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Efficient Synthesis of Peptides by Extension at the N- and C-Terminii of Arginine

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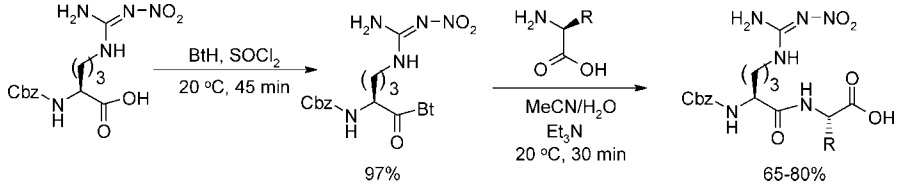

L-N^{ω}-Nitroarginine and L-arginine were coupled with *N*-(Cbz-α-aminoacyl)benzotriazoles and *N*-Cbz-dipeptidoylbenzotriazoles to provide arginine LL-dipeptides **9a**−**e**, **11a**−**d**; LLL-tripeptides **18a**−**c**, **20**; and diastereomeric mixtures (**9b**+**9b′**), (**9c**+**9c′**), (**11b**+**11b′**) and (**18c**+**18c′**) [compound numbers written within parentheses represent a diastereomeric mixture or racemate; compound numbers without parentheses represent an achiral compound or a single enantiomer] by extension at the N-terminus of arginine, in isolated yields of 66−95% with complete retention of chirality as evidenced by NMR and HPLC analysis. Arginine LL-dipeptides **15a**−**d** were synthesized by extension at the C-terminus of arginine in isolated yield of 66−80%, using benzotriazole activated arginine L-$^{\omega}NO_2$-Arg-Bt, **13**. Our methodology has also been used to synthesize the protected RGD peptide (Cbz$^{\alpha}$-L-$^{\omega}NO_2$-Arg-Gly-L-Asp-$(OH)_2$) **21**.

Introduction

The essential amino acid L-arginine with its guanidine group is involved in numerous diverse biological processes connected *inter alia* with cell division, healing wounds, removal of ammonia, immune functions, and hormone release.[1]

L-Arginine is an immediate precursor of nitric oxide (NO) in a reaction catalyzed by nitric oxide synthase (NOS) isoforms.[2] Nitric oxide is a potent biological signal for diverse physiological processes within the cardiovascular, immune, and nervous systems.[3] Overproduction of NO can lead to chronic neurodegenerative diseases including Alzheimer's, Parkinson,[4a–e] and inflammatory diseases such as arthritis[5] and colitis.[6] On the other hand, impaired NO production can cause hypertension[7] and atherosclerosis.[8] Therefore, many studies have been conducted on novel substrates and isoform-selective NOS inhibitors in attempts to find treatments for pathological NO production in biological systems. N^{ω}-Methyl-L-arginine and N^{ω}-ethyl-L-arginine show limited selective inhibition of the NOS isoforms;[9] however, high selectivity was estimated for dipeptides and dipeptide esters containing N^{ω}-nitroarginine and phenylalanine.[10] Important selective inhibitors of neuronal NO synthase (nNOS) over endothelial cell NO synthase (eNOS) include nonbiological dipeptide amides and peptidomimetics, built on an L-N^{ω}-nitroarginine scaffold (**1a**−**c**, Figure 1).[4e]

Arginine is the preferred residue at the P1 position of serine protease substrates[11] such as trypsin,[12] factor Xa,[13] and others of the coagulation cascade; it is an essential residue in the integrin recognition sequence Arg-Gly-Asp.[14a,b]

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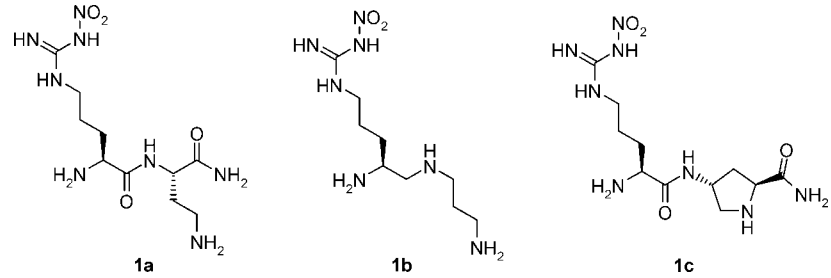

FIGURE 1. Selective inhibitors based on L-N^{ω}-nitroarginine scaffold **1a**−**c**.

Its geometry, charge distribution, and ability to form multiple hydrogen bonds make arginine ideal for binding negatively charged groups; when located on the outside of a protein it can interact effectively with a polar environment. Certain peptides containing high percentages of cationic amino acids efficiently translocate through a cell membrane, as can short oligomers of arginine.[15a,b] Synthetic arginine-rich peptides are efficient transporters of diverse biomolecules including nucleic acids,[17a,b] peptides, and proteins[18] into the cytoplasmic and nuclear regions of living cells.[16] As a result of their extensive physiological functions, arginine-containing peptides and conjugates show diverse activities as therapeutic agents,[19] e.g., as drugs in anticancer therapy.[20a–d]

Considerable effort has been devoted to the synthesis of arginine peptides and peptidomimetics[21a,b] utilizing solution and solid phase methodologies.[22a–e] The highly basic nature and nucleophilic character of the guanidine moiety in arginine often requires appropriate protection before chemical manipulations. The mixed anhydride method was used to prepare various C-terminal arginine peptides from Cbz-L-N^{ω}-nitroarginine,[23a–d] *N*-acetylated peptides from L-N^{ω}-nitroarginine ester,[24] the selective anticoagulant tripeptide D-Phe-Pro-Arg[25] and peptides from tribenzoxycarbonyl-L-arginine.[26a,b] Other approaches utilized Arg(Cbz)$_2$-OH with TFFH/collidine in CH_2Cl_2;[27] coupling agents were DCC,[28] DCC/DNP, DCC/HOBt, DPPA,[29] and *N*-carboxyanhydride[30] or pyrophosphate.[31] Protected Leu-Arg-Pro tripeptides are prepared with NMM/pivaloyl chloride/HOBt in DMF or CH_2Cl_2.[32] Unprotected arginine couples with activated amino acid pentafluorophenyl ester (Pfp) in DMF and utilization of orthogonal protection affords free tripeptide **4** (Scheme 1).[33]

With carboxyl-activated protected arginines, intramolecular δ-lactam formation competes with coupling[29,34] to an extent depending on both the nature of the carboxyl activation and the amino acid component. Mixed anhydride coupling favors δ-lactam formation[29,34a] as also does EDC/HOBt/NMM in MeCN (Scheme 2).[34b] Lactam formation was favored by deprotection of the guanidino function[27] and minimized by the DPPA method.[29]

We have used *N*-acylbenzotriazoles extensively for N-acylation of amines[35a–e] and amides,[36] for C-acylation,[37a–c] and

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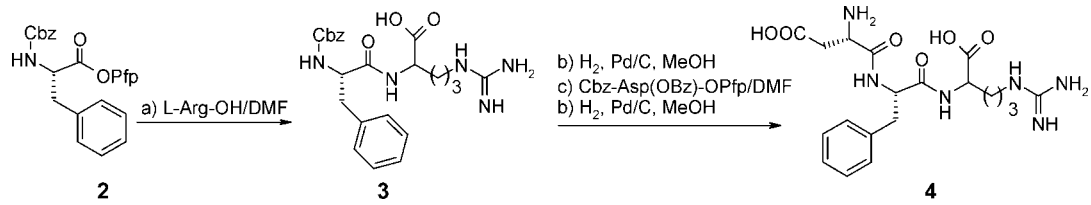
SCHEME 1



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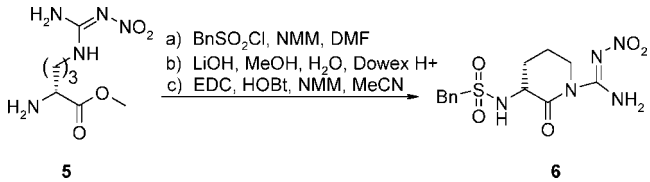

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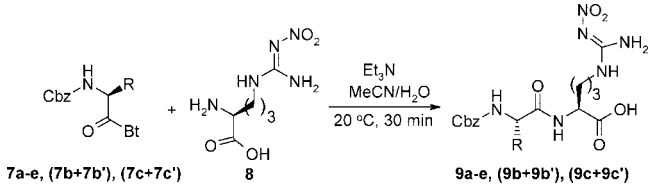

for O-acylation.[38a,b] Chiral di-, tri-, and tetrapeptides were prepared using (α-Boc-, Cbz-, and Fmoc-aminoacyl)benzotriazoles in aqueous acetonitrile in good to high yields from carboxyl-unprotected amino acids, both without side chain functionality (Ala, Phe, Val, Leu)[39a] and with unprotected (Trp, Tyr, Gln, Ser, Cys, Asn)[39b–d] and protected (N^{ω}-Cbz-L-Lys-OH) side chain functionality.[40] The original chirality was preserved in all cases (>95% as evidenced by NMR and >99% by HPLC).

Herein, we present convenient procedures for the coupling of arginine and N^{ω}-nitroarginine to the C- and N-termini of amino acids and peptides using benzotriazole methodology.

Results and Discussion

Preparation of LL-Dipeptides 9a−e and Diastereomeric Mixtures (9b+9b′) and (9c+9c′) by Extension at the α-N-Terminus of L-N^{ω}-NO_2-Arg-OH. L-N^{ω}-NO_2-Arg-OH **8** couples with *N*-(α-Cbz-aminoacyl)benzotriazoles **7a−e** derived from chiral L-Phe, L-Met, L-Ala, L-Trp, and di-Cbz-L-cystine-di-Bt and corresponding racemic mixtures (**7b+7b′**) and (**7c+7c′**) in aqueous acetonitrile (CH_3CN/H_2O, 1:2) containing Et_3N in 30 min at 20 °C to give dipeptides **9a−e** and diastereomeric mixtures (**9b+9b′**) and (**9c+9c′**) (75−95%), all isolated without column chromatography (Scheme 3, Table 1). Diastereomeric mixtures were prepared to confirm that the original chirality of the amino acids and peptides used is maintained during coupling reactions by means of HPLC and NMR analysis.

^{1}H NMR analysis of dipeptides **9a−e** revealed that each LL-dipeptide displayed two sets of doublets for two amide NH protons ranging from 7.30 to 8.40 ppm, supporting their enantiopurity. However, for each of the diastereomeric mixtures (**9b+9b′**) and (**9c+9c′**), one of the amide NH protons showed as a multiplet. The ^{13}C NMR of (**9b+9b′**) and (**9c+9c′**) each showed doubled signals for each aliphatic and carbonyl carbon. The guanidine NH_2 protons all appeared as broad signals ranging from 7.30 to 8.30 ppm. The NH proton of the guanidine group is a broad singlet at ∼8.50 ppm.

HPLC Analysis. The enantiopurity of each of the LL-dipetides **9a−e** was further supported by HPLC analyses by using a Chirobiotic T column [detection at 220 nm, flow rate 0.4−1.0 mL/min; eluting with MeOH/H_2O (1:1) for **9a**,**c**,**d** and (**9c+9c′**), MeOH/H_2O (9:1) for **9b** and (**9b+9b′**); MeOH for **9e**]. Enantiopure compound **9b** showed a single retention time at 10.13 min, whereas the corresponding diastereomeric mixture (**9b+9b′**) showed two retention times with equal intensity at 9.42 and 10.03 min. Similarly in the case of compound **9c** one single retention time at 10.79 min was observed, whereas the diastereomeric mixture (**9c+9c′**) showed two retention times at 8.64 and 10.45 min. Enantiopure compounds **9a**, **9d**, and **9e** showed single retention times at 11.16, 11.12, and 3.04 min, respectively.

Preparation of LL-Dipeptides 11a−d and Diastereomeric Mixture (11b+11b′) by Chain Elongation at the α-N-Terminus of L-Arg-OH. L-Arginine-containing dipeptides **11a−d** and diastereomeric mixture (**11b+11b′**) were prepared by extension at the N-terminus of L-arginine **10** by coupling with *N*-(Cbz-α-aminoacyl)benzotriazoles **7a−d**, (**7b+7b′**) in aqueous acetonitrile without Et_3N at 20 °C for 6 h (the strongly basic guanidine group remains protonated at all pH values up to pH 9). After evaporation of solvent, the residue was purified by reprecipitation from MeOH/Et_2O. Repetition of this procedure three times afforded complete removal of byproduct BtH and gave pure dipeptides **11a−d** and the diastereomeric mixture (**11b+11b′**) in yields of 75−83% (Scheme 4, Table 2). NMR analysis of the compounds revealed no detectable racemization (<5%). For each of the enantiopure compounds **11a−d,** two sets of doublets were observed for the amide NH protons. For the diastereomeric mixture (**11b+11b′**), one of the amide NH protons appeared as a multiplet and the ^{13}C NMR spectrum also showed doubling of the signals for the aliphatic and carbonyl carbons.

HPLC Analysis. HPLC analyses (flow rate 1.0 mL/min with MeOH as eluent) of LL-dipeptides **11a**, **11c**, and **11d** each showed single retention times at 1.91, 1.92, and 2.88 min, respectively.

Preparation from Cbz$^{\alpha}$-L-$^{\omega}$$NO_2$-Arg-Bt 13 of Arginine LL-Dipeptides 15a−c and Diastereomeric Mixture (15a+15a′) by Extension at the C-Terminus of L-Arginine. To extend at the arginine C-terminus, Cbz$^{\alpha}$-L-$^{\omega}$$NO_2$-Arg-OH **12** was treated with

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TABLE 1. Preparation of Arginine Dipeptides 9a−e, (9b+9b′), and (9c+9c′) from L-N^{ω}-NO_2-Arg-OH, 8

reactant	product	yield[a] (%)	mp (°C)	$[\alpha]^{23}_D$	t_R[f] (min)
Cbz-L-Phe-Bt **7a**	Cbz-L-Phe-L-$^{\omega}NO_2$-Arg-OH **9a**[b]	90	173−175	−6.2	11.16
Cbz -L-Met-Bt **7b**	Cbz-L-Met-L-$^{\omega}NO_2$-Arg-OH **9b**[c]	80	141−143	−4.2	10.13
Cbz-DL-Met-Bt (**7b**+**7b′**)	Cbz-DL-Met-L-$^{\omega}NO_2$-Arg-OH (**9b**+**9b′**)	95	61−64	−2.7	9.42, 10.03
Cbz-L-Ala-Bt **7c**	Cbz-L-Ala-L-$^{\omega}NO_2$-Arg-OH **9c**[d]	93	168−169	+3.8	10.79
Cbz-DL-Ala-Bt (**7c**+**7c′**)	Cbz-DL-Ala-L-$^{\omega}NO_2$-Arg-OH (**9c**+**9c′**)	92	133−134	−2.3	8.64, 10.45
Cbz-L-Trp-Bt **7d**	Cbz-L-Trp-L-$^{\omega}NO_2$-Arg-OH **9d**[e]	80	65−68	−19.6	11.12
Di-Cbz-L-cystine-di-Bt **7e**	Di-Cbz-L-cystine-di-L-$^{\omega}NO_2$-Arg-OH **9e**	75	105−108	−84.3	3.04

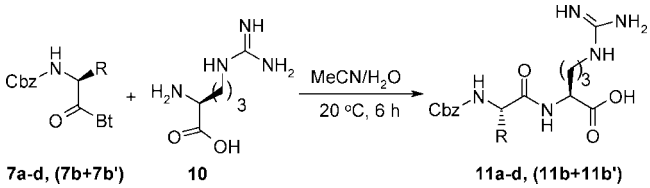
[a] Isolated yields. [b] Lit.[23d] mp 174−176 °C. [c] Lit.[23d] mp 140−143 °C. [d] Lit.[23b] mp 171−172 °C. [e] Lit.[23d] mp 68−75 °C. [f] Retention time for HPLC

TABLE 2. Preparation of Arginine Dipeptides 11a−d and (11b+11b′) from L-Arg-OH, 10

reactant	product	yield[a] (%)	mp (°C)	$[\alpha]^{23}_D$
Cbz-L-Phe-Bt **7a**	Cbz-L-Phe-L-Arg-OH **11a**[b]	83	130−132	−10.4
Cbz-L-Met-Bt **7b**	Cbz-L-Met-L-Arg-OH **11b**	81	143−144	−7.2
Cbz-DL-Met-Bt (**7b**+**7b′**)	Cbz-DL-Met-L-Arg-OH (**11b**+**11b′**)	76	121−124	+5.9
Cbz-L-Trp-Bt **7c**	Cbz-L-Trp-L-Arg-OH **11c**[c]	83	135−137	−18.3
Cbz-L-Val-Bt **7d**	Cbz-L-Val-L-Arg-OH **11d**[d]	75	121−123	−1.0

[a] Isolated yields, [b] Lit.[33b] mp 131−133 °C. [c] Reference 33c. [d] Reference 33d.

SCHEME 4



1*H*-benzotriazole and thionyl chloride in THF at 20 °C to give benzotriazole derivative Cbz$^{\alpha}$-L-$^{\omega}NO_2$-Arg-Bt **13** (97%); this reaction completed in 45 min without formation of side products. Coupling Cbz$^{\alpha}$-L-$^{\omega}NO_2$-Arg-Bt **13** with free amino acids **14a-d**, (**14a**+**14a′**) gave chiral N-terminal arginine dipeptides **15a−d** and the diastereomeric mixture (**15a**+**15a′**) in yields of 65−80% (Scheme 5, Table 3). The procedure was similar to that utilized above for the preparation of **9a−c** (Scheme 3). Purification of crude product by reprecipitation in MeOH/Et_2O gave pure dipeptides **15a−c**, (**15a**+**15a′**), while **15d** was isolated by acidifying at −15 °C. NMR analysis showed no detectable racemization (<5%) for the LL-dipeptides **15a−d** and diastereomeric mixture (**15a**+**15a′**). However monitoring by TLC disclosed that a side product **16** (10−30%) was formed in all of these reactions and could be isolated from the filtrate. The structure of **16** was revealed (by ^{1}H and ^{13}C NMR) to be the intramolecular cyclization product (δ-lactam); **16** formed competitively with the expected dipeptide during coupling of benzotriazole activated nitroarginine **13** with free amino acids, other than Gly-OH.

HPLC Analysis. HPLC analyses for the enantiopure LL-peptides **15a−d** showed single retention times, while diastereomeric mixture (**15a**+**15a′**) showed two retention times at 3.16 and 3.94 min (Table 3).

Preparation of C-Terminal Arginine Tripeptides 18a−c and (18c+18c′) by Extension at the α-N-Terminus of L-N^{ω}-NO_2-Arg-OH 8. *N*-Cbz-dipeptidoylbenzotriazoles **17a**,**b** were obtained as reported previously.[40] Analogs **17c** and (**17c**+**17c′**) were prepared similarly to a described procedure[39d] from Cbz-L-Asp(OBz)Bt **7f** (synthesis of **7f** is described in Supporting Information). L-N^{ω}-NO_2-Arg-OH **8** was coupled with **17a−c**, (**17c**+**17c′**) in aqueous acetonitrile in the presence of Et_3N for 2 h to give tripeptides **18a−c** and diastereomeric mixture (**18c**+**18c′**) in 66−85% yields (Scheme 6, Table 4).

The ^{1}H and ^{13}C NMR spectra of the optically pure LLL-tripeptides **18a−c** showed the absence of racemization (<5%). ^{1}H NMR showed three sets of doublets for the amide NH protons for each of the enantiopure compounds. In the case of the diastereomeric mixture (**18c**+**18c′**), each set of amide NH protons appeared as split doublets and ^{13}C NMR showed doubling of the signals for the aliphatic and carbonyl carbons. The room temperature ^{1}H NMR for **18b** showed the existence of two rotameric forms, which underwent coalescence in a high temperature ^{1}H NMR experiment. HPLC analysis of tripeptide **18a** shows one retention time at 3.17 min supporting its enantiopurity.

Tripeptide **18c** is a protected analogue of H-Asp-Phe-Arg-OH **4**, recently adopted as a catalyst for asymmetric Michael addition reactions.[33] The classical method[33] for the preparation of **4** (Scheme 1), utilizing the pentafluorophenyl ester of amino acid in DMF, requires prolonged reaction times and complicated isolation procedures; our methodology advantageously includes coupling of benzotriazole activated amino acid in aqueous media, short reaction times, and simple workup procedures affording final chiral tripeptide **18c** and diastereomeric mixture (**18c**+**18c′**) in 82−84% yields.

Application of Benzotriazole Methodology in the Synthesis of Protected RGD Peptide. We used benzotriazole methodology to synthesize protected arginyl-glycyl-α-aspartyl "RGD" tripeptide Cbz-Arg(NO_2)-Gly-α-Asp$(OH)_2$ **21** (Scheme 7). The recent literature[41] preparation of dibenzyl ester derivative Cbz-Arg(NO_2)-Gly-α-Asp(β-OBz)OBz utilizes amino acid esters and requires low temperatures of −5 to +8 °C, prolonged reaction times (14−16 h), and coupling reagents (HOBt, DCCI). Dibenzyl ester protected tripeptide was finally deprotected by a conventional method to give Arg-Gly-Asp-$(OH)_2$.[41] We now show that our methodology utilizing benzotriazole activated amino acid **13** enables synthesis of protected RGD peptide **21** from Cbz$^{\alpha}$-L-$^{\omega}NO_2$-Arg-Gly-Bt **19** and free aspartic acid **20** by modification of the coupling procedure (THF was added to the MeCN/H_2O solvent system) adopted for the preparation of other tripeptides.

(41) Abo-Ghalia, M.; Abd El-Rahman, S.; El-Kafrawy, A.; Kalomuch, A. *Amino Acids* **2003**, *24*, 405–411.

SCHEME 5

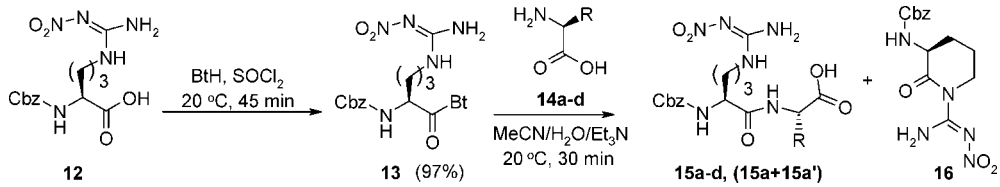


TABLE 3. Preparation from Cbz$^{\alpha}$-L-$^{\omega}$NO$_2$-Arg-Bt 13 of Arginine Dipeptides 15a−d, (15a+15a′)

reactant	product	yield[a] (%)	mp (°C)	$[\alpha]^{23}_D$	t_R[c] (min)
L-Phe-OH **14a**	Cbz$^{\alpha}$-L-$^{\omega}$NO$_2$-Arg-L-Phe-OH **15a**	68	217−219	+5.3	3.01[d]
DL-Phe-OH (**14a**+**14a′**)	Cbz$^{\alpha}$-L-$^{\omega}$NO$_2$-Arg-DL-Phe-OH (**15a**+**15a′**)	65	205−207	+8.1	3.16, 3.94[d]
L-Met-OH **14b**	Cbz$^{\alpha}$-L-$^{\omega}$NO$_2$-Arg-L-Met-OH **15b**	66	144−146	−4.6	3.34[d]
L-Ser-OH **14c**	Cbz$^{\alpha}$-L-$^{\omega}$NO$_2$-Arg-L-Ser-OH **15c**	65	83−85	−5.6	3.57[d]
Gly-OH **14d**	Cbz$^{\alpha}$-L-$^{\omega}$NO$_2$-Arg-Gly-OH **15d**[b]	80	115−117	−1.5	4.15[d]

[a] Isolated yields. [b] Lit.[41] mp 114−117 °C. [c] Retention time. [d] Flow rate 1.0 mL/min, eluent MeOH.

SCHEME 6

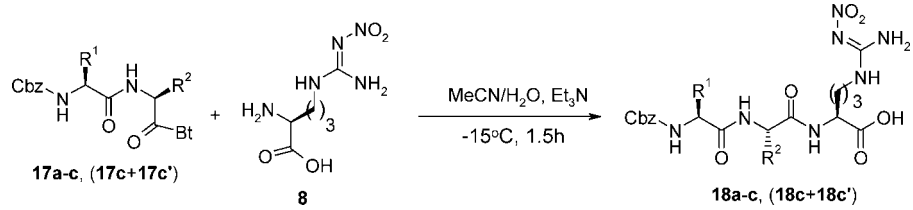
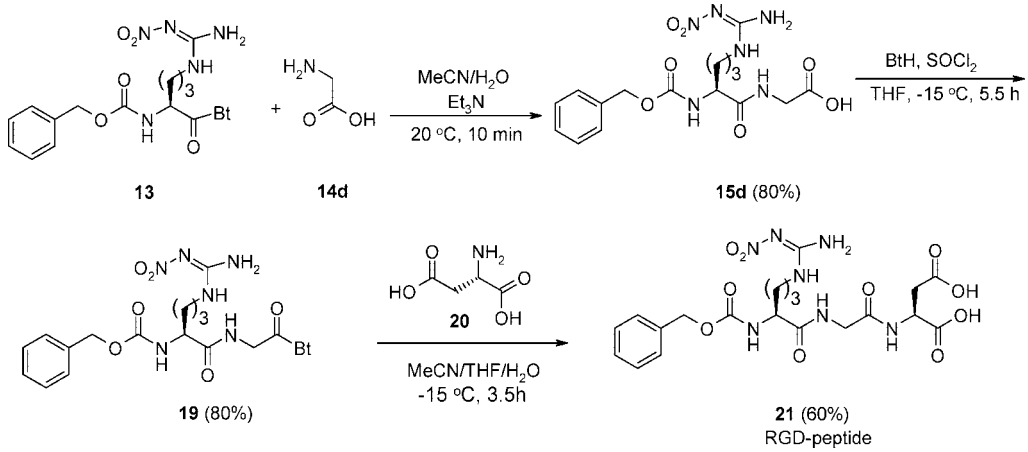


TABLE 4. Preparation of L-Arginine Tripeptides 18a−c and (18c+18c′)

reactant	product	yield[a] (%)	mp (°C)	$[\alpha]^{23}_D$
Cbz-L-Ala-L-Trp-Bt **17a**	Cbz-L-Ala-L-Trp-L-$^{\omega}$NO$_2$-Arg-OH **18a**	66	150−151	−10.2
Cbz-L-Phe-L-Met-Bt **17b**	Cbz-L-Phe-L-Met-L-$^{\omega}$NO$_2$-Arg-OH **18b**	75	77−79	−8.0
Cbz-L-Asp(OBz)-L-Phe-Bt **17c**[b]	Cbz-L-Asp(OBz)-L-Phe-L- $^{\omega}$NO$_2$-Arg-OH **18c**	84	128−129	−11.7
Cbz-L-Asp(OBz)-DL-Phe-Bt (**17c**+**17c′**)[b]	Cbz-L-Asp(OBz)-DL-Phe-L-$^{\omega}$NO$_2$-Arg-OH (**18c**+**18c′**)	82	60.4−65.0	−6.3

[a] Isolated yields; [b] Syntheses of **17c** and (**17c**+**17c′**) are described in Supporting Information.

SCHEME 7. Preparation of Protected RGD Peptide 21



In comparison, our methodology offers simple preparative and workup procedures, takes less time to complete, uses inexpensive reagents, gives high yields, and allows the use of free amino acids as coupling components, affording **21** in good yield.

Conclusions

The methodology described in the present paper provides for the convenient and efficient preparation of arginine di- and tripeptides in short reaction times utilizing simple preparative and workup procedures, inexpensive reagents, and free amino acids as coupling components. The peptides can be prepared by chain elongation at either the N- or C-terminus of L-arginine. We have successfully synthesized protected RGD peptide sequence with our benzotriazole methodology.

Experimental Section

General Procedure for the Preparation of LL-Dipeptides 9a−e and Diastereomeric Mixtures (9b+9b′), (9c+9c′). *N*-(Cbz-

α-aminoacyl)benzotriazoles **7a**−**d** and (**7b**+**7b′**) (0.5 mmol) were added at 20 °C to a solution of L-$^{\omega}NO_2$-Arg-OH **2** (0.5 mmol) in MeCN (5 mL)/H_2O (10 mL) in the presence of Et_3N (0.6 mmol). The reaction mixture was then stirred at 20 °C until the starting material was completely consumed as observed by TLC using EtOAc/hexanes (1:2) as the eluent. After addition of 4 N HCl (1 mL), the solution was concentrated under reduced pressure to remove acetonitrile. Residue was extracted with EtOAc (20 mL), and the organic extract was washed with 4 N HCl (5 mL) and saturated NaCl (10 mL) and then dried over anhydrous $MgSO_4$. Evaporation of the solvent gave the desired product in pure form, which was further recrystallized from MeOH/Et_2O unless specified otherwise.

(*S*)-2-((*S*)-2-Benzyloxycarbonylamino-3-phenylpropanoylamino)-5-nitroguanidinopentanoic Acid (Cbz-L-Phe-L-$^{\omega}NO_2$-Arg-OH, 9a). White microcrystals (90%), mp 173−175 °C (lit.[23d] 174−176 °C), $[\alpha]^{23}_D = -6.2$ (*c* 1.0, DMF); ^{1}H NMR (DMSO-d_6) δ 1.40−1.70 (m, 3H), 1.70−1.87 (m, 1H), 2.60−2.80 (m, 1H), 2.90−3.05 (m, 1H), 3.05−3.25 (m, 2H), 4.10−4.35 (m, 2H), 4.92 (s, 2H), 7.00−7.40 (m, 10H), 7.51 (d, J = 8.4 Hz, 1H), 7.60−8.25 (m, 2H), 8.32 (d, J = 7.4 Hz, 1H), 8.56 (br s, 1H), 12.65 (br s, 1H). ^{13}C NMR (DMSO-d_6) δ 24.9, 28.3, 37.4, 40.2, 51.7, 56.0, 65.2, 126.3, 127.5, 127.7, 128.1, 128.3, 129.2, 137.0, 138.1, 155.9, 159.3, 171.8, 173.4. Anal. Calcd for $C_{23}H_{28}N_6O_7$: C, 55.19; H, 5.64; N, 16.79. Found: C, 54.88; H, 5.76; N, 16.63.

General Procedure for the Preparation of LL-Dipeptides 11a−d and Diastereomeric Mixture (11b+11b′). *N*-(Cbz-α-aminoacyl-)benzotriazoles **7a**−**d** and (**7b**+**7b′**) (0.5 mmol) were added at 20 °C to a solution of L-Arg-OH 10 (0.5 mmol) in MeCN (5 mL)/H_2O (3 mL) The reaction mixture was then stirred at 20 °C until the starting material was completely consumed as observed by TLC using EtOAc/hexanes (1:2) as the eluent. The reaction mixture was evaporated under reduced pressure, the residue was dissolved in a minimum amount of MeOH, and then product was reprecipitated with Et_2O. This was repeated three times to remove all BtH from reaction mixture.

(*S*)-2-((*S*)-2-Benzyloxycarbonylamino-3-phenyl-propionylamino)-5-guanidinopentanoic Acid (Cbz-L-Phe-L-Arg-OH, 11a). White microcrystals (83%), mp 131−132 °C (lit.[33b] 131−133 °C), $[\alpha]^{23}_D = -10.4$ (*c* 1.0, DMF); ^{1}H NMR (DMSO-d_6) δ 1.35−1.75 (m, 4H), 1.65−1.80 (m, 1H), 2.95−3.18 (m, 3H), 3.85−4.00 (m, 1H), 4.12−4.25 (m, 1H), 4.93 (s, 2H), 7.05−7.35 (m, 10H), 7.35−7.55 (m, 3H), 7.55−7.70 (m, 2H), 9.41 (br s, 1H). ^{13}C NMR (DMSO-d_6) δ 25.3, 29.8, 37.4, 40.4, 53.7, 56.7, 65.2, 126.2, 127.3, 127.7, 128.1, 128.3, 129.2 137.1, 138.4, 155.9, 157.4, 170.4, 175.5.

Synthetic Procedure for Preparation of Cbz$^{\alpha}$-L-$^{\omega}NO_2$-Arg-Bt, 13. Cbz$^{\alpha}$-L-$^{\omega}NO_2$-Arg-Bt was prepared from Cbz$^{\alpha}$-L-$^{\omega}NO_2$-Arg-OH using a reported procedure.[40] Reaction was completed in 45 min.

Benzyl *N*-[(1*S*)-4-{[Amino(nitroimino)methyl]amino}-1-(1*H*-1,2,3-benzotriazol-1-ylcarbonyl)-butyl]carbamate (Cbz$^{\alpha}$-L-$^{\omega}NO_2$-Arg-Bt, 13). White microcrystals (97%), mp 146−148 °C, $[\alpha]^{23}_D = -16.2$ (*c* 1.0, DMF); ^{1}H NMR (DMSO-d_6) δ 1.58−2.10 (m, 4H), 3.08−4.23 (m, 2H), 5.05 (s, 2H), 5.43−5.55 (m, 1H), 7.28−7.44 (m, 5H), 7.66 (t, J = 7.7 Hz, 1H), 7.83 (t, J = 7.7 Hz, 1H), 7.74−8.16 (m, 2H), 8.20−8.36 (m, 3H), 8.45 (br s, 1H). ^{13}C NMR (DMSO-d_6) δ 24.8, 28.1, 40.2, 54.1, 65.9, 114.0, 120.3, 126.8, 127.9, 128.0, 128.4, 130.6, 131.2, 136.7, 145.4, 156.4, 159.3, 171.8. Anal. Calcd for $C_{20}H_{22}N_8O_5$: C, 52.86; H, 4.88; N, 24.66. Found: C, 52.96; H, 4.89; N, 24.50.

(*S*)-2-((*S*)-2-Benzyloxycarbonylamino-5-(nitroguanidino)pentanoylamino)-3-phenylpropanoic Acid (Cbz$^{\alpha}$-L-$^{\omega}NO_2$-Arg-L-Phe-OH, 15a). White microcrystals (68%), mp 220−222 °C (lit.[23a] 225−226 °C), $[\alpha]^{23}_D = +5.3$ (*c* 1.0, DMF); ^{1}H NMR (DMSO-d_6) δ 1.30−1.80 (m, 4H), 2.80−2.98 (m, 1H), 2.98−3.22 (m, 3H), 3.90−4.15 (m, 1H), 4.37−4.54 (m, 1H), 5.02 (s, 2H), 7.07−7.50 (m, 11H), 7.60−8.25 (m, 2H), 8.12 (d, J = 7.4 Hz, 1H), 8.48 (br s, 1H), 12.78 (br s, 1H). ^{13}C NMR (DMSO-d_6) δ 24.7, 29.2, 36.7, 53.3, 54.2, 65.5, 126.5, 127.7, 128.2, 128.4, 129.2, 137.0, 137.4, 155.9, 159.3, 171.7, 172.8. Anal. Calcd for $C_{23}H_{28}N_6O_7$: C, 55.19; H, 5.64; N, 16.79. Found: C, 54.84; H, 5.76; N, 16.63.

(*S*)-2-[(*S*)-2-((*S*)-2-Benzyloxycarbonylaminopropanoylamino)-3-(1*H*-indol-3-yl)propanoyl-amino]-5-nitroguanidinopentanoic Acid (Cbz-L-Ala-L-Trp-L-$^{\omega}NO_2$-Arg-OH, 18a). White microcrystals (66%), mp 150−151 °C, $[\alpha]^{23}_D = -10.2$ (*c* 1.0, DMF); ^{1}H NMR (DMSO-d_6) δ 1.13 (d, J = 7.1 Hz, 3H), 1.43−1.85 (m, 4H), 2.90−3.15 (m, 1H), 3.15−3.25 (m, 3H), 3.95−4.10 (m, 1H), 4.15−4.20 (m, 1H), 4.50−4.65 (m, 1H), 4.98 (d, J = 12.4 Hz, 1H, A part of AB system,), 5.03 (d, J = 12.6 Hz, 1H, B part of AB system), 6.96 (t, J = 7.4 Hz, 1H), 7.05 (t, J = 7.4 Hz, 1H), 7.15 (s, 1H), 7.20−7.40 (m, 6H), 7.41 (d, J = 7.4 Hz, 1H), 7.58 (d, J = 7.7 Hz, 1H), 7.65−8.40 (m, 2H), 7.96 (d, J = 8.0 Hz, 1H), 8.23 (d, J = 8.0 Hz, 1H), 8.54 (br s, 1H), 10.82 (s, 1H), 12.71 (br s, 1H). ^{13}C NMR (DMSO-d_6) δ 18.1, 24.8, 27.5, 28.4, 50.1, 51.6, 53.1, 65.4, 109.8, 111.2, 118.2, 118.4, 120.8, 123.6, 127.4, 127.8, 128.4, 136.0, 137.0, 155.7, 159.3, 171.5, 172.3, 173.3. Anal. Calcd for $C_{28}H_{34}N_8O_8 \cdot H_2O$: C, 53.50; H, 5.77; N; 17.82. Found: C, 53.18; H, 5.70; N, 17.37. HRMS calcd for $[C_{28}H_{34}N_8O_8 + Na]^+$: 633.2391. Found: 633.2371.

Procedure for Preparation of Cbz$^{\alpha}$-L-$^{\omega}NO_2$-Arg-Gly-L-Asp-OH, 21. Cbz$^{\alpha}$-L-$^{\omega}NO_2$-Arg-Gly-Bt (**19**, 0.5 mmol) was dissolved in a minimum amount of THF and added dropwise at −15 °C to a solution of L-Asp-OH (0.55 mmol) in MeCN (5 mL/3 mL) in the presence of Et_3N (2.1 mmol). The reaction mixture was stirred at −15 °C, and progress was monitored using TLC by disappearance of **19**. After 3.5 h reaction mixture was concentrated under reduced pressure to remove MeCN. The reaction mixture was acidified with 4 N HCl (2 mL) under cold condition, and the solution was extracted with EtOAc (100 mL) after adding solid NaCl to the acidified solution. The organic extract was washed with 4 N HCl and saturated NaCl (10 mL) and then dried over anhydrous $MgSO_4$. Evaporation of solvent under reduced pressure gave the product, which was purified by reprecipitation from MeOH/Et_2O.

(*S*)-2-(2-((*S*)-2-(Benzyloxycarbonylamino)-5-(2-nitroguanidino)-pentanamido)acetamido)succinic Acid (Cbz$^{\alpha}$-L-$^{\omega}NO_2$-Arg-Gly-L-Asp-$(OH)_2$, 21). White microcrystals (60%), mp 75−79 °C, $[\alpha]^{23}_D = -13.7$ (*c* 1.0, DMF); ^{1}H NMR (DMSO-d_6) δ 1.40−1.80 (m, 4H), 2.59 (dd, J = 16.8, 6.6 Hz, 1H), 2.70 (dd, J = 16.5, 5.5 Hz, 1H), 3.03−3.22 (m, 2H), 3.65−3.83 (m, 2H), 3.95−4.08 (m, 1H), 4.50−4.62 (m, 1H), 5.00 (d, J = 12.6 Hz, A part of AB system, 1H), 5.05 (d, J = 12.6 Hz, B part of AB system, 1H), 7.25−7.42 (m, 5H), 7.52 (d, J = 7.4 Hz, 1H), 7.55−8.10 (m, 2H), 8.07−8.30 (m, 2H), 8.48 (br s, 1H) 12.60 (br s, 2H). ^{13}C NMR (DMSO-d_6): δ 24.7, 29.0, 36.1, 41.6, 45.6, 48.5, 54.4, 65.0, 127.8, 128.4, 136.9, 156.1, 159.3, 168.6, 171.6, 172.0, 172.3. HRMS calcd for $[C_{21}H_{31}N_7O_{10} + 2Na]^+$: 570.1531. Found: 570.1531.

Acknowledgment. We thank Dr. Parul Angrish for her initial help and Dr. C. Dennis Hall for helpful suggestions.

Supporting Information Available: Compound characterization data for **7f**, **9b**−**e**, (**9b**+**9b′**), (**9c**+**9c′**), **11b**−**d**, (**11b**+**11b′**), **15b**−**d**, (**15a**+**15a′**), **17c**, (**17c**+**17c′**), **18b**,**c**, (**18c**+**18c′**), **19**, **22a**, (**22a**+**22a′**). This material is available free of charge via the Internet at http://pubs.acs.org.

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