Pergamon

Tetrahedron Letters 39 (1998) 8241–8244

TETRAHEDRON LETTERS

A Reversible Affinity Tag for the Purification of N-Glycolyl Capped Peptides

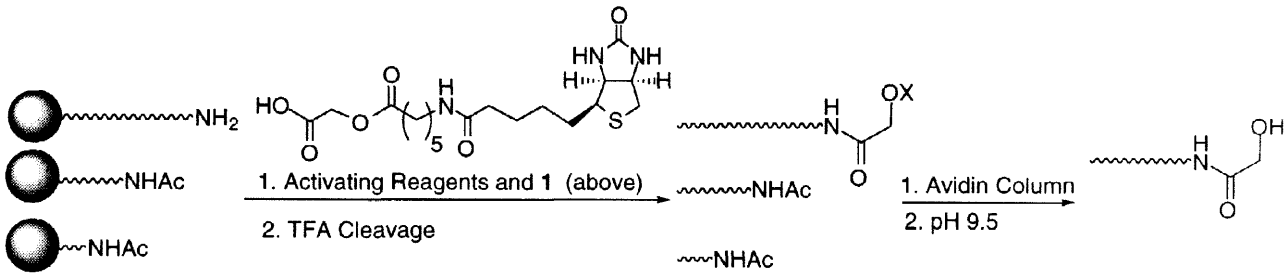
Michael A. Shogren-Knaak and Barbara Imperiali*

Division of Chemistry and Chemical Engineering, California Institute of Technology
Pasadena, California 91125

Received 26 June 1998; revised 3 August 1998; accepted 12 August 1998

Abstract: A reversible biotin affinity tag, 2-[*N*-(Biotin)-6-aminocaproate]-acetate (**1**), has been generated for the efficient purification of peptides requiring *N*-terminal derivatization. This methodology is compatible with solid phase peptide synthesis techniques.

While solid phase peptide synthesis reagents for the rapid affinity purification of peptides containing a free amino terminus have been developed,[1-11] no analogous agents exist for the efficient purification of peptides capped with simple, neutral, nonreactive *N*-terminal capping groups. Synthetic peptides are commonly capped at the N-terminus to prevent the reactive and charged terminal amine from interfering with the structure and function of a peptide. In the course of generating related families of peptides for structure/function analyses, we have developed compound **1** (Scheme 1), a general, reversible affinity tagging agent for the rapid generation of purified *N*-terminal capped peptides. Compound **1** integrates the powerful binding properties of biotin[12] into a derivatization agent which also includes a 6-amino-caproic acid linker (to maximize the biotin binding to avidin during the purification steps)[13] and glycolate, a molecule that ultimately serves as the *N*-terminal capping group. Compound **1** was designed to provide a strong and highly specific affinity interaction, facile peptide coupling, and compatibility with solid phase peptide synthesis and resin deprotection until affinity purification is complete (Scheme 1). The properties of the glycolate ester allow for efficient Fmoc-based peptide synthesis, which employs acid-based deprotection schemes, followed by a mild basic treatment after affinity purification to liberate peptide products. Synthesis and use of **1** by affinity purification indicate that the compound is an attractive agent for purification of *N*-terminal capped peptides.

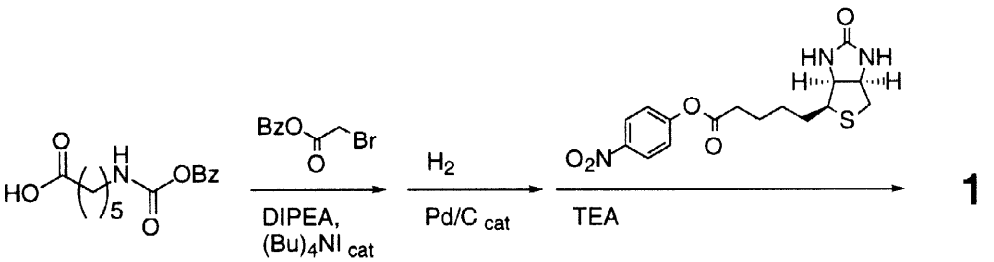


Scheme 1. The reversible biotin affinity capping reagent and use of this tag to purify *N*-terminal glycolate-capped peptides.

Affinity tag, **1**, was generated in 3 steps from commercially available starting materials (Scheme 2).

0040-4039/98/$ - see front matter
PII: S0040-4039(98)01780-8

Affinity tag, **1**, was generated in 3 steps from commercially available starting materials (Scheme 2).



Scheme 2. Synthetic scheme for reversible affinity tag **1**.

The glycolate ester was formed by *O*-alkylation of *N*-benzyloxycarbonyl-6-aminohexanoic acid with benzyl-2-bromoacetate (1.0 equiv.) in DMF at 50 °C for 12 hrs with an equivalent of diisopropylethylamine and a catalytic amount of tetrabutylammonium iodide. The product from this reaction was isolated by silica gel chromatography (70% yield), dissolved in 3:1 THF/water, and deprotected under a hydrogen atmosphere in the presence of 5 mole% Pd/C as catalyst at 0 °C. After a total of 20 hrs, the amine product was isolated by filtration through Celite and dried (63% yield). The free amine was dissolved in water and then coupled to an activated nitrophenyl biotin ester (1.0 equivalent) in DMF (2:1 DMF/water) in the presence of TEA (2.0 equiv.). After three hours at room temperature, a single product was seen by TLC and the water was removed under vacuum. The concentrated solution of **1** was brought to a concentration of 0.216 M by addition of DMF and was either directly coupled to peptide or stored at -80°C until required. For these experiments the affinity functionality included a biotin derivative. In principle however, any compound with a carboxylate could be linked through an amide bond in the final synthetic step to afford a different reversible affinity group.

Compound **1** was attached to full length peptides by amide bond formation. Peptides tested for coupling and for subsequent steps were synthesized by standard techniques from N^{α}-(9-fluorenylmethyloxycarbonyl) (Fmoc) amino acids on an automated peptide synthesizer, where an acetylation step was included after every round of amino acid coupling to insure selective coupling of compound **1** to full length peptides only.[14] Peptides tested included representative short peptide **P1** (Glc-F-S-R-S-NH_2) and long peptide **P2** (Glc-F-S-R-S-D-E-L-A-K-L-L-R-L-H-A-G-NH_2). Coupling was performed manually and typically proceeded as follows: The Fmoc group of resin bound peptide (45.0 mg, 8.0 μmoles) was removed by incubating the resin in 2 mL of 20% piperidine in DMF twice for 30 min. After the resin was washed with DMF (15 mL), a solution of 2-(1H-benzotriazole-1-yl)-1,1',3,3',-tetramethyluronium hexafluorophosphate (HBTU) (12.2 mg, 31.9 μmol), 1-hydroxybenzotriazole (HOBT) (4.9 mg, 31.9 μmol), excess diisopropylethylamine (DIPEA) (0.03 mL, 175.5 μmol), and the 0.216 M solution of affinity tag in DMF (0.11 mL, 23.9 μmol) was added. The mixture was agitated continuously, and coupling was complete by 4 hrs as determined by the Kaiser test.[15] The resin was washed with 15 mL DMF, resuspended in dichloromethane, and dried *in vacuo*. Side chain protecting groups were removed and crude peptide cleaved from the resin with reagent K (33:2:2:2:1 trifluoroacetic acid/water/thioanisole/phenol/ethane- dithiol) for 2 hrs.[16] Filtered supernatant was reduced to a minimal volume.

triturated, and washed with 2:1 ether/hexanes (6 × 1 mL), and lyophilized from water (1 mL) to afford the crude affinity tag capped peptide.

Reagent **1** was selectively coupled to the amino terminus of full length peptides. Coupling to the resin-bound peptide proceeded efficiently; analysis using a Kaiser test indicated that coupling was nearly complete in under four hours. Furthermore, HPLC and ESI-MS analysis of the crude products cleaved from the resin indicated that the affinity cap was coupled cleanly, with little or no uncapped or miscapped peptide present. Additionally, the peptide cap survived acidic cleavage from resin bound support. HPLC and ESI-MS analysis of crude product from reagent K cleavage shows that side products from loss or decomposition of the capping group are not present at significant levels.

Tagged peptide was isolated by avidin-based affinity column chromatography and released by cleavage of the ester bond under mildly basic conditions to yield glycolate-capped peptide. Typically, avidin resin (0.5 mL of 3.6 mg avidin/mL agarose resin) was washed with 10 mL of pH 7.0 buffer (20 mM sodium phosphate, 500 mM NaCl, 0.05% NaN_3). The crude peptide solution (excess peptide relative to the number of avidin binding sites), was resuspended in 0.5 mL pH 7.0 buffer, added to the column, mixed, and allowed to equilibrate over three hours. The crude peptide solution and a 5 mL water wash of the resin were collected and stored at – 80 °C for use in future purifications. The avidin column was washed with 5 mL of 1:1 water/acetonitrile, and 5 mL water. 1 mL of 0.075 M 3-(Cyclohexylamino)-2-hydroxypropanesulfonic acid (CAPSO) pH 9.5 buffer was added to the resin and the mixture was agitated and left for 24 h. Collection of the column eluent and a 1.5 mL of water wash afforded *N*-terminal glycolate-capped peptide. Pure peptide was recovered at 76% yield as determined by HPLC analysis of the peptide solutions before and after affinity column purification and was the only high molecular weight compound observed as determined by HPLC and ESI-MS analysis (Figure 1). Purified peptides were either used directly, or stored in solution at – 80 °C.

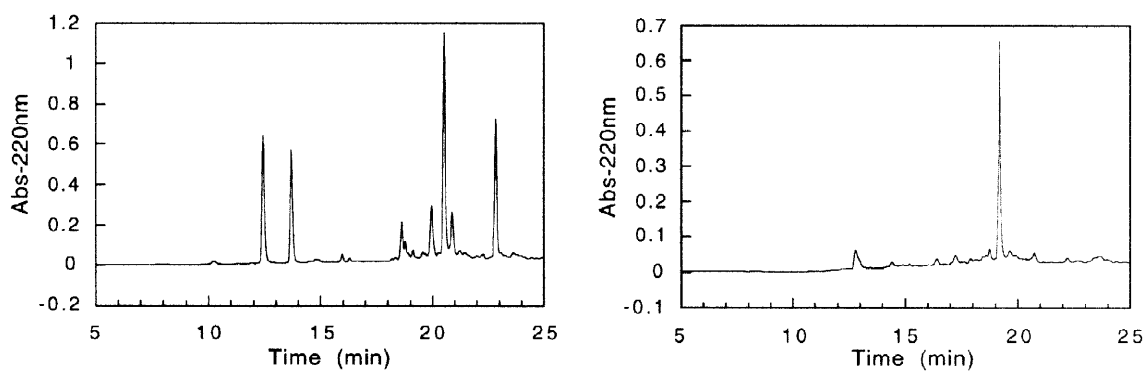


Figure 1. HPLC trace of peptide P2 before and after avidin purification.
0-70% gradient of acetonitrile/water/0.1% trifluoroacetic acid

The removable affinity capping agent **1** generates glycolate-capped peptides with good recovery and purity. Current work is directed at using the advantages of this reagent to aid in the rapid screening of large families of peptides.

Acknowledgements

This work was supported by NIH grant number GM 53098. Additional support for MSK was provided by a predoctoral fellowship from the Howard Hughes Medical Institute.

References

1. Canne, L. E.; Winston, R. L.; Kent, S. B. H. *Tetrahedron Lett.* **1997**, *38*, 3361-3364.
2. Kellman, B.; Chan, W. C.; Chhabra, S. R.; Bycroft, B. W. *Tetrahedron Lett.* **1997**, *38*, 5391-5394.
3. Roggero, M. A.; Corradin, S. G. *FEBS Lett.* **1997**, *408*, 285-288.
4. Ball, H.; Mascagni, P. *Int. J. Pep. Protein Res.* **1996**, *48*, 31-47.
5. Garcia-Echeverria, C. *J. Chem. Soc, Chem. Commun.* **1995**, *7*, 779-780.
6. Kazmierski, W. M.; McDermed, J. *Tetrahedron Lett.* **1995**, *36*, 9097-9100.
7. Brown, A. R.; Irving, S. L.; Ramage, R. *Tetrahedron Lett.* **1993**, *34*, 7129-7132.
8. Funakoshi, S.; Fujii, N. *J. Chrom.* **1993**, *638*, 21-27.
9. Merrifield, R. B.; Bach, A. E. *J. Org. Chem.* **1978**, *43*, 4808-4816.
10. Krieger, D. E.; Erickson, B. W.; Merrifield, R. B. *PNAS* **1976**, *73*, 3160-3164.
11. Thiele, C.; Fahrenholz, F. *Anal. Biochem.* **1994**, *218*, 330-337.
12. Green, N. M. *Adv. Prot Chem.* **1975**, *29*, 85-.
13. Wilcheck, M.; Bayer, E. A. Avidin-Biotin Technology. In *Methods in Enzymology*; Wilcheck, M. Bayer, E. A. Eds.; Academic Press Inc.; San Diego, 1990; pp 123-139.
14. Fields, G. B.; Noble, R. L. *Int. J. Peptide Protein Res.* **1990**, *35*, 161-214.
15. Kaiser, E.; Colescott, R. L.; Bossinger, C. D.; Cook, P. I. *Anal. Biochem.* **1970**, *34*, 595-598.
16. King, D. S.; Fields, C. G.; Fields, G. B. *Int. J. Pept. Protein Res.* **1990**, *36*, 255-266.