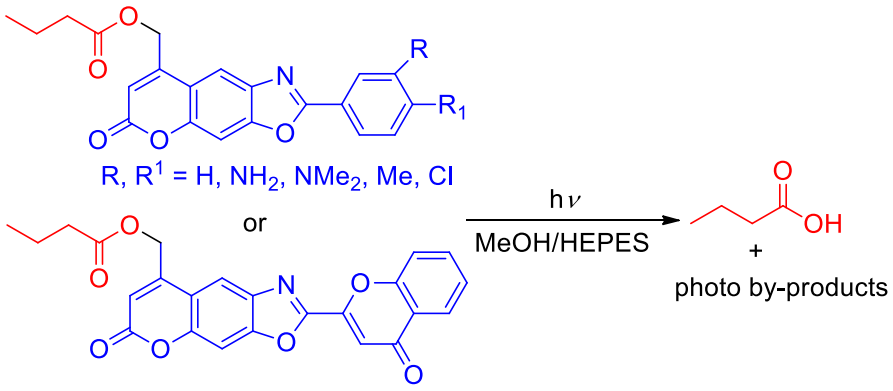
GRAPHICAL ABSTRACT

Photoactivatable prodrugs of butyric acid based on coumarin new fused oxazole heterocycles

Ana M. S. Soares,[a] Graham Hungerford,[b] Susana P. G. Costa[a] and M. Sameiro T. Gonçalves[a] *

[a]*Centre of Chemistry, University of Minho, Campus of Gualtar, 4710-057 Braga, Portugal*

[b]*HORIBA Jobin Yvon IBH Ltd, 133 Finnieston Street, Glasgow G3 8HB, UK*



*Corresponding author. Fax: +351 253 604382; *e-mail:* msameiro@quimica.uminho.pt

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[a]*Centre of Chemistry, University of Minho, Campus of Gualtar, 4710-057 Braga, Portugal*

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HIGHLIGHTS

- Chloromethylated coumarin fused oxazoles were obtained and efficiently used in the derivatisation of butyric acid, as model carboxylic acid drug.

- The new seven cages based on 6-oxo-6*H*-benzopyrano[6,7-*d*]oxazol-8-yl)methyl groups revealed to be photo-responsive units upon irradiation at various selected wavelengths.

- Irradiation of the cages resulted in the complete release of the expected butyric acid, being the coumarin fused oxazole with phenyl or chromone substituents at the position 2 of the polycyclic system especially pertinent for bioapplications.

- This study shows new promising alternative moieties for the development of photoactivable fluorescent butyric acid prodrugs.

Photoactivatable prodrugs of butyric acid based on new coumarin fused oxazole heterocycles

Ana M. S. Soares,[a] Graham Hungerford,[b] Susana P. G. Costa[a] and M. Sameiro T. Gonçalves[a] *

[a]*Centre of Chemistry, University of Minho, Campus of Gualtar, 4710-057 Braga, Portugal*

[b]*HORIBA Jobin Yvon IBH Ltd, 133 Finnieston Street, Glasgow G3 8HB, UK*

Abstract: New coumarin fused oxazoles were investigated as photosensitive units for carboxylic acid groups using butyric acid as a model compound. 6-Oxo-6*H*-benzopyrano[6,7-*d*]oxazol-8-yl)methyl derivatives possessing various (hetero)aromatic substituents at position 2 of the heterocyclic system were used in the synthesis of ester conjugates of butyric acid. Photolysis at selected wavelengths in methanol/HEPES buffer (80:20) solutions, monitored by HPLC/UV and ^{1}H NMR, produced the complete release of butyric acid. The shorter irradiation times for cleavage at longer wavelengths occurred for the conjugate with a 4-oxo-4*H*-benzopyran-2-yl substituent and thus (6-oxo-2-(4-oxo-4*H*-benzopyran-2-yl)-6*H*-benzopyrano[6,7-*d*]oxazol-8-yl)methyl has potential as a candidate photosensitive moiety for butyric acid prodrugs.

Keywords: Prodrugs; Coumarins; Oxazoles; Butyric acid; Photocleavable protecting groups; Photolysis.

*Corresponding author. Fax: +351 253 604382; *e-mail:* msameiro@quimica.uminho.pt

1. Introduction

A diversity of light-sensitive moieties have been reported in recent years to target molecules including amines and amino acid neurotransmitters, nucleic acids, enzyme substrates and inhibitors, proteins, biochemical sensors, as well as to be used as triggers for the creation of biomaterials. The use of light in combination with these moieties enables the behavioural manipulation of organisms, control of cell biochemistry and the treatment of a variety of anomalous physiological conditions and manifestations of disease [1-6]. Moreover, "phototherapeutics" possesses wide-ranging potential applications in cancer therapy, tissue engineering and surgery [7]. The preparation of light-sensitive species usually involves the covalent modification of a functional group essential for the biological activity of the compound of interest. This modification results from the use of a photocleavable protecting group, also designated as a phototrigger or caging group. Carboxylates, amines and alcohols are typical reactive sites for substitution by photocleavable units, usually through ester, carbonates, carbamate and anhydride linkages [3,8,9]. The implementation of a caging strategy provides both spatial and temporal control over the release of molecules triggered by ultraviolet and visible light.

The occurrence of severe side effects, drug resistance, along with other diverse factors that influence the efficacy of drug activity are responsible for the development of innovative methodologies to circumvent these limitations. Butyric acid can be taken as an example, as it is a pleotropic anticancer agent that has a specific effect on the inhibition of nuclear histone deacetylase enzyme(s), leading to an increase in the acetylation level of H3 and H4 histones. However, *in vivo* it displays low potency because of rapid metabolisation [10-12]. In order to bypass this problem, butyric acid prodrugs, including those that are photoactivatable, have been described in the literature [13,14].

Our research has been involved in studies related with the design, synthesis and evaluation of light-sensitive moieties for the release of bioactive molecules, including butyric acid [1,2,15-20].

Recently, we have reported on coumarin, benzocoumarin thio(benzo)coumarin, coumarin fused with julolidine and amino-substituted benzocoumarin cages [19-21]. Also, our studies with photoactive prodrugs of butyric acid were initiated with the use of naphthoxazoles and coumarin fused oxazoles; namely naphtho[2,3-*d*]oxazole, naphtho[1,2-*d*]oxazole and 6-oxo-6*H*-benzopyrano[6,7-*d*]oxazole (with a linkage between the heterocycle and the active molecule through oxopyran or oxazole moieties) [22]. In this regard, and considering that the more promising results were found with benzopyranoxazole with a linkage to butyric acid through the pyranone ring, we describe here the synthesis of a new set of benzopyranoxazoles, with an improved capability for the photorelease of butyric acid using longer wavelength light to initiate the release. Longer wavelengths are advantageous as they help to avoid the absorption of light by intrinsic species (biological material including amino acids, proteins and nucleic acids), and enable the resulting conjugates to be addressed using two-photon excitation.

In this work, novel 6-oxo-6*H*-benzopyrano[6,7-*d*]oxazol-8-yl)methyl groups were synthesised and used in the caging of butyric acid. The resulting ester cages were irradiated at 254, 300 and 350 nm in a photochemical reactor in a solution of methanol/HEPES buffer (80:20). Because the caging groups exhibit fluorescence, it is possible to make use of this fact and employ fluorescence techniques, principally time-resolved methods to characterise their photophysical properties. Since the photocleavage proceeds via intermediate species (eg ion pairs) it is helpful to ascertain if the substituent groups have any marked effect on processes that can be elucidated using changes in their fluorescence behaviour. The determination of decay associated spectra enables both spectral (energetic) and decay kinetics to be compared.

2. Experimental section

2.1. Material and instruments

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 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 fluorometer. Time-resolved fluorescence measurements were performed on a HORIBA Scientific DeltaFlex with a DeltaDiode excitation source emitting at 336 nm (DD-340). All reagents were used as received.

*2.2. General procedure for the synthesis of oxo-benzopyranoxazoles **3a-g***

To a solution of 6-amino-4-(chloromethyl)-7-hydroxy-2-oxo-2*H*-benzopyran **1** (1 equiv.) in polyphosphoric acid (0.500 g), the acid derivative **2** (2 equiv.) was added, and the mixture was stirred at 130 °C for 4 h. The reaction mixture was poured into iced water and stirred for 1 h to give a fine grey precipitate. The solid was collected by filtration, washed with cold water and dried in a vacuum oven.

2.2.1. 8-(Chloromethyl)-2-phenyl-6-oxo-6H-benzopyrano[6,7-d]oxazole, ***3a***

Starting from benzopyran **1** (0.095 g, 0.42 mmol) in polyphosphoric acid (0.500 g) and benzoic acid **2a** (0.101 g, 0.83 mmol), compound **3a** was obtained as a grey solid (0.080 g, 62 %). mp = 251.1-251.9 ºC. λ_{max} (MeOH-HEPES 80/20)/nm 341 (log ε 3.9). ^{1}H NMR (DMSO-d_6, 400 MHz): δ = 5.11 (s, 2 H, CH_2), 6.69 (s, 1 H, H-7), 7.61-7-67 (m, 3 H, H-3', H-4' and H-5'), 7.92 (s, 1 H, H-4), 8.21 (dd, J = 7.6 and 1.6 Hz, 2 H, H-2' and H-6'), 8.28 (s, 1 H, H-9) ppm. ^{13}C NMR (DMSO-d_6, 100.6 MHz): δ = 41.47 (CH_2), 99.58 (C-4), 114.13 (C-7), 114.69 (C-8a), 115.46 (C-9), 125.65 (C-1'), 127.28 (C-2' and C-6'), 129.20 (C-3' and C-5'), 132.27 (C-4'), 138.36 (C-9a), 150.75 (C-8), 151.64 (C-4a), 151.93 (C-3a), 159.03 (C-6), 163.91 (C-2) ppm. IR (KBr 1%): ν_{max} 3377, 2922, 2315, 1714 (br), 1632, 1595, 1554, 1489, 1438, 1406, 1353, 1267, 1137, 1043, 1018, 961, 886, 843, 778, 729, 700, 664 cm^{-1}. HRMS: m/z (EI): Found [M^+]: 311.03585; $C_{17}H_{10}NO_3Cl$ requires [M^+]: 311.03492.

2.2.2. 2-(3'-Aminophenyl)-8-(chloromethyl)-6-oxo-6H-benzopyrano[6,7-d]oxazole, ***3b***

Starting from benzopyran **1** (0.050 g, 0.22 mmol) in polyphosphoric acid (0.500 g) and 3-aminobenzoic acid **2b** (0.060 g, 0.22 mmol), compound **3b** was obtained as a grey solid (0.065 g, 89 %). mp = 242.5-243.5 ºC. λ_{max} (MeOH-HEPES 80/20)/nm 340 (log ε 3.90). ^{1}H NMR (DMSO-d_6, 400 MHz): δ 5.13 (s, 2 H, CH_2), 6.70 (s, 1 H, H-7), 7.02 (d, J = 7.6 Hz, 1 H, H-4'), 7.36 (t, J = 7.6 Hz, 1 H, H-5'), 7.53 (dd, J = 7.6 and 1.6 Hz, 1 H, H-6'), 7.60 (s, 1 H, H-2'), 7.96 (s, 1 H, H-4), 8.26 (s, 1 H, H-9) ppm. ^{13}C NMR (DMSO-d_6, 100.6 MHz): δ = 41.61 (CH_2), 99.70 (C-4), 114.36 (C-7), 114.65 (C-2'), 114.83 (C-8a), 115.66 (C-9), 117.92 (C-6'), 120.22 (C-4'), 126.48 (C-1'), 130.29 (C-5'), 138.56 (C-9a), 145.52 (C-3'), 151.10 (C-8), 151.80 (C-4a), 152.06 (C-3a), 159.70 (C-6), 164.36 (C-2) ppm. IR (KBr 1%): ν_{max} 3385, 2965, 2320, 1720 (br), 1635, 1593, 1557, 1487, 1442, 1410, 1328, 1143, 1094, 995, 877, 744, 666 cm^{-1}. HRMS: m/z (EI): Found [M^+]: 326.04501; $C_{17}H_{11}N_2O_3Cl$ requires [M^+]: 326.04582.

2.2.3. 2-(3'-Amino-4'-methylphenyl)-8-(chloromethyl)-6-oxo-6H-benzopyrano[6,7-d]oxazole, ***3c***

Starting from benzopyran **1** (0.100 g, 0.44 mmol) in polyphosphoric acid (0.500 g) and 4-methyl-3-aminobenzoic acid **2c** (0.066 g, 0.44 mmol), compound **3c** was obtained as a grey solid (0.080 g, 54 %). mp = 201.3-202.0 ºC. λ_{max} (MeOH-HEPES 80/20)/nm 342 (log ε 3.46). ^{1}H NMR (DMSO-d_6, 400 MHz): δ = 5.12 (s, 2 H, CH_2), 6.69 (s, 1 H, H-7), 7.20 (d, J = 7.6 Hz, 1 H, H-5'), 7.41 (d, J = 7.6 Hz, 1 H, H-6'), 7.58 (s, 1 H, H-2'), 7.92 (s, 1 H, H-4), 8.22 (s, 1 H, H-9) ppm. ^{13}C NMR (DMSO-d_6, 100.6 MHz): δ = 17.57 (CH_3), 41.67 (CH_2), 99.60 (C-4), 113.74 (C-2'), 114.11 (C-7), 114.72 (C-8a), 115.24 (C-9), 117.14 (C-6'), 123.90 (C-4'), 128.01 (C-1'), 131.00 (C-5'), 138.57 (C-9a), 144.88 (C-3'), 150.98 (C-8), 151.53 (C-4a), 151.88 (C-3a), 159.57 (C-6), 164.55 (C-2) ppm. IR (KBr 1%): ν_{max} 3377, 2970, 2316, 1719 (br), 1635, 1585, 1558, 1489, 1438, 1406, 1328, 1141, 1094, 993, 876, 743, 663 cm^{-1}. HRMS: m/z (EI): Found [M^+]: 340.06272; $C_{18}H_{13}N_2O_3Cl$ requires [M^+]: 340.06147.

2.2.4. 2-(3'-Amino-4'-chlorophenyl)-8-(chloromethyl)-6-oxo-6H-benzopyrano[6,7-d]oxazole, ***3d***

Starting from benzopyran **1** (0.100 g, 0.44 mmol) in polyphosphoric acid (0.500 g) and 4-chloro-3-aminobenzoic acid **2d** (0.075 g, 0.44 mmol), compound **3d** was obtained as a grey solid (0.110 g, 70 %). mp = 323.4-324.2 ºC. λ_{max} (MeOH-HEPES 80/20)/nm 341 (log ε 3.85). ^{1}H NMR (DMSO-d_6, 400 MHz): δ 5.13 (s, 2 H, CH_2), 6.70 (s, 1 H, H-7), 7.32 (dd, J = 7.6 and 2.0 Hz, 1 H, H-6'), 7.41 (d, J = 7.6 Hz, 1 H, H-5'), 7.64 (d, J = 2.0 Hz, 1 H, H-2'), 7.95 (s, 1 H, H-4), 8.25 (s, 1 H, H-9) ppm. ^{13}C NMR (DMSO-d_6, 100.6 MHz): δ = 41.70 (CH_2), 99.76 (C-4), 113.58 (C-2'), 114.27 (C-7), 114.76 (C-8a), 115.54 (C-6'), 115.56 (C-9), 121.04 (C-4'), 125.03 (C-1'), 130.08 (C-5'), 138.46 (C-9a), 145.40 (C-3'), 150.98 (C-8),

151.73 (C-4a), 151.96 (C-3a), 159.59 (C-6), 163.83 (C-2) ppm. IR (KBr 1%): ν_{max} 3380, 2970, 2316, 1722 (br), 1638, 1600, 1567, 1498, 1440, 1406, 1335, 1151, 1084, 997, 882, 745, 666 cm^{-1}. HRMS: m/z (EI): Found $[M^+]$: 360.00710; $C_{17}H_{10}N_2O_3Cl_2$ requires $[M^+]$: 360.00685.

2.2.5. 2-(3'-Dimethylaminophenyl)-8-(chloromethyl)-6-oxo-6H-benzopyrano[6,7-d]oxazole, ***3e***

Starting from benzopyran **1** (0.043 g, 0.19 mmol) in polyphosphoric acid (0.200 g), and 3-dimethylaminobenzoic acid **2e** (0.060 g, 0.19 mmol), compound **3e** was obtained as a yellow solid (0.047 g, 70 %). mp = 211.2-212.1 ºC. λ_{max} (MeOH-HEPES 80/20)/nm 339 (log ε 4.02). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 3.09 (s, 6 H, $N(CH_3)_2$), 4.75 (s, 2 H, CH_2), 6.61 (s, 1 H, H-7), 6.95 (dd, J = 2.2 and 8.2 Hz, 1 H, H-4'), 7.41 (t, J = 8.0 Hz, 1 H, H-5'), 7.58-7.61 (m, 3 H, H-4, H-2', H-6'), 8.06 (s, 1 H, H-9) ppm. ^{13}C NMR ($CDCl_3$, 100.6 MHz): δ = 40.79 ($N(CH_3)_2$), 41.61 (CH_2), 99.84 (C-4), 111.35 (C-2'), 114.52 (C-9), 114.72 (C-7), 114.83 (C-8a), 116.62 (C-4'), 116.72 (C-6'), 126.75 (C-1'), 129.88 (C-5'), 139.42 (C-9a), 149.78 (C-8), 150.40 (C-3'), 152.07 (C-4a), 152.62 (C-3a), 160.16 (C-6), 165.77 (C-2) ppm. IR (KBr 1%): ν_{max} 2969, 2315, 1715 (br), 1633, 1594, 1553, 1502, 1436, 1406, 1349, 1285, 1239, 1140, 1055, 991, 949, 918, 875, 842, 777, 724, 680 cm^{-1}. HRMS: m/z (EI): Found $[M^+]$: 354.07808; $C_{19}H_{15}N_2O_3Cl$ requires $[M^+]$: 354.07712.

2.2.6. 2-(4'-Dimethylaminophenyl)-8-(chloromethyl)-6-oxo-6H-benzopyrano[6,7-d]oxazole, ***3f***

Starting from benzopyran **1** (0.060 g, 0.26 mmol) in polyphosphoric acid (0.200 g), 4-dimethylaminobenzoic acid **2f** (0.043 g, 0.26 mmol), compound **3f** was obteined as a yellow solid (0.064 g, 69 %). mp = 221.3-222.1 ºC. λ_{max} (MeOH-HEPES 80/20)/nm 361 (log ε 4.02). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 3.11 (s, 6 H, $N(CH_3)_2$), 4.74 (s, 2 H, CH_2), 6.58 (s, 1

H, H-7), 6.95 (dd, *J* = 2.0 and 7.2 Hz, 2 H, H-3' and H-5'), 7.53 (s, 1 H, H-4), 7.94 (s, 1 H, H-9), 8.10 (dd, *J* = 2.4 and 7.2 Hz, 2 H, H-2' and H-6') ppm. ^{13}C NMR ($CDCl_3$, 100.6 MHz): δ = 40.11 ($N(CH_3)_2$), 41.69 (CH_2), 99.42 (C-4), 111.61 (C-3' and C-5'), 112.82 (C-1'), 113.17 (C-9), 114.28 (C-7), 114.44 (C-8a), 129.47 (C-2' and C-6'), 140.09 (C-9a), 149.93 (C-8), 151.54 (C-4a), 152.68 (C-3a), 152.85 (C-4'), 160.45 (C-6), 166.24 (C-2) ppm. IR (KBr 1%): ν_{max} 2918, 2849, 2315, 1727 (br), 1630, 1607, 1511, 1436, 1373, 1345, 1262, 1190, 1138, 1041, 944, 867, 820, 735, 696 cm^{-1}. HRMS: m/z (EI): Found [M^+]: 354.07744; $C_{19}H_{15}N_2O_3Cl$ requires [M^+]: 354.07712.

2.1.7. 8-(Chloromethyl)-2-(4'-oxo-4H-benzopyran-2'-yl)-6H-benzopyrano[6,7-d]oxazole, ***3g***

Starting from benzopyran **1** (0.100 g, 0.44 mmol) in polyphosphoric acid (0.500 g) and chromone-2-carboxylic acid **2h** (0.167 g, 0.87 mmol), compound **3g** was obtained as a yellow solid (0.086 g, 51 %). mp = 224.5-225.5 ºC. λ_{max} (MeOH-HEPES 80/20)/nm 306 (log ε 3.85). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 4.77 (s, 2 H, CH_2), 6.67 (s, 1 H, H-7), 7.38 (s, 1 H, H-3'), 7.52 (dt, *J* = 1.0 and 7.6 Hz, 1 H, H-6'), 7.71 (s, 1 H, H-4), 7.33 (dd, *J* = 7.6 and 1.0 Hz, 1 H, H-8'), 7.82 (dt, *J* = 1.6 and 7.6 Hz, 1 H, H-7'), 8.25 (s, 1 H, H-9), 8.28 (dd, *J* = 7.6 and 1.6 Hz, 1 H, H-5') ppm. ^{13}C NMR ($CDCl_3$, 100.6 MHz): δ = 41.48 (CH_2), 100.55 (C-4), 113.23 (C-3'), 115.76 (C-7), 116.12 (C-8a), 116.68 (C-9), 118.54 (C-8'), 124.54 (C-4a'), 126.00 (C-5'), 126.22 (C-6'), 134.02 (C-7'), 138.32 (C-8) 149.35 (C-9a), 150.43 (C-2'), 152.42 (C-4a), 153.37 (C-3a), 156.14 (C-8a'), 157.56 (C-2), 159.40 (C-6), 177.35 (C-4') ppm. IR (KBr 1%): ν_{max} 3429, 2921, 1738, 1648, 1462, 1438, 1390, 1346, 1286, 1198, 1022, 951, 900, 870, 842, 776, 750, 732 cm^{-1}. HRMS: m/z (EI): Found [M^+]: 379.02551; $C_{20}H_{10}NO_5Cl$ requires [M^+]: 379.02475.

*2.3. General procedure for the synthesis of conjugates **5a-g***

The chloromethyl precursor **3a-g** was dissolved in dry DMF (3 mL), potassium fluoride (3 equiv) and butyric acid (1 equiv) were added. The reaction mixture was stirred at room temperature for 24 h. The solvent was removed by evaporation under reduced pressure and the required conjugate was obtained as a solid. The crude residue of compounds **5a**, **5c** and **5g** were purified by column chromatography using chloroform/methanol (95:5) as eluent.

*2.3.1. (6-Oxo-2-phenyl-6H-benzopyrano[6,7-d]oxazol-8-yl)methyl butyrate, **5a***

Starting from compound **3a** (0.090 g, 0.29 mmol) in dry DMF (3 mL), potassium fluoride (3 equiv, 0.050 g, 0.86 mmol) and butyric acid (1 equiv, 0.026 mL, 0.29 mmol), the ester conjugate **5a** was obtained as a yellow solid (0.050 g, 48 %). mp = 246.5-247.3 ºC. λ_{max} (MeOH-HEPES 80/20)/nm 339 (log ε 3.86). ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 1.01 (t, J = 7.6 Hz, 3 H, CH_3), 1.75 (sext, J = 7.6 Hz, 2 H, β-CH_2), 2.48 (t, J = 7.2 Hz, 2 H, α-CH_2), 5.38 (s, 2 H, CH_2), 6.52 (s, 1 H, H-7), 7.54-7.61 (m, 4 H, H-4, H-3', H-4' and H-5'), 7.91 (s, 1 H, H-9), 8.25-8.28 (m, 2 H, H-2' and H-6') ppm. ^{13}C NMR ($CDCl_3$, 100.6 MHz): δ = 13.20 (CH_3), 17.88 (β-CH_2), 35.44 (α-CH_2), 60.74 (CH_2), 99.37 (C-4), 111.85 (C-7), 113.51 (C-9), 114.36 (C-8a), 125.72 (C-1'), 127.41 (C-2' and C-6'), 128.64 (C-3' and C-5'), 131.86 (C-4'), 138.87 (C-9a), 148.99 (C-8), 151.48 (C-4a), 152.06 (C-3a), 159.79 (C-6), 164.49 (C-2), 172.24 (C=O ester) ppm. IR (KBr 1%): ν_{max} 3355, 2970, 2404, 1727 (br), 1634, 1608, 1556, 1484, 1443, 1405, 1354, 1300, 1263, 1189, 1140, 1095, 993, 872, 747, 704, 523 cm^{-1}. HRMS: m/z (ESI): Found [M^++1]: 364.11693; $C_{21}H_{18}NO_5$ requires [M^++1]: 364.11795.

*2.3.2. (2-(3'-Aminophenyl)-6-oxo-6H-benzopyrano[6,7-d]oxazol-8-yl)methyl butyrate, **5b***

Starting from compound **3b** (0.083 g, 0.25 mmol) in dry DMF (3 mL), potassium fluoride (3 equiv, 0.044 g, 0.75 mmol) and butyric acid (1 equiv, 0.023 mL, 0.25 mmol), the ester conjugate **5b** was obtained as a yellow solid (0.030 g, 46 %). mp = 252.3-253.1 ºC. λ_{max}

(MeOH-HEPES 80/20)/nm 343 (log ε 3.36). ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 1.01 (t, J = 7.6 Hz, 3 H, CH_3), 1.68 (sext, J = 7.6 Hz, 2 H, β-CH_2), 2.47 (t, J = 7.6 Hz, 2 H, α-CH_2), 5.37 (s, 2 H, CH_2), 6.51 (s, 1 H, H-7), 6.88 (dd, J = 2.0 and 7.6 Hz, 1 H, H-4'), 7.32 (t, J = 7.6 Hz, 1 H, H-5'), 7.55 (t, J = 1.6 Hz, 1 H, H-2'), 7.57 (s, 1 H, H-4), 7.63 (dd, J = 1.6 and 7.6 Hz, 1H, H-6'), 7.87 (s, 1 H, H-9) ppm. ^{13}C NMR ($CDCl_3$, 100.6 MHz): δ = 13.59 (CH_3), 18.20 (β-CH_2), 35.91 (α-CH_2), 61.22 (CH_2), 99.76 (C-4), 112.23 (C-7), 113.70 (C-2'), 113.84 (C-9), 114.75 (C-8a), 118.08 (C-6'), 118.91 (C-4'), 126.95 (C-1'), 130.06 (C-5'), 139.31 (C-9a), 147.00 (C-3'), 149.49 (C-8), 151.88 (C-4a), 152.48 (C-3a), 160.32 (C-6), 165.22 (C-2), 171.72 (C=O ester) ppm. IR (KBr 1%): ν_{max} 3377, 2969, 2315, 1719 (br), 1634, 1595, 1557, 1489, 1438, 1406, 1328, 1141, 1094, 993, 876, 743, 663 cm^{-1}. HRMS: m/z (ESI): Found [M^+ +1]: 379.12757; $C_{21}H_{19}N_2O_5$ requires [M^++1]: 379.12885.

2.3.3. (2-(3'-Amino-4'-methylphenyl)-6-oxo-6H-benzopyrano[6,7-d]oxazol-8-yl)methyl butyrate, ***5c***

Starting from compound **3c** (0.100 g, 0.44 mmol) in dry DMF (3 mL), potassium fluoride (3 equiv, 0.077 g, 1.32 mmol) and butyric acid (1 equiv, 0.067 mL, 0.44 mmol), the ester conjugate **5c** was obtained as a yellow solid (0.080g, 69 %). mp = 232.0-234.0 ºC. λ_{max} (MeOH-HEPES 80/20)/nm 344 (log ε 3.78). ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 1.01 (t, J = 7.6 Hz, 3 H, CH_3), 1.75 (sext, J = 7.6 Hz, 2 H, β-CH_2), 2.27 (s, 3 H, CH_3), 2.47 (t, J = 7.6 Hz, 2 H, α-CH_2), 5.38 (s, 2 H, CH_2), 6.52 (s, 1 H, H-7), 7.22 (d, J = 7.6 Hz, 1 H, H-5'), 7.54-7.63 (m, 3 H, H-4, H-5'and H-6'), 7.88 (s, 1 H, H-9) ppm. ^{13}C NMR ($CDCl_3$, 100.6 MHz): δ = 13.67 (CH_3), 17.65 (CH_3), 18.35 (β-CH_2), 35.91 (α-CH_2), 61.24 (CH_2), 99.69 (C-4), 112.15 (C-7), 113.48 (C-9), 113.60 (C-2'), 114.65 (C-8a), 118.19 (C-6'), 124.71 (C-1'), 127.26 (C-4'), 131.14 (C-5'), 139.45 (C-9a), 145.17 (C-3'), 149.52 (C-8), 151.7 (C-4a), 152.49 (C-3a), 160.37 (C-6), 165.46 (C-2), 172.72 (C=O ester) ppm. IR (KBr 1%): ν_{max} 3462, 3357, 2966, 2934, 2875, 2315, 1734, 1718, 1634, 1606, 1557, 1522, 1491, 1437,

1420, 1333, 1302, 1247, 1166, 1140, 1058, 995, 960, 870, 842, 811, 755, 739, 726, 664 cm^{-1}. HRMS: m/z (ESI): Found $[M^+ +1]$: 393.14611; $C_{22}H_{21}N_2O_5$ requires $[M^+ +1]$: 393.14513.

2.3.4. (2-(3'-Amino-4'-chlorophenyl)-6-oxo-6H-benzopyrano[6,7-d]oxazol-8-yl)methyl butyrate, ***5d***

Starting from compound **3d** (0.050 g, 0.14 mmol) in dry DMF (3 mL), potassium fluoride (3 equiv, 0.024 g, 0.42 mmol) and butyric acid (1 equiv, 0.013 mL, 0.14 mmol), the ester conjugate **5d** was obtained as a yellow solid (0.050 g, 70 %). mp = 201.3-202.0 ºC. λ_{max} (MeOH-HEPES 80/20)/nm 345 (log ε 4.04). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.01 (t, J = 7.6 Hz, 3 H, CH_3), 1.75 (sext, J = 7.6 Hz, 2 H, β-CH_2), 2.47 (t, J = 7.6 Hz, 2 H, α-CH_2), 5.38 (s, 2 H, CH_2), 6.52 (s, 1 H, H-7), 7.55 (dd, J = 2.0 and 7.6 Hz, 1 H, H-6'), 7.58 (s, 1 H, H-4), 7.65 (d, J = 2.0 Hz, 1 H, H-2') 7.88 (s, 1 H, H-9) ppm. ^{13}C NMR ($CDCl_3$, 100.6 MHz): δ = 13.66 (CH_3), 18.34 (β-CH_2), 35.90 (α-CH_2), 61.19 (CH_2), 99.80 (C-4), 112.35 (C-7), 113.93 (C-9), 114.32 (C-2'), 114.88 (C-8a), 118.16 (C-6'), 123.31 (C-4'), 125.51 (C-1'), 130.20 (C-5'), 139.24 (C-9a), 143.51 (C-3'), 149.42 (C-8), 151.97 (C-4a), 152.47 (C-3a), 160.21 (C-6), 164.41 (C-2), 172.71 (C=O ester) ppm. ν_{max}/cm^{-1} 3450, 3365, 2966, 2931, 2875, 2317, 1733 (br), 1634, 1599, 1558, 1503, 1439, 1328, 1264, 1167, 1139, 1098, 1046, 996, 955, 871, 840, 815, 740, 726, 664. HRMS: m/z (ESI): Found $[M^+ +1]$: 413.08861; $C_{21}H_{18}N_2O_5Cl$ requires $[M^+ +1]$: 413.08988.

2.3.5. (2-(3'-(Dimethylamino)phenyl)-6-oxo-6H-benzopyrano[6,7-d]oxazol-8-yl)methyl butyrate, ***5e***

Starting from compound **3e** (0.030 g, 0.08 mmol) in dry DMF (3 mL), potassium fluoride (3 equiv, 0.050 g, 0.24 mmol) and butyric acid (1 equiv, 0.007 mL, 0.08 mmol), the ester conjugate **5e** was obtained as a yellow solid (0.024 g, 70 %). mp = 198.4-199.2 ºC. λ_{max} (MeOH-HEPES 80/20)/nm 341 (log ε 3.98). ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 1.01 (t, J =7.6

Hz, 3 H, CH_3), 1.75 (sext, *J* = 7.6 Hz, 2 H, β-CH_2), 2.47 (t, *J* = 7.6 Hz, 2 H, α-CH_2), 3.07 (s, 6 H, $N(CH_3)_2$), 5.36 (s, 2 H, CH_2), 6.49 (s, 1 H, H-7), 6.92 (dd, *J* = 8.0 and 2.4 Hz, 1 H, H-4'), 7.39 (t, *J* = 8.0 Hz, 1 H, H-5'), 7.53-7.55 (m, 2 H, H-2' and H-6'), 7.57 (s, 1 H, H-4), 7.87 (s, 1 H, H-9) ppm. ^{13}C NMR ($CDCl_3$, 100.6 MHz): δ = 13.66 (CH_3), 18.34 (β-CH_2), 35.89, (α-CH_2), 40.45 ($N(CH_3)_2$), 61.21 (CH_2), 99.72 (C-4), 110.90 (C-2'), 112.13 (C-7), 113.71 (C-9), 114.65 (C-8a), 115.81 (C-6'), 116.61 (C-4'), 126.62 (C-1'), 129.74 (C-5'), 139.37 (C-9a), 149.49 (C-8), 150.68 (C-3'), 151.79 (C-4a), 152.48 (C-3a), 160.34 (C-6), 165.80 (C-2), 172.69 (C=O ester) ppm. IR (KBr 1%): ν_{max} 3414, 2963, 2301, 1730 (br), 1633, 1599, 1552, 1498, 1438, 1403, 1360, 1328, 1263, 1141, 1061, 990, 959, 886, 781, 723, 679 cm^{-1}. HRMS: m/z (ESI): Found [M^++1]: 407.15928; $C_{23}H_{23}N_2O_5$ requires [M^++1]: 407.16015.

2.3.6. (2-(4'-(Dimethylamino)phenyl)-6-oxo-6H-benzopyrano[6,7-d]oxazol-8-yl)methyl butyrate, ***5f***

Starting from compound **3f** (0.025 g, 0.07 mmol) in dry DMF (3 mL), potassium fluoride (3 equiv, 0.012 g, 0.21 mmol) and butyric acid (1 equiv, 0.006 mL, 0.07 mmol), the ester conjugate **5f** was obtained as a yellow solid (0.020 g, 71 %). mp = 200.4-201.4 ºC. λ_{max} (MeOH-HEPES 80/20)/nm 361 (log ε 3.90). ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 1.01 (t, *J* = 7.6 Hz, 3 H, CH_3), 1.72 (sext, *J* = 7.6 Hz, 2 H, β-CH_2), 2.47 (t, *J* = 7.6 Hz, 2 H, α-CH_2), 5.37 (s, 2 H, CH_2), 6.49 (s, 1 H, H-7), 6.78 (d, *J* = 7.6 Hz, 2 H, H-3' and H-5'), 7.53 (s, 1 H, H-4), 7.79 (s, 1 H, H-9), 8.10 (d, *J* = 7.6 Hz, 2 H, H-2' and H-6') ppm. ^{13}C NMR ($CDCl_3$, 100.6 MHz): δ = 13.67 (CH_3), 18.35 (β-CH_2), 35.89, (α-CH_2), 40.08 ($N(CH_3)_2$), 61.30 (CH_2), 99.36 (C-4), 111.57 (C-3' and C-5'), 112.50 (C-1'), 112.60 (C-9), 112.80 (C-7), 114.32 (C-8a), 129.46 (C-2'and C-6'), 140.05 (C-9a), 149.67 (C-8), 151.34 (C-4a), 152.57 (C-3a), 152.85 (C-4'), 160.64 (C-6), 166.19 (C-2), 172.74 (C=O ester) ppm. IR (KBr 1%): ν_{max} 3416, 2964, 2300, 1729 (br), 1635, 1595, 1549, 1490, 1440, 1401, 1359, 1328, 1261, 1130,

1059, 992, 960, 885, 783, 725, 669 cm^{-1}. HRMS: m/z (ESI): Found [M^++1]: 407.15900; $C_{23}H_{23}N_2O_5$ requires [M^++1]: 407.16015.

2.3.7. (6-Oxo-2-(4'-oxo-4H-benzopyran-2'-yl)-6H-benzopyrano[6,7-d]oxazol-8-yl)methyl butyrate, ***5g***

Starting from compound **3g** (0.031 g, 0.08 mmol) in dry DMF (3 mL), potassium fluoride (3 equiv, 0.015 g, 0.26 mmol) and butyric acid (1 equiv, 0.008 mL, 0.08 mmol), the ester conjugate **5g** was obtained as a yellow solid (0.030 g, 85%). mp = 237.4-238.4 ºC. λ_{max} (MeOH-HEPES 80/20)/nm 348 (log ε 3.53). ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 1.01 (t, J = 7.6 Hz, 3 H, CH_3), 1.75 (sext, J = 7.6 Hz, 2 H, β-CH_2), 2.47 (t, J = 7.6 Hz, 2 H, α-CH_2), 5.39 (s, 2 H, CH_2), 6.56 (s, 1 H, H-7), 7.34 (s, 1 H, H-3'), 7.50 (dt, J = 7.2 and 1.2 Hz, 1 H, H-6'), 7.69 (s, 1 H, H-4), 7.71 (dd, J = 8.4 and 0.8 Hz, 1 H, H-8'), 7.80 (dt, J = 7.6 and 1.6 Hz, 1 H, H-7'), 8.07 (s, 1 H, H-9), 8.25 (dd, J = 1.6 and 8.0 Hz, 1 H, H-5') ppm. ^{13}C NMR ($CDCl_3$, 100.6 MHz): δ = 13.65 (CH_3), 18.32 (β-CH_2), 35.85 (α-CH_2), 61.08 (CH_2) 100.43 (C-4), 113.15 (C-3'), 113.31 (C-7), 115.86 (C-9), 116.03 (C-8a), 118.50 (C-8'), 124.48 (C-4a'), 125.92 (C-6'), 126.17 (C-5'), 134.86 (C-7'), 138.25 (C-8), 149.05 (C-9a), 150.38 (C-2'), 152.28 (C-4a), 153.13 (C-3a), 156.06 (C-8a'), 157.43 (C-2), 159.52 (C-6), 172.64 (C=O ester), 177.25 (C-4') ppm. IR (KBr 1%): ν_{max} 3441, 2963, 2285, 1734 (br), 1649 (br), 1466, 1439, 1385, 1329, 1248, 1127, 1051, 955, 871, 843, 815, 778, 754 cm^{-1}. HRMS: m/z (ESI): Found [M^++1]: 432.10646; $C_{24}H_{18}NO_7$ requires [M^++1]: 432.10778.

2.4. Photolysis general

A 1×10^{-4} M methanol/HEPES (80:20) solution of compounds **5a**-**g** (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 at 254 nm, 300 nm and 350 nm (± 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 for all compounds, previously filtered through a Millipore, type HN 0.45 µm filter and degassed by ultra-sound for 30 min. The chromatograms were traced by detecting UV absorption at the wavelength of maximum absorption for each compound (retention time: **5a**, 6.6; **5b**, 7.0; **5c**, 6.7; **5d**, 6.5; **5e**, 9.6; **5f**, 11.9; **5g**, 7.3 min).

3. Results and Discussion

*3.1. Synthesis of butyric acid conjugates **5a**–**g***

The synthesis of chloromethylated 6-oxo-6*H*-benzopyrano[6,7-*d*]oxazoles **3a**-**f** was achieved by a condensation reaction between 6-amino-4-(chloromethyl)-7-hydroxy-2*H*-benzopyran-2-one [23] **1** and benzoic acid **2a** or its derivatives; namely 3-aminobenzoic acid **2b**, 3-amino-4-methylbenzoic acid **2c**, 3-amino-4-chlorobenzoic acid **2d**, 3-(dimethylamino)benzoic acid **2e** and 4-(dimethylamino)benzoic acid **2f**, mediated by polyphosphoric acid (PPA) at 130º C according to a known procedure [23]. Reaction of precursor **1** with 4-oxo-4*H*-benzopyran-2-carboxylic acid **2g** in the same conditions gave compound **3g** (Scheme 1, Table 1).

<Scheme 1>

Compounds **3a-g** were used in the derivatisation of butyric acid **4**, in the presence of potassium fluoride in *N*,*N*-dimethylformamide at room temperature, resulting in the ester prodrugs **5a-g** in moderate to good yields (Scheme 2, Table 1).

All compounds were new and fully characterized by high-resolution mass spectrometry, IR, ^{1}H and ^{13}C NMR spectroscopy. The IR spectra of compounds **5a-g** displayed stretching bands of the ester carbonyl groups from 1727 to 1734 cm^{-1}. ^{1}H NMR spectra showed signals of butyric acid, the methyl (δ 1.01 ppm) and two methylenes (δ 1.68-1.76 and 2.47-2.48 ppm, respectively). The heterocycle methylene group, adjacent to the ester link, was visible for all compounds (δ 5.36-5.40 ppm). The newly formed ester linkages were confirmed by ^{13}C NMR spectra signals of the carbonyl group, at about δ 171.7-172.7 ppm.

<Scheme 2>

<Table 1>

*3.2 Evaluation of the photophysical properties of butyric acid conjugates **5a-g***

Fundamental UV–vis photophysical characterisation of conjugates **5a-g** was carried out to acquire the parameters required for monitoring the photolytic process and to assess their sensitivity to light. The absorption and emission spectra of degassed 10^{-5} M solutions in methanol/HEPES buffer (80:20) solution of conjugates **5a-g** and precursors **3a-g** were measured and the corresponding data are presented in Table 1. Relative fluorescence quantum yields (Φ_F) were calculated using 9,10-diphenylanthracene in ethanol (Φ_F 0.95) [24] as standard. For the Φ_F determination, the fluorescence standard was excited at the wavelengths of maximum absorption found for each compound and in all fluorimetric measurements the absorbance of the solution did not exceed 0.1. Maximum absorption wavelengths (λ_{abs}) in methanol/HEPES buffer (80:20) solutions of the new conjugates **5a-g** displayed a bathochromic shift from 13 to 35 nm (λ_{abs} 339-361 nm) in comparison with compound **6**, previously obtained by our research group

(Figure 1) [22]. The fluorescence spectra in the same solvent revealed that emission maxima (λ_{em}) of conjugates **5a**-**g** occurred in the range 418-463 nm, with relative fluorescent quantum yields inferior to the of analogue **6**, with exception of conjugate **5g** (~ four times superior), and good large Stokes' shifts (79-125 nm).

<Figure 1>

The conjugates were further characterised using time-resolved fluorescence spectroscopy and after an initial study monitoring the decay at 450 nm revealed that the decay kinetic was multiexponential (data not shown) a determination of decay associated spectra was made by measuring the fluorescence decay at 5 nm increments, over the range 360 nm to 625 nm, for equal time periods. A global analysis of each dataset was then made, with the need to use the sum of three exponential decay components in each case to give a satisfactory fit to the data. The data, given in Figure 2, show that the shorter-wavelength spectrum is associated with the shorter-lived decay and the longer-lived decay with the longer wavelength spectrum. This is consistent with the emissions having the same origin [25] and previously we have reported these to relate to the conjugate, ion pair and photocleaved species [16]. It is expected that the light intensities present in this time-correlated single-photon counting experiment should not cause significant photocleaveage of the conjugates. However, as this cannot be completely ruled out these data are indicative of the states present and via the lifetimes the rates ($1/\tau$) of the decay processes.

<Figure 2>

Although the decay associated spectra are good to identify the species present and their contribution to the overall fluorescence emission it should be kept in mind that since they are basically the pre-exponential weighted by the lifetime, they do not represent the relative concentrations of the species

present. This is better represented by the normalised pre-exponential values and this is illustrated in Figure 3 for the decay monitored at 425 nm, which is close to the peak emission.

<Figure 3>

These data show that the decay kinetic, in the main, is dominated by the shorter-lived component (shorter wavelength species) which is most likely relates to the conjugate. The minor contribution comes from the longer-lived (longer wavelength species) and hence can relate to the cleaved species. In this case the intermediate species would be the ion pair.

Coumarin-caged esters, phosphates, carboxylates, and sulfonates as well as carbonate, carbamate, and anhydride derivatives have been reported as interesting photosensitive compounds with an emphasis on biological applications [26-30]. Ever since the introduction of coumarins as photoactivatable releasing groups, several alterations in the coumarin skeleton have been carried out; namely the nature of substituents at positions 6 and 7, as well as by the addition of a third aromatic ring, a benzene nucleus or an oxazole moiety [3,15,16,19-22]. The later modifications were carried out by our research group, and coumarin fused oxazoles (eg. 6-oxo-6*H*-benzopyrano[6,7-*d*]oxazoles) were used in the caging of butyric acid [22]. The most advantageous results were found with (2-methyl-6-oxo-6*H*-benzopyran[6,7-*d*]oxazol-8-yl)methyl group with a linkage to butyric acid through pyranone ring. However, improvements on the release parameters of the active molecule at longer wavelengths are still desired for bioapplications. As a result, a new set of 6-oxo-6*H*-benzopyrano[6,7-*d*]oxazoles were synthesised. Instead of possessing the methyl group (Figure 1), (hero)aromatic substituents at position 2 of the heterocyclic system were employed. The groups used were phenyl, 3-aminophenyl, 3-amino-4-methylphenyl, 3-amino-4-chlorophenyl, 3-(dimethylamino)phenyl and 4-oxo-4*H*-benzopyran-2-yl. Evaluation of the new coumarin fused oxazoles as photosensitive units for carboxylic acids, using butyric acid as a model compound was

achieved. Accordingly, conjugates **5a**-**g** were irradiated at 254 nm, 300 nm and 350 nm in mixtures of methanol with aqueous HEPES buffer in 80:20 solutions, in a Rayonet RPR-100 reactor, and kinetic data were collected. The course of the photolytic 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 chosen wavelengths. The peak areas were determined by HPLC, which revealed a gradual decrease with time and were taken as the average of three runs. The irradiation time given 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, based on HPLC data, the plot of ln *A versus* irradiation time showed a linear correlation for the disappearance of the starting material. This is indicative of 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 extinction coefficients (ε) and the incident photon flux (I_0), which was determined by potassium ferrioxalate actinometry [31].

The results at the various irradiation wavelengths revealed the significant influence of the (hetero)aromatic substitution on the oxazole ring; namely at position 2 of the oxo-benzopyranoxazole, in the irradiation time (t_{irr}) necessary to release butyric acid (Table 2). In comparison with conjugate **6**, bearing a methyl group, the most relevant result is related to the decrease of irradiation times at 350 nm for all cages (except for **5b**, which is similar). In the case of compound **5a** with the phenyl group (t_{irr} 55 min) this was more than five times and about twenty-five times in the case of compound **5g**, which possesses the 4-oxo-4*H*-benzopyran-2-yl group. These results are advantageous for biological purposes. On the other hand, by comparing conjugates **5c** and **5d**, the presence at the benzene ring of a methyl group promotes faster photolysis than the chlorine atom in all wavelengths of irradiation. It is interesting to note that the shortest-lived decay component (exciting at 336 nm) occurs in compounds **5f** and **5g**, which signifies that non-radiative processes are more dominant in these compounds. Considering the practical applications of the present compounds, although

they cleaved readily at 254 nm (the fastest were **5a** with 13 min and **5g** with 18 min), and also at 300 nm (the fastest again were **5g** with 14 min, followed by **5a** with 46 min), photolysis at these shorter wavelengths (higher energies) can be harmful to biological media. Hence, photolysis at 350 nm and longer wavelengths is preferable and the results obtained encourage us to continue the development of new oxo-benzopyranoxazoles as alternative photosensitive carboxylic acid caging compounds.

<Table 2>

As stated earlier, the photolysis process was monitored by HPLC/UV detection. Nevertheless, the release of butyric acid, as the expected product of the cage photolysis, was also followed by ^{1}H NMR in a methanol-d_4/D_2O (80:20) solution to provide further evidence. Upon irradiation at 350 nm of a solution of butyric acid conjugate **5a**, the signal due to the benzylic-type CH_2 at position 4 of the pyran ring, observable at about δ 5.3 ppm gradually decreased with time. The same observation occurred with the signals related to the butyric acid in the conjugated form at about δ 2.5, 1.7 and 1.0 ppm, giving rise to a close set of signals corresponding to butyric acid in its free form at about δ 2.3, 1.6 and 0.90 ppm, respectively (see Figure 4 as representative example). NMR monitoring was carried out with a 4.4×10^{-4} 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. In the reported conditions, at the various wavelengths of irradiation, no formation of side or rearrangement products was detected in the monitoring by NMR.

<Figure 4>

Coumarinyl esters are thought to photocleave through an ionic mechanism that involves both homolytical or heterolytical fission of the O-CH_2 bond, although the latter is energetically favoured

(Scheme 3). The homolytic cleavage of the O-C bond, followed by electron transfer, can yield the ion pair (a methylenic coumarin carbocation and the leaving group anion), whereas the heterolytic cleavage of the O-C bond directly affords the already mentioned ion pair. Once formed, the methylenic coumarin carbocation can undergo nucleophilic attack by the solvent to form the final products. The ion pair may also recombine to the starting material [3]. The presence of these species is consistent with what is observed in the time-resolved fluorescence data.

The behaviour of a variety of coumarinyl methyl esters as photocleavable protecting groups for a butyric acid derivative, namely γ-aminobutyric acid (GABA), has also been explored in previous work by the authors [2,15,18,32,33]. The previous findings also confirmed the applicability of such heterocycles as phototriggers for GABA at different wavelengths and their behaviour is closely related to the one described in the present report, with varying irradiation times to ensure complete release of the active molecule, according to the structure of the heterocycle.

4. Conclusion

Chloromethylated coumarin fused oxazoles were synthesised and efficiently used in the derivatisation of butyric acid, as a model carboxylic acid drug. The seven new cages based on 6-oxo-6*H*-benzopyrano[6,7-*d*]oxazol-8-yl)methyl groups with various (hetero)aromatic substituents at position 2 of the heterocyclic system were revealed to be photo-responsive units upon irradiation at various selected wavelengths (254, 300 and 350 nm). Irradiation resulted in the complete release of the expected butyric acid. The influence of the substitution at position 2 was confirmed, and in comparison with previously reported (2-methyl-6-oxo-6*H*-benzopyran[6,7-*d*]oxazol-8-yl)methyl group, was found to provide a significant improvement in relation to the photolysis data. Especially pertinent when considering the longer wavelengths more suited for bioapplications. Overall, the presence of the phenyl group and 4-oxo-4*H*-benzopyran-2-yl, namely (6-oxo-2-phenyl-6*H*-

benzopyrano[6,7-*d*]oxazol-8-yl)methyl and (6-oxo-2-(4-oxo-4*H*-benzopyran-2-yl)-6*H*-benzopyrano[6,7-*d*]oxazol-8-yl)methyl groups displayed the best results, with the corresponding ester cages activated using short irradiation times (11 min, at 350 nm). Thus, resulting in promising moieties for the development of photoactivable fluorescent butyric acid prodrugs.

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CAPTIONS

Scheme 1. Synthesis of chloromethylated 6-oxo-6*H*-benzopyrano[6,7-*d*]oxazoles 3a-g.

Scheme 2. Synthesis of butyric acid cages based on 6-oxo-6*H*-benzopyrano[6,7-*d*]oxazol-8-yl)methyl groups 5a-g.

Scheme 3. Proposed mechanism for the photolysis of butyric acid cages **5**.

Table 1. Synthesis, UV/vis absorption and emission data for precursors **3a-g** and their conjugates **5a-g**, in methanol/HEPES buffer (80:20) solutions.

Table 2. Irradiation times (t_{irr}, in min), and photochemical quantum yields (Φ_{Phot}, $\times 10^{-3}$) for the photolysis of conjugates **5a-g** at different wavelengths in methanol/HEPES buffer (80:20) solution.

Figure 1. Structure of (2-methyl-6-oxo-6*H*-benzopyran[6,7-*d*]oxazol-8-yl)methyl butyrate **6** [22].

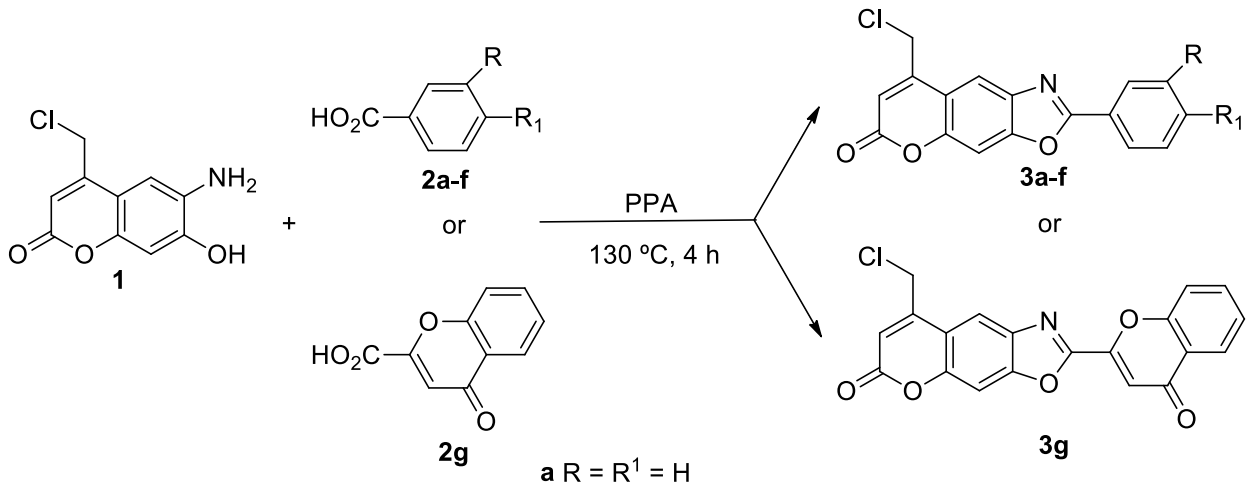
Figure 2. Decay associated spectra for compounds **5a** to **5g**, excited at 336 nm. The dotted lines signify the overall emission spectrum (sum of the decay associated spectra).

Figure 3. Normalised pre-exponential values for compounds **5a** to **5g**, excited at 336 nm, with the decay monitored at 425 nm.

Figure 4. Partial ^{1}H NMR spectra in methanol-d_4/D_2O (80:20) of the photolysis of conjugate **5a** (C = 4.4×10^{-4} M) at 350 nm: (a) before irradiation; (b) after irradiation for 30 min; (c) after irradiation for 60 min; (d) sample of free butyric acid.

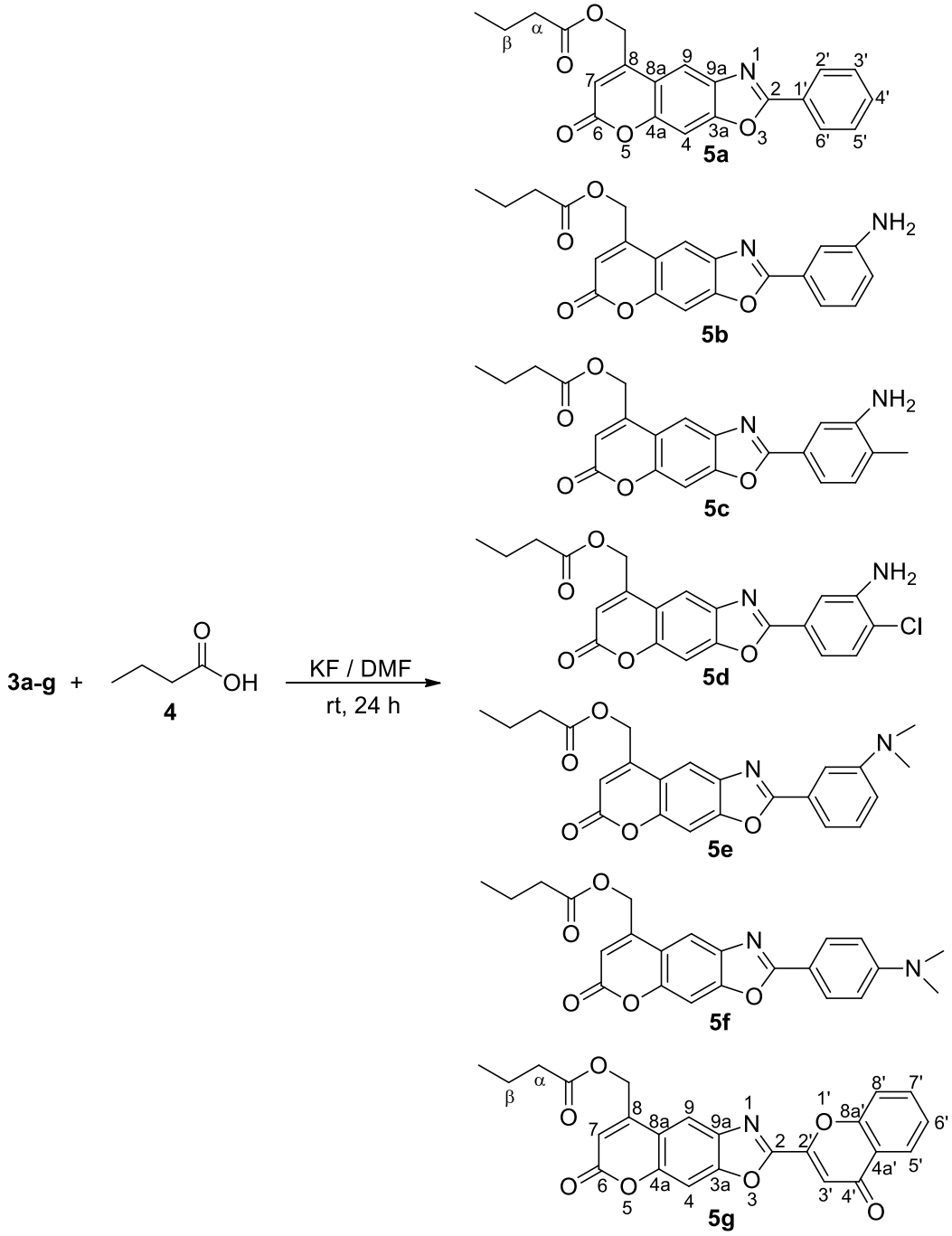
SCHEMES

Scheme 1

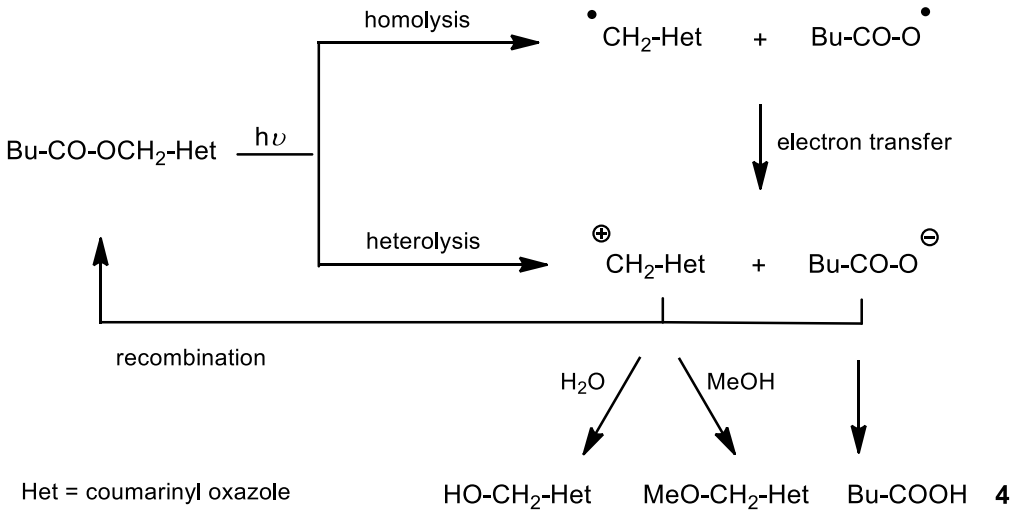


a R = R^1 = H
b R = NH_2, R^1 = H
c R = NH_2, R^1 = Me
d R = NH_2, R^1 = Cl
e R = NMe_2, R^1 = H
f R = H, R^1 = NMe_2

Scheme 2



Scheme 3


homolysis
CH2-Het + Bu-CO-O
Bu-CO-OCH2-Het
hυ
electron transfer
heterolysis
CH2-Het + Bu-CO-O
recombination
H2O
MeOH
Het = coumarinyl oxazole
HO-CH2-Het
MeO-CH2-Het
Bu-COOH
4

TABLES

Table 1

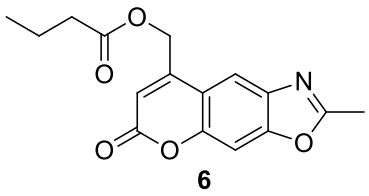
Compound	Yield (%)	Absorption		Emission		
		λ_{max} (nm)	log ε	λ_{em} (nm)	Φ_F	Stokes' shift (nm)
3a	62	341	3.98	397	0.06	56
3b	89	340	3.90	428	0.01	88
3c	54	342	3.46	423	0.02	81
3d	70	341	3.85	418	0.01	77
3e	70	339	4.02	465	0.004	127
3f	69	361	4.02	417	0.02	56
3g	51	306	3.85	444	0.04	138
5a	48	339	3.86	418	0.07	79
5b	46	337	3.36	426	0.03	83
5c	69	345	4.04	430	0.01	85
5d	70	344	3.78	456	0.07	112
5e	70	341	3.98	463	0.003	125
5f	71	361	3.90	455	0.02	116
5g	85	347	3.53	451	0.39	103
6 [22]	95	326	3.83	424	0.10	98

Table 2

Compound	254 nm		300 nm		350 nm	
	t_{irr}	Φ_{Phot}	t_{irr}	Φ_{Phot}	t_{irr}	Φ_{Phot}
5a	13	0.486	46	0.220	55	0.132
5b	30	1.42	149	0.166	291	0.080
5c	35	0.510	127	0.071	160	0.052
5d	58	0.322	195	0.042	193	0.042
5e	79	0.152	170	0.355	243	0.207
5f	68	0.203	58	0.124	40	0.158
5g	18	1.80	14	0.213	11	0.153
6[22**]**	45	6.59	33	6.59	285	1.04

FIGURES

Figure 1



6

Figure 2

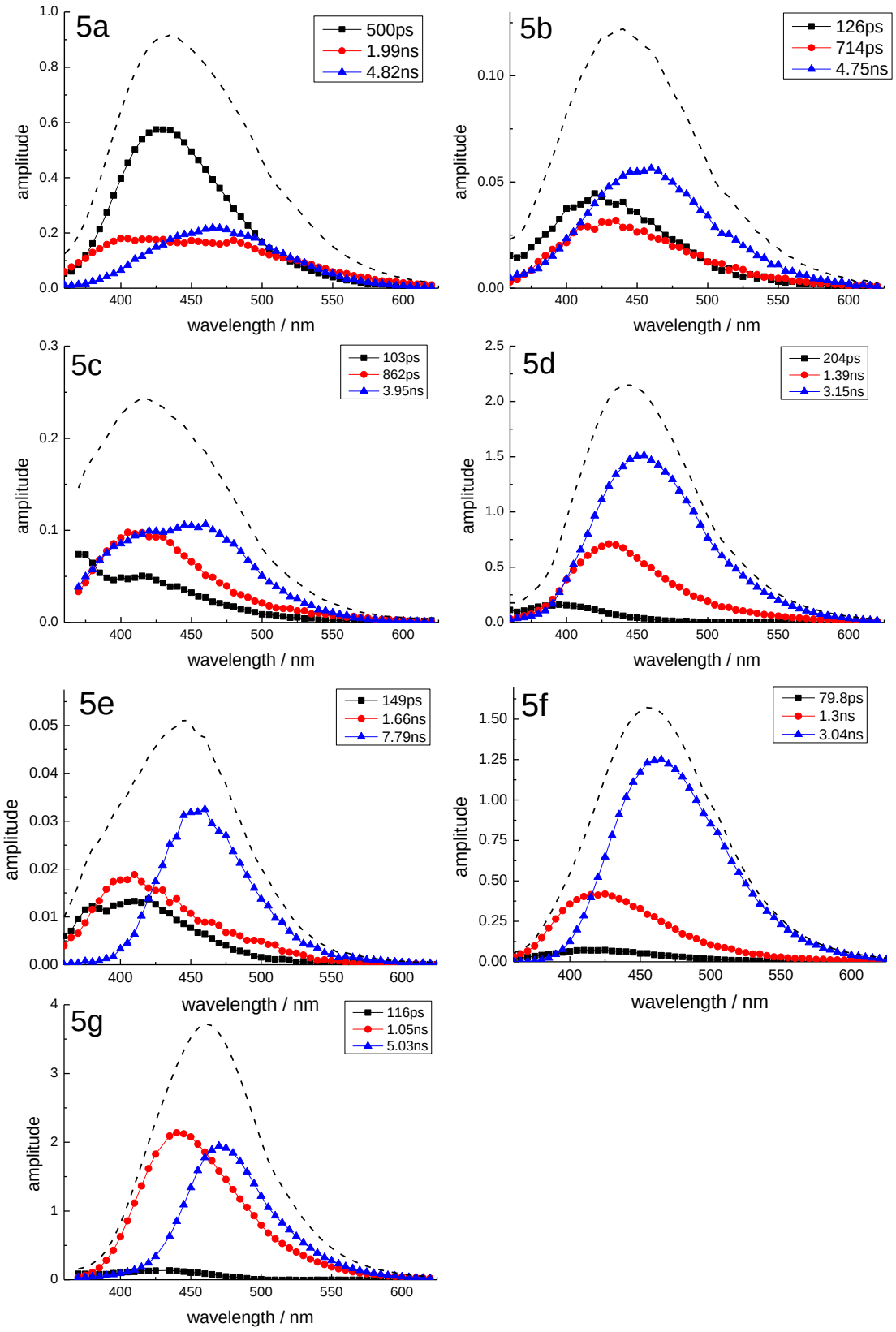

5a
500ps
1.99ns
4.82ns
amplitude
wavelength / nm
5b
126ps
714ps
4.75ns
amplitude
wavelength / nm
5c
103ps
862ps
3.95ns
amplitude
wavelength / nm
5d
204ps
1.39ns
3.15ns
amplitude
wavelength / nm
5e
149ps
1.66ns
7.79ns
amplitude
wavelength / nm
5f
79.8ps
1.3ns
3.04ns
amplitude
wavelength / nm
5g
116ps
1.05ns
5.03ns
amplitude
wavelength / nm

Figure 3

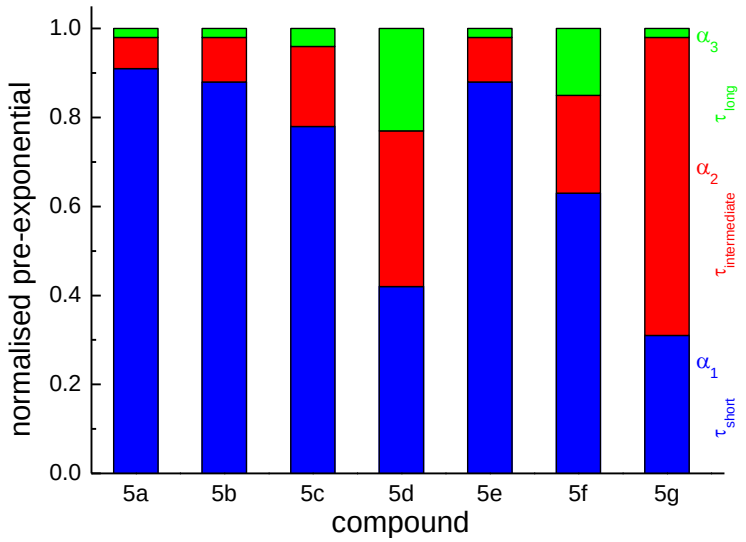
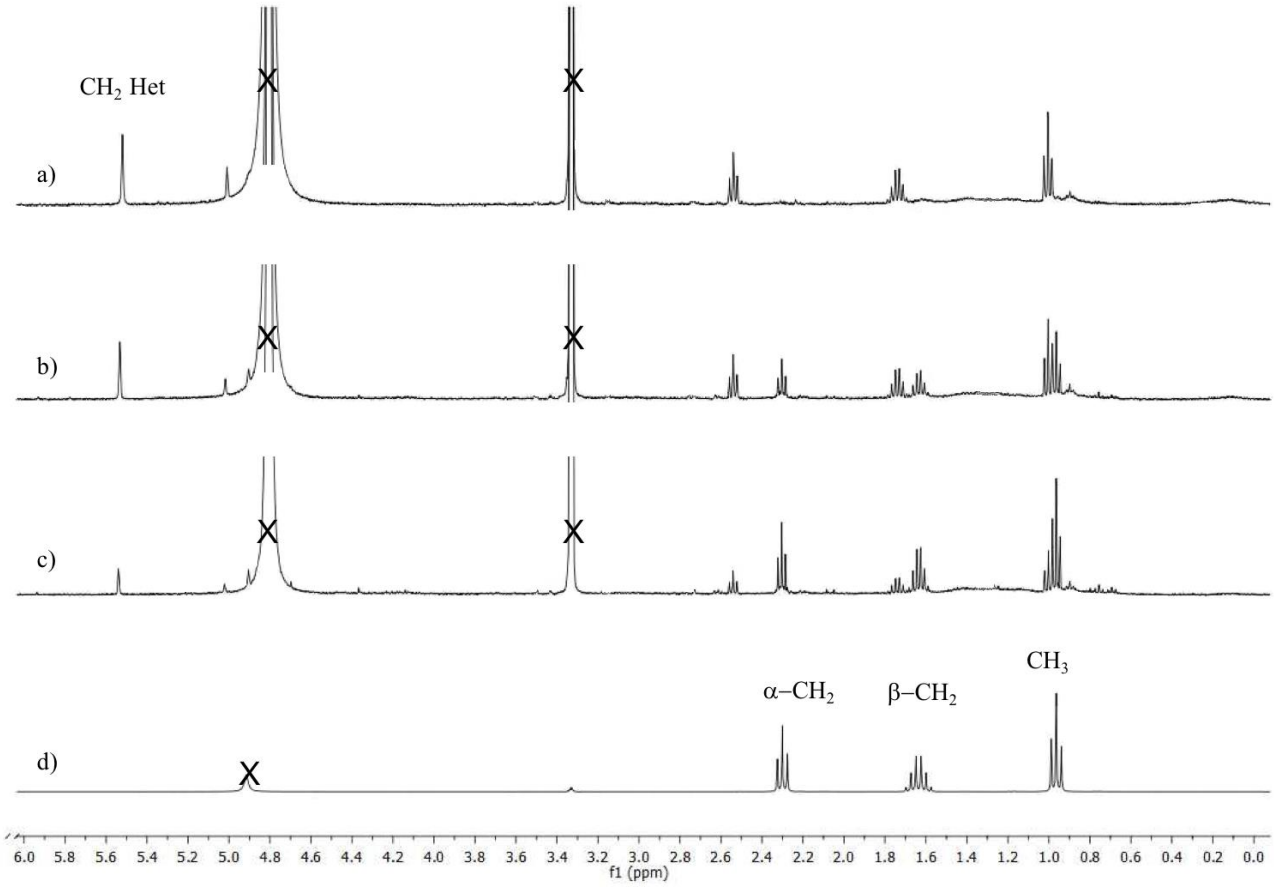

normalised pre-exponential
1.0
0.8
0.6
0.4
0.2
0.0
5a
5b
5c
5d
5e
5f
5g
compound
α3
τlong
α2
τintermediate
α1
τshort

Figure 4


CH2 Het
a)
b)
c)
d)
α–CH2
β–CH2
CH3
6.0 5.8 5.6 5.4 5.2 5.0 4.8 4.6 4.4 4.2 4.0 3.8 3.6 3.4 3.2 3.0 2.8 2.6 2.4 2.2 2.0 1.8 1.6 1.4 1.2 1.0 0.8 0.6 0.4 0.2 0.0
f1 (ppm)