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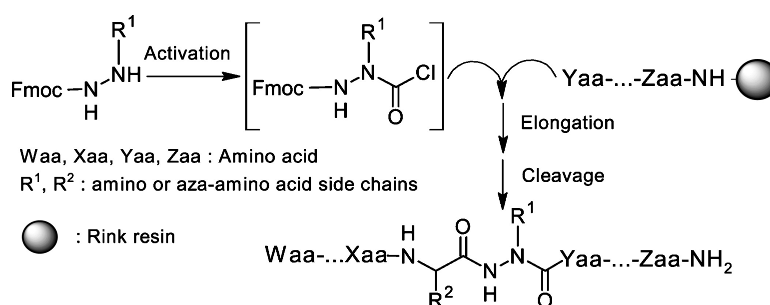

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Aza-Amino Acid Scanning of Secondary Structure Suited for Solid-Phase Peptide Synthesis with Fmoc Chemistry and Aza-Amino Acids with Heteroatomic Side Chains

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Aza-peptides, peptide analogues in which the α-carbon of one or more of the amino acid residues is replaced with a nitrogen atom, exhibit a propensity for adopting *β*-turn conformations. A general Fmoc-protection protocol for the stepwise solid-phase synthesis of aza-peptides has now been developed based on the activation of *N*′-alkyl fluoren-9-ylmethyl carbazates with phosgene for coupling the aza-amino acid residues. This method has proven effective for introducing aza-amino acid residues with aliphatic (Ala, Leu, Val, and Gly) and aromatic (Phe, Tyr, and Trp) side chains. Acid promoted loss of aromatic side chains was noted with aza-Trp and aza-Tyr residues during peptide cleavage and suppressed by temperature control in the case of the latter. In addition, aza-peptides with heteroatomic side chain residues (Lys, Orn, Arg, and Asp) were conveniently synthesized using this protocol. Partial aza-amino acid scans were performed on three biologically active peptides: the potent tetrapeptide melanocortin receptor agonist, Ac-His-D-Phe-Arg-Trp-NH_2; the growth hormone secretagogue hexapeptide, GHRP-6, His-D-Trp-Ala-Trp-D-Phe-Lys-NH_2; and the human calcitonin gene-related peptide (*h*CGRP) antagonist, FVPTDVGPFAF-NH_2. This practical procedure for aza-amino acid scanning using Fmoc-based solid-phase synthesis should find general utility for probing the existence and importance of *β*-turn conformations in bioactive peptides.

Introduction

Peptide mimics are desired to retain the biological effects of natural peptides and, at the same time, remove undesirable properties such as poor bioavailability, rapid metabolism, and nonselective receptor affinity. The design of such peptide mimics requires understanding of the backbone and the side chain structure and geometry of native peptides. Among tools for developing peptide mimics, aza-peptides,[1] formed by the replacement of one or more amino acids by an aza-amino acid in which C_α is replaced by nitrogen, have been demonstrated to induce turn conformations in peptides by spectroscopic,[2−6] crystallographic,[6−10] as well as computational[2,5,11−14] studies (Figure 1).

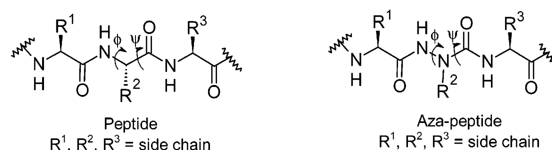

Figure 1. Peptide and aza-peptide.

A reduction of the flexibility of the parent linear peptide has been predicted to occur in aza-peptides due to replacement of the rotatable C_α−C(O) bond by a more rigid urea N_α−C(O) structure. Moreover, electronic repulsion of the lone pairs of the two adjacent nitrogens restricts motion about the dihedral angle ϕ.[15,16] Incorporation of aza-amino acids into peptides has been shown to favor type I, type II, and type VI *β*-turn geometry possessing the aza-residue at the $i + 1$ or $i + 2$ position in an intramolecularly hydrogen bonded *β*-turn conformation[2,5,11,14] contingent on the aza-residue structure and peptide sequence.

Numerous aza-analogues of biologically active peptides have been prepared, and some have exhibited improved pharmacological properties relative to their parent peptide. Increased duration of action, potency, and/or selectivity has been observed in aza-analogues of angiotensin II,[17] oxytocin,[18] eledoisin,[19] enkephalin,[20] lubilerin (LHRH),[21] thyrotropin-releasing hormone (TRH),[22] as well as somatostatin.[23] Aza-amino acids have also been employed as components of serine and cysteine protease inhibitors exhibiting competitive,[24,25] reversible,[26−38] and irreversible[39−41] mechanism-based inhibitory activity. In addition, aza-amino acids have also been employed in small molecule ligands of membrane bound protein receptors such as the G-protein coupled receptor[42,43] and integrin receptor families.[44]

A variety of methods have been devised to introduce aza-amino acids into peptide analogues of varying length. However, only two strategies have been reported for preparing aza-peptides on solid support (Figure 2). Attempts to synthesize an aza-peptide on solid support by conversion of the N-terminal amino group of a growing peptide into an isocyanate or active carbamate,[45,46] followed by reaction with a suitable *N*′-alkyl fluoren-9-ylmethyl carbazate afforded the desired aza-peptide contaminated with significant amounts of hydantoin from intramolecular nucleophilic attack on the

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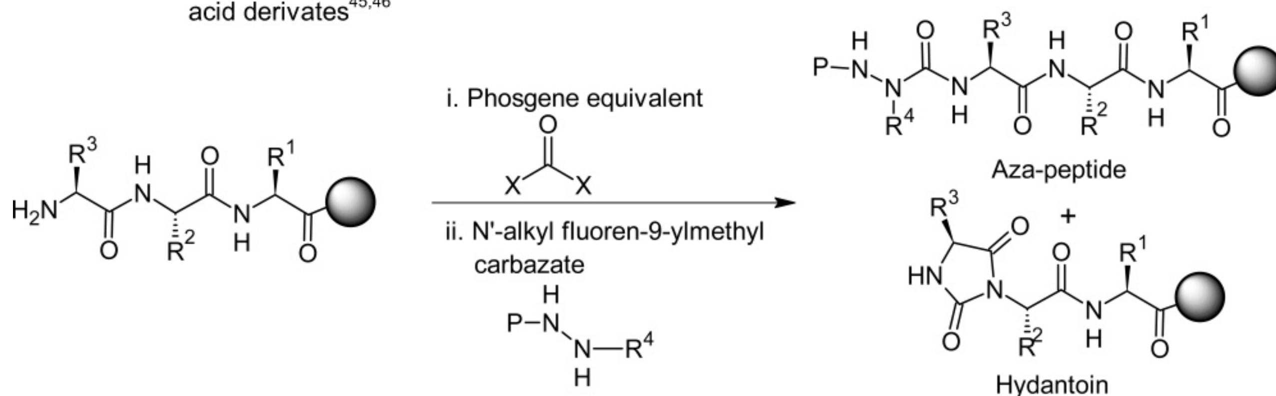
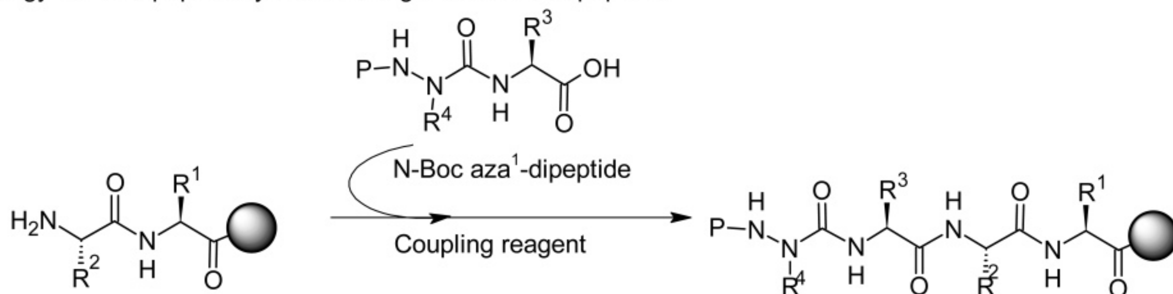




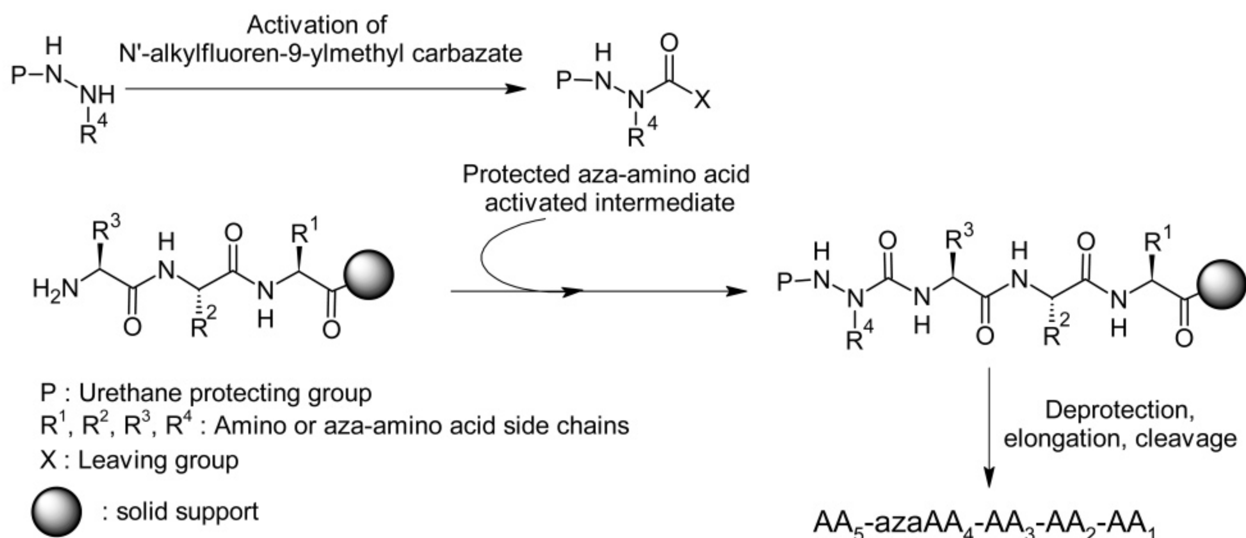
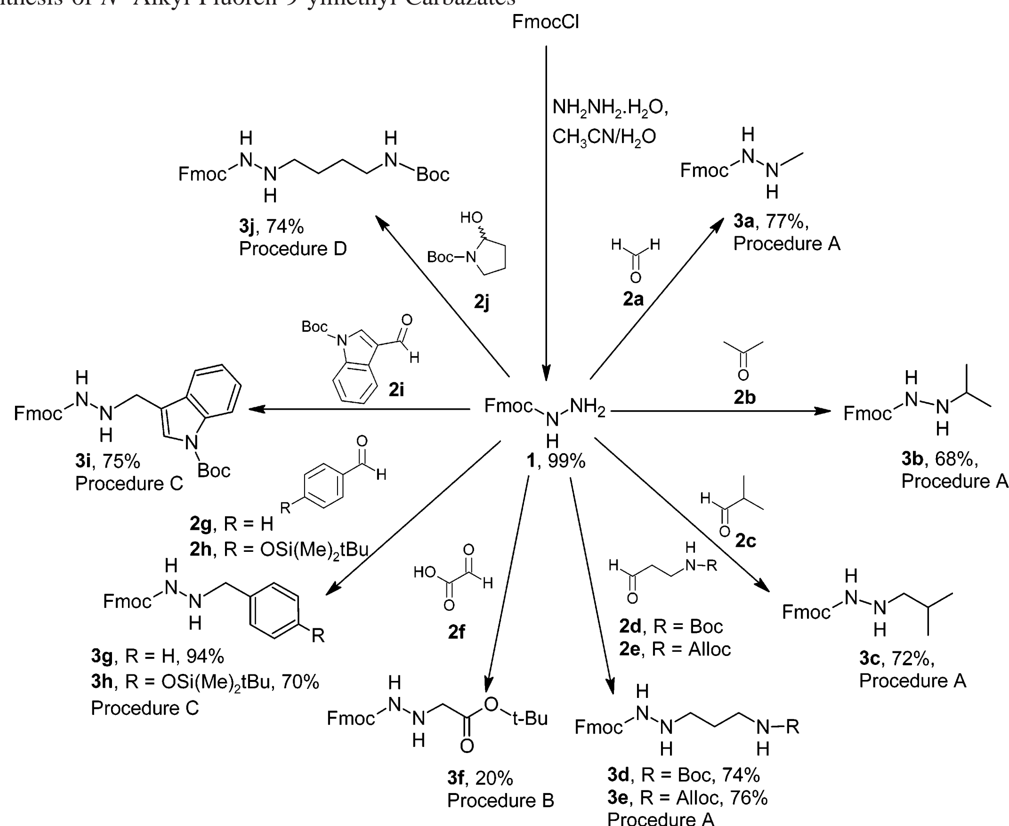


Figure 2. Solid-phase procedures for the introduction of aza-residues into peptide chains.

activated intermediate by the secondary nitrogen of the preceding C-terminal residue in the peptide chain (Strategy 1, Figure 2). This limitation, which resulted in low yields and difficult purifications of the aza-peptide products, could be prevented by blocking the intramolecular attack through protection of the preceding amide with a *N*-2-hydroxy-4-methoxybenzyl group, albeit with the inconvenience of two additional synthetic steps.[47] The second approach (Strategy 2, Figure 2) consisted of coupling aza-tri[48] or dipeptide[49,50] fragments that were prepared in solution to the N-terminus of a resin-bonded peptide. In particular, *N*-(Boc)aza^1-dipeptides have been shown to be configurationally stable building blocks for solution and solid-phase peptide synthesis and have been used successfully for aza-amino acid scanning.[50] Although employment of preformed aza-peptide building blocks in solid-supported synthesis circumvented hydantoin formation, their preparation has required multiple synthetic steps in solution. Moreover, this approach has been limited to Boc/Bzl solid-phase peptide synthesis.

A straightforward method for aza-peptide synthesis would involve the activation and reaction of N-protected aza-amino acid building blocks onto the growing chain of a resin-bound peptide (Strategy 3, Figure 2). Several reagents have been explored for preparing activated *N*-(Boc)aza-amino acid building blocks from *N′*-alkyl *t*-butyl carbazate, including *p*-nitrophenyl chloroformate,[51] bis(2,4-dinitrophenyl) carbon-

Scheme 1. Synthesis of *N*′-Alkyl Fluoren-9-ylmethyl Carbazates[a]



[a] Reagents and conditions: procedure A: (i and ii); procedure B: (i−iii); procedure C: (i and iv); procedure D: (v and ii). (i) Aldehyde or ketone **2a**−**i**, EtOH 78 °C, 2 h; (ii) $NaBH_3CN$, AcOH, THF, RT, 1 h; EtOH, 78 °C, 1 h; (iii) $CCl_3C(OtBu)NH$, DCM, RT, 24 h; (iv) H_2 $Pd(OH)_2$, 100 Psi, THF, 16 h; and (v) masked aldehyde **2j**, *p*-TsOH, EtOH 78 °C, 2h.

ate, carbonyldiimidazole,[38] pentafluorophenyl carbonate,[20a,43,52] and triphosgene.[25,49] The resulting nitrophenylcarbazates and imidazolides were not very reactive and required long coupling times and high temperatures to provide aza-peptides in poor yield contaminated with numerous side products.[1b] Pentafluorophenylcarbazate analogues of *N*-(Boc)-aza-amino acids have been shown to be more efficient in solution-phase synthesis of aza-peptides; [43,52] however, they failed to react with hydrazine derivates to form azatides.[52] On the other hand, *N*-(Boc) or *N*-(Cbz)-aza-amino acid chloride analogues possessing Ala and protected Asp side chains have been generated in situ from their corresponding *N*′-alkyl *t*-butyl and benzyl carbazates using triphosgene and employed successfully in the synthesis of immunogenic aza-peptides in solution.[49] Attempts failed, however, to couple a secondary amine to the activated aza-amino acid building block by treatment of *N*′-3-phenylpropyl *t*-butylcarbazate with triphosgene; instead, polymerization of the reactants was reported.[43] Finally, a stepwise synthesis of aza-peptide on solid support in this manner has, to the best of our knowledge, been investigated once. Activated *N*-Fmoc-aza-amino acid building blocks used successfully for aza-Gly and aza-Ala by employing fluorenylmethyl carbazate and *N*′-methyl fluorenylmethylcarbazate and phosgene.[42,44] Considering that aza-amino scanning would constitute an effective means for identifying the importance of turn structure for activity in native peptides,[50] a more general and efficient method was pursued for the solid-phase preparation of aza-peptides. In light of the reported precedents and the convenience of Fmoc-based solid-phase synthesis, we decided to further explore the application of *N*-Fmoc-aza-amino acid chlorides as building blocks for aza-peptide synthesis.

A variety of N′-substituted fluorenylmethyl carbazates were synthesized, activated with phosgene, and employed in coupling reactions on Rink resin to prepare aza-peptides with aliphatic, aromatic, and heteroatomic side chains. Loss of the aromatic side chains of aza-Trp and aza-Tyr residues was detected during treatments with TFA and suppressed by temperature control. A practical process for aza-peptide synthesis has been developed that should greatly enhance the application of aza-peptides in peptide science and medicinal chemistry.

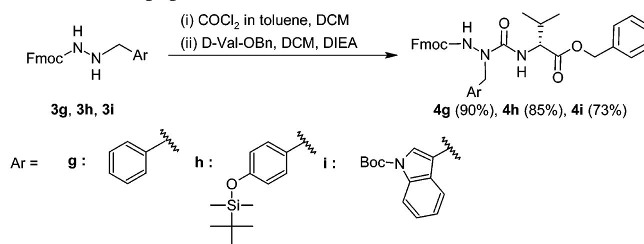
Results and Discussion

Solution-Phase Synthesis of *N*′-Alkyl Fluoren-9-ylmethyl Carbazates in Solution. The assembly of aza-peptides on solid support using Fmoc-aza-amino acid building blocks requires the activation of the respective *N*′-alkyl fluoren-9-ylmethyl carbazate precursors. Although *N*-Boc protected hydrazine derivatives have been prepared for making aza-analogues of most of the proteinogenic amino acids,[53,54] to the best of our knowledge, only fluoren-9-ylmethyl carbazate **1** and its *N*′-methyl analogue **3a** were reported in the literature at the start of our investigation.[44,55] The precursor for aza-Gly, fluoren-9-ylmethyl carbazate **1**, was prepared by acylation of excess hydrazine with fluoren-9-ylmethyl chloroformate.[55] Fluoren-9-ylmethyl carbazate **1** was subsequently used to synthesize **3a** and nine new *N*′-alkyl fluoren-9-ylmethyl carbazates **3b**−**j** (Scheme 1). One

general pathway to the desired hydrazines involved condensations of protected hydrazine **1** with an appropriate aldehyde or ketone **2a−j** in ethanol at reflux to give an acyl hydrazone intermediate that was reduced by catalytic hydrogenation and hydride addition, respectively, for aromatic **3g−i** and aliphatic **3a−f** and **3j** substituents. After reduction with cyanoborohydride, decomplexation of the amino borane intermediate was accomplished by heating in ethanol at reflux. β-Amino aldehydes **2d** and **2e** for the synthesis of aza-Orn analogues were obtained from reduction of their corresponding β-alanine morpholinamide with $LiAlH_4$. The protected hydrazine for the synthesis of aza-Lys was made by condensation of fluoren-9-ylmethyl carbazate **1** with *N-t*-butyloxycarbonyl 2-hydroxypyrrolidine **2j** in the presence of *p*-toluenesulfonic acid (*p*-TsOH). Aminol **2j** was obtained by the reduction of *N-t*-butyloxycarbonylpyrrolidinone with diisobutylaluminum hydride (DIBAL).[56] Introduction of the aspartic acid side chain was achieved by the reduction of the condensation product from glyoxylic acid and fluoren-9-ylmethyl carbazate **1**. A 5-fold excess of cyanoborohydride was required to ensure complete and clean reduction of the acylhydrazone to the substitued carbazate as monitored by RP-HPLC. The resulting acid was then protected as *t*-butyl ester **3f** in low yield using *t*-butyl trichloroacetimidate[57] in DCM at room temperature such that the overall yield from **1** was 20%. (The isobutylene[58,59] method for introducing *t*-butyl groups was uneffective in our hands.) The other substituted carbazates were obtained in 68−94% yield. This condensation/reduction reaction sequence allowed us to synthesize conveniently protected hydrazines for the preparation of aza-amino acids possessing aliphatic (Ala, **3a**; Val, **3b**; Leu, **3c**), aromatic (Phe, **3g**; Trp, **3i**; Tyr, **3h**), acid (Asp, **3f**), and basic (Orn, **3d**; Lys, **3j**) amino acid side chains. Furthermore, the synthesis of an ornithine carbazate precursor **3e** containing orthogonal ω-amine Alloc protection allowed the introduction of aza-arginine into peptide sequences.

Solution and Solid-Phase Synthesis of Aza-Peptides using Activated *N*-Fmoc-Aza-amino Acid Building Blocks. With *N*′-alkyl carbazates **3a−j** in hand, we examined next their incorporation into peptides as activated aza-amino acids. Carbonyl activation reagents that have been successful for forming aza-peptide bonds in solution include carbonyldiimidazole,[38] bis(pentafluorophenyl)carbonate,[25,43,52] bis-(trichloromethyl)carbonate (BTC),[25,30,49] *p*-nitrophenylchloroformate,[43,51] as well as phosgene[44] in toluene solution. In our hands, activation of the *N*′-alkyl carbazate was achieved conveniently by treating carbazates **3a−j** in DCM with a 2-fold excess of a phosgene solution in toluene at room temperature. Using these conditions, *N*′-alkyl fluoren-9-ylmethyl carbazates **3a−j**, all could be cleanly converted to their corresponding activated Fmoc-aza-amino acid building blocks. After 15 min, the starting carbazate was no longer observed by TLC, and the phosgene excess was removed with the other volatiles under reduced pressure to yield the activated building block that was employed without further purification. Activated Fmoc-aza-amino acids from **3g**, **3h**, and **3i** possessing aromatic side chains were first tested in solution (Scheme 2). These activated building blocks were allowed to react with D-valine benzyl ester (Val-Obn) in

Scheme 2. Solution Phase Synthesis of Fmoc-Aza-dipeptides



DCM with DIEA as base to produce Fmoc-aza-dipeptides **4g**, **4h**, and **4i** in 90, 85, and 73% yields, respectively (Scheme 2).

Encouraged by these results, we next employed Fmoc-aza-amino acid building blocks in solid-phase aza-peptide synthesis (Figure 3). Acid-labile Rink[60] resin was used in standard Fmoc/*t*Bu protocols[61] except in the case of aza-amino acid residues. Coupling reactions with Fmoc amino acids were performed with 300 mol % of the appropriate amino acid, HBTU, and DIEA in DMF for 45 min. The Fmoc group was removed using an 80:20 (v/v) DMF/piperidine solution for two 15 min periods. Treatment of the free amine of the growing resin-bound peptide with a 3-fold excess of the activated aza-amino acid building block for 6 h in DCM in the presence of DIEA afforded the desired aza-peptide. In certain cases, the aza-amino acid coupling was repeated to ensure complete conversion as monitored by the Kaiser ninhydrin[62] and TNBS tests.[63] The Fmoc protecting group of the aza-residue was then removed by treating the resin with a DMF/piperidine solution (80:20, v/v), and the resulting free aza-amino acid residue was acylated by the next Fmoc-amino acid. The aza-amino acid residue was found to be much less reactive than a typical amino acid residue[48] and difficult to acylate. For example, acylation was not observed using HBTU as a coupling reagent. Acylation was achieved using *N*-Fmoc-amino acid chlorides. Treatment of the resin in THF with a 3-fold excess of Fmoc-amino acid chloride, generated in situ with bis-(trichloromethyl)-carbonate (BTC)[64] and 2,4,6-collidine, usually gave clean and complete conversion to the desired supported aza-peptide. Of particular note, couplings on aza-amino acid residues could not be monitored by the Kaiser ninhydrin test; instead, conversion was monitored by the cleavage of resin aliquots with TFA followed by LC/MS analysis of the crude product. Peptide synthesis was then continued as described previously. Final cleavage of the aza-peptide from the support and deprotection of side chain protecting groups were performed by treating the resin with a freshly made TFA/TES/H_2O (95:2.5:2.5, v/v/v) solution. Aza-peptides were isolated by precipitation with Et_2O, dissolved in a 1:1 acetonitrile/H_2O solution, and lyophilized to white foams that were directly analyzed by RP-HPLC to assess purity (Table 1).

Synthesis of Aza-Analogues of Biologically Active Peptides. The general strategy described previously and depicted in Figure 3 was used to synthesize aza-analogues of three different biologically active peptides: melanocortin receptor (MCR) ligand **5**, growth hormone secretagogue **10**,

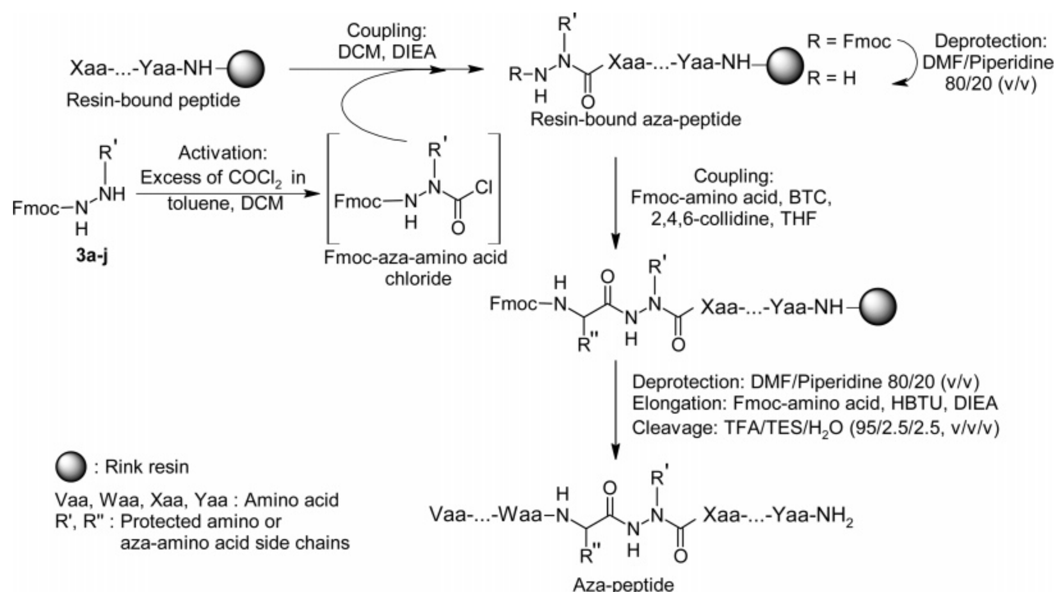


Figure 3. General protocol used for synthesis of aza-peptides.

Table 1. Yields and Purities of Aza-Peptides and Corresponding Parent Peptides **5**, **10**, **16**, and **20**

compound	t_R (min)[a]	HPLC purity at 214 nm	yield %
(**5**) Ac-His-D-Phe-Arg-Trp-NH_2	13.31	84[b]	95[c]
(**6**) Ac-(D/L)-His-azaPhe-Arg-Trp-NH_2	13.36	56[b]	70[c]
(**7**) Ac-His-D-Phe-azaLys-Trp-NH_2	13.15	74[b]	91[c]
(**8**) Ac-His-D-Phe-azaOrn-Trp-NH_2	13.28	66[b]	75[c]
(**9**) Ac-His-D-Phe-azaArg-Trp-NH_2	13.63	72[b]	75[c]
(**10**) His-D-Trp-Ala-Trp-D-Phe-Lys-NH_2	15.73	93[b]	76[c]
(**11**) His-D-Trp-azaAla-Trp-D-Phe-Lys-NH_2	15.84	83[b]	80[c]
(**12**) His-D-Trp-Ala-Trp-azaPhe-Lys-NH_2	16.69	>99[d]	15[e]
(**13**) His-D-Trp-Ala-Trp-D-Phe-azaLys-NH_2	15.68	90[b]	81[c]
(**14**) (D/L)-His-azaTyr-Ala-Trp-D-Phe-Lys-NH_2	14.93; 15.01	83[b]	72[c]
(**15**) His-D-Trp-Ala-azaTyr-D-Phe-Lys-NH_2	8.69	82[b]	55[c]
(**16**) [D^{31},P^{34},F^{35}]$CGRP_{29-37}$,PTDVGPFAF-NH_2	16.32	93[b]	67[c]
(**17**) [D^{31},P^{34},F^{35},azaA^{36}]$CGRP_{29-37}$,PTDVGPFazaAF-NH_2	16.27	96[b]	66[c]
(**18**) [D^{31},azaG^{33},P^{34},F^{35}]$CGRP_{29-37}$, PTDVazaGPFAF-NH_2	15.76	90[b]	70[c]
(**19**) [azaD^{31},P^{34},F^{35}]$CGRP_{29-37}$, PTazaDVGPFAF-NH_2	14.16	86[b]	57[c]
(**20**) [D^{31},P^{34},F^{35}]$CGRP_{27-37}$,FVPTDVGPFAF-NH_2	17.63	95[b]	68[c]
(**21**) [D^{31},P^{34},F^{35},azaA^{36}]$CGRP_{27-37}$, FVPTDVGPFazaAF-NH_2	17.52	95[b]	60[c]
(**22**) [D^{31},azaG^{33},P^{34},F^{35}]$CGRP_{27-37}$, FVPTDVazaGPFAF-NH_2	17.09	86[b]	60[c]
(**23**) [azaD^{31},P^{34},F^{35}]$CGRP_{27-37}$, FVPTazaDVGPFAF-NH_2	16.29	86[b]	57[c]

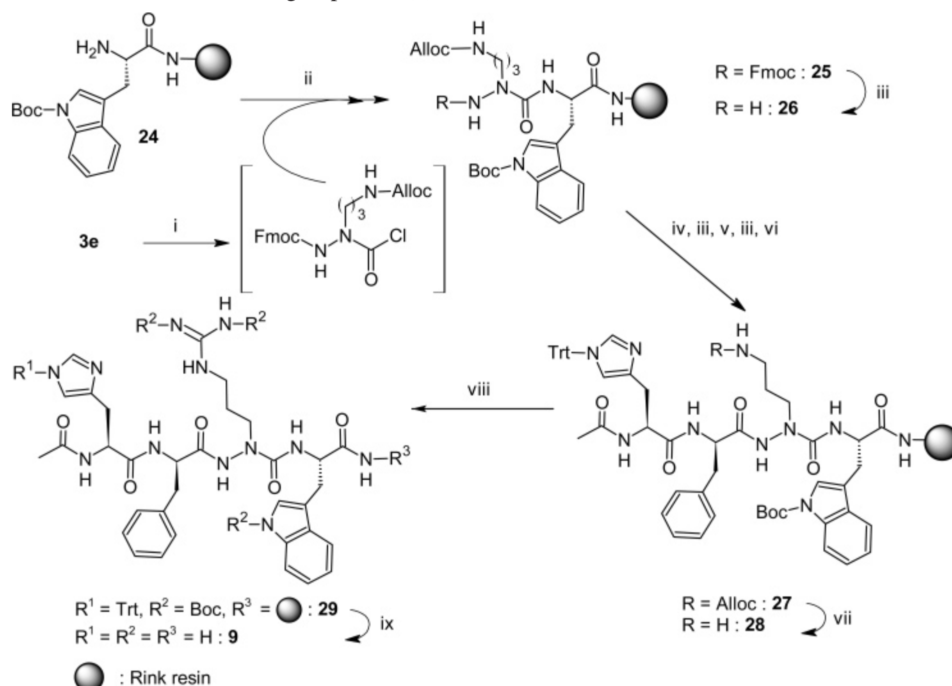
[a] Analytical HPLC analyses were performed on a TARGA column from Higgins Analytical, Inc. (4.6 × 250 mm, 5 μm, C_{18}) with a flow rate of 1.5 mL/min using a 40 min linear gradient from water (0.1% TFA) to CH_3CN (0.1% TFA). [b] HPLC purity at 214 nm of the crude peptide. [c] Crude yield according to manufacturer's reported loading of the rink resin. [d] HPLC purity at 214 nm of the purified peptide. [e] Yields after purification by RP-HPLC are based on manufacturer's reported loading for Rink resin.

and calcitonin gene-related peptide (CGRP) antagonists **16** and **20** (Table 1).

Investigating the synthesis of aza-peptides having basic amino acid residues, Ac-His-D-Phe-Arg-Trp-NH_2 (**5**) was scanned because of its potent melanocortin receptor (MCR) agonist activity.[65] Five melanocortin receptor subtypes have been characterized as seven transmembrane G-protein coupled receptors (GPCRs) known to be involved in feeding behavior, obesity, metabolism, energy homeostasis (MC3R and MCR4R), as well as in skin pigmentation and animal coat pigmentation (MC1R). Aza-amino acid scanning of the central residues of peptide **5** was performed to study the importance of turn geometry for receptor affinity and selectivity. Four aza-peptide analogues were synthesized possessing aza-Phe[3] (**6**), aza-Lys[4] (**7**), aza-Orn[4] (**8**), and aza-Arg[4] (**9**) residues, respectively. Crude purities ranged from 56 to 72% as assessed by RP-HPLC analysis at 214 nm. For comparison, the native peptide **5** was obtained with a purity of 84%. As in the case of Fmoc-amino acid chlorides of Orn and Lys bearing ω-amines protected as carbamates, activation of aza-Orn and aza-Lys by treatment of **3d**, **3e**, or **3j** with phosgene caused no intramolecular cyclization leading to cyclic urea side products during couplings to the resin-bound Trp residue **24**. The aza-Arg residue was assembled on resin after peptide synthesis by removal of the Alloc protection using tetrakis(triphenylphosphine)palladium ($Pd(PPh_3)_4$) and an excess of dimethyl barbituric acid as a scavenger in THF[66,67] followed by guanylation using *N*,*N'*-bis-Boc-1-guanylpyrazole[68,69] in DMF with DIEA (Scheme 3).

Aza-amino acid scanning was next employed to study a member of the growth hormone releasing peptide family, namely, GHRP-6 (His-D-Trp-Ala-Trp-D-Phe-Lys-NH_2, **10**). This hexapeptide[70] acts at the growth hormone secretagogue

Scheme 3. Synthesis of Ac-His-D-Phe-azaArg-Trp-NH_2 (**9**)[a]



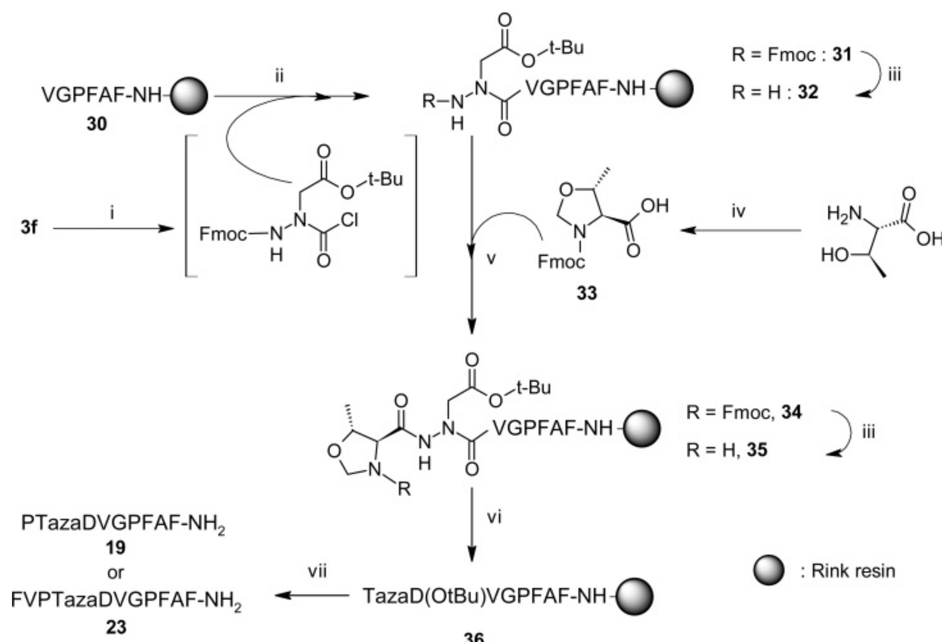
[a] Reagents and conditions: (i) excess of $COCl_2$ in toluene, DCM; (ii) DCM, DIEA; (iii) DMF/piperidine 80:20 (v/v); (iv) Fmoc-D-Phe-OH, BTC, 2,4,6-collidine, THF; (v) Fmoc-His(Trt)-OH, HBTU, DIEA; (vi) Ac_2O, DCM, Py; (vii) $Pd(PPh_3)_4$, THF, dimethylbarbituric acid; (viii) *N*,*N*′-bis-Boc-1-guanylpyrazole, DMF, DIEA, 2×; and (ix) TFA/TES/H_2O 95:2.5:2.5 (v/v/v).

receptor-1a (GHSR-1a, another GPCR) and stimulates the release of growth hormones from the pituitary.[71] Employing carbazates **3a**, **3g**, and **3j**, aza-analogues of GHRP-6 possessing aza-Ala[3] **(11)**, aza-Phe[5] **(12)**, and aza-Lys[6] **(13)** residues, respectively, were successfully synthesized with crude purities ranging from 82 to 90% as ascertained by LC/MS analysis of the crude cleavage products (Table 1). Attempts to synthesize aza-Trp[4] analogues of GHRP-6 were not successful using the procedure described previously, and issues concerning synthesis of aza-Trp containing peptides will be addressed next.

As a third example, we continued our aza-amino acid scan of [D[31],P[34],F[35]]$CGRP_{29-37}$ **16** and [D[31],P[34],F[35]]$CGRP_{27-37}$ **20**, antagonists of the potent vasodilator human calcitonin gene-related peptide (*h*CGRP), a 37-amino acid neuropeptide (ACDTATCVTHRLAGLLSRSGGVVKNNFVPTNVGSK-AF-NH_2) that exhibits activity at a series of GPCRs.[72,73] The syntheses of aza-Phe[27], aza-Pro[34], and aza-Phe[35] peptide analogues of these antagonists were previously reported by our group[50] using *N*-(Boc)-aza[1]-dipeptide building blocks. The aza-amino acid scan of both peptides has now been continued with the synthesis of aza-Ala[36] (**17** and **21**), aza-Gly[33] (**18** and **22**), and aza-Asp[31] (**19** and **23**) analogues. Aza-Ala residues had been introduced into peptides by using an Fmoc-aza-amino acid chloride in a similar way as described in this paper. Aza-Gly was previously introduced into peptides by way of a 1,3,4-oxadiazol-2(*3H*)-one.[44] Syntheses of [azaD[31],P[34],F[35]]$CGRP_{29-37}$ **19** and [azaD[31],P[34],F[35]]$CGRP_{27-37}$ **23** were performed to demonstrate tolerance of a side chain carboxylate (Scheme 4). The aza-Asp residue was also readily introduced using our general protocol; however, acylation of the aza-amino acid residue with threonine proved to be more troublesome than in the parent Asp residue. Attempts failed to acylate the aza-Asp(OtBu) residue of the resin bound peptide **32** using Fmoc-Thr(OtBu)-OH and a number of activation methods and reagents, such as BTC,[64] HATU,[74] PyBrop,[75] BOPCl,[76] and DIC[77] (symmetric anhydride method). For comparison, acylation of the corresponding Asp(OtBu) residue by Fmoc-Thr(O*t*Bu)-OH was effectively accomplished with HBTU as a coupling reagent. Because traces of the expected acylated product were sometimes observed by LC/MS analysis, we considered that steric hindrance was preventing activated Fmoc-Thr(O*t*Bu)-OH from acylating the less reactive resin-bound aza-peptide **32** and decided to protect the hydroxyl group in oxazolidine **33**. Fmoc protected oxazolidine **33** was synthesized from L-threonine on treatment with an aqueous formaldehyde solution in the presence of sodium carbonate followed by a solution of FmocCl in acetonitrile.[78] Using the BTC protocol, we were then able to couple oxazolidine carboxylate **33** onto resin **32**. After removal of the Fmoc protecting group, the oxazolidine ring was hydrolyzed by treating the resin **34** with a THF/H_2O (80:20, v/ v) solution in the presence of *p*-TsOH and yielded unprotected resin-bound threonine **35**. Aza-peptide sequences were completed using the previously described Fmoc/*t*Bu coupling and deprotection procedures (Scheme 5). Aza-peptides **19** and **23** were both obtained with 86% HPLC purity as measured at 214 nm (Table 1).

Tryptophan and Tyrosine Containing Aza-Peptides. Certain aza-amino acids were expected to be unstable, such as those related to Ser, Thr, and Cys, because of their aminal-like structure (Figure 4). On the other hand, the stability of aza-amino acid residues possessing aromatic residues was less obvious and thus merited further study. Aza-Phe had previously been successfully introduced into aza-peptides by our laboratory[50] using Boc/Bzl chemistry, demonstrating that the benzyl side chain was relatively stable to acid. The *p*-hydroxy group in aza-Tyr and the indole group in aza-

Scheme 4. Synthesis of [AzaD,[31]P,[34]F[35]]CGRP$_{29-37}$ and [AzaD,[31]P,[34]F[35]]CGRP$_{27-37}$[a]



[a] Reagents and conditions: (i) excess of $COCl_2$ in toluene, DCM; (ii) DCM, DIEA, ×2; (iii) DMF/piperidine 80:20 (v/v); (iv) $(CHO)_n$ aq., Na_2CO_3, then FmocCl, ACN; (v) BTC, 2,4,6-collidine, THF; (vi) THF/H_2O 80:20 (v/v), *p*-TsOH, ×2; (vii) deprotection: DMF/piperidine 80:20 (v/v); elongation: HBTU, DIEA, *N*-(Fmoc) amino acid; cleavage: TFA/TES/H_2O 95:2.5:2.5 (v/v/v).

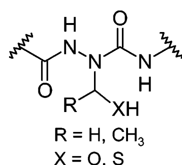


Figure 4. Structure of unstable aza-amino acids.

Trp were, however, expected to facilitate loss of the aromatic side chain under acidic conditions due to their potential to better stabilize their respective carbocation.

As mentioned previously, attempts to synthesize the aza-Trp[4] analogue of GHRP-6 failed using our general solid-phase synthesis protocol; instead, a complex mixture of unidentified products was observed by analysis of the crude cleavage products using LC/MS. Activated Fmoc-aza-tryptophan had been previously used in solution to obtain aza-dipeptide **4i.** Moreover, the coupling and elongation steps in the synthesis of the aza-Trp-peptide appeared normal by the Kaiser ninhydrin test and by the strong UV absorption at 254 nm of the dibenzofulvene-piperidine adduct released in the deprotection step. The acidic conditions employed during cleavage of the aza-Trp peptide from the solid support were suspected to cause loss of the indole stabilized methyl carbocation. To study this hypothesis, aza-Trp-dipeptide **4i** was treated with a TFA/TES/H_2O (95:2.5:2.5, v/v/v) solution at 0 °C. Analysis of the reaction mixture after 30 min by LC/MS revealed the formation of three major new compounds. Two of the new products had the expected mass for the desired aza-Trp peptides without the Boc group. The third new product exhibited a mass corresponding to loss of both the indole methylene and the Boc group. Loss and intramolecular migration of the indole methylene during treatment with TFA may be explained by formation of a indole methyl carbocation as in gramine-like chemistry.[79]

A similar experiment was performed on aza-Tyr-dipeptide **4h** to examine the stability of the aza-tyrosine moiety under acidic conditions. In contrast to aza-Trp dipeptide **4i**, deprotection of **4h** with the TFA/TES/H_2O conditions at 0 °C cleanly provided the expected product. This result in hand, two additional aza-peptides, aza-Tyr[2] (**14**) and aza-Tyr[4]-GHRP-6 (**15**)**,** were synthesized to demonstrate the feasibility of introducing aza-Tyr residues into peptides using our general solid-phase synthesis approach. Synthesis of these aza-peptides was conducted in a similar way as described earlier with the exception of the cleavage protocol that was performed at 0 °C using a freshly made TFA/TES/H_2O (95:2.5:2.5) solution. At room temperature, cleavage produced an aza-Tyr peptide product that by LC/MS analysis was found to be contaminated with 2−10% of the corresponding aza-Gly peptide from loss of the *p*-hydroxybenzyl group. Using the 0 °C conditions for cleavage and side chain deprotection, aza-Tyr[2] and aza-Tyr[4]-GHRP-6 analogues **14** and **15** were obtained with 83 and 82% HPLC purity, respectively, without traces of products from loss of the *p*-hydroxybenzyl group.

Conclusion

Activated Fmoc-aza-amino acids with aliphatic (Ala, Val, Leu), aromatic (Phe, Tyr, Trp), and heteroatomic side chains (Orn, Lys, Asp) were generated from their corresponding *N*′-alkyl fluoren-9-ylmethyl carbazates using a solution of phosgene in toluene and demonstrated to be valuable building blocks for the assembly of aza-peptides in solution and on solid phase. The utility of the methodology was illustrated by the solid-phase synthesis of aza-peptide analogues of a MCR agonist, a GHS-R secretagogue and a *h*CGRP antagonist, which are currently being evaluated for biological activity. Considering the effectiveness of this approach for constructing libraries of aza-peptides to scan for the impor-

tance of backbone structure and geometry of biologically active peptides, this method should be of general use for exploring peptide biology and creating peptide mimics.

Experimental Procedures

General Methods. Rink resin (0.65 mmol/g) was purchased from Advanced Chemtech Inc., and the manufacturer's reported loading of the resin was used in the calculation of the yields of the final products. The 20% solution of phosgene in toluene was purchased from Fluka. Melting points were uncorrected. ^{1}H and ^{13}C NMR spectra were recorded, respectively, at 400 and 100 MHz in $CDCl_3$ or DMSO as the solvent and internal reference. Thin-layer chromatography was performed on silica gel 60 F_{254} plates from Merck. Flash chromatography[80] was performed on silica gel 60 (230−400 Mesh ASTM) from Merck. Analytical HPLC analyses were performed on a TARGA column from Higgins Analytical, Inc. (4.6 mm × 250 mm, 5 μm, C_{18}) with a flow rate of 1.5 mL/min using a 40 min linear gradient from water (0.1% TFA) to CH_3CN (0.1% TFA). Retention times (t_R) from analytical RP-HPLC are reported in minutes. Peptides **5**−**9** were purified using semipreparative LC/MS (Previal C18 column, 22 mm^2 × 250 mm^2, particle size 5 μm) with solvent A, H_2O (0.1% TFA), and solvent B, acetonitrile (0.1% TFA) using a gradient of 20−40% of A over 20 min at a flow rate of 15 mL/min.

9-*H*-Fluoren-9-ylmethyl Carbazate (1). To a well-stirred solution of hydrazine hydrate (19 g, 386 mmol) in 150 mL of CH_3CN/H_2O (1:1, v/v), a solution of FmocCl (10 g, 38.65 mmol) in 600 mL of CH_3CN was added dropwise at 0 °C over 2 h. The reaction mixture was then allowed to warm to room temperature and stirred for an additional 12 h, concentrated in vacuo to 150 mL, and filtered to yield the title compound as a white solid, which was washed with water and hexane and dried to a constant weight in vacuo (9.74 g, 99%): mp 172−173 °C (lit,[55] mp = 170 °C); ^{1}H NMR (DMSO) δ 4.08 (brs, 2H), 4.21 (t, J = 7.2 Hz, 1H), 4.28 (d, J = 7.2 Hz, 2H), 7.32 (t, J = 7.4 Hz, 2H), 7.42 (t, J = 7.4 Hz, 2H), 7.69 (d, J = 7.4 Hz, 2H), 7.89 (d, J = 7.4 Hz, 2H), 8.36 (brs, 1H); ^{13}C NMR (DMSO) δ 47.7, 66.7, 121.1 (2C), 126.3 (2C), 128.1 (2C), 128.7 (2C), 141.7 (2C), 144.9 (2C), 159.2.

3-(*t*-Butyloxycarbonylamino)propanal (2d). A stirred suspension of β-alanine (2 g, 22.4 mmol) in a dioxane/water solution (1:1 v/v, 120 mL) was treated with $NaHCO_3$ (3.77 g, 44.9 mmol, 2 equiv), stirred for 30 min, treated with Boc_2O (4.89 g, 22.4 mmol, 1 equiv), and stirred at room temperature for 3 days. The mixture was acidified with an aqueous $KHSO_4$ solution (1 M). The resulting solution was extracted twice with EtOAc. The combined organic layers were washed with brine, dried over Na_2SO_4, and concentrated in vacuo to yield Boc-β-Ala-OH (3.84 g, 21 mmol, 93%), which was used in the next reaction without further purification. To a stirred solution of Boc-β-Ala-OH (3.83 g, 20.2 mmol) and NMM (4.45 mL, 40.5 mmol, 2 equiv) in THF (60 mL) at 0 °C, isobutylchloroformate (2.75 mL, 21.3 mmol, 1.05 equiv) was added. After 15 min, the mixture was filtered, and the filtrate was treated at 0 °C under agitation with morpholine (1.77 mL, 20.2 mmol, 1.0 equiv). The reaction mixture was stirred at 0 °C for 15 min and for an additional 1 h at room temperature and was then concentrated to yield a residue that was dissolved in EtOAc. The organic layer was washed successively with an aqueous $KHSO_4$ solution (1 M), a saturated aqueous $NaHCO_3$ solution, and brine, dried over Na_2SO_4, and concentrated in vacuo. *N*-Boc-β-Alaninylmorpholinamide (4.30 g, 1.66 mmol, 82%) was isolated as a white solid after flash chromatography using 80% EtOAc in hexane as eluant. R_f = 0.25 (80% EtOAc in hexane); mp 63−64 °C; ^{1}H NMR ($CDCl_3$) δ 1.41 (s, 9H), 2.49 (t, J = 5.9 Hz, 2H), 3.38−3.67 (m, 10H), 5.28 (bs, 1H). ^{13}C NMR ($CDCl_3$) δ 28.8 (3C), 33.6, 36.7, 42.2, 46.1, 66.9, 67.2, 79.6, 156.4, 170.6. LRMS (EI) 259.0 $(M + H)^+$, 281.1 $(M + Na)^+$. *N*-Boc-β-Alaninylmorpholinamide (1.5 g, 5.81 mmol) in THF (29 mL) was cooled to 0 °C, treated with $LiAlH_4$ (231.4 mg, 6.1 mmol, 1.05 equiv), stirred for 1 h, treated with an aqueous $KHSO_4$ solution, and extracted twice with EtOAc. The combined organic layers were washed with an aqueous $KHSO_4$ solution (1 M) and brine, dried over Na_2SO_4, and concentrated in vacuo. Aldehyde **2d** (409 mg, 2.36 mmol, 41%) was isolated as a colorless oil by flash chromatography using 20% EtOAc in hexane as eluant. R_f =0.41 (30% EtOAc in hexane); ^{1}H NMR ($CDCl_3$) δ 1.43 (s, 9H), 2.71 (t, J = 5.9 Hz, 2H), 3.42 (q, J = 5.9 Hz, 2H), 4.90 (bs, 1H), 9.81 (s, 1H).

3-(Allyloxycarbonylamino)propanal (2e). A suspension of β-alanine (4 g, 44.9 mmol) in a dioxane/water solution (1:1 v/v, 120 mL) was stirred at room temperature, treated with $NaHCO_3$ (7.54 g, 89.8 mmol, 2 equiv) followed 30 min later with AllocCl (4.82 g, 40 mmol), stirred at room temperature for 3 days, acidified with an aqueous $KHSO_4$ solution (1 M), and extracted twice with EtOAc. The combined organic layers were washed with brine, dried over Na_2SO_4, and concentrated in vacuo to yield Alloc-β-Ala-OH (6.28 g, 36.3 mmol, 91%), which was used in the next reaction without further purification. A stirred solution of Alloc-β-Ala-OH (6.28 g, 36.3 mmol) and NMM (7.97 mL, 72.5 mmol, 2 equiv) in THF (120 mL) at 0 °C was treated with isobutylchloroformate (4.94 mL, 38.1 mmol, 1.05 equiv), stirred for 15 min, and filtered. The filtrate was agitated at 0 °C, treated with morpholine (3.48 mL, 40 mmol, 1.10 equiv) at 0 °C for 15 min and for an additional 1 h at room temperature, and concentrated to yield a residue, which was dissolved in EtOAc and washed successively with an aqueous $KHSO_4$ solution (1 M), a saturated aqueous $NaHCO_3$ solution, and brine, dried over Na_2SO_4, and concentrated in vacuo. *N*-Alloc-β-Alaninylmorpholinamide (5.02 g, 20.72 mmol, 57%) was isolated by flash chromatography using 70% EtOAc in hexane as a white solid: R_f =0.17 (70% EtOAc in hexane); mp 72−73 °C; ^{1}H NMR ($CDCl_3$) δ 2.52 (t, J = 6.0 Hz, 2H), 3.43 (t, J = 4.8 Hz, 2H), 3.49 (t, J = 4.8 Hz, 2H), 3.60 (m, 2H), 3.63−3.71 (m, 4H), 4.54 (d, J = 5.6 Hz, 2H), 5.19 (dd, J = 10.4 Hz, J = 1.2 Hz, 1H), 5.29 (dd, J = 17.2 Hz, J = 1.2 Hz, 1H), 5.56 (bs, 1H), 5.90 (m, 1H); ^{13}C NMR ($CDCl_3$) δ 33.5, 37.0, 42.2, 46.1, 66.8, 66.9, 67.2, 117.9, 133.3, 156.7, 170.4. LRMS (EI) 243.0 $(M + H)^+$. A stirred solution of *N*-Alloc-β-alaninylmorpholinamide (2.0 g, 8.25 mmol) in THF (20 mL) and Et_2O (60 mL) was treated at −10 °C with $LiAlH_4$ (329 mg, 8.7 mmol, 1.05

equiv), then allowed to warm slowly to room temperature. After 2 h, the reaction was quenched by addition of an aqueous $KHSO_4$ solution. The reaction mixture was extracted twice with Et_2O. The combined organic layers were washed with an aqueous $KHSO_4$ solution (1 M) and brine, dried over Na_2SO_4, and concentrated in vacuo. Aldehyde **2e** (409 mg, 2.36 mmol, 41%) was obtained as a colorless oil that was used in the next step without further purification. $R_f = 0.41$ (30% EtOAc in hexane); ^{1}H NMR ($CDCl_3$) δ 2.75 (t, J = 5.9 Hz, 2H), 3.49 (q, J = 5.9 Hz, 2H), 4.55 (d, J = 5.2 Hz, 2H), 5.21 (d, J = 10.4 Hz, 1H), 5.30 (d, J = 17.2 Hz, 1H), 5.54 (bs, 1H), 5.90 (m, 1H), 9.81 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 34.8, 44.5, 66.0, 118.1, 133.2, 156.7, 201.6.

1-*t*-Butyloxycarbonyl-2-hydroxypyrrolidine (2j). A solution of pyrrolidinone (3.0 g, 35.2 mmol) in acetonitrile (170 mL) was treated successively with DMAP (431 mg, 3.52 mmol, 0.1 equiv) and Boc_2O (7.69 g, 35.2 mmol, 1.0 equiv), stirred overnight at room temperature, and concentrated in vacuo to yield a residue. 1-*t*-Butyloxycarbonylpyrrolidin-2-one (5.23 g, 28.2 mmol, 80%) was isolated as a colorless oil by flash chromatography using 30% EtOAc in hexane as eluant: $R_f = 0.30$ (40% EtOAc in hexanes); ^{1}H NMR (DMSO) δ 1.44 (s, 9H), 1.90 (quint, J = 7.4 Hz, 2H), 2.39 (t, J = 7.9 Hz, 2H), 3.62 (t, J = 7.5 Hz, 2H); ^{13}C NMR (DMSO) δ 17.9, 28.7 (3C), 33.5, 47.1, 82.4, 150.8, 174.6. LRMS (EI) 86.2 $(M + H - 100)^+$, 130.1 $(M + H - 56)^+$, 186.1 $(M + H)^+$. A stirred solution of 1-*t*-butyloxycarbonylpyrrolidin-2-one (1.5 g, 8.1 mmol) in THF (45 mL) at −78 °C was treated dropwise with a solution of DIBAL in toluene (1 M, 12.1 mmol, 12.1 mL, 1.5 equiv). After 1.5 h, the reaction was partitioned between a saturated NH_4Cl solution and Et_2O. The mixture was let stand at room temperature for 30 min, and the jelly was filtered through a pad of Celite. The organic filtrate was separated, washed with an aqueous saturated NH_4Cl solution and brine, dried over Na_2SO_4, and concentrated in vacuo to yield a residue. Aminol **2j** was isolated as a colorless oil by flash chromatography using 30% EtOAc in hexane as eluant (1.14 g, 6.09 mmol, 75%). $R_f =$ 0.41 (40% EtOAc in hexane); ^{1}H NMR (DMSO) showed a 1:1 mixture of carbamate conformers δ 1.40 (s, 9H), 1.64 (m, 1H), 1.69−1.77 (m, 2H), 1.92 (m, 1H), 3.12 (m, 1H), 3.32 (m, 1H), 5.22−5.32 (m, 1H), 5.54 (d, J = 4.4 Hz, 1H); ^{13}C NMR (DMSO) δ 22.3 (conf1), 23.2 (conf2), 29.2 (3C), 34.6 (conf1), 35.4 (conf2), 46.0 (conf1), 46.3 (conf2), 79.4, 81.0, 154.3. LRMS (EI) 114.1 $(M + H - 56 - H_2O)^+$, 170.1 $(M + H - H_2O)^+$.

General Procedure A for the Synthesis of *N*′-Alkyl Fluorenylmethyl Carbazates, 3a−e. A suspension of 9-*H*-fluoren-9-ylmethyl carbazate **1** in EtOH (0.2 M) was treated with 100 mol % of aldehyde **(2a−e)**, heated at reflux for 2 h, and concentrated in vacuo. The hydrazone was dissolved in THF (0.2 M), treated successively with 110 mol % AcOH and 110 mol % $NaBH_3CN$, stirred for 1 h, and treated with additional $NaBH_3CN$ if necessary until completion of the reaction was observed by TLC. The mixture was concentrated in vacuo. The residue was dissolved in EtOAc, washed with aqueous $KHSO_4$ (1 M) and brine, dried over Na_2SO_4, and concentrated under reduced pressure to yield a white solid that was dissolved in EtOH and heated at reflux for 1 h. The mixture was concentrated under reduced pressure to yield a residue that was isolated by flash chromatography to yield the carbazate **(3a−e)**.

***N*′-Methyl-fluorenylmethyl Carbazate (3a).** Product from the reaction of carbazate **1** (7.1 mmol) and aldehyde **2a** (37% aqueous formaldehyde solution) (7.1 mmol) was isolated in 77% yield by flash chromatography using a 30% EtOAc in hexane eluant as a white solid: $R_f = 0.54$ (40% EtOAc in hexanes); mp 155−156 °C; ^{1}H NMR (DMSO) δ 2.43 (s, 3H), 4.22 (t, J = 6.8 Hz, 1H), 4.31 (d, J = 6.8 Hz, 2H), 4.46 (brs, 1H), 7.32 (t, J = 7.4 Hz, 2H), 7.41 (t, J = 7.4 Hz, 2H), 7.69 (d, J = 7.4 Hz, 2H), 7.88 (d, J = 7.4 Hz, 2H), 8.67 (brs, 1H); ^{13}C NMR (DMSO) δ 39.3, 47.7, 66.4, 121.1 (2C), 126.2 (2C), 128.1 (2C), 128.7 (2C), 141.8 (2C), 144.9 (2C), 157.8. LRMS (EI) 179.2 $(M + H - 100)^+$, 268.9 $(M + H)^+$, 291.7 $(M + Na)^+$; HRMS (EI) m/z for $C_{16}H_{17}N_2O_2$ $(M + H)^+$, calcd 269.1285, found 269.1291.

***N*′-Isopropyl-fluorenylmethyl Carbazate (3b).** Product from the reaction of carbazate **1** (3.9 mmol) and ketone **2b** (39 mmol) was isolated in 68% yield by flash chromatography using a 30% EtOAc in hexane eluant as a white solid: $R_f = 0.20$ (30% EtOAc in hexanes); mp 163−164 °C; ^{1}H NMR (DMSO) δ 0.91 (d, J = 5.9 Hz, 6H), 2.51 (t, J = 6.0 Hz, 1H), 4.22 (t, J = 6.6 Hz, 1H), 4.31 (d, J = 6.6 Hz, 2H), 4.36 (brs, 1H), 7.32 (t, J = 7.4 Hz, 2H), 7.41 (t, J = 7.4 Hz, 2H), 7.71 (d, J = 7.4 Hz, 2H), 7.89 (d, J = 7.4 Hz, 2H), 8.68 (brs, 1H); ^{13}C NMR (DMSO) δ 21.5 (2C), 47.6, 50.3, 66.3, 121.0 (2C), 126.1 (2C), 127.9 (2C), 128.5 (2C), 141.6 (2C), 144.7 (2C), 157.8. LRMS (EI) 296.9 $(M + H)^+$. HRMS (EI) m/z for $C_{18}H_{21}N_2O_2$ $(M + H)^+$, calcd 297.1598, found 297.1599.

***N*′-2-Isobutyl-fluorenylmethyl Carbazate (3c).** Product from the reaction of carbazate **1** (3.6 mmol) and aldehyde **2c** (3.6 mmol) was isolated in 72% yield by flash chromatography using a 20% EtOAc in hexane eluant as a white solid: $R_f = 0.30$ (20% EtOAc in hexanes); mp 124−125 °C; ^{1}H NMR (DMSO) δ 0.87 (d, J = 4.0 Hz, 6H), 1.63 (brs, 1H), 2.50 (brs, 2H), 4.22 (t, J = 6.3 Hz, 1H), 4.29 (d, J = 6.3 Hz, 2H), 4.49 (brs, 1H), 7.32 (t, J = 7.4 Hz, 2H), 7.40 (t, J = 7.4 Hz, 2H), 7.68 (d, J = 7.4 Hz, 2H), 7.88 (d, J = 7.4 Hz, 2H), 8.66 (brs, 1H); ^{13}C NMR (DMSO) δ 21.5 (2 C), 27.1, 47.6, 59.6, 66.2, 121.0 (2C), 126.1 (2C), 127.9 (2C), 128.5 (2C), 141.6 (2C), 144.7 (2C), 157.7. LRMS (EI) 310.9 $(M + H)^+$. HRMS (EI) m/z for $C_{19}H_{23}N_2O_2$ $(M + H)^+$, calcd 311.1754, found 311.1761.

***N*′-1-(3-(*t*-Butyloxycarbonylamino)propanyl)-fluorenylmethyl Carbazate (3d).** Product from the reaction of carbazate **1** (1.8 mmol) and aldehyde **2d** (1.8 mmol) was isolated in 74% yield by flash chromatography using 50% EtOAc in hexane as eluant: white foam; $R_f = 0.13$ (40% EtOAc in hexanes); ^{1}H NMR (DMSO) δ 1.36 (s, 9H), 1.45 (t, J = 6.8 Hz, 2H), 2.65 (brs, 2H), 2.94 (quint, 2H), 4.22 (t, J = 6.3 Hz, 1H), 4.29 (d, J = 6.3 Hz, 2H), 4.52 (brs, 1H), 6.78 (t, J = 5.5 Hz, 1H), 7.32 (t, J = 7.4 Hz, 2H), 7.40 (t, J = 7.4 Hz, 2H), 7.68 (d, J = 7.4 Hz, 2H), 7.88 (d, J = 7.4 Hz, 2H), 8.65 (brs, 1H); ^{13}C NMR (DMSO) δ 28.7, 29.1 (3C), 38.9, 47.6, 49.3, 66.3, 78.2, 121.0 (2C), 126.1 (2C), 127.9 (2C), 128.5 (2C), 141.6 (2C), 144.7 (2C), 156.4, 157.7. LRMS (EI) 312.1 $(M + H - Boc)^+$, 412.0 $(M + H)^+$, 434.4

$(M + Na)^+$. HRMS (EI) *m/z* for $C_{23}H_{30}N_3O_4$ $(M + H)^+$, calcd 412.2231, found 412.2233.

***N′*-1-(3-(*t*-Allyloxycarbonylamino)propanyl)-fluorenylmethyl Carbazate (3e).** Product from the reaction of carbazate **1** (2.5 mmol) and aldehyde **2e** (2.5 mmol) was isolated in 76% yield by flash chromatography using 50% EtOAc in hexane as eluant: white foam; $R_f = 0.26$ (50% EtOAc in hexanes); ^{1}H NMR (DMSO) δ 1.50 (m, 2H), 2.69 (brs, 2H), 3.03 (q, $J = 6.3$ Hz, 2H), 4.22 (t, $J = 6.3$ Hz, 1H), 4.29 (d, $J = 6.3$ Hz, 2H), 4.46 (d, $J = 4.4$ Hz, 2H), 4.55 (brs, 1H), 5.16 (dd, $J = 10.5$ Hz, 1.3 Hz, 1H), 5.27 (dd, $J = 1.6$ Hz, 17.2 Hz, 1H), 5.91 (m, 1H), 7.19 (t, $J =$ 5.4 Hz, 1H), 7.33 (t, $J = 7.4$ Hz, 2H), 7.42 (t, $J = 7.4$ Hz, 2H), 7.69 (d, $J = 7.4$ Hz, 2H), 7.89 (d, $J = 7.4$ Hz, 2H), 8.66 (brs, 1H); ^{13}C NMR (DMSO) δ 28.6, 39.3, 47.6, 49.2, 65.0, 66.3, 117.7, 121.0 (2C), 126.1 (2C), 127.9 (2C), 128.5 (2C), 134.7, 141.6 (2C), 144.7 (2C), 156.8, 157.7. LRMS (EI) 396.1 $(M + H)^+$, 418.2 $(M + Na)^+$. HRMS (EI) *m/z* for $C_{22}H_{26}N_3O_4$ (M + H)+, calcd 396.1918, found 396.1919.

***N′*-*t*-Butyl Acetate Fluorenylmethyl Carbazate (3f).** A suspension of 9-*H*-fluoren-9-ylmethyl carbazate **1** in EtOH (0.2 M) was treated with 100 mol % glyoxilic acid monohydrate **2f**, heated at reflux for 2 h, and then concentrated in vacuo. The hydrazone was dissolved in THF (0.2 M), treated successively with 200 mol % AcOH and 100 mol % $NaBH_3CN$, stirred for 1 h, and treated with additional $NaBH_3$-CN (300 mol %) until completion of the reaction was observed by RP-HPLC (starting material: t_R 23.52 min; expected product: t_R 21.49 min, 40 min linear gradient from water 0.1% TFA to acetonitrile 0.1% TFA) and concentrated in vacuo to a residue. The residue was dissolved in EtOAc, washed with aqueous $KHSO_4$ (1 M) and brine, dried over Na_2SO_4, and concentrated under reduced pressure to yield a white solid. This solid was dissolved in EtOH, heated at reflux for 1 h, and concentrated under reduced pressure to a white solid that was then dissolved in DCM (0.1 M) and treated with *t*-butyl trichloroacetimidate (100 mol %). After stirring overnight at room temperature, the reaction mixture was concentrated in vacuo to yield a white residue that was purified by flash chromatography using a 30% EtOAc in hexane eluant to provide **3f** as a white foam: Yield 20%; $R_f = 0.28$ (30% EtOAc in hexanes); ^{1}H NMR (DMSO) δ 1.42 (s, 9H), 3.39 (brs, 2H), 4.21 (t, $J = 6.8$ Hz, 1H), 4.29 (d, $J = 6.8$ Hz, 2H), 4.78 (brs, 1H), 7.32 (t, $J = 7.4$ Hz, 2H), 7.42 (t, $J = 7.4$ Hz, 2H), 7.69 (d, $J = 7.4$ Hz, 2H), 7.88 (d, $J = 7.4$ Hz, 2H), 8.72 (brs, 1H); ^{13}C NMR (DMSO) δ 28.8 (3C), 47.7, 53.8, 66.7, 81.4, 121.1 (2C), 126.3 (2C), 128.1 (2C), 128.7 (2C), 141.8 (2C), 144.8 (2C), 157.7, 170.6. LRMS (EI) 368.8 $(M + H)^+$, 391.0 $(M + Na)^+$. HRMS (EI) *m/z* for $C_{21}H_{25}N_2O_4$ $(M + H)^+$, calcd 369.1809, found 369.1813.

General Procedure B for the Synthesis of *N′*-Alkyl Fluorenylmethyl Carbazates (3g−i). A suspension of 9-*H*-fluoren-9-ylmethyl carbazate **1** in EtOH (0.2 M) was treated with 100 mol % of aldehyde **2g−i** heated at reflux for 2 h, let cool, and concentrated in vacuo. The hydrazone was dissolved in THF (0.2 M), treated with a suspension of 10 mol % of $Pd(OH)_2$ on carbon (20 wt %) in THF, placed under H_2 gas at 100 psi, and stirred at room temperature overnight. The reaction mixture was filtered over Celite. The filtrate was evaporated on a rotary evaporator. The *N′*-alkyl fluorenylmethyl carbazate, **3g−i**, was isolated by flash chromatography.

***N′*-Benzyl-fluorenylmethyl Carbazate (3g).** Product from the reaction of carbazate **1** (7.8 mmol) and aldehyde **2g** (7.8 mmol) was isolated as a white solid in 94% yield by flash chromatography using a 30% EtOAc in hexane eluant: $R_f =$ 0.27 (30% EtOAc in hexanes); mp 143−145 °C; ^{1}H NMR (DMSO) δ 3.88 (brs, 2H), 4.21 (t, $J = 6.8$ Hz, 1H), 4.31 (d, $J = 6.8$ Hz, 2H), 4.95 (brs, 1H), 7.15−7.35 (m, 7H), 7.41 (t, $J = 7.4$ Hz, 2H), 7.67 (d, $J = 7.6$ Hz, 2H), 7.89 (d, $J =$ 7.6 Hz, 2H), 8.71 (brs, 1H); ^{13}C NMR (DMSO) δ 47.7, 55.2, 66.4, 121.1 (2C), 126.3 (2C), 127.9, 128.1 (2C), 128.7 (2C), 129.1 (2C), 129.5 (2C), 139.8, 141.8 (2C), 144.8 (2C), 157.9. LRMS (EI) 344.9 $(M + H)^+$, 688.9 $(2M + H)^+$. HRMS (EI) *m/z* for $C_{22}H_{21}N_2O_2$ $(M + H)^+$, calcd 345.1598, found 345.1603.

***N′*-(4-(*t*-Butyldimethylsilyloxy)-benzyl)-fluorenylmethyl Carbazate (3h).** Product from the reaction of carbazate **1** (5.90 mmol) and aldehyde **2h** (5.90 mmol) was isolated in 70% yield as a colorless oil by flash chromatography using a 20% EtOAc in hexane eluant: $R_f = 0.30$ (20% EtOAc in hexanes); ^{1}H NMR (DMSO) δ 0.18 (s, 6H), 0.93 (s, 9H), 3.80 (brs, 2H), 4.20 (t, $J = 6.5$ Hz, 1H), 4.29 (d, $J = 6.5$ Hz, 2H), 4.83 (brs, 1H), 6.77 (d, $J = 8.3$ Hz, 2H), 7.18 (d, $J = 8.0$ Hz, 2H), 7.31 (t, $J = 7.5$ Hz, 2H), 7.41 (t, $J = 7.5$ Hz, 2H), 7.67 (d, $J = 7.4$ Hz, 2H), 7.88 (d, $J = 7.5$ Hz, 2H), 8.69 (brs, 1H); ^{13}C NMR (DMSO) δ −3.5 (2C), 19.0, 26.6 (3C), 47.7, 54.8, 66.5, 120.4 (2C), 121.1 (2C), 126.3 (2C), 128.1 (2C), 128.7 (2C), 130.8 (2C), 132.4, 141.8 (2C), 144.8 (2C), 155.0, 157.9. HRMS (EI) *m/z* for $C_{28}H_{35}N_2O_3Si$ $(M + H)^+$, calcd 475.2412, found 475.2414.

***N′*-3-((*N*-Boc-Indolyl)methyl)-fluorenylmethyl Carbazate (3i).** Product from the reaction of carbazate **1** (1.96 mmol) and aldehyde **2i** (1.96 mmol) was isolated as a colorless foam in 76% yield by flash chromatography using a 20% EtOAc in hexane eluant: $R_f = 0.22$ (20% EtOAc in hexanes); ^{1}H NMR (DMSO) δ 1.60 (s, 9H), 4.04 (s, 2H), 4.22 (t, $J = 6.6$ Hz, 1H), 4.31 (d, $J = 6.6$ Hz, 2H), 4.98 (brs, 1H), 7.20−7.45 (m, 6H), 7.60 (brs, 1H), 7.65−7.75 (m, 3H), 7.90 (d, $J = 7.4$ Hz, 2H), 8.05 (d, $J = 8.3$ Hz, 2H), 8.83 (brs, 1H); ^{13}C NMR (DMSO) δ 28.4 (3C), 46.4, 47.6, 66.4, 84.3, 115.5, 118.7, 120.7, 121.0 (2C), 123.3, 124.8, 125.2, 126.1 (2C), 127.9 (2C), 128.5 (2C), 130.7, 135.8, 141.6 (2C), 144.6 (2C), 149.9, 157.8. LRMS (EI) 483.8 $(M + H)^+$. HRMS (EI) *m/z* for $C_{29}H_{30}N_3O_4$ $(M + H)^+$, calcd 484.2231, found 484.2223.

***N′*-1-(4-(*t*-Butyloxycarbonylamino)butyl)-fluorenylmethyl Carbazate (3j).** A suspension of 9-*H*-fluoren-9-ylmethyl carbazate (**1**, 692 mg, 2.7 mmol) in EtOH (0.2 M) was treated with 100 mol % 1-*t*-butyloxycarbonyl 2-hydroxypyrrolidine (510 mg, 2.7 mmol) **2j** and 30 mol % *p*-TsOH (155 mg, 0.8 mmol). The mixture was then heated at reflux for 2 h and concentrated in vacuo to provide hydrazone, which was dissolved in THF (0.1 M, 27 mL) and treated successively with 110 mol % AcOH (2.9 mmol, 172 μL) and 110 mol % $NaBH_3CN$ (2.9 mmol, 190 mg). The solution was stirred for 1 h, and additional $NaBH_3CN$

was added if necessary until completion of the reaction was observed by TLC. The mixture was concentrated in vacuo. The residue was dissolved in EtOAc and washed with aqueous $KHSO_4$ (1 M) and brine, dried over Na_2SO_4, and concentrated under reduced pressure to yield a white solid that was dissolved in EtOH, heated at reflux for 1 h, and concentrated under reduced pressure to yield a residue. *N′*-Alkyl carbazate **3j** was isolated in 74% yield as a white solid by flash chromatography using a 50% EtOAc in hexane eluant: $R_f = 0.42$ (50% EtOAc in hexanes); mp 142−144 °C; ^{1}H NMR (DMSO) δ 1.34−1.36 (s, 13H), 2.66 (brs, 2H), 2.90 (q, $J = 6.5$ Hz, 2H), 4.22 (t, $J = 6.8$ Hz, 1H), 4.30 (d, $J = 6.8$ Hz, 2H), 4.47 (brs, 1H), 6.77 (t, $J = 5.5$ Hz, 1H), 7.32 (t, $J = 7.4$ Hz, 2H), 7.41 (t, $J = 7.4$ Hz, 2H), 7.69 (d, $J = 7.4$ Hz, 2H), 7.88 (d, $J = 7.4$ Hz, 2H), 8.65 (brs, 1H); ^{13}C NMR (DMSO) δ 25.7, 28.2, 29.3 (3C), 40.5, 47.8, 51.5, 66.4, 78.3, 121.1 (2C), 126.3 (2C), 128.1 (2C), 128.7 (2C), 141.8 (2C), 144.8 (2C), 156.6, 157.9. LRMS (EI) 326.1 $(M + H - 100)^+$, 370.1 $(M + H - 56)^+$, 426.0 $(M + H)^+$, 448.1 $(M + Na)^+$. HRMS (EI) *m/z* for $C_{24}H_{32}N_3O_4$ $(M + H)^+$, calcd 426.2387, found 426.2389.

Fmoc-azaPhe-D-Val-OBn (4g). To a solution of *N′*-benzyl fluoren-9-ylmethyl carbazate **3g** (1.0 g, 2.97 mmol) in dry DCM (30 mL) under argon at 0 °C was added dropwise a solution of phosgene in toluene (3.0 mL, 5.93 mmol, 1.93 M). After complete consumption of starting material **3g** (usually after 15 min as indicated by TLC), the reaction mixture was concentrated in vacuo to yield the corresponding Fmoc-aza-amino acid chloride, which was employed without further purification. The resulting Fmoc-aza-amino acid chloride was suspended in dry DCM (10 mL), treated with DIEA (5.93 mmol, 1.03 mL) to obtain a clear solution, and transferred to a vessel containing D-Val-OBn (400 mg, 1.98 mmol) in dry DCM (10 mL). The solution was stirred for 90 min, and the mixture was concentrated in vacuo. The residue was dissolved in EtOAc and washed with aqueous $KHSO_4$ (1 M), $NaHCO_3$ (sat), and brine, dried over Na_2SO_4, and concentrated under reduced pressure to yield a residue. Fmoc-azaPhe-D-Val-OBn **4g** was isolated by flash chromatography using a 20% EtOAc in hexane eluant as a white foam in 98% yield (1.13 g): $R_f = 0.44$ (30% EtOAc in hexane), $[\alpha]_D^{22}$ −4.1 (c 1.1, MeOH); ^{1}H NMR (DMSO) δ 0.78 (bs, 3H), 0.81 (bs, 3H), 2.05 (m, 1H), 4.05−4.45 (m, 6H), 5.14 (m, 2H), 6.38 (bs, 1H), 7.15−7.45 (m, 14H), 7.66 (bs, 2H), 7.89 (d, 2H, $J = 7.5$ Hz), 9.60 (bs, 1H); ^{13}C NMR (DMSO) δ 19.1, 20.0, 31.4, 47.6, 51.9, 59.8, 67.0, 67.2, 121.2 (2C), 126.2 (2C), 128.1 (2C), 128.2 (2C), 128.7 (2C), 129.1 (4C), 129.2, 129.3, 129.4 (2C), 136.9, 138.4, 141.8 (2C), 144.5 (2C), 156.2, 158.4, 173.0. LRMS (EI) 578.1 $(M + H)^+$, 600.2 $(M + Na)^+$. HRMS (FAB) *m/z* for $C_{35}H_{36}N_3O_5$ $(M + H)^+$, calcd 578.2655, found 578.2643.

Fmoc-azaTyr(O*t*BDMS)-D-Val-OBn (4h). To a solution of *N′*-(4-(*t*-butyldimethylsilyloxy))-benzyl)-fluorenylmethyl carbazate (**3h**, 564 mg, 1.19 mmol) in dry DCM (12 mL) under argon at 0 °C, a solution of phosgene in toluene (1.23 mL, 2.37 mmol, 1.93 M) was added dropwise. After complete consumption of starting material, **3h** was indicated by TLC (usually after 15 min), and the reaction mixture was concentrated in vacuo to yield the corresponding Fmoc-aza-amino acid chloride that was employed without further purification. The resulting Fmoc-aza-amino acid chloride was suspended in dry DCM (5 mL), treated with DIEA (2.97 mmol, 517 μL) to obtain a clear solution, and transferred to a vessel containing D-Val-OBn (200 mg, 0.99 mmol) in dry DCM (5 mL). The solution was stirred for 1.5 h, and the mixture was concentrated in vacuo. The residue was dissolved in EtOAc and washed with aqueous $KHSO_4$ (1 M), $NaHCO_3$ (sat), and brine, dried over Na_2SO_4, and concentrated under reduced pressure to yield a residue. Fmoc-aza-Tyr(OtBDMS)-D-Val-OBn **4h** was isolated in 85% yield (600 mg) as a white foam by flash chromatography using a 15% EtOAc in hexane eluant: $R_f = 0.18$ (15% EtOAc in hexane), $[\alpha]_D^{22}$ −6.2 (c 1.1, Me0H); ^{1}H NMR (DMSO) δ 0.11 (s, 6H), 0.78 (bs, 3H), 0.81 (bs, 3H), 0.92 (s, 9H), 2.05 (m, 1H), 4.09−4.40 (m, 6H), 5.13 (m, 2H), 6.35 (bs, 1H), 6.73 (d, $J = 8.4$ Hz, 2H), 7.06 (d, $J = 8.4$ Hz, 2H), 7.25−7.45 (m, 9H), 7.66 (bs, 2H), 7.88 (d, 2H, $J = 7.5$ Hz), 9.53 (bs, 1H); ^{13}C NMR (DMSO) δ −3.6 (2C), 18.9, 19.2, 20.0, 26.6 (3C), 31.3, 47.6, 51.2, 59.8, 66.9, 67.2, 120.5 (2C), 121.2 (2C), 122.4, 126.2 (2C), 128.1 (2C), 128.7 (2C), 129.1 (2C), 129.4 (2C), 130.8 (2C), 131.0, 136.9, 141.8 (2C), 144.5 (2C), 155.3,156.3, 158.4, 173.0. LRMS (EI) 708.1 $(M + H)^+$, 730.2 $(M + Na)^+$. HRMS (FAB) *m/z* for $C_{41}H_{50}N_3O_6Si$ $(M + H)^+$, calcd 708.3469, found 708.3483.

Fmoc-azaTrp(Boc)-D-Val-OBn (4i). To a solution of *N′*-3-(*N*-Boc-indolyl)-fluorenylmethyl carbazate (**3i**) (250 mg, 0.52 mmol) in dry DCM (5 mL) under argon at 0 °C, a solution of phosgene in toluene (535 μL, 1.03 mmol, 1.93 M) was added dropwise. After complete consumption of starting material, **3i** was indicated by TLC (usually after 15 min), and the reaction mixture was concentrated in vacuo to yield the corresponding Fmoc-aza-amino acid chloride that was employed without further purification. The resulting Fmoc-aza-amino acid chloride was suspended in dry DCM (3 mL), treated with DIEA (1.55 mmol, 270 μL) to obtain a clear solution, and transferred to a vessel containing D-Val-OBn (105 mg, 0.52 mmol) in dry DCM (3 mL). The solution was stirred for 90 min, and the mixture was concentrated in vacuo. The residue was dissolved in EtOAc and washed with aqueous $KHSO_4$ (1 M), $NaHCO_3$ (sat), and brine, dried over Na_2SO_4, and concentrated under reduced pressure to yield a residue. Fmoc-azaTrp(Boc)-D-Val-OBn **4i** was isolated in 73% yield (272 mg) as a white foam by flash chromatography using a 20% EtOAc in hexane eluant: $R_f = 0.18$ (20% EtOAc in hexane), $[\alpha]_D^{22}$ +9.2 (c 1.3, MeOH); ^{1}H NMR (DMSO) δ 0.79 (bs, 3H), 0.84 (bs, 3H), 1.57 (s, 9H), 2.08 (m, 1H), 4.00−4.40 (m, 5H), 5.16 (m, 2H), 6.44 (bs, 1H), 7.19 (t, $J = 8$ Hz, 1H), 7.24−7.46 (m, 11H), 7.50−7.72 (m, 4H), 7.89 (d, $J = 8$ Hz, 2H), 8.05 (d, $J = 8$ Hz, 1H), 9.61 (bs, 1H); ^{13}C NMR (DMSO) δ 18.2, 19.1, 27.7 (3C), 30.4, 41.9, 46.6, 58.9, 66.1, 66.6, 83.7, 114.8, 116.7, 120.0, 120.2 (2C), 122.7, 124.5, 125.2, 125.3, 127.2 (2C), 127.8 (2C), 128.1 (2C), 128.2 (2C), 128.5 (2C), 129.7, 135.1, 136.0, 140.8 (2C), 143.7 (2C), 149.1, 155.4, 157.3, 172.1. LRMS (EI) 739.1 $(M + Na)^+$. HRMS (ESI) *m/z* for $C_{42}H_{44}N_4O_7Na$ $(M + Na)^+$, calcd 739.3102, found 739.3105.

(4S,5*R*)-3-[(9*H*-Fluoren-9-ylmethoxy)carbonyl]-5-methyl-1,3-oxazolidine-4-carboxylic Acid (Fmoc-Oxazolidine

Carboxylic Acid, 31). L-Threonine (1.227 g, 10.3 mmol) was dissolved in an aqueous Na_2CO_3 solution (32 mL, 2.5 M), treated with an aqueous formaldehyde solution (19.3 mL, 260 mmol, 37%) dropwise with vigorous stirring over 2 h, stirred overnight at 4 °C, treated dropwise over a period of 2 h with a solution of FmocCl (2.67 g, 10.3 mmol) in acetonitrile (15 mL), stirred for 1 h at room temperature, and cooled to 0 °C. The solution was acidified with concentrated hydrochloric acid to pH 3 and extracted with EtOAc twice. The combined organic layers were washed with an aqueous $KHSO_4$ (1 M) solution and brine, dried over Na_2SO_4, and concentrated in vacuo to yield a white residue. The protected threonine **31** was isolated as a white solid in 55% yield by flash chromatography using a 50% EtOAc in hexane eluant followed by a chloroform/methanol/acetic acid (180:10:5, v/v/v) eluant. Compound **31** was dissolved in an 1:1 acetonitrile/water solution and lyophilized to yield a white solid: R_f = 0.49 (chloroform/methanol/acetic acid, 180:10:5); mp 124−125 °C; $[\alpha]_D^{22}$ −64.4 (c 1.2, MeOH); ^{1}H NMR (DMSO) showed a 1:1 mixture of conformers δ 1.29 (d, J = 5.2 Hz, 1.5H, conf1), 1.37 (d, J = 6.0 Hz, 1.5H, conf2), 3.63 (d, J = 5.6 Hz, 0.5H, conf1), 3.83 (d, J = 5.6 Hz, 0.5 H, conf2), 3.98−4.40 (m, 4H), 4.67 (d, J = 3.2 Hz, 0.5H, conf1), 4.71 (d, J = 3.2 Hz, 0.5H, conf2), 4.92 (d, J = 3.2 Hz, 0.5 H, conf1), 5.06 (d, J = 3.2 Hz, 0.5H, conf2), 7.36 (t, J = 7.6 Hz, 2H), 7.40 (t, J = 7.6 Hz, 2H), 7.60−7.80 (m, 2H), 7.87−7.90 (m, 2H). ^{13}C NMR (DMSO) δ 19.53 (conf 1), 19.64 (conf2), 46.71, 65.31 (conf1), 65.49 (conf2), 66.50 (conf1), 66.84 (conf2), 77.67 (conf1), 78.43 (conf2), 78.75 (conf1), 79.81 (conf2), 120.11 (conf 1), 120.22 (conf2), 125.22 (conf1), 125.75 (conf2), 127.28 (conf1), 127.74 (conf2), 140.68 (conf1), 140.83 (conf2), 143.75 (conf1), 143.97 (conf2), 152.40 (conf1), 153.15 (conf2), 172.30 (conf1), 172.48 (conf2). LRMS (EI) 353.9 $(M + H)^+$. HRMS (ESI) m/z for $C_{20}H_{20}NO_5$ $(M + H)^+$, calcd 354.1336, found 354.1336.

Aza-Peptide Synthesis. General Procedure for Fmoc Deprotection and HBTU Couplings. Peptide synthesis was performed under standard conditions[77] in an automated shaker using Rink resin. Couplings of amino acids (3 equiv) were performed in DMF using HBTU (3 equiv) as a coupling reagent and DIEA (3 equiv initially followed by 1 equiv 20 min after the start of the coupling reaction) as base. Fmoc deprotections were performed by treating the resin with 20% piperidine in DMF for periods of 10 and 20 min. Resin was washed after each coupling and deprotection step alternatively with DMF (2×), MeOH (2×), and DCM (2×).

General Procedure for Introduction of Aza-Amino Acid on Resin and Coupling of the Next Amino Acid. To a 0.1 M solution of N'-alkyl fluoren-9-ylmethyl carbazate **3a−j** (300 mol % relative to resin loading) in dry DCM under argon at 0 °C, a solution of phosgene in toluene (20% by wt, 600 mol %) was added dropwise. After complete consumption of starting material **3a−j** (usually after 15 min as indicated by TLC), the reaction mixture was concentrated in vacuo to yield the Fmoc-aza-amino acid chloride that was employed without further purification.

Fmoc-Aza-amino Acid Chloride Derived from N'-Benzyl-fluorenylmethyl Carbazate (3 g). R_f = 0.41 (20% EtOAc in hexane); ^{1}H NMR (DMSO) δ 4.20−4.40 (m, 2H), 4.52 (d, J = 8 Hz, 2H), 4.96 (d, J = 17 Hz, 1H), 7.10−7.50 (m, 9H), 7.67 (d, J = 9.6 Hz, 2H), 7.88 (d, J = 9.6 Hz, 2H), 10.55 (bs, 1H); ^{13}C NMR (DMSO) δ 47.5, 55.9, 67.1, 121.0 (2C), 126.0 (2C), 128.0, 128.6 (2C), 128.9 (2C), 129.4, 129.5, 135.5, 141.7 (2C), 144.2 (2C), 152.3, 155.7. The resulting Fmoc-aza-amino acid chloride (300 mol %) was suspended in dry DCM (0.15 M), treated with DIEA (600 mol %) to obtain a clear solution, and transferred to a vessel containing the resin-bound N-terminal amine swollen in dry DCM. The mixture was shaken overnight at room temperature under argon. The solution was filtered, the resin was washed twice with dry DCM, and the aza-amino acid coupling procedure described previously was repeated again. The resin was then treated under the conditions to remove the Fmoc group that were described previously. The aza-amino acid resin was then swollen in dry THF and treated with a solution of Fmoc-amino acid (300 mol %) in THF (0.15 M), followed sequentially by BTC (100 mol %) and 2,4,6-collidine (1400 mol %). The reaction mixture was shaken for 3 h under argon, and the resin was filtered. The resin was washed alternatively with DMF (2×), MeOH (2×), and DCM (2×), and peptide synthesis was continued.

General Procedure for Side Chain Deprotection and Aza-Peptide Cleavage. Aza-peptide resin was treated with a freshly made solution of TFA/H_2O/TES (95:2.5:2.5, v/v/v, 20 mL/g of aza-peptide resin) for 2 h at room temperature. The cleavage mixture was filtered, and the resin was washed with neat TFA. The filtrate was then concentrated to about 1 mL and treated with Et_2O. The resulting aza-peptide precipitate was filtered, washed with Et_2O, and dissolved in an acetonitrile/H_2O (1:1, v/v) solution and lyophilized to yield a light foam or powder.

Ac-His-D-Phe-Arg-Trp-NH$_2$, (5). t_R =13.31; ^{1}H NMR (DMSO) δ 1.27 (m, 2H); 1.42 (m, 1H); 1.60 (m, 1H); 1.79 (s, 3H); 2.65−2.81 (m, 2H); 2.86 (dd, J = 15.2 Hz, 4.8 Hz, 1H); 2.91−3.05 (m, 4H); 3.14 (dd, J = 14.4 Hz, 5.2 Hz, 1H); 4.22 (m, 1H); 4.44 (m, 1H); 4.53−4.64 (m, 2H); 6.96 (t, J = 7.6 Hz, 1H); 7.00−7.08 (m, 3H); 7.10−7.27 (m, 6H); 7.31 (d, J = 8 Hz, 1H); 7.34 (bs, 1H); 7.49 (t, J = 5.2 Hz, 1H); 7.59 (d, J = 8.0 Hz, 1H); 7.97 (d, J = 8.0 Hz, 1H); 8.11 (d, J = 8.0 Hz, 1H); 8.31 (d, J = 8.0 Hz, 1H); 8.38 (d, J = 7.6 Hz, 1H); 8.93 (s, 1H); 10.76 (bs, 1H); LRMS (EI) calcd for $C_{34}H_{43}N_{12}O_5$ $(M + H)^+$, 686.3 found m/z 343.9 $(M + 2H)^{2+}$, 686.4 $(M + H)^+$, 708.4 $(M + Na)^+$, 821.9 $(M + Na + CF_3CO_2H)^+$.

Ac-His-azaPhe-Arg-Trp-NH$_2$, (6). t_R = 13.36, LRMS (EI) calcd for $C_{33}H_{43}N_{12}O_5$ $(M + H)^+$, 687.3 found m/z 344.3 $(M + 2H)^{2+}$, 687.2 $(M + H)^+$, 709.2 $(M + Na)^+$, 822.8 $(M + Na + CF_3CO_2H)^+$

Ac-His-D-Phe-azaLys-Trp-NH$_2$, (7). t_R = 13.15; ^{1}H NMR (DMSO) δ 1.00 (m, 2H); 1.32 (m, 2H); 1.83 (s, 3H); 2.57−2.74 (m, 2H); 2.81−3.13 (m, 6H); 3.19 (d, J = 13.2 Hz, 1H); 3.30 (m, 1H); 4.19 (m, 1H); 4.32 (m, 1H); 4.58 (m, 1H); 6.54 (bs, 1H); 6.89 (t, J = 7.2 Hz, 1H); 7.02−7.10 (m, 2H); 7.15−7.37 (m, 10H); 7.56 (d, J = 8 Hz, 1H); 7.61−7.77 (m, 3H); 8.08 (d, J = 8.4 Hz, 1H); 8.69 (s, 1H); 8.99 (s, 1H); 10.36 (bs, 1H); 10.84 (bs, 1H); LRMS (EI) calcd

for $C_{33}H_{43}N_{10}O_5$ (M + H)$^+$, 659.3 found *m/z* 330.3 (M + 2H)$^{2+}$, 659.3 (M + H)$^+$, 681.3 (M + Na)$^+$.

Ac-His-D-Phe-aza-Orn-Trp-NH$_2$, (8). t_R = 13.28; ^{1}H NMR (DMSO) δ 1.40 (m, 2H); 1.83 (s, 3H); 2.65 (bs, 2H); 2.78−3.13 (m, 6H); 3.15−3.32 (m, 2H); 4.23 (m, 1H); 4.32 (m, 1H); 4.66 (m, 1H); 6.58 (bs, 1H); 6.98 (t, J = 7.2 Hz, 1H); 7.06 (t, J = 7.2 Hz, 1H); 7.11 (bs, 1H); 7.15−7.37 (m, 9H); 7.56 (d, J = 8 Hz, 1H); 7.61−7.77 (m, 3H); 8.10 (d, J = 8.8 Hz, 1H); 8.70 (s, 1H); 8.97 (s, 1H); 10.42 (bs, 1H); 10.84 (bs, 1H); LRMS (EI) calcd for $C_{32}H_{41}N_{10}O_5$ (M + H)$^+$, 645.3 found *m/e* 323.3 (M + 2H)$^{2+}$, 645.3 (M + H)$^+$, 667.2 (M + Na)$^+$.

Deprotection of the Alloc Group and Guanylation of Resin-Bound Aza-Orn Residue, Synthesis of Ac-His-D-Phe-azaArg-Trp-NH$_2$, (9). To Fmoc-D-Phe-azaOrn(Alloc)-Trp(Boc)NH-Rink-bounded resin (392 mg, 0.41 mmol/g, 162.5 μmol) swollen in dry THF, a solution of $Pd(PPh_3)_4$ (28.2 mg, 24 μmol, 0.15 equiv) and dimethylbarbutiric acid (76.2 mg, 0.48 mmol, 3 equiv) in dry THF (3 mL) was added, and the resin was shaken overnight at room temperature under argon. The resin was first washed with THF (2×) followed by alternating DMF (2×), MeOH (2×), and DCM (2×) solutions. The resulting resin-bound amine was swollen in DMF and treated with a solution of *N,N′*-bisBoc-1-guanylpyrazole (252 mg, 0.812 mmol, 5.0 equiv) and DIEA (283 μL, 1.62 mmol, 10 equiv) in DMF for 3 h at room temperature and washed with DMF. The resin was resubmitted to the guanylation reaction conditions and washed with DMF (2×), MeOH (2×), and DCM (2×) prior to peptide synthesis and cleavage as described previously.

Ac-His-D-Phe-aza-Arg-Trp-NH$_2$, (9). t_R =13.63; ^{1}H NMR (DMSO) δ 1.17 (m, 2H); 1.83 (s, 3H); 2.72−3.13 (m, 8H); 3.20 (d, J = 12.8 Hz, 1H); 3.30 (m, 1H); 4.22 (m, 1H); 4.32 (m, 1H); 4.66 (m, 1H); 6.57 (m, 1H); 6.98 (t, J = 7.6 Hz, 1H); 7.06 (t, J = 7.4 Hz, 1H); 7.09 (bs, 1H); 7.14−7.44 (m, 13H); 7.56 (d, J = 8.0 Hz, 1H); 8.12 (d, J = 8.8 Hz, 1H); 8.71 (d, J = 4 Hz, 1H); 8.98 (bs, 1H); 10.41 (bs, 1H); 10.83 (bs, 1H); LRMS (EI) calcd for $C_{33}H_{43}N_{12}O_5$ (M + H)$^+$, 687.3 found *m/e* 344.3 (M + 2H)$^{2+}$, 687.3 (M + H)$^+$, 822.9 (M + Na + CF_3CO_2H)$^+$.

His-D-Trp-Ala-Trp-D-Phe-Lys-NH$_2$, (10). t_R = 15.73; LRMS (EI) calcd for $C_{46}H_{57}N_{12}O_6$ (M + H)$^+$, 873.4 found *m/z* 437.4 (M + 2H)$^{2+}$, 873.4 (M + H)$^+$, 895.4 (M + Na)$^+$.

His-D-Trp-aza-Ala-Trp-D-Phe-Lys-NH$_2$, (11). t_R = 15.84; LRMS (EI) calcd for $C_{45}H_{56}N_{13}O_6$ (M + H)$^+$, 874.4 found *m/z* 437.9 (M + 2H)$^{2+}$, 874.4 (M + H)$^+$, 896.4 (M + Na)$^+$.

His-D-Trp-Ala-Trp-D-aza-Phe-Lys-NH$_2$, (12). t_R = 16.69; LRMS (EI) calcd for $C_{45}H_{56}N_{13}O_6$ (M + H)$^+$, 874.4 found *m/z* 437.9 (M + 2H)$^{2+}$, 874.3 (M + H)$^+$, 896.3 (M + Na)$^+$.

His-D-Trp-Ala-Trp-D-Phe-aza-Lys-NH$_2$, (13). t_R = 15.68; LRMS (EI) calcd for $C_{45}H_{56}N_{13}O_6$ (M + H)$^+$, 874.4 found *m/z* 437.9 (M + 2H)$^{2+}$, 874.5 (M + H)$^+$, 896.4 (M + Na)$^+$.

(D/L)-His-aza-Tyr-Ala-Trp-D-Phe-aza-Lys-NH$_2$, (14). t_R = 14.93 and 15.01; LRMS (EI) calcd for $C_{43}H_{55}N_{12}O_7$ (M + H)$^+$, 851.4 found *m/z* 426.4 (M + 2H)$^{2+}$, 851.3 (M + H)$^+$, 873.2 (M + Na)$^+$.

His-D-Trp-Ala-azaTyr-D-Phe-aza-Lys-NH$_2$, (15). t_R = 8.69; LRMS (EI) calcd for $C_{43}H_{55}N_{12}O_7$ (M + H)$^+$, 851.4 found *m/z* 426.4 (M + 2H)$^{2+}$, 851.3 (M + H)$^+$, 873.3 (M + Na)$^+$.

[D^{31},P^{34},F^{35}]CGRP$_{29-37}$,PTDVGPFAF-NH$_2$ (16). t_R = 16.32; LRMS (EI) calcd for $C_{46}H_{65}N_{10}O_{12}$ (M + H)$^+$, 949.5 found *m/z* 949.4 (M + H)$^+$.

[D^{31},P^{34},F^{35},azaA^{36}]CGRP$_{29-37}$,PTDVGPFazaAF-NH$_2$ (17). t_R = 16.27; LRMS (EI) calcd for $C_{45}H_{64}N_{11}O_{12}$ (M + H)$^+$, 950.5 found *m/z* 950.4 (M + H)$^+$, 972.4 (M + Na)$^+$.

[D^{31},azaG^{33},P^{34},F^{35}]CGRP$_{29-37}$,PTDVazaGPFAF-NH$_2$ (18). t_R = 15.76; LRMS (EI) calcd for $C_{45}H_{64}N_{11}O_{12}$ (M + H)$^+$, 950.5 found *m/z* 950.4 (M + H)$^+$, 972.4 (M + Na)$^+$.

[azaD^{31},P^{34},F^{35}]CGRP$_{29-37}$,PTazaDVGPFAF-NH$_2$ (19). t_R = 14.16; LRMS (EI) calcd for $C_{45}H_{64}N_{11}O_{12}$ (M + H)$^+$, 950.5 found *m/z* 950.5 (M + H)$^+$, 972.5 (M + Na)$^+$.

[D^{31},P^{34},F^{35}]CGRP$_{27-37}$,FVPTDVGPFAF-NH$_2$ (20). t_R = 17.63; LRMS (EI) calcd for $C_{60}H_{83}N_{12}O_{14}$ (M + H)$^+$, 1195.6 found *m/z* 1195.6 (M + H)$^+$, 1217.6 (M + Na)$^+$.

[D^{31},P^{34},F^{35},azaA^{36}]CGRP$_{27-37}$,FVPTDVGPFazaAF-NH$_2$ (21). t_R = 17.52; LRMS (EI) calcd for $C_{59}H_{82}N_{13}O_{14}$ (M + H)$^+$, 1196.6 *m/z* 1196.5 (M + H)$^+$, 1218.5 (M + Na)$^+$.

[D^{31},azaG^{33},P^{34},F^{35}]CGRP$_{27-37}$,FVPTDVazaGPFAF-NH$_2$ (22). t_R =17.09; LRMS (EI) calcd for $C_{59}H_{82}N_{13}O_{14}$ (M + H)$^+$, 1196.6 *m/z* 1196.6 (M + H)$^+$, 1218.6 (M + Na)$^+$.

[azaD^{31},P^{34},F^{35}]CGRP$_{27-37}$,FVPTazaDVGPFAF-NH$_2$ (23). t_R = 16.29; LRMS (EI) calcd for $C_{59}H_{82}N_{13}O_{14}$ (M + H)$^+$, 1196.6 *m/z* 1196.6 (M + H)$^+$, 1218.6 (M + Na)$^+$.

Supporting Information Available. Complete ref 27 and spectral data for compounds **1**, **3a-3j**, **4 g-4i**, **5−9**, **and 33**. This material is available free of charge via the Internet at http://pubs.acs.org.

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