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Native Chemical Ligation,Thiol−Ene Click: A Methodology for the Synthesis of Functionalized Peptides

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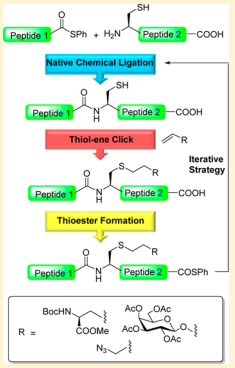
Supporting Information

ABSTRACT: The sequential combination of native chemical ligation and thiol−ene radical chemistry (NCL-TEC) on the resulting cysteine thiol has been investigated as a methodology for rapidly accessing functionalized peptides. Three sequential cycles of native chemical ligation and subsequent thiyl radical reactions (including a free-radical-mediated desulfurization reaction) were carried out on a peptide backbone demonstrating the iterative nature of this process. The versatility of the thiyl radical reaction at cysteine was demonstrated through the introduction of a number of different side chains including an amino acid derivative, a carbohydrate group, and an alkyl azide. Conditions were developed that allowed the sequential NCL-TEC process to proceed in high yield.



INTRODUCTION

Covalent modification of peptides and proteins is important for understanding biological function and for the development of new therapeutics. Free-radical-based methodologies at cysteine are emerging as powerful tools for the chemoselective modification of both peptides and proteins.[1,2] The alkene hydrothiolation reaction, also called the thiol−ene coupling reaction (TEC), has allowed the site-specific elaboration of peptides and proteins with a range of biomolecules including carbohydrates[3,4] and lipids.[5] The methodology is high yielding and is compatible with oxygen and an aqueous environment. This methodology has been demonstrated to be efficient on large peptides[3] and can function as a synthetic equivalent to post translational modification of proteins. The resulting thioether linkage is robust and biologically stable.[6]

The ligation reaction between an unprotected peptide thioester fragment and a second unprotected peptide possessing an *N*-terminal cysteine that results in formation of an amide bond is called native chemical ligation (NCL).[7−10] This ligation methodology results in peptide fragments linked by a cysteine residue and has allowed for the assembly of peptides and proteins beyond the scope of current synthetic SPPS methods.[11,12] Because of their low abundance in nature, the cysteine residues are often unwanted and are transformed by desulfurization reactions to furnish the more abundant alanine residue.[13,14] However, the cysteine residue also provides a synthetic handle for free-radical-based peptide functionalization.[3,5]

As part of an ongoing research program into the synthesis of glycosylated therapeutics[15] and functionalized nanomaterials,[16] we became interested in accessing functionalized peptides containing both carbohydrate units and fluorescent probes. We set out to investigate if a combination of native chemical ligation and thiol−ene click chemistry could be applied to the general synthesis of highly functionalized S-linked peptides, suitable for further conjugation onto nanomaterials. Simple amino acid building blocks were employed for the initial model studies, but we envisaged that SPPS could be used to access more complex building blocks for further study.

Here we report the results of our investigation into sequential NCL-thiyl radical reactions on a short peptide backbone. The ultimate goal of this study was to realize an iterative methodology that would allow continuous cycles of NCL and thiyl radical chemistry to be carried out efficiently on synthetic peptides. While there exists an abundance of methodologies for the functionalization of peptides and proteins at cysteine,[17] the hydrothiolation reaction was chosen due to its high yield and general applicability. Sequential NCL-TEC, as a methodology for the synthesis of functionalized peptides and proteins, is highly desirable in that both reactions are high yielding and can be carried out in an aqueous environment. Scheme 1 outlines the general iterative strategy for the NCL-TEC process.

Following the NCL reaction between two peptides, the resulting cysteine thiol is functionalized using a thiyl radical reaction to introduce a robust thiother linkage. This can be considered to be a thio-analogue of serine, a common glycosylation site in glycoproteins. The carboxyl terminus on the new glycopeptide is then converted to the reactive thioester for the next sequential NCL step. Iterative cycles of NCL and TEC can be repeated in order to furnish the extended functionalized peptide or protein. Sequential NCL reactions have previously been applied to the synthesis of long chain

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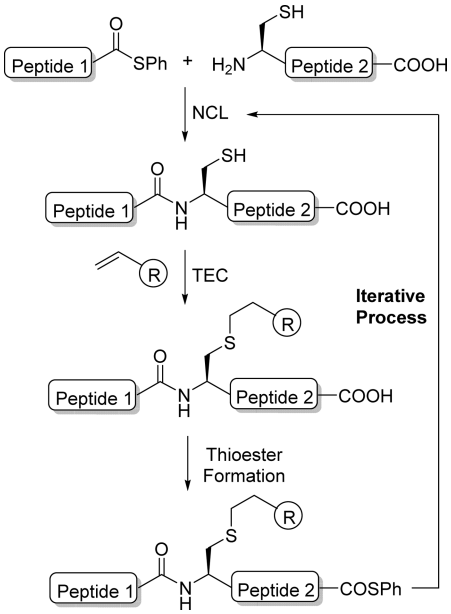
Scheme 1. Strategy for Sequential NCL-TEC Methodology

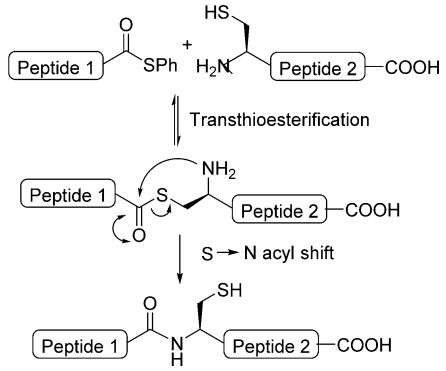


peptides[18−20] and methodology has been developed to introduce thioesters onto the C-terminus of the growing peptide chain in the presence of a range of functional groups. To the best of our knowledge, sequential NCL-thiol−ene coupling reactions remain unexplored. In this study, we aimed to carry out three successive NCL-thiyl radical mediated reactions on a peptide backbone in order to demonstrate the iterative nature of this process.

The sequential NCL-desulfurization reaction to furnish alanine linked peptide fragments has been reported by several groups; indeed, Danishefsky and co-workers have reported a mild desulfurization reaction that proceeds by way of a thiyl radical intermedicate in the conversion of cysteine to alanine following the NCL process.[2,21,22] This strategy has been widely adopted in peptide and protein synthesis.[23−25] We reasoned that the free-radical-based specific desulfurization of cysteine was appropriate to include within this study since its mechanism involves a thiyl radical intermediate, and this reaction can occur readily in the presence of the phosphine reagents used in NCL. We were also interested in demonstrating that the conditions employed for the radical-mediated desulfurization reaction would not affect the thioether linkage formed in the thiol−ene reaction. It has previously been demonstrated by Danishefsky and co-workers that the free-radical desulfurization of cysteine is compatible with thioethers.[2] Initially, with a view toward developing a one-pot procedure for the NCL-TEC sequence we first considered the proposed mechanism of NCL,[7,8] which is outlined in Scheme 2.

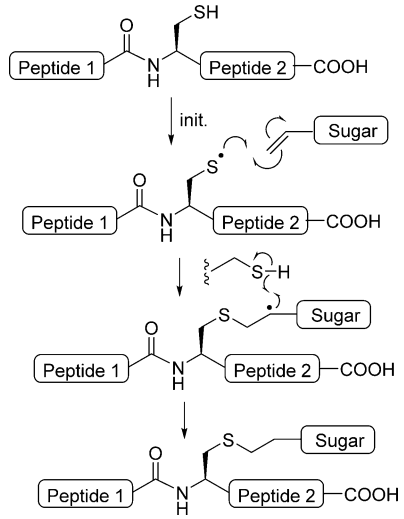
First, trans-thioesterification with the thiol side chain of an N-terminal cysteine residue results in formation of a thioester-linked intermediate. This thioester immediately undergoes a spontaneous rearrangement to give the native peptide bond at the ligation site. The reaction is normally carried out in aqueous media and at physiological pH in the presence of a phosphine-reducing reagent to avoid disulfide formation which would inhibit the ligation methodology. Byproducts generated by the NCL process include phosphine oxides and aromatic thiols. Danishefsky[2] and others[26−28] have reported that phosphorus-containing reagents react with thiyl radicals to give desulfurized

Scheme 2. Proposed Mechanism of Native Chemical Ligation



products.. We anticipated that any residual phosphine compounds, phosphine oxides, free thiol byproducts, or disulfides present following NCL could interfere with the thiol−ene reaction outlined in Scheme 3.

Scheme 3. Mechanism of Thiol−Ene "Click" Reaction



RESULTS AND DISCUSSION

We first investigated the sequential NCL-TEC process on a model system (Scheme 4). The NCL reaction between the two amino acids, Boc-protected alanine thioester **1** and cysteine methyl ester **2**, was first optimized. Native chemical ligation is usually applied to long peptide fragments but is equally effective for short peptides. Kent et al. determined that the rate of NCL is dependent on both the identity of the C-terminal amino acid derivatives and the thioester.[7,8] Alanine was selected at the C-terminal as it has a small side chain (Me) and should therefore readily undergo ligation. Tributyl phosphine was employed as a reducing agent. The NCL reaction proceeded in a yield of 96% to furnish the dipeptide **3**. In order to complete the sequential NCL-TEC cycle, the dipeptide **3** was subjected to a thiol−ene coupling reaction with a non-natural amino acid derivative (fully protected L-homoallylglycine) **4**. This residue had previously been employed for thiol−ene coupling reactions to furnish glycoproteins.[4] The thiol−ene coupling reaction gave the desired thioether-linked compound **5** in a yield of 63% (unoptimized). This reaction completed the first sequential

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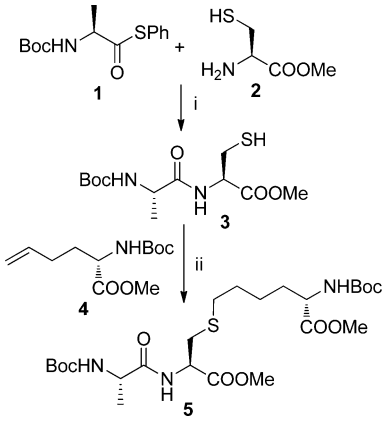
Scheme 4. NCL-TEC Sequence on a Model System[a]



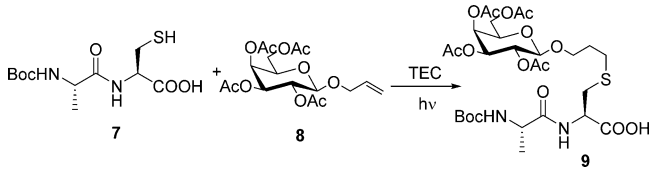
[a]Initial NCL-TEC cycle: (i) MeOH, rt, 1 h, Bu_3P, 96%; (ii) 4,4-azobis(4-cyanovaleric acid) (ACVA) (0.6 equiv), DMF, *hν*, rt, 2 h, 63%.

NCL-TEC cycle for this system and demonstrated that the methodology is viable for functionalized peptide synthesis.

Once the thiol−ene reaction between **3** and **4** had been completed, we carefully studied the effect of NCL byproducts on the thiol−ene reaction to see if the methodologies were compatible for a one-pot procedure. First, tributylphosphine was added to the TEC reaction, and as expected, the competing desulfurization process was found to dominate. Addition of tributylphosphine oxide also resulted in significantly diminished yields of the thiol−ene product. Addition of thiophenol, the byproduct from the trans-thioesterification step, also inhibited the TEC reaction. An attempt to couple arylthiols to an alkene using TEC was unsuccessful. It is likely that they form stable, unreactive thiyl radicals that inhibit the radical chain process. We concluded from this model study that a sequential NCL-TEC process was feasible provided that all traces of NCL byproducts had been removed since even trace quantities of phosphorus reagents or thiol contaminants would inhibit the radical reaction.

In order to further investigate the synthetic scope of this methodology and to demonstrate its synthetic utility for the preparation of functionalized peptides, we set out to complete three sequential NCL-thiyl radical steps on a short peptide backbone. For the iterative system, it was necessary to use unprotected cysteine where the C-terminus could be converted into a thioester following the NCL-thiyl radical sequence (Scheme 1). For the initial NCL-TEC sequence, peptide ligation followed by introduction of a carbohydrate group bearing a terminal alkene at the anomeric position onto the peptide backbone was investigated. Dondoni and co-workers have previously demonstrated the thiol−ene coupling reaction between glycans and cysteine-containing peptides and it has been reported to proceed in good yield without racemization of the cysteine.[6] Following NCL between **1** and **6**, purification of dipeptide **7** proved difficult and the residual phosphine oxide hindered the subsequent coupling reaction. Sodium borohydride was investigated as an alternative reducing agent for the NCL-TEC process since there would be no possibility of trace phosphine inhibiting the subsequent radical ligation step. A number of solvent systems were examined for their utility in the NCL reaction. Sodium borohydride in MeOH was found to be the most efficient system. When sodium borohydride was employed as a reducing agent for the NCL reaction the purification step was greatly simplified and the thiol−ene coupling reaction between **7** and **8** was optimized to furnish glycopeptide **9** in a 95% isolated yield (Table 1, entry 15).

Table 1. Optimization of TEC Conditions between Peptide 7 and Glycan 8 To Furnish Glycopeptide 9



entry	peptide (equiv)	sugar (equiv)	initiator	MAP[a]	conversion[b] (%)
1	1.0	2	0.4[c]	X	36
2	3.0	1	0.4[c]	X	78
3	3.0	1	0.6[c]	X	85
4	3.0	1	0.6[c]	0.6	87
5	3.0	1	0.3[d]	X	>99
6	2.0	1	0.2[d]	X	>99
7	1.0	1	0.1[d]	X	70
8	1.0	1	0.1[d]	0.1	77
9	1.1	1	0.1[d]	0.1	83
10	1.1	1	0.2[d]	0.2	84
11	1.3	1	0.13[d]	0.13	92
12	1.5	1	0.15[d]	0.15	>95
13	1.0	3	0.1[d]	X	84
14	1.0	3	0.1[d]	0.1	93
15	1.3	1	0.13[d]	0.13	95[e]

[a]4-Methoxyacetophenone (MAP). [b]Conversions determined by ^1H NMR. [c]4,4-Azobis(4-cyanovaleric acid) (ACVA) as radical initiator. [d]2,2-Dimethoxy-2-phenyl-acetophenone (DPAP) as radical initiator. [e]Isolated yield.

The optimization study of this reaction addressed some of the drawbacks associated with recent reports of TEC reactions for glycopeptides synthesis. In particular, the reported requirement for a 3-fold excess of the thiol reagent[6] was found to be unnecessary for this system. Indeed, in our hands, it was determined that 1.0:1.3 ratio of alkene **8** to thiol **7** furnished the desired product in 95% yield (entry 15). Using an excess of the sugar and reducing the equivalents of initiator allowed the product to be formed in >90% conversion. This means that in conjugation reactions where a large peptide or protein is employed that an excess of carbohydrate can be used without diminishing ligation yields. If the carbohydrate is the limiting reagent then an excess or stoichiometric equivalent of the peptide can be used. 4-Methoxyacetophenone (MAP) was employed as a photosensitizer and found to generally improve yields of the photochemical reaction. Following the successful TEC reaction the glycopeptide **9** was converted into the corresponding thioester **10** for the next NCL-Thiyl radical cycle (Scheme 5).

The second NCL-thiyl radical process involved a chemical ligation step between glycopeptide **10** and amino acid **6** followed by a thiyl-radical-mediated desulfurization of the resulting cysteine linkage to alanine to furnish glycopeptide **12** (Scheme 6). As outlined in the Introduction, Danishefsky and co-workers have previously reported a sequential NCL-radical desulfurization reaction on a glycopeptide,[2] and we felt this reaction, which is becoming widely used in peptide synthesis and which also involves formation a thiyl radical intermediate at cysteine, was appropriate to include within this study. As was

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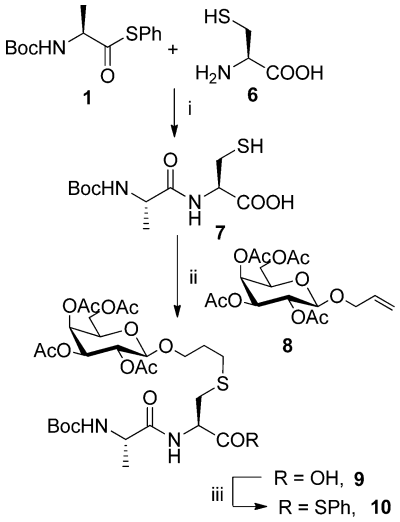
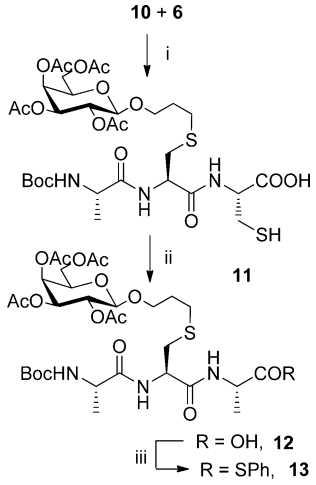
Scheme 5. NCL-TEC Cycle To Furnish Glycopeptide 10[a]



[a]Key: (i) MeOH, rt, 1 h, $NaBH_4$, 96%; (ii) 4-methoxyacetophenone (MAP) (0.1 equiv), 2,2-dimethoxy-2-phenylacetophenone (DPAP) (0.1 equiv), DMF, *hν*, rt, 2 h, 87%; (iii) THF, 0 °C to rt, isobutyl chloroformate (1.1 equiv), PhSH (1.2 equiv), *N*-methylmorpholine (NMM) (1.2 equiv), 79%.

Scheme 6. NCL-Desulfurization Cycle To Furnish Glycopeptides 12[a]

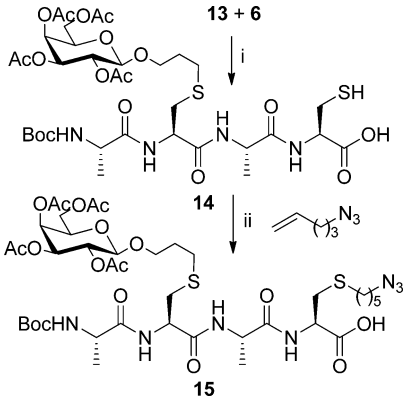


[a]Key: (i) MeOH, rt, 1 h, $NaBH_4$, 87%; (ii) PBu_3, 4,4-azobis(4-cyanovaleric acid) (ACVA), DMF, 79%; (iii) THF, 0 °C to rt, isobutyl chloroformate (1.1 equiv), PhSH (1.2 equiv), NMM (1.2 equiv), 60%.

previously reported by Danishefsky,[2] the radical-mediated desulfurization reaction did not affect the thioether bond, therefore demonstrating that desulfurization reactions can be carried out in the presence of this linkage. As part of the iterative NCL-TEC pathway, conditions can therefore be selected to allow for a functionalization reaction via the thiyl radical or a desulfurization reaction to alanine to be carried out at the point of ligation. The glycopeptide **12** was subsequently converted to thioester **13** for the final NCL-thiyl radical cycle.

The first two NCL-thiyl radical cycles had demonstrated the introduction of a a carbohydrate at the ligation site and a desulfurization at the ligation site. For the final NCL-TEC cycle, we considered how thiol−ene methodology could be used to introduce a photosensitive fluorophore at the ligation site. Mannose-containing glycopeptides labeled with fluorescein have previously been used in cellular immunology screens for the development of carbohydrate vaccines.[29] The thermally initiated thiol−ene reaction is inefficient for peptide functionalization,[3] and the conditions required for the photochemical thiol−ene reaction would result in decomposition of the photosensitive fluorescein. We therefore reasoned that a two-step procedure would be the most efficient strategy for the introduction of the fluorophore. In the first step an alkyl azide group was introduced at the ligation site using the thiol−ene reaction on 5-azidopent-1-ene to furnish the azide-functionalized glycopeptide **15** (Scheme 7).

Scheme 7. NCL-TEC Cycle To Introduce the Azido Group[a]



[a]Key: (i) MeOH, rt, 1 h, $NaBH_4$, 85%; (ii) 4-methoxyacetophenone (MAP) (0.1 equiv), 2,2-dimethoxy-2-phenylacetophenone (DPAP) (0.1 equiv), DMF, *hν*, rt, 1 h, 83%.

Despite literature reports that thiyl radicals can add to the central nitrogen of azides,[30] this competing side reaction was not observed and the desired thiol−ene coupling reaction proceeded in good yield. The direct photochemical functionalization of cysteine to furnish an azide-functionalized peptide or protein has general applications for peptide and protein modification reactions. The final thiol−ene reaction concluded the third cycle of NCL-TEC process and demonstrated how the iterative approach could be applied to functionalized peptide synthesis. The resulting azide-functionalized glycopeptide **15** was then further modified via a copper-catalyzed azide−alkyne cycloaddition reaction[31] that could be carried out in the absence of light to furnish the fluorescein-labeled glycopeptide **17** (Scheme 8)

■ CONCLUSION

In summary, we have developed a methodology that demonstrates the compatibility of native chemical ligation and thiyl radical chemistry. We have demonstrated that thiyl radical chemistry may be used to functionalize the ligation site to access highly functionalized peptides. The versatility of the thiyl radical click reaction was demonstrated through the introduction of amino acid side chains, carbohydrate groups, and alkyl azides. The thioether products were found to be compatible with the well-established thiyl-radical-mediated desulfurization chemistry. The presence of any trace phosphine or phosphine oxide reagents was found to be detrimental to the thiylene radical reaction. The use of sodium borohydride as a reducing agent in the NCL step was found to have no adverse effect on the subsequent radical reaction. A sequence of three

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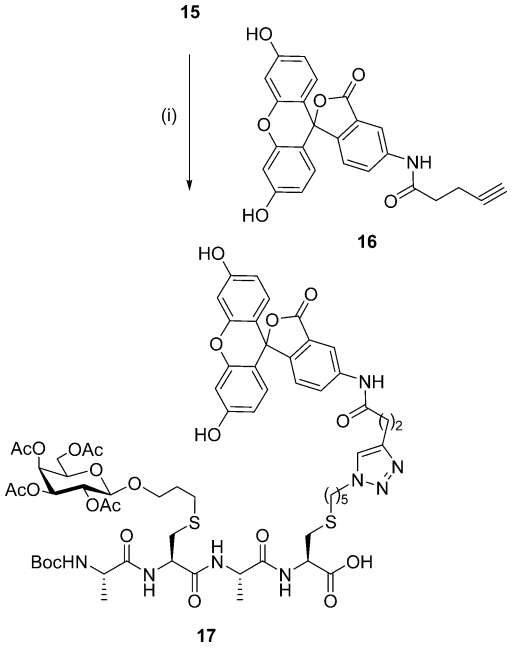
Scheme 8. Synthesis of Fluorescein-Labeled Glycopeptide 17[a]



[a]Key: (i) sodium ascorbate, tris(3-hydroxypropyltriazolylmethyl)-amine (THPTA), EtOH/H_2O, $Cu(MeCN)_4PF_6$, 60 °C, 16 h, 65%.

iterative cycles has been demonstrated for the synthesis of a fluorescein labeled glycopeptide **17**. A solution to the problem of introducing a photosensitive fluorophore is presented. In the presence of phosphine reagents, the desulfurization reaction dominates and the thiyl radical is inhibited by the presence of aromatic thiols or disulfides. It is anticipated that this methodology could be used in combination with solid phase peptide synthesis (SPPS) methods to allow access longer chain peptides of biological interest and that the methodology should find general applications in functionalized peptide and protein synthesis.

EXPERIMENTAL SECTION

General Experimental Methods. For NMR spectra, a 400 MHz spectrometer was employed for ^1H (400.13 MHz) and ^{13}C (100.61 MHz) spectra, a 600 MHz spectrometer was employed for ^1H (600.13 MHz) and ^{13}C (150.90 MHz) spectra. Resonances δ, are in ppm units downfield from an internal reference in $CDCl_3$ (δ_H = 7.26 ppm, δ_C = 77.0 ppm), or MeOH (δ_H = 3.31 ppm, δ_C = 49.0 ppm). ^1H and ^{13}C NMR assignments were confirmed by 2D COSY, HSQC, HMBC, and NOESY experiments. Mass spectrometry analysis was performed with Maldi-quadrupole time-of-flight (Q-Tof) mass spectrometer equipped with Z-spray electrospray ionization source (ESI). Silica gel (200 mesh) was used for column chromatography. Analytical thin-layer chromatography was performed using silica gel (precoated sheets, 0.2 mm thick, 20 cm ×20 cm) and visualized by UV irradiation or molybdenum staining (heating with a phosphomolybdic acid reagent). DCM, MeOH, THF and toluene were dried over flame-dried 3 or 4 Å sieves. Dimethylformamide (DMF), triethylamine (Et_3N), and trifluoroacetic acid (TFA) were used dry from Sure/Seal bottles. Other reagents were purchased from an industrial supplier. All UV reactions were carried out in a Luzchem photoreactor, LZC-EDU (110 V/60 Hz), containing 10 UVA lamps centered at 350 nm.

General Procedure for the Synthesis of Thioesters.[32] Peptide-OH (1.0 equiv), *N*-methylmorpholine (1.2 equiv), and THF (0.5 mmol/mL) were added to a flame-dried flask and dried over 4 Å molecular sieves. The solution was cooled to 0 °C, isobutyl chloroformate (1.1 equiv) was added, and the reaction mixture was stirred for 15 min. Thiophenol (1.2 equiv) was added followed by *N*-methylmorpholine (1.2 equiv). The reaction mixture was slowly warmed to room temperature and stirred overnight. The reaction mixture was filtered to remove molecular sieves, and the solvent was removed in vacuo. The reaction mixture was redissolved in DCM and washed with $NaHCO_3$, H_2O, and brine. The organic phase was dried ($MgSO_4$) and concentrated in vacuo and the product purified by column chromatography.

General Procedure for Native Chemical Ligation (NCL). Cysteine·HCl (1.0 equiv) was added to MeOH (0.03 mmol/mL). $NaBH_4$ (2.0 equiv) was added slowly, and the mixture was stirred for 1 h under N_2. Thioester (1.0 equiv) was added in MeOH, and the reaction mixture was stirred until TLC showed the disappearance of starting materials (1−4 h). Silica gel was added to the reaction mixture and solvent removed in vacuo. The product was purified by column chromatography (60−100% EtOAc/hexane +1% AcOH).

(2S)-2-[(*tert*-Butyloxycarbonyl)amino]thiopropionic Acid *S*-Phenyl Ester (1).[33] The general procedure for the synthesis of thioesters was carried out to prepare **1**. The product was purified by column chromatography (hexane/EtOAc, 9:1, R_f = 0.42) to yield a white solid (21.22 g, 74%): ^1H NMR (400 MHz, $CDCl_3$) δ 7.41 (5H, br s, CH Ar), 5.01 (1H, d, J = 7.5 Hz, NH), 4.52 (1H, app t, J = 7.5 Hz, $CHCH_3$), 1.49 (9H, s, $C(CH_3)_3$), 1.44 (3H, d, J = 7.0 Hz, CH_3); HRMS (ES^+) m/z calcd for $C_{14}H_{19}NO_3SNa$ $[M + Na]^+$ 304.0983, found 304.0976.

L-Cysteine Methyl Ester Hydrochloride (2).[34] Methanol (150 mL) was added to a flame-dried round-bottom flask equipped with a Teflon stir bar. The solvent was stirred and cooled to 0 °C, and acetyl chloride (26.4 mL, 0.37 mol) was added dropwise. The solution was stirred for an additional 10 min. L-Cysteine hydrochloride monohydrate (4.35 g, 24.7 mmol) was added, the ice bath was removed, and the reaction was stirred at room temperature for 24 h. The solvent was removed in vacuo, and the resulting residue was dissolved in methanol (40 mL) and water (8 mL). Tributylphosphine (3.0 mL, 12.4 mmol) was added and the solution stirred at room temperature for 3.5 h. The reaction mixture was diluted with water (50 mL) and the aqueous phase washed with diethyl ether (4 × 25 mL). Solvent was removed in vacuo and the product recrystallized in ethanol/ether and washed with 5% ethanol/ether to furnish **2** (2.89 g, 68%) as a white solid: ^1H NMR (400 MHz, MeOD) δ 4.36 (1H, t, J = 5.0 Hz, CH), 3.89 (3H, s, OCH_3), 3.11 (2H, d, J = 5.0 Hz, CH_2); HRMS (ES^+) m/z calcd for $C_4H_9NO_2SNa$ $[M + Na]^+$ 158.0252, found 158.0263.

(*R*)-Methyl 2-[(*S*)-2-[(*tert*-Butoxycarbonyl)amino]-propanamido]-3-mercaptopropanoate (3).[35] Compounds **1** (0.166 g, 0.59 mmol) and **2** (0.101 g, 0.59 mmol) and tributylphosphine (0.09 mL, 0.35 mmol) were stirred in MeOH (6 mL) under N_2 at room temperature for 1 h. Solvent was removed in vacuo, and purification by column chromatography (20−40% EtOAc/hexane) furnished **3** as a white solid (0.17 g, 96%): ^1H NMR (400 MHz, $CDCl_3$) δ 6.99 (1H, br s, $NHCHCH_2$), 5.06 (1H, br s, $NHBoc$), 4.88 (1H, m, $CHCH_2SH$), 4.21 (1H, m, $CHCH_3$), 3.82 (3H, s, OCH_3), 3.04 (2H, m, CH_2SH), 1.48 (9H, s, $(C(CH_3)_3)$), 1.40 (3H, d, J = 7.0 Hz, CH_3CH); HRMS (ES^+) m/z calcd for $C_{12}H_{22}N_2O_5SNa$ $[M + Na]^+$ 329.1147, found 329.1146.

(*S*)-Methyl 2-[(*tert*-Butoxycarbonyl)amino]hex-5-enoate (4).[36,37] Compound **11** was synthesized according to the reported literature procedure. Zinc dust (0.298 g, 4.56 mmol) was added to iodine (0.012 g, 0.047 mmol) in a three-neck round-bottomed flask and heated under vacuum for 10 min. The flask was flushed with nitrogen and evacuated three times. *N*-(*tert*-Butoxycarbonyl)-3-iodo-L-alanine methyl ester (0.50 g, 1.52 mmol) was dissolved in dry DMF (5 mL) and added to the zinc slurry at 0 °C. The reaction mixture was stirred at room temperature for 1 h. While the zinc insertion reaction was in progress, CuBr−DMS (0.041 g, 0.20 mmol) was placed in a three-necked flask and gently dried under vacuum until a color change from white to green was observed. Dry DMF (4 mL) and allyl chloride (0.151 g, 1.98 mmol) were added, and the reaction was cooled to −15 °C. Once zinc insertion was complete (TLC), stirring of the reaction mixture was ceased to allow the zinc to settle, and the supernatant was

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removed and added dropwise to the electrophile and Cu catalyst. The cold bath was removed, and the reaction mixture was stirred at room temperature for 2 days. EtOAc (100 mL) was added, and the reaction was stirred for 15 min. The reaction mixture was washed with 1 M $Na_2S_2O_3$ (100 mL), water (2 × 100 mL), and brine (100 mL) and dried ($MgSO_4$), and solvent was removed in vacuo. Purification by column chromatography (95:5 hexane/ether) furnished **4** as a yellow oil (0.143 g, 39%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.84−5.68 (1H, m, CH_2CH), 5.10−4.93 (2H, m, CH_2CH), 4.30 (1H, m, CHNHBoc), 3.72 (3H, s, OCH_3), 2.10 (2H, m, CH_2), 1.97−1.56 (4H, m, CH_2), 1.42 (9H, s, $C(CH_3)_3$); HRMS (ES$^+$) m/z calcd for $C_{12}H_{21}NO_4Na$ [M + Na]$^+$ 266.1368, found 266.1371.

(6*S*,9*R*,16*S*)-Methyl 16-[(*tert*-Butoxycarbonyl)amino]-9-(methoxycarbonyl)-2,2,6-trimethyl-4,7-dioxo-3-oxa-11-thia-5,8-diazaheptadecan-17-oate (5). Compounds **3** (63.0 mg, 0.21 mmol) and **4** (100.0 mg, 0.41 mmol) and 4,4-azobis(4-cyanovaleric acid) (ACVA) (12.2 mg, 0.04 mmol) were dissolved in DMF (1 mL) and irradiated in a UV reactor for 1 h (after this time, TLC showed incomplete reaction). ACVA (12.2 mg, 0.04 mmol was added and the reaction mixture placed in the UV reactor for 1 h. DMF was removed in vacuo, and purification by column chromatography (40% EtOAc/hexane) furnished **5** as a white solid (71 mg, 63%): $[\alpha]^{20}_D = -8.0$ (c = 0.1 in $CHCl_3$); IR ν_{max} (thin film) 3320 (NH), 1742, 1672 (C═O), 1505 (CH),1161 (CO) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.96 (1H, m, NHCHCH$_2$), 5.14 (2H, br s, NHBoc), 4.80 (1H, m, CHCH$_2$S), 4.31(1H, m, CHCH$_2$CH$_2$), 4.24 (1H, m, CHCH$_3$), 3.79, 3.77 (3H, s, OCH_3), 3.03−2.92 (2H, m, CHCH_2S), 2.53 (2H, t, J = 7.0 Hz, SCH_2CH$_2$), 1.82 - 1.57 (4H, m, CH$_2$), 1.52−1.36 (23H, m, 2 × C(CH$_3$)$_3$, CHCH_3, CH$_2$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.0, 172.0, 170.6, 155.0, 154.9 (C═O), 79.7, 79.5 (C(CH$_3$)$_3$), 52.8 (CHCH$_2$), 52.2, 51.8 (OCH$_3$), 51.3 (CHCH$_2$CH$_2$), 49.6 (CHCH$_3$), 33.7, 31.8, 31.7, 28.3, 23.8 (CH$_2$), 28.4, 28.4, 28.4, 28.4, 28.4, 28.4, 28.4, (C(CH$_3$)$_3$), 17.6 (CH$_3$); HRMS (ES$^+$) m/z calcd for $C_{24}H_{43}N_3O_9SNa$ [M + Na]$^+$ 572.2618, found 572.2637.

***N*-(*tert*-Butoxycarbonyl)-L-alanyl-L-cysteine (7).**[38] Following the general procedure for native chemical ligation furnished **7** as a white solid (16.0 g, 96%): $[\alpha]^{23}_D = -29$ (c = 0.1 g cm^{-1} in MeOH); IR ν_{max} (thin film) 3319 (NH), 2978 (OH), 1718, 1655 (C═O), 1159 (CH) cm^{-1}; ^{1}H NMR (400 MHz, MeOD) δ 4.57 (1H, t, J = 4.7 Hz, CHCH$_2$), 4.10 (1H, q, J = 7.1 Hz, CHCH$_3$), 3.00 (2H, m, CHCH_2), 1.44 (9H, s, C(CH$_3$)$_3$), 1.33 (3H, d, J = 7.1 Hz, CHCH_3); ^{13}C NMR (100 MHz, MeOD) δ 174.5, 171.4, 156.4 (C═O), 79.3 (C(CH$_3$)$_3$), 54.1 (CHCH$_2$), 50.2 (CHCH$_3$), 27.2, 27.2, 27.2(C(CH$_3$)$_3$), 25.4 (CH$_2$), 16.6 (CH$_3$); HRMS (ES$^+$) m/z calcd for $C_{11}H_{20}N_2O_5SNa$ [M + Na]$^+$ 315.0993, found 315.0995.

Allyl 2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranoside (8).[39] Compound **8** was synthesized according to literature procedures. β-D-Galactose pentaacetate (5.00 g, 12.80 mmol) and allyl alcohol (1.75 mL, 25.6 mmol) were dissolved in dry DCE (50 mL) and stirred over 4 Å molecular sieves for 2 h under N_2. The reaction was cooled to −15 °C, and tin tetrachloride (1.60 mL, 13.35 mmol) was added and stirred for 2 h. The reaction mixture was warmed to 5 °C, $NaHCO_3$ (100 mL) was added, and the precipitate was filtered over Celite. DCM (200 mL) was added to the filtrate, which was washed with $NaHCO_3$ (200 mL), water (200 mL), and brine (200 mL), dried ($MgSO_4$), and concentrated in vacuo. Purification by column chromatography (35% EtOAc/hexane) furnished **8** as a white solid (3.20 g, 64%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.90−5.77 (1H, m, CH═CH$_2$), 5.37 (1H, app d, J = 3.0 Hz, H4), 5.30−5.15 (3H, m, H2, CH═CH_2), 5.00 (1H, dd, J = 10.5 Hz, J = 3.5 Hz, H3) 4.50 (1H, d, J = 7.9 Hz, H1), 4.38−4.30 (1H, dd, J = 13.3 Hz, J = 5.0 Hz, H6′), 4.22−4.04 (3H, m, CH_2CH═CH$_2$, H6), 3.88 (1H, app t, J = 6.7 Hz, H5), 2.14, 2.05, 2.04, 1.96 (3H, s, OAc); HRMS (ES$^+$) m/z calcd for $C_{17}H_{24}O_{10}Na$ [M + Na]$^+$ 411.1267, found 411.1260.

(*R*)-2-[(*S*)-2-[(*tert*-Butoxycarbonyl)amino]propanamido]-3-[[3-[[(2*R*,3*R*,4*S*,5*S*,6*R*)-3,4,5-triacetoxy-6-(acetoxymethyl)-tetrahydro-2*H*-pyran-2-yl]oxy]propyl]thio]propanoic Acid (9). Compounds **7** (2.45 g, 8.37 mmol) and **8** (2.50 g, 6.44 mmol), 2,2-dimethoxy-2-phenylacetophenone (DPAP) (0.215 g, 0.837 mmol), and MAP (0.163 g, 0.837 mmol) were dissolved in DMF (17 mL, 0.5 mmol/mL). The reaction was irradiated for 1 h at room temperature. EtOAc (200 mL) was added, and the reaction was washed with brine (6 × 100 mL). The organic phase was dried ($MgSO_4$) and filtered, and silica gel was added to the organic solution. Solvent was removed in vacuo, and the product was purified by column chromatography (60−80% EtOAc/hexane + 1% AcOH) to furnish **9** as a white solid (4.16 g, 95%): $[\alpha]^{23}_D = 13$ (c = 0.1 g cm^{-1} in $CHCl_3$); IR ν_{max} (thin film) 3345 (NH), 2979 (OH), 1743 (C═O), 1215 (CH), 1043 (COC) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.06 (1H, d, J = 7.5 Hz, NH), 5.38 (1H, d, J = 3.0 Hz, H4), 5.18 (2H, dd, J = 11.0 Hz, J = 8.0 Hz, H2, NH), 5.03 (1H, dd, J = 11.0 Hz, J = 3.5 Hz, H3), 4.78 (1H, m, CHCH$_2$S), 4.44 (1H, d, J = 8.0 Hz, H1), 4.30−4.06 (3H, m, CHCH$_3$, H6), 3.92 (2H, m, OCH_2CH$_2$, H5), 3.52 (1H, m, OCH_2CH$_2$), 3.05 (2H, m, CHCH_2S), 2.70−2.43 (2H, m, SCH_2CH$_2$), 2.15, 2.11, 2.04, 1.98 (3H, s, OAc), 1.94−1.69 (2H, m, SCH$_2$CH_2), 1.44 (9H, s, C(CH_3)$_3$), 1.38 (3H, d, J = 7.1 Hz, CHCH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.7, 172.1, 171.2, 170.6, 170.3, 170.2, 155.6 (C═O), 101.2 (C1), 80.2 (C(CH$_3$)$_3$), 70.7 (C-5), 70.6 (C3), 69.3 (C2), 68.4 (OCH$_2$CH$_2$), 67.0 (C4), 61.2 (C6), 51.5 (CHCH$_2$S), 50.0 (CHCH$_3$), 33.6 (CHCH$_2$S), 30.1 (SCH$_2$CH$_2$), 30.0 (SCH$_2$CH$_2$), 28.2, 28.2, 28.2 (C(CH$_3$)$_3$), 21.0, 20.7, 20.7, 20.6 (C(O)CH$_3$), 18.3 (CHCH$_3$); HRMS (ES$^-$) m/z calcd for $C_{28}H_{44}N_2O_{15}S$ [M − H]$^-$ 679.2383, found 679.2390.

(2*R*,3*S*,4*S*,5*R*,6*R*)-2-(Acetoxymethyl)-6-[[(6*S*,9*S*)-2,2,6-trimethyl-4,7-dioxo-9-[(phenylthio)carbonyl]-3-oxa-11-thia-5,8-diazatetradecan-14-yl]oxy]tetrahydro-2*H*-pyran-3,4,5-triyl Triacetate (10). Following the general procedure for the synthesis of thioester, **10** was purified by column chromatography (15−50% EtOAc/Hexane) to furnish a white solid (3.41 g, 79%): $[\alpha]^{23}_D = -44$ (c = 0.1 g cm^{-1} in $CHCl_3$); IR ν_{max} (thin film) 3316 (NH), 2935 (OH), 1745, 1654 (C═O), 1513 (C═C Ar), 1217 (CH), 1044 (COC), 753 (CH Ar) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.41 (5H, m, CH Ar), 7.12 (1H, br s, NH), 5.39 (1H, d, J = 3.1 Hz, H4), 5.19 (1H, dd, J = 11.0 Hz, J = 8.0 Hz, H2), 5.09 (1H, br s, NH), 5.00 (1H, dd, J = 11.0 Hz, J = 3.1 Hz, H3), 4.95 (1H, m, CHCH$_2$S), 4.45 (1H, d, J = 8.0 Hz, H1), 4.30 (1H, br s, CHCH$_3$), 4.22−4.07 (2H, m, H6), 3.91 (2H, m, OCH_2CH$_2$, H5), 3.60 (1H, m, OCH_2CH$_2$), 3.09−2.89 (2H, m, CHCH_2S), 2.58 (2H, t, J = 7.2 Hz, SCH_2CH$_2$), 2.12, 2.05, 2.05, 1.98 (3H, s, OAc), 1.84 (2H, m, OCH$_2$CH_2), 1.46 (12H, br s, CHCH_3, C(CH_3)$_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 197.8, 172.9, 170.5, 170.3, 170.2, 169.6, 155.6 (C═O), 134.6, 134.6, 129.8, 129.3, 129.3, 126.9 (C Ar), 101.2 (C1), 80.6 (C(CH$_3$)$_3$), 70.9 (C3), 70.7 (C5), 68.8 (C2), 68.0 (OCH$_2$CH$_2$), 67.0 (C4), 61.3 (C6), 58.0 (CHCH$_3$), 49.9 (CHCH$_3$), 34.1 (CHCH$_2$S), 29.2 (OCH$_2$CH$_2$), 28.9 (SCH$_2$CH$_2$), 28.4, 28.4, 28.4 (C(CH$_3$)$_3$), 20.8, 20.7, 20.7, 20.6 (C(O)CH$_3$), 17.8 (CHCH$_3$); HRMS (ES$^+$) m/z calcd for $C_{34}H_{48}N_2O_{14}S_2Na$ [M + Na]$^+$ 795.2447, found 795.2452.

(6*S*,9*R*,12*R*)-12-(Mercaptomethyl)-2,2,6-trimethyl-4,7,10-trioxo-9-[[[3-[[(2*R*,3*R*,4*S*,5*S*,6*R*)-3,4,5-triacetoxy-6-(acetoxymethyl)tetrahydro-2*H*-pyran-2-yl]oxy]propyl]thio]-methyl]-3-oxa-5,8,11-triazatridecan-13-oic Acid (11). Compound **11** was prepared according to the general procedure for native chemical ligation to furnished **11** as a white solid (2.73 g, 87%): $[\alpha]^{23}_D$ = 6 (c = 0.1 g cm^{-1} in $CHCl_3$); IR ν_{max} (thin film) 3316 (NH), 2935 (OH), 1745, 1655 (C═O), 1217 (CH), 1044 (COC) cm^{-1}; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.65 (1H, d, J = 7.3 Hz, NH), 7.37 (1H, br s, NH), 5.41 (1H, d, J = 3.0 Hz, H4), 5.29−5.16 (2H, m, H2, NHBoc), 5.06 (1H, dd, J = 10.5 Hz, J = 3.0 Hz, H3), 4.86 (1H, br s, CHCH$_2$SH), 4.71 (1H, m, CHCH$_2$S), 4.51 (1H, d, J = 8.0 Hz, H1), 4.32−4.09 (3H, m, CHCH$_3$, H6), 3.96 (2H, br s, H5, OCH_2CH$_2$), 3.63 (1H, br s, OCH_2CH$_2$), 3.17−2.81 (4H, m, CHCH_2S, CHCH_2SH), 2.65 (2H, m, SCH_2CH$_2$), 2.18, 2.09, 2.08, 2.01 (3H, s, OAc), 1.88 (2H, m, SCH$_2$CH$_2$), 1.47 (9H, s, C(CH_3)$_3$), 1.41 (3H, d, J = 6.7 Hz, CHCH_3); ^{13}C NMR (150 MHz, $CDCl_3$) δ 173.1, 170.6, 170.2, 170.2, 170.1, 170.0, 169.7, 155.7 (C═O), 101.2 (C1), 80.6 (C(CH$_3$)$_3$), 70.7 (C5), 70.5 (C3), 68.8 (C2), 68.0 (OCH$_2$CH$_2$), 66.9 (C4), 61.1 (C6), 54.4 (CHCH$_2$SH), 52.4 (CHCH$_2$S), 50.4 (CHCH$_3$), 33.6 (CHCH$_2$S), 29.1 (OCH$_2$CH$_2$), 28.7 (SCH$_2$CH$_2$), 28.2, 28.2, 28.2 (C(CH$_3$)$_3$), 26.1 (CHCH$_2$SH), 20.8, 20.6, 20.6, 20.5 (C(O)CH$_3$), 17.9

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($CHCH_3$); HRMS (ES^-) m/z calcd for $C_{31}H_{49}N_3O_{16}S_2$ $[M - H]^-$ 782.2475, found 782.2474.

(6*S*,9*R*,12*S*)-2,2,6,12-Tetramethyl-4,7,10-trioxo-9-[[[3-[[(2*R*,3*R*,4*S*,5*S*,6*R*)-3,4,5-triacetoxy-6-(acetoxymethyl)-tetrahydro-2*H*-pyran-2-yl]oxy]propyl]thio]methyl]-3-oxa-5,8,11-triazatridecan-13-oic Acid (12). Compound **11** (1.32 g, 1.68 mmol) was dissolved in DMF (3.5 mL), and tributylphosphine (0.42 mL, 1.68 mmol) was added. DPAP (0.043 g, 0.168 mmol) and MAP (0.033 g, 0.168 mmol) were added, and the reaction was irradiated for 1 h. EtOAc (100 mL) was added, the organic layer was washed with brine (100 mL × 6) and dried ($MgSO_4$), silica gel was added, and the solvent was removed in vacuo. The product was purified by column chromatography (75−100% EtOAc/hexane + 1% AcOH) to furnish **12** as a white solid (1.00 g, 79%): $[\alpha]^{23}_D = -12$ (c = 0.1 g cm^{-1} in $CHCl_3$); IR ν_{max} (thin film) 3317 (NH), 2933 (OH), 1745, 1654 (C═O), 1217 (CH), 1044 (COC) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.58 (1H, d, J = 7.0 Hz, NH), 7.39 (1H, d, J = 7.0 Hz, NH), 5.38 (1H, d, J = 3.1 Hz, H4), 5.29 (1H, br s, NHBoc), 5.16 (1H, dd, J = 10.8 Hz, J = 8.0 Hz, H2), 5.03 (1H, dd, J = 10.8 Hz, J = 3.5 Hz, H3), 4.72−4.50 (2H, m, $CHCH_3$, $CHCH_2S$), 4.48 (1H, d, J = 8.0 Hz, H1), 4.25−4.07 (3H, m, H6, $CHCH_3$), 3.93 (2H, m, H5, OCH_2), 3.60 (1H, m, OCH_2), 3.13−2.74 (2H, m, $CHCH_2S$), 2.60 (2H, m, SCH_2CH_2), 2.15, 2.06, 2.05, 1.98 (3H, s, OAc), 1.86 (2H, m, OCH_2CH_2), 1.50 (3H, d, J = 7.30 Hz, $CHCH_3$), 1.45 (9H, s, $C(CH_3)_3$), 1.39 (3H, d, J = 7.0 Hz, $CHCH_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.3, 170.1, 169.9, 169.8, 169.6, 169.5, 169.4, 155.3 (C═O), 100.8 (C1), 80.4 ($C(CH_3)$, 70.4 (C3), 70.1 (C5), 68.5 (C2), 67.7 (OCH_2CH_2), 66.6 (C4), 60.8 (C6), 51.9 ($CHCH_2S$), 51.5 ($CHCH_3$), 48.2 ($CHCH_3$), 33.1 ($CHCH_2S$), 29.3 (OCH_2CH_2), 28.8 (SCH_2CH_2), 27.8, 27.8, 27.8 ($C(CH_3)_3$), 20.4, 20.3, 20.2, 20.2 ($C(O)CH_3$), 17.6, 16.9 ($CHCH_3$); HRMS (ES^-) m/z calcd for $C_{31}H_{49}N_3O_{16}S$ $[M - H]^-$ 750.2755, found 750.2748.

(2*R*,3*S*,4*S*,5*R*,6*R*)-2-(Acetoxymethyl)-6-[[(6*S*,9*R*)-2,2,6-trimethyl-4,7-dioxo-9-[[(*S*)-1-oxo-1-(phenylthio)propan-2-yl]-carbamoyl]-3-oxa-11-thia-5,8-diazatetradecan-14-yl]oxy]-tetrahydro-2*H*-pyran-3,4,5-triyl Triacetate (13). Compound **13** was prepared according to the general procedure for the synthesis of thioesters. Purification by column chromatography (60% EtOAc/hexane) furnished **13** as a white solid (1.24 g, 65%): $[\alpha]^{23}_D = -8$ (c = 0.1 g cm^{-1} in $CHCl_3$); IR ν_{max} (thin film) 3314 (NH), 2977 (OH), 1747, 1656 (C═O), 1510 (C═C Ar), 1217 (CH), 1044 (COC), 749 (CH Ar) cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) δ 7.49 (1H, br s, NH), 7.42−7.36 (5H, m, CH Ar), 6.93 (1H, d, J = 7.9 Hz, NH), 5.38 (1H, d, J = 3.0 Hz, H4), 5.17 (1H, dd, J = 10.3 Hz, J = 8.0 Hz, H2), 5.01 (2H, dd, J = 10.3 Hz, J = 3.0 Hz, H3, NHBoc), 4.77 (1H, q, J = 7.3 Hz, $CHCH_3$), 4.63 (1H, q, J = 6.4 Hz, $CHCH_2S$), 4.44 (1H, d, J = 8.0 Hz, H1), 4.20 - 4.06 (3H, m, H6, $CHCH_3$), 3.98 - 3.85 (2H, m, OCH_2CH_2, H5), 3.58 (1H, m, OCH_2CH_2), 3.05 (1H, br s, $CHCH_2S$), 2.88 (1H, dd, J = 14.1 Hz, J = 6.4 Hz, $CHCH_2S$), 2.63 (2H, t, J = 6.9 Hz, CH_2CH_2S), 2.13, 2.07, 2.05, 1.98 (3H, s, OAc), 1.87 (2H, m, OCH_2CH_2), 1.50 (3H, d, J = 7.3 Hz, $CHCH_3$), 1.42 (9H, s, $C(CH_3)_3$), 1.40 (3H, d, J = 7.2 Hz, $CHCH_3$); ^{13}C NMR (150 MHz, $CDCl_3$) δ 198.6, 172.9, 170.4, 170.2, 170.1, 170.0, 169.8, 155.6 (C═O), 134.7, 134.7 (CH Ar), 129.5 (C Ar), 129.2, 129.2, 127.2 (CH Ar), 101.5 (C1), 80.5 ($C(CH_3)_3$), 70.8 (C3), 70.7 (C5), 70.0 (C2), 68.1 (OCH_2CH_2), 67.1 (C4), 61.3 (C6), 55.4 ($CHCH_3$), 52.1 ($CHCH_2S$), 50.9 ($CHCH_3$), 33.3 ($CHCH_2S$), 29.4 (OCH_2CH_2), 28.5 (CH_2CH_2S), 28.3, 28.3, 28.3 ($C(CH_3)_3$), 20.8, 20.7, 20.6, 20.6 ($C(O)CH_3$), 18.7, 17.7 ($CHCH_3$); HRMS (ES^+) m/z calcd for $C_{37}H_{53}N_3O_{15}S_2Na$ $[M + Na]^+$ 866.2818, found 866.2823.

(6*S*,9*R*,12*S*,15*R*)-15-(Mercaptomethyl)-2,2,6,12-tetramethyl-4,7,10,13-tetraoxo-9-[[[3-[[(2*R*,3*R*,4*S*,5*S*,6*R*)-3,4,5-triacetoxy-6-(acetoxymethyl)tetrahydro-2*H*-pyran-2-yl]oxy]propyl]thio]-methyl]-3-oxa-5,8,11,14-tetraazahexadecan-16-oic Acid (14). Compound **14** was prepared according to the general procedure for native chemical ligation to furnish **14** as a white solid (0.583 g, 85%): $[\alpha]^{23}_D = -23$ (c = 0.1 g cm^{-1} in $CHCl_3$); IR ν_{max} (thin film) 3303 (NH), 2927 (OH), 1745, 1641 (C═O), 1218 (CH), 1044 (COC) cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) δ 7.63 (1H, d, J = 5.9 Hz, NH), 7.58 (1H, d, J = 6.7 Hz, NH), 7.36 (1H, d, J = 6.6 Hz, NH), 5.39 (1H, d, J = 3.7 Hz, H4), 5.26 (1H, br s, NH), 5.17 (1H, dd, J = 11.0 Hz, J = 8.1 Hz, H2), 5.04 (1H, dd, J = 11.0 Hz, J = 3.2 Hz, H3), 4.83−4.64 (2H, m, $CHCH_3$, $CHCH_2SH$), 4.55−4.45 (2H, m, H1, $CHCH_2S$), 4.25 (1H, br s, $CHCH_3$), 4.22−4.08 (2H, m, H6), 3.94 (2H, m, OCH_2CH_2, H5), 3.60 (1H, m, OCH_2CH_2) 3.10 - 2.99 (3H, m, 1 × $CHCH_2S$, 2 × $CHCH_2SH$), 2.84 (1H, m, $CHCH_2S$), 2.59 (2H, m, SCH_2CH_2), 2.15, 2.07,2.05, 1.98 (3H, s, OAc),1.93−1.78 (2H, m, OCH_2CH_2), 1.50−1.35 (15H, br s, $CHCH_3$, $CHCH_3$, $C(CH_3)_3$); ^{13}C NMR (150 MHz, $CDCl_3$) δ 173.4, 172.6, 171.5, 170.5, 170.5, 170.2, 170.1, 169.7, 156.3 (C═O), 101.2 (C1), 81.3 ($C(CH_3)_3$), 70.8 (C3), 70.6 (C5), 69.0 (C2), 68.0 (OCH_2CH_2), 67.0 (C4), 61.2 (C6), 54.8 ($CHCH_2SH$), 52.9 ($CHCH_2S$), 51.3 ($CHCH_3$), 49.4 ($CHCH_3$), 33.3 (SCH_2CH), 29.3 (OCH_2CH_2), 28.6 (SCH_2CH_2), 28.4, 28.4, 28.4 ($C(CH_3)_3$), 26.2 ($CHCH_2SH$), 20.8, 20.7, 20.6, 20.6 ($C(O)CH_3$), 18.0, 17.9 ($CHCH_3$); HRMS (ES^-) m/z calcd for $C_{34}H_{54}N_4O_{17}S_2$ $[M - H]^-$ 853.2846, found 853.2856.

(6*S*,9*R*,12*S*,15*R*)-15-[[(5-Azidopentyl)thio]methyl]-2,2,6,12-tetramethyl-4,7,10,13-tetraoxo-9-[[[3-[[(2*R*,3*R*,4*S*,5*S*,6*R*)-3,4,5-triacetoxy-6-(acetoxymethyl)tetrahydro-2*H*-pyran-2-yl]oxy]-propyl]thio]methyl]-3-oxa-5,8,11,14-tetraazahexadecan-16-oic Acid (15). Compound **14** (0.134 g, 0.157 mmol) was dissolved in DMF (1 mL). MAP (2.83 μL, 15.7 μmol), DPAP (4.0 mg, 15.7 μmol), and 5-azidopent-1-ene (52.0 mg, 0.471 mmol) were added. The reaction mixture was irradiated for 1 h. EtOAc (50 mL) was added, the reaction mixture was washed with brine (6 × 25 mL) and dried ($MgSO_4$), silica gel was added, and solvent was removed in vacuo. The product purified by column chromatography (0−10% MeOH/EtOAc + 1% AcOH) furnished **15** as a white solid (0.126 g, 83%): $[\alpha]^{24}_D$ = −20 (c = 0.1 g cm^{-1} in $CHCl_3$); IR ν_{max} (thin film) 3301 (NH), 2925 (OH), 2097 (N_3), 1747, 1640 (C═O), 1218 (CH), 1045 (COC); ^1H NMR (600 MHz, $CDCl_3$) δ 7.61, 7.54, 7.37 (1H, br s, NH), 5.42 (1H, d, J = 3.0 Hz, H4), 5.28 (1H, br s, NHBoc), 5.20 (1H, m, H2), 5.07 (1H, dd, J = 10.8 Hz, J = 3.0 Hz, H3), 4.66−4.40 (4H, m, $CHCH_3$, $CHCH_2S$, $CHCH_2S$, H1), 4.23−4.15 (3H, m, $CHCH_3$, H6), 4.03−3.88 (2H, m, OCH_2CH_2, H5), 3.64 (1H, m, OCH_2CH_2), 3.29 (2H, t, J = 6.8 Hz, CH_2N_3), 3.15−2.83 (4H, m, $CHCH_2S$, $CHCH_2S$), 2.72−2.51 (4H, m, SCH_2CH_2, SCH_2CH_2), 2.18, 2.10, 2.08, 2.01 (3H, s, OAc), 1.87 (2H, m, OCH_2CH_2), 1.62 (4H, m, $SCH_2CH_2CH_2CH_2$), 1.55−1.34 (15H, br s, $C(CH_3)_3$, $CHCH_3$, $CHCH_3$), 1.27 (2H, brs, $SCH_2CH_2CH_2$); ^{13}C NMR (150 MHz, $CDCl_3$) δ 173.5, 172.5, 171.2, 170.5, 170.2, 170.2, 170.1, 169.7, 156.3 (C═O), 101.2 (C1), 80.9 ($C(CH_3)_3$), 70.8 (C3), 70.5 (C5), 68.9 (C2), 68.1 (OCH_2CH_2), 67.0 (C4), 61.2 (C6), 52.8, 52.5 ($CHCH_2S$), 51.2 ($CHCH_3$), 50.9 (CH_2N_3), 49.4 ($CHCH_3$), 33.6, 33.4 ($CHCH_2S$), 32.2 (SCH_2CH_2), 29.2 (OCH_2CH_2), 28.9 (SCH_2CH_2), 28.6 (SCH_2CH_2), 28.4 ($CH_2CH_2N_3$), 28.3, 28.3, 28.3 ($C(CH_3)_3$), 25.8 ($CH_2CH_2CH_2N_3$), 20.8, 20.6, 20.6, 20.6 ($C(O)CH_3$), 18.2, 18.1 ($CHCH_3$); HRMS (ES^-) m/z calcd for$C_{39}H_{63}N_7O_{17}S_2$ $[M - H]^-$ 964.3643, found 964.3664.

Alkynyl Fluorescein Dye 16.[40] Alkynyl fluoresceine dye was prepared according to the literature. Briefly, fluoresceinamine (0.600 g, 1.73 mmol) was dissolved in pyridine (8 mL), and DCC (0.536 g, 2.60 mmol) and pentynoic acid (0.339 g, 3.46 mmol) were added. The reaction mixture was stirred overnight, and DCU was filtered off. The filtrate was poured into ice-cold water (30 mL). The pH was adjusted to 2 with the addition of HCl (5 M) and the precipitate filtered off and washed with water (3 mL). The product was purified by column chromatography (0−6% MeOH/DCM) to furnish an orange solid (0.465 g, 63%): ^1H NMR (400 MHz, MeOD) δ 8.32 (1H, d, J = 1.8 Hz, CH Ar), 7.90 (1H, dd, J = 8.3, J = 1.8 Hz, CH Ar), 7.17 (1H, d, J = 1.8 Hz, CH Ar), 6.75−6.70 (4H, m, CH Ar), 6.60−6.56 (2H, m, CH Ar), 2.67−2.60 (4H, m, CH_2CH_2), 2.33 (1H, t, J = 6.8 Hz, C≡CH); HRMS (ES^-) m/z calcd for$C_{25}H_{17}NO_6$ $[M - H]^-$ 428.1134, found 428.1132.

Fluorescein-Functionalized Glycopeptide (17). Compound **15** (88.4 mg, 91.5 μmol), alkynylfluoresceine dye (86.0 mg, 201.0 μmol), sodium ascorbate (36.2 mg, 182.4 μmol), and THPTA (39.7 mg, 91.5 μmol) were dissolved in EtOH (20 mL) and water (5 mL). Argon was bubbled through the reaction mixture before the addition of $Cu(MeCN)_4PF_6$ (6.8 mg, 18.2 μmol). The reaction was heated to 60 °C under argon for 16 h until TLC showed the consumption of starting material (R_f = 0.7, 7:2:1 EtOAc/EtOH/H_2O). The reaction

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mixture was concentrated in vacuo, and EtOAc (40 mL) was added. This was washed with water (30 mL), and the aqueous phase extracted with EtOAc (3 × 40 mL). The combined organics were dried ($MgSO_4$), and silica gel was added. Solvent was removed in vacuo and the product purified by column chromatography (0−20% 2:1 EtOH/H_2O/EtOAc + 1% AcOH) to furnish a yellow solid (82.8 mg, 65%): $[\alpha]^{23}_D$ = −40 (c = 0.1 g cm^{-1} in MeOH); IR ν_{max} (thin film) 3358 (NH), 2934 (OH), 1745, 1654 (C═O), 1220 (CH Alkyl), 1048 (COC), 850, 696 (CH-Ar) cm^{-1}; ^{1}H NMR (600 MHz, d-DMSO) δ 8.25 (1H, br s, NH), 7.97 (1H, s, NCH Ar), 7.92 (1H, d, J = 7.9 Hz, NH), 7.59 (2H, d, J = 8.4 Hz, NH, CH Ar), 6.97−6.87 (4H, m, NH, 3 × CH Ar), 6.85 (1H, d, J = 8.8 Hz, CH Ar), 6.76 (1H, d, J = 8.4 Hz, CH Ar), 6.66 (1H, dd, J = 8.8 Hz, J = 1.9 Hz, CH Ar), 6.55 (1H, m, CH Ar), 6.29 (1H, br s, NH), 6.13 (1H, d, J = 1.6 Hz, CH Ar), 5.25 (1H, d, J = 3.7 Hz, H4), 5.16 (1H, dt, J = 10.4 Hz, J = 3.7 Hz, H3), 4.92 (1H, m, H2), 4.69 (1H, apt t, J = 7.4 Hz, H1), 4.45 (1H, br s, $CHCH_2S$), 4.34 (2H, t, J = 7.3 Hz, CH_2N), 4.26 (1H, br s, $CHCH_3$), 4.19 (1H, t, J = 6.5 Hz, H5), 4.05−3.95 (4H, m, H6, $CHCH_3$, $CHCH_2S$), 3.78 (1H, m, OCH_2), 3.56 (1H, m, OCH_2), 3.04−2.89 (6H, m, $CCH_2CH_2C(O)$, CH_2S, CH_2S), 2.83 (1H, m, CH_2S), 2.66 (1H, m, CH_2S), 2.56−2.49 (4H, br s, SCH_2CH_2, SCH_2CH_2), 2.11, 2.02, 2.00, 1.91 (3H, s, $C(O)CH_3$), 1.83 (2H, quint, J = 7.3 Hz, CH_2CH_2N), 1.74 (2H, t, J = 6.0 Hz, OCH_2CH_2), 1.58 (2H, t, J = 6.6 Hz, SCH_2CH_2), 1.38 (9H, s, $C(CH_3)_3$), 1.33 (2H, m, $CH_2CH_2CH_2N$), 1.23 (3H, d, J = 7.0 Hz, $CHCH_3$), 1.19 (3H, d, J = 7.1 Hz, $CHCH_3$); ^{13}C NMR (150 MHz, d-DMSO) δ 172.8 (C═ONH), 172.5 (C═OOH), 172.1, 172.5, 170.8 (C═ONH), 170.0, 169.9 (C═OCH_3), 169.8 (C═OBoc), 169.5, 169.2 (C═OCH_3), 168.9 (ArCC═O), 160.3, 160.2 (ArCOCAr), 156.2 (ArCC═O), 155.8 (Ar CNH), 151.2, 150.8 (Ar COH), 145.1 (Ar CN), 128.9, 128.6 (ArCH), 126.1 (Ar CH), 122.1 (Ar CHN), 117.8 (ArCHCOH), 115.6 (ArCHCNH), 113.4 (ArCHCOH), 111.6, 110.0, 109.9 (ArCCO), 105.5 (ArCHCNH), 102.4, 102.4 (ArCHCOH),100.0 (C1), 79.9 (CAr), 78.2 ($C(CH_3)_3$), 70.3 (C3), 69.8 (C5), 68.7 (C2), 67.8 (OCH_2), 67.4 (C4), 61.2 (C6) 53.7 ($CHCOOH$), 52.2 ($CHCH_2S$), 49.9 ($CHNHBoc$), 49.1 (CH_2N), 48.6 ($CHCH_3$), 33.8, 33.7 ($CHCH_2S$), 33.2 (CH_2CONH), 31.3 (SCH_2), 29.4 (CH_2CH_2N), 29.0 (OCH_2CH_2), 28.3 (SCH_2CH_2), 28.2, 28.2, 28.2 ($C(CH_3)_3$), 27.7 ($OCH_2CH_2CH_2S$), 24.8 ($CH_2CH_2CH_2N$), 20.7 (CH_2CH_2CONH), 20.5, 20.5, 20.4, 20.3 ($COCH_3$), 18.3, 18.2 ($CHCH_3$); HRMS (ES$^-$) m/z calcd for $C_{64}H_{80}N_8O_{23}S_2$ $[M - H]^-$ 1391.4700, found 1391.4741.

■ ASSOCIATED CONTENT

Supporting Information

^{1}H and ^{13}C NMR spectra for all compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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