Synthesis and photophysical properties of *meso*-aminophenyl-substituted heptamethine dyes as potential leads to new contrast agents

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Abstract

The series of rigid *meso*-aminophenyl substituted heptamethine dyes presented herein exhibit surprising fluorescence properties, demonstrating larger Stokes shifts when compared to both structurally-similar rigid *meso*-chlorophenyl and linear heptamethine dyes. Based on their photophysical properties, these are of considerable importance to the development of contrast agents, within biology and medicine.

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Introduction

The detection, imaging and quantification of biomolecules play a vital part within biomedicine especially in areas relating to the non-invasive monitoring of diseased tissues.[1] Indeed, the use of fluorescent probes within the biosciences has revolutionised the way biomolecules are visualised.[2] Fluorescent probes with spectra within the near infrared region (700-1000nm) offer significant advantages over those which absorb within the visible region. Such advantages include minimal tissue auto-fluorescence, resulting mainly from flavins, flavoproteins and NADH, that can often hinder the visualisation of the probe. Other advantages includes, sharper optical contrast with less scatter, deeper light penetration into tissues and the possibility of specific chemotargeting through the attachment of sugars, amino acids, monoclonal antibodies or aptamers, all of which are advantageous for cell and tissue imaging.[3]

The clinically established heptamethine dyes such as Indocyanine Green (ICG) and New Indocyanine Green (IR-820) have proved to be excellent molecular probes; both possess reasonable fluorescent quantum yields, low photo-bleaching, good aqueous solubility, high signal to noise ratio, low toxicity and rapid clearance. [4] Both probes absorb within the near infrared 800-850nm range, which again is advantageous as this is well away from endogenous absorbers such as haemoglobin and melanin. The shift into the near infrared can be attributed to the extension of conjugation due to benzene fusion in the dihydrobenzo[*e*]indolyl groups which increases the bathochromic shift of these dyes by approximately 30nm,[5] in comparison to dyes such as IR-746 and IR-783, neither of which possesses the fused aromatic system. IR-820 also has a chloro-substituted cyclohexyl moiety incorporated into the polymethine backbone, allowing for increased photostability.[6] It also allows the prospect of addition of nucleophilic moieties, which may not only alter the photophysical properties, but also an option of adding groups such as amino acids, oligopeptides or sugars for specific sub-cellular targeting. Such a pathway would be advantageous as discussed above.

Inspired by this, we report the synthesis and photophysical properties for a series of structurally related non-targeting NIR heptamethine cyanine dyes, bearing fused and non-fused benzene subunits. These contain either a polymethine backbone that is partially rigidified by the inclusion of a central cyclic moiety with a chlorine atom in the *meso* position, or alternatively an aminophenyl moiety at the *meso* position. Our intention was to compare the photophysical properties (absorption and fluorescence spectra, fluorescence quantum yields and Stokes shifts) of the dyes bearing an aniline moiety against those bearing a chlorine atom at the *meso* position. These were also compared to the simple linear heptamethine conjugated chain analogues, in order to investigate the effects of replacing the *meso*-chlorine atom with a phenylamino moiety, in addition to determining the effect of the fused benzene ring on the dyes' absorption characteristics.

Experimental

General information

NMR spectra were recorded on either a Bruker DPX 250 MHz, Bruker Avance-III 300 MHz or a Bruker Avance 400 MHz spectrometer at ambient temperature. Chemical shifts are reported in ppm relative to residual protic solvent (^{1}H NMR d_6-DMSO, 2.500 ppm; ^{13}C NMR d_6-DMSO, 39.520 ppm). Low and high resolution mass spectra (HRMS) were obtained using electrospray ionization (ESI) mass spectrometry on a hybrid linear ion trap-fourier transform mass spectrometer. Accurate mass measurements were carried out for novel compounds by the EPSRC National mass spectrometry service research group using the Thermofisher LTQ Orbitrap XL. Stock solutions for UV-Vis spectroscopy of dyes were prepared in methanol. The absorbance and fluorescence spectra of each of the dyes were measured sequentially to reduce photobleaching and solubility issues. The fluorescence quantum yields of the dyes were calculated using the relative method i.e. integrated fluorescence peak area versus fraction of light absorbed at the excitation wavelength were plotted for both the standards and cyanine dyes.[1] Fluorescence quantum yields ($\boldsymbol{\phi}$) were measured using a fluorimeter based on an Innovative Photonic Solutions 785 nm diode laser, operating with a power output of 8 mW, an Andor Shamrock SR-303i spectrograph and an Andor iDus CCD detector (model DU420A-BR-DD). Fluorescence was detected at right angles without any filters. The spectral response of the system was corrected following the method outlined by Kosch and coworkers.[2] Absorbance values (A) of solutions at 785 nm were measured using a Perkin Elmer Lambda 25 spectrophotometer. Corrected fluorescence spectra for a series of solutions of increasing concentration were rescaled in energy and the integrated intensity was plotted versus $1\text{-}10^{-A}$, with A (785 nm) ≤ 0.1. The quantum yields were obtained from the relative slopes of such plots compared with that from solutions of ICG in dimethyl sulfoxide. For determinations in different solvents, the refractive index (n^2) correction was applied. Thin Layer Chromatography (TLC) was carried out on Machery-Nagel polygramSil/G/UV254 pre-coated plates. Melting point (m.p) analysis was carried out using a Griffin melting point apparatus. Infra-red spectra (1800-800 cm^{-1}) were recorded on a Perkin Elmer Spectrum RX 1 with a Specac Golden Gate™ ATR accessory and values are quoted in wavenumbers. All chemicals were purchased from commercial sources and used without further purification. Purification of the final dyes were accomplished via column chromatography on Silca gel eluting with the solvent blends listed within the experimental methods as well as the supporting information.

The synthesis of a **1e**, **2e** and the reactive imine from the Vilsmeier-Haack formylation of cyclohexanone (**VMI**) are shown in the experimental section below for clarity. More information concerning the synthesis of these dyes, the reactive pyridinium salt and the linear hepthamethine dyes (**4a-j**) is shown in Supporting Information. The full synthesis of compounds 3a – 3j is given below.

1-Benzyl-2,3,3-trimethyl-3*H*-indol-1-ium bromide (1e)

To a refluxing solution of benzyl bromide (0.80 mL, 6.74 mmol) in acetonitrile (20.0 mL) was added dropwise 2,3,3-trimethylindolenine (1.00 g, 6.28 mmol) in acetonitrile (20.0 mL) and the reaction was held at reflux with constant stirring for 2 days. Upon cooling, the precipitate produced was isolated at the pump, washed with *n*-hexane and dried *in vacuo* to give the product **1k** (1.65 g, 79.62%) as a red hygroscopic solid.

^{1}H NMR (d_6-DMSO, 250 MHz) δ 7.89 (d, *J*=7.0 Hz, 1H, Ar-H), 7.84 (d, *J*=7.0 Hz, 1H, Ar-H), 7.67-7.59 (m, 2H, Ar-H), 7.45-7.38 (m, 5H, Ar-H), 5.87 (s, 2H, N-CH_2), 3.02(s, 3H, N-C-CH_3), 1.55 (s, 6H C-$(CH_3)_2$). ^{13}C NMR (d_6-DMSO, 62.8 MHz) δ 198.7, 142.5, 141.6, 132.7, 130.1, 129.5, 129.3, 128.1, 124.2, 116.5, 55.1, 51.2, 22.7, 15.1, 9.8. IR (ATR) 2969, 1603, 1454, 931, 741, 701, 567 cm^{-1}. MS (ESI) m/z: 250 $[M]^+$.

***N*-[5-Anilino-3-chloro-2,4-(propane-1,3-diyl)-2,4-pentadiene]anilinium chloride (VMI)**

To a stirred solution of anhydrous DMF (13.0 mL, 168 mmol) held at 0°C was added dropwise $POCl_3$ (11.0 mL, 117mmol). After 30 min, cyclohexanone (5.50 mL, 53.1 mmol) was added and the mixture was heated under reflux for 1 h. The reaction was cooled to 20 ºC and with constant stirring, a mixture of aniline/EtOH [1:1 (v/v), 18.0 mL] was added dropwise. The reaction was continued for 30 min at 20 ºC with vigorous stirring and the deep purple solution was poured into H_2O/HCl [10:1 (v/v), 110 mL]. After two hours of the solution being held in an ice bath, the crystals formed were isolated at the pump and washed with cold H_2O and Et_2O. The solid was allowed to dry at the pump to yield *N*-[5-anilino-3-chloro-2,4-(propane-1,3-diyl)-2,4-pentadiene]anilinium chloride (15.4 g, 81%) as a dark purple solid.

^{1}H NMR (d_6-DMSO, 250 MHz) δ 11.2 (br s, 2H, NH), 8.55 (d, *J*=14.0 Hz, 2H, CH*alkene*), 7.68 (d, *J*=7.0 Hz, 4H, Ar-H), 7.44 (t, *J*=7.0 Hz, 4H, Ar-H), 7.30-7.28 (m, 2H, Ar-H), 3.03 (t, *J*=6.0 Hz, 4H, CH_2-CH_2), 2.05-1.98 (m, 2H, CH_2-CH_2). IR (ATR) 1609, 1562, 1457, 1267, 1179, 752, 683, 565 cm-1. MS (ESI) m/z: 323.22 $[M]^+$.

1-Benzyl-2-[(*E*)-2-[(3*E*)-3-{2-[(2*E*)-1-benzyl-3,3-dimethyl-2,3-dihydro-1*H*-indol-2-ylidene]ethylidene}-2-chlorocyclohex-1-en-1-yl]ethenyl]-3,3-dimethyl-3*H*-indol-1-ium bromide (2e).

To a solution of **1e** (0.66 g, 2 mmol) and anhydrous sodium acetate (0.16 g, 1.95 mmol) in EtOH (30.0 mL) was added *N*-[5-anilino-3-chloro-2,4-(propane-1,3-diyl)-2,4-pentadiene]anilinium chloride (0.36 g, 1 mmol) with constant stirring, and the solution was heated under reflux for 4 h. Upon cooling, the solution was evaporated to dryness under reduced pressure and the crude product was purified by column chromatography on silica gel eluting with $CHCl_3$ 9: MeOH 1 to obtain **2e** (0.10 g, 14.87%) as a shiny purple solid.

^{1}H NMR (d_6-DMSO, 400 MHz) δ 8.25 (d, *J*=14.0 Hz, 2H, CH_{alkene}), 7.41- 7.28 (m, 18H, Ar-H), 6.35 (d, *J*=14.0 Hz, 2H, CH_{alkene}), 4.95 (br s, 4H, N-CH_2-Ph), 2.50-2.45 (m, 4H, CH_2-CH_2), 1.69 (s, 12H, C-CH_3), 1.30-1.26 (m, 2H, CH_2-CH_2-CH_2).IR (ATR) 1543, 1363, 1225, 1099, 896, 784, 668, 597 cm^{-1}. MS (ESI) m/z: 635 $[M]^+$.λ_{max} = 760 nm.

1,3,3-Trimethyl-2-[(*E*)-2-[(3*E*)-2-(phenylamino)-3-{2-[(2*E*)-1,3,3-trimethyl-2,3-dihydro-1*H*-indol-2-ylidene]ethylidene}cyclohex-1-en-1-yl]ethenyl]-3*H*-indol-1-ium iodide (3a)

Aniline (1.00 mmol) was added by syringe to a solution of **2a** (0.10 mmol) dissolved in anhydrous DMF (10.0 mL) under nitrogen. The reaction mixture was stirred at 85°C overnight under nitrogen. The solvent was removed under reduced pressure, the crude product was purified by column chromatography on silica gel eluting with DCM 9 : MeOH 1, then and treated with propan-2-ol to afford **3a** (20 mg, 30%) as a dark green solid.

^{1}H NMR (d_6-DMSO, 250 MHz) δ 8.62 (br s, 1H, Ar-NH), 7.93 (d, *J*=14.0 Hz, 2H, CH_{alkene}), 7.49 (d, *J*=6.0 Hz, 2H, Ar-H), 7.30-7.21 (m, 9H, Ar-H), 6.90 (d, *J*=6.0 Hz, 2H, ArH), 6.05 (d, *J*=14.0 Hz, 2H, CH_{alkene}), 3.65 (s, 6H, N-CH_3), 2.62 (t, *J*=6.0 Hz, 4H, CH_2), 1.85-1.79 (m, 2H, CH_2), 1.03 (s, 12H, C-CH_3). IR (ATR) 2928, 1555, 1484, 1436, 1349, 1308, 1206, 1149, 915, 786 cm^{-1}. MS (ESI) m/z: 540 $[M]^+$. λ_{max} = 736 nm. m.p. 213-215°C.

1-Ethyl-2-[(*E*)-2-[(3*E*)-3-{2-[(2*E*)-1-ethyl-3,3-dimethyl-2,3-dihydro-1*H*-indol-2-ylidene]ethylidene}-2-(phenylamino)cyclohex-1-en-1-yl]ethenyl]-3,3-dimethyl-3*H*-indol-1-ium iodide (3b).

3b was synthesised as for **3a** using **2b** (0.10 mmol), aniline (1.00 mmol), and anhydrous DMF (10.0 mL). The solvent was removed under reduced pressure, the crude product was purified by column chromatography on silica gel eluting with DCM 9 : MeOH 1 to afford **3b** (24 mg, 35%) as a shiny dark blue solid.

^{1}H NMR ($CDCl_3$, 400 MHz) δ 9.05 (br s, 1H, Ar-NH), 8.16 (d, *J*=14.0 Hz, 2H, CH_{alkene}), 7.41-7.35 (m, 5H, ArH), 7.22 (t, *J*=8.0 Hz, 4H, Ar-H), 7.09 (t, *J*=7.0 Hz, 2H, Ar-H), 6.92 (d, *J*=8.0 Hz, 2H, Ar-H), 6.80 (t, *J*=7.0 Hz, 2H, Ar-H), 5.79 (d, *J*=14.0 Hz, 2H, CH_{alkene}), 3.56 (q, *J*=9.0 Hz, 4H, NCH_2), 2.57 (t, *J*=6.0 Hz, 4H, CH_2), 1.92-1.84 (m, 2H, CH_2), 1.72 (t, *J*=6.0 Hz, 6H, CH_3), 1.36 (s, 12H, C-CH_3). IR (ATR) 2922, 1560, 1484, 1436, 1342, 1318, 1206, 1129, 905, 783 cm^{-1}. MS (ESI) m/z 568 $[M]^+$. λ_{max} = 735 nm. m.p. 217-219°C.

2-[(*E*)-2-[(3*E*)-3-{2-[(2*E*)-3,3-Dimethyl-1-propyl-2,3-dihydro-1*H*-indol-2-ylidene]ethylidene}-2-(phenylamino)cyclohex-1-en-1-yl]ethenyl]-3,3-dimethyl-1-propyl-3*H*-indol-1-ium iodide (3c).

3c was synthesised as for **3a** using **2c** (0.10 mmol), aniline (1.00 mmol) and anhydrous DMF (10.0 mL). The solvent was removed under reduced pressure, the crude product was purified by column chromatography on silica gel eluting with DCM 9 : MeOH 1 to afford **3c** (31 mg, 43%) as a shiny dark blue solid.

^{1}H NMR ($CDCl_3$, 400 MHz) δ 9.02 (br s, 1H, Ar-NH), 8.14 (d, *J*=14.0 Hz, 2H, CH_{alkene}), 7.45-7.38 (m, 3H, ArH), 7.18-7.12 (m, 5H, Ar-H), 7.09 (t, *J*=7.0 Hz, 2H, Ar-H), 6.90 (d, *J*=8.0 Hz, 2H, Ar-H), 6.78 (t, *J*=7.0 Hz, 1H, Ar-H), 5.79 (d, *J*=14.0 Hz, 2H, CH_{alkene}), 3.88 (t, *J*=7.0 Hz, 4H, NCH_2), 2.56 (t, *J*=6.0 Hz, 4H, CH_2), 1.93 (quin, *J*=6.0 Hz, 2H, CH_2), 1.83-1.76 (m, 4H, CH_2), 1.38 (s, 12H, C-CH_3), 1.01 (t, *J*=7.0 Hz, 6H, CH_2-CH_3). IR (ATR) 2920, 1549, 1431, 1344, 1232, 1151, 944, 787 cm^{-1}. MS (ESI) m/z: 596 $[M]^+$. λ_{max} = 746 nm. m.p. 215-217°C.

2-[(*E*)-2-[(3*E*)-3-{2-[(2*E*)-3,3-Dimethyl-1-(4-sulfonatobutyl)-2,3-dihydro-1*H*-indol-2-ylidene]ethylidene}-2-(phenylamino)cyclohex-1-en-1-yl]ethenyl]-3,3-dimethyl-1-(4-sulfonatobutyl)-3*H*-indol-1-ium (3d).

3d was synthesised as for **3a** using **2a** (0.10 mmol), aniline (1.00 mmol) and anhydrous DMF (10.0 mL). The solvent was removed under reduced pressure, the crude product was purified by column chromatography on silica gel eluting with $CHCl_3$ 8 : MeOH 2 to afford **3d** (31 mg, 40%) as a dark green solid.

^{1}H NMR (d_6-DMSO, 400 MHz) δ 8.65 (br s, 1H, Ar-NH), 7.98 (d, *J*=14.0 Hz, 2H, CH_{alkene}), 7.43 (d, *J*=7.0 Hz, 2H, ArH), 7.33-7.28 (m, 4H, Ar-H), 7.22 (t, *J*=8.0 Hz, 2H, Ar-H), 7.13-6.99 (m, 2H, Ar-H), 6.93 (d, *J*=7.0 Hz, 2H, Ar-H), 6.71 (d, *J*=7.0 Hz, 1H, Ar-H), 6.10 (d, *J*=14.0 Hz, 2H, CH_{alkene}), 4.05 (t, 4H, *J*=7.0 Hz, N-CH_2), 2.63 (t, *J*=6.0 Hz, 4H, CH_2), 2.47 (t, *J*=7.0 Hz, 4H, CH_2), 1.89 (br s, 2H, CH_2), 1.71-1.63 (m, 8H, CH_2), 1.28 (s, 12H, C-CH_3). IR (ATR) 2928, 1508, 1437, 1366, 1097, 1101, 908, 790 cm^{-1}. MS (ESI) m/z: 784 $[M+2H]^+$. λ_{max} = 745 nm. m.p. 179-181°C.

1-Benzyl-2-[(*E*)-2-[(3*E*)-3-{2-[(2*E*)-1-benzyl-3,3-dimethyl-2,3-dihydro-1*H*-indol-2-ylidene]ethylidene}-2-(phenylamino)cyclohex-1-en-1-yl]ethenyl]-3,3-dimethyl-3*H*-indol1-ium bromide (3e).

3e was synthesised as for **3a** using 2e (0.10 mmol), aniline (1.00 mmol) and anhydrous DMF (10.0 mL). The solvent was removed under reduced pressure, the crude product was purified by column chromatography on silica gel eluting $CHCl_3$ 9 : MeOH 1 to afford **3e** (13 mg, 17%) as a dark shiny blue solid.

^{1}H NMR (d_6-DMSO, 400 MHz) δ 8.87 (br s, 1H, Ar-NH), 7.95 (d, *J*=14.0 Hz, 2H, CH_{alkene}), 7.48 (d, *J*=7.0 Hz, 2H, ArH), 7.33-7.32 (m, 4H, Ar-H), 7.31-7.28 (m, 6H, Ar-H), 7.22-7.19 (m, 6H, Ar-H), 7.14 (t, *J*=7.0 Hz, 2H, Ar-H), 6.95 (d, *J*=7.0 Hz, 2H, Ar-H), 6.78 (t, *J*=7.0 Hz, 1H, Ar-H), 6.07 (d, *J*=14.0 Hz, 2H, CH_{alkene}), 5.36 (s, 4H, N-CH_2), 2.43 (t, *J*=5.0 Hz, 4H, CH_2), 1.73 (quin, *J*=6.0 Hz, 2H, CH_2), 1.33 (s, 12H, CH_3). IR (ATR) 2921, 1504, 1436, 1361, 1224, 1005, 897, 787 cm^{-1}. MS (ESI) m/z: 692 $[M]^+$. HRMS(ESI): Calcd for $C_{50}H_{52}N_3$ $[M]^+$ 692.3978, found 692.3980. λ_{max} = 736 nm. m.p. 210-212 °C.

1,1,3-Trimethyl-2-[(*E*)-2-[(3*E*)-2-(phenylamino)-3-{2-[(2*E*)-1,1,3-trimethyl-1*H*,2*H*,3*H*-benzo[*e*]indol-2-ylidene]ethylidene}cyclohex-1-en-1-yl]ethenyl]-1*H*-benzo[*e*]indol-3-ium iodide (3f).

3f was synthesised as for **3a** using **2f** (0.10 mmol), aniline (1.00 mmol) and anhydrous DMF (10.0 mL). The solvent was removed under reduced pressure, the crude product was purified by column chromatography on silica gel eluting with DCM 9 : MeOH 1 to afford **3f** (34 mg, 44%) as a shiny dark blue solid.

^{1}H NMR (d_6-DMSO, 300 MHz) δ 8.76 (br s, 1H, NH), 8.37 (t, *J*=9.0 Hz, 2H, Ar-H), 8.16 (d, *J*=9.0 Hz, 2H, Ar-H), 7.77-7.67 (m, 4H, Ar-H), 7.66-7.63 (m, 5H, Ar-H), 7.46 (t, *J*=9.0 Hz, 2H, Ar-H), 7.32 (t, *J*=9.0 Hz, 1H, Ar-H), 7.25 (t, *J*=9.0 Hz, 2H, Ar-H), 6.59 (d, *J*=12.0 Hz, 2H, CH_{alkene}), 5.42

(d, *J*=12.0 Hz, 2H, CH_{alkene}), 3.70 (s, 6H, N-CH_3), 2.83-2.66 (m, 2H, CH_2), 2.28 (t, *J*=6.0 Hz, 4H, CH_2), 1.20 (s, 12H, CH_3). IR (ATR) 2992, 1515, 1441, 1350, 1228, 1011, 929, 747 cm^{-1}. MS (ESI) m/z: 638 $[M]^+$. HRMS(ESI): Calcd for $C_{46}H_{46}N_3$ $[M]^+$ 638.3530, found 638.3529. λ_{max} = 780 nm. m.p. 188-190°C.

3-Ethyl-2-[(*E*)-2-[(3*E*)-3-{2-[(2*E*)-3-ethyl-1,1-dimethyl-1*H*,2*H*,3*H*-benzo[*e*]indol-2-ylidene]ethylidene}-2-(phenylamino)cyclohex-1-en-1-yl]ethenyl]-1,1-dimethyl-1*H*-benzo[*e*]indol-3-ium iodide (3g).

3g was synthesised as for **3a** using **2g** (0.10 mmol), aniline (1.00 mmol) and anhydrous DMF (10.0 mL). The solvent was removed under reduced pressure, the crude product was purified by column chromatography on silica gel eluting with DCM 9 : MeOH 1 to afford **3g** (27 mg, 34%) as a shiny dark blue solid.

^{1}H NMR (d_6-DMSO, 300 MHz) δ 8.74 (br s, 1H, NH), 8.14-8.01 (m, 8H, Ar-H), 7.67 (d, *J*=9.0 Hz, 2H, Ar-H), 7.60 (t, *J*=9.0 Hz, 2H, Ar-H), 7.47 (t, *J*=9.0 Hz, 2H, Ar-H), 7.31 (d, *J*=15.0 Hz, 2H, CH_{alkene}), 7.03 (d, *J*=9.0 Hz, 2H, Ar-H), 6.79 (t, *J*=6.0 Hz, 1H, Ar-H), 6.14 (d, *J*= 15.0 Hz, 2H, CH_{alkene}), 4.25 (q, *J*=6.0 Hz, 4H, N-CH_2), 2.70 (t, *J*=6.0 Hz, 4H, CH_2), 1.96-1.87 (m, 4H, CH_2), 1.62 (s, 12H, CH3), 1.32 (t, *J*=9.0 Hz, 6H, CH3). IR (ATR) 2921, 1546, 1430, 1346, 1226, 1001, 901, cm^{-1}. MS (ESI) m/z: 668 $[M]^+$. HRMS(ESI): Calcd for $C_{48}H_{50}N_3[M]^+$ 668.3999, found 668.3993. λ_{max} = 776 nm. m.p. 188-190°C.

2-[(*E*)-2-[(3*E*)-3-{2-[(2*E*)-1,1-imethyl-3-propyl-1*H*,2*H*,3*H*-benzo[*e*]indol-2-ylidene]ethylidene}-2-(phenylamino)cyclohex-1-en-1-yl]ethenyl]-1,1-dimethyl-3-propyl-1*H*-benzo[*e*]indol-3-ium iodide (3h).

3h was synthesised as for **3a** using **2h** (0.10 mmol), aniline (1.00 mmol) and anhydrous DMF (10.0 mL). The solvent was removed under reduced pressure, the crude product was purified by column chromatography on silica gel eluting with DCM 9 : MeOH 1 to afford **3h** (31 mg, 38%) as a shiny dark blue solid.

^{1}H NMR (d_6-DMSO, 300 MHz) δ 8.76 (br s, 1H, NH), 8.13-8.07 (m, 4H, Ar-H), 8.02-7.98 (m, 4H, Ar-H), 7.68 (d, *J*=9.0 Hz, 2H, Ar-H), 7.57 (t, *J*=9.0 Hz, 2H, Ar-H), 7.46 (t, *J*=9.0 Hz, 2H, Ar-H), 7.31 (d, *J*=15.0 Hz, 2H, CH_{alkene}), 7.03 (d, *J*=9.0 Hz, 2H, Ar-H), 6.78 (t, *J*=6.0 Hz, 1H, Ar-H), 6.14 (d, *J*= 15.0 Hz, 2H, CH_{alkene}), 4.20 (t, *J*=9.0 Hz, 4H, N-CH_2), 2.68 (t, *J*=6.0 Hz, 4H, CH_2), 1.92-180 (m, 2H, CH_2), 1.78-1.61 (m, 4H, N-CH_2), 0.97 (s, 12H, CH_3), 0.97 (t, *J*=9.0 Hz, 6H, CH_3). IR (ATR) 2922, 1547, 1430, 1342, 1225, 1001, 897, 711 cm^{-1}. MS (ESI) m/z: 696 $[M]^+$. HRMS(FAB): Calcd for $C_{50}H_{54}N_3[M]^+$ 696.4312, found 696.4304. λ_{max} = 778 nm. m.p. 188-190°C.

2-[(*E*)-2-[(3*E*)-3-{2-[(2*E*)-1,1-Dimethyl-3-(4-sulfonatobutyl)-1*H*,2*H*,3*H*-benzo[*e*]indol-2-ylidene]ethylidene}-2-(phenylamino)cyclohex-1-en-1-yl]ethenyl]-1,1-dimethyl-3-(4-sulfonatobutyl)-1*H*-benzo[*e*]indol-3-ium (3i).

3i was synthesised as for **3a** using **2i** (0.10 mmol), aniline (1.00 mmol), and anhydrous DMF (10.0 mL). The solvent was removed under reduced pressure, the crude product was

purified by column chromatography on silica gel eluting with DCM 9 : MeOH 1 to afford **3i** (35 mg, 40%) as a green solid.

^{1}H NMR (d_6-DMSO, 300 MHz) δ 8.75 (brs, 1H, NH), 8.12-8.08 (m, 4H, Ar-H), 8.00 (d, *J*=6.0 Hz, 4H, Ar-H), 7.69 (d, *J*=9.0 Hz, 2H, Ar-H), 7.56 (t, *J*=15.0 Hz, 2H, CH_{alkene}), 7.45 (t, *J*=9.0 Hz, 2H, Ar-H), 7.30 (t, *J*=6.0 Hz, 2H, Ar-H), 7.03 (d, *J*=9.0 Hz, 2H, Ar-H), 6.77 (t, *J*=6.0 Hz, 1H, Ar-H), 6.16 (d, *J*= 15.0 Hz, 2H, CH_{alkene}), 4.19 (t, *J*=6.0 Hz, 4H, N-CH_2), 2.73 (t, *J*=6.0 Hz, 4H, CH_2), 2.48-2.35 (m, 4H, CH_2), 1.93-187 (m, 2H, CH_2), 1.76-1.65 (m, 8H, CH_2), 1.60 (s, 12H, CH_3). IR (ATR) 2928, 1594, 1433, 1349, 1227, 1004, 886, 714 cm^{-1}. MS (ESI) m/z: 884 $[M]^+$. λ_{max} = 779 nm. m.p. 230-232°C.

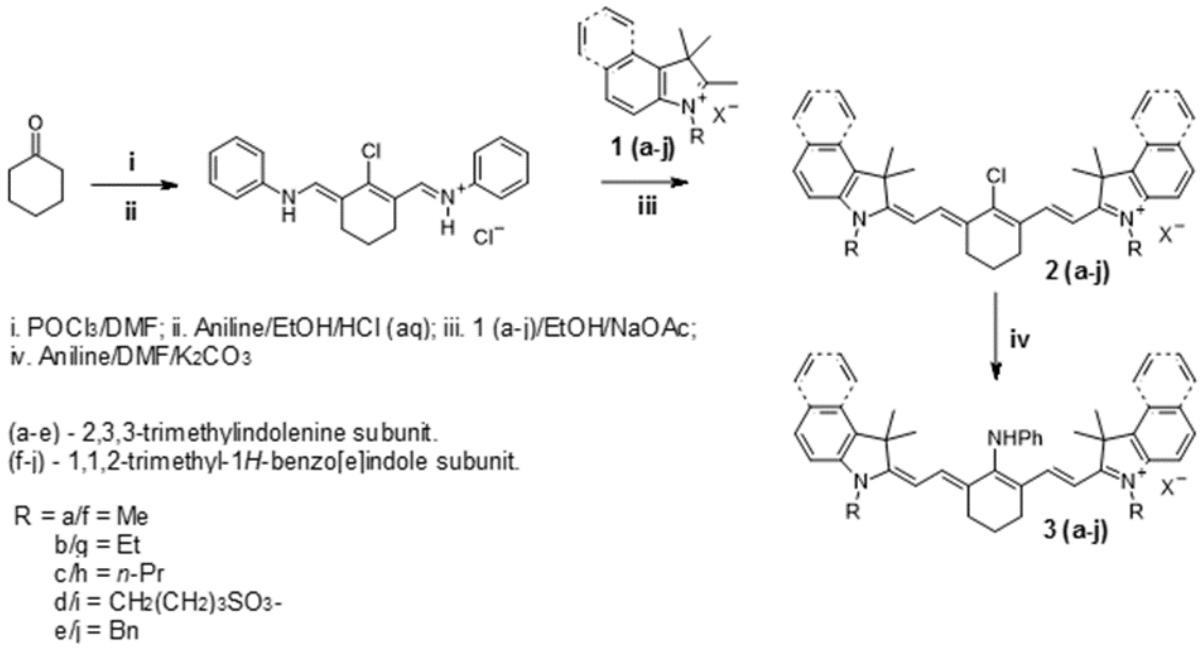
3-Benzyl-2-[(*E*)-2-[(3*E*)-3-{2-[(2*E*)-3-benzyl-1,1-dimethyl-1*H*,2*H*,3*H*-benzo[*e*]indol-2-ylidene]ethylidene}-2-(phenylamino)cyclohex-1-en-1-yl]ethenyl]-1,1-dimethyl-1*H*-benzo[*e*]indol-3-ium bromide (3j).

3j was synthesised as for **3a** using **2j** (0.10 mmol), aniline (1.00 mmol and anhydrous DMF (10.0 mL). The solvent was removed under reduced pressure, the crude product was purified by column chromatography on silica gel eluting with DCM 9 : MeOH 1 to afford **3j** (31 mg, 34%) as a shiny dark blue solid.

^{1}H NMR (d_6-DMSO, 300 MHz) δ 8.93 (brs, 1H, NH), 8.32 (s, 1H, Ar-H), 8.16 (d, *J*=9.0 Hz, 2H, Ar-H), 8.08 (d, *J*=15.0 Hz, 2H, CH_{alkene}), 7.99-7.90 (m, 4H, Ar-H), 7.62-7.33 (m, 4H, Ar-H), 7.46 (t, *J*=9.0 Hz, 2H, Ar-H), 7.31-7.23 (m, 11H, Ar-H), 7.03 (d, *J*=9.0 Hz, 2H, Ar-H), 6.83 (t, *J*=6.0 Hz, 1H, Ar-H), 6.11 (d, *J*= 15.0 Hz, 2H, CH_{alkene}), 5.50 (br s, 4H, N-CH_2), 2.64-2.56 (m, 4H, CH_2), 1.78 (quin, *J*=6.0 Hz, 2H, CH_2), 1.65 (s, 12H, CH_3). IR (ATR) 2924, 1592, 1433, 1345, 1271, 1093, 886, 664 cm^{-1}. MS (ESI) m/z: 792 $[M]^+$. HRMS(FAB): Calcd for $C_{58}H_{54}N_3[M]^+$ 792.4312, found 792.4307. λ_{max} = 769 nm. m.p. 214-216°C.

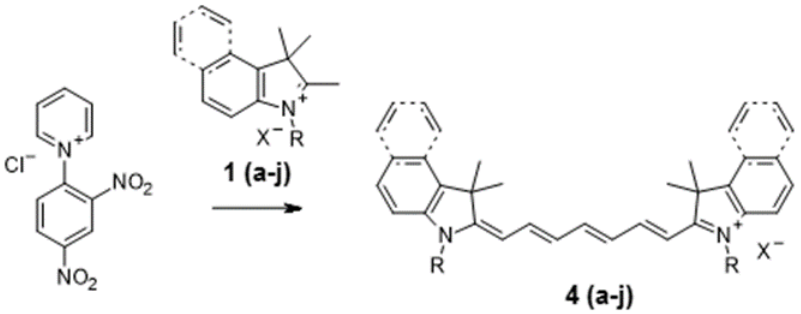
Results and Discussion

The synthesis of both the rigid (**2a-j – 3a-j**) and linear (**4a-j**) cyanine dyes was straightforward and required no harsh or unusual synthetic methodologies.[7-8] The majority of the *meso*-aminophenyl substituted heptamethine dyes presented in this manuscript are novel and have been analysed by NMR as well as HMRS. The salts of 2,3,3-trimethylindolenine (**1a-e**) and 1,1,2-trimethyl-1*H*-benzo[*e*]indole (**1f-j**) were readily prepared via an *N*-alkylation with the corresponding alkyl/benzyl halides or 1,4-butanesultone.[9] In order to synthesise the rigid cyanine dyes (**2a-j** and **3a-j**), a Vilsmeier-Haack formylation of cyclohexanone followed by reaction with aniline produced the reactive imine (**VHI**) in 97% yield. This was followed by an aldol-like condensation of the *N*-alkylated salt precursors with this imine under basic conditions. Upon purification via column chromatography, the rigid *meso*-chloro substituted dyes (**2a-j**) were isolated in moderate to low yields. Refluxing (**2a-j**) in ten equivalents of aniline in anhydrous DMF yielded the crude rigid *meso*-aminophenyl substituted dyes (**3a-j**) which were purified again by column chromatography to give the target compounds, also in moderate to low yields. The synthetic route to these dyes is shown in Scheme 1.



Scheme 1: Route to the heptamethine dyes 3 (a-j). Commercial dyes: IR-746 = 2d; IR-820 = 2i; IR-783 = 4d; ICG = 4i. Note X for compounds a-c and f-h are iodide salts, e/j are bromide salts and d/I are charge balanced inner salts. Purification of the final dyes was accomplished via column chromatography on silica gel eluting with the solvent blends listed within the experimental section as well as the supporting information.

The linear cyanine dyes (**4a-j**) were produced as shown in Scheme 2, via an *in-situ* cascade reaction, via the ring opening of *N*-(2,4-dinitrophenyl)-pyridinium chloride with aniline to produce 5-anilino-*N*-phenyl-2,4-pentadienylideniminium chloride. This was immediately followed by the direct substitution of the aniline subunit with the *N*-alkylated substituted indolene salt under basic conditions with the whole reaction taking place at room temperature over a period of 12 hours.[5] The crude dyes were purified via column chromatography using silica gel to obtain the dyes. For all dyes, NMR confirmed their structure and it was noted from the NMR spectra that the dyes were free from solvent and other impurities with high-resolution mass spectrometry confirming the molecular weight.
The photophysical properties of both the linear and rigid NIR heptamethine cyanine dyes used in this study are summarised in Table 1, all in methanol solution. ICG was used as the quantum yield standard producing a value of 0.13 in DMSO. The absorbance and fluorescence spectra of each of the dyes were measured sequentially to reduce photobleaching and solubility issues.



i. subunits a-j; EtOH; NaOAc and Aniline

(a-e) - 2,3,3-trimethylindolenine subunit.
(f-j) - 1,1,2-trimethyl-1*H*-benzo[e]indole subunit.

R = a/f = Me
b/g = Et
c/h = *n*-Pr
d/i = $CH_2(CH_2)_3SO_3$-
e/j = Bn

Scheme 2: Route to the heptamethine dyes 4 (a-j). Note X for compounds a-c and f-h are iodide salts, e/j are bromide salts and d/I are charge balanced inner salts. Purification of the final dyes were accomplished via column chromatography on silica gel, eluting with the solvent blends listed within the supporting information.

The fluorescence quantum yields of the dyes were calculated using the relative method, i.e. from plots for standard and cyanine dyes of their individual integrated fluorescence peak areas versus fraction of light absorbed at the excitation wavelength.[10-11] In this study, emphasis was focused on the effects of structural diversity of the synthesised NIR heptamethine cyanine dyes on their photophysical properties. Firstly, the effect of substituting the *meso*-chlorine (**2a-j**) atom with an aminophenyl (**3a-j**) moiety was investigated, results indicating hypsochromic shifts in absorption wavelength when compared to the *meso*-chloro analogue. The dyes bearing the phenylamino- moiety exhibited larger Stokes shifts, ranging from 38-58 nm in comparison to the parent *meso*-chloro dye, indicating a large structural change between the ground and excited singlet states for these examples. The difference in Stokes shift could be attributed to the nitrogen atom within the aniline participating in an excited-state intramolecular charge transfer (ICT).[12] One notable drawback with most of the established heptamethine probes is their small Stokes shifts. It is well known that increased Stokes shifts play a key role in avoiding self-quenching and excitation light scattering, thereby extending the potential applications of the current examples as contrast agents.[13] It was also noted that the relative fluorescence quantum yields of some of the meso-aminophenyl dyes with the greatest Stokes shifts have quantum yields comparable to ICG. It is interesting to note that the largest Stokes shift was observed in both **3e** and **3j** where the substituent is a benzyl group and this may be due to an effect of coplanarity of the core indole rings.

Compounds			Fluorescence Studies		
Code	**R**	**Absorption (nm)**	**Emission (nm)*[1]**	**Stokes Shift (nm)**	**Relative Fluorescence Quantum Yield*[2]**
2a	**Me**	**775**	**793**	**18**	**0.073**
2b	**Et**	**777**	**796**	**19**	**0.080**
2c	**n-Pr**	**781**	**798**	**17**	**0.074**
2d/IR783	***$CH_2(CH_2)_3SO_3^-$***	***782***	***802***	***20***	***0.085***
2e	**Bn**	**775**	**793**	**18**	**0.084**
2f	**Me**	**813**	**834**	**21**	**0.028**
2g	**Et**	**815**	**833**	**18**	**0.035**
2h	**n-Pr**	**818**	**838**	**20**	**0.029**
2i/IR820	***$CH_2(CH_2)_3SO_3^-$***	***820***	***840***	***20***	***0.032***
2j	**Bn**	**822**	**845**	**23**	**0.029**
3a	**Me**	**736**	**786**	**50**	**0.030**
3b	**Et**	**735**	**786**	**51**	**0.027**
3c	**n-Pr**	**746**	**795**	**49**	**0.054**
3d	**$CH_2(CH_2)_3SO_3^-$**	**745**	**791**	**46**	**0.047**
3e	**Bn**	**736**	**794**	**58**	**0.015**
3f	**Me**	**780**	**818**	**38**	**0.049**
3g	**Et**	**776**	**820**	**44**	**0.062**
3h	**n-Pr**	**778**	**823**	**45**	**0.063**
3i	***$CH_2(CH_2)_3SO_3^-$***	**779**	**824**	**45**	**0.067**
3j	**Bn**	**769**	**826**	**57**	**0.048**
4a	**Me**	**740**	**770**	**30**	**0.09**
4b	**Et**	**742**	**770**	**28**	**0.11**
4c	**n-Pr**	**746**	**776**	**30**	**0.13**
4d/IR746	***$CH_2(CH_2)_3SO_3^-$***	***747***	***775***	***28***	***0.13***
4e	**Bn**	**748**	**776**	**28**	**0.15**
4f	**Me**	**778**	**807**	**29**	**0.064**
4g	**Et**	**781**	**809**	**28**	**0.072**
4h	**n-Pr**	**782**	**811**	**29**	**0.071**
4i/ICG	***$CH_2(CH_2)_3SO_3^-$***	***784***	***813***	***29***	***0.071***
4j	**Bn**	**786**	**814**	**28**	**0.076**

Table 1: Photophysical data for dyes within methanol solution. *[1] Excitation at 785nm. *[2] Quantum yields ± 10%, λ_{max} ± 1 nm. Relative to ICG.

Although research has been published concerning the addition of heteroatoms directly to the meso- position on the rigid heptamethine dyes, it is noteworthy that there are few

examples which show the attachment of aromatic amine systems such as aniline. Indeed, Ma *et al.* have reported the direct attachment of pyridin-4-ol and pyridin-4-thiol directly to the *meso*-position of IR-786, through the alcohol and thiol groups respectively, both giving marginal Stokes shifts of 13 and 21nm respectively,[13] i.e. much less than those described here. Other publications have focused on adding a small range of differently-substituted anilines to a rigid heptamethine zwitterionic fluorophore with varying Stokes shifts being identified.[14-15]

The effect of an extra fused benzene on the heterocyclic system was also investigated and data indicated that the dyes with the extra benzo[*e*]-fusion exhibited longer absorption and emission wavelengths than the indolium-based dyes. It is recognised that the increase in the conjugation of the chromophore system also increases the wavelengths of NIR dyes along with the planarity of the dyes.[16]

Conclusions

The small library of rigid *meso*-aminophenyl substituted dyes prepared exhibited a 1.5 - 2 fold increase in Stokes shift when compared against *meso*-chloro or linear analogues. Furthermore, compound **3i** demonstrated a 2-fold increase in relative fluorescence quantum yield compared to the structural standard dye IR820, whilst a greater than 2-fold increase was seen in its Stokes shift. Based on their photophyscial properties, further development and investigation of amino-substituted hepthamethine dyes is clearly of importance to the contrast agent field.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

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