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ORIGINAL ARTICLE

Synthesis of bis-amino acid derivatives by Suzuki cross-coupling, Michael addition and substitution reactions

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Abstract Several bis-amino acids were prepared using a bis-Suzuki coupling (compounds **4**–**8**, **10**), a sequential Michael addition and bis-Suzuki coupling (compounds **12**, **13**) and a Michael addition followed by a substitution reaction (compounds **18**, **19**). Thus, the pure stereoisomer of the methyl esters of *N*-(*tert*-butoxycarbonyl)-*β*-bromodehydroaminobutyric acid and dehydrophenylalanine and of *N*-benzyloxycarbonyl-*β*-bromodehydroaminobutyric acid were reacted with 1,4-phenylene-bis-boronic acid or 9,9-dioctyl-9*H*-fluorene-2,7-bis-boronic acid using modified Suzuki coupling conditions. The corresponding bis-dehydroamino acid derivatives were obtained in good to high yields maintaining the stereochemistry of the starting materials. This reaction was also applied successfully to a brominated dehydrodipeptide and 1,4-phenylene-bis-boronic acid showing that it could be used to create cross-links in peptide chains. An *N,N*-diacyldehydroalanine derivative was used in a sequential Michael addition and bis-Suzuki coupling giving a *p*-terphenyl bis-amino acid and a fluorenyl bis-amino acid in good yields. Two bis-α,*β*-diamino acids were obtained by a Michael addition of 1,2,4-triazole to the methyl esters of *N*-(4-toluenesulfonyl), *N*-(*tert*-butoxycarbonyl) dehydroamino acids followed by treatment with ethylenediamine.

Keywords Dehydroamino acids · Bis-amino acids · Bis-Suzuki coupling · Michael addition · Substitution reaction

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Introduction

Bis-amino acids can be used as cross-linking elements for the control of peptide secondary structure, for stabilization of peptides against degradation and denaturation and in the synthesis of new analogues of biologically active peptides most commonly being employed as replacements for cystine (Schumann et al. 2000; Hoven et al. 2002). Several bis-amino acids have been found in nature: meso-diaminopimelic acid is found in several bacteria and plant sources, being a biosynthetic precursor of the essential amino acid L-lysine and a cross-linking element of the cell wall of all Gram-negative and some Gram-positive bacteria (Jurgens 1992); dityrosine occurs naturally in fungal cell wall proteins and in elastin and collagen (Labella et al. 1968; Smail et al. 1995); isotyrosine is found in plant cell wall proteins (Brady et al. 1998); lanthionine, which is a thioether analogue of cystine is found in a class of peptidic antibiotics known as lantibiotics (Bregant and Tabor 2005). Thus, the development of synthetic methods that allow the preparation of bis-amino acids has attracted significant interest in the last few years. For example, Porzi and Sandri et al. carried out a simple and efficient enantioselective synthesis of α,α′-diamino dicarboxylic acids, including (+)- and (−)-2,6-diaminopimelic acids, starting from a chiral diketopiperazine derivative (Paradisi et al. 2000a, b). Other strategies proposed for the synthesis of bis-amino acids include palladium catalyzed cross-couplings followed by hydrogenation of the bis-dehydroamino acids obtained. Frejd et al. used the Heck reaction for the synthesis of several bis-dehydroamino acids by reacting a dehydroalanine derivative with a dihalogenated benzene (Carlstrom and Frejd 1991; Ritzén et al. 1998). Hutton et al. developed an efficient synthesis of dityrosine from 3-iodo-L-tyrosine employing a tandem

Miyaura borylation–Suzuki coupling strategy (Hutton and Skaff 2003; Skaff et al. 2005).

In our laboratories, we have developed an efficient method for the synthesis of α,β-dehydroamino acids (Ferreira et al. 1999). These have been used as substrates for the preparation of new non-proteinogenic amino acids via several types of reactions, namely, Michael additions, substitution reactions and palladium catalyzed cross-couplings (Ferreira et al. 2000a, b, 2001; Abreu et al. 2003a, b, 2004; Ferreira and Monteiro 2006). In the course of this work we have synthesized bis-amino acids using Michael additions and substitution reactions (Ferreira et al. 2003; Ferreira and Monteiro 2006). Now we report the high yield synthesis of bis-dehydroamino acids using a bis-Suzuki cross coupling of β-bromodehydroamino acids and bis-boronic acids and by a sequential Michael addition and substitution reaction of *N,N*-diacyldehydroamino acids with 1,2,4-triazole and ethylenediamine. The couplings were carried out using the general conditions developed by our group for the synthesis of β,β-diaryldehydroalanines from a β,β-dibromodehydroalanine and aryl or heteroaryl boronic acids (Abreu et al. 2004; Queiroz et al. 2007). The preparation of bis-amino acids was carried out by a sequential Michael addition–bis-Suzuki coupling of an *N,N*-diprotected dehydroalanine with 3-iodobenzylamine and a bis-boronic acid.

Materials and methods

Melting points (°C) were determined in a Gallenkamp apparatus and are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded on a Varian Unity Plus at 300 and 75.4 MHz, respectively. ^{1}H–^{1}H spin–spin decoupling and DEPT θ 45° were used. Chemical shifts are given in ppm and coupling constants in Hz. MS and HRMS data were recorded by the mass spectrometry service of the University of Vigo, Spain. Elemental analysis was performed on a LECO CHNS 932 elemental analyzer. The reactions were monitored by thin layer chromatography (TLC). Column chromatography was performed on Macherey-Nagel silica gel 230–400 mesh. Petroleum ether refers to the boiling range 40–60°C.

Synthesis of compounds **Z-1** (Silva et al. 2002), **E-1** (Silva et al. 2002), **Z-2** (Abreu et al. 2004), **E-2** (Abreu et al. 2004), **Z-3** (Ferreira et al. 2007), **Z-9** (Ferreira et al. 2007), **11** (Abreu et al. 2003a), **14** (Ferreira et al. 1998), **Z-15** (Ferreira et al. 1999), **E-16** (Ferreira et al. 2000a) and **E-17** (Ferreira et al. 2000a)

The synthesis of these compounds is described elsewhere.

General procedure for the synthesis of bis-dehydroamino acid derivatives

To a solution of the β-bromodehydroamino acid derivative (0.625 mmol) in THF/H_2O (1:1, 10 mL), bis-boronic acid (0.25 mmol), $PdCl_2dppf.CH_2Cl_2$ (1:1, 10 mol%) and Cs_2CO_3 (1.4 eq.) were added. The reaction mixture was heated at 70°C for 3 h (the reaction was followed by TLC). The solvent was removed under reduced pressure and the residue dissolved in ethyl acetate (100 mL). The organic layer was washed with water and brine (2 × 30 mL each), dried over $MgSO_4$ and the solvent removed. The residue was submitted to column chromatography using a solvent gradient from neat petroleum ether to mixtures of ether/petroleum ether, increasing 10% of ether each time until the isolation of the product.

Synthesis of (2Z,2′Z)-dimethyl, 3,3′-(1,4-phenylene), bis[2-(*tert*-butoxycarbonylamino)]but-2-enoate (**Z,Z-4**)

The general procedure described above was used with compound **Z-1** and 1,4-phenylene-bis-boronic acid to afford compound **Z,Z-4** (98 mg, 78%) as a white solid. M.p. 114.0–115.0°C (from ethyl acetate/*n*-hexane). ^{1}H NMR (300 MHz, $CDCl_3$): 1.41 (s, 18 H, CH_3 Boc), 2.27 (s, 6 H, γCH_3), 3.86 (s, 6 H, OCH_3), 5.79 (s, 2 H, NH), 7.27 (s, 4 H, ArH) ppm. ^{13}C NMR (75.4 MHz, $CDCl_3$): 20.49 (γCH_3), 28.12 [$C(CH_3)_3$], 52.03 (OCH_3), 80.63 [$OC(CH_3)_3$], 123.95 (=C), 128.00 (CH), 135.66 (C), 139.83 (=C), 153.21 (C=O), 165.92 (C=O) ppm. MS (EI): *m/z* (%) = 505.98 (9.34) [M^++1], 504.25 (4.56) [M^+], 404.20 (8.48) [M^+-Boc], 304.00 (100) [M^+-2Boc]. HRMS: calcd for $C_{26}H_{36}N_2O_8$ was 504.2472, found: 504.2481.

Synthesis of (2E,2′E)-dimethyl, 3,3′-(1,4-phenylene), bis[2-(*tert*-butoxycarbonylamino)]but-2-enoate (**E,E-4**)

The general procedure described above was used with compound **E-1** and 1,4-phenylene-bis-boronic acid to afford compound **E,E-4** (91 mg, 72%) as a white solid. M.p. 153.5–154.0°C (from diethyl ether/*n*-hexane). ^{1}H NMR (300 MHz, $CDCl_3$): 1.49 (s, 18 H, CH_3 Boc), 2.13 (s, 6 H, γCH_3), 3.48 (s, 6 H, OCH_3), 6.04 (s, 2 H, NH), 7.13 (s, 4 H, ArH) ppm. ^{13}C NMR (75.4 MHz, $CDCl_3$): 21.19 (γCH_3), 28.19 [$C(CH_3)_3$], 51.68 (OCH_3), 80.90 [$OC(CH_3)_3$], 124.44 (=C), 127.02 (CH), 137.30 (C), 140.34 (=C), 153.20 (C=O), 165.92 (C=O) ppm. Anal. Calcd for $C_{26}H_{36}N_2O_8$ (504.57): C 61.89; H 7.19; N 5.55; found: C 61.53; H 7.30; N 5.20.

Synthesis of (2Z,2′Z)-dimethyl, 3,3′-(1,4-phenylene), bis[2-(*tert*-butoxycarbonylamino]-3-phenylacrylate (**Z,Z-5**)

The general procedure described above was used with compound **Z-2** and 1,4-phenylene-bis-boronic acid to produce compound **Z,Z-5** (138.5 mg, 88%) as a white solid. M.p. 194.5–195.0°C (from ethyl acetate/*n*-hexane). ^{1}H NMR (300 MHz, $CDCl_3$): 1.45 (s, 18 H, CH_3 Boc), 3.52 (s, 6 H, OCH_3), 6.15 (s, 2 H, NH), 7.12–7.16 (m, 4 H, ArH), 7.23 (s, 4 H, ArH), 7.30–7.34 (m, 6 H, ArH) ppm. ^{13}C NMR (75.4 MHz, $CDCl_3$): 28.13 [C(CH_3)$_3$], 52.00 (OCH_3), 81.38 [OC(CH_3)$_3$], 126.07 (C), 128.00 (CH), 128.17 (CH), 130.14 (CH), 133.58 (=C), 138.57 (=C), 139.55 (C), 152.73 (C=O), 166.39 (C=O) ppm. Anal. Calcd for $C_{36}H_{40}N_2O_8$ (628.72): C 68.77; H 6.41; N 4.46; found: C 68.75; H 6.45; N 4.45.

Synthesis of (2E,2′E)-dimethyl, 3,3′-(1,4-phenylene), bis[2-(*tert*-butoxycarbonylamino)]-3-phenylacrylate (**E,E-5**)

The general procedure described above was used with compound **E-2** and 1,4-phenylene-bis-boronic acid to produce compound **E,E-5** (105 mg, 67%) as a white solid. M.p. 210.0–211.0°C (from diethyl ether/*n*-hexane). ^{1}H NMR (300 MHz, $CDCl_3$): 1.45 (s, 18 H, CH_3 Boc), 3.56 (s, 6 H, OCH_3), 6.06 (s, 2 H, NH), 7.03 (s, 4 H, ArH), 7.20–7.40 (m, 10 H, ArH) ppm. ^{13}C NMR (75.4 MHz, $CDCl_3$): 28.08 [C(CH_3)$_3$], 51.95 (OCH_3), 81.29 [OC(CH_3)$_3$], 125.95 (C), 126.50 (C), 128.37 (C), 128.75 (CH), 128.85 (CH), 129.62 (C), 129.81 (CH), 132.96 (C), 138.25 (C), 138.28 (C), 139.34 (C), 152.70 (C=O), 166.36 (C=O) ppm. MS (EI): *m/z* (%) = 628.27 (4.1) [M$^+$], 528.23 (16.4) [M$^+$-Boc], 428.16 (100) [M$^+$-2Boc]. HRMS: calcd for $C_{36}H_{40}N_2O_8$ 628.2785;found: 628.2790.

Synthesis of (2Z,2′Z)-dimethyl, 3,3′-(9,9-dioctyl-9*H*-fluorene-2,7-diyl), bis[2-(*tert*-butoxycarbonylamino)] but-2-enoate (**Z,Z-6**)

The general procedure described above was used with compound **Z-1** and 9,9-dioctyl-9*H*-fluorene-2,7-bis-boronic acid to produce compound **Z,Z-6** (197 mg, 97%) as a white solid. M.p. 154.0–155.0°C (from diethyl ether/*n*-hexane). ^{1}H NMR (300 MHz, $CDCl_3$): 0.58–0.72 (m, 4 H, CH_2), 0.82 (t, J = 6.9 Hz, 6 H, CH_3), 1.00–1.24 (m, 20 H, CH_2), 1.41 (s, 18 H, CH_3 Boc), 1.90–1.96 (m, 4 H, CH_2), 2.29 (s, 6 H, γCH_3), 3.88 (s, 6 H, OCH_3), 5.82 (s, 2 H, NH), 7.22 (d, J = 8.1 Hz, 2 H, ArH), 7.23 (s, 2 H, ArH), 7.71 (d, J = 8.1 Hz, 2 H, ArH) ppm. ^{13}C NMR (75.4 MHz, $CDCl_3$): 14.03 (CH_3), 20.65 (γCH_3), 22.56 (CH_2), 23.99 (CH_2), 28.15 [C(CH_3)$_3$], 29.18 (CH_2), 29.22 (CH_2), 30.01 (CH_2), 31.70 (CH_2), 40.08 (CH_2), 52.03 (OCH_3), 55.30 (C), 80.71 [OC(CH_3)$_3$], 120.07 (CH), 122.34 (CH), 123.87 (C), 126.47 (CH), 134.50 (C), 138.90 (C), 140.23 (C), 151.44 (C), 153.20 (C=O), 166.12 (C=O) ppm. Anal. Calcd for $C_{49}H_{72}N_2O_8$ (817.10): C 72.03; H 8.88; N 3.43; found: C 72.33; H 8.93; N 3.59.

Synthesis of (2E,2′E)-dimethyl, 3,3′-(9,9-dioctyl-9*H*-fluorene-2,7-diyl), bis[2-(*tert*-butoxycarbonylamino)] but-2-enoate (**E,E-6**)

The general procedure described above was used with compound **E-1** and 9,9-dioctyl-9*H*-fluorene-2,7-bis-boronic acid to produce compound **E,E-6** (183 mg, 90%) as a white solid. M.p. 131.0–132.0°C (from diethyl ether/*n*-hexane). ^{1}H NMR (300 MHz, $CDCl_3$): 0.58–0.62 (m, 4 H, CH_2), 0.81 (t, J = 6.9 Hz, 6 H, CH_3), 1.02–1.21 (m, 20 H, CH_2), 1.50 (s, 18 H, CH_3 Boc), 1.88–1.94 (m, 4 H, CH_2), 2.18 (s, 6 H, γCH_3), 3.40 (s, 6 H, OCH_3), 6.08 (s, 2 H, NH), 7.12 (d, J = 8.1 Hz, 2 H, ArH), 7.13 (s, 2 H, ArH), 7.60 (d, J = 8.1 Hz, 2 H, ArH) ppm. ^{13}C NMR (75.4 MHz, $CDCl_3$): 14.02 (CH_3), 21.76 (γCH_3), 22.55 (CH_2), 23.68 (CH_2), 28.20 [C(CH_3)$_3$], 29.22 (CH_2), 29.29 (CH_2), 30.00 (CH_2), 31.72 (CH_2), 40.39 (CH_2), 51.56 (OCH_3), 55.10 (C), 80.84 [OC(CH_3)$_3$], 119.28 (CH), 121.76 (CH), 123.98 (C), 125.99 (CH), 139.01 (C), 140.15 (C), 140.24 (C), 150.73 (C), 153.31 (C=O), 166.16 (C=O) ppm. Anal. Calcd for $C_{49}H_{72}N_2O_8$ (817.10): C 72.03; H 8.88; N 3.43; found C 72.21; H 8.97; N 3.54.

Synthesis of (2Z,2′Z)-dimethyl, 3,3′-(9,9-dioctyl-9*H*-fluorene-2,7-diyl), bis[2-(*tert*-butoxycarbonylamino)]-3-phenylacrylate (**Z,Z-7**)

The general procedure described above was used with compound **Z-2** and 9,9-dioctyl-9*H*-fluorene-2,7-bis-boronic acid to produce compound **Z,Z-7** (179 mg, 76%) as a white solid. M.p. 165.5–166.5°C (from diethyl ether/*n*-hexane). ^{1}H NMR (300 MHz, $CDCl_3$): 0.56–0.62 (m, 4 H, CH_2), 0.85 (t, J = 6.9 Hz, 6 H, CH_3), 1.01–1.26 (m, 20 H, CH_2), 1.48 (s, 18 H, CH_3 Boc), 1.83–1.87 (m, 4 H, CH_2), 3.57 (s, 6 H, OCH_3), 6.14 (s, 2 H, NH), 7.10–7.30 (m, 14 H, ArH), 7.66 (d, J = 7.8 Hz, 2 H, ArH) ppm. ^{13}C NMR (75.4 MHz, $CDCl_3$): 14.05 (CH_3), 22.55 (CH_2), 24.07 (CH_2), 28.17 [C(CH_3)$_3$], 29.18 (CH_2), 29.33 (CH_2), 30.05 (CH_2), 31.72 (CH_2), 40.08 (CH_2), 52.03 (OCH_3), 55.23 (C), 81.29 [OC(CH_3)$_3$], 120.12 (CH), 124.64 (CH), 125.39 (C), 127.92 (CH), 128.05 (CH), 129.13 (CH), 129.21 (CH), 133.96 (C), 137.70 (C), 139.97 (C), 140.55 (C), 151.38 (C), 152.82 (C=O), 166.68 (C=O) ppm. Anal. Calcd for $C_{59}H_{76}N_2O_8$ (941.24): C 75.29; H 8.14; N 2.98; found C 74.89; H 8.02; N 2.98.

Synthesis of (2E,2′E)-dimethyl, 3,3′-(9,9-dioctyl-9*H*-fluorene-2,7-diyl), bis[2-(*tert*-butoxycarbonylamino)]-3-phenylacrylate (**E,E-7**)

The general procedure described above was used with compound **E-2** and 9,9-dioctyl-9*H*-fluorene-2,7-bis-boronic acid to afford compound **E,E-7** (212 mg, 90%) as a white solid, M.p. 156.0–156.5°C (from diethyl ether/*n*-hexane). ^{1}H NMR (300 MHz, $CDCl_3$): 0.48–0.57 (m, 4 H, CH_2), 0.84 (t, $J = 6.9$ Hz, 6 H, CH_3), 0.96–1.26 (m, 20 H, CH_2), 1.46 (s, 18 H, CH_3 Boc), 1.79–1.84 (m, 4 H, CH_2), 3.51 (s, 6 H, OCH_3), 6.12 (s, 2 H, NH), 7.03–7.10 (m, 4 H, ArH), 7.22–7.36 (m, 10 H, ArH), 7.58 (d, $J = 7.8$ Hz, 2 H, ArH) ppm. ^{13}C NMR (75.4 MHz, $CDCl_3$): 14.04 (CH_3), 22.54 (CH_2), 23.74 (CH_2), 28.13 [C(CH_3)$_3$], 29.16 (CH_2), 29.31 (CH_2), 29.90 (CH_2), 31.73 (CH_2), 40.22 (CH_2), 51.90 (OCH_3), 55.01 (C), 81.24 [OC(CH_3)$_3$], 119.43 (CH), 123.86 (CH), 125.23 (C), 128.27 (CH), 128.61 (CH), 128.93 (CH), 129.94 (CH), 134.45 (C), 137.40 (C), 138.83 (C), 139.06 (C), 140.38 (C), 150.82 (C), 152.95 (C=O), 166.72 (C=O) ppm. Anal. Calcd for $C_{59}H_{76}N_2O_8$ (941.24): C 75.29; H 8.14; N 2.98; found: C 74.66; H 8.16; N 3.12.

Synthesis of (2Z,2′Z)-dimethyl, 3,3′-(1,4-phenylene), bis(2-benzyloxycarbonylamino)but-2-enoate (**Z,Z-8**)

The general procedure described above was used with compound **Z-3** and 1,4-phenylene-bis-boronic acid to produce compound **Z,Z-8** (71 mg, 50%) as a white solid, M.p. 171.0–172.0°C (from ethyl acetate/*n*-hexane). ^{1}H NMR (300 MHz, $CDCl_3$): 2.27 (s, 6 H, γCH_3), 3.86 (s, 6 H, OCH_3), 5.08 (s, 4 H, CH_2 Z), 5.98 (s, 2 H, NH), 7.20–7.33 (s, 14 H, ArH) ppm. ^{13}C NMR (75.4 MHz, $CDCl_3$): 20.62 (γCH_3), 52.16 (OCH_3), 67.33 (CH_2), 123.56 (=C), 127.96 (CH), 128.18 (CH), 128.33 (CH), 128.49 (CH), 135.80 (C), 139.66 (=C), 154.00 (C=O), 165.49 (C=O) ppm. Anal. Calcd for $C_{32}H_{32}N_2O_8$ (572.61): C 67.12; H 5.63; N 4.89; found: C 67.04; H 5.67; N 5.14.

Synthesis of (2Z,2′Z)-dimethyl, 3,3′-(1,4-phenylene), bis{2-[2-(*tert*-butoxycarbonylamino)acetamido]}but-2-enoate (**Z,Z-10**)

The general procedure described above was used with compound **Z-9** and 1,4-phenylene-bis-boronic acid to produce compound **Z,Z-10** (116 mg, 75%) as a white solid, M.p. 195.0–195.5°C (from ethyl acetate/*n*-hexane). ^{1}H NMR (300 MHz, DMSO): 1.35 (s, 18 H, CH_3 Boc), 2.20 (s, 6H, γCH_3), 3.46 (d, $J = 5.7$ Hz, 4 H, CH_2), 3.64 (s, 6 H, OCH_3), 6.85 (t, $J = 5.7$ Hz, 2 H, NH Gly), 7.30 (s, 4 H, ArH), 9.08 (s, 2 H, NH ΔAbu) ppm. ^{13}C NMR (75.4 MHz, DMSO): 19.91 (γ-CH_3), 28.15 [C(CH_3)$_3$], 42.54 (CH_2), 51.57 (OCH_3), 78.00 [OC(CH_3)$_3$], 123.83 (C), 127.79 (CH), 137.23 (C), 139.28 (CH), 155.68 (C=O), 165.60 (C=O), 169.10 (C=O) ppm. Anal. Calcd for $C_{30}H_{42}N_4O_{10}$ (618.66): C 58.24; H 6.84; N 9.06; found: C 57.70; H 6.80; N 8.79.

Synthesis of compound **12**

The general procedure described above was used with compound **11** and 1,4-phenylene-bis-boronic acid to produce compound **12** (149 mg, 67%) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 1.45$ (s, 36 H, CH_3 Boc), 3.53 (s, 3 H, OCH_3), 3.95 (d, $J = 6.0$ Hz, 2 H, CH_2), 5.23 (br s, 1 H, NH), 7.35 (br s, 5 H, ArH), 8.20 (br s, 1 H, NH) ppm. ^{13}C NMR (75.4 MHz, $CDCl_3$): 28.30 [C(CH_3)$_3$], 44.64 (CH_2), 52.59 (OCH_3), 80.93 [OC(CH_3)$_3$], 117.89 (C), 128.27 (CH), 128.36 (C), 128.88 (CH), 129.43 (CH), 136.99 (C), 156.22 (C=O), 163.27 (C=O), 167.83 (C=O) ppm. Anal. Calcd for $C_{48}H_{66}N_4O_{12}$ (890.47): C 64.70; H 7.45; N 6.29; found C 64.88; H 7.63; N 6.24.

Synthesis of compound **13**

The general procedure described above was used with compound **11** and 9,9-dioctyl-9*H*-fluorene-2,7-bis-boronic acid to produce compound 13 (159 mg, 53%) as a yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$): 1.45 (s, 36 H, CH_3 Boc), 3.53 (s, 3 H, OCH_3), 3.95 (d, $J = 6.0$ Hz, 2 H, CH_2), 5.23 (br s, 1 H, NH), 7.35 (br s, 5 H, ArH), 8.20 (br s, 1 H, NH) ppm. ^{13}C NMR (75.4 MHz, $CDCl_3$): 28.30 [C(CH_3)$_3$], 44.64 (CH_2), 52.59 (OCH_3), 80.93 [OC(CH_3)$_3$], 117.89 (C), 128.27 (CH), 128.36 (C), 128.88 (CH), 129.43 (CH), 136.99 (C), 156.22 (C=O), 163.27 (C=O), 167.83 (C=O) ppm. Anal. Calcd for $C_{71}H_{102}N_4O_{12}$ (1202.75): C 70.85; H 8.54; N 4.64; found: C 70.85; H 8.56; N 4.62.

General procedure for the synthesis of compounds **E,E-18** and **E,E-19**

To a solution of the β-(1,2,4-triazol-1-yl) dehydroamino acid derivative in methanol (0.1 mol dm^{-3}), 1,2-ethylenediamine (10 eq.) was added. The reaction was left overnight, stirring at room temperature. The solvent was removed at reduced pressure and the residue obtained was dissolved in ethyl acetate (100 mL). The solution was washed with $NaHCO_3$ 1 mol dm^{-3} (2 × 30 mL) and brine (2 × 30 mL). The organic layer was dried with $MgSO_4$ and the solvent removed under reduced pressure. The residue obtained was added to a solution of β-(1,2,4-triazol-1-yl) dehydroamino acid derivative (0.8 eq.) in methanol (0.1 mol dm^{-3}) followed by the addition of triethylamine (10 eq.) The reaction was left overnight, stirring at room temperature and the work-up procedure described above was repeated to give an oil.

Synthesis of compound **E,E-18**

The general procedure described above was used with compound **E-16** (134 mg, 0.5 mmol) and 1,2-ethylenediamine to produce compound **E,E-18** (115 mg, 50%) as a white solid. M.p. 139.0–141.0°C (from ethyl acetate/*n*-hexane). ^{1}H NMR (300 MHz, $CDCl_3$): 1.45 (s, 18 H, CH_3 Boc), 3.32 (br s, 4 H, CH_2), 3.71 (s, 6 H, OCH_3), 5.46 (br s, 2 H, NH), 5.95 (br s, 2 H, NH), 7.15 (br s, 2 H, β-CH) ppm. ^{13}C NMR (75.4 MHz, $CDCl_3$): 28.21 [C(CH_3)$_3$], 49.53 (CH_2), 51.36 (OCH_3), 80.25 [OC(CH_3)$_3$], 98.93 (C), 141.46 (CH), 154.31 (C=O), 166.50 (C=O) ppm. MS (EI): *m/z* (%) = 458 (22.0) [M^+], 358 (16.3) [M^+-Boc], 258 (100) [M^+-2xBoc], HRMS: calcd for $C_{20}H_{34}N_4O_8$: 458.2376; found: 458.2370.

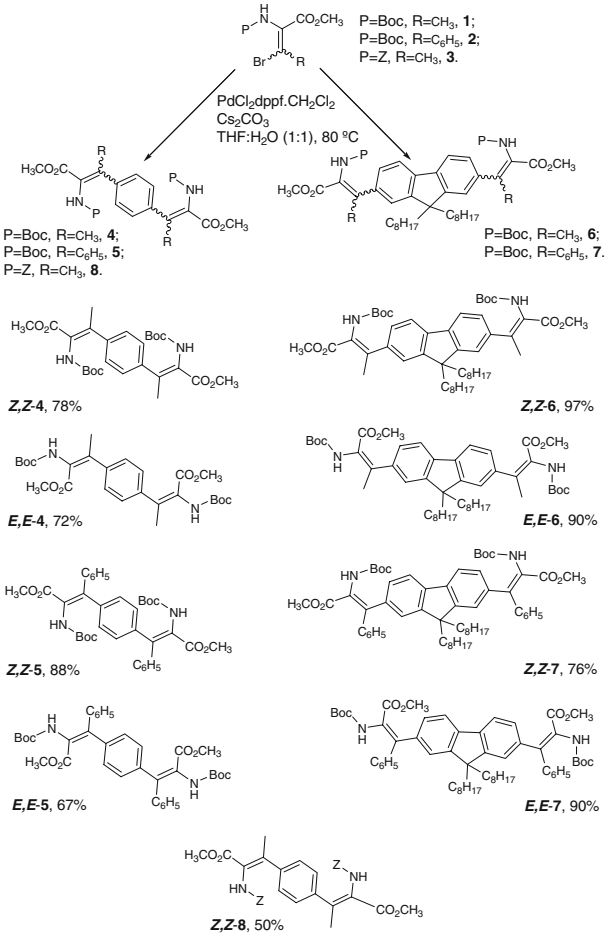
Synthesis of compound **E,E-19**

The general procedure described above was used with compound **E-17** (141 mg, 0.5 mmol) and 1,2-ethylenediamine to produce compound **E,E-19** (151 mg, 62%) as a white solid. M.p. 197.0–198.0°C (from ethyl acetate/*n*-hexane). ^{1}H NMR (400 MHz, $CDCl_3$): 1.47 (s, 18 H, CH_3 Boc), 1.95 (s, 6 H, γ-CH_3), 3.40 (br s, 4 H, CH_2), 3.65 (s, 6 H, OCH_3), 5.65 (br s, 2 H, NH), 9.13 (br s, 2 H, NH) ppm. ^{13}C NMR (75.4 MHz, $CDCl_3$): 13.88 (γ-CH_3), 28.34 [C(CH_3)$_3$], 44.39 (CH_2), 50.74 (OCH_3), 79.30 [OC(CH_3)$_3$], 93.65 (C), 156.22 (C=O), 161.97 (C), 169.32 (C=O) ppm. Anal. Calcd for $C_{22}H_{38}N_4O_8$ (486.27): C 54.31; H 7.87; N 11.51; found: C 54.06; H 7.69; N 11.78.

Results and discussion

In view of our past experience in bis-Suzuki couplings (Abreu et al. 2003a, b; Queiroz et al. 2007), we decided to use this methodology for cross-linking dehydroaminobutyric acid and dehydrophenylalanine derivatives using a phenyl or a fluorenyl linker. The latter was chosen due to the interesting properties of fluorene derivatives, which make them useful in the development of light emitting diodes (Grisorio et al. 2007) and photovoltaic devices (Wang et al. 2005). The pure stereoisomers of the methyl esters of *N*-(*tert*-butoxycarbonyl)-β-bromodehydroaminobutyric acid and *N*-(*tert*-butoxycarbonyl)-β-bromodehydrophenylalanine prepared according to the procedure already described by us (Abreu et al. 2004; Silva et al. 2002) were coupled in a one-pot bis-Suzuki coupling with 1,4-phenylene-bis-boronic or 9,9-dioctyl-9*H*-fluorene-2,7-bis-boronic acids. The conditions used were: 0.4 eq. of bis-boronic acid, 20 mol% of $PdCl_2dppf.CH_2Cl_2$, and Cs_2CO_3 as base (1.4 eq.) in THF.H_2O (1:1) at 70°C for 2 h (Scheme 1). The bis-dehydroamino acids were stereoselectively obtained in good to high yields (compounds **4**–**7**, Scheme 1). To test the applicability of this bis-Suzuki coupling with dehydroamino acid derivatives with different protecting groups, the *Z*-isomer of *N*-benzyloxycarbonyl-β-bromodehydroaminobutyric acid was also reacted with 1,4-phenylene-bis-boronic acid to give the corresponding cross-linked dehydroamino acid in 50% yield (compound **Z,Z-8**, Scheme 1).

The stereochemistry of the bis-dehydroamino acids was determined by NOE difference experiments by irradiation of the α-NH and OCH_3 protons. In the case of the *Z,Z*-isomers, when the OCH_3 protons were irradiated an NOE enhancement was observed on the γ-CH_3 protons or on the C_6H_5 protons of the dehydroaminobutyric acid and dehydrophenylalanine residues, respectively. The *E,E*-isomers showed an NOE enhancement on the signals of the same protons when the α-NH proton was irradiated. As reported by other authors (Yamada et al. 1996; Brown and Smale 1969), proton NMR chemical shifts of the γ-CH_3 protons of the *E,E*-isomers of bis-dehydroaminobutyric acid derivatives were observed at a higher field relative to that of the *Z,Z*-isomers (Table 1). The methyl ester protons



Scheme 1 Synthesis of bis-dehydroamino acids by Suzuki coupling

Table 1 ^{1}H NMR Chemical shifts of the γ-CH_3 and OCH_3 protons of bis-dehydroaminobutyric acid and dehydrophenylalanine derivatives in $CDCl_3$

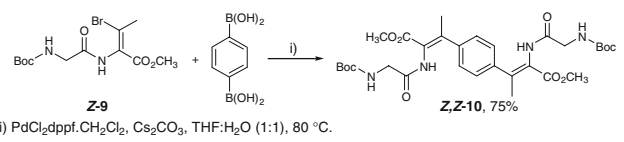
Compound	δ/ppm		Compound	δ/ppm	
	γ-CH_3	OCH_3		γ-CH_3	OCH_3
Z,Z-4	2.27	3.86	**E,E-4**	2.13	3.48
Z,Z-6	2.29	3.88	**E,E-6**	2.18	3.40
Z,Z-5	–	3.52	**E,E-5**	–	3.56
Z,Z-7	–	3.57	**E,E-7**	–	3.51
Z,Z-8	2.27	3.86	–	–	–

of the *E,E*-isomers of compounds **4** and **6** appear at a higher field than that of the corresponding *Z,Z*-isomers probably because of the shielding anisotropic effect of the aromatic ring in the β-position. This effect was observed by Nunami et al. on the *Z*- and *E*-isomers of β-bromodehydrophenylalanines (Yamada et al. 1996). The chemical shift of the OCH_3 protons of the *Z,Z*- and *E,E*-isomers of compounds **5** and **7** are similar due to the influence of an adjacent aromatic ring in both isomers.

The brominated dehydrodipeptide Boc-Gly-ΔAbu(β-Br)-OMe (compound **Z-9**) had been previously prepared by us by reacting the corresponding dehydrodipeptide with NBS and Et_3N (Ferreira et al. 2007). The stereoisomers obtained were separated by column chromatography. The *Z*-isomer of **Z-9** was coupled with 1,4-phenylene-bis-boronic acid under bis-Suzuki cross-coupling conditions to give the corresponding bis-dehydrodipeptide in good yield and maintaining the stereochemistry of the starting material (Scheme 2).

The setereoselective hydrogenation of compounds **4–8** and **10** would allow their use in the synthesis of cross-linked or cyclic peptides.

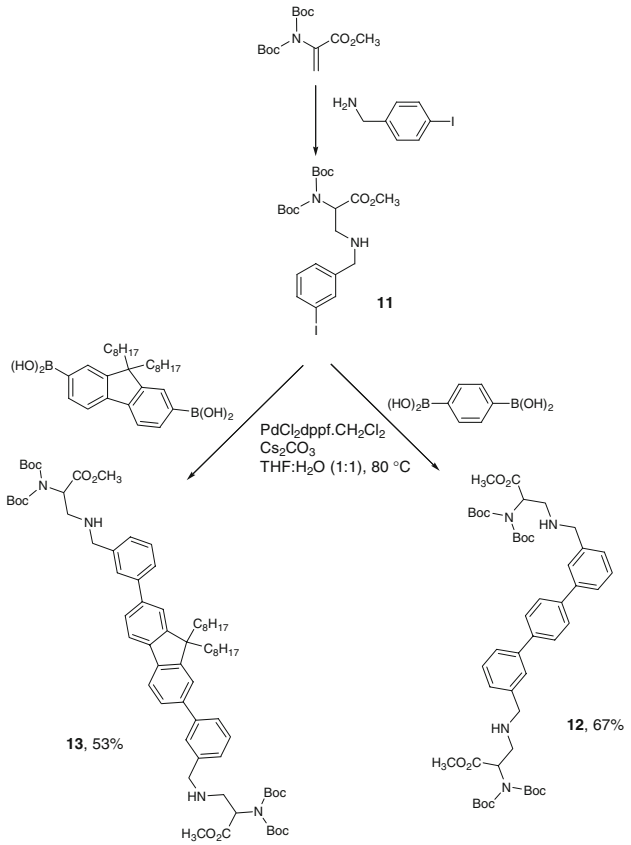
In order to increase the chemical diversity of our bis-amino acid derivatives, we took advantage of our previously described method for the synthesis of β-substituted amino acids by Michael addition to *N,N*-diacyldehydroamino acids. Thus, two bis-amino acids were prepared by sequential Michael addition and bis-Suzuki coupling. Compound **11** (Abreu et al. 2003a), was already prepared in our group by Michael addition of 3-iodoaniline to the methyl ester of *N,N*-bis(*tert*-butoxycarbonyl) dehydroalanine and submitted to a bis-Suzuki coupling reaction with 1,4-phenylene-bis-boronic acid or 9,9-dioctyl-9*H*-fluorene-2,7-bis-boronic acid to give cross-linked α,β-diamino acids (**12** and **13**) in good yields (67 and 53%, respectively, Scheme 3).



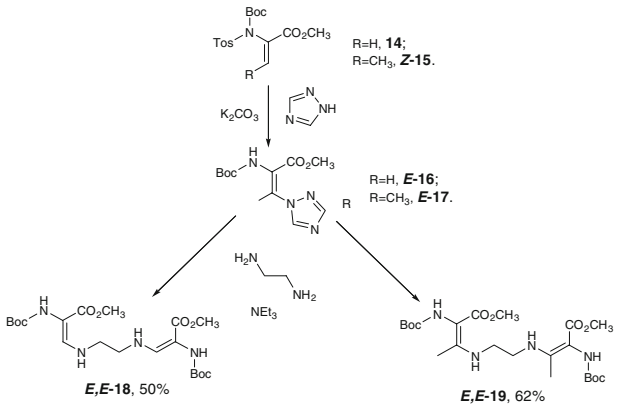
Scheme 2 Synthesis of a bis-dehydrodipeptide by Suzuki coupling

Using a substitution reaction previously developed for the synthesis of α,β-diaminodehydroamino acids (Ferreira et al. 2003) we were able to prepare in good yields bis-dehydroamino acids linked by a diaminoethylene moiety (Scheme 4). Thus, the methyl esters of *N*-(*tert*-butoxycarbonyl), β-(1,2,4-triazol-1-yl) dehydroalanine (compound **E-16**) and of *N*-(*tert*-butoxycarbonyl), β-(1,2,4-triazol-1-yl) dehydroaminobutyric acid (compound **E-17**) were reacted with 1,2-ethylenediamine in a two-step reaction to give the corresponding bis-dehydroalanine and bis-dehydroaminobutyric acid derivatives (compounds **E,E-18** and **E,E-19**, respectively). The stereochemistry of the products was determined by NOE difference experiments by irradiation of the α-NH and observing an NOE effect on the β-CH or on γ-CH_3 protons of the dehydroalanine and dehydroaminobutyric acid derivatives, respectively.

In conclusion, several new bis-dehydroaminobutyric acid and bis-dehydrophenylalanine derivatives were prepared in good to high yields and maintaining the stereochemistry of the starting materials, by a one-pot Suzuki bis-coupling of the corresponding β-brominated



Scheme 3 Synthesis of bis-amino acids by sequential Michael addition and Suzuki coupling



Scheme 4 Synthesis of bis-dehydroamino acids from β-(1,2,4-triazol-1-yl)dehydroamino acid derivatives

dehydroamino acids with bis-boronic acids. The same methodology was applied successfully for the preparation of a cross-linked dehydrodipeptide using a β-bromodehydrodipeptide and 1,4-phenylene-bis-boronic acid showing that this strategy could also be applied in the synthesis of cross-linked peptides. Using a sequential Michael addition and bis-Suzuki coupling it was possible to prepare cross-linked β-substituted alanines. This strategy has the advantage of giving directly bis-amino acids avoiding a hydrogenation step. However, the products were obtained as diastereomeric mixtures. Bis-dehydroalanine and bis-dehydroaminobutyric acid were also obtained by a Michael addition followed by a substitution reaction. By changing the diamine linker it is possible to prepare several bis-α,β-diamino acid derivatives. This work shows the versatility of bis-Suzuki couplings and substitution reactions in the synthesis of several cross-linked amino acids.

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