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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

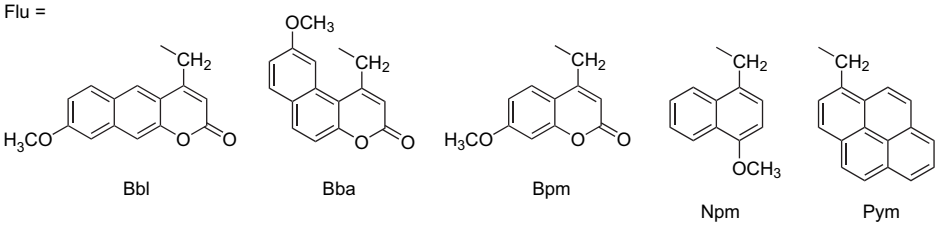
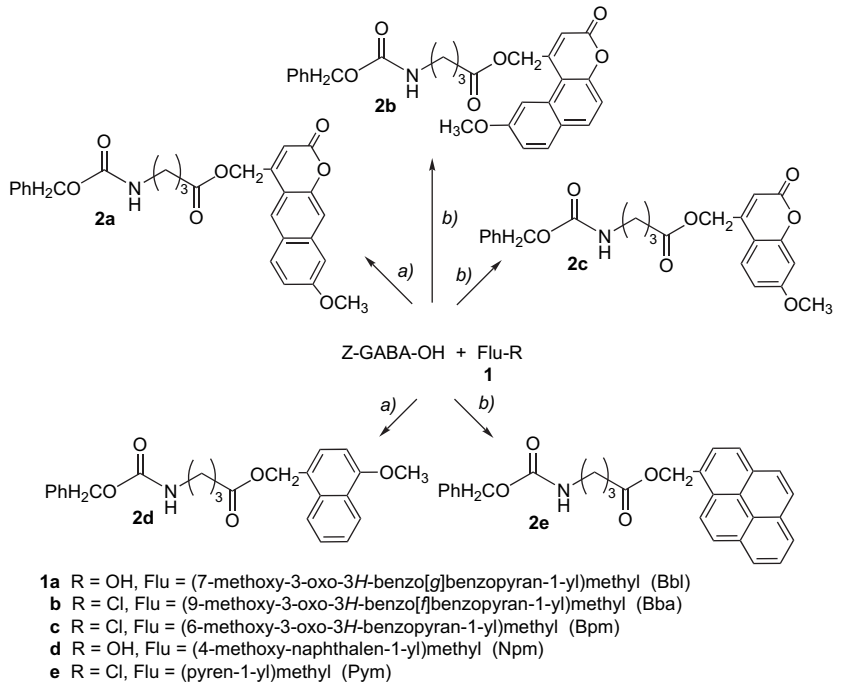


Figure 1. Structure and three letter code of fluorophores **1a**–**e**.



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

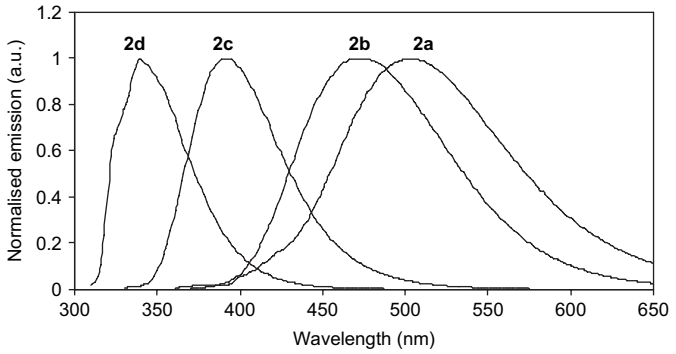


Figure 2. Normalised emission spectra of GABA conjugates **2a**–**d** in absolute ethanol ([**2a**]=1.0×10^{-5} M, λ_{exc}=345 nm; [**2b**]=1.0×10^{-5} M, λ_{exc}=345 nm; [**2c**]=5.2×10^{-6} M, λ_{exc}=320 nm; [**2d**]=1.0×10^{-5} M, λ_{exc}=295 nm).

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

Compound		254 nm		300 nm		350 nm	
		Irr time	Φ_{phot}	Irr time	Φ_{phot}	Irr time	Φ_{phot}
2a	Z–GABA–OBbl	479	0.037	1148	0.024	790	0.007
2b	Z–GABA–OBba	169	0.095	124	0.112	84	0.062
2c	Z–GABA–OBpm	131	0.418	90	0.805	986	0.015
2d	Z–GABA–ONpm	20	0.570	7	1.330	4284	0.192
2e	Z–GABA–OPym	17	0.521	19	0.564	158	0.065

(ca. 68 h in the case of compound **2e**, and higher for the other compounds), 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 for conjugates Z–GABA–OBbl (**2a**) and Z–GABA–OBba (**2b**), in which the label was a linear or an angular 3-oxobenzobenzopyran, respectively, the cleavage for the angular derivative was faster, for all wavelengths of irradiation. Comparison between Z–GABA–OBpm (**2c**) with compound **2b**, which differ in the number of fused aromatic rings, showed that the irradiation times are comparable at 254 and 300 nm, with **2c** cleaving slightly faster. However, at 350 nm, this trend is reversed (**2b** cleaves approximately 12 times faster) probably due to the proximity of the wavelength of maximum absorption of **2b** to the irradiation wavelength. Regarding the polyaromatic or polyheteroaromatic nature of the label, it was found that the cleavage of compounds **2d**–**e** (aromatic) was faster than that of compounds **2a**–**c** (heteroaromatic), at 254 and 300 nm.

As reported before,[29c] the *N*-blocking group was stable in the tested conditions, no cleavage being detected. The photochemical quantum yields were calculated as previously described[24,29e] and are given in Table 2. Although the efficiency of the photocleavage process was not high, due to fluorescence deactivation and other photophysical processes, which limit the overall quantum yield of deprotection, regarding the low irradiation times, these fluorescent labels can be considered as suitable photocleavable protecting groups in organic synthesis.

3. Conclusions

Fluorescent γ-aminobutyric acid ester conjugates **2a**–**e** were prepared in low to excellent yields by using general synthetic methods, involving chloromethyl or hydroxymethyl precursors (oxobenzopyran, naphthalene and pyrene derivatives) and the

C-*terminus* of *N*-benzyloxycarbonyl-protected GABA. The photophysical studies showed that all labels are appropriate fluorogenic reagents for the derivatisation of non-fluorescent molecules, being 1-chloromethyl-9-methoxy-3-oxo-3*H*-benzo[*f*]benzopyran (**1b**), together with the other heteroaromatic moieties, the most interesting fluorophores.

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. Depending on 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 350 nm, benzo[*f*]benzopyran (**1b**), at 300 nm naphthalene (**1d**) and at 254 nm pyrene (**1e**). Irradiation times at 419 nm were too long and not convenient for practical applications.

In summary, all labels considered lead to conjugates, which required low irradiation times for photocleavage to occur, making them appropriate to use as photolabile protecting groups for organic molecules, including amino acids and other relevant biomolecules, in addition to their usefulness in fluorescent labelling due to the high Stokes' shifts and moderate to excellent fluorescence quantum yields.

4. Experimental section

4.1. General

All melting points were measured on a Gallenkamp melting point apparatus and are uncorrected. TLC analyses were carried out on 0.25 mm thick precoated silica plates (Merck Fertigplatten Kieselgel $60F_{254}$) and spots were visualised under UV light. Chromatography on silica gel was carried out on Merck Kieselgel (230–240 mesh). IR spectra were determined on a Perkin–Elmer FTIR-1600 using KBr discs. UV/visible spectra were run on a Hitachi U-2000 spectrophotometer. ^{1}H NMR spectra were recorded on a Varian 300 spectrometer in $CDCl_3$ or DMSO-d_6 at 300 MHz at 25 °C. All chemical shifts are given in parts per million using δ_H Me_4Si=0 ppm as reference and *J* values are given in hertz. ^{13}C NMR spectra were run in the same instrument at 75.4 MHz using the solvent peak as internal reference. Assignments were made by comparison of chemical shifts, peak multiplicities and *J* values and were supported by spin decoupling-double resonance and bidimensional heteronuclear HMBC and HMQC correlation techniques. Mass spectrometry analyses were performed at the 'C.A.C.T.I.—Unidad de Espectrometria de Masas', at University of Vigo, Spain. Fluorescence spectra were collected using a Spex Fluorolog 1680 Spectrometer. Photolyses were carried out using a Rayonet RPR-100 chamber reactor equipped with 10 lamps of 254 (35 W), 300 (21 W), 350 (24 W) and 419 (14 W) nm. HPLC analyses were performed using a Licrospher 100 RP18 (5 μm) column in a HPLC system composed by a Jasco PU-980 pump, a UV/vis Shimadzu SPD-GAV detector and a Shimadzu C-RGA Chromatopac register.

N-Benzyloxycarbonyl-L-γ-aminobutyric acid (Z–GABA–OH) was purchased from Senn Chemicals, 1-chloromethylpyrene (Pym–Cl) from TCI and other reagents from Sigma-Aldrich. All reagents were used as received.

4.2. Synthesis of 1-hydroxymethyl-7-methoxy-3-oxo-3H-benzo[g]benzopyran, Bbl–OH (*1a*)

4.2.1. 1-Methyl-7-hydroxy-3-oxo-3H-benzo[g]benzopyran

2,7-Dihydroxynaphthalene (0.506 g, 3.16 mmol) and ethyl acetoacetate (0.60 mL, 4.68 mmol), catalysed by 80% aqueous sulfuric acid, were stirred at room temperature for 3 days. After column chromatography using chloroform as eluent, the compound was obtained as a light green solid (0.616 g, 41%). Mp=262.3–264.5 °C. [lit. not quoted]. ^{1}H NMR (DMSO-d_6): δ=2.47 (d, *J* 0.9 Hz, 3H, CH_3), 6.31 (d, *J* 1.2 Hz, 1H, H-2), 7.11 (dd, *J* 9.0 and 2.4 Hz, 1H, H-8), 7.15 (d, *J* 2.4 Hz, 1H, H-6), 7.58 (s, 1H, H-5), 7.92 (d, *J* 9.0 Hz, 1H, H-9), 8.23 (s, 1H, H-10), 10.23 (s, 1H, OH).

4.2.2. 1-Methyl-7-methoxy-3-oxo-3H-benzo[g]benzopyran

The previous compound (0.450 g, 1.99 mmol) was reacted with potassium carbonate (1.100 g, 7.96 mmol) and methyl iodide (1.51 mL, 9.95 mmol) in DMF (5 mL), at 50–60 °C for 4 h under a nitrogen atmosphere. Water (15 mL) was added and the aqueous phase extracted with ethyl acetate (4×10 mL). The organic phases were combined and washed with NaCl saturated solution, dried with magnesium sulfate, filtered and evaporated under reduced pressure. After column chromatography using chloroform as eluent, the methoxy derivative was obtained as a yellow solid (0.360 g, 75%). Mp=220.6–223.0 °C. ^{1}H NMR ($CDCl_3$): δ=2.51 (d, *J* 0.9 Hz, 3H, CH_3), 3.95 (s, 3H, OCH_3), 6.27 (d, *J* 1.2 Hz, 1H, H-2), 7.09 (d, *J* 2.4 Hz, 1H, H-6), 7.14 (dd, *J* 9.0 and 2.7 Hz, 1H, H-8), 7.56 (s, 1H, H-5), 7.80 (d, *J* 9.0 Hz, 1H, H-9), 7.97 (s, 1H, H-10). ^{13}C NMR ($CDCl_3$): δ=18.64 (CH_3), 55.41 (OCH_3), 104.51 (C-6), 111.59 (C-5), 114.53 (C-2), 117.87 (C-10a), 119.42 (C-8), 124.63 (C-10), 125.54 (C-4a), 130.13 (C-9), 136.38 (C-5a), 150.82 (C-9a), 152.11 (C-1), 159.50 (C-7), 160.95 (C-3). IR (KBr 1%, cm^{-1}): ν=3055, 3005, 2937, 1703, 1625, 1484, 1434, 1400, 1388, 1374, 1313, 1264, 1233, 1209, 1183, 1147, 1134, 1057, 1030, 919, 896, 882, 865, 821, 803, 793, 755, 693. UV/vis (EtOH, nm): λ_{max} (log ε)=343 (4.16), 286 (4.24). MS (EI, %): *m*/*z*=240 ($[M]^+$, 100), 212 (21), 197 (17), 169 (25). HRMS (EI): calcd for $C_{15}H_{12}O_3$ $[M]^+$ 240.0786; found 240.0782.

4.2.3. 1-Formyl-7-methoxy-3-oxo-3H-benzo[g]benzopyran

The previous compound (0.300 g, 1.25 mmol) was reacted with selenium dioxide (0.420 g, 3.75 mmol) in chlorobenzene (30 mL), at reflux for 48 h. The mixture was filtered hot and the solvent was removed by rotary evaporation. The crude residue, was used in the next reaction without purification (yellow solid). Mp=273.8–275.6 °C. ^{1}H NMR (DMSO-d_6): δ=3.90 (s, 3H, OCH_3), 6.51 (s, 1H, H-2), 7.17 (dd, *J* 8.7 and 2.1 Hz, 1H, H-8), 7.37 (d, *J* 2.1 Hz, 1H, H-6), 7.81 (s, 1H, H-5), 8.02 (d, *J* 8.7 Hz, 1H, H-9), 9.00 (s, 1H, H-10), 10.20 (s, 1H, CHO). ^{13}C NMR (DMSO-d_6): δ=55.54 (OCH_3), 105.01 (C-6), 110.99 (C-2), 111.42 (C-5), 119.17 (C-8), 124.44

(C-10a), 125.07 (C-9a), 126.69 (C-10), 130.66 (C-9), 135.84 (C-5a), 143.43 (C-1), 151.05 (C-4a), 159.58 (C-7), 160.76 (C-3), 193.70 (CHO). IR (KBr 1% cm^{-1}): ν=1705, 1628, 1617, 1561, 1487, 1401, 1235, 1167, 1131, 1041, 1026, 930, 902, 883, 810, 725. UV/vis (EtOH, nm): λ_{max} (log ε)=343 (4.16), 285 (4.25). MS (EI, %): *m/z*=254 ($[M]^+$, 21), 137 (18), 121 (17), 95 (19), 81 (57), 69 (100). HRMS (EI): calcd for $C_{15}H_{10}O_4$ $[M]^+$ 254.0579; found 254.0589.

The previous compound (0.252 g, 0.991 mmol) was reacted with sodium borohydride (0.026 g, 0.69 mmol) in ethanol (5 mL) for 3 days at room temperature, affording Bbl–OH (**1a**) as a light pink solid (0.087 g, 34%). Mp=202.5–206.0 °C. ^{1}H NMR (DMSO-d_6): δ=3.89 (s, 3H, OCH_3), 4.86 (d, *J* 5.4 Hz, 2H, CH_2), 5.44 (s, 1H, H-2), 5.70 (t, *J* 5.4 Hz, 1H, OH), 7.16 (dd, *J* 9.0 and 2.4 Hz, 1H, H-8), 7.35 (d, *J* 2.7 Hz, 1H, H-6), 7.70 (s, 1H, H-5), 7.93 (d, *J* 9.0 Hz, 1H, H-9), 8.22 (s, 1H, H-10). ^{13}C NMR (DMSO-d_6): δ=60.55 (OCH_3), 64.20 (CH_2), 110.06 (C-6), 115.25 (C-2), 116.10 (C-5), 120.15 (C-10a), 124.22 (C-8), 129.54 (C-10), 130.30 (C-9a), 135.57 (C-9), 141.12 (C-5a), 155.52 (C-4a), 161.56 (C-1), 164.25 (C-7), 165.42 (C-3). IR (KBr 1%, cm^{-1}): ν=3380, 2977, 1707, 1630, 1483, 1458, 1444, 1435, 1408, 1333, 1235, 1181, 1147, 1129, 1083, 1024, 980, 972, 939, 899, 879, 859, 842, 808. UV/vis (EtOH, nm): λ_{max} (log ε)=346 (4.02), 288 (4.05). MS (EI, %): *m/z*=256 ($[M]^+$, 50), 254 (32), 288 (31), 227 (27), 240 (100), 212 (26), 211 (18), 199 (20), 198 (17), 197 (21), 183 (16), 169 (36). HRMS (EI): calcd for $C_{15}H_{12}O_4$ $[M]^+$ 256.0736; found 256.0736.

*4.3. Synthesis of 1-hydroxymethyl-4-methoxy-naphthalene, Npm–OH (**1d**)*

4-Methoxy-1-naphthaldehyde (0.500 g, 2.69 mmol) was dissolved in ethanol (5 mL) and sodium borohydride (0.071 g, 1.88 mmol), dissolved previously in ethanol (6 mL), was added dropwise while stirring in an ice bath. The reaction mixture was stirred at room temperature for 3 days. The solvent was evaporated to dryness and the residue submitted to column chromatography (eluent: ethyl acetate/hexane, 1:3). Compound **1d** was obtained as a colourless solid (0.481 g, 95%). Mp=65.4–68.0 °C. ^{1}H NMR ($CDCl_3$): δ=4.02 (s, 3H, OCH_3), 5.04 (s, 2H, CH_2), 6.74 (d, *J* 8.1 Hz, 1H, H-3), 7.39 (d, *J* 7.8 Hz, 1H, H-2), 7.52 (dt, *J* 6.9 and 1.2 Hz, 1H, H-6), 7.58 (dt, *J* 6.9 and 1.2 Hz, 1H, H-7), 8.13 (dd, *J* 8.4 and 1.5 Hz, 1H, H-8), 8.33 (dd, *J* 8.4 and 1.5 Hz, 1H, H-5). ^{13}C NMR ($CDCl_3$): δ=55.46 (OCH_3), 63.61 (CH_2), 102.85 (C-3), 122.51 (C-5), 123.61 (C-8), 125.19 (C-6), 125.92 (C-4a), 126.14 (C-2), 126.83 (C-7), 128.45 (C-1), 132.27 (C-8a), 155.66 (C-4). IR (KBr 1%, cm^{-1}): ν=3375, 3299, 3079, 3014, 2995, 2965, 2934, 2872, 2840, 1583, 1514, 1463, 1450, 1426, 1391, 1338, 1305, 1277, 1249, 1225, 1156, 1091, 1064, 1026, 1010, 999, 822, 767, 738, 616, 510. UV/vis (EtOH, nm): λ_{max} (log ε)=296 (3.70). MS (EI, %): *m/z*=188 ($[M]^+$, 95), 187 (18), 172 (75), 171 (80), 156 (18), 144 (39), 129 (68), 128 (100), 127 (46), 115 (48). HRMS (EI): calcd for $C_{12}H_{12}O_2$ $[M]^+$ 188.0837; found 188.0840.

*4.4. N-Benzyloxycarbonyl-L-γ-aminobutyric acid (7-methoxy-3-oxo-3H-benzo[g]benzopyran-1-yl)methyl ester, Z–GABA–OBbl (**2a**)*

N-Benzyloxycarbonyl-L-γ-aminobutyric acid, Z–GABA–OH (0.100 g; 0.42 mmol) was reacted with 1-hydroxymethyl-7-methoxy-3-oxo-3*H*-benzo[*g*]benzopyran, Bbl–OH (**1a**) (0.050 g; 0.197 mmol) in DMF (3 mL) using a standard DCC/HOBt coupling. The solvent was removed by rotary evaporation under reduced pressure and the crude residue was purified by column chromatography on silica gel (chloroform/methanol, 100:1). Compound **2a** was obtained as a yellow solid (0.025 g, 27%). Mp=194.0–195.6 °C. ^{1}H NMR ($CDCl_3$): δ=1.92–1.96 (m, 2H, β-CH_2), 2.56 (t, *J* 7.2 Hz, 2H, α-CH_2), 3.28–3.35 (m, 2H, γ-CH_2), 3.97 (s, 3H, OCH_3), 4.90 (br s, 1H, NH), 5.11 (s, 2H, CH_2 Bbl), 5.41 (s, 2H, CH_2 Z), 6.49 (s, 1H, H-2), 7.13 (d, *J* 2.4 Hz, 1H, H-6), 7.17 (dd, *J* 9.3 and 2.7 Hz, 1H, H-8), 7.31–7.36 (m, 5H, 5×Ph–*H*), 7.64 (s, 1H, H-5), 7.81 (d, *J* 9.0 Hz, 1H, H-9), 7.91 (s, 1H, H-10). ^{13}C NMR ($CDCl_3$): δ=25.17 (β-CH_2), 31.18 (α-CH_2), 40.23 (γ-CH_2), 55.49 (OCH_3), 61.22 (CH_2 Z), 66.77 (CH_2 Bbl), 104.61 (C-6), 112.06 (C-5), 112.66 (C-2), 114.81 (C-10a), 119.85 (C-8), 123.59 (C-10), 125.55 (C-4a), 128.14 (2×Ph–*C*), 128.51 (3×Ph–*C*), 130.23 (C-9), 136.48 (C-5a), 136.51 (C1 Ph), 148.76 (C-1), 150.71 (C-9a), 156.46 (C=O urethane), 159.83 (C-7), 160.61 (C-3), 172.41 (C=O ester). IR (KBr 1%, cm^{-1}): ν=3327, 2928, 2851, 1732, 1721, 1688, 1627, 1575, 1486, 1455, 1312, 1262, 1232, 1171, 1089, 1028. UV/vis (MeOH/HEPES, 80:20, nm): λ_{max} (log ε)=345 (3.89). MS (FAB, %): *m/z*=476 ($[M+H]^+$, 3), 475 (M^+, 2), 307 (12), 226 (16), 225 (100), 155 (15), 154 (50). HRMS (FAB): calcd for $C_{27}H_{26}NO_7$ $[M+H]^+$ 476.1709; found 476.1711.

*4.5. N-Benzyloxycarbonyl-L-γ-aminobutyric acid (4-methoxy-naphthalen-1-yl)methyl ester, Z–GABA–ONpm (**2d**)*

Starting from Z–GABA–OH (0.134 g, 0.567 mmol) and 1-hydroxymethyl-4-methoxy-naphthalene Npm–OH (**1d**) (0.050 g, 0.266 mmol), and following a similar procedure as described for **2a**, compound **2d** was obtained as a yellow oil (0.057 g, 52%). ^{1}H NMR ($CDCl_3$): δ=1.79–1.85 (m, 2H, β-CH_2), 2.38 (t, *J* 7.5 Hz, 2H, α-CH_2), 3.18–3.25 (m, 2H, γ-CH_2), 4.01 (s, 3H, OCH_3), 4.92 (br s, 1H, NH), 5.08 (s, 2H, CH_2 Z), 5.50 (s, 2H, CH_2 Npm), 6.76 (d, *J* 8.1 Hz, 1H, H-3), 7.31–7.38 (m, 5H, 5×Ph–*H*), 7.45 (d, *J* 7.8 Hz, 1H, H-2), 7.51 (dt, *J* 6.9 and 1.2 Hz, 1H, H-6), 7.57 (dt, *J* 6.9 and 1.2 Hz, 1H, H-7), 7.95 (dd, *J* 8.1 and 1.8 Hz, 1H, H-8), 8.33 (dd, *J* 8.1 and 1.8 Hz, 1H, H-5). ^{13}C NMR ($CDCl_3$): 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 (C1 Ph), 156.23 (C=O urethane), 156.37 (C-4), 173.18 (C=O ester). IR (KBr 1%, cm^{-1}): ν=3418, 3033, 2942, 1726, 1626, 1585, 1515, 1464, 1455, 1394, 1250, 1164, 1093, 818, 768. UV/vis (MeOH/HEPES,

80:20, nm): λ_{max} (log ε)=295 (3.75). MS (FAB, %): *m/z*=408 ([M+H]$^+$, 34), 407 (M$^+$, 12), 307 (100), 155 (18), 154 (44). HRMS (FAB): calcd for $C_{24}H_{26}NO_5$ [M+H]$^+$ 408.1813; found 408.1805.

4.6. N-Benzyloxycarbonyl-L-γ-aminobutyric acid (9-methoxy-3-oxo-3H-benzo[f]benzopyran-1-yl)methyl ester, Z–GABA–OBba (2b)

Z–GABA–OH (0.095 g; 0.40 mmol) was reacted with 1-chloromethyl-9-methoxy-3-oxo-3*H*-benzo[*f*]benzopyran, Bba-Cl (**1b**) (0.110 g; 0.40 mmol) and potassium fluoride (0.070 g, 1.2 mmol) in dry DMF (3 mL). The reaction mixture was stirred at room temperature for 3 days. The solvent was removed by rotary evaporation under reduced pressure and the crude residue was purified by column chromatography with chloroform. Compound **2b** was obtained as a light brown solid (0.154 g, 81%). Mp=120.7–121.9 °C. ^{1}H NMR ($CDCl_3$): δ=1.91–1.96 (m, 2H, β-CH_2), 2.57 (t, *J* 7.2 Hz, 2H, α-CH_2), 3.28–3.34 (m, 2H, γ-CH_2), 3.95 (s, 3H, OCH_3), 5.00 (br s, 1H, NH), 5.10 (s, 2H, CH_2 Z), 5.66 (s, 2H, CH_2 Bba), 6.65 (s, 1H, H-2), 7.22 (dd, *J* 8.7 and 2.1 Hz, 1H, H-8), 7.31–7.35 (m, 6H, 5×Ph–*H* and H-5), 7.42 (d, *J* 2.1 Hz, 1H, H-10), 7.83 (d, *J* 9.3 Hz, 1H, H-7), 7.91 (d, *J* 8.7 Hz, 1H, H-6). ^{13}C NMR ($CDCl_3$): δ=25.04 (β-CH_2), 31.12 (α-CH_2), 40.16 (γ-CH_2), 55.43 (OCH_3), 64.21 (CH_2 Bba), 66.76 (CH_2 Z), 105.79 (C-10), 111.88 (C-4b), 112.61 (C-2), 115.26 (C-5), 116.53 (C-8), 126.34 (C-6a), 128.10 (2×Ph–*C*), 128.47 (3×Ph–*C*), 130.55 (C-6b), 131.31 (C-7), 133.80 (C-6), 136.35 (C1 Ph), 151.02 (C-1), 155.49 (C-4a), 156.54 (C=O urethane), 159.62 (C-9), 160.39 (C-3), 172.33 (C=O ester). IR (KBr 1%, cm^{-1}): ν=3329, 2927, 2851, 1739, 1721, 1691, 1626, 1553, 1456, 1365, 1265, 1229, 1182, 1100, 942, 837. UV/vis (MeOH/HEPES, 80:20, nm): λ_{max} (log ε)=350 (3.90). MS (FAB, %): *m/z*=476 ([M+H]$^+$, 22), 475 (M$^+$, 5), 432 (10), 314 (28), 276 (14), 240 (10), 230 (14), 193 (12), 192 (61), 175 (22), 174 (42), 154 (25). HRMS (FAB): calcd for $C_{27}H_{26}NO_7$ [M+H]$^+$: 476.1709; found: 476.1705.

4.7. N-Benzyloxycarbonyl-L-γ-aminobutyric acid (6-methoxy-3-oxo-3H-benzopyran-1-yl)methyl ester, Z–GABA–OBpm (2c)

Starting from Z–GABA–OH (0.112 g; 0.42 mmol) and 1-chloromethyl-6-methoxy-3-oxo-3*H*-benzopyran, Bpm-Cl (**1c**) (0.097 g; 0.42 mmol), and following a similar procedure as described for **2b**, compound **2c** was obtained as a off-white solid (0.152 g, 85%). Mp=117.8–118.5 °C. ^{1}H NMR ($CDCl_3$): δ=1.86–1.95 (m, 2H, β-CH_2), 2.51 (t, *J* 7.2 Hz, 2H, α-CH_2), 3.26–3.31 (m, 2H, γ-CH_2), 3.87 (s, 3H, OCH_3), 4.97 (br s, 1H, NH), 5.09 (s, 2H, CH_2 Z), 5.24 (s, 2H, CH_2 Bpm), 6.31 (s, 1H, H-2), 6.84 (dd, *J* 7.1 and 2.4 Hz, 1H, H-7), 6.88 (d, *J* 2.4 Hz, 1H, H-5), 7.27–7.40 (m, 6H, 5×Ph–*H* and H-8). ^{13}C NMR ($CDCl_3$): δ=25.08 (β-CH_2), 31.10 (α-CH_2), 40.18 (γ-CH_2), 55.75 (OCH_3), 61.22 (CH_2 Z), 66.68 (CH_2 Bpm), 101.17 (C-5), 109.99 (C-2), 110.53 (C-8a), 112.60 (C-7), 124.40 (C-8), 128.08 (2×Ph–*C*), 128.46 (3×Ph–*C*), 136.42 (C1 Ph), 149.05 (C-1), 155.48 (C-4a), 156.43 (C=O urethane), 160.79 (C-3), 162.85 (C-6), 172.30 (C=O ester). IR (KBr 1%, cm^{-1}): ν=3311, 3083, 3004, 2948, 1716, 1689, 1622, 1513, 1435, 1411, 1328, 1295, 1207, 1152, 1104, 1063, 1035, 998, 894, 846, 807, 742, 696. UV/vis (MeOH/HEPES, 80:20, nm): λ_{max} (log ε)=325 (4.10). MS (FAB, %): *m/z*=426 ([M+H]$^+$, 54), 455 (M$^+$, 8), 307 (32), 289 (15), 155 (31), 154 (100). HRMS (FAB): calcd for $C_{23}H_{24}NO_7$ [M+H]$^+$: 426.1553; found: 426.1555.

4.8. General photolysis procedure

A 1×10^{-4} M MeOH/HEPES buffer (80:20) solution of compounds **2a–e** (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 or 1.2 mL/min (for compound **2e**), 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**, 4.6 min; **2b**, 4.4 min; **2c**, 3.3 min; **2d**, 5.7 min; **2e**, 8.8 min).

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