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Preparation of Biological Labels with Acetylenic Linker Arms

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Abstract: The preparation of a series of fluorescent labels based on acridone, pyrene, fluorescein, dimethylaminonaphthalenesulphonamide and tris-1,10-phenanthroline ruthenium and an enzymic label based on biotin with spacers containing a terminal acetylene is described.

INTRODUCTION

The detection of biomolecules at concentrations in the subnanogram range is now routinely achieved with labels based on enzymes, fluorophores and luminophores.[1] Techniques based on luminescent labels are replacing those based on radioisotopes which have contributed greatly to the elucidation of biochemical mechanisms and the routine availability of immunoassays.[2] Detection systems based on the quantitative measure of emitted or absorbed light generally consist of three components, the biomolecule, the spacer arm and the signal generator or label (**Figure 1**).

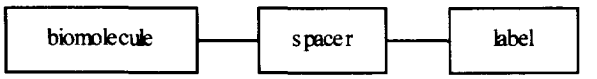


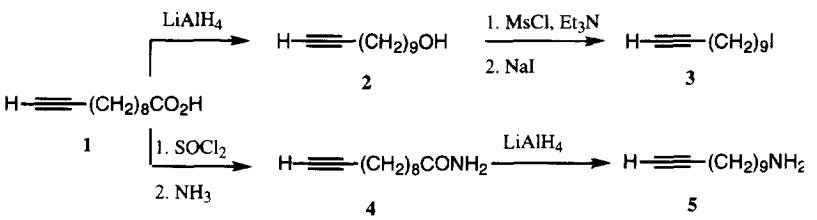
Figure 1

The spacer arm provides the crucial bridge between the biomolecule which is being detected and the signal molecule whose concentration can be quantified. Traditional spacers have consisted of aliphatic and/or aromatic hydrocarbons containing two reactive funtional groups on the termini. The length of the spacer is usually 6 - 15 carbons so as to prevent steric interference between the signal generator and the biomolocule.[3] The functional groups are usually matched with those on the biomolecule such that a complementary pair of nucleophile and leaving group is available to form the required covalent bond.[1] We wish to report in this paper the synthesis of a representative set of fluorescent labels based on acridone, pyrene, fluorescein, dimethylaminonaphthalene- sulfonamide and 1,10-phenanthroline and an enzymic probe based on biotin attached to spacers containing a terminal alkyne which can be used in a palladium-catalysed coupling with suitably derivatised biomolecules[4]. The terminal alkyne group offers the opportunity to form a non-polar covalent bond between the linker arm and the biomolecule and to position the label remote from essential functional groups on the biomolecule.

RESULTS AND DISCUSSION

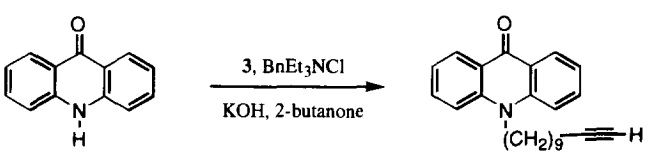
The synthesis of the alkynyl linker arms was based on the readily available undec-10-ynoic acid[5], **1**, which could be converted to a number of key intermediates (**Scheme 1**). Reduction of **1** by $LiAlH_4$ in diethyl ether furnished undec-10-yn-1-ol[6], **2**, in 87% yield. Conversion of **2** to the corresponding iodide **3** was accomplished in 70% overall yield via a standard two step procedure of mesylation and subsequent displacement with NaI in 2-butanone.[7]

Preparation of aminoalkyne **5** proceeded via the reaction of carboxylic acid **1** with $SOCl_2$ to give undec-10-ynoyl chloride which was added directly to a cold (-15°) saturated solution of ammonia and yielded the amide **4** as a white precipitate in 84%. The amide **4** was only partially soluble in Et_2O, hence it was placed in the thimble of a Soxhlet extraction apparatus for reduction with $LiAlH_4$. After heating at reflux for 36 hours the amide had completely reacted and the amine was recovered in 85% yield. The overall yield for the two-step synthesis of **5** from **1** was 73%. These sequences are summarised in **Scheme 1** and readily provided the spacer arms for attachment to the biological labels.



Scheme 1

N-Alkylacridone derivatives have been prepared previously by alkylations under phase transfer conditions.[8] Using a modification of these conditions **3** was added dropwise to a mixture of acridone, 50% aqueous KOH, benzyltriethylammonium chloride (0.02 eq) and 2-butanone at 80° (**Scheme 2**). The expected product **6** was recovered in 59% yield.



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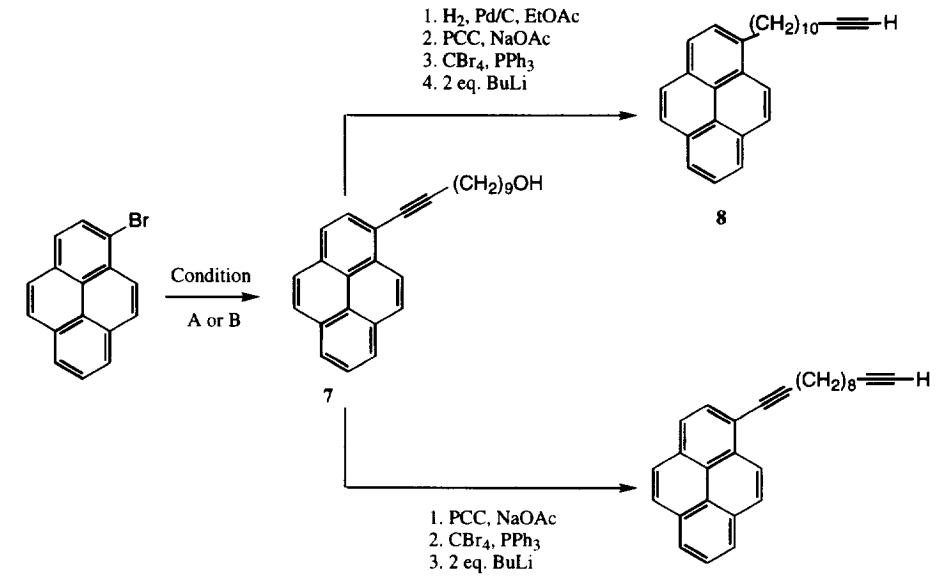
Scheme 2

Hydrocarbon spacer arms could be readily attached to the fluorescent label pyrene by taking advantage of the facile palladium-catalysed coupling of bromoaromatics with terminal alkynes.[9] Coupling of 1-bromopyrene with alkynol **2** under the influence of catalytic $Pd(PPh_3)_4$ and CuI in piperidine[10] (**Scheme 3**, Condition A) gave, after heating at reflux overnight, **7** in a yield of 72%.

An alternative procedure based on the reaction of 1-bromopyrene and **2** with only catalytic $Pd(PPh_3)_4$ in pyrrolidine[11] at 80° for 20 hours (**Scheme 3**, Condition B) also afforded **7** in a good yield of 79%.

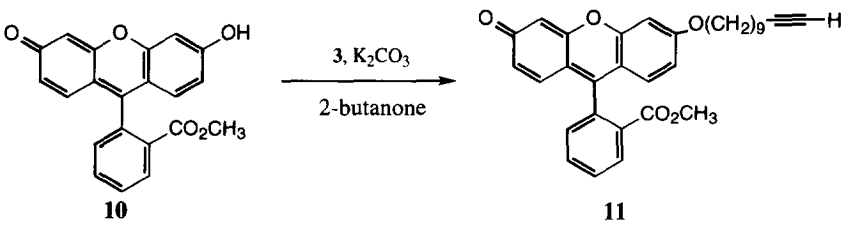


Condition A: **2**, $Pd(PPh_3)_4$, CuI, PPh_3, piperidine
Condition B: **2**, $Pd(PPh_3)_4$, pyrrolidine

Scheme 3

Preparation of the saturated pyrene label **8** was achieved in good overall yield (57%) by initial reduction of **7** with hydrogen and 5% Pd/C, followed by selective oxidation of the primary alcohol to the corresponding aldehyde which could be converted to the terminal alkyne **8** via the method of Corey and Fuchs[12]. Treatment of CBr_4 with PPh_3 in CH_2Cl_2 at -15° for 30 minutes gave rise to an orange solution to which the aldehyde was added and the mixture stirred for 60 minutes at 0° to give the dibromo alkene intermediate. Dehydrohalogenation of the dibromo alkene was facilitated by *n*-BuLi in THF (30 minutes at -78° and room temperature for 2 hours), followed by protonation of the resultant lithium acetylide with saturated NH_4Cl to give the alkyne **8** in 64% yield (**Scheme 3**). The unsaturated pyrene label **9** was also synthesised in good overall yield (61%) by selective oxidation of **7** with PCC to give the corresponding aldehyde in 96% yield followed by conversion to the terminal alkyne **9** via dehydrohalogenation of the dibromo alkene as described for **8** (**Scheme 3**).

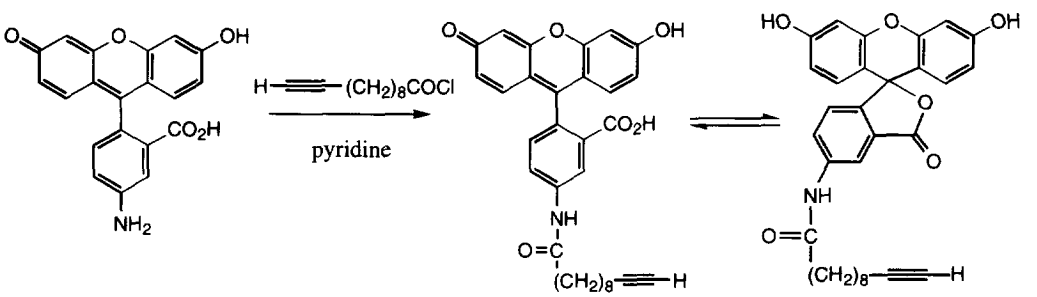
The methyl ester of fluorescein **10** was prepared by heating a methanol solution of fluorescein with H_2SO_4 as catalyst for 72 hours.[13] The mono- and dimethylated byproducts were removed by dissolving the crude product in 1M NaOH solution, extracting the aqueous solution with EtOAc to remove the less polar dimethylated compounds, protonating with 10% HCl to precipitate the crude product, and finally recrystallisation from methanol to give **10** in 25% yield. Alkylation of **10** with alkynyl iodide **3** in the presence of K_2CO_3 in 2-butanone gave the expected product **11** in 79% yield (**Scheme 4**).



10 11

Scheme 4

Attachment of a spacer molecule to a functionalised fluorescein derivative, such as 5-amino fluorescein allows the highly fluorescent phenolic anion to be retained. Hence label **12** was prepared in 65% yield by the reaction of 5-aminofluorescein with undec-10-ynoyl chloride in pyridine (**Scheme 5**). Alkynyl fluorescein **12** existed in the lactone form in DMSO[d^6] as shown by the presence of 6 aromatic resonances (3 which integrate for 2H and 3 which integrate for 1H) in the ^{1}H NMR spectrum. This facile interconversion was also manifested when **12** was dissolved in solvents of different polarity. Solutions of **12** in protic solvents such as water, methanol and ethanol are highly coloured and fluorescent whereas solutions of **12** in aprotic solvents such as ethyl acetate and dimethylsulfoxide are colorless and non fluorescent.



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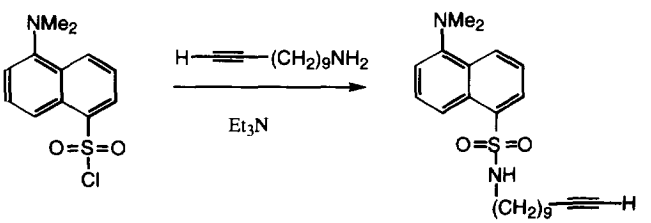
Scheme 5

The dansyl label **13** was prepared in 72% yield by the reaction of dansyl chloride with aminoalkyne **5** and Et_3N in CH_2Cl_2 at room temperature (**Scheme 6**).



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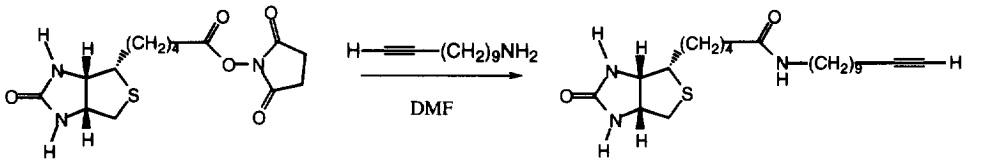
Scheme 6

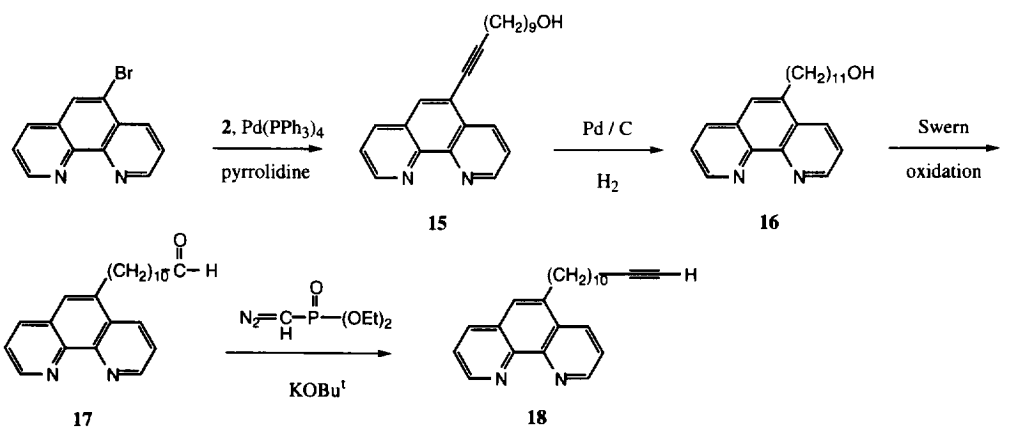
A common method for preparing biotin conjugates is via reaction of the *N*-hydroxysuccinimide ester with an amine to give a *N*-substituted biotin amide derivative.[14] Preparation of **14** in 90% yield was accomplished by reaction of biotin N-hydroxysuccinimide ester with aminoalkyne **5** in DMF (**Scheme 7**). Compound **14** was found to gelate ethyl acetate and other low polarity organic solvents.



14

Scheme 7

The bromination of 1,10-phenanathroline in fuming sulphuric acid (60% oleum) gave 5-bromo-1,10-phenanathroline as described in the literature.[15] Previous palladium catalysed coupling reactions of bromo-substituted 1,10-phenanthrolines have used CuI as a co-catalyst, however complexation of Cu(I) to the phenanthroline moiety reduces the yield of the reaction and the use of sonication to disrupt complexation or treatment with aqueous KCN was used to increase the yield of the coupling.[16] The use of copper iodide as a co-catalyst is not necessary for couplings performed in pyrrolidine at reflux, hence 5-bromo-1,10-phenanthroline was reacted with alkynol **2** to give the adduct **15** in 66% yield (**Scheme 8**). Treatment of **15** under acidic conditions with 5% Pd/C under an atmosphere of hydrogen overnight gave the saturated compound **16** in 78% yield. Hydrogenation under neutral conditions gave a mixture of the cis alkene analogue and **16**.



15 **16**

17 **18**

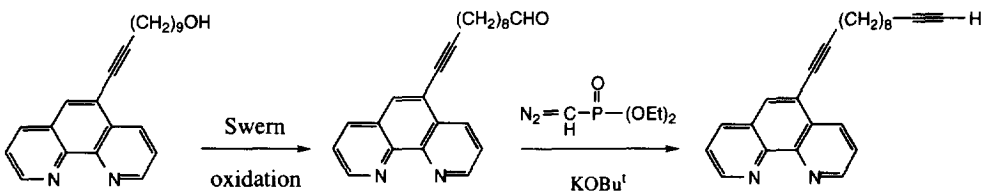
Scheme 8

Conversion of the alcohol **16** to the aldehyde **17** by a standard Swern oxidation using DMSO, oxalyl chloride and Et_3N (**Scheme 8**) afforded the expected product **17** in 71% yield The aldehyde was unstable to chromatography on silica and alumina and so was used in crude form for the next step.

Conversion of **17** to the alkyne **18** was initially attempted using the procedure of Corey and Fuchs[12] in which treatment of **17** with CBr_4 and PPh_3 gave the dibromoolefin in low yield. Subsequent

dehydrohalogenation and halogen exchange with 2 equivalents of *n*-BuLi gave a complex mixture of products with only a small amount of alkyne. Dialkylphosphinodiazomethanes have been shown to convert aldehydes to alkynes in average to good yields.[17] Hence treatment of **17** with base and diethoxyphosphinodiazomethane gave the alkyne **18** directly in a 47% yield.

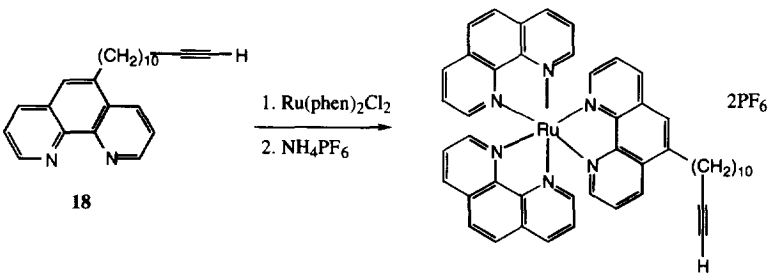
Using an analogous route the unsaturated analogue **20** was synthesised from **15** by an initial Swern oxidation (69% yield) and subsequent treatment of the resulting aldehyde **19** with diethoxyphosphinodiazomethane and base in 60% yield (**Scheme 9**).



15 **19** **20**

Scheme 9

Formation of the ruthenium complex **21** was achieved by stirring **18** with $Ru(phen)_2Cl_2$ in a mixture of methanol and water at 40° for 48 hours (**Scheme 10**).[18] The dark purple colour of the neutral ruthenium (II) complex in solution changed to a deep red-brown as the reaction proceeded. After filtering out a black precipitate (presumably ruthenium metal), concentration of the reaction mixture and addition of aqueous NH_4PF_6, red-orange crystals of the ruthenium (II) salt **21** were obtained. Both ^1H NMR and TLC showed impurities present however attempted recrystallisation from various solvents were unsuccessful. Purification was effected by chromatography on alumina (silica gel caused decomposition) and the product was recovered in an average yield of 68%. LSIMS showed an intense peak at m/z 951 ($^{102}RuM^{2+}.PF_6^-$), confirming incorporation of **18** into the ruthenium complex. As the parent *tris*-1,10- phenanthroline ruthenium (II) complex is chiral[18], formation of diastereoisomers upon addition of the non-symmetric ligand, **18**, was expected. The ^1H NMR showed two overlapping triplets at δ 3.22 and δ 3.24 (total integration two protons) instead of a single triplet for the methylene hydrogens adjacent to the aromatic moiety, confirming the presence of diastereoisomers which could not be separated by column chromatography. A fluorescence spectrum of a solution of **21** showed a maximum absorption at 578 nm (λ_{ex} = 449 nm).



18 **21**

Scheme 10

EXPERIMENTAL

All reactions were performed in oven dried glassware under a nitrogen atmosphere (unless in aqeuous solution). Melting points were recorded on a Reichhert hot stage apparatus and are uncorrected. Proton and carbon NMR spectra were recorded on a Bruker ACP-300 or a Varian Gemini 200 spectrometer in $CDCl_3$ as solvent with tetramethylsilane as an internal standard. Mass spectra were recorded on VG ZAB 2HF mass spectrometer with either electron impact (EI) or fast atom bombardment (FAB) ionisation, or on an AEI-GEC MS 3074 instrument with EI ionisation. Ultraviolet spectra were recorded on a Pye Unicam SP8-100 spectrometer. Fluorescence spectra were recorded on a Perkin Elmer 3000 spectrometer. IR spectra were recorded on a Hitachi 270-30 spectrometer. Triethylamine, pyrrolidine, piperidine and CH_2Cl_2 were distilled from CaH_2 under nitrogen and stored over 4Å molecular sieves. DMF was distilled from CaH_2 (*ca.* 80° at 20mmHg) and stored over 4Å molecular sieves. Et_2O and THF were freshly distilled from sodium and benzphenone under nitrogen. Methanol was fractionally distilled under nitrogen and stored over 3Å molecular sieves. Analytical thin layer chromatography was carried out using Merck aluminium sheets precoated with kieselgel 60 F_{254} or (when stated) with Alumina 150 F_{254}, and visualised using either a 254 nm or 365 nm lamp, or with a 4% solution of phosphomolybdic acid in ethanol. Flash chromatography was carried using Merck kieselgel 60 (230-400 mesh) or (when stated) on Alumina UG, and solvents used were distilled before use. The following compounds were synthesised according to literature procedures: undec-10-ynoic acid[5], undec-10-yn-1-ol[6], fluorescein methyl ester (**10**)[13], biotin NHS ester[14], 5-bromo-1,10-phenanthroline[15], diethylmethyldiazophosphonate[17], *bis*-1,10-phenanthrolineruthenium(II)dichloride[18] and 1-bromopyrene[19].

11-Iodoundec-1-yne (3)

To a stirred mixture of **2** (5.00g, 30 mmol) and Et_3N (3.01g, 30 mmol) in CH_2Cl_2 (50ml) was added dropwise methanesulphonyl chloride (3.41g, 30mmol). The mixture was stirred at room temperature for 24 hours, filtered to remove precipitated triethylammonium chloride, washed with water (50ml), dried over $MgSO_4$ and the solvent removed. The crude undec-10-yn-1-*O*-methanesulphonate was recovered as a pale yellow oil in 6.42g (88%) yield, IR (film): ν_{max} 3292, 2928, 2852, 2116, 1468, 1354, 1176 cm^{-1} ^1H NMR: δ 1.30-1.41 (m, 14H, $(CH_2)_7$); 1.75 (quintet, 2H, J = 7.2 Hz, CH_2-CH_2-OMs); 1.95 (t, 1H, J = 2.6 Hz, C-H); 2.19 (dt, 2H, J = 2.6, 6.9 Hz, CH_2-C); 3.01 (s, 3H, CH_3-SO_3); 4.22 (t, 2H, J = 6.6 Hz, CH_2-OMs.). The crude mesylate (6.00g, 24 mmol) was dissolved in dry methyl ethyl ketone (100ml), anhydrous NaI (30.0g, 200 mmol) was added, the mixture heated to reflux for 24 hours, filtered to remove inorganic salts and the solvent

removed. The dark yellow residue was purified by squat chromatography (eluant hexane) to give the title compound[7] as a colourless oil in 5.06g (75%) yield. HRMS Calculated for $C_{11}H_{19}I$: 278.0532. Found: 278.0539. MS: 278 (M^+), 151 (M^+- I). IR (thin film): ν_{max} 3300, 2924, 2852, 2116, 1464, 1432, 1220, 1178, 720 cm^{-1}. ^1H NMR: δ 1.25-1.61 (m, 12H, $(CH_2)_6$); 1.82 (quintet, J = 7.0 Hz, 2H, CH_2-CH_2-I); 1.95 (t, J = 2.6 Hz, H-C); 2.19 (dt, J = 2.6, 6.9 Hz, CH_2-C); 3.19 (t, J = 7.0 Hz, 2H, CH_2-I). ^{13}C NMR: δ 7.18 (CH_2-I), 18.32, 28.37, 28.40, 28.59, 29.19, 29.22, 30.39, 33.46, 68.06, 84.50.

Undec-10-ynamide (4)

Thionyl chloride (7.30g, 4.5ml, 61 mmol) was added dropwise to stirred undec-10-ynoic acid (**1**) (9.33g, 51 mmol) at 40°. The mixture was heated at reflux for 60 minutes, cooled to room temperature and added dropwise to concentrated NH_3 solution held at -15° (dry ice-MeOH bath). The precipitated white product was collected, washed with water (20ml), air dried and recrystallised from CH_2Cl_2 (charcoal). The product was recovered as colourless crystals[20] mp 94-95° in 7.84g (in two crops) (84%) yield. HRMS Calculated for $C_{11}H_{19}NO$: 181.1467. Found: 181.1459. MS: 182 ($M+H^+$), 181 (M^+), 122 (21), 72 (19), 59 (100). IR (nujol): ν_{max} 3356, 3280, 3184, 2150, 1662, 1632 cm^{-1}. ^1H NMR: δ 1.31-1.65 (m, 12H, $(CH_2)_6$); 1.93 (t, 1H, J = 2.6 Hz, H-C); 2.12-2.24 (m, 4H, CH_2-C and CH_2-$CONH_2$); 5.56 and 5.89 (2 x bs, 1H, $CONH_2$). ^{13}C NMR: δ 20.36, 27.48, 30.41, 30.64, 30.79, 31.00, 31.15, 37.92, 70.10, 86.71 (alkynyl), 177.83.

Undec-10-ynyl-1-amine (5)

A Soxhlet apparatus charged with undec-10-ynamide (**4**) (5.00g, 28 mmol) and fitted to a flask containing a suspension of $LiAlH_4$ (1.22g, 33 mmol) in Et_2O (200ml). The suspension was heated at reflux and the amide extracted until dissolved (approximately 30 hours), then lithium complexes were decomposed by the sequential addition of water (1.2ml), 15% NaOH solution (1.2ml) and water (3.6ml). The grey precipitate was removed by filtration, the solvent removed and the residue distilled bp 70-72° at 0.10 mmHg to give the title compound as a colourless oil in 3.92g (85%) yield. The oil slowly solidified to give a colourless solid mp 53-55°. HRMS Calculated for $C_{11}H_{22}N$ ($M+H^+$): 168.1752. Found: 168.1756. MS: 168 ($M+H^+$), 138 (48), 110 (39), 96 (43), 86 (100). IR (film): ν_{max} 3300 , 3304, 2116 cm^{-1} . ^1H NMR: δ 1.21-1.48 (m, 14 H, $(CH_2)_7$); 1.86 (t, 1H, J = 2.7 Hz, H-C); 2.09 (dt, 2H, J = 2.7, 6.9, Hz, CH_2-C); 2.60 (t, 2H, J = 6.6 Hz, CH_2-NH_2). ^{13}C NMR: δ 20.33, 28.81, 30.41, 30.66, 30.98, 31.38, 35.81, 44.20; 70.02, 86.64 (alkynyl).

10-(Undec-10-ynyl)-9-(10*H*)-acridone (6)

Acridone (1.00g, 5.1 mmol), benzyltriethylammonium chloride (50mg, 0.2 mmol), 50% aqueous KOH (10ml) and 2-butanone (10ml) were stirred at 60° for 30 minutes, then 11-iodoundec-1-yne (**3**) (2.00g, 7.1 mmol) was added dropwise over 10 minutes. The temperature was raised to 80° and the mixture stirred for 5 hours until analytical TLC indicated the absence of starting material. The reaction mixture was poured into hot water (100ml), allowed to cool to room temperature and then placed in an ice bath. The solid which formed was collected and recrystallised from EtOH to give the title compound as light green needle crystals mp 95-97° in 1.06g (59%) yield. HRMS Calculated for $C_{24}H_{27}NO$: 345.2095. Found 345.2101. MS: 345 (M^+), 208 (100), 195 (37), 81 (35). IR (nujol): ν_{max} 3280, 1638 , 1602, 1496, 1264, 1180, 754 cm^{-1}. UV (EtOH) λ_{max} : 208 (46 500), 253 (66 300), 385 (8 900), 404 (9 800) nm. Fluorescence (EtOH, λ_{ex} = 385 nm): 421 (100), 444 (75) *sh*. ^1H NMR: δ 1.29-1.52 (m, 12H, $(CH_2)_6$); 1.76 (quintet, 2H, *J* = 7.5 Hz, N-CH_2-CH_2); 1.97 (t, 1H, *J* = 2.1 Hz, C-H); 2.16 (dt, 2H, *J* = 2.3, 6.6 Hz, CH_2-C); 4.11 (t, 2H, *J* = 7.8 Hz, N-CH_2); 7.17 (t, 2H, *J* = 7.5 Hz); 7.33 (d, 2H, *J* = 8.4 Hz); 7.60 (t, 2H, *J* = 8.1 Hz); 8.48 (d, 2H, *J* = 7.9 Hz). ^{13}C NMR: δ 18.1, 26.5, 26.7, 28.1, 28.3, 28.7, 28.9, 29.1, 45.7, 68.0, 84.3 (alkyne); 114.2, 120.7, 121.9, 127.4, 133.4, 141.2, 177.4.

11-(1-Pyrenyl)undec-10-yn-1-ol (7)

To a stirred mixture of 1-bromopyrene (1.00g, 3.6 mmol) in pyrrolidine (10ml) was added undec-10-yn-1-ol (**2**) (0.72g, 4.3mmol), and $Pd(PPh_3)_4$ (0.20g, 0.215mmol). The reaction mixture was stirred at 80° for six hours, cooled to room temperature, poured into saturated NH_4Cl solution (50ml), the mixture extracted with CH_2Cl_2 (2 x 25ml), the combined organic extracts washed with 10% citric acid (25ml), brine (25ml), dried ($MgSO_4$) and solvent removed. The residue was separated by flash chromatography with 30/70 EtOAc/hexanes as the eluant and recrystallised from hexane to give the product as colourless crystals mp 46-48° in 0.85g (65%) yield. HRMS Calculated for $C_{27}H_{28}O$: 368.2140. Found: 368.2126. MS: 368 (M^+, 100), 239 (50). ^1H NMR: δ 1.48-1.52 (m, 11H, $(CH_2)_5$ and OH); 1.56 (quintet, 2H, *J* = 6.5 Hz, alkyne-CH_2-CH_2-); 1.77 (quintet, 2H, *J* = 7.2 Hz, -CH_2-CH_2-OH); 2.64 (t, 2H, *J* = 7.0 Hz, CH_2-alkyne); 3.62 (t, 2H, *J* = 6.5 Hz, CH_2-OH); 7.97-8.20 (m, 8H, Ar-H); 8.56 (d, 1H, *J* = 9.0 Hz, Ar-H). ^{13}C NMR: δ 19.9, 25.7, 28.9, 29.0, 29.1, 29.4, 29.5, 32.6, 63.0, 79.6, 96.4 (alkyne), 118.8, 124.3, 124.4, 125.3, 125.7, 126.1, 127.2, 127.7, 127.9, 130.6, 131.2, 131.8.

1-(1-Pyrenyl)dodeca-11-yne (8)

A mixture of **7** (1.00g, 2.7 mmol), 5% Pd/C (300mg) and EtOAc (100ml) was stirred overnight under an atmosphere of hydrogen. The reaction mixture was filtered through celite, the solvent removed and the residue recrystallised from hexanes to give 11-(1-pyrenyl)undecan-1-ol as colourless crystals mp 48° in 0.89g (89%) yield. HRMS Calculated for $C_{27}H_{32}O$: 372.2453. Found 372.2465. MS: 372 (M^+, 100), 215 (24). IR (nujol mull): ν_{max} 3416, 3050, 1610, 1520, 1054, 836 cm^{-1}. ^1H NMR: δ 1.25-1.52 (m, 16H, $(CH_2)_8$); 1.66 (bs, 1H (exchanges with D_2O), -OH); 1.81 (quintet, 2H, J = 7.5 Hz, CH_2-CH_2-Ar); 3.28 (t, 2H, J = 7.7 Hz, Ar-CH_2); 3.57 (t, 2H, J = 6.6 Hz, CH_2-OH); 7.82 (d, 1H, J = 7.8 Hz, Ar-H); 7.91-8.13 (m, 7H, Ar-H); 8.23 (d, 1H, J = 9.3 Hz, Ar-H). ^{13}C NMR: δ 25.7, 29.4, 29.5, 29.7, 29.8, 31.9, 32.7, 33.6, 63.0, 123.5, 124.5, 124.7, 125.0, 125.7, 126.4, 127.0, 127.2, 127.5, 128.5, 129.6, 130.8, 131.4, 137.3. Pyridinium chlorochromate (1.74g, 8.00 mmol) and anhydrous NaOAc (0.66g, 8.0 mmol) were suspended in CH_2Cl_2 (15ml) and a solution of the 11-(1-pyrenyl)undecan-1-ol (1.49g, 4.0 mmol) in CH_2Cl_2 (10ml) was added and stirred for 4 hours, at which time analytical TLC of the dark brown mixture showed the absence of the alcohol. The reaction mixture was filtered, the solvent removed and the residue purified by flash chromatography, eluant 20/80 EtOAc/hexanes. 11-(1-Pyrenyl)undecanal was isolated in 0.89g (89%) yield as colourless crystals mp 43°. HRMS Calculated for $C_{27}H_{30}O$: 370.2297. Found: 370.2290. MS: 370 (M^+, 79), 215 (100). ^1H NMR: δ 1.26-1.48 (m, 14H, $(CH_2)_7$); 1.83 (quintet, 2H, J = 7.7 Hz, CH_2-CH_2-Ar); 2.36 (dt, 2H, J = 7.3, 1.9 Hz, CH_2-CHO); 3.31 (t, 2H, J = 7.6 Hz, CH_2-Ar); 7.83-8.28 (m, 9H, Ar-H); 9.72 (t, 1H, J = 1.9 Hz, CHO). ^{13}C NMR: 22.1, 29.1, 29.3, 29.4, 29.5, 29.8, 31.9, 33.6, 123.5, 124.6, 124.8, 125.1, 125.8, 126.5, 127.1, 127.2, 127.5, 128.6, 129.7, 130.9, 131.4, 137.3, 203.0. Triphenylphosphine (6.02g, 22.9 mmol) was dissolved in CH_2Cl_2 (20ml) and cooled to -15° in an dry ice/MeOH bath. A solution of CBr_4 (3.80g, 11.5 mmol) in CH_2Cl_2 (20ml) was added, the cooling bath replaced by an ice bath and the mixture stirred at 0° for 30 minutes. A solution of 11-(1-pyrenyl)undecanal (1.40g, 3.8 mmol) in CH_2Cl_2 (10ml) was added, and the mixture stirred for 1 hour. The solvent was removed and the residue purified by flash chromatography with EtOAc/hexanes 5/95 as the eluant. 1,1-Dibromo-12-(1-pyrenyl)dodec-1-ene was recovered as colourless crystals in 0.87g (80%) yield, mp 41-43° . MS: 524/526/528 (1:2:1, 100, M^+), 215 (29). ^1H NMR: δ 1.28-1.48 (m, 14H, $(CH_2)_7$); 1.87 (quintet, 2H, J = 7.4 Hz, CH_2-CH_2-Ar); 2.08 (q, 2H, J = 7.0 Hz, CH_2-CH=); 3.35 (t, 2H, CH_2-Ar); 6.38 (t, 1H, J = 7.3 Hz, H-C=); 7.86-8.32 (m, 9H, Ar-H). ^{13}C NMR: δ 27.8, 29.0, 29.3, 29.5, 29.5, 31.9, 33.0, 33.6, 88.4 (Br_2C=), 123.5, 124.6, 124.8, 125.7, 126.5, 127.1, 127.2, 127.5, 128.6, 129.7, 130.9, 131.5, 137.3, 138.9. 1,1-Dibromo-12-(1-pyrenyl)dodec-1-ene (0.80g, 1.5mmol) was dissolved in THF (30ml), cooled to

-78° in a dry ice/acetone bath and *n*-butyllithium (1.5ml, 2.5M in hexanes, 3.8mmol) added dropwise via syringe. The red mixture was stirred for 2 hours at -78°, 1 hour at room temperature and the reaction quenched by the addition of saturated aqueous NH_4Cl (7ml). The mixture was separated, dried (Na_2SO_4) and the solvent removed. The residue was purified by flash chromatography using EtOAc/hexanes 1/99 as eluent. The title compound was isolated in 430mg (71%) yield as colourless crystals, mp 50-51°. HRMS Calculated for $C_{28}H_{30}$: 366.2348. Found 366.2334. MS: 366 (M^+, 49), 215 (100). IR (nujol): ν_{max} 3308, 3040, 1600, 1180, 842 cm^{-1}. UV (EtOH) λ_{max}: 205 (107 000), 234 (143 000), 243 (191 000), 256 (48 300), 266 (96 700), 277 (160 000), 313 (40 000), 327 (86 700), 343 (117 000). Fluorescence (EtOH, λ_{ex} = 343 nm): 377 (100), 397 (59), 416 (20). ^1H NMR: δ 1.25-1.53 (m, 14H, $(CH_2)_7$); 1.81 (quintet, 2H, *J* = 7.6 Hz, Ar-CH_2-$\mathbf{CH_2}$); 1.93 (t, 1H, *J* = 2.7 Hz, C≡C-H); 2.15 (dt, 2H, *J* = 7.0, 2.7 Hz, -CH_2-alkyne); 3.31 (t, 1H, *J* = 7.8 Hz, Ar-CH_2); 7.82 (d, 1H, *J* = 7.8 Hz, Ar-H); 7.92-8.13 (m, 7H, Ar-H); 8.24 (d, 1H, *J* = 9.3 Hz, Ar-H). ^{13}C NMR: δ 18.4, 28.4, 28.7, 29.1, 29.5, 29.8, 31.9, 33.6, 68.1, 84.8 (alkyne), 123.4, 124.6, 124.7, 125.0, 125.7, 126.3, 126.4, 127.0, 127.2, 127.5, 128.5, 129.6, 130.9, 131.4, 137.3, 138.3.

1-(1-Pyrenyl)dodeca-1,11-diyne (9)

Pyridinium chlorochromate (1.74g, 8.00 mmol) and anhydrous NaOAc (0.66g, 8.0 mmol) were suspended in CH_2Cl_2 (15ml) and a solution of the alcohol **7** (1.49g, 4.0 mmol) in CH_2Cl_2 (10ml) was added and the mixture stirred for 4 hours. The reaction mixture was filtered, the solvent removed and the residue purified by flash chromatography using 20/80 EtOAc/hexanes as eluant . 11-(1-Pyrenyl)undec-10-ynal was recovered as a viscous oil which slowly solidified to a colourless solid, mp 58-59° in 1.42g (96%) yield. HRMS Calculated for $C_{27}H_{26}O$: 366.1984. Found: 366.1989. MS: 366 (M^+, 100), 253 (15), 239 (48). IR (nujol): ν_{max} 3100, 2750, 2240, 1720, 846 cm^{-1}. ^1H NMR: δ 1.36-1.69 (m, 12H, $(CH_2)_6$); 1.77 (quintet, 2H, *J* = 7.2 Hz, $\mathbf{CH_2}$-CH_2-alkyne); 2.42 (dt, 2H, *J* = 7.3, 1.8 Hz, $\mathbf{CH_2}$-CHO); 2.65 (t, 2H, *J* = 6.9 Hz, CH_2-alkyne); 7.98-8.21 (m, 8H, Ar-H); 8.56 (d, 1H, *J* = 9.2 Hz, Ar-H); 9.75 (t, 1H, *J* = 1.8 Hz, CHO). ^{13}C NMR: δ 19.9, 22.1, 28.3, 28.9, 29.1, 29.3, 43.9, 79.7, 96.3 (alkynyl), 118.9, 124.4, 124.4, 124.5, 125.3, 125.7, 126.1, 127.2, 127.7, 127.9, 129.6, 130.6, 131.1, 131.3, 131.8, 202.8. The aldehyde was treated in an analogous manner as described under **8** to give the title compound as an oil which slowly solidified to a colourless solid, mp 40-41° in 0.86g (64%) yield. HRMS Calculated for $C_{28}H_{26}$: 362.2034. Found: 362.2032. MS: 362 ($M^{+\cdot}$, 15), 255 (38), 198 (62), 181 (100), 153 (58). IR (thin film): ν_{max} 3300, 3040, 2928, 2852, 2245, 2140, 1600, 1582, 1506, 1490, 1466, 1436, 1186, 846, 718 cm^{-1}. UV ($CHCl_3$) λ_{max}: 249 (47 000), 264 (13 900), 274 (29 400), 285 (54 900), 329 (17 200), 345 (39 600), 364 (56 000) nm.

Fluorescence ($CHCl_3$, λ_{ex} = 364 nm): 386 (100), 396 (63), 406 (68). ^{1}H NMR: δ 1.37-1.60 (m, 10H, $(CH_2)_6$); 1.76 (quintet, 2H, *J* = 7.2 Hz, C**H**$_2$CH$_2$-alkyne); 1.95 (t, 1H, *J* = 2.6 Hz, C≡C-H); 2.19 (dt, 2H, *J* = 6.9, 2.6 Hz, C**H**$_2$-alkyne); 2.64 (t, 2H, *J* = 7.2 Hz, CH_2-C≡CAr); 7.96-8.19 (m, 8H, Ar-H); 8.55 (d, 1H, *J* = 9.2 Hz, Ar-H). ^{13}C NMR: δ 18.4, 19.9, 28.7, 28.9, 29.00, 29.1, 68.1, 79.6, 84.7, 96.3 (alkyne), 118.8, 124.3, 124.4, 125.7, 126.1, 127.2, 127.7, 127.9, 129.6, 130.6, 131.1, 131.2, 131.8.

6-*O*-(1-Undec-10-ynyl)fluorescein methyl ester (11)

A mixture of fluorescein methyl ester (**10**) (1.00g, 2.89 mmol), 11-iodoundec-1-yne (**3**) (1.20g, 4.33mmol), K_2CO_3 (0.80g, 5.78 mmol) and 2-butanone (100ml) was heated at reflux for 8 hours. The mixture was filtered, the solvent removed and the residue dissolved in $CHCl_3$ (50ml). The organic solution was washed with 1.5N NaOH (50ml), water (50ml), brine (50ml), dried (Na_2SO_4) and the solvent removed. The residue was recrystallised from CH_2Cl_2/hexanes to give 1.19g (79%) of the title compound as bright orange crystals, mp 83-84°. HRMS Calculated for $C_{32}H_{32}O_5$: 496.2250. Found: 496.2234. MS: 496 (M^+, 97); 446 (79); 360 (100); 258 (48). IR (nujol mull): ν_{max} 3296, 3180, 2145, 1730, 1644, 1590, 1260, 852 cm^{-1}. UV (EtOH) λ_{max}: 234 (135 000), 256 (62 700), 277 (60 500), 311 (29 400), 364 (29 500), 437 (74 000), 459 (94 400), 489 (80 600) nm. Fluorescence (EtOH, λ_{ex} = 489 nm): 517 nm. ^{1}H NMR: 1.34-1.56 (m, 12H, $(CH_2)_6$); 1.83 (quintet, 2H, *J* = 6.6 Hz, C**H**$_2$-CH_2-O); 1.94 (t, *J* = 2.7 Hz, H-C≡); 2.19 (dt, 2H, *J* = 6.9, 2.7 Hz, CH_2-alkyne); 3.64 (s, 3H, CH_3-O); 4.06 (t, 2H, *J* = 7.0 Hz, CH_2-O-Ar); 6.46 (d, *J* = 1.9 Hz, C4-H); 6.54 (dd, *J* = 9.7, 1.9 Hz, C2-H); 6.73 (dd, *J* = 8.6, 2.4 Hz, C7-H); 6.85 (d, *J* = 9.7 Hz, C1-H); 6.88 (d, *J* = 8.6 Hz, C8-H); 6.94 (d, *J* = 2.4 Hz, C5-H); 7.31 (dd, *J* = 7.4, 1.4 Hz, C3'-H); 7.67 (dt, *J* = 7.4, 1.3 Hz, C5'-H); 7.74 (dt, *J* = 7.4, 1.3 Hz, C4'-H); 8.25 (dd, *J* = 7.4, 1.3 Hz, C6'-H). ^{13}C NMR: δ 18.4, 25.9, 28.4, 28.7, 28.9, 29.0, 29.2, 29.9, 52.4, 68.1, 68.9, 87.7, 100.5, 105.7, 113.9, 114.6, 117.4, 128.8, 129.6, 129.8, 130.2, 130.3, 131.1, 132.7, 134.7, 150.4, 154.3, 159.0, 163.7, 165.7, 185.7.

***N*-(1-Oxoundec-10-ynyl)-5-aminofluorescein (12)**

To a stirred solution of 5-aminofluorescein (0.50g, 1.44 mmol) in pyridine (5ml) was added dropwise undec-10-ynoyl chloride (0.58g, 2.88 mmol, 2eq). The reaction mixture was stirred for 48 hours, poured into water (50ml), acidified with 10% HCl until pH<2, the bright orange precipitate collected, washed with water (5ml), air dried, dissolved in a small quantity of EtOAc and precipitated by the addition of hexane to give the title compound as bright orange crystals, mp 138-140° in 0.63g (65%) yield. Slow recrystallisation from EtOAc/hexanes gave crystals composed of a mixture of lactone (colourless) and acid (orange) forms. HRMS

(LSIMS) Calculated for $C_{31}H_{30}NO_6$ ($M+H^+$): 512.2073. Found: 512.1987. MS: 512 ($M+H^+$, 100), 347 (16), 207 (19). IR (nujol): ν_{max} 3500-2500, 3296, 2140, 1738, 1666, 1610 cm^{-1} UV (EtOH) λ_{max}: 231 (48 500), 255 (24 900), 455 (6 840), 483 (6 670) nm. Fluorescence (EtOH, λ_{ex} = 483 nm): 516 nm. NMR data for the lactone structure. ^1H NMR (d_6-DMSO): δ 1.30-1.44 (m, 10H, $(CH_2)_5$); 1.63 (quintet, 2H, *J* = 6.6 Hz, CH_2-CH_2-C(O)N); 2.14 (dt, 2H, *J* = 6.8, 2.5 Hz, CH_2-alkyne); 2.37 (t, 2H, *J* = 7.3 Hz, $CH_2C(O)N$); 2.73 (t, 1H, *J* = 2.5 Hz, H-C≡C); 6.54 (dd, 2H, *J* = 8.6, 2.1 Hz, C2-H); 6.59 (d, 2H, *J* = 8.6 Hz, C1-H); 6.66 (d, 2H, *J* = 2.1 Hz, C4-H); 7.19 (d, 1H, *J* = 8.3 Hz, C5'-H); 7.82 (dd, 1H, *J* = 8.3, 1.8 Hz, C6'-H); 8.33 (d, 1H, *J* = 1.8 Hz, C5'-H); 10.11 (bs, 2H, Ar-OH); 10.35 (bs, 1H, C(O)NH). ^{13}C NMR (d_6-DMSO): δ 19.5, 26.8, 29.7, 29.9, 30.2, 30.4, 30.5, 38.3, 72.8, 84.8, 86.3, 104.0, 111.6, 114.3, 115.1, 126.1, 128.0, 128.7, 130.9, 142.6, 148.4, 153.7, 161.2, 170.4, 173.7.

5-Dimethylamino-*N*-(11-undec-1-ynyl)-1-naphthalenesulphonamide (13)

To a stirred mixture of dansyl chloride (200mg, 0.74 mmol) and Et_3N (75mg, 100ml, 0.74 mmol) in CH_2Cl_2 (5ml) was added undec-10-yn-1-amine (**5**) (124mg, 0.74 mmol). The reaction mixture was stirred for 60 minutes, the solvent removed and the residue separated by flash chromatography eluant 15:85 EtOAc/hexanes to give the title compound as a fluorescent green oil in 215mg (72%) yield. When stored at -18° the oil solidified to give a fluorescent green solid, mp 58°. HRMS Calculated for $C_{23}H_{32}N_2O_2S$: 400.2184. Found: 400.2200. MS (EI, % relative abundance): 400 (M^+, 77), 171 (100). IR (thin film): ν_{max} 3300, 3052, 2116, 1586, 1574, 1464, 1322, 1144, 790 cm^{-1}. UV (EtOH) λ_{max}: 220 (29 900), 253 (12 400), 337 (4 000) nm. Fluorescence (EtOH, λ_{ex} = 337 nm): 506 nm. ^1H NMR: 1.09-1.26 (m, 12H, $(CH_2)_5$); 1.48 (quintet, *J* = 7.5 Hz, CH_2-CH_2-NH); 1.94 (t, *J* = 2.7 Hz, H-C≡); 2.16 (dt, 2H, *J* = 7.0, 2.7 Hz, CH_2-alkyne); 2.88 (q, 2H, *J* = 6.8 Hz, CH_2-NH); 2.89 (s, 6H $(CH_3)_2$-N); 4.59 (bt, 1H, *J* = 5.2 Hz, SO_2NH); 7.19 (d, 1H, *J* = 7.7 Hz, C6-H); 7.53 (dd, 1H, *J* = 8.4, 7.4 Hz, C3-H); 7.57 (dd, 1H, *J* = 8.6, 7.7 Hz, C7-H); 8.25 (dd, 1H, *J* = 7.3, 1.3 Hz, C4-H); 8.29 (d, 1H, *J* = 8.6 Hz, C8-H); 8.54 (dd, 1H, *J* = 8.4, 1.0 Hz, C2-H). ^{13}C NMR: δ 18.3, 26.3, 28.4, 28.6, 28.8, 29.2, 29.4, 43.3, 45.4, 68.1, 84.7 (alkynyl), 115.1, 118.6, 123.2, 128.4, 129.7, 129.8, 130.4, 134.7, 152.0.

Biotin-*N*-(undec-10-ynyl)amide (14)

To a stirred solution of biotin-NHS ester (300mg, 0.29 mmol) in DMF (6ml) was added the amine **5** (147mg, 0.29 mmol). A white precipitate formed within 10 minutes. The mixture was stirred overnight, the solvent removed *in vacuo* (oil pump), the residue purified by flash chromatography using 8/92 MeOH/CH_2Cl_2 as

eluant, recrystallised from MeOH/H_2O and dried under vacuum to give the title compound as a colourless solid, mp 176-178° in 312mg (90%) yield. HRMS: Calculated for $C_{21}H_{35}N_3O_2S$: 393.2450. Found: 393.2458. MS: 393 (M^+, 3), 333 (28), 160 (100). IR (nujol): ν_{max} 3700-3200, 3292, 2140, 1692, 1648, 1548 cm^{-1}. ^1H NMR: 1.18-1.67 (*m*, 20H, $(CH_2)_{10}$); 1.87 (*t*, 1H, *J* = 2.6 Hz, H-C≡); 2.08-2.15 (*m*, 4H, CH_2-CON and CH_2-C=); 2.66 (*d*, 1H, J_{gem} = 12.9 Hz, C5-Hβ); 2.86 (*dd*, 1H, J_{gem} = 12.9, 4.9 Hz, C5-Hα); 3.10 (*dt*, 1H, *J* = 7.1, 4.6 Hz, C2-H); 3.16 (*q*, 2H, *J* = 6.9 Hz, CH_2-NHCO); 4.26 (*dd*, 1H, *J* = 7.7, 4.9 Hz, C4-H); 4.45 (*dd*, 1H, *J* = 7.7 Hz, 4.6 Hz, C3-H); 4.56 (*bs*, 1H, N1'-H); 5.12 (*bs*, 1H, N3'-H); 5.45 (*bt*, 1H, *J* = 5.2 Hz, -NHCO). ^{13}C NMR: δ 16.5, 24.0, 25.1, 26.5, 26.6, 26.7, 26.8, 27.2, 27.4, 27.6, 27.8, 34.0, 37.3, 38.6, 54.1, 58.0, 59.8, 67.9, 101.0 (alkynyl), 161.6, 170.9.

5-(11-Hydroxyundec-1-ynyl)-1,10-phenanthroline (15)

A mixture of 5-bromo-1,10-phenanthroline[15] (2.59g, 10.0 mmol), undec-10-yn-1-ol **2** (2.01g, 12.0 mmol) and $Pd(PPh_3)_4$ (0.58g, 0.50 mmol) was stirred at 70° in pyrrolidine (40ml) for 7 hours, at which time analytical TLC showed the absence of starting material at R_f= 0.80 (alumina plates, MeOH/CH_2Cl_2 10/90). The reaction mixture was poured into saturated NH_4Cl solution (100ml), extracted with CH_2Cl_2 (2 x 100ml), the extracts combined, washed with saturated NH_4Cl solution (3 x 50ml), brine (25ml), dried ($MgSO_4$) and solvent removed. The residue was purified by flash chromatography on alumina, using $CHCl_3$/hexanes 50/50 as eluant. The product was recovered as a colourless oil which solidified when stored at -18° to give colourless crystals, mp 52-54° in 2.31g (66%) yield. HRMS: calculated for $C_{23}H_{26}N_2O$: 346.2045. Found: 346.2032. MS: 346 (M^+, 8), 260 (13), 232 (33), 219 (62), 194 (21), 83 (100). IR (thin film): ν_{max} 3600-3100, 3050, 2220 cm^{-1}. ^1H NMR: δ 1.34-1.72 (m, 14H, $(CH_2)_7$); 2.58 (t, 2H, *J* = 6.9Hz, CH_2-alkyne); 3.63 (t, 2H, CH_2-OH); 7.60 (dd, 1H, *J* = 8.1, 4.4Hz, C3-H); 7.68 (dd, 1H, *J* = 8.3, 4.3Hz, C8-H); 7.92 (s, 1H, H6); 8.16 (dd, 1H, *J* = 8.1, 1.7Hz, C4-H); 8.72 (dd, 1H, *J* = 8.3, 1.7Hz, C7-H); 9.14 (dd, 1H, *J* = 4.4, 1.7Hz, C2-H); 9.19 (dd, 1H, *J* = 4.4, 1.8Hz, C9-H). ^{13}C NMR: δ 19.5, 25.6, 28.5, 28.8, 29.1, 29.2, 29.3, 32.6, 62.6, 77.0, 96.8 (alkyne); 120.58, 123.0, 123.1, 127.9, 128.4, 129.8, 134.6, 135.3, 145.6, 145.7, 150.2, 150.2.

5-(11-Hydroxyundecyl)-1,10-phenanthroline (16)

A mixture of alkyne **15** (1.00g, 2.89 mmol), 10% Pd/C (0.50g), 10% HCl (5ml) and MeOH (50ml) was stirred under a hydrogen atmosphere for 24 hours. The mixture was filtered through celite, the organic solvent removed and the pH of the aqueous residue adjusted to ~12 with 1M NaOH. The aqueous solution was

extracted with $CHCl_3$ (50ml), the organic extract washed with water (50ml), dried ($MgSO_4$), the solvent removed and the residue purified by flash chromatography on alumina, eluting first with 50/50 hexanes/$CHCl_3$ to remove impurities, then $CHCl_3$. The product was recrystallised from CCl_4 to give the title compound as colourless crystals, mp 117.5-119.0°, in 0.79g (78%) yield. HRMS Calculated for $C_{23}H_{30}N_2O$: 350.2358. Found: 350.2362. MS: 350 (M^+, 53), 349 (39), 319 (60), 207 (94), 193 (100). IR (thin film): ν_{max} 3600-3100, 3050, 1620, 1564, 1424 cm^{-1}. ^1H NMR: δ 1.21-1.42 (m, 14H, $(CH_2)_7$); 1.49 (quintet, 2H, *J* = 6.9 Hz, CH_2-CH_2-Ar); 1.70 (quintet, 2H, *J* = 7.4 Hz, CH_2-CH_2-OH); 2.07 (br s, 1H, R-OH); 2.99 (t, 2H, *J* = 7.7 Hz, CH_2-Ar); 3.58 (t, 2H, *J* = 6.6 Hz, CH_2-OH); 7.48 (s, 1H, C6-H); 7.50 (dd, 1H, *J* = 8.0, 4.4 Hz, C3-H); 7.56 (dd, 1H, *J* = 8.4, 4.3 Hz, C8-H); 8.07 (dd, 1H, *J* = 8.0, 1.7 Hz, C4-H); 8.33 (dd, 1H, *J* = 8.4, 1.6 Hz, C7-H); 9.05 (dd, 1H, *J* = 4.4, 1.7 Hz, C2-H); 9.11 (dd, 1H, *J* = 4.3, 1.6 Hz, C9-H). ^{13}C NMR: δ 27.7, 31.2, 31.3, 31.4, 31.4, 31.5, 31.6, 32.2, 34.6, 34.8, 64.9, 124.6, 124.9, 126.8, 130.0,130.4, 134.2, 137.3, 139.4, 147.5, 148.5, 151.7, 151.8.

5-(11-Oxoundecyl)-1,10-Phenanthroline (17)

Oxalyl chloride (0.22ml, 2.6 mmol) was dissolved in CH_2Cl_2 (25ml) and cooled in a dry ice/acetone bath. DMSO (0.36ml, 5.1 mmol) was added and the reaction mixture was stirred for 5 minutes. A solution of the alcohol **16** (815mg, 2.3 mmol) in CH_2Cl_2 (10ml) was added, the mixture stirred for 15 minutes, Et_3N (1.62ml, 11.6 mmol) added and stirring continued for 5 minutes. The cooling bath was removed and the reaction mixture allowed to come to room temperature. Water (25ml) was added, the organic layer was separated, washed with brine (25ml), dried ($MgSO_4$) and solvent removed. The residue was separated by flash chromatography on alumina, eluant $CHCl_3$ to give the title compound in 559mg (69%) yield. HRMS (LSIMS) Calculated for $C_{23}H_{29}N_2O$ ($M+H^+$): 349.2280. Found: 349.2263. MS: 349 ($M+H^+$, 72), 321 (53), 207 (82), 194 (100). IR (thin film): ν_{max} 3030, 2716, 1724, 1424, 744 cm^{-1}. ^1H NMR: δ 1.21-1.49 (m, 12H, $(CH_2)_7$); 1.61 (quintet, 2H, *J* = 7.1 Hz, CH_2-CH_2-CHO); 1.79 (quintet, 2H, *J* = 7.2 Hz, CH_2-CH_2-Ar); 2.41 (dt, 2H, *J* = 7.3, 1.9 Hz, CH_2-CHO); 3.11 (t, 2H, *J* = 7.5 Hz, CH_2-Ar); 7.60 (s, 1H, C6-H); 7.61 (dd, 1H, J = 8.1, 4.4 Hz, C3-H); 7.67 (dd, 1H, *J* = 8.4, 4.3 Hz, C8-H); 8.18 (dd, 1H, *J* = 8.1, 1.4 Hz, C4-H); 8.44 (dd, 1H, *J* = 8.4, 1.5 Hz, C7-H); 9.13 (dd, 1H, *J* = 4.3, 1.5 Hz, C9-H); 9.19 (dd, 1H, *J* = 4.4, 1.4 Hz, C2-H); 9.75 (t, 1H, *J* = 1.7 Hz, CHO). ^{13}C NMR: δ 21.8, 24.8, 28.8, 29.1, 29.2, 29.4, 29.9, 32.3, 43.6, 122.5, 122.9, 124.6, 127.8, 128.2, 132.0, 135.1, 137.2, 145.2, 146.2, 149.2, 149.3, 202.7.

5-(Dodec-11-ynyl)-1,10-phenanthroline (18)

To a stirred slurry of potassium *tert*-butoxide (35mg, 0.32 mmol) in THF (10ml) at -78° was added dropwise diethylmethyldiazophosponate (56mg, 0.32 mmol). The mixture was stirred for 10 minutes then a solution of the aldehyde **17** (100mg, 0.29 mmol) in THF (5ml) was added dropwise. The reaction mixture was stirred overnight as the cooling bath warmed to room temperature, quenched by the addition of saturated $NaHCO_3$ solution (2ml) and water (15ml) was added. The mixture was extracted with CH_2Cl_2 (2 x 10ml), the combined extracts washed with brine (20ml), dried ($MgSO_4$) and the solvent removed. The yellow residue was purified by flash chromatography on alumina with $CHCl_3$/hexanes 30:70 as eluant, and recrystallised from hexanes to give the title compound as colourless crystals in 47mg (48%) yield, mp 73-74°. Calculated for $C_{24}H_{28}N_2$: 344.2252. Found: 344.2245. MS: 344 (M^+, 100), 217 (15), 207 (19), 193 (40), 145 (51), 105 (83). IR (nujol mull): ν_{max} 3172, 2130, 1512, 872, 740 cm^{-1}. ^1H NMR: δ 1.27-1.53 (m, 14H, $(CH_2)_7$); 1.77 (quintet, 2H, J = 7.7 Hz, CH_2-CH_2-Ar); 1.92 (t, 1H, J = 2.6 Hz, H-C≡C); 2.14 (dt, 2H, J = 6.9, 2.6 Hz, CH_2-alkyne); 3.08 (t, 2H, J = 7.5 Hz, CH_2-Ar); 7.57 (s, 1H, C6-H); 7.58 (dd, 1H, J = 8.4, 4.2 Hz, C8-H); 7.64 (dd, 1H, J = 8.4, 4.2 Hz, C3-H); 8.15 (dd, 1H, J = 8.1, 1.7 Hz, C4-H); 8.41 (dd, 1H, J = 8.4, 1.6 Hz, C7-H); 9.11 (dd, 1H, J = 4.3, 1.7 Hz, C2-H); 9.17 (dd, 1H, J = 4.2, 1.6 Hz, C9-H). ^{13}C NMR: δ 18.4, 28.7, 29.1, 29.4, 29.5, 29.5, 29.6, 29.7, 30.1, 32.6, 68.0, 84.8 (alkynyl), 122.7, 123.1, 124.8, 128.1, 128.4 132.3, 135.3, 132.4, 145.5, 146.5, 149.5, 149.6.

5-(11-Oxoundec-1-ynyl)-1,10-phenanthroline (19)

Reaction of alcohol **15** under the same conditions as outlined for **17** gave the product as colourless crystals, mp 50-51° in 69% yield. HRMS Calculated for $C_{23}H_{24}N_2O$: 344.1889. Found: 344.1886. MS: 344 (M^+, 8), 315 (7), 259 (52), 231 (69), 219 (70), 91 (100). IR (nujol): ν_{max} 2720, 2212, 1722, 1504, 1420, 744 cm^{-1}. ^1H NMR: δ 1.33-1.73 (m, 12H, $(CH_2)_6$); 2.38 (dt, 2H, J = 7.3, 1.7 Hz, CH_2-CHO); 2.55 (t, 2H, J = 7.0 Hz, CH_2-alkyne); 7.56 (dd, 1H, J = 8.1, 4.3 Hz, C3-H); 7.65 (dd, 1H, J = 8.2 Hz, 4.3 Hz, C8-H); 7.88 (s, 1H, C6-H); 8.12 (dd, 1H, J = 8.1, 1.7 Hz, C4-H); 8.67 (dd, 1H, J = 8.2, 1.7 Hz, C7-H); 9.11 (dd, 1H, J = 4.3, 1.7 Hz, C2-H); 9.16 (dd, 1H, J = 4.3, 1.7 Hz, C9-H); 9.72 (t, 1H, J = 1.6 Hz, CHO). ^{13}C NMR: δ 19.2, 21.3, 28.2, 28.3, 28.4, 28.5, 28.6, 28.8, 43.4, 67.7, 96.4 (alkynyl), 120.3, 122.8, 122.9, 128.8, 129.2, 129.6, 134.4, 135.1, 145.4, 145.5, 150.0, 150.1, 202.3.

5-(Dodec-1,11-diynyl)-1,10-phenanthroline (20)

The aldehyde **19** was treated as described for **17** to give the title compound as colourless crystals, mp 86-88°

in 60% yield. HRMS: Calculated for $C_{24}H_{24}N_2$: 340.1939. Found: 340.1937. MS: 340 (M^+, 88), 245 (38), 219 (87), 231 (48), 217 (75), 190 (24), 149 (19), 41 (100). IR (thin film): ν_{max} 3300, 3020, 2928, 2852, 2220, 2120, 1606, 1590, 1506, 1424, 742 cm^{-1}. ^1H NMR: 1.26-1.68 (m, 10H, $(CH_2)_5$); 1.75 (quintet, 2H, J =7.2 Hz, CH_2-CH_2-C=C-Ar); 1.95 (t, 1H, J = 2.6 Hz, H-C≡C); 2.20 (dt, 2H, J = 6.9, 2.6 Hz, CH_2-C≡CH); 2.61 (t, 2H, J = 7.0 Hz, CH_2-C≡C-Ar); 7.63 (dd, 1H, J = 8.1, 4.4 Hz, C3-H); 7.71 (dd, 1H, J = 8.3, 4.4 Hz, C8-H); 7.95 (s, 1H, C6-H); 8.19 (dd, 1H, J = 8.3, 1.8 Hz, C4-H); 8.74 (dd, 1H, J = 8.3, 1.8 Hz, C7-H); 9.17 (dd, 1H, J = 4.4, 1.8 Hz, C2-H); 9.20 (dd, 1H, J = 4.4, 1.8 Hz, C9-H). ^{13}C NMR: δ 18.4, 19.7, 28.4, 28.7, 28.7, 28.9, 29.0, 68.1, 77.2, 84.7, 96.9 (alkynyl), 120.2, 123.2, 123.3, 128.1, 128.6, 130.0, 134.8, 135.5, 145.8, 145.9, 150.4, 150.5.

Bis-1,10-Phenanthroline-5-[(dodec-11-ynyl)-1,10-phenanthroline]ruthenium(II) hexafluorophosphate (21)

A mixture of *bis*-1,10-phenanthrolineruthenium(II)dichloride (100mg, 0.19 mmol), 5-(dodec-11-ynyl)-1,10-phenanthroline **18** (65mg, 0.19 mmol), water (2ml) and MeOH (1ml) was stirred at 50° for 48 hours. The dark red-brown mixture was filtered to remove a black precipitate, the filtrant concentrated under reduced pressure and a solution of ammonium hexafluorophosphate (500mg) in water (2.5ml) added. The precipitated orange crystals were collected, air dried and purified by flash chromatography on alumina (the compound decomposes on silica), eluant $CHCl_3$ to give the title compound as a dark red glass mp >150° in 140mg (68%) yield. Attempted recrystallisations from various solvents were unsuccessful. HRMS Calculated for $C_{48}H_{44}F_6N_6P^{102}Ru$ (M-PF_6^+): 951.2313. Found: 951.2321. MS (LSIMS): 951 ($^{102}Ru\ M^{2+}.PF_6^-$, 100), 806 ($^{102}Ru\ MH^+$, 61), 625 ($^{102}RuM^+$ - phen, 22). IR (nujol): ν_{max} 3300, 1640, 1040, 840, 722 cm^{-1}. UV ($CHCl_3$): 257 (31800), 423 (11600), 449 (12300) nm. Fluorescence: 578 nm. ^1H NMR (d_6-DMSO): δ 1.22-1.48 (m, $(CH_2)_6$); 1.79 (quintet, 2H, J = 7.2 Hz, CH_2-CH_2-Ar); 2.11 (dt, 2H, J = 6.8, 2.6 Hz, CH_2-C≡C); 2.70 (t, 1H, J = 2.6 Hz, H-C≡C); 3.24 (m, 2H, CH_2-Ar); 7.70-7.78 (m, 6H, C3-H, C3'-H, C8-H and C8'-H); 7.99 (d, 1H, J = 5.3 Hz, C4'-H); 8.06 (m, 5H, C7'-H, C4-H and C7-H); 8.19 (s, 1H, C6'-H), 8.37 (s, 4H, C5-H and C6-H); 8.66 (d, 1H, J = 7.2 Hz, C2'-H); 8.76 (d, 4H, J = 8.2 Hz, C2-H and C9-H); 8.84 (d, 1H, J = 7.4 Hz, C9'-H). ^{13}C NMR (d_6-DMSO): δ 17.6, 27.9, 28.0, 28.4, 28.8, 28.8, 28.9, 29.8, 31.3, 70.8, 84.5 (alkynyl), 125.2, 126.1, 127.9, 130.0, 130.1, 130.4, 133.8, 134.7, 135.4, 136.6, 136.9, 140.0, 146.4, 147.2, 147.6, 151.5, 151.9, 152.3, 153.0, 153.9.

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