Photoactivable heterocyclic cages in a comparative release study of butyric acid as a model drug

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Abstract

Aiming at the improvement of the photorelease of butyric acid - a model carboxylic acid drug, a set of heteroaromatic compounds based on acridine, naphtho[2,1-*b*]pyran, 3*H*-benzopyran fused julolidine and thioxo-naphtho[2,1-*b*]pyran were evaluated as benzyl-type phototriggers, in comparison with the well-known *o*-nitrobenzyl group. The corresponding ester cages were irradiated in a photochemical reactor at 254, 300, 350 and 419 nm, in two solvent systems (methanol or acetonitrile in 80:20 mixtures with HEPES buffer). Photolysis studies showed that, for some of the cages, the release of the active molecule occurred with short irradiation times at 419 nm. Time-resolved fluorescence was used to elucidate their photophysical properties and determine the decay kinetics. Studies were also carried out to assess the suitability of using two-photon excitation to address these compounds, which is advantageous if their use in biological systems is to be considered.

Keywords

Prodrugs; Butyric acid; Coumarin; Acridine; *o*-Nitrobenzyl group; Phototriggers.

1. Introduction

The use of several drugs in clinical practice is dependent on the improvement of their therapeutic effects, bioavailability, physicochemical properties and the minimizing of other undesirable side effects. Prodrugs, pharmacologically latent derivatives of active agents, can be designed to undergo

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activation through a specific stimulus. In addition to chemical and/or enzymatic triggers, light is an appealing tool to use for conversion of prodrugs to active agents in a spatially and temporally controlled manner [1-8]. Butyric acid, a saturated unbranched monocarboxylic acid, is one of the short-chain fatty acids. The effects of butyric acid include the disruption of cell proliferation and induction of apoptosis; modification of cell morphology and alteration of gene expression [9]. The presence of carboxylic acids or other ionisable polar groups in drugs can result in poor absorption from the gastrointestinal tract owing to lipophilicity/solubility issues.

Light triggerable benzyl or heterocyclic benzyl esters represent a large family of photolabile protecting groups. *o*-Nitrobenzylic derivatives have been widely used in various applications, as they combine a satisfactory photosensitivity with a stability for handling and synthesis purposes [10-12]. Nevertheless, *o*-nitrobenzyl cages exhibit some limitations, since the wavelength of excitation required for the uncaging is not necessarily the most suitable for bioapplications. The search for protecting groups with improved photochemical properties and even displaying fluorescence has motivated the incorporation of heterocyclic moieties in their design, with coumarinyl methyl groups as relevant examples. It is possible to find a wide range of derivatives possessing different combinations of substituents and/or fusion ring units for the release of various active molecules [13-15]. Recently, acridinyl methyl esters have also proved to possess the required photosensitivity for the release of carboxylic acid compounds [16].

Considering the know-how of the authors in the field of fluorescent photoactivable molecules based on aromatic and heteroaromatic skeletons for the release of bioanalytes [17-24], in connection with the interest in the development of alternative light sensitive prodrugs, and following the previous work regarding butyric acid, a new set of oxygen and nitrogen heterocyclic cages were synthesised. The use of these heterocyclic moieties can produce longer maximum wavelengths of absorption, allowing the photorelease of butyric acid at longer wavelengths, not detrimental to bioapplications. It also opens the way to use two-photon excitation (TPE), where if sufficient photon flux is present two longer wavelength photons can be used to excite a sample. This is advantageous as it only occurs in a fentolitre volume and the longer wavelength photons are less likely to interact with biological material. Thus, the present work evaluates the behaviour of (acridin-9-yl)methyl, (5-methoxy-3-oxo-3*H*-naphtho[2,1-*b*]pyran-1-yl)methyl, (8-methoxy-3-oxo-3*H*-naphtho[2,1-*b*]pyran-1-yl)methyl, and [11-oxo-2,3,5,6,7,11-hexahydro-1*H*-pyrano[2,3-*f*]pyrido[3,2,1-*ij*]quinolin-9-yl]methyl groups, in comparison with the well-known *o*-nitrobenzyl group in the light-induced release of butyric acid. Additionally, bearing in mind that the replacement of a carbonyl by a thiocarbonyl group results in

an improvement in the photolytic release [23,24], thionated groups; namely (5-methoxy-3-thioxo-3*H*-naphtho[2,1-*b*]pyran-1-yl)methyl, (8-methoxy-3-thioxo-3*H*-naphtho[2,1-*b*]pyran-1-yl)methyl, and (9-methoxy-3-thioxo-3*H*-naphtho[2,1-*b*]pyran-1-yl)methyl were tested. The ester cages, in two solvent systems (methanol or acetonitrile in 80:20 mixtures with HEPES buffer), were irradiated at 254, 300, 350 and 419 nm in a photochemical reactor. The fact that the groups exhibit fluorescence enabled time-resolved fluorescence measurements to be employed to elucidate their photophysical properties and determine the decay kinetics, along with their suitability for two-photon excitation. This last point can be important if their use in biological systems is to be considered

2. Experimental

2.1. Synthesis general

All melting points were measured on a Stuart SMP3 melting point apparatus. 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 BOMEM MB 104 spectrophotometer. UV/visible absorption spectra (200 – 700 nm) were obtained using a Shimadzu UV/2501PC spectrophotometer. NMR spectra were obtained on a Bruker Avance III 400 at an operating frequency of 400 MHz for ^{1}H and 100.6 MHz for ^{13}C using the solvent peak as internal reference at 25 ºC. All chemical shifts are given in ppm using δ_H Me_4Si = 0 ppm as reference and *J* values are given in Hz. Assignments were made by comparison of chemical shifts, peak multiplicities and *J* values and were supported by spin decoupling-double resonance and bidimensional heteronuclear 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 FluoroMax-4 spectrofluorometer. All reagents were used as received. Compounds **5**, **6** and **11** were synthesised as previously reported [20,25,26].

*2.2. Synthesis of 1-chloromethyl-5-methoxy-3-oxo-3H-naphtho[2,1-b]pyran **3***. To a solution of 3-methoxy-2-naphthol (0.104 g, 5.97×10^{-4} mol) in 70% aqueous sulphuric acid (5 mL), ethyl 4-chloro-3-oxobutanoate (0.089 mL, 6.57×10^{-4} mol) was added. The reaction was followed by TLC (ethyl acetate/*n*-hexane, 1:4), and stirred at room temperature for 96 h. The mixture was poured into ice water and stirred for 2 h to give a fine pale precipitate. The solid was collected by filtration, washed with cold water, dried and purified by column chromatography, using ethyl acetate/ light

petroleum as eluent, with mixtures of increasing polarity. Compound **3** was obtained as pale brown solid (0.012 g, 7%). Mp 169.5-171.8 °C. R_f = 0.50 (ethyl acetate/*n*-hexane, 1:4). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H = 4.03 (s, 3 H, OCH_3), 5.05 (s, 2 H, CH_2), 6.75 (s, 1 H, H-2), 7.36 (s, 1 H, H-6), 7.54-7.57 (m, 2 H, H-8 and H-9), 7.82-7.84 (m, 1 H, H-7), 8.30-8.32 (m, 1 H, H-10). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ_C = 45.79 (CH_2), 56.16 (OCH_3), 111.17 (C-6), 113.51 (C-4b), 117.74 (C-2), 123.69 (C-6b), 124.71 (C-10), 125.95 (C-9), 126.17 (C-8), 128.46 (C-7), 131.25 (C-6a), 146.75 (C-5), 147.21 (C-4a), 151.33 (C-1), 159.22 (C-3). IR (KBr 1%): ν = 1728, 1627, 1599, 1556, 1513, 1486, 1462, 1439, 1422, 1378, 1348, 1325, 1264, 1217, 1168, 1140, 1122, 1046, 1024, 1001, 938, 896, 866, 844, 778, 737, 704 cm^{-1}. HRMS (ESI) for $C_{15}H_{12}{}^{37}ClO_3$ [M^++H]: calculated 277.04456, found 277.04478; for $C_{15}H_{12}{}^{35}ClO_3$ [M^++H]: calculated 275.04756, found 275.04770.

2.3. Synthesis of 1-chloromethyl-8-methoxy-3-oxo-3H-naphtho[2,1-b]pyran ***4***.To a solution of 6-methoxy-2-naphthol (0.248 g, 1.42 × 10^{-3} mol) in 70% aqueous sulphuric acid (5 mL), ethyl 4-chloro-3-oxobutanoate (0.288 mL, 2.13 × 10^{-3} mol) was added. The reaction was followed by TLC (ethyl acetate/*n*-hexane, 1:4), and stirred at room temperature for 72 h. The mixture was poured into ice water and stirred for 2 h to give a fine pale precipitate. The solid was collected by filtration, washed with cold water, dried and purified by column chromatography, using ethyl acetate/light petroleum as eluent, with mixtures of increasing polarity. Compound **4** was obtained as pale yellow solid (0.145 g, 37%). Mp 207.3-209.9 °C. R_f = 0.50 (ethyl acetate/*n*-hexane, 1:4). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H = 3.97 (s, 3 H, OCH_3), 5.03 (s, 2 H, CH_2), 6.72 (s, 1 H, H-2), 7.26 (d, J = 2.8 Hz, 1 H, H-7), 7.36 (dd, J = 9.6 and 2.8 Hz, 1 H, H-9), 7.46 (d, J = 8.8 Hz, 1 H, H-5), 7.92 (d, J = 8.8 Hz, 1 H, H-6), 8.33 (d, J = 9.2 Hz, 1 H, H-10). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ_C = 45.84 (CH_2), 55.41 (OCH_3), 108.61 (C-7), 112.70 (C-4b), 117.51 (C-2), 118.18 (C-5), 120.09 (C-9), 123.44 (C-6b), 126.36 (C-10), 132.97 (C-6a), 133.16 (C-6), 150.98 (C-1), 153.76 (C-4a), 157.13 (C-8), 160.09 (C-3). IR (KBr 1%): ν = 1722, 1611, 1553, 1514, 1468, 1422, 1362, 1318, 1264, 1213, 1182, 1154, 1118, 1034, 1015, 998, 912, 897, 878, 858, 816, 737, 704 cm^{-1}. HRMS (ESI) for $C_{15}H_{12}{}^{37}ClO_3$ [M^++H]: calculated 277.04456, found 277.04449; for $C_{15}H_{12}{}^{35}ClO_3$ [M^++H]: calculated 275.04756, found 275.04751.

2.4. Synthesis of 2-nitrobenzyl butyrate, ***7***. To a solution of butyric acid (0.263 mL, 2.88 × 10^{-3} mol) in dry DMF (4 mL) at 0 °C, 1-hydroxybenzotriazole (HOBt) (0.072 g, 5.33 × 10^{-4} mol) was added. After stirring for 10 min, *N,N'*-dicyclohexylcarbodiimide (DCC) (0.114 g, 5.52 × 10^{-4} mol) was added, followed by (2-nitrophenyl)methanol **1** (0.402 g, 2.62 × 10^{-3} mol). The reaction mixture was

stirred at room temperature for 72 h and followed by TLC (ethyl acetate/light petroleum, 1:4). The solid was filtered and the residue was evaporated under vacuum. Cold acetone was added and the dicyclohexylurea precipitate was filtered. The solvent was removed by rotary evaporation under reduced pressure and the crude residue was purified by column chromatography using ethyl acetate/light petroleum, with mixtures of increasing polarity as eluent. Compound **7** was obtained as an orange oily solid (0.169 g, 29 %). R_f = 0.88 (ethyl acetate/light petroleum, 1:4). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H = 0.92 (t, *J* = 7.6 Hz, 3 H, C*H*$_3$-CH$_2$-CH$_2$), 1.65 (sext, *J* = 7.2 Hz, 2 H, CH$_3$-C*H*$_2$-CH$_2$), 2.36 (t, *J* = 7.2 Hz, 2 H, CH$_3$-CH$_2$-C*H*$_2$), 5.47 (s, 2 H, CH$_2$), 7.44 (dt, *J* = 7.4 and 1.6 Hz, 1 H, H-4), 7.55 (dd, *J* = 7.6 and 0.8 Hz, 1 H, H-6), 7.62 (dt, *J* = 7.4 and 0.8 Hz, 1 H, H-5), 8.02 (dd, *J* = 8.0 and 0.8 Hz, 1 H, H-3). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ_C = 13.43 (*C*H$_3$-CH$_2$-CH$_2$), 18.19 (CH$_3$-*C*H$_2$-CH$_2$), 35.80 (CH$_3$-CH$_2$-*C*H$_2$), 62.54 (CH$_2$), 124.79 (C-3), 128.56 (C-4), 128.84 (C-6), 132.06 (C-1), 133.54 (C-5), 147.39 (C-2), 172.80 (C=O). IR (KBr 1%): *v* = 2964, 2931, 2851, 1711, 1613, 1574, 1525, 1476, 1445, 1434, 1366, 1338, 1305, 1251, 1187, 1144, 1085, 1037, 989, 858, 792, 726 cm^{-1}. HRMS (ESI) for $C_{11}H_{14}NO_4$ [M$^+$+H]: calculated 224.09232, found: 224.09240.

2.5. Synthesis of (acridin-9-yl)methyl butyrate, ***8***. To a solution of 9-(bromomethyl)acridine **2** (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 was followed by TLC (ethyl acetate/light petroleum, 1:1), and stirred at room temperature for 15 h. The solvent was removed by rotary evaporation under reduced pressure and the crude residue was purified by column chromatography using ethyl acetate/light petroleum, mixtures of increasing polarity as eluent. Compound **8** was obtained as a brown oily solid (0.027 g, 69%). R_f = 0.48 (ethyl acetate/light petroleum, 1:1). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H = 0.89 (t, *J* = 7.2 Hz, 3 H, C*H*$_3$-CH$_2$-CH$_2$), 1.63 (sext, *J* = 7.2 Hz, 2 H, CH$_3$-C*H*$_2$-CH$_2$), 2.32 (t, *J* = 7.2 Hz, 2 H, CH$_3$-CH$_2$-C*H*$_2$), 6.12 (s, 2 H, CH$_2$), 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 (100.6 MHz, $CDCl_3$): δ_C = 13.54 (*C*H$_3$-CH$_2$-CH$_2$), 18.34 (CH$_3$-*C*H$_2$-CH$_2$), 35.96 (CH$_3$-CH$_2$-*C*H$_2$), 57.31 (CH$_2$), 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-4b), 173.42 (C=O). IR (KBr 1%): *v* = 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 cm^{-1}. HRMS (ESI) for $C_{18}H_{18}NO_2$ [M$^+$+H]: calculated 280.13384, found: 280.13403.

2.6. Synthesis of (5-methoxy-3-oxo-3H-naphtho[2,1-b]pyran-1-yl)methyl butyrate, ***9***. To a solution of 1-chloromethyl-5-methoxy-3-oxo-3*H*-naphtho[2,1-*b*]pyran **3** (0.100 g, 3.64×10^{-4} mol) in dry DMF (4 mL), potassium fluoride (0.063 g, 1.09×10^{-3} mol) and butyric acid (0.033 mL, 3.64×10^{-4} mol) were added. The reaction was followed by TLC (ethyl acetate/light petroleum, 1:4), and stirred at room temperature for 42 h. The solvent was removed by rotary evaporation under reduced pressure and the crude residue was purified by column chromatography using mixtures of ethyl acetate/light petroleum as eluent. Compound **9** was obtained as an oily solid (0.083 g, 70%). R_f = 0.22 (ethyl acetate/light petroleum, 1:4). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H = 1.01 (t, *J* = 7.2 Hz, 3 H, C*H*$_3$-CH$_2$-CH$_2$), 1.70-1.80 (m, 2 H, CH$_3$-C*H*$_2$-CH$_2$), 2.48 (t, *J* = 7.2 Hz, 2 H, CH$_3$-CH$_2$-C*H*$_2$), 4.04 (s, 3H, OCH$_3$), 5.64 (d, *J* = 1.2 Hz, 2 H, CH$_2$), 6.70 (t, *J* = 1.2 Hz, 1 H, H-2), 7.30 (s, 1 H, H-6), 7.47-7.53 (m, 2 H, H-8 and H-9), 7.76-7.81 (m, 1 H, H-10), 7.95-8.10 (m, 1 H, H-7). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ_C = 13.62 (*C*H$_3$-CH$_2$-CH$_2$), 18.32 (CH$_3$-*C*H$_2$-CH$_2$), 35.95 (CH$_3$-CH$_2$-*C*H$_2$), 56.06 (OCH$_3$), 63.94 (CH$_2$), 110.81 (C-6), 113.14 (C-2), 113.43 (C-4b), 123.94 (C-6b), 124.40 (C-7), 125.89 (C-8), 125.98 (C-9), 128.42 (C-10), 131.09 (C-6a), 146.65 (C-4a), 146.81 (C-5), 151.46 (C-1), 159.43 (C-3), 172.65 (C=O). IR (KBr 1%): *v* = 3426, 3060, 2964, 2878, 2836, 1733, 1627, 1599, 1559, 1513, 1460, 1425, 1346, 1322, 1264, 1147, 1121, 1085, 1005, 932, 864, 835, 780, 738, 703 cm^{-1}. HRMS (ESI) for $C_{19}H_{19}O_5$ [M^++H]: calculated 327.12327, found: 327.12356.

2.7. Synthesis of (8-methoxy-3-oxo-3H-naphtho[2,1-b]pyran-1-yl)methyl butyrate, ***10***. To a solution of 1-chloromethyl-8-methoxy-3-oxo-3*H*-naphtho[2,1-*b*]pyran **4** (0.305 g 1.11×10^{-3} mol) in dry DMF (5 mL), potassium fluoride (0.193 g, 3.33×10^{-3} mol) and butyric acid (0.101 mL, 1.11×10^{-3} mol) were added. The reaction was followed by TLC (ethyl acetate/light petroleum, 1:4), and stirred at room temperature for 44 h. The solvent was removed by rotary evaporation under reduced pressure and the crude residue was purified by column chromatography using mixtures of ethyl acetate/light petroleum as eluent. Compound **10** was obtained as oil (0.257 g, 71%). R_f = 0.28 (ethyl acetate/light petroleum, 1:4). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H = 0.99 (t, *J* = 7.2 Hz, 3 H, C*H*$_3$-CH$_2$-CH$_2$), 1.69-1.78 (m, 2 H, CH$_3$-C*H*$_2$-CH$_2$), 2.45 (t, *J* = 7.2 Hz, 2 H, CH$_3$-CH$_2$-C*H*$_2$), 3.90 (s, 3H, OCH$_3$), 5.52 (d, *J* = 1.2 Hz, 2 H, CH$_2$), 6.58 (t, *J* = 1.2 Hz, 1H, H-2), 7.14 (d, *J* = 2.4 Hz, 1 H, H-7), 7.22 (dd, *J* = 9.2 and 2.8 Hz, 1 H, H-9), 7.31 (d, *J* = 9.2 Hz, 1 H, H-10), 7.78 (d, *J* = 8.8 Hz, 1 H, H-5), 7.87 (d, *J* = 9.6 Hz, 1H, H-6). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ_C = 13.54 (*C*H$_3$-CH$_2$-CH$_2$), 18.22 (CH$_3$-*C*H$_2$-CH$_2$), 35.82 (CH$_3$-CH$_2$-*C*H$_2$), 55.22 (OCH$_3$), 63.78 (CH$_2$), 108.43 (C-7), 112.43 (C-4b), 112.72 (C-2), 117.89 (C-10), 119.86 (C-9), 123.50 (C-6b), 125.86 (C-6), 132.62 (C-5), 132.66 (C-1), 150.91 (C-4a), 153.14 (C-6a), 156.82 (C-8), 160.05 (C-3), 172.52 (C=O). IR (neat): *v* = 3425, 2965,

2877, 2840, 1725, 1614, 1556, 1519, 1468, 1364, 1308, 1249, 1205, 1170, 1122, 1086, 1035, 999, 916, 857, 812, 734, 700, 675 cm^{-1}. HRMS (ESI) for $C_{19}H_{19}O_5$ [M^++H]: calculated 327.12327, found: 327.12319.

2.8. Synthesis of (11-oxo-2,3,5,6,7,11-hexahydro-1H-pyrano[2,3-f]pyrido[3,2,1-ij]quinolin-9-yl)methyl butyrate, ***12***. To a solution of 9-(chloromethyl)-2,3,6,7-tetrahydro-1*H*-pyrano[2,3-*f*]pyrido[3,2,1-*ij*]quinolin-11(5*H*)-one **6** (0.042 mg, 1.45×10^{-4} mol) in dry DMF (4 mL), potassium fluoride (0.025 g, 4.35×10^{-4} mol) and butyric acid (0.014 mL, 1.59×10^{-4} mol) were added. The reaction was followed by TLC (ethyl acetate/light petroleum, 1:4), and stirred at room temperature for 14 h. The solvent was removed by rotary evaporation under reduced pressure and the crude residue was purified by column chromatography using mixtures of ethyl acetate/light petroleum as eluent. Compound **12** was obtained as oil (0.017 g, 34 %). R_f = 0.58 (ethyl acetate/light petroleum, 1:4). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H = 0.99 (t, *J* = 7.2 Hz, 3 H, C*H*$_3$-CH$_2$-CH$_2$), 1.73 (sext, *J* = 7.2 Hz, 2 H, CH$_3$-C*H*$_2$-CH$_2$), 1.91-2.03 (m, 4H, H-2 and H-6), 2.42 (t, *J* = 7.2 Hz, 2 H, CH$_3$-CH$_2$-C*H*$_2$), 2.76 (t, *J* = 6.4 Hz, 2 H, H-7), 2.89 (t, *J* = 6.4 Hz, 2 H, H-1), 3.18-3.33 (m, 4 H, H-3 and H-5), 5.20 (s, 2 H, CH$_2$), 6.10 (s, 1 H, H-10), 6.88 (s, 1 H, H-8). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ_C = 13.66 (*C*H$_3$-CH$_2$-CH$_2$), 18.37 (CH$_3$-*C*H$_2$-CH$_2$), 20.39 (C-2), 20.55 (C-1), 21.45 (C-6), 27.67 (C-7), 36.00 (CH$_3$-CH$_2$-*C*H$_2$), 49.48 (C-3), 49.88 (C-5), 61.17 (CH$_2$), 105.49 (C-10), 105.80 (C-8a), 106.96 (C-12b), 118.20 (C-7a), 120.40 (C-8), 145.95 (C-7b), 149.68 (C-9), 151.17 (C-12a), 162.26 (C-11), 172.93 (C=O). IR (neat): *v* = 2958, 2933, 2873, 2856, 1720, 1605, 1558, 1522, 1437, 1382, 1343, 1312, 1203, 1174, 1124, 1075, 1019, 884, 851, 753, 738, 701 cm^{-1}. HRMS (ESI) for $C_{20}H_{24}NO_4$ [M^++H]: calculated 342.17062, found: 342.17031.

2.9. Synthesis of (5-methoxy-3-thioxo-3H-naphtho[2,1-b]pyran-1-yl)methyl butyrate, ***13***. Lawesson's reagent (0.268 g, 6.62×10^{-4} mol) was added to a solution of (5-methoxy-3-oxo-3*H*-naphtho[2,1-*b*]pyran-1-yl)methyl butyrate **9** (0.072 g, 2.20×10^{-4} mol) in toluene (8 mL). The reaction mixture was refluxed for 9 h and the process was followed by TLC (ethyl acetate/light petroleum, 1:4). The solvent was removed by rotary evaporation under reduced pressure and the crude residue was purified by column chromatography using dichlorometane/light petroleum mixtures of increasing polarity as eluent. Compound **13** was obtained as oil (0.004 g, 5 %). R_f = 0.71 (ethyl acetate/light petroleum, 1:4). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H = 1.02 (t, *J* = 7.2 Hz, 3 H, C*H*$_3$-CH$_2$-CH$_2$), 1.70-1.81 (m, 2 H, CH$_3$-C*H*$_2$-CH$_2$), 2.50 (t, *J* = 7.2 Hz, 2 H, CH$_3$-CH$_2$-C*H*$_2$), 4.10 (s, 3 H, OCH$_3$), 5.66 (d, *J* = 1.2 Hz, 2 H, CH$_2$), 7.39 (s, 1 H, H-6), 7.52-7.62 (m, 3 H, H-2, H-8 and H-9), 7.81-7.86 (s, 1 H,

H-7), 8.03-8.10 (m, 1 H, H-10). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ_C = 13.66 (CH$_3$-CH$_2$-CH$_2$), 18.39 (CH$_3$-CH$_2$-CH$_2$), 35.99 (CH$_3$-CH$_2$-CH$_2$), 56.24 (OCH$_3$), 63.76 (CH$_2$), 111.27 (C-6), 116.07 (C-4b), 123.76 (C-6b), 125.02 (C-10), 126.24 (C-9), 126.73 (C-8), 127.80 (C-2), 128.57 (C-7), 131.63 (C-6a), 142.49 (C-1), 146.33 (C-5), 150.29 (C-4a), 172.78 (C=O), 194.49 (C-3). IR (neat): ν = 2964, 2932, 2875, 1738, 1623, 1593, 1545, 1513, 1460, 1425, 1323, 1297, 1256, 1233, 1218, 1164, 1125, 1104, 1004, 913, 866, 835, 778, 739 cm^{-1}. HRMS (ESI): calculated for $C_{19}H_{19}O_4S$ [M^++H]: 343.10047, found: 343.10079.

2.10. Synthesis of (8-methoxy-3-thioxo-3H-naphtho[2,1-b]pyran-1-yl)methyl butyrate, ***14***. Lawesson's reagent (0.383 g, 9.47 × 10^{-4} mol) was added to a solution of (8-methoxy-3-oxo-3*H*-naphtho[2,1-*b*]pyran-1-yl)methyl butyrate **10** (0.103 g, 3.16 × 10^{-4} mol) in toluene (8 mL). The reaction mixture was refluxed for 23 h and followed by TLC (ethyl acetate/light petroleum, 1:4). The solvent was removed by rotary evaporation under reduced pressure and the crude residue was purified by column chromatography using ethyl acetate/light petroleum mixtures of increasing polarity as eluent. Compound **14** was obtained as an orange solid (0.087 g, 80%). M.p. 144.9–146.5 ºC. R_f = 0.64 (ethyl acetate/light petroleum, 1:4). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H = 1.02 (t, J = 7.2 Hz, 3 H, C$H$$_3$-CH$_2$-CH$_2$), 1.70-1.80 (m, 2 H, CH$_3$-C$H$$_2$-CH$_2$), 2.49 (t, J = 7.2 Hz, 2 H, CH$_3$-CH$_2$-C$H$$_2$), 3.96 (s, 3 H, OCH$_3$), 5.57 (d, J = 0.8 Hz, 2 H, CH$_2$), 7.24 (d, J = 2.8 Hz, 1 H, H-7), 7.29-7.35 (m, 1 H, H-9), 7.51 (s, 1 H, H-2), 7.55 (d, J = 9.2 Hz, 1 H, H-10), 7.91 (d, J = 9.2 Hz, 1 H, H-5), 8.02 (d, J = 9.6 Hz, 1 H, H-6). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ_C = 13.63 (CH$_3$-CH$_2$-CH$_2$), 18.34 (CH$_3$-CH$_2$-CH$_2$), 35.93 (CH$_3$-CH$_2$-CH$_2$), 55.43 (OCH$_3$), 63.66 (CH$_2$), 108.83 (C-7), 115.09 (C-4b), 117.77 (C-10), 120.24 (C-9), 123.26 (C-6b), 126.60 (C-6), 127.22 (C-2), 133.23 (C-6a), 133.39 (C-5), 142.29 (C-1), 156.87 (C-4a), 157.56 (C-8), 172.71 (C=O), 195.02 (C-3). IR (KBr 1%): ν = 3061, 2961, 2877, 1903, 1743, 1609, 1542, 1524, 1468, 1454, 1426, 1367, 1292, 1266, 1208, 1163, 1139, 1097, 1035, 978, 936, 908, 858, 810, 736 cm^{-1}. HRMS (ESI) for $C_{19}H_{19}O_4S$ [M^++H]: calculated 343.10047, found: 343.10096.

2.11. Synthesis of (9-methoxy-3-thioxo-3H-naphtho[2,1-b]pyran-1-yl)methyl butyrate, ***15***. Lawesson's reagent (0.166 g, 4.11 × 10^{-4} mol) was added to a solution of (9-methoxy-3-oxo-3*H*-benzo[*f*]benzopyran-1-yl)methyl butyrate, **11** (0.067g, 2.05 × 10^{-4} mol) in toluene (5 mL). The reaction mixture was heated at reflux for 48 h and the process was followed by TLC (ethyl acetate/ light petroleum, 1:4). The solvent was removed by rotary evaporation under reduced pressure and the crude residue was purified by column chromatography using ethyl acetate/light petroleum, with

mixtures of increasing polarity as eluent. Compound **15** was obtained as an orange solid (0.049 g, 70%). Mp 132.5-134.7 ºC. R_f = 0.86 (ethyl acetate/light petroleum, 1:4). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H = 1.01 (t, *J* = 7.2 Hz, 3 H, C*H*$_3$-CH$_2$-CH$_2$), 1.75 (sext, *J* = 7.2 Hz, 2 H, CH$_3$-C*H*$_2$-CH$_2$), 2.49 (t, *J* = 7.6 Hz, 2 H, CH$_3$-CH$_2$-C*H*$_2$), 3.97 (s, 3 H, OCH$_3$), 5.60 (s, 2 H, CH$_2$), 7.24 (dd, *J* = 8.8 and 2.4 Hz, 1 H, H-8), 7.43 (d, *J* = 9.2 Hz, 1 H, H-5), 7.48 (d, *J* = 2.4 Hz, 1 H, H-10), 7.49 (s, 1 H, H-2), 7.83 (d, *J* = 8.8 Hz, 1 H, H-7), 7.93 (d, *J* = 8.8 Hz, 1 H, H-6). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ_C = 13.63 (*C*H$_3$-CH$_2$-CH$_2$), 18.28 (CH$_3$-*C*H$_2$-CH$_2$), 35.92 (CH$_3$-CH$_2$-*C*H$_2$), 55.47 (OCH$_3$), 63.69 (CH$_2$), 106.25 (C-10), 114.30 (C-4b), 114.90 (C-5), 117.22 (C-8), 126.56 (C-6a), 126.97 (C-2), 130.29 (C-6b), 131.38 (C-7), 134.25 (C-6), 142.39 (C-1), 158.69 (C-4a), 159.86 (C-9), 172.76 (C=O), 195.27 (C-3). IR (KBr 1%): *v* = 3074, 2964, 2935, 2875, 2836, 1742, 1623, 1606, 1592, 1538, 1485, 1461, 1443, 1431, 1364, 1296, 1229, 1216, 1163, 1139, 1096, 1056, 1025, 1008, 982, 917, 870, 838, 803, 706 cm^{-1}. HRMS (ESI) for $C_{19}H_{19}O_4S$ [M^++H]: calculated 343.10047, found: 343.10074.

2.12. Photolysis general

A 1 × 10^{-4} M methanol or acetonitrile/HEPES (80:20) solution of compounds **7**-**15** (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. HEPES buffer solution was prepared in distilled water with HEPES (4-(2-hydroxyethyl)-1-piperazine ethanesulfonic acid) (10 mM), sodium chloride (120 mM), potassium chloride (3 mM), calcium chloride (1 mM) and magnesium chloride (1mM) and pH adjusted to 7.2 with aqueous 1 M sodium hydroxide solution.

Aliquots of 100 µL were taken at regular intervals and analysed by RP-HPLC using a Licrospher 100 RP18 (5 µm) column in a JASCO HPLC system composed by a PU-2080 pump and a UV-2070 detector with ChromNav software. The eluent was acetonitrile/water, 75:25 at a flow rate of 0.8 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: **7**, 5.2; **8**, 8.7; **9**, 6.1; **10**, 6.6; **11**, 6.5; **12**, 8.4; **13**, 9.4; **14**, 10.4; **15**, 8.5 min).

2.13. Time-resolved fluorescence measurements

Time-resolved fluorescence measurements with one photon excitation made use of the time-correlated single-photon counting (TCSPC) technique and were performed using a HORIBA Scientific DeltaFlex, equipped with a DeltaDiode laser excitation source emitting at 378 nm and a

PPD-850 detector. Analysis of each dataset was performed using DAS6 software and the decays were reconvoluted with the instrumental response and fitted to the sum of exponentials (equation 1).

$$I(t) = \sum_{i=1}^{n} \alpha_i exp^{-t/\tau_i} \quad (1)$$

Where α_i are the normalised pre-exponential factors and the average lifetime was obtained from

$$\tau_{ave} = \Sigma \alpha_i \tau_i \quad (2)$$

The goodness of fit was judged in terms of a chi-squared value and weighted residuals.

Two-photon excitation (TPE) TCSPC measurements were performed using Ti:sapphire 80MHz pulsed laser (Chameleon®, Coherent, UK) equipped with a pulse selector (A.P.E. PulseSelect) picking 1:20 pulses generating an emission train at 4MHz. The fluorescence was recorded using a Horiba Scientific FluoroCube synchronized to the pulse train by the driving unit of the pulse selector. The emission signal was recorded using a blue green copper sulphate bandpass filter. The detector used was a Horiba Scientific TBX-850 and data were analysed in a similar manner to the one photon excitation data using DAS6 software. The TPE cross-sections, expressed in Göppert-Meyer units (GM) with 1 GM = 10^{-50}cm^4 s photon^{-1} [27], were calculated relative to a reference fluorophore in a manner that we have previously reported [28]. Briefly to obtain the raw data the Ti:sapphire laser was tuned from 690nm to 850nm and the counts-per-second on the Horiba TBX detector recorded.

3. Results and discussion

3.1. Synthesis of butyric acid conjugates 7-15

Butyric acid was chosen as a model carboxylic acid drug to study the behaviour towards irradiation of the corresponding cages with several light sensitive *o*-nitrobenzyl or heterocyclic benzyl type esters **7**-**12**. Their synthesis started with the preparation of the 2-nitrobenzyl ester cage **7**, by a *N,N'*-dicyclohexylcarbodiimide (DCC)/1-hydroxybenzotriazole (HOBt) mediated coupling of butyric acid with (2-nitrophenyl)methanol **1** in DMF at room temperature. For the assembly of the heterocyclic benzyl type ester cages **8**-**12**, several bromo- or chloromethylated heteroaromatics based on acridine **2**, naphtho[2,1-*b*]pyran **3**-**5** and a 3*H*-benzopyran fused julolidine **6** were reacted with the model acid in the presence of potassium fluoride in DMF at room temperature (Scheme 1, Table 1). Fused pyrans **3**-**6** resulted from a Pechmann condensation between ethyl 4-chloro-3-oxobutanoate and 3-methoxy-2-naphthol, 6-methoxy-2-naphthol, 7-methoxy-2-naphthol [25] and 1,2,3,5,6,7-

hexahydropyrido[3,2,1-*ij*]quinolin-8-ol (trivially named 8-hydroxyjulolidine) [26], respectively. With the purpose of replacing the carbonyl by a thiocarbonyl group at the naphtho[2,1-*b*]pyran conjugates **9-11**, these compounds were reacted with Lawesson's reagent in toluene, under reflux conditions [29], affording the corresponding thionaphthopyran conjugates **13-15**.

< Scheme 1>

< Table 1>

All compounds were fully characterised by high resolution mass spectrometry, IR, ^{1}H and ^{13}C NMR spectroscopy. The IR spectra of compounds **7-15** displayed stretching vibration bands of the ester carbonyl group from 1711 to 1743 cm^{-1}. ^{1}H NMR spectra showed signals of butyric acid, the methyl (δ 0.89-1.02 ppm) and two methylenes (δ 1.58-1.81 and 2.32-2.50 ppm). The heterocycle methylene group, adjacent to the ester link, was visible for all compounds (δ 5.20-6.12 ppm). The newly formed ester linkages were confirmed by ^{13}C NMR spectra signals of the carbonyl group, at about δ 172.52-173.42 ppm. The thiocarbonyl group in compounds **13-15** affected the chemical shift of the pyran proton H-2, which appeared downfield in the range δ 7.51-7.59 ppm, while in the precursor compounds **9-11**, with a carbonyl group, it occurred at δ 6.58-6.70 ppm. The presence of the new C=S bond (C-3) at the heterocyclic ring was also confirmed by ^{13}C NMR spectra signals at δ 194.49-195.27 ppm, when compared to the carbonyl group, which occurred at δ 159.43-160.31 ppm. The chemical shift of the pyran carbon C-2 was also influenced by the carbon-sulphur double bond, being in the range δ 126.97-127.80 ppm for compounds **13-15**, and δ 112.72 or 113.14 ppm for compounds **9-11**.

3.2. Evaluation of the photophysical properties of butyric acid conjugates 7-15

Fundamental UV/visible photophysical characterisation was carried out to obtain the parameters required for monitoring the photolytic process. The absorption and emission spectra of degassed 10^{-5} M solutions in absolute ethanol, a methanol/HEPES and acetonitrile/HEPES buffer (80:20) solutions of ester conjugates **7-15** were measured and the corresponding data, absorption and emission maxima, molar absorption coefficients and relative fluorescence quantum yields are reported in Table 1. Relative fluorescence quantum yields were calculated using 9,10-diphenylanthracene in ethanol (Φ_F 0.95) [30] or a 0.05 M solution of quinine in sulphuric acid (Φ_F 0.546) [31] as standards. For the Φ_F determination, the fluorescence standard was excited at the wavelengths of maximum

absorption found for each compound to be tested and in all fluorimetric measurements the absorbance of the solution did not exceed 0.1.

Regarding the maximum absorption wavelengths (λ_{abs}) of conjugates **7**-**15**, in all the tested solvents, it was found that the nitrobenzyl conjugate **7** displayed the lowest values (at about 260 nm), in comparison with the polycyclic compounds **8**-**12** (344-394 nm), whereas the thiocarbonyl conjugates **13**-**15**, displayed even larger bathochromic shifts (404-431 nm). For naphtho[2,1-*b*]pyrans **9**-**11**, which differ in the relative position of the electron donor methoxy group, the λ_{abs} was longer for substitution in position 8 (compound **10**), with a bathochromic shift between 15 to 23 nm, when compared with substitution in positions 5 and 9 (compounds **9** and **11**, respectively). Nevertheless, the largest shift was observed for the 3*H*-benzopyran fused julolidine **12**, with maximum absorption wavelengths in the range 394-399 nm, in the various solvents. Furthermore, the replacement of the carbonyl group in the pyran ring (compounds **9**-**11**) by a thiocarbonyl (compounds **13**-**15**) also resulted in a shift in the λ_{abs}, by tuning absorption to values in the visible region (λ_{abs} 404-431 nm).

Concerning the fluorescence spectra, in all the tested solvents, it was observed that emission maxima (λ_{em}) of conjugates **8**-**15** occurred in the range (410-497 nm), being the 3*H*-benzopyran fused julolidine **12** associated to the longer wavelengths, and the naphtho[2,1-*b*]pyran **10** the most emissive (Φ_F 0.45 to 0.53). The nitrobenzyl conjugate **7** displayed the lowest values (λ_{em} at about 300 nm), as was expected due to its structure. The emission of thiocarbonyl conjugates **13**-**15** was hypsochromically shifted with lower fluorescent quantum yields, in comparison with the corresponding carbonyl precursors.

3.3. Photolysis studies of butyric acid conjugates 7-15

The present work intended to profit from our gathered knowledge in the application of different (hetero)aromatic benzyl type systems as photoremovable protecting groups for a variety of relevant biomolecules. In particular, our previous findings in the photolytic release of butyric acid from different naphthoxazole, naphthopyran and oxazole fused pyran cages, revealed that these heterocyclic systems were promising mainly at short wavelengths. In order to develop a system that allows the delivery of butyric acid at longer wavelengths, heterocyclic compounds that showed interesting results with other biomolecules, as previously reported [16,18,24], as well as new naphthopyrans methoxylated at different positions of the polycyclic ring, and the corresponding thionated derivatives were used in the caging of butyric acid. Photolytic studies of all the ester cages **7**-**15** were carried out in a Rayonet RPR-100 reactor at 254, 300, 350 and 419 nm in mixtures of methanol or acetonitrile with aqueous HEPES buffer in 80:20 solutions, with collection of kinetic

data. The course of the photocleavage reaction was followed by reverse phase HPLC with UV detection. The plots of peak area (*A*) 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. The photochemical quantum yields (Φ_{Phot}) were calculated based on half-lives ($t_{1/2}$), molar absorptivities (ε) and the incident photon flux (I_0), which was determined by potassium ferrioxalate actinometry [30].

The results at various wavelengths of irradiation revealed the significant influence of the photoactive unit structure in the irradiation time (t_{irr}) necessary to release butyric acid (Table 2). Although a study at short wavelengths (254 and 300 nm) was carried out for comprehensiveness and the results are included in Table 2, the main focus of the present work was the performance at 350 and 419 nm. At these wavelengths, considering the nitrogen heterocyclic system, namely acridine cage **8**, it was found that the release occurred with very short t_{irr} in methanol/HEPES when compared to acetonitrile/HEPES, which suggested the influence of the organic solvent character in this photoreaction. For the remaining oxygen heterocyclic conjugates, naphtho[2,1-*b*]pyrans **9**-**11**, irradiation times were comparable in both solvent systems, with compound **9** cleaving faster at 350 nm and compound **11** at 419 nm, which can be related to the position of the methoxy substituent.

However, the best results were obtained with the 3*H*-benzopyran fused julolidine **12**, at both wavelengths and solvent systems, as butyric acid was completely released within 4 to 6 min of irradiation. The bathochromic shift in the maximum absorption wavelengths related to the presence of the thiocarbonyl group in cages **13**-**15** lead to significantly shorter irradiation times at 419 nm due to an increase in the efficiency of the photolysis, in comparison to the carbonyl precursors **9**-**11**, as expected.

<Table 2>

Additionally to monitoring the photolysis process by HPLC/UV detection, the release of butyric acid was also followed by ^{1}H NMR in an acetonitrile-d_3/D_2O (80:20) solution. Upon irradiation at 419

nm of a solution of 3*H*-benzopyran fused julolidine cage **12**, the signal due to the benzylic-type CH_2 at position 9 of the heterocyclic ring, visible at about δ 5.17 ppm gradually decreased with time. The same observation occurred with the signals related to the butyric acid in the conjugated form at about δ 2.45, 1.65 and 0.95 ppm, giving rise to a close set of signals corresponding to butyric acid in its free form at about δ 2.10, 1.55 and 0.90 ppm, respectively (Figure 1). NMR monitoring was carried out with a 1.17×10^{-2} M solution, which led to an expected increase in the photolysis time for the complete release of the molecule, when compared to the irradiation times in Table 2 obtained with dilute solutions.

3.4. Time-resolved studies of butyric acid conjugates 8-15

To help elucidate the photophysical processes occurring in these compounds time-resolved fluorescence measurements were performed on samples that were freshly prepared (after an initial check of the absorption and emission steady state spectra). To facilitate future (accessible using 2-photon excitation) studies, an excitation wavelength of 378nm for the compounds in acetonitrile/HEPES buffer (80:20) solution was chosen. This wavelength is between the two longer ones employed in the photoreactor and provides an excitation into the main absorption bands for each of the compounds. Measurements were made close to the peak emission and in every case the decay was found to require the sum of 3 exponential components (apart from 2 exponentials for **8**). This is indicative of the presence of different excited state species and the outcome of these measurements is presented in Table 3. The behaviour seen is in keeping with our previous observations [19,20,26] and the longer-lived emission can relate to unquenched fluorophore. The shorter-lived decays are attributed to charge transfer states, caused by the presence of the conjugate upon excitation, with the possibility of the formation of an ion pair that can either recombine or progress to a cleaved pair. Thus, the contribution of each of the fluorescing components gives an insight into the species present.

Here we aim to ascertain if the fluorescence lifetime can help provide complementary information to that of the photolysis study and assume that the amount of photocleavage during the measurement is not too significant. From Table 3 it can be noted that the compound giving the highest photochemical yield (**12**) also exhibits the shortest average lifetime, showing that overall the non-radiative processes, ie charge transfer, can be higher in this compound. On the other hand, compound **8**, considering the 350 nm values in Table 2, has one of the lowest photochemical yields and exhibits the longest-lived average lifetime. It is a moot point in this case whether the quantity of the longest-lived component relates to cleaved photoproduct, as we have previously shown using decay associated spectra and a kinetic measurement [26], or can reflect on the strength of coupling to the

conjugate. This will be the basis of further work, although the fact that **12** exhibits a high photochemical yield, while displaying the shortest average lifetime may indicate the latter. Here the photophysics will be used to assess the effect of treatment of Lawesson's reagent (ie comparison of **9**,**10**,**11** with **13**,**14**,**15** respectively). Considering the data in Table 3, it does not appear that treatment dramatically affects the lifetime values, minor changes are observed and their trend between compounds similar before and after treatment with Lawesson's reagent. However there are some differences in the contribution of the lifetimes to the overall emission. It would appear that the use of Lawesson's reagent has generally had the desired effect of enabling longer wavelength irradiation to be employed without significantly altering the photophysics of these compounds.

Having established that longer wavelength excitation could be employed, and since this fits within the wavelength range of Ti:sapphire lasers making use of two-photon excitation, a study was performed to ascertain the compounds two-photon absorption cross-section. Initially to investigate the extent of two-photon excitation the relation between the fluorescence intensity and the excitation power was plotted. Figure 2a shows an example of the intensity squared dependence of the fluorescence signal form the measurement of compound **11**. The slope of the curve lies close to 2, which is indicative of a two-photon excitation process. Measurements for the other compounds yielded similar dependencies between the fluorescence intensity and the excitation power. An example of a decay measurement (this time compound **10**) obtained using 750 nm excitation is shown in Figure 2b. The lifetime values recovered (τ_1 = 378 ± 56 ps, α_1 = 0.29; τ_2 = 4948 ± 174 ps, α_2 = 0.44; τ_3 = 9635 ± 84 ps, α_3 = 0.27) are in keeping with those obtained from the one-photon excitation measurement. It should be noted that the pulses appearing in the instrumental response arise from suppression of the fundamental 80MHz pulse train by the pulse picker. Their presence has been reduced to ~1% of the principal pulse and appear accounted for in the fitting process, as judged by the weighted residuals.

<Figure 2>

To further characterise their suitability the two-photon absorption cross-section were obtained, and these are given in Figure 3. The probability for two-photon-excitation (TPE) depends on both spatial and temporal overlap of the incident photons. TPE spectra will usually differ from one-photon spectra as transitions that are forbidden in one case may be allowed in the other [33]. The cross sections for TPE are usually small and very high photon flux is needed for excitation. The measured GM for different fluorophores can range from 1 to 1000 GM [34]. From Figure 3, peak values of

about 8 GM for compounds **10** and **11** are obtained. Weaker cross-sections are obtained for the compounds treated with Lawesson's reagent, with TPE characteristics reaching up to 2, although their spectral response extends to longer wavelengths. However, it should also be noted that because of the uncertainty reported for the reference values [35] and subsequent error progression in the calculation these values should be treated as good estimates. Nevertheless, TPE cross-sections falling in the range of 1 to 10 GM are in good agreement with commonly used dyes [34, 36], although weaker than those exhibited by engineered fluorescent proteins [33]. Thus it is apparent that these compounds are indeed suitable for two-photon excitation.

<Figure 3>

4. Conclusions

Acridine, naphtho[2,1-*b*]pyran, 3*H*-benzopyran fused julolidine and thioxo-naphtho[2,1-*b*]pyran ester cages of butyric acid were studied for the controlled delivery of the active molecule by photolysis at selected wavelengths (254, 300, 350 and 419 nm), in comparison with the well-known *o*-nitrobenzyl photolabile group. Irradiation was carried out in a Rayonet RPR-100 photochemical reactor in mixtures of organic solvents (methanol or acetonitrile) with aqueous HEPES buffer and monitored by HPLC-UV and ^{1}H NMR. Overall, the obtained results, combined with time-resolved fluorescence data, showed that for naphtho[2,1-*b*]pyrans the attachment position of the methoxy substituent as well as the presence of the thiocarbonyl group influenced the behaviour towards light of the corresponding cage. Furthermore, the release of butyric acid was faster in the case of using (acridin-9-yl)methyl, and [11-oxo-2,3,5,6,7,11-hexahydro-1*H*-pyrano[2,3-*f*]pyrido[3,2,1-*ij*]quinolin-9-yl]methyl (3*H*-benzopyran fused julolidine) groups as phototriggers. The suitability to be addressed using two-photon excitation was also demonstrated with promising TPE cross-sections.

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CAPTIONS

Figure 1. Partial ^{1}H NMR spectra in acetonitrile-d_3/D_2O (80:20) of the photolysis of 3*H*-benzopyran fused julolidine **12** (C = 1.17 × 10^{-2} M) at 419 nm: (a) before irradiation; (b) after irradiation for 4 h; (c) after irradiation for 10 h; (d) butyric acid.

Figure 2. (a) Plot of intensity against power, showing the data for compound **11** and the slopes calculated for all the compounds. The values obtained (error within 15%) close to 2 showed a quadratic dependency and are demonstrative of a two-photon process. (b) Fluorescence decay (from **10**) showing the instrumental response, decay, fitted function (3 exponential) and weighted residuals. Note that the pulse selection process has left the fundamental (80MHz) train suppressed to a level ~1% that of the selected pulse.

Figure 3. Plot of the two-photon excitation cross-sections *versus* wavelength for the different compounds.

Table 1. Yields, UV/visible absorption and fluorescence data for compounds **7**-**15** in absolute ethanol, methanol/HEPES buffer (80:20) and acetonitrile/HEPES buffer (80:20) solutions.
[a] in nm. [b] Data in ethanol and methanol/HEPES buffer (80:20) solution was previously reported [20].
[c] The excitation was carried out at 326 nm.

Table 2. Irradiation times (t_{irr} in min), and photochemical quantum yields (Φ_{Phot}, × 10^{-3}) for the photolysis of conjugates **7**-**15** at 254, 300, 350 and 419 nm in methanol/HEPES buffer (80:20) and acetonitrile/HEPES buffer (80:20) solutions. [a] Data in ethanol and methanol/HEPES buffer (80:20) solution was previously reported [20].

Table 3. Time-resolved fluorescence decay parameters, lifetime (τ_i, in ps), and normalised pre-exponential values (α_i), calculated average lifetime and goodness of fit (χ^2), for compounds **8**-**15** in acetonitrile/HEPES buffer (80:20). The excitation wavelength was 378 nm and the emission wavelengths (λ_{em}) are also in nm.

Scheme 1. Synthesis of ester cages of butyric acid **7-15**. Reagents and conditions: *a)* DCC/HOBt, DMF, rt; *b)* KF, DMF, rt; *c)* Lawesson's reagent, toluene, reflux.

TABLES

Table 1.

Compd	Yield	Ethanol					Methanol/HEPES (80:20)					Acetonitrile/HEPES (80:20)				
	(%)	λ_{abs}[a]	log ε	λ_{em}[a]	Φ_F	$\Delta\lambda$[a]	λ_{abs}[a]	log ε	λ_{em}[a]	Φ_F	$\Delta\lambda$[a]	λ_{abs}[a]	log ε	λ_{em}[a]	Φ_F	$\Delta\lambda$[a]
7	29	256	3.77	303	0.08	47	259	3.73	304	0.07	45	260	3.74	304	0.13	44
8	69	360	3.71	410	0.34	50	360	4.12	427	0.32	67	360	4.15	427	0.27	67
9	70	344	3.54	464	0.18	120	343	3.93	479	0.21	136	343	3.96	472	0.20	129
10	71	316, 365	4.07, 3.85	459	0.53	94	316, 366	3.99, 3.80	470	0.49	104	314, 364	4.18, 3.97	464	0.45	100
11[b]	80	350	3.92	466	0.45	116	352	4.22	484	0.46	132	345	3.86	474	0.40	129
12	34	394	4.28	485	0.19	91	399	4.13	497	0.16	98	397	4.06	494	0.18	97
13	5	310, 404	3.83, 3.70	447	0.03	43	308, 406	3.94, 3.86	448	0.03	42	308, 405	4.04, 3.95	437	0.03	32
14[c]	80	283, 326, 431	4.19, 4.28, 4.21	464	0.06	138	326, 428	4.15, 4.12	477	0.05	151	326, 430	4.26, 4.23	466	0.01	140
15	70	407	4.20	462	0.002	55	412	3.97	470	0.001	58	412	4.21	470	0.001	58

Table 2.

Compound	Methanol/HEPES (80:20)								Acetonitrile/HEPES (80:20)							
	254 nm		300 nm		350 nm		419 nm		254 nm		300 nm		350 nm		419 nm	
	t_{irr}	Φ_{Phot}	t_{irr}	Φ_{Phot}	t_{irr}	Φ_{Phot}	t_{irr}	Φ_{Phot}	t_{irr}	Φ_{Phot}	t_{irr}	Φ_{Phot}	t_{irr}	Φ_{Phot}	t_{irr}	Φ_{Phot}
7	11	1.71	26	0.37	76	0.12	2217	0.004	14	1.49	41	0.225	106	0.085	1198	0.008
8	4	1.88	7	0.60	2	1.64	83	0.044	58	0.129	120	0.03	328	0.011	2499	0.001
9	192	0.063	230	0.027	182	0.033	5985	0.001	186	0.062	184	0.033	172	0.031	4288	0.001
10	432	0.039	546	0.015	555	0.014	3324	0.0024	457	0.025	481	0.012	493	0.012	9989	0.001
11[a]	178	0.034	253	0.011	263	0.011	490	0.006	278	0.054	294	0.024	270	0.026	520	0.013
12	6	1.41	9	0.426	6	0.79	5	0.790	5	1.87	7	0.691	4	1.04	4	1.03
13	55	0.262	178	0.039	210	0.032	142	0.047	53	0.202	129	0.044	292	0.018	193	0.029
14	212	0.040	371	0.001	526	0.007	351	0.010	153	0.042	585	0.005	308	0.010	296	0.010
15	90	0.129	184	0.029	296	0.018	98	0.129	57	0.114	111	0.020	203	0.023	76	0.040

Table 3.

compound	λ_{em}	τ_1	τ_2	τ_3	α_1	α_2	α_3	τ_{ave}	χ^2
8	410	511 ± 60	10791 ± 27		0.31	0.69		7655	0.98
9	475	113 ± 39	2631 ± 507	3591 ± 75	0.24	0.34	0.42	2416	1.14
10	460	155 ± 150	4954 ± 174	10379 ± 67	0.13	0.53	0.34	6121	1.08
11	470	65 ± 39	3522 ± 543	7208 ± 33	0.38	0.09	0.53	4142	1.09
12	470	240 ± 30	2241 ± 99	5108 ± 60	0.46	0.35	0.19	1835	1.20
13	470	216 ± 54	2991 ± 75	5831 ± 175	0.28	0.64	0.08	2434	1.16
14	470	91 ± 39	4812 ± 162	10065 ± 93	0.42	0.35	0.23	4100	1.08
15	470	118 ± 129	2445 ± 339	6893 ± 24	0.25	0.11	0.64	4701	1.10

FIGURES

Figure 1.

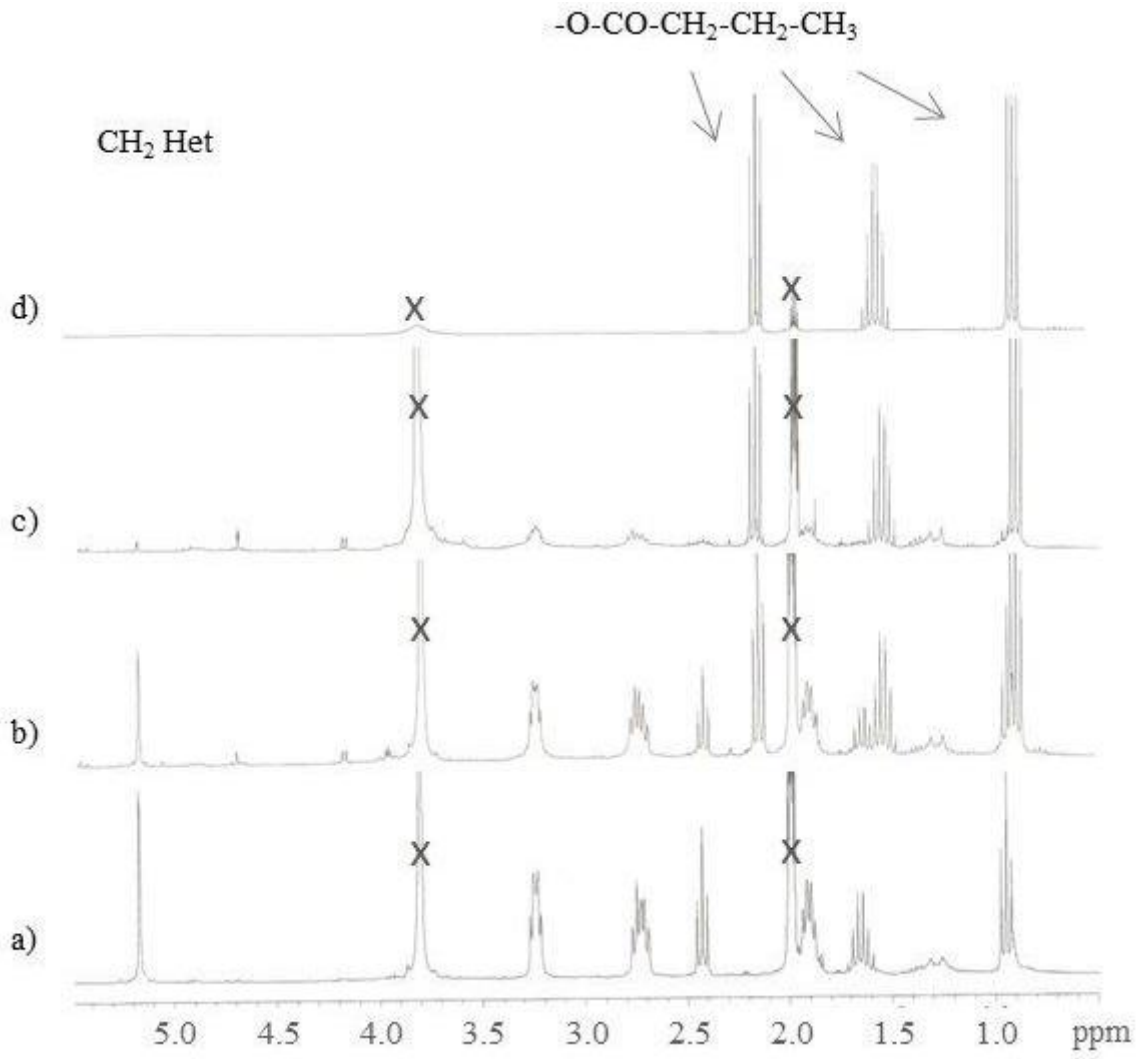
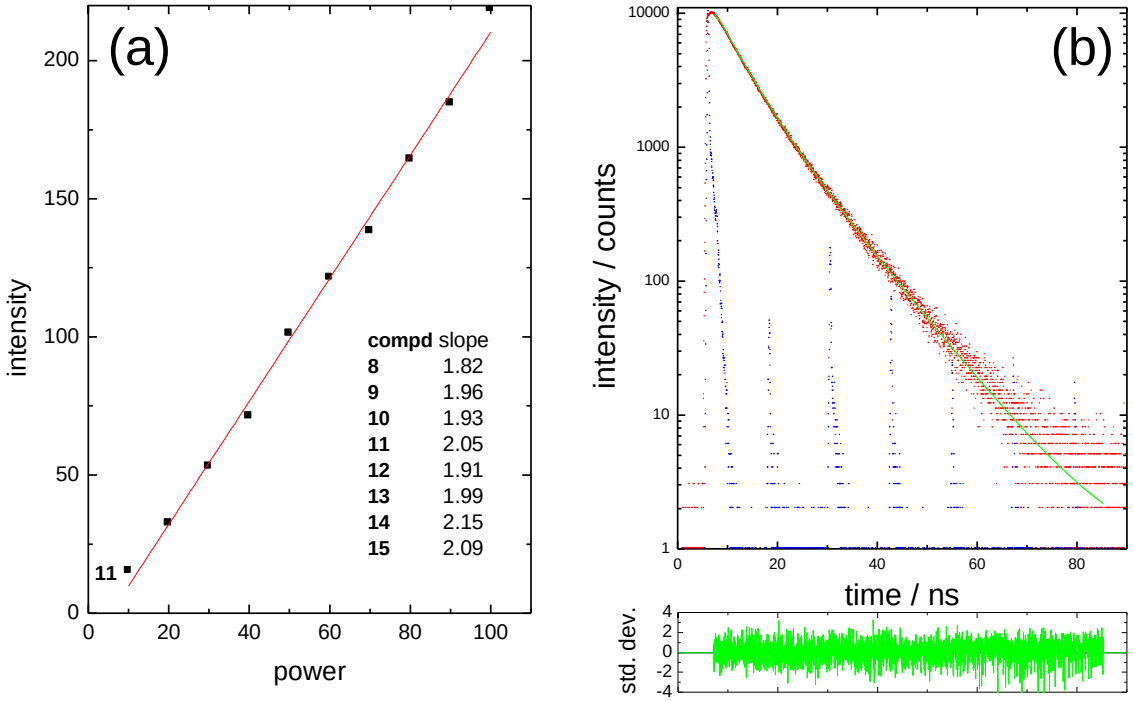


Figure 2.


(a)
intensity
power
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50
100
150
200
0
20
40
60
80
100
11
compd slope
8 1.82
9 1.96
10 1.93
11 2.05
12 1.91
13 1.99
14 2.15
15 2.09
(b)
intensity / counts
time / ns
std. dev.
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10
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0
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40
60
80
4
2
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-2
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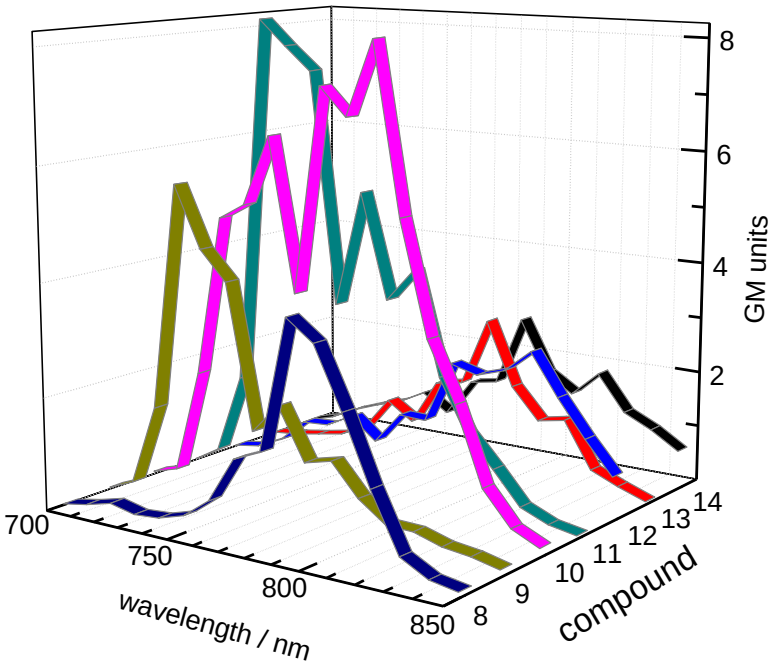
Figure 3.


8
6
4
2
GM units
700
750
800
850
wavelength / nm
8
9
10
11
12
13
14
compound

SCHEMES

Scheme 1.