

Synthesis of Thiophenylalanine-containing Peptides via Cu(I)-mediated Cross-Coupling

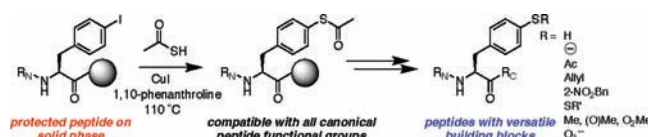
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ABSTRACT



Aryl thiolates have unique reactive, redox, electronic, and spectroscopic properties. A practical approach to synthesize peptides containing thiophenylalanine has been developed via a novel Cu(I)-mediated cross-coupling reaction between thioacetic acid and iodophenylalanine-containing peptides in the solid phase. This approach is compatible with all canonical proteinogenic functional groups, providing general access to aryl thiolates in peptides. Peptides containing thiophenylalanine (pK_a 6.4) were readily elaborated to contain methyl, allyl, and nitrobenzyl thioethers, disulfides, sulfoxides, sulfones, or sulfonates.

Cysteine residues play critical roles in protein folding, structure, catalysis, response to oxidative stress, metal binding, modification, and cell signaling.¹ These uses are dependent on the redox properties, acidity, and nucleophilicity of thiols and thiolates. The multiple oxidation states accessible to thiols are critical in their versatility in function. Aryl thiols may perform in similar roles but with reduced pK_a and electronically tunable properties.² Considering the special role of tyrosine in biomolecular recognition, thiophenylalanine represents an intriguing hybrid of tyrosine and cysteine residues.³

Thiophenylalanine and related amino acids have diverse potential applications in fields including medicinal chemistry, enzymology, protein design, materials science, and nanotechnology. However, there are very few published examples of thiophenylalanine-containing peptides. The first such description, applied by Escher in

angiotensin II analogues, employed sulfonylation of phenylalanine, followed by tin reduction, thiol protection, and amine protection to generate the Boc amino acids.⁴ DeGrado extended this approach to prepare Fmoc-thiophenylalanine and employed its disulfide in a peptide as a light-based switch of protein folding.⁵ In an alternative synthetic approach, Still used diazonium chemistry to access thiophenylalanine-containing peptides that were subsequently photocoupled to prepare thioether analogues of diaryl ether antibiotics.⁶ Recently, Hecht incorporated thiophenylalanine in place of an active site tyrosine of

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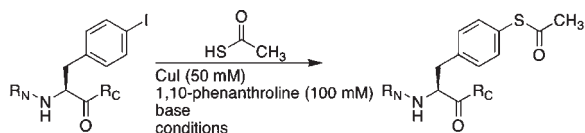
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topoisomerase I to examine the dependence of catalysis on the nucleophilic phenolate.⁷

In all of these cases, significant effort was required to synthesize protected thiophenylalanine. The development of a practical synthetic approach to thiophenylalanine, ideally employing commercially available amino acids without separate, additional purification steps, could greatly expand the applications of this amino acid. In view of the recent advances in metal-catalyzed cross-coupling reactions between aryl halides and thiols,^{8,9} we sought to develop an alternative approach to protected thiophenylalanine employing commercially available 4-iodophenylalanine and modification of a peptide via reaction on solid phase.

Copper catalysts have been most broadly employed for cross-coupling reactions with thiols, typically using aryl iodides. Therefore, we examined reaction conditions on solid phase within the protected model peptide Ac-T(4-I-Phe)PN-NH₂, which we have previously used to understand aromatic electronic effects in peptides.¹⁰

Table 1. Reaction Development



entry	phase	base	solvent	temp, °C	time, h	4-SAc-Phe	total cross-coupled products ^a
1	Solution	0.5 M Na ₂ CO ₃	<i>t</i> -amyl-OH	90	6	44%	59%
2	Solution	0.1 M DIPEA	<i>t</i> -amyl-OH	90	6	15%	26%
3	Solution	0.5 M DIPEA	<i>t</i> -amyl-OH	90	6	11%	60%
4	Solution	1 M DIPEA	<i>t</i> -amyl-OH	90	6	22%	58%
5	Solution	1 M DIPEA	<i>t</i> -amyl-OH	90	11	29%	65%
6	Solid	1 M DIPEA	<i>t</i> -amyl-OH	90	6	40%	45%
7	Solid	1 M DIPEA	<i>t</i> -amyl-OH	100	6	59%	69%
8	Solid	1 M DIPEA	<i>t</i> -amyl-OH	100	24	53%	90%
9	Solid	1 M DIPEA	<i>t</i> -amyl-OH	110	16	0%	99%
10	Solid	1 M DIPEA	toluene	100	18	57%	67%
11	Solid	1 M DIPEA	toluene	110	16	22%	99%

^a All cross-coupled products, including overacetylated side products in solution phase reactions, or disulfide products in solid phase reactions. R_N = Ac-Thr(*t*Bu)- (solid) or Ac-Thr- (solution). R_C = -Pro-Asn(Trt)-NH-Rink (solid) or -Pro-Asn-NH₂ (solution). Solid phase reactions were followed by TFA cleavage/deprotection.

Initial efforts to employ *t*-BuSH proceeded without success.¹¹ We next examined cross-coupling with thioacetic acid (Table 1), which could be subjected to thiolysis or hydrolysis in solution or on solid phase to yield the free thiophenylalanine-containing peptide.⁹ The conditions of Sawada et al., using 1,10-phenanthroline as a ligand and

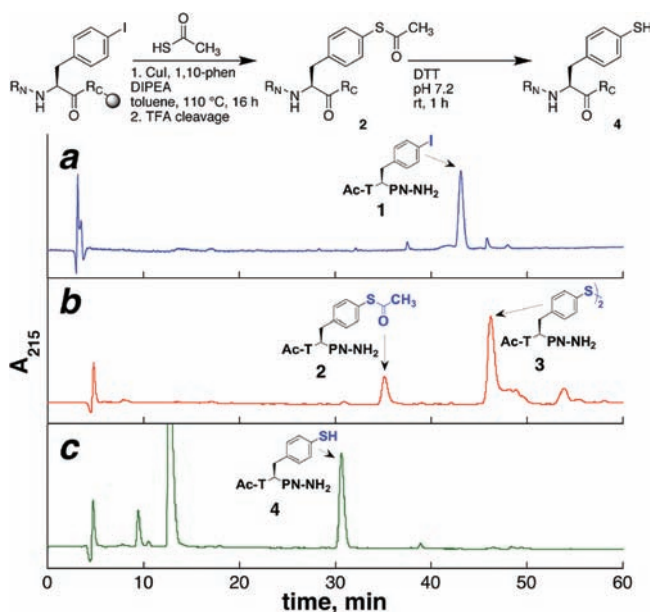


Figure 1. HPLC chromatograms of (a) Ac-T(4-I-Phe)PN-NH₂ peptide starting material (1); (b) peptide after cross-coupling reaction and peptide cleavage and deprotection, showing a mixture of thioacetyl (2) and disulfide (3) products; and (c) the result of treatment of the solution in (b) with DTT to effect thiolysis and reduction of disulfide, showing clean overall conversion to the 4-thiophenylalanine-containing product (4).

DIPEA as a base in toluene or *t*-amyl alcohol at 90–110 °C, resulted in good conversion on solid phase to a mixture of cross-coupled thioester and disulfide products. This mixture could be cleanly converted to the thiophenylalanine-containing peptide during standard cleavage/deprotection/purification steps (Figure 1). At 110 °C in toluene, iodophenylalanine underwent nearly complete conversion to cross-coupled products in 16 h. If the thioester product was desired, maximum yield was obtained by running the reaction at 100 °C, which decreased thiolysis and disulfide formation. The scope of this reaction with different thiols was briefly examined (Table 2). The reaction proceeded readily with thiophenol.⁶ Alkyl thiols reacted poorly under these conditions.

To further examine the scope of this reaction, a series of peptides, which included all canonical amino acid functional groups, was synthesized and examined for cross-coupling under the optimized reaction conditions (Figure 2). The peptides included model peptides as well as the trp cage miniprotein, with Tyr₃ replaced by 4-I-Phe.¹² Reaction on the trp cage provides a critical test to confirm that

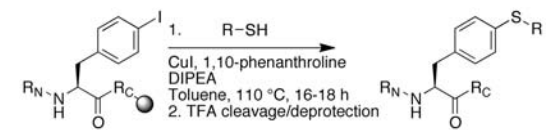
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





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Table 2. Thiol Scope of Reactions in the Solid Phase


entry	thiol (R-SH)	product	4-SR-Phe product	total cross-coupled products ^a
1		2	22%	99%
2		5	90%	90%
3		6	2%	2%
4			0%	0%
5		7	21% ^b	45% ^b
6		2	57% ^b	67% ^b

^a Includes disulfide products. ^b At 100 °C. R_N = Ac-Thr(*t*Bu)- (solid) or Ac-Thr- (solution). R_C = -Pro-Asn(Trt)-NHRink (solid) or -Pro-Asn-NH₂ (solution).

the reaction conditions do not modify peptide structure in a subtle manner that would not be identified by HPLC or MS.

Analysis by HPLC indicated clean reactions and high conversions to cross-coupled product in all cases (Figure 2). Trityl-protected cysteine residues exhibited evidence of Cys deprotection and acetylation under reaction conditions. After workup with DTT, both thiophenylalanine and Cys were generated cleanly as free thiols. Alternatively, to allow differentiation of Cys and thiophenylalanine, the peptide with *t*-butyl-protected Cys was subjected to reaction conditions, cleanly generating the peptide with free aryl thiol and protected Cys.

Analysis of the thiophenylalanine-containing trp cage peptide by circular dichroism (Figure S31) indicated that its structure and stability were similar to that of the native tyrosine-containing peptide. In total, these data indicate that the cross-coupling reaction may be employed to modify complex peptides with diverse amino acid substitutions and implies generality in the application of this reaction to synthesize peptides containing aryl thiols.

The UV–vis spectra of T(4-SH-Phe)PN revealed a strong dependence on pH, with a λ_{\max} of 249 nm as the thiol and an intense λ_{\max} of 276 nm as the thiolate ($\epsilon_{276} = 17500$) (Figure S1). The pK_a of thiophenylalanine was 6.4, significantly lower than cysteine (pK_a ~8–8.5). Notably, thiophenylalanine is primarily anionic at physiological pH. Thiophenylalanine was weakly fluorescent as the thiolate ($\lambda_{\max} = 405$ nm).¹³

The reactivity of thiols provides the opportunity to modify peptides to incorporate a range of functional groups, which was examined in T(4-SH-Phe)PN peptides. While alkyl thiols were poor substrates in cross-coupling reactions, the aryl thiolate was readily alkylated with methyl iodide or allyl iodide to generate the respective thioethers (Scheme 1).

(13) See the Supporting Information for details.

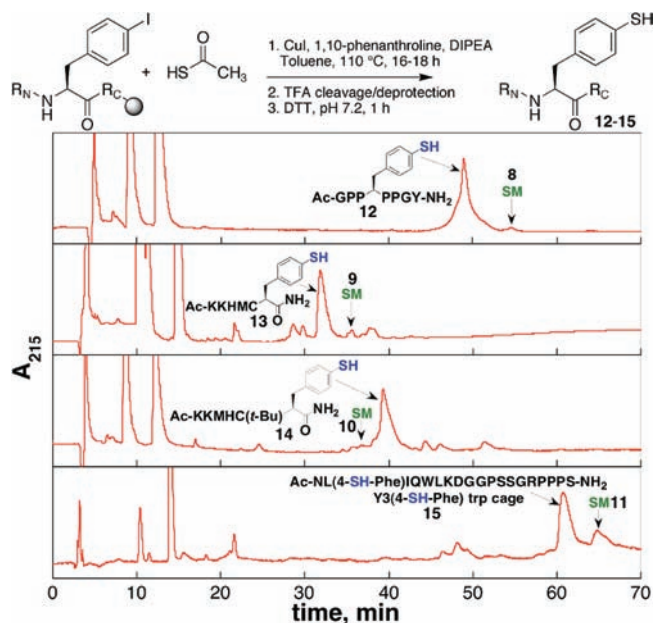
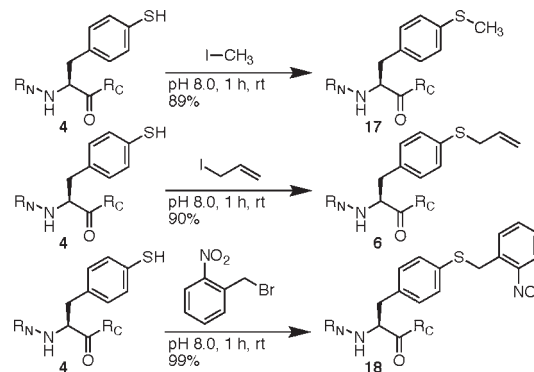


Figure 2. Crude HPLC chromatograms of cross-coupling reactions with different peptides. SM = starting material peptide.¹³

A photocleavable analogue of thiophenylalanine was also prepared by alkylation with 2-nitrobenzyl bromide.¹⁴ In addition the thioester could be deprotected and alkylation effected on solid phase (Scheme 2).¹³

Scheme 1. Solution Alkylation Reactions on 4^a



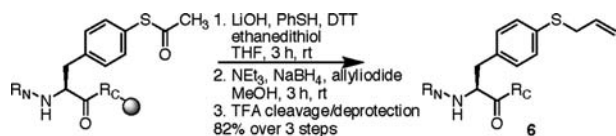
^a R_N = Ac-Thr-, R_C = -Pro-Asn-NH₂.

Allyl thioethers have been shown by Davis to be superior substrates to allyl ethers for cross-metathesis reactions.¹⁵ Aryl allyl thioethers thus have potential as reactive substrates for bioorthogonal cross-metathesis reactions.^{15c}

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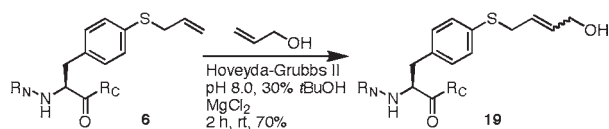
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Scheme 2. Solid Phase Alkylation of Ac-TXPN-NH₂



To explore this possibility, the *S*-allyl-thiophenylalanine-containing peptide **6** was subjected to Davis' cross-metathesis conditions and found to react with allyl alcohol at room temperature (Scheme 3).¹³

Scheme 3. Cross-metathesis on Ac-T(4-SAllyl-Phe)PN-NH₂



Thiol oxidation state dramatically impacts protein structure and function.^{1,16} Thiophenylalanine-containing peptides were subjected to a variety of oxidation reactions, including formation of the activated disulfide, glutathionylated mixed disulfide, sulfoxide, sulfone, or sulfonate (Scheme 4).¹⁷

Aryl electronics can significantly affect protein structure and function.^{10,18} The derivatives described above represent a continuum from strongly electron-donating to electron-withdrawing groups. In the TXPN (X = aryl) context, the strength of an aromatic-proline CH/ π interaction is manifested in stabilization of a *cis* amide bond by more electron-donating substituents. All model peptides were examined by NMR and $K_{\text{trans/cis}}$ quantified to identify the structural effects of aryl substitution (Table 3). The data indicate that thiophenylalanine and its modified variants exhibit a range of electronic properties that might be exploited for control of peptide structure.

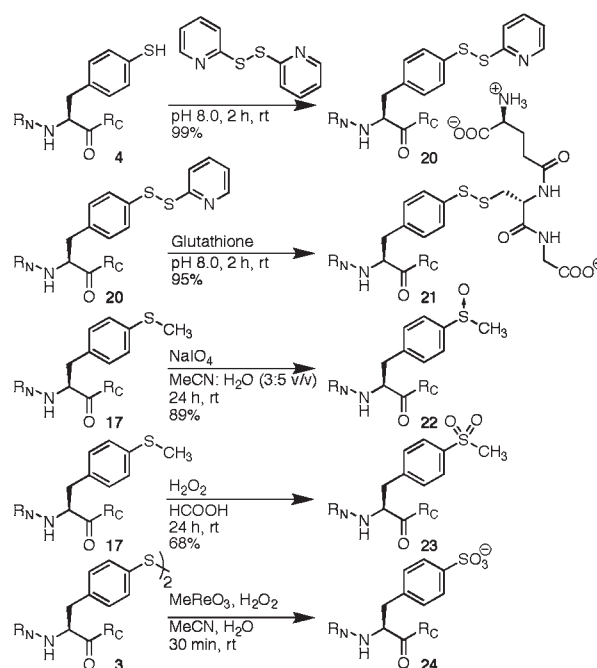
We have described a practical approach to the synthesis of thiophenylalanine-containing peptides that is compatible with all proteinogenic functional groups. The method employs the commercially available amino

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Scheme 4. Solution Phase Peptide Oxidation Reactions^a



^a R_N = Ac-Thr-, R_C = Pro-Asn-NH₂.

Table 3. Electronic Effects of Aryl Substitution on Peptide Structure ($K_{\text{trans/cis}}$ of the prolyl amide bond)

Ac-TXPN-NH ₂ , X =	$K_{\text{trans/cis}}$	$\Delta G_{\text{trans/cis}}$ kcal mol ⁻¹	$\Delta\Delta G_{\text{trans/cis}}$ kcal mol ⁻¹
4-S ⁻ -Phe (4 , pH 8.5)	2.2	-0.47	0.22
4-S-SPyridyl-Phe (20)	2.9	-0.63	0.06
4-SAllyl-Phe (6)	3.3	-0.71	-0.02
4-SH-Phe (4 , pH 4.0)	3.5	-0.74	-0.05
4-SCH ₃ -Phe (17)	3.5	-0.74	-0.05
4-SPh-Phe (5)	3.6	-0.76	-0.07
4-S-SGlutathione-Phe (21)	3.7	-0.77	-0.08
4-SAc-Phe (2)	4.3	-0.86	-0.17
4-SO ₃ ⁻ -Phe (24)	4.4	-0.88	-0.19
4-S(2-Nitrobenzyl)-Phe (18)	4.4	-0.88	-0.19
4-S(O)Me-Phe (22)	4.7	-0.92	-0.23
4-SO ₂ Me-Phe (23)	5.9	-1.05	-0.36
Phe(F) ^{10c}	3.2	-0.69	0.00
Tyr(Y) ^{10c}	2.7	-0.59	0.10

acid 4-iodophenylalanine and is readily applied to fully synthesized peptides, providing convenient access to versatile thiol-modified aromatic amino acids.

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Supporting Information Available. Experimental procedures, HPLC chromatograms, and spectral and characterization data. This material is available free of charge via the Internet at <http://pubs.acs.org>.