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Photorelease of amino acid neurotransmitters from pyrenylmethyl ester conjugates

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Abstract—The linkage of model neurotransmitter L-amino acids, such as glycine, alanine, β-alanine, glutamic acid and γ-aminobutyric acid, to a pyrenylmethyl group as the fluorescent moiety through an ester bond at their carboxylic functions at the main and side chains (in the case of glutamic acid) was investigated. The behaviour of the resulting fluorescent conjugates towards photocleavage was studied in different cleavage conditions, namely the wavelength of irradiation and the use of different solvents, in a photochemical reactor equipped with lamps of 254, 300, 350 and 419 nm, followed by HPLC/UV monitoring.

1. Introduction

Neurodegenerative and neuropsychiatric disorders such as Alzheimer's disease, schizophrenia, Down's syndrome and Parkinson's disease are related to unbalanced action of particular neurotransmitters.[1–4] As a result, the quantification of neurotransmitters, such as amino acids, nucleotides and physiological amines, in biological samples may offer a means for diagnosis of disease as well as possible treatment. Amino acids play a major role in neuronal communication at the central nervous system (CNS) and their function has been the focus of steadily growing research in recent years. Aspartate (Asp) and glutamate (Glu) are the major excitatory amino acid neurotransmitters and are responsible for normal synaptic neurotransmission, whereas γ-aminobutyric acid (GABA) and glycine (Gly) acts as the main inhibitory transmitters within the mammalian CNS. Most of the neurotransmitter amino acids are small aliphatic molecules, which do not absorb or fluoresce strongly in the ultraviolet/visible region. Thus, derivatisation of such analytes is necessary to enhance the sensitivity of detection. Fluorescent labelling is a widely applied methodology, as it is the most suitable for analytical purposes for being far more sensitive than common UV techniques, thus overcoming problems of low detection limits. Examples are found in many diverse areas such as cell biology and biochemistry, to investigate the functionality and action of biomolecules inside living cells,[5] in combinatorial chemistry, to allow the rapid screening of large libraries of compounds[6] and in analytical chemistry.[7]

The use of photocleavable protecting groups, which only require light for the cleavage to occur, in the controlled temporal and spatial release of biologically active species (*caged compounds*) has become relevant in the investigation of signal transduction mechanisms at cellular level and in drug delivery. In addition, fluorescent photolabile protecting groups have advantages over other photolabile groups, because they can also act as temporary fluorescent labels.

Pyrene, a well known fluorophore, has been used in a varied range of applications that make use of its strong fluorescence, for example, in ligands for the measurement of the proteolytic activity of enzymes,[8] for high-affinity targeting of RNA and DNA using modified oligonucleotides,[9] as probes for site-specific photocleavage of proteins,[10] in supramolecular systems for chiral recognition,[11] in the elaboration of electroluminescent materials for optical devices[12] and in the preparation of film sensors for copper(II) salts.[13] Furthermore, the use of pyrene as a photochemically removable protecting group has been reported for alcohols,[14] carboxylic acids,[15] phosphates[16] and amines.[17]

Taking these facts into consideration together with our ongoing research related to the area of fluorescent heterocycle synthesis, amino acid labelling and the design and application of fluorescent photocleavable groups,[18] we decided to investigate the use of a pyrenylmethyl group in the derivatisation of the carboxylic function of amino acid neurotransmitters. Considering the fluorescence properties of the pyrene moiety, this group could act as a fluorescent photocleavable

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protecting group, in addition to a temporary fluorescent label. Thus, the synthesis and characterisation of new fluorescent neurotransmitter conjugates were carried out. Absorption and emission properties of all compounds were measured and the results showed that these conjugates exhibited good fluorescence quantum yields. Photocleavage of these fully protected conjugates was achieved by using radiation of 254, 300, 350 and 419 nm. The consumption of starting materials was monitored by RP-HPLC with UV monitoring.

2. Results and discussion

The linkage of model neurotransmitters to a strongly fluorescent moiety like pyrene is advantageous as it allows easier visualisation and isolation of these non-fluorescent systems whenever necessary during the course of organic reactions or in photorelease applications. Thus, 1-chloromethylpyrene was reacted with the carboxylic acid function of *N*-benzyloxycarbonyl protected neurotransmitter amino acids **1a–f**, such as glycine, alanine, β-alanine, glutamic acid and γ-aminobutyric acid, resulting in the corresponding fluorescent conjugates **2a–f** bearing the fluorogenic polycyclic aromatic system linked through an ester bond at the main or side chain, in the case of glutamic acid conjugate **2f**. The derivatisation of neurotransmitters **1a–f** was carried out with potassium fluoride,[19] in DMF, at room temperature, and after purification by column chromatography on silica gel, fluorescent conjugates **2a–f** were obtained in excellent yields (>90%) and fully characterised by the usual spectroscopic techniques (Scheme 1, Table 1). The pyrenylmethyl group will be designated in this report by a three letter code (Pym) for simplicity of naming the various fluorescent conjugates and pyrene will be designated by Py.

The IR spectra of conjugates **2a–f** showed bands due to stretching vibrations of the ester carbonyl group of the fluorophore–amino acid linkage from 1715 to 1739 cm^{-1}. The spectra also showed the carbonyl band of the *N*-benzyloxycarbonyl (Z) protecting group (1681–1710 cm^{-1}).

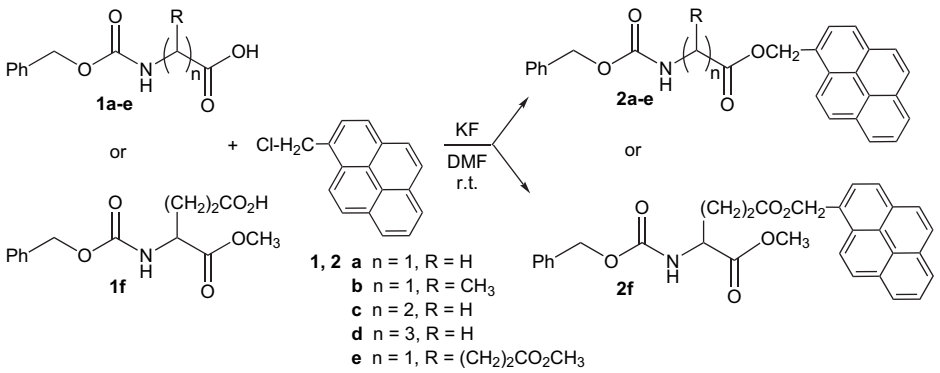
^1H NMR spectra showed signals of the amino acid residues, such as the α-CH (δ 4.42–4.55 ppm, for Ala and Glu) or α-CH_2 (δ 2.45–4.05 ppm, for Gly, β-Ala and GABA), as well as the characteristic protons of the methylene group adjacent to the pyrene (δ 5.82–5.98 ppm). The confirmation of the presence of the newly formed ester linkage was also supported by ^{13}C NMR spectral signals of the carbonyl group, which were found between δ 170 and 173 ppm. The signals for the carbonyl of the Z protecting group were found at about δ 155 ppm.

Table 1. Yields, UV/vis and fluorescence data for fluorescent neurotransmitter conjugates **2a–f** in absolute ethanol

Compound		Yield (%)	UV/vis		Fluorescence		
			λ_{max} (nm)	log ε	λ_{max} (nm)	Stokes' shift (nm)	Φ_F
2a	Z–Gly–OPym	91	342	4.62	375	33	0.19
2b	Z–Ala–OPym	93	342	4.62	374	32	0.14
2c	Z–β-Ala–OPym	99	342	4.63	374	32	0.20
2d	Z–GABA–OPym	98	342	4.61	374	32	0.15
2e	Z–Glu(OMe)–OPym	98	342	4.61	374	32	0.16
2f	Z–Glu(OPym)–OMe	99	342	4.63	374	32	0.18

Pyrene, widely known for its strong fluorescence, is a fluorogenic reagent when used for the derivatisation of non-fluorescent molecules, so the characterisation of the absorption and emission properties of the synthesised conjugates **2a–f** was carried out.

The electronic absorption and emission spectra of 10^{-6} M solutions of compounds **2a–f** were measured in absolute ethanol: absorption and emission maxima, molar absorptivities and fluorescence quantum yields (Φ_F) are reported in Table 1. The Φ_F were calculated using 9,10-diphenylanthracene as standard (Φ_F=0.95 in ethanol).[20] Absorption spectra showed a strong absorption band centred at 342 nm, with log ε at about 4.62. The maximum absorption wavelength and the shape of the absorption curve were similar for all compounds, which was due to the presence of the pyrene moiety in the structure. The nature of the amino acid neither influenced the position of the wavelength of maximum absorption and emission nor the shape of the absorption and emission bands of conjugates **2a–f**. The starting *N*-benzyloxycarbonyl amino acids **1a–f** presented a broad band with an absorption maximum at around 254 nm and the spectra of the conjugates displayed the pyrene fine vibronic structure (e.g., absorption spectrum of amino acid Z–GABA–OH **1d** compared to the absorption and emission spectra of conjugate Z–GABA–OPym **2d** (Fig. 1)). The wavelength of maximum emission was 374 nm for all compounds (except for **2a**, which was 375 nm). All labelled amino acids **2a–f** exhibited good quantum yields ($0.14<\Phi_F<0.20$) and Stokes' shifts of 32 nm, which can make them suitable for using as fluorescent probes.



Scheme 1. Synthesis of fluorescent neurotransmitter conjugates **2a–f**.

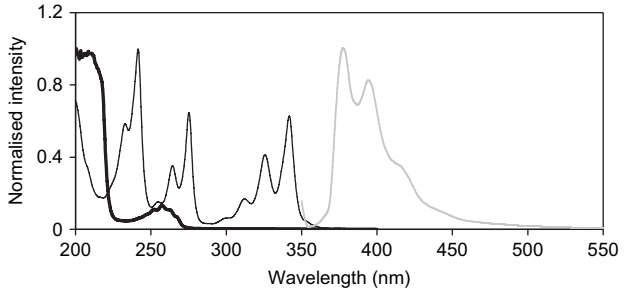


Figure 1. Normalised absorption spectra of Z–GABA–OH **1d** (thick line), absorption (thin line) and emission (grey line) spectra of Z–GABA–OPym **2d** in absolute ethanol ([**2d**]=2.0×10^{-6} M, λ_{exc}=342 nm).

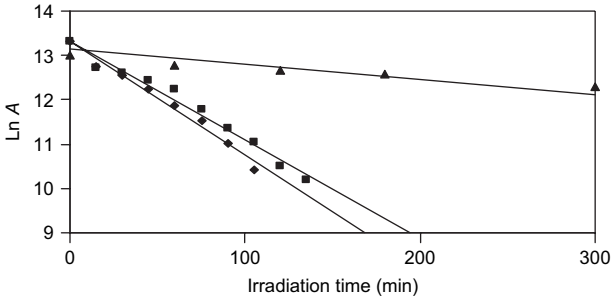


Figure 2. Plot of ln *A* versus irradiation time for the photolysis of Z–GABA–OPym **2d** in acetonitrile at 254 (◆), 300 (■) and 350 (▲) nm (for better visualisation, time scale is shown only up to 300 min, although photolysis at 350 nm proceeded until ca. 1000 min).

In order to assess the potential application of pyrenylmethyl as a versatile photocleavable protecting group in organic synthesis and/or as a phototrigger for the caging of biologically relevant molecules in appropriate conditions, photocleavage studies of neurotransmitter conjugates **2a–f** were carried out. Solutions of the mentioned compounds in acetonitrile (1×10^{-4} M) were irradiated in a Rayonet RPR-100 reactor, at 254, 300 and 350 nm, to confirm the influence of the irradiation wavelength on the photocleavage reaction. 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 RP-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, at all considered wavelengths (Table 2).

For each compound 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. Figure 2 summarises the behaviour of GABA conjugate **2d** at 254, 300 and 350 nm.

Concerning the influence of the wavelength of irradiation on the rate of the photocleavage reactions of pyrenylmethyl conjugates **2a–f** in acetonitrile, it can be seen that there was an increase in the irradiation time as the wavelength of irradiation increased for all conjugates (except for compound **2e**), which was more pronounced at 350 nm (from 654 to 1055 min) when compared to those obtained at 254 and 300 nm (from 121 to 140 and from 110 to 170 min, respectively).

Table 2. Irradiation times for the photolysis of compounds **2a–f** at different wavelengths in acetonitrile solution (1×10^{-4} M)

Compound	Irradiation time (min)		
	254 nm	300 nm	350 nm
2a	121	167	738
2b	129	146	654
2c	125	148	912
2d	138	159	990
2e	122	110	978
2f	140	170	1055

Taking into consideration the influence of the structure of the conjugates on the photocleavage rates, it was found that for conjugates Z–Glu(OMe)–OPym **2e** and Z–Glu(OPym)–OMe **2f**, in which the pyrenylmethyl group was linked through the carboxylic acid group at the main or side chain, respectively, the cleavage at the main chain ester bond (compound **2e**) was faster, for all wavelengths of irradiation. As for the number of methylene groups between the amino and carboxylic terminals in compounds Z–Gly–OPym **2a** (*n*=1), Z–β-Ala–OPym **2c** (*n*=2) and Z–GABA–OPym **2d** (*n*=3), there was an increase of the irradiation time as the number of methylene groups increased. For conjugates Z–Ala–OPym **2b** and Z–β-Ala–OPym **2c**, a significative difference in the cleavage rates was seen at 350 nm, as the cleavage of conjugate **2b** was ca. 28% faster than that of conjugate **2c**. The corresponding cleavage rates at 254 and 300 nm were very similar. At the same time, the study of the stability of the *N*-benzyloxycarbonyl protected amino acids **1a–f** was carried out under the above reported photolytic conditions. HPLC studies showed that the *N*-blocking group was stable in the tested conditions, no cleavage being detected. These results supported the fact that the disappearance of conjugates **2a–f** was associated with the cleavage of the ester linkage between the fluorophore and the carboxyl group of the amino acid, as expected. The Pym ester solutions in acetonitrile were found to be stable to prolonged storage at room temperature, in the dark.

We also studied the influence of the solvent on the course of the photocleavage reaction of GABA conjugate **2d**, by using other organic solvents of different polarity and character such as methanol and tetrahydrofuran in mixtures with aqueous 4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid (HEPES) buffer solution or water. A solution of HEPES was used in order to simulate biological conditions. The absorption spectra as well as the wavelength of maximum absorption of conjugate **2d** did not vary with the nature of the solvent system. Solutions of compound **2d** were irradiated in a manner analogous to that reported above for the acetonitrile solutions. Similar analysis of the collected data revealed that the water content in the aqueous mixtures strongly influenced the photocleavage rate, as the increase in the water percentage led to a dramatic increase in the cleavage rates. By comparison of entries 1–3 (Table 3) in which acetonitrile was mixed with 0, 30 and 50% aqueous solution, respectively, it was concluded that in the conditions of entry 3, the ester bond of conjugate **2d** cleaved 8–11 times

Table 3. Irradiation time for the photolysis of compound **2d** at different wavelengths in different solvent systems (1×10^{-4} M)

Entry	Solvent	Irradiation time (min)			
		254 nm	300 nm	350 nm	419 nm
1	ACN	138	159	990	—
2	ACN/HEPES (70:30)	73	60	820	—
3	ACN/HEPES (50:50)	18	14	91	2371
4	MeOH/HEPES (80:20)	17	19	158	—
5	THF/H_2O (50:50)	19	20	124	—

faster when compared to the conditions in entry 1. Considering entries 2 and 3, at 254 and 300 nm, the relative increase in the photocleavage rate was the same (4 times), whereas at 350 nm the cleavage was 9 times faster.

The character of the solvent also played a role on the time necessary to cleave at least 95% of the starting material, as the irradiation time with an aqueous mixture with a protic solvent like methanol (entry 4, Table 3) was considerably smaller (ca. 4 times) when compared to a similar mixture with an aprotic solvent like acetonitrile (entry 2, Table 3). This fact may be related to the nature of the mechanism, which is thought to be ionic.[21] Comparing entries 3 and 5, where mixtures of polar aprotic solvents (acetonitrile and tetrahydrofuran) were tested, the influence of the solvent was not perceptible at 254 nm as there was no difference in the cleavage rates. At 300 and 350 nm a slight difference was noted as cleavage in THF/H_2O went 1.4 times faster than the cleavage in ACN/HEPES.

Regarding the stability of the pyrenylmethyl conjugates, in the dark, in aqueous solvent systems, no hydrolysis products were detected by HPLC monitoring upon storage over an extended period of time (ca. 2 weeks).

The photochemical quantum yields were calculated using an expression[22] based on half-lives ($t_{1/2}$), molar absorptivities (ε) and the incident photon flux (I_0), which were determined by potassium ferrioxalate actinometry[23] (at 254, 300, 350 and 419 nm, 1.22×10^{17}, 2.54×10^{17}, 2.63×10^{17} and 2.61×10^{17} photons $s^{-1}\,cm^{-2}$, respectively) (Tables 4 and 5).

Taking into account the determined photochemical quantum yields, the photocleavage process of compounds **2a–f** was not as efficient as desirable, probably due to the dissipation of part of the absorbed energy via fluorescence pathways that compete with the photocleavage reaction, as well as the type of reactor used (open chamber reactor) and the low power of the lamps (14–35 W).

Table 4. Photolytic data for the photolysis of compounds **2a–f** at different wavelengths in acetonitrile solution

Compound	254 nm			300 nm			350 nm		
	ε_{254}[a]	$t_{1/2}$[b]	Φ_{phot}[c]	ε_{300}[a]	$t_{1/2}$[b]	Φ_{phot}[c]	ε_{350}[a]	$t_{1/2}$[b]	Φ_{phot}[c]
2a	10,500	2112	0.067	4750	2748	0.055	3050	12,468	0.018
2b	11,850	2226	0.057	5100	2424	0.058	3400	11,640	0.017
2c	11,650	2526	0.051	5000	2982	0.048	4150	11,722	0.014
2d	11,650	2592	0.049	5000	3086	0.046	4150	17,523	0.009
2e	11,140	2478	0.054	4555	4638	0.034	4505	15,096	0.010
2f	11,650	2052	0.062	5000	1998	0.072	4150	18,366	0.009

[a] Molar absorption coefficient (in $mol^{-1}\,L\,cm^{-1}$) at the irradiation wavelength.
[b] Half-life under experimental photolytic conditions (in seconds).
[c] Photochemical quantum yield ($\times10^{-3}$) of the photocleavage reaction. Calculated as indicated in Ref. 23.

In terms of applicability of the pyrenylmethyl unit as a fluorescent photocleavable protecting group for the carboxylic function, considering the results presented so far and in particular for the GABA conjugate **2d**, it appeared that the most promising solvent of irradiation would be the ACN/HEPES 50:50 solution since the irradiation times at 350 nm were smaller (91 min) than that for the other solvents (124–990 min). As for the wavelength of irradiation, we decided to test also the photocleavage at 419 nm to see if an acceptable irradiation time could be achieved at a wavelength already in the visible region. Thus, by following a similar irradiation procedure to that previously described, it was found that at 419 nm there was a very large increase in the irradiation time (2371 min, ca. 40 h necessary to cleave 95% of the starting material), which is not of use for practical applications (entry 3, Table 3).

Comparison of the photochemical quantum yields for the photocleavage reaction of compound **2d** in acetonitrile and in different solvent systems showed a noticeable increase for all the aqueous systems tested, the most relevant being THF/H_2O, 50:50, at 254 nm (26 times) and ACN/HEPES, 50:50, at 300 nm (25 times) and 350 nm (15 times).

The mechanism for cleavage with ultraviolet radiation of polycyclic aromatic systems like anthracene, phenanthrene and pyrene[22] linked to carboxylic acids through ester bonds is thought to involve the homolytic cleavage of the C–O bond, followed by electron transfer that yields a methylenic carbocation, which can undergo nucleophilic attack by a solvent molecule, resulting in the hydroxymethylated compound and free acid or concurrently, the heterolytic cleavage of the C–O bond that could directly afford the already mentioned methylenic carbocation. In our case, the fact that the photocleavage rate increased for solvent systems with higher

Table 5. Photolytic data for the photolysis of compound **2d** at different wavelengths in different solvent systems

Solvent system	254 nm			300 nm			350 nm		
	ε_{254}[a]	$t_{1/2}$[b]	Φ_{phot}[c]	ε_{300}[a]	$t_{1/2}$[b]	Φ_{phot}[c]	ε_{350}[a]	$t_{1/2}$[b]	Φ_{phot}[c]
ACN/HEPES (70:30)	8570	893	0.197	3438	845	0.246	3215	8218	0.026
ACN/HEPES (50:50)	10,180	137	1.080	4055	154	1.143	4280	1157	0.139
MeOH/HEPES (80:20)	11,120	260	0.521	4643	273	0.564	3930	2688	0.065
THF/H_2O (50:50)	3482	332	1.302	3571	301	0.665	4063	2015	0.084

[a] Molar absorption coefficient (in $mol^{-1}\,L\,cm^{-1}$) at the irradiation wavelength.
[b] Half-life under experimental photolytic conditions (in seconds).
[c] Photochemical quantum yield ($\times10^{-3}$) of the photocleavage reaction. Calculated as indicated in Ref. 23.

water percentage and with a polar protic solvent like methanol suggests an ionic mechanism. In this work, amino acids **1a–f** and 1-hydroxymethylpyrene would be expected as photoproducts. Although the amount of the photoproducts obtained was not quantified, for the photolysis of the GABA conjugate **2d** in different aqueous solvent systems containing water it was possible to identify the peak corresponding to 1-hydroxymethylpyrene at a retention time of 4.1 min (see Section 4 for HPLC conditions). In the case of the MeOH/HEPES 80:20 system, it was also visible another peak at a retention time of 9.6 min, which may be attributed to 1-methoxymethylpyrene, which resulted from methanol attack to the intermediary carbocation.

3. Conclusions

A series of fluorescent neurotransmitter ester conjugates **2a–f** were synthesised in excellent yields by a straightforward procedure, between commercially available 1-chloromethylpyrene and the C-terminus of *N*-benzyloxycarbonyl protected L-amino acid neurotransmitters (for glycine, alanine, β-alanine, glutamic acid protected at the side chain with a methyl ester and γ-aminobutyric acid) or the side chain carboxyl group (for glutamic acid methyl ester). The photophysical properties displayed by these conjugates showed that pyrenylmethyl is a versatile fluorogenic reagent for the derivatisation of non-fluorescent molecules. The photocleavage studies of the fluorescent conjugates, in acetonitrile and mixtures of acetonitrile, methanol and tetrahydrofuran with aqueous HEPES buffer or water, at 254, 300, 350 and 419 nm showed that the pyrenylmethyl group could be used as photolabile protecting group for organic molecules, including amino acids and other relevant biomolecules. The type of solvent system used in the photolysis also had a marked influence on the time of irradiation required to cleave the ester link: the use of a polar protic solvent like methanol and a higher percentage of water lead to a significant increase in the photocleavage rate, at all tested wavelengths of irradiation.

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 recorded on a Perkin–Elmer FTIR-1600 using KBr discs. UV/vis 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 an 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 spectrometric analyses were performed at the 'C.A.C.T.I.—Unidad de Espectrometria de Masas', at University of Vigo, Spain. Elemental analyses were carried out in a Leco CHNS 932 instrument. Fluorescence spectra were recorded 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 an HPLC system composed by a Jasco PU-980 pump, an UV/vis Shimadzu SPD-GAV detector and a Shimadzu C-RGA Chromatopac register.

1-Chloromethylpyrene was purchased from TCI and *N*-protected amino acid neurotransmitters **1a–f** from Senn Chemicals.

4.2. General procedure for the synthesis of compounds 2a–f

1-Chloromethylpyrene (1 equiv) was dissolved in dry DMF (2 mL), potassium fluoride (3 equiv) and the corresponding amino acid neurotransmitters **1a–f** (1 equiv) were added. The reaction mixture was stirred at room temperature for 3–4 days. The solvent was removed by rotary evaporation under reduced pressure and the crude residue was purified by column chromatography with chloroform.

4.2.1. *N*-(Benzyloxycarbonyl)-L-glycine (pyren-1-yl)-methyl ester, Z–Gly–OPym (2a). Starting from 1-chloromethylpyrene (0.100 g, 3.99 mmol) and Z–Gly–OH **1a** (0.084 g, 3.99 mmol), compound **2a** was obtained as a colourless solid (0.154 g, 91%). Mp=112.9–114.6 °C. ^{1}H NMR ($CDCl_3$): δ=4.05 (d, *J*=5.7 Hz, 2H, CH_2 Gly), 5.14 (s, 2H, CH_2 Z), 5.33 (t, *J*=4.8 Hz, 1H, NH), 5.89 (s, 2H, CH_2 Pym), 7.34 (s, 5H, 5×Ph–*H*), 8.01–8.23 (m, 9H, 9×Py–*H*). ^{13}C NMR ($CDCl_3$): δ=42.83 (CH_2 Gly), 65.59 (CH_2 Pym), 67.07 (CH_2 Z), 122.60 (Py–*C*), 124.49 (Py–*C*), 124.51 (Py–*C*), 124.75 (Py–*C*), 125.49 (Py–*C*), 125.56 (Py–*C*), 126.07 (Py–*C*), 127.23 (Py–*C*), 127.80 (Py–*C*), 127.86 (Ph–*C*), 127.92 (2×Ph–*C*), 128.04 (Py–*C*), 128.12 (Py–*C*), 128.32 (2×Ph–*C*), 128.46 (Py–*C*), 129.49 (Py–*C*), 130.55 (Py–*C*), 131.08 (Py–*C*), 131.85 (Py–*C*), 136.11 (C1 Ph), 156.24 (C=O urethane), 169.98 (C=O ester). IR (KBr 1%, cm^{-1}): ν=3307 (NH), 3035, 2948, 1715 (C=O), 1688 (C=O), 1589, 1538, 1467, 1455, 1417, 1386, 1355, 1316, 1270, 1245, 1196, 1105, 1061, 973, 943, 848, 823, 784, 757, 713, 695. UV/vis (ethanol, nm): λ_{max} (log ε)=342 (4.62), 326 (4.43), 311 (4.03), 275 (4.65), 264 (4.40), 241 (4.86). MS (FAB, %): *m*/*z*=424 ([M+H]$^+$, 12), 423 (M$^+$, 36), 216 (23), 215 (100), 154 (27). Anal. Calcd for $C_{27}H_{21}NO_4$ (423.47): C 76.58, H 5.00, N 3.31. Found: C 76.59, H 4.89, N 3.36.

4.2.2. *N*-(Benzyloxycarbonyl)-L-alanine (pyren-1-yl)-methyl ester, Z–Ala–OPym (2b). Starting from 1-chloromethylpyrene (0.100 g, 3.99 mmol) and Z–Ala–OH **1b** (0.089 g, 3.99 mmol), compound **2b** was obtained as a colourless solid (0.162 g, 93%). Mp=157.5–158.9 °C. ^{1}H NMR ($CDCl_3$): δ=1.41 (d, *J*=7.2 Hz, 3H, β-CH_3), 4.50 (t, *J*=7.5 Hz, 1H, α-H), 5.12 (s, 2H, CH_2 Z), 5.40 (d, *J*=7.2 Hz, 1H, NH), 5.82–5.96 (m, 2H, CH_2 Pym), 7.34 (s,

5H, 5×Ph–*H*), 8.04–8.23 (m, 9H, 9×Py–*H*). ^{13}C NMR ($CDCl_3$): δ=18.57 (β-CH_3), 49.79 (α-C), 65.71 (CH_2 Pym), 66.87 (CH_2 Z), 122.62 (Py–*C*), 124.51 (Py–*C*), 124.78 (Py–*C*), 125.47 (Py–*C*), 125.54 (Py–*C*), 126.06 (Py–*C*), 127.24 (Py–*C*), 127.71 (Py–*C*), 127.88 (Py–*C* and 3×Ph–*C*), 128.03 (Py–*C*), 128.08 (Py–*C*), 128.25 (2×Ph–*C*), 128.44 (Py–*C*), 129.45 (Py–*C*), 130.56 (Py–*C*), 131.09 (Py–*C*), 131.81 (Py–*C*), 136.16 (C1 Ph), 155.57 (C=O urethane), 172.91 (C=O ester). IR (KBr 1%, cm^{-1}): ν=3305 (NH), 3049, 2929, 1739 (C=O), 1688 (C=O), 1543, 1450, 1391, 1343, 1312, 1265, 1216, 1184, 1096, 1060, 935, 869, 848, 841, 824, 779, 750. UV/vis (ethanol, nm): λ_{max} (log ε)=342 (4.62), 326 (4.44), 312 (4.07), 275 (4.67), 264 (4.40), 241 (4.81). MS (FAB, %): *m*/*z*=438 ($[M+H]^+$, 15), 437 (M^+, 45), 216 (23), 215 (100), 154 (48). Anal. Calcd for $C_{28}H_{23}NO_4$ (437.49): C 76.87, H 5.30, N 3.20. Found: C 76.65, H 5.25, N 3.24.

4.2.3. *N*-(Benzyloxycarbonyl)-L-β-alanine (pyren-1-yl)-methyl ester, Z–β-Ala–OPym (2c). Starting from 1-chloromethylpyrene (0.100 g, 3.99 mmol) and Z–β-Ala–OH **1c** (0.089 g, 3.99 mmol), compound **2c** was obtained as an off-white solid (0.174 g, 99%). Mp=93.2–95.0 °C. ^{1}H NMR ($CDCl_3$): δ=2.64 (t, *J*=6.0 Hz, 2H, α-CH_2), 3.50 (q, *J*=6.3 Hz, 2H, β-CH_2), 5.07 (s, 2H, CH_2 Z), 5.29 (br s, 1H, NH), 5.85 (s, 2H, CH_2 Pym), 7.33 (s, 5H, 5×Ph–*H*), 8.04–8.24 (m, 9H, 9×Py–*H*). ^{13}C NMR ($CDCl_3$): δ=34.52 (α-CH_2), 36.54 (β-CH_2), 65.00 (CH_2 Pym), 66.66 (CH_2 Z), 122.63 (Py–*C*), 124.56 (Py–*C*), 124.81 (Py–*C*), 125.48 (Py–*C*), 125.55 (Py–*C*), 126.07 (Py–*C*), 127.27 (Py–*C*), 127.74 (Py–*C*), 127.87 (Py–*C*), 128.04 (Py–*C*), 128.06 (2×Ph–*C*), 128.32 (2×Ph–*C*), 128.38 (Ph–*C*), 128.46 (2×Py–*C*), 129.45 (Py–*C*), 130.59 (Py–*C*), 131.12 (Py–*C*), 131.78 (Py–*C*), 136.39 (C1 Ph), 156.20 (C=O urethane), 172.23 (C=O ester). IR (KBr 1%, cm^{-1}): ν=3346 (NH), 3041, 2955, 1722 (C=O), 1710 (C=O), 1605, 1589, 1530, 1515, 1455, 1417, 1384, 1368, 1352, 1317, 1246, 1179, 1139, 1072, 1065, 1003, 845, 819, 755, 698. UV/vis (ethanol, nm): λ_{max} (log ε)=342 (4.63), 326 (4.45), 312 (4.09), 275 (4.67), 264 (4.40), 241 (4.81). MS (FAB, %): *m*/*z*=438 ($[M+H]^+$, 10), 437 (M^+, 32), 231 (16), 216 (23), 215 (100). Anal. Calcd for $C_{28}H_{23}NO_4$ (437.49): C 76.87, H 5.30, N 3.20. Found: C 76.65, H 5.25, N 3.24.

4.2.4. *N*-(Benzyloxycarbonyl)-L-γ-aminobutyric acid (pyren-1-yl)methyl ester, Z–GABA–OPym (2d). Starting from 1-chloromethylpyrene (0.100 g, 3.99 mmol) and Z–GABA–OH **1d** (0.095 g, 3.99 mmol), compound **2d** was obtained as an off-white solid (0.180 g, 98%). Mp=120.5–121.6 °C. ^{1}H NMR ($CDCl_3$): δ=1.82–1.92 (m, 2H, β-CH_2), 2.45 (t, *J*=7.5 Hz, 2H, α-CH_2), 3.20–3.27 (m, 2H, γ-CH_2), 4.87 (br s, 1H, NH), 5.08 (s, 2H, CH_2 Z), 5.84 (s, 2H, CH_2 Pym), 7.33 (s, 5H, 5×Ph–*H*), 8.03–8.30 (m, 9H, 9×Py–*H*). ^{13}C NMR ($CDCl_3$): δ=25.11 (β-CH_2), 31.48 (α-CH_2), 40.30 (γ-CH_2), 64.79 (CH_2 Pym), 66.60 (CH_2 Z), 122.75 (Py–*C*), 124.56 (Py–*C*), 124.80 (Py–*C*), 125.42 (Py–*C*), 125.49 (Py–*C*), 126.05 (Py–*C*), 127.29 (Py–*C*), 127.71 (Py–*C*), 127.80 (Py–*C*), 128.03 (2×Ph–*C*), 128.20 (2×Ph–*C*), 128.45 (2×Py–*C* and Ph–*C*), 128.67 (Py–*C*), 129.43 (Py–*C*), 130.60 (Py–*C*), 131.13 (Py–*C*), 131.70 (Py–*C*), 136.45 (C1 Ph), 156.34 (C=O urethane), 173.10 (C=O ester). IR (KBr 1%, cm^{-1}): ν=3343 (NH), 3030, 2883, 1721 (C=O), 1688 (C=O), 1605, 1537, 1467, 1394, 1358, 1319, 1258, 1246, 1180, 1141, 1100, 1062, 1036, 966, 941, 907, 849, 819, 778. UV/vis (ethanol, nm): λ_{max} (log ε)=342 (4.61), 325 (4.42), 312 (4.03), 275 (4.65), 264 (4.38), 241 (4.75). MS (FAB, %): *m*/*z*=452 ($[M+H]^+$, 8), 451 (M^+, 19), 392 (22), 391 (80), 307 (25), 289 (15), 215 (53), 167 (21), 166 (15), 155 (31), 154 (100), 152 (18). Anal. Calcd for $C_{29}H_{25}NO_4$ (451.52): C 77.14, H 5.58, N 3.10. Found: C 76.77, H 5.59, N 3.06.

4.2.5. 2-(*N*-Benzyloxycarbonyl)amino-5-methyl-1-(pyren-1-yl)methyl pentanedioate, Z–Glu(OMe)–OPym (2e). Starting from 1-chloromethylpyrene (0.100 g, 3.99 mmol) and Z–Glu(OMe)–OH **1e** (0.118 g, 3.99 mmol), compound **2e** was obtained as a colourless solid (0.203 g, 98%). Mp=125.5–126.6 °C. ^{1}H NMR ($CDCl_3$): δ=1.94–2.05 (m, 1H, γ-CH_2), 2.16–2.39 (m, 3H, β-CH_2 and γ-CH_2), 3.51 (s, 3H, OCH_3), 4.45–4.55 (m, 1H, α-H), 5.10 (s, 2H, CH_2 Z), 5.47 (d, *J*=7.5 Hz, 1H, NH), 5.86–5.98 (m, 2H, CH_2 Pym), 7.32 (s, 5H, 5×Ph–*H*), 8.07–8.26 (m, 9H, 9×Py–*H*). ^{13}C NMR ($CDCl_3$): δ=27.48 (γ-CH_2), 29.73 (β-CH_2), 51.62 (OCH_3), 53.48 (α-C), 65.91 (CH_2 Pym), 67.03 (CH_2 Z), 122.62 (Py–*C*), 124.54 (Py–*C*), 124.81 (Py–*C*), 125.53 (Py–*C*), 125.58 (Py–*C*), 126.10 (Py–*C*), 127.27 (Py–*C*), 127.88 (Py–*C*), 127.94 (2×Ph–*C*), 128.05 (Py–*C*), 128.13 (Py–*C*), 128.37 (2×Ph–*C*), 128.46 (Ph–*C* and 2×Py–*C*), 129.51 (Py–*C*), 130.60 (Py–*C*), 131.11 (Py–*C*), 131.90 (Py–*C*), 136.06 (C1 Ph), 155.87 (C=O urethane), 171.76 (C=O ester), 172.96 (C=O methyl ester). IR (KBr 1%, cm^{-1}): ν=3330 (NH), 3043, 2950, 2851, 2791, 1806, 1749 (C=O), 1731 (C=O), 1681 (C=O), 1604, 1588, 1538, 1458, 1446, 1435, 1420, 1395, 1378, 1360, 1297, 1270, 1206, 1102, 1052, 1036, 983, 964, 911, 893, 842. UV/vis (ethanol, nm): λ_{max} (log ε)=342 (4.61), 326 (4.43), 312 (4.02), 275 (4.66), 264 (4.38), 241 (4.77). MS (FAB, %): *m*/*z*=510 ($[M+H]^+$, 12), 509 (M^+, 29), 216 (23), 215 (100), 154 (43). Anal. Calcd for $C_{31}H_{27}NO_6$ (509.56): C 73.07, H 5.34, N 2.75. Found: C 73.04, H 5.21, N 2.79.

4.2.6. 4-(*N*-Benzyloxycarbonyl)amino-5-methyl-1-(pyren-1-yl)methyl pentanedioate, Z–Glu(OPym)–OMe (2f). Starting from 1-chloromethylpyrene (0.100 g, 3.99 mmol) and Z–Glu–OMe **1f** (0.118 g, 3.99 mmol), compound **2f** was obtained as a yellow solid (0.201 g, 99%). Mp=162.2–163.9 °C. ^{1}H NMR ($CDCl_3$): δ=1.97–2.09 (m, 1H, γ-CH_2), 2.22–2.33 (m, 1H, γ-CH_2), 2.41–2.57 (m, 2H, β-CH_2), 3.71 (s, 3H, OCH_3), 4.42–4.49 (m, 1H, α-H), 5.09 (s, 2H, CH_2 Z), 5.43 (d, *J*=7.8 Hz, 1H, NH), 5.84 (s, 2H, CH_2 Pym), 7.32 (s, 5H, 5×Ph–*H*), 8.04–8.30 (m, 9H, 9×Py–*H*). ^{13}C NMR ($CDCl_3$): δ=27.63 (γ-CH_2), 30.18 (β-CH_2), 52.50 (OCH_3), 53.24 (α-C), 64.96 (CH_2 Pym), 67.03 (CH_2 Z), 122.79 (Py–*C*), 124.56 (Py–*C*), 124.81 (Py–*C*), 125.44 (Py–*C*), 125.51 (Py–*C*), 126.07 (Py–*C*), 127.30 (Py–*C*), 127.75 (Py–*C*), 127.83 (Py–*C*), 128.04 (2×Ph–*C*), 128.14 (Py–*C*), 128.24 (Py–*C*), 128.47 (Py–*C* and 2×Ph–*C*), 128.55 (Ph–*C*), 129.49 (Py–*C*), 130.63 (Py–*C*), 131.15 (Py–*C*), 131.74 (Py–*C*), 136.06 (C1 Ph), 155.86 (C=O urethane), 172.24 (C=O methyl ester), 172.57 (C=O ester). IR (KBr 1%, cm^{-1}): ν=3331 (NH), 3035, 2951, 2897, 1905, 1743 (C=O), 1717 (C=O), 1694 (C=O), 1605, 1591, 1527, 1466, 1448, 1432, 1395, 1330, 1301, 1276, 1255, 1216, 1167, 1055, 998, 974, 962, 933, 901, 850, 819, 796, 783. UV/vis (ethanol, nm): λ_{max} (log ε)=342 (4.63), 325 (4.42), 312 (4.03), 275 (4.65), 264

(4.36), 241 (4.78). MS (FAB, %): m/z=510 ([M+H]$^+$, 6), 509 (M$^+$, 11), 307 (33), 289 (16), 215 (35), 155 (31), 154 (100). Anal. Calcd for $C_{31}H_{27}NO_6$ (509.56): C 73.07, H 5.34, N 2.75. Found: C 73.32, H 5.25, N 2.81.

4.3. General photolysis procedure

A 1×10^{-4} M solution in acetonitrile of compounds **2a–f** (5 mL) and various solvent systems of compound **2d** were placed in a quartz tube and irradiated in the 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 1.2 mL/min, previously filtered through a Millipore, type HN 0.45 μm filter and degassed by ultrasound for 30 min. The chromatograms were traced by detecting UV absorption at the wavelength of maximum absorption for each compound (retention time: **2a**, 6.5; **2b**, 8.8; **2c**, 8.0; **2d**, 8.8; **2e**, 7.5; **2f**, 8.4 min).

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References and notes

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