ORGANIC LETTERS

2001
Vol. 3, No. 20
3181−3184

Improved Synthesis of C-Terminal Peptide Thioesters on "Safety-Catch" Resins Using LiBr/THF

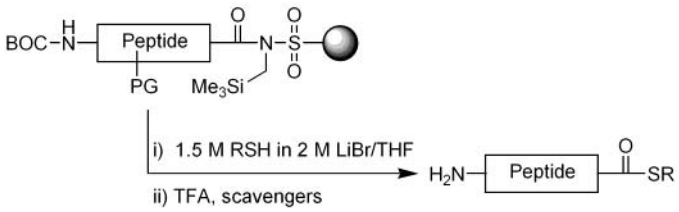
Richard Quaderer and Donald Hilvert*

Laboratory of Organic Chemistry, Swiss Federal Institute of Technology (ETH), Universitätstrasse 16, CH-8092 Zürich, Switzerland

hilvert@org.chem.ethz.ch

Received July 25, 2001

ABSTRACT



The alkanesulfonamide "safety-catch" resin has proven useful for Fmoc-based synthesis of C-terminal peptide thioesters. We now report that the yield of isolated thioester can increase significantly when the cleavage reaction is carried out in 2 M LiBr/THF rather than DMF or THF. The largest effects are seen with problematic peptides that aggregate or form secondary structures on the resin.

The broad utility of C-terminal peptide thioesters in protein chemistry[1] has stimulated a search for improved methods of synthesis. Until recently, these derivatives were not accessible via standard 9-fluorenylmethoxycarbonyl (Fmoc)-based solid-phase peptide synthesis (SPPS)[2] because resin-bound thioesters are unstable to repeated exposure to piperidine. They are also subject to epimerization under basic conditions.

Several strategies to address these problems have been reported. For example, thioester-compatible Fmoc cleavage cocktails have been developed and successfully applied.[3] Thioesters can also be obtained directly by cleaving protected peptides, prepared in standard ways, from commercially available Wang or PAM resins with an excess of Me_2AlCl or Me_3Al in the presence of thiol.[4]

Special linkers have also been implemented for SPPS of peptide thioesters.[5,6] An increasingly popular approach involves the use of an acid- and base-stable alkanesulfonamide "safety-catch" linker (Scheme 1).[6] Following normal Fmoc-SPPS, the sulfonamide is activated by alkylation with iodoacetonitrile or trimethylsilyldiazomethane (TMS-CHN_2), allowing the fully protected peptide to be cleaved from the solid support with a thiol nucleophile.[7,8] This strategy has been used to prepare thioesters of several peptides,[7,9] including a 24-residue glycopeptide.[8]

Although the safety-catch resin is easy to implement and potentially broadly useful, some peptide sequences fail to

(1) For a recent review, see: Dawson, P. E.; Kent, S. B. H. *Annu. Rev. Biochem.* **2000**, *69*, 923−960.

(2) (a) Chan, W. C.; White, P. D. *Fmoc Solid-Phase Peptide Synthesis: A Practical Approach*; Oxford University Press: Oxford, 2000; Vol. 222. (b) Fields, G. B.; Noble, R. L. *Int. J. Pept. Protein Res.* **1990**, *35*, 161−214.

(3) Li, X.; Kawakami, T.; Aimoto, S. *Tetrahedron Lett.* **1998**, *39*, 8669−8672.

(4) (a) Swinnen, D.; Hilvert, D. *Org. Lett.* **2000**, *2*, 2439−2442. (b) Sewing, A.; Hilvert, D. *Angew. Chem.*, in press.

(5) (a) Alsina, J.; Yokum, T. S.; Albericio, F.; Barany, G. *J. Org. Chem.* **1999**, *64*, 8761−8769. (b) Jensen, K. J.; Alsina, J.; Songster, M. F.; Vagner, J.; Alberico, F.; Barany, G. *J. Am. Chem. Soc.* **1998**, *120*, 5441−5452. (c) Alsina, J.; Yokum, T. S.; Albericio, F.; Barany, G. *Tetrahedron Lett.* **2000**, *41*, 7277−7280.

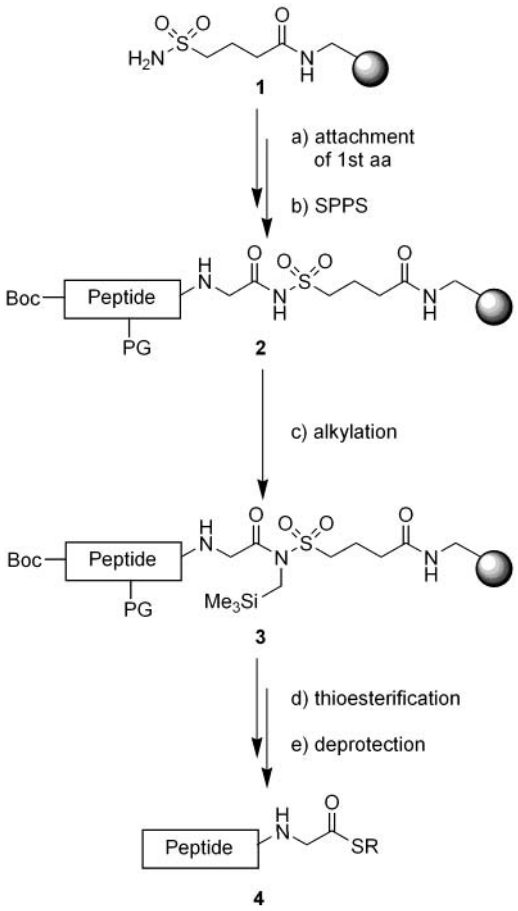
(6) (a) Backes, B. J.; Virgilio, A. A.; Ellman, J. A. *J. Am. Chem. Soc.* **1996**, *118*, 3055−3056. (b) Backes, B. J.; Ellman, J. A. *J. Org. Chem.* **1999**, *64*, 2322−2330.

(7) Ingenito, R.; Bianchi, E.; Fattori, D.; Pessi, A. *J. Am. Chem. Soc.* **1999**, *121*, 11369−11374.

(8) Shin, Y.; Winans, K. A.; Backes, B. J.; Kent, S. B. H.; Ellman, J. A.; Bertozzi, C. R. *J. Am. Chem. Soc.* **1999**, *121*, 11684−11689.

10.1021/ol016492h CCC: $20.00
Published on Web 09/07/2001

Scheme 1. Preparation of C-Terminal Peptide Thioesters on a Safety-Catch Resin[a]



[a] Conditions: (a) Fmoc-Gly-OH (5 equiv), PyBOP (5 equiv), DIEA (10 equiv) in CH_2Cl_2, 8 h from −20 °C to rt; (b) Fmoc-AA-OH, HBTU/HOBt/DIEA; (c) 1 M TMS-CHN_2 (100 equiv) hexane/THF 1/1 (v/v), 2 h; (d) NaSPh (0.5 equiv), 1 M $HSCH_2CH_2CO_2Et$ (200 equiv) in DMF, (R = $CH_2CH_2CO_2Et$), 24 h; (e) TFA and scavengers 2−4 h. PG = protecting groups; peptide = ARIIRYFYNAKAGLCQTFVYG (**2a**−**4a**); Ac-GLCQTFVYG (**2b**−**4b**); Ac−REDLIAYLKKATN (**2c**−**4c**); (Ac = acetyl).

yield the desired product[10] or afford only low yields of thioester.[9a] We encountered such problems in the preparation of a thioester corresponding to a 22-residue fragment of bovine pancreatic trypsin inhibitor (BPTI), presumably because of the peptide's tendency to aggregate. We now report that significant improvements in yield can be achieved by including lithium bromide (LiBr) in the cleavage step.

The peptide ARIIRYFYNAKAGLCQTFVYGG [BPTI-(16−37)] comprises two strands of β-sheet in native BPTI.[11] Attempts to synthesize this fragment as an activated thioester were carried out on a 4-sulfamylbutyryl aminomethyl polystyrene (AM) resin (**1**) with standard Fmoc chemistry,[2] and thiol cleavage was initially performed according to published protocols (see Scheme 1).[7] After completion of the synthesis, the resin was activated with 1 M TMS-CHN_2 (100 equiv) in hexane/THF 1/1 (v/v) for 3 h. It was washed twice with THF, twice with DMF, and then suspended in a 1.5 M solution of ethyl 3-mercaptopropionate ($HSCH_2CH_2$-CO_2Et, 200 equiv) containing a catalytic amount of sodium thiophenolate (NaSPh, 0.5 equiv) in DMF for 24 h. The resin was filtered and washed, and the combined filtrates were collected and dried to give the crude peptide. The side-chain protecting groups were removed by treating the dried residue with reagent B (88% TFA, 5% H_2O, 5% phenol, 2% triisopropylsilane)[12] for 2.5 h. After removal of TFA, ether precipitation, and drying, only 3 mg of crude product was obtained from 177 mg of peptide-resin **2a** at a loading of 0.19 mmol/g. This material was analyzed by reversed-phase HPLC (Figure 1A). The product mixture was extremely complex. Characterization of the lyophilized sample by liquid chromatography−mass spectrometry (LC-MS) did not reveal any peak with the expected mass for thioester **4a**.

Because of well-known synthetic difficulties associated with β-sheet-forming peptides,[13] it seemed possible that BPTI(16−37) might form secondary structures and/or aggregate on the solid support. Peptide aggregation can have deleterious effects on the coupling steps in SPPS.[13] It could also lower the efficiency of the cleavage process by hindering access of the alkylating agent or the nucleophilic thiol to their respective sites of reaction.

Seebach and co-workers have shown that addition of inorganic salts can dramatically increase the solubility of peptides in nonpolar organic solvents[14] as well as improve the efficiency of coupling reactions in solution[15] and on solid supports.[16] Lansbury has exploited this phenomenon to great effect in peptide synthesis,[17] demonstrating that the yield of nucleophilic cleavage of protected and highly aggregating β-sheet-forming peptides from the Kaiser oxime resin[18] can be substantially increased by carrying out the reaction in 2 M LiBr in anhydrous THF. This solvent system effectively disrupts secondary structures, as was shown by FT-IR,[17a] making the reaction site accessible to the incoming nucleophile.

The swelling properties of derivatized resin **1** in the presence and absence of lithium salts support the notion that

(9) (a) Huse, M.; Holford, M. N.; Kuriyan, J.; Muir, T. W. *J. Am. Chem. Soc.* **2000**, *122*, 8337−8338. (b) Kohli, R. M.; Trauger, J. W.; Schwarzer, D.; Marahiel, M. A.; Walsh, C. T. *Biochemistry* **2001**, *40*, 7099−7108. (c) Gieselman, M. D.; Xie, L.; van der Donk, W. A. *Org. Lett.* **2001**, *3*, 1331−1334.

(10) Marcaurelle, L. A.; Mizoue, L. S.; Wilken, J.; Oldham, L.; Kent, S. B. H.; Handel, T. M.; Bertozzi, C. R. *Chem. Eur. J.* **2001**, *7*, 1129−1132.

(11) Parkin, S.; Rupp, B.; Hope, H. *Acta Crystallogr., Sect. D* **1996**, *52*, 18−29.

(12) Solé, N. A.; Barany, G. *J. Org. Chem.* **1992**, *57*, 5399−5403.

(13) (a) Kent, S. B. H. In *Peptides; Structure and Function, Proceedings of the Ninth American Peptide Symposium*; Deber, C. M., Hruby, V. J., Kopple, K. D., Eds.; Pierce Chemical Company: Rockford, 1985; pp 407−414. (b) Kent, S. B. H. *Annu. Rev. Biochem.* **1988**, *57*, 957−989. (c) Tam, J. P.; Lu, Y. A. *J. Am. Chem. Soc.* **1995**, *117*, 12058−12063.

(14) Seebach, D.; Thaler, A.; Beck, A. K. *Helv. Chim. Acta* **1989**, *72*, 857−867.

(15) Thaler, A.; Seebach, D.; Cardinaux, F. *Helv. Chim. Acta* **1991**, *74*, 617−627.

(16) Thaler, A.; Seebach, D.; Cardinaux, F. *Helv. Chim. Acta* **1991**, *74*, 628−643.

(17) (a) Hendrix, J. C.; Halverson, K. J.; Jarrett, J. T.; Lansbury, P. T. *J. Org. Chem.* **1990**, *55*, 4517−4518. (b) Hendrix, J. C.; Jarrett, J. T.; Anisfeld, S. T.; Lansbury, P. T. *J. Org. Chem.* **1992**, *57*, 3414−3420.

(18) Kaiser, E. T. *Acc. Chem. Res.* **1989**, *22*, 47−54.

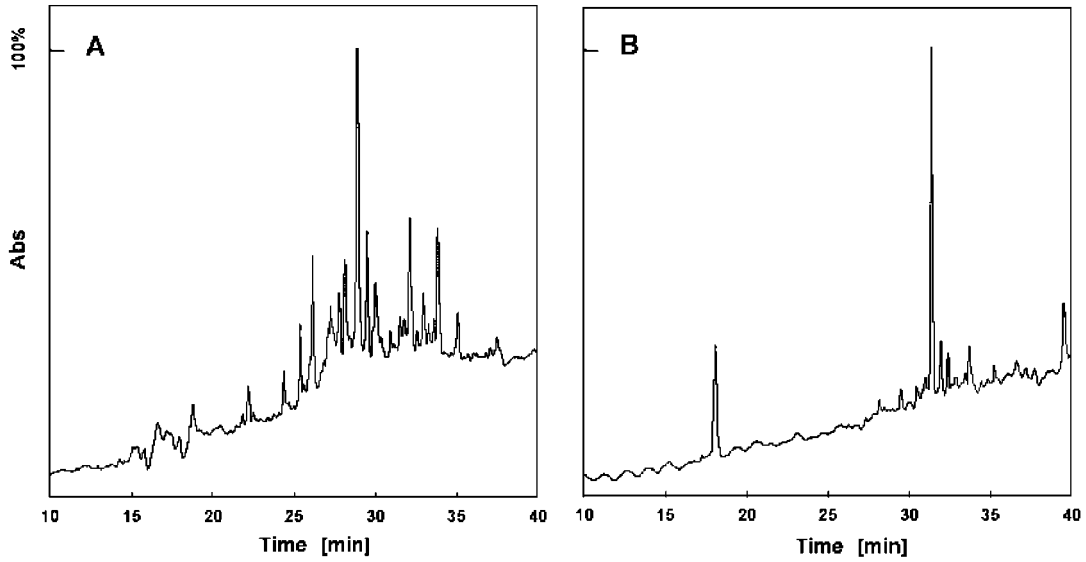


Figure 1. Analytical HPLC traces of the crude reaction products obtained upon cleavage of BPTI(16−37) from the safety-catch resin. The sulfonamide linker was first activated with 1 M TMS-CHN_2 (100 equiv) in hexane/THF 1/1 (v/v) for 3 h and then cleaved with either a 1.5 M solution of $HSCH_2CH_2CO_2Et$ in DMF (A) or 2 M LiBr/THF (B). Protecting groups were removed using reagent B.[12] In both cases, the same batch of peptide-resin **2a** (177 mg) was used. The total yield of crude product in (A) was 3 mg; the peak with a retention time of 31 min in (B) was isolated by preparative HPLC to give 11.5 mg of pure thioester **4a** (11% yield). The peak with retention time of 18 min in (B) is a volatile low molecular weight impurity. For experimental details see Supporting Information.

BPTI(16−37) adopts higher-order structures on the solid support. As shown in Table 1, resin loaded with this peptide (**2a**) swelled significantly in 2 M LiBr/THF, with the increase in volume roughly twice that in DMF or THF alone. In contrast, the same resin derivatized with Fmoc-Gly, swelled the most in DMF, followed by 2 M LiBr/THF and THF alone. These results suggest that 2 M LiBr/THF substantially aids solubilization of resin-bound BPTI(16−37) and thus might have a positive effect on the cleavage reaction.

Table 1. Swelling of Peptide-Resins

	volume ratio[b]		
resin[a]	DMF	THF	2 M LiBr/THF
Fmoc-Gly	4.0	2.9	3.5
2a[c]	2.5	2.2	4.5

[a] For both samples, 4-sulfamylbutyryl AM resin (**1**) was used. [b] Volume ratio = swollen resin/dry resin. [c] Resin-bound BPTI(16−37) was fully protected in these experiments.

Indeed, addition of LiBr greatly enhanced the efficiency of thiol cleavage. Activation of the peptide-resin **2a** as before with 1 M TMS-CHN_2 (100 equiv) in hexane/THF 1/1 (v/v), followed by 24 h reaction with 200 equiv of $HSCH_2CH_2CO_2Et$ in 2 M LiBr/THF instead of DMF, gave the desired thioester **4a** in high purity. Analysis of the crude product by HPLC (Figure 1B) showed predominantly a single peak with a retention time (t_R) of 31 min. This material was isolated (11.5 mg, 11% yield) and characterized by electrospray-ionization mass spectrometry (ESI-MS). The measured mass of 2760.3 Da is in excellent agreement with the expected mass of **4a** (as a mixed disulfide between Cys30 and ethyl 3-mercaptopropionate, 2760.3 Da calculated for $C_{127}H_{191}N_{31}O_{32}S_3$). Comparison of Figure 1A and 1B dramatically illustrates the benefit of using LiBr/THF in the substitution step. In the absence of LiBr, the full-length peptide **4a** is largely uncleaved from the resin and only contaminating side products of the reaction are observed. Upon addition of the structure-breaking agent, the desired thioester **4a** is released cleanly from the solid support.

The generality of this approach was examined using two other peptide sequences. The BPTI fragment Ac-GLCQTFVYGG [BPTI(28−37)], which was expected to be less problematic than BPTI(16−37) because of its shorter length, was synthesized on the same 4-sulfamylbutyryl AM resin, and the efficiency of thiol cleavage under standard conditions in DMF and in 2 M LiBr/THF was assessed. The crude C-terminal thioester **4b** was obtained with comparable purity in both cases, although the yield of isolated product was higher for the reaction carried out in the presence of LiBr (in DMF, 17%; in LiBr/THF, 25%). An unrelated 14-residue peptide taken from cytochrome C (Ac-REDLIAYLKKATNG), which has been reported as being difficult to synthesize as a result of secondary structure formation on the resin,[19] was also investigated. In this case, the yield of the corresponding thioester (**4c**) was 4-fold higher when the cleavage reaction was carried out in 2 M LiBr/THF rather than DMF (in DMF, 4%; in LiBr/THF, 17%).

(19) Atherton, E.; Woolley, V.; Sheppard, R. C. *J. Chem. Soc., Chem. Commun.* **1980**, 970−971.

The beneficial effect of LiBr is associated primarily with nucleophilic cleavage of the peptide from the resin to give the thioester. When both alkylation of the sulfonamide and the thiol cleavage steps were carried out with **2a** in the presence of LiBr, no additional benefit was observed. In fact, the reaction occurred cleanly as before, but the yields of isolated peptide were about 4-fold lower. Apparently, LiBr/THF adversely affects alkylation of the linker with TMS-CHN_2.

All three peptides examined here demonstrate the positive influence of LiBr/THF on the yield of C-terminal peptide thioesters. We anticipate that this solvent system could significantly widen the scope and general applicability of the safety-catch approach for the preparation of many other C-terminal thioester peptides.

Acknowledgment. We are grateful to Prof. Pier Luigi Luisi for generously providing access to the LC-MS device. This work was supported by the ETH-Zürich and Novartis Pharma AG.

Supporting Information Available: Experimental procedures for compounds **4a**−**c**. This material is available free of charge via the Internet at http://pubs.acs.org.

OL016492H