



Synthesis and photophysical characterization of new fluorescent bis-amino acids bearing a heterocyclic bridge containing benzoxazole and thiophene

Susana P.G. Costa*, Rosa M.F. Batista, M. Manuela M. Raposo

Centre of Chemistry, University of Minho, Campus of Gualtar, 4710-057 Braga, Portugal

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ABSTRACT

A series of new bis-alanine derivatives bearing (oligo)thiophene and benzoxazole units as the hetero-aromatic bridge were synthesized in moderate to good yields. The photophysical characterization of these bis-amino acids was performed by UV–vis absorption and fluorescence emission studies and revealed that the compounds displayed exceptionally high fluorescence quantum yields, making them good candidates for application as fluorescent probes when incorporated into peptides, as well as peptide conformation-restricting and cross-linking elements.

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1. Introduction

Bis-amino acids have been the subject of significant research in recent years because they can be used in the synthesis of analogues of biologically active peptides¹ and also in biomimetic chemistry as cross-linking elements for the control of peptide secondary structure, leading to stabilized α -helical, β -turn, and γ -turn conformations.² Bis-amino acids can be regarded as a replacement for cysteine residues in the cross-linking between peptide chains, and other residues like lysine, tyrosine and aspartic, and glutamic acids, which can also undergo cross-linking in the presence of appropriate reagents. Cross-linking is a key feature in (bio)polymer and biomaterials sciences and food chemistry.³

The insertion of noncoded amino acids into the backbone of both natural and synthetic polymers is a very appealing area of research since it can lead to the development of macromolecules possessing biomimetic characteristics, with unique structural and biological properties. By synthetic manipulation at the side chain of coded amino acids, new functions and functional relationships can be generated as well as altered physicochemical properties, such as luminescence, conducting ability, and metal ion and other analyte recognition ability, among other properties.⁴

The molecular design of functional peptides can thus be achieved by judicious choice of noncoded amino acids of natural and

synthetic origin, which are effective building blocks for peptide design considering the following two points: (i) use of rigid unnatural amino acids to control the conformation and function of the resulting peptides and (ii) incorporation of functional unnatural amino acids into peptides resulting in the appearance of the inherent functions.⁵

Benzoxazoles display excellent optical properties (broad spectral windows, high molar absorptivity coefficients and fluorescence quantum yields) and they have been described as fluorescent probes and sensing materials, namely as fluorescent and/or colorimetric sensors for metals, anionic species and in pharmaceutical analysis.⁶ Thiophene and its derivatives also possess important electroluminescent properties with wide application in polymer science, which has prompted their application as energy transfer and light-harvesting systems, for optical and electronic devices,⁷ as sensors and as fluorescent markers.⁸

The work on bis-amino acids reported so far deals with the synthesis of aliphatic- and aromatic-bridged bis-amino acids and, to the best of our knowledge, this is the first report on the synthesis of heteroaromatic-bridged bis-amino acids, apart from those concerning histidinoalanine.⁹ Therefore, there is a practical interest on the synthesis of new and more complex systems and in order to contribute to and expand the body of work published in this area in the last years, we decided to design new bis-amino acid-based systems consisting on functionalized alanines containing thiophene and benzoxazole as the heterocyclic bridge, as a result of the previously mentioned applications for these heterocycles. These bis-alanine derivatives represent both a conformation-restricting

* Corresponding author. Tel.: +351 253 604054; fax: +351 253 604382.

E-mail address: spc@quimica.uminho.pt (S.P.G. Costa).

and a functional amino acid, in view of their fluorescence. The resulting bis-(oligo)thienylbenzoxazole-alanine derivatives, because of the presence of amino and carboxyl groups, could be incorporated into peptide chains and as such used as an energy donor in conformational studies of peptides by means of fluorescence or be used as fluorescence markers. Following our previous research on the synthesis and characterization of unnatural amino acids,¹⁰ benz-X-azole derivatives with interesting optical properties¹¹ and heterocyclic colorimetric/fluorimetric chemosensors containing (oligo)thiophene, benzoxazole, and amino acid moieties,¹² we now report the synthesis of a new highly fluorescent family of alanine derived bis-amino acids connected through an (oligo)thienylbenzoxazole bridge, also having in mind the future studies of these compounds as fluorimetric peptide-based chemosensors. Amino acids and peptides are known to bind a variety of metal ions as they contain a large number of potential donor atoms through the peptide backbone and amino acid side chains and the insertion of an extended heterocyclic system between the two ends of the bis-amino acid will provide an UV-active and highly fluorescent chromophore, as well as additional binding sites through the donor atoms in the conjugated π -bridge. With the study of these new bis-(oligo)thienylbenzoxazolyl-alanines we also intended to evaluate the effect of the length of the π -conjugated (oligo)thienyl system at position 2 of the benzoxazole moiety in the photophysical properties of the resulting compounds.

2. Results and discussion

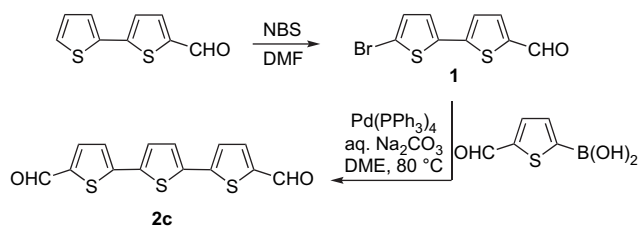
2.1. Synthesis

The new bis-(oligo)thienylbenzoxazolyl-alanines **4a–d** with thiophene, bithiophene, terthiophene, and phenylthiophene units acting as linking π -conjugated bridges between the two benzoxazolyl-alanine systems were synthesized in moderate to good yields, by a multistep synthesis. Different (oligo)thiophenes were used in order to study the influence of the structure modification (i.e., the increase of the π -conjugated bridge) on the overall optical properties of compounds **4a–d** and also in order to compare with our recently reported systems containing only one benzoxazolyl-alanine system.^{10c,12b}

2.1.1. Synthesis of diformyl-terthiophene **2c**

The diformyl-terthiophene **2c** was synthesized through a Suzuki cross-coupling reaction of 5'-bromo-5-formyl-2,2'-bithiophene **1**

with 5-formylthiophene boronic acid in 75% yield. Precursor **1** was obtained from commercial 5-formyl-2,2'-bithiophene by bromination with NBS in DMF in 98% yield (Scheme 1).



Scheme 1. Synthesis of precursor diformyl-terthiophene **2c**.

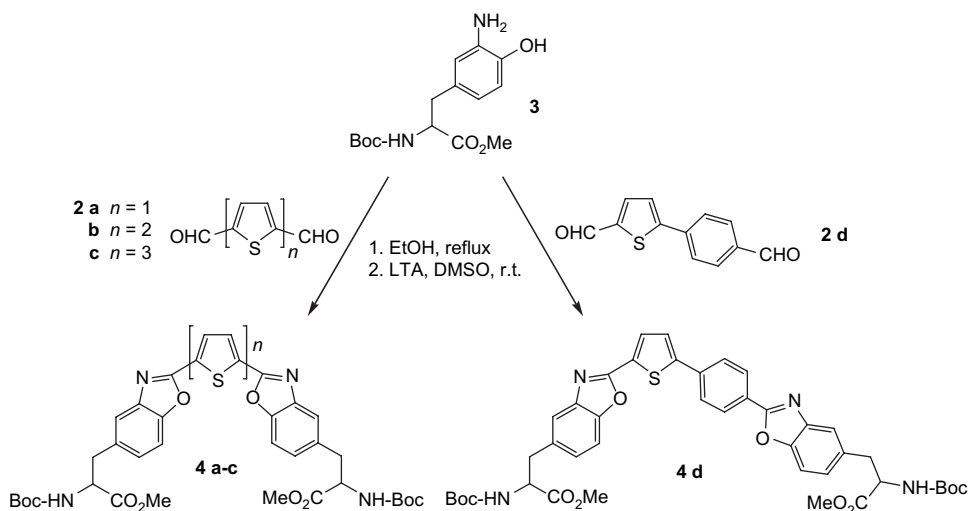
The synthesis of compound **2c** has been reported earlier by Giannetas et al.¹³ through Vilsmeier formylation of terthiophene. This reaction gave a mixture of three products, which were isolated by column chromatography: the starting material, the mono-formyl derivative (65%) and the diformyl-terthiophene **2c** in 20% yield.

In comparison to the method described earlier, compound **2c** was obtained by us in higher yield from low cost commercially available reagents and using simple work-up procedures, allowing the good yielding preparation and ease of isolation of this derivative. Attempt to synthesize compound **2c** using the same method described by Giannetas gave, in our hands, a complex mixture of compounds, which was not possible to separate neither by recrystallization nor by chromatography.

2.1.2. Synthesis of bis-[2-(oligothienyl)benzoxazol-5-yl]-alanine derivatives **4a–d**

Starting from commercially available 3-nitro-L-tyrosine, *N*-Boc-3-amino-L-tyrosine methyl ester **3** was obtained by using simple synthetic procedures.^{12b} Condensation of compound **3** with diformylated (oligo)thiophenes **2a–d**, namely thiophene, bithiophene, terthiophene, and phenylthiophene, resulted in the corresponding imines, which by subsequent oxidation and cyclization with lead tetraacetate in DMSO afforded the bis-[2-(oligothienyl)benzoxazol-5-yl]-alanine derivatives **4a–d** in moderate to good yields (Scheme 2, Table 1). These compounds were fully characterized by the usual spectroscopic techniques.

The IR spectra of bis-amino acids **4a–d** showed the characteristic bands due to stretching vibrations of the carbonyl of the C- and N-terminal protecting groups: the methyl ester from 1744 to 1749 cm^{-1} and the *N*-butyloxycarbonyl from 1693 to 1715 cm^{-1} . A



Scheme 2. Synthesis of N- and C-terminus protected bis-(oligo)thienylbenzoxazolyl-alanine derivatives **4a–d**.

Table 1

Yields, UV-vis absorption and emission data for bis-(oligo)thienylbenzoxazolyl-alanines **4a–d** in absolute ethanol and comparison with data for (oligo)thienylbenzoxazolyl-alanines **5a**^{12b} and **5b**, **c**^{10c}

Cpd.	π -Bridge	Yield (%)	UV-vis		Fluorescence		Cpd.	UV-vis		Fluorescence		Φ_F	
			λ_{\max} (nm)	ϵ (mol ⁻¹ L cm ⁻¹)	λ_{em} (nm)	Stokes' shift (cm ⁻¹)		λ_{\max} (nm)	ϵ (mol ⁻¹ L cm ⁻¹)	λ_{em} (nm)	Stokes' shift (cm ⁻¹)		
4a	Thiophene	75	371	47,210	430	3698	0.89	5a ^{12b}	315	21,300	394	6365	0.80
4b	Bithiophene	54	388	46,641	469	4451	0.59	5b ^{10c}	365	33,884	445	4925	0.46
4c	Terthiophene	60	416	46,454	514	4583	0.14	5c ^{10c}	400	37,153	490	4592	0.10
4d	Arylthiophene	76	363	51,800	440	4821	0.77	—	—	—	—	—	—

band related to the oxazole ring (N=C stretching) appeared at 1633–1668 cm⁻¹.

¹H NMR spectra showed signals that were attributed to the benzoxazole, in the form of doublets at about δ 7.48 and 7.53 ppm for H7 and H4, respectively, and double doublets for H6 around δ 7.15 ppm. The confirmation of the occurrence of the oxidative cyclization was also supported by ¹³C NMR spectra, where signals of the oxazole C2 carbon were visible at about δ 159 ppm.

2.2. Photophysical study

The absorption and emission spectra of bis-(oligo)thienylbenzoxazolyl-alanines **4a–d** were measured in absolute ethanol (10⁻⁶ to 10⁻⁵ M solution) (Table 1). The nature of the thiophenic bridge at position 2 of the benzoxazole had a clear influence on the absorption and emission bands of compounds **4a–d**. Comparing the absorption data for compounds **4b** and **4d**, it can be seen that the replacement of the second thiophene ring by a phenyl ring leads to a small hypsochromic shift, related to the bathochromic effect of sulfur and an increased π -overlap between the thiophene units in compound **4b**. Also, compounds **4a–d** possess very large molar absorptivity coefficients (higher than 46,000 mol⁻¹ L cm⁻¹).

The wavelength of maximum absorption for compounds **4a–c** was shifted to longer wavelengths as the number of thiophene units increased, as expected from the increase in conjugation. The same trend was observed in the emission spectra of these compounds as the position of the wavelength of maximum emission was red-shifted, ca. 40 nm for each added thiophene (Fig. 1). The synthesized compounds showed large Stokes' shift (the lowest being 3698 cm⁻¹ for **4a** and the highest 4821 cm⁻¹ for **4d**). As Stokes' shifts directly relate to energy differences, an increasing trend could be observed along the series **4a–d**. A large Stokes' shift is an interesting characteristic for a fluorescent probe that allows an improved separation of the light inherent to the matrix and the light dispersed by the sample.¹⁴

The relative fluorescence quantum yields were determined using a 10⁻⁶ M solution of 9,10-diphenylanthracene in ethanol as standard ($\Phi_F=0.95$)¹⁵ and bis-(oligo)thienylbenzoxazolyl-alanines **4a–d** exhibited good to excellent fluorescence quantum yields. For

the Φ_F determination, the fluorescence standard was excited at the wavelengths of maximum absorption found for each one of the compounds to be tested and in all fluorimetric measurements the absorbance of the solution did not exceed 0.1. The highest values were obtained for bis-alanines **4a**, **4b** and **4d**, with a thiophene, bithiophene and a phenylthiophene at position 2 of the benzoxazole, respectively, which were found to be strongly emissive (0.59 < Φ_F < 0.89) while compound **4c**, with a terthiophene bridge, displayed much lower quantum yield ($\Phi_F=0.14$).

At this stage, a comparison could be made between the photophysical data of the synthesized bis-alanines **4a–c** and the corresponding (oligo)thienylbenzoxazolyl-alanines **5a–c**, recently reported by us,^{10c,12b} bearing only one benzoxazolyl-alanine system (Fig. 2, Table 1). For all compounds, it was found that the introduction of a second benzoxazolyl-alanine system resulted in a bathochromic shift of both the absorption and the emission wavelength maxima, an increase in the molar absorptivity value and, more importantly, in an enhancement of the fluorescence quantum yield. Also, the new bis-alanines **4a–c** had smaller Stokes' shifts than the mono-alanine series **5a–c**, a fact that can potentially be rationalized by the more polar nature of the mono-alanine compounds when compared to the inherently less polar nature of the bis-alanine compounds.

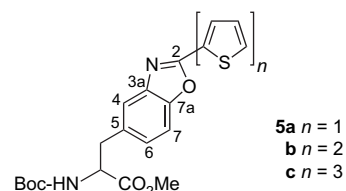


Figure 2. Structure of (oligo)thienylbenzoxazolyl-alanine derivatives **5a–c**.^{12b,10c}

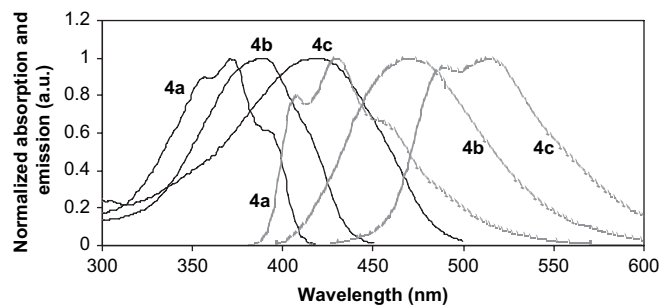


Figure 1. Normalized UV-vis absorption and emission spectra of compounds **4a–c** in absolute ethanol at $T=298$ K (**4a**, $\lambda_{\text{exc}}=371$ nm; **4b**, $\lambda_{\text{exc}}=388$ nm; **4c**, $\lambda_{\text{exc}}=416$ nm) (absorption, full line; emission, grey).

Our results revealed a decrease in the fluorescence quantum yield as the number of thiophene rings at the π -conjugated bridge increased. Nevertheless, the fluorescence quantum yield of non-substituted (oligo)thiophenes is expected to increase as the oligothiophene chain length increases, due to a further extension of the conjugated π -system. On the other hand, the heavy atom induced spin-orbit coupling by the sulfur atoms can give rise to a very efficient intersystem crossing mechanism, thus lowering the emission.¹⁶ Moreover, azomethine nitrogens contribute to the heavy atom effect concomitant with the increased degree of conjugation.^{16c} Also, the different chains (thiophene, bithiophene, and terthiophene) should exhibit different degrees of torsion between the thiophene units, which lead to variations in the effective conjugation length, affecting the planarity of the whole heteroaromatic system.^{16d} In our case, we believe that a combination of the above mentioned effects could be responsible for the trend observed in our results.

Keeping in mind, further applications of these amino acids as emissive probes for energy transfer or FRET (Fluorescence Resonance Energy Transfer) studies when incorporated in peptides

chains, in Figure 1 can also be seen the good superposition of some absorption and emission spectra (for example, compounds **4c** and **4a**), which opens up a very wide range of potential interesting applications to be explored.

3. Conclusions

In summary, we have achieved for the first time the synthesis of new fluorescent heteroaromatic bis-amino acid derivatives **4a–d** containing (oligo)thiophene and benzoxazole moieties combined with an alanine residue by simple procedures in moderate to good yields and their photophysical properties were evaluated. Due to their interesting photophysical properties, namely their strongly emissive character, these heterocyclic alanine derivatives could find application as useful building blocks for the cross-linking of peptide chains with the extra value of inserting a UV-active and fluorescent chromophore, adding functionality to the resulting peptide. These heteroaromatic bis-amino acids could also be used as fluorescent markers and probes for FRET studies in peptides.

4. Experimental

4.1. Synthesis general

All melting points were measured on a Gallenkamp melting point apparatus and are uncorrected. TLC analyses were carried out on 0.25 mm thick precoated silica plates (Merck Fertigplatten Kieselgel 60F₂₅₄) and spots were visualized under UV light. Chromatography on silica gel was carried out on Merck Kieselgel (230–240 mesh). IR spectra were determined on a BOMEM MB 104 spectrophotometer using KBr discs or Nujol. UV–vis absorption spectra (200–800 nm) were obtained using a Shimadzu UV/2501PC spectrophotometer. ¹H NMR spectra were recorded on a Varian 300 spectrometer in CDCl₃ at 300 MHz at 25 °C. All chemical shifts are given in parts per million using δ_H Me₄Si=0 ppm as reference and *J* values are given in hertz. ¹³C NMR spectra were run in the same instrument at 75.4 MHz using the solvent peak as internal reference. Assignments were made by comparison of chemical shifts, peak multiplicities, and *J* values, and were supported by spin decoupling-double resonance and bidimensional heteronuclear HMBC and HMQC correlation techniques. Mass spectra were obtained on a Finnigan LXQ MS spectrometer (ESI technique, positive mode). Elemental analyses were carried out on a Leco CHNS 932 instrument. Fluorescence spectra were collected using a Spex Fluorolog 1680 spectrometer. All reagents were used as received. 5-Formyl-2,2'-bithiophene, 3-nitro-*L*-tyrosine, and 2,5-diformylthiophene **2a** are commercially available (Aldrich). The synthesis of 5,5'-diformyl-2,2'-bithiophene **2b**^{11g} and 2-formyl-5-(4'-formylphenyl)-thiophene **2d** was described elsewhere.^{11c}

4.2. Procedure for the synthesis of diformyl-terthiophene **2c**

4.2.1. Synthesis of 5'-bromo-5-formyl-2,2'-bithiophene **1**

To a stirred solution of 5-formyl-2,2'-bithiophene (0.8 g, 4.1 mmol) in DMF (1 mL), in the dark, was added NBS (0.73 g, 4.1 mmol) at –20 °C during 4 h. After this time the reaction mixture was poured on ice and stirred till room temperature and a light yellow solid precipitated, which was isolated by filtration. Recrystallization from dichloromethane–light petroleum gave the pure compound as a light yellow solid (1.09 g, 98%). Mp=137–139 °C. IR (KBr): ν=1651 (C=O), 1510, 1455, 1278, 1230, 1193, 1050, 975, 879, 800, 784, 751, 666, 644 cm⁻¹. ¹H NMR (CDCl₃): δ=7.05 (d, 1H, *J*=3.9 Hz, H-3'), 7.12 (d, 1H, *J*=3.9 Hz, H-4'), 7.19 (d, 1H, *J*=3.9 Hz, H-3), 7.67 (d, 1H, *J*=3.9 Hz, H-4), 9.88 (s, 1H, CHO) ppm. C₉H₅BrOS₂: calcd C 39.57, H 1.84, S 23.48; found C 39.65, H 1.75, S 23.42.

4.2.2. Synthesis of 5,5'-diformyl-2,2':5'2''-terthiophene **2c** through Suzuki cross-coupling

5'-Bromo-5-formyl-2,2'-bithiophene **1** (0.60 g, 2.1 mmol) was coupled to 5-formylthiophene boronic acid (0.42 g, 2.7 mmol), in a mixture of DME (35 mL) and aqueous 2 M Na₂CO₃ (2 mL) and Pd(PPh₃)₄ (6 mol %) at 80 °C under a argon atmosphere during 20 h. After cooling the mixture was filtered. Ethyl acetate and a saturated solution of NaCl were added and the phases were separated. The organic phase was washed with water (3×75 mL) and an aqueous solution of NaOH (10%). The organic phase obtained was dried (MgSO₄), filtered, and solvent removal gave the crude product, which was recrystallized affording the pure diformyl derivative **2** as an orange solid (0.38 g, 60%). Mp=203.6–205.2 °C (lit.¹³ 224 °C). IR (Nujol): ν=1664 (C=O), 1505, 1319, 1219, 1164, 1047, 798, 759, 668 cm⁻¹. ¹H NMR (CDCl₃): δ=7.28–7.38 (m, 4H, H-3, H-3', H-3'', and H-4''), 7.70 (d, 2H, *J*=4.2 Hz, H-4 and H-4''), 9.89 (s, 2H, 2×CHO) ppm.

4.3. General procedure for the synthesis of bis-(oligo)-thienylbenzoxazolyl-alanines **4a–d**

N-*t*-Butyloxycarbonyl-3-amino-*L*-tyrosine methyl ester **3** (2 equiv) was stirred with the corresponding diformylated (oligo)-thiophene **2** (1 equiv) and heated in ethanol overnight at reflux (5 mL/equiv). The solvent was evaporated and the crude imine used in the next step without further purification. The corresponding crude imine and lead tetraacetate (3 equiv) were stirred at room temperature in DMSO (5 mL/equiv) for 3 days. The mixture was poured over water and extracted with ethyl acetate (3×10 mL). After drying with anhydrous magnesium sulfate and evaporation of the solvent under reduced pressure, the crude compound was submitted to column chromatography with silica gel by elution with dichloromethane and dichloromethane/methanol, 100:1. The fractions containing the pure compound were combined and evaporated under reduced pressure.

4.3.1. bis-[*N*-*t*-Butyloxycarbonyl [2-(thien-2'-yl)benzoxazol-5-yl]-*L*-alanine methyl ester] (**4a**)

The product was isolated as a yellow solid (0.19 g, 75%). Mp=106.2–108.0 °C. IR: ν=3355, 2968, 2940, 1749, 1693, 1640, 1583, 1524, 1476, 1446, 1392, 1365, 1255, 1166, 1056, 1016, 813, 794, 714 cm⁻¹. ¹H NMR (CDCl₃): δ=1.43 (s, 18H, 2×C(CH₃)₃), 3.16–3.31 (m, 4H, 2×βCH₂), 3.75 (s, 6H, 2×CH₃), 4.62–4.67 (m, 2H, 2×αH), 5.06 (d, *J*=7.8 Hz, 2H, 2×NH), 7.16 (dd, *J*=1.8 and 8.4 Hz, 2H, 2×H-6), 7.50 (d, *J*=8.4 Hz, 2H, 2×H-7), 7.53 (d, *J*=1.8 Hz, 2H, 2×H-4), 7.93 (dd, *J*=1.2 and 3.9 Hz, 2H, 2×H-3') ppm. ¹³C NMR (CDCl₃): δ=28.20 (C(CH₃)₃), 38.28 (βCH₂), 52.31 (CH₃), 54.53 (αC), 79.95 (C(CH₃)₃), 110.25 (C7), 120.30 (C4), 126.18 (C6), 130.02 (C2'), 130.17 (C3'), 132.83 (C5), 142.27 (C3a), 149.61 (C7a), 155.14 (C=O Boc), 159.50 (C2), 171.97 (C=O ester) ppm. UV–vis (ethanol, nm): λ_{max} (ε)=371 (47,210). MS: *m/z* (ESI, %): 721 ([M+H]⁺, 31), 665 (100), 609 (32). C₃₆H₄₀N₄O₁₀S: calcd C 59.99, H 5.59, N 7.77; found C 60.04, H 5.67, N 7.61.

4.3.2. bis-[*N*-*t*-Butyloxycarbonyl[2-(bithien-5'-yl)benzoxazol-5-yl]-*L*-alanine methyl ester] (**4b**)

The product was isolated as an orange solid (0.09 g, 54%). Mp=111.7–113.9 °C. IR (KBr): ν=3415, 2965, 2932, 1745, 1714, 1633, 1579, 1473, 1394, 1369, 1260, 1164, 1053, 1019, 936, 863, 801, 713 cm⁻¹. ¹H NMR (CDCl₃): δ=1.43 (s, 18H, 2×C(CH₃)₃), 3.18–3.28 (m, 4H, 2×βCH₂), 3.75 (s, 6H, 2×CH₃), 4.60–4.65 (m, 2H, 2×α-H), 5.03 (d, *J*=8.1 Hz, 2H, 2×NH), 7.15 (dd, *J*=1.2 and 8.4 Hz, 2H, 2×H-6), 7.36 (d, *J*=4.2 Hz, 2H, 2×H-4'), 7.48 (d, *J*=8.4 Hz, 2H, 2×H-7), 7.51 (d, *J*=1.2 Hz, 2H, 2×H-4), 7.85 (d, *J*=4.2 Hz, 2H, 2×H-3') ppm. ¹³C NMR (CDCl₃): δ=28.22 (C(CH₃)₃), 38.25 (βCH₂), 52.32 (CH₃), 54.60 (αC), 80.04 (C(CH₃)₃), 110.22 (C7), 120.23 (C4), 126.45 (C4'), 126.28 (C6),

127.38 (C2'), 130.91 (C3'), 132.85 (C5), 143.84 (C5'), 142.37 (C3a), 149.65 (C7a), 155.09 (C=O Boc), 159.01 (C2), 172.10 (C=O ester) ppm. UV–vis (ethanol, nm): $\lambda_{\max}(\epsilon)=388(46,641)$. MS: m/z (ESI, %): 803 ($[M+H]^+$, 34), 747 (30), 681 (78), 509 (30), 451 (100). $C_{40}H_{42}N_4O_{10}S_2$: calcd C 59.84, H 5.27, N 6.98; found C 59.78, H 5.27, N 7.11.

4.3.3. bis-[N-t-Butyloxycarbonyl[2-(terthien-5'-yl)benzoxazol-5-yl]-L-alanine methyl ester] (4c)

The compound was isolated as an orange solid (0.07 g, 60%). Mp=99.1–101.3 °C. IR (KBr): $\nu=3428, 2976, 2929, 1744, 1714, 1662, 1577, 1516, 1496, 1439, 1392, 1366, 1256, 1218, 1165, 1060, 1018, 913, 863, 792\text{ cm}^{-1}$. $^1\text{H NMR}$ (CDCl_3): $\delta=1.44$ (s, 18H, $2\times\text{C}(\text{CH}_3)_3$), 3.15–3.24 (m, 4H, $2\times\beta\text{CH}_2$), 3.75 (s, 6H, $2\times\text{CH}_3$), 4.60–4.66 (m, 2H, $2\times\alpha\text{-H}$), 5.04 (d, $J=8.1\text{ Hz}$, 2H, $2\times\text{NH}$), 7.14 (dd, $J=1.8$ and 8.1 Hz , 2H, $2\times\text{H-6}$), 7.34 (d, $J=3.9\text{ Hz}$, 2H, $2\times\text{H-4}'$), 7.48 (d, $J=8.1\text{ Hz}$, 2H, $2\times\text{H-7}$), 7.51 (d, $J=1.8\text{ Hz}$, 2H, $2\times\text{H-4}$), 7.71 (d, $J=3.9\text{ Hz}$, 2H, $2\times\text{H-3}'$), 7.86 (s, 2H, $2\times\text{H-3''}$) ppm. $^{13}\text{C NMR}$ (CDCl_3): $\delta=28.23$ ($\text{C}(\text{CH}_3)_3$), 38.17 (βCH_2), 52.24 (CH_3), 54.60 (αC), 80.03 ($\text{C}(\text{CH}_3)_3$), 110.17 (C7), 120.16 (C4), 124.30 (C4'), 125.74 (C3''), 126.30 (C6), 127.26 (C2'), 130.81 (C3'), 132.78 (C5), 137.83 (C2''), 141.93 (C5'), 142.28 (C3a), 149.67 (C7a), 155.11 (C=O Boc), 158.91 (C2), 172.12 (C=O ester) ppm. UV–vis (ethanol, nm): $\lambda_{\max}(\epsilon)=416(46,454)$. MS: m/z (ESI, %): 885 ($[M+H]^+$, 22), 509 (100). $C_{44}H_{44}N_4O_{10}S_3$: calcd C 61.97, H 5.20, N 6.57; found C 61.88, H 5.27, N 6.72.

4.3.4. bis-[N-t-Butyloxycarbonyl[2-(phen-4''-yl-thien-2'-yl)-benzoxazol-5-yl]-L-alanine methyl ester] (4d)

The compound was isolated as a yellow solid (0.14 g, 76%). Mp=96.00–98.5 °C. IR (KBr): $\nu=3435, 2977, 2930, 1746, 1715, 1668, 1615, 1575, 1505, 1479, 1367, 1257, 1168, 1056, 1018, 802\text{ cm}^{-1}$. $^1\text{H NMR}$ (CDCl_3): $\delta=1.44$ (s, 18H, $2\times\text{C}(\text{CH}_3)_3$), 3.20–3.25 (m, 4H, $2\times\beta\text{CH}_2$), 3.75 (s, 6H, $2\times\text{CH}_3$), 4.62–4.66 (m, 2H, $2\times\alpha\text{-H}$), 5.05 (d, $J=8.4\text{ Hz}$, 2H, $2\times\text{NH}$), 7.15 (dd, $J=1.2$ and 8.1 Hz , 2H, $2\times\text{H-6}$), 7.48 (d, $J=8.1\text{ Hz}$, 2H, $2\times\text{H-7}$), 7.52 (d, $J=1.2\text{ Hz}$, 2H, $2\times\text{H-4}$), 7.56 (d, $J=3.9\text{ Hz}$, 1H, H-4'), 7.81–7.87 (m, 3H, H-3', H-2'', and H-6''), 8.31 (d, $J=8.1\text{ Hz}$, 2H, H-3'' and H-5'') ppm. $^{13}\text{C NMR}$ (CDCl_3): $\delta=28.30$ ($\text{C}(\text{CH}_3)_3$), 38.31 (βCH_2), 52.35 (CH_3), 54.57 (αC), 80.00 ($\text{C}(\text{CH}_3)_3$), 110.25 (C7), 120.28 (C4), 126.38 (C4'), 126.30 (C6), 127.41 (C2'), 130.48 (C2'' and C6''), 130.93 (C3'), 132.84 (C5), 136.41 (C3'' and C5''), 138.49 (C1''), 144.00 (C5'), 142.44 (C3a), 149.80 (C7a), 151.78 (C4''), 155.11 (C=O Boc), 159.10 (C2), 172.03 (C=O ester) ppm. UV–vis (ethanol, nm): $\lambda_{\max}(\epsilon)=363(51,800)$. MS: m/z (ESI, %): 797 ($[M+H]^+$, 43), 740 (100). $C_{42}H_{44}N_4O_{10}S$: calcd C 63.30, H 5.57, N 7.03; found C 63.23, H 5.43, N 7.12.

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