Journal of Photochemistry & Photobiology A: Chemistry 364 (2018) 679–685

Contents lists available at ScienceDirect

Journal of Photochemistry & Photobiology A: Chemistry

journal homepage: www.elsevier.com/locate/jphotochem

New non-protienogenic fluorescent amino acids: Benzoxazol-5-yl-alanine derivatives containing acetylene unit. Synthesis, spectral and photophysical properties

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ARTICLE INFO

Keywords:
Non-proteinogenic amino acids
Benzoxazoles
Acetylene derivatives
Absorbance
Fluorescence
Fluorophore

ABSTRACT

New derivatives of non-proteinogenic amino acids benzoxazol-5-yl-alanine containing substituted acetylene derivative were synthesized according to Sonogashira coupling reaction. All of the obtained compounds are fluorescent. They are characterized by high or moderate molar absorption coefficients, large Stokes shifts, high fluorescence quantum yields and very high brightness. All of these parameters as well as the positions of absorption and emission bands depend on the type and size of substituent and the solvent polarity. Their high brightness enables working with low concentrations, simple and easy detection of spectral absorption and fluorescence analyzes. Moreover, amino acid part of studied compounds allow to use them as covalently attached to a peptide or protein fluorescent probes in biological system studies.

1. Introduction

The subject of the presented studies are 2-phenylbenzoxazol-5-ylo-alanine derivatives with triple bond in position 4 of phenyl ring and 2-pyridinbenzoxazol-5-ylo-alanine derivative with triple bond in position 5 of benzoxazole ring. The presence of a triple bond results in an increased electron coupling which facilitates the charge transfer without causing steric hindrance. It makes such derivatives to be an interesting subject of research from their spectroscopic properties point of view. In the past decades benzoxazoles are the unfailing subject of research as they are an important class of heterocyclic compounds with huge potential application in many areas such as chemistry, medicine, technology and industry. They are known as photostable, highly efficient UV dyes, laser dyes [1], organic brightening agents [2], organic plastic scintillators [3], photonic devices [4], light emitting diodes [5,6] and chemosensors [7–10]. Moreover, benzoxazole skeleton occurs in a number of biologically active compounds [11–13] and natural products [14,15]. Their antifungal [16], antibacterial [17], anti-inflammatory [18], antitumour [19], anti-HIV [20] activities have been reported. A benzoxazole derivatives are also used as a fluorescent probe for intracellular imaging in living cells [21–24].

On the other hand introduction of the triple bond to the system results in electron relocation (blurring) due to the coupling extension. An acetylene unit is also used between a donor and an acceptor as a linker (D-π-A) in so-called push-pull compounds which show a large nonlinear optical response (NLO) [25]. Hydrocarbon aromatic derivatives of acetylene exhibit bathochromic shift of the absorption and emission spectra, large Stokes shifts, high molar absorption coefficients and high quantum fluorescence yields [26]. Asymmetrically substituted acetylene derivatives containing aromatic or heteroaromatic substituents are fluorescent compounds. They can be applied as polarity-sensitive probes [27], sensors of metal or pH [28], fluorescence switches [29,30], for labelling biological molecules [31] or as oligonucleotide analogues [32].

Benzoxazole derivatives containing substituents with unsaturated carbon bond, such as styrylbenzoxazoles, especially their Pt(II) complexes, have shown biological activity (cytotoxicity) [33,34]. They are also applied as sensitizers for photographic halide emulsion [35]. 2-(4-Dimethylaminostyryl)benzoxazole can be used in electroluminescence devices [36].

All the information mentioned above indicates that the benzoxazole derivatives containing acetylene unit seems to be an interesting combination with potential new application of obtained derivatives. Hence, we have synthesized and measured spectral and photophysical properties of six new derivatives of 2-phenylbenzoxazol-5-ylo-alanine (BoxPh) and one derivative of 2-pyridynylbenzoxazol-5-ylo-alanine (Fig. 1). We also included into the study BoxPhBr, which was the substrate for most of the obtained derivatives and at the same time is a good reference for investigating the effect of acetylene on the studied properties. It is worth emphasizing that derivatives under study as non-

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https://doi.org/10.1016/j.jphotochem.2018.07.012
Received 25 April 2018; Received in revised form 3 July 2018; Accepted 6 July 2018
Available online 07 July 2018
1010-6030/

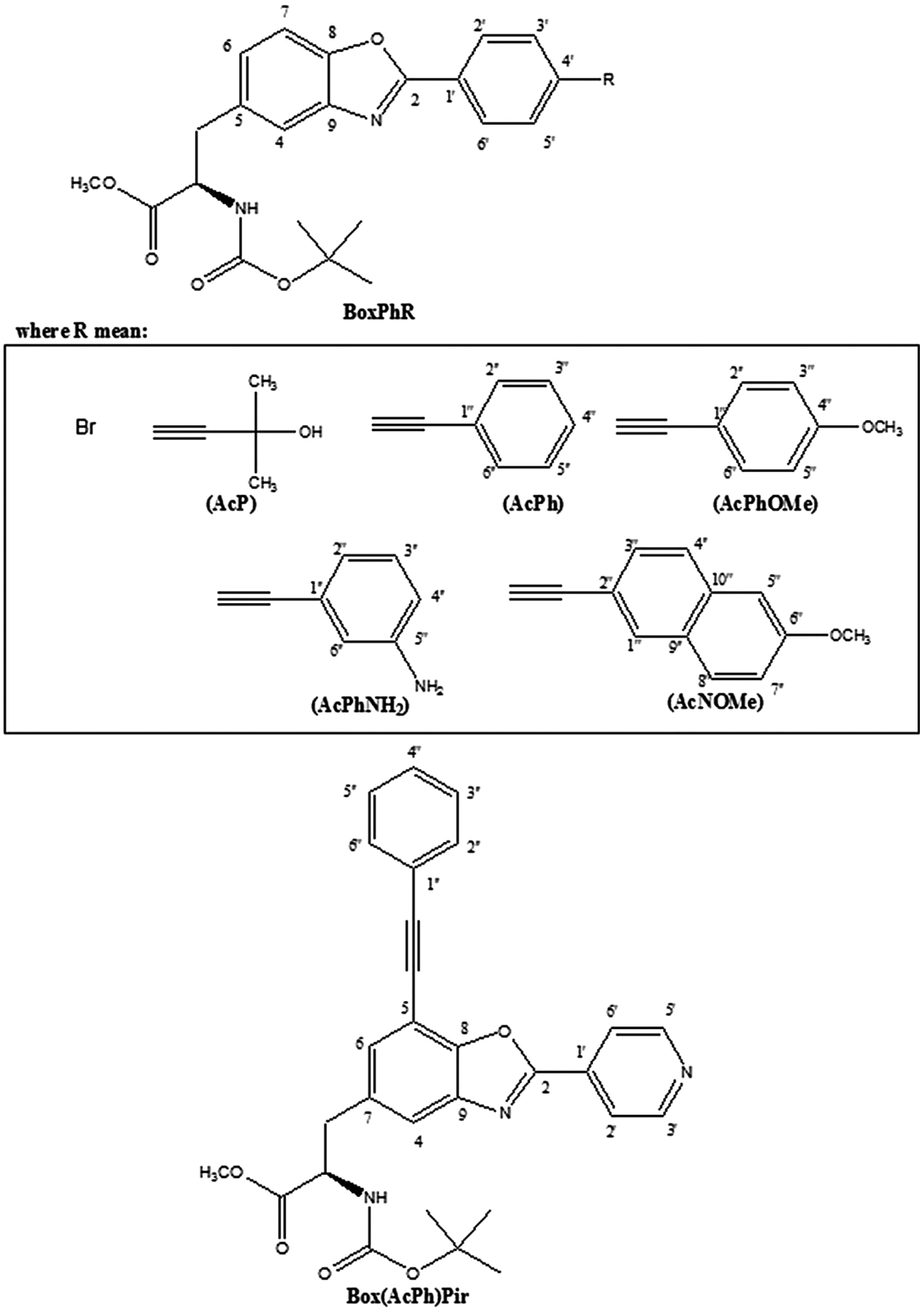
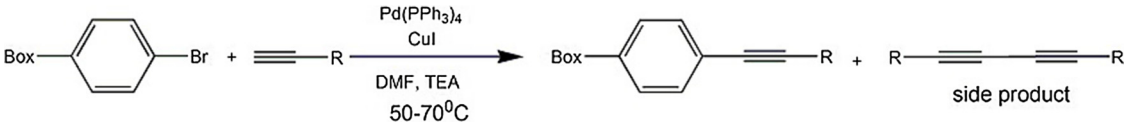


Fig. 1. Structures of synthesized acetylene derivatives of 3-(2-benzoxazol-5-yl)alanine.



Scheme 1. Scheme of synthesis of acetylene derivatives of 3-(2-benzoxazol-5-yl)alanine.

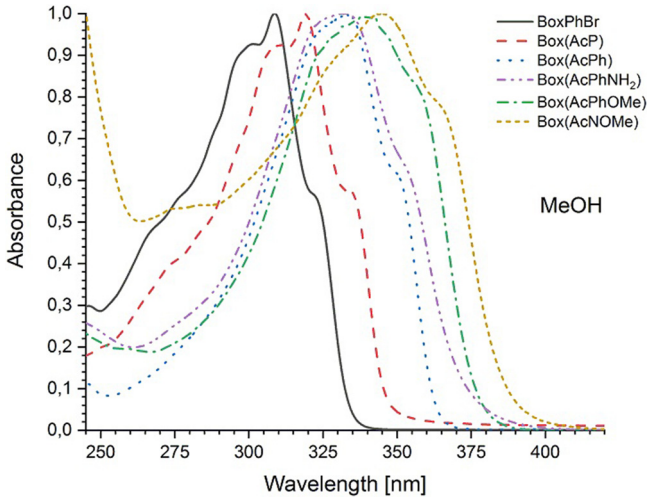


Fig. 2. Normalized absorption spectra of 2-phenylbenzoxazol-5-ylo-alanine in MeOH.

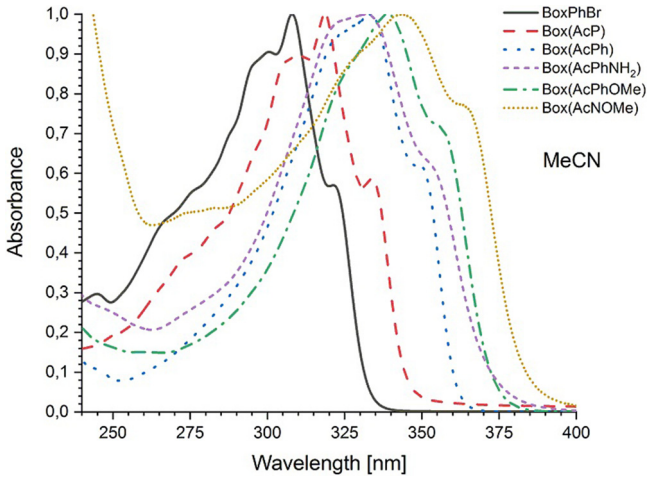


Fig. 3. Normalized absorption spectra of 2-phenylbenzoxazol-5-ylo-alanine in MeCN.

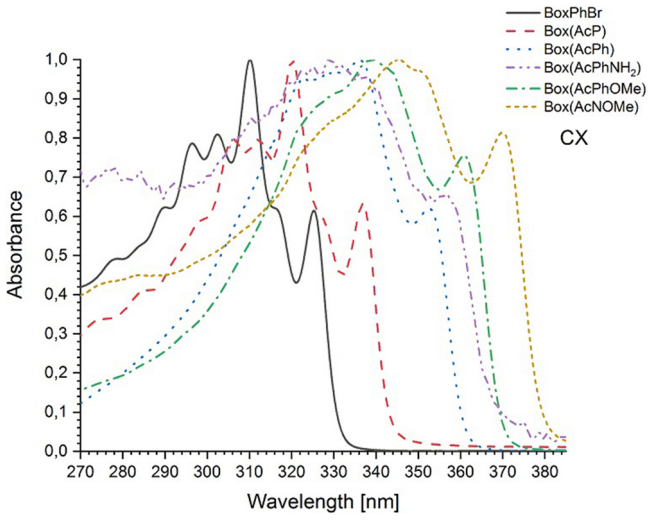


Fig. 4. Normalized absorption spectra of 2-phenylbenzoxazol-5-ylo-alanine in CX.

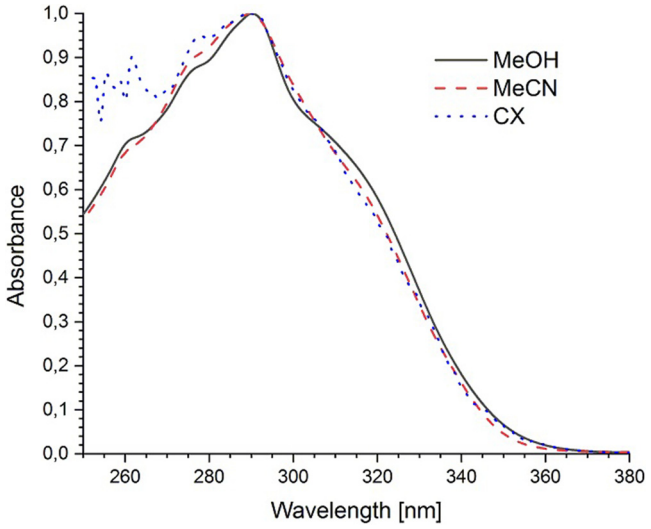


Fig. 5. Normalized absorption spectra of (Box(AcPh)Pir) in MeOH, MeCN and CX.

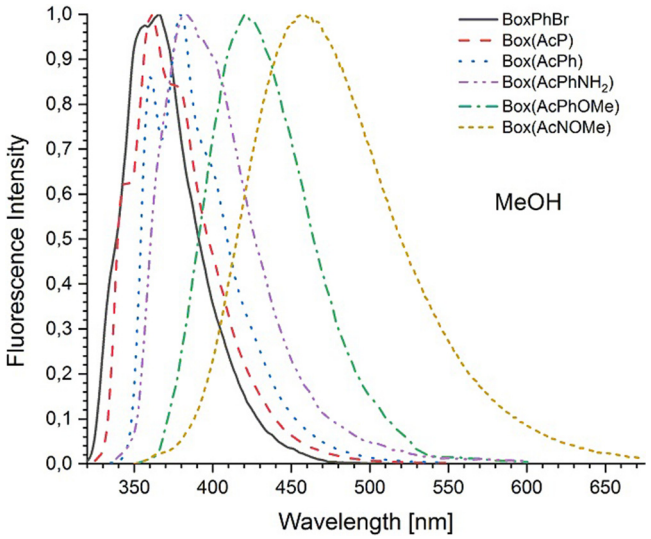


Fig. 6. Normalized emission spectra of 2-phenylbenzoxazol-5-ylo-alanine in MeOH.

proteinogenic amino acids possess reactive functional groups (amino and carboxyl) which allows to incorporate them into a peptide chain and conduct research in biological systems e.g. fluorescence conformational analysis of peptide or enzymatic kinetics assays as a fluorescence probe connected with a substrate [37].

2. Materials and methods

2.1. Synthesis

N-Boc-3-[2-(4-bromo)phenyl)benzoxazol-5-yl]alanine methyl ester (BoxPhBr; reaction yield 66%), and 5 acetylene derivatives of 2-phenylbenzoxazol-5-yl-alanine: *N*-Boc-3-[2-(4-ethynylphenyl)phenyl)benzoxazol-5-yl]alanine methyl ester (Box(AcPh)Ph; reaction yield 22%), *N*-Boc-3-[2-(4-(4-ethynylanizol)phenyl)benzoxazol-5-yl]alanine methyl ester (Box(AcPhOMe)Ph; reaction yield 10%), *N*-Boc-3-[2-(4-ethynyl-(3-aminophenyl)phenyl)benzoxazol-5-yl]alanine methyl ester (Box ($AcPhNH_2$)Ph; reaction yield 25%), (*N*-Boc-3-[2-(4-(4-(3-hydroxy-3-methylbut-1-ynyl)pheny))benzoxazol-5-yl]alanine methyl ester (Box (AcP)Ph; reaction yield 53%), *N*-Boc-3-[2-(4-(4-ethynyl(2-(6-methoxy)

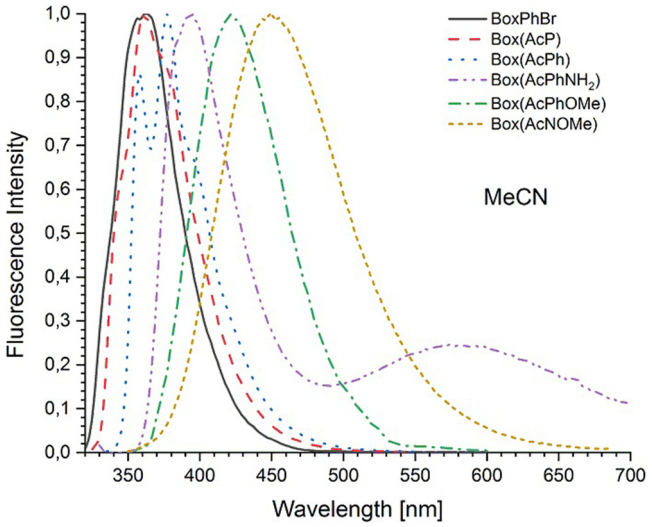


Fig. 7. Normalized emission spectra of 2-phenylbenzoxazol-5-ylo-alanine in MeCN.

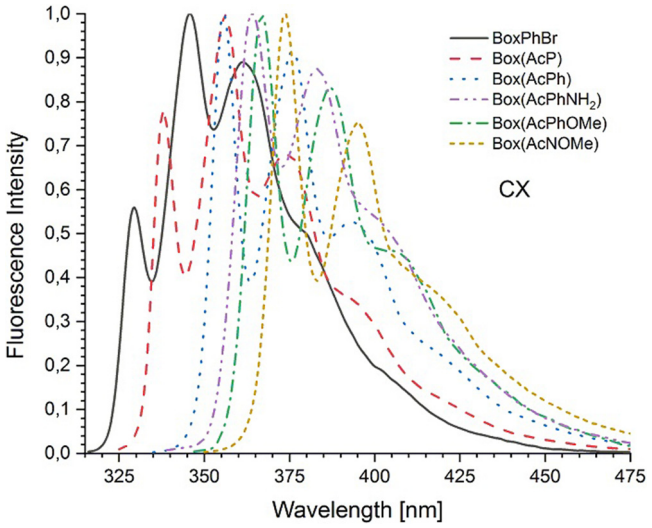


Fig. 8. Normalized emission spectra of 2-phenylbenzoxazol-5-ylo-alanine in CX.

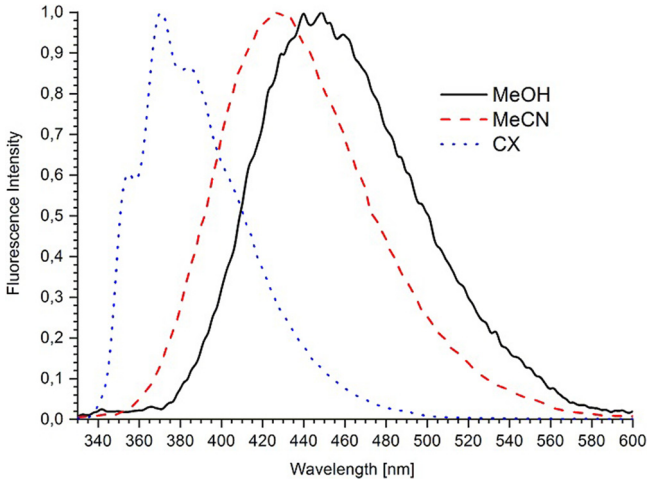


Fig. 9. Normalized emission spectra of (Box(AcPh)Pir) in MeOH, MeCN and CX.

naphtyl))phenyl)benzoxazol-5-yl]alanine methyl ester (Box(AcNOMe)Ph; overstated reaction yield due to the oily form of the compound), and 1 derivative of 2-pyridynylbenzoxazol-5-yl-alanine: *N*-Boc-3-[7-(2-phenylethynyl)-2-(pyridyn-4-yl)benzoxazol-5-yl]alanine methyl ester (Box(AcPh)Pir; reaction yield 24%) were synthetized.

2-Methylbut-3-yn-2-ol, 1-ethynylbenzene, 1-ethynyl-4-methoxybenzene, 3-ethynyl-benzenamine, 2-ethynylpyridine, 2-ethynyl-6-methoxynaphthalene, tetrakis(triphenyl-phosphine)palladium(0) were commercially available and used without further purification. Copper (I) iodide was synthesized using standard procedure based on copper(II) reduction, *N*-Boc-3-[2-(4-bromo)phenyl)benzoxazol-5-yl]alanine methyl ester (BoxPhBr) and N-(*tert*-butyloxycarbonyl)3-[7-bromo-2-(2-pyridynyl)benzoxazol-5-yl]-alanine methyl ester (BoxBrPir) were synthesized using published procedure [38]. All of acetylene derivatives of benzoxazol-5-yl-alanine were synthesized according to Sonogashira coupling reaction [39] of appropriate acetylene derivatives with BoxPhBr or BoxBrPir in case of Box(AcPh)Pir (Scheme 1) in the presence of tetrakis(triphenylphosphine)palladium(0) and copper(I) iodide. The reaction were carried out in DMF with the addition of trimethylamine in an inert gas atmosphere (argon) (Scheme 1).

The progress of all reactions was monitored by TLC using Merck plates (Kieselgel 60 F254). The products were isolated by means of column chromatography (Merck Kieselgel 60 (0.040–0.063 mm)) and/or semi-preparative RP-HPLC (Kromasil column, C-8, 5 µm, 250 mm long, i.d. 20 mm or Jupiter Phenomenex® column, C-18, 5 µm, 250 mm long, i.d. 4.6 mm). The compounds were obtained with yields from 10 to 66% (the exception is Box(AcNOMe)Ph derivative obtained in the form of an oil for which yield was inflated). In addition to the desired acetylene Box derivatives, side-products (dimers) resulting from homo-coupling of terminal acetylene derivatives were isolated in all syntheses. Simultaneous competitive reaction of homo-coupling reduced the value of the reaction yields. The identification of the product was based on: ^{1}H and ^{13}C NMR spectra (Varian, Mercury-400 BB spectrometer, ^{1}H NMR - 400 MHz and ^{13}C NMR - 100 MHz) in $CDCl_3$ or DMSO-d_6, mass spectra (Bruker Daltonics HCT Ultra instrument or Bruker Biflex III (MALDI-TOF)) and IR spectra (Bruker IFS-66 instrument). Detailed information on the identification of synthesized compounds are included in supporting information.

2.2. Spectroscopic properties

Absorption spectra were measured using Perkin-Elmer Lambda-40 P spectrophotometer whereas fluorescence spectra using FluoroMax-4 (Horriba Yobin-Yvon) spectrofluorimeter. The solvents used in studies: methanol (MeOH), acetonitrile (MeCN) and cyclohexane (CX) were either spectroscopic or HPLC grade. Fluorescence quantum yields were calculated with quinine sulphate in 0.5 M H_2SO_4 ($\phi = 0.53 \pm 0.02$) as reference and were corrected for different refractive indices of solvents [40].

The fluorescence lifetimes were measured with a time-correlated single-photon counting apparatus Edinburgh CD-900 equipped with NanoLed N16 (UV LED 339 nm) or NanoLed N05 A (UV LED 460 nm) from IBH as the excitation source. The half width of the response function of the apparatus, measured using a Ludox solution as a scatter, was about 1.0 ns. The emission wavelengths were isolated using a monochromator. Fluorescence decay data were fitted by the iterative convolution to the sum of exponents according to eq.:

$$I(t) = \sum_i \alpha_i \exp(-t/\tau_i)$$

where α_i is the pre-exponential factor and τ_i the decay time of the ith component, using a software supported by the manufacturer. The adequacy of the exponential decay fitting was judged by visual inspection of the plots of weighted residuals as well as by the statistical parameter χ_R^2 and shape of the autocorrelation function of the weighted residuals and serial variance ratio (SVR).

Table 1
Range of absorption, absorption maximum and molar absorption coefficient values of studied derivatives in MeOH, MeCN and CX.

Compounds	MeOH			MeCN			CX		
	Range [nm]	λ_{max} [nm]	ε [M^{-1} cm^{-1}]	Range [nm]	λ_{max} [nm]	ε [M^{-1} cm^{-1}]	Range [nm]	λ_{max} [nm]	ε [M^{-1} cm^{-1}]
BoxPhBr	235 –340–	309	25,200	235–340	308	24,240	235–335	310	28,500
Box(AcPh)Ph	250–370	333	58,400	250–370	333	44,000	250–370	336	60,988
Box(AcPhOMe)Ph	260–390	340	57,330	260–385	339	52,340	260–375	340	56,445
Box(AcPhNH_2)Ph	260–400	332	22,170	260–400	332	25,608	260–420	329	–
Box(AcP)Ph	240–355	319	26,700	240–355	319	26,200	240–350	320	27,000
Box(AcNOMe)Ph	260–410	345	34,500	260–410	344	33,000	260–400	345	29,400
Box(AcPh)Pir	240–370	290	33,040	240–370	289	28,585	240–400	290	–

Table 2
Range of emission, emission maximum and fluorescence quantum yield of studied derivatives in MeOH, MeCN and CX.

Compounds	MeOH			MeCN			CX		
	Range [nm]	λ_{max} [nm]	ϕ	Range [nm]	λ_{max} [nm]	ϕ	Range [nm]	λ_{max} [nm]	ϕ
BoxPhBr	320–475	366	0.30	320–475	362	0.29	315–465	346	0.22
Box(AcPh)Ph	335–520	379	0.86	335–520	377	0.87	335–505	356	0.89
Box(AcPhOMe)Ph	360–560	428	1	355–560	421	1	350–515	367	1
Box(AcPhNH_2)Ph	340–580	384	0.05	340–700	383	0.08	340–505	364	1
Box(AcP)Ph	325–500	361	0.78	325–500	362	0.78	325–490	356	0.85
Box(AcNOMe)Ph	355–685	456	0.72	355–685	449	0.78	350–540	374	0.84
Box(AcPh)Pir	360–600	449	0.09	340–600	426	0.32	335–515	371	1

Table 3
Brightness of selected fluorophores.

Fluorophore	Solvent	ε × ϕ [M^{-1} cm^{-1}]	Ref.
phenylalanine	H_2O	5	[40]
tyrosine	H_2O	210	[40]
tryptophan	H_2O	820	[40]
quinine	pH 2	3000	[43]
Cy5	pH 7	36,000	[44]
Rh_{110}	pH 7.5	68,000	[45]
BODIPY-FL	MeOH	86,000	[46]
fluorescein	pH 9	88,000	[40]
Box(AcPhOMe)Ph	*MeOH*	57,330	
	MeCN	52,340	
	CX	*56,445*	
Box(AcPh)Ph	*MeOH*	*50,224*	
	MeCN	*38,280*	
	CX	*54,280*	

Table 4
The fluorescence lifetimes (τ_i) of studied derivatives in MeOH, MeCN and CX.

	Solvent	τ_i [ns]	χ_R^2
Box(AcPh)Ph	MeOH	0.87	1.10
	MeCN	0.88	0.97
	CX	0.76	1.16
Box(AcPhOMe)Ph	MeOH	1.24	0.94
	MeCN	1.16	1.14
	CX	0.75	0.82
Box(AcP)Ph	MeOH	1.20	0.95
	MeCN	1.20	1.00
	CX	1.00	1.19
Box(AcNOMe)Ph	MeOH	1.52	1.14
	MeCN	1.50	1.16
	CX	0.75	1.17

3. Results and discussion

3.1. Spectral properties

Absorption and emission spectra of all obtained compounds were measured in MeOH, MeCN and CX, solvents of different polarity and ability to form hydrogen bonds. Normalized spectra of 2-phenylbenzoxazol-5-ylo-alanine grouped by solvents (MeOH, MeCN and CX) are presented in respectively Figs. 2–4 (absorption) and Figs. 6–8 (emission). The absorption and emission spectra of (Box(AcPh)Pir) in all studied solvent are presented respectively in Figs. 5 and 9. Additionally, the range of long wavelength absorption band and wavelength of absorption maximum as well as molar absorption coefficients are given in Table 1. In Table 2 the appropriate parameters for emission are presented.

Maxima of long-wavelength absorption bands of studied compounds are within the range 310–345 nm. The exception is Box(AcPh)Pir for which the absorption maximum is at about 290 nm. The vibronic structure of the spectrum for all studied compounds is well resolved only in the case of nonpolar solvent (cyclohexane), however, for Box (AcP)Ph and BoxPhBr, it is a clearer one. The introduction of an aromatic hydrocarbon substituent blurs the vibronic structure, indicating the contribution of various conformers associated with the rotation of the aromatic substituent. The polarity of the solvent practically does not affect the position of the absorption bands. Only a slight long wavelength shift of the spectrum can be seen as the polarity of the solvent increases. However, the effect of the size of aromatic substituent attached via the triple bond to the 3-[2-(phenyl)benzoxazol-5-yl]alanine derivative on its spectral properties is observed. The larger substituent causes the bathochromic shift of the absorption bands, which is related to the increase of the electron coupling system in the molecule. The same trend is observed in the presence of electron donating substituent (EDS) (as methoxy group in the phenyl ring) (Figs. 2–5).

The molar absorption coefficient values are diverse (Table 1) – from relatively high (over 60 000 M^{-1} cm^{-1}) to moderate (about 22 200 M^{-1} cm^{-1}). In two cases, for Box(AcPhNH_2)Ph and Box(AcPh)Pir, the values of molar absorption coefficients in cyclohexane could not be estimated due to the low solubility of the aforementioned compounds. In most cases, the increase in polarity of the solvent causes a decrease in molar absorption coefficients. Substitution of bromine atom in starting substrate with phenylacetylene and its derivatives causes appreciable increase of these values (Table 1).

A comparison of spectral properties of Box(AcPh)Ph with the methyl ester of N-Boc-3-[2-(4-biphenyl)benzoxazol-5-yl)alanine described in the literature [] allowed to assess the impact of introducing a triple

bond into the molecule. The presence of the acetylene unit shifts the absorption bands by 15 nm to longer wavelength, which may be due to the elongation of the conjugated bond system. In addition, spectral parameters of the acetylene derivative depend more on the properties of the solvent used.

3.2. Photophysical properties

The measured excitation spectra of all studied derivatives overlap with respective absorption spectra, which indicates that the emitting species is present in the ground state.

Maxima of emission bands of the studied derivatives are in the range of 346–456 nm (Table 2). The spectra in polar solvents have blurred vibronic structure. The only exception are Box(AcPh)Ph and Box(AcP)Ph for which even in polar solvents the vibronic structure is noticeable. Emission spectra are more sensitive to the polarity of the solvent than the absorption one. In more polar solvents the emission bands are shifted to longer wavelength. This effect is most visible in the case of derivatives with methoxy group (Box(AcPhOMe)Ph and Box(AcNOMe)Ph) and for Box(AcPh)Pir, for which the differences between the positions of the maxima in CX and MeOH are respectively 61, 81 and 78 nm. Increase of hydrocarbon substituent size also causes bathochromic shift of emission bands. Similarly, the presence of EDS shifts spectrum by about 10 nm in the case of CX and by 50 nm in polar solvent (Figs. 6–9). For Box(AcPhOMe)Ph, Box(AcNOMe)Ph and Box(AcPh)Pir large Stokes shift is observed, especially in polar solvents (5994, 7089, 12,174 cm^{-1} in MeOH and 5772, 6832, 11,080 cm^{-1} in MeCN, respectively). An interesting case is Box($AcPhNH_2$)Ph which shows dual fluorescence (Figs. 6–8). It should be noted that long-wave band does not come from excimer because measurements of the ten times diluted solution spectrum does not change the intensity ratio of the short-wave and long-wave band. This phenomenon assign two bands to the two excited states, one being related to the fluorescence from a local excited state and the other from the CT state. The presence of two fluorescence bands for acetylene derivatives with an amino substituent (aminophenyl (phenyl)acetylene) has already been described previously by Hirata et al. [41] and also for N,*N*-dimethyl-4-(phenylethynyl)aniline [42]. Observation of this phenomenon, requiring the presence of both the acceptor and the donor connected with a single bond, for Box ($AcPhNH_2$)Ph indicates that the coupled system with labile π-type electrons favors the phenomenon of CT, and benzoxazole is a strong acceptor of the charge.

Studied compounds are characterized by high fluorescence quantum yields (ϕ) in the range from 0.22 to 1.0, however limiting the analysis to acetylene derivatives (bypassing the BoxPhBr derivative) from 0.72-1.0. The exception are: Box($AcPhNH_2$)Ph and Box(AcPh)Pir. However, even in the case of these derivatives ϕ in the non-polar solvent (CX) for which in all cases the values are the highest, is equal to 1.0. Substitution of bromine with aromatic acetylene derivative results in remarkable increase in the fluorescence quantum yield. An increase in the size of the aromatic substituent lowers a little the fluorescence quantum yield, whereas the introduction of the EDS results in a significant increase in ϕ (Table 2). Some of the studied compounds, especially Box(AcPhOMe)Ph and Box(AcPh)Ph, show high brightness determined as a product of the molar extinction coefficient and fluorescence quantum yield. Brightness values of Box(AcPhOMe) and Box(AcPh)Ph are comparable to brightness of known fluorophores e.g. rhodamine 110 (Table 3). This enables work with low concentrations, simple and easy detection of spectral absorption and fluorescence analyzes.

The fluorescence intensity decay of selected studied compounds were measured in three solvents used. The fluorescence lifetimes and pre-exponential factors are presented in Table 4. The fluorescence intensity decays of the studied compounds in all used solvents are mono-exponential with relatively short life times (about 1 ns). An increase in the size of the aromatic substituent and the introduction of EDS results in a longer fluorescence decay time. Moreover, the increase in polarity of the solvent extends lifetime in all cases, which is characteristic for intramolecular charge transfer systems.

In comparison to the methyl ester of N-Boc-3-[2-(4-biphenyl)benzoxazol-5-yl)alanine the derivative containing triple bond (Box(AcPh)Ph) is characterized by a bathochromically shifted emission spectrum (by about 8 nm), higher fluorescence quantum yield and a shorter fluorescence lifetimes what indicates on a greater fluorescence rate constant. In addition, the acetylene derivative shows a greater dependence of photophysical parameters on the properties of the solvent [].

4. Conclusion

Synthesized using known procedures with moderate or low reaction yields and studied for spectroscopic properties 3-(2-benzoxazol-5-yl) alanine derivatives containing acetylene unit are a group of new fluorescent non-proteinogenic amino acids. Their photophysical properties are dependent on the size and type of hydrocarbon substituent. They show high fluorescence quantum yields, which, combined with high values of molar absorption coefficients, gives high brightness values and make them suitable as the fluorescence probes.

These compounds, due to the presence of functional groups (amino acid part of compounds), may be used as fluorescent probes covalently attached to a peptide or protein in biological system studies or may be used to synthesize linear or branched homo- or heterobiopolymers. In addition, the presence of a triple bond in the 3-[2-(phenyl)benzoxazol-5-yl]alanine derivatives, increasing the hydrophobic character of the compound and may also favorably affect the permeability of this type of systems through lipid membranes, constituting a useful fluorescent sensor in biophysical studies.

Acknowledgements

This work was financially supported by Ministry of Science and Higher Education, Poland, grant NN204 126137.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jphotochem.2018.07.012.

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