Triurethane-Protected Guanidines and Triflyldiurethane-Protected Guanidines: New Reagents for Guanidinylation Reactions

Konrad Feichtinger,† Heather L. Sings, Tracy J. Baker, Kenneth Matthews, and Murray Goodman*

Department of Chemistry and Biochemistry, University of California, San Diego, La Jolla, California, 92093-0343

Received July 22, 1998

New guanidinylation reagents are reported. These reagents consist of *N*,*N*′,*N*″-tri-Boc-guanidine (**1**) and *N*,*N*′,*N*″-tri-Cbz-guanidine (**2**), which allow for the facile conversion of alcohols to substituted guanidines. A series of arginine analogues were synthesized via condensation of a primary or secondary alcohol with the guanidinylation reagents **1** or **2**, under Mitsunobu conditions,[1] to produce protected alkylated guanidines. In addition, an extended study of the previously reported reagents[2] *N*,*N*′-di-Boc-*N*″-triflylguanidine (**3**) and *N*,*N*′-di-Cbz-*N*″-triflylguanidine (**4**) is presented. The triflyldiurethane-protected guanidine **3** was utilized to guanidinylate primary and secondary amines under mild conditions with high yield in both solution and on solid phase.

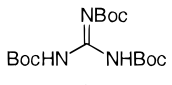
Introduction

The guanidine unit of arginine residues plays an essential role in biologically active proteins, peptides, and peptidomimetics.[3,4] Guanidine itself, the imine of urea, has a strongly basic character (pK_a of guanidinium ion ∼13.5) which renders the side chain of arginine to be fully protonated under physiological conditions. The positive charge thus imposed on the molecule forms the basis for specific interactions between ligand and receptor or enzyme and substrate, mediated by hydrogen bonds and/or electrostatic interactions. In addition to simple arginine-containing peptides, a wide array of structurally diverse molecules that incorporate single or multiple guanidine units have been isolated from microorganisms, terrestrial invertebrates, and marine and freshwater organisms as well as higher plants.[3] Many of these novel metabolites possess unprecedented biological activity ranging from antimicrobial, antiviral, and antifungal to neurotoxic. Hence, guanidine-containing bioactive molecules, particularly the analogues or derivatives of natural products, are notable targets for drug design and discovery. Consequently, procedures that allow for the preparation of guanidines with high yield and under mild conditions are of great interest in medicinal chemistry, and much effort has been directed toward developing efficient synthetic routes for the preparation of these compounds.

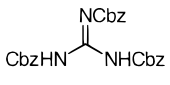
Typically, the synthesis of guanidine-containing compounds involves treatment of an amine with an electrophilic amidine species. The most commonly used reagents include derivatives of pyrazole-1-carboxamidine, *S*-alkylisothioureas, and protected thiourea derivatives, the last mostly used in conjunction with mercury salts or the Mukaiyama reagent.[5−14] Despite recent progress, a completely satisfactory guanidinylating reagent has not yet been developed. For example, most of the guanidinylating reagents present in the literature are limited by the availability of the starting amines, though some examples of the conversion of alcohols to guanidines have been reported.[8] In addition, most of the known guanidinylating reagents are incompatible with solid-phase synthesis. It is apparent that a completely versatile guanidinylating reagent must meet several basic criteria: (1) the reagent should not be limited to simple amines, (2) it should be amenable to solid phase synthesis, (3) it should furnish high yields for a comprehensive array of substrates in a number of organic and/or aqueous solvents, and (4) the reagent should be readily available from inexpensive starting materials.

In a recent report, we introduced the diprotected triflylguanidines, a new class of guanidinylation reagents. These reagents, *N*,*N*′-di-Boc-*N*″-triflylguanidine (**3**) and *N*,*N*′-di-Cbz-*N*″-triflylguanidine (**4**), were utilized to

Triurethane-Protected Guanidines:

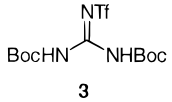


1

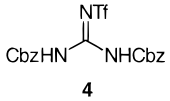


2

Triflyl-Diurethane-Protected Guanidines:



3



4

* To whom correspondence should be addressed. Phone: (619) 534-4466. Fax: (619) 534-0202. E-mail: mgoodman@ucsd.edu.

† Current address: Cytel Corporation, 3525 John Hopkins Ct., San Diego, CA.

(1) Mitsunobu, O.; Yamada, M.; Mukaiyama, T. *Bull. Chem. Soc. Jpn.* **1967**, *40*, 935.

(2) Feichtinger, K.; Zaph, C.; Sings, H. L.; Goodman, M. *J. Org. Chem.* **1998**, *63*, 3804.

(3) Berlinck, R. G. S. *Fortschr. Chem. Org. Naturst.* **1995**, *66*, 119.

(4) Berlinck, R. G. S. *Nat. Prod. Rep.* **1996**, 337.

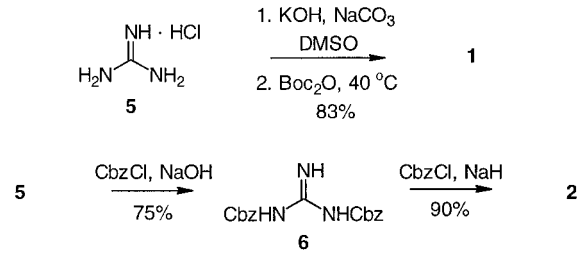
(5) Poss, M. A.; Iwanowicz, E.; Reid, J. A.; Lin, J.; Gu, Z. *Tetrahedron Lett.* **1992**, *33*, 5933.

(6) Bernatowicz, M. S.; Wu, Y.; Matsueda, G. R. *Tetrahedron Lett.* **1993**, *34*, 3389.

(7) Kim, K. S.; Qian, L. *Tetrahedron Lett.* **1993**, *34*, 7677.

10.1021/jo9814344 CCC: $15.00 © 1998 American Chemical Society
Published on Web 10/27/1998

Scheme 1



guanidinylate primary and secondary amines under mild conditions with high yield.[2] We have now modified this class of compounds to include the new guanidinylation reagents *N*,*N*′,*N*″-tri-Boc-guanidine (**1**) and *N*,*N*′,*N*″-tri-Cbz-guanidine (**2**), which allow for the facile conversion of alcohols to substituted guanidines. The scope and limitations of *N*,*N*′,*N*″-tri-Boc-guanidine and *N*,*N*′,*N*″-tri-Cbz-guanidine, as well as an extended examination of the previously described reagents **3** and **4** are reported herein.

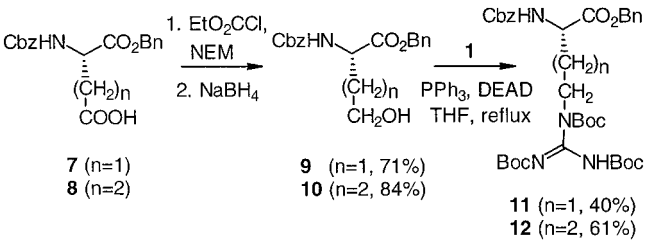
Results and Discussion

Triurethane-Protected Guanidines: Conversion of Alcohols to Guanidines. The triurethane-protected guanidines, *N*,*N*′,*N*″-tri-Boc-guanidine (**1**) and *N*,*N*′,*N*″-tri-Cbz-guanidine (**2**), may be used to convert alcohols to guanidines in one step. The synthetic routes toward these symmetrical trisubstituted guanidines are shown in Scheme 1. The tri-Boc derivative **1** was prepared from guanidine hydrochloride (**5**) in a one-pot procedure with yields up to 83%. Guanidine hydrochloride was added to a solution of potassium hydroxide and sodium carbonate in DMSO. After the addition of di-*tert*-butyl dicarbonate, the mixture was allowed to stir for 60 h at 40 °C. The product was then precipitated by pouring the reaction mixture into cold water and purified by recrystallization from acetonitrile.

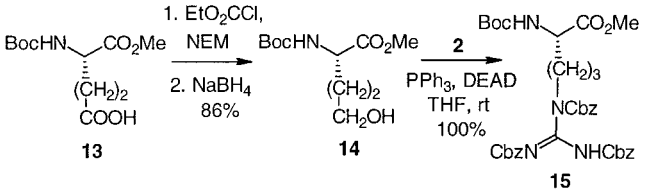
The structure of reagent **1** was confirmed by X-ray analysis. This analysis was necessary to unambiguously determine that reagent **1** was a symmetric molecule, with one Boc group on each of the nitrogen atoms (see Supporting Information). All of the atoms were found to lie in a single plane with the exception of the *tert*-butyl groups. The three carbon−nitrogen bonds of the central guanidine core all have the same bond length, indicating a fully conjugated system.

The synthesis of *N*,*N*′,*N*″-tri-Cbz-guanidine (**2**) was likewise facile, with the diprotection of guanidine hydrochloride accomplished in one step in good yield (75%). The diprotected guanidine **6** was then treated with 2 equiv of sodium hydride under anhydrous conditions followed by acylation of the resulting anion to afford reagent **2**. The crude product was purified by column chromatography.

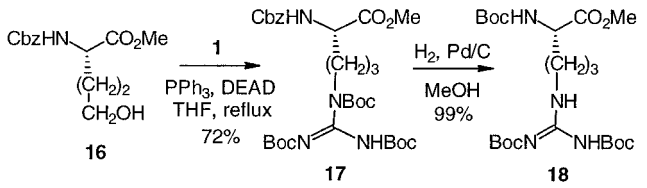
Scheme 2



Scheme 3



Scheme 4



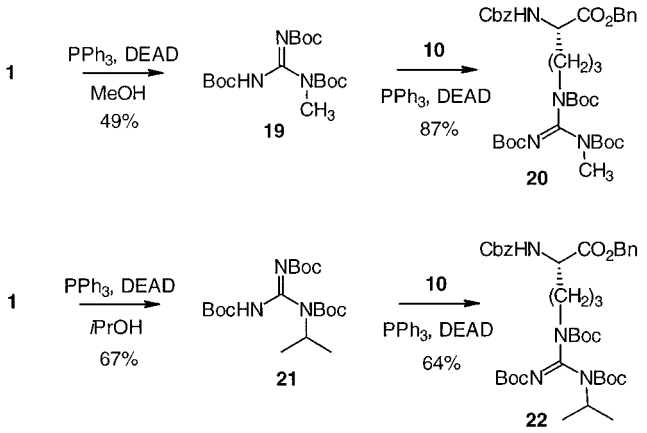
Guanidinylation reagents of the triurethane type react with primary alcohols under Mitsunobu conditions to produce protected alkylated guanidines.[1,8] This was exemplified in the synthesis of several orthogonally protected arginine analogues from suitable precursor molecules (Schemes 2−4). The precursor alcohols **9**,[15] **10**,[15] **14**,[16] and **16** were synthesized from an appropriately protected aspartic or glutamic acid following the procedure of Kokotos.[17] For example, commercially available *N*-Cbz-Asp-OBn (**7**) was first converted to the mixed anhydride derivative with ethyl chloroformate and *N*-ethylmorpholine (NEM). The anhydride was then reduced with sodium borohydride to afford alcohol **9** (Scheme 2).

Introduction of the fully protected guanidine unit was accomplished by condensation of 1 mol equiv of the alcohol with 3.0 equiv of reagent **1** or **2** and 1.5 equiv each of triphenylphosphine and diethylazodicarboxylate (DEAD). Reactions with the tri-Boc derivative **1** were most effectively carried out in refluxing THF or toluene, and yields of up to 72% were obtained (Schemes 2 and 4). If reagent **2** was used as the guanidinylating species, the reaction could be carried out at room temperature in THF (Scheme 3). In addition, yields for reactions with the tri-Cbz derivative **2** were somewhat higher than those for comparable reactions with the tri-Boc derivative **1**, ranging from 86% to quantitative yield. In contrast to the traditional syntheses of guanidine-containing compounds, this new synthesis uses an alcohol as the starting material, thus opening an additional route to a wide range of arginine analogues. In addition, the optical integrity of the α-carbon stereocenter is maintained.

During the initial studies on the conversion of alcohol **10** to the protected arginine analogue **12**, a side product

(8) Dodd, D. S.; Kozikowski, A. P. *Tetrahedron Lett.* **1994**, *35*, 977.
(9) Drake, B.; Patek, M.; Lebl, M. *Synthesis* **1994**, 579.
(10) Katriztky, A. R.; Parris, R. L.; Allin, S. M.; Steel, P. S. *Synth. Commun.* **1995**, *25*, 1173.
(11) Kent, D. R.; Cody, W. L.; Doherty, A. M. *Tetrahedron Lett.* **1996**, *37*, 8711.
(12) Levallet, C.; Lerpiniere, J.; Ko, S. Y. *Tetrahedron* **1997**, *53*, 5291.
(13) Yong, Y. F.; Kowalski, J. A.; Lipton, M. A. *J. Org. Chem.* **1997**, *62*, 1540.
(14) Bergeron, R. J.; McManis, J. S. *J. Org. Chem.* **1987**, *52*, 1700.
(15) Rosenthal, G. A.; Dahlman, D. L.; Crooks, P. A.; Phuket, S. N.; Trifonov, L. S. *J. Agric. Food Chem.* **1995**, *43*, 2728.
(16) Akimoto, H.; Ootsu, K.; Ito, F. *JP Patent 06220060*; Japan, p 32.
(17) Kokotos, G. *Synthesis* **1990**, 299.

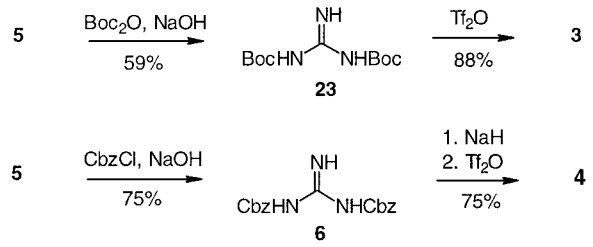
Scheme 5



was isolated that resulted from condensation of compound **12** and a second equivalent of alcohol **10**. The overconsumption of compound **10** could be avoided if an excess (3.0 equiv) of the guanidinylation reagent was employed. Nevertheless, this observation led to the examination of the inherent potential of compound **1** to generate N′-alkylated arginine analogues. Many biologically interesting guanidines contain two different alkyl groups in a *N*,*N*′-substitution pattern, which are now easily accessible from reagent **1** through two consecutive Mitsunobu reactions. An example is given in Scheme 5 with the synthesis of protected *ω*-methylarginine (**20**), an important inhibitor of nitric oxide synthethase. The tri-Boc reagent **1** was converted to the *N*-methyl derivative **19** by reaction of **1** with MeOH, PPh_3, and DEAD. This derivative was then used in a second Mitsunobu reaction with alcohol **10** to afford compound **20** in good yield (87%). As an additional example, isopropyl alcohol was used to produce the alkylated arginine derivative **22** (Scheme 5). The ability to generate dialkylated guanidines provides an excellent tool for the preparation of novel alkylated arginine analogues. It should be noted that some highly reactive alcohols, such as prenyl and benzyl alcohol, react with the tri-Boc reagent **1** to produce the dialkylated side product exclusively.

Deprotection of the arginine analogues can be achieved either through acidolysis or hydrogenolysis. The ability to use either the Boc derivative **1** or the Cbz derivative **2** allows for greater choice in protection and deprotection strategies. It should be noted that when *N*-Cbz-L-arginine methyl ester (**17**) was subjected to catalytic hydrogenation, L-*N*-Boc-*ω*,*ω*′-di-Boc-arginine methyl ester (**18**) was isolated in nearly quantitative yield (Scheme 4). Compound **18** appears to result from transfer of the *δ*-Boc group of the guanidine side chain to the free amine, which is generated upon removal of the Cbz group. This rearrangement may occur in either an intermolecular reaction or via an intramolecular pseudo-seven-membered transition state. The positions of the three Boc groups as shown for compound **18** were confirmed via total correlation spectroscopy (TOCSY). The TOCSY spectrum showed correlation between the *δ*-NH and the α, *β*, *γ*, and *δ* protons of this amino acid derivative, confirming that the Boc groups of the guanidine side chain existed in a *ω*,*ω*′-substitution pattern. This side reaction can be avoided if arginine is not the *N*-terminal residue or if no free amine is present in the molecule, as in the cases described for the conversion of methanol and isopropyl alcohol to the guanidine derivatives **19** and **21**.

Scheme 6



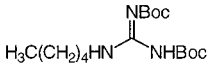
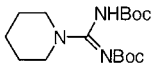
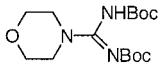
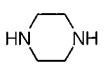
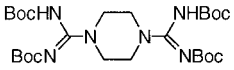
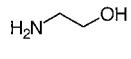
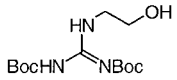
Clearly, the outstanding feature of this new class of guanidinylation reagents is that primary and secondary alcohols may be converted to a fully protected guanidine derivative in one step in good to excellent yield.

Triflyldiurethane-Protected Guanidines: Conversion of Amines to Guanidines. In a previous report, we introduced the triflyldiurethane-protected guanidines *N*,*N*′-di-Boc-*N*″-triflylguanidine (**3**) and *N*,*N*′-di-Cbz-*N*″-triflylguanidine (**4**).[2] We have found these triflylguanidines to be the most reactive among the reagents synthesized thus far, and initial kinetic studies propound that they are superior to existing guanidinylating reagents.[2] To further demonstrate the utility of these reagents, we have synthesized a number of different arginine and simple amine derivatives in both solution and on solid phase.

The guanidinylation agents, *N*,*N*′-di-Boc-*N*″-triflylguanidine and *N*,*N*′-di-Cbz-*N*″-triflylguanidine, were obtained in two steps from guanidine hydrochloride (**5**) as previously reported (Scheme 6).[2] Reaction of compound **5** with Boc-anhydride under strongly alkaline conditions produced the intermediate *N*,*N*′-di-Boc-guanidine (**23**),[8] which was easily converted to the target compound **3** with triflic anhydride. In a similar sequence of reactions, the synthesis of reagent **4** commenced with the conversion of guanidine hydrochloride to *N*,*N*′-di-Cbz-guanidine (**6**). The diprotected guanidine **6** was then deprotonated with sodium hydride, followed by treatment of the resulting anion with triflic anhydride to generate compound **4** in good yield (75%). The triflyldiurethane-protected guanidines **3** and **4** are stable, crystalline substances that have been stored at room temperature for at least 3 months with no apparent loss of activity; hence, they should remain stable indefinitely if refrigerated. The X-ray crystal structure of reagent **3** (see Supporting Information) revealed that the triflyl and Boc groups reside on separate nitrogen atoms and, of the three central carbon−nitrogen bonds, the one that bears the triflyl group has the shortest bond length (1.31 Å). Although the remaining two carbon−nitrogen bonds are predicted to have the same length, the crystal structure showed them to be significantly different (1.37 vs 1.34 Å). The difference in bond length might be due to intra- and/or intermolecular hydrogen bonding, both of which were observed in the packing model of the crystal structure.

In a recent communication,[2] a series of structurally different amines was subjected to reaction with *N*,*N*′-di-Boc-*N*″-triflylguanidine (**3**) or *N*,*N*′-di-Cbz-*N*″-triflylguanidine (**4**). In a typical reaction, a slight excess of the amine was added in one portion to a solution of either **3** or **4** and 1 equiv of triethylamine, and the course of the reaction was monitored by TLC. After the reaction was complete, triethylamine and the byproduct triflic amide were removed during a simple aqueous workup proce-

Table 1. Guanidinylation of Amines Using *N,N′*-Di-Boc-*N″*-triflylguanidine (3)

entry	Amine	Product[a]	Yield (%)[b]
1	$H_3C(CH_2)_4NH_2$		98
2[c]			85[d]
3[e]			86
4			100
5			100

[a] See experimental section for reaction conditions. [b] Isolated yield after chromatography. [c] Reference 13. [d] Guanidinylation of piperidine with reagent 4 resulted in a 15% yield of the corresponding product. [e] Reference 9.

dure. Typically, the crude products obtained in this way were greater than 95% pure as evidenced by TLC and ^{1}H NMR. To summarize the results of our previous findings, reagent **3** reacted rapidly with primary and secondary amines under mild conditions. The reactions were typically carried out at room temperature and were usually complete within 1 h. Difficulties were only observed with highly sterically hindered amines. For example, *tert*-butylamine reacted rather sluggishly at room temperature though a good yield was obtained in refluxing dichloromethane. Equally impressive were the results obtained with reagent **4**. If a direct comparison is made between the triflyldiurethane-protected guanidines **3** and **4**, little difference is observed for the guandinylation of benzylamine, though compound **4** gave a slightly higher yield for aniline.[2]

Results from further investigation of these reagents are shown in Tables 1 and 2. For example, reagent **3** may be successfully employed in the guanidinylation of compounds containing more than one amine in quantitative yield (entry 4, Table 1). In addition, reagent **3** displayed compatibility with functional groups such as ethers (entry 3, Table 1) and amino alcohols (entry 5, Table 1). However, the guanidinylation of secondary amines using di-Cbz-triflylguanidine **4** was less efficient than that with di-Boc-triflylguanidine **3**. Piperidine, for example, showed minimal product formation using reagent **4**, though an acceptable yield was obtained with reagent **3** (entry 2, Table 1) under similar conditions. In general, *N,N′*-di-Cbz-*N″*-triflylguanidine (**4**) is an excellent reagent for the guanidinylation of unreactive aromatic amines, though for practical purposes, *N,N′*-di-Boc-*N″*-triflylguanidine (**3**) is superior overall.

To understand the reactivity of the triflyldiurethane-protected guanidines **3** and **4**, one must consider that they not only behave as electrophiles but also behave as weak acids, which may explain the variation in reaction rates in solvents of differing polarity. Polar solvents may facilitate deprotonation of **3** or **4** by the amine, thus slowing the guanidinylation process. Furthermore, the di-Cbz reagent **4** is slightly more acidic than the di-Boc reagent **3**, which may clarify the decreased product formation observed with reagent **4** and piperidine in CH_2Cl_2. Addition of 1 equiv of a tertiary base was found to accelerate the reaction in most cases; hence, triethylamine is added as a standard procedure. However, even with the addition of triethylamine, reactions using either **3** or **4** are still slower in protic solvents than in nonpolar solvents. Therefore, the guanidinylation reactions are preferably carried out in CH_2Cl_2 or $CHCl_3$.

Application to Solid Phase Synthesis. The guanidinylation of three separate resin-bound amines was examined using the general procedure as described for reagent **3**, and these results are summarized in Table 2. For example, the hexapeptide Boc-Gly-Orn(Mtt)-Gly-Asp(*t*Bu)-Ser(*t*Bu)-Pro (entry 1, Table 2) was assembled on a Rink-amide[18] resin by successive coupling of Fmoc-protected amino acids using Fastmoc chemistry on an Applied Biosystems peptide synthesizer. The 4-methyltrityl (Mtt) protecting group of the Orn residue was removed with TFA, and the free amino function was guanidinylated by treatment with a solution of *N,N′*-di-Boc-*N″*-triflylguanidine (**3**) and triethylamine in CH_2Cl_2 for 8 h. A negative Kaiser test[19] indicated that the reaction went to completion, and the peptide was cleaved from the resin with concomitant removal of the protecting groups by treatment with TFA. The resin was removed via filtration, and the combined filtrates were concentrated and cooled on an ice bath, and the peptide was precipitated with cold ether. The peptide was isolated by centrifugation to afford H-Gly-Arg-Gly-Asp-Ser-Pro-NH_2 as a white powder (77%), which was characterized by HPLC and HRMS. In addition, the same peptide sequence was assembled on a phenylacetamidomethyl resin (PAM)[20] using Boc-protected amino acids (entry 2, Table 2). The free amino functionality of the Orn residue was guanidinylated as described for the Rink-amide-bound peptide. The peptide was cleaved from the resin using HF, and the product, H-Gly-Arg-Gly-Asp-Ser-Pro-OH, was isolated in quantitative yield by centrifugation as described above. The peptide was then characterized by HPLC and HRMS.

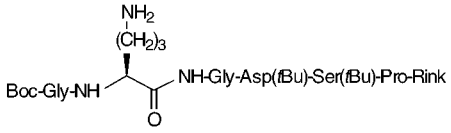
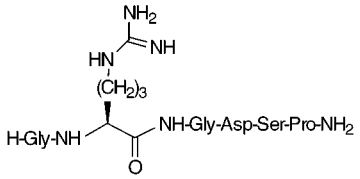
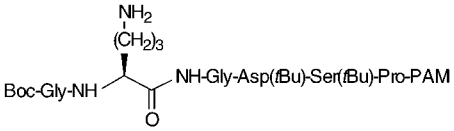
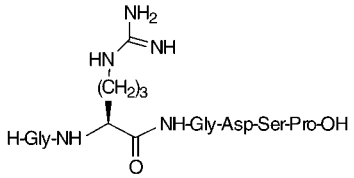
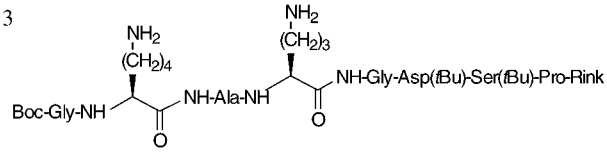
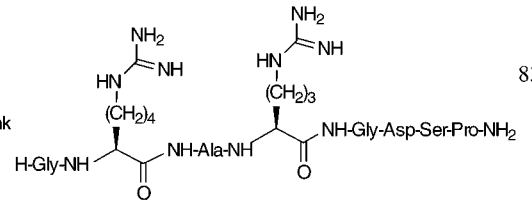
As a final example of the ease and efficiency of using reagent **3** in the guanidinylation of resin-bound amines, the octapeptide Boc-Gly-Lys(Mtt)-Ala-Orn(Mtt)-Gly-Asp(*t*Bu)-Ser(*t*Bu)-Pro, which contains two potential sites for guanidinylation, was assembled on a Rink-amide resin (entry 3, Table 2). The peptide was assembled as described for H-Gly-Arg-Gly-Asp-Ser-Pro-NH_2 and the product H-Gly-hArg-Ala-Arg-Gly-Asp-Ser-Pro-NH_2 was isolated as an off-white powder (83%).

Conclusion

In summary, new routes for the guanidinyation of amines and alcohols have been reported. The preparation and product isolation for the triurethane-protected guanidines (**1** and **2**) and the triflyldiurethane-protected guanidines (**3** and **4**) are facile and less demanding than some known guanidinylation reagents. Efficient routes

(18) Rink, H. *Tetrahedron Lett.* **1987**, *28*, 3787.
(19) Kaiser, E.; Colescott, R. L.; Bossinger, C. D.; Cook, P. I. *Anal. Biochem.* **1970**, *34*, 595.
(20) Mitchell, A. R.; Erickson, B. W.; Ryabtsev, M. N.; Hodges, R. S.; Merrifield, R. B. *J. Am. Chem. Soc.* **1976**, *98*, 7357.
(21) Data not shown.

Table 2. Guanidinylation of Resin-Bound Amines Using *N,N′*-Di-Boc-*N″*-triflylguanidine (3)

Entry	Amine	Product	Yield (%)[a]
1			77
2			100
3			83

[a] Total yield of peptide after cleavage from resin. HPLC analysis indicated that these molecules were greater than 95% pure.

for the conversion of both alcohols and amines using reagents **1**−**4** have been presented, and we have shown reagent **3** to be well suited for solid-phase organic synthesis. Reactions using compound **3** may be carried out successfully in a wide range of solvents such as benzene,[21] chloroform, dichloromethane, and acetonitrile.[21] Remarkably, we have been successful in the guanidinylation of several aminoglycosides using reagent **3** in aqueous media and these results will be presented in a future publication. We believe that our new reagents will find widespread use in the synthesis of both simple and highly complex guanidine-containing compounds.

Experimental Section

General. Unless otherwise noted, all materials were obtained from commercial suppliers and used without further purification. Analytical thin-layer chromatography was carried out on precoated silica gel plates (Kieselgel 60 F254, E. Merck & Co, Germany). Flash column chromatography was performed using silica gel (230−400 mesh) from J. T. Baker. All NMR spectra were obtained on a 360 MHz spectrometer assembled at UCSD with a pulse programmer, digitizer, and an Oxford Instruments superconducting magnet. Chemical shifts (δ) are in ppm; multiplicities are indicated by s (singlet), br s (broad singlet), d (doublet), t (triplet), q (quartet), or m (multiplet). Coupling constants (J) are reported in hertz. Mass spectra were obtained at the Scripps Research Institute, La Jolla, CA. Elemental analysis was performed by Desert Analytics, Tucson, AZ. Independent analysis of purity for noncrystalline material was achieved using analytical HPLC (Vydac C_{18}, 25 cm × 0.46 cm, 5 μm) performed on a Millennium 2010 system consisting of a Waters 715 Ultra WISP sample processor, a Waters TM 996 photodiode array detector, two Waters 510 pumps, and a NEC Power Mate 486/33I computer. Solvents used in HPLC analysis were as follows: solvent A, H_2O/0.1%TFA; solvent B, CH_3CN/0.1% TFA. Flow rate = 1.0 mL/min. X-ray crystallography was performed at UCSD.

General Procedure A (GPA) for the Mitsunobu Reaction Using Reagent 1. In a typical reaction, a solution of the alcohol (1.0 mmol), reagent **1** (3.0 mmol), and PPh_3 (1.5 mmol) in anhydrous THF (20 mL) was cooled to −5 °C under an inert atmosphere. DEAD (1.5 mmol) was added dropwise at a rate such that the reaction mixture was completely colorless before addition of the next drop. After the addition was complete, the reaction mixture was heated at reflux for 15−18 h. (Note: reagent **1** is only partially soluble in THF at room temperature. The solution becomes clear in refluxing THF). The solution was then cooled to room temperature, and the precipitate of excess **1** that formed was collected by filtration and washed with a mixture of THF/hexanes 1:1. The filtrate was concentrated in vacuo, and the product was isolated by flash column chromatography on silica gel. Modifications of the molar concentrations described in GPA are indicated in the experimental text.

General Procedure B (GPB) for the Guanidinylation of Amines and/or Amino Acids Using Reagent 3 or 4. In a typical reaction, the amine (0.5 mmol) was added neat to a solution of **3** or **4** (0.45 mmol) and triethylamine (0.5 mmol) in CH_2Cl_2 (2 mL, filtered over neutral alumina) or $CHCl_3$, and the mixture was allowed to stir at room temperature until **3** or **4** was consumed as evidenced by TLC. After the reaction was complete, the mixture was diluted with CH_2Cl_2 or $CHCl_3$ (3 mL) and washed with 2 M sodium bisulfate, saturated sodium bicarbonate, and brine. The organic extract was then dried over sodium sulfate and filtered, and the solvent was removed under reduced pressure. The crude products were further purified by flash column chromatography to afford material suitable for elemental analysis or reversed-phase analytical HPLC.

***N,N′,N″*-Tri-Boc-guanidine (1).** Potassium hydroxide pellets (2.81 g, 50 mmol) and sodium carbonate (5.30 g, 50 mmol) were finely ground in a mortar and transferred to a three-necked round-bottomed flask equipped with a magnetic stirrer and a reflux condenser. DMSO (50 mL) was added, and the resulting suspension was stirred for 5 min at room temperature. Guanidine hydrochloride (**5**, 4.78 g, 50 mmol) was added, and the mixture was stirred for 5 min. After the addition of di-*tert*-butyl dicarbonate (51.7 g, 225 mmol), the mixture was stirred for 60 h at 40 °C. The white precipitate obtained by pouring the cold reaction mixture into 1 L of water was collected by filtration, washed with H_2O, and dried overnight in vacuo. Recrystallization from acetonitrile yielded colorless needles (14.9 g, 83%). mp 147−150 °C. ^{1}H NMR ($CDCl_3$) δ 1.48 (s, 27H). FABMS *m/z* (relative intensity) 360 (100, {M + H}$^+$), 304 (34), 260 (10), 248 (74). Anal. Calcd for

$C_{16}H_{29}N_3O_6$: C, 53.47%; H, 8.13%; N, 11.69%. Found: C, 53.48%; H, 8.34%; N, 11.86%.

***N,N′,N″*-Tri-Cbz-guanidine (2).** Sodium hydride (400 mg, 60% dispersion in mineral oil) was added in small portions to a suspension of **6** (1.65 g, 5.0 mmol) in anhydrous THF (20 mL) at −45 °C under an argon atmosphere. After the addition was complete, the mixture was allowed to stir for 1 h at −45 °C. Benzyl chloroformate (0.82 mL, 5.0 mmol) was added, and the mixture was allowed to warm to room temperature and stir overnight. The solvent was evaporated in vacuo, and the residue was dissolved in a mixture of CH_2Cl_2 (50 mL) and H_2O (25 mL). The phases were separated, and the aqueous layer was extracted with CH_2Cl_2 (2 × 50 mL). The extracts were combined, washed with 1 N HCl and H_2O, and dried with magnesium sulfate. After filtration and evaporation of the solvent, the crude product was purified by flash column chromatography on silica gel (eluent, CH_2Cl_2/ethyl ether 98:2). *N,N′,N″*-Tri-Cbz-guanidine (**2**, 2.07 g, 90%) was obtained as a white powder. mp 111−112 °C. ^{1}H NMR (DMSO-d_6) δ 10.55 (s, 2H), 7.36 (s, 10H), 5.11 (br s, 6H). FABMS *m/z* (relative intensity) 484 (100, $\{M + Na\}^+$), 462 (24, $\{M + H\}^+$). Anal. Calcd for $C_{25}H_{23}N_3O_6$: C, 65.07%; H, 5.02%; N, 9.11%. Found: C, 64.89%; H, 4.74%; N, 8.82%.

***N,N′*-Di-Boc-*N″*-trifluoromethanesulfonylguanidine (3).** A solution of *N,N′*-di-Boc-guanidine (**23**, 0.52 g, 2.0 mmol) and triethylamine (0.29 mL, 2.1 mmol) in anhydrous CH_2Cl_2 (10 mL) was cooled to −78 °C under an inert atmosphere. Triflic anhydride (0.35 mL, 2.1 mmol) was added dropwise at a rate such that the reaction temperature did not exceed −65 °C. After the addition was complete, the mixture was allowed to warm to room temperature within 4 h. The solution was transferred to a separation funnel, washed with 2 M sodium bisulfate and H_2O, and dried with anhydrous sodium sulfate. After filtration and removal of the solvent under reduced pressure, the crude product was purified by flash column chromatography on silica gel (eluent, CH_2Cl_2). *N,N′*-Di-Boc-*N″*-trifluoromethanesulfonylguanidine (**3**, 686 mg, 88%) was obtained as pale-yellow crystals. The product was further purified by recrystallization from hexanes. mp 114−115 °C. ^{1}H NMR (DMSO-d_6) δ 11.45 (br s, 2H), 1.45 (s, 18H). FABMS *m/z* (relative intensity) 414 (16, $\{M + Na\}^+$), 392 (13, $\{M + H\}^+$), 336 (43), 280 (100), 236 (9). Anal. Calcd for $C_{12}H_{20}F_3N_3O_6S$: C, 36.83%; H, 5.15%; N, 10.74%; F, 14.56%; S, 8.19%. Found: C, 36.93%; H, 5.21%; N, 10.66%; F, 14.80%; S, 8.33%.

***N,N′*-Di-Cbz-*N″*-trifluoromethanesulfonylguanidine (4).** Sodium hydride (400 mg, 60% dispersion in mineral oil) was added to a solution of *N,N′*-di-Cbz-guanidine (**6**, 1.65 g, 5.0 mmol) in anhydrous chlorobenzene (50 mL) at 0 °C under an argon atmosphere. After being stirred for 1 h at 0 °C, the mixture was cooled to −45 °C. Triflic anhydride (0.84 mL, 5.0 mmol) was added, and the mixture was allowed to warm to room temperature and stir overnight. The solvent was removed under reduced pressure, and the residue was dissolved in a mixture of ethyl acetate (100 mL) and 2 M sodium bisulfate (25 mL). The phases were separated, and the organic layer was washed with H_2O and brine and dried with magnesium sulfate. After filtration and removal of the solvent under reduced pressure, the crude product was purified by flash column chromatography on silica gel (eluent, CH_2Cl_2/ethyl ether 95:5). Compound **4** (1.58 g, 75%) was obtained as a pale-yellow oil that crystallizes in vacuo. mp 74−75 °C. ^{1}H NMR (DMSO-d_6) 11.55 (br s, 2H), 7.45−7.28 (m, 10H), 5.20 (s, 4H). Electrospray MS *m/z* (relative intensity) 498 (30, $\{M + K\}^+$), 482 (100, $\{M + Na\}^+$), 460 (2, $\{M + H\}^+$). Anal. Calcd for $C_{18}H_{16}F_3N_3O_6S$: C, 47.06%; H, 3.51%; N, 9.15%; F, 12.41%; S, 6.98%. Found: C, 47.37%; H, 3.35%; N, 8.67%; F, 12.79%; S, 6.92%.

***N,N′*-Di-cbz-guanidine (6).** CH_2Cl_2 (80 mL) was added to a solution of guanidine hydrochloride (**5**, 3.82 g, 40 mmol) and sodium hydroxide (8.0 g, 0.20 mol) in H_2O (40 mL), and the resulting mixture was cooled to 0 °C. Benzyloxycarbonyl chloride (17.1 mL, 120 mmol) was added dropwise with vigorous stirring over a period of 45 min. After the addition was complete, stirring was continued for 20 h at 0 °C. The mixture was diluted with CH_2Cl_2 (100 mL), the layers were separated, and the aqueous layer was extracted with CH_2Cl_2 (100 mL). The extracts were combined, washed with H_2O, and dried with magnesium sulfate. After filtration and removal of the solvent under reduced pressure, the crude product was recrystallized from methanol. Compound **6** (9.85 g, 75%) was obtained as colorless crystals. mp 149−150 °C. ^{1}H NMR (DMSO-d_6) δ 10.88 (br s, 1H), 8.67 (br s, 2H), 7.40−7.25 (m, 10H), 5.10 (s, 4H); Anal. Calcd for $C_{17}H_{17}N_3O_4$: C, 62.38%; H, 5.23%; N, 12.84%. Found: C, 62.26%; H, 5.01%; N, 12.79%.

L-*N*-Cbz-*δ,ω,ω′*-tri-Boc-desmethylenoarginine Benzyl Ester (11). To a solution of **1** (1.08 g, 3.0 mmol) and PPh_3 (400 mg, 1.5 mmol) in dry toluene (25 mL) was added **9** (340 mg, 1.0 mmol) in toluene (4.1 mL). A solution of DEAD (0.24 mL, 1.5 mmol) in toluene was added dropwise to the above solution at a rate such that the reaction mixture was completely colorless before addition of the next drop. After the addition was complete, the reaction mixture was allowed to stir at 60 °C for 4 h. The reaction mixture turned clear after ca. 3 h. Upon cooling the solution to room temperature, a precipitate of excess *N,N′,N″*-tri-Boc-guanidine formed, which was collected by filtration and washed with toluene. The filtrate was concentrated in vacuo and the product (**11**, colorless oil, 272 mg, 40%) was isolated by flash column chromatography on silica gel (eluent, EtOAc/hexanes 1:4). ^{1}H NMR (DMSO-d_6) δ 10.62 (s, 1H), 7.38−7.29 (m, 10H), 6.25 (d, 1H, J = 8.6 Hz), 5.14 (br s, 2H), 5.09 (br s, 2H), 4.44 (m, 1H), 3.85 (q, 2H, J = 7.2 Hz), 2.19 (m, 2H), 1.45 (s, 9H), 1.42 (s, 18H). HRFABMS calcd for $C_{35}H_{48}N_4O_{10}$ (M_r) 817.2425 $(M + Cs)^+$, found 817.2445 Δ = 2.4 ppm. Analytical HPLC conditions: 220 nm, 30:70 A/B to 10:90 A/B over 30 min, t_R = 27.0 min.

L-*N*-Cbz-*δ,ω,ω′*-tri-Boc-arginine Benzyl Ester (12). Compound **12** was prepared from (*S*)-*N*-Cbz-2-amino-5-hydroxyvaleric acid benzyl ester (**10**) according to GPA. The product **12** was isolated as a colorless oil (0.426 g, 61%; eluent, CH_2Cl_2/ethyl ether 92:8). ^{1}H NMR (DMSO-d_6) δ 10.20 (s, 1H), 7.75 (d, 1H, J = 7.5 Hz), 7.36−7.29 (m, 10H), 5.04 (d, 2H, J = 12.9 Hz), 4.99 (d, 2H, J = 12.9 Hz), 4.05 (m, 1H), 3.47 (m, 2H), 1.75−1.53 (m, 4H), 1.39−1.37 (3 s, 27 H). HRFABMS calcd for $C_{36}H_{50}N_4O_{10}$ (M_r) 721.3425 $(M + Na)^+$, found 721.3445 Δ = 2.8 ppm. Analytical HPLC conditions: 220 nm, 50:50 A/B to 5:95 A/B over 30 min, t_R = 24.6 min.

L-*N*-Boc-*δ,ω,ω′*-tri-Cbz-arginine Methyl Ester (15). To a solution of *(S)*-*N*-Boc-2-amino-5-hydroxy-valeric acid methyl ester (**14**, 80 mg, 0.33 mmol) in THF (4 mL) were added **2** (450 mg, 0.98 mmol) and PPh_3 (130 mg, 0.49 mmol) under an inert atmosphere. The mixture was cooled to 0 °C, and DEAD (0.10 mL, 0.49 mmol) was added dropwise at a rate such that the reaction mixture was completely colorless before addition of the next drop. After the addition was complete, the reaction mixture was allowed warm to room temperature and stir overnight. The reaction was quenched with H_2O, and the solvent was evaporated under reduced pressure. The product (**15**, colorless oil, 220 mg, 100%) was isolated by flash column chromatography on silica gel (eluent, CH_2Cl_2/ethyl ether 94:6). ^{1}H NMR (DMSO-d_6) δ 10.89 (s, 1H), 7.39−7.30 (m, 15H), 7.20 (d, 1H, J = 7.8 Hz), 5.09 (br s, 2H), 5.04 (br s, 2H), 4.99 (br s, 2H), 3.90 (m, 1H), 3.52 (br s, 5H), 1.67−1.50 (m, 4H), 1.34 (s, 9H). HRFABMS calcd for $C_{36}H_{42}N_4O_{10}$ (M_r) 691.2979 $(M + H)^+$, found 691.2952 Δ = 3.9 ppm. Analytical HPLC conditions: 220 nm, 30:70 A/B to 10:90 A/B over 30 min, t_R = 7.2 min.

L-*N*-Cbz-*δ,ω,ω′*-tri-Boc-arginine Methyl Ester (17). Compound **17** was prepared and purified according to GPA with the following modifications: *(S)*-*N*-Cbz-2-amino-5-hydroxyvaleric acid methyl ester (**16**, 0.92 g, 3.27 mmol), **1** (5.9 g, 6.4 mmol), PPh_3 (1.3 g, 5.9 mmol), THF (50 mL), DEAD (0.93 mL, 5.9 mmol). The product (**17**) was isolated as a colorless oil (1.46 g, 72%; eluent, CH_2Cl_2/ethyl ether 9:1). mp 37−41 °C. ^{1}H NMR (DMSO-d_6) δ 10.18 (s, 1H), 7.72 (d, 1H, J = 7.9 Hz), 7.40−7.26 (m, 5H), 5.01 (s, 2H), 4.03−3.94 (m, 1H), 3.6 (s, 3H), 3.45 (t, 3H, J = 5.8 Hz), 1.73−1.45 (m, 4H), 1.39−1.37 (3 s, 27H). HRFABMS calcd for $C_{30}H_{46}N_4O_{10}$ (M_r) 645.3112 (M +

$Na)^+$, found 645.3125 $\Delta = 2.0$ ppm. Analytical HPLC conditions: 220 nm, 60:40 A/B to 20:80 A/B over 30 min, t_R= 27.4 min.

L-*N*-Boc-*ω,ω′*-di-Boc-arginine Methyl Ester (18). Compound **17** (1.46 g, 2.3 mmol) was dissolved in MeOH (20 mL). After 10% Pd/C (150 mg) was added, the solution was allowed to stir under an atmosphere of H_2 for 4 h. The product was filtered over Celite, and the solvent was evaporated to give an oil, which was precipitated with ether to give **18** as a white foam (1.05 g, 99%). ^{1}H NMR ($CDCl_3$) δ 11.39 (s, 1H), 8.88 (br s, 1H), 4.85 (m, 2H), 3.73 (s, 3H), 3.15 (m, 2H), 1.82 (m, 2H), 1.49 (m, 2H), 1.49−1.41 (3 s, 27H). HRFABMS calcd for $C_{22}H_{40}N_4O_8$ (M_r) 511.2744 $(M + Na)^+$, found 511.2754 $\Delta = 2.0$ ppm. Analytical HPLC conditions: 220 nm, 40:60 A/B to 20:80 A/B over 30 min, t_R = 8.7 min.

***N*-Methyl-*N,N′,N″*-tri-Boc-guanidine (19).** Compound **19** was prepared according to GPA with the following modifications: anhydrous methanol (0.04 mL, 1.0 mmol), **1** (539 mg, 1.5 mmol), PPh_3 (393 mg, 1.5 mmol), DEAD (0.24 mL, 1.5 mmol). The mixture was allowed to cool to room temperature, and the solvent was evaporated under reduced pressure. The crude product was suspended in MeOH (10 mL), and the precipitate of excess *N,N′,N″*-tri-Boc-guanidine that formed was collected by filtration and washed with MeOH. The filtrate was concentrated in vacuo, and the product (**19**) was isolated as a colorless oil (182 mg, 49%; eluent, CH_2Cl_2/ethyl ether 98:2). FABMS *m/z* (relative intensity) 396 (100, $\{M + Na\}^+$), 374 (13, $\{M + H\}^+$); ^{1}H NMR (DMSO-d_6) δ 10.17 (s, 1H), 2.94 (s, 3H), 1.43−1.36 (3 s, 27H). HRFABMS calcd for $C_{17}H_{31}N_3O_6$ (M_r) 374.2291 $(M + H)^+$, found 374.2298 $\Delta = 1.9$ ppm. Analytical HPLC conditions: 220 nm, 50:50 A/B to 5:95 A/B over 30 min, t_R = 14.8 min.

L-*N*-Cbz-*ω*-methyl-*δ,ω,ω′*-tri-Boc-arginine Benzyl Ester (20). Compound **20** was prepared according to GPA with the following modifications: *(S)*-*N*-Cbz-2-amino-5-hydroxyvaleric acid benzyl ester (**10**, 97 mg, 0.273 mmol), **19** (66 mg, 0.177 mmol), PPh_3 (0.11 g, 0.408 mmol), anhydrous THF (3.5 mL), DEAD (84 μL, 0.408 mmol). The reaction mixture was allowed to cool to room temperature and the solvent was evaporated in vacuo. The product (**20**) was isolated as a colorless oil (113 mg, 87%; eluent, ethyl acetate/hexanes 1:3). ^{1}H NMR (DMSO-d_6) δ 7.80 (d, 1H, J = 7.9 Hz), 7.39−7.28 (m, 10H), 5.10 (s, 2H), 5.06−4.94 (m, 2H), 4.11−4.00 (m, 1H), 3.53−3.44 (m, 2H), 2.89 (s, 3H), 1.75−1.50 (m, 4H), 1.40−1.34 (3 s, 27H). HRFABMS calcd for $C_{37}H_{52}N_4O_{10}$ (M_r) 845.2738 $(M + Cs)^+$, found 845.2709 $\Delta = 3.4$ ppm. Analytical HPLC conditions: 220 nm, 30:70 A/B to 10:90 A/B over 30 min, t_R = 12.2 min.

***N*-Propyl-*N,N′,N″*-tri-Boc-guanidine (21).** Compound **20** was prepared according to GPA with the following modifications: isopropyl alcohol (0.07 mL, 0.92 mmol), **1** (0.50 g, 1.4 mmol), PPh_3 (0.365 g, 1.4 mmol), DEAD (0.22 mL, 1.4 mmol). The filtrate was concentrated in vacuo, and the product (**21**) was isolated as a colorless oil (249 mg, 67%; eluent, EtOAc/hexanes 1:4). ^{1}H NMR ($CDCl_3$) δ 1.50−1.44 (2 s, 27H), 1.48 (m, 1H, beneath *tert*-butyl groups), 1.34 (d, 6H, J = 6.7 Hz). HRFABMS calcd for $C_{19}H_{35}N_3O_6$ (M_r) 402.2604 $(M + H)^+$, found 402.2613 $\Delta = 2.2$ ppm. Analytical HPLC conditions: 220 nm, 50:50 A/B to 5:95 A/B over 30 min, t_R = 18.6 min.

L-*N*-Cbz-*ω*-propyl-*δ,ω,ω′*-tri-Boc-arginine Benzyl Ester (22). Compound **22** was prepared according to GPA with the following modifications. To a solution of PPh_3 (0.11 g, 0.402 mmol) and (*S*)-*N*-Cbz-2-amino-5-hydroxyvaleric acid benzyl ester (**10**, 96.0 mg, 0.268 mmol) in THF (4 mL) was added **21** (0.243 g, 0.604 mmol) via cannulation. After the addition of DEAD (84 μL, 0.402 mmol) was complete, the reaction mixture was heated at reflux for 15 h. The mixture was allowed to cool to room temperature, and the solvent was evaporated under reduced pressure. The filtrate was concentrated in vacuo, and the product (**22**) was isolated as a colorless oil (126 mg, 64%; eluent, EtOAc/hexanes 1:4). ^{1}H NMR ($CDCl_3$) δ 7.36−7.26 (m, 10H), 5.73 (br s, 1H), 5.16 (br s, 2H), 5.09 (br d, 2H, J = 2.9 Hz), 4.06 (m, 1H), 3.61 (m, 2H), 1.86 (m, 1H), 1.74−1.68 (m, 4H), 1.45−1.41 (3 s, 27H), 1.23 (d, 6H, J = 6.5 Hz). HRFABMS calcd for $C_{39}H_{56}N_4O_{10}$ (M_r) 873.3051 $(M + Cs)^+$, found 873.3019 $\Delta = 3.7$ ppm. Analytical HPLC conditions: 220 nm, 70:30 A/B to 10:90 A/B over 30 min, t_R = 16.6 min.

***N,N′*-Bis(*tert*-butoxycarbonyl)guanidine (23).** 1,4-Dioxane (50 mL) was added to a solution of guanidine hydrochloride (**5**, 2.39 g, 25 mmol) and sodium hydroxide (4.0 g, 0.1 mol) in H_2O (25 mL), and the resulting mixture was cooled to 0 °C. Di-*tert*-butyl dicarbonate (12.0 g, 55 mmol) was added in one portion, and the reaction mixture was allowed to warm to room temperature within 2 h. After being stirred for 20 h, the mixture was concentrated in vacuo to one-third of its original volume. The resulting suspension was diluted with H_2O (50 mL) and extracted with ethyl acetate (3 × 50 mL). The crude product is a mixture of *N*-Boc-guanidine, *N,N′*-bis-(*tert*-butoxycarbonyl)guanidine (**23**), and *N,N′,N″*-tri-Boc-guanidine (**1**). The combined extracts were washed with 10% citric acid (*N*-Boc-guanidine remains in the acidic layer and may be recovered by cooling the acidic layer in an ice bath, adjusting the pH to 9.0, and extracting with EtOAc), H_2O, and brine and dried with magnesium sulfate. After filtration and removal of the solvent under reduced pressure, the crude product was purified by flash column chromatography on silica gel (eluent, 100% CH_2Cl_2 to CH_2Cl_2/MeOH 97:3). *N,N′*-Bis-(*tert*-butoxycarbonyl)guanidine (**23**, 3.84 g, 59%) was obtained as a colorless powder. mp 144−145 °C. ^{1}H NMR (DMSO-d_6) 10.42 (br s, 1H), 8.47 (br s, 2H), 1.39 (s, 18H). FABMS *m/z* (relative intensity) 260 (50, $\{M + H\}^+$), 204 (48), 148 (100). Anal. Calcd for $C_{11}H_{21}N_3O_4$: C, 50.95%; H, 8.16%; N, 16.21%. Found: C, 50.83%; H, 8.04%; N, 16.26%.

***N,N′*-Bis(*tert*-butyloxycarbonyl)-*N″*-pentylguanidine (Entry 1, Table 1).** *N,N′*-Bis(*tert*-butyloxycarbonyl)-*N″*-pentylguanidine was prepared according to GPB (solvent = CH_2Cl_2, reaction time = 1 h, rt). mp 73−74 °C. ^{1}H NMR (DMSO-d_6) δ 11.48 (s, 1H), 8.26 (br s, 1H), 3.25 (q, 2H, J = 8.5 Hz), 1.52−1.16 (m, 6H), 1.46 (s, 9H), 1.37 (s, 9H), 0.85 (t, 3H, J = 8.5 Hz). Anal. Calcd for $C_{16}H_{31}N_3O_4$: C, 58.34%; H, 9.49%; N, 12.76%. Found: C, 58.36%; H, 9.60%; N, 13.09%.

Di[*N,N′*-bis-(*tert*-butyloxycarbonyl)]piperazine-1,4-carboxamidine (Entry 4, Table 1). Di[*N,N′*-bis-(*tert*-butyloxycarbonyl)]piperazine-1,4-carboxamidine was prepared according to GPB (solvent = $CHCl_3$, reaction time = 20 h, rt). ^{1}H NMR ($CDCl_3$) δ 3.64 (br s, 8H), 1.46 (br s, 36H). Anal. Calcd for $C_{26}H_{46}N_6O_8$: C, 54.72%; H, 8.13%; N, 14.73%. Found: C, 54.59%; H, 7.85%; N, 14.64%.

***N,N′*-Bis-(*tert*-butyloxycarbonyl)-*N″*-2-hydroxyethylguanidine (Entry 5, Table 1).** *N,N′*-Bis-(*tert*-butyloxycarbonyl)-*N″*-2-hydroxyethylguanidine was prepared according to GPB (solvent = CH_2Cl_2, reaction time = 2 h, rt). ^{1}H NMR (DMSO-d_6) δ 11.48 (s, 1H), 8.42 (br s, 1H), 4.88 (t, 1H, J = 5.3 Hz), 3.47 (q, 2H, J = 5.1 Hz), 3.33 (m, 2H), 1.46 (s, 9H), 1.37 (s, 9H). Anal. Calcd for $C_{13}H_{25}N_3O_5$: C, 51.48%; H, 8.31%; N, 13.86%. Found: C, 51.23%; H, 8.33%; N, 13.60%.

H-Gly-Arg-Gly-Asp-Ser-Pro-NH_2 (Entry 1, Table 2). The hexapeptide Boc-Gly-Orn(Mtt)-Gly-Asp(*t*Bu)-Ser(*t*Bu)-Pro was assembled on a Rink-amide[18] resin (179 mg, 0.1 mmol) by successive coupling of Fmoc-protected amino acids using Fastmoc chemistry on an Applied Biosystems automated peptide synthesizer. After coupling of Boc-Gly-OH in the last step, the synthesis was completed manually as described below. The resin was washed with CH_2Cl_2 (5 × 10 mL) and with a solution of 1% TFA and 2.5% triisopropylsilane (TIS) in CH_2Cl_2 (2 × 10 mL). The Mtt group was removed by shaking the resin for 2 h in a solution of 1% TFA and 2.5% TIS in CH_2Cl_2. The resin was washed with 10% DIEA in CH_2Cl_2 (3 × 10 mL) and with CH_2Cl_2 (5 × 10 mL). The free amino function was guanidinylated by treatment with a solution of **3** (180 mg, 0.5 mmol) and triethylamine (70 μL, 0.5 mmol) in CH_2Cl_2 (2 mL) for 8 h. A negative Kaiser test[19] indicated that the reaction had gone to completion. The resin was washed with CH_2Cl_2 (5 × 10 mL), methanol, and CH_2Cl_2 (3 × 10 mL) and dried in vacuo over KOH. The peptide was cleaved from the resin by treatment with a solution of 2.5% TIS and 2.5% H_2O in TFA for 1 h. The resin was removed by filtration and washed with TFA (3 × 2 mL). The filtrates were combined and concentrated under reduced pressure to a small volume (ca. 0.5 mL). The solution was cooled in an ice bath,

and the peptide was precipitated by adding ice cold ether (10 mL). The precipitate was isolated by centrifugation and washed with ethyl ether (3 × 10 mL). After drying in vacuo, the product H-Gly-Arg-Gly-Asp-Ser-Pro-NH_2 (entry 5, Table 2; 54 mg, 77%) was obtained as an off-white powder. HR-FABMS calcd for $C_{22}H_{38}N_{10}O_9$ (M_r) 719.1878 $(M + Cs)^+$, found 719.1899 Δ =2.9 ppm. Analytical HPLC conditions: 220 nm, 100:0 A/B to 85:15 A/B over 15 min, t_R = 12.6 min.

H-Gly-Arg-Gly-Asp-Ser-Pro-OH (entry 2, Table 2). Boc-Pro-PAM resin (114 mg, 0.1 mequiv) was washed with CH_2Cl_2 (5 × 10 mL), and the resin was treated with TFA/anisole/CH_2Cl_2 (25:5:70) for 2 min. The solution was filtered, and the Boc group was removed by treatment with TFA/anisole/CH_2Cl_2 (25:5:70) for 30 min. The resin was washed with CH_2Cl_2 (3 × 10 mL), 2-propanol, CH_2Cl_2, 2-propanol, and CH_2Cl_2 (3 × 10 mL). The resin was treated with 10% DIEA in CH_2Cl_2 (3 × 10 mL) for 2 min. The solution was filtered, and the resin was washed with CH_2Cl_2 (3 × 10 mL) and DMF (3 × 10 mL). A solution of Boc-Ser(Bn)-OH (148 mg, 0.5 mmol), 1-hydroxybenzotriazole (0.5 mmol), and 2-dimethylaminoisopropyl chloride (0.5 mmol) in DMF (2 mL) was added, and the mixture was allowed to stir for 4 h. The solution was filtered, and the resin was washed with DMF (3 × 10 mL), CH_2Cl_2 (3 × 10 mL), 2-propanol, and CH_2Cl_2 (3 × 10 mL). The above cycle was repeated with Boc-Asp(cHex)-OH (156 mg, 0.5 mmol), Boc-Gly-OH (88 mg, 0.5 mmol), Boc-Orn(Fmoc)-OH (227 mg, 0.5 mmol), and Boc-Gly-OH (88 mg, 0.5 mmol). After completion of the sequence, the resin was washed with DMF (3 × 10 mL) and with a 20% solution of piperidine in DMF. The Fmoc group was removed by shaking the resin for 30 min in a 20% solution of piperidine in DMF. The resin was then washed with DMF (3 × 10 mL) and CH_2Cl_2 (3 × 10 mL). The free amino function was guanidinylated by treatment with a solution of **3** (180 mg, 0.5 mmol) and triethylamine (70 μL, 0.5 mmol) in CH_2Cl_2 (2 mL) for 8 h. A negative Kaiser test[19] was obtained, indicating the reaction had gone to completion. The resin was washed with CH_2Cl_2 (3 × 10 mL) followed by TFA/anisole/CH_2Cl_2 (25:5:70) for 2 min. The solution was filtered, and the Boc group was removed by treatment with TFA/anisole/CH_2Cl_2 (25:5:70) for 30 min. The resin was washed with CH_2Cl_2 (3 × 10 mL), methanol (3 × 10 mL), and CH_2Cl_2 (3 × 10 mL), and dried in vacuo over KOH. The resin was placed in a Teflon reaction vessel, and anisole (0.2 mL) was added as a scavenger. The reaction vessel was flushed with nitrogen and cooled to −78 °C. HF (ca. 5 mL) was condensed into the vessel, and the mixture was stirred for 1 h at 0 °C. The HF was evaporated by flushing with nitrogen, and the vessel containing the resin was dried in vacuo over KOH. The resin was washed with TFA (3 × 2 mL) and filtered. The combined filtrates were concentrated in vacuo to ca. 0.5 mL and cooled to 0 °C. Ice cold ether (10 mL) was added, and the precipitate was isolated by centrifugation and washed with ether (3 × 10 mL). After drying in vacuo the product H-Gly-Arg-Gly-Asp-Ser-Pro-OH (entry 6, Table 2; 59 mg, 100%) was obtained as an off-white powder. HRFABMS calcd for $C_{22}H_{37}N_9O_{10}$ (M_r) 720.1718 $(M + Cs)^+$, found 720.1743 Δ =3.5 ppm. Analytical HPLC conditions: 214 nm, 100:0 A/B to 80:20 A/B over 20 min, t_R = 11.6 min.

H-Gly-hArg-Ala-Arg-Gly-Asp-Ser-Pro-NH_2 (Entry 3, Table 2). The hexapeptide Boc-Gly-Lys(Mtt)-Ala-Orn(Mtt)-Gly-Asp(*t*Bu)-Ser(*t*Bu)-Pro was assembled on a Rink-amide[18] resin (0.1 mmol, 179 mg) as described for H-Gly-Arg-Gly-Asp-Ser-Pro-NH_2. The free amino functions were guanidinylated by treatment with a solution of **3** (359 mg, 1.0 mmol) and triethylamine (140 μL, 1.0 mmol) in CH_2Cl_2 (2 mL) for 16 h. The peptide was cleaved from the resin as described for H-Gly-Arg-Gly-Asp-Ser-Pro-NH_2. After being dried in vacuo, the product H-Gly-hArg-Ala-Arg-Gly-Asp-Ser-Pro-NH_2 (entry 3, Table 2; 88 mg, 83%) was obtained as an off-white powder. HRFABMS calcd for $C_{32}H_{57}N_{15}O_{11}$ (M_r) 828.4440 $(M + H)^+$, found 828.4468 Δ = 3.4 ppm. Analytical HPLC conditions: 220 nm, 100:0 A/B to 80:20 A/B over 20 min, t_R = 15.2 min).

Acknowledgment. We thank NIGU-Chemie GmBH, Germany, for financial support.

Supporting Information Available: ORTEP diagrams, tables of crystallographic data, bond lengths and angles, atomic coordinates, and anisotropic thermal parameters are available for compounds **1** and **3** (7 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

JO9814344