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Synthesis of a Versatile Purification Handle for Use with Boc Chemistry Solid Phase Peptide Synthesis

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Abstract: The synthesis of a versatile handle for the purification of synthetic peptides is described. The Boc-aminoethylsulfonylethyloxycarbonyl handle is coupled to the NH_2-terminus of the resin-bound peptide. Removal of the Boc group affords a free amine which can be derivatized with any of a variety of functionalities. Cleavage from the resin gives a peptide functionalized for covalent chemoselective reaction with the column support. Desired full length peptide is eluted from the column by cleavage of the handle by treatment with base. © 1997 Elsevier Science Ltd.

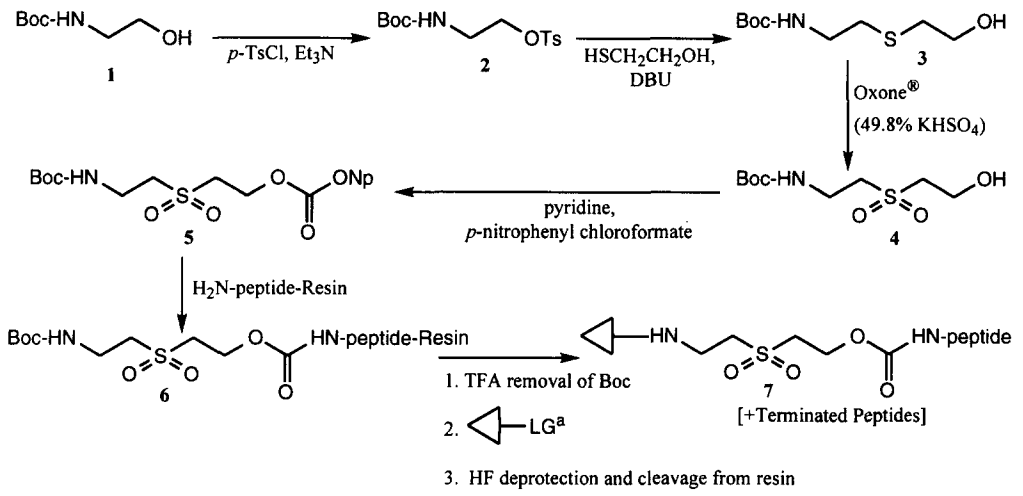
The accumulation of minor side products during each cycle of stepwise solid phase synthesis (SPPS) of lengthy peptides (>40 residues) can pose a major obstacle to the purification of the desired full length peptides. We have shown that when using highly optimized HBTU/DIEA *in situ* neutralization coupling cycles for Boc chemistry stepwise SPPS,[1] the majority of remaining side products are 'terminated,' that is peptides blocked at their N-terminus, and not deletions or internally modified peptides.[2] By coupling a functionalized, removable handle as the final step in SPPS, we are able to chemically tag peptides that have a free N-terminal amine, primarily the full length peptide. After cleavage from the resin, the desired full length peptide is separated from the crude SPPS mixture by chemoselective reaction or affinity chromatography.

A variety of purification handles have been reported previously for use in SPPS, including Fmoc-based handles for use with ion exchange,[3] hydrophobic columns[4] and avidin[5] and aminoethylsulfonylethyloxycarbonyl-based handles for use with iodoacetamide,[6] avidin,[7] and hydrophobic columns.[8] We report an alternative synthesis to the previously reported pre-derivatized aminoethylsulfonylethyloxycarbonyl-based handles:[6] a versatile Boc-aminoethylsulfonylethyloxycarbonyl handle (**5**) which is ideal for use with Boc chemistry. After removal of the Boc group, the handle can be modified with a variety of functional groups or ligands while still attached to the peptide-resin. Because resins with a wide variety of functional groups (amino, carboxy, formyl, boronate, metal chelating, hydrophobic, ion exchange, etc...) are now commercially available, it is desirable to have a versatile handle that can be easily modified to accommodate a variety of chemical strategies for covalent or affinity purification.

The synthesis, subsequent coupling to the peptide-resin, and modification of the Boc-aminoethylsulfonylethyloxycarbonyl handle (**5**) is outlined in **Scheme 1**. Boc-ethanolamine (**1**) is converted to the tosylate **2,** which is then reacted with 2-mercaptoethanol to produce the thioether **3**. Compound **3** is oxidized to the corresponding sulfone **4** with Oxone®.[9] The hydroxyl of **4** is reacted with *p*-nitrophenyl chloroformate[10] to form the corresponding *p*-nitrophenyl carbonate **5**. At the completion of the stepwise SPPS of the target sequence, handle **5** is added to the Boc-deprotected peptide-resin to produce the modified peptide-resin **6**. Subsequent removal of the Boc group from the peptide-resin (**6**) permits elaboration of the

amino-handle with the moiety of choice, affording, after deprotection and cleavage from the resin in anhydrous HF, a product mixture consisting of functionalized full-length peptide **7** plus terminated peptide coproducts.

Scheme 1



[a]LG = leaving group, ◁ = chemoselective functional group for reaction with complimentary group on column

Scheme 2 shows the binding, by covalent chemoselective reaction or non-covalent affinity interaction, of peptide **7** to the complimentary functional group of the column support used for purification. After binding is complete, the unbound terminated peptides are removed in a wash step. The desired full length peptide (**9**) is obtained in an unmodified form following base cleavage of the aminoethylsulfonylethyloxycarbonyl handle.

Scheme 2

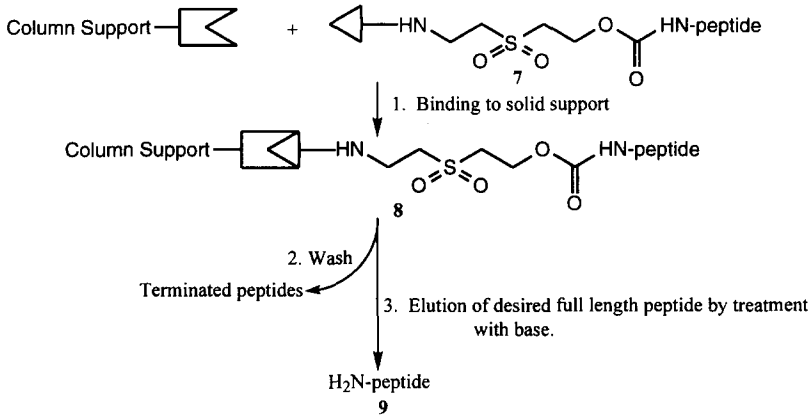


Figure 1 demonstrates the utility of this method. In this particular case, 4-oxopentanoic acid was coupled to the N^{α}Boc deprotected handle form of the modified peptide-resin **6**. The column support was functionalized with aminooxyacetic acid. The ketone "tagged" full length peptide was covalently bound to the purification column through the formation of an oxime link between the aminooxy on the solid support and the ketone on the peptide.[11,12] Panel **A** shows an analytical HPLC of the crude peptide products prior to chemoselective covalent purification. Panel **B** shows an analytical HPLC of the peptide eluted from the purification column after treatment with base.

The NH_2-functionalized cleavable handle described here provides a versatile intermediate for removal of terminated coproducts from peptides prepared by stepwise Boc chemistry solid phase synthesis.

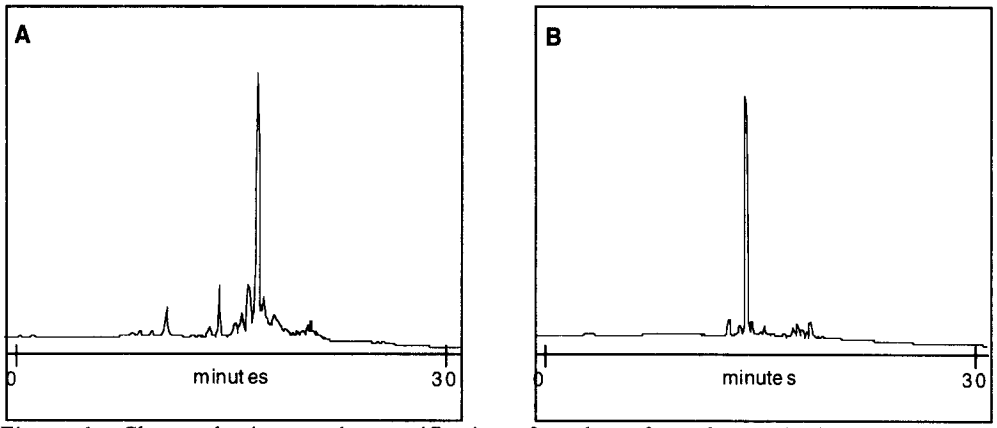


Figure 1. Chemoselective covalent purification of products from the synthesis of the sequence NAIIKNAYKKGE by stepwise Boc chemistry SPPS. Panel **A** shows an HPLC trace of the crude products before purification. Panel **B** shows the same peptide after purification by chemoselective reaction and cleavage of the functionalized handle.

Experimental Procedures

N-*t*-Boc-(O-*p*-tosyl)-2-aminoethanol (2). N-*t*-Boc-ethanolamine (**1**)(20.2 g, 125 mmol), triethylamine (39.0 ml, 280 mmol), and dichloromethane (DCM) (180 ml) were combined in a 500 ml round bottom flask and cooled in an icebath. *p*-Toluenesulfonyl chloride (26.6 g, 139 mmol) dissolved in DCM (180 ml) was added and the flask removed from the icebath. The mixture was allowed to stir at ambient temperature for 48 hr. Solids were filtered and washed with DCM. The filtrate was washed with 10% citric acid (3 x 100 ml), saturated NaCl (1 x 100 ml) and dried over $MgSO_4$. Volatiles were removed *in vacuo* and the resulting oil triturated with hexane/ethyl acetate to give a white solid. Solids were filtered, washed with hexane and dried to give 31.6 g (100 mmol, 80%) of **2**. ^{1}H NMR ($CDCl_3$): δ 7.79 (d, 2H, J=8.1 Hz), 7.35 (d, 2H, J=8.1 Hz), 4.89 (br m, 1H, ex D_2O), 4.07 (t, 2H, J=5.0 Hz), 3.38 (m, 2H), 2.45 (s, 3H), 1.41 (s, 9H).

2-[S-[(N-*t*-Boc)-2-aminoethyl]]mercaptoethanol (3). 2-Mercaptoethanol (4.4 ml, 63 mmol), 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) (9.4 ml, 63 mmol), **2** (15.7 g, 50 mmol) and benzene (240 ml) were combined in a 500 ml round bottom flask and stirred at ambient temperature overnight. The reaction mixture was washed with H_2O (1 x 50 ml), 1 N HCl (2 x 50 ml), saturated NaCl (1 x 50 ml) and dried over Na_2SO_4. Volatiles were removed *in vacuo* to give 10.0 g (45 mmol, 91%) of **3** as a clear, colorless oil. ^{1}H NMR ($CDCl_3$): δ 4.94 (br m, 1H, ex D_2O), 3.75 (t, 2H, J=5.7), 3.33 (m, 2H), 2.75 (t, 2H, J=5.7 Hz), 2.67 (t, 2H, 6.6 Hz), 2.38 (br s, 1H, ex D_2O), 1.45 (s, 9H).

2-[[(N-*t*-Boc)-2-aminoethyl]sulfonyl]ethanol (4). Compound **3** was oxidized by the method of Trost and Curran.[9] Compound **3** (10.0 g, 45 mmol) was dissolved in methanol (150 ml) in a 500 ml round bottom flask

and cooled in an icebath. Oxone® (49.8% $KHSO_4$) (42.2 g, 137 mmol $KHSO_4$) in H_2O (150 ml) was added. The reaction mixture was removed from the icebath and stirred at ambient temperature for 3 hr. The reaction mixture was diluted with H_2O (600 ml) and extracted with ethyl acetate (5 x 100 ml). The combined organic extractions were washed with saturated NaCl (1x 100 ml) and dried over Na_2SO_4. Volatiles were removed *in vacuo* to give 8.8 g (35 mmol, 77%) of **4** as a clear, colorless oil. ^1H NMR ($CDCl_3$): δ 5.25 (br m, 1H, ex D_2O), 4.13 (t, 2H, J=5.1), 3.66 (m, 2H), 3.35 (t, 2H, J=6.1 Hz), 3.26 (t, 2H, 5.1 Hz), 2.39 (br s, 1H, ex D_2O), 1.45 (s, 9H).

2-[[(N-*t*-Boc)-2-aminoethyl]sulfonyl]ethyl *p*-nitrophenyl carbonate (5). Compound **4** was converted to the *p*-nitrophenyl carbonate by the method of Tesser and Balvert-Geers.[10] Compound **4** (4.57 g, 18.0 mmol) and pyridine (15.0 ml) were combined in a 50 ml round bottom flask and cooled in an icebath. *p*-Nitrophenyl chloroformate (3.67 g, 18.2 mmol) was added. The mixture was removed from the icebath and stirred at ambient temperature overnight. The reaction mixture was poured into cold 1 N HCl (300 ml) and extracted with ethyl acetate (4 x 50 ml). The combined organic phases were washed with 1 N HCl (2 x 25 mls), H_2O (1 x 25 mls), saturated NaCl (1 x 30 ml) and dried over $MgSO_4$. Volatiles were removed *in vacuo* and the resulting white solid crystallized from ethyl acetate/hexane to give 3.83 g (9.2 mmol, 51%) of **5** as a white solid. ^1H NMR ($CDCl_3$): δ 8.30 (d, 2H, J=9.1 Hz), 7.41 (d, 2H, J=9.1 Hz), 5.13 (br m, 1H, ex D_2O), 4.74 (t, 2H, J=5.6), 3.70 (m, 2H), 3.47 (t, 2H, J=5.6 Hz), 3.35 (t, 2H, 6.3 Hz), 1.44 (s, 9H); FAB MS (cesium ion): calcd for [$C_{16}H_{22}N_2O_9S$, H^+] 551.0100, found 551.0121. Anal. calcd for $C_{16}H_{22}N_2O_9S$: C, 45.93; H, 5.30; N, 6.70; S, 7.66. Found: C, 46.26; H, 5.10; N, 6.95; S, 8.05.

Peptide Synthesis. Synthesis of NAIIKNAYKKGE was carried out manually on *p*-methylbenzhydrylamine resin using standard *in situ* neutralization protocols.[1] Compound **5** (0.17 mmol in minimal DMF and 5% DIEA) was coupled to the Boc-deprotected target peptide (0.0765 mmol) to give peptide-resin **6**. Following Boc-deprotection, 4-oxopentanoic acid (0.55 mmol in 0.5 M HBTU/DMF and 5% DIEA) was coupled to peptide-resin **6** to yield the ketone tagged peptide-resin. The tagged peptide-resin was deprotected and cleaved in anhydrous HF to give free peptide **7** and coproducts. The crude peptide was dissolved in 150 mM phosphate buffer, pH 7.2 and equilibrated with aminooxy-derivatized sepharose beads for 2 hrs. After washing with 1.0 M NaCl, 150 mM phosphate buffer, full length underivatized peptide (**9**) was eluted by adjusting the pH to 12 with NaOH for 3 minutes. The eluant was quenched by adjusting the pH to 5 with aqueous HCl and immediately assayed by analytical reverse phase HPLC.

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