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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. © 1997, Elsevier Science Ltd. All rights reserved.

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**).



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 biomolecules⁴. 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 acid⁵, 1, which could be converted to a number of key intermediates (**Scheme 1**). Reduction of 1 by LiAlH₄ in diethyl ether furnished undec-10-yn-1-ol⁶, 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 SOCl₂ 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 LiAlH₄. 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.





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.



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(PPh₃)₄ and CuI in piperidine¹⁰ (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 pyrrolidine¹¹ at 80° for 20 hours (Scheme 3, Condition B) also afforded 7 in a good yield of 79%.



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 Fuchs¹². Treatment of CBr₄ with PPh₃ in CH₂Cl₂ 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_4CI 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).



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[d⁶] as shown by the presence of 6 aromatic resonances (3 which integrate for 2H and 3 which integrate for 1H) in the ¹H 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.



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).



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.



The bromination of 1,10-phenanathroline in fuming sulphuric acid (60% oleum) gave 5-bromo-1,10phenanathroline as described in the literature.¹⁵ Previous palladium catalysed coupling reactions of bromosubstituted 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-1,10phenanthroline 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**.



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 Fuchs¹² in which treatment of 17 with CBr_4 and PPh₃ 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).



Formation of the ruthenium complex 21 was achieved by stirring 18 with Ru(phen)₂Cl₂ 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 (102RuM2+.PF6⁻), confirming incorporation of 18 into the ruthenium complex. As the parent tris-1,10- phenanthroline ruthenium (II) complex is chiral¹⁸, 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 ($\lambda_{ex} = 449$ nm).



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 CDCl₃ 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 CH₂Cl₂ were distilled from CaH₂ under nitrogen and stored over 4Å molecular sieves. DMF was distilled from CaH₂ ($ca. 80^{\circ}$ at 20mmHg) and stored over 4Å molecular sieves. Et₂O 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 acid⁵, undec-10-yn-1-ol6, fluorescein methyl ester (10)¹³, biotin NHS ester¹⁴, 5-bromo-1,10-phenanthroline¹⁵, diethylmethyldiazophosphonate¹⁷, bis-1,10-phenanthrolineruthenium(II)dichloride¹⁸ and 1-bromopyrene¹⁹.

11-Iodoundec-1-yne (3)

To a stirred mixture of **2** (5.00g, 30 mmol) and Et₃N (3.01g, 30 mmol) in CH₂Cl₂ (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 MgSO₄ 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): v_{max} 3292, 2928, 2852, 2116, 1468, 1354, 1176 cm⁻¹ ⁻¹H NMR: δ 1.30-1.41 (m, 14H, (CH₂)₇); 1.75 (quintet, 2H, J = 7.2 Hz, CH₂-CH₂-OMs); 1.95 (t, 1H, J = 2.6 Hz, C-H); 2.19 (dt, 2H, J = 2.6, 6.9 Hz, CH₂-C); 3.01 (s, 3H, CH₃-SO₃); 4.22 (t, 2H, J = 6.6 Hz, CH₂-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 compound⁷ 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): v_{max} 3300, 2924, 2852, 2116, 1464, 1432, 1220, 1178, 720 cm⁻¹. ¹H NMR: δ 1.25-1.61 (m, 12H, (CH₂)₆); 1.82 (quintet, J = 7.0 Hz, 2H, CH₂-CH₂-I); 1.95 (t, J = 2.6 Hz, H-C); 2.19 (dt, J = 2.6, 6.9 Hz, CH₂-C); 3.19 (t, J = 7.0 Hz, 2H, CH₂-I). ¹³C NMR: δ 7.18 (CH₂-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 NH₃ solution held at -15° (dry ice-MeOH bath). The precipitated white product was collected, washed with water (20ml), air dried and recrystallised from CH₂Cl₂ (charcoal). The product was recovered as colourless crystals²⁰ mp 94-95° in 7.84g (in two crops) (84%) yield. HRMS Calculated for C₁₁H₁₉NO: 181.1467. Found: 181.1459. MS: 182 (M+H⁺), 181 (M⁺), 122 (21), 72 (19), 59 (100). IR (nujol): v_{max} 3356, 3280, 3184, 2150, 1662, 1632 cm⁻¹. ¹H NMR: δ 1.31-1.65 (m, 12H, (CH₂)₆); 1.93 (t, 1H, *J* = 2.6 Hz, H-C); 2.12-2.24 (m, 4H, CH₂-C and CH₂-CONH₂); 5.56 and 5.89 (2 x bs, 1H, CONH₂). ¹³C 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 LiAlH₄ (1.22g, 33 mmol) in Et₂O (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 C₁₁H₂₂N (M+H⁺): 168.1752. Found: 168.1756. MS: 168 (M+H⁺), 138 (48), 110 (39), 96 (43), 86 (100). IR (film): v_{max} 3300 , 3304, 2116 cm⁻¹ . ¹H NMR: δ 1.21-1.48 (m, 14 H, (CH₂)₇); 1.86 (t, 1H, *J* = 2.7 Hz, H-C); 2.09 (dt, 2H, *J* = 2.7, 6.9, Hz, CH₂-C); 2.60 (t, 2H, *J* = 6.6 Hz, CH₂-NH₂). ¹³C 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-(10H)-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): v_{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, (CH₂)₆); 1.76 (quintet, 2H, *J* = 7.5 Hz, N-CH₂-CH₂); 1.97 (t, 1H, *J* = 2.1 Hz, C-H); 2.16 (dt, 2H, *J* = 2.3, 6.6 Hz, CH₂-C); 4.11 (t, 2H, *J* = 7.8 Hz, N-CH₂); 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). ¹³C 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(PPh₃)₄ (0.20g, 0.215mmol). The reaction mixture was stirred at 80° for six hours, cooled to room temperature, poured into saturated NH₄Cl solution (50ml), the mixture extracted with CH₂Cl₂ (2 x 25ml), the combined organic extracts washed with 10% citric acid (25ml), brine (25ml), dried (MgSO₄) 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 C₂₇H₂₈O: 368.2140. Found: 368.2126. MS: 368 (M⁺, 100), 239 (50). ¹H NMR: δ 1.48-1.52 (m, 11H, (CH₂)₅ and OH); 1.56 (quintet, 2H, *J* = 6.5 Hz, alkyne-CH₂-CH₂-); 1.77 (quintet, 2H, *J* = 7.2 Hz, -CH₂-CH₂-OH); 2.64 (t, 2H, *J* = 7.0 Hz, CH₂-alkyne); 3.62 (t, 2H, *J* = 6.5 Hz, CH₂-OH); 7.97-8.20 (m, 8H, Ar-H); 8.56 (d, 1H, *J* = 9.0 Hz, Ar-H). ¹³C 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 C₂₇H₃₂O: 372.2453. Found 372.2465. MS: 372 (M⁺, 100), 215 (24). IR (nujol mull): v_{max} 3416, 3050, 1610, 1520, 1054, 836 cm⁻¹. ¹H NMR: δ 1.25-1.52 (m, 16H, (CH₂)₈); 1.66 (bs, 1H (exchanges with D₂O), -OH); 1.81 (quintet, 2H, J = 7.5 Hz, CH₂-CH₂-Ar); 3.28 (t, 2H, J = 7.7Hz, Ar-CH₂); 3.57 (t, 2H, J = 6.6 Hz, CH₂-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). ¹³C 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 CH₂Cl₂ (15ml) and a solution of the 11-(1-pyrenyl)undecan-1-ol (1.49g, 4.0 mmol) in CH₂Cl₂ (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 C₂₇H₃₀O: 370.2297. Found: 370.2290. MS: 370 (M+, 79), 215 (100). ¹H NMR: δ 1.26-1.48 (m, 14H, (CH₂)₇); 1.83 (quintet, 2H, J = 7.7 Hz, CH₂-CH₂-Ar); 2.36 (dt, 2H, J = 7.3, 1.9 Hz, CH₂-CHO); 3.31 (t, 2H, J = 7.6 Hz, CH₂-Ar); 7.83-8.28 (m, 9H, Ar-H); 9.72 (t, 1H, J = 1.9 Hz, CHO). ¹³C 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 CH₂Cl₂ (20ml) and cooled to -15° in an dry ice/MeOH bath. A solution of CBr₄ (3.80g, 11.5 mmol) in CH₂Cl₂ (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 CH₂Cl₂ (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-(1pyrenyl)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, (CH₂)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 = 7.3Hz, H-C=); 7.86-8.32 (m, 9H, Ar-H). ¹³C NMR: δ 27.8, 29.0, 29.3, 29.5, 29.5, 31.9, 33.0, 33.6, 88.4 (Br₂C=), 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 NH₄Cl (7ml). The mixture was separated, dried (Na₂SO₄) 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 C₂₈H₃₀: 366.2348. Found 366.2334. MS: 366 (M⁺, 49), 215 (100). IR (nujol): v_{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, (CH₂)₇); 1.81 (quintet, 2H, *J* = 7.6 Hz, Ar-CH₂-CH₂); 1.93 (t, 1H, *J* = 2.7 Hz, C≡C-H); 2.15 (dt, 2H, *J* = 7.0, 2.7 Hz, -CH₂-alkyne); 3.31 (t, 1H, *J* = 7.8 Hz, Ar-CH₂); 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). ¹³C 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 CH₂Cl₂ (15ml) and a solution of the alcohol 7 (1.49g, 4.0 mmol) in CH₂Cl₂ (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 C27H26O: 366.1984. Found: 366.1989. MS: 366 (M⁺, 100), 253 (15), 239 (48). IR (nujol): v_{max} 3100, 2750, 2240, 1720, 846 cm⁻¹. ¹H NMR: δ 1.36-1.69 (m, 12H, (CH₂)₆); 1.77 (quintet, 2H, J = 7.2 Hz, CH₂-CH₂-alkyne); 2.42 (dt, 2H, J = 7.3, 1.8 Hz, CH₂-CHO); 2.65 (t, 2H, J = 6.9 Hz, CH₂-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). ¹³C 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 C₂₈H₂₆: 362.2034. Found: 362.2032. MS: 362 (M⁺, 15), 255 (38), 198 (62), 181 (100), 153 (58). IR (thin film): v_{max} 3300, 3040, 2928, 2852, 2245, 2140, 1600, 1582, 1506, 1490, 1466, 1436, 1186, 846, 718 cm⁻¹. UV (CHCl₃) 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 (CHCl₃, $\lambda_{ex} = 364$ nm): 386 (100), 396 (63), 406 (68). ¹H NMR: δ 1.37-1.60 (m, 10H, (CH₂)₆); 1.76 (quintet, 2H, J = 7.2 Hz, CH₂CH₂-alkyne); 1.95 (t, 1H, J = 2.6 Hz, C=C-H); 2.19 (dt, 2H, J = 6.9, 2.6 Hz, CH₂-alkyne); 2.64 (t, 2H, J = 7.2 Hz, CH₂-C=CAr); 7.96-8.19 (m, 8H, Ar-H); 8.55 (d, 1H, J = 9.2 Hz, Ar-H). ¹³C 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, 4.33 mmol), 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 CHCl3 (50ml). The organic solution was washed with 1.5N NaOH (50ml), water (50ml), brine (50ml), dried (Na₂SO₄) and the solvent removed. The residue was recrystallised from CH₂Cl₂/hexanes to give 1.19g (79%) of the title compound as bright orange crystals, mp 83-84°. HRMS Calculated for C₃₂H₃₂O₅: 496.2250. Found: 496.2234. MS: 496 (M⁺, 97); 446 (79); 360 (100); 258 (48). IR (nujol mull): v_{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, $\lambda_{ex} = 489$ nm): 517 nm. ¹H NMR: 1.34-1.56 (m, 12H, $(CH_2)_6$; 1.83 (quintet, 2H, J = 6.6 Hz, CH_2 -CH₂-O); 1.94 (t, J = 2.7 Hz, H-C=); 2.19 (dt, 2H, J = 6.9, 2.7 Hz, CH₂-alkyne); 3.64 (s, 3H, CH₃-O); 4.06 (t, 2H, J = 7.0 Hz, CH₂-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, 1.4 Hz, C3'-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). ¹³C 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): v_{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, (CH₂)₅); 1.63 (quintet, 2H, J = 6.6 Hz, CH₂-CH₂-C(O)N); 2.14 (dt, 2H, J = 6.8, 2.5 Hz, CH₂-alkyne); 2.37 (t, 2H, J = 7.3 Hz, CH₂C(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). ¹³C 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 Et₃N (75mg, 100ml, 0.74 mmol) in CH₂Cl₂ (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 C₂₃H₃₂N₂O₂S: 400.2184. Found: 400.2200. MS (EI, % relative abundance): 400 (M⁺, 77), 171 (100). IR (thin film): v_{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, (CH₂)₅); 1.48 (quintet, *J* = 7.5 Hz, CH₂-CH₂-NH); 1.94 (t, *J* = 2.7 Hz, H-C≡); 2.16 (dt, 2H, *J* = 7.0, 2.7 Hz, CH₂-alkyne); 2.88 (q, 2H, *J* = 6.8 Hz, CH₂-NH); 2.89 (s, 6H (CH₃)₂-N); 4.59 (bt, 1H, *J* = 5.2 Hz, SO₂NH); 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* = 8.4, 1.0 Hz, C2-H). ¹³C 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/CH₂Cl₂ as

eluant, recrystallised from MeOH/H₂O and dried under vacuum to give the title compound as a colourless solid, mp 176-178° in 312mg (90%) yield. HRMS: Calculated for C₂₁H₃₅N₃O₂S: 393.2450. Found: 393.2458. MS: 393 (M⁺, 3), 333 (28), 160 (100). IR (nujol): v_{max} 3700-3200, 3292, 2140, 1692, 1648, 1548 cm⁻¹. ¹H NMR: 1.18-1.67 (*m*, 20H, (CH₂)₁₀); 1.87 (*t*, 1H, J = 2.6 Hz, H-C=); 2.08-2.15 (*m*, 4H, CH₂-CON and CH₂-C=); 2.66 (*d*, 1H, $J_{gem} = 12.9$ Hz, C5-H β); 2.86 (*dd*, 1H, $J_{gem} = 12.9$, 4.9 Hz, C5-H α); 3.10 (*dt*, 1H, J = 7.1, 4.6 Hz, C2-H); 3.16 (*q*, 2H, J = 6.9 Hz, CH₂-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). ¹³C 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-phenanthroline¹⁵ (2.59g, 10.0 mmol), undec-10-yn-1-ol **2** (2.01g, 12.0 mmol) and Pd(PPh₃)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/CH₂Cl₂ (2 x 100ml), The reaction mixture was poured into saturated NH₄Cl solution (100ml), extracted with CH₂Cl₂ (2 x 100ml), the extracts combined, washed with saturated NH₄Cl solution (3 x 50ml), brine (25ml), dried (MgSO₄) and solvent removed. The residue was purified by flash chromatography on alumina, using CHCl₃/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 C₂₃H₂₆N₂O: 346.2045. Found: 346.2032. MS: 346 (M⁺, 8), 260 (13), 232 (33), 219 (62), 194 (21), 83 (100). IR (thin film): v_{max} 3600-3100, 3050, 2220 cm⁻¹. ¹H NMR: δ 1.34-1.72 (m, 14H, (CH₂)₇); 2.58 (t, 2H, *J* = 6.9Hz, CH₂-alkyne); 3.63 (t, 2H, CH₂-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). ¹³C 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.

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 CHCl₃ (50ml), the organic extract washed with water (50ml), dried (MgSO₄), the solvent removed and the residue purified by flash chromatography on alumina, eluting first with 50/50 hexanes/CHCl₃ to remove impurities, then CHCl₃. The product was recrystallised from CCl₄ to give the title compound as colourless crystals, mp 117.5-119.0°, in 0.79g (78%) yield. HRMS Calculated for C₂₃H₃₀N₂O: 350.2358. Found: 350.2362. MS: 350 (M⁺, 53), 349 (39), 319 (60), 207 (94), 193 (100). IR (thin film): v_{max} 3600-3100, 3050, 1620, 1564, 1424 cm⁻¹. ¹H NMR: δ 1.21-1.42 (m, 14H, (CH₂)₇); 1.49 (quintet, 2H, *J* = 6.9 Hz, CH₂-CH₂-Ar); 1.70 (quintet, 2H, *J* = 7.4 Hz, CH₂-CH₂-OH); 2.07 (br s, 1H, R-OH); 2.99 (t, 2H, *J* = 7.7 Hz, CH₂-Ar); 3.58 (t, 2H, *J* = 6.6 Hz, CH₂-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). ¹³C 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 CH₂Cl₂ (10ml) was added, the mixture stirred for 15 minutes, Et₃N (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 (25m), dried $(MgSO_4)$ and solvent removed. The residue was separated by flash chromatography on alumina, eluant CHCl₃ to give the title compound in 559mg (69%) yield. HRMS (LSIMS) Calculated for C₂₃H₂₉N₂O (M+H⁺): 349.2280. Found: 349.2263. MS: 349 (M+H⁺, 72), 321 (53), 207 (82), 194 (100). IR (thin film): v_{max} 3030, 2716, 1724, 1424, 744 cm⁻¹. ¹H NMR: δ 1.21-1.49 (m, 12H, (CH₂)₇); 1.61 (quintet, 2H, J = 7.1 Hz, CH₂-CH₂-CHO); 1.79 (quintet, 2H, J = 7.2 Hz, CH₂-CH₂-Ar); 2.41 (dt, 2H, J = 7.3, 1.9 Hz, CH₂-CHO); 3.11 (t, 2H, J = 7.5 Hz, CH₂-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, 1.4Hz, 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, 2H); = 4.4, 1.4 Hz, C2-H); 9.75 (t, 1H, J = 1.7 Hz, CHO). ¹³C 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 NaHCO3 solution (2ml) and water (15ml) was added. The mixture was extracted with CH₂Cl₂ (2 x 10ml), the combined extracts washed with brine (20ml), dried (MgSO₄) and the solvent removed. The yellow residue was purified by flash chromatography on alumina with CHCl₃/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): v_{max} 3172, 2130, 1512, 872, 740 cm⁻¹. ¹H NMR: δ 1.27-1.53 (m, 14H, (CH₂)₇); 1.77 (quintet, 2H, J = 7.7 Hz, CH₂-CH₂-Ar); 1.92 (t, 1H, J = 2.6 Hz, H-C=C); 2.14 (dt, 2H, J = 6.9, 2.6 Hz, CH₂-CH₂-Ar); alkyne); 3.08 (t, 2H, J = 7.5 Hz, CH₂-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, C4-Hz); 8.41 (dd, 1H, J = 8.4, 1.6 Hz, C4-Hz); 8.41 (dd, 1H, J = 8.4, 1.6 Hz, C4-Hz); 8.41 (dd, 1H, J = 8.4, 1.6 Hz, C4-Hz); 8.41 (dd, 1H, J = 8.4, 1.6 Hz, C4-Hz); 8.41 (dd, 1H, J = 8.4, 1.6 Hz, C4-Hz); 8.41 (dd, 1H, J = 8.4, 1.6 Hz, C4-Hz); 8.41 (dd, 1H, J = 8.4, 1.6 Hz, C4-Hz); 8.41 (dd, 1H, J = 8.4, 1.6 Hz, C4-Hz); 8.41 (dd, 1H, J = 8.4, 1.6 Hz, C4-Hz); 8.41 (dd, 1H, J = 8.4, 1.6 Hz, C4-Hz); 8.41 (dd, 1H, J = 8.4, 1.6 Hz); 8.41 (dd, 1H, J = 8.41 (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). ¹³C 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 C₂₃H₂₄N₂O: 344.1889. Found: 344.1886. MS: 344 (M⁺, 8), 315 (7), 259 (52), 231 (69), 219 (70), 91 (100). IR (nujol): v_{max} 2720, 2212, 1722, 1504, 1420, 744 cm⁻ ¹. ¹H NMR: δ 1.33-1.73 (m, 12H, (CH₂)₆); 2.38 (dt, 2H, J = 7.3, 1.7 Hz, CH₂-CHO); 2.55 (t, 2H, J = 7.0 Hz, CH₂-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). ¹³C 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): v_{max} 3300, 3020, 2928, 2852, 2220, 2120, 1606, 1590, 1506, 1424, 742 cm⁻¹. ¹H NMR: 1.26-1.68 (m, 10H, (CH₂)₅); 1.75 (quintet, 2H, *J* =7.2 Hz, CH₂-CH₂-C=C-Ar); 1.95 (t, 1H, *J* = 2.6 Hz, H-C=C); 2.20 (dt, 2H, *J* = 6.9, 2.6 Hz, CH₂-C=CH); 2.61 (t, 2H, *J* = 7.0 Hz, CH₂-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). ¹³C 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)-1,10phenanthroline 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 CHCl₃ 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 C₄₈H₄₄F₆N₆P¹⁰²Ru (M-PF₆⁺): 951.2313. Found: 951.2321. MS (LSIMS): 951 (¹⁰²Ru M²⁺.PF₆⁻, 100), 806 (102 Ru MH⁺, 61), 625 (102 RuM⁺ - phen, 22). IR (nujol): v_{max} 3300, 1640, 1040, 840, 722 cm⁻¹. UV (CHCl₃): 257 (31800), 423 (11600), 449 (12300) nm. Fluorescence: 578 nm. ¹H NMR (*d*₆-DMSO): δ 1.22-1.48 (m, (CH₂)₆); 1.79 (quintet, 2H, J = 7.2 Hz, CH₂-CH₂-Ar); 2.11 (dt, 2H, J = 6.8, 2.6 Hz, CH₂-C≡C); 2.70 (t, 1H, J = 2.6 Hz, H-C≡C); 3.24 (m, 2H, CH₂-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). ¹³C 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.

REFERENCES

- 1. Cook, A. F.; Vuocolo, E.; Brakel, C. L. Nucl. Acids Res. 1988, 16, 4077-4095. Mayer, A.; Neuenhofer, S. Angew. Chem. Int. Ed. Engl. 1994, 33, 1044-1072.
- Slater, R. J. Ed. Radioisotopes in Biology: A Practical Approach. Oxford University Press; Oxford; 1989. Rashtchian, A.; Mackey, J. in Nonradioactive Labeling and Detection of Biomolecules; Kessler, C. Ed. Springer-Verlag, Berlin, 1992; Chapter 4.
- McInnes, J. L.; Symons, R. H. in DNA Probes, Symons R. H. Ed., CRC, Boca Raton, 1989.
 Rashtchian, A.; Mackey, J. in Nonradioactive Labeling and Detection of Biomolecules; Kessler, C. Ed. Springer-Verlag, Berlin, 1992; Chapter 4.
- 4. Crisp, G.T.; Gore, J. Tetrahedron following paper.
- Vogel, A. I. Vogel's Textbook of Practical Organic Chemistry, 5th Ed.; Longman, London, 1989; p 510.
- Oserko, A. Chem. Ber. 1937, 70B, 55-61, (CA 1937, 31, 2580). Oehlschlager, A. C.; Czyzewska, E.; Aksela, R.; Pierce Jr., H. D. Can. J. Chem. 1986, 64, 1407-1413.
- Knapp, F. F.; Goodman, M. M.; Callahan, A. P.; Ferren, L. A.; Kabalka, G.W.; Sastry, K. A. R. J. Med. Chem. 1985, 26, 1293-1300.
- Nishi, H.; Kohno, H.; Kano, T. Bull. Chem. Soc. Jpn. 1981, 54, 1897-1898. Katrisky, A. R.; Ramer, W. H. J. Org. Chem. 1985, 50, 852-856.
- Heck, R. F. Palladium Reagents in Organic Synthesis Academic Press, London, 1985; p 299. Sonogashira, K.; Tohda, Y.; Hagihara, N. Tetrahedron Lett. 1975, 4467-4470.
- Brandsma, L.; van den Heuvel, H. G. M.; Verkruijsse, H. D. Synth. Commun. 1990, 20, 1889-1892.
- 11. Alami, M.; Ferri, F.; Linstrumelle, G. Tetrahedron Lett. 1993, 34, 6403-6406.
- 12. Corey, E. J.; Fuchs, P. L. Tetrahedron Lett. 1972, 3769-3772.
- 13. von Liebig, H. J. Prakt. Chem. 86, 472-516, (CA 1913, 7, 778-779).
- 14. Coughlin, R. W.; Baclaski, J. B. Biotechnol. Prog. 1990, 6, 307-309. McCormick, D. B. J. Heterocycl. Chem. 1973, 235-237.
- 15. Mlochowski, J. Roucz. Chem. 1974, 48, 2145-2155.
- 16. Suffert, J.; Ziessel, R. Tetrahedron Lett. 1991, 32, 757-760.
- Gilbert, J. C.; Weerasooriya, U. J. Org. Chem. 1979, 44, 4997-4998. McAlanan, H.; Stevenson,
 P. J. Tetrahedron: Asymmetry 1995, 6, 239-244. Regitz, M.; Liedhegener, A.; Eckstein, U.; Martin, M.; Anschutz, W. Liebigs Ann. Chem. 1971, 748, 207-210.
- Bannwarth, W.; Schmidt, D.; Stallard, R. L.; Hornung, C.; Knorr, R.; Muller, F. Helv. Chim. Acta. 1988, 71, 2085-2099. Bosnich, B.; Dwyer, F. P. Aust. J. Chem. 1966, 19, 2229-2240.
- 19. Lock, G. Chem. Ber. 1937, 70B, 926-930.
- Sandman, D. J.; Velazquez, C. S.; Hamill, G. P.; Preses, J. M.; Weston Jr, R. E. Mol. Cryst. Liq. Cryst. 1988, 156, 103-108.

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