Pergamon

Tetrahedron Letters, Vol. 36, No. 8, pp. 1217-1220, 1995
Elsevier Science Ltd
Printed in Great Britain
0040-4039/95 $9.50+0.00

0040-4039(95)00037-2

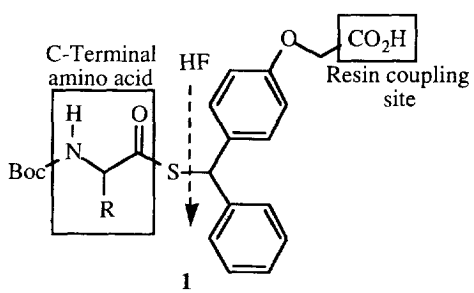
A General Method for the Synthesis of Thioester Resin Linkers for Use in the Solid Phase Synthesis of Peptide-α-Thioacids

Lynne E. Canne,* Sharon M. Walker, and Stephen B.H. Kent
The Scripps Research Institute, 10666 North Torrey Pines Rd,
La Jolla, CA 92037

Abstract: A generalized procedure for the synthesis of thioester linkers for use in stepwise solid phase peptide synthesis is reported. The linkers are compatible with Boc chemistry and, upon cleavage in HF, generate peptide C-terminal thioacids.

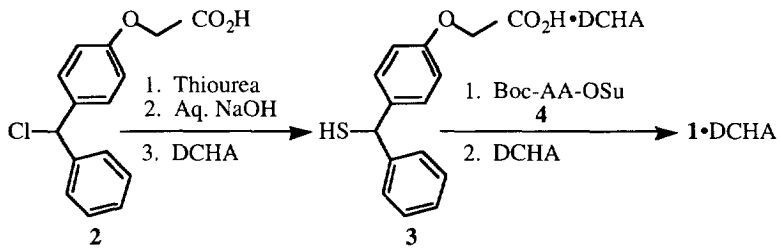
Peptides of up to 60 amino acids can be efficiently produced using stepwise solid phase peptide synthesis (SPPS).[1] Beyond this range, the accumulation of low level by-products makes purification of substantial amounts of the desired peptide difficult.[2] Recently, techniques for the chemical ligation of unprotected peptide segments have been developed that overcome the length limitations of SPPS.[3-7] This approach uses chemoselective reaction between two unique, mutually reactive functionalities to join unprotected peptide segments and gives the target product directly in final form. Several examples of functional proteins made by chemical ligation employ a C-terminal thioacid as a reactive functionality on one of the peptide segments to be reacted.[3-5,8]

The generation of peptide C-terminal thioacids in Boc chemistry SPPS requires the use of a special thioester linker between the peptide and the resin (**1**).[9,10] The appropriate amino acyl form of this linker is coupled to the resin support via the indicated carboxyl group. The desired peptide is then assembled in a stepwise fashion from the C-terminal amino acid which was incorporated into the linker prior to coupling to



1

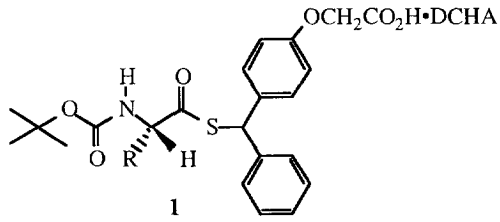
Scheme 1



the resin. Acidolytic cleavage in HF generates a peptide with a C-terminal -$^{\alpha}$COSH.[3-5,8-10]

We report a generalized, more convenient version of the Blake[9] and Yamashiro[10] procedure for the synthesis of thioacid-producing linkers. The Blake and Yamashiro method requires the conversion of Boc-amino acid succinimide esters to the corresponding Boc-amino thioacids with hydrogen sulfide. We have developed an alternative method, based on that of Hojo and Aimoto,[11] that uses the Boc-amino acid succinimide esters directly, avoiding the inconvenience and hazards of hydrogen sulfide gas. In this method (**Scheme 1**), thiol **3** is generated from the reaction of chloride **2**[10] with thiourea, followed by

Table 1. Boc-L-Amino thioester linkers.



Starting material (**4**)[14]	Product (**1**) R	% yield of **1** based on **3**	Product (**1**) formula[a]
a. Boc-L-Gly-OSu	-H[15]	57	$C_{34}H_{48}N_2O_6S$
b. Boc-L-Ala-OSu	-CH_3[16]	74	$C_{35}H_{50}N_2O_6S$
c. Boc-L-Aba-OSu[b]	-CH_2CH_3[17]	70	$C_{36}H_{52}N_2O_6S$
d. Boc-L-Leu-OSu	-$CH_2CH(CH_3)_2$[18]	65	$C_{38}H_{56}N_2O_6S$

[a]Compounds were analyzed for C, H, N, S. Values were within ±0.4% of the theoretical value for the indicated molecular formula. [b]Aba = α-amino-n-butyric acid.

hydrolysis of the resulting thiouronium salt in aqueous base.[12] Thiol **3** is a general intermediate which can then be reacted with any of a wide range of Boc-amino acid succinimide esters (**4**) to produce the desired thioester linker (**1**) which is conveniently isolated as the dicyclohexylamine (DCHA) salt.

This method has been employed in the synthesis of several different Boc-amino thioester linkers, examples of which are shown in **Table 1**. This convenient new method is a simple and safe alternative to the previously published procedures,[9,10] and in combination with new chemical ligation strategies[3,5,13,19] will find a wide application in peptide chemistry.

Experimental Procedures

4-(α-Mercaptobenzyl)phenoxyacetic acid, DCHA (3). A mixture of **2**[10] (7.5 g, 27 mmol), thiourea (2.3 g, 30 mmol), and ethanol (100 mL) were heated to reflux.[12] After 4 hours, conversion to the thiouronium salt was essentially complete as shown by TLC (90:5:5 $CHCl_3$:MeOH:AcOH). 10N NaOH (30 ml) was added and the reflux continued for 2-3 hours. After cooling to room temperature, the reaction mixture was concentrated *in vacuo* to approximately half the original volume, acidified with concentrated HCl (to pH 2.0), and extracted with EtOAc (4 x 30 mL). The combined EtOAc extracts were washed with saturated NaCl (1 x 30 mL) and dried over $MgSO_4$. The volatile materials were removed *in vacuo*. The resulting oil was dissolved in EtOAc (100 mL) and any insoluble material filtered. DCHA (6.0 mL, 30 mmol) was added to the filtrate with stirring. Within a few minutes, a white solid began to precipitate. Et_2O (150 mL) was added and the suspension cooled at -20 °C for several hours. The resulting white solid was filtered, washed with Et_2O, and dried under vacuum to give **3** (10.3 g, 23 mmol, 84%): ^1H NMR ($CDCl_3$): δ 7.30 (m, 7H), 6.82 (d, 2H, J=8.7 Hz), 5.39 (br s, 1H), 4.40 (s, 2H), 2.81 (m, 2H), 2.23 (br s, 1H, ex D_2O), 1.88-1.02 (comp m, 20H); FAB MS (cesium ion): calc for [$C_{27}H_{37}NO_3S$, H^+] 456.2572, found 456.2572. Anal. Calcd for $C_{27}H_{37}NO_3S$: C, 71.17; H, 8.18; N, 3.07; S, 7.04. Found: C, 71.11; H, 8.41; N, 3.08; S, 7.09.

General Synthesis of Boc-amino thioester linker (1), DCHA salt. A mixture of **3** (3.67 mmol), Boc-AA-OSu (**4**) (3.68 mmol), DIEA (5.74 mmol), DMF (35 mL) and CH_2Cl_2 (4 mL) was stirred at room temperature. After several hours, the initial white suspension completely dissolved to give a clear, colorless solution. After 24 hours, the reaction mixture was poured into 1N HCl (150 mL) and extracted with EtOAc (4 x 35 mL). The combined EtOAc extracts were washed with 1N HCl (2 x 30 mL), H_2O (1 x 30 mL), saturated NaCl (1 x 30 mL) and dried over $MgSO_4$. Volatiles were removed *in vacuo*. The resulting oil was purified by flash chromatography (925:50:25 $CHCl_3$:MeOH:AcOH) to give an oil contaminated with AcOH. To remove residual AcOH, the oil was dissolved in $CHCl_3$ (40 mL) and washed with 0.1 N HCl (7 x 10 mL), saturated NaCl (1 x 10 mL) and dried over $MgSO_4$. Volatiles were removed *in vacuo* to give **1** as an oil. This oil was dissolved in Et_2O (10 mL) to which was added DCHA (1 equiv). Hexane (100 mL) was added with stirring to separate the DCHA salt of **1** as a thick oil from any unreacted DCHA. Solvents were decanted from the oil and the oil dissolved in CH_2Cl_2 (30-40 mL). The resulting solution was concentated *in vacuo* to give the DCHA salt **1** as a white foamy solid.

Acknowledgment

This work was supported by funds from the NIH (S.B.H.K.). SMW was supported by NIH grant PO1 GM48870. We gratefully acknowledge the assistance of: Dr. Gary Suizdak and Dr. Raj K. Chandra, The Scripps Research Institute, for FAB MS measurements and elemental analyses, respectively.

References and Notes

(1) Schnölzer, M.; Alewood, P.; Jones, A.; Alewood, D.; Kent, S. B. H. *Int. J. Pept. Protein Res.* **1992**, *40*, 180-193.
(2) Kent, S. B. H. *Ann. Rev. Biochem.* **1988**, *57*, 957-989.
(3) Schnölzer, M.; Kent, S. B. H. *Science* **1992**, *256*, 221-225.
(4) Dawson, P. E.; Kent, S. B. H. *J. Am. Chem. Soc.* **1993**, *115*, 7263-7266.
(5) Baca, M.; Kent, S. B. H. *Proc. Natl. Acad. Sci.* **1993**, *90*, 11638-11642.
(6) Rose, K. *J. Am. Chem. Soc.* **1994**, *116*, 30-34.
(7) Muir, T. W.; Williams, M. J.; Ginsberg, M. H.; Kent, S. B. H. *Biochemistry* **1994**, *33*, 7701-7708.
(8) Williams, M. J.; Muir, T. W.; Ginsberg, M. H.; Kent, S. B. H. *J. Am. Chem. Soc.* **in press** (1994).
(9) Blake, J.; C.H., L. *Proc. Natl. Acad. Sci. USA* **1981**, *78*, 4055-4058.
(10) Yamashiro, D.; Li, C. H. *Int. J. Pept. Protein Res.* **1988**, *31*, 322-334.
(11) Hojo, H.; Aimoto, S. *Bull. Chem. Soc. Jpn.* **1991**, *64*, 111-117.
(12) Koenig, N. H.; Sasin, G. S.; Swern, D. *J. Org. Chem.* **1958**, *23*, 1525-1530.
(13) Dawson, P. E.; Muir, T. W.; Clark-Lewis, I.; Kent, S. B. H. *Science* **1994**, *266*, 776-779.
(14) Boc-AA-OSu esters were purchased from Novabiochem (LaJolla, CA). Any succinimide esters not commercially available were made from the corresponding free acids by the method of Anderson [Anderson, G.W.; Zimmerman, J.E.; Callahan, F.M. *J. Am. Chem. Soc.* **1964**, *86*, 1839-1842.] Boc-Aba-OSu: ^{1}H NMR ($CDCl_3$): δ 5.00 (m, 1H, ex D_2O), 4.62 (m, 1H), 2.83 (s, 4H), 1.89 (m, 2H), 1.45 (s, 9H), 1.07 (t, 3H).
(15) **1a**: ^{1}H NMR ($CDCl_3$): δ 7.24 (m, 7H), 6.80 (d, 2H, J=8.6 Hz), 5.88 (s, 1H), 5.06 (m, 1H, ex D_2O), 4.39 (s, 2H), 4.05 (d, 2H, J=5.7 Hz), 2.81 (m, 2H), 1.87-1.05 (comp m, 29H); FAB MS (cesium ion): calc for [$C_{34}H_{48}N_2O_6S$, H^+] 613.3311, found 613.3308.
(16) **2a**: ^{1}H NMR ($CDCl_3$): δ 7.24 (m, 7H), 6.80 (d, 2H, J=8.6 Hz), 5.81 (s, 1H), 4.95 (m, 1H, J=8.0 Hz, ex D_2O), 4.39 (m, 3H), 2.82 (m, 2H), 1.88-1.06 (comp m, 31H); FAB MS (cesium ion): calc for [$C_{35}H_{50}N_2O_6S$, H^+] 627.3468, found 627.3445.
(17) **3a**: ^{1}H NMR ($CDCl_3$): δ 7.24 (m, 7H), 6.80 (d, 2H, J=8.0 Hz), 5.82 (s, 1H), 4.94 (m, 1H, J=7.5 Hz, ex D_2O), 4.39 (m, 3H), 2.83 (m, 2H), 1.92-1.09 (comp m, 31H), 0.89 (t, 3H, J=7.4 Hz).
(18) **4a**: ^{1}H NMR ($CDCl_3$): δ 7.22 (m, 7H), 6.81 (d, 2H, J=7.5 Hz), 5.80 (s, 1H), 4.78 (d, 1H, J=8.1 Hz, ex D_2O), 4.39 (m, 3H), 2.82 (m, 2H), 1.88-1.06 (comp m, 32H), 0.90 (d, 6H, J=6.0 Hz); FAB MS (cesium ion): calc for [$C_{38}H_{56}N_2O_6S$, H^+] 669.3937, found 669.3960.
(19) Canne, L.E.; Ferré-D'Amaré, A.R.; Burley, S.K.; Kent, S.B.H. *J. Am, Chem. Soc.* **in press** (1995).

(*Received in USA* 10 *November* 1994; *accepted* 19 *December* 1994)