Sterically Hindered $C^{\alpha,\alpha}$-Disubstituted α-Amino Acids: Synthesis from α-Nitroacetate and Incorporation into Peptides

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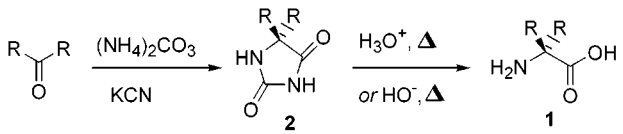
The preparation of sterically hindered and polyfunctional $C^{\alpha,\alpha}$-disubstituted α-amino acids (ααAAs) via alkylation of ethyl nitroacetate and transformation into derivatives ready for incorporation into peptides are described. Treatment of ethyl nitroacetate with *N*,*N*-diisopropylethylamine (DIEA) in the presence of a catalytic amount of tetraalkylammonium salt, followed by the addition of an activated alkyl halide or Michael acceptor, gives the doubly *C*-alkylated product in good to excellent yields. Selective nitro reduction with Zn in acetic acid or hydrogen over Raney Ni gives the corresponding amino ester that, upon saponification, can be protected with the fluorenylmethyloxycarbonyl (Fmoc) group. The first synthesis of an orthogonally protected, tetrafunctional $C^{\alpha,\alpha}$-disubstituted analogue of aspartic acid, 2,2-bis(*tert*-butylcarboxymethyl)glycine (Bcmg), is described. Also, the sterically demanding $C^{\alpha,\alpha}$-dibenzylglycine (Dbg) has been incorporated into a peptide using solid-phase synthesis. It was found that once sterically congested Dbg is at the peptide *N*-terminus, further chain extension becomes very difficult using uronium or phosphonium salts (PyAOP, PyAOP/HOAt, HATU). However, preformed amino acid symmetrical anhydride couples to *N*-terminal Dbg in almost quantitative yield in nonpolar solvent (dichloroethane−DMF, 9:1).

Introduction

The discovery of $C^{\alpha,\alpha}$-disubstituted α-amino acids (ααAAs, **1**) in biological systems and their propensity to induce secondary structure even into short peptides has resulted in an increased interest in novel methods for their synthesis.[1,2] The most common methods of ααAA preparation include the Bucherer−Bergs and the Strecker syntheses.[3] We have found the Bucherer−Bergs method (Scheme 1) convenient and effective for preparation of a number of hydantoins **2** that can be hydrolyzed to ααAAs. Also, we and others have developed milder methods for cleaving the hydantoin ring so that side-chain protected ααAAs can be obtained directly from hydantoin hydrolysis.[4] However, several more sterically hindered hydantoins, including dibenzyl hydantoin **2** (R = Bn), have been impossible to hydrolyze to the free amino acids even under the most extreme conditions.[5] Also, we have found that the Bucherer−Bergs route cannot be extended to a number of noncyclic difunctionalized ketones due to their resistance to hydantoin formation.[6]

Scheme 1



These and other limitations of the hydantoin approach have led to the development of alternative syntheses of ααAAs, including glycine anion and cation equivalents.[7] Nitroacetate esters have long been recognized as potentially useful synthetic intermediates for the preparation of amino acids as well as other biologically significant compounds.[8] Alkylations of nitroacetate esters have usually resulted in racemic monoalkylations, and the predominant products were often results of *C*- and/or *O*-alkylation.[8,9] The previously reported *C*-dialkylation of ethyl nitroacetate with benzyl bromide employed an inorganic base, and the reaction took a prolonged time.[10] Electrochemically generated anions have also been used on a small scale to perform mono- and dialkylations of

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(5) Hydrolysis of hydantoin **2** (R = Bn) with either strong base (6 M KOH, 20 psi, 180 °C, 2 days) or strong acid (10 N HBr, reflux, 2 days) gave starting material as the only isolable product.

(6) It was observed that a number of symmetrical difunctionalized ketones, such as 1,3-dihydroxy 2-propanone, 4-oxoheptanedinitrile, 1,5-diamino-3-pentanone, dimethyl 3-oxoglutarate, dimethyl 4-oxopimelate, and 4-ketopimelic acid do not form hydantoins to an appreciable extent under standard Bucherer−Bergs conditions. Hammarström, L. G. J. Synthetic Peptides: Design, Structure & Biological Function. Dissertation, Louisiana State University, 2001; Ch. 2, pp 20−43.

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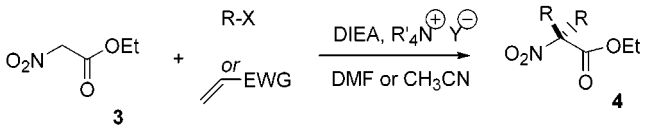
Scheme 2



nitroacetate esters.[11] The strategy and conditions proposed herein employ the commercially available ethyl nitroacetate (**3**) along with 2 equiv each of a tertiary amine base and an electrophile to provide a variety of novel $C^{\alpha,\alpha}$-dialkyl nitroacetate esters (Scheme 2). Furthermore, methods for preparing Fmoc-protected ααAAs, including tetrafunctional ααAAs, suitable for solid-phase peptide synthesis are elaborated.

The main application of these protected ααAAs will be for the synthesis of peptide analogues which are conformationally constrained (limited ϕ and ψ space) into turn, helix or extended conformations.[2,12] Incorporation of ααAAs such as Aib,[13] Deg, and Dpg into peptides is generally accomplished by standard carbodiimide protocols[14] or in more difficult cases coupling is accomplished with preformed acid fluorides,[15] or in situ activation with uronium (e.g., HATU)[16] or phosphonium salts (e.g., PyBOP).[17] More sterically hindered ααAAs such as Dbg are much more difficult to incorporate. For example, reported acylation of the *N*-terminus of Dbg only gave a low to moderate coupling yield after prolonged reaction time.[18] In this paper, we report an efficient method for readily incorporating amino acid residues to sterically hindered *N*-terminus of Dbg.

Results and Discussion

Synthesis of N^{α}-Fmoc-Protected ααAAs. We have found that treatment of ethyl nitroacetate with 2 equiv of DIEA in the presence of catalytic amounts of tetraalkylammonium salts, followed by the addition of an activated alkyl halide or Michael acceptor, gave the disubstituted nitroacetate ester in good to excellent yields (Table 1). DIEA was employed based on evidence which suggests that ethyl nitroacetate anion exhibits high nucleophilic reactivity in the presence of a quaternary ammonium cation that does not tend to form a strong ion pair.[11] The reactivity of the anion was further increased when a catalytic amount of tetraalkylammonium salt was added to the reaction. The benzyl bromides gave rapid conversion to dialkylated products **4**[19] in a matter of minutes to hours. Reactions were initially exothermic—benzyl bromides having electron-withdrawing substituents (Table 1, entries 2−4) needed to be cooled initially to control the reaction. Unlike benzyl bromides, dialkylation with *tert*-butyl 2-bromoacetate (Table 1, entry 5) required longer reaction time at slightly elevated temperature (50 °C) to provide **4e** in 79% yield.[20] Allyl bromide gave only trace amounts of the diallylated product **4f** even after prolonged reaction times and heating. The major product of this reaction was the mono-*C*-allylated derivative, ethyl 2-nitro-4-pentenoate (65%). Higher yields of **4f** could be obtained using allyl iodide as the electrophile; however, this reaction still required 24 h at 60 °C to provide product, which was still admixed with monoallylated ester. Double Michael-type addition to a variety of activated alkenes provided tetrafunctional nitroesters **4g**−**i** in good yields.

Table 1. Alkylation of Ethyl Nitroacetate 3 to $C^{\alpha,\alpha}$-Dialkylated Nitroacetates 4

entry	electrophile	method[a]	time (h)	temp (°C)	prod. no.	yield, %[b]
1	$PhCH_2Br$	A	1	25−60[c]	**4a**	63
2	p-O_2NPhCH_2Br	B	2	0−25	**4b**	75
3	p-$NCPhCH_2Br$	B	2	0−25	**4c**	82
4	p-$CH_3O_2CPhCH_2Br$	B	2	0−25	**4d**	72
5	$(CH_3)_3CO_2CCH_2Br$	B	24	50	**4e**	79
6	$CH_2{=}CHCH_2Br$	A	24	60	**4f**	trace[d]
7	$CH_2{=}CHCH_2I$	A[e]	24	60	**4f**	45
8	$CH_2{=}CHSO_2Ph$	C[f]	30	25	**4g**	70
9	$CH_2{=}CHCO_2t$-Bu	C	48	25	**4h**	89
10	$CH_2{=}CHCN$	C	48	25	**4i**	87

[a] Method A: Solvent: DMF; 1.5 M **3**, 0.15 M Bu_4NBr, 2.1 equiv of DIEA, 2.1, equiv of electrophile added last. Method B: Solvent: DMF; 1.5 M **3**, 0.15 M Bu_4NBr, 2.1, equiv of electrophile, 2.1 equiv of DIEA added last. Method C: Solvent: CH_3CN; 1.5 M **3**, 0.15 M Et_4NBr, 2.1 equiv of DIEA, 2.1, equiv of electrophile added last. [b] All percentage yields refer to isolated, analytically pure products. [c] Spontaneous exotherm to 60 °C. [d] Major product is monoallylated product, ethyl 2-nitro-4-pentenoate. [e] Bu_4NI is substituted for Bu_4NBr. [f] Bu_4NBr and Et_4NBr give the same yield.

The high yields for these dialkylation reactions make this a particularly viable route to symmetrically substituted ααAAs (vide infra) with or without side chain functionality. As examples of the utility of these α-amino acid precursors, the dibenzyl derivative **4a** and di-*tert*-butyl acetate derivate **4e** were converted to N^{α}-fluorenylmethyloxycarbonyl (Fmoc)-protected $C^{\alpha,\alpha}$-dibenzylglycine (Dbg) **7a** and 2,2-bis(*tert*-butylcarboxymethyl)-glycine (Bcmg(O*t*Bu)$_2$−OH) **7e**, respectively (Scheme 3). First, several methods were investigated for reduction of the nitro group to an amino group. Nitro ester **4a** was readily reduced with Zn/acetic acid to afford the amino ester **5a** in high yield (Table 2, entry 1). Results for catalytic hydrogenation approaches to reduce the nitro

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(13) Abbreviations: Aib, 2-aminoisobutyric acid; CCA, α-cyano-4-hydroxycinnamic acid; DBU, 1,8-Diazabicyclo[5.4.0]undec-7-ene; Deg, $C^{\alpha,\alpha}$-diethylglycine; DIEA, *N*,*N*-diisopropylethylamine; Dpg, $C^{\alpha,\alpha}$-dipropylglycine; FAB-MS, fast atom bombardment mass spectrometry; HATU, *O*-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium; HOAt, 1-hydroxy-7-azabenzotriazole; MALDI-MS, matrix assisted laser desorption ionization mass spectrometry; PAL, 5-(4-aminomethyl-3,5-dimethoxyphenoxy)valeric acid; PEG-PS, poly(ethylene glycol)−polystyrene graft; PyAOP, 7-azabenzotriazol-1-yloxytris(pyrrolidino)phosphonium hexafluorophosphate; PyBOP, 1-benzotriazolyoxytris(pyrrolidino)phosphonium hexafluorophosphate; TFA, trifluoroacetic acid; TPS, triisopropylsilane.

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Scheme 3

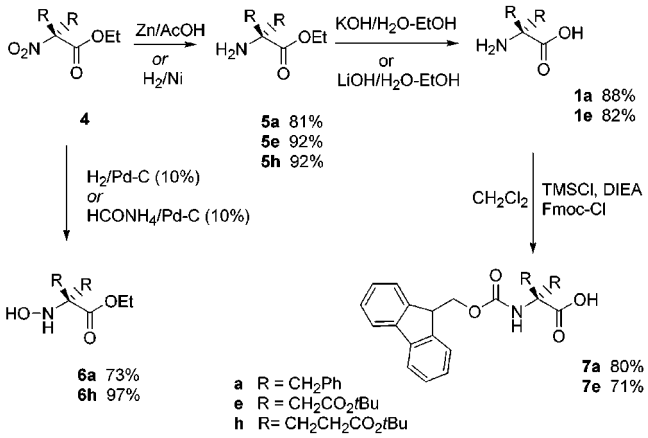

Table 2. Reduction of $C^{\alpha,\alpha}$-Disubstituted Nitro Esters 4a, 4e, and 4h

entry	nitro ester	conditions[a]	product	yield %[b]
1	**4a**	Zn/AcOH	**5a**	81
2	**4a**	HCO_2NH_4,10% Pd−C	**6a**	73
3	**4e**	H_2/Raney Ni	**5e**	92
4	**4h**	H_2/Raney Ni	**5h**	92
5	**4h**	H_2/10% Pd−C	**6h**	97
6	**4h**	HCO_2NH_4,10% Pd−C	**6h**	97

[a] Hydrogenation over Pd or Ni were carried out in hydrogenation flask at 50 psi; reduction with Zn/AcOH or HCO_2NH_4 in the presence of Pd−C was performed in a regular round-bottom flask. [b] All yields refer to isolated pure products.

group were mixed. Reduction of the nitro group of $C^{\alpha,\alpha}$-disubstituted nitro triester **4h**, an analogue of **4e**, with hydrogen (50 psi) over T-1 Raney Ni afforded the desired amino triester **5h** in excellent yield (Table 2, entry 4). In contrast, Pd/C-catalyzed hydrogenation or catalytic hydrogen transfer with ammonium formate in the presence of Pd resulted in almost quantitative conversion of **4h** to the corresponding hydroxylamine **6h** (Table 2, entries 5 and 6). Similarly catalytic transfer hydrogenation of **4a** also produced the hydroxylamine product **6a** (data not shown). It has been suggested[21] that Pd catalysts are preferable for partial reduction of aliphatic nitro groups to hydroxylamines. Boger et al.[22] reported hydrogenation of tertiary aliphatic nitro groups over a variety of catalysts. They found that hydrogenation over Raney Ni provided the amino derivatives with variable amounts of corresponding hydrocarbon from loss of the nitro group, while reduction using H_2−Pd/C, HCO_2NH_4−Pd/C, and H_2−PtO_2 afforded mixtures including the hydroxylamine and hydrocarbon. The observed partial reduction of $C^{\alpha,\alpha}$-disubstituted nitro ester **4h** into the hydroxylamine ester may be attributed to the relatively low activity of Pd catalysts and the intramolecular hydrogen bonding which enhanced the stability of the hydroxylamine derivative (Figure 1). On the basis of these experimental results, reduction of nitro triester **4e** was carried out using H_2/Ni at 50 psi, and the desired amino ester **5e** was obtained as a clear crystalline solid[23] in excellent yield (Table 2, entry 3).

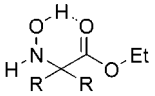

Figure 1. Intramolecular hydrogen bonding in hydroxylamine.

To make the desired Fmoc-protected amino acids, the ester of the α-carboxylate must first be removed. For amino ester **5a** this was readily accomplished by saponification under strongly basic conditions (2 M KOH) in refluxing H_2O−EtOH (v/v, 2:1) to give the free ααAA **1a** in 88% yield (Scheme 3). Unlike saponification of **5a**, hydrolysis of the ethyl ester of triester **5e** required more precise conditions to avoid concurrent *tert*-butyl ester hydrolysis. Selective hydrolysis was realized by treatment of **5e** with 3 equiv of lithium hydroxide in H_2O−EtOH (v/v, 1:2) at 50 °C to give the side-chain protected ααAA **1e** in 82% yield. Acid-catalyzed hydrolysis of **5e** with HCl, nucleophilic dealkylation, and saponfication using KOH failed to selectively hydrolyze the ethyl ester. The low selectivity of ethyl vs *tert*-butyl ester hydrolysis in this compound is most likely due to the steric congestion of the ethyl ester resulting from its proximity to the quaternary α-carbon. The free ααAAs are N^{α}-protected to allow for SPPS. Protection of the amine with Fmoc-Cl using an in situ silylation procedure[24] provided Fmoc-Dbg-OH (**7a**) in 80% yield and Fmoc-Bcmg(O*t*Bu)$_2$-OH (**7e**) in 71% yield. The synthetic route allows facile synthesis of sterically hindered ααAAs, even those with sensitive side-chain functionality, in good yields (57% for **7a** from **4a**, 54% for **7e** from **4e**).

Incorporation of Dbg into Peptides Using SPPS. Dbg has been previously incorporated into peptides using solution-phase peptide synthesis, and the prevalent method was using 2-trifluoromethyl-4,4-dibenzyloxazolin-5-one as the Dbg precusor.[18a,25] The sterically hindered Dbg inhibits efficient peptide bond formation. In our work, the coupling of the carboxyl group of Dbg using PyAOP proved relatively efficient, but acylation of the *N*-terminal Dbg turned out to be very difficult. The same problem was also encountered in solution-phase synthesis of peptides containing a Dbg residue.[18] In our studies, coupling of Fmoc-Lys(Boc)-OH onto the *N*-terminus of Dbg on PAL resin using PyAOP or PyAOP/HOAt were ineffective (Table 3, entries 1, 2), even though PyAOP has been shown to be efficient for acylation of ααAAs with less-hindered side chains, such as dipropylglycine[26] and Aib.[27] The acylation of Dbg using HATU also gave a low yield as determined by UV analysis of the Fmoc-deprotection (Table 3, entry 3).

Several other groups had previously found that secondary amino acids acylate poorly with in situ reagents

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Table 3. Coupling of Fmoc-Lys(Boc)-OH onto H-Dbg-Ala-Dpg-Glu(O*t*Bu)-PAL-PEG-PS

entry	coupling method	base	solvent	time (h)	temp °C	yield (%)[a]
1	PyAOP	DDIEA	DCE−DMF (1:1)	8	50	20
2	PyAOP−HOAt	DIEA	DCE−DMF (1:1)	8	25	25
3	HATU	DIEA	DMF	8	25	18
4	symmetrical anhydride[b]	no base	DCE−DMF (9:1)	3	50	95
5	symmetrical anhydride	no base	DCE−DMF (1:1)	3	50	81
6	symmetrical anhydride	no base	DMF	3	50	52

[a] The coupling yield was determined by UV analysis of the Fmoc-deprotection.[15b] [b] For entries 4−6, the preformed Fmoc-Lys(Boc)-symmetrical anhydride was used in the coupling. Symmetrical anhydride was prepared by treatment of 2 equiv of Fmoc-Lys(Boc)-OH with 1 equiv of DCC in CH_2Cl_2 at room temperature for 2 h. DCU was removed by filtration. After removal of CH_2Cl_2 by evaporation, the symmetrical anhydride was taken up in the described solvent for coupling.

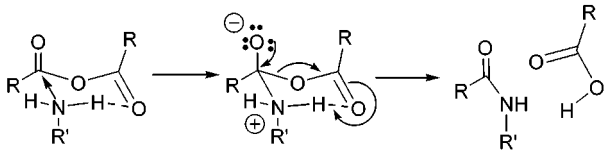
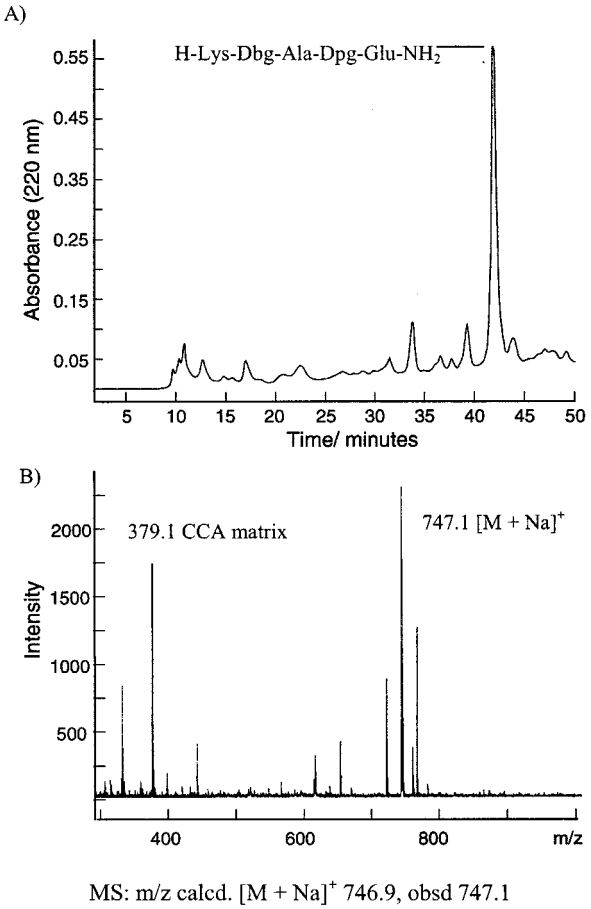


Figure 2. Potential anchimeric assistance provided by the H-bond donation of the symmetrical anhydride.

(DCC/HOBt; HOBt/HOAt-derived uronium or phosphonium salts), but more readily react with symmetrical anhydrides in nonpolar solvents such as dichloromethane.[28,29] To our surprise, coupling of the symmetrical anhydride of Fmoc-Lys(Boc)-OH with the *N*-terminus of Dbg in DCE-DMF (v/v, 9:1) in the *absence of base* gave an almost quantitative yield (Table 3, entry 4). It is not as yet understood why the symmetrical anhydride method is so much more efficient at acylating the sterically hindered Dbg residue than methods based on uronium or phosphonium salts. One possiblility is that the symmetrical anyhdride may possess an enhanced reactivity in the aprotic nonpolar solvent for which anchimeric assistance from H-bonding is more pronounced (Figure 2). The results bear this out in that coupling of the symmetrical anhydride in DCE−DMF (9:1) gives a 95% coupling yield (Table 3, entry 4). In contrast reaction of the symmetrical anhydride in the more H-bond accepting solvent mixture of DCE−DMF (1:1) or in pure DMF (Table 3, entries 5, 6), gave systematic decreases in coupling efficiency. The fact that the nonpolar solvent swells the PAL-PEG-PS resin more efficiently than pure DMF may also attribute to the successful coupling. The pentapeptide containing Dbg was prepared using the optimized coupling conditions and cleaved from the resin by 88% TFA and analyzed by reverse-phase HPLC (Figure 3).



MS: m/z calcd. $[M + Na]^+$ 746.9, obsd 747.1

Figure 3. (A) HPLC profile of crude peptide (H-Lys-Dbg-Ala-Dpg-Glu-NH_2). Column: Delta-Pak C_{18}, 15 μm 300 Å, 8 × 100 mm; eluent A: 0.05% TFA in H_2O; eluent B: 0.05% TFA in CH_3CN; gradient: 10−60% B over 50 min; flow rate 1 mL/min. (B) MALDI-MS spectrum of the main peak on HPLC.

Conclusion

In summary, we have outlined the synthesis of sterically hindered ααAAs via the mild dialkylation of nitroacetate. The method has been shown to be successful for the synthesis of highly sterically encumbered ααAAs (Fmoc-Dbg-OH), as well as the synthesis of chemically sensitive ααAAs, such as the N^{α}- and side-chain-protected tetrafunctional derivatives (Fmoc-Bcmg(O*t*Bu)$_2$-OH). This methodology should prove invaluable for the preparation of previously unavailable ααAAs with and without side chain functionality. Also, we have presented the first example of Dbg being incorporated into peptides via solid-phase approach and an efficient method for readily incorporating amino acid residues onto the sterically hindered *N*-terminus of ααAAs. This facile and convenient coupling method may be extended to a variety of sterically hindered ααAAs for solid-phase or solution-phase peptide synthesis.

Experimental Section

General Methods. All melting points were measured with a Fisher-Johns melting point apparatus and are uncorrected. ^1H and ^{13}C spectra were recorded on a 250 and 500 MHz spectrometers. Flash column chromatography was performed

(28) (a) Coupling to *N*-MePhe derivative: Rich, D. H.; Bhatnagar, P.; Mathiaparanam, P.; Grant, J. A.; Tam, J. P. *J. Org. Chem.* **1978**, *43*, 296−302. (b) Resin-bound *N*-benzyl("BAL")-amino acid: Jensen, K. J.; Alsina, J.; Songster, M. F.; Vagner, J.; Albericio, F., Barany, G. *J. Am. Chem. Soc.* **1998**, *120*, 5441−5452, and refs cited therein.

(29) For an excellent review of coupling methods for sterically hindered amino acids, see: Humphrey, J. M.; Chamberlain, A. R. *Chem. Rev.* **1997**, 2243-2266

using silica gel 60 (230−400 mesh). Elemental analyses were performed by M−H−W Laboratories (Phoenix, AZ). TLC was performed on Merck silica gel 60 F_{254} plates and visualized by UV lamp. The peptide was synthesized in a two-necked round-bottom flask, one neck fitted with frit suitable for filtration. A Brinkmann rotary evaporator and a Labquake shaker were used for rotating and shaking the flask containing resin. UV analysis of the Fmoc-deprotection was performed on a Gilford UV spectrometer. The peptide was purified on a Waters HPLC system (Delta-Pak C_{18} column, 15 μm 300 Å, 8 × 100 mm for analytical scale, flow rate 1 mL/min; 25 × 100 mm for preparative scale, flow rate 10 mL/min). Peptide mass was verified by mass spectrometry on a Bruker ProFLEX III MALDI-MS.

Ethyl 2-Benzyl-2-nitro-3-phenylpropanoate (4a). To a solution of ethyl nitroacetate **3** (1.0 g, 7.51 mmol) in dry DMF (5 mL) were added DIEA (2.0 g, 15.4 mmol) and $Bu_4N^+Br^-$ (0.24 g, 0.75 mmol). To this clear yellow solution was added benzyl bromide (2.6 g, 15.4 mmol), and the reaction spontaneously warmed to 60 °C over 5 min. After 20 min, DIEA·HBr precipitated out of solution. After 1 h, the solution was filtered to remove DIEA·HBr, the salt was washed with Et_2O (100 mL), and the combined filtrates were washed with H_2O (5 × 50 mL). The organic layer was dried (Na_2SO_4) and evaporated at 0 °C to provide a yellow oil, which was of sufficient purity to be used in further reactions or could be purified to homogeneity by silica gel chromatography using Et_2O−pentane (1:4) to provide a white solid. Yield, 1.48 g (63%); mp 81−82 °C (lit.[10] mp 79−80 °C); ^{1}H NMR (250 MHz, DMSO-d_6) δ 7.38−7.22 (m, 10H), 4.10 (q, J = 7.1 Hz, 2H), 3.52 (d, J = 14.2 Hz, 2H), 3.46 (d, J = 14.2 Hz, 2H), 1.07 (t, J = 7.1 Hz, 3H); ^{13}C NMR (60 MHz, $CDCl_3$) δ 166.5, 133.4, 130.3, 128.8, 128.0, 97.4, 62.9, 40.2, 13.7; HRMS (FAB) calcd for $C_{18}H_{19}NO_4$ $(M + H)^+$ 314.1392, found 314.1395. Anal. Calcd for $C_{18}H_{19}NO_4$: C, 68.99; H, 6.11; N, 4.47. Found: C, 69.09; H, 5.94; N, 4.32.

Ethyl 2-Nitro-2-(4-nitrobenzyl)-3-(4-nitrophenyl)propanoate (4b). To a solution of ethyl nitroacetate **3** (1.0 g, 7.51 mmol) in dry DMF (5 mL) was added $Bu_4N^+Br^-$ (0.24 g, 0.75 mmol) and 4-nitrobenzyl bromide (3.3 g, 15.4 mmol). To this cooled solution, DIEA (2.0 g, 15.4 mmol) was added dropwise, and the solution was allowed to stir for 2 h. The resulting solution was diluted with diethyl ether (100 mL) and washed successively with 1 N HCl (2 × 15 mL), saturated aqueous sodium carbonate solution (2 × 15 mL; to remove mono-alkylated product), and water (5 × 15 mL). The solvent was removed in vacuo, and the resulting light yellow solid was recrystallized from ethanol to provide 2.27 g (75%) of **4b** as white crystals. mp 194−196 °C; ^{1}H NMR (250 MHz, DMSO-d_6) δ 8.21 (d, J = 8.7 Hz, 4H), 7.51 (d, J = 8.7 Hz, 4H), 4.11(q, J = 7.1 Hz, 2H), 3.74 (d, J = 14.2 Hz, 2H), 3.70 (d, J = 14.2 Hz, 2H), 1.05 (t, J = 7.1 Hz, 3H); ^{13}C NMR (60 MHz, DMSO-d_6) δ 165.0, 147.1, 141.1, 131.6, 123.4, 96.9, 63.0, 39.6, 13.3; HRMS (FAB) calcd for $C_{18}H_{17}N_3O_8$ $(M + H)^+$ 404.1094, found 404.1094. Anal. Calcd for $C_{18}H_{17}N_3O_8$: C, 53.60; H, 4.25; N, 10.42. Found: C, 53.40; H, 4.51; N 10.31.

Ethyl 2-(4-Cyanobenzyl)-2-nitro-3-(4-cyanophenyl)-propanoate (4c). This compound was prepared from 4-cyanobenzyl bromide in 82% yield by using the same procedure as described for **4b**. mp 138−139 °C; ^{1}H NMR (250 MHz, DMSO-d_6) δ 7.83 (d, J = 8.3 Hz, 4H), 7.41 (d, J = 8.3 Hz, 4H), 4.08 (q, J = 7.1 Hz, 2H), 3.76 (d, J = 14.2 Hz, 2H), 3.69 (d, J = 14.2 Hz, 2H), 1.03 (t, J = 7.1 Hz, 3H); ^{13}C NMR (60 MHz, DMSO-d_6) δ 165.0, 139.0, 132.3, 131.2, 118.5, 110.7, 96.9, 62.9, 39.9, 13.3; HRMS (FAB) calcd for $C_{20}H_{17}N_3O_4$ $(M + H)^+$ 364.1297, found 364.1298. Anal. Calcd for $C_{20}H_{17}N_3O_4$: C, 66.10.; H, 4.72; N, 11.56. Found: C, 65.93; H, 4.97; N 11.55.

Ethyl 2,2-Bis(methylcarboxybenzyl)-2-nitroacetate (4d). This compound was prepared from methyl 4-(bromomethyl)-benzoate in 72% yield by using the same procedure as described for **4b**. mp 116−117 °C; ^{1}H NMR (250 MHz, DMSO-d_6) δ 8.01−7.97 (m, 4H), 7.26−7.21 (m, 4H), 4.14 (q, J = 7.2 Hz, 2H), 3.92 (s, 6H), 3.53 (s, 4H), 1.13 (t, J = 7.2 Hz, 3H); ^{13}C NMR (60 MHz, DMSO-d_6) δ 165.8, 165.2, 138.7, 130.6, 129.2, 129.0, 96.9, 62.8, 52.1, 39.9, 13.3; HRMS (FAB) calcd for $C_{22}H_{23}NO_8$ $(M + H)^+$ 430.1502, found 430.1492. Anal. Calcd for $C_{22}H_{23}NO_8$: C, 61.53; H, 5.40; N, 3.26. Found: C, 61.66; H, 5.62; N 3.26.

Bis(*tert*-butyl) 3-Ethylcarboxy-3-nitroglutarate (4e). This compound was prepared from *tert*-butyl bromoacetate in 79% yield by using the same procedure as described for **4b**. ^{1}H NMR (250 MHz, $CDCl_3$) δ 4.25 (q, J = 7.2 Hz, 2H), 3.42 (d, J = 16.8 Hz, 2H), 3.30 (d, J = 16.8 Hz, 2H), 1.42 (s, 18 H), 1.24 (t, J = 7.2 Hz, 3H); ^{13}C NMR (60 MHz, $CDCl_3$) δ 167.4, 165.0, 90.0, 82.6, 63.5, 39.9, 28.1, 13.9; Anal. Calcd for $C_{16}H_{27}NO_8$: C, 53.18; H, 7.53; N, 3.88. Found: C, 53.04; H, 7.33; N 3.81.

Ethyl 2,2-Bis(allyl)-2-nitroacetate (4f). This compound was previously prepared by Pd-mediated approaches.[30] In this work **4f** was prepared from allyl iodide by using the same procedure as described for **4a** followed by flash chromatography (hexanes−ether, 1:3) to provide **4f** in 45% yield. ^{1}H NMR (250 MHz, $CDCl_3$) δ 5.68−5.57 (m, 2H), 5.24−5.17 (m, 4H), 4.27 (q, J = 7.2 Hz, 2H), 2.97−2.90 (m, 4H), 1.29 (t, J = 7.2 Hz, 3H); ^{13}C NMR (60 MHz, $CDCl_3$) δ 166.8, 129.5, 121.6, 95.0, 62.9, 38.2, 14.1; MS (FAB) calcd for $C_{10}H_{15}NO_4Na$ $(M + Na)^+$ 236.1, found 236.1. Anal. Calcd for $C_{10}H_{15}NO_4$: C, 56.32; H, 7.09; N, 6.57. Found: C, 56.41; H, 6.98; N 6.53.

Ethyl 2,2-Bis(2-phenylsulfonyl ethyl)-2-nitroacetate (4g). To a solution of ethyl nitroacetate (0.4 g, 3.0 mmol) in acetonirile (5 mL) were added DIEA (0.79 g, 6.15 mmol) and tetraethylammonium bromide (0.09 g, 0.3 mmol). To this solution was added phenyl vinyl sulfone (1.03 g, 6.15 mmol) dropwise, and the solution was stirred at room temperature for 30 h. The acetonitrile was then removed in vacuo, and the residue was dissolved in ethyl acetate (25 mL). The resulting solution was washed with 10% aqueous K_2CO_3 (2 × 10 mL) and dried over anhydrous Na_2SO_4, and the solvent was removed in vacuo to give **4g** as a light yellow solid. Yield, 0.98 g (70%). mp 88−89 °C; ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.92−7.59 (m, 10H), 4.25 (q, J = 7.1 Hz, 2H), 3.17−3.10 (m, 4H), 2.60−2.53 (m, 4H), 1.26 (t, J = 7.1 Hz, 3H); ^{13}C NMR (60 MHz, $CDCl_3$) δ 164.3, 138.1, 134.2, 129.5, 127.8, 92.8, 63.6, 49.3, 26.6, 13.3; HRMS (FAB) calcd for $C_{20}H_{23}NO_8S_2$ $(M + H)^+$ 470.0977, found 470.0936. Anal. Calcd for $C_{20}H_{23}NO_8S_2$: C, 51.16; H, 4.94; N, 2.98; S, 13.66. Found: C, 51.11; H, 5.07; N, 3.00; S, 13.51.

Bis(*tert*-butyl)-4-ethylcarboxy-4-nitropimelate (4h). This compound was prepared from *tert*-butyl acrylate in 89% yield by using the same procedure as described for **4g**. ^{1}H NMR (250 MHz, DMSO-d_6) δ 4.24 (q, J = 7.1 Hz, 2H), 2.41−2.20 (m, 8H), 1.40 (s, 18H), 1.21 (t, J = 7.1 Hz, 3H); ^{13}C NMR (60 MHz, DMSO-d_6) δ 170.3, 165.8, 94.5, 80.3, 62.9, 29.0, 28.3, 27.6, 13.5; HRMS (FAB) calcd for $C_{18}H_{31}NO_8$ $(M + H)^+$ 390.2128, found 390.2118. Anal. Calcd for $C_{18}H_{31}NO_8$: C, 55.51; H, 8.02; N, 3.60. Found: C, 55.31; H, 7.83; N, 3.87.

4-Ethylcarboxy-4-nitropimelonitrile (4i). This compound was prepared from acrylonitrile in 87% yield by using the same procedure as described for **4g**. mp 52−53 °C (lit.[11] mp 52−53 °C); ^{1}H NMR (250 MHz, DMSO-d_6) δ 4.27 (q, J = 7.1 Hz, 2H), 2.70−2.54 (m, 8H), 1.24 (t, J = 7.1 Hz, 3H); ^{13}C NMR (60 MHz, DMSO-d_6) δ 164.7, 118.9, 93.2, 63.5, 28.5, 13.4, 11.7; HRMS (FAB) calcd for $C_{10}H_{13}N_3O_4Na$ $(M + Na)^+$ 262.0804, found 262.0809. Anal. Calcd for $C_{10}H_{13}N_3O_4$: C, 50.20; H, 5.48; N, 17.56. Found: C, 50.07; H, 5.36; N, 17.61.

Ethyl 2,2-Dibenzylglycine Ester (5a). To a solution of nitro ester **4a** (1.0 g, 3.19 mmol) in glacial AcOH (15 mL) at 15 °C was added Zn dust (0.63 g, 9.57 mmol) in several portions over 1 h. The resulting mixture was allowed to warm to room temperature and vigorously stirred for 24 h. The solids were filtered off and washed with 1.0 M HCl (2 × 5 mL), and the combined filtrates were concentrated to an oil. This oil was dissolved in a mixture of ethyl acetate (10 mL) and aqueous 1 M NaOH (15 mL) and stirred for 24 h. The organic layer was separated, dried ($MgSO_4$), and evaporated in vacuo to provide amino ester **5a** as a white solid (0.73 g, 81% yield). mp 93−95 °C; ^{1}H NMR (250 MHz, DMSO-d_6) δ 7.30−7.16 (m, 10H), 4.0

(30) (a) Lopez, A.; Moreno-Manas, M.; Pleixats, R.; Roglans, A. *An. Quim. Int. Ed.* **1997**, *93*, 355−362. (b) Ferroud, D.; Genet, J. P.; Muzart, J. *Tetrahedron Lett.* **1984**, *25*, 4379−4382.

(q, J = 7.2 Hz, 2H), 3.18 (d, J = 12.9 Hz, 2H), 2.77 (d, J = 12.9 Hz, 2H), 1.43 (s, 2H), 1.16 (t, J = 7.2 Hz, 3H), ^{13}C NMR (60 MHz, DMSO-d_6) δ 174.8, 136.6, 130.0, 128.0, 126.6, 62.6, 60.3, 45.9, 14.0; HRMS (FAB) calcd for $C_{18}H_{21}NO_2$ $(M + H)^+$ 284.1650, found 284.1637. Anal. Calcd for $C_{18}H_{21}NO_2$: C, 76.29; H, 7.47; N, 4.94. Found: C, 76.37; H, 7.31; N, 4.83.

Ethyl 2,2-Bis(*tert*-butylcarboxylmethyl)glycine Ester (5e). To a solution of **4e** (1.3 g, 3.92 mmol) in absolute ethanol (10 mL) were added glacial acid (1 mL) and a 50% (w/w) slurry of Raney nickel in water (1.0 g). The reaction was hydrogenated over H_2 gas (50 psi) for 24 h. The resulting solution was filtered carefully over Celite and the Celite cake washed with EtOH (30 mL). The solvent was removed in vacuo, and the crude was dissolved in diethyl ether (30 mL). After washed with saturated sodium carbonate (30 mL) and brine, the organic fraction was dried (Na_2SO_4) and rotary evaporated to yield **5e** in 92% yield. ^{1}H NMR (250 MHz, $CDCl_3$) δ 4.12 (q, J = 7.2 Hz, 2H), 2.68 (d, J = 15.5 Hz, 2H), 2.48(d, J = 15.5 Hz), 2.26 (s, 2H), 1.37 (s, 18H), 1.20 (t, J = 7.2 Hz, 3H); ^{13}C NMR (60 MHz, $CDCl_3$) δ 174.9, 169.7, 81.5, 61.5, 57.7, 45.0, 28.1, 14.2. Anal. Calcd for $C_{16}H_{29}NO_6$: C, 57.99; H, 8.82; N, 4.23. Found: C, 58.07; H, 8.86; N, 4.03.

Ethyl 2,2-Bis(*tert*-butylcarboxyethyl)glycine Ester (5h). This compound was prepared from **4h** in 92% yield by using the same procedure as described for **5e**. ^{1}H NMR (250 MHz, DMSO-d_6) δ 4.09 (q, J = 7.2 Hz, 2H), 2.34−1.64 (m, 8H), 1.81 (s, 2H), 1.38 (s, 18H), 1.18 (t, J = 7.2 Hz, 3H); ^{13}C NMR (60 MHz, DMSO-d_6) δ 175.2, 172.0, 79.5, 60.4, 59.2, 34.2, 29.8, 27.6, 14.0; MS (FAB) calcd for $C_{18}H_{33}NO_6$ 359.46, found 359.35.

Ethyl 2,2-Bis(*tert*-butylcarboxyethyl)-*N*-hydroxyglycine Ester (6h). To a solution of **4h** (1.0 g, 2.57 mmol) in absolute ethanol (15 mL) was added carefully 10% (w/w) palladium on carbon (0.1 g). The reaction was hydrogenated over H_2 gas (50 psi) for 24 h. The resulting solution was filtered carefully over Celite and the Celite cake washed with EtOAc (30 mL). The organic filtrate was dried (Na_2SO_4) and rotary evaporated to yield **5k** in 97% yield. ^{1}H NMR (250 MHz, DMSO-d_6) δ 7.23 (s, 1H), 5.69 (s, 1H), 4.06 (q, J = 7.2 Hz, 2H), 2.17−2.13 (m, 4H), 1.74−1.70 (m, 4H), 1.37 (s, 18H), 1.17 (t, J = 7.2 Hz, 3H); ^{13}C NMR (60 MHz, DMSO-d_6) δ 173.6, 172.0, 79.5, 66.6, 60.5, 29.1, 27.7, 26.3, 14.0; MS (FAB) calcd for $C_{18}H_{33}NO_7$ $(M + H)^+$ 376.47, found 376.77.

2,2-Dibenzylglycine (1a). A suspension of **5a** (2.0 g, 7.1 mmol) in 2 M KOH (50 mL) and ethanol (25 mL) was refluxed under N_2 for 24 h. The resulting mixture was concentrated to 20 mL in vacuo, and the mixture was acidified to pH 6.5 with concentrated HCl. The white precipitate was filtered, washed with water (5 mL), and evaporated to provide the amino acid **6a** (1.58 g, 6.2 mmol) in 88% yield (mp > 300 °C). ^{1}H NMR (250 MHz, CD_3OD) δ 7.27 (bs, 10H), 3.36 (d, J = 13.3 Hz, 2H), 2.85 (d, J = 13.3 Hz, 2H), 1.27 (s, 2H), HRMS (FAB) calcd for $C_{16}H_{17}NO_2Na$ $(M + Na)^+$ 278.1157, found 278.1159. Anal. Calcd for $C_{16}H_{17}NO_2$: C, 75.27; H, 6.71; N, 5.48. Found: C, 75.34; H, 6.64; N, 5.30.

2,2-Bis(*tert*-butylcarboxymethyl)glycine (1e). To a solution of **5e** (2.0 g, 6.03 mmol) in ethanol (20 mL) was added LiOH (0.4 g, 18.1 mmol) in water (10 mL). The reaction was stirred at 50 °C for 3 h followed by the same workup procedure as described for **1a** to provide the amino acid as a white solid. Yield, 1.53 g (82%). ^{1}H NMR (250 MHz, CD_3OD) δ 2.78 (d, J = 17.2 Hz, 2H), 2.69 (d, J = 17.2 Hz), 1.37 (s, 18H); ^{13}C NMR (125 MHz, CD_3OD) δ 175.8, 173.7, 85.7, 62.7, 43.3, 30.5; HRMS (FAB) calcd for $C_{14}H_{25}NO_6$ $(M + H)^+$ 304.1760, obsd 304.1760.

***N*$^\alpha$-(9-Fluorenylmethoxycarbonyl)-2,2-dibenzylglycine (7a).** TMSCl (0.85 g, 7.84 mmol) was added to a suspension of amino acid **1a** (1.0 g, 3.92 mmol) in dry CH_2Cl_2 (20 mL) and refluxed under N_2 for 8 h. The mixture was cooled to 0 °C, and DIEA (1.01 g, 7.84 mmol) and Fmoc-Cl (1.01 g, 3.92 mmol) were added. The reaction was allowed to warm to 25 °C and stirred for 30 h. The resulting mixture was concentrated in vacuo to provide a yellow solid which was dissolved in aqueous 10% $NaHCO_3$. This solution was washed with Et_2O (3 × 15 mL), and the aqueous layer was acidified to pH 2.0 with HCl and extracted with EtOAc (3 × 35 mL). The combined EtOAc layers were dried ($MgSO_4$) and concentrated in vacuo to provide a light yellow solid. The crude product was purified by crystallization from Et_2O−hexanes to give the protected ααAA **7a** as a white solid (1.5 g, 80% yield). mp 184−185 °C; ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.81−7.10 (m, 18H), 5.50 (s, 1H), 4.49 (d, J = 7.0 Hz, 2H), 4.29 (t, J = 7.0 Hz, 1H), 3.90 (d, J = 13.6 Hz, 2H), 3.26 (d, J = 13.6 Hz, 2H); ^{13}C NMR (60 MHz, $CDCl_3$) δ 177.2, 155.0, 144.2, 141.7, 136.1, 130.1, 128.8, 128.1, 127.5, 125.6, 120.4, 67.1, 66.9, 47.7, 41.5; HRMS (FAB) calcd for $C_{31}H_{27}NO_4$ $(M + H)^+$ 478.2018, found 478.1996. Anal. Calcd for $C_{31}H_{27}NO_4$: C, 77.96; H, 5.70; N, 2.93. Found: C, 78.15; H, 5.85; N 2.99.

***N*$^\alpha$-(9-Fluorenylmethyloxycarbonyl)-2,2-bis(*tert*-butylcarboxymethyl)glycine (7e).** This compound was prepared from **1e** in 71% yield by using the same procedure as described for **7a**. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.76−7.24 (m, 8H), 6.33 (s, 1H), 4.32 (d, J = 7.0 Hz, 2H), 4.20 (t, J = 7.0 Hz, 1H), 3.37 (d, J = 15.1 Hz, 2H), 2.85 (d, J = 15.1 Hz, 2H), 1.38 (s, 18H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 171.9, 168.4, 153.9, 143.7, 140.7, 127.6, 127.0, 125.1, 120.1, 80.1, 65.6, 57.9, 46.5, 39.7, 27.6; Anal. Calcd for $C_{29}H_{35}NO_8$: C, 66.27; H, 6.71; N, 2.66. Found: C, 66.48; H, 6.87; N 2.56.

Solid-Phase Peptide Synthesis. (1) Preparation of Resin-Bound H-Dbg-Ala-Dpg-Glu(O*t*Bu)-PAL-PEG-PS. The peptide was prepared using Fmoc methodology[16] with Fmoc-PAL-PEG-PS resin (0.20 mmol/g loading) as a solid support. To ensure a high degree of coupling efficiency, all amino acids were coupled using PyAOP in the manual protocol as a coupling reagent. The peptide chain was assembled at 0.4 mmol scale, and each coupling consisted of the following steps: (i) removal of Fmoc protection group with DBU−piperidine−DMF (2:5:93; 20 mL) at room temperature for 15 min. (ii) Coupling of the Fmoc-amino acid (1.6 mmol, 4 equiv) onto resin using PyAOP (1.6 mmol, 4 equiv) and DIEA (3.2 mmol, 8 equiv) in the solvent of DCE−DMF (1:1; 8 mL) at 50 °C for 2 h. After each coupling step, coupling efficiency was examined by UV analysis of the Fmoc-deprotection (~8 mg of resin in 1 mL of deblocking solution for 10 min).

Coupling of Fmoc-Lys(Boc)-OH onto H-Dbg-Ala-Dpg-Glu(O*t*Bu)-PAL-PEG-PS. Coupling of Fmoc-Lys(Boc)-OH onto resin-bounded peptide was performed on 0.1 mmol scale resin under different conditions. (i) Coupling using PyAOP and DIEA was carried out under the same conditions as described above. Acylation yield, 20%. (ii) Coupling of Fmoc-Lys(Boc)-OH (0.4 mmol, 4 equiv) using PyAOP (0.4 mmol, 4 equiv), HOAt (0.4 mmol, 4 equiv), and DIEA (0.8 mmol, 8 equiv) in DCE−DMF (1:1; 2 mL) at room temperature for 8 h gave a 25% coupling yield. (iii) The acylation of *N*-terminal Dbg with Fmoc-Lys(Boc)-OH (0.2 mmol, 4 equiv) using HATU (0.2 mmol, 4 equiv) and DIEA (0.4 mmol, 8 equiv) in DMF (2 mL) at room temperature gave an 18% coupling yield. (iv) The symmetrical anhydride method was performed by adding preformed (Fmoc-Lys(Boc))$_2$O (0.3 mmol, 3 equiv) to the resin-bound peptide in DCE−DMF (9;1; 4 mL) in the absence of base. The reaction was carried out at 50 °C for 2 h to give a 95% coupling yield determined by UV analysis. After acylation, the Fmoc group on Lys was removed, and the resin was suitable for cleavage.

Peptide Cleavage and Purification. The resin was treated with a cleavage solution of TFA/TPS/phenol/water (88:2:5:5, v/v/v, 10 mL) at room temperature for 2 h. After filtration, the filtrate was diluted with cold 30% acetic acid (50 mL) and extracted with cold ether (4 × 30 mL). The aqueous fraction was then lyophilized overnight to provide the crude peptide. Peptide purification was performed by reversed-phase HPLC on C_{18} column. The peptide homogeneity (>99%) was determined by analytical HPLC using the same solvents with a gradient of 10−40% (v/v) acetonitrile over 30 min. Peptide was identified by MALDI-MS, m/z calcd $[M + Na]^+$ 746.9, obsd 747.1.

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Supporting Information Available: NMR spectra for **4a**-**4i**, **5a**, **5e**, **5h**, **6h**, **1a**, **1e**, **7a**, and **7e**; ORTEPs of **4e** and **5e**, tables of crystallographic data, bond lengths and angles, atomic coordinates and anisotropic thermal parameters for structures for **4e** and **5e**; analytical HPLC profile for isolated pure peptide H-Lys-Dbg-Ala-Dpg-Glu-NH_2. This material is available free of charge via the Internet at http://pubs.acs.org.

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