



9-7-2015

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Recommended Citation

Pardo, A., Hogenauer, T. J., Cai, Z., Vellucci, J. A., Castillo, E. M., Dirk, C. W., Franz, A. H., & Michael, K. (2015). Efficient photochemical synthesis of peptide- α -phenylthioesters. *ChemBioChem: A European Journal of Chemical Biology, Synthetic Biology and Bio-nanotechnology*, 16(13), 1884–1889. DOI: [10.1002/cbic.201500266](https://doi.org/10.1002/cbic.201500266)
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Efficient Photochemical Synthesis of Peptide- α -Phenylthioesters

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Low yields and substantial epimerization of peptide- α -thioesters often compromise the overall efficiency of native chemical ligation (NCL). Peptide arylthioesters are more reactive than peptide alkylthioesters in NCL, but are also more difficult to handle due to their propensity to hydrolyze, and are therefore often generated in situ. However, pre-prepared peptide arylthioesters are required for some NCL applications. Here we present a 7-nitroindoline-based photochemical method that generates protected peptide phenylthioesters under neutral reaction conditions via their activated esters from photoreactive peptide precursors in high isolated yields, and with low levels of epimerization. This method is fully compatible with Fmoc-strategy solid-phase peptide synthesis. Global deprotection with trifluoroacetic acid furnishes peptide phenylthioesters for NCL. Photoreactive peptide precursors can also be converted into their hydrazides in two steps by this method.

Native chemical ligation (NCL) has been extensively used for the preparation of large peptides,^[1] and is particularly attractive for the synthesis of homogeneous polypeptides and proteins with post-translational modifications.^[2] Typically, two unprotected peptide segments, one with a C-terminal thioester and another with an N-terminal cysteine, are condensed in aqueous buffer at near-neutral pH. Different NCL strategies for the sequential ligation of three or more peptide segments have also been developed.^[2a,f,h,i,3] For example, a peptide arylthioester can be selectively ligated with the N-terminal cysteine of a peptide alkylthioester, and the ligation product can undergo another ligation at the C terminus.^[3a] Furthermore, cysteine-free variants of NCL using cysteine mimetics have been established.^[2d,e,4]

The common denominator of all NCL variants is the requirement of a peptide- α -thioester, which can be synthesized and isolated, or generated in situ.^[3d,f,5] Peptide- α -alkyl and -arylthioesters can be prepared by solid-phase peptide synthesis (SPPS) with high chiral integrity by using the *tert*-butyloxycarbonyl

(Boc) strategy on a thioester resin,^[1a,6] but the repeated exposure to trifluoroacetic acid and exposure to hydrofluoric acid for cleavage from the resin, makes this method less suitable for peptide thioesters with acid-labile modifications. Therefore, several fluorenylmethoxycarbonyl (Fmoc) compatible methods have been developed and reviewed.^[7] Examples include the use of non-nucleophilic Fmoc-deblocking reagents that leave thioesters intact,^[8] cleavage of the peptide from the resin by thiolysis of a linker,^[2a,9] thiolysis of a cyclic sulfonamide with benzyl mercaptan followed by cleavage from the resin,^[10] hydrolysis of a thioimide linker,^[11] thiolysis of peptidyl ureas with 4-mercaptophenylacetic acid in solution,^[12] coupling of peptide acids with benzyl mercaptan or an aryl mercaptan by using coupling reagents,^[13] and a variety of N-to-S acyl transfer methods.^[7b] Unfortunately, substantial C-terminal epimerization (up to 30%) and low peptide thioester yields are quite common.^[7b,11,13a,14] New methods for the synthesis of peptide thioesters capable of overcoming these problems would reduce purification challenges and increase overall NCL efficiencies.

Here we describe a photochemical peptide phenylthioester synthesis that produces these compounds in high yield and with high levels of chiral integrity. Pre-prepared peptide arylthioesters are essential starting materials in kinetically controlled sequential NCL in the presence of peptide alkylthioesters.^[3a] However, for most NCL applications reported in the literature, the more easily accessible peptide alkylthioesters serve as starting materials. Although the NCL of alkylthioesters can be effectively accelerated with high concentrations of 4-(carboxymethyl)thiophenol, no thiol additive is required when peptide arylthioesters are employed directly.^[15]

Previously we reported the photochemical synthesis of tri- and tetrapeptide- α -ethylthioesters through the esterification of ethyl mercaptan by photoreactive *N*-peptidyl-7-nitroindoline precursors.^[16] The peptides were assembled according to a Fmoc-strategy SPPS by using a photoreactive nitroindoline linker^[16–17] to connect the peptide's C terminus and the indoline's ring nitrogen through an amide bond. Illumination of the photoreactive peptides in *N,N,N',N'*-tetramethylurea/ethyl mercaptan (5:1) in the presence of HOBt afforded peptide- α -thioesters, but their yields were lowered by the formation of significant amounts (~25%) of peptide acid by-products. In addition, the method was limited to ethyl thioesters; attempts to prepare peptide phenylthioesters failed completely. These shortcomings can be rationalized by a closer examination of the photochemistry.

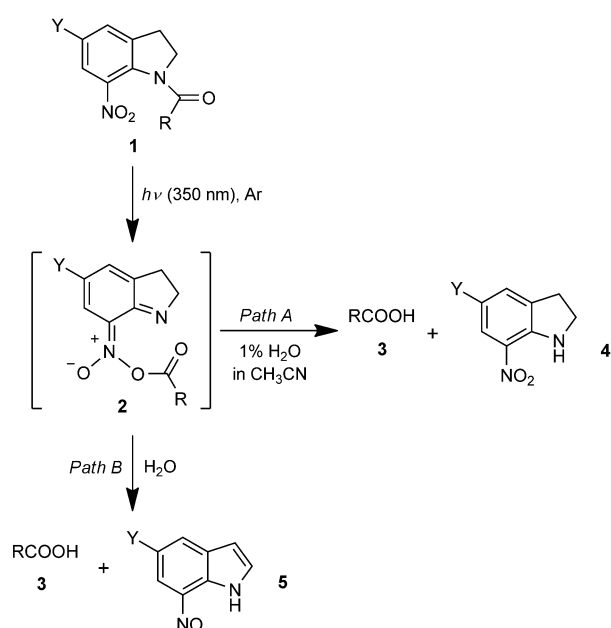
Several reports show that *N*-acyl-7-nitroindoline derivatives can be activated toward nucleophilic acylation by near UV

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Supporting information for this article is available on the WWW under <http://dx.doi.org/10.1002/cbic.201500266>.

light.^[18] Mechanistic studies of the photochemistry displayed by *N*-acyl-7-nitroindolines suggest that, upon excitation, the latent *N*-acyl-7-nitroindoline **1** rearranges into the nitronic anhydride **2** (Scheme 1).^[19,20] Corrie and co-workers discovered that the photochemical reaction path is solvent dependent, that is, in a 1% solution of water in acetonitrile, carboxylic acid **3** and nitroindoline **4** are produced (path A), but in 100% water, carboxylic acid **3** and nitrosoindole **5** are produced (path B).^[19a] Photolysis experiments on an *N*-acyl-7-nitroindoline in H₂¹⁸O have shown that the carboxylic acid produced does not contain ¹⁸O, thus suggesting that, in water, an oxygen from the nitro group becomes part of the carboxyl group of **3**.^[21] Thus, in path A the nitronic anhydride is hydrolyzed, but in path B a redox reaction takes place that is believed to be triggered by protonation of the nitronic anhydride **2** by the protic solvent.^[19d] This is consistent with our observation that

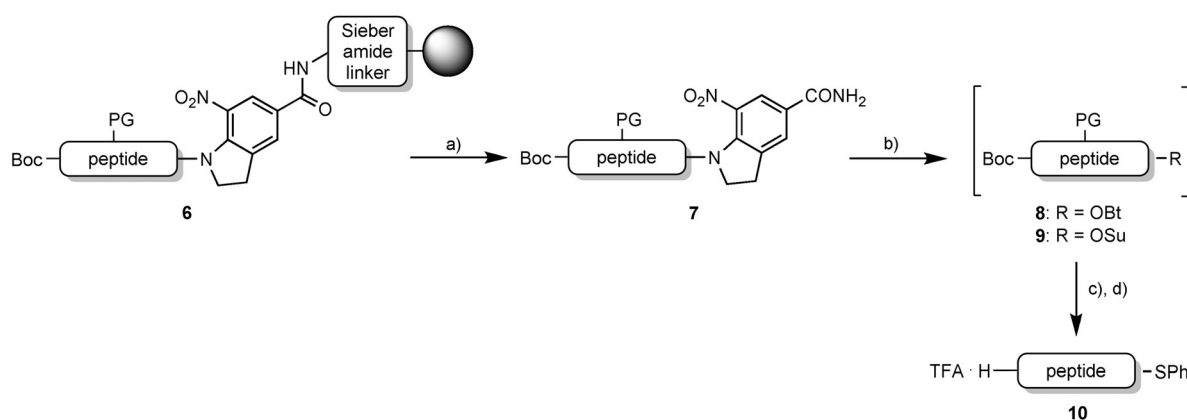


Scheme 1. Solvent-dependent photolysis of *N*-acyl-7-nitroindoline.^[19a] Y = CH₂COOCH₃.

attempts to photoacylate ethyl mercaptan with an *N*-peptidyl-7-nitroindoline in neat anhydrous ethyl mercaptan does not give the thioester, but produces the peptide acid quantitatively. Photoacylations in mixtures of ethyl mercaptan and dichloromethane under anhydrous conditions lead to mixtures of peptide acids and peptide thioesters; this suggests mixed mechanistic pathways.^[16,19a]

We hypothesized that performing the photolysis step in the presence of an auxiliary nucleophile in an aprotic solvent should afford the peptide-activated ester. Upon completion of the photoacylation, thiophenol could be added to the reaction mixture to form the peptide phenylthioester under mild conditions. As neither the formation of the peptide-activated ester nor the thioesterification would be performed under basic conditions, little or no C-terminal epimerization would be expected. Scheme 2 provides a general overview of the envisioned light-induced peptide phenylthioester synthesis. First, a peptide with a photoreactive nitroindoline linker (**6**) is constructed by Fmoc-based SPPS on Sieber amide resin.^[16,22] The fully protected photoreactive peptide **7** is cleaved off in a dilute solution of TFA, then purified by chromatography prior to the one-pot photochemical acylation and thioesterification. In an aprotic anhydrous solvent, such as THF or DMF, the photoreactive peptide **7** is illuminated at 350 nm in the presence of 1.3–3.0 equivalents of *N*-hydroxybenzotriazole (HOBt) or *N*-hydroxysuccinimide (HOSu) to give the activated ester **8** or **9**, respectively.^[23] Then thiophenol is added, and the resulting peptide phenylthioester is globally deprotected to give the unprotected peptide phenylthioester **10**. Because the photochemical reaction step is performed without a protic solvent, the formation of peptide acid (path B in Scheme 1) is avoided.^[24]

This concept was put to a test by synthesizing two model peptide phenylthioesters that are partial sequences of human erythropoietin (hEPO), the hexapeptide Boc-Ala¹-Pro-Pro-Arg(Pbf)-Leu-Ile⁶-SPh (**19**), and the hencosapeptide Boc-Lys(Boc)¹⁴⁰-Leu-Phe-Arg(Pbf)-Val-Tyr(tBu)-Ser(tBu)-Asn(Trt)-Phe-Leu-Arg(Pbf)-Gly-Lys(Boc)-Leu-Lys(Boc)-Leu-Tyr(tBu)-Thr(tBu)-Gly-Glu(OtBu)-Ala¹⁶⁰-SPh (**20**). They were produced from photoreactive peptide precursors, which were prepared by Fmoc/*t*Bu-based SPPS using a 7-nitroindoline linker attached to

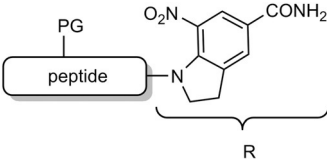
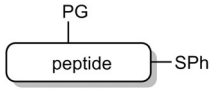


Scheme 2. Photochemical synthesis of peptide- α -phenylthioesters via a peptide-OBt or OSu ester intermediate (not isolated). a) 1% TFA in CH₂Cl₂; b) *h* ν (350 nm), argon, THF or DMF, HOBt or HOSu; c) PhSH; d) TFA/H₂O/TIS (95:2.5:2.5). TIS = triisopropylsilane.

Sieber Amide resin through an amide bond.^[16] The photochemical reaction can be carried out in the presence of HOBt or HOSu, and the activated ester intermediates are observable by mass spectrometry.^[25]

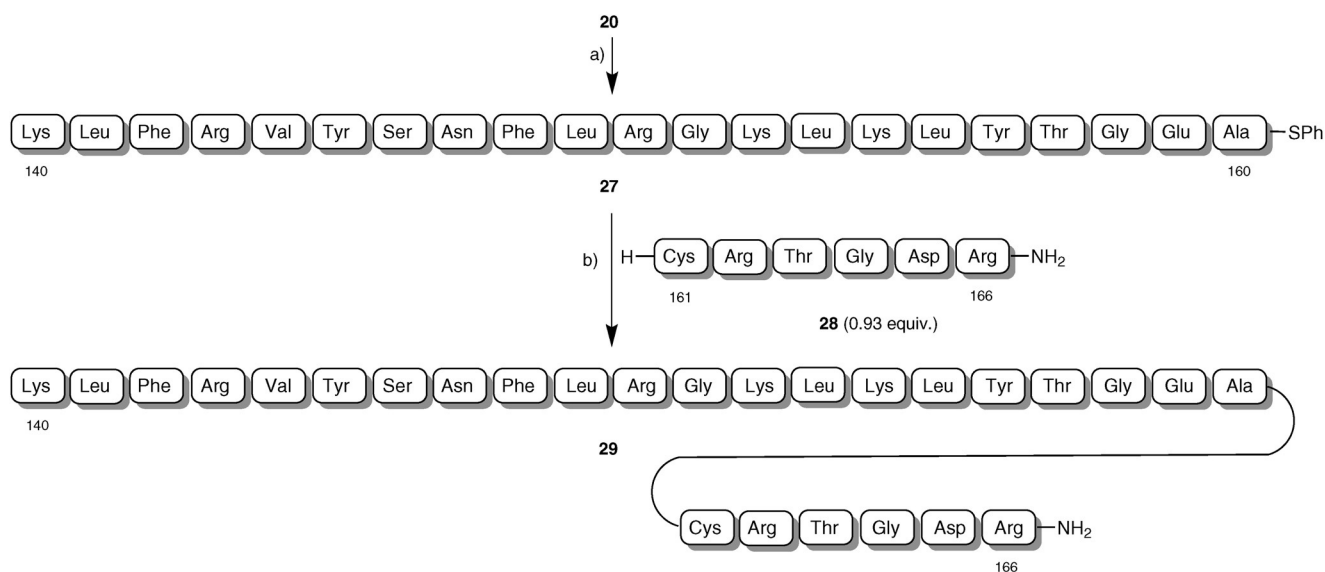
To study the utility of this method with respect to different C-terminal amino acids, short peptides with different C termini comprising polar, nonpolar, rigid, or sterically hindered amino acids were explored, and an epimerization study was conducted. The photoreactive Fmoc amino acid precursors were synthesized by acylation of 5-allylcarboxylate-7-nitroindoline,^[16] with Fmoc amino acid chlorides generated in situ,^[26] followed by Pd⁰-catalyzed deallylation and coupling to the solid support under standard conditions.^[16] All photoreactive peptides were synthesized by SPPS on Sieber amide resin (peptides **11** and **12**) or Rink amide resin (peptides **13**–**18**). The short photoreactive tri- and tetrapeptides **13**–**18** were equipped with an N-terminal Fmoc protecting group for the sole purpose of facilitating detection for reaction monitoring and chromatography. After completion of the SPPS by Fmoc/tBu strategy,^[22b] cleavage from the resin, and chromatography, the photoreactive peptides **11**–**18** were illuminated with near-UV light (350 nm) in the presence of HOBt or HOSu followed by removal of the light source and addition of ten equivalents of thiophenol. Table 1 summarizes the isolated yields of the photoreactive peptides **11**–**18** (36–90%), and the peptide- α -phenylthioester products **19**–**26** (69–89% over two steps). Typical minor by-products are the peptide acids.

Peptide phenylthioester **20** was globally deprotected with TFA to afford **27**, which was purified by reversed-phase FPLC and subjected to NCL with hexapeptide **28** to give the C-terminal hEPO peptide **29** (140–166) following Kent's NCL protocol,^[15] except that peptides **27** and **28** were used in nearly equimolar amounts (Scheme 3). The ligation was >90% completed within 45 min (Figure 1), and the yield of the FPLC-purified product **29** was 88%.

Photoreactive peptide	Yield [%]	Peptide- α -phenylthioester	Yield [%]
			
Boc-Ala-Pro-Pro-Arg(Pbf)-Leu-Ile-R (11)	80 ^[a]	Boc-Ala-Pro-Pro-Arg(Pbf)-Leu-Ile-SPh (19)	83 ^[a]
Boc-Lys(Boc)-Leu-Phe-Arg(Pbf)-Val-Tyr(tBu)-Ser(tBu)-Asn(Trt)-Phe-Leu-Arg(Pbf)-Gly-Lys(Boc)-Leu-Lys(Boc)-Leu-Tyr(tBu)-Thr(tBu)-Gly-Glu(OtBu)-Ala-R (12)	62 ^[a,c]	Boc-Lys(Boc)-Leu-Phe-Arg(Pbf)-Val-Tyr(tBu)-Ser(tBu)-Asn(Trt)-Phe-Leu-Arg(Pbf)-Gly-Lys(Boc)-Leu-Lys(Boc)-Leu-Tyr(tBu)-Thr(tBu)-Gly-Glu(OtBu)-Ala-SPh (20)	69 ^[a,d]
Fmoc-Phe-Ala-Pro-R (13)	90 ^[a]	Fmoc-Phe-Ala-Pro-SPh (21)	88 ^[a]
Fmoc-Lys(Tfa)-Leu-Phe-R (14)	82 ^[a]	Fmoc-Lys(Tfa)-Leu-Phe-SPh (22)	84 ^[a]
Fmoc-Glu(OBn)-Ala-Lys(Tfa)-R (15)	88 ^[a]	FFmoc-Glu(OBn)-Ala-Lys(Tfa)-SSPh (23)	82 ^[a]
Fmoc-Thr(Bn)-Ile-Thr(Bn)-R (16)	84 ^[a]	Fmoc-Thr(Bn)-Ile-Thr(Bn)-SPh (24)	89 ^[a]
Fmoc-Leu-Gly-Ile-R (17)	86 ^[a]	Fmoc-Leu-Gly-Ile-SPh (25)	79 ^[a]
Fmoc-Gly-Gln-Gln-Ala-R (18)	36 ^[b]	Fmoc-Gly-Gln-Gln-Ala-SPh (26)	85 ^[b]

[a] Purified by flash chromatography. [b] Reversed-phase FPLC. [c] Gel permeation chromatography. [d] HPLC.

The epimerization that can potentially occur during the photochemical acylation of HOBt and the subsequent thioesterification was studied by using dipeptides with C-terminal amides of 5-bromo-7-nitroindoline^[18b] as photoreactive model peptides



Scheme 3. NCL of peptide phenylthioester **27** (4.6 mM) with hexapeptide **28** (4.3 mM). a) TFA/TIS/H₂O (95:2.5:2.5); b) Gdm-HCl (6 M), phosphate (0.2 M), pH 7.1, TCEP (20 mM), RT.

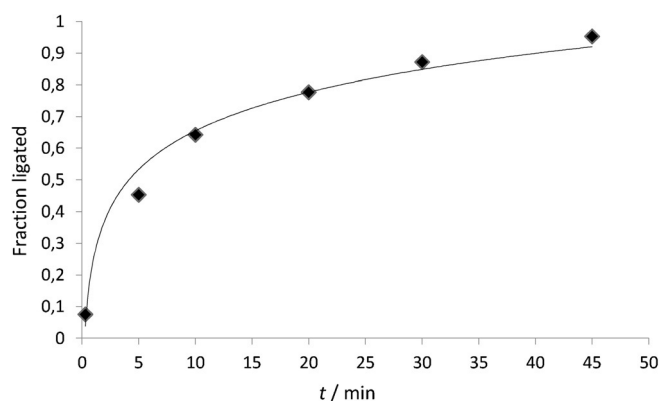
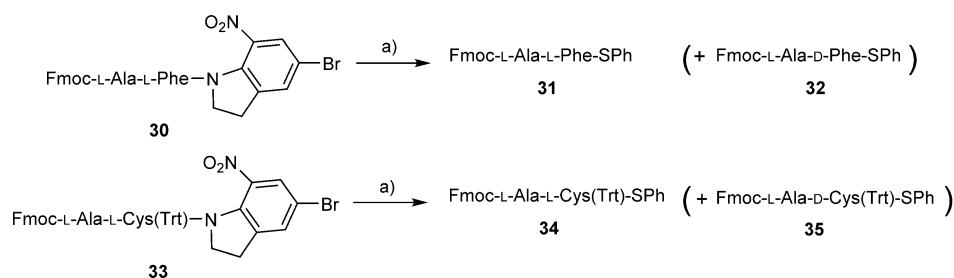


Figure 1. Progression of the ligation of peptide phenylthioester **27** and peptide **28** to the hEPO partial sequence **29**.

(Scheme 4). *N*-Dipeptidyl-5-bromo-7-nitroindolines can be readily prepared in solution by acylation of commercially available 5-bromo-7-nitroindoline with an Fmoc-amino acid chloride generated in situ,^[26] followed by removal of the Fmoc group and coupling to the N-terminal Fmoc-alanine with 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDCI) under standard conditions.^[27] The epimerization study was conducted on peptides with a C-terminal phenylalanine (**30**) and a C-terminal



Scheme 4. C-terminal epimerization study on model dipeptides. a) 1) $h\nu$ (350 nm), HOBt, argon, THF, molecular sieves 4 Å, 4 h; 2) PhSH, 12 h.

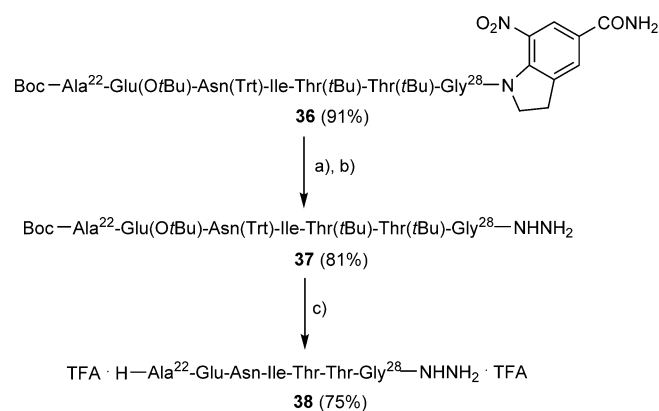
terminal cysteine (**33**). A higher level of epimerization was expected with **33** because of the well-known racemization propensity of cysteine.^[27]

The extent of epimerization was studied using ^1H NMR spectroscopy by integration of suitable H- α or - β signals of the diastereomeric peptide thioesters in the crude reaction mixtures.^[25] The conversion of photoreactive dipeptide **30** produces thioesters **31** and **32** in a ratio of 97:3.^[28] This level of epimerization is among the lowest reported for peptide thioesters synthesized by Fmoc strategy,^[12, 13b, 29] and is in agreement with that observed for a photochemically synthesized peptide ethylthioester with a C-terminal alanine from an *N*-peptidyl-5-carboxamide-7-nitroindoline precursor.^[16] The cysteine-containing photoreactive dipeptide **33** produces thioesters **34** and **35** in a ratio of 94:6. As expected a slightly larger amount of epimerized peptide thioester product is observed (6%). To the best of our knowledge, the epimerization of a peptide phenylthioester

with a C-terminal cysteine has not yet been reported. These epimerization studies also demonstrate that the activated ester intermediates, which exist in solution for several hours, also show high chiral integrity. Presumably, the comparably low levels of epimerized peptide thioester products can be attributed to the neutral reaction conditions during the photochemical acylation and thioesterification steps.

The scope of photochemical acylation with *N*-peptidyl-7-nitroindolines can be expanded to other peptide derivatives relevant for peptide ligations. Peptide hydrazides have been used as starting materials for the generation of peptide-activated esters that could be used for peptide segment condensation.^[30] Recently, the in situ synthesis of peptide thioesters from peptide hydrazides via peptide azides has gained popularity.^[3d] Treatment of photoreactive hEPO partial sequence **36**,^[31] synthesized in 91% yield by SPPS using our 7-nitroindoline linker, with *N*-hydroxysuccinimide and then hydrazine, produced peptide hydrazide **37** in 81% yield (Scheme 5). Deprotection under standard conditions afforded peptide hydrazide **38** in 75% yield.

In summary, the photochemistry of *N*-acyl-7-nitroindolines can be exploited for the efficient preparation of the most powerful peptide starting materials used in modern NCL, that is, peptide- α -phenylthioesters and peptide- α -hydrazides. The direct photochemical synthesis of peptide- α -phenylthioesters has failed in the past, but when *N*-peptidyl-7-nitroindolines are first converted into their activated esters by UV light then thioesterified, both under neutral conditions, protected peptide- α -phenylthioesters are obtained in high yield and with low levels of epimerization. The synthesis of the photoreactive peptides is fully compatible with Fmoc-strategy SPPS and is successful with a number of different C-terminal amino acids with different steric



Scheme 5. Photochemical synthesis of a peptide hydrazide. a) $h\nu$ (350 nm), DMF, argon, 1.5 h, HOSu (3 equiv); b) hydrazine (5 equiv.); c) TFA/ H_2O /TIS (95:2.5:2.5).

demands and polarities as well as for a 21-residue peptide. The photoreactive peptides can easily be stored in the dark between 4 °C and –50 °C for many months. Our combined results of high isolated reaction yields and low levels of epimerization demonstrate that the preparation and handling of peptide phenylthioesters does not have to be difficult. Although this method requires the installation of the thioester on fully protected peptides, it holds great potential for the preparation of sensitive glycopeptide thioesters that are particularly prone to acid-catalyzed hydrolysis. For those compounds the peptide hydrazide method might not be suitable because it requires conversion into an azide under acidic aqueous conditions prior to the thioesterification.

Experimental Section

Procedure for the conversion of the photoreactive peptide 12 into peptide- α -phenylthioester 20: Peptide 12 was prepared from *N*-(Fmoc-Ala)-5-carboxylic acid-7-nitroindoline^[16] by Fmoc/tBu SPPS on Sieber amide resin^[22a] and purified by gel-permeation chromatography on Sephadex LH20 in DMF followed by silica gel flash chromatography with a gradient of 1–10% MeOH in CH₂Cl₂; $R_f = 0.40$ (MeOH/CH₂Cl₂ 1:9). Compound 12 (17 mg, 4.04 μ mol) and HOSu (1.3 mg, 11 μ mol) were dissolved in anhydrous DMF (0.2 mL) in a test tube with molecular sieves (4 Å) under argon. The test tube was sealed with a rubber septum and illuminated with UV light (350 nm) at 25 °C for 2 h in a Rayonet photoreactor. The reaction was monitored by ESI-TOF-MS until peptide 12 had been consumed. The reaction container was removed from the photoreactor, and PhSH (4 μ L, 40.4 μ mol) was added to the mixture. After 12 h of stirring, the mixture was concentrated under reduced pressure and dried in vacuum; this gave 18 mg of a colored remainder. The crude (13 mg) was purified by silica gel flash chromatography using 10% MeOH in CHCl₃ followed by isocratic HPLC on a silica column with 7% MeOH in CHCl₃; this afforded 9 mg of peptide phenylthioester 20 (69%); $R_f = 0.34$ (MeOH/CH₂Cl₂ 1:9); ESI-TOF HR MS: *m/z* calcd: 2012.6119 [M+2H]²⁺; obs.: 2012.6266.

Acknowledgement

The authors thank Professor Luis Echegoyen and Maira Cerón for the HPLC analysis. This work was supported by the following National Science Foundation grants: CHE-0719538 and CHE-0840525 (K.M.); A University of Texas System Louis Stokes Alliance for Minority Participation–Bridge to the Doctorate scholarship, grant HRD-0832951 (A.P.); and Student Mentoring to Achieve Retention: Triads in Science scholarships, grant DUE-1153832 (A.P. and E.M.C.).

Keywords: acylation · hydrazides · peptides · photochemistry · thioesters

- [1] a) P. E. Dawson, T. M. Muir, I. Clark-Lewis, S. B. H. Kent, *Science* **1994**, *266*, 776–779; b) P. E. Dawson, S. B. H. Kent, *Annu. Rev. Biochem.* **2000**, *69*, 923–960.
[2] a) Y. Shin, K. A. Winans, B. J. Backes, S. B. H. Kent, J. A. Ellman, C. R. Bertozzi, *J. Am. Chem. Soc.* **1999**, *121*, 11684–11689; b) S. Mezzato, M. Schaffrath, C. Unverzagt, *Angew. Chem. Int. Ed.* **2005**, *44*, 1650–1654; *Angew. Chem.* **2005**, *117*, 1677–1681; c) Y. Kajihara, N. Yamamoto, R. Okamoto, K. Hirano, T. Murase, *Chem. Rec.* **2010**, *10*, 80–100; d) R. J.

- Payne, C.-H. Wong, *Chem. Commun.* **2010**, *46*, 21–43; e) C. P. R. Hackenberger, D. Schwarzer, *Angew. Chem. Int. Ed.* **2008**, *47*, 10030–10074; *Angew. Chem.* **2008**, *120*, 10182–10228; f) C. Unverzagt, Y. Kajihara, *Chem. Soc. Rev.* **2013**, *42*, 4408–4420; g) P. Wang, S. Dong, J. Brailsford, K. Iyer, S. D. Townsend, Q. Zhang, T. C. Hendrickson, J. Shieh, M. A. S. Moore, S. J. Danishefsky, *Angew. Chem. Int. Ed.* **2012**, *51*, 11576–11584; *Angew. Chem.* **2012**, *124*, 11744–11752; h) P. Wang, S. Dong, J.-H. Shieh, E. Peguero, R. Hendrickson, M. A. S. Moore, S. J. Danishefsky, *Science* **2013**, *342*, 1357–1360; i) M. Murakami, R. Okamoto, M. Izumi, Y. Kajihara, *Angew. Chem. Int. Ed.* **2012**, *51*, 3567–3572; *Angew. Chem.* **2012**, *124*, 3627–3632.
[3] a) D. Bang, B. L. Pentelute, S. B. H. Kent, *Angew. Chem. Int. Ed.* **2006**, *45*, 3985–3988; *Angew. Chem.* **2006**, *118*, 4089–4092; b) D. Bang, S. B. H. Kent, *Angew. Chem. Int. Ed.* **2004**, *43*, 2534–2538; *Angew. Chem.* **2004**, *116*, 2588–2592; c) F.-K. Deng, Y.-T. Wang, O. Schneewind, S. B. H. Kent, *Angew. Chem. Int. Ed.* **2014**, *53*, 4662–4666; *Angew. Chem.* **2014**, *126*, 4750–4754; d) J.-S. Zheng, S. Tang, Y.-C. Huang, L. Liu, *Acc. Chem. Res.* **2013**, *46*, 2475–2484; e) N. Ollivier, J. Vicogne, A. Vallin, H. Drobecq, R. Desmet, O. E. Mahdi, B. Leclercq, G. Goormachtigh, V. Fafeur, O. Melnyk, *Angew. Chem. Int. Ed.* **2012**, *51*, 209–213; *Angew. Chem.* **2012**, *124*, 213–217; f) G.-M. Fang, Y.-M. Li, F. Shen, Y.-C. Huang, J.-B. Li, Y. Lin, H.-C. Cui, L. Liu, *Angew. Chem. Int. Ed.* **2011**, *50*, 7645–7649; *Angew. Chem.* **2011**, *123*, 7787–7791.
[4] a) B. Wu, J. Chen, J. D. Warren, G. Chen, Z. Hua, S. J. Danishefsky, *Angew. Chem. Int. Ed.* **2006**, *45*, 4116–4125; *Angew. Chem.* **2006**, *118*, 4222–4231; b) J. Offer, *Biopolymers* **2010**, *94*, 530–541; c) R. Okamoto, S. Souma, Y. Kajihara, *J. Org. Chem.* **2009**, *74*, 2494–2501; d) C. Kan, J. D. Trzupke, B. Wu, G. Chen, Z. Tan, Y. Yuan, S. J. Danishefsky, *J. Am. Chem. Soc.* **2009**, *131*, 5438–5443; e) C. Haase, H. Rohde, O. Seitz, *Angew. Chem. Int. Ed.* **2008**, *47*, 6807–6810; *Angew. Chem.* **2008**, *120*, 6912–6915; f) J. Chen, Q. Wan, Y. Yuan, J. Zhu, S. J. Danishefsky, *Angew. Chem. Int. Ed.* **2008**, *47*, 8521–8524; *Angew. Chem.* **2008**, *120*, 8649–8652; g) Q. Wan, S. J. Danishefsky, *Angew. Chem. Int. Ed.* **2007**, *46*, 9248–9252; *Angew. Chem.* **2007**, *119*, 9408–9412; h) L. Z. Yan, P. E. Dawson, *J. Am. Chem. Soc.* **2001**, *123*, 526–533; i) D. Crich, A. Banerjee, *J. Am. Chem. Soc.* **2007**, *129*, 10064–10065; j) X. Guan, M. Drake, Z. Tan, *Org. Lett.* **2013**, *15*, 6128–6131; k) K. M. Cergol, R. E. Thompson, L. R. Malins, P. Turner, R. J. Payne, *Org. Lett.* **2014**, *16*, 290–293.
[5] a) P. Botti, M. Villain, S. Manganiello, H. Gaertner, *Org. Lett.* **2004**, *6*, 4861–4864; b) J. D. Warren, J. S. Miller, S. J. Keding, S. J. Danishefsky, *J. Am. Chem. Soc.* **2004**, *126*, 6576–6578; c) W. Hou, X. Zhang, F. Li, C.-F. Liu, *Org. Lett.* **2011**, *13*, 386–389; d) R. Okamoto, K. Morooka, Y. Kajihara, *Angew. Chem. Int. Ed.* **2012**, *51*, 191–196; *Angew. Chem.* **2012**, *124*, 195–200.
[6] a) D. Bang, B. L. Pentelute, Z. P. Gates, S. B. Kent, *Org. Lett.* **2006**, *8*, 1049–1052; b) N. Yamamoto, Y. Tanabe, R. Okamoto, P. E. Dawson, Y. Kajihara, *J. Am. Chem. Soc.* **2008**, *130*, 501–510; c) S. Aimoto, *Biopolymers* **1999**, *51*, 247–265.
[7] a) F. Mende, O. Seitz, *Angew. Chem. Int. Ed.* **2011**, *50*, 1232–1240; *Angew. Chem.* **2011**, *123*, 1266–1274; b) J. Kang, D. Macmillan, *Org. Biomol. Chem.* **2010**, *8*, 1993–2002.
[8] X. Li, T. Kawakami, S. Aimoto, *Tetrahedron Lett.* **1998**, *39*, 8669–8672.
[9] a) A. Sewing, D. Hilvert, *Angew. Chem. Int. Ed.* **2001**, *40*, 3395–3396; *Angew. Chem.* **2001**, *113*, 3503–3505; b) R. Ingenito, E. Bianchi, D. Fattori, A. Pessi, *J. Am. Chem. Soc.* **1999**, *121*, 11369–11374.
[10] F. Mende, M. Beisswenger, O. Seitz, *J. Am. Chem. Soc.* **2010**, *132*, 11110–11118.
[11] I. Sharma, D. Crich, *J. Org. Chem.* **2011**, *76*, 6518–6524.
[12] J. B. Blanco-Canosa, P. E. Dawson, *Angew. Chem. Int. Ed.* **2008**, *47*, 6851–6855; *Angew. Chem.* **2008**, *120*, 6957–6961.
[13] a) A. R. Mezo, R. P. Cheng, B. Imperiali, *J. Am. Chem. Soc.* **2001**, *123*, 3885–3891; b) A. C. Nagalingam, S. E. Radford, S. L. Warriner, *Synlett* **2007**, 2517–2520; c) I. Sakamoto, K. Tezuka, K. Fukae, K. Oshii, K. Taduru, M. Maeda, M. Ouchi, K. Yoshida, Y. Nambu, J. Igarashi, N. Hayaishi, T. Tiuji, Y. Kajihara, *J. Am. Chem. Soc.* **2012**, *134*, 5428–5431; d) Y. Kajihara, A. Yoshihara, K. Hirano, N. Yamamoto, *Carbohydr. Res.* **2006**, *341*, 1333–1340; e) T. Leta Aboye, R. J. Clark, D. J. Craik, U. Göransson, *ChemBioChem* **2008**, *9*, 103–113.
[14] S. E. O'Connor, J. Pohlmann, B. Imperiali, I. Saskiawan, K. Yamamoto, *J. Am. Chem. Soc.* **2001**, *123*, 6187–6188.
[15] E. C. B. Johnson, S. B. H. Kent, *J. Am. Chem. Soc.* **2006**, *128*, 6640–6646.

- [16] T. J. Hogenauer, Q. Wang, A. K. Sanki, A. J. Gammon, C. H. Chu, C. M. Kaneshiro, Y. Kajihara, K. Michael, *Org. Biomol. Chem.* **2007**, *5*, 759–762.
- [17] K. C. Nicolaou, B. S. Safina, N. Winssinger, *Synlett* **2001**, 900–903.
- [18] a) B. Amit, D. A. Ben-Efraim, A. Patchornik, *J. Am. Chem. Soc.* **1976**, *98*, 843–844; b) S. Pass, B. Amit, A. Patchornik, *J. Am. Chem. Soc.* **1981**, *103*, 7674–7675; c) J.-L. Débieux, C. G. Bochet, *J. Phys. Org. Chem.* **2010**, *23*, 272–282; d) C. Helgen, C. G. Bochet, *Synlett* **2001**, 1968–1970; e) J.-L. Débieux, C. G. Bochet, *J. Org. Chem.* **2009**, *74*, 4519–4524.
- [19] a) J. Morrison, P. Wan, J. E. T. Corrie, G. Papageorgiou, *Photochem. Photobiol. Sci.* **2002**, *1*, 960–969; b) G. Papageorgiou, D. Ogden, G. Kelly, J. E. T. Corrie, *Photochem. Photobiol. Sci.* **2005**, *4*, 887–896; c) A. D. Cohen, C. Helgen, C. G. Bochet, J. P. Toscano, *Org. Lett.* **2005**, *7*, 2845–2848; d) J. E. Mendez, N. J. Westfall, K. Michael, C. W. Dirk, *Trends Photochem. Photobiol.* **2012**, *14*, 75–91.
- [20] The proposed nitronic anhydride **2** seems to decompose quickly as it could not be isolated or characterized by NMR spectroscopically, even when *N*-acyl-7-nitroindoline **1** was irradiated in the absence of a nucleophile.
- [21] G. Papageorgiou, D. C. Ogden, A. Barth, E. E. T. Corrie, *J. Am. Chem. Soc.* **1999**, *121*, 6503–6504.
- [22] a) P. Sieber, *Tetrahedron Lett.* **1987**, *28*, 2107–2110; b) W. C. Chan, P. D. White, *Fmoc Solid Phase Peptide Synthesis: A Practical Approach*, Oxford University Press, Oxford, **2000**.
- [23] We have found photoacylations to be successful in THF, CH₂Cl₂, CH₃CN, DMF, DMSO, *N*-methylpyrrolidone (NMP), and *N,N,N',N'*-tetramethylurea.
- [24] At 2.7 equivalents HOSu in 0.2 mL DMF (55 mM, 0.7%; see the Experimental Section), HOSu behaves like a protic solvent at very low concentration; this results in its photoacylation (analogous to path A in Scheme 1) rather than protonation of the nitronic anhydride (analogous to path B).
- [25] See the Supporting Information.
- [26] The Fmoc amino acid chlorides were synthesized in situ from Fmoc amino acids with thionyl chloride, see ref. [18b], or by Appel reaction, see W. Pluemanupat, O. Chantarasriwong, P. Taboonpong, D. O. Jang, W. Chavasiri, *Tetrahedron Lett.* **2007**, *48*, 223–226.
- [27] M. Bodanszky, *Principles of Peptide Synthesis*, 2nd ed., Springer, Berlin, **1993**.
- [28] This ratio remains constant after seven days at room temperature in [D₆]DMSO.
- [29] P. Wang, L. P. Miranda, *Int. J. Pept. Protein Res. Therapeutics* **2005**, *11*, 117–123.
- [30] P. Wang, R. Layfield, M. Landon, R. J. Mayer, R. Ramage, *Tetrahedron Lett.* **1998**, *39*, 8711–8714.
- [31] Even after **36** had been stored in the dark at –50 °C for ten months, its FPLC profile hardly changed; very little of the photoreactive heptapeptide had decomposed. See the Supporting Information.

Manuscript received: May 25, 2015

Final article published: July 17, 2015