ELSEVIER

Journal of Photochemistry and Photobiology A: Chemistry 143 (2001) 135–139

Journal of Photochemistry and Photobiology A:Chemistry

www.elsevier.com/locate/jphotochem

Influence of a substituent on amide nitrogen atom on fluorescence efficiency quenching of Tyr(Me) by amide group

Joanna Łukomska, Alicja Rzeska, Joanna Malicka, Wiesław Wiczk*

Faculty of Chemistry, University of Gdańsk, Sobieskiego 18, 80-952 Gdańsk, Poland

Received 26 February 2001; received in revised form 6 June 2001; accepted 3 July 2001

Abstract

An amide group quenches the *O*-methyl-tyrosine fluorescence with higher efficiency than that of tyrosine because of a lower ionization potential of the former. The influence of methyl substituents on an amide nitrogen atom of Tyr(Me) amide, as well as distance dependence of the efficiency of fluorescence quenching by an additional amide group in Tyr(Me)-Gly dipeptide supported suggestion that the photo-induced electron transfer from the excited fluorophore to an amide group is responsible for the fluorescence quenching of aromatic amino acid residue by an amide (peptide) group. The mono-exponential fluorescence intensity decay of Tyr(Me)-NHMe and much lower quenching efficiency than that observed for other Tyr(Me) derivatives studied, indicate that specific hydration of the whole molecule plays a crucial role in the fluorescence quenching process.

Keywords: *O*-methyl-tyrosine; Tyrosine derivatives; Fluorescence quenching; Time-resolved fluorescence

1. Introduction

The fluorescence of aromatic amino acids (phenylalanine, tyrosine and tryptophan) and their residues incorporated into a peptide or protein chain is a subject of extensive studies because of their use as internal probes in conformational analysis [1–3]. In the case of the tyrosine zwitterion and tyrosine derivatives with an ionized α-carboxyl group mono-exponential fluorescence decays were observed. The conversion of the α-carboxyl group into the corresponding amide or its protonation result in a complex fluorescence decay [1–5]. An explanation of this behavior was offered by the Gauduchon and Whal rotamer model [6]. This model assumes the existence of well-defined rotamers about the C^{α}–C^{β} bond, where interconversion time is considerably longer than the excited-state lifetimes of the rotamers. This rotamer model has been further extended to the C^{β}–C^{γ} bond conformers [7–10]. The different lifetimes of the rotamers arise from the interaction between the phenol fluorophore and the quenching groups. The quenching efficiency is controlled by the orientation of both groups and is distance dependent. In the case of tyrosine analogues and derivatives the rotamer model showed that the rotamer in which the phenol ring can come into the closest contact with the carbonyl group had the shortest fluorescence lifetime. Both, Cowgill [11] and Tournon et al. [12] suggested that the fluorescence quenching of an aromatic amino acid by the peptide (amide) group occurred by a charge transfer between the excited aromatic chromophore (phenol ring), as a donor, and electrophilic units in the amino-acid backbone (the carbonyl of the amide group), as an acceptor.

In this paper, we present the results of our studies on the influence of the substituent on the amide nitrogen atom on the fluorescence quenching efficiency of Tyr(Me) by an amide or peptide group in water solution.

2. Experimental

2.1. Synthesis

Boc-Tyr(Me)-OH was purchased from Bachem and used without further purification.

2.2. Synthesis of Boc-Tyr(Me)-NH_2

An amount of 0.89 g (3 mmol) of Boc-Tyr(Me)-OH was dissolved in anhydrous THF and cooled to 0°C in water–ice bath. To this solution 0.46 ml (3.3 mmol) of triethylamine (TEA) and 0.43 ml (3.3 mmol) of isobutylchloroformate were added. After 30 min, 0.70 ml (9 mmol) of 25% aqueous solution of NH_3 was added and reaction mixture was stirred for additional 3 h. After that time, the solvent was

* Corresponding author. Tel.: +48-58-34-50-353; fax: +48-58-34-10-357.
E-mail address: ww@chemik.chem.univ.gda.pl (W. Wiczk).

1010-6030/01/$ – see front matter
PII: S1010-6030(01)00521-4

evaporated under vacuum and residue was dissolved in ethyl acetate and washed with water (three times), 5% aqueous solution of Na_2CO_3 (three times), 1 M solution of $KHSO_4$ (three times) and water again. The organic layer was separated and dried over anhydrous $MgSO_4$. The solvent was evaporated under vacuum and residue was purified using column chromatography (Kieselgel 60, 0.063–0.2, Macherey Nagel) and EtOH/CH_2Cl_2 (1:9, v/v) as eluent.

The Boc-protection removal, from Boc-Tyr(Me) and all synthesized compounds, was accomplished by the action of 4 M HCl–dioxane at room temperature. The homogeneity of amino acid derivatives and dipeptides were assessed by thin layer chromatography (TLC) (*n*-BuOH/AcOH/H_2O = 4:1:1, $CHCl_3$/MeOH/AcOH = 85:10:5), analytical reversed phase high performance liquid chromatography (RP-HPLC) (a linear gradient of 0–80% CH_3CN with 0.1% TFA in H_2O within 60 min at flow rate of 1 ml min^{-1}, column Kromasil C-18, 4.6 mm × 250 mm, 5 μm).

2.2.1. ^1H NMR ($CDCl_3$, $(CD_3)_2SO$, in ppm) for the HCl × Tyr(Me)-NH_2

$\delta(C^{\beta}H)$ 2.95 (dd); $\delta(C^{\beta}H')$ 3.06 (dd); $\delta(OCH_3)$ 3.76 (s); $\delta(C^{\alpha}H)$ 3.91 (t); δ(Ar C^3H, C^5H) 6.92 (d); δ(Ar C^2H, C^6H) 7.21 (d); δ(CONH) 7.56 (s); δ(CONH′) 7.95 (s); $\delta(NH_3^+)$ 8.17 (br, s).

2.3. Synthesis of Boc-Tyr(Me)-NHMe and Boc-Tyr(Me)-$N(Me)_2$

Boc-Tyr(Me)-NHMe and Boc-Tyr(Me)-$N(Me)_2$ were obtained in the same way as described above using 0.23 (3.3 mmol) HCl × NH_2Me or 0.27 g (3.3 mmol) HCl × $N(Me)_2$, respectively.

2.3.1. ^1H NMR ($(CD_3)_2SO$, in ppm) for the HCl × Tyr(Me)-NHMe

$\delta(NCH_3)$ 2.75 (d); $\delta(C^{\beta}H)$ 2.97 (dd); $\delta(C^{\beta}H')$ 3.05 (dd); $\delta(OCH_3)$ 3.83 (s); $\delta(C^{\beta}H)$ 3.93 (t); δ(Ar C^3H, C^5H) 6.85 (d); δ(Ar C^2H, C^6H) 7.15 (d); δ(CONH) 6.10 (s); $\delta(NH_3^+)$ 8.14 (br, s).

2.3.2. ^1H NMR ($(CD_3)_2SO$, in ppm) for the HCl × Tyr(Me)-$N(Me)_2$

$\delta(NCH_3)$ 2.65 (s); $\delta(NCH_3')$ 2.85 (s); $\delta(C^{\beta}H)$ 2.94 (d); $\delta(C^{\beta}H')$ 3.03 (d); $\delta(OCH_3)$ 3.78 (s); $\delta(C^{\alpha}H)$ 3.88 (t); δ(Ar C^3H, C^5H) 6.97 (d); δ(Ar C^2H, C^6H) 7.15 (d); $\delta(NH_3^+)$ 8.10 (br, s).

2.4. Synthesis of Boc-Tyr(Me)-Gly-NH_2

An amount of 0.89 g (3 mmol) of Boc-Tyr(Me)-OH was dissolved in DMF and cooled to 0°C in water–ice bath. To this solution 0.46 g (3.3 mmol) of HOBt, 1.06 g (3.3 mmol) of TBTU and 1.25 ml (9.0 mmol) of TEA were added. After 30 min, the solvent was evaporated under vacuum and solid residue was isolated as described previously. The compound was purified by means of column chromatography (Kieselgel 60, 0.063–0.2, Macherey Magel) using EtOH/CH_2Cl_2 (1:9, v/v) as eluent.

2.4.1. ^1H NMR ($(CD_3)_2SO$, in ppm) for the HCl × Tyr(Me)-Gly-NH_2

δ(Tyr, $C^{\beta}H$) 2.90 (dd); δ(Tyr, $C^{\beta}H'$) 3.05 (dd); δ(Gly, $C^{\alpha}H$) 3.64 (dd); δ(Tyr, OCH_3) 3.73 (s); δ(Gly, $C^{\alpha}H'$) 3.77 (dd); δ(Tyr, $C^{\alpha}H$) 4.02 (dd); δ(Tyr, C^3H, C^5H) 6.88 (d); δ(Gly, CONH) 7.12 (br, s); δ(Tyr, C^2H, C^6H) 7.19 (d); δ(Gly, CONH′) 7.33 (br, s); δ(Tyr, NH_3^+) 8.13 (br, s), δ(Gly, NH) 8.69 (t).

2.5. Synthesis of Boc-Tyr(Me)-Gly-OCH_3

This compound was synthesized and purified as described above for Boc-Tyr(Me)-Gly-NH_2, using 0.36 g (3.3 mmol) HCl × $GlyOCH_3$.

2.5.1. ^1H NMR ($(CD_3)_2SO$, in ppm) for the Boc-Tyr(Me)-Gly-OCH_3

$\delta(C(CH_3)_3)$ 1.40 (s); δ(Tyr, $C^{\beta}H$) 2.90 (dd); δ(Tyr, $C^{\beta}H'$) 3.05 (dd); δ(Gly, OCH_3) 3.60 (s); δ(Gly, $C^{\alpha}H$) 3.66 (dd); δ(Tyr, OCH_3) 3.73 (s); δ(Gly, $C^{\alpha}H'$) 3.77 (dd); δ(Tyr, $C^{\alpha}H$) 4.02 (dd); δ(NH, urethane) 5.42 (d); δ(Tyr, C^3H, C^5H) 6.88 (d); δ(Tyr, C^2H, C^6H) 7.19 (d); δ(Gly, NH) 8.69 (t).

The ester group of Boc-Tyr(Me)-Gly-OCH_3 was removed by saponification in 1 M KOH in MeOH [13]. The progress of the reaction was monitored by means of TLC (*n*-BuOH/AcOH/H_2O = 4:1:1, $CHCl_3$/MeOH/AcOH = 85:10:5).

^1H NMR spectrum of Boc-Tyr(Me)-Gly-OH was similar to that of Boc-Tyr(Me)-Gly-OCH_3 except for lack of Gly, OCH_3 signal at 3.60 ppm.

All prepared compounds were purified by means of semi-preparative RP-HPLC, Kromasil C-8 column (10 mm × 250 mm, 5 μm) and their purity was checked by means of analytical RP-HPLC using a linear gradient of 0–80% CH_3CN with 0.1% TFA in H_2O within 60 min at flow rate of 1 ml min^{-1} and Kromasil C-18 column (4.6 mm × 250 mm, 5 μm).

2.6. Spectroscopic measurements

Photophysical parameters were obtained as described in our previous similar studies of 7-hydroxy-tetrahydroisoquinoline-3-carboxylic acid and its derivatives [14]. The absorption spectra were obtained using a Lambda 18 Perkin-Elmer spectrophotometer. The steady-state spectra were obtained on a Perkin-Elmer LS-50B spectrofluorimeter with 2.5 nm bandwidth for excitation and emission. The excitation wavelength was 275 nm. The fluorescence quantum yields were measured relative to the value for tyrosine in water (0.14) at room temperature [15].

Fluorescence decays were collected by the time-correlated single-photon counting techniques on an Edinburgh Analytical Instrument type CD-900 fluorometer interfaced with an IBM PC AT. The excitation source was a flash lamp filled with hydrogen (0.5 atm), operated at 40 kHz with about 6.5 kV across a 1 mm gap. The half width of the instrument response was about 1.0 ns. The excitation (275 nm) and emission (310 nm) wavelengths were selected by means of monochromators (about 10 nm bandwidth). The sample concentrations were about 7×10^{-5} M for steady-state measurements and 1.5×10^{-4} M in time-resolved experiments. All measurements were performed in double deionized water (Milli Q, Millipore) (pH = 5.5) at room temperature.

All spectroscopic measurements were done for non de-aerated water to keep consistency with published data.

3. Results

3.1. Absorption and steady-state fluorescence

The absorption spectrum of *O*-methylated tyrosine is blue-shifted by 2 to 3 nm as compared with that of tyrosine. The conversion of a carboxylic into an amide group does not change the shape and position of the absorption band (figure not shown). The fluorescence spectrum of Tyr(Me) like the absorption spectrum is blue-shifted with respect to that of tyrosine. For all the Tyr(Me) derivatives studied the conversion of the carboxylic group into the appropriate amide (peptide) group influenced on fluorescence intensity only, without changing the position and shape of the fluorescence spectrum (Fig. 1). The measured fluorescence quantum yields of Tyr(Me) and its derivatives are presented in Table 1. The conversion of the hydroxyl group of tyrosine into the methoxy group increases the fluorescence quantum yield of Tyr(Me) from 0.14 observed for Tyr to 0.201. The conversion of the carboxyl group to an amide group or to a peptide bond decreases the fluorescence quantum yield, depending on the type and the number of the substituents on the amide nitrogen atom. The lowest fluorescence quantum yield was observed for the Tyr(Me)-Gly-NH_2 dipeptide (0.041); whereas higher fluorescence quantum yields were observed for Tyr(Me)-Gly-OH and Tyr(Me) amides containing one or two methyl substituents on the amide nitrogen atom. The highest fluorescence quantum yield was observed for Tyr(Me)-NHMe (0.055). Lower, than for parent compound, fluorescence quantum yields of Tyr(Me) derivatives indicate high quenching efficiency of *O*-methyl-tyrosine fluorescence by the amide (peptide) group.

Table 1
Fluorescence quantum yield of Tyr(Me) derivatives in water at pH = 5.5

Compound	Quantum yield
Tyr(Me)	0.201
Tyr(Me)-NH_2	0.048
Tyr(Me)-NHMe	0.090
Tyr(Me)-$N(Me)_2$	0.055
Tyr(Me)-Gly	0.049
Tyr(Me)-Gly-NH_2	0.041

3.2. Time-resolved fluorescence

The fluorescence intensity decay of Tyr(Me) like that of tyrosine is mono-exponential with fluorescence lifetime equal to 5.02 ns. A very similar result (5.06 ns) was obtained

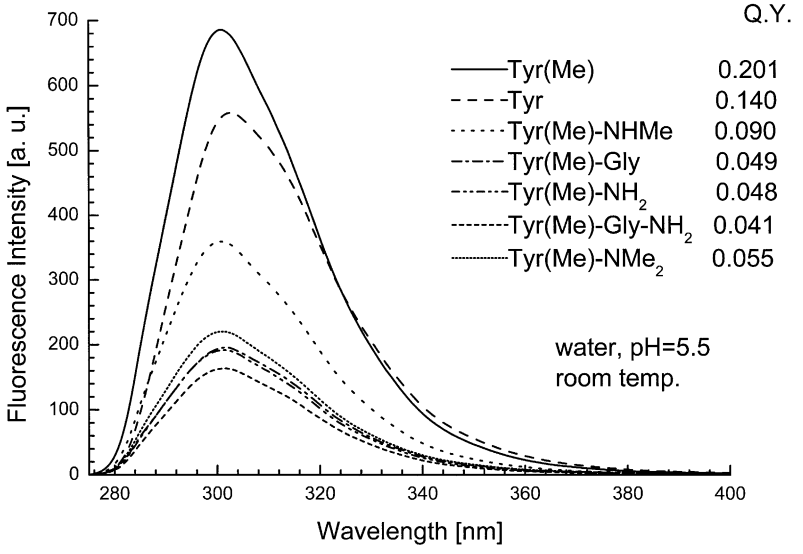

Fig. 1. Fluorescence spectra of Tyr(Me) and its derivatives in water at pH = 5.5. Tyr fluorescence spectrum is included for comparison.

Table 2
Fluorescence lifetimes (τ), pre-exponential factors (α), quality of fit (χ_R^2), average lifetime ($\langle\tau\rangle$), fluorescence (k_f) and non-radiative (k_{nr}) rate constants of Tyr(Me) derivatives

Compound	τ_1 (ns)	α_1	τ_2 (ns)	α_2	χ_R^2	$\langle\tau\rangle$ (ns)	$k_f \times 10^{-7}$ (s^{-1})	$k_{nr} \times 10^{-8}$ (s^{-1})
Tyr(Me)	5.02	1.000	–	–	1.04	5.02	4.00	1.6
Tyr(Me)-NH_2	1.23	1.000	–	–	1.36			
	1.46	0.567	0.18	0.433	1.03	1.35	3.6	7.1
Tyr(Me)-NHMe	2.46	1.000	–	–	1.03	2.46	3.7	3.7
Tyr(Me)-$N(Me)_2$	1.23	1.000	–	–	2.98			
	1.90	0.138	1.00	0.862	1.09	1.58	3.5	6.1
Tyr(Me)-Gly	1.53	1.000	–	–	3.03			
	1.61	0.564	0.30	0.436	1.08	1.45	3.4	6.6
Tyr(Me)-Gly-NH_2	1.34	1.000	–	–	2.51			
	1.46	0.534	0.44	0.467	1.07	1.25	3.6	7.7

by Ross et al. [4] and Laws et al. [5]. The fluorescence intensity decay of Tyr(Me) derivatives are more diversified. For all derivatives studied, except Tyr(Me)-NHMe, the fluorescence intensity decays are bi-exponential with diversified fluorescence lifetimes (Table 2). The shortest average fluorescence lifetime was observed for the Tyr(Me)-Gly-NH_2 dipeptide (1.25 ns). For the dipeptide with ionized glycin carboxyl group the fluorescence lifetime increased to the value of 1.45 ns. The substitution of hydrogen atom(s) by methyl group(s) on the amide nitrogen atom made the differences in the heterogeneity of fluorescence decay, as well as in the average fluorescence lifetimes. For the Tyr(Me)-NH_2, the average fluorescence lifetime is the shortest one among all of amides studied. The substitution of one hydrogen atom in the amide group by a methyl group causes that the fluorescence intensity decay of this compound becomes mono-exponential with relatively long fluorescence lifetime (2.46), while for di-substituted amide (Tyr(Me)-$N(Me)_2$) the heterogeneous fluorescence intensity decay appears ($\chi_R^2 = 2.98$), so bi-exponential function is needed to get correct fit. The average fluorescence lifetime for the compound mentioned above is longer (1.58 ns) than for unsubstituted Tyr(Me)-NH_2 (1.35 ns).

4. Discussion

The higher fluorescence quantum yield as well as the longer fluorescence lifetime of Tyr(Me) than that of unmodified Tyr indicate that the interaction of the hydroxyl group of Tyr with water is an efficient process of excited state deactivation. The formation of hydrogen bond-network between the phenol hydroxyl as well as amino acid moiety and the solvent molecules leads to a creation of non-fluorescent species. For tyrosine, the fluorescence rate constant is nearly equal to that of Tyr(Me), whereas the non-radiative rate constant is almost twice higher.

The decrease of the fluorescence quantum yield of Tyr(Me) derivatives in comparison to that of the parent molecule indicates that the amide (peptide) bond, like in the case of tyrosine [1–5] efficiently quenches methoxybenzene fluorescence. The calculated values of the fluorescence and non-radiative rate constants indicate that the amide group causes a small decrease of the k_f and simultaneously increases, about four times, the non-radiative rate constant (k_{nr}) (Table 2). Comparing the fluorescence quantum yields and the average fluorescence lifetimes of Tyr(Me) and Tyr(Me)-NH_2 (Tables 1 and 2), one can observe that the quenching efficiency of Tyr(Me) by amide group (express as a ratio of the fluorescence quantum yield or the fluorescence lifetime of the parent molecule to that of the amide derivatives), is about 4, whereas for tyrosine and its amide derivative, based on published fluorescence lifetimes, is about 3.2 [4,5] or 2.2 [6]. Also, higher quenching efficiency of Tyr(Me) fluorescence by an amino-acid residue in dipeptides Tyr(Me)-Gly-OH and Tyr(Me)-Gly-NH_2 (3.5 and 4.0, respectively) than for Tyr-Gly-OH and Tyr-Gly-NH_2 (2.5 [6], 1.5 [16] and 3.1 [6], respectively) was observed. Thus, the lower ionization potential of Tyr(Me) (8.05 eV [17], 8.09 eV [18] for *p*-methyl-anisole) as compared with that of tyrosine (8.22 eV [18], 8.23 eV [19] for *p*-methyl-phenol) causes higher quenching efficiency by the amide group. Observed by Klapper and co-workers [20–22], the dependence of the electron transfer rate constant between N- or C-terminal tyrosine residue or tyrosine with ionizated hydroxyl group and tryptophan radical, separated by peptide chain, on the ionization potential of tyrosine derivatives as well as our above-mentioned results, support the suggestion of Cowgill [11] and Tournon et al. [12] that the fluorescence quenching of an aromatic amino acid by the peptide (amide) group occurs by a charge (electron) transfer between the excited aromatic chromophore and the amide (peptide) group. This suggestion is also supported by the dependence of the quenching efficiency on the distance between the fluorophore and the quenching group. The conversion of the C-terminal carboxylic group of the Tyr(Me)-Gly or the Tyr-Gly dipeptide into an amide group to give Tyr(Me)-Gly-NH_2 or Tyr-Gly-NH_2 results in some additional quenching. However, this quenching is

substantially smaller than the quenching due to the presence of the peptide bond in the dipeptide with a free carboxylic group mentioned above or by an amide group in Tyr(Me)-NH_2 or Tyr-NH_2. Moreover, the lower quenching efficiency of an additional amide group can be explained by the fact that the peptide group is an electron delocalized one [22].

According to Seidel et al. [16] the electron-withdrawing or electron-donating groups modulate the fluorescence quenching efficiency of the peptide group. In the case of Tyr(Me)-$N(Me)_2$, two methyl substituents possess the electron-donating character thereby lowering the quenching efficiency (3.2) as compared with Tyr(Me)-NH_2 (3.70). The decrease of the electron transfer rate constant observed in tripeptide Tyr-Xx-$Trp^{\bullet}$ after replacement of Xx (Gly or Glu or Lys) by Pro residue [20] supports this explanation.

This coherent picture supporting the mechanism of fluorescence quenching of aromatic amino acids by an amide (peptide) group based on the photo-induced electron transfer from the excited fluorophore (donor) to an amide group (acceptor) is disturbed by the results obtained for the Tyr(Me)-NHMe. For this compound, the fluorescence intensity decay, in contrary to that of all Tyr(Me) derivatives studied, is mono-exponential with relatively large fluorescence lifetime (2.46 ns) and high fluorescence quantum yield (0.090). It caused the substantial decrease of the k_{nr} rate constant without changing the fluorescence rate constant (Table 2). Regarding the electron-donating character of a methyl substituent on the amide nitrogen atom the photophysical properties of this compound should be similar to that of Tyr(Me)-Gly-OH. The rotamer populations determined by ^1H NMR spectroscopy for Tyr-NH_2 by Ross et al. [4], Laws et al. [5] and for Tyr-NHMe by Kobayashi et al. [23] do not differ substantially. Also the conversion of the tyrosine hydroxyl group into the methoxy group do not alter the rotamer populations of Tyr [4,5], in the range of experimental error. Thus, the unusual photophysical properties of the Tyr(Me)-NHMe are difficult to explain. The specific hydration of the molecule as a consequence of forming a hydrogen bond-network between the fluorophore and the *N*-methyl amide quenching group as well as a protonated amino group could result in a specific electron donor–acceptor orientation which determine the electron transfer rate [24].

Acknowledgements

This work was supported by Grant 0369/T09/98/15 from the State Committee for Scientific Research (KBN Poland).

References

[1] J.M. Beechem, L. Brand, Ann. Rev. Biochem. 54 (1985) 43.
[2] M.R. Eftink, in: C.H. Schulter (Ed.), Methods in Biochemical Analysis, Vol. 35, Protein Structure Determination, Wiley, New York, 1991.
[3] J.B.A. Ross, W.R. Laws, K.W. Rousslang, H.R. Wyssbrod, in: J.R. Lakowicz (Ed.), Topics in Fluorescence Spectroscopy, Vol. 3, Biochemical Applications, Plenum Press, New York, 1992.
[4] J.B.A. Ross, W.R. Laws, J.C. Sutherland, A. Buku, P.G. Katsoyannis, I.L. Schwartz, H.R. Wyssbrod, Photochem. Photobiol. 44 (1986) 365.
[5] W.R. Laws, J.B.A. Ross, H.R. Wyssbrod, J.M. Beechem, L. Brand, J.C. Sutherland, Biochemistry 25 (1986) 599.
[6] P. Gauduchon, P. Whal, Biophys. Chem. 8 (1978) 87.
[7] D.M. Rayner, A.G. Szabo, Can. J. Chem. 56 (1978) 743.
[8] D.M. Rayner, A.G. Szabo, J. Am. Chem. Soc. 102 (1980) 554.
[9] R.A. Engh, L.X.-Q. Chen, G.R. Fleming, Chem. Phys. Lett. 126 (1986) 365.
[10] H.R. Gordon, H.C. Jarrell, A.G. Szabo, K.J. Willis, R.L. Somorjai, J. Phys. Chem. 96 (1992) 1915.
[11] R.W. Cowgill, Biochim. Biophys. Acta 133 (1967) 6.
[12] J.E. Tournon, E. Kuntz, M.A. ElBayoumi, Photochem. Photobiol. 16 (1972) 425.
[13] M. Bodansky, A. Bodansky, The Practice of Peptide Synthesis, Springer, Berlin, 1984, pp. 177–178.
[14] W. Wiczk, K. Stachowiak, P. Skurski, L. Łankiewicz, A. Michniewicz, A. Rój, J. Am. Chem. Soc. 118 (1996) 8300.
[15] R.F. Chen, Anal. Lett. 1 (1967) 35.
[16] C. Seidel, A. Orth, R.O. Greulich, Photochem. Photobiol. 58 (1993) 178.
[17] R.C. Cookson, D.E. Sadler, K. Salisbury, J. Chem. Soc., Perkin Trans. 2 (1981) 774.
[18] Y. Yagchi, W. Schnabel, A. Wilert, J. Bending, J. Chem. Soc., Faraday Trans. 90 (1994) 287.
[19] S. Spange, K. Maenz, D. Stadermann, Leibigs Ann. Chem. 10 (1992) 1033.
[20] M. Fraggi, M.R. DeFelippis, M.H. Klapper, J. Am. Chem. Soc. 111 (1989) 5141.
[21] M.R. DeFelippis, M. Fraggi, M.H. Klapper, J. Am. Chem. Soc. 112 (1990) 5641.
[22] A.K. Mishara, R. Chandrasekar, M. Fraggi, M.H. Klapper, J. Am. Chem. Soc. 116 (1994) 1414.
[23] J. Kobayashi, T. Higashijima, U. Nagi, T. Miyazawa, Biochim. Biophys. Acta 621 (1980) 190.
[24] Y. Sakata, S. Nakashima, Y. Goto, H. Tatemitsu, S. Misumi, T. Asahi, M. Hagihara, S. Nishkawa, T. Okata, N. Mataga, J. Am. Chem. Soc. 111 (1989) 8979.