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HMDO-Promoted Peptide and Protein Synthesis in Ionic Liquids

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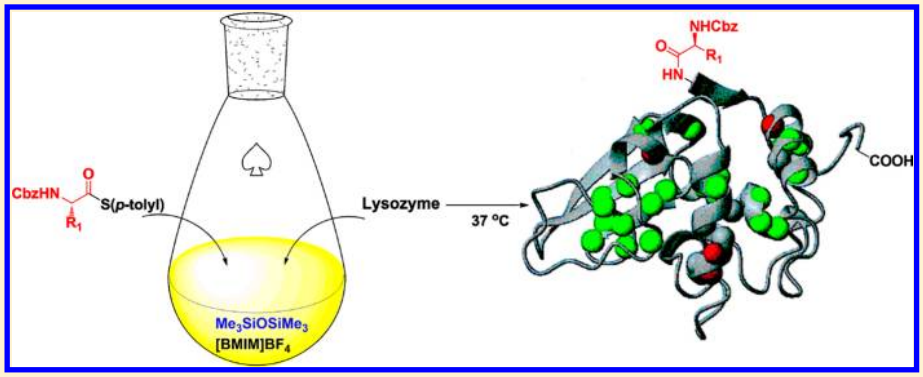
Supporting Information



ABSTRACT: Hexamethyldisiloxane (HMDO) has been developed to efficiently promote the metal-free direct coupling of an amino function of one *cysteine-free* peptide or protein and a C-terminal thioester of the second peptide in ionic liquids. The amide-coupling reaction proceeds smoothly under mild conditions to afford the corresponding products in good to excellent yields (63−94%). Peptide couplings were also achieved using in-situ-generated thioesters by the thioesterification of oxo esters.

INTRODUCTION

The development of methods for reliable, chemoselective conjugation of synthetic molecules to peptides and proteins is an important pursuit in chemical biology.[1] These conjugation methods have made possible the successful preparation of semisynthetic proteins in large amounts for the study of the protein−protein interactions, protein functionalities and time-resolved localization in living cells.[2] The formation of native amide bonds plays a crucial role in the ligation and modification of peptides and proteins,[3] and many research groups have been developing new strategies for building up amide bonds.[4] Native chemical ligation (NCL) was originally developed by Kent and co-workers[5] in 1994, and it is one of the most widely used chemoselective ligation tools to join two peptide fragments under mild reaction conditions and without the need for protecting groups. However, a particular limitation of NCL is its intrinsic reliance on the presence of a cysteine residue or a Cys-mimicking auxiliary residue at the ligation juncture. Cysteine is uncommon, comprising only 1.7% of all residues in proteins (Scheme 1A).[6] Additionally, the NCL technique presents challenges for proteins with more than ∼100 amino acids, and Cys residues can undergo disulfide-bond formation and influence the overall folding pathway.[7] Therefore, it remains a challenging task to overcome the limitations associated with the need for a terminal cysteine. A more general ligation technology to affect direct amidation of natural or unnatural cysteine-free peptides under mild conditions would greatly expand the utility of total protein synthesis.

Proteins containing a C-terminal thioester are very important intermediates in protein modification. It is well-known that C-terminal $^{\alpha}$thioesters can be selectively prepared by engineered inteins/Sortase-mediated protein ligation, also referred to as expressed protein ligation (EPL). This approach is an extension of NCL, and hence any soluble protein could be converted to the respective peptide thioester by the robust intein (Scheme 1B) or Sortase (Scheme 1C).[8] Several peptide backbone modifications using thioesters have been reported, but these methods use either some toxic metals or organic solvents,[9] which could lead to epimerization of amino acids. From the perspective of biological chemistry, it is desirable to design chemical reactions for peptides and proteins that proceed at physiological pH, temperature and pressure.[10] Inspired by the above *trans*-thioesterification reaction between the peptide/protein thioesters, we set out to develop a general ligation that

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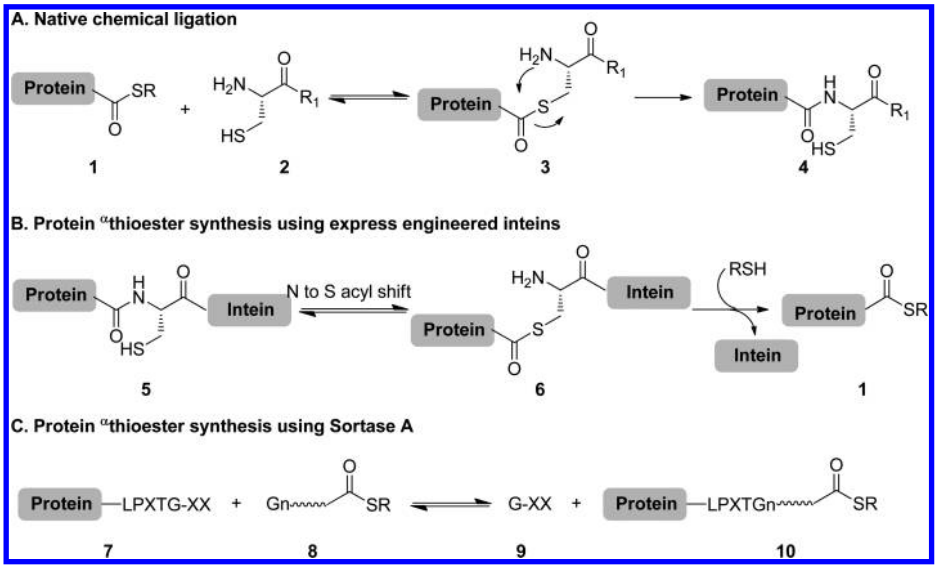
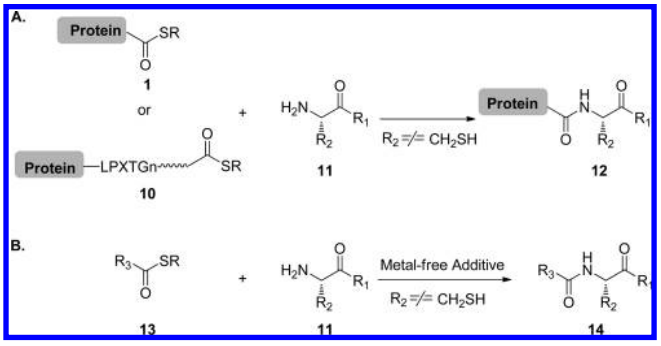
Scheme 1. Native Chemical Ligation and Protein $^{\alpha}$Thioester Synthesis Using Express Engineered Inteins and Sortase



Scheme 2. A New Strategy for the Modification of Peptides and Proteins: (A) Conceptual Depiction of Thioester-Mediated Ligation and (B) Approach Discussed in the Present Work



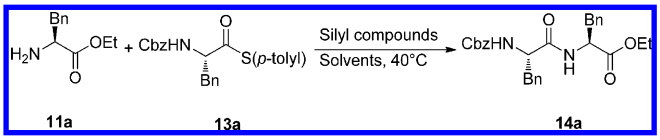
could be used as a new strategy for the modification of peptides and proteins (Scheme 2A).

The thioester substrates for the new chemoselective peptide/protein modification would be obtained through thiolysis of C-terminally intein or Sortase A. The key feature of the new methodology concerns the selective installation of the peptide thioester into the peptide backbone without cysteine residue in the second coupling component (Scheme 2B). Recently, our group reported on the metal-free direct amidation of thioester peptides with amino acid esters without cysteine residues in the presence of bistrimethylsilylacetamide (BSA) in EtOH under mild conditions.[11] Liebeskind and co-workers published similar results at the same time for peptide synthesis with thioacids as the starting materials in toxic organic solvents.[12] In our efforts to develop a more efficient synthesis of peptides and proteins via their thioesters or CoA esters or even oxo esters under such mild conditions, we have discovered that hexamethyldisiloxane (HMDO) promotes effectively the metal-free direct amide-coupling reaction between a C-terminal thioester of one peptide or protein and an amino function of the second *cysteine-free* peptide under mild conditions in ionic liquids. Herein, we report on such a method for the modification of peptides and proteins.

RESULTS AND DISCUSSION

Our earlier method for the peptide synthesis involved EtOH as the solvent,[11] and this solvent is not a suitable choice in native biological systems. Therefore, we investigated the modification using ionic liquids instead of EtOH. Because of their unique properties, ionic liquids are fascinating to chemists and biochemists in various fields. Besides their potential for green chemistry (mainly due to their catalytic ability and safety for the biological system), many organic synthesis reactions have been carried out in ionic liquids, and these include amino acid and peptide synthesis.[13] The catalyst BSA is an expensive and unstable reagent, and we therefore pursued to find a new catalytic system to modify peptides and proteins (Table 1). At first, the modification of L-Phe-OEt with *N*-Cbz-L-Phe thioester was evaluated at various reaction conditions (Table 1). It is noteworthy that ionic liquids and HMDO can promote the reactions individually in good yields in an extended time (Table 1, entries 4 and 5). Optimization studies revealed that the best results were obtained by the combination of HMDO (1 equiv)

Table 1. Optimization for the Synthesis of Peptide 14a



entry	silyl compounds	solvents (2 mL)	time (h)	yield (%)[a]
1	BSA (1 equiv)	EtOH	72	65
2	HMDO (1 equiv)	EtOH	72	74
3	HMDO (1 equiv)	*i*-PrOH	72	86
4	HMDO (2 mL)	–	72	87
5	–	[BMIM]PF_6	72	45
6	HMDO (0.5 equiv)	[BMIM]PF_6	24	54
7	HMDO (1 equiv)	[BMIM]PF_6	24	70
8	**HMDO (1 equiv)**	**[BMIM]PF_6**	**48**	**93**
9	HMDO (1 equiv)	[BMIM]BF_4	24	75
10	HMDO (1 equiv)	[BMIM]BF_4:H_2O = 4:1	48	63
11	HMDO (1 equiv)	[BMIM]BF_4:H_2O = 2:1	48	42

[a]Isolated yield based on the thioester.

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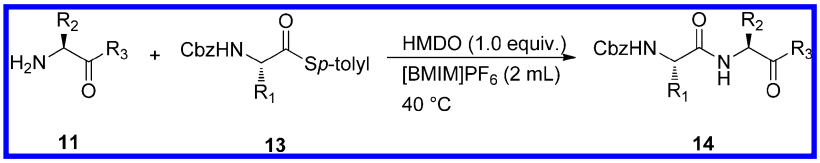
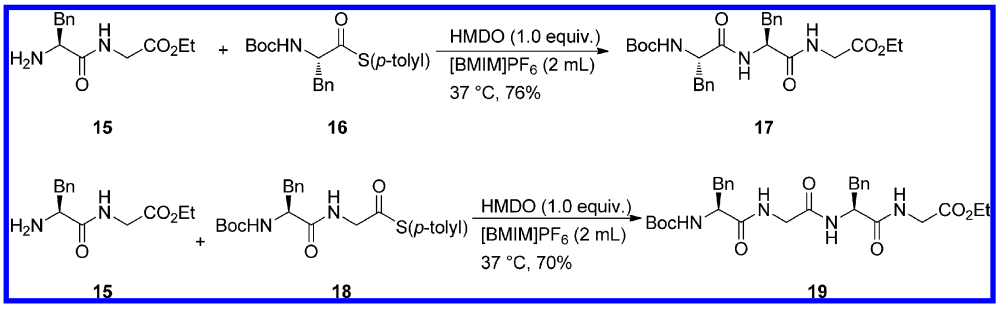
Table 2. Peptide Synthesis via HMDO-Mediated Ligation[a]



entry	α-amino acid ester (**11**)	thioester (**13**)	dipeptide (**14**)	reaction time (h)	yield (%)[b]
1	L-Phe-OEt (**11a**)	Cbz-L-Phe-S(*p*-tolyl) (**13a**)	Cbz-L-Phe-L-Phe-OEt (**14a**)	48	93
2	D-Phe-OMe (**11b**)	Cbz-L-Phe-S(*p*-tolyl) (**13a**)	Cbz-L-Phe-D-Phe-OMe (**14b**)	46	82
3	D-Phg-OEt (**11c**)	Cbz-L-Phe-S(*p*-tolyl) (**13a**)	Cbz-L-Phe-D-Phg-OEt (**14c**)	48	80
4	L-Leu-OMe (**11d**)	Cbz-L-Phe-S(*p*-tolyl) (**13a**)	Cbz-L-Phe-L-Leu-OMe (**14d**)	24	83
5	L-Ser-OEt (**11e**)	Cbz-L-Phe-S(*p*-tolyl) (**13a**)	Cbz-L-Phe-L-Ser-OEt (**14e**)	48	85
6	L-Leu-OMe (**11d**)	Cbz-L-Trp-S(*p*-tolyl) (**13b**)	Cbz-L-Trp-L-Leu-OMe (**14f**)	24	63
7	L-His-OMe (**11f**)	Cbz-L-Trp-S(*p*-tolyl) (**13b**)	Cbz-L-Trp-L-His-OMe (**14g**)	48	63
8	L-Phe-OEt (**11a**)	Cbz-L-Trp-S(*p*-tolyl) (**13b**)	Cbz-L-Trp-L-Phe-OEt (**14h**)	24	89
9	L-Trp-OMe (**11g**)	Cbz-L-Trp-S(*p*-tolyl) (**13b**)	Cbz-L-Trp-L-Trp-OMe (**14i**)	48	82
10	L-Ser-OEt (**11e**)	Cbz-L-Trp-S(*p*-tolyl) (**13b**)	Cbz-L-Trp-L-Ser-OEt (**14j**)	48	86
11	L-Leu-OMe (**11d**)	Cbz-L-Pro-S(*p*-tolyl) (**13c**)	Cbz-L-Pro-L-Leu-OMe (**14k**)	27	63
12	L-Trp-OMe (**11g**)	Cbz-L-Pro-S(*p*-tolyl) (**13c**)	Cbz-L-Pro-L-Trp-OMe (**14l**)	48	79
13	L-Phe-OEt (**11a**)	Cbz-L-Ala-S(*p*-tolyl) (**13d**)	Cbz-L-Ala-L-Phe-OEt (**14m**)	29	80
14	Gly-OEt (**11h**)	Cbz-L-Met-S(*p*-tolyl) (**13e**)	Cbz-L-Met-Gly-OEt (**14n**)	12	91
15	Gly-OEt (**11h**)	Cbz-D-Phe-S(*p*-tolyl) (**13f**)	Cbz-D-Phe-Gly-OEt (**14o**)	12	90[c]
16	Gly-OEt (**11h**)	Ac-D-Phe-S(*p*-tolyl) (**13g**)	Ac-D-Phe-Gly-OEt (**14p**)	12	91[d]
17	D-Phe-OMe (**11i**)	Cbz-Gly-S(*p*-tolyl) (**13h**)	Cbz-Gly-D-Phe-OMe (**14q**)	12	94
18	Gly-OH (**11j**)	Cbz-L-Phe-S(*p*-tolyl) (**13a**)	Cbz-L-Phe-Gly-OH (**14r**)	12	92[e]

[a]All reactions performed at 0.5 mmol in $[BMIM]PF_6$ (2 mL) at 40 °C. [b]Isolated yield based on the thioester. [c]**13f** (>99% ee); **14o** (>99% ee); HPLC conditions: Chiral Pak AD-H, 5 μm, 4.6 mm × 250 mm, *n*-hexane/2-propanol = 70:30, 210 nm, flow rate = 1.0 mL min^{-1}. [d]**13g** (50.4% ee), **14p** (49.4% ee), Chiral Pak AD-H, 5 μm, 4.6 mm × 250 mm, *n*-hexane/2-propanol = 70:30, 210 nm, flow rate = 1.0 mL min^{-1}. [e]Thioester (1.0 equiv), Gly-OH (2.00 equiv), NaOH (2.0 equiv).

Scheme 3. Synthesis of Tripeptide 17 and Tetrapeptide 19 via HMDO-Mediated Ligation



and ionic liquids such as 1-butyl-3-methylimidazolium hexafluorophosphate ($[BMIM]PF_6$) and 1-butyl-3-methylimidazolium tetrafluoroborate ($[BMIM]BF_4$) (Table 1, entries 8–9). It was also found that the HMDO loading presents a critical determinant of the reaction efficiency; increasing the amount of HMDO from 0 to 1 equiv can significantly boost the yield from 45 to 70% (Table 1, entries 5–7). Peptide bond formation was also accomplished in aqueous media ($[BMIM]BF_4$:water >4:1 v/v), and the reactions become much slower with increasing amounts of water (Table 1, entries 10–11).

Encouraged by this promising result, the scope of the reaction was tested with a number of thioesters and α-amino acid esters (Table 2). To our delight, every thioester substrate tested produced the desired products in good to excellent yields (63–94%) in $[BMIM]PF_6$, though some α-substituted amino acids required an extended reaction time (48 h) (Table 2, entries 1 and 4–13). Treatment of the thioester **13a** with unnatural α-amino acid esters (**11b**, **11c**) resulted in the formation of unnatural dipeptides (**14b**, **14c**) in excellent yields (Table 2, entries 2–3). The amidation reaction of α-unsubstituted amino acid esters such as Gly-OEt **11h** proceeds very well to provide the desired *N*-Cbz or *N*-Ac peptides **14n**–**14p** in 90–91% yields (Table 2, entries 14–16). When the *N*-Cbz protected α-unsubstituted thioester such as **13h** was utilized as the coupling partner with the α-substituted amino acid ester, the desired peptide **14q** was obtained in 94% yield (Table 2, entry 17). The amino acid can be directly used in the coupling reaction to give the desired product **14r** in 92% yield (Table 2, entry 18). In order to examine the extent of racemization, an enantiomeric mixture of **14o** was synthesized in the same manner, and HPLC data revealed that no detectable racemization occurred for the synthesis of D-**14o** in the HMDO-mediated coupling reaction. For the *N*-Ac protected thioester **13g**, the enantiomeric excess of the corresponding peptide **14p** is almost identical to that of the thioester **13g**. Moreover, tripeptide **17** and tetrapeptide **19** were successfully synthesized via this ligation (Scheme 3). It is noteworthy that this modification reaction is compatible with a free hydroxyl or thiol group, and hence, the method has potential for applications to glycopeptide and polypeptide

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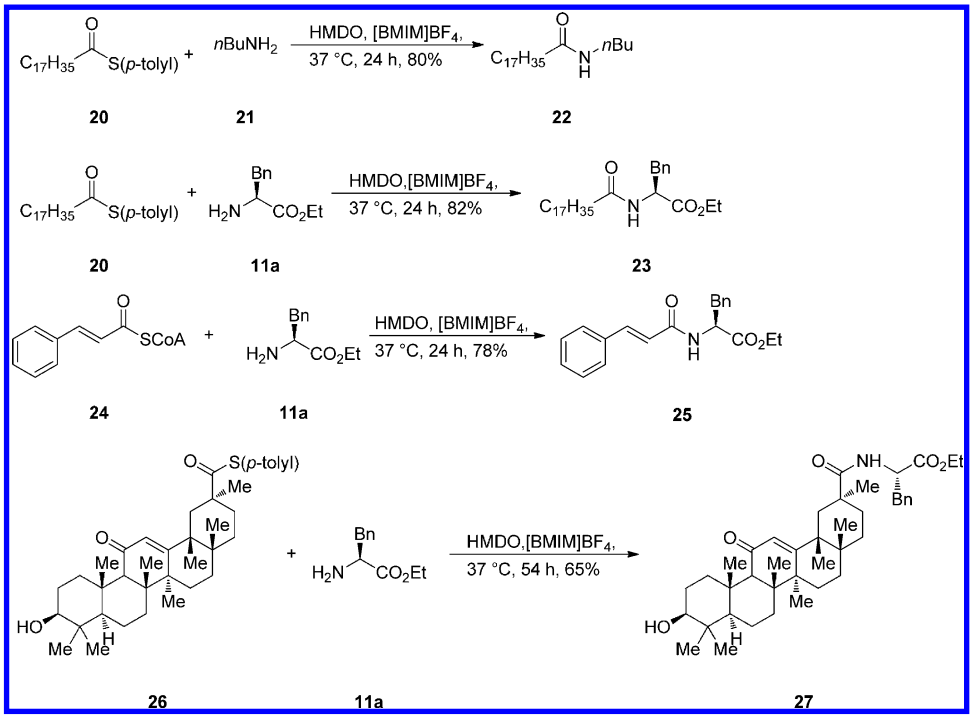
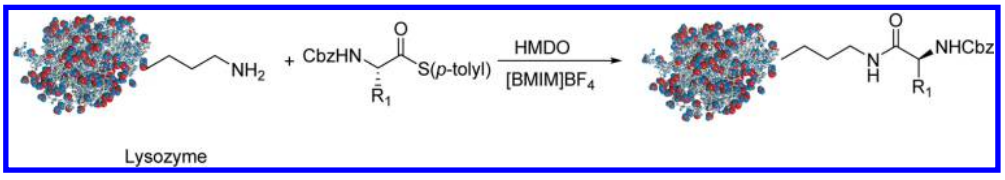
Scheme 4. Modification of Stearic Acid Thioester 20, Cinnamoyl-CoA 24, and Glycyrrhizic Acid Thioester 26 via HMDO-Mediated Ligation



Scheme 5. Proposed Approach for Lysozyme Modification



synthesis. Though the reaction mechanism remains under investigation, we speculate that HMDO not only can react with amines to form *N*-silylamine intermediates but also activates the carbonyl group of the thioester in the polar solvent system, and that both of these effects synergistically facilitate peptide amide bond formation.[12,14]

Further, the reaction was tested on stearic acid thioester **20** and cinnamoyl-CoA **24** to examine their potential use as probes to study protein dynamics (Scheme 4). Very pleasingly, the reactions proceeded smoothly and provided the desired products **22**, **23** and **25** in excellent yields. The reaction of cinnamoyl-CoA **24** was monitored by HPLC analysis (see Supporting Information). The α-amino acid esters or amines could be used as the chemical chain terminator to intercept and off-load truncated biosynthetic species from acyl carrier protein (ACP) domains of polyketide synthase (PKSs).[15] Interestingly, modification of the glycyrrhizic acid α-trisubstituted thioester **26** via HMDO-mediated ligation afforded the desired product **27** in 65% yield.

In light of these results, the functionalization of proteins was attempted. The lysine residue of proteins has attracted much attention due to its importance in many post-translational modifications in cell physiology and pathology. The amino group of a lysine residue can be modified by activated esters, sulfonyl chlorides and isocyanates.[10a,16] We envisioned that the thioesters could be used to modify lysine residues of proteins via our HMDO-mediated ligation methodology in ionic liquids. Lysozyme was chosen as the model to test this protein modification because of its excellent chemical and thermal stabilities (Scheme 5).

Using the optimized conditions, lysozyme was reacted with *N*-Cbz-L-Phe-S(*p*-tolyl) (**13a**) at 37 °C and analyzed by LTQ Orbitrap XL-MS. Analysis of the reaction mixture after 24 h confirmed consumption of lysozyme, along with a single peak (m/z 1621.6776) corresponding to the mass of *N*-Cbz-L-Phe-lysozyme (Figure 1B). In order to test our methodology is applicable for other small molecules modification lysozyme, we also tested the ability of *N*-Cbz-L-Trp-S(*p*-tolyl) (**13b**) to modify lysozyme (Figure 1C).

Finally, various oxo esters instead of thioesters were tested to expand the general reaction scope of the substrates. The coupling reactions of oxo esters are quite different and much slower compared to that of the thioesters. This may in part be attributed to the higher thermodynamic stability and lower activity of oxo esters.[17] However, the reaction was much faster using a catalytic amount of *p*-tolylSH (0.1 equiv) (Table 3). We expected the thioesters to be more reactive intermediates in the direct addition of amino acid esters toward oxo esters (Scheme 6). In our mechanistic hypothesis, the thiol would play the part of a catalytic mediator, and then the in situ-generated thioesters by the thioesterification of oxo esters would be converted into the peptide in the presence of HMDO in ionic liquids. Analysis of the reaction mixture by LTQ Orbitrap XL-MS indicated the

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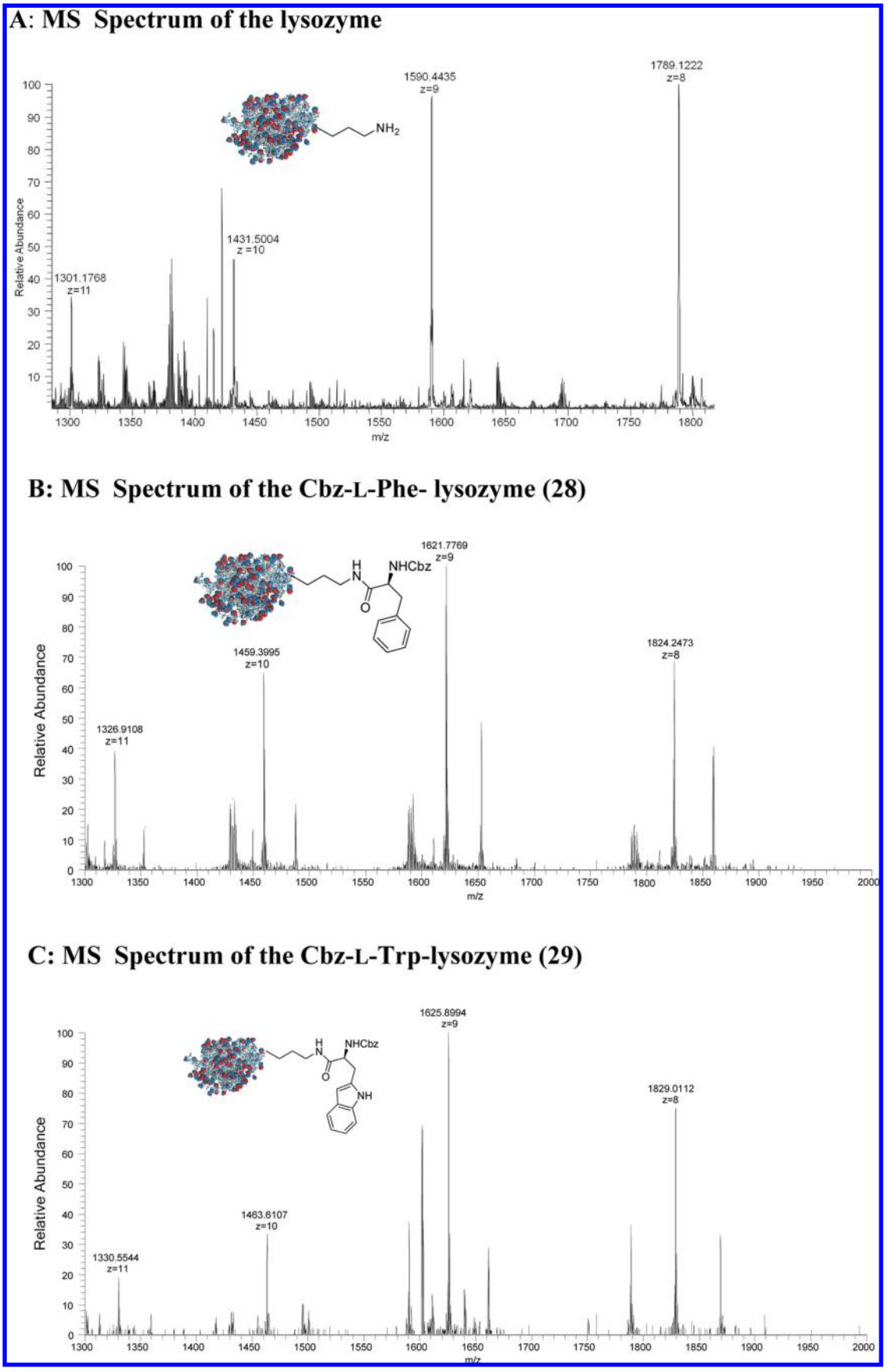

Figure 1. LTQ Orbitrap XL-MS spectrum of the lysozyme (A), *N*-Cbz-L-Phe-lysozyme (**28**) (B), and *N*-Cbz-L-Trp-lysozyme (**29**) (C).

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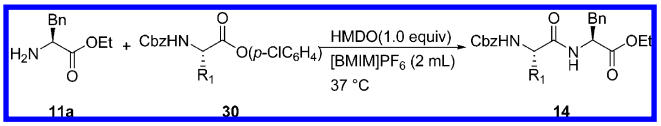
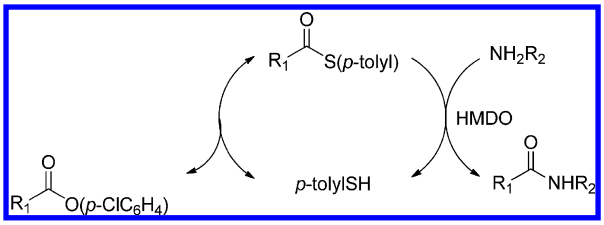
Table 3. Peptide Synthesis of Oxo Esters via HMDO-Mediated Ligation



entry	oxo ester (**30**)	*p*-tolylSH (equiv)	reaction time (h)	dipeptide (**14**)	yield (%)[a]
1	Cbz-L-Phe-oxo ester (**30a**)	0	72	**14a**	20
2	Cbz-L-Phe-oxo ester (**30a**)	0.1	62	**14a**	72
3	Cbz-L-Phe-oxo ester (**30a**)	0.5	55	**14a**	68
4	Cbz-L-Ala-oxo ester (**30a**)	0	72	**14m**	25
5	Cbz-L-Ala-oxo ester (**30a**)	0.1	60	**14m**	70
6	Cbz-L-Ala-oxo ester (**30a**)	0.5	48	**14m**	71

[a]Isolated yield based on oxo ester.

Scheme 6. Possible Pathway for the 1,2-Addition of Amino Acid Esters to Oxo Esters by the Thioester Intermediates



formation of the thioester intermediate (*m*/*z* 406.1482, Figure 2).

CONCLUSIONS

In summary, we have developed an innovative strategy for the modification of peptides and proteins that relies on HMDO-mediated ligation in ionic liquids and proceeds smoothly under mild conditions. The peptide coupling scope is expanded using in situ-generated thioesters by the thioesterification of oxo esters. This method is environmental friendly and does not require a cysteine residue to form a native amide bond, and the new method therefore presents a significant expansion to extant NCL strategies. The approach will be particularly useful in combination with the chemoselective thioester formation through thiolysis of C-terminally fused inteins or enzyme Sortase A. We are currently exploring this approach for several proteins without cysteine residue, and progress will be reported in due course.

EXPERIMENTAL SECTION

General Information. ^{1}H and ^{13}C NMR spectra were recorded on 400 MHz spectrometers in deuteriochloroform ($CDCl_3$) unless otherwise stated. All ^{1}H chemical shifts are reported in ppm (δ) relative to TMS (0.00); ^{13}C shifts are reported in ppm (δ) relative to $CDCl_3$ (77.0). Data are reported in the following order: chemical shifts are given (δ); multiplicities are indicated as br (broadened), s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), app (apparent); coupling constants, *J*, are reported (Hz); integration is provided. Optical rotation values were measured at 20 °C with different solvents. Melting points were taken without calibration. Analytical thin-layer chromatography (TLC) was performed on silica gel aluminum sheets with F-254 indicator. Visualization was accomplished by UV light or with solutions of K_2CO_3/$KMnO_4$ in water. Purification by chromatography was performed using 230−400 mesh SiO_2 with compressed air as a source of positive pressure. HPLC analyses were carried out with a binary pump on Symmetry C18 (4.6 × 150 mm, 5 μm) and CHIRAL PAK AD-H (4.6 mm I.D. × 250 mm, 5 μm). Solvents for reactions and chromatography were reagent grade and used as received. "Brine" refers to a saturated aqueous solution of NaCl. Solution of $NaHCO_3$ refers to saturated aqueous solutions. Solvents used as reaction media were purchased in >99% purity without further purification, unless otherwise specified. High Resolution MS was analyzed by Thermo LTQ XL Orbitrap.

Starting Materials. Cinnamoyl CoA was prepared according to the literature.[18] Thioesters were prepared from the corresponding *N*-Cbz-L-amino acids.[11]

General Procedure A: Preparation of Thioesters Derived from *N*-Cbz-L-Amino Acids. 4-Methylbenzenethiol (10.5 mmol) and 1-hydroxybenzotriazole (15 mmol) were added to a solution of the *N*-Cbz-L-amino acid (10 mmol) in ethyl acetate (10 mL) at 0 °C followed by *N*,*N*′-diisopropylcarbodiimide (10 mmol). The mixture was stirred for 24 h at room temperature, and the reaction progress was monitored by TLC analysis. At the end of the reaction a few drops of 50% acetic acid in ethyl acetate were added. The mixture was filtered through Celite, and the organic phase was washed with $NaHCO_3$ solution and brine, dried over $MgSO_4$, filtered, and concentrated in vacuo. The crude product was purified by flash chromatography affording the desired products.

*N-Cbz-L-Phenylalanine p-toluene thioester (**13a**).* Following the general procedure A, the reaction performed with *N*-Cbz-L-phenylalanine (2.990 g, 10 mmol) afforded the desired product as a white solid (3.244 g, 80% yield): mp 114−116 °C (lit.[11] 116−117 °C); $[\alpha]_D^{20}$ −66.6 (*c* 0.42, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.38−7.19 (m, 14H), 5.27 (d, *J* = 8.0 Hz, 1H), 5.16 (s, 2H), 4.89−4.84 (m, 1H), 3.24 (m, 2H), 2.41 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ

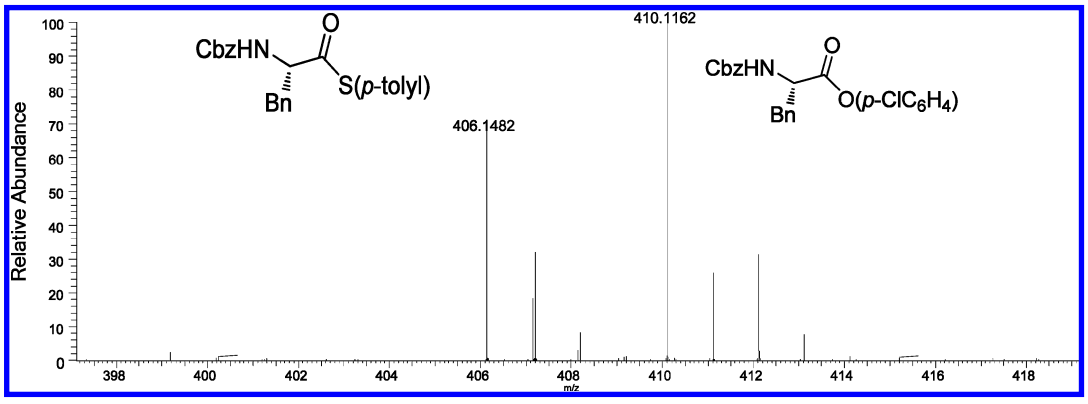


Figure 2. LTQ Orbitrap XL-MS spectrum of the thioesterification reaction mixture.

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= 199.1, 155.6, 139.9, 136.0, 135.3, 134.5, 130.1, 129.4, 128.7, 128.6, 128.3, 128.1, 127.3, 123.4, 67.3, 61.3, 38.5, 21.4; HRMS (ESI) Calcd for $C_{24}H_{24}NO_3S$ ([M + H]$^+$) 406.1471, found 406.1483.

*N-Cbz-L-tryptophan p-toluene thioester (**13b**).* Following the general procedure A, the title compound was prepared as a pale yellow solid (3.779 g, 85% yield): mp 50−52 °C (lit.[11] 51−52.5 °C); $[\alpha]_D^{20}$ −82.3 (*c* 0.50, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.12 (s, 1H), 7.59 (d, *J* = 8.0 Hz, 1H), 7.41−7.34 (br, 6H), 7.26−7.12 (m, 6H), 7.06 (d, *J* = 4.0 Hz, 1H), 5.37 (d, *J* = 8.0 Hz, 1H), 5.16 (s, 2H), 4.93−4.88 (m, 1H), 3.46−3.31 (m, 2H), 2.40 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ = 139.8, 136.2, 134.6, 130.0, 128.5, 128.2, 128.1, 123.2, 122.4, 119.9, 111.2, 109.5, 67.2, 61.0, 28.3, 21.3; HRMS (ESI) Calcd for $C_{26}H_{25}N_2O_3S$ ([M + H]$^+$) 445.1580, found 445.1589.

*N-Cbz-L-Proline p-toluene thioester (**13c**).* Following the general procedure A, the title compound was prepared as a white crystal. (2.318 g, 65% yield): mp 57−59 °C (lit.[11] 61−62 °C); $[\alpha]_D^{20}$ −105 (*c* 0.50, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.45−7.32 (m, 6H), 7.25−7.14 (m, 3H), 5.34−5.12 (m, 2H), 4.69−4.57 (m, 1H), 3.74−3.61(m, 2H), 2.40 (s, 3H), 2.31−2.15 (m, 3H), 2.10−1.98 (m, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ = 200.6, 200.1, 155.1, 154.5, 139.7, 139.6, 136.6, 136.4, 134.6, 134.5, 130.0, 128.5, 128.4, 128.0, 127.9, 123.8, 123.6, 127.9, 123.8, 123.6, 67.3, 66.4, 65.9, 47.3, 46.9, 31.7, 30.7, 24.1, 23.4, 21.3; HRMS (ESI) Calcd for $C_{20}H_{22}NO_3S$ ([M + H]$^+$) 356.1315, found 356.1321.

*N-Cbz-L-Alanine p-toluene thioester (**13d**).* Following the general procedure A, the title compound was prepared as a white solid (2.505 g, 76% yield): mp 61−63 °C (lit.[11] 58−62 °C); $[\alpha]_D^{20}$ −28.6 (*c* 0.7, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.42−7.24 (m, 9H), 5.32−5.19 (m, 1H), 5.16 (s, 2H), 4.66−4.59 (m, 1H), 2.40 (s, 3H), 1.50 (d, *J* = 8.0 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ = 199.8, 155.5, 139.9, 134.6, 130.1, 128.6, 128.3, 128.2, 123.3, 67.3, 56.6, 21.4, 19.0; HRMS (ESI) Calcd for $C_{18}H_{20}NO_3S$ ([M + H]$^+$) 330.1158, found 330.1160.

*N-Cbz-L-Methionine p-toluene thioester (**13e**).* Following the general procedure A, the title compound was prepared as a white solid (3.116 g, 80% yield): mp 141−142 °C (lit.[11] 143−144 °C); $[\alpha]_D^{20}$ −35.2 (*c* 0.50, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.41−7.35 (m, 5H), 7.31−7.24 (m, 4H), 5.54 (d, *J* = 8.0 Hz, 1H), 5.20 (s, 2H), 4.77−4.71 (m, 1H), 2.67−2.57 (m, 2H), 2.40 (s, 3H), 2.31−2.30 (m, 1H), 2.27 (s, 3H), 2.25−1.99 (m, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ = 199.1, 155.8, 140.0, 136.0, 135.4, 134.5, 130.1, 128.6, 128.3, 128.2, 123.1, 67.4, 60.0, 32.0, 30.0, 21.4, 15.4; HRMS (ESI) Calcd for $C_{20}H_{24}NO_3S_2$ ([M + H]$^+$) 390.1192, found 390.1199.

*N-Cbz-D-Phenylalanine p-toluene thioester (**13f**).* Following the general procedure A, the reaction performed with *N*-Cbz-D-phenylalanine (2.990 g, 10 mmol) afforded the desired product as a white solid (3.163 g, 78% yield): mp 110−112 °C; $[\alpha]_D^{20}$ +18.6 (*c* 0.52, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.36−7.15 (m, 14H), 5.26 (d, *J* = 8.0 Hz, 1H), 5.12 (s, 2H), 4.85−4.80 (m, 1H), 3.20−3.10 (m, 2H), 2.37 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ = 199.2, 155.6, 139.9, 136.1, 135.4, 134.6, 130.2, 129.5, 128.8, 128.6, 128.3, 128.1, 127.3, 123.4, 110.0, 67.3, 61.3, 38.4, 21.4; HRMS (ESI) Calcd for $C_{24}H_{24}NO_3S$ ([M + H]$^+$) 406.1471, found 406.1488.

*N-Ac-D-Phenylalanine p-toluene thioester (**13g**).* Following the general procedure A, the title compound was prepared as a white solid (2.347 g, 75% yield): mp 158−159 °C; $[\alpha]_D^{20}$ +56.4 (*c* 0.65, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.74−7.65 (m, 1H), 7.36−7.10 (m, 8H), 5.92 (d, *J* = 8.0 Hz, 1H), 5.06−5.00 (m, 1H), 3.10 (d, *J* = 8.0 Hz, 2H), 2.30 (s, 3H), 1.93 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ = 198.7, 169.8, 140.0, 135.3, 134.6, 130.0, 129.3, 128.7, 127.3, 59.3, 38.3, 23.2, 21.4; HRMS (ESI) Calcd for $C_{18}H_{20}NO_2S$ ([M + H]$^+$) 314.1209, found 314.1216.

*N-Cbz-Glycine p-toluene thioester (**13h**).* Following the general procedure A, the title compound was prepared as a white solid (2.625 g, 83% yield): mp 93−95 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.37−7.21 (m, 10H), 5.41 (br, 1H), 5.16 (s, 2H), 4.41 (d, *J* = 4.0 Hz, 2H), 2.37 (s, 3H), 1.93 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ = 196.5, 140.1, 134.7, 130.3, 128.6, 128.3, 128.2, 122.7, 67.4, 50.5, 21.3; HRMS (ESI) Calcd for $C_{17}H_{18}NO_3S$ ([M + H]$^+$) 316.1002, found 316.1015.

*N-Boc-Phenylalanine p-toluene thioester (**16**).* Following the general procedure A, the title compound was prepared as a white solid (3.195 g, 86% yield): mp 166−167 °C; $[\alpha]_D^{20}$ −38.4 (*c* 0.60, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.27−7.11 (m, 9H), 4.92 (br, 1H), 4.70 (br, 1H), 3.11−3.00 (m, 2H), 2.30 (s, 3H), 1.36 (s, 9H); ^{13}C NMR (101 MHz, $CDCl_3$) δ = 155.0, 139.8, 135.7, 134.6, 130.1, 129.5, 128.7, 127.1, 123.7, 80.5, 60.8, 42.3, 38.4, 23.4, 21.4; HRMS (ESI) Calcd for $C_{21}H_{26}NO_3S$ ([M + H]$^+$) 372.1628, found 372.1633.

*N-Boc-Phenylalanine-glycine p-toluene thioester (**18**).* Following the general procedure A, the title compound was prepared as a white solid (3.215 g, 75% yield): mp 186−188 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.31−7.21 (m, 9H), 6.71 (br, 1H), 4.98 (br, 1H), 4.43 (br, 1H), 4.31−4.14 (m, 2H), 3.19−3.02 (m, 2H), 2.38 (s, 3H), 1.39 (s, 9H); ^{13}C NMR (101 MHz, $CDCl_3$) δ = 140.1, 136.5, 134.6, 130.2, 129.3, 128.7, 127.0, 122.6, 48.8, 29.7, 28.2, 21.3; HRMS (ESI) Calcd for $C_{23}H_{29}N_2O_4S$ ([M + H]$^+$) 429.1843, found 429.1856.

*Stearic acid p-toluene thioester (**20**).* Following the general procedure A, the title compound was prepared as a white solid (3.315 g, 85% yield): mp 92−94 °C (lit.[19] 46.5 °C); ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.32−7.23 (m, 4H), 2.67 (t, *J* = 12.0 Hz, 2H), 2.39 (s, 3H), 1.76−1.68 (m, 2H), 1.28 (s, 28H), 0.92 (t, *J* = 12.0 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ = 198.2, 139.3, 134.2, 130.1, 124.3, 43.6, 31.9, 29.7, 29.6, 29.4, 29.3, 29.2, 29.0, 21.3, 14.0; IR (film) 2918, 2850,1710, 1534, 1469, 1400, 1043, 947, 806, 762 cm^{-1}; HRMS (ESI) Calcd for $C_{25}H_{43}OS$ ([M + H]$^+$) 391.3029, found 391.3029.

*Glycyrrhizic acid p-toluene thioester (**26**).* Following the general procedure A, the title compound was prepared as a white solid (5.015 g, 85% yield): mp 260−262 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.30−7.22 (m, 4H), 7.13 (d, *J* = 8.0 Hz, 2H), 6.04 (d, *J* = 8.0 Hz, 2H), 5.63 (s, 1H), 4.92−4.87 (m, 1H), 4.25−4.20 (m, 2H), 3.24−3.05 (m, 4H), 2.80 (d, *J* = 16.0 Hz, 1H), 2.31 (s, 1H), 2.18−2.12 (m, 1H), 2.05−1.96 (m, 1H), 1.90−1.56 (m, 10H), 1.41−1.22 (m, 34H), 1.15−1.11 (m, 10H), 1.05 (s, 3H), 1.00 (s, 4H), 0.88−0.80 (m, 16H), 0.75 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ = 171.7, 129.2, 128.6, 127.2, 78.8, 61.8, 61.7, 54.9, 52.6, 47.7, 45.3, 43.6, 43.1, 41.7, 39.1, 37.8, 37.2, 37.0, 32.7, 31.9, 31.8, 29.6, 29.4, 28.3, 28.1, 27.3, 26.3, 23.5, 23.4, 18.6, 17.5, 16.7, 16.4, 15.6, 14.2, 14.1; IR (film) 3310, 3034, 2951, 1732, 1660, 1529, 1447, 1344, 1254, 1048, 744, 700 cm^{-1}; HRMS (ESI) Calcd for $C_{37}H_{53}O_3S$ ([M + H]$^+$) 577.3710, found 577.3710.

*N-Cbz-L-Phenylalanine oxo ester (**30a**).* 4-Chlorophenol (10.5 mmol) and 1-hydroxybenzotriazole (15 mmol) were added to a solution of the *N*-Cbz- L-amino acid (10 mmol) in ethyl acetate (10 mL) at rt followed by *N*,*N*′-diisopropylcarbodiimide (10 mmol). The mixture was stirred for 24 h at room temperature, and the reaction progress was monitored by TLC analysis. At the end of the reaction, a few drops of 50% acetic acid in ethyl acetate were added. The mixture was filtered through Celite, and the organic phase was washed with $NaHCO_3$ solution and brine, dried over $MgSO_4$, filtered, and concentrated in vacuo. The crude product was purified by flash chromatography affording the desired products; the title compound was prepared as a white solid (3.485 g, 85% yield): mp 136−138 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.35−7.31 (m, 12H), 7.21−7.19 (d, *J* = 12.0 Hz, 2H), 6.92−6.89 (d, *J* = 12.0 Hz, 2H), 5.31 (br, 1H), 5.13 (s, 2H), 4.88 (br, 1H), 3.25−3.24 (d, *J* = 4.0 Hz, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ = 170.1, 135.1, 131.6, 129.5, 129.4, 128.8, 128.6, 128.3, 128.2, 127.5, 122.6, 67.2, 55.0, 38.2; IR (film) 3310, 3034, 2951, 1732, 1660, 1529, 1447, 1344, 1254, 1048, 744, 700 cm^{-1}; HRMS (ESI) Calcd for $C_{23}H_{21}ClNO_4$ ([M + H]$^+$) 410.1154, found 410.1138.

*N-Cbz-L-Alanine oxo ester (**30b**).* Following the above general procedure, the title compound was prepared as a white solid (2.939 g, 88% yield): mp 104−107 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.37−7.33 (m, 8H), 7.05−7.03 (d, *J* = 8.0 Hz, 2H), 7.92−7.89 (d, *J* = 12.0 Hz, 2H), 5.34 (br, 1H), 5.14 (s, 2H), 4.63 (br, 1H), 1.61 (d, *J* = 8 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ = 171.5, 155.6, 131.6, 129.6, 128.6, 128.2, 122.7, 67.1, 49.8, 18.4; IR (film) 3336, 1777, 1692, 1531, 1488, 1456, 1359, 1298, 1261, 1202, 1152, 1120, 1072 cm^{-1}; HRMS (ESI) Calcd for $C_{17}H_{17}ClNO_4$ ([M + H]$^+$) 334.0841, found 334.0852.

General Method B: Preparation of Amino Acid Ester Free Amine (11a−11i).[20] The amino acid ester hydrochloride salt (10 mmol) was mixed with tetrahydrofuran (THF) (20 mL). Triethyl-

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amine (200 mmol) was added dropwise and stirred overnight at room temperature. The reaction mixture was filtered and washed with THF (10 mL). The solution was concentrated under a vacuum to afford the amino acid ester free amine.

General Method C: Modification of Amines with Thiol Esters in the Presence of HMDO. *N*-Cbz-L-amino acid thioester or other thioester (0.5 mmol, 1.0 equiv) was dissolved in ionic liquids (2 mL). HMDO (0.5 mmol, 1.0 equiv) and amino acid ester free amine (1.0 mmol, 2.0 equiv) were added stepwise. The reaction mixture was stirred at 37 °C for 24−48 h, cooled to room temperature, and concentrated under reduced pressure. The remaining residue was dissolved in ethyl acetate (20 mL) and washed with 1 M aqueous HCl (5 mL), 2 M aqueous NaOH (5 mL), and brine (5 mL). The organic layer was dried over Na_2SO_4, filtered, and concentrated to dryness. The crude product was purified by flash chromatography.

N-Cbz-L-Phe-L-Phe-OEt (**14a**). Following the general procedure C, the title compound was prepared as a white solid (220 mg, 93% yield): mp 142−144 °C (lit.[21] 138−139 °C); $[\alpha]_D^{20}$ −9.9 (*c* 0.5, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.36−7.15 (m, 13H), 7.04−6.96 (m, 2H), 6.45 (br, 1H), 5.36 (br, 1H), 5.09 (s, 2H), 4.84−4.76 (m, 1H), 4.45 (br, 1H), 4.16−4.13 (m, 2H), 3.08−3.06 (m, 4H), 1.24−1.19 (m, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ = 170.9, 170.4, 155.9, 136.2, 135.6, 129.4, 129.3, 128.7, 128.5, 128.2, 128.0, 127.1, 67.1, 61.5, 53.4, 53.1, 38.0, 29.0, 14.1; HRMS (ESI) Calcd for $C_{28}H_{31}N_2O_5$ ([M + H]$^+$) 475.2227, found 475.2228.

N-Cbz-L-Phe-D-Phe-OMe (**14b**). Following the general procedure C, the title compound was prepared as a white solid (188 mg, 82% yield): mp 141−142 °C; $[\alpha]_D^{20}$ −24.6 (*c* 0.5, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.36−7.13 (m, 13H), 6.91−6.89 (m, 2H), 6.31 (br, 1H), 5.28 (br, 1H), 5.06 (s, 2H), 4.86−4.81 (m, 1H), 4.45 (br, 1H), 4.16−4.13 (m, 2H), 3.66 (s, 3H), 3.08−2.89 (m, 4H); ^{13}C NMR (101 MHz, $CDCl_3$) δ = 171.4, 170.4, 136.2, 135.5, 129.3, 129.2, 128.7, 128.6, 128.5, 128.2, 128.0, 127.2, 127.1, 67.1, 56.2, 53.0, 52.3, 38.5, 37.8; HRMS (ESI) Calcd for $C_{27}H_{29}N_2O_5$ ([M + H]$^+$) 461.2071, found 461.2070.

N-Cbz-L-Phe-D-Phg-OEt (**14c**). Following the general procedure C, the title compound was prepared as a white solid (184 mg, 80% yield): mp 146−148 °C; $[\alpha]_D^{20}$ −32.8 (*c* 0.5, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.36−7.30 (m, 9H), 7.19−7.15 (m, 5H), 7.07 (br, 2H), 6.71 (br, 1H), 5.48 (d, *J* = 8.0 Hz, 1H), 5.08 (s, 2H), 4.49 (br, 1H), 4.21−4.08 (m, 2H), 3.05−3.00 (m, 2H), 1.20 (t, *J* = 8.0 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ = 170.4, 170.0, 136.0, 129.3, 128.9, 128.7, 128.5, 128.2, 128.0, 127.2, 110.0, 61.9, 56.0, 13.9; IR (film) 3734, 3006, 1732, 1695, 1651, 1536, 1451, 1377, 1317, 1258, 1214, 1136, 1041, 744, 698; HRMS (ESI) Calcd for $C_{27}H_{29}N_2O_5$ ([M + H]$^+$) 461.2071, found 461.2071.

N-Cbz-L-Phe-L-Leu-OMe (**14d**). Following the general procedure C, the title compound was prepared as a white solid (176.7 mg, 83% yield): mp 110−112 °C. (lit.[22] 109−110 °C); $[\alpha]_D^{20}$ −23.4 (*c* 0.5, $CHCl_3$) ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.37−7.21 (m, 10H), 6.23 (br, 1H), 5.37 (br, 1H), 5.11 (s, 2H), 4.58−4.47 (m, 2H), 3.71 (s, 3H), 3.16−3.05 (m, 2H), 1.60−1.43 (m, 3H), 0.92−0.86 (m, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ = 172.7, 170.5, 156.0, 136.2, 129.4, 128.7, 128.5, 128.2, 128.0, 127.0, 67.1, 56.0, 52.3, 50.8, 41.5, 38.3, 24.7, 22.7; HRMS (ESI) Calcd for $C_{24}H_{31}N_2O_5$ ([M + H]$^+$) 427.2227, found 427.2212.

N-Cbz-L-Phe-L-Ser-OEt (**14e**). Following the general procedure C, the title compound was prepared as a white solid (176.1 mg, 85% yield): mp 97−99 °C (lit.[11] 100−103 °C); $[\alpha]_D^{20}$ +12.3 (*c* 0.5, $CHCl_3$) ^{1}H NMR (400 MHz, CD_3OD-d_6) δ = 7.62 (d, *J* = 8.0 Hz, 1H), 7.36−7.28 (m, 6H), 7.14−7.09 (m, 3H), 7.03−6.99 (m, 1H), 5.03 (s, 2H), 4.56−4.53 (m, 1H), 4.50−4.48 (m, 1H), 4.20−4.12 (m, 2H), 3.89−3.85 (m, 1H), 3.78−3.74 (m, 1H), 3.34−3.29 (m, 2H), 3.16−3.10 (m, 1H), 1.27 (t, *J* = 8.0 Hz, 3H); ^{13}C NMR (101 MHz, CD_3OD-d_6) δ = 173.2, 170.1, 156.9, 136.7, 136.6, 128.0, 127.5, 127.4, 127.3, 123.4, 121.0, 118.4, 117.9, 110.9, 109.4, 66.2, 61.5, 61.2, 55.9, 54.9, 27.8, 13.1; HRMS (ESI) Calcd for $C_{22}H_{27}N_2O_6$ ([M + H]$^+$) 415.1864, found 415.1863.

N-Cbz-L-Trp-L-Leu-OMe (**14f**). Following the general procedure C, the title compound was prepared as a pale yellow solid (147 mg, 63% yield): mp 70−72 °C (lit.[11] 73−75 °C); $[\alpha]_D^{20}$ +46.4 (*c* 0.5, CHCl3) ^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.16 (s, 1H), 7.73 (br, 1H), 7.39−7.11 (m, 10H), 6.11 (br, 1H), 5.56 (br, 1H), 5.14 (s, 2H), 4.54−4.50 (m, 2H), 3.67 (s, 3H), 3.39 (br, 1H), 3.22 (br, 1H), 1.71 (br, 2H), 1.49 (br, 1H), 0.87 (t, *J* = 8.0 Hz, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ = 172.8, 171.0, 136.2, 128.5, 128.2, 128.1, 123.5, 122.3, 119.8, 118.9, 111.2, 52.2, 50.8, 41.5, 24.6, 22.6, 21.9; HRMS (ESI) Calcd for $C_{26}H_{32}N_3O_5$ ([M + H]$^+$) 466.2336, found 466.2344.

N-Cbz-L-Trp-L-His-OMe (**14g**). Following the general procedure C, the title compound was prepared as a white solid (154.0 mg, 63% yield): mp 163−165 °C; $[\alpha]_D^{20}$ −22.8 (*c* 0.5, $CHCl_3$) ^{1}H NMR (400 MHz, CD_3OD-d_6) δ = 8.45 (s, 1H), 7.59 (d, *J* = 8.0 Hz, 1H), 7.34−7.30 (m, 6H), 7.19 (s, 1H), 7.12 (t, *J* = 6.0 Hz, 2H), 7.03 (t, *J* = 8.0 Hz, 1H), 5.08−5.07 (m, 2H), 4.72−4.70 (m, 1H), 4.39 (t, *J* = 6.0 Hz, 1H), 3.65 (s, 3H), 3.25−3.19 (m, 2H), 3.10−3.02 (m, 2H); ^{13}C NMR (101 MHz, CD_3OD-d_6) δ = 173.3, 170.3, 156.9, 136.8, 136.6, 136.5, 133.7, 129.7, 128.0, 127.5, 127.2, 123.3, 120.9, 118.4, 117.8, 110.8, 109.2, 27.4, 26.6; HRMS (ESI) Calcd for $C_{26}H_{28}N_5O_5$ ([M + H]$^+$) 490.2085, found 490.2080.

N-Cbz-L-Trp-L-Phe-OEt (**14h**). Following the general procedure C, the title compound was prepared as a pale yellow solid (228.2 mg, 89% yield): mp 158−160 °C; $[\alpha]_D^{20}$ +9.2 (*c* 0.5, $CHCl_3$) ^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.06 (s, 1H), 7.71 (br, 1H), 7.38−7.34 (m, 6H), 7.24−7.14 (m, 5H), 7.02 (br, 1H), 6.68 (d, *J* = 8.0 Hz, 2H), 6.18 (d, *J* = 8.0 Hz, 1H), 5.49 (br, 1H), 5.13 (s, 2H), 4.75−4.73 (m, 1H), 4.52 (br, 1H), 4.12−4.05 (m, 2H), 3.38 (br, 1H), 3.18 (q, 1H), 2.98 (d, *J* = 8.0 Hz, 2H), 1.69 (br, 1H), 1.22 (t, *J* = 8.0 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ = 170.8, 170.7, 136.2, 135.6, 129.2, 128.5, 128.4, 128.2, 128.0, 127.0, 123.4, 122.3, 119.9, 118.9, 111.2, 61.4, 53.3, 37.8, 28.5, 14.0; HRMS (ESI) Calcd for $C_{30}H_{32}N_3O_5$ ([M + H]$^+$) 514.2336, found 514.2334.

N-Cbz-L-Trp-L-Trp-OMe (**14i**). Following the general procedure C, the title compound was prepared as a pale yellow solid (231.2 mg, 82% yield): mp 185−187 °C (lit.[23] 175 °C); $[\alpha]_D^{20}$ +13.4 (*c* 0.5, $CHCl_3$) ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.93 (d, *J* = 8.0 Hz, 1H), 7.26−6.96 (m, 15H), 6.47 (d, *J* = 8.0 Hz, 1H), 6.38 (d, *J* = 8.0 Hz, 1H), 5.16 (br, 1H), 4.98 (m, 2H), 4.81 (m, 1H), 4.38 (br, 1H), 3.56 (d, *J* = 12.0 Hz, 3H), 3.21 (m, 1H), 3.06 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ = 171.8, 170.4, 136.3, 129.4, 129.3, 128.7, 128.6, 128.2, 128.1, 128.0, 123.0, 122.2, 119.7, 118.4, 111.2, 109.4, 52.8, 52.5, 52.4, 27.5; HRMS (ESI) Calcd for $C_{31}H_{31}N_4O_5$ ([M + H]$^+$) 539.2289, found 539.2290.

N-Cbz-L-Trp-L-Ser-OEt (**14j**). Following the general procedure C, the title compound was prepared as a white solid (195.7 mg, 86% yield): mp 118−119 °C; $[\alpha]_D^{20}$ +16.4 (*c* 0.5, $CHCl_3$) ^{1}H NMR (400 MHz, DMSO-d_6) δ = 8.47 (d, *J* = 8.0 Hz, 1H), 7.53 (d, *J* = 8.0 Hz, 1H), 7.33−7.09 (m, 10H), 5.15 (br, 1H), 4.94 (s, 2H), 4.41 (br, 2H), 4.12−4.06 (m, 2H), 3.78 (br, 1H), 3.68 (br, 1H), 3.07−3.00 (m, 1H), 2.78−2.69 (m, 1H), 1.21 (t, *J* = 8.0 Hz, 3H); ^{13}C NMR (101 MHz, DMSO-d_6) δ = 172.4, 170.9, 156.3, 138.6, 137.4, 129.7, 128.7, 128.4, 128.1, 127.8, 126.6, 65.6, 61.0, 56.3, 55.2, 37.9, 14.5; IR (film) 3737, 3401, 2922, 2856, 1703, 1671, 1525, 1457, 1342, 1258, 1136, 1023, 847 cm^{-1}; HRMS (ESI) Calcd for $C_{24}H_{28}N_3O_6$ ([M + H]$^+$) 454.1973, found 454.1974.

N-Cbz-L-Pro-L-Leu-OMe (**14k**). Following the general procedure C, the title compound was prepared as a pale yellow solid (118 mg, 63% yield): mp 73−75 °C (lit.[24] 78−80 °C); $[\alpha]_D^{20}$ −56.7 (*c* 0.5, $CHCl_3$) ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.37 (br, 5H), 7.12 (br, 0.5H), 6.34 (br, 0.33H), 5.23−5.14 (m, 2H), 4.55 (br, 1H), 4.39 (br, 1H), 3.73−3.45 (m, 5H), 2.36−2.17 (m, 1H), 1.92 (s, 3H), 1.61 (br, 3H), 0.91 (d, *J* = 4.0 Hz, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ = 173.2, 171.4, 136.5, 128.6, 128.0, 127.8, 67.3, 60.7, 60.3, 52.2, 50.9, 47.5, 46.9, 41.2, 24.9, 24.6, 22.7, 21.9, 14.1; HRMS (ESI) Calcd for $C_{20}H_{29}N_2O_5$ ([M + H]$^+$) 377.2071, found 377.2070.

N-Cbz-L-Pro-L-Trp-OMe (**14l**). Following the general procedure C, the title compound was prepared as a colorless oil (177.3 mg, 79% yield): $[\alpha]_D^{20}$ −32.4 (*c* 0.5, $CHCl_3$) ^{1}H NMR (400 MHz, CD_3OD-d_6) δ = 7.55−7.49 (m, 1H), 7.38−7.31 (m, 3H), 7.23 (br, 3H), 7.10−7.08 (m, 1H), 7.04−7.0 (m, 1H), 5.15−5.08 (m, 1H), 5.00−4.95(m, 2H), 4.73−4.71 (m, 1H), 4.32−4.29 (m, 1H), 3.66 (d, *J* = 12.0 Hz, 3H), 3.50−3.43 (m, 2H), 3.28−3.10 (m, 2H), 2.22−2.12 (m, 1H), 1.96−

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1.79 (m, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ = 170.8, 170.5, 136.2, 135.6, 128.5, 128.2, 128.0, 127.0, 123.4, 122.3, 119.8, 118.8, 111.2, 61.4, 53.3, 42.2, 37.8, 23.5, 14.1; HRMS (ESI) Calcd for $C_{25}H_{28}N_3O_5$ ([M + H]$^+$) 450.2023, found 450.2022.

N-Cbz-L-Ala-L-Phe-OEt (**14m**). Following the general procedure C, the title compound was prepared as a white solid (159 mg, 80% yield): mp 97−99 °C (lit.[25] 97−98 °C) $[\alpha]_D^{20}$ +18.4 (*c* 0.5, $CHCl_3$) ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.37−7.24 (m, 8H), 7.13 (d, *J* = 8.0 Hz, 2H), 6.55 (br, 1H), 5.36 (br, 1H), 5.15 (q, 2H), 4.87−4.82 (m, 1H), 4.26−4.16 (m, 3H), 3.18−3.07 (m, 2H), 1.36 (d, *J* = 4.0 Hz, 3H), 1.27 (t, *J* = 4.0 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ = 171.8, 171.2, 155.8, 136.1, 135.7, 129.3, 128.5, 128.2, 128.0, 127.1, 67.0, 61.6, 53.2, 50.4, 37.8, 18.5, 14.1; HRMS (ESI) Calcd for $C_{22}H_{27}N_2O_5$ ([M + H]$^+$) 399.1914, found 399.1918.

N-Cbz-L-Met-Gly-OEt (**14n**). Following the general procedure C, the title compound was prepared as a white solid (167 mg, 91% yield): mp 74−76 °C (lit.[11] 74−76 °C); $[\alpha]_D^{20}$ −19.5 (*c* 0.2, EtOH) ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.61−7.27 (m, 5H), 6.70 (s, 1H), 5.57 (d, *J* = 7.9 Hz, 1H), 5.20−5.04 (m, 2H), 4.44 (dd, *J* = 14.1 Hz, 6.8, 1H), 4.21 (q, 2H), 4.03 (ddd, *J* = 23.1 Hz, 18.3 Hz, 5.4 Hz, 2H), 2.60 (t, *J* = 7.0 Hz, 2H), 2.34−1.85 (m, 5H), 1.28 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ = 171.3, 169.5, 156.1, 136.1, 128.6, 128.3, 128.1, 67.2, 61.7, 53.7, 41.3, 31.5, 29.9, 15.1, 14.1; HRMS (ESI) Calcd for $C_{17}H_{25}N_2O_5S$ ([M + H]$^+$) 369.1479, found 369.1493.

N-Cbz-D-Phe-Gly-OEt (**14o**). Following the general procedure C, the title compound was prepared as a white solid (173 mg, 90% yield): mp 109−111 °C; $[\alpha]_D^{20}$ −17.5 (*c* 0.57, EtOH) ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.37−7.18 (m, 10H), 6.34 (br, 1H), 5.33 (br, 1H), 5.08 (s, 2H), 4.50 (br, 2H), 4.21 (q, *J* = 8.0 Hz, 2H), 3.11 (d, *J* = 12.0 Hz, 2H), 1.28 (t, *J* = 8.0 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ = 171.0, 169.3, 136.2, 129.3, 128.7, 128.6, 128.2, 128.0, 127.1, 67.2, 61.6, 41.3, 14.1; HRMS (ESI) Calcd for $C_{21}H_{25}N_2O_5$ ([M + H]$^+$) 385.1758, found 385.1757.

N-Ac-D-Phe-Gly-OEt (**14p**). Following the general procedure C, the title compound was prepared as a white solid (132 mg, 91% yield): mp 133−136 °C; $[\alpha]_D^{20}$ +2.0 (*c* 0.50, EtOH) ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.29−7.20 (m, 5H), 6.70 (br, 1H), 6.42 (br, 1H), 4.78 (d, *J* = 8.0 Hz, 2H), 4.20−4.15 (m, 2H), 3.98−3.91 (m, 2H), 3.09−3.06 (m, 2H), 1.96 (s, 3H), 1.28 (t, *J* = 8.0 Hz, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ = 171.3, 170.2, 169.3, 136.5, 129.2, 128.6, 127.0, 61.5, 54.2, 41.3, 38.2, 23.1, 14.1; HRMS (ESI) Calcd for $C_{15}H_{21}N_2O_4$ ([M + H]$^+$) 293.1496, found 293.1495.

N-Cbz-Gly-D-Phe-OMe (**14q**). Following the general procedure C, the title compound was prepared as a colorless oil (174 mg, 94% yield): $[\alpha]_D^{20}$ +14.8 (*c* 1.0, EtOH) ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.34−7.22 (m, 9H), 7.08 (d, *J* = 8.0 Hz, 2H), 6.57 (br, 1H), 5.48 (br, 1H), 5.10 (s, 2H), 4.90−4.85 (m, 1H), 3.90−3.79 (m, 2H), 3.70 (s, 3H), 3.13−3.06 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ = 171.7, 168.6, 136.1, 135.6, 129.2, 128.6, 128.5, 128.3, 128.1, 127.2, 67.2, 53.1, 52.4, 44.5, 37.8; HRMS (ESI) Calcd for $C_{20}H_{23}N_2O_5$ ([M + H]$^+$) 371.1601, found 371.1626.

N-Cbz-L-Phe-Gly-OH (**14r**). *N*-Cbz-L-Phe *p*-toluene thiol ester (156 mg, 0.50 mmol) was dissolved in [BMIM]PF_6 (2 mL), followed by adding HMDO (81 mg, 0.50 mmol), Gly-OH (75 mg, 1.00 mmol) and NaOH (40 mg). The mixture was heated at 37 °C for 12 h and then cooled to room temperature. After workup, the title compound was prepared as a white solid (163 mg, 92% yield): mp 118−120 °C; $[\alpha]_D^{20}$ −8.7 (*c* 1.0, EtOH) ^{1}H NMR (400 MHz, DMSO-d_6) δ = 8.45−8.40 (m, 1H), 7.58−7.19 (m, 10H), 4.98−4.90 (m, 2H), 4.34 (t, *J* = 8.0 Hz, 1H), 3.83−3.77 (m, 2H), 3.07 (d, *J* = 12.0 Hz, 1H), 2.78 (t, *J* = 12.0 Hz, 1H); ^{13}C NMR (101 MHz, DMSO-d_6) δ = 171.9, 171.1, 155.8, 138.2, 136.9, 129.2, 128.2, 128.0, 127.7, 127.4, 126.2, 65.1, 56.0, 40.7, 37.5; HRMS (ESI) Calcd for $C_{19}H_{21}N_2O_5$ ([M + H]$^+$) 357.1445, found 375.1445.

L-Phe-Gly-OEt (**15**). Following the general procedure C, *N*-Boc-L-Phe-Gly-OEt (**15a**) was prepared as a white solid (154 mg, 88% yield): $[\alpha]_D^{20}$ +7.2 (*c* 1.0, EtOH) ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.23−7.13 (m, 5H), 6.63 (br, 1H), 5.14 (d, *J* = 8.0 Hz, 1H), 4.38 (br, 1H), 4.13 (d, *J* = 8.0 Hz, 2H), 3.97−3.82 (m, 2H), 3.09−2.96 (m, 2H), 1.31 (s, 9H), 1.21 (t, *J* = 8.0 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ = 171.7, 169.5, 136.7, 129.3, 128.6, 126.8, 61.5, 41.3, 28.2, 14.1; HRMS (ESI) Calcd for $C_{18}H_{27}N_2O_5$ ([M + H]$^+$) 351.1914, found 351.1918.

N-Boc-L-Phe-Gly-OEt. Title compound (350 mg, 1 mmol) was dissolved in DCM (3 mL). The reaction mixture was cooled at 0 °C and then added with TFA (1 mL). The reaction mixture was stirred at 0 °C for 40 min and then diluted with DCM, washing with $NaHCO_3$ and brine. The organic layer was dried over $MgSO_4$ and then concentrated to get the crude product as yellow oil. The crude product used directly in the next reaction without purification

N-Boc-L-Phe-L-Phe-Gly-OEt (**17**). Following the general procedure C, the title compound was prepared as a white solid (190 mg, 76% yield): mp 164−167 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ = 7.55 (t, *J* = 8 Hz, 1H), 7.43 (t, *J* = 8 Hz, 1H), 7.27−7.17 (m, 10H), 6.89 (br, 1H), 4.60 (br, 1H), 4.13 (q, *J* = 8.0 Hz, 3H), 3.86 (br, 2H), 3.07−3.03 (m, 1H), 2.87 (d, *J* = 16.0 Hz, 2H), 2.69 (s, 2H), 1.28 (s, 9H), 1.21 (t, *J* = 8 Hz, 3H), ^{13}C NMR (101 MHz, DMSO-d_6) δ = 171.3, 169.5, 155.0, 138.0, 137.5, 129.2, 129.1, 128.0, 127.9, 127.2, 126.2, 126.0, 124.4, 119.0, 109.6, 78.0, 60.4, 55.7, 53.3, 28.0, 14.0; HRMS (ESI) Calcd for $C_{27}H_{36}N_3O_6$ ([M + H]$^+$) 498.2599, found 498.2603.

N-Boc-L-Phe-Gly-L-Phe-Gly-OEt (**19**). Following the general procedure C, the title compound was prepared as a white solid (193 mg, 70% yield): mp 178−180 °C; ^{1}H NMR (400 MHz, MeOD) δ = 7.31−7.21 (m, 10H), 4.70 (br, 1H), 4.60 (br, 3H), 4.31−4.27 (m, 1H), 4.22−4.17 (q, *J* = 8.0 Hz, 2H), 3.94−3.89 (m, 3H), 3.67 (d, *J* = 12.0 Hz, 1H), 3.28−3.11 (m, 2H), 2.98−2.81 (m, 2H), 1.38 (s, 9H), 1.30 (t, *J* = 8.0 Hz, 3H); ^{13}C NMR (101 MHz, MeOD) δ = 175.0, 173.9, 171.1, 138.5, 130.4, 130.3, 129.5, 129.4, 127.8, 127.7, 62.3, 57.6, 56.0, 43.5, 42.1, 38.9, 38.7, 28.7, 14.5; HRMS (ESI) Calcd for $C_{29}H_{39}N_4O_7$ ([M + H]$^+$) 555.2813, found 555.2825.

Compound **22**. Following the general procedure C, the reaction performed with stearic acid thiol ester (185 mg, 0.50 mmol) afforded the title compound as a white solid (135 mg, 80% yield): mp 123−124 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 5.41 (br, 1H), 3.30 (q, 2H), 2.19 (t, *J* = 8.0 Hz, 2H), 1.66−1.62 (m, 4H), 1.27 (s, 30H), 0.96−0.88 (m, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ = 173.0, 39.2, 36.9, 31.9, 31.7, 29.7, 29.6, 29.5, 29.4, 29.3, 25.8, 22.7, 20.0, 14.1, 13.7; IR (film) 3315, 2966, 2921, 2853, 1688, 1618, 1571, 1526, 1449, 1381, 1260, 1171, 1039 cm^{-1}; HRMS (ESI) Calcd for $C_{22}H_{46}NO$ ([M + H]$^+$) 340.3574, found 340.3575.

Compound **23**. Following the general procedure C, the title compound was prepared as a white semisolid (188 mg, 82% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.33−7.26 (m, 3H), 7.14 (d, *J* = 8.0 Hz, 2H), 5.91 (d, *J* = 8.0 Hz, 1H), 4.93 (q, 1H), 4.22 (q, 2H), 3.16 (t, *J* = 4.0 Hz, 2H), 2.39 (t, *J* = 8.0 Hz, 0.4H), 2.21 (t, *J* = 8.0 Hz, 1.6H), 1.68−1.59 (m, 2H), 1.28 (s, 31H), 0.92 (t, *J* = 8.0 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ = 172.6, 171.7, 135.9, 129.3, 128.5, 127.0, 61.5, 52.9, 37.9, 36.6, 31.9, 29.7, 29.6. 29.5, 29.4, 29.3, 29.2, 25.6, 22.7, 14.1; HRMS (ESI) Calcd for $C_{29}H_{50}NO_3$ ([M + H]$^+$) 460.3785, found 460.3784.

Compound **25**. Following the general procedure C, cinnamoyl-CoA (10 mg, 1.1 μmol), HMDO (50 μL) and L-Phe-OEt (10.6 mg, 55 μmol) were added to [BMIM]BF_4 (0.3 mL). The reaction mixture was monitored by HPLC analysis (see Supporting Information): ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.76 (d, *J* = 4.0 Hz, 2H), 7.55−7.52 (m, 1H), 7.47−7.43 (m, 2H), 7.33−7.16 (m, 6H), 7.18 (d, *J* = 8.0 Hz, 2H), 6.62 (br,1H), 5.91 (d, *J* = 8.0 Hz, 1H), 5.12 (q, *J* = 8.0 Hz, 1H), 5.91 (q, *J* = 8.0 Hz, 2H), 3.34−3.24 (m, 2H), 1.32 (t, *J* = 8.0 Hz, 3H).^{13}C NMR (101 MHz, $CDCl_3$) δ = 171.6, 135.9, 131.7, 129.4, 128.6, 128.5, 127.1, 127.0, 61.6, 53.5, 37.9, 14.1; HRMS (ESI) Calcd for $C_{20}H_{22}NO_3$ ([M + H]$^+$) 324.1594, found 324.1595.

Compound **27**. Following the general procedure C, the title compound was prepared as a white solid (214.5 mg, 65% yield): mp 256−258 °C.^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.31−7.19 (m, 6H), 7.12 (d, *J* = 4.0 Hz, 2H), 6.01 (d, *J* = 12.0 Hz, 1H), 5.63 (s, 2H), 4.92−4.87 (m, 1H), 4.25−4.14 (m, 3H), 3.88−3.80 (m, 2H), 3.24−3.06 (m, 4H), 2.89−2.77 (m, 1H), 2.31 (s, 1H), 2.12−2.11 (m, 1H), 2.01−1.96 (m, 1H), 1.89−1.80 (m, 2H), 1.71−1.58 (m, 8H), 1.34−1.00 (m, 36H), 0.80 (s, 3H), 0.75 (s, 3H), 0.70 (d, *J* = 12.0 Hz, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ = 129.2, 128.6, 52.6, 47.4, 43.6, 43.1, 39.1, 37.0,

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31.8, 28.4, 28.1, 26.5, 23.5, 23.4, 23.3, 18.6, 15.6, 14.2; IR (film) 3504, 3395, 3340, 2965, 2927, 2870, 1738, 1715, 1654, 1618, 1574, 1520, 1456, 1385, 1326, 1248, 1206, 1034 cm^{-1}; HRMS (ESI) Calcd for $C_{41}H_{60}NO_5$ ($[M]^+$) 646.4466, found 646.4470.

Modification of Lysozyme with Thioesters. Following the general procedure C, thioester (0.10 mmol) was added to [BMIM]-BF_4/$CH_3CO_2NH_4$ buffer solution (20 mM, pH = 7.0, 0.5 mL), followed by addition of HMDO (20 μL) and lysozyme (20 mg). The reaction mixture was completed at 37 °C for 24 h and then cooled to room temperature. The desired product was analyzed by HRMS.

Reaction of Oxo Ester with Amines. The oxo ester and a catalytic amount of thiol (0.1 equiv) were added to [BMIM]PF_6 in a round-bottom flask. The mixture was heated at 37 °C. After the completion, the reaction mixture was cooled to room temperature and concentrated under reduced pressure. The residue was diluted with ethyl acetate (15 mL) and washed with 1 M aqueous HCl (4 mL), 2 M aqueous NaOH (4 mL), and brine (4 mL), successively. The organic layer was collected and dried over anhydrous Na_2SO_4, filtered, and concentrated to dryness. The crude product was purified by flash chromatography to provide the product (25% ethyl acetate in petroleum ether and then 10% MeOH in DCM).

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

NMR spectra for all compounds. HPLC data for compounds **14o**, **14p**, **19**, and **20**. This information 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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■ DEDICATION

Dedicated to the memory of Professor Richard Loeppky. Deceased on April 21, 2012.

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