Sonogashira Cross-Couplings of Dehydroamino Acid Derivatives and Phenylacetylenes

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Several phenylacetylenes were coupled under Sonogashira cross-coupling conditions with the methyl esters of *N*-(*tert*-butoxycarbonyl)-(*E*)-β-bromo- or -β,β-dibromodehydroalanine, to give β-substituted or β,β-disubstituted dehydroalanines, respectively. The β-substituted dehydroalanines were obtained in good to high yields (60−90%) under the usual Sonogashira conditions (1 equiv. of the phenylacetylene, 1 mol % of $[Pd(PPh_3)_4]$, 2 mol % of CuI, 18 equiv. of NEt_3 in acetonitrile, 24 h at room temp.), with retention of stereochemistry. The β,β-disubstituted dehydroalanines were, in turn, obtained in moderate to good yields (44−63%) under modified Sonogashira conditions (4 equiv. of the phenylacetylene,10 mol % of $[PdCl_2(PPh_3)_2]$, 20 mol % of CuI, 1.4 equiv. of Cs_2CO_3 in acetonitrile, 2 h at reflux). In the latter reactions, some phenylacetylene dimer and the (*E*) isomer of the monosubstituted coupled products were also isolated to some extent. The Sonogashira products obtained from the 4-bromophenylacetylene were allowed to react with functionalized benzo[*b*]thiophenes under C−C or C−N palladium-catalyzed cross-coupling conditions. Preliminary fluorescence studies were performed for mono- and disubstituted (4-aminophenyl)acetylenic dehydroamino acids and for the benzo[*b*]-thiophene derivatives. The results showed that some of the dehydroalanines prepared can be used as fluorescent probes.

Introduction

In recent years, dehydroamino acids have shown to be versatile substrates for the synthesis of novel amino acids.[1,2]

We have been interested in the synthesis of benzo[*b*]thienylamino acids that may have biological activity or may be used as fluorescent probes when inserted into peptides.[3] Using several differently functionalized benzo[*b*]thiophenes, we have been able to synthesize new amino acids and dehydroamino acids either by Suzuki coupling or by sequential Michael addition and C−C (Suzuki) or C−N palladium-catalyzed cross-couplings.[4a−4c]

Some ethynylamino acid derivatives have been isolated from microorganisms or prepared by various methods, and have shown biological activities.[5] Sonogashira cross-coupling reactions have been used in the synthesis of alkynylamino acid derivatives having the triple bond either in the amino acid side chain[6,7] or in its amine function.[8]

Herein, we describe the use of Sonogashira cross-coupling for the synthesis of dehydroamino acids, in which β-bromo- or β,β-dibromodehydroalanine derivatives were coupled with several phenylacetylenes. Some of the coupled products were allowed to react with functionalized benzo[*b*]thiophenes by palladium-catalyzed (C−C or C−N) cross-couplings. The acetylenic dehydroamino acids obtained may have biological activity, and some of them may be used as probes when inserted into peptides, due to their fluorescence properties.

Results and Discussion

The methyl esters of *N*-(*tert*-butoxycarbonyl)-(*E*)-β-bromo-[4a] and -β,β-dibromodehydroalanine[4c] were coupled with several phenylacetylenes under different Sonogashira conditions to give the corresponding coupled products (Scheme 1 and 2).

The coupled products (*E*)-**1a**−**c** were obtained in good to high yields (60−90%) from the methyl ester of *N*-(*tert*-butoxycarbonyl)-(*E*)-β-bromodehydroalanine, under the usual Sonogashira coupling conditions[6,9] (Scheme 1). In this reaction, NEt_3 is usually used as base and solvent (ca. 18 equiv.), but in our case, it was necessary to add a small amount of acetonitrile, due to the low solubility of the starting materials in NEt_3.

The stereochemistry of the products was determined by NOE difference experiments, observing an enhancement of the β-CH signal when α-NH was irradiated.

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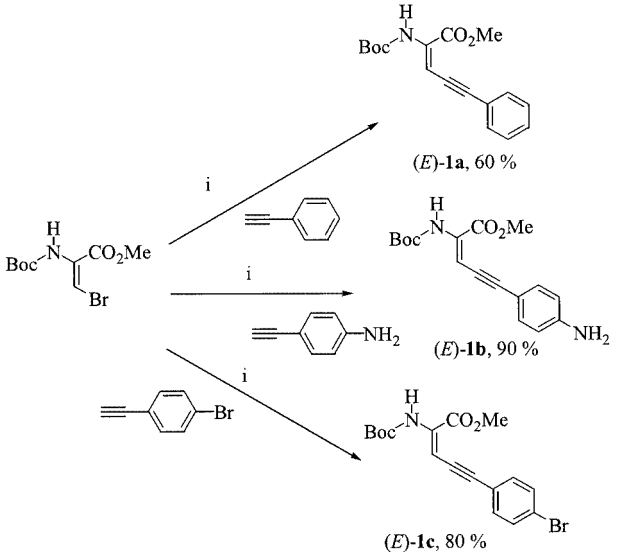
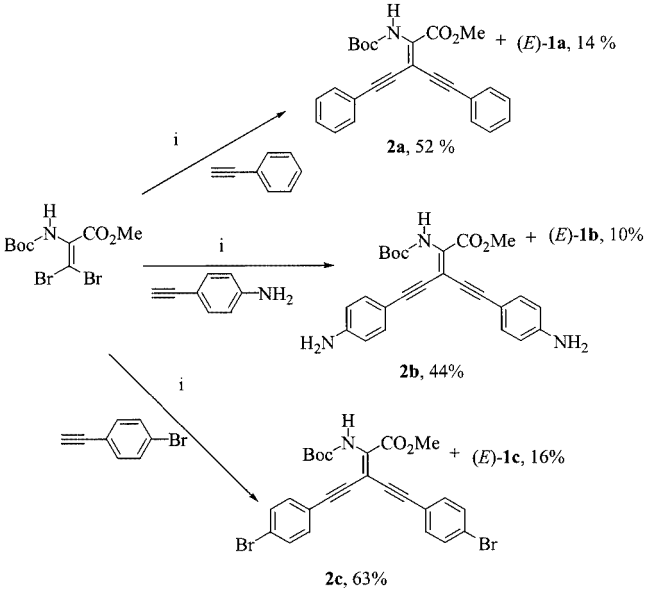


Scheme 1. i) 1 equiv. of the phenylacetylene, 1 mol% of $[Pd(PPh_3)_4]$, 2 mol% of CuI, 18 equiv. of NEt_3 in acetonitrile, 24 h at room temp.



Scheme 2. i) 4 equiv. of the phenylacetylene, 10 mol% of $[PdCl_2(PPh_3)_2]$, 20 mol% of CuI, 1.4 equiv. of Cs_2CO_3, 2 h at reflux of acetonitrile

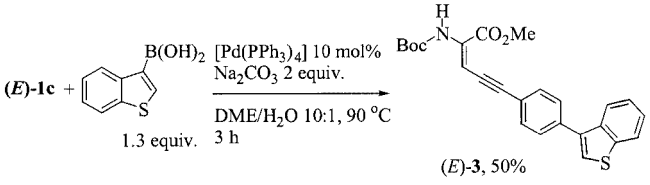
The reaction yields significantly increased when the phenylacetylene was *para*-substituted either with a bromine atom or an amino group.

The same reaction conditions applied to the coupling of the methyl ester of *N*-(*tert*-butoxycarbonyl)-β,β-dibromodehydroalanine with (4-aminophenyl)acetylene (2 equiv.) gave only the monosubstituted derivative (*E*)-**1b** in 35% yield, along with a significant amount of the corresponding phenylacetylene dimer. The isolation of the (*E*) isomer could be due to the fact that the first oxidative addition occurs at the less hindered side of the carbon−halogen bonds, as has been observed by other authors for the case of 1,1-dibromo-1-alkenes.[10]

The best conditions to obtain disubstituted coupled products from the β,β-dibromodehydroalanine derivative, found after several experiments, are shown in Scheme 2. Compounds **2a**−**c** were isolated in moderate to good yields, together with a small amount of the corresponding monosubstituted (*E*) isomer and the phenylacetylene dimers. The change of NEt_3 to Cs_2CO_3 was crucial for the formation of the disubstituted coupled products. In fact, even when the amount of NEt_3 was lowered to 9 equiv. in the coupling of phenylacetylene with the dibromo compound, only the monosubstituted compound (*E*)-**1a** and the phenylacetylene dimer were isolated. The use of 1.4 equiv. of Cs_2CO_3 gave the best yields of the disubstituted compounds. In the coupling of (4-aminophenyl)acetylene using 2.8 equiv. of Cs_2CO_3, the disubstituted product **2b** was only obtained in 10% yield, together with 20% of the monosubstituted (*E*)-**1b** and an increased amount of the corresponding dimer. Higher yields of the disubstituted products were obtained when the reactions were carried out in refluxing acetonitrile instead of room temperature, and when $[PdCl_2(PPh_3)_2]$ was used. Compound **2a** was obtained in only 25% yield, together with (*E*)-**1a** in 27% yield and the corresponding acetylene dimer, using the best conditions but stirring at room temperature for 18 h.

The differences in the reaction yields for compounds **2a**−**c** (Scheme 2) are due to the different amounts of the corresponding phenylacetylene dimers obtained in the reactions (see Exp. Sect.).

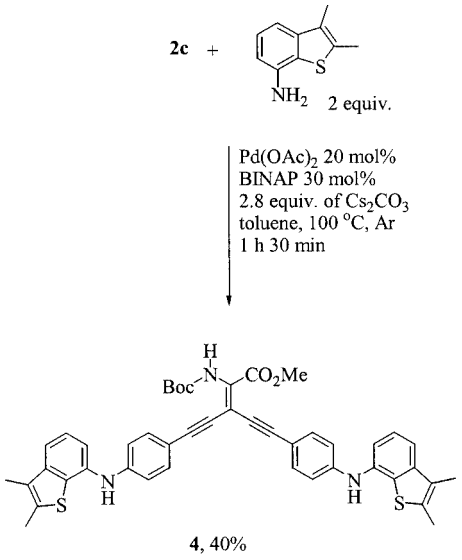
Compounds (*E*)-**1c** and **2c** were allowed to undergo palladium-catalyzed C−C and C−N cross-couplings with functionalized benzo[*b*]thiophenes. Benzo[*b*]thiophene-3-boronic acid reacted under Suzuki cross-coupling conditions[4b] with compound (*E*)-**1c** to give compound **3** in 50% yield, along with 3,3′-bi(benzo[*b*]thienyl) (18%) (Scheme 3). Compound **2c** reacted with 7-amino-2,3-dimethylbenzo[*b*]thiophene[4b] under C−N palladium-catalyzed cross-coupling conditions[4b] to give compound **4** in 40% yield, together with the starting aminobenzo[*b*]thiophene (40%), due to some decomposition of the starting dehydroamino acid (Scheme 4).



Scheme 3

The UV/Vis absorption and fluorescence properties of compounds (*E*)-**1b**, **2b**, (*E*)-**3** and **4** were studied with the aim of using these compounds as fluorescent probes.

The absorption spectral features of compounds (*E*)-**1b** and **2b** in dichloromethane are very different (Figure 1). The red-shifted absorption of (*E*)-**1b** suggests a large delocalization of the (aminophenyl)acetylenic electron distribution over the entire molecule, lowering the Franck−Condon energy gap. The linkage of a second (4-aminophenyl)acetylene strongly perturbs the spectral profile of compound **2b**, shifting its lowest energy absorption band to shorter wave-



Scheme 4

lengths, and splitting it into two components. These findings suggest the occurrence of a strong electronic coupling between the two phenylacetylenic groups in compound **2b**. The exciton-like character of this interaction is shown by the splitting of the lowest energy absorption band, while the shift to shorter wavelengths indicates the predominant stabilization of the chromophore ground state with respect to the electronic excited state.

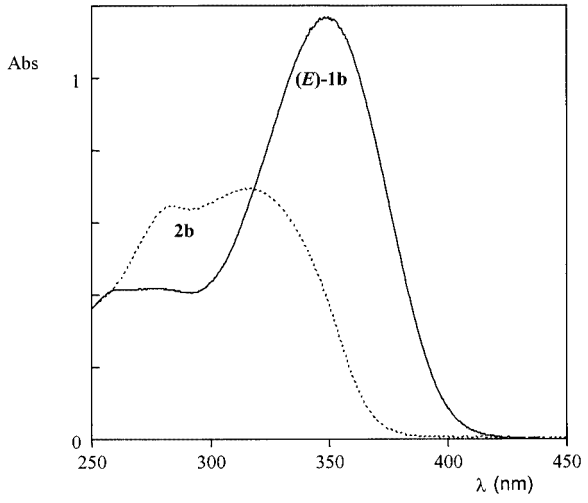


Figure 1. Absorption spectra of compounds (*E*)-**1b** and **2b** in dichloromethane

This interaction is also responsible for the different fluorescence properties of compounds (*E*)-**1b** and **2b** (Figure 2). The emission of (*E*)-**1b** is hardly detectable, implying that its lowest energy excited state decays through very efficient non-radiative relaxation processes. This is probably due to the electronic coupling of the phenylacetylene moiety with non-radiative electronic states of the dehydroamino acid (internal conversion).

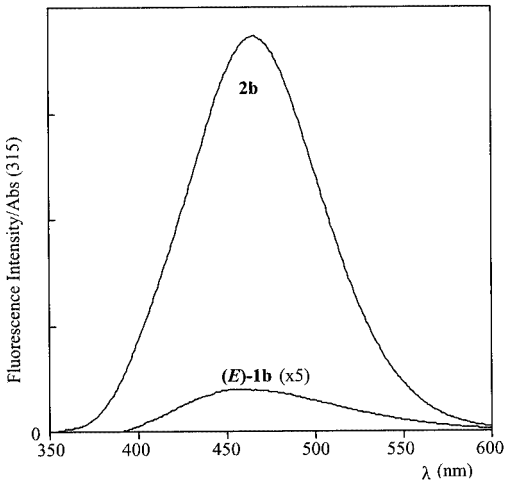


Figure 2. Fluorescence spectra of compounds (*E*)-**1b** and **2b** in dichloromethane

The high fluorescence quantum yield of compound **2b** suggests that the linkage of a second (4-aminophenyl)acetylene weakens the electronic coupling with the dehydroamino acid moiety, decreasing the efficiency of non-radiative processes. The polar character of the new emissive state is proved by the strong solvent dependence of compound **2b** emission (Figure 3). Lower quantum yields and longer lifetimes in polar solvents are typical of radiative charge-transfer states;[11] the fluorescence properties of compound **2b** in acetonitrile show these features (Table 1). The time-resolved fluorescence decay of the latter compound is described by a function containing two exponential components in both solvents studied, implying that at least two different electronic states contribute to its emission.

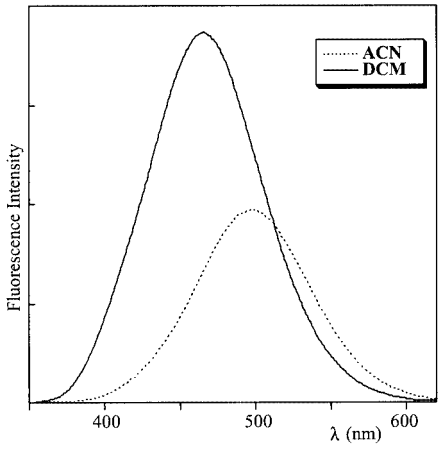


Figure 3. Fluorescence spectra of compound **2b** in dichloromethane (DCM) and acetonitrile (MeCN)

The absorption spectra of compound (*E*)-**3** is dominated at low energies by the π,π* transitions of the phenylacetylene moiety, as shown in Figure 4. The absorption profile of compound **4** shows a broader band, with respect to the spectrum

Table 1. The fluorescence lifetimes (τ_i), pre-exponential factors (α_i) and quantum yields (ϕ) for compound **2b**

Solvent	λ_{em} (max.) [nm]	Φ[a]	τ_1 [ns]	α_1	τ_2 [ns]	α_2	$<\tau>$ [ns][b]
Dichloromethane	467	0.03	0.27	0.98	1.94	0.02	0.31
Acetonitrile	498	0.01	0.74	0.98	2.66	0.02	0.78

[a] With respect to anthracene in ethanol (Φ = 0.27±0.01). [b] $<\tau> = \alpha_1\tau_1 + \alpha_2\tau_2$.

of the compound (*E*)-**3**, suggesting the occurrence of a second spectral component, associated with the aminobenzothiophene group at shorter wavelengths.

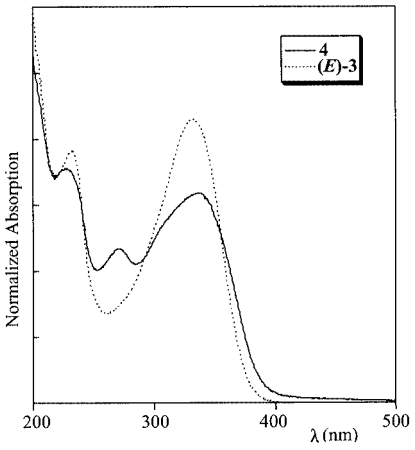


Figure 4. Absorption spectra of compounds (*E*)-**3** and **4** in acetonitrile (MeCN)

The fluorescence time decay of compound (*E*)-**3** is described by a single exponential function (Table 2) and its emission quantum yield is notably higher than that one of compound **4**, as shown in Figure 5. These findings suggest that the excited state electronic distribution of (*E*)-**3** is localized on the phenylacetylene moiety.

Table 2. Fluorescence data of compounds (*E*)-**3** and **4** in acetonitrile

Compound	λ_{em} (max) [nm]	Φ[a]	τ_1 [ns]	α_1	τ_2 [ns]	α_2
(*E*)-**3**	471	0.14	1.2	1.0	–	–
4	471	0.006	0.8	0.96	2.5	0.04

[a] With respect to anthracene in ethanol (Φ = 0.27±0.01).

The emission quantum yield of compound **4** is very low, and its time decay is described by two time components (Table 2). Due to extensive overlap between the wavelengths of aminobenzothiophene fluorescence emission and the phenylacetylene absorption, a complete intramolecular energy transfer process occurs, leading to emission only from the phenylacetylene moiety. The presence in compound **4** of an amino group (a strong electron donor) bridging the electron distributions of the benzothiophene and the phenylacetylene groups favors the formation of an intramolecular charge transfer (ICT) state. ICT states are characterized by very low quantum yield and relatively slow decay times,[11] as revealed

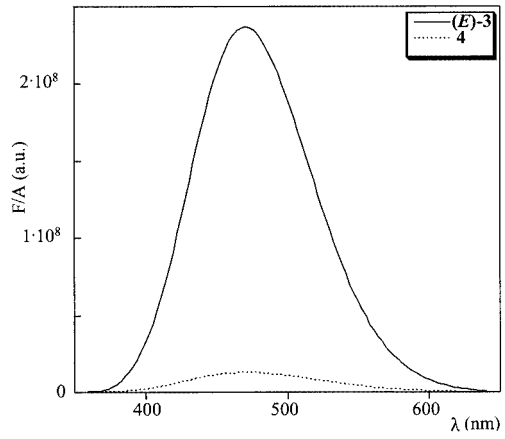


Figure 5. Fluorescence spectra of compounds (*E*)-**3** and **4** in MeCN (λ_{exc} 334 nm)

by the second time component (Table 2) found in the time-resolved fluorescence measurement. This interaction has been found in other aminobenzothiophene-based compounds, and is currently under investigation in our laboratory.

These preliminary results suggest that compounds **2b** and (*E*)-**3** can be used as fluorescent probes when inserted into peptides. A proper selection of the chromophores and the fine-tuning of the interactions that involve the aromatic moieties could be usefully exploited to modulate the fluorescence properties of the newly designed compounds.

Conclusions

Several new acetylenic α,β-dehydroamino acids were prepared using Sonogashira cross-coupling reactions. Different conditions were established to obtain either eneyne or "enediyne" amino acids. The dehydroamino acids having a bromine atom in their side chains, (*E*)-**1c** and **2c**, were coupled with benzo[*b*]thiophenes by C−C (Suzuki) or C−N palladium-catalyzed cross-couplings to give the benzo[*b*]thienyl amino acids (*E*)-**3** and **4**.

The photophysical properties of four of the compounds obtained were studied. The results suggest that two of them can be used as fluorescent markers.

Experimental Section

General Remarks: Melting points were determined with a Gallenkamp apparatus and are uncorrected. The ^{1}H NMR spectra were measured with a Varian Unity Plus at 300 MHz. Spin-spin decoupling techniques were used to assign the signals. NOE experiments were performed to determine the stereochemistry of the products. The ^{13}C NMR spectra were measured with the same instrument at 75.4 MHz (using DEPT, θ = 45°). Elemental analyses were determined with a LECO CHNS 932 elemental analyser. Mass spectra (EI and FAB) and HRMS data were recorded by the mass spectrometry service of University of Vigo-Spain. Steady-state fluorescence spectra were recorded with a Fluoromax spectrofluorimeter (Jobin−Yvon, France), operating in SPC (Single Photon Counting) mode. Quantum yields were obtained by using anthracene in ethanol as reference: $\Phi_r = 0.27 \pm 0.01$. The quantum yields of the samples are given by $\Phi_s = [(A_r F_s n_s^2)/(A_s F_r n_r^2)]\Phi_r$, where A is the absorbance at the excitation wavelength, F the integrated emission area and n the refraction index of the solvent used. Subscripts refer to the reference (r) or sample (s) compound. Nanosecond time decays were measured by a CD-900 (Edinburgh Instruments, Edinburgh, U.K.) lifetime apparatus with SPC detection. Excitation in the UV region was achieved by a flashlamp filled with ultrapure hydrogen (300 Torr), working at a repetition rate of 30 kHz. Under these conditions, the full width at half maximum (FWHM) of the excitation profile was 1.2 ns. Experimental decay curves were fitted by a non-linear least-squares analysis to exponential functions or time distributions through an iterative deconvolution method, using standard software licensed by Edinburgh Instruments. All fluorescence experiments were carried out in quartz cells, using solutions previously bubbled with ultrapure nitrogen for 20 min. All solutions for fluorescence measurements were freshly prepared at micromolar concentrations [absorbances < 0.1 (l = 1 cm) to avoid inner filter effects]. Excitation wavelengths used were as follows. (*E*)-**1b**: λ_{exc} = 360 nm; **2b**: λ_{exc} = 321 nm; (*E*)-**3**: λ_{exc} = 334 nm; **4**: λ_{exc} = 334 nm. Column chromatography was performed on Macherey−Nagel silica gel 230−400 mesh. Petroleum ether refers to the boiling range 40−60 °C. Ether refers to diethyl ether. When a solvent gradient was used, the polarity was gradually increased from neat petroleum ether to mixtures of ether/petroleum ether by 10% increments of ether until the product could be isolated.

General Procedure for Sonogashira Cross-Coupling Using the Methyl Ester of *N*-(*tert*-Butoxycarbonyl)-(*E*)-β-bromodehydroalanine and Phenylacetylenes: CuI (2 mol %, 4.00 mg) and [Pd(PPh$_3$)$_4$] (1 mol %, 11.0 mg) were added to a solution of Boc-(*E*)-ΔAla(β-Br)−OMe (1 mmol, 280 mg) in triethylamine (18 equiv.), with rapid stirring under argon at room temperature, and then a solution of the alkyne (1 equiv.) in acetonitrile (0.5 mL) was added. The reaction mixture was left stirring for 24 h. The acetonitrile was removed under reduced pressure and the residue was dissolved in 30 mL of ethyl acetate. The organic phase was then washed with water and brine (2 × 15 mL each), dried with MgSO$_4$, and the solvents were evaporated at reduced pressure giving an oil which was submitted to column chromatography.

Boc-(*E*)-ΔAla{β-[2-(phenyl)ethynyl]}−OMe [(*E*)-1a]: Column chromatography using a solvent gradient from neat petroleum ether to 50% ether/petroleum ether gave product (*E*)-**1a** (178 mg, 60%) as an oil. Recrystallization from ether/petroleum ether gave yellow crystals, m.p. 108.8−109.5 °C. ^{1}H NMR (CDCl$_3$): δ = 1.49 (s, 9 H, CH$_3$ Boc), 3.84 (s, 3 H, OCH$_3$), 6.28 (s, 1 H, βCH), 6.60 (broad s, 1 H, NH), 7.33−7.35 (m, 3 H, ArH), 7.46−7.49 (m, 2 H, ArH) ppm. ^{13}C NMR (CDCl$_3$): δ = 28.02 [C(*C*H$_3$)$_3$], 52.63 (OCH$_3$), 81.40 [O*C*(CH$_3$)$_3$], 83.93 (C), 102.49 (C), 105.85 (CH), 122.41 (C), 128.33 (CH), 129.01 (CH), 131.65 (CH), 134.86 (C), 151.71 (C=O), 164.36 (C=O) ppm. $C_{17}H_{19}NO_4$ (301.34): calcd. C 67.76, H 6.35, N 4.65; found C 67.50, H 6.34, N 4.75.

Boc-(*E*)-ΔAla{β-[2-(4-aminophenyl)ethynyl]}−OMe [(*E*)-1b]: Column chromatography using ether as solvent gave product (*E*)-**1b** (284 mg, 90%) as a yellow solid. Recrystallization from ether gave yellow crystals, m.p. 107.8−108.8 °C. ^{1}H NMR (CDCl$_3$): δ = 1.50 (s, 9 H, CH$_3$ Boc), 3.83 (s, 3 H, OCH$_3$), 3.91 (br. s, 2 H, NH$_2$), 6.30 (s, 1 H, βCH), 6.45 (br. s, 1 H, NH), 6.62 (d, J = 8.4 Hz, 2 H, ArH *ortho* to NH$_2$), 7.29 (d, J = 8.4 Hz, 2 H, ArH *meta* to NH$_2$) ppm. ^{13}C NMR (CDCl$_3$): δ = 28.04 [C(*C*H$_3$)$_3$], 52.49 (OCH$_3$), 81.18 [O*C*(CH$_3$)$_3$], 82.50 (C), 104.35 (C), 107.49 (CH), 111.32 (C), 114.53 (CH), 133.27 (CH), 133.35 (C), 147.58 (C), 151.97 (C=O), 164.56 (C=O) ppm. $C_{17}H_{20}N_2O_4$ (316.36): calcd. C 64.54, H 6.37, N 8.86; found C 64.27, H 6.09, N 8.84.

Boc-(*E*)-ΔAla{β-[2-(4-bromophenyl)ethynyl]}−OMe [(*E*)-1c]: Column chromatography using a solvent gradient from neat petroleum ether to 40% ether/petroleum ether gave product (*E*)-**1c** (304 mg, 80%) as a solid. Recrystallization from ether/petroleum ether gave colourless crystals, m.p. 123.1−124.1 °C. ^{1}H NMR (CDCl$_3$): δ = 1.49 (s, 9 H, CH$_3$ Boc), 3.84 (s, 3 H, OCH$_3$), 6.27 (s, 1 H, βCH), 6.59 (br. s, 1 H, NH), 7.32 (d, J = 8.4 Hz, 2 H, ArH *meta* to Br), 7.47 (d, J = 8.4 Hz, 2 H, ArH *ortho* to Br) ppm. ^{13}C NMR (CDCl$_3$): δ = 28.04 [C(*C*H$_3$)$_3$], 52.75 (OCH$_3$), 81.46 [O*C*(CH$_3$)$_3$], 85.22 (C), 101.34 (C), 105.41 (CH), 121.45 (C), 123.37 (C), 131.65 (CH), 133.03 (CH), 134.88 (C), 151.48 (C=O), 164.29 (C=O) ppm. $C_{17}H_{18}BrNO_4$ (380.24): calcd. C 53.70, H 4.77, N 3.68; found C 53.85, H 4.89, N 3.72.

General Procedure for Sonogashira Cross-Coupling Using the Methyl Ester of *N*-(*tert*-Butoxycarbonyl)-β,β-dibromodehydroalanine and Phenylacetylenes: CuI (20 mol %, 20.0 mg), PdCl$_2$(PPh$_3$)$_2$ (10 mol %, 36.0 mg) and Cs$_2$CO$_3$ (1.4 equiv., 228 mg) were added to a solution of compound Boc-ΔAla(β,β-Br)−OMe (0.5 mmol, 180 mg) in acetonitrile (5 mL), and then the alkyne (4 equiv.) was added. The reaction mixture was stirred rapidly at 85 °C under argon for 1 h 30 min. The acetonitrile was removed under reduced pressure and the residue was dissolved in 15 mL of ethyl acetate. The organic phase was then washed with water and brine (2 × 15 mL each), dried with MgSO$_4$ and the solvents were evaporated at reduced pressure giving an oil which was submitted to column chromatography on silica.

Boc-ΔAla{β,β-bis[2-(phenyl)ethynyl]}−OMe (2a): Column chromatography using a solvent gradient from neat petroleum ether to 80% ether/petroleum ether, gave, as a less polar product, the dimer of the phenylacetylene (22.0 mg) as a white solid, m.p. 85−87 °C. ^{1}H NMR (CDCl$_3$): δ = 7.35−7.38 (m, 6 H, ArH), 7.53−7.56 (m, 4 H, ArH) ppm, followed by compound **2a** (104 mg, 52%) as an oil. Recrystallization from ether/petroleum ether gave colourless crystals, m.p. 113.7−114.0 °C. ^{1}H NMR (CDCl$_3$): δ = 1.50 (s, 9 H, CH$_3$ Boc), 3.95 (s, 3 H, OCH$_3$), 6.96 (br. s, 1 H, NH), 7.32−7.34 (m, 3 H, ArH), 7.38−7.40 (m, 3 H, ArH), 7.46−7.49 (m, 2 H, ArH), 7.53−7.56 (m, 2 H, ArH) ppm. ^{13}C NMR (CDCl$_3$): δ = 28.00 [C(*C*H$_3$)$_3$], 52.68 (OCH$_3$), 81.97 [O*C*(CH$_3$)$_3$], 82.69 (C), 83.19 (C), 92.64 (C), 98.35 (C), 121.84 (C), 122.61 (C), 128.26 (CH), 128.45 (CH), 128.56 (CH), 129.23 (CH), 131.53 (CH), 131.72 (CH), 140.53 (C), 150.74 (C=O), 163.33 (C=O) ppm. $C_{25}H_{23}NO_4$ (401.46): calcd. C 74.80, H 5.77, N 3.49; found C 74.69, H 5.87, N 3.53. Compound (*E*)-**1a** (22.0 mg, 14%) was isolated as a more polar product.

Boc-ΔAla{β,β-bis[2-(4-aminophenyl)ethynyl]}−OMe (2b): Column chromatography using a solvent gradient from neat petroleum ether to 80% ether/petroleum ether, gave, as a less polar product, compound (*E*)-**1b** (16 mg, 10%), followed by the dimer of (4-aminopheny-

l)acetylene as a solid (50.0 mg), m.p. 220−222 °C. ^{1}H NMR ($CDCl_3$): δ = 3.88 (br. s, 4 H, 2 × NH_2), 6.60 (d, *J* = 9 Hz, 4 H, 2 × ArH *ortho* to NH_2) 7.32 (d, *J* = 9 Hz, 4 H, 2 × ArH *meta* to NH_2) ppm. $C_{16}H_{12}N_2$ (232.28): calcd. C 82.73, H 5.21, N 12.06; found C 82.47, H 5.49, N 12.05. As a more polar product, compound **2b** was isolated as an oil (93.0 mg, 44%). Recrystallization from diethyl ether/*n*-hexane gave a yellow solid, m.p. 129.6−131.8 °C. ^{1}H NMR ($CDCl_3$): δ = 1.43 (s, 9 H, CH_3 Boc), 3.84 (br. s, 4 H, NH_2), 3.93 (s, 3 H, OCH_3), 6.26 (s, 1 H, NH), 6.63 (d, *J* = 8.7 Hz, 2 H, ArH *ortho* to NH_2), 6.68 (d, *J* = 8.7 Hz, 2 H, ArH *ortho* to NH_2), 7.23 (d, *J* = 8.7 Hz, 2 H, ArH *meta* to NH_2), 7.34 (d, *J* = 8.7 Hz, 2 H, ArH *meta* to NH_2) ppm. ^{13}C NMR ($CDCl_3$): δ = 27.19 [C(CH_3)$_3$], 51.72 (OCH_3), 80.88 [O*C*(CH_3)$_3$], 85.37 (C), 94.23 (C), 112.70 (C), 113.84 (C), 114.35 (CH), 114.65 (CH), 120.81 (C), 124.34 (C), 130.15 (CH), 132.93 (CH), 139.05 (C), 146.66 (C), 146.91 (C), 149.26 (C), 160.51 (C=O), 170.71 (C=O) ppm. MS: *m/z* (%) = 431 (20) [M^+], 331 (100) [M^+ − Boc], 299 (49), 270 (42). HRMS: calcd. for $C_{25}H_{25}N_3O_4$ [M^+] 431.1844; found 431.1845.

Boc-ΔAla{β,β-bis[2-(4-bromophenyl)ethynyl]}−OMe (2c): Column chromatography using a solvent gradient from neat petroleum ether to 20% ether/petroleum ether, gave, as a less polar product, the dimer of (4-bromophenyl)acetylene as a solid (10 mg), m.p. 257−258 °C. ^{1}H NMR ($CDCl_3$): δ = 7.39 (d, *J* = 8.7 Hz, 4 H, 2 × ArH *meta* to Br) 7.49 (d, *J* = 8.7 Hz, 4 H, 2 × ArH *ortho* to Br) ppm; $C_{16}H_8Br_2$ (360.05): calcd. C 53.37, H 2.24; found C 53.28, H 2.27, followed by compound **2c**, which was isolated as a white solid (177 mg, 63%). Recrystallization from ether/petroleum ether gave white crystals, m.p. 175.6−176.1 °C. ^{1}H NMR ($CDCl_3$): δ = 1.50 (s, 9 H, CH_3 Boc), 3.93 (s, 3 H, OCH_3), 6.93 (br. s, 1 H, NH), 7.32 (d, *J* = 8.4 Hz, 2 H, ArH *meta* to Br), 7.39 (d, *J* = 8.4 Hz, 2 H, ArH *meta* to Br), 7.46 (d, *J* = 8.4 Hz, 2 H, ArH *ortho* to Br), 7.52 (d, *J* = 8.4 Hz, 2 H, ArH *ortho* to Br) ppm. ^{13}C NMR ($CDCl_3$): δ = 28.01 [C(CH_3)$_3$], 52.79 (OCH_3), 82.83 (C), 82.98 (C), 84.12 (C), 91.66 (C), 97.35 (C), 120.78 (C), 121.50 (C), 122.95 (C), 123.74 (C), 131.60 (CH), 131.80 (CH), 132.94 (CH), 133.11 (CH), 141.09 (C), 150.60 (C), 163.17 (C=O), 170.76 (C=O) ppm. $C_{25}H_{21}Br_2NO_4$ (559.25): calcd. C 53.69, H 3.78, N 2.50; found C 53.68, H 4.01, N 2.60. As a more polar product, compound (*E*)-**1c** (31 mg, 16%) was isolated.

Suzuki Coupling

Synthesis of Boc-(*E*)-ΔAla(β-{2-[4-(benzo[*b*]thien-3-yl)phenyl]-ethynyl})−OMe [(*E*)-3]: Compound (*E*)-**1c** (0.300 mmol, 114 mg) was coupled with benzo[*b*]thiophene-3-boronic acid (0.330 mmol, 59.0 mg), using $Pd(PPh_3)_4$ (0.0300 mmol, 35.0 mg) and Na_2CO_3 (0.600 mmol, 63.6 mg) in DME/H_2O (10:1) at 90 °C for 3 h. After cooling, water and ethyl acetate were added and the phases were separated. The organic phase was washed with brine, dried with $MgSO_4$, filtered, and the solvents were evaporated at reduce pressure to give an oil. Column chromatography on silica using a solvent gradient from neat petroleum ether to 30% diethyl ether/petroleum ether, gave product (*E*)-**3** (65.0 mg, 50%) as a white solid. Recrystallization from diethyl ether/petroleum ether gave colourless crystals, m.p. 142.8−144.0 °C. ^{1}H NMR ($CDCl_3$): δ = 1.53 (s, 9 H, CH_3 Boc), 3.87 (s, 3 H, OCH_3), 6.32 (s, 1 H, βCH), 6.58 (s, 1 H, NH), 7.40−7.44 (m, 2 H, ArH), 7.46 (s, 1 H, ArH), 7.60 (s, 4 H, ArH), 7.90−7.95 (m, 2 H, ArH) ppm. ^{13}C NMR ($CDCl_3$): δ = 28.10 [C(CH_3)$_3$], 52.72 (OCH_3), 81.47 [O*C*(CH_3)$_3$], 84.78 (C), 102.45 (C), 105.77 (CH), 121.58 (C), 122.66 (CH), 122.98 (CH), 124.12 (CH), 124.50 (CH), 124.56 (CH), 128.59 (CH), 132.10 (CH), 134.85 (C), 136.70 (C), 137.12 (C), 137.40 (C), 140.69 (C), 151.66 (C=O), 164.39 (C=O) ppm. MS: *m/z* (%) = 433 (18) [M^+], 333 (100) [M^+ − Boc], 273 (30), 245 (23). HRMS: calcd. for $C_{25}H_{23}NO_4S$ [M^+] 433.1328; found 433.1348.

C−N Cross-Coupling

Synthesis of Boc-ΔAla(β,β-bis{2-[4-amino(2,3-dimethylbenzo[*b*]-thien-7-yl)phenyl]ethynyl})−OMe (4): A dried Schlenk tube was charged under Ar with dry toluene (1.5 mL) and compound **2c** (0.150 mmol, 84.0 mg), and the mixture was heated at 80 °C for 10 min. $Pd(OAc)_2$ (0.0300 mmol, 6.73 mg), BINAP (0.0450 mmol, 28.0 mg) and Cs_2CO_3 (0.420 mmol, 137 mg) were added and the mixture was heated at 80 °C for another 10 min. 7-Amino-2,3-dimethylbenzo[*b*]thiophene (0.300 mmol, 53 mg) was added in dry toluene (1.5 mL), and the mixture was heated with stirring at 100 °C under Ar for ca. 1 h 30 min. After cooling, water and diethyl ether were added, the phases were separated, and then the aqueous phase was washed with diethyl ether (3 × 10 mL). The organic phase was collected, dried with $MgSO_4$, filtered, and then the solvent was evaporated at reduced pressure giving a brown oil, which was subjected to column chromatography after traces of toluene were evaporated using MeOH. A solvent gradient from neat petroleum ether to 50% diethyl ether/petroleum ether was used, giving product **4** (44 mg, 40%) as a brown oil. ^{1}H NMR ($CDCl_3$): δ = 1.47 (s, 9 H, CH_3 Boc), 2.33 (s, 6 H, 2 × CH_3), 2.50 (s, 6 H, 2 × CH_3), 3.95 (s, 3 H, OCH_3), 5.73 (br. s, 2 H, 2 × NH), 6.33 (s, 1 H, NH), 6.97 (d, *J* = 8.7 Hz, 2 H, ArH), 7.02 (d, *J* = 8.7 Hz, 2 H, ArH), 7.23 (br. dd, *J* = 6.3, 2.4 Hz, 2 H, ArH), 7.34 (m, 6 H, ArH), 7.43 (d, *J* = 8.7 Hz, 2 H, ArH) ppm. ^{13}C NMR ($CDCl_3$): δ = 11.65 (CH_3), 13.84 (CH_3), 27.28 [C(CH_3)$_3$], 51.79 (OCH_3), 81.59 [O*C*(CH_3)$_3$], 85.55 (C), 94.02 (C), 113.53 (CH), 113.67 (C), 114.13 (CH), 114.48 (CH), 114.78 (C), 116.25 (CH), 116.29 (CH), 116.40 (CH), 116.45 (CH), 122.81 (C), 124.76 (C), 124.95 (CH), 128.12 (C), 128.15 (C), 130.04 (CH), 130.53 (C), 130.74 (C), 132.82 (CH), 133.26 (C), 133.34 (C), 135.83 (C), 136.08 (C), 138.71 (C), 142.78 (C), 143.54 (C), 143.81 (C), 149.20 (C=O), 160.50 (C=O) ppm. MS (FAB): *m/z* (%) = 752 (26) [M^+ + H], 751 (26) [M^+] 652 (36) [M^+ + H − Boc] 651 (53) [M^+ − Boc]. HRMS: calcd. for $C_{45}H_{42}N_3O_4S_2$ [M^+ + H]$^+$ 752.2617; found 752.2635.

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