS). It was obtained as a 1:1 mixture of the mixed selenosulfide [**1a**, (av. isotope comp. for $C_{64}H_{103}N_{19}O_{23}S_2Se$) = 1649.7 Da; found = 1650.0 Da] and Mob-protected selenol [**1b**, (av. isotope comp. for $C_{70}H_{107}N_{19}O_{24}SSe$) = 1709.7 Da; found = 1710.0 Da], resulting from incomplete deprotection. Treatment of **1b** with 1 M trimethylsilyl bromide in TFA containing *m*-cresol and thioanisole[14] under argon at 4 °C for 1 h yielded the diselenide dimer **1c** [(av. isotope comp. for $C_{124}H_{196}N_{38}O_{46}S_2Se_2$) = 3177.2 Da; found = 3177.0 Da].

Linear peptides **1b** and **1c** cleanly and chemoselectively cyclized in phosphate buffer (0.1 M, pH 7.5) containing 3% thiophenol (v/v). Thiophenol is needed to generate the reactive selenol *in situ* and to activate the thioester *via* transthioesterification. After 3 h, both reactions were complete as judged by HPLC. The desired cyclized peptide was formed quantitatively as a mixture of the diselenide dimer [**2a**, (av. isotope comp. for $C_{120}H_{184}N_{38}O_{46}Se_2$) = 3052.9 Da; found = 3054.0 Da] and the mixed selenosulfide with thiophenol [**2b**, (av. isotope comp. for



Scheme 1 *Reagents and conditions*: (a) 1 M TMSBr in TFA/thioanisole/*m*-cresol (750:120:50), 4 °C, 1 h, quant.; (b) 0.1 M phosphate, pH 7.5, thiophenol 3% (v/v), 3 h, quant.; (c) 0.1 M phosphate, pH 7.6/acetonitrile (1:1), TCEP, ICH_2CONH_2, 6.5 h, 35%; (d) H_2O_2, water/acetonitrile (9:1), quant.; (e) 0.2 M phosphate, pH 8/acetonitrile (1:1), thiophenol 4% (v/v); (f) Raney Ni, H_2 atm., TCEP, AcOH 20% (aq.), 6 h, 94%.

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$C_{66}H_{97}N_{19}O_{23}SSe$) = 1635.6 Da; found = 1635.8 Da] in a 71 to 29 ratio.

An attractive feature of selenocysteine-mediated ligation is that it provides a convenient handle for subsequent chemical manipulation. The unique reactivity of selenium makes tailored modification of the selenocysteine side chain possible. To demonstrate this versatility, we have prepared several derivatives of peptides **2a** and **2b** *via* alkylation, oxidative elimination, and reduction of the selenol (Scheme 1).

The high nucleophilicity[15] and low pK_a[16] of selenols can be exploited to introduce a wide range of electrophiles, including mechanistic or spectroscopic probes, into peptides in a site-selective fashion. For example, alkylation of peptide **2b** (3.3 mg, 2 μmol) with a ninefold excess of iodoacetamide in degassed phosphate buffer (0.1 M, pH 7.6) containing 50% acetonitrile and tris(2-carboxyethyl)phosphine (TCEP, 1.4 mg, 5 μmol) as a reducing agent yielded peptide **3** [(av. isotope comp. for $C_{62}H_{96}N_{20}O_{24}Se$) = 1584.5 Da; found = 1585.0 Da]. This compound possesses a novel seleno ether analog of homoglutamine at the original site of ligation.

Selenocysteine derivatives also undergo fast and mild oxidative elimination to generate dehydroalanine (Dha),[17] which is found in many natural products and is also a useful starting point for further elaboration.[18] Indeed, as previously seen with aryl selenides, the diselenide-containing cyclic peptide **2a** reacts with hydrogen peroxide in H_2O/CH_3CN (9 : 1) at room temperature to give the Dha derivative **4** in quantitative yield. The final product exhibits diagnostic olefinic peaks at δ 5.72 and 5.81 ppm in the ^{1}H-NMR spectrum and has the molecular mass expected for the product of oxidative elimination [(av. isotope comp. for $C_{60}H_{91}N_{19}O_{23}$) = 1446.5 Da; found = 1445.9 Da]. The overall transformation proceeds *via* an unstable intermediate, which is relatively long-lived under the reaction conditions ($\tau_{1/2} \approx 60$ min) and is readily observed both by HPLC and NMR spectroscopy.† In accord with the known reactivity of diselenides toward hydrogen peroxide,[16] we speculate that the unstable precursor to **4** is a seleninic acid derivative of the starting peptide which undergoes subsequent β-elimination. However, attempts to characterize it by ESI-MS or LC-MS were unsuccessful, presumably because of the rapid elimination of selenium during analysis. Like other Dha derivatives,[18] compound **4** is susceptible to Michael addition by nucleophiles. Thus, it reacts readily with thiophenol in a 1 : 1 mixture of acetonitrile and 0.2 M phosphate buffer (pH 8) to give thioether **5** [(av. isotope comp. for $C_{66}H_{97}N_{19}O_{23}S$) = 1556.7 Da; found = 1556.0 Da].

Finally, the selenocysteine was chemoselectively converted to alanine by reduction of the relatively weak C–Se bond. Peptide **2a** (2.1 mg, 0.7 μmol) was cleanly reduced to peptide **6** when vigorously stirred under a hydrogen atmosphere in the presence of *ca*. 10 mass equivalents of Raney nickel and TCEP (0.6 mg, 2 μmol) in 20% AcOH (aq., 2 mL) for 6 hours. The identity of the isolated peptide (2 mg, 94%) was confirmed by ESI-MS [(av. isotope comp. for $C_{60}H_{93}N_{19}O_{23}$) = 1448.5 Da; found = 1448.0 Da]. Without TCEP, which is used to form the free selenol, conversion to alanine was not quantitative. Use of Pd on Al_2O_3 with model compounds was similarly efficacious, whereas PdO-catalyzed reductions gave inferior results with and without the addition of TCEP. Similar findings have been obtained for the reduction of cysteine to alanine.[19] Since side chain protected cysteine derivatives like Cys(Acm) and Cys(tBu) are stable to the reductive conditions needed to convert selenocysteine to alanine,‡ sequential selenocysteine- and cysteine-mediated ligations can be used to create longer peptides containing both Xaa-Ala and Yaa-Cys ligation sites. Our findings thus extend the cysteine ligation/reduction strategy recently introduced by Dawson.[19]

In summary, the work presented in this communication broadens the utility of selenocysteine in peptide and protein chemistry. Selenocysteine-mediated native chemical ligation provides a facile means of cyclizing peptides. The versatile post-ligation chemistry accessible to the selenocysteine side chain makes this method especially valuable, particularly in the context of efforts to join peptides at a wide variety of different ligation sites. Of particular note in this regard is the facile reduction of selenocysteine to alanine.

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Notes and references

† The peptides were resolved by RP-HPLC on a C_{18} column eluted with 5% acetonitrile containing 0.05% TFA in water containing 0.1% TFA for 5 min, followed by a gradient in which the acetonitrile fraction was increased to 45% over 45 min. The retention time of the intermediate was 27.4 min, compared to 34.3 min and 30.1 min for **2a** and **4**, respectively. All three compounds are clearly distinguishable by ^{1}H-NMR spectroscopy at 500 MHz. For example, the chemical shift of the aromatic protons of the Tyr side chain appear at δ 7.00 (*d*, J = 8.4 Hz) and 7.30 (*d*, J = 8.4 Hz) for **2a**, δ 7.03 (*d*, J = 8.5 Hz) and 7.32 (*d*, J = 8.6 Hz) for the intermediate, and 7.03 (*d*, J = 8.4 Hz) and 7.34 (*d*, J = 8.5 Hz) for final product **4**.

‡ H-Cys(Acm)-OH (10 mg, 52 μmol) and TCEP (28 mg, 100 μmol) were dissolved in 20% aq. AcOH (25 mL). Raney nickel (*ca*. 10 mass equiv.) was added and the mixture vigorously stirred at room temperature under a hydrogen atmosphere for 6 h. Raney nickel was filtered off and the amino acid isolated from an ion-exchange resin and lyophilized. The ^{1}H-NMR spectrum (200 MHz, 1 M DCl/D_2O) of this material was identical to that of the starting material. The same procedure was performed with H-Cys(tBu)-OH.

1 P. Brazeau, W. Vale, R. Burgus, N. Ling, M. Butcher, J. Rivier and R. Guillemi, *Science*, 1973, **179**, 77; Y. A. Ovchinnikov and V. T. Ivanov, *Tetrahedron*, 1975, **31**, 2177; J. Rizo and L. M. Gierasch, *Annu. Rev. Biochem.*, 1992, **61**, 387; S. Fernandez-Lopez, H. S. Kim, E. C. Choi, M. Delgado, J. R. Granja, A. Khasanov, K. Kraehenbuehl, G. Long, D. A. Weinberger, K. M. Wilcoxen and M. R. Ghadiri, *Nature*, 2001, **412**, 452.
2 P. Wipf, *Chem. Rev.*, 1995, **95**, 2115; A. V. R. Rao, M. K. Gurjar, K. L. Reddy and A. S. Rao, *Chem. Rev.*, 1995, **95**, 2135; J. N. Lambert, J. P. Mitchell and K. D. Roberts, *J. Chem. Soc., Perkin Trans. 1*, 2001, 471.
3 P. E. Dawson and S. B. H. Kent, *Annu. Rev. Biochem.*, 2000, **69**, 923; J. P. Tam, J. X. Xu and K. D. Eom, *Biopolymers*, 2001, **60**, 194.
4 L. S. Zhang and J. P. Tam, *Tetrahedron Lett.*, 1997, **38**, 3.
5 J. A. Camarero and T. W. Muir, *Chem. Commun.*, 1997, 1369.
6 P. Botti, T. D. Pallin and J. P. Tam, *J. Am. Chem. Soc.*, 1996, **118**, 10018.
7 J. A. Camarero, G. J. Cotton, A. Adeva and T. W. Muir, *J. Pept. Res.*, 1998, **51**, 303.
8 J. A. Camarero, J. Pavel and T. W. Muir, *Angew. Chem. Int. Edit.*, 1998, **37**, 347.
9 J. P. Tam, Y. A. Lu and Q. T. Yu, *J. Am. Chem. Soc.*, 1999, **121**, 4316.
10 R. Quaderer, A. Sewing and D. Hilvert, *Helv. Chim. Acta*, 2001, **84**, 1197; R. J. Hondal, B. L. Nilsson and R. T. Raines, *J. Am. Chem. Soc.*, 2001, **123**, 5140; M. D. Gieselman, L. Xie and W. A. van der Donk, *Org. Lett.*, 2001, **3**, 1331; S. M. Berry, M. D. Gieselman, M. J. Nilges, W. A. van der Donk and Y. Lu, *J. Am. Chem. Soc.*, 2002, **124**, 2084.
11 R. Knorr, A. Trzeciak, W. Bannwarth and D. Gillessen, *Tetrahedron Lett.*, 1989, **30**, 1927.
12 D. Besse and L. Moroder, *J. Pept. Sci.*, 1997, **3**, 442.
13 D. Swinnen and D. Hilvert, *Org. Lett.*, 2000, **2**, 2439; A. Sewing and D. Hilvert, *Angew. Chem., Int. Ed.*, 2001, **40**, 3395.
14 T. Koide, H. Itoh, A. Otaka, H. Yasui, M. Kuroda, N. Esaki, K. Soda and N. Fujii, *Chem. Pharm. Bull.*, 1993, **41**, 502.
15 R. G. Pearson, H. Sobel and J. Songstad, *J. Am. Chem. Soc.*, 1968, **90**, 319; J. C. Pleasants, W. Guo and D. L. Rabenstein, *J. Am. Chem. Soc.*, 1989, **111**, 6553; R. Singh and G. M. Whitesides, *J. Org. Chem.*, 1991, **56**, 6931.
16 *The Chemistry of Organic Selenium and Tellurium Compounds*, eds. S. Patai and Z. Rappoport, John Wiley & Sons, Chichester, 1986, vol. 1.
17 R. Walter and J. Roy, *J. Org. Chem.*, 1971, **36**, 2561.
18 M. D. Gieselman, Y. Zhu, H. Zhou, D. Galonic and W. A. van der Donk, *ChemBioChem*, 2002, **3**, 709.
19 L. Z. Yan and P. E. Dawson, *J. Am. Chem. Soc.*, 2001, **123**, 526.