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An alternate preparation of thioester resin linkers for solid-phase synthesis of peptide C-terminal thioacids

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Abstract

An alternative preparation of thioester resin linkers for solid-phase synthesis of peptide C-terminal thioacids is presented.

Keywords: thioacids; thioesters; thiopeptides; supported reagents/reactions; peptide analogues/mimetics.

1. Introduction

The solid phase peptide synthesis of large peptides (greater than 60 amino acids) is problematic in that it suffers from low yields and purification problems. To overcome these problems, researchers have covalently joined unprotected, large peptide fragments using 'native chemical ligation' strategies. In one of the first approaches developed, a globally deprotected C-terminal thioacid peptide is coupled to a halogenated 'N-terminal' peptide in aqueous media.[1,2] The newly created peptide contains a thioester peptidomimetic bond that connects the fragments. The difficulty with this approach lies in the chemical synthesis of the appropriate C-terminal thioacid-containing peptide. In a previously reported synthesis, thioester (**1**) was prepared from 4-hydroxybenzophenone (**2**) via intermediate dibenzyl alcohol (**3**). This alcohol was transformed to the corresponding chloride, which in turn could be converted to thioester (**1**) by a one- or two-step route.[3,4] The thioester (**1**) can be attached to an aminomethyl resin and elongated using standard Boc-chemistry with the ultimate cleavage from the resin effected by treatment with HF to give the C-terminal peptide thioacid. In our studies involving a novel peptide vaccine delivery system based on lipid tubules, it became necessary to find a more reliable and efficient way to generate the thioester (**1**).

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2. Discussion

Repeated attempts to prepare the substituted dibenzyl alcohol (**3**) using the reported synthetic pathway were unsuccessful. The substituted benzophenone derivative would not reduce under the given conditions; therefore, an alternative pathway was devised (Fig. 1). 4-Hydroxybenzophenone (**2**) is initially reduced to the corresponding alcohol by treatment with sodium borohydride in refluxing dioxane/water. The phenol can then be selectively alkylated upon treatment with potassium carbonate and ethyl iodoacetate. The resulting ester is then converted to the corresponding carboxylic acid (**3**) by saponification.

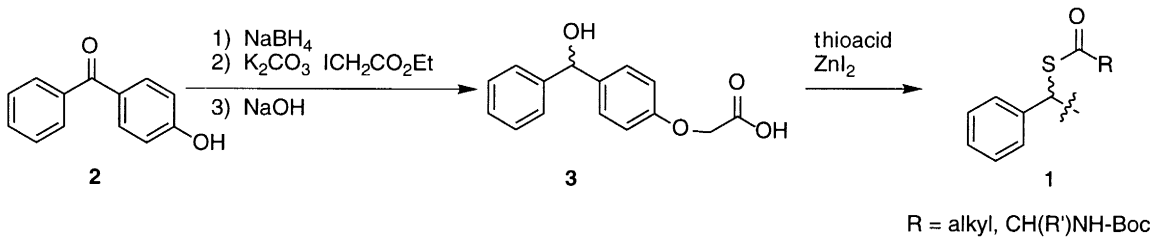

Fig. 1.

The formation of the chloride, the next step in the reported thioester synthesis, also proved problematic. We were unable to purify this very reactive intermediate. Instead, we generated the thioester linker (**1**) directly by Lewis acid (zinc iodide) catalyzed coupling of the alcohol (**3**) with a thioacid.[5] The required aminothioacids were formed from the corresponding *N*-hydroxysuccinimidyl-esters (NHS-ester) either by treatment with anhydrous H_2S in triethylamine/dioxane[4] (method A) or with NaHS in methanol (method B). Both the aminothioacid and thioester linker formation conditions are quite mild. The synthesis is amenable to a wide variety of chemical functionalities (Table 1) and should be compatible with all protected amino acids commonly used in standard BOC chemistry. The optical rotations of the thioester linkers (**1**) made directly from the alcohol (**3**) are identical to those made by coupling the NHS-ester to a thiol linker.[3]

Table 1

Thioacid	Thioacid Formation Method	% Yield Thioacid	% Yield Thioester
Thiolacetic acid	--	--	93
BocLeuSH	A	95	66
	B	98	67
BocArg(Z)$_2$SH	A	95	53
BocLys(2-Cl-Z)SH	A	95	78
BocAsp(OcHex)SH	B	95	77
BocSer(OBn)SH	A	98	72

3. Experimental

^{1}H NMR spectra were obtained in $CDCl_3$ using a Bruker 300 MHz NMR spectrometer with tetramethylsilane as an internal standard. Silica gel (EM Science Silica Gel 60, 230–400 Mesh) was used for all flash chromatography. TLC was performed using plates coated with 250 μm Silica Gel 60 F_{254} (EM Science). All reagents were used as received.

3.1. Formation of thioacids[6]

Method A: A solution of triethylamine (3 equiv.) in *p*-dioxane at 0°C was saturated with anhydrous H_2S (gentle bubbling for ~25 min). The NHS-ester (1 equiv., 13 mg/mL) was added and the solution stirred for 40 min while warming to room temperature. The yellow solution was partially neutralized with 1N HCl. By bubbling N_2 through the solution for 20 min, excess H_2S was removed and the mixture lightened in color. The mixture was concentrated to quarter the volume and then acidified to pH=3 with 1N HCl. The solution was diluted with 20 mL H_2O and then extracted twice with 15 mL portions of EtOAc. The organic layers were combined and washed with 3×15 mL H_2O and once with 15 mL saturated NaCl (aq.). The solvent was removed and the residue purifed by flash chromatography to provide the thioacids as clear oils.

Method B: To NHS-ester in anhydrous MeOH (1 equiv., 10 mg/mL) was added $(NaHS)\cdot(H_2O)_{1.5}$ (1 equiv.). The solution was stirred for 3 h and then acidified to pH=4 with 1N HCl. Water (20 mL) was added and the solution was extracted with 30 mL EtOAc. The organic layers were combined and washed with 10 mL H_2O and 10 mL saturated NaCl. The solvent was removed and the residue purifed by flash chromatography to provide the thioacids as clear oils.

*3.2. Preparation of 4-benzoylphenoxyacetic acid (**3**)*

To 4-hydroxybenzophenone (1.0 g, 5.0 mmol) in 100 mL 19:1 *p*-dioxane:H_2O was added sodium borohydride (0.4 g, 10.1 mmol). The solution was maintained at reflux for 14 h and then concentrated to half the volume. The solution was acidified with 1 M H_2SO_4 until pH=3 and an additional 100 mL H_2O was added. The solution was extracted with 3×50 mL EtOAc and the combined organics were washed with 50 mL saturated NaCl (aq.). The solvent was removed by rotary evaporation and the residue partially purified by flash chromatography (25:1–8:1, $CHCl_3$:MeOH) and those fractions containing the reduced material were dried and then recrystallized from acetone/H_2O to provide 4-benzoylphenol as white needles (0.6 g, 60%): mp 160–162°C; R_f (9:1, $CHCl_3$:MeOH) 0.48; ^{1}H NMR 7.36–7.28 (m, 7H), 6.87 (d, 2H, ArOH, J=8.8 Hz), 5.80 (s, 1H, ArCH), 4.79 (s, 1H, ArOH), 2.19 (s, 1H, OH).

3.3. 4-Benzyloxyphenoxyacetic acid ethyl ester

To 4-benzoylphenol (0.6 g, 3.0 mmol) in 35 mL anhyd. DMSO was added anhydrous potassium carbonate (0.8 g, 6.0 mmol) and ethyl iodoacetate (354 μL, 3.3 mmol). After stirring overnight, the slurry was acidified to pH=4 with 1 M H_2SO_4. Water (25 mL) was added and the solution extracted with 3×20 mL EtOAc. The organic layers were combined and washed with 25 mL 1 M H_2SO_4 and 25 mL H_2O. The solvent was removed under reduced pressure and the residue was purified by flash chromatography (20:1–9:1, $CHCl_3$:MeOH) to provide the desired material as a clear oil (0.6 g, 68%): R_f (9:1, $CHCl_3$:MeOH) 0.58; ^{1}H NMR 7.28 (m, 7H), 6.87 (d, 2H, J=8.8 Hz), 5.80 (s, 1H), 4.60 (s, 2H), 4.23 (q, 2H, J=7.3 Hz), 2.19 (s, 1H, OH), 1.29 (t, 3H, J=7.3 Hz).

*3.4. 4-Benzoylphenoxyacetic acid (**3**)*

To 4-(α-hydroxybenzyl)phenoxyacetic ethyl ester (0.6 g, 2.1 mmol) in 100 mL MeOH and 5 mL H_2O was added sodium hydroxide (0.2 g, 5 mmol). After stirring overnight the mixture was acidified to pH=4 with 1 M H_2SO_4. Approximately half of the solvent was removed by rotary evaporation whereupon an additional 75 mL H_2O was added. The mixture was extracted with 3×50 mL EtOAc. The organic layers were combined and washed with 40 mL saturated NaCl (aq). The solvent was removed under reduced pressure and the residue purified by flash chromatography (20:1–9:1, $CHCl_3$:MeOH) to provide the desired material as a clear film (0.5 g, 100%): R_f (9:1:1 $CHCl_3$:MeOH:HOAc) 0.56; ^{1}H NMR 7.25 (m, 7H), 6.80 (d, 2H, J=8.8 Hz), 5.80 (s, 1H), 4.60 (s, 2H).

*3.5. Formation of thioester linker (**1**)*

To thioacid (1.1 equiv., 30 μmol/mL) and alcohol 3 (1.0 equiv.) in dry CH_2Cl_2 was added zinc iodide (0.5 equiv.). The heterogeneous mixture was refluxed in an inert atmosphere for at least 17 h during which it turned a light pink to red color. After cooling to room temperature the solution was filtered, and the solvent removed under reduced pressure. The residue was purified by flash chromatography (30:1–8:1, $CHCl_3$:MeOH) to provide the thioester as oils.[7]

References

1. Blake, J.; Li, C. H. *Proc. Natl. Acad. Sci. USA* **1981**, *78*, 4055–4058.
2. Schnolzer, M.; Kent, S. B. H. *Science* **1992**, *256*, 221–225
3. Canne, L. E.; Walker, S. M.; Kent, S. B. H. *Tetrahedron Lett.* **1995**, *38*, 1217–1220.
4. Yamashiro, D.; Li, C. H. *Int. J. Peptide Protein Res.* **1988**, *31*, 322–334.
5. Gauthier, J. Y.; Bourdon, F.; Young, R. N. *Tetrahedron Lett.* **1986**, *27*, 15–18.
6. All aminothioacids have an identical R_f and ^{1}H NMR to their oxyacid analogues.
7. All thioester linkers (**1**) have an R_f of 0.56 in 9:1:1 $CHCl_3$:MeOH:HOAc. Their ^{1}H NMR are identical to the superimposition of the *N*-BOC-aminothioacid with 4-benzoylphenoxyacetic acid (**3**).