JOC Note
pubs.acs.org/joc

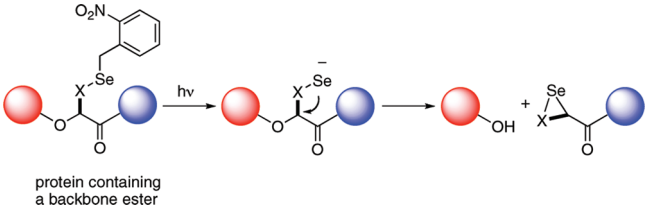
A Selenide-Based Approach to Photochemical Cleavage of Peptide and Protein Backbones at Engineered Backbone Esters

Amy L. Eastwood,[†] Angela P. Blum,[†] Niki M. Zacharias, and Dennis A. Dougherty*

Division of Chemistry and Chemical Engineering, California Institute of Technology, Pasadena, California 91125.
[†]*These authors contributed equally to this work.*

dadougherty@caltech.edu

Received June 29, 2009



A strategy for photochemical cleavage of peptide and protein backbones is described, which is based on a selenide-mediated cleavage of a backbone ester moiety. Studies in model systems establish the viability of the chemistry and suggest the method could be a valuable tool for chemical biology studies of proteins.

We describe here a new strategy for photochemical cleavage of peptide and protein backbones and model studies intended to evaluate the viability of the novel chemistry involved. Strategies for preparing photoresponsive biomolecules are finding increasing use in chemical biology.[1,2] Some time ago we introduced a strategy for photochemically initiating backbone cleavage of a protein, employing the unnatural amino acid 2-nitrophenylglycine (Npg, Figure 1).[3] After incorporation of Npg into a protein or peptide, the well-known *o*-nitrobenzyl "deprotection" of the peptide bond nitrogen results in cleavage of the protein backbone, a site-specific, nitrobenzyl-induced, photochemical proteolysis (SNIPP). Other strategies for photochemical cleavage of protein and peptide backbones have recently appeared. Imperiali and Kron have both employed *o*-nitro-β-phenylalanine as a linker between two protein or peptide fragments,[4,5] and Muir has employed an expanded nitrobenzyl linker.[6] Otaka and co-workers introduced a novel system based on a nitrobenzyl-caged phenol and the "trimethyl lock" motif, which promoted intramolecular cleavage of the backbone amide after the phenol was decaged.[7] Schultz and co-workers demonstrated a novel cleavage mediated by 2-nitrophenylalanine.[8]

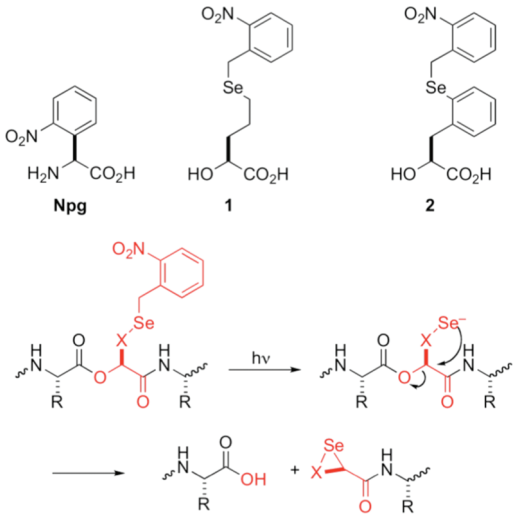


FIGURE 1. Npg and the second-generation SNIPP unnatural α-hydroxy acids, **1** and **2**.

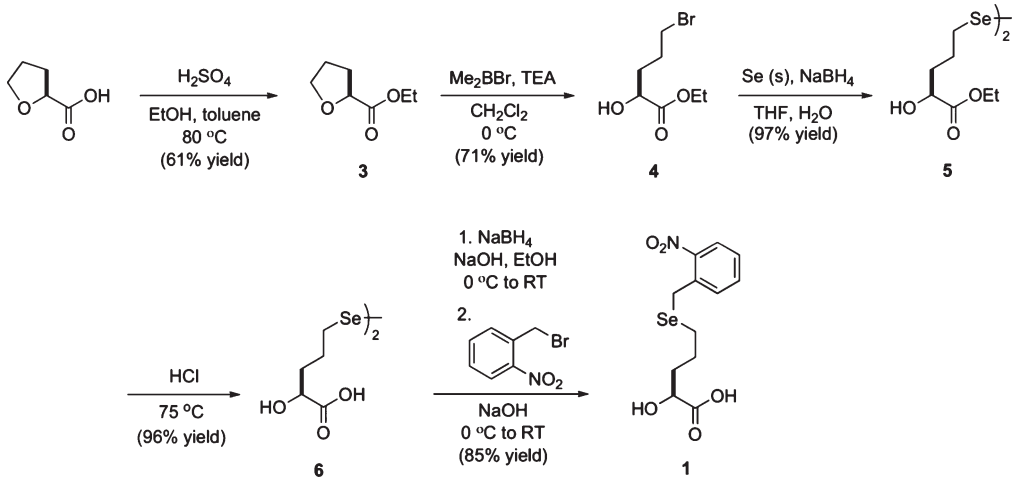
All of these strategies have advantages and disadvantages. The Npg approach has been employed in several contexts[3,9,10] and is compatible with both solid-phase peptide synthesis (SPPS) and *in vivo* incorporation into full proteins via nonsense suppression. However, the photochemical efficiency of the cleavage is not high, with a ~50% cleavage yield after 4 h of photolysis.[3] Also, the incorporation into proteins of the relatively crowded, β-branched residue is often not efficient. Similarly low photoefficiency is seen with 2-nitrophenylalanine.[8] The other strategies can show more efficient cleavage of peptides but are not compatible with *in vivo* incorporation into proteins.

We envisioned an alternative strategy in which the key cleavage reaction is based on a novel, intramolecular S_N2 reaction, shown schematically in Figure 1. Selenide is one of the most potent nucleophiles known, and at physiological pH a selenol (pK_a ~5−6) should be predominantly in the selenide form. The essential reaction, S_N2 cleavage of an ester carbon with a carboxylate as the leaving group, has ample precedent.[11−15] Formation of the ester leaving group

(1) Mayer, G.; Heckel, A. *Angew. Chem., Int. Ed.* **2006**, *45*, 4900–4921.
(2) Young, D. D.; Deiters, A. *Org. Biomol. Chemi.* **2007**, *5*, 999–1005.
(3) England, P. M.; Lester, H. A.; Davidson, N.; Dougherty, D. A. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 11025–11030.
(4) Bosques, C. J.; Imperiali, B. *J. Am. Chem. Soc.* **2003**, *125*, 7530–7531.
(5) Parker, L. L.; Kurutz, J. W.; Kent, S. B.; Kron, S. J. *Angew. Chem., Int. Ed. Engl.* **2006**, *45*, 6322–6325.
(6) Pellois, J. P.; Muir, T. W. *Angew. Chem., Int. Ed.* **2005**, *44*, 5713–5717.
(7) Shigenaga, A.; Tsuji, D.; Nishioka, N.; Tsuda, S.; Itoh, K.; Otaka, A. *ChemBioChem* **2007**, *8*, 1929–1931.
(8) Peters, F. B.; Brock, A.; Wang, J. Y.; Schultz, P. G. *Chem. Biol.* **2009**, *16*, 148–152.
(9) Endo, M.; Nakayama, K.; Kaida, Y.; Majima, T. *Angew. Chem., Int. Ed.* **2004**, *43*, 5643–5645.
(10) England, P. M.; Zhang, Y.; Dougherty, D. A.; Lester, H. A. *Cell* **1999**, *96*, 89–98.
(11) Scarborough, R. M.; Smith, A. B. *Tetrahedron Lett.* **1977**, 4361–4364.
(12) Liotta, D.; Markiewicz, W.; Santiesteban, H. *Tetrahedron Lett.* **1977**, *18*, 4365–4367.
(13) Liotta, D.; Santiesteban, H. *Tetrahedron Lett.* **1977**, *18*, 4369–4372.
(14) Liotta, D.; Sunay, U.; Santiesteban, H.; Markiewicz, W. *J. Org. Chem.* **1981**, *46*, 2605–2610.
(15) Nazari, M.; Movassagh, B. *Tetrahedron Lett.* **2009**, *50*, 438–441.

DOI: 10.1021/jo901368g Published on Web 11/10/2009

SCHEME 1. Synthesis of Selenide α-Hydroxy Acid 1



requires incorporation of an α-hydroxy acid (rather than an α-amino acid), but such backbone esters can be efficiently incorporated into peptides by SPPS[16,17] and into proteins by nonsense suppression.[10,18−22] In addition, selenium-containing natural amino acids such as selenocysteine and selenomethionine are well-known to be efficiently incorporated into proteins. Finally, "caging" the selenide with an *o*-nitrobenzyl group allows the process to be initiated photochemically.

The process proposed in Figure 1, however, raises many questions. Like all S_N2 reactions, the selenide-induced ester displacement is sensitive to steric effects. As such, the reaction is typically applied to methyl esters, although under optimal conditions and with heating, ethyl, benzyl, and even isopropyl esters along with many lactones are reactive. Certainly, the α-carbon of the α-hydroxy acid that is incorporated will be sterically crowded. The question is whether the intramolecularity of the process, perhaps aided by the inductive effect of the neighboring amide carbonyl, will overcome the steric burden. Caged selenides are not common, and so there is the question of the efficiency of the photochemical step. In addition, selenides are sensitive to oxidation, more so for aliphatic than aromatic (selenophenol) derivatives. On the other hand, an aliphatic selenide might be expected to be the stronger nucleophile, but an aryl selenide has fewer rotatable bonds that need to be restricted in the cyclization reaction.

Given these chemical uncertainties, it seemed prudent to first evaluate the viability of the chemistry proposed in Figure 1 before proceeding with chemical biology studies. In the present work we evaluate two structures that are meant to provide such a test. We describe the synthesis and characterization of aliphatic (**1**) and aromatic (**2**) variants of the design, along with mechanistic characterization.

Synthesis of enantiopure **1** (Scheme 1) began with conversion of *S*-(−)-tetrahydro-2-furoic acid to the ring-opened bromide **4** as previously described.[23,24] Conversion to the diselenide and ester hydrolysis then produced **6**.[25,26] Acid diselenide **6** was directly reduced with sodium borohydride, and the product was alkylated with *o*-nitrobenzyl bromide to give the target compound **1**.[27]

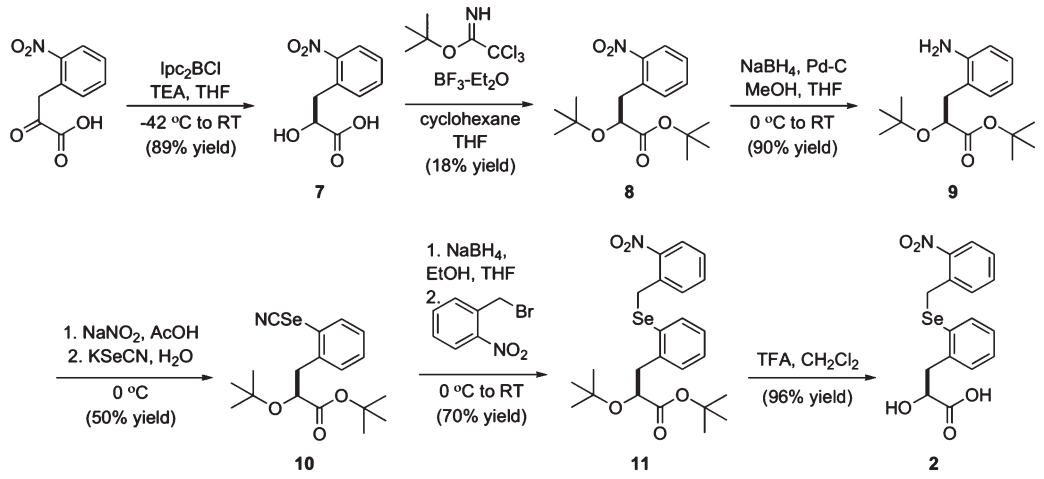
The synthesis of **2** (Scheme 2) began with the known reduction of 2-nitrophenylpyruvic acid by (+)-*B*-chlorodiisopinocampheylborane (Ipc_2BCl) to yield **7** in 94% ee.[28] The selenocyanate was prepared by a modification of the standard sequence, and the nitrobenzyl group was introduced by reductive alkylation. The bulky *tert*-butyl protecting groups were installed to discourage intramolecular cyclization, which was seen when **7** was subjected to reducing conditions, as well as to improve the solubility and ease of purification of subsequent compounds in the sequence.

To evaluate whether the proposed cleavage mechanism was viable, studies in model systems were performed. Depsipeptides **12** and **14** were chosen for synthetic accessibility and because they introduce a UV chromophore into the carboxylate cleavage product. They were prepared through standard solution-phase coupling procedures, employing PyBop/*N*-methylmorpholine and DCC/DMAP for the hydroxypeptide- and depsipeptide-forming reactions, respectively.

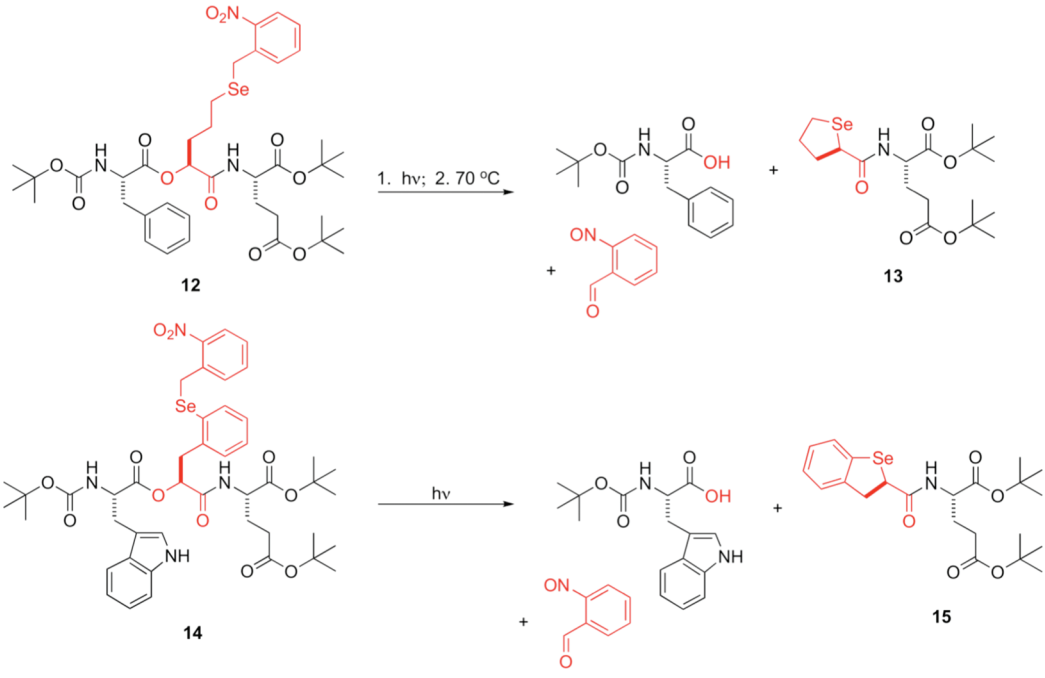
Mass spectrometric analysis of initial photolysis studies indicated the formation of the mechanistically revealing selenacyclopentane (**13**, **15**) and the appropriate carboxylic acid but also suggested several complicating side reactions. Both **12** and **14** produced product *m*/*z* ratios consistent with the dimer of the deprotected selenide, including the characteristic isotope pattern for a structure with two selenium atoms. In addition, the aliphatic variant **12** showed *m*/*z* ratios consistent with a depsipeptide containing

(16) Blankenship, J. W.; Balambika, R.; Dawson, P. E. *Biochemistry* **2002**, *41*, 15676–15684.
(17) Deechongkit, S.; Nguyen, H.; Powers, E. T.; Dawson, P. E.; Gruebele, M.; Kelly, J. W. *Nature* **2004**, *430*, 101–105.
(18) Bain, J.; Wacker, D. A.; Kuo, E. E.; Chamberlain, R. *Tetrahedron* **1991**, *47*, 2389–2400.
(19) Ellman, J. A.; Mendel, D.; Schultz, P. G. *Science* **1992**, *255*, 197–200.
(20) Fahnestock, S.; Rich, A. *Science* **1971**, *173*, 340–343.
(21) Koh, J. T.; Cornish, V. W.; Schultz, P. G. *Biochemistry* **1997**, *36*, 11314–11322.
(22) England, P. M.; Lester, H. A.; Dougherty, D. A. *Biochemistry* **1999**, *38*, 14409–14415.
(23) Fujima, Y.; Hirayama, Y.; Ikunaka, M.; Nishimoto, Y. *Tetrahedron: Asymmetry* **2003**, *14*, 1385–1391.
(24) Guindon, Y.; Therien, M.; Girard, Y.; Yoakim, C. *J. Org. Chem.* **1987**, *52*, 1680–1686.
(25) Klayman, D. L.; Griffin, T. S. *J. Am. Chem. Soc.* **1973**, *95*, 197–200.
(26) Stocking, E. M.; Schwarz, J. W.; Senn, H.; Salzmann, M.; Silks, L. A. *J. Chem. Soc., Perkin Trans. 1* **1997**, 2443–2447.
(27) Andreadou, I.; Menge, W. M.; Commandeur, J. N.; Worthington, E. A.; Vermeulen, N. P. *J. Med. Chem.* **1996**, *39*, 2040–2046.
(28) Wang, Z.; La, B.; Fortunak, J. M.; Meng, X. J.; Kabalka, G. W. *Tetrahedron Lett.* **1998**, *39*, 5501–5504.

SCHEME 2. Synthesis of Selenide α-Hydroxy Acid 2



SCHEME 3. Depsipeptide Cleavage Reactions[a]



[a]Conditions are described in Supporting Information.

dehydronorvaline (i.e., an allyl side chain), presumably formed by elimination of the selenoxide produced by air oxidation. These undesirable reactions could be suppressed by running the photolyses in the presence of excess dithiothreitol (DTT),[29] which was expected to discourage both dimerization and oxidation.

For aryl selenide **14** an unanticipated side product, identified by mass spectrometry, was the depsipeptide in which the original nitrobenzylselenyl group had been replaced by a hydrogen atom. While this work was in progress, Kitahara and co-workers reported that short-wavelength photolysis of arylalkyl selenides can lead to C−Se bond cleavage.[30] As suggested by that study, we find that changing the photolysis wavelength from >300 nm (pyrex filter) to >330 nm (uranium glass filter) eliminated this side reaction.

Of course, the novel process of Scheme 1 cannot be considered validated without unambiguous confirmation that the selenacyclopentanes **13** and **15** were formed. Using the knowledge gained from the preliminary studies, preparative-scale photolyses were performed (Scheme 3). For both depsipeptides the selenacyclopentane product could be isolated after irradiation, providing clear support for the proposed scheme; mass spectrometry and NMR spectroscopy confirm product identities. We find that cleavage is more efficient with the aryl selenide (**14**) than the alkyl (**12**), in that isolated yields of the selenacyclopentane are consistently higher (72% vs 29%). In addition, obtaining significant yields of the selenacyclopentane from the aliphatic system

(29) Besse, D.; Siedler, F.; Diercks, T.; Kessler, H.; Moroder, L. *Angew. Chem., Int. Ed. Engl.* **1997**, *36*, 883–885.
(30) Ouchi, A.; Liu, S.; Li, Z.; Kumar, S. A.; Suzuki, T.; Hyugano, T.; Kitahara, H. *J. Org. Chem.* **2007**, *72*, 8700–8706.

(**12**) required heating the photolysis mixture to ~70 °C, which was not necessary in the case of aryl selenide **14** under optimal photolysis conditions. These observations, coupled with the lack of complications due to olefin formation, suggest that aryl selenide **2** may be the better system to incorporate for subsequent protein and peptide studies.

In summary, we describe novel chemistry in which a photochemically liberated selenide undergoes an intramolecular S_N2 reaction, cleaving an ester that results from the incorporation of the α-hydroxy acids **1** or **2** into a peptide. These studies suggest that for chemical biology applications, aryl selenide **2** is the preferred substrate. Two side reactions have been discovered that must be considered in possible chemical biology applications: dimerization and oxidation. For studies involving peptides prepared by SPPS, adding DTT and controlling the concentration might be appropriate. For *in vivo* nonsense suppression expression experiments, where protein concentrations are typically low, dimerization may be less likely. In addition, the reducing conditions inside cells likely will discourage dimerization and oxidation. Future studies will evaluate the use of **1** and **2** in full proteins expressed in living cells.

Experimental Section

Representative Procedure for Selenacyclopentane Formation (13 or 15). One equivalent of depsipeptide **12** (0.0722 g; 0.088 mmol) or **14** (0.0230 g; 0.0250 mmol) was placed in a pyrex reaction vessel and dissolved in acetonitrile (125 mL). To this was added dithiothreitol (100 or 10 equiv for **12** and **14**, respectively) and 125 mL of pH 8 water (with 20 mM phosphate buffer for **12**). The resulting solution was stirred under N_2 (g), and a 450 W medium-pressure mercury-vapor UV immersion lamp, filtered with a glass absorption sleeve and equipped with a water cooling jacket, was assembled and attached to the reaction vessel. The progress of the reaction was followed by ESI-MS. Following photolysis, the reaction of **12** was heated to 70 °C for 3 h. Following aqueous workup, the crude product was purified by flash column chromatography to afford the selenacyclopentane product. Yield of **13** was 29%. R_f = 0.28 (33% EtOAC in hexanes); $[\alpha]^{24}{}_D = -16.0°$ (*c* 1, $CHCl_3$); ^{1}H NMR of **13** (500 MHz, $CDCl_3$, 298 K) δ 7.08 (1H, d, J = 13.5 Hz), 4.44 (1H, dt, J = 13.5, 8 Hz), 4.08 (1H, m), 3.15 (1H, m), 2.97 (1H, m), 2.37 (1.5H, m), 2.26 (1.5H, m), 2.14 (4H, m), 1.92 (1H, m), 1.47 (9H, s), 1.44 (9H, s); ^{13}C NMR of **13** (125 MHz, $CDCl_3$, 298 K) 172.3, 172.1, 170.8, 82.3, 80.7, 52.8, 45.4, 37.4, 32.4, 31.5, 28.1, 28.0, 27.5, 27.1. HRMS (TOF) of **13** m/z calcd for $C_{18}H_{31}NO_5Se$ [M + H] 422.1446, found 422.1465. Yield of **15** was 72%. R_f = 0.59 (30% EtOAC in hexanes); $[\alpha]^{24}{}_D = -80.5°$ (*c* 1, $CHCl_3$); ^{1}H NMR of **15** (300 MHz, $CDCl_3$, 298 K) δ 7.30 (1H, m), 7.20 (1H, m), 7.12 (2H, m), 7.29 (2H, m), 6.92 (1H, d, J = 7.41 Hz), 4.50 (1H, dd, J = 8.2, 5.2 Hz), 4.43 (1H, dd, J = 7.8, 4.7 Hz), 2.25 (2H, m), 2.15 (1H, m), 1.91 (1H, m), 1.44 (9H, s), 1.41 (9H, s); ^{13}C NMR of **15** (75 MHz, $CDCl_3$, 298 K) δ 172.4, 171.4, 170.7, 141.7, 135.3, 128.0, 125.9, 125.7, 125.5, 82.6, 81.0, 53.1, 45.9, 41.7, 31.6, 29.9, 28.3, 28.1; HRMS of **15** (ESI) m/z calcd for $C_{22}H_{31}NO_5Se$ [M + H] 470.1446, found 470.1469.

Acknowledgment. We thank Rick Gerhart for the gift of the uranium glass filter, Mona Shahgholi for her assistance with the mass spectrometery, Michael Krout for his knowledge about the immersion lamp, and Nyssa Puskar for thoughtful discussions. This work was supported by NIH (NS 34407) and an NIH training grant for A.L.E. (NRSA 5-T32-GM07616).

Supporting Information Available: General experimental methods and copies of ^{1}H and ^{13}C NMR spectra for all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.