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Long-Range Intramolecular S → N Acyl Migration: A Study of the Formation of Native Peptide Analogues via 13-, 15-, and 16-Membered Cyclic Transition States

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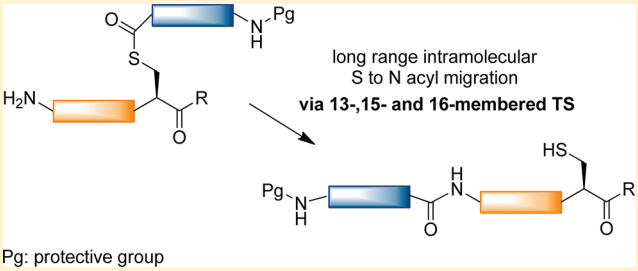
Supporting Information

ABSTRACT: The intramolecular long-range S → N acyl migration via 13-, 15-, and 16-membered cyclic transition states to form native tetra- and pentapeptide analogues was studied on S-acylcysteine peptides containing β- or γ-amino acids. The pH-dependency study of the acyl migration via a 15-membered cyclic transition state indicated that the reaction is favored at a pH range from 7.0 to 7.6. Experimental observations are supported by structural and computational investigations.



INTRODUCTION

Native chemical ligation (NCL), the most common form of chemical ligation, has become a widely used chemoselective technique to synthesize large peptides based on a capture/rearrangement concept involving S-acylation by a peptide-thioester of the N-terminal cysteine residue of another peptide, followed by a S → N acyl shift to form a native amide linkage.[1,2] NCL has been extensively studied in peptidic compounds bearing a cysteine residue at the N-terminus.[3] Modified cysteine scaffolds have also been incorporated for the syntheses of novel peptides and proteins[4−8] and for surface immobilization.[9] For instance, N-acylcysteines[10] have found utility as photoactivatable analogues of glutathione[11] and for the synthesis of oxytocin-like peptides[12] or glycopeptides.[13−15]

Recently, Haase and Seitz reported that the chemical ligation of peptides bearing internal cysteine residues is significantly accelerated in comparison to peptides lacking cysteine. In particular, the highest ligation rates and yields were obtained when the cysteine residue was incorporated at the fifth or sixth position from the N-terminus of the C-terminal coupling segment. However, the method required relatively long reaction times (48−72 h) and the ligation products were neither isolated nor characterized.[16] Sugar-assisted ligation (SAL) has also been introduced as an approach to overcome problems in the synthesis of glycopeptides. The technique uses a glycopeptide which undergoes thioester exchange with a peptide thioester, followed by a long-range S → N acyl migration to afford a native peptide linkage. SAL showed high sequence tolerance at the ligation junction, therefore expanding the number of glycoproteins potentially accessible.[17−19]

Our group has recently studied the feasibility of chemical ligation of S-acylated cysteine peptides via 5-, 8-, 11-, and 14-membered cyclic transition states,[20,21] leading to the corresponding native di-, tetra-, and pentapeptides without the use of auxiliaries. Additionally, this work was extended to peptides containing nonterminal cysteine units, thus affording the synthesis of diverse peptidic sequences.[22] It was demonstrated that chemical ligation of S-acyl peptides via an 8-membered cyclic transition state is disfavored, whereas the 11- and 14-membered cyclic transition states are relatively favored. Computational studies demonstrated that hydrogen bonding provides additional stabilization and thus facilitates ligation to form native peptides. The conformational preorganization was demonstrated to be particularly effective on tetra- and higher peptides.[23]

Unnatural amino acids have been used to prepare peptide analogues with enhanced enzymatic stability,[24,25] improved pharmacodynamics,[26] and bioavailability.[27] β-Amino acid residues, which are often encountered in natural products,[28] have been used for obtaining peptidomimetics[29] and for affecting secondary and

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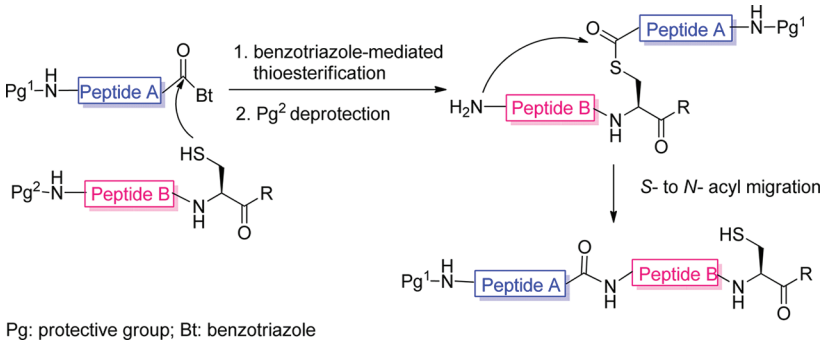
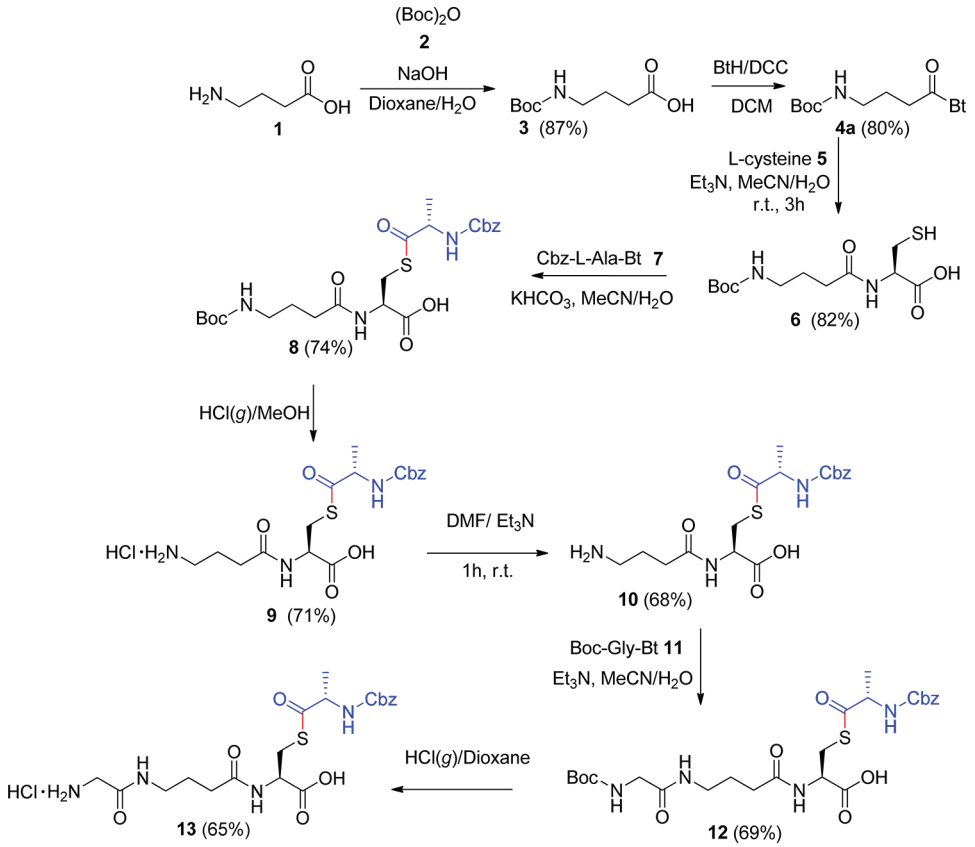


Figure 1. Long-range S → N acyl migration to form native peptide analogues.

Scheme 1. Preparation of S-Acyl Tripeptide 13



tertiary structures of synthetic peptides.[30] Peptides containing γ-amino acids have shown antianxiety, anticonvulsive, and relaxing effects.[31,32]

To investigate the further applicability of our methodology to access peptide analogues,[20−23] we study now long-range S → N acyl migrations of S-acylated cysteine peptides containing β- or γ-amino acid residues via 13-, 15-, and 16-membered cyclic transition states (Figure 1), leading to the formation of the corresponding native tetra- and pentapeptide analogues. The impact of pH on the long-range S → N acyl migration is exemplified using a S-acyl tetrapeptide. Reaction mechanisms and governing parameters are discussed in our computational study.

RESULTS AND DISCUSSION

Demonstration of S → N Acyl Migration in S-Acyl Tripeptide 13 via a 13-Membered Cyclic Transition State. The preparation of starting tripeptide **13** for S → N acyl migration via a 13-membered cyclic transition state is illustrated in Scheme 1. Boc protection of γ-aminobutyric acid **1** was carried out according to a reported procedure[33] (Scheme 1), affording N-Boc-γ-aminobutyric acid **3** in 87% yield. Compound **3** was converted into the corresponding N-(Boc-aminoacyl)benzotriazolide **4a** (80%). Coupling of **4a** with L-cysteine (**5**) was carried out in a CH_3CN/H_2O mixture (8:2) in the presence of Et_3N during 3 h at 20 °C, leading to the N-Boc protected cysteine dipeptide **6** in 82% yield. S-Acylation of dipeptide **6** with Cbz-L-alanyl benzotriazole (**7**) in a acetonitrile−water mixture (10:1) gave S-acyl dipeptide **8** in 74% yield. Boc deprotection of dipeptide **8** using HCl(g) in methanol gave the HCl salt **9**, which was neutralized with triethylamine in DMF to afford S-acyl dipeptide **10**. The final coupling of unprotected S-acyl dipeptide **10** with Boc-Gly-Bt **11**, followed by Boc-deprotection gave S-acyl tripeptide **13** (65%).

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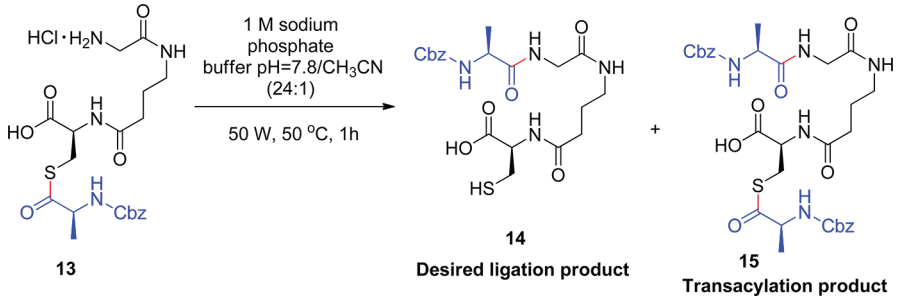
Table 1. Characterization Data of Ligation Product Mixtures

			product ratio[b] (%)		[M + H]$^{+c}$	
entry	starting material	crude[a] yield (%)	ligated peptide	transacylation product	ligated peptide[d]	transacylation product
1	**13**	88	28, **14**	72, **15**	935	674
2	**23a**	92	35, **24**	65, **25**	1183	809
3	**23b**	94	64, **26a**	36, **27a**	1257	835
4	**23c**	82	57, **26b**	43, **27b**	1257	835

[a]The combined crude yield of ligated product was calculated according to the following equation: combined crude yield = ([ligated peptide] + 2 × [transacylation product])/[starting material]. The S-deacylated peptide side products were removed during the workup. [b]Determined by HPLC–MS semiquantitative. The area of ion-peak resulting from the sum of the intensities of the $[M + H]^+$ and $[M + Na]^+$ ions of each compound was integrated. [c]HPLC–MS (ESI). [d]Analyzed as disulfide dimer.

Scheme 2. Chemical Ligation of S-Acyl Tripeptide 13[a]



[a]The ligation compound **14** is drawn as a monomer for clarity.

In our first set of ligation experiments, a solution of tripeptide **13** in a mixture of NaH_2PO_4/Na_2HPO_4 buffer and acetonitrile (pH = 7.8) was subjected to microwave irradiation at 37 °C. HPLC–MS (ESI) analysis of the crude reaction mixture revealed that the reaction does not complete after 3 h (50% conversion of **13**). However, when carried out at 50 W and 50 °C, the ligation reaction proceeded essentially to completion within 1 h. HPLC–MS (ESI) analysis demonstrated that the expected ligation product **14** is formed together with the intermolecular transacylation product **15** in a 28:72 ratio (Table 1, Scheme 2). The desired ligation product **14** was detected as its disulfide dimer, producing a *m/z* 935 $[M + H]^+$ ion which allows unambiguous distinction between the ligation product **14** and the starting tripeptide **13** (molecular ion $[M + H]^+$, *m/z* = 469). It is well-known that small peptides containing a Cys residue easily dimerize to the corresponding disulfide dimers in solution in the absence of reducing agent.[34] This product mixture was subsequently purified by semipreparative HPLC which enabled the isolation and the characterization of the native peptide **14**. Ligated product **14** was then further characterized by analytical HPLC and HRMS analysis (Table 2).

Table 2. Characterization Data of the Isolated Ligation Products 14, 24, 26a,b

	after semipreparative HPLC		HRMS $[M + Na]^{+b}$		
ligation product	purity[a]	isolated yield (%)	calculated	found	retention time[c]
14	>92%	18	957.3093	957.3117	16.95
24	>98%	31	1183.4774	1183.4733	19.42
26a	>99%	42	1279.4774	1279.4718	20.89
26b	>95%	37	1279.4774	1279.4775	18.64

[a]Purity based on analytical HPLC. [b]Analyzed as disulfide dimer. [c]254 nm, MeOH/H_2O (65:35), 0.15 mL/min.

Demonstration of S → N Acyl Migration in S-Acyl Tetrapeptide 23a via a 15-Membered Cyclic Transition State. For the S → N ligation via a 15-membered cyclic transition state, the starting material **23a** was obtained in a six-step procedure starting from the L-cystine dimethyl ester dihydrochloride (**16**). The protected dipeptide dimer **17a** was prepared in 67% yield from the mixed anhydride coupling of **16** with Boc-Gly-OH following a literature procedure (Scheme 3).[35] The deprotection of **17a** using HCl(g) in methanol gave **18a** in 84% yield. The coupling of **18a** with Boc-protected dipeptide Boc-*β*-Ala-L-Leu-OH (**19a**) provided the tetrapeptide dimer **20a** in 65% yield, which was subsequently treated with tributylphosphine to afford the tetrapeptide monomer **21a** in 75% yield. S-Acylation of N-Boc-protected cysteine tetrapeptide **21a** with Cbz-L-Ala-Bt (**7**) in acetonitrile–water (10:1) in the presence of triethylamine gave S-acyl tetrapeptide **22a** in 86% yield. Finally, compound **22a** was Boc-deprotected using HCl(g) in methanol to give the HCl salt **23a** in 82% yield (Scheme 3).

The intramolecular S → N acyl migration experiment **23a** → **24** would proceed through a 15-membered-ring transition state. Although attempted **13** to **14** acyl migration via a 13-membered-ring transition state favored the intermolecular transacylation reaction to give **15**, precedents[30−32] for successful S → N acyl shift in sugar-assisted ligation (SAL) proceeding (Figure 2) through transition states with 15-membered rings encouraged us to pursue this approach.

The chemical ligation experiment was carried out at 2 mM concentration of **23a** under microwave irradiation at 50 °C and 50 W for 1 h at pH = 7.6 (Scheme 4). HPLC–MS (ESI) analysis of the crude reaction mixture revealed the presence of two major products in a 35:65 ratio (Table 1). The HPLC–MS (ESI) analysis identified the major reaction products as the desired ligation product **24** and the intermolecular transacylation product **25** (Table 1). Compound **24** was isolated by semi-preparative HPLC and fully characterized by ^1H and ^{13}C NMR

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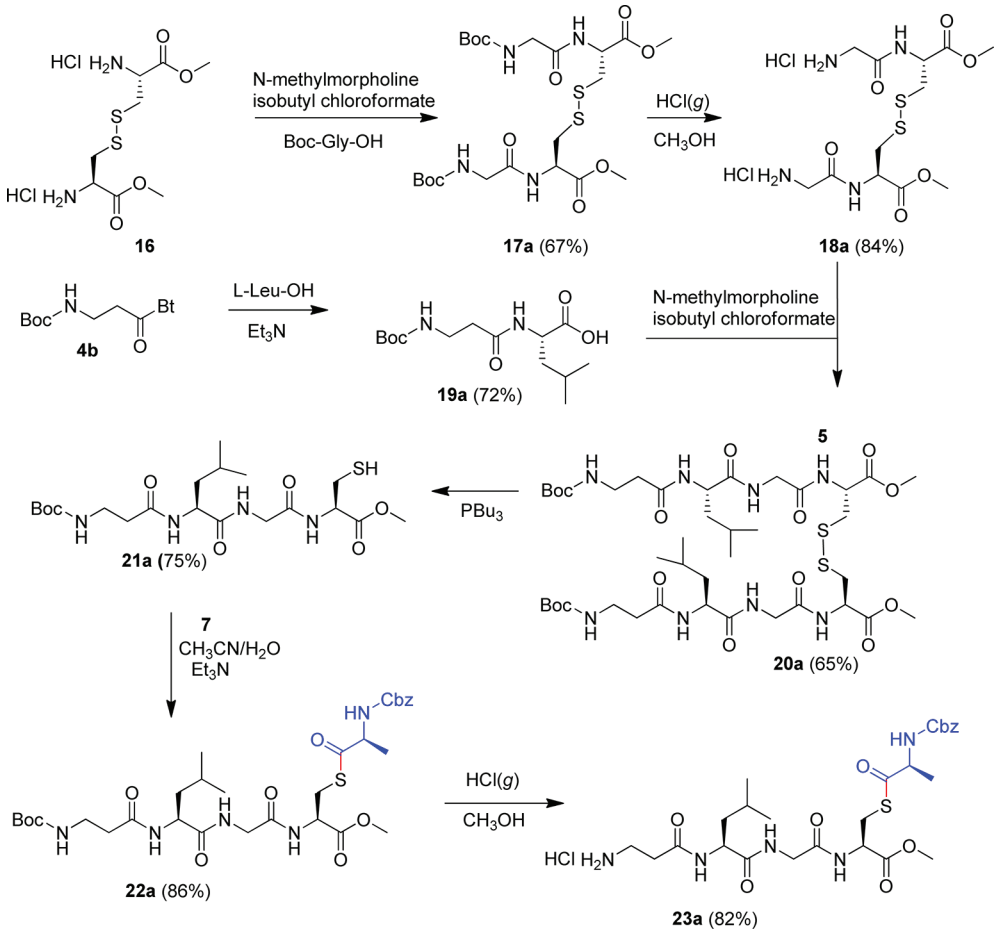
Scheme 3. Preparation of S-Acyl Tetrapeptide 23a



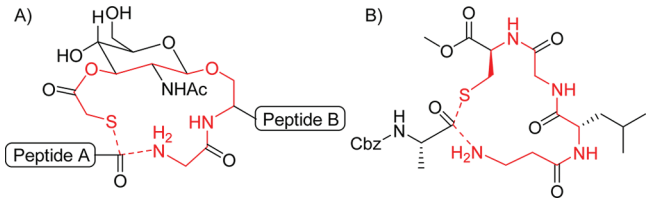
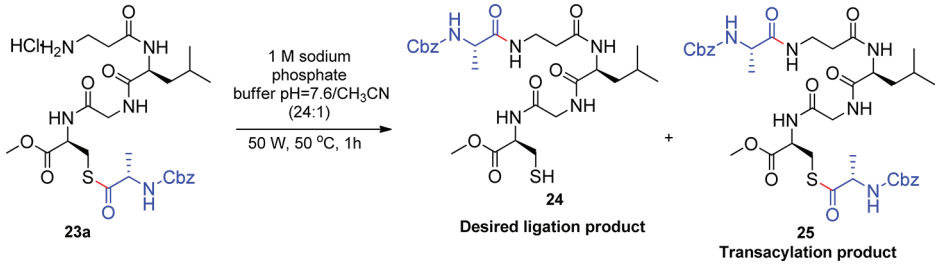


Figure 2. Proposed 15-membered cyclic transition states of (A) the second-generation sugar-assisted ligation[30] (SAL) and (B) the long-range intramolecular acyl-migration.

spectroscopy, HRMS (ESI), and analytical HPLC (Table 2). ^{1}H NMR spectra indicated the formation of the desired ligation product **24** (appearance of five amide proton signals), while ^{13}C NMR provided further evidence. Indeed, S-aminoacyltetrapeptide **23a** has four typical amide ^{13}C signals with chemical shifts ranging from δ 171.5 to 175.4 ppm and a very typical thioester carbonyl ^{13}C observed at δ 203.2 ppm. In contrast, compound **24** showed distinct ^{13}C NMR resonances for five amide bonds at δ 169.1, 170.7, 170.8, 172.4, and 172.5 ppm (Figure 3). These results clearly confirmed the S → N migration of Cbz-alanine to the N-terminus (Scheme 4).

An additional set of two reactions using different concentrations in substrate **23a** were carried out under the same conditions. Interestingly, HPLC–MS (ESI) analysis revealed that the concentration of the substrate does not have a significant influence on the ligation/transacylation ratio. At a 2 mM concentration of **23a**, the measured ligation/transacylation ratio was 45:55, while at 0.5 mM and 6 mM the ratios were 43:57 and 55:45, respectively.

Scheme 4. Chemical Ligation of S-Acyl Tetrapeptide 23a[a]



[a]The ligation compound **24** is drawn as a monomer for clarity.

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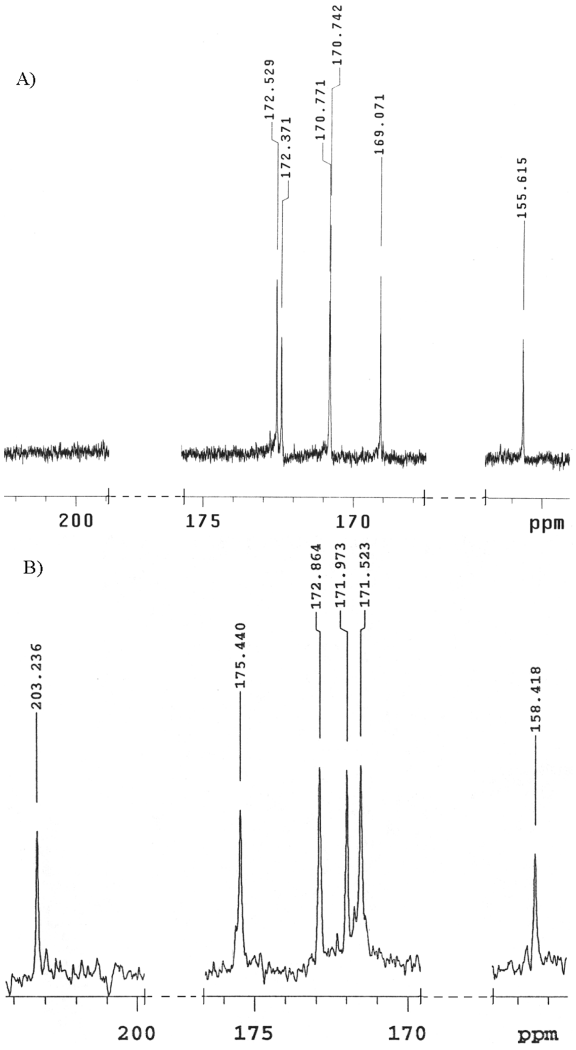

Figure 3. ^{13}C NMR carbonyl signals in (A) ligated pentapeptide **24** and in (B) starting S-acylated tetrapeptide **23a**.

Demonstration of S → N Acyl Migrations in S-Acyl Tetrapeptides 23b and 23c Each via Distinct Isomeric 16-Membered Cyclic Transition States. To study the chemical ligation via a 16-membered cyclic transition state, we first prepared the tetrapeptide dimer **20b** in 52% yield by coupling amino-unprotected cystine dipeptide dimer **18b** with Boc-β-Ala-L-Phe-OH (**19b**). Then, tetrapeptide dimer **20b** was treated with tributylphosphine to release the tetrapeptide monomer **21b** in 68% yield. S-Acylation of N-Boc-protected cysteine tetrapeptide **21b** with Cbz-L-Ala-Bt (**7**) in a acetonitrile–water mixture (10:1) in the presence of triethylamine afforded S-acyl tetrapeptide **22b** in 88% yield. Boc deprotection of **22b** using HCl(g) in methanol gave the hydrochloride **23b** in 81% yield (Scheme 5).

Similarly, **18b** was coupled with Boc-GABA-L-Phe-OH (**19c**) to afford Boc-protected tetrapeptide dimer **20c** in 57% yield. Cleavage of the disulfide bond in **20c** furnished the monomer **21c** (66%), which was S-acylated with Cbz-L-Ala-Bt (**7**) to provide the Boc-protected S-acyl tetrapeptide **22c** (92%). Final Boc deprotection afforded the desired amino-unprotected S-acylated tetrapeptide **23c** in 87% yield (Scheme 5).

We then investigated S → N acyl migrations for compounds **23b** and **23c** each of which proceeds via distinct isomeric 16-membered cyclic transition states. Chemical ligation on **23b** was carried out under microwave irradiation at 50 °C and 50 W for 1 h in NaH_2PO_4/Na_2HPO_4 buffer (1 M, pH 7.6) and acetonitrile (8:1 mixture). HPLC–MS (ESI) analysis of the crude ligation mixture revealed the major component (64%) to be the expected ligation product **26a** (molecular ion of the disulfide dimer $[M + H]^+$ m/z 1257 vs 630 for the $[M + H]^+$ molecular ion of the starting S-(Pg-α-aminoacyl)tetrapeptide **23b**). The HPLC–MS (ESI) confirmed that the second major product is **27a** formed by intermolecular trans-acylation (Table 1, Scheme 6). Separation of **26a** by semipreparative HPLC allowed isolation of 42% of pure pentapeptide **26a**, which was further characterized by analytical HPLC and HRMS analysis (Table 2).

Similarly, the acyl migration experiment of S-acylpeptide **23c** was carried out under the conditions described above. HPLC–MS (ESI) analysis showed the presence of two main products in a 57:43 ratio (Table 1, Scheme 7). As expected, the major product of this experiment was the desired ligation product **26b**. The subsequent separation of **26b** by semipreparative HPLC provided the purified ligation product **26b** in 37% yield. The product was characterized by HPLC analytical and HRMS (Table 2).

Study of pH Dependence on the Ligation Experiment. Chemical ligation is pH sensitive: ligation usually proceeds rapidly around pH 7 but is rendered less efficient at pH < 5.5.[32,36] In addition, in some cases the yields for traceless Staudinger ligation in water increased at higher pH but decreased drastically at pH ≥ 8.5.[37] The ratio of the ligation product **24** versus the intermolecular transacylated compound **25** was studied under different pH conditions (Table 3, Figure 4). Chemical ligation experiments on **23a** were carried out under microwave irradiation at 50 °C and 50 W for 1 h in 1 M phosphate buffer under pH values ranging from 6.2 to 8.2. After workup, the crude ligation mixtures were subjected to HPLC–MS (ESI) analysis. The results are summarized in Table 3 and Figure 4. HPLC–MS (ESI) analysis of the crude ligation mixture at pH = 6.2 showed the presence of the major intermolecular transacylation product **25** and the desired ligation product **24** in a 80:20 ratio. When the ligation experiment was conducted at pH = 8.2, HPLC–MS (ESI) analysis identified the same two products: the transacylated product **25** and the desired ligated product **24** in a similar 85:15 ratio. In contrast, when ligation was carried out at pH 7.0 and at pH 7.6, HPLC–MS (ESI) showed a significant increase in ligation product **24** with calculated **24**/**25** ratios of 45:55 and 36:64, respectively. In the case of a lower pH, the starting material **23a** exists partially in the unreactive protonated form and the acyl migration reaction is relatively slow, which favors the intermolecular S-acylation of **24** by the excess of starting material **23a** to form the undesired product **25**. At pH > 8, the thiol group of the ligation product **24** is partially deprotonated, which again favors the formation of the transacylation product **25** in a subsequent intermolecular reaction of **24** with unreacted **23a**. At pH = 7.3, a **24**/**25** ratio of 43:57 was obtained. The S → N acyl migration in S-acyl tetrapeptide **23a** via a 15-membered cyclic transition state to give **24** is therefore favored at a pH range from 7.0 to 7.6.

Computational Investigation. The elementary step of the chemical ligation was investigated by computational means for the S-acylated tripeptide **13** and for tetrapeptides **23a** and **23c** (Cbz group was modeled by a methyl carbamate and the Phe residue in tetrapeptide **23c** was replaced by an Ala residue). Tetrapeptide **23c** was considered as a representative model for

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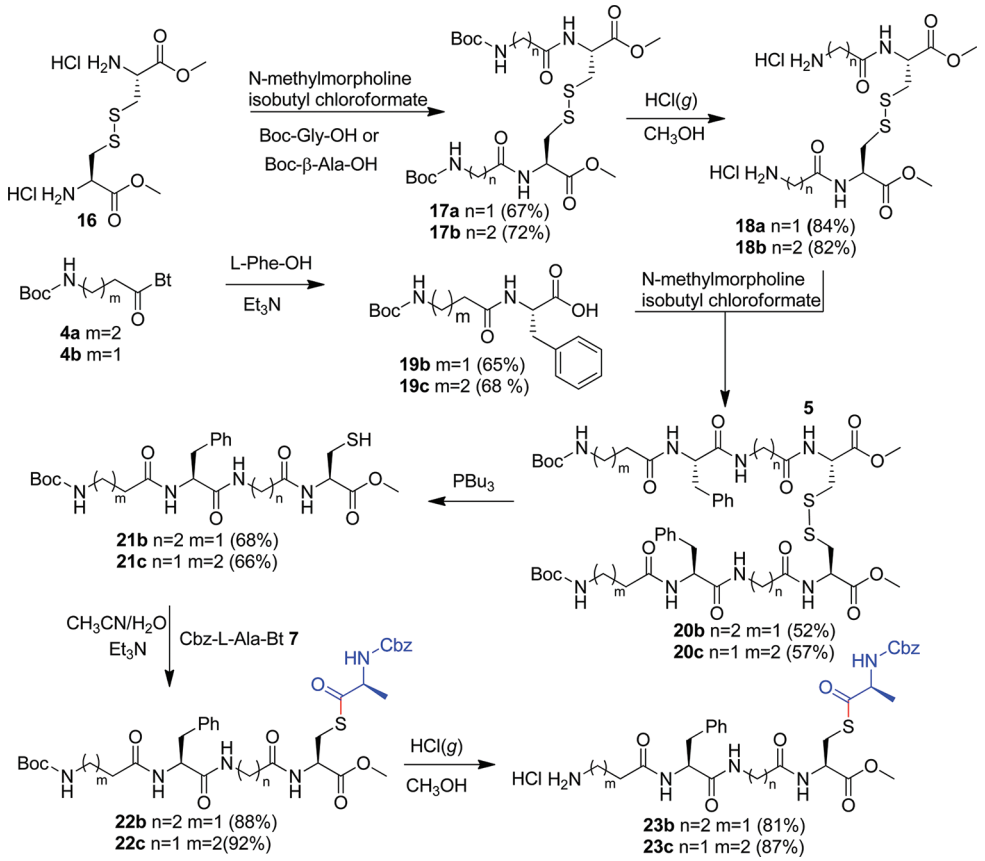
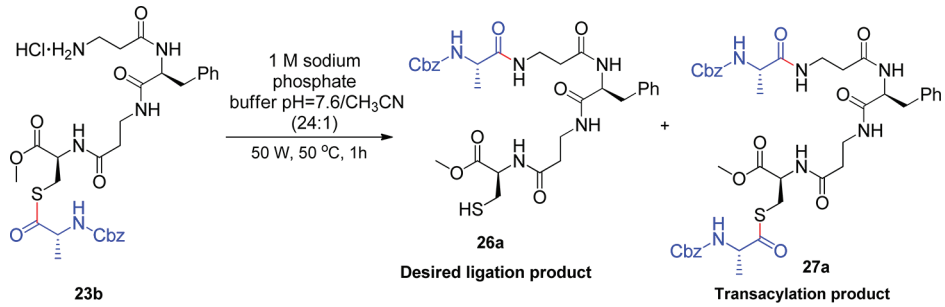
Scheme 5. Preparation of S-Acylated Tetrapeptides 23b and 23c

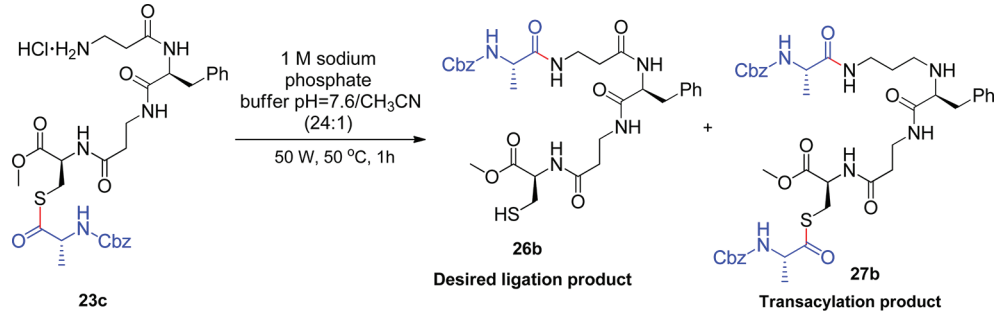


Scheme 6. Acyl Migration of S-Acyl Tetrapeptides 23b[a]



[a]The ligation product compound **26a** is drawn as its monomer for clarity.

Scheme 7. Acyl Migration of S-Acyl Tetrapeptide 23c[a]



[a]The ligation product compound **26b** is drawn as a monomer for clarity.

the 16-membered TS series. A full conformational search was performed using the MMX force field by the PC Model program package. Full geometry optimization and verification of the Hessian have been performed by the G03 program package

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Table 3. Dependence of pH on the Product Ratio 24/25 in the Ligation Reaction of 23a

		product ratio[a]	
entry	pH	ligated peptide[b] (**24**)	transacylation product (**25**)
1	6.2	20	80
2	7.0	45	55
3	7.3	43	57
4	7.6	36	64
5	8.2	15	85

[a]Determined by HPLC–MS semiquantitation. The area of the ion-peak resulting from the sum of the intensities of the $[M + H]^+$ and $[M + Na]^+$ ions of each compound was integrated. [b]Analyzed as disulfide dimer.

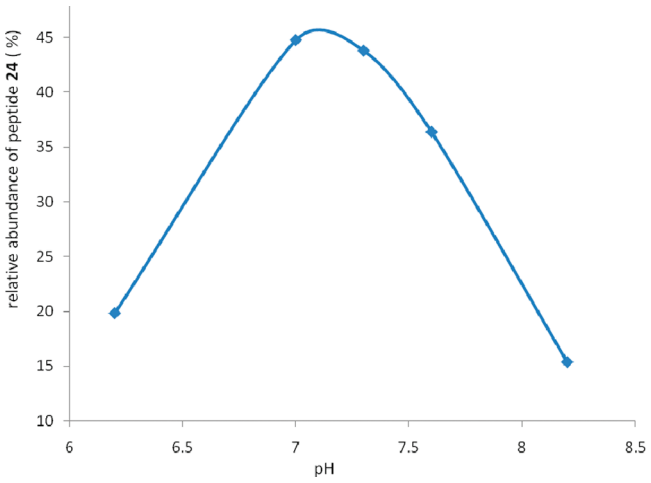

Figure 4. Effect of pH on S → N acyl migration in S-acyl tetrapeptide **23a**.

(revision E.01).[38] Transition states were optimized and localized at the HF/6-31G* level with zero point energy correction and were verified by frequency and IRC calculations. All reactants were optimized in the gas phase at the HF/6-31G* level. For the 13-membered cyclic case, TSs were also isolated at the HF/6-31+G* and B3LYP/6-31G*. Neither the inclusion of diffuse functions nor electronic correlation fundamentally altered the nature of the TS. Single point calculations were carried out for some representative examples using Tomasi's Polarizable Continuum (PCM) with dielectric constants of 78.4 (water phase), 36.6 (acetonitrile), and 7.6 (tetrahydrofuran) to provide an indication of the sensitivity of the reaction toward solvent polarity.

A full conformational analysis was performed on tripeptide **13** plus tetrapeptides **23a** and **23c** using the MMX force field. In complete agreement with our previous study,[23] the most stable conformers of compounds **13**, **23a**, and **23b** are folded into a cyclic ready-to-ligate structure. The transition states (TSs) for the long-range intramolecular S → N acyl migration via 13-, 15-, and 16-membered cyclic transition states were located and studied. As a common feature (Table 4 and Figure 5), these TSs are late, in the Hammond postulate sense, i.e., product-like with a net transfer of the alanine residue from the S-cysteine terminus (average bond length in rupture, 2.42 Å) to the N-terminus (average bond length in formation, 1.56 Å). The average advancement of the 13-, 15-, and 16-membered TSs is about 90% among the reaction coordinate. These

Table 4. Bond Formation and Rupture (Å) among 13-, 15-, and 16-Membered TSs

	TS		reactants	products
ring size	N···C(=O)	(O=C)···S	(O=C)–S	N–C(=O)
13	1.56	2.42	1.79	1.35
15	1.56	2.49	1.78	1.35
16	1.56	2.35	1.79	1.35 h

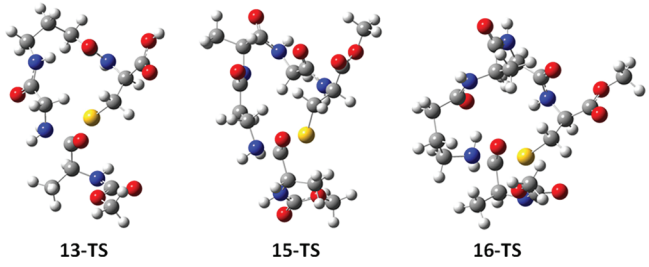

Figure 5. Pictures of the 13-, 15-, and 16-membered TSs associated with the long-range intramolecular S → N acyl transfer for the model peptides **13**, **23a**, and **23c**.

reactions are exothermic, the enthalpy of reaction ranging from 53.9 to 67.6 kJ mol^{-1}.

The computed activation barriers for the 13-, 15-, and 16-membered TSs associated with the long-range intramolecular S → N acyl transfer for the model peptides **13**, **23a**, and **23c** are shown in Table 5. Values obtained using Tomasi's PCM model

Table 5. Computed Activation Barriers for 13-, 15-, and 16-TSs (kJ mol^{-1})

	$\Delta E^{\ddagger}$ (gas)
13-TS	165.0
15-TS	224.8
16-TS	186.0

are indicative of the reaction sensitivity to solvent polarity: the activation barriers obtained for the tripeptide **13** leveled off at 122.7, 137.5, and 136.5 kJ mol^{-1} in water, acetonitrile, and tetrahydrofuran, respectively.

For the long-range intramolecular S → N acyl transfer of model peptide **13**, we were also able to isolate a TS including two molecules of water involved in an "active" H-bonding network (Figure 6): the first one stabilizes the sulfur anion in formation while the second one seems to interact with the N–H terminus in order to facilitate the proton transfer and hence acylation of the N terminus (see the Supporting Information for details).

The activation barriers presented in Table 5 show some trends. The lowest activation barrier was computed for the chemical ligation of tripeptide **13** (13-membered cyclic TS), even though the observed yield of the corresponding ligation product was the lowest of the series. This result is probably linked to the formation of a tight zwitterionic pair between the N-terminus and the free carboxylic acid on the cysteyl residue (tripeptide **13** is the only of the series to bear a free carboxylic acid) that competes with the intramolecular acylation and hence gives the desired ligation production in low yield (Table 1). Notably, the TSs associated with the chemical ligation of tripeptide **13** (13-TS) and tetrapeptide **23c** (16-TS) display stabilizing hydrogen bonding (short contacts ranging from 1.87

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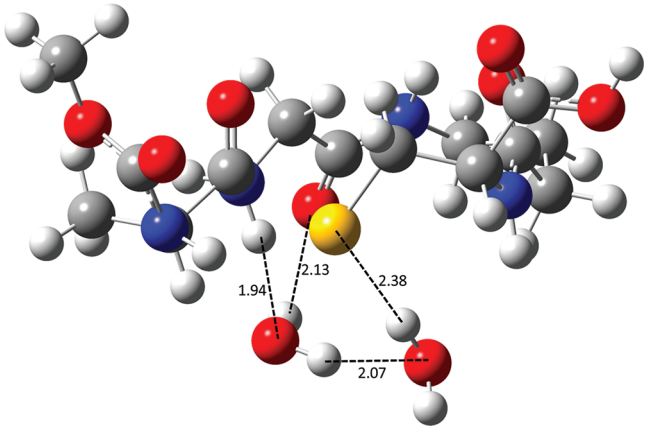

Figure 6. Pictures of the 13-membered TSs for the long-range intramolecular S → N acyl transfer catalyzed by water for the model peptide **13**. Distances are indicated in Å.

to 2.20 Å observed between the CO and NH of the GABA residue), while the TS associated to tetrapeptide **23a** (15-TS) does not include this feature. The absence of an electrostatic stabilizing interaction in the latter is consistent with the higher computed activation barrier (Table 5) and, hence, the lower yield observed experimentally (Table 1). In the 16-TS, the above-mentioned short-contact not only folds the structure-bringing the reactive moiety to a closer proximity but also increases the nucleophilicity (increased negative NBO charge) of the N-terminus, in agreement with the superior ligation yield experimentally observed for this series (Table 1). These results are in good agreement with the experimental observations and strongly suggest that the intramolecular long-range acyl migration of **23c** is favored compared to chemical ligation for compounds **13** and **23a**.

CONCLUSIONS

In this paper, we successfully apply our methodology to synthesize peptide analogues and also provide mechanistic evidence for the ligation process. A series of novel S-acyl peptides containing β- and/or γ-amino acid residues, which are useful intermediates in various synthetic and biological applications, were synthesized according to original protocols. The ligation step was investigated under MW heating and was found to be sensitive to pH. The observed variation in ligation yield allows for reactivity scaling. The native peptides obtained after chemical ligation of tripeptide **13** and tetrapeptides **23a**–**c** via 13-, 15-, and 16-membered cyclic transition states were isolated in modest to good yields. Interestingly, the ligation products **26a** and **26b** derived through isomeric 16-membered cyclic TSs were obtained in similar yields. In a representative example, the native peptide was isolated and fully characterized. The experimental results show that the extent of chemical ligation follows the following scale: 13-TS ≤ 15-TS ≪ 16-TS. Our computational study revealed that the chemical ligation associated with 13-TS has the lowest activation barrier; but in this case the formation of a tight zwitterionic pair may hamper the long-range intramolecular S → N acyl transfer thus decreasing the yield. The chemical ligation proceeding through 16-TS appeared to be highly favored in comparison with the 15-TS. The importance of stabilization by intramolecular short contacts has been illustrated.

EXPERIMENTAL SECTION

General Methods. Melting points were determined on a capillary point apparatus equipped with a digital thermometer. ^{1}H NMR and ^{13}C NMR spectra were recorded in $CDCl_3$, DMSO-d_6, acetone-d_6, or CD_3OD-d_4 using a 300 or 500 MHz spectrometer (with TMS as an internal standard). The following abbreviations are used to describe spin multiplicity: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, brs = broad singlet, dd = doublet of doublets, ddd = doublet of doublets of doublets. All microwave assisted reactions were carried out with a single mode cavity Discover Microwave Synthesizer (CEM Corporation, NC). The reaction mixtures were transferred into a 10 mL glass pressure microwave tube equipped with a magnetic stirrer bar. The tube was closed with a silicon septum and the reaction mixture was subjected to microwave irradiation (Discover mode; run time: 60 s.; PowerMax-cooling mode). HPLC–MS analyses were performed on a reverse phase gradient using 0.2% acetic acid in H_2O/methanol as mobile phases; wavelength = 254 nm; and mass spectrometry was done with electrospray ionization (ESI). Ether refers to diethyl ether.

Boc-GABA-OH (3). Di-*tert*-butyl dicarbonate (4.37 g, 20 mmol) was added to a stirred solution of 4-aminobutyric acid (20 mmol, 2.06 g) in 1,4-dioxane (60 mL), NaOH (1 M, 60 mL), and H_2O (60 mL) at 0 °C. The reaction mixture was stirred for 1 h at room temperature and was concentrated under reduced pressure. The residual solution was acidified with a 10% aqueous citric acid to pH 2–3 in an ice bath. This aqueous phase was extracted with EtOAc (4 × 60 mL), and the organic layers were combined and washed with H_2O (2 × 50 mL) and saturated brine (20 mL). The organic solution was dried over anhydrous Na_2SO_4, filtered from drying agent, and concentrated under reduced pressure. Recrystallization from EtOAc gave Boc-GABA-OH. White microcrystals, 3.54 g, 87% yield; mp 54–56 °C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.44 (s, 9H), 1.82 (quint, J = 7.1 Hz, 2H), 2.39 (t, J = 7.2 Hz, 2H), 3.10–3.25 (m, 2H), 4.77 (br s, 1H), 10.60 (br s, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 25.3, 28.6, 31.5, 40.0, 79.7, 156.4, 178.5. Anal. Calcd for $C_9H_{17}NO_4$: C 53.19, H 8.43, N 6.89. Found: C 53.26, H 8.73, N 6.77.

Boc-GABA-Bt (4a). A solution of *N*-Boc-GABA-OH (3.23 g, 15.9 mmol) in CH_2Cl_2 (30 mL) was added to a solution of dicyclohexylcarbodiimide (3.27 g, 15.9 mmol) and *1H*-benzotriazole (1.89 g, 15.9 mmol) in CH_2Cl_2 (10 mL). The reaction mixture was stirred at room temperature for 1.5 h. The precipitate was filtered, and the solution was passed through 6.00 g of Celite. The solvent was evaporated under reduced pressure, and the crude mixture obtained was dissolved in EtOAc (50 mL). The organic layer was washed with saturated Na_2CO_3 (3 × 30 mL), brine (20 mL) and dried over $MgSO_4$. Recrystallization from CH_2Cl_2–hexanes gave Boc-GABA-Bt. White microcrystals, 4.68 g, 97% yield; mp 108–110 °C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.41 (br s, 9H), 2.12 (p, J = 7.0 Hz, 2H), 3.29–3.36 (m, 2H), 3.49 (t, J = 7.2 Hz, 2H), 4.72 (br s, 1H), 7.52 (ddd, J = 8.2, 7.1, 1.1 Hz, 1H), 7.67 (ddd, J = 8.2, 7.2, 1.0 Hz, 1H), 8.13 (ddd, J = 8.2, 0.8, 0.8 Hz, 1H), 8.29 (ddd, J = 8.6, 1.1, 0.8 Hz, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 25.0, 28.5, 32.9, 39.9, 79.5, 114.6, 120.3, 126.3, 130.6, 131.3, 146.3, 156.2, 172.2. Anal. Calcd for $C_{15}H_{20}N_4O_3$: C 59.20, H 6.62, N 18.41. Found: C 59.34, H 6.71, N 18.39.

Boc-GABA-L-Cys-OH (6). Boc-GABA-Bt (0.50 g, 1.6 mmol) was suspended in acetonitrile (20 mL), and a solution of L-cysteine (0.20 g, 1.6 mmol) in water containing triethylamine (0.19 mL, 1.5 mmol) was added. The mixture was stirred at 20 °C until the TLC revealed complete consumption of the starting materials. The solvent was removed under reduced pressure, and the residue was taken in ethyl actetate. Then the solution was washed with 3 N HCl (3 × 15 mL) and brine (10 mL). Recrystallization from ethyl acetate–hexanes yielded Boc-GABA-L-Cys-OH. Sticky white solid, 0.41 g, 84% yield. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.37 (br s, 9H), 1.54–1.62 (m, 2H), 1.99 (s, 1H), 2.10 (t, J = 7.4 Hz, 2H), 2.85–2.94 (m, 3H), 3.12 (dd, J = 13.7, 4.7 Hz, 1H), 4.43–4.50 (m, 1H), 6.80 (br s, 1H), 8.25 (d, J = 8.2 Hz, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 26.9, 28.5, 32.7, 40.0, 53.8, 80.0, 156.9, 172.9, 173.8. Anal. Calcd for $C_{12}H_{22}N_2O_5S$: C 47.04, H 7.24, N 9.14. Found: C 47.04, H 6.97, N 9.38.

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Boc-GABA-Cys(*S*-L-Cbz-Ala)-OH (8). To a solution of Boc-GABA-L-Cys-OH (1.0 g, 3.26 mmol) in acetonitrile (15 mL), $KHCO_3$ (0.32 g, 6.5 mmol) in water was added dropwise at room temperature. After 15 min L-Cbz-Ala-Bt (1.05 g, 3.26 mmol) was added slowly to the reaction mixture. The solution was further stirred at room temperature for 5 h. Acetonitrile was removed under reduced pressure, and the residue formed was taken in ethyl acetate (30 mL) and washed with 3 N HCl (3 × 15 mL) and brine (10 mL). Then, the solution was concentrated under reduced pressure, and hexane was added until the solution was turbid and the compound was left to crystallize in the freezer. The solid obtained was filtered and dried to give Boc-GABA-Cys(*S*-L-Cbz-Ala)-OH. White microcrystal, 1.23 g, 74% yield; mp 70−72 °C. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.25 (d, J = 7.0 Hz, 3H), 1.37 (s, 9H), 1.52−1.62 (m, 2H), 2.08 (t, J = 7.2 Hz, 2H), 2.84−2.92 (m, 2H), 3.02 (dd, J = 13.4, 8.6 Hz, 1H), 3.27 (dd, J = 13.6, 5.0 Hz, 1H), 4.15−4.24 (m, 1H), 4.26−4.36 (m, 1H), 4.90−5.10 (m, 2H), 6.80 (br s, 1H), 7.22−7.46 (m, 5H), 8.07 (d, J = 7.3 Hz, 1H), 8.20 (d, J = 8.0 Hz, 1H). ^{13}C NMR (DMSO-d_6, 75 MHz): δ 17.3, 25.8, 28.3, 29.6, 32.6, 51.3, 56.6, 65.7, 77.4, 127.7, 128.4, 136.7, 155.7, 155.8, 171.7, 171.9, 201.7. Anal. Calcd for $C_{23}H_{33}N_3O_8S$: C 54.00, H 6.50, N 8.21. Found: C 53.63, H 6.59, N 8.45.

GABA-L-Cys(*S*-L-Cbz-Ala)-OH Hydrochloride (9). HCl(g) was passed through a solution of Boc-GABA-Cys(*S*-L-Cbz-Ala)-OH (0.5 g, 0.97 mmol) in methanol (8 mL) for 30 min at room temperature. The solvent was then evaporated under reduced pressure, and the residue was recrystallized from methanol−diethyl ether to yield the product GABA-L-Cys(*S*-L-Cbz-Ala)-OH hydrochloride. Sticky white solid, 0.41 g, 89% yield. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.25 (d, J = 7.3 Hz, 3H), 1.72−1.84 (m, 2H), 2.17−2.26 (m, 2H), 2.71−2.82 (m, 2H), 3.04 (dd, J = 13.5, 8.5 Hz, 1H), 3.28 (dd, J = 13.7, 5.2 Hz, 1H), 4.15−4.25 (m, 1H), 4.28−4.37 (m, 1H), 5.06 (s, 2H), 7.26−7.40 (m, 5H), 7.99 (br s, 3H), 8.10 (d, J = 7.4 Hz, 1H), 8.4 (d, J = 8.0 Hz, 1H). ^{13}C NMR (DMSO-d_6, 75 MHz): δ 17.3, 23.1, 29.5, 31.8, 38.3, 51.5, 56.7, 65.7, 127.7, 127.9, 128.4, 136.8, 155.8, 171.4, 171.6, 201.8. Anal. Calcd for $C_{18}H_{26}ClN_3O_6S{\cdot}1.5H_2O$: C 45.52, H 6.15, N 8.85. Found: C 45.19, H 6.59, N 8.95.

GABA-L-Cys(*S*-L-Cbz-Ala)-OH (10). Triethylamine (0.24 g, 2.4 mmol) was added dropwise to a solution of GABA-L-Cys(*S*-L-Cbz-Ala)-OH hydrochloride (0.5 g, 1.2 mmol) in DMF (7 mL), and the reaction mixture was stirred at room temperature for 30 min. A white precipitate was filtered, washed with diethyl ether, and dried under vacuum to give pure GABA-L-Cys(*S*-L-Cbz-Ala)-OH. White microcrystal, 0.38 g, 1.6 mmol, 77% yield; mp 110−114 °C. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.25 (d, J = 7.3 Hz, 3H), 1.76−1.84 (m, 2H), 2.18−2.24 (m, 2H), 2.74−2.86 (m, 2H), 3.00 (dd, J = 12.8, 7.8 Hz, 1H), 3.33 (dd, J = 13.0, 4.7 Hz, 1H), 3.23−3.44 (m, 2H), 4.08−4.12 (m, 1H), 4.12−4.20 (m, 1H), 5.01−5.09 (m, 2H), 7.30−7.40 (m, 5H), 7.90 (d, J = 7.3 Hz, 1H), 7.99 (d, J = 7.6 Hz, 1H). ^{13}C NMR (DMSO-d_6, 75 MHz): δ 17.5, 23.4, 31.2, 32.4, 45.1, 52.7, 56.6, 65.7, 127.7, 127.8, 128.4, 136.8, 155.7, 170.9, 172.4, 202.1. Anal. Calcd for $C_{18}H_{25}N_3O_6S{\cdot}0.5H_2O$: C 51.42, H 6.23, N 9.99. Found: C 51.04, H 6.18, N 9.65.

Boc-Gly-GABA-L-Cys(*S*-L-Cbz-Ala)-OH (12). Triethylamine (0.02 g, 0.29 mmol) was added dropwise to a solution of GABA-L-Cys(*S*-L-Cbz-Ala)-OH (0.10 g, 0.24 mmol) in acetonitrile−water (10:2) at room temperature. After 10 min, Boc-Gly-Bt (0.06 g, 0.24 mmol) was added slowly to the reaction mixture. The solution was further stirred at room temperature for 4 h. Acetonitrile was removed under reduced pressure, and the residue was taken in ethyl acetate (30 mL) and washed with 3 N HCl (2 × 15 mL) and brine (10 mL). Then ethyl acetate was removed under reduced pressure, and the compound was recrystallized from ethyl acetate−hexanes. The solid obtained was filtered and dried to give Boc-Gly-GABA-Cys(*S*-L-Cbz-Ala)-OH. White solid, 0.09 g, 66% yield; mp 110−112 °C. ^{1}H NMR (CD_3OD, 300 MHz): δ 1.35 (d, J = 7.2 Hz, 3H), 1.45 (s, 9H), 1.74−1.84 (m, 2H), 2.24 (t, J = 7.3 Hz, 2H), 3.12−3.26 (m, 3H), 3.50 (dd, J = 13.9, 4.8 Hz, 1H), 3.67 (s, 2H), 4.25−4.33 (m, 1H), 4.56−4.65 (m, 1H), 5.12 (s, 2H), 7.26−7.40 (m, 5H), 7.44−7.52 (m, 2H), 7.80−7.98 (m, 2H). ^{13}C NMR (CD_3OD, 75 MHz): δ 18.0, 26.6, 28.8, 31.0, 34.1, 39.9, 44.8, 53.2, 58.4, 67.9, 80.9, 128.9, 129.1, 129.6, 138.2, 158.5, 172.7, 173.3, 175.5, 203.3. Anal. Calcd for $C_{25}H_{36}N_4O_2S$: C 52.80, H 6.38, N 9.85. Found: C 53.12, H 6.65, N 10.20.

Gly-GABA-L-Cys(*S*-L-Cbz-Ala)-OH Hydrochloride (13). Boc-Gly-GABA-Cys(*S*-L-Cbz-Ala)-OH (0.3 g, 0.52 mmol) was dissolved in dioxane/HCl(g) solution (8 mL), and the reaction mixture was stirred at room temperature. After 1 h, the solvent was removed under reduced pressure, and the residue was crystallized from methanol−diethyl ether to yield the title compound, Gly-GABA-Cys(*S*-L-Cbz-Ala)-OH hydrochloride. White solid, 0.17 g, 65% yield; mp 80−83 °C. ^{1}H NMR (CD_3OD, 300 MHz): δ 1.35 (d, J = 6.7 Hz, 3H), 1.80−1.88 (m, 2H), 2.23−2.32 (m, 2H), 3.19 (dd, J = 13.8, 7.6 Hz, 1H), 3.28 (br s, 2H), 3.50 (dd, J = 13.7, 3.5 Hz, 1H), 3.71 (br s, 2H), 4.28−4.30 (m, 1H), 4.60−4.64 (m, 1H), 5.09 (br s, 2H), 7.28−7.34 (m, 5H), 7.53−7.54 (m, 3H), 7.90−7.92 (m, 3H). ^{13}C NMR (CD_3OD, 75 MHz): δ 18.4, 26.8, 31.2, 34.4, 40.4, 42.1, 53.6, 58.8, 68.3, 128.1, 129.1, 130.0, 138.4, 158.8, 167.7, 173.6, 175.8, 203.8. Anal. Calcd for $C_{20}H_{29}ClN_4O_7S{\cdot}0.5H_2O$: C 46.74, H 5.88, N 10.90. Found: C 46.68, H 5.96, N 10.85.

Boc-β-Ala-Bt (4b). A solution of *N*-Boc-β-Ala-OH (3.00 g, 15.9 mmol) in CH_2Cl_2 (30 mL) was added to a solution of dicyclohexylcarbodiimide (3.27 g, 15.9 mmol) and *1H*-benzotriazole (1.89 g, 15.9 mmol) in CH_2Cl_2 (10 mL). The reaction mixture was stirred at room temperature for 1.5 h. The precipitate was filtered, and the solution was passed through 6.00 g of Celite. The solution was evaporated under reduced pressure, and the crude mixture obtained was dissolved in EtOAc (50 mL). The organic layer was washed with saturated Na_2CO_3 (3 × 30 mL) and brine (20 mL) and dried over $MgSO_4$. Concentration under reduced pressure produced the final product. Recrystallization from CH_2Cl_2−hexanes gave Boc-β-Ala-Bt. White microcrystals, 3.46 g, 63% yield; mp 112−115 °C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.43 (s, 9H), 3.66−3.70 (m, 4H), 5.11 (br s, 1H), 7.53 (t, J = 7.6 Hz, 1H), 7.68 (t, J = 7.6 Hz, 1H), 8.14 (d, J = 8.2 Hz, 1H), 8.28 (d, J = 8.2 Hz, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 28.5, 35.8, 36.6, 79.8, 114.4, 120.4, 126.4, 130.7, 131.1, 146.3, 155.9, 171.5. Anal. Calcd for $C_{14}H_{18}N_4O_3$: C 57.92, H 6.25, N 19.30. Found: C 58.00, H 6.33, N 19.31.

Boc-β-Ala-L-Leu-OH (19a). A solution of L-leucine (0.81 g, 6.20 mmol) and triethylamine (0.63 g, 6.20 mmol) in acetonitrile (10 mL) and water (5 mL) was added to the suspension of Boc-β-Ala-Bt (1.50 g, 5.17 mmol) in acetonitrile (30 mL). The solution was stirred for 15 h, and then the solvent was evaporated under reduced pressure. The crude product was dissolved in ethyl acetate. The organic phase was washed with 2 N HCl (3 × 10 mL) and brine (10 mL). The solution was dried over $MgSO_4$, filtered, and then concentrated under vacuum. The solid was recrystallized from ethyl acetate−hexanes to yield Boc-β-Ala-L-Leu-OH. White microcrystals, 1.13 g, 72% yield; mp 124−127 °C. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 0.84 (d, J = 6.4 Hz, 3H), 0.88 (d, J = 6.4 Hz, 3H), 1.37 (s, 9H), 1.45−1.53 (m, 2H), 1.54−1.68 (m, 1H), 2.21−2.32 (m, 2H), 3.10 (dd, J = 13.3, 7.2 Hz, 2H), 4.15−4.23 (m, 1H), 6.68 (t, J = 5.2 Hz, 1H), 8.11 (d, J = 7.9, Hz 1H), 12.50 (br s, 1H). ^{13}C NMR (DMSO-d_6, 75 MHz): δ 21.3, 22.9, 24.3, 28.2, 35.5, 36.7, 39.9, 50.1, 77.6, 155.4, 170.4, 174.2. Anal. Calcd for $C_{14}H_{26}N_2O_5$: C 55.61, H 8.67, N 9.26. Found: C 55.98, H 8.91, N 9.22.

(Boc-Gly-L-Cys-OCH$_3$)$_2$ (17a). A solution of Boc-Gly-OH (1.13 g, 6.4 mmol) in dry THF (10 mL) under argon was cooled to −15 °C in an ice bath with stirring. *N*-methylmorpholine (0.65 g, 6.4 mmol), followed by isobutyl chloroformate (0.84 g, 6.4 mmol) were added. After 5 min, a solution of L-cystine dimethyl ester dihydrochloride (1.00 g, 2.9 mmol) and *N*-methylmorpholine (0.65 g, 6.4 mmol) in DMF (5 mL) were added. The ice bath was removed after 5 min, and the solution was allowed to stir for 24 h at room temperature. The solution was concentrated under vacuum; the residue was taken up in ethyl acetate (20 mL) and 2 N HCl (5 mL). The organic phase was washed successively with saturated Na_2CO_3 (3 × 10 mL) and 2 N HCl (3 × 10 mL). The solution was dried over dry $MgSO_4$, filtered, and then concentrated under vacuum. The peptide was recrystallized from diethyl ether−hexanes to give (Boc-Gly-L-Cys-OCH_3)$_2$. White microcrystals, 1.13 g, 66% yield; mp 51−54 °C. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.38 (br s, 18H), 2.96 (dd, J = 13.9, 8.3 Hz, 2H), 3.12 (dd, J = 13.4, 4.8 Hz, 2H), 3.58 (d, J = 5.8 Hz, 4H), 3.65 (s, 6H), 4.55−4.65 (m, 2H),

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6.94 (t, J = 5.8 Hz, 2H), 8.35 (d, J = 7.9 Hz, 2H). ^{13}C NMR (DMSO-d_6, 75 MHz): δ 28.2, 39.1, 42.9, 51.2, 52.2, 78.1, 155.7, 169.6, 170.8. Anal. Calcd for $C_{22}H_{38}N_4O_{10}S_2$: C 45.35, H 6.57, N 9.62. Found: C 45.64, H 6.76, N 9.45.

(Gly-L-Cys-OCH$_3$)$_2$ Hydrochloride (18a). HCl gas was passed through a solution of (Boc-Gly-L-Cys-OCH$_3$)$_2$ (0.58 g, 1.0 mmol) in methanol (15 mL) for 30 min. The methanol solution was concentrated under vacuum, and ether was added. The turbid solution was left to crystallize in the freezer overnight. The solid formed was filtered and washed with dry diethyl ether (10 mL) and dried to give the corresponding (Gly-L-Cys-OCH$_3$)$_2$ hydrochloride. White microcrystals, 0.38 g, 83% yield; mp 185−189 °C. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 2.98 (dd, J = 13.9, 8.5 Hz, 2H), 3.15 (dd, J = 13.7, 5.0 Hz, 2H), 3.60 (s, 4H), 3.66 (s, 6H), 4.57−4.68 (m, 2H), 8.36 (s, 6H), 9.31 (d, J = 7.4 Hz, 2H). ^{13}C NMR (DMSO-d_6, 75 MHz): δ 39.9, 51.5, 52.4, 166.3, 170.4. Anal. Calcd for $C_{12}H_{24}Cl_2N_4O_6S_2{\cdot}H_2O$: C 30.45, H 5.54, N 11.84. Found: C 30.33, H 5.53, N 11.98.

(Boc-β-Ala-L-Leu-Gly-L-Cys-OCH$_3$)$_2$ (20a). A solution of Boc-β-Ala-L-Leu-OH (0.91 g, 3 mmol) in dry THF (10 mL) under argon was cooled to −15 °C in an ice bath with stirring. *N*-Methylmorpholine (0.33 g, 3.2 mmol), followed by isobutyl chloroformate (0.45 g, 3.2 mmol) were added. After 4 min, a solution of (Gly-L-Cys-OCH$_3$)$_2$ hydrochloride (0.68 g, 1.5 mmol) and *N*-methylmorpholine (0.7 g, 1.6 mmol) in DMF (5 mL) was added. The ice bath was removed after 5 min, and the solution was allowed to stir for 12 h at room temperature. The solution was concentrated under vacuum; the residue was taken up in ethyl acetate (30 mL) and water (5 mL). The organic phase was washed successively with saturated Na_2CO_3 (2 × 15 mL), water (10 mL), 2 N HCl (15 mL), and water (10 mL). The solution was dried over $MgSO_4$, filtered, and then concentrated under vacuum. The peptide was recrystallized from ethyl acetate−hexanes to give (Boc-β-Ala-L-Leu-Gly-L-Cys-OCH$_3$)$_2$. White microcrystals, 0.93 g, 65% yield; mp 105−111 °C. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 0.83 (d, J = 6.4 Hz, 6H), 0.88 (d, J = 6.6 Hz, 6H), 1.36 (s, 18H), 1.44 (t, J = 7.1, 4H), 1.53−1.63 (m, 2H), 2.25−2.31 (m, 4H), 2.95 (dd, J = 13.8, 8.5 Hz, 2H), 3.07−3.16 (m, 6H), 3.65 (s, 6H), 3.70−3.75 (m, 4H), 4.19−4.26 (m, 2H), 4.58 (dd, J = 13.3, 8.2 Hz, 2H), 6.70 (t, J = 5.5 Hz, 2H), 8.07 (d, J = 7.3 Hz, 2H), 8.22 (t, J = 5.6 Hz, 2H), 8.30 (d, J = 7.9 Hz, 2H). ^{13}C NMR (acetone-d_6, 75 MHz): δ 19.4, 22.2, 23.4, 25.4, 28.7, 36.9, 37.8, 40.6, 41.3, 43.5, 52.8, 53.4, 78.8, 156.7, 170.2, 171.5, 173.2, 174.1. Anal. Calcd for $C_{40}H_{70}N_8O_{14}S_2$: C 50.51, H 7.42, N 11.78. Found: C 50.35, H 7.56, N 11.39.

Boc-β-Ala-L-Leu-Gly-L-Cys-OCH$_3$ (21a). (Boc-β-Ala-L-Leu-Gly-L-Cys-OCH$_3$)$_2$ (1.36 g, 1.43 mmol) was treated with P(t-Bu)$_3$ (0.58 g, 0.71 mL, 2.86 mmol) in 20 mL of 9:1 MeOH:water for 2 h at rt under argon gas. The reaction mixture was concentrated under vacuum and the residue was taken up in ethyl acetate (15 mL). The solution was dried over $MgSO_4$, and then concentrated under vacuum. The peptide was recrystallized from ethyl acetate:hexane to give Boc-β-Ala-L-Leu-Gly-L-Cys-OCH$_3$. White microcrystals, 1.02 g, 75% yield; mp 140−142 °C. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 0.83 (d, J = 6.4 Hz, 3H), 0.88 (d, J = 6.4 Hz, 3H), 1.25 (br s, 1H), 1.37 (s, 9H), 1.44 (t, J = 7.3 Hz, 2H), 1.53−1.65 (m, 1H), 2.23−2.34 (m, 2H), 2.74−2.90 (m, 2H), 3.11 (dd, J = 12.9, 7.0 Hz, 2H), 3.65 (s, 3H), 3.73 (d, J = 4.6 Hz, 2H), 4.16−4.24 (m, 1H), 4.46−4.53 (m, 1H), 6.72 (t, J = 6.1 Hz, 1H), 8.08−8.13 (m, 2H), 8.32 (t, J = 6.1 Hz, 1H). ^{13}C NMR (acetone-d_6, 75 MHz): δ 22.2, 23.4, 25.4, 26.7, 28.7, 36.9, 37.8, 41.1, 43.5, 52.6, 53.6, 55.5, 78.8, 156.7, 169.9, 171.3, 173.2, 173.8. Anal. Calcd for $C_{20}H_{36}N_4O_7S$: C 50.40, H 7.61, N 11.76. Found: C 50.45, H 7.85, N 11.38.

Boc-β-Ala-L-Leu-Gly-L-Cys(*S*-L-Cbz-Ala)-OCH$_3$ (22a). (Boc-β-Ala-L-Leu-Gly-L-Cys-OCH$_3$ (500 mg, 1.25 mmol) was suspended in acetonitrile (15 mL), and a solution of Cbz-L-Ala-Bt **7** (596 mg, 1.25 mmol) in acetonitrile−water (10 mL of 4:1) containing an equivalent amount of triethylamine (127 mg, 1.26 mmol) was added. The mixture was stirred at 25 °C for 3 h until completion. Acetonitrile was removed under reduced pressure, and the residue was taken in ethyl acetate (30 mL) and extracted with 2 N HCl (2 × 20 mL), water (15 mL), and brine (10 mL). Ethyl acetate was concentrated under reduced pressure, and hexane was added; the turbid solution was left to crystallize overnight at −20 °C. The solid obtained was filtered, washed with diethyl ether (3 mL) and CH_2Cl_2 (3 mL), and dried to give the corresponding Boc-β-Ala-L-Leu-Gly-L-Cys(*S*-L-Cbz-Ala)-OCH$_3$. White microcrystals, 739 mg, 87% yield; mp 100−105 °C. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 0.83 (d, J = 6.4 Hz, 3H), 0.83 (d, J = 6.4 Hz, 3H), 1.25 (d, J = 7.3 Hz, 3H), 1.36 (s, 9H), 1.44 (t, J = 7.2 Hz, 2H), 1.52−1.64 (m, 1H), 2.28 (t, J = 6.5 Hz, 2H), 3.06−3.15 (m, 3H), 3.24 (dd, J = 14.1, 6.4 Hz, 1H), 3.62 (s, 3H), 3.70 (t, J = 6.1 Hz, 2H), 4.14−4.26 (m, 2H), 4.36−4.43 (m, 1H), 5.06 (s, 2H), 6.70 (t, J = 5.0 Hz, 1H), 7.30−7.42 (m, 5H), 8.07 (t, J = 6.8 Hz, 1H), 8.18 (t, J = 5.6 Hz, 1H), 8.33 (d, J = 7.6 Hz, 1H). ^{13}C NMR (acetone-d_6, 75 MHz): δ 18.0, 22.2, 23.4, 25.4, 28.7, 37.8, 41.2, 43.2, 52.7, 53.3, 57.9, 67.1, 78.8, 128.7, 129.3, 138.0, 156.7, 156.9, 169.9, 171.2, 173.0, 173.4, 202.0. Anal. Calcd for $C_{31}H_{47}N_5O_{10}S$: C 54.61, H 6.95, N 10.27. Found: C 54.58, H 6.80, N 10.08.

β-Ala-L-Leu-Gly-L-Cys(*S*-L-Cbz-Ala)-OCH$_3$ Hydrochloride (23a). HCl gas was passed through a solution of Boc-β-Ala-L-Leu-Gly-L-Cys(*S*-L-Cbz-Ala)-OCH$_3$ (600 mg, 0.88 mmol) dissolved in methanol for 1.5 h at rt. The solvent was then evaporated under reduced pressure, and the solid was recrystallized from methanol−ether to yield β-Ala-L-Leu-Gly-L-Cys(*S*-L-Cbz-Ala)-OCH$_3$ hydrochloride. White microcrystals, 0.45 g, 82% yield; mp 174−185 °C. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 0.86 (dd, J = 12.9, 6.4 Hz, 6H), 1.25 (d, J = 7.2 Hz, 3H), 1.42−1.50 (m, 2H), 1.54−1.64 (m, 1H), 2.92−3.00 (m, 2H), 3.09 (dd, J = 13.4, 7.4 Hz, 1H), 3.23 (dd, J = 14.6, 6.7 Hz, 1H), 3.62 (br s, 3H), 3.65 (br s, 2H), 3.70−3.76 (m, 2H), 4.14−4.22 (m, 1H), 4.29 (dd, J = 15.0, 7.5 Hz, 1H), 4.32 (q, J = 6.8 Hz, 1H), 5.06 (s, 2H), 7.30−7.42 (m, 5H), 7.90 (br s, 3H), 8.10 (d, J = 7.6 Hz, 1H), 8.26 (t, J = 5.4 Hz, 1H), 8.33−8.37 (m, 1H), 8.40 (d, J = 7.3 Hz, 1H). ^{13}C NMR (CD_3OD, 75 MHz): δ 18.0, 22.2, 23.5, 26.0, 30.7, 32.8, 37.2, 41.4, 41.9, 43.3, 53.3, 54.0, 58.4, 67.9, 128.8, 129.2, 129.6, 138.2, 158.4, 171.5, 172.0, 172.9, 175.4, 203.2. Anal. Calcd for $C_{26}H_{40}ClN_5O_8S{\cdot}3H_2O$: C 46.46, H 6.90, N 10.42. Found: C 46.53, H 6.72, N 10.54.

Boc-β-Ala-L-Phe-OH (19b). The compound was prepared according to the method for preparation of Boc-β-Ala-L-Leu-OH (**19a**). White microcrystals, 1.02 g, 65% yield; mp 154 −157 °C. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.37 (br s, 9H), 2.19−2.25 (m, 2H), 2.84 (dd, J = 13.7, 9.5, Hz, 1H), 3.00−3.11 (m, 3H), 4.37−4.44 (m, 1H), 6.63 (br s, 1H), 7.17−7.30 (m, 5H), 8.23 (d, J = 8.0 Hz, 1H). ^{13}C NMR (DMSO-d_6, 75 MHz): δ 28.3, 35.5, 36.6, 36.7, 53.4, 77.6, 126.4, 128.1, 129.1, 137.7, 155.4, 170.3, 173.0. Anal. Calcd for $C_{17}H_{24}N_2O_5$: C 60.70, H 7.19, N 8.33. Found: C 60.29, H 7.22, N 8.19.

(Boc-β-Ala-L-Cys-OCH$_3$)$_2$ (17b). The compound was prepared according to the method for preparation of (Boc-Gly-L-Cys-OCH$_3$)$_2$ (**17a**). White microcrystals, 1.27 g, 72% yield; mp 117−119 °C. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.37 (s, 18H), 2.29 (t, J = 7.3 Hz, 4H), 2.92 (dd, J = 13.8, 8.8 Hz 2H), 3.06−3.13 (m, 6H), 3.64 (s, 6H), 4.53 (dd, J = 13.2, 8.1 Hz, 2H), 6.72 (br s, 2H), 8.46 (d, J = 7.4 Hz, 2H). ^{13}C NMR (DMSO-d_6, 75 MHz): δ 28.2, 35.4, 36.5, 51.1, 52.2, 77.6, 155.4, 170.6, 170.9. Anal. Calcd for $C_{24}H_{42}N_4O_{10}S_2$: C 47.20, H 6.93, N 9.17. Found: C 47.33, H 7.12, N 9.01.

(β-Ala-L-Cys-OCH$_3$)$_2$ hydrochloride (18b). The compound was prepared according to the method for preparation of (Gly-L-Cys-OCH$_3$)$_2$ hydrochloride (**18a**). White microcrystals, 0.20 g, 82% yield; mp 87−92 °C. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 2.58 (t, J = 7.4 Hz, 4H), 2.92−3.00 (m, 6H), 3.12 (dd, J = 14.1, 4.9 Hz, 2H), 3.66 (br s, 6H), 4.53−4.60 (m, 2H), 8.04 (br s, 6H), 8.81 (d, J = 7.7 Hz, 2H). ^{13}C NMR (DMSO-d_6, 75 MHz): δ 32.6, 32.7, 51.9, 52.9, 170.3, 171.4. Anal. Calcd for $C_{14}H_{28}Cl_2N_4O_6S_2{\cdot}0.75H_2O$: C 33.84, H 5.98, N 11.27. Found: C 34.06, H 6.01, N 10.88.

(Boc-β-Ala-L-Phe-β-Ala-L-Cys-OCH$_3$)$_2$ (20b). The compound was prepared according to the method for preparation of (Boc-β-Ala-L-Leu-Gly-L-Cys-OCH$_3$)$_2$ (**20a**). White microcrystals, 0.45 g, 52% yield; mp 187−197 °C. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.35 (s, 18H), 2.13−2.23 (m, 4H), 2.28 (t, J = 7.5 Hz, 4H), 2.73 (dd, J = 13.6, 9.6 Hz, 2H), 2.91−3.03 (m, 8H), 3.10 (dd, J = 13.7, 5.3 Hz, 2H), 3.16−3.27 (m, 4H), 3.63 (s, 6H), 4.38−4.45 (m, 2H), 4.52−4.59 (m, 2H), 6.61 (t, J = 5.3 Hz, 2H), 7.12−7.27 (m, 10H), 8.09 (bs, 2H), 8.15 (d, J = 8.5 Hz, 2H), 8.57 (d, J = 7.6 Hz, 2H). ^{13}C NMR (DMSO-d_6,

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75 MHz): δ 28.2, 34.8, 35.2, 35.5, 36.6, 37.8, 51.2, 52.2, 54.0, 77.6, 126.2, 127.9, 129.1, 138.0, 155.3, 170.1, 170.6, 170.9, 171.1. Anal. Calcd for $C_{48}H_{70}N_8O_{14}S_2$: C 55.05, H 6.74, N 10.70. Found: C 55.00, H 7.15, N 10.72.

Boc-β-Ala-L-Phe-β-Ala-L-Cys-OCH$_3$ (21b). The compound was prepared according to the method for preparation of Boc-β-Ala-L-Leu-Gly-L-Cys-OCH$_3$ (**21a**). White microcrystals, 0.21 g, 68% yield; mp 137−142 °C. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 0.84−0.94 (m, 1H), 1.36(br s, 9H), 2.10−2.25 (m, 2H), 2.31 (t, J = 7.2 Hz, 2H), 2.68−2.86 (m, 3H), 2.93−3.04 (m, 3H), 3.15−3.30 (m, 2H), 3.64 (s, 3H), 4.38−4.49 (m, 2H), 6.62 (bs, 1H), 7.17−7.27 (m, 5H), 8.04 (bs, 1H), 8.12 (d, J = 8.6 Hz, 1H), 8.43 (d, J = 7.6 Hz, 1H). ^{13}C NMR (CD$_3$OD, 75 MHz): δ 26.7, 28.9, 36.3, 37.1, 37.2, 38.0, 39.1, 53.2, 56.3, 56.4, 80.3, 127.9, 129.6, 130.4, 138.7, 158.3, 172.3, 173.7, 173.9. Anal. Calcd for $C_{24}H_{36}N_4O_7S$: C 54.95, H 6.92, N 10.68. Found: C 55.02, H 7.01, N 11.00.

Boc-β-Ala-L-Phe-β-Ala-L-Cys(*S*-L-Cbz-Ala)-OCH$_3$ (22b). The compound was prepared according to the method for preparation of Boc-β-Ala-L-Leu-Gly-L-Cys(*S*-L-Cbz-Ala)-OCH$_3$ (**22a**). White microcrystals, 0.25 g, 81% yield; mp 158−162 °C. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.24 (d, J = 7.2 Hz, 3H), 1.35 (br s, 9H), 2.12−2.30 (m, 4H), 2.71 (dd, J = 13.6, 9.2 Hz, 1H), 2.91−3.10 (m, 4H), 3.16−3.26 (m, 3H), 3.61 (s, 3H), 4.14−4.22 (m, 1H), 4.34−4.48 (m, 2H), 5.05 (s, 2H), 6.61 (br s, 1H), 7.16−7.30 (m, 5H), 7.30−7.42 (m, 5H), 7.98−8.02 (m, 1H), 8.07−8.12 (m, 2H), 8.47 (d, J = 7.6 Hz, 1H). ^{13}C NMR (CD$_3$OD, 75 MHz): δ 18.0, 28.9, 30.7, 36.2, 37.1, 37.3, 38.0, 39.1, 53.3, 53.4, 56.4, 58.4, 67.9, 80.3, 127.9, 128.9, 129.2, 129.6, 130.4, 138.2, 138.7, 158.4, 172.2, 173.8, 203.3. Anal. Calcd for $C_{35}H_{47}N_5O_{10}S$: C 57.60, H 6.49, N 9.60. Found: C 57.42, H 6.70, N 9.44.

β-Ala-L-Phe-β-Ala-L-Cys(*S*-L-Cbz-Ala)-OCH$_3$ Hydrochloride (23b). The compound was prepared according to the method for preparation of β-Ala-L-Leu-Gly-L-Cys(*S*-L-Cbz-Ala)-OCH$_3$ hydrochloride. White microcrystals, 0.16 g, 81% yield; mp 158−162 °C. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.24 (d, J = 7.2 Hz, 3H), 2.24−2.30 (m, 2H), 2.40 (dd, J = 15.4,7.7 Hz, 2H), 2.74 (dd, J = 13.5, 9.6 Hz, 1H), 2.82−2.88 (m, 2H), 2.97 (dd, J = 13.7, 4.7 Hz, 1H), 3.08 (dd, J = 13.6, 8.0 Hz, 1H), 3.18−3.29 (m, 4H), 3.62 (br s, 3H), 4.13−4.23 (m, 1H), 4.34−4.48 (m, 2H), 5.06 (s, 2H), 7.16−7.28 (m, 5H), 7.28−7.37 (m, 5H), 7.91 (br s, 3H), 8.10 (d, J = 7.3 Hz, 1H), 8.17 (t, J = 5.4 Hz, 1H), 8.43 (d, J = 8.5 Hz, 1H), 8.53 (d, J = 7.7 Hz, 1H). ^{13}C NMR (CD$_3$OD, 75 MHz): δ 17.9, 30.6, 32.8, 36.1, 36.9, 37.1, 38.9, 53.2, 56.5, 58.4, 67.8, 127.9, 128.8, 129.1, 129.5, 130.3, 138.1, 138.5, 158.4, 172.1, 173.8, 203.5. Anal. Calcd for $C_{30}H_{40}ClN_5O_8S\cdot H_2O$: C 52.66, H 6.19, N 10.24. Found: C 52.64, H 6.62, N 10.13.

Boc-GABA-L-Phe-OH (19c). The compound was prepared according to the method for preparation of Boc-β-Ala-L-Leu-OH (**19a**). White microcrystals, 1.82 g, 68% yield; mp 78−81 °C. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.37 (br s, 9H), 1.49 (p, J = 7.2 Hz, 2H), 1.98−2.05 (m, 2H), 2.79−2.87 (m, 3H), 3.04 (dd, J = 13.9, 5.0 Hz, 1H), 4.36−4.44 (m, 1H), 6.76 (t, J = 5.5 Hz, 1H), 7.16−7.30 (m, 5H), 8.15 (d, J = 8.2 Hz, 1H). ^{13}C NMR (DMSO-d_6, 75 MHz): δ 26.0, 28.4, 32.7, 36.9, 53.5, 77.7, 126.5, 128.3, 129.2, 137.8, 155.7, 172.1, 173.3. Anal. Calcd for $C_{18}H_{26}N_2O_5$: C 61.70, H 7.48, N 7.99. Found: C 61.32, H 7.55, N 7.59.

(Boc-GABA-L-Phe-Gly-L-Cys-OCH$_3$)$_2$ (20c). The compound was prepared according to the method for preparation of (Boc-β-Ala-L-Leu-Gly-L-Cys-OCH$_3$)$_2$ (**20a**). White microcrystals, 0.33 g, 52% yield; mp 110−115 °C. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.37 (br s, 18H), 1.41−1.52 (m, 4H), 2.02 (t, J = 7.2 Hz, 4H), 2.70−2.82 (m, 6H), 2.93−3.06 (m, 4H), 3.15 (dd, J = 13.7, 4.3 Hz, 2H), 3.65 (br s, 6H), 3.72 (dd, J = 16.5, 5.0 Hz, 2H), 3.82 (dd, J = 17.0, 5.8 Hz, 2H), 4.43−4.54 (m, 2H), 4.55−4.64 (m, 2H), 6.74 (br s, 2H), 7.14−7.32 (m, 10H), 8.12 (d, J = 8.2 Hz, 2H), 8.33 (br s, 2H), 8.40 (d, J = 7.7 Hz, 2H). ^{13}C NMR (DMSO-d_6, 75 MHz): δ 25.7, 28.3, 32.6, 37.4, 41.6, 51.3, 52.3, 54.1, 77.4, 126.2, 128.0, 129.1, 138.0, 155.5, 169.0, 170.7, 171.7, 172.0. Anal. Calcd for $C_{48}H_{70}N_8O_{14}S_2$: C 55.05, H 6.74, N 10.70. Found: C 55.12, H 6.46, N 10.56.

Boc-GABA-L-Phe-Gly-L-Cys-OCH$_3$ (21c). The compound was prepared according to the method for preparation of Boc-β-Ala-L-Leu-Gly-L-Cys-OCH$_3$ (**21a**). White microcrystals, 0.27 g, 66% yield; mp 84−90 °C. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 0.88 (dd, J = 13.5, 7.1 Hz, 1H), 1.37 (br s, 9H), 1.42−1.51 (m, 2H), 2.02 (t, J = 7.4 Hz, 2H), 2.71−2.88 (m, 5H), 3.02 (dd, J = 13.5, 4.2 Hz, 1H), 3.65 (br s, 3H), 3.69−3.83 (m, 2H), 4.46−4.53 (m, 2H), 6.75 (br s, 1H), 7.15−7.26 (m, 5H), 8.15 (d, J = 8.0 Hz, 1H), 8.19 (d, J = 7.9 Hz, 1H), 8.38 (d, J = 5.4 Hz, 1H). ^{13}C NMR (DMSO-d_6, 75 MHz): δ 25.4, 25.7, 28.3, 32.6, 37.3, 41.8, 51.2, 54.2, 54.5, 77.4, 126.2, 128.0, 129.1, 138.0, 155.6, 16.9, 170.5, 171.9, 172.2. Anal. Calcd for $C_{24}H_{36}N_4O_7S$: C 54.95, H 6.92, N 10.68. Found: C 54.84, H 6.59, N 10.67.

Boc-GABA-L-Phe-Gly-L-Cys(*S*-L-Cbz-Ala)-OCH$_3$ (22c). The compound was prepared according to the method for preparation of Boc-β-Ala-L-Leu-Gly-L-Cys(*S*-L-Cbz-Ala)-OCH$_3$ (**22a**). White microcrystals, 0.27 g, 87% yield; mp 91−96 °C. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.26 (d, J = 7.2 Hz, 3H), 1.37 (br s, 9H), 1.42−1.50 (m, 2H), 2.01 (t, J = 7.4 Hz, 2H), 2.70−2.82 (m, 3H), 3.02 (dd, J = 14.0, 3.8 Hz, 1H), 3.10 (dd, J = 13.7, 7.7 Hz, 1H), 3.25 (dd, J = 12.9, 5.0 Hz, 1H), 3.63 (br s, 3H), 3.65−3.82 (m, 2H), 4.15−4.25 (m, 1H), 4.38−4.54 (m, 2H), 5.06 (br s, 2H), 6.74 (br s, 1H), 7.14−7.40 (m, 10H), 8.08−8.11 (m, 2H), 8.29 (t, J = 5.4 Hz, 1H), 8.40 (d, J = 7.5 Hz, 1H). ^{13}C NMR (acetone-d_6, 75 MHz): δ 18.1, 26.9, 28.8, 33.4, 37.9, 40.3, 52.8, 56.3, 57.9, 67.1, 78.7, 127.3, 128.7, 129.2, 129.3, 130.1, 138.0, 138.8, 156.9, 157.2, 169.9, 171.3, 172.6, 174.0, 202.1. Anal. Calcd for $C_{35}H_{47}N_5O_{10}S$: C 57.60, H 6.49, N 9.60. Found: C 57.49, H 6.66, N 9.46.

GABA-L-Phe-Gly-L-Cys(*S*-L-Cbz-Ala)-OCH$_3$ Hydrochloride (23c). The compound was prepared according to the method for preparation of β-Ala-L-Leu-Gly-L-Cys(*S*-L-Cbz-Ala)-OCH$_3$ hydrochloride (**23a**). White microcrystals, 0.17 g, 87% yield; mp 125−131 °C. ^{1}H NMR (CD$_3$OD, 300 MHz): δ 1.36 (d, J = 7.2 Hz, 3H), 1.79−1.88 (m, 2H), 2.24−2.41 (m, 2H), 2.76−2.85 (m, 2H), 2.91 (dd, J = 13.7, 9.4 Hz, 1H), 3.16−3.27 (m, 2H), 3.43 (dd, J = 13.8, 5.3 Hz, 1H), 3.67−3.74 (m, 5H), 4.29 (q, J = 7.3 Hz, 1H), 4.56−4.72 (m, 2H), 5.06−5.16 (m, 2H), 7.18−7.36 (m, 10H). ^{13}C NMR (CD$_3$OD, 75 MHz): δ 18.0, 24.3, 30.7, 33.5, 38.6, 40.3, 43.3, 53.4, 56.7, 58.4, 68.0, 128.0, 128.8, 129.2, 129.6, 130.4, 138.2, 138.6, 158.5, 171.4, 171.9, 17.3, 174.8, 203.3. Anal. Calcd for $C_{30}H_{40}ClN_5O_8S\cdot 3H_2O$: C 50.03, H 6.44, N 9.72. Found: C 50.06, H 6.24, N 9.55.

General Procedure for Long-Range Acyl-Migration of N-Terminus-Unprotected *S*-(Pg-α-aminoacyl)-tripeptide 13 and *S*-(Pg-α-aminoacyl)tetrapeptide 23a,b,c to Form Native Peptides Analogues 14, 24, 26a,b. The N-terminus unprotected *S*-(Pg-α-aminoacyl)peptide (**13**, **23a**−**c**; 0.02 mmol) was suspended in degassed phosphate buffer (NaH_2PO_4/Na_2HPO_4) (1 M, pH = 7.8 for **13**, 6.2−8.2 for **23a**, and 7.6 for **26a,b**; 9.6 mL), and acetonitrile (0.4 mL) was added dropwise until the starting material was dissolved. The mixture was subjected to microwave irradiation (50 °C, 50 W, 1 h). The reaction was allowed to cool to rt, and acetonitrile was removed under reduced pressure and the residue was acidified with 2 N HCl to pH = 1. The mixture was extracted with ethyl acetate (3 × 10 mL), the combined organic extracts were dried over $MgSO_4$, and the solvent was removed under reduced pressure. Native peptide analogues **14**, **24**, **26a**, or **26b** were subsequently isolated as disulfide dimers by semipreparative HPLC.

Cbz-L-Ala-Gly-GABA-L-Cys-OH (14). Colorless oil, 1.7 mg, 18% yield. ESI-MS *m*/*z*: 935 (M + H). HRMS (ESI) calcd for $C_{40}H_{54}N_8O_{14}S_2Na$ $[M + Na]^+$ 957.3093, found 957.3117.

Cbz-L-Ala-β-Ala-L-Leu-Gly-L-Cys-OCH$_3$ (24). The compound was prepared from β-Ala-L-Leu-Gly-L-Cys(*S*-L-Cbz-Ala)-OCH$_3$ hydrochloride according to the general procedure for long-range acyl migration. Cbz-L-Ala-β-Ala-L-Leu-Gly-L-Cys-OCH$_3$ was subsequently isolated as a disulfide dimer by semipreparative HPLC. Colorless oil, 3.1 mg, 31% yield. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 0.83−0.88 (m, 12H), 1.17 (d, J = 7.2 Hz, 6H), 1.45 (t, J = 7.2 Hz, 4H), 1.56−1.64 (m, 2H), 2.26−3.36 (m, 4H), 2.97 (dd, J = 13.8, 8.4 Hz, 2H), 3.13 (dd, J = 13.9, 5.1 Hz, 2H), 3.21−3.28 (m, 4H), 3.65 (br s, 6H), 3.69−3.80 (m, 4H), 3.95−4.01 (m, 2H), 4.25 (q, J = 7.4 Hz, 2H), 4.57−4.61 (m, 2H), 4.97−5.05 (m, 4H), 7.28−7.32 (m, 2H), 7.32−7.38 (m, 10H), 7.87 (t, J = 5.8 Hz, 2H), 8.09 (d, J = 7.4 Hz, 2H), 8.21 (t, J = 5.8 Hz, 2H), 8.32 (d, J = 7.7 Hz, 2H). ^{13}C NMR (DMSO-d_6, 75 MHz): δ 18.2,

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21.5, 23.0, 24.2, 35.0, 35.3, 40.5, 41.5, 50.0, 51.2, 51.3, 52.2, 65.4, 127.7, 127.8, 128.3, 137.0, 155.6, 169.1, 170.7, 170.8, 172.4, 172.5. ESI-MS *m/z*: 1183 (M + Na). HRMS (ESI) calcd for $C_{52}H_{76}N_{10}O_{16}S_2Na$ $[M + Na]^+$ 1183.4774, found 1183.4733.

Cbz-L-Ala-β-Ala-L-Phe-β-Ala-L-Cys-OCH$_3$ (26a). The compound was prepared from β-Ala-L-Phe-β-Ala-L-Cys(*S*-L-Cbz-Ala)-OCH$_3$ hydrochloride according to the general procedure for long-range acyl migration. Cbz-L-Ala-β-Ala-L-Phe-β-Ala-L-Cys-OCH$_3$ was subsequently isolated as the disulfide dimer by semipreparative HPLC. Colorless oil, 2.2 mg, 42% yield. ESI-MS *m/z*: 1256 (M + H). HRMS (ESI) calcd for $C_{60}H_{76}N_{10}O_{16}S_2Na$ $[M + Na]^+$ 1279.4774, found 1279.4718.

Cbz-L-Ala-GABA-L-Phe-Gly-L-Cys-OCH$_3$ (26b). The compound was prepared from GABA-L-Phe-Gly-L-Cys(*S*-L-Cbz-Ala)-OCH$_3$ hydrochloride according to the general procedure for long-range acyl migration. Cbz-L-Ala-GABA-L-Phe-Gly-L-Cys-OCH$_3$ was subsequently isolated as a disulfide dimer by semipreparative HPLC. Colorless oil, 1.9 mg, 42% yield. ESI-MS *m/z*: 1256 (M + H). HRMS (ESI) calcd for $C_{60}H_{76}N_{10}O_{16}S_2Na$ $[M + Na]^+$ 1279.4774, found 1279.4775.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Detailed experimental procedures, copies of ^1H and ^{13}C NMR for all the novel compounds, copies of the chromatograms from the HPLC experiments, and computational details. 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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