Photoresponsive prodrugs of butyric acid based on amino naphthopyranones

Ana M. S. Soares, Susana P. G. Costa and M. Sameiro T. Gonçalves*

Centro de Química, Universidade do Minho, Campus de Gualtar, 4710-057 Braga, Portugal
E-mail: msameiro@quimica.uminho.pt

Abstract: In order to evaluate the application of new 7-amino-naphtho[1,2-*b*]pyran-2-ones as photoactive groups in the preparation of carboxylic acid derivatives, model butyric ester conjugates were synthesised by reaction with the chloromethylated heterocyclic precursor. Photocleavage studies of the ester conjugates in methanol/HEPES buffer (80:20) solution at different wavelengths of irradiation (254, 300, 350 and 419 nm) confirmed the quantitative release of the model acid in short irradiation times.

Keywords: prodrugs, butyric acid, coumarin, naphthopyranone, photolysis.

Introduction

The use of pharmacologically inert drug derivatives (the so called prodrugs) are useful to overcome common drawbacks associated with the intake of drugs (i.e. low oral absorption, chemical instability or toxicity) and have gained increasing importance in the development of improved prodrugs [1]. Photoresponsive prodrugs contain a photoactive group that can be cleaved upon irradiation with a suitable wavelength thus controlling its reactivity and such derivatives have been proposed recently for the optimisation of drug delivery with light as triggering agent also allowing spatial and temporal control of the release process [2]. Given the promising results obtained so far, this strategy could allow for a more patient-friendly drug delivery and availability scheme, involving less complex dosing schedules and with a lower potential for side effects.

There is an interest in developing prodrugs of butyric acid, which is a short chain fatty acid involved in the regulatory mechanisms for gene expression known to promote markers of cell differentiation, apoptosis and cell growth control [3].

We have been engaged for some years in the design of novel photolabile protecting groups based on oxygen and nitrogen heterocycles for temporary protection of carboxylic acids and amines for

organic synthesis and caging applications [4], and the present communication intends to give an account of the use of such heterocycles in the area of photoresponsive prodrugs.

Thus, in order to evaluate the differences in the photolytic release of butyric acid from the heterocyclic cage, several ester conjugates of butyric acid were prepared by reaction with an amino naphthopyranone, which was further *N*-alkylated. Photolysis studies were carried out under irradiation at different wavelengths (250, 300, 350 and 419 nm) in a Rayonet RPR-100 photochemical reactor in a mixture of methanol and HEPES buffer solution in a 80:20 proportion.

Experimental

Synthesis of 7-amino-4-(chloromethyl)-naphtho[1,2-*b*]pyran-2-one 1.

To a solution of 5-aminonaphthalen-1-ol (1 equiv, 0.500 g, 3.14 mmol) in 70% aqueous sulphuric acid (5 mL), ethyl chloroacetoacetate (1.5 equiv, 0.64 mL, 4.71 mmol) was added and stirred at room temperature for 24 h. The reaction mixture were poured into ice water and stirred for 2 h to give a fine greenish precipitate. The solid was collected by filtration, washed with cold water and dried in a vacuum oven (0.490 g, 60%). Mp = 312.0-313.2 ºC. ^{1}H NMR (300 MHz, DMSO-d_6): δ = 5.11 (2H, d, *J* 0.6 Hz, CH_2), 6.76 (1H, s, H3), 7.02 (1H, dd, *J* 8.1 and 0.6 Hz, H8), 7.46 (1H, t, *J* 8.1 Hz, H9), 7.83 (1H, d, *J* 8.6 Hz, H5), 7.74 (1H, d, *J* 8.6 Hz, H6), 8.01 (1H, dd, *J* 8.1 and 0.6 Hz, H10). ^{13}C NMR (75.4 MHz, DMSO-d_6): δ= 41.65 (CH_2), 112.98 (C4a), 114.58 (C6), 115.08 (C3), 116.36 (C8), 118.51 (C10), 119.94 (C5), 123.39 (C10a), 125.28 (C6a), 127.96 (C9), 137.91 (C7), 150.42 (C4), 151.42 (C10b), 159.56 (C2). FTIR (KBr 1%, cm^{-1}): ν = 3388, 2926, 2620, 1758, 1714, 1630, 1610, 1563, 1513, 1472, 1439, 1387, 1282, 1225, 1149, 1117, 1085, 1064, 970, 851, 805, 755, 734, 674, 616.

Synthesis of (7-amino-2-oxo-naphtho[1,2-*b*]pyran-4-yl)methyl butyrate 2a.

The chloromethyl precursor **1** (1 equiv, 0.070 g, 0.27 mmol) was dissolved in dry DMF (3 mL), potassium fluoride (3 equiv, 0.047 g, 0.81 mmol) and butyric acid (1 equiv, 0.024 mL, 0.27 mmol) were added. The reaction mixture was stirred at room temperature for 24 h. The solvent was removed by evaporation under reduced pressure and the crude residue was purified by column chromatography using mixtures of ethyl acetate and *n*-hexane as eluent. Conjugate **2a** was obtained as a yellow solid (0.047 g, 50%). Mp = 190.8-191.8. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.01 (3H, t, *J* 7.4 Hz, $CH_2CH_2CH_3$), 1.73-1.79 (2H, m, $CH_2CH_2CH_3$), 2.48 (2H, t, *J* 7.4 Hz, $CH_2CH_2CH_3$), 4.20 (2H, br s, NH_2), 5.40 (2H, d, *J* 1.5 Hz, OCH_2), 6.58 (1H, t, *J* 1.5 Hz, H3), 6.96 (1H, dd, *J* 7.4 and *J* 0.9 Hz, H8), 7.44 (1H, d, *J* 8.9 Hz, H5), 7.46 (1H, t, *J* 8.2 Hz, H9), 7.69 (1H, dd, *J* 8.9 and 0.9 Hz,

H6), 8.03 (1H, d, *J* 8.2 Hz, H10). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ = 13.68 ($CH_2CH_2\mathit{C}H_3$), 18.39 ($CH_2\mathit{C}H_2CH_3$), 35.97 ($\mathit{C}H_2CH_2CH_3$), 61.27 (OCH_2), 112.31 (C3), 112.39 (C4a), 112.96 (C5), 113.35 (C10), 117.56 (C6), 117.65 (C8), 124.24 (C10a), 124.43 (C6a), 128.01 (C9), 142.19 (C7), 150.02 (C4), 151.28 (C10b), 160.66 (C2), 172.79 (C=O, ester). FTIR (KBr 1%, cm^{-1}): ν = 3442, 3350, 3257, 2960, 2872, 1734, 1714, 1641, 1608, 1564, 1510, 1477, 1436, 1410, 1381, 1355, 1330, 1300, 1254, 1224, 1178, 1162, 1115, 1057, 1028, 980, 957, 935, 860, 798, 759, 750, 718, 678, 554.

Synthesis of (7-(methylamino)-2-oxo-naphtho[1,2-*b*]pyran-4-yl)methyl butyrate 2b.

To a solution of compound **2a** (1 equiv, 0.118 g, 0.38 mmol) in dry DMF (5 mL), methyl iodide (1.3 equiv, 0.031 mL, 0.49 mmol) was added and the reaction mixture was heated at reflux for 4 h. The solvent was evaporated and the crude residue dissolved in ethyl acetate, washed with saturated sodium hydrogencarbonate aqueous solution and water. The organic layer was dried with magnesium sulphate and concentrated by evaporation. The crude residue was purified by column chromatography using mixtures of ethyl acetate and *n*-hexane as eluent, and two products were isolated, **2b** (monomethylated) and **2c** (dimethylated).

Product **2b** was obtained as a yellow solid (0.070 g, 57%). Mp = 172.6-173.3. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.01 (3H, t, *J* 7.2 Hz, $CH_2CH_2C\mathit{H}_3$), 1.72-1.77 (2H, m, $CH_2C\mathit{H}_2CH_3$), 2.46 (2H, t, *J* 7.2 Hz, $C\mathit{H}_2CH_2CH_3$), 3.04 (1H, s, $NHC\mathit{H}_3$), 5.35 (2H, d, *J* 1.3 Hz, OCH_2), 6.52 (1H, t, *J* 1.3 Hz, H3), 6.85 (1H, d, *J* 7.6 Hz, H8), 7.37 (1H, d, *J* 9.0 Hz, H5), 7.52 (1H, t, *J* 8.4 Hz, H9), 7.67 (1H, d, *J* 9.0 Hz, H6), 7.94 (1H, d, *J* 8.4 Hz, H10). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ = 13.66 ($CH_2CH_2\mathit{C}H_3$), 18.35 ($CH_2\mathit{C}H_2CH_3$), 31.38 ($NH\mathit{C}H_3$), 35.92 ($\mathit{C}H_2CH_2CH_3$), 61.27 (OCH_2), 107.97 (C8), 112.10 (C3), 112.32 (C10), 116.74 (C6), 117.51 (C5), 123.96 (C6a), 124.39 (C10a), 128.24 (C9), 143.67 (C7), 149.94 (C4), 151.09 (C10b), 160.61 (C2), 172.74 (C=O, ester). FTIR (KBr 1%, cm^{-1}): ν = 3415, 2962, 2932, 1743, 1712, 1636, 1605, 1568, 1467, 1383, 1290, 1175, 1132, 1090, 1074, 1024, 966, 861, 792, 750, 717.

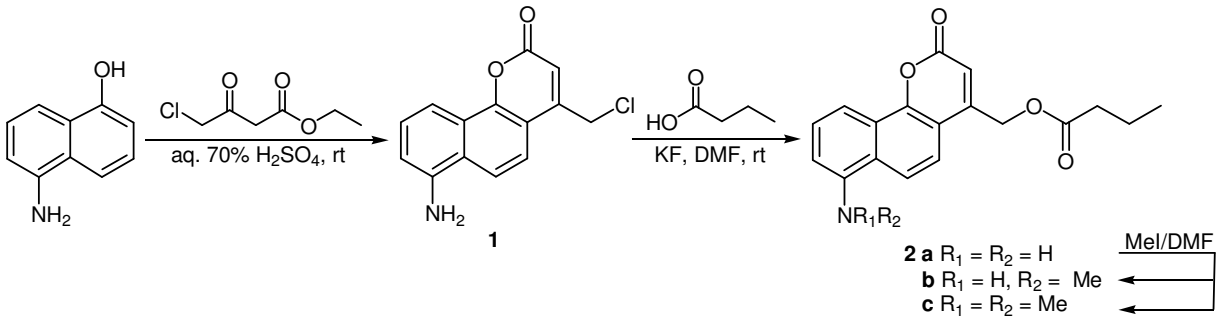
Product **2c**, (7-(dimethylamino)-2-oxo-naphtho[1,2-*b*]pyran-4-yl)methyl butyrate, was obtained as a yellow solid (0.010 g, 8%). Mp = 105.3-106.2 ºC. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.02 (3H, t, *J* 7.2 Hz, $CH_2CH_2C\mathit{H}_3$), 1.73-1.78 (2H, m, $CH_2C\mathit{H}_2CH_3$), 2.48 (2H, t, *J* 7.2 Hz, $C\mathit{H}_2CH_2CH_3$), 2.93 (6H, s, $N(CH_3)_2$), 5.42 (2H, d, *J* 1.6 Hz, OCH_2), 6.58 (1H, t, *J* 1.2 Hz, H3), 7.28 (1H, d, *J* 8.5 Hz, H8), 7.37 (1H, d, *J* 9.0 Hz, H5), 7.52 (1H, t, *J* 8.5 Hz, H9), 7.67 (1H, d, *J* 9.0 Hz, H6), 7.94 (1H, d, *J* 8.5 Hz, H10). FTIR (KBr 1%, cm^{-1}): ν = 3427, 2965, 2934, 2833, 2803, 2786, 1746, 1729, 1634, 1605, 1565, 1502, 1469, 1418, 1402, 1380, 1344, 1317, 1299, 1269, 1258, 1225, 1181, 1128, 1106, 1073, 1045, 1020, 985, 709.

General photolysis procedure

A 1×10^{-4} M methanol/HEPES buffer (80:20) solution of conjugates **2a**-**c** and 1×10^{-4} M acetonitrile/HEPES buffer (80:20) solution of **2b** (5 mL) was placed in a quartz tube and irradiated in a Rayonet RPR-100 reactor at the desired wavelength. The lamps used for irradiation were of 254, 300, 350 and 419 ± 10 nm. HEPES buffer solution was prepared in distilled water with HEPES (4-(2-hydroxyethyl)-1-piperazine ethanesulfonic acid) (10 mM), NaCl (120 mM), KCl (3 mM), $CaCl_2$ (1 mM) and $MgCl_2$ (1mM) and pH adjusted to 7.2. Aliquots of 100 µL were taken at regular intervals and analysed by RP-HPLC. The eluent was acetonitrile/water (3:1) at a flow rate of 0.8 mL/min for all compounds, previously filtered through a Millipore, type HN 0.45 µm filter and degassed by ultra-sound for 30 min. The chromatograms were traced by detecting UV absorption at the wavelength of maximum absorption (retention time: 4.4 min, **2a**; 6.5 min, **2b**; 9.3 min, **2c**).

Results and Discussion

7-Amino-4-(chloromethyl)-naphtho[1,2-*b*]pyran-2-one **1** was obtained by a Pechmann reaction between 5-aminonaphthalen-1-ol and ethyl chloroacetoacetate, catalised by acid. This compound bearing a reactive chloromethyl group was used in the derivatization at the carboxylic acid function of butyric acid in the presence of potassium fluoride. The resulting ester conjugate **2a** was obtained in moderate yield (Scheme 1, Table 1). Compound **2a** was *N*-alkylated with methyl iodide yielding the monoalkylated **2b** and dialkylated **2c** derivatives.



Scheme 1. Synthesis of butyric acid conjugates **2a**-**c**.

UV-visible spectroscopic characterization was carried out to obtain the parameters needed for monitorization during photolysis. Absorption spectra of degassed 10^{-5} M solutions in methanol/HEPES buffer (80:20) solution of conjugates **2a**-**c** were measured (Table 1).

Table 1. Yields, UV/visible absorption and emission data for precursor **1** and conjugates **2a**-**c** in methanol/HEPES buffer (80:20) solution.

Compound	Yield (%)	Absorption		Emission		
		λ_{max} (nm)	log ε	λ_{max} (nm)	ϕ_F	Stokes's shift (nm)
1	60	290	3.92	384	0.08	94
2a	50	293	4.53	340	0.07	47
2b	57	291	4.36	339	0.08	48
2c	8	297	4.26	339	0.16	42

The release of butyric acid from conjugates **2a**-**c** was carried out by photolysis at different wavelengths. Solutions of the mentioned compounds (1×10^{-4} M) in methanol/HEPES buffer (80:20) were irradiated in a Rayonet RPR-100 reactor at 254, 300, 350 and 419 nm, in order to determine the most favourable cleavage conditions. The course of the photocleavage reaction was followed by reverse phase HPLC with UV detection. The determined irradiation time represents the time necessary for the consumption of the starting materials until less than 5% of the initial area was detected (Table 2).

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

Compound	254 nm		300 nm		350 nm		419 nm	
	t_{Irr}	k	t_{Irr}	k	t_{Irr}	k	t_{Irr}	k
2a	69	4.29	73	4.13	215	1.39	288	1.02
2b	6	3.12	25	11.97	68	4.43	259	1.12
2c	41	7.21	93	58.28	186	1.62	1151	0.26

Considering the data in Table 2, *N*-alkylation can be considered advantageous for promoting a faster cleavage as the alkylated derivatives generally displayed shorter irradiation times at all wavelengths of irradiation. Between the mono and dialkyl conjugates, it was found that the monoalkylated derivative **2b** was always the fastest.

Considering the present compounds for practical applications, although they cleaved readily at 254 nm (the fastest being **2b** with 6 min), photolysis at this wavelength can be damaging in biological

media. Therefore, photolysis at 300 nm and longer wavelengths is always preferable. A compromise can be achieved either at 300 (25 min for **2b**) or 350 nm (68 min for **2b**), depending on the media where it will be applied. At 419 nm, although possessing much lower sensitivity to irradiation at this wavelength, the irradiation time is long but still feasible (259 min for **2b**).

For naphtho[1,2-*b*]pyranone conjugate **2b**, photolysis was also attempted in a different solvent in order to see if it was possible to further improve the above result at 300 nm. Instead of a protic solvent like methanol, acetonitrile was used in an acetonitrile/HEPES buffer (80:20) solution and it was found that the irradiation time (27 min) was very similar to the previously reported for the methanol containing mixture.

Furthermore, monitorisation of the photolysis process at 300 nm was also carried out by ^{1}H NMR in a methanol-d_4/D_2O (80:20) solution for all conjugates in a concentration of 9.0×10^{-3} M, which is several times larger than the concentration used in the experiments followed by HPLC, leading to an increase in the photolysis time for the complete release of the model acid (see Figure 1 for **2b** as a representative example). It was observed that the irradiation process lead to progressively decreased of the the signals related to the linked acid, with simultaneous increase of its signals in the released form, as well as signals due to aromatic by-products related to the heterocyclic group.

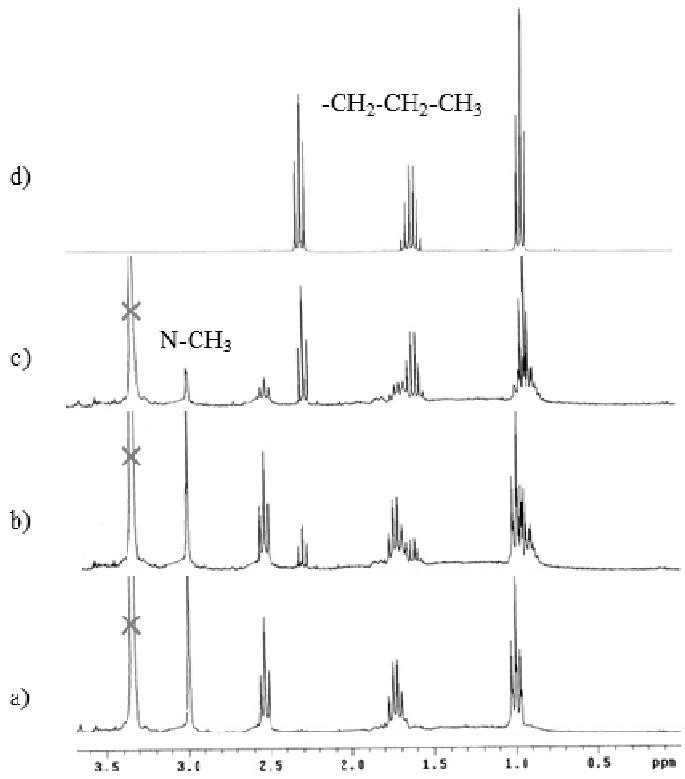


Figure 1. Partial ^{1}H NMR spectra in methanol-d_4/D_2O (80:20) of the photolysis of conjugate **2b** (C = 9.0×10^{-3} M) at 300 nm: a) before irradiation; b) after irradiation for 15 min; c) after irradiation for 90 min; d) butyric acid (free form).

Conclusions

The results of the evaluation of naphtho[1,2-*b*]pyranones as photoactive moieties for the preparation of butyric acid prodrugs revealed that quantitative release of the acid from the corresponding conjugates was possible under irradiation at 254, 300 and 350 nm in short times as well as at 419 nm but with longer values. Overall, this preliminary study suggests the feasibility of using an *N*-alkylated aminonaphtho[1,2-*b*]pyranone as a photocleavable unit for the carboxylic acid function.

Acknowledgements

Thanks are due to the *Fundação para a Ciência e Tecnologia* (FCT, Portugal) for financial support to the NMR portuguese network (PTNMR, Bruker Avance III 400-Univ. Minho), FCT and FEDER (European Fund for Regional Development)-COMPETE-QREN-EU for financial support to the Research Centre CQ/UM [PEst-C/QUI/UI0686/2011 (FCOMP-01-0124-FEDER-022716)]. A PhD grant to A.M.S.S. (SFRH/BD/80813/2011) is also acknowledged.

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