JOC Article
pubs.acs.org/joc

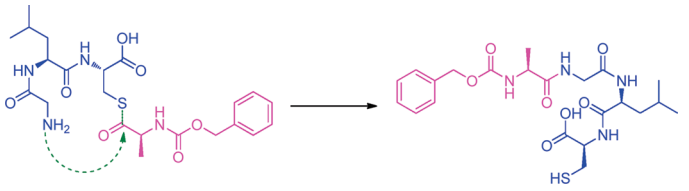
Chemical Ligation of S-Scylated Cysteine Peptides to Form Native Peptides via 5-, 11-, and 14-Membered Cyclic Transition States‡

Alan R. Katritzky,*[,§] Srinivasa R. Tala,[§] Nader E. Abo-Dya,[§,∥] Tarek S. Ibrahim,[§,∥] Said A. El-Feky,[∥] Kapil Gyanda,[§] and Keyur M. Pandya[§]

[§]*Center for Heterocyclic Compounds, Department of Chemistry, University of Florida, Gainesville, Florida 32611-7200, United States, and* [∥]*Department of Pharmaceutical Organic Chemistry, Faculty of Pharmacy, Zagazig University, Zagazig-44519, Egypt*

katritzky@chem.ufl.edu

Received August 16, 2010



Cysteine-containing dipeptides **3a**−**l**, (**3b**+**3b′**) (compound numbers in parentheses are used to indicate racemic mixtures; thus (**3b**+**3b′**) is the racemate of **3b** and **3b′**), and tripeptide **13** were synthesized in 68−96% yields by acylation of cysteine with *N*-(Pg-α-aminoacyl)- and *N*-(Pg-α-dipeptidoyl)benzotriazoles (where Pg stands for protecting group in the nomenclature for peptides throughout the paper) in the presence of Et_3N. Cysteine-containing peptides **3a**−**l** and **13** were S-acylated to give *S*-(Pg-α-aminoacyl)dipeptides **5a**−**l** and *S*-(Pg-α-aminoacyl)tripeptide **14** without racemization in 47−90% yields using *N*-(Pg-α-aminoacyl)benzotriazoles **2** in CH_3CN-H_2O (7:3) in the presence of $KHCO_3$. (In our peptide nomenclature, the prefixes di-, tri-, etc. refer to the number of amino acid residues in the main peptide chain; amino acid residues attached to sulfur are designated as *S*-acyl peptides. Thus we avoid use of the prefix "iso".) Selective S-acylations of serine peptide **3k** and threonine peptide **3l** containing free OH groups were thus achieved in 58% and 72% yield, respectively. *S*-(Pg-α-aminoacyl)cysteines **4a**,**b** underwent native chemical ligations to form native dipeptides **3f**,**i** via 5-membered cyclic transition states. Microwave irradiation of *S*-(Pg-α-aminoacyl)tripeptide **15** and *S*-(Pg-α-aminoacyl)tetrapeptide **17** in the presence of NaH_2PO_4/Na_2HPO_4 buffer solution at pH 7.8 achieved chemical ligations, involving intramolecular migrations of acyl groups, via 11- and 14-membered cyclic transition states from the S-atom of a cysteine residue to a peptide terminal amino group to form native peptides **19** and **20** in isolated yields of 26% and 23%, respectively.

Introduction

Peptides and proteins play important roles in biological and physiological processes in living organisms.[1] The design and synthesis of peptides with defined conformational properties is important for understanding the mechanisms of peptide structure formation and its biological importance.[2]

S-Acyl proteins and peptides are useful in regulating the dynamics of multiprotein assemblies[3] and in elucidating the structural and biophysical properties of the numerous proteins of mammalian cells.[4]

Published methods describe S-acylations using acyl chlorides[4,5] or coupling reagents like EDC[5a] of peptides devoid of free amino groups and of free hydroxyl groups in threonine, tyrosine, or serine residues. Acyl chlorides in neat

‡ Some of this work was published as a communication in Organic & Biomolecular Chemistry; see ref 11.

(1) Kimmerlin, T.; Seebach, D. *J. Peptide Res.* **2005**, *65*, 229–260.

(2) Schmuck, C.; Wennemers, H. *Highlights in Bioorganic Chemistry*; Wiley-VCH Verlag GmbH & Co.: Weinheim, 2004.

(3) Yang, W.; Vizio, D. D.; Kirchner, M.; Steen, H.; Freeman, M. R. *Mol. Cell. Proteomics* **2010**, *9*, 54–70.

(4) Schroeder, H.; Leventis, R.; Rex, S.; Schelhaas, M.; Nägele, E.; Waldmann, H.; Silvius, J. R. *Biochemistry* **1997**, *36*, 13102–13109.

(5) (a) Stöber, P.; Schelhaas, M.; Nägele, E.; Hagenbuch, P.; Rétey, J.; Waldmann, H. *Bioorg. Med. Chem.* **1997**, *5*, 75–83. (b) Schmittberger, T.; Waldmann, H. *Bioorg. Med. Chem.* **1999**, *7*, 749–762. (c) Kadereit, D.; Waldmann, H. *ChemBioChem.* **2000**, *1*, 200–203.

DOI: 10.1021/jo1015757 Published on Web 12/15/2010

TABLE 1. Conversion of *N*-(Pg-α-amino) Acids 1a−l into *N*-(Pg-α-aminoacyl)benzotriazoles 2a−l

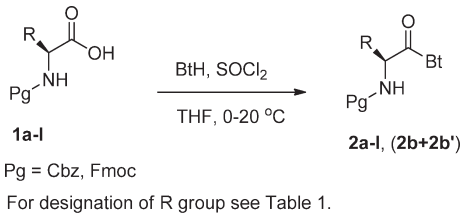
entry	reagent **1**	product **2**	yield (%)	mp (°C)
1	*N*-Z-L-Trp-OH (**1a**)	*N*-Z-L-Trp-Bt (**2a**)[a]	92 (95)[c]	98−100 (100−101)[c,e]
2	*N*-Z-L-Ala-OH (**1b**)	*N*-Z-L-Ala-Bt (**2b**)[b]	79 (91)[c]	113−115 (114−115)[c]
3	*N*-Z-L-Leu-OH (**1c**)	*N*-Z-L-Leu-Bt (**2c**)	80	68−70
4	*N*-Z-L-Tyr-OH (**1d**)	*N*-Z-L-Tyr-Bt (**2d**)[a]	86 (86)[c]	169−171 (165−166)[c]
5	*N*-Z-L-Met-OH (**1e**)	*N*-Z-L-Met-Bt (**2e**)[a]	86 (95)[c]	108−109 (105−107)[c]
6	*N*-Z-DL-Ala-OH (**1b**+**1b′**)	*N*-Z-DL-Ala-Bt (**2b**+**2b′**)[b]	90 (94)[c]	(DL-mixture)
7	*N*-Fmoc-L-Phe-OH (**1f**)	*N*-Fmoc-L-Phe-Bt **2f**[d]	80 (85)[c]	136−137 (159−160)[c]
8	*N*-Fmoc-L-Ala-OH **1g**	*N*-Fmoc-L-Ala-Bt (**2g**)[d]	50 (72)[c]	160−161 (160−161)[c]
9	*N*-Fmoc-L-Trp-OH (**1h**)	*N*-Fmoc-L-Trp-Bt (**2h**)[d]	95 (90)[c]	88−90 (92−94)[c]
10	*N*-Fmoc-L-Met-OH (**1i**)	*N*-Fmoc-L-Met-Bt (**2i**)[d]	91 (82)[c]	98−100 (122−123)[c]
11	*N*-Fmoc-Gly-OH (**1j**)	*N*-Fmoc-Gly-Bt (**2j**)[d]	80 (88)[c]	160−162 (161−162)[c]
12	*N*-Z-L-Ser-OH (**1k**)	*N*-Z-L-Ser-Bt (**2k**)	76	67−68
13	*N*-Z-L-Thr-OH (**1l**)	*N*-Z-L-Thr-Bt (**2l**)	75	142−143

[a]Previously reported.[10b] [b]Previously reported.[10g] [c]Literature yields and melting points in parentheses. [d]Previously reported.[10h] See the Supporting Information for characterization data of **2c**, **2k**.

trifluoroacetic acid can selectively S-acylate cystine residues, avoiding acylation of amino groups, but hydroxyl groups must be protected.[6] Novel protecting groups were used for the synthesis of selectively S-acylated peptides.[7] However, these specially protected amino acids used in these approaches are not always available, and the required synthetic procedures are long. Long-chain acyl-coenzyme A thioesters (acyl-CoAs) in micelles or lipid bilayers were used as acyl donors for the selective S-acylation of small quantities of unprotected peptides;[8] however, such acyl-CoAs are costly and prone to hydrolysis during storage. Acyl thioesters were used for the selective S-acylation of unprotected peptides, but purification techniques such as flash chromatography were needed in the purification of acyl thioesters.[9] Hence, there is a need for a mild, efficient, and selective general method for S-acylation of peptides having free carboxyl, hydroxyl, or amino groups.

N-Acylbenzotriazoles, which can be synthesized easily without purification by column chromatography, are advantageous for N-, O-, C-, and S-acylation,[10] especially where the corresponding acid chlorides are unstable or difficult to prepare and/or store.[10k] We previously demonstrated that peptide coupling of *N*-(Pg-α-aminoacyl)benzotriazoles with unprotected amino acids in MeCN−H_2O occurs with total preservation of the original chirality.[10b,i] A recent paper[11] described the selective S-acylation of cysteine peptides having carboxyl and/or hydroxyl groups and their chemical ligation. We now report (i) full details of the use of *N*-(Pg-α-aminoacyl)-benzotriazoles for the selective isolation and characterization of *S*-(Pg-α-aminoacyl)peptides (including successful S-acylations of peptides containing free carboxyl, hydroxyl or amino groups) under mild conditions in good yields without racemization, (ii) the isolation and characterization of native peptides by microwave assisted chemical ligation of unprotected amino *S*-(Pg-α-aminoacyl)peptides via 11- and 14-membered transition states, and (iii) competitive ligation experiments, which support the intramolecular nature of the ligation of N-terminus unprotected *S*-(Pg-α-aminoacyl)peptides to form native peptides.

SCHEME 1. Preparation of *N*-(Pg-α-aminoacyl)benzotriazoles 2a−l and (2b+2b′)



Results and Discussion

Preparation of *N*-(Pg-α-aminoacyl)benzotriazoles 2a−l from *N*-(Pg-α-amino) Acids 1a−l. The *N*-(Pg-α-amino) acids **1a**−**l** and (**1b**+**1b′**) were treated as previously reported[10b] with a mixture of 4 equiv of 1*H*-benzotriazole and 1 equiv of thionyl chloride in anhydrous THF at 25 °C for 2 h to afford the desired *N*-(Pg-α-aminoacyl)benzotriazoles **2a**−**l** and (**2b**+**2b′**) (Scheme 1) in yields of 50−95% (Table 1). In the case of **2k**, the reaction was carried out at 0 °C, and Z-Thr-Bt **2l** was prepared using 3 equiv of benzotriazole and 3 equiv of $(i\text{-Pr})_2$EtN at −30 °C.

Preparation of *N*-Protected Cysteine-Containing Dipeptides 3a−l. N-Protected cysteine-containing dipeptides **3a**−**l** and (**3b**+**3b′**) were synthesized by coupling the appropriate *N*-(Pg-α-aminoacyl)benzotriazoles **2a**−**l** and (**2b**+**2b′**) with 1 equiv of L-cysteine in aqueous acetonitrile solution (MeCN−H_2O, 7:3) in the presence of 1 equiv of Et_3N for 1 h at room temperature in 68−98% yields (Scheme 2, Table 2).[10b] Novel dipeptides **3b**−**l**

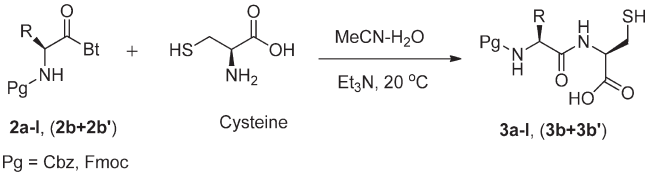
(6) Yousefi-Salakdeh, E.; Johansson, J.; Strömberg, R. *Biochem. J.* **1999**, *343*, 557–562.
(7) (a) Kadereit, D.; Deck, P.; Heinemann, I.; Waldmann, H. *Chem.—Eur. J.* **2001**, *7*, 1184–1193. (b) Ludolph, B.; Eisele, F.; Waldmann, H. *J. Am. Chem. Soc.* **2002**, *124*, 5954–5955. (c) Ludolph, B.; Waldmann, H. *Chem.—Eur. J.* **2003**, *9*, 3683–3691.
(8) (a) Duncan, J. A.; Gilman, A. G. *J. Biol. Chem.* **1996**, *271*, 23594–23600. (b) Quesnel, S.; Silvius, J. R. *Biochemistry* **1994**, *33*, 13340–13348. (c) Bharadwaj, M.; Bizzozero, O. A. *J. Neurochem.* **1995**, *65*, 1805–1815. (d) Bano, M. C.; Jackson, C. S.; Magee, A. I. *Biochem. J.* **1998**, *330*, 723–731. (e) Belanger, C.; Ansanay, H.; Qanbar, R.; Bouvier, M. *FEBS Lett.* **2001**, *499*, 59–64. (f) Bizzozero, O. A.; Bixler, H. A.; Pastuszyn, A. *Biochem. Biophys. Acta* **2001**, *1545*, 278–288.
(9) Leung, S.; Sang, W.; Silvius, J. *J. Peptide Res.* **2005**, *66*, 169–180.
(10) (a) Wang, X.; Zou, X.; Li, J.; Hu, Q. *Synlett* **2005**, 3042–3046. (b) Katritzky, A. R.; Angrish, P.; Suzuki, K. *Synthesis* **2006**, 411–424. (c) Katritzky, A. R.; Suzuki, K.; Singh, S. K.; He, H.-Y. *J. Org. Chem.* **2003**, *68*, 5720–5723. (d) Katritzky, A. R.; He, H.-Y.; Suzuki, K. *J. Org. Chem.* **2000**, *65*, 8210–8213. (e) Katritzky, A. R.; Wang, M.; Yang, H.; Zhang, S.; Akhmedov, N. G. *ARKIVOC* **2002**, *viii*, 134–142. (f) Katritzky, A. R.; Shestopalov, A. A.; Suzuki, K. *ARKIVOC* **2005**, *vii*, 36–55. (g) Katritzky, A. R.; Suzuki, K.; Singh, S. K. *Synthesis* **2004**, 2645–2652. (h) Katritzky, A. R.; Singh, A.; Haase, D.; Yoshioka, M. *ARKIVOC* **2009**, *viii*, 47–56. (i) Katritzky, A. R.; Angrish, P.; Todadze, E. *Synlett* **2009**, 2392–2411. (j) Katritzky, A. R.; Avan, I.; Tala, S. R. *J. Org. Chem.* **2009**, *74*, 8690–8694. (k) Katritzky, A. R.; Belyakov, S. A. *Aldrichim. Acta* **1998**, *31*, 35–46.
(11) Katritzky, A. R.; Abo-Dya, N. E.; Tala, S. R.; Abdel-Samii, Z. K. *Org. Biomol. Chem.* **2010**, *8*, 2316–2319.

TABLE 2. Preparation of *N*-Protected Cysteine Peptides 3a−l and (3b+3b′) from *N*-(Pg-α-aminoacyl)benzotriazoles 2a−l and (2b+2b′) and L-cysteine

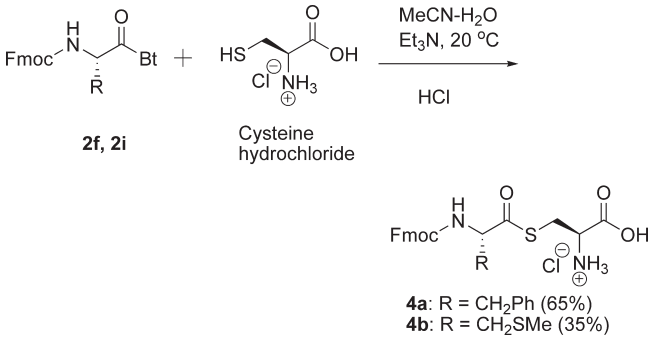
entry	reactant	product **3**	yield (%)	mp (°C)	$[\alpha]^{23}_D$
1	**2a**	*N*-Z-L-Trp-L-Cys-OH (**3a**)[a]	78	82−86	−9.2
2	**2b**	*N*-Z-L-Ala-L-Cys-OH (**3b**)	79	169−172	−21.3
3	**2c**	*N*-Z-L-Leu-L-Cys-OH (**3c**)	79	61−65	−17.6
4	**2d**	*N*-Z-L-Tyr-L-Cys-OH (**3d**)	96	105−107	−13.3
5	**2e**	*N*-Z-L-Met-L-Cys-OH (**3e**)	87	135−137	−14.2
6	(**2b+2b′**)	*N*-Z-DL-Ala-L-Cys-OH (**3b+3b′**)	75	(DL-mixture)	(DL-mixture)
7	**2f**	*N*-Fmoc-L-Phe-L-Cys-OH (**3f**)	98	164−166	−12.6
8	**2g**	*N*-Fmoc-L-Ala-L-Cys-OH (**3g**)	68	167−169	−7.6
9	**2h**	*N*-Fmoc-L-Trp-L-Cys-OH (**3h**)	78	106−107	−12.4
10	**2i**	*N*-Fmoc-L-Met-L-Cys-OH (**3i**)	88	97−99	−9.0
11	**2j**	*N*-Fmoc-Gly-L-Cys-OH (**3j**)	84	90−91	+6.3
12	**2k**	*N*-Z-L-Ser-L-Cys-OH (**3k**)	70	82−84	−9.2
13	**2l**	*N*-Z-L-Thr-L-Cys-OH (**3l**)	74	68−70	−20.0

[a]Previously reported, lit.[10b] mp 140−144 °C.

SCHEME 2. Preparation of *N*-Protected Cysteine-Containing Dipeptides 3a−l and (3b+3b′)



SCHEME 3. Preparation of *S*-(Fmoc-α-aminoacyl)cysteines 4a,b



and (**3b**+**3b**′) were characterized by ^{1}H and ^{13}C NMR spectroscopy and elemental analysis.

Preparation of *S*-(Fmoc-α-aminoacyl)cysteines 4a,b. Recently, we reported selective S-acylations and N-acylations of cysteine using *N*-acylbenzotriazoles.[12] We have now achieved selective S-acylation of cysteine hydrochloride with *N*-(Fmoc-α-aminoacyl)benzotriazoles **2f** and **2i** in aqueous acetonitrile (MeCN−H_2O in 9:1) in the presence of *1 equiv* of Et_3N for 2 h at 25 °C to form novel *S*-(Fmoc-α-aminoacyl)cysteine hydrochloride salts **4a**,**b** (35−65%) (Scheme 3). Salts **4a**,**b** were characterized by ^{1}H and ^{13}C NMR spectroscopy and elemental analysis.

Convenient Preparation of *S*-(Pg-α-aminoacyl)dipeptides 5a−l and (5c+5c′). Treatment of cysteine-containing dipeptides **3a−l** with the appropriate *N*-acylbenzotriazoles and *N*-(Pg-α-aminoacyl)benzotriazoles at 0−5 °C in the presence of $KHCO_3$ enabled convenient preparations of *S*-(Pg-α-aminoacyl)dipeptides **5a−l** and (**5c**+**5c**′) in average yields of 47−90% (Scheme 4, Table 3). This procedure included the selective S-acylation of peptides **3k** and **3l** containing serine and threonine amino acid residues possessing free hydroxyl groups to provide **5j** and **5k**, respectively (see Scheme 4, Table 3).

S-(Pg-α-aminoacyl)dipeptides **5a−l** were each characterized by ^{1}H and ^{13}C NMR and elemental analysis. Retention of diastereomeric purity of *S*-(Pg-α-aminoacyl)dipeptides **5c** and (**5c**+**5c**′) was supported by Chiral HPLC analysis using a Chirobiotic T column (detection at 254 nm, flow rate 0.5 mL/min, and MeOH−H_2O (98:2) as eluent). Thus, the diastereomeric mixture (**5c**+**5c**′) showed two peaks at 3.98 and 4.29, whereas the single diastereomer **5c** showed one single peak at 4.28.

Studies on Chemical Ligation/Rearrangement. Native chemical ligation (NCL)[13] is a powerful tool widely applied for the synthesis of small proteins, which provides the researcher with complete atom-by-atom control over the covalent structure of the protein molecule.[14] As shown in Scheme 5, it utilizes chemoselective reactions between two unprotected fragments, a C-terminal thioester (peptide A) **6** and N-terminal cysteine (peptide B) **7**. Trans-thioesterification of **6** with **7** results intermediate **8**, after which "classical" "S" to "N" acyl transfer takes place forming an amide bond to give the new peptide **9**. The "S" to "N" acyl transfer from **8** to **9** is assisted by the proximity of the amino group in **8** to the thioester functionality and the 5-membered intramolecular transition state.

Native Chemical Ligation in *S*-(Fmoc-α-aminoacyl)cysteines 4a,b. *S*-(Fmoc-α-aminoacyl)cysteines **4a** and **4b** were stirred at room temperature in acetonitrile−water (3:1) in the presence of equimolar amounts of triethylamine. Classical "S" to "N" acyl transfer takes place forming an amide bond to give native cysteine dipeptides **3f** and **3i**, respectively, in 80−89% yields (Scheme 6). The "S" to "N" acyl transfer involves a five-membered transition state allowed by the proximity of the amino group in **4a** and **4b** to the thioester functionality. Native cysteine dipeptides **3f**,**i** were characterized by ^{1}H and ^{13}C NMR

(12) Katritzky, A. R.; Tala, S. R.; Abo-Dya, N. E.; Gyanda, K.; El-Gendy, B. E.-D. M.; Abdel-Samii, Z. K.; Steel, P. J. *J. Org. Chem.* **2009**, *74*, 7165–7167.

(13) (a) Bang, D.; Pentelute, B. L.; Kent, S. B. H. *Angew. Chem., Int. Ed.* **2006**, *45*, 3985–3988. (b) Pentelute, B. L.; Kent, S. B. H. *Org. Lett.* **2007**, *9*, 687–690. (c) Richardson, J. P.; Macmillan, D. *Org. Biomol. Chem.* **2008**, *6*, 3977–3982. (d) Anderson, S. *Langmuir* **2008**, *24*, 13962–13968. (e) Wan, Q.; Chen, J.; Tuan, Y.; Danishefsky, S. J. *J. Am. Chem. Soc.* **2008**, *130*, 15814–15816. (f) Moyle, P. M.; Olive, C.; Ho, M.-F.; Burgess, M.; Karpati, L.; Good, M. F.; Toth, I. *J. Org. Chem.* **2006**, *71*, 6846–6850.

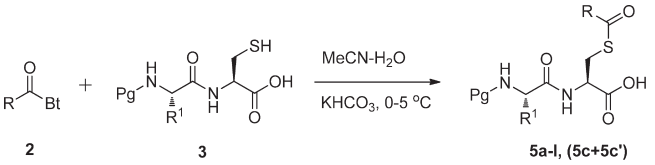
(14) Johnson, E. C. B.; Kent, S. B. H. *J. Am. Chem. Soc.* **2006**, *128*, 6640–6646.

TABLE 3. Preparation of *S*-(Pg-α-aminoacyl)dipeptides 5a−l

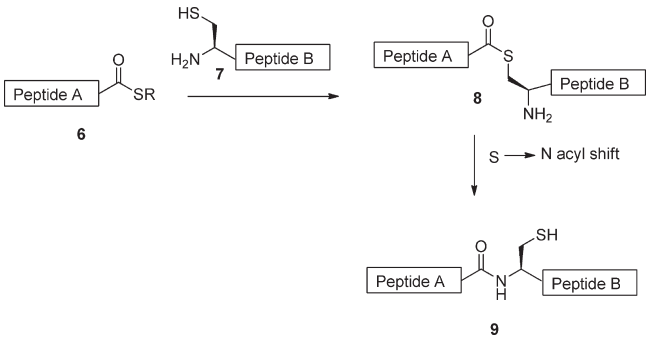
entry	dipeptide	RCOBt used	product **5**	yield (%)	mp (°C)	$[\alpha]^{23}_D$
1	**3a**	**2a**	Z-L-Trp-L-Cys(Z-L-Trp)-OH (**5a**)	88	73−76	−59.4
2	**3a**	**2b**	Z-L-Trp-L-Cys(Z-L-Ala)-OH (**5b**)	90	63−66	−38.2
3	**3b**	**2a**	Z-L-Ala-L-Cys(Z-L-Trp)-OH (**5c**)	90	65−67	−44.3
4	(**3b**+**3b′**)	**2a**	Z-DL-Ala-L-Cys(Z-L-Trp)-OH (**5c**+**5c′**)	90	(DL-mixture)	(DL-mixture)
5	**3d**	**2c**	Z-L-Tyr-L-Cys(Z-L-Leu)-OH (**5d**)	85	84−88	−47.8
6	**3e**	**2e**	Z-L-Met-L-Cys(Z-L-Met)-OH (**5e**)	90	146−150	−41.5
7	**3a**	**2m**[a]	Z-L-Trp-L-Cys(COPh)-OH (**5f**)	70	156−158	−56.1
8	**3f**	**2n**[b]	Fmoc-L-Phe-L-Cys(COC_6H_4Me-*p*)-OH (**5g**)	78	152−154	−71.8
9	**3f**	**2f**	Fmoc-L-Phe-L-Cys(Fmoc-L-Phe)-OH (**5h**)	47	171−173	−40.4
10	**3i**	**2c**	Fmoc-L-Met-L-Cys(Z-L-Leu)-OH (**5i**)	83	84−86	−65.0
11	**3k**	**2f**	Z-L-Ser-L-Cys(Fmoc-L-Phe)-OH (**5j**)	58	77−80	−35.0
12	**3l**	**2f**	Z-L-Thr-L-Cys(Fmoc-L-Phe)-OH (**5k**)	72	175−177	−79.0
13	**3j**	**2b**	Fmoc-Gly-L-Cys(Z-L-Ala)-OH (**5l**)	87	159−161	−10.7

[a] **2m** = PhCOBt. [b] **2n** = 4-Me-PhCOBt.

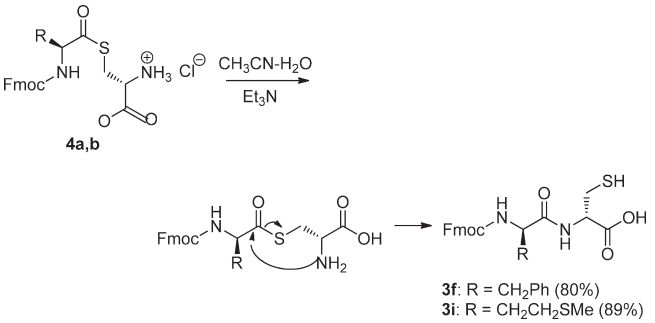
SCHEME 4. Preparation of *S*-(Pg-α-aminoacyl)dipeptides 5a−l and (5c+5c′)



SCHEME 5. Native Chemical Ligation Mechanism



SCHEME 6. Native Chemical Ligation of *S*-(Fmoc-α-aminoacyl)-cysteines 4a,b



spectroscopy and elemental analysis and compared with authentic samples (see Table 2).

Synthesis of N-Terminal Unprotected *S*-(Acyl)dipeptides 10a−c. The NCL method for chemical ligation requires an N-terminal cysteine residue on a peptide or glycopeptide fragment; although thiol auxiliary ligation[15] and sugar-assisted ligation[16] can overcome this limitation, such auxiliaries can sterically hinder the chemical ligation. We have now explored the possibility of *S*-(Pg-α-aminoacyl) group intramolecular migration in *S*-(Pg-α-aminoacyl)peptides to form native peptides via 8-, 11-, and 14-membered ligation transition states without use of auxiliaries. For this we prepared N-terminal unprotected *S*-(acyl)dipeptides **10a−c**, *S*-(Pg-α-aminoacyl)tripeptide **15**, and *S*-(Pg-α-aminoacyl)tetrapeptide **17** as shown in Schemes 7, 9, and 10, respectively. Removal of the Fmoc protecting group from protected *S*-(acyl)dipeptides **5g**, **5i**, and **5l** proceeded on treatment with 2 equiv of DBU in dry THF to afford N-terminal unprotected *S*-(acyl)dipeptides **10a−c** (75−90%), each possessing a free terminal amino group (Scheme 7, Table 4). N-Terminal unprotected *S*-(acyl)-dipeptides **10a−c** were each characterized by ^{1}H and ^{13}C NMR and elemental analysis.

SCHEME 7. Fmoc Deprotection of *S*-(Acyl)dipeptides 5g, 5i, and 5l

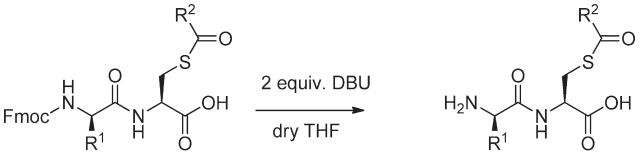


TABLE 4. Preparation of N-Terminal Unprotected *S*-(Acyl)dipeptides 10a−c

entry	product **10**	yield (%)	mp (°C)	$[\alpha]^{23}_D$
1	L-Phe-L-Cys(COC_6H_4-Me-*p*)-OH (**10a**)	75	200−202	−79.0
2	L-Met-L-Cys(Z-L-Leu)-OH (**10b**)	90	148−150	−57.0
3	L-Gly-L-Cys(Z-L-Ala)-OH (**10c**)	88	188−190	−25.0

To facilitate monitoring of the ligation experiments to be conducted on **10b**,**c**, two of the expected native tripeptide ligation products **12a**,**b** were each synthesized independently using benzotriazole methodology (Scheme 8);[17] **12a**,**b** were

(15) (a) Offer, J.; Boddy, C. N. C.; Dawson, P. E. *J. Am. Chem. Soc.* **2002**, *124*, 4642–4646. (b) Wu, B.; Chen, J.; Warren, J. D.; Chen, G.; Hua, Z.; Danishefsky, S. J. *Angew. Chem., Int. Ed.* **2006**, *45*, 4116–4125. (c) Lutsky, M.-Y.; Nepomniaschiy, N.; Brik., A. *Chem. Commun.* **2008**, 1229–1231.

(16) (a) Brik, A.; Yang, Y.-Y.; Ficht, S.; Wong, C.-H. *J. Am. Chem. Soc.* **2006**, *128*, 5626–5627. (b) Brik, A.; Ficht, S.; Yang, Y.-Y.; Bennett, C. S.; Wong, C.-H. *J. Am. Chem. Soc.* **2006**, *128*, 15026–15033. (c) Payne, R. J.; Ficht, S.; Tang, S.; Brik, A.; Yang, Y.-Y.; Case, D. A.; Wong, C.-H. *J. Am. Chem. Soc.* **2007**, *129*, 13527–13536.

(17) Katritzky, A. R.; Abo-Dya, N. E.; Tala, S. R.; Gyanda, K.; Abdel-Samii, Z. K. *Org. Biomol. Chem.* **2009**, *7*, 4444–4447.

SCHEME 8. Preparation of the Expected Native Tripeptides 12a,b

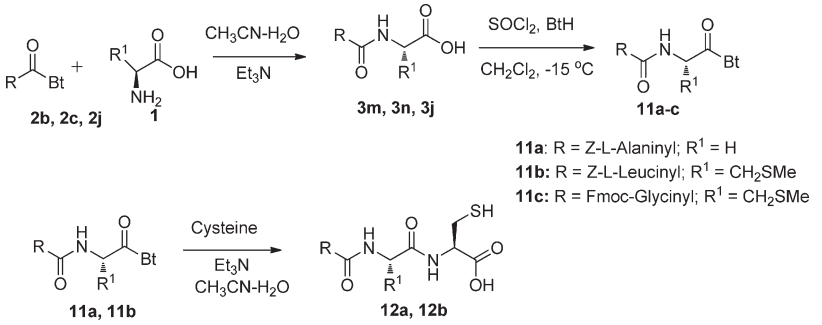


TABLE 5. Preparation of the Expected Native Tripeptides 12a,b

entry	product **12**	yield (%)	mp (°C)	$[\alpha]^{23}_D$
1	Z-L-Ala-Gly-L-Cys-OH (**12a**)	80	78−80	−19.0
2	Z-L-Leu-L-Met-L-Cys-OH (**12b**)	90	96−98	−34.0

characterized by ^{1}H and ^{13}C NMR spectroscopy and elemental analysis (Table 5).

Synthesis of *S*-(Pg-α-aminoacyl)tripeptide 15. *N*-(Fmoc-glycyl-leucyl)benzotriazole **11c**[17] was reacted with cysteine in acetonitrile−water (10:3) in the presence of 1 equiv of Et_3N to give Fmoc-Gly-Leu-L-Cys-OH **13**. Compound **13** was then S-acylated using Z-Ala-Bt in acetonitrile−water (7:3) in the presence of 1 equiv of $KHCO_3$ to give *S*-(Z-α-aminoacyl)-tripeptide **14**. Fmoc-deprotection of **14** was achieved using DBU in dry THF to provide *S*-(Pg-α-aminoacyl)tripeptide **15** (Scheme 9). Compounds **13**−**15** were each isolated and characterized by ^{1}H and ^{13}C NMR spectroscopy and elemental analysis. *S*-(Pg-α-Aminoacyl)tripeptide **15** was further characterized using HPLC−MS (Figure 1, Supporting Information), in which compound **15** (m/z = 496) showed a retention time of 29.31 min.

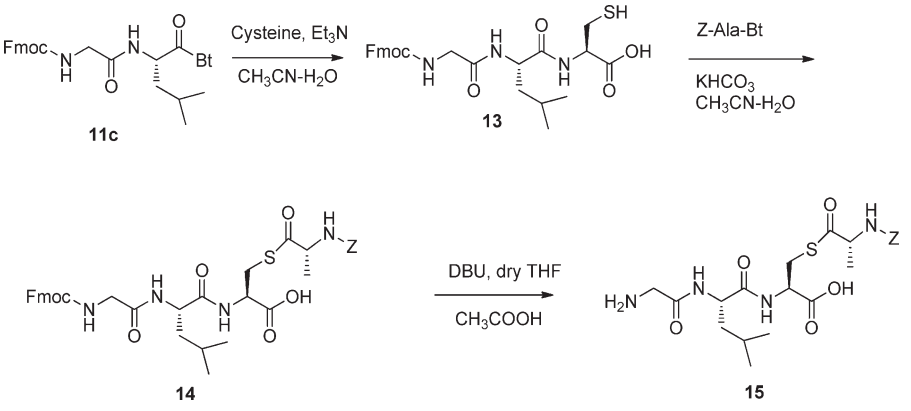
Synthesis of N-Terminus Unprotected *S*-(Pg-α-aminoacyl)-tetrapeptide 17. Treatment of L-Gly-L-Cys(Z-L-Ala)-OH (**10c**) with *N*-(Fmoc-Glycyl-leucyl)benzotriazole **11c** afforded *S*-(Pg-α-aminoacyl)tetrapeptide **16** (80%) as shown in Scheme 10; compound **16** was characterized by ^{1}H and ^{13}C NMR spectroscopy and elemental analysis. Deprotection of **16** was achieved using DBU in dry THF to give N-terminus unprotected *S*-(Pg-α-aminoacyl)tetrapeptide **17** (70%), characterized by ^{1}H NMR and HPLC−MS analysis (Figure 2, Supporting Information, m/z = 553), which showed a retention time of 27.22 min and HRMS (ESI) (Scheme 10).

Studies on Non-native Chemical Ligations: Acyl Group Migration from S→N. N-Terminus-unprotected *S*-(Pg-α-aminoacyl)dipeptide **10c** was suspended in NaH_2PO_4/Na_2HPO_4 (2 M, pH 7.8) and subjected to microwave irradiation (50 W, 50 °C) for 1 h. HPLC−MS analysis of the crude isolated product revealed the expected ligation product **12a** having a retention time of 27.91 (independent synthesis of **12a** is described in Scheme 8) was only a minor component (ca. 3%, M^+ = 383 m/z); the major product was **18** (ca. 85%, M^+ = 588 m/z) having a retention time of 37.73 formed by intermolecular trans-acylation (Scheme 11; Figure 4, Supporting Information).

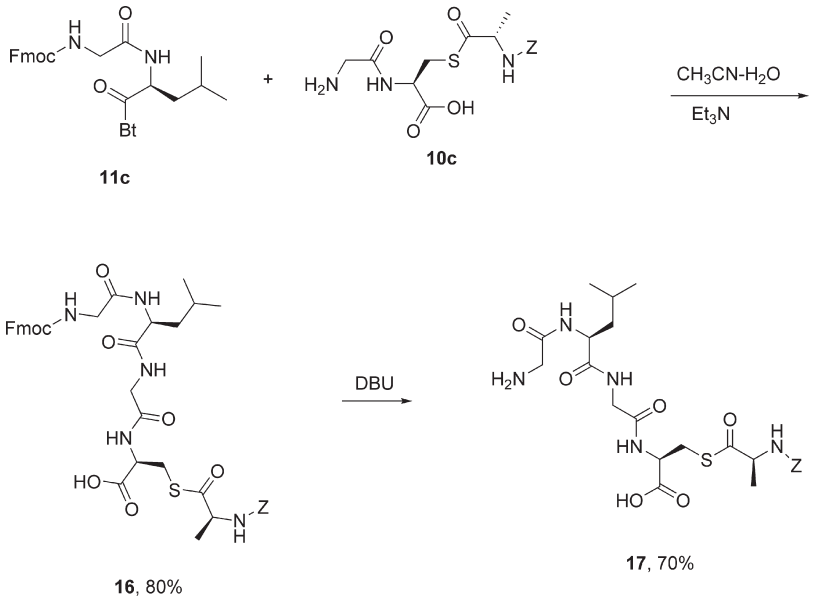
We have treated N-terminus-unprotected *S*-(acyl)dipeptide **10a** under conditions similar to those described above. However, HPLC−MS analysis of the crude product showed no ligation or intermolecular trans-acylation products; this may be because of the lower solubility of N-terminus-unprotected *S*-(acyl)dipeptide **10a**. In the case of N-terminus-unprotected *S*-(acyl)dipeptide **10b**, we have not attempted the ligation experiment because of the lower solubility of this compound.

However, when we suspended N-terminus unprotected *S*-(Z-α-aminoacyl)tripeptide **15** in NaH_2PO_4/Na_2HPO_4 (2 M, pH 7.8) and subjected the suspension to microwave irradiation (50 W, 50 °C) for 1 h, after workup we isolated 95% of the crude product. HPLC−MS analysis of the crude isolated product revealed the major component (67%) to be the expected ligation product **19** (M^+ = 496 m/z) with a retention time at 25.23 (Figure 5 in Supporting Information). This retention time distinguishes ligation product **19** from N-terminus unprotected *S*-(Pg-α-aminoacyl)tripeptide **15**,

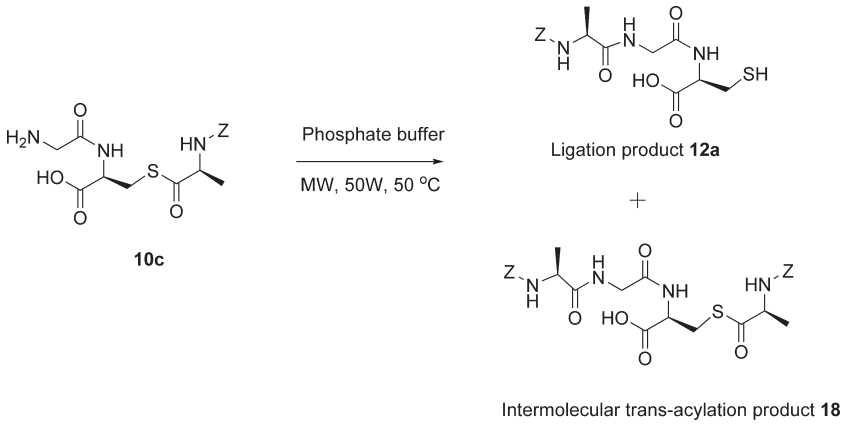
SCHEME 9. Preparation of *S*-(Pg-α-aminoacyl)tripeptide 15



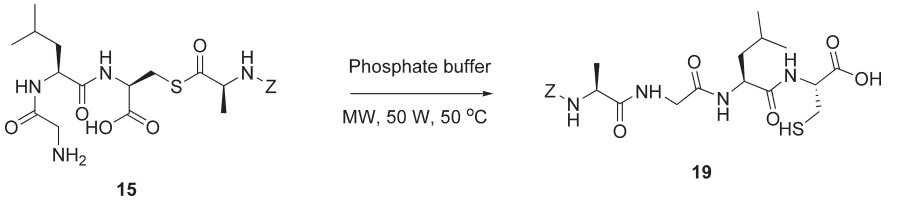
SCHEME 10. Preparation of N-Terminus Unprotected *S*-(Pg-α-aminoacyl)tetrapeptide 17



SCHEME 11. Attempted Ligation under Microwave Irradiation of N-Terminus Unprotected *S*-(Z-α-aminoacyl)dipeptide 10c



SCHEME 12. Chemical Ligation of N-Terminus Unprotected *S*-(Pg-α-aminoacyl)tripeptide 15



which has a retention time of 29.31 (Scheme 12; Figure 1, Supporting Information). Separation of **19** by semipreparative HPLC provided 26% of pure tetrapeptide **19**, which was characterized by analytical HPLC and HRMS (Table 6).

N-Terminus-unprotected *S*-(Pg-α-aminoacyl)tetrapeptide **17** was similarly suspended in NaH_2PO_4/Na_2HPO_4 (2 M, pH 7.8) and subjected to microwave irradiation (50 W, 50 °C) for 1 h; this afforded 90% of crude isolated product, HPLC−MS analysis of which revealed the major component (61%) to be the expected ligation product **20** (M^+ = 553 *m*/*z*) with retention time at 24.19 (Figure 6, Supporting Information). This retention time is significantly different from N-terminus-unprotected *S*-(Pg-α-aminoacyl)tetrapeptide **17**, which has a retention time of 27.22 (Scheme 13; Figure 2, Supporting Information). Separation of **20** by semipreparative HPLC provided 23% of pure pentapeptide **20**, which was characterized by analytical HPLC and HRMS (Table 6).

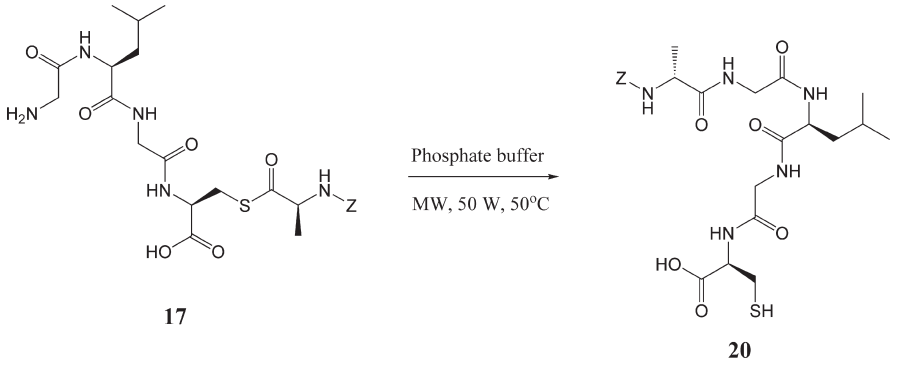
Thus, of the four non-native chemical ligations conducted, two succeeded. The ligation of *S*-(Pg-α-aminoacyl)tripeptide **15** gave **19** (95% crude product of 67% purity, i.e., 64% yield from which 26% of pure **19** was isolated). The ligation of *S*-(Pg-α-aminoacyl)tetrapeptide **17** gave **20** (90% crude

TABLE 6. Ligated Native Peptides Formed by Chemical Ligation

			crude		after preparative HPLC		HRMS $[M + H]^+$	
entry	ligated peptide	t_R (min)	yield[a] (%)	purity[b] (%)	yield[a] (%)	purity[b] (%)	calcd	found
1	Z-Ala-Gly-Cys-OH (**12a**)	27.91	85	3	nd[c]	nd[c]	nd[c]	nd[c]
2	Z-Ala-Gly-Leu-Cys-OH (**19**)	25.23	95	67	26	>95	497.2064	497.2078
3	Z-Ala-Gly-Leu-Gly-Cys-OH (**20**)	24.19	90	61	23	>95	554.2279	554.2285

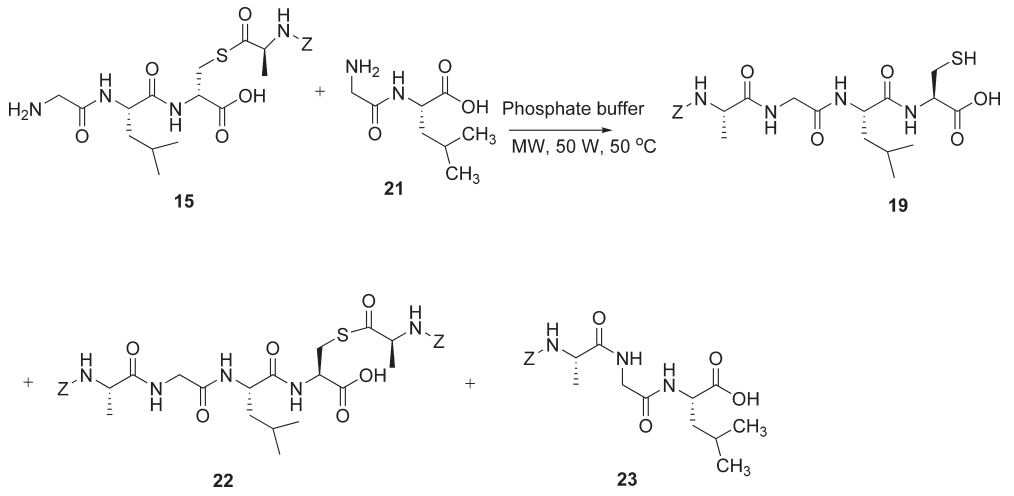
[a]Isolated yield. [b]Determined from analytical HPLC. [c]Not determined.

SCHEME 13. Chemical Ligation of N-Terminus Unprotected *S*-(Pg-α-aminoacyl)tripeptide 17



17 **20**

SCHEME 14. Competitive Experiment 1



15 **21** **19**

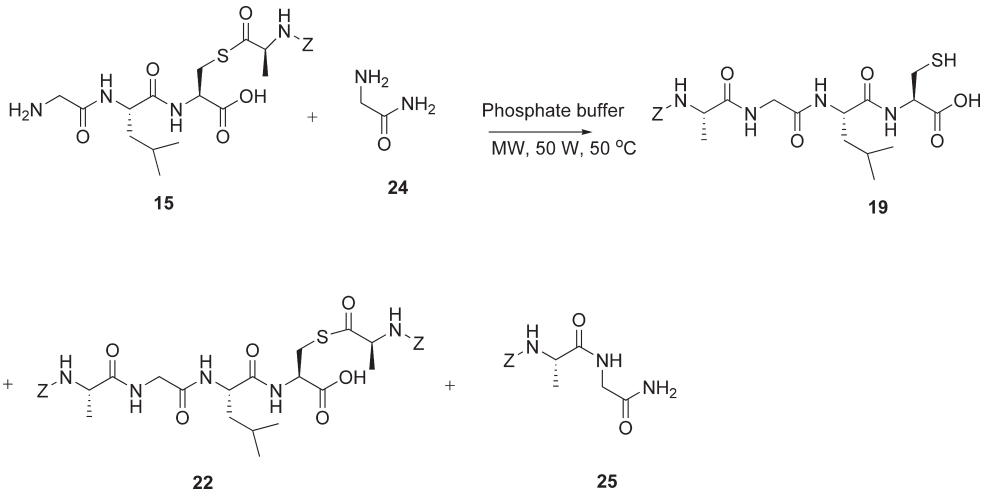
22 **23**

product of 61% purity, i.e., 55% yield from which 23% pure **20** was isolated). Thus, native tetrapeptide **19** is formed by ligation from **15** through an 11-membered cyclic transition state, native pentapeptide **20** is formed by ligation from **17** through a 14-membered cyclic transition state (Table 6). By contrast, *S*-(Pg-α-aminoacyl)dipeptide **10c** gave only ca. 3% of ligated native tripeptide **12a** but 85% of intermolecular trans-acylation product **18** (Table 6). This strongly suggests that, for *S*-(Pg-α-aminoacyl)dipeptide **10c**, the 8-membered transition state required for intramolecular chemical ligation is disfavored compared to intermolecular trans-acylation. Finally, attempts with **10a** failed because of insolubility.

Competitive Ligation Experiments. To further support the intramolecular nature of the ligation of N-terminus unprotected *S*-(Pg-α-aminoacyl)tripeptide **15** to form native tetrapeptide **19** by chemical ligation via an 11-membered transition state, we carried out the chemical ligation of N-terminus unprotected *S*-(Pg-α-aminoacyl)tripeptide **15** in the presence of 5 equiv of dipeptide **21** (H-Gly-Leu-OH) under similar reaction conditions (Scheme 14). HPLC−MS analysis of the isolated crude product (Figure 7, Supporting Information) confirmed the formation of approximately 70% of the desired ligation product **19** having aretention time at 29.93 along with 10% of a mixture of intermolecular trans-acylation product **22** having a retention time at 38.75. Compound **23**, which is the *N*-acylated product of dipeptide **21**, was not observed in the HPLC−MS analysis.

In a second competition experiment, ligation of N-terminus-unprotected *S*-(Pg-α-aminoacyl)tripeptide **15** was conducted in the presence of 20 equiv of a glycinamide **24** under similar reaction conditions (Scheme 15). HPLC−MS analysis of the isolated crude product (Figure 8, Supporting Information) confirmed formation of approximately 75% of ligation product **19** having a retention time of 28.73 along with ca. 10% of intermolecular trans-acylation product **22**

SCHEME 15. Competitive Experiment 2



having a retention time of 37.92 and ca. 2% of acylation product **25** of glycinamide **24** having a retention time of 27.08. These experiments strongly support the intramolecular characteristic nature of the chemical ligation of N-terminus-unprotected *S*-(Pg-α-aminoacyl)tripeptide **15** to give native tetrapeptide **19** via an 11-membered transition state.

Conclusion

In conclusion, we have successfully synthesized cysteine-containing peptides having free COOH, SH, NH_2, and OH functional groups. We have described selective syntheses of *S*-(Pg-α-aminoacyl) peptides having free hydroxyl, COOH, and/or amino groups in good yields under mild conditions. Microwave-assisted chemical ligation accelerates ligation and demonstrates that the migration of (Pg-α-aminoacyl) groups can take place from a cysteine sulfur to N-terminal amino groups in a peptide via 11- and 14-membered transition states.

The results open many avenues for further investigations, both in the general area of peptide chemistry and also beyond. In peptide chemistry, the selective S-acylation achieved via *N*-acylbenzotriazoles should be of considerable value.

Beyond the question of the viability of cyclic transition states, apart from the well-studied case of transition states with solely carbon atoms in the cycle, a large number of combinations of different atoms in the transition state cycle can now be studied.

The present results indicate that surprises are to be expected and that trans-annular interactions are probably a major factor why the 11- and 14-membered transition states are relatively favored, whereas an 8-membered transition state is significantly disfavored in the range of compounds studied in this present paper.

Experimental Section

Melting points were uncorrected. ^{1}H (300 MHz) and ^{13}C (75 MHz) NMR spectra were recorded on a 300 MHz spectrometer in $CDCl_3$ or DMSO-d_6 with tetramethylsilane as internal standard. THF was distilled from sodium benzophenone ketyl prior to use. All the reactions were performed under a nitrogen atmosphere unless otherwise stated and in oven-dried glassware. N-Cbz- and Fmoc-amino acids were purchased from commercial sources and used without further purification. Microwave experiments were performed on a Discover Benchmate in 10 mL vials from CEM Corp. HPLC−MS analyses were performed on a reversed-phase gradient (2 × 150 mm; 4 um; S/N = 106273−5) plus C18 guard column (2 mm × 4 mm) or dC18 (2.1 × 150 mm; 3 μm; no guard column) using 0.2% acetic acid in H_2O, acetonitrile/methanol as mobile phases; wavelength = 254 nm; and mass spectrometry was done with electrospray ionization (ESI). Optical rotations were measured in methanol at a concentration of 1.0. Elemental analyses of compounds **2c**, **2k**, **3l**, and **16** were outside of 0.4% limits as shown in their experimental data. However, we used these specimens to prepare compounds **3c**, **3k**, **5k**, and **17**, respectively, which passed their elemental analysis/high-resolution mass analysis.

General Procedure for *N*-(Z- and Fmoc-aminoacyl)benzotriazoles 2a−k. Thionyl chloride was added to a solution of benzotriazole in anhydrous THF at 25 °C with stirring. After the mixture was stirred for 30 min, N-protected amino acid was added in one portion, and stirring was continued for 2 h at room temperature. The white precipitate formed during the reaction was filtered and washed with THF. The filtrate was concentrated under reduced pressure. The residue was diluted with ethyl acetate, the solution was washed with 6 N HCl solution or sodium carbonate and dried over $MgSO_4$, and the solvent was removed under reduced pressure. The crude product was recrystallized from ether chloroform−hexanes or ethyl acetate−hexanes.

***N*-Z-L-Thr-Bt (2l).** To the mixture of 3 equiv of benzotriazole and 3 equiv of diisopropylethylamine in dry THF at −30 °C was added 1 equiv of $SOCl_2$, and the solution was stirred for 10 min. One equivalent of *N*-Z-L-Thr-OH was added to the above mixture, and the solution was stirred at −30 °C for 1 h. THF was evaporated, and ethyl acetate was added. After the solution was washed with 4 N HCl and saturated sodium carbonate solution, the organic layer was dried over sodium sulfate and evaporated to give *N*-Z-L-Thr-Bt (**2l**) in 75% yield as white microcrystals: mp 142−143 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.26 (d, J = 6.3 Hz, 3H), 4.30−4.52 (m, 1H), 5.11 (m, 2H), 5.57 (dd, J = 3.3, 8.1 Hz, 1H), 7.25−7.49 (m, 5H), 7.62−7.71 (m, 1H), 7.76 (d, J = 8.1 Hz, 1H), 7.78−7.86 (m, 1H), 8.25 (d, J = 8.1 Hz, 1H), 8.30 (d, J = 8.1 Hz, 1H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 20.1, 60.6, 65.9, 66.7, 113.9, 120.2, 126.7, 127.7, 127.9, 128.3, 130.6, 131.1, 136.7, 145.2, 156.5, 170.0; HRMS (ESI) calcd for $C_{18}H_{18}N_4O_4$ $[M + Na]^+$ 377.1220, found 377.1208.

General Procedure for N-Protected Cysteine-Containing Dipeptides. N-Protected (aminoacyl)benzotriazole was added at room temperature to a solution of L-cysteine in MeCN/H_2O (7:3 mL) in the presence of triethylamine. The reaction mixture

was then stirred at room temperature until the starting material was completely consumed, as observed on TLC using hexanes−ethyl acetate (2:1) as the elutent. Aqueous 6 N HCl solution was then added, and the solvent was removed under reduced pressure. The residue obtained was dissolved in ethyl acetate, and the organic extract was washed with 6 N HCl solution and satd NaCl solution and dried over $MgSO_4$. Evaporation of the solvent gave the desired product, which was recrystallized from ether chloroform/hexanes or ethyl acetate/hexanes.

Z-L-Ala-L-Cys-OH (3b): white microcrystals; yield 79%; mp 169−172 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.22 (d, J = 7.2 Hz, 3H), 2.40 (t, J = 8.7 Hz, 1H), 2.77−2.88 (m, 2H), 4.10−4.15 (m, 1H), 4.40−4.45 (m, 1H), 5.02 (s, 2H), 7.35 (br s, 5H), 7.49 (d, J = 7.3 Hz, 1H), 8.11 (d, J = 7.8 Hz); ^{13}C NMR (75 MHz, DMSO-d_6) δ 18.1, 25.5, 49.9, 54.2, 65.4, 127.7, 127.8, 128.3, 137.0, 155.7, 171.5, 172.6. Anal. Calcd for $C_{14}H_{18}N_2O_5S$: C, 51.52; H, 5.56; N, 8.58. Found: C, 51.89; H, 5.57; N, 8.26.

Z-DL-Ala-L-Cys-OH (3b+3b′): white microcrystals; yield 75%; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.23 (s, 3H), 2.30 (t, J = 8.1 Hz, 1H), 2.41 (t, J = 8.4 Hz, 1H), 2.80 (br s, 1H), 2.80−2.9 (m, 1H), 4.12 (t, J = 6.6 Hz, 1H), 4.40 (br s, 1H), 5.02 (s, 2H), 7.35 (s, 5H), 7.52 (d, J = 5.7 Hz, 1H), 8.08−8.32 (m, 1H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 18.2, 18.6, 25.7, 25.8, 50.1, 54.3, 54.4, 65.6, 127.9, 128.0, 128.5, 137.1, 155.9, 171.6, 172.9. Anal. Calcd for $C_{14}H_{18}N_2O_5S$: C, 51.52; H, 5.56 ; N, 8.52. Found: C, 51.80 ; H, 5.67 ; N, 8.61.

Z-L-Leu-L-Cys-OH (3c): white microcrystals; yield 79%; mp 61−65 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.87 (s, 6H), 1.35−1.52 (m, 2H), 1.55−1.74 (m, 1H), 2.35−2.50 (m, 1H), 2.70−2.95 (m 1H), 4.01- 4.15 (m, 1H), 4.35- 4.45 (m, 1H), 5.02 (s, 2H), 7.35 (br s, 5H), 7.48 (d, J = 8.1 Hz, 1H), 8.15 (d, J = 8.2 Hz, 1H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 21.6, 23.2, 24.3, 25. 6, 40.7, 53.1, 54.5, 65.5, 127.8, 127.9, 128.5, 137.2, 156.1, 171.6,172.6. Anal. Calcd for $C_{17}H_{24}N_2O_5S$: C, 55.42; H, 6.57; N, 7.60. Found: C, 55.42; H, 7.00; N, 7.73.

Z-L-Tyr-L-Cys-OH (3d): white microcrystals; yield 96%; mp 105−107 °C; ^{1}H NMR (300 MHz, DMSO-d_6) (rotameric forms) δ 2.38−2.49 (m, 2H), 2.59−2.68 (m, 2H), 2.74−2.94 (m, 4H), 4.21−4.28 (m, 1H), 4.40−4.45 (m, 1H), 4.95 (s, 2H), 6.65 (d, J = 8.3 Hz, 2H), 7.08 (d, J = 8.3 Hz, 2H), 7.20−7.40 (m, 5H), 7.47 (d, J = 8.7 Hz, 1H), 8.28 (d, J = 7.7 Hz, 1H), 9.19 (s, 1H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 25.7, 36.7, 54.5, 56.5, 65.3, 115.0, 127.5, 127.8, 128.1, 128.4, 130.3, 137.1, 155.9, 156.0, 171.6, 172.0. Anal. Calcd for $C_{20}H_{22}N_2O_6S$: C, 57.40; H, 5.30; N, 6.69. Found: C, 57.19; H, 5.41; N, 6.84.

Z-L-Met-L-Cys-OH (3e): white microcrystals; yield 87%; mp 135−137 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.75−1.90 (m, 2H), 2.03 (s, 3H), 2.40−2.50 (m, 2H), 2.65−2.95 (m, 2H), 4.10−4.20 (m, 1H), 4.35−4.45 (m, 1H), 5.03 (s, 2H), 7.35 (br s, 5H), 7.55 (d, J = 7.8 Hz, 1H), 8.20 (d, J = 7.8 Hz, 1H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 14.7, 25.5, 29.6, 31.8, 53.8, 54.4, 65.5, 127.8, 127.9, 128.4, 137.0, 156.0, 171.6, 171.7. Anal. Calcd for $C_{16}H_{22}N_2O_5S_2$: C, 49.72; H, 5.74; N, 7.25. Found: C, 49.20; H, 5.61; N, 7.21.

Fmoc-L-Phe-L-Cys-OH (3f): white microcrystals; yield 98%; mp 164−166 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.44 (t, J = 8.5 Hz, 1 H), 2.77−2.97 (m, 3H), 3.06 (dd, J = 13.6, 3.3 Hz, 1H), 3.99−4.07 (m, 1H), 4.15−4.19 (m, 3H), 4.33−4.38 (m, 1H), 4.44−4.50 (m, 1H), 7.17−7.44 (m, 9H), 7.62−7.71 (m, 3H), 7.87 (d, J = 7.6 Hz, 2H), 8.35 (d, J = 7.7 Hz, 1H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 25.6, 37.4, 46.6, 54.4, 56.0, 59.8, 65.7, 120.1, 125.3, 125.4, 126.3, 127.1, 127.7, 128.1, 129.3, 138.2, 140.7, 143.8, 143.8, 155.8, 171.5, 171.8. Anal. Calcd for $C_{27}H_{26}N_2O_5S$: C, 66.10; H, 5.34; N, 5.71. Found: C, 65.79; H, 5.61; N, 5.41.

Fmoc-L-Ala-L-Cys-OH (3g): white microcrystals; yield 68%; mp 167−169 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.24 (d, J = 6.7 Hz, 3H), 2.41 (t, J = 8.2 Hz, 1H), 2.75−2.88 (m, 2H), 4.13 (t, J = 7.4 Hz, 1H), 4.19−4.28 (m, 3H), 4.38−4.45 (m, 1H), 7.32 (t, J = 7.1 Hz, 2H), 7.41 (t, J = 7.4 Hz, 2H), 7.58 (d, J = 7.7 Hz, 1H), 7.72 (t, J = 6.4 Hz, 2H), 7.88 (d, J = 7.4 Hz, 2H), 8.13 (d, J = 8.0 Hz, 1H), 12.87 (br s, 1H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 18.1, 25.5, 46.6, 49.8, 54.2, 65.6, 120.1, 125.3, 127.1, 127.7, 140.7, 143.8, 143.9, 155.7, 171.5, 172.7. Anal. Calcd for $C_{21}H_{22}N_2O_5S$: C, 60.85; H, 5.35; N, 6.76. Found: C, 60.70; H, 5.41; N, 6.58.

Fmoc-L-Trp-L-Cys-OH (3h): white microcrystals; yield 78%; mp 106−107 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.40 (t, J = 8.5 Hz, 1H), 2.78−3.01 (m, 3H), 3.12−3.18 (m, 1H), 4.16 (m or s, 3H), 4.38 (m, 1H), 4.44−4.49 (m, 1H), 6.98 (t, J = 7.6 Hz, 2H), 7.06 (t, J = 6.7 Hz, 2H), 7.20−7.43 (m, 6H), 7.56−7.70 (m, 4H), 7.87 (d, J = 7.4 Hz, 2H), 8.34 (d, J = 7.8 Hz, 1H), 10.88 (br s, 1H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 25.5, 27.9, 46.6, 51.6, 54.5, 55.4, 59.8, 65.7,100.1, 110.2, 110.3, 111.3, 118.2, 118.7, 120.1, 120.9, 121.2, 124.0, 125.4, 127.1, 127.3, 127.6, 129.5, 135.4, 136.1, 140.6, 143.7, 143.8, 155.8, 170.4, 171.5, 171.9, 172.2, 172.3; HRMS (ESI) calcd for $C_{29}H_{27}N_3O_5S$ $[M + Na]^+$ 552.1564, found 552.1562.

Fmoc-L-Met-L-Cys-OH (3i): white microcrystals; yield 88%; mp 97−99 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.83−0.86 (m, 1H), 1.24 (br s, 1H), 1.85−1.92 (m, 2H), 1.95−2.05 (m, 3H), 2.42−2.47 (m, 1H), 2.79−2.90 (m, 2H), 4.18−4.35 (m, 4H), 4.42−4.44 (m, 1H), 7.33 (t, J = 6.6 Hz, 2H), 7.43 (t, J = 7.2 Hz, 2H), 7.65 (d, J = 8.1 Hz, 1H), 7.74 (t, J = 5.7 Hz, 2H), 7.90 (d, J = 4.5 Hz, 2H), 8.22 (d, J = 7.5 Hz, 1H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 21.3, 22.8, 24.2, 37.4, 46.5, 50.3, 55.9, 65.6, 120.0, 125.2, 125.3, 126.2, 127.0, 127.6, 128.0, 129.2, 138.1, 140.6, 143.7, 143.8, 155.7, 171.6, 173.9. Anal. Calcd for $C_{23}H_{26}N_2O_5S_2$: C, 58.21; H, 5.52; N, 5.90. Found: C, 58.48; H, 5.53; N, 5.64.

Fmoc-Gly-L-Cys-OH (3j): white microcrystals; yield 84%; mp 90−91 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.42 (t, J = 8.7 Hz, 1H), 2.79−2.88 (m, 2H), 3.70−3.71 (m, 2H), 4.24−4.30 (m, 3H), 4.42−4.50 (m, 1H), 7.33 (t, J = 7.5 Hz, 2H), 7.42 (t, J = 7.5 Hz, 2H), 7.57−7.61 (m, 1H), 7.72 (d, J = 7.5 Hz, 2H), 7.89 (d, J = 7.5 Hz, 2H), 8.18 (d, J = 7.5 Hz, 1H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 25.7, 43.2, 46.6, 54.2, 65.8, 120.1, 125.2, 127.0, 127.6, 140.7, 144.0, 156.5, 169.1, 171.4. Anal. Calcd for $C_{20}H_{20}N_2O_5S$: C, 59.99; H, 5.03; N, 7.00. Found: C, 59.65; H, 4.94; N, 7.01.

Z-L-Ser-L-Cys-OH (3k): white microcrystals; yield 70%; mp 82−84 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.18 (t, J = 7.2 Hz, 1H), 2.97 (br s, 1H), 3.08 (d, J = 6.9 Hz, 1H), 3.18 (br s, 1H), 3.40−3.59 (m, 1H), 3.62 (br s, 1H), 4.13 (s, 1H), 4.50 (s, 1H), 5.04 (s, 2H), 7.24 (br s, 1H), 7.26−7.50 (m, 6H), 8.24 (s, 1H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 25.68, 45.79, 54.43, 57.31, 61.87, 65.66, 127.81, 128.45, 136.99, 156.04, 170.26, 171.45; HRMS (ESI) calcd for $C_{14}H_{18}N_2O_6S$ $[M + Na]^+$ 377.1220, found 365.1208.

General Procedure for Preparation of *S*-(Pg-α-aminoacyl)-cysteines 4a,b. To a suspension of *N*-(protected-α-aminoacyl)-benzotriazoles **2a**,**b** (1 mmol) in acetonitrile (9 mL) was added a solution of cysteine HCl (157 mg, 1 mmol) in water (1 mL) and triethylamine (0.14 mL, 1 mmol). The heterogeneous reaction mixture was stirred at room temperature for 2 h. Concentrated HCl (0.1 mL) was added, and acetonitrile was removed. The residue was filtered, washed with ethyl acetate, and dried to give the corresponding *S*-(Pg-α-aminoacyl)cysteine hydrochlorides **4a**,**b**.

Fmoc-L-Phe-S-L-Cys(NH_3Cl)-OH (4a): white microcrystals; yield 65%; mp 158−160 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.85 (t, J = 11.7 Hz, 1H), 3.17 (dd, J = 13.5, 3.0 Hz, 1H), 3.39 (d, J = 5.7 Hz, 2H), 4.16 (d, J = 6.9 Hz, 2H), 4.25 (d, J = 6.3 Hz, 2H), 4.38- 4.46 (m, 1H), 7.18−7.36 (m, 7 H), 7.42 (t, J = 7.05 Hz, 2H), 7.64 (t, J = 6.0 Hz, 2H),7.89 (d, J = 7.5 Hz, 2H), 8.30 (d, J = 8.7 Hz, 1H), 8.66 (br s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 28.0, 36.1, 46.6, 51.6, 62.6, 65.9, 120.1, 125.3, 126.5, 127.1, 127.7, 128.3, 129.1, 137.4, 140.7, 143.6, 155.9, 169.0, 200.0 Anal. Calcd for $C_{27}H_{27}ClN_2O_5S$: C, 61.53; H, 4.97; N, 5.32. Found: C, 61.86; H, 5.10; N, 5.30.

Fmoc-L-Met-S-L-Cys(NH_3Cl)-OH (4b): white microcrystals; yield 35%; mp 150−152 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.80−1.94 (m, 2H), 1.94−1.99 (m, 1H), 2.04 (s, 3H), 2.41−2.45 (m, 1H), 3.32−3.34 (m, 2H), 4.10−4.14 (m, 1H), 4.26, (t, J = 6.9 Hz, 1H), 4.38−4.39 (m, 3H), 7.34 (t, J = 6.9 Hz, 2H), 7.43 (t, J = 7.2 Hz, 2H), 7.73 (d, J = 6.3 Hz, 2H), 7.91 (d, J = 7.5 Hz, 2H), 8.18 (d, J = 7.5 Hz, 1H), 8.53 (br s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 14.5, 27.8, 29.5, 30.3, 46.7, 51.5, 59.9, 65.8, 120.1, 125.2, 127.1, 127.7, 140.7, 143.6, 143.7, 156.1, 169.0, 200.5. Anal. Calcd for $C_{23}H_{27}ClN_2O_5S_2$: C, 54.05; H, 5.33; N, 5.48. Found: C, 54.05; H, 5.36; N, 5.44.

General Procedure for S-Acylations of Cysteine-Containing Peptides with *N*-Acylbenzotriazoles and *N*-(Pg-α-aminoacyl)-benzotriazoles To Give 5a−l. To a precooled solution of cysteine-containing peptides in MeCN/H_2O (7 mL:3 mL) at 10 °C was added a solution of *N*-acylbenzotriazoles or *N*-(Pg-α-aminoacyl)benzotriazoles in MeCN with stirring followed by addition of $KHCO_3$ for 10 min in four installments. After additional stirring for 20 min at room temperature, the reaction mixture was acidified with 5 N HCl. The solution was then extracted with ethyl acetate, washed with water, and dried over sodium sulfate. Evaporation of the solvent gave the desired product, which was recrystallized from chloroform−hexanes or ethyl acetate−hexanes.

Z-L-Trp-L-Cys-S-(Z-L-Trp)-OH (5a): white microcrystals; yield 88%; mp 73−76 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.85−3.01 (m, 3H), 3.12−3.21 (m, 4H), 4.30− 4.50 (m, 3H), 4.89 (s, 2H), 4.96 (s, 2H), 6.96 (t, J = 8.7 Hz, 3H), 7.06 (t, J = 7.5 Hz, 3H), 7.16−7.21 (m, 6H), 7.27−7.41 (m, 8H), 7.52 (d, J = 9.7 Hz, 1H), 7.66 (d, J = 7.7 Hz, 1H), 8.15 (d, J = 7.1 Hz, 1H), 8.50 (d, J = 7.6 Hz, 1H), 10.83 (d, J = 12.6 Hz, 2H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 20.0, 27.9, 29.6, 51.6, 55.5, 62.2, 65.2, 65.6, 109.6, 110.2, 111.3, 111.5, 118.0, 118.2, 118.5, 120.8, 121.0, 123.9, 126.9, 127.3, 127.4, 127.6, 127.7, 128.3, 128.4, 136.1, 136.7, 136.9, 155.8, 156.0, 171.6, 172.1, 201.0; HRMS (ESI) calcd for $C_{41}H_{39}N_5O_8S$ $[M + Na]^+$ 784.2412, found 784.2431.

Z-L-Trp-L-Cys-S-(Z-L-Ala)-OH (5b): white microcrystals; yield 90%; mp 63−66 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.27(s, 3H), 2.86−3.36 (m, 4H), 4.15−4.50 (m, 3H), 4.80−5.15 (m, 4H), 6.97−7.45 (m, 15H), 7.67 (d, J = 8.2 Hz, 1H), 7.91 (br s, 1H), 8.12 (d, J = 8.3 Hz, 1H), 8.48 (d, J = 7.2 Hz, 1H), 10.8 (s, 1H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 14.1, 17.3, 22.1, 27.9, 29.4, 31.1, 51.6, 55.5, 56.7, 65.3, 65.8, 110.2, 111.4, 118.2, 118.6, 120.9, 124.0, 127.3, 127.5, 127.7, 127.9, 128. 3, 136.1, 136.7, 137.0, 155.8, 155.9, 171.6, 172.1, 201.9; HRMS (ESI) calcd for $C_{33}H_{34}N_4O_8S$ $[M + H_2O + 2H]^+$ 666.2359, found 666.2465.

Z-L-Ala-L-Cys-S-(Z-L-Trp)-OH (5c): white microcrystals; yield 90%; mp 65−67 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.15−1.30 (m, 4H), 1.99 (s, 1H), 2.85−3.05 (m, 2H), 3.06−3.25 (m, 3H), 3.95−4.19 (m, 1H), 4.38 (br s, 1H), 4.85−5.15 (m, 4H), 7.00 (d, J = 7.5 Hz, 1H), 7.08 (t, J = 7.2 Hz, 1H), 7.17 (s, 1H), 7.25 (s, 1H), 7.33 (br s, 8H), 7.46 (d, J = 6.9 Hz, 1H), 7.54 (d, J = 7.2 Hz, 1H), 8.15 (d, J = 7.8 Hz, 1H), 8.29 (d, J = 7.2 Hz, 1H), 10.88 (s, 1H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 18.4, 27.2, 29.7, 50.1, 51.7, 62.3, 65.6, 109.7, 111.7, 118.1, 118.7, 121.2, 124.1, 127.1, 127.5, 127.9, 128.5, 136.3, 136.9, 137.0, 155.8, 156.2, 171.7, 172.8, 201.0; HRMS (ESI) calcd for $C_{33}H_{34}N_4O_8S$ $[M + Na]^+$ 669.1995, found 669.1995.

Z-DL-Ala-L-Cys-S-(Z-L-Trp)-OH (5c+5c′): white microcrystals; yield 90%; ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.86 (s, 1H), 1.23 (s, 4 H), 2.96 (br s, 1H), 3.03−3.30 (m, 3H), 4.10 (br s, 1H), 4.37 (br s, 2H), 4.99 (d, J = 8.1 Hz, 3H), 7.00 (d, J = 6.9 Hz, 1H), 7.08 (br s, 1H), 7.17 (s, 1H), 7.19−7.42 (m, 10H), 7.48 (t, J = 7.8 Hz, 2H), 8.15 (d, J = 7.2 Hz, 1H), 8.22−8.42 (m, 1H) 10.89 (s, 1H), 13.02 (s, 1H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 18.45, 18.63, 27.2, 30.1, 50.2, 51.5, 62.3, 65.7, 65.8, 109.7, 111.7, 118.1, 118.7, 121.3, 124.1, 127.1, 127.6, 127.9, 128.6, 129.4, 136.3, 136.9, 137.1, 155.8, 156.2, 171.7, 172.8, 201.0 Anal. Calcd for $C_{33}H_{34}N_4O_8S$: C, 61.29; H, 5.30; N, 8.66. Found: C, 61.04; H, 5.70; N, 9.15.

Z-L-Tyr-L-Cys-S-(Z-L-Leu)-OH (5d): white microcrystals; yield 85%; mp 84−88 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.83−0.87 (m, 6H), 1.45−1.80 (m, 3H), 2.85−3.20 (m, 4H), 4.10−4.40 (m, 3H), 4.85−5.15 (m, 4H), 6.63 (d, J = 8.3 Hz, 2H), 7.07 (d, J = 8.3 Hz, 2H), 7.19−7.50 (m, 10H), 8.9 (d, J = 7.8 Hz, 1H), 8.41 (d, J = 8.1 Hz, 1H), 9.20 (s, 1H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 21.1, 21.3, 22.8, 22.9, 24.2, 29.4, 36.9, 51.6, 56.5, 59.7, 65.2, 65.8, 114.9, 127.4, 127.5,127.7, 127.8, 127.9, 128.3, 128.4, 130.2, 136.8, 137.1, 155.8, 156.2, 171.6, 171.9, 201.8. Anal. Calcd for $C_{34}H_{39}N_3O_9S$: C, 61.34; H, 5.90; N, 6.31. Found: C, 61.66; H, 6.28; N, 5.95.

Z-L-Met-L-Cys-S-(Z-L-Met)-OH (5e): white microcrystals; yield 90%; mp 146−150 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.75−2.00 (m, 3H), 2.01 (br s, 6H), 2.40−2.65 (br m, 5H), 3.07−3.15 (m, 1H), 3.21−3.35 (m, 1H), 4.10−4.20 (m, 1H), 4.25−4.45 (m, 2H), 5.01−5.15 (m, 4H), 7.35 (br s, 10H), 7.49 (d, J = 8.3 Hz, 1H), 8.13 (d, J = 7.8 Hz, 1H), 8.33 (d, J = 7.8 Hz, 1H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 14.5, 14.7, 29.5, 29.6, 30.6, 31.8, 51.6, 53.8, 60.0, 65.5, 65.9, 127.8, 127.9, 128.4, 136.8, 137.0, 156.0, 156.2, 171.5, 171.6, 173.8, 201.2. Anal. Calcd for $C_{29}H_{37}N_3O_8S_3$: C, 53.44; H, 5.72; N, 6.45. Found: C, 53.63; H, 5.82; N, 6.76.

Z-L-Trp-L-Cys-S-(PhCO)-OH (5f): pink microcrystals; yield 70%; mp 156−158 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.97−2.89 (m, 1H), 3.19−3.13 (m, 2H), 3.67 (dd, J = 13.1,5 Hz, 1H), 4.41−4.35 (m, 1H), 4.59−4.51 (m, 1H), 4.95 (s, 2H), 7.00 (t, J = 7.1 Hz, 1H), 7.1 (t, J = 7.4 Hz, 1H), 7.19−7.38 (m, 7H), 7.46 (d, J = 8.2 Hz, 1H), 7.76−7.56 (m, 4H), 7.96 (d, J = 7.1 Hz, 1H), 8.56 (d, J = 7.6 Hz, 1H), 10.86 (br s, 1H), 13.05 (br s, 1H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 27.8, 29.9,51.6, 55.4, 65.2, 110.1, 111.3, 118.2, 118.5, 120.9, 123.9, 126.9, 127.2, 127.4, 127.7, 128.3, 129.1, 134.1, 136.1, 136.2, 136.9, 155.8, 171.6, 172.1, 190.6. Anal. Calcd for $C_{29}H_{27}N_3O_6S$: C, 63.84; H, 4.99; N, 7.70. Found: C, 63.58; H, 5.19; N, 7.54.

Fmoc-L-Phe-L-Cys-S-(*p*-toluoyl)-OH (5g): white microcrystals; yield 78%; 152−154 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.34 (s, 3H), 2.79 (t, J = 12.6 Hz, 1H), 3.05 (dd, J = 13.2, 3.0 Hz, 1H), 3.29−3.37 (m, 1H), 3.62 (dd, J = 4.8, 13.5 Hz, 1H), 4.07−4.14 (m, 3H), 4.28−4.38 (m, 1H), 4.48−4.53 (m, 1H), 7.19 (d, J = 6.9 Hz, 1H), 7.23−7.32 (m, 9H), 7.40 (t, J = 7.5 Hz, 2H), 7.62 (t, J = 6.0 Hz, 2H), 7.68 (d, J = 8.7 Hz, 1H), 7.79 (d, J = 7.8 Hz, 2H), 7.88 (d, J = 7.2 Hz, 2H), 8.54 (d, J = 8.1 Hz, 1H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 21.2, 29.3, 37.4, 38.8, 46.6, 51.7, 56.1, 65.8, 120.1, 125.3, 125.4, 126.3, 126.7, 127.0, 127.1, 127.7, 128.1, 129.3, 129.6, 133.7, 138.2, 140.7, 143.7, 143.8, 144.6, 155.8, 171.5, 171.8, 190.0. Anal. Calcd for $C_{35}H_{32}N_2O_6S$: C, 69.06; H, 5.30; N, 4.60. Found: C, 68.70; H, 4.92; N, 4.75.

Fmoc-Phe-L-Cys-S-(Fmoc-L-Phe)-OH (5h): white microcrystals; yield 47%; mp 171−173 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.72−2.90 (m, 2H), 3.02−3.19 (m, 4H), 4.06−4.21 (m, 6H), 4.27−4.44 (m, 3H), 7.16−7.41 (m, 18H), 7.56−7.64 (m, 5H), 7.87 (d, J = 7.4 Hz, 4H), 8.23 (d, J = 8.2 Hz, 1H), 8.53 (d, J = 7.7 Hz, 1H), 13.03 (br s, 1H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 29.6, 37.3, 37.5, 46.5, 51.5, 56.0, 59.8, 62.7, 65.7, 65.8, 120.1, 125.3, 125.4, 126.3, 127.1, 127.6, 128.0, 128.2, 129.1, 129.3, 137.4, 138.1, 140.6, 140.7, 143.6, 143.7, 143.8, 155.8, 155.8, 171.6, 171.8, 200.6; HRMS (ESI) calcd for $C_{51}H_{45}N_3O_8S$ $[M + Na]^+$ 882.2820, found 882.2820.

Fmoc-L-Met-L-Cys-S-(Z-L-Leu)-OH (5i): white microcrystals; yield 83%; mp 84−86 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.81−0.86 (m, 6H), 1.24 (br s, 1H), 1.47−1.62 (m, 2H), 1.83−1.93(m, 2H), 2.04 (s, 3H), 2.45−2.48 (m, 1H), 3.05−3.13 (m, 3H), 3.26−3.42 (m, 1H), 4.15−4.31 (m, 5H), 5.07 (s, 2H), 7.30−7.35 (m, 5H), 7.42 (t, J = 7.5 Hz, 2H), 7.59 (d, J = 8.1 Hz, 1H), 7.72 (t, J = 6.9 Hz, 2H), 7.89 (d, J = 7.2 Hz, 2H), 8.07 (d, J = 7.5 Hz, 1H), 8.33 (d, J = 7.5 Hz, 1H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 14.6, 20.9, 22.8, 24.2, 29.3, 29.6, 31.9, 46.7, 51.5, 53.7, 59.6, 65.7, 120.1, 125.3, 127.0, 127.6, 127.8, 128.3, 136.8, 140.7, 143.7, 143.9, 155.8, 156.1, 171.4, 171.5,

201.6. Anal. Calcd for $C_{37}H_{43}N_3O_8S_2$: C, 61.56; H, 6.00; N, 5.82. Found: C, 61.80; H, 6.26; N, 5.45.

Z-L-Ser-L-Cys-S-(Fmoc-L-Phe)-OH (5j): white microcrystals; yield 58%; mp 77−80 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.21 (s, 1H), 2.56−3.00 (m, 1H), 3.05−3.20 (m, 1H), 3.70 (d, J = 4.8 Hz, 1H), 4.05−4.35 (m, 6H), 4.35−4.60 (m, 1H), 5.06 (s, 2H), 5.74 (d, J = 0.3 Hz, 1H), 7.16−7.23 (m, 1H), 7.24−7.33 (m, 8H), 7.34−7.39 (m, 7H), 7.38−7.48 (m, 2H), 7.65 (t, J = 6.6 Hz, 2H), 7.77 (d, J = 8.7 Hz, 1H), 7.87 (d, J = 7.5 Hz, 2H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 21.4, 23.1, 24.3, 36.5, 46.6, 51.5, 53.1, 62.7, 65.5, 120.1, 125.3, 126.6, 127.1, 127.7, 128.4, 129.2, 137.0, 140.7, 143.6, 155.9, 171.6, 172.5, 200.6; HRMS (ESI) calcd for $C_{38}H_{37}N_3O_9S$ $[M + H]^+$ 712.2323, found 712.2355.

Z-L-Thr-L-Cys-S-(Fmoc-L-Phe)-OH (5k): white microcrystals; yield 72%; mp 175−177 °C; ^{1}H NMR (300 MHz, acetone-d_6) δ 1.18 (d, J = 5.7 Hz, 3H), 2.92−3.00 (m, 1H), 3.27−3.38 (m, 2H), 3.55 (dd, J = 13.5, 4.5 Hz, 1H), 4.13−4.16 (m, 1H), 4.25−4.31 (m, 4H), 4.53−4.60 (m, 1H), 4.72−4.80 (m, 1H), 5.02−5.14 (m, 1H), 6.33 (d, J = 7.5 Hz, 1H), 7.20−7.42 (m, 16 H), 7.61 (t, J = 8.7 Hz, 2H), 7.68 (d, J = 8.1 Hz, 1H), 7.84 (d, J = 7.5 Hz, 2H); ^{13}C NMR (75 MHz, acetone-d_6) δ 19.7, 38.4, 48.4, 53.0, 61.0, 64.1, 64.2, 67.5, 67.9, 68.4, 121.2, 126.6, 128.0, 128.4, 129.0, 129.1, 129.7, 130.6, 138.4, 138.6, 142.5, 145.3, 145.4, 157.4, 157.7, 171.6, 172.0, 202.0. Anal. Calcd for $C_{39}H_{41}N_3O_{10}S\cdot H_2O$: C, 62.97; H, 5.56; N, 5.65. Found: C, 62.83; H, 5.29; N, 5.76.

Fmoc-Gly-L-Cys-S-(Z-L-Ala)-OH (5l): white microcrystals; yield 87%; mp 159−161 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.26 (d, J = 6.6 Hz, 3H), 3.08 (dd, J = 13.2, 9 Hz, 1H), 3.25−3.39 (m, 2H), 3.65 (d, J = 5.1 Hz, 2H), 4.19−4.29 (m, 4H), 4.34−4.38 (m, 1H), 5.03−5.07 (m, 2H), 7.31−7.45 (m, 9H), 7.57 (t, J = 6 Hz, 1H), 7.72 (d, J = 7.2 Hz, 2H), 7.90 (d, J = 7.5 Hz, 2H), 8.09 (d, J = 7.2 Hz, 1H), 8.29 (d, J = 7.5 Hz, 1H); ^{13}C NMR (75 MHz, acetone-d_6) δ 18.3, 31.0, 45.0, 48.3, 52.9, 58.4, 67.6, 67.9, 121.2, 126.6, 128.3, 128.9, 129.1, 129.6, 138.2, 142.4, 145.4, 157.4, 157.9, 170.5, 171.9, 202.5. Anal. Calcd for $C_{31}H_{31}N_3O_8S$: C, 61.48; H, 5.16; N, 6.94. Found: C, 61.12; H, 5.22; N, 6.77.

General Procedure for Chemical Ligation of *S*-(Pg-α-aminoacyl)-cysteine Hydrochlorides 4a,b To Form Native Dipeptides 3a,b. The *N*-(Pg-α-aminoacyl)cysteine hydrochloride **4a**,**b** (0.25 mmol) was dissolved in a deoxygenated mixture of water (2 mL), acetonitrile (6 mL), and triethylamine (0.035 mL, 0.25 mmol). The mixture was stirred at room temperature for 1 h under argon. The reaction was acidified to pH 1 using 2 N HCl and extracted with ethyl acetate (10 mL). The ethyl acetate layer was washed with 2 N HCl (2 × 2 mL) and brine (1 × 2 mL) and dried over sodium sulfate. Hexanes (5 mL) were added to ethyl acetate, and the turbid solution was left to crystallize in the freezer overnight. The solid formed was filtered and dried in desiccators to give the corresponding native dipeptide **3a**,**b**.

General Procedure for the Preparation of Dipeptidoyl Benzotriazoles 11a−c. Dipeptides **3j**,**3m**, and **3n** (1 mmol) were treated with 1*H*-benzotriazole (4 mmol) and thionyl chloride (1 mmol) in dichloromethane (20 mL) at −15 °C for 3 h. The resulting mixture was washed with water (5 mL), aqueous sodium carbonate (3 × 10 mL), and brine solution (5 mL) and dried over sodium sulfate. The solvent was evaporated under reduced pressure, and the residue was recrystallized from methylene chloride-hexanes to give dipeptidoyl benzotriazoles **11a−c**.

Z-L-Ala-Gly-Bt (11a): white microcrystals; yield 85%; mp 162−164 °C; ^{1}H NMR (DMSO-d_6) δ 1.33 (d, J = 7.2 Hz, 3H), 4.24 (t, J = 7.5 Hz, 1H), 4.96 (d, J = 5.7 Hz, 1H), 4.99 (d, J = 6 Hz, 1H), 5.05−5.11 (m, 2H), 7.31−7.39 (m, 5H), 7.59 (d, J = 8.1 Hz, 1H), 7.63 (td, J = 0.9, 7.2 Hz, 1H), 7.80 (td, J = 0.9, 6.9 Hz, 1H), 8.22 (d, J = 8.1 Hz, 1H), 8.28 (dt, J = 0.9, 6.6 Hz, 1H), 8.64 (t, J = 5.7 Hz, 1H); ^{13}C NMR (DMSO-d_6) δ 18.2, 42.4, 49.8, 65.3, 113.6, 120.0, 126.5, 127.6, 128.2, 130.4, 130.8, 136.9, 145.1, 155.6, 168.4, 173.4. Anal. Calcd for $C_{19}H_{19}N_5O_4$: C, 59.84; H, 5.02; N, 18.36. Found: C, 60.20; H, 4.84; N, 18.50.

Z-L-Leu-L-Met-Bt (11b): white microcrystals; yield 90%; mp 96−98 °C; ^{1}H NMR (acetone-d_6) δ 0.93 (t, J = 6.3 Hz, 6 H), 1.59−1.66 (m, 2H), 1.72−2.05 (m, 1H), 2.22 (br s, 3H), 2.23−2.28 (m, 1H), 2.70−2.82 (m, 2H), 4.35 (q, J = 5.4 Hz, 1H), 5.09 (s, 2H), 5.99−6.05 (m, 1H), 6.59 (d, J = 9 Hz, 1H), 7.31−7.42 (m, 5H), 7.63 (td, J = 0.9, 7.2 Hz, 1H), 7.79 (td, J = 0.9, 8.4 Hz, 1H), 8.20 (dd, J = 1.2, 8.1 Hz, 1H), 8.27 (d, J = 8.1 Hz, 1H); ^{13}C NMR (acetone-d_6) δ 15.3, 22.3, 23.67, 25.6, 232.1, 42.2, 53.5, 54.4, 67.1, 115.3, 121.2, 127.6, 128.8, 129.4, 131.8, 138.4, 147.1, 157.3, 172.3, 174.0. Anal. Calcd for $C_{25}H_{31}N_5O_4S$: C, 60.34; H, 6.28; N, 14.07. Found: C, 60.55; H, 6.59; N, 13.77.

General Procedure for the Preparation of Tripeptides 12a,b and 13. To dipeptidoyl benzotriazoles **11a−c** (0.5 mmol) in acetonitrile (10 mL) was added a solution of cysteine (0.5 mmol) and triethylamine (0.5 mL) in water (3 mL). The reaction mixture was stirred for 30 min. The reaction mixture was acidified by 6 N HCl and extracted with ethyl acetate (10 mL). The organic layer was washed with 6 N HCl (3 mL) and brine solution (5 mL) and dried over anhydrous sodium sulfate. After evaporation of solvent, the residue was triturated with ether−hexanes (1:1) and the solid formed was filtered and dried under vacuum to give tripeptides **12a**,**b** and **13**.

Z-L-Leu-L-Met-L-Cys-OH (12b): white microcrystals; yield 90%; mp 96−98 °C; ^{1}H NMR (acetone-d_6) δ 0.92 (t, J = 6.0 Hz, 6 H), 1.58−1.66 (m, 2H), 1.69−1.81 (m, 1H), 1.92−1.99 (m, 1H), 2.05 (br s, 3H), 2.08−2.19 (m, 1H), 2.52−2.59 (m, 2H), 2.94−3.01 (m, 2H), 4.25 (dd, J = 8.1, 8.1 Hz, 1H), 4.58−4.66 (m, 1H), 4.71−4.75 (m, 1H), 5.09 (s, 2H), 6.65 (d, J = 7.8 Hz, 1H), 7.29−7.38 (m, 5H), 7.61 (d, J = 7.5 Hz, 1H), 7.71 (d, J = 7.8 Hz, 1H); ^{13}C NMR (acetone-d_6) δ 14.8, 21.6, 23.1, 24.3, 25.5, 29.4, 32.0, 40.6, 51.7, 53.2, 54.4, 65.5, 127.7, 127.8, 128.4, 137.1, 156.0, 171.1, 171.4, 172.5. Anal. Calcd for $C_{22}H_{33}N_3O_6S_2$: C, 52.89; H, 6.66 ; N, 8.41. Found: C, 52.74; H, 6.67; N, 8.11.

Fmoc-Gly-L-Leu-L-Cys-OH (13): white microcrystals; yield 88%; mp 170−172 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.85−0.89 (m, 6H), 1.31−1.63 (m, 2H), 1.63−1.70 (m, 1H), 2.46−2.48 (m, 1H), 2.72−2.92 (m, 2H), 3.66 (d, J = 5.4 Hz, 2H), 4.15−4.32 (m, 3H), 4.37−4.49 (m, 2H), 7.34 (t, J = 7.2 Hz, 2H), 7.43 (t, J = 7.2 Hz, 2H), 7.57 (t, J = 5.7 Hz, 1H), 7.73 (d, J = 7.2 Hz, 2H), 7.90 (d, J = 7.5 Hz, 2H), 8.15 (d, J = 8.1 Hz, 1H), 8.26 (d, J = 7.8 Hz, 1H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 21.7, 23.0, 24.0, 25.3, 41.0, 43.3, 46.6, 50.8, 54.4, 65.7, 120.1, 125.2, 127.1, 127.6, 140.7, 143.8, 156.5, 168.8, 171.4, 172.1. Anal. Calcd for $C_{26}H_{31}N_3O_6S$: C, 60.80; H, 6.08; N, 8.18. Found: C, 60.56; H, 6.21; N, 8.20.

Fmoc-Gly-L-Leu-L-Cys-S-(Z-L-Ala)-OH (14). Compound **14** was prepared according to the method for preparation of compounds **5a−l**: white microcrystals; yield 75%; 174−176 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.85 (t, J = 6.0 Hz, 6H), 1.26 (d, J = 6.6 Hz, 3H), 1.40−1.46 (m, 2H), 1.55−1.64 (m, 1H), 3.07 (dd, J = 13.5, 8.4 Hz, 1H), 3.24−3.23 (m, 1H), 3.66 (d, J = 6.0 Hz, 3H), 4.12−4.28 (m, 5H), 4.35−4.42 (m, 1H), 5.01−5.11 (m, 2H), 7.31−7.45 (m, 9H), 7.54 (t, J = 6.3 Hz, 1H), 7.72 (d, J = 7.5 Hz, 2H), 7.90 (d, J = 7.2 Hz, 2H), 7.94 (d, J = 9.3 Hz, 1H), 8.08 (d, J = 7.2 Hz, 1H), 8.39 (d, J = 7.8 Hz, 1H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 17.2, 21.6, 23.0, 24.0, 29.2, 41.2, 43.3, 46.6, 50.6, 51.5, 56.6, 65.7, 120.1, 125.2, 127.1, 127.6, 127.7, 127.9 128.4, 136.7, 140.7, 143.8, 155.8, 156.5, 168.7, 171.3, 172.0, 201.7. Anal. Calcd for $C_{37}H_{42}N_4O_9S$: C, 61.82; H, 5.89; N, 7.79. Found: C, 61.47; H, 6.08; N, 7.68.

General Procedure for Fmoc Deprotection of *N*-Fmoc-cysteine Peptides 5g, 5i, 5l, 14, and 16. *N*-Fmoc-cysteine peptide **5g**, **5i**, **5l**, **14**, or **16** was dissolved in dry THF at 0 °C under argon. Then, DBU (2 equiv) was added dropwise. The solution was stirred for 15 min, THF was evaporated, the sticky solid was dissolved in 2 N HCl, and the pH of the solution was adjusted to 5 using

Na_2HPO_4. The solid formed was filtered off, washed with water, methanol, and diethyl ether, and dried to give the corresponding unprotected *S*-(Pg-α-aminoacyl)peptides **10a-c**, **15**, or **17**.

Z-L-Phe-Cys-S-(P-toluyl)-OH (10a): white microcrystals; yield 75%; mp 200−202 °C; ^{1}H NMR (DMSO-d_6) δ 2.37 (s, 3H), 2.77 (dd, J = 8.1, 13.2 Hz, 1H), 3.07 (dd, J = 4.5, 13.8 Hz, 1H), 3.34 (dd, J = 6.6, 13.2 Hz, 1H), 3.58 (dd, J = 4.8, 13.2 Hz, 1H), 3.75 (br s, 1H), 4.36 (brs, 1H), 4.73 (br s, 2H), 7.19−7.24 (m, 5H), 7.34 (d, J = 8.1 Hz, 2H), 7.79 (d, J = 7.8 Hz, 2H), 8.54 (br s, 1H); ^{13}C NMR (DMSO-d_6−TFA-d) δ 21.3, 30.0, 37.0, 51.9, 53.4, 127.2, 127.4, 128.8, 129.8, 33.8, 134.9, 145.0, 168.4, 171.1, 190.0; HRMS (ESI) calcd for $C_{20}H_{22}N_2O_4S$ $[M + Na]^+$ 4409.1193, found 409.1180.

H-L-Met-L-Cys-S-(Z-L-Leu)-OH (10b): white microcrystals; yield 90%; mp 148−150 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.83−089 (m, 6H), 1.53 (dd, J = 1.6, 8.7 Hz, 2H), 1.59−1.64 (m, 1H), 1.81−1.92 (m, 3H), 2.04 (s, 3H), 2.51−2.58 (m, 1H), 3.11 (dd, J = 12.6, 6.6 Hz, 1H), 3.31 (dd, J = 12.6, 7.5 Hz, 1H), 3.59 (br s, 1H), 4.14−4.18 (m, 2H), 4.50 (br s, 2H), 5.04−5.13 (m, 2H), 7.33−7.38 (m, 5H), 8.04 (d, J = 8.1 Hz, 1H), 8.37 (br s, 1H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 14.5, 20.9, 22.9, 24.2, 28.8, 30.8, 32.7, 52.4, 52.6, 59.6, 65.7, 127.6 127.8, 128.3, 136.8, 156.1, 170.7, 171.3, 201.9. Anal. Calcd for $C_{22}H_{33}N_3O_6S_2 \cdot H_2O$: C, 51.04; H, 6.81; N, 8.12. Found: C, 50.72; H, 6.68; N, 7.75.

H-Gly-L-Cys-S-(Z-L-Ala)-OH (10c): white microcrystals; yield 88%; mp 188−190 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.25 (d, J = 7.2 Hz, 3H), 3.07 (dd, J = 12.3, 6.6 Hz, 1H), 3.29 (dd, J = 13.5, 3.9 Hz, 1H), 3.47 (d, J = 16.5 Hz, 1H), 3.54 (d, J = 13.8 Hz, 1H), 4.15−4.19 (m, 2H), 5.06 (dd, J = 18.6, 12.3 Hz, 2H), 7.30−7.37 (m, 5H), 8.04 (d, J = 7.8 Hz, 1H), 8.29 (br s, 1H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 17.5, 31.4, 40.9, 52.9, 56.6, 65.7, 127.8, 128.4, 136.8, 155.8, 166.8, 171.4, 202.1. Anal. Calcd for $C_{16}H_{21}N_3O_6S$: C, 50.12; H, 5.52; N, 10.96. Found: C, 50.43; H, 5.67; N, 10.52.

H-Gly-L-Leu-L-Cys-S-(Z-L-Ala)-OH (15): white microcrystals; yield 90%; mp 200−202 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.83−0.89 (m, 6H), 1.24 (d, J = 7.2 Hz, 3H), 1.41−1.65 (m, 3H), 3.06 (dd, J = 12.6, 6 Hz, 1H), 3.30 (dd, J = 12.9, 4.8 Hz, 1H), 3.98−4.01 (m, 2H), 4.14−4.24 (m, 3H), 5.06 (dd, J = 21.3, 11.7 Hz, 2H), 7.30−7.37 (m, 5H), 7.71 (d, J = 6.3 Hz, 1H), 8.01 (d, J = 7.5 Hz, 1H), 8.69 (d, J = 8.1 Hz, 1H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 17.5, 21.4, 23.0, 24.2, 31.1, 40.9, 51.9, 52.5, 56.6, 65.7, 127.7, 128.3, 136.7, 155.7, 167.3, 1701.1, 171.8, 201.8; t_R = 21.75 min; HRMS (ESI) calcd for $C_{22}H_{32}N_4O_7S$ $[M + H]^+$ 497.2064, found 497.2065.

H-Gly-L-Leu-Gly-L-Cys-S-(Z-L-Ala)-OH (17): white microcrystals; yield 70%; ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.85 (br s, 6H), 1.25 (br s, 3H), 1.40−1.70 (m, 3H), 1.80−2.10 (m, 1H), 3.08 (br s, 1H), 3.25 (br s, 1H), 3.68 (br s, 3H), 4.10−4.40 (m, 5H), 5.07 (br s, 2H), 7.37 (br s, 5H), 7.73 (br s, 1H), 7.71 (br s, 1H), 7.98−8.20 (m, 2H); t_R = 27.22 min; HRMS (ESI) calcd for $C_{24}H_{35}N_5O_8S$ $[M + H]^+$ 554.2279, found 554.2270.

General Procedure for Chemical Ligation of N-Terminus-Unprotected *S*-(Pg-α-aminoacyl)dipeptide 10c, *S*-(Pg-α-aminoacyl)-tripeptide 15, and *S*-(Pg-α-aminoacyl)tetrapeptide 17. N-Terminus-unprotected *S*-(Pg-α-aminoacyl)peptide (**10a**, **10c**, **15**, or **17**) (0.10 mmol) was suspended in deoxygenated phosphate buffer (NaH_2PO_4/ Na_2HPO_4) (pH 7.8, 2 mL) containing acetonitrile (0.5 mL) as a cosolvent. The mixture was microwave irradiated (50−70 °C, 50 W, 1 h) in a microwave tube under argon. The reaction was cooled to room temperature, quenched with aqueous solution of TCEP (1 M, 0.1 mL), and stirred for 20 min before acidification with 2 N HCl to pH 1. After extraction with ethyl acetate and the usual workup, the solid obtained was weighed as crude yield. A solution in methanol (2 mL) was then made and analyzed by HPLC−MS.

Acknowledgment. We thank Dr. C. D. Hall for helpful discussions. We also thank Dr. Jodie Johnson for his help in HLPC−MS analyses and Prof. Kenneth B. Wagener and Mr. Bora Inci for their help in preparative HPLC analyses.

Supporting Information Available: HPLC−MS chromatograms, characterization data of compounds **2c**, **2k**, **3a**, **3l**, **12a**, and **16**, and ^{1}H and ^{13}C NMR spectra of compounds. This material is available free of charge via the Internet at http://pubs.acs.org.