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Photocleavage studies of γ-aminobutyric acid (GABA) conjugates

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Abstract- Polycyclic aromatic labels such as naphthalene and pyrene were coupled to the C-*terminus* of *N*-benzyloxycarbonyl protected γ-aminobutyric acid (GABA). The photophysical properties of the corresponding fluorescent conjugates were evaluated, as well as their behaviour towards photocleavage by irradiation in MeOH/HEPES buffer solution (80:20), in a photochemical reactor at different wavelengths (254, 300, 350 and 419 nm), followed by HPLC/UV monitoring.

Keywords*:* Naphthalene; Pyrene; γ-Aminobutyric acid (GABA); Photocleavable protecting groups.

1. Introduction

Photochemically removable protecting groups represent a very important tool in both synthetic and biological chemistry, as cleavage only requires light, which is a soft deprotection strategy and usually compatible with base or acid sensitive groups. Photolabile goups have found extensive application in organic synthesis, particularly that involving polyfunctional molecules,[1] photoactive precursors of neurotransmitters[2] and in time-resolved studies in cell biology.[3]

In recent years, photoreleasable groups based on 2-nitrobenzyl,[4] benzyl,[5] benzoin,[6] phenacyl,[7] cynnamyl,[8] vinylsilane[9] groups and their derivatives, have been developed and applied in the protection of several classes of compounds, with 2-nitrobenzyl derivatives being the most extensively studied. Also, the use of polycyclic aromatics, namely anthraquinon-2-ylmethoxycarbonyl,[10] anthraquinon-2-ylethyl-1',2'-diol,[11] pyren-1-ylmethoxycarbonyl,[12] phenanthren-9-ylmethoxycarbonyl,[10] anthracene-9-methanol,[13] and oxobenzopyrans (trivially known as coumarins)[14] has been reported for the protection of alcohols, amines, phosphates, carboxylic acids, aldehydes and ketones. Polycyclic aromatics usually display fluorescence and fluorescent phototriggers are more interesting than non-fluorescent protecting groups, since they may act as temporary fluorescent labels, during the course of reaction, and also allow tracing of the location of caged molecules inside living cells by fluorescent techniques

as well as the visualisation of processes during *in situ* synthesis of oligonucleotides and peptides.[15]

The significance of photorelease applications in neurological sciences, for studying the chemical mechanisms and the kinetics of synaptic transmission,[16] has grown steadily in recent years. Considering the role played by amino acids in neuronal communication at the central nervous system (CNS), γ-aminobutyric acid (GABA) has a strong inhibitory function within the mammalian CNS and is one of the most extensively studied neurotransmitter amino acids.

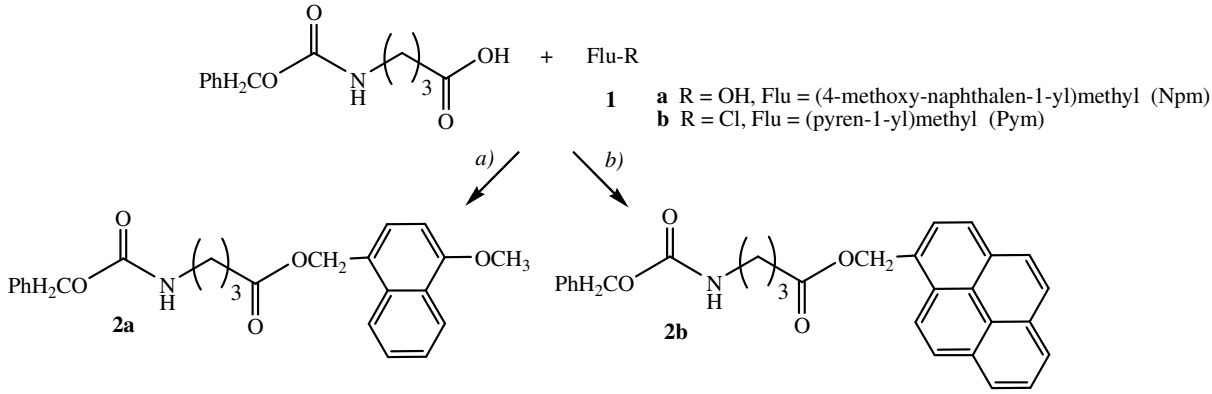
Taking these facts into consideration in connection with our research interests in the development of new fluorescent heterocyclic compounds and their applications as photoreleasable protecting groups,[17] we now report the use of fluorophores of aromatic nature, namely naphthalene and pyrene, in the preparation of fluorescent conjugates of GABA, with the aim of undertaking a comparative study of their performance as photolabile groups.

2. Results and discussion

1-Hydroxymethyl-4-methoxy-naphthalene **1a** was obtained by reduction of the formyl group of 4-methoxy-naphthaldehyde, with sodium borohydride. 1-Chloromethylpyrene **1b** was commercially available. Fluorophores **1a**,**b** will be designated in this report by a three letter code for simplicity of naming the fluorescent conjugates, as indicated in Table 1.

Our purpose being the investigation of compounds **1a**,**b** as fluorescent photocleavable protecting groups for neurotransmitter amino acids, namely γ-aminobutyric acid (GABA), we synthesised the corresponding conjugates in order to do a comparative study of the behaviour to photolysis conditions of the ester linkage between fluorophores **1a**,**b** and the carboxylic function of GABA.

Derivatisation at the C-*terminus* of *N*-benzyloxycarbonyl-protected GABA with labels **1a**,**b** was carried out in DMF, at room temperature, with the aid of *N,N*'-dicyclohexylcarbodiimide (DCC) assisted by 1-hydroxybenzotriazole (HOBt) under standard conditions[18] (for **1a**, 17% yield) or by using potassium fluoride[19] (for **1b**, 98% yield), yielding fluorescent GABA conjugates **2a**,**b** (Scheme 1). All conjugates were characterised by IR, ^{1}H and ^{13}C NMR spectroscopy and elemental analyses or high resolution mass spectrometry.



Scheme 1. Synthesis of fluorescent GABA ester conjugates **2a**,**b**. *Reagents and conditions: a)* DCC, HOBt, DMF, rt; *b)* KF, DMF, rt.

The UV/Vis absorption and emission spectra of degassed 10^{-5}-10^{-6} M solutions in absolute ethanol of compounds **2a**,**b** were measured, absorption and emission maxima, molar absorptivities and fluorescence quantum yields are also reported (Table 1). Fluorescence quantum yields were calculated using 9,10-diphenylanthracene as standard (Φ_F = 0.95 in ethanol).[20] The wavelength of maximum absorption and emission was red-shifted for the conjugate bearing the pyrene label, due to more extensive conjugation. Labelled GABA **2a**,**b** exhibited moderate quantum yields ($0.15 < \Phi_F < 0.20$), and moderate Stokes' shift (33 to 44 nm).

Table 1. UV/Vis and fluorescence data for GABA ester conjugates **2a**,**b** in absolute ethanol.

Compound		UV/Vis		Fluorescence		
		λ_{max} (nm)	log ε	λ_{max} (nm)	Stokes' shift (nm)	Φ_F
2a	Z-GABA-ONpm	295	3.80	339	44	0.20 ± 0.02
2b	Z-GABA-OPym[17c]	342	4.61	375	33	0.15 ± 0.01

Considering that the main goal of this research was to compare the performance of compounds **1a**,**b** as fluorescent photocleavable protecting groups, photolysis studies of GABA conjugates **2a**,**b** were carried out. Solutions of the mentioned compounds in

methanol/HEPES buffer 80:20 solution were irradiated in a Rayonet RPR-100 reactor, at 254, 300, 350 and 419 nm, in order to determine the best cleavage conditions. The course of the photocleavage reaction was followed by reverse phase HPLC with UV detection.

The plots of peak area of the starting material *versus* irradiation time were obtained for each compound, at the considered wavelengths. Peak areas were determined by HPLC, which revealed a gradual decrease with time, and were the average of 3 runs. The determined irradiation time represents the time necessary for the consumption of the starting materials until less than 5% of the initial area was detected (Table 2).
For each compound and based on HPLC data, the plot of ln *A versus* irradiation time showed a linear correlation for the disappearance of the starting material, which suggested a first order reaction, obtained by the linear least squares methodology for a straight line.

Table 2. Irradiation times (in min) and photochemical quantum yield (Φ_{phot}, $\times 10^{-3}$) for the photolysis of compounds **2a**,**b** at different wavelengths in MeOH/HEPES buffer (80:20) solution.

		2a Z-GABA-ONpm	**2b** Z-GABA-OPym
254 nm	Irr time	20	17
	Φ_{phot}	0.057	0.521
300 nm	Irr time	7	19
	Φ_{phot}	1.330	0.564
350 nm	Irr time	4284	158
	Φ_{phot}	0.192	0.065

Concerning the influence of the wavelength of irradiation on the rate of the photocleavage reactions of GABA conjugates **2a**,**b** in methanol/HEPES buffer 80:20 solution, it was found that the most suitable was 300 nm, resulting in shorter irradiation times. Cleavage at 419 nm lead to a very large increase in the irradiation time (*ca* 68 h in the case of compound **2b**, and higher for compound **2a**) which is not useful for practical applications.

Taking into consideration the influence of the structure of the conjugates on the photocleavage rates, it was found that the irradiation times were comparable at 254 nm (*ca*. 20 min), while that at 300 nm, compound **2a** cleaved *ca*. 3 times faster (7 min). However at 350 nm, this trend was reversed (**2b** cleaved approximately 27 times faster).

As reported before,[17a] the *N*-blocking group was stable in the tested conditions, no cleavage being detected. The photochemical quantum yields were calculated as previously described[15c,17c] and are given in Table 2. Although the efficiency of the photocleavage process was not high, nevertheless and considering the low irradiation times, these fluorescent labels can be considered as suitable photocleavable protecting groups in organic synthesis.

3. Conclusions

By using general synthetic methods, fluorescent γ-aminobutyric acid ester conjugates **2a**,**b** were prepared through reaction of hydroxymethylnaphthalene or chloromethylpyrene and the C-*terminus* of *N*-benzyloxycarbonyl-protected GABA. The photophysical studies showed that the labels are appropriate fluorogenic reagents for the derivatisation of non-fluorescent molecules, due to their fluorescence properties.

Regarding the photocleavage studies of the fluorescent conjugates, in methanol/ HEPES buffer solution (80:20), at 254, 300 and 350 nm, it was possible to conclude that the irradiation time depended on the structure of the label. Regarding the stability to radiation of the analyte, a choice of the protecting group can be made based on the wavelength of irradiation, *i.e.* at 300 nm, naphthalene (**1a**) and at 254 and 350 nm, pyrene (**1b**). Irradiation times at 419 nm were too long and not convenient for pratical applications.

4. Experimental

4.1 Synthesis of N-benzyloxycarbonyl-L-γ-aminobutyric acid (4-methoxy-naphthalen-1-yl)methyl ester, Z-GABA-ONpm **2a**: *N*-Benzyloxycarbonyl-L-γ-aminobutyric acid, Z-GABA-OH (0.529 g; 2.23 mmol) was reacted with 1-hydroxymethyl-4-methoxy-naphthalene, Npm-OH (**1a**) (0.200 g; 1.06 mmol) in DMF (4 mL) using a standard DCC/HOBt coupling. After chromatography on silica gel (chloroform/methanol, 100:1), conjugate **2a** was obtained as a yellow oil (0.075 g, 17 %). IR (neat): υ_{max} 3357, 3033, 2942, 1726, 1585, 1515, 1464, 1455, 1395, 1251, 1231, 1164, 1093, 1066, 1027, 818 cm^{-1}. ^1H NMR ($CDCl_3$, 300 MHz): δ = 1.79-1.89 (2H, m, β-CH_2), 2.39 (2H, t, *J* 7.2 Hz, α-CH_2), 3.18-3.27 (2H, m, γ-CH_2), 4.02 (3H,

s, OCH_3), 4.81 (1H, broad s, NH), 5.08 (2H, s, CH_2 Z), 5.50 (2H, s, CH_2 Npm), 6.76 (1H, d, *J* 8.1 Hz, H-3), 7.27-7.36 (5H, m, 5 × Ph-*H*), 7.48 (1H, d, *J* 8.1 Hz, H-2), 7.52 (1H, dt, *J* 9.3 and 2.7 Hz, H-6), 7.58 (1H, dt, *J* 9.3 and 2.7 Hz, H-7), 7.95 (1H, dd, *J* 9.0 and 2.7 Hz, H-8), 8.32 (1H, dd, *J* 9.0 and 2.7 Hz, H-5) ppm. ^{13}C NMR ($CDCl_3$, 75.4 MHz): δ = 25.06 (β-CH_2), 31.47 (α-CH_2), 40.28 (γ-CH_2), 55.48 (OCH_3), 64.79 (CH_2 Npm), 66.58 (CH_2 Z), 102.83 (C-3), 122.62 (C-5), 123.34 (C-8), 125.25 (C-6), 125.81 (C-4a), 127.06 (C-7), 128.02 (2 × Ph-*C*), 128.44 (3 × Ph-*C*), 128.45 (C-1), 128.59 (C-2), 132.62 (C-8a), 136.45 (C-1 Ph), 156.23 (C=O urethane), 156.37 (C-4), 173.18 (C=O ester) ppm. UV/Vis (MeOH/HEPES, 80:20, nm): λ_{max} (log ε) = 295 (3.80). $C_{24}H_{25}NO_5$: calc. C, 70.75; H, 6.18; N, 3.44; found C, 70.85; H, 6.14; N, 3.67%.

4.2 General photolysis procedure: A 1 × 10^{-4} M MeOH/HEPES buffer (80:20) solution of compounds **2a**,**b** (5 mL) were placed in a quartz tube and irradiated in a Rayonet RPR-100 reactor at the desired wavelength. The lamps used for irradiation were of 254, 300, 350 and 419 ± 10 nm.

Aliquots of 100 μL were taken at regular intervals and analysed by RP-HPLC. The eluent was acetonitrile/water, 3:1, at a flow rate of 1.0 (for **2a**) or 1.2 (for **2b**) mL/min, previously filtered through a Millipore, type HN 0.45 μm filter and degassed by ultra-sound for 30 min. The chromatograms were traced by detecting UV absorption at the wavelength of maximum absorption for each compound (retention time: **2a**, 5.7; **2b**, 8.8 min.).

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