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TETRAHEDRON

Conjugate Addition of Amino Acid Side Chains to Dyes Containing Alkynone, Alkynoic Ester and Alkynoic Amide Linker Arms

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Abstract: Derivatives of fluorescein, 7-hydroxycoumarin, Sudan 1 and dansyl chloride with linker arms containing a conjugated terminal alkyne have been prepared. The Michael addition of the sulfanyl group of protected cysteine, the hydroxyl group of protected serine and the ε-amino group of protected lysine to the conjugated alkyne gave the expected heterosubstituted vinyl producs.

INTRODUCTION

Conjugate addition of the nucleophilic groups present in the side chains of amino acids in proteins to α,β-unsaturated carbonyls and sulfones has been used as an analytical tool[1a] for the elucidation of enzyme mechanisms by providing information on the microenvironment of the active site[1b] and for structure determination in proteins by analysing conformational changes[1c] and the spatial relationship between labelled subunits[1d].

Conjugate addition of protein-derived nucleophiles to reactive dyes has also been used for the covalent attachment of colorfast dyes to wool[2] as alternatives to the use of chromium-based mordant dyes [3]. Previous studies have identified the reactive sites in keratin and their relative reactivities as cysteine thiol > lysine ε-amino > serine hydroxyl.[4] Although the cysteine thiol group has the greatest reactivity in this series, lysine is more important for the formation of covalent bonds because of its greater abundance in keratin.

We have reported in the preceeding paper the conjugate addition of the sulfanyl group of protected cysteine, the ε-amino group of protected lysine and the hydroxyl group of protected serine to a series of model α,β-unsaturated alkynes.[5] In view of the importance of heteronucleophilic conjugate addition to dyes containing α,β-unsaturated carbonyls and sulfones we have investigated, and report herein, on the reactions between suitably protected serine, cysteine and lysine derivatives and a variety of dyes containing alkynes which can act as Michael acceptors.

Fluorescent labels and reactive dyes usually consist of three sections: the dye, a spacer unit and a reactive functional group. We required the functionalisation of the dyes with a linker arm terminating in a conjugated alkyne and so four representative dyes were chosen to act as models. Fluorescein **1a, 1b** is a xanthene dye which has found extensive use in both wool dyeing and as a fluorescent probe for biological systems[6]. 7-Hydroxycoumarin **2** is a fluorescent dye which is commonly used in fluorescent labelling experiments[6]. Sudan 1 **3** is a member of the family of azo derivatives which are used extensively in wool dyeing[2] and dansyl chloride **4** is used as a fluorescent label to probe the local environment of the label in

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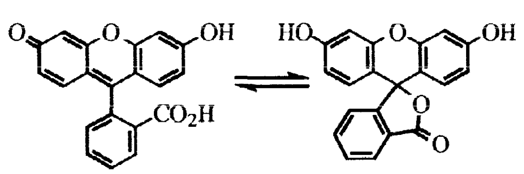
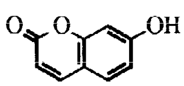
biological systems. Fluorescein **1a**, **1b**, 7-hydroxycoumarin **2** and Sudan 1 **3** contain the reactive hydroxyl groups for the attachment of the linker arm while dansyl chloride **4** contains a reactive sulfonyl chloride.


1a **1b**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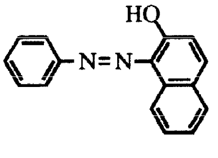
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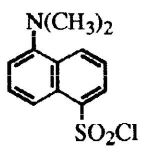
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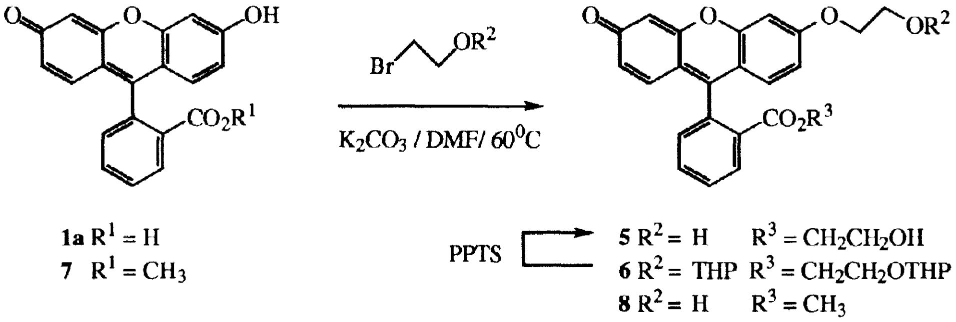
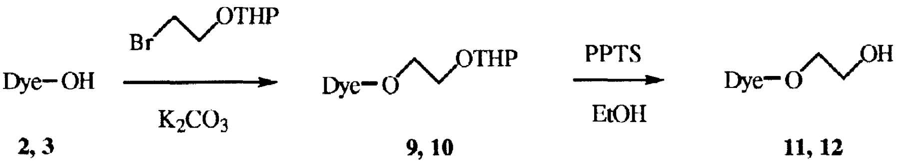
RESULTS AND DISCUSSION

Attachment of the Linker Arms to the Dyes. Fluorescein exists in two tautomeric forms, the orange, fully conjugated **1a**, and colourless lactone **1b** (favoured under acidic conditions[7]). Since the fluorescent form **1a** is needed for labelling experiments, basic conditions were chosen for the alkylation. Accordingly **1a**, 2-bromoethanol and K_2CO_3 were heated in DMF to give the expected disubstituted fluorescein **5** as the major product in 54%, Scheme 1. Unfortunately, diol **5** proved to be very polar and difficult to purify, so the THP adduct of 2-bromoethanol was used for the alkylation of **1a** and gave the readily purified **6** in 95% yield. Subsequent removal of the THP protecting groups by treatment with PPTS[8] in ethanol gave **5** as an orange solid in 63%, Scheme 1. In order to prepare a monoalkylated fluorescein derivative, methyl ester **7** was alkylated with 2-bromoethanol to produce **8** in 52%, Scheme 1.



Scheme 1

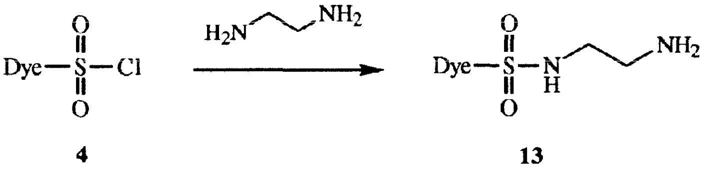
Derivatives of 7-hydroxy coumarin and Sudan 1 were alkylated in a similar manner. Thus **2** and **3** were alkylated with the THP derivative of 2-bromoethanol and K_2CO_3 to form the expected products **9** and **10**, which were subsequently deprotected by PPTS in ethanol to give **11** (99%) and **12** (99%), Scheme 2.



2, 9, 11 Dye-OH = 7-hydroxy coumarin
3, 10, 12 Dye-OH = Sudan 1

Scheme 2

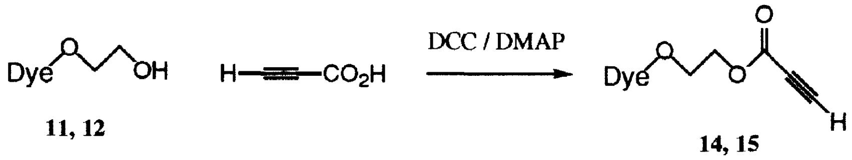
Dansyl chloride **4** was reacted with an excess of ethylenediamine to produce **13**, Scheme 3. As this material was not stable at room temperature, partially decomposing within 1 week, it was prepared immediately before use and the crude material used for further reactions.



4, 13 Dye = 5-dimethylamino-1-naphthyl

Scheme 3

The attachment of the Michael acceptor, propiolic acid, to the dye-linker complex was achieved by an esterification using DCC as the coupling agent. Addition of DMAP and DCC to propiolic acid at room temperature caused polymerisation of the alkyne. Cooling a solution of propiolic acid and **11** to -20°C prior to the addition of DCC and DMAP gave the alkynyl ester derivative **14** in a modest 31% yield. At this temperature the coupling was relatively slow and polymerization of propiolic acid occurred. The coupling between **12** and propiolic acid was more rapid and gave the expected product **15** in a respectable 73% yield, along with a 14% recovery of the starting alcohol **12**, Scheme 4.

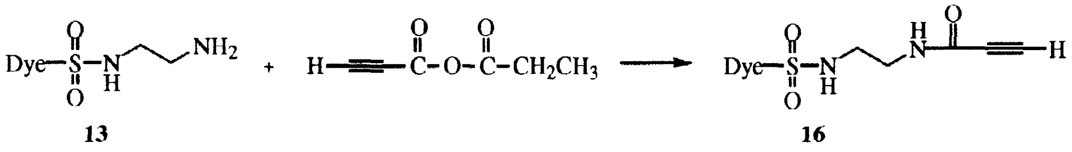


11, 14 Dye = 7-substituted coumarin
12, 15 Dye = O-substituted Sudan 1

Scheme 4

The DCC mediated coupling reactions of the di- and mono-alkylated fluorescein derivatives, **5** and **8**, with propiolic acid gave none of the expected products as the reactions were relatively slow and polymerization of the alkyne occurred. Attempted coupling of **5** with the acid chloride derivative of propiolic acid also produced none of the required esters.

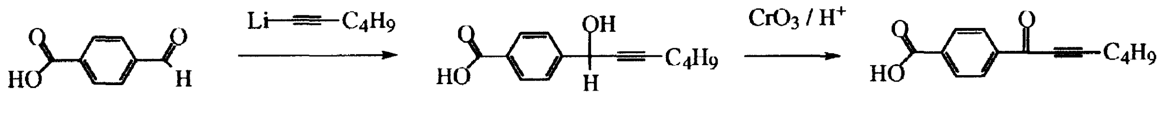
Dansyl derivative **13** was reacted with the mixed anhydride formed from propiolic acid and ethyl chloroformate and gave the required conjugated amide **16** in 58%, Scheme 5



13, 16 Dye = 5-dimethylamino-1-naphthyl

Scheme 5

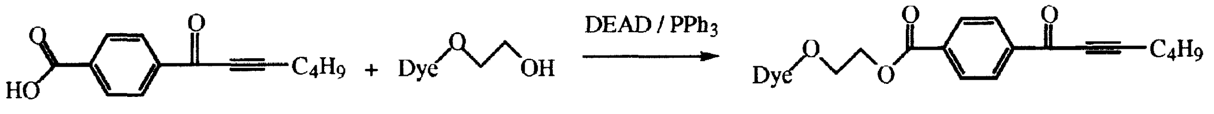
In order to prepare an alkynylketone linker arm a difunctional compound was required which contained both a conjugated, non-terminal acetylenic ketone and a carboxylic acid capable of forming an amide or ester bond with the dye. Compound **18** was chosen and so two equivalents of butyllithium were added to 1-hexyne at -78°C in THF containing two equivalents of DMPU and subsequent addition of 4-carboxybenzaldehyde gave the benzyl alcohol **17** in 40-60%. Jones oxidation of **17** produced **18** as a cream coloured solid in 80%, Scheme 6.



17 **18**

Scheme 6

Acid **18** was coupled to both 7-hydroxycoumarin derivative **11** and the Sudanl derivative **12** using a standard Mitsunobu coupling.[9] The corresponding products, **19** and **20**, were isolated in a 54% and 58% yield respectively, Scheme 7. In an attempt to increase the yields of the couplings, the reaction of the Sudan 1 derivative **12** and the acid **18** was also performed under standard DCC / DMAP conditions, resulting in a similar yield of the alkynyl ketone **20**, (51%).

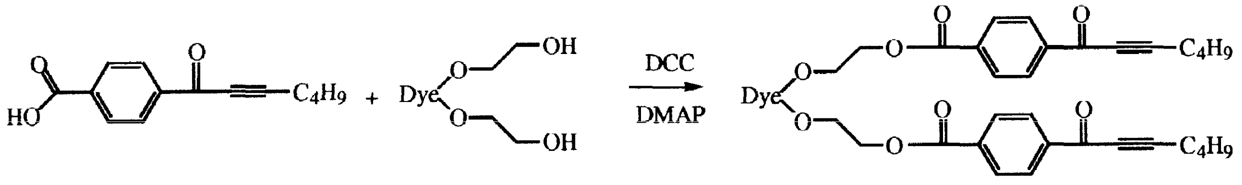


18 **11, 12** **19, 20**

11, 19 Dye = 7-substituted coumarin
12, 20 Dye = O-substituted Sudan 1

Scheme 7

DCC mediated coupling of dialkylated fluorescein **5** and conjugated alkyne **18** for 18h gave only decomposition products. Isolating the product after only 2h at room temperature, however, gave the expected coupling product **21** in 50%, Scheme 8. When the mono-alkylated fluorescein methyl ester **8** and carboxylic acid **18** were subjected to the DCC mediated coupling conditions described above, only decomposition resulted.



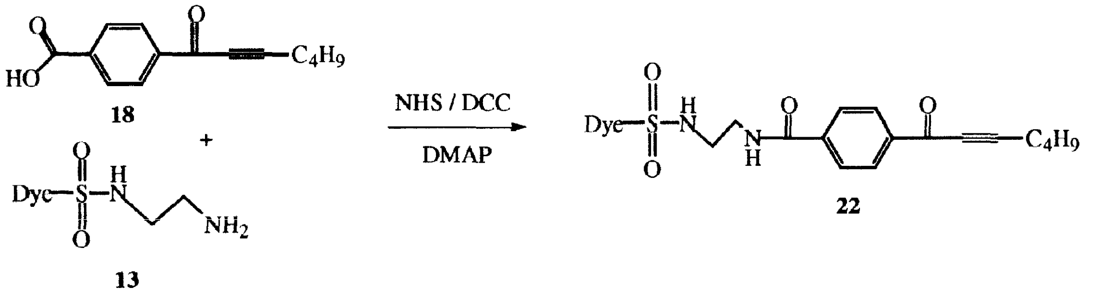
18 **5** **21**

5, 21 Dye = O, CO_2-substituted fluorescein

Scheme 8

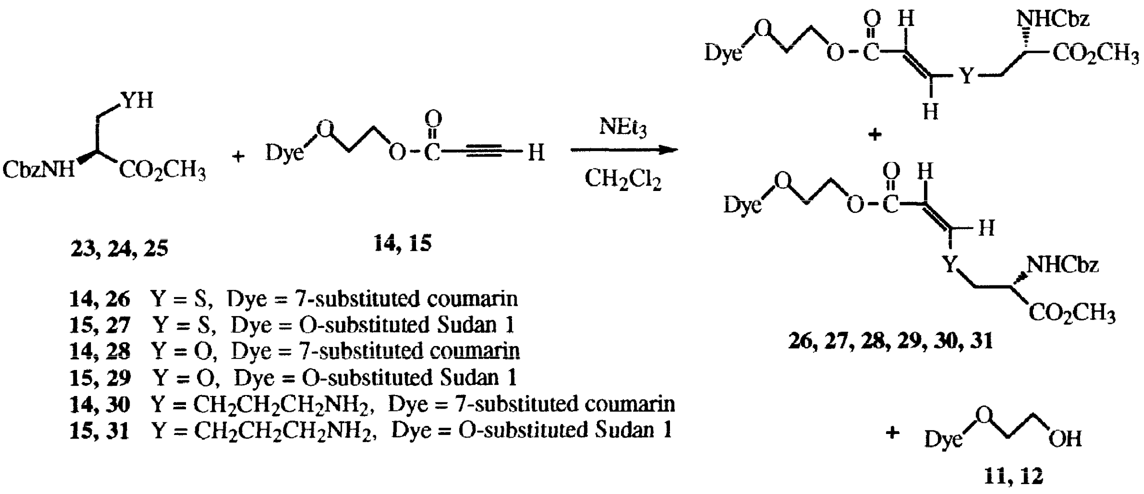
Dansy derivative **13** was reacted with the N-hydroxy succinimde ester of **18** in the presence of DCC and DMAP and gave the expected coupled product 22 in a modest 30%, Scheme 9. This compound was unstable and decomposed within one week at room temperature.

Michael Addition to Dyes. To probe the Michael addition of amino acid nucleophiles to activated acetylenes the reactions of the three protected amino acids *N*-Cbz cysteine methyl ester **23**, *N*-Cbz serine methyl ester **24**, and N_α-Cbz lysine methyl ester **25**, were examined. Our previous model reactions[5] had indicated that a catalytic quantity of NEt_3 was required to initiate the Michael additions. The addition of the cysteine **23** to coumarin **14** in $CHCl_3$ with a catalytic quantity of NEt_3 at 0°C (Procedure A) gave predominantly the *E* isomer **26** (66% isolated yield), the stereochemistry indicated by a vicinal coupling constant of $J = 15$ Hz for the two vinylic protons. Traces of a second isomer were observed by ^1H NMR in the crude reaction mixtures. Similarly, the addition of cysteine **23** to Sudan 1 **15** using Procedure A gave only the *E* isomer (vicinal coupling constant of $J = 15$ Hz) of **27** in 73%, Scheme 10. Again traces of the *Z* isomer were observed by ^1H NMR in the crude reaction mixtures. The isomeric ratio observed in the products was unexpected, as in the model reactions reported previously[5] addition of **23** to ethyl propiolate gave both the *E* and the *Z* isomers in a 4 to 1 ratio. This suggests that the bulky dye moiety is affecting the relative stabilities of the two intermediate anions, such that the *E* anion is greatly preferred over the less stable *Z* anion.



13, 22 Dye = 5-dimethylamino-1-naphthyl

Scheme 9



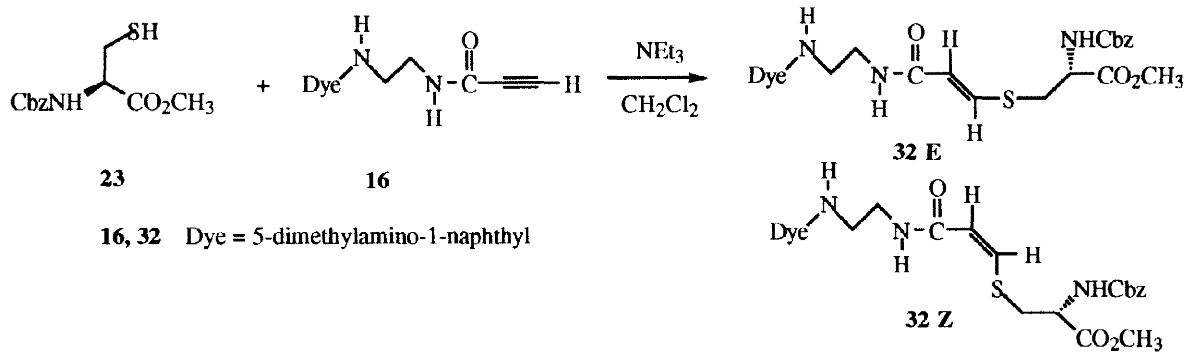
14, 26 Y = S, Dye = 7-substituted coumarin
15, 27 Y = S, Dye = O-substituted Sudan 1
14, 28 Y = O, Dye = 7-substituted coumarin
15, 29 Y = O, Dye = O-substituted Sudan 1
14, 30 Y = $CH_2CH_2CH_2NH_2$, Dye = 7-substituted coumarin
15, 31 Y = $CH_2CH_2CH_2NH_2$, Dye = O-substituted Sudan 1

Scheme 10

In an analogous manner serine **24** was reacted with **14** under Procedure A and produced the expected 1,4-addition product **28** (29%) and the unexpected alcohol **11** (30%), Scheme 10. The vinylic protons of **28** were observed as two doublets at δ 5.26 and at δ 7.55, ($J = 12.5$ Hz), similar to the model reactions reported for the addition of serine **24** and ethyl propiolate ($J = 12$ Hz)[5], confirming that **28** had *E* stereochemistry. Addition of **24** to Sudan 1 derivative **15** also gave two products after purification, the major product 29 (vicinal coupling J = 12 Hz) in 44% and alcohol **12** in 30%. This unexpected 1,2-addition was not observed in the model reactions[5] which suggests that the presence of the bulky dye is slowing the rate of the Michael addition sufficiently to cause base induced hydrolysis to occur. As the nucleophile is postulated to approach the β-carbon of the alkyne along a carbon-carbon-nucleophile angle of 60°[10] it is likely that the large bulk of the dye is partially blocking this route, thus making the normally less accessible 1,2-addition possible. This was not seen for the reactions of the cysteine derivative **23** discussed above since the sulfanyl group has a higher reactivity compared to the hydroxyl group.

The reactions of N_α-Cbz lysine methyl ester **25** with the two alkynyl esters **14** and **15** in chloroform (without the addition of NEt_3) produced almost identical results to the model reaction between ethyl propiolate and **25**. In each case the addition reaction produced both the *E* and *Z* isomers as an inseparable mixture. The products **30*E*** (vicinal coupling J = 13 Hz) and **30*Z*** (vicinal coupling J = 8 Hz) from the addition of lysine **25** to coumarin **14** were formed in a 1.5:1 ratio and in 55%. The addition products **31*E*** (vicinal coupling J = 13 Hz)and **31*Z*** (vicinal coupling J = 8 Hz) from the reaction of the amine **25** with Sudan 1 derivative **15** were produced in a ratio of 1.1:1 in 51% overall yield.

The reaction of the compound where a dansyl group was attached to a terminal acetylenic amide **16** was only carried out with the *N*-Cbz cysteine methyl ester **23**, as the model reactions[5] had demonstrated that serine and lysine were unreactive under these conditions. Cysteine **23** was added to amide **16** using Procedure A and, as expected, two products **32*E*** (vicinal coupling J = 15 Hz) and **32*Z*** (vicinal coupling J = 10 Hz), were recovered, in 55% overall yield, in a 1.6:1 ratio, Scheme 11. Addition of **23**-**25** to **22** using Procedure A gave no isolable products and only decomposition of the alkynone was observed.

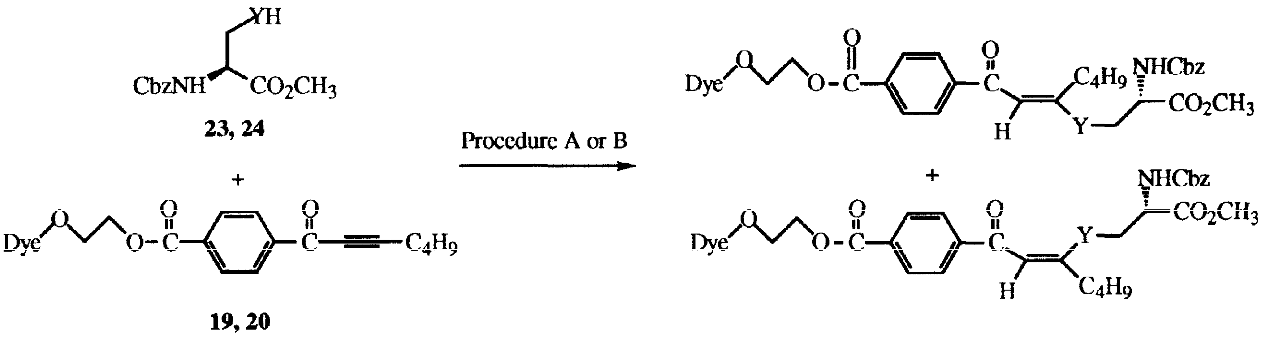


16, 32 Dye = 5-dimethylamino-1-naphthyl

Scheme 11

The reaction of between **19** and **23** using Procedure A gave the two isomers **33*E*** and **33*Z*** in a ratio of 2:1 and in 66% isolated yield, Scheme 12. The vinylic protons of **33*E*** and **33*Z*** were observed as singlets at δ 6.74 and δ 7.00 respectively, consistent with the chemical shifts reported for the model reactions[5]. The addition of **23** to Sudan 1 **20** using Procedure A also produced two isomers **34*E*** and **34*Z***, in a 2.5:1 ratio and 78% isolated yield, Scheme 12. This ratiois in contrast to the model studies which showed a 1:2 E:Z ratio.[5] This reversal in stability is almost certainly due to steric interactions by the bulky dye destabilizing the intermediate *Z* anion. The stereochemistry of the isomers was assigned by comparison with the ^1H NMR spectra from the products of the model reaction reported previously[5].

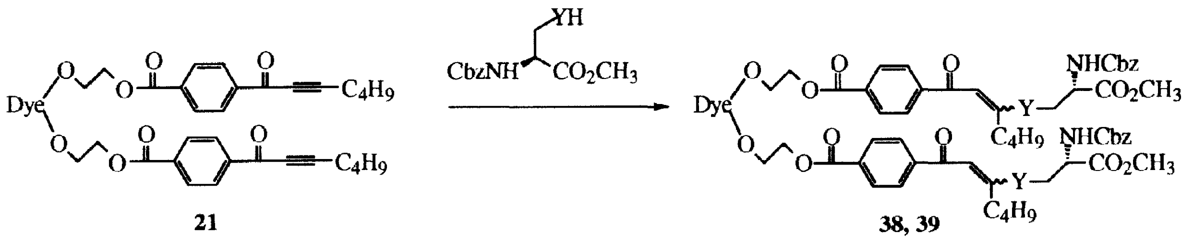
The reaction of serine **24** with **19** did not take place using Procedure A, as anticipated from the model studies[5], so Procedure B in which a 20% molar ratio of tributylphosphine[11] catalyses the addition was used, Scheme 12. The reaction produced the expected Michael addition product **35** which had an almost identical R_f to the starting amino acid **24**, and hence could isolated in pure form. Sudan 1 derivative **20** and serine **24** ireacted using Procedure B and gave **36** in 43% yield. In both cases the chemical shift for the vinylic proton was used to assign stereochemistry by analogy with the model studies.[5] Lysine **25** did not react with Sudan 1 **20** at room temperature using Procedure A however heating the mixture to 60°C did produce a low yield of the expected addition product **37**.



19, 33 Y = S, Dye = 7-substituted coumarin
20, 34 Y = S, Dye = Sudan 1
19, 35 Y = O, Dye = 7-substituted coumarin
20, 36 Y = O, Dye = Sudan 1
20, 37 Y = $CH_2CH_2CH_2NH_2$, Dye = Sudan 1
Procedure A: NEt_3 in CH_2Cl_2
Procedure B: PPh_3 in $CHCl_3$

Scheme 12

The addition of cysteine **23** to the dialkylated fluorescein **21** was undertaken using Procedure A and tlc and hplc analysis of the crude mixture indicated that all of the four possible isomers of the addition product **38** had formed in a 48% combined yield, Scheme 13. The structure of compound **38** was confirmed by LSIMS where a molecular ion at 1382 was observed. The complexity of the ^{1}H NMR spectrum of the mixture prevented the identification of the individual isomers. The reaction of fluorescein **21** with serine **24** using Procedure B produced a single isomer **39** in only 12% isolated yield, Scheme 13. Tlc analysis of the crude reaction mixture showed large amounts of material at low R_f, possibly from the further reaction of the initially formed phosphine-alkyne adduct. The structure of **39** was confirmed by a molecular ion at 1352 by LSIMS. Vinylic resonances were observed as singlets at δ 6.07 and δ 6.16 in the ^{1}H NMR spectrum. This indicates that **39** possesses *E* stereochemistry about both double bonds by analogy with the products **35** and **36**.



21, 38 Y = S, Dye = O, CO_2-substituted fluorescein, Procedure A
21, 39 Y = O, Dye = O, CO_2-substituted fluorescein, Procedure B

Scheme 13

The studies described in this paper illustrate the applicability of conjugated alkynes as Michael acceptors for the attachment of labels to the side-chains of amino acids. A number of labelled amino acids have been prepared in moderate to good yields.

EXPERIMENTAL

Triethylamine, chloroform, dichloromethane and DMF (80°C at 20 mmHg) were distilled from calcium hydride under nitrogen and stored over 4Å molecular sieves. THF was freshly distilled from sodium and benzophenone under nitrogen. Other reagents were purified according to literature procedures.[12] All organic extracts were dried over anhydrous magnesium sulfate unless otherwise specified. 2-(2′-Bromo ethoxy)tetrahydro-2*H*-pyran [13], *N*-carbobenzyloxycysteine methyl ester(**7**)[14], *N*-carbobenzyloxyserine methyl ester(**8**)[15] and *N*-carbobenzyloxy lysine methyl ester(**9**)[14] and were all prepared by literature methods. When a reaction produced more than one stereoisomer, the mass spectrum and analytical data were determined on the mixture.

O-(2′-Hydroxy)ethyl fluorescein (2′′-hydroxy)ethyl ester 5

The tetrahydropyranyl protected alcohol **6** was dissolved in ethanol (100 ml) with PPTS (50 mg, 0.2 mmol) and warmed to 50°C for 3 hours. Removal of the solvent and flash chromatography (10% methanol / 90% dichloromethane) produced the title compound in 63% (0.72g) yield from **6**. ^{1}H NMR δ (d^6-DMSO) 3.76 (m, 2H, 2xCH-O), 3.97 (t, *J* = 5 Hz, 2H, 2xCH-O), 4.16 (m, 2H, 2xCH-O), 4.73 (t, *J* = 5.5 Hz, 1H, CH-O), 4.97 (t, *J* = 5 Hz, 1H, CH-O), 6.23 (d, *J* = 1.5 Hz, 1H, Ar), 6.38 (d, *J* = 9.5 Hz, 1H, Ar), 6.86 (m, 3H, Ar), 7.22 (d, *J* = 1.8 Hz, 1H, Ar), 7.50 (d, *J* = 7 Hz, 1H, Ar), 7.80 (m, 2H, Ar), 8.26 (d, *J* = 7 Hz, 1H, Ar); ^{13}C NMR δ (d^6-DMSO) 58.6, 59.3, 66.6, 70.7, 100.9, 104.5, 113.9, 114.3, 116.7., 128.9, 129.3, 129.8, 129.9, 130.4, 130.6, 130.7, 125.1, 125.8, 150.1, 153.6, 158.4, 163.4, 164.9, 183.9; IR ν_{max} (CDCl$_3$) 2500(br), 2950, 2920, 2850, 1715, 1600, 1500, 1460, 1415 cm^{-1}; UV λ_{max} (EtOH) 204 (50590), 225 (70490), 256 (29430), 439 (35315), 460 (49250), 488 (40640) nm; MS (EI) m/z 420 (M$^{+\cdot}$, 50), 377 (10), 253 (15), 305 (15), 287 (20), 259 (20), 203 (12), 105 (100); HRMS Calcd for $C_{24}H_{20}O_7$ 420.12090. Found 420.12212.

2-(tetrahydro-2H-2-pyranyloxy)ethyl 2-{3-oxo-6-[2-(tetrahydro-2H-2-pyranyloxy)ethoxy]-3H-9-xanthenyl}benzoate 6

2-(2′-Bromoethoxy)tetrahydro-2*H*-pyran (1.40g, 6.62 mmol) was added to fluorescein (1.0g, 3.01 mmol) and potassium carbonate (0.21g, 1.51 mmol) in DMF (50 ml) and the solution heated to 60°C for 12 hours. After this period the mixture was cooled and water (150 ml) was added. The solution was extracted with ethyl acetate (5x30 ml) and the combined organic layers washed with water (4x30 ml) and brine (30 ml). Drying, removal of the solvent and flash chromatography (5% EtOAc / 95% hexane) on the residue yielded the THP protected alcohol **6**, (1.61g, 91%). ^{1}H NMR δ 1.45-1.75 (m, 12H, 12xCH$_2$), 3.4-3.6 (m, 3H, 3xCH-O), 3.70 (m, 2H, CH$_2$O), 3.87 (m, 2H, CH$_2$O), 4.1-4.3 (m, 5H, CH-O), 4.43 (m, 1H, OCHO), 4.71 (m, 1H, OCHO), 6.45 (d, *J* = 2 Hz, 1H, Ar), 6.54 (dd, *J* = 2, 10 Hz, 1H, Ar), 6.7-6.9 (m, 2H, Ar), 7.0 (d, *J* = 2 Hz, 1H, Ar), 7.31 (d, *J* = 7 Hz, 1H, Ar), 7.68-7.75 (m, 2H, Ar), 8.01 (s, 1H, Ar), 8.29 (dd, *J* = 1, 7.5 Hz, 1H, Ar).

7-(2-hydroxyethoxy)-2H-2-chromenone 11

A mixture of potassium carbonate (1.3 g, 9.3 mmol), 2-(2′-bromoethoxy)tetrahydro-2*H*-pyran (2.6 g, 12 mmol) and 7-hydroxycoumarin (1.0 g, 6.2 mmol) was heated to 60°C overnight in DMF (50 ml). After cooling to room temperature water (150 ml) was added to the solution, followed by extraction with ethyl acetate (4x30 ml). The organic layer was washed with water (3x30 ml) and brine (30 ml), dried and the solvent removed on a rotary evaporator. Flash chromatography (30% EtOAc / hexane) gave 7-[2-(tetrahydro-2H-2-pyranyloxy)ethoxy]-2H-2-chromenone **9** as a white solid (1.78 g, 99%). ^{1}H NMR δ 1.5-1.8 (m, 6H, 3xCH_2), 3.58 (m, 1H, CH-O), 3.87 (m, 2H, OCH_2), 4.1-4.3 (m, 3H, 3xCH-O), 4.72 (m, 2H, OCHO), 6.26 (d, *J* = 9.5 Hz, 1H, =CH), 6.87 (m, 2H, Ar), 7.39 (d, *J* = 8.5 Hz, 1H, Ar), 7.65 (d, *J* = 9.5 Hz, 1H, =CH). Compound **9** (1.78 g, 6.1 mmol) was dissolved in dichloromethane (10 ml) and added to a solution of PPTS (50 mg, 0.2 mmol) in ethanol (30ml). The mixture heated at 50°C until tlc analysis showed no starting material remaining. At this time the solvent was removed under vacuum and the resulting material purified by flash chromatography (70% EtOAc / 30% hexane) to yield the title compound as a white solid in quantitative yield (1.26 g). Mp 92.5-93.5°C; ^{1}H NMR δ 4.02 (m, 2H, OCH_2), 4.15 (m, 2H, OCH_2), 6.25 (d, *J* = 9.5 Hz, 1H, =CH), 6.85 (m, 2H, Ar), 7.35 (d, *J* = 8.5 Hz, 1H, Ar), 7.64 (d, *J* = 9.5 Hz, 1H, =CH); ^{13}C NMR δ 58.3, 68.6, 99.8, 110.9, 111.0, 111.1, 127.6, 142.4, 154.0, 159.0. 160.6; IR ν_{max} ($CDCl_3$) 3600, 3430, 3010, 2940, 2875, 1710, 1620, 1560, 1510, 1405 cm^{-1}; UV λ_{max} (CH_2Cl_2) 232 (23801), 323 (21476) nm; MS (EI) m/z 206 ($M^{+\cdot}$, 50), 162 (40), 134 (100), 105 (15), 89 (15), 69 (12), 43 (8); HRMS Calcd for $C_{11}H_{10}O_4$ 206.05791. Found 206.05872.

2-({1-[(E)-2-phenyl-1-diazenyl]-2-naphthyl}oxy)-1-ethanol 12

Sudan 1 (1.0 g, 4.0 mmol), 2-(2′-bromoethoxy)tetrahydro-2*H*-pyran (1.7 g, 8.0 mmol) and potassium carbonate (1.1 g, 8.0 mmol) were heated to 80°C in DMF (50 ml) for twelve hours. After cooling, water (150 ml) was added and the solution extracted with ether (4x40 ml). The organic layer was washed with water (3x30 ml), dried and the solvent removed. Flash chromatography (20% EtOAc / 80% hexane) on the crude material yielded 1.51 g (99.5%) of 1-phenyl-2-{2-[2-(tetrahydro-2H-2-pyranyloxy)ethoxy]-1-naphthyl}-1-diazene as a red oil. ^{1}H NMR δ 1.3-1.8 (m, 6H, 3xCH_2), 3.38 (m, 1H, CH-O), 3.75 (m, 2H, 2xCH-O), 4.00 (m, 1H, CH-O), 4.31 (t, *J* = 5 Hz, 2H, OCH_2), 4.62 (m, 1H, OCHO), 6.9-7.1 (m, 1H, Ar), 7.35-7.55 (m, 5H, Ar), 7.80 (m, 2H, Ar), 8.01 (2x d, *J* = 8, 8 Hz, 2H, Ar), 8.32 (d, *J* = 7 Hz, 1H, Ar). PPTS (50 mg 0.2 mmol) was added to a solution of **10** (1.51 g, 4.0 mmol) in ethanol (30 ml) with dichloromethane (10 ml) to solubilise the protected alcohol. The solution was heated at 50°C for 5 hours before cooling and removal of the solvent under vacuum. Purification by chromatography (40% EtOAc / 60% hexane) gave the expected alcohol in 99% yield (1.17 g) as a red oil. ^{1}H NMR δ 3.88 (m, 2H, CH_2O), 4.35 (m, 2H, CH_2O), 7.35-7.6 (m, 6H, Ar), 7.84 (m, 2H, Ar), 8.01 (m, 2H, Ar), 8.45 (d, *J* = 8 Hz, 1H, Ar); ^{13}C NMR δ 60.9, 72.7, 117.5, 122.5, 123.1, 124.9, 127.5, 127.8, 129.2, 129.6, 130.4, 131.2, 131.5, 136.6, 145.8, 153.1; IR ν_{max} ($CHCl_3$) 3400, 3015, 2950, 2840, 1620, 1600, 1590, 1500, 1450, 1430 cm^{-1}; UV λ_{max} (CH_2Cl_2) 236 (30695), 282 (11985), 378 (7855) nm; MS (EI) m/z 292 ($M^{+\cdot}$, 22), 248 (17), 246 (13), 170(13), 159 (17), 143 (20), 136 (25), 115 (59), 106 (40), 103 (68), 85 (100); HRMS Calcd for $C_{18}H_{16}N_2O_2$ 292.12177. Found 292.12074.

N1-(2-aminoethyl)-5-(dimethylamino)-1-naphthalenesulfonamide 13

A solution of dansyl chloride (1.0 g, 3.7 mmol) in dichloromethane (10 ml) was slowly added to ethylene diamine (0.99 ml, 1.5 mmol) in dichloromethane (50 ml). After stirring for 30 minutes the solvent and the excess ethylene diamine were removed to yield the title compound as a pale green solid along with a small amount of the dimer. Further purification was not carried out as this was mixture added directly to further reactions. ^{1}H NMR δ 2.89 (s, 6H, 2xCH_3), 3.03 (m, 2H, CH_2), 3.19 (m, 2H, CH_2), 7.19 (d, *J* = 7 Hz, 1H, CH), 7.55 (m, 2H, 2xCH), 8.17 (d, *J* = 7 Hz, 1H, CH), 8.34 (d, *J* = 8.5 Hz, 1H, CH), 8.53 (d, *J* = 8.5 Hz, 1H, CH); MS (EI) m/z 293 ($M^{+\cdot}$, 52), 235 (100), 171 (60), 170 (80), 168 (28), 154 (20), 127 (18).

2-[(2-oxo-2H-7-chromenyl)oxy]ethyl propiolate 14

DCC (1.0 g, 4.9 mmol) and DMAP (25 mg, 0.2 mmol) were added slowly to a solution of the coumarin derivative **11** (1.0 g, 4.9 mmol) and propiolic acid (0.30 ml, 4.9 mmol) in dichloromethane (50 ml) at -20°C. After stirring at room temperature overnight the solution was filtered, the solvent removed and the resultant material purified by chromatography (60% EtOAc / 40% hexane) to yield 0.39 g (31%) of the title compound as a white solid. Mp 84-86°C; ^{1}H NMR δ 2.96 (s, 1H, ∫-H), 4.28 (m, 2H, CH_2O), 4.58 (m, 2H, CH_2O), 6.27 (d, *J* = 9.5 Hz, 1H, =CH), 6.86 (m, 2H, Ar), 7.39 (d, *J* = 8.5 Hz, 1H, Ar), 7.65 (d, *J* = 9.5 Hz, 1H, =CH); ^{13}C NMR δ 63.9, 65.9, 76.0, 101.7, 112.9, 113.0, 113.1, 113.5, 129.0, 143.4, 152.5, 155.8, 161.0, 161.4; IR ν_{max} ($CHCl_3$) 2500, 3030, 2125, 1730, 1615, 1510, 1410, 1280, 1230 cm^{-1}; UV λ_{max} (CH_2Cl_2) 225 (7359), 319 (4519) nm; MS (EI) m/z 258 ($M^{+\cdot}$, 20), 134 (18), 97 (100), 89 (12), 69 (13), 53 (66), 43 (10); HRMS Calcd for $C_{14}H_{10}O_5$ 258.05282. Found 258.05247.

2-({1-[(E)-2-phenyl-1-diazenyl]-2-naphthyl}oxy)ethyl propiolate 15

DCC (0.72 g, 3.5 mmol) and 4-dimethylaminopyridine (50 mg, 0.4 mmol) were added to a solution of the alcohol **12** (1.0 g, 3.4 mmol) and propiolic acid (0.22 ml, 3.5 mmol) in ether (50 ml) at -20°C. After stirring at room temperature overnight the reaction was filtered, the solvent was removed and the resultant oil chromatographed (30% EtOAc / 70% hexane) to yield 0.86 g (75%) of the title compound as a red oil. ^{1}H NMR δ 2.86 (s, 1H, ∫-H), 4.24 (m, 2H, CH_2O), 4.41 (m, 2H, CH_2O), 7.22 (d, *J* = 9 Hz, 1H, Ar), 7.35-7.53 (m, 5H, Ar), 7.30 (m, 2H, Ar), 8.00 (d, *J* = 7 Hz, 2H, Ar), 8.36 (d, *J* = 8 Hz, 1H, Ar); ^{13}C NMR δ 64.4, 69.1, 74.2, 117.8, 122.5, 122.7, 123.4, 125.0, 127.5, 127.8, 128.6, 128.9, 129.1, 129.9, 130.7, 131.0, 138.0, 146.6, 152.4, 153.3; IR ν_{max} ($CHCl_3$) 2500, 3030, 2125, 1720, 1600, 1515, 1460, 1230, 1090 cm^{-1}; UV λ_{max} (CH_2Cl_2) 236 (30155), 278 (13495), 372 (8155), 466 (1600) nm; MS (FAB) m/z 345 (MH^+, 6), 154 (10), 136 (6), 109 (13), 107 (14), 97 (53), 83 (38), 81 (40), 69 (75), 57 (87), 55 (100); HRMS Calcd for $C_{21}H_{16}N_2O_3$ 344.11609. Found 344.11566.

N1-[2-({[5-(dimethylamino)-1-naphthyl]sulfonyl}amino)ethyl]-2-propynamide 16

Propiolic acid (0.24 ml, 3.8 mmol) was added slowly to lithium hydride (0.03 g, 4.0 mmol) and THF (50 ml) at ~5°C. After stirring for three hours the solution was cooled to -10°C and ethylchloroformate (0.38 ml, 4.0 mmol) added. The mixture was allowed to warm to room temperature and stirred for a further hour before the addition of the amine **13** (3.7 mmol, see above for preparation). The solution was stirred overnight, the solvent removed and the resulting oil chromatographed (60% EtOAc / 40% hexane) to yield 0.74 g (58%) of the conjugated alkynyl amide **16** as a pale green oil. ^{1}H NMR δ 2.81 (s, 1H, C≡CH), 2.82 (s, 6H, 2xCH_3), 3.05 (m, 2H, CH_2N), 3.25 (m, 2H, CH_2N), 6.40 (t, *J* = 6 Hz, 1H, NH), 7.10 (d, *J* = 8 Hz, 1H, Ar), 7.24 (t, *J* = 6 Hz, 1H, NH), 7.47 (m, 2H, Ar), 8.18 (dd, *J* = 1, 7 Hz, 1H, Ar), 8.27 (d, *J* = 8 Hz, 1H, Ar), 8.49 (d, *J* = 8 Hz, 1H, Ar); ^{13}C NMR δ 39.9, 42.4, 45.4, 74.6, 77.0, 115.4, 118.8, 123.3, 128.7, 129.4, 129.5, 129.9, 130.6, 134.5, 152.0, 153.2; IR ν_{max} ($CHCl_3$) 3430, 2590, 2500, 3025, 2950, 2870, 2835, 2790, 2115, 1660, 1575, 1515, 1480, 1455, 1355 cm^{-1}; UV λ_{max} (CH_2Cl_2) 232 (19690), 257 (25075), 347 (9395) nm; MS (FAB) m/z 346 (MH^+, 4), 345 (5), 170 (12), 154 (10), 137 (10), 136 (12), 107 (18), 95 (32), 83 (32), 81 (41), 69 (80), 67 (41), 57 (74), 55 (100).

4-(2-heptynoyl)benzoic acid 18

Butyl lithium (5.46 ml, 2.5 M, 13.7 mmol) was added slowly to a solution of 1-hexyne (1.54 ml, 13.3 mmol) and DMPU (1.6 ml, 13.3 mmol) in THF (50 ml) at -78°C. The solution was stirred for 20 minutes before the addition of 4-carboxy benzaldehyde (1.0 g, 6.66 mmol). After stirring for 3 more hours the excess butyllithium was quenched by the careful addition of water (5 ml) and the THF removed under vacuum. The resulting aqueous solution was acidified with 10% sulfuric acid (as indicated by pH paper), followed by extraction with ethyl acetate (4x40 ml). The organic layer was washed with water (2x40 ml) and brine (40 ml) and then dried. After removal of the solvent the resulting yellow solid was chromatographed (1% AcOH / 30% EtOAc / 69 % hexane) to yield between 0.64 g and 0.96 g (40-60%) of 4-(1-hydroxy-2-heptynyl) benzoic acid **17** as a white solid. ^{1}H NMR δ 0.91 (t, *J* = 7 Hz, 3H, CH_3), 1.38-1.57 (m, 2H, 2xCH_2), 2.28 (t, *J* = 7 Hz, 2H, CH_2), 5.52 (s, 1H, CH-O), 7.63 (d, *J* = 7.5 Hz, 2H, Ar), 8.11 (d, *J* = 7.5 Hz, 2H, Ar); IR ν_{max} ($CHCl_3$) 3250, 2950, 2900, 2850, 2650, 2535, 1690, 1600, 1570, 1500, 1455, 1420 cm^{-1}. Jones reagent was added to a solution of **17** (1.0 g, 4.3 mmol) in acetone (125 ml) at 0°C until the reaction remained orange in colour for more than 10 minutes. The solution was then filtered and the acetone removed. The solution was extracted with ethyl acetate (3x40 ml) and the organic layer washed with 10% sulfuric acid (40 ml) and saturated ammonium chloride (40 ml). After removal of the ethyl acetate the resulting material was purified by flash chromatography (1% AcOH / 35% EtOAc / 64% hexane) to yield 0.79 g (80%) of the title compound as a white solid. Mp 146-149°C; ^{1}H NMR δ 0.98 (t, *J* = 7 Hz, 3H, CH_3), 1.51 (m, 2H, CH_2), 1.69 (m, 2H, CH_2), 2.54 (t, *J* = 7 Hz, 2H, CH_2), 8.17, 8.21 (AB pattern, *J* = 9 Hz, 4H, Ar); ^{13}C NMR δ 13.6, 19.0, 22.2, 29.8, 79.7, 98.6, 129.6, 130.7, 125.7, 140.7, 177.4; IR ν_{max} ($CHCl_3$) 2950, 2860, 2680, 2560, 2200, 1690, 1660, 1610, 1500, 1470, 1430 cm^{-1}; MS (FAB) m/z 231 (MH$^+$, 12), 185 (30), 149 (30), 105 (10), 93 (100), 75 (30), 57 (30).

2-[(2-oxo-2H-7-chromenyl)oxy]ethyl 4-(2-heptynoyl)benzoate 19

Diethylazodicarboxylate (0.38 ml, 2.4 mmol) was added slowly to a solution of triphenylphosphine (0.76 g, 2.9 mmol) in THF (25 ml). When the yellow colour of the DEAD reagent had disappeared, the coumarin **11** (0.5 g, 2.4 mmol) was added in a THF solution (5 ml) and the mixture stirred for a further 20 minutes. At this time the acid **18** (0.55 g, 2.4 mmol) was added to the reaction. The reaction was stirred for 60 minutes, the solvent removed under vacuum and the resulting orange oil chromatographed (40% EtOAc / 60% hexane) to yield 0.46 g (54%) of the expected ester **19** as a white solid. Mp 106-108°C; ^{1}H NMR δ 0.95 (t, *J* = 7 Hz, 3H, CH_3), 1.50 (m, 2H, CH_2), 1.67 (m, 2H, CH_2), 4.41 (t, *J* = 4.5 Hz, 2H, CH_2O), 4.75 (t, *J* = 4.5 Hz, 2H, CH_2O), 6.26 (d, *J* = 9.5 Hz, 1H, =CH), 6.88 (m, 2H, Ar), 7.41 (d, *J* = 8.5 Hz, 1H, Ar), 7.66 (d, *J* = 9.5 Hz, 1H, =CH), 8.16 (m, 4H, Ar); ^{13}C NMR δ 13.4, 18.8, 21.9, 29.6, 63.2, 66.2, 79.4, 98.2, 101.5, 112.7, 112.8, 113.2, 128.8, 129.2, 129.7, 125.8, 140.0, 143.2, 155.6, 160.9, 161.4, 165.4, 177.2; IR ν_{max} ($CHCl_3$) 3030, 2990, 2960, 2940, 2200, 1730, 1640, 1615, 1505, 1405 cm^{-1}; UV λ_{max} (CH_2Cl_2) 263 (25260), 311 (16608) nm; MS (EI) m/z 418 (M$^{+\cdot}$, 45), 257 (40), 257 (100), 213 (23), 104 (12), 43 (8); HRMS Calcd for $C_{25}H_{22}O_6$ 418.14164. Found 418.14147.

2-({1-[(Z)-2-phenyl-1-diazenyl]-2-naphthyl}oxy)ethyl 4-(2-heptynoyl)benzoate 20

Method A: The DEAD reagent (0.27 ml, 1.7 mmol) was added to triphenylphosphine (0.70 g, 2.7 mmol) in THF (25 ml). After stirring until the yellow colour faded, **12** (0.5 g, 1.7 mmol) was added to the solution, followed by after a further 20 minutes the acid **18** (0.39 g, 1.7 mmol). The solvent was removed under vacuum and the residue purified by chromatography (20% EtOAc / 80% hexane) to produce the title compound **20** in 58% yield (0.50 g) as a red oil.

Method B: Dicyclohexylcarbodiimide (0.35 g, 1.7 mmol) and DMAP (25 mg, 0.2 mmol) were added to a solution of **12** (0.5 g, 1.7 mmol) and the acid **18** (0.39 g, 1.7 mmol) in chloroform (25 ml) and the mixture was stirred overnight. Filtration of the reaction followed by removal of the solvent and chromatography (as above) yielded 0.44 g (51%) of the expected product **20**. ^{1}H NMR δ 0.97 (t, J = 7 Hz, 3H, CH_3), 1.50 (m, 2H, CH_2), 1.67 (m, 2H, CH_2), 2.53 (t, J = 7 Hz, 2H, CH_2), 4.50 (m, 2H, CH_2O), 4.65 (m, 2H, CH_2O), 7.01 (m, 1H, Ar), 7.45 (m, 5H, Ar), 7.8-8.3 (m, 8H, Ar), 8.32 (d, J = 9 Hz, 1H, Ar); ^{13}C NMR δ 13.4, 18.9, 22.0, 29.7, 63.9, 69.5, 79.6, 97.9, 117.7, 122.6, 123.3, 124.9, 127.5, 127.8, 128.6, 129.0, 129.1, 129.2, 129.4, 129.5, 129.6, 129.8, 130.6, 131.0, 134.0, 138.2, 139.8, 146.5, 153.2, 165.4, 177.3; IR ν_{max} ($CHCl_3$) 3025, 2960, 2935, 2875, 2200, 1720, 1640, 1595, 1505, 1455, 1410 cm^{-1}; UV λ_{max} (CH_2Cl_2) 235 (47790), 268 (41735), 364 (11317) nm; MS (FAB) m/z 505 (MH$^+$, 6), 257 (25), 213 (12), 154 (20), 137 (20), 136 (19), 97 (32), 83 (40), 81 (38), 67 (41), 69 (83), 57 (100), 55 (95); HRMS Calcd for $C_{32}H_{28}N_2O_4$ 504.20491. Found 504.20536.

2-{[4-(2-heptynoyl)benzoyl]oxy}ethyl 2-[6-(2-{[4-(2-heptynoyl)benzoyl]oxy}ethoxy)-3-oxo-3H-9-xanthenyl]benzoate 21

A solution of the dialkylated fluorescein derivative **5** (0.5 g, 1.2 mmol), the alkynyl acid **18** (0.82 g, 3.6 mmol), DCC (0.74 g, 3.6 mmol), and DMAP (25 mg, 0.1 mmol) in chloroform (50 ml) was stirred for 2 hours at room temperature. The reaction was then filtered and the organic layer washed with 10% hydrochloric acid (30 ml) and 10% potassium bicarbonate (30 ml), before the solution was dried and the solvent removed. Purification by flash chromatography (100% EtOAc) of the residue yielded 0.51g (50%) of the ester **21** as an orange solid. ^{1}H NMR δ 0.95 (m, 6H, 2xCH_3), 1.50 (m, 4H, 2x$CH_2$0), 1.68 (m, 4H, 2xCH_2), 2.53 (m, 4H, 2xCH_2), 4.30 (m, 2H, 2xCH-O), 4.40 (m, 4H, 4xCH-O), 4.74 (m, 2H, 2xCH-O), 6.36 (d, J = 2 Hz, 1H, Ar), 6.50 (dd, J = 2, 10 Hz, 1H, Ar), 6.72 (dd, J = 2, 9 Hz, 1H, Ar), 6.90 (m, 3H, Ar), 7.32 (dd, J = 1, 7.5 Hz, 1H, Ar), 7.73 (m, 2H, Ar), 8.00 (d, J = 8 Hz, 2H, Ar), 8.17 (m, 6H, Ar), 8.29 (dd, J = 1, 7Hz, 1H, Ar); ^{13}C NMR δ 13.4, 18.8, 22.0, 24.8, 25.5, 29.7, 25.6, 49.2, 62.9, 63.0, 63.1, 66.5, 79.5, 98.3, 98.4, 101.0, 105.5, 114.0, 115.1, 117.6, 129.1, 129.3, 129.4, 129.6, 129.7, 129.9, 130.5, 131.4, 125.0, 125.7, 125.8, 134.1, 134.2, 140.1, 151.6, 154.2, 157.6, 158.9, 163.1, 164.8, 165.1, 165.4, 168.1, 177.2, 185.3; IR ν_{max} ($CHCl_3$) 3010, 2935, 2860, 2340, 2200, 1720, 1640, 1600, 1530, 1500, 1410 cm^{-1}; UV λ_{max} (CH_2Cl_2) 234 (47650), 262 (41735), 434 (13690) nm; MS (FAB) m/z 846 (MH$^+$, 9), 431 (25), 257 (53), 213 (30), 149 (44), 83 (39), 81 (40), 71 (48), 69 (62), 57 (96), 55 (100).

N1-[2-({[5-(dimethylamino)-1-naphthyl]sulfonyl}amino)ethyl]-4-(2-heptynoyl)benzamide 22

DCC (0.38 g, 1.9 mmol) and DMAP (25 mg, 0.1 mmol) were slowly added to a solution of *N*-hydroxy succinimide (0.21 g, 1.9 mmol) and the acid **18** (0.42 g, 1.9 mmol) in chloroform (25 ml) at -20°C and the reaction allowed to stir for 5 hours while warming to room temperature. At this stage the mixture was filtered and added to the previously prepared dansyl amine derivative **13** (1.9 mmol). After stirring overnight the solvent was removed and the residue purified by flash chromatography (55% EtOAc / 45% hexane) to produce the title compound as a pale green oil in 30% overall yield (0.27g) from dansyl chloride **22**. ^{1}H NMR δ 0.97 (t, J = 7 Hz, 3H, CH_3), 1.51 (m, 2H, CH_2), 1.69 (m, 2H, CH_2), 2.53 (t, J = 7 Hz, 2H, CH_2), 2.86 (s, 6H, 2xCH_3), 3.17 (m, 2H, CH_2N), 3.53 (m, 2H, CH_2N), 6.09 (t, J = 6 Hz, 1H, NH), 7.09 (s, 1H, Ar), 7.13 (t, J = 5 Hz, 1H, NH), 7.49 (m, 2H, Ar), 7.71 (d, J = 8.5 Hz, 2H, Ar), 8.04 (d, J = 8.5 Hz, 2H, Ar), 8.24 (m, 2H, Ar), 8.50 (d, *J* = 8.5 Hz, 1H, Ar); IR ν_{max} ($CHCl_3$) 2590, 3030, 3010, 2960, 2935, 2200, 1740, 1610, 1530, 1405 cm^{-1}; UV λ_{max} (CH_2Cl_2) 231 (37920), 260 (44062), 253 (13000) nm; MS (FAB) m/z 506 (MH^+, 27), 505 (24), 213 (15), 170 (31), 149 (25), 129 (27), 113 (20), 71 (63), 69 (29), 56 (100), 54 (38).

General Procedure for the base catalysed reactions between the protected amino acids and the dyes attached to conjugated alkynes, Procedure A

Triethylamine (catalytic) was added to the protected amino acid (25 mg) and the conjugated alkyne (1.0 molar equivalent) dissolved in chloroform at either room temperature or 0°C, as stated. The reaction was stirred until tlc analysis revealed none of the derivatised dye remaining, at which time the solvent was removed. Purification of the crude material by flash chromatography then yielded the addition product(s).

2-[(2-oxo-2H-7-chromenyl)oxy]ethyl (E)-3-[(2-{[(benzyloxy)carbonyl]amino}-3-methoxy-3-oxo propyl)sulfanyl]-2-propenoate 26

The reaction between the coumarin derivative **14** and the protected cysteine **23** was carried out at 0°C following Procedure A. Flash chromatography (50% EtOAc / 50% hexane) produced 66% (32 mg) of the *E* alkene. ^{1}H NMR δ 3.25 (m, 2H, CH_2S), 3.78 (s, 3H, OCH_3), 4.24 (m, 2H, CH_2O), 4.51 (m, 2H, CH_2O), 4.70 (m, 1H, α-CH), 5.10, 5.13 (AB pattern, *J* = 12 Hz, 2H, OC$\underline{H}_2$Ph), 5.68 (d, *J* = 7.5 Hz, 1H, NH), 5.88 (d, *J* = 15 Hz, 1H, =CH), 6.26 (d, *J* = 9 Hz, 1H, =CH (Coumarin)), 6.85 (m, 2H, Ar), 7.25 (m, 6H, Ar), 7.63 (d, *J* = 15 Hz, 1H, =CH), 7.64 (d, *J* = 9 Hz, 1H, =CH (Coumarin)); IR ν_{max} ($CHCl_3$) 3420, 2930, 1715, 1605, 1590, 1490, 1450, 1395, 1340, 1300, 1115, 1050, 985, 940, 890, 830 cm^{-1}; UV λ_{max} (CH_2Cl_2) 280 (55695), 322 (39035) nm; MS (LSIMS) m/z 528 (MH^+, 100), 484 (70), 376 (25), 366 (26), 307 (26), 286 (70), 225 (86), 216 (69), 189 (43); HRMS Calcd for $C_{26}H_{25}NO_9S$ 527.12500. Found 527.12419.

2-({1-[(Z)-2-phenyl-1-diazenyl]-2-naphthyl}oxy)ethyl 4-{(E)-3-[(2-{[(benzyloxy)carbonyl]amino}-3-methoxy-3-oxopropyl)sulfanyl]-2-propenoyl}benzoate 27

The reaction between the alkynyl ester **15** and the thiol **23** was undertaken at 0°C following Procedure A. Flash chromatography (40% EtOAc / 60% hexane) produced 73% (48 mg) of **27**. ^{1}H NMR δ 3.20 (m, 2H, CH_2S), 3.72 (s, 3H, OCH_3), 4.36 (m, 2H, CH_2O), 4.42 (m, 2H, CH_2O), 4.65 (m, 1H, α-CH), 5.09, 5.12 (AB pattern, *J* = 12 Hz, 2H, OC$\underline{H}_2$Ph), 5.62 (d, *J* = 7.5 Hz, 1H, NH), 5.79 (d, *J* = 15 Hz, 1H, =CH), 6.89 (m, 1H, Ar), 7.08 (m, 1H, Ar), 7.3-7.6 (m, 9H, Ar), 7.35 (d, *J* = 15 Hz, 1H, =CH), 7.82 (m, 2H, Ar), 7.99 (m, 2H, Ar), 8.25 (d, *J* = 8 Hz, 1H, Ar); IR ν_{max} ($CHCl_3$) 3430, 2950, 1720, 1590, 1500, 1460, 1350, 1310, 1155, 1070, 995, 955 cm^{-1}; UV λ_{max} (CH_2Cl_2) 230 (43660), 272 (34890) nm; MS (FAB) m/z 614 (MH^+, 18), 366 (23), 307 (12), 154 (100), 137 (58), 136 (78), 91 (98), 87 (40); HRMS Calcd for $C_{25}H_{32}N_3O_7S$ (MH^+) 614.19610. Found 614.19782.

2-[(2-oxo-2H-7-chromenyl)oxy]ethyl (E)-3-(2-{[(benzyloxy)carbonyl]amino}-3-methoxy-3-oxo prop oxy)-2-propenoate 28

The addition of the alkyne **14** and the alcohol **24** was undertaken at 0°C using Procedure A. Purification of the crude material by flash chromatography on silica (60% EtOAc / 40% hexane) yielded 15 mg, (29%) of **28**. ^{1}H NMR δ 3.79 (s, 3H, OCH_3), 4.13 (m, 1H, CH-O), 4.28 (m, 3H, 3xCH-O), 4.50 (m, 2H, CH_2O), 4.66 (m, 1H, α-CH), 5.13 (s, 2H, OC$\underline{H}_2$Ph), 5.26 (d, *J* = 12.5 Hz, 1H, =CH), 5.65 (m, 1H, NH), 6.27 (d, *J* = 9.5 Hz, 1H, =CH (Coumarin)), 6.85 (m, 2H, Ar), 7.35 (s, 5H, Ph), 7.55 (d, *J* = 12.5 Hz, 1H, =CH), 7.64 (d, *J* = 9.5 Hz, 1H, =CH (Coumarin)); IR ν_{max} ($CHCl_3$) 3430, 3000, 2950, 1725, 1610, 1500, 1275, 1230, 1210, 1120, 1060, 790, 740 cm^{-1}; UV λ_{max} (CH_2Cl_2) 236 (30100), 322 (27318) nm; MS (FAB) m/z 512

(MH^+, 100), 468 (50), 391 (20), 307 (37), 289 (43), 259 (60), 225 (65), 207 (60); HRMS Calcd for $C_{26}H_{26}NO_{10}$ (MH^+) 512.15567. Found 512.15425.

2-({1-[(Z)-2-phenyl-1-diazenyl]-2-naphthyl}oxy)ethyl 4-[(E)-3-(2-{[(benzyloxy)carbonyl]amino}-3-methoxy-3-oxopropoxy)-2-propenoyl]benzoate 29

The addition reaction between **15** and **24** was undertaken using Procedure A at 0°C. Purification of the crude material by flash chromatography (40% EtOAc / 60% hexane) yielded 26 mg, (44%) of the expected addition product. ^1H NMR δ 3.77 (s, 3H, OCH_3), 3.89 (m, 2H, OCH_2), 4.26 (m, 2H, OCH_2), 4.40 (m, 2H, OCH_2), 4.66 (m, 1H, α-CH), 5.07 (d, *J* = 12 Hz, 1H, =CH), 5.20 (m, 2H, $OC\underline{H}_2Ph$), 5.75 (m, 1H, NH), 6.9-7.15 (m, 2H, Ar), 7.3-7.65 (m, 9H, Ar), 7.25 (d, *J* = 12 Hz, 1H, =CH), 7.91 (m, 2H, Ar), 8.05 (m, 2H, Ar), 8.46 (d, *J* = 8.5 Hz, 1H, Ar); IR ν_{max} ($CHCl_3$) 3450, 2950, 1725, 1630, 1600, 1510, 1465, 1345, 1250, 1280, 1140, 1090, 1070, 980 cm^{-1}; UV λ_{max} (CH_2Cl_2) 234 (90255), 279 (29324), 368 (17790) nm; MS (EI) m/z 597 ($M^{+\cdot}$, 10), 489 (10), 362 (10), 292 (50), 248 (30), 242 (57), 198 (22), 115 (62), 91 (100), 87 (22); HRMS Calcd for $C_{25}H_{31}N_3O_8$ 597.21111. Found 597.21187.

2-[(2-oxo-2H-7-chromenyl)oxy]ethyl (E)-3-[(5-{[(benzyloxy)carbonyl]amino}-6-methoxy-6-oxo hexyl) amino]-2-propenoate 30*E* and 30*Z*

The hydrochloride salt of the protected lysine **25** was removed prior to the reaction by washing with 10% sodium bicarbonate solution, and the reaction of the lysine derivative **25** with the alkyne **14** was undertaken without added base at room temperature. Purification of the crude material by flash chromatography (60% EtOAc / 40% hexane) yielded 27 mg, (55%) of the two alkenes, **105*E*** and **105*Z***, in a 1.5:1 ratio, as an inseparable mixture. ^1H NMR δ 1.38 (m, 2H, CH_2), 1.5-1.8 (m, 4H, $2xCH_2$), 3.02 (m, 2H, CH_2N (*E*)), 3.15 (m, 2H, CH_2N (*Z*)), 3.75 (s, 6H, $2xOCH_3$), 4.25 (m, 2H, CH_2O), 4.42 (m, 2H, CH_2O), 4.51 (d, *J* = 8 Hz, 1H, =CH (*Z*)), 4.73 (d, *J* = 13 Hz, 1H, =CH (*E*)), 5.10 (s, 4H, $2xOC\underline{H}_2Ph$), 5.39 (d, *J* = 8 Hz, 1H, NH), 6.25 (d, *J* = 9.5 Hz, 1H, =CH (Coumarin)), 6.63 (dd, *J* = 8, 13 Hz, 1H, =CH (*Z*)), 6.86 (m, 2H, Ar), 7.35 (m, 6H, Ar), 7.54 (dd, *J* = 8, 13 Hz, 1H, =CH (*E*)), 7.63 (d, *J* = 9.5 Hz, 1H, =CH (Coumarin)), 7.7 (m, 1H, NH); IR ν_{max} ($CHCl_3$) 3440, 2950, 1725, 1615, 1600, 1555, 1500, 1450, 1395, 1380, 1125, 1060, 995, 835 cm^{-1}; UV λ_{max} (CH_2Cl_2) 277 (40810), 321 (31120) nm; MS (LSIMS) m/z 553 (MH^+, 60), 391 (21), 347 (100), 295 (51); HRMS Calcd for $C_{29}H_{32}N_2O_9$ 552.21078. Found 552.20958.

2-({1-[(Z)-2-Phenyl-1-diazenyl]-2-naphthyl}oxy)ethyl 4-{(E)-3-[(5-{[(benzyloxy)carbonyl]amino}-6-methoxy-6-oxohexyl)amino]-2-propenoyl}benzoate 31*E* and 31*Z*

The addition reaction between the Sudan 1 derivative **15** and the amine **25** was undertaken at room temperature after the initial removal of the hydrochloride salt by washing the protected lysine with 10% sodium bicarbonate solution. Purification of the crude material by flash chromatography (50% EtOAc / 50% hexane) yielded 51% (30 mg) of the two isomers **31*E*** and **31*Z*** (1.1:1) as an inseparable mixture. ^1H NMR δ 1.3-1.85 (m, 6H, $3xCH_2$), 2.90 (m, 2H, CH_2N (*E*)), 3.10 (m, 2H, CH_2N (*Z*)), 3.72 (s, 3H, OCH_3), 3.73 (s, 3H, OCH_3), 4.21 (m, 1H, α-CH), 4.38 (m, 4H, $2xCH_2$), 4.49 (d, *J* = 8 Hz, 1H, =CH (*Z*)), 4.60 (d, *J* = 13 Hz, 1H, =CH (*E*)), 5.10 (s, 4H, $2xOC\underline{H}_2Ph$), 5.25 (m, 1H, NH), 6.54 (dd, *J* = 8, 13 Hz, 1H, =CH (*Z*)), 6.9-7.1 (m, 2H, Ar), 7.25-7.6 (m, 9H, Ar), 7.62 (dd, *J* = 8, 13 Hz, 1H, =CH (*E*)), 7.83 (m, 2H, Ar), 8.00 (m, 2H, Ar), 8.36 (d, *J* = 8 Hz, 1H, Ar); IR ν_{max} ($CHCl_3$) 3440, 2940, 1720, 1665, 1615, 1500, 1455, 1345, 1305, 1275, 1140, 1065, 980, 900 cm^{-1}; UV λ_{max} (CH_2Cl_2) 225 (56110), 278 (45910) nm; MS (FAB) m/z 639 (MH^+, 15), 551 (18), 513 (18), 446 (10), 405 (13), 391 (23), 307 (10), 154 (100), 137 (60), 136 (80), 107 (32), 91 (52), 77 (48), 38 (46); HRMS Calcd for $C_{36}H_{39}N_4O_7$ (MH^+) 639.28187. Found 639.28251.

Methyl 2-{[(benzyloxy)carbonyl]amino}-3-[((E)-3-{[2-({[5-(dimethylamino)-1-naphthyl]sulfonyl} amino)ethyl]amino}-3-oxo-1-propenyl)sulfanyl]propanoate 32*E* and 32*Z*

The addition reaction between the conjugated alkynyl amide **16** and the protected cysteine **23** was undertaken at room temperature using Procedure A. Purification of the crude material by flash chromatography (40% EtOAc / 60% hexane) yielded 31 mg, (55%) of the expected addition products in a ratio of 1.6:1 favouring the *E* isomer. **32*E* / 32*Z*** MS (FAB) m/z 615 (MH^+, 22), 614 (21), 506 (10), 446 (22), 170 (45), 154 (95), 137 (53), 136 (74), 91 (100), 57 (37); HRMS Calcd for $C_{29}H_{34}N_4O_7S_2$ 614.18689. Found 614.18611. **32*E*** ^{1}H NMR δ 2.89 (s, 6H, $2xNCH_3$), 3.03 (m, 2H, CH_2N), 3.27 (m, 4H, CH_2N+CH_2S), 3.79 (s, 3H, OCH_3), 4.69 (m, 1H, α-CH), 5.13 (s, 2H, $OC\underline{H_2}Ph$), 5.66 (d, *J* = 15 Hz, 2H, =CH+NH), 5.79 (d, *J* = 8 Hz, 1H, NH), 5.93 (t, *J* = 6 Hz, 1H, NH), 7.18 (d, *J* = 8 Hz, 1H, Ar), 7.36 (m, 5H, Ph), 7.39 (d, *J* = 15 Hz, 1H, =CH), 7.55 (m, 2H, Ar), 8.23 (m, 2H, Ar), 8.54 (d, *J* = 8.5 Hz, 1H, Ar); IR ν_{max} ($CHCl_3$) 3425, 3030, 2930, 2855, 1720, 1510, 1230, 750, 740, 715, 670 cm^{-1}; IR λ_{max} (CH_2Cl_2) 264 (107482), 256 (23032) nm. **32*Z*** ^{1}H NMR δ 2.88 (s, 6H, $2xNCH_3$), 3.03 (m, 2H, CH_2N), 3.20 (d, *J* = 5 Hz, 2H, CH_2S), 3.25 (m, 2H, CH_2N), 3.74 (s, 3H, OCH_3), 4.63 (m, 1H, α-CH), 5.10 (s, 2H, $OC\underline{H_2}Ph$), 5.57 (d, *J* = 10 Hz, 1H, =CH), 5.73 (t, *J* = 6 Hz, 1H, NH), 5.83 (d, *J* = 7.5 Hz, 1H, NH), 6.03 (t, *J* = 5.5 Hz, 1H, NH), 6.67 (d, *J* = 10 Hz, 1H, =CH), 7.18 (d, *J* = 7.5 Hz, 1H, Ar), 7.25 (s, 5H, Ph), 7.51 (m, 2H, Ar), 8.21 (m, 2H, Ar), 8.53 (d, *J* = 8.5 Hz, 1H, Ar); IR ν_{max} ($CHCl_3$) 3430, 3030, 2945, 2930, 2870, 1720, 1650, 1575, 1510, 1410, 1340, 1320, 1260, 1230, 1145, 1060, 795, 775, 740, 720 cm^{-1}; UV λ_{max} (CH_2Cl_2) 263 (46576), 342 (11260) nm.

2-[(2-Oxo-2H-7-chromenyl)oxy]ethyl 4-{(E)-3-[(2-{[(benzyloxy)carbonyl]amino}-3-methoxy-3-oxo propyl)sulfanyl]-2-heptenoyl}benzoate 33*E* and 33*Z*

The addition reaction between the coumarin derivative **19** and the thiol **23** was carried out by Procedure A at room temperature. Purification of the crude material by flash chromatography (55% EtOAc / 45% hexane) yielded 66% (42 mg) of the expected addition products in a 2:1 ratio in favour of the *E* isomer. **33*E* / 33*Z*** MS (FAB) m/z 688 (MH^+, 8), 644 (5), 552 (8), 526 (9), 451 (14), 257 (100), 257 (15), 219 (28), 149 (83), 105 (76); HRMS Calcd for $C_{37}H_{38}NO_{10}S$ (MH^+) 688.22164. Found 688.22096. **33*E*** ^{1}H NMR δ 0.94 (t, *J* = 7 Hz, 3H, CH_3), 1.43 (m, 2H, CH_2), 1.61 (m, 2H, CH_2), 2.87 (m, 2H, CH_2), 3.37 (m, 2H, CH_2S), 3.78 (s, 3H, OCH_3), 4.39 (m, 2H, CH_2O), 4.73 (m, 3H, CH_2O+α-CH), 5.08 (m, 2H, $OC\underline{H_2}Ph$), 5.68 (d, *J* = 7.5 Hz, 1H, NH), 6.27 (d, *J* = 9.5 Hz, 1H, =CH (Coumarin)), 6.74 (s, 1H, =CH), 6.88 (m, 2H, Ar), 7.32 (m, 6H, Ar), 7.64 (d, *J* = 9.5 Hz, 1H, =CH (Coumarin)), 8.00 (d, *J* = 8 Hz, 2H, Ar), 8.13 (d, *J* = 8 Hz, 2H, Ar); IR ν_{max} ($CHCl_3$) 3430, 3040, 2960, 1720, 1620, 1555, 1505, 1410, 1255, 1270, 1230, 1200, 1125, 1105, 1060, 840, 800 cm^{-1}; UV λ_{max} (CH_2Cl_2) 230 (34840), 254 (25810), 324(49960) nm. **33*Z*** ^{1}H NMR δ 0.96 (t, *J* = 7 Hz, 3H, CH_3), 1.43 (m, 2H, CH_2), 1.60 (m, 2H, CH_2), 2.59 (t, *J* = 7 Hz, 2H, CH_2), 3.40 (m, 2H, CH_2S), 3.79 (s, 3H, OCH_3), 4.38 (m, 2H, CH_2O), 4.70 (m, 3H, CH_2O+α-CH), 5.11 (s, 2H, $OC\underline{H_2}Ph$), 5.65 (d, *J* = 7.5 Hz, 1H, NH), 6.27 (d, *J* = 9.5 Hz, 1H, =CH (Coumarin)), 6.88 (m, 2H, Ar), 7.00 (s, 1H, =CH), 7.34 (m, 6H, Ar), 7.65 (d, *J* = 9.5 Hz, 1H, =CH (Coumarin)), 7.97 (d, *J* = 8 Hz, 2H, Ar), 8.12 (d, *J* = 8 Hz, 2H, Ar); IR ν_{max} ($CHCl_3$) 3430, 3020, 2950, 1725, 1610, 1500, 1270, 1230, 1200, 1105, 910, 840, 800 cm^{-1}; UV λ_{max} (CH_2Cl_2) 231 (12880), 254 (13550), 324 (16660) nm.

2-({1-[(E)-2-Phenyl-1-diazenyl]-2-naphthyl}oxy)ethyl 4-{(E)-3-[(2-{[(benzyloxy)carbonyl]amino}-3-methoxy-3-oxopropyl)sulfanyl]-2-heptenoyl}benzoate 34*E* and 34*Z*

The addition of the non terminal conjugated alkyne **20** and the thiol **23** was undertaken at room temperature following Procedure A. Purification of the crude material by flash chromatography on silica (35% EtOAc / 65% hexane) yielded 56 mg, (78%) of the alkenes in a 2.5:1 ratio for ***34E*** : ***34Z***. ***34E* / *34Z***

MS (FAB) m/z 774 (MH^+, 65), 526 (100), 436 (27), 358 (5), 291 (15), 289 (15), 257 (36), 213 (15); HRMS Calcd for $C_{44}H_{44}N_3O_8S$ (MH^+) 774.28491. Found 774.28507. ***34E*** ^1H NMR δ 0.95 (t, J = 7 Hz, 3H, CH_3), 1.45 (m, 2H, CH_2), 1.62 (m, 2H, CH_2), 2.87 (m, 2H, CH_2), 3.37 (m, 2H, CH_2S), 3.76 (s, 3H, OCH_3), 4.51 (m, 2H, CH_2O), 4.66 (m, 2H, CH_2O), 4.77 (m, 1H, α-CH), 5.07 (m, 2H, OCH_2Ph), 5.62 (d, J = 7.5 Hz, 1H, NH), 6.69 (s, 1H, =CH), 7.04 (m, 1H, Ar), 7.05-7.50 (m, 10H, Ar), 7.82 (m, 4H, Ar), 7.96 (m, 4H, Ar), 8.30 (d, J = 8 Hz, 1H, Ar); IR ν_{max} ($CHCl_3$) 3420, 3030, 3015, 2950, 2930, 2870, 1720, 1650, 1550, 1505, 1340, 1280, 1270, 1250, 1245, 1230, 1060, 800 cm^{-1}; UV λ_{max} (CH_2Cl_2) 236 (47925), 256 (36385), 328 (24835) nm. ***34Z*** ^1H NMR δ 0.97 (t, J = 7 Hz, 3H, CH_3), 1.42 (m, 2H, CH_2), 1.59 (m, 2H, CH_2), 2.59 (m, 2H, CH_2), 3.40 (m, 2H, CH_2S), 3.79 (s, 3H, OCH_3), 4.51 (m, 2H, CH_2O), 4.65 (m, 2H, CH_2O), 4.71 (m, 1H, α-CH), 5.11 (s, 2H, $OC\underline{H}_2Ph$), 5.65 (d, J = 7.5 Hz, 1H, NH), 6.85-7.05 (m, 2H, Ar), 6.95 (s, 1H, =CH), 7.3-7.5 (m, 9H, Ar), 7.8-7.95 (m, 8H, Ar), 8.25 (d, J = 8 Hz, 1H, Ar); IR ν_{max} ($CHCl_3$) 3430, 3020, 2500, 2960, 1720, 1650, 1505, 1270, 1235, 1230, 1200, 800 cm^{-1}; UV λ_{max} (CH_2Cl_2) 238 (46960), 260 (35065), 342 (25925) nm.

General Procedure for the tri-*n*-butylphosphine catalysed reactions between the protected amino acids and the dyes attached to conjugated alkynes. Procedure B

Tri-*n*-butylphosphine (0.2 molar equivalents) was added to the amino acid (25mg) and the conjugated alkyne (1 molar equivalent) in chloroform at room temperature. The solution was stirred until tlc analysis revealed none of the derivatised dye remaining, at which time the solvent was removed and the resulting material chromatographed to yield the addition product.

2-[(2-oxo-2H-7-chromenyl)oxy]ethyl 4-[(E)-3-(2-{[(benzyloxy)carbonyl]amino}-3-methoxy-3-oxo prop oxy)-2-heptenoyl]benzoate 35

This reaction between the alkyne **19** and the alcohol **24** was carried using Procedure B. Flash chromatography (55% EtOAc / 45% hexane) allowed the recovery of the addition product as an inseparable mixture with the starting **24**. The yield of the reaction was calculated to be 31% from the integration in the ^1H NMR spectrum. ^1H NMR δ 0.90 (t, J = 7 Hz, 3H, CH_3), 1.25 (m, 2H, CH_2), 1.54 (m, 2H, CH_2), 2.70 (m, 1H, CHC=), 2.95 (m, 1H, CHC=), 3.78 (s, 3H, OCH_3), 4.01 (m, 2H, CH_2O), 4.30 (m, 2H, CH_2O), 4.44 (m, 1H, α-CH), 4.74 (m, 2H, CH_2O), 5.13 (s, 2H, $OC\underline{H}_2Ph$), 5.73 (d, J = 8 Hz, 1H, NH), 6.07 (s, 1H, =CH), 6.27 (d, J = 9.5 Hz, 1H, =CH (Coumarin)), 6.89 (m, 2H, Ar), 7.36 (m, 6H, Ar), 7.64 (d, J = 9.5 Hz, 1H, =CH (Coumarin)), 7.91 (d, J = 8.5 Hz, 2H, Ar), 8.11 (d, J = 8.5 Hz, 2H, Ar); IR ν_{max} ($CHCl_3$) 3430, 3025, 2960, 1725, 1615, 1580, 1510, 1270, 1235, 1200, 1180, 1120, 1105, 1060 cm^{-1}; UV λ_{max} (CH_2Cl_2) 230 (13420), 253 (15620), 293 (17505) nm; MS (FAB) m/z 672(MH^+, 31), 637 (6), 595 (8), 257 (43), 321 (35), 254 (57), 219 (100); HRMS Calcd for $C_{37}H_{38}NO_{11}$ (MH^+) 672.24448. Found 672.24386.

2-({1-[(E)-2-phenyl-1-diazenyl]-2-naphthyl}oxy)ethyl 4-[(E)-3-(2-{[(benzyloxy)carbonyl]amino}-3-methoxy-3-oxopropoxy)-2-heptenoyl]benzoate 36

This reaction of the non terminal alkynyl ketone **20** and the protected serine derivative **24** was undertaken following Procedure B. The crude reaction mixture was purified by flash chromatography on silica (35% EtOAc / 65% hexane) to yield 31 mg (43%) of the title compound, **35**. ^1H NMR δ 0.97 (t, J = 7 Hz, 3H, CH_3), 1.35 (m, 2H, CH_2), 1.55 (m, 2H, CH_2), 2.70(m, 1H, CHC=), 2.95 (m, 1H, CHC=), 3.81 (s, 3H,

OCH_3), 4.27 (m, 2H, CH_2O), 4.53 (m, 2H, CH_2O), 4.65 (m, 2H, CH_2O), 4.77 (m, 1H, α-CH), 5.16 (m, 2H, $OC\underline{H}_2Ph$), 5.73 (d, J = 8 Hz, 1H, NH), 6.04 (s, 1H, =CH), 7.00 (m, 1H, Ar), 7.35-7.55 (m, 10H, Ar), 7.75-8.00 (m, 8H, Ar), 8.32 (d, J = 8 Hz, 1H, Ar); IR ν_{max} ($CHCl_3$) 3430, 3030, 2960, 1720, 1660, 1580, 1510, 1280, 1265, 1245, 1235, 1230, 1180, 1105, 800 cm^{-1}; UV λ_{max} (CH_2Cl_2) 232 (127050), 285 (75070), 303 (14130) nm; MS (FAB) m/z 758 (MH^+, 55), 510 (100), 420 (20), 275 (45), 267 (25), 230 (18), 219 (20); HRMS Calcd for $C_{44}H_{44}N_3O_9$ (MH^+) 758.30775. Found 758.30826.

2-({1-[(E)-2-phenyl-1-diazenyl]-2-naphthyl}oxy)ethyl 4-{(E)-3-[(5-{[(benzyloxy)carbonyl]amino}-6-methoxy-6-oxohexyl)amino]-2-heptenoyl}benzoate 37

The addition reaction of the amine **25** to the conjugated alkyne **21** was undertaken using Procedure B. The solution was stirred for 48 hours followed by heating at reflux for a further 48 hours. The reaction mixture was allowed to cool and the solvent removed. Purification of the crude material by flash chromatography (40% EtOAc / 60% hexane) produced the expected addition product in very low yield (>5%). ^{1}H NMR δ 0.98 (t, J = 7 Hz, 3H, CH_3), 1.40-1.75 (m, 4H, $2xCH_2$), 2.29 (t, J = 7 Hz, 2H, CH_2), 3.25 (m, 2H, NCH_2), 3.75 (s, 3H, OCH_3), 4.45 (m, 1H, α-CH), 4.50 (m, 2H, CH_2O), 4.62 (m, 2H, CH_2O), 5.11 (s, 2H, $OC\underline{H}_2Ph$), 5.46 (d, J = 8 Hz, 1H, NH), 5.63 (s, 1H, =CH), 6.91 (m, 1H, Ar), 7.02 (m, 2H, Ar), 7.3-7.5 (m, 7H, Ar), 7.7-8.0 (m, 9H, Ar), 8.30 (d, J = 7 Hz, 1H, Ar), 11.67 (m, 1H, enamine NH).

2-[(4-{(E)-3-[(2-{[(benzyloxy)carbonyl]amino}-3-methoxy-3-oxopropyl)sulfanyl]-2-heptenoyl} benzoyl)oxy]ethyl 2-(6-{2-[(4-{(E)-3-[(2-{[(benzyloxy)carbonyl]amino}-3-methoxy-3-oxopropyl) sulfanyl]-2-heptenoyl}benzoyl)oxy]ethoxy}-3-oxo-3H-9-xanthenyl)benzoate 38

The addition reaction between the alkyne **21** and the thiol **23** was undertaken following Procedure A. Purification of the crude material by flash chromatography (30% EtOAc / 70% hexane) yielded 48% of the four alkenes. MS (LSIMS) m/z 1382 (MH^+, 50), 526 (85), 291 (57), 257 (100), 225 (70). The ^{1}H NMR spectrum of the mixture was too complicated for individual assignments to be made.

2-({4-[(E)-3-(2-{[(benzyloxy)carbonyl]amino}-3-methoxy-3-oxopropoxy)-2-heptenoyl]benzoyl} oxy) ethyl 2-{6-[2-({4-[(E)-3-(2-{[(benzyloxy)carbonyl]amino}-3-methoxy-3-oxopropoxy)-2-heptenoyl] benzoyl}oxy)ethoxy]-3-oxo-3H-9-xanthenyl}benzoate 39

The addition of the dialkylated fluorescein derivative **21** and the protected serine **24** was undertaken by Procedure B. The crude material was purified by flash chromatography on silica (25% EtOAc / 75% hexane) to give **39** in 12% yield. ^{1}H NMR δ 0.95 (m, 6H, $2xCH_3$), 1.1-2.0 (m, 8H, $4xCH_2$), 2.70 (m, 2H, CH_2), 2.92 (m, 2H, CH_2), 3.47 (m, 4H, $2xCH_2S$), 3.79 (s, 6H, $2xOCH_3$), 4.0-4.5 (m, 10H, $5xCH_2O$), 4.72 (m, 4H, CH_2O+2xα-CH), 5.14 (s, 4H, $2xOC\underline{H}_2Ph$), 5.78 (m, 1H, NH), 5.87 (d, J = 7 Hz, 1H, NH), 6.07 (s, 1H, =CH), 6.16 (s, 1H, =CH), 6.31 (m, 1H, Ar), 6.51 (dd, J = 2, 9.5 Hz, 1H, Ar), 6.75 (m, 1H, Ar), 6.82 (d, J = 1 Hz, 1H, Ar), 6.87 (m, 3H, Ar), 7.37 (m, 10H, Ar), 7.69 (m, 2H, Ar), 7.91 (m, 6H, Ar), 8.11 (m, 2H, Ar), 8.30 (dd, J = 1, 9 Hz, 1H, Ar); MS (LSIMS) m/z 1352 (MH^+, 60), 1144 (85), 510 (15), 494 (10), 390 (60), 349 (13), 303 (20), 275 (17), 273 (15), 225 (85), 219 (100).

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