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Structure−Affinity Relationships of Glutamine Mimics Incorporated into Phosphopeptides Targeted to the SH2 Domain of Signal Transducer and Activator of Transcription 3

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In cancer cells, signal transducer and activator of transcription 3 (Stat3) participates in aberrant growth, survival, angiogenesis, and invasion signals and is a validated target for anticancer drug design. We are targeting its SH2 domain to prevent docking to cytokine and growth factor receptors and subsequent signaling. One of the important elements of the recognition sequence, pTyr-Xxx-Xxx-Gln, is glutamine. We incorporated novel Gln mimics into a lead peptide, pCinn-Leu-Pro-Gln-NHBn, and found that a linear, unconstrained side chain and carboxamide are necessary for high affinity, and the benzamide can be eliminated. Replacement of Gln-NHBn with (*R*)-4-aminopentanamide or 2-aminoethylurea produced inhibitors with equal or greater potency than that of the lead, as judged by fluorescence polarization (IC_{50} values were 110 and 130 nM, respectively). When Pro was replaced with *cis*-3,4-methanoproline, the glutamine mimic, (4*R*,5*S*)-4-amino-5-benzyloxyhexanamide resulted in an IC_{50} of 69 nM, the highest affinity Stat3 inhibitor reported to date.

Introduction

Signal transducer and activator of transcription 3 (Stat3) transmits signals from IL-6 family cytokines, epidermal growth factor, plate-derived growth factor, leptin, and vascular epithelial growth factor directly from their receptors on the cell surface to the nucleus.[1−5] On binding of these extracellular signaling proteins, Stat3 is recruited to phosphotyrosine residues on their receptors to which it binds via its SH2 domain. Stat3 then becomes phosphorylated on Tyr705, a process known as activation, either by the tyrosine kinase activity of the receptor or that of associated Janus or Src-family kinases. The phosphorylated protein dimerizes via reciprocal interactions between the SH2 domains and pTyr705 residues, and the activated complex is translocated to the nucleus where it binds to promoters of genes involved in cell survival, cell cycling, invasion and migration, and angiogenesis. Constitutively activated Stat3 has been detected in tumor samples from numerous cancers.[6] Inhibition of Stat3 activity by antisense oligonucleotides, decoy oligonucleotides, and siRNA results in apoptosis and reduced cell growth of tumor cells. Thus Stat3 is a target for antitumor drug design.[2,4]

We[7−11] and others[12−21] have been targeting the SH2 domain of Stat3 with phosphopeptides and related peptidomimetics to break up preformed dimers and to prevent initial docking to receptors and the subsequent events of activation, dimerization, nuclear transport, and expression of downstream genes. The recognition determinant for this target is pTyr-Xxx-Xxx-Gln.[7,22−24] Of particular importance is the requirement for glutamine three residues C-terminal to the phosphotyrosine, pY+3. From a drug design perspective, the hydrophilic nature of this amino acid is likely to impart specificity for inhibitors of Stat3 since non-Stat SH2 domains typically recognize hydrophobic residues at this position.[25−30] In a screen of putative receptor docking sites for Stat3, our laboratory found that pTyr-Leu-Pro-Gln-Thr-Val-NH_2 (**1**) was a high-affinity ligand and it possessed glutamine at pY+3.[7] Extensive studies that probed the interactions between each amino acid and Stat3 were conducted.[7−11] Modification of glutamine, for example, by side chain N-methylation, conversion to carbamoyl threonine, replacement with methionine sulfoxide, etc., provided information that supported a model of phosphopeptide−Stat3 binding in which the side chain fits tightly into a groove on the surface of the protein at the junction of β-strand D and the STAT protein-specific helix, $\alpha B'$ (Figure 1).[31] Direct hydrogen bonds are hypothesized to occur between the NH_2 group of Gln and main chain oxygens of Glu638 and Pro639 as well as between the C=O of Gln and the side chain NH_2 of Gln644. A water-mediated hydrogen bond may also form between the C=O of Gln and the side chain carboxyl group of Glu638. In addition, a hydrogen bond is predicted to exist between the backbone NH of the residue C-terminal to Gln and the side chain hydroxyl group of Tyr640 of Stat3.

Glutamine can be considered to be 4-carboxy-4-aminobutyramide. In this paper, we incorporated a library of novel 4-aminobutyramide (Aba)[a] derivatives into a phosphopeptide

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[a] Abbreviations: Aba, 4-aminobutyramide; 4-Abu, 4-aminobutyric acid; DIEA, diisopropylethylamine; DIPCDI, diisopropylcarbodiimide; Fmoc, 9-fluorenylmethoxycarbonyl; FMPB, 4-(4-formyl-3-methyoxyphenoxy)butyryl; HOBt, 1-hydroxybenzotriazole; homoGlu, homoglutamic acid; Met(O), methionine sulfoxide; Met(O2), methionine sulfone; mPro, *cis*-3,4-methanoproline; pCinn, 4-phosphoryloxycinnamic acid; PyBOP, 1*H*-benzotriazol-1-yloxytripyrrolidinophosphonium hexafluorophosphate; SAR, structure−activity relationship; TES, triethylsilane; TIS, triisopropylsilane.

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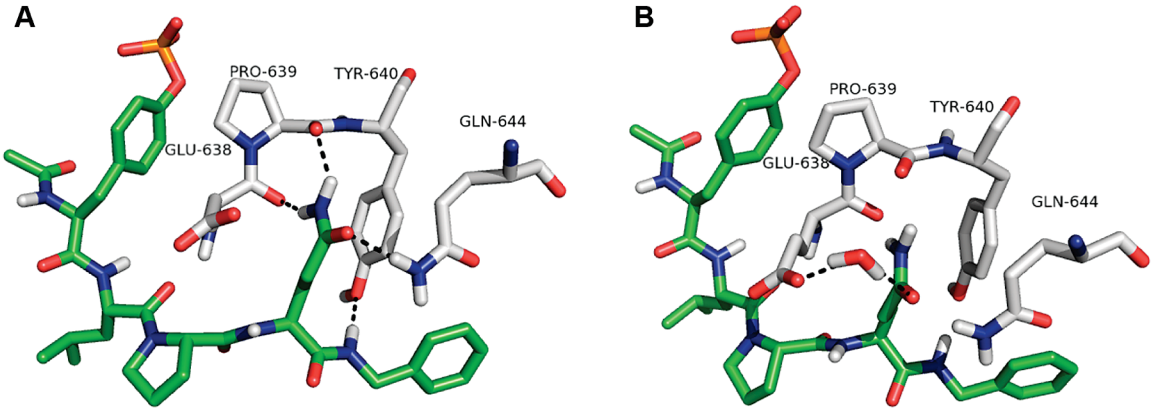
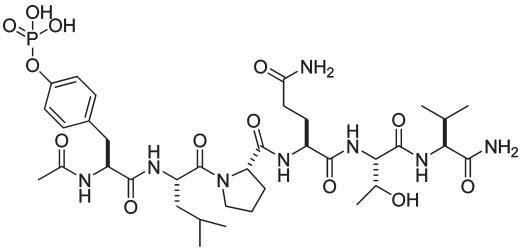
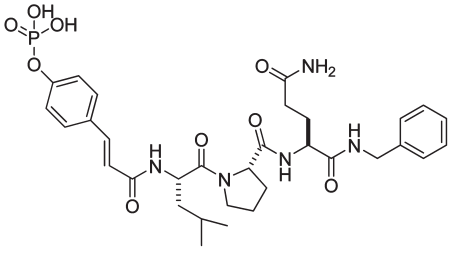


Figure 1. Hydrogen-bonding interactions between Ac-pTyr-Leu-Pro-Gln-NHBn and Stat3 from the model described in ref 31. (A) Direct hydrogen bonds between the side chain NH_2 and the main chain carbonyl groups of Glu638 and Pro639, the side chain C=O of Gln and Gln644, and the NH of benzylamide and Tyr640. (B) A water-mediated hydrogen bond between the side chain C=O of Gln and the carboxyl group of Glu638. Peptide 2 is depicted in the green coloring scheme: carbon is in green, nitrogen is in blue, oxygen is in red, and hydrogen is in white. Amino acids from Stat3 are depicted in the white coloring scheme with carbon being white and the other elements the same as in the ligand.

Chart 1. Structures of the Starting Phosphopeptide Inhibitor of Stat3, **1**, and the Modified Lead, **2**[a]



1, Ac-pTyr-Leu-Pro-Gln-Thr-Val-NH_2 IC_{50} = 290 ± 63 nM

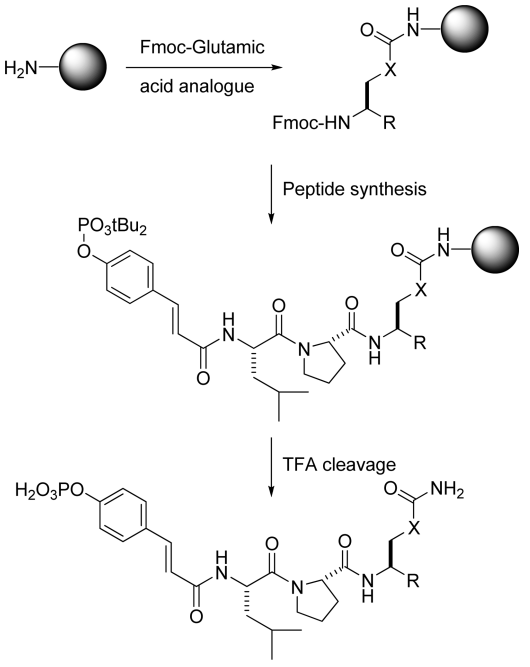


2, pCinn-Leu-Pro-Gln-NHBn IC_{50} = 138 ± 8 nM

[a] The IC_{50} value of **1** was reported in ref 8 and that of **2** in ref 10.

to serve as Gln mimics to (1) further understand peptide−protein interactions and (2) reduce the peptidic nature of the inhibitor with the goal of increased stability to proteases and possibly glutaminases. The lead inhibitor for these studies, pCinn-Leu-Pro-Gln-NHBn (**2**)[10,11] (Chart 1), was chosen for its ease of synthesis and for its high affinity. In **2** the phosphotyrosine of peptide **1** was replaced with 4-phosphoryloxycinnamic acid (pCinn), and the C-terminal Thr-Val-NH_2 was substituted with a benzyl group. Conversion of the pTyr to pCinn increased affinity,[10,11] and the benzyl group likely fits into a hydrophobic pocket on the surface of Stat3.[31] Peptide **2** exhibited an IC_{50} of 138 nM in a fluorescence polarization assay, as compared to 290 nM for peptide **1**.[10,11] The studies described herein revealed that replacement of the C-terminal Gln-NHBn unit of **2** with simpler mimics, 4-aminopentanamide or 2-aminoethylurea, led to inhibitors that were equipotent with the lead. However, when proline was substituted with *cis*-3,4-methanoproline,[8]

Scheme 1. General Synthesis Scheme for Inhibitors Possessing the New, Modified Glutamine Mimics



replacement of the C-terminal CONHBn with the isosteric 1-benzyloxyethyl group resulted in an IC_{50} value of 69 nM, the highest affinity inhibitor of Stat3 reported to date.

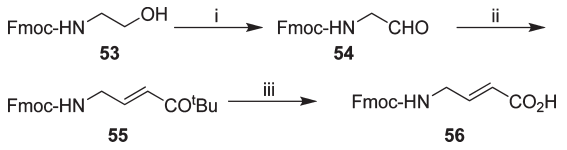
Chemistry[32]

Peptide Synthesis. The novel glutamine mimics were derived from corresponding N^{α}-Fmoc glutamic acid analogues, the syntheses of which are described below. Phosphopeptides were synthesized manually via attachment of the side chain of the glutamic acid derivatives to Rink amide resin so that upon cleavage from the support the final products possessed glutamine mimics (Scheme 1). The Fmoc protection scheme was used. Couplings were mediated with either diisopropylcarbodiimide (DIPCDI)/1-hydroxybenzotriazole (HOBt) or 1*H*-benzotriazol-1-yloxytripyrrolidinophosphonium hexafluorophosphate (PyBOP)/HOBt/DIEA.

Table 1. Effect of the Length of the Side Chain of Glutamine Analogues in the Peptide pCinn-Leu-Pro-NHCH(R)CO-NHBn

peptide	R	IC_{50} (nM)
2	$(CH_2)_2CONH_2$	138 ± 8
3	H	4940 ± 1220
4	CH_2CONH_2	874 ± 189
5	$(CH_2)_3CONH_2$	1400 ± 189

Scheme 2[a]



[a] Reagents and conditions: (i) DMSO, oxalyl chloride, DIEA, CH_2Cl_2, −78 °C; (ii) $PPh_3CHCO_2{}^tBu$, CH_2Cl_2; (iii) TFA.

Fmoc removal was accomplished with three treatments of 20% piperidine in DMF for 6 min each. All sequences were capped with 4-(di-*tert*-butylphosphoryl)cinnamic acid.[11] Peptides were cleaved from solid supports with TFA/triisopropylsilane/H_2O (TFA/TIS/H_2O) (95:2.5:2.5)[33] and were purified by reverse-phase HPLC.

For the synthesis of inhibitor **3** in which Gln-NHBn was replaced with Gly-NHBn (Table 1), benzylamine was attached to FMPB aldehyde resin through reductive amination with $NaBH_3CN$ and 1% AcOH/DMF.[34] The resulting resin-bound secondary amine was then acylated with Fmoc-Gly-OH using PyBOP/HOBt/DIEA. The remaining amino acids were coupled using DIPCDI/HOBt. Inhibitors **2**, **4**, and **5** (Table 1) were initiated by coupling Fmoc-Glu-NHBn (**50**), Fmoc-Asp-NHBn (**51**), and Fmoc-homoGlu-NHBn (**52**, homoGlu = homoglutamic acid), respectively, to Rink amide resin via their side chains using PyBOP/HOBt/DIEA. Amino acids **51** and **52** were synthesized as described for **50**.[8]

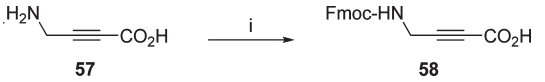
Synthesis of Inhibitors Possessing Constrained Glutamine Mimics. (*E*)-4-Amino-2-butenamide was used as a constrained glutamine mimic in inhibitor **9** (Table 3). The N-Fmoc protected amino acid precursor, **56**, was prepared as depicted in Scheme 2. Commercially available Fmoc-aminoethanol (**53**) was oxidized to the aldehyde (**54**) by Swern oxidation[35] followed by Wittig coupling with $PPh_3CHCO_2{}^t$Bu to give **55**. Hydrolysis of the *tert*-butyl ester with TFA gave **56,** which was attached to Rink amide resin to start the synthesis of **9**.

Fmoc-4-aminotetrolic acid (**58**), used in the synthesis of inhibitor **10** (Table 3), was prepared by protecting the amino group of 4-aminotetrolic acid (**57**)[36] with Fmoc-OSu/10% Na_2CO_3/1,4-dioxane (Scheme 3). Throughout the synthesis of inhibitors **9** and **10**, Fmoc removal was accomplished with 5% DBU/DMF to avoid Michael addition with piperidine.

For inhibitors **12** and **13** (Table 3), commercially available Fmoc-(2*S*,4*S*)-4-amino-1-Boc-pyrrolidine-2-carboxylic acid and Fmoc-(2*S*,4*R*)-4-amino-1-Boc-pyrrolidine-2-carboxylic acid, respectively, were coupled to Rink amide resin using DIPCDI/HOBt.

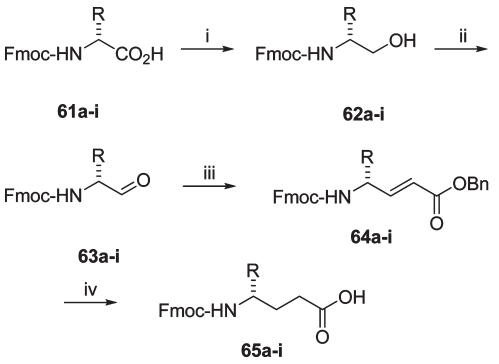
Substitution of the Side Chain Carboxamide of 4-Aminobutyramide (Aba). Inhibitors **8** (Table 3) and **14** (Table 4) were synthesized by initial attachment of Fmoc-4-Abu-OH (Abu = 4-aminobutyric acid) and Fmoc-3-aminopropanesulfonyl chloride (**59**), respectively, to Rink amide resin followed by assembly of the remainder of the amino acids using standard coupling methods. The latter was prepared by

Scheme 3[a]

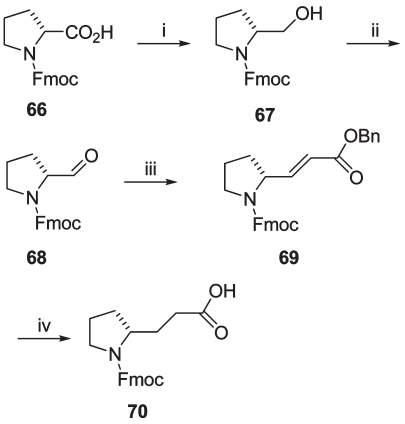


[a] Reagents and conditions: (i) Fmoc-OSu, 10% Na_2CO_3, 1,4-dioxane.

Scheme 4[a]



R = a) Me; b) (*S*)Me; c) iPr; d) iBu; e) nBu; f) $CH_2Ph(4\text{-}O^tBu)$; g) CH_2OBn; h) CH(Me)OBn; i) CH(Me)O^{t}Bu;

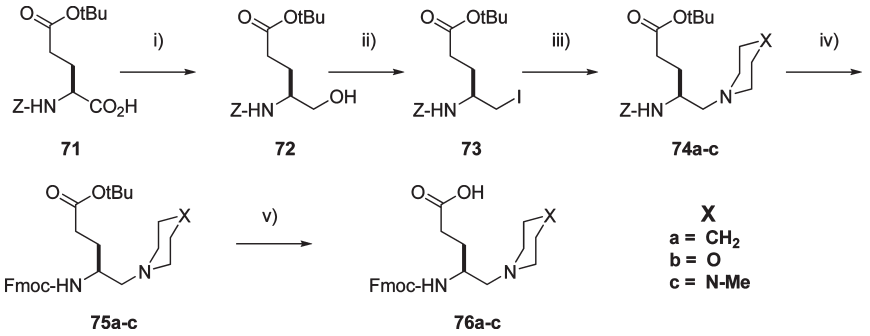


[a] Reagents and conditions: (i) (a) 2-mercaptopyridine, DCC, EtOAc; (b) $NaBH_4$, THF−H_2O; (ii) DMSO, oxalyl chloride, DIEA, CH_2Cl_2, −78 °C; (iii) PPh_3CHCO_2Bn, CH_2Cl_2; (iv) TES, Pd/C, MeOH.

treating the sodium salt of Fmoc-3-aminopropanesulfonic acid (**60**) with $SOCl_2$/DMF (cat).[37] Peptide **15** was initiated by coupling of Fmoc-4-Abu-OH to hydroxylamine resin using DIPCDI/HOBt, followed by assembly of the rest of the amino acids. For inhibitor **16**, FMPB aldehyde resin was first treated with hydrazine to make the corresponding hydrazone, which was then coupled with Fmoc-4-Abu-OH followed by assembly of the remainder of the amino acids. The peptide was cleaved from the resin with 6 N HCl/MeCN and purified by HPLC.

Synthesis of Inhibitors Possessing 4-Alkyl Aba Analogues. We demonstrated that during triethylsilane (TES)/Pd−C mediated hydrogenation, the Fmoc group is stable, which allowed selective deprotection of benzyl-based protecting groups.[38] This observation formed the basis of our strategy for the synthesis of the 4-alkyl-substituted Aba analogues used in inhibitors **17**−**24** (Table 5) and **42**−**49** (Table 7). A modification of the procedure of Loukas et al.[39] was employed for the synthesis of 4-alkyl-substituted Aba analogues **64a**−**h** and **68** (Scheme 4). The carboxyl groups of

Scheme 5[a]



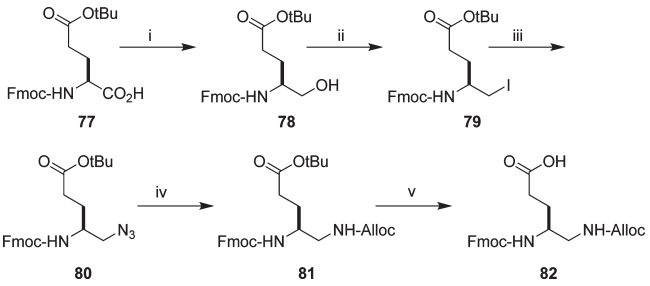
[a] Reagents and conditions: (i) (a) 2-mercaptopyridine, DCC, EtOAc; (b) $NaBH_4$, THF−H_2O; (ii) PPh_3/I_2/imidazole/CH_2Cl_2 (91%); (iii) piperidine or morpholine or *N*-methylpiperazine, THF; (iv) (a) TES/Pd−C, 2% $CHCl_3$/MeOH; (b) Fmoc-OSu, 10% Na_2CO_3, THF; (v) TFA.

Fmoc-D-amino acids, **61a**−**i**, were converted to their corresponding alcohols (**62a**−**i**) via formation of 2-thiopyridyl esters followed by reduction with sodium borohydride. The alcohols were oxidized to aldehydes **63a**−**i** by Swern oxidation,[35] and these were elongated by Wittig coupling with Ph_3PCHCO_2Bn to **64a**−**i**. Concurrent reduction of the double bond and hydrogenolysis of the benzyl group with TES and Pd/C in MeOH gave Fmoc-protected glutamic acid analogues (**65a**−**i**) ready for coupling to Rink amide resin. In this scheme, the side chains of D-amino acids end up as carboxyl group replacements in L-Gln analogues. This method was also used to prepare the constrained glutamic acid analogue, Fmoc-3-(2-pyrrolidino)propionate (**70**), used in the synthesis of **11** (Table 3).

Syntheses of inhibitors **26**−**28** (Table 5) were initiated by coupling of 5-piperidino-4-(9-fluorenylmethoxycarbonylamino)pentanoic acid and analogues (**76a**−**c**) to Rink resin. To synthesize **76a**−**c** (Scheme 5), the carboxyl group of Z-L-Glu(OtBu)-OH (**71**) was reduced to an alcohol (**72**) via sodium borohydride mediated reduction of the intermediate 2-thiopyridine ester. Intermediate **72** was then transformed to the corresponding iodide (**73**) by treatment with the PPh_3/I_2/imidazole complex.[40] Nucleophilic substitution with piperidine, morpholine, and 4-methylpiperazine afforded *tert*-butyl 5-piperidino-4-benzyloxyaminopentanoate **74a**, *tert*-butyl 5-morpholino-4-benzyloxyaminopentanoate **74b**, and *tert*-butyl 5-piperazino-4-benzyloxyaminopentanoate **74c**, respectively. TES/Pd−C mediated hydrogenation of **74a**−**c** in 2% $CHCl_3$/MeOH followed by Fmoc protection yielded **75a**−**c**. Treatment of **75a**−**c** with neat TFA followed by triturating with ether−hexane gave **76a**−**c** as white powders.

For the synthesis of substituted 4-aminomethyl Aba-containing inhibitors (**29**−**31**, Table 5), *tert*-butyl 4-(9-fluorenyloxycarbonylamino)-5-iodopentanoate (**79**) was synthesized from Fmoc-Glu(OtBu)-OH using an analogous scheme as for **73** (Scheme 6). Iodide **79** was treated with tetrabutylammonium azide, and the resulting azide, **80**, was reduced with TES/Pd−C to give the free amine, which was then protected with the Alloc group to give **81**. Removal of the side chain *tert*-butyl group with neat TFA gave **82**, which was then attached to Rink amide resin. After addition of the pCinn, Leu, and Pro units, the Alloc group was removed with $Pd(PPh_3)_4/CHCl_3$/AcOH/NMM[41], and the resin was split into three portions. The first was not derivatized and led to inhibitor **30**. The second was acetylated with acetic anhydride, leading to inhibitor **31**. Treatment of the final portion with a 3-fold excess of benzaldehyde in the presence of

Scheme 6[a]



[a] Reagents and conditions: (i) (a) 2-mercaptopyridine, DCC, EtOAc; (b) $NaBH_4$, THF−H_2O; (ii) PPh_3/I_2/imidazole/CH_2Cl_2 (88%); (iii) nBu_4NN_3/CH_2Cl_2 (81%); (iv) (a) TES/Pd−C, 2% $CHCl_3$/MeOH; (b) allyl chloroformate, DIEA, CH_2Cl_2 (64%); (v) TFA.

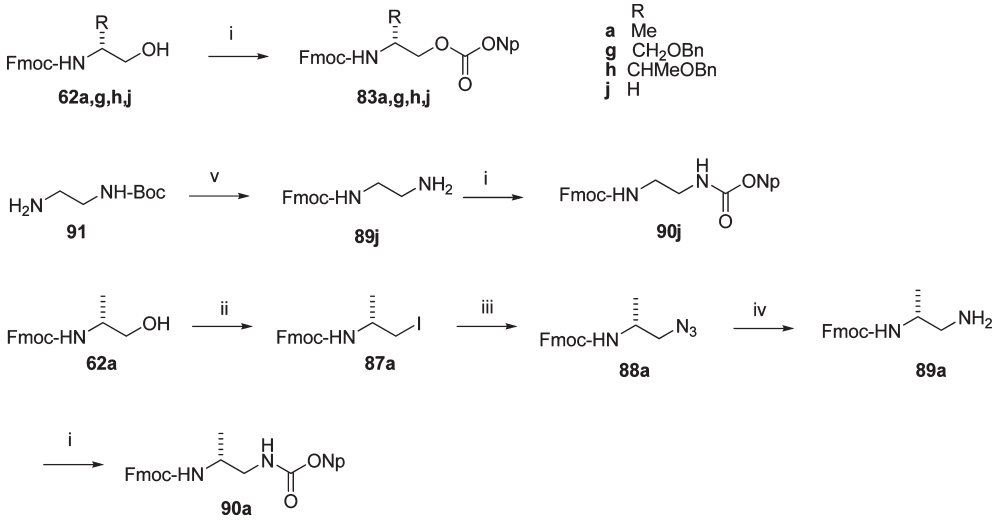
$NaCNBH_3$/AcOH resulted in complete alkylation of the amine, leading to inhibitor **29**.

Syntheses of Carbamate-Containing Phosphopeptides. Fmoc-amino alcohols **62a**, **62g**, and **62h** (Scheme 4) and Fmoc-aminoethanol (**62j**) were converted to the corresponding 4-nitrophenyl carbonates (**83a**,**g**,**h**,**j**) by treatment with nitrophenyl chloroformate[10] (Scheme 7). These were coupled to Rink amide resin in the presence of DIEA to initiate the synthesis of the inhibitors. After addition of the pCinn, Leu, and Pro residues, cleavage from the resin yielded the carbamate-containing inhibitors **34**−**37** (Table 6).

Inhibitor **25** (Table 5), possessing a 4-amino-5-carbamoylpentamide on the C-terminus, was synthesized by initial attachment of **86** to Rink amide resin, followed by subsequent coupling of the remainder of the amino acids. Nitrophenyl carbonate **86** was obtained from reducing the carboxyl group of Fmoc-Gln(Trt) (**84**) to the corresponding alcohol **85**, followed by treatment with 4-nitrophenyl chloroformate (Scheme 8).

Synthesis of Urea-Containing Inhibitors 38 and 39. Syntheses of urea-containing inhibitors **38** and **39** (Table 6) started with the coupling of Fmoc-aminoethylnitrophenylurethanes **90a**,**j** to Rink amide resin to give resin-bound ureas. As shown in Scheme 7, commercially available Boc-diaminoethane (**91**) was treated with Fmoc-OSu followed by HCl in EtOAc to prepare Fmoc-diaminoethane, **89j**. Treatment with nitrophenyl chloroformate provided the corresponding nitrophenylurethane, **90j**. The synthesis of 2-methyl-2-aminonitrophenylurethane, **90a**, was carried out by a different route than that reported by Boeijen et al.[42] Fmoc-alaninol, **62a** (Scheme 4), was converted to the iodide, **87a**, as described for the 4-piperidinomethyl Aba analogues

Scheme 7[a]



[a] Reagents and conditions: (i) nitrophenyl chloroformate, pyridine, CH_2Cl_2; (ii) PPh_3/I_2/imidazole/CH_2Cl_2 (91%); (iii) Bu_4NN_3/CH_2Cl_2 (84%); (iv) TES, Pd/C, 2% $CHCl_3$/MeOH; (v) (a) Fmoc-OSu, $NaHCO_3$; (b) HCl/EtOAc.

Table 2. Probes of Potential Interactions of the Backbone Atoms at pY+3 and Stat3

peptide	sequence	IC_{50} (nM)
2	pCinn-Leu-Pro-Gln-NHBn	138 ± 8
6	pCinn-Leu-Pro-NH_2	11400 ± 1600
7	pCinn-Leu-Pro-Ala-NH_2	7800 ± 730

described above, which was then followed by azide substitution with tetrabutylammonium azide to give **88a**. The use of tetrabutylammonium azide resulted in a higher yield than sodium azide[32] for this step. Reduction of **88a** with TES/Pd−C[38] gave the diamine, **89a**, which was converted to the nitrophenylurethane **90a** with nitrophenyl chloroformate. The urethane intermediates were added to Rink amide resin to give solid-supported ureas. After adding the remainder of the amino acids, cleavage from the resin with TFA gave urea-containing inhibitors **38** and **39**.

Synthesis of Inhibitor 47. Inhibitor **47** (Table 7) was initiated by coupling of 4-[[(9*H*-fluoren-9-ylmethoxy)carbonyl]amino]-5-acetoxypentanoate **93** (Scheme 9) to Rink resin. Hydroxypentanoate **78** (Scheme 6) was acetylated with acetic anhydride and the *tert*-butyl ester deprotected to give the starting amino acid.

Results

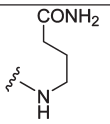
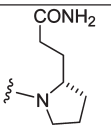
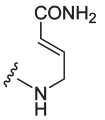
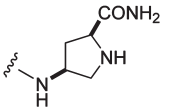
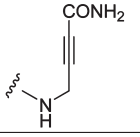
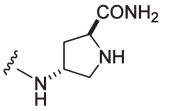
Alteration of the Length of the Glutamine Side Chain. To establish the importance of the distance of the side chain carboxamide group from the main chain peptide, Gln was replaced with glycine, asparagine, and homoglutamine (Table 1). Substitution with glycine (**3**), without a side chain, decreased affinity 50-fold, reiterating the importance of the alkylcarboxamide at pY+3. Peptides containing asparagine (**4**), with a one carbon contraction, and homoglutamine (**5**), with a one carbon extension with respect to glutamine, showed reduced activity by 7- and 10-fold, respectively, indicating that the position of the amide group is critical for attaining high affinity. The reduced affinity of **4** is in keeping with a similar result in which Asn substitution for Gln in **1** reduced the ability of the peptide to inhibit DNA binding in an electrophoretic mobility shift assay.[7]

Main Chain Interactions at Position pY+3. To probe for main chain interactions between the pY+3 residue and Stat3, Gln was either removed (peptide **6**) or replaced with Ala (peptide **7**). Peptide **7** displayed higher affinity than **6**, which is consistent with hydrogen bonding of the peptide bond C-terminal to the pY+3 residue and Tyr640 of Stat3 (Figure 1, Table 2).[31] As in the case of **3**, the absence of the Gln side chain accounts for the reduced affinity of **7**.

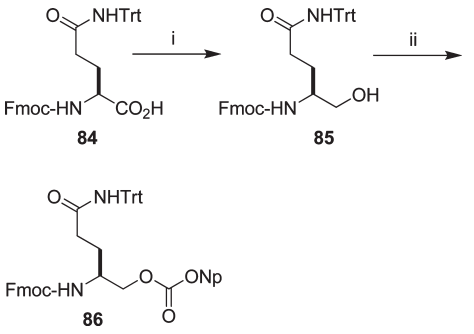
Conformationally Constrained Aba Analogues. Peptides with a properly constrained side chain will exhibit higher affinity due to reduced entropy penalty on binding. The side chain of glutamine has three rotatable C−C bonds. We prepared a series of constrained Aba analogues as models to probe the effect of constraining the Gln side chain (Table 3). Note that Aba (**8**), exhibiting an IC_{50} value of 0.57 μM, results in a 3-fold loss of affinity as compared to Gln-NHBn in peptide **2**. Incorporation of double and triple bonds in Aba (**9** and **10**, respectively) reduced affinity ca. 2-fold compared to **8**. Cyclizing C(4)−N(4) and C(2)−C(4) caused 6-fold reductions compared to Aba (**11**−**13**). Taken together with the loss in affinity observed when Gln-Thr-NH_2 was replaced with 3-acetamidopyrrolidine, nipecotamide, aminocyclohexane-3-carboxamide, and 4-acetamidopiperazine reported previously,[8] we conclude that when bound to Stat3, the alkyl chain of Gln adopts a conformation not readily mimicked by chemical modification.

Importance of the Side Chain Carboxamide Group. Previously,[8] we found that replacement of Gln by the isosteric analogue, methionine sulfoxide, as well as the oxidized version, methionine sulfone, resulted dramatic losses of inhibition. Mono- and dimethylation of the side chain nitrogen of glutamine also reduced affinity. Taken together, these results suggested a role for the amide hydrogens in hydrogen-bonding interactions with the protein. To determine if sulfonamide protons could interact with Stat3, the side chain carboxamide of Aba was substituted with a SO_2NH_2 group (**14**). This modification resulted in very low affinity (IC_{50} = 68 μM). In an attempt to mimic the water molecule involved in the water-mediated hydrogen bond observed in the computational model (Figure 1),[31] one of the amide protons of Aba was substituted with either a

Table 3. Effect of Constrained Gaba Analogues on the Affinity of pCinn-Leu-Pro-Xxx

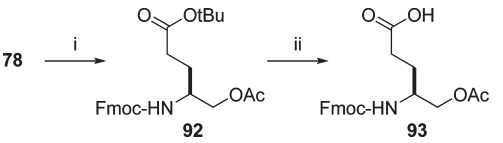
Peptide	Xxx	IC_{50} (nM)	Peptide	Xxx	IC_{50} (nM)
8		574 ± 130	**11**		3,310 ± 392
9		1,110 ± 430	**12**		3,210 ± 269
10		1,140 ± 110	**13**		3,600 ± 1,270

Scheme 8[a]



[a] Reagents and conditions: (i) (a) 2-mercaptopyridine, DCC, EtOAc; (b) $NaBH_4$, $THF-H_2O$; (ii) 4-nitrophenyl chloroformate, pyridine, CH_2Cl_2 (62%).

Scheme 9[a]



[a] Reagents and conditions: (i) Ac_2O, DIEA, DMAP (cat), CH_2Cl_2; (ii) TFA.

hydroxyl group (peptide **15**) or an amino group (peptide **16**). In both cases, 3−5-fold reduced affinity was observed (Table 4). The reduction of affinity may be due to steric crowding in the glutamine binding pocket or loss of intermolecular H-bonding. Thus, a carboxamide group with both amide protons intact is optimal for high affinity.

4-Alkyl Aba Analogues. To further probe for interactions between the main chain atoms at pY+3 and Stat3, and to search for new ones, a series of glutamine analogues in which the backbone carboxyl group was substituted with alkyl groups was used to replace Gln-NHBn in peptide **2** (Table 5). A methyl group produced the most potent inhibitor, increasing affinity 5-fold (**17**) over the hydrogen of Aba (**8**). Interestingly, compound **17** (IC_{50} = 110 nM) was equipotent with **2**. The configuration of C(4) is important as the *S* enantiomer of 4-methyl Aba (inhibitor **18**) resulted in lower affinity than **17**. The larger, simple alkyl substitutions in **19**−**22** were not tolerated as well as the methyl group. Benzyloxymethyl and benzyloxyethyl groups were appended to C(4) of Aba, which resulted in IC_{50} values of 294 (**23**) and 272 nM (**24**), respectively. These groups are nearly isosteric to the CONHBn group in compound **2**, and **23** and **24** reduce affinity by ca. 2-fold.

Attachment of an amino group to the γ-methyl carbon (**30**) resulted in an IC_{50} of 1.2 μM, 10-fold lower affinity than the unsubstituted methyl group. Piperidinomethyl Gaba analogues were synthesized with the long-term goal of solubility of prodrug versions of phosphopeptide inhibitors of Stat3. Compounds **26**−**28**, with IC_{50} values ranging from 1.2 to 1.5 μM, showed almost 10-fold reduced binding affinity compared to the unsubstituted methyl group. The acyclic tertiary amine containing inhibitor (**29**) also came out with 7-fold decreased affinity. However, acetylation of the amino group of **31** partially restored activity (compound **31**). It appears that a charged amine at this position may be deleterious for activity. Addition of a carbamate at the C-terminus, **25**, gave an IC_{50} value of 612 nM, similar to the acetamide **31**.

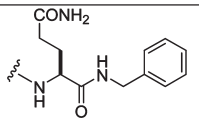
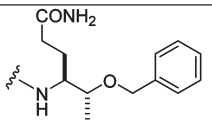
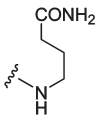
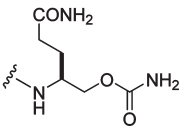
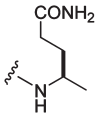
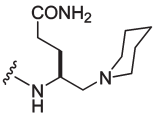
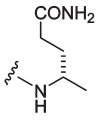
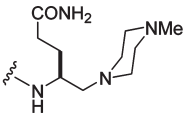
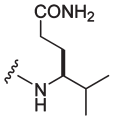
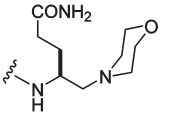
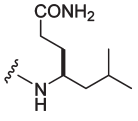
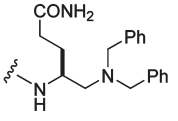
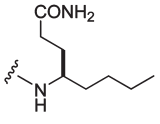
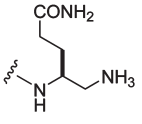
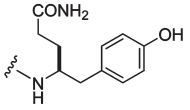
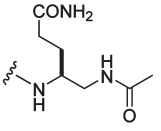
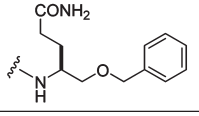
Table 4. Importance of the Side Chain Carboxamide Group in pCinn-Leu-Pro-Xxx

peptide	Xxx	IC_{50} (nM)
8	$NH(CH_2)_3CONH_2$	574 ± 130
14	$NH(CH_2)_3SO_2NH_2$	68200 ± 23500
15	$NH(CH_2)_3CONHOH$	2640 ± 45
16	$NH(CH_2)_3CONHNH_2$	1830 ± 770

Taken together, these results suggest that the binding surface for the backbone CONH atoms of glutamine of **2** is polar and that the alkyl groups do not make good contact. This is in keeping with the proposed model in which the phenolic hydroxyl group of Tyr640 is within hydrogen-bonding distance of this group (Figure 1). However, in spite of the polar surface, formal positive charge provided by amines is not tolerated well.

Substitution of Glutamine with Carbamate and Ureas. Previously, we reported the replacement of the γ-methylene group of glutamine with oxygen to give side chain carbamate

Table 5. Effect of Substitutions at C(4) of Aba in pCinn-Leu-Pro-Xxx

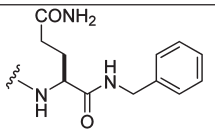
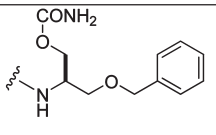
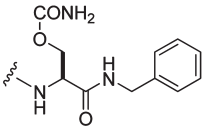
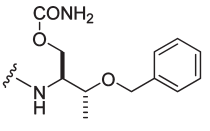
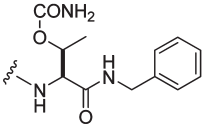
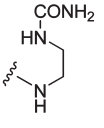
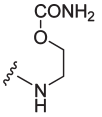
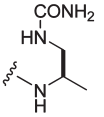
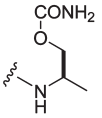
Peptide	Xxx	IC_{50} (nM)	Peptide	Xxx	IC_{50} (nM)
2		138 ± 8	**24**		272 ± 7
8		574 ± 130	**25**		615 ± 30
17		110 ± 30	**26**		1,210 ± 190
18		1,180 ± 30	**27**		1,310 ± 228
19		1,880 ± 220	**28**		1,530 ± 70
20		4,900 ± 610	**29**		1,080 ± 360
21		1,350 ± 360	**30**		1,240 ± 215
22		848 ± 126	**31**		589 ± 174
23		294 ± 40			

analogues.[10] *O*-Carbamoylserine (**32**) and *O*-carbamoylthreonine (**33**) resulted in 3- and 6-fold losses of affinity compared to peptide **2**. To further study this functional group, a series of Aba analogues was converted to 2-aminoethylcarbamates (Table 6). In contrast to the loss in binding of Aba vs Gln-NHBn (**2** and **8**) discussed above (Table 5), the Aba analogue, 2-aminoethylcarbamate (**34**) results in affinity equal to that of the carbamoylserine benzylamide (**32**). Substitution of C(2) of the ethyl group (C(4) of Aba) results in loss of activity, even those possessing benzyloxymethyl and benzyloxyethyl groups, **36**, and **37**, respectively. Replacement of Gln-NHBn with unsubstituted 2-aminoethylurea (**38**) results in equal affinity, 131 nM. Addition of a methyl group (**39**) resulted in a 10-fold loss in activity.

Glutamine Analogues in Peptides Containing *cis*-3,4-Methanoproline. In an earlier publication we reported that substitution of proline with *cis*-3,4-methanoproline (mPro) increased affinity by a factor of 2 and that hydrocinnamoyl-pTyr-Leu-mPro-Gln-NHBn exhibited an IC_{50} value of 125 nM in our FP assay.[8] mPro is a rather expensive starting material so the studies discussed above were conducted on peptides containing proline. Adding to the cost is the fact commercially available Fmoc-*cis*-3,4-methanoPro is sold as a racemic mixture of (2*S*,3*R*,4*S*) and (2*R*,3*S*,4*R*) enantiomers, and syntheses of peptides with this amino acid produce two diastereomers. Only one-half of the material gives the high-affinity interaction with Stat3. To test the effect of some of the Gaba analogues discussed above, we substituted mPro for Pro. Only the high-affinity diastereomers are reported here.

Incorporation of *cis*-3,4-methanoPro in peptide **2** resulted in a 2-fold increase in affinity consistent with our previous report[8] (**40**, Table 7). pCinn-Leu-*cis*-3,4-methanoPro-Gln-NHBn

Table 6. Inhibition of Stat3 by Carbamates and Ureas as Glutamine Mimics in pCinn-Leu-Pro-Xxx

Peptide	Xxx	IC_{50} (nM)	Peptide	Xxx	IC_{50} (nM)
2		138 ± 8	**36**		454 ± 21
32		379 ± 49[a]	**37**		6,900 ± 350
33		850 ± 85[a]	**38**		131 ± 14
34		360 ± 37	**39**		1,350 ± 250
35		797 ± 29			

[a] From ref 10.

exhibited an IC_{50} value of 68 nM, the highest affinity Stat3 inhibitor reported to date. γ-Alkyl Gaba analogues were also appended onto the C-terminus of pCinn-Leu-*cis*-3,4-methanoPro and the IC_{50}'s determined by FP. Substituting the benzylamide moiety with hydrogen (compound **41**) resulted in a loss of affinity. In contrast to the proline analogues, γ-methyl Gaba (**42**) was not equipotent to the Gln-NHBn analogue, **40**. Larger alkyl groups reduced affinity. The benzyloxymethyl group (**48**) restored binding to 94 nM. However, the isosteric benzyloxyethyl group (**49**) was equipotent with the benzylamide, exhibiting an IC_{50} value of 69 nM. Thus compound **49** is a very high affinity peptidomimetic inhibitor of Stat3 in which pTyr is replaced by pCinn, proline is replaced with 3,4-*cis*-methanoproline, and glutamine is replaced with γ-(2-benzyloxyethyl) Aba. With only one natural amino acid remaining, leucine, we have made great strides in the development of a nonpeptide inhibitor of Stat3.

Conclusions

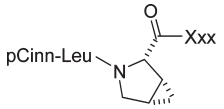
We have probed the glutamine binding pocket of Stat3 with a series of glutamine and Aba analogues. A nonconstrained, linear side chain is necessary for high affinity, as is the carboxamide group. These findings are consistent with the model of Gln fitting into a tight pocket on the surface of the Stat3 SH2 domain and the side chain amide participating in hydrogen-bonding interactions.[31] Glutamine can be substituted with nonpeptide analogues. Replacement of the C-terminal Gln-NHBn unit of pCinn-Leu-Pro-Gln-NHBn with either (*R*)-4-aminopentamide or 2-aminoethylurea leads to modified peptides with IC_{50} values of ca. 110 and 130 nM, respectively, equipotent with the lead inhibitor. However, replacement of proline with *cis*-3,4-methanoproline and Gln-NHBn with (4*R*,5*S*)-4-amino-5-benzyloxyhexamide leads to a modified peptide with enhanced affinity; the IC_{50} value of **49** was 69 nM. Conversion of these phosphopeptide mimics into cell permeable inhibitors will be reported under separate cover.

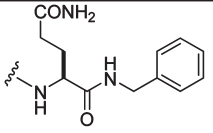
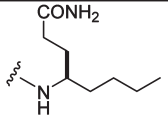
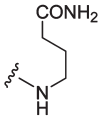
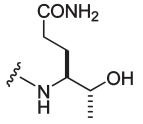
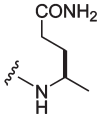
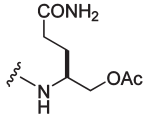
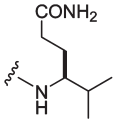
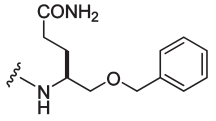
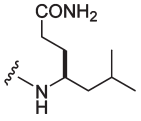
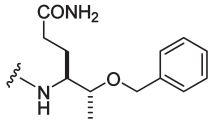
Experimental Section

Chemistry: General. N^{α}-Protected amino acids were purchased from Advanced Chemtech, NovaBiochem, ChemImpex, or AnaSpec. HOBt was from ChemImpex. Fmoc-*cis*-3,4-methanoPro was from EMD Biosciences (formerly NovaBiochem). Rink amide resin was purchased from Advanced Chemtech, loaded between 0.6 and 0.7 mmol/g. Anhydrous DMF for amino acid solutions was from Baker. Other solvents were of reagent grade and were used without further purification. Peptides were purified by reverse-phase HPLC on a Rainin rabbit HPLC or a Varian Dynamax HPLC using a Vydac 2.5 × 25 cm peptide and protein C18 column or a 2.1 × 25 cm Phenomenex Luna 10 μM C18(2). Gradients of MeCN in H_2O (both containing 0.1% TFA) or MeCN in 0.01 M NH_4OAc (pH 6.5) at 10 mL/min were employed. Peptides were tested for purity by reverse-phase HPLC on an Agilent 1090 HPLC or an Agilent 1100 HPLC using a Vydac 4.6 × 250 mm C18 peptide/protein column or a 4.6 × 250 mm Phenomenex 5 μM C18(2) in two systems: (A) 10−50% MeCN in H_2O with 0.1% TFA in both solvents and (B) 0−40% MeCN in 0.01 M NH_4OAc, pH 6.5. The flow rate for both was 1.5 mL/min. Peptides were always >95% pure and were often >98% pure as judged by analytical HPLC. Before evaluation by fluorescence polarization, peptides were dried in vacuo over P_2O_5 at 37 °C for 24 h. NMR spectra were recorded on Bruker 300 MHz DPX or 500 MHz DRX spectrometers.

Solid-Phase Peptide Synthesis: Manual Method. Manual solid-phase synthesis was carried out on aliquots of 0.20 g of Rink resin (0.6 mmol/g). Fmoc groups were removed with 2 × 5 mL of 20% piperidine for 3 and 7 min each. After removing the Fmoc group from the resin, synthesis of inhibitors was initiated by coupling of the 3-fold excesses of the new Fmoc-glutamic acid

Table 7. Effect of Substitution in pCinn-Leu-*cis*-3,4-methanoPro-Xxx



Peptide	Xxx	IC_{50} (nM)	Peptide	Xxx	IC_{50} (nM)
40		68 ± 8	**45**		699 ± 62
41		303 ± 48	**46**		437 ± 4
42		175 ± 35	**47**		290 ± 41
43		511 ± 67	**48**		94 ± 11
44		1,630 ± 132	**49**		69 ± 10

analogues, PyBop and HOBt, along with 6-fold excesses of DIEA in 5 mL of (1:1 v/v) DMF/CH_2Cl_2. The nitrophenyl carbonates (**83a**,**h**,**g**,**j**) and nitrophenyl carbamates (**90a**,**j**) were added to the resin in 3-fold excess along with a 3-fold excess of DIEA in 5 mL of (1:1 v/v) DMF/CH_2Cl_2 as described.[10] Reactions were monitored with ninhydrin. After coupling and deprotection steps, resins were washed with 5 × 5 mL of DMF/CH_2Cl_2. Cleavage was accomplished with three treatments of the resins with 5 mL of TFA/TIS/H_2O (95:2.5:2.5)[33] for 10 min each. The volumes of the solvents were reduced, and the solutions were dropped into ice-cold Et_2O. After 30 min the precipitates were collected by filtration and were washed 2× more with Et_2O. Crude peptides were dried, and peptides were purified by reverse-phase HPLC as described in the general methods. All peptides were dried over P_2O_5 in vacuo at 37 °C for 24 h before testing. Peptide yields, HPLC retention times, and mass spectra are tabulated in Supporting Information Table S1.

Synthesis of Fmoc-Asp-NHBn (51). Starting with 0.5 g of Fmoc-Asp(tBu)-OH the procedure described by Coleman et al.[8] for Fmoc-Glu-NHBn was employed. Yield 0.48 g (89%), white powder. ^{1}H NMR (DMSO-d_6, 500 MHz) δ 2.56 (dd, J = 9.0, 16.5 Hz, 1H), 2.27 (dd, J = 5.5, 16.5 Hz, 1H), 4.22−4.33 (m, 5H), 4.42 (m, 1H), 7.2−7.35 (m, 7H), 7.43 (t, J = 7.0 Hz, 2H), 7.7 (d, J = 8.0 Hz, 1H), 7.73 (d, J = 7.0 Hz, 2H), 7.9 (d, J = 8.0 Hz, 2H), 8.42 (t, J = 6.0 Hz, 1H). ^{13}C NMR (DMSO-d_6, 125 MHz) δ 36.9, 42.6, 47.1, 51.9, 66.3, 120.6, 125.8, 127.1, 127.4, 127.6, 128.1, 128.6, 139.8, 141.2, 144.3, 156.3, 171.2, 172.3. HRMS (M + H) calcd, 445.1763; found, 445.1772. Anal. ($C_{26}H_{24}N_2O_5$) C, H, N. Calcd: C, 70.26; H, 5.44; N, 6.30. Found: C, 69.11; H, 5.35; N, 6.24. Note: % C is >0.4%.

Fmoc-homoGlu-NHBn (52). Starting with 0.5 g of Fmoc-homoGlu(tBu)-OH the procedure described by Coleman et al.[8] for Fmoc-Glu-NHBn was employed. Yield 0.49 g (82%), white powder. ^{1}H NMR (DMSO-d_6, 500 MHz) δ 1.56−1.74 (m, 4H), 2.72 (t, J = 7.0 Hz, 2H), 4.1 (m, 1H), 4.26−4.37 (m, 5H), 7.25−7.38 (m, 7H), 7.47 (t, J = 7.5 Hz, 2H), 7.58 (d, J = 8.5 Hz, 1H), 7.78 (d, J = 7.0 Hz, 2H), 7.94 (d, J = 7.5 Hz, 2H), 8.47 (t, J = 5.5 Hz, 1H). ^{13}C NMR (DMSO-d_6, 125 MHz) δ 21.6, 31.9, 33.8, 42.1, 47.2, 54.9, 66.1, 120.6, 125.8, 127.2, 127.6, 128.1, 128.7, 139.8, 141.2, 144.3, 144.4, 156.5, 172.3, 174.7. Anal. ($C_{28}H_{28}N_2O_5$) C, H, N. Calcd: C, 71.17; H, 5.97; N, 5.93. Found: C, 70.41; H, 5.92; N, 5.87. HRMS (M + H) calcd, 473.2076; found, 473.2115. Note: % C is >0.4%.

Synthesis of 4-[[(9*H*-Fluoren-9-ylmethoxy)carbonyl]amino]-2-butenoic Acid (56). Commercially available Fmoc-glycinol (**53**) was oxidized to Fmoc-glycinal (**54**) via Swern oxidation.[43] A solution of **54** (1.0 g, 3.55 mmol) and *tert*-butyl (triphenylphosphoranylidene)acetate (1.3 g, 3.91 mmol) in dry CH_2Cl_2 (20 mL) was stirred for 2 h. The solvent was removed in vacuo, and the residue was purified by silica gel column chromatography (15% EtOAc−hexane, v/v) to get **55**. Yield 85% (1.20 g). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.4 (s, 9H), 3.86 (m, 2H), 4.13 (t, J = 6.5 Hz, 1H), 4.35 (d, J = 6.5 Hz, 2H), 4.9 (m, 1H), 5.76 (d, J = 15.5 Hz, 1H), 6.71 (m, 1H), 7.22 (m, 2H), 7.31 (m, 2H), 7.5 (d, J = 7.5 Hz, 2H), 7.67 (d, J = 7.5 Hz, 2H). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 28.1, 41.7, 47.2, 66.9, 80.7, 120.1, 123.5, 125.0, 127.1, 127.8, 141.4, 142.8, 143.8, 156.2, 165.3. HRMS (M + H) calcd, 380.1862; found, 380.1856.

Compound **55** (1.0 g) was treated with 5.0 mL of neat TFA for 1 h. The TFA was removed under vacuum, and residual acid was removed by the addition and evaporation of toluene (3 × 5 mL).

Trituration with ether−hexane resulted in a white precipitate which was collected by filtration and dried over P_2O_5 yielding 0.81 g of **56** as a white powder, 95%. ^{1}H NMR (DMSO-d_6, 500 MHz) δ 3.8 (m, 2H), 4.25 (m, 1H), 4.33 (d, J = 6.5 Hz, 2H), 5.81 (d, J = 15.5 Hz, 1H), 6.76 (m, 1H), 7.34 (m, 2H), 7.42 (m, 2H), 7.66 (t, J = 5.5 Hz, 1H), 7.72 (d, J = 7.5 Hz, 2H), 7.9 (d, J = 7.5 Hz, 2H). ^{13}C NMR (DMSO-d_6, 125.0 MHz) δ 41.4, 47.2, 66.0, 120.6, 122.0, 125.6, 127.5, 128.1, 141.2, 144.3, 145.5, 156.6, 167.4. Anal. ($C_{19}H_{17}NO_4$) C, H, N. Calcd: C, 70.58; H, 5.30; N, 4.33. Found: C, 70.38; H, 5.56; N, 4.25. HRMS (M + H) calcd, 324.1236; found, 324.1164.

Synthesis of 4-[[(9*H*-Fluoren-9-ylmethoxy)carbonyl]amino]tetrolic Acid (58). 4-Aminotetrolic acid (1.0 g, 7.37 mmol) (**57**), prepared as described by Ahern et al.,[36] was treated with 10 mL of 10% Na_2CO_3 and Fmoc-OSu (2.2 g, 6.6 mmol) in 20 mL of 1,4-dioxane overnight. The solution was washed with 20 mL of EtOAc and the aqueous layer then acidified with concentrated HCl. It was the extracted with EtOAc (3 × 30 mL), and the combined organic layers were washed with brine, dried ($MgSO_4$), and evaporated. The residue was triturated with ether−hexane, and the precipitate was collected by filtration and dried over P_2O_5. Yield 1.3 g (41%). ^{1}H NMR (DMSO-d_6, 500 MHz) δ 4.04 (d, J = 5.0 Hz, 2H), 4.29 (t, J = 6.5 Hz, 1H), 4.41 (d, J = 6.5 Hz, 2H), 7.39 (m, 2H), 7.47 (m, 2H), 7.75 (d, J = 7.5 Hz, 2H), 7.93−7.95 (m, 3H). ^{13}C NMR (DMSO-d_6, 125 MHz) δ 30.3, 47.1, 66.3, 75.4, 84.7, 120.6, 125.6, 127.6, 128.1, 141.2, 144.2, 154.4, 156.5. Anal. ($C_{19}H_{15}NO_4$) C, H, N. Calcd: C, 71.02; H, 4.71; N, 4.36. Found: C, 70.37; H, 4.67; N, 4.37. HRMS (M + H) calcd, 322.1079; found, 322.1083.

Synthesis of Sodium 3-[[(9*H*-Fluoren-9-ylmethoxy)carbonyl]amino]propanesulfonate (60). To a stirred aqueous solution (30 mL) of 3-aminopropanesulfonic acid (1 g, 7.18 mmol) and NaOH (0.3 g, 7.5 mmol) was added Fmoc-OSu (2.9 g, 8.6 mmol) in portions over 10 min. Stirring was continued overnight. Excess Fmoc-OSu was removed by washing with EtOAc (2 × 10 mL). The aqueous layer was lyophilized, and the residual solid was triturated with ether−hexane. The product was collected by filtration and dried in vacuo over P_2O_5. Yield 1.9 g (72%). ^{1}H NMR (DMSO-d_6, 500 MHz) δ 1.77 (m, 2H), 2.5 (t, J = 7.5 Hz, 2H), 3.1 (m, 2H), 4.26 (m, 1H), 4.3 (d, J = 7.0 Hz, 2H), 7.37−7.41 (m, 3H), 7.46 (t, J = 7.5 Hz, 2H), 7.74 (d, J = 7.5 Hz, 2H), 7.93 (d, J = 7.5 Hz, 2H). ^{13}C NMR (DMSO-d_6, 125 MHz) δ 26.3, 47.2, 49.6, 65.8, 120.6, 125.7, 127.6, 128.1, 141.2, 144.4, 156.6. HRMS (M + H) calcd, 384.0882; found, 384.0914.

Synthesis of 3-[[(9*H*-Fluoren-9-ylmethoxy)carbonyl]amino]propanesulfonyl Chloride (59).[37] To a mixture of **60** (1.00 g, 2.6 mmol) and thionyl chloride (10 mL) was added DMF (1 mL) dropwise. The mixture was heated at reflux for 5 h. The volatiles were removed by evaporation, followed by coevaporation with toluene 3 × 5 mL. Compound **59** was coupled directly to Rink amide resin.

General Procedure for *N*-[(9*H*-Fluoren-9-ylmethoxy)carbonyl]-amino Alcohols (62a−i and 67). A solution of Fmoc-amino acid (5.0 mmol), DCC (6.0 mmol), and 2-mercaptopyridine (5.5 mmol) in 100 mL of EtOAc was stirred for 3 h. The white precipitate was filtered off, and the filtrate was concentrated under vacuum. It was then diluted with 50 mL of THF, and the solution was added slowly to a suspension of $NaBH_4$ (10.0 mmol) in 20 mL of THF and 10 mL of water at 0 °C. After 30 min, the reaction was quenched by slow addition of ice-cold 5% HCl(aq) (50 mL) and extracted with ether (3 × 150 mL). The combined organic layers were washed with aqueous 10% $NaHCO_3$ (3 × 40 mL), water (2 × 50 mL), and brine (1 × 40 mL). After drying (Na_2SO_4) and concentration under vacuum the crude residue was purified either by recrystallization from hexane−ether or by silica gel column chromatography.

Fmoc-D-alaninol (62a). Yield 1.32 g (90%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.07 (m, 3H), 3.43 (m, 1H), 3.55 (m, 1H), 3.73 (m, 1H), 4.11 (m, 1H), 4.33 (m, 2H), 4.75 (br s, 1H), 7.22 (m, 2H), 7.3 (m, 2H), 7.48 (d, J = 7.5 Hz, 2H), 7.66 (d, J = 7.5 Hz, 2H). HRMS (M + H) calcd, 298.1443; found, 298.1247.

Fmoc-L-alaninol (62b). Yield 1.4 g (92%), white powder, mp 100−102 °C. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.07 (m, 3H), 3.43 (m, 1H), 3.55 (m, 1H), 3.72 (m, 1H), 4.11 (m, 1H), 4.33 (m, 2H), 4.78 (br s, 1H), 7.21 (m, 2H), 7.3 (m, 2H), 7.48 (d, J = 7.5 Hz, 2H), 7.66 (d, J = 7.5 Hz, 2H). HRMS (M + H) calcd, 298.1443; found, 298.1356.

Fmoc-D-valinol (62c). Yield 1.5 g (91%), white powder, mp 126 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.93 (m, 6H), 1.84 (m, 1H), 3.64 (m, 2H), 4.2 (t, J = 6.6 Hz, 1H), 4.43 (m, 2H), 4.9 (m, 1H), 7.28−7.42 (m, 4H), 7.58 (d, J = 7.2 Hz, 2H), 7.75 (d, J = 7.2 Hz, 2H). HRMS (M + H) calcd, 326.1756; found, 326.1629.

Fmoc-D-leucinol (62d). Yield 1.55 g (90%), white powder, mp 138 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.92 (d, J = 5.7 Hz, 6H), 1.32 (m, 2H), 1.63 (m, 1H), 3.46−3.76 (m, 3H), 4.21 (m, 1H), 4.44 (m, 2H), 4.73 (m, 1H), 7.28−7.42 (m, 4H), 7.58 (d, J = 7.5 Hz, 2H), 7.76 (d, J = 7.5 Hz, 2H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 22.1, 23.0, 24.8, 40.4, 47.4, 66.5, 120.0, 125.0, 127.0, 127.7, 141.4, 143.9. HRMS (M + H) calcd, 340.1913; found, 340.1699.

Fmoc-D-norleucinol (62e). Yield 1.6 g (92%), white powder, mp 140 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.92 (m, 3H), 1.34−1.6 (m, 6H), 3.58−3.68 (m, 3H), 4.24 (m, 1H), 4.46 (m, 2H), 4.82 (m, 1H), 7.31−7.45 (m, 4H), 7.61 (d, J = 7.5 Hz, 2H), 7.78 (d, J = 7.5 Hz, 2H). HRMS (M + H) calcd, 340.1913; found, 340.1739.

Fmoc-D-tyrosinol *tert*-Butyl Ether (62f). Yield 2.0 g (92%), mp 108 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.34 (s, 9H), 2.82 (m, 2H), 3.67 (m, 2H), 3.91 (m, 1H), 4.21 (m, 1H), 4.42 (m, 2H), 4.94 (m, 1H), 6.92 (d, J = 8.4 Hz, 2H), 7.3−7.45 (m, 4H), 7.58 (d, J = 7.5 Hz, 2H), 7.78 (d, J = 7.5 Hz, 2H). HRMS (M + H) calcd, 446.2331; found, 446.2057.

Fmoc-D-serinol Benzyl Ether (62g). Yield 1.61 g (78%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 3.46−3.83 (m, 5H), 4.2 (t, J = 6.6 Hz, 1H), 4.4 (m, 2H), 4.5 (s, 2H), 5.43 (m, 1H), 7.23−7.41 (m, 9H), 7.58 (d, J = 7.2 Hz, 2H), 7.74 (d, J = 7.2 Hz, 2H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 47.3, 52.0, 66.8, 70.5, 73.6, 120.0, 125.1, 127.1, 127.7, 128.0, 128.6, 137.6, 141.4, 143.9. HRMS (M + H) calcd, 404.1862; found, 404.1855.

Fmoc-D-threoninol Benzyl Ether (62h). Yield 1.74 g (81%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.22 (d, J = 7.2 Hz, 3H), 2.65 (m, 1H), 3.7 (m, 2H), 3.83 (m, 1H), 4.2 (t, J = 6.6 Hz, 1H), 4.35−4.45 (m, 3H), 4.61 (d, J = 11.4, 1H), 5.32 (m, 1H), 7.28−7.4 (m, 9H), 7.58 (d, J = 7.2 Hz, 2H), 7.73 (d, J = 7.2 Hz, 2H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 16.2, 47.3, 56.7, 63.9, 66.9, 70.9, 74.4, 120.0, 125.1, 127.1, 127.7, 127.9, 128.0, 128.6, 138.0, 141.4, 144.0. HRMS (M + H) calcd, 418.2018; found, 418.1696.

Fmoc-D-threoninol *tert*-Butyl Ether (62i). Yield 1.6 g (82%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.06 (d, J = 6.0 Hz, 3H), 1.11 (s, 9H), 3.52−3.57 (m, 3H), 3.85 (m, 1H), 4.12 (t, J = 7.0 Hz, 1H), 4.26−4.35 (m, 2H), 5.22 (br s, 1H), 7.21 (m, 2H), 7.29 (m, 2H), 7.5 (d, J = 7.5 Hz, 2H), 7.65 (d, J = 7.5 Hz, 2H). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 20.1, 28.7, 47.3, 57.4, 66.9, 74.3, 120.0, 125.1, 127.1, 127.7, 141.4, 143.9, 157.1. HRMS (M + H) calcd, 384.2175; found, 384.1983.

Fmoc-D-prolinol (67). Yield 1.45 g (89%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.6−1.9 (m, 4H), 3.3−3.4 (m, 2H), 3.6 (d, J = 4.5 Hz, 2H), 3.9 (m, 1H), 4.1 (m, 1H), 4.2 (t, J = 6.5 Hz, 1H), 4.3−4.44 (m, 2H), 7.24−7.35 (m, 4H), 7.54 (d, J = 7.5 Hz, 2H), 7.7 (d, J = 7.5 Hz, 2H). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 24.0, 28.4, 47.2, 60.5, 63.5, 66.1, 67.5, 120.0, 125.1, 127.1, 127.8, 141.4, 144.0, 156.8. HRMS (M + H) calcd, 324.1600; found, 324.1588.

General Procedure for the Synthesis of *N*-[(9*H*-Fluoren-9-ylmethoxy)carbonyl]amino Aldehydes (63a−i and 68). Fmoc-amino aldehydes were synthesized by Swern oxidation of corresponding Fmoc-D-amino alcohols.[35] To a solution of oxalyl chloride (8.0 mmol) in 30 mL of dry CH_2Cl_2, stirred at −78 °C under argon, was added DMSO (16.0 mmol) via syringe dropwise with vigorous stirring. After 20 min, a solution of Fmoc-D-amino alcohol (5.0 mmol) in 20 mL of dry CH_2Cl_2 was added while maintaining the bath temperature at −78 °C. Stirring was continued further for 30 min. Dry and distilled DIEA (30 mmol) was then added using a syringe and the reaction mixture then allowed to warm to room temperature without removing the

bath. The reaction mixture then quenched with 20 mL of ice-cold water and extracted with CH_2Cl_2 (3 × 80 mL). The combined organic layers were washed with 1 N HCl (3 × 30 mL) and brine (1 × 30 mL), dried ($MgSO_4$), and concentrated under vacuum. The crude aldehydes were used immediately for the next step without characterization.

General Procedure for Synthesis of *N*-[(9*H*-Fluoren-9-ylmethoxy)carbonyl]-γ-amino-α,β-unsaturated Benzyl Esters (64a−i, 69). A mixture of Fmoc-amino aldehyde (4.0 mmol) and benzyl (triphenylphosphoranylidene)acetate (4.4 mmol) in dry CH_2Cl_2 (20 mL) was stirred for 3 h. The progress of reaction was monitored by thin-layer chromatography. After completion of reaction, the solvent was removed in vacuo, and the residue was purified by silica gel chromatography using EtOAc in hexane.

Benzyl (*R*)-4-[[(9*H*-Fluoren-9-ylmethoxy)carbonyl]amino]-(*E*)-2-pentenoate (64a). Prepared as in Mandal et al.[38]

Benzyl (*S*)-4-[[(9*H*-Fluoren-9-ylmethoxy)carbonyl]amino]-(*E*)-2-pentenoate (64b). Yield 1.52 g (87%), white powder, mp 130−131 °C. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.18 (m, 3H), 4.1 (t, *J* = 7.0 Hz, 1H), 4.35 (m, 2H), 4.65 (br s, 1H), 5.1 (s, 2H), 5.84 (d, *J* = 16.0 Hz, 1H), 6.81 (d, *J* = 15.0 Hz, 1H), 7.2−7.3 (m, 9H), 7.48 (d, *J* = 7.0 Hz, 2H), 7.65 (d, *J* = 7.0 Hz, 2H). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 20.2, 47.3, 66.4, 120.0, 120.2, 125.0, 127.1, 127.7, 128.3, 128.4, 128.6, 135.8, 141.4, 143.8, 143.9, 149.3, 155.4, 166.0. HRMS (M + H) calcd, 428.1862; found, 428.1876.

Benzyl (*R*)-5-Methyl-4-[[(9*H*-fluoren-9-ylmethoxy)carbonyl]-amino]-(*E*)-2-hexenoate (64c). Yield 1.55 g (85%), white powder, mp 141 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.94 (m, 6H), 1.89 (m, 1H), 4.23 (m, 2H), 4.48 (m, 2H), 4.75 (d, *J* = 9.0 Hz, 1H), 5.21 (s, 2H), 5.96 (d, *J* = 15.9 Hz, 1H), 6.93 (m, 1H), 7.31−7.42 (m, 9H), 7.60 (d, *J* = 7.2 Hz, 2H), 7.77 (d, *J* = 7.2 Hz, 2H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 18.0, 18.9, 32.2, 47.3, 66.4, 120.0, 121.5, 124.9, 127.1, 127.7, 128.3, 128.6, 135.8, 141.4, 143.8, 147.4, 155.8, 165.9. Anal. ($C_{29}H_{29}NO_4$) C, H, N. Calcd: C, 76.46; H, 6.42; N, 3.07. Found: C, 75.74; H, 6.45; N, 3.32. HRMS (M + H) calcd, 456.2175; found, 456.1540.

Benzyl (*R*)-6-Methyl-4-[[(9*H*-fluoren-9-ylmethoxy)carbonyl]-amino]-(*E*)-2-heptenoate (64d). Yield 1.6 g (82%), white powder, mp 118 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.94 (d, *J* = 6.3 Hz, 6H), 1.42 (m, 2H), 1.67 (m, 1H), 4.22 (t, *J* = 6.3 Hz, 1H), 4.41−4.5 (m, 3H), 4.65 (m, 1H), 5.2 (s, 2H), 5.96 (d, *J* = 15.3 Hz, 1H), 6.88 (m, 1H), 7.31−7.42 (m, 9H), 7.60 (d, *J* = 7.2 Hz, 2H), 7.77 (d, *J* = 7.2 Hz, 2H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 22.7, 24.7, 43.7, 47.3, 50.4, 66.4, 120.0, 120.4, 124.9, 127.1, 127.7, 128.3, 128.4, 128.6, 135.9, 141.4, 143.8, 148.9, 155.6, 166.0. Anal. ($C_{30}H_{31}NO_4$) C, H, N. Calcd: C, 76.73; H, 6.65; N, 2.98. Found: C, 76.56; H, 6.71; N, 3.02. HRMS (M + H) calcd, 470.2331; found, 470.2342.

Benzyl (*R*)-4-[[(9*H*-Fluoren-9-ylmethoxy)carbonyl]amino]-(*E*)-2-octenoate (64e). Yield 1.52 g (78%), white powder, mp 116 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.93 (m, 3H), 1.33 (m, 4H), 1.53 (m, 2H), 4.23 (m, 1H), 4.35 (m, 1H), 4.48 (m, 2H), 4.7 (m, 1H), 5.21 (s, 2H), 5.96 (d, *J* = 15.6 Hz, 1H), 6.9 (m, 1H), 7.3−7.43 (m, 9H), 7.6 (d, *J* = 7.5 Hz, 2H), 7.78 (d, *J* = 7.5 Hz, 2H). ^{13}C NMR ($CDCl_3$, 75.0 MHz) δ 13.9, 22.3, 27.7, 34.2, 47.3, 52.0, 66.4, 120.0, 120.6, 124.9, 127.1, 127.7, 128.3, 128.4, 128.6, 135.9, 141.4, 143.8, 143.9, 148.7, 155.7, 166.0. Anal. ($C_{30}H_{31}NO_4$) C, H, N. Calcd: C, 76.73; H, 6.65; N, 2.98. Found: C, 76.61; H, 6.71; N, 2.99. HRMS (M + H) calcd, 470.2331; found, 470.2338.

Benzyl (*R*)-5-(4-*tert*-Butoxyphenyl)-4-[[(9*H*-fluoren-9-ylmethoxy)carbonyl]amino]-(*E*)-2-pentenoate (64f). Yield 1.9 g (81%), white powder, mp 82 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.33 (s, 9H), 2.87 (m, 2H), 4.2 (t, *J* = 6.6 Hz, 1H), 4.4 (m, 2H), 4.63−4.74 (m, 2H), 5.2 (s, 2H), 5.88 (d, *J* = 15.9 Hz, 1H), 6.91−6.94 (m, 3H), 7.02−7.1 (m, 2H), 7.30−7.42 (m, 10H), 7.55 (m, 2H), 7.77 (d, *J* = 7.5 Hz, 2H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 28.8, 47.2, 66.4, 66.7, 78.4, 120.0, 121.1, 124.2, 124.9, 127.1, 127.7, 128.2, 128.3, 128.6, 129.8, 135.8, 141.3, 143.7, 147.7, 154.5, 155.5, 165.8. Anal. ($C_{37}H_{37}NO_5$) C, H, N. Calcd: C, 77.19; H, 6.48; N, 2.43. Found: C, 77.09; H, 6.46; N, 2.54. HRMS (M + H) calcd, 576.2750; found, 576.2092.

Benzyl (*S*)-5-Benzyloxy-4-[[(9*H*-fluoren-9-ylmethoxy)carbonyl]-amino]-(*E*)-2-pentenoate (64g). Yield 1.53 g (72%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 3.5 (m, 2H), 4.15 (t, *J* = 7.0 Hz, 1H), 4.4−4.6 (m, 2H), 4.5 2 (m, 1H), 5.15 (s, 2H), 5.34 (d, *J* = 8.0 Hz, 1H), 6.0 (d, *J* = 15.5 Hz, 1H), 6.93 (m, 1H), 7.24−7.34 (m, 14 H), 7.54 (d, *J* = 7.0 Hz, 2H), 7.7 (d, *J* = 7.0 Hz, 2H). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 47.3, 52.00, 66.5, 70.9, 73.4, 120.1, 122.0, 122.1, 125.1, 127.2, 127.8, 128.1, 128.2, 128.4, 128.5, 128.6, 128.7, 136.0, 137.5, 141.4, 143.9, 144.0, 146.2, 146.3, 155.9, 165.9. Anal. ($C_{34}H_{31}NO_5$) C, H, N. Calcd: C, 76.53; H, 5.86; N, 2.62. Found: C, 76.42; H, 5.96; N, 2.66. HRMS (M + H) calcd, 534.2280; found, 534.2291.

Benzyl (4*S*,5*S*)-5-Benzyloxy-4-[[(9*H*-fluoren-9-ylmethoxy)carbonyl]amino]-(*E*)-2-hexenoate (64h). Yield 1.55 g (68%), white powder. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.22 (d, *J* = 5.7 Hz, 3H), 3.7 (m, 1H), 4.2 (t, *J* = 6.6 Hz, 1H), 4.3−4.45 (m, 4H), 4.58 (d, *J* = 11.7 Hz, 1H), 5.2 (s, 2H), 5.98 (d, *J* = 15.3 Hz, 1H), 6.98 (m, 1H), 7.28−7.38 (m, 14H), 7.58 (d, *J* = 7.5 Hz, 2H), 7.74 (d, *J* = 7.5 Hz, 2H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 16.5, 47.3, 66.3, 66.9, 71.1, 75.1, 120.0, 121.7, 125.0, 127.0, 127.7, 127.8, 127.9, 128.3, 128.5, 128.6, 135.9, 137.7, 141.3, 143.8, 143.9, 147.2, 165.8. Anal. ($C_{35}H_{33}NO_5$) C, H, N. Calcd: C, 76.76; H, 6.07; N, 2.56. Found: C, 76.61; H, 6.06; N, 2.55. HRMS (M + H) calcd, 548.2437; found, 548.2451.

Benzyl (4*S*,5*S*)-5-*tert*-Butoxy-4-[[(9*H*-fluoren-9-ylmethoxy)-carbonyl]amino]-(*E*)-2-hexenoate (64i). Yield 1.4 g (65%) as an oil. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 0.99−1.06 (m, 12H), 3.72 (m, 1H), 4.14 (m, 1H), 4.2 (m, 1H), 4.35−4.42 (m, 2H), 5.1 (s, 2H), 5.16 (d, *J* = 9.0 Hz, 1H), 5.87 (d, *J* = 15.5 Hz, 1H), 6.9 (m, 1H), 7.22−7.3 (m, 9H), 7.51 (d, *J* = 7.0 Hz, 2H), 7.66 (d, *J* = 7.0 Hz, 2H). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 20.4, 28.6, 47.3, 57.5, 66.4, 66.9, 68.2, 74.3, 120.0, 121.2, 125.1, 127.1, 127.8, 128.2, 128.3, 128.6, 136.0, 141.4, 143.8, 147.9, 156.4, 166.0. HRMS (M + H) calcd, 514.2593; found, 514.1597.

***N*-[[(9*H*-Fluoren-9-ylmethoxy)carbonyl]benzyl]-3-pyrrolidin-2-yl-(*E*)-propenoate (69).** Yield 1.2 g (62%) as an oil. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.7−1.96 (m, 4H), 3.36 (m, 2H), 4.1 (m, 0.5H), 4.14−4.2 (m, 1H), 4.3 (m, 0.5H), 4.4 (m, 1.5H), 4.48 (m, 0.5H), 5.57 (d, *J* = 15.5 Hz, 0.5H), 5.8 (d, *J* = 15.5 Hz, 0.5H), 6.6 (m, 1H), 6.68 (m, 0.5H), 6.8 (m, 0.5H), 7.1−7.3 (m, 9H), 7.42 (m, 1H), 7.5 (m, 1H), 7.6−7.68 (m, 2H). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 22.6, 23.5, 30.8, 31.7, 46.5, 46.8, 47.3, 57.9, 58.2, 66.5, 67.5, 120.0, 120.4, 120.7, 125.0, 127.1, 127.7, 128.5, 135.8, 141.3, 143.7, 143.9, 147.9, 148.1, 155.1, 155.4, 158.2, 158.6, 166.1, 166.3. HRMS (M + H) calcd, 454.2018; found, 454.1068.

General Procedure for the Synthesis of 4-Alkyl-Substituted 4-(9-Fluorenylmethyloxycarbonylamino)butyric Acids. To a stirred suspension of Fmoc-protected γ-amino-α,β-unsaturated benzyl esters (2.0 mmol) and 10% Pd−C (10% by wt) in 15 mL of methanol−THF (4:1 v/v) was added TES (20.0 mmol) dropwise under argon atmosphere. The reaction started with evolution of hydrogen gas. After completion of the reaction (TLC), the mixture was filtered through Celite, and the solvent was removed in vacuo. The crude product was purified either by triturating with ether−hexane or by short silica gel column chromatography, eluting with 2% hexane−EtOAc, followed by hexane−EtOAc−MeOH (3:6:1 v/v/v).

(*R*)-4-[[(9*H*-Fluoren-9-ylmethoxy)carbonyl]amino]-2-pentanoic Acid (65a). Yield 0.6 g (88%), white powder, mp 140−141 °C. Prepared as in Mandal et al.[38]

(*S*)-4-[[(9*H*-Fluoren-9-ylmethoxy)carbonyl]amino]-2-pentanoic Acid (65b). Yield 0.59 g (87%), white powder, mp 128−130 °C. ^{1}H NMR (DMSO-d_6, 500 MHz) δ 0.94 (d, *J* = 6.5 Hz, 3H), 1.52 (m, 2H), 2.1 (m, 2H), 3.41 (m, 1H), 4.1 (m, 1H), 4.14−4.23 (m, 2H), 7.05 (d, *J* = 8.5 Hz, 1H), 7.22 (m, 2H), 7.31 (m, 2H), 7.59 (d, *J* = 7.5 Hz, 2H), 7.78 (d, *J* = 7.5 Hz, 2H). ^{13}C NMR (DMSO-d_6, 125.0 MHz) δ 21.3, 31.0, 31.7, 46.4, 47.3, 65.5, 120.6, 125.5, 127.5,

128.1, 141.2, 144.4, 156.1, 174.8. HRMS (M + H) calcd, 340.1549; found, 340.1566.

(*S*)-5-Methyl-4-[[(9*H*-fluoren-9-ylmethoxy)carbonyl]amino]-2-hexanoic Acid (65c). Yield 0.66 g (89%), white powder, mp 120 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.9 (m, 6H), 1.5−1.78 (m, 3H), 2.36 (t, J = 7.5 Hz, 2H), 3.5 (m, 1H), 4.22 (m, 1H), 4.42 −4.6 (m, 3H), 7.3−7.43 (m, 4H), 7.60 (d, J = 7.5 Hz, 2H), 7.77 (d, J = 7.5 Hz, 2H). ^{13}C NMR ($CDCl_3$, 75 MHz) 17.8, 19.0, 27.4, 31.1, 32.5, 47.5, 56.1, 66.4, 1120.0, 125.0, 127.0, 127.7, 141.4, 143.9, 156.6, 178.8. Anal. ($C_{22}H_{25}NO_4$) C, H, N. Calcd: C, 71.91; H, 6.86; N, 3.81. Found: C, 71.60; H, 6.82; N, 3.80. HRMS (M + H) calcd, 368.1862; found, 368.1276.

(*S*)-6-Methyl-4-[[(9*H*-fluoren-9-ylmethoxy)carbonyl]amino]-2-heptanoic Acid (65d). Yield 0.68 g (88%), white powder, mp 150 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.91 (d, J = 6.3 Hz, 6H), 1.24 (m, 2H), 1.53−1.62 (m, 2H), 1.85 (m, 1H), 2.35 (t, J = 7.2 Hz, 2H), 3.73 (m, 1H), 4.2 (m, 1H), 4.44−4.5 (m, 3H), 7.31−7.42 (m, 4H), 7.60 (d, J = 7.2 Hz, 2H), 7.77 (d, J = 7.2 Hz, 2H). ^{13}C NMR ($CDCl_3$, 75.0 MHz) δ 22.2, 23.0, 24.8, 30.8, 45.0, 47.4, 49.1, 66.3, 119.95, 125.0, 127.0, 127.7, 141.4, 143.9, 156.3, 178.2. Anal. ($C_{23}H_{27}NO_4$) C, H, N. Calcd: C, 72.42; H, 7.13; N, 3.67. Found: C, 72.14; H, 7.25; N, 3.80. HRMS (M + H) calcd, 382.2018; found, 382.1545.

(*R*) 4-[[(9*H*-Fluoren-9-ylmethoxy)carbonyl]amino]-2-octanoic Acid (65e). Yield 0.67 g (87%), white powder. ^{1}H NMR (DMSO-d_6, 500 MHz) δ 0.85 (s, 3H), 1.2−1.4 (m, 6H), 1.53 (m, 1H), 1.66 (m, 1H), 2.18 (m, 2H), 3.4 (m, 1H), 4.22 (m, 1H), 4.3 (m, 2H), 7.1 (d, J = 8.0 Hz, 1H), 7.3−7.43 (m, 4H), 7.7 (d, J = 7.0 Hz, 2H), 7.9 (d, J = 7.0 Hz, 2H). ^{13}C NMR (DMSO-d_6, 125 MHz) δ 14.0, 22.5, 27.9, 30.5, 30.8, 35.3, 47.4, 51.0, 66.4, 120.0, 125.0, 127.1, 127.7, 141.4, 143.9, 156.4, 178.2. HRMS (M + H) calcd, 382.2018; found, 382.1975. Anal. ($C_{23}H_{27}NO_4$) C, H, N. Calcd: C, 72.42; H, 7.13; N, 3.67. Found: C, 72.65; H, 7.25; N, 4.19.

(*R*)-5-(4-*tert*-Butoxyphenyl)-4-[[(9*H*-fluoren-9-ylmethoxy)carbonyl]amino]-2-pentanoic Acid (65f). Yield 0.86 g (89%), white powder, mp 127 °C. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.22 (s, 9H), 1.53 (m, 1H), 1.76 (m, 1H), 2.3 (m, 2H), 2.6 (m, 2H), 3.8 (m, 1H), 4.3 (m, 2H), 4.6 (d, J = 9.0 Hz, 1H), 6.8 (d, J = 8.0 Hz, 2H), 6.9 (d, J = 8.0 Hz, 2H), 7.2−7.3 (m, 4H), 7.5 (d, J = 7.0 Hz, 2H), 7.7 (d, J = 7.0 Hz, 2H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 29.1, 30.8, 40.8, 47.3, 51.9, 66.5, 78.4, 120.0, 124.1, 125.0, 127.1, 127.7, 129.8, 132.1, 141.3, 143.8, 154.0, 156.1, 178.3. Anal. ($C_{30}H_{33}NO_5$) C, H, N. Calcd: C, 73.90; H, 6.82; N, 2.87. Found: C, 72.71; H, 6.69; N, 2.95. HRMS (M + H) calcd, 488.2437; found, 488.2423.

(*S*)-5-Benzyloxy-4-[[(9*H*-fluoren-9-ylmethoxy)carbonyl]amino]-2-pentanoic Acid (65g). Yield 0.69 g (77%), white powder. ^{1}H NMR (DMSO-d_6, 500 MHz) δ 1.58 (m 1H), 1.80 (m, 1H), 2.24 (m, 2H), 3.38 (m, 2H), 3.67 (m, 1H), 4.23 (m, 1H), 4.3−4.37 (m, 2H), 4.48 (s, 2H), 7.23−7.35 (m, 8H), 7.42 (m, 2H), 7.71 (d, J = 7.0 Hz, 2H), 7.89 (d, J = 7.5 Hz, 2H), 12.1 (s, 1H). ^{13}C NMR (DMSO-d_6, 125 MHz) δ 26.9, 30.7, 47.2, 50.3, 65.7, 72.3, 72.4, 120.5, 120.6, 125.7, 127.4, 127.6, 127.8, 128.0, 128.2, 128.5, 128.8, 138.9, 141.2, 144.4, 156.4, 174.7. Anal. ($C_{27}H_{27}NO_5$) C, H, N. Calcd: C, 72.79; H, 6.11; N, 3.14. Found: C, 72.76; H, 6.23; N, 3.09. HRMS (M + H) calcd, 446.1967; found, 446.1984.

(4*S*,5*S*)-5-Benzyloxy-4-[[(9*H*-fluoren-9-ylmethoxy)carbonyl]amino]-2-hexanoic Acid (65h). Yield 0.75 g (82%), white powder. ^{1}H NMR (DMSO-d_6, 500 MHz) δ 1.07 (d, J = 6.9 Hz, 3H), 1.63 (m, 1H), 1.8 (m, 1H), 2.2−2.3 (m, 2H), 3.53 (m, 1H), 3.62 (m, 1H), 4.22 (m, 1H), 4.26−4.31 (m, 3H), 4.5 (m, 2H), 7.2 (d, J = 5.4 Hz, 1H), 7.27−7.43 (m, 9 H), 7.72 (d, J = 7.5 Hz, 2H), 7.9 (d, J = 7.5 Hz, 2H), 12.04 (s, 1H). ^{13}C NMR (DMSO-d_6, 125 MHz) δ 15.6, 24.9, 31.0, 47.3, 53.9, 65.7, 70.4, 76.3, 120.6, 125.7, 127.5, 127.7, 127.8, 128.1, 128.6, 129.2, 139.4, 141.2, 144.4, 156.7, 174.8. Anal. ($C_{28}H_{29}NO_5$) C, H, N. Calcd: C, 73.18; H, 6.36; N, 3.05. Found: C, 72.52; H, 6.38; N, 3.01. HRMS (M + H) calcd, 460.2124; found, 460.2141.

(4*S*,5*S*)-5-*tert*-Butoxy-4-[[(9*H*-fluoren-9-ylmethoxy)carbonyl]-amino]-2-hexanoic Acid (65i). Yield 0.71 g (83%) as an oil. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.03 (d, J = 6.0 Hz, 3H), 1.12 (s, 9H), 1.65−1.76 (m, 2H), 2.31 (t, J = 7.0 Hz, 2H), 3.48 (m, 1H), 3.6 (m, 1H), 4.14 (m, 1H), 4.34−4.41 (m, 2H), 4.5 (m, 1H), 5.0 (d, J = 9.5 Hz, 1H), 7.25 (m, 2H), 7.32 (m, 2H), 7.53 (d, J = 7.0 Hz, 2H), 7.68 (d, J = 7.5 Hz, 2H), 10.1 (s, 1H). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 20.0, 27.7, 28.7, 31.0, 47.4, 56.0, 66.7, 68.4, 73.9, 120.0, 125.1, 127.1, 127.7, 141.4, 143.8, 157.2, 178.9. HRMS (M + Na) calcd, 448.2100; found, 448.2134.

***N*-[(9*H*-Fluoren-9-ylmethoxy)carbonyl]-3-pyrrolidin-2-ylpropionic Acid (70).** Yield 0.59 g (80%) as an oil. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.53−2.07 (m, 6H), 2.36 (m, 1H), 3.3−3.45 (m, 2.5H), 3.95 (m, 0.5H), 4.0 (m, 1H), 4.35−4.46 (m, 1H), 4.58 (m, 1H), 7.28−7.38 (m, 4H), 7.77 (m, 2H), 7.74 (m, 2H), 9.4 (m, 2H). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 22.8, 23.6, 28.9, 29.4, 30.2, 31.2, 46.4, 47.3, 57.1, 66.8, 67.4, 119.95, 125.0, 127.1, 127.7, 141.4, 143.9, 155.8, 178.5. HRMS (M + H) calcd, 366.1705; found, 366.1651.

Synthesis of *tert*-Butyl 4-(Benzyloxycarbonylamino)-5-hydroxypentanoate (72). To a stirred solution of Z-Glu(OtBu)-OH (5.0 g, 14.8 mmol) and DCC (3.46 g, 16.8 mmol) in 150 mL of EtOAc was added 2-mercaptopyridine (1.81 g, 16.33 mmol). Stirring was continued for 4 h at which time the precipitate was filtered off and the solvent was removed under vacuum. The residue was dissolved in 100 mL of 1,4-dioxane, cooled to 0 °C and treated with $NaBH_4$ (2.0 g). When the reaction was finished, as monitored by TLC, approximately after 30 min, it was then quenched slowly by adding 2% $KHSO_4$(aq). The mixture was extracted with Et_2O (3 × 150 mL). The combined organic layers were washed with 5% $NaHCO_3$ (2 × 50 mL) and brine, dried ($MgSO_4$), and concentrated under vacuum. The crude product was purified by silica gel column chromatography eluting with 35% EtOAc−hexane (v/v) to give 4.2 g of **72** as colorless oil. Yield 88%. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.45 (s, 9H), 1.77 (m, 1H), 1.87 (m, 1H), 2.34 (m, 2H), 2.76 (br s, 1H), 3.6 (m, 1H), 3.65−3.7 (m, 2H), 5.1 (s, 2H), 5.25 (d, J = 6.5 Hz, 1H), 7.36 (s, 5H). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 28.0, 28.1, 66.8, 80.9, 128.0, 128.2, 128.4, 128.6, 136.4, 156.6, 173.3. HRMS (M + H) calcd, 324.1811; found, 324.0416.

Synthesis of *tert*-Butyl 4-(Benzyloxycarbonylamino)-5-iodopentanoate (73). A solution of PPh_3 (4.8 g, 18.6 mmol), I_2 (2.4 g, 18.6 mmol), and imidazole (2.1 g, 31.0 mmol) in dry CH_2Cl_2 (30 mL) was stirred for 30 min under inert atmosphere at room temperature. To this solution was added a solution of **62** (2.0 g, 6.2 mmol) in 15 mL of dry CH_2Cl_2, and stirring continued for 3 h. The solvent was removed, and the crude product was purified by silica gel chromatography eluting 15% EtOAc−hexane (v/v). A white solid was obtained (2.45 g, 91%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.45 (s, 9H), 1.85 (m, 2H), 2.3 (m, 2H), 3.33 (m 1H), 3.42 (m, 1H), 3.5 (m, 1H), 5.1 (br s, 3H), 7.37 (s, 5H). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 28.1, 30.0, 31.8, 66.9, 80.8, 127.9, 128.1, 128.2, 128.3, 128.5, 128.7, 136.3, 155.7, 172.3. HRMS (M + H) calcd, 434.0828; found, 434.0541.

Synthesis of *tert*-Butyl 4-(Benzyloxycarbonylamino)-5-(1-piperidino)pentanoate (74a). A solution of **73** (1.0 g, 2.3 mmol) in 10 mL of dry THF was treated with piperidine (0.7 mL, 6.92 mmol) overnight under argon. The reaction mixture was diluted with 100 mL of EtOAc and was washed with water and brine. After drying ($MgSO_4$) and concentration under vacuum the crude product was purified by silica gel chromatography eluting with 50% EtOAc−hexane (v/v) to give the desired N-alkylated product. Yield 0.74 g, 82%. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.45 (s, 9H), 1.53−1.58 (m, 4H), 1.7 (m, 1H), 1.93 (m, 1H), 2.28−2.40 (m, 8H), 3.73 (m, 1H), 5.03 (br s, 1H), 5.12 (s, 2H), 7.37 (s, 5H). ^{13}C NMR ($CDCl_3$, 125.0 MHz) δ 24.3, 26.0, 28.0, 28.1, 28.3, 32.0, 54.9, 66.5, 80.3, 127.8, 128.1, 128.4, 128.6, 129.1, 136.8, 156.4, 172.9. Anal. ($C_{22}H_{34}N_2O_4$) C, H, N. Calcd: C, 67.66; H, 8.78; N, 7.17. Found: C, 67.89; H, 8.91; N, 7.21. HRMS (M + H) calcd, 391.2597; found, 391.0992.

***tert*-Butyl 4-(Benzyloxycarbonylamino)-5-(4-morpholiino)pentanoate (74b).** The same procedure as for **74a** was used. Yield 0.76 g (84%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.45 (s, 9H), 1.67 (m, 1H), 1.94 (m, 1H), 2.3−2.48 (m, 8H), 3.65 (s, 4H), 3.77 (m, 1H), 4.9 (br s, 1H), 5.11 (s, 2H), 7.36 (s, 5H). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 28.0, 28.1, 32.0, 48.4, 53.9, 62.8, 66.9, 67.3, 80.4, 127.9, 128.1, 128.4, 128.6, 136.6, 156.4, 172.8. Anal. ($C_{21}H_{32}N_2O_5$) C, H, N. Calcd: C, 64.26; H, 8.22; N, 7.14. Found: C, 63.85; H, 8.26; N, 7.07. HRMS (M + H) calcd, 393.2389; found, 393.1541.

***tert*-Butyl 4-(Benzyloxycarbonylamino)-5-(4-methyl-1-piperazino)pentanoate (74c).** The same procedure as for **74a** was used. Yield 0.73 g (78%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.44 (s, 9H), 1.67 (m, 1H), 1.92 (m, 1H), 2.27 (s, 3H), 2.3−2.57 (m, 12H), 3.74 (m, 1H), 4.94 (m, 1H), 5.13 (s, 2H), 7.36 (s, 5H). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 28.0, 28.1, 32.0, 45.4, 45.9, 46.4, 48.6, 53.2, 55.0, 62.1, 66.5, 80.4, 127.9, 128.1, 128.4, 128.5, 128.6, 136.7, 156.4, 172.8. Anal. ($C_{22}H_{35}N_3O_4$) C, H, N. Calcd: C, 65.16; H, 8.70; N, 10.36. Found: C, 64.50; H, 8.71; N, 10.05. HRMS (M + H) calcd, 406.2706; found, 406.1604.

Synthesis of *tert*-Butyl 4-[[(9*H*-Fluoren-9-ylmethoxy)carbonyl]-amino]-5-(1-piperidino)pentanoate (75a). To a suspension of **74a** (1.0 g, 2.56 mmol) and 10% Pd−C (0.2 g) in 2% $CHCl_3$−MeOH (20 mL) was added TES (4.2 mL, 26.0 mmol) dropwise under argon atmosphere. After completion (TLC monitoring), the catalyst was filtered off through Celite and the filter cake washed with 10 mL of methanol. The combined filtrates were concentrated under vacuum, and residual methanol was removed by the addition and evaporation of toluene (2 × 10 mL). The residue was dissolved in 10 mL of 10% Na_2CO_3 and 20 mL of 1,4-dioxane. Fmoc-OSu (1.3 g, 3.84 mmol) was added, and the mixture was stirred overnight. The mixture was extracted with EtOAc (2 × 40 mL), and the combined organic layers were washed with water followed by brine. The organic part was dried ($MgSO_4$) and concentrated under vacuum. The crude product was purified by silica gel column chromatography eluting with 10% methanol−chloroform (v/v) to give desired product. Yield 0.94 g, 76%. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.3−1.4 (m, 10H), 1.65 (m, 1H), 1.7−1.87 (m, 6H), 2.00 (m, 1H), 2.26 (m, 2H), 2.52 (m, 1H), 2.62 (m, 1H), 2.81 (m, 1H), 3.35 (m, 1H), 3.4 (m, 1H), 3.65 (m, 1H), 4.1−4.2 (m, 4H), 4.3 (m, 1H), 6.9 (d, J = 9.5 Hz, 1H), 7.23 (m, 2H), 7.3 (m, 2H), 7.54 (m, 2H), 7.66 (d, J = 7.5 Hz, 2H). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 22.0, 22.4, 28.0, 28.7, 31.1, 45.5, 47.0, 51.6, 55.6, 59.2, 67.4, 81.0, 119.8, 125.3, 125.5, 127.1, 127.6, 127.7, 141.3, 143.7, 144.1, 156.8, 172.1. HRMS (M + H) calcd, 479.2910; found, 479.1051.

***tert*-Butyl 4-[[(9*H*-Fluoren-9-ylmethoxy)carbonyl]amino]-5-(1-morpholino)pentanoate (75b).** The same procedure as for **75a** was employed. Yield 0.91 g (74%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.35 (s, 9H), 1.66 (m, 1H), 1.77 (m, 1H), 2.25 (m, 2H), 2.78−2.86 (m, 3H), 3.41 (m, 2H), 3.67 (m, 1H), 3.91 (m, 4H), 4.12 (m, 2H), 4.21−4.3 (m, 2H), 6.37 (d, J = 9.5 Hz, 1H), 7.24 (m, 2H), 7.31 (m, 2H), 7.52 (m, 2H), 7.68 (d, J = 7.5 Hz, 2H). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 28.0, 28.1, 30.9, 45.2, 46.9, 51.1, 53.9, 60.3, 63.6, 67.5, 81.2, 114.8, 117.1, 119.9, 125.2, 125.3, 127.1, 127.7, 127.8, 141.2, 141.3, 143.5, 144.1, 156.7, 162.1, 162.3, 172.0. HRMS (M + H) calcd, 481.2702; found, 481.1066.

***tert*-Butyl [[(9*H*-Fluoren-9-ylmethoxy)carbonyl]amino]-5-(4-methy1-1-piperazino)pentanoate (75c).** The same procedure as for **75a** was employed. Yield 0.78 g (64%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.35 (s, 9H), 1.6 (m, 1H), 1.75 (m, 1H), 2.22 (m, 2H), 2.74 (s, 3H), 2.88 (m, 1H), 3.13 (m, 1H), 3.33−3.5 (m, 8H), 3.97 (m, 1H), 4.11 (t, J = 6.5 Hz, 1H), 4.28 (d, J = 7.0 Hz, 2H), 5.7 (d, J = 9.0 Hz, 1H), 7.23 (m, 2H), 7.32 (m, 2H), 7.5 (m, 2H), 7.68 (m, 2H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 27.8, 28.0, 28.3, 31.2, 43.3, 46.5, 46.9, 50.6, 60.6, 67.3, 81.2, 112.7, 115.0, 117.3, 119.9, 125.11, 127.2, 127.7,127.9, 141.3, 143.4, 144.1, 156.8, 162.1, 162.4, 162.7, 171.9. HRMS (M + H) calcd, 494.3019; found, 494.2492.

Synthesis of 4-[[(9*H*-Fluoren-9-ylmethoxy)carbonyl]amino]-5-(1-piperidino)pentanoic Acid (76a). Compound **75a** (0.7 g) was treated with 95% TFA/CH_2Cl_2 for 1 h, and the solvent was removed under vacuum. Traces of TFA were removed by addition and evaporation of toluene (2 × 10 mL). The product then triturated with ether−hexane to give a white solid (0.56 g, 90%). ^{1}H NMR (DMSO-d_6, 500 MHz) δ 1.36 (m, 1H), 1.56−1.76 (m, 7H), 2.21 (m, 2H), 2.88 (m, 2H), 3.05 (m, 1H), 3.16 (m, 1H), 3.37 (m, 1H), 3.5 (m, 1H), 3.88 (m, 1H), 4.25 (m, 1H), 4.32 (m, 1H), 4.52 (m, 1H), 7.34 (m, 2H), 7.42 (m, 3H), 7.7 (d, J = 7.5 Hz, 2H), 7.9 (d, J = 7.5 Hz, 2H), 9.24 (s, 1H). ^{13}C NMR (DMSO-d_6, 125 MHz) δ 21.6, 22.5, 28.3, 30.2, 46.2, 47.3, 52.4, 53.6, 59.6, 65.8, 120.6, 125.5, 125.6, 127.5, 128.1, 141.3, 144.2, 144.3, 156.5, 158.5, 158.7, 174.3. HRMS (M + H) calcd, 423.2284; found, 423.2716.

4-[[(9*H*-Fluoren-9-ylmethoxy)carbonyl]amino]-5-(1-morpholino)pentanoic Acid (76b). The same procedure as for **76a** was employed. Yield 0.56 g (90%). ^{1}H NMR (DMSO-d_6, 500 MHz) δ 1.61 (m, 1H), 1.74 (m, 1H), 2.21 (m, 2H), 3.1−3.48 (m, 6H), 3.73 (m, 2H), 3.91 (m, 3H), 4.24−4.32 (m, 2H), 4.5 (m, 1H), 7.34 (m, 2H), 7.43 (m, 3H), 7.7 (d, J = 7.5 Hz, 2H), 7.9 (d, J = 7.5 Hz, 2H), 9.9 (br s, 1H). ^{13}C NMR (DMSO-d_6, 125 MHz) δ 28.1, 30.2, 45.8, 47.2, 60.0, 63.5, 65.9, 120.6, 125.5, 125.6, 127.5, 128.1, 141.3, 144.2, 144.3, 156.5, 158.6, 158.8, 174.3. HRMS (M + H) calcd, 425.2076; found, 425.2117.

4-[[(9*H*-Fluoren-9-ylmethoxy)carbonyl]amino]-5-(4-methy1-1-piperazino)pentanoic Acid (76c). The same procedure as for **76a** was employed. Yield 0.45 g (72%). ^{1}H NMR (DMSO-d_6, 500 MHz) δ 1.55 (m, 1H), 1.78 (m, 1H), 2.22 (m, 2H), 2.66 (m, 2H), 2.8 (s, 3H), 2.9−3.5 (m, 8H), 3.71 (m, 1H), 4.24−4.3 (m, 2H), 4.41 (m, 1H), 7.25 (d, J = 8.0 Hz, 1H), 7.34 (m, 2H), 7.42 (m, 2H), 7.7 (m, 2H), 7.9 (d, J = 7.5 Hz, 2H). ^{13}C NMR (DMSO-d_6, 125 MHz) δ 28.1, 30.5, 42.5, 47.3, 47.4, 49.6, 65.7, 115.6, 117.9, 120.6, 125.6, 127.5, 128.1, 141.3, 144.3, 144.4, 156.5, 158.6, 158.9, 159.2, 159.4, 174.6. HRMS (M + H) calcd, 438.2393; found, 438.2433.

Synthesis of *tert*-Butyl 4-[[(9*H*-Fluoren-9-ylmethoxy)carbonyl]-amino]-5-hydroxypentanoate (78). Fmoc-Glu(OtBu)-OH (**77**) (4.0 g, 9.4 mmol) was treated as described for the preparation of **72** to give **78**. Yield 3.2 g (83%). NMR same as in ref 44. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.37 (s, 9H), 1.71 (m, 1H), 1.8 (m, 1H), 2.25 (m, 2H), 3.5 (m, 1H), 3.6 (m, 2H), 4.13 (m, 1H), 4.33 (m, 2H), 5.12 (br s, 1H), 7.24 (m, 2H), 7.33 (m, 2H), 7.52), 7.52 (d, J = 7.5 Hz, 2H), 7.68 (d, J = 7.5 Hz, 2H). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 25.7, 28.1, 31.9, 47.3, 53.0, 64.7, 66.7, 81.0, 120.0, 125.1, 127.1, 127.7, 141.3, 143.9, 155.9, 173.3. HRMS (M + H) calcd, 412.2124; found, 412.2132.

Synthesis of *tert*-Butyl 4-[[(9*H*-Fluoren-9-ylmethoxy)carbonyl]-amino]-5-iodopentanoate (79). The same procedure was followed as described for 7**3** starting with 2.0 g (4.86 mmol) of **78**. Yield 2.3 g (88%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.38 (s, 9H), 1.78 (m, 2H), 2.2−2.3 (m, 2H), 3.24 (m, 1H), 3.35 (m, 1H), 3.4 (m, 1H), 4.15 (m, 1H), 4.3 (m, 1H), 4.4 (m, 1H), 4.95 (d, J = 7.5 Hz, 1H), 7.25 (m, 2H), 7.33 (m, 2H), 7.53 (d, J = 7.0 Hz, 2H), 7.7 (d, J = 7.5 Hz, 2H). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 28.1, 29.9, 31.8, 47.3, 50.5, 66.8, 80.9, 120.0, 125.0, 125.1, 127.1, 127.7, 141.3, 143.8, 155.7, 172.4. HRMS (M + H) calcd, 522.1141; found, 522.1157.

Synthesis of *tert*-Butyl 4-[[(9*H*-Fluoren-9-ylmethoxy)carbonyl]-amino]-5-azidopentanoate (80).[40] To a solution of **79** (2.0 g, 3.84 mmol) in 30 mL of dry CH_2Cl_2 was added nBu_4NN_3 (3.3 g, 11.5 mmol) in portions. After completion of the reaction (ca. 8 h, monitored by TLC), the solvent was removed in vacuo, and the product was purified by silica gel chromatography (10% EtOAc−hexane v/v). Yield 1.45 g (86%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.37 (s, 9H), 1.73 (m, 2H), 2.2−2.3 (m, 2H), 3.35 (m, 2H), 3.71 (m, 1H), 4.13 (m, 1H), 4.3−4.4 (m, 2H), 4.93 (d, J = 8.0 Hz, 1H), 7.24 (m, 2H), 7.33 (m, 2H), 7.51 (d, J = 7.5 Hz, 2H), 7.68 (d, J = 7.5 Hz, 2H). ^{13}C NMR ($CDCl_3$, 125.0 MHz) δ 28.1, 32.0, 46.8, 51.2, 54.8, 66.8, 80.9, 120.1, 125.1, 127.1, 127.7, 127.8, 141.3, 143.8, 155.9, 172.5. HRMS (M + H) calcd, 437.2189; found, 437.2208.

Synthesis of *tert*-Butyl 4-[[(9*H*-Fluoren-9-ylmethoxy)carbonyl]-amino]-5-(allyloxycarbonyl)aminopentanoate (81). To a stirred suspension of **80** (1.0 g, 2.3 mmol) and 10% Pd−C (0.1 g) in 15 mL of

1% $CHCl_3$ in MeOH was added TES (4.0 mL) dropwise under argon atmosphere. After completion of ~20 min, the mixture was filtered through Celite and the filtrate then concentrated under vacuum. The residue was dissolved in 15 mL of CH_2Cl_2 and was treated with allyl chloroformate (0.5 mL, 4.6 mmol) and DIEA (1.2 mL, 6.9 mmol) for 3 h at 0 °C. The solution was transferred to a separatory funnel with an additional 50 mL of CH_2Cl_2 and was washed with 5% HCl (2 × 15 mL), 5% $NaHCO_3$ (20 mL), and brine and dried ($MgSO_4$). The solvent was removed, and the crude product was purified by silica gel chromatography (25% EtOAc−hexane, v/v) yielding **81** as a white powder. Yield 0.73 g (64%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.46 (s, 9H), 1.75 (m, 1H), 1.84 (m, 1H), 2.34 (m, 2H), 3.28−3.36 (m, 2H), 3.73 (m, 1H), 4.22 (m, 1H), 4.35−4.45 (m, 2H), 4.55 (m, 2H), 5.17−5.3 (m, 4H), 5.90 (m, 1H), 7.33 (m, 2H), 7.42 (m, 2H), 7.61 (d, J = 7.0 Hz, 2H), 7.78 (d, J = 7.0 Hz, 2H). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 27.3, 28.1, 32.0, 45.1, 47.3, 52.0, 65.7, 66.7, 80.8, 117.7, 120.0, 125.1, 127.1, 127.7, 132.8, 141.3, 143.9, 156.6, 156.9, 172.7. HRMS (M + H) calcd, 495.2495; found, 495.2478. Anal. ($C_{28}H_{35}N_2O_6$) C, H, N. Calcd: C, 68.00; H, 6.93; N, 5.66. Found: C, 67.93; H, 6.88; N, 5.62.

Synthesis of 4-[[(9*H*-Fluoren-9-ylmethoxy)carbonyl]amino]-5-(allyloxycarbonyl)aminopentanoic Acid (82). Compound **81** (0.6 g, 1.2 mmol) was treated with neat TFA for 1 h. The TFA was removed under vacuum, and the residue was triturated with ether−hexane to form a white solid. The product was collected by filtration and dried in vacuo over P_2O_5. Yield 0.44 g (82%). ^{1}H NMR (DMSO-d_6, 500 MHz) δ 1.5 (m, 1H), 1.7 (m, 1H), 2.2 (m, 2H), 3.03 (m, 2H), 3.5 (m, 1H), 4.19−4.3 (m, 3H), 4.45 (m, 2H), 5.14 (dd, J = 2.0, 17.0 Hz, 1H), 5.26 (dd, J = 2.0, 28.5 Hz, 1H), 5.87 (m, 1H), 7.15 (d, J = 14.5, 1H), 7.23 (t, J = 20.0 Hz, 1H), 7.32 (m, 2H), 7.42 (m, 2H), 7.68 (d, J = 12.0 Hz, 2H), 7.88 (d, J = 12.0 Hz, 2H), 12.04 (br s, 1H). ^{13}C NMR (DMSO-d_6, 125 MHz) δ 27.3, 30.7, 44.6, 47.3, 50.8, 64.7, 65.7, 117.3, 120.6, 125.7, 127.5, 128.1, 134.2, 141.2, 144.3, 144.4, 156.4, 156.6, 174.6. HRMS (M + H) calcd, 439.1869; found, 439.1836. Anal. ($C_{24}H_{27}N_2O_6$) C, H, N. Calcd: C, 65.74; H, 5.98; N, 6.39. Found: C, 65.56; H, 5.93; N, 6.28.

Synthesis of *tert*-Butyl 4-[[(9*H*-Fluoren-9-ylmethoxy)carbonyl]-amino]-5-acetoxypentanoate (92). Acetic anhydride (1.4 mL, 14.6 mmol) was added to a solution of **78** (1.0 g, 2.43 mmol), DIEA (0.85 mL, 4.86 mmol), and DMAP (0.03 g, 0.24 mmol) in dry CH_2Cl_2 (15 mL). After completion of the reaction (TLC), 10 mL of water was added and the mixture extracted with CH_2Cl_2 (3 × 30 mL). The combined organic layers were washed with 5% HCl (2 × 20 mL) and saturated $NaHCO_3$ (2 × 20 mL). After drying ($MgSO_4$) and concentration under vacuum, the crude product was purified by silica gel column chromatography eluting with 25% EtOAc−hexane (v/v) to give 1.0 g (93%) of **92**. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.37 (s, 9H), 1.64−1.77 (m, 2H), 1.98 (s, 3H), 2.23 (m, 2H), 3.84 (m, 1H), 4.00 (m, 2H), 4.13 (m, 1H), 4.34 (m, 2H), 4.88 (d, J = 9.0 Hz, 1H), 7.24 (m, 2H), 7.32 (m, 2H), 7.5 (d, J = 7.5 Hz, 2H), 7.68 (d, J = 7.5 Hz, 2H). ^{13}C NMR ($CDCl_3$, 125.0 MHz) δ 28.1, 31.9, 46.8, 47.2, 50.4, 66.0, 66.6, 80.8, 119.9, 120.1, 125.0, 127.0, 127.2, 127.6, 127.8, 141.3, 143.9, 143.8, 156.0, 170.9, 172.5. HRMS (M + H) calcd, 454.2230; found, 454.2240.

Synthesis of 4-[[(9*H*-Fluoren-9-ylmethoxy)carbonyl]amino]-5-acetoxypentanoic Acid (93). Compound **92** (0.5 g) was treated with 2 mL of neat TFA for 1 h. The TFA was removed in vacuo, and residual acid was removed by addition and evaporation of toluene (2 × 5 mL). Trituration with ether−hexane resulted in a white solid which was collected by filtration and dried in vacuo over P_2O_5. Yield 0.4 g (90%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.77 (m, 1H), 1.89 (m, 1H), 2.1 (s, 3H), 2.42 (m, 2H), 3.96 (m, 1H), 4.06 (m, 1H), 4.14 (m, 1H), 4.22 (m, 1H), 4.46 (m, 2H), 4.95 (d, J = 8.5 Hz, 1H), 7.33 (m, 2H), 7.42 (m, 2H), 7.6 (m, 2H), 7.78 (d, J = 7.5 Hz, 2H). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 26.6, 30.4, 31.6, 47.2, 50.1, 66.0, 66.7, 119.8, 120.0, 120.1, 125.0, 127.0, 127.2, 127.6, 127.9, 141.3, 143.8, 156.2, 171.0, 177.9. HRMS (M + Na) calcd, 420.1423; found, 420.1442.

General Procedure for 4-Nitrophenyl Fmoc-aminocarbonates: Synthesis of 4-Nitrophenyl 2-[(9*H*-Fluoren-9-ylmethoxy)carbonyl]aminoethylcarbonate (83j). 4-Nitrophenyl chloroformate (1.6 g, 7.8 mmol) in 10 mL of dry CH_2Cl_2 was added dropwise to a stirred solution of Fmoc-aminoethanol (2.0 g, 7.06 mmol) and pyridine (0.9 mL, 10.6 mmol) in 20 mL of dry CH_2Cl_2 at 0 °C under an atmosphere of argon. Upon completion (TLC), the solution was transferred to a separatory funnel with an additional 20 mL of CH_2Cl_2. The organic solution was washed with 10% HCl (3 × 30 mL), 10% Na_2CO_3 (3 × 30 mL), and brine (50 mL) and was dried ($MgSO_4$). The crude mixture was purified by silica gel chromatography eluting with 40% EtOAc−hexane to give 2.6 g (82%) of desired product as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.6 (m, 2H), 4.25−4.48 (m, 5H), 5.2 (m, 1H), 7.28−7.45 (m, 6H), 7.6 (d, J = 7.2 Hz, 2H), 7.8 (d, J = 7.5 Hz, 2H), 8.28 (d, J = 8.4 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 39.9, 47.2, 66.9, 68.2, 120.0, 121.6, 121.7, 124.9, 125.3, 125.5, 127.0, 127.7, 141.4, 143.8, 145.5, 152.4, 155.4. Anal. ($C_{24}H_{20}N_2O_7$) C, H, N, O. Calcd: C, 64.28; H, 4.50; N, 6.25; O, 24.98. Found: C, 63.91; H, 4.42; N, 6.36. HRMS (M + H) calcd, 454.2230; found, 454.2240.

4-Nitrophenyl 2-[(9*H*-Fluoren-9-ylmethoxy)carbonyl]amino-3-benzyloxybutylcarbonate (83g). The procedure for **83j** was used. Compound **62g** (1.0 g, 2.4 mmol) yielded 1.1 g of **83g** (77%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.16 (d, J = 6.0 Hz, 3H), 3.68 (m, 1H), 3.97 (m, 1H), 4.11 (m, 1H), 4.20 (d, J = 6.5 Hz, 2H), 4.27−4.33 (m, 3H), 4.55 (m, 1H), 5.16 (d, J = 9.5 Hz, 1H), 7.17−7.27 (m, 11H), 7.47 (d, J = 7.0 Hz, 2H), 7.64 (d, J = 7.0 Hz, 2H), 8.08 (d, J = 8.5 Hz, 2 Hz). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 16.0, 47.3, 54.1, 67.1, 68.6, 70.8, 72.0, 120.1, 121.8, 125.1, 125.3, 127.2, 127.8, 128.0, 128.1, 128.6, 137.8, 141.4, 143.8, 145.4, 152.4, 155.5, 156.8. HRMS (M + H) calcd, 449.1349; found, 449.1357.

4-Nitrophenyl 2-[(9*H*-Fluoren-9-ylmethoxy)carbonyl]amino-3-benzyloxypropylcarbonate (83f). The procedure for **83j** was used. Compound **62f** (1.0 g, 2.47 mmol) yielded 1.2 g of **83g** (81%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 3.64 (m, 2H), 4.25 (m, 2H), 4.46 (m, 4H), 4.58 (s, 2H), 5.27 (d, J = 8.4 Hz, 1H), 7.31−7.45 (m, 13H), 7.61 (d, J = 7.5 Hz, 2H), 7.79 (d, J = 7.5 Hz, 2H), 8.24 (m, 2H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 47.2, 49.5, 67.0, 68.1, 68.5, 73.5, 120.1, 121.7, 125.0, 125.3, 127.1, 127.8, 128.1, 128.6, 137.5, 141.4, 143.8, 145.4, 152.3, 155.4, 156.2. Anal. ($C_{32}H_{28}N_2O_8$) C, H, N. Calcd: C, 67.60; H, 4.96; N, 4.93. Found: C, 67.62; H, 4.86; N, 4.96. HRMS (M + H) calcd, 569.1924; found, 569.1937.

4-Nitrophenyl 2-[(9*H*-Fluoren-9-ylmethoxy)carbonyl]aminopropylcarbonate (83a). The procedure for **83j** was used. Compound **62a** (1.0 g, 3.36 mmol) yielded 1.3 g of **83a** (84%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.20 (s, 3H), 4.09−4.22 (m 4H), 4.36 (m, 2H), 4.78 (br s, 1H), 7.22−7.34 (m, 6H), 7.51 (d, J = 7.5 Hz, 2H), 7.69 (d, J = 7.5 Hz, 2H), 8.17 (d, J = 9.0 Hz, 2H). ^{13}C NMR ($CDCl_3$, 125.0 MHz) δ 17.3, 45.8, 47.2, 66.8, 71.6, 120.1, 121.7, 124.9, 125.3, 127.1, 127.8, 141.4, 143.8, 145.5, 152.5, 155.4, 155.7. Anal. ($C_{25}H_{22}N_2O_7$) C, H, N. Calcd: C, 64.93; H, 4.80; N, 6.06. Found: C, 64.78; H, 4.69; N, 6.04. HRMS (M + H) calcd, 463.1505; found, 463.1507.

Synthesis of *N*-Triphenylmethyl 4-[[(9*H*-Fluoren-9-ylmethoxy)-carbonyl]amino]-5-hydroxypentanamide (85). Starting with 2.0 g (3.27 mmol) of Fmoc-Gln(Trt)-OH (**84**), the same procedure as described above for the preparation of amino alcohols **62a−i** was followed to give **85**. Yield 1.2 g (63%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.71 (m, 1H), 1.83 (m, 1H), 2.2−2.34 (m, 2H), 3.36−3.44 (m, 2H), 3.58 (m, 1H), 4.22 (t, J = 6.5 Hz, 1H), 4.43 (d, J = 6.5 Hz, 2H), 5.46 (d, J = 8.5 Hz, 1H), 7.1 (s, 1H), 7.24−7.32 (m, 17H), 7.4−7.42 (m, 2H), 7.61 (d, J = 7.5 Hz, 2H), 7.77 (d, J = 7.5 Hz, 2H). HRMS (M + H) calcd, 597.2753; found, 597.2763.

Synthesis of 4-Nitrophenyl [*N*-Triphenylmethyl-4-[[(9*H*-fluoren-9-ylmethoxy)carbonyl]amino]pentanamide-5-yl]carbonate (86). To a stirred solution of **85** (1.0 g, 1.67 mmol) and pyridine (0.27 mL, 3.34 mmol) in 20 mL of dry CH_2Cl_2 at 0 °C was added a solution of nitrophenyl chloroformate (0.37 g, 1.84 mmol) in 10 mL of dry

CH_2Cl_2 dropwise. The reaction was slowly warmed to room temperature and stirred for 4 h. It was then diluted with an additional 100 mL of CH_2Cl_2 and transferred to a separatory funnel. The organic layer was washed with water (20 mL) and brine (20 mL) and dried ($MgSO_4$). The solvent was removed, and the concentrated crude product was purified by silica gel chromatography (20% EtOAc−hexane, v/v). Yield 0.79 g (62%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.74 (m, 1H), 1.8 (m, 1H), 2.26 (m, 2H), 3.88 (m, 1H), 4.1−4.15 (m, 3H), 4.33 (m, 1H), 4.40 (m, 1H), 5.08 (d, J = 8.5 Hz, 1H), 6.7 (s, 1H), 7.1−7.22 (m, 19H), 7.25−7.31 (m, 2H), 7.47 (d, J = 7.5 Hz, 2H), 7.64 (d, J = 7.5 Hz, 2H), 8.11 (d, J = 9.0 Hz, 2H). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 26.6, 33.4, 47.4, 49.7, 66.6, 70.7, 96.2, 120.0, 120.1, 121.6, 121.8, 125.0, 125.3, 127.0, 127.2, 127.7, 127.9, 128.1, 128.6, 128.8, 141.4, 143.7, 143.8, 144.6, 145.4, 152.4, 155.4, 156.4, 171.1. HRMS (M + H) calcd, 762.2815; found, 762.3604.

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Supporting Information Available: Mass spectral and HPLC characterization of peptides **3**−**49**. This material is available free of charge via the Internet at http://pubs.acs.org.

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