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Comparative study of polyaromatic and polyheteroaromatic fluorescent photocleavable protecting groups

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Abstract

Fluorescent conjugates of *N*-benzyloxycarbonyl protected γ-aminobutyric acid were prepared by coupling to its C-*terminus* several polyheteroaromatic, based on the oxobenzopyran skeleton (trivially known as coumarin) and polyaromatic labels, such as naphthalene and pyrene. Photophysical properties 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: Oxobenzopyran; Oxobenzobenzopyran; Coumarin; γ-Aminobutyric acid (GABA); Photocleavable protecting groups

1. Introduction

Initially reported by Barltrop and Schofield in 1962,[1] photolabile protecting groups have found extensive application in both synthetic and biological chemistry, being responsible for recent advances in various areas and technologies, such as in organic synthesis, particularly that involving polyfunctional molecules,[2,3] photoactive calcium chelators,[4,5] photoactive precursors of neurotransmitters[6–8] and in time-resolved studies for a wide range of subjects, varying from cell biology[9] to X-ray crystallography.[10]

Light-sensitive molecules, including groups like 2-nitrobenzyl,[11] benzyl,[12] benzoin,[13] phenacyl,[14] cinnamyl,[15] vinylsilane[16] and their derivatives, have been developed and used as photoreleasable groups. Polycyclic aromatics, namely anthraquinon-2-ylmethoxycarbonyl,[17] anthraquinon-2-ylethyl-1′,2′-diol,[18] pyren-1-ylmethyl,[19,20] pyren-1-ylmethoxycarbonyl,[17] phenanthren-9-ylmethoxycarbonyl,[17] anthracene-9-methanol,[21] and oxobenzopyrans (coumarins)[22] have also been applied in the protection of alcohols, amines, phosphates, carboxylic acids, aldehydes and ketones. Within this last class of heterocycles, 7-methoxycoumarin-4-yl derivatives are some of the most widely used photoreleasable groups in cell biology and biophysics.[23] These compounds are fluorophores, which are more convenient as phototriggers than non-fluorescent protecting groups, since they may be useful in monitoring the course of reaction and thus 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 the functioning of peptides.[24–26] Although fluorescence deactivation may be an inconvenience in some photochemical processes, in recent years, the direction of improvement on photoreleasable groups has been towards the development and application of polycyclic structures (both benzene and heterocycle derived), which are fluorophores in most cases. These compounds have been reported as having improved properties as photolabile protecting groups.

γ-Aminobutyric acid (GABA), one of the main transmitters in the central nervous system, has been widely used in photorelease applications in neurological sciences, for studying the chemical mechanisms and the kinetics of synaptic transmission.[27,28] Bearing in mind these facts in connection with our current research interests in the development of new fluorescent heterocyclic compounds and their applications as labels and as

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photoreleasable protecting groups,[29] we now report the use of different recognised and new fluorophores of aromatic, namely naphthalene and pyrene, and heteroaromatic nature, such as oxobenzopyran derivatives, 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-7-methoxy-3-oxo-3*H*-benzo[*g*]benzopyran **1a** was synthesised through a Pechmann reaction, between 2,7-dihydroxynaphthalene and ethyl acetoacetate, catalysed by sulfuric acid at room temperature,[30] followed by methylation of the resulting 1-methyl-7-hydroxy-3-oxo-3*H*-benzo[*g*]benzopyran with methyl iodide. The methyl group was oxidised to the aldehyde, by reaction with selenium dioxide, which was then reacted with sodium borohydride, affording the hydroxymethyl group.[31] 1-Chloromethyl-9-methoxy-3-oxo-3*H*-benzo[*f*]benzopyran **1b** and 1-chloromethyl-6-methoxy-3-oxo-3*H*-benzopyran **1c** were obtained by a similar procedure from the reaction of 7-methoxy-2-naphthol and 3-methoxyphenol with ethyl chloroacetoacetate.[29b] Starting from 4-methoxy-naphthaldehyde, 1-hydroxymethyl-4-methoxy-naphthalene **1d** was obtained by reduction of the formyl group with sodium borohydride. 1-Chloromethylpyrene **1e** was commercially available. Fluorophores **1a**–**e** will be designated in this report by a three letter code for simplicity of naming the various fluorescent conjugates, as indicated in Table 1 and Figure 1.

Our purpose being the investigation of compounds **1a**–**e** 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 in photolysis conditions of the ester linkage between fluorophores **1a**–**e** and the carboxylic function of GABA.

Table 1
Yields, UV/vis and fluorescence data for GABA ester conjugates **2a**–**e** in absolute ethanol

Compound		Yield (%)	UV/vis		Fluorescence		
			λ_{max} (nm)	log ε	λ_{max} (nm)	Stokes' shift (nm)	Φ_F
2a	Z–GABA–OBbl	27	345	3.91	503	158	0.21±0.03
2b	Z–GABA–OBba	81	345	3.95	472	127	0.76±0.02
2c	Z–GABA–OBpm	85	320	4.22	393	73	0.27±0.03
2d	Z–GABA–ONpm	17	295	3.80	339	44	0.20±0.02
2e	Z–GABA–OPym[29e]	98	342	4.61	375	33	0.15±0.01

Derivatisation at the C-*terminus* of *N*-benzyloxycarbonyl-protected GABA with labels **1a**–**e** was carried out in DMF, at room temperature, with the aid of *N*,*N*′-dicyclohexylcarbodiimide (DCC) assisted by 1-hydroxybenzotriazole (HOBt) under standard conditions[32] (for **1a**,**d**) or by using potassium fluoride[33] (for **1b**,**c**,**e**), yielding fluorescent GABA conjugates **2a**–**e** (Scheme 1, Table 1). All conjugates were characterised by IR, ^{1}H and ^{13}C NMR spectroscopy and elemental analyses or high resolution mass spectrometry.

The UV/vis absorption and emission spectra of degassed 10^{-5}–10^{-6} M solutions in absolute ethanol of compounds **2a**–**e** were measured, absorption and emission maxima, molar absorptivities and fluorescence quantum yields are also reported (Table 1, Fig. 2). Fluorescence quantum yields were calculated using 9,10-diphenylanthracene as standard (Φ_F=0.95 in ethanol).[34] Labelled GABA **2a**–**e** exhibited moderate to excellent quantum yields ($0.15<\Phi_F<0.76$), and Stokes' shift from 33 to 158 nm, the highest values being associated with the heteroaromatic moieties.

Considering that the main goal of this research was to compare the performance of compounds **1a**–**e** as fluorescent photocleavable protecting groups, photolysis studies of GABA conjugates **2a**–**e** 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 three 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.

Concerning the influence of the wavelength of irradiation on the rate of the photocleavage reactions of GABA conjugates **2a**–**e** in methanol/HEPES buffer 80:20 solution, it was found that the most suitable was 254 nm (compound **2a**), 300 nm (compounds **2c**–**e**) and 350 nm (compound **2b**). Cleavage at 419 nm lead to a very large increase in the irradiation time

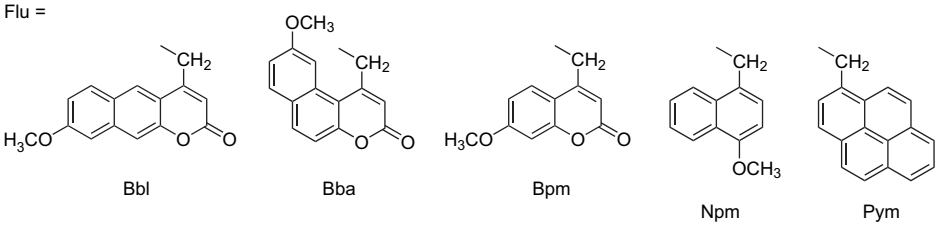

Figure 1. Structure and three letter code of fluorophores **1a**–**e**.