The Nonradioactive Labelling of Biologically Important Molecules

A Thesis Submitted Towards the Degree of
Doctor of Philosophy

by

Jeffrey Gore B.Sc.(Hons)

Department of Chemistry

The University of Adelaide

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Contents

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It is easy to lose sight of the realities of life during such an artificial situation as studying for a higher degree, and so to my spouse Jane and children Antonia, Peter, Max and Madeleine, thankyou for your love, support, patience and for remembering who I am.

Statement

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

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Abstract

Radioisotopes, due to their radiation hazard and inherent instability, are being replaced as reporters in biochemical systems by molecules which have luminescent or other properties. The reporter molecules are covalently bound to the biomolecule of interest generally via a nucleophilic/electrophilic interaction, hence the label is generally attached to a hydrophilic site. The development and application of the palladium catalysed coupling reaction between aryl/vinyl halides/triflates and terminal alkynes to various biomolecule derivatives suggested the possibility of using this methodolgy for the attachment of labels to lipophilic positions in biomolecules. Hence fluorescent labels were synthesised from acridone, pyrene, fluorescein and 5-aminofluorescein by attachment to one terminus of a hydrocarbon spacer arm, which had at the other terminus either an alkyne or a functional group capable of being converted to an alkyne . Also synthesised similarly were a *tris*-phenanthrolineruthenium(II) complex for use as a time resolved fluorescence reporter, and a biotin label for use in the avidin/strepavidin reporting system. Suitable biomolecule derivatives of amino acids (4-iodophenylalanine, 3-iodotyrosine, 5-*O*-triflyltryptophan and 4-*O*-triflyltyrosine, propargyl glycine), nucleosides (5-iododeoxyuridine, 8-bromoadenosine, 8-bromoguanosine) and steroids (estrone and epiandrosterone triflate derivatives) were prepared and labelling attempted with the above alkynes. It was found that palladium catalysed cross coupling was an efficient and mild method for the introduction of the labels to the biomolecules (except for tryptophan).

Unexpectedly, it was found that the biotin label **107** gelled some low polarity organic solvents at low concentrations. Concentration dependent ^{1}H NMR spectroscopy of the gel in $CDCl_3$ and variable temperature studies of the gels in $CDCl_3$ and $^{2}[H]_8$-toluene were performed. In an attempt to define the scope of gelation by biotin compounds, a series of saturated alkyl biotin esters and amides with varying chain lengths from *n*-propyl to *n*-hexadecyl were synthesised and tested for gelation in many solvents. Gelation was observed in hexane and paraffin oil for some derivatives.

Abbreviations

18C-6	18-Crown-6
dansyl	5-dimethylamino-1-naphthalenesulphonyl
DCC	dicyclohexylcarbodiimide
DMAP	4-dimethylaminopyridine
DMF	dimethylformamide
DMSO	dimethylsulphoxide
DNA	deoxyribonucleic acid
FABMS	fast atom bombardment mass spectrometry
HRP	horseradish peroxidase
LAH	lithium aluminium hydride
LSIMS	liquid secondary ion mass spectometry
MEK	methyl ethyl ketone
mesyl, Ms	methanesulphonyl
NHS	*N*-hydroxysuccinimide
PCC	pyridinium chlorochromate
PdCC	palladium catalysed coupling
RNA	ribonucleic acid
RT	room temperature
TBAF	tetrabutylammonium fluoride
TBDMS	*t*-butyldimethylsilyl
TEMPO	2,2,6,6-tetramethyl-1-piperidinyloxy free radical
THF	tetrahydrofuran
TLC	thin layer chromatography
TRF	time resolved fluorescence
triflate	trifluoromethanesulphonate
triflyl	trifluoromethanesulphonyl
tosyl, Ts	*p*-toluenesulphonyl

Chapter 1. Introduction

1.1 Radioisotopic Labelling

The elucidation of biochemical processes is fundamental to an understanding of the nature of life[1]. With the discovery of methods in the 1930s for the artificial transmutation of stable atoms into non-stable isotopes, and for the collection of naturally occuring low abundance isotopes, the availability of *labelled* elements and compounds allowed researchers to trace the fate of vital substances *in vivo*, and hence deduce facts about the mechanisms which had occurred. In 1960 Broda wrote in his monograph[2]:

The importance of isotopic methods in biology has frequently been compared with that of the microscope. Just as the invention of the microscope in the 17th century advanced the science of living tissues by tremendous strides, and made possible the later discovery of cells and microbes, so does the employment of isotopic methods put us in a position to investigate the details of metabolism in a reliable and highly sensitive manner.

From the end of the Second World War Broda had seen the use of isotopic methods increase dramatically[3] and answer decisively many of the unresolved problems in biological science. For example, in classic experiments the use of differentially labelled bacteriophage (^{32}P incorporated into the DNA and ^{35}S into the protein coat) confirmed that DNA was the genetic material[4], and the incorporation of ^{15}N into *E. coli* bacteria showed the semiconservative nature of DNA replication[5], consistent with Watson and Crick's hypothesis[6].

The use of radioisotopically labelled compounds for research in biochemistry laboratories is well established[7]. Substrates labelled with radionuclides can be detected in very low amounts, and when the radionuclide replaces an atom of the same element the chemistry of the system is unchanged (assuming the radioisotope is not involved in the rate determing step and the concentration of radioisotope is low enough to ignore radiation-chemical effects). Detection of the emitted radiation is easy given the correct instrumentation, the literature for introducing radionuclides into compounds is extensive, and a large range of labelled compounds is commercially available. A survey of commonly used isotopes and relevant data is shown in **Table 1**. However, there are many disadvantages of

radionuclide labelling. The labelled compounds are a radiation hazard, requiring special storage, handling and disposal facilities. Compounds labelled with short half-life isotopes (e.g. ^{32}P, ^{125}I) have a limited life before activity levels drop below useful detection limits. Laboratories and their workers which deal in radioisotopes are required to be government approved. Quantitation of radioactivity levels in experiments may require a lengthy counting period. Although research laboratories will continue to use radioisotopes when necessary, the disadvantages have spurred development of methods for the detection of labelled molecules by nonradioactive means, especially for use in diagnostic laboratories[8] where a longer shelf life and shorter detection times are distinct advantages.

Table 1. Commonly Used Radioisotopes in the Biological Sciences[9].

	^{3}H	^{14}C	^{35}S	^{32}P	^{125}I	^{131}I
$T_{1/2}$	12.3 yr	5730 yr	87.4 days	14.3 days	60.0 days	8.04 days
decay mode	β	β	β	β	γ	$\beta + \gamma$
external shielding	none	perspex 1cm	perspex 1cm	perspex 1cm	lead 0.25mm	lead 13mm
toxicty (ref. 2)	slight	slight	moderate	moderate	high	high

1.2 Nonradioisotopic Labelling of Biomolecules

A nonradioactively labelled biomolecule can be considered to consist of three main parts; the biomolecule, a spacer-linker arm and the label (a diagrammatic representation is shown in **Figure 1**). When designing a labelled biomolecule, the main criterion is that the "interaction of interest" (e.g. the ability of a labelled molecule to be recognised at the binding site on a receptor, or to be used as a substrate by an enzyme) should not be significantly affected. Most biomolecules have many potential points of attachment for the spacer or spacer-label adduct; a judicious choice allows one to minimise the disruption to the biomolecule's normal interactions. Secondary considerations, such as the type of label and detection system to be used, and the relative polarity and solubility of the labelled biomolecule compared to the unlabelled are also important, as the physicochemical properties should not be changed significantly.

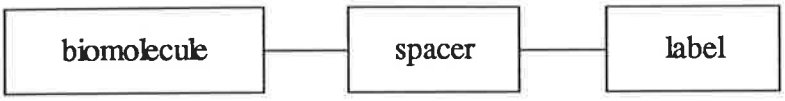


Figure 1.

The spacer arm provides the reactive groups (as detailed below) for covalently linking the label and biomolecule, and a means of preventing, or at least minimising, the steric interaction between the label and the substrate. Depending on the label and biomolecule to be joined, the reactive groups may be the same or different; in the latter case chemical compatibility is necessary. The length of the spacer may vary from zero to sixteen or more[10], the choice in some cases being a compromise between the requirements of the biomolecule and the label. For example, biotinylated nucleotides with shorter spacer arms are incorporated more readily by DNA polymerases into DNA than those with longer spacer arms, however detection of the biotin by avidin/strepavidin conjugates increases with increasing spacer arm length[11]. Functionalities such as amide and ester linkages are commonly incorporated to modify characteristics (such as the improvement of water solubility[8]) of the labelled biomolecule. The order of attachment of biomolecule and label is normally determined by synthetic considerations and the use of the labelled molecule.

1.3 Characteristics and Detection of Labels

Labelled biomolecules may be detected via direct or indirect systems (**Figure 2**). Direct detection is used when the biomolecule is covalently bound to the reporter; the most common reporter groups are fluorescent dyes or marker enzymes (which catalyse the formation of the detection signal) coupled with a luminescent compound. These systems have the advantage that detection occurs in a single step, however an individual labelled biomolecule must be synthesised for each application, and detection systems must be available for all reporters. Indirect detection occurs when the reporter group is not linked covalently to the biomolecule, but indirectly through a non-covalent interaction between a modification of the probe, and a molecule which binds specifically to that modification. The most common modification group is biotin, and it is commonly detected via binding to avidin

or strepavidin which is covalently bound to a reporter enzyme. A disadvantage is that detection requires an extra step to allow the specific non-covalent interaction between the modification group and the specific binding partner to occur, however the reporter group and the detection system may be the same in all biomolecular systems. Hence indirect systems tend to be used in basic research, and also in applied areas such as genetic engineering where the detection of different target molecules is necessary.

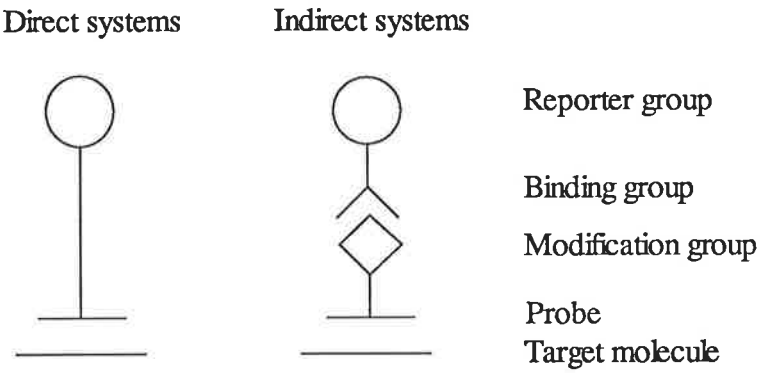
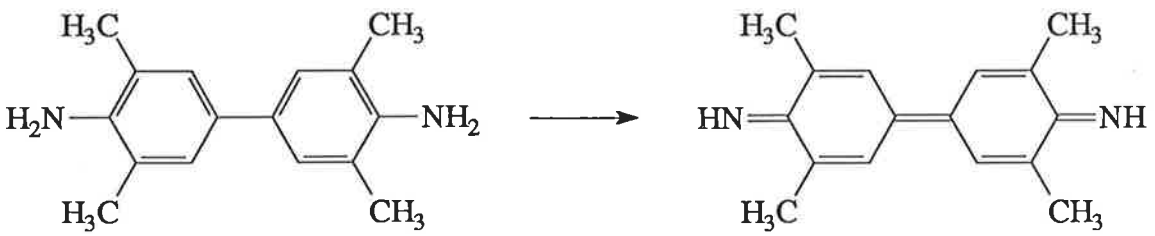


Figure 2.[12]

Generally either an enzyme (commonly alkaline phosphatase (AP) or horseradish peroxidase (HRP)) or a fluorescent reporter is covalently bound to avidin for detection purposes. The enzyme catalyses a chemical reaction (which depends on the type of enzyme) which generally involves the conversion of a colourless compound into a strongly coloured derivative, which is the detection signal. For example, 3,3',5,5'-tetramethylbenzidine **1** in the presence of HRP and hydrogen peroxide is oxidised to coloured **2** (**Scheme 1**), thus allowing localisation of the labelled biomolecule.



1: λ_{max} = 285 nm **2:** λ_{max} = 450 nm

Scheme 1. HRP, H_2O_2.

Luminescence[13], the emission of photons from electronically excited states, is the property most commonly used for direct detection, and sometimes (*via* conjugation of a luminophore to the binding group) in indirect detection. Molecules in electronically excited states may be generated either by irradiation with photons of the required energy, or as part of a chemical reaction (*chemiluminescence*). The absorption of a photon occurs in approximately 10^{-15} second, and gives a molecule generally in a vibrational level (v_n) of the first (S_1) or second (S_2) electronic states. Molecules in condensed phases usually relax rapidly (approximately 10^{-12} second) to the lowest vibrational level v_0 of S_1 (*internal conversion*), and many processes are now available for relaxation to the ground state S_0 (**Figure 3**, Jablonski diagram). *Fluorescence* occurs when emission of a photon from S_1 allows the molecule to regain S_0; the transition is spin allowed and hence occurs quickly (the average lifetime of fluorescent states are near 10^{-8} second). *Intersystem crossing* to the triplet state T_1 may also occur, but now the transition to S_0 is spin forbidden and emission of a photon occurs slowly (triplet lifetimes generally are between 10^{-3} to 10^{1} seconds); the molecule exhibits *phosphorescence.* Other processes, such as quenching, solvent relaxation, and reactions in the excited state are also possible, and may decrease or negate the luminescent yield.

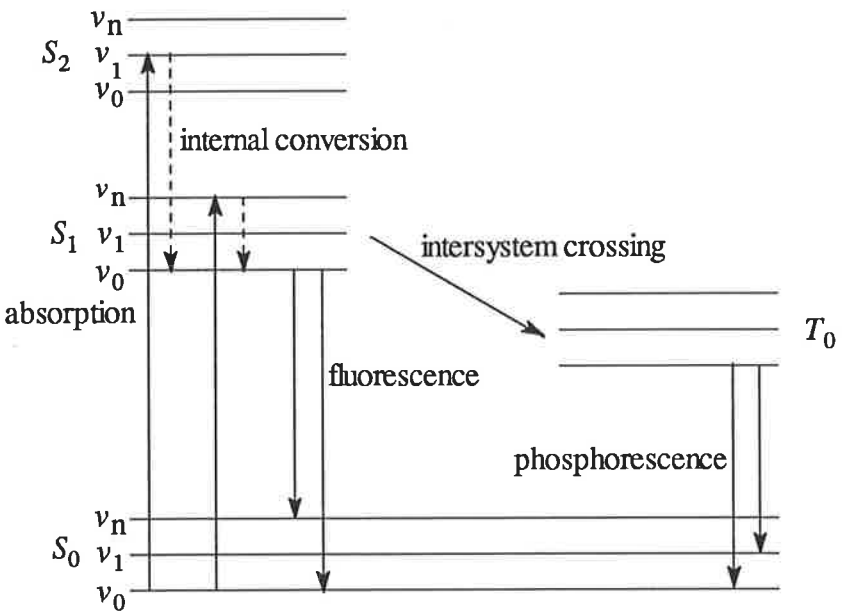
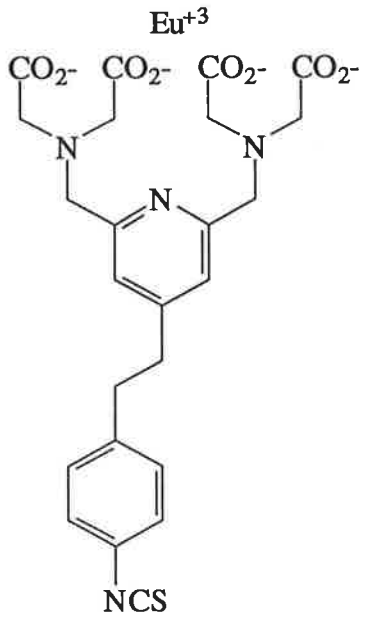


Figure 3. Jablonski diagram

The most important optical properties of fluorescent molecules[14] for use as reporters are the wavelengths of maximum absorption and fluorescence emission, absorbance molar extinction coefficient ε, and fluorescence quantum yield ϕ. The matrix may exhibit background fluorescence at particular wavelengths and hence the reporter should be chosen such that its fluorescence maximum is sufficiently different. Many specialised instruments use a single fixed wavelength (usually an argon laser which emits at 488 nm), and consequently the reporter must have significant absorption at this value. A large Stokes shift (the separation of the absorbance and fluorescence wavelength maxima) is necessary to allow the fluorescence signal to be isolated from backscattered excitation radiation. The fluorescence intensity per reporter molecule, which is proportional to the product of ε and ϕ, indicates the potential sensitivity available from a given molecule. Other information apart from spatial and temporal location, and concentration, may be obtained from fluorescent molecules as fluorescence spectra and ϕ are affected by environmental factors including solvent polarity, local environment (i.e. position in membranes, cells, proteins etc.), proximity of quenching species and pH of aqueous media. Also, some fluorescent molecules show enhancement of ϕ upon binding to a biomolecular target, as quenching by the aqueous medium is diminished.

Another technique used is time resolved fluorescence (TRF), which is a system of detection that has a potentially high degree of sensitivity. Certain europium (III) chelates (e.g. **3**) have been used as reporters[15] as they have a long fluorescent lifetime of 10^{-6} to 10^{-3} second, which enables the differentiation of the reporter's fluorescence signals from any background or native fluorescence. After short pulse excitation of the system (generally by a laser) a time delay of 200 to 400μs occurs before the start of detection. This allows the background fluorescence (with an average lifetime of 10^{-8} second) to decay to a negligible level. As the longer lifetime fluorescent species are still emitting photons they are readily detected. Pulsing can be repeated and the signal summed. However, the thermodynamic stability of some europium complexes is low, which results in ligand dissociation at low concentrations. Bannwarth *et. al.*[16] have used ruthenium (II) complexes such as **4** as reporters attached to probes; they are thermodynamically very stable, chemically inert and are strongly

fluorescent for a relatively long time. The detection limit for DNA probes labelled with **4** is below 10^{-14} M, which is similar to ^{32}P-labelled probes prepared by nick translation.



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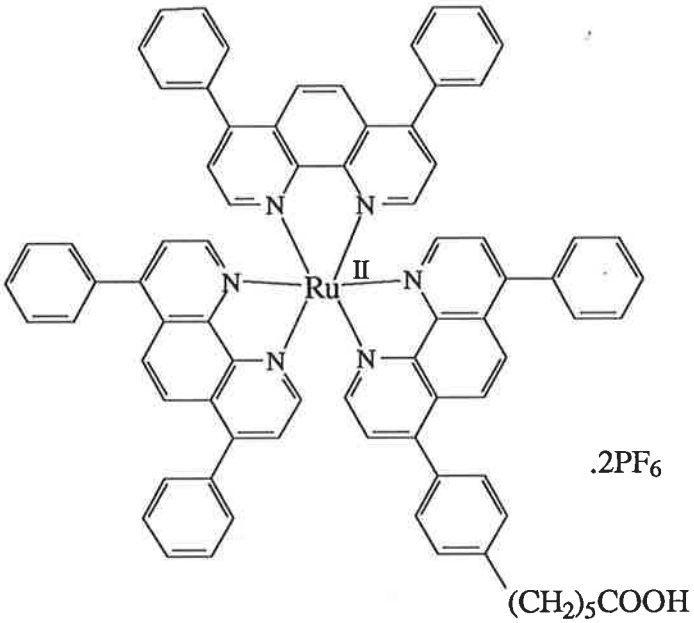

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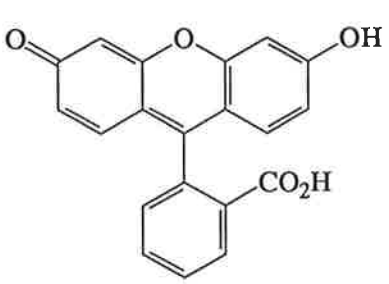
Classes of molecules that are commonly used as fluorescent reporters and probes[30] are shown below. *Xanthene dyes* (e.g. fluorescein **5**, rhodamine B **6**) generally have high extinction coefficients and quantum yields, and are used mainly as reporter compounds. Their characteristics include good water solubility, pH sensitivity and susceptibility to photobleaching (reduction in fluorescence intensity due to chemical reaction of the electronically excited species). *Polycyclic aromatic hydrocarbons* (e.g. pyrene **7**, anthracene **8**) have a high extinction coefficient and quantum yield. They are used as probes in lipophilc systems as the fluorescence spectrum is altered by enviromental factors, and hence information about the the probe's local environment may be obtained. Also, they may be used as indicators of complexation[17] or structure[18] due to the formation of excimers (**excited** state di**mers**), which are formed due to proximity of probe molecules and show a significant shift in λ_{max} in the fluorescence spectrum. Aminosulphonyl derivatives of polycyclic aromatic hydrocarbons (e.g. mansyl **9** and dansyl **10** sulphonamide adducts) are formed by reaction of the corresponding sulphonyl chloride with an amine. Although the extinction coefficient

value (and hence the overall fluorescence output) is only moderate, the spectral characteristics are environmentally sensitive, hence **9** and **10** may be used to probe the local environment of the label. Dansyl chloride is commonly used for the determination of the N-terminal residue of amino acids, and to prepare fluorescent derivatives of drugs, amino acids, oligonucleotides and proteins. Acridine **11** derivatives, in particular acridine orange **13**, have been used for many years as fluorescent biological stains[19]. Derivative **15** has been attached to oligonucleotides to increase the stability of the duplex formed upon binding to a complementary strand[20], as the acridine moiety inserts (intercalates) between the nucleobases. Acridone **12** is easily alkylated on the nitrogen[21], and fluorescence is maintained. Recently fluorophores based on the 4,4-difluoro-4-bora-3a,4a-diaza-s-indacene moiety (**14**) (BODIPY™) have been introduced, which are claimed to have superior spectral characteristics to existing dyes[22].



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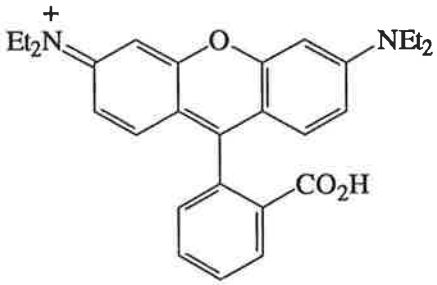

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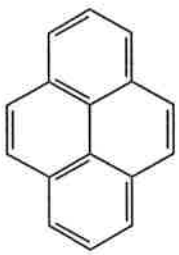

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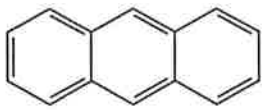

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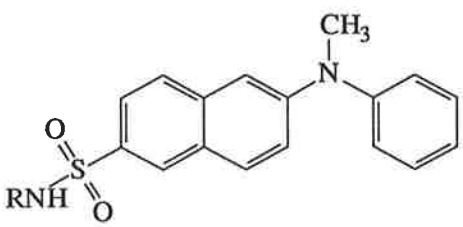

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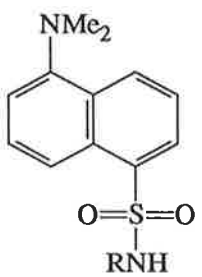

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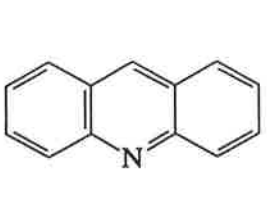

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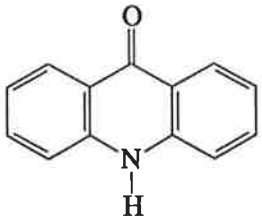

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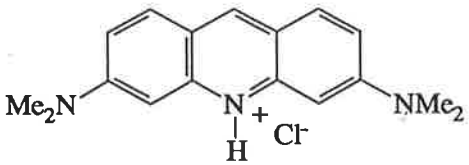

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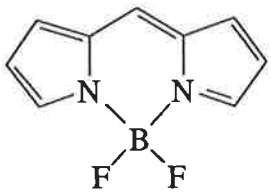

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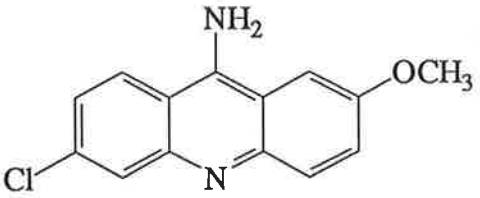

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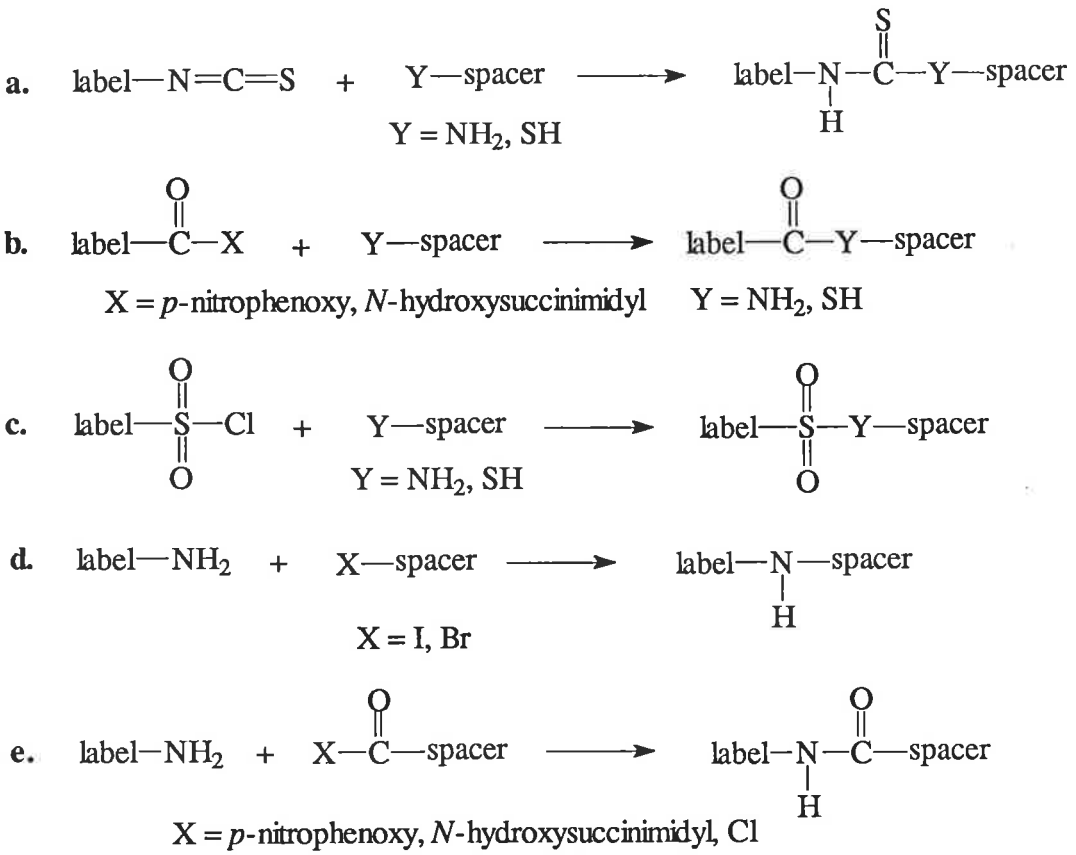
Labels are generally attached to spacer molecules via a nucleophilic/electrophilic interaction, hence the label molecule may require modification (either pre- or postsynthetic) to introduce a suitably reactive functionality. Common electrophilic groups included in labels are isothiocyanates (**Scheme 1a**), activated esters (such as *N*-hydroxysuccinimide and *p*-nitrophenol esters) (**Scheme 1b**) and sulphonyl chlorides (**Scheme 1c**), which react most readily with amines and sulphydryls. The most common nucleophilic moiety introduced is an amine, which generally is alkylated (**Scheme 1d**) or acylated (**Scheme 1e**). Once the label-spacer adduct is formed, covalent bonding to a biomolecule may occur by many methods, which are discussed in the next section.



Scheme 1. General methods of label-spacer attachment.

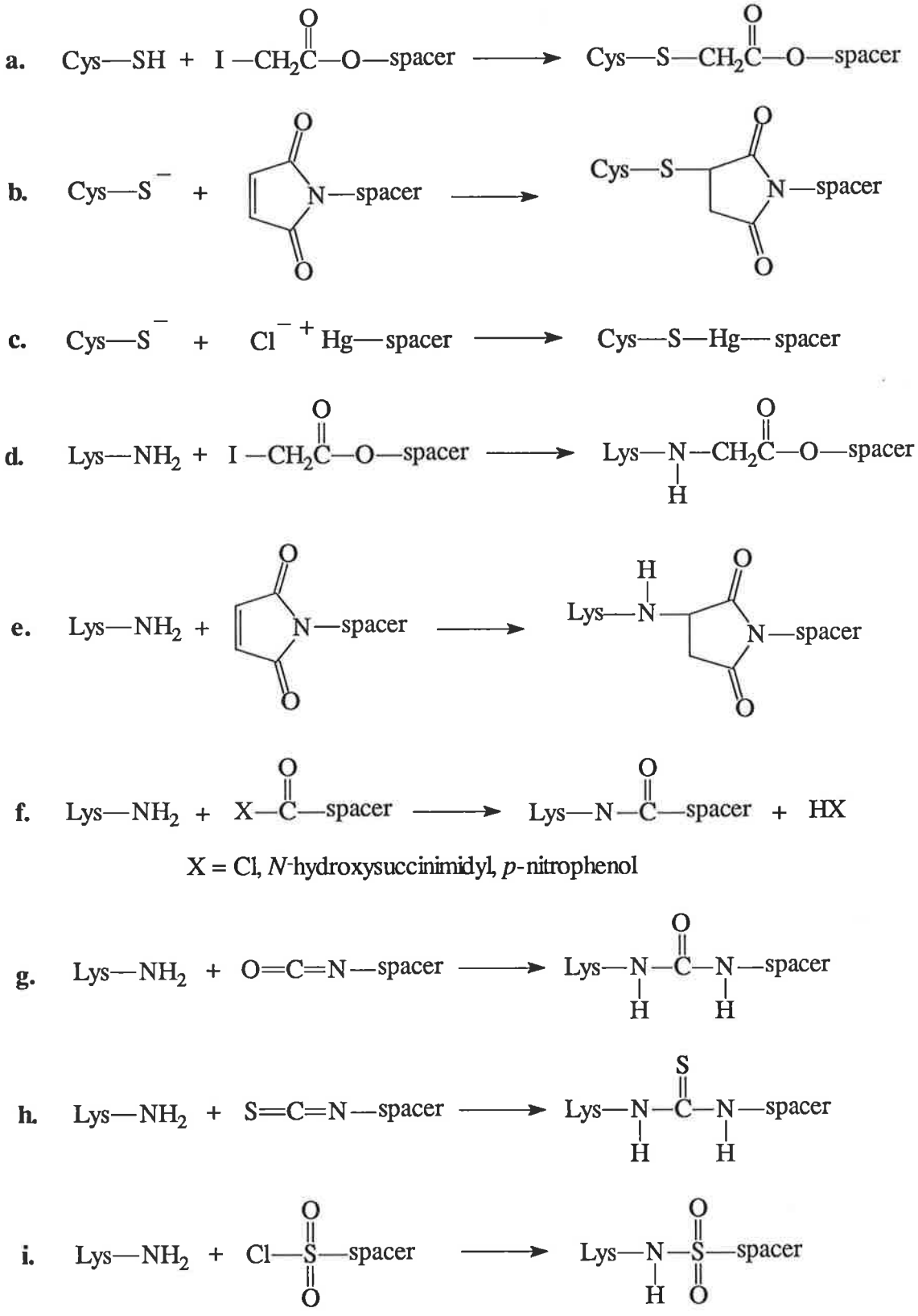
1.4. Attachment of Nonradioactive Labels to Biomolecules.

The nonradioactive labelling of naturally occurring proteins, peptides and nucleic acids is well described[8,11,14,19,23,25]. Labelling reactions occur mainly at discrete residues in the biopolymer, and the chemistry of the interaction is described in the labelling of residues section below. *Native proteins* and *peptides* are generally labelled via a nucleophilic group on the hydrophilic sidechain of an amino acid residue reacting with an electrophilic group at the terminus of the spacer in an alkylation or acylation reaction, although many other procedures are used less commonly[23]. The most reactive nucleophile is the sulphydryl group of cysteine, followed by the amino group of lysine; selectivity for the amino group of lysine in the presence of a free sulphydryl is difficult to achieve. Serine and threonine are rarely used for labelling as the nucleophilicity of the hydroxyl group is low compared to sulphydryl and amino groups (which react preferentially). Also, the substrates are generally in aqueous solution, and reagents of sufficient reactivity are decomposed by the solvent. By appropriate choice of functional group on the spacer and reaction conditions selectivity for most other

reactive functional groups in the protein may be achieved. *Synthetic peptides* may be labelled as previously, or may incorporate a modified residue which is either labelled or may easily be attached to a label. *RNA* and *DNA*[24] are generally labelled via enzymatic incorporation of a modified nucleotide triphosphate using nick translation or random primer labelling; photochemical reaction with an azide compound or a photoreactive intercalating compound (such as psoralen); or via chemical modification (e.g. bromide derivation with NBS and subsequent reaction with an amine). *Synthetic oligonucleotides* may incorporate a modified phosphoramidite[25] at the 5'-terminus (or less commonly the 3'-terminus, the internucleotidic phosphate linkage or C2' of the sugar) which is attached to a label, or a nucleotide which is either labelled or is modified so that it can be easily attached to a label[25].

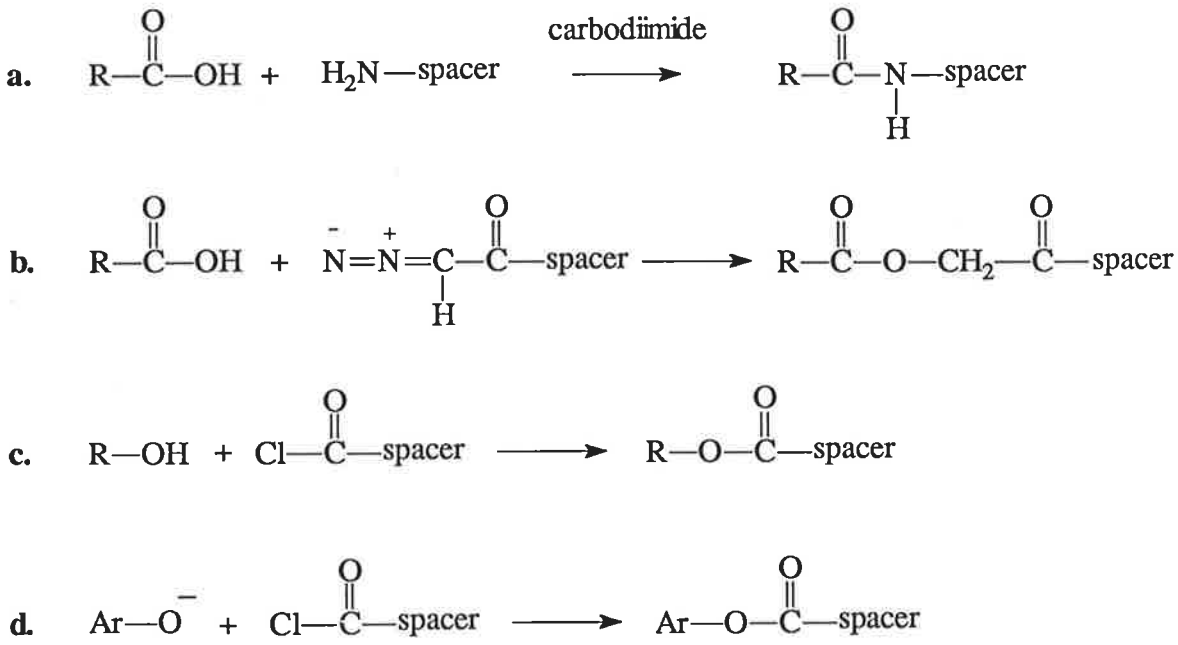
Amino acids (as mentioned above) are generally labelled via a nucleophilic group on a sidechain. A larger range of coupling reactions are available for single residues or small peptides as they may be soluble in organic solvents, hence more reactive functional groups (which are hydrolysed in aqueous solutions) are available on the spacer unit; also they may be subjected to more forcing conditions than proteins, which may be denatured. The stability of the functional group formed upon reaction with the spacer needs to be considered with respect to the proposed use of the labelled residue. The sulphydryl moiety of *cysteine* reacts fastest with common electrophilic moieties. Reaction with α-haloacetamides (**Scheme 2a**) occurs via an S_N2 displacement of the iodide leaving group, while reaction with *N*-maleimides (**Scheme 2b**) is via a Michael addition. The most specific groups for sulphydryl moieties are the mercurial compounds (**Scheme 2c**), as the formation of a strong sulphur-mercury bond results.

The nucleophilic ε-amino moiety of *lysine* reacts with α-haloacetyl and *N*-maleimide compounds similarly to cysteine, although at a slower rate (**Schemes 2d** and **2e**). Reaction also occurs with compounds with activated acid derivatives such as acid chlorides, *N*-hydroxysuccinimde esters and *p*-nitrophenol esters (**Scheme 2f**) to give acyl derivatives. Isocyanates (**Scheme 2g**), isothiocyanates (**Scheme 2h**) and sulphonyl chlorides (**Scheme 2i**) also react with lysine to give *N*-alkylurea, *N*-alkylthiourea and *N*-sulphonamide derivatives respectively. A free N-terminus of a small peptide reacts similarly.



Scheme 2. Reactions of cysteine (**a-c**) and lysine (**d-i**)

Amino acids with an acidic side chain (*glutamic* and *aspartic acids*) have been reacted with carbodiimide derivatives and amines to form amides (**Scheme 3a**), and also diazoacetate ester and diazoacetamide derivatives (**Scheme 3b**) resulting in esters. The C-terminus of a small peptide may be labelled similarly. *Serine* and *threonine* may be labelled via the hydroxy moiety with the more reactive acylating reagents such as acid chlorides to form esters (**Scheme 3c**). Similarly, tyrosine may be labelled via the phenolic hydroxyl under basic conditions (**Scheme 3d**). The other hydrophilic amino acids are generally not labelled, as the resulting functional groups do not have sufficient chemical stability.

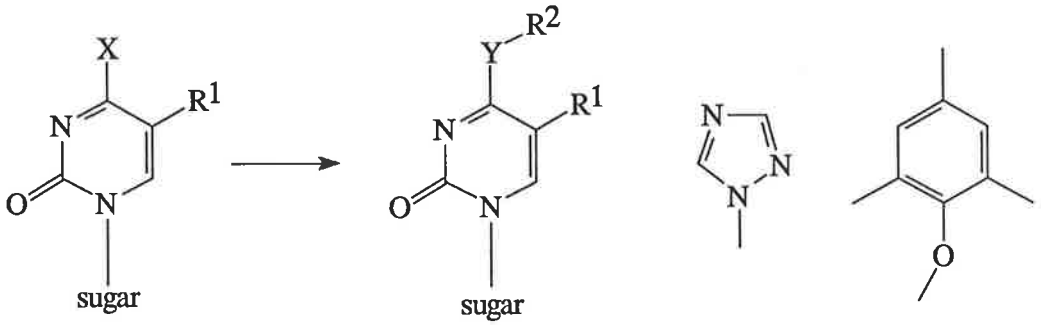


Scheme 3. Reactions of carboxylic acid (**a**, **b**), hydroxyl (**c**) and tyrosine (**d**) sidechains.

Nucleotides[25] may have labels attached at the sugar (generally the 2'-hydroxy of ribose[26]), or more commonly, at the nucleobase. Moieties involved in hydrogen bonding which are used for molecular recognition should not be affected, and importantly, the modified nucleotide must be a substrate for any enzymes used in the processes in which it (the nucleotide) is involved. Attachment of the spacer or label-spacer adduct to the nucleobase may be realised by a displacement reaction of a leaving group by a nucleophile (generally an amine or sulphydryl group) of the spacer. Hence reaction of the good leaving

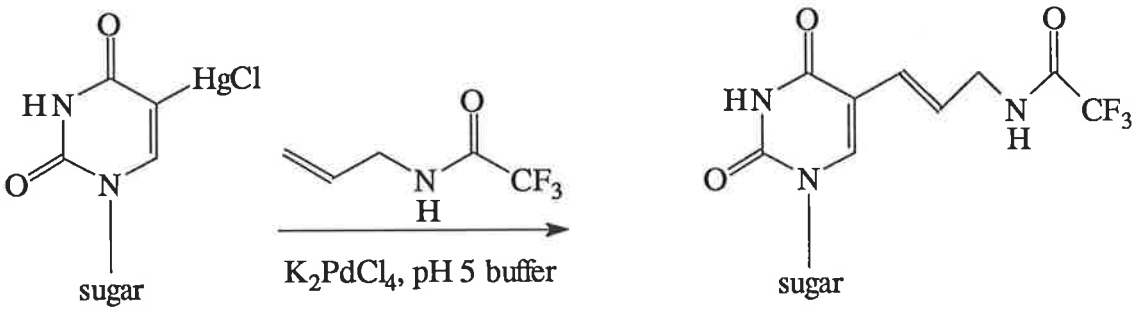
groups triazole **20**[27] and 2,4,6-trimethylphenol **21**[28] at C4 of both cytosine **16** and thymidine derivatives **18** (**Scheme 4**) gave the labelled adducts **17** and **19**; other leaving groups have also been used[29]. Also, 5-thymidine derivatives **23** and **25** have been prepared by the palladium mediated allylation[30] of **22** in poor yield, and by the palladium catalysed coupling of protected propargylamine to 5-iodouridine derivatives[31] **24** (**Schemes 5** and **6** respectively).

Adenosine derivatives **27** and **29** have been synthesised in the 8-position via nucleophilic displacement of bromide from 8-bromoadenosine **26** by amines[32] and thiolate groups[33] (**Scheme 7**), and in the 6-position by reaction of amines with 6-iododeoxyadenosine **28**[34] (**Scheme 8**). Palladium catalysed coupling of an N-protected propargylamine to 7-deazadideoxypurines **30** and **32** gave the adducts[35] **31** and **33** (**Schemes 9** and **10** respectively), which were then deprotected and reacted with fluorescent labels to give compounds suitable for use as terminators in an automated Sanger dideoxynucleotide sequencing protocol[9].



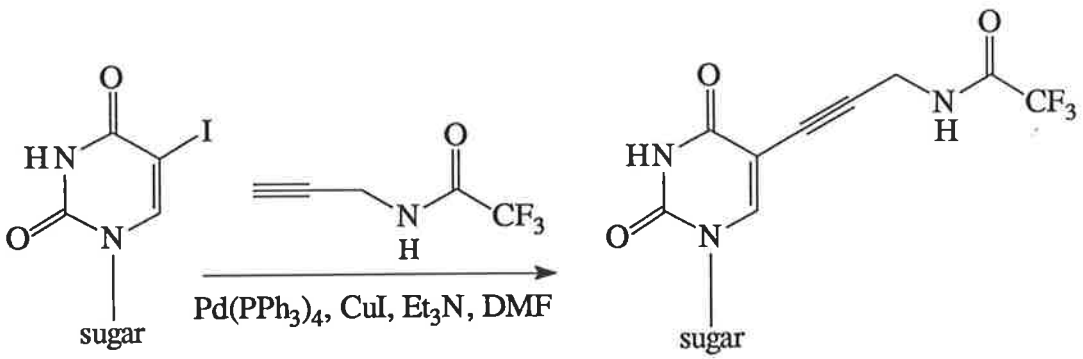
16: $R^1 = H$ **17**: $R^1 = H$ **20** **21**
18: $R^1 = CH_3$ **19**: $R^1 = CH_3$

Scheme 4: R^2 = spacer; X = **20** or **21**; Y = NH or S.



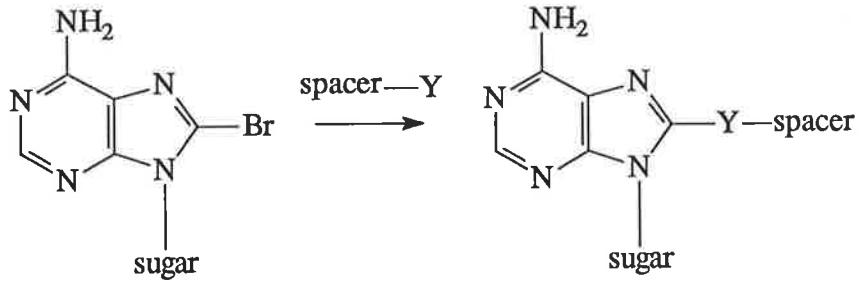
22 **23**

Scheme 5.



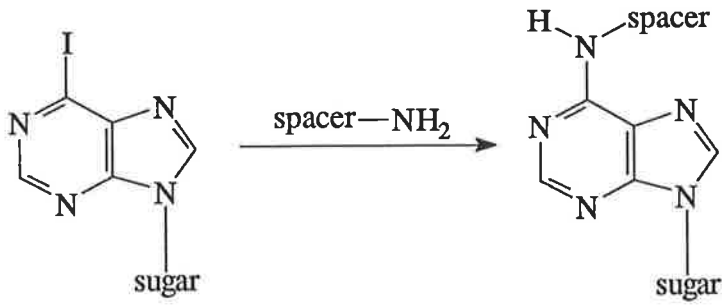
24 **25**

Scheme 6.



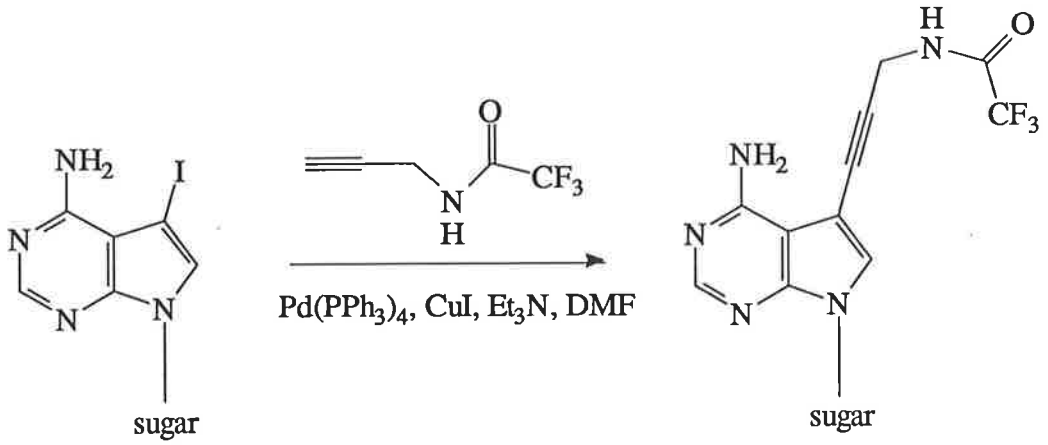
26 **27**

Scheme 7.



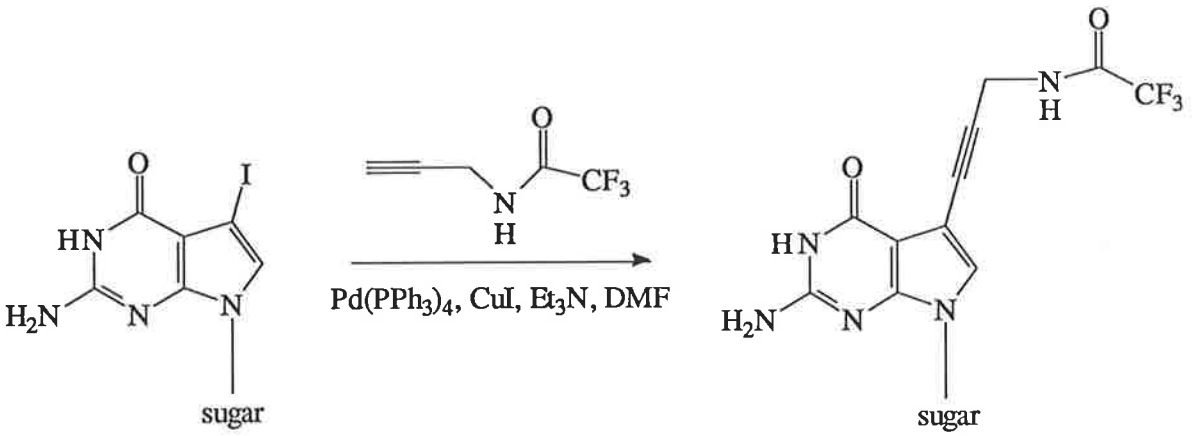
28 **29**

Scheme 8.



30 **31**

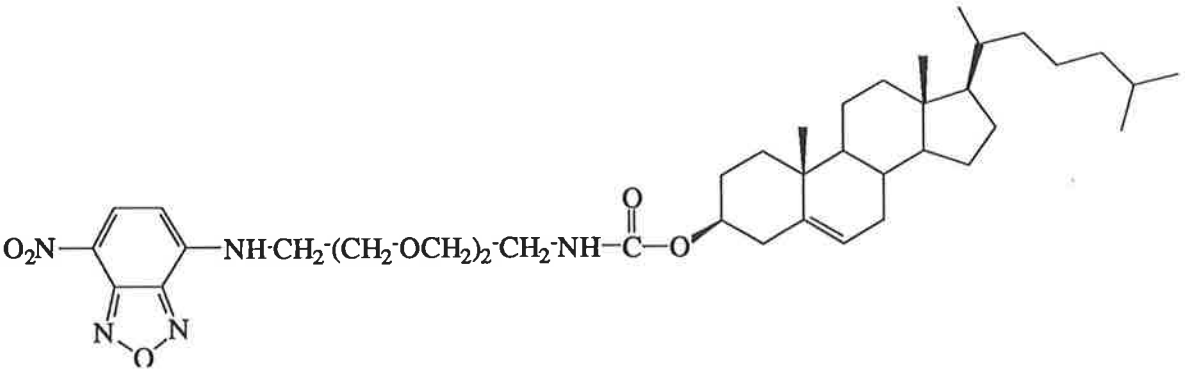
Scheme 9.



32 **33**

Scheme 10.

Steroid derivatives are generally monomeric and so a wider range of chemistry may be utilised in the labelling reactions, although labels are commonly attached via interaction with either hydroxyl groups or carbonyl groups. Attachment of a fluorescent label via a hydroxyl group is common and simple (e.g. **34**)[36], however a free hydroxyl group may be necessary to maintain the physicochemical properties of the unlabelled molecule. Formation of carbon-carbon bonds via a carbonyl group, although synthetically more involved, may enable attachment of labels with minimal alteration of the physical properties. For example, addition of phenyl lithium to **35** (**Scheme 11**) enabled formation of organometallic derivative **36**, which was suitable for studying the hormone-receptor interaction for estradiol[37]. Also, addition of a phosphonate ylid to **37** enabled the synthesis of a fluorescent cholesterol analogue **38** (**Scheme 12**), suitable for use as a probe in membrane studies[38].



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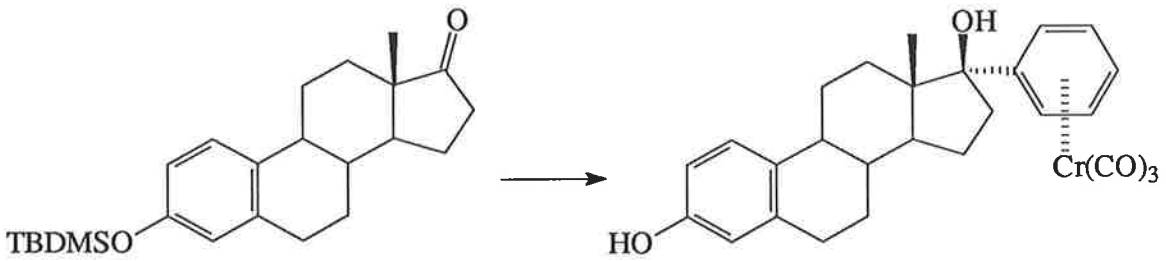
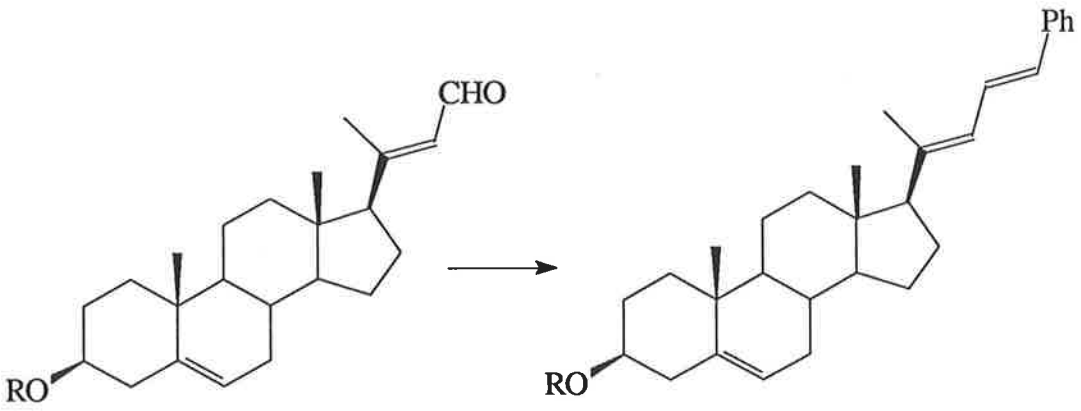


35 **36**

Scheme 10. (i) PhLi, -70°. (ii) $ISiMe_3$. (iii) $Cr(CO)_6$.

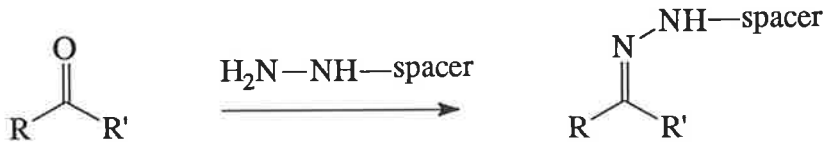


37 **38**

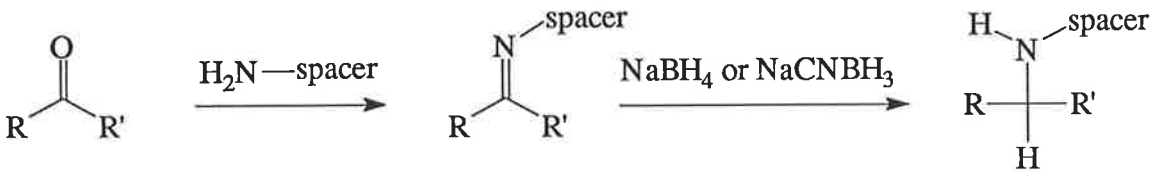
Scheme 11. (i) (*E*)-$(EtO)_2P(O)CH_2CH{=}CHPh$, *n*-BuLi, THF, -78°; (ii) **37**; (iii) TBAF, THF.

Polysaccharides are commonly reacted with hydrazine and amine derivatives to introduce labels[39]. Addition of hydrazines to an aldehyde or ketone functionality in the polysaccharide (which may be introduced by periodate oxidation of the commonly occurring

vicinal diols, if not originally present) gives a stable hydrazone adduct (**Scheme 13**). However, addition of amines to the carbonyl groups is reversible and gives unstable Schiff bases; subsequent reduction by $NaBH_4$ or $NaCNBH_3$ results in stable amine conjugates (**Scheme 14**).



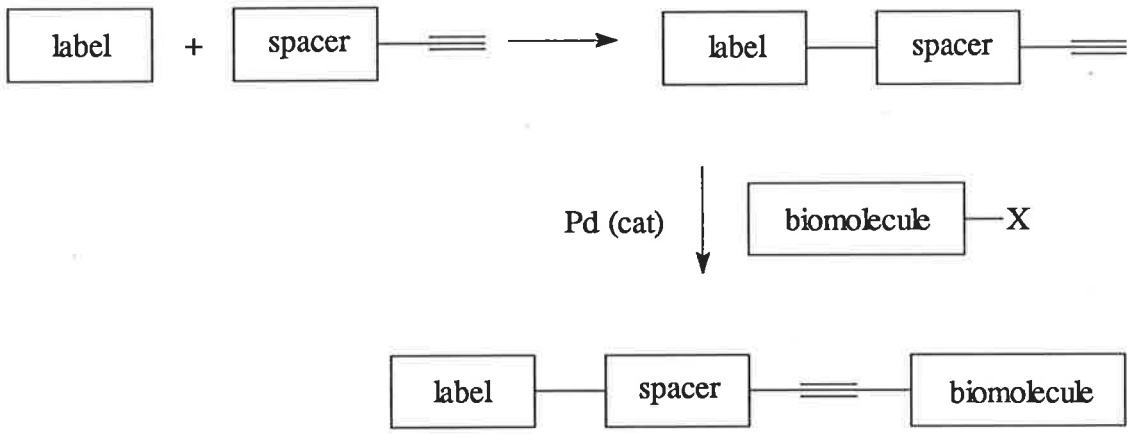
Scheme 13.



Scheme 14.

As has been shown, the formation of a covalent bond between a label and a biomolecule generally requires a nucleophilic-electrophilic interaction. However, with the application of modern transition-metal mediated chemistry such as the palladium-catalysed cross coupling reaction of terminal alkynes with aryl or vinyl halides (as shown in **Schemes 6**, **9** and **10**), low polarity covalent bonds may be formed readily between suitable carbon atoms. Hence a label molecule may be attached to one terminus of a spacer which is functionalised at the alternate terminus with a terminal alkyne (or a group which is converted easily to a terminal alkyne), and then reacted under palladium catalysis with a bromo-, iodo- or triflyl- arene or alkene functionality on a biomolecule (**Scheme 15**). The use of this type of reaction for labelling biomolecules offers the primary advantage in that lipophilic sites on biomolecules may be labelled, which is in general complementary to current methods. Thus the aromatic sidechains of aminoacids such as phenylalanine, tyrosine and tryptophan, the nucleobases of adenosine, guanosine and deoxyuridine and derivatives of the steroids estrone and epiandrosterone should be able to reacted with label-spacer adducts, as suitable halogenated or triflated derivatives are either commercially available or readily synthesised.

Another advantage is that label-spacer adducts synthesised with a terminal alkyne functionality may be coupled to any suitable biomolecule derivative in a one-step reaction; it is a general method. Also, a hydrocarbon spacer arm may be used to link the label and biomolecule, which should be more resistant to enzymatic hydrolysis if used *in vivo* than the functional groups (e.g. esters and amides) which result from standard labelling reactions.



Scheme 14. X = I, Br, OTf

1.5. Palladium Catalysed Couplings of Nonradioactive Labels and Biomolecules

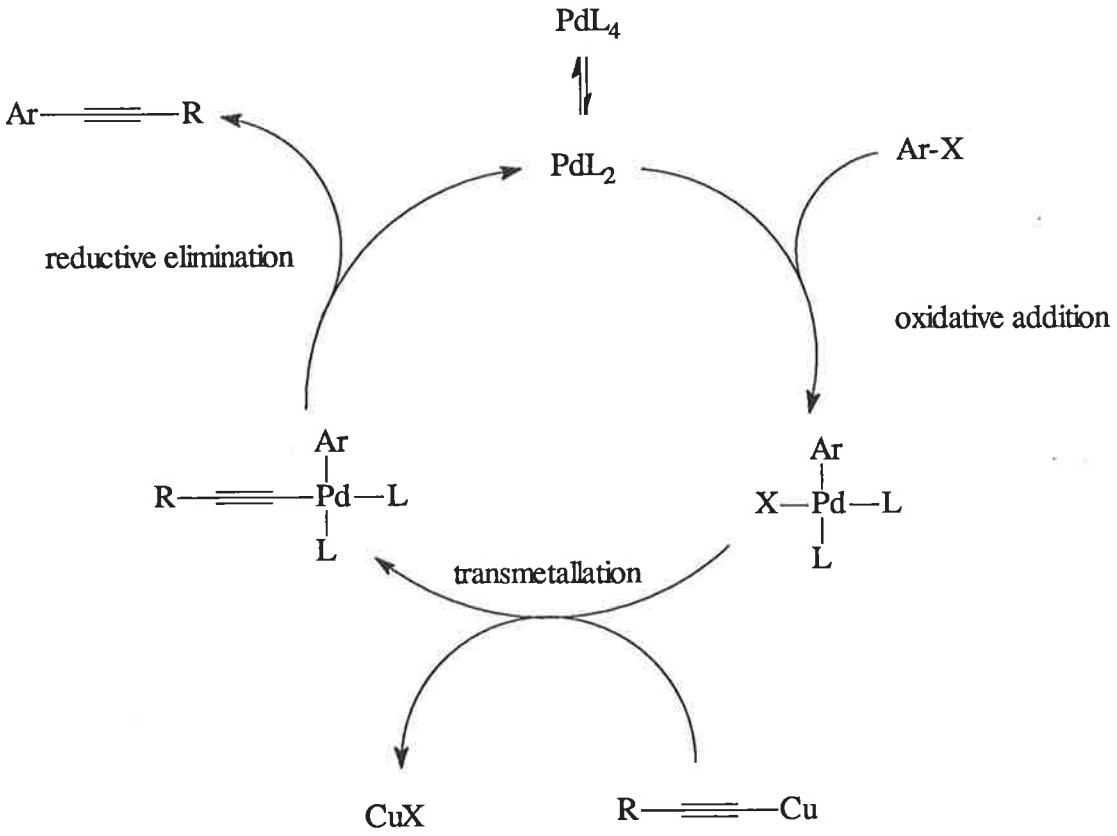
The use of palladium catalysis in the formation of carbon-carbon bonds in the synthesis of complex organic molecules is well established[40]. The reactions are generally high yielding, chemoselective, occur under mild conditions, are compatible with a wide range of functional groups, and have been shown not to cause racemisation of amino acid derivatives[47]. A new bond is formed between an electrophilic sp^2- or sp^3-hybridised carbon atom (a halo- or triflyl- derivative) and a nucleophilic (alkene or alkyne) carbon atom or derivative, the major derivaives being cuproacetylides[41], organoboranes[42] (the "Suzuki" coupling) and organostannanes[43] (the "Stille" coupling). The cross-coupling reaction between acetylenes and aryl or vinyl halides under palladium catalysis was first reported by Heck[44] and Cassar[45] independently in 1975. A typical coupling between iodobenzene and phenylacetylene in Et_3N (**Scheme 16,** Conditions A) required elevated temperatures to

proceed. Sonogashira's use of a copper iodide co-catalyst[46] (e.g. **Scheme 16,** conditions B) (which forms a nucleophilic copper acetylide) allowed this reaction to occur at room temperature. Reaction of substrates which were not soluble in the amines initially used as solvents can take place in polar aprotic solvents such as DMF and DMSO. Many complex organic molecules have been constructed using this methodology as a key synthetic step[47].

Conditions A: $Pd(OAc)_2$ (0.01 eq), PPh_3 (0.02 eq), Et_3N, 100°, 1.5 hours, 73%.
Conditions B: $Pd(PPh_3)_2Cl_2$ (0.10 eq), CuI (0.05 eq), Et_2NH, RT, 6 hours, 85%.

Scheme 16.

The catalytic cycle for the cross-coupling between aryl/vinyl halides/triflates and cuproacetylides[41] is shown in **Scheme 17.** The palladium species may be introduced in an oxidation state of zero ($Pd(PPh_3)_4$) or two (e.g. $Pd(PPh_3)_2Cl_2$). In both cases the active catalyst is the 14 electron, coordinately unsaturated, catalytic species PdL_2 (L = PPh_3) which is formed by either the dissociation of two ligands from PdL_4 upon dissolution in the reaction solvent, or by the reduction of $Pd^{II}L_2Cl_2$ via homocoupling of the alkyne. Oxidative addition of the electrophilic carbon occurs most quickly for iodides; bromides and triflates add at a similar rate and chlorides are the slowest. Transmetallation of the alkynylcuprate (generated in catalytic quantities from the reaction between CuI, Et_3N and alkyne) with the catalytic species and reductive elimination of the coupled product regenerates the catalyst.



Scheme 17.

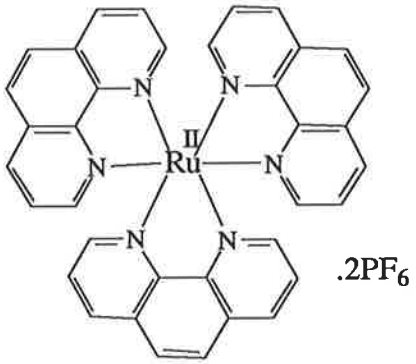
A potential problem of the reaction is the production of the homocoupled alkyne in preference to the cross-coupled product. Recovery of the homocoupled alkyne in a significant amount has been reported when the oxidative addition of the aryl halide species is slow[48] (generally due to the use of an aryl bromide or electron rich aryl iodide). Reaction in piperidine[49] or pyrrolidine[50] at reflux gives a higher yield of cross coupled product, and hence less homocoupled alkyne, however this approach is limited by the stability of the substrate under the reaction conditions. Another problem is chelation of the catalytic metal ions to the substrate or product, or both; a sluggish reaction may result due to the reduced availability of the catalyst[51]. Treatment of a solution of a crude purine nucleoside cross-coupled product with H_2S gas to precipitate the chelated metals as metal sulphides was necessary for isolation of the desired product[52]. These disadvantages are readily overcome by suitable choices of

reaction conditions and/or substrate protection, and purification methods. Hence the palladium catalysed cross-coupling of aryl or vinyl halides or triflates with terminal alkynes should provide an efficient method of attaching a reporter compound to a suitable biomolecule derivative.

1.6. Aim of Project

The aim of this project is to ascertain if the palladium catalysed cross-coupling reaction is a suitable method for the introduction of label-spacer adducts, which are functionalised with a terminal alkyne group, to iodo, bromo and triflyl derivatives of biomolecules. The synthetic work consists of three main parts; (i) synthesis of spacer molecules and label-spacer adducts, (ii) synthesis of halogenated or triflated biomolecule derivatives, and (iii) reaction of biomolecule derivatives and label-spacer adducts under PdCC conditions to give the labelled biomoleclues.

Undec-10-enoic acid was selected as a suitable starting material for *spacer molecules* (which need to be α,ω-difunctional hydrocarbon chains) as it was inexpensive, readily available and of medium length. Interconversion of the functional groups to those suitable for attachment to both the label and the biomolecule (which requires an alkyne or a group easily converted to one) should be readily accomplished by standard chemistry. The *fluorescent labels* selected for use in this study were fluorescein **5**, pyrene **7**, dansyl sulphonamide derivative **10** and acridone **12**. The complex *tris*(phenanthroline)ruthenium (II) hexafluorophosphate **39** was selected as a model *time resolved fluorescence* reporter in place of the bathophenanthroline ruthenium complex **27** as the ligand **40** is less expensive, bromo derivatives of 1,10-phenanthroline **40** are easily synthesised from literature procedures, and the coupling ability under palladium catalysis of both complexes should be similar. Also 1,10-phenanthroline derivatives and complexes have many other interesting chemical and physical properties which have been intensively studied[53], and the methodology developed may have applications in those areas. Finally, a *biotin* **41** label was tested for coupling under PdCC conditions.



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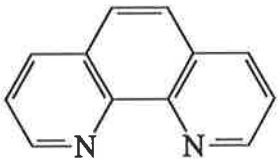

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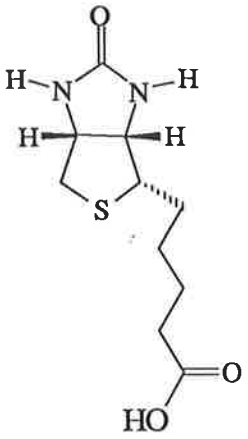
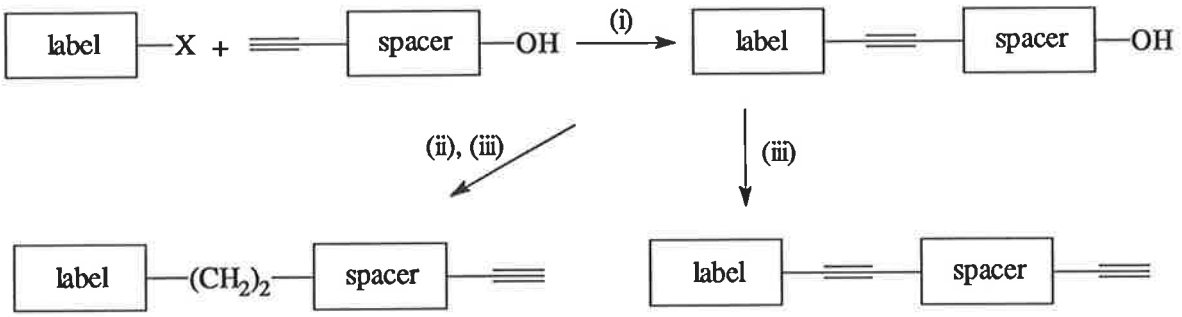


41

Synthesis of the label-spacer adducts involving the aromatic moities **7** and **40** should occur via PdCC of halogenated derivatives with an alkynyl spacer (**Scheme 18**). After coupling, the internal alkyne may be either hydrogenated to the saturated analogue, or left *in situ*, which offers labels with possibly different spectral characteristics. Finally, functional group interconversion (FGI) to the terminal alkyne gives the labels **42** and **43** which should be suitable for coupling.

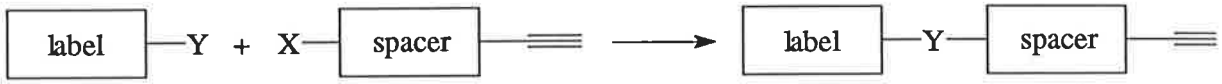


42 **43**

Scheme 18. X = I, Br. (i) PdCC. (ii) [H]. (iii) FGI

Synthesis of the other labels involves electrophilic-nucleophilic interaction between suitable functionalities of the label and spacer molecules; attack on an electrophilic alkynyl spacer for the labels (acridone, fluorescein) which may possess a suitable nucleophilic site such as an anionic heteroatom (**Scheme 19**) to give **44**, or attack by a nucleophilic spacer on

an electrophilic label (dansyl chloride, biotin NHS ester) (**Scheme 20**) to give **45**.



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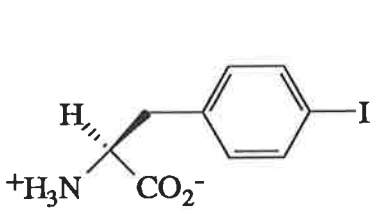
Scheme 19. Y = ArO^-, N^-; X = I.

45

Scheme 20. Y = NH_2; X = Cl, NHS.

The biomolecules selected for labelling are halogenated or hydroxy derivatives (for conversion to triflates using standard methodology[54]) of amino acids and nucleosides which are commercially available. Functional groups such as the hydroxyls of the sugars of nucleosides and the carboxylate and amino groups of amino acids will be protected; although not necessary for the PdCC reaction, protection will aid purification by chromatography and characterisation of the labelled biomolecule. The halogenated amino acids selected to be trialled were 4-iodophenylalanine **46** and 3-iodotyrosine **47**; tyrosine **48** and 5-hydroxytryptophan **49** were to be converted to the respective triflates before labelling was attempted. Also, reactions of protected propargyl glycine **50** (which is readily resolved into both enantiomers, and has been shown to couple with aromatic and vinyl halides and triflates[47]) with 1-halopyrenes to give a labelled glycine derivative will be studied. Halogenated nucleosides are readily available, and those selected for trial were 5-iododeoxyuridine **51**, 8-bromoadenosine **52** and 8-bromoguanosine **53**. Finally, as steroidal triflate derivatives have been shown to couple readily to terminal alkynes[48,55], the estrone triflate derivative **54** and epiandrosterone triflate derivative **55** were selected for trial in labelling reactions.



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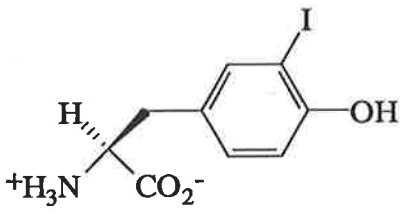

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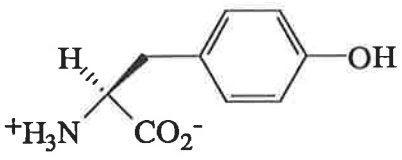

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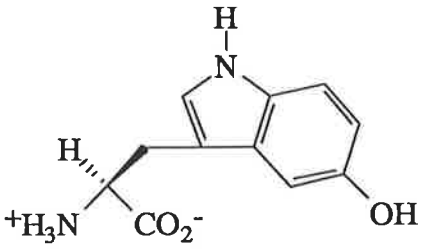

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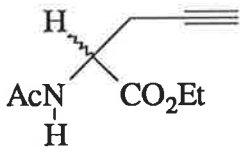

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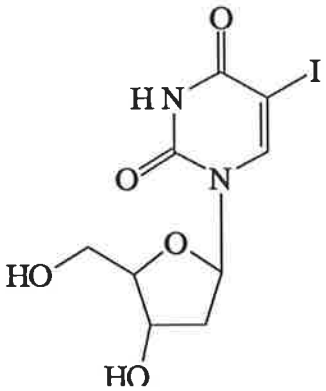

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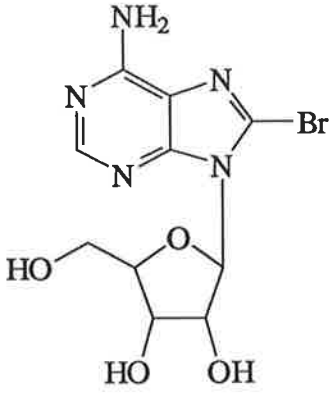

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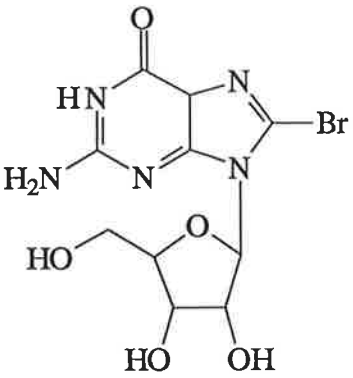

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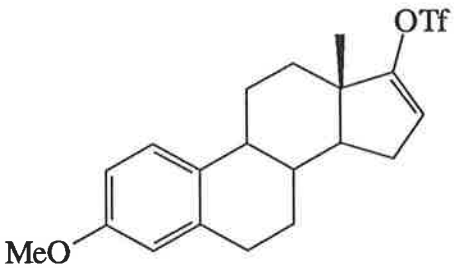

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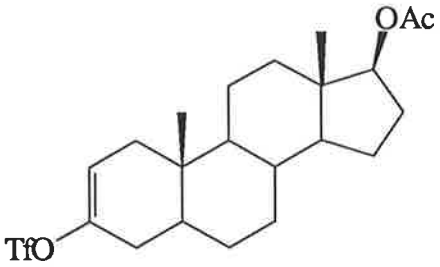


55

If these biomolecules are successfully labelled using PdCC, the number of possible labelling sites will be increased, in a method complementary to existing protocols. There are, however, other advantages. The conformational change difference between labelled and unlabelled polypeptides may be reduced as hydrogen bonding interactions of the sidechains are not disrupted by heteroatoms being used for attachment of the label. Also, the charge

and/or the pKa of the heteroatom, and hence the polypeptide, is affected by the covalent bonding of the label. Both these problems may be overcome by attachment of the label to the sidechain of a lipophilic residue. The use of a hydrocarbon spacer may offer advantages when synthesising labelled biomolecules for use in lipophilic systems. Compared to the labelling reactions for steroids (see chapter 1.3, *Labelling of Biomolecules*), use of this methodology does not require the presence of heteroatoms for attachment (*c.f.* **19**) and so the adduct should be less polar. Although some nucleosides have used PdCC for the introduction of spacer molecules, labelling of nucleosides via this methodology should be simpler as the spacer-label adduct is introduced in one step, compared to the three steps required for coupling of the protected spacer, deprotection and coupling of the label as shown in **Schemes 5**, **8** and **9**.

Successful exploitation of palladium catalysed coupling methodology would allow the facile preparation of a large range of labelled biomolecules, with the incorporation of a hydrocarbon spacer arm which is attached to a lipophilic portion of the biomolecule, in high yields. The chemoselectivity of the coupling process should allow different label-spacer adducts to couple to different biomolecules, hence the synthesis of novel labelled biomolecules should be possible. This methodology is, in general, complementary to existing labelling protocols.

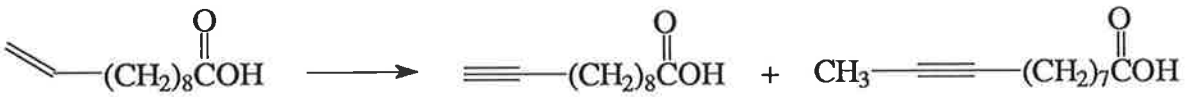
Chapter 2. Synthesis of Label-spacer Molecules.

Chapter 2.1. Synthesis of Linker-spacer Molecules.

Linker-spacer molecules were required to be α,ω-functionalised alkyl chains with an alkyne occupying one of the termini, so as to be attached to a halogenated or triflated label derivative or the biomolecule. The other terminus could be a functional group suitable for either attachment to the label, or for conversion to an alkyne which is then attached to the biomolecule. The choice depends on whether a purely hydrocarbon linker arm is required or whether some functionality is permitted. When PdCC is necessary for attachment of the label the functional group at the other terminus should be inert to the coupling reaction conditions, and if required subsequent conversion to an alkyne should be facile. When an electrophilic label is required to be attached, nucleophilic attack by the spacer with an amine functionality is preferred as the resultant functional group (e.g. an amide) generally is stable under projected reaction conditions. Conversely, attack on the spacer by a nucleophilic label requires an electrophilic group such as an iodide. The chemistry involved in the functional group interconversions should not depend on the length of the alkyl chain, and hence be applicable to any alkyl length chosen. Undec-10-enoic acid **56** is an inexpensive, readily available, α,ω-difunctionalised molecule of medium length and was selected to provide the starting material for the spacer-linker molecules.

Conversion of **56** to undec-10-ynoic acid **57** was via a literature method[56]. Bromination of **56** in CCl_4 at 0° gave the vicinal dibromide, which was then subjected to double dehydrohalogenation. Dehydrohalogenations to give terminal alkynes have been achieved using many methods[57], most commonly with $NaNH_2$ in liquid ammonia or with aqueous KOH. The basicity of $NaNH_2$ is strong enough to form the salt of the terminal alkyne (which is thermodynamically more stable than the internal isomer[58]), and hence upon protonation in the workup the major product is the terminal alkyne. The alternative method of KOH in H_2O was chosen due to its ease of workup; the low literature yield (32%) is acceptable due to the low cost of **56**. After reaction of the vicinal dibromide with KOH for 8 hours (**Scheme 21**) at 150°, and isolation of the product by distillation and recrystallisation

from hexane, the product was recovered in 30% yield. ^{1}H NMR analysis showed resonances at δ 1.94 (*t*, 1H, *J* 2.6 Hz, HC≡C) and 2.18 (*dt*, 2H, *J* 7.0, 2.6 Hz, CH_2-C≡) which were consistent with **57**; also observed was an unexpected resonance at δ 1.78 (*t*, *J* 2.4 Hz). A ^{13}C NMR spectrum of the product showed alkyne resonances at δ 68.0 (terminal) and 84.6 (internal) which were consistent with **57**, and low intensity resonances at δ 74.5 and 79.1. Isomerisation of a terminal alkyne to the thermodynamically more stable internal alkyne[58] has been shown to occur under basic conditions[59] (presumably via an allene intermediate); here the unexpected resonance at δ 1.78 was consistent with the terminal methyl group[59] of the 9-alkyne isomer **58**, and the ^{13}C resonances also consistent with an internal alkyne[60]. Integration of the terminal methyl group resonance with reference to the methylene next to the carboxyl group (with allowance for the contribution from **58**) showed the ratio of **57** to **58** to be 90:10. Repeated fractional distillations through a 150mm Vigreaux column could not increase the ratio and so modifications to the reaction conditions were made to reduce the amount of **58**.



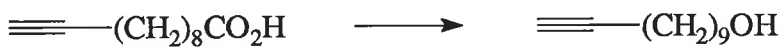
56 **57** **58**

(i) Br_2, CCl_4, 0°. (ii) KOH (aq), 150°, 8 hours, 30%.

Scheme 21.

Reducing the temperature and/or time of reaction should lead to an increase in the ratio of the kinetic product **57** to thermodynamic product **58**, albeit with a reduction in overall yield. Consequently repeating the reaction at 130° for 8 hours gave a ratio of 98:2 for **57** to **58**, with a yield of 24%, and reaction at 120° for 6 hours gave only **57** in a yield of 18%. After distillation of the product, the majority of reaction mixture remained as high boiling point residue, which was presumably a mixture of the bromoalkenes which are intermediates in the reaction. As the reaction was performed on a large (100g) scale from starting material, the poor yield was acceptable, in terms of quantity of material obtained.

Reduction of **57** to the alcohol **59** occurred readily in very good yield using LAH in refluxing Et_2O (**Scheme 22**). Synthesis was confirmed by IR (3400-3100 cm^{-1}, O-H str., and absence of carbonyl absorption at 1720 cm^{-1}) and ^1H NMR (δ 3.62, *t*, 2H, CH_2-OH).

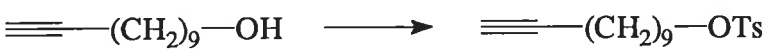


57 **59**

LAH, Et_2O, reflux overnight, 87%.

Scheme 22.

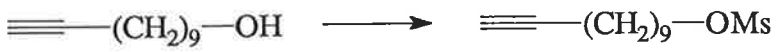
Conversion of **59** to the iodide **62** was via the standard two step procedure; conversion of the alcohol to a good leaving group (ie the tosylate **60** or the mesylate **61**) and subsequent displacement with NaI in 2-butanone[61]. Reaction of **59** with TsCl in pyridine (**Scheme 23**) gave an excellent crude yield of the corresponding tosylate **60**. Reaction of **59** with MsCl in pyridine gave the expected mesylate **61** also in excellent yield (**Scheme 24,** Conditions A). The method of choice however, was reaction of 59 with MsCl and Et_3N in CH_2Cl_2 to give **61** (**Scheme 24,** Conditions B), then conversion to **62**. Although the yield of crude mesylate was slightly lower than tosylate, (88% to 93%), the advantages were that no pyridine was used, and that the isolation procedure was easiest. Displacement of the tosylate or mesylate groups (**Scheme 25**) occurred readily to give the iodide **62** as a colourless oil in good yield; synthesis was confirmed by mass spectrometry (M^+ 278) and the distinctive high field chemical shift in the ^{13}C NMR spectrum (δ 7.18) of the carbon attached to the iodide.



59 **60**

TsCl (2 eq), pyridine, 0° to RT overnight; 94%.

Scheme 23.



59 **61**

Conditions A: MsCl (1 eq), pyridine, 0°, 4 hours; 93%.
Conditions B: MsCl (1 eq), Et_3N (1 eq), CH_2Cl_2, RT, 24 hours, 88%.

Scheme 24.

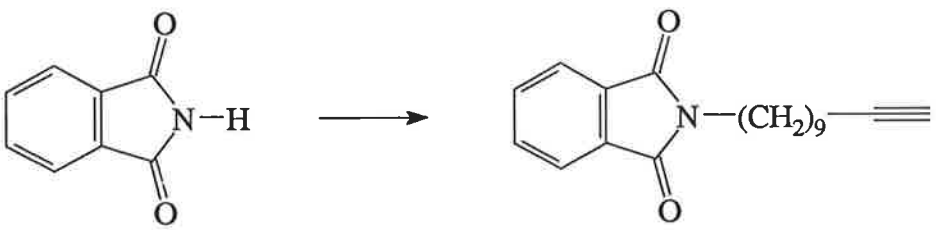
60: R = OTs; 69%
61: R = OMs; 75%

62

NaI (5 eq), 2-butanone, reflux 12 hours.

Scheme 25.

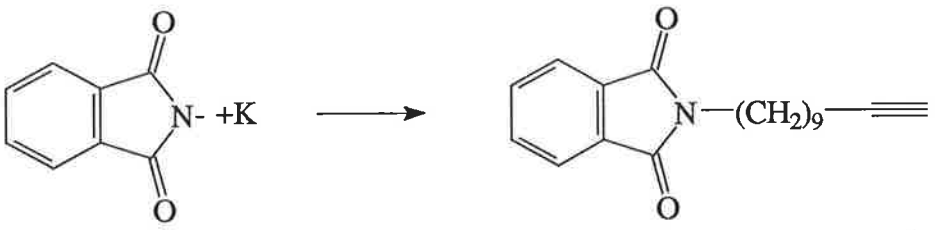
Preparation of aminoalkyne **66** was envisaged to occur by a standard Gabriel synthesis[62]. Reaction of **62** with the sodium anion of phthalimide **63** in DMF (**Scheme 26**, Conditions A) gave **64** in a poor yield of 41%. TLC (20/80 EtOAc/hexanes) of the reaction mixture after 3 hours stirring at 100° showed a large amount of phthalimide at R_f 0.22, and a new UV active spot at R_f 0.54 corresponding to product; the spot corresponding to **62** was absent. Synthesis was confirmed by mass spectrometry (M^+ 297) and IR (1772 and 1710 cm^{-1}, imide C=O stretch). The yield was increased to 78% by the reaction between **62**, **63** and K_2CO_3 in refluxing 2-butanone (**Scheme 26**, Conditions B). In an attempt to improve the yield a phase transfer catalysed reaction[63] was performed, however reaction of potassium phthalimide **65** and iodide **62** in toluene with PTC catalyst 18C-6 (**Scheme 27**) gave upon workup only a poor yield of 40%.



63 **64**

Conditions A: (i) NaH (1.1 eq), DMF. (ii) Iodide **62**, 100°, 3 hours, 41%.
Conditions B: Iodide **62** (1.2 eq), K_2CO_3 (1.2 eq), MEK, reflux 24 hours, 78%.

Scheme 26.

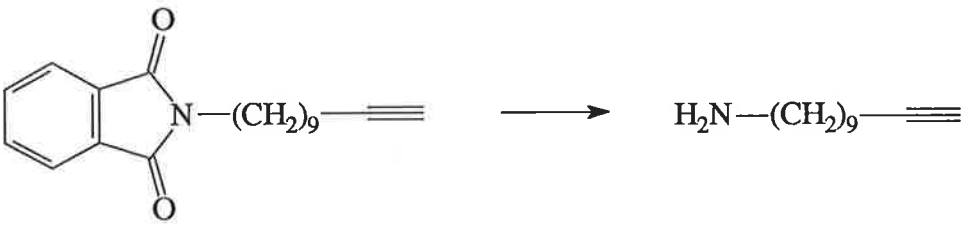


65 **64**

Iodide **62** (1.2 eq), 18C-6 (0.1 eq), toluene, 90°, 8 hours, 40%.

Scheme 27.

Reaction of **64** with 3 equivalents of hydrazine hydrate in EtOH at room temperature and standard workup (**Scheme 28**) gave the amine **66** in an average yield of 64%. Synthesis was confirmed by IR (3500-3100 cm^{-1} N-H stretch) and mass spectrometry ($M+H^+$ 168). Varying the amount of hydrazine (1, 2 and 5 equivalents) did not improve the yield, neither did reaction at a higher temperature. As the amine is four synthetic steps from the acid **57** with an overall yield of 34%, the alternative path of conversion of **57** to the amide **68** and reduction to **66** with LAH was explored.



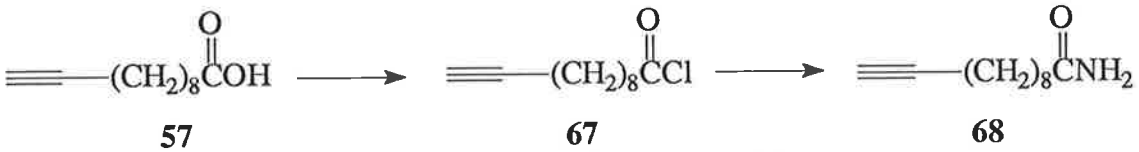
64 **66**

(i) $H_2NNH_2.H_2O$ (3 eq), EtOH, RT, 48 hours. (ii) HCl (aq). (iii) NaOH (aq).

Scheme 28.

Reaction of acid **57** with $SOCl_2$ gave the acid chloride **67** (**Scheme 29** (i)) in good yield. The IR spectrum of **67** showed the absence of the O-H stretch between 3350 and 2500 cm^{-1}, and a strong absorption at 1796 cm^{-1} consistent with the carbonyl group of an acid chloride. The ^1H NMR chemical shift of the methylene next to the acyl chloride was δ 2.88, compared with δ 2.35 in **57**. Addition of **67** to a cold (-15°) saturated solution of ammonia

(**Scheme 29** (ii)) precipitated the amide **68** in very good yield. The IR spectrum showed distinctive primary amide N-H stretch absorptions at 3356 and 3184 cm^{-1}, and strong absorptions at 1662 (C=O str.) and 1632 cm^{-1} (NH_2 def.).

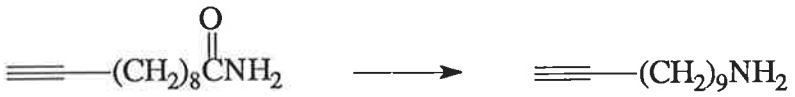


57 **67** **68**

(i) $SOCl_2$, reflux, 60 minutes (ii) conc. aq. NH_3, -15°; 84%.

Scheme 29.

The amide **68** was only partially soluble in Et_2O, hence it was placed in the thimble of a Soxhlet extraction apparatus for reduction to the amine **66** by LAH in Et_2O (**Scheme 30**). After reflux for 36 hours the amide had dissolved and the amine was recovered in very good yield; the IR spectrum showed a broad absorption between 3500 and 3100 cm^{-1} (N-H str.) and the ^1H NMR a resonance at δ 2.60 (t, 2H, *J* 6.6 Hz) consistent with CH_2NH_2. The overall yield for the two-step synthesis of **66** from the acid **57** was 73%, a marked improvement compared with the previous four-step synthesis of 34%.



68 **66**

LAH (1 eq), Et_2O, reflux, 36 hours, 85%.

Scheme 30.

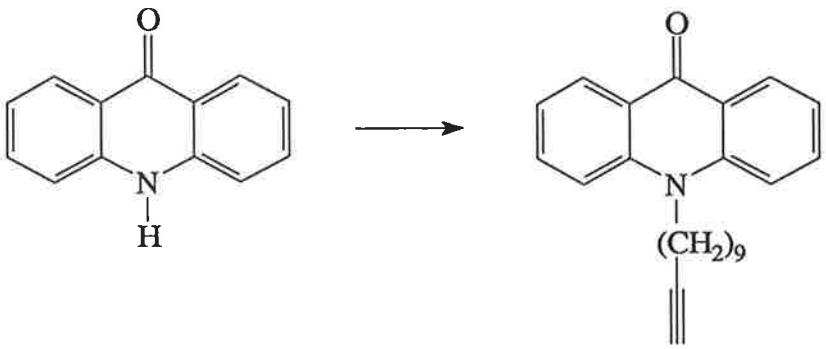
Hence the preparation of α-alkynyl-ω-functionalised linker-spacer compounds was accomplished using standard chemistry in good yields. It was envisaged that alkynyl alcohol **59** would be used in PdCC reactions (with the hydroxy function subsequently being converted to an alkyne), alkynyl acid chloride **62** and alkynyl iodide **67** in coupling to labels which incorporate a nucleophilic group, and alkynyl amine **66** for coupling to electrophilic groups of labels. The use of these compounds in the preparation of label-spacer molecules is described in the remaining sections of this Chapter.

Chapter 2.2. Synthesis of Fluorescent Label-spacer Molecules.

The coupling step between label and spacer molecules for the construction of fluorescent label-spacer adducts may occur via a palladium catalysed coupling of a terminal alkyne of the spacer with a halogenated derivative of the pyrene **7** moiety (**Scheme 18**, *Introduction*), or via nucleophilic/electrophilic interactions for the acridone **12**, fluorescein **5** and dansyl **10** moieties (**Schemes 19** and **20**, *Introduction*). Previous work[64] had established conditions for the preparation of the acridone label **69** and pyrene label **78**, however the yields were only moderate. Hence an improvement in yield for the preparation of **69** and **78**, and the development of conditions suitable for the preparation of label-spacer adducts based on the fluorescein **5** and dansyl derivative **10** molecules were the objectives of this section.

The acridone label **69**, as it has previously been synthesised easily in 59% yield (**Scheme 31**, Conditions A), was selected to be a test label for optimising conditions for the labelling reactions with the halogenated and triflated biomolecule derivatives. The reaction was repeated with 1-iodoundec-10-yne **62** as the alkylating agent in an attempt to improve the yield of **69** (**Scheme 31**, Conditions B). TLC of the reaction mixture between **12** and NaH in DMF after stirring for 60 minutes at 50° suggested formation of the anion of **12** as shown by the disappearance of the spot corresponding to **12** at R_f 0.17 (EtOAc/hexanes 40/60) and the appearance of a new spot on the baseline. Also, the reaction mixture turned a fluorescent yellow-green which is consistent with formation of the anion[65]. Addition of **62** dropwise over 10 minutes discharged the fluorescent colour and gave a dark green solution. TLC of the reaction mixture after 60 minutes stirring at room temperature showed a new spot at R_f 0.69 corresponding to product **69**, a large spot corresponding to **12** and the absence of the baseline spot. After workup and removal of traces of DMF under vacuum, **69** was recovered in 23% yield; the balance of **12** appeared to be reprotonated. This result suggested that **62** was reacting via an E_2 process and reprotonating the anion of **12**. *N*-Alkylacridone derivatives have also been synthesised in phase transfer catalysed reactions[66], the best yields and mildest conditions reported by Nishi *et. al.*[67] TLC of the reaction mixture after dropwise addition of **62** to a stirred mixture of **12**, 50% aqueous KOH, benzyltriethylammonium chloride (0.02 eq) and 2-butanaone (**Scheme 31**, Conditions C) at 80° showed after 6 hours a large spot for **69**

and the absence of **62**. The product was recovered after an easy workup in 59% yield and in high purity; the remainder of the anion of **12** presumably remained in the basic aqueous layer. The spectral data were identical with those obtained previously, although the melting point of 95-97° was higher (lit.[64] 88-90°). Although the yield of **69** was not significantly different to the previous work, these reaction conditions were preferred as isolation of the pure compound was easiest.



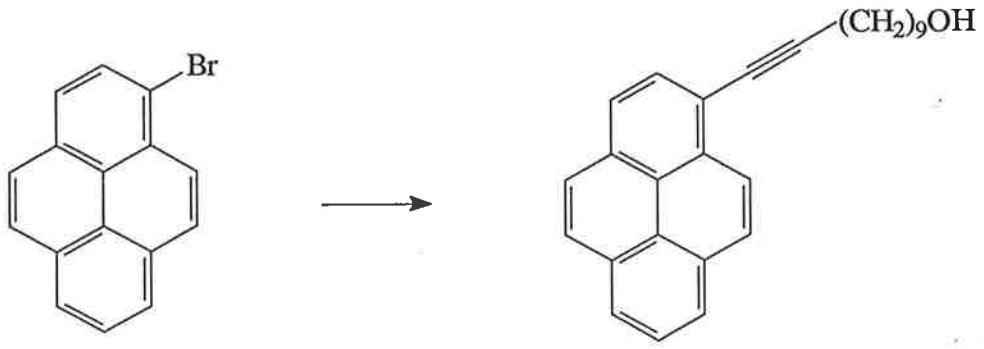
12 **69**

Conditions A: (i) NaH, DMF, 50° (ii) $HC{\equiv}C\text{-}(CH_2)_9Br$; 59%.
Conditions B: (i) NaH, DMF, 50°. (ii) Iodide **62**; 23%.
Conditions C: Iodide **62** (1.5 eq), $BzEt_3NCl$ (0.02 eq), 50% KOH, 2-butanone; 59%

Scheme 31.

The pyrene label-spacer adduct **71** has been synthesised previously[64] in 60% yield by the palladium catalysed coupling of 1-bromopyrene (**70**) and undec-10-yn-1-ol (**59**) (**Scheme 32**, Conditions A). The moderate yield is typical of the cross coupling reaction between aryl bromides and terminal alkynes under these conditions. Piperidine, when used as the reaction solvent and base, has been shown to enhance the slow oxidative addition step of the aryl bromide to the palladium (O) catalytic species and give an increase in yield[49]. Reaction of **70** and **59** with palladium and copper catalysts in piperidine (**Scheme 32**, Conditions B) gave, after overnight reflux and workup, **71** in a yield of 72%. An alternative system for the activation of aryl bromides using pyrrolidine has also been reported[50]. Reaction of **70** and **59** with only a palladium catalyst in pyrrolidine at 80° for 20 hours (**Scheme 32**, Conditions C) and workup afforded **71** in a good yield of 79%. The more reactive conditions also enable best use of the alkyne; less is needed if the coupling occurs quickly as the slower, competitive

homocoupling process is not favoured. The spectral data were identical with those obtained previously.

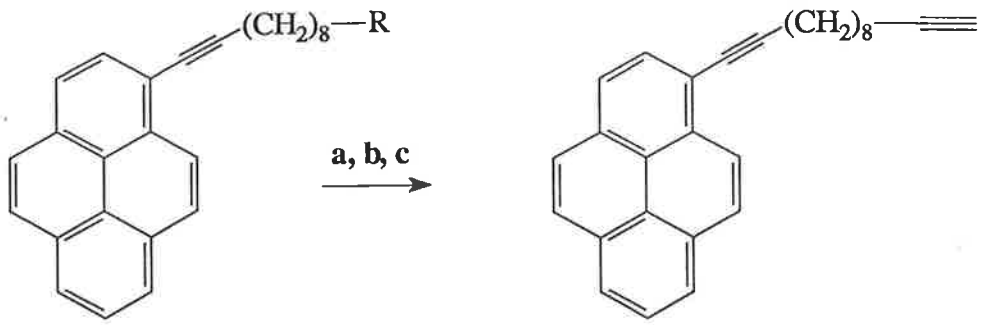


70 **71**

Conditions A: **59** (2.5 eq), $Pd(PPh_3)_2Cl_2$ (0.05 eq), CuI (0.1 eq), PPh_3 (0.1 eq), Et_3N, pyridine, 90°, 24 hours; 60%.
Conditions B: **59** (1.2 eq), $Pd(PPh_3)_4$ (0.025 eq), CuI (0.05 eq), PPh_3 (0.05 eq), piperidine, reflux ON; 72%
Conditons C: **59** (1.2 eq), $Pd(PPh_3)_4$ (0.025 eq), pyrrolidine; 79%.

Scheme 32.

The unsaturated pyrene label **74** was synthesised in good overall yield (61%) from **71**. Oxidation with PCC (**Scheme 33a**) gave the aldehyde **72** in excellent yield. Synthesis was confirmed by IR (ν_{max} 1720 cm^{-1}) and the appearance in ^1H NMR spectra of a resonance at δ 9.75 (*t*, 1H, *J* 1.8 Hz) indicative of the aldehydic proton. Conversion of **72** to the terminal alkyne **74** was via the method of Corey and Fuchs[68]. Treatment of CBr_4 with PPh_3 in CH_2Cl_2 at -15° for 30 minutes (**Scheme 33b**) gave rise to an orange solution which is thought to contain the ylid $Br_2C{=}PPh_3$. Addition of **72** to the reaction mixture and stirring for 60 minutes at 0° allowed attack on the carbonyl group via a Wittig-type process to give **73**. TLC (10/90 EtOAc/hexanes) of the dark brown reaction mixture showed the absence of **72** at about R_f 0.20 and a new fluorescent spot at about R_f 0.80 corresponding to **73**.
Dehydrohalogenation and halogen exchange of **73** 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 solution (**Scheme 33c**) gave the alkyne **74** in good yield. Synthesis was confirmed by the disappearance in the ^1H NMR of the dibromoalkene proton resonance; a strong sharp absorption in the IR at 3300 cm^{-1} and a new resonance at δ 1.95 (*t*, 1H, *J* 1.8 Hz) were both indicative of a terminal alkyne.

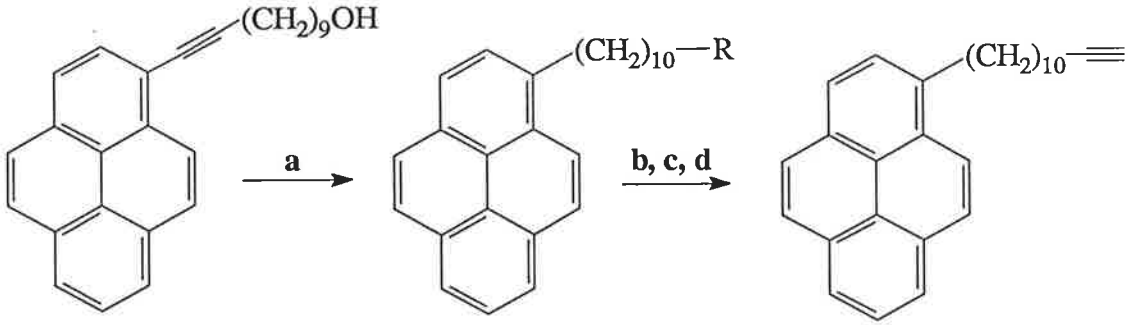


71: R = CH_2OH
72: R = CHO
73: R = $HC{=}CBr_2$

74

a. PCC (1.5 eq), NaOAc (2 eq), CH_2Cl_2; 96%.
b. CBr_4, PPh_3, CH_2Cl_2, -10°.
c. (i) *n*-BuLi, THF, -78° to RT. (ii) NH_4Cl (sat), 64% (overall for steps **b** and **c**).

Scheme 33.



71

75: R = CH_2OH
76: R = CHO
77: R = $HC{=}CBr_2$

78

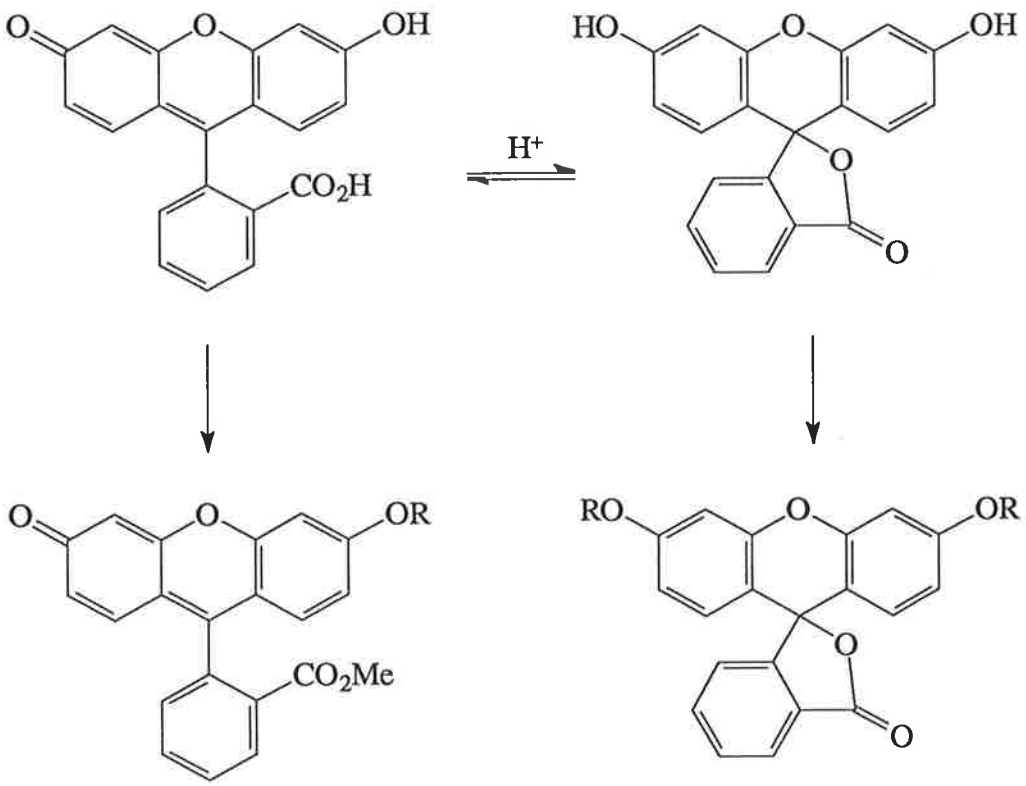
a. H_2, 5% Pd/C, EtOAc, 89%.
b. PCC (1.5 eq), NaOAc (2 eq), CH_2Cl_2; 89%.
c. CBr_4, PPh_3, CH_2Cl_2,-10°; 80%. **d.** (i) *n*-BuLi, THF, -78° to RT. (ii) NH_4Cl (sat), 71%.

Scheme 34.

Preparation of the saturated analogue **78** required reduction of the internal alkyne of **71** to give the saturated analogue **75**. This was readily achieved by stirring **71** with a 5% Pd/C catalyst under a hydrogen atmosphere (**Scheme 34a**). Filtration of the catalyst, removal of the solvent and recrystallisation gave the saturated compound in excellent yield. Conversion of **75** to the alkyne **78** via the aldehyde **76** was by the method for the unsaturated

analogue **74**, with the yields being comparable. The spectral data for the alkyne were identical to those obtained previously[64].

Attachment of a spacer molecule to fluorescein **5** to give a label-spacer adduct could theoretically be achieved via either the carboxylic acid to give an ester or amide, or alkylation of the phenolic hydroxyl to give an ether. However the small pKa difference between the acidic and phenolic protons ($pK_1 = 5.05$, $pK_2 = 7.00$)[69], and lactone isomerisation to **79** in non-polar solvents or under acidic conditions makes selective alkylation of either group difficult to achieve[70]. Also, the unreactivity of the carboxylic acid to carbodiimide reagents such as DCC[71] prevents attachment of the spacer via an amide linkage. A possible approach was then to prepare the known fluorescein methyl ester **80**, which should be easily separable from other products in the reaction mixture, and to subsequently alkylate the phenolic hydroxyl with the iodide **62**.



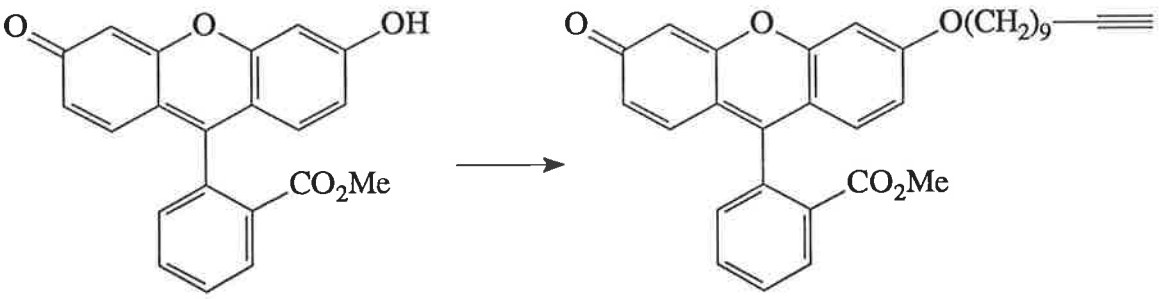
80: R = H.

H_2SO_4 (cat.), MeOH, reflux 72 hours; 25%.

Scheme 35.

The methyl ester of fluorescein **80** was prepared by refluxing **5** in MeOH with H_2SO_4 as catalyst for 72 hours (**Scheme 35**). The mono- and dimethylated byproducts which formed were removed by dissolving the crude product in 1M NaOH solution, extracting the aqueous solution with EtOAc to remove the less polar dimethylated compounds, reprotonation with 10% HCl to precipitate the crude product, and finally recrystallisation from MeOH. The methyl ester's identity was confirmed by mass spectrometry (M^+ 346) and melting point (282°; lit.[72] 282°), and was recovered in 25% yield.

Alkylation to give the label **81** was first attempted by deprotonation of **80**, and reaction of the anion with the iodide **62** (**Scheme 36**). Addition of NaH to a suspension of **80** in DMF caused effervescence and the formation of a dark red mixture. After 15 minutes the evolution of gas had ceased and the substrate was in a dark red solution; presumably the phenolic anion had formed. Compound **62** was added dropwise and the mixture stirred for 60 minutes, then TLC (EtOAc) showed large spots for starting material at R_f 0.10 and product at R_f 0.24. After workup and recrystallisation the product was recovered in 36% yield. A molecular ion at m/z 496, a strong sharp absorption in the IR at 3296 cm^{-1} (H-C≡ stretch) and a ^1H NMR resonance at 4.06 (*t*, 2H, *J* 7.0 Hz, CH_2-OAr) confirmed alkylation had occured.



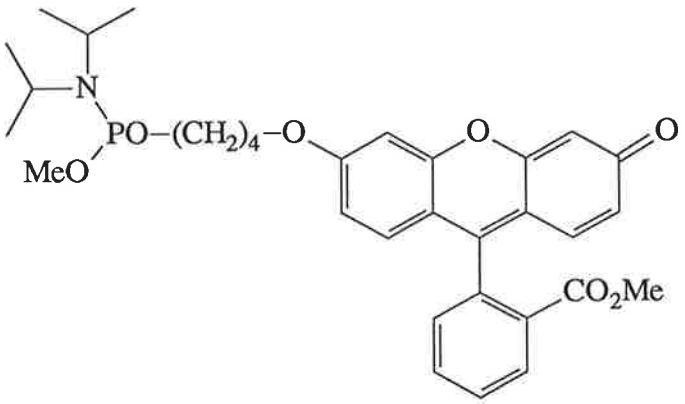
80 **81**

Conditions A: (i) NaH (1.1 eq), DMF. (ii) **62** (1.1 eq).
Conditions B: **62** (1.5 eq), K_2CO_3 (2 eq), 2-butanone, reflux 24 hours.

Scheme 36.

As presumably the starting material evident by TLC in the previous reaction was reformed by the abstraction of a proton from **62**, a base mild enough to exist with **62** and yet deprotonate **80** was required. With an increase in the amount of **62**, a higher yield should

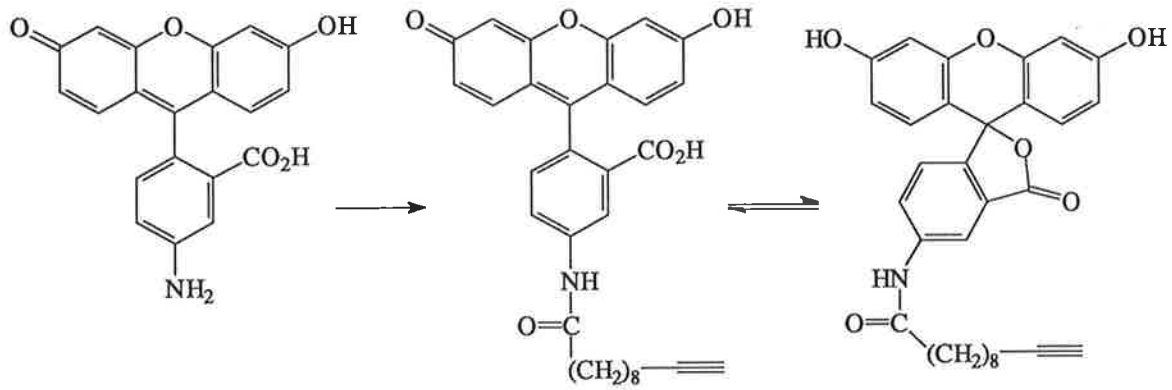
result. Reaction of **80** with **62** and K_2CO_3 in 2-butanone at reflux overnight (**Scheme 36,** Conditions B), workup and recrystallisation gave compound **80** in a good yield of 79%. The product prepared by this method has the disadvantage that the most fluorescent form of fluorescein, in which the phenolic group is deprotonated, is not available. Even so, the neutral form is sufficiently fluorescent such that a modified phosphoramidite **82** which included an *O*-alkylated fluorescein ether methyl ester has been used to label oligodeoxynucleotides[73] successfully.



82

Attachment of a spacer molecule to a functionalised fluorescein derivative, such as 5-aminofluorescein **83** allows the highly fluorescent phenolic anion to be formed. Hence label **84** was synthesised by the reaction of **83** with acid chloride **67** in pyridine (**Scheme 37**). After dissolution of **83** to give a deep green solution, **67** was added dropwise. After stirring at room temperature for 48 hours, TLC (10/90 MeOH/CH_2Cl_2) showed a trace of **83** at R_f 0.33, a small byproduct spot at R_f 0.87 and a large spot corresponding to product **84** at R_f 0.54. After workup the product was recovered in 65% yield as bright orange crystals. Acylation was confirmed by mass spectrometry (M^+ 511) and ^1H NMR spectroscopy in $[^2H]_6$-DMSO, which showed resonances at δ 2.37 (*t*, 2H, *J* 7.3, CH_2-CONH) and δ 2.73 (*t*, 1H, *J* 2.5 Hz, H-C≡). Also **84** was shown to exist in the lactone form **85** in DMSO. The aromatic part of the ^1H spectrum for the non-symmetrical, open chain form **84** would be expected to have 12 resonances, each of which integrates to 1H (for the aromatic and acidic

protons). However only 8 resonances (4 with integration 2H, the remainder 1H) are observed, which is consistent with the plane of symmetry in **85**. In addition, the ^{13}C NMR spectrum showed 25 resonances rather than 31, which is again consistent with **85**. This facile isomerisation explains the fact that a solution of **84** in a protic solvent (eg water, MeOH, EtOH) is coloured and fluorescent whereas a solution of **84** in an aprotic solvent (eg EtOAc, DMSO) is neither.



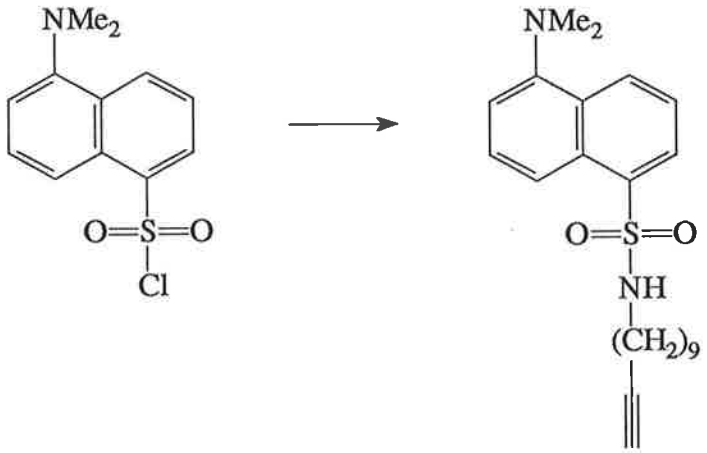
83 **84** **85**

Acid chloride **67** (1.2 eq), pyridine, 48 hours, room temperature; 65%.

Scheme 37.

The dansyl label **87** was prepared by reaction of dansyl chloride **86**, the aminoalkyne **66** and Et_3N in CH_2Cl_2 at room temperature (**Scheme 38**). After stirring for 60 minutes (although presumably the reaction was over much sooner) TLC (20/80 EtOAc/hexanes) showed the absence of non-fluorescent **86** at R_f 0.71 and a new fluorescent spot at R_f 0.42 corresponding to product. After chromatography **87** was recovered in 72% yield with MS showing a molecular ion at m/z 400, and ^1H NMR showing a resonance at δ 4.59 (*bt*, 1H) consistent with the proton of the sulphonamide group.

With the preparation of the dansyl label-spacer adduct **87** all of the selected fluorescent moieties had been attached to spacer molecules in average to good yields. Fluorescence data of the compounds is shown in **Table 2**, and coupling reactions with the biomolecule derivatives are described in Chapter 3.



86 **87**

Amine **66** (1 eq), Et_3N (1 eq), CH_2Cl_2; 72%

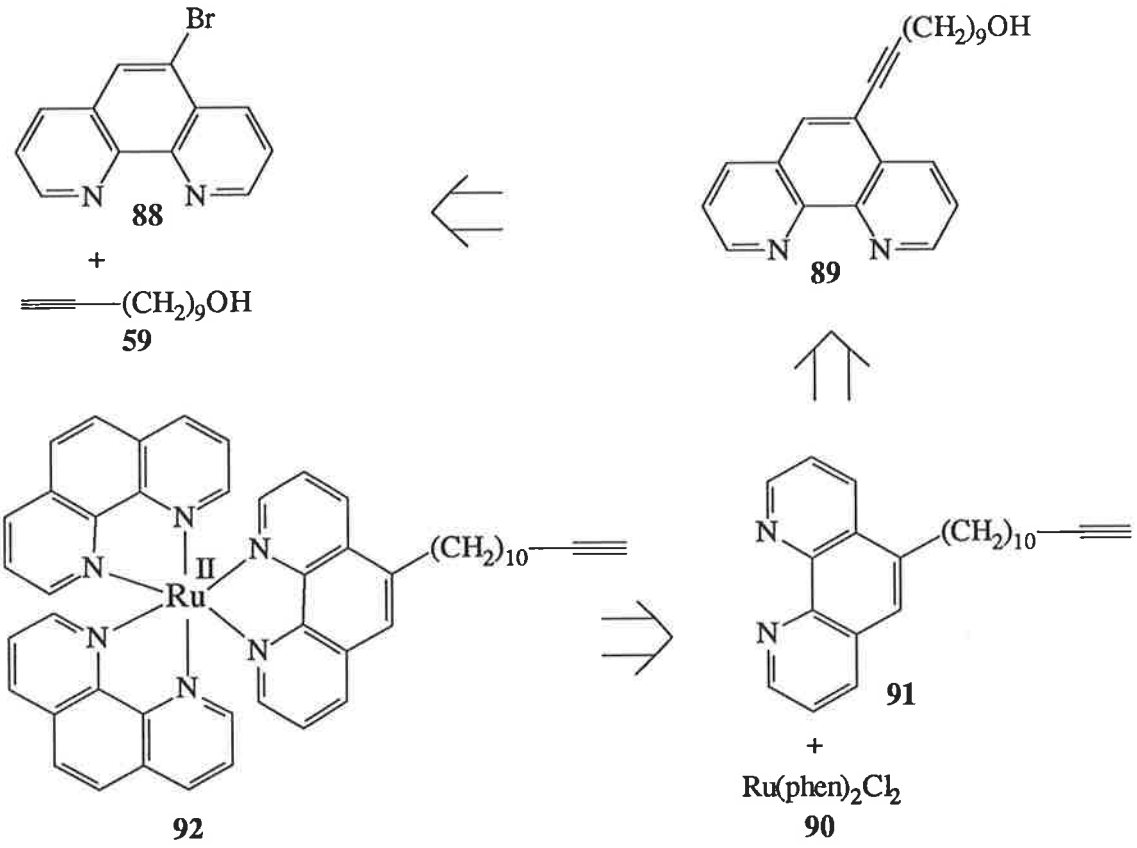
Scheme 38.

Table 2. Fluorescence Data for Label-spacer Adducts.

Compound	Solvent	λ_{ex} nm	λ_{max} nm
69	EtOH	385	421, 444
74	$CHCl_3$	364	386, 396, 406
78	$CHCl_3$	343	377, 397, 416
81	EtOH	489	517
84	EtOH	482	516
87	EtOH	337	506

Chapter 2.3. Synthesis of Time Resolved Fluorescence Label-spacer Molecules.

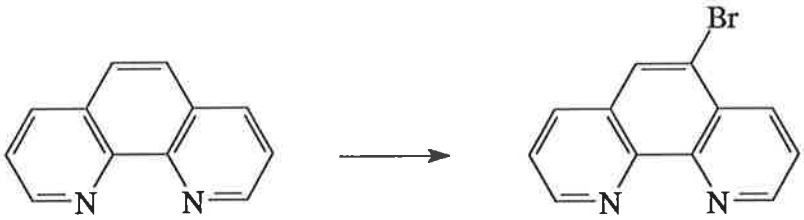
The synthesis of a time resolved fluorescence (TRF) reporter compound **92** required the incorporation of an alkynyl-alkyl chain-modified-1,10-phenanthroline ligand **91** into ruthenium (II) *bis*-1,10-phenanthroline dichloride **90**. Retrosynthetic analysis (**Scheme 39**) showed that palladium catalysed coupling of the alkynol **59** with 5-bromo-1,10-phenanthroline **88**, and subsequent functional group interconversion from a terminal alcohol to a terminal alkyne was necessary. The internal alkyne of the adduct **91** can either be reduced to give a saturated link to the 1,10-phenanthroline moiety, or retained to modify the fluorescent behaviour of the label.



Scheme 39.

The preparation of 5-bromo-1,10-phenanthroline **88** has been achieved via a Skraup synthesis from 8-amino-6-bromoquinoline[74] and by the bromination of 1,10-phenanathroline

40 in fuming sulphuric acid (60% oleum)[75] (**Scheme 40**), both methods reporting an average yield. The latter method was preferred as the substrate and reagents were readily available, and did not require the toxic arsenic pentoxide. Initial attempts under the reported conditions gave only a poor yield, with a large amount of dark red byproduct (which was not characterised). Heating anhydrous **40** at 120° at 0.01 mmHg for three hours prior to reaction under the previous conditions to ensure anhydrous starting material reduced the amount of byproduct, and increased the yield to 52%. The product was purified by chromatography on alumina as pronounced streaking occurred on silica TLC; slow elution from a short column gave the best separation from unreacted starting material. Mass spectrometry confirmed monobromination (m/z 258/260); melting point and ^{1}H NMR were in accordance with the literature[75].

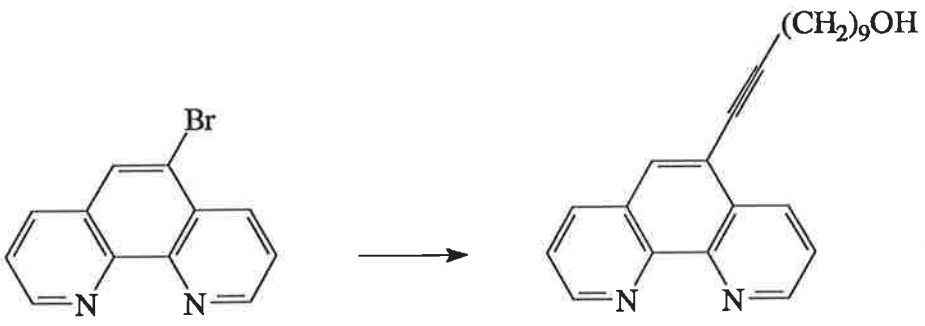


40 **88**

Br_2, fuming H_2SO_4, 125°, 12 hours; 52%.

Scheme 40.

Previous palladium catalysed coupling reactions of bromo-substituted 1,10-phenanthrolines have used CuI as a co-catalyst[76], however complexation of Cu(I) to the phenanthroline moiety reduced the yield of the reaction. The use of sonication to disrupt complexation, and treatment with aqueous KCN were necessary to allow a good yield. The use of copper iodide as a co-catalyst is not necessary (*introduction*, p 20) if the substrate can withstand the higher temperatures of pyrrolidine at reflux, hence **88** was reacted with alkyne alcohol **59** to give the adduct **89** in 66% yield (**Scheme 41**). Coupling was confirmed by MS (M^+ 346), the absence of the terminal alkyne triplet at δ 1.97 and the presence of a new triplet at δ 2.68 (2H, *J* 7.1 Hz) indicative of Ar-≡-CH_2.



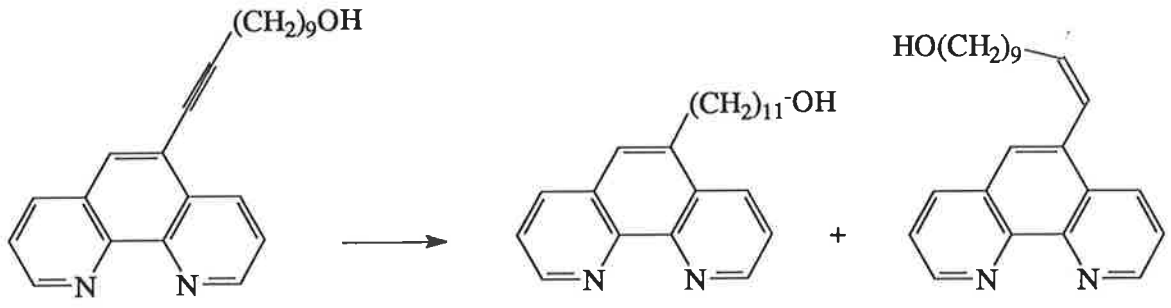
88 **89**

59 (1.2 eq), $Pd(PPh_3)_4$ (0.05 eq), pyrrolidine, 80°, 6 hours; 66%.

Scheme 41.

Treatment of **89** with 5% Pd/C under an atmosphere of hydrogen overnight (**Scheme 42**, Conditions A) showed no reaction. Increasing the amount of palladium to 10% and stirring for 48 hours (**Scheme 42**, Conditions B) gave a mixture of starting **89** and the alkene **94** (in a ratio of approximately 1:7), as indicated by vinylic resonances at δ 6.02 (*dt*, *J* 11.5, 7.5 Hz, CH_2-**H**C=) and δ 6.77 (*dd*, *J* 11.5, 1.2 Hz, Ar-HC=). As the vinylic coupling constant was 11.5 Hz, **94** was assumed to be the expected *Z* isomer[77]. Increasing the reaction temperature to 55° and stirring overnight removed the alkene resonances, however the aromatic region was now very complex, indicating a mixture of products. Presumably complexation of the 1,10-phenanthroline moiety to the catalyst was the cause of the sluggish reaction. Increasing the temperature to decrease complexation caused overreduction. Protonation of amines is a common way of preventing complexation to the catalyst in hydrogenation reactions[78], hence reaction of **89** under acidic conditions (**Scheme 42**, Conditions C) gave the saturated compound **93** in 78% yield. Mass spectrometry showed a molecular ion at m/z 350, and ^{1}H NMR showed the absence of the resonance at δ 2.68, and a new resonance at δ 2.99 (*t*, 2H, *J* 7.7 Hz, CH_2-Ar) which were consistent with hydrogenation having occurred. The remainder of the starting material appeared to be converted to a red polar compound which could not be eluted from the top of the alumina column used for purification. Repeating the reaction using sulphuric acid gave a yield of 52%, and with chloroacetic acid a yield of 52% was obtained. The reduction step occasionally did not go to

completion (even with catalyst renewal) and in such cases the partially reduced compound could not be separated by column chromatography.



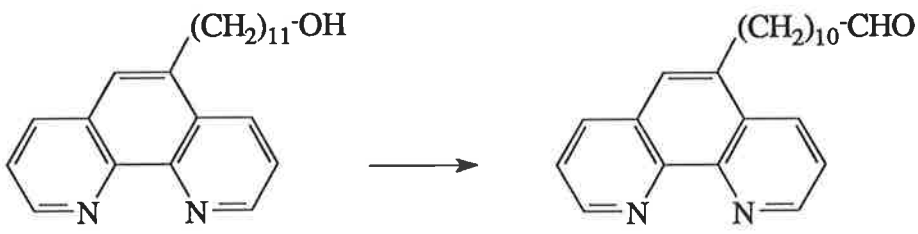
89 **93** **94**

Conditions A: H_2, 5% Pd/C, MeOH, O/N.
Conditions B: H_2, 10% Pd/C, MeOH, 48 hours.
Conditions C: H_2, 5% Pd/C, 10% HCl, MeOH; 78%.

Scheme 42.

Conversion of the alcohol **93** to the aldehyde **95** using PCC (**Scheme 43**, Conditions A) was unsuccessful. TLC analysis of the reaction mixture showed only a large amount of baseline material, suggesting that complexation of the 1,10-phenanthroline moiety to the reagent had occurred. Recently, TEMPO (2,2,6,6-tetramethyl-1-piperidinyloxy free radical) has been used as a catalyst for the large scale oxidation of alcohols to carbonyl compounds[79]. The advantages of this method have been reported to include no moisture sensitivity, ease of workup and ease of reaction on a large scale. When **93** was reacted with NaOCl, NaBr and TEMPO (**Scheme 43**, Conditions B) a low yield of **95** was obtained, with TLC analysis showing a small amount of starting material remaining. Oxidation was confirmed by the absence in the IR of broad O-H stretch between 3600 and 3100 cm^{-1}, and a new intense IR absorption at 1724 cm^{-1} consistent with a C=O stretch. The majority of the starting material was presumably converted to the corresponding acid, which was not isolated from the biphasic reaction mixture. Reverting to the standard Swern oxidation[80] using DMSO, oxalyl chloride and Et_3N (**Scheme 43**, Conditions C) afforded the aldehyde **95** in good yield; ^1H NMR of the crude product showed conversion was effected relatively cleanly. The aldehyde

was partially unstable to chromatography on alumina hence the isolated yield was average, and so the crude aldehyde was used in the next synthetic step.

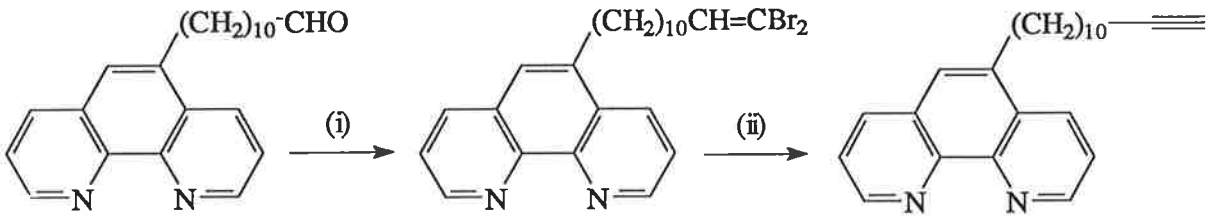


93 **95**

Conditions A: PCC (1.5 eq), NaOAc (2 eq), CH_2Cl_2, 0%.
Conditions B: TEMPO (0.1 eq), NaOCl (1.1 eq), NaBr (1.1 eq), CH_2Cl_2, H_2O; 38%.
Conditions C: ClCOCOCl (1.1 eq), DMSO (2.2 eq), Et_3N (5 eq), CH_2Cl_2, -78° to RT; 71%

Scheme 43.

Conversion of **95** to the alkyne **91** was to occur via the method of Corey and Fuchs[81], which involves reaction of **95** with the Wittig-type reagent derived from CBr_4 and PPh_3 to give the dibromoolefin **96**, followed by dehydrohalogenation and halogen exchange with 2 equivalents of *n*-BuLi to give the lithium acetylide and protonation to give the alkyne (**Scheme 44**). The dibromoolefin **96** was recovered in a poor yield of 35%, and contaminated with triphenylphosphine oxide (in a ratio of 95:5), which could not be removed by repeated chromatography or recrystallisation. Formation of **96** was confirmed by ^1H resonances at δ 6.34 (*t*, 1H, $HC{=}CBr_2$) and δ 2.04 (*q*, 2H, CH_2-$CH{=}CBr_2$).

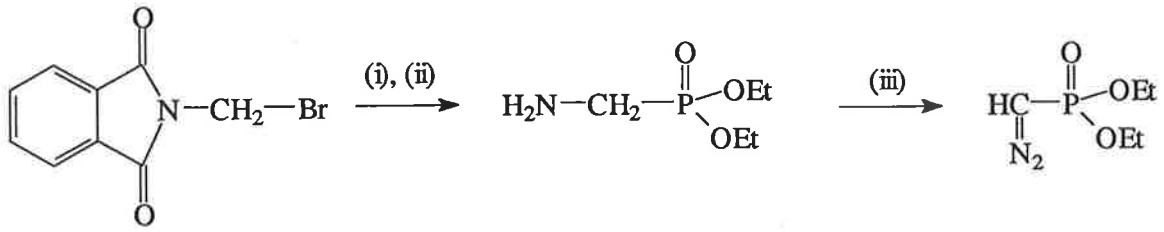


95 **96** **91**

(i) CBr_4, PPh_3,-10°, 3 hrs, 35%. (ii) (a) *n*-BuLi (2.2 eq), THF, -78°- RT, 2 hrs; (b) NH_4Cl (sat)

Scheme 44.

^{1}H NMR analysis of the reaction mixture of **96** with *n*-BuLi (2.2eq) showed a different and complex aromatic region, suggesting the lithium reagent had reacted with the 1,10-phenanthroline moiety to give many products. Nucleophilic addition of alkyl lithium species to C2 and C4 of pyridine systems is well known[82], and occurs readily at room temperature. Repeating the reaction but quenching at -78° again gave a complex aromatic region in the ^{1}H NMR spectrum and many products by TLC. Given the poor yield and purification problems of the previous step an alternative method for the conversion of **95** to **91** was necessary.

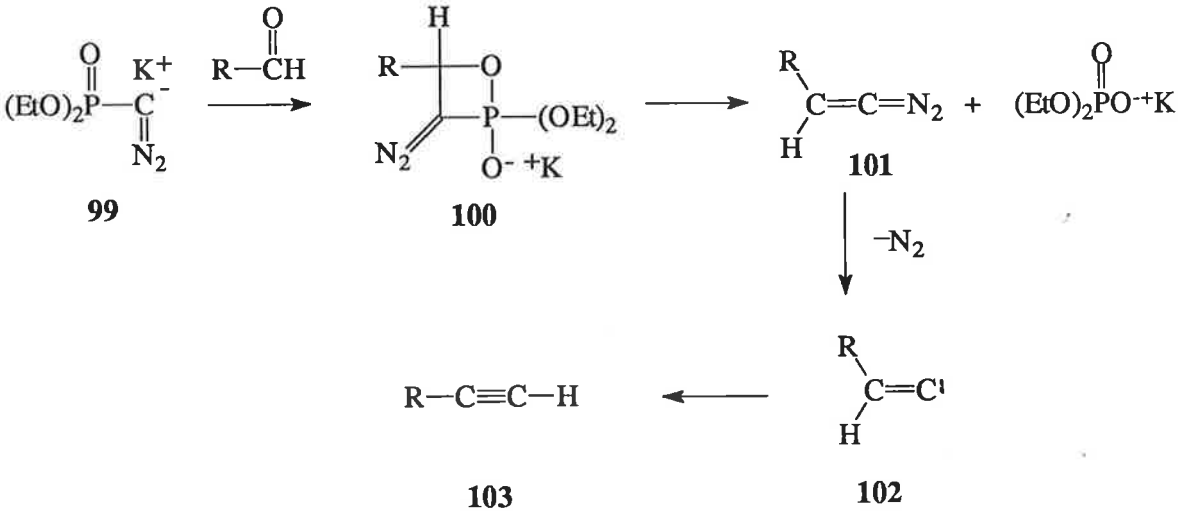


97 **98**

(i) $P(OEt)_3$, *p*-xylene, Δ, 48%. (ii) $N_2H_4.H_2O$, AcOH. (iii) $NaNO_2$, AcOH, 63%.

Scheme 45.

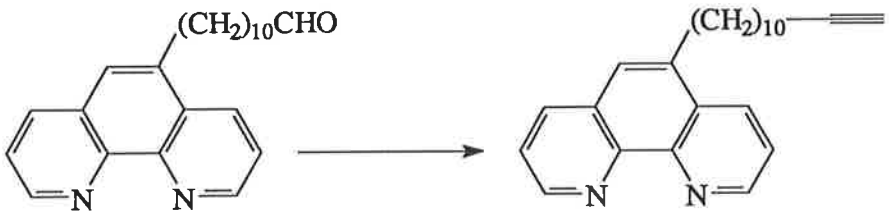
Dialkylphosphinodiazomethanes such as **98** have been shown to convert aldehydes to alkynes in average to good yields[83]. Compound **98** was readily prepared by the reaction of commercially available *N*-(bromomethyl)phthalimide **97** with triethylphosphite, removal of the phthalimido group with hydrazine hydrate and diazotisation with $NaNO_2$ under acidic conditions (**Scheme 45**)[84]. Although a diazo compound, **98** is relatively stable due to the electron withdrawing influence of the phosphino group, and purification by distillation was possible. The postulated mechanism for the reaction (**Scheme 46**)[85] of **98** with aldehydes involves nucleophilic addition of the diazo compound anion **99** to the carbonyl group, and formation of a Wittig-type intermediate **100** which loses potassium diethyl phosphate to give a diazoalkene **101**. Loss of nitrogen from **101** results in a alkylidenecarbene **102**, which undergoes hydrogen migration to give the alkyne **103**.



99 **100** **101** **102** **103**

Scheme 46.

Hence treatment of **95** with **98** was attempted (**Scheme 47**, Conditions A), and gave the alkyne **91** in 47% yield. Synthesis was confirmed by ^{1}H NMR resonances at 1.95 (*t*, 1H, HC≡C) and 2.23 (*dt*, 2H, CH_2-C≡CH), and IR absorptions at 3300 cm^{-1} (*s*, H-C≡ str) and 2060 cm^{-1} (*w*, C≡C str). Recently the use of dimethyl-(1-diazo-2-oxopropyl)phosphonate ($CH_3C(O)C(N_2)P(O)(OMe)_2$) for the conversion of aldehydes to alkynes under mild conditions has been described[86] . Using this reagent for the conversion of **95** to **91** (**Scheme 47**, Conditions B) gave a yield of 60%.

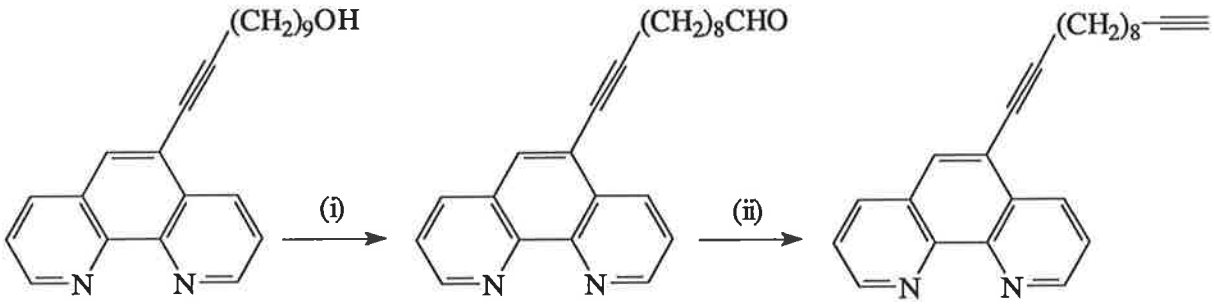


95 **91**

Conditions A: (i) **98** (1.1 eq), KOBu^t (1.1 eq), THF, -78°,10 mins;
(ii) RCHO, 18 hrs,T to RT.
Conditions B: $CH_3C(O)C(N_2)P(O)(OMe)_2$ (1.1 eq), K_2CO_3 (1.1 eq), MeOH, 0°, 12 hours.

Scheme 47.

Using an analogous route the unsaturated ligand **105** was synthesised by the procedures developed for **91** (**Scheme 48**). Alcohol **89** was converted to the aldehyde **104** using a Swern oxidation, and subsequent reaction with **98** gave the alkyne **105**. The IR spectrum of **104** showed the absence of the absorption between 3600 and 3100 cm^{-1} (O-H stretch), a strong absorption at 1722 cm^{-1} (C=O) and the aldehyde resonance in the ^1H NMR spectrum at δ 9.72 (*t*, *J* 1.6 Hz). Conversion to the alkyne **105** was confirmed by the characteristic absorption in the IR at 3300 cm^{-1} (H-C≡ str), absence of C=O absorption at 1722 cm^{-1} and the alkyne proton resonance at δ 1.95 (*t*, *J* 2.6 Hz).



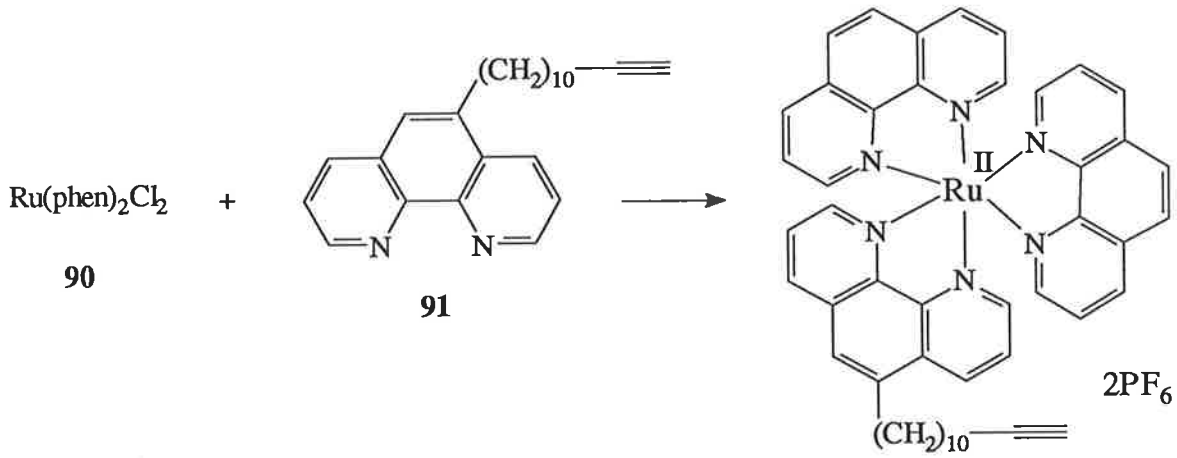
89 **104** **105**

(i) $(COCl)_2$ (1.1 eq), DMSO (2.2 eq), Et_3N (5 eq), -78° to RT, 69%.
(ii) (a) $HC(N_2)P(O)(OEt)_2$ (1.1 eq), $KOBu^t$ (1.1 eq), THF, -78°, 10 mins;
(b) RCHO, stir 18 hours, then warm to RT, 60%.

Scheme 48.

Formation of the ruthenium complex **92** was achieved by stirring **91** with $Ru(phen)_2Cl_2$ **90** in a mixture of MeOH and H_2O at 40° for 48 hours (**Scheme 49**). The dark purple colour of the neutral ruthenium (II) complex in solution changed to a deep red-brown as the reaction progressed. 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 **92** were obtained. Both ^1H NMR and TLC showed impurities present however attempted recrystallisations from various solvents were unsuccessful; the compound continued to oil out of solution. 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 **91** into the ruthenium complex. As the parent *tris*-1,10-phenanthroline ruthenium (II) complex is chiral, formation of diastereomers upon addition of the non-symmetrical modified ligand was expected. The ^{1}H 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 adjacent to the aromatic moiety, confirming non-separable (by column chromatography) diastereomers. As the coupling properties of the alkyne under palladium catalysis were of interest, separation by a higher resolution method (i.e. HPLC) was not attempted.



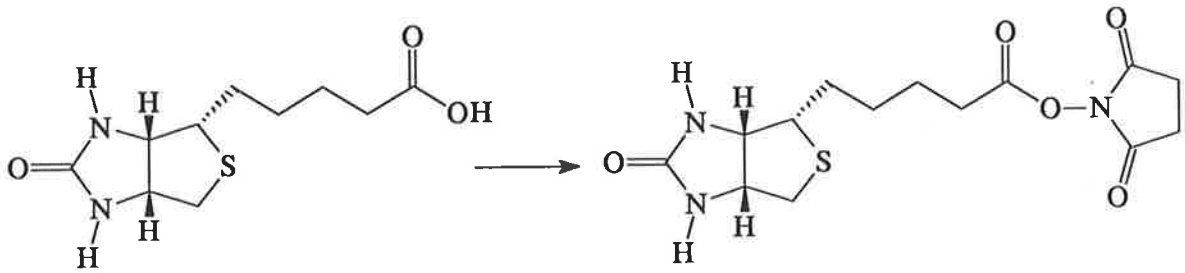
$MeOH/H_2O$ (1:2), 40°, 48 hours, 68%.

Scheme 49.

With the incorporation of the modified 1,10-phenanthroline ligand **91** the synthesis of the target reporter compound **92** was complete. Fluorescence spectroscopy of a solution of **92** showed a maximum at 578 nm (λ_{ex} = 449 nm). The unsaturated ligand **105** was not incorporated into a ruthenium complex due to time constraints. Reaction of the reporter **92** with a phenylalanine derivative under palladium catalysis is described in Chapter 3.1 (*Coupling of Label-spacer Molecules to Aminoacid Derivatives*).

Chapter 2.4. Synthesis of Biotin-spacer Label.

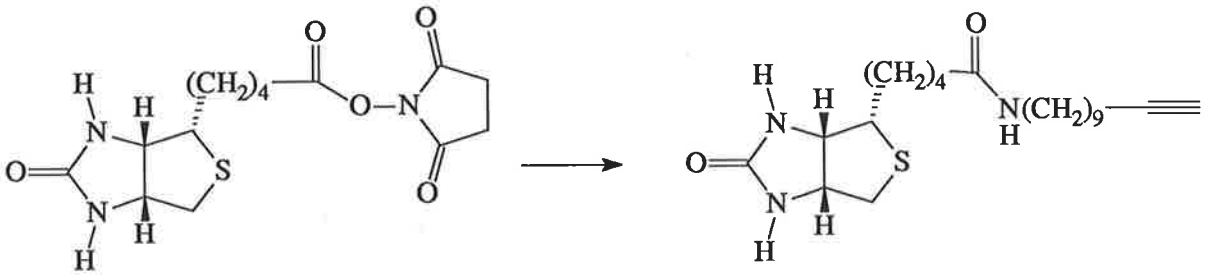
A common method of synthesising biotin conjugates is via reaction of an activated biotin ester (e.g. the *N*-hydroxysuccinimide ester **106**) with an amine to give a *N*-substituted biotin amide derivative. Preparation of **106** by reaction of **41** with NHS and DCC in DMF (**Scheme 50**) gave the product in average yield[87]. The melting point and spectral data of **106** were consistent with the literature[88].



41 **106**

Scheme 50. NHS, DCC, DMF; 51%.

Displacement of the NHS group by the amine **66** occurred readily in DMF (**Scheme 51**). Filtration to remove precipitated NHS, removal of the solvent and purification of the residue by flash chromatography and recrystallisation from MeOH/H_2O gave the biotin undecynyl amide **107** in very good yield. Synthesis was confirmed by MS (M^+ 393); the NMR data were consistent with the expected structure. Unexpectedly, when testing for a suitable recrystallisation solvent, the compound was found to gelate EtOAc and other low polarity organic solvents. An investigation into the gelling properties of **107** and other biotin analogues is reported in Chapter 4.



106 **107**

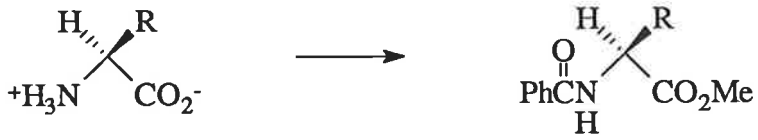
Amine **66** (1 eq), DMF, RT, 15 hours; 90%.

Scheme 51.

Chapter 3. Preparation and Coupling of Biomolecules

Chapter 3.1. Coupling of Label-spacer Adducts to Aminoacid Derivatives

This section describes the preparation of the halogenated and triflated amino acids **108**, **109**, **110** and **111**, and their behaviour under PdCC conditions to a selection of the developed label-spacer adducts. Also, the reaction of propargyl glycine derivative **126** with 1-bromopyrene and 1-iodopyrene is reported. Protection of the amino and carboxyl functionalities of the amino acid derivatives, although not necessary during palladium catalysed couplings, was undertaken for reasons of ease of purification and characterisation. Esterification of the amino acid derivatives with MeOH/$SOCl_2$ gave the methyl ester hydrochlorides (**Scheme 52(i)**), generally in excellent crude yields. Removal of the solvent, treatment of the crude products with benzoyl chloride under Schotten-Baumann conditions (**Scheme 52(ii)**) and column chromatography on silica gel followed by recrystallisation gave the protected amino acids in average to good yields.

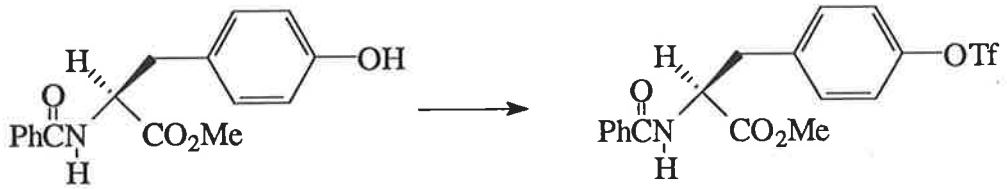


(i) MeOH, $SOCl_2$. (ii) PhCOCl, K_2CO_3, CH_2Cl_2, H_2O.

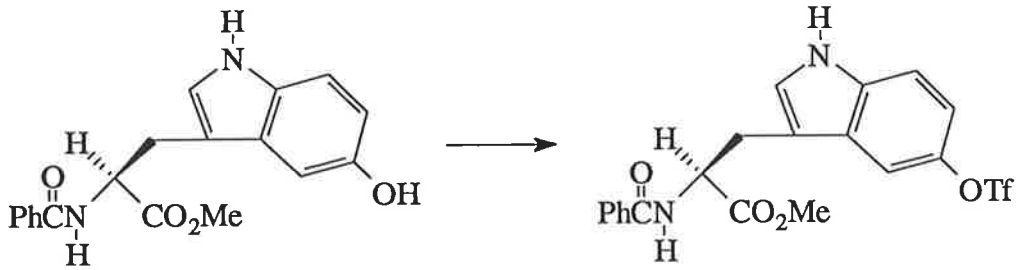
Amino acid	Product	yield %
4-iodophenylalanine (**46**)	**108**	73
3-iodotyrosine (**47**)	**109**	59
tyrosine (**48**)	**110**	55
5-hydroxyltryptophan (**49**)	**111**	50

Scheme 52.

Hydroxy amino acids **110** and **111** were converted to the triflates **112** and **113** using N-phenyltriflimide ($PhNTf_2$) and Et_3N in CH_2Cl_2 (**Scheme 53**). Purification by flash chromatography and recrystallisation gave the products in high yields. Triflate **112** was a known compound[89] and melting point and spectral data were in agreement. Formation of **112** was confirmed by MS (M^+ 470) and IR (absence of O-H stretch).



110 **112**: 99%.



111 **113**: 91%.

$PhNTf_2$ (1.1 eq), Et_3N (1.1 eq), CH_2Cl_2, 0° to RT, overnight.

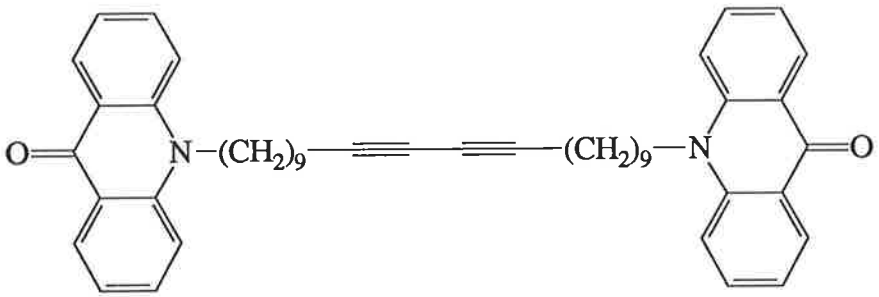
Scheme 53.

The reaction of the acid derivatives with the label-spacer adducts were first attempted under standard conditions, which are shown in **Scheme 54**. Modifications to the conditions, such as changing temperature, catalyst, solvent and/or base were made if the coupled product was not obtained in an acceptable yield. Reactions of the triflate **112** with the acridone label **69** are described first.

Ar-X (X =I, OTf) (1.0 eq), label (1.2 eq), $Pd(PPh_3)_4$ (0.1 eq), CuI (0.2 eq), Et_3N, DMF, RT.

Scheme 54: Standard Conditions

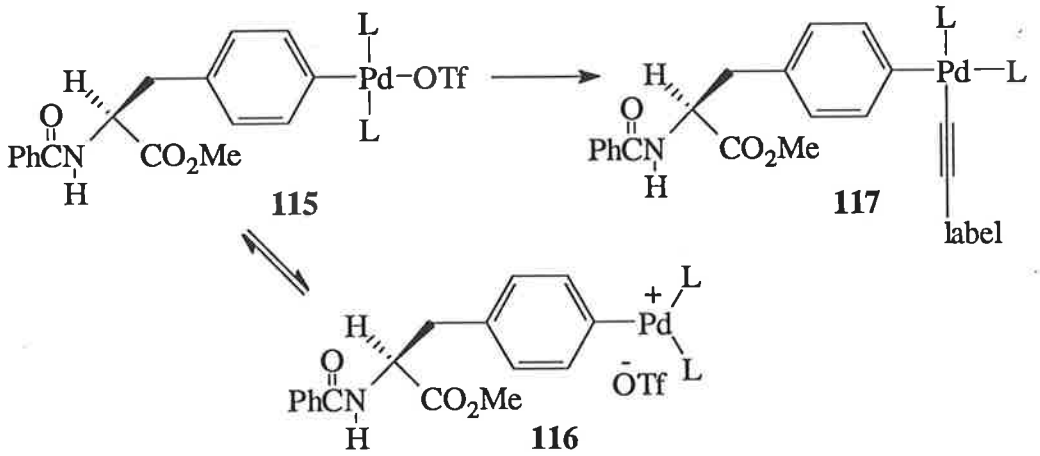
Reaction of **112** with **69** under standard conditions at room temperature (**Scheme 55**, Conditions A) showed no coupled product (by TLC comparison of product synthesised from the iodide **108**) after 60 minutes. Increasing the temperature sequentially to 35°, 50° and 60° after 60 minute periods again showed no reaction. The only new fluorescent spot was due to the homocoupled alkyne dimer **114** in increasing quantity; triflate **112** was still present. Repeated reactions with increasing amounts of catalyst (10%, 15% and 30%), and increasing temperature again gave only the homocoupled product.



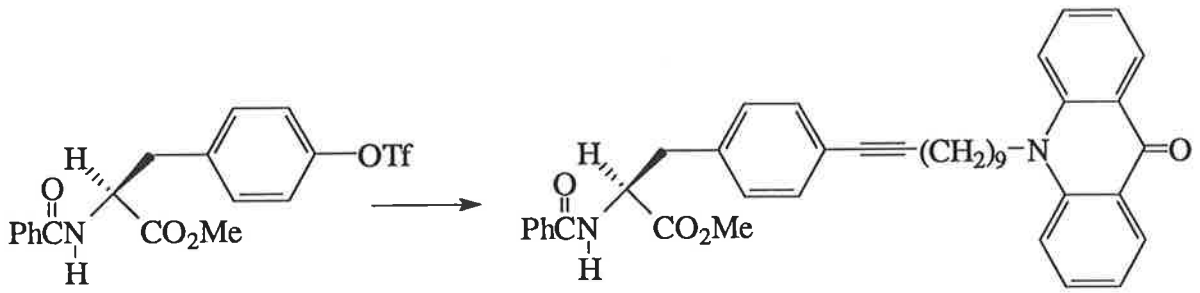
114

As **112** was still present in the reaction mixture, this showed that homocoupling of the alkyne was occurring at a faster rate than formation of the cross-coupled product. In the cross coupling reaction, the rate determining step usually is either the oxidative addition of the electrophilic carbon species to PdL_2, or transmetallation by the nucleophilic carbon onto the palladium (II) intermediate **115**. The use of $Pd_2dba_3/AsPh_3$ catalyst system has been shown to increase the rate of the transmetallation step as the rate of the ligand dissociation from the intermediate **115** is increased[90], hence facilitating formation of the transmetallated intermediate **117**. However TLC analysis of the reaction mixture between **112** and **69** (stirred at room temperature overnight) under these conditions (**Scheme 55**, Conditions B) showed only alkyne dimer **114**; **112** and **69** were still present. Increasing the temperature to 50° and stirring until the catalyst decomposed (about 30 hours in total) showed only an increase in the amount of **114** and the absence of **112** and **69**. A spot corresponding to the desired coupled product **118** was not observed. Removal of the solvent and separation of the residue by chromatography gave **114** in 61% yield (from starting alkyne). Increasing the

amount of catalyst to 10% with stirring at 50°, and workup as previously gave only **114** in 67% yield. The lack of coupled product **118** suggested that oxidative addition was probably the rate determining step in this system.



The rate of oxidative additon of aryl bromides in palladium catalysed cross-coupling reactions is relatively slow[91] compared to aryl iodides, and so conditions reported to effect efficient coupling of aryl bromides were tested[49] (**Scheme 55**, Conditions C). Reaction of **112** and **69** at reflux under these conditions gave only unreacted starting alkyne (28%) and the dimer **114** (32%). The triflate was consumed in the reaction but the resultant compound(s) were not identified. A large amount of baseline material was observed suggesting the decomposition of **112** under the reaction conditions.



112 **118**

Conditions A: **69** (1.5 eq), $Pd(PPh_3)_4$ (0.1eq), CuI (0.2 eq), Et_3N, DMF.
Conditions B: **69** (2.5 eq), Pd_2dba_3 (0.025 eq), $AsPh_3$ (0.2 eq), CuI (0.1 eq), Et_3N, DMF.
Conditions C: **69** (1.5 eq), $Pd(PPh_3)_4$ (0.1 eq), CuI (0.2 eq), PPh_3 (0.2 eq), piperidine.
Conditions D: (i) $Pd(PPh_3)_4$ (1.0 eq), DMF. (ii) **69** (1.5 eq), CuI (0.2 eq), Et_3N; 68%.
Conditions E: **69** (1.5 eq), $Pd(PPh_3)_4$ (0.1 eq), Et_3N, DMF, 90°.
Conditions F: **69** (1.5 eq), $Pd(PPh_3)_4$ (0.1eq), CuI (0.2 eq), Et_3N, DMSO; 86%.

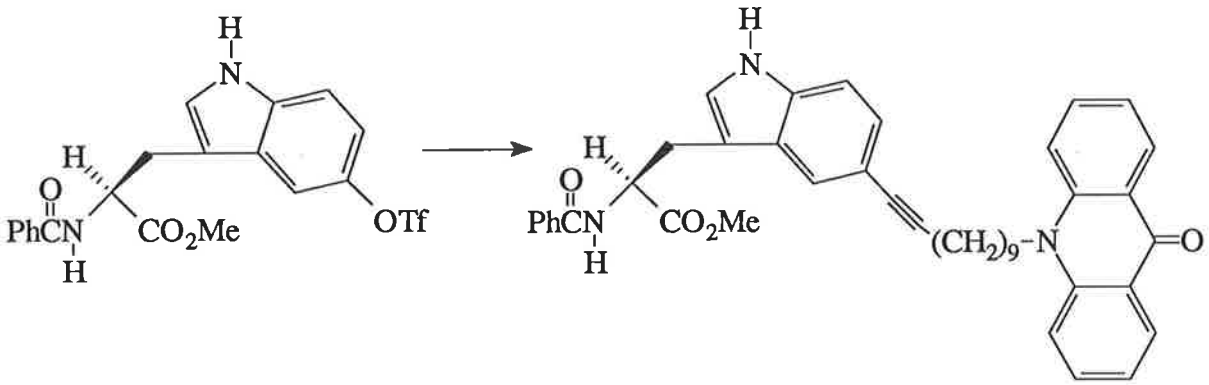
Scheme 55.

Thus it appears that the oxidative addition step of the aryl triflate is slow, which allows the (normally) slower homocoupling process (which is catalysed by copper and palladium species[92]) to predominate. This problem may be overcome by reacting the triflate stoichiometrically with $Pd(PPh_3)_4$ to form the σ-bonded intermediate **115** (which is thought to be in equilibrium with the ionic palladium species **116**[93]), and is then reacted with the label under standard conditions.

Reaction of **112** with a stoichiometric amount of $Pd(PPh_3)_4$ in DMF (**Scheme 55,** Conditions D) at room temperature gave rise to a green precipitate (which decomposed upon exposure to the atmosphere). After 3.5 hours TLC analysis showed the absence of **112** at R_f = 0.25 and a new spot at R_f = 0.05. As presumably the σ-complex had been formed the label, CuI and Et_3N were added. The green precipitate was immediately consumed and the reaction mixture turned dark brown. After stirring at 50° for 30 minutes, TLC analysis (EtOAc/hexanes 50/50) showed the absence of the low R_f spot, and spots corresponding to unreacted **69** (R_f = 0.63), dimer **114** (R_f = 0.37) and coupled product **118** (R_f = 0.22). After separation by flash chromatography **118** was recovered in 68% yield; 48% of the label was converted to the dimer **114.** The structure of the product was confirmed by mass spectrometry (M^+ 626); the appearance of the ^1H spectrum was a superimposition of the spectra of **112** and **69**, apart from the absence of the resonances for the terminal alkyne and adjacent methylene, and a new resonance at δ 2.40 (*t*, 2H, *J* 7.0 Hz) consistent with a methylene attached to an ethynylbenzene group.

Although the stoichiometric coupling of triflate **112** was successful in average yield, conditions for catalytic couplings were still sought as palladium reagents are expensive, and isolation of the product is easier when a catalytic amount is used. Chen and Yang[94] reported that phenyl triflate was coupled with a variety of terminal alkynes under palladium catalysis in DMF at 90°; no CuI was used (**Scheme 55,** Conditions E). However reaction of **112** with **69** under these conditions resulted in alkyne dimer **114** in 33% yield of starting alkyne and small amounts of unreacted starting triflate and label. The remainder of the starting materials appeared to undergo decomposition.

DMSO has been used as a solvent in coupling reactions where the reaction temperature has been raised to facilitate reaction of an unreactive substrate[95] (**Scheme 55**, Conditions F), and so reaction of **112** with **69** at 70° gave the coupled product in 86% yield. A small amount of alkyne dimer **114** (3%) was also recovered. Repeating the reaction in DMF under standard conditions (**Scheme 55**, Conditions A) with the temperature at 70° from the start of the reaction resulted in a yield of 80% of the desired coupled product **118**, showing that the temperature of reaction is critical to the coupling. Presumably as the temperature rises the rate of oxidative addition of the aryl triflate to the palladium catalyst becomes significant and hence can react further in the catalytic cycle to give the coupled product. This approach is, however, limited by the thermal stabilities of the substrate, coupling compound and catalyst.



113 **119**

Scheme 56.

Conditions A: Acridone label (1.5 eq), $Pd(PPh_3)_4$ (0.1 eq), CuI (0.2), Et_3N, DMF.
Conditions B: Acridone label (1.5 eq), $Pd(PPh_3)_4$ (0.1 eq), CuI (0.2 eq), PPh_3 (0.2 eq), piperidine; 8%.
Conditions C: (i) $Pd(PPh_3)_4$ (1 eq), DMF, 50°. (ii) acridone label (1.5 eq), CuI (0.2 eq), Et_3N.

As **112** has been successfully coupled to the acridone label **69**, the reaction of tryptophan triflate derivative **113** with **69** was explored by reaction under standard conditions (**Scheme 56**, Conditions A) at 50°. After 60 minutes TLC analysis (EtOAc/hexanes 40/60) showed alkyne at R_f 0.57, triflate **113** at R_f 0.16, and alkyne dimer **114** at R_f 0.30. After stirring overnight the amount of alkyne dimer had increased; other starting materials were

still present. The reaction mixture developed a large amount of black precipitate (presumably palladium metal) after 24 hours indicating the catalyst had decomposed. Separation of the reaction mixture by flash chromatography gave the alkyne dimer in 25% yield, and 75% of the starting triflate was recovered. The balance of starting materials appeared to have decomposed, as suggested by the large amount of low Rf material left on the silica column.

As the majority of triflate **113** was recovered in the previous reaction, **113** was reacted with **69** in pyrrolidine at reflux (**Scheme 56**, Conditions B) in an attempt to increase the rate of oxidative addition of the triflate to the active catalytic palladium species. After 5 hours at reflux, TLC (40/60 EtOAc/hexanes) showed the absence of **113** at R_f 0.18, unreacted **69** at R_f 0.60, dimer **114** at R_f 0.33 and a new fluorescent spot at R_f 0.11. Separation by flash chromatography gave the coupled product **119** in a poor yield of 8% and the alkyne dimer in 39% yield (calculated from starting alkyne). Mass spectrometry (M^+ 689) and ^1H NMR confirmed the synthesis of **119**. Again, the balance of starting materials appeared to have decomposed. The reaction was repeated at room temperature in an attempt to lessen decomposition, however after overnight stirring **113** was completely consumed, and the only fluorescent spots were due to **69** and **114**. Evidently **113** was not stable under the reaction conditions.

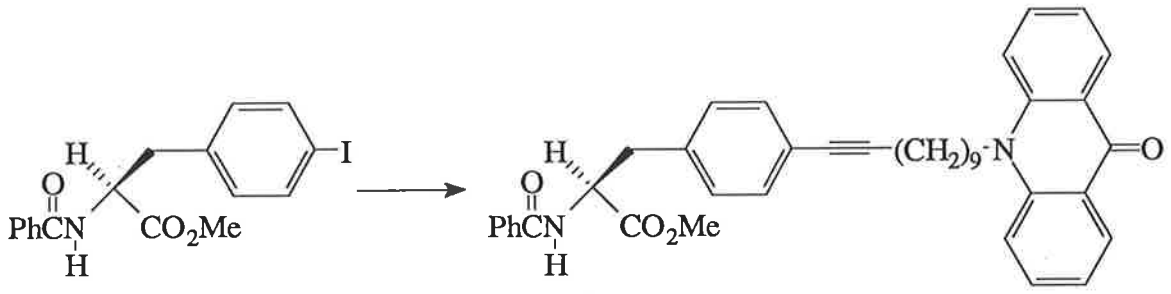
Given the success of the stoichiometric coupling of **112**, **113** was stirred with an equivalent of $Pd(PPh_3)_4$ in DMF (**Scheme 56**, Conditions C) at room temperature. TLC after 90 minutes showed no reaction, so the temperature was raised to 50°. A green precipitate slowly formed as the initial dark brown colour was discharged. After 4 hours, TLC showed the absence of **113** at R_f 0.81 and a large spot at R_f 0.14; a small amount of baseline material was also observed. As the σ-bonded intermediate had presumably formed, **69**, CuI and Et_3N were added, the green compound was consumed and the reaction mixture stirred for 60 minutes. TLC analysis of the reaction mixture showed the absence of the intermediate, a large amount of **114**, and no trace of product.

As reaction of **112** with the label occurred under standard conditions at higher temperature in DMF and DMSO, **113** was reacted with **69** in DMF at 70° (**Scheme 56**, Conditions A). TLC after 90 minutes showed starting materials, and a small spot possibly for

product. After overnight stirring TLC showed the absence of **113**, a large spot for the dimer **114** and a small fluorescent spot at low Rf. The desired product **119** was recovered in a poor yield of 21% after workup and chromatography (two passages through a silica column were required due to the large amount of decomposition). The label dimer **114** was not isolated.

It appeared unlikely that the desired product could be obtained in good yield without modification of **113** (possibly protection of the indole nitrogen) or the label (functionalisation of the alkyne to a more reactive group, such as a borane or stannane derivative). These were not considered, as it was desired to develop general reaction conditions which facilitated labelling of all selected biomolecules and labels. Hence attempted coupling reactions of **113** were discontinued, and reaction of the 4-iodophenylalanine derivative **108** with labels commenced.

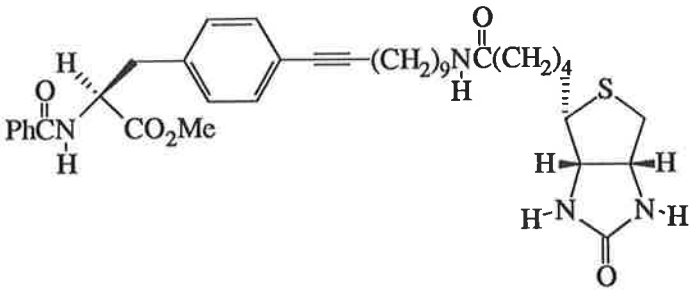
Reaction of **108** with the acridone label **69** proceeded at room temperature under standard conditions (**Scheme 57**). The reaction was left overnight for convenience; TLC (EtOAc/hexanes 50/50) the next morning showed the absence of **108** at R_f 0.65, major fluorescent spots at R_f 0.51 and 0.37 and a small fluorescent spot at R_f 0.77 corresponding to unreacted **69**. Separation by flash chromatography and ^{1}H NMR analysis showed the compound with R_f 0.51 was the alkyne dimer **114**; the compound with R_f 0.37 was the product **118**. Although the sizes of the spots on the chromatogram were similar, **118** was recovered in an excellent yield of 96% whereas **114** was recovered in only 3% (from conversion of starting alkyne).



108 **118**

69 (1.5 eq), $Pd(PPh_3)_4$ (0.1 eq), CuI (0.2), Et_3N, DMF: 96%.

Scheme 57.



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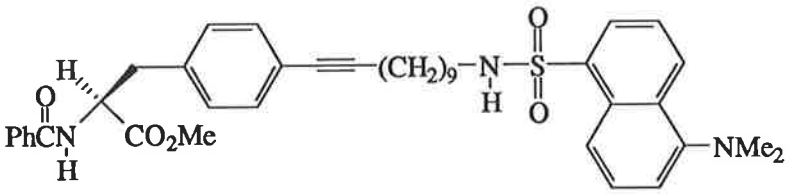

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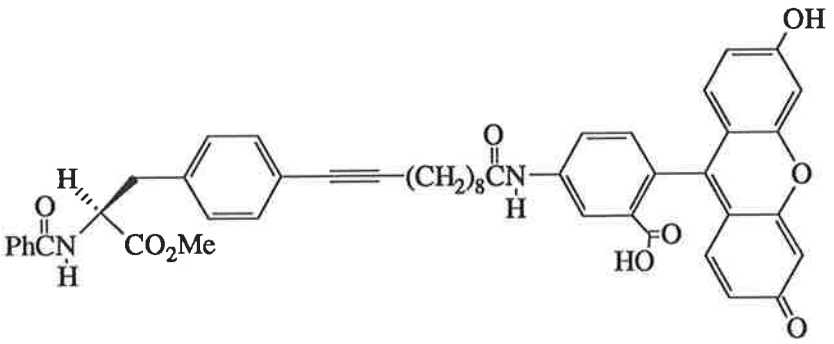

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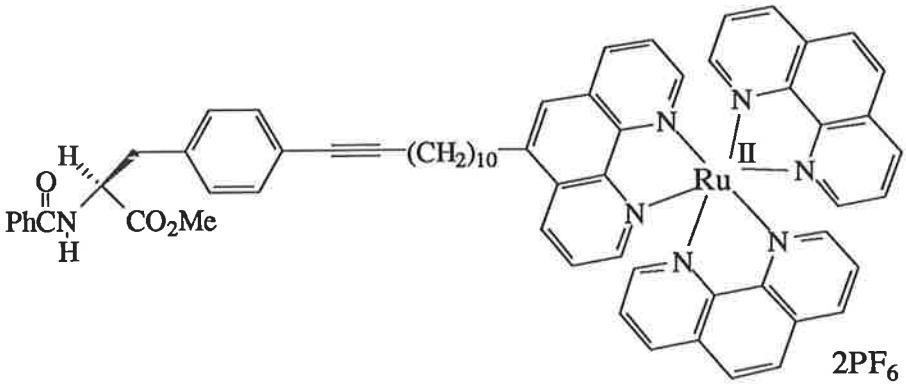
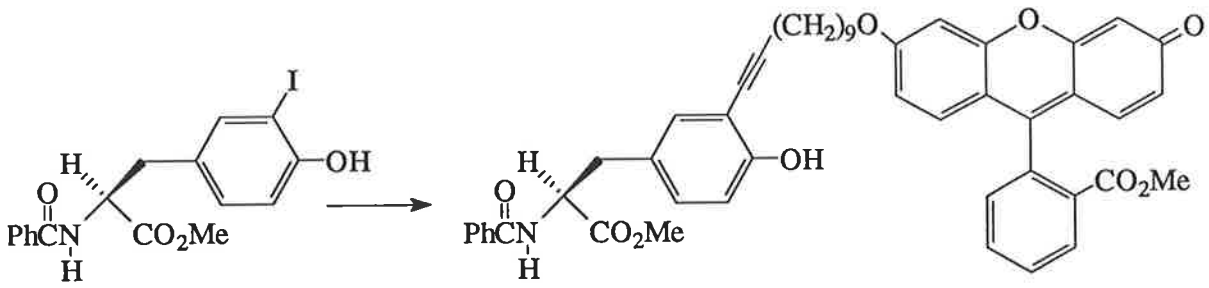


123

Reaction of **108** with the biotin **107**, dansyl **87**, aminofluorescein **84** and *tris*-1,10-phen- anthrolineruthenium **88** labels all proceeded under standard conditions at room temperature in less than four hours to give the coupled products **120** (in 84%), **121** (82%), **122** (75%) and **123** (65% yield) respectively. Analytical TLC showed the absence of **108** and new spots for the coupled products; the alkyne dimer was not observed in all cases.

Purification by flash chromatography gave the labelled products in good to excellent yields, and mass spectrometry and spectral data were consistent with the expected structures. As **108** proved to be a suitable substrate for coupling a wide range of labels with different functionalities, testing of the iodotyrosine derivative **109** was commenced.

Reaction of **109** with the fluorescein label **81** proceeded at room temperature under standard conditions (**Scheme 58**). The reaction was completed within 4 hours, as indicated by the absence of starting material by TLC (R_f 0.71, 10/90 MeOH/CH_2Cl_2) and a new major fluorescent (λ = 365 nm) spot at R_f 0.33. A small fluorescent spot presumably corresponding to the alkyne dimer was also observed at R_f 0.21 but not isolated. Purification by flash chromatography gave the labelled compound **124** in 91% yield. The identity of the product was confirmed by MS (M^+ 793) and the spectral data were consistent with the expected structures, in particular the appearance in the ^1H spectra of a triplet at δ 2.48 (2H, *J* 7.0 Hz) indicative of a methylene group next to an alkyne attached to a benzene ring.

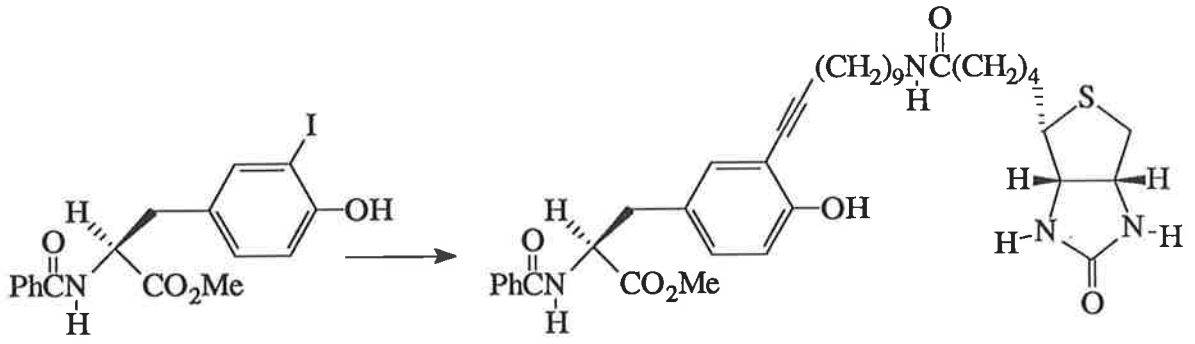


109 **124**

81 (1.2 eq), $Pd(PPh_3)_4$ (0.1 eq), CuI (0.2 eq), Et_3N, DMF: 91%.

Scheme 58.

Reaction of **109** with the biotin label **107** under standard conditions (**Scheme 59**) again proceeded at room temperature. The reaction was complete within 2 hours, TLC (10/90 MeOH/CH_2Cl_2) showing the absence of **109** at R_f 0.84, a major new spot at R_f 0.38 corresponding to product and a minor spot at R_f 0.15 presumably corresponding to the alkyne dimer. Purification by flash chromatography gave the labelled compound **125** in 76% yield. Coupling was confirmed by mass spectrometry (M^+ 690), and the spectral data were consistent with the expected structure.



109 **125**

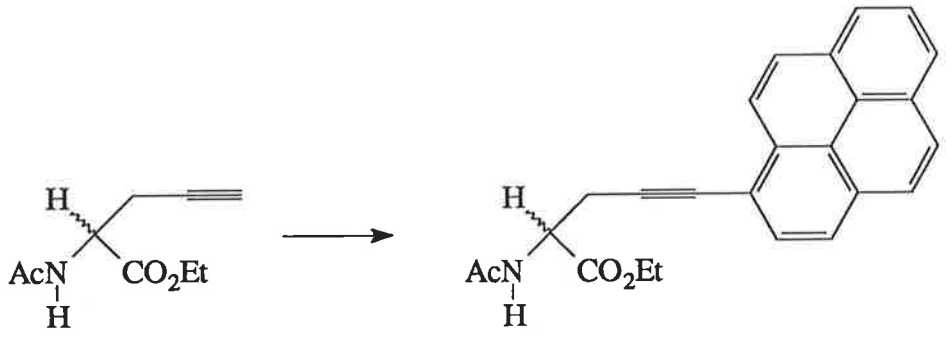
107 (1.2 eq), $Pd(PPh_3)_4$ (0.1 eq), CuI (0.2 eq), Et_3N, DMF: 76%.

Scheme 59.

With the successful labelling of **109**, all of the aromatic amino acids selected had been tested for labelling suitability under PdCC. With the exception of the tryptophan derivative **113** conditions were found where the substrates reacted readily with the labels, to give the adducts in good to excellent yields and under mild conditions. The remaining amino acid derivative to label was the propargyl glycine derivative **126**, and these reactions are described next.

Reaction of **126** with 1-bromopyrene **70** at 55° overnight under standard conditions (**Scheme 60**, Conditions A) gave the labelled compound **127** in 49% yield. TLC (60/40 EtOAc/hexanes) showed the absence of **126** at R_f 0.36, unreacted **70** at R_f 0.89, a new fluorescent spot at R_f 0.29 (corresponding to product) and a large non-UV active spot at R_f 0.08 (presumably the alkyne dimer **128**). Mass spectrometry (M^+ 383) confirmed the coupling and NMR spectra were consistent with the structure. As the yield was average, the reaction was repeated in refluxing piperidine (**Scheme 60**, Conditions B). TLC of the reaction mixture after 60 minutes at reflux showed the absence of **70** and a fluorescent spot for **127**. After chromatography **127** was recovered in 51% yield. As the yield was not significantly better, coupling of **126** to 1-iodopyrene was attempted under standard conditions (**Scheme 60**, Conditions A). The coupled product was obtained in a good yield of 72% after stirring at room temperature overnight. Repeating the coupling of 1-iodopyrene in refluxing

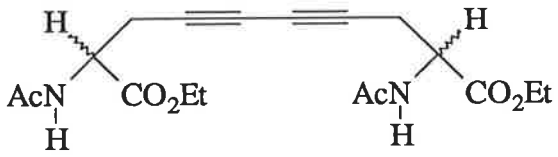
piperidine (**Scheme 60**, Conditions B) gave a yield of 70%.



126 **127**

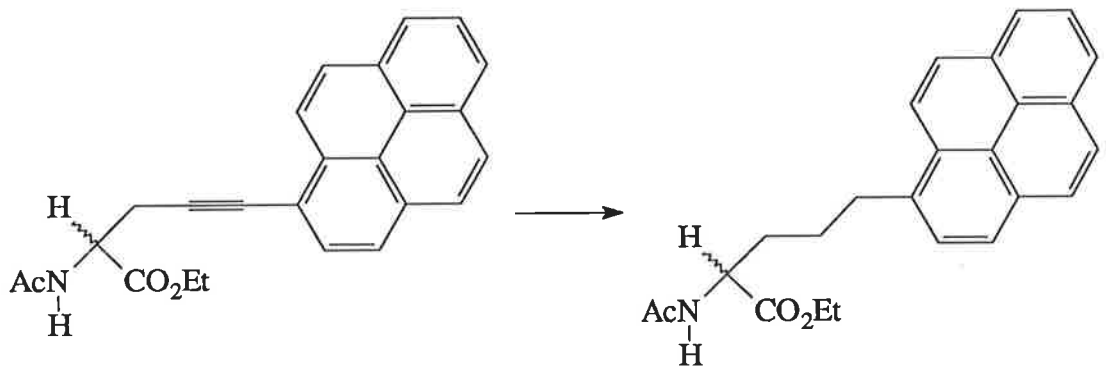
Conditions A: **70** (0.66 eq), $Pd(PPh_3)_4$ (0.1 eq), CuI (0.2 eq), Et_3N, DMF; 49%
Conditions B: **70** (0.66 eq), $Pd(PPh_3)_4$ (0.1 eq), CuI (0.2 eq), PPh_3 (0.2 eq), piperidine; 51%

Scheme 60.



128

Catalytic hydrogenation of **127** proceeded in EtOAc overnight in 94% yield (**Scheme 61**). Reduction to the saturated compound **129** was confirmed by MS (M^+ 387), the disappearance in the ^1H NMR of **127** of the resonance at δ 3.25 (*d*, 2H, *J* 4.7 Hz) corresponding to CH_2-C≡C and the appearance in the ^1H spectrum of **129** of resonances at 1.75-2.05 (*m*, 4H) and 3.26-3.42 (*m*, 2H).

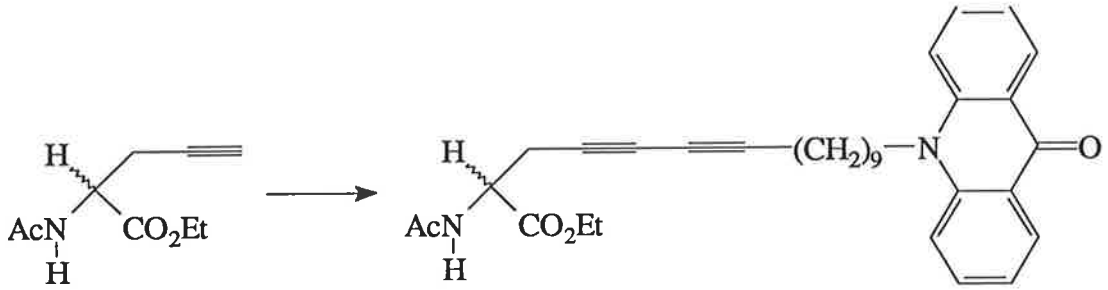


127 **129**

H_2, 5% Pd/C, EtOAc; 94%

Scheme 61.

As homocoupled alkyne dimers may be formed to a significant extent in PdCC reactions, the coupling of **126** to **69** was attempted in order to synthesise the cross-coupled product[96] **130 (Scheme 62)**. Subsequent hydrogenation of **130** would then give a labelled glycine derivative which incorporates a longer spacer unit. However TLC of the mixture after reaction under standard conditions at room temperature overnight showed spots for unreacted **69**, alkyne dimer **114** and (presumably) acid dimer **128**; no other fluorescent spot for **130** was found. This suggested that the kinetic acidity[97] of the alkyne proton on **127** was probably much greater than for **69** and that for successful cross-coupling[98] of the alkynes activation of one of the alkynes as a halogen or stannane[99] derivative would be necessary. As it appeared unlikely that other labels would give a different result, experimentation with **127** was concluded.

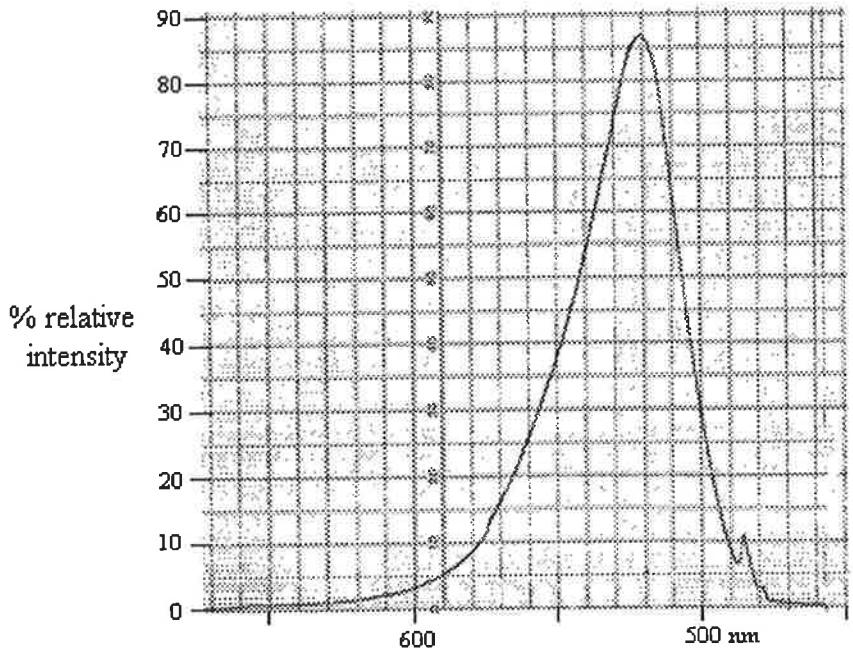


127 **130**

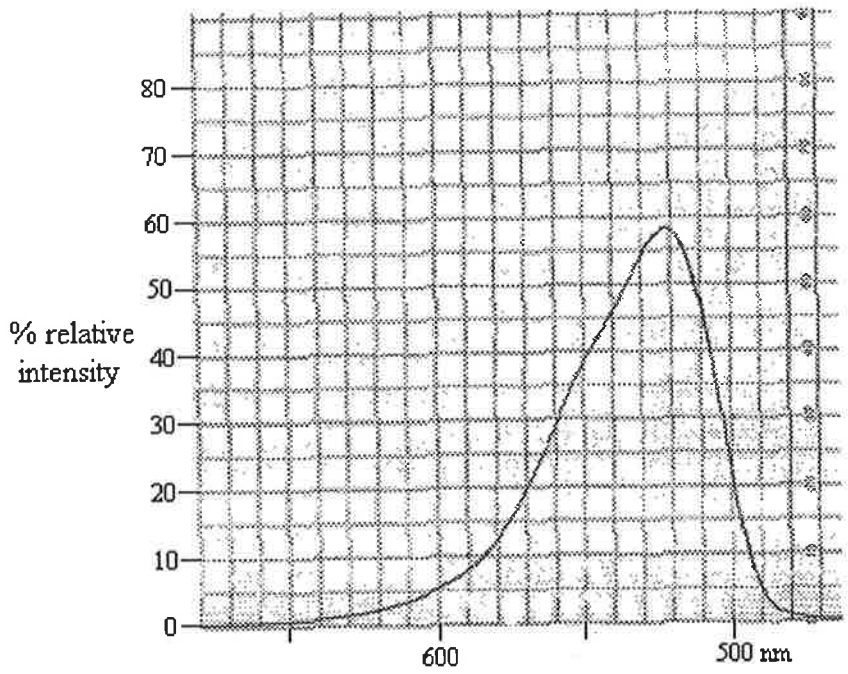
69 (1 eq), $Pd(PPh_3)_4$ (0.1 eq), CuI (0.2 eq), Et_3N, DMF.

Scheme 62.

It has been shown that the palladium catalysed cross-coupling reaction of terminal alkynes and aryl iodides/triflates is an efficient method for the preparation of labelled aromatic amino acid derivatives (except for the tryptophan derivative **113**). Also, the pyrene-glycine derivative **127** was prepared in good yield using this methodology. Fluorescence was not quenced upon coupling of the labels to the amino acids; a comparison of the fluorescein label **84** and the corresponding phenylalanine adduct **122** is shown in **Figure 4**. Such labelled amino acids (suitably protected) should be able to be used in the construction of modified small synthetic peptides (using both automated and manual methods) which display the advantages previously mentioned (*Introduction*, p 18).



(a) Compound **84** (c = 5.7 x 10^{-6} mol dm^{-3})



(b) Compound **122** (c = 6.9 x 10^{-6} mol dm^{-3})

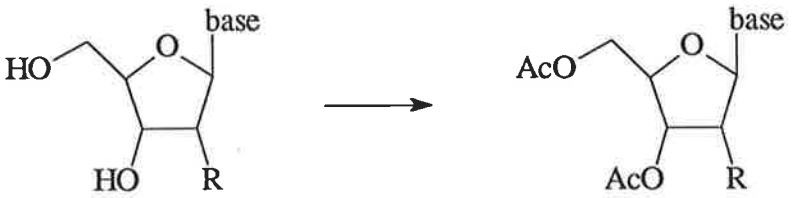
Fluorescence Spectra of Compounds **84** and **122** (ethanol solutions, λ_{ex} = 483 nm).

Figure 4.

Chapter 3.2. Coupling of Label-spacer Molecules to Nucleoside Derivatives

The palladium catalysed cross coupling reaction between 5-halo or 5-triflyluridine derivatives and terminal alkynes has been used to prepare 5-alkynyl substituted compounds, which may have antiviral or anticancer activity[51,92] and in the preparation of 5-propargylamine adducts, which were reacted with fluorescent labels[31]. Also, 8-alkynyl substituted adenosine and guanosine derivatives have been synthesised from the respective 8-bromo derivatives, which has allowed the preparation of compounds which have been tested for cytokinin activity[95] and A_1 adenosine receptor activity[52].

The reactions generally occured in high yields and under mild conditions; protection of the sugar hydroxyl groups, although not necessary for the coupling reaction, was generally used for chromatographic and characterisation purposes. Hence the nucleoside derivatives **51**, **52** and **53** selected for coupling reactions were protected as the di- or triacetate by reaction with acetic anhydride in pyridine (**Scheme 63**) to give the protected compounds **131**, **132** and **133** respectively. The melting points of the products were comparable with the literature, and FABMS, ^1H and ^{13}C spectroscopy were consistent with the proposed structures.

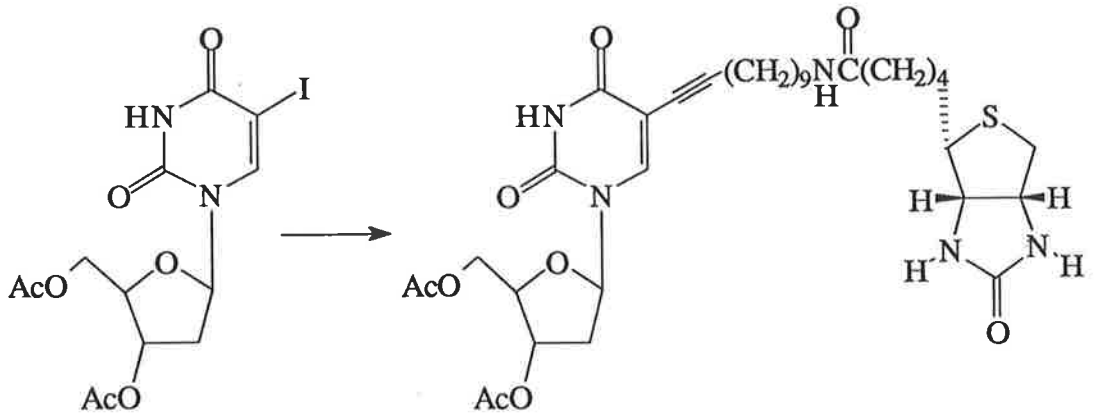


Base = 5-Iodouracil	**51**: R = H	2 eq Ac_2O	**131**: R = H
Base = 8-Bromoadenine	**52**: R = OH	3 eq Ac_2O	**132**: R = OAc
Base = 8-Bromoguanosine	**53**: R = OH	3 eq Ac_2O	**133**: R = OAc

Scheme 63. Ac_2O (2 or 3 eq), pyridine, 0° to RT overnight.

The reaction of protected 5-iododeoxyuridine **131** with the biotin label **107** was considered first. The production of cyclised isomer **135** (which previous reports have indicated is catalysed by copper salts[51b]) is a potential problem, however reaction in DMF has been shown to reduce the amount of cyclised isomer formed[100]. A mole ratio of 2:1 copper to

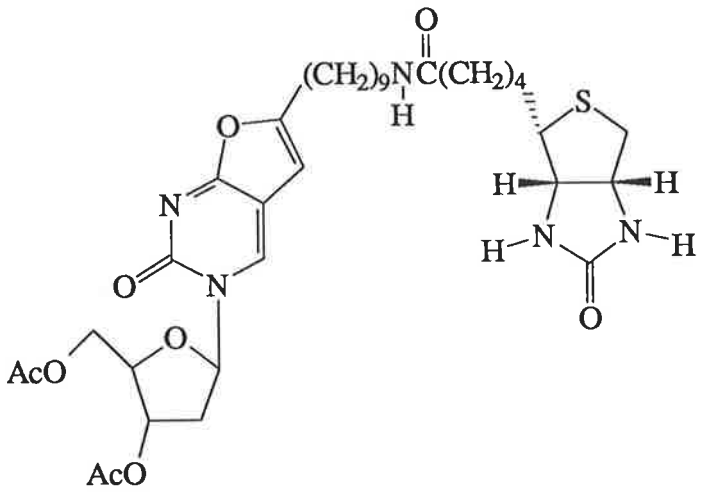
palladium has been shown to offer best coupling conditions with the minimal production of sideproducts[31c]. Hence the initial reaction conditions used for the couping of the biotin label **107** are shown in **Scheme 64** (Conditions A). TLC (10/90 $MeOH/CH_2Cl_2$) of the dark brown reaction mixture after stirring at room temperture for 3 hours showed the absence of **131** at R_f 0.64, and a new fluorescent spot (λ_{ex} = 365 nm) at R_f 0.20. After workup and chromatography, ^1H NMR showed the expected resonances for the product **134**; also, an unexpected low intensity resonance at δ 8.19 was observed. The resonance was assigned to the vinylic proton in the furan ring of the cyclised isomer **135**, and integration relative to C4 showed a ratio of **134** to **135** of approximately 4:1. Repeated column chromatography was unable to effect separation, and as reducing the amount of base from 2.5 to 1.2 eq has been shown to reduced the amount of cyclisation product[92] , the reaction was repeated under these modified conditions (**Scheme 64**, Conditions B). Interestingly, the reaction mixture turned a light yellow colour after the addition of the $Pd(PPh_3)_4$, and remained a light colour throughout the reaction. The adduct **134** was recovered in 86% yield, and no trace of fluorescent **135** was observed; by TLC, the spot corresponding to **134** was no longer fluorescent. Synthesis was confirmed by FABMS ($M+H^+$ 704); the ^1H and ^{13}C spectra were consistent with the proposed structure. Similarly, reaction of **131** with the fluorescein label **81** (**Scheme 65**) gave the adduct **136** in 76% yield, and there was no evidence for the formation of any cyclised byproduct. Coupling was confirmed by FABMS ($M+H^+$ 807), and the ^1H and ^{13}C NMR spectra were consistent with the proposed structure.



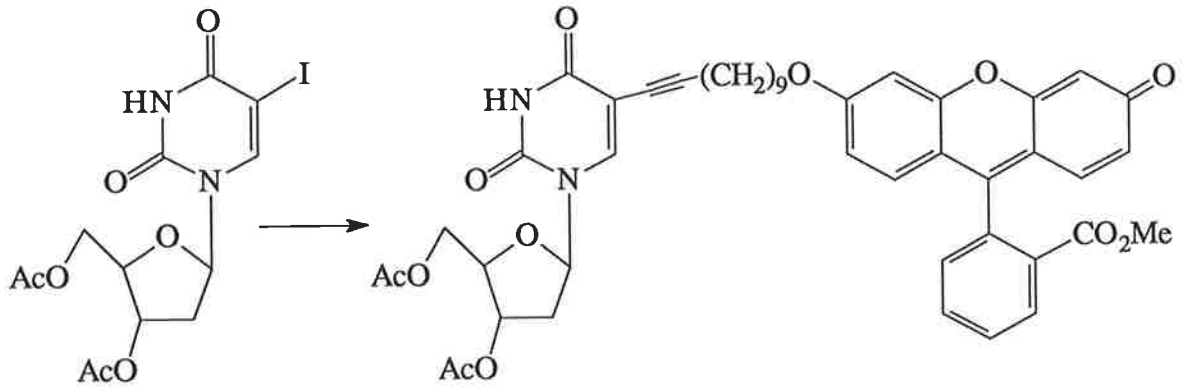
131 **134**

Conditions A: **107** 1.5 eq, $Pd(PPh_3)_4$ (0.1 eq), CuI (0.2 eq), Et_3N (2.5 eq), DMF, RT; 56%.
Conditions B: **107** 1.2 eq, $Pd(PPh_3)_4$ (0.1 eq), CuI (0.2 eq), Et_3N (1.2 eq), DMF, 40°; 86%.

Scheme 64.



135

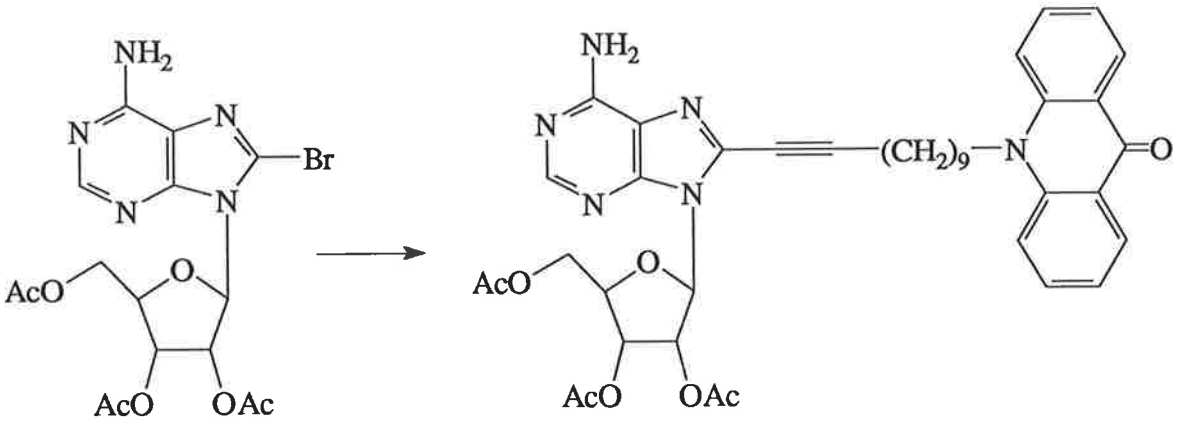


131 **136**

81 (1.2 eq), $Pd(PPh_3)_4$ (0.1 eq), CuI (0.2 eq), Et_3N (1.2 eq), DMF, 40°; 76%.

Scheme 65.

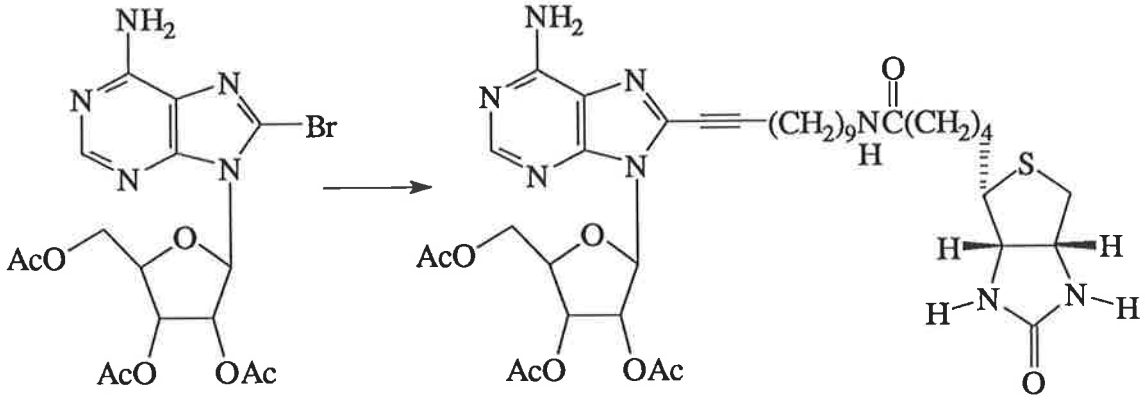
Coupling of protected 8-bromoadenosine **132** with the acridone label **69** (**Scheme 66**) was attempted next. TLC (EtOAc) of the reaction mixture after 24 hours stirring at room temperature showed the absence of **132** at R_f 0.42, a small amount of label at R_f 0.78 and a new spot corresponding to product at R_f 0.38. After workup the product **137** was recovered in 89% yield; coupling was confirmed by LSIMS ($M+H^+$ 737), and ^1H and ^{13}C NMR spectra were consistent with the expected structure. Similarly, reaction of **132** with the biotin label **107** under the same conditions (**Scheme 67**) gave the biotin adduct **138** in 88% yield. Synthesis was confirmed by LSIMS ($M+H^+$ 785), and ^1H and ^{13}C NMR spectra were consistent with the expected structures.



132 **137**

69 (1.5 eq), $Pd(PPh_3)_4$ (0.1 eq), CuI (0.2 eq), Et_3N (2.5 eq), DMF, RT, 24 hr: 89%.

Scheme 66.

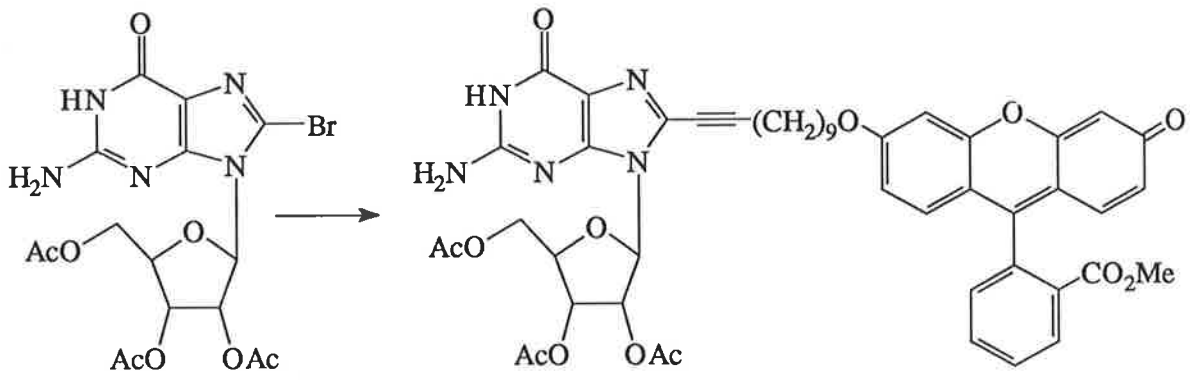


132 **138**

107 (1.2 eq), $Pd(PPh_3)_4$ (0.1 eq), CuI (0.2 eq), Et_3N (eq), DMF, 50°, ON: 88%.

Scheme 67.

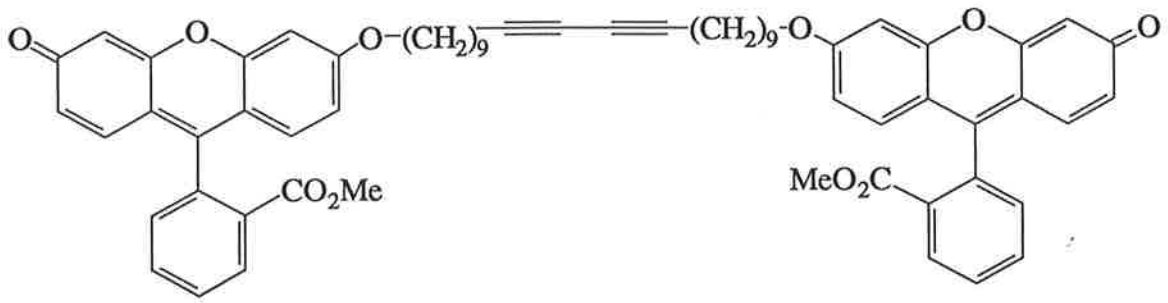
Guanosine derivative **133** was reacted with the fluorescein label **81** under standard conditions (**Scheme 68**). After 5 hours stirring, TLC (10/90 $MeOH/CH_2Cl_2$) showed yellow fluorescent spots at R_f 0.68, 0.27 and 0.17 corresponding to the label, homocoupled label dimer and product respectively, and the absence of a spot at R_f 0.22 corresponding to **133**. After removal of solvent, chromatographic separation of the residue gave the fluorescein dimer **140** in 12% yield (based on starting label), followed by the coupled product **139**, which was impure. A large amount of low R_f material was observed on the column, which failed to elute with a higher polarity solvent mixture. Upon repeating the chromatography, **139** was obtained in 51% yield as a bright orange glass. The low yield may be due to the partial complexation of the metal catalysts. Any such complex formed would be charged and hence presumably would bind strongly to silica; this may account for the majority of the mass balance of starting materials. Synthesis was confirmed by LSIMS ($M+H^+$ 904); ^1H and ^{13}C spectra were consistent with the coupled product **139**. Similarly, reaction of **133** with the biotin label **107** under the previous conditions (**Scheme 69**) and workup gave the labelled compound **141** in 58% yield. Synthesis was confirmed by LSIMS ($M+H^+$ 801) and ^1H and ^{13}C NMR.



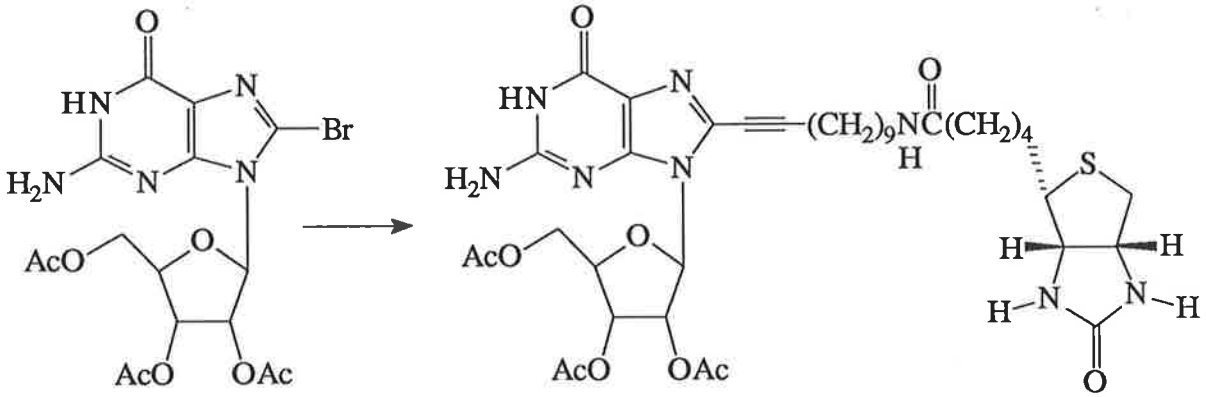
133 **139**

81 (1.5 eq), $Pd(PPh_3)_4$ (0.1 eq), CuI (0.2 eq), Et_3N (eq), DMF, 50°, 5 hours: 51%.

Scheme 68.



140



133 **141**

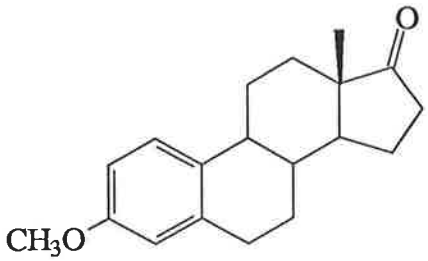
107 (1.5 eq), $Pd(PPh_3)_4$ (0.1 eq), CuI (0.2 eq), Et_3N (eq), DMF, 50°, 4 hours: 58%.

Scheme 69.

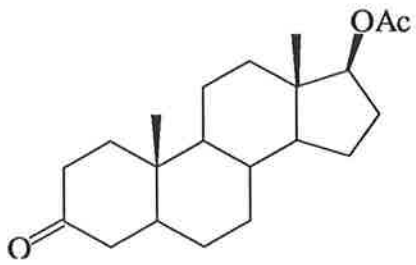
The results of these coupling reactions show that large label-spacer adducts may be coupled easily and in very good yields for the 5-iododeoxyuridine derivative **131** and 8-bromoadenosine derivative **132**; however the 8-bromoguanosine derivative **133** couples in only average yields. The preparation of labelled deoxynucleosides for incorporation into oligonucleotides via standard solid phase methodology should easily be achieved. This methodology may also be applicable to preparation of oligonucleotides which are functionalised with intercalating moities from a nucleobase[20].

Chapter 3.3. Coupling of Label-spacer Molecules to Steroid Derivatives

Steroids with carbonyl groups such as **142** and **143** are easily converted to triflate derivatives[101] and have been shown to undergo a variety of palladium catalysed processes[102]; the cross coupling with terminal alkynes has been well investigated[48,55]. As labelled steroidal derivatives have been used as probes in biological systems[37,103] (particularly in the study of membrane structures[36,38]), the possibility of coupling estrone triflate derivative **54** and epiandrosterone triflate derivative **55** with the developed pyrene labels **74** and **78**, and biotin label **107** was investigated.

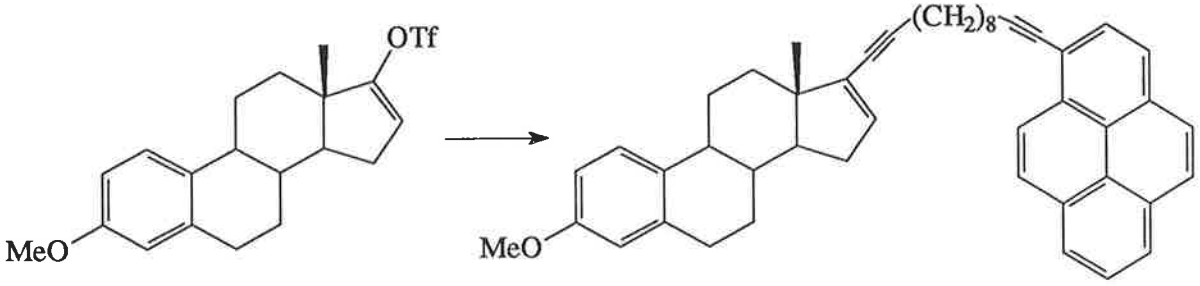


142



143

Reaction of **54** with pyrene label **74** under standard conditions (**Scheme 70**) proceeded readily. TLC (20/80 CH_2Cl_2/hexanes) of the reaction mixture after 3 hours stirring at room temperature showed a new fluorescent spot at R_f 0.31 corresponding to product, and the absence of the triflate at R_f 0.10. After workup and chromatography, the product **144** was recovered in 92% yield. MS showed a molecular ion at m/z 616 and ^1H and ^{13}C NMR spectra were consistent with the expected structure.

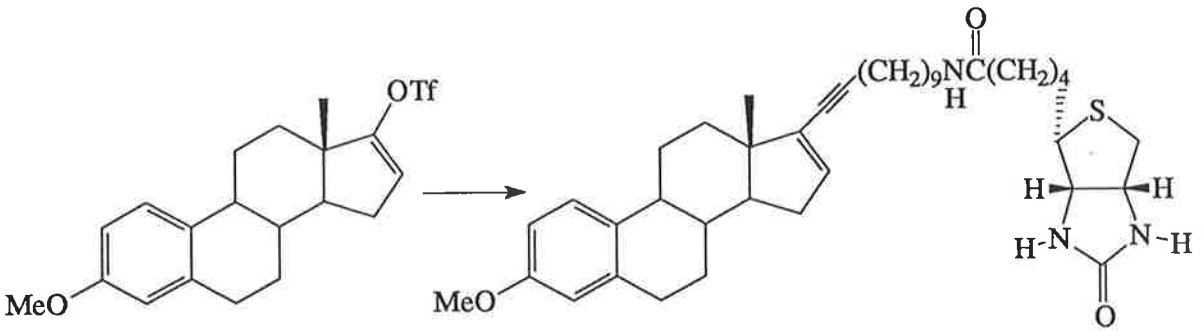


54 **144**

Conditions: **74** (1.2 eq), $Pd(PPh_3)_4$ (0.1 eq), CuI (0.2 eq), Et_3N, DMF, 3 hours RT; 92%.

Scheme 70.

Similarly, reaction of **54** with biotin label **107** under standard conditions (**Scheme 71**) gave the labelled product **145** in 89% yield. The reaction occurred in less than three hours, as indicated by the absence of starting triflate by TLC, and the spectral data (MS, ^{1}H, ^{13}C NMR) were in agreement with the expected structure.

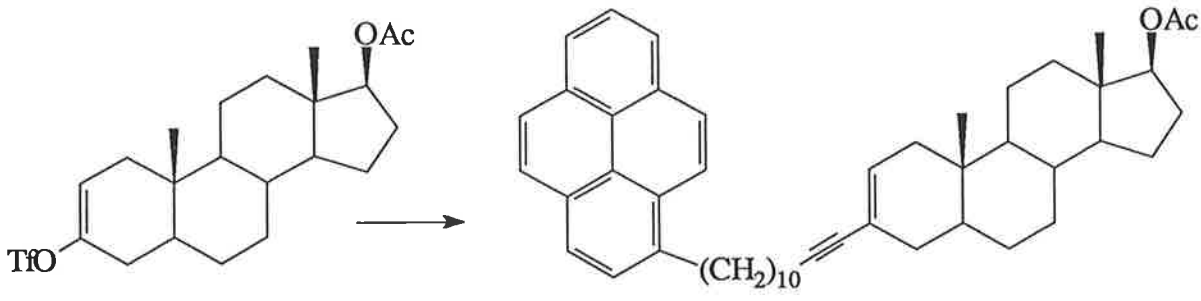


54 **145**

Conditions: **107** (1.2 eq), $Pd(PPh_3)_4$ (0.1 eq), CuI (0.2 eq), Et_3N, DMF, 4 hours RT; 89%.

Scheme 71.

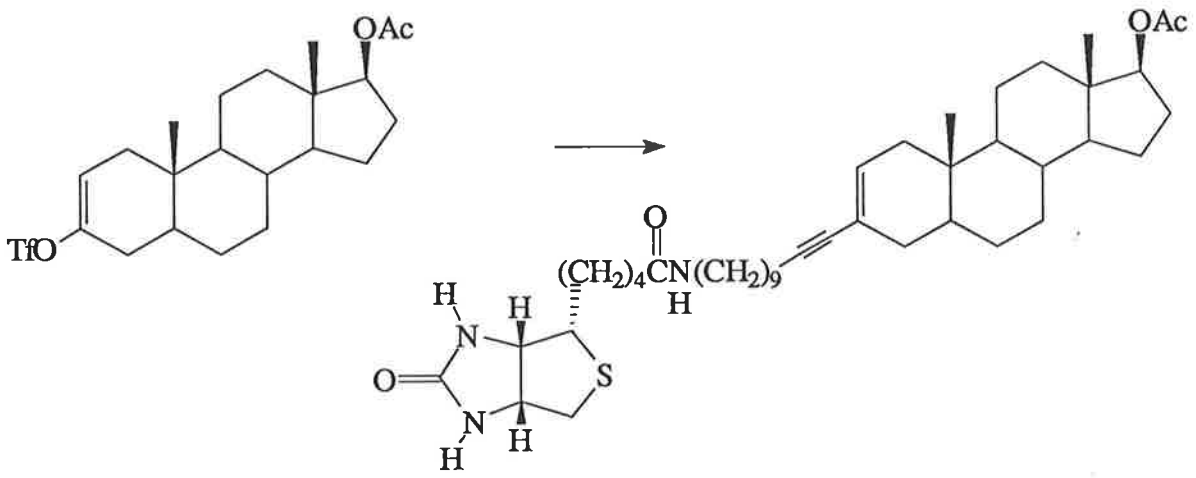
Reaction of androsterone derivative **55** with pyrene label **78** under standard conditions (**Scheme 72**) to give **146** occurred in 85% yield, and the spectral data were consistent with the proposed structure. Repeating the reaction with the biotin label **107** (**Scheme 73**) gave the adduct in 88% yield; again the spectral data were consistent with the expected product.



55 **146**

Conditions: **78** (1.2 eq), $Pd(PPh_3)_4$ (0.1 eq), CuI (0.2 eq), Et_3N, DMF, 4 hours RT; 85%.

Scheme 72.



55 **147**

Conditions: **107** (1.2 eq), $Pd(PPh_3)_4$ (0.1 eq), CuI (0.2 eq), Et_3N, DMF, 4 hours RT; 88%.

Scheme 73.

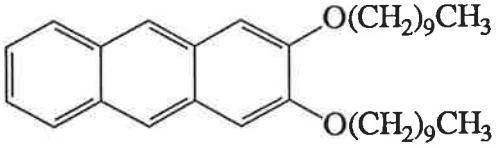
These results showed that the selected steroidal triflates may be labelled readily and in high yield using PdCC methodology. Other steroidal triflates have been shown to have similar reactivity[48,55,102], and so this may be a general method for the labelling of steroids which are functionalised with a carbonyl group. As a hydrocarbon spacer unit can be incorporated with the label, the lipophilicity of adducts may be enhanced, which could prove advantageous in some systems.

Chapter 4. Gelation of Organic Solvents by Biotin Amides and Esters.

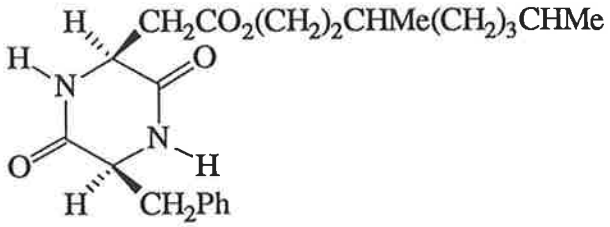
Gelation is a well known phenomenon[104]. It occurs when a liquid displays "solid-like" behaviour due to the presence of a small amount of solid forming a network which incorporates and retains the liquid (which is the major component). Defining a gel is difficult due to the diverse nature of systems which display gel-like behaviour, however the following criteria include almost all systems which are currently catagorised as gels[105]: (i) A gel consists of a three-dimensional network composed of basic elements connected in some way and swollen by a solvent; (ii) Gel formation or gel melting should proceed via a first order transition (which implies there exists a well-defined temperature (the gelation temperature, T_g) below which the solute-solvent mixture has solid behaviour; (iii) A gel immersed in an excess of preparation solvent should be unaffected or swell but not dissolve or disaggregate; (iv) A gel is a system which can be removed from the vessel in which it has been prepared without losing its shape or integrity.

The molecules of the network which entrap the solvent may be connected by covalent bonds (*chemical gels*) or by hydrogen bonding and van der Waals interactions (*physical gels*). When chemical gels are heated to the point that the covalent bonds break, irreversible degredation impedes the reformation of a similar system. In contrast, heating a physical gel disrupts the secondary bonds which can reform upon cooling, thus physical gels are also called *thermoreversible* gels. Physical gels of gelatin or other biopolymers in aqueous solution have been known for a long time, and it was believed that gelation occurred only for such systems. However the first thermoreversible gels formed in the organic solvents 2-butanone and cyclohexanone by poly(vinylchloride) polymers were reported in 1947[106]. Recently the gelation of organic solvents by low molecular weight compounds has been an active area of investigation. The recovery of spilled solvents, disposal of used cooking oil and use in drug delivery systems[107,108] have been suggested as possible applications. About 12 groups of compounds (not including biotin derivatives) are known to gelate organic solvents[108]. They can be divided into two classes; compounds which associate via hydrogen bonding and van der

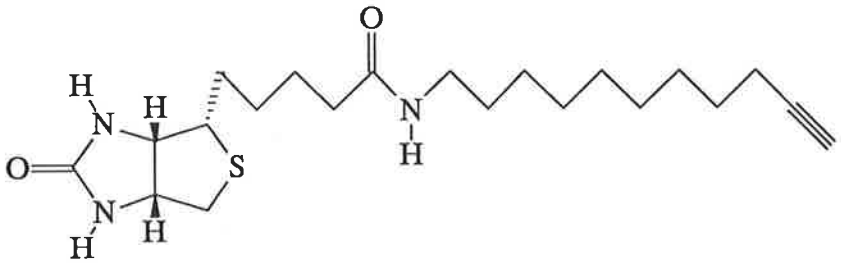
Waal interactions to form gels[109,110,111] (e.g. **149**), and compounds which form gels via van der Waal interactions only[112,113,114] (e.g. **107**).



148



149



107

The observation that the attempted recrystallisation of biotin-*N*-(11-undec-1-yn)-amide (**107**) from EtOAc gave rise to a gel was unexpected. Formation of the gel was achieved by heating **107** (about 150mg) in boiling EtOAc (about 35 ml). As the compound dissolved, the solution thickened markedly and upon being allowed to cool to room temperature gelled. The gel was transparent, stable to mechanical inversion and retained shape upon lifting with a spatula, although vigorous treatment caused degradation of the gel to solvent and crystals of **107**. Reheating the mixture reformed the gel, i.e. it was thermoreversible. Storage in a sealed vial prolonged the life of the gel; upon removal of the seal solvent was slowly lost, the gel shrank and a xerogel was formed. The gel did not show any structure when viewed under an optical microscope, and both the gel and xerogel did not transmit light when placed between crossed polarising filters (i.e. were not birefringent). Numerous other solvents were tested for gelation, and the results are summarized in **Table 3**. Toluene formed the most mechanically stable gel, and at the lowest concentration. The gel was unchanged even after it was left

uncovered in a fume cupboard for 30 days. Subsequent mechanical agitation of the gel resulted in the solvent being lost overnight.

Table 3. Concentration and Molar gelation ratios of **107** with several solvents.

Solvent	Concentration (gdm^{-3})	Molar ratio
Ethyl acetate	4.2	960
Benzene	3	1,470
Toluene	3.2	1,040
Chloroform	12	410

^1H NMR spectroscopic analysis of a series of increasing concentrations of **107** in $CDCl_3$ showed the chemical shifts of the amide protons moved markedly downfield, consistent with aggregation which may lead to formation of a hydrogen bonded network (**Table 4**). The spectra were relatively well resolved (**Figure 5a**) although as the concentration increased resonances were broadened slightly, and smaller poorly resolved resonances became apparent (**Figure 5b**), presumably due to the network . As the solvent was gelled at these concentrations, it was anticipated that a loss of resolution would have occurred[115]. The apparent resolution may be explained by considering that **107** exists in two distinct states[116]. The first is as monomeric or intermolecularly hydrogen bonded species in solution which rotate quickly enough to exhibit well resolved resonances, and the second is in the hydrogen bonded network whose resonances are broadened due to their restricted rotation. The spectra at higher concentrations are superpositions of the two states. Molecular modelling studies suggest that the energy minimised structure of a possible dimeric species, which is shown in **Figure 6**, has strong intermolecular and intramolecular hydrogen bonds, with an oxygen-hydrogen distance of about 2.1 Angstroms.

The relatively well resolved spectra in $CDCl_3$ is in contrast to the spectrum of a $[^2H_8]$-toluene gel which was poorly resolved at room temperature (**Figure 7a**). Increasing the temperature of the sample in 20° steps showed the resolution improved betweeen 80° and 100°, presumably when the gelation temperature (T_{gel}) was exceeded (**Figure 7b**). Increasing the

temperature of a 10mg/0.6ml gel in $CDCl_3$ showed large upfield changes in the chemical shifts (**Table 5**) and broadening of the resonances for the amide protons, consistent with disruption in the hydrogen bonding which exists between the presumed dimeric, trimeric etc. associations of molecules.

Table 4 Chemical shift of amide protons of **107** vs concentration.

Concentration[a]	N1'-H	N3'-H	CH_2NHCO
0.5mg	4.56	5.12	5.45
2.0mg	4.85	5.57	5.59
5.0mg[b]	5.13	5.95	5.73
10.0mg[b]	5.31	6.16	5.82
25.0mg[b]	5.35	6.19	5.84

(notes: [a]per 0.6ml $CDCl_3$; [b]gel formed)

Table 5 Chemical shift of amide protons of **107** (δ ppm) vs Temperature (K)

T (K)	N1'-H	N3'-H	CH_2NHCO
293	5.62	6.23	5.91
303	5.23	6.01	5.82
313	5.09	5.79	5.71
323	4.97	5.61	5.61

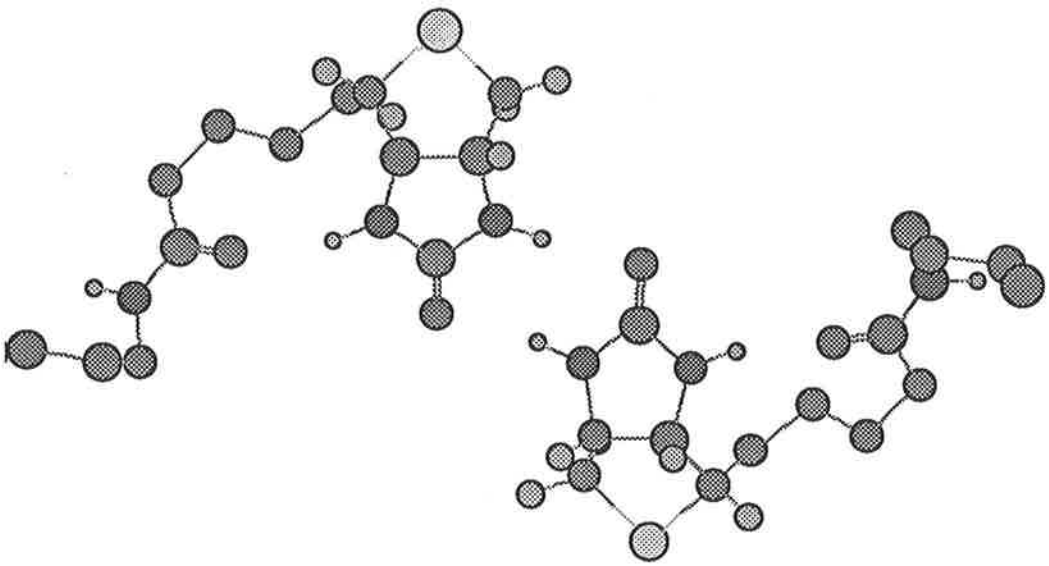
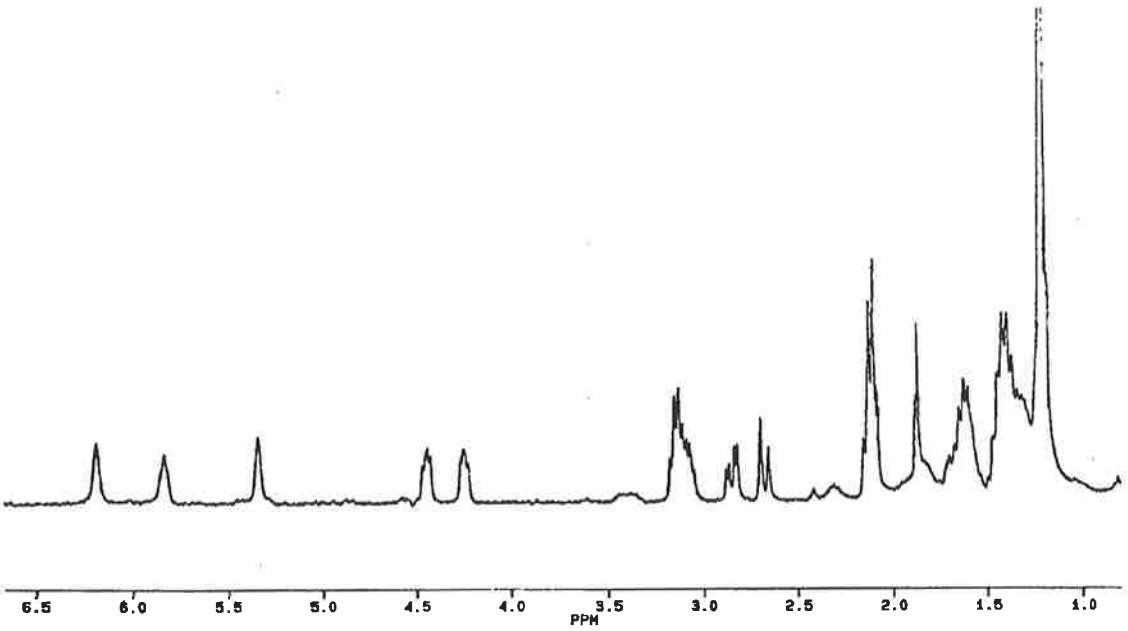
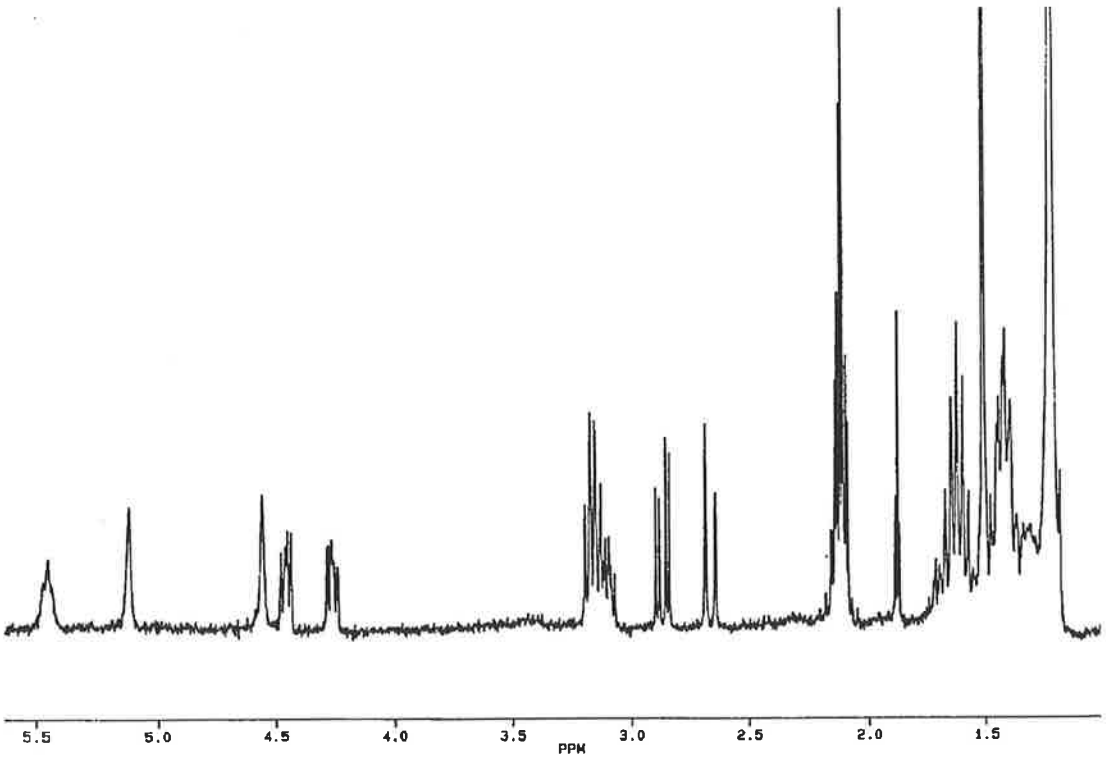


Figure 6. Possible Dimer formed by Biotin Amide derivatives.

[Side chain hydrogens (apart from amide proton) removed for clarity.]

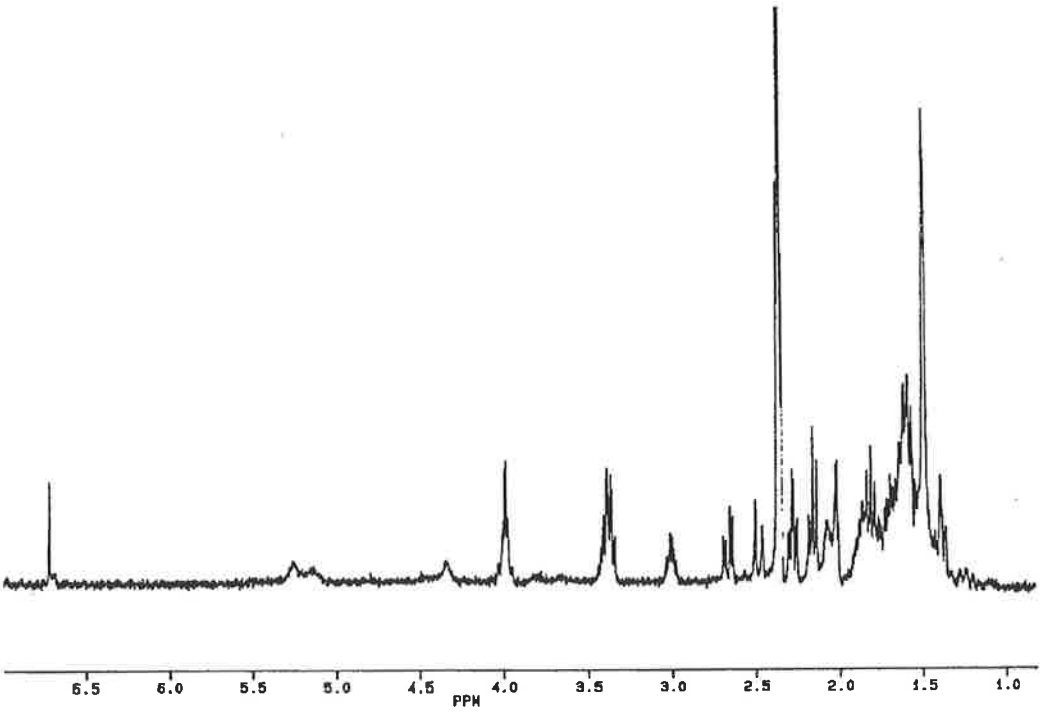


(b) 25mg / 0.6 ml

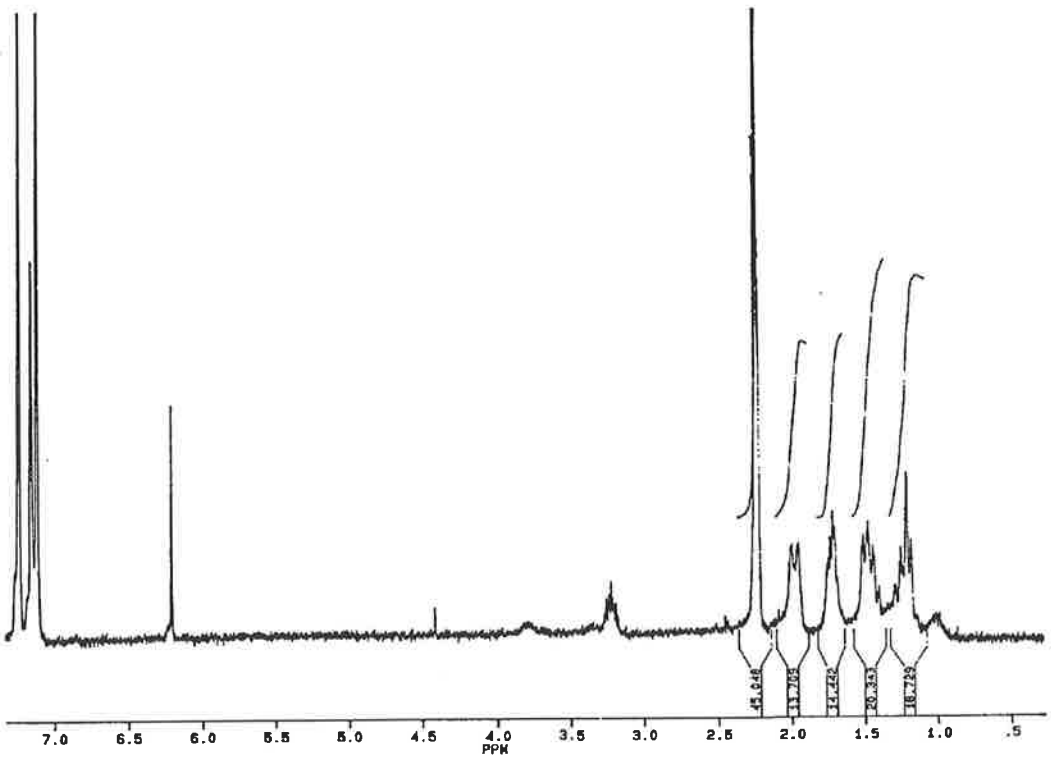


(a) 0.5mg / 0.6ml

Figure 5. ^{1}H NMR spectrum of compound **107** in $CDCl_3$ gel at 293K



(b) 373 K



(a) 293 K

Figure 7. ^{1}H NMR of compound **107** in $^{2}[H]_{8}$-toluene (10 mg / 0.6 ml)

Urea derivatives are well known hydrogen bond donor and acceptor moieties[117], and have often been used for molecular recognition purposes[118]. The self assembly of five-membered cyclic urea derivatives, in which the amide protons are constrained *syn* to the carbonyl group is not surprising given their symmetrical nature. Possibly an extended linear network (a "ribbon") composed of the 2-oxoimidazole rings intermolecularly hydrogen bonded is formed. A model on the molecular scale is shown in **Figure 8**, and energy minimised molecular modelling suggests the hydrogen bonding distance to be about 2.1 Angstroms. As gelation has been proposed to be a form of incomplete crystallisation[119], irregularity in the network will help stabilise the gel state. This is achieved as the tetrahydrothiophene ring may be incorporated above or below the plane of the hydrogen bonded network. The long alkyl chain solubilises the molecule in organic solvents, and enhances the gelation process by its conformational flexibility.

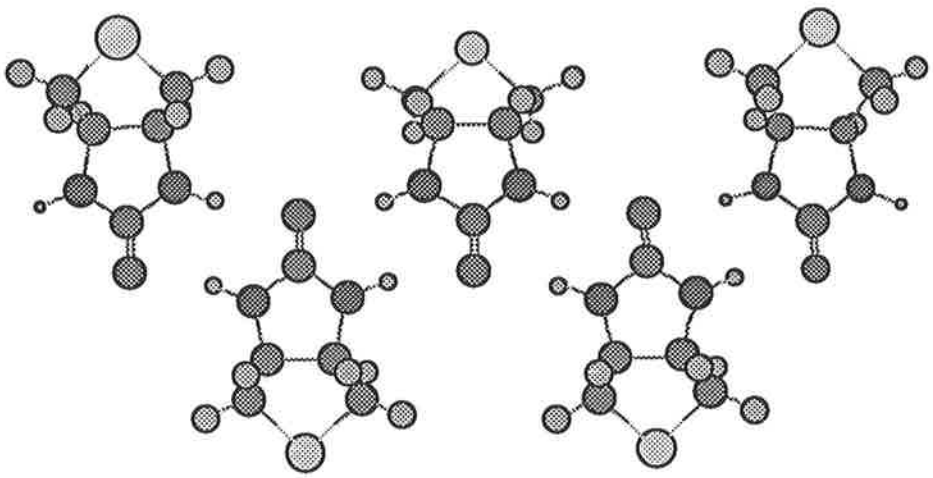
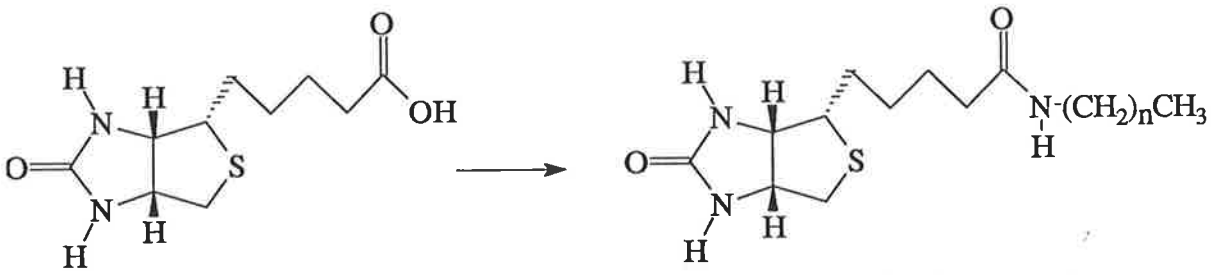


Figure 8. Possible hydrogen bonding network formed by the bicyclic rings of biotin.

The possible uses of compounds which gelate organic solvents may necessitate the inclusion of low polarity solvents such as aliphatic hydrocarbons. The amide **107** was not soluble in hexane, however it was realised that a more lipophilic analogue may be and could possibly form gels. The most obvious modifications would be to either lengthen the alkyl chain of the amide, connect the alkyl moiety via an ester, or both. Hence a series of biotin amides and biotin esters with varying alkyl chain lengths (*n*-propyl, *n*-hexyl, *n*-octyl, *n*-undecyl,

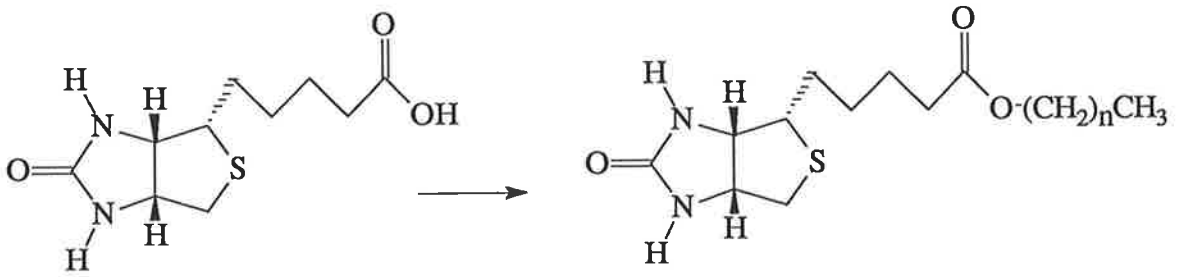
n-dodecyl, *n*-hexadecyl) were synthesised in order to determine the scope of gelation of solvents by biotin derivatives. Reaction of biotin NHS ester **106** with the amine in DMF (**Scheme 74**) gave the amides in good yields. The reactions were generally over (as indicated by absence of **106** by TLC) within 2 hours. Removal of the precipitated NHS by filtration, removal of the solvent *in vacuo*, chromatography and recrystallisation gave the amides as colourless amorphous solids. Biotin esters were synthesised by reaction of biotin (**41**) with 5 equivalents of alcohol in refluxing toluene, catalysed by *p*-toluenesulphonic acid (**Scheme 75**). Heating at reflux for 48 hours, removal of the solvent *in vacuo*, filtration to remove unreacted biotin, and chromatography gave the esters in poor to excellent yields. The propyl ester **161** was prepared in a similar manner, however the reaction solvent was propan-1-ol and the period of reflux was 6 hours (**Scheme 76**). The undec-10-yn-1-ol ester **162** was prepared by reaction of **106** with the alcohol **59** in DMF, catalysed by DMAP (**Scheme 77**). The products were recovered after chromatography generally as colourless amorphous solids. Gelation tests were performed by placing a weighed amount of compound (15-20mg) in a preweighed vial and heating with the solvent to be tested. After dissolution (if not insoluble) and cooling at room temperature for 60 minutes the outcome of the experiment was noted. A gel was considered to have formed if a transparent mass which did not flow upon inversion of the vial resulted. The total mass of the vial and solvent was determined and the concentration of gelator calculated. Gelation was generally complete by the time room temperature was reached; more vigorous attempts to achieve gelation such as cooling the gel in cold water or a refrigerator[113b] were not attempted. If the compound was soluble at room temperature, the amount of solvent was reduced by evaporation and the concentrated solution studied for gelation as above. The results of the gelation tests are shown in **Table 6** for the amides and **Table 7** for the esters.



(i) NHS (1 eq), DCC (1.1 eq), DMF, 80° to RT, 2 hours. (ii) $CH_3(CH_2)_nNH_2$.

Scheme 74.

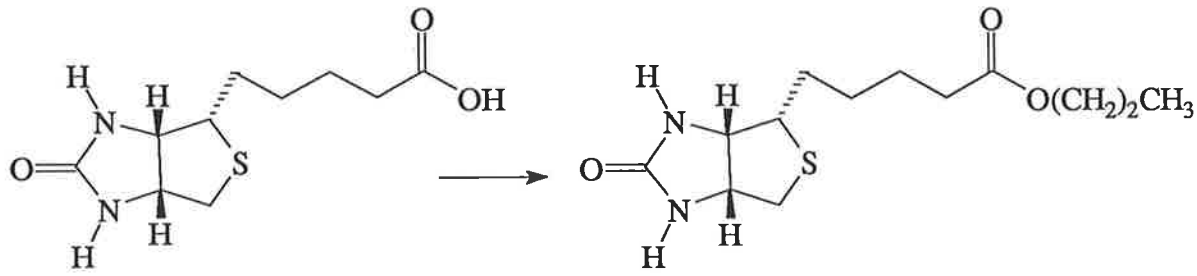
n	compound	yield %
15	**150**	83
11	**151**	71
10	**152**	56
7	**153**	67
5	**154**	66
2	**155**	73



$CH_3(CH_2)_nOH$ (5 eq), *p*-TsOH (0.1 eq), toluene, reflux 48 hours.

Scheme 75.

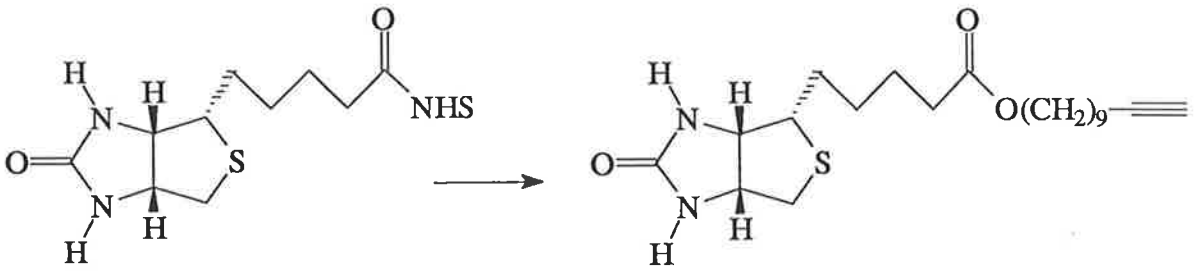
n	compound	yield %
15	**156**	38
11	**157**	80
10	**158**	79
7	**159**	98
5	**160**	81



41 **161**

$CH_3(CH_2)_2OH$, *p*-TsOH (0.1 eq), reflux 6 hours, 62%.

Scheme 76.



41 **162**

Compound **59**, DMAP (1 eq), DMF, 46%.

Scheme 77.

The results of the gelation tests showed (i) the optimal length of the alkyl substituent is between 8 and 12 carbons (*n*-octyl to *n*-dodecyl), i.e. an overall (including pentanoic chain of biotin and heteroatom) length of 14 to 18 atoms; (ii) esters are less polar than amides, and the *n*-dodecyl, *n*-undecyl and *n*-octyl esters (**157**, **158** and **159** respectively) gel hexane when predissolved in CH_2Cl_2; (iii) the octyl ester **158** gelled light paraffin oil when predissolved in CH_2Cl_2, however crystallisation occurred after 14 days; (iv) a terminal alkyne is important in enhancing the gelation process. Terminal alkynes are known to undergo weak hydrogen bonding[120], however the chemical shift of the acetylenic proton showed no significant change in either the variable concentration or variable temperature experiments. How the alkyne enhances the gelation process is unclear. As the undecylamide is not such an efficient gelator this shows how a small difference in structure leads to a large difference in gelator behaviour.

Further work is required to determine the structure of the hydrogen bonded network on both the molecular and macroscopic scales for this new class of thermoreversible gelator compounds. The compounds are readily synthesised, gel at low concentrations, may form very stable gels and can be designed to gel hexane. Analogues incorporating branched alkyl chains, dialkylamines, terminal alkynes and/or various alkyl lengths or other features which may give rise to enhanced gelation should be easily synthesised.

Table 6: Gelation of Solvents by Biotin Amide Derivatives

Solvent	(16)	(12)	(11)	(8)	(6)	(3)	(undecyn)
CCl_4	s	c	c	c	i	i	i
$CHCl_3$	s	18	21	22	c	c	12
CH_2Cl_2	i	c	c	c	i	i	i
EtOAc	i	c	c	c	c	c	4.2
hexanes	i	i	i	i	i	i	i
toluene	s	c	c	c	c	i	3.2
acetonitrile	c	c	c	c	c	c	c
ether	s	i	i	i	i	i	i
MeOH	c	c	c	s	s	c	c
acetone	i	c	c	c	c	c	c
benzene							3

(*i* insoluble, *c* crystallise, *s* soluble, number = conc (gdm^{-3}) of gel)

Table 7: Gelation of Organic Solvents by Biotin Esters

Solvents	(16)	(12)	(11)	(8)	(6)	(3)	undecyn
CCl_4	c	s	s	43	9	17	s
$CHCl_3$	s	s	s	s	s	s	s
CH_2Cl_2	s	s	s	s	s	s	s
EtOAc	c	s	s	s	s	s	s
hexanes	i	c	c	i	i	i	i
toluene	c	s	s	s	s	s	s
acetonitrile	c	c	s	s	s	s	s
ether	i	c	i	i	i	i	i
MeOH	s	s	s	s	s	s	s
acetone	c	s	s	s	s	s	s
CH_2Cl_2/hex	c	5.3	c	9.9	c	c	*
paraffin				2.4			

*formed a transparent polymeric mass

(*i* insoluble, *c* crystallise, *s* soluble, number = conc (gdm^{-3}) of gel)

Chapter 5. Summary

The synthesis of novel label-spacer molecules in which a hydrocarbon chain is attached to a reporter moiety (acridone, biotin, dansyl, fluorescein, pyrene, and tris(1,10-phenanthroline)ruthenium) at one terminus, and is functionalised at the other terminus with an alkyne, has been achieved. The palladium catalysed cross coupling reaction between aryl/vinyl halides/triflates and terminal alkynes has proved to be an efficient method for the introduction of the label-spacer molecules to halogenated and triflated derivatives of biomolecules (amino acids (apart from tryptophan), nucleosides and steroids).

The biotin label **107** was found to gelate benzene, chloroform, ethyl acetate and toluene. Variable temperature ^{1}H NMR spectroscopy of gels in $CDCl_3$ and $^{2}[H]_8$-toluene suggested the formation of a polymeric hydrogen bonded network, presumably formed by the cyclic urea moiety of **107**, which entraps solvent. Some *n*-alkyl biotin ester and amide derivatives were found to form gels in hexane and paraffin oil at low concentrations.

Experimental

All reactions were routinely performed in oven dried glassware under a nitrogen atmosphere (unless in aqeuous solution), and palladium catalysed cross coupling reactions were performed in Schlenk glassware. 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. $CDCl_3$ was used as a solvent unless otherwise stated, with tetramethylsilane used 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. Accurate mass determinations using EI or Liquid Secondary Ion MS (LSIMS) were made by the Organic Mass Spectrometry Facility at the University of Tasmania, or using EI at the Department of Chemistry, University of Melbourne. 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 and data processor.

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. Other regents were purified according to according to literature procedures[121].

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[122] was carried using Merck kieselgel 60 (230-400 mesh) or (when stated) on alumina UG, and solvents used were distilled before use.

Compounds synthesised according to literature procedures: 1-bromopyrene[123], 1-iodopyrene[124], *N*-phenyltriflimide[125], *bis*-1,10-phenanthrolineruthenium(II)dichloride[126],

tetrakis(triphenylphosphine)palladium[127], estrone triflate[102a], androsterone triflate[102a]. A sample of dimethyl-(1-diazo-2-oxopropyl)phosphonate[86] was a gift from Mr. C. de Savi.

The following abbreviations have been used in defining peak shape for the various spectra: ^{1}H and ^{13}C NMR; *b* = broad, *d* = doublet, *m* = multiplet, *q* = quartet, *s* = singlet, *t* = triplet. IR: *b* = broad, *m* = medium, *s* = strong, *w* = weak; UV and fluorescence spectra; *sh* = shoulder. Unless otherwise stated $J = {}^3J$ for ^{1}H NMR. The numbering on structures is for NMR resonance assignment only. Mass spectral data are reported: m/z ratio (% relative abundance). UV data are reported: λ_{max} (ε). Fluorescence data are reported: λ_{max} (% relative intensity).

Due to the lack of reproducibility with microanalytical data the labelled biomolecules were characterised by ^{1}H and ^{13}C NMR, with high resolution mass spectroscopy (EI and LSIMS) confirming the molecular formulae.

Experimental Described in Chapter 2.1.

Undec-10-ynoic acid (57)

Redistilled 10-undecenoic acid (108g, 0.59 mol) was dissolved in dry CCl_4 (300ml) and cooled in an ice/methanol bath to approximately -10°. Bromine (96g, 31ml, 0.60 mol) was added dropwise over 90 minutes, the cooling bath removed, the reaction mixture allowed to come to room temperature and the solvent removed. A solution of KOH (272g, 4.85 mol) in water (160ml) was added and the mixture stirred at 125° (oil bath temperature) for 9 hours. After cooling to room temperature, the mixture was poured into water (1500ml), acidified to pH 1 with 10% H_2SO_4 (approximately 500ml) and extracted with CH_2Cl_2 (4 x 250ml). The organic extracts were combined, washed with saturated brine (500ml), dried (Na_2SO_4) and the solvent removed. The residue was distilled through a 150mm Vigreaux column, the fraction boiling between 128-136° at 0.02mmHg being collected. Recrystallisation from hexanes gave the title compound as colourless crystals mp 43° (lit[56]. 43°) in 18.1g (17%) yield.

IR (film): 3350-2500*br* (O-H), 3300*s* (≡C-H), 2140*w* (C≡C), 1720*s* (C=O). ^{1}H NMR: 1.33-1.67, *m*, 16H, methylene protons; 1.94 (*t*, 1H, *J* 2.6 Hz, ≡C-H); 2.18 (*dt*, 2H, *J* 7.0, 2.6 Hz, CH_2-C≡); 2.35 (*t*, 2H, *J* 7.3 Hz, CH_2-CO_2H); 9.95 (*bs*, 1H, COOH). ^{13}C NMR: 18.8, 25.1, 29.1, 29.2, 29.3, 29.4, 29.6, 34.6 (alkyl), 68.6, 85.2 (alkynyl), 180.7 (CO_2H).

Undec-10-yn-1-ol (59)

A solution of undec-10-ynoic acid (**57**) (12.0g, 66 mmol) in Et_2O (100ml) was added dropwise to a stirred suspension of $LiAlH_4$ (2.50g, 66 mmol) in Et_2O (150ml). The mixture was heated to reflux overnight. After cooling to room temperature, the reaction mixture was hydrolysed by the dropwise addition of water (2.7ml), 15% NaOH (2.7ml) and water (2.7ml). After filtration to remove the lithium salts, the solvent was removed and the residue distilled, bp 75-76° at 0.02mmHg (lit[128]. 111-112° at 4mmHg) to give the title compound as a colourless oil in 9.60g (87%) yield. IR (film): 3400-3100*br* (O-H), 3304*s* (H-C≡), 2924*s*, 2852*s*, 2110*w* (C≡C), 1466*m*, 1058*s*. ^{1}H NMR: 1.29-1.59, (*m*, 14H, methylene protons); 1.93 (*t*, 1H, *J* 2.6 Hz, ≡C-H); 2.17 (*dt*, 2H, *J* 7.0, 2.6 Hz, CH_2-C≡); 3.62 (*t*, 2H, *J* 6.6 Hz, CH_2-OH). ^{13}C NMR: 20.4, 27.7, 30.4, 30.7, 31.0, 31.3, 31.4, 31.5, 34.7 (alkyl); 70.1, 86.7 (alkynyl).

Undec-10-yn-1-*O*-*p*-toluenesulphonate (60)

To a stirred solution of *p*-toluenesulphonyl chloride (11.33g, 59.4mmol, 2 eq) in pyridine (75 ml) at 0° (icebath), was added dropwise a solution of the alcohol **59** (5.00g, 29.7 mmol) in pyridine (25 ml) over 60 minutes. Stirring was continued as the ice bath warmed to room temperature overnight, then the reaction mixture was poured into water (100ml) and extracted with Et_2O (4 x 50ml). The organic extracts were combined, washed with 10% HCl (50ml), water (50ml), dried (Na_2SO_4) and the solvent removed to give the crude product in 9.02g (94%) yield. The crude product was converted immediately to the iodide as described below.

Undec-10-yn-1-*O*-methanesulphonate (61): Method A.

Alcohol **59** (5.00g, 29.7 mmol) was dissolved in pyridine (20ml) and cooled in an ice bath. Methanesulphonyl chloride (3.40g, 29.7 mmol) was added dropwise over 30 mins and the mixture stirred for 4 hours. The mixture was poured into water (100ml), the organic layer separated, diluted with CH_2Cl_2 (50ml), and washed successively with 10% aqueous HCl (3 x 50ml), water (50ml), brine (50ml), dried over Na_2SO_4 and the solvent was removed to give the crude title compound in 6.82g (93%) yield. IR (film): 3292*s* (H-C≡), 2928*s*, 2852*s*, 2116*w* (C≡C), 1468*m*, 1354*s*, 1176*s*. ^{1}H NMR: 1.30-1.41 (*m*, 14H, methylene protons); 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* 6.9, 2.6 Hz, CH_2-C≡); 3.01 (*s*, 3H, CH_3-SO_3); 4.22 (*t*, 2H, *J* 6.6 Hz, CH_2-OMs.)

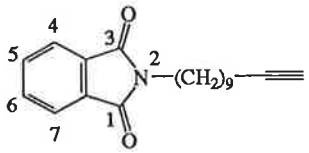
Undec-10-yn-1-*O*-methanesulphonate (61): Method B.

To a stirred mixture of **59** (5.00g, 30 mmol) and Et_3N (3.01g, 30 mmol, 1 eq) in CH_2Cl_2 (50ml) was added dropwise methanesulphonyl chloride (3.41g, 30mmol, 1 eq). 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 product was recovered as a pale yellow oil in 6.42g (88%) yield, with ^{1}H and IR spectral data as before.

11-Iodoundec-1-yne[129] (62).

The crude tosylate (7.70g, 24 mmol) or mesylate (6.00g, 24 mmol) was dissolved in dry MEK (100ml), anhydrous NaI (30.0g, 200 mmol) was added, the mixture refluxed 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 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^+, 100), 151 (M^+- I, 30). IR (thin film): 3300s (H-C≡), 2924, 2852, 2116 (C≡C), 1464, 1432, 1220, 1178, 720. ^{1}H NMR: 1.25-1.61 (*m*, 12H, methylene protons); 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* 6.9, 2.6 Hz, CH_2-C≡); 3.19 (*t*, *J* 7.0 Hz, 2H, CH_2-I). ^{13}C NMR: 7.18 (CH_2-I), 18.32, 28.37, 28.40, 28.59, 29.19, 29.22, 30.39, 33.46 (alkyl), 68.06, 84.50 (alkyne).

***N*-(Undec-10-yn-1-yl)phthalimide (64):** Method A.



To a stirred solution of phthalimide (265mg, 1.80 mmol) in DMF (10ml) was added NaH (60mg, 80% suspension in oil, 1.98 mmol, 1.1eq). The mixture was stirred for 15 minutes then 1-iodoundec-10-yne (**62**) (500mg, 1.80 mmol) was added and stirring continued for 3 hours at 100°. After cooling to room temperature the mixture was poured into water (100ml), the aqueous mixture extracted with CH_2Cl_2 (3 x 30ml), the extracts combined, washed with water (50ml), dried (Na_2SO_4) and solvent removed. Residual DMF was removed under vacuum (oil pump). The residue was separated by flash chromatography, eluant 15/85 EtOAc/hexanes to give the product as a colourless oil which slowly solidified to white crystals mp 45-48° in 221mg (41%) yield. HRMS Calculated for $C_{19}H_{23}NO_2$: 297.1729. Found: 297.1724. MS: 297 (M^+, 42), 149 (100). IR: 3284*s* (H-C≡), 3056*w* (Ar-H), 2120*w* (C≡C), 1772*s* and 1710*s* (imide C=O), 1616*m* (Ar C=C). ^{1}H NMR: 1.30-1.35 (*m*, 10H, methylene protons); 1.51 (*quintet*, 2H, *J* 7.1 Hz, CH_2-CH_2-C≡C); 1.67 (*quintet*, 2H, *J* 7.3 Hz, CH_2-CH_2-phthalimide); 1.95 (*t*, 1H, *J* 2.6 Hz, H-C≡); 2.17 (*dt*, 2H, *J* 7.1, 2.6 Hz, CH_2-C≡); 3.68 (*t*, 2H, *J* 7.3 Hz, CH_2-phthalimide); 7.68-7.74 (*m*, 2H, C3-H and C4-H); 7.82-7.87 (*m*, 2H, C2-H and C5-H). ^{13}C NMR: 18.38, 26.81, 28.44, 28.57, 28.67, 28.98, 29.10, 29.30, 38.05 (alkyl), 68.06, 84.74 (alkynyl), 123.14, 132.20, 133.82 (aryl), 168.45 (carbonyl).

***N*-(Undec-10-yn-1-yl)phthalimide (64):** Method B.

A mixture of potassium phthalimide (1.64g, 8.85 mmol, 1.2 eq), 1-iodoundec-10-yne (**62**) (2.05g, 8.02 mmol), 18C-6 (0.21g, 0.8 mmol, 0.1 eq) and toluene (13 ml) was stirred for 24 hours at 90°. After cooling to room temperature the reaction mixture was poured into water (25 ml), extracted with CH_2Cl_2 (25ml), the organic extract washed with brine (25ml), dried (Na_2SO_4) and solvent removed. The residue was separated by squat chromatography eluant EtOAc/hexanes 20/80 to give the product in 0.95g (40%) yield. The spectral data were identical to those in the previous method.

***N*-(Undec-10-yn-1-yl)phthalimide (64):** Method C.

A mixture of phthalimide (400mg, 2.72 mmol), 1-iodoundec-10-yne (**62**) (0.907mg, 3.26 mmol, 1.2 eq), anhydrous K_2CO_3 (564mg, 4.14 mmol, 1.5 eq) and 2-butanone (20ml) was stirred at reflux for 48 hours. After cooling to room temperature the rection mixture was poured into water (50ml), the aqueous mixture extracted with CH_2Cl_2 (3 x 25ml), the organic extracts combined, dried (Na_2SO_4) and solvent removed. The method of purification and the spectral data were identical with those in Method A. The product was recovered in 614mg (78%) yield.

Undec-10-ynyl-1-amine (66): Method A.

A Soxhlet apparatus charged with undec-10-ynamide (**68**) (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 refluxed and the amide extracted until dissolved (approximately 30 hours), then lithium complexes 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 ($M+H^+$, 26), 138 (48), 110 (39), 96 (43), 86 (100). IR (film): 3500-3100*m* (N-H str), 3304*s* (H-C≡), 2116*w* (C≡C str). ^{1}H NMR: 1.21-1.48 (*m*, 14 H, methylene protons); 1.86 (*t*, 1H, *J* 2.7 Hz, H-C≡); 2.09 (*dt*, 2H, *J* 6.9, 2.7 Hz, CH_2-C≡); 2.60 (*t*, 2H, *J* 6.6 Hz, CH_2-NH_2). ^{13}C NMR: 20.33, 28.81, 30.41, 30.66, 30.98, 31.38, 35.81, 44.20 (alkyl); 70.02, 86.64 (alkynyl).

Undec-10-ynyl-1-amine (66): Method B.

A mixture of *N*-(undec-10-yn-1-yl)phthalimide (**64**) (1.21g, 4.07 mmol), hydrazine hydrate (0.51g, 0.50ml, 10.1 mmol) and EtOH (30ml) was strirred at room temperature for 24 hours. The thick white precipitate was dissolved by the addition of 10% HCl to pH<1, the mixture stirred for 15 minutes and precipitated phthalhydrazide filtered off. The filtrant was adjusted to pH>12 with 1M NaOH, the aqueous layer extracted with CH_2Cl_2 (2 x 25ml), the extracts

combined, dried (Na_2SO_4), solvent removed and the residue distilled to give the product in 0.43g (64%) yield. The physical data (^1H, IR) were identical with those in the previous method.

Undec-10-ynoyl chloride (67)

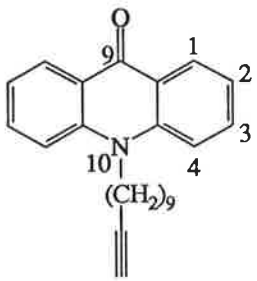
A mixture of undec-10-ynoic acid (**57**) (5.0g, 27 mmol) and $SOCl_2$ (4.0g, 2.5ml, 34 mmol) was refluxed for 60 minutes, allowed to cool to room temperature and excess $SOCl_2$ removed *in vacuo*. The residue was fractionally distilled bp 67-68° at 0.015 mmHg to give the title compound as a colourless oil in 4.30g (76%) yield. MS: 165 ($M\text{-}Cl^+$, 0.5), 135 (4), 94 (37), 79 (100). IR (thin film): 3300*s* (H-C≡), 2928*s*, 2852*s*, 2140*w* (C≡C), 1796*s* (C=O). ^1H NMR: 1.32-1.55 (*m*, 10H, methylene protons); 1.70 (*quintet*, 2H, *J* 7.2 Hz, CH_2-CH_2-COCl); 1.93 (*t*, 1H, *J* 2.6 Hz, HC≡); 2.18 (*dt*, 2H, *J* 6.9, 2.6 Hz, CH_2-C≡C); 2.88 (*t*, 2H, *J* 7.2 Hz, CH_2-COCl). ^{13}C NMR: 18.33, 25.00, 28.33, 28.54, 28.74, 28.89, 29.04, 47.06 (alkyl); 68.14, 84.58 (alkynyl); 173.77 (carbonyl).

Undec-10-ynamide (68)

Thionyl chloride (7.30g, 4.5ml, 61 mmol) was added dropwise to stirred undec-10-ynoic acid (**57**) (9.33g, 51 mmol) at 40°. The mixture was refluxed for 60 minutes, cooled to room temperature and added dropwise to concentrated NH_3 solution held at -15° (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 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 ($M+H^+$, 21), 181 (M^+, 2), 122 (21), 72 (19), 59 (100). IR (nujol): 3356 and 3184 *s* (N-H), 3280 *s* (H-C≡), 2150 *w* (C≡C), 1662 and 1632 *s* (C=O amide). ^1H NMR: 1.31-1.65 (*m*, 12H, methylene protons); 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: 20.36, 27.48, 30.41, 30.64, 30.79, 31.00, 31.15, 37.92 (alkyl), 70.10, 86.71 (alkynyl), 177.83 (carbonyl).

Experimental Described in Chapter 2.2.

10-(Undec-10-ynyl)-9-(10*H*)-acridone (69): Method A.

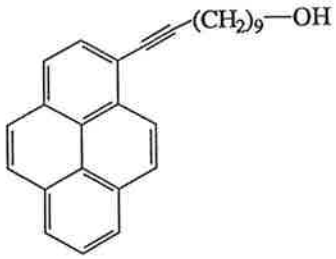


To a stirred suspension of acridone (**12**) (1.00g, 5.1 mmol) in DMF (25ml) was added NaH (80% suspension in oil, 169 mg, 5.6 mmol, 1.1 eq). The reaction mixture turned a fluorescent yellow-green as stirring was continued at 50° for 60 minutes. The iodide (**62**) (1.57g, 5.6 mmol, 1.1 eq) was added dropwise, and stirring continued at room temperature for 60 minutes. TLC (40/60 EtOAc/hexanes) of the dark green reaction mixture showed spots for product (R_f 0.55) and acridone (R_f 0.18); a fluorescent spot on the baseline was assumed to be the acridone salt. After 3 hours stirring the baseline spot was absent. The reaction mixture was poured into water (100ml), the aqueous mixture extracted with $CHCl_3$ (2 x 25 ml), organic extracts combined, washed with water (50ml), dried (Na_2SO_4), the solvent removed and the residue recrystallised from EtOH to give the title compound as green needle crystals in 0.40g (23%) yield mp 94-95°. HRMS Calculated for $C_{24}H_{27}NO$: 345.2095. Found 345.2101. MS: 345 (M^+, 32), 208 (100), 195 (37), 81 (35). IR (nujol): 3280*s* (H-C≡), 1638*s* (C=O), 1602*s*, 1496*s*, 1264*s*, 1180*s*, 754*s*. UV (EtOH): 208 (46 500), 253 (66 300), 385 (8 900), 404 (9 800). Fluorescence (EtOH, λ_{ex} = 385 nm): 421 (100), 444 (75) *sh*. ^{1}H NMR: 1.29-1.52 (*m*, 12H, methylene protons); 1.76 (*quintet*, 2H, *J* 7.5 Hz, N-CH_2-CH_2); 1.97 (*t*, 1H, *J* 2.1 Hz, C≡C-H); 2.16 (*dt*, 2H, *J* 6.6, 2.3 Hz, CH_2-C≡C); 4.11 (*t*, 2H, *J* 7.8 Hz, N-CH_2); 7.17 (*t*, 2H, *J* 7.5 Hz, C2-H); 7.33 (*d*, 2H, *J* 8.4 Hz, C4-H); 7.60 (*t*, 2H, *J* 8.1 Hz, C3-H); 8.48 (*d*, 2H, *J* 7.9 Hz, C1-H). ^{13}C NMR: 18.1, 26.5, 26.7, 28.1, 28.3, 28.7, 28.9, 29.1, 45.7 (alkyl); 68.0, 84.3 (alkyne); 114.2, 120.7, 121.9, 127.4, 133.4, 141.2 (aromatic); 177.4 (carbonyl).

10-(Undec-10-ynyl)-9-(10*H*)-acridone (69): Method B.

Acridone (**12**) (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 (**62**) (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, with spectral data identical with those reported in the previous procedure.

11-(1-Pyrenyl)-undec-10-yn-1-ol (70)



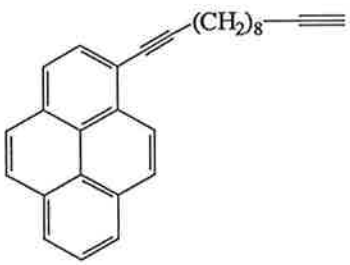
To a stirred mixture of 1-bromopyrene (1.00g, 3.6 mmol) in pyrrolidine (10ml) was added undec-10-yn-1-ol (**59**) (0.72g, 4.3mmol, 1.2eq), and $Pd(PPh_3)_4$ (0.20g, mmol, 0.05eq). The reaction mixture was stirred at 80° for six hours, cooled to room temperature, poured into saturated NH_4Cl solution (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). ^{1}H NMR: 1.48-1.52 (*m*, 11H, methylene and hydroxyl protons); 1.56 (*quintet*, 2H, *J* 6.5 Hz, Ar-C≡C-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-C≡C-Ar); 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: 19.9, 25.7, 28.9, 29.0,

29.1, 29.4, 29.5, 32.6, 63.0 (alkyl); 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 (aromatic).

11-(1-Pyrenyl)-undec-10-ynal (72)

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 **70** (1.49g, 4.0 mmol) in CH_2Cl_2 (10ml) was added with stirring. Stirring was continued 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. The aldehyde 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): 3100*w* (Ar-H), 2750*w* (C-H aldehyde), 2240*w* (C≡C), 1720*s* (C=O), 846*s*. ^{1}H NMR: 1.36-1.69 (*m*, 12H, methylene protons); 1.77 (*quintet*, 2H, *J* 7.2 Hz, CH_2-CH_2-C≡); 2.42 (*dt*, 2H, *J* 7.3, 1.8 Hz, CH_2-CHO); 2.65 (*t*, 2H, *J* 6.9 Hz, CH_2-C≡C-Ar); 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: 19.9, 22.1, 28.3, 28.9, 29.1, 29.3, 43.9 (alkyl), 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 (aryl), 202.8 (C=O).

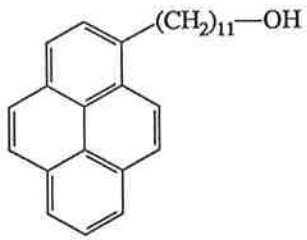
1-(1-Pyrenyl)-dodeca-1,11-diyne (74)



Triphenylphosphine (6.02g, 22.9 mmol) was dissolved in CH_2Cl_2 (20ml) and cooled to -15° in an 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 the aldehyde **72** (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. The product **73**, which was recovered in 1.96g (98%) yield, was dissolved in THF (30ml), cooled to -78° in a dry ice/acetone bath and *n*-butyllithium (3.0ml, 2.5M in hexanes, 7.5mmol) 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, eluant EtOAc/hexanes 1/99, 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): 3300*s* (H-C≡), 3040*m* (Ar-H), 2928s, 2852*s*, 2245*w* (-C≡C-), 2140*w* (H-C≡C), 1600*m*, 1582*w*, 1506*w*, 1490*w*, 1466*m*, 1436*m*, 1186*m*, 846*s*, 718*s*. UV ($CHCl_3$): 249 (47 000), 264 (13 900), 274 (29 400), 285 (54 900), 329 (17 200), 345 (39 600), 364 (56 000). Fluorescence ($CHCl_3$, λ_{ex} = 364 nm): 386 (100), 396 (63), 406 (68). ^{1}H NMR: 1.37-1.60 (*m*, 10H, methylene protons); 1.76 (*quintet*, 2H, *J* 7.2 Hz, CH_2CH_2-≡-Ar); 1.95 (*t*, 1H, *J* 2.6 Hz, C≡C-H); 2.19 (*dt*, 2H, *J* 6.9, 2.6 Hz, CH_2-≡); 2.64 (*t*, 2H, *J* 7.2 Hz, CH_2-≡-Ar); 7.96-8.19 (*m*, 8H, Ar-H); 8.55 (*d*, 1H, *J* 9.2 Hz, Ar-H). ^{13}C NMR: 18.4, 19.9, 28.7, 28.9, 29.00, 29.1 (alkyl), 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 (aromatic).

11-(1-Pyrenyl)-undecan-1-ol (75)



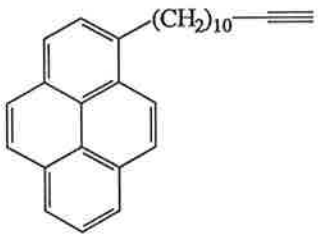
A mixture of 11-(1-pyrenyl)undec-10-yn-1-ol (**70**) (1.00g, 2.7 mmol), 5% Pd/C (300mg) and EtOAc (100ml) was stirred overnight under an atmosphere of hydrogen. The reaction mixture was filtered through celite, the solvent removed and the residue recrystallised from hexanes to give the product as colourless 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): 3416*br s* (O-H), 3050*w* (Ar-H), 1610*s*, 1520*s*, 1054*s*, 836*s*. ^{1}H NMR: 1.25-1.52 (*m*, 16H, methylene protons); 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: 25.7, 29.4, 29.5, 29.7, 29.8, 31.9, 32.7, 33.6, 63.0 (alkyl); 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 (aromatic).

11-(1-Pyrenyl)-undecanal (76)

The alcohol **75** was converted to the title compound by the method used for compound **72**. The title compound 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). ^{1}H NMR: 1.26-1.48 (*m*, 14H, methylene protons); 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 (alkyl), 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 (aryl), 203.0 (carbonyl).

1-(1-Pyrenyl)-dodeca-11-yne (78)



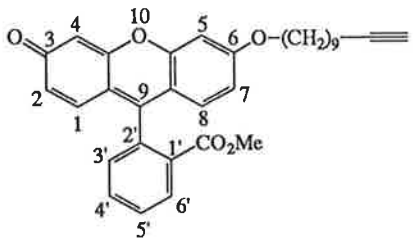
The aldehyde **76** was converted to the title compound by the method used for compound **74**. The intermediate **1,1-dibromo-12-(1-pyrenyl)-dodec-1-ene** (**77**) was isolated as colourless crystals mp 41-43° in 0.87g (80%) yield. MS: 524/526/528 (1:2:1, 100, M^+), 215 (29). ^{1}H NMR: 1.28-1.48 (*m*, 14H, methylene protons); 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: 27.8, 29.0, 29.3, 29.5, 29.5, 31.9, 33.0, 33.6 (alkyl),

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 (aryl), 138.9 (HC=). 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): 3308*s* (H-C≡), 3040*w* (Ar-H), 1600*s*, 1180*s*, 842*s*. UV (EtOH): 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). ^{1}H NMR: 1.25-1.53 (*m*, 14H, methylene protons); 1.81 (*quintet*, 2H, *J* 7.6 Hz, Ar-CH_2-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-C≡C); 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: 18.4, 28.4, 28.7, 29.1, 29.5, 29.8, 31.9, 33.6 (alkyl); 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 (aromatic).

Fluorescein methyl ester (80)

Fluorescein (**5**) (5.00g, 15 mmol), concentrated H_2SO_4 (3ml) and MeOH (100ml) were refluxed for 30 hours, and left at room temperature for 2 days. The orange crystals which precipitated were collected, dissolved in 1.5N NaOH (50ml), extracted with EtOAc (50ml) to remove the dimethyl derivatives, acidified to pH 1 with 10% HCl, the red precipitate collected and recrystallised from MeOH to give 1.32g (25%) of the title compound as red microcrystals mp 282° (lit.[130] 282°). MS: 346 (M^+, 100), 258 (46).

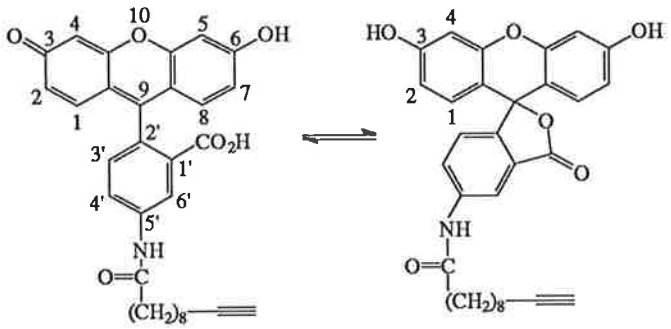
6-*O*-(1-Undec-10-ynyl)fluorescein methyl ester (81)



A mixture of fluorescein methyl ester (**80**) (1.00g, 2.89 mmol), 11-iodoundec-1-yne (**62**) (1.20g, 4.33mmol, 1.5 eq.), K_2CO_3 (0.80g, 5.78 mmol, 2 eq.) and 2-butanone (100ml) was refluxed 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): 3296*s* (H-C≡), 3180*w* (Ar-H), 2145*w* (C≡C), 1730*s* (C=O), 1644*s*, 1590*s*, 1260*s*, 852*s*. UV (EtOH): 234 (135 000), 256 (62 700), 277 (60 500), 311 (29 400), 364 (29 500), 437 (74 000), 459 (94 400), 489 (80 600). Fluorescence (EtOH, λ_{ex} = 489 nm): 517 nm. ^{1}H NMR: 1.34-1.56 (*m*, 12H, methylene protons); 1.83 (*quintet*, 2H, *J* 6.6 Hz, CH_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-C≡C); 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: 18.4, 25.9, 28.4, 28.7, 28.9, 29.0, 29.2, 29.9, 52.4 (alkyl), 68.1 (HC≡C), 68.9, 87.7 (C≡CH), 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 (alkene and aromatic), 165.7 (ester), 185.7 (carbonyl).

_N_-(1-Oxoundec-10-ynyl)-5-aminofluorescein (84)

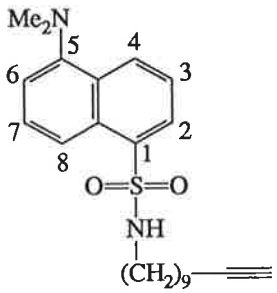


84 **85**

To a stirred solution of 5-aminofluorescein (**83**) (0.50g, 1.44 mmol) in pyridine (5ml) was added dropwise undec-10-ynoyl chloride (**67**) (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): 3500-2500*m* (O-H), 3296*s* (H-C≡), 2140*w* (C≡C), 1738*s*, 1666*s* (C=O), 1610*s*. UV (EtOH): 231 (48 500), 255 (24 900), 455 (6 840), 483 (6 670). Fluorescence (EtOH, λ_{ex} = 483 nm): 516 nm. *The NMR data are for the lactone structure* (**85**). ^{1}H NMR (d_6-DMSO): 1.30-1.44 (*m*, 10H, methylene protons); 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-C≡); 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): 19.5, 26.8, 29.7, 29.9, 30.2, 30.4, 30.5, 38.3 (alkyl), 72.8 (HC≡C), 84.8, 86.3 (HC≡C), 104.0, 111.6, 114.3, 115.1, 126.1, 128.0, 128.7, 130.9, 142.6, 148.4, 153.7, 161.2 (aryl), 170.4, 173.7 (carbonyl).

5-Dimethylamino-*N*-(11-undec-1-ynyl)-1-naphthalenesulphonamide (87)

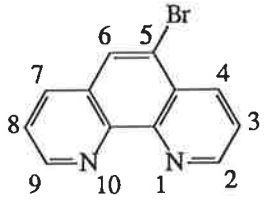


To a stirred mixture of dansyl chloride (**86**) (200mg, 0.74 mmol) and Et_3N (75mg, 100µl, 0.74 mmol, 1eq) in CH_2Cl_2 (5ml) was added undec-10-yn-1-amine (**66**) (124mg, 0.74 mmol, 1 eq). The reaction mixture was stirred for 60 minutes, the solvent removed and the residue separated by flash chromatography 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): 3300*br*

s (H-C≡ and N-H), 3052*w* (Ar-H), 2116*w* (C≡C), 1586*s*, 1574*s*, 1464*s*, 1322*s* and 1144*s* (O=S=O), 790*s*. UV (EtOH): 220 (29 900), 253 (12 400), 337 (4 000). Fluorescence (EtOH, λ_{ex} = 337 nm): 506 nm. ^{1}H NMR: 1.09-1.26 (*m*, 12H, methylene protons); 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-C≡C); 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: 18.3, 26.3, 28.4, 28.6, 28.8, 29.2, 29.4, 43.3, 45.4 (alkyl), 68.1, 84.7 (alkynyl), 115.1, 118.6, 123.2, 128.4, 129.7, 129.8, 130.4, 134.7, 152.0 (aryl).

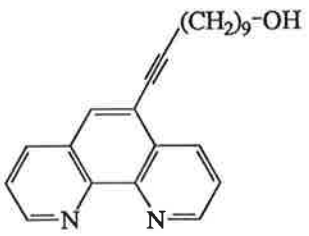
Experimental Described in Chapter 2.3.

5-Bromo-1,10-phenanthroline (88).



Anhydrous 1,10-phenanthroline (6.20g, 34 mmol) was dried at 120° at 0.01mmHg for 3 hours, and cooled in a ice/MeOH bath. Fuming H_2SO_4 (60% oleum) (30ml) was added slowly, and the mixture allowed to come to room temperature with stirring. When the phenanthroline had dissolved, Br_2 (2.75g, 0.89ml, 17 mmol) was added and the mixture heated at 120° in an oil bath for 19 hours. After cooling to room temperature the reaction mixture was poured onto ice (250g) and neutralised with concentrated ammonium hydroxide solution. The aqueous mixture was extracted with $CHCl_3$ (2 x 100ml), the organic extracts combined, dried ($MgSO_4$) and the solvent removed. The residue was separated by flash chromatography on alumina, eluant $CHCl_3$/hexanes 50/50, and recrystallised from dry $CHCl_3$. The title compound was recovered as colourless crystals mp 117-118° (lit[75]. 118°) in 4.62g (52%) yield. MS: 258/260 (M^+, 100), 179 (M^+-Br, 99), 152 (40), 125 (28). ^{1}H NMR: 7.65 (*dd*, 1H, *J* 8.1, 4.3Hz, C3-H); 7.74 (*dd*, 1H, *J* 8.3, 4.4Hz, C8-H); 8.14 (*s*, 1H, C6-H); 8.18 (*dd*, 1H, *J* 8.2, 1.8 Hz, C7-H); 8.66 (*dd*, 1H, *J* 8.3, 1.6 Hz, C4-H); 9.20 (*dd* (partially obscured), 1H, *J* 4.4, 1.8 Hz, C9-H); 9.21 (*dd* (partially obscured), 1H, *J* 4.3, 1.6 Hz, C2-H). ^{13}C NMR: 120.6, 123.4, 123.6, 124.2, 127.7, 128.6, 129.4, 134.9, 135.7, 136.9, 150.5, 150.7.

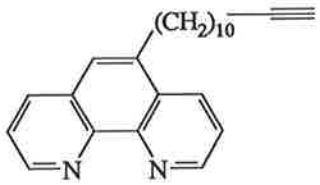
5-(11-Hydroxyundec-1-ynyl)-1,10-phenanthroline (89)



A mixture of 5-bromo-1,10-phenanthroline **88** (2.59g, 10.0 mmol), undec-10-yn-1-ol **59** (2.01g, 12.0 mmol, 1.2 eq.) and $Pd(PPh_3)_4$ (0.58g, 0.50 mmol, 0.05eq) 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): 3600-3100*s* (O-H), 3050*w* (Ar-H), 2220*w* (C≡C). ^{1}H NMR: 1.34-1.72 (*m*, 14H, methylene protons); 2.58 (*t*, 2H, *J* 6.9Hz, CH_2-C≡); 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: 19.5, 25.6, 28.5, 28.8, 29.1, 29.2, 29.3, 32.6, 62.6 (alkyl); 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 (aromatic).

5-(Dodec-11-ynyl)-1,10-phenanthroline (91): Method A.



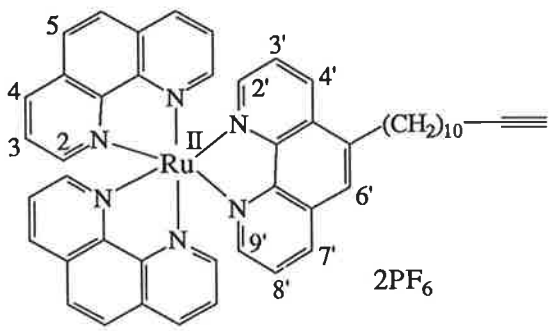
To a stirred slurry of potassium *tert*-butoxide (35mg, 0.32 mmol, 1.1 eq) in THF (10ml) at -78° was added dropwise diethylmethyldiazophosponate **98** (56mg, 0.32 mmol, 1.1 eq). The mixture was stirred for 10 minutes then a solution of the aldehyde **95** (100mg, 0.29 mmol) in THF (5ml) was added dropwise. 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): 3172*s* (H-C≡), 2130*w* (C≡C), 1512*m*, 872*m*, 740*s*. ^{1}H NMR: 1.27-1.53 (*m*, 14H, methylene protons); 1.77 (*quintet*, 2H, *J* 7.7 Hz, CH_2-CH_2-Ar); 1.92 (*t*, 1H, *J* 2.6 Hz, H-C≡); 2.14 (*dt*, 2H, *J* 6.9, 2.6 Hz, CH_2-C≡); 3.08 (*t*, 2H, *J* 7.5 Hz, CH_2-Ar); 7.57 (*s*, 1H, C6-H); 7.58 (*dd* (obscured), 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: 18.4, 28.7, 29.1, 29.4, 29.5, 29.5, 29.6, 29.7, 30.1, 32.6 (alkyl), 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 (aryl).

5-(Dodec-11-ynyl)-1,10-phenanthroline (91): Method B.

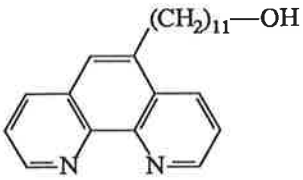
To a stirred mixture of the aldehyde **95** (0.58g, 1.7 mmol) and dimethyl-(1-diazo-2-oxo-propyl)phosphonate (0.48g, 2.5 mmol, 1.5 eq) in dry MeOH (7 ml) at 0° was added K_2CO_3 (0.46g, 0.34 mmol, 2 eq). The mixture was stirred for 1 hour at 0° then at room temperature overnight. After quenching with saturated NH_4Cl solution (4ml), the organic solvent was removed, CH_2Cl_2 (25ml) and water (25ml) added, the organic layer separated, dried ($MgSO_4$), solvent removed and the residue purified as in the previous procedure. The product was recovered in 0.34g (58%) yield with ^{1}H NMR data identical with those reported in the previous procedure.

***Bis* -1,10-Phenanthroline-5-[(dodec-11-ynyl)-1,10-phenanthroline]ruthenium(II) hexafluorophosphate (92)**



A mixture of *bis*-1,10-phenanthrolineruthenium(II)dichloride **90** (100mg, 0.19 mmol, 1eq), 5-(dodec-11-ynyl)-1,10-phenanthroline **91** (65mg, 0.19 mmol, 1eq), 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): 3300*w* (H-C≡), 1640*m*, 1040*m*, 840*s* (P-F), 722*s*. UV ($CHCl_3$): 257 (31800), 423 (11600), 449 (12300). Fluorescence: 578 nm. ^{1}H NMR (d_6-DMSO): 1.22-1.48 (*m*, methylene protons); 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≡); 2.70 (*t*, 1H, *J* 2.6 Hz, H-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): 17.6, 27.9, 28.0, 28.4, 28.8, 28.8, 28.9, 29.8, 31.3 (alkyl), 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.

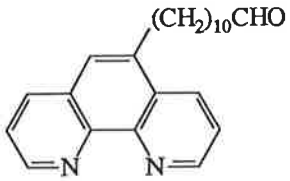
5-(11-Hydroxyundecyl)-1,10-phenanthroline (93)



A mixture of alkyne **89** (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 ([M-H]$^+$, 39), 319 ([M-H_2C=OH^+], 60), 207 (94), 193 (100). IR (thin film): 3600-3100*s* (O-H), 3050*w* (Ar-H), 1620*s*, 1564*s*, 1424*s*. ^{1}H NMR: 1.21-1.42 (*m*, 14H, methylene protons); 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: 27.7, 31.2, 31.3, 31.4, 31.4, 31.5, 31.6, 32.2, 34.6, 34.8, 64.9 (alkyl), 124.6, 124.9, 126.8, 130.0,130.4, 134.2, 137.3, 139.4, 147.5, 148.5, 151.7, 151.8 (aromatic).

5-(11-Oxoundecyl)-1,10-Phenanthroline (95): Method A.



To a vigorously stirred mixture of the alcohol **94** (573mg, 1.64 mmol), NaBr (382mg, 1.64 mmol, 1.0 eq.), TEMPO (25mg, 0.16 mmol, 0.1 eq.), CH_2Cl_2 (10ml) and water (2ml) at 0° was added dropwise over 40 minutes a mixture of 0.35M NaOCl (4.7ml, 1.64 mmol, 1.0 eq.) and $NaHCO_3$ (382mg, 4.28 mmol, 3.0 eq.). The mixture was stirred for 20 minutes, the layers separated, the aqueous layer extracted with CH_2Cl_2 (10ml), the organic layers combined, washed succesively with aqueous KI solution (0.25g in 10ml), 10% NaS_2O_3 solution (10ml), brine (10ml) and dried ($MgSO_4$). The solvent was removed and the residue purified by flash chromatography on alumina using $CHCl_3$ as eluant, to give the title compound as cream crystals mp 52-54° in 217mg (38%) 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

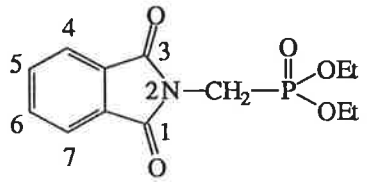
(82), 194 (100). IR (thin film): 3030*w* (Ar-H), 2716*w* (aldehyde C-H), 1724*s* (C=O), 1424*s*, 744*s*. ^{1}H NMR: 1.21-1.49 (*m*, 12H, methylene protons); 1.61 (*quintet*, 2H, *J* 7.1 Hz, CH_2-CH_2-CHO); 1.79 (*quintet*, 2H, *J* 7.2 Hz, CH_2-CH_2-Ar); 2.41 (*dt*, 2H, *J* 7.3, 1.9 Hz, CH_2-CHO); 3.11 (*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: 21.8, 24.8, 28.8, 29.1, 29.2, 29.4, 29.9, 32.3, 43.6 (alkyl), 122.5, 122.9, 124.6, 127.8, 128.2, 132.0, 135.1, 137.2, 145.2, 146.2, 149.2, 149.3 (aryl), 202.7 (carbonyl).

5-(11-Oxoundecyl)-1,10-Phenanthroline (95): Method B.

Oxalyl chloride (0.22ml, 2.6 mmol. 1.1 eq) was dissolved in CH_2Cl_2 (25ml) and cooled in a dry ice/acetone bath. DMSO (0.36ml, 5.1 mmol, 2.2 eq) was added and the reaction mixture was stirred for 5 minutes. A solution of the alcohol **94** (815mg, 2.3 mmol) in CH_2Cl_2 (10ml) was added, the mixture stirred for 15 minutes, Et_3N (1.62ml, 11.6 mmol, 5 eq) 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. The physical data were identical with those in the previous method.

Diethylmethyldiazophosphonate (98)

(a) Phthalimidodiethylphosphonomethane



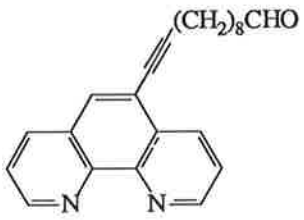
To a stirred solution of bromomethylphthalimide (6.00g, 25 mmol) in xylene (mixture of isomers, 20ml) at 130° was added dropwise triethylphosphite (4.15g, 25 mmol). The byproduct ethyl bromide was distilled from the reaction mixture over 60 minutes, the

temperature raised to 150° and stirring continued overnight. After cooling to room temperature hexane (20ml) was added and the reaction mixture stored at -20°. The precipitated crude product was collected, washed with hexane, air dried and recrystallised from ether. The title compound was recovered as colourless crystals mp 63-65° (lit[131]. 64-65°) in 3.83g (in two crops, 48%) yield. ^{1}H NMR: 1.32 (*t*, 6H, *J* 7.1 Hz, CH_3-CH_2); 4.10 (*d*, 2H, $^2J_{PH}$ 11.5 Hz, P-CH_2-N); 4.20 (*m*, 4H, O-CH_2-CH_3); 7.73 (*dd*, 2H, *J* 5.6, 3.0 Hz, C5-H and C6-H); 7.87 (*dd*, 2H, *J* 5.6, 3.0 Hz, C4-H and C5-H). ^{13}C NMR: 16.3 ($^3J_{CP}$ 6.3 Hz, CH_3), 33.2 ($^1J_{CP}$ 156 Hz, P-CH_2-N), 62.8 ($^2J_{CP}$ 5.4 Hz, O-CH_2-CH_3), 123.5, 131.9, 134.2 (aromatic), 166.9 (carbonyl).

(b) Diethylmethyldiazophosphonate (**98**)

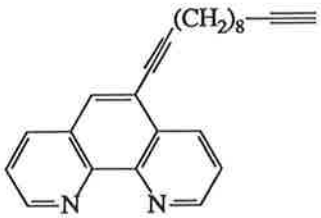
A mixture of phthalimidodiethylphosphonomethane (2.5g, 8.4 mmol), hydrazine hydrate (0.42g, 8.4 mmol), acetic acid (1.01g, 16.8 mmol) and MeOH (10ml) was refluxed for 2 hours. After cooling to 0°, the precipitated phthalhydrazide was filtered off and the solvent removed. The residue was dissolved in a mixture of acetic acid (1.0ml) and water (8.5ml). Dichloromethane (6ml) was added, the mixture cooled to -10° (ice-MeOH bath) and a solution of $NaNO_2$ (0.58g, 8.4 mmol) in water (1.5ml) added dropwise. The cooling bath was replaced by an ice bath and the reaction mixture stirred for 90 minutes. The organic layer was separated, the aqueous layer extracted with CH_2Cl_2 (3 x 10ml), the combined extracts washed with saturated $NaHCO_3$ solution (20ml), brine (20ml), dried ($MgSO_4$) and solvent removed. The residue was distilled behind a safety shield, bp 64° at 0.012mmHg (Kugelrohr; air temperature; lit.[131] 51° at 0.1mmHg) to give the title compound as a yellow oil in 0.94g (63%) yield. IR (thin film): 2984*s*, 2112*s* (N_2), 1300*s* and 1250*s* (P=O). ^{1}H NMR: 1.34 (*t*, 6H, *J* 7.1 Hz, CH_3-CH_2); 3.77 (*d*, 1H, $^2J_{HP}$ 11.0 Hz, HC=N_2); 4.13 (*m*, 4H, O-CH_2-CH_3).

5-(11-Oxoundec-1-ynyl)-1,10-phenanthroline (104)



The title compound was prepared by Method B used for compound **95.** Reaction of alcohol **91** (1.50g, 4.33 mmol) gave the product as colourless crystals mp 50-51° in 1.11g (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): 2720*w* (C-H aldehyde), 2212*w* (C=C), 1722*s* (C=O), 1504*m*, 1420*m*, 744*m*. ^{1}H NMR: 1.33-1.73 (*m*, 12H, methylene protons); 2.38 (*dt*, 2H, *J* 7.3, 1.7 Hz, CH_2-CHO); 2.55 (*t*, 2H, *J* 7.0 Hz, CH_2-C≡C-Ar); 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: 19.2, 21.3, 28.2, 28.3, 28.4, 28.5, 28.6, 28.8, 43.4 (alkyl), 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 (aryl), 202.3 (carbonyl).

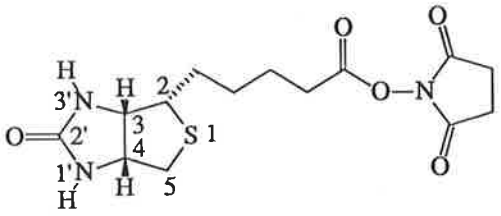
5-(Dodec-1,11-diynyl)-1,10-phenanthroline (105)



Prepared by the method used for compound **95** from aldehyde **104** to give the title compound as colourless crystals mp 86-88° in 300mg (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): 3300*m* (H-C≡), 3020*w* (Ar-H), 2928*s*, 2852*s*, 2220*w* (-C≡C-), 2120*w* (H-C≡C), 1606*m*, 1590*m*, 1506*s*, 1424*s*, 742*s*. ^{1}H NMR: 1.26-1.68 (*m*, 10H, methylene protons); 1.75 (*quintet*, 2H, *J* 7.2 Hz, CH_2-CH_2-≡-Ar); 1.95 (*t*, 1H, *J* 2.6 Hz, H-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-≡-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: 18.4, 19.7, 28.4, 28.7, 28.7, 28.9, 29.0 (alkyl), 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 (aryl).

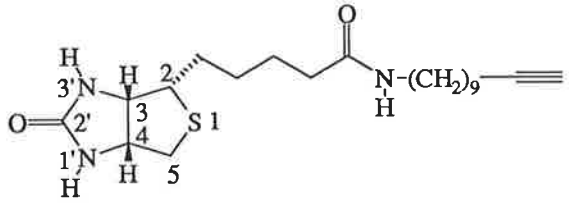
Experimental Described in Chapter 2.4.

Biotin *N*-hydroxysuccinimide ester (106)



Biotin **41** (0.97g, 4.0 mmol) was dissolved in DMF (12ml) at 80° and the solution removed from heating. NHS (0.47g, 4.1 mmol) and DCC (0.93g, 4.5 mmol, 1.1 eq) were added and the reaction mixture stirred at room temperature for 2 hours, filtered to removed precipitated DCU and the solvent removed under vacuum (oil pump). The residue was recrystallised from isopropanol to give the title compound as cream crystals mp 207-209° (lit.[88] 210°) in 0.71g (51%) yield. MS: 341 (M^+, 31), 227 (32), 166 (11), 97 (49), 55 (100). IR (nujol): 3232*m* (N-H), 1750*s*, 1730*s*, 1704*s* (3 x C=O), 1216*s*, 1072*s*. ^{1}H NMR (d_6-DMSO): 1.41-1.68 (*m*, 6H, methylene protons); 2.56 (*d*, 1H, J_{gem} 12.4 Hz, C5-Hb); 2.65 (*t*, 2H, *J* 7.3 Hz, CH_2-CO_2); 2.81 (*dd*, J_{gem} 12.4, 4.6 Hz, C5-Ha); 3.10 (*dt*, 1H, *J* 7.3, 4.6 Hz, C2-H); 4.13 (*dd*, 1H, *J* 7.0, 4.7 Hz, C4-H); 4.29 (*dd*, 1H, *J* 7.0, 4.7 Hz, C3-H); 6.36 (*bs*, 1H, N1'-H); 6.42 (*bs*, 1H, N3'-H). ^{13}C NMR (d_6-DMSO): 24.2, 25.3, 27.4, 27.7, 29.8, 39.8, 55.0, 59.0, 60.8 (alkyl), 162.5, 168.8, 170.2 (carbonyl).

Biotin-*N*-(undec-10-ynyl)amide (107)



To a stirred solution of biotin-NHS ester (**106**) (300mg, 0.29 mmol) in DMF (6ml) was added the amine **66** (147mg, 0.29 mmol, 1eq). 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): 3700-3200*m* (N-H), 3292*s* (H-C≡), 2140*w* (C≡C), 1692*s*, 1648*s* (C=O), 1548*s*. ^{1}H NMR: 1.18-1.67 (*m*, 20H, methylene protons); 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-Hb); 2.86 (*dd*, 1H, J_{gem} 12.9, 4.9 Hz, C5-Ha); 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: 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 (alkyl), 67.9, 101.0 (alkynyl), 161.6, 170.9 (carbonyl).

Experimental Described in Chapter 3.1.

Protection of Amino Acids: General Procedure

The crude amino acid was suspended in dry MeOH (10ml/g) and $SOCl_2$ (1eq) added dropwise with stirring. After stirring for 24 hours, the solvent was removed *in vacuo*, and the crude amino acid methyl ester hydrochloride suspended in a mixture of CH_2Cl_2 (25ml/g) and benzoyl chloride (1eq). A solution of Na_2CO_3 (1eq) in water (5ml/g) was added dropwise and the reaction mixture stirred for 48 hours. The organic layer was separated, washed with saturated $NaHCO_3$ solution, brine, dried ($MgSO_4$), the solvent removed and the residue purified by recrystallisation or chromatography.

N-Benzoyl-3-iodo-L-tyrosine methyl ester (108)

The crude product was recrystallised from EtOAc/hexanes to give the title compound as a colourless glass in 0.56g (59%) yield. Calculated for $C_{17}H_{16}INO_4$: 425.0124. Found: 425.0135. MS: 425 (M^+, 0.1), 305 (M^+-PhCONH, 100), 233 (58). IR (nujol): 3500-3000*m* (O-H), 1732*s* (C=O ester), 1640*s* (C=O amide), 1532s. ^{1}H NMR: 3.06 and 3.14 (2 x *dd*, 1H, *J* 14.9, 5.4 Hz, CH_2-Ar); 3.71 (*s*, 3H, CO_2CH_3); 4.96 (*dt*, 1H, *J* 7.4, 5.4 Hz, αC-H); 5.41 (*bs*, 1H, Ar-OH); 6.54 (*d*, 1H, *J* 7.4 Hz, NH); 6.81-7.69 (*m*, 8H, Ar-H). ^{13}C NMR: 36.6, 52.6, 53.6 (alkyl); 85.4 (Ar-I), 115.1, 127.1, 128.7, 129.7, 130.9, 132.0, 133.6, 139.1, 154.4 (aromatic); 167.1, 171.9 (carbonyl).

N-Benzoyl-4-iodo-L-phenylalanine methyl ester (109)

The crude compound was recrystallised from CH_2Cl_2/hexanes to give the title compound as colourless crystals mp 147-148° in 0.22g (73%) yield. Calculated for $C_{17}H_{16}INO_3$: 409.0175. Found 409.0160. MS: 409 (M^+, 7), 349 (M^+-CO_2Me, 10), 289 (M^+-PhCONH, 34), 287 (100), 257 (31), 217 (21). IR (nujol): 3308*m* (N-H), 1746*s* and 1640*s* (C=O), 1528*s*. ^{1}H NMR: 3.18 and 3.26 (2 x *dd*, 1H, *J* 13.9, 5.6 Hz, CH_2-Ar); 3.78 (*s*, 3H, CH_3-O_2C); 5.08 (*dt*, 1H, *J* 5.6, 7.3 Hz, αC-H); 6.15 (*d*, 1H, *J* 7.3 Hz, PhCONH); 6.88 (*d*, 2H, *J* 8.2 Hz, C3-H, C5-H); 7.42-7.55 (*m*, 3H, **Ph**CON); 7.61 (*d*, 2H, *J* 8.2 Hz, C2-H, C6-H); 7.72-7.75 (*m*, 2H,

PhCON). ^{13}C NMR: 37.3, 52.5, 53.3 (alkyl); 92.7 (Ar-I), 126.9, 128.3, 128.6, 130.0, 131.3, 131.6, 131.9, 133.3, 133.6, 135.5, 137.6 (aromatic); 166.8, 171.7 (carbonyl).

_N_-Benzoyl-L-tyrosine methyl ester (110)

The crude compound was recrystallised from EtOAc/hexanes to give the title compound as colourless crystals mp 149-152° (lit.[132] 151-153°) in 12.9g (55%) yield. MS: 299 (M^+, 13), 282 (20), 240 (33), 178 (63), 122 (29), 105 (100). IR (nujol): 3340*bs* (O-H) 3284*s* (N-H), 3070*w*, 3050*w*, 1712*s* (C=O ester), 1640 (C=O amide), 1598*s*. ^{1}H NMR: 3.12 and 3.22 (2 x *dd*, 1H, 13.8, 5.6 Hz, CH_2-Ar); 3.77 (*s*, 3H, CO_2CH_3); 5.06 (*dt*, 1H, *J* 7.7, 5.6 Hz, αC-H); 6.20 (*bs*, 1H, Ar-OH); 6.66 (*bd*, 1H, *J* 7.7 Hz, CONH-); 6.70-6.77 (*m*, 2H, C3-H and C5-H); 6.94-7.01 (*m*, 2H, C2-H and C6-H); 7.37- 7.75 (*m*, 5H, **Ph**CON). ^{13}C NMR (d_6-DMSO): 37.3, 53.6, 56.5(alkyl), 116.8, 129.2, 129.5, 130.0, 131.8, 133.2, 135.5, 157.7 (aryl), 168.2, 174.1 (carbonyl).

_N_-Benzoyl-5-hydroxy-L-tryptophan methyl ester (111)

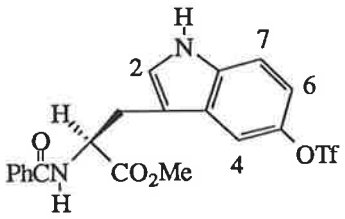
The crude compound was purified by flash chromatography eluant EtOAc/hexanes 50/50 to give the title compound as a colourless glass in 0.77g (50%) yield. HRMS Calculated for $C_{19}H_{18}N_2O_4$: 338.1267. Found: 338.1267. MS: 337 (M^+, 32), 278 (5), 216 (58), 145 (100). IR (nujol): 3600-3200*m* (Ar-OH str), 1732*s* (C=O ester), 1642*s* (C=O amide). ^{1}H NMR: 3.30 and 3.38 (2 x *dd*, 1H, *J* 11.7, 5.3 Hz, CH_2-Ar); 3.70 (*s*, 3H, CH_3O_2C); 5.11 (*dt*, *J* 7.7, 5.3 Hz, αC-H); 5.86 (*bs*, 1H, Ar-OH); 6.75 (*d*, 1H, *J* 7.6 Hz, C(O)NH); 6.78 (*dd*, 1H, *J* 8.7, 2.4 Hz, C6-H); 6.97 (*m*, 2H, C2-H and C4-H); 7.21 (*d*, 1H, *J* 8.7 Hz, C7-H); 7.36 (*m*, 3H and 7.69, *m*, 2H, **Ph**CON); 8.14 (*bs*, 1H, N1-H). ^{13}C NMR: 27.8, 52.5, 53.6 (alkyl), 103.0, 109.3, 112.0, 112.3, 123.8, 127.1, 128.3, 128.6, 131.2, 131.8, 133.6, 150.2 (aryl), 167.3, 172.6 (carbonyl).

_N_-Benzoyl-(4-_O_-trifluoromethanesulphonyl)-L-phenylalanine methyl ester (112)

To a cold (0°) stirred solution of *N*-benzoyltyrosine methyl ester (**110**) (1.00g, 3.7 mmol) and Et_3N (0.34g, 0.47ml, 3.3 mmol) in dry CH_2Cl_2 (15ml) was added N-phenyltriflimide (1.43g,

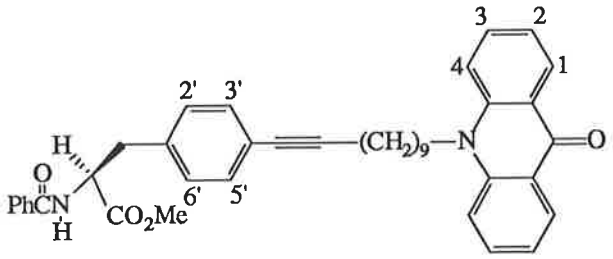
4.0 mmol). The reaction mixture was stirred overnight as the ice bath melted and came to room temperature. The solvent was removed and the residue separated by flash chromatography with 30:70 EtOAc/hexanes as eluant to give the title compound as colourless crystals mp 114-115° (lit.[89] 112°) in 1.44g (99%) yield. MS: 431 (M^+, 0.1), 372 (M^+-CO_2Me, 7), 310 (M^+-CF_3SO_2, 100), 225 (55), 192 (14), 177 (35). IR (nujol): 3320*m* (N-H str), 3050*w* (Ar-H str), 1736*s* (C=O ester), 1638*s* (C=O amide), 1534*s*. ^{1}H NMR: 3.26 and 3.34 (2 x *dd*, 1H, *J* 13.9, 5.6 Hz, CH_2-Ar); 3.77 (*s*, 3H, CO_2CH_3); 5.09 (*dt*, 1H, *J* 7.2, *J* 5.6 Hz, αC-H); 6.65 (*d*, 1H, *J* 7.2 Hz, N-H); 7.17-7.75 (*m*, 9H, Ar-H). ^{13}C NMR: 37.3, 52.6, 53.4 (alkyl), 115.5, 121.4, 121.9 and 128.6 (central peaks of *q*, CF_3, J_{CF} 334 Hz), 126.9, 128.7, 131.1, 132.0, 133.6, 136.8, 148.6 (aryl), 166.9, 171.7 (carbonyl).

_N_-Benzoyl-5-_O_-trifluoromethanesulphonyl-L-tryptophan methyl ester (113)



The title compound was prepared as described for compound **112**. The crude product was separated by flash chromatography eluant 40/60 EtOAc/hexanes and recrystallised from CH_2Cl_2/hexanes to give the title compound as colourless needle crystals in 0.63g (91%) yield mp 109-110°. Calculated for $C_{20}H_{16}F_3N_2O_6S$: 470.0759. Found: 470.0744. MS: 470 (M^+, 9), 411 (M^+-CO_2Me, 5), 349 (M^+-$PhCONH_2$, 100), 278 (90), 225 (60). IR (nujol): 3403*s* (Ar-N-H), 3350*m*(N-H), 3050*w*, 1734*s* (C=O ester), 1636*s* (C=O amide). ^{1}H NMR: 3.38 and 3.46 (2 x *dd*, 1H, *J* 14.7, 5.3 Hz, CH_2-Ar); 3.73 (*s*, 3H, CH_3O_2C); 5.13 (*dt*, 1H, *J* 7.3, 5.3 Hz, αC-H); 6.78 (*d*, 1H, *J* 7.3 Hz, PhCONH); 7.03 (*dd*, *J* 8.8, 2.4 Hz, C6-H); 7.08 (*d*, 1H, *J* 2.4 Hz, C-4H); 7.26-7.72 (*m*, 7H, Ar-H); 8.68 (*bs*, 1H, N1-H). ^{13}C NMR: 27.5, 52.7, 53.3 (alkyl), 110.8, 111.1, 112.5, 115.4, 123.3 and 127.1, (central peaks of *q*, CF_3, J_{CF} 283 Hz), 125.6, 127.0, 127.9, 128.7, 129.5, 132.0, 133.5, 143.5 (aromatic), 167.2, 172.3 (carbonyl).

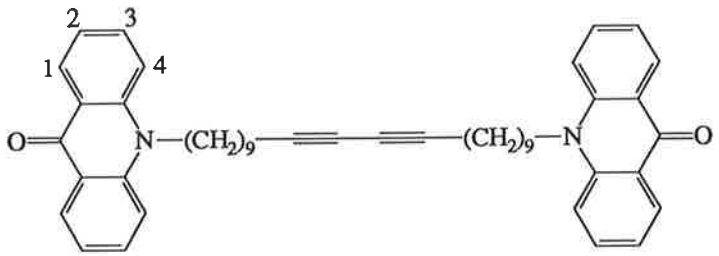
N-Benzoyl-4-{[11-(10-(10*H*)-9-acridonyl)]undec-1-ynyl}-L-phenylalanine methyl ester (118): Method A.



To a stirred solution of triflate **112** (50mg, 0.16 mmol) in DMF (1.5ml) was added $Pd(PPh_3)_4$ (134mg, 0.16 mmol). A green/brown suspension slowly formed. TLC analysis after 3.5 hours showed a minor amount of starting material at Rf = 0.32 (30/70 EtOAc/hexanes) and a major spot on baseline. Alkyne **69** (60mg, 0.17mmol, 1.5eq), CuI (4.4 mg, 0.023mmol, 0.2eq) and Et_3N (0.5ml) were added and stirring continued at 50° for 30 minutes. The solvent was removed *in vacuo* and the residue separated by flash chromatography eluant 50/50 EtOAc/hexanes, to give the title compound as light green crystals in 51mg (68%) yield mp 66-67°. HRMS Calculated for $C_{41}H_{42}N_2O_4$: 626.3145. Found: 626.3148. MS: 626 (M^+, 5), 208 (9), 195 (100), 167 (25). IR (nujol): 3272*w* (N-H), 1732*s* (C=O ester), 1642*s* (C=O amide), 1606*s*, 1538*s*. UV (EtOH): 257 (87 700), 387 (10 400), 405 (12 400). Fluorescence (EtOH, λ_{ex} = 387 nm): 420 (100), 440 (70). ^{1}H NMR: 1.17-1.65 (*m*, 12H, methylene protons); 1.92 (*quintet*, 2H, *J* 7.6 Hz, CH_2-CH_2-acridone); 2.40 (*t*, 2H, *J* 7.0 Hz, CH_2-≡-Ar); 3.19 and 3.27 (2 x *dd*, 1H, *J* 13.8, 5.6 Hz, CH_2-Ar); 3.75 (*s*, 3H, CH_3O_2C); 4.31 (*m*, 2H, CH_2-N); 5.07 (*dt*, 1H, *J* 7.4, 5.6 Hz, αC-H); 6.67 (*d*, 1H, *J* 7.4 Hz, N-H); 7.04 (*d*, 2H, *J* 8.2 Hz, C3'-H and C5'-H); 7.25-7.33 (*m*, 4H, C2-H, C2'-H and C6'-H); 7.38-7.52 (*m*, 5H, C4-H and **Ph**CON); 7.69-7.75 (*m*, 4H, C3-H and **Ph**CON); 8.57 (*dd*, 2H, *J* 8.0, 1.6 Hz, C1-H). ^{13}C NMR: 19.3, 26.8, 27.1, 28.6, 28.8, 29.0, 29.2, 29.4, 37.6, 46.1, 52.4, 53.4 (alkyl), 80.2, 90.6 (alkynyl), 114.5, 121.1, 122.4, 122.8, 126.9, 127.9, 128.6, 129.2, 131.6, 131.8, 133.7, 133.8, 135.3, 141.7 (aryl), 166.7, 171.8, 177.9 (carbonyl).

N-Benzoyl-4-{[11-(10-(10*H*)-9-acridonyl)]undec-1-ynyl}-L-phenylalanine methyl ester (118): Method B.

To a stirred mixture of DMF (1.0 ml) and Et_3N (0.2 ml) was added sequentially the iodide **109** (50mg, 0.12 mmol), alkyne **69** (63mg, 0.18 mmol, 1.5 eq), $Pd(PPh_3)_4$ (14mg, 0.012mmol, 0.1eq), and CuI (4.5mg, 0.024mmol, 0.2eq). After stirring overnight at room temperature TLC (EtOAc/hexanes 50/50) showed the absence of of **109** at Rf 0.65 and new compounds at Rf 0.51 and Rf 0.37. The mixture was separated by flash chromatography using EtOAC/hexanes 40/60 as eluant. First to elute was the alkyne dimer **114** in 2.0mg yield. [^{1}H NMR (300 MHz, δ ppm): 1.23-1.54 (*m*, 6H, methylene protons); 1.95 (*quintet*, 1H, *J* 7.8 Hz, CH_2CH_2-N); 2.24 (*t*, 1H, *J* 6.7 Hz, CH_2-≡); 4.33 (*m*, 1H, CH_2-N); 7.29 (*t*, 2H, *J* 7.1 Hz, C2-H); 7.49 (*d*, 2H, *J* 8.7 Hz, C4-H); 7.74 (*ddd*, 2H, *J* 8.7, 7.1, 1.7 Hz, C3-H); 8.59 (*dd*, 2H, *J* 8.1, 1.7 Hz, C1-H.)] Next to elute was the title compound, which after recrystallisation from CH_2Cl_2/hexanes in 74mg (96%) yield. The ^{1}H NMR data were identical with those reported in the previous method.



114

Compounds **119** (Method A), **120, 121, 122, 123, 124** and **125** were prepared in a similar manner, except for the difference stated.

Attempted formation of 118 by reaction of triflate 112 with alkyne 69 using Pd_2dba_3/$AsPh_3$ catalyst.

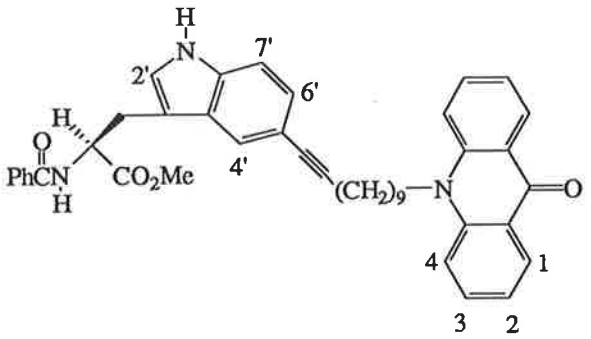
A mixture of Pd_2dba_3 (24mg, 0.026 mmol), $AsPh_3$ (52mg, 0.176 mmol) and DMF (3ml) was stirred at room temperature until a yellow-brown solution was formed, then triflate **112** (100mg, 0.23 mmol), alkyne **69** (200mg, 0.579mmol), CuI (18mg, 0.095mmol) and Et_3N (100µl, 0.69mmol) were added and the reaction mixture stirred at room temperature for 60 minutes. TLC analysis showed the absence of product, so the temperature was increased to

50° and stirring continued overnight. TLC analysis then showed the absence of triflate, no spot for coupled product **118** and a large spot corresponding to the coupled alkyne **114.**

Attempted formation of 118 by reaction of triflate 112 with alkyne 69 using piperidine. A mixture of triflate **112** (100mg, 0.23mmol), alkyne **69** (96 mg, 0.28 mmol, 1.2 eq), $Pd(PPh_3)_4$ (20mg, 0.017 mmol), CuI (20mg, 0.11 mmol), PPh_3 (20 mg, 0.08 mmol) and piperidine (4ml) was refluxed for 4 hours, at which time TLC showed the absence of triflate. The reaction mixture was diluted with CH_2Cl_2 (25ml), the organic layer washed with 10% HCl (2 x 20ml), water (20ml), dried (Na_2SO_4) and solvent removed. TLC analysis showed the presence of starting alkyne **69** and alkyne dimer **114**, and the absence of the coupled product **118.**

***N*-benzoyl-5-{11-[10-(10*H*)-9-acridonyl]undec-1-ynyl}tryptophan methyl ester (119):**

Method A



Reaction of triflate **113** (23mg, 49 μmol) with alkyne **69** (32mg, 92μmol) under the conditions described for compound **118** (Method B) at 70° overnight, removal of solvents *in vacuo*, and separation by flash chromatography, gradient eluant 40/60 to 60/40 EtOAc/hexanes gave the title compound in 7.3mg (21%) yield. MS: 689 ($M+H^+$, 35), 453 (100). ^{1}H NMR: 1.40-1.62 (*m*, 12H, methylene protons); 1.92 (*quintet*, 2H, *J* 7.6 Hz, CH_2CH_2-N); 2.39 (*t*, 2H, *J* 6.9 Hz, CH_2-≡); 3.38 (2 x *dd*, 1H, *J* 15.0, 5.2 Hz, CH_2-Ar); 3.72 (*s*, 3H, CH_3O_2C); 4.30 (*m*, 2H, CH_2-N); 5.12 (*dt*, 1H, *J* 7.6, 5.2 Hz, αC-H); 6.70 (*bd*, 1H, *J* 7.6 Hz, PhCONH); 6.97 (*d*, 1H, *J* 2.3 Hz, C4'-H); 7.18 (*d*, 2H, *J* 0.9 Hz, Ar-H); 7.27 (*t*, 2H, *J* 7.5 Hz, C2-H); 7.35-7.47 (*m*, 4H, Ar-H); 7.48 (*d*, 2H, *J* 8.1 Hz, C4-H); 7.62 (*s*, 1H, C4-H);

7.69-7.75 (*m*, 4H, Ar-H); 8.50 (*bs*, 1H, N1'-H); 8.57 (*dd*, 2H, *J* 8.1, 1.8 Hz, C1-H). ^{13}C NMR: 19.4, 26.8, 27.1, 27.5, 28.8, 28.8, 28.9, 29.2, 29.3, 46.2, 52.4, 53.4 (alkyl), 81.5, 87.5 (alkynyl), 109.9, 111.3, 114.6, 115.1, 121.2, 122.3, 122.4, 123.7, 125.7, 127.1, 127.4, 127.9, 128.5, 131.6, 133.8, 133.9, 135.3, 141.7 (aryl), 167.0, 172.2, 179.5 (carbonyl).

_N_-benzoyl-5-{11-[10-(10*H*)-9-acridonyl]undec-1-ynyl}tryptophan methyl ester (119):

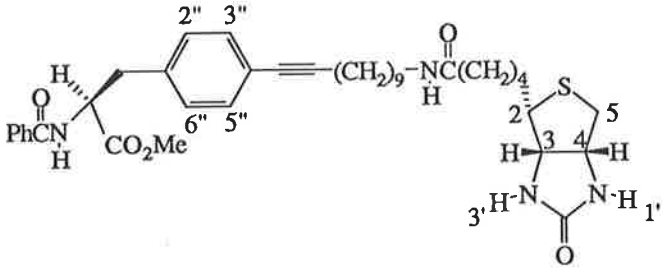
Method B

A mixture of triflate **113** (100mg, 0.21 mmol), alkyne **69** (110mg, 0.32 mmol, 1.5 eq), $Pd(PPh_3)_4$ (25mg, 0.021 mmol, 0.1 eq), CuI (8.1mg, 0.042 mmol, 0.2 eq), PPh_3 (11mg, 0.042 mmol, 0.2 eq) and piperidine (5ml) was refluxed for 5 hours, at which time TLC showed the absence of the triflate. The solvent was removed *in vacuo* and the residue subjected to flash chromatography using eluant 40/60 EtOAc/hexanes. First compound to elute was the acridone label dimer **114** in 45mg yield. Increasing the eluant polarity to 60/40 eluted the title compound in 10.8mg (8%) yield, with ^{1}H NMR data identical with those given in the previous procedure.

Attempted formation of 119 by reaction between triflate 113 and alkyne 69 using 1 equivalent tetrakistriphenylphosphine palladium

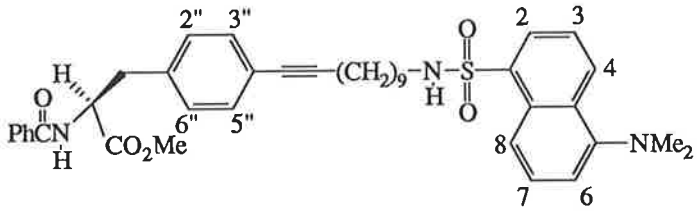
To a stirred solution of triflate **113** (50mg, 0.107 mmol) in DMF (2 ml) was added $Pd(PPh_3)_4$ (123mg, 0.107 mmol, 1 eq). A green suspension slowly formed. TLC analysis of the reaction mixture after 2.5 hours showed the absence of triflate at $R_f = 0.24$ and a new spot at $R_f = 0.18$ (50/50 EtOAc/hexanes). Triethylamine (0.5ml), CuI (4.1mg, 0.02 mmol, 0.2 eq) and alkyne **69** (37mg, 0.107 mmol, 1eq) were added and stirring continued. TLC analysis of the dark brown reaction mixture after 30 minutes showed the absence of the green intermediate, formation of the alkyne dimer **114** ($R_f = 0.45$) and unreacted alkyne **69** ($R_f = 0.80$). No fluorescent spot at *ca* $R_f = 0.10$ corresponding to coupled product **118** was observed.

N-Benzoyl-4-[(N-biotinyl)-11-aminoundec-1-ynyl]-L-phenylalanine methyl ester (120)



Eluant 5/95 MeOH/CH_2Cl_2; recrystallisation from MeOH; light yellow crystals mp 142°; (84%) yield. HRMS Calculated for $C_{38}H_{50}N_4O_5S$: 674.3502. Found: 674.3520. MS: 674 (M^+, 9), 615 (11), 521 (14), 461 (12), 227 (12), 105 (100). IR (nujol): 3296*br* (N-H), 1742*m*, 1706*s* and 1642*s* (C=O), 1538*s*. ^{1}H NMR: 1.29-1.76 (*m*, 22H, methylene protons); 2.17 (*t*, 2H, *J* 7.5 Hz, CH_2CON); 2.38 (*t*, *J* 6.9 Hz, CH_2-≡-Ar); 2.76 (*d*, 1H, J_{gem} 12.8 Hz, C5-Hb); 2.86 (*dd*, 1H, J_{gem} 12.8, 4.9 Hz, C5-Ha); 3.09-3.31 (4H, *m*, CH_2-Ar and CH_2NHCO); 3.75 (*s*, 3H, CH_3O_2C); 4.28 (*dd*, 1H, *J* 7.2, 4.5 Hz, C4-H); 4.48 (*dd*, 1H, *J* 7.6, 4.9 Hz, C3-H); 5.06 (*dt*, 1H, *J*7.4, 5.8 Hz, αC-H); 5.55 (*bs*, 1H, N1'-H); 6.01 (*t*, 1H, *J* 5.6 Hz, CH_2-NHC=O); 6.33 (*bs*, 1H, N3'-H); 6.85 (*d*, 1H, *J* 7.5 Hz, C2-NHCO); 7.06 (*d*, 2H, *J* 8.1 Hz, C3-H and C5-H); 7.31 (*d*, 2H, *J* 8.1 Hz, C2-H and C6-H); 7.39-7.75 (*m*, 5H, **Ph**CONH). ^{13}C NMR: 19.4, 25.7, 26.9, 28.1, 28.2, 28.7, 28.8, 29.1, 29.3, 29.4, 29.6, 36.0, 37.6, 39.5, 40.5, 46.2, 52.5, 53.5, 55.6, 60.2, 61.8 (alkyl), 80.2, 90.8 (alkynyl), 122.9, 127.1, 128.6, 129.2, 131.7, 131.8, 133.7, 135.4 (aryl), 163.8, 166.9, 172.2, 173.1 (carbonyl).

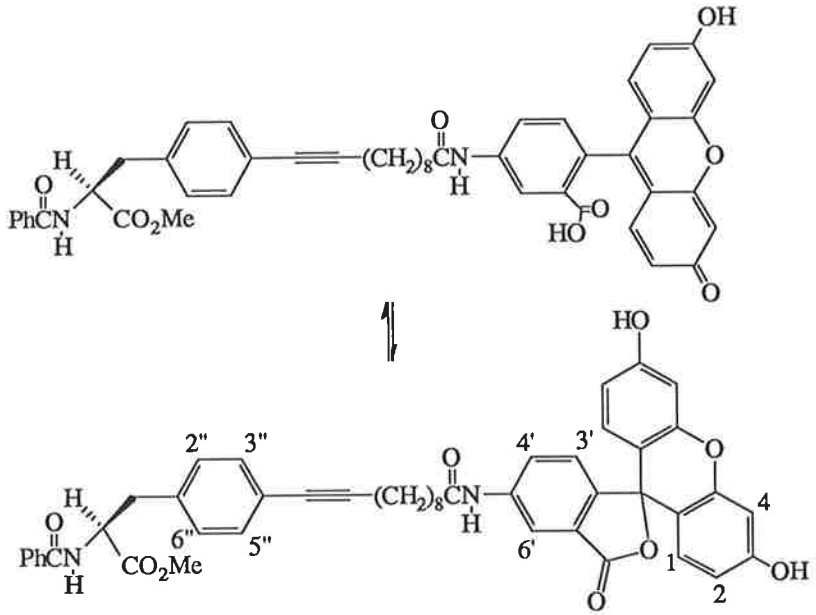
N-benzoyl-4-[N-(5-dimethylamino-1-naphthalenesulphonyl)-11-aminoundec-1-ynyl]-L-phenylalanine methyl ester (121)



Eluant 40/60 EtOAc/hexanes; fluorescent green viscous oil; 82% yield. Calculated for $C_{40}H_{47}N_3O_5S$: 681.3236. Found: 681.3253. MS: 681 (M^+, 68), 649 (12), 560 (22), 203 (55), 169 (100), 105 (79). IR (thin film): 3300*br m* (N-H), 1736*s*, 1652*s* (C=O), 1578*s*, 1512*s*, 1316*s* and1144*s* (O=S=O), 910*s*, 738*s*. UV (EtOH): 227 (36 200), 247 (39 600), 338

(5 700). Fluorescence (EtOH, λ_{ex} = 338 nm): 503 nm. ^{1}H NMR: 1.30-1.21 (*m*, 12H, methylene protons); 1.47 (*quintet*, 2H, *J* 7.3 Hz, CH_2-CH_2-$NHSO_2$); 2.29 (*t*, 2H, *J* 7.1 Hz, CH_2-≡-Ar); 2.79 (*q*, 2H, *J* 6.5 Hz, CH_2-$NHSO_2$); 2.80 (*s*, 6H, $(CH_3)_2$-N); 3.13 and 3.21 (2 x *dd*, 1H, *J* 13.8, 5.6 Hz, C3-H); 3.69 (*s*, 3H, CH_3-O_2C); 4.58 (*t*, 1H, *J* 6.5 Hz, SO_2NH-CH_2); 5.01 (*dt*, 1H, *J* 7.5, 5.6 Hz, αC-H); 6.53 (*d*, 1H, *J* 7.5 Hz, CONH); 6.97 (*d*, 2H, *J* 8.2 Hz, C3'-H and C5'-H); 7.10 (*d*, 1H, *J* 7.4 Hz, C6-H); 7.24 (*d*, 2H, *J* 8.2 Hz, C2'-H and C6'-H); 7.32-7.51 (*m*, 5H, Ar-H); 7.63-7.67 (*m*, 2H, Ar-H); 8.17 (*dd*, 1H, *J* 7.5, 1.1 Hz, C3-H); 8.21 (*d*, 1H, *J* 8.6 Hz, C4-H); 8.46 (*d*, 1H, *J* 7.5 Hz, C2-H). ^{13}C NMR: 19.3, 26.3, 28.6, 28.7, 28.8, 28.9, 29.1, 29.4, 37.7, 43.2, 45.4, 52.4, 53.4 (alkyl), 80.2, 90.7 (alkynyl), 115.1, 118.7, 122.9, 123.1, 127.0, 128.3, 128.6, 129.2, 129.2, 129.6, 129.8, 130.3, 131.7, 131.8, 133.7, 134.7, 135.3, 152.0 (aryl), 166.8, 171.9 (carbonyl).

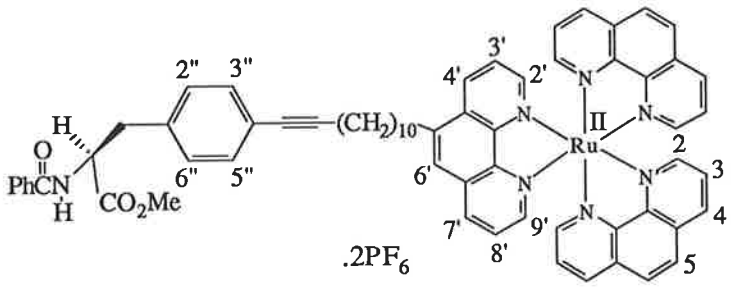
_N_-Benzoyl-4-[11-(_N_-5'-fluoresceinyl)-11-carbonylaminoundec-1-ynyl]-L-phenylalanine methyl ester (122)



Eluant 5/95 MeOH/CH_2Cl_2; bright orange glass; 75%. HRMS (LSIMS) Calculated for $C_{48}H_{45}N_2O_9$ ($M+H^+$): 793.3125. Found: 793.3096. MS: 793 ($M+H^+$, 100), 594 (19), 402 (22), 347 (18). IR ($CDCl_3$): 3500-3000*br w* (Ar-OH), 3080*w* (Ar-H), 1742*s* and 1644*s* (C=O), 1602*s* (Ar C=C). UV (EtOH): 231 (39 900), 257 (31 100), 457 (5 600), 483 (5 900). Fluorescence (EtOH, λ_{ex} = 483 nm): 518 nm. *The NMR data are for the lactone structure.*

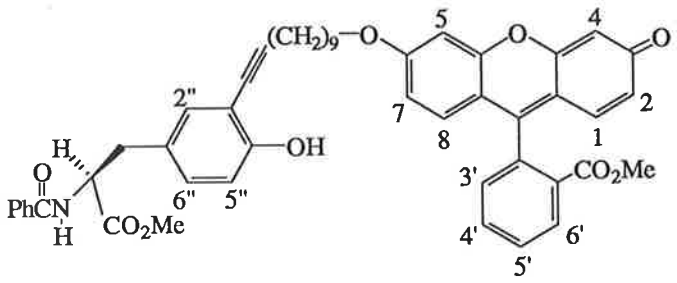
^{1}H NMR (d_6-DMSO): 1.21-1.61 (*m*, 12H, methylene protons); 2.36 (*t*, 2H, *J* 6.7 Hz, CH_2-≡-Ar); 3.08 and 3.16 (2 x *dd*, 1H, *J* 13.7, 5.4 Hz, CH_2-Ar); 3.63 (*s*, 3H, CH_3O_2C); 4.64 (*dt*, 1H, *J* 7.9, 5.4 Hz, αC-H); 6.52 (*dd*, 2H, *J* 8.7, 1.9 Hz, C2-H); 6.58 (*d*, 2H, *J* 8.7 Hz, C1-H); 6.61 (*d*, 2H, *J* 1.9 Hz, C4-H); 7.17 (*d*, 1H, *J* 8.4 Hz, C3'-H); 7.26 (*bs*, 4H, C2"-H, C3"-H, C5"-H, C6"-H); 7.41-7.54 (*m*, 3H, **Ph**CON); 7.77 (*m*, 2H, **Ph**CON); 7.82 (*dd*, 1H, *J* 8.4, 1.6 Hz, C4'-H); 8.33 (*d*, 1H, *J* 1.6 Hz, C6'-H); 8.85 (*d*, 1H, *J* 7.9 Hz, PhCON**H**); 10.20 (*bs*, 2H, Ar-OH); 10.40 (*bs*, 1H, 5'-NH). ^{13}C NMR (d_6-DMSO): 18.6, 25.0, 28.2, 28.3, 28.4, 28.6, 28.7, 29.0, 36.0, 36.5, 54.0, 51.9 (alkyl), 80.5, 83.0, 90.4, 102.1, 109.8, 112.8, 121.5, 126.2, 127.2, 127.6, 128.3, 129.1, 129.5, 130.9, 131.3, 131.5, 133.6, 137.6, 140.9, 152.0, 159.9 (aryl), 166.4, 168.7, 172.0, 172.1 (carbonyl).

_N_-Benzoyl-4-(12-{5-[1,10-phenanthroline-*bis*-1,10-phenanthrolineruthenium(II)]dodec-1-ynyl})phenylalanine methyl ester hexafluorophospate (123)



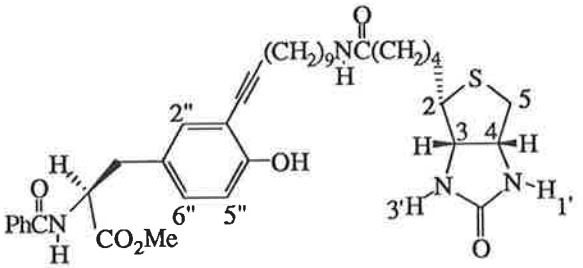
Chromatography on alumina with eluant $CHCl_3$; bright orange glass; 65%. MS (LSIMS): 1232 ([^{102}RuM-PF_6]$^+$, 82), 1087 (^{102}RuM-2PF_6, 100). UV ($CHCl_3$): 263 (87 600), 418 (12 000), 451 (12 800). Fluorescence ($CHCl_3$, λ_{ex} = 451 nm): 573 nm. ^{1}H NMR (300 MHz, δ ppm): 1.22-1.61, *m*, 14H, methylene protons; 1.86, *quintet*, 2H, *J* 7.2 Hz, CH_2-CH_2-Ar; 2.38, *t*, 2H, *J* 6.9 Hz, CH_2-≡-Ar; 3.17-3.31, *m*, 4H, CH_2-Ar and CH_2-phen; 3.76, *s*, 3H, CH_3O_2C; 5.05, *dt*, 1H, *J* 7.3, 5.8 Hz, C-H; 6.58, *bd*, 1H, *J* 7.3 Hz, PhCONH; 7.05, *d*, 2H, *J* 8.0 Hz, C3"-H and C5"-H; 7.31, *d*, 2H, *J* 8.0 Hz, C2"-H and C6"-H; 7.40-7.86, *m*, H; 7.89, *s*, 1H, C6-H; 8.04, *d*, 1H, *J* 5.3 Hz, Cx-H; 8.10-8.13, *m*, 8H; 8.35, *d*, 1H, *J* 8.2 Hz, C2'-H; 8.45, *d*, 4H, *J* 8.2 Hz, C2-H and C9-H; 8.57, *d*, 1H, *J* 8.6 Hz, C9'-H.

_N_-Benzoyl -3-({11-[6-_O_-(methyl)fluoresceinyl]}undec-1-ynyl)-L-tyrosine methyl ester (124)



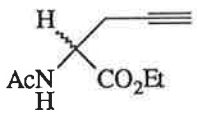
Eluant 8/92 MeOH/CH_2Cl_2; bright orange glass; 91% yield. HRMS Calculated for $C_{49}H_{47}NO_9$: 793.3251. Found: 793.3241. MS: 793 (M^+, 31), 734 (100), 673 (37), 601 (30), 326 (78). IR ($CDCl_3$): 3436*w* (N-H), 1726*s* (C=O ester), 1644*s* (C=O amide), 1598*s*, 1516*s*. UV (EtOH): 225 (72 500), 253 (36 200), 276 (20 600), 301 (13 900), 365 (9 200), 441 (23 700), 460 (33 000), 489 (26 100). Fluorescence (EtOH, λ_{ex} = 489 nm): 521 nm. ^{1}H NMR: 1.35-1.50 (*m*, 10H, methylene protons); 1.65 (*quintet*, 2H, *J* 7.2 Hz, CH_2CH_2-≡); 1.86 (*quintet*, 2H, *J* 6.8 Hz, CH_2CH_2-OAr); 2.48 (*t*, 2H, *J* 7.0 Hz, CH_2-≡-Ar); 3.13 and 3.21 (2 x dd, 1H, *J* 14.0, 5.6 Hz, CH_2-Ar); 4.09 (*t*, 2H, *J* 6.5 Hz, CH_2-OAr); 5.05 (*dt*, 1H, *J* 7.3, 5.5 Hz, αH-C); 6.12 (*bs*, 1H, Ar-OH); 6.49 (*d*, 1H, *J* 1.9 Hz, C4-H); 6.57 (*dd*, 1H, *J* 9.7, 1.9 Hz, C2-H); 6.65 (*bd*, 1H, *J* 7.3 Hz, PhCONH); 6.75 (*dd*, 1H, *J* 8.9, 2.4 Hz, C7-H); 6.86-7.00 (*m*, 5H, C1-H, C5-H, C8-H, C5"-H, C6"-H); 7.11 (*d*, 1H, *J* 2.1 Hz, C2"-H); 7.33 (*dd*, 1H, *J* 7.3, 1.2 Hz, C3'-H); 7.42-7.56 (*m*, 3H, PhCON); 7.68 (*dt*, 1H, *J* 7.5, 1.4 Hz, C5'-H); 7.73 (*dd*, 1H, *J* 5.9, 1.5 Hz, C4'-H); 7.75-7.79 (*m*, 2H, PhCON); 8.27 (*dd*, 1H, *J* 7.6, 1.3 Hz, C6'-H). ^{13}C NMR: 19.6, 25.9, 28.4, 28.9, 29.0, 29.1, 29.2, 29.3, 36.9, 52.4, 53.7, 68.9 (alkyl), 74.9, 97.3 (alkynyl), 100.7, 105.7, 110.7, 114.0, 114.6, 114.8, 117.4, 127.1, 127.2, 128.4, 128.6, 128.8, 129.7, 129.9, 130.0, 130.3, 130.3, 130.5, 131.1, 131.7, 132.5, 132.7, 133.9, 134.6, 150.7, 154.4, 156.1, 159.0, 163.8 (aryl), 165.6, 166.9, 172.0, 185.6 (carbonyl).

N-Benzoyl-3-[N-(biotinyl)-11-aminoundec-1-ynyl]-L-tyrosine methyl ester (125)



Eluant 8/92 MeOH/CH_2Cl_2; 76%; light yellow crystals mp 88-90°. HRMS Calculated for $C_{38}H_{50}N_4O_6S$: 690.3451. Found: 690.3464. MS: 690 (M^+, 14), 631 (14), 569 (12), 537 (33), 477 (50), 413 (37), 105 (100). IR ($CDCl_3$): 3500*br w* (N-H), 1706*s* and 1656*s* (C=O), 1470*s*. ^1H NMR: 1.26-1.73 (*m*, 20H, methylene protons); 2.17 (*t*, 2H, *J* 7.4 Hz, CH_2-C=O); 2.45 (*t*, 2H, *J* 6.8 Hz, CH_2-≡-Ar); 2.68 (*d*, 1H, J_{gem} 12.8 Hz, C5-Hb); 2.86 (*dd*, 1H, J_{gem} 12.8, 4.8 Hz, C5-Ha); 3.07-3.24 (*m*, 5H, C2-H, CH_2-NHCO and CH_2-Ar); 3.78 (*s*, 3H, CH_3O_2C); 4.27 (*dd*, 1H, *J* 7.5, 4.8 Hz, C4-H); 4.46 (*dd*, 1H, *J* 7.5, 4.9 Hz, C3-H); 4.99 (*dt*, 1H, *J* 7.5, 5.9 Hz, C2'-H); 5.46 (*bs*, 1H, N1'-H); 6.18 (*t*, 1H, *J* 5.6 Hz, CH_2-NHCO); 6.26 (*bs*, 1H, N3'-H); 6.74 (*bs*, 1H, Ar-OH); 6.85 (*d*, 1H, *J* 8.4 Hz, C5"-H); 6.92 (*d*, 1H, *J* 7.5 Hz, NHCOAr); 6.96 (*dd*, 1H, *J* 8.4, *J* 2.1 Hz, C6"-H); 7.10 (*d*, 1H, *J* 2.1 Hz, C2"-H); 7.40-7.53 (*m*, 3H) and 7.74-7.77 (*m*, 2H, PhCONH). ^{13}C NMR: 19.5, 25.6, 26.8, 28.0, 28.1, 28.5, 28.6, 28.8, 29.0, 29.2, 29.5, 35.9, 36.8, 39.5, 40.5, 50.8, 52.4, 53.8, 55.4, 60.1, 61.8 (alkyl), 75.0, 97.2 (alkynyl), 110.7, 114.8, 127.1, 127.3, 128.6, 130.2, 131.8, 132.5, 133.8, 156.0 (aryl), 163.7, 167.1, 172.3, 173.2 (carbonyl).

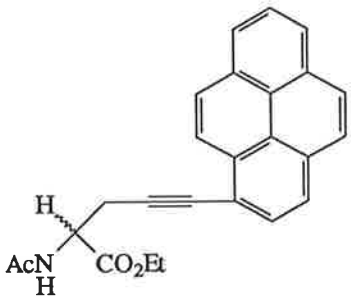
Ethyl 2-acetamidopent-4-ynoate (126)



A solution of diethyl acetamido malonate (30.1g, 0.139 mol) in DMF (50ml) was added dropwise over 90 minutes to a cooled (ice bath) suspension of hexane washed NaH (80% in mineral oil, 6.90g, 0.173 mol, 1.25 eq). The mixture was stirred at room temperature for 30 minutes, then cooled in an ice bath and propargyl bromide (80% wt. in toluene, 24.7g, 0.166 mol, 1.2 eq) was added dropwise over 60 minutes. After stirring at 70° overnight, TLC analysis (50/50 EtOAc/hexanes) showed a trace of starting material at R_f 0.32 and a large spot

corresponding to product at R_f 0.56. The dark brown reaction mixture was cooled to room temperature, filtered to remove precipitated inorganic salts (Buchner), LiCl (5.88g, 0.139 mmol, 1.0eq) and water (2.49g, 0.139 mmol, 1.0eq) were added and the mixture stirred overnight at 145°. After cooling to room temperature, the reaction mixture was poured into water (500ml), the aqueous mixture extracted with CH_2Cl_2 (250ml), the organic extract washed with water (3 x 250ml), dried ($MgSO_4$) and solvent removed. Residual DMF was removed under vacuum (oil pump), the residue separated by squat chromatography eluant 50/50 EtOAc/hexanes and recrystallised from CH_2Cl_2/hexanes to give the title compound as colourless needle crystals mp 71-72° (lit.[48] 73°) in 7.24g (28%) yield. MS: 183 (M^+, 5), 144 (22), 110 (66), 104 (59), 68 (100). IR (nujol): 3312*s* (N-H), 3264*s* (H-C≡), 1728*s* (C=O ester), 1634*s* (C=O amide), 1554*s*, 1232*s*. ^{1}H NMR: 1.30 (*t*, 3H, *J* 7.0 Hz, CH_3-CH_2-); 2.03 (*t*, 1H, *J* 2.7 Hz, H-C≡); 2.06 (*s*, 3H, CH_3CO); 2.78 (2H, *dd*, *J* 4.5, 2.6 Hz, CH_2-C≡); 4.25 (*m*, 2H, *J* 7.2 Hz, -O**C**H_2CH_3); 4.72 (*dt*, 1H, *J* 7.8, 4.6 Hz, αCH-); 6.33 (*d*, 1H, *J* 5.6 Hz, N-H). ^{13}C NMR: 14.0, 22.3, 22.3, 50.5, 61.8 (alkyl), 71.4, 78.4 (alkynyl), 169.7, 170.3 (carbonyl).

Ethyl 5-(1-pyrenyl)-2-acetamidopent-4-ynoate (127): Method A.



A mixture of the alkyne **126** (100mg, 0.55 mmol, 1.5 eq), 1-bromopyrene (102mg, 0.37 mmol, 1.0 eq), $Pd(PPh_3)_4$ (63mg, 0.055 mmol, 0.15 eq), CuI (21mg, 0.011 mmol, 0.3 eq), Et_3N (1ml) and DMF (2ml) was stirred overnight at 50°, at which time the catalyst had decomposed. The solvent was removed under vacuum (oil pump), the residue separated by flash chromatography eluant 60/40 EtOAc/hexanes and recrystallised from CH_2Cl_2/hexanes to give the title compound as cream crystals mp 155-156° in 63mg (44%) yield. HRMS Calculated for $C_{25}H_{21}NO_3$: 383.1521. Found: 383.1509. MS: 383 (M^+, 11), 323 (71), 238 (100). IR (nujol): 3320s (N-H), 1736 (C=O ester), 1644 (C=O amide). ^{1}H NMR; 1.35 (*t*, J

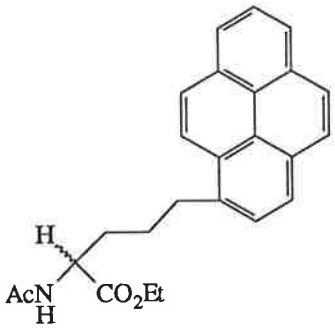
7.4 Hz, CH_3-CH_2); 2.12 (*s*, 3H, CH_3-CON); 3.25 (*d*, 2H, *J* 4.7 Hz, CH_2-C≡); 4.36 (*m*, 2H, OCH_2CH_3); 4.95 (*dt*, 1H, *J* 7.5, 4.7 Hz, αC-H); 6.57 (*d*, 1H, *J* 7.5 Hz, N-H); 7.99-8.49 (*m*, 9H, Ar-H). ^{13}C NMR: 14.3, 23.3, 24.1, 51.2, 62.1 (alkyl), 82.6, 89.5 (alkynyl), 124.4, 125.1, 125.3, 125.5, 126.2, 126.8, 127.2, 127.8, 128.1, 128.3, 129.7, 130.3, 130.4, 131.0, 131.1, 132.0 (aromatic), 169.9, 170.8 (carbonyl).

When the reaction was repeated using 1-iodopyrene the yield of product was 72%.

Ethyl 5-(1-pyrenyl)-2-acetamidopent-4-ynoate (127): Method B.

A mixture of the alkyne **125** (78mg, 0.43 mmol, 1.2 eq), 1-bromopyrene (100mg, 0.36 mmol, 1.0 eq), $Pd(PPh_3)_4$ (41mg, 0.036 mmol, 0.1 eq), CuI (13mg, 0.072mmol, 0.2 eq), PPh_3 (19mg, 0.072 mmol, 0.2 eq) and piperidine (10ml) was refluxed for 60 minutes, at which time analytical TLC showed the absence of the aryl bromide. The solvent was removed under reduced pressure and the residue separated by flash chromatography eluant 60/40 EtOAc/hexanes to give the title compound as white crystals in 76mg (51%) yield. After recrystallisation from CH_2Cl_2/hexanes the physical data were identical with that from the previous method. When the reaction was repeated using 1-iodopyrene the yield was 70%.

Ethyl 5-(1-pyrenyl)-2-acetamidopentanoate (129)



A mixture of the alkyne **126** (294mg, 0.77mmol), 5% Pd/C (100mg) and EtOAc (40ml) was stirred under a hydrogen atmosphere overnight. The reaction mixture was filtered through celite, the solvent removed and the residue recrystallised from EtOAc/hexanes to give the title compound as white microneedle crystals mp 155-156° in 278mg (94%) yield. HRMS Calculated for $C_{25}H_{25}NO_3$: 387.1834. Found: 387.1824. MS: 387 (M^+, 68), 255 (27), 228 (32), 215 (100), 149 (22). IR (nujol): 3316*s* (N-H), 1748*s* (C=O ester), 1650*s* (C=O amide),

844*s*. UV (EtOH): 205 (12 200), 234 (19 700), 256 (6 300), 266 (13 500), 278 (20 200), 302 (2 600), 313 (6 100), 327 (12 300), 343 (14 800). Fluorescence (EtOH, λ_{ex} = 343 nm): 375 (100), 395 (61), 415 (19). ^{1}H NMR: 1.19 (*t*, 3H, *J* 7.1 Hz, CH_3-CH_2); 1.75-2.05 (*m*, 4H, C4-H and C5-H); 2.00 (*s*, 3H, CH_3-CO); 3.26-3.42 (*m*, 2H, C5-H); 4.14 (*q*, 2H, *J* 7.1 Hz, O-CH_2-CH_3); 4.70 (*dt*, 1H, *J* 7.7, 6.7 Hz, C2-H); 5.98 (*d*, 1H, *J* 7.7 Hz, CONH); 7.82-8.26 (*m*, 9H, Ar-H). ^{13}C NMR: 14.1, 23.2, 27.2, 32.5, 32.9, 52.0, 61.5 (alkyl), 123.3, 124.7, 124.8, 124.9, 125.9, 126.7, 127.2, 127.3, 127.5, 128.6, 131.4, 135.9 (aryl), 169.8, 172.6 (carbonyl).

Experimental Described in Chapter 3.2.

Acetylation of Nucleosides: General Method.

To a cold (0°) stirred solution of the nucleoside in pyridine (10ml/g) was added acetic anhydride (2 eq. or 3 eq. as required) dropwise over 30 minutes. The icebath was removed and the mixture stirred overnight. After removal of solvent *in vacuo* the residue was dissolved in $CHCl_3$, washed with water, dried ($MgSO_4$) and solvent removed. The residue was purified by recrystallisation to give the protected nucleoside.

5-Iodo-3',5'-di-*O*-acetyldeoxyuridine (131)

Reaction of **51** (1.00g, 2.8 mmol) under standard protection conditions and recrystallisation from EtOH gave the title compound as colourless crystals mp 163° (lit.[133] 163-164°) in 1.18g (95%) yield. MS (FAB): 439 ($M+H^+$, 7), 413 (18), 207 (31), 115 (78), 93 (100). ^{1}H NMR: 2.11 and 2.21 (*s*, 3H, $CH_3C=O$); 2.15-2.22 (*m* (obscured), 1H, C2'-H); 2.55 (*ddd*, 1H, J_{gem} 14.3, 5.6, 2.0 Hz, C2'-H); 4.29-4.45 (*m*, 3H, C4'-H and C5'-H); 5.24 (*dt*, 1H, *J* 6.5, 2.0 Hz, C3'-H); 6.30 (*dd*, 1H, *J* 8.2Hz, 5.6 Hz, C1'-H); 7.98 (*s*, 1H, C6-H); 8.44 (*bs*, 1H, N5-H). ^{13}C NMR: 19.8, 20.0 ($CH_3C=O$), 36.6, 62.8, 68.6, 73.2, 81.3 (alkyl), 84.2, 142.8, 149.1 (aromatic), 159.4, 169.0, 169.1 (carbonyl).

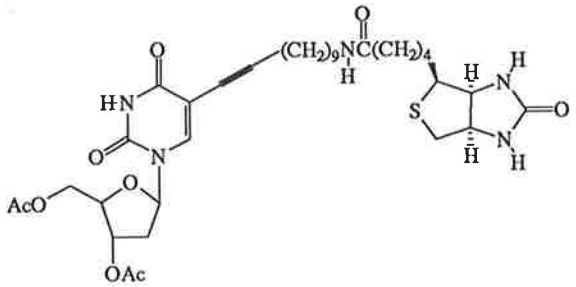
8-Bromo-2',3',5'-tri-*O*-acetyladenosine (132)

Reaction of **52** (500mg, 1.4 mmol) under standard protection conditions and recrystallisation from THF gave the title compound as colourless crystals mp 186-187° (lit[134]. 187-188° dec.) in 0.63g (92%) yield. MS (FAB): 472/474 (1:1, $M+H^+$, 64), 389 (45), 258 (100). ^{1}H NMR: 2.05, 2.12 and 2.16 (3 x *s*, 3H, $CH_3C=O$); 4.30-4.42 (*m*, 2H, C4'-H, C5'-Hb); 4.53 (*dd*, 1H, J_{gem} 11.2, 3.0 Hz. C5'-Ha); 5.66 (*bs*, 2H, C5-NH_2); 5.95 (*t*, 1H, *J* 6.0 Hz, C3'-H); 6.11 (*d*, 1H, *J* 4.3 Hz, C1'-H); 6.35 (*dd*, 1H, *J* 6.0, 4.3 Hz, C2'-H); 8.32 (*s*, 1H, C4-H). ^{13}C NMR (d_6-DMSO): 19.3, 19.4, 19.5 (CH_3CO), 61.6, 69.0, 70.6, 78.7, 87.2 (alkyl), 119.3, 125.2, 149.3, 152.0, 153.9 (aryl), 168.2, 168.3, 169.1 (carbonyl).

8-Bromo-2',3',5'-tri-*O*-acetylguanosine (133)

Reaction of **53** (1.00g, 2.5 mmol) under standard protection conditions and recrystallisation from acetone/water gave the title compound as colourless crystals mp 214-217° (lit.[134] 216-218°) in 1.01g (82%) yield. MS (FAB): 488/490 (1:1, M+H$^+$, 16), 259 (100). ^{1}H NMR: 1.72, 1.77, 1.79 (3 x *s*, 3H, CH_3CO); 3.95-4.04 (*m*, 2H, C4'-H and C5'-Hb); 4.18 (*m*, 1H, C5'-Ha); 5.58 (*t*, 1H, *J* 5.9 Hz, C3'-H); 5.62 (*d*, 1H, *J* 4.3 Hz, C1'-H); 5.64 (*bs*, 2H, C2-NH_2); 5.85 (*dd*, 1H, *J* 5.9, 4.3 Hz, C2'-H); 10.38 (*bs*, 1H, N1-H). ^{13}C NMR (d_6-DMSO): 19.0, 19.1, 19.2 (CH_3CO), 61.4, 68.8, 70.3, 78.1, 86.7 (alkyl), 116.5, 119.0, 150.5, 152.4 (aryl), 154.6 (C2), 167.8, 167.9, 168.8 (carbonyl).

5-[*N*-(Biotinyl)-1-aminoundec-1-ynyl]-3',5'-di-*O*-acetyl-2'-deoxyuridine (134)

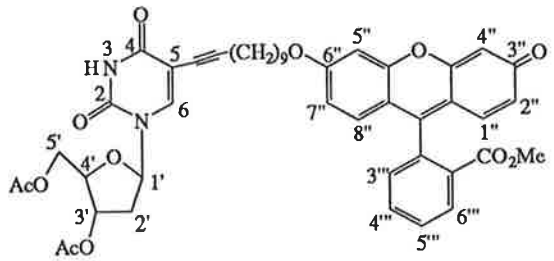


To a stirred mixture of DMF (1.0 ml) and Et_3N (14mg, 0.14 mmol, 1.2 eq) at room temperature were added sequentially 5-iodo-2',3'-di-O-acetyldeoxyuridine **131** (50mg, 0.11 mmol), the alkyne **107** (54mg, 0.14 mmol, 1.2 eq), $Pd(PPh_3)_4$ (13mg, 0.011 mmol, 0.1 eq) and CuI (4.3mg, 0.022 mmol, 0.2 eq). The mixture was stirred at room temperature until TLC indicated the absence of the nucleoside. The solvent was removed *in vacuo*, the residue separated by flash chromatography using 10/90 MeOH/CH_2Cl_2 as eluant to give the title compound as a colourless glass in 60mg (76%) yield. HRMS Calculated for $C_{34}H_{50}N_5O_9S$ (M+H$^+$): 704.3329. Found: 704.3325. MS (FAB): 704 (M+H$^+$, 31), 504 (24), 394 (100), 307 (48), 227 (29). ^{1}H NMR: 1.09-1.61 (*m*, 18H, methylene protons); 1.95 and 2.01 (2 x *s*, 3H, CH_3CO); 1.96-2.13 (*m*, 3H, CH_2CONH and C2'-Hb); 2.21 (*t*, 2H, *J* 7.0 Hz, CH_2-≡); 2.31 (*ddd*, 1H, J_{gem} 14.3, 5.9, 2.4 Hz, C2'-Ha); 2.58 (*d*, 1H, J_{gem} 12.8 Hz, C5"-Hb); 2.74 (*dd*, 1H, J_{gem} 12.8, 5.0 Hz, C5"-Ha); 2.96-3.06 (*m*, 3H, C2"-H and CH_2NHCO); 4.10-4.15 (*m*, 2H, C4'-H and C4"-H); 4.19 (*d*, 2H, *J* 3.2 Hz, C5'-H); 4.31 (*dd*, 1H, *J* 7.7, 5.1 Hz, C3"-H); 5.08

(*dt*, 1H, *J* 6.7, 3.9 Hz, C3'-H); 5.42 (*bs*, 1H, N1'-H); 5.56 (*bs*, 1H, N3'-H); 6.15 (*dd*, 1H, *J* 7.9, 5.9 Hz, C1'-H); 6.39 (*bt*, 1H, *J* 5.2 Hz, CH_2NHCO); 7.54 (*s*, 1H, C6-H); 11.20 (*bs*, 1H, N3-H). ^{13}C NMR: 18.8, 20.5, 20.7, 25.3, 26.4, 28.0, 28.2, 28.3, 28.5, 28.8, 28.9, 29.2, 35.2, 36.2, 38.3, 39.8, 45.9, 55.4, 59.2, 61.0, 63.5, 72.5, 73.8, 81.5, 84.8 (alkyl), 93.5, 99.7 (alkynyl), 142.1 (aryl), 149.3, 161.5, 162.7, 169.8, 169.9, 171.8 (carbonyl).

Compounds **136**, **137**, **138**, **139** and **141** were prepared using the appropriate alkyne in a similar manner, except for the differences stated.

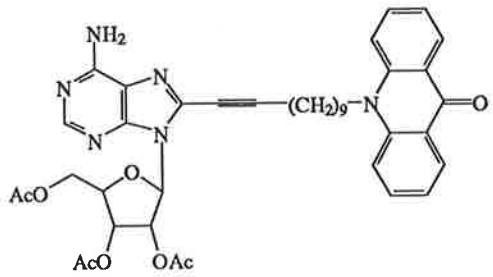
5-{11-[6-*O*-(Methyl)fluoresceinyl]-1-undec-1-ynyl}-3', 5'-di-*O*-acetyl-2'-deoxyuridine (136)



Eluant 7:93 MeOH:CH_2Cl_2; bright orange glass; 76%. HRMS Calculated for $C_{45}H_{16}N_2O_{12}$ ($M+H^+$): 807.3129. Found: 807.3115. MS (FAB): 807 ($M+H^+$, 100), 747 (10), 607 (22), 347 (80). IR ($CDCl_3$): 3400*w* (N-H), 1760-1680*s* (C=O), 1644s, 1598s. UV (EtOH): 232 (66 400), 279 (28 900), 435 (23 000), 460 (32 900), 489 (25 800). Fluorescence (EtOH) (λ_{ex} = 489 nm): 521 nm. ^1H NMR: 1.36-1.62 (*m*, 12H, methylene protons); 1.85 (*quintet*, 2H, *J* 7.2 Hz, CH_2-CH_2-O-Ar); 2.14 and 2.20 (2 x *s*, 3H, CH_3CO); 2.24 (*dd*, 1H, J_{gem} 14.4, 6.7 Hz, C2'-Hb); 2.41 (*t*, 2H, *J* 7.1 Hz, CH_2-≡-Ar); 2.54 (*ddd*, 1H, J_{gem} 14.4, 5.8 Hz, 2.4 Hz, C2'-Ha); 3.66 (*s*, 3H, CH_3-O_2C); 4.08 (*t*, 2H, *J* 6.6 Hz, CH_2-OAr); 4.30 (*q*, 1H, *J* 2.9 Hz, C4'-H); 4.38 (*d*, 2H, *J* 3.0 Hz, C5'-H); 5.26 (*dt*, 1H, *J* 6.4 Hz, 2.4 Hz, C3'-H); 6.32 (*dd*, 1H, *J* 8.0, 5.8 Hz, C1'-H); 6.47 (*d*, 1H, *J* 1.9 Hz, C4"-H); 6.55 (*dd*, 1H, *J* 9.7, 1.9 Hz, C2"-H); 6.73 (*dd*, 1H, *J* 8.8, 2.4 Hz, C7"-H); 6.85 (*d*, 1H, *J* 9.3 Hz, C1"-H); 6.88 (*d*, 1H, *J* 8.7 Hz, C8"-H); 6.95 (*d*, 1H, *J* 2.4 Hz, C5"-H); 7.31 (*dd*, 1H, *J* 7.5, 1.4 Hz, C3'''-H); 7.67 (*dt*, 1H, *J* 7.5, 1.4 Hz, C5'''-H); 7.72 (*s*, 1H, C4-H); 7.74 (*dt*, 1H, *J* 7.4, 1.5 Hz, C4'''-H); 8.23 (*bs*, 1H, N3-H); 8.25

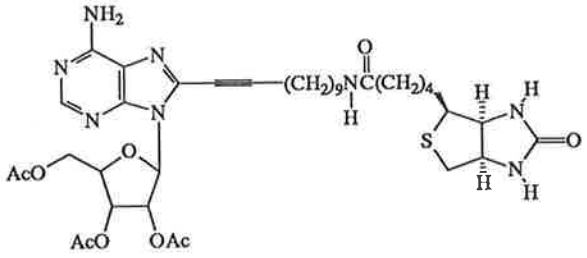
(*dd*, 1H, *J* 7.7, 1.2 Hz, C6'"-H). ^{13}C NMR: 19.5, 20.7, 20.8, 25.8, 28.4, 28.8, 28.9, 29.0, 29.2, 29.3, 38.0, 52.4, 63.8, 68.8, 71.0, 74.0, 82.4 (alkyl), 85.2, 95.4 (alkynyl), 100.6, 101.4, 105.6, 113.8, 114.6, 117.4, 128.7, 129.6, 129.7, 130.1, 130.2, 130.5, 131.0, 132.6, 134.6. 140.5, 149.2, 150.3, 154.3, 158.9 (aryl), 161.4, 163.6, 165.6, 170.0, 170.3, 185.6 (carbonyl).

8-[11-(10-(10*H*)-9-Acridonyl)undec-1-ynyl]-2', 3',5'-tri-*O*-acetyladenosine (137)



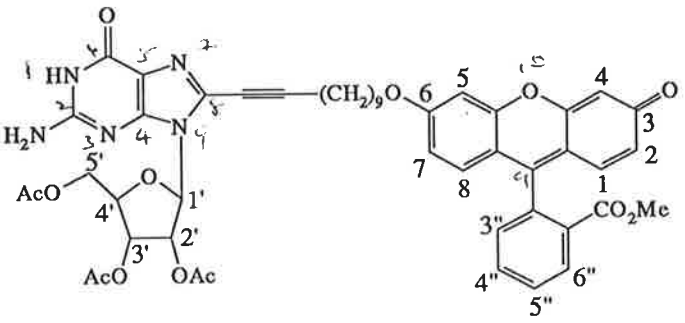
Reaction at 50° for 24 hours; Eluant 50/50 EtOAc/hexanes; pale green glass; 89%. HRMS (LSIMS) Calculated for $C_{40}H_{45}N_6O_8$ ($M+H^+$): 737.3299. Found: 737.3295. MS: 737 ($M+H^+$, 54), 479 (100), 208 (23). IR (nujol): 3312*br m*, 3164*br m*, 2232*w*, 1748*s*, 1634*s*, 1600*s*, 1496*s*, 1232*s*. UV (EtOH): 218 (30 700), 234 (30 100), 257 (46 700), 293 (17 800), 386 (7 060), 405 (7 830). Fluorescence (EtOH, λ_{ex} = 386 nm): 418 (100), 439 (70). ^{1}H NMR: 1.41-1.61 (*m*, 10H, methylene protons); 1.73 (*quintet*, 2H, *J* 7.1 Hz, CH_2CH_2-≡); 1.97 (*quintet*, 2H, *J* 7.9 Hz, CH_2CH_2-N); 2.06, 2.11 and 2.15 (3 x *s*, 3H, CH_3CO); 2.58 (*t*, 2H, *J* 7.1 Hz, CH_2-≡-Ar); 4.31-4.43 (*m*, H,); 4.54 (*dd*, 1H, *J* 11.4, 3.3 Hz,); 5.73 (*bs*, 2H, C2-NH_2); 5.99 (*m*, 1H, C3'-H); 6.24-6.27 (*m*, 2H, C1'-H and C2'-H); 7.32 (*dd*, 2H, *J* 8.7, 7.8 Hz, C2"-H); 7.52 (*d*, 2H, *J* 8.7 Hz, C4"-H); 7.75 (*ddd*, 2H, *J* 8.7, 6.9, 1.5 Hz, C3"-H); 8.37 (*s*, 1H, C4-H); 8.61 (*dd*, 2H, *J* 7.8, 1.5 Hz, C1"-H). ^{13}C NMR: 19.54, 20.46, 20.54, 20.70, 26.91, 27.20, 27.86, 28.88, 28.98, 29.32, 29.40, 46.15, 63.12, 69.70, 70.43, 72.51, 79.73, 87.32, 98.91, 114.51, 121.19, 122.46, 128.01, 133.88, 134.77, 141.74, 149.24, 153.76, 155.00, 169.28, 169.46, 170.60, 177.98.

8-[*N*-(Biotinyl)-11-aminoundec-1-ynyl]-2',3',5'-tri-*O*-acetyladenosine (138)



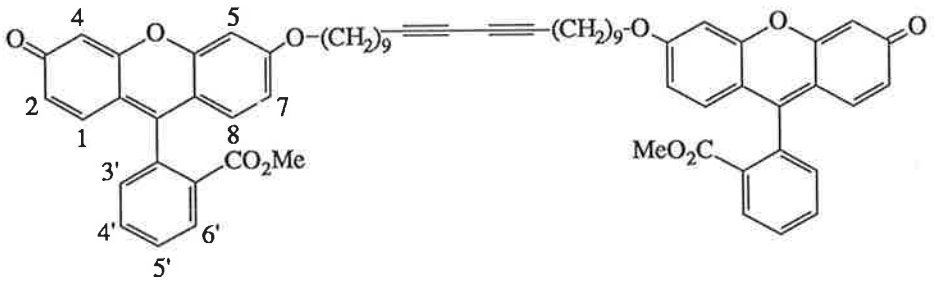
Eluant 10/90 MeOH/CH_2Cl_2; recrystallisation from EtOAc/hexanes; pale yellow crystals mp 88-90°; 88%. Calculated for $C_{37}H_{51}N_8O_9S$ ($M+H^+$): 785.3656. Found: 785.3680. MS (FAB): 785 ($M+H^+$, 62), 527 (100), 301 (12), 259 (13). IR ($CDCl_3$): 3500*w*, 1740*s*, 1708*s*, 1632*s*, 1470*s*. ^1H NMR: 1.23-1.75 (*m*, 20H, methylene protons); 2.04, 2.10 and 2.13 (3 x *s*, 3H, CH_3CO); 2.17 (*t*, 2H, *J* 7.3 Hz, CH_2CONH); 2.54 (*t*, 2H, *J* 6.9 Hz, CH_2-≡-Ar); 2.74 (*d*, 1H, J_{gem}, 12.7 Hz, C5"-Hb); 2.89 (*dd*, 1H, J_{gem} 12.7, 4.8 Hz, C5"-Ha); 3.14 (*dt*, 1H, *J* 6.0, 4.6 Hz, C2"-H); 3.19 (*q*, 2H, *J* 6.6 Hz, CH_2NHCO); 4.28-4.40 (*m*, 3H, C4'-H, C5b'-H and C4"-H); 4.51 (*m*, 2H, C5'-Ha and C3"-H); 5.95 (*t*, 1H, *J* 5.5 Hz, C3'-H); 6.08 (*bs*, 1H, N1"-H); 6.17 (*bt*, 1H, *J* 5.4 Hz, CH_2NHCO); 6.22 (*m*, 2H, C1'-H and C2'-H); 6.33 (*bs*, 2H, C2-NH_2); 6.52 (*bs*, 1H, N3"-H); 8.30 (*s*, 1H, C4-H). ^{13}C NMR (75 MHz, δ ppm): 19.46, 20.43, 20.51, 20.67, 25.60, 26.80, 27.76, 28.06, 28.19, 28.74, 28.85, 29.10, 29.21, 29.51, 36.02, 39.40, 40.56, 55.62, 60.16, 61.85, 63.08, 69.62, 70.40, 72.39, 79.69 (alkyl), 87.28, 99.00 (alkynyl), 119.25, 134.54, 149.05, 153.69, 155.26 (aryl), 164.22, 169.30, 169.46,170.60, 173.07 (carbonyl).

8-{11-[6-*O*-(Methyl)fluoresceinyl]-1-undec-1-ynyl}-tri-*O*-acetylguanosine (139)



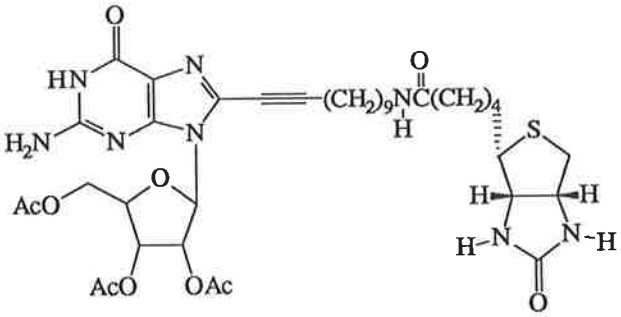
Reaction at 50° for 5 hours; eluant 7.5/92.5 MeOH/CH_2Cl_2; recrystallisation from MeOH; bright orange glass; 51%. HRMS (LSIMS) Calculated for $C_{48}H_{50}N_5O_{13}$ ($M+H^+$): 904.3405.

Found: 904.3411. MS: 904 ($M+H^+$, 100), 646 (32), 347 (88), 259 (27). IR ($CDCl_3$): 3132*m* (N-H), 1748*s*, 1720*s*, 1688*s* (C=O), 1644*s*, 1596*s*. UV (EtOH) λ_{max} (ε): 230 (25 200), 279 (18 400), 363 (3 900), 437*sh* (9 600), 460 (13 600), 489 (10 800). Fluorescence (EtOH, λ_{ex} = 489 nm): 522 nm. ^{1}H NMR: 1.26-1.48 (*m*, 10H, methylene protons); 1.65 (*quintet*, 2H, *J* 7.2 Hz, CH_2-CH_2-≡); 1.84 (*quintet*, 2H, *J* 7.1, CH_2-CH_2-OAr); 2.05, 2.11 and 2.12 (3 x *s*, 3H, CH_3CO); 2.51 (*t*, 2H, *J* 6.9 Hz, CH_2-≡-Ar); 3.65 (*s*, 3H, CH_3-OAr); 4.32 (*m*, 2H, C4'-H and C5'-Hb); 4.51 (*m*, 1H, C5'-Ha); 5.88 (*br s*, 2H, C2-NH_2); 5.93 (*t*, 1H, *J* 5.8 Hz, C3'-H); 6.09 (*m*, 2H, C1'-H and C2'-H); 6.39 (*d*, 1H, *J* 1.5 Hz, C4-H); 6.48 (*dd*, 1H, *J* 9.8, 1.5 Hz, C2-H); 6.76 (*dd*, 1H, *J* 9.0, 2.2 Hz, C7-H); 6.86 (*d*, 1H, *J* 7.7 Hz, C1-H); 6.89 (*d*, 1H, *J* 7.1 Hz, C8-H); 6.99 (*d*, 1H, *J* 2.3 Hz, C5-H); 7.34 (*d*, 1H, *J* 7.3 Hz, C3"-H); 7.70 (*t*, 1H, *J* 7.0 Hz, C5"-H); 7.78 (*t*, 1H, *J* 6.9 Hz, C4"-H); 8.25 (*d*, 1H, *J* 7.8 Hz, C6"-H); 10.69 (*bs*, 1H, N1-H). ^{13}C NMR (d_6-DMSO): 19.2, 20.4, 20.4, 20.6, 25.7, 27.8, 28.6, 28.7, 28.8, 29.0, 29.2, 52.2, 53.7, 62.9, 68.8, 70.0, 70.2, 72.1, 78.1, 79.2, 87.0, 96.3, 100.7, 105.2, 113.8, 114.4, 117.0, 117.4, 128.8, 129.7, 129.9, 130.3, 130.4, 131.0, 132.8, 134.3, 150.8, 153.8, 154.1, 156.6, 158.9, 163.7, 165.4, 169.1, 169.3, 170.4, 185.1. Also recovered was the diyne **140** in 9.3mg (12%) yield. ^{1}H NMR: 1.25-1.47 (*m*, 12H, methylene protons); 1.45 (*quintet*, 2H, *J* 6.8 Hz, CH_2CH_2-OAr); 2.17 (*t*, 2H, *J* 6.8 Hz, CH_2-≡); 3.56 (*s*, 3H, CH_3O_2C); 3.98 (*t*, 2H, *J* 6.5 Hz, CH_2-OAr); 6.39 (*d*, 1H, *J* 1.9 Hz, C4-H); 6.47 (*dd*, 1H, *J* 9.6, 1.9 Hz, C2-H); 6.65 (*dd*, 1H, *J* 8.7, 2.3 Hz, C7-H); 6.77 (*d*, 1H, *J* 9.3 Hz, C1-H); 6.80 (*d*, 1H, *J* 8.7 Hz, C8-H); 6.87 (*d*, 1H, *J* 2.3 Hz, C5-H); 7.23 (*dd*, 1H, *J* 7.4, 1.2 Hz, C3'-H); 7.60 (*dt*, 1H, *J* 6.2, 1.4 Hz, C5'-H); 7.67 (*dt*, 1H, *J* 7.4, 1.4 Hz, C4'-H); 8.17 (*dd*, 1H, *J* 7.6, 1.2 Hz, C6'-H).



140

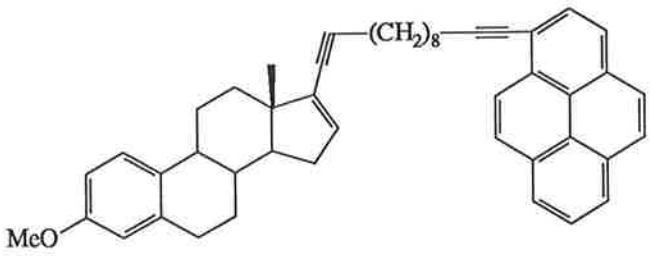
8-[*N*-(Biotinyl)-11-aminoundec-1-ynyl]-2',3',5'-tri-*O*-acetylguanosine (141)



Reaction at 50° for 6 hours; Eluant 10/90 MeOH/CH_2Cl_2; recrystallisation from MeOH; pale yellow crystals mp 157-160°; 58%. HRMS (LSIMS) Calculated for $C_{37}H_{53}N_8O_{10}S$ ($M+H^+$): 801.3605. Found: 801.3597. MS: 801 ($M+H^+$, 65), 543 (29), 261 (100). ^1H NMR ($CDCl_3/d_6$-DMSO): 1.20-1.58 (*m*, 20H, methylene protons); 1.99, 2.05, 2.09 (3 x *s*, 3H, CH_3CO); 2.03 (*t*, 2H, *J* 7.8 Hz, CH_2CONH); 2.53 (*t*, 2H, CH_2-≡-Ar); 2.64 (*d*, 1H, J_{gem} 12.3 Hz, C5"-Hb); 2.80 (*dd*, 1H, J_{gem} 12.3, 5.1 Hz, C5"-Ha); 2.99 (*q*, 1H, *J* 6.2 Hz, C2"-H); 3.06 (*q*, 2H, *J* 7.2 Hz, CH_2NHCO); 4.09-4.31 (*m*, 4H, C3"-H, C4"-H, C5'-Hb, C4'-H); 4.41 (*dd*, 1H, *J* 11.7, 3.8 Hz, C5'-Ha); 5.58 (*t*, 1H, *J* 6.0 Hz, C3'-H); 5.97 (*m*, 2H, C1'-H and C2'-H); 6.35 (*bs*, 1H, N1"-H); 6.42 (*bs*, 1H, N3"-H); 6.73 (*bs*, 2H, C2-NH_2); 7.74 (*bt*, 1H, *J* 5.5 Hz, CH_2NHCO); 10.97 (*bs*, 1H, N1-H). ^{13}C NMR (d_6-DMSO): 8.5 18.50, 20.2, 20.4, 25.3, 26.4, 27.5, 28.0, 28.2, 28.5, 28.7, 28.9, 29.2, 35.2, 38.4, 45.5, 55.5, 59.2, 61.0, 62.8, 70.2, 78.9, 96.3, 116.4, 129.3, 150.6, 154.2, 156.0, 162.7, 169.3, 169.4, 170.1, 171.8.

Experimental Described in Chapter 3.3.

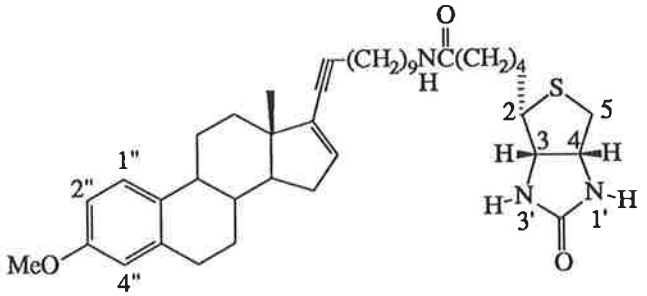
17-[12-(1-Pyrenyl)dodeca-1,11-diynyl]-3-*O*-methylestr-16-ene (144)



To a stirred mixture of DMF (1.0 ml) and Et_3N (0.2 ml) was added sequentially the triflate **54** (50mg, 0.12 mmol), alkyne **74** (52mg, 0.14mmol, 1.2eq), $Pd(PPh_3)_4$ (14mg, 0.012 mmol, 0.1 eq) and CuI (4.6mg, 0.024 mmol, 0.2 eq). The reaction mixture was stirred at room temperature for 3 hours, at which time TLC (30/70 CH_2Cl_2/hexanes) indicated the absence of the triflate. The solvent was removed *in vacuo* and the residue subjected to flash chromatography using 30/70 CH_2Cl_2/hexanes as eluant to give the title compound as a viscous oil in 69mg (92%) yield. HRMS Calculated for $C_{47}H_{48}O$: 628.3705. Found: 628.3700. MS: 628 (M^+, 100), 239 (80). IR (thin film): 3040*m* (Ar-H), 2250 and 2216*w* (C≡C), 1610*s* (C=C), 1502*s*, 1256*s*, 908*s*, 846*s*, 734*s*. UV ($CHCl_3$): 251 (29 400), 265 (11 900), 275 (23 900), 285 (28 700) 316*sh* (4 800), 331 (12 400), 348 (21 600), 365 (23 700). Fluorescence ($CHCl_3$, λ_{ex} = 365 nm): 388 (100), 396*sh* (75), 407 (80). ^{1}H NMR: 0.84 (*s*, 3H, CH_3-); 1.37-2.31 (many *m*, methylene and methine protons); 2.38 (*t*, 2H, *J* 6.6 Hz, CH_2-≡-HC=); 2.65 (*t*, 2H, *J* 7.0 Hz, CH_2-≡-Ar); 2.85 (*m*, 2H, CH_2-CH=); 3.74 (*s*, 3H, CH_3-OAr); 5.91 (*t*, 1H, *J* 1.2 Hz, HC=); 6.61 (*d*, 1H, *J* 2.6 Hz, C4-H); 6.65 (*dd*, 1H, *J* 8.4, 2.6 Hz, C2-H); 7.15 (*d*, 1H, *J* 8.4 Hz, C1-H). ^{13}C NMR: 16.2, 19.7, 20.0, 26.6, 27.8, 28.8, 29.0, 29.0, 29.1, 29.1, 29.2, 29.5, 31.6, 34.7, 37.6, 44.3, 48.1, 55.2, 55.3 (alkyl), 76.1, 77.6, 79.7, 93.9 (alkynyl), 96.4, 111.4, 113.9, 118.9, 124.5, 125.3, 125.4, 125.7, 126.1, 126.1, 127.3, 127.7, 128.0, 129.6, 130.7, 131.2, 131.3, 131.9, 132.9, 133.6 137.91, 138.0, 157.4 (aryl).

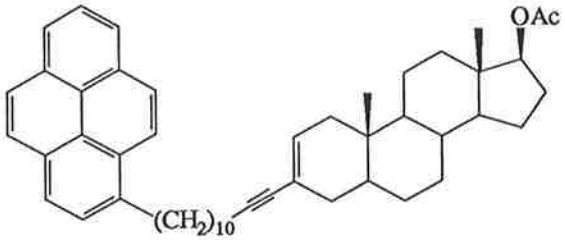
Compounds **145**, **146** and **147** were prepared in a similar manner as that described for compound **144**, apart from the differences stated.

17-[*N*-(Biotinyl)-11-aminoundec-1-ynyl]-3-*O*-methylestr-16-ene (145)



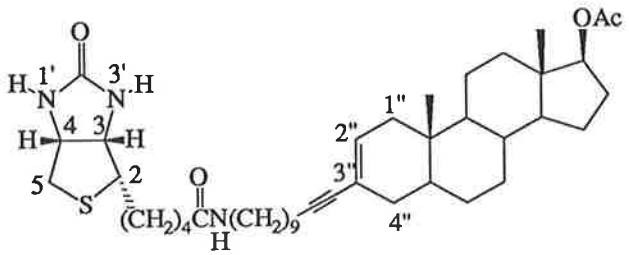
Reaction of compound **54** (50mg, 0.12mmol) with alkyne **107** (57mg, 0.14mmol, 1.2eq) under standard conditions and purification by flash chromatography using 6/94 MeOH/CH_2Cl_2 as eluant gave the title compound as colourless crystals mp 150-152° in 70mg (89%) yield. HRMS Calculated for $C_{40}H_{57}N_3O_3S$: 659.4121. Found: 659.4087. MS : 659 (M^+, 29), 644 (M^+-CH_3, 40), 459 (15), 416 (100). ^{1}H NMR: 0.86 (*s*, 3H, CH_3-); 1.22-2.35 (many *m*, methylene and methine protons); 2.20 (*t*, 2H, *J* 7.5 Hz, CH_2-CONH); 2.36 (*t*, 2H, *J* 6.9 Hz, CH_2-≡); 2.73 (*d*, 1H, *J* 12.9 Hz, C5-Hb); 2.91 (*m*, 3H, C5-Ha and CH_2-C=); 3.16 (*dt*, 1H, *J* 7.3, 4.5 Hz, C2-H); 3.23 (*q*, 2H, *J* 6.5 Hz, CH_2-NH); 3.79 (*s*, 3H, CH_3-OAr); 4.31 (*dd*, 1H, *J* 7.5, 4.7 Hz, C4-H); 4.52 (, *dd*, 1H, *J* 7.5, 4.8 Hz, C3-H); 5.21 (*br s*, 1H, N1'-H); 5.93 (*br t*, 1H, *J* 5.7 Hz, NHCO); 5.93 (*m*, 1H, HC=); 6.03 (*br s*, 1H, N3'-H); 6.64 (*d*, 1H, *J* 2.7 Hz, C4"-H); 6.72 (*dd*, 1H, *J* 8.6, 2.7 Hz, C2"-H); 7.21 (*d*, 1H, *J* 8.6 Hz, C1"-H). ^{13}C NMR (d_6-DMSO): 16.0, 18.7, 25.2, 25.3, 26.0, 26.4, 27.2, 28.0, 28.1, 28.2, 28.3, 28.4, 28.7, 28.9, 29.1, 29.2, 31.1, 34.2, 35.2, 37.1, 38.3, 43.7, 47.6, 54.8, 54.8, 55.4, 59.2, 61.0 (alkyl), 76.0, 93.7 (alkynyl), 111.4, 113.4, 125.8, 132.0, 133.2, 137.2, 137.3, 157.0 (vinyl and aryl), 162.6, 171.7 (carbonyl).

3-[12-(1-pyrenyl)dodec-1-ynyl]-17β-acetyloxyandrost-2-ene (146)



Reaction of triflate **55** (mixture of Δ-2 and Δ-3 isomers, *ca.* 5:1 ratio) (50mg, 0.11mmol) with alkyne **78** (48mg, 0.13 mmol, 1.2eq) under standard conditions and purification by flash chromatography eluant 40/60 CH_2Cl_2/hexanes gave the title compound as a fluorescent light green viscous oil in 65mg (85%) yield. HRMS Calculated for $C_{49}H_{60}O_2$: 680.4593. Found: 680.4611. MS: 680 (M^+, 76), 620 (87), 326 (69), 215 (100). IR (thin film): 3040*m* (Ar-H), 1732*s* (C=O), 1446*m*, 1250*s*, 1030*s*, 844*s*, 740*s*. UV ($CHCl_3$): 248 (35 000), 258 (11 800), 269 (22 400), 279 (35 300), 317 (10 300), 330 (21300), 346 (26 600). Fluorescence ($CHCl_3$, λ_{ex} = 346 nm): 379 (100), 397 (67), 418*sh* (22). ^{1}H NMR: 0.73 and 0.76 (2 x *s*, 3H, CH_3-); 0.56-2.19 (many *m*, methylene and methine protons); 2.05 (*s*, 3H, CH_3CO_2); 2.30 (*t*, 2H, *J* 6.9 Hz, CH_2-C≡); 3.34 (*t*, 2H, *J* 7.8 Hz, CH_2-Ar); 4.57 (*dd*, 1H, *J* 9.1, 7.8 Hz, CH-OAc); 5.91 (*d*, 1H, *J* 4.9 Hz, HC=); 7.86-8.30 (*m*, 9H, Ar-H). ^{13}C NMR: 11.8, 12.0, 12.1, 19.3, 20.4, 21.2, 23.4, 27.4, 28.1, 28.8, 28.9, 29.1, 29.4, 29.5, 29.8, 31.0, 31.3, 33.6, 34.0, 34.1, 34.4, 35.2, 36.8, 40.1, 41.2, 42.4, 50.5, 53.5 (alkyl), 81.8, 82.8, 87.5, 119.7 (alkynyl and vinyl), 123.5, 124.6, 124.7, 125.0, 125.7, 126.4, 127.0, 127.2, 127.5, 128.6, 129.6, 130.9, 131.4, 131.8, 137.2, 137.3 (aryl), 171.2 (carbonyl).

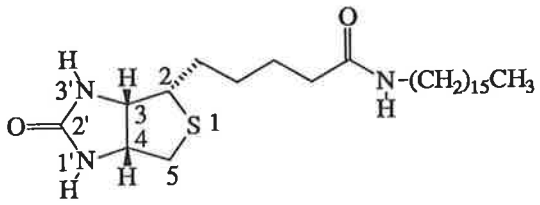
3-[*N*-(Biotinyl)-11-aminoundec-1-ynyl]-17β-acetyloxyandrost-2-ene (147)



Reaction of triflate **55** (mixture of Δ-2 and Δ-3 isomers, *ca.* 5:1 ratio) (50mg, 0.11 mmol) with alkyne **107** (51mg, 0.13 mmol, 1.2eq) under standard conditions, purification by flash chromatography eluant 5/95 MeOH/CH_2Cl_2 and recrystallisation from MeOH gave the title compound as colourless crystals mp 162-164° in 76mg (88%) yield. HRMS Calculated for $C_{42}H_{65}N_3O_4S$: 707.4696. Found: 707.4696. MS: 707 (M^+, 18), 632 (26), 354 (1100), 227 (29), 147 (27). IR (nujol mull): 3296*br m* (N-H), 1738*m* and 1704*s* (C=O), 1642*s* (urea C=O), 1246*s*. ^{1}H NMR: 0.75 and 0.78 (2 x *s*, 3H, CH_3-); 0.65-2.20 (many *m*, methylene and methine protons; 2.04 (*s*, 3H, CH_3CO); 2.18 (*t*, 2H, *J* 7.3 Hz, CH_2CONH); 2.28 (*t*, 2H, *J* 7.0 Hz, CH_2-≡); 2.74 (*d*, 1H, *J* 12.8 Hz, C5-Hb); 2.93 (*dd*, 1H, *J* 12.8, 5.0 Hz, C5-Ha); 3.16 (*dt*, 1H, *J* 7.2, 4.9 Hz, C2-H); 3.22 (*q*, 2H, *J* 6.5 Hz, CH_2-NHCO); 4.33 (*dd*, 1H, *J* 7.4, 4.9 Hz, C3-H); 4.52 (*dd*, 1H, *J* 7.4, 5.0 Hz, C4-H); 4.58 (*dd*, 1H, *J* 9.1, 7.8 Hz, CH-OAc); 5.07 (*br s*, 1H, N1'-H); 5.64 (*s*, HC= Δ-3 isomer); 5.73 (*br t*, 1H, *J* 5.5 Hz, NHCO); 5.84 (*br s*, 1H, N3'-H); 5.91 (*br d*, 1H, *J* 4.8 Hz, HC=, Δ-2 isomer). ^{13}C NMR: 11.8, 11.9, 19.2, 20.4, 21.1, 23.4, 25.6, 26.9, 27.4, 27.7, 28.0, 28.1, 28.8, 28.9, 29.0, 29.2, 29.3, 29.6, 31.1, 34.1, 34.4, 35.3, 36.0, 36.8, 39.5, 40.2, 40.4, 41.3, 42.4, 50.6, 53.6, 55.5, 60.1, 61.7 (alkyl), 81.7, 82.8, 87.4, 131.8 (alkynyl and vinyl), 163.8, 171.1, 173.0 (carbonyl).

Experimental Described in Chapter 4.

Biotin-*N*-(1-hexadecyl)-amide (150)



To a stirred solution of biotin-NHS ester (**106**) (100mg, 0.29 mmol) in DMF (2ml) was added 1-hexadecylamine (71mg, 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 10/90 MeOH/CH_2Cl_2 as eluant, recrystallised from MeOH and dried under vacuum to give the title compound as a colourless solid in 115mg (83%) mp 196-198°. HRMS: Calculated for $C_{26}H_{49}N_3O_2S$: 467.3544. Found: 467.3536. MS: 467 (M^+, 34), 450 (22), 407 (44), 166 (29), 97 (37), 43 (100). ^{1}H NMR: 0.88 (*t*, 3H, *J* 6.5 Hz, CH_3-CH_2); 1.25-1.77 (*m*, methylene protons); 2.20 (*t*, 2H, J 6.2 Hz, CH_2-CON); 2.74 (*d*, 1H, J_{gem} 12.8 Hz, C5-Hb); 2.93 (*dd*, 1H, J_{gem} 12.8, 5.0 Hz, C5-Ha); 3.17 (*dt*, 1H, *J* 7.3, 4.7 Hz, C2-H); 3.23 (*q*, 2H, *J* 6.6 Hz, CH_2-NHCO); 4.33 (*dd*, 1H, *J* 7.7, 5.0 Hz, C4-H); 4.52 (*dd*, 1H, *J* 7.7 Hz, 4.7 Hz, C3-H); 4.91 (bs, 1H, N1'-H); 5.63 (*bs*, 1H, N3'-H); 5.65 (*bt*, 1H, *J* 5.3 Hz, -NHCO). ^{13}C (CD_3OD, 323K): 14.2, 23.6, 26.8, 28.0, 29.5, 29.7, 30.3, 30.4, 30.6, 30.6, 32.9, 36.9, 40.5, 41.0, 56.9, 61.7, 63.5 (alkyl), 175.9, 184.9.

Compounds **151**, **152**, **153**, **154** and **155** were prepared in a similar manner, varying only in the amine used, product yield and recrystallisation solvent.

Biotin-*N*-(1-dodecyl)-amide (151)

Recrystallised from MeOH. Recovered 86mg (71%) of colourless amorphous solid mp 193-196° (lit.[88]194-198°). HRMS Calculated for $C_{22}H_{41}N_3O_2S$: 411.2919. Found: 411.2904. MS: 411 (M^+, 13), 351 (100), 227 (68), 186 (61), 166 (77), 148 (90). ^{1}H ($CDCl_3$/d_6-DMSO): 0.89 (*t*, 3H, J 6.4 Hz, CH_3-CH_2); 0.96-1.47 (*m*, methylene protons); 1.89, *t*, 2H, *J* 7.4 Hz,

CH_2CONH); 2.44 (*d*, 1H, J_{gem} 12.7 Hz, C5-Hb); 2.60 (*dd*, 1H, J_{gem} 12.7, 4.9 Hz, C5-Ha); 2.82-2.90 (*m*, 3H, CH_2CON and C2-H); 3.97 (*dd*, 1H, *J* 7.8, 4.9 Hz, C4-H); 4.45 (*dd*, 1H, *J* 7.8 Hz, 4.8 Hz, C3-H); 5.53 (*bs*, 1H, N1'-H); 5.56 (*bs*, 1H, N3'-H); 6.51 (*bt*, 1H, *J* 5.2 Hz, -NHCO). ^{13}C (d_6-DMSO): 14.0, 22.1, 25.4, 26.5, 28.1, 28.2, 28.7, 28.8, 29.1, 29.2, 31.3, 35.3, 38.6, 40.7, 55.8, 59.2, 61.1 (alkyl), 162.7, 171.8 (carbonyl).

Biotin-*N*-(1-undecyl)-amide (152)

Recrystallised from MeOH. Recovered 65mg (56%) of colourless amorphous solid mp 183-186°. HRMS Calculated for $C_{21}H_{39}N_3O_2S$: 397.2763. Found: 397.2758. MS: 397 (M^+, 10), 337 (100), 226 (49), 172 (55), 166 (75), 97 (72). ^1H NMR: 0.89 (*t*, 3H, *J* 6.4 Hz, CH_3-CH_2-); 1.26-1.77 (*m*, methylene protons); 2.21 (*t*, 2H, *J* 7.5 Hz, CH_2-CON); 2.74 (*d*, 1H, J_{gem} 12.8 Hz, C5-Hb); 2.93 (*dd*, 1H, J_{gem} 12.8, 4.9 Hz, C5-Ha); 3.16 (*dt*, 1H, *J* 7.3, 4.8 Hz, C2-H); 3.16 (*dt*, 1H, *J* 7.3, 4.8 Hz, C2-H); 3.23 (*q*, 2H, *J* 6.3 Hz, CH_2-NHCO); 4.33 (*dd*, 1H, *J* 7.6, 4.6 Hz, C4-H); 4.52 (*dd*, 1H, *J* 7.6 Hz, 4.9 Hz, C3-H); 5.16 (*bs*, 1H, N1'-H); 5.77 (*bt*, 1H, *J* 5.4 Hz, -NHCO); 5.98 (*bs*, 1H, N3'-H). ^{13}C NMR: 14.1, 22.7, 25.4, 25.6, 27.0, 28.0, 28.1, 29.3, 29.6, 29.1, 31.9, 36.0, 39.6, 40.5, 55.4, 60.2, 61.8 (alkyl), 163.7, 173.0 (carbonyl).

Biotin-*N*-(1-octyl)-amide (153)

Recrystallised from MeOH/water. Recovered 70mg (67%) of colourless amorphous solid mp 193-196°. HRMS Calculated for $C_{18}H_{33}N_3O_2S$: 355.2293. Found: 355.2293. MS: 355 (M^+, 7), 338 (5), 311 (9), 295 (100), 227 (30), 184 (56), 166 (55), 130 (68), 100(64). ^1H NMR: 0.88 (*t*, 3H, *J* 6.6 Hz, CH_3-CH_2); 1.28-1.77 (*m*, 18H, methylene protons); 2.20, *t*, 2H, *J* 7.3 Hz, CH_2-CON); 2.74 (*d*, 1H, J_{gem} 12.8 Hz, C5-Hb); 2.93 (*dd*, 1H, J_{gem} 12.8, 4.9 Hz, C5-Ha); 3.17 (*dt*, 1H, *J* 7.2, 4.7 Hz, C2-H); 3.23 (*q*, 2H, *J* 7.1 Hz, CH_2-NHCO); 4.34 (*dd*, 1H, *J* 7.7, 4.9 Hz, C4-H); 4.53 (*dd*, 1H, *J* 7.7 Hz, 4.7 Hz, C3-H); 4.92 (*bs*, 1H, N1'-H); 5.63 (*bs*, 2H, N3'-H and -NHCO). ^{13}C NMR: 14.1, 22.6, 25.6, 26.9, 28.1, 29.3, 29.5, 29.6, 31.7, 31.8, 36.0, 39.6, 40.5, 55.4, 60.1, 61.8 (carbonyl), 163.4, 173.0 (carbonyl).

Biotin-*N*-(1-hexyl)-amide (154)

Recrystallised from MeOH. Recovered 63mg (66%) of colourless morphous solid mp 183-186°. HRMS Calculated for $C_{16}H_{29}N_3O_2S$: 327.1980. Found: 327.1971. MS: 327 (M^+, 2), 310 (3), 283 (4), 267 (40), 184 (68), 166 (23), 156 (28), 143 (26), 116 (22), 100 (100). ^1H NMR: 0.89 (*t*, 3H, J 6.7 Hz, CH_3-CH_2); 1.29-1.80 (*m*, 14H, methylene protons); 2.20 (*t*, 2H, *J* 7.4 Hz, CH_2-CON); 2.74 (*d*, 1H, J_{gem} 12.9 Hz, C5-Hb); 2.93 (*dd*, 1H, J_{gem} 12.9, 5.0 Hz, C5-Ha); 3.17 (*dt*, 1H, *J* 7.1, 4.6 Hz, C2-H); 3.23 (*q*, 2H, *J* 7.0 Hz, CH_2-NHCO); 4.34 (*dd*, 1H, *J* 7.6, 5.0 Hz, C4-H); 4.45 (*dd*, 1H, *J* 7.6 Hz, 4.6 Hz, C3-H); 4.85 (*bs*, 1H, N1'-H); 5.54 (*bs*, 1H, N3'-H); 5.63 (*bt*, 1H, *J* 5.2 Hz, -NHCO). ^{13}C NMR (d_6-DMSO): 13.8, 22.0, 25.2, 26.0, 27.9, 28.1, 29.0, 30.9, 35.1, 38.2, 38.4, 55.3, 59.1, 60.9 (alkyl), 162.6, 171.6 (carbonyl).

Biotin-*N*-(1-propyl)-amide (155)

Recrystallised from water to give a colourless amorphous solid mp 193-194° (lit.[87b] 195-200°) in 61mg (73%) yield. HRMS Calculated for $C_{13}H_{23}N_3O_2S$: 285.1511. Found: 285.1511. MS: 285 (M^+, 4), 225 (67), 166 (26), 142 (45), 114 (39), 100 (100). ^1H NMR: 0.92 (*t*, 3H, CH_3-CH_2-); 1.41-1.83 (*m*, 8H, methylene protons); 2.21 (*t*, 2H, *J* 7.4 Hz, CH_2-CONH); 2.75 (*d*, 1H, J_{gem} 12.9 Hz, C5-Hb); 2.83 (*dd*, 1H, J_{gem} 12.9, 5.0 Hz, C5-Ha); 3.16 (*dt*, 1H, *J* 7.4, 4.8 Hz, C2-H); 3.21 (*q*, 2H, *J* 7.0 Hz, CH_2-NHCO); 4.34 (*dd*, 1H, *J* 7.6, 5.0 Hz, C4-H); 4.45 (*dd*, 1H, *J* 7.6 Hz, 4.8 Hz, C3-H); 5.12 (*bs*, 1H, N1'-H); 5.72 (*bt*, 1H, *J* 5.2 Hz, -NHCO); 5.83 (*bs*, 1H, N3'-H). ^{13}C NMR (d_6-DMSO): 11.7, 23.6, 27.0, 29.5, 29.8, 36.9, 41.0, 42.2, 47.8, 57.0, 61.7, 63.4 (alkyl), 166.1, 176.0 (carbonyl).

Biotin hexadecan-1-ol ester (156)

A mixture of biotin (100mg, 0.41 mmol), hexadecan-1-ol (496mg, 2.05 mmol, 5 eq). *p*-toluenesulphonic acid (8mg, 0.04 mmol, 0.1 eq.) and toluene (5ml) was stirred at reflux for 48 hours. The reaction mixture was cooled to room temperature, unreacted biotin removed by filtration, solvent removed *in vacuo* and the residue purified by flash chromatography, eluant $MeOH/CH_2Cl_2$ 5:95, to give the title compound as a colourless solid in 72mg (38%) yield mp 113-118°. HRMS Calculated for $C_{26}H_{48}N_2O_3S$: 468.3386. Found: 468.3369. MS:

468 (M^+, 100), 296 (20), 227 (22), 97 (24). ^{1}H NMR: 0.90 (*t*, 3H, *J* 6.4 Hz, CH_3-CH_2); 1.28-1.77 (*m*, methylene protons); 2.35 (*t*, 2H, *J* 7.2 Hz, CH_2-CO_2); 2.76 (*d*, 1H, J_{gem} 12.8 Hz, C5-Hb); 2.95 (*dd*, 1H, J_{gem} 12.8, 5.0 Hz, C5-Ha); 3.19 (*dt*, 1H, *J* 8.0, 4.7 Hz, C2-H); 4.08 (*t*, 2H, *J* 6.8 Hz, CH_2-O_2C); 4.34 (*dd*, 1H, *J* 7.5, 4.5 Hz, C4-H); 4.54 (*dd*, 1H, *J* 7.5, 5.0 Hz, C3-H); 4.88 (*bs*, 1H, N1'-H); 5.18 (*bs*, 1H, N3'-H). ^{13}C NMR: 14.1, 22.7, 24.8, 25.9, 28.2, 28.3, 28.6, 29.3, 29.4, 29.5, 29.6, 29.6, 29.7, 31.9, 33.9, 40.5, 55.4, 60.1, 61.9, 64.6 (alkyl), 163.4, 173.8 (carbonyl).

Biotin esters **157**, **158**, **159** and **160** were prepared in a similar manner, varying only in the alcohol used and yield obtained..

Biotin dodecan-1-ol ester (157)

Recovered in 136mg (80%) yield as a colourless amorphous solid mp 117-118°. HRMS Calculated for $C_{22}H_{40}N_2O_3S$: 412.2760. Found: 412.2761. MS: 412 (M^+, 4), 352 (15), 227 (40), 166 (100), 143 (16). ^{1}H NMR: 0.89 (*t*, 3H, *J* 6.6 Hz, CH_3-); 1.27-1.76 (*m*, methylene protons); 2.34 (*t*, 2H, *J* 7.6 Hz, CH_2-CONH); 2.75 (*d*, 1H, *J* 12.8 Hz, C5-Hb); 2.93 (*dd*, 1H, *J* 12.8, 4.9 Hz, C5-Ha); 3.17 (*dt*, 1H, *J* 7.9, 4.8 Hz, C2-H); 4.06 (*t*, 2H, *J* 6.8 Hz, CH_2-O_2C); 4.32 (*dd*, 1H, *J* 7.4, 5.0 Hz, C3-H); 4.52 (*dd*, 1H, *J* 7.4, 5.0 Hz, C4-H); 5.15 (*bs*, 1H, N1'-H); 5.51 (*bs*, 1H, N3'-H). ^{13}C NMR: 14.1, 22.7, 24.8, 25.9, 28.2, 28.3, 28.6, 29.2, 29.3, 29.5, 29.6, 29.6, 31.9, 33.9, 40.6, 55.4, 60.2, 61.9, 64.6 (alkyl), 165.0, 173.8 (carbonyl).

Biotin-undecan-1-ol ester (158)

Recovered in 129mg (79%) yield as a colourless amorphous solid mp 110-113°. HRMS: Calculated for $C_{21}H_{38}N_2O_3S$: 398.2603. Found: 398.2584. MS: 398 (M^+, 5), 338 (24), 227 (24), 166 (100), 97 (46). ^{1}H NMR: 0.91 (3H, *J* 6.4 Hz, CH_3-CH_2); 1.29-1.77 (*m*, methylene protons); 2.36 (*t*, 2H, *J* 7.3 Hz, CH_2-CO_2); 2.76 (*d*, 1H, J_{gem} 12.8 Hz, C5-Hb); 2.96 (*dd*, 1H, J_{gem} 12.8, 4.9 Hz, C5-Ha); 3.19 (*dt*, 1H, *J* 7.6, 4.9 Hz, C2-H); 4.09 (*t*, 2H, *J* 6.8 Hz, CH_2-O_2C); 4.35 (*dd*, 1H, *J* 6.6, 4.6 Hz, C4-H); 4.52 (*dd*, 1H, *J* 6.6, 4.9 Hz, C3-H); 4.66 (*bs*,

1H, N1'-H); 4.88 (*bs*, 1H, N3'-H). ^{13}C NMR: 14.1, 22.6, 24.8, 25.9, 28.2, 28.3, 28.6, 29.2, 29.3, 29.5, 29.5, 31.8, 33.9, 40.5, 55.4, 60.1, 61.9, 64.5, (alkyl), 163.7, 173.8 (carbonyl).

Biotin octan-1-ol ester (159)

Recovered 147mg (98%) as a colorless amorphous solid mp 121°. HRMS Calculated for $C_{18}H_{32}N_2O_3S$: 356.2134. Found: 356.2128. MS: 356 (M^+, 100), 329 (26), 287 (51), 227 (52), 166 (82), 97 (71). ^{1}H NMR: 0.89 (*t*, 3H, *J* 6.5 Hz, CH_3-CH_2); 1.28-1.76 (*m*, 18H, methylene protons); 2.35 (*t*, 2H, *J* 7.2 Hz, CH_2-CO_2); 2.74 (*d*, 1H, J_{gem} 12.8 Hz, C5-Hb); 2.92 (*dd*, 1H, J_{gem} 12.8, 4.7 Hz, C5-Ha); 3.16 (*dt*, 1H, *J* 6.9, 4.8 Hz, C2-H); 4.06 (*t*, 2H, *J* 6.8 Hz, CH_2-O_2C); 4.32 (*dd*, 1H, *J* 7.7, 4.8 Hz, C4-H); 4.52 (*dd*, 1H, *J* 7.7, 4.8 Hz, C3-H); 5.21 (*bs*, 1H, N1'-H); 5.59 (*bs*, 1H, N3'-H). ^{13}C NMR: 14.1, 22.7, 24.8, 25.9, 28.2, 28.3, 28.6, 29.3, 29.4, 29.5, 29.6, 29.6, 29.7, 31.9, 33.9, 40.5, 55.4, 60.1, 61.9, 64.6 (alkyl), 163.4, 173.8 (carbonyl).

Biotin hexan-1-ol ester (160)

Recovered 110mg (81%) as a colourless amorphous solid mp 119-120°. HRMS Calculated for $C_{16}H_{28}N_2O_3S$: 328.1821. Found: 328.1827. MS: 328 (M^+, 19), 268 (14), 227 (19), 166 (39), 105 (100). ^{1}H NMR: 0.89 (*t*, 3H, *J* 6.3 Hz, CH_3-CH_2); 1.29-1.71 (*m*, 14H, methylene protons); 2.33 (*t*, 2H, *J* 7.0 Hz, CH_2-CO_2); 2.73 (*d*, 1H, J_{gem} 12.8 Hz, C5-Hb); 2.91 (*dd*, 1H, J_{gem} 12.8, 4.8 Hz, C5-Ha); 3.15 (*dt*, 1H, *J* 7.4, 4.8 Hz, C2-H); 4.05 (*t*, 2H, *J* 6.7 Hz, CH_2-O_2C); 4.30 (*dd*, 1H, *J* 7.7, 4.8 Hz, C4-H); 4.51 (*dd*, 1H, *J* 7.7, 4.8 Hz, C3-H); 5.38 (*bs*, 1H, N1'-H); 5.78 (*bs*, 1H, N3'-H). ^{13}C NMR: 13.9, 22.5, 24.8, 25.5, 28.2, 28.4, 28.6, 31.4, 33.9, 40.5, 55.4, 60.1, 61.9, 64.5 (alkyl), 163.8, 173.8 (carbonyl).

Biotin propan-1-ol ester (161)

A mixture of biotin (**41**) (100mg, 4.1 mmol), propan-1-ol (3ml) and *p*-TsOH (7.8mg, 0.04 mmol, 0.1eq) was refluxed for 6 hours, at which time a homogeneous mixture was formed. The reaction mixture was cooled to room temperature, crystallised unreacted biotin removed by filtration, the solvent removed *in vacuo* and the residue purified by flash chromatography

eluant MeOH/CH_2Cl_2 5:95 to give the title compound as colourless crystals mp 126-127° in 73mg (62 %) yield. HRMS: Calculated for $C_{13}H_{22}N_2O_3S$: 286.1351. Found: 286.1362. MS: 286 (M^+, 7), 227 (82), 166 (84), 97 (100). ^{1}H NMR: 0.95 (*t*, 3H, *J* 7.5 Hz, CH_3-CH_2); 1.43-1.78 (*m*, 8H, methylene protons); 2.34 (*t*, 2H, *J* 7.3 Hz, CH_2-CO_2-); 2.74 (*d*, 1H, J_{gem} 12.8 Hz, C5-Hb); 2.93 (*dd*, 1H, J_{gem} 12.8, 4.9 Hz, C5-Ha); 3.17 (*dt*, 1H, *J* 8.0, 4.7 Hz, C2-H); 4.04 (*t*, 2H, *J* 6.8 Hz, CH_2-O_2C); 4.33 (*dd*, 1H, *J* 7.6, 4.7 Hz, C4-H); 4.53 (*dd*, 1H, *J* 7.6, 4.7 Hz, C3-H); 5.00 (*bs*, 1H, N1'-H); 5.34 (*bs*, 1H, N3'-H). ^{13}C NMR: 10.3, 21.9, 24.7, 28.2, 28.3, 33.9, 40.5, 55.4, 60.1, 61.9, 65.9 (alkyl), 163.9, 173.8 (carbonyl).

Biotin-undec-10-yn-1-ol ester (162)

A mixture of biotin-NHS ester (**106**) (100mg, 0.29 mmol), undec-10-yn-1-ol (**59**) (49mg, 0.29 mmol) and DMAP (3.6mg, 0.03 mmol, 0.1 eq) was stirred in DMF (0.5ml) for 48 hours at 50°. Silica gel (1g) was added and the solvent removed under vacuum (oil pump). The residue was purified by flash chromatography eluant MeOH/CH_2Cl_2 6/94 to give the title compound as a colourless amorphous solid mp 84-86° in 52.6mg (46%) yield. HRMS Calculated for $C_{21}H_{34}N_2O_3S$: 394.2290. Found: 394.2298. MS: 394 (M^+, 6), 334 (18), 227 (62), 166 (37), 97 (100). IR ($CDCl_3$ solution): 3476*br m* (N-H), 3304*s* (H-C≡), 2140*w* (C≡C), 1710*br s* (C=O). ^{1}H NMR: 1.25-1.77 (*m*, methylene protons); 1.94 (*t*, 1H, *J* 2.6 Hz, H-C≡); 2.18 (*dt*, 1H, *J* 6.8, 2.6 Hz, CH_2-C≡); 2.29 (*t*, 2H, *J* 7.1 Hz, CH_2-CO_2); 2.74 (*d*, 1H, *J* 12.8 Hz, C5-Hb); 2.92 (*dd*, 1H, *J* 12.8, 4.8 Hz, C5-Ha); 3.16 (*dt*, 1H, *J* 7.4, 4.7 Hz, C2-H); 4.05 (*t*, 2H, *J* 6.6 Hz, CH_2-O_2C); 4.31 (*dd*, 1H, *J* 7.6, 4.7 Hz, C4-H); 4.51 (*dd*, 1H, *J* 7.6, 4.8 Hz, C3-H); 5.43 (*bs*, 1H, N1'-H); 5.81 (*bs*, 1H, N3'-H). ^{13}C NMR: 20.4, 26.8, 27.9, 30.3, 30.4, 30.5, 30.6, 30.7, 31.0, 31.2, 31.3, 35.9, 42.5, 57.4, 62.1, 64.0, 66.6 (alkyl), 70.1, 86.7 (alkynyl), 165.5, 175.7 (carbonyl).

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