An efficient method for the preparation of peptide alcohols†

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N-Protected LL-dipeptide alcohols **3a–p**, diastereomeric mixture (**3d** + **3d′**) and tripeptide alcohols **6a–c** were synthesized by treatment of various amino alcohols with *N*-protected(α-aminoacyl)benzotriazoles **1a–c**, **1f–m**, (**1a** + **1a′**) and *N*-protected(α-dipeptidoyl)benzotriazoles **5a**, **5b** respectively in good yields with complete retention of chirality.

Introduction

Peptide alcohols are synthetic intermediates for peptide aldehydes which are inhibitors of m-calpain,[1a] Subtilisin 72,[1b] α-Chymotrypsin,[1b] HIV-protease,[1c,d] multicatalytic proteinase complex (MPC)[1e] and other biologically and pharmaceutically active molecules.[1f,g] Peptide alcohols serve as reversible activators of catalytic activity of the 20S proteasome.[2] Peptide alcohols are also used as ligands or precursors for ligands in asymmetric catalysis,[3a–g] and in the preparation of a prototype calix[4]arene-based receptor for carbohydrate recognition.[4]

Reported preparations of peptide alcohols have utilized: (i) coupling reactions with DCC/HOBT,[1c,3d] EDC/HOBT,[3g] HATU, DIPEA,[1a] NMM, Bu^{i}OCOCl,[3b] DEPBT,[5] (ii) aminoacyl chlorides,[6] (iii) activated esters,[3b,e] (iv) reduction of esters,[4,7] ketones,[6] (v) enzymatic synthesis,[8] and (vi) solid phase synthesis.[9]

Utilization of these methods involves: (i) cumbersome reaction conditions,[3b,3e,6] (ii) producing mixtures of diastereomeric alcohols on reduction,[6] (iii) long reaction times (up to 53 hours),[8] (iv) use of expensive reagents and (v) low yields.[6] Thus a mild and efficient approach to peptide alcohols would be of utility.

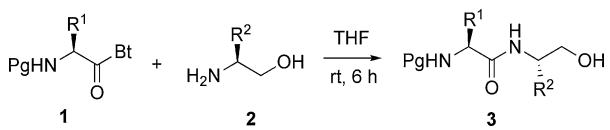
N-Acylbenzotriazoles are advantageous for N-, O-, C-, S-acylation,[10a–j] especially where the corresponding acid chlorides are unstable or difficult to prepare.[10k,l] *N*-Fmoc-(α-aminoacyl)-benzotriazoles and their Boc- and Cbz- analogs enabled the preparation of chiral di-, tri- and tetra-peptides in good yields from natural amino acids in the solution phase.[10b,11a–b] Recently, we have also prepared tri-, tetra-, penta-, hexa-, and hepta-peptides in 71% average crude yields by microwave-assisted solid phase peptide synthesis utilizing *N*-Fmoc-(α-aminoacyl)-benzotriazoles and *N*-Fmoc-protected(α-dipeptidoyl)benzotriazoles.[12a,b] We now report the preparation of di- and tri-peptide alcohols in solution phase by reaction of *N*-protected(α-aminoacyl)benzotriazoles and *N*-protected(α-dipeptidoyl)benzotriazoles with amino alcohols.

Results and discussion

1. Preparation of dipeptide alcohols 3a–p and diastereomeric mixture (3d + 3d′)

The starting *N*-protected(α-aminoacyl)benzotriazoles **1a–e**, **1g–m** and (**1a** + **1a′**) (*N*-Cbz-L-Ala-Bt **1a**, *N*-Cbz-DL-Ala-Bt (**1a** + **1a′**), *N*-Fmoc-L-Val-Bt **1b**, *N*-Fmoc-L-Met-Bt **1c**, *N*-Fmoc-L-Phe-Bt **1d**, *N*-Fmoc-Gly-Bt **1e**, *N*-Cbz-L-Phe-Bt **1g**, *N*-Fmoc-L-Glu(O^{t}Bu)-Bt **1h**, *N*-Fmoc-L-Asp(O^{t}Bu)-Bt **1i**, *N*-Fmoc-L-Lys(Boc)-Bt **1j**, *N*-Fmoc-L-Trp-Bt **1k**, *N*-Fmoc-L-His(Trt)-Bt **1l** and *N*-Fmoc-L-Cys(Trt)-Bt **1m**) were prepared in 75–90% yield from corresponding *N*-protected amino acids following our previously published one-step procedure.[10b] *N*-Boc-L-Val-Bt **1f** was prepared as described previously.[10e]

Reaction of *N*-protected(α-aminoacyl)benzotriazoles **1a–c**, **1f–m** and racemic mixture (**1a** + **1a′**) with various amino alcohols **2** in THF at room temperature for 6 h gave *N*-protected dipeptide alcohols **3a–p** and diastereomeric mixture (**3d** + **3d′**) in 46–89% yield, all isolated without column chromatography (Scheme 1, Table 1). The dipeptide alcohols **3a–p** were characterized by ^{1}H-NMR, ^{13}C-NMR, HRMS and elemental analysis. Diastereomeric mixtures were prepared to confirm that the original chirality of the amino acid used was maintained during the formation of peptide alcohol by means of chiral HPLC analysis. Chiral HPLC (detection at 254 nm, flow rate 1 mL/min, hexanes–isopropanol (9:1) as solvent using Whelk-O1 chiral column) showed a single peak for **3d** at 21.12 min. By contrast, two peaks were observed for the corresponding racemic mixtures (**3d** + **3d′**) at 19.25 and 21.12 min, confirming the enantiopurity of **3d**.



Scheme 1 Preparation of dipeptide alcohols.

2. Preparation of *N*-Fmoc-α-dipeptides 4a, 4b from *N*-Fmoc-(α-aminoacyl)benzotriazoles 1d, 1e

N-Fmoc-protected dipeptides **4a** and **4b** were prepared by reaction of *N*-Fmoc-protected(α-aminoacyl)benzotriazoles **1d** and **1e**, respectively, with the corresponding amino acids in

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Table 1 Preparation of dipeptide alcohols **3a–p** and (**3d** + **3d′**)

Entry	**1**	Amino alcohol **2**	Product **3**, yield (%)[a]
1	**1f**	L-Phenylalaninol	*N*-Boc-L-Val-L-Phenylalaninol **3a**, 86
2	**1f**	L-Leucinol	*N*-Boc-L-Val-L-Leucinol **3b**, 77
3	**1f**	L-*t*-Leucinol	*N*-Boc-L-Val-L-*t*-Leucinol **3c**, 81
4	**1a**	L-Phenylalaninol	*N*-Cbz-L-Ala-L-Phenylalaninol **3d**, 88
5	(**1a** + **1a′**)	L-Phenylalaninol	*N*-Cbz-DL-Ala-L-Phenylalaninol (**3d** + **3d′**), 89
6	**1a**	L-Leucinol	*N*-Cbz-L-Ala-L-Leucinol **3e**[b], 82
7	**1c**	L-Phenylalaninol	*N*-Fmoc-L-Met-L-Phenylalaninol **3f**, 60
8	**1b**	L-Phenylglycinol	*N*-Fmoc-L-Val-L-Phenylglycinol **3g**, 54
9	**1a**	L-Phenylglycinol	*N*-Cbz-L-Ala-L-Phenylglycinol **3h**, 85
10	**1g**	L-Phenylglycinol	*N*-Cbz-L-Phe-L-Phenylglycinol **3i**, 60
11	**1g**	L-Leucinol	*N*-Cbz-L-Phe-L-Luecinol **3j**, 46
12	**1h**	L-Phenylalaninol	*N*-Fmoc-L-Glu(O^{t}Bu)-L-Phenylalaninol **3k**, 80
13	**1i**	L-Phenylglycinol	*N*-Fmoc-L-Asp(O^{t}Bu)-L-Phenylglycinol **3l**, 77
14	**1j**	L-Leucinol	*N*-Fmoc-L-Lys(Boc)-L-Leucinol **3m**, 85
15	**1k**	L-Phenylalaninol	*N*-Fmoc-L-Trp-L-Phenylalaninol **3n**, 83
16	**1l**	L-*t*-Leucinol	*N*-Fmoc-L-His(Trt)-L-*t*-Leucinol **3o**, 60
17	**1m**	L-Leucinol	*N*-Fmoc-L-Cys(Trt)-L-Leucinol **3p**, 70

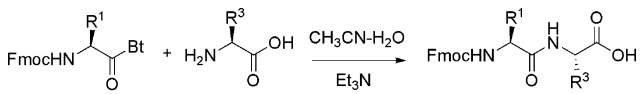
[a] Isolated yield. [b] Lit.,[13] mp 90 °C.

Table 2 Preparation of *N*-Fmoc-α-dipeptides **4a**, **4b** from *N*-Fmoc-protected(α-aminoacyl)benzotriazoles **1d**, **1e**

Entry	**1**	Amino acid	Product **4**, yield (%)[a]	**4**, mp (°C)
1	**1d**	L-Ala-OH	*N*-Fmoc-L-Phe-L-Ala-OH **4a**, 87	208–210[b]
2	**1e**	L-Leu-OH	*N*-Fmoc-Gly-L-Leu-OH **4b**, 85	133–134[c]

[a] Isolated yield. [b] Lit.,[12b] mp 208.7–210.6 °C. [c] Lit.,[14] mp 133–135 °C.

acetonitrile–water (3:1) in the presence of triethylamine at room temperature in 85–87% yield as described previously (Scheme 2, Table 2).[10b]

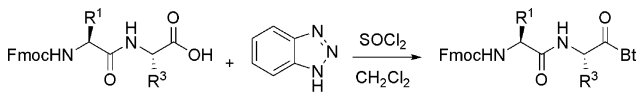


1d, 1e **4a, 4b**

Scheme 2 Preparation of *N*-Fmoc-α-dipeptides **4a**, **4b** from *N*-Fmoc-protected(α-aminoacyl)benzotriazoles **1d**, **1e**.

3. Preparation of *N*-Fmoc-(α-dipeptidoyl)benzotriazoles 5a, 5b

N-Fmoc-(α-dipeptidoyl)benzotriazoles **5a** and **5b** were prepared by treatment of *N*-Fmoc-α-dipeptides **4a** and **4b**, respectively, with benzotriazole and thionyl chloride in CH_2Cl_2 at −15 °C for 3 h in 78–82% yield as described previously (Scheme 3, Table 3).[12b] Compounds **5a**, **5b** were characterized by ^1H-NMR, ^{13}C-NMR and elemental analysis. The *N*-Fmoc-(α-dipeptidoyl)benzotriazoles **5a**, **5b** were utilized for the synthesis of *N*-Fmoc-protected tripeptide alcohols **6a–c**.



4a, 4b **5a, 5b**

Scheme 3 Preparation of *N*-Fmoc-(α-dipeptidoyl)benzotriazoles **5a**, **5b** from *N*-Fmoc-α-dipeptides **4a**, **4b**.

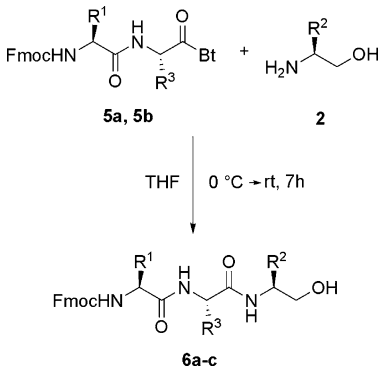
Table 3 Preparation of *N*-Fmoc-(α-dipeptidoyl)benzotriazoles **5a**, **5b**

Entry	**4**	Product **5**	**5**, yield (%)[a]	**5**, mp (°C)
1	**4a**	*N*-Fmoc-L-Phe-L-Ala-Bt, **5a**	**5a**, 82	155–156[b]
2	**4b**	*N*-Fmoc-Gly-L-Leu-Bt, **5b**	**5b**, 78	165–167

[a] Isolated yield. [b] Lit.,[12b] mp 155.2–156.9 °C.

4. Preparation of tripeptide alcohols 6a–c

N-Fmoc-protected tripeptide alcohols **6a–c** were prepared in solution phase by treatment of *N*-Fmoc-(α-dipeptidoyl)benzotriazoles **5a** and **5b** with amino alcohols **2** in THF for 4 h at 0 °C followed by 3 h at room temperature in good yields, all isolated without column chromatography (Scheme 4, Table 4). These compounds were characterized by ^1H-NMR, ^{13}C-NMR, and elemental analysis. No detectable racemization of the tripeptide alcohols was observed in chiral HPLC analysis.



5a, 5b **2**

6a-c

Scheme 4 Preparation of tripeptide alcohols **6a–c** from *N*-Fmoc-(α-dipeptidoyl)benzotriazoles **5a**, **5b**.

In comparison our methodology offers: (1) mild and simple reaction and work-up conditions: we isolated all products under milder reaction conditions and by simply washing or recrystallization; (2) high yields: most of our products were obtained in

Table 4 Preparation of tripeptide alcohols **6a–c** from *N*-Fmoc-(α-dipeptidoyl)benzotriazoles **5a**, **5b**

Entry	5	Amino alcohol **2**	Product **6**, yield (%)[a]
1	**5a**	L-Phenylalaninol	*N*-Fmoc-L-Phe-L-Ala-L-Phenylalaninol[b] **6a**, 69
2	**5a**	L-Phenylglycinol	*N*-Fmoc-L-Phe-L-Ala-L-Phenylglycinol[b] **6b**, 65
3	**5b**	L-Phenylalaninol	*N*-Fmoc-Gly-L-Leu-L-Phenylalaninol[b] **6c**, 69

[a] Isolated yield. [b] Retention times for **6a**, 28.7; **6b**, 13.65; and **6c**, 26.67 min.

high yield (46–89%); (3) no racemization occurred; (4) less time to complete; and (5) uses inexpensive reagents.

Conclusions

In conclusion, a convenient and efficient method for the preparation of di-, and tri-peptide alcohols under mild and simple reaction conditions has been developed by reacting *N*-protected(α-aminoacyl)benzotriazoles and *N*-protected(α-dipeptidoyl)benzotriazoles with amino alcohols at room temperature. All the peptide alcohols were obtained in moderate to good yields with no detectable racemization for chiral compounds.

Experimental

Starting materials and solvents were purchased from commercial sources and used without further purification. Melting points were determined on a Fisher melting apparatus and are uncorrected. Column chromatography was carried out using silica gel 200–425 mesh. HPLC analyses were performed on Shimadzu SPD-20-A using a Whelk-O1 chiral column with detection at 254 nm, a flow rate of 1.0 mL/min and hexanes–isopropanol (9:1) as the eluting solvent. ^{1}H NMR (300 MHz) and ^{13}C NMR (75 MHz) spectra were recorded on a 300 MHz NMR spectrometer with $CDCl_3$ or DMSO-d_6 as solvents. *J* values are given in Hz. $[\alpha]_D$ values are given in 10^{-1} deg cm^2 g^{-1}. Elemental analyses were performed on a Carlo Erba-1106 instrument.

Preparation of dipeptide alcohols 3a–p and (3d + 3d′); general procedure

To a mixture of *N*-protected(α-aminoacyl)benzotriazoles **1** (1 mmol) in dry THF (10 mL) was added the α-amino alcohol **2** (1 mmol) and the mixture was stirred for 6 h at room temperature. The solvent was evaporated under vacuum and the residue was dissolved in ethyl acetate (15 mL), washed with Na_2CO_3 (3 × 5 mL), brine (1 × 5 mL), water (1 × 5 mL) and dried over Na_2SO_4. The solvent was evaporated and the solid residue was crystallized from ethyl acetate–hexanes to afford the corresponding dipeptide alcohols **3**.

N-Boc-L-Val-L-Phenylalaninol 3a[1a]**.** (Yield 0.19 g, 86%.) White microcrystals; mp 145.0–146.0 °C (EtOAc–hexanes). $[\alpha]_D^{24}$ –49.4 (*c* 1.0 in MeOH). (Found: C, 65.26; H, 8.94; N, 7.99. Calc. for $C_{19}H_{30}N_2O_4$: C, 65.12; H, 8.63; N, 7.99%.) δ_H (300 MHz; $CDCl_3$; Me_4Si): 7.37–7.26 (5 H, m, Ar*H*), 6.32 (1 H, d, *J* 7.2, N*H*), 4.98 (1 H, br s, N*H*), 4.24 (1 H, br s, O*H*), 3.89 (1 H, t, *J* 6.0, CH_2CH), 3.77–3.59 (2 H, m, $PhCH_2$), 3.00–2.86 (2 H, m, OCH_2), 2.22–2.11 (1 H, m, $(CH_3)_2CH$), 1.82 (1 H, br s, O*H*), 1.50 (9 H, s, 3 × CCH_3), 0.96 (3 H, d, *J* 6.9, $CH(CH_3)$) and 0.87 (3 H, d, *J* 5.7, $CH(CH_3)$); δ_C (75 MHz; $CDCl_3$; Me_4Si): 172.0, 156.2, 137.8, 129.3, 128.6, 126.6, 80.2, 63.4, 60.6, 52.9, 37.0, 30.6, 28.4, 19.3 and 17.9.

Preparation of tripeptide alcohols 6a–c; general procedure

To a solution of *N*-Fmoc-(α-dipeptidoyl)benzotriazole **5** (0.29 mmol) in THF (5 mL), amino alcohol (0.29 mmol) was added at 0 °C. The reaction mixture was stirred at 0 °C for 4 h followed by 3 h at room temperature. Hexanes (5 mL) were added and the solid separated was washed with diethyl ether (2 × 10 mL) and dried to give the corresponding tripeptide alcohol **6**.

Fmoc-L-Phe-L-Ala-L-Phenylalaninol 6a. (Yield 0.11 g, 69%.) White microcrystals; mp 185.0–186.0 °C (from tetrahydrofuran–hexanes). $[\alpha]_D^{24}$ –67.0 (*c* 1.0 in MeOH). (Found: C, 71.29; H, 6.25; N, 6.83. Calc. for $C_{36}H_{37}N_3O_5H_2O$: C, 70.92; H, 6.12; N, 6.89%.) δ_H (300 MHz; DMSO-d_6; Me_4Si): 8.12 (1 H, d, *J* 7.2, N*H*), 7.87 (2 H, d, *J* 7.5, Ar*H*), 7.73 (1 H, d, *J* 8.1, N*H*), 7.61–7.66 (2 H, m, Ar*H*), 7.41(2 H, t, *J* 8.1, Ar*H*), 7.15–7.34 (13 H, m, Ar*H*, *N*H), 4.81 (1 H, br s, C*H*), 4.25–4.29 (2 H, m, CH_2), 4.13–4.17 (4 H, m, 2 × CH_2), 3.82–3.89 (1 H, m, C*H*), 2.62–3.01 (5 H, m, CH_2, 2 × C*H*, O*H*) and 1.18 (3 H, d, *J* 6.9, CH_3); δ_C (75 MHz: DMSO-d_6; Me_4Si): 171.8, 171.2, 155.9, 143.8, 140.7, 138.3, 129.3, 129.2, 128.2, 128.1, 127.7, 127.2, 126.3, 126.0, 125.3, 120.2, 65.7, 62.1, 56.1, 52.4, 48.41, 46.6, 37.5, 36.4 and 18.6.

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