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4*R*- and 4*S*-iodophenyl hydroxyproline, 4*R*-pentynoyl hydroxyproline, and *S*-propargyl-4-thiolphenylalanine: conformationally biased and tunable amino acids for bioorthogonal reactions†

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Bioorthogonal reactions allow the introduction of new functionalities into peptides, proteins, and other biological molecules. The most readily accessible amino acids for bioorthogonal reactions have modest conformational preferences or bases for molecular interactions. Herein we describe the synthesis of 4 novel amino acids containing functional groups for bioorthogonal reactions. (2*S*,4*R*)- and (2*S*,4*S*)-iodophenyl ethers of hydroxyproline are capable of modification *via* rapid, specific Suzuki and Sonogashira reactions in water. The synthesis of these amino acids, as Boc-, Fmoc- and free amino acids, was achieved through succinct sequences. These amino acids exhibit well-defined conformational preferences, with the 4*S*-iodophenyl hydroxyproline crystallographically exhibiting β-turn (ϕ, $\psi \sim -80°$, 0°) or relatively extended (ϕ, $\psi \sim -80°$, +170°) conformations, while the 4*R*-diastereomer prefers a more compact conformation ($\phi \sim -60°$). The aryloxyproline diastereomers present the aryl groups in a highly divergent manner, suggesting their stereospecific use in molecular design, medicinal chemistry, and catalysis. Thus, the 4*R*- and 4*S*-iodophenyl hydroxyprolines can be differentially applied in distinct structural contexts. The pentynoate ester of 4*R*-hydroxyproline introduces an alkyne functional group within an amino acid that prefers compact conformations. The propargyl thioether of 4-thiolphenylalanine was synthesized *via* copper-mediated cross-coupling reaction of thioacetic acid with protected 4-iodophenylalanine, followed by thiolysis and alkylation. This amino acid combines an alkyne functional group with an aromatic amino acid and the ability to tune aromatic and side chain properties *via* sulfur oxidation. These amino acids provide novel loci for peptide functionalization, with greater control of conformation possible than with other amino acids containing these functional groups.

Introduction

Bioorthogonal reactions have generated unprecedented opportunities for peptide and protein modification, with applications in medicinal chemistry, biological probes, materials, and imaging.[1] While the copper-mediated azide–alkyne cycloaddition has been the most widely employed bioorthogonal reaction, other key reactions include strain-promoted azide–alkyne cycloaddition, 1,2-aminothiol/thioester reaction (native chemical ligation), Diels–Alder (diene/dienophile) reaction, tetrazine/*trans*-cyclooctene or cyclopropene inverse-electron-demand Diels–Alder reaction, Staudinger ligation (azide/phosphine), oxime and hydrazone formation (aldehyde or ketone/hydroxylamine or hydrazine), Suzuki reaction (aryl halide/boronic acid), Sonogashira reaction (aryl halide/alkyne), and Grubbs cross-metathesis (alkene/alkene).[2] Bioorthogonal reaction chemistries have also been applied to the synthesis of diverse, constrained, and macrocyclic peptides, which can result in rigid medium-sized molecules with protein-like stability and function.[3]

The applications of bioorthogonal reactions are dependent on the ability to site-selectively introduce reactive functional groups into molecules. Functional groups may be incorporated *via* unnatural amino acids, *via* chemical modification of peptides or proteins (cysteine or lysine modification, derivatization at the N-terminus), or *via* genetic incorporation (expression with unnatural amino acids either using suitable

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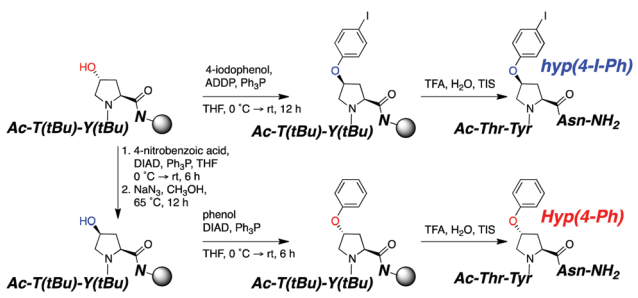
†Electronic supplementary information (ESI) available: Synthetic procedures, characterization data, and ^{1}H and ^{13}C NMR spectra for all new compounds; peptide synthesis details and MS and NMR characterization data; additional crystal structure information; and CIF files for crystal structures. CCDC 1438138–1438140. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/c5ob02473k

auxotrophs or through amber codon approaches).[2i,4] These approaches typically involve modification at relatively flexible sites on peptides or proteins, including the use of long-chain amino acids or flexible linkers. While these approaches have been broadly used, for some applications tight control of structure at the site of functionalization would be preferable, particularly within compact recognition motifs and macrocycles. Azidoprolines and aminoxyprolines are the best-developed examples of amino acids that both introduce bioorthogonal functional groups and impose inherent structural control.[5] Proline residues are particularly well suited as sites of modification, as prolines are often solvent-exposed at surface loops and turns in proteins.[6] In addition, proline plays important structural roles due to the conformational constraint of the cyclic amino acid. Moreover, further fine-tuning of conformation is possible *via* the stereospecific 4-substitution of prolines. Proline substitution allows predictable control of proline ring pucker, which is coupled to peptide main chain conformation, providing the possibility of significant conformational stabilization *via* the modification.[7]

We previously developed a method, termed proline editing, that allows the stereospecific incorporation of diverse functional groups into proline amino acids within fully synthesized peptides. This approach provides the possibility for the introduction of functional groups with defined structural control.[7d,e,8] Within this work, we demonstrated the incorporation of a series of bioorthogonal groups at proline within peptides, including azide, alkyne, 1,2-aminothiol, aryl bromide, aryl iodide, dienophile, hydroxylamine, ketone, and tetrazine, and the subsequent application of these groups in reactions on peptides in water.[7e]

Among these groups, the aryl iodides and the alkynes seemed particularly appealing for further development, due to the fast and selective reactions of aryl halides in aqueous Suzuki and Sonogashira reactions using the ligands of Davis and Lin and the relatively smaller number of methods to incorporate both functional groups within peptides with structural control.[2i,j] Both the 4*R*- and 4*S*-diastereomers of the aryl ethers of hydroxyproline are readily synthetically accessible (Scheme 1),[7e,9] and both exhibit substantially distinct conformational preferences (Fig. 1 and 2, Table 1), including strong biases on the ϕ, ψ, and ω torsion angles. Moreover, the iodophenyl ethers of



Scheme 1 Synthesis of peptides containing hyp(4-I-Ph) and Hyp(4-Ph) *via* proline editing.[7e] The 4*R*-hydroxyproline was incorporated in the peptide as a free alcohol (*via* Fmoc-Hyp-OH), *in situ* protected on solid phase using trityl chloride, the peptide synthesis completed, and the trityl group selectively removed to yield the peptide with the free proline hydroxyl.[7d,e,8]

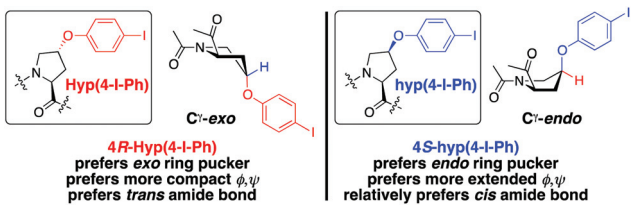


Fig. 1 4*R*- and 4*S*-substituted hydroxyproline iodophenyl ethers and the general conformational preferences for proline derivatives with electron-withdrawing 4-substituents. Hyp (upper case) indicates 4*R*-hydroxyproline, while hyp (lower case) indicates 4*S*-hydroxyproline.

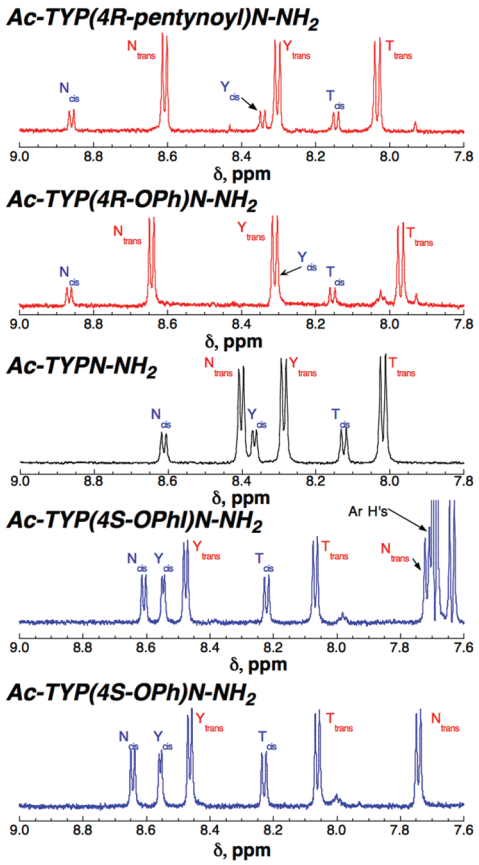
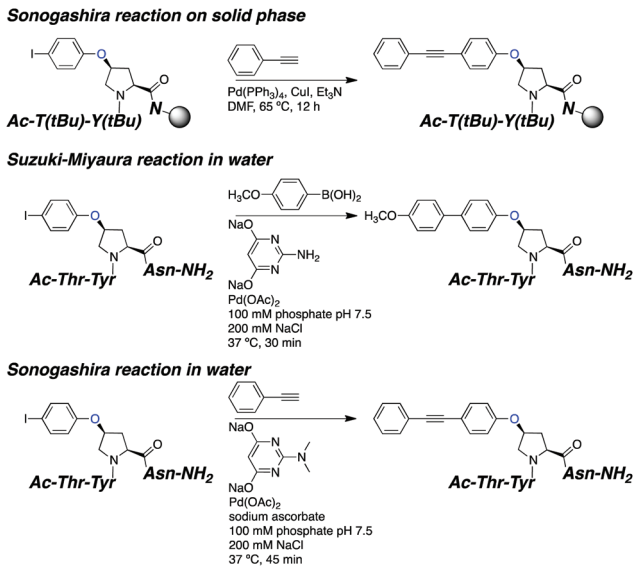


Fig. 2 NMR spectra (amide region) of Ac-TY*Prox*N-NH_2 peptides containing proline and the proline derivatives Hyp(C(O)CH_2CH_2CCH), Hyp(Ph), hyp(Ph), and hyp(4-I-Ph) (*Prox* = proline or substituted proline) (data derived from ref. 7*e*). The ratio of proline *trans* to *cis* amide bond ($K_{trans/cis}$) provides a readout of the conformational bias of the 4-substituent, which depends on the stereochemistry, electronic, and steric properties of the substituent. In addition, the *exo versus endo* ring pucker preferences are seen in the chemical shifts and coupling constants to the Hβ and Hδ hydrogens, with greater dispersion between the diastereotopic Hβ protons and between the diastereotopic Hδ protons in the *endo* conformation compared to the *exo* conformation (see *e.g.* ref. 7*e*, page S25;† see also refs).[40]

Table 1 Properties of Ac-TZ*Prox*N-NH_2 peptides in 90% H_2O/10% D_2O with 5 mM phosphate pH 4 and 25 mM NaCl at 298 K as determined by NMR spectroscopy.[7e,8,10] $K_{trans/cis}$ = ratio of the population of species with *trans* amide bond to species with *cis* amide bond. $\Delta G = -RT \ln K_{trans/cis}$. $\Delta\Delta G = \Delta G_{peptide} - \Delta G_{TYPN}$. $^3J_{\alpha N}$ = coupling constant between amide H and Hα, which can be correlated to the backbone torsion angle ϕ *via* a parametrized Karplus equation.[47] Smaller values of $^3J_{\alpha N}$ indicate more compact conformations at this residue, while larger values indicate more extended conformations. Changes in δ are associated with changes in the conformations of the peptides as a function of the 4-substituent. n.d. = not determined due to spectral overlap

Ac-TZ*Prox*N-NH_2 Z=	*Prox*=	$K_{trans/cis}$	ΔG, kcal mol^{-1}	$\Delta\Delta G$, kcal mol^{-1}	$^3J_{\alpha N}$, Tyr$_{cis}$	δ, Tyr$_{cis}$	δ, Asn$_{trans}$
Tyr	Hyp(C(O)CH_2CH_2CCH)	5.8	−1.04	−0.45	7.8	8.34	8.61
Tyr	Hyp(Ph)	4.8	−0.93	−0.34	n.d.	8.31	8.64
4-*S*-Allyl-Phe	Pro	3.4	−0.72	−0.13	6.7	8.40	8.43
Tyr	Pro	2.7	−0.59	0.00	6.1	8.37	8.40
Tyr	hyp(4-I-Ph)	2.1	−0.44	0.15	5.4	8.58	7.72
Tyr	hyp(Ph)	1.7	−0.31	0.26	4.6	8.56	7.74

hydroxyproline readily undergo Suzuki and Sonogashira reactions (Scheme 2) in solution phase, using the conditions of Davis or Lin,[2i,j] or on solid phase,[9c] suggesting that these derivatives could find significant use as loci for modification with structural control.



Scheme 2 Solid-phase Sonogashira reaction and solution-phase aqueous Suzuki–Miyaura and Sonogashira reactions with peptides containing hyp(4-I-Ph).[7e]

Propargylglycine is the most common alkyne amino acid typically employed for click chemistry applications (Fig. 3). Most other commercially available or readily synthesized alkyne derivatives are relatively flexible or lacking in fine conformational control.[5g] In prior work, we demonstrated that the pentynoate of 4*R*-hydroxyproline exhibits a strong bias for an *exo* proline ring pucker and *trans* amide bonds, suggesting its application when this conformation is preferred (Scheme 3, Fig. 2 and Table 1).[7e] In addition, we have previously developed methods for the synthesis of the amino acid 4-thiolphenylalanine, and derivatives thereof, including the *S*-allyl substitution (Scheme 4).[10] Alkylated 4-thiolphenylalanine differs from tyrosine in the smaller C–S–C bond angle (typically ~100°) than the C–O–C bond angle of tyrosine (~120°), resulting in a different presentation of substituents and a different conformation when present within a macrocycle (Fig. 3). In addition, sulfur provides an additional aspect of tunability in conformation (loss of coplanarity with the aromatic ring) and in effects on the electronics of the aromatic ring[10,11] *via* oxidation to the sulfoxide or sulfone, states that are not possible for tyrosine. Using similar synthetic methods, the *S*-propargyl derivative should be readily synthesized, and is expected to have similar structural effects as the *S*-allyl derivative previously described. Thus, the pentynoate of 4*R*-hydroxyproline and 4-*S*-propargyl-thiolphenylalanine are distinct alkyne amino acids which could find unique applications due to their conformational constraint or tunable structural control.

Our previous efforts in the use of unnatural amino acids have focused on the development of practical solid-phase

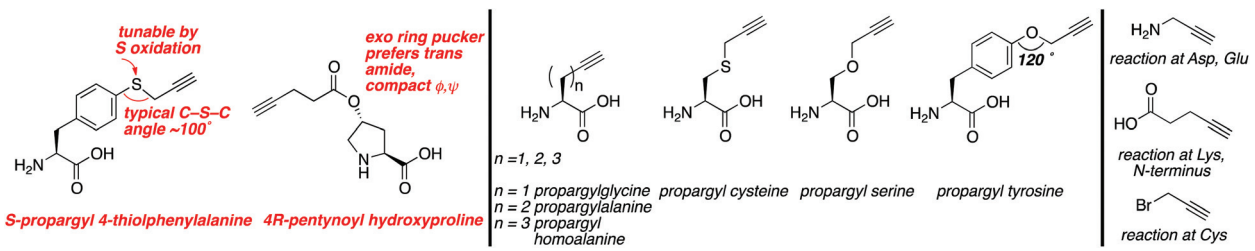
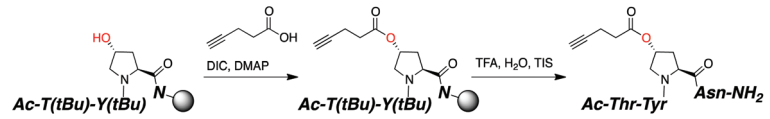
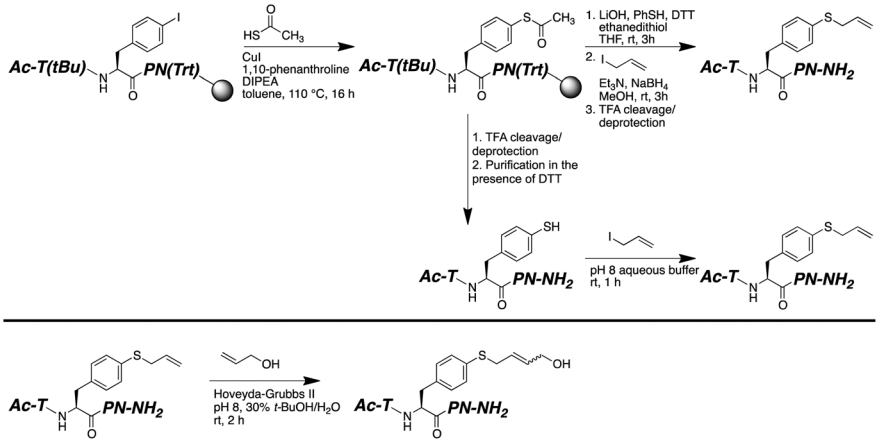


Fig. 3 *S*-Propargyl 4-thiolphenylalanine, 4*R*-pentynoyl hydroxyproline, commercially available alkyne-containing α-amino acids, and commonly used reagents for peptide and protein modification to incorporate alkynes.[41]



Scheme 3 Synthesis of peptide containing 4*R*-hydroxyproline pentynoate *via* proline editing.[7e] The hydroxyproline was incorporated as a free alcohol, *in situ* protected on solid phase using trityl chloride, the peptide synthesis completed, and the trityl group selectively removed to yield the protected peptide with a free hydroxyl.[7d,e,8]



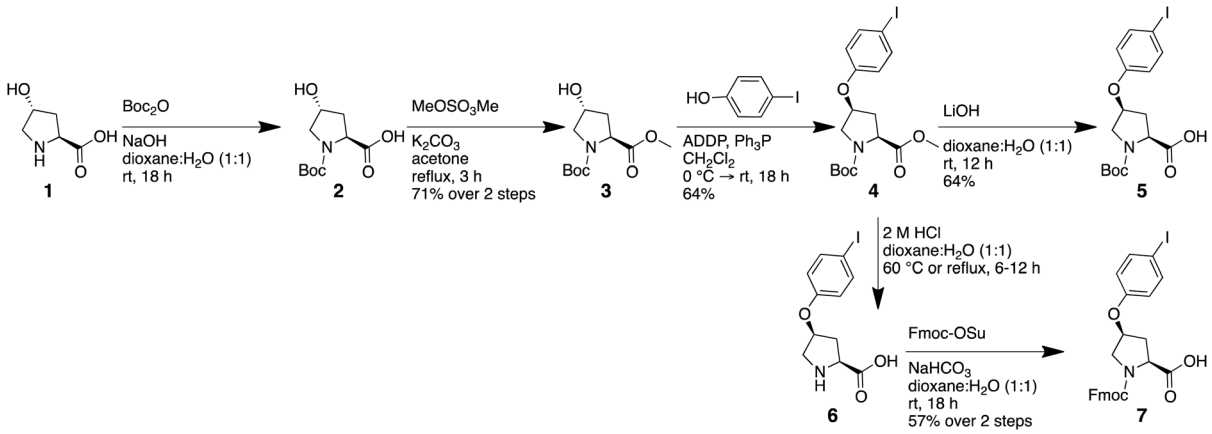
Scheme 4 (top) Synthesis of peptide containing *S*-allyl 4-thiolphenylalanine *via* cross-coupling reaction on peptide on solid phase followed by thiolysis and alkylation on solid phase.[10] The thiolysis and alkylation reaction sequence was alternatively conducted in solution phase. (bottom) Grubbs cross-methathesis[45] of peptide containing *S*-allyl 4-thiolphenylalanine.[10] Due to chalcogen effects on cross-metathesis, this reaction proceeded under milder conditions (room temperature, *versus* 37 °C; no added salt, *versus* 180 mM $MgCl_2$) than the reaction on peptides with *O*-allyl tyrosine.[2k,4f,46]

peptide modification chemistries that allow the synthesis of diverse amino acids using commercially available precursors. In our efforts to synthesize novel cyclic proteins with bioorthogonal functional groups and containing multiple disulfide bonds, we sought alternative solution-phase approaches to these amino acids, in order to maximize the chemical flexibility in the incorporation of these amino acids *via* peptide synthesis. Therefore, we sought to develop solution-phase approaches to the Fmoc amino acids for bioorthogonal reactions, for the broadest possible range of their applications.

Results

The iodophenyl ether of 4*S*-hydroxyproline **4** was synthesized *via* Mitsunobu reaction of iodophenol with the methyl ester of Boc-4*R*-hydroxyproline **3** (Scheme 5), which is readily synthesized from inexpensive commercial sources (**1** or **2**). The Boc-protected methyl ester of **4** and other proline derivatives synthesized herein were analyzed by NMR for comparison of their conformational properties (Table 2). Ester hydrolysis generated the Boc 4*S*-iodophenyl hydroxyproline amino acid **5**. This amino acid crystallized from ethyl acetate/hexanes (Fig. 4 and Table 3). The crystal structure exhibited an *endo* proline ring pucker, a *cis* Boc-proline bond, and main chain torsion angles ϕ, ψ that were ideal for β-turn formation (ϕ, ψ = −77°, +1°, near the ideal values for the i + 2 residue of type I (−90°, 0°), type II′ (−80°, 0°), or type VIa1 (−90°, 0°) β-turns).[12] This conformation was also observed in the crystal structure of Ac-hyp-OMe (CSD: EMITEA; ϕ, ψ = −84°, +18°).[13] β-Turns are central components of protein structure,[12a,b,14] and are also common recognition motifs in protein–protein interactions,[15] suggesting the use of this amino acid (and other 4*S*-substituted prolines) in the stabilization of β-turn motifs in peptides and proteins and in molecular recognition.[16]

The Boc-protected methyl ester **4** was alternatively readily converted into the free amino acid **6** and the Fmoc amino acid **7** *via* standard approaches (Scheme 5). The Fmoc amino acid 4*S*-iodophenyl hydroxyproline **7** crystallized from methanol, interestingly revealing an alternative favorable conformation for this amino acid (Fig. 5 and Table 3). Here, the 4*S*-amino acid adopts an *endo* ring pucker with a more extended conformation of proline (ϕ, ψ = −83°, +174°). This conformation is consistent with the typical conformation observed for 4*S*-substituted prolines with electron-withdrawing substituents,



Scheme 5 Synthesis of Boc- and Fmoc-hyp(4-I-Ph).

Table 2 NMR properties ($CDCl_3$) of Boc-protected methyl esters of proline derivatives

Boc-*Prox*-OMe *Prox*=	Compound	$K_{trans/cis}$	$\Delta G_{trans/cis}$, kcal mol^{-1}
Hyp	**3**	1.5	−0.24
Hyp(4-I-Ph)	**10**	1.5	−0.24
hyp(4-I-Ph)	**4**	1.3	−0.16
hyp(4-NO_2-Bz)	**8**	1.2	−0.11
hyp	**9**	1.1	−0.06

including 4*S*-fluoroproline, which stabilizes collagen mimetic peptides when at the Xaa (Pro) position of the canonical Pro-Hyp-Gly collagen repeats, where an *endo* ring pucker and more extended proline conformation are preferred.[7b,17] Collectively, the crystal structures of **5** and **7** demonstrate the strong conformational preferences of 4*S*-substituted prolines containing electron-withdrawing groups to adopt defined proline conformations in ϕ, while simultaneously presenting aryl halide functional groups within structurally defined environments.

The 4*R*-iodophenyl hydroxyproline was synthesized following a standard sequence for 4*R*-substituted prolines, in which the protected 4*R*-hydroxyproline **3** was subjected to Mitsunobu reaction with 4-nitrobenzoic acid to invert the stereochemistry, followed by azidolysis (Scheme 6).[5a,18] The resulting protected 4*S*-hydroxyproline **9** was then subjected to a second Mitsunobu reaction with 4-iodophenol to generate the doubly inverted 4*R*-hydroxyproline iodophenyl ether. This second Mitsunobu reaction with iodophenol required ADDP to proceed in reasonable yield, as was also the case in the synthesis of the 4*S*-iodophenyl hydroxyproline (Scheme 5).[19] The Boc-protected methyl ester **10** was then subjected to acid hydrolysis to generate the free amino acid, followed by Fmoc protection under standard conditions to generate the Fmoc 4*R*-hydroxyproline iodophenyl ether **12**. This amino acid has been previously synthesized within an inhibitor of the hepatitis C virus NS3 protease.[9c] Interestingly, within this inhibitor series, the halogens showed a periodic trend in IC_{50}, with the aryl iodide the most potent

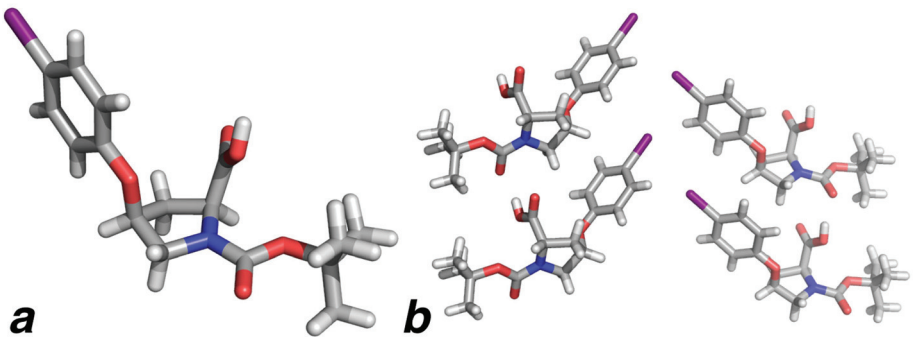


Fig. 4 (a) X-ray crystal structure of Boc-4*S*-(4-iodophenyl) hydroxyproline (Boc-hyp(4-I-Ph)-OH) (**5**). The structure exhibits a *cis* Boc-proline bond (ω = +1.4°), an *endo* proline ring pucker, and (ϕ, ψ) = (−76.5°, +1.4°). These data are consistent with the ability of this amino acid to promote relatively more extended proline conformations in ϕ, to function as the *i* + 2 residue of type I, type II′, and type VIa1 β-turn conformations (ideal ϕ = −80° to −90°, ψ = 0°), and to promote *cis* amide bonds (ω = 0°). (b) The crystal assembly is mediated by hydrogen bonding between the carbamate carbonyl and the carboxylic acid, as well as by halogen–halogen interactions[20,42] between iodines (I–I distance 3.959 Å, I–I–C angle 76.7°, I–I–I angle 110.3°).

Table 3 Crystallographic data and refinement details

	5	7	10
Empirical formula	$C_{16}H_{20}INO_5$	$C_{27}H_{26}INO_6$	$C_{17}H_{22}INO_5$
Resolution (Å)	0.77	0.76	0.74
formula weight	433.23	587.39	447.25
T (K)	200(2)	200(2)	200(2)
Wavelength (Å)	0.71073	0.71073	0.71073
Crystal system, space group	Orthorhombic, $P2_12_12_1$	Monoclinic, $P2_1$	Monoclinic, $P2_1$
Unit cell dimensions (Å, °)	a = 6.4983(17), α = 90° b = 10.457(3), β = 90° b = 27.274(7), β = 90°	a = 9.8340(9), α = 90° b = 8.9990(8), β = 106.4220(14)° b = 14.7646(14), β = 90°	a = 6.5131(13), α = 90° b = 16.5.81(3), β = 95.961(3)° b = 8.9446(18), β = 90°
Volume (Å^3)	1853.4(8)	1253.3(2)	960.7(3)
Z, Z', calcd density (g cm^{-3})	4, 0, 1.553	2, 0, 1.556	2, 0, 1.546
Absorption coeffiecient (mm^{-1})	1.75	1.32	1.691
F(000)	864	592	448
Crystal size (mm)	0.248 × 0.210 × 0.078	0.324 × 0.281 × 0.180	0.266 × 0.123 × 0.114
θ range for data collection	1.493 to 27.637°	2.159 to 27.780°	2.289 to 28.677°
Limiting indices	$-7 \le h \le 8$, $-13 \le k \le 13$, $-31 \le l \le 35$	$-12 \le h \le 12$, $-11 \le k \le 11$, $-19 \le l \le 19$	$-8 \le h \le 8$, $-22 \le k \le 22$, $-12 \le l \le 12$
Reflns collected/unique	14 104/4306 [R(int) = 0.0694]	19 303/5883 [R(int) = 0.306]	15 874/4955 [R(int) = 0.0432]
Completeness to θ = 25.242	100.00%	99.90%	100.00%
Absorption correction	Multiscan	Multiscan	Multiscan
Max and min transmission	0.7456 and 0.5887	0.7970 and 0.6740	0.7458 and 0.6471
Refinement method	Full-matrix least-squares on F^2	Full-matrix least-squares on F^2	Full-matrix least-squares on F^2
Data/restraints/parameters	4306/0/211	5883/2/323	7955/1/221
goodness-of-fit on F^2	1.017	1.017	0.964
Final R indices [$I > 2\sigma(I)$]	R = 0.0454, wR^2 = 0.0756	R = 0.0281, wR^2 = 0.0590	R = 0.0374, wR^2 = 0.0725
R indices (all data)	R = 0.0905, wR^2 = 0.0924	R = 0.0352, wR^2 = 0.0626	R = 0.0480, wR^2 = 0.0765
Absolute structure parameter	−0.02(2)	−0.023(7)	−0.031(14)
Largest diff peak and hole (e Å^{-3})	0.408 and −0.738 e Å^3	0.502 and −0.394 e Å^3	0.856 and −0.385 e Å^3

derivative, 2.5-, 5-, and 10-fold more potent than the bromide, chloride, and fluoride, respectively, potentially due to halogen bonding effects that favor interactions with iodides.[20] This amino acid thus could also find applications in molecular design based on halogen bonding.

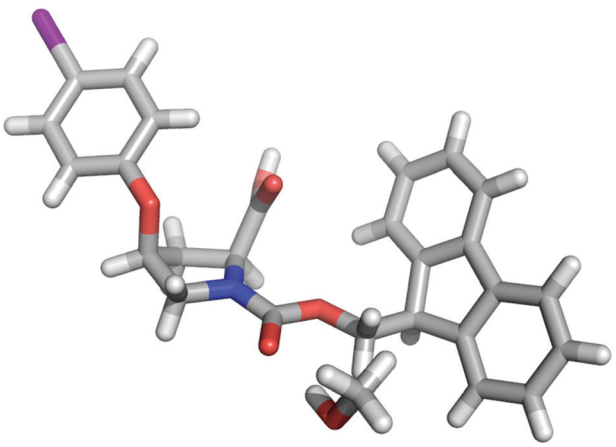
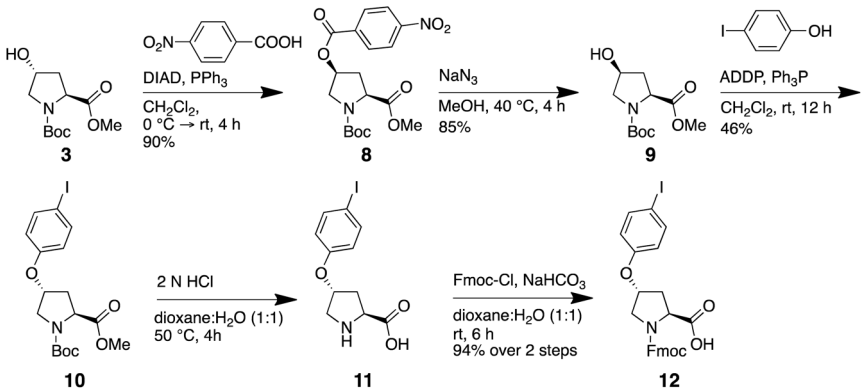


Fig. 5 X-ray crystal structure of Fmoc-4*S*-(4-iodophenyl) hydroxyproline (Fmoc-hyp(4-I-Ph)-OH) (**7**). The structure exhibits a *cis* Fmoc-proline bond (ω = +8.6°), an *endo* proline ring pucker, and (ϕ, ψ) = (−83.1°, +174.0°). These data are consistent with the ability of this amino acid to promote relatively extended conformations of proline and/or *cis* amide bonds (ω = 0°). In addition, one proline Hβ exhibits a close intramolecular C−H/π interaction (2.65 Å) with the *ipso* carbon of the iodophenyl ring which could potentially restrict the iodophenyl ether conformation.[11,43] Crystal assembly is promoted by hydrogen bonding of the carbamate carbonyl and carboxylic acid to methanol and T-shaped stacking of the iodophenyl and Fmoc aromatic rings of adjacent molecules.[44]

The methyl ester of Boc-4*R*-iodophenyl hydroxyproline **10** crystallized from chloroform. The crystal structure (Fig. 6) revealed an *exo* ring pucker, a *trans* Boc-proline bond, and a compact conformation of proline (ϕ, ψ = −55.9°, −39.2°). The structure also exhibited a short distance between adjacent carbonyls indicative of a favorable n → π* interaction (2.836 Å O···C distance, below the 3.22 Å sum of the van der Waals radii of O and C; 109.7° O···C=O angle, near the ideal Bürgi-Dunitz trajectory) that is promoted by 4*R*-substituted prolines and that stabilizes α-helix and PPII conformations.[7a,b,21] These data, including the compact $\phi \sim -60°$, suggest the application of 4*R*-iodophenyl hydroxyproline in contexts where a more compact proline conformation (α_R or PPII) is preferred, including at the i + 1 position of type I, type II, or type VIa1 β-turns.[12a,b,14,22] Indeed, Raines and coworkers observed that related 4*R*-hydroxyproline derivatives (–OH, –OMe, –OAc) can alternatively adopt α_R or PPII conformations, with both conformations exhibiting favorable n → π* interactions.[22] These data also emphasize the conformational control possible with the judicious use of appropriate 4*R*- and 4*S*-substituted prolines, with each stereoisomer exhibiting divergent presentation of its functional group and distinct conformational preferences, suitable for structurally orthogonal applications (Fig. 7).[7,8,16b,c,17c,23]

In order to further characterize the conformational effects of this amino acid, it was incorporated into a peptide in the Ac-TY*Prox*N-NH_2 model system used to quantify stereoelectronic effects of 4-substitutions on proline.[7e,8] The resultant



Scheme 6 Synthesis of Fmoc-Hyp(4-I-Ph).

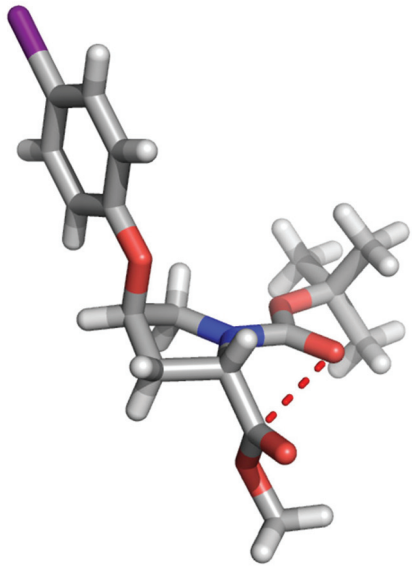


Fig. 6 X-ray crystal structure of Boc-4*R*-(4-iodophenyl) hydroxyproline methyl ester (Boc-Hyp(4-I-Ph)-OMe) (**10**). The structure exhibits a *trans* Boc-proline bond (ω = +180.0°), an *exo* proline ring pucker, and (ϕ, ψ) = (−55.9°, −39.2°). This amino acid also exhibits an n → π^* interaction between adjacent carbonyls (O···C═O distance 2.836 Å, angle 109.7°). The α-helical conformation (α_R) of this amino acid is consistent with its application at the N-terminus of α-helices and at the *i* + 1 position of type I β-turns. This ϕ torsion angle would also be compatible with polyproline II conformation and with the conformation at the *i* + 1 position of type II and type VIa1 β-turns ((ϕ, ψ) ∼ (−60°, +120°)), which is the observed (ϕ, ψ) for other proline amino acids with electron-withdrawing 4*R*-substitutents.[7a,e,17a,22a,23a] These data indicate the ability of this amino acid to promote compact conformations of proline and *trans* amide bonds (ω = 180°).

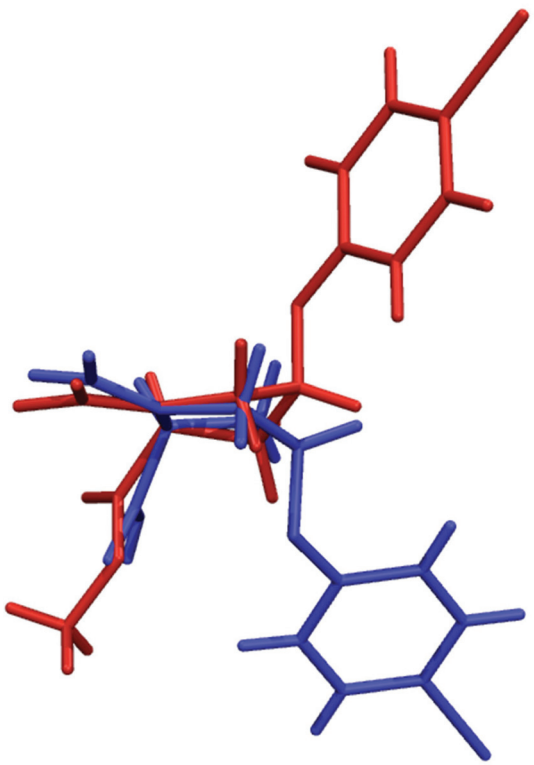


Fig. 7 Superposition of the crystal structures of Fmoc-4*S*-(4-iodophenyl) hydroxyproline (Fmoc-hyp(4-I-Ph)-OH) (**7**) (blue) and Boc-4*R*-(4-iodophenyl) hydroxyproline methyl ester (Boc-Hyp(4-I-Ph)-OMe) (**10**) (red). The Boc and Fmoc groups were removed beyond the carbonyl for clarity. The 4*R*-derivative (red) exhibits an *exo* ring pucker and compact ϕ, ψ, while the 4*S*-derivative (blue) exhibits an *endo* ring pucker and extended ϕ, ψ. The changes in stereochemistry, ring pucker, and main chain torsion angles result in divergent presentation of the iodophenyl functional group, with the ether oxygens 3.8 Å apart, the aryl *ipso* carbons 5.5 Å apart, the aryl *para* carbons 9.8 Å apart, and the iodines 13.2 Å apart from each other in this superposition.

peptide exhibited a typical NMR spectrum of a 4*R*-oxy substituent, promoting a *trans* amide bond relative to proline and exhibiting an NMR signature indicative of preference for an *exo* ring pucker (Table 4 and ESI†), consistent with crystallographic data on **10**. Comparison of the $K_{trans/cis}$ of 4*R* *versus* 4*S* diastereomers of 4-substituted prolines can be used to quantify the overall balance of steric and stereoelectronic effects of the substituent.[7e] The diastereomeric iodophenyl hydroxyprolines exhibit an overall $\Delta\Delta G_{trans/cis}$ (= $\Delta G_{trans/cis(4R)} - \Delta G_{trans/cis(4S)}$) of

Table 4 NMR properties of Ac-TYHyp(4-I-Ph)N-NH_2, Ac-TPhe(4-*S*-propargyl)PN-NH_2, Ac-TPhe(4-S(O)-propargyl)PN-NH_2, and Ac-TPhe(4-SO_2-propargyl)PN-NH_2 peptides within the model peptide Ac-TYPN-NH_2 context,[27] for comparison with data on other derivatives in Table 1. Experimental details and abbreviations are as in Table 1

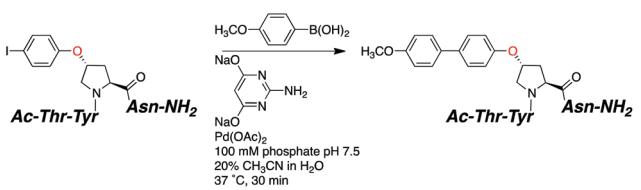
Ac-TZ*Prox*N-NH_2 Z=	*Prox*=	$K_{trans/cis}$	ΔG, kcal mol^{-1}	$\Delta\Delta G$, kcal mol^{-1}	$^3J_{\alpha N}$, Tyr$_{cis}$	δ, Tyr$_{cis}$	δ, Asn$_{trans}$
Tyr	Hyp(4-I-Ph)	4.8	−0.93	−0.34	8.0[a]	8.26	8.58
4-SO_2-propargyl-Phe	Pro	4.5	−0.89	−0.30	6.7	8.47	8.45
4-*S*(O)-propargyl-Phe[b]	Pro	4.3	−0.86	−0.27	n.d.[c]	8.42	8.52
4-*S*-propargyl-Phe	Pro	3.2	−0.69	−0.10	6.4	8.41	8.45

[a] Data were recorded at 277 K to resolve spectral overlap. [b] Observed as a mixture of sulfoxide diastereomers. [c] n.d. = not determined due to spectral overlap.

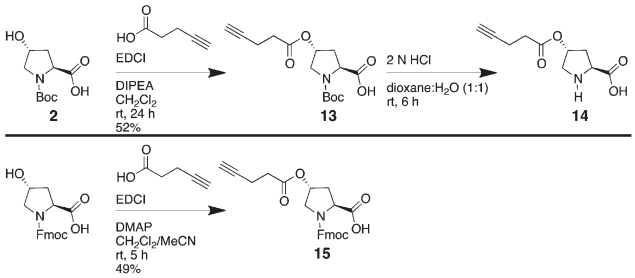
−0.49 kcal mol^{-1}. These data indicate similar net stereoelectronic effects for the hydroxyproline iodophenyl ethers compared to hydroxyproline ($\Delta\Delta G_{trans/cis}$ = −0.43 kcal mol^{-1} in an Ac-TY*Prox*N-NH_2 context, $\Delta\Delta G_{trans/cis}$ = −0.56 kcal mol^{-1} in an Ac-TA*Prox*N-NH_2 context).[7e,8,13,24] The smaller structural effects observed in the 4*R*-iodophenyl hydroxyproline ($K_{trans/cis}$ = 4.8) compared to the 4*R*-hydroxyproline ($K_{trans/cis}$ = 5.6) are potentially due to a modest steric demand of the phenyl group in the peptide context and the possibility of a steric interaction between tyrosine and the 4*R*-aryloxy group in the *cis* conformation that somewhat reduces the magnitude of the stereoelectronic effect.

This peptide was then subjected to aqueous Suzuki–Miyaura reaction with 4-methoxyphenyl boronic acid, using the Davis ligand (Scheme 7).[2i] The Suzuki–Miyaura reaction proceeded cleanly to product in high conversion in 30 minutes to 3 hours, depending on the concentration of boronic acid, exhibiting reactivity comparable to that previously observed for the diastereomeric peptide with the 4*S*-iodophenyl hydroxyproline (Scheme 2).[7e] Analysis of reaction progress under pseudo-first order conditions allowed the approximation of a second order rate constant of ~3 × 10^{-2} M^{-1} s^{-1} for the aqueous Suzuki–Miyaura reaction using these reagents under these conditions.[1d]

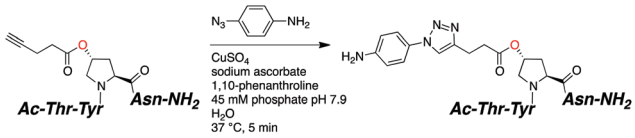
We subsequently examined the synthesis of alkyne-containing amino acids. We previously observed that the pentynoate ester of 4*R*-hydroxyproline exhibited a strong stereoelectronic effect within peptides (Table 1).[7e] This amino acid was synthesized directly in one step from either the commercially available Boc- or Fmoc-amino acid (Scheme 8). The free amino acid was synthesized in one additional step following Boc deprotection. The peptide containing this amino acid exhibited a



Scheme 7 Aqueous Suzuki–Miyaura reaction on a peptide with 4*R*-iodophenyl hydroxyproline. HPLC characterization of the reaction and kinetics are in the ESI (Fig. S29–S33†).



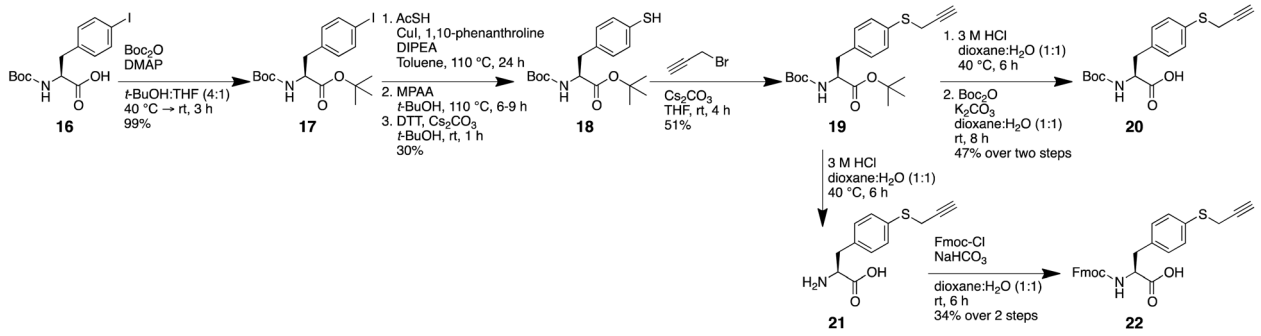
Scheme 8 Synthesis of Boc- and Fmoc-4*R*-pentynoyl hydroxyproline.



Scheme 9 Copper-mediated azide–alkyne cycloaddition reaction on a peptide with 4*R*-pentynoyl hydroxyproline. The major expected regioisomer is shown, although the regiochemistry of the click reaction was not confirmed. HPLC characterization of the reaction is in the ESI (Fig. S34†).

rapid and clean aqueous copper-mediated azide–alkyne cycloaddition reaction with the model azide 4-azidoaniline (Scheme 9). The highly practical synthetic sequence for Boc- or Fmoc-4*R*-pentynoyl hydroxyproline should promote the use of this amino acid in click chemistry applications where an ester functionality is tolerated or is desirable due to the possibility of subsequent hydrolysis by base or by esterases.

The propargyl thioether of 4-thiolphenylalanine represents a potentially unique alkyne handle within peptides and proteins, due to its different geometry compared to propargyl tyrosine (Fig. 3) and the ability to modulate its torsional angles and aromatic electronic properties *via* oxidation to the sulfoxide or sulfone.[10,11] This amino acid was synthesized from the commercially available amino acid Boc-4-iodophenylalanine (Scheme 10). After protection as the *tert*-butyl ester, the resultant amino acid **17** was subjected to copper-catalyzed cross-coupling reaction with thioacetic acid, using conditions similar to those we employed for this reaction on 4-iodophenylalanine-containing peptides on solid phase.[10,25]

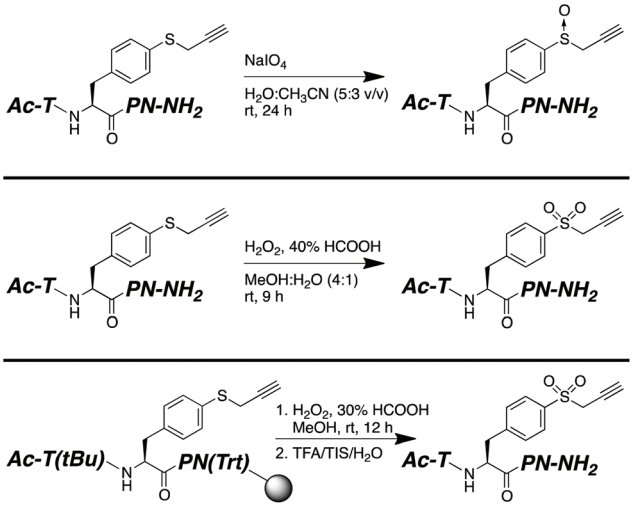


Scheme 10 Synthesis of Boc- and Fmoc-*S*-propargyl-4-thiolphenylalanine.

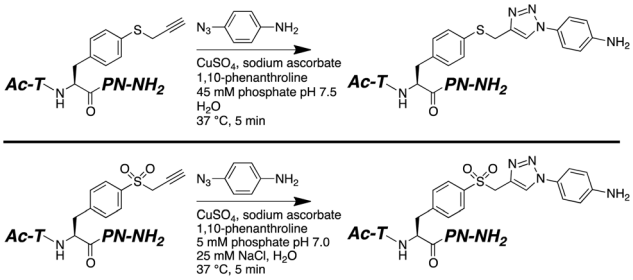
This reaction proceeded with high conversion to a mixture of thiolphenylalanine-derived products, including the expected thioester product as well as the disulfide product that resulted from subsequent reactivity of the thioester under reaction conditions. In addition, the product showed evidence of a strong interaction with copper, as was identified and crystallographically observed by Hartwig in a 2 : 2 : 2 complex of Cu(I) : phenanthroline : thiophenolate, and a strong iodine charge-transfer complex.[26] Therefore, the crude product mixture was subjected to an initial reaction with 4-mercaptophenylacetic acid (MPAA) to compete with the product for the copper(I) : phenanthroline complex, and then a second reductive step in the presence of DTT and base to induce thiolysis and to reduce disulfides to generate the desired protected thiolphenylalanine **18**. This reaction sequence proceeded with minimal epimerization (starting material 98% ee, product 94% ee). While the conversion in this cross-coupling reaction was excellent by TLC and by NMR of the crude reaction mixture, challenges in isolation led to reduced yield of the isolated product. Notably, for the previously described reactions to synthesize 4-thiolphenylalanine within peptides on the solid phase,[10] these reductive steps proceed cleanly, as reactants can be washed away, leading to significantly cleaner reactions for the solid-phase over the solution-phase cross-coupling reactions.

This amino acid was then subjected to alkylation with propargyl bromide, generating the Boc-protected *S*-propargyl thiolphenylalanine *tert*-butyl ester **19**. This product was converted to the Boc-, Fmoc-, and free amino acids using standard approaches. The overall sequence allowed the synthesis of either the Fmoc- or Boc-amino acid in 7 steps from commercially available starting material.

To examine the structural and aromatic electronic effects of the *S*-propargyl substituent, the Fmoc-amino acid was used in the synthesis of the Ac-TPhe(4-*S*-propargyl)PN-NH_2 model system peptide[8,11,27] by solid-phase peptide synthesis. The NMR spectrum of this peptide (Table 4, ESI†) indicated that the *S*-propargyl substituent was relatively electron-neutral, comparable to phenylalanine and to the *S*-allyl derivative (Table 1), as expected. In order to examine the structural effects of sulfur oxidation, this peptide was further oxidized to the sulfoxide and sulfone derivatives (Scheme 11). In addition, peptides containing *S*-propargyl phenylalanine and the sulfone derivative were subjected to copper-mediated azide–alkyne cycloaddition reactions, demonstrating rapid and clean reactions with a model azide (Scheme 12).



Scheme 11 Oxidation of the peptide Ac-TPhe(*S*-propargyl)PN-NH_2 to the sulfoxide (top) and to the sulfone (middle, bottom). Both diastereomeric sulfoxide products were observed by NMR, although they were not separable by HPLC. (top, middle) Solution-phase oxidation reactions. (bottom) Solid-phase oxidation reaction.



Scheme 12 Copper-mediated azide–alkyne cycloaddition reaction on peptides with 4-*S*-propargyl-phenylalanine and with 4-SO_2-propargyl-phenylalanine. The major expected regioisomers are shown, although the regiochemistry of the click reactions was not confirmed. HPLC characterization of the reactions is in the ESI (Fig. S35 and S36†).

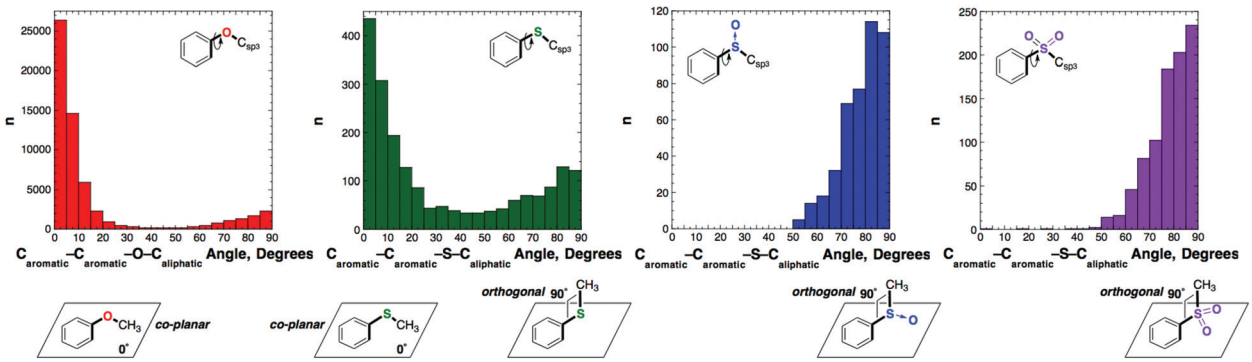


Fig. 8 Comparison of the extent of co-planarity *versus* orthogonality of aromatic rings and exocyclic chalcogen–carbon bonds with ether, thioether, sulfoxide, and sulfone substituents. Data were obtained *via* a search of the Cambridge Structural Database (CSD) for the torsion angle between the aromatic ring and the substituent, $C_{aromatic}$–$C_{aromatic}$–X–$C_{aliphatic}$, where X = O (ether), S (thioether), S(O) (sulfoxide), or SO_2 (sulfone). Consistent with prior analyses, these data indicate that ethers and thioethers prefer a co-planar structure, allowing conjugation of the ether or thioether lone pair with the aromatic ring, with a stronger preference for co-planarity for the ether. Deviations from co-planarity for ethers were most commonly observed for aromatic rings with *ortho* substituents that would sterically clash if the O–$C_{aliphatic}$ bond were co-planar. In contrast, the sulfoxide and sulfone exhibit no propensity for co-planarity of the substituent carbons with the aromatic ring and a strong preference for an orthogonal or near-orthogonal conformation. In addition, the oxygen-substituted (ether) and sulfur-substituted (thioether, sulfoxide, sulfone) derivatives exhibit significant differences in $C_{aromatic}$–X bond lengths and $C_{aromatic}$–X–$C_{aliphatic}$ bond angles, consistent with the known properties of oxygen *versus* sulfur: X = O (ether), 1.370 Å ± 0.016 Å C–O bond length, 117° ± 2° C–O–C bond angle); X = S (thioether), 1.769 Å ± 0.015 Å C–S bond length, 103° ± 2° C–S–C bond angle; X = S(O) (sulfoxide), 1.792 Å ± 0.014 Å C–S bond length, 98° ± 2° C–S–C bond angle; X = SO_2 (sulfone), 1.765 Å ± 0.019 Å C–S bond length, 103° ± 2° C–S–C bond angle. Ranges of bond angles and bond lengths indicate standard deviations for the 59 294 ethers, 1966 thioethers, 437 sulfoxides, and 887 sulfones that were identified in the CSD and examined.

As had been previously seen for the oxidized products of the methyl thioethers,[10,11] oxidation of *S*-propargyl phenylalanine to the sulfoxide and to the sulfone significantly modulated the electronics of the aromatic ring, inducing substantial electron-withdrawing substituent effects, which are observed as an increase in $K_{trans/cis}$ (Table 4) in the model peptide context upon oxidation. These data are consistent with the expected electronic effects of sulfoxides and sulfones based on Hammett substituent constants.[28]

In order to assess the conformational effects of sulfur oxidation, and to compare the expected side chain structural preferences of derivatives of thiolphenylalanine with propargyl tyrosine, an analysis was conducted of aryl–alkyl ethers, thioethers, sulfoxides, and sulfones in the Cambridge Structural Database (CSD) (Fig. 8).[29] As expected, ethers and thioethers exhibit a significant preference for a co-planar geometry of the O–$C_{aliphatic}$ or S–$C_{aliphatic}$ group with the aromatic ring ($C_{aromatic}$–$C_{aromatic}$–O/S–$C_{aliphatic}$ torsion angle ~0°), to allow conjugation of the O or S lone pair with the aromatic π orbitals, with a greater co-planar preference for ethers than thioethers.[30] The thioether C–S bonds exhibit smaller C–S–C bond angles (mean 102°) and longer $C_{aromatic}$–S bond lengths (mean 1.77 Å) than observed for ethers (C–O–C bond angle mean 117°; $C_{aromatic}$–O bond length mean 1.37 Å), as expected based on the known properties of sulfur *versus* oxygen. Notably, sulfur oxidation results in a dramatic change in the conformational preferences for the sulfoxides and sulfones compared to the thioethers. Both oxidized forms of sulfur exhibit a strong preference for the S–$C_{aliphatic}$ bond to be orthogonal or near-orthogonal to the aromatic ring ($C_{aromatic}$–$C_{aromatic}$–S–$C_{aliphatic}$ torsion angle ~90°), with almost no structures with the S–$C_{aliphatic}$ bond coplanar with the aromatic ring (torsion angle ~0°). These structural data emphasize the unique properties of *S*-propargyl thiolphenylalanine compared to propargyl tyrosine, with substantial structural differences (bond length, bond angle) between the thioether and ether and the special ability of sulfur oxidation to change the structural preferences of the substituent and the electronic properties of the aromatic ring.

Discussion

We have described the synthesis of 4 novel Fmoc amino acids for the incorporation of bioorthogonal functional groups within peptides. All amino acids were characterized within model peptides to ascertain their inherent conformational preferences (Tables 1 and 4), with the aim of generating a palette of amino acids optimized for applications in defined structural contexts.[31] 4*R*-Iodophenyl hydroxyproline and 4*S*-iodophenyl hydroxyproline include aryl iodides, for use in Suzuki and Sonogashira reactions or in molecular design based on halogen bonding.[2i,j,7e] Each amino acid exhibits distinct and strong conformational preferences that are divergent from the other diastereomer (4*R* favors more compact ϕ; 4*S* favors more extended ϕ and ψ and/or *cis* amide bonds), allowing their implementation to be molecularly tailored to a given structural fit (Fig. 7). 4*R*-Pentynoyl hydroxyproline introduces an alkyne functional group, for application in azide–alkyne coupling reactions, with greater conformational control than other alkyne-containing amino acids, in locations that accept a proline and a more compact ϕ torsion angle (α-helix or poly-

proline II helix). *S*-Propargyl thiolphenylalanine is a unique alkyne-containing aromatic amino acid that includes an aromatic functionality, bond lengths and bond angles (and thus presentation of the alkyne) that are distinct from the tyrosine analogue, and the ability to change both geometry and aromatic properties by sulfur oxidation. All derivatives described herein provide distinct potential advantages in conformational control over previously described amino acids containing these functional groups.

Crystal structures were obtained of both Boc- and Fmoc-4*S*-iodophenyl hydroxyproline. Consistent with expectations from NMR data within peptides, this amino acid exhibited an *endo* ring pucker and an enhanced tendency to form a *cis* carbonyl–nitrogen bond, which was observed with both carbamates. Proline derivatives that favor *endo* ring puckers typically favor more extended proline conformations.[7a,b,17a] Interestingly, both crystallographically observed structures of this amino acid adopted distinct conformations. One derivative exhibited a combination of ϕ and ψ ideal for the $i + 2$ residue of different β-turns (ϕ, $\psi \sim -80°$, 0°).[12a,b] β-Turn stabilization has been the subject of extensive work in protein design, medicinal chemistry, and biomaterials.[12a,b,14–16,32] This amino acid thus suggests the ability both to stabilize a β-turn and to employ this location as a site for functionalization for target optimization. The other derivative favored an extended conformation for proline (ϕ, ψ = −83°, +174°), typical of the structures of most 4*S*-substituted prolines with electron-withdrawing substituents, which have been extensively employed in tuning structure and stability *via* modification at the Xaa position of collagen.[5d,7a,b,17a,c,d,33]

There are two prior examples of crystal structures of hydroxyproline phenyl ethers.[3e,9a] Rose *et al.* described the use of 4*S*-substituted aryloxyprolines in the generation of templated, highly constrained macrocycles.[3e] Interestingly, the macrocyclic structure (CSD: HOSMEJ) exhibits a compact conformation at proline ((ϕ, ψ) = (−43°, −46°)) despite the Cγ-*endo* ring pucker, indicative of the ability of macrocyclization to significantly restrain conformation and promote disfavored conformations. Indeed, this structure exhibits significant disorder, consistent with the structural tension in the constrained molecule. Palkowitz *et al.* reported the structure of an angiotensin II receptor antagonist with a 2*R*,4*R*-aryloxyproline (*i.e.* with a D-proline derivative, enantiomeric to the 2*S*,4*S* L-proline derivative employed herein), which exhibited the aryloxy group pseudoaxial on a Cγ-*endo* ring pucker, (ϕ, ψ) = (+71°, +169°), and a *cis* proline amide bond (CSD: YIHJEE).[9a] These data are consistent with the data obtained herein, where the 4*S*-iodophenyl hydroxyproline exhibits an *endo* ring pucker and relatively more extended conformations for proline. Notably, the aryloxy substituents have opposite conformational preferences from the sulfur (thioether) analogues: the 4*S*-phenyl thioether prefers an *exo* ring pucker and compact ϕ, ψ (CSD: TUHMUE, of the angiotensin-converting enzyme inhibitor drug zofenopril), due to the balance of steric over stereoelectronic effects for the larger and less electronegative sulfur atom.[7e,34]

The 4*R*-iodophenyl hydroxyproline, in contrast, exhibited a more compact conformation of proline ((ϕ, ψ) = (−55.9°, −39.2°)) and a *trans* amide bond. This conformation is consistent in ϕ with that observed for 4*R*-hydroxyprolines in collagen and for various other prolines with electron-withdrawing 4*R*-substituents.[7a,b,17a,22,23,35] This work represents the first crystal structure of a *trans*-substituted diastereomer (*i.e.* 2*S*,4*R*- or 2*R*,4*S*-) of an aryloxyproline. In summary, the crystallographic data will be useful for the molecular design and engineering of peptides and proteins employing 4*R*- and 4*S*-aryloxyprolines. These stereospecific conformational data further emphasize the structural stabilization and defined functional group presentation that are possible with chi-space optimized application of stereochemically appropriate 4-substituted prolines (Fig. 7).[31] The 4-iodophenyl hydroxyprolines combine conformational control with sites for molecular recognition and/or bioorthogonal functionalization, suggesting broad potential application.

All Fmoc amino acids described herein were synthesized in relatively short synthetic sequences (1, 4, 6, and 7 steps) from commercially available starting materials, in addition to the prior synthesis of these or related derivatives within peptides *via* proline editing or *via* cross-coupling reaction on solid phase.[7e,10] The schemes for the synthesis of the iodophenyl ethers of hydroxyproline could also be applied to synthesize diverse proline phenyl ethers *via* the appropriate 4-substituted phenols, including the phenyl (constrained phenylalanine mimetic), aryl bromide (Suzuki reaction), and aryl nitrile (IR (ν_{max} = 2233 cm^{-1}) and fluorescence (emission λ_{max} = 295 nm, greater intensity than Tyr fluorescence)) functional groups whose utility we demonstrated previously.[7e] Aryloxyproline derivatives have also been employed in inhibitor development in medicinal chemistry.[9] 4*S*-Aryloxyprolines have also been used as components in asymmetric catalysts, with ee up to 97% and dr up to 99 : 1 observed in the aldol reaction of cyclohexanone with benzaldehyde derivatives.[36] The crystallographic results herein thus could be used to further enhance the application of 4-aryloxyprolines in catalyst design and structure-based drug design.

Conclusions

We have described the synthesis of 4 distinct amino acids with aryl iodide or alkyne bioorthogonal functional groups and demonstrated their application within peptides in aqueous Suzuki–Miyaura or azide–alkyne cycloaddition reactions. These amino acids have defined conformational preferences or the ability to tune their structural preferences. These amino acids provide novel sites for peptide functionalization with defined structural control. The intrinsic structural features and facile synthetic modification of this series of amino acids afford new possibilities in molecular design.

Experimental

Boc-(2*S*,4*R*)-4-hydroxyproline (2)

1 (15.2 g, 116 mmol) and Boc_2O (36 g, 165 mmol) were dissolved in 1,4-dioxane (300 mL). A solution of NaOH (1 M in

H_2O, 300 mL) was added to the solution. The solution was stirred at room temperature for 18 hours. Dioxane was removed under reduced pressure and the crude product was acidified with 4 M HCl (200 mL). The crude product was extracted with ethyl acetate (3 × 200 mL), the organic layers combined, and the solvent removed under reduced pressure. **2** was used as a crude reagent in the next step without purification. The NMR data corresponded to literature values.[37]

Boc-(2*S*,4*R*)-4-hydroxyproline methyl ester (3)

2 (24.9 g, 108 mmol), dimethyl sulfate (15.3 mL, 161 mmol), and K_2CO_3 (45.5 g, 329 mmol) were dissolved in acetone (1 L). The solution was heated at reflux for 3 hours. The solution was allowed to cool to room temperature and solvent was removed under reduced pressure. The crude product was dissolved in ethyl acetate (200 mL) and washed with distilled water (3 × 200 mL). The organic layer was dried over Na_2SO_4, filtered, and concentrated under reduced pressure to produce a colorless oil in 71% yield over two steps. **3** was used in the next step without purification. The NMR data corresponded to the literature values.[37]

Boc-(2*S*,4*S*)-*p*-iodophenyl-4-hydroxyproline methyl ester (4)

3 (500 mg, 2.04 mmol) was dissolved in anhydrous CH_2Cl_2 (20.4 mL). The solution was cooled to 0 °C and placed under nitrogen. Triphenylphosphine (Ph_3P) (963 mg, 3.67 mmol), ADDP (540 mg, 2.14 mmol), and 4-iodophenol (449 mg, 2.04 mmol) were added to the solution. The solution was stirred at 0 °C for 2 h. The solution was then removed from the ice bath, allowed to warm to room temperature, and stirred for an additional 16 hours. The mixture was washed with brine (3 × 75 mL). The organic layer was dried over Na_2SO_4 and filtered. The solvent was removed under reduced pressure and the crude product was redissolved in hexanes (15 mL). The crude product was purified *via* column chromatography (0 to 25% ethyl acetate in hexanes v/v) to yield **4** (583 mg, 1.30 mmol) as a colorless oil in 64% yield. NMR spectra reflected a mixture of *cis* and *trans* proline rotamers (1 : 1.3 ratio, respectively). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.55 (d, J = 8.8 Hz), 7.53 (d, J = 8.7 Hz) (sum of 7.55 ppm and 7.53 ppm resonances = 2H), 6.59 (d, J = 8.8 Hz), 6.57 (d, J = 8.8 Hz) (sum of 6.59 ppm and 6.57 ppm resonances = 2H), 4.89–4.85 (m, 1H), 4.54 (dd, J = 8.7 Hz, 2.4 Hz), 4.43 (dd, J = 7.6 Hz, 4.0 Hz) (sum of 4.54 ppm and 4.43 ppm resonances = 1H), 3.80–3.62 (m, 2H), 3.73 (s), 3.71 (s) (sum of 3.73 ppm and 3.71 ppm resonances = 2H), 2.51–2.37 (m, 2H), 1.48 (s), 1.43 (s) (sum of 1.48 ppm and 1.43 ppm resonances = 9H). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 172.45, 172.06, 156.39, 154.15, 153.77, 138.40, 138.39, 117.86, 83.66, 80.38, 80.28, 75.55, 74.50, 57.76, 57.39, 52.36, 52.22, 51.86, 51.46, 36.14, 35.25, 28.40, 28.29. HRMS (LIFDI-TOF) m/z: $[M + H]^+$ calcd for $C_{17}H_{22}INO_5$ 447.0543, found 447.0515.

Boc-(2*S*,4*S*)-*p*-iodophenyl-4-hydroxyproline (5)

4 (100 mg, 0.22 mmol) was dissolved in 1,4-dioxane (1.1 mL). LiOH (5.6 mg, 0.23 mmol) was dissolved in water (1.1 mL). The LiOH solution (210 mM) was added to the solution of **4**, and the resultant solution was stirred for 12 hours at room temperature. The mixture was acidified to pH 2 with 1 M HCl and extracted with ethyl acetate (3 × 10 mL). The organic layer was dried over Na_2SO_4 and filtered. The solvent was removed under reduced pressure and the resultant solid was redissolved in CH_2Cl_2. The crude product was purified *via* column chromatography (0 to 1.5% methanol in CH_2Cl_2 v/v) to obtain **5** (60 mg, 0.14 mmol) as an off-white solid in 64% yield. NMR spectra reflected a mixture of *cis* and *trans* proline rotamers (1 : 1.5 ratio, respectively). ^{1}H (400 MHz, CD_3OD) δ 7.45 (d, J = 8.7 Hz, 2H), 6.59 (d, J = 8.9 Hz), 6.58 (d, J = 8.9 Hz) (sum of 6.59 ppm and 6.58 ppm resonances = 2H), 4.88 (m, 1H), 4.34–4.28 (m, 1H), 3.67–3.61 (m, 1H), 3.52–3.47 (m, 1H), 2.49–2.36 (m, 1H), 2.29 (m, 1H), 1.37 (s), 1.34 (s) (sum of 1.37 ppm and 1.34 ppm resonances = 9H). ^{13}C NMR (100.6 MHz, CD_3OD) δ 175.45, 175.28, 158.03, 157.99, 156.05, 155.94, 139.49, 119.18, 119.14, 84.06, 81.57, 81.56, 76.95, 75.99, 59.12, 58.77, 53.31, 52.79, 36.78, 36.02, 28.67, 28.52. HRMS (LIFDI-TOF) m/z: $[M + H]^+$ calcd for $C_{16}H_{20}INO_5$ 433.0386, found 433.0384.

(2*S*,4*S*)-*p*-Iodophenyl-4-hydroxyproline (6)

4 (100 mg, 0.22 mmol) was dissolved in 1,4-dioxane (1.1 mL). A solution of 4 M HCl (1.1 mL) was added, and the resultant mixture was stirred at 60 °C for 6 h, or until the disappearance of **4** was confirmed *via* TLC. The solvent was removed under reduced pressure. The crude product **6** was used without further purification. ^{1}H (600 MHz, CD_3OD) δ 7.61 (d, J = 8.5 Hz, 2H), 6.75 (d, J = 8.5 Hz, 2H), 5.19 (s, 1H), 4.59 (d, J = 7.2 Hz, 1H), 3.68 (d, J = 12.4 Hz, 1H), 3.59 (dd, J = 12.8, 3.4 Hz, 1H), 2.72–2.57 (m, 2H). ^{13}C NMR (150.9 MHz, CD_3OD) δ 157.31, 139.85, 119.36, 85.13, 76.46, 52.53, 35.59. ESI-MS m/z: $[M + H]^+$ calcd for $C_{11}H_{12}INO_3$ 333.0, found 334.1.

Fmoc-(2*S*,4*S*)-*p*-iodophenyl-4-hydroxyproline (7)

4 (1.33 g, 2.98 mmol) was dissolved in 1,4-dioxane (15 mL). A solution of HCl (4 M, 15 mL) was added, and the resultant solution was heated to reflux and stirred for 12 hours. After verifying disappearance of the starting material *via* TLC, the mixture was neutralized with $NaHCO_3$ (approximately 7 g). Fmoc-OSu (1.20 g, 3.56 mmol) was added directly to the crude mixture, and the solution was stirred at room temperature for 18 h. The mixture was acidified to pH 2 with 1 M HCl and extracted with ethyl acetate (3 × 30 mL). The combined organic layers were dried over Na_2SO_4 and filtered. The solvent was removed under reduced pressure and the resultant solid was redissolved in CH_2Cl_2. The crude product was purified *via* column chromatography (0 to 1.5% methanol in CH_2Cl_2 v/v) to obtain **7** (940 mg, 1.69 mmol) as an off-white solid in 57% yield. NMR spectra reflected a mixture of *cis* and *trans* proline rotamers (1 : 1 ratio). ^{1}H (600 Hz, CD_3OD) δ 7.79 (d, J = 7.6 Hz), 7.77 (d, J = 7.8 Hz) (sum of 7.79 ppm and 7.77 ppm resonances = 2H), 7.62 (d, J = 7.8 Hz), 7.59 (d, J = 8.9 Hz), 7.60 (d, J = 8.4 Hz), 7.56 (d, J = 8.8 Hz) (sum of 7.62 ppm, 7.60 ppm, 7.59 ppm, and 7.56 ppm resonances = 4H), 7.38 (t, J = 7.6 Hz),

7.36 (t, J = 8.3 Hz), 7.29 (m), 7.24 (t, J = 7.4 Hz) (sum of 7.29 ppm and 7.24 ppm resonances = 2H), 6.70 (d, J = 8.6 Hz), 5.01 (br s, 1H), 4.50–4.43 (m), 4.41–4.35 (m), 4.35–4.30 (m) (sum of 4.47 ppm, 4.38 ppm, and 4.33 resonances = 3H), 4.27 (t, J = 6.6 Hz), 4.21 (t, J = 6.8 Hz) (sum of 4.27 ppm and 4.21 resonances = 1H), 3.78 (dd, J = 12.2 Hz, 4.8 Hz), 3.70 (dd, J = 12.2 Hz, 4.7 Hz) (sum of 3.78 ppm and 3.70 resonances = 1H), 3.66 (d, J = 12.2 Hz), 3.63 (d, J = 12.2 Hz) (sum of 3.66 ppm and 3.63 resonances = 1H), 2.58–2.40 (m, 2H). ^{13}C NMR (150.9 MHz, CD_3OD) δ 175.18, 174.89, 158.07, 156.62, 145.37, 145.17, 145.11, 142.70, 142.64, 142.58, 142.54, 139.62, 139.58, 128.84, 128.83, 128.24, 128.20, 126.24, 126.19, 126.15, 126.11, 120.94, 120.91, 119.51, 119.37, 84.25, 84.16, 77.07, 76.22, 69.08, 68.83, 59.26, 59.15, 53.50, 53.01, 48.49, 48.37, 37.04, 36.18. HRMS (LIFDI-TOF) m/z: $[M + Na]^+$ calcd for $C_{26}H_{22}INO_5$ 555.0543, found 555.0546.

Boc-(2*S*,4*S*)-4-nitrobenzoyl-hydroxyproline methyl ester (8)

3 (1.10 g, 4.48 mmol), triphenylphosphine (PPh_3, 1.41 g, 5.38 mmol), and *p*-nitrobenzoic acid (0.82 g, 4.93 mmol) were dissolved in anhydrous CH_2Cl_2 (100 mL), and the resultant solution was cooled to 0 °C under a nitrogen atmosphere. DIAD (1.09 g, 5.38 mmol) was added dropwise to the solution over 2 min under nitrogen. The solution was stirred at 0 °C for 1 hour, then allowed to warm to room temperature and stirred for an additional 3 hours. The solvent was removed under reduced pressure. To the crude product was added 100 mL of water, and the product was extracted with ethyl acetate (3 × 30 mL). The combined organic layers were washed with brine (1 × 15 mL), dried over anhydrous Na_2SO_4, and the solvent removed under reduced pressure. The crude product was purified by column chromatography using 15% ethyl acetate in hexanes (v/v) to yield **8** (1.58 g, 4.00 mmol) in 90% yield as thick oil that solidified to a white solid upon standing at room temperature. NMR data corresponded to the literature values.[5a]

Boc-(2*S*,4*S*)-4-hydroxyproline methyl ester (9)

8 (1.58 g, 4.01 mmol) was dissolved in methanol (50 mL). Sodium azide (520 mg, 8.02 mmol) was added to the reaction mixture, and the mixture stirred at 40 °C for 4 hours. The solution was allowed to cool to room temperature and methanol was removed under reduced pressure. Water (30 mL) was added to the crude dried mixture to dissolve unreacted sodium azide. The aqueous layer was extracted with ethyl acetate (3 × 10 mL). The organic layers were combined, then washed with brine and dried over anhydrous Na_2SO_4 and solvent removed under reduced pressure to yield the crude product. The compound was purified by column chromatography (0 to 3% methanol in CH_2Cl_2 (v/v)) to obtain **9** as colorless oil (840 mg, 3.42 mmol) in 85% yield. NMR data corresponded to literature values.[5a]

Boc-(2*S*,4*R*)-*p*-iodophenyl-4-hydroxyproline methyl ester (10)

9 (840 mg, 3.42 mmol), triphenylphosphine (PPh_3, 1.08 g, 4.12 mmol), 4-iodophenol (910 mg, 4.12 mmol), and ADDP (1.04 g, 4.12 mmol) were dissolved in anhydrous CH_2Cl_2 (50 mL). The solution was stirred at room temperature for 12 hours. The solvent was removed under reduced pressure. To the crude product was added 50 mL of water containing 1.5% NaOH, and the aqueous layer extracted with ethyl acetate (3 × 20 mL). The combined organic layers were washed with brine (1 × 15 mL), dried over anhydrous Na_2SO_4, and the solvent removed under reduced pressure. The product was purified by column chromatography using 15% ethyl acetate in hexanes (v/v) to yield **10** (696 mg, 1.56 mmol) as a colorless oil in 46% yield. NMR spectra reflected a mixture of *cis* and *trans* proline rotamers (1 : 1.5 ratio, respectively). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.57 (d, J = 8.7 Hz), 7.56 (d, J = 8.6 Hz) (sum of 7.57 ppm and 7.56 ppm = 2H), 6.64 (d, J = 8.9 Hz), 6.63 (d, J = 8.9 Hz) (sum of 6.64 ppm and 6.63 ppm = 2H), 4.92–4.80 (m, 1H), 4.48 (t, J = 7.7 Hz), 4.41 (t, J = 8.0 Hz) (sum of 4.48 ppm and 4.41 ppm = 1H), 3.80–3.77 (m, 2H), 3.75 (s), 3.76 (s) (sum of singlets at 3.75 ppm and 3.76 ppm = 3H), 2.59–2.45 (m, 1H), 2.24–2.20 (m, 1H), 1.45 (s), 1.42 (s) (sum of singlets 1.42 ppm and 1.45 ppm = 9H). ^{13}C NMR (150.9 MHz, $CDCl_3$) δ 175.33, 173.13, 156.75, 156.73, 154.29, 153.62, 138.48, 138.44, 117.85, 83.71, 80.55, 75.47, 74.77, 57.92, 57.53, 52.41, 52.20, 51.99, 51.76, 36.43, 35.49, 28.35, 28.24. HRMS (LIFDI-TOF) m/z: $[M + H]^+$ calcd for $C_{17}H_{22}INO_5$ 447.0543, found 447.0550.

(2*S*,4*R*)-*p*-Iodophenyl-4-hydroxyproline (11)

10 (490 mg, 1.10 mmol) was dissolved in a 20 mL solution of water and 1,4-dioxane (1 : 1, v/v) containing 2 M HCl. This mixture was stirred at 50 °C for 4 hours. Dioxane and water were removed under reduced pressure. The crude product (470 mg) was used in the subsequent reaction directly without further purification. ^{1}H NMR (600 MHz, CD_3OH) 7.88 (s, 1H), 7.63 (d, J = 8.8 Hz, 2H), 6.80 (d, J = 8.8 Hz, 2H), 5.21 (t, J = 4.0 Hz, 1H), 4.55 (dd, J = 11.0, 8.0 Hz, 1H), 3.67 (dd, J = 13.0 Hz, 4.1 Hz, 1H), 3.58 (d, J = 13.0 Hz, 1H), 2.69 (dd, J = 14.4 Hz, 7.1 Hz, 1H), 2.42 (ddd, J = 14.3 Hz, 10.4 Hz, 4.6 Hz, 1H). ^{13}C NMR (150.9 MHz, CD_3OH) 156.1, 138.50, 117.79, 83.71, 75.80, 58.49, 50.89, 34.53. HRMS (LIFDI-TOF) m/z: $[M + H]^+$ calcd for $C_{11}H_{12}INO_3$ 332.9862, found 332.9843.

Fmoc-(2*S*,4*R*)-*p*-iodophenyl-4-hydroxyproline (12)

11 (470 mg) was dissolved in a 20 mL solution of water and 1,4-dioxane (1 : 1, v/v), and $NaHCO_3$ was added to neutralize the residual HCl from the previous reaction. Fmoc-Cl (341 mg, 1.32 mmol) and $NaHCO_3$ (185 mg, 2.20 mmol) were added, and the reaction mixture was stirred at room temperature for 6 hours. The solution was acidified to pH 3 using 2 M HCl. The crude product was extracted with ethyl acetate (3 × 10 mL). The combined organic layers were washed with brine (1 × 10 mL), dried over Na_2SO_4, and the solvent removed under reduced pressure. The product was purified by column chromatography using 0–4% methanol in CH_2Cl_2 containing 0.5% acetic acid to yield **12** (572 mg, 1.03 mmol) as a white solid in 94% yield over two steps. NMR spectra reflected a mixture of *cis* and *trans* proline rotamers (1 : 2.5 ratio). ^{1}H (600 Hz, $CDCl_3$)

δ 7.72 (d, J = 7.7 Hz), 7.69 (d, J = 8.0 Hz), (sum of 7.72 ppm and 7.69 ppm resonances = 2H), 7.58 (d, J = 8.8 Hz), 7.54 (d, J = 8.5 Hz), 7.47 (d, J = 7.5 Hz), 7.43 (d, J = 7.5 Hz), 7.39–7.27 (m), 7.32–7.23 (m) (sum of 7.58 ppm, 7.54 ppm, 7.47 ppm, 7.43 ppm, 7.36 ppm, and 7.29 ppm resonances = 6H), 7.22 (t, J = 7.5 Hz), 7.17 (t, J = 7.4 Hz) (sum of 7.22 ppm and 7.17 ppm resonances = 2H), 6.60 (d, J = 8.7 Hz), 6.60 (d, J = 8.1 Hz) (sum of 6.60 ppm and 6.60 ppm resonances = 2H), 4.86–4.82 (m), 4.82–4.79 (m) (sum of 4.84 ppm and 4.81 ppm resonances = 1H), 4.56–4.29 (m, 3H), 4.20 (t, J = 7.3 Hz), 4.17 (t, J = 6.4 Hz) (sum of 4.20 ppm and 4.17 ppm resonances = 1H), 3.86 (d, J = 12.2 Hz), 3.80 (d, J = 12.1 Hz) (sum of 3.86 ppm and 3.80 ppm resonances = 1H), 3.76 (dd, J = 12.0 Hz, 4.4 Hz), 3.69 (dd, J = 12.0 Hz, 4.3 Hz) (sum of 3.76 ppm and 3.69 ppm resonances = 1H), 2.62–2.54 (m), 2.53–2.44 (m) (sum of 2.58 ppm and 2.49 ppm resonances = 1H), 2.44–2.34 (m), 2.31–2.23 (m) (sum of 2.39 ppm and 2.27 ppm resonances = 1H). ^{13}C NMR (150.9 MHz, $CDCl_3$) δ 175.18, 174.89, 156.53, 156.14, 143.53, 143.50, 141.27, 138.60, 138.51, 127.81, 127.72, 127.67, 127.11, 127.09, 125.07, 124.92, 120.02, 119.96, 117.88, 117.81, 83.95, 75.10, 74.65, 68.17, 67.71, 67.10, 58.51, 57.44, 52.37, 51.88, 47.19, 46.99, 36.60, 34.85. HRMS (LIFDI-TOF) m/z: $[M + H]^+$ calcd for $C_{26}H_{22}INO_5$ 555.0543, found 555.0532.

Boc-(2*S*,4*R*)-4′-pentynoyl-4-hydroxyproline (13)

2 (0.50 g, 2.16 mmol) was dissolved in anhydrous CH_2Cl_2 (20.0 mL) under a nitrogen atmosphere. 4-Pentynoic acid (212 mg, 2.16 mmol) was added to the solution, followed by EDCI (456 mg, 2.38 mmol). DIPEA (578 μL, 3.24 mmol) was added, and the reaction mixture was stirred vigorously for 24 h. The reaction was quenched by addition of 20 mL water, then acidified to pH 1–2 using 1 M HCl, and the aqueous phase extracted with CH_2Cl_2 (3 × 10 mL). The combined organic layers were washed once with brine (10 mL) and then dried over anhydrous Na_2SO_4. The organic solution was concentrated *in vacuo* and purified *via* column chromatography (0–2% methanol in CH_2Cl_2) to yield **13** (350 mg, 1.12 mmol) as viscous oil in 52% yield. NMR spectra reflected a mixture of *cis* and *trans* proline rotamers (1 : 1.9 ratio). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.35 (br s, 1H), 4.51 (dd, J = 8.1, 7.7 Hz), 4.39 (dd, J = 8.1, 7.8 Hz) (sum of 4.51 ppm and 4.39 ppm resonances = 1H), 3.71 (d, J = 2.2 Hz), 3.64 (d, J = 3.1 Hz) (sum of 3.71 ppm and 3.64 ppm resonances = 2H), 2.64–2.54 (m), 2.54–2.42 (m), 2.42–2.20 (m) (sum of 2.58 ppm, 2.49 ppm, and 2.31 ppm resonances = 5H), 1.49 (s), 1.44 (s) (sum of 1.49 ppm and 1.44 ppm resonances = 9H). ^{13}C NMR ($CDCl_3$, 100.6 MHz) 171.16, 82.66, 72.28, 69.36, 52.54, 34.12, 33.28, 28.33, 28.23, 14.38. HRMS (ESI) $[M]^-$ m/z calcd for $C_{15}H_{20}O_6N$ 310.1296, found 310.1298.

(2*S*,4*R*)-4′-Pentynoyl-4-hydroxyproline (14)

13 (250 mg, 0.80 mmol) was dissolved in 1,4-dioxane (4.0 mL), and dilute HCl (4 N, 4.0 mL) was added to the solution. The reaction mixture was stirred at room temperature for 6 h. The solvent was removed *in vacuo*, and the residue was washed with hexanes (3 × 5 mL) to yield **14** (140 mg, 0.66 mmol) in 83% yield as a viscous oil. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 5.27 (m, 1H), 4.42 (br s, 1H), 4.40–4.35 (m, 1H), 3.39–3.25 (m, 2H), 3.08 (s, 1H), 2.53–2.51 (m, 4H), and 2.28–2.02 (m, 2H); ^{13}C NMR (DMSO-d_6, 100 MHz) 171.1, 168.2, 79.8, 72.2, 69.1, 58.2, 53.7, 37.7, 34.6, 14.1. MS (ESI) m/z: $[M + H]^+$ calcd for $C_{10}H_{13}NO_4$ 211, found 212.

Fmoc-(2*S*,4*R*)-4′-pentynoyl-4-hydroxyproline (15)

4-Pentynoic acid (65 mg, 0.67 mmol) was dissolved in CH_2Cl_2 (10 mL) under a nitrogen atmosphere. EDCI (128 mg, 0.67 mmol) was added to the solution, followed by DMAP (24 mg, 0.20 mmol). 4-Pentynoic acid was activated in this reaction mixture for 30 min. Fmoc-(2*S*,4*R*)-4-hydroxyproline (200 mg, 0.57 mmol) was dissolved in acetonitrile (10 mL) and added dropwise to the activated pentynoic acid solution. This solution was allowed to stir at room temperature for 5 hours under nitrogen. The solvent was removed under reduced pressure. Water (50 mL) was added to the crude oil, and the product was extracted with ethyl acetate (3 × 10 mL). The combined organic layers were washed with brine (1 × 10 mL) and dried over Na_2SO_4. The crude product was purified by column chromatography using 0–4% methanol in CH_2Cl_2 containing 0.5% acetic acid to yield **15** (121 mg, 0.27 mmol) as a white solid in 49% yield. NMR spectra reflected a mixture of *cis* and *trans* proline rotamers (1 : 1.7 ratio). ^{1}H (600 Hz, $CDCl_3$) δ 7.77 (d, J = 7.7 Hz), 7.74 (d, J = 7.6 Hz) (sum of 7.77 ppm and 7.74 ppm resonances = 2H), 7.57 (t, J = 7.5 Hz), 7.55 (t, J = 6.8 Hz) (sum of 7.57 ppm and 7.55 ppm resonances = 2H), 7.42–7.35 (m, 2H), 7.34–7.28 (m, 2H), 5.39–5.34 (m), 5.34–5.31 (m) (sum of 5.36 ppm and 5.32 ppm resonances = 1H), 4.56–4.46 (m), 4.45–4.38 (m), 4.36 (t, J = 8.1 Hz) (sum of 4.51 ppm, 4.41 ppm, and 4.36 ppm resonances = 3H), 4.26 (t, J = 7.0 Hz), 4.18 (t, J = 6.3 Hz) (sum of 4.26 ppm and 4.18 ppm resonances = 1H), 3.81–3.69 (m, 2H), 2.60–2.37 (m), 2.31–2.23 (m) (sum of 2.50 ppm and 2.27 ppm resonances = 6H), 2.00–1.94 (m, 1H). ^{13}C NMR (150.9 MHz, $CDCl_3$) δ 171.10, 143.63, 141.36, 127.87, 127.72, 127.66, 127.14, 127.08, 124.98, 124.92, 124.77, 120.09, 119.99, 81.93, 72.61, 71.99, 69.42, 69.38, 68.19, 68.02, 52.53, 52.26, 47.19, 47.08, 36.69, 34.96, 33.30, 14.01. HRMS (LIFDI-TOF) m/z: $[M + H]^+$ calcd for $C_{25}H_{23}NO_6$ 433.1525, found 433.1528.

Boc-4-iodo-L-phenylalanine-*O*-*tert*-butyl ester (17)

Boc-4-iodo-L-phenylalanine (**16**, 300 mg, 0.77 mmol) was dissolved in tetrahydrofuran (307 μL). Boc_2O (434 mg, 1.99 mmol) and *tert*-butanol (1.23 mL) were added, and the mixture was warmed to 40 °C to allow **16** to completely dissolve. DMAP (28 mg, 0.23 mmol) was added, and the mixture was stirred at room temperature for 3 hours, or until the disappearance of **16** was observed *via* TLC. 1 M HCl (20 mL) was added, and the product was extracted with CH_2Cl_2 (3 × 20 mL). The combined organic layers were dried over sodium sulfate and filtered. The solvent was removed under reduced pressure. The crude product was purified *via* column chromatography (0–1% methanol in CH_2Cl_2 (v/v)) to yield **17** (340 mg, 0.76 mmol) as a colorless oil in 99% yield. Enantiopurity was verified *via* chiral

HPLC (see ESI†). The ^{1}H NMR and ^{13}C NMR spectra of the resultant product corresponded with literature values.[38]

Boc-4-thiol-L-phenylalanine-*O*-*tert*-butyl ester (18)

17 (400 mg, 0.89 mmol), copper(I) iodide (17 mg, 89 μmol), and 1,10-phenanthroline (32 mg, 180 μmol) were placed in an oven-dried glass vial with a stir bar. Toluene (1.8 mL) and DIPEA (470 μL, 2.7 mmol) were added, and the mixture was stirred at room temperature under nitrogen for 5 minutes. Thioacetic acid (128 μL, 1.8 mmol) was added to the solution at room temperature, the vial was sealed, and the mixture was heated to 110 °C in an oil bath and stirred for 24 hours. During this time, the reaction darkened to a red-brown color as iodine was formed. The solution was cooled to room temperature, and the solvent was removed under reduced pressure. The crude residue was redissolved in *t*-butanol (1.8 mL), and 4-mercaptophenylacetic acid (MPAA, 150 mg, 0.89 mmol) was added. The reaction mixture was stirred at 110 °C for 6–9 hours. The solution was allowed to cool to room temperature, and DTT (150 mg, 0.97 mmol) and cesium carbonate (320 mg, 0.98 mmol) were added. This mixture was stirred at room temperature for 1 hour. The crude mixture was diluted with ethyl acetate (10 mL) and added to dilute HCl (1 M, 20 mL). The crude product was extracted with ethyl acetate (3 × 25 mL) and the combined organic layers were dried over Na_2SO_4. The solvent was removed under reduced pressure, and the crude product was redissolved in CH_2Cl_2. The product was purified *via* column chromatography (0–10% ethyl acetate in hexanes (v/v)) to yield **18** as a separable mixture of free thiol and disulfides. The disulfide products of **18** were recovered, and the solvent removed under reduced pressure. The resultant yellow oil was redissolved in THF (1.8 mL), and DTT (150 mg, 0.97 mmol) and Cs_2CO_3 (320 mg, 0.98 mmol) were added. The solution was stirred for 1 hour at room temperature. The crude mixture was diluted with ethyl acetate (10 mL) and added to dilute HCl (1 M, 20 mL). The crude product was extracted with ethyl acetate (3 × 25 mL) and the combined organic layers were dried over Na_2SO_4. The solvent was removed under reduced pressure, and the crude product was redissolved in CH_2Cl_2. This additional **18** generated from recovered disulfide products was purified *via* column chromatography (0–10% ethyl acetate in hexanes (v/v)), and combined with **18** from the initial purification.

The free thiol product is prone to form a charge transfer complex with residual iodine from the reaction, resulting in a yellow oil that can rapidly form disulfides. In order to disrupt the charge transfer complex and remove the iodine, the combined product **18** was precipitated from hexanes, or recrystallized from 25% ethyl acetate in hexanes (v/v), and the white, crystalline solids were filtered. The product after reduction of recovered disulfides and removal of iodine was obtained in 30% yield (95 mg, 0.27 mmol). NMR spectra reflected a mixture of *cis* and *trans* rotamers (1 : 5.7 ratio) about the carbamate bond. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.20 (d, J = 8.1 Hz, 2H), 7.04 (d, J = 8.1 Hz, 2H), 4.98 (d, J = 8.0 Hz), 4.70 (br s) (sum of 4.98 ppm and 4.70 ppm resonances = 1H), 4.41 (ddd, J = 7.7 Hz, 6.1 Hz, 6.1 Hz), 4.22 (br s) (sum of 4.41 ppm and 4.22 ppm resonances = 1H), 3.41 (s, 1H), 3.00 (ddd, J = 13.8 Hz, 6.2 Hz, 6.0 Hz), 2.90 (br s) (sum of 3.00 ppm and 2.90 ppm resonances = 2H), 1.42 (s), 1.41 (s) (sum of 1.42 ppm and 1.41 ppm resonances = 18H). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 170.78, 155.02, 133.99, 130.28, 129.48, 128.85, 82.18, 79.71, 54.68, 37.89, 28.30, 27.95. HRMS (LIFDI-TOF) m/z: $[M + H]^+$ calcd for $C_{36}H_{52}N_2O_8S_2$ 353.1661, found 353.1673.

Boc-4-(*S*-propargyl)-thiol-L-phenylalanine-*O*-*tert*-butyl ester (19)

18 (180 mg, 0.51 mmol) was dissolved in THF (5.1 mL). Propargyl bromide (150 μL, 80 wt% in toluene, 1.4 mmol) and cesium carbonate (331 mg, 1.02 mmol) were added, and the mixture was stirred at room temperature for 4 h, or until the disappearance of **18** was confirmed *via* TLC. The reaction mixture was quenched with dilute HCl (1 M, 40 mL), and the crude product was extracted with ethyl acetate (3 × 40 mL). The combined organic layers were dried over Na_2SO_4 and filtered, and the solvent was removed under reduced pressure to produce a pale yellow oil. The crude product was dissolved in hexanes (10 mL) and purified *via* column chromatography (5–12% ethyl acetate in hexanes v/v) to obtain **19** (101 mg, 0.258 mmol) as a yellow oil in 51% yield. NMR spectra reflected a mixture of *cis* and *trans* rotamers (1 : 5.9 ratio) about the carbamate bond. ^{1}H (600 MHz, $CDCl_3$) δ 7.38 (d, J = 8.1 Hz, 2H), 7.14 (d, J = 8.2 Hz, 2H), 5.01 (d, J = 7.7 Hz), 4.73 (br s) (sum of 5.01 ppm and 4.73 ppm resonances = 1H), 4.44 (dd, J = 13.5 Hz, 6.3 Hz), 4.25 (br s) (sum of 4.44 ppm and 4.25 ppm resonances = 1H), 3.58 (d, J = 2.6 Hz, 2H), 3.03 (ddd, J = 13.9, 6.5, 6.3 Hz), 2.93 (br s) (sum of 3.03 ppm and 2.93 ppm resonances = 2H), 2.22 (t, J = 2.6 Hz, 1H), 1.42 (s), 1.40 (s) (sum of 1.42 ppm and 1.40 ppm resonances = 18H). ^{13}C NMR (150.9 MHz, $CDCl_3$) δ 170.80, 155.04, 135.60, 133.20, 130.34, 130.20, 82.17, 79.85, 79.73, 71.56, 54.73, 38.15, 28.33, 27.97, 22.77. HRMS (LIFDI-TOF) m/z: $[M + H]^+$ calcd for $C_{25}H_{31}SNO_4$ 391.1817, found 391.1809.

Boc-4-(*S*-propargyl)-thiol-L-phenylalanine (20)

19 (32 mg, 0.082 mmol) was dissolved in 1,4-dioxane (1.6 mL). A solution of HCl (6 M, 1.6 mL) was added, and the resultant solution was stirred at 40 °C for 6 hours to produce **21**. The crude mixture was dried *in vacuo*, and the residue was redissolved in water (330 μL) and 1,4-dioxane (330 μL). Potassium carbonate was added until the pH was 9 (approximately 460 mg). Boc_2O (35 mg, 0.16 mmol) was added, and the resultant mixture was stirred at room temperature for 8 h. 1 M HCl (20 mL) was added to the mixture, and the product was extracted with ethyl acetate (3 × 20 mL). The combined organic layers were dried over Na_2SO_4 and filtered. The solvent was removed under reduced pressure and the resultant solid was redissolved in CH_2Cl_2. The crude product was purified *via* column chromatography (0–6% methanol in CH_2Cl_2 v/v) to obtain **20** (13 mg, 0.039 mmol) as an off-white solid in 47% yield over two steps. ^{1}H (400 MHz, CD_3OD) δ 7.37 (d, J = 8.2 Hz, 2H), 7.20 (d, J = 8.1 Hz, 2H), 4.32 (dd, J = 4.9 Hz, 9.1 Hz), 4.25 (br m) (sum of 4.32 ppm and 4.25 ppm resonances = 1H),

3.62 (d, J = 2.6 Hz, 2H), 3.14 (dd, J = 4.9 Hz, 13.8 Hz, 1 H), 2.88 (dd, J = 9.2 Hz, 13.9 Hz, 1H), 2.54 (t, J = 2.5 Hz, 1H), 1.38 (s), 1.33 (s) (sum of 1.38 ppm and 1.33 ppm resonances = 9H). ^{13}C NMR (100.6 MHz, CD_3OD) δ 175.41, 157.83, 138.02, 137.65, 134.83, 132.20, 131.13, 131.02, 119.86, 80.99, 80.54, 72.56, 57.68, 56.25, 38.85, 38.30, 28.69, 28.47, 22.97. HRMS (LIFDI-TOF) m/z: $[M + H]^+$ calcd for $C_{17}H_{21}SNO_4$ 335.1191, found 335.1183.

4-(*S*-Propargyl)-thiol-L-phenylalanine (21)

19 (32 mg, 0.082 mmol) was dissolved in 1,4-dioxane (1.6 mL). A solution of 6 M HCl (1.6 mL) was added, and the mixture was stirred at 40 °C for 6 h, or until the disappearance of **19** was confirmed *via* TLC. The solvent was removed under reduced pressure. The crude product **21** was used without purification. ^{1}H (600 MHz, CD_3OD) δ 7.44 (d, J = 8.2 Hz, 2H), 7.27 (d, J = 8.1 Hz, 2H), 4.25 (dd, J = 7.6, 5.5 Hz, 1H), 3.68 (d, J = 2.6 Hz, 2H), 3.25 (dd, J = 8.3 Hz, 15.2 Hz), 3.13 (dd, J = 7.8 Hz, 14.6 Hz) (sum of 3.25 ppm and 3.13 resonances = 2H), 2.57 (t, J = 2.5 Hz, 1H). ^{13}C NMR (150.9 MHz, CD_3OD) δ 171.17, 136.69, 134.10, 131.51, 131.20, 131.14, 72.65, 55.03, 55.84, 47.82, 37.16, 36.92, 22.52. HRMS (LIFDI-TOF) m/z: $[M + H]^+$ calcd for $C_{12}H_{13}SNO_2$ 235.0667, found 235.0677.

Fmoc-4-(*S*-propargyl)-thiol-L-phenylalanine (22)

19 (0.10 g, 0.26 mmol) was dissolved in 1,4-dioxane (5.1 mL). A solution of HCl (6 M, 5.1 mL) was added, and the resultant solution was stirred at 40 °C for 6 hours to produce **21**. The crude mixture was concentrated *in vacuo*, the mixture was neutralized with $NaHCO_3$ (approximately 1.8 g), and then Fmoc-Cl (132 mg, 0.51 mmol) was added to the mixture, and the resultant solution was stirred at room temperature for 6 h. As needed, additional $NaHCO_3$ was added to maintain basic conditions (pH approximately 8). After confirming disappearance of **21** *via* TLC, 1 M HCl (20 mL) was added to the mixture, and the product was extracted with ethyl acetate (3 × 20 mL). The combined organic layers were dried over Na_2SO_4 and filtered. The solvent was removed under reduced pressure and the resultant solid was redissolved in CH_2Cl_2. The crude product was purified *via* column chromatography (0–5% methanol in CH_2Cl_2 v/v) to obtain **22** (40 mg, 0.088 mmol) as an off-white solid in 34% yield over two steps. ^{1}H (400 MHz, CD_3OD) δ 7.78 (d, J = 7.6 Hz, 2H), 7.60 (d, J = 7.4 Hz), 7.59 (d, J = 7.4 Hz) (sum of 7.60 ppm and 7.59 ppm resonances = 2H), 7.52 (d, J = 7.1 Hz, 0.3H), 7.38 (t, J = 7.5 Hz), 7.34 (d, J = 8.2 Hz), 7.29 (m) (sum of 7.38 ppm, 7.34 ppm, and 7.29 ppm resonances = 7H), 7.20 (d, J = 8.1 Hz, 2H), 7.04 (d, J = 7.3 Hz, 0.3H), 4.41 (dd, J = 4.7 Hz, 9.8 Hz, 1H), 4.31 (dd, J = 10.4 Hz, 7.0 Hz, 1H), 4.20 (dd, J = 10.3 Hz, 7.1 Hz, 1H), 4.14 (t, J = 6.8 Hz, 1H), 3.56 (d, J = 2.6 Hz, 2H), 3.20 (dd, J = 14.0 Hz, 4.7 Hz, 1H), 2.91 (dd, J = 13.8 Hz, 9.9 Hz, 1H), 2.49 (t, J = 2.6 Hz, 1H). ^{13}C NMR (100.6 MHz, CD_3OD) δ 175.02, 158.42, 145.25, 142.58, 137.62, 134.95, 131.13, 131.03, 128.80, 128.21, 126.39, 126.28, 120.93, 72.56, 67.98, 56.65, 38.11, 22.91. HRMS (LIFDI-TOF) m/z: $[M + H]^+$ calcd for $C_{30}H_{25}SNO_6$ 457.1348, found 457.1372.

Peptide synthesis and purification

The peptides Ac-TYHyp(4-I-Ph)N-NH_2 and Ac-TPhe(*S*-propargyl)PN-NH_2 were synthesized using the above-synthesized Fmoc amino acids *via* standard methods of solid-phase peptide synthesis. Peptides were purified to homogeneity *via* HPLC and characterized for purity, identity, and structural preferences *via* NMR spectroscopy and electrospray ionization mass spectrometry. Details are in the ESI.† The peptides Ac-TYHyp(4-I-Ph)N-NH_2 and Ac-TYHyp(C(O)CH_2CH_2CCH)N-NH_2 were synthesized previously.[7e]

Suzuki–Miyaura reaction on Ac-TYHyp(4-I-Ph)N-NH_2

The peptide Ac-TYHyp(4-I-Ph)N-NH_2 (approximately 50 nmol; 100 μM final concentration) was dissolved in phosphate buffer (500 μL, 50 mM aqueous phosphate pH 7.9) containing 20% acetonitrile. To this solution, $Pd(OAc)_2$·ligand (20 μL of 10 mM $Pd(OAc)_2$ and 10 mM ligand (the disodium salt of 2-amino-4,6-dihydroxypyrimidine) in 100 mM NaOH; 400 μM final concentration) and 4-methoxyphenyl boronic acid (7.6 mg, 50 μmol, 100 mM final concentration) were added. The resultant solution was allowed to incubate at 37 °C for 30 minutes, to produce the cross-coupled peptide product. The peptide was purified *via* HPLC using a linear gradient of 0–50% buffer B (20% H_2O, 80% MeCN, 0.05% TFA) in buffer A (98% H_2O, 2% MeCN, 0.06% TFA) over 60 minutes: t_R 43.0 min, exp. 732.3, obs. 755.4 $(M + Na)^+$. The above conditions were employed to directly compare to prior results on the diastereomeric peptide containing 4*S*-iodophenyl hydroxyproline.[7e] In addition, experiments were conducted using lower concentrations of boronic acid. These reactions were analyzed as a function of time. At 5 mM boronic acid, the reaction was 21% complete at 30 minutes, 76% complete at 2 hours, 94% complete at 3.5 hours, and 97% complete at 5 hours; at 10 mM boronic acid, the reaction was 51% complete at 30 minutes and 94% complete at 2 hours. These reactions were conducted under pseudo-first order conditions (100 μM peptide/aryl iodide). Therefore, these conversions allow an estimate of the second order rate constant as ~3 × 10^{-2} M^{-1} s^{-1} for the aqueous Suzuki–Miyaura reaction[2i] (at 400 μM catalyst with this ligand and this combination of aryl iodide and boronic acid), a potentially broadly useful bioorthogonal reaction whose rate constant was not included in a recent comparison of rate constants for bioorthogonal reactions.[1d] See the ESI† for details.

Copper-mediated azide–alkyne cycloaddition reaction with Ac-TYHyp(C(O)CH_2CH_2CCH)N-NH_2

The peptide Ac-TYHyp(C(O)CH_2CH_2CCH)N-NH_2 (approximately 50 nmol; 100 μM final concentration) was dissolved in phosphate buffer (500 μL of 50 mM phosphate buffer pH 7.9). To this solution, copper(II) sulfate (50 μL of a 10 mM solution in water), sodium ascorbate (5.0 mg), and 1,10-phenanthroline (10 μL of a 10 mM solution in DMSO) were added. To this solution was added to 4-azidoaniline·HCl (4.5 mg; 50 mM final concentration). The reaction mixture was allowed to incubate

at 37 °C for 5 minutes to generate the conjugated peptide. The crude solution was diluted with deionized water (300 μL), and diethyl ether (1 mL) was added. After thorough mixing, the ether layer was removed *via* pipet, and CH_2Cl_2 (1 mL) was added. After thorough mixing, the aqueous layer was removed and filtered through a nylon filter (0.45 μm). The peptide was purified *via* HPLC using a linear gradient of 0–15% buffer B in buffer A over 30 minutes: t_R 29.6 min, exp. 764.3, obs. 787.3 $(M + Na)^+$.

Oxidation of Ac-TPhe(*S*-propargyl)PN-NH_2 to the sulfoxide Ac-TPhe(S(O)-propargyl)PN-NH_2

The peptide Ac-TPhe(4-*S*-propargyl)PN-NH_2 (approximately 5 nmol) was dissolved in a mixture of H_2O : MeCN (5 : 3 v/v, 80 μL). $NaIO_4$ was then added (2.4 μL of a 12.3 mg mL^{-1} solution in water). The mixture was stirred at room temperature for 24 h to produce the peptide Ac-TPhe(4-S(O)-propargyl)PN-NH_2 as an inseparable mixture of sulfoxide diastereomers. The peptide was purified *via* HPLC using a gradient of 0–40% buffer B in buffer A over 60 minutes: t_R 18.9 min, exp. 604.2, obs. 627.1 $(M + Na)^+$.

Oxidation of Ac-TPhe(*S*-propargyl)PN-NH_2 to the sulfone Ac-TPhe(SO_2-propargyl)PN-NH_2

The peptide Ac-TPhe(4-*S*-propargyl)PN-NH_2 (approximately 5 nmol) was dissolved in methanol (100 μL). To this solution was added 95% formic acid in water (150 μL; final: 40% formic acid), followed by a solution of 30% H_2O_2 (100 μL). The resultant solution was allowed to incubate at room temperature for 9 hours to produce the peptide Ac-TPhe(4-SO_2-propargyl)PN-NH_2. The peptide was purified *via* HPLC using a linear gradient of 0–10% buffer B in buffer A over 30 minutes: t_R 26.1 min, exp. 620.2, obs. 643.1 $(M + Na)^+$.

This peptide was alternatively synthesized *via* solid-phase oxidization. MeOH was added to the protected peptide Ac-T(*t*-Bu)Phe(4-*S*-propargyl)PN(Trt)-NH-resin on solid phase in a disposable, fritted tube. 1 mL 90% formic acid (final: 30% formic acid) and 200 μL 30% H_2O_2 (final: 2% H_2O_2) in water were added, and the resultant solution was subjected to rotary mixing for 12 h at room temperature. The solution was removed and the resin washed with DMF (3×) and CH_2Cl_2 (3×) and dried, followed by standard TFA cleavage/deprotection and purification (ESI page S32†), to generate the peptide Ac-TPhe(4-SO_2-propargyl)PN-NH_2, which was identical by NMR to the peptide synthesized by solution phase oxidation.

Copper-mediated azide–alkyne cycloaddition reaction with Ac-TPhe(*S*-propargyl)PN-NH_2

The peptide Ac-TPhe(4-*S*-propargyl)PN-NH_2 (approximately 17 nmol; 70 μM final concentration) was dissolved in phosphate buffer (250 μL of 50 mM phosphate pH 7.5). To the peptide-containing solution, copper(II) sulfate (25 μL of a 10 mM solution in water), sodium ascorbate (2.5 mg), and 1,10-phenanthroline (5 μL of a 10 mM solution in DMSO) were added. To this solution was subsequently added 4-azidoaniline·HCl (2.7 mg, 60 mM final concentration), and the resultant solution allowed to incubate at 37 °C for 5 minutes to produce the conjugated peptide. The crude solution was diluted with deionized water (300 μL), and diethyl ether (1 mL) was added. After thorough mixing, the ether layer was removed *via* pipet, and CH_2Cl_2 (1 mL) was added. After thorough mixing, the aqueous layer was removed and filtered through a nylon filter (0.45 μm). The peptide was purified *via* HPLC using a linear gradient of 0–20% buffer B in buffer A over 60 minutes: t_R 48.5 min, exp. 722.3, obs. 745.4 $(M + Na)^+$.

X-ray crystallography

Crystals were mounted using viscous oil onto a plastic mesh and cooled to the data collection temperature. Data were collected on a Bruker-AXS APEX II DUO CCD diffractometer with Mo-Kα radiation (λ = 0.71073 Å) monochromated with graphite. Unit cell parameters were obtained from 36 data frames, 0.5° ω, from three different sections of the Ewald sphere. The systematic absences in the diffraction data are consistent with $P2_1$ and $P2_1/m$ for **7** and **10**; and, uniquely, for $P2_12_12_1$ for **5**. The absence of a molecular mirror, known chirality, and occupancy of two for **7** and **10** are consistent with the non-centrosymmetric option, P_{21}. The data-sets were treated with multi-scan absorption corrections (Apex3 software suite, Madison, WI, 2015). The structures were solved using direct methods and refined with full-matrix, least-squares procedures on F^2.[39] Refinement of the absolute structure parameter for each structure yielded nil, indicating that the true hand of the data has been determined. A molecule of methanol solvent was found in H-bonding association with the compound molecule in **7**. All non-hydrogen atoms were refined with anisotropic displacement parameters. H-atoms were placed in calculated positions with U_{iso} equal to 1.2 (1.5 for methyl H) U_{eq} of the attached atom. Atomic scattering factors are contained in the SHELXTL program library.[39] The CIF files have been deposited with the Cambridge Crystallographic Database under CCDC 1438138–1438140 (**5**, **7**, and **10**, respectively).

Analysis of aryl ethers, thioethers, sulfoxides, and sulfones in the CSD

Crystal structures for aryl ethers, aryl thioethers, aryl sulfoxides, and aryl sulfones in the Cambridge Structural Database (CSD) (version 5.36, released May 2015, analysis conducted 29 December 2015) were identified and analyzed using ConQuest (version 1.17, November 2014).[29] Only error-free, non-disordered structures where $R < 0.10$ were included, and powder pattern structures were excluded (search parameters are detailed in the ESI†). The search examined 6-membered carbon-based aromatic rings bonded to sulfur or oxygen atoms that were bonded to an sp^3-hybridized carbon (carbons with 4 attached substituents). This search resulted in 59 294 aryl ethers, 1966 aryl thioethers, 447 aryl sulfoxides, and 887 aryl sulfones. For each structure, the torsion angle $C_{aromatic}$–$C_{aromatic}$–X–$C_{aliphatic}$ (X = O, S, S(O), or SO_2), the $C_{aromatic}$–X bond length, and the $C_{aromatic}$–X–$C_{aliphatic}$ bond angle were obtained. The torsion angles originally obtained ranged from +180° to −180°; a logic function was applied so that all torsion

angles ranged from 0° to 90°, where 0° represents X–$C_{aliphatic}$ bonds that are co-planar with the aromatic ring, and 90° represents X–$C_{aliphatic}$ bonds that are orthogonal to the aromatic ring.

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