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Synthesis of pure stereoisomers of benzo[*b*]thienyl dehydrophenylalanines by Suzuki cross-coupling. Preliminary studies of antimicrobial activity

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Abstract—Several benzo[*b*]thienyldehydrophenylalanines were synthesized from pure stereoisomers of the methyl ester of *N*-(*tert*-butoxycarbonyl)-β-bromodehydrophenylalanine as an extension of our previously reported method for the synthesis of dehydrotryptophan analogues to dehydrophenylalanine derivatives. The latter were obtained in high yields by *N*-deprotection and bromination of *N*,*N*-bis-(*tert*-butoxycarbonyl)-(*Z*)-dehydrophenylalanine using TFA and NBS. This was carried out in two steps or in a one pot procedure resulting in different *E*/*Z* ratios. These compounds were coupled under Suzuki cross-coupling conditions [$Pd(PPh_3)_4$, Na_2CO_3, DME/H_2O] with several boronic benzo[*b*]thienyl acids in good to high yields maintaining the stereochemistry of the starting materials. The best yields were obtained when the boronic acid was in position 7 of the benzo[*b*]thiophene and with the *E* isomer of the brominated dehydrophenylalanine. In some cases it was possible to increase the lower yields by changing the Pd source to $PdCl_2(PPh_3)_2$. A model dipeptide was prepared coupling a benzo[*b*]thienyldehydrophenylalanine with the methyl ester of alanine. Preliminary antimicrobial studies were performed with both isomers of one of the β, β-diaryldehydroalanines obtained. The results show that the compounds are selective and very active (very low MICs) against Gram positive bacteria (*B. cereus* and *B. subtilis*) the *Z*-isomer being more active. The compounds are also active against *Candida albicans* presenting similar MICs.

1. Introduction

Dehydroamino acids have been found in several natural peptides from microbial or marine sources. The incorporation of α,β-dehydroamino acids constitutes a valuable tool in structure-activity relationship (SAR) studies, due to the conformational constraints they impose. These restrained analogues of amino acids mainly dehydrophenylalanine and dehydrotyrosine have been introduced in peptide sequences to probe the preferred orientations of these residues once bound to the receptors.[1] These molecules can also be very useful as pharmaceutical probes towards various proteases, namely HIV-proteases.[2]

The dehydrophenylalanine residue as a constrained phenylalanine mimic has gained much importance, in particular, because of its turn inducing as well as helix-forming propensity.[3] Studies have indicated that the α,β-double bond of dehydroamino acids does not in itself cause reversal in the peptide backbone and the preferred conformation of a dehydropeptide may be decided by the nature of the β-substituents. Thus, different dehydroamino acids can be used to introduce different kinds of constraints in peptides.[4]

Recently, we have been interested in the synthesis of new β-substituted dehydroamino acids either by Michael additions[5] or by palladium catalyzed cross-couplings.[6a–c] The fluorescence studies performed on β-benzo[*b*]thienyldehydroamino acids already prepared by us showed that some of them can also be used as fluorescent probes.[6c]

Here we describe the synthesis of benzo[*b*]thienyldehydrophenylalanines using Suzuki cross-coupling[7] of several boronic benzo[*b*]thienyl acids with pure stereoisomers of a β-bromodehydrophenylalanine derivative. Two of the β, β-diaryldehydroalanines obtained were tested for antimicrobial activity and showed to be active with low minimal inhibitory concentration (MIC). The insertion of this type of compounds in peptides was demonstrated preparing a model dipeptide using DCC/HOBt.

Keywords: Amino acids; Dehydrophenylalanines; Benzo[*b*]thiophenes; Suzuki coupling; Palladium; Antimicrobial.
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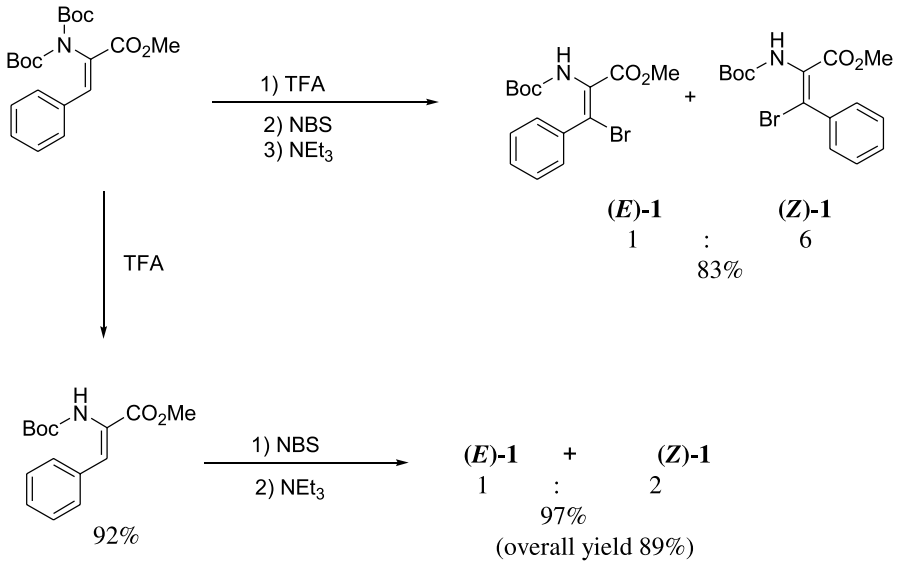


Scheme 1. Synthesis of compound (*E*)-**1** and (*Z*)-**1** in two steps or in a one pot procedure.

2. Results and discussion

The methyl ester of *N*,*N*-bis-(*tert*-butoxycarbonyl)-(*Z*)-dehydrophenylalanine[5] (Boc_2-ΔPhe-OMe) was N-monodeprotected[5] with TFA and β-brominated with NBS followed by treatment with NEt_3 to give the methyl ester of *N*-(*tert*-butoxycarbonyl)-β-bromodehydrophenylalanine [Boc-ΔPhe(β-Br)-OMe)] as a 1:2 E/Z mixture in an overall

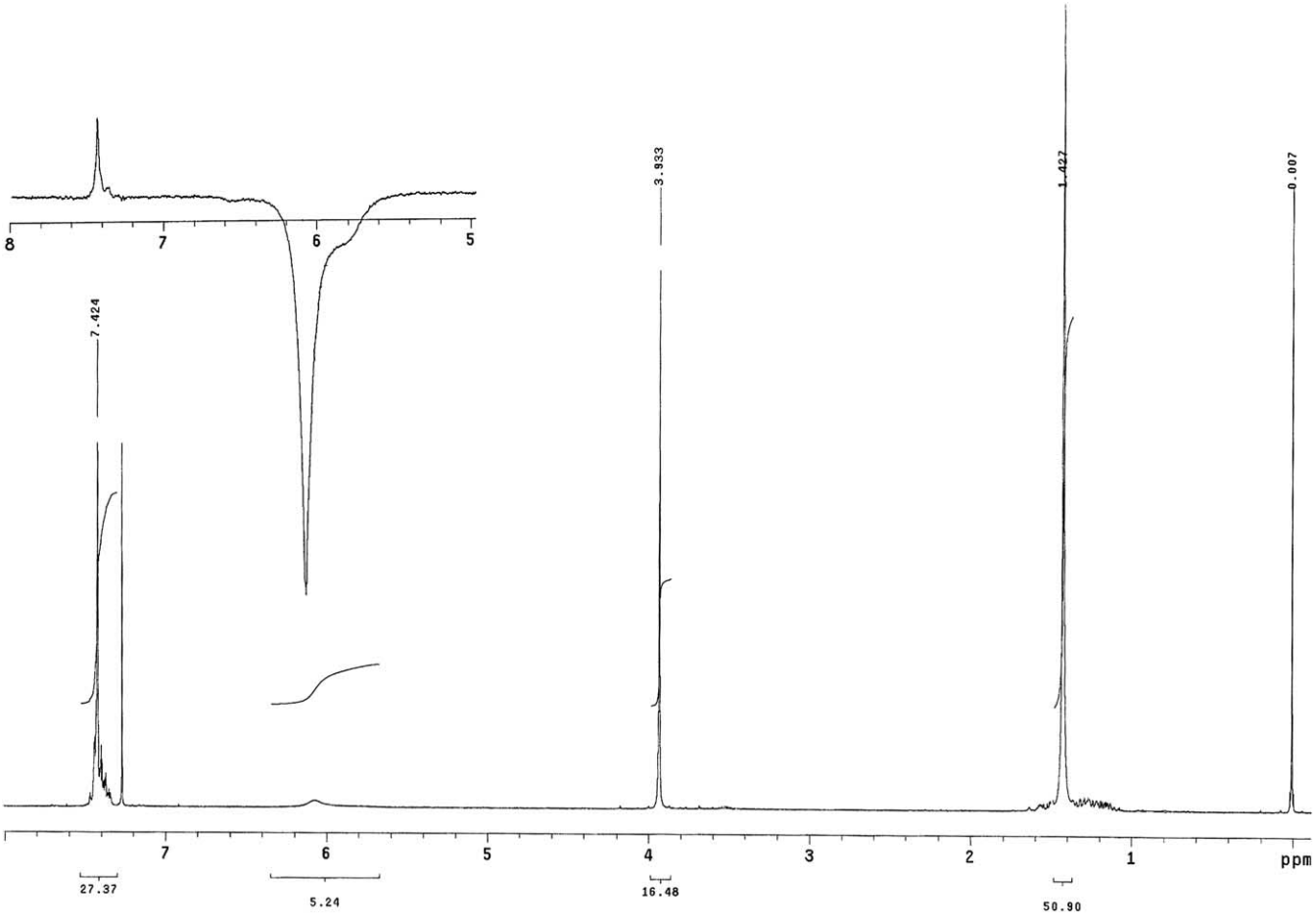


Figure 1. ^1H NMR spectrum ($CDCl_3$) of (*E*)-**1** and NOE difference experiment irradiating the α-NH and observing the effect on the signal of the phenyl protons.

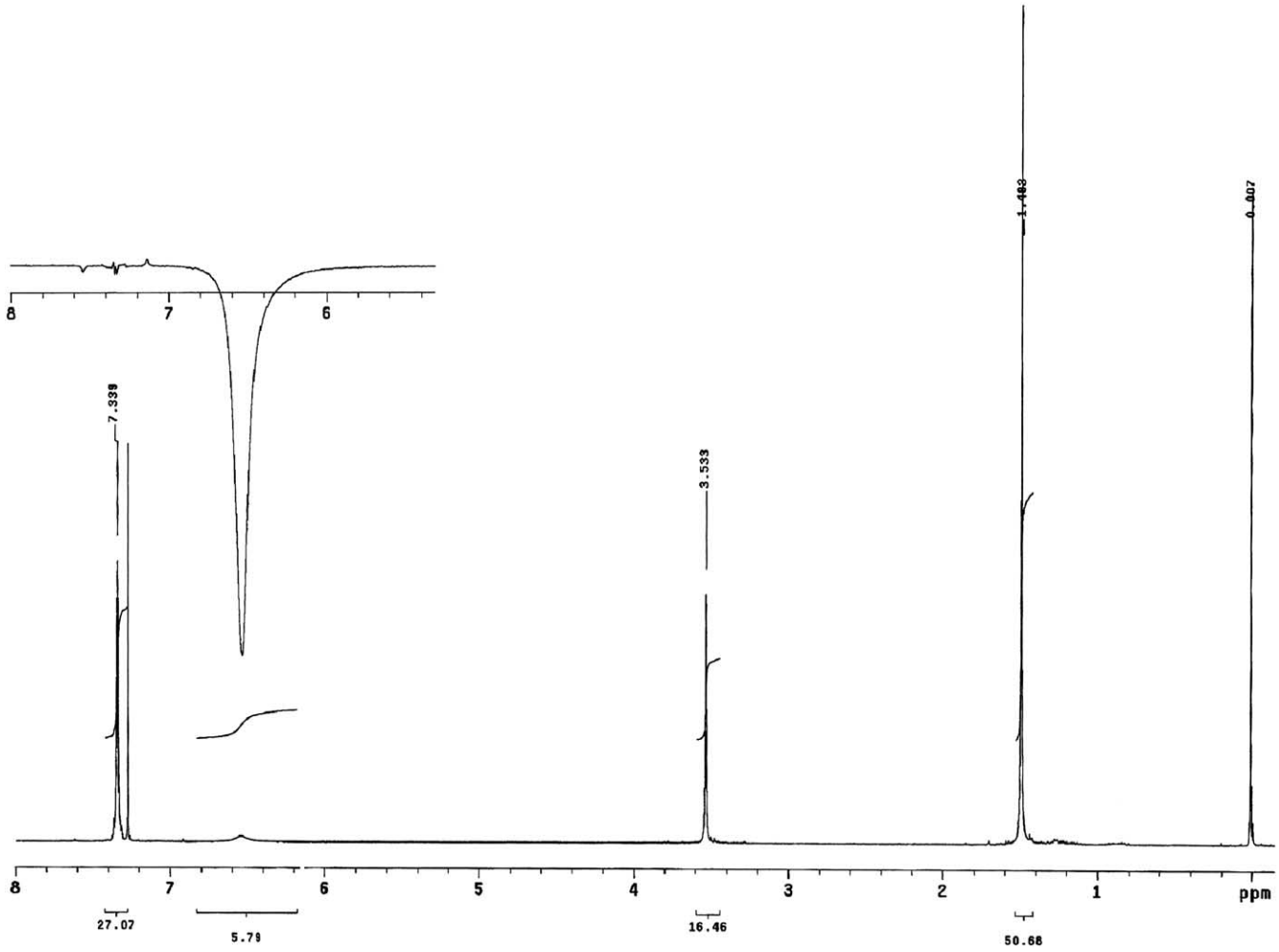


Figure 2. ^{1}H NMR spectrum ($CDCl_3$) of (*Z*)-**1** and NOE difference experiment irradiating the α-NH.

yield of 89%. The same reactions performed in a one pot procedure gave the products in a similar yield but resulted in a higher selectivity towards the *Z* isomer (1:6 *E*/*Z*) (Scheme 1). These results are similar to those obtained by us in the bromination of dehydroaminobutyric acid.[6a]

The stereochemistry of (*E*) and (*Z*)-**1** was determined by NOE difference experiments irradiating the α-NH and observing a NOE effect on the signal of the phenyl protons of (*E*)-**1** (Fig. 1) while for compound (*Z*)-**1** this was not observed (Fig. 2).

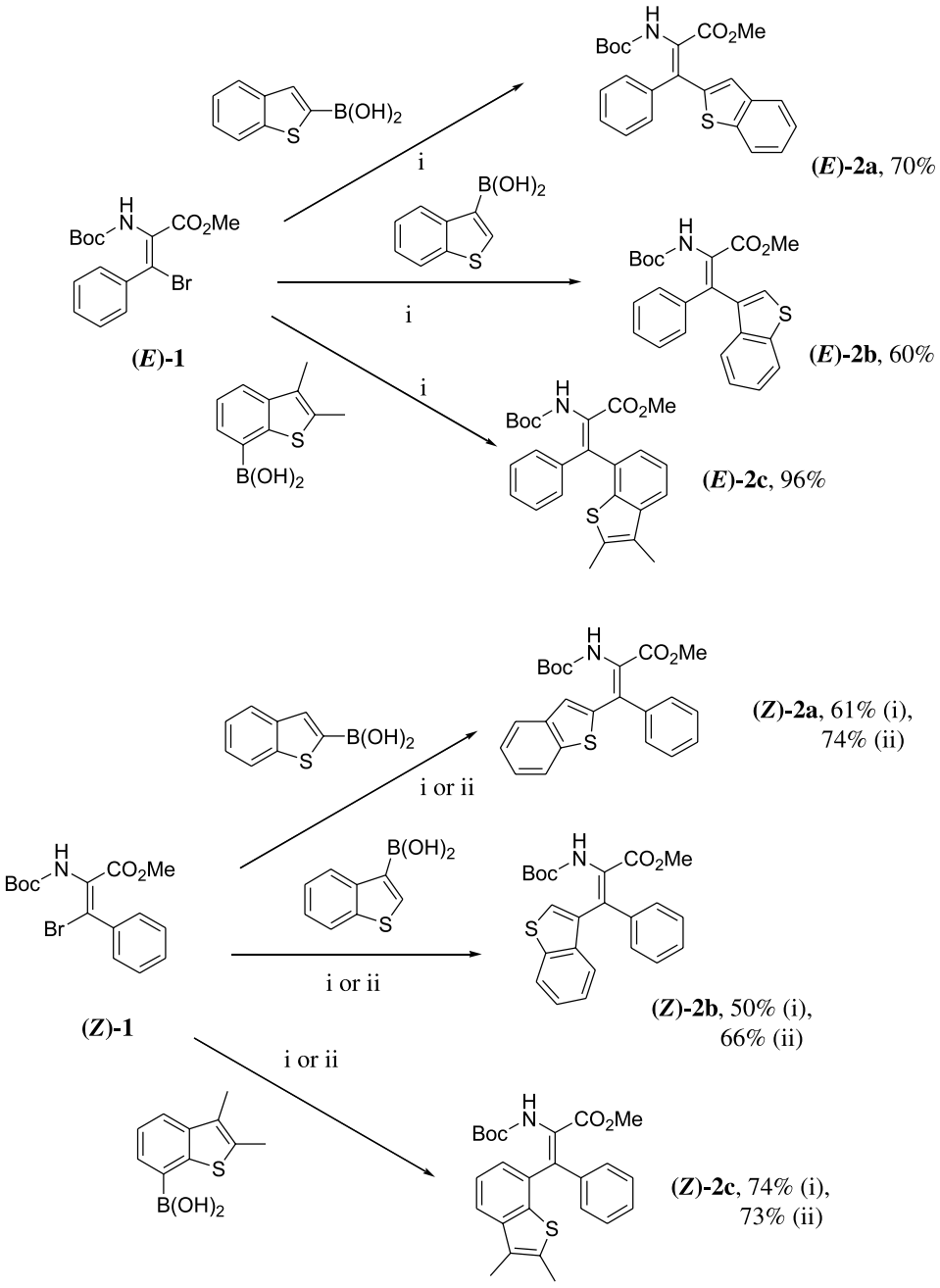
The pure stereoisomers (*E*) and (*Z*)-**1** were coupled with several boronic benzo[*b*]thienyl acids under Suzuki cross-coupling conditions[6a] to give β-benzo[*b*]thienyldehydrophenylalanines in good to high yields (Scheme 2). NOE difference experiments irradiating the α-NH confirmed that the coupled products maintained the stereochemistry of the starting materials.

The best yields were obtained when the boronic acid was in position 7 of the benzo[*b*]thiophene moiety. In all cases using the same catalytic conditions (i), the higher yields were obtained from compound (*E*)-**1** which can be due to the lower steric hindrance of this derivative.

In order to increase the yields of the *Z* isomers, another palladium catalyst [$PdCl_2(PPh_3)_2$], that had already given good results in the synthesis of β,β-bis-(benzo[*b*]thienyl)-dehydroalanines from a β,β-dibromodehydroalanine derivative, was used.[6c] In these conditions (ii), when the boronic acids are in the thiophene ring the yields increased from 61 to 74% in the synthesis of (*Z*)-**2a** and from 50 to 66% in the synthesis of (*Z*)-**2b**. This increase was not observed in the synthesis of compound (*Z*)-**2c** which was obtained in similar yields using both catalytic systems (Scheme 2).

A dipeptide was prepared from (*E*)-**2b** by C-deprotection and coupling with the methyl ester of alanine using DCC/HOBt (Scheme 3). This result in the synthesis of a model dipeptide shows that our β,β-diaryldehydroamino acids can be inserted into peptides.

A screening of antibacterial activities using two Gram negative (*Escherichia coli* and *Pseudomonas aeruginosa*) and two Gram positive bacteria (*Bacillus subtilis* and *Bacillus cereus*) and antifungal activity using *Candida albicans* as a representative of fungi was assessed for compounds (*Z*)-**2c** and (*E*)-**2c**. The MIC (in μg/mL) was determined (Table 1) using an adaptation of agar streak

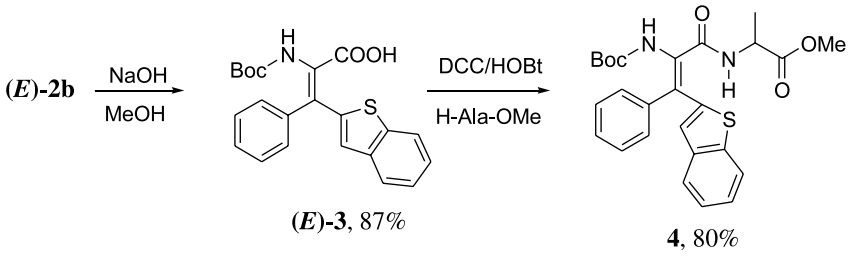


Scheme 2. Synthesis of compounds (*E*)-**2a–c** and (*Z*)-**2a–c** under Suzuki cross-coupling conditions. (i) $Pd(PPh_3)_4$ (10 mol%), Na_2CO_3 (2 equiv), boronic acid (1.3 equiv), DME/H_2O (4:1), 90 °C, 3–5 h. (ii) Same conditions but using $PdCl_2(PPh_3)_2$ (10 mol%).

dilution method based on radial diffusion.[8] In the same conditions, different concentrated solutions of Ampicillin (antibacterial) and Cyclohexamide (antifungal) were used as standards. The MIC was considered to be the lowest concentration of the tested compound which inhibits growth of bacteria or fungi on the plate. The compounds tested were inactive against the Gram- bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*). The diameters of the inhibition zones corresponding to the MICs for the Gram+ bacteria and for *C. albicans* are presented in Table 1.

From the inspection of Table 1 it is possible to conclude that the compounds tested are active against *B.cereus*, *B.subtilis* and *C. albicans* presenting MICs very much lower than those obtained with Ampicillin (antibacterial) and Cyclohexamide (antifungal).

Compound (*Z*)-**2c** shows lower MICs than (*E*)-**2c** for Gram+ bacteria but the results against *C. albicans* are similar for both isomers. These results indicate that the compounds are selective and very active (very low MICs) against the Gram+ bacteria tested and against a representative of fungi, thus showing very interesting antimicrobial properties.



Scheme 3. Synthesis of dipeptide **4** from (*E*)-**2b** and the methylester of alanine.

Table 1. Antimicrobial activity of compounds (*Z*)-**2c** and (*E*)-**2c**

Compounds	MIC (μg/mL) (zone of inhibition in mm)		
	Bacillus cereus CECT148	*Bacillus subtilis* CECT498	*Candida albicans* CECT 1394
(*E*)-**2c**	0.125 (13)	0.125 (15)	0.125 (6)
(*Z*)-**2c**	1.25×10^{-3} (15)	1.25×10^{-3} (11)	0.125 (5)
Ampicillin	3.13 (13)	12.5 (10)	—
Cyclohexamide	—	—	12.5 (5)

CECT—Spanish type culture collection of Valencia University.

3. Conclusion

Several β,β-diaryldehydroamino acids in the benzo[*b*]thiophene series were synthesized in good to high yields from pure stereoisomers of a β-bromodehydrophenylalanine derivative and benzo[*b*]thienylboronic acids using C–C palladium-catalyzed cross-couplings. From the results obtained we can conclude that the *E* isomer of the β-brominated dehydrophenylalanine (*E*)-**1** is more reactive under Suzuki cross coupling conditions then the *Z* isomer. It is also possible to conclude that the 7-benzo[*b*]thienylboronic acid is the most reactive, and its reactivity does not depend on the Pd source. The insertion of this type of compounds in peptides was tested preparing a model dipeptide in high yield. Preliminary antimicrobial studies were performed using both isomers of one of the β,β-diaryldehydroalanines obtained. The results show that the compounds are selective and very active (very low MICs) against Gram+bacteria (*B. cereus* and *B. subtilis*), the *Z*-isomer being more active. The compounds are also active against *Candida albicans* presenting similar MICs.

4. Experimental

4.1. General

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 analyser.

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. When solvent gradient was used, the increase in polarity was made from neat petroleum ether to mixtures of ether/petroleum ether, increasing 10% of ether each time until the isolation of the product.

4.2. Synthesis of Boc-(*E*)-ΔPhe(β-Br)-OMe (*E*)-1 and Boc-(*Z*)-ΔPhe(β-Br)-OMe (*Z*)-1

Boc-ΔPhe-OMe[5] (1.39 g, 5.00 mmol) was dissolved in dichloromethane (0.1 M) and 1.2 equiv of *N*-bromosuccinimide (0.980 g, 5.50 mmol) were added with vigorous stirring. After reacting for 16 h, triethylamine (1.5 equiv) was added and stirring continued for an additional hour. Dicloromethane (50 mL) was added and the organic phase was washed with water and brine (3×30 mL each). After drying over $MgSO_4$ the extract was taken to dryness at reduced pressure to give (*E*)-**1** and (*Z*)-**1** (1.73 g, 97%) as a 1:2 mixture. The diastereomers were separated by column chromatography using solvent gradient from neat petroleum ether to 20% diethyl ether/petroleum ether to give (*Z*)-**1** mp 101–103 °C (from diethyl ether/*n*-hexane). ^{1}H NMR ($CDCl_3$): 1.49 (9H, s, CH_3 Boc), 3.53 (3H, s, OCH_3), 6.55 (1H, s, αNH), 7.34 (5H, br s, ArH). ^{13}C NMR ($CDCl_3$): 28.06 ($C(CH_3)_3$), 52.43 (OCH_3), 82.10 ($OC(CH_3)_3$), 128.21 (CH), 128.41 (C), 128.94 (CH), 129.11 (CH), 129.31 (C), 137.39 (C), 151.89 (C=O), 163.47 (C=O) ppm. Anal. Calcd for $C_{15}H_{18}NO_4Br$ (356.22): C, 50.58; H, 5.09; N, 3.93. Found: C, 50.61; H, 5.12; N, 4.01. (*E*)-**1** mp 80–81 °C (from petroleum ether). ^{1}H NMR ($CDCl_3$): 1.43 (9H, s, CH_3 Boc), 3.93 (3H, s, OCH_3), 6.08 (1H, s, αNH), 7.43 (5H, br s, ArH). ^{13}C NMR ($CDCl_3$): 28.01 ($C(CH_3)_3$), 52.58 (OCH_3), 82.00 ($OC(CH_3)_3$), 128.58 (C), 128.94 (CH), 129.04 (CH), 129.47 (CH), 136.50 (C), 151.56 (C=O), 164.55 (C=O) ppm. Anal. Calcd for $C_{15}H_{18}NO_4Br$ (356.22): C, 50.58; H, 5.09; N, 3.93. Found: C, 50.74; H, 5.21; N, 4.09.

One pot procedure. Boc_2-ΔPhe-OMe[5] (1.86 g, 5.00 mmol) was dissolved in dichloromethane (0.1 M) and 2% of TFA was slowly added with vigorous stirring. The reaction was monitored by TLC and when no starting material was detected (≈1 h) 1.2 equiv of *N*-bromosuccinimide (1.34 g,

7.50 mmol) were added. After reacting for 16 h triethylamine (15.0 mmol) was added and stirring continued for an additional hour. Dichoromethane was added (50 mL) and the organic phase was then washed with water and brine (2×30 mL each). After drying over $MgSO_4$, the extract was taken to dryness at reduce pressure to afford a 1:6 mixture of (*E*)-**1** and (*Z*)-**1** (1.48 g, 83%).

4.3. General procedure for Suzuki cross couplings

To a solution of compound (*E*)-**1** or (*Z*)-**1** in DME/water (4:1), benzo[*b*]thienylboronic acids (1.1 equiv), Na_2CO_3 (2 equiv) and $Pd(PPh_3)_4$ (10 mol%) were added and the mixture was heated at 90 °C while the reaction was monitored by TLC. The DME was removed under reduced pressure and the residue was dissolved in ethyl acetate (15 mL). The organic layer was then washed with water and brine (3×5 mL) dried with $MgSO_4$ and the solvent evaporated at reduce pressure to give an oil which was submitted to column chromatography.

4.3.1. Boc-(*E*)-ΔPhe(β-benzo[*b*]thien-2-yl)-OMe ((*E*)-2a). The procedure described above was applied using compound (*E*)-**1** (107 mg, 0.300 mmol) and the 2-benzo-[*b*]thienylboronic acid (0.330 mmol, 59.0 mg) and heating for 3 h 30 min. Column chromatography using a solvent gradient from pure petroleum ether to 30% diethyl ether in petroleum ether, gave compound (*E*)-**2a** (86.0 mg, 70%) as an oil. Recrystallization from diethyl ether/petroleum ether afforded light yellow crystals, mp 97–99 °C. ^{1}H NMR ($CDCl_3$): 1.46 (9H, s, CH_3 Boc), 3.69 (3H, s, OCH_3), 6.17 (1H, br s, NH), 7.16 (1H, s, ArH), 7.29–7.43 (7H, m, ArH), 7.69–7.74 (2H, m, ArH) ppm. ^{13}C NMR ($CDCl_3$): 28.09 ($C(CH_3)_3$), 52.58 (OCH_3), 81.77 ($OC(CH_3)_3$), 122.05 (CH), 123.60 (CH), 124.33 (CH), 124.43 (CH), 124.55 (CH), 127.44 (C), 128.83 (CH), 129.02 (CH), 129.62 (CH), 137.33 (C), 139.31 (C), 140.72 (C), 141.97 (C), 152.12 (C=O), 166.01 (C=O) ppm. Anal. Calcd for $C_{23}H_{23}NO_4S$ (409.50): C, 67.46; H, 5.66; N, 3.42; S, 7.83. Found: C, 67.25; H, 5.93; N, 3.39; S, 7.36.

4.3.2. Boc-(*Z*)-ΔPhe(β-benzo[*b*]thien-2-yl)-OMe ((*Z*)-2a). The procedure described above was applied using compound (*Z*)-**1** (107 mg, 0.300 mmol) and the 2-benzo-[*b*]thienylboronic acid (59.0 mg, 0.330 mmol) and heating for 5 h. Column chromatography using a solvent gradient from pure petroleum ether to 30% diethyl ether in petroleum ether, gave compound (*Z*)-**2a** (75.0 mg, 61%) as an oil. Recrystallization from diethyl ether/petroleum ether afforded colourless crystals, mp 123–124 °C. ^{1}H NMR ($CDCl_3$): 1.52 (9H, s, CH_3 Boc), 3.49 (3H, s, OCH_3), 6.55 (1H, br s, NH), 7.08 (1H, s, ArH), 7.28–7.41 (7H, m, ArH), 7.67–7.76 (1H, m, ArH), 7.80–7.83 (1H, m, ArH) ppm. ^{13}C NMR ($CDCl_3$): 28.15 ($C(CH_3)_3$), 51.96 (OCH_3), 81.59 ($OC(CH_3)_3$), 122.01 (CH), 123.57 (C), 124.00 (CH), 124.59 (CH), 125.31 (CH), 125.87 (C), 127.80 (CH), 128.07 (CH), 128.24 (CH), 129.38 (CH), 138.79 (C), 138.85 (C), 140.77 (C), 152.84 (C=O), 165.89 (C=O) ppm. Anal. Calcd for $C_{23}H_{23}NO_4S$ (409.50): C, 67.46; H, 5.66; N, 3.42; S, 7.83. Found: C, 67.58; H, 5.74; N, 3.48; S, 7.70. The procedure described above using 0.5 mmol of (*Z*)-**1** and using $PdCl_2(PPh_3)_2$ gave compound (*Z*)-**2a** (150 mg, 74%).

4.3.3. Boc-(*E*)-ΔPhe(β-benzo[*b*]thien-3-yl)-OMe ((*E*)-2b). The procedure described above was applied using compound (*E*)-**1** (178 mg, 0.500 mmol) and the 3-benzo-[*b*]thienylboronic acid (98.0 mg, 0.550 mmol) and heating for 3 h. Column chromatography using a solvent gradient from pure petroleum ether to 30% diethyl ether in petroleum ether, gave compound (*E*)-**2b** (112 mg, 60%) as an oil. Recrystallization from diethyl ether/petroleum ether afforded colourless crystals, mp 171–172 °C. ^{1}H NMR ($CDCl_3$): 1.48 (9H, s, CH_3 Boc), 3.46 (3H, s, OCH_3), 6.33 (1H, br s, NH), 7.19 (1H, d, J=7.8 Hz, ArH), 7.26–7.40 (8H, m, ArH), 7.81 (1H, d, J=7.5 Hz, ArH) ppm ^{13}C NMR ($CDCl_3$): 28.09 ($C(CH_3)_3$), 52.09 (OCH_3), 81.46 ($OC(CH_3)_3$), 122.49 (CH), 123.21 (CH), 124.08 (CH), 124.30 (CH), 125.63 (CH), 127.57 (C), 128.45 (CH), 128.89 (CH), 129.20 (CH), 131.94 (C), 134.59 (C), 136.75 (C), 138.09 (C), 139.81 (C), 152.54 (C=O), 166.34 (C=O) ppm. Anal. Calcd for $C_{23}H_{23}NO_4S$ (409.50): C, 67.46; H, 5.66; N, 3.42; S, 7.83. Found: C, 67.30; H, 5.94; N, 3.51; S, 7.64.

4.3.4. Boc-(*Z*)-ΔPhe(β-benzo[*b*]thien-3-yl)-OMe ((*Z*)-2b). The procedure described above was applied using compound (*Z*)-**1** (107 mg, 0.300 mmol) and the 3-benzo-[*b*]thienylboronic acid (59.0 g, 0.330 mmol) and heating for 3 h. Column chromatography using a solvent gradient from pure petroleum ether to 30% diethyl ether in petroleum ether, gave compound (*Z*)-**2b** (62.0 mg, 50%) as an oil. Recrystallization from diethyl ether/petroleum ether afforded colourless crystals, mp 139–140 °C. ^{1}H NMR ($CDCl_3$): 1.40 (9H, s, CH_3 Boc), 3.61 (3H, s, OCH_3), 5.92 (1H, br s, NH), 7.16–7.20 (2H, m, ArH), 7.27–7.44 (6H, m, ArH), 7.58 (1H, d, J=8.1 Hz, ArH), 7.90 (1H, d, J=8.1 Hz, ArH) ppm. ^{13}C NMR ($CDCl_3$): 28.07 ($C(CH_3)_3$), 52.13 (OCH_3), 81.38 ($OC(CH_3)_3$), 122.79 (CH), 123.25 (C), 124.67 (CH), 124.82 (CH), 127.22 (C), 128.05 (CH), 128.17 (CH), 128.41 (CH), 128.70 (CH), 134.16 (C), 136.84 (C), 139.39 (C), 140.12 (C), 152.73 (C=O), 166.18 (C=O) ppm. Anal. Calcd for $C_{23}H_{23}NO_4S$ (409.50): C, 67.46; H, 5.66; N, 3.42; S, 7.83. Found: C, 67.53; H, 5.79; N, 3.50; S, 7.72. The procedure described above using $PdCl_2(PPh_3)_2$ gave compound (*Z*)-**2b** (81.0 mg, 66%).

4.3.5. Boc-(*E*)-ΔPhe(β-2,3-dimethylbenzo[*b*]thien-7-yl)-OMe ((*E*)-2c). The procedure described above was applied using compound (*E*)-**1** (178 mg, 0.500 mmol) and the 2,3-dimethyl-7-benzo[*b*]thienylboronic acid (113 mg, 0.550 mmol) and heating for 4 h 30 min. Column chromatography using a solvent gradient from pure petroleum ether to 30% diethyl ether in petroleum ether, gave compound (*E*)-**2c** (209 mg, 96%) as an oil. Recrystallization from petroleum ether afforded colourless crystals, mp 152–154 °C. ^{1}H NMR ($CDCl_3$): 1.47 (9H, s, CH_3 Boc), 2.26 (3H, s, $ArCH_3$), 2.36 (3H, s, $ArCH_3$), 3.41 (3H, s, OCH_3), 6.24 (1H, br s, NH), 7.15 (1H, br d, J=6.9 Hz, ArH), 7.31–7.36 (6H, m, ArH), 7.53 (1H, dd, J=8.1, 0.9 Hz, ArH) ppm. ^{13}C NMR ($CDCl_3$): 11.41 (CH_3), 13.63 (CH_3), 28.13 ($C(CH_3)_3$), 51.99 (OCH_3), 81.27 ($OC(CH_3)_3$), 120.89 (CH), 123.71 (CH), 124.47 (CH), 126.70 (C), 127.06 (C), 128.06 (C), 128.45 (CH), 128.76 (CH), 129.67 (CH), 133.50 (C), 134.48 (C), 136.60 (C), 138.07 (C), 141.48 (C), 152.70 (C=O), 166.03 (C=O) ppm. Anal. Calcd for $C_{25}H_{27}NO_4S$

(437.55): C, 68.62; H, 6.22; N, 3.20; S, 7.33. Found: C, 68.64; H, 6.44; N, 3.29; S, 7.02.

4.3.6. Boc-(*Z*)-ΔPhe(β-2,3-dimethylbenzo[*b*]thien-7-yl)-OMe ((*Z*)-2c). The procedure described above was applied using compound (*Z*)-**1** (178 mg, 0.500 mmol) and the 2,3-dimethyl-7-benzo[*b*]thienylboronic acid (113 mg, 0.550 mmol) and heating for 4 h 30 min. Column chromatography using a solvent gradient from pure petroleum ether to 30% diethyl ether in petroleum ether, gave compound (*E*)-**2c** (162 mg, 74%) as an oil. Recrystallization from petroleum ether afforded colourless crystals, mp 140–142 °C. ^{1}H NMR ($CDCl_3$): 1.42 (9H, s, CH_3 Boc), 2.31 (3H, s, $ArCH_3$), 2.42 (3H, s, $ArCH_3$), 3.60 (3H, s, OCH_3), 5.89 (1H, br s, NH), 7.08–7.21 (3H, m, ArH), 7.23–7.30 (3H, m, ArH), 7.38 (1H, t, J=8.1 Hz, ArH), 7.59 (1H, d, J=8.1 Hz, ArH) ppm. ^{13}C NMR ($CDCl_3$): 11.46 (CH_3), 13.67 (CH_3), 28.08 ($C(CH_3)_3$), 52.19 (OCH_3), 81.14 ($OC(CH_3)_3$), 121.26 (CH), 124.38 (CH), 125.33 (CH), 126.99 (C), 127.93 (CH), 128.08 (CH), 128.78 (CH), 129.69 (C), 132.00 (C), 134.87 (C), 137.87 (C), 138.59 (C), 141.78 (C), 152.67 (C=O), 166.30 (C=O) ppm. Anal. Calcd for $C_{25}H_{27}NO_4S$ (437.55): C, 68.62; H, 6.22; N, 3.20; S, 7.33. Found: C, 68.54; H, 6.35; N, 3.24; S, 7.05. The procedure described above using $PdCl_2(PPh_3)_2$ gave compound (*Z*)-**2c** (160 mg, 73%).

4.4. Synthesis of the model dipeptide Boc-(*E*)-ΔPhe-(β-benzo[*b*]thien-2-yl)-Ala-OMe

4.4.1. Synthesis of Boc-(*E*)-ΔPhe-(β-benzo[*b*]thien-2-yl)-OH ((*E*)-3). To a solution of Boc-*E*-ΔPhe(β-benzo[*b*]thien-2-yl)-OMe (0.34 mmol, 137 mg) in dioxane (3 mL), 1 equiv of NaOH 1 M was added. The solution was left stirring for 18 h at rt (the reaction was followed by TLC until no starting material was detected). The reaction mixture was acidified to pH 2–3 with $KHSO_4$ 1 M and the solid formed filtered. Crystallization from ethyl acetate/*n*-hexane afforded compound (*E*)-**3** (117 mg, 87%) as a light yellow solid, mp 189–191 °C (from ethyl acetate/*n*-hexane). ^{1}H NMR ($CDCl_3$): 1.43 (9H, s, CH_3 Boc), 6.18 (1H, s, NH), 7.29–7.40 (8H, m, ArH), 7.70–7.73 (2H, m, ArH) ppm. ^{13}C NMR ($CDCl_3$): 28.08 ($C(CH_3)_3$), 82.01 ($OC(CH_3)_3$), 114.48 (C), 122.09 (CH), 123.87 (CH), 124.38 (CH), 124.72 (CH), 125.23 (CH), 126.32 (C), 128.95 (CH), 129.02 (CH), 129.58 (CH), 137.46 (C), 139.36 (C), 141.05 (C), 141.34 (C), 151.32 (C=O), 152.56 (C=O) ppm.

4.4.2. Synthesis of Boc-(*E*)-ΔPhe-(β-benzo[*b*]thien-2-yl)-Ala-OMe (4). To a solution of Boc-(*E*)-ΔPhe(β-benzo-[*b*]thien-2-yl)-OH (0.20 mmol, 79.0 mg) in acetonitrile (5 mL), HOBt (0.22 mmol, 34 mg) and DCC (0.22 mmol, 44 mg) were added with vigorous stirring at 0 °C. After 15 min, HCl,H-Ala-OMe (0.2 mmol, 28 mg) and NEt_3 (0.2 mmol, 0.03 mL) were added. The reaction was left stirring for 18 h at rt. The urea was removed by filtration and the solvent removed at reduced pressure. The oily residue was dissolved in ethyl acetate (15 mL) and the solution washed with $KHSO_4$ 1 M (3×5 mL), $NaHCO_3$ 1 M (3×5 mL) and brine (3×5 mL). The organic layer was dried with $MgSO_4$ and solvent removed at reduced pressure giving an oil which was submitted to column chromatography with diethyl ether/petroleum ether (2:1). Compound **4** was isolated as a white solid (74 mg, 80%), mp 168–169 °C (from diethyl ether/*n*-hexane). ^{1}H NMR ($CDCl_3$): 1.10 (3H, d, J=7.2 Hz, βCH_3 Ala), 1.44 (9H, s, CH_3 Boc), 3.43 (3H, s, OMe), 4.54–4.63 (1H, m, αCH Ala), 6.06 (1H, s, NH), 6.42 (1H, d, J=7.5 Hz, NH), 7.27–7.41 (8H, m, ArH), 7.73–7.78 (2H, m, ArH) ppm. ^{13}C NMR ($CDCl_3$): 17.82 (CH_3), 28.13 ($C(CH_3)_3$), 48.33 (CH), 52.17 (OMe), 81.52 ($OC(CH_3)_3$), 122.07 (CH), 123.57 (C), 123.67 (CH), 124.45 (CH), 124.56 (CH), 125.18 (CH), 128.73 (CH), 128.96 (CH), 129.54 (CH), 131.11 (C), 137.50 (C), 139.44 (C), 140.82 (C), 141.44 (C), 152.16 (C=O), 164.29 (C=O), 172.60 (C=O) ppm. Anal. Calcd for $C_{26}H_{28}N_2O_5S$ (480.58): C, 64.98; H, 5.87; N, 5.83; S, 6.67. Found: C, 64.94; H, 5.95; N, 5.82; S, 6.66.

4.5. In vitro antimicrobial activity

Suspensions of the microorganism were prepared to contain approximately 10^8 cfu/mL and the plates were inoculated. A stock solution of the synthesized compound (1000 μg/mL) in DMSO was prepared and graded dilutions of the tested compounds were incorporated in a cavity (depth 3 mm, diameter 4 mm) made in the center of the Petri dish (nutrient agar for antibacterial activity and sabouraud dextrose agar medium for antifungal activity). The plates were incubated at 37 °C (for bacteria) and at 30 °C (for fungi) for 24 h in duplicate. Positive control using only inoculation and negative control using only DMSO in the cavity were carried out.

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