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ORIGINAL ARTICLE

Photolabile protection for amino acids: studies on the release from novel benzoquinolone cages

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Abstract The synthesis of a novel fused nitrogen heterocycle, benzoquinolone, for evaluation as a photocleavable protecting group is described for the first time by coupling to model amino acids (alanine, phenylalanine and glutamic acid). Conversion of the phenylalanine ester conjugate to the thionated derivative was accomplished by reaction with Lawesson's reagent. Photocleavage studies of the carbonyl and thiocarbonyl benzoquinolone conjugates in various solvents and at different wavelengths (300, 350 and 419 nm) showed that the most interesting result was obtained at 419 nm for the thioconjugate, revealing that the presence of the thiocarbonyl group clearly improved the photolysis rates, giving practicable irradiations times for the release of the amino acids (less than 1 min).

Keywords Quinolone · Coumarin · Amino acids · Phototriggers · Photolabile protecting groups

Introduction

There is a strong interest in the design of more efficient protecting groups that allow orthogonal cleavage/deprotection for application in organic synthesis, materials sciences and especially in the case of applications with biomolecules, where a rapid and clean cleavage under irradiation at longer wavelengths is envisaged (Klan et al. 2013; Ahmed and Fruk 2013; Cui et al. 2013). A shift in absorption towards longer wavelengths (higher than 400 nm) is desirable in biochemical and biophysical studies in order to minimise side reactions through absorption of radiation by other chromophores in the molecule under study. For a given light-controlled process that requires spatial and temporal resolution, this can be accomplished by substituent tailoring at the photoactive unit or by careful choice of the photolytic conditions.

Quinolone derivatives were recently reported by us for the first time for the protection of carboxylic acids, in the form of ester conjugates, by using an amino acid as model biomolecule (Fonseca et al. 2010). This nitrogen heterocycle, structurally related to coumarin, a well-known photocleavable protecting group (Hagen et al. 2010; Furuta et al. 2004; Piloto et al. 2011; Soares et al. 2010a, b), assured fast cleavage of the ester bond between the amino acid and the heterocycle at 350 and 419 nm. More recently, replacement of these heterocycles' carbonyl by a thiocarbonyl group resulted in a bathochromic shift of the molecule's absorption wavelength and, consequently, of the photoresponsive properties of the compounds at higher wavelengths, more appropriate for bioapplications (Piloto et al. 2012; Fonseca et al. 2012a, b, c; Fournier et al. 2013). Thiocarbonylated compounds have been reported as amino acid derivatives containing side-chain thioamides for the synthesis of photoactivable peptides, and thiocarbonyls have been used to photo-link peptides to modified RNA molecules (Singh et al. 1997; Renwick et al. 1995). Considering our current research interests which includes the design of new oxygen and nitrogen heterocycles, as well as polycyclic aromatics, and their application as photocleavable protecting groups for the amino and carboxylic functions (Soares et al. 2010a, b, 2012; Piloto et al. 2013, 2015; Fonseca et al. 2012a, b, c) we now report our efforts to further optimise the photolytic

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process at longer wavelengths by designing a polycyclic nitrogen heterocycle in an attempt to accomplish larger shifts of the maximum wavelength of absorption in the UV/Vis, that can influence the outcome of the photocleavage reaction (allowing photolysis at longer wavelengths and in shorter irradiation times). To the best of our knowledge, this is the first time that a benzoquinolone derivative and its thionated analogue are synthesised and tested as photocleavable protecting groups, by irradiation of model amino acid conjugates at 300, 350 and 419 nm in different solvent systems, consisting of mixtures of methanol or acetonitrile with aqueous HEPES buffer in various proportions. Monitoring of the photolysis of the benzoquinolone and thiobenzoquinolone amino acid conjugates was carried out by HPLC–UV and ^{1}H NMR detection and kinetic data was obtained.

Experimental section

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 in KBr discs. UV/Vis absorption spectra (200–700 nm) were obtained using a Shimadzu UV/2501PC spectrophotometer. NMR spectra were obtained on a Varian Unity Plus Spectrometer at an operating frequency of 300 MHz for ^{1}H and 75.4 MHz for ^{13}C or 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 °C. 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. Low- and high-resolution 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. Photolyses were carried out using a Rayonet RPR-100 chamber reactor equipped with 10 lamps of 254 (35 W), 300 (21 W), 350 (24 W) and 419 (14 W) nm. HPLC analyses were performed using a Licrospher 100 RP18 (5 μm) column in a HPLC system composed by a Jasco PU-980 pump, a Shimadzu SPD-GAV UV/Vis detector and a Shimadzu C-RGA Chromatopac register. All commercial reagents were used as received.

Synthesis of 7-methoxy-*N*-methylnaphthalen-2-amine *1* 7-Methoxynaphthalen-2-ol (1 equiv, 0.500 g, 2.87 mmol) was mixed with sodium metabisulfite (2.44 equiv, 1.331 g, 7.00 mmol), sodium hydroxide (5.2 equiv, 0.597 g, 14.9 mmol), water (12 mL) and methylamine hydrochloride (5.2 equiv, 1.010 g, 14.9 mmol). The reaction mixture was stirred, in an oil bath, at 90 °C for 3 days. The reaction mixture was extracted with ethyl acetate (3 × 10 mL) and the organic layer was dried with anhydrous magnesium sulphate, filtered and evaporated under reduced pressure. The resulting crude was purified by silica gel column chromatography using dichloromethane as eluent, yielding compound **1** as a pink solid (0.350 g, 1.87 mmol, 65 %). mp = 86.5–88.0 °C. ^{1}H NMR (DMSO-d_6, 300 MHz): δ_H = 2.74 (s, 3 H, NCH_3), 3.79 (s, 3 H, OCH_3), 5.91 (d, J = 4.8 Hz, 1 H, NH), 6.58 (d, J = 2.1 Hz, 1 H, H-1), 6.71–6.77 (m, 2 H, H-3 and H-6), 6.99 (d, J = 2.4 Hz, 1 H, H-8), 7.48 (d, J = 8.7 Hz, 1 H, H-4), 7.52 (d, J = 9.0 Hz, 1 H, H-5). ^{13}C NMR (DMSO-d_6, 75.4 MHz): δ_C = 30.75 (NCH_3), 54.94 (OCH_3), 101.63 (C-1), 104.36 (C-8), 113.05 (C-6), 115.62 (C-3), 121.74 (C-8a), 128.10 (C-4), 128.93 (C-5), 136.56 (C-4a), 148.07 (C-2), 157.66 (C-7). IR (KBr 1 %, cm^{-1}): ν = 3435, 2996, 2924, 2801, 1631, 1610, 1587, 1554, 1525, 1491, 1468, 1413, 1392, 1332, 1278, 1260, 1240, 1212, 1188, 1171, 1143, 1120, 1066, 1026, 968, 957, 857, 827, 788, 680, 660, 629, 615, 502. UV/Vis (ethanol, nm): λ_{max} (log ε) = 338 (3.03). MS *m/z* (ESI, %): 188 ([M$^+$+H], 100). HRMS: *m/z* (ESI) calc. for $C_{12}H_{14}NO$ 188.10699, found: 188.10723.

Synthesis of 9-methoxy-1,4-dimethylbenzo[*f*]quinolin-3(4*H*)-one *2* Ethyl 3-oxobutanoate (2 equiv, 0.27 mL, 2.14 mmol) was heated at 180 °C and compound **1** (1 equiv, 0.200 g, 1.07 mmol) was added. The reaction mixture was refluxed for 45 min. After evaporation of the excess ethyl 3-oxobutanoate under vacuum, the residue was stirred with aqueous 70 % sulphuric acid (5 mL) at 95 °C for 45 min. After cooling to room temperature, water (10 mL) was added to the mixture, followed by extraction with ethyl acetate (3 × 15 mL). The organic layer was dried with anhydrous magnesium sulphate, filtered and evaporated under reduced pressure. The resulting solid was purified by silica gel column chromatography using dichloromethane/methanol (100:1). Fractions containing the product were combined and evaporated yielding compound **2** as a pink solid (0.179 g, 0.71 mmol, 66 %). mp = 164.1–165.3 °C. ^{1}H NMR ($CDCl_3$, 400 MHz): δ_H = 2.99 (s, 3 H, CH_3), 3.89 (s, 3 H, NCH_3), 3.99 (s, 3 H, OCH_3), 6.79 (s, 1 H, H-2), 7.21 (dd, J = 8.8 and 2.4 Hz, 1H, H-8), 7.49 (d, J = 9.2 Hz, 1 H, H-5), 7.84 (d, J = 8.8 Hz, 1 H, H-7), 7.94 (d, J = 9.2 Hz, 1 H, H-6), 8.04 (d, J = 2.0 Hz, 1 H, H-10). ^{13}C NMR ($CDCl_3$, 100.6 MHz): δ_C = 26.78 (CH_3),

30.60 (NCH_3), 55.41 (OCH_3), 106.95 (C-10), 112.81 (C-5), 115.71 (C-8), 115.81 (C-10b), 122.27 (C-2), 125.20 (C-10a), 130.58 (C-7), 132.14 (C-6), 132.45 (C-6a), 140.78 (C-4a), 147.81 (C-1), 158.78 (C-9), 161.56 (C-3). IR (KBr 1 %, cm^{-1}): ν = 2957, 2929, 1735, 1651, 1624, 1576, 1547, 1519, 1455, 1432, 1414, 1367, 1322, 1232, 1170, 1096, 1042, 911, 861, 832, 797, 720, 691, 666, 588, 501. UV/Vis (ethanol, nm): λ_{max} (log ε) = 334 (3.42). MS *m/z* (ESI, %): 254 ([M^++H], 100). HRMS: *m/z* (ESI) calc. for $C_{16}H_{16}NO_2$ 254.11756, found 254.11728.

Synthesis of 1-formyl-9-methoxy-4-methylbenzo[*f*]quinolin-3(4*H*)-one *3* Compound **2** (1 equiv, 0.120 g, 0.47 mmol) was dissolved in chlorobenzene (30 mL) and selenium dioxide (4 equiv, 0.210 g, 1.89 mmol) was added. The reaction mixture was heated at reflux for 2 days. The mixture was filtered hot and the solvent was removed by rotary evaporation. The crude residue was purified by silica gel column chromatography using dichloromethane/methanol (98:2). Fractions containing the product were combined and evaporated, yielding compound **3** as a yellow solid (0.100 g, 0.37 mmol, 79 %). mp = 200.1–201.8 °C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ_H = 3.89 (s, 3 H, NCH_3), 3.94 (s, 3 H, OCH_3), 7.01 (s, 1 H, H-2), 7.12 (d, J = 2.1 Hz, 1 H, H-10), 7.23 (dd, J = 9.0 and 2.4 Hz, 1 H, H-8), 7.49 (d, J = 9.3 Hz, 1 H, H-5), 7.87 (d, J = 9.0 Hz, 1 H, H-7), 8.02 (d, J = 9.0 Hz, 1 H, H-6), 10.55 (s, 1 H, CHO). ^{13}C NMR ($CDCl_3$, 75.4 MHz): δ_C = 30.63 (NCH_3), 55.53 (OCH_3), 105.82 (C-10), 111.99 (C-10b), 112.35 (C-5), 117.4 (C-8), 122.64 (C-2), 124.72 (C-10a), 130.07 (C-6a), 130.71 (C-7), 132.91 (C-6), 141.31 (C-4a), 145.21 (C-1), 159.78 (C-9), 161.49 (C-3), 192.08 (CHO). IR (KBr 1 %, cm^{-1}): ν = 3027, 2976, 1682, 1658, 1622, 1576, 1551, 1513, 1473, 1460, 1413, 1378, 1366, 1303, 1261, 1227, 1171, 1132, 1088, 1028, 1016, 916, 883, 838, 827, 797, 771, 743, 679, 665, 611, 600, 504. UV/Vis (ethanol, nm): λ_{max} (log ε) = 353 (3.89). MS *m/z* (ESI, %): 268 ([M^++H], 100). HRMS: *m/z* (ESI) calc. for $C_{16}H_{14}NO_3$ 268.09682, found: 268.09653.

Synthesis of 1-hydroxymethyl-9-methoxy-4-methylbenzo[*f*]quinolin-3(4*H*)-one *4* Compound **3** (1 equiv, 0.090 g, 0.34 mmol) was dissolved in ethanol (10 mL) and sodium borohydride (0.7 equiv, 0.010 g, 0.24 mmol) was added. The reaction mixture was stirred for 6 days at room temperature. The mixture was filtered and the solvent was removed under reduced pressure. Compound **4** was obtained as yellow solid (0.081 g, 0.30 mmol, 90 %). mp = 213.2–215.0 °C. ^{1}H NMR (DMSO-d_6, 400 MHz): δ_H = 3.74 (s, 3 H, NCH_3), 3.92 (s, 3 H, OCH_3), 5.02 (s, 2 H, CH_2), 6.90 (s, 1 H, H-2), 7.20 (dd, J = 8.8 and 2.4 Hz, 1 H, H-8), 7.62 (d, J = 9.2 Hz, 1 H, H-5), 7.92 (d, J = 8.8 Hz,1 H, H-7), 7.96 (d, J = 2.0 Hz, 1 H, H-10), 8.05 (d, J = 9.2 Hz, 1 H, H-6). ^{13}C NMR (DMSO-d_6, 100.6 MHz): δ_C = 30.13 (NCH_3), 55.27 (OCH_3), 63.33 (CH_2), 106.92 (C-10), 113.45 (C-5), 113.83 (C-10b), 115.96 (C-8), 119.31 (C-2), 124.62 (C-6a), 130.46 (C-7), 131.12 (C-10a), 131.98 (C-6), 140.75 (C-4a), 150.74 (C-1), 158.75 (C-9), 160.78 (C-3). IR (KBr 1 %, cm^{-1}): ν = 3303, 2951, 1641, 1611, 1561, 1549, 1520, 1476, 1453, 1431, 1411, 1366, 1312, 1283, 1261, 1230, 1198, 1168, 1132, 1102, 1067, 1033, 1004, 946, 919, 865, 832, 824, 801, 776, 735, 692, 666, 602, 574, 510. UV/Vis (ethanol, nm): λ_{max} (log ε) = 337 (3.90). MS *m/z* (ESI, %): 270 ([M^++H], 100). HRMS: *m/z* (ESI) calc. for $C_{16}H_{16}NO_3$ 270.11247, found: 270.11220.

Synthesis of 1-chloromethyl-9-methoxy-4-methylbenzo[*f*]quinolin-3(4*H*)-one *5* Compound **4** (1 equiv, 0.060 g, 0.22 mmol) was dissolved in dichloromethane (10 mL) and thionyl chloride (20 equiv, 0.32 mL, 4.4 mmol) was added. The reaction mixture was stirred for 1 day at room temperature. After removal of the solvent under reduced pressure, compound **5** was obtained as beige solid (0.061 g, 0.21 mmol, 95 %). mp = 154.0–156.0 °C. ^{1}H NMR (MeOH-d_4, 400 MHz): δ_H = 3.91 (s, 3 H, NCH_3), 4.04 (s, 3 H, OCH_3), 5.20 (s, 2 H, CH_2), 6.96 (s, 1 H, H-2), 7.26 (dd, J = 9.2 and 2.0 Hz, 1 H, H-8), 7.68 (d, J = 9.2 Hz, 1H, H-5), 7.92 (d, J = 9.2 Hz,1 H, H-7), 8.04 (d, J = 2.0 Hz, 1 H, H-10), 8.09 (d, J = 9.2 Hz, 1 H, H-6). ^{13}C NMR (MeOH-d_4, 100.6 MHz): δ_C = 31.4 (NCH_3), 47.74 (CH_2), 56.21 (OCH_3), 107.43 (C-10), 114.11 (C-5), 115.38 (C-10b), 118.13 (C-8), 123.47 (C-2), 126.79 (C-6a), 131.85 (C-7), 132.11 (C-10a), 134.30 (C-6), 142.62 (C-4a), 148.53 (C-1), 161.03 (C-9), 163.48 (C-3). IR (liquid film, cm^{-1}): ν = 3055, 2917, 2849, 1728, 1707, 1652, 1623, 1575, 1548, 1519, 1463, 1432, 1417, 1367, 1267, 1232, 1172, 1133, 1117, 1086, 1033, 996, 832, 708, 665. UV/Vis (ethanol, nm): λ_{max} (log ε) = 342 (3.78). MS *m/z* (ESI, %): 290 ([M^++H] ^{37}Cl, 32), 288 ([M^++H] ^{35}Cl, 100). HRMS: *m/z* (ESI) calc. for $C_{16}H_{15}NO_2$ ^{37}Cl 290.07563, found: 290.07538; calc. for $C_{16}H_{15}NO_2$ ^{35}Cl 288.07858, found: 288.07832.

General procedure for the synthesis of amino acid-benzoquinolone conjugates 6a–c

Compound **5** was dissolved in dry *N,N*-dimethylformamide (1 mL/mmol) and the *N*-benzyloxycarbonyl-protected amino acid (1 equiv) and potassium fluoride (3 equiv) were added to the mixture. The reaction mixture was stirred at room temperature for 2 days. The mixture was filtered and the solvent was removed by rotary evaporation. The obtained crude solid was purified by silica gel column chromatography using dichloromethane/methanol (100:1) as eluent.

N*-Benzyloxycarbonyl-L-alanine (9-methoxy-4-methylbenzo[*f*]quinolin-3(4*H*)-one-1-yl) methyl ester *6a Starting from compound **5** (0.025 g, 0.09 mmol) and *N*-benzyloxycarbonyl-L-alanine (0.020 g, 0.09 mmol), after chromatography compound **6a** was obtained as a brown solid (0.015 g, 0.03 mmol, 35 %). mp = 157.5–158.5 °C. ^{1}H NMR ($CDCl_3$, 400 MHz): δ_H = 1.47 (d, J = 7.2 Hz, 3 H, CH_3), 3.85 (s, 3 H, NCH_3), 3.95 (s, 3 H, OCH_3), 4.50–4.54 (m, 1 H, αH), 5.08-5.15 (m, 2 H, CH_2 Z), 5.39 (d, J = 7.6 Hz, 1 H, NH), 5.66-5.78 (m, 2 H, CH_2), 6.98 (s, 1 H, H-2), 7.19 (dd, J = 2.4 and 11.2 Hz, 1 H, H-8), 7.31–7.35 (m, 5 H, 5 × Ph-*H* Z), 7.45 (d, J = 9.2 Hz, 1 H, H-5), 7.48 (s, 1 H, H-10), 7.82 (d, J = 8.8 Hz, 1 H, H7), 7.93 (d, J 9.2 Hz, 1 H, H-6) ppm. ^{13}C NMR ($CDCl_3$, 100.6 MHz): δ_C = 18.37 (CH_3), 30.60 (NCH_3), 49.78 (αC), 55.45 (OCH_3), 65.93 (CH_2), 67.03 (CH_2 Z), 106.07 (C-10), 112.68 (C-5), 113.87 (C-10b) 116.20 (C-8), 120.11 (C-2), 125.01 (C-6a), 128.10 (C-2′), 128.14 (C-6′), 128.47 (C-3′, C-4′ and C-5′), 130.77 (C-7), 131.15 (C-10a), 132.42 (C-6), 136.13 (C-1′), 141.06 (C-4a), 144.04 (C-1), 155.02 (C=O urethane), 159.35 (C-9), 161.39 (C-3), 172.50 (C=O ester). IR (KBr 1 %, cm^{-1}): ν = 3296, 3062, 3030, 2935, 1720, 1652, 1624, 1576, 1576, 1547, 1519, 1499, 1455, 1432, 1416, 1367, 1346, 1261, 1232, 1174, 1134, 1083, 1043, 911, 866, 833, 733, 698, 665, 512. MS *m/z* (ESI, %): 551 ([M^++H], 100). HRMS: *m/z* (ESI) calc. for $C_{27}H_{27}N_2O_6$ 475.18701, found: 475.18748.

N*-Benzyloxycarbonyl-L-phenylalanine (9-methoxy-4-methylbenzo[*f*]quinolin-3(4*H*)-one-1-yl) methyl ester *6b Starting from compound **5** (0.166 g, 0.35 mmol), and *N*-benzyloxycarbonyl-L-phenylalanine (0.104 g, 0.35 mmol), after chromatography compound **6b** was obtained as beige solid (0.115 g, 0.21 mmol, 60 %). mp = 86.5–88.0 °C. ^{1}H NMR ($CDCl_3$, 400 MHz): δ_H = 3.09–3.20 (m, 2 H, βCH$_2$), 3.86 (s, 3 H, NCH_3), 3.93 (s, 3 H, OCH_3), 4.75–4.80 (m, 1 H, α-CH), 5.05–5.12 (m, 2 H, CH_2 Z), 5.31 (d, J = 8.4 Hz, 1 H, NH), 5.66 (s, 2 H, CH_2), 6.91 (s, 1 H, H-2), 7.10 (dd, J = 8.0 and 1.6 Hz, 2 H, H-2′ and H-6′), 7.18-7.22 (m, 4 H, H-8 and 3 × Ph-*H* Phe), 7.27–7.32 (m, 5 H, 5 × Ph-*H* Z), 7.45 (d, J = 9.2 Hz 1H, H-5), 7.48 (s, 1 H, H-10), 7.82 (d, J = 8.8 Hz, 1 H, H-7), 7.93 (d, J = 9.2 Hz, 1 H, H-6). ^{13}C NMR ($CDCl_3$, 100.6 MHz): δ_C = 30.58 (NCH_3), 38.17 (βCH$_2$), 55.05 (αC), 55.40 (OCH_3), 66.05 (CH_2), 67.07 (CH_2 Z), 105.90 (C-10), 112.63 (C-5), 113.90 (C-10b), 116.31 (C-8), 120.85 (C-2), 124.89 (C-6a), 127.23 (C-4′), 128.08 (C-2″), 128.14 (C-6″), 128.46 (C-3″, C-4″ and C-5″), 128.67 (C-3′ and C-5′), 129.11 (C-2′ and C-6′), 130.70 (C-7), 131.13 (C-10a), 132.39 (C-6), 135.26 (C-1′), 136.08 (C-1′'), 141.06 (C-4a), 143.50 (C-1), 155.63 (C = O urethane), 159.35 (C-9), 161.30 (C-3), 171.23 (C = O ester). IR (KBr 1 %, cm^{-1}): ν = 3295, 3062, 3030, 2935, 1719, 1651, 1623, 1576, 1548, 1519, 1498, 1455, 1432, 1417, 1367, 1346, 1261, 1232, 1175, 1134, 1083, 1043, 911, 867, 832, 732, 698, 666, 513. MS *m/z* (ESI, %): 551 ([M^++H], 100). HRMS: *m/z* (ESI) calc. for $C_{33}H_{31}N_2O_6$ 551.21766, found 551.21663.

1-((9-Methoxy-4-methylbenzo[*f*]quinolin-3(4*H*)-one-1-yl))methyl 5-methyl 2-[(benzyloxycarbonyl)amino]-pentanedioate *6c* Starting from compound **5** (0.025 g, 0.09 mmol) and *N*-benzyloxycarbonyl-L-glutamic acid methyl ester (0.027 g, 0.09 mmol), after chromatography compound **6c** was obtained as a brown solid (0.006 g, 0.01 mmol, 13 %). mp = 162.4–163.7 °C. ^{1}H NMR ($CDCl_3$, 400 MHz): δ_H = 2.00–2.09 (m, 2 H, β-CH$_2$), 2.38–2.49 (m, 2 H, γ-CH$_2$), 3.64 (s, 3 H, ester CH_3), 3.87 (s, 3 H, NCH_3), 3.96 (s, 3 H, OCH_3), 4.52–4.57 (m, 1 H, α-H), 5.08–5.15 (m, 2 H, CH_2 Z), 5.50 (d, J = 7.6 Hz, 1 H, NH), 5.75 (s, 2 H, CH_2), 7.00 (s, 1 H, H-2), 7.20 (dd, J = 2.4 and 8.8 Hz, 1 H, H-8), 7.31–7.34 (m, 5 H, 5 × Ph-*H* Z), 7.47 (d, J = 9.2 Hz, 1 H, H-5), 7.50 (s, 1 H, H-10), 7.83 (d, J = 8.8 Hz, 1 H, H-7), 7.94 (d, J = 9.2 Hz, 1 H, H-6). ^{13}C NMR ($CDCl_3$, 100.6 MHz): δ_C = 27.25 (βCH$_2$), 29.93 (γCH$_2$), 30.64 (NCH_3), 51.85 (ester CH_3), 53.63 (α-C), 55.47 (OCH_3), 66.19 (CH_2), 67.18 (CH_2 Z), 106.05 (C-10), 112.69 (C-5), 113.91 (C-10b) 116.25 (C-8), 120.41 (C-2), 125.03 (C-6a), 128.13 (C-2′), 128.19 (C-6′), 128.50 (C-3′, C-4′ and C-5′), 130.79 (C-7), 131.17 (C-10a), 132.47 (C-6), 136.06 (C-1′), 141.12 (C-4a), 143.84 (C-1), 155.95 (C = O urethane), 159.40 (C-9), 161.38 (C-3), 171.46 (C=O ester), 173.00 (C=O methyl ester). IR (KBr 1 %, cm^{-1}): ν = 3330, 3043, 2935, 2851, 1749, 1721, 1651, 1623, 1576, 1547, 1520, 1497, 1456, 1432, 1415, 1367, 1346, 1260, 1233, 1176, 1133, 1085, 1083, 1044, 910, 867, 834, 733, 698, 666, 513. MS *m/z* (ESI, %): 551 ([M^++H], 100). HRMS: *m/z* (ESI) calc. for $C_{30}H_{32}N_2O_8$ 548.21596, found 548.21652.

Synthesis of *N*-benzyloxycarbonyl-L-phenylalanine (9-methoxy-4-methylbenzo[*f*]quinolin-3(4*H*)-thione-1-yl) methyl ester *7* Compound **6b** (1 equiv, 0.043 g, 0.08 mmol) was reacted with Lawesson's reagent (0.8 equiv, 0.025 g, 0.06 mmol) in dry toluene (10 mL) by heating at reflux for 2 days. The mixture was filtered hot and the solvent was removed under reduced pressure in a rotary evaporator. The solid crude was purified by column chromatography using chloroform/methanol (100:1) as eluent, yielding **7** as a light yellow solid (0.030 g, 0.04 mmol, 56 %). mp = 90.2–92.0 °C. ^{1}H NMR ($CDCl_3$, 400 MHz): δ_H = 3.10–3.18 (m, 2 H, β-CH$_2$), 3.94 (s, 3 H, OCH_3), 4.46 (s, 3 H, NCH_3), 4.74–4.79 (m, 1 H, α-H), 5.08–5.09

(m, 2 H, CH_2 Z), 5.28 (d, J = 6.0 Hz, 1 H, NH), 5.62 (s, 2 H, CH_2), 7.10 (dd, J = 8.4 and 1.6 Hz, 2 H, H-2′ and H-6′), 7.15-7.32 (m, 9H, H-8, 3 × Ph-*H* Phe and 5 × Ph-*H* Z), 7.56 (s, 1 H, H-10), 7.61 (d, J = 9.2 Hz, 1 H, H-5), 7.85 (d, J = 8.8 Hz, 1 H, H-7), 7.92 (s, 1 H, H-2), 7.98 (d, J = 9.2 Hz, 1 H, H-6). ^{13}C NMR ($CDCl_3$, 100.6 MHz): δ_C = 29.66 (β-CH_2), 31.46 (NCH_3), 55.02 (α-C), 55.50 (OCH_3), 65.84 (CH_2), 67.09 (CH_2 Z), 106.69 (C-10), 113.28 (C-5), 117.49 (C-8), 118.85 (C-10b), 125.41 (C-6a), 127.25 (C-4′), 128.08 (C-2″), 128.15 (C-6″), 128.46 (C-3″, C-4″ and C-5″), 128.71 (C-3′ and C-5′), 129.11 (C-2′ and C-6′), 130.72 (C-7), 130.74 (C-10a), 133.05 (C-6), 134.26 (C-2), 135.18 (C-1′), 136.01 (C-1″), 136.06 (C-1), 142.45 (C-4a), 155.61 (C=O urethane), 159.67 (C-9), 171.23 (C=O ester) 181.68 (C-3). IR (KBr 1 %, cm^{-1}): ν = 3401, 2929, 1719, 1650, 1621, 1592, 1542, 1521, 1456, 1364, 1338, 1305, 1229, 1185, 1104, 1075, 1049, 834, 746, 700, 665, 506. MS *m/z* (ESI, %): 567 ([M^++H], 100). HRMS: *m/z* (ESI) calc. for $C_{33}H_{31}N_2SO_5$ 567.19482, found 567.19446.

General photolysis procedure for amino acid conjugates 6, 7, 8 and 9

A 1 × 10^{-4} M methanol or acetonitrile/HEPES buffer (80:20 or 60:40) solution of amino acid conjugates **6–9** (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 300, 350 and 419 ± 10 nm. HEPES buffer solution was prepared in distiled 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 (1 mM), and the pH was adjusted to 7.2 with 1 M sodium hydroxide.

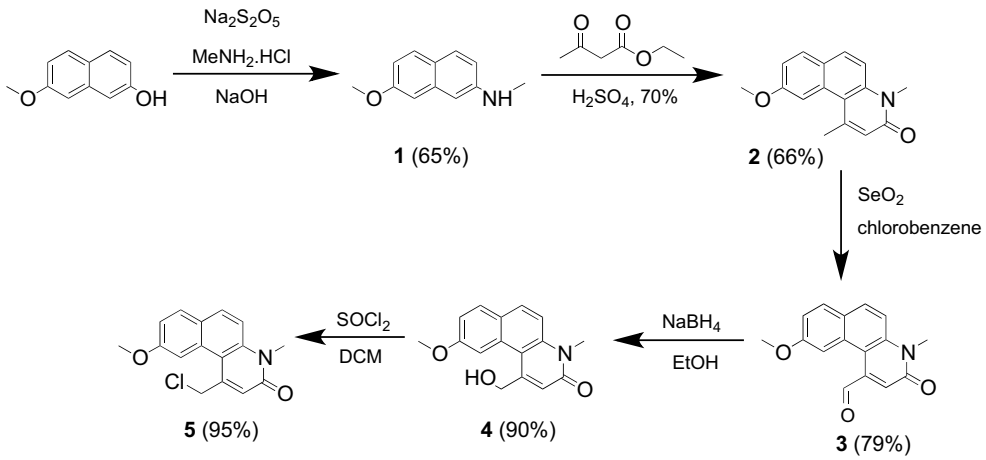
Aliquots of 100 µL were taken at regular intervals and analysed by RP-HPLC. The eluent was acetonitrile/water (3:1), previously filtered through a Millipore, type HN 0.45 µm filter and degassed by ultra-sound for 30 min. The chromatograms were traced with flow rate of 0.8 or 1.0 mL/min by detection at the wavelength of maximum absorption for each conjugate (retention time: **6a**, 5.5; **6b**, 6.8; **6c**, 5.1; **7**, 7.2; **8**, 7.7; **9**, 7.7 min).

Results and discussion

Synthesis of amino acids conjugates 6 and 7

7-Methoxy-*N*-methylnaphthalen-2-amine **1**, was obtained from 7-methoxynaphthalen-2-ol by reductive treatment with methylamine (Kim et al. 2007). Through a modified Knorr synthesis (Uray et al. 1999) between **1** and ethyl 3-oxobutanoate, in acidic media, 9-methoxy-1,4-dimethylbenzo[*f*]quinolin-3(4*H*)-one **2** was obtained. Its methyl group at position 1 was oxidised with selenium dioxide to the aldehyde **3**, which then underwent reduction to the hydroxymethyl derivative **4** by treatment with sodium borohydride. Conversion to the chloromethylated derivative, by treatment with thionyl chloride, yielded the desired 1-chloromethyl-9-methoxy-4-methylbenzo[*f*]quinolin-3(4*H*)-one **5** (Scheme 1; Table 1).

Compound **5** was used in the preparation of model amino acid ester conjugates **6a-c** (with alanine, phenylalanine and glutamic acid, respectively), by coupling to the carboxylic acid function of the *N*-benzyloxycarbonyl protected residues in *N,N*-dimethylformamide, at room temperature,



Scheme 1 Synthesis of functionalised benzoquinolone **5**

in the presence of potassium fluoride (Scheme 2; Table 1) (Tjoeng and Heavner 1981).

The synthesis of the thionated derivative **7** was accomplished by reaction of **6b** with Lawesson's reagent (LR), by refluxing in dry toluene (Jesberger et al. 2003). The desired thiobenzoquinolone was obtained in fair yield, after purification by silica gel column chromatography. All new compounds were fully characterised by the usual spectroscopic techniques. Benzoquinolone derivative **7** was prepared to allow comparison with other structure-related benzocoumarin and quinolone derivatives previously prepared by us (Fonseca et al. 2010; Piloto et al. 2012; Fonseca et al. 2012a, b, c). In the NMR spectra of compounds **6a–c** and **7**, the main differences resided in the chemical shift of H-2, close to the carbonyl/thiocarbonyl group, which appeared between δ 6.91 and 7.00, and at 7.91 ppm, respectively, and of the carbonyl/thiocarbonyl group C-3 which was visible between δ 161.30 and 161.39, and at 181.68 ppm, respectively.

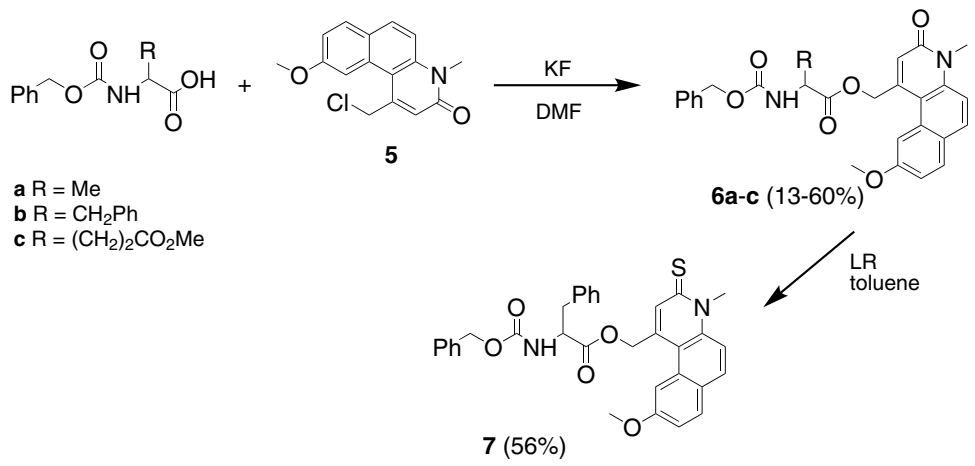
The photophysical properties (absorption and emission maxima, molar absorption coefficients and relative fluorescence quantum yields, ϕ_F) of the newly synthesised benzoquinolones **2–7** were evaluated by tracing the UV/Vis absorption and emission spectra of degassed 10^{-5} M solutions in absolute ethanol (Table 1). Relative fluorescence quantum yields were calculated using 9,10-diphenylanthracene as standard ($\phi_F = 0.95$ in ethanol) (Morris et al. 1976) for all compounds, except for thiobenzoquinolone **7** which required a 0.05 M solution of quinine in sulphuric acid as standard ($\phi_F = 0.546$) (Montalti et al. 2006). For the ϕ_F determination, the fluorescence standard was excited at the wavelengths of maximum absorption found for each of the compounds to be tested and in all fluorimetric measurements, the absorbance of the solution did not exceed 0.1.

By comparison of the data of quinolone derivatives **2–7**, it was found that the fluorescence quantum yield was influenced by the nature of the group at position 1: compounds with electron donor groups (**2**, **4**, **6** and **7**) displayed higher quantum yields than compounds bearing a formyl or chloromethyl group (**3** and **5**) as expected. Upon thionation, the fluorescence quantum yield decreased dramatically, with compound **7** being non-fluorescent, most likely due to the heavy atom-induced spin–orbit coupling by the sulphur that gives rise to an effective intersystem crossing mechanism that lowers the fluorescence emission (Seixas de Melo et al. 2003). This derivative also had the largest Stokes' shift of all compounds, which is an advantageous feature for fluorescence-based techniques. The nature of the amino acid residue did not alter the properties of the corresponding conjugates, as the wavelengths of maximum absorption and emission were the same with a very slight difference in the fluorescence quantum yields.

As for the maximum wavelengths of absorption and emission, a 68 and 115 nm bathochromic shift occurred after conversion of the carbonyl (**6b**) to a thiocarbonyl group (**7**), thus confirming the benefits of such change for shifting absorption and emission to longer wavelengths, bearing in mind possible biological applications of this

Table 1 UV/Vis absorption and fluorescence emission data for benzoquinolone derivatives **2–7** in absolute ethanol

Compound	Absorption		Fluorescence		
	λ_{max} (nm)	log ε	λ_{em} (nm)	ϕ_F	Stokes' shift (nm)
2	334	3.42	406	0.244	72
3	353	3.89	415	0.078	62
4	337	3.90	410	0.337	73
5	342	3.78	409	0.069	67
6a	340	3.42	417	0.186	77
6b	340	3.97	417	0.157	77
6c	340	3.49	417	0.177	77
7	408	3.83	532	0.004	124



a R = Me
b R = CH_2Ph
c R = $(CH_2)_2CO_2Me$

Scheme 2 Synthesis of amino acid-benzoquinolone conjugates **6a–c** and the thionated analogue **7**

Table 2 Irradiation times (t_{irr}, in min) and photochemical quantum yields (Φ_{Phot}) for the photolysis of amino acid conjugates **6**–**11** at different wavelengths and in different solvents: A- MeOH/HEPES (80:20); B- MeOH/HEPES (60:40); C- ACN/HEPES (80:20)

Compound	Eluent	300 nm		350 nm		419 nm	
		t_{irr}	Φ_{Phot}	t_{irr}	Φ_{Phot}	t_{irr}	Φ_{Phot}
6a	A	9.6	2.2×10^{-3}	10.4	1.9×10^{-3}	1181	1.1×10^{-3}
6a	B	9.1	1.6×10^{-3}	8.2	1.7×10^{-3}	505	1.7×10^{-3}
6b	A	17	4.9×10^{-4}	12	6.2×10^{-4}	877	2.3×10^{-4}
6b	B	6.1	1.0×10^{-3}	5.7	9.3×10^{-4}	503	3.1×10^{-4}
6b	C	44	1.6×10^{-4}	43	1.6×10^{-4}	2996	6.5×10^{-5}
6c	A	14.8	5.9×10^{-4}	11.5	7.0×10^{-4}	1038	4.7×10^{-4}
6c	B	7.2	2.2×10^{-3}	7.0	2.1×10^{-3}	436	1.9×10^{-3}
7	A	1.2	2.6×10^{-3}	1.1	7.5×10^{-3}	1.1	4.8×10^{-3}
7	B	0.4	1.3×10^{-2}	0.4	3.2×10^{-2}	0.3	3.2×10^{-2}
8[a]	A	34	8.5×10^{-4}	38	4.4×10^{-4}	301	6.6×10^{-4}
8	C	36	2.9×10^{-4}	31	1.2×10^{-4}	677	2.7×10^{-4}
9[a]	A	100	1.0×10^{-4}	354	7.1×10^{-5}	52	8.8×10^{-5}
9	C	64	7.8×10^{-5}	108	6.7×10^{-5}	48	7.3×10^{-5}
10[b]	A	1.2	5.5×10^{-2}	0.6	3.5×10^{-2}	34	1.6×10^{-1}
11[c]	A	0.7	8.7×10^{-3}	0.5	3.8×10^{-2}	0.4	2.1×10^{-2}

[a] Data previously reported (Fonseca et al. 2012a, b, c)

[b] Data previously reported (Fonseca et al. 2010)

[c] Data previously reported (Piloto et al. 2012)

type of compounds. Absorption with high molar absorption coefficients at longer wavelengths could enable more efficient photolysis at higher wavelengths with shorter irradiation times. Also, due to the negligible fluorescence of **7**, competition between bond scission and radiative and non-radiative relaxation after absorption could be minimised.

The evaluation of benzoquinolone and the thionated analogue as photocleavable protecting groups for amino acid models was carried out by photolysis of the corresponding ester conjugates **6a–c** and **7** under irradiation at different wavelengths (300, 350 and 419 nm). Solutions of the mentioned compounds (1×10^{-4} M) in various solvent systems, with mixtures of organic solvents (acetonitrile or methanol) of different character (aprotic/protic) and HEPES buffer, in varying proportion were irradiated in a Rayonet RPR-100 reactor 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 peak area (A) was detected. 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, with high correlation coefficients (Table 2). The photochemical quantum yields (Φ_{Phot}) were calculated based on half-lives ($t_{1/2}$), molar absorption coefficients at the irradiation wavelength (ε) and the incident photon flux (I_0), which was determined by potassium ferrioxalate actinometry (Muller et al. 2001). The photocleavage process was not as efficient as desirable (see Φ_{Phot} in Table 2), possibly by deactivation via fluorescence pathways that compete with the photochemical reaction.

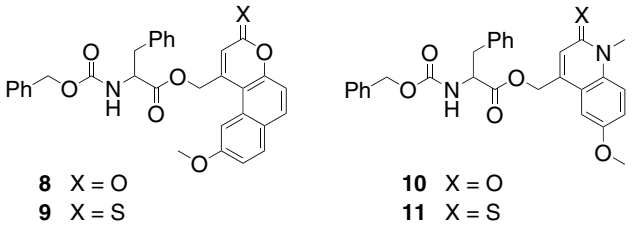


Fig. 1 Phenylalanine benzocoumarin **8**,**9** and quinolone **10**,**11** conjugates

The behaviour towards irradiation of the above-mentioned compounds was compared to a closely related benzocoumarin conjugate and its thionated derivative **8** and **9**, respectively, as well as to the parent quinolone conjugate and its thionated derivative **10** and **11**, respectively (Fig. 1), and newly obtained photolysis data as well as some previously reported data (Fonseca et al. 2010; Piloto et al. 2012; Fonseca et al. 2012a, b, c) are presented in Table 2 for easier comparison.

For phenylalanine-benzoquinolone conjugate **6b**, three different solvent systems were tested, namely methanol/HEPES (80:20), methanol/HEPES (60:40) and acetonitrile/

HEPES (80:20), whereas for the other benzoquinolone conjugates **6a** and **6c** the methanol/HEPES mixtures were used. It was found that the mixtures containing the protic solvent were beneficial for the cleavage and that higher water content also had a positive influence in the cleavage rates. Such behaviour might be explained by the nature of the photocleavage mechanism that could resemble that of the coumarin system, involving an ionic pair formed by homolytical (followed by electron transfer) or heterolytical cleavage of the ester O-CH_2 bond (Yamaji et al. 2009; Schmidt et al. 2007). In fact, this mechanism is in agreement with our earlier findings for quinolone and thioquinolone conjugates as the photolysis was also followed by ^{1}H NMR by irradiation at 350 and 419 nm (Piloto et al. 2012). The release of *N*-benzyloxycarbonylphenylalanine was confirmed as well as the formation of the heterocyclic by-products (in their hydroxymethylated or methyloxymethylated forms).

In the present work, efficient cleavage was seen by irradiation at 300 and 350 nm, whereas at 419 nm, the irradiation times were much longer and not useful for practical applications. Comparison of the data obtained for conjugates **6a–c** in the different solvents revealed that the nature of the residue did not influence significantly the irradiation times.

Comparison of the results obtained for **6b** with those of similar benzocoumarin conjugate **8** revealed opposite trends for different solvents: in methanol/HEPES buffer (80:20), **6b** cleaved more efficiently at 300 and 350 nm and slower at 419 nm; in acetonitrile/HEPES buffer (80:20), **6b** cleaved slower at all wavelengths. Comparison of the results for **6b** with those of the parent quinolone conjugate **10** in methanol/HEPES buffer (80:20) showed a better performance for the quinolone.

With regard to the thionated analogue **7**, once again the use of a methanol mixture with high water content afforded

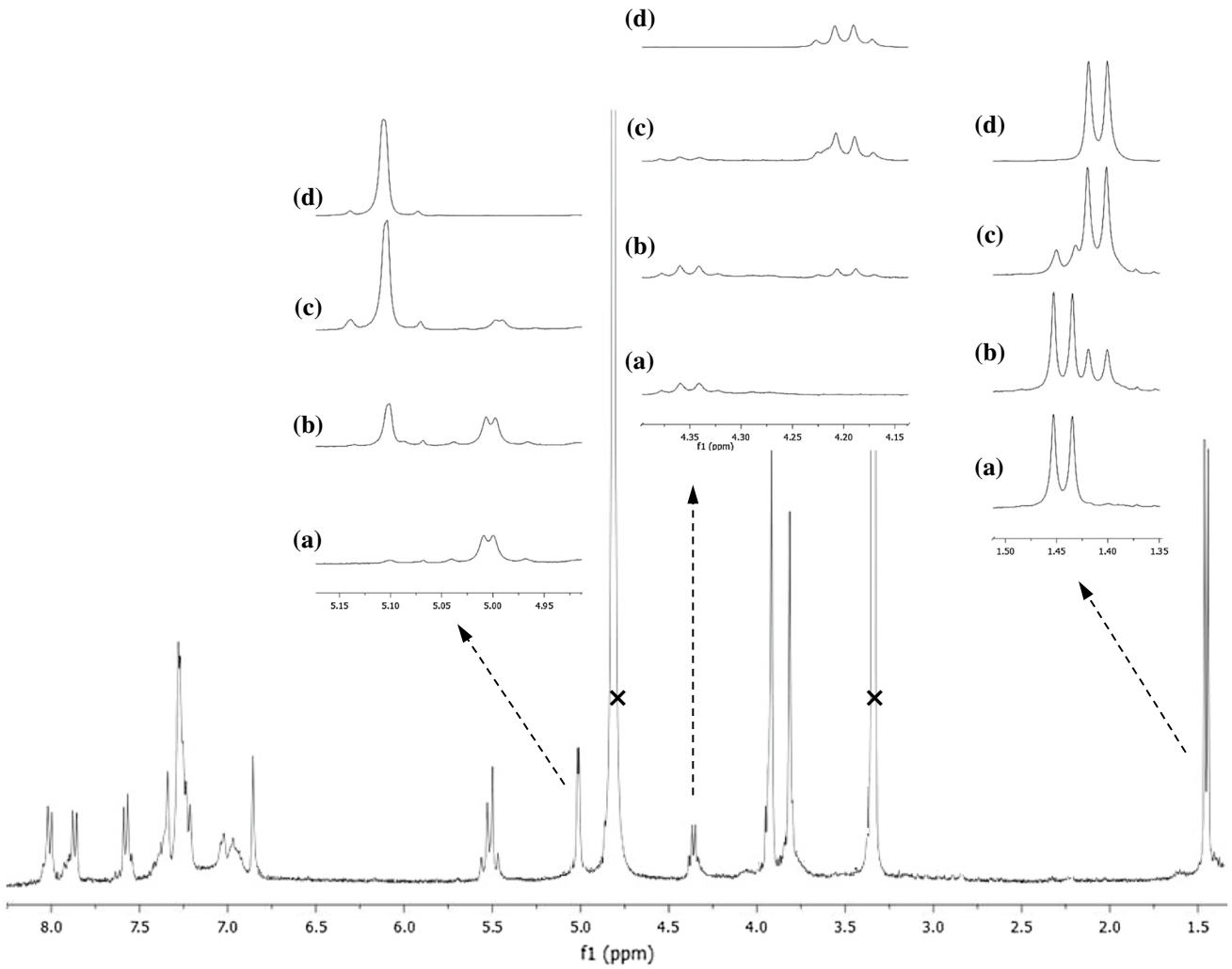


Fig. 2 ^{1}H NMR spectra in methanol-d_4/D_2O (60:40) of the photolysis of alanine-benzoquinolone conjugate **6a** ($C = 1 \times 10^{-3}$ M) at 350 nm: **a** before irradiation; **b** after irradiation for 30 min; **c** after irradiation for 2 h; **d** free Z-Ala-OH

the best results but this time cleavage at 419 nm was much more efficient and suitable for practical applications. Comparison with thionated benzocoumarin conjugate **9** in methanol/HEPES buffer (80:20) revealed that cleavage of the benzoquinolone conjugate **7** was exceedingly faster. Comparison with thionated quinolone conjugate **11** in methanol/HEPES buffer (80:20) showed very similar behaviour for both compounds.

In addition to monitoring the photolysis process by HPLC, the release of the model amino acid was also followed by ^{1}H NMR in an methanol-d_4/D_2O (60:40) solution. Fig. 2 shows, as a representative example, the irradiation at 350 nm of a solution of the alanine-benzoquinolone conjugate **6a**. The signals related to the amino acid in the conjugated form, at about δ 1.43 (CH_3), 4.33 (α-H) and 5.00 (CH_2 Z group) ppm, gradually decreased with time, giving rise to a close set of signals corresponding to free Z-protected alanine at about δ 1.41 (CH_3), 4.20 (α-H) and 5.10 (CH_2 Z group) ppm, respectively (Fig. 2). NMR monitoring was carried out with a 1×10^{-3} M solution, which led to an expected increase in the photolysis time for the complete release of the target molecule, when compared to the irradiation times in Table 2, obtained with dilute solutions, which can be related to the relatively high optical density.

Also, in order to evaluate the hydrolytic stability of the amino acid conjugates in aqueous media, solutions of conjugates **6a–c** and **7** were prepared in methanol/HEPES (80/20 and 60/40) and acetonitrile/HEPES (80/20) (the same conditions as the ones used for the photolysis experiments), and kept on the bench exposed to daylight and in the dark, at room temperature, for at least 7 days (a period of time that is longer than the irradiation times observed in the photolysis experiments). Aliquots were taken at regular intervals and analysed by HPLC using the same set of detection parameters. It was found that at the end of the seven-day period, dark hydrolysis did not exceed 2 %, whereas hydrolysis in day lighting did not exceed 5 %, for all the compounds studied. Therefore, it can be concluded that the newly obtained conjugates are hydrolytically stable in the present conditions.

Conclusions

The synthesis of a novel nitrogen heterocycle, benzoquinolone, for application as a photocleavable protecting group for amino acids was described for the first time. The functionalised heterocycle, synthesised by a sequence of good to high yield reactions, was coupled to model amino acids and conversion of a representative conjugate to the thionated derivative by reaction with Lawesson's reagent was accomplished. Photocleavage studies of the carbonyl and thiocarbonyl benzoquinolone amino acid conjugates in various solvents and at different wavelengths showed that the most interesting result was obtained at 419 nm for the thioconjugate, revealing that the presence of the thiocarbonyl group clearly improved the photolysis rates, giving practicable irradiations times (less than 1 min).

The newly reported (9-methoxy-4-methylbenzo[*f*]quinolin-3(4*H*)-thione-1-yl)methyl group can be considered an original and efficient photocleavable protecting group suitable for the fast release of carboxylic acids at wavelengths that are not detrimental to a variety of applications.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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