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ORIGINAL ARTICLE

Solid phase synthesis of peptides containing novel amino acids, substituted 3-benzimidazolealanines

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Abstract A direct solid-phase synthesis of a series of substituted benzimidazole-containing peptides is described. The method involves on-resin formation of new amino acids containing benzimidazole derivatives in the side chain. The heterocycle conjugates were obtained by reaction between aldehydes and peptides containing β-(3,4-diaminophenyl)alanine residue, immobilized on a polymeric solid support.

Keywords Solid phase peptide synthesis · Unnatural amino acids · 4-Amino-3-nitrophenylalanine · Benzimidazoles · Combinatorial synthesis

Introduction

It is known that short peptide sequences present a broad spectrum of biological activity (Lucchese et al. 2006). The incorporation of nonproteinaceous amino acid residues into such peptides offers a possibility of designing new molecules with improved activity, specificity and enzymatic stability. A peptide can be used as an address tag to deliver the function generated by unnatural amino acid residue to the receptor. In addition to this, a peptide backbone may serve as a scaffold to properly organize the introduced structural motifs. The modifications may be applied as, for example, markers in enzymatic assays (Hines et al. 2005), delivery systems (Frochot et al. 2007), or they may be introduced to change the physicochemical properties of peptides (Kotha et al. 2007).

Such an approach enables the increase in the diversity of peptide libraries by introduction of new building blocks. Recently, we focused our attention on the post-assembly on-resin modifications of peptides, used as a tool in the synthesis of novel immunomodulatory peptides. The direct transformations of the side chains of peptides attached to a solid support allowed us to synthesize peptides containing novel heterocyclic amino acids (Staszewska et al. 2005; Stefanowicz et al. 2007a, b). After introducing the quinoxaline moiety into peptides to modify their immunomodulatory activity and to promote the complexing abilities of such modified peptides (Szczepanik et al. 2007; Stefanowicz et al. 2007a, b), our attention was attracted by another privileged structure, the benzimidazole.

Benzimidazole scaffold frequently appears in molecules of biological importance, the derivatives are used as therapeutic agents, including antivirals, antibacterials, antiulcers, and anticancers (Alamgir et al. 2007; Bendsoe and Ronquist 2004). Special attention was recently given to the complexing abilities of 2-(pyridin-2-yl)benzimidazole, because its copper(II) complexes show potential as cancer chemotherapeutics against the hepatocellular carcinoma (Hep-G2) and kidney adenocarcinoma (A-498) cell lines (Devereux et al. 2007). The importance of benzimidazole moiety in medicinal chemistry is reflected by numerous approaches to the synthesis of this heterocyclic system, including several solid phase procedures employing insoluble (Mayer et al. 1998; Huang and Scarborough 1999; Wu et al. 2000) and soluble supports (Wu and Sun 2002; Bendale and Sun 2002).

Most methods involve formation of the *o*-nitroaniline intermediate obtained by treatment of the polymer bound *o*-fluoronitroaromatic compound with an amine. After the reduction of the nitro group, the product is further

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cyclocondensed with an aldehyde. Although benzimidazoles are pharmacologically relevant group of compounds, there are only few reports on peptides or amino acids bearing benzimidazole scaffold in the side chain. The 3-(1*H*-benzimidazol-5-yl)alanine was synthesized in solution by Milkowski et al. (1970) through condensation of 3,4-diaminophenylalanine with formic acid. Analogues of 3-(1*H*-benzimidazol-5-yl)alanine were also reported by Stone (1979). However, the synthetic procedures were performed only in solution and required harsh conditions. Another approach, based on cinchona alkaloid-catalyzed alkylation of glycinate, was used by Lin et al. (2004) for synthesis of 2,6-dichlorophenyl-substituted bezimidazol-5-yl-alanine. A similar method was used in synthesis of peptide-heterocycle hybride, designed as a result of modeling studies superimposing 2-aminobenzimidazole over lysine reside (McGrath et al. 2006).

This paper reports the first direct solid phase synthesis of benzimidazole-containing peptides. The substrate (4-amino-3-nitrophenyl)alanine is incorporated into the specified position of the peptide chain by the standard peptide synthesis protocol. The on-resin heterocycle formation consists of the reduction of the nitro group to form the β-(3,4-diaminophenyl)alanine residue and the final reaction with an aldehyde to form the benzimidazole moiety. A series of dipeptides (substituted benzimidazol-5-yl-alanylglycines) was synthesized to establish the procedure of benzimidazole scaffold formation using both aliphatic and aromatic aldehydes. The compatibility of this method with the Fmoc solid phase peptide synthesis protocols was proven by the synthesis of three oligopeptides, designed as analogues of immunosuppressory fragments of ubiquitin and HLA-DQ (Szewczuk et al. 1996, 2004).

Materials and methods

Peptides were prepared by manual solid-phase techniques. The synthesis was performed using standard Fmoc procedure on Wang (for all dipeptides and peptide P1) or amide Rink (for peptides P2 and P3) resins. The Wang resins preloaded with Fmoc-Gly (capacity 0.73 mmol/g) and Fmoc-Leu (capacity 0.65 mmol/g), and the Rink amide resin (capacity 0.69 mmol/g) were purchased from NovaBiochem. The 9-fluorenyl-methoxy-carbonyl (Fmoc) amino acids and the coupling reagent 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyl-uronium tetrafluoroborate (TBTU) were purchased from NovaBiochem. The side chain protecting group for Fmoc-amino acids were *t*-butyl (*t*-but) for Thr and Tyr, *t*-butyl ester for Asp and 2,2,5,7,8-pentamethylchroma-6-sulfonyl (Pbf) for Arg. *N*,*N*-diisopropylethylamine (DIEA) was purchased from Fluka.

Trifluoroacetic acid (TFA) and *N*,*N*-dimethylformamide (DMF) were obtained from Iris Biotech. HPLC-grade solvents were purchased from Fisher Scientific. Deuterated NMR solvents were purchased from Cambridge Isotope Labs and Isotech Inc. All other reagents were purchased from Aldrich.

The yield was calculated assuming that the crude product is isolated as a salt containing two molecules of TFA except for compounds 4f and 4g which contain three TFA molecules, and peptides P1–P3, which contain 4 TFA molecules. The purity of crude product was analyzed according to HPLC peak integrals at λ 220 nm on analytical HPLC.

Analytical HPLC was carried out on Thermo Separation Products chromatograph using Vydac RP C_{18} 4.6 × 250 mm column with UV detection at 220 nm. The flow rate was 1 mL/min, gradient 0–80% B in A in 40 min, where A is water with 0.1% TFA and B is acetonitrile with 0.1% TFA. Preparative HPLC was carried out on Varian (ProStar) chromatograph using TOSOH Bioscience RP C_{18} 21.5 × 300 mm column, flow rate—7 mL/min in the same solvent system.

The ^{1}H NMR spectra were obtained on a Bruker Avance (500 MHz) spectrometer. Chemical shifts (δ) are reported in parts per million (ppm) relative to residual solvent as an internal reference. The following abbreviations were used to explain the multiplicities: s, singlet; d, doublet; t, triplet; q, quartet; dd doublet of doublets; m, multiplet; b, broad.

High-resolution mass spectra were obtained on a Bruker micrOTOF-Q spectrometer, equipped with Apollo II electrospray ionization source with ion funnel, operated in the positive ion mode. The sample in 50:50:1 CH_3CN–H_2O–HCOOH (0.1 mg/mL) was infused at a flow rate of 3 μL/min. Before each run the instrument was calibrated externally with the Tunemix™ mixture (Bruker Daltonik, Germany) in quadratic regression mode.

Synthesis of 2′-substituted 3-(1*H*-benzimidazol-5-yl)alanine-containing dipeptides

As a base for the benzimidazole formation in a peptide we have developed a special phenylalanine derivative, the Fmoc-Phe(4-NH_2-3-NO_2)-OH, which was synthesized according to the method developed by our research group (Staszewska et al. 2005).

General procedure

The synthesis was performed on preloaded Fmoc-Gly-Wang resin (100 mg, capacity 0.73 mmol/g). After the deprotection of the N-terminal amino group with the aid of 25% piperidine in DMF, the coupling of Fmoc-Phe(4-NH_2-3-NO_2)-OH (69 mg, 2 equiv) was carried out in the presence of activation reagent TBTU (47 mg, 2 equiv) and DIEA (50 μL, 4 equiv) in DMF (2 mL) for 2 h at room

temperature. The successive on-resin reduction with 2 mL of 2 M tin(II) chloride dihydrate in DMF resulted after 48 h in immobilized *o*-phenylenediamine intermediate. The resin was thoroughly washed with DMF (5 × 3 mL) and treated with an aldehyde (0.11 mmol, 1.5 equiv) in 2 mL DMF for 1.5 h at room temperature, while being stirred by air bubbles. After the final removal of the Fmoc group, the peptide–resin was washed with DMF (3 × 5 mL), MeOH:DMF (1:1, v/v, 1 × 5 mL), MeOH (3 × 5 mL), and then dried in vacuo for 3 h. The final cleavage of the benzimidazole-containing dipeptides from the resin by incubation with TFA containing 5% of water for 2 h at room temperature afforded the crude dipeptides.

The compounds were purified by RP-HPLC. After lyophilization, the peptides were analyzed by ^{1}H NMR and high-resolution mass spectrometry.

The synthesis of benzimidazole moiety containing dipeptides is presented in Fig. 1. The structures of the resulting benzimidazole containing dipeptides (**4a-h**) are presented in Fig. 2.

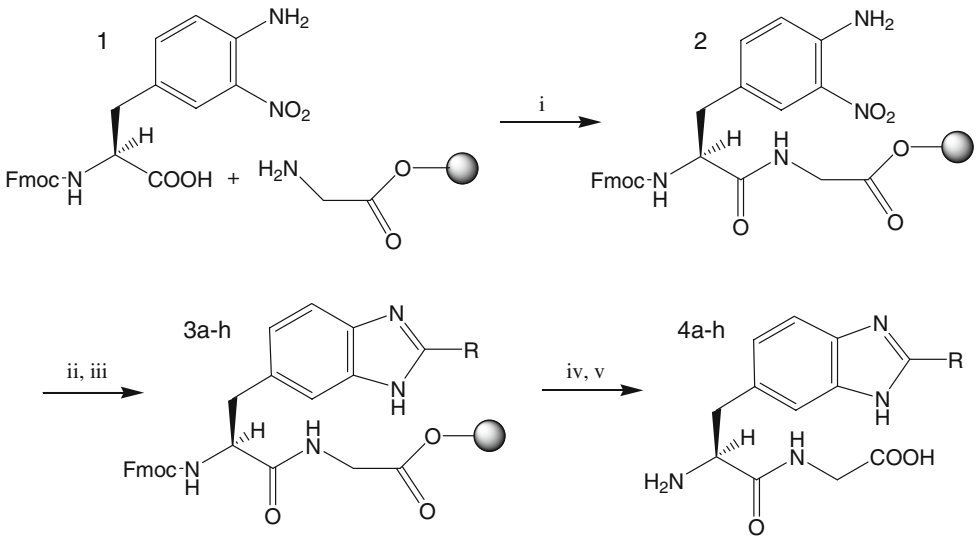

Fig. 1 Reagents and conditions: (*i*) **1** (2 equiv), TBTU (2 equiv), DIEA (4 equiv), DMF, 2 h, rt; (*ii*) 2 M $SnCl_2 \times 2H_2O$ in DMF, 48 h, rt; (*iii*) aldehyde (RCHO) (1.5 equiv), DMF, 1.5 h, rt; (*iv*) piperidine (25% in DMF), 2 × 7 min, rt; (*v*) TFA:water (95:5), 2 h, rt

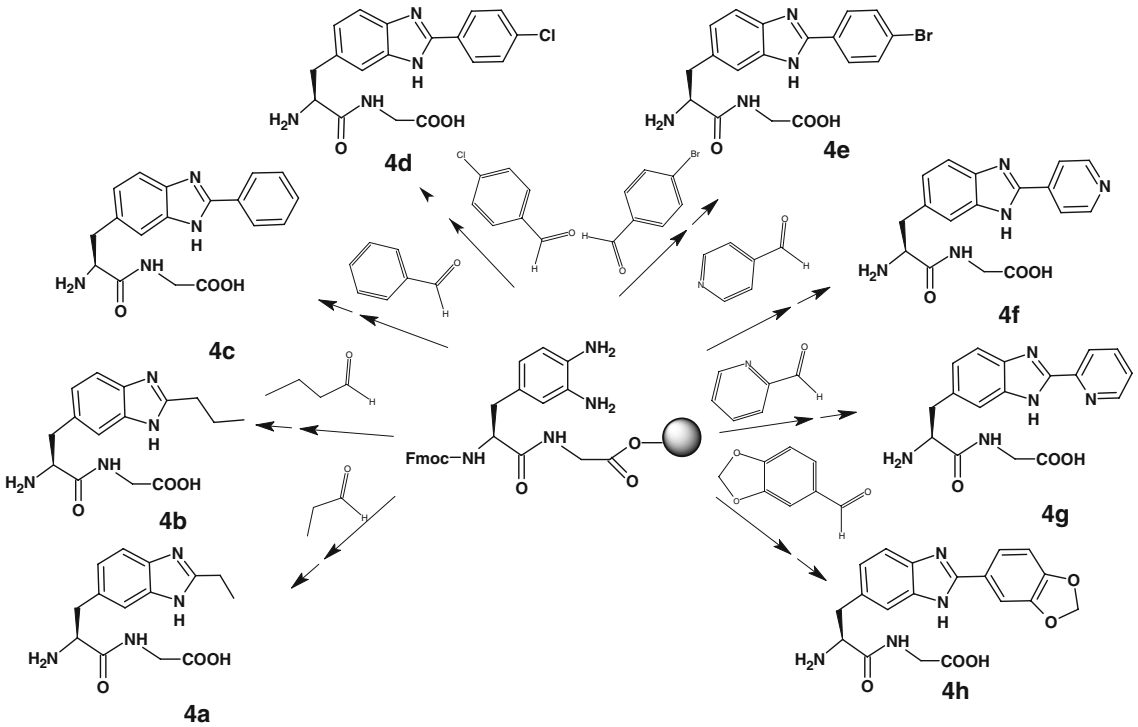

Fig. 2 Preparation of the benzimidazole-containing dipeptides

*2′-ethyl-3-(1H-benzimidazol-5-yl)alanylglycine (**4a**)*

Yield 55%; purity of crude product 70%, R_t 9.5 min; ^{1}H NMR (CD_3OD, 500 MHz) ppm: 7.65 (d, 1H, J = 8.4 Hz ArH), 7.63 (s, 1H ArH), 7.42 (dd, 1H, J = 1.2 Hz, J = 8.4 Hz ArH) 4.19 (t, 1H, J = 7.0 Hz CH_α Phe), 3.93 (d, 2H, J = 1.1 Hz CH_2 Gly), 3.40 (dd, 1H, J = 7.6 Hz, J = 14.1 Hz CH_β Phe), 3.25 (dd, 1H, J = 7.4 Hz, J = 13.8 Hz CH_β Phe), 3.13 (q, 2H, J = 7.6 Hz $\underline{CH_2}CH_3$), 1.48 (t, 3H, J = 7.6 Hz $CH_2\underline{CH_3}$); HR–ESI–MS *m/z* 291.1443; calcd. for $C_{14}H_{19}N_5O_3$ ($[M + H]^+$):291.1452.

*2′-propyl-3-(1H-benzimidazol-5-yl)alanylglycine (**4b**)*

Yield 67%; purity of crude product 50%; R_t 12.3 min; HR–ESI–MS *m/z* 305.1605; calcd. for $C_{15}H_{21}N_4O_3$ ($[M + H]^+$): 305.1608.

*2′-phenyl-3-(1H-benzimidazol-5-yl)alanylglycine (**4c**)*

Yield 72%; purity of crude product 70%; R_t 14.3 min; ^{1}H NMR (CD_3OD, 500 MHz) ppm: 8.10 (m, 2H, ArH), 7.67 (d, 1H, J = 8.2 Hz ArH), 7.63 (s, 1H ArH), 7.61 (m, 3H ArH), 7.33 (dd, 1H, J = 1.1 Hz, J = 8.3 Hz ArH), 4.19 (dd, 1H, J = 6.4 Hz, J = 7.9 Hz CH_α Phe), 3.96 (d, 2H, J = 4.0 Hz CH_2 Gly), 3.42 (dd, 1H, J = 6.2 Hz, J = 14.0 Hz CH_β Phe), 3.22 (dd, 1H, J = 8.5 Hz, J = 14.4 Hz CH_β Phe), N$\underline{H}$ and COO$\underline{H}$ invisible; HR–ESI–MS *m/z* 339.1448; calcd. for $C_{18}H_{19}N_4O_3$ ($[M + H]^+$): 339.1452.

*2′-(4-chlorophenyl)-3-(1H-benzimidazol-5-yl)alanylglycine (**4d**)*

Yield 64%; purity of crude product 56%; R_t 15.9 min; ^{1}H NMR (CD_3OD, 500 MHz) ppm: 8.09 (d, 2H, J = 8.5 Hz ArH), 7.71 (d, 2H, J = 8.4 Hz ArH), 7.67 (dd + s, 2H, J = 1.9 Hz, J = 6.6 Hz ArH), 7.41 (d, 1H, J = 8.2 Hz ArH), 4.21 (t, 1H, J = 7.1 Hz CH_α Phe), 3.96 (s, 2H CH_2 Gly), 3.43 (dd, 1H, J = 6.5 Hz, J = 14.1 Hz CH_β Phe), 3.34 (s, 1H), 3.25 (dd, 1H, J = 6.9 Hz, J = 14.3 Hz CH_β Phe); HR–ESI–MS *m/z* 373.1060; calcd. for $C_{18}H_{18}ClN_4O_3$ ($[M + H]^+$): 373.1062.

*2′-(4-bromophenyl)-3-(1H-benzimidazol-5-yl)alanylglycine (**4e**)*

Yield 74%; purity of crude product 60%; R_t 18.0 min; ^{1}H NMR (CD_3OD, 500 MHz) ppm: 8.02 (d, 2H, J = 8.6 Hz ArH(Br)), 7.84 (d, 2H, J = 8.6 Hz ArH(Br)), 7.72 (d, 1H, J = 8.4 Hz ArH), 7.68 (s, 1H ArH), 7.43 (dd, 1H, J = 1.1 Hz, J = 8.3 Hz ArH), 4.67 (s, 1H), 4.21 (t, 1H, J = 7.1 Hz CH_α Phe), 3.96 (d, 2H, J = 2.4 Hz CH_2 Gly), 3.43 (dd, 1H, J = 6.4 Hz, J = 14.2 Hz CH_β Phe), 3.25 (m, 1H CH_β Phe), 1.28 (s, br.s., 2H); HR–ESI–MS *m/z* 417.0575; calcd. for $C_{18}H_{18}BrN_4O_3$ ($[M + H]^+$): 417.0557.

*2′-(pyridin-4-yl)-3-(1H-benzimidazol-5-yl)alanylglycine (**4f**)*

Yield 64%; purity of crude product 80%; R_t 12.3 min; HR–ESI–MS *m/z* 340.1422; calcd. for $C_{17}H_{18}N_5O_3$ ($[M + H]^+$): 340.1406.

*2′-(pyridin-2-yl)-3-(1H-benzimidazol-5-yl)alanylglycine (**4g**)*

Yield 60%; purity of crude product 70%; R_t 13.0 min; HR–ESI–MS *m/z* 340.1278 calcd. for $C_{17}H_{18}N_5O_3$ ($[M + H]^+$): 340.1406.

*2′-(1,3-benzodioxol-5-yl)-3-(1H-benzimidazol-5-yl)alanylglycine (**4h**)*

Yield 70%; purity of crude product 75%, R_t 16.7 min; ^{1}H NMR (CD_3OD, 500 MHz) ppm: 7.58 (m, 3H ArH), 7.47 (d, 1H, J = 1.8 Hz ArH), 7.33 (d, 1H, J = 8.3 Hz ArH), 7.02 (m, 1H ArH), 6.05 (s, 2H OCH_2O, 4.57 (s, 1H), 4.11 (t, 1H, J = 7.1 Hz CH_α Phe), 3.86 (d, 2H, J = 4.3 Hz CH_2 Gly), 3.33 (dd, 1H, J = 6.6 Hz, J = 14.2 Hz CH_β Phe), 3.16 (m, 1H CH_β Phe), 1.19 (s, br.s. 2H); HR–ESI–MS *m/z* 383.1535; calcd. for $C_{19}H_{19}N_4O_5$ ($[M + H]^+$): 383.1350.

Synthesis of oligopeptides

The sequences of model oligopeptides were taken from ubiquitin (fragment 52–56) and HLA-DQ (fragment 167–172), respectively. The novel amino acid residue 3-(2-(pyridin-2-yl)benzimidazol-5-yl)alanine was abbreviated as Pba.

The crude peptides were purified by RP-HPLC. The identity of obtained peptides was proven using high-resolution mass spectrometry and MS/MS experiments.

Synthesis of H-Pba-Gly-Arg-Thr-Leu-OH (P1)

The synthesis was performed using the preloaded Fmoc-Leu-Wang (200 mg, capacity 0.65 mmol/g). The N-terminal amino group was deprotected with the aid of 25% piperidine in DMF. The successive couplings of Fmoc-Thr(*t*-but)-OH (103 mg, 2 equiv), Fmoc-Arg(Pbf)-OH (168 mg, 2 equiv), Fmoc-Gly-OH (297 mg, 2 equiv) and the Fmoc-Phe(4-NH_2-3-NO_2)-OH (124 mg, 2 equiv) in the presence of TBTU (114 mg, 2 equiv) and DIEA (100 μL, 4 equiv) in 4 mL of DMF resulted in the immobilized protected peptide. The benzimidazole

formation was achieved by the procedure described for dipeptide synthesis, with picolinaldehyde (17.2 μL, 1.5 equiv) in 4 mL DMF. After the final removal of the Fmoc group, the peptide–resin was washed with DMF (3 × 5 mL), MeOH:DMF (1:1, v/v, 1 × 5 mL); MeOH (3 × 5 mL), and then dried in vacuo for 5 h. The final cleavage from the resin by incubation with TFA containing 5% of phenol for 2 h at room temperature afforded the crude peptide.

Yield 75%; purity of crude product 80%; R_t 14.9 min; HR–ESI–MS *m/z* 710.3702; calcd. for $C_{33}H_{48}N_{11}O_7$ ($[M + H]^+$): 710.3738.

Synthesis of H-Arg-Gly-Asp-Val-Pba-NH$_2$ (P2)

The synthesis was performed using the Rink amide resin (200 mg, capacity 0.69 mmol/g). After the deprotection of the N-terminal amino group with the aid of 25% piperidine in DMF, the successive couplings of Fmoc-Phe(4-NH_2-3-NO_2)-OH (123 mg, 2 equiv), Fmoc-Val-OH (93 mg, 2 equiv), Fmoc-Asp(*t*-but)-OH (113 mg, 2 equiv), Fmoc-Gly-OH (82 mg, 2 equiv) and the Fmoc-Arg(Pbf)-OH (179 mg, 2 equiv) in the presence of TBTU (114 mg, 2 equiv) and DIEA (100 μL, 4 equiv) in 4 mL of DMF were achieved. The benzimidazole formation was performed according to the procedure described for dipeptide synthesis, with picolinaldehyde (19.8 μL, 1.5 equiv). The peptide was deprotected and cleaved from the resin according to the procedure described for peptide P1.

Yield 65%; purity of crude product 75%; R_t 14.3 min; HR–ESI–MS *m/z* 709.3503; calcd. for $C_{32}H_{45}N_{12}O_7$ ($[M + H]^+$): 709.3534.

Synthesis of H-Arg-Gly-Asp-Val-Tyr-Pba-NH$_2$ (P3)

The synthesis was performed using the Rink amide resin (200mg, capacity 0.69 mmol/g). After the deprotection of the N-terminal amino group with the aid of 25% piperidine in DMF, the successive couplings of Fmoc-Phe(4-NH_2-3-NO_2)-OH (123 mg, 2 equiv), Fmoc-Tyr(*t*-but)-OH (126 mg, 2 equiv), Fmoc-Val-OH (93 mg, 2 equiv), Fmoc-Asp(*t*-but)-OH (113 mg, 2equiv), Fmoc-Gly-OH (82 mg, 2 equiv) and the Fmoc-Arg(Pbf)-OH (179 mg, 2 equiv) in the presence of TBTU (114 mg, 2 equiv) and DIEA (100 μL, 4 equiv) in 4 mL of DMF were achieved. The benzimidazole formation was performed according to the procedure described for the dipeptide synthesis with picolinaldehyde (19.8 μL, 1.5 equiv). The peptide was deprotected and cleaved from the resin according to the procedure described for peptide P1.

Yield 67%; purity of crude product 75%; R_t 15.4 min; HR–ESI–MS *m/z* 872.4148; calcd. for $C_{41}H_{54}N_{13}O_9$ ($[M + H]^+$): 872.4167.

Results and discussion

Modified peptides are used for various purposes in chemical, biological and medical research, although their synthesis is often more complicated than the standard peptide synthesis protocols. The introduction of nonproteinaceous amino acid residues into peptides could be achieved by either using a suitably protected novel amino acid residue in classical peptide synthesis, or by the post-assembly modification of peptide side chains.

We developed the Fmoc-protected (4-amino-3-nitrophenyl)alanine as a substrate for the synthesis of quinoxaline-containing peptides (Staszewska et al. 2005). Herein, we report that such a precursor can be also used for the on-resin formation of benzimidazoles in side chains of peptides through the condensation with various aliphatic and aromatic aldehydes (Fig. 1). To investigate the range of possible substitutions in benzimidazole scaffold, a series of dipeptides were synthesized (Fig. 2), whereas the synthesis of longer peptides (P1–P3) proves that the method is appropriate for general solid phase peptide synthesis.

The applied procedure gave 2′-substituted 3-(1*H*-benzimidazol-5-yl)alanine-containing dipeptides (4a-h) with crude yield and purity in the range of 55–74 and 50–80%, respectively. The products were purified by HPLC and their identities were confirmed by ^{1}H NMR and HR–ESI–MS. The mass accuracy was better than 5 ppm, enabling, together with the true isotopic pattern (using SigmaFit), an unambiguous confirmation of the elemental composition of the synthesized compounds.

The HPLC analysis of the crude reaction products revealed in the case of compounds 4b-f and 4h the presence of minor side products. The molecular formulas deduced from the monoisotopic molecular masses of these compounds (361.2213; 429.1966; 497.1141; 585.0164; 431.1871 and 517.1703 for entries: 4b, 4c, 4d, 4e, 4f, and 4h, respectively) were identified by HR–ESI–MS and are consistent with the side products observed by Wu et al. (2000) (Fig. 3).

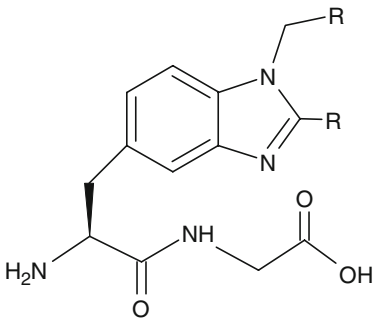

Fig. 3 The hypothetical structure of a side product observed during the solid phase synthesis of benzimidazole moiety containing dipeptides

For the synthesis of longer peptides, we selected the sequences of immunosuppressory fragments of ubiquitin (52–56) and HLA-DQ (167–172), investigated previously by us both as unmodified peptides (Stefanowicz et al. 2004) and as quinoxaline-peptide hybrides (Stefanowicz et al. 2007a, b). The peptides H-Pba-Gly-Arg-Thr-Leu-OH (P1), H-Arg-Gly-Asp-Val-Pba-NH_2 (P2) and H-Arg-Gly-Asp-Val-Tyr-Pba-NH_2 (P3) (where Pba is 3-(2-(pyridin-2-yl) benzimidazol-5-yl)alanine) were synthesized by the solid phase Fmoc procedure and their composition and purity was examined by HR-MS and HPLC, respectively.

The previously reported benzimidazole-containing amino acids were synthesized in solution by two strategies: one involved the modification of phenylalaniane to form the *o*-diaminoaryl derivative and the consecutive condensation (Milkowski et al. 1970), the second followed the procedures of amino acid synthesis either by alkylation of glycinate by a pre-formed heterocyclic derivative (Lin et al. 2004), or by a Wittig reaction of an aldehyde and protected phosphonoglycine (McGrath et al. 2006). The catalytic reduction of the nitro-precursor is one of the more challenging steps in the reaction due to the labile character of the diamino-compounds. In addition to this, the introduction of benzimidazole-containing amino acids into peptides may require an additional protection of the heterocyclic nitrogen atom.

In our approach, the Fmoc-protected (4-amino-3-nitrophenyl)alanine is introduced into the specific position of the peptide chain, and the further formation of benzimidazole scaffold takes place after the completion of peptide assembly, with the full advantage of the solid phase synthesis methods. The immobilized substrate is reduced with tin(II) chloride dihydrate and immediately condensed with a selected aldehyde, whereas washing with DMF replaces the isolation and purification of intermediates. The successful synthesis of the N-terminally substituted peptide P1 and the C-terminally substituted peptide P2 proves that the location of the precursor residue—(4-amino-3-nitrophenyl)alanine does not affect the benzimidazole formation.

The optical purity of the key intermediate in our synthesis, Fmoc-Phe(4-NH_2-3-NO_2)-OH, was previously confirmed by crystallography (Stefanowicz et al. 2006). The synthesis of peptide bonds, deprotection, and the cleavage reaction were performed utilizing standard, racemization-free procedures used in peptide synthesis (Atherton and Scheppart 1989). The benzimidazole moiety formation (including the reduction of the aromatic nitro group and condensation with an aldehyde) does not involve the stereogenic center of the amino acid residue. Therefore the configuration of the novel amino acid is determined by the configuration of the substrate phenylalanine derivative.

As the modification occurs after the peptide is assembled on resin, the main advantage of the approach is worth attention: a relatively simple chiral amino acid residue is introduced into the peptide, which opens the way to a variety of modifications, limited only by the set of available aldehydes. The method combines the procedures for peptide and heterocycle synthesis. The presented post-assembly on-resin method of benzimidazole formation may allow a synthesis of combinatorial peptide libraries using various commercially available aldehydes.

Conlusions

We have developed an efficient and straightforward method of benzimidazole formation in peptides attached to a solid support. The method is general and may be applied to the synthesis of longer peptides that contain various protecting groups. The methodology could be easily incorporated into combinatorial synthesis, being also suitable for automated application, since the entire synthetic sequence can be carried out in DMF at room temperature.

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