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Reversible and Versatile On-Tether Modification of Chiral-Center-Induced Helical Peptides

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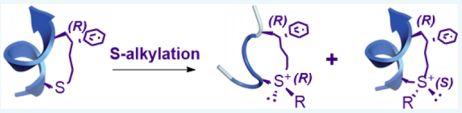
Supporting Information

ABSTRACT: Modification of the cross-linker of constrained peptides has recently received considerable attention. Here, we present a versatile approach to modifing the cross-linking tether of chiral-center-induced helical (CIH) peptides via the S-alkylation reaction. The alkylation process displayed high conversion efficiency, selectivity, and substrate tolerance. Notably, although on-tether S-alkylation could lead to a pair of peptide epimers, the major alkylated product retained the helical structure of its helical precursor peptide. This S-alkylation was readily reversible under reductive conditions, which provides a simple method for traceless modification. In addition to expanding the chemical space of CIH peptides, this strategy is the first on-tether modification platform with known retention of the peptides' original helicity.



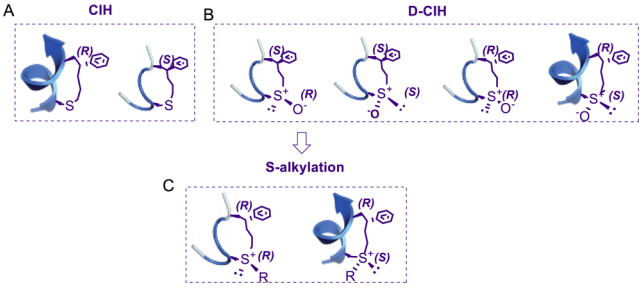
INTRODUCTION

The α-helix is one of the most abundant secondary protein structures and plays a critical role in the recognition of biomolecules.[1−3] A common approach to fine-tuning biophysical properties such as stability, target-binding affinity, and cellular uptake is to chemically constrain peptides into α-helical mimics. Constraint peptide ligands have now been widely utilized to interrupt several protein−protein interactions (PPIs),[2,4−6] which had previously been regarded as "undruggable" by small molecules.[7−10] State-of-the-art synthetic strategies have been developed for a variety of purposes, including disulfide-bond and lactam-bridge formation,[11−13] ring-closing alkene and alkyne metathesis,[14−17] click reactions,[18,19] cys alkylation,[20] and the incorporation of perfluoroarenes.[21,22]

In the development of constraint peptides, numerous studies reported that in some cases, the peptide tether could directly interact with the target protein despite its design on the solvent exposure face.[9,23−25] This observation has piqued interest for further research on tether modifications. Recently, deliberately designed modifications were shown to enhance the peptides' binding affinities and cellular uptakes.[26−29] In general, a modifiable functional group is purposely reserved on the tether to allow further modification, such as the incorporation of a ketone moiety for oxime ligation[30] or an alkyne for copper-catalyzed azide−alkyne cycloaddition.[31] Recently, Ley et al. developed a two-component i and i + 7 stapling method that enables the incorporation of an in-tether chiral center as well as post-stapling modification.[32] Although a peptide's biophysical properties are directly related to its secondary structure, little is known about how on-tether modifications can influence the backbone peptide's secondary structure. (In-tether means that the modification or chiral center is performed or generated before and during peptide synthesis, and on-tether modification means that the modification is an after-peptide-synthesis modification.) To the best of our knowledge, an elaborate on-tether modification approach that maintains the peptide's secondary structure is yet to be developed.

Methionine (Met) methylation is a well-known post-translational modification of proteins.[33] Alkylation of Met in short peptides has also been extensively studied by Deming et al. and others.[34−36] In 2016, we reported that a precisely positioned chiral center of *R* absolute configuration at the C-γ position to the peptide C-terminus in a seven-membered linker could effectively induce the helical conformation of the backbone peptide, while its *S* epimer was mainly in random coil conformation (illustrated in Scheme 1A). This chirality induced helicity (CIH) concept has since been extended from hydrocarbon to sulfoxide or sulfilimine chiral centers.[37,38] In a

Scheme 1. Schematic Overview of Different CIH Strategies and the Correlations between Stereocenter(s) and the Backbone Peptide's Secondary Structure[a]



[a](A) CIH strategy: an in-tether chiral center would largely determine the peptide's secondary structure. (B) Dual in-tether chiral centers: only the (N-γ *S*, C-γ *R*) epimer is helical. (C) S-alkylation of CIH peptides: a promising platform for structure-retaining on-tether modification.

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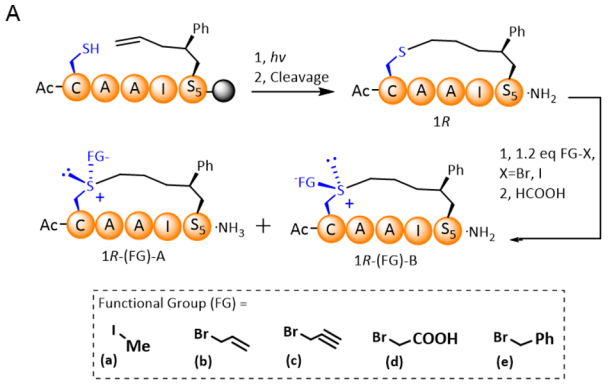

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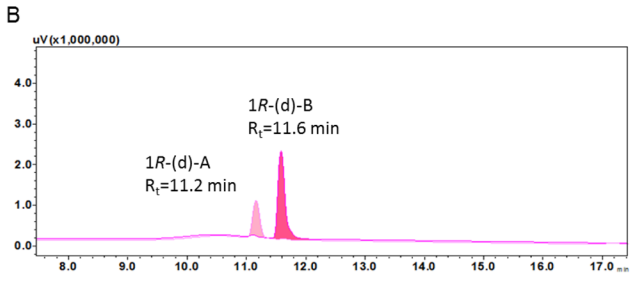
Entry	FG[a]	conversion	epimer B					ratio[c]
			$[\theta]_{215}$	$[\theta]_{207}$	$[\theta]_{190}$	$[\theta]_{215}/[\theta]_{207}$	Relative helicity[b]	
1*R*	-	-	-10955	-14347	35085	0.76	0.61	-
1*R*-(a)	Me	0.62	-6047	-7601	8290	0.80	0.33	1:1.5
1*R*-(b)		0.78	-8120	-13357	25167	0.61	0.45	1:1.8
1*R*-(c)		0.70	-9342	-17501	25735	0.53	0.52	1:1.5
1*R*-(d)	COOH	0.77	-12998	-18464	41006	0.70	0.72	1:2
1*R*-(e)	Ph	0.98	-5946	-8934	17030	0.67	0.33	1:3



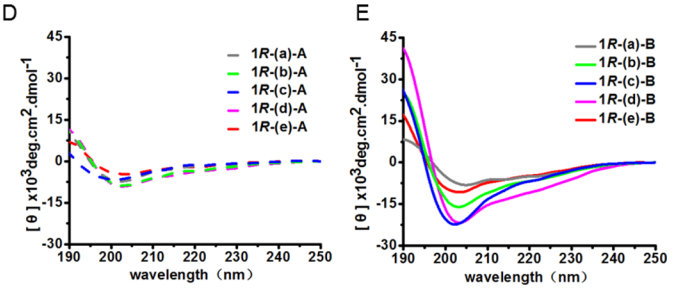


Figure 1. (A) Synthesis steps for S-alkylation on the CIH peptide tether. Peptide **1*R*** was synthesized via an intramolecular thiol−ene reaction for cyclization. Thioether-tethered peptides were alkylated in 0.2 M aqueous formic acid at RT to produce peptides **1*R*-(a−e)-A** or **-B**. (B) Representative high-performance liquid chromatography (HPLC) separation of peptide epimers **1*R*-(d)-A** or **-B**. (C) Alkylation conversion, epimer product ratios, molar ellipticities, and percentage of helical content of peptide **1*R*** modified with different functional groups (FG) a−e. [b]The α-helical content of each peptide was calculated as reported previously.[43] The final helical content shown is relative to peptide **2*R*-(d)-B**, which was defined as 100% helical. [c]The ratios were calculated based on the integration of the peak areas of peptide epimers Group A to Group B in HPLC. (D, E) CD spectra of peptides **1*R*-(a−e)-A** or **-B**, respectively. CD spectra were recorded at a peptide concentration of 25 μM in PBS buffer (50 mM, pH 7.4) at 20 °C.

Table 1. Sequence Tolerance of Sulfonium-Functionalized Pentapeptides[c]

Entry	FG	(2R) Ac-cyclo[CAAAS$_5$(Ph*R*)]-NH$_2$			(3R) Ac-cyclo[CAQAS$_5$(Ph*R*)]-NH$_2$			(4R) Ac-cyclo[CASAS$_5$(Ph*R*)]-NH$_2$			(5R) Ac-cyclo[CARAS$_5$(Ph*R*)]-NH$_2$			(6R) Ac-cyclo[CRRRS$_5$(Ph*R*)]-NH$_2$		
		Conversion	Ratio[a]	Relative helicity[b]	Conversion	Ratio	Relative helicity	Conversion	Ratio	Relative helicity	Conversion	Ratio	Relative helicity	Conversion	Ratio	Relative helicity
R	-	-	-	0.76	-	-	0.54	-	-	0.30	-	-	0.18	-	-	0.10
(a)	Me	0.58	only product	0.27	0.60	1:2.3	0.40	0.57	1:3	0.29	0.54	1:3	0.17	0.60	1:2.5	N/A
(b)		0.83	1:3.5	0.49	0.77	1:1.8	0.64	0.76	1:2	0.34	0.82	1:2	0.19	0.79	1:3	N/A
(c)		0.69	1:4.5	0.32	0.65	1:2.5	0.55	0.73	1:2.3	0.30	0.77	1:4	0.23	0.78	1:2.2	N/A
(d)	COOH	0.76	only product	1.00	0.75	1:2	0.60	0.67	only product	0.41	0.69	1:2.7	0.23	0.65	only product	0.16
(e)	Ph	0.99	1:5	0.21	0.98	1:2.5	0.25	0.98	1:1.2	0.27	0.95	1;5	0.08	0.94	1:2.4	N/A

[a]The ratios were calculated based on the integration of the peak areas of peptide epimers A/epimers B in HPLC. For those entries with only one epimer, the ratio was designated as "only product". [b]The α-helical contents of the B epimers (or the only products' helicity) were calculated as previously reported.[37] The final helical content presented is relative to peptide **2*R*-(d)-B**, which was defined as 100% helical. [c]The sequences of peptides **2*R*−6*R*** are shown at the top of the table. Alkylation conversion, epimer product ratios, and percentage of helical contents of pentapeptides with different sequences are also shown.

systematic study of peptide helicity induced by double on-tether chiral centers, we determined that only one of four possible peptide epimers (N-γ *S*, C-γ *R*) was helical (Scheme 1B). Whenever a preset helical CIH peptide was oxidized, the helical (N-γ *S*, C-γ *R*) epimer was always the main product (the only product in some cases).[39] Inspired by the reversible chemoselective Met functionalization,[40,41] we therefore speculated that a sulfur atom in the tether of a preset helical CIH peptide could act as a suitable modification site that may minimize the potential for structural perturbation (Scheme 1C).

RESULTS AND DISCUSSION

To validate this approach, we first examined the alkylation efficiency of the thioether-tethered peptide. We synthesized a model peptide with well-documented helical structure,[34] **1*R***, Ac-(cyclo-1,5)-CAAIS$_5$(Ph*R*)−NH$_2$ with an elaborated *R*-configured in-tether chiral center (Figure 1A). After various alkylating agents (a−e) of different nucleophilicities were tested under different conditions, the formic acid additive[34,42] resulted in the best reaction and conversion rates (Table S1). All of the reactions went to completion in less than 4 h at room temperature (RT) and were then analyzed by reverse-phase

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high-performance liquid chromatography (RP-HPLC). For most entries, we observed both epimers with distinguishable retention times and designated the epimers with the longer retention times as epimers B unless otherwise specified (Figure 1B). Next, we examined the secondary structures of all epimers in phosphate-buffered saline (PBS) by circular dichroism (CD) spectroscopy (Figure 1D,E). The conversion efficiency, epimer ratio, and relative helicity of epimer B for all five entries are summarized in Figure 1C. The B epimers were always the major product and more-helical than their A counterparts. Notably, the peptide's original helical structure was maintained, while different modifications clearly influenced the helical content, which provides a useful platform for studying the relationship between on-tether substitution groups and the secondary structures of the backbone peptide. We chose to characterize alkylating agents with carboxylic acid, alkene, and alkyne functional groups, which could provide handles for additional modifications. We were unable to get solvable crystals from the peptides synthesized in this study, but based on our previous DCIH study,[39] we believe that the B epimers in this study also possess (N-γ *S*, C-γ *R*) stereocenters.

Next, we prepared peptides **2*R*−*R*** (Ac-cyclo-[CXXXS$_5$(PhR)]-NH$_2$; X means amino acids) to test the method's sequence flexibility. All of the peptides showed good conversion, epimer selectivity, and helicity, maintained as summarized in Table 1. The CD spectra for all peptides were summarized in Figure S1. Similarly, all B epimers were more helical than their A counterparts. We also noticed that for some entries, such as **2*R*-(d)** and **4*R*-(d)**, only one enantiomer was obtained, and it could be a mixture of A and B enantiomers or the B enantiomer. Thus, this proof-of-concept study demonstrated that this strategy provides a facile platform toward sulfonium peptides for after-peptide-synthesis modification with maintenance of the peptides' helical structures.

We then evaluated the serum, pH, thermal and in-guanidine HCl stabilities of the alkylated sulfonium peptides (Figure 2).

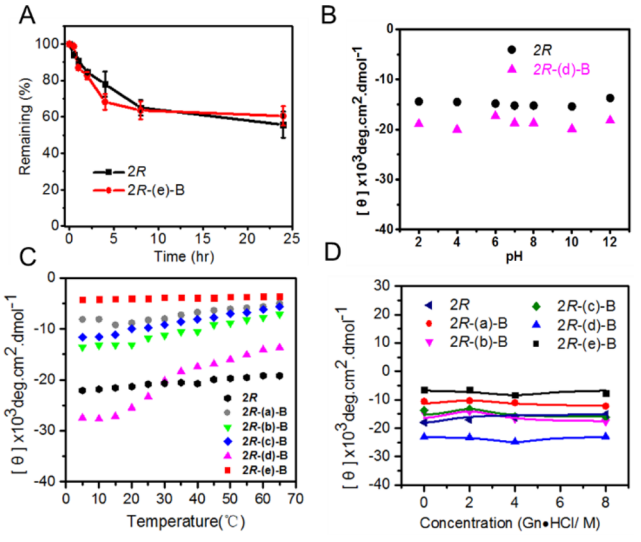

Figure 2. (A) In vitro serum digestion assay of peptides **2*R*** and **2*R*-(e)-B**. The percentage of intact peptide was recorded from HPLC integration. (B) The molar ellipticity at 215 nm of peptides **2*R*** and **2*R*-(d)-B** under different pH values. (C) Molar ellipticity at 215 nm of **2*R*** and **2*R*-(a−e)-B** at different temperatures from 5 to 65 °C. (D) Molar ellipticity at 215 nm of **2*R*** and **2*R*-(a−e)-B** at different concentrations of guanidine hydrochloride.

Peptides **2*R*** and **2*R*-(e)-B** were similarly stable in serum and remained more than 60% intact after 24 h (Figure 3A). We next

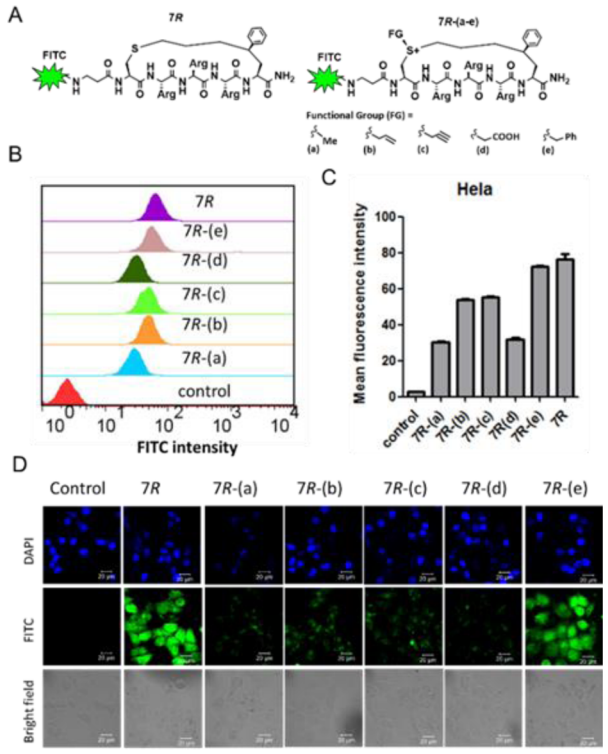

Figure 3. (A) The chemical structures of peptide **7*R*** and **7*R*-(a−e)-B.** (B) Flow-cytometry analysis of the cellular uptake of peptides by HeLa cells. HeLa cells were incubated with peptides (5 μM) for 2 h at 37 °C. (C) A histogram that represents the intracellular fluorescence signal of peptides measured by flow cytometry. Values represent the average of three independent experiments. (D) Confocal microscopy images of the **7*R*-(a−e)-B** peptides in HeLa cells. The cells were incubated with peptides (5 μM) at 37 °C for 2 h. Nuclei were stained with 4', 6-diamidino-2-phenylindole (blue), while the peptides were labeled with fluorescein isothiocyanate (FITC, green). The scale bars are 20 μm.

measured the molar ellipticity of peptide **2*R*-(d)-B** at 215 nm at different pH values; the precursor peptide **2*R*** was set as a control (Figure 2B). The **2*R*-(d)-B** conformation changed negligibly between pH 2−12, demonstrating a high pH stability of the sulfonium center. Thermal denaturation experiments of peptides **2*R*-(a−e)-B** demonstrated a temperature-dependent change in molar ellipticity at 215 nm that was consistent with a partial unwinding of the helix (Figures 2C and S2), while in comparison with **2*R***, these sulfonium peptides displayed more apparently decrease with elevated temperature. After reinjection of the peptide **2*R*-(c)-B** into HPLC after 30 min of heating at 70 °C, we found that approximately 5% of the peptides epimerized (Figure S3), which could also be attributed to the compromise of helical content. Finally, the molar ellipticity at 215 nm of peptides **2*R*-(a−e)-B** was measured in different concentrations of guanidine hydrochloride, and the results suggested that the sulfonium peptides retained helical conformation, even with a high concentration of denatured reagents (Figure 2D).

To test the effects on the peptides' biophysical properties, we evaluated the cellular uptakes of peptide **7*R*-(a−e)-B** by flow cytometry (Figure 3A). These peptides displayed significantly different cellular uptakes (Figure 3B−C). Unexpectedly, most

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alkylated peptides displayed less intracellular mean fluorescence intensity (MFI) than **7*R***. These results were further confirmed by confocal microscopy (Figure 3D). Deming et al. revealed that alkylation of polymethionine to sulfonium peptides could significantly enhance the peptide's cell permeability via the incorporation of sterically demanding hydrophilic cationic groups in polypeptides.[42] In our case, we hypothesized that the on-tether sulfonium structure might disrupt the hydrophobic interface formed by the aliphatic thioether tether, which counteracted the additional positive charge. Notably, peptide **7*R*-(e)-B** had a similar cellular uptake rate as peptide **7*R***, which supported our hypothesis and suggested that we could possibly tune the peptide's biophysical properties with this post-synthesis on-tether modification method. In addition, the handle group in the sulfonium chiral center can be further modified for preferable properties. We successfully conjugated a TAT peptide onto the tether via a copper-catalyzed alkyne−azide click reaction (Figure S4), and the conjugated peptide **8*R*** showed improved cell permeability over both peptides **7*R*-(c)-B** and **7*R*** (Figure S5).

To this end, we demonstrated that the alkylation on the thiol−ether tether of CIH peptides is a facile strategy to expand the chemical space of constraint peptides. The sulfonium helical peptides possessed good stability in different rigorous conditions. Comparing to the D-CIH helical peptides we reported previously,[39] the sulfonium peptides showed similar helical contents as D-CIH sulfoxide peptides. Interestingly, the sulfonium peptides were more stable in the presence of serum and denaturing reagents. The sulfonium peptides also displayed good tolerance to temperature variations. (The date is shown in Figure S8\) The sulfonium strategy is more practicable than the D-CIH sulfoxide system because it generates a modifiable sulfonium stereocenter. Different substituents could be installed to optimize for desired biophysical properties or for further modifications.

Traceless labeling of peptides or proteins is crucial for some biological applications, such as the release of therapeutic peptides from a carrier or the recovery of affinity-purified, tagged peptide fractions from protein digests for downstream proteomic analysis.[44−46] However, few of the existing selective peptide or protein tags, other than labile disulfides, are reversible to allow for the inducible regeneration of unmodified samples.[47−49] Recently, Deming et al. demonstrated that polymethionine alkylation can be reversible under certain reductive conditions.[34,42] Understanding the stability of different S-alkylation groups could allow efficient S-alkylation, accessible bio-orthogonal on-tag modification, and a controllable removal of the alkylation group. Therefore, we evaluated the sulfonium peptide stability in the presence of reducing agents using peptide **2*R*-(a−e)-B** as the model polypeptide (Figure 4A,B). Peptides **2*R*-b/c/e-B** showed time-dependent dealkylation into **2*R*** as the sole product in 0.1 M 4-mercaptopyridine (PyS) in dimethylformamide (DMF) (Figures 4A,B and S6), while peptides **2*R*-a/d-B** were inert in this condition. These results were consistent with previous studies by Deming and others.[34] Next, we tested the dealkylation property of peptide **7*R*-c-B** as the alkyne tag could enable further labeling. High-resolution mass spectrometry (HRMS) analysis of HEK293T lysed after culture with 10 μM **7*R*-(c)-B** for 24 h also identified the dealkylated peptide **7*R*** (Figure S7). Thus, to the best of our knowledge, this strategy is the first report of a traceless on-tether modification system.

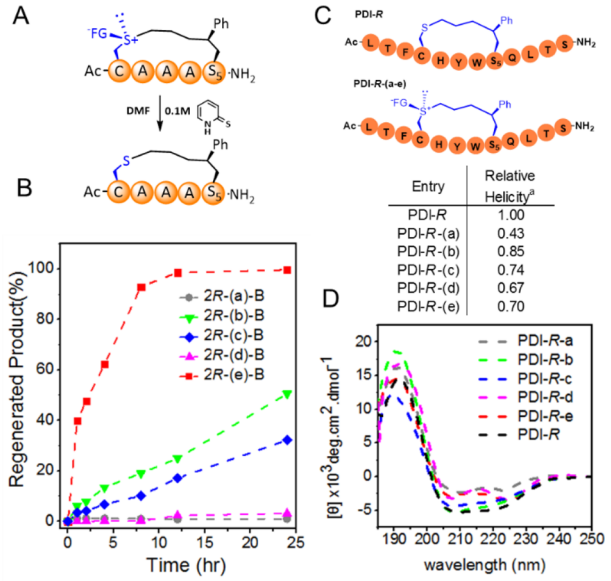

Figure 4. (A) Dealkylation process of the sulfonium peptides. (B) The dealkylation products of peptides **2*R*-(a−e)-B** treated with 0.1 M PyS in DMF detected by LC−MS at different times. (C) The chemical structures and calculated relative helicity of peptides **PDI-*R*** and **PDI-R-(a−e)**. (D) CD spectra for **PDI-*R*-(a−e)**. CD spectra were recorded at a peptide concentration of 25 μM in PBS buffer at 20 °C. [a]The final helical content presented is relative to peptide **PDI-*R***, which was defined as 100% helical.

To test this strategy's effects on longer peptides and its tolerance of multiple simultaneous functional groups, we prepared an E3 ligase protein MDM2-targeting sequence cyclo-LTFCHYWS$_5$(Ph*R*)QLTS (Figure 4C). In the presence of free hydroxyl, phenol, indole, and guanidinium groups, PDI-*R* was readily alkylated with high conversion efficiencies at RT. Notably, only one epimer was detected for all entries, and CD measurements indicated that all of them retained the helical structure of PDI-*R* (Figure 4D).

CONCLUSIONS

This proof-of-concept study demonstrated that the on-tether S-alkylation of CIH peptides can be utilized as a versatile on-tether modification method that maintains the peptide's secondary structure. This method promises to expand the chemical space of constraint peptides via the diversification of the tether in a convergent manner. This method is efficient under mild conditions and tolerant to multiple functional groups from various residues. This efficient post-synthesis modification system allows for the on-tether placement of important tags. Alkylation is reversible under reductive environments, including the intracellular circumstances. Different alkylation groups vary significantly in their modulation of a peptide's biophysical properties, which provides an intriguing venue for constructing peptides of preferable properties with this post-synthesis modification strategy. Therefore, this method has the potential to efficiently generate a vast array of peptide probes. Further study of this method is under current investigation in our laboratory.

MATERIALS AND METHODS

Reagents. All reagents, including amino acids and resins, were purchased from GL Biochem (Shanghai), Shanghai Hanhong Chemical Co., J&K Scientific, or Energy Chemical

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and were used without further purification. Unnatural amino acids were synthesized following reported procedures.[50] The detailed method can be accessed in the Supporting Information. NMP was purchased from Shenzhen Tenglong Logistics Co. and used without purification. All other solvents were purchased from Cantotech Chemicals, Ltd. Anhydrous solvents were purchased from J&K Scientific. Peptides were purified by HPLC (SHIMAZU Prominence LC-20AT) using reverse-phase C18 column Grace Vydac protein and peptide C18 250 × 10 mm at a flow rate of 5 mL/min or Grace Smart C18 (250× 4.6 mm) at a flow rate of 1 mL/min. Deionized H_2O (containing 0.1% TFA) and pure acetonitrile were used as solvents in linear gradient elution. HPLC fractions containing product (monitored by electrospray ionization) were combined and lyophilized. Molecular weights were measured using the SHIMAZU-SPD2020.

Peptide Alkylation Using Activated FG-X. Alkylated peptides were synthesized in 0.2 M aqueous formic acid (10 mg/mL). Alkyl halide (1.2 eq per peptide) was added. The reaction mixture was stirred at RT for less than 4 h. The pure product of alkylated peptide was obtained after HPLC purification. Typical yields of alkylated products were over 60%.

Circular Dichroism Spectroscopy. The peptides were dissolved at a concentration of 25 μM in PBS (50 mM, pH 7.4). CD spectra were obtained on an applied photophysics chirascan CD Spectrometer at 20 °C using the following standard parameters: wavelength, 185−260 nm; step resolution, 0.5 nm; speed, 20 nm/sec; accumulations, 10; response, 1 s; bandwidth, 1 nm; path 3 length, 0.1 cm. Every sample was scanned twice, and the final CD spectrum was averaged and smoothed. Thermal disruption curves were acquired by monitoring the signal at 215 nm while increasing the temperature stepwise by 5 °C with a 10 min equilibration between steps. The data were fit to a two-state folding model using Origin Pro 9.0. The pH-dependent effects on stability were assessed from pH 2 (0.01 M HCl) to pH 12 (0.01 M NaOH), prepared in distilled water. For longer peptides, percent helicity was calculated based on the equation described by Arora:[43] helicity (%) = $[\theta]_{215}/[\theta]_{max} \times 100$, where $[\theta]_{max} = (-44\,000 + 250T)(1 - k/n)$ for k is 4.0, n is number of amino acid residues in the peptide, and T = 20 °C. The final helical content was presented relative to peptide **PDI-*R***, which was defined as 100% helical. For pentapeptides, the helical content was first calculated based on the above equation and finally normalized into relative helicity, which fixed peptide **2*R*-(d)-B** as 100% helical.

Serum Stability Assay. Standard solutions of peptide **2*R*** and **2*R*-(e)-B** (1 mg/mL) were prepared in water. Each peptide (200 μL) was added to human serum (800 μL) and incubated at 37 °C. A total of 300 μL of an acetonitrile/water mixture (3:1) was added to 100 μL of serum aliquots at 0, 0.5, 1, 2, 4, 8, 12, and 24 h to precipitate serum proteins, which were then removed by centrifugation. The standard supernatant was analyzed by LC−MS with a Grace Smart C18 250 × 4.6 mm column using a 2% per minute linear gradient from 20% to 80% acetonitrile over 30 min. The amount of starting material left in each sample was quantified by LC−MS-based peak detection at 220 nm.

Flow Cytometry. HeLa cells were grown in Dulbecco's modified Eagle medium (DMEM) media supplemented with 10% fetal bovine serum (FBS) (v/v) in imaging dishes (50 000 cells per well) at 37 °C and 5% CO_2 for 2 days. Cells were incubated with fluorescently labeled peptides (5 μM) for up to 2 h at 37 °C. After washing with media, the cells were treated with trypsin (0.25%; Gibco) for 3 min at 37 °C, washed with PBS, and resuspended in PBS. Cellular fluorescence was analyzed using a BD FACSCalibur flow cytometer (Becton Dickinson).

Cell Imaging. HeLa cells were cultured in DMEM with 10% FBS (v/v) in imaging dishes (50 000 cells per well) at 37 °C and 5% CO_2 for 1 day until they were about 80% confluent. Peptides were first dissolved in DMSO to make a 1 mM stock solution and then added to cells to a final concentration of 5 μM. The cells were incubated with peptides for 1 h at 37 °C, washed 3 times with PBS, and then fixed with 4% formaldehyde (Alfa Aesar, MA) in PBS for 10 min. They were then washed 3 times with PBS and stained with 1 μg/mL 4′,6-diamidino-2-phenylindole (DAPI) (Invitrogen, CA) in PBS for 5 min. Images of peptide localization in cells were taken on a PerkinElmer confocal microscope. Image processing was done with the Volocity software package (Zeiss Imaging).

CuAAC Reaction for Peptide Modification. Pure, lyophilized peptide and $CuSO_4 \cdot 5H_2O$ (4.4 equiv) were dissolved in H_2O/*t*-BuOH (2:1) for a final peptide concentration of 1 mg/mL. Sodium L-ascorbate (4.4 equiv) dissolved in H_2O (2 mL) was added slowly. The reaction was stirred at RT for 60 min. The reaction product was then concentrated in vacuo and purified by RP-HPLC. The product conversion was about 40% as determined by HPLC.

In Vitro Dealkylation of Alkylated Peptide Assay. Alkylated peptides **2*R*-(a−e)-B** (0.5 mg, 0.8 mM) were dissolved in DMF, and 4-mercaptopyridine (11.1 mg, 0.1 M) was added. The reaction was stirred at RT. At different time points, an aliquot of each reaction was removed and monitored by LC−MS.

In Vivo Dealkylation of Alkylated Peptide Assay. HEK293-T cells were cultured as described above for 2 days and then treated with 10 μM alkylated peptides for 24 h. After that, the cells were exposed to trypsin (0.25%; Gibco) for 3 min at 37 °C, washed with PBS, and lysed in cell lysis buffer. The soluble peptide in the supernatant was separated with the precipitate after centrifugation. The dealkylation of peptide **7*R*-(c)-B** produced peptide **7*R***, which was analyzed by HRMS (QSTAR Elite).

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.bioconjchem.7b00321.

Figures showing a flow chart for amino acid synthesis, presentation of the solid-phase peptide sysntheses, CD spectra, temperature dependence of mean residue ellipticity, HPLC results, the click reaction, the dealkylation of peptides, and a comparison of biophysical properties of peptides. Tables showing conversion efficiency of the alkylation reaction, peptide characterization and a library of sulfonium-functionalized pentapeptides. HPLC and LC−MS data. PDF)

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Notes

The authors declare no competing financial interest.

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