Solid-Phase Synthesis of Arginine-Containing Peptides by Guanidine Attachment to a Sulfonyl Linker

H. Marlon Zhong,† Michael N. Greco, and Bruce E. Maryanoff*

Drug Discovery, The R. W. Johnson Pharmaceutical Research Institute, Spring House, Pennsylvania 19477

Received April 24, 1997

Combinatorial chemistry methods have unleashed a powerful strategy for the diversity-based discovery of new research leads with desired chemical, biological, or physical properties.[1] A centerpiece in this area is solid-phase organic synthesis, which has been used for many years in the effective preparation of oligopeptides and oligonucleotides, but has only recently come to the forefront in the preparation of small organic molecules. As a critical part of the strategy of solid-phase organic synthesis, one must be careful to select suitable polymer supports and anchoring groups. Recent attention has particularly focused on the development of new linker technologies.[1b,e−g,2]

In the course of a multistep resin-based synthesis, it is necessary that the anchor and other protecting groups be stable to the conditions employed, but be removable through relatively mild or, at least, chemoselective procedures. Typically, solid-phase synthesis of peptides and related compounds is conducted by anchoring a carboxy terminus to the resin and dressing up the amino terminus and/or various side chains with suitable protecting groups.[1b,e,g] Although linkage to the resin via an amino terminus or side chain groups is also quite useful, this approach is comparatively much less common.[1b,3] For the synthesis of arginine-containing derivatives, protection of the guanidine group with *N*-nitro, *N*-arenesulfonyl, and *N*-carbalkoxy moieties is frequently required.[4] However, it would seem expeditious to have a guanidine blocking group that can simultaneously serve as an anchor to the support. In seeking to develop an effective means for mounting the guanidine group of an arginine (or a related molecule for that matter) onto a solid support, for conducting subsequent chemical steps, and for releasing the ultimate product from the resin, the idea of a suitable arenesulfonyl linker came to mind. In this paper, we present our investigation of nitrogen attachment to a resin-bound arenesulfonyl linker that is stable to various reaction conditions and compatible with both Boc and Fmoc peptide chemistry.[5,6] Thus, four peptide products (**12**, **15**, **18**, and **19**) were constructed by addition of protected amino acids to either or both termini of the growing chain.

Results and Discussion

Given the general utility of electron-rich *N*-sulfonyl protecting groups for the guanidine moiety,[4] we designed a resin bearing a 4-(benzyloxy)benzenesulfonyl group, viz. **4**, which could be readily obtained from the standard Merrifield resin **1** (Scheme 1) and have useful loading levels. Conditions for the solid-phase chemistry were first established by performing a solution-phase model reaction. Thus, benzyl chloride was successfully reacted with sulfonate **2** in the presence of K_2CO_3 and KI to give the benzyl ether, and the corresponding sulfonyl chloride was prepared by using PCl_5. By extension, Merrifield resin **1** was reacted with **2** in a similar manner to introduce the arenesulfonyl functionality onto the resin. After any unreacted resin sites were capped with Et_2NH in DMF, sulfonic acid resin **3** was converted into desired sulfonyl chloride resin **4** with PCl_5 in DMF (Scheme 1). Reaction of **4** with 1,3-propanediamine, followed by the pentafluorophenyl ester of Fmoc-Gly, was intended to provide **6** for assessing the resin-loading level. However, the resin-loading of 0.14 mmol/g, determined spectrophotometrically by liberation of the Fmoc chromophore (dibenzofulvene) with piperidine in DMF ("Fmoc-echo" procedure),[7] was viewed as too low for further work, considering the loading level for the commercial Merrifield resin of 0.9 mmol/g.

To improve the loading level of novel resin **4**, we changed the base in the alkylation of **1** to NaOMe.[8] The sodium phenoxide salt of **2** was prepared with 1.2 equiv of NaOMe and reacted with **1** in *N*,*N*-dimethylacetamide to afford **3**, with a loading estimated at 0.70 mmol/g by the increase in weight. Sulfonyl chloride resin **4** was prepared from **3**, and **4** now had a loading level of 0.63 mmol/g by weight increase and 0.51 mmol/g by elemental analysis for Cl. Also, a loading level of 0.50 mmol/g was realized after conversion of **4** to **6** and Fmoc-echo analysis. This preferred NaOMe alkylation method (not depicted in Scheme 1) is described in the Experimental Section, and the improved batch of resin **4** was suitable for solid-phase synthesis operations.

Boc-arginine was mounted onto resin **4** by using 4.0 N KOH in 1,4-dioxane (Scheme 2) to give resin **7** with a loading of 0.40 mmol/g by weight increase and 0.34 mmol/g by elemental analysis for N. Cleavage of **7** was studied under different acidic conditions, such as CF_3CO_2H, HBr/CF_3CO_2H, triflic acid/CF_3CO_2H, and anhydrous HF in the presence of anisole as a cation scavenger. Most of the conditions tried (except for CF_3CO_2H, which gave minimal reaction) resulted in partial

* Address correspondence to this author. Fax: 215-628-4985.
† Johnson & Johnson Excellence in Science Postdoctoral Scientist.

(1) (a) Gordon, E. M.; Barrett, R. W.; Dower, W. J.; Fodor, S. P. A.; Gallop, M. A. *J. Med. Chem.* **1994**, *37*, 1385−1401. (b) Thompson, L. A.; Ellman, J. A. *Chem. Rev.* **1996**, *96*, 555−600. (c) Hermkens, P. H. H.; Ottenheijm, H. C. J.; Rees, D. *Tetrahedron* **1996**, *52*, 4527−4554. (d) Terrett, N. K.; Gardner, M.; Gordon, D. W.; Kobylecki, R. J.; Steele, J. *Tetrahedron.* **1995**, *51*, 8135−8173. (e) Früchtel, J. S.; Jung, G. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 17−42. (f) Balkenhohl, F.; von dem Bussche-Hünnefeld, C.; Lansky, A.; Zechel, C. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 2289−2337. (g) Fenniri, H. *Curr. Med. Chem.* **1996**, *3*, 343−378.

(2) (a) Chenera, B.; Finkelstein, J. A.; Veber, D. F. *J. Am. Chem. Soc.* **1995**, *117*, 11999−12000. (b) Plunkett, M. J.; Ellman, J. A. *J. Org. Chem.* **1995**, *60*, 6006−6007. (c) Backes, B. J.; Virgilio, A. A.; Ellman, J. A. *J. Am. Chem. Soc.* **1996**, *118*, 3055−3056. (d) Hughes, I. *Tetrahedron Lett.* **1996**, *37*, 7595−7598. (e) Boehm, T. L.; Showalter, H. D. H. *J. Org. Chem.* **1996**, *61*, 6498−6499.

(3) (a) Barlos, K.; Gatos, D.; Kallitsis, I.; Papaioannou, D.; Sotiriou, P. *Liebigs Ann. Chem.* **1988**, *11*, 1079−1081. (b) Hoekstra, W. J.; et al. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 2371−2376. (c) Hoekstra, W. J.; Greco, M. N.; Yabut, S. C.; Hulshizer, B. L.; Maryanoff, B. E. *Tetrahedron Lett.* **1997**, *38*, 2629−2632. (d) Dressman, B. A.; Spangle, L. A.; Kaldor, S. W. *Tetrahedron Lett.* **1996**, *37*, 937−940.

(4) For a review on different arginine protecting groups, see: Rzeszotarska, B.; Masinkiewicz, E. *Org. Prep. Proced. Int.* **1988**, *20*, 427−464.

(5) For a recent report on the use of a *p*-alkoxybenzenesulfonyl resin for guanidine attachment, see: Bonnat, M.; Bradley, M.; Kilburn, J. D. *Tetrahedron Lett.* **1996**, *37*, 5409−5412.

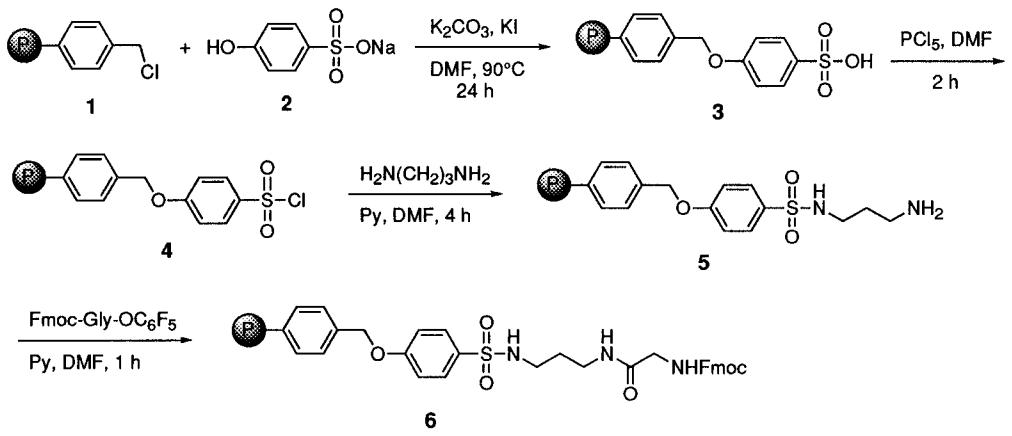
(6) For information on Boc- and Fmoc-based peptide synthesis, see: Bodansky, M., Ed. *Principles of Peptide Synthesis*; Springer-Verlag: Heidelberg, 1984.

(7) Chan, W. C.; Mellor, S. L. *J. Chem. Soc., Chem. Commun.* **1995**, 1475. This method measures the amount of dibenzfulvene produced.

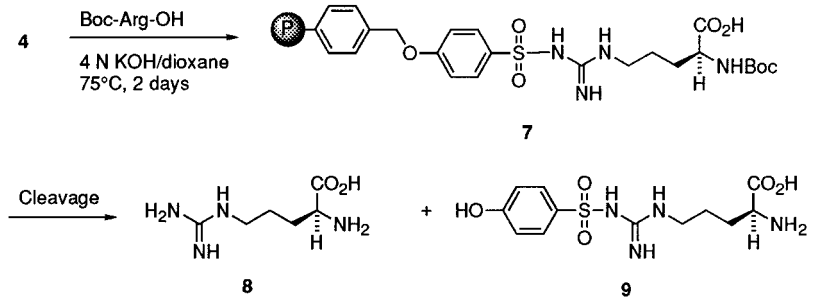
(8) Wang, S.-S. *J. Am. Chem. Soc.* **1973**, *95*, 1328−1333.

S0022-3263(97)00736-6 CCC: $14.00

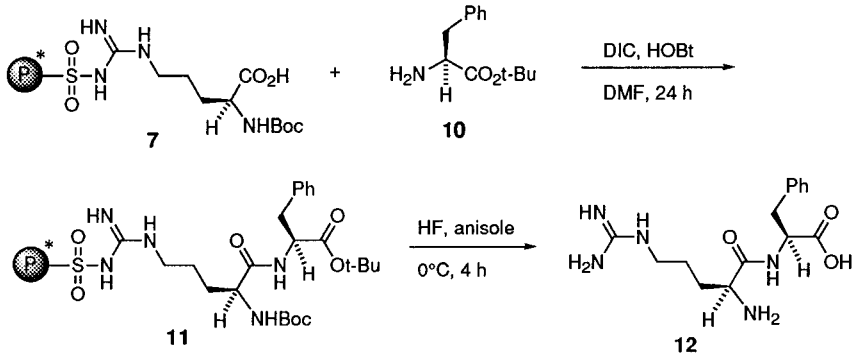
Scheme 1



Scheme 2



Scheme 3



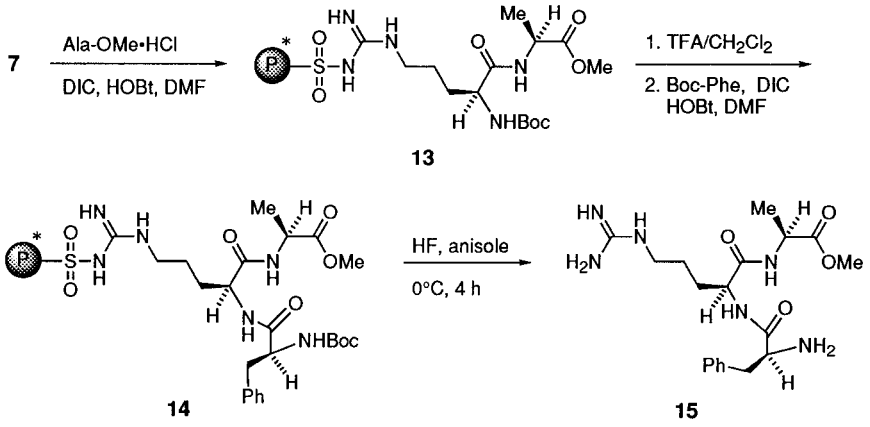
cleavage to form two products, **8** and **9**, which were identified by MS analysis. Only HF furnished a single species, which was desired product **8**. Apparently, the benzyl ether used to attach the requisite arenesulfonyl group is too labile to the stronger acids; however, the HF cleavage procedure was certainly adequate. Examination of **8** for stereochemical integrity by chiral HPLC revealed virtually no racemization (see Experimental Section).

We carried out the syntheses of three different model peptides starting with resin-bound arginine derivative **7** (Schemes 3−5). After HF cleavage, each target peptide was inspected as the crude product for diastereomers due to any stereomutation during coupling steps (HPLC/MS), although this is expected to be insignificant under DIC/HOBt conditions, and then purified. No diastereomeric species were detected in any of the crude products. The yield of dipeptide Arg-Phe, **12**, was 0.29 mmol/g of resin **7** (72% yield). It is particularly attractive to be able to build up the peptide chain by the coupling of amino acids to either or both of the termini (C or N), as illustrated by the preparation of tripeptide **15** (Scheme 4). Resin **7** was first coupled with Ala-OMe at the C-terminus in the presence of DIC and HOBt in DMF. After deprotection of the Boc group, Boc-Phe was introduced at the N-terminus to provide resin **14**. The yield of cleaved tripeptide **15**, Phe-Arg-Ala-OMe, was 0.20 mmol/g of resin **7** (50% yield).[9] The utility of resin **7** was further demonstrated by the synthesis of tetrapeptide **18**, Phe-Gly-Arg-Ala-OMe (Scheme 5). Notably, *one can perform both standard Boc and Fmoc processes on the same resin*, an advantage which results from the high stability of the sulfonyl linker. The three steps of coupling and three steps of deprotection furnished cleaved tetrapeptide **18**, Phe-Gly-Arg-Ala-OMe, with a yield of 0.16 mmol/g of resin **7** (40%).[9]

To test the versatility of our novel resin further, we prepared Ser-Phe-Leu-Leu-Arg-Asn-NH_2 (SFLLRN-NH_2, **19**), a biologically important hexapeptide representing the activation sequence for the human thrombin receptor (PAR-1).[10] Our synthesis involved peptide chain extension in both directions, by amino acid addition to both

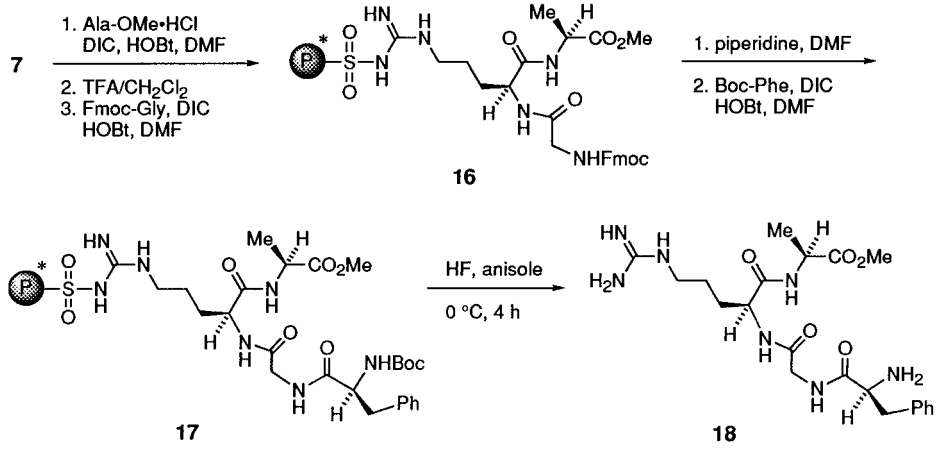
(9) ESI-MS examination of the minor constituents of the crude final product (separated by reverse-phase HPLC) did not show the presence of any diastereomeric peptides.

Scheme 4



13 14 15

Scheme 5



16 17 18

the C- and N-terminals, as well as both Boc and Fmoc protection, under standard, racemization-free coupling conditions (DIC, HOBt; 24 h). The sequence was initiated by attachment of Asn-NH_2 to resin **7**. The two Leu residues were installed via a Boc strategy, and then Fmoc-Phe and Boc-(O-*t*-Bu)Ser were sequentially coupled. Peptide-resin cleavage (HF, anisole) afforded the desired hexapeptide (16% overall yield from **7**), which was identical with a sample prepared by using a Boc strategy with a commercial methylbenzhydrylamine resin.[11]

Conclusion

We have developed a new Merrifield-type resin with an electron-rich arenesulfonyl linker, having a reasonably useful loading capacity of ca. 0.5 mmol/g, for the solid-phase organic synthesis of compounds bearing guanidine groups. In particular, we have applied this new resin to the synthesis of compounds containing arginine, in which the guanidine group of Arg was attached to the solid support. A sequence of chemical manipulations culminated in release of the product with anhydrous HF. The electron-rich arenesulfonyl linker was stable to various reaction conditions and compatible with both Boc and Fmoc peptide chemistry; however, it was labile to strong acids such as HBr and triflic acid because of the benzyl ether functionality. To have more convenient, mild conditions for the final cleavage process, we have recently employed the Wang *p*-nitrophenyl carbonate resin[3d,12] for anchoring amidines and guanidines, but this linker is not amenable to Boc chemistry on account of its acid lability.[13] For future improvements, one could also consider modifying the arenesulfonyl linker, such as by adding electron-releasing groups, to make it cleavable under more convenient conditions.

Our new resin potentially has other worthwhile uses. Besides the role in anchoring guanidine groups, it would be reasonable to apply the resin for nitrogen attachment with amidines, heteroaromatics (e.g., indoles), and amines,[14] although cleavage of a standard sulfonamide group would most likely require harsher reaction conditions.[15] Also, one can envision exploiting this resin where standard arenesulfonyl groups have found synthetic utility, such as for the activation of alcohols, oximes, or

(10) (a) Scarborough, R. M.; Naughton, M.; Teng, W.; Hung, D. T.; Rose, J.; Vu, T.; Wheaton, V. I.; Turck, C. W.; Coughlin, S. R. *J. Biol. Chem.* **1992**, *267*, 13146−13149. (b) Vu, T.-K. H.; Wheaton, V. I.; Hung, D. T.; Coughlin, S. R. *Cell* **1991**, *64*, 1057−1068. (c) Coughlin, S. R. *Trends Cardiovasc. Med.* **1994**, *4*, 77−83.

(11) The overall yield of SFLLRN-NH_2 from traditional synthesis on a commercial MBHA resin was 49%. We thank Kenway Hoey (RWJPRI−La Jolla) for this information, as well as a sample of the SFLLRN-NH_2 for comparison. Our hexapeptide sample was identical with this "reference" material by ^1H and ^{13}C NMR.

(12) This resin was purchased from NovaBiochem Corp.

(13) (a) Such chemistry has been successfully executed in our laboratory: Greco, M. N., unpublished results. Cleavage was easily effected by using trifluoroacetic acid in methylene chloride. (b) For a very recent paper in this area, see: Roussel, P.; Bradley, M.; Matthews, I.; Kane, P. *Tetrahedron Lett.* **1997**, *38*, 4861−4864.

(14) For solid-phase synthesis with nitrogen attachment via an acylsulfonamide "safety-catch" linker, see: (a) Backes, B. J.; Virgilio, A. A.; Ellman, J. A. *J. Am. Chem. Soc.* **1996**, *118*, 3055−3056. (b) Backes, B. J.; Ellman, J. A. *J. Am. Chem. Soc.* **1994**, *116*, 11171−11172.

(15) (a) Greene, T. W.; Wuts, P. G. M. *Protective Groups in Organic Chemistry*, 2nd ed.; Wiley-Interscience: New York, 1991. (b) Nyasse, B.; Grehn, L.; Ragnarsson, U. *Chem. Commun.* **1997**, 1017−1018 and references therein.

hydrazones.[16] In this regard, colleagues in our laboratories have performed displacement reactions with diverse nucleophiles on alcohols activated by **4** as resin-based sulfonates.[17]

Experimental Section

General. All chemicals and Merrifield resin were reagent grade and used as purchased without further purification. α-*N*−Boc-arginine was purchased from Aldrich Chemical Co.; the other amino acid derivatives were purchased from Bachem Bioscience Inc. Reactions involving resins were performed in an hourglass reaction vessel (Peptides International, catalog number SHG-21060-PI) with nitrogen gas being bubbled through the sintered glass frit for agitation. Proton and carbon-13 NMR spectra were obtained on a Bruker AC-300 or AC-400 spectrometer, as indicated, in MeOH-d_4 (s = singlet, d = doublet, t = triplet, m = multiplet). General MS analysis was performed on a Micromass Platform-II spectrometer with electrospray ionization (ESI); HR-MS data were obtained on a Finnigan MAT 900 double-focusing spectrometer with ESI. UV analyses for the Fmoc-echo procedure were performed on a Hewlett-Packard Model 8453 spectrometer. Optical rotations were determined on Perkin-Elmer 241 polarimeter. HPLC separations were performed on a Waters 600E instrument with three PrePak cartridges in series charges with Bondapak C18 (12−20 μM, 125 Å, 40 mm × 100 mm; gradient elution with MeCN (w/0.1% TFA) and water (w/2% TFA) starting at 5:95 and ending at 50:50, 40 mL/min over ca. 60 min; λ = 220 nm). Elemental microanalysis was performed by Robertson Microlit Laboratories, Inc. (% water by Karl Fischer analysis). Abbreviations: DIC = diisopropylcarbodiimide; HOBt = 1-hydroxybenzotriazole, Fmoc = 9-fluorenylmethoxycarbonyl, Boc = *tert*-butoxycarbonyl, TFA = trifluoroacetic acid.

Preparation of Novel Arenesulfonyl Resin 4 and Resin-Bound Arginine Derivative 7. To a suspension of Merrifield resin (**1**) (5.0 g, 4.9 mmol) and anhydrous 4-hydroxybenzenesulfonic acid sodium salt (2.94 g, 15 mmol; commercial material was dehydrated at 110 °C in vacuo for 8 h) in *N*,*N*-dimethylacetamide (50 mL) was added NaOMe (0.81 g, 15 mmol), and the mixture was stirred at 90 °C for 2 days. The resin product (**3**) was collected by filtration, washed (sequentially with DMF, 1.0 N HCl, MeOH, and CH_2Cl_2), and dried in a vacuum oven at 50 °C for 24 h. The resin was treated with 1:1 Et_2NH−DMF (50 mL) for 1 h and washed with DMF. To resin **3** in DMF (50 mL) was added PCl_5 (5.20 g, 25 mmol) in several portions, and the resulting suspension was stirred at 23 °C for 4 h. After the mixture was washed with DMF and CH_2Cl_2, the resin was dried in a vacuum oven at 50 °C overnight to afford the novel arenesulfonyl chloride resin, **4**, which was suitable for further applications. On the basis of weight increase, the loading level was 0.63 mmol/g, but elemental analysis indicated 1.8% Cl for a loading of 0.51 mmol/g. A sample of this resin (0.20 g) was suspended in DMF and treated with 1,3-propanediamine and pyridine (0.5 mL) at 23 °C for 18 h. The resin was washed with DMF (2 × 30 mL) and CH_2Cl_2 (2 × 30 mL), then suspended in DMF (15 mL), and treated with Fmoc-Gly-OC_6F_5 (0.278 g, 0.6 mmol) and pyridine (0.079 g, 1.0 mmol) for 3 h. After being washed with DMF and CH_2Cl_2, **6** was dried in vacuo, treated with 20% piperidine in DMF (10 mL) for 30 min, filtered, and washed with DMF (2 × 5 mL). The combined filtrate was measured by UV absorbance to determine the amount of Fmoc cleaved from resin. The loading of the resin was calculated as 0.50 mmol/g on the basis of this Fmoc-echo procedure.[7]

The resin (4.00 g, 36.0 mmol) was treated with α-*N*−Boc-arginine[18] (2.14 g, 7.8 mmol) in the presence of 4.0 N KOH (5 mL) in dioxane (20 mL) at 75 °C for 2 days;[19] the reaction was then cooled to 23 °C and filtered. The product resin (**7**) was washed with DMF and CH_2Cl_2 and dried in a vacuum oven at 50 °C for 24 h. A sample of this resin (0.20 g) was suspended in DMF (15 mL) and treated with 1,3-propanediamine (0.074 g, 1.0 mmol), HOBt (0.135 g, 1.0 mmol), and DIC (0.127 g, 1.0 mmol). After 16 h at 23 °C, the resin was washed with DMF (2 × 30 mL) and CH_2Cl_2 (2 × 30 mL), then suspended in DMF (15 mL), and treated with Fmoc-Gly-OC_6F_5 (0.278 g, 0.6 mmol) and pyridine (0.079 g, 1 mmol) for 3 h. After sequential washings with DMF and CH_2Cl_2, the resin was dried in vacuo, treated with 20% piperidine−DMF (10 mL) for 30 min, collected, and washed with DMF (2 × 5 mL). The combined filtrate was measured by UV absorbance to quantitate Fmoc cleavage. The loading was determined to be 0.40 mmol/g by Fmoc-echo,[7] but elemental analysis indicated 1.90% N for a loading of 0.34 mmol/g. To assess the stereochemical integrity of the attached arginine, we cleaved a sample of the *N*−Boc-Arg resin (HF/anisole; see below) and triturated the crude product with ether. The mixture was filtered, treated with water, and filtered again to remove the resin particles. Analysis of the aqueous solution by HPLC (Beckman analytical system) on a chiral column (15 cm × 4.6 mm Crownpak CR+ from Chiral Technologies, Inc.), eluting isocratically with aqueous $HClO_4$ at pH = 1.5 (0.8 mL/min, λ = 200 nm), indicated a 97:3 ratio of L-Arg:D-Arg,[20] which was virtually identical with the enantiomeric purity (96%) of the starting α-*N*−Boc-arginine.[18]

Arg-Phe Dipeptide 12.[21] To a suspension of resin **7** (0.50 g, 0.2 mmol) in DMF (30 mL), agitated by nitrogen gas, was added the HCl salt of Phe-O-*t*-Bu (0.294 g, 1.0 mmol), Et_3N (0.101 g, 1.0 mmol), HOBt (0.135 g, 1.0 mmol), and DIC (0.127 g, 1.0 mmol). After 24 h at 23 °C, resin **11** was washed with DMF (2 × 30 mL) and CH_2Cl_2 (2 × 30 mL) and then dried in a vacuum oven at 50 °C for 24 h. Resin **11** (0.30 g, 0.12 mmol) was suspended in anhydrous anisole (2 mL) in a Teflon reaction vessel, and HF (8 mL) was condensed into the vessel at −78 °C; the vessel was sealed and warmed to 0 °C. The solution was stirred at 0 °C for 4 h, and then the HF was removed under reduced pressure. The residue was triturated with Et_2O (30 mL) and filtered. The collected solid was treated with MeOH (25 mL) and filtered. The filtrate was concentrated to give the crude product, which was purified by reverse-phase HPLC and lyophilized to give **12** (28.0 mg, 72%) as a white fluffy solid: $[\alpha]^{23}_D$ +13.9° (*c* 1.00, H_2O); ^{1}H NMR (300.1 MHz) δ 7.27 (m, 5 H), 4.72 (dd, J = 9.3, 4.9 Hz, 1 H), 3.89 (t, J = 6.0 Hz, 1 H), 3.20 (m, 3 H), 3.00 (dd, J = 14.2, 9.3 Hz, 1 H), 1.90 (m, 2 H), 1.71 (m, 2 H); ^{13}C NMR (75.5 MHz) δ 174.5, 169.9, 158.8, 138.3, 130.2, 129.6, 128.0, 55.4, 53.7, 41.9, 38.0, 29.7, 24.8; HR-MS calcd for $C_{15}H_{24}N_5O_3$ (MH^+) 322.1879, found 322.1876.

Phe-Arg-Ala Tripeptide 15. To a suspension of resin **7** (0.50 g, 0.2 mmol) in DMF (30 mL), agitated by nitrogen gas, was added alanine methyl ester hydrochloride (0.139 g, 1.0 mmol), Et_3N (0.101 g, 1.0 mmol), HOBt (0.135 g, 1.0 mmol), and DIC (0.127 g, 1.0 mmol). After 24 h at 23 °C, resin **13** was washed with DMF and CH_2Cl_2. Resin **13** in CH_2Cl_2 (15 mL) was treated with TFA (15 mL) at 23 °C for 1 h, and the resulting resin was washed sequentially with CH_2Cl_2, 20% Et_3N in DMF, and DMF. The resin in DMF (30 mL) was treated with Boc-phenylalanine (0.266 g, 1.0 mmol), HOBt (0.135 g, 1.0 mmol), and DIC (0.135 g, 1.0 mmol). After 24 h at 23 °C, resin **14** was washed with DMF and CH_2Cl_2, and then dried in a vacuum oven at 50 °C for 24 h. Resin **14** (0.30 g, 0.12 mmol) in anhydrous anisole (2 mL) was treated with HF (8 mL) as described above. The residue from evaporation of volatiles was triturated with Et_2O (30 mL). After filtration, the collected solid was treated with MeOH (25 mL) and filtered. The filtrate was concentrated to give the crude product, which was purified by reverse-phase HPLC and lyophilized to give **15** (24.2 mg, 50%) as a white fluffy solid: $[\alpha]^{23}_D$ −9.7° (*c* 1.00, H_2O); ^{1}H NMR (300.1 MHz) δ 7.32 (m, 5 H), 4.39

(16) A polymeric "tosyl chloride" has been used as a polymer-bound "tosyl azide" reagent: Roush, W. R.; Feitler, D.; Rebek, J. *Tetrahedron Lett.* **1974**, 1391−1392.

(17) (a) Reitz, A. B., and co-workers *Tetrahedron Lett.*, in press. (b) This type of chemistry has recently been executed with a different Merrifield resin-based sulfonyl chloride; see: Hunt, J. A.; Roush, W. R. *J. Am. Chem. Soc.* **1996**, *118*, 9998−9999.

(18) The commercial material had an enantiomeric purity of 96% (L/D = 96:4) as determined by capillary electrophoresis (Beckman P/ACE System 2100; 50 μM × 50 cm fused silica capillary; 0.1 N ammonium acetate and 0.01 M hydroxypropyl-β-cyclodextrin; 18 kV). The D isomer was analyzed for confirmation.

(19) Loading onto the resin required these reaction conditions. In a separate experiment, the reaction was still incomplete (%Cl analysis) after 2 d at 65 °C.

(20) The well-shaped, baseline-resolved peaks for D-Arg (t_R = 2.5−2.6 min) and L-Arg (t_R = 4.3−4.4 min) were corroborated by spiking with authentic samples.

(21) Appel, R.; Hiester, E. *Chem. Ber.* **1981**, *114*, 2649.

(m, 2 H), 4.15 (dd, J = 8.1, 6.0 Hz, 1 H), 3.72 (s, 3 H), 3.21 (m, 2 H), 3.02 (dd, J = 14.2, 8.3 Hz, 1 H), 1.82 (m, 1 H), 1.71 (m, 3 H), 1.41 (d, J = 7.3 Hz, 3 H), 1.27 (m, 1 H); ^{13}C NMR (100.6 MHz) δ 174.6, 172.8, 169.5, 163.0, 158.7, 135.5, 130.5, 128.9, 55.5, 54.1, 52.8, 42.1, 38.6, 30.6, 26.0, 17.2; HR-MS calcd for $C_{19}H_{31}N_6O_4$ (MH^+) 407.2406, found 407.2409.

Phe-Gly-Arg-Ala Tetrapeptide 18. To a suspension of resin **7** (0.50 g, 0.2 mmol) in DMF (30 mL), agitated by nitrogen gas, was added Ala-OMe·HCl (0.139 g, 1.0 mmol), Et_3N (0.101 g, 1.0 mmol), HOBt (0.135 g, 1.0 mmol), and DIC (0.127 g, 1.0 mmol). After 24 h at 23 °C, the resin product was washed with DMF and CH_2Cl_2. The resin in CH_2Cl_2 (15 mL) was treated with TFA (15 mL) at 23 °C for 1 h, washed (CH_2Cl_2; 20% Et_3N in DMF; DMF), suspended in DMF (30 mL), and treated with Fmoc-glycine (0.297 g, 1.0 mmol), HOBt (0.135 g, 1.0 mmol), and DIC (0.135 g, 1.0 mmol). After 24 h at 23 °C, resin **16** was washed with DMF and treated with 20% piperidine in DMF (20 mL) for 1 h. After the mixture was washed with DMF, the resin in DMF (30 mL) was treated with Boc-phenylalanine (0.266 g, 1.0 mmol), HOBt (0.135 g, 1.0 mmol), and DIC (0.135 g, 1.0 mmol). After 24 h at 23 °C, resin **17** was washed (DMF; CH_2Cl_2) and dried in a vacuum oven at 50 °C for 24 h. Resin **17** (0.30 g, 0.12 mmol) in anhydrous anisole (2 mL) was treated with HF (8 mL) as described above. The residue from evaporation of volatiles was triturated with Et_2O (30 mL). After filtration, the collected solid was treated with MeOH (25 mL) and filtered. The filtrate was concentrated to give the crude product, which was purified by reverse-phase HPLC and lyophilized to give **18** (22 mg, 40%) as a white fluffy solid: $[\alpha]^{23}_D$ −8.8° (c 0.27, H_2O); ^{1}H NMR (300.1 MHz) δ 7.31 (m, 5 H), 4.40 (m, 2 H), 4.10 (dd, J = 8.1, 5.2 Hz, 1 H), 3.90 (d, J = 16.6 Hz, 1 H), 3.83 (d, J = 16.6 Hz, 1 H), 3.70 (s, 3 H), 3.30 (m, 2 H), 3.20 (m, 3 H), 3.07 (m, 1 H), 1.86 (m, 1 H), 1.69 (m, 3 H), 1.39 (d, J = 7.3 Hz, 3 H); ^{13}C NMR (75.5 MHz) δ 174.6, 173.4, 170.8, 170.3, 158.7, 135.6, 130.5, 130.2, 128.9, 55.8, 54.0, 52.8, 49.6, 43.2, 42.2, 38.5, 30.5, 26.0, 17.2; HR-MS calcd for $C_{21}H_{34}N_7O_5$ (MH^+) 464.2621, found 464.2633.

Ser-Phe-Leu-Leu-Arg-Asn-NH$_2$ (19).[10] To a suspension of **7** (0.80 g, 0.28 mmol) in DMF (30 mL) in a bubbler, agitated by nitrogen gas, was added Asn-NH_2·HCl (0.168 g, 1 mmol), Et_3N (0.102 g, 1 mmol), HOBt (0.162 g, 1.2 mmol), and DIC (0.151 g, 1.2 mmol). After 24 h at 23 °C, the resin was washed (DMF; CH_2Cl_2), suspended in CH_2Cl_2 (15 mL), treated with TFA (15 mL) at 23 °C for 30 min, washed sequentially with CH_2Cl_2, 20% Et_3N in DMF, and DMF, suspended in DMF (30 mL) in a bubbler, and treated with Boc-Leu·H_2O (0.25 g, 1.0 mmol), HOBt (0.162 g, 1.2 mmol), and DIC (0.151 g, 1.2 mmol). After 24 h at 23 °C, the resin was washed (DMF; CH_2Cl_2), suspended in CH_2Cl_2 (15 mL), treated with TFA (15 mL) at 23 °C for 30 min, and washed (CH_2Cl_2; 20% Et_3N in DMF; DMF). The resin in DMF (30 mL) was retreated in the same manner with Boc-Leu·H_2O. The washed resin was suspended in CH_2Cl_2 (15 mL), treated with TFA (15 mL) at 23 °C for 30 min, washed (CH_2Cl_2; 20% Et_3N in DMF; DMF), and treated in DMF (30 mL) with Fmoc-Phe (0.387 g, 1.0 mmol), HOBt (0.162 g, 1.2 mmol), and DIC (0.151 g, 1.2 mmol). After 24 h at 23 °C, the resin was washed with DMF, treated with 20% piperidine in DMF (20 mL) for 30 min, washed with DMF, and treated in DMF (30 mL) with Boc-(O-t-Bu)Ser (0.261 g, 1.0 mmol), HOBt (0.162 g, 1.2 mmol), and DIC (0.151 g, 1.2 mmol). After 24 h at 23 °C, the resin was washed (DMF; CH_2Cl_2) and dried in a vacuum oven at 23 °C for 24 h. The resin product (0.50 g, 0.17 mmol) in anhydrous anisole (2 mL) was treated with HF (8 mL) as described above. After evaporation of volatiles, the residue was triturated with Et_2O (30 mL) and filtered. The solid was treated with MeOH (25 mL) and filtered. The filtrate was concentrated to yield the crude product, which was purified by reverse-phase HPLC and lyophilized to give **19** (28.8 mg, 16%) as a white fluffy solid: $[\alpha]^{23}_D$ −6.3° (c 0.50, H_2O); ^{1}H NMR (400.1 MHz) δ 0.94 (m, 12 H), 1.65 (m, 8 H), 1.77 (m, 1 H), 1.88 (m, 1 H), 2.73 (d, J = 6.4 Hz, 2 H), 2.94 (dd, J = 14.2, 9.5 Hz, 1 H), 3.19, (m, 3 H), 3.90 (m, 3 H), 4.29 (m, 1.5 H), 4.37 (m, 1.5 H), 4.67 (m, 2 H), 7.21 (m, 1 H), 7.26 (m, 4 H); ^{13}C NMR (100.6 MHz) δ 21.99, 23.49, 23.53, 25.85, 25.93, 26.04, 29.64, 37.64, 38.30, 41.39, 41.68, 42.00, 51.41, 53.46, 53.67, 54.60, 55.87, 56.71, 61.86, 127.99, 129.64, 130.25, 138.20, 158.65, 168.82, 173.44, 173.51, 174.81, 174.98, 175.14, 175.57; HR-MS calcd for $C_{34}H_{58}N_{11}O_8$ (MH^+) 748.4470, found 748.4453.[11] Anal. Calcd for $C_{34}H_{57}N_{11}O_8$·2.2TFA·2.3H_2O: C, 44.34; H, 6.18; N, 14.81; F, 12.05; H_2O (KF), 3.98. Found: C, 44.09; H, 5.84; N, 14.47; F, 11.64; H_2O (KF), 3.82.

Acknowledgment. We thank Ralph Rivero for assistance with the "Fmoc-echo" procedure, Richard Dunphy for high-resolution mass spectrometry data, and Gregory Leo and Diane Gauthier for NMR spectral data. We are particularly grateful to Rekha Shah for chiral HPLC and capillary electrophoresis analyses.

Supporting Information Available: Proton and carbon-13 NMR spectra for **12**, **15**, **18**, and **19** (9 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.

JO970736N