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Conjugate Addition of Amino Acid Side Chains to Alkynones and Alkynoic Acid Derivatives

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Abstract: Suitably protected amino acids were used to investigate the Michael addition of the sulfanyl group of cysteine, the hydroxyl group of serine and the ε-amino group of lysine to a conjugated alkynone, alkynoic ester and alkynoic amide. The expected heterosubstituted vinyl product was formed in each case.

INTRODUCTION

The conjugate addition of heteronucleophiles to unsaturated alkynes has not been studied as extensively as the corresponding conjugate additions to unsaturated alkenes.[1] The conjugate additions of sulfur and selenium nucleophiles to alkynones and alkynoic acid derivatives have been the predominant examples[1] and these reactions can proceed with high stereoselectivity for the Z isomer in the derived vinyl sulfides[2] and selenides under suitable conditions. The observed isomer ratio shows wide variation depending upon the substituents and the solvent.[3] Intramolecular conjugate addition of thiolate anions to alkynones shows considerable potential for the synthesis of sulfur heterocycles[4] and dithioacetals have been prepared by the intermolecular double conjugate addition of thiols to methyl propiolates catalysed by tributylphosphine.[5]

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 for the elucidation of mechanisms and for structure determination in protein chemistry.[6] The thiol group of glutathione was used recently to model cysteine in a protein during a study of compounds which act as irreversible inhibitors of HIV-1 and HIV-2 proteases.[7] One of the inhibitors was an alkynone which underwent an initial rapid reaction with glutathione to give a vinyl sulfide, followed by a slower second addition to form a thioacetal. Conjugate addition of acetate anion to the alkynone was also used to model the reactivity of the carboxylate of aspartate.

Conjugate addition of protein-derived nucleophiles to reactive dyes has been used for the covalent attachment of colourfast dyes to wool.[8] Previous studies have identified the reactive sites in keratin as cysteine thiol > lysine ε-amino > serine hydroxyl.[9] 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.

In view of the importance of heteronucleophilic conjugate addition to alkynone and alkynoic acid derivatives we have investigated, and report herein, on the reactions between suitably protected serine, cysteine and lysine derivatives and a variety of alkynes acting as Michael acceptors.

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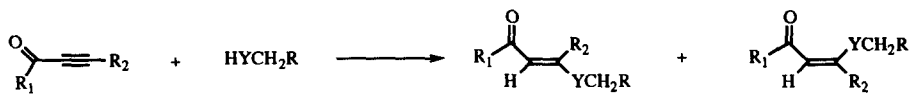
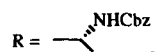
RESULTS AND DISCUSSION

A series of conjugated alkynes **1-6** was prepared by standard literature procedures (see Experimental) and reacted with derivatives of cysteine **7**, serine **8** and lysine **9** (Scheme 1). The addition of **7** to terminal alkyne **1** was found to proceed rapidly in chloroform at 0°C (significant polymerization of **1** occurred at room temperature) when catalysed by a small quantity of triethylamine (reaction time ~5 min), and generated **10*E*** and **10*Z***, in a 1.2:1 ratio and 71% (Scheme 1). The formation of a mixture of stereoisomers is consistent with literature reporoom temperatures for the base catalysed addition of thiols to alkynones under similar conditions.[10] Isomers **10*E*** (trans vinylic coupling of 15Hz) and **10*Z*** (cis vinylic coupling of 12.5Hz) had similar retention times on tlc, and were separated with difficulty by silica gel chromatography. The addition products were stable at room temperature for several weeks, but demonstrated *Z* to *E* isomerization in chloroform solution to yield a final *E* to *Z* ratio of 3.5:1.



1 R^1 = Ph, R^2 = H
2 R^1 = Ph, R^2 = Bu
3 R^1 = EtO, R^2 = H
4 R^1 = MeO, R^2 = Bu
5 R^1 = BnNH, R^2 = H
6 R^1 = BnNH, R^2 = Bu

7 Y = S
8 Y = O
9 Y = $HN(CH_2)_3$

R = 

10*E* R^1 = Ph, R^2 = H, Y = S
11*E* R^1 = Ph, R^2 = Bu, Y = S
12 R^1 = Ph, R^2 = Bu, Y = O
15*E* R^1 = EtO, R^2 = H, Y = S
16 R^1 = EtO, R^2 = H, Y = O
17*E* R^1 = EtO, R^2 = H, Y = $HN(CH_2)_3$
18*E* R^1 = MeO, R^2 = H, Y = S
19 R^1 = MeO, R^2 = Bu, Y = O
20*E* R^1 = BuNH, R^2 = H, Y = S

10*Z* R^1 = Ph, R^2 = H, Y = S
11*Z* R^1 = Ph, R^2 = Bu, Y = S
13 R^1 = Ph, R^2 = H, Y = $HN(CH_2)_3$
14 R^1 = Ph, R^2 = Bu, Y = $HN(CH_2)_3$
15*Z* R^1 = EtO, R^2 = H, Y = S
17*Z* R^1 = EtO, R^2 = H, Y = $HN(CH_2)_3$
18*Z* R^1 = MeO, R^2 = Bu, Y = S
20*Z* R^1 = BuNH, R^2 = H, Y = S

Scheme 1

The addition of **7** to the more hindered alkynone **2** was slower than for **1**, hence this reaction was performed at room temperature. The conjugate addition gave rise to the readily separable isomers **11*E*** and **11*Z*** in 53% and a ratio of 1:2 (Scheme 1). The stereochemistry of the isomers was confirmed by NOE difference measurements, whereby irradiation of the vinylic proton of **11*E*** showed an enhancement of 8% to the SCH_2 unit and irradiation of the vinylic proton of **11*Z*** showed a 5% enhancement to the CH_2 unit of the butyl group. Interestingly, **11*Z*** was a yellow oil with a maximum absorbance at 327nm in the UV-visible spectrum, whereas **11*E*** was colourless and showed a maximum at 320nm.

A rapid reaction between **8** and **1** took place in the presence of triethylamine but no identifiable products were isolated (Scheme 1). Serine **8** showed no addition products after being stirred with **2** for one week at room temperature or heating to reflux in chloroform with a catalytic amount of triethylamine. Serine **8** did react with **2** within 3 hours upon addition of 20% tributylphosphine[11] in THF generating the single stereoisomer **12** in 40% (Scheme 1). Irradiation of the vinylic proton of **12** showed a 12% enhancement to the OCH_2 unit by NOE difference measurements.

The addition of **9** to **1** occurred within 30 minutes, and was noticeably slower than the additions of **7** and **8**, both of which reacted in less than 5 minutes (Scheme 1). The addition of **9** produced only the *Z* isomer **13** in 50% yield with the stereochemistry being determined by a 6% enhancement between the vinylic

protons by NOE difference experiments. The formation of only a single stereoisomer for **13** is probably due to hydrogen bonding of the amino proton to the carbonyl oxygen in the intermediate anion in the *Z* configuration.[12] The amino group of **9** reacted with **2** in the presence of a catalytic amount of triethylamine at room temperature and gave one isomer **14** in 63% (Scheme 1). The structure of **14** was again confirmed by NOE difference measurements and as for **11*Z***, **14** was yellow and showed a maximum absorption at 348nm (ε = 28 215) in the UV-visible spectrum.

Additions to ethyl propiolate (**3**) should be slower than for the corresponding additions to alkynone **1** . The reaction of **7** with **3** occurred readily under basic conditions at 0°C within 15 minutes and gave **15*E*** (trans vinylic coupling of 15Hz) and **15*Z*** (cis vinylic coupling of 10Hz) in 75% total yield and a ratio of 4:1 (Scheme 1). Serine **8** also underwent rapid conjugate addition to **3** in chloroform at 0°C with a catalytic amount of triethylamine within 10 minutes and formed **16** as a colourless oil in 75% (Scheme 1). This result agrees with literature reporoom temperatures that the base catalysed addition of alcohols to methyl propiolate gives exclusively the *E* isomer.[13] The coupling constants between the vinyl protons (J = 12.7 Hz) in the ^{1}H NMR spectrum were not definitive for *trans* stereochemistry and so the stereochemistry of **16** was confirmed by a ROESY (rotational Overhauser effect spectroscopy) spectrum which showed a correlation between the vinyl proton α to the ester and the methylene protons adjacent to the oxygen.

The addition of triethylamine to a solution of **3** and lysine **9** in chloroform at 0°C caused a colour change from colourless to dark brown but no addition products were recovered. Triethylamine catalysed the self condensation of **3** rather than the conjugate addition of **9**. If the hydrochloride salt of **9** was washed with a sodium bicarbonate solution and no additional base added, the conjugate addition proceeded as expected, and two isomers **17*E*** and **17*Z*** were isolated in a total yield of 45% and a 1:1 ratio (Scheme 1). These isomers were inseparable by chromatography and a COSY spectrum of the mixture was used to assign the vinylic resonances to the two stereoisomers.

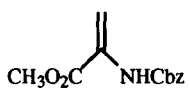
Reaction of **7** with **4** in chloroform with a catalytic amount of triethylamine gave, after 24 hours, the expected addition products **18*E*** and **18*Z*** in a total yield of 40% and a ratio of 2:1 (Scheme 1). The stereochemistry of the products was assigned by comparison with the ^{1}H NMR spectra for polymers prepared by Endo.[11] Addition of **8** to **4** did not occur with triethylamine as the catalyst, even after stirring for two weeks at room temperature. The use of a catalytic amount of tributylphosphine caused the reaction to occur at room temperature over a 12 hours period and gave a single isomer **19** in 35% with the stereochemistry being determined by NOE difference measurements which showed a 21% enhancement between the vinylic proton and the OCH_2 group.

The addition of **9** to **4** was attempted using both triethylamine and tributylphosphine as the catalyst, but in each case none of the expected addition product was isolated. When triethylamine was used the staroom temperatureing materials were recovered unchanged, whilst addition of tributylphosphine resulted in the recovery of products from the reaction of the initially formed phosphine-alkyne adduct. To determine if tributylphosphine could be used as a catalyst for the addition of nitrogen nucleophiles to conjugated alkynes, the reaction of **9** with **2**, which was known to occur under basic conditions, was attempted. When tributylphosphine was added to a solution of **9** and **2** at room temperature the reaction turned dark brown after a shoroom temperature period of time (~20 minutes) and neither tlc analysis nor a ^{1}H NMR spectrum of the solution showed any evidence for the expected addition product.

Cysteine **7** was added to amide **5** in chloroform with a catalytic amount of triethylamine and the

reaction was complete within three hours and produced two isomers **20*E*** and **20*Z*** in 58% and a ratio of 1.4:1 (Scheme 1). When triethylamine was added to **5** and **8** in chloroform, the major products recovered after 24 hours were dehydroalanine **21**, formed by the elimination of water from **8**, and unreacted **5**. As the rates of conjugate addition reactions are known to increase with increasing solvent polarity,[1] addition of **8** to **5** in acetonitrile was attempted but again only dehydroalanine **21** and amide **5** were recovered. The reaction was also attempted with one equivalent of DBU but only **21** and amide **5** were again recovered. It was apparent that the base catalysed addition of hydroxyl nucleophiles to acetylenic amides was too slow to compete with elimination. Addition of tributylphosphine to the solution instead of base caused staroom temperatureing materials to be consumed but gave no identifiable products. The reaction of **9** with **5** was carried out in acetonitrile to increase the rate of the conjugate addition, with triethylamine as the catalyst but after stirring for several days at room temperature only staroom temperatureing materials were present in the mixture.

Addition of a catalytic amount of triethylamine to **7** and amide **6** in acetonitrile at room temperature for 24 hours gave *N,N'*-bis(carbobenzyloxy)cystine dimethyl ester **(22)** as the major product. The acetylenic amide **6** was not recovered from the reaction mixture. The oxidation of the thiol to the disulfide was favored by the basic conditions and, unexpectedly, by the presence of **6**, as shown by the greatly decreased rate of the thiol oxidation in acetonitrile and triethylamine in the absence of **6**, (several days at room temperature produced only minor amounts of **22**). Degassing the solvent and repeating the reaction under a stream of nitrogen still resulted in the formation of **22** as the major product.



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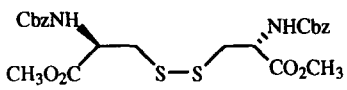

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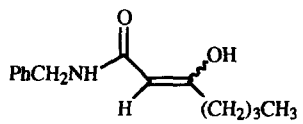

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Tetrabutylammonium hydroxide was then added to a solution of **7** and the amide **6** in acetonitrile under a nitrogen atmosphere in order to increase the proporoom temperatureion of thiolate anion present in solution. This resulted in the formation of dehydroalanine **21** and a second product, **23**, from the conjugate addition of hydroxide anion to **6**. The addition product **23** showed resonances in the ^{1}H NMR spectrum from both tautomeric forms. This result indicated the need for a strong base with little nucleophilic character, and so DBU was added to a solution of **7** and **6** in acetonitrile and stirred for 24 hours but yielded only staroom temperatureing materials with no cystine **22** being isolated. Stirring a mixture of **6** and **7** for 12 hours in the presence of tributylphosphine gave only staroom temperatureing materials.

The influence of solvent on the rate of conjugate addition and the stereochemistry of the products was examined for the reactions of the three most reactive conjugated alkynes, **1**, **2**, and **3**, with the most reactive of the amino acids, cysteine **7**. The results are summarised in Table 1 and show that reactions carried out in methanol all favour formation of the the *Z* stereoisomer, whereas those performed in chloroform ,THF or acetonitrile tend to favour formation of the *E* stereoisomer, except for **11*E*/11*Z*** where chloroform and THF tend to also favor formation of the Z stereoisomer.

Theoretical studies on the addition of nucleophiles to alkynes have shown that the nucleophile approaches the β carbon of the alkyne along a carbon-carbon-nucleophile angle of approximately 60°.[14] The

approach of the nucleophile is accompanied by the trans motion of the substituents on the acetylene, and will minimise the activation energy required for the formation of the vinyl anion. The addition of the nucleophile to the alkyne initially generates the *Z* anion due to the favoured trans bending of the acetylene. [15]

The initially formed *Z* anion can either, i) undergo a fast protonation to give the *Z* stereoisomer as the major product or, ii) undergo isomerisation *via* an allene intermediate, followed by protonation, to generate a thermodynamic mixture of E and *Z* stereoisomers. [16] The ratio of the isomers in the product will then depend on the proton donating ability of the solvent, for protic solvents such as methanol, the kinetic or *Z* stereoisomer should predominate, whereas in non-protic solvents such as THF or chloroform, equilibration can occur and the thermodynamically more stable isomer should predominate. [17] However, the ratio of the stereoisomers is also dependent on the ability of the carbonyl group to stabilise the allene intermediate by stabilising the α anion through resonance in the order ketones > esters > amides. [18] Hence, an increased ability to stabilise the allene should result in a greater proporoom temperatureion of the thermodynamically more stable anion.

The results shown in Table 1 are in agreement with this mechanism as reactions carried out in methanol all favoured the kinetic *Z* stereoisomer as predicted, while those performed in chloroform, THF or acetonitrile formed predominately the more stable *E* stereoisomer. The solvent effects observed for **11*E*** and **11*Z***, indicate that either the *Z* stereoisomer is the more stable or, more likely, that for very slow reaction rates the lifetimes of intermediates are altered and the polarity or proton donating ability of the solvent is no longer dominating the stereoselectivity.

Table 1: Effect of solvent on conjugate addition of **7** to **1**, **2** and **3**

Solvent	Product Ratio		Reaction Time/min	Product Ratio		Reaction Time/min	Product Ratio		Reaction Time/min
	10*E*	**10*Z***		**11*E***	**11*Z***		**15*E***	**15*Z***	
Chloroform	1.2	1	~5	1	2	300	4	1	~15
THF	2.5	1	15	1	1.1	1440	2	1	120
Methanol	1	2	10	1	3	~15	1	3.5	~5
Acetonitrile	3	1	10	7.5	1	~15	-	-	-

No isolable products were obtained when the reaction of **7** and **3** was carried out in acetonitrile and the reaction rapidly darkened and self condensation of **3** predominated. When methanol was used as the solvent, competing addition of the methoxide anion to the terminal alkynes **1** and **3** also occurred. As **10*E*** and **10*Z*** were still formed in the presence of a large excess of methanol as solvent, the thiol of cysteine is a much better nucleophile than the oxygen of methanol, and by analogy the oxygen of serine.

In all of the reactions discussed above no products were observed from a second conjugate addition onto the initially formed alkene. To determine if a second addition could occur under basic conditions, a mixture of **15*E*/15*Z*** was added to another equivalent of **7** in chloroform with either triethylamine or DBU to catalyse the reaction. In each case after 24 hours at room temperature no trace of a second addition product

was apparent by either tlc or in the ^{1}H NMR spectra of the mixtures.

A recent reporoom temperature by Endo *et al* discusses the tributylphosphine catalysed addition of sulfur nucleophiles to methyl propiolate[19] in which the ratio of di-addition to mono-addition was selectively controlled by varying the number of equivalents of both the thiol and the tributylphosphine catalyst. These studies also determined that the initial addition of the thiol to the unsaturated acetylene is rapid, whereas the addition of the thiol to the conjugated alkene was much slower. To determine if such a di-addition was possible for the sterically hindered amino acids used in this study, a 20% molar equivalent of tributylphosphine was added to **3** and two equivalents of **7** in a solution of THF. After 48 hours tlc analysis of the mixture showed only the two mono-addition products **15*E*** and **15*Z***. Addition of excess **7** caused no furoom temperatureher reactions suggesting that the second addition does not occur under these mild conditions.

The possibility of a second, reversible addition followed by a subsequent elimination was also considered as this could affect the isomeric ratios in the final product. Addition of a catalytic amount of triethylamine to **13** and **7** showed no evidence for the presence of either **10*E*** or **10*Z*** suggesting that if a reversible addition is possible then the time scale for this addition is such as to leave unaffected the isomeric ratios determined in the present studies.

In conclusion, the conjugate addition of amino acid side chains containing sulfanyl, hydroxyl and amino groups to alkynones, alkynoic esters and alkynoic amides occurs under mild conditions and gives the expected vinyl sulfides, vinyl ethers and enamines, respectively. The reactivity order thiol > lysine ε-amino > serine hydroxyl is consistent with previous reporoom temperatures from protein studies with α,β-unsaturated esters and sulfones.[6,9]

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.[20] All organic extracts were dried over anhydrous magnesium sulfate unless otherwise specified. *N*-Carbobenzyloxy serine and *N*-α-Carbobenzyloxy lysine were purchased from Aldrich. 1-Phenyl-2-propyn-1-one(**1**)[21], 1-phenyl-2-heptyn-1-one(**2**)[22], methyl-2-heptynoate(**4**)[23], *N*1-benzyl-2-propynamide(**5**)[24], *N,N'*-bis(carbobenzyloxy) cystine dimethyl ester(**22**)[25], 2-heptynoic acid [23], *N*–carbo benzyloxycysteine methyl ester(**7**)[26], *N*–carbobenzyloxyserine methyl ester(**8**)[27] and *N*–carbobenzyloxy lysine methyl ester(**9**)[26] 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.

N1-Benzyl-2-heptynamide (6)

Lithium hydride (0.016g, 2.0 mmol) was added to a solution of 2-heptynoic acid (0.25g, 1.98 mmol) in THF (8 mL) under nitrogen and stirred for 2 hours. The mixture was cooled to -8°C and ethyl chloroformate (0.19 mL, 1.98 mmol) in THF (3 mL) was slowly added. The solution was allowed to warm to room temperature and stirred for a furoom temperatureher 30 minutes before being cooled to approximately 5°C, at which time benzylamine (0.22 mL, 1.98 mmol) in THF (3 mL) was added, and the mixture stirred overnight. Solvent was removed, CH_2Cl_2 added and the solution washed with 10% sodium bicarbonate solution (30mL), 10%

HCl solution (30mL), dried and solvent removed. Flash chromatography (35% EtOAc/65% hexanes) yielded the expected product in 67% (0.28g). Mp 32-34.5°C; ^{1}H NMR δ 0.90 (t, J = 7 Hz, 3H, CH_3), 1.14 (m, 2H, CH_2), 1.51 (m, 2H, CH_2), 2.28 (t, J = 7 Hz, 2H, CH_2), 4.45 (d, J = 6 Hz, 2H, CH_2N), 6.20 (br s, 1H, NH), 7.30 (m, 5H, Ph); ^{13}C NMR δ 13.5, 18.3, 29.7, 43.8, 75.4, 88.0, 127.7, 127.8, 128.7, 19.6, 153.6; IR ν_{max} (nujol) 3270, 1700, 1615, 1600, 1515 cm^{-1}; MS (EI) m/z 215 ($M^{+\cdot}$), 179 (50), 178 (30), 171(1), 170 (15), 149 (100), 105 (100), 91 (45).

General Procedure for the base catalysed reactions between the protected amino acids and the conjugated alkynes (Procedure A)

Triethylamine (~5 drops, catalytic) was added to a mixture of the protected amino acid (50 mg) and the conjugated alkyne (1.2 molar equivalents) in the reaction solvent (normally chloroform, unless otherwise stated) at either room temperature or 0°C. The reaction was stirred until tlc analysis indicated the reaction was complete, at which time either the solvent was removed or the reaction was quenched with dilute hydrochloric acid followed by normal workup. Flash chromatography on silica then yielded the purified product(s).

General Procedure for the tri-*n*-butylphosphine catalysed reactions between the protected amino acids and the conjugated alkynes (Procedure B)

Tri-*n*-butylphosphine (0.2 molar equivalents) was added to the amino acid (50mg) and the conjugated alkyne (1.2 molar equivalents) in either THF or chloroform at room temperature. The solution was stirred until tlc analysis of the mixture showed the disappearance of the protected amino acid, then the solvent was removed and the resulting material chromatographed on silica to yield the addition product.

Methyl (2S)-2-{[(benzyloxy)carbonyl]amino}-3-{[(E)-3-oxo-3-phenyl-1-propenyl]sulfanyl} propanoate 10*E* and 10*Z*

The addition of **1** to **7** was undertaken at 0°C following Procedure A. After an acidic workup, purification of the crude material by flash chromatography (30% EtOAc / 70% hexanes) yielded 53 mg, (71%) of the expected addition products **10*E*** and **10*Z*** in a 1.2 : 1 ratio. Anal. Calcd for $C_{21}H_{21}NO_5S$: C, 63.14; H, 5.30; N, 3.51. Found C, 62.98; H, 5.9; N, 3.30. **10*E***: ^{1}H NMR δ 3.46 (m, 2H, CH_2S), 3.79 (s, 3H, OCH_3), 4.76 (m, 1H, α-CH), 5.9 (m, 2H, $OC\underline{H}_2Ph$), 5.70 (br d, J = 7 Hz, 1H, NH), 7.06 (d, J = 15 Hz, 1H, HC=), 7.3-7.6 (m, 8H, Ar), 7.80 (d, J = 15 Hz, 1H, HC=), 7.94 (d, J = 7 Hz, 2H, Ar); ^{13}C NMR δ 35.4, 53.0, 53.5, 67.3, 119.5, 128.0, 128.1, 128.3, 128.4, 128.5, 128.6, 132.7, 132.7, 135.8, 19.7, 147.4, 155.5, 170.1, 186.6; IR ν_{max} ($CHCl_3$) 1720, 1615, 1600, 1550, 1500 cm^{-1}; UV λ_{max} (CH_2Cl_2) 262 (11150), 316 (22750). **10*Z*:** ^{1}H NMR δ 3.8 (d, J = 4.5 Hz, 2H, CH_2S), 3.79 (s, 3H, OCH_3), 4.71 (m, 1H, α-CH), 5.14 (m, 2H, $OC\underline{H}_2Ph$), 5.68 (br d, J = 7 Hz, 1H, NH), 6.99 (d, J = 9.5 Hz, 1H, HC=), 7.2-7.5 (m, 8H, Ar), 7.93 (d, J = 9.5 Hz, 1H, HC=), 7.94 (d, J = 7 Hz, 2H, Ar); ^{13}C NMR δ 14.3, 53.0, 54.2, 67.2, 117.1, 128.0, 128.2, 128.3, 128.4, 128.56, 128.6, 132.6, 136.0, 19.6, 151.7, 155.6, 170.2, 189.0; IR ν_{max} ($CHCl_3$) 1735, 1650, 1610, 1590, 1515 cm^{-1}; UV λ_{max} (CH_2Cl_2) 260 (4995), 324 (24465).

Methyl (2S)-2-[[(benzyloxy)carbonyl]amino}-3-{[(E)-1-butyl-3-oxo-3-phenyl-1-propenyl]sulfanyl} propanoate 11*E* and 11*Z*

The reaction between **2** and **7** was undertaken at room temperature by Procedure A. Flash chromatography (25% EtOAc / 75% hexanes) produced 53% (45 mg) of the two alkenes in a ratio of 1:2 for **11*E*** and **11*Z***. MS (EI) m/z 455 ($M^{+\cdot}$), 364 (15), 269 (15), 232 (15), 219 (100); Anal. Calcd for $C_{25}H_{29}NO_5S$: C, 65.91; H, 6.41; N, 3.08; Found C, 66.06; H, 6.46; N, 2.95. **11*E*:** ^{1}H NMR δ 0.93 (t, *J* = 7 Hz, 3H, CH_3), 1.43 (m, 2H, CH_2), 1.61 (m, 2H, CH_2), 2.85 (m, 2H, CH_2), 3.9 (m, 2H, CH_2S), 3.77 (s, 3H, OCH_3), 4.78 (m, 1H, α-CH), 5.10, 5.01 (AB, *J* = 9 Hz, 2H, OCH_2Ph), 5.59 (d, *J* = 7.5 Hz, 1H, NH), 6.74 (s, 1H, HC=), 7.3-7.6 (m, 8H, Ar), 7.94 (d, *J* = 7 Hz, 2H, Ar); ^{13}C NMR δ 13.8, 22.7, 32.0, 33.6, 34.9, 52.8, 53.0, 67.3, 113.7, 127.9, 128.1, 128.3, 128.5, 128.6, 132.2, 139.2, 155.5, 165.4, 170.4; IR ν_{max} ($CDCl_3$) 1720, 1420 cm^{-1}; UV λ_{max} (EtOH) 257 (4955), 320 (12310). **11*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), 2.57 (t, *J* = 8 Hz, 2H, CH_2), 3.38(m, 2H, CH_2S), 3.78 (s, 3H, OCH_3), 4.69 (m, 1H, α-CH), 5.11 (s, 2H, OCH_2Ph), 5.67 (d, *J* = 8 Hz, 1H, NH), 7.01 (s, 1H, HC=), 7.3-7.5 (m, 8H, Ar), 7.92 (m, 2H, Ar); ^{13}C NMR δ 13.9, 22.3, 32.0, 32.5, 36.8, 53.0, 53.7, 67.2, 117.9, 128.0, 128.1, 128.2, 128.4, 128.5, 128.6, 132.2, 138.6, 155.7, 165.8, 170.2; IR ν_{max} ($CDCl_3$) 1720, 1530, 1500 cm^{-1}; UV λ_{max} (EtOH) 256 (7560), 327 (17130).

Methyl (2R)-2-[[(benzyloxy)carbonyl]amino}-3-{[(E)-1-butyl-3-oxo-3-phenyl-1-propenyl]oxy} propanoate 12

The addition of **2** to **8** was undertaken following Procedure B. The crude material was purified by flash chromatography on silica (1% EtOAc / 75% hexanes) to yield 28 mg, (40%) of **12**. ^{1}H NMR δ 0.90 (t, *J* = 7 Hz, 3H, CH_3), 1.35 (m, 2H, CH_2), 1.53 (m, 2H, CH_2), 2.68 (m, 1H, CH), 2.91 (m, 1H, CH), 3.80 (s, 3H, OCH_3), 4.2 (m, 2H, CH_2O), 4.74 (m, 1H, α-CH), 5.15 (s, 2H, OCH_2Ph), 5.67 (d, *J* = 7.5 Hz, 1H, NH), 6.09 (s, 1H, HC=), 7.3-7.5 (m, 8H, Ar), 7.86 (d, *J* = 7 Hz, 2H, Ar); ^{13}C NMR δ 13.9, 22.4, 29.4, 32.2, 52.9, 53.4, 67.3, 68.1, 97.1, 127.7, 128.1, 128.3, 128.4, 128.6, 132.0, 135.9, 115.1, 155.7, 169.6, 176.4, 189.8; IR ν_{max} ($CHCl_3$) 1750, 1725, 1660, 1600, 1590, 1510 cm^{-1}; MS (FAB) m/z 340 (MH^+, 30), 332 (9), 320 (8), 154 (8), 105 (20), 95 (20), 91 (100); Exact Mass Calcd for $C_{25}H_{29}NO_6$ 439.19949. Found 439.20043.

Methyl (2S)-2-[[(benzyloxy)carbonyl]amino}-6-{[(E)-3-oxo-3-phenyl-1-propenyl]amino} hexanoate 13

The addition of **1** to **9** was undertaken at room temperature following Procedure A. Purification of the crude material by flash chromatography (50% EtOAc / 50% hexanes) yielded 36 mg, (50%) of the expected addition product. ^{1}H NMR δ 1.42 (m, 2H, CH_2), 1.63 (m, 2H, CH_2), 1.87 (m, 2H, CH_2), 3.1 (m, 2H, NCH_2), 3.74 (s, 3H, OCH_3), 4.39(m, 1H, α-CH), 5.10 (s, 2H, OCH_2Ph), 5.34(d, *J* = 8 Hz, 1H, NH), 5.69 (d, *J* = 7.5 Hz, 1H, HC=), 6.90 (dd, *J* = 7.5, 13 Hz, 1H, HC=), 7.35 (m, 8H, Ar), 7.85 (m, 2H, Ar), 10.33 (m, 1H, enamine NH); ^{13}C NMR δ 22.2, 29.7, 30.5, 32.3, 48.8, 52.5, 53.6, 67.0, 90.2, 127.0, 128.1, 128.2, 128.5, 130.9, 136.2, 139.7, 154.2, 155.8, 172.7, 189.9; IR ν_{max} ($CHCl_3$) 1720, 1630, 1600, 1580, 1515, 1500 cm^{-1}; MS (LSIMS) m/z 425 (MH^+, 100); Exact Mass (EI) Calcd for $C_{24}H_{28}N_2O_5$ 424.19982. Found 424.1998.

Methyl (2S)-2-[[(benzyloxy)carbonyl]amino}-6-{[(E)-1-butyl-3-oxo-3-phenyl-1-propenyl]amino} hexanoate 14

The reaction between **2** and **9** was undertaken using Procedure A. Flash chromatography (1% EtOAc / 75%

hexane) produced 63% (51 mg) of **14**. ^{1}H NMR δ 0.95 (t, J = 7 Hz, 3H, CH_3), 1.4-1.7 (m, 10H, 5xCH_2), 2.30 (t, J = 7.5 Hz, 2H, CH_2C=), 3.30 (m, 2H, CH_2N), 3.74 (s, 3H, OCH_3), 4.45 (m, 1H, α-CH), 5.8 (s, 2H, OCH_2Ph), 5.51 (d, J = 8 Hz, 1H, NH), 5.66 (s, 1H, HC=), 7.37 (m, 8H, Ar), 7.85 (m, 2H, Ar), 11.56 (br s, 1H, enamine NH); ^{13}C NMR δ 13.8, 22.6, 29.6, 30.2, 32.2, 42.5, 52.4, 53.7, 67.0, 85.0, 91.0, 126.9, 128.1, 128.3, 128.5, 128.6, 130.3, 19.1, 136.3, 115.6, 168.9, 172.8, 187.7; IR ν_{max} ($CDCl_3$) 1730, 1600, 1550, 1520 cm^{-1}; UV λ_{max} (CH_2Cl_2) 246 (15530), 88 (28215); MS (EI) m/z 480 ($M^{+\cdot}$, 60), 463 (15), 451 (15), 438 (75), 333 (20), 258 (20), 216 (100); Anal. Calcd for $C_{28}H_{35}N_2O_5$: C, 69.97; H, 7.55; N, 5.83. Found C, 69.83; H, 7.52; N, 5.93.

Ethyl (E)-3- [((2S)-2-{[(benzyloxy)carbonyl]amino}-3-methoxy-3-oxopropyl)sulfanyl]-2- propenoate 15*E* and 15*Z*

The addition reaction between **3** and **7** was undertaken at 0°C following Procedure A. Acid workup, followed by purification of the crude material by flash chromatography (30% EtOAc / 70% hexane) yielded 51 mg, (75%) of the expected addition products in a ratio of 4:1 in favour of the *E* alkene. **15*E* / 15*Z*:** MS (EI) m/z 367 ($M^{+\cdot}$), 216 (90), 91 (M-CH_2Ph, 100); Anal. Calcd for $C_{17}H_{21}NO_6S$: C, 55.56; H, 5.76; N, 3.81. Found C, 55.45; H, 5.61; N, 4.21.

15*E*: ^{1}H NMR δ 1.29 (t, J = 7 Hz, 3H, CH_3), 3.35 (m, 2H, CH_2S), 3.80 (s, 3H, OCH_3), 4.18 (q, J = 7 Hz, 2H, CH_2O), 4.72 (m, 1H, α-CH), 5.15 (s, 2H, OCH_2Ph), 5.66 (br d, J = 8 Hz, 1H, NH), 5.87 (d, J = 15 Hz, 1H, HC=), 7.36 (s, 5H, Ph), 7.57 (d, J = 15 Hz, 1H, HC=); ^{13}C NMR δ 14.4, 36.2, 53.0, 53.5, 60.5, 67.5, 115.8, 128.2, 128.4, 128.7, 136.0, 145.5, 155.4, 165.2, 170.3; IR ν_{max} ($CDCl_3$) 1720, 1580, 1510 cm^{-1}. **15*Z*:** ^{1}H NMR δ 1.28 (t, J = 7 Hz, 3H, CH_3), 3.29 (m, 2H, CH_2S), 3.79 (s, 3H, OCH_3), 4.18 (q, J = 7 Hz, 2H, OCH_2), 4.70 (m, 1H, α-CH), 5.11 (m, 2H, OCH_2Ph), 5.65 (br d, J = 7.5 Hz, 1H, NH), 5.78 (d, J = 8 Hz, 1H, HC=), 6.92 (d, J = 8 Hz, 1H, HC=), 7.37 (s, 5H, Ph); ^{13}C NMR δ 14.6, 35.3, 52.6, 53.7, 60.6, 67.6, 116.0, 128.4, 128.5, 128.8, 136.2, 145.7, 155.8, 165.2, 170.4; IR ν_{max} ($CDCl_3$) 1720, 1600, 1500 cm^{-1}.

Ethyl (E)-3- [((2R)-2-{[(benzyloxy)carbonyl]amino}-3-methoxy-3-oxopropyl)oxy]-2- propenoate 16

The reaction of **3** with **8** was undertaken at 0°C using Procedure A. Acidic workup was followed by purification of the crude material by flash chromatography on silica (30% EtOAc / 70% hexane) to yield 39 mg, (75%) of **16**. ^{1}H NMR δ 1.3 (t, J = 7 Hz, 3H, CH_3), 3.79 (s, 3H, OCH_3), 4.2 (m, 4H, 2xOCH_2), 4.68 (m, 1H, α-CH), 5.15 (s, 2H, OCH_2Ph), 5.22 (d, J = 9.7 Hz, 1H, HC=), 5.68 (br d, J = 8 Hz, 1H, NH), 7.37 (s, 5H, Ph), 7.51 (d, J = 9.7 Hz, 1H, HC=); ^{13}C NMR δ 14.5, 53.1, 53.7, 60.1, 67.5, 70.6, 98.0, 126.3, 128.5, 128.7, 136.1, 155.9, 161.3, 167.3, 169.6; IR ν_{max} ($CDCl_3$) 1710, 1620, 1500 cm^{-1}; MS (EI) m/z 337 ($M^{+\cdot}$, 3), 260 (8), 198 (8), 90 (20), 91 (100).

Ethyl (E)-3- [((5S)-5-{[(benzyloxy)carbonyl]amino}-6-methoxy-6-oxohexyl)amino]-2- propenoate 17*E* and 17*Z*

The reaction of **3** with **9** was undertaken at room temperature after the initial removal of HCl by washing the protected lysine with 8% sodium bicarbonate solution. Purification of the crude material by flash chromatography (50% EtOAc / 50% hexanes) yielded 45% of the two isomers as an inseparable mixture in a 1:1 ratio. ^{1}H NMR δ 1.2 (m, 3H, CH_3), 1.4-1.9 (m, 6H, 3xCH_2), 2.98 (m, 2H, CH_2N (*E*)), 3.8 (m, 2H, CH_2N (*Z*)), 3.71 (s, 3H, OCH_3), 3.72 (s, 2H, OCH_3), 4.09 (2x q, J = 7 Hz, 4H, 2xOCH_2), 4.15 (m, 1H, α-

CH), 4.42 (d, J = 8 Hz, 1H, HC= (Z)), 4.55 (m, 1H, α-CH), 4.67 (d, J = 13 Hz, 1H, HC= (E)), 5.08 (s, 4H, 2xOC$\underline{H}_2$Ph), 5.7 (t, J = 8 Hz, 1H, NH), 6.55 (dd, J = 8, 13 Hz, 1H, HC= (Z)), 7.32 (s, 5H, Ph), 7.33 (s, 5H, Ph), 7.44 (dd, J = 8, 13 Hz, 1H, HC= (E)), 7.8 (m, 1H, NH enamine); IR ν_{max} ($CHCl_3$) 1720, 1650, 1600, 1500 cm^{-1}; MS (FAB) m/z 393 (MH^+, 73), 347 (100); Exact mass calcd for $C_{20}H_{29}N_2O_6$ (MH^+) 393.20256. Found 393.20220.

Methyl (E)-3- [((2S)-2-{[(benzyloxy)carbonyl]amino}-3-methoxy-3-oxopropyl)sulfanyl]-2-propenoate 18*E* and 18*Z*

The reaction between **4** and **7** was undertaken at room temperature following Procedure A. Purification of the crude material by flash chromatography (20% EtOAc / 80% hexane) yielded 40% of the expected addition products **18*E*** and **18*Z*** in a ratio of 2:1.

18*E* / 18*Z*: MS (FAB) m/z 410 ($M^{+\cdot}$), 378 (53), 270 (28), 107 (15), 91 (100); Anal. Calcd for $C_{20}H_{27}NO_6S$: C, 58.66; H, 6.65; N, 3.42. Found C, 58.59; H, 6.64; N, 3.13. **18*E***: Mp 86.5-88.5°C; ^{1}H NMR δ 0.91 (t, J = 7 Hz, 3H, CH_3), 1.*11* (m, 2H, CH_2), 1.57 (m, 2H, CH_2), 2.77 (t, J = 7 Hz, 2H, CH_2), 3.24 (m, 2H, CH_2S), 3.66 (s, 3H, OCH_3), 3.77 (s, 3H, OCH_3), 4.71 (m, 1H, α-CH), 5.12 (s, 2H, OC$\underline{H}_2$Ph), 5.57 (s, 1H, HC=), 5.64 (d, J = 7.5 Hz, 1H, NH), 7.34 (s, 5H, Ph); ^{13}C NMR δ 13.9, 22.6, 32.0, 33.5, 34.0, 51.0, 52.7, 52.9, 67.3, 108.8, 128.1, 128.3, 128.6, 136.1, 155.7, 162.7, 165.0, 170.5; IR ν_{max} ($CHCl_3$) 1750, 1715, 1600, 1500 cm^{-1}. **18*Z***: ^{1}H NMR δ 0.92 (t, J = 7 Hz, 3H, CH_3), 1.34 (m, 2H, CH_2), 1.52 (m, 2H, CH_2), 2.39 (t, J = 7 Hz, 2H, CH_2), 3.34 (m, 2H, CH_2S), 3.70 (s, 3H, OCH_3), 3.77 (s, 3H, OCH_3), 4.67 (m, 1H, α-CH), 5.11 (s, 2H, OC$\underline{H}_2$Ph), 5.64 (d, J = 8 Hz, 1H, NH), 5.83 (s, 1H, HC=), 7.34(s, 5H, Ph); ^{13}C NMR δ 13.8, 22.0, 31.2, 32.3, 36.2, 51.1, 52.9, 53.8, 67.2, 113.5, 128.1, 128.2, 128.5, 136.0, 155.6, 159.4, 166.3, 170.3; IR ν_{max} ($CHCl_3$) 1720, 1580, 1505 cm^{-1}.

Methyl (E)-3- [((2R)-2-{[(benzyloxy)carbonyl]amino}-3-methoxy-3-oxopropyl)oxy]-2-heptenoate 19

The addition of **4** to **8** was undertaken following Procedure B. Purification of the crude material by flash chromatography on silica (1% EtOAc / 75% hexane) yielded 14 mg, (35%) of **19**. ^{1}H NMR δ 0.90 (t, J = 7 Hz, 3H, CH_3), 1.7 (m, 2H, CH_2), 1.44 (m, 2H, CH_2), 2.57 (m, 1H, CHC=), 2.86 (m, 1H, CHC=), 3.67 (s, 3H, OCH_3), 3.77 (s, 3H, OCH_3), 4.09 (m, 2H, OCH_2), 4.68 (m, 1H, α-CH), 4.97 (s, 1H, HC=), 5.14 (s, 2H, OC$\underline{H}_2$Ph), 5.63 (d, J = 8 Hz, 1H, NH), 7.36 (s, 5H, Ph); ^{13}C NMR δ 13.8, 22.2, 29.4, 31.2, 50.9. 52.8, 53.3, 67.3, 67.9, 91.6, 128.2, 128.3, 128.6, 135.9, 155.7, 167.5, 169.7, 175.1; IR ν_{max} ($CHCl_3$) 1750, 1710, 1620, 1500 cm^{-1}; MS (FAB) m/z 394 (MH^+, 20), 362 (20), 308 (8), 236 (20); Anal. Calcd for $C_{20}H_{27}NO_7$: C, 61.05; H, 6.92; N, 3.56. Found C, 60.84; H, 7.9; N, 3.43.

Methyl (2R)-2-{[(benzyloxy)carbonyl]amino}-3-{[(E)-(butylamino)-3-oxo-1-propenyl]sulfanyl} propanoate 20*E* and 20*Z*

The reaction of **7** with **9** was undertaken at room temperature by Procedure A. Purification of the crude material by flash chromatography (50% EtOAc / 50% hexane) yielded 47 mg, (58%) of the two isomers **20*E*** and **20*Z*** in a ratio of 1.4:1.

20*E* / 20*Z*: MS (EI) m/z 428 ($M^{+\cdot}$), 337 (15), 206 (50), 192 (60), 160 (90), 106 (100); Anal. Calcd for $C_{22}H_{24}N_2O_5S$: C, 61.66; H, 5.64; N, 6.54. Found C, 61.90; H, 5.69; N, 6.2. **20*E***: Mp 134-137°C; ^{1}H NMR δ 3.3 (m, 2H, CH_2S), 3.77 (s, 3H, OCH_3), 4.47 (d, J = 6 Hz, 2H, CH_2N), 4.67 (m, 1H, α-CH), 5.08,

5.12 (AB, J = 9 Hz, 2H, OC$\underline{H}_2$Ph), 5.65 (br d, J = 7.5 Hz, 1H, NH), 5.73 (m, 1H, NH), 5.87 (d, J = 15 Hz, 1H, HC=), 7.1-7.35 (m, 5H, Ph), 7.49 (d, J = 15 Hz, 1H, HC=); ^{13}C NMR δ 35.2, 43.7, 53.0, 53.3, 67.3, 118.0, 127.5, 127.8, 128.1, 128.3, 128.6, 128.7, 138.1, 141.5, 155.9, 164.3, 170.6; IR ν_{max} ($CDCl_3$) 1730, 1660, 1590, 1510 cm^{-1}. **20*Z***: Mp 109-112°C; ^{1}H NMR δ 3.23 (m, 2H, CH_2S), 3.76 (s, 3H, OCH_3), 4.48 (d, J = 6 Hz, 2H, CH_2N), 4.67 (m, 1H, α-CH), 5.11 (s, 2H, OC$\underline{H}_2$Ph), 5.6 (m, 2H, 2xNH), 5.70 (d, J = 8 Hz, 1H, HC=), 6.73 (d, J = 8 Hz, 1H, HC=), 7.2-7.4 (m, 5H, Ph); ^{13}C NMR δ 38.6, 43.5, 52.9, 54.3, 67.1, 115.9, 127.55, 127.6, 127.9, 128.0, 128.1, 128.2, 128.5, 128.7, 136.1, 136.9, 138.2, 145.1, 155.6, 165.8, 170.3; IR ν_{max} ($CDCl_3$) 1710, 1615, 1570, 1500 cm^{-1}.

Methyl-2-{[(benzyloxy)carbonyl]amino}acrylate 21

N-Carbobenzyloxy dehydroalanine methyl ester[28] **21** was formed as a by-product from the reaction of triethylamine with either **7** or **8** with **6**, or from the reaction of **8** with **5**. ^{1}H NMR δ 3.84 (s, 3H, OCH_3), 5.17 (s, 2H, OC$\underline{H}_2$Ph), 5.80 (d, J = 1.4 Hz, 1H, HC=), 6.2 (s, 1H, HC=), 7.11 (s, 5H, Ph).

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