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Generation of a cysteine sulfinic acid analog for incorporation in peptides using solid phase peptide synthesis

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ABSTRACT

The sulfinic acid analog of aspartic acid, cysteine sulfinic acid, introduces a sulfur atom that perturbs the acidity and oxidation properties of aspartic acid. Cysteine sulfinic acids are often introduced in peptides and proteins by oxidation of cysteine, but this method is limited as all cysteine residues are oxidized and cysteine residues are often oxidized to sulfonic acids. To provide the foundation for the specific incorporation of cysteine sulfinic acids in peptides and proteins, we synthesized a 9-fluorenylmethyloxycarbonyl (Fmoc) benzothiazole sulfone analog. Oxidation conditions to generate the sulfone were examined and oxidation of the Fmoc-protected sulfide (**3**) with NbC in hydrogen peroxide provided the corresponding sulfone (**4**) in the highest yield and purity. Reduction with sodium borohydride generated the cysteine sulfinic acid (**5**) suggesting this approach may be an efficient method to incorporate a cysteine sulfinic acid in biomolecules. A model tripeptide bearing a cysteine sulfinic acid was synthesized using this approach. Future studies are aimed at using this method to incorporate cysteine sulfinic acids in peptide hormones and proteins for use in the study of biological function.

Chemical synthesis of modified amino acids and their incorporation in peptides and proteins are powerful tools in the study of biological function and in the development of pharamaceuticals.[e.g.,1–4] For example, peptide synthesis was used to site specifically incorporate chemical modifications to study cellular processes (*e.g.*, protein tyrosyl phosphorylation and sulfurylation).[5–9] Additional examples include the use of peptides bearing fluorescent labels,[e.g.,10–12] and proteins bearing unnatural amino acids to investigate enzyme function.[e.g.,13–16]

Cysteine plays an important role in the use of amino acid analogs in biochemical studies, as the sulfhydryl group is specifically modified within peptides and proteins (Fig. 1). Indeed, prior studies report using the cysteine sulfhydryl group as a handle to introduce functional groups in enzymatic studies, e.g., cysteine alkylation to introduce lysine analogs in the active site of ribonuclease A.[15] Additional examples include using cysteine to tether fluorescent dyes in peptide antigens and protein-glycoprotein conjugates.[15,16] Furthermore, Benesik et al. introduced a sulfinic acid in the active site of ketosteroid isomerase to serve as an analog of aspartic acid,[17] and more recently cysteine sulfenic and sulfinic acids were used to evaluate oxidative posttranslational modifications in regulation.[e.g.,22]

While cysteine sulfinic acid has been used as an aspartic acid analog as described above, methods to synthesize peptides and proteins bearing cysteine sulfinic acids are limited. Cysteine sulfinic acids are often generated using hydrogen peroxide or sodium periodate to oxidize cysteine.[17] However, the oxidation reaction typically generates a mixture of the sulfinic acid and sulfonic acid (Fig. 2). The oxidation reaction lacks specificity so all cysteine residues in the protein are susceptible to oxidation. Methionine is also susceptible to oxidation yielding methionine sulfoxide.[23,24] To overcome these limitations, herein we describe the synthesis and application of a benzothiazole analog to incorporate cysteine sulfinic acid in peptides via solid phase peptide synthesis (see Scheme 1).

Incorporation of cysteine sulfinic acids via solid phase synthesis requires that the sulfinic acid (or protected analog) be stable to amino acid coupling conditions (typically formation and coupling of an active ester such as HBTU or HATU), subsequent couplings, cleavage from the resin/side chain deprotection, and purification. Multiple cysteine protecting groups have been developed including trityl (Trt), *t*-butyl (tBu), and acetamidomethyl (Acm), but these protecting groups introduce a thioether and are not suitable for introducing a sulfinic acid.[18,19] The carboxylate acid equivalent, aspartic and glutamic acid, are also commonly protected using *t*-butyl or Trt groups. While these groups may serve as protecting

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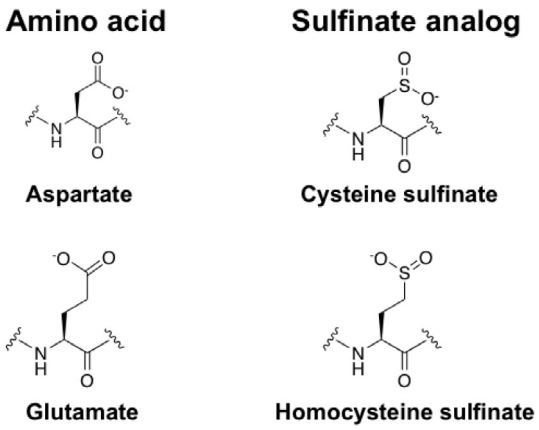


Fig. 1. Examples of amino acids and sulfinate analogs.

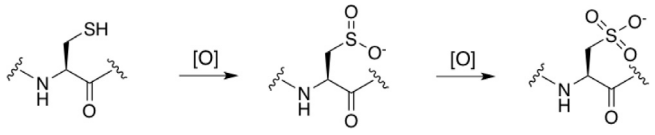


Fig. 2. Oxidation of the cysteine side chain to a sulfinate and sulfonic acid.

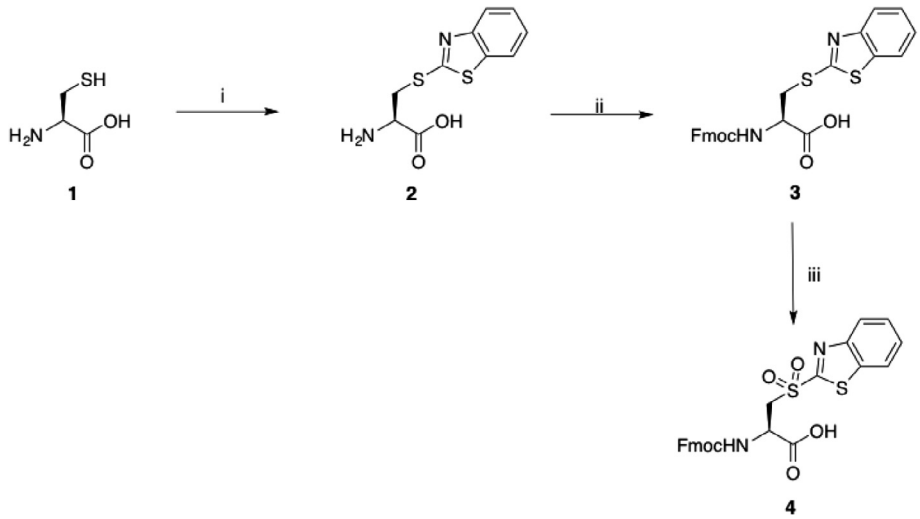
groups for the sulfinic acid, the sulfur is vulnerable to oxidation during peptide synthesis and purification.

Ueno et al. reported that 2-(alkylsulfonyl)benzothiazoles could serve as precursors of alkyl sulfinic acids.[20] The authors reported that methyl, ethyl, propyl, and phenyl sulfones were converted to the corresponding sulfinic acids upon treatment with sodium borohydride. Carruthers et al. later used this approach to generate sulfinic acid analogs of γ-aminobutyric acid, as the benzothiazole sulfone analogs of γ-aminobutryic acid were converted to the corresponding sulfinic acids following reaction with sodium borohydride.[21] Building on this work, we envisioned that cysteine benzothiazole sulfone could serve as a protecting group for introducing a cysteine sulfinic acid in a peptide. Following peptide synthesis, cleavage from the resin, and purification, the peptide could be treated with sodium borohydride to generate the sulfinic acid (Fig. 3).

Based on literature precedence, the sulfide was first generated by reacting l-cysteine with 2-cholorobenzothiazole in sodium methoxide.[22] l-Cysteine (*R*-stereoisomer) was used as the starting material to generate an analog bearing the same stereochemistry as l-aspartic acid (*S*-stereoisomer). It should be noted that while the *R/S* stereochemical designation differs for the carboxylate and sulfinate side chains, the stereochemistry remains the same. The amino group was then protected as the 9-fluorenylmethoxycarboyl (Fmoc) carbamate. The common conditions of 9-fluorenylmethyxyl carbonyl succinimide (Fmoc-OSu) and sodium carbonate or sodium bicarbonate in water and dioxane led to the Fmoc product but in ~50% yield. TLC analysis suggested the low yield was due to a large amount of unreacted starting material. Additional conditions were tested and we found that a reaction using 0.5 M boric acid pH 9.2 and acetonitrile as a cosolvent led to an increased yield of up to 80%.

Following protection of the amino group, the thioether was next oxidized to the corresponding sulfone. In the literature, Ueno et al. used potassium permanganate to oxidize the alkyl sulfides to the corresponding sulfones,[20] and Carruthers et al. later reported that higher yields were obtained using mCPBA.[21] We first followed the conditions reported by Carruthers et al., using two equivalents of mCPBA in dichloromethane. However, HPLC and TLC analysis suggested the oxidation reaction produced a mixture of sulfoxide and sulfone. The sulfone was generated as the major product when using ten equivalents mCPBA, but isolation of the product from mCPBA was problematic due to similar migrations of the amino acid and *meta*-chlorobenzoic acid during silica gel column chromatography.

To evaluate if different oxidation conditions might afford the sulfone product in higher yield, we investigated several recently reported conditions for the conversion of sulfides to sulfones. Tumula et al. recently reported the selective and high yielding oxidation of sulfides to sulfones using H_2O_2 in the presence of acetic acid and Amberlyst 15 as an acid catalyst.[23] In this study, the authors reported the quantitative conversion of a series of organic sulfides including dialkyl, dibenzyl, and diphenyl sulfides to the corresponding sulfones in 40–90 min at 50 °C. Based on this report, we tested oxidation of the Fmoc-protected cysteine sulfide with 30% H_2O_2 in the presence of acetic acid and Amberlyst 15. HPLC analysis of the reaction after 120 min suggested complete conversion of the starting material to a new compound, as a single new peak was observed and the peak corresponding to the starting



Scheme 1. Synthesis of amino acid analog **4**. Reagents and conditions: (1) 2-chlorobenzothiazole, NaOMe, MeOH, reflux 30 min then rt 16 h, 78%; (ii) Fmoc-OSu, 0.5 M boric acid pH 9.2/acetonitrile (2/3 v/v), rt, 16 h, 72%; (iii) NbC, 30% H_2O_2, EtOH, 50 °C, 2 h, 90%.