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Solid-Phase Synthesis of "Mixed" Peptidomimetics Using Fmoc-Protected Aza-β^3-amino Acids and α-Amino Acids

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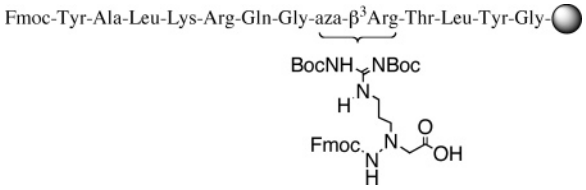

A solid-phase fluorenylmethyloxycarbonyl (Fmoc)-based synthesis strategy is described for "mixed" aza-β^3-peptides as well as a convenient general approach for their required building blocks, the aza-β^3-amino acid residues (aza-β^3-aa). These monomers allow the synthesis of relatively large quantities of pure mixed aza-β^3-peptides. The required Fmoc-substituted aza-β^3-amino acids are accessible by convenient synthesis, and a number of monomers including those containing side chains with functional groups have been synthesized. The method was applied toward the solid-phase synthesis of aza-β^3-peptide mimetics of a biologically active histone H4 sequence.

Introduction

Peptides and proteins play a crucial role in virtually all biological processes. Peptides are usually smaller than proteins and are often important starting molecules for the development of potential therapeutic agents.[1] Nevertheless, peptides have severe limitations for usage as therapeutic agents due to degradation by proteases and low membrane permeability. To overcome these limitations and to improve metabolic stability, bioavailability, and biological absorption, a number of backbone-modified peptides and peptide mimics have been the focus of research over the past several years.[2,3]

Potential classes of peptidomimetics for broad application are peptoids[4−7] and analogues such as azapeptoids,[8−14] ureapeptoids,[15,16] amino-oxypeptoids,[17] β-peptoids,[18] and hydrazinoazapeptoids.[19] The particularity of these analogues is the absence of chirality in the *N*-substituted residues. Peptoid analogues are peptide mimics in which

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the side chain has been shifted from the α-carbon atom in a peptide to the achiral nitrogen. Undoubtedly, this will have consequences not only on the structure but also on biological activity of these analogues as compared to the parent peptide.[20]

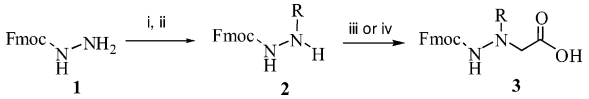
As part of our effort to explore new peptoid analogues with potentially useful biological properties, we have developed a synthetic strategy for hydrazinopeptoids or aza-β^3-peptides.[21] As is the case with the synthesis of other peptidomimetics, the synthesis of the required building blocks is just as important as the construction of the oligomeric structure. Recently, we developed a procedure for solution synthesis of aza-β^3-peptides using *tert*-butyloxycarbonyl[2] (Boc)-protected monomers.[22] Because of the usefulness of these peptide derivatives, we have been interested in the synthesis of "mixed" peptides using solid-phase synthesis based upon the fluorenylmethyloxycarbonyl (Fmoc)/*tert*-butyl strategy, because the Fmoc protection groups can easily be removed using mild basic conditions. Moreover, when using the Fmoc protection strategy, acid-labile groups can be used for protection of the side chains. Consequently, final deprotection of side chains and cleavage from the resin can be carried out under less vigorous conditions compared to using the Boc deprotection strategy. Employment of the Fmoc groups also makes these monomers fully compatible with usage of other standard Fmoc-protected amino acids or other Fmoc-protected building blocks. Thus, both aza-β^3-peptides and "mixed" peptidomimetics would easily be synthesized.

We describe in this article a convenient and general method for the synthesis of Fmoc-protected aza-β^3-amino acid monomers, including those with functional side chains. Validation of the solid-phase synthesis was demonstrated by the synthesis of mixed aza-β^3-peptides of a model peptide encompassing residues 88−99 of histone H4 (H_2N-^{88}Tyr-Ala-Leu-Lys-Arg-Gln-Gly-Arg-Thr-Leu-Tyr-^{99}Gly-OH). As part of our program to study the structural characteristics as well as the biological activities, we are especially interested in the synthesis of hybrid peptides using solid-phase synthesis, with the long-term challenge of creating libraries for structure−activity relationship and potentially drug discovery.

Results and Discussion

Preparation of Monomers. Two strategies have been employed for the synthesis of the required Fmoc-protected monomers **3**. Fmoc-carbazate **1** was converted to the corresponding N^β-Fmoc-protected N^α-substituted hydrazine **2** by condensation with an aldehyde and then reduction of the hydrazone. The first approach involves the use of nucleophilic substitution of *tert*-butyl or benzyl bromoacetates by the hydrazine **2**, which has been successfully applied for the synthesis of various aza-β^3-amino esters.[22] The required monomers **3** were then obtained in satisfactory to good yields by deprotection of the carboxy-protecting group. The synthesis of monomers containing nonfunctional side chains is straightforward and is outline in Scheme 1.

SCHEME 1[a]



[a] Reagents and conditions: (i) RCHO; (ii) $NaBH_3CN$; (iii) $BrCH_2CO_2Bn$, K_2CO_3, toluene, Δ, 24 h; H_2 (1 atm), 5% Pd/C or $BrCH_2CO_2t$-Bu, K_2CO_3, toluene, Δ, 24 h; CF_3CO_2H; $NaHCO_3$; (iv) $OHCCO_2H$, methanol, $NaBH_3CN$, HCl (2 N).

For the synthesis of functionalized monomers, the most commonly used acid-labile protections reported for solid-phase synthesis, using the Fmoc strategy, are Boc, *t*-Bu, or Trt group, depending on the functionality. Due to the acid-labile group, protecting group requiring acid conditions cannot be applied to protect the carboxy group. A benzyl group could be used, but a partial cleavage of the Fmoc protecting group can occur by catalytic hydrogenation during the benzyl deprotection. However, through carefully controlled hydrogenation condition we have shown that the benzyl group can be cleaved in the presence of Fmoc in 60% yield. Nevertheless, the nucleophilic substitution of benzyl bromoacetate with Fmoc carbazate proceeds with low yield (20%) and with Fmoc hydrazine in 40−60% yield, so to avoid the steps of substitution and benzyl deprotection, a second approach that relies on reductive amination of glyoxilic acid and N^β-Fmoc-protected N^α-substituted hydrazine **2** has been studied (Scheme 1, iv).

As an example to get the Fmoc-aza-β^3-tyrosine, the 4-hydroxybenzaldehyde was first protected either as a *tert*-butyl ether using *tert*-butyl trichloroacetimidate[23] or as a methoxy ethyl ether using chloromethyl ethyl ether,[24] after which condensation with Fmoc carbazate **1** and reduction with $NaBH_3CN$ afford the hydrazine **2** (R = $EtOCH_2O-C_6H_4-CH_2$). Reductive amination of glyoxilic acid and hydrazine **2** (R = $EtOCH_2O-C_6H_4-CH_2$) leads to Fmoc-aza-β^3-Tyr(OCH_2OEt)−OH **3** (R = $EtOCH_2O-C_6H_4-CH_2$) in 60% yield.

For the synthesis of the aza-β^3-alanine, the formaldehyde is not stable and must be used either in water, which is not favorable for a condensation with hydrazine, or as diethyl acetal. Thus, the corresponding Fmoc hydrazine **2** (R = CH_3) was prepared in using the enhanced nucleophilicity of the methylated *N*-atom of methylhydrazine. Methylhydrazine was treated first with an excess of di-*tert*-butyl dicarbonate (Boc_2O), the monoacylation occurred on the substituted nitrogen in 92% yield, no acylation was observed on the unsubstituted nitrogen, followed by addition of FmocCl and NEt_3.[25] The Boc-protecting group was then cleaved by acidification with trifluoroacetic acid (TFA), and then reductive amination of glyoxylic acid led to the Fmoc-aza-β^3-Ala-OH **3** (R = CH_3) (Scheme 2).

Because side reactions occurred during the coupling of the analogue Fmoc-aza-β^3-Gly-OH **3** (R = H), due to the formation of polymeric products during activation of

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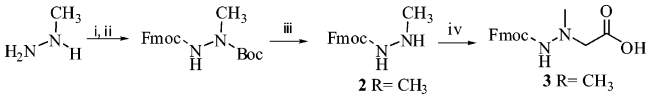
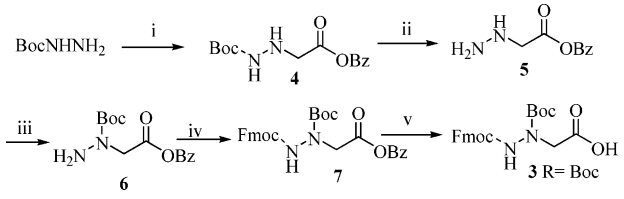
SCHEME 2[a]



[a] Reagents and conditions: (i) Boc_2O, $CHCl_3$; (ii) FmocCl, NEt_3; (iii) CF_3CO_2H, $NaHCO_3$; (iv) $OHCCO_2H$, methanol, $NaBH_3CN$, HCl (2 N).

SCHEME 3[a]



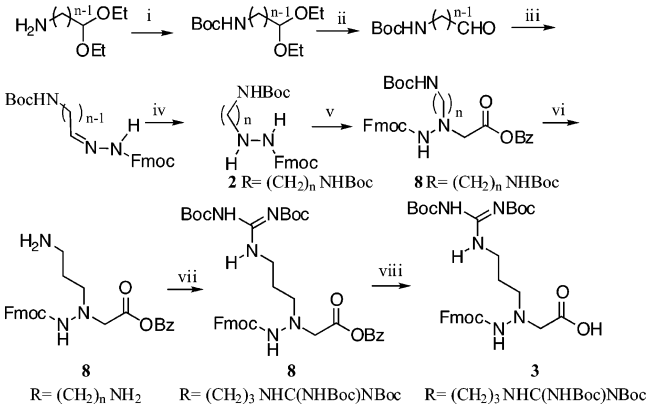
[a] Reagents and conditions: (i) $BrCH_2CO_2Bz$, DIEA, toluene, Δ, 48 h, 61%; (ii) CF_3CO_2H or HCl_g NEt_3, 85%; (iii) $(Boc)_2O/NEt_3$, CH_2Cl_2, 60%; (iv) FmocCl, 71%; (v) H_2 (1 atm), 10% Pd/C, 96%.

N^{α}-unprotected hydrazino acids,[26,27] this monomer needs to be N^{α}-protected. Due to the insolubility of the Fmoc-aza-β^3-Gly-OH **3** (R = H) in organic solvent, it was not possible to protect its N^{α}-postion. Therefore, we focused our attention on the synthesis of Boc-aza-β^3-Gly-OBn **4** by nucleophilic substitution of benzyl bromoacetate with Boc-carbazate, followed by deprotection of the N^{β}-Boc of **4**, affording **5**, which was acylated by using Boc_2O/NEt_3 in CH_2Cl_2. Bonnet et al.[27] have shown that the acylation of ethyl hydrazinoacetate hydrochloride using Boc_2O/*N*-methylmorpholine in a H_2O/EtOH mixture led only to a monoacylation in the N^{β}-position. In our conditions, it is interesting to note that the acylation occurred in the N^{α}-position in 60% yield together with monoacylation in N^{β}-position in 40% yield. Then, protection of **6** at the N^{β}-position with FmocCl in the presence of triethylamine and hydrogenolysis of **7** over 10% Pd/C gave the Fmoc-aza-β^3-Gly(Boc)-OH **3** (R = Boc) (Scheme 3).

The Fmoc-aza-β^3-Lys(Boc)-OH was prepared starting from the commercially available 4-aminobutylaldehyde diethyl acetal (Scheme 4, $n = 4$).[11] The amino group was initially protected with the *t*-butoxycarbonyl group by reaction with Boc_2O in chloroform. The Boc-protected diethyl acetal was then treated with aqueous HCl in tetrahydrofuran (THF) to give crude N^{α}-Boc-pyrrolidin-2-ol in good yield. The amide carbinol exists in equilibrium with the required aldehyde. Reaction between Fmoc hydrazine and N^{α}-Boc-pyrrolidin-2-ol in ether gave the hydrazone as a mixture of geometrical isomers. Reduction of this crude mixture with sodium cyanoborohydride gave the required *N*,*N*′-disubstituted hydrazine **2** (R = $(CH_2)_4$-NHBoc), and then reductive amination of glyoxilic acid led to the final product Fmoc-aza-β^3-Lys(Boc)-OH **3** (R = $(CH_2)_4$NHBoc).

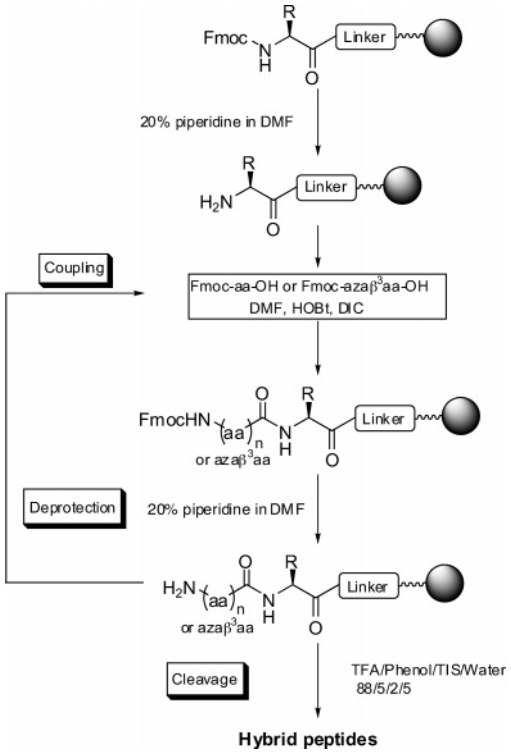
For the synthesis of the analogue of arginine, we need to conveniently protect the trifunctional guanidine group, which displays a strong nucleophilic character, otherwise side reactions such as intramolecular cyclization to

SCHEME 4[a]



[a] Reagents and conditions: (i) $(Boc)_2O/NEt_3$, 90%; (ii) $AcCO_2H$, 98%; (iii) $FmocNHNH_2$, 89%; (iv) $NaBH_3CN$, 82%; (v) $BrCH_2CO_2Bn$, K_2CO_3, toluene, Δ, 48 h, 60%; (vi) CF_3CO_2H, NEt_3, 95%; (vii) $(BocNH)_2NTf$, NEt_3, 84%; (viii) H_2 (1 atm) Pd/C, 96%.

SCHEME 5



δ-lactam derivatives can occur. Herein we used Goodman reagents *N*,*N*′-di-Boc-*N*″-trifluoromethane sulfonylguanidine as versatile guanidinating reagents.[28] Therefore, to get Fmoc-aza-β^3-Arg(Boc)-OH, we have to prepare first the Fmoc-aza-β^3-Orn(Boc)-OH, which is the homologue of the Fmoc-aza-β^3-Lys(Boc)-OH. Similarly, Fmoc hydrazine **1** and the commercially available diethylacetal of 3-aminopropanal were used as initial starting material (Scheme 4, $n = 3$). The latter was *N*-*t*-butoxy carbonyl-protected with Boc_2O, and the diacetal was hydrolyzed with diluted acetic acid.[29] Condensation of 3-*t*-butoxy

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TABLE 1. Amino Acid Sequence of Peptide Analogues 88−99 of H4

name	sequence	yield %
aza-β^3-Ala 89	Y *azaβ^3A* L K R Q G R T L Y G	36
aza-β^3-Leu 90	Y A *azaβ^3L* K R Q G R T L Y G	34
aza-β^3-Lys 91	YA L *azaβ^3K* R Q G R T L Y G	31
aza-β^3-Arg 92	YA L K *azaβ^3R* Q G R T L Y G	26
aza-β^3-Gly 94	YA L K R Q *azaβ^3G* R T L Y G	30
aza-β^3-Arg 95	YA L K R Q G *azaβ^3R* T L Y G	25
aza-β^3-Leu 97	YA L K R Q G R T *azaβ^3L* Y G	42
aza-β^3-Tyr 98	YA L K R Q G R T L *azaβ^3Y* G	17
aza-β^3-Gly 99	YA L K R Q G R T L Y *azaβ^3G*	24
aza-β^3-Ala 89-Leu 90	Y *azaβ^3A azaβ^3L* K R Q G R T L Y G	32
aza-β^3-Ala 89-Leu 90-Lys 91	Y *azaβ^3A azaβ^3L azaβ^3K* R Q G R T L Y G	20
aza-β^3-Leu97-Tyr 98	YA L K R Q G R T *azaβ^3L azaβ^3Y* G	15

TABLE 2. NMR Data (ppm) of Aza-β^3-L97 in DMSO (313 K)
H_2N-^{88}Tyr-Ala-Leu-Lys-Arg-Gln-Gly-Arg-Thr-Aza-β^3-Leu-Tyr-^{99}Gly-OH

residue	^{15}N	NH	C_αH	C_βH	others
Tyr 88	33.25		3.97	2.78, 3.00	NH_2 7.70, $CH_{oo'}$ 7.05, $CH_{mm'}$ 6.70, OH 9.29
Ala 89	123.48	8.59	4.43	1.25	
Leu 90	118.22	8.06	4.35	1.48, 1.49	C_γH 1.72, C_δH 0.88 0.91
Lys 91	117.00	7.88	4.28	1.68	C_γH 1.33, C_δH 1.55, C_ϵH 2.77, NH 7.99, N 38.50
Arg 92	118.48	7.96	4.30	1.51, 1.53	C_γH 1.71, C_δH 3.10, NH 7.52, N 85.13
Gln 93	118.33	7.95	4.28	1.78, 1.91	C_γH 2.14, NH 6.73 7.21
Gly 94	106.36	8.12	3.88, 3.69		
Arg 95	118.22	8.06	4.42	1.50, 1.54	C_γH 1.72, C_δH 3.11, NH 7.47, N 84.85
Thr 96	112.36	7.84	4.06	3.96	C_γH 1.03, OH 4.88
aza-β^3-Leu 97	156.12	8.92		2.42, 2.51	C_γH 1.63, C_δH 0.83 0.85
Tyr 98	118.27	8.26	4.43	2.69, 2.95	$CH_{oo'}$ 7.08, $CH_{mm'}$ 6.64, OH 9.10
Gly 99	106.36	8.24	3.77, 3.78		

carbonyl aminopropanal with the Fmoc hydrazine **1** and then reduction of the hydrazone with sodium cyanoborohydride afforded *N*,*N*′-disubstituted hydrazine **2** (R = $(CH_2)_3$NHBoc). As described previously, Fmoc-aza-β^3-Orn(Boc)-OBn **8** (R = $(CH_2)_3$NHBoc) was obtained by nucleophilic substitution of benzyl bromoacetate with hydrazine **2** (R = $(CH_2)_3$NHBoc). Then, selective deprotection of **8** (R = $(CH_2)_3$NHBoc) with trifluoroacetic acid (TFA) gave **8** (R = $(CH_2)_3NH_2$) in 90% yield. The crude amine **8** (R = $(CH_2)_3NH_2$) was guanidinylated with the Goodman reagents $(BocNH)_2NTf$,[28] and the protected analogue of arginine **8** (R = $(CH_2)_3$NHC(NHBoc)NBoc) was obtained in 85% yield. Finally, deprotection of the benzyl-protecting group with Pd/C (10%) in ethanol was effected successfully to give the expected Fmoc-aza-β^3-Arg$(Boc)_2$-OH **3** (R = $(CH_2)_3$NHC(NHBoc)NBoc).

Automated Solid-Phase Synthesis of Mixed Aza-β^3-peptides. The synthesis of hybrid peptides of the dodecapeptide (residues 88−99 of histone H4) was next examined using a commercial automatic peptide synthesizer by coupling Fmoc-α-amino acids or Fmoc-aza-β^3-amino acid on preloaded PEG-PS or Wang resin or on resin where we first attached a Fmoc-protected aza-β^3-amino acid. The general procedure for the solid-phase synthesis of hybrid peptides using Fmoc/*tert*-butyl strategy is depicted in Scheme 5. The Fmoc-Gly-OH on the preloaded resin was first liberated upon treatment with 20% piperidine in dimethylformamide (DMF). For elongating the peptide chain, the resin was treated with a solution of 4 equiv of Fmoc-α-amino acid or Fmoc-aza-β^3-amino acid activated by diisopropylcarbodiimide (DIC)/1-hydroxybenzotriazole (HOBt) at room temperature for 30−60 min (depending on the amino acid) to 80 min for the analogues. No problem was observed for the coupling of most analogues except for the time of coupling cycles, which must be longer. In comparison to the synthesis of β^3-amino acid-containing peptides in which coupling reactions for α/β + α peptides proceed for 3−24 h while those for α-peptides proceed for 1−24 h,[30] coupling cycles for Fmoc-aza-β^3-amino acid as well as coupling cycles for α-amino acid following the analogue proceed for 80 min to 2 h while those for Fmoc-α-amino acids proceed for 30−60 min. Coupling Fmoc-aza-β^3-Gly-OH affords polymeric products during activation of N^α-unprotected monomer; this is in accordance with the results described before during the coupling of hydrazino acids.[26] Guy et al. solved this problem by using N^β-Boc-N^α-Bn-hydrazino acids, and Bonnet et al. coupled a triprotected Boc monomer in the *N*-terminal position.[27] In our case, the coupling of Fmoc-aza-β^3-Gly(Boc)-OH leads to the expected peptide. Deprotection cycles were realized with 20% piperidine in DMF. The hybrid peptides were then deprotected and cleaved from the resin with TFA/phenol/EDT/H_2O (88/5/2/5), and after preparative HPLC, the hybrid peptides (25−45% yield) were isolated in >98% purity. During the cleavage of peptide analogue aza-β^3-Y98 either with a monomer Fmoc-aza-β^3-Tyr(Ot-Bu)-OH or with a monomer Fmoc-aza-β^3-Tyr(OCH_2OEt)-OH, we observed not only the deprotection of the protecting group but also a partial cleavage of the tyrosine chain, and thus cleavage was realized with CH_2Cl_2/TFA/phenol/TIS/H_2O (40/48/5/2/5) (where TIS is triisopropylsilane) and monitored by HPLC.

For the synthesis of the peptide analogue aza-β^3-G99, anchoring the first monomer Fmoc-aza-β^3-Gly(Boc)-OH was achieved by esterification of the Fmoc-aza-β^3-Gly-(Boc)-OH (5 equiv with respect to the resin loading) to

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the hydroxyl group of the resin with activation by DIC at room temperature in DMF for 2 h.[31] Completion of the coupling was confirmed by a 2,4,6-trinitrobenzenesulfonic acid test, and the loading was determined by Fmoc cleavage from a resin simple by measuring the UV absorption of the dibenzofulvene−piperidine adduct that is formed after cleavage.[32]

All the peptides (Table 1) were characterized by electrospray mass spectrometry (HR-MS, ESI) as well as NMR spectroscopy. The NH hydrazino protons are not detectable in D_2O/H_2O because of fast exchange with the water protons; however, they are readily observed in DMSO. We give as an example in Table 2 the chemical shifts in DMSO for the analogue aza-β3-Leu 97.

Conclusion

We have developed an efficient synthetic strategy for the synthesis of Fmoc-aza-β^3-amino acids. When suitable protecting groups were used, identical chains can be incorporated into amino acid analogues. Furthermore, an efficient solid-phase methodology for the synthesis of hybrid peptides using a fully automated peptide synthesizer has been developed. Fmoc-protected monomers enable the preparation of hybrid peptides on automated peptide synthesizers without any major adjustments in the synthesis protocols, since the Fmoc-protecting group is standard in automated synthesis. Thus, different "mixed" peptide-aza-β^3-peptides are easily accessible and other aza β^3-peptidomimetics could be envisaged. These peptide-aza-β^3-peptidomimetic hybrids have been used to evaluate their biological activity compared with the histone H4 dodecapeptide (residues 88−99). Evaluation of the biological properties of these analogues of histone H4 is currently being undertaken and will be published elsewhere.[33]

Experimental Section

***N*-Boc-*N*-methylhydrazine.**[25] To a solution of methylhydrazine (3.07 g, 66.7 mmol) in $CHCl_3$ 60 mL cooled in ice bath, Boc_2O (13.11 g, 60 mmol) in $CHCl_3$ was slowly added dropwise. The mixture was then stirred at room temperature (rt) overnight and washed respectively with aqueous $NaHCO_3$ 1 M, water, and brine and dried over Na_2SO_4. Evaporation of solvent gave crude Boc-*N*-methylhydrazine (8.10 g, 92%) suitable for the next step without purification. ^{1}H NMR ($CDCl_3$) δ 1.5 (s, 9H, CH_3), 3.09 (s, 3H, CH_3), 4.08 (brs, 2H, NH_2).

Fmoc-Protected Alanine Hydrazine 2 (R = Me). To a stirred solution of Boc-*N*-methylhydrazine (8.10 g, 55.4 mmol) in THF/water (70/70 mL) was first added solid $NaHCO_3$ (9.3 g, 2 equiv) and then, dropwise, a mixture of FmocCl (15.72 g, 1.1 equiv) in THF (70 mL). The mixture was stirred overnight at rt, and ether (100 mL) was added. The organic layer was washed with brine, dried over Na_2SO_4, and concentrated. The residual oil was taken up with dichloromethane (DCM) (200 mL) and HClg overnight to remove Boc protection. The mixture was concentrated and taken up with ether, giving by filtration the hydrochloride of **2** (R = Me) as a white powder. We generated hydrazine in solution of water−ethyl acetate (EtOAc) by adding saturated aqueous $NaHCO_3$. The organic layer was dried over Na_2SO_4 and concentrated, giving a white solid that was triturated with petroleum ether to afford a white powder (13.28 g, 90%): mp 160 °C; ^{1}H NMR ($CDCl_3$) δ 2.68 (s, 3H, CH_3), 4.28 (t, J = 6.6 Hz, 1H, CH), 4.50 (d, J = 6.6 Hz, 2H, CH_2), 6.33 (brs, 1H, NH), 7.30−7.83 (m, 8H, CH_{ar}). ^{13}C NMR ($CDCl_3$) δ 157.2 (s), 143.7 (s), 141.4 (s), 127.8 (d), 127.1 (d), 125.0 (d), 120.0(d), 67.0 (t), 47.2 (d), 39.2 (q). HRMS (ESI) *m/z* calcd for $C_{16}H_{16}N_2O_2Na$ $[M + Na]^+$: 291.11095, found: 291.1104 (2 ppm).

Fmoc-Aza-β^3-Ala-OH 3 (R = Me). To a stirred solution of **2** (R = Me) (1.50 g, 5.6 mmol) in DCM/MeOH (10/20 mL) was added glyoxylic acid monohydrate (0.62 g, 1.2 equiv). $NaBH_3$-CN (0.42 g, 1.2 equiv) was added to the above mixture, and pH was brought to 3 by slowly adding a solution of 2 N HCl. After being stirred for 0.5 h, the pH was adjusted to 1 during 10 min and increased to 4 with solid $NaHCO_3$. The mixture was filtrated, concentrated and taken up with EtOAc (50 mL), and washed with water and brine. The organic layer was dried over Na_2SO_4 and concentrated to afford the final product as a moss that was purified by chromatography on silica gel (ether/MeOH/AcOH 95/5/0.25) to give **3** R = Me: (1.68 g, 92%). ^{1}H NMR ($CDCl_3$) δ 2.75 (s, 3H, CH_3), 3.62 (s, 2H, NCH_2), 4.21 (t, J = 6.6 Hz, 1H, CH), 4.49 (d, J = 6.6, 2H, CH_2), 6.57 (brs, 1H, NH), 7.26−7.81 (m, 8H, CH_{ar}). ^{13}C NMR ($CDCl_3$) δ 173.1 (s), 156.6 (s), 143.5 (s), 141.3 (s), 127.9 (d), 127.2 (d), 125.1 (d), 120.1 (d), 67.4 (t), 59.0 (t), 47.0 (d), 45.1 (q). HRMS (ESI): *m/z* calcd for $C_{18}H_{17}N_2O_4Na_2$ $[M - H + 2Na]^+$: 371.09837, found: 371.0981 (1 ppm).

Fmoc-Aza-β^3-Leu-OH 3 (R = $CH_2CH(CH_3)_2$). Hydrazine (**2**, R = $CH_2CH(CH_3)_2$)[22] (2.00 g, 6.46 mmol), glyoxylic acid monohydrate (0.71 g, 1.2 equiv), and $NaBH_3CN$ (0.48 g, 1.2 equiv) were reacted according to the procedure of **3** R = CH_3. Yield: 64%; (1.52 g); mp 131 °C; ^{1}H NMR ($CDCl_3$) δ 0.93 (d, 6H, J = 6.4 Hz, CH_3), 1.59 (m, 1H, CH), 2.70 (d, 2H, J = 7.0 Hz, CH_2), 3.55 (s, 2H, CH_2), 4.23 (t, 1H, J = 6.1 Hz, CH), 4.58 (d, 2H, J = 6.1 Hz, CH_2), 6.02 (s, 1H, NH), 7.28−7.79 (m, 8H, CH_{ar}). ^{13}C NMR ($CDCl_3$) δ 173.5 (s), 157.1 (s), 143.6 (s), 141.4 (s), 127.8 (d), 127.2 (d), 125.1 (d), 120.1 (d), 67.1 (t), 65.8 (t), 59.4 (t), 47.2 (d), 26.5 (d), 20.6 (q). HRMS (ESI) *m/z* calcd for $C_{19}H_{22}N_2O_2Na$ $[M + Na]^+$: 333.15790, found: 333.1580 (0 ppm).

Fmoc-Protected Tyrosine Hydrazine 2 (R = $EtOCH_2O-C_6H_4-CH_2$). Fmoc carbazate (7.64 g, 30 mmol) was added to a stirred solution of 4-ethyloxymethylbenzaldehyde (5.41 g, 1 equiv) in DCM (100 mL) at rt. The reaction mixture was stirred for 24 h and concentrated under vacuum to give crude solid that was purified by crystallization from ethanol to afford (**1**) as a white powder (10.00 g, 80%): mp 159 °C; ^{1}H NMR ($CDCl_3$) δ 1.24 (t, 3H, J = 7.0 Hz, CH_3), 3.76 (q, 2H, J = 7.0 Hz, CH_2), 4.36 (t, 1H, J = 7.1 Hz, CH), 4.58 (d, 2H, J = 7.1 Hz, CH_2), 5.28 (s, 2H, CH_2), 7.08 (d, J = 8.2 Hz, 1H, CH), 7.32−8.1 (m, 12H, CH_{ar}). ^{13}C NMR ($CDCl_3$) δ 158.9 (s), 144.8 (s), 143.7 (s), 141.3 (s), 128.7 (d), 127.4 (d), 116.3 (d), 128.8 (d), 127.8 (d), 125.2 (d), 120.0 (d), 92.9 (t), 67.8 (t), 64.5 (t), 47.0 (d), 15.1 (q). HRMS (ESI) *m/z* calcd for $C_{25}H_{24}N_2O_4Na$ $[M + Na]^+$: 439.16338, found: 439.1630 (1 ppm).

Then Fmoc-protected tyrosine hydrazone (8.24 g, 19.8 mmol) was dissolved in a solution of DCM/MeOH 40/60 mL. Sodium cyanoborohydride (1.5 g, 1,2 equiv) was added, and we brought the pH to 3 or 4 by slowly adding a solution of 2 N HCl. The mixture was stirred for 0.5 h, and then the pH was adjusted to 1. After 10 min of being stirred, the solution was neutralized with solid $NaHCO_3$, the mixture was concentrated under vacuum, and the residue was taken up with EtOAc (50 mL) and washed with water and brine. The organic layer was dried over Na_2SO_4, and the solvent was removed to give crude oil that was purified by chromatography on silica gel (PE/EtOAc 1/1) to afford (**2** R = $EtOCH_2O-C_6H_4-CH_2$) as a white solide (6.12 g 69%): mp 109 °C; ^{1}H NMR ($CDCl_3$) δ 1.25 (t, 3H, J = 7.1 Hz, CH_3), 3.76 (q, 2H, J = 7.1 Hz, CH_2), 3.96 (brs, 2H, CH_2), 4.25 (t, 1H, J = 6.6 Hz, CH), 4.39 (d, 2H, J = 6.6 Hz, CH_2), 5.24 (s, 2H, CH_2), 6.28 (brs, 1H, NH), 7.01−7.82 (m, 12H,

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CH_{ar}). ^{13}C NMR ($CDCl_3$) δ 157.8 (s), 144.4 (s), 142.0 (s), 131.2 (s), 130.9 (s), 128.4 (d), 127.7 (d), 125.6 (d), 120.7 (d), 116.9 (d), 93.8 (t), 67.5 (t), 64.9 (t), 55.6 (t), 47.8 (t), 15.8 (q). HRMS (ESI) *m/z* calcd for $C_{25}H_{26}N_2O_4Na$ $[M + Na]^+$: 441.17903, found: 441.1788 (0 ppm).

Fmoc-Aza-β^3-Tyr(OCH_2OEt)-OH 3 (R = $EtOCH_2O-C_6H_4-CH_2$). Hydrazine **2** (R = $EtOCH_2O-C_6H_4-CH_2$) (1.50 g, 3.60 mmol), glyoxylic acid monohydrate (0.40 g, 1.2 equiv), and $NaBH_3CN$ (0.34 g, 1.5 equiv) were reacted according to the procedure of **3** R = CH_3. Yield: 60% (1.03 g); mp 114 °C; ^1H NMR ($CDCl_3$) δ 1.21 (t, 3H, J = 7.0 Hz, CH_3), 3.63 (s, 2H, CH_2), 3.70 (q, 2H, J = 7.0 Hz, CH_2), 4.00 (s, 2H, CH_2), 4.17 (br t, 1H, CH), 4.48 (br d, 2H, CH_2), 5.18 (s, 2H, CH_2), 6.39 (br s, 1H, NH), 7.00−7.80 (m, 12H, CH_{ar}). ^{13}C NMR ($CDCl_3$) δ 173.3 (s), 157.2 (s), 143.7 (s), 141.4 (s), 130.8 (s), 128.9 (d), 116.3 (d), 127.9 (d), 127.2 (d), 125.2 (d), 120.1 (d), 93.1 (t), 67.3 (t), 64.3 (t), 60.4 (t), 56.6 (t), 47.1 (d), 15.2 (q). HRMS (ESI) *m/z* calcd for $C_{27}H_{28}N_2O_6Na$ $[M + Na]^+$: 499.18451, found: 499.1843 (0 ppm).

Fmoc-Protected Lysine Hydrazine 2 (R = $(CH_2)_4NHBoc$). Fmoc carbazate **1** (9.50 g, 37.3 mmol) was added to a stirred solution of 4-*t*-butoxycarbonylaminobutanal[27] (7.00 g, 37.3 mmol) in DCM (150 mL) at rt. The reaction mixture was stirred for 24 h and concentrated under vacuum to give crude solid that was triturated in petroleum ether to afford as a white solid (14.3 g, 90%): mp 165 °C; ^1H NMR (DMSO) δ 1.38 (s, 9H, CH_3), 1.56 (m, 2H, CH_2), 2.16 (m, 2H, CH_2), 2.94 (m, 2H, CH_2), 4.26 (t, 1H, J = 6.9 Hz, CH), 4.39 (d, 2H, J = 6.9 Hz, CH_2), 6.84 (brs, 1H, NH), 7.29−7.92 (m, 9H, Ar + CH), 10.72 (s, 1H, NH). ^{13}C NMR (DMSO) δ 155.9 (s), 153.7 (s), 148.2 (s), 144.1 (s), 141.1 (s), 128.0 (d), 127.5 (d), 125.5 (d), 120.5 (d), 77.8 (s), 65.8 (t), 46.9 (d), 39.7 (t), 29.6 (t), 28.6 (q), 26.89 (CH_2). HRMS (ESI) *m/z* calcd for $C_{24}H_{29}N_3O_4Na$ $[M + Na]^+$: 446.20558, found: 446.2053 (1 ppm).

Then Fmoc-protected lysine hydrazone (1.25 g, 2.94 mmol) was reduced as described before with sodium cyanoborohydride (0.53 g, 1.5 equiv). The crude solid was purified by chromatography on silica gel (EtOAc/DCM 4/6) to give **2** (R = $(CH_2)_4$-NHBoc) as a white solid (0.70 g 56%): mp 150 °C; ^1H NMR ($CDCl_3$) δ 1.46 (s, 9H, CH_3), 1.52 (m, 4H, CH_2), 2.88 (m, 2H, CH_2), 3.16 (m, 2H, CH_2), 4.00 (brs, 1H, NH), 4.24 (t, 1H, J = 6.6 Hz, CH), 4.47 (d, 2H, J = 6.9 Hz, CH_2), 4.62 (brs, 1H, NH), 6.37 (brs, 1H, NH), 7.29−7.80 (m, 8H, Ar). ^{13}C NMR ($CDCl_3$)δ 157.3 (s), 156.1 (s), 143.7 (s), 141.3 (s), 127.8 (d), 127.1 (d), 124.0 (d), 120.0 (d), 79.1 (s), 66.9 (t), 51.5 (t), 47.21 (d), 40.40 (t), 28.5 (q), 27.6 (t), 24.8 (t). HRMS (ESI) calcd for $C_{24}H_{31}N_3O_4$-Na $[M + Na]^+$: 448.22123, found: 448.2222 (2 ppm).

Fmoc-Aza-β^3-Lys(Boc)-OH 3 (R = $(CH_2)_4NHBoc$). Hydrazine (**2**, R = $(CH_2)_4NHBoc$) (2.89 g, 6.76 mmol), glyoxylic acid monohydrate (0.75 g, 1.2 equiv), and $NaBH_3CN$ (0.51 g, 1.2 equiv) were reacted according to the procedure of **3** R = CH_3. Yield 59% (2.00 g); ^1H NMR ($CDCl_3$) δ 1.44 (s, 9H, CH_3), 1.40−1.60 (m, 4H, CH_2), 2.89 (m, 2H, CH_2), 3.09 (m, 2H, CH_2), 3.66 (s, 2H, CH_2), 4.21 (br t, 1H, CH), 4.46 (br d, 2H, CH_2), 4.87 (br s, 1H, NH), 7.04 (br s, 1H, NH), 7.29−7.80 (m, 8H, Ar), 9.72 (brs, 1H, CO_2H). ^{13}C NMR ($CDCl_3$) δ 173.0 (s), 157.3 (s), 156.1 (s), 143.6 (s), 141.3 (s), 127.8 (d), 127.1 (d), 125.0 (d), 120.0 (d), 79.3 (s), 67.0 (t), 58.4 (t), 56.5 (t), 47.2 (d), 40,2 (d), 28.4 (q), 27.2 (t), 24.3 (t). HRMS (ESI) *m/z* calcd for $C_{26}H_{33}N_3O_6$-Na $[M + Na]^+$: 506.22671, found: 506.2276 (2 ppm).

Boc-Aza-β^3-Gly-OBn (4). To a solution of Boc carbazate (2.00 g, 15.13 mmol) in toluene (25 mL) were added diisopropylethylamine (DIEA) (1.96 g, 1 equiv) and benzyl 2-bromoacetate (3.47 g, 1 equiv). The mixture was stirred at 75 °C for 4 days, filtrated, and concentrated to give a crude oil that was purified by chromatography on silica gel (EtOAc/DCM 1/9) to give a colorless oil (2.60 g, 61%) that slowly crystallized; mp 40 °C. ^1H NMR ($CDCl_3$) δ 1.47 (s, 9H, CH_3), 3.72 (s, 2H, CH_2), 5.19 (s, 2H, CH_2), 6.43 (br s, 1H, NH), 7.31−7.42 (m, 5H, CH_{ar}). ^{13}C NMR ($CDCl_3$) δ 171.5 (s), 156.7 (s), 135.8 (s), 129.0 (d), 128.8 (d), 128.7 (d), 80.9 (s), 67.0 (t), 53.2 (t), 28.7(q). HRMS (ESI) *m/z* calcd for $C_{14}H_{20}N_2O_4Na$ $[M + Na]^+$: 303.13208, found: 303.1327 (2 ppm).

Aza-β^3-Gly(Boc)-OBn (6). A solution of **4** (2.23 g, 7.95 mmol) in DCM 30 mL was saturated by HClg and stirred at rt overnight. The mixture was concentrated and taken up with ether, giving by filtration the hydrochloride of **5** as a white powder (1.47 g), which was added into a stirred solution of Et_3N (0.76 g, 1.1 equiv) in 20 mL of DCM. Boc_2O (1.47 g, 1 equiv) was added, and the mixture was stirred overnight, concentrated, and taken up with ether. After cooling for 4 h, the hydrochloride of Et_3N was removed by filtration, and the filtrate was concentrated. The crude oil was purified by chromatography on silica gel (EtOAc/EP 3/7) to give **6** (1.15 g, 52%) as a colorless oil. ^1H NMR ($CDCl_3$) δ 1.40 (s, 9H, CH_3), 4.17 (s, 2H, CH_2), 5.17 (s, 2H, CH_2), 7.34−7.41 (m, 5H, CH_{ar}). ^{13}C NMR ($CDCl_3$) δ 170.4 (s), 158.0 (s), 136.1 (s), 129.2 (d), 129.0 (d), 128.9 (d), 81.9 (s), 67.4 (t), 54.1 (t), 28.8 (q). HRMS (ESI) *m/z* calcd for $C_{14}H_{20}N_2O_4Na$ $[M + Na]^+$: 303.13208, found: 303.1309 (4 ppm).

Fmoc-Aza-β^3-Gly(Boc)-OBn (7). To a stirred solution of **6** (1.83 g, 6.52 mmol) in THF/water (15/15 mL) was first added solid $NaHCO_3$ (1.09 g, 2 equiv) and then, dropwise, a mixture of FmocCl (2.02 g, 1.2 equiv) in THF (15 mL). The mixture was stirred overnight at rt, and then ether (50 mL) was added. The organic layer was washed with brine, dried over Na_2SO_4, and concentrated to afford an oil that was purified by chromatography on silica gel (EP/EtOAc 9/1 and 7/3) to give 2.31 g (71%) of **7** as a white powder: mp 140 °C; ^1H NMR ($CDCl_3$) δ 1.44 (s, 9H, CH_3), 4.26 (brs, 2H, CH_2), 4.35 (brs, 1H, CH), 4.47 (br, 2H, CH_2), 5.21 (s, 2H, CH_2), 6.96 (br s, 1H, NH), 7.30−7.79 (m, 13H, CH_{ar}). ^{13}C NMR ($CDCl_3$) δ 170.0 (s), 156.5 (s), 155.4 (s), 144.1 (s), 141.9 (s), 135.8 (s), 129.3 (d), 129.2 (d), 129.0 (d), 128.9 (d), 128.5 (d), 125.6 (d), 120.7 (d), 83.1 (s), 68.6 (t), 67.8 (t), 53.5 (t), 47.7 (d), 28.7 (q). HRMS (ESI) *m/z* calcd for $C_{29}H_{30}N_2O_6Na$ $[M + Na]^+$: 525.20016, found: 525.2001 (0 ppm).

Fmoc-Aza-β^3-Gly(Boc)-OH 3 (R = Boc). Catalytic hydrogenolyse with 10% Pd/C (80 mg) of **7** (1.40 g, 2.79 mmol) afforded 1.10 g (96%) of **3** (R = Boc) as a moss. ^1H NMR ($CDCl_3$) δ 1.47 (m, 9H, CH_3), 4.05−4.38 (br m, 3H, CH + CH_2), 4.52 (d, 2H, J = 6.8 Hz, CH_2), 5.21 (s, 2H, CH_2), 6.96 (br s, 1H, NH), 7.30−7.79 (m, 8H, CH_{ar}). ^{13}C NMR ($CDCl_3$) δ 169.8 (s), 156.5 (s), 155.2 (s), 144.0 (s), 141.7 (s), 135.6 (s), 129.1 (d), 128.9 (d), 128.8 (d), 128.3 (d), 127.6 (d), 125.5 (d), 120.5 (d), 82.9 (s), 68.4 (t), 67.6 (t), 53.3 (t), 47.4 (d), 28.49 (q). HRMS (ESI) *m/z* calcd for $C_{22}H_{24}N_2O_6Na$ $[M + Na]^+$: 435.1532, found: 435.1533 (0 ppm).

1-Boc-amino-3,3-diethoxypropane.[29] A mixture of Boc_2O (9 g, 40 mmol) in dioxane (40 mL) was added dropwise to a stirred solution of 1-amino-3,3-diethoxypropane (5.52 g, 37 mmol) and Et_3N (4.04 g, 40 mmol) in dioxane (5 mL) at 0 °C. After 2 h, the mixture was allowed to warm to rt. Stirring was continued overnight, and the solvent was evaporated. The residual oil was taken up in water (10 mL), and the mixture was acidified with aqueous solution of 1 N HCl (pH 3−4) and extracted with EtOAc (60 mL × 3). The organic layer was dried (Na_2SO_4) and evaporated to give crude oil of 1-Boc-amino-3,3-diethoxypropane 8.89 g (90%) suitable for further work without purification. ^1H NMR ($CDCl_3$) δ 1.25 (t, 6H, J = 8.8 Hz, CH_3), 1.48 (s, 9H, CH_3), 1.85 (q, 2H, J = 6.3 Hz, CH_2), 3.26 (q, 2H, J = 6.1 Hz, CH_2), 3.22−3.77 (m, 6H, CH_2), 4.58 (t, 1H, J = 6.3 Hz, CH), 4.95 (br s, 1H, NH).

3-Boc-aminopropanal.[29] A solution of 1-Boc-amino-3,3-diethoxypropane (8.89 g, 36 mmol) in AcOH (15 mL) and H_2O (4 mL) was stirred at rt for 10 h, neutralized with Na_2CO_3, taken up in ether, and washed with water and brine. The organic phase was evaporated under vacuum to give a yellow oil used as such in the next step (6.31 g, 98%). ^1H NMR ($CDCl_3$) δ 1.47 (s, 9H, CH_3), 2.75 (t, 2H, CH_2), 3.46 (m, 2H, CH_2), 4.95 (br s, 1H, NH), 9.85 (s, 1H, CHO).

Fmoc-Protected Ornitine Hydrazine 2 (R = $(CH_2)_3$-NHBoc). Fmoc carbazate **1** (9.24 g, 36.4 mmol) was added to

a stirred solution of 3-Boc-aminopropanal (6.31 g, 36.4 mmol) in DCM (150 mL) at rt. The reaction mixture was stirred for 12 h and concentrated under vacuum to give crude solid that was triturated with petroleum ether to afford the hydrazone as a white solid (13.2 g, 89%). ^{1}H NMR (DMSO) δ 1.38 (s, 9H, CH_3), 2.31 (m, 2H, CH_2), 3.11 (m, 2H, CH_2), 4.28 (t, 1H, J = 6.8 Hz, CH), 4.41 (d, 2H, J = 6.8 Hz, CH_2), 6.91 (br, 1H, NH), 7.28−7.93 (m, 9H, Ar + CH), 10.84 (s, 1H, NH). ^{13}C NMR (DMSO) δ 155.5 (s), 153.2 (s), 146.2 (s), 143.7 (s), 140.72 (s), 127.6 (d), 127.0 (d), 125.1 (d), 120.1 (d), 77.5 (s), 65.4 (t), 46.6 (d), 37.3 (t), 28.8 (t), 28.2 (q).

Then, Fmoc-protected hydrazone (3 g, 7.31 mmol) was reduced with sodium cyanoborohydride (0.55 g, 1.2 equiv) as described previously. The crude solid was triturated with petroleum ether (PE) to afford a white solid of **2** (R = $(CH_2)_3$-NHBoc) (2.48 g 82%): mp 101 °C; ^{1}H NMR (DMSO) δ 1.40 (s, 9H, CH_3), 1.50 (m, 2H, CH_2), 2.68 (m, 2H, CH_2), 2.98 (m, 2H, CH_2), 4.24 (t, 1H, J = 6.9 Hz, CH), 4.32 (d, 2H, J = 6.9 Hz, CH_2), 4.70−4.85 (br s, 1H, NH), 6.78 (br s, 1H, NH), 6.80 (br s, 1H, NH), 7.25−7.90 (m, 8H, Ar). ^{13}C NMR (DMSO) δ 157.2 (s), 155.9 (s), 144.2 (s), 142.9 (s), 128.0 (d), 127.6 (d), 125.6 (d), 120.5 (d), 77.7 (s), 65.8 (t), 47.1 (d), 38.4 (t), 28.6 (q), 28.2 (t). Anal. Calcd for $C_{23}H_{29}N_3O_4$ (411.22): C, 67.12; H, 7.11; N, 10.22. Found: C, 67.22; H, 7.19; N, 10.24.

Fmoc-Aza-β^3-Orn(NBoc)-OBn (8, R = $(CH_2)_3$NHBoc)). A mixture of Fmoc-protected ornitine hydrazine **2** (R = $(CH_2)_3$-NHBoc) (3.7 g, 9 mmol), benzyl 2-bromoacetate (2.66 g, 11.6 mmol), toluene (20 mL), and dry K_2CO_3 (870 mg, 0.7 equiv) was refluxed under stirring for 28 h. Usual workup afforded after chromatography on silica gel (EtOAc/EP 1/3) 3.02 g (60%) of **8** (R = $(CH_2)_3$NHBoc) as a colorless oil that slowly crystallized in ether: mp 107 °C; ^{1}H NMR ($CDCl_3$) δ 1.50 (s, 9H, CH_3), 1.62 (m, 2H, CH_2), 2.97 (m, 2H, CH_2), 3.24 (m, 2H, CH_2), 3.75 (m, 2H, CH_2), 4.20 (t, 1H, J = 6.9 Hz, CH), 4.50 (d, 2H, J = 6.9 Hz, CH_2), 5.19 (s, 2H, CH_2), 6.88 (brs, 1H, NH), 7.25−7.90 (m, 13H, Ar). ^{13}C NMR ($CDCl_3$) δ 171.23 (s), 156.55 (s), 155.50 (s), 144.13, 141.77 (s), 135.54 C (s), 129.13, 129.04, 128.84 (d), 128.14, 127.47, 125.44, 120.40 (d), 79.38 (s), 67.09 (t), 64.47 (t), 57.63 (t), 54.56 (t), 47.67 (d), 38.83 (t), 28.85 (q), 27.83 (t). Anal. Calcd for $C_{32}H_{37}N_3O_6$ (559.27): C, 68.66; H, 6.67; N, 7.51. Found: C, 68.59; H, 6.86; N, 7.28.

Fmoc-Aza-β^3-Arg(Boc)$_2$OBn (8, R = $(CH_2)_3$NHC(NHBoc)-NBoc). To a solution of **8** (R = $(CH_2)_3$NHBoc) (1 g, 1.79 mmol) in DCM (5 mL), 5 mL of TFA was added. After stirring at rt overnight, 25 mL of DCM and 10 mL of water were added. The mixture was neutralized with solid Na_2CO_3 (pH 8−9), and the organic layer was washed with brine, dried over Na_2SO_4, and partly concentrated, giving 10−15 mL of DCM solution. Then NEt_3 (1.1 equiv, 251 μL) and $(BocNH)_2C{=}NTf$ (0.9 equiv, 0.64 g) were added. After being stirred at rt for 15 h, the solution was washed, respectively, with a 2 N aqueous solution of $NaHSO_4$ (10 mL), Na_2CO_3 (10 mL), and brine (10 mL). The organic layer was dried over Na_2SO_4 and concentrated, giving a crude oil that was purified by chromatography (EP/EtOAc 7/3) to afford the guanidine **8** (R = $(CH_2)_3$NHC(NHBoc)NBoc) (1.00 g, 80%). ^{1}H NMR ($CDCl_3$) δ 1.39 (s, 18H, CH_3), 1.60 (m, 2H, CH_2), 2.80 (m, 2H, CH_2), 3.32 (m, 2H, CH_2), 3.66 (m, 2H, CH_2), 4.24 (t, 1H, J = 7.1 Hz, CH), 4.45 (d, 2H, J = 7.1 Hz, CH_2), 5.05 (s, 2H, CH_2), 6.95 (brs, 1H, NH), 7.27−7.82 (m, 13H, Ar), 8.28 (br s, 1H, NH), 11.45 (br s, 1H, NH). ^{13}C NMR $CDCl_3$ δ 171.45 (s), 170.80 (s), 163.97 (s), 156.57 (s), 153.59 (s), 144.17 (s), 141.73 (s), 135.67 (d), 129.06, 128.91, 128.78 (d), 128.09, 127.45, 125.47, 120.34 (d), 83.35 (s), 79.48 (s), 66.93 (t), 60.74 (t), 58.04 (t), 53.89 (t), 47.64 (d), 38.83 (t), 28.67 (q), 28.44 (q), 27.45 (t). Anal. Calcd for $C_{38}H_{47}N_5O_8$ (701.34): C, 65.02; H, 6.75; N, 9.98. Found: C, 65.10; H, 6.83; N, 9.98.

Fmoc-Aza-β^3-Arg(Boc)$_2$−OH 3 (R = $(CH_2)_3$NHC(NHBoc)-NBoc). Catalytic hydrogenolysis with 10% Pd/C (50 mg) of **8** (R = $(CH_2)_3$NHC(NHBoc)NBoc) (0.86 g, 0.5 mmol) afforded 0.72 g (96%) of **7** as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 1.38 (s, 9H, CH_3), 1.39 (s, 9H, CH_3), 1.62 (m, 2H, CH_2), 2.89 (m, 2H, CH_2), 3.38 (m, 2H, CH_2), 3.60 (m, 2H, CH_2), 4.14 (t, 1H, J = 7.1 Hz, CH), 4.40 (d, 2H, J = 7.1 Hz, CH_2), 7.27−7.82 (m, 8H, Ar), 7.95 (brs, 1H, NH), 8.50 (brs,1H, NH), 11.50 (brs, 1H, NH). ^{13}C NMR ($CDCl_3$) δ 171.35 (s), 170.70 (s), 163.90 (s), 157.45 (s), 153.57 (s), 143.96 (s), 141.75 (s), 128.18 (d), 127.52 (d), 125.44 (d), 120.38 (d), 83.35 (s), 79.60 (s), 67.42 (t), 58.04 (t), 53.89 (t), 47.61 (d), 38.83 (t), 28.59 (q), 28.46 (q), 27.47 (t). HRMS *m/z* calcd for $C_{31}H_{41}N_5O_8$ (M^+): 611.2955, found: 611.2958. Anal. Calcd for $C_{31}H_{41}N_5O_8$ (611.29): C, 60.85; H, 6.76; N, 11.45. Found: C, 60.95; H, 6.78; N, 11.47.

H$_2$N-Tyr-Aza-β^3-Ala-Leu-Lys-Arg-Gln-Gly-Arg-Thr-Leu-Tyr-Gly-OH (Aza-β^3-A89). Aza-β^3-A89 peptide was synthesized on a resin loaded with the first amino acid: Fmoc-Gly-PEG-PS (1 g, 0.2 mmol; loading 0.2 mmol/g) on the Millipore 9050 Plus PepSynthesizer. The resin was swelled with DMF for 3 min with 1.5 mL/min flow rate. After cleavage of the Fmoc group using 20% piperidine in DMF (1 and 5 min, 5 mL/min), the resin was washed with DMF during 7 min (5 mL/min). Subsequently, 4 equiv of the appropriate amino acid were dissolved in HOBt/DMF (1.4 mL, 0.6 M) and DIC/DMF (1.4 mL, 0.6 M), and the activated amino acid was then transferred to the reaction vessel. After 30−80 min for the analogue the reaction vessel was washed by DMF during 4 min (5 mL/min). The deprotection and the coupling were followed by monitoring the dibenzofulvene−piperidine adduct at 301 nm. The peptide backbone was elongated step by step as described in Scheme 5. Finally, the Fmoc protective group was removed by treatment with 20% piperidine in DMF (1 and 5 min, 5 mL/min), and the resin was washed with DMF (7 min, 5 mL/min), removed from the reaction vessel, washed with DCM and ether, and dried in vacuo. The anchored hybrid peptide thus obtained was cleaved from the solid support by treatment with TFA/phenol/TIS/water (88/5/2/5) for 2 h. The mixture was then filtered, and the resin was washed thoroughly with TFA and then with DCM. The total filtrate was concentrated in vacuo to a volume of approximately 1 to 2 mL, and then cold ether (10 mL) was added to precipitate the peptide. The precipitate peptide was collected by filtration through a fritted glass funnel and dried in vacuo. The crude product was purified by reversed-phased preparative HPLC (XTerra RP18 19 × 300 10 μm) (5−65% B in 30 min). HPLC fraction was freeze-dried to give the target hybrid peptide as a bis-trifluoroacetate salt (118 mg, 36%). Analytical RP-HPLC (5−65% B in 30 min) t_r = 17.45 min, purity > 99%. HRMS (ESI) *m/z* calcd for $C_{22}H_{24}N_2O_6Na$ $[M + H]^+$: 1440.8076, found: 1440.8074.

H$_2$N-Tyr-Ala-Leu-Lys-Aza-β^3-Arg-Gln-Gly-Arg-Thr-Leu-Tyr-Gly-OH (aza-β^3-R92). Aza-β^3-R92 was synthesized on a resin loaded with the first amino acid (Fmoc-Gly-Wang) (200 mg, 0.60 mmol) using the Advanced Chem Tech 440 Mos synthesizer. A quantity of 200 mg of resin was swelled with DMF (2.5 mL) during 15 min. After cleavage of the Fmoc group by 20% piperidine solution in DMF (2 × 2.5 mL; 5 and 10 min), the resin was washed with DMF (5 × 2.5 mL). Subsequently, 4 equiv of the appropriate amino acid were dissolved in DMF/HOBt (960 μL, 0.5 M), and the activated amino acid was then transferred to the reaction vessel as well as DIC in DMF (960 μL, 0.5 M). After 30−80 min for the analogue, the reaction vessel was drained and the resin was washed by DMF (5 × 2.5 mL). The peptide backbone was elongated step by step as described in Scheme 5. Finally, the Fmoc protective group was removed by the treatment with 20% piperidine in DMF (2 × 2.5 mL; 5 and 10 min), and the resin was washed with DMF (5 × 2.5 mL) and DCM (6 × 2.5 mL), removed from the reaction vessel, washed with ether, and dried in vacuo. The anchored hybrid peptide thus obtained was cleaved from the solid support and purified as described before to afford the peptide showing a purity > 99% (84 mg, 26%). Analytical RP-HPLC (5−65% B in 30 min) t_r = 18.01 min, purity > 99%. HRMS (ESI) *m/z* calcd for $C_{64}H_{106}N_{21}O_{17}$ $[M + H]^+$: 1440.8076, found: 1440.8053 (2 ppm).

H$_2$N-Tyr-Ala-Leu-Lys-Arg-Gln-Gly-Arg-Thr-Leu-Tyr-Aza-β^3-Gly-OH (Aza-β^3-G99). Fmoc-aza-β^3-Gly(Boc)-OH was first loaded on resin. All manipulations were carried out under

N_2. Wang resin (1 g, 200−400 mesh, 0.68 mmol/g) was swelled in DMF (10 mL) for 30 min. Fmoc-aza-β^3-Gly(Boc)-OH (1.12 g, 4 equiv relative to resin loading) was dissolved in dry DCM (10 mL) (one or two drops of DMF may be needed to aid complete dissolution). DIC (5 equiv) was added to the solution, which was stirred for 20 min at 0 °C under N_2. The DCM was removed by evaporation, and the residue was dissolved in the minimum of DMF (3 mL) and added to the resin with 0.1 equiv of DMAP (8 mg). The mixture was mixed for 2 h by N_2 bubbling. A resin sample was removed to determine the level of Fmoc-aza-β^3-Gly(Boc)-OH attachment by Fmoc cleavage. The dry Fmoc-aza-β^3-Gly(Boc) resin was weighed (3−5 mg) into each of two UV cells, 20% of piperidine in DMF (3 mL) was added, and the solution was agitated for 3 min. The absorbance of the UV absorption of the dibenzofulvene−piperidine adduct gave the estimate loading of the Fmoc-aza-β^3-Gly-OH monomer, which is 0.61 mmol/g. Aza-β^3-G99 was then synthesized in a procedure analogous to that of aza-β^3-R92 (80 mg, 24%). Analytical RP-HPLC (5−65% B in 30 min) t_r = 18.08 min, purity > 99%. HRMS (ESI) *m/z* calcd for $C_{64}H_{106}N_{21}O_{17}$ $[M + H]^+$: 1440.8076, found: 1440.8057 (2 ppm).

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Note Added after ASAP Publication. There were errors in Scheme 3, the footnotes of Schemes 3 and 4, the discussion of compounds **6** and **7** below Scheme 3, and the compound numbering for **8** in the Experimental Section in the version published ASAP November 8, 2005; the corrected version was published ASAP November 17, 2005.

Supporting Information Available: General considerations, procedure used for "mixed" peptide synthesis, and analytical data for aza-β^3-L90, aza-β^3-K91, aza-β^3-G94, aza-β^3-R95, aza-β^3-L97, aza-β^3-Y98, aza-β^3-A89-L90, aza-β^3-A89-L90-K91, and aza-β^3-L97-Y98. ^{1}H NMR spectra of *N*-Boc-*N*-methylhydrazine, 1-Boc-amino-3,3-diethoxypropane, and 3-Boc-aminopropanal and ^{1}H, ^{13}C NMR of Fmoc-protected ornithine hydrazone (R = $(CH_2)_3$NHBoc), and ^{13}C NMR spectra of Aza-β^3Leu97. This material is available free of charge via the Internet at http://pubs.acs.org.

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