Available online at www.sciencedirect.com
ScienceDirect
Dyes and Pigments 75 (2007) 298–305
DYES and PIGMENTS
www.elsevier.com/locate/dyepig

Synthesis and characterization of mono- and dicarboxyalkyloxacarbocyanines

R.E.F. Boto [a,b], P.F. Santos [c,d], L.V. Reis [c,d], P. Almeida [a,b,*]

[a] Departamento de Química, Universidade da Beira Interior, 6201-001 Covilhã, Portugal
[b] Unidade de I & D de Materiais Têxteis e Papeleiros, Universidade da Beira Interior, 6201-001 Covilhã, Portugal
[c] Departamento de Química, Vila Real, Universidade de Trás-os-Montes e Alto Douro, Apartado 1013, 5001-801 Vila Real, Portugal
[d] Centro de Química - Vila Real, Universidade de Trás-os-Montes e Alto Douro, Apartado 1013, 5001-801 Vila Real, Portugal

Received 20 May 2006; received in revised form 4 June 2006; accepted 6 June 2006
Available online 25 July 2006

Abstract

Several novel asymmetric *N*-carboxyalkyl-*N′*-alkyloxacarbocyanines and symmetric *N*,*N′*-dicarboxyalkyloxacarbocyanines have been synthesized and characterized using ^{1}H and ^{13}C NMR, FTIR and Visible spectroscopy and HR-FAB-MS.

Keywords: Oxacarbocyanines; Carboxyalkyl; Asymmetric carbocyanines; Dyes; Spectroscopic characterization

1. Introduction

Recently we have described a series of novel asymmetric *N*-carboxyalkyl-*N′*-alkylthiacarbocyanines and symmetric *N*,*N′*-dicarboxyalkylthiacarbocyanines, as well as the corresponding methyl esters, regarding their use as biospecific ligands for affinity chromatography (AC) [1,2].

Following this, we report here the synthesis and full spectroscopic characterization of new asymmetric *N*-carboxyalkyl-*N′*-alkyloxacarbocyanines and symmetric *N*,*N′*-dicarboxyalkyloxacarbocyanines.

So far, carboxyalkylcarbocyanines derived from benzoxazole have been used mainly for photographic emulsions [3], as electrophotographic layer sensitizers [4], as labelling dyes for amino groups in biological substrates [5] and, lately, as energy transfer labelling agents for DNA sequencing and multiplex genetic analyses [6–13]. The ability of oxacarbocyanines bearing pendant carboxyalkyl groups to act as selective ligands for AC, taking advantage of the diversity of possible specific interactions between this type of dyes and the biomolecules to be purified, remains unexplored.

The carboxyalkyl chains, apart from acting as spacer arms that potentially enhance the efficiency of the purification process [14], offer a convenient way of coupling the dye to typical chromatographic matrices with hydroxyl reactive groups such as cellulose and agarose. For this purpose, an expeditious heterogeneous esterification method to link dyes with carboxylic groups to cellulose has been recently developed by our group [15].

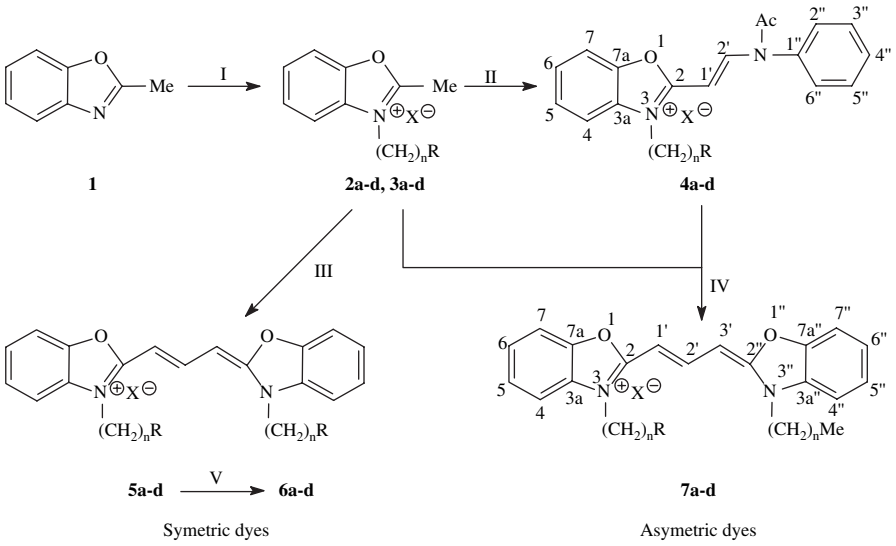
The fixation of the synthesized dyes to cellulose and the evaluation of the purification performance of the resulting affinity supports are currently being undertaken and will be reported elsewhere.

2. Results and discussion

Symmetric and asymmetric oxacarbocyanines **5–7** possessing pendant *N*-alkyl, *N*-carboxyalkyl and *N*-methoxycarbonylalkyl chains were synthesized as depicted in Scheme 1, based on the optimized synthetic routes previously described for the corresponding thiacarbocyanines analogues [2].

* Corresponding author. Departamento de Química, Universidade da Beira Interior, Rua Marques d.Avilae Bolama, 6201-001 Covilhã, Portugal. Tel.: +351 275319761; fax: +351 275319730.
E-mail addresses: rboto@ubi.pt (R.E.F. Boto), psantos@utad.pt (P.F. Santos), lvrfisco@utad.pt (L.V. Reis), paulo.almeida@ubi.pt (P. Almeida).

0143-7208/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.dyepig.2006.06.002



Scheme 1. Reagents and conditions. (I) **2a–d**: I(CH$_2$)$_n$Me (2.5–4.0 eq.), MeCN, reflux; **3a–d**: Br(CH$_2$)$_n$CO$_2$H (2.0–2.3 eq.), 1,2-dichlorobenzene, 120 °C; (II) **4a–d**: PhN=CHNHPh (1.1–1.2 eq.), Ac$_2$O, reflux; (III) **5a–d**: CH(OEt)$_3$ (2.0 eq.), pyridine, reflux; (IV) **7a–d**: triethylamine (2.0–2.1 eq.), ethanol, reflux; (V) **6a–d**: MeOH, H_2SO_4 (1.5–2.0% v/v), reflux.

Symmetric *N,N′*-dicarboxyalkyloxacarbocyanines **5a–d** were prepared by the classical base catalysed condensation of the *N*-carboxyalkylbenzoxazolium salts **3a–d**, readily obtained by quaternisation of 2-methylbenzoxazole (**1**) with a suitable bromoalkanoic acid [1,2,13] (method I), with triethyl orthoformate in dry pyridine (method III) [16], in moderate to good yields. Dyes **5a–d** were additionally characterized by means of the corresponding methyl ester derivatives **6a–d**, which were obtained upon Fisher's esterification by treatment with 1.5–2.0% methanolic sulphuric acid solution under reflux (method V).

The vinylacetanilide salts **4a–d** required for the synthesis of the asymmetric dyes **7a–d** were obtained by reaction of the *N*-alkylbenzoxazolium iodides **2**, easily achieved by alkylation of 2-methylbenzoxazole (**1**) with a suitable alkyl iodide [17], with *N,N′*-diphenylformamidine, in acetic anhydride (method II) [2]. Subsequently, condensation of the *N*-carboxyalkylbenzoxazolium salts **3a–d** with the intermediate vinylacetanilide iodides **4a–d** in ethanol, in the presence of triethylamine (method IV) [2,13], furnished the desired asymmetric oxacarbocyanines **7a–d** with good yields.

The zwitterionic dyes possessing carboxylic groups are considerably insoluble in the reaction solvent and can be collected as such, or converted to the comparatively more soluble hydrogensulphate or iodide salts by treatment with 5% aqueous H_2SO_4 or 14% aqueous KI, respectively. For each dye only one of those different forms (inner salt, hydrogensulphate salt or iodide salt) is described, since they uniquely differ in the solubility, melting point and yield, being the spectroscopic data practically indistinguishable.

Since the majority of the precursors and dyes described have not yet been referred in the literature, their full spectroscopic characterization, including ^{1}H and ^{13}C NMR shift assignments, which were established with the aid of HMQC (heteronuclear multiple quantum coherence), HMBC (heteronuclear multiple bond correlation) and COSY (correlated spectroscopy) experiments, is reported.

3. Experimental

3.1. General

All reagents were of the highest purity available, purchased from Sigma–Aldrich, and used as received. Solvents were of analytical grade. Acetonitrile, 1,2-dichlorobenzene, ethanol, methanol, pyridine and triethylamine were dried over 3 Å molecular sieves prior to use.

All reactions were monitored by thin-layer chromatography (TLC) on aluminum plates precoated with Merck silica gel 60 F_{254} (0.25 mm) using dichloromethane or dichloromethane/methanol (5–10%) and the spots were examined under 254, 312 and 365 nm UV light. Column chromatography was performed on silica gel 60 (70–230 mesh) from Macherey-Nagel.

^{1}H and ^{13}C NMR spectra were recorded on a Brücker ACP 250 (250.13 and 62.90 MHz) or Brücker ARX 400 (400.13 and 100.61 MHz) spectrometers. Chemical shifts are reported in parts per million relative to residual solvent signals or Me_4Si and coupling constants (J) are given in Hertz.

HMQC, HMBC and COSY spectra were acquired on the Brücker ARX 400 spectrometer.

Infrared spectra (IR) were performed on a Mattson 5000-FTS FTIR spectrophotometer. Solid samples were prepared by mixing FTIR-grade KBr with 1% (w/w) compound and grinding to a fine powder. Spectra were recorded over the 400–4000 cm^{-1} range without baseline corrections. More intensive bands are given in cm^{-1}.

Visible spectra (Vis) were recorded on a Perkin–Elmer Lambda 6 spectrophotometer using ethanol as solvent. Wavelength of maximum absorption is reported in nanometers.

High resolution fast atom bombardment mass spectra (HR-FAB-MS) were recorded in a Micromass AutoSpec M, operating at 70 eV, using a matrix of 3-nitrobenzyl alcohol (3-NBA). All new dyes were determined to be >95% pure by ^1H NMR.

Melting points were determined in open capillary tubes in a Büchi 530 melting point apparatus and are uncorrected.

3.2. Synthesis of N-alkylbenzoxazolium salts **2**

3.2.1. 2-Methyl-3-undecylbenzoxazol-3-ium iodide (**2d**): typical procedure

A solution of 2.24 g (2.00 ml, 16.8 mmol) of 2-methylbenzoxazole (**1**) and 12.20 g (10.00 ml, 43.2 mmol) of 1-iodoundecane in 25 ml of dry acetonitrile was refluxed for 4 days. After cooling to room temperature, diethyl ether was added to allow complete precipitation. The resulting crystalline product was collected by filtration under reduced pressure, washed with diethyl ether and recrystallized from dry acetonitrile. Yield: 30%. M.p. 59–60 °C. ^1H NMR (250.13 MHz, $CDCl_3$) δ (ppm): 0.83 (3H, t, $J = 6.9$ Hz, CH_2CH_3), 1.20–1.45 (16H, m, CH_2), 1.98 (2H, qui, $J = 7.3$ Hz, 3-NCH_2CH_2), 3.37 (3H, s, 2-CH_3), 4.68 (2H, t, $J = 7.6$ Hz, 3-NCH_2), 7.64–7.72 (2H, m, 5-CH, 6-CH), 7.77–7.83 (1H, m, 4-CH), 7.85–7.90 (1H, m, 7-CH). ^{13}C NMR (62.90 MHz, $CDCl_3$) δ (ppm): 13.9 (CH_2CH_3), 16.6 (2-CH_3), 22.5 (CH_2CH_3), 26.6 (3-$N(CH_2)_8CH_2$), 28.1 (3-$N(CH_2)_7CH_2$), 28.8 (3-$N(CH_2)_6CH_2$), 29.1 (3-$N(CH_2)_5CH_2$), 29.3 (3-$N(CH_2)_4CH_2$), 29.3 (3-$N(CH_2)_3CH_2CH_2$), 31.6 (3-NCH_2CH_2), 49.0 (3-NCH_2), 113.2 (4-CH), 114.4 (7-CH), 128.3 (6-CH), 129.2 (5-CH), 129.5 (3a-C), 147.7 (7a-C), 167.1 (2-C). IR (KBr) ν (cm^{-1}): 2918, 2850, 1648, 1582 (C=C), 1496, 1416, 1195, 750. HR-FAB-MS (3-NBA) $C_{19}H_{30}NO$: calcd., 288.2327; found, 288.2324.

The *N*-alkylbenzoxazolium iodides **2a–c** were prepared using a similar procedure (1–4 days). The spectroscopic characterization of **2a** and **2c** has been previously reported [17].

3.2.2. 2-Methyl-3-propylbenzoxazol-3-ium iodide (**2b**)

Yield: 50%. M.p. 195–197 °C. ^1H NMR (250.13 MHz, $CDCl_3$) δ (ppm): 1.11 (3H, t, $J = 7.4$ Hz, CH_2CH_3), 2.02–2.18 (2H, m, CH_2CH_3), 3.42 (3H, s, 2-CH_3), 4.71 (2H, t, $J = 7.4$ Hz, 3-NCH_2), 7.71–7.77 (2H, m, 5-CH, 6-CH), 7.84–7.88 (1H, m, 4-CH), 8.00–8.04 (1H, m, 7-CH). ^{13}C NMR (62.90 MHz, $CDCl_3$) δ (ppm): 11.5 (CH_2CH_3), 16.8 (2-CH_3), 21.9 (CH_2CH_3), 50.4 (3-NCH_2), 113.4 (4-CH), 114.8 (7-CH), 128.5 (6-CH), 129.4 (5-CH), 129.7 (3a-C), 147.9 (7a-C), 167.4 (2-C). IR (KBr) ν (cm^{-1}): 3018, 2961, 2946, 2875, 1592 (C=C), 1391, 1190, 1027, 770. HR-FAB-MS (3-NBA) $C_{11}H_{14}NO$: calcd., 176.1075; found, 176.1068.

3.3. Synthesis of N-carboxyalkylbenzoxazolium salts **3**

3.3.1. 3-(10-Carboxydecyl)-2-methylbenzoxazol-3-ium bromide (**3d**): typical procedure

A mixture of 2.24 g (2.00 ml, 16.8 mmol) of 2-methylbenzoxazole (**1**), 9.11 g (34.4 mmol) of 11-bromoundecanoic acid and 20 ml of 1,2-dichlorobenzene was heated at 120 °C for 3 days. After cooling to room temperature, diethyl ether was added to the reaction mixture until complete precipitation. The crystalline product so formed was collected by filtration under reduced pressure, washed with diethyl ether and recrystallized from dry acetonitrile. Yield: 29%. M.p. 130–132 °C. ^1H NMR (250.13 MHz, $CDCl_3$/5% CH_3OD) δ (ppm): 1.28–1.64 (14H, m, CH_2), 1.97–2.08 (2H, m, 3-NCH_2CH_2), 2.28 (2H, t, $J = 7.5$ Hz, CH_2COOH), 3.26 (3H, s, 2-CH_3), 4.66 (2H, t, $J = 7.5$ Hz, 3-NCH_2), 7.75–7.81 (2H, m, 5-CH, 6-CH), 7.90–7.97 (2H, m, 4-CH, 7-CH). ^{13}C NMR (62.90 MHz, $CDCl_3$/5% CH_3OD) δ (ppm): 14.2 (2-CH_3), 25.1 (CH_2), 26.9 (CH_2), 28.6 (CH_2), 29.3 (CH_2), 29.4 (CH_2CH_2), 29.5 (CH_2CH_2), 34.4 (CH_2COOH), 48.3 (3-NCH_2), 113.5 (4-CH), 114.5 (7-CH), 128.8 (6-CH), 129.7 (5-CH), 129.8 (3a-C), 148.4 (7a-C), 168.1 (2-C), 176.8 (CO). IR (KBr) ν (cm^{-1}): 2922, 2852, 1722 (C=O), 1596 (C=C), 1463, 1400, 1170, 769, 754. HR-FAB-MS (3-NBA) $C_{19}H_{28}NO_3$: calcd., 318.2069; found, 318.2062.

The *N*-carboxyalkylbenzoxazolium bromides **3a–c** were prepared using a similar procedure (1–3 days).

3.3.2. 3-Carboxymethyl-2-methylbenzoxazol-3-ium bromide (**3a**)

Yield: 75%. M.p. 236–237 °C. ^1H NMR (250.13 MHz, $CDCl_3$/5% CH_3OD) δ (ppm): 3.20 (3H, s, 2-CH_3), 5.68 (2H, s, 3-NCH_2COOH) 7.79–7.84 (2H, m, 5-CH, 6-CH), 8.06–8.08 (1H, m, 4-CH), 8.09–8.11 (1H, m, 7-CH). ^{13}C NMR (62.90 MHz, $CDCl_3$/5% CH_3OD) δ (ppm): 14.0 (2-CH_3), 48.5 (3-NCH_2COOH), 114.2 (4-CH), 115.8 (7-CH), 129.5 (6-CH), 130.5 (5-CH), 131.1 (3a-C), 149.2 (7a-C), 167.2 (2-C), 171.7 (CO). IR (KBr) ν (cm^{-1}): 3022, 2960, 2926, 2730, 2544, 2420, 1760 (C=O), 1734 (C=O), 1582 (C=C), 1463, 1220, 762. HR-FAB-MS (3-NBA) $C_{10}H_{10}NO_3$: calcd., 192.0661; found, 192.0653.

3.3.3. 3-(2-Carboxyethyl)-2-methylbenzoxazol-3-ium bromide (**3b**)

Yield: 80%. M.p. 213–214 °C. ^1H NMR (250.13 MHz, $CDCl_3$/5% CH_3OD) δ (ppm): 3.15 (2H, t, $J = 6.2$ Hz, CH_2COOH), 3.28 (3H, s, 2-CH_3), 4.89 (2H, t, $J = 6.2$ Hz, 3-NCH_2), 7.76–7.83 (2H, m, 5-CH, 6-CH), 7.95–7.99 (1H, m, 4-CH), 8.09–8.15 (1H, m, 7-CH). ^{13}C NMR (62.90 MHz, $CDCl_3$/5% CH_3OD) δ (ppm): 14.3 (2-CH_3), 31.9 (CH_2COOH), 44.0 (3-NCH_2), 113.8 (4-CH), 115.4 (7-CH), 129.1 (6-CH), 130.0 (5-CH, 3a-C), 148.9 (7a-C), 170.1 (2-C), 172.6 (CO). IR (KBr) ν (cm^{-1}): 3397, 2954,

1733 (C=O), 1590 (C=C), 1461, 1390, 1197, 807, 769. HR-FAB-MS (3-NBA) $C_{11}H_{12}NO_3$: calcd., 206.0817; found, 206.0818.

3.3.4. 3-(4-Carboxybutyl)-2-methylbenzoxazol-3-ium bromide (**3c**)

Yield: 45%. M.p. 111–113 °C. ^{1}H NMR (400.13 MHz, $CDCl_3$/5% CH_3OD) δ (ppm): 1.54–1.64 (2H, m, 3-N$(CH_2)_2CH_2$), 1.88 (2H, qui, $J = 7.7$ Hz, 3-NCH_2CH_2), 2.22 (2H, t, $J = 7.0$ Hz, CH_2COOH), 3.06 (3H, s, 2-CH_3), 4.48 (2H, t, $J = 7.6$ Hz, 3-NCH_2), 7.53–7.58 (2H, m, 5-C*H*, 6-C*H*), 7.67–7.72 (1H, m, 4-C*H*), 7.77–7.81 (1H, m, 7-C*H*). ^{13}C NMR (100.61 MHz, $CDCl_3$/5% CH_3OD) δ (ppm): 13.6 (2-*C*H_3), 21.2 (3-N$(CH_2)_2$*C*H_2), 27.0 (3-NCH_2*C*H_2), 32.4 (*C*H_2COOH), 47.2 (3-N*C*H_2), 112.7 (4-*C*H), 114.0 (7-*C*H), 128.1 (6-*C*H), 129.0 (5-*C*H), 129.2 (3a-*C*), 147.6 (7a-*C*), 167.7 (2-*C*), 173.5 (*C*O). IR (KBr) ν (cm^{-1}): 3322, 2956, 2587, 1728 (C=O), 1585 (C=C), 1458, 1195, 775. HR-FAB-MS (3-NBA) $C_{13}H_{16}NO_3$: calcd., 234.1130; found, 234.1132.

3.4. Synthesis of acetanilide derivatives **4a–d**

3.4.1. 2-[2-(Acetylphenylamino)vinyl]-3-ethylbenzoxazol-3-ium iodide (**4a**): typical procedure

A solution of 1.06 g (3.67 mmol) of 3-ethyl-2-methylbenzoxazol-3-ium iodide (**2a**) and 0.811 g (4.14 mmol) of *N*,*N*′-diphenylformamidine in 10 ml of acetic anhydride was heated under reflux for 30 min. After cooling to room temperature, diethyl ether was added to the reaction mixture until complete precipitation. The crystalline product so formed was collected by filtration under reduced pressure, washed with diethyl ether and recrystallized from dry acetonitrile. Yield: 75%. M.p. 214–216 °C. ^{1}H NMR (250.13 MHz, DMSO-d_6) δ (ppm): 1.25 (3H, t, $J = 7.1$ Hz, CH_2CH_3), 2.06 (3H, s, COCH_3), 4.34 (2H, q, $J = 7.1$ Hz, 3-NCH_2CH_3), 5.50 (1H, d, $J = 13.8$ Hz, 1′-C*H*), 7.54 (2H, d, $J = 7.9$ Hz, 2″-C*H*, 6″-C*H*), 7.64–7.74 (5H, m, 5-C*H*, 6-C*H*, 3″-C*H*, 4″-C*H*, 5″-C*H*), 7.99–8.09 (2H, m, 4-C*H*, 7-C*H*), 9.06 (1H, d, $J = 13.7$ Hz, 2′-C*H*). ^{13}C NMR (62.90 MHz, DMSO-d_6) δ (ppm): 12.9 (CH_2CH_3), 23.3 (COCH_3), 41.6 (3-NCH_2CH_3), 87.1 (1′-*C*H), 112.4 (7-*C*H), 113.6 (4-*C*H), 127.2 (5-*C*H), 127.9 (6-*C*H), 128.0 (2″-*C*H, 6″-*C*H), 130.0 (4″-*C*H), 130.1 (3a-*C*), 130.7 (3″-*C*H, 5″-*C*H), 136.7 (1″-*C*), 146.6 (2′-*C*H), 146.9 (7a-*C*), 163.1 (2-*C*), 170.3 (*C*O). IR (KBr) ν (cm^{-1}): 3088, 3063, 3011, 1721 (C=O), 1615 (C=C), 1588 (C=C), 1360, 1256, 1225, 760. Vis (EtOH): 348 nm. HR-FAB-MS (3-NBA) $C_{19}H_{19}N_2O_2$: calcd., 307.1447; found, 307.1458.

The *N*-acetanilide derivatives **4b–d** were prepared using a similar procedure (0.5–2 h).

3.4.2. 2-[2-(Acetylphenylamino)vinyl]-3-propylbenzoxazol-3-ium iodide (**4b**)

Yield: 64%. M.p. 209–210 °C. ^{1}H NMR (400.13 MHz, DMSO-d_6) δ (ppm): 0.78 (3H, t, $J = 7.5$ Hz, CH_2CH_3), 1.67 (2H, q, $J = 7.4$ Hz, CH_2CH_3), 2.07 (3H, s, COCH_3), 4.28 (2H, t, $J = 7.4$ Hz, 3-NCH_2), 5.45 (1H, d, $J = 13.9$ Hz, 1′-C*H*), 7.53 (2H, d, $J = 7.5$ Hz, 2″-C*H*, 6″-C*H*), 7.62–7.74 (5H, m, 5-C*H*, 6-C*H*, 3″-C*H*, 4″-C*H*, 5″-C*H*), 8.00–8.09 (2H, m, 4-C*H*, 7-C*H*), 9.06 (1H, d, $J = 13.7$ Hz, 2′-C*H*). ^{13}C NMR (62.90 MHz, DMSO-d_6) δ (ppm): 10.8 (CH_2CH_3), 21.2 (CH_2CH_3), 23.3 (COCH_3), 46.6 (3-NCH_2), 87.4 (1′-*C*H), 112.5 (7-*C*H), 113.8 (4-*C*H), 127.2 (5-*C*H), 128.1 (6-*C*H), 128.2 (2″-*C*H, 6″-*C*H), 130.3 (4″-*C*H), 130.5 (3a-*C*), 130.6 (3″-*C*H, 5″-*C*H), 136.7 (1″-*C*), 146.7 (2′-*C*H), 146.8 (7a-*C*), 163.5 (2-*C*) 170.3 (*C*O). IR (KBr) ν (cm^{-1}): 3430, 3040, 2971, 2937, 1721 (C=O), 1616 (C=C), 1589 (C=C), 1464, 1275, 1238, 1217. Vis (EtOH): 349 nm. HR-FAB-MS (3-NBA) $C_{20}H_{21}N_2O_2$: calcd., 321.1603; found, 321.1591.

3.4.3. 2-[2-(Acetylphenylamino)vinyl]-3-pentylbenzoxazol-3-ium iodide (**4c**)

Yield: 41%. M.p. 129–131 °C. ^{1}H NMR (400.13 MHz, DMSO-d_6) δ (ppm): 0.79 (3H, t, $J = 6.5$ Hz, CH_2CH_3), 1.14–1.20 (4H, m, 3-N$(CH_2)_2CH_2$, CH_2CH_3), 1.65 (2H, qui, $J = 6.7$ Hz, 3-NCH_2CH_2), 2.09 (3H, s, COCH_3), 4.29 (2H, t, $J = 7.0$ Hz, 3-NCH_2), 5.40 (1H, d, $J = 13.7$ Hz, 1′-C*H*), 7.54 (2H, d, $J = 8.0$ Hz, 2″-C*H*, 6″-C*H*), 7.64–7.73 (5H, m, 5-C*H*, 6-C*H*, 3″-C*H*, 4″-C*H*, 5″-C*H*), 8.02 (1H, d, $J = 8.9$ Hz, 4-C*H*), 8.07 (1H, d, $J = 9.0$ Hz, 7-C*H*), 9.06 (1H, d, $J = 13.7$ Hz, 2′-C*H*). ^{13}C NMR (100.61 MHz, DMSO-d_6) δ (ppm): 13.6 (CH_2CH_3), 21.3 (CH_2CH_3), 23.1 (COCH_3), 27.1 (3-N$(CH_2)_2CH_2$), 27.6 (3-NCH_2*C*H_2), 45.0 (3-NCH_2), 87.2 (1′-*C*H), 112.3 (7-*C*H), 113.6 (4-*C*H), 127.1 (5-*C*H), 127.9 (6-*C*H), 128.0 (2″-*C*H, 6″-*C*H), 130.1 (4″-*C*H), 130.2 (3a-*C*), 130.5 (3″-*C*H, 5″-*C*H), 136.6 (1″-*C*), 146.6 (2′-*C*H), 146.7 (7a-*C*), 163.7 (2-*C*), 170.0 (*C*O). IR (KBr) ν (cm^{-1}): 3430, 3067, 3007, 2965, 2930, 2857, 1723 (C=O), 1620 (C=C), 1589 (C=C), 1215. Vis (EtOH): 348 nm. HR-FAB-MS (3-NBA) $C_{22}H_{25}N_2O_2$: calcd., 349.1916; found, 349.1925.

3.4.4. 2-[2-(Acetylphenylamino)vinyl]-3-undecylbenzoxazol-3-ium iodide (**4d**)

Yield: 58%. M.p. 56–57 °C. ^{1}H NMR (250.13 MHz, DMSO-d_6) δ (ppm): 0.85 (3H, t, $J = 7.2$ Hz, CH_2CH_3), 1.12–1.28 (16H, m, CH_2), 1.62 (2H, qui, $J = 7.0$ Hz, 3-NCH_2*C*H_2), 2.08 (3H, s, COCH_3), 4.27 (2H, t, $J = 7.0$ Hz, 3-NCH_2), 5.39 (1H, d, $J = 13.7$ Hz, 1′-C*H*), 7.50–7.56 (2H, m, 2″-C*H*, 6″-C*H*), 7.62–7.75 (5H, m, 5-C*H*, 6-C*H*, 3″-C*H*, 4″-C*H*, 5″-C*H*), 7.97–8.02 (1H, m, 4-C*H*), 8.04–8.11 (1H, m, 7-C*H*), 9.05 (1H, d, $J = 13.6$ Hz, 2′-C*H*). ^{13}C NMR (62.90 MHz, DMSO-d_6) δ (ppm): 14.0 (CH_2CH_3), 22.2 (CH_2CH_3), 23.2 (COCH_3), 25.8 (3-N$(CH_2)_8CH_2$), 27.4 (CH_2), 28.4 (CH_2), 28.8 (CH_2), 28.9 (CH_2), 29.0 (CH_2), 29.0 (CH_2), 31.3 (3-NCH_2*C*H_2), 48.2 (3-NCH_2), 87.3 (1′-*C*H), 112.5 (7-*C*H), 113.7 (4-*C*H), 127.3 (5-*C*H), 128.1 (6-*C*H), 128.2 (2″-*C*H, 6″-*C*H), 130.2 (4″-*C*H), 130.4 (3a-*C*), 130.6 (3″-*C*H, 5″-*C*H), 136.7 (1″-*C*), 146.7 (2′-*C*H), 146.8 (7a-*C*), 163.3 (2-*C*), 170.2 (*C*O). IR (KBr) ν (cm^{-1}): 3441, 3086, 3063, 3036, 2924, 2853, 1709 (C=O), 1616 (C=C), 1588 (C=C), 1468, 1225. Vis (EtOH): 351 nm. HR-FAB-MS (3-NBA) $C_{28}H_{37}N_2O_2$: calcd., 433.2855; found, 433.2865.

3.5. Synthesis of symmetric carboxyalkyloxacarbocyanine dyes **5**

3.5.1. 3-(10-Carboxydecyl)-2-{3-[3-(10-carboxydecyl)-3H-benzoxazol-2-ylidene]propenyl}benzoxazol-3-ium inner salt (**5d**): typical procedure

A solution of 0.504 g (1.27 mmol) of 3-(10-carboxydecyl)-2-methylbenzoxazol-3-ium bromide (**3d**), 0.384 g (0.430 ml, 2.59 mmol) of triethyl orthoformate and 5 ml of dry pyridine was heated under reflux for 18 h. After cooling to room temperature, diethyl ether was added and the resulting mixture was refrigerated to allow complete precipitation. The crystalline product obtained was collected by filtration under reduced pressure, washed with diethyl ether and recrystallized from dry acetonitrile.

Yield: 34%. M.p. 191–193 °C. ^{1}H NMR (250.13 MHz, DMSO-d_6) δ (ppm): 1.08–1.46 (28H, m, CH_2), 1.66–1.76 (4H, m, 3-NCH$_2$CH_2, 3″-NCH$_2$CH_2), 2.11 (4H, t, $J = 7.4$ Hz, CH_2COO), 4.15 (4H, t, $J = 7.2$ Hz, 3-NCH_2, 3″-NCH_2), 6.07 (2H, d, $J = 13.7$ Hz, 1′-C*H*, 3′-C*H*), 7.31–7.47 (4H, m, 5-C*H*, 6-C*H*, 5″-C*H*, 6″-C*H*), 7.61–7.74 (4H, m, 4-C*H*, 7-C*H*, 4″-C*H*, 7″-C*H*), 8.21 (1H, t, $J = 13.4$ Hz, 2′-C*H*). ^{13}C NMR (62.90 MHz, DMSO-d_6) δ (ppm): 24.8 (3-N(CH$_2$)$_8$CH_2, 3″-N(CH$_2$)$_8$CH_2), 26.2 (3-N(CH$_2$)$_2$CH_2, 3″-N(CH$_2$)$_2$CH_2), 27.7 (3-NCH$_2$CH_2, 3″-NCH$_2$CH_2), 28.8 (CH_2), 29.0 (CH_2), 29.0 (CH_2), 29.0 (CH_2), 29.1 (CH_2), 34.0 (CH_2COO), 44.1 (3-NCH_2, 3″-NCH_2), 85.5 (1′-C*H*, 3′-C*H*), 111.2 (7-C*H*, 7″-C*H*), 111.7 (4-C*H*, 4″-C*H*), 125.5 (6-C*H*, 6″-C*H*), 126.3 (5-C*H*, 5″-C*H*), 131.6 (3a-C, 3a″-C), 146.6 (2′-C*H*), 146.7 (7a-C, 7a″-C), 161.9 (2-C, 2″-C), 175.0 (C*O*). IR (KBr) ν (cm^{-1}) 2924, 2852, 1739 (C=O), 1716 (C=O), 1563 (C=C), 1503, 1452, 1193, 1114, 1083, 746. Vis (EtOH): 487 nm. HR-FAB-MS (3-NBA) $C_{39}H_{53}N_2O_6$: calcd., 645.3904; found, 645.3886.

The oxacarbocyanine dyes **5a–c** were prepared using a similar procedure (2–18 h).

3.5.2. 3-Carboxymethyl-2-[3-(3-carboxymethyl-3H-benzoxazol-2-ylidene)propenyl]-benzoxazol-3-ium inner salt (**5a**)

Yield: 34%. M.p. 260–262 °C. ^{1}H NMR (250.13 MHz, DMSO-d_6) δ (ppm): 5.19 (4H, s, 3-NCH_2COO, 3″-NCH_2COOH), 6.06 (2H, d, $J = 13.2$ Hz, 1′-C*H*, 3′-C*H*), 7.37–7.47 (4H, m, 5-C*H*, 6-C*H*, 5″-C*H*, 6″-C*H*), 7.67 (2H, d, $J = 7.5$ Hz, 4-C*H*, 4″-C*H*), 7.77 (2H, d, $J = 7.9$ Hz, 7-C*H*, 7″-C*H*), 8.31 (1H, d, $J = 13.2$ Hz, 2′-C*H*). ^{13}C NMR (62.90 MHz, DMSO-d_6) δ (ppm): 44.9 (3-NCH$_2$COO, 3″-NCH$_2$COOH), 85.6 (1′-C*H*, 3′-C*H*), 111.2 (7-C*H*, 7″-C*H*), 111.6 (4-C*H*, 4″-C*H*), 125.5 (6-C*H*, 6″-C*H*), 126.3 (5-C*H*, 5″-C*H*), 131.4 (3a-C, 3a″-C), 146.3 (2′-C*H*), 147.0 (7a-C, 7a″-C), 162.6 (2-C, 2″-C), 167.7 (C*O*). IR (KBr) ν (cm^{-1}): 3066, 1727 (C=O), 1568 (C=C), 1512, 1467, 1240, 1205, 1123, 1093, 905, 745. Vis (EtOH): 489 nm. HR-FAB-MS (3-NBA) $C_{21}H_{17}N_2O_6$: calcd., 393.1087; found, 393.1082.

3.5.3. 3-(2-Carboxyethyl)-2-{3-[3-(2-carboxyethyl)-3H-benzoxazol-2-ylidene]propenyl}-benzoxazol-3-ium inner salt (**5b**)

Yield: 27%. M.p. 202–203 °C. ^{1}H NMR (250.13 MHz, DMSO-d_6) δ (ppm): 2.79 (4H, t, $J = 7.0$ Hz, CH_2COO), 4.32 (4H, t, $J = 7.0$ Hz, 3-NCH_2, 3″-NCH_2), 6.06 (2H, d, $J = 13.3$ Hz, 1′-C*H*, 3′-C*H*), 7.27–7.40 (4H, m, 5-C*H*, 6-C*H*, 5″-C*H*, 6″-C*H*), 7.60–7.69 (4H, m, 4-C*H*, 7-C*H*, 4″-C*H*, 7″-C*H*), 8.17 (1H, d, $J = 13.5$ Hz, 2′-C*H*). ^{13}C NMR (62.90 MHz, DMSO-d_6) δ (ppm): 32.2 (CH_2COO), 40.4 (3-NCH_2, 3″-NCH_2), 86.1 (1′-C*H*, 3′-C*H*), 111.3 (7-C*H*, 7″-C*H*), 112.3 (4-C*H*, 4″-C*H*), 125.6 (6-C*H*, 6″-C*H*), 126.4 (5-C*H*, 5″-C*H*), 131.6 (3a-C, 3a″-C), 146.8 (2′-C*H*), 146.9 (7a-C, 7a″-C), 162.2 (2-C, 2″-C), 172.3 (C*O*). IR (KBr) ν (cm^{-1}): 3063, 1715 (C=O), 1571 (C=C), 1513, 1455, 1397, 1203, 1116, 1089, 757. Vis (EtOH): 488 nm. HR-FAB-MS (3-NBA) $C_{23}H_{21}N_2O_6$: calcd., 421.1400; found, 421.1415.

3.5.4. 3-(4-Carboxybutyl)-2-{3-[3-(4-carboxybutyl)-3H-benzoxazol-2-ylidene]propenyl}-benzoxazol-3-ium inner salt (**5c**)

Yield: 19%. M.p. 184–186 °C. ^{1}H NMR (400.13 MHz, DMSO-d_6) δ (ppm): 1.59–1.66 (4H, m, 3-N(CH$_2$)$_2$CH_2, 3″-N(CH$_2$)$_2$CH_2), 1.76–1.83 (4H, m, 3-NCH$_2$CH_2, 3″-NCH$_2$CH_2), 2.30 (4H, t, $J = 7.1$ Hz, CH_2COO), 4.23 (4H, t, $J = 7.1$ Hz, 3-NCH_2, 3″-NCH_2), 6.11 (2H, d, $J = 13.3$ Hz, 1′-C*H*, 3′-C*H*), 7.40 (2H, t, $J = 7.7$ Hz, 6-C*H*, 6″-C*H*), 7.45 (2H, t, $J = 7.6$ Hz, 5-C*H*, 5″-C*H*), 7.71 (2H, d, $J = 7.7$ Hz, 4-C*H*, 4″-C*H*), 7.76 (2H, d, $J = 7.9$ Hz, 7-C*H*, 7″-C*H*), 8.28 (1H, t, $J = 13.3$ Hz, 2′-C*H*). ^{13}C NMR (100.61 MHz, DMSO-d_6) δ (ppm): 21.5 (3-N(CH$_2$)$_2$CH$_2$, 3″-N(CH$_2$)$_2$CH$_2$), 26.8 (3-NCH$_2$CH$_2$, 3″-NCH$_2$CH$_2$), 33.1 (CH$_2$COO), 43.5 (3-NCH$_2$, 3″-NCH$_2$), 85.0 (1′-C*H*, 3′-C*H*), 110.8 (7-C*H*, 7″-C*H*), 111.3 (4-C*H*, 4″-C*H*), 124.9 (6-C*H*, 6″-C*H*), 125.8 (5-C*H*, 5″-C*H*), 131.2 (3a-C, 3a″-C), 146.1 (2′-C*H*), 146.3 (7a-C, 7a″-C), 161.6 (2-C, 2″-C), 174.0 (C*O*). IR (KBr) ν (cm^{-1}): 3422, 3028, 2955, 1732 (C=O), 1564 (C=C), 1501, 1454, 1393, 1192, 1115, 1082, 748. Vis (EtOH): 488 nm. HR-FAB-MS (3-NBA) $C_{27}H_{29}N_2O_6$: calcd., 477.2026; found, 477.2014.

3.6. Synthesis of symmetric methoxycarbonylalkyloxacarbocyanine dyes **6**

The methoxycarbonylalkyloxacarbocyanines **6a–d** were prepared by heating under reflux a solution of the corresponding carboxyalkyloxacarbocyanines **5a–d** in 1.5–2.0% (v/v) methanolic H_2SO_4 for 1–4 h. After concentration of the reaction mixture under reduced pressure and precipitation with diethyl ether, dyes **6a** and **6b** were collected by filtration under reduced pressure, washed with diethyl ether and recrystallized from dry acetonitrile. For dyes **6c** and **6d** the reaction mixture was evaporated to dryness and the resulting oil was dissolved in dichloromethane, washed with water, dried over anhydrous sodium sulphate and then purified by column chromatography (silica gel, CH_2Cl_2/MeOH: 0–10%).

All these dyes, as well as **5a–d** and **7a–d**, readily undergo counter-ion exchange to hydrogensulphate or iodide by dissolution in a minimum amount of acetonitrile, followed by addition of excess 5% (v/v) aqueous H_2SO_4 or 14% (w/v) aqueous KI, respectively. After heating to reflux, the solution or

suspension obtained was cooled to room temperature and refrigerated overnight. The resulting crystalline product was collected by filtration under reduced pressure, washed with diethyl ether and recrystallized from dry acetonitrile or dry methanol.

*3.6.1. 3-Methoxycarbonylmethyl-2-{3-[3-methoxycarbonylmethyl-3H-benzoxazol-2-ylidene]propenyl}benzoxazol-3-ium hydrogensulphate (**6a**)*

Yield: 33%. M.p. 199–201 °C. ^{1}H NMR (250.13 MHz, DMSO-d_6) δ (ppm): 3.78 (6H, s, COOCH_3), 5.36 (4H, s, 3-NCH_2COOCH$_3$, 3″-NCH_2COOCH$_3$), 6.08 (2H, d, $J = 13.5$ Hz, 1′-CH, 3′-CH), 7.41–7.52 (4H, m, 5-CH, 6-CH, 5″-CH, 6″-CH), 7.67–7.74 (2H, m, 4-CH, 4″-CH), 7.78–7.84 (2H, m, 7-CH, 7″-CH), 8.31 (1H, d, $J = 13.2$ Hz, 2′-CH). ^{13}C NMR (62.90 MHz, DMSO-d_6) δ (ppm): 44.6 (3-NCH_2COOCH$_3$, 3″-NCH_2COOCH$_3$), 53.0 (COOCH$_3$), 85.9 (1′-CH, 3′-CH), 111.2 (7-CH, 7″-CH), 111.6 (4-CH, 4″-CH), 125.6 (6-CH, 6″-CH), 126.2 (5-CH, 5″-CH), 131.1 (3a-C, 3a″-C), 146.2 (7a-C, 7a″-C), 147.1 (2′-CH), 162.6 (2-C, 2″-C), 166.9 (CO). IR (KBr) ν (cm^{-1}): 2948, 1752 (C=O), 1569 (C=C), 1505, 1467, 1252, 1205, 1125, 1093, 1013, 904, 762. Vis (EtOH): 486 nm. HR-FAB-MS (3-NBA) $C_{23}H_{21}N_2O_6$: calcd., 421.1400; found, 421.1393.

*3.6.2. 3-(2-Methoxycarbonylethyl)-2-{3-[3-(2-methoxycarbonylethyl)-3H-benzoxazol-2-ylidene]propenyl}benzoxazol-3-ium hydrogensulphate (**6b**)*

Yield: 49%. M.p. 161–162 °C. ^{1}H NMR (400.13 MHz, DMSO-d_6) δ (ppm): 2.94 (4H, t, $J = 6.5$ Hz, CH_2COOCH$_3$), 3.60 (6H, s, COOCH_3), 4.43 (4H, t, $J = 6.5$ Hz, 3-NCH_2, 3″-NCH_2), 6.13 (2H, d, $J = 13.3$ Hz, 1′-CH, 3′-CH), 7.35–7.48 (4H, m, 5-CH, 6-CH, 5″-CH, 6″-CH), 7.70–7.76 (4H, m, 4-CH, 7-CH, 4″-CH, 7″-CH), 8.25 (1H, d, $J = 13.3$ Hz, 2′-CH). ^{13}C NMR (62.90 MHz, DMSO-d_6) δ (ppm): 31.3 (CH$_2$COOCH$_3$), 39.6 (3-NCH_2, 3″-NCH_2), 51.8 (COOCH$_3$), 85.6 (1′-CH, 3′-CH), 110.8(7-CH, 7″-CH), 111.7 (4-CH, 4″-CH), 125.0 (6-CH, 6″-CH), 125.8 (5-CH, 5″-CH), 131.1 (3a-C, 3a″-C), 146.3 (2′-CH, 7a-C, 7a″-C), 161.7 (2-C, 2″-C), 170.7 (CO). IR (KBr) ν (cm^{-1}): 2954, 1731 (C=O), 1568 (C=C), 1505, 1467, 1444, 1192, 1118, 1087, 1055, 983, 749. Vis (EtOH): 487 nm. HR-FAB-MS (3-NBA) $C_{25}H_{25}N_2O_6$: calcd., 449.1713; found, 449.1718.

*3.6.3. 3-(4-Methoxycarbonylbutyl)-2-{3-[3-(4-methoxycarbonylbutyl)-3H-benzoxazol-2-ylidene]propenyl}benzoxazol-3-ium hydrogensulphate (**6c**)*

Yield: 53%. Orange-coloured oil. ^{1}H NMR (250.13 MHz, DMSO-d_6) δ (ppm): 1.56–1.68 (4H, m, 3-N(CH$_2$)$_2$CH_2, 3″-N(CH$_2$)$_2$CH_2), 1.73–1.85 (4H, m, 3-NCH$_2$CH_2, 3″-NCH$_2$CH_2), 2.38 (4H, t, $J = 7.1$ Hz, CH_2COOCH$_3$), 3.55 (6H, s, COOCH_3), 4.20 (4H, t, $J = 7.7$ Hz, 3-NCH_2, 3″-NCH_2), 6.09 (2H, d, $J = 13.5$ Hz, 1′-CH, 3′-CH), 7.32–7.45 (4H, m, 5-CH, 6-CH, 5″-CH, 6″-CH), 7.65–7.75 (4H, m, 4-CH, 7-CH, 4″-CH, 7″-CH), 8.24 (1H, d, $J = 13.7$ Hz, 2′-CH). ^{13}C NMR (62.90 MHz, DMSO-d_6) δ (ppm): 21.6 (3-N(CH$_2$)$_2$$CH_2$, 3″-N(CH$_2$)$_2$$CH_2$), 27.0 (3-NCH$_2$$CH_2$, 3″-NCH$_2$$CH_2$), 32.8 ($CH_2$COOCH$_3$), 43.6 (3-NC$H_2$, 3″-NC$H_2$), 51.4 (COO$CH_3$), 85.1 (1′-C$H$, 3′-C$H$), 110.9 (7-C$H$, 7″-C$H$), 111.4 (4-C$H$, 4″-C$H$), 125.1 (6-C$H$, 6″-C$H$), 125.9 (5-C$H$, 5″-C$H$), 131.3 (3a-$C$, 3a″-$C$), 146.2 (2′-C$H$), 146.4 (7a-$C$, 7a″-$C$), 161.7 (2-$C$, 2″-$C$), 173.1 ($C$O). IR (neat) ν (cm^{-1}): 2952, 1715 (C=O), 1634, 1566 (C=C), 1510, 1460, 1194, 1081, 1007, 753. Vis (EtOH): 488 nm. HR-FAB-MS (3-NBA) $C_{29}H_{33}N_2O_6$: calcd., 505.2339; found, 505.2336.

*3.6.4. 3-(10-Methoxycarbonyldecyl)-2-{3-[3-(10-methoxycarbonyldecyl)-3H-benzoxazol-2-ylidene]propenyl}benzoxazol-3-ium hydrogensulphate (**6d**)*

Yield: 41%. Orange-coloured oil. ^{1}H NMR (250.13 MHz, DMSO-d_6) δ (ppm): 1.18–1.39 (24H, m, CH_2), 1.45–1.55 (4H, m, CH_2), 1.72–1.83 (4H, m, 3-NCH$_2$CH_2, 3″-NCH$_2$CH_2), 2.24 (4H, t, $J = 7.4$ Hz, CH_2COOCH$_3$), 3.55 (6H, s, COOCH_3), 4.20 (4H, t, $J = 7.2$ Hz, 3-NCH_2, 3″-NCH_2), 6.10 (2H, d, $J = 13.3$ Hz, 1′-CH, 3′-CH), 7.35–7.48 (4H, m, 5-CH, 6-CH, 5″-CH, 6″-CH), 7.67–7.75 (4H, m, 4-CH, 7-CH, 4″-CH, 7″-CH), 8.30 (1H, t, $J = 13.3$ Hz, 2′-CH). ^{13}C NMR (62.90 MHz, DMSO-d_6) δ (ppm): 24.3 (3-N(CH$_2$)$_8$$CH_2$, 3″-N(CH$_2$)$_8$$CH_2$), 25.9 ($CH_2$), 27.4 ($CH_2$), 28.3 ($CH_2$), 28.5 ($CH_2$), 28.6 ($CH_2$), 28.7 ($CH_2$), 28.8 ($CH_2$), 33.1 ($CH_2$COOCH$_3$), 43.7 (3-NC$H_2$, 3″-NC$H_2$), 51.0 (COO$CH_3$), 85.0 (1′-C$H$, 3′-C$H$), 110.8 (7-C$H$, 7″-C$H$), 111.2 (4-C$H$, 4″-C$H$), 124.9 (6-C$H$, 6″-C$H$), 125.8 (5-C$H$, 5″-C$H$), 131.1 (3a-$C$, 3a″-$C$), 146.1 (2′-C$H$), 146.3 (7a-$C$, 7a″-$C$), 161.5 (2-$C$, 2″-$C$), 173.1 ($C$O). IR (neat) ν (cm^{-1}): 2927, 2854, 1737 (C=O), 1565 (C=C), 1505, 1455, 1198, 1114, 1085, 1010, 750. Vis (EtOH): 488 nm. HR-FAB-MS (3-NBA) $C_{41}H_{57}N_2O_6$: calcd., 673.4217; found, 673.4218.

*3.7. Synthesis of asymmetric oxacarbocyanine dyes **7***

*3.7.1. 3-Carboxymethyl-2-[3-(3-ethyl-3H-benzoxazol-2-ylidene)propenyl]benzoxazol-3-ium inner salt (**7a**): typical procedure*

A solution of 0.131 g (0.481 mmol) of 3-carboxymethyl-2-methylbenzoxazol-3-ium bromide (**3a**), 0.201 g (0.464 mmol) of the acetanilide derivative **4a** and 0.094 g (0.13 ml, 0.93 mmol) of triethylamine in 5 ml of dry ethanol was refluxed for 3 h. After cooling to room temperature, diethyl ether was added and the resulting mixture was refrigerated to allow complete precipitation. The crystalline product obtained was collected by filtration under reduced pressure, washed with diethyl ether and recrystallized from dry acetonitrile. Yield: 97%. M.p. 198–199 °C. ^{1}H NMR (400.13 MHz, DMSO-d_6) δ (ppm): 1.38 (3H, t, $J = 7.0$ Hz, CH$_2$CH_3), 4.30 (2H, q, $J = 7.2$ Hz, 3″-NCH_2CH$_3$), 5.15 (2H, s, 3-NCH_2COO), 5.99 (1H, d, $J = 13.1$ Hz, 1′-CH), 6.20 (1H, d, $J = 13.4$ Hz, 3′-CH), 7.36–7.52 (4H, m, 5-CH, 6-CH, 5″-CH, 6″-CH), 7.65–7.81 (4H, m, 4-CH, 7-CH, 4″-CH, 7″-CH), 8.32 (1H, t, $J = 13.3$ Hz, 2′-CH). ^{13}C NMR (100.61 MHz, DMSO-d_6) δ (ppm): 12.8 (CH$_2$$CH_3$), 39.1.4 (3″-NC$H_2CH_3$), 44.6 (3-NC$H_2$COO), 84.0 (1′-C$H$), 86.6 (3′-C$H$), 110.7 (7-C$H$),

111.0 (4-*C*H, 7″-*C*H), 111.5 (4″-*C*H), 124.9 (6-*C*H), 125.4 (6″-*C*H), 125.8 (5-*C*H), 126.0 (5″-*C*H), 130.6 (3a″-*C*), 131.3 (3a-*C*), 146.0 (7a-*C*), 146.3 (2′-*C*H), 146.5 (7a″-*C*), 161.5 (2″-*C*), 162.1 (2-*C*), 167.5 (*C*O). IR (KBr) ν (cm^{-1}): 2974, 2938, 2739, 2678, 2491, 1725 (C=O), 1615, 1566 (C=C), 1511, 1464, 1398, 1205, 1118, 1090, 1036, 747. Vis (EtOH): 487 nm. HR-FAB-MS (3-NBA) $C_{21}H_{19}N_2O_4$: calcd., 363.1345; found, 363.1350.

The asymmetric oxacarbocyanine dyes **7b–d** were prepared using a similar procedure (3–18 h). The partial spectroscopic characterization of **7b** has been previously described [13].

3.7.2. 3-(2-Carboxyethyl)-2-[3-(3-propyl-3H-benzoxazol-2-ylidene)propenyl]benzoxazol-3-ium inner salt (*7b*)

Yield: 87%. M.p. 182–183 °C. ^{1}H NMR (400.13 MHz, DMSO-d_6) δ (ppm): 0.97 (3H, t, $J = 7.3$ Hz, CH_2CH_3), 1.76–1.85 (2H, m, CH_2CH_3), 2.80 (2H, t, $J = 6.8$ Hz, CH_2COO), 4.18 (2H, t, $J = 6.8$ Hz, 3″-NCH_2), 4.35 (2H, t, $J = 6.5$ Hz, 3-NCH_2), 6.11 (1H, d, $J = 13.9$ Hz, 1′-C*H*), 6.14 (1H, d, $J = 14.0$ Hz, 3′-C*H*), 7.36–7.48 (4H, m, 5-C*H*, 6-C*H*, 5″-C*H*, 6″-C*H*), 7.70–7.78 (4H, m, 4-C*H*, 7-C*H*, 4″-C*H*, 7″-C*H*), 8.29 (1H, t, $J = 13.4$ Hz, 2′-C*H*). ^{13}C NMR (100.61 MHz, DMSO-d_6) δ (ppm): 10.8 (CH_2CH_3), 20.8 (CH_2CH_3), 32.0 (CH_2COO), 40.1 (3-NCH_2), 45.1 (3″-NCH_2), 85.2 (1′-*C*H), 85.4 (3′-*C*H), 110.7 (7-*C*H), 110.9 (7″-*C*H), 111.4 (4-*C*H), 111.7 (4″-*C*H), 124.9 (6-*C*H), 125.1 (6″-*C*H), 125.7 (5-*C*H), 125.9 (5″-*C*H), 131.1 (3a-*C*), 131.3 (3a″-*C*), 146.2 (2′-*C*H), 146.3 (7a-*C*), 146.4 (7a″-*C*), 161.6 (2-*C*), 161.7 (2″-*C*), 171.9 (*C*O). IR (KBr) ν (cm^{-1}): 3441, 2974, 2938, 2762, 2739, 2679, 2492, 1724 (C=O), 1568 (C=C), 1510, 1464, 1452, 1204, 750. Vis (EtOH): 487 nm. HR-FAB-MS (3-NBA) $C_{23}H_{23}N_2O_4$: calcd., 391.1658; found, 391.1654.

3.7.3. 3-(4-Carboxybutyl)-2-[3-(3-pentyl-3H-benzoxazol-2-ylidene)propenyl]benzoxazol-3-ium inner salt (*7c*)

Yield: 32%. M.p. 94–96 °C. ^{1}H NMR (250.13 MHz, DMSO-d_6) δ (ppm): 0.86 (3H, t, $J = 6.4$ Hz, CH_2CH_3), 1.28–1.39 (4H, m, CH_2), 1.55–1.83 (6H, m, CH_2), 2.29 (2H, t, $J = 6.7$ Hz, CH_2COO), 4.26–4.39 (4H, m, 3-NCH_2, 3″-NCH_2), 6.09 (1H, d, $J = 13.3$ Hz, 1′-C*H*), 6.12 (1H, d, $J = 13.3$ Hz, 3′-C*H*), 7.35–7.48 (4H, m, 5-C*H*, 6-C*H*, 5″-C*H*, 6″-C*H*), 7.69–7.77 (4H, m, 4-C*H*, 7-C*H*, 4″-C*H*, 7″-C*H*), 8.25 (1H, t, $J = 13.3$ Hz, 2′-C*H*). ^{13}C NMR (62.90 MHz, DMSO-d_6) δ (ppm): 13.8 (CH_2CH_3), 21.6 (CH_2), 21.8 (CH_2), 26.9 (CH_2), 27.1 (CH_2), 28.1 (CH_2), 33.1 (CH_2COO), 43.5 (3″-NCH_2), 43.8 (3-NCH_2), 85.0 (1′-*C*H, 3′-*C*H), 110.9 (7-*C*H, 7″-*C*H), 111.3 (4-*C*H, 4″-*C*H), 125.0 (6-*C*H, 6″-*C*H), 125.8 (5-*C*H, 5″-*C*H), 131.2 (3a-*C*, 3a″-*C*), 146.1 (2′-*C*H), 146.3 (7a-*C*, 7a″-*C*), 161.5 (2-*C*, 2″-*C*) 174.3 (*C*O). IR (KBr) ν (cm^{-1}): 2935, 2678, 1725 (C=O), 1565 (C=C), 1511, 1452, 1391, 1355, 1192, 1112, 1081, 748. Vis (EtOH): 488 nm. HR-FAB-MS (3-NBA) $C_{27}H_{31}N_2O_4$: calcd., 447.2284; found, 447.2267.

3.7.4. 3-(10-Carboxydecyl)-2-[3-(3-undecy)-3H-benzoxazol-2-ylidene)propenyl]benzoxazol-3-ium inner salt (*7d*)

Yield: 79%. M.p. 81–83 °C. ^{1}H NMR (400.13 MHz, DMSO-d_6) δ (ppm): 0.79 (3H, t, $J = 7.0$ Hz, CH_2CH_3), 1.09–1.45 (30H, m, CH_2), 1.70–1.78 (4H, m, CH_2), 2.14 (2H, t, $J = 7.1$ Hz, CH_2COO), 4.15–4.22 (4H, m, 3-NCH_2, 3″-NCH_2), 6.12 (2H, d, $J = 13.5$ Hz, 1′-C*H*, 3′-C*H*), 7.35–7.46 (4H, m, 5-C*H*, 6-C*H*, 5″-C*H*, 6″-C*H*), 7.66–7.77 (4H, m, 4-C*H*, 7-C*H*, 4″-C*H*, 7″-C*H*), 8.25 (1H, t, $J = 13.5$ Hz, 2′-C*H*). ^{13}C NMR (100.61 MHz, DMSO-d_6) δ (ppm): 13.9 (CH_2CH_3), 22.1 (CH_2), 24.4 (CH_2), 25.9 (CH_2), 27.4 (CH_2), 28.5 (CH_2), 28.7 (CH_2), 28.8 (CH_2), 28.8 (CH_2), 28.9 (CH_2), 28.9 (CH_2), 31.3 (CH_2), 33.6 (CH_2COO), 43.8 (3-NCH_2, 3″-NCH_2), 85.2 (1′-*C*H, 3′-*C*H), 110.9 (7-*C*H, 7″-*C*H), 111.4 (4-*C*H, 4″-*C*H), 125.1 (6-*C*H, 6″-*C*H), 125.9 (5-*C*H, 5″-*C*H), 131.3 (3a-*C*, 3a″-*C*), 146.2 (2′-*C*H), 146.4 (7a-*C*, 7a″-*C*), 161.6 (2-*C*, 2″-*C*), 174.3 (*C*O). IR (KBr) ν (cm^{-1}): 3439, 2974, 2934, 2853, 2758, 2739, 2679, 2492, 1713 (C=O), 1566 (C=C), 1510, 1462, 1397, 1200, 1115, 1086, 1036, 746. Vis (EtOH): 487 nm. HR-FAB-MS (3-NBA) $C_{39}H_{55}N_2O_4$: calcd., 615.4162; found, 615.4135.

4. Conclusions

Several new mono- and dicarboxyalkylcarbocyanines derived from benzoxazole have been synthesized, using an optimized method previously developed for the benzothiazole analogues, and were fully spectroscopically characterized.

The presence of a terminal carboxylic group in the pendant *N*-alkyl chain of these oxacarbocyanine dyes turns them suitable candidates as ligands for AC. The different lengths of the *N*-carboxyalkyl moities, together with the asymmetry due to the presence of one vs two bounding groups, could play an important role as spacer arms in AC supports.

Acknowledgements

Thanks are due to *Fundação para a Ciência e Tecnologia*, Portugal, for the funding of the Project "Development of New Supports for Dye-Affinity Chromatography" (POCTI/QUI/44776/2002).

References

[1] Boto REF, Oliveira AS, Vieira Ferreira LF, Almeida P. A study of *N,N′*-dicarboxyalkylthiacarbocyanines as cyanine direactive dyes covalently bound to cellulose. Dyes Pigments 2001;48(2):71–84.
[2] Boto REF, El-Shishtawy RM, Santos PF, Reis LV, Almeida P. Synthesis and characterization of novel mono- and dicarboxyalkylthiacarbocyanines and their ester derivatives. Dyes Pigments 2007;73(2):195–205.
[3] Brooker LGS, Smith LA. US: Eastman Kodak Company. US 2231658; 1941.
[4] Fujimura N, Ishikawa S. Japan: Canon KK. JP 61110145; 1986.
[5] Nakayama H, Myasaki K, Mitsumata T. Japan: Jpn Kokai Tokkyo Koho. JP 06313117, 1994; Chem Abstr; 1995; 122:108670v.
[6] Kumar S, Nampalli S, Khot M. US: Amersham Biosciences Corp. US 6967250B1; 2005.

[7] Kumar S, Nampalli S, Khot M. US: Amersham Pharmacia Biotech Inc. WO 00/13026; 2000.
[8] Flick P. US: Amersham Biosciences Corp. WO 99/39203; 1999.
[9] Glazer AN, Mathies RA, Hung S-C, Jue J. US: The Regents of the University of California. WO 98/14612; 1998.
[10] Hung S-C, Mathies RA, Glazer AN. Comparison of fluorescent energy transfer primes with different donor–acceptor dye combinations. Anal Biochem 1998;255(1):32–8.
[11] Yiwen W, Hung S-C, Linn JF, Steiner G, Glazer AN, Sidransky D, et al. Microsatellite-based cancer detection using capillary array electrophoresis and energy-transfer fluorescent primers. Electrophoresis 1997;18(19): 1742–9.
[12] Hung S-C, Mathies RA, Glazer AN. Optimization of spectroscopic and electrophoretic properties of energy transfer primers. Anal Biochem 1997;252(1):78–88.
[13] Hung S-C, Mathies RA, Glazer AN. Cyanine dyes with high absorption cross section as donors chromophores in energy transfer primers. Anal Biochem 1996;243(1):15–27.
[14] Hermanson GT, Mallia AK, Smith PK. In: Hermanson GT, editor. Immobilized affinity ligand techniques. San Diego: Academic Press; 1992. p. 137–57.
[15] Silva A, Boto REF, El-Shishtawy RM, Almeida P. Rhodamine B as ligand for affinitty chromatography. Fixation studies onto cellulose by a curing method. Eur Polym J 2006, accepted for publication.
[16] Hamer FM. In: Weissberger A, editor. The cyanine dyes and related compounds – the chemistry of heterocyclic compounds, vol. 18. New York: Interscience; 1971.
[17] Pardal AC, Ramos SS, Santos PF, Reis LV, Almeida P. Synthesis and spectroscopic characterization of *N*-alkyl quaternary ammonium salts typical precursors of cyanines. Molecules 2002;7:320–30.