# Practical and Efficient Synthesis of Orthogonally Protected Constrained 4-Guanidinoprolines†

Makoto Tamaki, Guoxia Han, and Victor J. Hruby*

*Department of Chemistry, University of Arizona, Tucson, Arizona 85721*

*hruby@u.arizona.edu*

*Received August 27, 2000*

## Introduction

The design, synthesis, and use of conformationally constrained amino acids has emerged as a critical strategy for understanding the interaction of peptides and proteins with their receptors/acceptors. Among the novel amino acids, 4-substituted prolines have been widely studied due in part to the readily available starting material 4-*trans*-hydroxyproline, which has been used as a versatile building block for many biologically important compounds.[1] Of the 20 naturally occurring α-amino acids, proline is the only one with a secondary amino group. When it is incorporated into a peptide or protein, it can induce a reverse turn which can provide enhanced bioactivity. Furthermore, proline residues are present in many peptide/protein sequences having important bioactivities, including α-melanotropin (α-MSH, Ac-Ser-Tyr-Ser-Met-Glu-His-Phe-Arg[8]-Trp-Gly-Lys-Pro[12]-Val-$NH_2$),[2−5] neurotension (NT, Glu-Leu-Tyr-Met-Glu-Asn-Lys-Pro[8]-Arg[9]-Arg[10]-Pro[11]-Tyr-Ile-Leu-OH),[6−10] and substance P (SP, Arg[1]-Pro[2]-Lys-Pro[4]-Gln-Gln-Phe-Phe-Gly-Leu-Met-$NH_2$).[11−15] Hence, design and synthesis of conformationally constrained proline analogues is an important tool to study ligand−receptor interactions.

In addition to proline, arginine also plays an important role in the biological activities of α-MSH, NT, and SP. The importance of arginine often is due to the strong basic guanidino group (**1**), which is almost always protonated at physiological pH and plays an important role in molecular recognition either through hydrogen bonding or electrostatic interactions. For these reasons, we have developed the synthesis of constrained amino acids (**2**) based on proline and arginine (Figure 1).

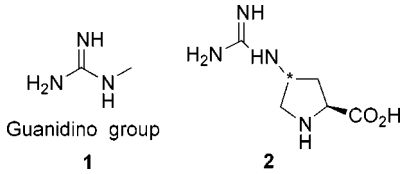



**Figure 1.** Structures of guanidino group and designed proline derivative.

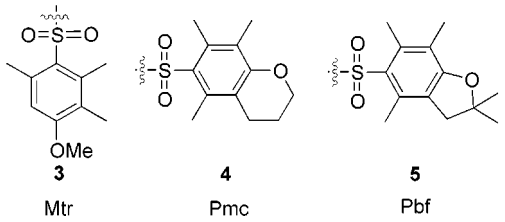


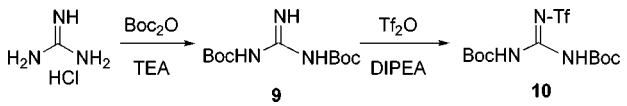

**Figure 2.** Common protecting groups for the guanidino group of Arg used in Fmoc chemistry.

**Retrosynthetic Pathways.** There are several choices [e.g., Mtr (**3**), Pmc (**4**), and Pbf (**5**)] to orthogonally protect the guanidino group of Arg for $N^{\alpha}$-Fmoc chemistry (Figure 2). Due to side reactions, which can occur during the cleavage of the Mtr and Pmc groups, these groups have been replaced, to some extent, by Pbf which has proven to be a better guanidino protecting group of Arg.[16−20] However, recently we have found that Pbf also could be problematic during the peptide synthesis of γ-MSH.[21] The *tert*-butyloxycarbonyl (Boc) group has been extensively used for the protection of amino groups but has seldom been used for the protection of the guanidino group of Arg in the Fmoc chemistry. Recently, Feichtinger et al. prepared *N*,*N*′-diBoc-*N*″-trifluoromethanesulfonylguanidine (**10**)[22] as a versatile guanidinating reagent.[23−29] We have investigated the use of this reagent

* To whom correspondence should be addressed. Tel: 520-621-8617. Fax: 520-621-8407.

† Part of this work was presented at the 2000 Spring ACS meeting in San Francisco, CA.

(1) Remuzon, P. *Tetrahedron* **1996**, *52*, 13803−13835.
(2) Alvaro, J. D.; Tatro, J. B.; Duman, R. S. *Life Sci.* **1997**, *61*, 1−9.
(3) Joost, H. G. *Nieren Hochdruckkr.* **1998**, *27*, 113−117.
(4) Versteeg, D. H. G.; Van Bergen, P.; Adan, R. A. H.; De Wildt, D. J. *Eur. J. Pharmacol.* **1998**, *360*, 1−14.
(5) Schallreuter, K. U. *Cell. Mol. Biol.* **1999**, *45*, 943−949.
(6) Kousseff, B. G.; Hoover, D. L. *Am. J. Med. Genet.* **1999**, *87*, 1−5.
(7) Tritos, N. A.; Maratos-Flier, E. *Neuropeptides* **1999**, *33*, 339−349.
(8) Vincent, J. P.; Mazella, J.; Kitabgi, P. *Trends Pharmacol. Sci.* **1999**, *20*, 302−309.
(9) Hermans, E.; Maloteaux, J. M. *Pharmacol. Ther.* **1998**, *79*, 89−104.
(10) Jolas, T.; Aghajanian, G. K. *Prog. Neurobiol.* **1997**, *52*, 455−468.
(11) Argiolas, A. *Neurosci. Biobehav. Rev.* **1999**, *23*, 1127−1142.
(12) Yew, D. T.; Chan, W. Y.; Luo, C. B.; Zheng, D. R.; Yu, M. C. *Biol. Signals Recept.* **1999**, *8*, 149−159.
(13) James, D. E.; Nijkamp, F. P. *Clin. Exp. Allergy* **1999**, *29*, 1309−1319.
(14) Mau, S. E. *Dan. Med. Bull.* **1999**, *46*, 35−56.
(15) Walsh, D. A. *Rheumatology* **1999**, *38*, 103−112.
(16) Carpino, L. A.; Shroff, H.; Triolo, S. A.; Mansour, E. M. E.; Wenschuh, H.; Albericio, F. *Tetrahedron Lett.* **1993**, *34*, 7829−7832.
(17) Nishino, N.; Xu, M.; Mihara, H.; Fujimoto, T.; Ueno, Y.; Kumagai, H. *Tetrahedron Lett.* **1992**, *33*, 1479−1482.
(18) Stierandova, A.; Sepetov, N. F.; Nikiforovich, G. V.; Lebl, M. *Int. J. Pept. Protein Res.* **1994**, *43*, 31−38.
(19) Prochazka, Z.; Jost, K.; Blaha, K. *Chem. Listy* **1981**, *75*, 699−722.
(20) Fields, C. G.; Fields, G. B. *Tetrahedron Lett.* **1993**, *34*, 6661−6664.
(21) Balse, P.; Hruby, V. J. Unpublished results.
(22) Feichtinger, K.; Zapf, C.; Sings, H. L.; Goodman, M. *J. Org. Chem.* **1998**, *63*, 3804−3805. *N*,*N*′-Di-Boc-guanidine (**9**) was synthesized following a slight modification of the literature.[22] We were able to obtain much better yields than those reported by carefully monitoring the reaction by TLC.

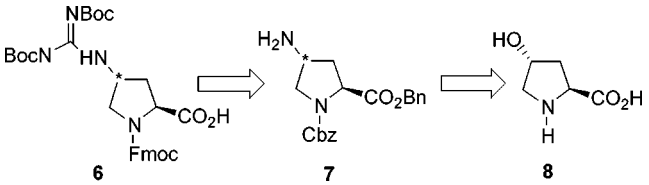



Nucleophilic addition to triflic anhydride gave *N*,*N*′-di-Boc-*N*″-Tf-guanidine(**10**, yield: 90%) which was purified by flash chromatography. We have found that the yield improved if diisopropylethylamine (DIPEA) is used instead of triethylamine (TEA) as the base. In addition, the reaction times were much shorter when DIPEA was used as the base.

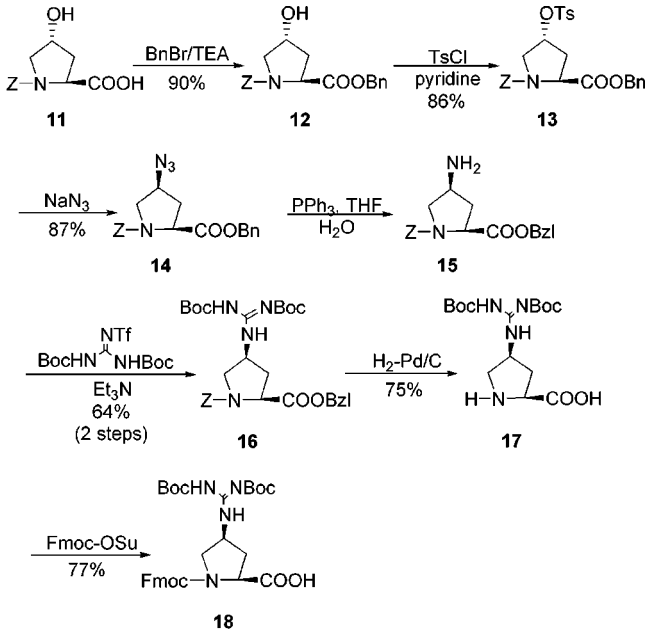
(23) Taylor, E. C.; Liu, B. *Tetrahedron Lett.* **1999**, *40*, 5291−5294.
(24) Baker, T. J.; Goodman, M. *Synthesis* **1999**, 1423−1426.

10.1021/jo005626m CCC: $20.00 
Published on Web 01/16/2001

**Scheme 1. Retrosynthetic Pathway to the Synthesis of Fmoc Proline Derivatives**



**Scheme 2. Synthesis Protected 4-*cis*-Guanidinoproline Derivative**

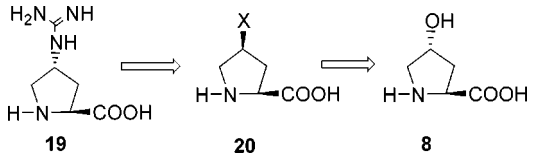


in the synthesis of 4-guanidinoprolines **6** (Scheme 1). To keep the diBoc protection intact during the synthesis, proper protection of the α-amino and acidic groups was essential. For example, groups requiring acid catalysis during protection and deprotection cannot be applied during the synthesis, as it would remove the Boc groups. Moreover, nucleophilic base-sensitive protecting groups for α-amino and acidic groups could not be used, because these would interfere with the guanidination step. In addition, the amino group of the key intermediates, the 4-amino-proline derivatives (**7**, Scheme 1), could cleave the protecting groups. Hence, protecting groups which could be cleaved under neutral condition such as hydrogenolysis were needed, and we chose the benzyloxycarbonyl (Cbz or Z) and benzyl (Bn) protecting groups.

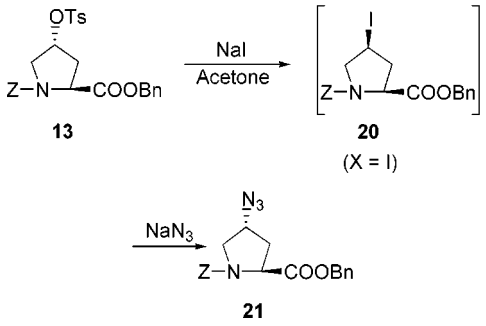
## Results and Discussion

**Synthesis $N^{\alpha}$-Fmoc-4-*cis*-(*N*,*N*′-di-Boc)guanidinoproline.** $N^{\alpha}$-Cbz-4-*trans*-hydroxyproline (**11**, Scheme 2) was first protected as its benzyl ester. The benzyl ester **12** was essentially pure after workup. Tosylation of the 4-hydroxyl group to give **13** was carried out in pyridine.

**Scheme 3. Retrosynthetic Pathway for 4-*trans*-Substituted Proline Derivative**



**Scheme 4. Synthesis of 4-*trans*-Azidoproline Derivative**



$SN_2$ displacement of the tosylate with azide resulted in the 4-*cis*-azide **14** with an inverted chiral center at the 4 position.[30]

Reduction of azide was accomplished under neutral conditions with $PPh_3$ in THF and water. Other possible methods were not suitable for this substrate. TLC showed that the crude product was almost pure and the crude amine **15** was used directly without purification for preparing the di-Boc-protected guanidino proline derivative **16** (Scheme 2).

Guanidino replacement at 4 position was accomplished by the new guanidilating reagent in a very good yield. Due to steric hindrance, the displacement reaction progressed slowly. However, by maintaining the reaction in an inert atmosphere, no significant side reactions occurred, and after 3 days, the reaction was essentially complete. After a quick flash column purification, benzyl $N^{\alpha}$-Cbz-4-*cis*-(*N*,*N*′-di-Boc)guanidinoprolinate **16** was obtained as a colorless oil (64%).

Hydrogenolysis of the fully protected derivative removed the Cbz and Bn groups giving the desired product, 4-*cis*-(N, N′-di-Boc)guanidinoprolinate (**17**, yield: 75%).

The free α-amino group was then protected with Fmoc using Fmoc-OSu and $Na_2CO_3$ in a mixed solvent system. The conditions presented here gave the best yield (75%) among several methods tried. In fact, a poor yield was obtained when TEA (commonly used in Fmoc protection of proline) was used.

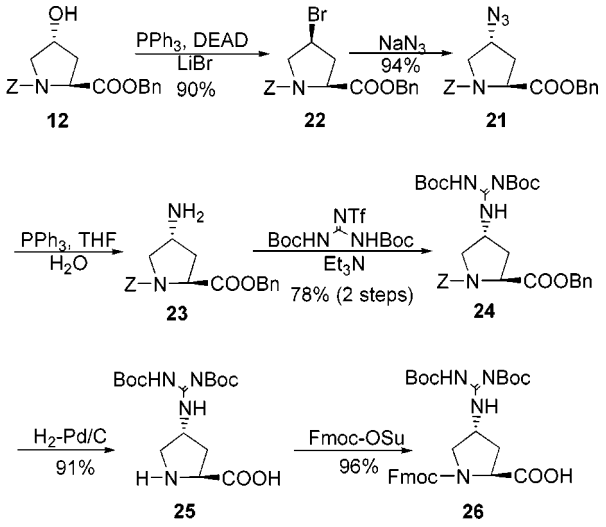
**Synthesis $N^{\alpha}$-Fmoc-4-*trans*-(*N*,*N*′-di-Boc)guanidinoproline.** To synthesize the 4-*trans*-proline isomer **19**, we proposed an $SN_2$ displacement of the 4-*cis*-halide via azide (Scheme 3), followed by reduction of the 4-trans azide to the corresponding *trans*-amine. Thus, the synthesis of the *cis*-4-haloproline became our first objective.

To make the synthesis simple and economical, displacement of the *trans*-4-TsO-proline (**13**) by iodide was carried out (Scheme 4). To our surprise, a mixture of products was obtained. Without separating the crude mixture, azide displacement was carried out immediately. However, the desired 4-*trans*-azide product **21** was

(25) Gothe, R.; Seyfarth, L.; Schumann, C.; Agricola, I.; Reissmann, S.; Lifferth, A.; Birr, C.; Filatova, M. P.; Kritsky, A.; Kibirev, V. *J. Prakt. Chem.* **1999**, *341*, 369−377.
(26) Golding, B. T.; Mitchinson, A.; Clegg, W.; Elsegood, M. R. J.; Griffin, R. J. *J. Chem. Soc., Perkin Trans. 1* **1999**, 349−356.
(27) Watson, S. E.; Markovich, A. *Heterocycles* **1998**, *48*, 2149−2155.
(28) Josey, J. A.; Tarlton, C. A.; Payne, C. E. *Tetrahedron Lett.* **1998**, *39*, 5899−5902.
(29) Dubey, I.; Pratviel, G.; Meunier, B. *Bioconjugate Chem.* **1998**, *9*, 627−632.

(30) It should be noted that, for the tosylation reaction, no significant difference was observed when the crude benzyl ester was used instead of the column purified material.

**Scheme 5. Synthesis of 4-*trans*-Guanidinoproline Derivative**




obtained in a low yield, ~30%. The reaction was repeated, using bromide. Unfortunately, slow rates and low yields of the desired *trans*-product led us to abandon this approach.

An alternative method to invert a chiral center of an alcohol is the Mitsunobu reaction (Scheme 5). When *trans*-4-hydroxy proline derivative **12** was subjected to a Mitsunobu reaction, the corresponding *cis*-bromoproline **22** was obtained in a high yield (90%) after purification.

Azide displacement of Br with $NaN_3$ in DMF afforded the 4-*trans*-azide proline derivative (**21**, 94% after purification). Reduction of azide by $PPh_3$ in THF and water gave the amine **23** which was used directly without purification. Nucleophilic addition of 4-*trans*-aminoproline using the guadinino reagent **10** was much faster than with the *cis* counterpart. The reaction time was 1 day with an improved yield of almost 80% after purification. Hydrogenolysis of the fully protected guanidino compound **24** removed the Cbz and Bn protecting groups and afforded the free proline derivative with free α-amino and acidic groups **25**. Fmoc protection (same protocol as discussed above) provided fully orthogonally protected $N^{\alpha}$-Fmoc 4-*trans*-(*N*,*N*′-di-Boc)guanidinoproline (**26**) in an almost quantitative yield (96%).

In summary, we have successfully synthesized enantiomerically pure proline analogues with 4-guanidino substitutions. These analogues are fully protected for Fmoc chemistry for peptide and protein synthesis. There are a number of advantages over the analogues made for Boc chemistry.[31] Applications of these compounds in the design of novel bioactive ligands are underway.

## Experimental Procedures

All reagents, unless otherwise noted, were purchased from Aldrich Chemical Co. Flash chromatography was performed using Merck silica gel 60 (70−230 mesh). TLC was performed on Whatman silica gel 60A MK6F plates and was developed with ninhydrin in ethanol followed by heating. Optical rotation values were obtained using an automatic polarimeter. High-resolution mass spectra were obtained using the FAB method. NMR spectra were obtained on a 500 MHz instrument equipped with gradient shimming. Solvents used were one of the following deuterated solvents: $CDCl_3$, DMSO-$d_6$, or $CD_3OD$, all of which were purchased from Cambridge Isotopes or Aldrich.

**(2*S*,4*S*)-$N^{\alpha}$-Cbz-4-hydroxylproline Benzyl Ester (12).** Triethylamine (7.7 mL, 55 mmol) was added to the solution of (2*S*,4*S*)-1-(benzyloxycarbonyl)-4-hydroxyl-L-proline (**11**, 13.25 g, 50 mmol) and benzyl bromide (9.4 g, 55 mmol) in tetrahydrofuran (50 mL) at 0 °C. After the mixture was stirred for 18 h at room temperature, the solvent was evaporated in vacuo. The residue was dissolved in 100 mL of $CH_2Cl_2$, washed with HCl (1 N), $H_2O$, $Na_2CO_3$ (5%), and $H_2O$, and then dried over $MgSO_4$ overnight. Evaporation of the solvent under reduced pressure yielded a light yellow oil, which was almost pure after standing overnight under high vacuum. It was further purified by flash chromatoghraphy (eluent, $CHCl_3$/MeOH = 20:1) to give a colorless oil (**12**). Yield: 16.0 g (90%). $[\alpha]^{25}_D = -58.0$ (*c* 1.40, $CHCl_3$). $^1$H NMR ($CDCl_3$): 7.33−7.19 (10 H), 5.21−5.00 (4H), 4.58−4.50 (1H), 4.41 (1H), 3.66−3.51 (2H), 2.59 (1H), 2.31−2.22 (1H), 2.07−2.01 (1H). $^{13}$C NMR (cis and trans conformers): 172.51, 172. 31, 155.03, 154.56, 136.35, 136.15, 135.51, 135.30, 128.48, 128.40, 128.34, 128.28, 128.20, 128.05, 128.00, 127.91, 127.77, 127.71, 69.91, 69.18, 67.19, 66.88, 66.75, 58.03, 57.79, 55.15, 54.55, 39.07, 38.26. HR-MS: calcd for $C_{20}H_{22}NO_5$, $[M + H]^+$ =356.1498, found 356.1503. $R_f = 0.24$ (hexane/ethyl acetate = 5:5).

**(2*S*,4*S*)-$N^{\alpha}$-Cbz-4-[(*p*-Toulenesulfonyl)oxy]proline Benzyl Ester (13).** *p*-Toluenesulonyl chloride (9.45 g, 50 mmol) was added portionwise to a solution of alcohol **12** (16.0 g, 45 mmol) in pyridine (70 mL) at 0 °C. After the mixture was stirred for 12 h at room temperature, the solvent was evaporated in vacuo. The residue was poured into an ice bath (50 g), and the mixture was extracted with ethyl acetate (100 mL × 3). The extract was washed with HCl (0.5 mL, 0.5 M), $H_2O$, $Na_2CO_3$ (10%), $H_2O$, and then dried over $Na_2SO_4$. Concentration of the solvent in vacuo gave an oily residue, which was purified by column chromatography (hexanes−ethyl acetate 7:3) to give as a colorless oil (**13**). Yield: 19.4 g (86%), $[\alpha]^{30}_D = -35.3$ (*c* 1.18, $CHCl_3$). $^1$H NMR ($CDCl_3$): 7.79−7.76 and 7.40−7.20 (14H), 5.23−5.50 (4H), 4.68−4.49 (1H), 3.78−3.65 (2H), 2.61−2.45 (1H and 3H), 2,23−2.12 (1H). $^{13}$C NMR: 171.68, 171.49, 154.42, 153.85, 145.33, 136.12, 136.02, 135.30, 135.08, 133.38, 133.30, 130.09, 130.05, 128.60, 128.51, 128.45, 128.39, 128.20, 128.12, 127.90, 127.74, 78.67, 78.03, 67.43, 67.20, 67.08, 57.62, 57.33, 52.43, 52.11, 37.25, 36.03, 21.66. HR-MS: calcd for $C_{27}H_{28}NO_7S$ $[M + H]^+ = 510.1586$, found 510.1592. $R_f = 0.25$ (hexane/ethyl acetate = 7:3).

**(2*S*,4*S*)-$N^{\alpha}$-Cbz-4-azidoproline Benzyl Ester (14).** Sodium azide (3.30 g, 51 mmol) was added to a solution of tosylate **13** (6.46 g, 12.7 mmol) in DMF (30 mL). The mixture was heated at 65−70 °C for 8 h. The reaction mixture was diluted with water, and the mixture was extracted with ethyl acetate. The extract was washed with brine and then dried over $Na_2SO_4$ overnight. Concentration of the solvent in vacuo gave an oily residue, which was purified by column chromatography (hexanes−ethyl acetate 8:2) to give **14** as a colorless oil, which solidified as a white powder from an ether−hexane solution. Yield: 4.42 g (87%). Mp: 66−68 °C. $[\alpha]^{25}_D = -47.3$ (*c* 0.96, $CHCl_3$). $^1$H NMR ($CDCl_3$): 7.39−7.38 (10H), 5.29−5.06 (4H), 4.60−4.49 (1H), 4.21 (1H), 3.85−3.76 (1H), 3.64−3.56 (1H), 2.52−2.42 (1H), 2.28−2.24 (1H). $^{13}$C NMR: 171.11, 170.85, 154.10, 136.30, 136.22, 135.49, 135.33, 128.56, 128.45, 128.39, 128.30, 128.25, 128.14, 128.06, 128.00, 127.91, 67.40, 67.33, 67.14, 59.26, 58.32, 57.93, 57.68, 51.51, 51.21, 36.14, 35.12. HR-MS: calcd for $C_{20}H_{21}N_4O_4$ $[M + H]^+ = 381.1563$, found 381.1573. $R_f = 0.21$ (hexane/ethyl acetate = 7:3).

**(2*S*,4*S*)-$N^{\alpha}$-Cbz-4-aminoproline Benzyl Ester (15).** A solution of azide **14** (838 mg, 2.2 mmol), tetrahydrofuran (10 mL), triphenylphosphine (1.15 g, 4.4 mmol), and water (0.08 mL, 4.4 mmol) was refluxed for 6 h with stirring and then concentrated in vacuo. The residue was dissolved in diethyl ether (30 mL) and HCl (0.1 N, 20 mL). The aqueous layer was extracted with diethyl ether (30 mL × 2) and was subsequently neutralized with $Na_2CO_3$ (10%), and then the mixture was extracted with dichloromethane (30 mL × 3). The combined $CH_2Cl_2$ layers were dried over $MgSO_4$ overnight, filtered, and concentrated in vacuo to afford amine **15** as a colorless oil, which was used to the next reaction without further purification. A small portion of the crude product was further purified by flash chromatography for analysis. $^1$H NMR ($CDCl_3$): 7.45−7.22 (10H), 5.25−5.00 (4H),

(31) Webb, T. R.; Eigenbrot, C. *J. Org. Chem.* **1991**, *56*, 3009−3016.

4.44−4.35 (1H), 3.77−3.68 (1H), 3.60−3.51 (1H), 3.34−3.28 (1H), 2.47−2.38 (1H), 1.87−1.80 (1H). $^{13}$C NMR: 172.86, 172.73, 154.88, 154.25, 141.20, 136.54, 136.40, 135.59, 135.38, 132.11, 132.04, 131.95, 131.93, 128.61, 128.55, 128.52, 128.49, 128.47, 128.41, 128.36, 128.30, 128.27, 128.23, 128.16, 128.06, 128.01, 127.96, 127.88, 127.83, 127.45, 126.92, 67.32, 67.28, 67.16, 67.14, 67.01, 66.93, 65.08, 65.06, 65.05, 58.44, 58.15, 55.55, 55.11, 51.07, 50.22, 39.46, 38.66. HR-MS: calcd for $C_{20}H_{23}N_2O_4$ $[M + H]^+$ = 355.1658, found 355.1654. $R_f$ = 0.40 ($CHCl_3/CH_3OH$ = 9:1).

**(2*S*,4*S*)-*N*$^\alpha$-Cbz-4-*N*,*N*′-di-Boc-guanidinoproline Benzyl Ester (16).** Compound **15** (2.2 mmol, crude product was used directly) in $CH_2Cl_2$ (5 mL) was added in one portion to a solution of the guanidination reagent **7** (860 mg, 2.2 mmol) and triethylamine (0.31 mL, 2.2 mmol) in $CH_2Cl_2$ (15 mL) at 0 °C, stirred for 3 days at room temperature, and then concentrated in vacuo. The residue was purified by flash column chromatography on silica gel (eluent, hexane/ethyl acetate = 7:3) to give a colorless oil (**16**). Yield: 850 mg (65% from compound **14**, two steps). $[\alpha]^{25}_D$ = −24.0 (*c* 1.06, $CHCl_3$). $^1$H NMR ($CDCl_3$): 8.60−8.55 (1H, NH), 7.32−7.18 (10H), 5.32−4.84 (4H), 4.80−4.77 (1H), 4.48−4.39 (1H), 3.94−3.87 (1H), 3.52−3.43 (1H), 2.62−2.53 (1H), 2.08−1.99 (1H), 1.51−1.45 (18H). $^{13}$C NMR: 172.02, 171.91, 163.10, 155.48, 154.61, 154.05, 152.73, 136.09, 135.97, 135.42, 135.28, 128.40, 128.28, 128.16, 128.06, 127.98, 127.92, 127.87, 127.82, 127.63, 83.27, 79.45, 67.30, 67.20, 66.98, 66.90, 58.11, 57.83, 52.39, 52.00, 49.19, 48.41, 36.54, 35.55, 28.10, 27.87, 27.64. HR-MS: calcd for $C_{31}H_{41}N_4O_8$ $[M + H]^+$ = 597.2924, found 597.2913. $R_f$ = 0.24 (hexane/ethyl acetate = 7:3).

**(2*S*,4*S*)-4-*N*,*N*′-Di-Boc-guanidinoproline (17).** Compound **16** (800 mg, 1.34 mmol) was hydrogenated over palladium on carbon (5%, 80 mg) in methanol (40 mL) for 7 h at room temperature under $H_2$ atmosphere (3 atm). The catalyst was filtered off, and the filtrate was concentrated in vacuo to give a residue which was purified by the recrystallization from methanol/ether/hexane. Yield: 374 mg (75%). Mp: >300 °C (dec, getting dark starting at 285 °C). $[\alpha]^{25}_D$ = −32.5 (*c* 0.31, $CH_3OH$). $^1$H NMR ($CD_3OD$): 4.61−4.59 (1H), 4.01−3.98 (1H), 3.53−3.50 (1H), 3.39−3.36 (1H), 2.73−2.67 (1H), 2.14−2.09 (1H), 1.50 (9H), 1.45 (9H). $^{13}$C NMR ($CD_3OD$): 171.95, 162.61, 155.58, 152.38, 83.33, 79.24, 60.80, 49.88, 49.83, 34.56, 30.88, 27.06, 26.77. HR-MS: calcd for $C_{16}H_{29}N_4O_6$ $[M + H]^+$ = 373.2089, found 373.2083. $R_f$ = 0.43 (*n*-BuOH/AcOH/$H_2O$ = 4:1:1).

**(2*S*,4*S*)-*N*$^\alpha$-Fmoc-4-*N*,*N*′-di-Boc-guanidinoproline (18).** Compound **17** (200 mg, 0.54 mmol) and sodium carbonate (113 mg, 1.08 mmol) were suspended in water (10 mL) and cooled in an ice bath. A solution of Fmoc-OSu (268 mg, 0.8 mmol) in dimethylformamide (10 mL) was added in one portion at 0 °C and stirred at room temperature for 15 min. The mixture was diluted with water (20 mL) and acidified to pH = 2 with HCl (3 M in water). The mixture was extracted with ethyl acetate (3 × 50 mL). The extract was washed with water, dried with $Na_2SO_4$, and evaporated. The crude product was purified by flash chromatography on silica gel (eluent, hexane/ethyl acetate = 7:3 and chloroform/methanol = 20:1) and recrystallization from ether/hexane. Yield: 247 mg (77%). Mp: >300 °C (dec, getting dark starting at 220 °C). $[\alpha]^{25}_D$ = −25.2 (*c* 0.50, $CHCl_3$). $^1$H NMR (DMSO-$d_6$): 8.43−8.39 (1H), 7.87−7.28 (8H), 4.61−4.60 (1H), 4.38−4.12 (1H and 3H), 3.79−3.74 (1H), 3.37−3.32 (1H), 2.64−1.92 (2H), 1.44−1.38 (18H). $^{13}$C NMR: 174.52, 174.08, 163.33, 155.21, 154.19, 152.12, 144.13, 144.05, 141.17, 141.10, 128.18, 128.13, 127.61, 125.65, 120.60, 83.50, 78.89, 67.67, 65.36, 58.16, 57.74, 52.86, 52.26, 49.88, 48.98, 47.11, 46.99, 36.21, 35.07, 28.42, 28.04. HR-MS: calcd for $C_{31}H_{39}N_4O_8$ $[M + H]^+$ = 595.2768, found: 595.2771. $R_f$ = 0.43 ($CHCl_3/CH_3OH$ = 9:1).

**(2*S*,4*R*)-*N*$^\alpha$-Cbz-4-bromoproline Benzyl Ester (22).** Diethyl azodicarboxylate (10.3 g, 59 mmol) was added dropwise with stirring to a solution of triphenylphosphine (15.7 g, 60 mmol) in anhydrous tetrahydrofuran (100 mL) under argon. After 20 min, lithium bromide (10.4 g, 120 mmol) was added to the nearly colorless solution followed by the alcohol **12** (4.27 g, 12 mmol) in anhydrous tetrahydrofuran (50 mL). The mixture was stirred at room temperature for 20 h. After evaporation of the solvent, the residue was poured into water and extracted twice with diethyl ether. The combined ether layer was washed with brine and dried over anhydrous $Na_2SO_4$. After removal of the solvent under reduced pressure, a solid residue was obtained. Purification by flash column chromatography on silica gel (eluent, hexane/ethyl acetate = 8:2) afforded product **22** as a white solid. Yield: 4.52 g (90%). Mp: 82−83 °C. $[\alpha]^{25}_D$ = −42.5 (*c* 1.12, $CHCl_3$). $^1$H NMR ($CDCl_3$): 7.40−7.28 (10H), 5.30−5.04 (4H), 4.58−4.47 (1H), 4.36−4.31 (1H), 4.21−4.11 (1H), 3.88−3.80 (1H), 2.90−2.82 (1H), 2.55−2.46 (1H). $^{13}$C NMR: 171.09, 170.83, 154.28, 153.80, 136.20, 136.14, 135.40, 135.23, 128.54, 128.45, 128.40, 128.32, 128.27, 128.17, 128.08, 128.04, 127.93, 67.48, 67.40, 67.27, 67.20, 58.42, 58.20, 55.90, 55.54, 41.99, 41.30, 40.93, 39.90. HR-MS: calcd for $C_{20}H_{21}BrN_{11}O_4$ $[M + H]^+$ = 420.0652, found 420.0636. $R_f$ = 0.43 (hexane/ethyl acetate = 7:3).

**(2*S*,4*R*)-*N*$^\alpha$-Cbz-4-azidoproline Benzyl Ester (21).** The $SN_2$ reaction between **22** (1.25 g, 3 mmol) and sodium azide (1.32 g, 20 mmol) was performed by the same method as used to prepare **14**. This reaction was performed at room temperature for 1 day. The purification of **21** was performed by column chromatography (hexane/ethyl acetate = 8:2) to give a colorless oil. Yield: 2.15 g (94%). $[\alpha]^{25}_D$ = −51.7 (*c* 1.63, $CHCl_3$). $^1$H NMR ($CDCl_3$): 7.38−7.19 (10H), 5.24−5.00 (4H), 4.55−4.45 (1H), 4.19−4.18 (1H), 3.78−3.73 (1H), 3.68−3.54 (1H), 2.38−2.30 (1H), 2.21−2.14 (1H). $^{13}$C NMR: 171.88, 171.70, 154.56, 154.00, 136.24, 136.13, 135.38, 135.17, 128.63, 128.61, 128.60, 128.55, 128.53, 128.49, 128.45, 128.40, 128.22, 128.15, 128.08, 127.95, 127.89, 67.46, 67.39, 67.19, 67.07, 59.21, 58.64, 57.95, 57.66, 51.81, 51.32, 36.30, 35.26. HR-MS: calcd for $C_{20}H_{21}N_4O_4$ $[M + H]^+$ = 381.1563, found 381.1574. $R_f$ = 0.58 (hexane/ethyl acetate = 7:3).

**(2*S*,4*R*)-*N*$^\alpha$-Cbz-4-aminoproline Benzyl Ester (23).** The selective hydrogenation of azide in **21** (1.52 g, 4 mmol) was performed by the same method as used to prepare **15**. Compound **23**, obtained as a colorless oil, was used to the next reaction without further purification. A small portion of the crude product was further purified by flash chromatography. $^1$H NMR ($CDCl_3$): 7.64−7.18 (10H), 5.21−4.92 (4H), 4.55−4.48 (1H), 3.79−3.73 (1H), 3.70−3.65 (1H), 3.30−3.17 (1H), 2.17−2.11 (1H), 2.08−1.96 (1H), 1.64 (2H). HR-MS: calcd for $C_{20}H_{23}N_2O_4$ $[M + H]^+$ = 355.1658, found 355.1660. $R_f$ = 0.42 ($CHCl_3/CH_3OH$ = 9:1).

**(2*S*,4*R*)-*N*$^\alpha$Cbz-4-*N*,*N*′-di-Boc-guanidinoproline (24).** The coupling between **23** (4 mmol) and guanidination reagent **7** (1.56 g, 4 mmol) was performed by the same method as used to prepare **16**. DIPEA (517 mg, 4 mmol) was used as a base instead of triethylamine. This reaction was performed for 1 day at room temperature. The purification of **24** was performed by flash column chromatography and recrystallization from ether/hexane. Yield: 1.87 g (78% from compound **21**). Mp: 118−119 °C. $[\alpha]^{25}_D$ = −27.7 (*c* 0.50, $CHCl_3$). $^1$H NMR ($CDCl_3$): 11.42 (1H), 8.48−8.47 (1H), 7.34−7.20 (10H), 5.22−5.00 (4H), 4.78−4.76 (1H), 4.56−4.46 (1H), 3.91−3.88 (1H), 3.58−3.43 (1H), 2.32−2.26 (2H), 1.48−147 (18H). $^{13}$C NMR: 171.78, 171.53, 163.22, 155.63, 154.78, 154.16, 136.35, 136.22, 135.44, 135.25, 128.56, 128.46, 128.40, 128.27, 128.16, 128.10, 128.00, 127.82, 83.54, 83.48, 79.54, 67.27, 67.10, 66.97, 57.94, 57.67, 52.60, 52.15, 49.38, 48.75, 36.80, 35.79, 28.22, 28.01. HR-MS: calcd for $C_{31}H_{41}N_4O_8$: $[M + H]^+$ = 597.2924, found 597.2925. $R_f$ = 0.43 (hexane/ethyl acetate = 7:3).

**(2*S*,4*R*)-4-*N*,*N*′-Di-Boc-guanidinoproline (25).** The hydrogenation of **24** (1.50 g, 2.5 mmol) was performed by the same method as used to prepare **17**. Yield: 840 mg (91%). Mp: >300 °C (dec, getting dark starting at 290 °C). $[\alpha]^{25}_D$ = −7.31 (*c* = 0.56, $CH_3OH$). $^1$H NMR (DMSO-$d_6$): 8.24−8.23 (1H), 4.45−4.44 (1H), 3.77−3.74 (1H), 3.43−3.40 (1H), 3.04−3.00 (1H), 2.24−2.06 (2H), 1.45−1.37 (18H). HR-MS: calcd for $C_{16}H_{29}N_4O_6$ $[M + H]^+$= 373.2087, found 373.2085. $R_f$ = 0.5 (*n*-BuOH/AcOH/$H_2O$ = 4:1:1).

**(2*S*,4*R*)-*N*$^\alpha$-Fmoc-4-*N*,*N*′-di-Boc-guanidinoproline (26).** The coupling between **25** (500 mg, 1.34 mmol) and Fmoc-OSu (670 mg, 2 mmol) was performed by the same method as used to prepare **18**. Yield: 770 mg (96%). Mp: 87−89 °C. $[\alpha]^{30}_D$ = −17.0 (*c* 0.55, $CHCl_3$). $^1$H NMR (DMSO-$d_6$): 8.26−8.23 (1H), 7.92−7.27 (8H), 4.55−4.54 (1H), 4.42−4.14 (1H and 3H), 3.73−3.69 (1H), 3.33−3.28 (1H), 2.45−2.13 (2H), 1.44−1.38 (18H). $^{13}$C NMR: 173.95, 173.55, 163.32, 162.73, 155.57, 155.49, 154.32, 154.12, 152.43, 144.19, 144.11, 144.04, 141.17, 141.09, 128.16, 127.61, 127.51, 125.72, 125.67, 125.53, 120.56, 83.63, 79.02, 67.59, 67.34, 58.04, 57.80, 51.83, 51.35, 49.43, 48.50, 47.08, 46.99, 36.21, 35.74, 34.66, 31.40, 31.20, 28.40, 18.10, 22.50. HR-MS: calcd for $C_{31}H_{39}N_4O_8$ $[M + H]^+$ = 595.2768, found: 595.2773. $R_f$ = 0.28 ($CHCl_3/CH_3OH$ = 9:1).

**Acknowledgment.** We thank Dr. Doyle for the use of the polarimeter in his laboratory. This research was supported by grants from the US Public Health Service, Grant Nos. DA06284 and DK17420. The views expressed are those of authors and not necessarily the reflection of those of the USPHS. Mass Spectrometer and NMR facilities in the Department of Chemistry at the University of Arizona were partially funded by grants from the NSF. The help of Dr. M. Kavanara with the manuscript is appreciated.

JO005626M