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SYNTHESIS AND PHOTOPHYSICS OF ACRIDINE DERIVATIVES

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Highly fluorescent acridine derivatives were prepared by a multistep synthesis starting from 2-chlorobenzoic acid and the appropriate (aminophenyl)alkanoic acid by means of a modified Ullmann-Jourdan reaction followed by a cyclodehydration step, and by amination in the case of aminoacridine analogues. The obtained derivatives were subjected to photophysical studies (absorption and fluorescence). The compounds displayed interesting absorption behavior and high quantum yield of fluorescence. Acridine analogues bearing a free carboxylic group can serve as effective fluorescent probes in conformation analysis of peptides.

Keywords: acridone, 9-aminoacridine, fluorescence, photophysics.

INTRODUCTION

Acridine and its derivatives belong to heterocyclic analogues of anthracene, central ring containing nitrogen instead of a carbon atom. Derivatives of acridine, due to their specific construction (large, planar structure of tricycle, acceptor/donor properties of nitrogen atom), can interact with nucleic acids (RNA and DNA), which means that they can be used as a base for anticancer and anti-inflammatory drug design [1-5]. Additionally, acridine and some of its derivatives have interesting photophysical properties, i.e., high fluorescence quantum yield and high molar absorption coefficients [6-9]. Moreover, for some acridine derivatives, a shift of the absorption spectra towards longer wavelengths ("red shift") is observed, which allows one to extend the range of excitation wavelengths and as such to avoid interference with other chromophores. Such a photophysical profile of the compounds allows one to use them as effective chromophores, especially as energy donors, in conformational studies of biopolymers (peptides, nucleic acids) by estimating the energy transfer between donor and acceptor, which leads to estimation of the interchromophoric distance [10], and in studies of enzymes (kinetics and mechanism of action and inhibition) [11].

There is a need in our laboratory for new, effective, and easily synthesized chromophores that can be used in conformational and enzymatic studies. Therefore we decided to evaluate acridine derivatives as potential chromophores. To perform our studies we prepared from substituted anthranilic acids **1a-d** acridine derivatives bearing the carboxyl group, which allowed us to use them as acylating agent for peptide amino groups (compounds **2a-d,** Scheme 1). Starting from the appropriate acridones, compounds **3a-d** containing the amino group were synthesized and used as amino components to be acylated by carboxy groups of peptides (Scheme 2).

RESULTS AND DISCUSSION

Syntheses of acridine derivatives, both acridone analogues such as 9,10-dihydro-9-oxoacridine-2-carboxylic acid (**2a**), -2-acetic acid (**2b**), 3-(9,10-dihydro-9-oxoacridine-2-yl)propionic acid (**2c**), 9,10-dihydro-9-oxo-acridine-4-carboxylic acid (**2d**) and aminoacridine analogues, *viz.* 9-amino-2-carboamoylacridine

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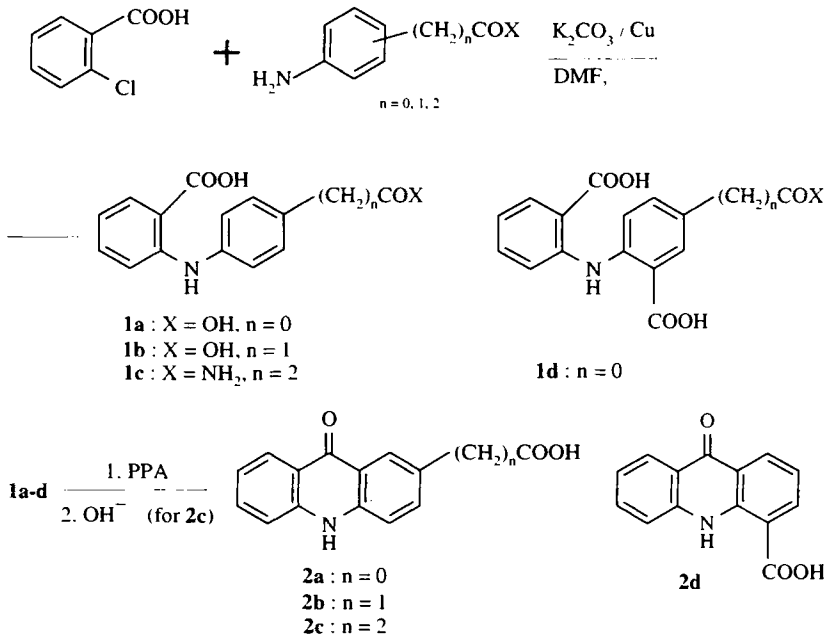
hydrochloride (**3a**), 9-amino-2-(carbamoylmethyl)acridine hydrochloride (**3b**), (2-carboxyamide)ethyl-9-amino-2-(2-carbamoylethyl)acridine hydrochloride (**3c**), 4-carboxyamide-9-amino-4-carbamoylacridine hydrochloride (**3d**), were performed as outlined in Schemes 1 and 2.

Scheme 1



General procedures for preparation of the compounds are included in the Experimental section, whereas the parameters of the syntheses and final products are presented in Tables 1-3. Starting compounds for the acridine derivatives (substituted anthranilic acids **1a-d**, Scheme 1) were prepared by a modified Ullmann-Jourdan reaction [12]. The cyclodehydration process was accomplished by heating with polyphosphoric acid. Synthesis of aminoacridine derivatives from the appropriate acridones was achieved by reaction with thionyl chloride followed by the action of phenol and ammonia. Such obtained free bases were finally converted into hydrochloride salts by

Scheme 2

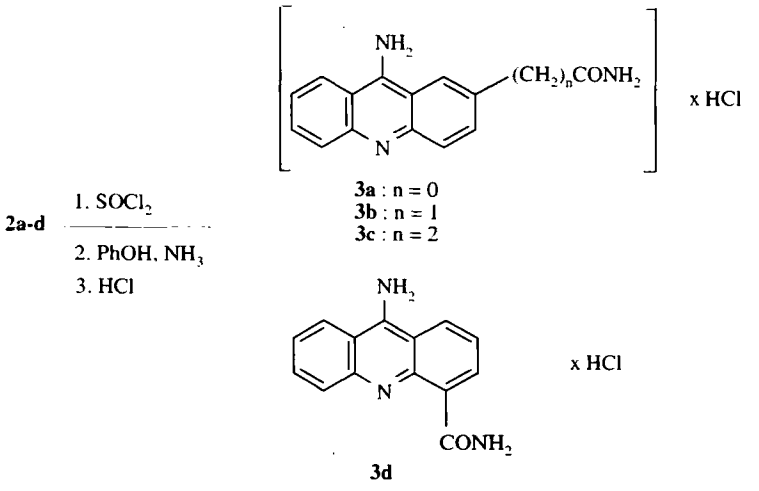


TABLE 1. Characteristics of Acridone Derivatives **2a-d**

Compound (formula)	Yield*, %	mp, °C (ref. mp)	MS $[M+1]^+$	IR spectrum, cm^{-1}	^1H NMR spectrum, ppm (CD_3OD)
2a $C_{14}H_9NO_3$	48.4	>340 dec. (>360 [1])	240	3262 (NH), 1678 (COOH), 1632 (CO)	7.35-8.42 (m, 7H, arom.)
2b $C_{15}H_{11}NO_3$	45.6	303-306 dec. (299-301 [1])	254	3296 (NH), 1697 (COOH), 1637 (CO)	3.77 (s, 2H, CH_2); 7.32-8.45 (m, 7H, arom.)
2c $C_{16}H_{13}NO_3$	45.9	301-304 dec.	268	3274 (NH), 1687 (COOH), 1630 (CO)	2.61 (t, 2H, $\underline{CH_2}CH_2CO$, J = 7.8 Hz); 3.05 (t, 2H, $CH_2\underline{CH_2}CO$, J = 7.8 Hz); 7.28-8.34 (m, 7H, arom.)
2d $C_{14}H_9NO_3$	92.7	330-334 dec. (324-325 [14])	240	3226 (NH), 1694 (COOH), 1620 (CO)	7.43-8.48 (m, 7H, arom.)

* From 4(or 2)-(2-carboxyphenylamino)benzenealkanoic acid **1**.

TABLE 2. Characteristics of 9-Aminoacridine Derivatives **3a-d**

Compound (formula)	Yield*, %	mp, °C	MS $[M+1]^+$	IR spectrum, cm^{-1}	^1H NMR spectrum, ppm (CD_3OD)
3a $C_{14}H_{11}ClN_3O$	24.6	>340 dec.	238	3384 (NH), 3106 (NH), 1669 (amide 1, CO), 1634 (amide 2, NH)	7.55-8.52 (m, 7H, arom.)
3b $C_{15}H_{13}ClN_3O$	45.2	>340 part. dec.	252	3365 (NH), 3096 (NH), 1669 (amide 1, CO), 1634 (amide 2, NH)	3.92 (s, 2H, CH_2) 7.62-8.64 (m, 7H, arom.)
3c $C_{16}H_{15}ClN_3O$	43.9	>240 dec.	266	3380 (NH), 3135 (NH), 1661 (amide 1, CO), 1638 (amide 2, NH)	2.92 (t, 2H, $\underline{CH_2}CH_2CO$, J = 7.9 Hz); 3.21 (t, 2H, $CH_2\underline{CH_2}CO$, J=7.9 Hz); 7.65-8.55 (m, 7H, arom.)
3d $C_{14}H_{11}ClN_3O$	27.2	224-227 part.dec.	238	3360 (NH), 3124 (NH), 1675 (amide 1, CO), 1626 (amide 2, NH)	7.63-8.65 (m, 7H, arom.)

* From compounds **2a-d**.

treatment with hydrogen chloride. The yields are rather moderate mostly due to the fact that they are multistep processes and some of the steps required many recrystalizations and/or column chromatography. Our attempt to hydrolyze the amide moiety in aminoacridines **3a-d** failed because during that process the 9-amino group was also hydrolyzed.

Photophysical parameters of the compounds are presented in Table 3 and in Figs. 1 and 2 (absorption) and Figs. 3 and 4 (fluorescence). As was expected, in the case of absorption, carboxy and carbamoyl substituents at position 2 of the acridine system caused a small bathochromic shift. The influence of an alkyl spacer was observed only for acridone analogues, but there was no significant difference between methylene and ethylene groups. On the other hand, the substituents at position 4 resulted in a stronger bathochromic shift (Figs. 1, 2).

The fluorescence spectra of all the studied compounds were recorded with excitation at λ = 380 nm and they are presented at Figs. 3 and 4.

Apart from 4-substituted derivatives, all the investigated acridine analogues displayed high quantum yield of fluorescence (Table 3). The much lower quantum yield of fluorescence found for the 4-substituted acridine analogues is due to the possibility of excited state proton transfer between the acridine nitrogen atom and the carboxylate moiety [13]. Additional proof of the proton transfer process came from the decrease in short-wave fluorescence and observation of an extra fluorescence band with maximum at 510 nm for the acridone analogue. Maxima of the fluorescence spectra are shifted towards the long-wave region (420 - 480 nm) in comparison with the acridine spectrum, which can be useful for conformational studies of peptides and proteins by means of fluorescence methods. The quantum yield of fluorescence of the appropriate pair of acridone and aminoacridine was found to be generally similar. From the point of view of application in conformational studies of peptides and proteins, aminoacridines are more suitable than acridines because their solubility is higher. At the same time, aminoacridines are less stable since they form acridones upon hydrolysis.

TABLE 3. Photophisical Data of Acridone and 9-Aminoacridine Derivatives

Compound (concentration in MeOH)	Absorption		Fluorescence	
	λ_{max}* [nm]	log ε	λ_{max}* [nm]	QY
2-(COOH)-Acridone **2a**	317	3.67	413	0.925
(c = 2.926 × 10^{-5} M)	379	3.61	435	
	396	3.63		
2-(CH_2COOH)-Acridone **2b**	383	3.86	419	0.714
(c = 4.028 · 10^{-5} M)	401	3.87	441	
2-(CH_2CH_2COOH)-Acridone **2c**	384	3.85	419	0.702
(c = 3.292 · 10^{-5} M)	402	3.86	441	
4-(COOH)-Acridone **2d**	391	3.97	425	0.268
(c = 4.013 × 10^{-5} M)	408	4.06	449	
			505	
2-($CONH_2$)-9-Aminoacridine·HCl **3a**	383	3.66	433	0.732
(c = 3.142 · 10^{-5} M)	402	3.86	459	
	424	3.81	485	
2-(CH_2CONH_2)-9-Aminoacridine·HCl **3b**	384	3.65	435	0.765
(c = 3.267 × 10^{-5} M)	403	3.84	460	
	424	3.82	487	
2-($CH_2CH_2CONH_2$)-9-Aminoacridine·HCl **3c**	384	3.73	435	0.698
(c = 2.982 · 10^{-5} M)	403	3.89	460	
	425	3.80	487	
4-($CONH_2$)-9-Aminoacridine·HCl **3d**	392	3.57	449	0.439
(c = 3.215 · 10^{-5} M)	410	3.79	472	
	432	3.77		

* λ_{max} denotes position of vibrational bands.

The prepared acridine analogues, because of their interesting photophysical properties, can be used in many aspects of studies of biopolymers. In particular, their high fluorescence quantum yields and the possibility of direct incorporation into the peptide chain by either a carboxyl or an amino group are worthy of mention. Moreover, synthesis of the compounds is quite straightforward, which make them additionally useful as chromophoric markers.

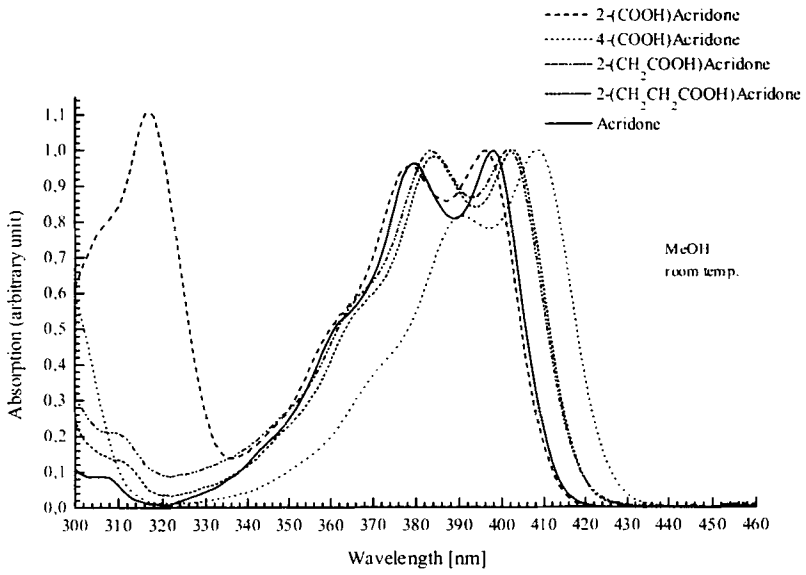


Fig. 1. Normalized absorption spectra of acridone derivatives **2a-d** (MeOH, room temp.).

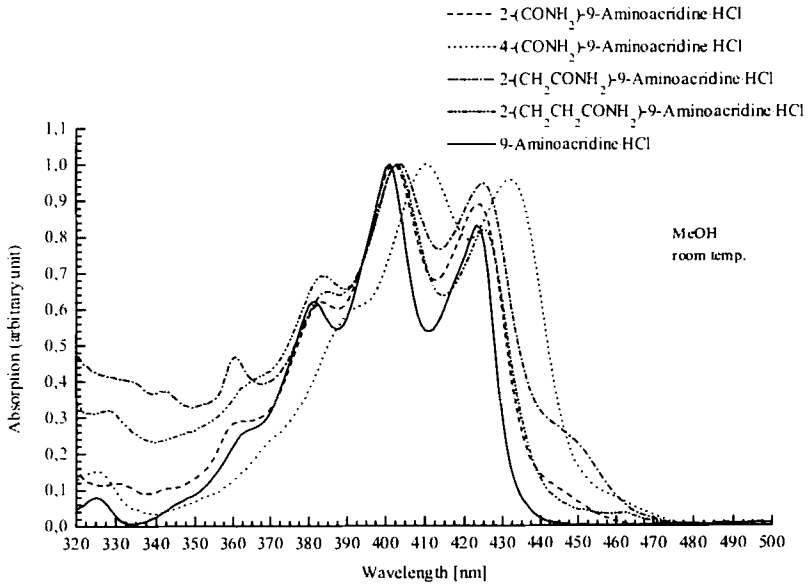


Fig. 2. Normalized absorption spectra of 9-aminoacridine derivatives **3a-d** (MeOH, room temp.).

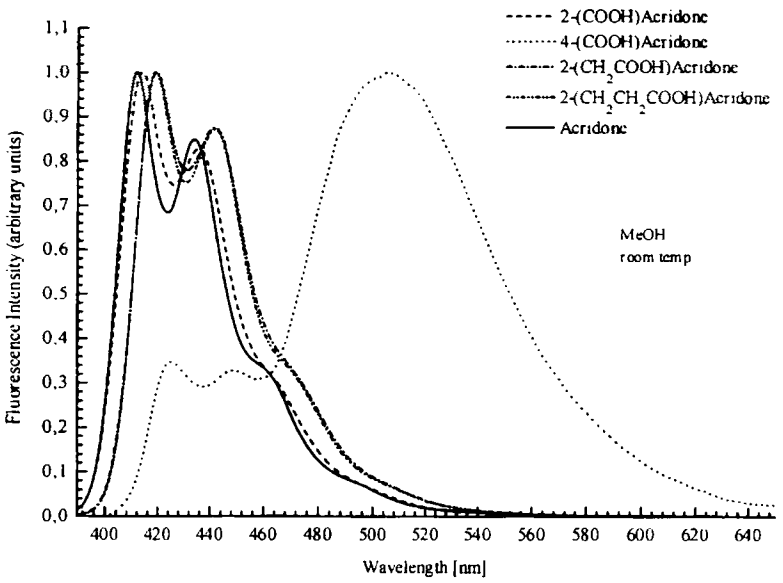


Fig. 3. Normalized fluorescence spectra of acridone derivatives **2a-d** (MeOH, room temp.).

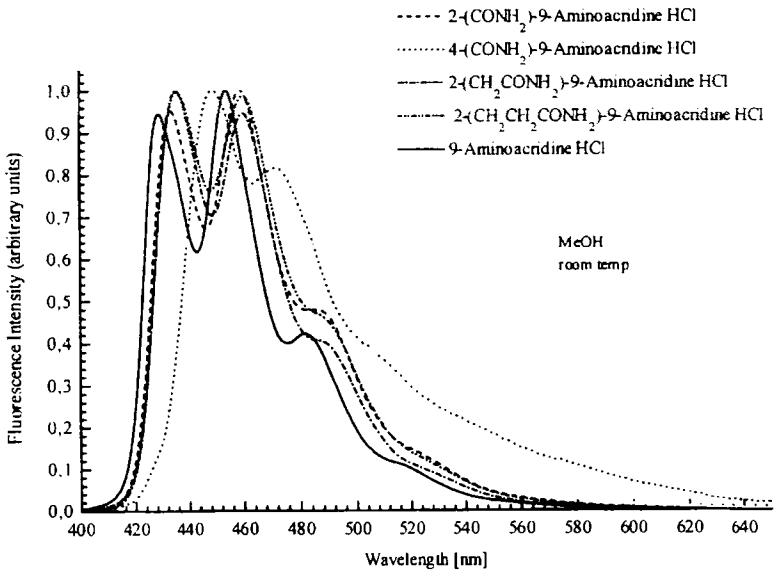


Fig. 4. Normalized fluorescence spectra of 9-aminoacridine derivatives **3a-d** (MeOH, room temp.).

EXPERIMENTAL

^{1}H NMR spectra were recorded on a Varian Mercury 400 Hz apparatus in CD_3OD with TMS as internal standard. IR spectra were obtained with a Bruker IFS 66 spectrophotometer in KBr pellets. Recording of absorption spectra was performed using a Perkin Elmer Lambda 18 spectrophotometer. Fluorescence spectra were recorded on a Perkin Elmer LS-50 spectrophotometer with 2.5 nm spectral width. The quantum yield was calculated relative to quinine sulfate using 1M H_2SO_4 as a standard (QY = 0.55). Mass spectra were obtained

using a VG Masslab Trio-3 spectrometer. The homogeneity of compounds was accessed by TLC (aluminum sheets precoated with silicagel 60 F-254, Merck) and by HPLC analysis on a Chromsil column 4.6×250 mm, C-8, 5 μm, using gradient 0-100% B (B = 80% CH_3CN + 0.08% TFA, A = H_2O + 0.1% TFA). Melting points are given uncorrected.

2-Chlorobenzoic acid, 4-aminophenylacetic acid, and polyphosphoric acids were purchased from Lancaster; anthranilic acid and 4-aminobenzoic acid were purchased from Aldrich. All compounds used were p.a. grade.

Preparation of 4-(2-Carboxyphenylamino)benzenealkanoic Acids (1a-c) (according to optimized procedure [1]). A mixture of 2-chlorobenzoic acid (1 eq.), 4-aminophenylalkanoic acid (1.1 eq.), anhydrous potassium carbonate (3 eq.), and freshly prepared copper powder (0.2 eq.) in dimethylformamide was heated with stirring at 140-145°C for 6-10 h under a stream of argon. The resulting mixture was then cooled to 60°C and the reaction was quenched with ice-water. The dark solution was decolorized with activated carbon, filtered, and carefully neutralized with 6M HCl (in an ice bath). The precipitate formed was filtered off or centrifuged, washed with water, and dried in a desiccator over P_2O_5. The crude product was recrystallized from aqueous ethanol or dimethylformamide/water. In the case of compound **1c**, 4-aminophenylpropionic acid amide was used instead of the free acid. The final 4-(2-carboxyphenylamino)phenylpropionic acid amide was purified by multiple crystallization from EtOH/H_2O.

N-(2-Carboxyphenyl)anthranilic Acid (1d) was prepared by the reaction of 2-chlorobenzoic acid with anthranilic acid using the procedure described for **1a-c.**

Preparation of 9,10-Dihydro-9-oxoacridine-2(or 4)-alkanoic Acids (2a-d) (cf. optimized literature procedure [1]). Polyphosphoric acid (10 ml/g of substrate) was heated with stirring to 80-90°C, and 4-(2-carboxyphenylamino)benzenealkanoic acid was added during 10-15 min. The reaction mixture was stirred at this temperature for an additional 1 to 1.5 h (in the case of 9,10-dihydro-9-oxoacridine-2-propionic carboxamide for 0.5 h), then cooled to room temperature and poured slowly into ice-water. The resulting suspension was boiled for 5 min, chilled to room temperature, and finally filtered. The crude product was recrystallized from glacial acetic acid. In the case of compound **2c** the crude product was purified by column chromatography on silicagel using CH_2Cl_2:MeOH (10:1) as an eluent to give pure 9,10-dihydro-9-oxoacridine-2-propionic acid amide. This compound was next hydrolyzed to the acid derivative with boiling 1M NaOH. The characteristics of resulting products **2a-d** are presented in Table 1.

Preparation of 2(or 4)-Carbamoylalkyl-9-aminoacridine Hydrochloride Salts (3a-d) (general procedure). A suspension of the appropriate acridone derivative in thionyl chloride (1 g, 3 ml) containing a catalytic amount of DMF was refluxed with stirring for 1-2 h. The solution was evaporated to dryness *in vacuo*. Removal of traces of $SOCl_2$ was accomplished by additional evaporation with toluene (3 times). The obtained 9-chloro derivative was dissolved in phenol and heated slowly to 100°C for 15 min. The resulting mixture was then cooled to 50°C and a stream of dry ammonia was passed through the solution while the temperature was raised to 110-120°C and held for 0.5-1 h. The mixture was then cooled and diluted with of 5M NaOH. The resulting solid was collected and crystallized from aqueous EtOH. The free base was then dissolved in MeOH, treated with HCl/dioxane (2 eq), evaporated to dryness, and finally crystallized from MeOH/Et_2O. The characteristics of products **3a-d** are presented in Table 2.

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REFERENCES

1. B. Taraporewala and J. M. Kaufmann, *J. Pharm. Sci.*, **79**, 173 (1990).
2. G. J. Atwell, B. F. Cain, B. C. Baguley, G. J. Finlay, and W. A. Denny, *J. Med. Chem.*, **27**, 1481 (1984).
3. D. W. Rewcastle, G. J. Atwell, D. Chambers, B. C. Baguley, and W. A. Denny, *J. Med. Chem.*, **29**, 472 (1986).
4. G. J. Atwell, G. W. Rewcastle, B. C. Baguley, and W. A. Denny *J. Med. Chem.*, **30**, 653 (1987).

5. W. A. Denny, G. J. Atwell, R. F. Anderson, and W. R. Wilson, *J. Med. Chem.*, **33**, 1288 (1990).
6. Y. Kubota and Y. Motoda, *J. Phys. Chem.* **84**, 2855 (1980).
7. C-H. Tung, T. Zhu, H. Lackland, and S. Stein, *Peptide Res.* **5**, 115 (1992).
8. C. Z. Dong, H. De Rocquigny, E. Remy, S. Mellac, M. C. Fournie-Zaluski, and P. B. Roques, *J. Peptide Res.* **50**, 269 (1997).
9. N. Bahr, E. Tierney, and J.-L. Reymond, *Tetrahedron Lett.*, **38**, 1489 (1997).
10. L. Jankiewicz, J. Malicka, and W. Wiczk, *Acta Biochim. Pol.*, **44**, 277 (1997).
11. D. Dwojakowska, A. Debrowska, L. Jankiewicz, W. Wiczk, and K. Stachowiak, *Peptides 1998, Proceedings of the 25th European Peptide Symposium*, Ed. S. Bajusz and F. Hudecz, Akademiai Kiado, Budapest, 1998, p. 632.
12. J. R. Pfister, I. T. Harrison, and J. H. Fried, U.S. Patent 3 835 139, 1974; *Chem. Abstr.* **82**, 16711 (1979).
13. L. G. Arnaut and S. J. Formosinho, *J. Photochem. Photobiol. A: Chem.*, **75**, 1 (1993).
14. G. W. Rewcastle and A. Denny, *Synthesis*, 217 (1985).