JOC The Journal of Organic Chemistry

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Benzotriazole-Mediated Synthesis of Aza-peptides: En Route to an Aza-Leuenkephalin Analogue

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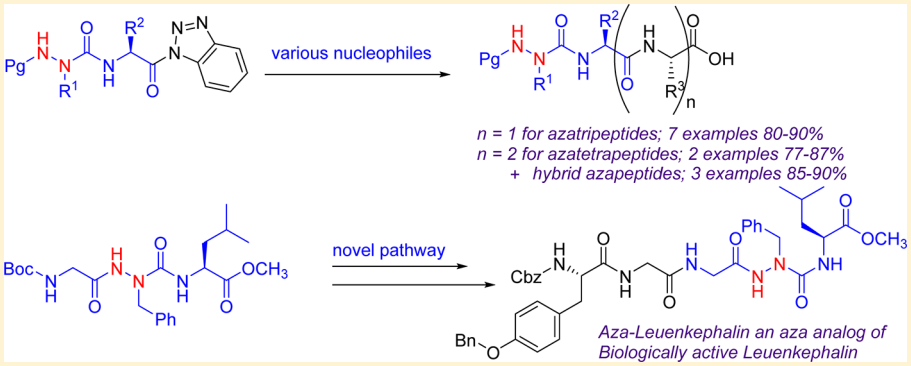
Supporting Information



ABSTRACT: Novel *N*-(*N*-Pg-azadipeptidoyl)benzotriazoles **20a−e** couple efficiently with α-amino acids **21a−e**, dipeptides **22a−c**, aminoxyacetic acid **23a**, depsidipeptide **23b**, and α-hydroxy-β-phenylpropionic acid **27** yielding, respectively, azatripeptides **24a−g**, azatetrapeptides **25a,b**, a hybrid azatripeptide with an oxyamide bond **26a**, a hybrid azatetrapeptide with an ester bond **26b**, and a hybrid azatripeptide with an ester bond **28**. A new protocol for the synthesis of *N*-Pg-azatripeptides **33a,b** and **35a,b**, each containing a natural amino acid at the *N*-terminus, avoids the low coupling rates of the aza-amino acid residue and enables the solution-phase synthesis of an azaphenylalanine analogue of Leu-enkephalin **40**.

INTRODUCTION

Azapeptides are peptidomimetics in which the α-CH groups of one or more amino acid residues are replaced by a nitrogen atom (Figure 1A, B). This decreases the electrophilicity of the

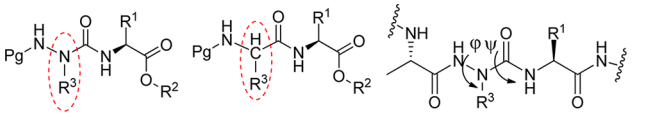


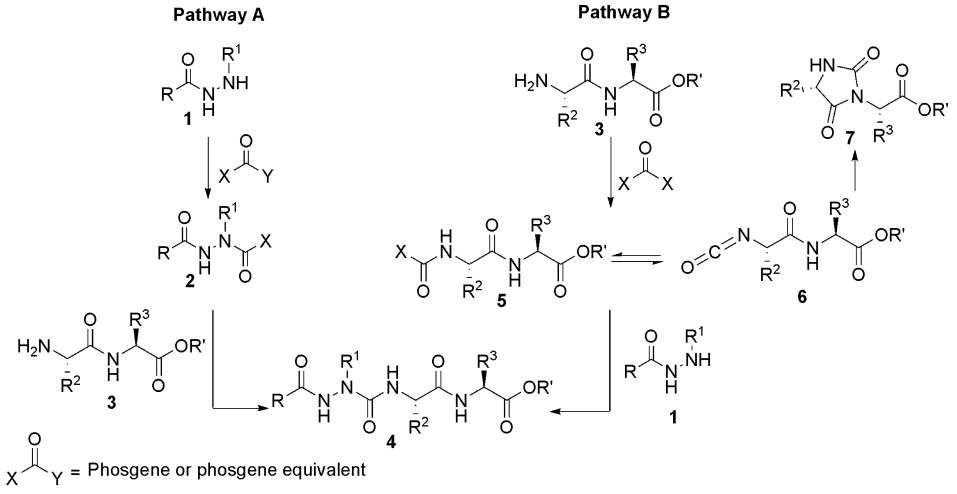
Figure 1. Comparison of azadipeptide and native dipeptide.

−(NR)CO− carbonyl group and changes the geometry at the α positions from tetrahedral to trigonal-planar, hence eliminating chirality. Relative to the natural peptides, azapeptides occupy a limited conformational space with dihedral angles (φ = 90° ± 30° or −90° ± 30° and ψ = 0° ± 30° or 180° ± 30°) close to those in the polyproline type II helix (φ = −78°, ψ = 149°) and other types of β-turns (Figure 1C).[1−4] The effect of the geometry of an aza-amino acid is similar to that of a β-turn in changing the chemical and biological properties of the parent peptide. Azapeptides may exhibit better interactions with protein receptors and enhanced stability to enzymatic and chemical degradation.[5,6] Azapeptides are therefore leads for the generation of receptor ligands, enzyme inhibitors, and clinically approved drugs.[7] Azapeptides possessing electrophilic moieties also act as inhibitors of cysteine proteases.[8−10]

Azapeptides can be synthesized both in solution and on solid phase by combining hydrazine chemistry and conventional peptide synthesis.[11] The nitrogen atoms of hydrazine need to be differentiated to produce *N*′-alkyl-*N*-Pg-hydrazines **1**; phosgene (or an equivalent) introduces the carbonyl group into the skeleton by reacting with either the hydrazine derivative **1** giving **2** which then reacts with dipeptide ester **3** (Scheme 1A). Alternatively, the phosgene reacts with the peptide *N*-terminus of dipeptide **3** (Scheme 1B).[12−14] Route B

Received: October 23, 2012
Published: February 1, 2013

Scheme 1. Construction of an Azapeptide by (A) a Hydrazine Pathway and (B) a Peptide *N*-Terminus Pathway



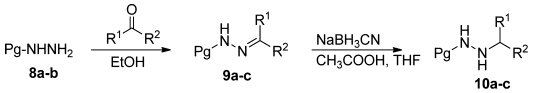
can produce a hydantoin byproduct **7** by intramolecular nucleophilic attack of the secondary amide nitrogen of the preceding amino acid residue on the activated isocyanate **6**. Lubell et al. coupled *N*-Boc-azadi- and tripeptide segments to the amine terminus of a growing peptide chain to circumvent formation of the hydantoin byproduct.[13]

N-Acylbenzotriazoles are advantageous for *N*-, *O*-, *C*-, and *S*-acylation, especially when the corresponding acid chlorides are unstable or difficult to prepare, and we have demonstrated the use of benzotriazolides for the synthesis of peptidomimetics such as aminoxypeptides and depsipeptides.[15−17] In the present work, stable, crystalline, and easy to handle *N*-(*N*-Pg-α-azadipeptidoyl)benzotriazoles **20a−e** were prepared, and their synthetic utility was demonstrated by the synthesis of *N*-Pg-azatripeptides **24a−h**, *N*-Pg-azatetrapeptides **25a,b**, and hybrid azapeptides containing oxyamide **26a** or depsi bonds **26b** and **28**. In addition, we developed a new route toward *N*-Pg-azatripeptides containing a natural amino acid at their *N*-terminus **33a,b** and **35a,b**. The novel pathway enabled the solution phase synthesis of aza-Leu-enkephalin analogue **40**.

RESULTS AND DISCUSSION

Preparation of *N*′-Alkyl-*N*-(Pg)-hydrazines. Reaction of Boc-$NHNH_2$ **8a** and Fmoc-$NHNH_2$ **8b** with the appropriate aldehyde or ketone followed by reduction with $NaCNBH_3$ furnished *N*′-alkyl *tert*-butyl carbazates **10a,b** and *N*′-alkyl fluorenyl-9-ylmethyl carbazates **10c** (Scheme 2). Selective protection of *N*-methyl hydrazine with $(Boc)_2O$ **11** afforded *N*′-Boc-*N*′-methyl hydrazine **12** which was coupled with benzyl chloroformate and fluorenyl-9-ylmethyl chloroformate to give *N*′-Boc-*N*′-methyl-*N*-Cbz-hydrazine **13a** and *N*′-Boc-*N*′-methyl-*N*-Fmoc-hydrazine **13b**. Subsequent Boc deprotection of **13a,b** afforded *N*′-methyl benzyl carbazates **14a** and *N*′-methyl fluorenyl-9-ylmethyl carbazates **14b** as their HCl salts in 70−73% overall yield (Scheme 3). Reaction of Cbz-$NHNH_2$ **13c** and methyl 2-bromoacetate in dry DMF in the presence of DIPEA gave benzyl 2-(2-methoxy-2-oxoethyl)hydrazine-1-carboxylate **14c** (Scheme 3).

Scheme 2. Preparation of *N*′-Alkyl-*N*-(Pg)-hydrazines 10a−c

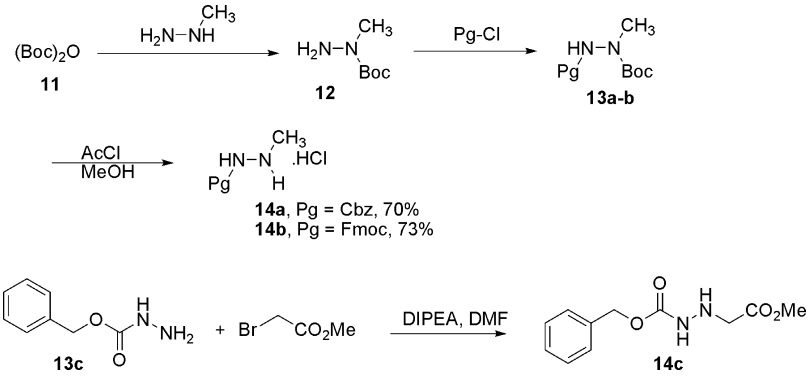


10a, Pg = Boc, $R^1 = R^2 = CH_3$, 70%
10b, Pg = Boc, R^1 = H, $R^2 = C_6H_5$, 75%
10c, Pg = Fmoc, $R^1 = R^2 = CH_3$, 68 %

Construction of Protected Azadipeptides. The activation of *N*′-alkyl *tert*-butyl carbazates into *N*-(Boc)aza-amino acid building blocks using *p*-nitrophenyl chloroformate,[18] bis(2,4-dinitrophenyl) carbonate, and carbonyldiimidazole (CDI)[9] gave nitrophenylcarbazates and imidazolides which required long reaction times and high temperatures to provide aza-peptides in poor yields. Phosgene and triphosgene activate *N*′-alkyl *tert*-butyl carbazates and *N*′-substituted fluorenylmethyl carbazates efficiently, but these reagents are extremely toxic. Although several literature papers describe synthetic protocols, a general, high-yielding, and efficient synthetic method has yet to be reported. After examination of the aza-peptide synthetic literature,[5] we chose to synthesize azadipeptides via activation of the amino acid ester hydrochloride salts **15a−e** by carbonyldiimidazole (CDI) in the presence of 2.5 equiv of DIPEA (Hünig's base) in dry DCM to afford the active carbamates **16a−e**. Stirring **16a−e** with *N*′-alkyl-*N*-Pg-hydrazines **10a−c** or **14a,b** at 20 °C for 16 h in dry THF containing 1.0 equiv of DIPEA provided protected azadipeptides **18a−h** (Scheme 4). DIPEA establishes an equilibrium between the active carbamates **16** and the isocyanate intermediates **17** which react quickly with *N*′-alkyl-*N*-Pg-hydrazines **10a−c** or **14a,b** to afford **18a−h**. The method tolerated *N*′-alkyl-*N*-Fmoc-hydrazines **10c** and **14b** without any sign of *N*-Fmoc deprotection. A simple extractive workup using 2 N HCl gave rise to *N*-Pg-azadipeptide esters **18a−h** (Table 1) displaying satisfactory ^1H NMR and ^{13}C NMR and were used in the next step without full characterization. In an attempt to show general applicability of the protocol, we chose to synthesize an azadipeptide containing a polar side chain. Compound **14c** was subjected to react with **16b**, which gave the protected azadipeptide **18i** with the polar side chain (Cbz-AzaAsp-Val-O^{t}Bu). It proves the methodology is general, and by choosing appropriate coupling partners and protection/deprotection methods when reacting with **16a−e**, other azapeptides with the polar side chains can be achieved.

dx.doi.org/10.1021/jo302251e | J. Org. Chem. 2013, 78, 3541−3552

Scheme 3. Preparation of *N*′-Methyl Benzyl Carbazates 14a, *N*′-Methyl Fluorenyl-9-ylmethyl Carbazates 14b, and Benzyl 2-(2-Methoxy-2-oxoethyl)hydrazine-1-carboxylate 14c



Scheme 4. Preparation of Protected Azadipeptides 18a–i

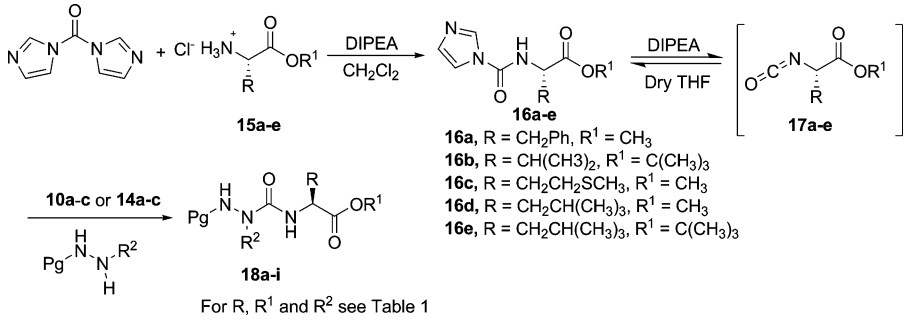


Table 1. Preparation of Protected Azadipeptides 18a–i

entry	Pg	R^2	R	R^1	**18**, yield[a] (%)
a	Cbz	CH_3	CH_2Ph	CH_3	**18a**, 90
b	Cbz	CH_3	$CH(CH_3)_2$	$C(CH_3)_3$	**18b**, 82
c	Boc	$CH(CH_3)_2$	$CH_2CH(CH_3)_2$	CH_3	**18c**, 89
d	Boc	$CH(CH_3)_2$	$CH_2CH_2SCH_3$	CH_3	**18d**, 90
e	Boc	CH_2Ph	$CH_2CH(CH_3)_2$	CH_3	**18e**, 95
f	Boc	H	CH_2Ph	CH_3	**18f**, 80
g	Fmoc	CH_3	$CH(CH_3)_2$	$C(CH_3)_3$	**18g**, 85
h	Fmoc	$CH(CH_3)_2$	$CH_2CH(CH_3)_2$	$C(CH_3)_3$	**18h**, 78
i	Cbz	$CH_2CO_2CH_3$	$CH(CH_3)_2$	$C(CH_3)_3$	**18i**, 92

[a]Isolated yield over two steps.

Preparation of *N*-(*N*-Pg-azadipeptidoyl)benzotriazoles 20a–e. *N*-Pg-Azadipeptides **19a–h** were prepared either by hydrolysis of *N*-Pg-azadipeptides methyl esters **18a,c–f** using lithium hydroxide in methanol/water mixture (10:1, v/v) or cleavage of the *tert*-butyl group of **18b,g,h** with trifluoroacetic acid in DCM (1:1 v/v). *N*-(*N*-Pg-azadipeptidoyl)benzotriazoles **20a–e** were synthesized in 81–92% yields by treatment of *N*-Pg-azadipeptides **19a–d,h** with 3.0 equiv of 1*H*-benzotriazole, 1.0 equiv of thionyl chloride, and 2.0 equiv of DIPEA in DCM at −30 °C (Scheme 5, Table 2). The presence of DIPEA neutralizes the HCl liberated from the reaction of benzotriazole and thionyl chloride, thus preventing *N*-Boc-deprotection. *N*-(*N*-Pg-azadipeptidoyl)benzotriazoles **20a–e** were characterized by ^{1}H NMR, ^{13}C NMR, and elemental analysis.

Coupling of *N*-(*N*-Pg-azadipeptidoyl)benzotriazoles 20a–e with Amino Acids 21a–e, Dipeptides 22a,b, Aminoxyacetic Acid 23a, Depsidipeptide 23b, and α-

Scheme 5. Preparation of *N*-(*N*-Pg-azadipeptidoyl)benzotriazoles 20a–e

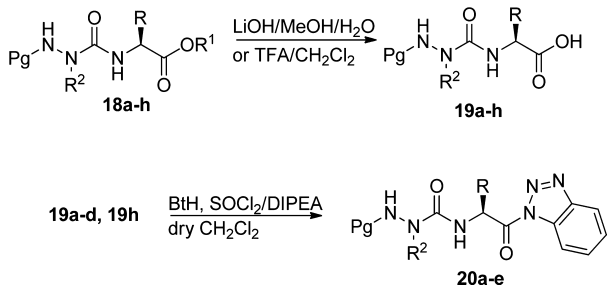


Table 2. Preparation of *N*-Pg-azadipeptide-OH 19a–h and *N*-(*N*-Pg-azadipeptidoyl)benzotriazoles 20a–e

entry	*N*-Pg-azadipeptide-OH, **19a–h**	yield[a] (%)	*N*-(*N*-Pg-azadipeptidoyl) benzotriazoles, **20a–e**	yield[a] (%)
A	Cbz-AzaAla-Phe -OH **19a**	90	Cbz-AzaAla-Phe-Bt **20a**	87
B	Cbz-AzaAla-Val -OH **19b**	83	Cbz-AzaAla-Val-Bt **20b**	81
C	Boc-AzaVal-Leu -OH **19c**	79	Boc-AzaVal-Leu-Bt **20c**	90
D	Boc-AzaVal-Met -OH **19d**	86	Boc-AzaVal-Met-Bt **20d**	85
E	Boc-AzaPhe-Leu -OH **19e**	89	(not attempted)	
F	Boc-AzaGly-Phe -OH **19f**	76	(not attempted)	
G	Fmoc-AzaAla-Val -OH **19g**	69	(not attempted)	
H	Fmoc-AzaVal-Leu -OH **19h**	80	Fmoc-AzaVal-Leu-Bt **20e**	92

[a]Isolated yield.

dx.doi.org/10.1021/jo302251e | J. Org. Chem. 2013, 78, 3541–3552

Scheme 6. Coupling Reactions of *N*-(*N*-Pg-azadipeptidoyl)benzotriazoles 20a−e

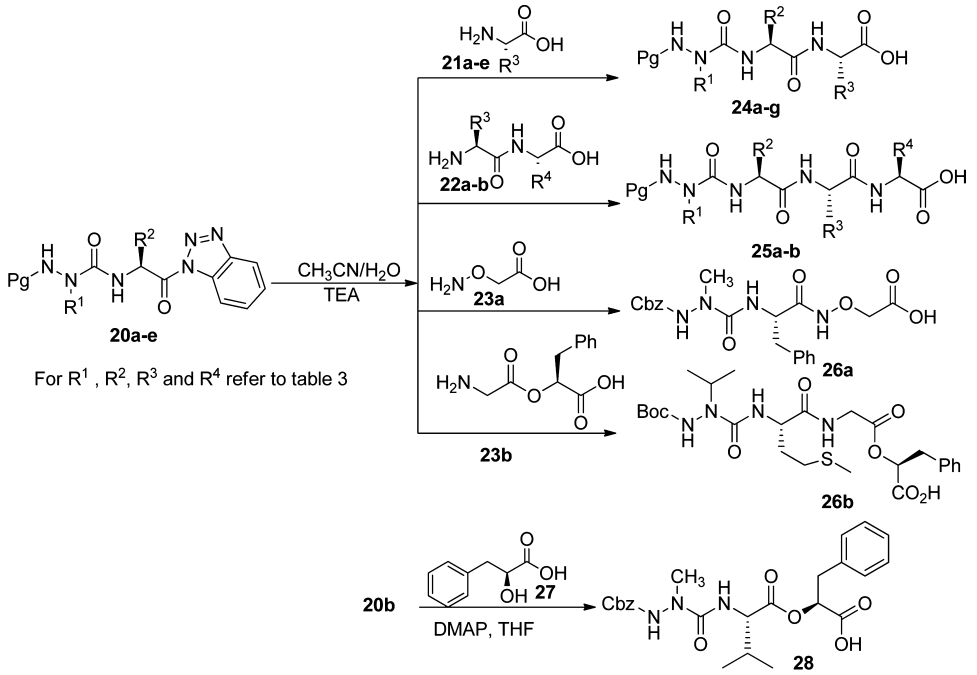


Table 3. Preparation of Azapeptides 24a−g, 25a,b, 26a,b, and 28

entry	20	nucleophiles, 21a−e, 22a,b, 23a,b, and 27	products 24a−g, 25a,b, 26a,b, and 28	yield[a] (%)
1	**20a**	H-Cys-OH **21a**	Cbz-AzaAla-Phe-Cys-OH **24a**	80
2	**20b**	H-Ser-OH **21b**	Cbz-AzaAla-Val-Ser-OH **24b**	80
3	**20b**	H-Cys-OH **21a**	Cbz-AzaAla-Val-Cys-OH **24c**	83
3′	**20b**	(D,L) H-Cys-OH **21a** + **21a′**	Cbz-AzaAla-Val-(D,L)Cys-OH **24c** + **24c′**	85
4	**20c**	H-Trp-OH **21c**	Boc-AzaVal-Leu-Trp-OH **24d**	89
5	**20d**	H-Ser-OH **21b**	Boc-AzaVal-Met-Ser-OH **24e**	83
6	**20c**	H-Asp-(OBn)-OH **21d**	Boc-AzaVal-Leu-Asp-(OBn)-OH **24f**	90
7	**20e**	H-Pro-OH **21e**	Fmoc-AzaVal-Leu-Pro-OH **24g**	86
8	**20a**	H-Gly-Gly-OH **22a**	Cbz-AzaAla-Phe-Gly-Gly-OH **25a**	77
9	**20c**	H-Gly-Phe-OH **22b**	Boc-AzaVal-Leu-Gly-Phe-OH **25b**	87
10	**20a**	H-*AO*Gly-OH **23a**	Cbz-AzaAla-Phe-*AO*Gly-OH **26a**	90
11	**20d**	H-Gly-*O*Phe **23b**	Boc-AzaVal-Met-Gly-*O*Phe-OH **26b**	85
12	**20b**	HO-Phe-OH **27**	Cbz-AzaAla-Val-*O*Phe-OH **28**	87

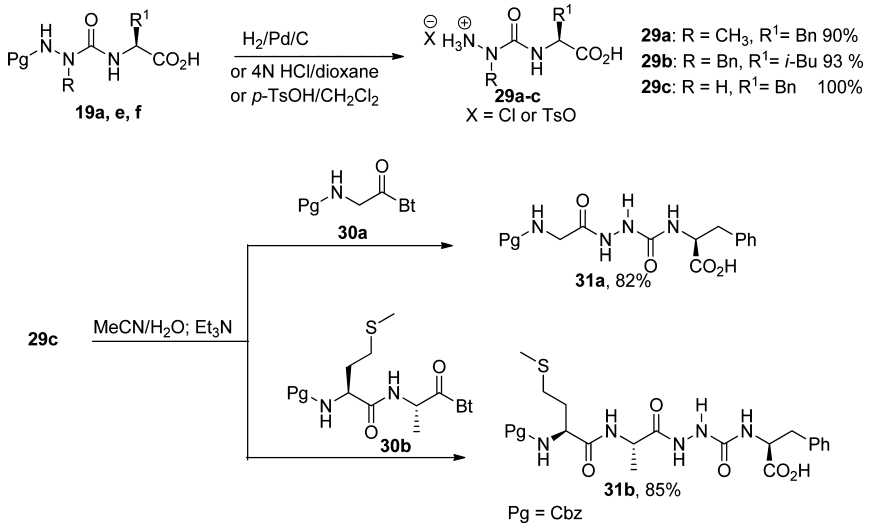
[a]Isolated yield.

Hydroxy-β-phenylpropionic Acid 27. Dipeptides are useful building blocks for longer peptide analogues; however, the functions and applications of dipeptides have been previously neglected, probably because of lack of an efficient protocol for the synthesis of dipeptides.[19] However, we now find that *N*-(*N*-Pg-azadipeptidoyl)benzotriazoles **20a−e** may be used to attain longer azapeptide sequences by coupling with (i) α-amino acids **21a−e**, (ii) dipeptides **22a,b**, (iii) aminoxyacetic acid **23a**, and (iv) depsidipeptide **23b** in aqueous acetonitrile−triethylamine (TEA) at 20 °C for 0.5−2 h. These coupling reactions afford, respectively, (i) *N*-Pg-azatripeptides **24a−g**, (ii) *N*-Pg-azatetrapeptides **25a,b**, (iii) hybrid *N*-Pg-azatripeptides containing the oxyamide bond **26a**, and (iv) hybrid azatetrapeptide with an ester bond **26b** in 77−90% yields (Scheme 6, Table 3). The coupling of α-hydroxy-β-phenylpropionic acid **27** with **20b** in dry THF containing 2.0 equiv of 4-dimethylaminopyridine (DMAP) gave hybrid peptide **28**. The target compounds were all characterized by ^{1}H NMR, ^{13}C NMR, and elemental analysis.

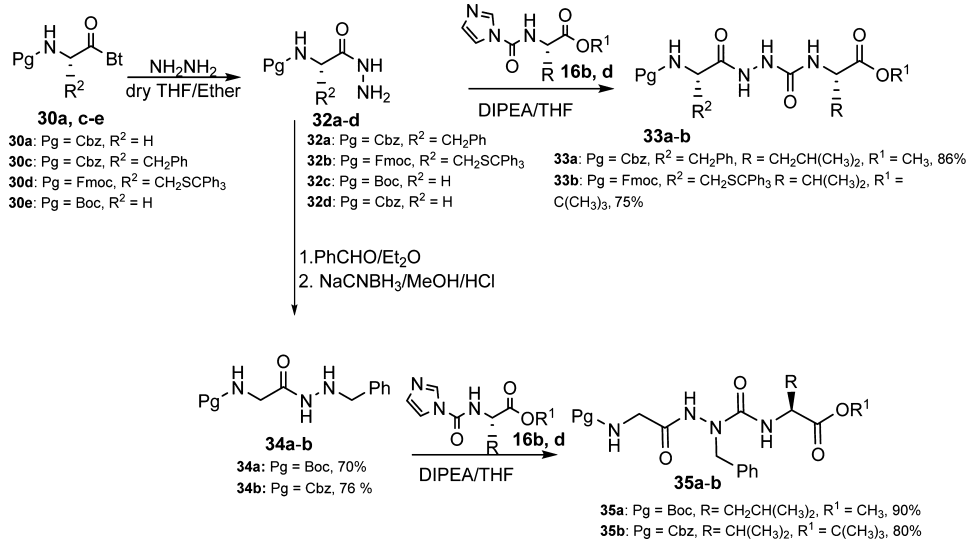
To show that no racemization occurs in the synthetic protocol used in the paper, we conducted reactions between the active benzotriazole dipeptide species **20b** and amino acid cysteine (both L and DL forms). The absence of racemization in the azapeptide (**24c** + **24c′**) was deduced from the ^{1}H NMR, where the −SH proton signal showed two separated triplets split in the D,L-cysteine moiety at 1.63 ppm (J = 9.0 Hz) and 1.49 ppm (J = 9.0 Hz), while compound **24c** showed a clear triplet at 1.61 ppm (J = 9.0 Hz). Retention of the chirality in the products was further confirmed by chiral HPLC analysis using a (S,S) Welk-O1 column (MeCN/H_2O 50:50, flow rate 0.25 mL/min, detection at 210 nm). The diastereomer **24c** showed a single retention-time peak in chiral HPLC at 10.2 min, while its corresponding diastereomeric mixture (**24c** + **24c′**) showed two peaks at 9.3 and 10.1 min. In previous studies on benzotriazole based peptide coupling we also demonstrated chirality of the reaction is maintained on *N*-acylation with *N*-acylbenzotriazoles for peptides[17a] depsipeptides[16] and aminoxy hybrid-peptides.[17b]

dx.doi.org/10.1021/jo302251e | J. Org. Chem. 2013, 78, 3541−3552

Scheme 7. Preparation of Free Azapeptides 29a–c and Coupling with *N*-(*N*-Pg-α-aminoacyl)benzotriazoles To Prepare 31a,b



Scheme 8. Preparation of Azatripeptide Starting from *N*-(*N*-Pg-α-aminoacyl)benzotriazoles 30



Preparation of Free-Amino Azadipeptides 29a–c and Their Coupling with *N*-(*N*-Pg-α-aminoacyl)benzotriazoles. The addition of a new unit to a peptide (or peptidomimetics) chain is accomplished chemically by coupling. However, chain extension of a peptidomimetic by *N*-acylation of an aza-amino acid residue is hampered by the low reactivity of the semicarbazide nitrogen. Such coupling difficulties have previously been addressed by prolonged coupling time[20] mixed coupling reagents and double coupling.[7,13] In the present study, we have investigated the ability of *N*-acylbenzotriazoles to acylate hydrochloride or *p*-TsOH salts of free-amino azadipeptides **29a–c**. Salts **29a–c** were prepared in 90–100% yields according to Scheme 7. Azadipeptide **29c** was conveniently acylated with *N*-(*N*-Pg-α-aminoacyl)benzotriazoles **30a,b** in acetonitrile/water (3:1 v/v) in the presence of 2.0 equiv of Et_3N to give azatripeptides **31a,b**. However, azapeptides **29a,b** were recovered unreacted upon treatment with **30a,b** under the same reaction conditions, and hydrolysis of **30a** was the only reaction observed when the coupling between **29a** and **30a** was attempted under microwave irradiation (1 h, 50 W, 70 °C) (Scheme 7).

Alternative Facile Route to the Synthesis of *N*-Pg-azatripeptides 33a,b and 35a,b. The low reactivity of **29a,b** is due to greater stabilization of the amide electron delocalized contributing structure by the *N*-alkyl groups thus inductively reduces the electron density at the terminal amino group and makes it less nucleophilic. Also steric hindrance arising from substitution on adjacent nitrogen could play a part. To bypass the low coupling rates of the aza-amino acid residue, we developed a novel route to azatripeptides: first we converted *N*-(*N*-Pg-α-aminoacyl)benzotriazoles **30a,c–e** to the corresponding hydrazides **32a–d**. Coupling active carbamates **16b,d** with hydrazides **32a,b** furnished azatripeptides **33a,b** containing an azaglycine residue. Alternatively, hydrazides **32c,d** were benzylated by reductive amination to give *N*′-benzyl-*N*-(*N*-Pg-glycyl)hydrazines **34a,b** which upon coupling with **16b,d** gave azatripeptides **35a,b** (Scheme 8).

Solution-Phase Synthesis of Hybrid Azapeptide 40: Analogue of Leu-enkephalin. Leu-enkephalin is an endogenous opioid peptide neurotransmitter found naturally in the brains of many animals, including humans. The amino acid sequence of Leu-enkephalin is Tyr-Gly-Gly-Phe-Leu.

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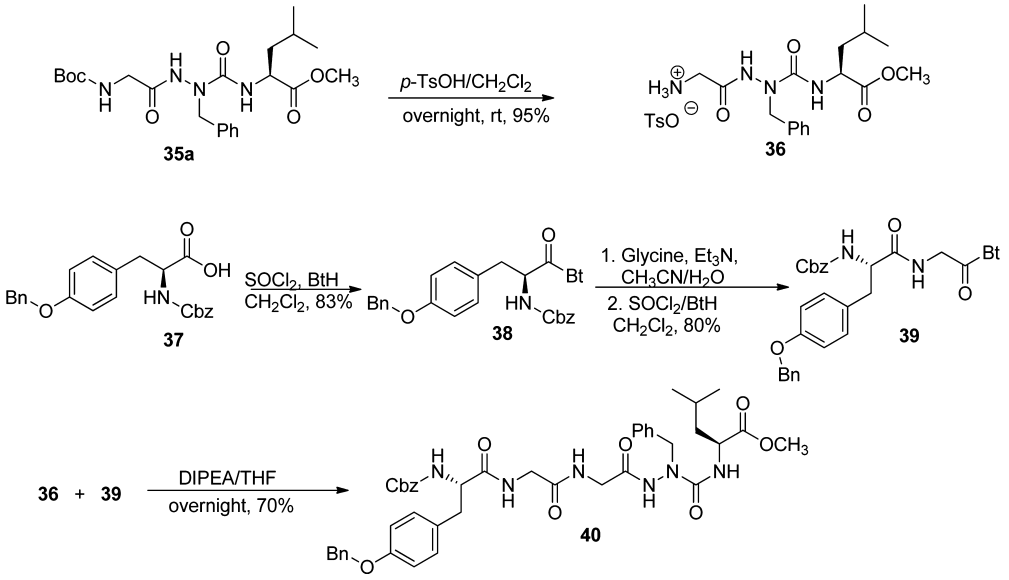
Scheme 9. Solution-Phase Synthesis of Azapeptide Analogue of Leu-enkephalin 40



Azapeptide mimetics of Leu-enkephalin were synthesized, and their binding affinity was examined in the context of monoclonal antibody 3-E7 known to strongly bind the [Leu^5] enkephalin sequence.[21] Our new route to the synthesis of azatripeptides (Scheme 8) inserted the aza-amino acid in the middle of azatripeptides, thus enabling the synthesis of the yet unknown AzaLeu-enkephalin analogue **40** by segment condensation of free-amino azatripeptide fragment **36** and the benzotriazolide **39** (Scheme 9).

■ CONCLUSIONS

In conclusion, a mild and efficient method for the preparation of *N*-Pg-azatripeptides, *N*-Pg-azatetrapeptides, hybrid *N*-Pg-azatripeptides, and hybrid *N*-Pg-azatetrapeptide has been developed by reacting *N*-(*N*-Pg-azadipeptidoyl)benzotriazoles with α-aminoacids, α-dipeptides, α-aminoxyacetic acid, a depsidipeptide, and α-hydroxy-β-phenylpropionic acid. These azatripeptides and tetrapeptides could be valuable building blocks for the synthesis of longer or cyclic azapeptides. In addition, *N*-(α-aminoacyl)benzotriazoles were easily converted to *N*-Pg-α-amino acid hydrazides which were used either directly or after alkylation to construct *N*-Pg-azatripeptides, thus inserting an aza-amino acid in the middle of the azatripeptide unit. An aza-leu-enkephalin analogue was synthesized by adopting this novel protocol. The new method avoids the low coupling rates of aza amino acids and provides an excellent alternative to the construction of azapeptides starting from *N*-Boc- and *N*-Fmoc hydrazides.

■ EXPERIMENTAL SECTION

Melting points were determined on a capillary melting point apparatus equipped with a digital thermometer and are uncorrected. NMR spectra were recorded in acetone-d_6, $CDCl_3$, and DMSO-d_6 with TMS for ^{1}H (300 MHz) and ^{13}C (75 MHz) as an internal reference. DCM was dried and distilled over CaH_2, whereas tetrahydrofuran (THF) was used after distillation over Na-benzophenone. Carbonyldiimidazole (CDI), Boc-hydrazide **8a**, $(Boc)_2O$ **11**, L-amino methyl/*tert*-butyl ester hydrochloride **15a−e**, amino acids **21a−e**, free dipeptides H-Gly-Gly-OH **22a**, H-Gly-Phe-OH **22b**, α-aminoxyacetic acid **23a**, and α-hydroxy-β-phenylpropionic acid **27** were purchased from chemical supply companies and used without further purification. Free despsipeptide H-Gly-*O*Phe.HCl **23b** was prepared according to the literature method.[16]

Fmoc-NH-NH$_2$ (8b). Compound **8b** was prepared according to the literature method:[11] white microcrystals (8.756 g, 89%); mp 170.0−172.0 °C (lit.[11] mp 172.0−173.0 °C); ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.36 (br s, 1H), 7.90 (d, J = 7.8 Hz, 1H), 7.70 (d, J = 7.2 Hz, 2H), 7.42 (t, J = 6.9 Hz, 1H), 7.33 (t, J = 6.9 Hz, 1H), 4.36−4.16 (m, 3H), 4.09 (br s, 2H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 158.2, 143.8, 140.7, 127.6, 127.0, 125.2, 120.1, 65.6, 46.7.

Boc-NH-N=CMe$_2$ (9a). Compound **9a** was prepared according to the literature method:[13] white microcrystals (1.156 g, 89%); mp 102.0−104.0 °C (lit.[22] mp 103.0−104.0 °C); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.43 (br s, 1H), 2.05−1.98 (m, 3H), 1.83−1.76 (m, 3H), 1.52−1.45 (m, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 152.8, 149.7, 80.8, 28.2, 25.3, 15.9.

Boc-NH-N=CHPh (9b). Compound **9b** was prepared according to the literature method:[13] white microcrystals (2.980 g, 89%); mp 199−202 °C (lit.[23] mp 203 °C); ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.00 (br s, 1H), 7.69−7.49 (m, 2H), 7.48−7.28 (m, 3H), 1.47 (s, 9H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 152.3, 143.0, 134.6, 129.3, 128.7, 126.4, 79.4, 28.1.

Fmoc-NH-N=CMe$_2$ (9c). To a solution of benzaldehyde (10.0 mmol, 1.0 equiv) in diethyl ether (50 mL) were added (9*H*-fluoren-9-yl)methyl hydrazinecarboxylate **8b** (10.0 mmol, 1.0 equiv) and two drops of glacial acetic acid. The reaction mixture was heated under reflux for 2−3 h and then cooled to room temperature. The white solid precipitate was collected by filtration, washed with cold diethyl ether, and dried under vacuum to yield **9c**. Compound **9c** was characterized by ^{1}H and ^{13}C NMR and taken to the next step without further purification: white microcrystals (2.796 g, 95%); mp 147.0−149.0 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.76 (d, J = 7.7 Hz, 2H), 7.63 (d, J = 7.1 Hz, 2H), 7.39 (t, J = 7.5 Hz, 2H), 7.30 (t, J = 7.5 Hz, 2H), 4.52 (d, J = 7.2 Hz, 2H), 4.30 (t, J = 7.1 Hz, 1H), 2.07 (d, J = 2.1 Hz, 3H), 1.84 (d, J = 3.0 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 151.1, 148.8, 143.6, 141.2, 127.7, 127.0, 125.1, 119.9, 67.2, 47.0, 25.4, 16.2. Compound **9c** was partially characterized by ^{1}H and ^{13}C NMR and taken to the next step without further purification.

***tert*-Butyl 2-Isopropylhydrazinecarboxylate (10a).** Compound **10a** was prepared according to the literature method:[13] white microcrystals (0.364 g, 70%); mp 45.0−47.0 °C (lit.[13] mp 47.0−49.0 °C); ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.42 (br s, 1H), 3.19−2.97 (m, 1H), 1.43 (s, 9H), 1.00 (d, J = 6.6 Hz, 3H), 0.98 (d, J = 6.3 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 156.8, 80.2, 50.7, 28.3, 20.5.

***tert*-Butyl 2-Benzylhydrazinecarboxylate (10b).** Compound **10b** was prepared according to the literature method:[13] colorless oil

dx.doi.org/10.1021/jo302251e | J. Org. Chem. 2013, 78, 3541−3552

(0.310 g, 75%); lit.[13] reported as low melting solid; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.41−7.21 (m, 5H), 6.13 (br s, 1H), 4.21 (br s, 1H), 3.99 (s, 2H), 1.46 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 156.6, 137.6, 128.9, 128.4, 127.4, 80.5, 55.8, 28.3.

(9*H*-Fluoren-9-yl)methyl 2-Isopropylhydrazinecarboxylate (10c). Compound **10c** was prepared according to the literature method:[11] white microcrystals (0.786 g, 68%); mp 161.0−163.0 °C (lit.[11] mp 163.0−164.0 °C); ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.63 (br s, 1H), 7.84 (d, J = 7.4 Hz, 2H), 7.65 (d, J = 7.5 Hz, 2H), 7.36 (t, J = 7.5 Hz, 2H), 7.27 (t, J = 7.4 Hz, 2H), 4.42−4.21 (m, 3H), 4.18 (d, J = 6.5 Hz, 1H), 3.05−2.82 (m, 1H), 0.86 (d, J = 5.6 Hz, 6H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 156.8, 143.8, 140.7, 127.6, 127.0, 125.2, 120.1, 65.4, 49.5, 46.7, 20.6.

Benzyl 2-Methylhydrazinecarboxylate Hydrochloride (14a). Compound **14a** was prepared according to the literature method:[24] white microcrystals (3.00 g, 70%); mp 174.0−177.0 °C (lit.[24] mp 175.0−177.0 °C); ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.76 (s, 1H), 7.55−7.05 (m, 5H), 5.13 (s, 2H), 2.67 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 155.4, 136.3, 129.1, 129.0, 128.7, 67.9, 36.4.

(9*H*-Fluoren-9-yl)methyl 2-methylhydrazinecarboxylate Hydrochloride (14b). Compound **14b** was prepared according to the literature method:[25] white microcrystals (0.850 g, 73%); mp 160.0−161.0 °C (lit.[25] mp 160.0 °C); ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.79 (br s, 1H), 7.90 (d, J = 7.4 Hz, 2H), 7.72 (d, J = 7.4 Hz, 2H), 7.43 (t, J = 7.5 Hz, 2H), 7.34 (t, J = 7.4 Hz, 2H), 4.52 (d, J = 6.5 Hz, 2H), 4.29 (t, J = 6.6 Hz, 1H), 2.70 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 155.5, 143.9, 141.4, 128.4, 127.8, 125.8, 120.9, 67.7, 47.0, 36.3.

General Methods for the Preparation of 16a−e. To a suspension of L-amino methyl/*tert*-butyl ester hydrochloride **15a−e** (10.0 mmol, 1.0 equiv) in DCM (20 mL) at 20 °C were added 2.5 equiv of DIPEA and CDI (carbonyldiimidazole, 1.1 equiv). The reaction mixture was stirred for 3 h at rt, and the organic layer was washed with water (2 × 20 mL), $NaHCO_3$ (3 × 20 mL) and brine solution (2 × 20 mL). The organic layer was dried over $MgSO_4$ and evaporated under vacuum to give oily mono substituted imidazole derivative **16a−e**.

Im-Phe-OMe (16a). Compound **16a** was prepared according to the given general procedure for **16a−e**: colorless oil[26] (2.596 g, 95%); $[\alpha]^{20}_D$ −20.0 (*c* 1.0, CH_3OH); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.02 (s, 1H), 7.34−7.17 (m, 3H), 7.15−7.03 (m, 2H), 7.00 (d, J = 1.7 Hz, 1H), 6.71 (d, J = 8.1 Hz, 1H), 4.90−4.80 (m, 1H), 3.75 (s, 3H), 3.30−3.11 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.8, 148.7, 136.3, 130.4, 129.3, 129.0, 128.6, 127.6, 116.3, 55.0, 53.0, 37.6.

Im-Val-O^{t}Bu (16b). Compound **16b** was prepared according to the given procedure for **16a**: oil (2.406 g, 90%); $[\alpha]^{20}_D$ −28.0 (*c* 1.0, CH_3OH); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.15 (s, 1H), 7.38 (d, J = 1.5 Hz, 1H), 7.06 (d, J = 1.8 Hz, 1H), 6.69 (d, J = 8.1 Hz, 1H), 4.45 (dd, J = 8.1, 4.8 Hz, 1H), 2.30−2.18 (m, 1H), 1.48 (s, 9H), 0.99 (d, J = 6.9 Hz, 3H), 0.96 (d, J = 6.9 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.7, 148.7, 136.0, 130.5, 115.8, 83.0, 59.0, 31.4, 28.0, 18.8, 17.8. Compound **16b** was partially characterized by ^{1}H, ^{13}C NMR and taken to the next step without further purification.

Im-Met-OMe (16c). Compound **16c** was prepared according to the given procedure for **16a**; colorless oil[27] (2.419 g, 94%); $[\alpha]^{20}_D$ −19 (*c* 1.0, CH_3OH); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.19 (s, 1H), 7.73 (d, J = 7.8 Hz, 1H), 7.44 (d, J = 1.2 Hz, 1H), 7.04 (d, J = 1.5 Hz, 1H), 4.79−4.71 (m, 1H), 3.77 (s, 3H), 2.62−2.55 (m, 2H), 2.31−2.01 (m, 5H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.9, 148.7, 136.1, 130.1, 116.2, 60.4, 53.1, 30.4, 30.1, 15.4.

Im-Leu-OMe (16d). Compound **16d** was prepared according to the given procedure for **16a**: white microcrystals (2.297 g, 96%); mp 80.0−82.0 °C (lit.[27] mp 80.0−81.0 °C); $[\alpha]^{20}_D$ −47.0 (*c* 1.0, CH_3OH); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.15 (s, 1H), 8.00 (d, J = 6.0 Hz, 1H), 7.44 (d, J = 3.0 Hz, 1H), 6.95 (d, J = 3.0 Hz, 1H), 4.69−4.39 (m, 1H), 3.69 (s, 3H), 1.82−1.41 (m, 3H), 0.89 (d, J = 6.0 Hz, 3H), 0.86 (d, J = 6.0 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.3, 148.9, 136.1, 129.7, 116.4, 52.5, 52.3, 40.4, 24.8, 22.8, 21.4.

Im-Leu-O^{t}Bu (16e). Compound **16e** was prepared according to the given procedure for **16a**: colorless oil (2.589 g, 92%); $[\alpha]^{20}_D$ −23.0 (*c* 1.0, CH_3OH); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.09 (d, J = 1.1 Hz, 1H), 7.34 (s, 1H), 7.04 − 6.97 (m, 1H), 6.99 − 6.89 (m, 1H), 4.58 − 4.43 (m, 1H), 1.76 − 1.52 (m, 4H), 1.45 (s, 12H), 0.94 (d, J = 4.2 Hz, 4H), 0.91 (d, J = 4.3 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.7, 148.7, 136.0, 130.5, 115.8, 83.0, 59.0, 53.4, 31.4, 28.0, 18.8, 17.8. Compound **16e** was partially characterized by ^{1}H, ^{13}C NMR and taken to the next step without further purification.

General Methods for the Preparation of *N*-Pg-azadipeptide 18a−h. The residue **16a−e** (1.0 equiv) was dissolved in dry DCM (20 mL) and reacted with *N*′-alkyl-*N*-Pg-hydrazines **10a−c** or **14a,b** (1.0 equiv; Pg = Boc-, Fmoc-, Cbz-) in the presence of DIPEA (1.0 equiv) at 20 °C overnight. The reaction mixture was poured into a separatory funnel, washed with water (2 × 20 mL), 2 N HCl (3 × 20 mL), brine solution (2 × 20 mL), dried over $MgSO_4$, and evaporated under vacuum to give both $-NH_2$ and $-CO_2H$ side protected azadipeptide **18a−h**, which were used in the next step without further purification.

Cbz-AzaAla-Phe-OMe (18a). Compound **18a** was prepared according to the given general procedure for **18a−h**: white microcrystals (1.734 g, 90%); mp 51.0−53.0 °C (lit.[28] mp not reported); $[\alpha]^{20}_D$ −25 (*c* 1.0, CH_3OH); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.31 (s, 5H), 7.27−7.11 (m, 3H), 7.02 (dd, J = 7.2, 2.4 Hz, 2H), 6.86 (s, 1H), 5.77 (d, J = 8.1 Hz, 1H), 5.13 (s, 2H), 4.72−4.64 (m, 1H), 3.62 (s, 3H), 3.13−2.94 (m, 5H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.6, 157.0, 155.2, 135.9, 135.3, 129.2, 128.6, 128.5, 128.4, 128.1, 126.9, 67.9, 54.2, 52.1, 38.1, 35.8. Anal. Calcd for $C_{20}H_{23}N_3O_5$: C, 62.33; H, 6.01; N, 10.90. Found: C, 62.42; H, 6.17; N, 10.84.

Cbz-AzaAla-Val-O^{t}Bu (18b). Compound **18b** was prepared according to the given procedure for **18a**: low melting solid (1.556 g, 82%); $[\alpha]^{20}_D$ −22.0 (*c* 1.0, CH_3OH); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.47−7.18 (m, 5H), 7.07 (s, 1H), 5.81 (d, J = 8.8 Hz, 1H), 5.18 (s, 2H), 4.28 (dd, J = 8.8, 4.5 Hz, 1H), 3.10 (s, 3H), 2.20−1.96 (m, 1H), 1.43 (s, 9H), 0.87 (d, J = 6.0 Hz, 3H), 0. 78 (d, J = 6.6 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.8, 163.2, 157.4, 135.3, 128.5, 128.4, 128.2, 81.7, 67.9, 58.4, 35.9, 31.5, 28.0, 18.8, 17.3. Compound **18b** was partially characterized by ^{1}H, ^{13}C NMR and taken to the next step without further purification.

Boc-AzaVal-Leu-OMe (18c). Compound **18c** was prepared according to the given procedure for **18a**: white microcrystals (1.537 g, 89%); mp 60.0−62.0 °C; $[\alpha]^{20}_D$ −27.0 (*c* 1.0, CH_3OH); ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.65 (br s, 1H), 5.57 (d, J = 9.1 Hz, 1H), 4.66−4.53 (m, 1H), 4.47 (q, J = 7.7 Hz, 1H), 3.68 (s, 3H), 1.76−1.48 (m, 3H), 1.44 (s, 9H), 1.09 (d, J = 6.6 Hz, 3H), 1.04 (d, J = 5.7 Hz, 3H), 0.95−0.83 (m, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 174.9, 157.0, 156.2, 82.0, 52.4, 51.9, 48.1, 41.8, 28.2, 24.9, 23.2, 22.0, 19.9, 19.5. Anal. Calcd for $C_{16}H_{31}N_3O_5$: C, 55.63; H, 9.05, N, 12.16. Found: C, 55.99; H, 9.18; N, 12.09.

Boc-AzaVal-Met-OMe (18d). Compound **18d** was prepared according to the given procedure for **18a**: colorless oil (1.636 g, 90%); $[\alpha]^{20}_D$ −35.0 (*c* 1.0, CH_3OH); ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.26 (br s, 1H), 5.90 (br s, 1H), 4.75−4.42 (m, 2H), 3.74 (s, 3H), 2.54 (t, J = 7.5 Hz, 2H), 2.29−1.90 (m, 5H), 1.49 (s, 9H), 1.11 (d, J = 5.7 Hz, 3H), 1.08 (d, J = 7.8 Hz, 3H). Compound **18d** was partially characterized by ^{1}H NMR and taken to the next step without further purification.

Boc-AzaPhe-Leu-OMe (18e). Compound **18e** was prepared according to the given procedure for **18a**: low melting solid (1.869 g, 95%); lit.[6] mp not reported; $[\alpha]^{20}_D$ −25.0 (*c* 1.0, CH_3OH); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.47−7.18 (m, 6H), 6.20 (s, 1H), 5.82 (d, J = 8.6 Hz, 1H), 4.60−4.51 (m, 1H), 3.75−3.65 (m, 5H), 1.70−1.51 (m, 3H), 1.44 (s, 9H), 0.96 (d, J = 6.0 Hz, 3H), 0.94 (d, J = 6.3 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 174.1, 157.1, 154.2, 135.9, 128.8, 128.6, 127.7, 82.2, 51.8, 41.8, 27.9, 24.7, 22.9, 21.8.

Boc-AzaGly-Phe-OMe (18f). Compound **18f** was prepared according to the given procedure for **18a**: colorless oil (1.350 g, 80%); $[\alpha]^{20}_D$ −16.0 (*c* 1.0, CH_3OH); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.29−7.17 (m, 3H), 7.13−7.05 (m, 2H), 6.71 (s, 1H), 5.91 (d, J = 8.1 Hz, 1H), 5.50 (d, J = 8.2 Hz, 1H), 4.80−4.71 (m, 1H), 3.65 (d, J = 7.2 Hz, 3H), 3.07 (d, J = 5.7 Hz, 1H), 3.01 (d, J = 6.0 Hz, 1H), 1.44 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.9, 158.3, 156.3, 136.3, 129.5, 128.6, 127.0, 81.8, 54.1, 52.4, 38.4, 28.3. Compound **18f** was partially

dx.doi.org/10.1021/jo302251e | *J. Org. Chem.* 2013, 78, 3541−3552

characterized by ^{1}H, ^{13}C NMR and taken to the next step without further purification.

*Fmoc-AzaAla-Val-O^{t}Bu (**18g**).* Compound **18g** was prepared according to the given procedure for **18a**: low melting solid (1.987 g, 85%); $[\alpha]^{20}_D$ −10.0 (*c* 1.0, CH_3OH); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.75 (d, *J* = 7.5 Hz, 2H), 7.58 (t, *J* = 6.1 Hz, 2H), 7.39 (t, *J* = 7.5 Hz, 2H), 7.31 (t, *J* = 7.5 Hz, 2H), 7.13 (s, 1H), 5.85 (d, *J* = 7.8 Hz, 1H), 4.62−4.44 (m, 2H), 4.30 (dd, *J* = 8.1, 3.8 Hz, 1H), 4.23 (t, *J* = 6.9 Hz, 1H), 3.09 (s, 3H), 2.20−2.00 (m, 1H), 1.42 (s, 9H), 0.91 (d, *J* = 6.9 Hz, 3H), 0.84 (d, *J* = 6.6 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.9, 157.4, 155.5, 143.2, 141.2, 127.8, 127.1, 124.9, 120.0, 81.8, 67.9, 58.5, 46.9, 35.9, 31.5, 28.0, 18.9, 17.5. Compound **18g** was partially characterized by ^{1}H and ^{13}C NMR and taken to the next step without further purification.

*Fmoc-AzaVal-Leu-O^{t}Bu (**18h**).* Compound **18h** was prepared according to the given procedure for **18a**: low melting solid (1.988 g, 78%); $[\alpha]^{20}_D$ −4.0 (*c* 1.0, CH_3OH); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.77 (d, *J* = 7.5 Hz, 2H), 7.60 (t, *J* = 6.6 Hz, 2H), 7.41 (t, *J* = 7.4 Hz, 2H), 7.32 (t, *J* = 7.4 Hz, 2H), 6.81−6.48 (m, 0.5H), 5.67−5.42 (m, 0.5H), 4.63−4.36 (m, 3H), 4.31−4.07 (m, 1H), 1.78−1.50 (m, 3H), 1.45 (s, 9H), 1.07 (d, *J* = 6.7 Hz, 6H), 1.00−0.84 (m, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.7, 164.9, 157.0, 143.6, 141.6, 128.1, 127.4, 125.3, 120.3, 81.9, 52.7, 48.6, 47.3, 42.5, 28.2, 25.1, 23.0, 22.4, 19.6. Anal. Calcd for $C_{29}H_{39}N_3O_5$: C, 68.34; H, 7.71, N, 8.24. Found: C, 68.04; H, 8.14; N, 8.47.

*Cbz-AzaAsp(OMe)-Val-O^{t}Bu (**18i**).* Compound **18i** was prepared according to the given procedure for **18a**: low melting solid (2.012 g, 92%); $[\alpha]^{20}_D$ −21.0 (*c* 1.0, CH_3OH); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.38−7.18 (m, 5H), 5.98 (d, *J* = 8.7 Hz, 1H), 5.13 (s, 2H), 4.22 (dd, *J* = 8.7, 4.5 Hz, 1H), 3.67−3.57 (m, 5H), 2.27 −1.71 (m, 1H), 1.38 (s, 9H), 0.83 (d, *J* = 6.8 Hz, 3H), 0.77 (d, *J* = 7.1 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.5, 170.2, 156.9, 155.4, 135.4, 128.8, 128.7, 128.4, 82.0, 68.3, 58.8, 52.4, 52.2, 31.9, 28.2, 19.0, 17.7; HRMS (ESI) calcd for $C_{21}H_{31}N_3O_7Na$ $[M + Na]^+$ 460.2054, found 460.2075.

General Methods for the −OMe and −O^{t}Bu Group Deprotection. *Method 1.* −OMe deprotection: *N*-Pg-AzaAA$_1$-AA$_2$-OMe (4.0 mmol, 1.0 equiv) was dissolved in a solution of MeOH and water (10 mL, 9:1 v/v). LiOH (8.0 mmol, 2.0 equiv) was added, and the mixture was stirred for 2 h at room temperature. After completion of the reaction (followed by TLC), MeOH was evaporated, water (5.0 mL) was added, and the aqueous layer was washed with ether (2 × 20 mL) and then acidified with 2 N HCl. The aqueous layer was extracted with EtOAc (2 × 10 mL) solution, and the organic layer was dried over anhydrous $MgSO_4$ and evaporated to give *N*-Pg-azadipeptide.

Method 2. −O^{t}Bu deprotection: *N*-Pg-AzaAA$_1$-AA$_2$-O^{t}Bu (4.0 mmol) was dissolved in a solution of dry DCM (10 mL) containing trifluoroacetic acid (5.0 mL) and stirred for 2 h at room temperature. After completion of the reaction [followed by TLC] and TFA were evaporated, 5 mL of water was added, the aqueous layer was extracted with EtOAc (10 mL × 2) solution, and the organic layer was dried over anhydrous $MgSO_4$ and evaporated to give white solid. Compounds **19b,d** were characterized by ^{1}H and ^{13}C NMR and taken to the next step without further purification.

*Cbz-AzaAla-Phe-OH (**19a**).* Compound **19a** was prepared from **18a** according to the given method 1 for −OMe deprotection. White microcrystals (1.337 g, 90%); mp 58.0− 60.0 °C; $[\alpha]_D^{20}$ −30.0 (*c* 1.0, CH_3OH); ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.73 (br s, 1H), 9.50−9.39 (m, 1H), 7.47−7.29 (m, 5H), 7.27−7.15 (m, 5H), 6.67−6.50 (m, 1H), 5.13 (s, 2H), 4.36−4.28 (m, 1H), 3.00 (d, *J* = 6.5 Hz, 2H), 2.93 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 173.5, 157.2, 155.5, 137.6, 136.3, 129.3, 128.5, 128.3, 128.1, 127.9, 126.4, 66.4, 54.5, 39.9, 36.9; Anal. Calcd for $C_{19}H_{21}N_3O_5$: C, 61.45; H, 5.70, N, 11.31. Found: C, 61.13; H, 5.90; N, 11.18.

*Cbz-AzaAla-Val-OH (**19b**).* Compound **19b** was prepared from **18b** according to the given method 2 for −O^{t}Bu deprotection: white microcrystals (1.074 g, 83%); mp 65.0−67.0 °C; $[\alpha]^{20}_D$ −15.0 (*c* 1.0, CH_3OH); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.63−7.41 (m, 1H), 7.34 (s, 5H), 5.99−5.90 (m, 1H), 5.19 (s, 2H), 4.30−4.20 (m, 1H), 3.09 (s, 3H), 2.18−2.07 (m, 1H), 0.89 (d, *J* = 7.5 Hz, 3H), 0.80 (d, *J* = 6.6 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.1, 163.4, 157.7, 135.6, 128.8, 128.6, 128.4, 68.1, 58.7, 36.2, 31.7, 19.1, 17.6. Compound **19b** was partially characterized by ^{1}H, ^{13}C NMR and taken to the next step without further purification.

*Boc-AzaVal-Leu-OH (**19c**).* Compound **19c** was prepared from **18c** according to the given method for **19a**: white microcrystals (1.047 g, 79%); mp 56.0−58.0 °C; $[\alpha]^{20}_D$ −25.0 (*c* 1.0, CH_3OH); ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.81 (s, 0.6H), 8.39 (s, 0.3H), 6.05−5.90 (m, 1H), 4.50−4.25 (m, 1H), 4.24−3.97 (m, 1H), 1.80−1.46 (m, 3H), 1.41 (s, 9H), 0.97 (d, *J* = 6.4 Hz, 6H), 0.88−0.83 (m, 6H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 174.9, 156.6, 155.4, 79.4, 51.3, 47.2, 27.9, 24.0, 23.0, 22.1, 21.5, 20.1, 19.4; Anal. Calcd for $C_{15}H_{29}N_3O_5$: C, 54.36; H, 8.82, N, 12.68. Found: C, 54.02; H, 9.15; N, 12.30.

*Boc-AzaVal-Met-OH (**19d**).* Compound **19d** was prepared from **18d** according to the given method for **19a**: low melting point solid (1.202 g, 86%); $[\alpha]^{20}_D$ −20.0 (*c* 1.0, CH_3OH); ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.84 (br s, 1H), 8.43 (br s, 1H), 6.60−6.12 (m, 1H), 4.52−3.98 (m, 2H), 2.44 (d, *J* = 7.4 Hz, 2H), 2.11−1.86 (m, 5H), 1.42 (s, 9H), 0.99 (d, *J* = 6.3 Hz, 6H). Compound **19d** was partially characterized by ^{1}H NMR and taken to the next step without further purification.

*Boc-AzaPhe-Leu-OH (**19e**).* Compound **19e** was prepared from **18e** according to the given method for **19a**: white microcrystals (1.351 g, 89%); mp 60.0−62.0 °C; $[\alpha]^{20}_D$ −22.0 (*c* 1.0, CH_3OH); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.46−7.11 (m, 5H), 5.94 (d, *J* = 8.0 Hz, 1H), 4.60−4.38 (m, 1H), 1.78−1.49 (m, 3H), 1.40 (s, 9H), 0.96 (d, *J* = 6.6 Hz, 4H), 0.94 (d, *J* = 6.6 Hz, 3H); ^{13}C NMR (75 MHz, acetone-d_6) δ 174.1, 158.0, 154.4, 137.7, 128.9, 128.4, 127.4, 80.5, 52.0, 41.4, 27.6, 24.6, 22.8, 21.3. Compound **19e** was partially characterized by ^{1}H and ^{13}C NMR.

*Boc-AzaGly-Phe-OH (**19f**).* Compound **19f** was prepared from **18f** according to the given method for **19a**: white microcrystals (0.983 g, 76%); mp 159.0−161.0 °C (lit.[13] mp 163.0− 164.0 °C); $[\alpha]^{20}_D$ −69.0 (*c* 1.0, CH_3OH); ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.77 (br s, 1H), 8.55 (s, 1H), 7.84 (s, 1H), 7.42−7.06 (m, 5H), 6.26 (d, *J* = 7.6 Hz, 1H), 4.45−4.32 (m, 1H), 3.08−2.86 (m, 2H), 1.39 (s, 9H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 173.3, 157.5, 155.9, 137.2, 129.3, 128.2, 126.5, 79.0, 53.7, 37.4, 28.1. Anal. Calcd for $C_{15}H_{20}LiN_3O_5$: C, 54.71; H, 6.12, N, 12.76. Found: C, 54.70; H, 6.41; N, 12.30.

*Fmoc-AzaAla-Val-OH (**19g**).* Compound **19g** was prepared from **18g** according to the given method for **19b**: white microcrystals (1.136 g, 69%); mp 178.0−180.0 °C; $[\alpha]^{20}_D$ −7.0 (*c* 1.0, CH_3OH); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.71 (d, *J* = 7.5 Hz, 2H), 7.53 (d, *J* = 7.1 Hz, 2H), 7.35 (t, *J* = 7.0 Hz, 2H), 7.29−7.18 (m, 2H), 4.57−4.30 (m, 1H), 4.18 (t, *J* = 6.8 Hz, 2H), 3.03 (s, 3H), 2.23−2.00 (m, 1H), 0.89 (d, *J* = 6.3 Hz, 3H), 0.82 (d, *J* = 6.6 Hz, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 174.1, 158.0, 156.1, 144.1, 141.4, 128.3, 127.7, 125.8, 120.8, 58.9, 47.2, 36.1, 31.3, 30.9, 19.7, 18.6. Compound **19g** was partially characterized by ^{1}H and ^{13}C NMR.

*Fmoc-AzaVal-Leu-OH (**19h**).* Compound **19h** was prepared from **18h** according to the given method for **19b**: white microcrystals (1.451 g, 80%); mp 157.0−159.0 °C; $[\alpha]^{20}_D$ −16.0 (*c* 1.0, CH_3OH); ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.25 (d, *J* = 10.9 Hz, 1H), 7.90 (d, *J* = 7.2 Hz, 2H), 7.76 (d, *J* = 7.3 Hz, 2H), 7.42 (t, *J* = 7.2 Hz, 2H), 7.33 (t, *J* = 7.1 Hz, 2H), 6.40−6.20 (m, 1H), 6.29 (dd, *J* = 33.4, 8.1 Hz, H), 4.65−3.93 (m, 5H), 1.80−1.12 (m, 3H), 0.97 (dd, *J* = 13.2, 5.7 Hz, 6H), 0.82 (d, *J* = 6.7 Hz, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 174.9, 156.9, 156.5, 140.7, 127.7, 127.1, 125.3, 120.1, 66.1, 64.9, 51.4, 47.6, 46.6, 24.2, 22.9, 21.5, 19.8, 19.4. Anal. Calcd for $C_{25}H_{31}N_3O_5$: C, 66.21; H, 6.89, N, 9.26. Found: C, 65.90; H, 6.98; N, 9.21.

General Method for the Preparation of *N*-(*N*-Pg-azadipeptidoyl)benzotriazoles 20a−e. Benzotriazole (6.0 mmol, 3.0 equiv) was dissolved in dry DCM (50 mL). $SOCl_2$ (2.2 mmol) was added by syringe, and the mixture was stirred for 15 min at rt under argon. The solution temperature was lowered to −30 to −40 °C (dry ice + acetone), and 2.0 equiv of TEA was added. After 5 min of stirring, *N*-Pg-AzaAA$_1$-AA$_2$-OH (2.0 mmol) was added and the mixture stirred for 2 h keeping the temperature at −30 to −40 °C. After completion of the reaction, ice-cold water (20 mL) was added, and the organic layer was washed with water (20 mL × 2), $NaHCO_3$

dx.doi.org/10.1021/jo302251e | J. Org. Chem. 2013, 78, 3541−3552

(20 mL × 4), and then brine (20 mL × 2). The organic layer was dried over $MgSO_4$ and evaporated to yield a white solid **20a−e**.

*Cbz-AzaAla-Phe-Bt (**20a**).* Compound **20a** was prepared according to the general method for the preparation of *N*-(*N*-Pg-azadipeptidoyl)-benzotriazoles **20a−e**: white microcrystals (0.822 g, 87%); mp 70.0−72.0 °C; $[\alpha]^{20}_D$ −15.0 (*c* 1.0, CH_3OH); ^1H NMR (300 MHz, $CDCl_3$) δ 8.04 (d, *J* = 8.4 Hz, 1H), 8.01 (d, *J* = 8.1 Hz, 1H), 7.51 (t, *J* = 7.8 Hz, 1H), 7.40 (t, *J* = 7.7 Hz, 1H), 7.30−7.15 (m, 5H), 7.09−6.92 (m, 5H), 6.14 (d, *J* = 7.8 Hz, 1H), 6.04−5.98 (m, 1H), 5.01 (s, 2H), 3.30 (dd, *J* = 14.0, 5.2 Hz, 1H), 3.12 (dd, *J* = 14.0, 7.5 Hz, 1H), 2.98 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.5, 157.2, 155.3, 145.7, 135.2, 130.8, 130.5, 129.0, 128.5, 128.3, 127.9, 127.1, 126.3, 120.1, 114.1, 67.7, 55.1, 38.2, 35.7. Anal. Calcd for $C_{25}H_{24}N_6O_4$: C, 63.55; H, 5.12, N, 17.79. Found: C, 63.23; H, 5.14; N, 18.00.

*Cbz-AzaAla-Val-Bt (**20b**).* Compound **20b** was prepared according to the given method for **20a**: white microcrystals (0.688 g, 81%); mp 57.0−59.0 °C; $[\alpha]^{20}_D$ −12.0 (*c* 1.0, CH_3OH); ^1H NMR (300 MHz, $CDCl_3$) δ 8.09 (d, *J* = 8.2 Hz, 1H), 7.98 (d, *J* = 8.2 Hz, 1H), 7.51 (t, *J* = 7.7 Hz, 1H), 7.38 (t, *J* = 7.4 Hz, 1H), 7.30−7.13 (m, 5H), 6.16 (d, *J* = 7.7 Hz, 1H), 5.69 (dd, *J* = 8.9, 5.4 Hz, 1H), 5.07 (s, 2H), 3.04 (s, 3H), 2.40−2.26 (m, 1H), 0.91 (d, *J* = 6.9 Hz, 3H), 0.77 (d, *J* = 6.6 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.0, 157.6, 155.4, 145.7, 135.2, 130.8, 130.4, 128.4, 128.2, 128.0, 126.2, 120.0, 114.1, 67.7, 58.8, 35.8, 31.4, 19.5, 17.2; HRMS (ESI) calcd for $C_{21}H_{24}N_6O_4Na$ $[M + Na]^+$ 447.1751, found 447.1771.

*Boc-AzaVal-Leu-Bt (**20c**).* Compound **20c** was prepared according to the given method for **20a**: white microcrystals (0.779 g, 90%); mp 92.0−95.0 °C; $[\alpha]^{20}_D$ −34.0 (*c* 1.0, CH_3OH); ^1H NMR (300 MHz, $CDCl_3$) δ 8.21 (d, *J* = 8.2 Hz, 1H), 8.09 (d, *J* = 8.1 Hz, 1H), 7.62 (t, *J* = 7.7 Hz, 1H), 7.48 (t, *J* = 7.7 Hz, 1H), 6.52−6.25 (m, 1H), 5.94−5.78 (m, 2H), 4.70−4.56 (m, 1H), 1.92−1.65 (m, 3H), 1.49 (s, 9H), 1.18−1.0 (m, 9H), 0.94 (d, *J* = 4.3 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.6, 157.0, 146.1, 131.4, 130.7, 126.5, 120.4, 114.6, 82.2, 53.1, 48.4, 41.9, 28.3, 25.5, 23.5, 21.7, 19.8, 19.5; HRMS (ESI) calcd for $C_{21}H_{32}N_6O_4Na$ $[M + Na]^+$ 455.2377, found 455.2399.

*Boc-AzaVal-Met-Bt (**20d**).* Compound **20d** was prepared according to the given method for **20a**: white microcrystals (0.766 g, 85%); mp 80.0−82.0 °C; $[\alpha]^{20}_D$ −22.0 (*c* 1.0, CH_3OH); ^1H NMR (300 MHz, $CDCl_3$) δ 8.23 (d, *J* = 7.7 Hz, 1H), 8.11 (d, *J* = 8.3 Hz, 1H), 7.64 (t, *J* = 8.1 Hz, 1H), 7.50 (t, *J* = 8.1 Hz, 1H), 6.54−6.24 (m, 1H), 4.67−4.59 (m, 1H), 2.68 (q, *J* = 7.2, 6.1 Hz, 2H), 2.21−2.10 (m, 3H), 2.07−2.05 (m, 3H), 1.50 (s, 9H), 1.13−1.06 (m, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.0, 165.2, 156.7, 145.9, 131.1, 130.6, 126.4, 120.2, 114.3, 83.1, 53.4, 48.2, 32.1, 30.1, 28.1, 19.6, 19.2, 15.4. Anal. Calcd for $C_{20}H_{30}N_6O_4S$: C, 53.32; H, 6.71, N, 18.65. Found: C, 53.04; H, 6.78; N, 18.33.

*Fmoc-AzaVal-Leu-Bt (**20e**).* Compound **20e** was prepared according to the given method for **20a**: white microcrystals (1.021 g, 92%); mp 100.0−102.0 °C; $[\alpha]^{20}_D$ −19.0 (*c* 1.0, CH_3OH); ^1H NMR (300 MHz, DMSO-d_6) δ 9.26 (d, *J* = 4.3 Hz, 1H), 8.28 (d, *J* = 8.1 Hz, 1H), 8.22 (d, *J* = 8.1 Hz, 1H), 7.90 (d, *J* = 7.4 Hz, 2H), 7.85−7.70 (m, 3H), 7.63 (t, *J* = 7.7 Hz, 2H), 7.44−7.39 (m, 2H), 7.33 (t, *J* = 7.5 Hz, 2H), 7.23−6.90 (m, 1H), 5.64−5.56 (m, 1H), 4.56−4.47 (m, 1H), 4.44−3.98 (m, 3H), 1.99−1.41 (m, 3H), 1.07−0.89 (m, 9H), 0.86−0.76 (m, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 173.3, 157.2, 156.4, 145.3, 143.7, 143.5, 140.7, 131.0, 130.6, 127.7, 127.1, 126.6, 125.4, 125.2, 120.1, 120.1, 113.9, 65.9, 52.5, 48.0, 46.7, 24.6, 22.9, 21.0, 19.8, 19.2; HRMS (ESI) calcd for $C_{31}H_{34}N_6O_4Na$ $[M + Na]^+$ 577.2534, found 577.2526.

General Procedure for the Coupling of *N*-(*N*-Pg-azadipeptidoyl)benzotriazoles 20a−e To Prepare 24a−g, 25a,b, and 26a,b. Different amino acids **21a−e** or dipeptides **22a,b**, **23a,b** (1.2 mmol, 1.2 equiv), and TEA (1.2 mmol, 1.2 equiv) were dissolved in a minimum amount of cold water (5.0 mL). Acetonitrile (10 mL) was added, and the solutions were cooled to 10 °C. A solution of *N*-Pg-azadipeptidoyl-Bt (1.0 mmol, 1.0 equiv) in acetonitrile (5.0 mL) was added and the mixture stirred for 2 h at 20 °C. The reaction mixture was monitored by TLC [EtOAc−hexanes (1:2)]. After completion of reaction, the solvent was evaporated. The residue was dissolved in DCM (30 mL) and washed with 2 N HCl solution (4 × 10 mL), water (10 mL), and brine (10 mL). The solvent was dried over $MgSO_4$ and evaporated to give various *N*-Pg-aza-tri- and -tetrapeptides.

*Cbz-AzaAla-Phe-Cys-OH (**24a**).* Compound **24a** was prepared according to the general procedure for the coupling of *N*-(*N*-Pg-azadipeptidoyl)benzotriazoles **20a−e** with various nucleophiles: white microcrystals (0.379 g, 80%); mp 89.0−92.0 °C; $[\alpha]^{20}_D$ −25.0 (*c* 1.0, CH_3OH); ^1H NMR (300 MHz, DMSO-d_6) δ 9.47 (br s, 1H), 8.25 (br s, 1H), 7.48−7.33 (m, 5H), 7.28−7.08 (m, 6H), 6.62 (br s, 1H), 5.13 (s, 2H), 4.70−4.30 (m, 2H), 3.06−2.78 (m, 7H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 171.5, 171.4, 157.1, 155.6, 137.7, 136.2, 129.4, 129.2, 128.5, 128.1, 127.9, 126.2, 66.4, 54.6, 54.4, 37.6, 35.5, 25.7. Anal. Calcd for $C_{22}H_{26}N_4O_6S$: C, 55.68; H, 5.52; N, 11.81. Found: C, 55.56; H, 5.64; N, 11.78.

*Cbz-AzaAla-Val-Ser-OH (**24b**).* Compound **24b** was prepared according to the given method for **24a**: white microcrystals (0.328 g, 80%); mp 50.0−52.0 °C; $[\alpha]^{20}_D$ −5.0 (*c* 1.0, CH_3OH); ^1H NMR (300 MHz, $CDCl_3$) δ 8.07 (br s, 1H), 7.95−7.70 (m, 1H), 7.35−7.25 (m, 6H), 5.24−5.07 (m, 2H), 4.67−4.54 (m, 1H), 4.33−4.16 (m, 1H), 4.06−3.81 (m, 2H), 3.03 (*s*, 3H), 2.20−1.92 (m, 1H), 0.85 (d, *J* = 6.3 Hz, 3H), 0.77 (d, *J* = 5.4 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 175.5, 173.1, 172.4, 158.4, 138.5, 135.3, 128.5, 128.0, 67.9, 62.5, 59.8, 54.7, 36.1, 31.2, 19.1, 17.9; HRMS (ESI) calcd for $C_{18}H_{25}N_4O_7$ $[M - H]^-$ 409.1729, found 409.1726.

*Cbz-AzaAla-Val-Cys-OH (**24c**).* Compound **24c** was prepared according to the given method for **24a**: white microcrystals (0.354 g, 83%); mp 113.0−115.0 °C; $[\alpha]^{20}_D$ −17.0 (*c* 1.0, CH_3OH); ^1H NMR (300 MHz, $CDCl_3$) δ 7.75−7.50 (m, 2H), 7.28−7.22 (m, 5H), 5.10 (s, 2H), 4.73−4.63 (m, 1H), 4.16 (t, *J* = 8.1 Hz, 1H), 3.00 (s, 3H), 2.96−2.78 (m, 1H), 2.07−1.81 (m, 1H), 1.61 (t, *J* = 9.0 Hz, 1H), 0.81 (d, *J* = 6.0 Hz, 3H), 0.74 (d, *J* = 6.6 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 179.5, 173.0, 172.0, 158.2, 135.2, 128.5, 128.4, 128.0, 67.9, 54.2, 53.4, 36.3, 30.6, 26.3, 19.1, 18.2; HRMS (ESI) calcd for $C_{18}H_{25}N_4O_6S$ $[M - H]^-$ 425.1500, found 425.1521.

*Cbz-AzaAla-Val-(D,L)Cys-OH (**24c** + **24c′**).* Compound **24c** + **24c′** was prepared according to the given method for **24a**: white microcrystals (0.363 g, 85%); mp 90.0−92.0 °C; ^1H NMR (300 MHz, $CDCl_3$) δ 7.71 (d, *J* = 7.1 Hz, 1H), 7.39 −7.27 (m, 5H), 5.18 (s, 2H), 4.89−4.56 (m, 1H), 4.46−4.13 (m, 1H), 3.09 (s, 3H), 3.04−2.79 (m, 2H), 2.10−1.94 (m, 1H), 1.63 (t, *J* = 9.0 Hz, 0.5H), 1.49 (t, *J* = 8.9 Hz, 0.5H), 0.90 (d, *J* = 6.6 Hz, 3H), 0.83 (d, *J* = 4.9 Hz, 3H); ^{13}C NMR was similar to the compound **24c**. Anal. Calcd for $C_{18}H_{26}N_4O_6S$: C, 50.69; H, 6.15; N, 13.14. Found: C, 50.48; H, 6.21; N, 13.08.

*Boc-AzaVal-Leu-Trp-OH (**24d**).* Compound **24d** was prepared according to the given method for **24a**: white microcrystals (0.461 g, 89%); mp 110.0−113.0 °C; $[\alpha]^{20}_D$ −4.0 (*c* 1.0, CH_3OH); ^1H NMR (300 MHz, DMSO-d_6) δ 10.66 (s, 1H), 8.58 (br s, 1H), 7.94 (d, *J* = 6.2 Hz, 1H), 7.52 (d, *J* = 7.7 Hz, 1H), 7.32 (d, *J* = 7.8 Hz, 1H), 7.12 (s, 1H), 7.05 (t, *J* = 7.5 Hz, 1H), 6.97 (t, *J* = 7.4 Hz, 1H), 5.89 (br s, 1H), 4.53−4.50 (m, 1H), 4.37 (qt, *J* = 6.6 Hz, 1H), 4.28−4.13 (m, 1H), 3.18 (dd, *J* = 14.7, 5.9 Hz, 1H), 3.05 (dd, *J* = 14.7, 7.4 Hz, 1H), 1.66−1.55 (m, 1H), 1.50−1.38 (m, 10H), 1.00 (d, *J* = 6.6 Hz, 6H), 0.85 (d, *J* = 6.3 Hz, 6H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 173.1, 172.6, 156.2, 155.3, 136.0, 127.2, 123.6, 120.9, 118.3, 118.2, 111.4, 109.7, 79.6, 52.9, 51.7, 47.1, 42.0, 27.9, 27.1, 23.7, 23.4, 22.0, 19.8, 19.3. Anal. Calcd for $C_{26}H_{39}N_5O_6$: C, 60.33; H, 7.59, N, 13.53. Found: C, 60.48; H, 8.07; N, 13.17.

*Boc-AzaVal-Met-Ser-OH (**24e**).* Compound **24e** was prepared according to the given method for **24a**: white microcrystals (0.362 g, 83%); mp 48.0−50.0 °C; $[\alpha]^{20}_D$ −21.0 (*c* 1.0, CH_3OH); ^1H NMR (300 MHz, Acetone-d_6) δ 8.17−8.10 (m, 1H), 7.83−7.73 (m, 1H), 7.56 (t, *J* = 8.2 Hz, 1H), 7.39−7.31 (m, 1H), 6.41−6.22 (m, 1H), 4.42 (dt, *J* = 14.8, 7.0 Hz, 3H), 3.84 (d, *J* = 11.7 Hz, 1H), 3.74 (d, *J* = 11.1 Hz, 1H), 2.43 (q, *J* = 7.4 Hz, 2H), 2.16−1.68 (m, 5H), 1.34 (s, 9H), 0.97 (d, *J* = 7.5 Hz, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.9, 172.7, 157.9, 157.7, 62.5, 55.1, 53.9, 48.9, 31.2, 30.3, 28.4, 19.7, 15.5; HRMS (ESI) calcd for $C_{17}H_{31}N_4O_7S$ $[M - H]^-$ 435.1919, found 435.1935.

*Boc-AzaVal-Leu-Asp(OBn)-OH (**24f**).* Compound **24f** was prepared according to the given method for **24a**: white microcrystals (0.483 g,

dx.doi.org/10.1021/jo302251e | J. Org. Chem. 2013, 78, 3541−3552

90%); mp 108.0−110.0; $[\alpha]^{20}_D$ −12.0 (*c* 1.0, CH_3OH); ^1H NMR (300 MHz, DMSO-d_6) δ 8.91 (s, 1H), 8.65−8.10 (m, 1H), 7.40−7.30 (m, 5H), 6.13− 5.86 (m, 1H), 5.10 (s, 2H), 4.62−4.56 (m, 1H), 4.40−4.35 (m, 1H), 4.26−4.21 (m, 1H), 2.87 (dd, *J* = 17.1, 6.9 Hz, 1H), 2.73 (dd, *J* = 16.5, 7.2 Hz, 1H), 1.89−1.45 (m, 3H), 1.41 (s, 9H), 0.97 (d, *J* = 6.6 Hz, 6H), 0.86 (d, *J* = 7.1 Hz, 6H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 172.7, 172.0, 170.0, 156.4, 155.4, 136.0, 128.4, 128.0, 127.9, 79.6, 65.8, 51.7, 48.6, 42.2, 35.9, 27.9, 23.2, 21.9, 19.7, 19.3; HRMS (ESI) calcd for $C_{26}H_{39}N_4O_8$ $[M - H]^-$ 535.2773, found 535.2782.

*Fmoc-AzaVal-Leu-Pro-OH (**24g**).* Compound **24g** was prepared according to the given method for **24a**: white microcrystals (0.474 g, 86%); mp 210.0−212.0 °C; $[\alpha]^{20}_D$ −37.0 (*c* 1.0, CH_3OH); ^1H NMR (300 MHz,DMSO-d_6) δ 0.75 (d, 6 Hz, 3H), 1.30−1.48 (m, 2H), (1.50−2.0, m, 4H), 2.0−2.2 (m, 1H), 3.7 (s, 1H), 4.1−4.4 (m, 4H), 4.44 (s, 2H), 6.10−6.40 (rotamars, 1H), 7.33 (t, 10 Hz, 7.42 (t, 7.5 Hz), 7.75 (d, 7.2 Hz, 2 H), 7.89 (d, 7.2 Hz, 2H), 9.30 (d, 13.2 Hz, 1H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 172.6, 171.2, 156.8, 156.5, 143.7, 143.6, 143.5, 140.8, 129.0, 127.8, 127.4, 127.2, 125.4, 125.3, 121.5, 120.2, 120.1, 66.2, 52.3, 48.1, 47.7, 46.8, 40.8, 24.1, 23.3, 21.8, 19.8, 19.4, 18.4. Anal. Calcd for $C_{30}H_{38}N_4O_6$: C, 65.44; H, 6.96, N, 10.17. Found: C, 65.65; H, 7.36; N, 10.01.

*Cbz-AzaAla-Phe-Gly-Gly-OH (**25a**).* Compound **25a** was prepared according to the given method for **24a**: white microcrystals (0.374 g, 77%); mp 72.0−74.0 °C; $[\alpha]^{20}_D$ = −22.0 (*c* 1.0, CH_3OH); ^1H NMR (300 MHz, DMSO-d_6) δ 9.45 (br s, 1H), 8.30−8.10 (m, 2H), 7.44−7.30 (m, 5H), 7.25−7.10 (m, 5H), 6.70 (br s, 1H), 5.12 (s, 2H), 4.45−4.35 (m, 1H), 3.89−3.59 (m, 4H), 3.00 (d, *J* = 6.3 Hz, 1H), 2.94 (d, *J* = 6.3 Hz, 1H), 2.91 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 171.9, 171.1, 169.1, 157.3, 155.6, 137.9, 136.2, 129.3, 128.5, 128.1, 128.0, 127.9, 126.2, 66.5, 55.2, 41.9, 40.7, 37.6, 35.5; HRMS (ESI) calcd for $C_{46}H_{53}N_{10}O_{14}$ $[2M - H]^-$ 969.3748, found 969.3740.

*Boc-AzaVal-Leu-Gly-Phe-OH (**25b**).* Compound **25b** was prepared according to the given method for **24a**: white microcrystals (0.466 g, 87%); mp 82.0−85.0; $[\alpha]^{20}_D$ −6.0 (*c* 1.0, CH_3OH); ^1H NMR (300 MHz, DMSO-d_6) δ 8.85 (d, *J* = 11.1 Hz, 0.7H), 8.37 (s, 0.3H), 8.24−7.94 (m, 2H), 7.35−7.15 (m, 5H), 6.30 (d, *J* = 8.1 Hz, 0.5H), 5.99 (d, *J* = 8.1 Hz, 0.5H), 4.45−4.35 (m, 2H), 4.25−4.16 (m, 1H), 3.77 (dd, *J* = 16.9, 6.2 Hz, 1H), 3.59 (dd, *J* = 17.0, 5.2 Hz, 1H), 3.04 (dd, *J* = 13.7, 4.8 Hz, 1H), 2.87 (dd, *J* = 13.7, 8.7 Hz, 1H), 1.73−1.43 (m, 3H), 1.42 (s, 9H), 0.97 (d, *J* = 6.6 Hz, 3H), 0.94 (d, *J* = 6.3 Hz, 3H), 0.88−0.77 (m, 6H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 173.1, 172.7, 168.6, 156.5, 155.3, 137.4, 129.1, 128.2, 126.4, 79.5, 53.5, 52.1, 47.1, 41.6, 36.9, 27.9, 23.9, 23.2, 21.8, 20.0, 19.3. Anal. Calcd for $C_{26}H_{41}N_5O_7$: C, 58.30; H, 7.72, N, 13.07. Found: C, 58.00; H, 7.98; N, 12.87.

*Cbz-AzaAla-Phe-AOGly-OH (**26a**).* Compound **26a** was prepared according to the given method for **24a**: white microcrystals (0.400 g, 90%); mp 57.0−59.0 °C; $[\alpha]^{20}_D$ = −21.0 (*c* 1.0, CH_3OH); ^1H NMR (300 MHz, DMSO-d_6) δ 11.45 (br s, 1H), 9.40 (s, 1H), 7.92 (s, 1H), 7.49−7.38 (m, 5H), 7.25−7.15 (m, 5H), 6.62 (s, 1H), 5.13 (s, 2H), 4.40−4.20 (m, 3H), 3.42−2.75 (m, 5H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 170.7, 169.4, 157.6, 156.1, 137.9, 136.9, 129.9, 129.1, 128.7, 128.4, 127.0, 72.3, 67.0, 53.5, 38.6, 36.1; HRMS (ESI) calcd for $C_{42}H_{47}N_8O_{14}$ $[2M - H]^-$ 887.3217, found 887.3214.

*Boc-AzaVal-Met-Gly-OPhe-OH (**26b**).* Compound **26b** was prepared according to the given method for **24a**: white microcrystals (0.471 g, 85%); mp 44.0−46.0 °C; $[\alpha]^{20}_D$ −15.0 (*c* 1.0, CH_3OH); ^1H NMR (300 MHz, $CDCl_3$) δ 7.74−7.34 (m, 1H), 7.35−6.91 (m, 5H), 6.80−6.23 (m, 1H), 5.23 (dd, *J* = 8.6, 4.1 Hz, 1H), 4.75−4.30 (m, 2H), 4.21−3.63 (m, 2H), 3.04 (dd, *J* = 18.6, 4.1 Hz, 2H), 2.65−2.34 (m, 2H), 2.20−1.80 (m, 5H), 1.47 (s, 9H), 1.19−1.11 (m, 6H); ^{13}C NMR (75 MHz, acetone-d_6) δ 174.8, 171.5, 170.2, 169.4, 157.4, 136.7, 129.8, 128.8, 127.2, 73.6, 73.2, 53.5, 48.3, 42.9, 41.0, 37.3, 32.9, 28.0, 19.7, 19.4, 14.8; HRMS (ESI) calcd for $C_{25}H_{37}N_4O_8S$ $[M - H]^-$ 553.2338, found 553.2350.

*Cbz-AzaAla-Val-OPhe-OH (**28**).* 4-(Dimethylamino)pyridine (1.2 mmol) was added to a stirred solution of Cbz-AzaAla-Val-Bt **20b** (1.0 mmol) and α-hydroxycarboxylic acid **27** (1.0 mmol) in dry THF (5.0 mL) at 4 °C. The reaction mixture was stirred for 4 h at room temperature until the reaction was complete by TLC [EtOAc−hexanes (1:2)]. After completion of the reaction, the solvent was evaporated. The residue was dissolved in DCM, washed with 2 N hydrochloric acid solution (3 × 5 mL) and brine (5 mL) and dried over $MgSO_4$. The solvent was evaporated to yield the hybrid peptides: colorless oil (0.410 g, 87%); $[\alpha]^{20}_D$ −13.0 (*c* 1.0, CH_3OH); ^1H NMR (300 MHz, $CDCl_3$) δ 7.50 (br s, 1H), 7.23−7.05 (m, 10H), 6.05−5.80 (m, 1H), 5.10 (t, *J* = 5.4 Hz, 1H), 5.04 (s, 2H), 4.34−4.25 (m, 1H), 3.03−2.79 (m, 5H), 2.01 (s, 1H), 0.71 (d, *J* = 6.9 Hz, 3H), 0.64 (d, *J* = 5.7 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 176.5, 175.5, 171.8, 157.9, 136.3, 135.3, 129.3, 129.1, 128.3, 128.1, 126.9, 126.6, 73.2, 70.9, 67.9, 39.9, 36.8, 30.9, 18.6, 17.0; HRMS (ESI) calcd for $C_{24}H_{28}N_3O_7$ $[M - H]^-$ 470.1933, found 470.1942.

*H-AzaAla-Phe-OH (**29a**).* Cbz-AzaAla-Phe-OH **19a** (0.75 g, 2.0 mmol) was dissolved in THF (20 mL), treated with a suspension of 10 mol % of Pd on carbon (20 wt %) in THF, placed under hydrogen gas at pressure, and stirred at room temperature overnight. The reaction mixture was filtered through Celite. The filtrate was evaporated on a rotary evaporator to give H-AzaAla-Phe-OH (**29a**): white microcrystals (0.427 g, 90%); mp 63.0−65.0 °C (lit.[29] mp not reported); $[\alpha]^{20}_D$ −3.0 (*c* 1.0, CH_3OH); ^1H NMR (300 MHz, $CDCl_3$) δ ^1H NMR (300 MHz, DMSO-d_6) δ 7.32−7.08 (m, 5H), 7.07−6.87 (m, 1H), 4.44−4.07 (m, 1H), 3.03 − 2.73 (m, 5H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 174.2, 159.0, 138.2, 129.8, 128.9, 127.1, 55.0, 38.0, 37.8; HRMS (ESI) calcd for $C_{11}H_{15}N_3O_3Na$ $[M + Na]^+$ 260.1006, found. 260.1010.

*H-AzaPhe-Leu-OH·HCl (**29b**).* Compound **19e** (1.0 mmol) was dissolved in a solution of dry 4 N HCl/dioxane (10 mL) solution and stirred for 2 h at room temperature. After completion of the reaction (followed by TLC) ether (5 mL) was added and the white solid was filtered off to give H-AzaPhe-Leu-OH·HCl (**29b**): white microcrystals (0.294 g, 93%); mp 170.0−172.0 °C; $[\alpha]^{20}_D$ −31.0 (*c* 1.0, CH_3OH); ^1H NMR (300 MHz, DMSO-d_6) δ 7.75 (d, *J* = 7.9 Hz, 1H), 7.47−7.16 (m, 5H), 4.92 (s, 2H), 4.17−4.07 (m, 1H), 1.82−1.40 (m, 3H), 0.87 (d, *J* = 6.6 Hz, 3H), 0.82 (d, *J* = 6.1 Hz, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 174.3, 157.0, 135.2, 128.5, 128.3, 127.9, 66.4, 52.1, 51.9,24.3, 23.1, 21.0; HRMS (ESI) calcd for $C_{14}H_{20}N_3O_3$ $[M - H]^-$ 278.1583, found 278.1564.

*H-AzaGly-Phe-OH·TsOH (**29c**).* Compound **19f** (3.0 mmol, 1.0 equiv) was dissolved in a solution of dry DCM (10 mL) containing *p*-TsOH (1.0 equiv) and stirred for 12 h at room temperature. After completion of the reaction [followed by TLC] ether (5 mL) was added and the white solid was filtered off to give H-AzaGly-Phe-OH·TsOH (**29c**): white microcrystals (1.186 g, 100%); mp 159.0−160.0 °C; $[\alpha]^{20}_D$ −36.0 (*c* 1.0, CH_3OH); ^1H NMR (300 MHz, DMSO-d_6) δ 9.88 (s, 2H), 8.72 (s, 1H), 7.51 (d, *J* = 8.0 Hz, 2H), 7.36 (d, *J* = 8.1 Hz, 1H), 7.32−7.18 (m, 5H), 7.14 (d, *J* = 8.3 Hz, 2H), 4.42−4.32 (m, 1H), 3.10 (dd, *J* = 13.9, 4.5 Hz, 1H), 2.90 (dd, *J* = 13.9, 9.4 Hz, 1H), 2.30 (s, 3H); ^{13}C (75 MHz, DMSO-d_6) δ 173.0, 156.7, 145.4, 137.8, 137.4, 129.2, 128.3, 128.1, 126.5, 125.5, 54.3, 36.8, 20.9. Anal. Calcd for $C_{17}H_{21}N_3O_6S$: C, 51.64; H, 5.35, N, 10.63. Found: C, 51.85; H, 5.25; N, 10.52.

*Cbz-Gly-AzaGly-Phe-OH (**31a**).* Tosylate salt of free aza-dipeptide **29c** (1.0 mmol, 1.0 equiv) and TEA (1.0 mmol, 1.0 equiv) were dissolved in minimum amount of cold water (5.0 mL). Acetonitrile (10 mL) was added to this solution and cooled to 10 °C. A solution of Cbz-Gly-Bt **30a** (1.0 mmol, 1.0 equiv) in acetonitrile (5.0 mL) was added and stirred for 2 h at 20 °C. The reaction mixture was monitored by TLC [EtOAc−hexanes (1:2)]. After completion of reaction, the solvent was evaporated. The residue was dissolved in DCM (30 mL) and washed with 2 N HCl solution (4 × 10 mL), water (10 mL) and brine (10 mL). The solvent was dried over $MgSO_4$ and evaporated to give Cbz-Gly-AzaGly-Phe-OH (**31a**): white microcrystals (0.340 g, 82%); mp 205.0−207.0 °C (lit.[30] mp not reported); $[\alpha]^{20}_D$ −12.0 (*c* 1.0, CH_3OH); ^1H NMR (300 MHz, DMSO-d_6) δ 12.70 (s, 1H), 9.59 (d, *J* = 2.1 Hz, 1H), 7.96 (s, 1H), 7.46 (t, *J* = 6.1 Hz, 1H), 7.32 - 7.11 (m, 10H), 6.38 (d, *J* = 8.0 Hz, 1H), 4.98 (s, 2H), 4.56 - 4.16 (m, 1H), 3.60 (d, *J* = 6.0 Hz, 2H), 3.29 (s, 3H), 2.97 (dd, *J* = 13.7, 5.2 Hz, 1H), 2.86 (dd, *J* = 13.7, 7.4 Hz, 1H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 173.3, 168.9, 157.2, 156.5, 137.3, 137.0, 129.3, 128.4, 128.2, 127.8, 127.7, 126.5, 65.5, 53.9, 42.1, 37.3. Anal. Calcd for

dx.doi.org/10.1021/jo302251e | J. Org. Chem. 2013, 78, 3541−3552

$C_{20}H_{22}N_4O_6$: C, 57.97; H, 5.35, N, 13.52. Found: C, 57.73; H, 5.17; N, 13.49.

*Cbz-Met-Ala-AzaGly-Phe-OH (**31b**).* Compound **31b** was prepared according to the method for preparation of Cbz-GlyAza-Gly-Phe-OH (**31a**): white microcrystals (0.476 g, 85%); mp 185.0−187.0 °C; $[\alpha]^{20}_D$ = −40.0 (*c* 1.0, CH_3OH); ^1H NMR (300 MHz, DMSO-d_6) δ 9.70 (d, *J* = 11.0 Hz, 1H), 8.20−7.96 (m, 2H), 7.59−7.49 (m, 1H), 7.40 − 7.16 (m, 10H), 6.38 (dd, *J* = 12.5, 7.9 Hz, 1H), 5.03 (s, 2H), 4.42 − 4.20 (m, 2H), 4.18 − 4.05 (m, 1H), 3.10−2.90 (m, 2H), 2.56 − 2.47 (m, 2H), 2.02 (s, 3H), 1.96 − 1.73 (m, 2H), 1.23 (d, *J* = 6.5 Hz, 3H); HRMS (ESI) calcd for $C_{26}H_{32}N_5O_7S$ $[M - H]^-$ 558.2028, found 558.2029.

*General Method for the Preparation of **33a,b**.* Hydrazine hydrate (1.0 mmol, 1.0 equiv) and DIPEA (1.0 mmol, 1.0 equiv) were dissolved in ether (5.0 mL). A solution of Cbz-Phe-Bt **30c** or Fmoc-Cys(S-trt)-Bt **30d** (1.0 mmol, 1.0 equiv) in ether (5.0 mL) was added and the mixture stirred for 10 min at 20 °C. The white precipitate **32a,b** was formed and collected by filtration. Compounds **32a,b** were used for the next step without further purification.[31] The residue was dissolved in dry DCM (20 mL) and reacted with **16b,d** (1.0 equiv) in the presence of DIPEA (1.0 equiv) at 20 °C overnight. The reaction mixture was poured into a separatory funnel, washed with water (2 × 20 mL), $NaHCO_3$ (3 × 20 mL), and brine solution (2 × 20 mL), dried over $MgSO_4$, and evaporated under vacuum to give both $-NH_2$ and $-CO_2H$ side protected aza-tripeptides **33a,b**.

*Cbz-Phe-AzaGly-Leu-OMe (**33a**).* Compound **33a** was prepared according to the general method given for the preparation of **33a,b**: white microcrystals (0.417 g, 86%); mp 179.0−181.0 °C; $[\alpha]^{20}_D$ −20.0 (*c* 1.0, CH_3OH); ^1H NMR (300 MHz, $CDCl_3$) δ 9.35 (s, 0.5H), 7.97 (s, 0.5H), 7.34−7.02 (m, 11H), 6.40 (d, *J* = 7.9 Hz, 0.5H), 6.05 (d, *J* = 7.0 Hz, 0.5H), 4.99 (d, *J* = 12.6 Hz, 1H), 4.85 (d, *J* = 12.3 Hz, 1H), 4.63−4.27 (m, 2H), 3.59 (s, 3H), 3.13 (dd, *J* = 13.9, 5.4 Hz, 1H), 2.92 (dd, *J* = 14.0, 9.0 Hz, 1H), 1.77−1.42 (m, 3H), 0.85 (d, *J* = 6.2 Hz, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 174.9, 170.9, 157.8, 156.6, 136.5, 136.2, 129.5, 128.7, 128.6, 128.2, 128.0, 127.0, 67.2, 55.1, 52.5, 51.8, 41.4, 38.1, 24.9, 23.0, 22.0; HRMS (ESI) calcd for $C_{25}H_{32}N_4O_6Na$ $[M + Na]^+$ 507.2214, found 507.2232.

*Fmoc-Cys(S-trt)-AzaGly-Val-O^{t}Bu (**33b**).* Compound **33b** was prepared according to the method for preparation of **33a**: white microcrystals (0.599 g, 75%); mp 79.0−81.0 °C; $[\alpha]^20_D$ −23.0 (*c* 1.0, CH_3OH); ^1H NMR (300 MHz, $CDCl_3$) δ 7.71 (dd, *J* = 7.4, 3.9 Hz, 2H), 7.59−7.46 (m, 2H), 7.45−7.30 (m, 8H), 7.30−7.08 (m, 12H), 6.74 (s, 1H), 6.23−6.02 (m, 1H), 4.45−4.24 (m, 3H), 4.14−4.10 (m, 2H), 2.80−2.55 (m, 2H), 1.50−1.38 (m, 9H), 1.29−1.23 (m, 1H), 0.93 (d, *J* = 6.6 Hz, 3H), 0.87 (d, *J* = 6.9 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.1, 170.1, 166.4, 158.2, 144.2, 143.6, 141.2, 129.5, 128.1, 127.7, 126.9, 125.0, 119.9, 82.0, 67.1, 65.8, 58.1, 47.0, 31.6, 31.3, 28.0, 19.0, 17.5; HRMS (ESI) calcd for $C_{47}H_{50}N_4O_6SNa$ $[M + Na]^+$ 821.3343, found 821.3358.

General Method for the Preparation of 35a,b. To a solution of benzaldehyde (1.0 equiv) in diethyl ether (10 mL) were added **32c,d** (prepared by using literature method)[31] (1.0 equiv) and two drops of glacial acetic acid. The reaction mixture was heated under reflux for 2−3 h and then cooled to room temperature. The white solid precipitate was collected by filtration, washed with cold diethyl ether and dried under vacuum to yield the desired hydrazone. The hydrazone intermediate (1.0 equiv) was treated with sodium cyanoborohydride ($NaBH_3CN$) (1.1 equiv) in absolute methanol (20 mL). HCl (1 N) in MeOH was added dropwise over 1 h to maintain the reaction pH in between 3.5−5.0. After being stirred at room temperature overnight, the solvent was removed and the residue was partitioned between ether (30 mL) and brine. The organic phase was washed with saturated $NaHCO_3$ (2 × 20 mL) and brine (2 × 10 mL), dried over $MgSO_4$, and evaporated to give the **34a,b** (yield 70−76%). Compounds **34a,b** were taken to the next step without further purification. Each residue was dissolved in dry DCM (20 mL) and reacted with **16b,d** (1.0 equiv) in the presence of TEA (1.0 equiv.) at 20 °C overnight. The reaction mixture was poured into a separatory funnel, washed with water (2 × 20 mL), $NaHCO_3$ (3 × 20 mL), and brine solution (2 × 20 mL), dried over $MgSO_4$, and evaporated under vacuum to give aza-tripeptide **35a,b**.

*Boc-Gly-AzaPhe-Leu-OMe (**35a**).* Compound **35a** was prepared according to the general method given for the preparation of **35a,b**: white microcrystals (0.405 g, 90%); mp 167.0−170.0 °C (lit.[32] mp not reported); $[\alpha]^{20}_D$ −9.0 (*c* 1.0, CH_3OH); ^1H NMR (300 MHz, acetone-d_6) δ 9.41 (s, 1H), 7.50−7.17 (m, 5H), 6.47 (d, *J* = 7.2 Hz, 2H), 4.54−4.29 (m, 1H), 3.69 (d, *J* = 5.5 Hz, 2H), 3.64 (s, 3H), 3.30 (s, 2H), 1.88−1.49 (m, 3H), 1.40 (s, 9H), 0.92 (d, *J* = 6.6 Hz, 3H), 0.90 (d, *J* = 6.6 Hz, 3H); 174.8, 168.9, 157.4, 156.8, 136.7, 128.9, 128.8, 127.9, 81.1, 52.5, 52.4, 51.3, 44.0, 41.2, 28.5, 24.9, 23.1, 21.9; HRMS (ESI) calcd for $C_{22}H_{34}N_4O_6Na$ $[M + Na]^+$ 473.2371, found 473.2395.

*Cbz-Gly-AzaPhe-Val-O^{t}Bu (**35b**).* Compound **35b** was prepared according to the method for preparation of **35a**: white microcrystals (0.410 g, 80%); mp 48.0−49.0 °C; $[\alpha]^{20}_D$ −12.0 (*c* 1.0, CH_3OH); ^1H NMR (300 MHz, $CDCl_3$) δ 8.24 (s, 0.5H), 7.37−7.14 (m, 11H), 5.86 (d, *J* = 8.6 Hz, 1H), 5.61 (s, 0.5H), 5.15−4.94 (m, 2H), 4.96−4.77 (m, 0.5H), 4.49 (d, *J* = 14.6 Hz, 0.5H), 4.30 (d, *J* = 8.7 Hz, 1H), 4.28 (d, *J* = 8.7 Hz, 1H), 3.69 (d, *J* = 5.5 Hz, 2H), 2.19−1.98 (m, 1H), 1.40 (s, 9H), 0.90 (d, *J* = 6.9 Hz, 3H), 0.83 (d, *J* = 6.9 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.4, 169.0, 157.3, 136.7, 136.1, 129.9, 128.9, 128.8, 128.7, 128.5, 128.3, 127.9, 82.1, 67.6, 59.2, 51.5, 43.9, 31.7, 28.3, 19.2, 18.0; HRMS (ESI) calcd for $C_{27}H_{36}N_4O_6Na$ $[M + Na]^+$ 535.2527, found 535.2560.

*H-Gly-AzaPhe-Leu-OMe·TsOH (**36**).* Compound **36** was prepared according to the method for preparation of **29c**: white microcrystals (0.372 g, 95%); mp 155.0−157.0 °C; $[\alpha]^{20}_D$ −26.0 (*c* 1.0, CH_3OH); ^1H NMR (300 MHz, DMSO-d_6) δ 8.01 (s, 1H), 7.43 (d, *J* = 8.1 Hz, 2H), 7.34−7.14 (m, 5H), 7.07 (d, *J* = 7.8 Hz, 2H), 4.25−4.04 (m, 1H), 3.74−3.52 (m, 7H), 2.24 (s, 3H), 1.70−1.37 (m, 3H), 0.82 (d, *J* = 6.3 Hz, 3H), 0.79 (d, *J* = 6.3 Hz, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 174.2, 166.7, 157.4, 146.2, 138.3, 138.2, 128.8, 128.7, 128.6, 126.1, 119.9, 79.0, 52.4, 51.7, 43.0, 28.8, 24.7, 23.4, 22.0, 21.5. Compound **36** was partially characterized by ^1H and ^{13}C NMR and used for the next step without further purification

*Cbz-Tyr-(OBn)-Bt (**38**).* Benzotriazole (6.0 mmol, 3.0 equiv) was dissolved in dry DCM (50 mL). $SOCl_2$ (2.2 mmol) was added by syringe, and the mixture was stirred for 15 min at rt under argon. The solution temp was lowered to −30 to −40 °C (dry ice + acetone), and 2.0 equiv of TEA was added. After 5 min of stirring, Cbz-Tyr-(OBn)-OH **37** (2.0 mmol, 1.0 equiv) was added and the mixture stirred for 2 h keeping the temperature at −30 to −40 °C. After completion of the reaction, ice-cold water (20 mL) was added and the organic layer was washed with water (20 mL × 2), $NaHCO_3$ (20 mL × 4), and then brine (20 mL × 2). The organic layer was dried over $MgSO_4$ and evaporated to yield a white solid: white microcrystals (0.841 g, 83%); mp 87.0−89.0 °C; $[\alpha]^{20}_D$ −20.0 (*c* 1.0, CH_3OH); ^1H NMR (300 MHz, $CDCl_3$) δ 8.20 (d, *J* = 8.2 Hz, 1H), 8.11 (d, *J* = 8.6 Hz, 1H), 7.63 (t, *J* = 7.6 Hz, 1H), 7.50 (t, *J* = 7.5 Hz, 1H), 7.41 − 7.23 (m, 10H), 7.03 (d, *J* = 8.2 Hz, 3H), 6.82 (dd, *J* = 8.8, 3.1 Hz, 2H), 6.15 − 5.88 (m, 1H), 5.68 (d, *J* = 8.1 Hz, 1H), 5.07 (d, *J* = 3.5 Hz, 2H), 4.96 (d, *J* = 2.8 Hz, 2H), 3.40 (dd, *J* = 13.9, 5.2 Hz, 1H), 3.14 (dd, *J* = 14.1, 7.6 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.8, 158.0, 155.7, 145.9, 136.7, 135.9, 130.9, 130.6, 130.2, 128.4, 128.0, 127.8, 127.3, 127.1, 126.4, 120.2, 114.9, 114.2, 69.8, 67.1, 55.7, 37.8. Compound **38** was partially characterized by ^1H and ^{13}C NMR and taken to the next step without further purification.

*Cbz-Tyr-(OBn)-Gly-Bt (**39**).* Gly (1.2 mmol, 1.2 equiv) and TEA (1.2 mmol, 1.2 equiv) were dissolved in a minimum amount of cold water (5.0 mL). Acetonitrile (10 mL) was added, and the solution was cooled to 10 °C. A solution of Cbz-Tyr-(OBn)-Bt **38** (1.0 mmol, 1.0 equiv) in acetonitrile (5.0 mL) was added and the mixture stirred for 2 h at 20 °C. The reaction mixture was monitored by TLC [EtOAc−hexanes (1:2)]. After completion of reaction, the solvent was evaporated. The residue was dissolved in DCM (30 mL) and washed with 2 N HCl solution (4 × 10 mL), water (10 mL), and brine (10 mL). The solvent was dried over $MgSO_4$ and evaporated to give Cbz-Tyr-(OBn)-Gly-OH, which was directly taken to the next step without further purification. Benzotriazole (3.0 mmol, 3.0 equiv) was dissolved

dx.doi.org/10.1021/jo302251e | J. Org. Chem. 2013, 78, 3541−3552

in dry DCM (50 mL). $SOCl_2$ (1.1 mmol) was added by syringe, and the mixture was stirred for 15 min at rt. under argon. The solution temperature was lowered to −30 to −40 °C (dry ice + acetone), and 2.0 equiv of TEA was added. After 5 min of stirring, Cbz-Tyr-(OBn)-Gly-OH was added, and the mixture was stirred for 2 h keeping the temperature at −30 to −40 °C. After completion of the reaction, ice-cold water (20 mL) was added, and the organic layer was washed with water (20 mL × 2); $NaHCO_3$ (20 mL × 4) and then brine (20 mL × 2). The organic layer was dried over $MgSO_4$ and evaporated to yield Cbz-Tyr-(OBn)-Gly-Bt (**39**): white microcrystals (0.451 g, 80%); mp 132−133 °C; $[\alpha]^{20}_D$ −19.0 (*c* 1.0, CH_3OH); ^1H NMR (300 MHz, $CDCl_3$) δ 8.10 (d, *J* = 9.0 Hz, 1H), 8.06 (d, *J* = 8.1 Hz, 1H), 7.58 (t, *J* = 7.1 Hz, 1H), 7.46 (t, *J* = 7.1 Hz, 1H), 7.36−7.29 (m, 5H), 7.28−7.22 (m, 6H), 7.19−7.00 (m, 3H), 6.91−6.75 (m, 2H), 5.74 (d, *J* = 7.1 Hz, 1H), 5.18−4.99 (m, 4H), 4.93 (s, 2H), 4.75−4.44 (m, 1H), 3.24−2.96 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.6, 167.7, 158.1, 156.6, 146.1, 139.0, 137.1, 136.1, 131.0, 130.6, 128.7, 128.3, 128.1, 127.6, 126.7, 126.2, 120.5, 115.2, 114.2, 70.1, 67.5, 56.6, 43.5, 37.9. Compound **39** was partially characterized by ^1H, ^{13}C NMR and taken to the next step without further purification.

*Leu-enkephalin-aza Analogue (**40**).* The tosylate salt of free aza-tripeptide **36** (0.5 mmol, 1.0 equiv) and DIPEA (1.0 mmol, 2.0 equiv) were dissolved in dry THF. A solution of Cbz-Tyr-(OBn)-Gly-Bt **39** (0.5 mmol, 1.0 equiv) in THF (5.0 mL) was added and the mixture stirred for 12 h at 20 °C. The reaction mixture was monitored by TLC [EtOAc−hexanes (1:2)]. After completion of the reaction, the solvent was evaporated. The residue was dissolved in DCM (30 mL) and washed with 2 N HCl solution (4 × 10 mL), water (10 mL), and brine (10 mL). The solvent was dried over $MgSO_4$ and evaporated to give Leu-enkephalin-aza analogue (**40**): white microcrystals (0.278 g, 70%); mp 89.0−91.0 °C; $[\alpha]^{20}_D$ −13.0 (*c* 1.0, CH_3OH); ^1H NMR (300 MHz, DMSO-d_6) δ 9.84 (s, 1H), 8.17−7.91 (m, 2H), 7.42−7.25 (m, 20H), 6.90 (dd, *J* = 5.8, 2.7 Hz, 2H), 6.46 (d, *J* = 7.9 Hz, 1H), 5.06 (s, 2H), 5.03−4.82 (m, 3H), 4.77−4.37 (m, 2H), 4.32−4.16 (m, 2H), 3.93−3.68 (m, 4H), 3.62 (s, 3H), 3.01 (dd, *J* = 13.9, 4.2 Hz, 2H), 2.94−2.65 (m, 2H), 1.77−1.41 (m, 3H), 0.91−0.84 (m, 6H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 174.2, 172.8, 172.5, 170.3, 168.9, 157.4, 156.5, 138.4, 137.8, 137.5, 131.1, 130.9, 130.8, 129.0, 128.8, 128.7, 128.5, 128.4, 128.2, 128.1, 128.0, 127.9, 127.6, 127.5, 127.3, 114.9, 70.5, 69.7, 67.0, 65.9, 57.0, 52.3, 42.7, 42.0, 37.1, 31.8, 24.6, 23.5, 21.9; HRMS (ESI) calcd for $C_{43}H_{50}N_6O_9Na$ $[M + Na]^+$ 817.3531, found 817.3551.

■ ASSOCIATED CONTENT

Supporting Information

^1H and/or ^{13}C spectra of all compounds listed in the Experimental Section. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This paper was funded by King Abdulaziz University under Grant No. D- 006/431. The authors, therefore, acknowledge technical and financial support of KAU. We thank Dr. C. D. Hall and Mr. Z. Wang for helpful discussions.

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