Azaheterocycles as caging groups: synthesis and photolysis studies with a model carboxylic acid

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Abstract: The (acridin-9-yl)methyl and (9-ethyl-9*H*-carbazol-3-yl)methyl groups were evaluated as photocleavable protecting groups by using butyric acid as a model carboxylic acid. Photocleavage studies of the corresponding ester conjugates were conducted in a Rayonet RPR-100 photochemical reactor in a mixture of methanol or acetonitrile with aqueous HEPES buffer (in a 80:20 proportion) at different wavelengths of irradiation. It was found that the most efficient release of butyric acid occurred from the corresponding (acridin-9-yl)methyl ester conjugate in methanol/HEPES buffer (80:20) solutions.

Keywords: protecting group; carbazole; acridine; caging; photolysis.

Introduction

The search for light-sensitive moieties which allow a controlled spatio-temporal triggering of chemical and biological relevant molecules within cellular systems represents an important challenge in chemical biology. Caging strategies make use of photochemically removable protecting groups to mask the activity of biologically relevant molecules and release the bioactive species upon irradiation with light of appropriate wavelength [1]. The use of fluorescent caging groups allows the visualization, quantification, and the follow-up of spatial distribution, localization, and depletion of the active compound through the monitoring of its fluorescent caged precursor using fluorescent techniques.

Examples of photocleavable groups most usually employed in caging applications and also in organic synthesis are aromatics such as 2-nitrobenzyl, benzoin, phenacyl, cinnamyl, 3-nitro-2-naphthalenemethanol, anthracene-9-methanol, phenanthren-9-ylmethoxycarbonyl, anthraquinon-2-ylmethoxycarbonyl, 2-(1'-hydroxyethyl)-anthraquinon, anthraquinon-2-ylethyl-1',2'-diol, pyren-1-ylmethyl and pyren-1-ylmethoxycarbonyl [2] and heteroaromatics like acylnitroindolines, xanthones, coumarins, benzocoumarins, quinolines and quinolones [3].

As a continuation of our research work concerning the synthesis of new fluorescent heterocyclic compounds, their application on the design of fluorescent conjugates of biologically relevant molecules and studies on their photorelease, the present work aims to give a contribution to the development of novel photoactivable groups through the evaluation of azaheterocycles, namely acridine and carbazole. A model carboxylic acid was used for the preparation of novel ester conjugates of acridine and carbazole, in order to be compared with the corresponding 2-nitrobenzyl derivative (a widely known caging group). The resulting compounds were studied in a photochemical reactor under irradiation at different wavelengths and the monitoring of the photocleavage process was carried out by HPLC and ^{1}H NMR.

Experimental

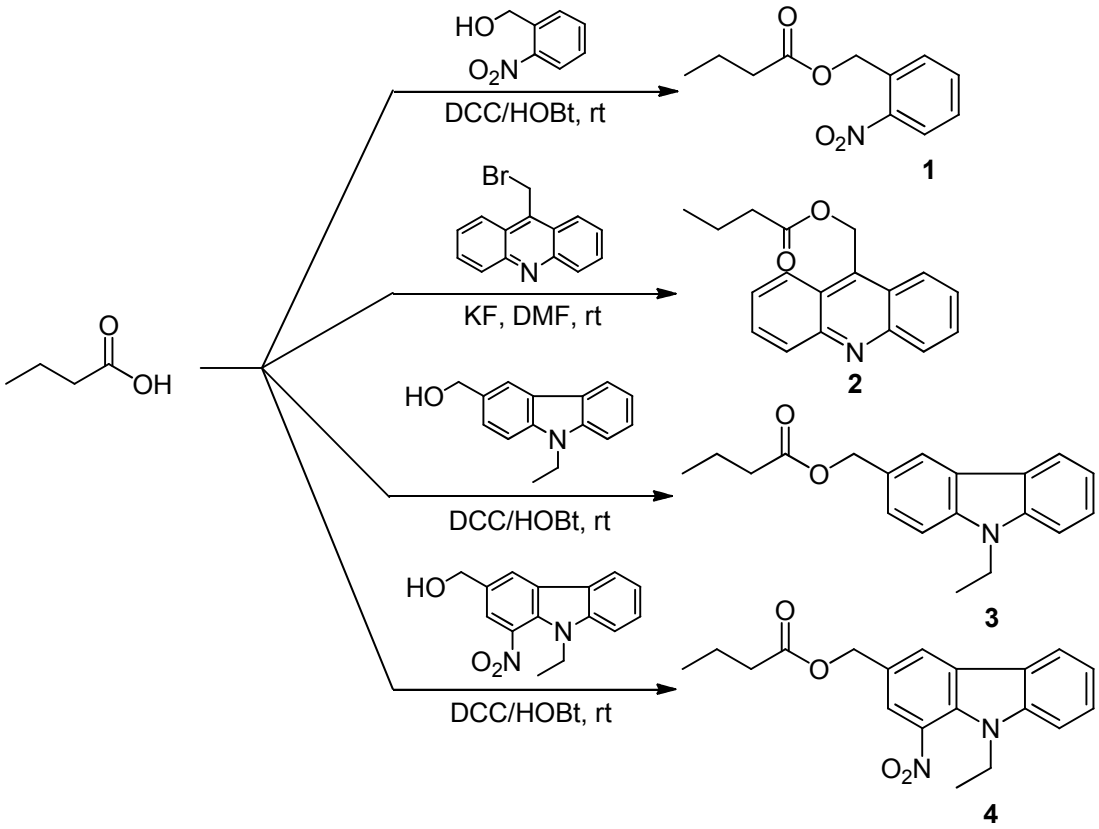
Synthesis of acridin-9-ylmethyl butyrate 2. To a solution of 9-(bromomethyl)acridine (0.038 g, 1.39×10^{-4} mol) in dry DMF (3 mL), potassium fluoride (0.073 g, 4.17×10^{-4} mol) and butyric acid (0.014 mL, 1.52×10^{-4} mol) were added. The reaction mixture was stirred at room temperature for 15 h. Potassium fluoride was removed by filtration, the solvent was evaporated under reduced pressure and the crude residue was purified by column chromatography using mixtures of ethyl acetate and light petroleum as eluent. Conjugate **2** was obtained as a brown oily solid (0.027 g, 69 %). Rf = 0.48 (ethyl acetate/light petroleum, 1:1). ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 0.89 (t, *J* 7.6 Hz, 3 H, $CH_2CH_2CH_3$), 1.58-1.69 (m, 2 H, $CH_2CH_2CH_3$), 2.32 (t, *J* 7.2 Hz, 2 H, $CH_2CH_2CH_3$), 6.12 (s, 2 H, OCH_2), 7.62 (dt, *J* 7.6 and 1.2 Hz, 2 H, H-2 and H-7), 7.79 (dt, *J* 7.8 and 1.2 Hz, 2 H, H-3 and H-6), 8.28 (d, *J* 8.6 Hz, 2 H, H-4 and H-5), 8.34 (d, *J* 9.0 Hz, 2 H, H-1 and H-8). ^{13}C NMR ($CDCl_3$, 100.6 MHz): δ = 13.54 ($CH_2CH_2CH_3$), 18.34 ($CH_2CH_2CH_3$), 35.96 ($CH_2CH_2CH_3$), 57.31 (OCH_2), 124.03 (C-1 and C-8), 125.32 (C-8a and C-9a), 126.68 (C-2 and C-7), 129.97 (C-4 and C-5), 130.08 (C-3 and C-6), 137.53 (C-9), 148.46 (C-4a and C-10a), 173.42 (C=O ester). IR (KBr 1%, cm^{-1}): ν =3185, 3067, 2965, 2934, 2875, 1737, 1692, 1629, 1603, 1557, 1519, 1498, 1461, 1441, 1416, 1382, 1352, 1303, 1286, 1249, 1165, 1100, 1058, 1040, 1018, 976, 911, 862, 753, 733, 643.

General photolysis procedure. A 1×10^{-4} M methanol/HEPES buffer (80:20) solution and acetonitrile/HEPES buffer (80:20) solution of conjugates **1**-**4** (5 mL) was 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. HEPES buffer solution was prepared in distilled water with HEPES (4-(2-hydroxyethyl)-1-piperazine ethanesulfonic acid) (10 mM), NaCl (120 mM), KCl (3 mM), $CaCl_2$ (1 mM) and $MgCl_2$ (1mM) and pH adjusted to 7.2. 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 0.8 mL/min, for all compounds, 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 (5.1 min, **1**; 8.7 min, **2**; 10.1 min, **3**; 10.2 min, **4**).

Results and discussion

Butyric acid was chosen as the model carboxylic acid for the derivatization with (2-nitrophenyl)methanol, 9-(bromomethyl)acridine, (9-ethyl-9*H*-carbazol-3-yl)methanol and its nitrated derivative (9-ethyl-1-nitro-9*H*-carbazol-3-yl)methanol, yielding the corresponding ester conjugates **1**-**4** (Scheme 1). Due to the nature of the function present at the potential protecting group, a bromomethyl or a hydroxymethyl group, the synthesis of conjugate **2** was achieved by a potassium fluoride mediated coupling in DMF, at room temperature, while for conjugates **1**,**3**-**4** a *N,N*'-dicyclohexylcarbodiimide (DCC) assisted by 1-hydroxybenzotriazole (HOBt) under standard conditions in DMF, at room temperature, was carried out (Table 1).



Scheme 1. Synthesis of butyric acid conjugates **1-4**.

The UV-visible spectroscopic characterization was also carried out to obtain the parameters needed for monitoring during photolysis. Absorption spectra of degassed 10^{-6} M solutions in methanol/HEPES buffer (80:20) and acetonitrile/HEPES buffer (80:20) of conjugates **1**-**4** were measured (Table 1), as well as the fluorescence spectra, due to their emissive nature (Figure 1).

Table 1. Yields, absorption and fluorescence data for conjugates **1**-**4** in methanol/HEPES and acetonitrile/HEPES buffer (80:20) solutions.

		Methanol/HEPES					Acetonitrile/HEPES				
		Absorption		Emission			Absorption		Emission		
Cpd	Yield (%)	λ_{max} [a]	log ε	λ_{max} [a]	ϕ_F	$\Delta\lambda$ [a]	λ_{max} [a]	log ε	λ_{max} [a]	ϕ_F	$\Delta\lambda$ [a]
1	28	259	3.73	304	0.07	45	260	3.74	304	0.13	44
2	69	360	4.12	427	0.32	67	360	4.15	427	0.27	67
3	57	295	3.89	370	0.18	75	294	3.89	372	0.14	78
4	39	282	3.64	345	0.09	63	282	4.03	345	0.04	63

[a] in nm.

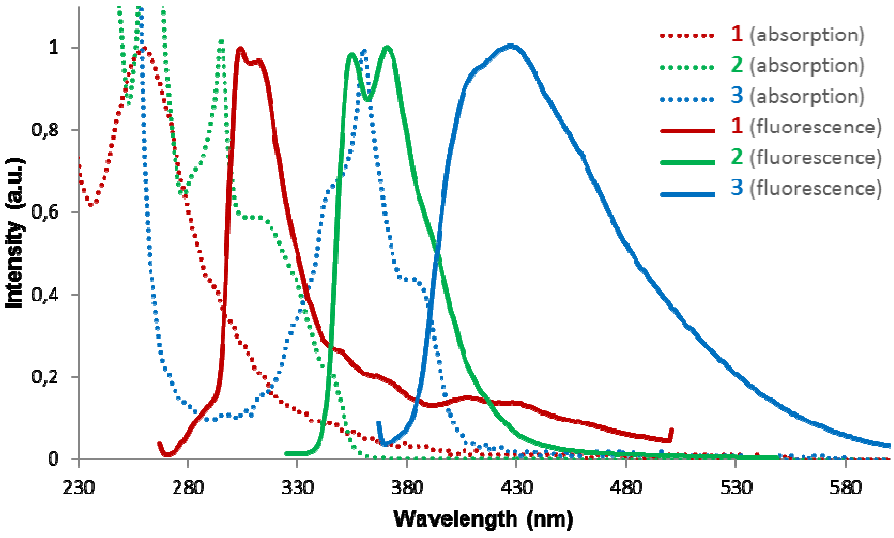


Figure 1. Normalised UV-visible absorption and fluorescence spectra of conjugates **1-3** in methanol/HEPES buffer (80:20) solution (C ≈ 1 × 10^{-6} M; **1**, λ_{exc} = 260 nm; **2**, λ_{exc} = 360 nm; **3**, λ_{exc} = 295 nm).

Conjugate **1**, bearing an *o*-nitrobenzyl group acting as the model photocleavable moiety, and conjugates **2**-**4** bearing an azaheterocycle as potential photocleavable protecting group, were irradiated in a Rayonet RPR-100 photochemical reactor in a mixture of methanol or acetonitrile

with aqueous HEPES buffer (in a 80:20 proportion) at different wavelengths (254, 300, 350 and 419 nm), in order to determine the most favourable cleavage conditions. The course of the photocleavage reaction was followed by reverse phase HPLC with UV detection. 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).

Table 2. Irradiation times (t_{Irr}, min) and rate constants (k, $\times 10^{-2}$ min^{-1}) for the photolysis of conjugates **1** and **2**, at different wavelengths (λ_{Irr}) in methanol/HEPES buffer (80:20) solutions.

λ_{Irr} (nm)		Compound	
		1	**2**
254	t_{Irr}	11	4
	k	26.64	71.64
300	t_{Irr}	26	7
	k	11.40	45.51
350	t_{Irr}	76	2
	k	3.94	127.39
419	t_{Irr}	2217	112
	k	0.135	2.69

It was found that the release of butyric acid was faster from conjugate **2** bearing the acridin-9-yl-methyl unit at all wavelengths of irradiation in methanol/HEPES buffer (80:20) solutions, being the best result at 350 nm. Although at 419 nm the photolysis required more time to occur, the value obtained for conjugate **2** is also suitable for practical applications, whereas for compound **1**, possessing the *o*-nitrobenzyl group, the irradiation time is inadequate. Irradiation in acetonitrile/HEPES buffer (80:20) solutions was found to be unfavourable for the photolysis of compound **2** resulting in longer irradiation times.

Concerning (9-ethyl-9*H*-carbazol-3-yl)methyl and its nitrated derivative (9-ethyl-1-nitro-9*H*-carbazol-3-yl)methyl, evaluation of their photosensitivity included photolysis of the corresponding conjugates (**3** and **4**), in both solvent systems mentioned, under irradiation at 254, 300 and 350 nm. Monitoring of the processes, namely by ^{1}H NMR in a methanol-d_4 or acetonitrile-d_3/D_2O (80:20) (C = 1.1×10^{-4} M) revealed that no release of butyric acid was detected after at least 5h of irradiation.

Owing to the fluorescence properties (Table 1) in addition to these preliminary results, (9-ethyl-9*H*-carbazol-3-yl)methyl and the related nitro derivative can be considered permanent fluorescent labels in processes involving irradiation in tested conditions.

Conclusions

Several ester conjugates were obtained by a potassium fluoride or a *N,N*'-dicyclohexylcarbodiimide with 1-hydroxybenzotriazole mediated coupling between acridine and carbazole azaheterocycles, as well as (2-nitrophenyl)methanol and butyric acid. Photolysis studies revealed the suitability of the (acridin-9-yl)methyl unit to act as photolabile protecting group, including for caging aplications, being more favourable than the well-known *o*-nitrobenzyl group in methanol/HEPES buffer (80:20) solutions. On the contrary, the carbazole based conjugates appeared to be stable upon irradiation having the possibility of being used as permanent fluorescent labels in the tested conditions.

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