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Synthesis and photophysical studies of new pyrenylamino acids

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ABSTRACT

Two new pyrenylamino acid derivatives were synthesized from β-bromodehydroalanine derivatives in good yields using addition and elimination reactions.
The photophysical properties of the two new pyrenylamino acids were studied in several solvents. Steady-state and time-resolved fluorescence measurements revealed that the bipyrenylamino acid undergoes excimer formation, this process being solvent dependent. Rate constants for excimer formation and dissociation were calculated. The monopyrenylamino acid exhibits a photophysical behavior similar to that of pyrene, including the sensitivity to solvent polarity.
The results point to a potential use of these new pyrenyl derivatives as fluorescent probes for peptides and proteins.

1. Introduction

Most of fluorescence studies concerning peptides and proteins make use of the natural fluorescent amino acids, tyrosine, tryptophan, and phenylalanine. However, the development of new fluorescent non-natural amino acids that can allow selective excitation and detection and act as site specific probes constitutes an important area of research in peptide chemistry.[1–3]

Pyrene has unique photophysical properties, such as high fluorescence quantum yield, long excited-state lifetimes and the ability to form excimers when two pyrene moieties are in close proximity.[4,5] There are several reports describing the synthesis and applications of pyrenylalanine as a fluorescent probe in peptides and proteins.[6–8] Previously, we have reported the synthesis of several new pyrenylamino acid derivatives.[9,10] These compounds were prepared from dehydroamino acid derivatives using several types of reactions, namely Michael additions, substitution reactions and palladium catalyzed cross-couplings. The photophysical properties of some of these compounds showed their potential utility as fluorescence probes for biological systems.[9,10] Continuing our work concerning the synthesis of non-proteinogenic fluorescent amino acids, we decided to prepare three new pyrenylamino acids using as substrates β,β-disubstituted dehydroalanine derivatives and a strategy previously developed by us.[11,12] Recently, we described the synthesis of α-substituted-β,β-dibromoalanines from a β,β-dibromodehydroalanine by treatment with oxygen nucleophiles and primary amines. Furthermore, by reacting a β-bromo-β-triazolyldehydroalanine with primary amines it was possible to prepare several α-amino-β-iminoalanines.[12] These compounds afforded the α-aminoglycines when treated with silica in dichloromethane.[12]

In this work, this methodology was applied to the synthesis of a α-(pyren-1-yl)methylamino-β,β-dibromoalanine, a α-(pyren-1-yl)methylamino-β-iminoalanine, and a α-(pyren-1-yl)methylaminoglycine. The photophysical properties of these compounds were studied in several solvents of different polarity and time-resolved fluorescence studies were performed.

2. Results and discussion

The methyl ester of the *N*-*tert*-butoxycarbonyl-β,β-dibromodehydroalanine (**1**) was treated with pyren-1-ylmethanamine hydrochloride in the presence of triethylamine (NEt_3) in methanol to give the α-addition product the methyl 3,3-dibromo-2-(*tert*-butoxycarbonylamino)-2-[((pyren-1-yl)methylamino)]propanoate **2** in 86% yield (Scheme 1). Compound **1** was obtained from the methyl ester of *N*-*tert*-butoxycarbonyldehydroalanine by treatment with *N*-bromosuccinimide (NBS) followed by NEt_3. In the ^1H NMR spectrum of compound **2** it is possible to observe the high chemical shift of the β-CH proton (6.32 ppm) due to the electron-withdrawing effect of the two bromine atoms.

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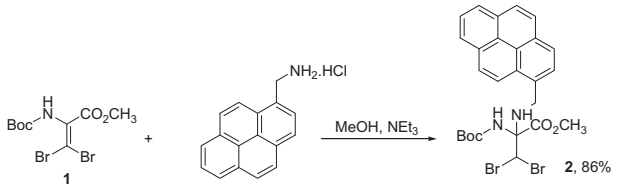
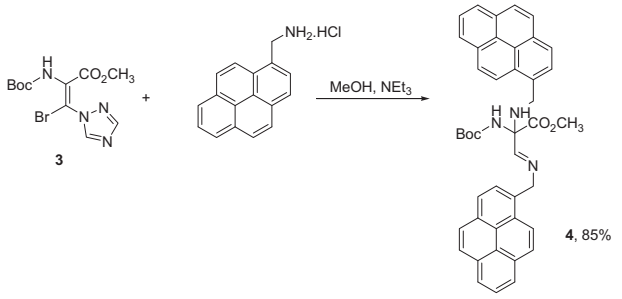


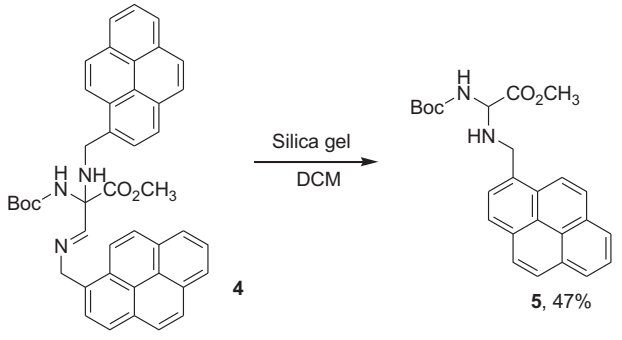
Scheme 1. Synthesis of the methyl 3,3-dibromo-2-(*tert*-butoxycarbonylamino)-2-[(pyren-1-yl)methylamino]propanoate (**2**).

The *Z*-isomer of the β-bromo,β-triazolyldehydroalanine (***Z*-3**) reacted with pyren-1-ylmethanamine hydrochloride in the presence of NEt_3 in methanol to afford the (*E*)-methyl 2-(*tert*-butoxycarbonylamino)-2-[(pyren-1-yl)methylamino]-3-[(pyren-1-yl) methylimino]propanoate (**4**) (Scheme 2). Compound ***Z*-3** was obtained by a sequential Michael addition reaction between the methyl ester of the *N*-(4-toluenesulfonyl)-*N*-(*tert*-butoxycarbonyl) dehydroalanine and 1,2,4-triazole, followed by halogenation with NBS and NEt_3.[12] Compound **4** was obtained after α-addition, β-substitution, and β-elimination. The stereochemistry of compound **4** was determined by NOE difference experiments by irradiating the β-CH and observing a NOE enhancement on the $=NCH_2$ protons.[12]



Scheme 2. Synthesis of (*E*)-methyl 2-(*tert*-butoxycarbonylamino)-2-(pyren-1-ylmethylamino)-3-(pyren-1-ylmethylimino)propanoate (**4**).

Compound **4** afforded the corresponding methyl 2-(*tert*-butoxycarbonylamino)-2-[(pyren-1-yl)methylamino]acetate (**5**) by treatment with silica in dichloromethane (Scheme 3). The mechanism proposed for this reaction involves the addition of water to the imine carbon atom followed by elimination of an amide.[12]



Scheme 3. Synthesis of 2-(*tert*-butoxycarbonylamino)-2-[(pyren-1-yl)methylamino] acetate (**5**).

The photophysical properties of compounds **4** and **5** were studied in several solvents of different polarity. The maximum absorption (λ_{abs}) and emission wavelengths (λ_{em}), molar absorption coefficients (ε) and fluorescence quantum yields (Φ_F) are presented in Table 1. The normalized absorption and fluorescence spectra of compounds **4** and **5** are presented in Figs. 1 and 2, respectively.

The absorption spectra of compounds **4** and **5** show intense bands with high molar absorption coefficients at the lowest energy peak ($\varepsilon \geq 2\times10^4$ M^{-1} cm^{-1}, Table 1), typical of a $\pi-\pi^*$ transition.[13]

Compound **4** displays a prominent excimer emission band with maximum near 460 nm, in all solvents studied (Fig. 1), as this compound possesses two pyrene moieties. The absorption spectra and the emission in the monomer region are roughly similar to those of pyrene.[4] This is due to the absence of conjugation between the aromatic moieties, caused by the presence of the single bonds linking the pyrenyl groups. A distinct behavior resulting in the total loss of the vibrational structure of pyrene emission was previously observed for a pyrenylindole[10] and for pyrene-dimethylaniline derivatives,[13,14] due to strong charge transfer in the excited-state.

Compound **5** exhibits absorption and emission spectra (Fig. 2) that resemble those of pyrene,[4] with generally high fluorescence quantum yields (Φ_F=0.58 for pyrene in cyclohexane).[16] A similar behavior was observed for the methyl ester of *N*,*N*-bis(*tert*-butoxycarbonyl)-β-[(pyren-1-yl)methylamino]alanine, previously synthesized by some of us.[9]

The fluorescence quantum yield values, Φ_F, are very reasonable for both pyrenylamino acids **4** and **5**, but a significant decrease is detected in methanol (Table 1). In this case, solute–solvent hydrogen-bonding interactions may play an important role, leading to an enhance of singlet-triplet intersystem crossing efficiency.[17] The two pyrenylamino acids have the possibility of establishing hydrogen bonds with several solvents, through NH groups (donors) and ester groups (acceptors) of Boc or acetate moieties. This is also a common behavior with the previously obtained *N*,*N*-bis(*tert*-butoxycarbonyl)-β-[(pyren-1-yl)methylamino]alanine methyl ester.[9]

For compound **4**, the ratio of emission intensities between excimer and monomer, I_E/I_M (with I_M taken in the first vibronic peak of the monomer emission) is solvent dependent (values are presented in Table SD1 in Supplementary data). Dynamic excimer formation is a diffusion-influenced process and viscosity plays an important role. If the rate of excimer formation is proportional to the diffusion coefficient (and assuming that *D* is proportional to $1/\eta$), the plot of ln (I_E/I_M) versus ln η should be linear.[18] In fact, this plot is roughly linear with a negative slope, if methanol is not considered (Fig. SD1 in Supplementary data). However, we also need to address the formation of static excimers (excimers originated from pre-associated pyrene dimers in the ground state). To predict how pyrene dimers influence the results, the excitation spectra were recorded at 376 nm (monomer emission) and 480 nm (excimer emission), for both compounds **4** and **5** (Figs. 3 and 4). As expected, for compound **5** no differences are observed in excitation spectra (Fig. 4) collected at different wavelengths. For compound **4**, some differences are detected (Fig. 3), but a clear evidence of pre-associated dimer formation is not obtained from steady-state results. The formation of ground state dimers usually causes a shift between the excitation spectra recorded at different emission wavelengths, as well as changes in the peak-to-valley intensity ratio.[5,19]

Time-resolved decays can give more relevant information about the excited-state kinetics of this compound, as described below.

Fluorescence emission of compound **5** displays a solvent sensitivity through variations of the relative intensities of the first and third vibronic bands (I_1/I_3), as already reported for pyrene (the widely used Py scale of solvent polarities).[20] Table 2 shows the comparison between the intensities ratio I_1/I_3 for pyrene and for the new pyrenylamino acid **5**. It can be observed that for both molecules the I_1/I_3 values follow the same trend (Fig. 5), the values being higher for compound **5**, may be due to the groups directly