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Synthesis and photophysical properties of 3-[2-(pyridyl)benzoxazol-5-yl]-L-alanine derivatives

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Abstract—The syntheses of *N*-(*tert*-butyloxycarbonyl)-3-[2-(4-pyridyl)benzoxazol-5-yl]-L-alanine methyl ester and *N*-(*tert*-butyloxycarbonyl)-3-[2-(2-pyridyl)benzoxazol-5-yl]-L-alanine methyl ester using different oxidizing agents (NBS, lead tetraacetate, Mitsunobu reaction) in oxidative cyclization of Schiff base are described. The compounds obtained are fluorescent. The position of the emission band and the fluorescence quantum yield depend on solvent polarity. The fluorescence decays of all the compounds studied are heterogeneous. © 2002 Elsevier Science Ltd. All rights reserved.

1. Introduction

Benzoxazoles are a very interesting group of heterocyclic compounds which are widely used in chemistry, industry and medicine. The 2-phenylbenzoxazoles are known as photostable, highly efficient UV dyes[1] and are used as organic brightening agents,[2] laser dyes,[1] and organic plastic scintillators.[3] The benzoxazole moiety is also found in biologically active compounds.[4] Benzoxazoles have been indicated as 5-HT_3 receptor partial agonists,[5] HIV protease inhibitors,[6] thrombin inhibitors,[7] and α_2-antagonist/5-HT uptake inhibitors.[8] Because of their photophysical properties and biological activity we are developing benzoxazole derivatives possessing reactive functional groups (amino and carboxyl) which allow to incorporate them into a peptide chain. This makes them suitable for use in fluorescence conformational analysis of peptides or enzymatic kinetics assays as a fluorescence probe incorporated in a substrate.[9]

Benzoxazoles can be synthesized according to various procedures described in the literature.[3,10–19] However, not all of the methods can be used to obtain derivatives possessing an amino acid moiety. The most suitable approach is oxidative cyclization of azomethines (Schiff bases) obtained from aromatic 1-amino-2-hydroxy compounds (in our case *N*-Boc-3-nitro-L-tyrosine methyl ester) and appropriate aldehydes.[16–18] To find the most efficient method of synthesis *N*-(*tert*-butyloxycarbonyl)-3-[2-(4-pyridyl)benzoxazol-5-yl]-L-alanine methyl ester (**4b**) was synthesized using three different oxidizing agents. *N*-(*tert*-Butyloxycarbonyl)-3-[2-(2-pyridyl)benzoxazol-5-yl]-L-alanine methyl ester (**4a**) was prepared using only one method. Their photophysical properties were also measured.

2. Results and discussion

2.1. Synthesis

N-(*tert*-Butyloxycarbonyl)-3-[2-(2-pyridyl)benzoxazol-5-yl]-L-alanine methyl ester (**4a**) and *N*-(*tert*-butyloxycarbonyl)-3-[2-(4-pyridyl)benzoxazol-5-yl]-L-alanine methyl ester (**4b**) were prepared according to the procedures published previously using as oxidizing agent either lead tetraacetate or *N*-bromosuccinimide (NBS).[20] In the case of using lead tetraacetate, the influence of the solvent on the reaction yield of **4b** was checked. Additionally, cyclization of the Schiff base to **4b** was performed by means of the Mitsunobu reaction using typical reagents, triphenylphosphine (TPP) and diisopropylazodicarboxylate (DIAD) (Fig. 1).[19,21] Also the removal of the protecting groups was performed generating compounds **6** and **7** (Fig. 2).

In the case of using NBS the reaction yield was rather low (about 10%). Furthermore, a side-product was also isolated (bromo-derivative). A better yield (about 25%), without by-product, was obtained under the mild conditions of the Mitsunobu reaction.

Lead tetraacetate was also quite an effective oxidizing

Keywords: benzoxazoles; pyridylbenzoxazol-5-yl-L-alanine; Mitsunobu reaction; fluorescence.

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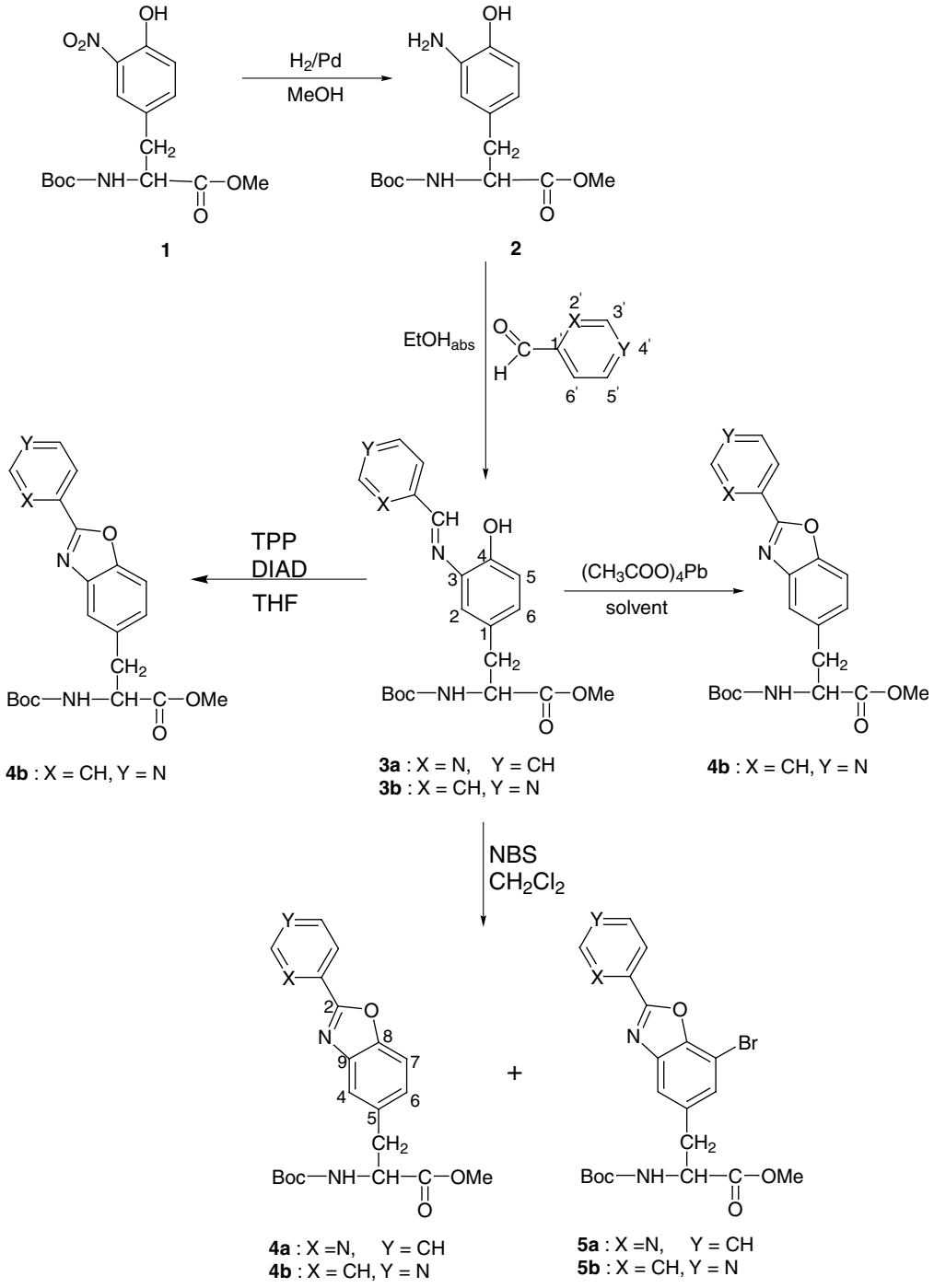

Figure 1. Reaction scheme.

agent, but the reaction yield depended on the solvent used. The least efficient synthesis was that performed in dimethoxyethane (about 15% of the product), while the syntheses in propylene carbonate and DMSO were the most efficient (about 80% of the product). The syntheses in the other solvents, except for DMF (70%), gave the desired product in about 60%. The reaction was run for ca. 25 h and monitored by RP-HPLC hold after 1 h and at the end. The prolongation of the reaction time (above 1 h) did not substantially change the percentage of the product in the reaction mixture.

2.2. Photophysical properties

Absorption spectra of compounds **4a** and **4b** were measured in MeOH, MeCN and methylcyclohexane and those obtained for **4b** are presented in Fig. 3.

In all solvents studied the absorption maxima are at about 300–310 nm. The vibronic structure of the spectrum is well resolved only in the case of methylcyclohexane, the least polar among the solvents used. The molar absorption coefficient values are quite high, about 20 000 $dm^3\ mol^{-1}\ cm^{-1}$.

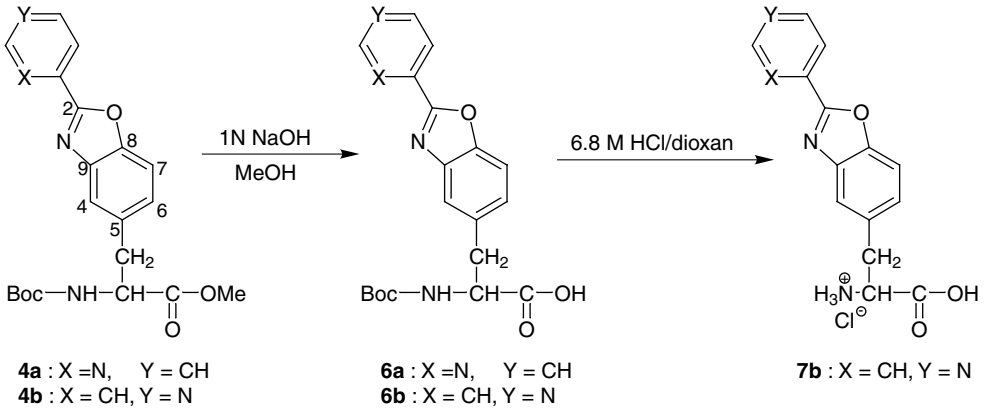

Figure 2. Scheme for protecting group removal.

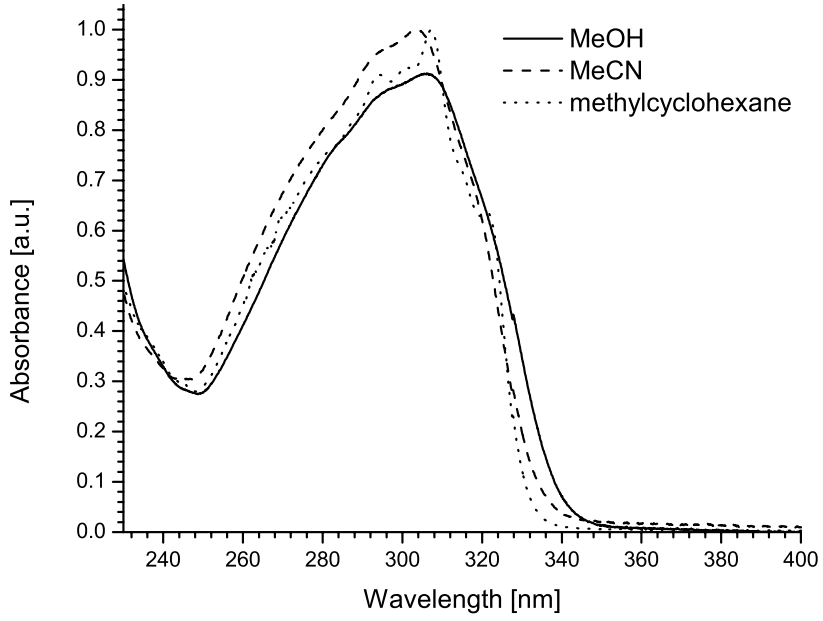

Figure 3. Absorption spectra of *N*-(*tert*-butyloxycarbonyl)-3-[2-(4-pyridyl)benzoxazol-5-yl]-L-alanine methyl ester in methanol, acetonitrile and methylcyclohexane.

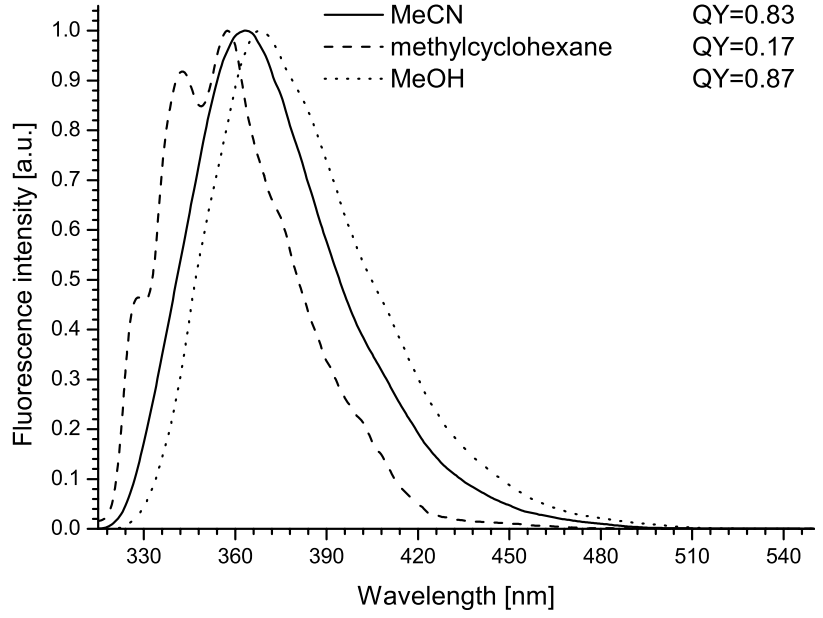

Figure 4. Emission spectra of *N*-(*tert*-butyloxycarbonyl)-3-[2-(2-pyridyl)benzoxazol-5-yl]-L-alanine methyl ester in methanol, acetonitrile and methylcyclohexane.

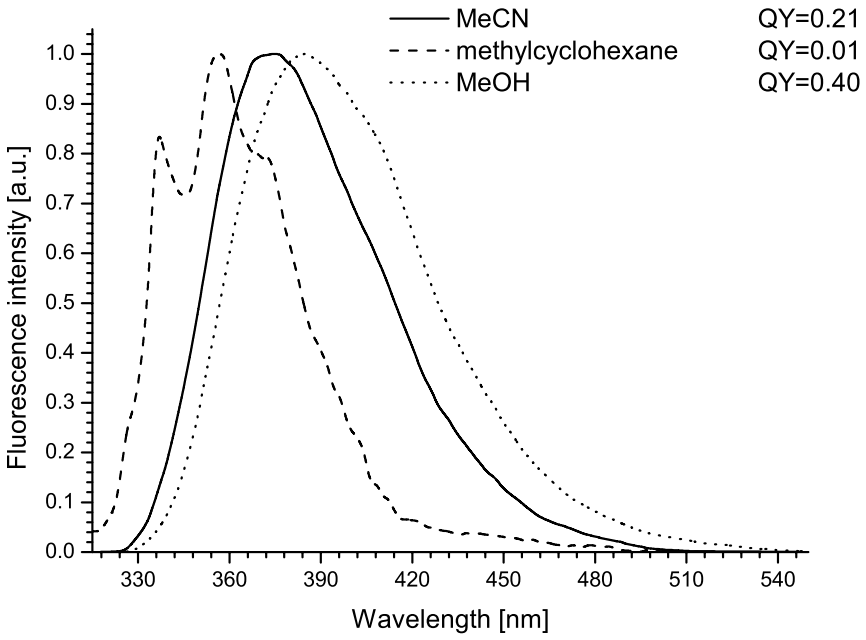

Figure 5. Emission spectra of *N*-(*tert*-butyloxycarbonyl)-3-[2-(4-pyridyl)benzoxazol-5-yl]-L-alanine methyl ester in methanol, acetonitrile and methylcyclohexane.

The fluorescence spectra of **4a**, **4b** and **7b** are presented in Figs. 4–6, respectively. The bromo-derivatives are non-fluorescent.

Similar to the absorption spectrum, the emission spectra of all the above-mentioned compounds exhibits well-resolved vibronic structure only in methylcyclohexane. The position of the emission band, as well as the fluorescence quantum yield, depends on the solvent polarity. In more polar solvents, the emission band is shifted to longer wavelength, and the fluorescence quantum yield is higher. The emission bands of **7b** are shifted slightly to shorter wavelength compared to those of **4b**. However, the fluorescence spectrum of **7b** in water shows an emission maximum at 403 nm. The emission maxima of **4b** are at a longer wavelength as compared with those of **4a** in the same solvents. The highest fluorescence quantum yield is displayed by **4a** in MeOH (Quantum yield (QY)=0.87), while the lowest quantum yield are displayed by **4b** and **7b** in methylcyclohexane (QY=0.01).

The fluorescence lifetimes, pre-exponential factors and the fluorescence QY of the compounds studied are presented in Table 1.

The fluorescence intensity decays of the compounds studied

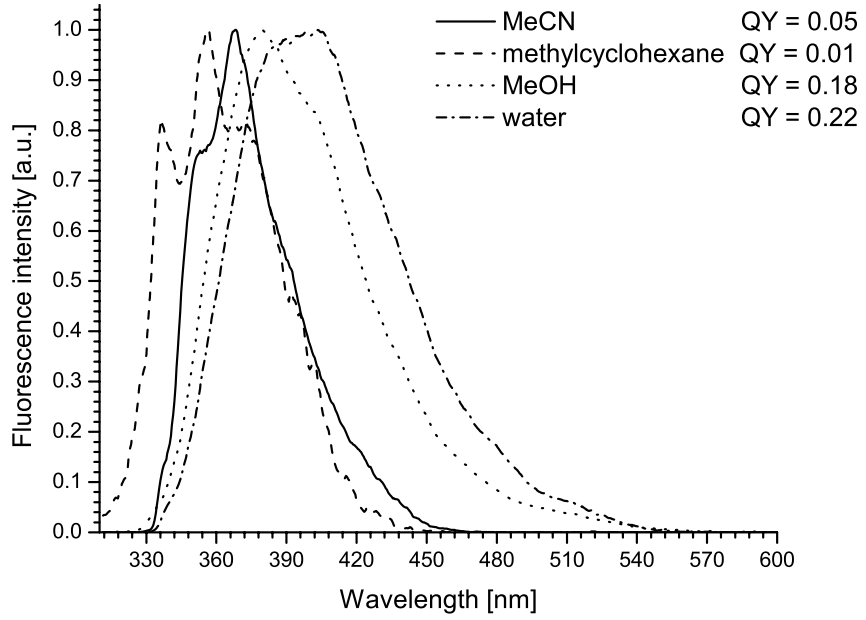

Figure 6. Emission spectra of 3-[2-(4-pyridyl)benzoxazol-5-yl]-L-alanine in methanol, acetonitrile, methylcyclohexane, and water.

Table 1. The fluorescence lifetimes (τ_i), pre-exponential factors (α_i) and the fluorescence quantum yields (QY) of benzoxazol-5-yl-L-alanine derivatives in methanol, acetonitrile, methylcyclohexane and water

Compound	τ_1 (ns)	τ_2 (ns)	τ_3 (ns)	α_1	α_2	α_3	χ_R^2	QY
MeOH								
4b	2.165			1.0000			1.168	0.40
	2.188	0.686		0.9355	0.0645		1.099	
7b	0.742			1.0000			1.851	0.18
	0.686	1.332		0.9559	0.0441		0.994	
6a	1.189			1.0000			1557	0.04
	1.399	0.276		0.1931	0.8069		2.692	
	1.620	0.587	0.102	0.1067	0.2400	0.6533	1.202	
MeCN								
4b	1.188			1.0000			34.247	0.21
	1.081	0.546		0.7677	0.2323		1.116	
7b	6.095			1.0000			5.045	0.05
	4.782	0.841		0.7590	0.2410		1.053	
6a	1.917			1.0000			440.35	0.20
	1.314	0.120		0.4472	0.5528		1.093	
Methylcyclohexane								
4b	0.609			1.0000			37.038	0.01
	1.615	0.028		0.0061	0.9939		2.225	
	0.999	0.013	0.194	0.0076	0.9729	0.0194	1.058	
7b	3.122			1.0000			58.126	0.01
	3.032	0.988		0.1557	0.8443		1.065	
6a	2.900			1.0000			2072	0.20
	1.028	0.104		0.1884	0.8116		1.081	
Water								
7b	0.999			1.0000			21.633	0.22
	0.443	1.057		0.2754	0.7246		1.112	

in different solvents are mostly two-exponential. Only in the case of **6a** in MeOH and **4b** in methylcyclohexane the decays are three-exponential.

3. Conclusion

All the described methods of synthesis of benzoxazol-5-yl-L-alanine were effective and gave the desired product. However, the worst method was that using NBS as the oxidizing agent because of the presence of side-product. The Mitsunobu reaction gave better yields, but it was the most time-consuming method. So the best approach seems to be method using lead tetraacetate in DMSO or propylene carbonate.

The properties of the obtained compounds are very interesting and make them suitable as the fluorescence probes to study the micro-environmental polarity of peptides.

4. Experimental

4.1. General

2-Pyridinecarboxaldehyde, 4-pyridinecarboxaldehyde, triphenylphosphine (TPP) and diisopropylazodicarboxylate (DIAD) were purchased from Aldrich, lead tetraacetate and NBS from Lancaster and 3-nitro-L-tyrosine from Fluka. The following compounds were prepared according to literature procedures: 3-nitro-L-tyrosine methyl ester[22] and *N*-Boc-3-nitro-L-tyrosine methyl ester.[23] TLC was carried out on Merck silica gel plates (Kieselgel 60 F_{254}). The spots were revealed using a UV lamp (254 nm, 366 nm). All solvent ratios are in volume parts. The purification was carried out by means of column chromatography (Merck, Silica gel 60, 0.040–0.063 mm) and semi-preparative RP-HPLC (Kromasil column, C-8, 5 μm, 250 mm long, ID=20 mm). The purity of the obtained compounds was checked by means of analytical RP-HPLC (Kromasil column, C-18, 5 μm, 250 mm long, ID=4.5 mm) with detection at 223 nm. Melting points (mp) were determined in capillary tubes and are uncorrected. ^{1}H NMR, ^{13}C NMR and ^{1}H–^{1}H COSY spectra were recorded on a Varian, Mercury-400 BB spectrometer (400 MHz). They were taken in $CDCl_3$ or DMSO-d_6. δ was referenced internally to the residual proton resonance of $CDCl_3$ (7.27 ppm) or DMSO-d_6 (3.29 ppm), respectively, or to $SiMe_4$ (=0.00 ppm) for ^{1}H and to DMSO-d_6 (39.51 ppm for centerline) for ^{13}C, respectively. Infrared spectra were recorded on a Bruker IFS-66 instrument. Mass spectra were recorded on a MASSLAB TRIO-3 instrument. Elemental analyses were taken on a Carlo Erba CNSO Eager 200 instrument. Absorption spectra were measured on a Perkin–Elmer Lambda 18 spectrophotometer. Fluorescence spectra were measured on a Perkin–Elmer LS-50B spectrofluorimeter. The solvents used in our studies: MeOH, MeCN, methylcyclohexane were of either spectroscopic or HPLC grade. QY were calculated using as a reference quinine sulphate in 0.5 M H_2SO_4 (QY=0.53±0.02[24]) or tryptophan in water (QY=0.14[25]). The fluorescence lifetimes were measured using a time-correlated single-photon counting apparatus (the pico/femtosecond laser system, Ti:Sapphire 'Tsunami' laser pumped with an argon ion laser 'Beammlok' 2060 and R3809U-05 MCP-PTM; a half-width of the response function was about 35 ps) at the Laboratory of Ultrafast Laser

Spectroscopy, Adam Mickiewicz University, Poznań, Poland.[26] Fluorescence decay data were fitted by the iterative convolution to the sum of exponents

$$I(t) = \sum_i \alpha_i \exp(-t/\tau_i), \quad (1)$$

where α_i is the pre-exponential factor obtained from the fluorescence intensity decay analysis and τ_i is the decay time of the ith component. The adequacy of the exponential decay fitting was judged by visual inspection of the plots of weighted residuals and by the statistical parameter ${\chi_R}^2$ and shape of the autocorrelation function of the weighted residuals and serial variance ratio (SVR).

4.2. *N*-(*tert*-Butyloxycarbonyl)-3-[2-(2-pyridyl)-benzoxazol-5-yl]-L-alanine methyl ester (4a)

4.2.1. *N*-Boc-3-amino-L-tyrosine methyl ester (2). A mixture of *N*-Boc-3-nitro-L-tyrosine methyl ester (**1**) (1.02 g, 3 mmol, mp 90–91°C) and 5% palladium on active carbon in MeOH (30 mL) was stirred under a hydrogen atmosphere at room temperature for 90 min (TLC monitoring (CH_2Cl_2/MeOH/AcOH 100:10:1), R_f=0.9 (**1**), R_f=0.72 (**2**)). The catalyst was filtered off and the solvent evaporated in vacuo to give the brownish, oily title compound **2**, which was used at once without any additional purification in the next step of the synthesis.

4.2.2. *N*-Boc-3-[(pyrid-2-ylmethylene)amino]-L-tyrosine methyl ester (3a). To a solution of **2** (3 mmol) in absolute EtOH (6 mL), 2-pyridinecarboxaldehyde (0.321 g, 0.29 mL, 3 mmol) was added. The mixture was stirred at rt overnight (TLC monitoring (AcOEt), R_f=0.62; no substrate left). After this time the solvent was removed by evaporation and the brown, oily title compound **3a** was used at once without any additional purification in the next step of the synthesis.

4.2.3. *N*-(*tert*-Butyloxycarbonyl)-3-[2-(2-pyridyl)benzoxazol-5-yl]-L-alanine methyl ester (4a) and *N*-(*tert*-butyloxycarbonyl)-3-[7-bromo-2-(2-pyridyl)benzoxazol-5-yl]-L-alanine methyl ester (5a)—cyclization of 3a in the presence of NBS. To a solution of **3a** (2.5 mmol) in CH_2Cl_2 (10 mL) NBS (0.49 g, 2.75 mmol) was added and the reaction mixture was stirred for about 30 min (TLC monitoring (AcOEt/petroleum ether 2:3), R_f=0.14 (**4a**)). Then the solvent was removed by evaporation. The brown, oily residue was dissolved in ethyl acetate (30 mL) and washed with water (2×10 mL), a 5% solution of $NaHCO_3$ (2×10 mL) and a saturated aqueous solution of NaCl (2×10 mL), dried over anhydrous $MgSO_4$ and the solvent evaporated in vacuo. Recrystallizations of the crude mixture from AcOEt/petroleum ether 2:3 and then from ethanol gave the title compound **5a** (208.4 mg, 0.44 mmol, 18%) as a white solid. The filtrates were collected, evaporated and the residue was purified twice by means of column chromatography using as eluents AcOEt/petroleum ether (2:3) and AcOEt/petroleum ether (1:1). Then **4a** was isolated by means of semi-preparative HPLC. The mobile phase was a gradient running from 0 to 80% of an aqueous solution of acetonitrile over 120 min. This gave the title compound **4a** (108.3 mg, 0.27 mmol, 11%) as a cream solid. The purity of the obtained compounds was checked by means of analytical RP-HPLC. The mobile phase was a gradient running from 0 to 80% of an aqueous solution of acetonitrile with addition of 0.1% trifluoroacetic acid (TFA) over 60 min (t_R=43.14 min (**4a**), t_R=48.94 min (**5a**)).

Compound **4a**: mp 102–103.5°C; (Found: C, 63.35; N, 10.34; H, 5.70. $C_{21}H_{23}N_3O_5$ requires C, 63.46; N, 10.57; H, 5.83%); R_f (67% AcOEt/petroleum ether) 0.14; ν_{max} (liquid film) 3345.8 (w), 2979.1 (m), 1744.3 (s), 1710.4 (s), 1250.0 (s), 1167.3 (s), 754.2 (s) cm^{-1}; δ_H (400 MHz, $CDCl_3$) 8.81 (1H, d, J=4.4 Hz, $C^{3'}H$), 8.33 (1H, d, J=8.0 Hz, $C^{6'}H$), 7.89 (1H, t, J=8.0 Hz, $C^{5'}H$), 7.58 (2H, d, J=8.4 Hz, $C^{4'}H$, C^7H), 7.45 (1H, dd, J=5.2, 7.2 Hz, C^4H), 7.17 (1H, d, J=8.8 Hz, C^6H), 5.06 (1H, d, J=8.0 Hz, NH), 4.64 (1H, dd, J=5.6, 13.2 Hz, $C^{\alpha}H$), 3.72 (3H, s, OCH_3), 3.17–3.29 (2H, m, $C^{\beta}H_2$), 1.41 (9H, s, $(CH_3)_3$); δ_C (100 MHz, DMSO-d_6) 172.40 ($C^{\alpha}COO$), 161.47 (HNCOO), 155.34 (C^2), 150.14 ($C^{1'}$), 149.30 ($C^{3'}$), 145.21 (C^8), 141.20 (C^9), 137.62 ($C^{5'}$), 134.73 (C^5), 127.41 (C^6), 126.11 ($C^{6'}$), 123.48 ($C^{4'}$), 120.67 (C^4), 110.71 (C^7), 78.23 ($C(CH_3)_3$), 55.39 (C^{α}), 51.75 (OCH_3), 36.28 (C^{β}), 28.01 ($C(CH_3)_3$); *m/z* (FAB) 398 (25, MH^+), 342 (100), 298 (22), 238 (68), 210 (70%).

Compound **5a**: mp 173–175°C; (Found: C, 52.92; N, 8.75; H, 4.62. $C_{21}H_{22}N_3O_5Br$ requires C, 52.95; N, 8.82; H, 4.66%); ν_{max} (KBr) 3351.3 (m), 2983.0 (w), 1737.7 (s), 1686.4 (s), 1526.4 (s), 1250.3 (s), 1166.6 (s) cm^{-1}; δ_H (400 MHz, DMSO-d_6) 8.81–8.83 (1H, m, $C^{3'}H$), 8.33 (1H, d, J=8.0 Hz, $C^{6'}H$), 8.07 (1H, td, J=1.6, 7.6, 7.8 Hz, $C^{5'}H$), 7.76 (1H, d, J=1.2 Hz, C^6H), 7.61–7.69 (2H, m, C^4H, $C^{4'}H$), 7.34 (1H, d, J=8.0 Hz, NH), 4.26–4.32 (1H, m, $C^{\alpha}H$), 3.65 (3H, s, OCH_3), 2.96–3.23 (2H, m, $C^{\beta}H_2$), 1.29 (9H, s, $(CH_3)_3$); each proton signal was confirmed by ^1H–^1H COSY spectrum; δ_C (100 MHz, DMSO-d_6) 172.16 ($C^{\alpha}COO$), 161.59 (HNCOO), 155.30 (C^2), 150.23 ($C^{1'}$), 148.96 ($C^{3'}$), 147.35 (C^8), 141.81 (C^9), 137.71 ($C^{5'}$), 136.72 (C^5), 130.02 (C^6), 126.48 ($C^{6'}$), 123.79 ($C^{4'}$), 120.27 (C^4), 101.28 (C^7), 78.26 ($C(CH_3)_3$), 55.00 (C^{α}), 51.82 (OCH_3), 35.86 (C^{β}), 27.97 ($C(CH_3)_3$); *m/z* (FAB) 477 and 479 (28 and 30, MH^+), 420 and 422 (76 and 72), 399 (50), 376 (20), 342 (100), 316 (37%).

4.3. *N*-(*tert*-Butyloxycarbonyl)-3-[2-(4-pyridyl)-benzoxazol-5-yl]-L-alanine methyl ester (4b)

4.3.1. *N*-Boc-3-amino-L-tyrosine methyl ester (2). This was synthesized according to the above-mentioned procedure (Section 4.2.1) using 4 mmol (1.36 g) of *N*-Boc-3-nitro-L-tyrosine methyl ester (**1**).

4.3.2. *N*-Boc-3-[(pyrid-4-ylmethylene)amino]-L-tyrosine methyl ester (3b). To a solution of **2** (4 mmol) in absolute EtOH (6 mL) 4-pyridinecarboxaldehyde (0.428 g, 0.38 mL, 4 mmol) was added. After about 30 min, a yellow precipitate formed. The mixture was stirred at rt overnight (TLC monitoring (AcOEt): no substrate left). The precipitate was filtered off, washed with cold ethanol and used without any additional purification in the next step of the synthesis (0.95 g, 2.38 mmol, 59.5%, t_R=27.96 min).

Compound **3b**: mp 164–165°C; (Found: C, 63.31; N, 10.32;

H, 6.27. $C_{21}H_{25}N_3O_5$ requires C, 63.14; N, 10.52; H, 6.31%); R_f (AcOEt) 0.63; ν_{max} (KBr) 3376.7 (m), 2978.5 (w), 1751.1 (s), 1680.4 (s), 1599.5 (m), 1502.5 (s), 1219.0 (s), 1165.9 (s) cm^{-1}; δ_H (400 MHz, $CDCl_3$) 8.78 (2H, dd, J=1.6, 4.4 Hz, $C^{3'}H$, $C^{5'}H$), 8.66 (1H, s, N=CH), 7.74 (2H, dd, J=1.6, 4.4 Hz, $C^{2'}H$, $C^{6'}H$), 7.13 (1H, d, J=1.2 Hz, OH), 7.02 (2H, br. d, J=8.4 Hz, C^2H, C^6H), 6.96 (1H, d, J=8.4 Hz, C^5H), 5.00 (1H, br. s, NH), 4.58 (1H, br. s, $C^{\alpha}H$), 3.72 (3H, s, OCH_3), 3.01–3.12 (2H, m, $C^{\beta}H_2$), 1.42 (9H, s, $(CH_3)_3$); *m/z* (FAB) 401 (100, $(M+2)^+$), 344 (95), 302 (30), 212 (78%).

4.3.3. *N*-(*tert*-Butyloxycarbonyl)-3-[2-(4-pyridyl)benzoxazol-5-yl]-L-alanine methyl ester (4b) and *N*-(*tert*-butyloxycarbonyl)-3-[7-bromo-2-(4-pyridyl)benzoxazol-5-yl]-L-alanine methyl ester (5b)

4.3.3.1. Cyclization of 3b in the presence of NBS. To a solution of **3b** (0.798 g, 2 mmol) in CH_2Cl_2 (8 mL) NBS (0.39 g, 2.2 mmol) was added and the reaction mixture was stirred for about 30 min (TLC monitoring (AcOEt), R_f=0.65 (**4b**)). Then the solvent was removed by evaporation. The brown, oily residue was dissolved in ethyl acetate (30 mL) and washed with water (2×10 mL), a 5% solution of $NaHCO_3$ (2×10 mL) and a saturated aqueous solution of NaCl (2×10 mL), dried over anhydrous $MgSO_4$ and evaporated. Recrystallizations of the crude mixture from AcOEt gave **5b** as a white solid (830 mg, 1.7 mmol, 87%). The filtrates were collected, evaporated and the residue was purified by means of column chromatography using as an eluent AcOEt. Recrystallization from ethanol gave the title compound **4b** as a dark beige solid (77.8 mg, 0.19 mmol, 10%). The purity of obtained compounds was checked by means of analytical RP-HPLC. The mobile phase was a gradient running from 0 to 80% of aqueous solution of acetonitrile with addition of 0.1% TFA over 60 min (t_R=35.96 min (**4b**), t_R=41.88 min (**5b**)).

Compound **4b**: mp 137–139°C; (Found: C, 63.25; N, 10.67; H, 5.88. $C_{21}H_{23}N_3O_5$ requires C, 63.46; N, 10.57; H, 5.83%); R_f (AcOEt) 0.65; ν_{max} (KBr) 3354.1 (m), 2980.6 (m), 1737.7 (s), 1689.2 (s), 1637.4 (m), 1524.8 (m), 1254.1 (m), 1169.1 (s), 1059.3 (m), 721.8 (m) cm^{-1}; δ_H (400 MHz, $CDCl_3$) 8.81 (2H, dd, J=1.2, 4.6 Hz, $C^{3'}H$, $C^{5'}H$), 8.06 (2H, dd, J=1.2, 4.6 Hz, $C^{2'}H$, $C^{6'}H$), 7.57 (1H, s, C^4H), 7.55 (1H, d, J=8.4 Hz, C^7H), 7.21 (1H, dd, J=1.6, 8.8 Hz, C^6H), 5.14 (1H, d, J=8.0 Hz, NH), 4.66 (1H, dd, J=5.6, 13.6 Hz, $C^{\alpha}H$), 3.74 (3H, s, OCH_3), 3.18–3.31 (2H, m, $C^{\beta}H_2$), 1.42 (9H, s, $(CH_3)_3$); δ_C (100 MHz, DMSO-d_6) 172.56 ($C^{\alpha}COO$), 160.43 (HNCOO), 154.82 (C^2), 150.84 ($C^{3'}$, $C^{5'}$), 144.02 (C^8), 141.45 (C^9), 135.01 (C^5), 133.12 ($C^{1'}$), 127.78 (C^6), 120.71 ($C^{2'}$, $C^{6'}$), 120.68 (C^4), 110.72 (C^7), 79.10 ($C(CH_3)_3$), 55.35 (C^{α}), 51.75 (OCH_3), 36.21 (C^{β}), 28.00 ($C(CH_3)_3$); *m/z* (FAB) 398 (85, MH^+), 342 (100), 298 (35), 258 (24), 237 (58%).

Compound **5b**: mp 186–187°C; (Found: C, 52.82; N, 8.76; H, 4.62. $C_{21}H_{22}N_3O_5Br$ requires C, 52.95; N, 8.82; H, 4.66%); ν_{max} (KBr) 3334.0 (m), 2985.7 (w), 1741.0 (s), 1683.4 (s), 1529.5 (m), 1254.4 (m), 1164.6 (m), 1057.3 (m) cm^{-1}; δ_H (400 MHz, DMSO-d_6) 8.86 (1H, dd, J=1.6, 4.4 Hz, $C^{3'}H$, $C^{5'}H$), 8.08 (2H, dd, J=1.6, 4.4 Hz, $C^{2'}H$, $C^{6'}H$), 7.77 (1H, br. s, C^6H), 7.64 (1H, br. s, C^4H), 7.34 (1H, br. d, J=8.4 Hz, NH), 4.25–4.31 (1H, m, $C^{\alpha}H$), 3.65 (3H, s, OCH_3), 2.98–3.25 (2H, m, $C^{\beta}H_2$), 1.29 (9H, s, $(CH_3)_3$); each proton signal was confirmed by ^1H–^1H COSY spectrum; δ_C (100 MHz, DMSO-d_6) 172.13 ($C^{\alpha}COO$), 160.61 (HNCOO), 155.30 (C^2), 150.90 ($C^{3'}$, $C^{5'}$), 147.24 (C^8), 141.76 (C^9), 136.95 (C^5), 132.91 ($C^{1'}$), 130.33 (C^6), 120.83 ($C^{2'}$, $C^{6'}$), 120.34 (C^4), 101.26 (C^7), 78.28 ($C(CH_3)_3$), 54.99 (C^{α}), 51.82 (OCH_3), 35.83 (C^{β}), 27.98 ($C(CH_3)_3$); *m/z* (FAB) 477 and 479 (75 and 60, MH^+), 420 and 422 (78 and 86), 376 (25), 319 (30), 290 (35), 221 (100%).

4.3.3.2. Cyclization of 3b by means of the Mitsunobu reaction. To a solution of **3b** (0.798 g, 2 mmol) in anhydrous THF (30 mL) cooled to 0°C under argon, triphenylphospine (1.05 g, 4 mmol) was added. After stirring for about 5 min, diisopropylazodicarboxylate (0.81 g, 0.79 mL, 4 mmol) was added dropwise. The reaction mixture was stirred for about 20 min. Afterwards the ice bath was removed and the mixture was stirred under argon at rt for 3 days (TLC monitoring (AcOEt), R_f=0.65 (**4b**)). Then the solvent was removed by evaporation. The brown, oily residue was dissolved in diethyl ether (30 mL). The obtained white precipitate (triphenylphosphine oxide) was filtered off and washed with diethyl ether. The filtrate was evaporated and purified by means of column chromatography using as an eluent AcOEt. After recrystallization from ethanol 196.1 mg of **4b** (0.49 mmol, 25%) was isolated.

4.3.3.3. The influence of a solvent on the reaction yield of cyclization 3b in the presence of lead tetraacetate: general procedure. To a solution of **3b** (15.9 mg, 40 μmol) in a solvent (4 mL of toluene or CH_2Cl_2 or THF or dimethoxyethane or propylene carbonate or acetic acid) lead tetraacetate (26.8 mg, 60 μmol) was added and the reaction mixture was stirred at rt. In the case of DMF and DMSO (1 mL) 4 mg (10 μmol) of **3b** and 6.7 mg (15 μmol) of lead tetraacetate were used in the reaction. The progress of the reaction was monitored by means of analytical RP-HPLC after 60 min and about 25 h. The mobile phase was a gradient running from 0 to 80% of aqueous solution of acetonitrile with addition of 0.1% TFA over 60 min (t_R=27.96 min (**3b**), t_R=35.96 min (**4b**)).

The percentage of the product in the reaction mixture was determined basing on analytical RP-HPLC (10 μL of the each reaction mixture was dissolved in 100 μL of EtOH and 50 μL of the prepared solution was injected) and was as follows: toluene (58%), CH_2Cl_2 (60%), THF (58%), dimethoxyethane (15%), propylene carbonate (80%), acetic acid (59%), DMF (70%), DMSO (80%).

4.4. The removal of protecting groups: general procedure

4.4.1. Methyl ester group removal: *N*-(*tert*-butyloxycarbonyl)-3-[2-(2-pyridyl)benzoxazol-5-yl]-L-alanine (6a) and *N*-(*tert*-butyloxycarbonyl)-3-[2-(4-pyridyl)benzoxazol-5-yl]-L-alanine (6b). **4a** or **4b** was dissolved in a 1 M solution of NaOH in MeOH and stirred at rt for about an hour (TLC monitoring (AcOEt): no substrate left). Then the solvent was removed by evaporation. The residue was

dissolved in AcOEt and washed twice with a 1 M solution of $KHSO_4$ and three times with a saturated aqueous solution of NaCl, dried over anhydrous $MgSO_4$ and evaporated. Recrystallization of the crude mixture from EtOH gave the product (**6a** as a white solid: 28%; **6b** as a beige solid: 25%). The purity of the obtained compounds was checked by means of analytical RP-HPLC. The mobile phase was a gradient running from 0 to 80% of aqueous solution of acetonitrile with addition of 0.1% TFA over 60 min (t_R=36.64 min (**6a**), t_R=29.96 min (**6b**)).

Compound **6a**: mp 145–147°C; (Found: C, 62.45; N, 10.74; H, 5.60. $C_{20}H_{21}N_3O_5$ requires C, 62.65; N, 10.96; H, 5.52%); ν_{max} (KBr) 3372.7 (m), 2972.5 (w), 2936.4 (w), 1725.5 (s), 1679.2 (s), 1521.1 (s), 1465.6 (m), 1442.6 (m), 1290.4 (m), 1252.7 (m), 1163.7 (m), 1043.7 (w) cm^{-1}; δ_H (400 MHz, $CDCl_3$) 11.78 (1H, br. s, OH), 8.82 (1H, d, J=4.4 Hz, $C^{3'}H$), 8.31 (1H, d, J=8.0 Hz, $C^{6'}H$), 7.90 (1H, t, J=8.0 Hz, $C^{5'}H$), 7.57 (2H, d, J=8.4 Hz, $C^{4'}H$, $C^{7}H$), 7.48 (1H, dd, J=5.2, 7.2 Hz, $C^{4}H$), 7.19 (1H, d, J=8.8 Hz, $C^{6}H$), 5.10 (1H, d, J=8.0 Hz, NH), 4.63 (1H, dd, J=5.6, 13.2 Hz, $C^{\alpha}H$), 3.15–3.29 (2H, m, $C^{\beta}H_2$), 1.41 (9H, s, $(CH_3)_3$); δ_C (100 MHz, DMSO-d_6) 172.41 ($C^{\alpha}COO$), 161.44 (HNCOO), 155.40 (C^{2}), 150.11 ($C^{1'}$), 149.35 ($C^{3'}$), 145.24 (C^{8}), 141.22 (C^{9}), 137.61 ($C^{5'}$), 134.75 (C^{5}), 127.43 (C^{6}), 126.14 ($C^{6'}$), 123.46 ($C^{4'}$), 120.66 (C^{4}), 110.73 (C^{7}), 78.21 ($C(CH_3)_3$), 55.36 (C^{α}), 36.26 (C^{β}), 28.00 ($C(CH_3)_3$); *m/z* (FAB) 384 (25, MH^+), 329 (100), 284 (52), 238 (68), 210 (50%).

Compound **6b**: mp 252–253°C; (Found: C, 62.47; N, 10.84; H, 5.66. $C_{20}H_{21}N_3O_5$ requires C, 62.65; N, 10.96; H, 5.52%); ν_{max} (KBr) 3325.2 (m), 3075.4 (w), 2976.0 (m), 2931.6 (m), 2455.0 (br), 1912.4 (br), 1708.0 (s), 1618.0 (m), 1519.6 (s), 1365.3 (m), 1294.2 (m), 1234.7 (s), 1173.3 (s), 1058.5 (m), 1010.8 (m), 832.3 (m), 707.9 (m) cm^{-1}; δ_H (400 MHz, DMSO-d_6) 8.84 (2H, dd, J=1.6, 4.6 Hz, $C^{3'}H$, $C^{5'}H$), 8.09 (2H, dd, J=1.6, 4.4 Hz, $C^{2'}H$, $C^{6'}H$), 7.76 (2H, s, $C^{4}H$, NH), 7.41 (1H, d, J=8.4 Hz, $C^{7}H$), 7.14 (1H, d, J=8.4 Hz, $C^{6}H$), 4.13–4.19 (1H, m, $C^{\alpha}H$), 2.95–3.21 (2H, m, $C^{\beta}H_2$), 1.29 (9H, s, $(CH_3)_3$); δ_C (100 MHz, DMSO-d_6) 172.99 ($C^{\alpha}COO$), 160.01 (HNCOO), 155.04 (C^{2}), 150.48 ($C^{3'}$, $C^{5'}$), 148.82 (C^{8}), 140.76 (C^{9}), 135.04 (C^{5}), 133.12 ($C^{1'}$), 127.44 (C^{6}), 120.42 ($C^{2'}$, $C^{6'}$), 120.35 (C^{4}), 110.32 (C^{7}), 77.65 ($C(CH_3)_3$), 55.02 (C^{α}), 35.93 (C^{β}), 27.69 ($C(CH_3)_3$); *m/z* (FAB) 384 (45, MH^+), 329 (100), 284 (58), 238 (61), 210 (52), 168 (50), 148 (100%).

4.4.2. Boc group removal: 3-[2-(4-pyridyl)benzoxazol-5-yl]-L-alanine (7b). 6b was dissolved in 6.8 M HCl in dioxan and stirred at rt for about 30 min (TLC monitoring (CH_2Cl_2/EtOH/AcOH 100:5:1): no substrate left). Then the solvent was removed by evaporation. Recrystallization from EtOH gave the title compound **7b** as a light beige solid (40%). The purity of the obtained compound was checked by means of analytical RP-HPLC. The mobile phase was a gradient running from 0 to 80% of aqueous solution of acetonitrile with addition of 0.1% TFA over 60 min (**7b**: t_R=15.68 min).

Compound **7b**: mp 253–254°C; (Found: C, 63.74; N, 14.85; H, 4.67. $C_{20}H_{21}N_3O_5$ requires C, 63.60; N, 14.83; H, 4.63%); ν_{max} (KBr) 3439.0 (br), 2936.7 (br), 2095.7 (w), 1595.2 (s), 1545.7 (m), 1416.9 (m), 1346.1 (m), 1318.5 (m), 1200.0 (m), 1058.6 (m), 825.5 (m), 702.1 (m), 511.6 (m) cm^{-1}; δ_H (400 MHz, DMSO-d_6) 8.83 (2H, dd, J=1.6, 4.6 Hz, $C^{3'}H$, $C^{5'}H$), 8.15 (3H, br. s, NH_3^+), 8.10 (2H, dd, J=1.6, 4.4 Hz, $C^{2'}H$, $C^{6'}H$), 7.75 (2H, s, $C^{4}H$), 7.44 (1H, d, J=8.4 Hz, $C^{7}H$), 7.16 (1H, d, J=8.4 Hz, $C^{6}H$), 4.12–4.19 (1H, m, $C^{\alpha}H$), 2.96–3.22 (2H, m, $C^{\beta}H_2$); δ_C (100 MHz, DMSO-d_6) 172.97 ($C^{\alpha}COO$), 155.06 (C^{2}), 150.45 ($C^{3'}$, $C^{5'}$), 148.84 (C^{8}), 140.77 (C^{9}), 135.04 (C^{5}), 133.10 ($C^{1'}$), 127.44 (C^{6}), 120.42 ($C^{2'}$, $C^{6'}$), 120.35 (C^{4}), 110.31 (C^{7}), 55.06 (C^{α}), 35.91 (C^{β}); *m/z* (FAB) 284 (5, MH^+), 277 (100), 259 (14), 241 (100), 223 (18), 207 (40%).

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