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Palladium-Catalysed Attachment of Labels with Acetylenic Linker Arms to Biological Molecules

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Abstract: Palladium-catalysed coupling has been used to attach a series of fluorescent (acridonyl, pyrenyl, fluoresceinyl, dansyl and ruthenium phenanthrolinyl) and enzymic (biotinyl) labels containing a terminal alkyne to electophilic biological molecules based on amino acids, nucleosides and steroids.

INTRODUCTION

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

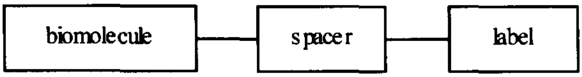


Figure 1

The formation of a covalent bond between a label and a biomolecule generally requires a nucleophilic substitution or electrophilic addition type of reaction in which polar functional groups are used.[3] However, the use of palladium-catalysed couplings of terminal alkynes with aryl or vinyl halides and triflates[4] should allow low polarity covalent bonds to be formed between the label and the biomolecule. Hence a label molecule may be attached to one terminus of a spacer which is further functionalised with a terminal alkyne and then reacted under palladium catalysis with a bromo-, iodo- or triflato arene or alkene on a derivatised biomolecule. The use of this type of reaction for labelling biomolecules offers a complementary methodology to the existing protocols for labelling lipophilic sites on biomolecules.[5] Thus the aromatic side-chains of amino acids such as phenylalanine or tyrosine, the purine and pyrimidine bases of nucleosides and the A and D rings of steroids should all be able to be derivatised with alkynyl labels. A predominately hydrocarbon spacer arm should be more resistant to enzymatic hydrolysis if used *in vivo* compared to functional groups such as esters and amides which result from standard labelling protocols.

In addition, for peptides conformational differences between the labelled and unlabelled biomolecules may be reduced if potential hydrogen bonding interactions of the side-chains caused by the linking heteroatoms are minimised by hydrocarbon spacer arms. The use of a hydrocarbon spacer may offer advantages when preparing labelled biomolecules for use in lipophilic systems such as steroids.

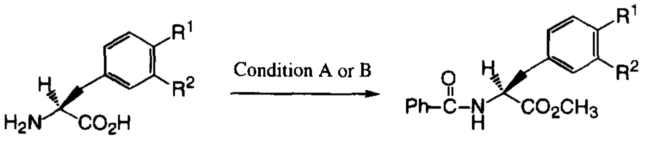
A potential problem with the palladium-catalysed coupling of an alkyne with an organic electrophile is the production of the homocoupled alkyne in preference to the cross-coupled product. Recovery of the homocoupled alkyne in a significant amount has been reported when the oxidative addition of the aryl halide species is slow (generally due to the use of an aryl bromide or electron rich aryl iodide).[6]

We wish to report in this paper the palladium-catalysed coupling of a representaive group of labels based on acridone, pyrene, fluorescein, dimethylaminonaphthalenesulfonamide, a ruthenium complex of 1,10-phenanthroline and an enzymatic probe based on biotin attached to spacers containing a terminal alkyne[7] with suitably derivatised biomolecules. The terminal alkyne group offers the opportunity to form a non-polar covalent bond between the linker arm and the biomolecule and to position the label in a remote position from essential functional groups on the biomolecule.

RESULTS AND DISCUSSION

Coupling with Amino Acids

The protection of the amino and carboxylic acid groups was accomplished by the standard esterification with methanol and thionyl chloride followed by reaction with benzoyl chloride under Schotten-Bauman conditions (**Scheme 1**, Condition B)[8].



$R^1 = OH, R^2 = H$
$R^1 = I, \quad R^2 = H$
$R^1 = OH, R^2 = I$

1 $R^1 = OTf, R^2 = H$
2 $R^1 = I, \quad R^2 = H$
3 $R^1 = OH, \ R^2 = I$

Condition A: (i) CH_3OH, $SOCl_2$; (ii) PhCOCl, K_2CO_3, CH_2Cl_2, H_2O; (iii) $PhNTf_2$, NEt_3, CH_2Cl_2.
Condition B: (i) CH_3OH, $SOCl_2$; (ii) PhCOCl, K_2CO_3, CH_2Cl_2, H_2O.

Scheme 1

The conversion of the hydroxyl group of L-tyrosine to the corresponding triflate followed a standard literature procedure (**Scheme 1**, Condition A).[9] The labels used to attach to the biomolecules are shown in **Figure 2**. They include a selection of fluoresent labels (**4, 5, 6, 7, 8** and **11**), a time-resolved fluorescent label (**9**) and the well used biotin label (**10**). The preparation of these alkyne containing labels was discussed in the preceding paper.[7]

Reaction of triflate **1** with acridone **4** under standard alkyne coupling conditions at room temperature[4] (**Scheme 2**, Condition A) showed no coupled product after 60 minutes (TLC examination of the mixture showed only starting materials). Increasing the temperature sequentially to 35°, 50° and 60° at 60 minute intervals again showed only one new fluorescent spot due to the homocoupled alkyne dimer **12** in increasing quantity. Repeating the reactions with either increasing amounts of catalyst (10%, 15% and 30%) or increasing temperature gave only the homocoupled product **12**.

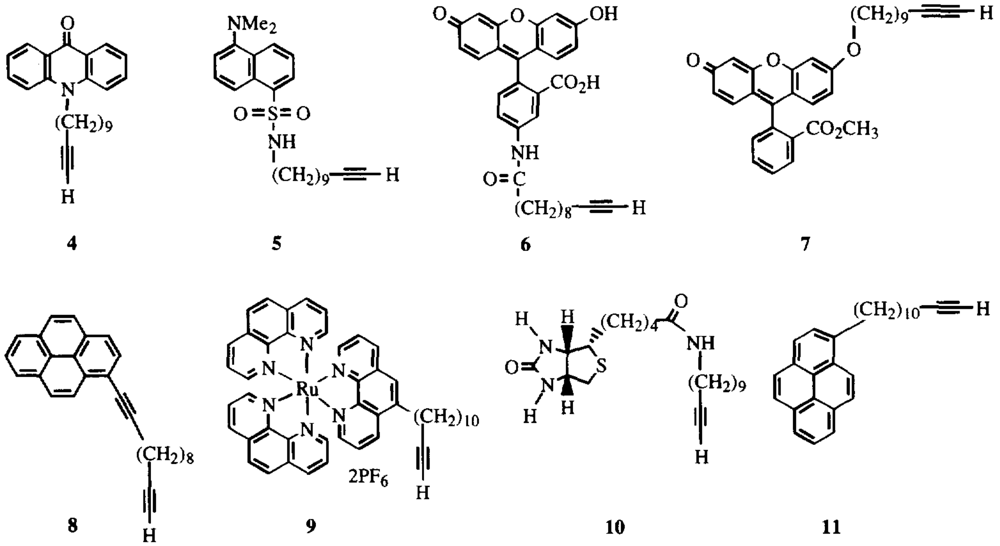
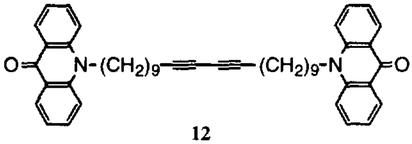


Figure 2

As triflate **1** was still present in the reaction mixture, the homocoupling of the alkyne was occurring at a rate faster than formation of the cross-coupled product. In palladium-catalysed cross coupling reactions, the rate determining step is either the oxidative addition of the electrophile to PdL_2, or transmetallation of the nucleophile onto a palladium (II) intermediate.[10] The use of the catalytic system $Pd_2dba_3/AsPh_3$ has been shown to increase the rate of the transmetallation step.[11] However TLC analysis of the mixture from the reaction between **1** and **4** (stirred at room temperature overnight) in the presence of this catalyst (**Scheme 2**, Condition B) showed only alkyne dimer **12** and residual **1** and **4**. Increasing the temperature to 50° and stirring until the catalyst decomposed (about 30 hours) showed only an increase in the amount of **12** and the consumption of **1** and **4**.

The rate of oxidative additon of aryl triflates and aryl bromides in palladium catalysed cross-coupling reactions is relatively slow compared to aryl iodides, and so conditions reported to effect efficient coupling of aryl bromides were tested[12] (**Scheme 2,** Condition C). Reaction of **1** and **4** at 100° in piperidine gave only unreacted starting alkyne (28%) and dimer **12** (32%). The triflate was consumed in the reaction but no identifiable compound(s) were isolated.



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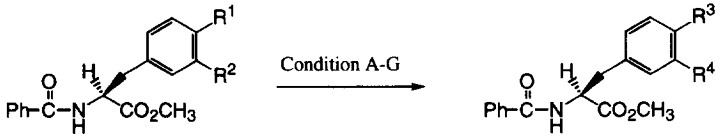
Thus it appeared that the oxidative addition of the aryl triflate was slow, which allowed the (normally) slower homocoupling process to predominate.[13] This problem may be overcome if the stoichiometric reaction between the triflate and $Pd(PPh_3)_4$ to form the σ-bonded intermediate were possible, followed by reaction with the alkyne label.[14] Reaction of **1** with a stoichiometric amount of $Pd(PPh_3)_4$ in DMF (**Scheme 2,** Condition D) at room temperature gave rise to a green precipitate (which decomposed upon exposure to the atmosphere). After 3.5 hours TLC (EtOAc/hexanes 50/50) analysis showed the absence of **1** at $R_f = 0.25$ and a new spot at $R_f = 0.05$. Assuming this new spot to be the required σ-complex the acridone label **4**, CuI and Et_3N were added to the mixture. The green precipitate was immediately consumed and the reaction turned dark brown. After stirring at 50° for 30 minutes, TLC analysis showed the absence of the low R_f spot, and spots corresponding to unreacted **4** ($R_f = 0.63$), dimer **12** ($R_f = 0.37$) and coupled product **13** ($R_f = 0.22$). After separation by flash chromatography **13** was recovered in 68% yield.

Although the stoichiometric coupling of triflate **1** was successful, conditions for catalytic couplings were still sought as palladium reagents are expensive. A literaure report revealed that phenyl triflate could be coupled with a variety of terminal alkynes under palladium catalysis in DMF at 90° without CuI.[15] However reaction of **1** with **4** under these conditions resulted in alkyne dimer **12** being isolated in 33% as the only identifiable product (**Scheme 2,** Condition E). The remainder of the starting materials appeared to undergo decomposition.

DMSO has been used as a solvent in coupling reactions where the temperature has been raised to facilitate reaction of an unreactive substrate[16], and so reaction of **1** with **4** at 70° under these conditions gave the coupled product **13** in 86% yield (**Scheme 2,** Condition F). A small amount of alkyne dimer **12** (3%) was also recovered. Repeating the reaction in DMF under standard conditions (**Scheme 2**, Conditions G) with the temperature at 70° from the beginning of the reaction resulted in a yield of 80% of the desired coupled product **13**, showing that the temperature of reaction is critical to the coupling.



1 R^1 = OTf, R^2 = H
2 R^1 = I, R^2 = H
3 R^1 = OH, R^2 = I

13 R^3 = **4**-H, R^4 = H
14 R^3 = **5**-H, R^4 = H
15 R^3 = **6**-H, R^4 = H
16 R^3 = **9**-H, R^4 = H
17 R^3 = **10**-H, R^4 = H
18 R^3 = OH, R^4 = **7**-H
19 R^3 = OH, R^4 = **10**-H

Condition A: Label (1.5 eq), $Pd(PPh_3)_4$ (0.1eq), CuI (0.2 eq), Et_3N, DMF, 20°.
Condition B: Label (2.5 eq), Pd_2dba_3 (0.025 eq), $AsPh_3$ (0.2 eq), CuI (0.1 eq), Et_3N, DMF.
Condition C: Label (1.5 eq), $Pd(PPh_3)_4$ (0.1 eq), CuI (0.2 eq), PPh_3 (0.2 eq), piperidine.
Condition D: (i) $Pd(PPh_3)_4$ (1.0 eq), DMF. (ii) Label (1.5 eq), CuI (0.2 eq), Et_3N.
Condition E: Label (1.5 eq), $Pd(PPh_3)_4$ (0.1 eq), Et_3N, DMF, 90°.
Condition F: Label (1.5 eq), $Pd(PPh_3)_4$ (0.1eq), CuI (0.2 eq), Et_3N, DMSO.
Condition G: Label (1.5 eq), $Pd(PPh_3)_4$ (0.1eq), CuI (0.2 eq), Et_3N, DMF, 70°.

Scheme 2

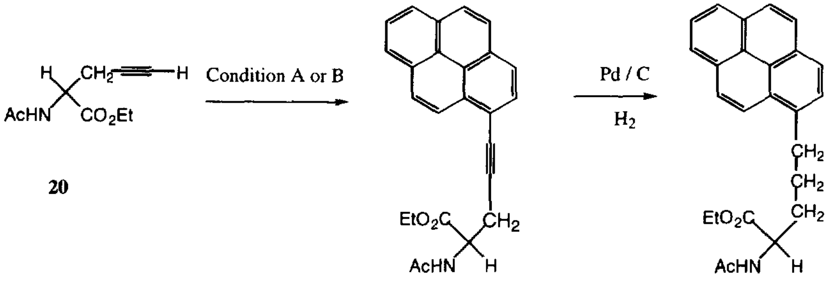
Presumably, as the temperature rises the rate of oxidative addition of the aryl triflate to the palladium catalyst becomes significant and hence can react further in the catalytic cycle to give the coupled product. This approach is, however, limited by the thermal stabilities of the substrates and catalyst.

Reaction of iodophenylalanine **2** with the acridone label **4** proceeded at room temperature under the standard conditions (**Scheme 2**, Condition A) and gave the expected coupled product **13** in an excellent yield of 96% and only 3% of **12**. Reaction of **2** with the dansyl **5**, aminofluorescein **6**, *tris*-1,10- phenanthroline ruthenium **9** and biotin **10**, labels all proceeded under the standard conditions (**Scheme 2**, Condition A) at room temperature in less than four hours to give the coupled products **14** (82%), **15** (75%), **16** (65%) and **17** (in 84%), respectively.

Reaction of **3** with the fluorescein label **7** proceeded at room temperature under the standard conditions (**Scheme 2**, Condition A). The reaction was complete 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 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 was not isolated. Purification by flash chromatography gave the labelled compound **18** in 91% yield. Reaction of **3** with the biotin label **10** under the standard conditions (**Scheme 2**, Condition A) again proceeded at room temperature and gave the labelled compound **19** in 76% yield. Thus conditions were identified where both the iodo and triflated substrates reacted readily with the labels to give the adducts in good to excellent yields and under mild conditions.

Pyrene derivatives[17] of propargylglycine could be readily prepared from coupling with the corresponding halopyrene. Thus reaction of **20**[6] with 1-bromopyrene at 50° overnight under standard conditions (**Scheme 3**, Condition A) gave the labelled compound **21** in 44% yield. TLC (60/40 EtOAc/hexanes) showed the absence of **20** at R_f 0.36, unreacted 1-bromopyrene at R_f 0.89, a new fluorescent spot at R_f 0.29 (corresponding to **21**) and a large non-UV active spot at R_f 0.08 (presumably the alkyne dimer). As the yield was only moderate, the reaction was repeated in piperidine at 100° (**Scheme 3**, Condition B). TLC of the reaction mixture after 60 minutes showed the absence of 1-bromopyrene and a fluorescent spot for **21**. After chromatography **21** was recovered in 51% yield. As the yield had not significantly improved, coupling of **20** with 1-iodopyrene was attempted(**Scheme 3**, Condition A). The coupled product was obtained in a good yield of 72% after stirring at room temperature overnight. The saturated derivative **22** was readily prepared in 94% yield by catalytic hydrogenation of **21**.

Fluorescence was not quenced upon coupling of the labels to the amino acids and such labelled amino acids (suitably protected) should be able to be used in the construction of modified synthetic peptides (using both automated and manual methods).



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21

22

Condition A: 1-halopyrene (0.66 eq), $Pd(PPh_3)_4$ (0.1 eq), CuI (0.2 eq), Et_3N, DMF, 50°
Condition B: 1-halopyrene (0.66 eq), $Pd(PPh_3)_4$ (0.1 eq), CuI (0.2 eq), PPh_3 (0.2 eq), piperidine, 100°

Scheme 3

Coupling with Nucleosides

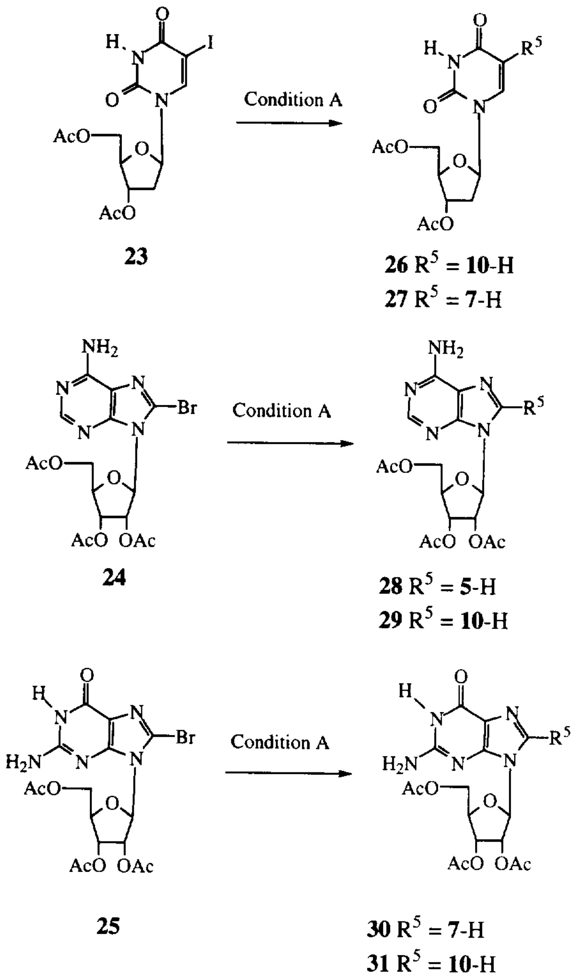
The palladium catalysed cross coupling reactions between 5-halo or 5-triflyluridine derivatives and terminal alkynes has been used to prepare 5-alkynyl substituted compounds, which may have antiviral or anticancer activity[18,13] and in the preparation of 5-propargylamine adducts, which were reacted with fluorescent labels[16]. 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[16] and A_1 adenosine receptor activity[19]. These reactions generally occured in high yields and under mild conditions; protection of the sugar hydroxyl groups, characterisation purposes.[20]

Thus the nucleoside derivatives 5-iodouridine, 8-bromoadeninosine and 8-bromoguanosine were selected for coupling reactions and protected as their di- or triacetates by reaction with acetic anhydride in pyridine[21,22] to give the protected compounds **23**, **24** and **25** respectively. A mole ratio of 2:1 copper to palladium has been shown to offer the best coupling conditions for alkynes with uridine derivatives as the production of sideproducts is minimised[13,18,16]. The reaction of protected 5-iododeoxyuridine **23** with the biotin label **10** as shown in **Scheme 4** (Condition A) gave a dark brown reaction mixture after stirring at 40° for 3 hours. TLC (10/90 $MeOH/CH_2Cl_2$) showed the absence of **23** 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 **26** which was formed in 86% yield, and no trace of products resulting from unwanted cyclization[13]. Similarly, reaction of **23** with the fluorescein label **7** (**Scheme 4**) gave the adduct **27** in 76% yield. Coupling of protected 8-bromoadenosine **24** with the acridone label **5** (**Scheme 4**, Condition A) gave the product **28** in 89% yield. Similarly, reaction of **24** with the biotin label **10** under the same conditions (**Scheme 4**) gave the biotin adduct **29** in 88% yield.

Guanosine derivative **25** was reacted with the fluorescein label **7** under standard conditions (**Scheme 4**, Condition A). 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 **25**. After removal of solvent, chromatographic separation of the residue gave the fluorescein dimer in 12% yield (based on starting label), followed by the coupled product **30**, 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, **30** 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. Similarly, reaction of **25** with the biotin label **10** under the previous conditions (**Scheme 4**) and workup gave the labelled compound **31** in 58% yield.

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 **23** and 8-bromoadenosine derivative **24**; however the 8-bromoguanosine derivative **25** couples in only average yields. The preparation of labelled deoxynucleosides for incorporation into oligonucleotides via standard solid phase methodology should easily be achieved.



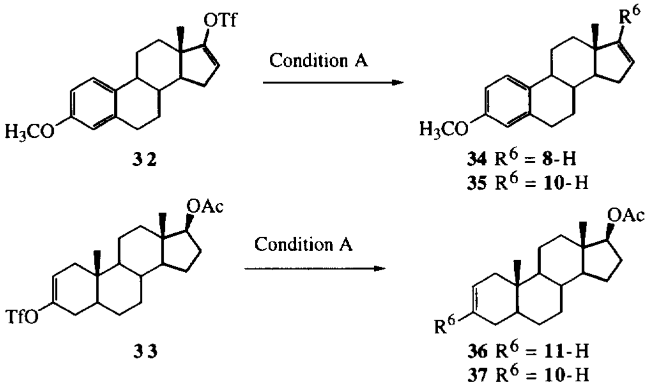
Condition A: Label 1.2 eq, $Pd(PPh_3)_4$ (0.1 eq), CuI (0.2 eq), Et_3N (1.2 eq), DMF, 40°

Scheme 4

Coupling with Steroids

Steroids with carbonyl groups such as estrone and epiandosterone are easily converted to triflate derivatives and have been shown to undergo a variety of palladium catalysed processes; the cross coupling with terminal alkynes has been well investigated[23]. As labelled steroidal derivatives have been used as probes in biological systems[24], (particularly in the study of membrane structures[25]), the possibility of coupling estrone triflate derivative **32** and epiandrosterone triflate derivative **33** with the developed pyrene labels **8** and **11**, and biotin label **10** was investigated.

Reaction of **32** with pyrene label **8** under standard conditions (**Scheme 5**) 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 **34** was recovered in 92% yield. Similarly, reaction of **32** with biotin label **10** under standard conditions (**Scheme 5**) gave the labelled product **35** 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.



Condition A: Label 1.2 eq, $Pd(PPh_3)_4$ (0.1 eq), CuI (0.2 eq), Et_3N (1.2 eq), DMF, 40°

Scheme 5

Reaction of androsterone derivative **33** with pyrene label **11** under standard conditions (**Scheme 5**) to give **36** occurred in 85% yield, and the spectral data were consistent with the proposed structure. Repeating the reaction with the biotin label **10** (**Scheme 5**) gave the adduct **37** in 88% yield; again the spectral data were consistent with the expected product.

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[26], 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.

EXPERIMENTAL

All reactions were performed in oven dried glassware under a nitrogen atmosphere (unless in aqeuous solution). Melting points were recorded on a Reichhert hot stage apparatus and are uncorrected. Proton and carbon NMR spectra were recorded on a Bruker ACP-300 or a Varian Gemini 200 spectrometer in $CDCl_3$ as solvent with tetramethylsilane as an internal standard. Mass spectra were recorded on VG ZAB 2HF mass spectrometer with either electron impact (EI) or fast atom bombardment (FAB) ionisation, or on an AEI-GEC MS 3074 instrument with EI ionisation. Ultraviolet spectra were recorded on a Pye Unicam SP8-100 spectrometer. Fluorescence spectra were recorded on a Perkin Elmer 3000 spectrometer. IR spectra were recorded on a Hitachi 270-30 spectrometer. Triethylamine, pyrrolidine, piperidine and CH_2Cl_2 were distilled from CaH_2 under nitrogen and stored over 4Å molecular sieves. DMF was distilled from CaH_2 (*ca.* 80° at 20mmHg) and stored over 4Å molecular sieves. Analytical thin layer chromatography was carried out using Merck aluminium sheets precoated with kieselgel 60 F_{254} or (when stated) with Alumina 150 F_{254}, and visualised using either a 254 nm or 365 nm lamp, or with a 4% solution of phosphomolybdic acid in ethanol. Flash chromatography was carried using Merck kieselgel 60 (230-400 mesh) or (when stated) on Alumina UG, and solvents used were distilled before use. The following compounds were synthesised according to literature procedures: 1-bromopyrene[27], 1-iodopyrene[28] N-benzoyl-4-iodo-L-phenylalanine methyl ester (**2**)[29], N-benzoyl-3-iodo-L-tyrosine methyl ester (**3**)[8], N-benzoyl-L-tyrosine methyl ester[30], N-benzoyl(4-O trifluoromethanesulfonyl)-L-phenylalanine methyl ester (**1**)[31], ethyl 2-acetamidopent-4-ynoate (**20**)[16], 5-iodo-3',5'-di-O-acetyldeoxyuridine (**23**)[21], 8-bromo-2',3',5'-tri-O-acetyl adedenosine (**24**)[22], 8-bromo-2',3',5'-tri-O-acetylguanosine (**25**)[22], estrone triflate(**32**)[32], androsterone triflate(**33**)[26] 10-(undec-10-ynyl)-9-(10*H*)-acridone (**4**)[7], 11-(1-pyrenyl)undec-10-yn-1-ol (**7**)[7], 1-(1-pyrenyl)dodeca-11-yne (**8**)[7] 1-(1-pyrenyl)-dodeca-1,11-diyne (**9**)[7], 6-*O*-(1-undec-10-ynyl)fluorescein methyl ester (**11**)[7], *N*-(1-oxoundec-10-ynyl)-5-aminofluorescein (**12**)[7], 5-dimethylamino-*N*-(11-undec-1-ynyl)-1-naphthalenesulphonamide (**13**)[7], biotin-*N*-(undec-10-ynyl)amide (**14**)[7], *bis* -1,10-phenanthroline-5-[(dodec-11-ynyl)-1,10-phenanthroline]ruthenium(II) hexafluorophosphate (**21**)[7].

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

***N*-Benzoyl-4-{[11-(10-(10*H*)-9-acridonyl)]undec-1-ynyl}-L-phenylalanine methyl ester (13):** Method A.

To a stirred solution of triflate **1** (50mg, 0.16 mmol) in DMF (1.5ml) was added $Pd(PPh_3)_4$ (29mg, 0.16 mmol). A green/brown suspension slowly formed. TLC analysis after 3.5 hours showed a minor amount of starting material at $R_f = 0.32$ (30/70 EtOAc/hexanes) and a major spot on baseline. Alkyne **5** (60mg, 0.17mmol), CuI (4.4 mg, 0.023mmol) 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 $C_{41}H_{42}N_2O_4$: 626.339. Found: 626.3148. MS: 626 (M^+, 5), 208, 195, 167. IR (nujol): ν_{max} 3272, 1732, 1642, 1606, 1538 cm^{-1}. UV (EtOH):λ_{max} 257 (87 700), 387 (10 400), 405 (12 400) nm (ε). Fluorescence (EtOH, λ_{ex} = 387 nm): 420 (100), 440 (70) nm. ^{1}H NMR: δ 1.17-1.65 (*m*, 12H, $(CH_2)_6$); 1.92 (*quintet*, 2H, *J* = 7.6 Hz, CH_2-CH_2-acridone); 2.40 (*t*, 2H, *J* 7.0 Hz, CH_2-C≡C-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, C-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 PhCON); 7.5-7.75 (*m*, 4H, C3-H and PhCON); 8.57 (*dd*, 2H, *J* = 8.0, 1.6 Hz, C1-H). ^{13}C NMR: δ 19.3, 26.8, 27.1, 28.6, 28.8, 29.0, 29.2, 29.4, 37.6, 46.1, 52.4, 53.4, 80.2, 90.6 (alkynyl), 114.5, 17.1, 18.4, 18.8, 22.9, 23.9, 24.6, 25.2, 26.6, 26.8, 28.7, 28.8, 30.3, 36.7, 166.7, 171.8, 177.9.

N-Benzoyl-4-{[11-(10-(10*H*)-9-acridonyl)]undec-1-ynyl}-L-phenylalanine methyl ester (13): Method B.

To a stirred mixture of DMF (1.0 ml) and Et_3N (0.2 ml) was added sequentially the iodide **3** (50mg, 0.12 mmol), alkyne **5** (63mg, 0.18 mmol), $Pd(PPh_3)_4$ (14mg, 0.012mmol), and CuI (4.5mg, 0.024mmol). After stirring overnight at room temperature TLC (EtOAc/hexanes 50/50) showed the absence of of **3** at R_f 0.65 and new compounds at R_f 0.51 and R_f 0.37. The mixture was separated by flash chromatography using EtOAC/hexanes 40/60 as eluant. First to elute was the alkyne dimer **12** in 2.0mg yield. [^{1}H NMR δ 1.23-1.37 (*m*, 12H, $(CH_2)_6$); 1.95 (*quintet*, 2H, *J* = 7.8 Hz, CH_2CH_2-N); 2.24 (*t*, 1H, *J* = 6.7 Hz, CH_2-C≡C); 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 was recrystallised from CH_2Cl_2/hexanes in 74mg (96%) yield. The ^{1}H NMR data were identical with those reported in the previous method.

Compounds **14**, **16**, **17**, **18**, **19**, **20** and **21** were prepared in a similar manner by Method A except for the difference stated. The scale was based on 50mg of the iodide or triflate.

N-benzoyl-4-[N-(5-dimethylamino-1-naphthalenesulphonyl)-11-aminoundec-1-ynyl]-L-phenylalanine methyl ester (14)

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, 560, 203, 169, 105. IR (thin film): ν_{max} 3300, 1736, 1652, 1578, 1512, 1316, 1144, 910, 738 cm^{-1}. UV (EtOH): λ_{max} 227 (36 200), 247 (39 600), 338 (5 700) nm. Fluorescence (EtOH, λ_{ex} = 338 nm): 503 nm. ^{1}H NMR: δ 1.30-1.21 (*m*, 12H, $(CH_2)_6$); 1.47

(*quintet*, 2H, J = 7.3 Hz, CH_2-CH_2-$NHSO_2$); 2.29 (*t*, 2H, J = 7.1 Hz, CH_2-C≡C-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); 3.5 (*s*, 3H, CH_3O_2C); 4.58 (*t*, 1H, J = 6.5 Hz, SO_2NH-CH_2); 5.01 (*dt*, 1H, J = 7.5, 5.6 Hz, C-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: δ 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, 80.2, 90.7 (alkynyl), 115.1, 13.7, 18.9, 19.1, 23.0, 24.3, 24.6, 25.2, 25.2, 25.6, 25.8, 130.3, 26.7, 26.8, 28.7, 29.7, 30.3, 152.0, 166.8, 171.9.

N-Benzoyl-4-[11-(N-5'-fluoresceinyl)-11-carbonylaminoundec-1-ynyl]-L-phenylalanine methyl ester (15)

Eluant 5/95 MeOH/CH_2Cl_2; bright orange glass; 75%. HRMS (LSIMS) Calculated for $C_{48}H_{45}N_2O_9$ (M+H$^+$): 793.321. Found: 793.3096. MS: 793 (M+H$^+$, 100), 594, 402, 347. IR ($CDCl_3$): ν_{max} 3500-3000, 3080, 1742, 1644, 1602 cm^{-1}. UV (EtOH): λ_{max} 231 (39 900), 257 (31 100), 457 (5 600), 483 (5 900) nm (ε). Fluorescence (EtOH, λ_{ex} = 483 nm): 518 nm. *The NMR data are for the lactone structure.* ^1H NMR (d_6-DMSO): δ 1.21-1.61 (*m*, 12H, $(CH_2)_6$); 2.36 (*t*, 2H, J = 6.7 Hz, CH_2-C≡C-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, C-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); 7.41-7.37 (*m*, 3H, **PhCON**); 7.77 (*m*, 2H, **PhCON**); 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): δ 18.6, 25.0, 28.2, 28.3, 28.4, 28.6, 28.7, 29.0, 36.0, 36.5, 37.0, 51.9, 80.5, 83.0, 90.4, 102.1, 3.8, 1.8, 17.5, 22.2, 23.2, 23.6, 24.3, 25.1, 25.5, 130.9, 26.3, 26.5, 28.6, 32.6, 35.9, 152.0, 159.9 , 166.4, 168.7, 172.0, 172.1.

N-Benzoyl-4-(12-{5-[1,10-phenanthroline-*bis*-1,10-phenanthrolineruthenium(II)]dodec-1-ynyl})phenylalanine methyl ester hexafluorophospate (16)

Chromatography on alumina with eluant $CHCl_3$; bright orange glass; 65%. MS (LSIMS): 192 ([^{102}RuM-PF_6]$^+$, 82), 37 (^{102}RuM-2PF_6, 100). UV ($CHCl_3$): λ_{max} 263 (87 600), 418 (12 000), 451 (12 800) nm (ε). Fluorescence ($CHCl_3$, λ_{ex} = 451 nm): 573 nm. ^1H NMR: δ 1.22-1.61 (*m*, 14H, $(CH_2)_7$); 1.86 (*quintet*, 2H, J = 7.2 Hz, CH_2-CH_2-Ar); 2.38 (*t*, 2H, J = 6.9 Hz, CH_2-C≡C-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-4-[(*N*-biotinyl)-11-aminoundec-1-ynyl]-L-phenylalanine methyl ester (17)

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, 521, 461, 227, 105. IR (nujol): ν_{max} 3296, 1742, 1706, 1642, 1538 cm^{-1}. ^{1}H NMR: δ 1.29-1.76 (*m*, 22H, $(CH_2)_{11}$); 2.17 (*t*, 2H, *J* = 7.5 Hz, CH_2CON); 2.38 (*t*, *J* = 6.9 Hz, CH_2-C$\equiv$C-Ar); 2.76 (*d*, 1H, J_{gem} = 12.8 Hz, C5-Hβ); 2.86 (*dd*, 1H, J_{gem} = 12.8, 4.9 Hz, C5-Hα); 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, C-H); 5.40 (*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, PhCONH). ^{13}C NMR: δ 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, 40.6, 60.2, 61.8, 80.2, 90.8 (alkynyl), 18.9, 23.1, 24.6, 25.2, 26.7, 26.8, 28.7, 30.4, 163.8, 166.9, 172.2, 173.1.

N-Benzoyl -3-({11-[6-*O*-(methyl)fluoresceinyl]}undec-1-ynyl)-L-tyrosine methyl ester (18)

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, 673, 601, 326. IR ($CDCl_3$): ν_{max} 3436, 1726, 1644, 1598, 1516 cm^{-1}. UV (EtOH): λ_{max} 225 (72 500), 253 (36 200), 276 (20 600), 301 (13 900), 365 (9 200), 441 (23 700), 460 (33 000), 489 (26 100) nm (ε). Fluorescence (EtOH, λ_{ex} = 489 nm): 521 nm. ^{1}H NMR: δ 1.35-1.50 (*m*, 10H, $(CH_2)_5$); 1.65 (*quintet*, 2H, *J* = 7.2 Hz, CH_2CH_2-C$\equiv$C); 1.86 (*quintet*, 2H, *J* = 6.8 Hz, CH_2CH_2-OAr); 2.48 (*t*, 2H, *J* = 7.0 Hz, CH_2-C$\equiv$C-); 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, H-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: δ 19.6, 25.9, 28.4, 28.9, 29.0, 29.1, 29.2, 29.3, 36.9, 52.4, 53.7, 68.9.

74.9, 97.3 (alkynyl), 100.7, 105.7, 110.7, 114.0, 114.6, 114.8, 117.4, 23.1, 23.2, 24.4, 24.6, 24.8, 25.7, 25.9, 130.0, 130.3, 130.3, 130.5, 26.1, 26.7, 27.5, 27.7, 28.9, 29.6, 150.7, 137.4, 156.1, 159.0, 163.8, 165.6, 166.9, 172.0, 185.6.

N-Benzoyl-3-[N-(biotinyl)-11-aminoundec-1-ynyl]-L-tyrosine methyl ester (19)

Eluant 8/92 MeOH/CH_2Cl_2; 76%; light yellow crystals mp 88-90°. HRMS Calculated for $C_{38}H_{50}N_4O_6S$: 50.3451. Found: 50.3464. MS: 50 (M^+, 14), 631, 537, 477, 413, 105. IR ($CDCl_3$): ν_{max} 3500, 1706, 1656, 420 cm^{-1}. ^{1}H NMR: δ 1.26-1.73 (*m*, 20H, $(CH_2)_{10}$); 2.17 (*t*, 2H, *J* = 7.4 Hz, CH_2-C=O); 2.45 (*t*, 2H, *J* = 6.8 Hz, CH_2-C≡C); 2.68 (*d*, 1H, J_{gem} = 12.8 Hz, C5-Hβ); 2.86 (*dd*, 1H, J_{gem} = 12.8, 4.8 Hz, C5-Hα); 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, 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: δ 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, 40.4, 60.1, 61.8, 75.0, 97.2 (alkynyl), 110.7, 114.8, 23.1, 23.3, 24.6, 130.2, 26.8, 27.5, 28.8, 156.0, 163.7, 167.1, 172.3, 173.2.

Ethyl 5-(1-pyrenyl)-2-acetamidopent-4-ynoate (21): Method A.

A mixture of the alkyne **20** (100mg, 0.40 mmol), 1-bromopyrene (102mg, 0.37 mmol), $Pd(PPh_3)_4$ (63mg, 0.040 mmol), CuI (21mg, 0.011 mmol), 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 140-156° in 63mg (44%) yield. HRMS Calculated for $C_{25}H_{21}NO_3$: 383.1521. Found: 383.1509. MS: 383 (M^+, 11), 323, 238. IR (nujol): ν_{max} 3320, 1736, 1644 cm^{-1}. ^{1}H NMR: δ 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≡C); 4.36 (*m*, 2H, OCH_2CH_3); 4.95 (*dt*, 1H, *J* = 7.5, 4.7 Hz, C-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, 82.6, 89.5 (alkynyl), 20.4, 21.1, 21.3, 21.5, 22.2, 22.8, 23.2, 23.8, 24.1, 24.3, 25.7, 130.3, 130.4, 26.0, 26.1, 27.0, 15.9, 170.8.

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

Ethyl 5-(1-pyrenyl)-2-acetamidopent-4-ynoate (21): Method B.

A mixture of the alkyne **20** (78mg, 0.43 mmol), 1-bromopyrene (100mg, 0.36 mmol), $Pd(PPh_3)_4$ (41mg, 0.036 mmol), CuI (13mg, 0.072mmol), PPh_3 (19mg, 0.072 mmol) and piperidine (10ml) was heated at reflux 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 (22)

A mixture of the alkyne **21** (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 140-156° in 278mg (94%) yield. HRMS Calculated for $C_{25}H_{25}NO_3$: 387.1834. Found: 387.1824. MS: 387 (M^+, 68), 240, 228, 215, 149. IR (nujol): ν_{max} 3316, 1748, 1650, 844 cm^{-1}. UV (EtOH): λ_{max} 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) nm (ε). Fluorescence (EtOH, λ_{ex} = 343 nm): 375 (100), 395 (61), 415 (19). ^1H NMR: δ 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_2CH_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, 19.3, 20.7, 20.8, 20.9, 21.9, 22.7, 23.2, 23.3, 23.5, 24.6, 26.4, 30.9, 15.8, 172.6.

5-[*N*-(Biotinyl)-1-aminoundec-1-ynyl]-3',5'-di-*O*-acetyl-2'-deoxyuridine (26)

To a stirred mixture of DMF (1.0 ml) and Et_3N (14mg, 0.14 mmol) at room temperature were added sequentially 5-iodo-2',3'-di-O-acetyldeoxyuridine **23** (50mg, 0.11 mmol), alkyne **10** (37mg, 0.14 mmol), $Pd(PPh_3)_4$ (13mg, 0.011 mmol) and CuI (4.3mg, 0.022 mmol). 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, 394, 307, 227. ^1H NMR: δ 1.09-1.61 (*m*, 18H, $(CH_2)_9$); 1.95 and 2.01 (2 x *s*, 3H, CH_3CO); 1.96-2.13 (*m*, 3H, CH_2CONH and C2'-Hβ); 2.21 (*t*, 2H, *J* = 7.0 Hz, CH_2-C$\equiv$C); 2.31 (*ddd*, 1H, J_{gem} = 14.3, 5.9, 2.4 Hz, C2'-Hα); 2.58 (*d*, 1H, J_{gem} = 12.8 Hz, C5''-Hβ); 2.74 (*dd*, 1H, J_{gem} = 12.8, 5.0 Hz, C5''-Hα); 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); 7.37 (*s*, 1H, C6-H); 11.20 (*bs*, 1H, N3-H). ^{13}C NMR: δ 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, 40.4, 59.2, 61.0, 63.5, 72.5, 73.8, 81.5, 84.8, 93.5, 99.7 (alkynyl), 142.1, 149.3, 161.5, 162.7, 15.8, 15.9, 171.8.

Compounds **27**, **28**, **29**, **30** and **31** were prepared using the appropriate alkyne in a similar manner, except for the differences stated. The scale was based on 50mg of the nucleoside base.

5-{11-[6-*O*-(Methyl)fluoresceinyl]-1-undec-1-ynyl}-3', 5'-di-*O*-acetyl-2'-deoxyuridine (27)

Eluant 7:93 MeOH:CH_2Cl_2; bright orange glass; 76%. HRMS Calculated for $C_{45}H_{16}N_2O_{12}$ ($M+H^+$): 807.325. Found: 807.3115. MS (FAB): 807 ($M+H^+$, 100), 747, 607, 347. IR ($CDCl_3$): ν_{max} 3400, 1760-1680, 1644, 1598 cm^{-1}. UV (EtOH): λ_{max} 232 (66 400), 279 (28 900), 435 (23 000), 460 (32 900), 489 (25 800) nm (ε). Fluorescence (EtOH) (λ_{ex} = 489 nm): 521 nm. ^1H NMR: δ 1.36-1.62 (*m*, 12H, $(CH_2)_6$); 1.85 (*quintet*, 2H, *J* = 7.2 Hz, CH_2CH_2O); 2.14 and 2.20 (2 x *s*, 3H, CH_3CO); 2.24 (*dd*, 1H, J_{gem} = 14.4, 6.7 Hz, C2'-Hβ); 2.41 (*t*, 2H, *J* = 7.1 Hz, CH_2-C≡C); 2.37 (*ddd*, 1H, J_{gem} = 14.4, 5.8, 2.4 Hz, C2'-Hα); 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, 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); 6.40 (*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, 85.2, 95.4 (alkynyl), 100.6, 101.4, 105.6, 2.8, 114.6, 117.4, 24.7, 25.6, 25.7, 130.1, 130.2, 130.5, 26.0, 27.6, 29.6. 35.5, 149.2, 150.3, 137.3, 158.9, 161.4, 163.6, 165.6, 170.0, 170.3, 185.6.

8-[11-(10-(10*H*)-9-Acridonyl)undec-1-ynyl]-2', 3',5'-tri-*O*-acetyladenosine (28)

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 ($M+H^+$, 37), 479, 208. IR (nujol): ν_{max} 3312, 3164, 2232, 1748, 1634, 1600, 1496, 192 cm^{-1}. UV (EtOH): λ_{max} 218 (30 700), 234 (30 100), 257 (46 700), 293 (17 800), 386 (7 060), 405 (7 830) nm (ε). Fluorescence (EtOH, λ_{ex} = 386 nm):

418 (100), 439 (70) nm. ^{1}H NMR: δ 1.41-1.61 (*m*, 10H, $(CH_2)_5$); 1.73 (*quintet*, 2H, *J* = 7.1 Hz, CH_2CH_2-$C\equiv C$); 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-$C\equiv C$); 4.31-4.43 (*m*, H,); 4.37 (*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: δ 19.37, 20.46, 20.37, 20.70, 26.91, 27.20, 27.86, 28.88, 28.98, 29.32, 29.40, 46.15, 63.12, 5.70, 70.43, 72.51, 79.73, 87.32, 98.91, 114.51, 17.19, 18.46, 24.01, 28.88, 29.77, 36.74, 149.24, 153.76, 140.00, 15.28, 15.46, 170.60, 177.98.

8-[*N*-(Biotinyl)-11-aminoundec-1-ynyl]-2',3',5'-tri-*O*-acetyladenosine (29)

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, 301, 259. IR ($CDCl_3$): ν_{max} 3500, 1740, 1708, 1632, 420 cm^{-1}. ^{1}H NMR: δ 1.23-1.75 (*m*, 20H, $(CH_2)_{10}$); 2.04, 2.10 and 2.13 (3 x *s*, 3H, CH_3CO); 2.17 (*t*, 2H, *J* = 7.3 Hz, CH_2CONH); 2.37 (*t*, 2H, *J* = 6.9 Hz, CH_2-$C\equiv C$); 2.74 (*d*, 1H, J_{gem} = 12.7 Hz, C5''-Hβ); 2.89 (*dd*, 1H, J_{gem} = 12.7, 4.8 Hz, C5''-Hα); 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, C5β'-H and C4''-H); 4.51 (*m*, 2H, C5'-Hα 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 δ 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, 40.62, 60.16, 61.85, 63.08, 5.62, 70.40, 72.39, 79.5, 87.28, 99.00 (alkynyl), 14.25, 29.37, 149.05, 153.5, 140.26, 164.22, 15.30, 15.46,170.60, 173.07.

8-{11-[6-*O*-(Methyl)fluoresceinyl]-1-undec-1-ynyl}-tri-*O*-acetylguanosine (30)

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, 347, 259. IR ($CDCl_3$): ν_{max} 327, 1748, 1720, 1688, 1644, 1596 cm^{-1}. UV (EtOH) λ_{max} : 230 (25 200), 279 (18 400), 363 (3 900), 437*sh* (9 600), 460 (13 600), 489 (10 800) nm (ε). Fluorescence (EtOH, λ_{ex} = 489 nm): 522 nm. ^{1}H NMR: δ 1.26-1.48 (*m*, 10H, $(CH_2)_5$); 1.65 (*quintet*, 2H, *J* = 7.2 Hz, $CH_2CH_2C{=}C$); 1.84 (*quintet*, 2H, *J* = 7.1, CH_2CH_2OAr); 2.05, 2.11 and 2.12 (3 x *s*, 3H, CH_3CO); 2.51 (*t*, 2H, *J* = 6.9 Hz, CH_2-$C\equiv C$); 3.65 (*s*, 3H, CH_3OAr); 4.32 (*m*, 2H, C4'-H and C5'-Hβ); 4.51 (*m*, 1H, C5'-Hα); 5.88 (*br s*, 2H, C3-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); 10.5 (*bs*, 1H, N3-H). ^{13}C NMR (d_6-DMSO): δ 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, 2.8, 114.4, 117.0, 117.4, 24.8, 25.7, 25.9, 130.3, 130.4, 26.0, 27.8, 29.3, 150.8, 153.8, 137.1, 156.6, 158.9, 163.7, 165.4, 15.1, 15.3, 170.4, 185.1.

8-[*N*-(Biotinyl)-11-aminoundec-1-ynyl]-2',3',5'-tri-*O*-acetylguanosine (31)

Reaction at 50° for 6 hours; Eluant 10/90 MeOH/CH$_2$Cl$_2$; 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), 373, 261. ^{1}H NMR (CDCl$_3$/d_6-DMSO): 1.20-1.58 (*m*, 20H, (CH$_2$)$_{10}$); 1.99, 2.05, 2.09 (3 x *s*, 3H, CH$_3$CO); 2.03 (*t*, 2H, J = 7.8 Hz, CH$_2$CONH); 2.53 (*t*, 2H, J = 6.2 Hz, CH$_2$-C$\equiv$C); 2.64 (*d*, 1H, J_{gem} = 12.3 Hz, C5"-Hβ); 2.80 (*dd*, 1H, J_{gem} = 12.3, 5.1 Hz, C5"-Hα); 2.99 (*q*, 1H, J = 6.2 Hz, C2"-H); 3.06 (*q*, 2H, J = 7.2 Hz, CH$_2$NHCO); 4.09-4.31 (*m*, 4H, C3"-H, C4"-H, C5'-Hβ, C4'-H); 4.41 (*dd*, 1H, J = 11.7, 3.8 Hz, C5'-Hα); 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, C3-NH$_2$); 7.74 (*bt*, 1H, J = 5.5 Hz, CH$_2$NHCO); 10.97 (*bs*, 1H, N3-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, 40.5, 59.2, 61.0, 62.8, 70.2, 78.9, 96.3, 16.4, 25.3, 150.6, 137.2, 156.0, 162.7, 15.3, 15.4, 170.1, 171.8.

17-[12-(1-Pyrenyl)dodeca-1,11-diynyl]-3-*O*-methylestr-16-ene (34)

To a stirred mixture of DMF (1.0 ml) and Et$_3$N (0.2 ml) was added sequentially the triflate **32** (50mg, 0.12 mmol), alkyne **8** (52mg, 0.14mmol), Pd(PPh$_3$)$_4$ (14mg, 0.012 mmol) and CuI (4.6mg, 0.024 mmol). The reaction mixture was stirred at room temperature for 3 hours, at which time TLC (30/70 CH$_2$Cl$_2$/hexanes) indicated the absence of the triflate. The solvent was removed *in vacuo* and the residue subjected to flash chromatography using 30/70 CH$_2$Cl$_2$/hexanes as eluant to give the title compound as a viscous oil in 5mg (92%) yield. HRMS Calculated for $C_{47}H_{48}O$: 628.3705. Found: 628.3700. MS: 628 (M$^+$, 100), 239. IR (thin film): 3040, 2250, 2216, 1610, 1502, 216, 908, 846, 734 cm^{-1}. 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) nm (ε). Fluorescence (CHCl$_3$, λ_{ex} = 365 nm): 388 (100), 396*sh* (75), 407 (80). ^{1}H NMR: δ 0.84 (*s*, 3H, CH$_3$-);

1.37-2.31 (*m*, CH_2 and CH); 2.38 (*t*, 2H, *J* = 6.6 Hz, CH_2-C$\equiv$C); 2.65 (*t*, 2H, *J* = 7.0 Hz, CH_2-C$\equiv$C); 2.85 (*m*, 2H, CH_2-CH=); 3.74 (*s*, 3H, CH_3-OAr); 5.91 (*t*, 1H, *J* = 1.2 Hz, HC$\equiv$C); 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: δ 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, 40.2, 40.3, 76.1, 77.6, 79.7, 93.9 (alkynyl), 96.4, 111.4, 2.9, 13.9, 20.5, 21.3, 21.4, 21.7, 22.1, 22.1, 23.3, 23.7, 24.0, 25.6, 130.7, 26.2, 26.3, 26.9, 27.9, 28.6 32.91, 33.0, 157.4.

Compounds **35**, **36** and **37** were prepared in a similar manner to that described for compound **34**, apart from the differences stated.

17-[*N*-(Biotinyl)-11-aminoundec-1-ynyl]-3-*O*-methylestr-16-ene (35)

Reaction of compound **32** (50mg, 0.12mmol) with alkyne **10** (57mg, 0.14mmol) 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 $C_{40}H_{57}N_3O_3S$: 659.417. Found: 659.4087. MS : 659 (M$^+$, 29), 644, 459, 416. ^{1}H NMR: δ 0.86 (*s*, 3H, CH_3-); 1.22-2.35 (*m*, CH_2 and CH); 2.20 (*t*, 2H, *J* = 7.5 Hz, CH_2-CONH); 2.36 (*t*, 2H, *J* = 6.9 Hz, CH_2-C$\equiv$C); 2.73 (*d*, 1H, *J* = 12.9 Hz, C5-Hβ); 2.91 (*m*, 3H, C5-Hα 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, 37.8, 37.8, 40.4, 59.2, 61.0, 76.0, 93.7 (alkynyl), 111.4, 2.4, 21.8, 27.0, 28.2, 32.2, 32.3, 157.0, 162.6, 171.7.

3-[12-(1-Pyrenyl)dodec-1-ynyl]-17β-acetyloxyandrost-2-ene (36)

Reaction of triflate **33** (mixture of Δ^2 and Δ^3 isomers, *ca.* 5:1 ratio) (50mg, 0.11mmol) with alkyne **11** (48mg, 0.13 mmol) 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, 326, 215. IR (thin film): 3040, 1732, 386, 210, 1030, 844, 740 cm^{-1}. UV ($CHCl_3$): λ_{max} 248 (35 000), 258 (11 800), 25 (22 400), 279 (35 300), 317 (10 300), 330 (21300), 346 (26 600) nm (ε). Fluorescence ($CHCl_3$, λ_{ex} = 346 nm): 379 (100), 397 (67), 418*sh* (22). ^{1}H NMR: δ 0.73 and 0.76 (2 x *s*, 3H, CH_3-); 0.56-2.19 (*m*, CH_2 and CH); 2.05 (*s*, 3H, CH_3CO_2); 2.30 (*t*, 2H, *J* = 6.9 Hz, CH_2-C≡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, 81.8, 82.8, 87.5, 14.7 (alkynyl and vinyl), 19.5, 20.6, 20.7, 21.0, 21.7, 22.4, 23.0, 23.2, 23.5, 24.6, 25.6, 130.9, 26.4, 26.8, 32.2, 32.3, 171.2.

3-[*N*-(Biotinyl)-11-aminoundec-1-ynyl]-17β-acetyloxyandrost-2-ene (37)

Reaction of triflate **33** (mixture of Δ^2 and Δ^3 isomers, *ca.* 5:1 ratio) (50mg, 0.11 mmol) with alkyne **10** (51mg, 0.13 mmol) 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 $C_{42}H_{65}N_3O_4S$: 707.456. Found: 707.456. MS: 707 (M^+, 18), 632, 337, 227, 42. IR (nujol mull): ν_{max} 3296, 1738, 1704, 1642, 706 cm^{-1}. ^{1}H NMR: δ 0.75 and 0.78 (2 x *s*, 3H, CH_3-); 0.65-2.20 (*m*, CH_2 and CH); 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-C≡C); 2.74 (*d*, 1H, *J* = 12.8 Hz, C5-Hβ); 2.93 (*dd*, 1H, *J* = 12.8, 5.0 Hz, C5-Hα); 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: δ 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, 40.5, 60.1, 61.7, 81.7, 82.8, 87.4, 26.8, 163.8, 171.1, 173.0.

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