Synthesis of the suicide substrate D-propargylglycine stereospecifically labelled with deuterium and investigation of its oxidation by D-amino acid oxidase[1]

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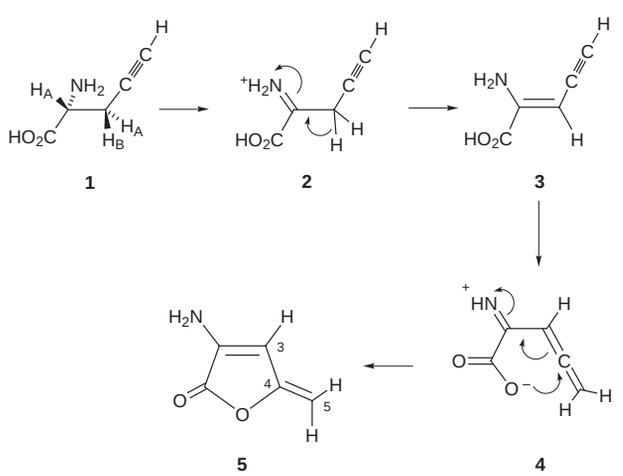
Stereospecifically deuteriated samples of D-propargylglycine 1 have been prepared by reaction of the labelled Pmc-protected aziridine free acids 22 with a lithium acetylide followed by deprotection. These samples have been used to show that D-amino acid oxidase, in converting D-propargylglycine to the lactone 5, deprotonates C-3 in a non-stereospecific manner. This strongly supports the idea that non-enzymic deprotonation is a key step in the formation of this compound.

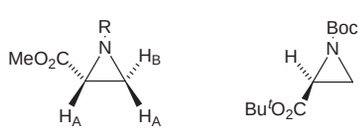
D-Propargylglycine **1** (D-prop-2-ynylglycine) and its corresponding L-isomer are substrates for the enzymes D-amino acid oxidase (EC 1.4.3.3) and L-amino acid oxidase (EC 1.4.3.2) respectively.[2] D-Propargylglycine acts both as an inhibitor of and as a substrate for D-amino acid oxidase, whereas L-propargylglycine acts only as a substrate for the L-oxidase.[2] The same species accumulates on incubation of either D- or L-propargylglycine with the appropriate enzyme and this has been shown[3] to be 2-amino-4-hydroxypenta-2,4-dienoate-γ-lactone **5**. The mechanism shown in Scheme 1 has been suggested[3] to account for the formation of this compound.



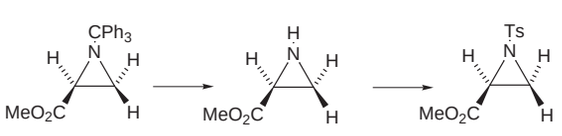
Scheme 1

By this mechanism, initial oxidation to the iminium species **2** would be followed by tautomerism to the enamine **3** and rearrangement to the allene **4**. The allene **4** would then cyclise to the lactone **5**. Proton loss at C-3 in this process might either be enzyme catalysed or non-enzymatic and Walsh[3] has indicated that the kinetics are more in keeping with the latter process. Since proton loss would be expected to be stereospecific in an enzyme catalysed process and would not be stereospecific if the step were not enzyme catalysed, we have completed a synthesis of samples of the suicide substrate D-propargylglycine **1** which are stereospecifically labelled at C-3 with deuterium and have used these to investigate the enzyme catalysed conversion of these compounds to the lactone **5**.

We chose the stereospecifically labelled aziridines **6**, $H_B = {}^2H$ and **6**, $H_A = {}^2H$ as the starting point for our synthesis, having previously developed a chemico-enzymatic synthesis for a series of these compounds.[4] We had shown these to be opened regiospecifically at C-3 with a variety of heteroatom nucleophiles,[4] a reaction which was dependent on the nature of the substituent R. For the trityl derivative **6**, R = CPh_3, aqueous perchloric acid ring opening afforded serine derivatives whilst for sulfur and halogen nucleophiles, we required a stronger electron withdrawing group and **6**, R = CO_2CH_2Ph was used. Thus if ring opening could be achieved with the carbon nucleophile, acetylene, then stereospecifically labelled samples of the suicide inhibitor might be accessed. The literature on opening N-substituted aziridine-2-carboxylic esters with carbon nucleophiles, however, indicated that such reactions were not entirely successful in achieving total regiospecific control[5–7] although, since our preliminary publication,[1] Baldwin has reported that the aziridine **7** will react regioselectively at C-3 in 'copper' catalysed Grignard reactions.[8]

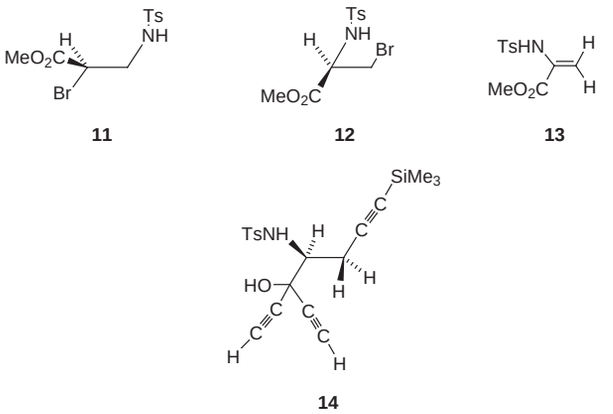


In our initial experiments, we used the more available[9] L-tritylaziridine **8**, and converted it into the tosylate **10** in 70% yield by initial deprotection to the unstable product **9** with trifluoroacetic acid and then reaction of this with toluene-*p*-sulfonyl chloride in pyridine (Scheme 2). Initially we reacted the



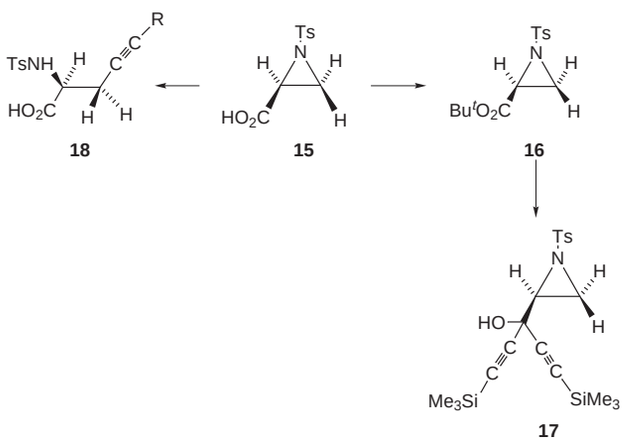
Scheme 2

N-tosylaziridine ester **10** with 1.1 equivalents of ethynylmagnesium bromide but obtained a mixture of the product **11** of α-attack of bromide in 54% yield together with a 15% yield of the β-adduct **12** and 9% of a crude compound which we assigned as the product of elimination **13** from its ^{1}H NMR spectrum. The tosylate **10** was then reacted with lithium trimethylsilylacetylide but this led to reaction at both ester and aziridine groups, yielding the mono(trimethylsilyl) tris-adduct **14** in 50% yield. Hydrolysis of the *N*-tosyl methyl ester **10** to the corresponding



11 **12** **13** **14**

acid **15** in 100% yield using sodium hydroxide allowed the *tert*-butyl ester **16** to be prepared in 72% yield using *tert*-butyl alcohol dicyclohexylcarbodiimide and dimethylaminopyridine (Scheme 3). However when this ester was reacted with lithium



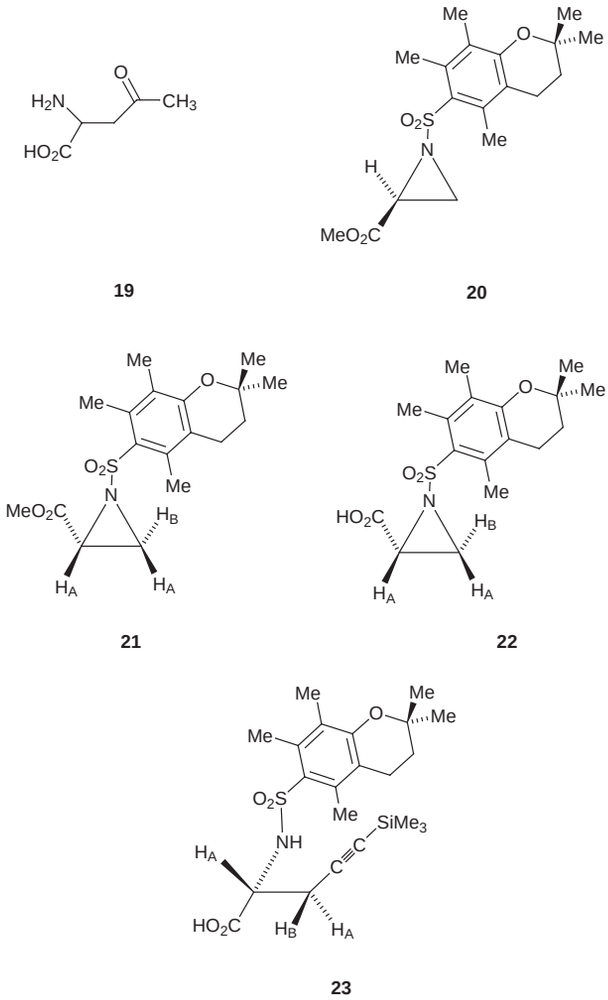
18 **15** **16** **17**

Scheme 3

trimethylsilylacetylide, unaccountably reaction occurred only at the ester group, the aziridine remaining untouched. The diacetylene **17** was obtained in 88% yield. Other work[7] has also indicated that Grignard and organolithium reagents attack the ester function in such aziridines.

Reasoning that carboxylate attack might be prevented and that β-regiospecificity in the ring opening reaction might be enforced if the anion of the acid **15** were used as the electrophile, the acid **15** was reacted with excess lithium trimethylsilylacetylide to yield a mixture of the products **18**, R = $SiMe_3$ (49%) and **18**, R = H (30%). Although both the free acetylene **18**, R = H and the trimethylsilylacetylene **18**, R = Me_3Si were obtained, it was evident that we had succeeded in directing attack entirely to C-3.

Unfortunately, attempts to convert the *N*-tosyl derivatives **18** into the target free amino acid were accompanied by reduction of the acetylene or hydrolysis to the methyl ketone **19** and so it was evident that we required to functionalise the nitrogen of the aziridine with a group which would not only have the electron withdrawing properties required for the ring opening reaction but which would subsequently be more easily removed. Ramage's arginine protecting group 2,2,5,7,8-pentamethylchroman-6-ylsulfonyl (Pmc) group[10] seemed appropriate for this purpose and so we prepared the labelled *N*-Pmc ester **20** from the (2*S*)-aziridine **8**, and **21**, $H_B = {}^2H$ and **21**, $H_A = {}^2H$ from the *N*-tritylaziridines **6**, R = CPh_3[4] in 30% yield by deprotection to the aziridines and reaction with Pmc chloride under Schotten–Baumann conditions. Saponification led to the labelled acids **22** in quantitative yield and reaction with lithium trimethylsilylacetylide gave the clean protected amino acids **23** in 30% yield. Deprotection was achieved using refluxing tri-



19 **20** **21** **22** **23**

fluoroacetic acid, giving the target compounds (2*R*)-2-{(1*R*)-[1-$^2H_1$]propynyl}glycine and (2*R*)-2-{(1*S*)-[1-$^2H_1$]propynyl}-[2-$^2H_1$]glycine, **1**, $H_B = {}^2H$ and **1**, $H_A = {}^2H$ respectively, contaminated with a small amount of the methyl ketones **19**. The ^1H NMR spectra of these samples of the suicide substrate showed them to be spectrospecifically labelled.

When unlabelled D-propargylglycine **1** was incubated with 20% w/w D-amino acid oxidase and 2% w/w catalyse in 0.02 M HEPES buffer at pH 8.03 and 20 °C for 3 hours and the solution was extracted with C^2HCl_3, the ^1H NMR spectrum (Fig. 1a) showed the characteristic peaks due to the lactone **5** as reported by Walsh.[3] When the labelled samples of D-propargylglycine **1**, $H_B = {}^2H$ or **1**, $H_A = {}^2H$ were used as substrates then in each case, from integration of the ^1H NMR spectra of the lactone products **5** (Fig. 1b and 1c, respectively), the hydrogen on the endocyclic double bond was 80–90% deuteriated and 10–20% protium was present. This result is in keeping with the operation of a primary isotope effect in the deprotonation step subsequent upon enzymic oxidation to the imine **2**. This step is evidently non-stereospecific, and so the result is in keeping with the second step in the mechanism being a non-enzyme catalysed process.

Two further experiments were carried out in the hope that the enzyme catalysed conversion of propargylglycine **1** to the lactone **5** might be faster than chemical exchange of the acetylenic proton. If this were the case then the stereochemical course of the reaction at the *exo*-methylene carbon might be assessed. Thus the reaction of unlabelled propargylglycine **1** with enzyme was carried out in ^2H_2O but *both* hydrogen atoms at C-5 in the resultant lactone **5** were extensively deuteriated. Further the enzymic reaction was carried out in H_2O after deuterium exchange at C-5 but now both hydrogens at C-5 in the resultant lactone **5** were unlabelled.

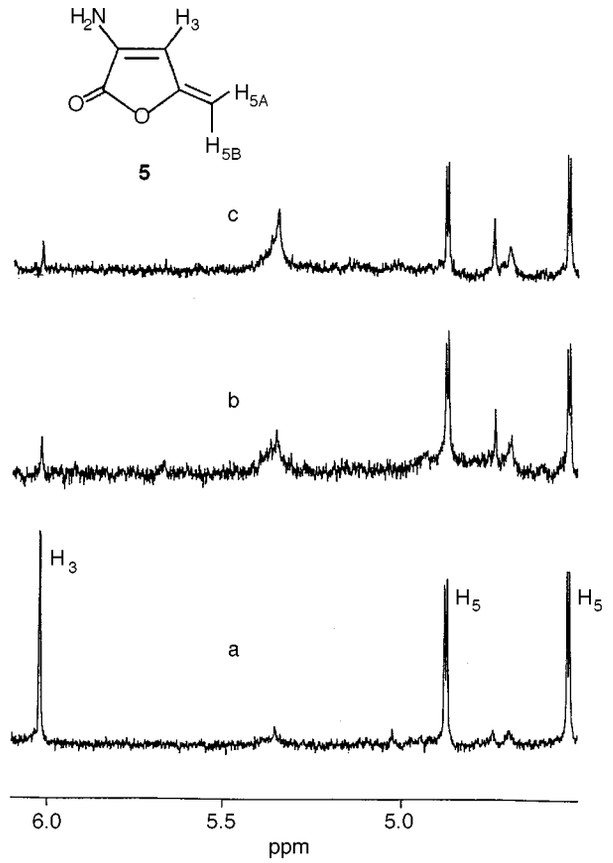


Fig. 1 ^{1}H NMR spectra (360 MHz; C^2HCl_3) of the lactones **5** formed on incubation of D-amino acid oxidase with (**a**) (2*R*)-propargylglycine; (**b**) (2*R*)-2-{(1*R*)-[1-^{2}H$_1$]propynyl}glycine; and (**c**) (2*R*)-2-{(1*S*)-[1-^{2}H$_1$]-propynyl}[2-^{2}H$_1$]glycine

Experimental

Melting points were determined on a Kofler hot-stage apparatus and are uncorrected. Optical rotations (given in units of 10^{-1} deg cm^{-2} g^{-1}) were measured on a Perkin-Elmer 241 polarimeter using a 1 dm path length micro cell. IR spectra were recorded on a Perkin-Elmer 1710 Fourier transform instrument, and UV spectra on a Unicam UV2-100 UV–VIS spectrophotometer. Mass spectra were recorded by Mr A. Greenway using a Kratos MS80RF instrument and Dr A. Al Sada at Sussex University using a Fisons VG Autospec instrument, and by Drs S. Chotai and D. Dell at the Wellcome Research Laboratories, Beckenham using Kratos Concept 1S and MS50 instruments. The electrospray mass spectrum was recorded on a Fisons BIO Q instrument by Dr D. Cooper at the Wellcome Research Laboratories, Beckenham. Accurate mass measurements were performed by Dr S. Chotai at the Wellcome Research Laboratories, Beckenham using a Kratos Concept 1S instrument. ^{1}H NMR spectra were recorded on a Bruker WM360 instrument (360 MHz), ^{13}C NMR spectra were recorded by Dr A. G. Avent on a Bruker AMX 500 instrument (125.8 MHz) and by Mr C. M. Dadswell on a Bruker AC-P250 instrument (62.9 MHz), and ^{29}Si NMR spectra on a Bruker WM360 instrument (99.4 MHz) by Dr A. G. Avent. *J* Values are given in Hz. 3-Trimethylsilylpropanesulfonic acid (DSS), located at δ 0.0, was used as the internal standard for samples run in 20% ^{2}HCl in ^2H_2O. For all other NMR spectra the residual solvent peak was used as the reference. Thin layer chromatography was carried out on Merck Kieselgel 60 F_{254} pre-coated silica gel glass-backed plates of thickness 0.25 mm (Art 5719). Column chromatography was carried out using Merck Kiesegel 60 (230–400 mesh-Art 9385). Microanaylses were performed by Miss M. Patel at Sussex University, and by Ms C. Lawless and W. C. Man at the Wellcome Research Laboratories, Beckenham. 2,2,5,7,8-Pentamethylchromane-6-sulfonyl chloride was purchased from Bachem. D-Amino acid oxidase (EC 1.4.3.3) was purchased from Sigma (Dorset). Light petroleum refers to the fraction boiling in the range 60–80 °C.

(2*S*)-Methyl *N*-*p*-tolylsulfonylaziridine-2-carboxylate 10[11]

(2*S*)-Methyl *N*-triphenylmethylaziridine-2-carboxylate **8**[9] (250 mg; 0.73 mmol) was dissolved in chloroform (0.9 ml) and methanol (0.9 ml) and cooled to 0 °C in an ice bath. Trifluoroacetic acid (0.9 ml; 11.7 mmol) was added dropwise over 1–2 minutes and the reaction mixture was stirred at 0 °C under nitrogen for 2.5 hours. The solvents were removed *in vacuo* at 0 °C and the resulting solid residue was azeotroped five times with diethyl ether (5 × 2 ml) at 0 °C and partitioned between diethyl ether (15 ml) and water (15 ml). The ether layer was washed with water (10 ml) and the combined aqueous fractions were neutralised with solid sodium hydrogen carbonate (250 mg; 2.98 mmol). The aqueous fractions were diluted with ethyl acetate (25 ml) and the mixture was cooled to 0 °C in an ice bath. Toluene-*p*-sulfonyl chloride (139 mg; 0.73 mmol) was added and the reaction mixture was stirred vigorously at room temperature for 1.5 hours. The layers were separated and the aqueous layer was extracted with ethyl acetate (20 ml). The solvent was removed from the combined organic fractions *in vacuo* and the product was purified by chromatography on silica gel using light petroleum–ethyl acetate (4:1) as eluent. The product (2S)-*methyl* N-p-*tolylsulfonylaziridine*-2-*carboxylate* **10** was a clear oil (86 mg, 46%); $[a]_D^{23}$ −50.2 (*c* 1, $CHCl_3$) (Found C, 51.4; H, 5.3; N, 5.0%. $C_{11}H_{13}NO_4S$ requires C, 51.75; H, 5.1; N, 5.5%); *m*/*z* [+ve FAB (thioglycerol)] 256 ([M + H]$^+$); ν_{max}(film)/cm^{-1} 1752 (ester); δ_H(360 MHz, C^2HCl_3) 2.45 (3H, s, C*H*$_3$-Ar), 2.55 (1H, d, $J_{3A,2}$ 4.1, *H*-3A), 2.76 (1H, d, $J_{3B,2}$ 7.1, *H*-3B), 3.33 (1H, dd, $J_{2,3A}$ 4.1, $J_{2,3B}$ 7.1, *H*-2), 3.73 (3H, s, OC*H*$_3$), and 7.38 and 7.85 (2 × 2H, 2 × d, Ar-*H*); δ_C(125.8 MHz, C^2HCl_3) 21.64 (*C*H$_3$-Ar), 31.96 (*C*-3) 35.70 (*C*-2), 52.82 (*C*H$_3$-O), 128.20 and 129.86 (*ortho* and *meta* Ar-*C*), 134.08 and 145.22 (2 × *ipso* Ar-*C*) and 167.20 (*C*O$_2$CH$_3$).

Investigation of the reaction between (2*S*)-methyl *N*-*p*-tolylsulfonylaziridine-2-carboxylate 10 and ethynylmagnesium bromide

(2*S*)-Methyl *N*-*p*-tolylsulfonylaziridine-2-carboxylate **10** (79 mg, 0.3 mmol) was dissolved in diethyl ether (5 ml) and cooled to −78 °C. Ethynylmagnesium bromide (0.5 M in THF, 1.9 ml, 0.9 mmol) was added and the reaction was stirred at −78 °C under argon for 1 hour. The reaction was allowed to warm to room temperature and was stirred for a further 4 hours before being quenched using saturated aqueous ammonium chloride (20 ml). The layers were separated and the aqueous portion was extracted with diethyl ether (3 × 10 ml). The combined organic layers were dried (Na_2SO_4) and the solvent was removed *in vacuo*. The crude product was purified by chromatography on silica gel using chloroform–light petroleum–ethyl acetate (10:3:1) as eluent. The first product to be eluted was the crude *enamine* **13** (7 mg, 9%); δ_H(360 MHz, C^2HCl_3) 2.4 (3H, s, C*H*$_3$Ar), 3.7 (3H, s, OC*H*$_3$), 5.6 and 5.7 (2 × 1H, 2 × s, *H*-3), 7.1 (1H, s, N*H*, exchangeable in ^2H_2O), 7.3 (2H, d, *J* 8.1, Ar-*H*) and 7.8 (2H, d, *J* 8.1, Ar-*H*). The second product to be eluted was (2S)-*methyl N*-p-*tolylsulfonyl*-3-*bromoalaninate* **12** as a colourless oil (15 mg, 15%); *m*/*z* [+ve FAB (3-nba)] 336 and 338 (1:1) ([M + H]$^+$); ν_{max}(film)/cm^{-1} 1746 (ester); δ_H(360 MHz, C^2HCl_3) 2.4 (3H, s, ArC*H*$_3$), 3.6 (1H, dd, $J_{3A,2}$ 3.9, $J_{3A,3B}$ 11, *H*-3A), 3.7 (3H, s, OC*H*$_3$), 3.9 (1H, dd, $J_{3B,2}$ 3.1, $J_{3B,3A}$ 11, *H*-3B), 4.3 (1H, m, *H*-2), 5.5 (1H, d, $J_{NH,2}$ 6.9, N*H*, exchangeable in ^2H_2O), 7.3 (2H, d, *J* 8.1, Ar*H*) and 7.8 (2H, d, *J* 8.1, Ar-*H*). The third product from the column was *methyl* N-p-*tolylsulfonyl*-2-*bromo*-β-*alaninate* **11**, obtained as a yellow oil (54 mg, 54%) (Found C, 40.4; H, 3.9; N, 4.6%. $C_{11}H_{14}O_4NSBr$ requires C, 39.4; H, 4.2; N, 4.2%); *m*/*z* [FAB (3-nba)] 336 and 338 (1:1) ([M + H]$^+$); ν_{max}(film)/cm^{-1} 1747 (ester); δ_H(360 MHz, C^2HCl_3)

2.4 (3H, s, ArCH_3), 3.4 (1H, m, *H*-3A), 3.5 (1H, m, *H*-3B), 3.8 (3H, s, OCH_3), 4.4 (1H, dd, $J_{2,3A}$ 5.9, $J_{2,3B}$ 8.4, *H*-2), 5.5 (1H, t, $J_{NH,3}$ 6.7, N*H*, exchangeable in ^2H_2O), 7.3 (2H, d, *J* 8.1, Ar-*H*) and 7.8 (2H, d, *J* 8.1, Ar-*H*); addition of ^2H_2O caused the multiplets at 3.4 (1H, dd, $J_{3A,2}$ 5.9, $J_{3A,3B}$ 14.2) and 3.5 (1H, dd, $J_{3B,2}$ 8.4, $J_{3B,3A}$ 14.2) to simplify; δ_C(125.8 MHz, C^2HCl_3) 21.5 (Ar*C*H_3), 41.6 (*C*-2), 45.8 (*C*-3), 53.3 (O*C*H_3), 127.0 and 130.0 (Ar-*C*H), 136.7 and 144.0 (quaternary Ar-*C*) and 169.0 (*C*O_2CH_3).

(4*S*)-1-Trimethylsilyl-(*N*-*p*-tolylsulfonylamino)-5-hydroxy-5-ethynylhepta-1,6-diyne 14

n-Butyllithium (1.6 M in hexane, 3.9 ml, 6.3 mmol) was added to a cooled (0 °C) solution of trimethylsilylacetylene (1.1 ml, 7.8 mmol) in THF (8 ml) and the reaction was stirred under argon at 0 °C for 30 min. (2*S*)-Methyl *N*-*p*-tolylsulfonylaziridine-2-carboxylate **10** (0.2 g, 0.8 mmol) in THF (4 ml) was added, followed by hexamethylphosphoric triamide (1.1 ml, 6.3 mmol). The reaction was allowed to warm up to room temperature and left under argon for 2 hours. The reaction was cooled to 0 °C, and was quenched by addition of saturated aqueous ammonium chloride (20 ml). The layers were separated and the aqueous phase was extracted with ethyl acetate (3 × 20 ml). The combined organic layers were dried (Na_2SO_4) and the solvent was removed *in vacuo* to yield an oil. The crude product was purified by chromatography on silica gel using ethyl acetate–light petroleum (1:2) as eluent to yield (4S)-1-*trimethylsilyl*-4-(N-p-*tolylsulfonylamino*-5-*hydroxy*-5-*ethynylhepta*-1,6-*diyne* **14** as an amber oil (0.15 g, 51%); $[\alpha]_D^{29}$ +49.6 (*c* 0.3, $CHCl_3$) (*m*/*z* found 373.114 90; $C_{19}H_{23}O_3NSSi$ requires 373.116 80); *m*/*z* [+ve CI (NH_3)] 374 ($[M + H]^+$); ν_{max}(film)/cm^{-1} 3752 (OH), 3290 (NH) and 2180 (C≡C); δ_H(500 MHz, C^2HCl_3) 0.1 [9H, s, Si(CH_3)$_3$], 2.4 (3H, s, ArCH_3), 2.7 (1H, dd, $J_{3A,2}$ 5.3, $J_{3A,3B}$ 17.6, *H*-3A), 2.8 (1H, dd, $J_{3B,2}$ 5.9, $J_{3B,3A}$ 17.6, *H*-3B), 3.7 (1H, m, *H*-2), 3.8 (1H, br s, O*H*, exchangeable in ^2H_2O), 5.2 (1H, d, $J_{NH,2}$ 9.2, N*H*, exchangeable in ^2H_2O), 7.3 (2H, d, *J* 8.1, Ar-*H*) and 7.8 (2H, d, *J* 8.1, Ar-*H*); δ_C(125.8 MHz, C^2HCl_3) 0.2 [Si(*C*H_3)$_3$], 21.5 (Ar*C*H_3), 23.0 (*C*-3), 59.4 (*C*-2), 66.4 (*C*-OH), 74.1 and 74.8 (C≡*C*H), 80.3 (2 × *C*≡CH), 90.0 [*C*≡CSi(CH$_3$)$_3$], 100.8 [*C*≡CSi(CH$_3$)$_3$], 127.5 and 129.6 (Ar-*C*H), and 137.2 and 143.8 (2 × quaternary Ar-*C*).

(2*S*)-*N*-*p*-Tolylsulfonylaziridine-2-carboxylic acid 15

(2*S*)-Methyl *N*-*p*-tolylsulfonylaziridine-2-carboxylate **10** (1 g, 3.9 mmol) was dissolved in THF (10 ml), and the solution was cooled to 0 °C in an ice bath. 1 M Aqueous NaOH (6.3 ml, 6.3 mmol) was added, and the reaction was stirred at room temperature for 3 hours. The solvent was removed *in vacuo*, and the resultant gum was partitioned between ethyl acetate (10 ml) and saturated aqueous sodium hydrogen carbonate (10 ml). The layers were separated and the aqueous fraction was cooled on ice and acidified to pH 4 with 10% aqueous citric acid. The acidified aqueous layer was extracted with ethyl acetate (3 × 20 ml), and the combined organic fractions were dried (Na_2SO_4) and the solvent was removed *in vacuo* to yield (2S)-N-p-*tolylsulfonylaziridine*-2-*carboxylic acid* **15** as a colourless oil (0.9 g, 96%); $[\alpha]_D^{27}$ −52.3 (*c* 1, $CHCl_3$) (*m*/*z* found 241.040 43; $C_{10}H_{11}O_4NS$ requires 241.040 88); *m*/*z* [+ve FAB (3-nba)] 242 ($[M + H]^+$); ν_{max}(film)/cm^{-1} 1736 (CO_2H); δ_H(360 MHz, C^2HCl_3) 2.4 (3H, s, ArCH_3), 2.5 (1H, d, $J_{3A,2}$ 4.0, *H*-3A), 2.8 (1H, d, $J_{3B,2}$ 7.1, *H*-3B), 3.3 (1H, dd, $J_{2,3A}$ 4.0, $J_{2,3B}$ 7.1, *H*-2), 7.4 (2H, d, *J* 8.2, Ar-*H*), 7.8 (2H, d, *J* 8.2, Ar-*H*) and 10.0 (1H, br s, CO_2H, exchangeable in ^2H_2O); δ_C(125.8 MHz, C^2HCl_3) 21.6 (Ar*C*H_3), 32.2 (*C*-3), 35.4 (*C*-2), 128.1 and 129.9 (Ar-*C*H), 133.4 and 145.5 (2 × quaternary Ar-*C*) and 171.8 (*C*O_2H).

(2*S*)-*tert*-Butyl *N*-*p*-tolylsulfonylaziridine-2-carboxylate 16

(2*S*)-*N*-*p*-Tolylsulfonylaziridine-2-carboxylic acid **15** (0.1 g, 0.4 mmol) was dissolved in dry dichloromethane (3 ml) and the solution was cooled to 0 °C in an ice bath. *tert*-Butyl alcohol (0.12 ml, 1.3 mmol), dimethylaminopyridine (5 mg, 0.04 mmol) and dicyclohexylcarbodiimide (0.9 g, 0.5 mmol) were added successively, and the mixture was kept at 0 °C for 10 min before being allowed to warm to room temperature and stirred under nitrogen for 20 hours. The mixture was filtered through Celite and the filtrate was washed with 0.05% aqueous citric acid (3 × 10 ml) and water (3 × 10 ml). The organic fraction was dried (Na_2SO_4). Removal of the solvent *in vacuo* yielded (2S)-tert-*butyl* N-p-*tolylsulfonylaziridine*-2-*carboxylate* **16** as a white solid which was recrystallised from light petroleum (90 mg, 72%); mp 97–100 °C; $[\alpha]_D^{27}$ −55.3 (*c* 0.7, $CHCl_3$) (Found C, 56.6; H, 6.6; N, 4.7%. $C_{14}H_{19}O_4NS$ requires C, 56.5; H, 6.4; N, 4.7%); *m*/*z* [+ve FAB (3-nba)] 298 ($[M + H]^+$); ν_{max}(KBr)/cm^{-1} 1741 (ester); δ_H(360 MHz, C^2HCl_3) 1.4 [9H, s, C(CH_3)$_3$] 2.4 (3H, s, ArCH_3), 2.5 (1H, d, $J_{3A,2}$ 4.2, *H*-3A), 2.6 (1H, d, $J_{3B,2}$ 7.1, *H*-3), 3.2 (1H, dd, $J_{2,3A}$ 4.2, $J_{2,3B}$ 7.1, *H*-2), 7.3 (2H, d, *J* 8.1, Ar-*H*) and 7.8 (2H, d, *J* 8.1, Ar-*H*); δ_C(125.8 MHz, C^2HCl_3) 21.6 (Ar*C*H_3), 27.7 [OC(*C*H_3)$_3$], 31.8 (*C*-3), 36.7 (*C*-2), 82.9 [O*C*(CH$_3$)$_3$], 128.1 and 129.7 (Ar-*C*H), 134.2 and 145.0 (2 × quaternary Ar-*C*) and 165.6 [*C*O_2C(CH$_3$)$_3$].

***N*-*p*-Tolylsulfonyl-2-[3-hydroxy-1,5-bis(trimethylsilyl)penta-1,4-diyn-3-yl]aziridine 17**

n-Butyllithium (1.6 M in hexane, 1.1 ml, 2.0 mmol) was added to a cooled (0 °C) solution of trimethylsilylacetylene (0.3 ml, 2.1 mmol) in THF (3 ml) under argon. The reaction was left to stir at 0 °C for 30 min, and (2*S*)-*tert*-butyl *N*-*p*-tolylsulfonylaziridine-2-carboxylate **16** (63 mg, 0.2 mmol) in THF (1 ml) was added. The reaction was allowed to warm to room temperature and was left to stir under argon for 2 hours. The reaction was cooled to 0 °C and was quenched by the addition of saturated aqueous ammonium chloride (10 ml). The layers were separated and the aqueous phase was extracted with ethyl acetate (3 × 20 ml). The combined organic layers were dried (Na_2SO_4) and the solvent was removed *in vacuo* to yield an oil. The crude product was purified by chromatography on silica gel using ethyl acetate–light petroleum (1:2) as eluent to yield N-p-*tolylsulfonyl*-2-[3-*hydroxy*-1,5-*bis*(*trimethylsilyl*)*penta*-1,4-*diyn*-3-*yl*]*aziridine* **17** as an amber oil (78 mg, 88%); $[\alpha]_D^{27}$ −7.1 (*c* 0.9, $CHCl_3$) (Found C, 57.4; H, 7.0; N, 3.4%. $C_{20}H_{29}O_3NSSi_2$ requires C, 57.3; H, 6.9; N, 3.3%); *m*/*z* [+ve FAB (3-nba)] 420 ($[M + H]^+$); ν_{max}(film)/cm^{-1} 3453 (OH) and 2178 (C≡C); δ_H(360 MHz, C^2HCl_3) 0.1 [18H, s, 2 × Si(CH_3)$_3$], 2.4 (3H, s, ArCH_3), 2.5 (1H, d, $J_{3A,2}$ 4.2, *H*-3A), 2.7 (1H, d, $J_{3B,2}$ 6.8, *H*-3B), 3.3 (1H, dd, $J_{2,3A}$ 4.2, $J_{2,3B}$ 6.8, *H*-2), 7.3 (2H, d, *J* 8.1, Ar-*H*) and 7.9 (2H, d, *J* 8.1, Ar-*H*); δ_C(125.8 MHz, C^2HCl_3) 0.5 [2 × Si(*C*H_3)$_3$], 21.6 (Ar*C*H_3), 30.4 (*C*-3), 46.2 (*C*-2), 61.6 (*C*-OH), 90.2 and 90.6 [2 × *C*≡CSi(CH$_3$)$_3$], 100.0 and 101.3 [C≡*C*Si(CH$_3$)$_3$], 128.2 and 129.7 (Ar-*C*H), and 134.4 and 144.7 (2 × quaternary Ar-*C*); δ_{Si}(99.4 MHz, C^2HCl_3) −16.28 and −16.33 [2 × Si(*C*H_3)$_3$].

Preparation of (2*S*)-*N*-*p*-tolylsulfonyl-2-(3-trimethylsilylprop-2-ynyl)glycine 18, R = SiMe$_3$ and (2*S*)-*N*-*p*-tolylsulfonyl-2-(prop-2-ynyl)glycine 18, R = H

n-Butyllithium (1.6 M in hexane; 31 ml; 49.8 mmol) was added to a cold (0 °C) solution of trimethylsilylacetylene (8.8 ml; 62.2 mmol) in THF (20 ml), and the mixture was stirred at 0 °C under argon for 30 min. (2*S*)-*N*-*p*-Tolylsulfonylaziridine-2-carboxylic acid **15** (1.5 g, 6.22 mmol) in THF (20 ml) was added gradually, and the reaction was allowed to warm to room temperature and stirred under argon for a further 3 hours. The reaction was quenched at 0 °C by addition of saturated aqueous ammonium chloride (20 ml), and the layers were separated. The aqueous phase was cooled on ice and acidified to pH 4 using 10% aqueous citric acid, and extracted with ethyl acetate (3 × 20 ml). The organic layers were combined and dried (Na_2SO_4) and the solvent was removed *in vacuo* to yield an oil. The crude product was purified by chromatography on silica gel using (chloroform–methanol–water–acetic acid 7:3:0.6:0.3)–ethyl acetate (1:2) as eluent to yield N-p-*tolylsulfonyl*-2-(3-*trimethyl*-

silylprop-2-*ynyl*)*glycine* **18**, R = $SiMe_3$ as the first product. This was a pale yellow solid which was recrystallised from ether and light petroleum (1.0 g, 49%); mp 141–145 °C; $[\alpha]_D^{23}$ +12.5 (*c* 1.2, $CHCl_3$) (*m/z* found 339.097 45; $C_{15}H_{21}O_4NSSi$ requires 339.096 06); ν_{max}(KBr)/cm^{-1} 2185 (C≡C) and 1741 (CO_2H); δ_H(360 MHz, C^2HCl_3)† 0.0 [9H, s, $Si(CH_3)_3$], 2.4 (3H, s, $ArCH_3$), 2.7 (1H, dd, $J_{3A,2}$ 5.4, $J_{3A,3B}$ 17.1, *H*-3A), 2.8 (1H, dd, $J_{3B,2}$ 4.9, $J_{3B,3A}$ 17.1, *H*-3B), 4.1 (1H, m, *H*-2), 5.6 (1H, d, $J_{NH,2}$ 8.8, N*H*, exchangeable in ^2H_2O), 7.3 (2H, d, *J* 8.1, Ar-*H*) and 7.8 (2H, d, *J* 8.1, Ar-*H*); δ_C(125.8 MHz, C^2HCl_3) 0.0 [$Si(CH_3)_3$], 21.5 ($ArCH_3$), 24.8 (*C*-3), 54.2 (*C*-2), 87.0 [$C{\equiv}CSi(CH_3)_3$], 106.0 [$C{\equiv}CSi(CH_3)_3$], 127.2 and 129.7 (Ar-*C*H), 136.8 and 143.6 (2 × quaternary Ar-*C*) and 143.9 (CO_2H). (2S)-N-p-*Tolylsulfonyl*-2-(*prop*-2-*ynyl*)*glycine* **18**, R = H was the second product obtained from the above column as a yellow solid, which was recrystallised from ether and light petroleum (0.5 g, 30%); mp 95–100 °C; $[\alpha]_D^{23}$ +13.0 (c 1, $CHCl_3$) (*m/z* found 267.056 80; $C_{12}H_{13}O_4NS$ requires 267.056 53); *m/z* [EI] 267 ($[M]^+$); ν_{max}(KBr)/cm^{-1} 2124 (C≡CH) and 1729 (CO_2H); δ_H(360 MHz, C^2HCl_3) 2.1 (1H, t, $J_{5,3}$ 2.8, *H*-5), 2.4 (3H, s, $ArCH_3$), 2.6–2.7 (1H, ddd, $J_{3A,5}$ 2.8, $J_{3A,2}$ 5.2, $J_{3A,3B}$ 17.0, *H*-3A), 2.7–2.8 (1H, ddd, $J_{3B,5}$ 2.8, $J_{3B,2}$ 4.5, $J_{3B,3A}$ 17.0, *H*-3B), 4.2 (1H, m, *H*-2), 5.6 (1H, d, $J_{NH,2}$ 8.7, N*H*, exchangeable in ^2H_2O), 7.3 (2H, d, *J* 8.1, Ar-*H*) and 7.8 (2H, d, *J* 8.1, Ar-*H*); δ_C(125.8 MHz, C^2HCl_3) 21.5 ($ArCH_3$), 23.4 (*C*-3), 54.3 (*C*-2), 72.3 (C≡*C*H), 77.9 (*C*≡CH), 127.2 and 129.7 (Ar-*C*H), 136.7 and 143.9 (2 × quaternary Ar-*C*) and 174.8 (CO_2H).

(2*S*)-Methyl *N*-(2,2,5,7,8-pentamethylchroman-6-ylsulfonyl)-aziridine-2-carboxylate 20

(2*S*)-Methyl *N*-triphenylmethylaziridine-2-carboxylate **8** (0.5 g, 1.5 mmol) was dissolved in a mixture of chloroform (2 ml) and methanol (2 ml), and cooled to 0 °C in an ice bath. Trifluoroacetic acid (1.8 ml, 23.4 mmol) was added dropwise over a period of 10 min, and the mixture was allowed to stir at 0 °C under argon for 4–6 hours. The solvents were removed *in vacuo* at 0 °C, and the resultant residue was azeotroped with diethyl ether (3 × 30 ml) and partitioned between diethyl ether (30 ml) and water (30 ml). The ether layer was extracted with water (3 × 30 ml), and the combined aqueous fractions were neutralised with solid sodium hydrogen carbonate (0.5 g, 6.0 mmol). Ethyl acetate (100 ml) was added to the aqueous portion and the mixture was cooled to 0 °C. 2,2,5,7,8-Pentamethylchromane-6-sulfonyl chloride (700 mg, 2.3 mmol) was added, and the mixture was stirred at room temperature overnight under argon. The aqueous layer was extracted with ethyl acetate (3 × 30 ml). The combined organic layers were dried (Na_2SO_4) and the solvent was removed *in vacuo* to yield a colourless oil. The crude product was purified by chromatography on silica gel using light petroleum–ethyl acetate (4 : 1) as eluent to yield (2S)-*methyl* N-(2,2,5,7,8-*pentamethylchroman*-6-*ylsulfonyl*)*aziridine*-2-*carboxylate* **20** as a colourless oil (118 mg, 22%); $[\alpha]_D^{29}$ −39.6 (*c* 0.4, $CHCl_3$) (Found C, 58.5; H, 6.8; N, 3.7%. $C_{18}H_{25}O_5NS$ requires C, 58.8; H, 6.8; N, 3.9%); *m/z* [+ve FAB (3-nba)] 368 ($[M + H]^+$); ν_{max}(film)/cm^{-1} 1752 (ester); δ_H(360 MHz, C^2HCl_3) 1.3 (6H, s, 2 × CH_3), 1.9 (2H, t, *J* 6.8, CH_2), 2.1 (3H, s, CH_3), 2.5 (1H, d, $J_{3S,2}$ 4.1, *H*-3*S*), 2.6 [2 × 3H, 2 × s, C-$(CH_3)_2$], 2.7 (2H, t, *J* 6.8, CH_2), 2.8 (1H, d, $J_{3R,2}$ 7.1, *H*-3*R*), 3.3 (1H, dd, $J_{2,3S}$ 4.1, $J_{2,3R}$ 7.1, *H*-2) and 3.7 (3H, s, OCH_3); δ_C(125.8 MHz, C^2HCl_3) 12.2 (CH_3), 17.6 and 18.6 (2 × CH_3), 21.4 (*C*-3′), 26.7 [C-$(CH_3)_2$], 31.8 (*C*-3), 32.6 (*C*-4′), 34.8 (*C*-2), 52.7 (OCH_3), 74.3 [quaternary *C*-$(CH_3)_2$], 118.6, 124.8, 126.6, 137.7, 137.8 and 155.6 (quaternary Ar-*C*) and 167.8 (CO_2CH_3).

(2*R*)-Methyl *N*-(2,2,5,7,8-pentamethylchroman-6-ylsulfonyl)-aziridine-2-carboxylate 21

This was prepared as described above using (2*R*)-methyl *N*-triphenylmethylaziridine-2-carboxylate **6**, R = Ph_3C (3.3 g, 9.7 mmol) and was obtained as a colourless oil (1.1 g, 30%); $[\alpha]_D^{29}$ +42.6 (*c* 0.4, $CHCl_3$) (Found C, 58.2; H, 6.8; N, 3.6%. $C_{18}H_{25}O_5NS$ requires C, 58.8; H, 6.8; N, 3.9%); *m/z* [+ve FAB (3-nba)] 368 ($[M + H]^+$); ν_{max}(film)/cm^{-1} 1752 (ester); δ_H(360 MHz, C^2HCl_3) 1.4 (6H, s, 2 × CH_3), 1.8 (2H, t, *J* 6.8, CH_2), 2.1 (3H, s, CH_3), 2.5 (1H, d, $J_{3R,2}$ 4.0, *H*-3*R*), 2.6 [2 × 3H, 2 × s C-$(CH_3)_2$], 2.7 (2H, t, *J* 6.8, CH_2), 2.8 (1H, d, $J_{3S,2}$ 7.1, *H*-3*S*), 3.4 (1H, dd, $J_{2,3R}$ 4.0, $J_{2,3S}$ 7.1, *H*-2) and 3.8 (3H, s, OCH_3); δ_C(125.8 MHz, C^2HCl_3) 12.2 (CH_3), 17.6 and 18.6 (2 × CH_3), 21.4 (*C*-3′), 26.7 [C-$(CH_3)_2$], 31.8 (*C*-3), 32.6 (*C*-4′), 34.8 (*C*-2), 52.7 (OCH_3), 74.3 [*C*-$(CH_3)_2$], 118.6, 124.8, 126.7, 137.7, 137.8 and 155.6 (quaternary Ar-*C*) and 167.8 (CO_2CH_3).

(2*R*,3*R*)-Methyl *N*-(2,2,5,7,8-pentamethylchroman-6-ylsulfonyl)-[3-$^2H_1$]aziridine-2-carboxylate 21, H_B = ^2H

This was prepared as described above using (2*R*,3*R*)-methyl *N*-triphenylmethyl[3-$^2H_1$]aziridine-2-carboxylate **6**, R = Ph_3C, H_B = ^2H (ref. 4) (3.1 g, 9.0 mmol) and was a colourless oil (0.7 g, 20%); $[\alpha]_D^{24}$ +44.9 (*c* 0.7, $CHCl_3$); *m/z* [+ve FAB (3-nba)] 369 ($[M + H]^+$); ν_{max}(film)/cm^{-1} 1752 (ester); δ_H(360 MHz, C^2HCl_3) 1.4 (6H, s, 2 × CH_3), 1.8 (2H, t, *J* 6.8, CH_2), 2.1 (3H, s, CH_3), 2.6 [2 × 3H, 2 × s, C-$(CH_3)_2$], 2.7 (2H, t, *J* 6.8, CH_2), 2.8 (1H, d, $J_{3S,2}$ 7.1, *H*-3*S*), 3.4 (1H, d, $J_{2,3S}$ 7.1, *H*-2) and 3.7 (3H, s, OCH_3); δ_C(125.8 MHz, C^2HCl_3) 12.2 (CH_3), 17.6 and 18.6 (2 × CH_3), 21.4 (*C*-3′), 26.7 [C-$(CH_3)_2$], 31.5 (t, *C*-3), 32.5 (*C*-4′), 34.7 (*C*-2), 52.7 (OCH_3), 74.2 [*C*-$(CH_3)_2$], 118.6, 124.8, 126.6, 137.7, 137.8 and 155.6 (quaternary Ar-*C*) and 167.8 (CO_2CH_3).

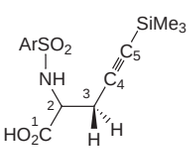
(2*R*,3*S*)-Methyl *N*-(2,2,5,7,8-pentamethylchroman-6-ylsulfonyl)-[2,3-$^2H_2$]aziridine-2-carboxylate 21, H_A = ^2H

This was prepared as above using (2*R*,3*S*)-methyl *N*-triphenylmethyl[2,3-$^2H_2$]aziridine-2-carboxylate **6**, R = Ph_3C, H_A = ^2H (ref. 4) (2.2 g, 6.4 mmol) and was a colourless oil (0.7 g, 30%); $[\alpha]_D^{26}$ +34.1 (*c* 0.7, $CHCl_3$); *m/z* [+ve FAB (3-nba)] 370 ($[M + H]^+$); ν_{max}(film)/cm^{-1} 1759 (ester); δ_H(360 MHz, C^2HCl_3) 1.4 (6H, s, 2 × CH_3), 1.9 (2H, t, *J* 6.8, CH_2), 2.1 (3H, s, CH_3), 2.5 (1H, s, *H*-3*R*), 2.6 [2 × 3H, 2 × s, C-$(CH_3)_2$], 2.7 (2H, t, *J* 6.8, CH_2) and 3.7 (3H, s, OCH_3); δ_C(125.8 MHz, C^2HCl_3) 12.2 (CH_3), 17.6 and 18.6 (2 × CH_3), 21.4 (*C*-3′), 26.7 [C-$(CH_3)_2$], 31.5 (t, *C*-3), 32.5 (*C*-4′), 34.5 (t, *C*-2), 52.7 (OCH_3), 74.2 [*C*-$(CH_3)_2$], 118.5, 124.8, 126.6, 137.7, 137.8 and 155.6 (quaternary Ar-*C*) and 169.0 (CO_2CH_3).

(2*S*)-*N*-(2,2,5,7,8-Pentamethylchroman-6-ylsulfonylaziridine-2-carboxylic acid *ent*-22

(2*S*)-Methyl *N*-(2,2,5,7,8-pentamethylchroman-6-ylsulfonyl)-aziridine-2-carboxylate **20** (42 mg, 0.1 mmol) was dissolved in THF (2 ml), and cooled to 0 °C in an ice bath. 1 M Aqueous NaOH (0.18 ml, 0.18 mmol) was added, and the reaction was stirred at room temperature for 3 hours. The solvent was removed *in vacuo*, and the resultant gum was partitioned between ethyl acetate (10 ml) and saturated aqueous sodium hydrogen carbonate (10 ml). The aqueous fraction was cooled on ice and acidified to pH 4 with 10% aqueous citric acid. The acidified aqueous layer was extracted with ethyl acetate (3 × 20 ml), and the combined organic fractions were dried (Na_2SO_4). The solvent was removed *in vacuo* to yield (2S)-N-(2,2,5,7,8-*pentamethylchroman*-6-*ylsulfonyl*)*aziridine*-2-*carboxylic acid ent*-**22** as a white solid which was recrystallised from light petroleum (39 mg, 97%); mp 122–126 °C (Found C, 57.8; H, 6.7; N, 3.9. $C_{17}H_{23}O_5NS$ requires C, 57.8; H, 6.5; N, 4.0%); *m/z*

† The numbering Scheme used in the NMR assignments for compounds **18** and **23** is as shown below.



[+ve FAB (3-nba)] 354 ([M + H]$^+$); ν_{max}(KBr)/cm^{-1} 1730 (CO_2H); δ_H(360 MHz, C^2HCl_3) 1.3 (6H, s, 2 × CH_3), 1.9 (2H, t, *J* 6.8, CH_2), 2.1 (3H, s, CH_3), 2.5 (1H, d, $J_{3S,2}$ 3.9, *H*-3*S*), 2.6 [2 × 3H, 2 × s, C-(CH_3)$_2$], 2.7 (2H, t, *J* 6.8, CH_2), 2.8 (1H, d, $J_{3R,2}$ 7.1, *H*-3*R*), 3.3 (1H, dd, $J_{2,3S}$ 3.9, $J_{2,3R}$ 7.1, *H*-2) and 7.1 (1H, s, CO_2H, exchangeable in ^2H_2O); δ_C(125.8 MHz, C^2HCl_3) 12.2 (CH_3), 17.6 and 18.6 (2 × CH_3), 21.4 (*C*-3′), 26.7 [C-(CH_3)$_2$], 32.0 (*C*-3), 32.5 (*C*-4′), 34.7 (*C*-2), 74.3 [*C*-(CH_3)$_2$], 118.7, 125.0, 126.3, 137.8, 137.8 and 155.7 (quaternary Ar-*C*) and 171.9 (CO_2H).

(2*R*)-*N*-(2,2,5,7,8-Pentamethylchroman-6-ylsulfonyl)aziridine-2-carboxylate 22

This was prepared as described above using (2*R*)-methyl *N*-(2,2,5,7,8-pentamethylchroman-6-ylsulfonyl)aziridine-2-carboxylate **21** (0.8 g, 2.2 mmol) and was a solid (0.77 g, 100%); mp 121–124 °C; $[\alpha]_D^{22}$ +38.3 (*c* 0.4, $CHCl_3$) (Found C, 57.8; H, 6.7; N, 3.9%. $C_{17}H_{23}O_5NS$ requires C, 57.8; H, 6.5; N, 4.0%); *m*/*z* [+ve FAB (3-nba)] 354 ([M + H]$^+$); ν_{max}(KBr)/cm^{-1} 1714 (CO_2H); δ_H(360 MHz, C^2HCl_3) 1.3 (6H, s, 2 × CH_3), 1.9 (2H, t, *J* 6.8, CH_2), 2.1 (3H, s, CH_3), 2.5 (1H, d, J_{3R2} 3.9, *H*-3*R*), 2.6 [2 × 3H, 2 × s, C-(CH_3)$_2$], 2.7 (2H, t, *J* 6.8, CH_2), 2.9 (1H, d, $J_{3S,2}$ 7.1, *H*-3*S*) and 3.4 (1H, dd, $J_{2,3R}$ 3.9, $J_{2,3S}$ 7.1, *H*-2); δ_C(125.8 MHz, C^2HCl_3) 12.2 (CH_3), 17.6 and 18.6 (2 × CH_3), 21.4 (*C*-3′), 26.7 [C-(CH_3)$_2$], 32.0 (*C*-3), 32.5 (*C*-4′), 34.7 (*C*-2), 74.3 [*C*-(CH_3)$_2$], 118.7, 125.0, 126.3, 137.8, 137.9 and 155.8 (quaternary Ar-*C*) and 171.9 (CO_2H).

(2*R*,3*R*)-*N*-(2,2,5,7,8-Pentamethylchroman-6-ylsulfonyl[3-$^2H_1$]-aziridine-2-carboxylate 22 H_B = ^2H

This was prepared as described above using (2*R*,3*R*)-methyl *N*-(2,2,5,7,8-pentamethylchroman-6-ylsulfonyl)[3-$^2H_1$]aziridine-2-carboxylate **21**, H_B = ^2H (0.5 g, 1.4 mmol) and was a solid (0.5 g, 100%); mp 124–127 °C; $[\alpha]_D^{25}$ +29.3 (*c* 0.3, $CHCl_3$); *m*/*z* [+ve FAB (3-nba)] 355 ([M + H]$^+$); ν_{max}(KBr)/cm^{-1} 1733 (CO_2H); δ_H(360 MHz, C^2HCl_3) 1.3 (6H, s, 2 × CH_3), 1.9 (2H, t, *J* 6.8, CH_2), 2.1 (3H, s, CH_3); 2.6 [2 × 3H, 2 × s, C-(CH_3)$_2$], 2.7 (2H, t, *J* 6.8, CH_2), 2.9 (1H, d, $J_{3S,2}$ 7.1, *H*-3*S*) and 3.4 (1H, d, $J_{2,3S}$ 7.1, *H*-2); δ_C(125.8 MHz, C^2HCl_3) 12.2 (CH_3), 17.6 and 18.6 (2 × CH_3), 21.4 (*C*-3′), 26.7 [C-(CH_3)$_2$], 31.5 (t, *C*-3), 32.5 (*C*-4′), 34.7 (*C*-2), 74.2 [*C*-(CH_3)$_2$], 118.6, 124.8, 126.7, 137.7, 137.8 and 155.6 (quaternary Ar-*C*) and 167.8 (CO_2H).

(2*R*,3*S*)-*N*-(2,2,5,7,8-Pentamethylchroman-6-ylsulfonyl)[2,3-$^2H_2$]aziridine-2-carboxylate 22 H_A = ^2H

This was prepared as described above using (2*R*,3*S*)-methyl *N*-(2,2,5,7,8-pentamethylchroman-6-ylsulfonyl)[2,3-$^2H_2$]aziridine-2-carboxylate **21**, H_A = ^2H (0.3 g, 0.8 mmol), and was a solid (0.3 g, 100%); mp 116–120 °C; $[\alpha]_D^{27}$ +33.1 (*c* 0.4, $CHCl_3$); *m*/*z* [+ve FAB (3-nba)] 356 ([M + H]$^+$); ν_{max}(KBr)/cm^{-1} 1726 (CO_2H); δ_H(360 MHz, C^2HCl_3) 1.3 (6H, s, 2 × CH_3), 1.9 (2H, t, *J* 6.7, CH_2), 2.1 (3H, s, CH_3), 2.5 (1H, s, *H*-3*R*), 2.6 [2 × 3H, 2 × s, C-(CH_3)$_2$] and 2.7 (2H, t, *J* 6.7, CH_2); δ_C(125.8 MHz, C^2HCl_3) 12.2 (CH_3), 17.6 and 18.6 (2 × CH_3), 21.4 (*C*-3′), 26.7 [C-(CH_3)$_2$], 31.7 (t, *C*-3), 32.5 (*C*-4′), 34.3 (t, *C*-2), 74.3 [*C*-(CH_3)$_2$], 118.7, 125.0, 126.3, 137.8, 137.9 and 155.7 (quaternary Ar-*C*) and 172.5 (CO_2H).

(2*S*)-*N*-(2,2,5,7,8-Pentamethylchroman-6-ylsulfonyl)-2-(3-trimethylsilylprop-2-ynyl)glycine *ent*-23

n-Butyllithium (1.6 M in hexane, 0.6 ml, 0.9 mmol) was added to a cold (0 °C) solution of trimethylsilylacetylene (0.2 ml, 1.4 mmol) in THF (2 ml) and the mixture was stirred under argon for 30 min. (2*S*)-*N*-(2,2,5,7,8-Pentamethylchroman-6-ylsulfonyl)aziridine-2-carboxylate *ent*-**22** (39 mg, 0.1 mmol) in THF (10 ml) was added, and the mixture was stirred at 0 °C for 2.5 hours under argon, and then allowed to warm slowly to room temperature over 1 hour. The reaction was quenched by addition of saturated aqueous ammonium chloride (20 ml), and the aqueous phase was cooled on ice, acidified to pH 4 with 10% aqueous citric acid and extracted with ethyl acetate (3 × 15 ml). The combined organic phases were dried (Na_2SO_4), and the solvent was removed *in vacuo* to yield an amber oil. The crude product was purified by chromatography on silica gel using (chloroform–methanol–water–acetic acid 7:3:0.6:0.3)–ethyl acetate (1:2) as eluent to yield (2S)-N-(2,2,5,7,8-*pentamethylchroman*-6-*ylsulfonyl*)-2-(3-*trimethylsilylprop*-2-*ynyl*)*glycine ent*-**23** as a pale yellow solid which was recrystallised from ethyl acetate and light petroleum (15 mg, 33%); mp 140–145 °C; $[\alpha]_D^{34}$ +30.8 (*c* 0.2, $CHCl_3$); *m*/*z* [+ve FAB (3-nba)] 474 ([M + Na]$^+$) 452 ([M + H]$^+$) (*m*/*z* found 451.18446; $C_{22}H_{33}O_5NSSi$ requires 451.18488); ν_{max}(KBr)/cm^{-1} 2180 (C≡C) and 1720 (CO_2H); δ_H(360 MHz, C^2HCl_3) 0.1 [9H, s, (CH_3)$_3$Si], 1.3 (6H, s, 2 × CH_3), 1.9 (2H, t, *J* 6.8, CH_2), 2.1 (3H, s, CH_3), 2.5 (1H, obscured m, *H*-3*S*), 2.6 [2 × 3H, 2 × s, C-(CH_3)$_2$], 2.7 (2H, t, *J* 6.8, CH_2), 2.7–2.8 (1H, dd, $J_{3R,2}$ 4.0, $J_{3R,3S}$ 17.1, *H*-3*R*), 4.1 (1H, m, *H*-2) and 5.4 (1H, d, $J_{NH,2}$ 8.5, N*H*).

(2*R*)-*N*-(2,2,5,7,8-Pentamethylchroman-6-ylsulfonyl)-2-(3-trimethylsilylprop-2-ynyl)glycine 23

This was prepared as described above using (2*R*)-*N*-(2,2,5,7,8-pentamethylchroman-6-ylsulfonyl)aziridine-2-carboxylate **22** (0.7 g, 1.8 mmol), and was a solid (0.3 g, 36%); mp 112–115 °C; $[\alpha]_D^{23}$ −29.2 (*c* 1.5, $CHCl_3$) (Found C, 58.2; H, 7.5; N, 3.1%. $C_{22}H_{33}O_5NSSi$ requires C, 58.5; H, 7.3; N, 3.1%); *m*/*z* [+ve FAB (glycerol)] 452 ([M + H]$^+$); ν_{max}(KBr)/cm^{-1} 2182 (C≡C), 1718 (CO_2H); δ_H(360 MHz, $C^2H_3O^2H$) 0.0 [9H, s, Si(CH_3)$_3$], 1.3 (6H, s, 2 × CH_3), 1.8 (2H, t, *J* 6.8, CH_2), 2.1 (3H, s, CH_3), 2.5–2.6 (1H, obscured m, *H*-3*R*), 2.6 [2 × 3H, 2 × s, C-(CH_3)$_2$], 2.6–2.7 (1H, dd, $J_{3S,2}$ 6.0, $J_{3S,3R}$ 17.0, *H*-3*S*), 2.7 (2H, t, *J* 6.7, CH_2) and 3.8 (1H, dd, $J_{2,3R}$ 6.2, $J_{2,3S}$ 6.0, *H*-2); δ_C(125.8 MHz, $C^2H_3O^2H$) 0.2 [Si(CH_3)$_3$], 12.2 (CH_3), 17.3 and 18.3 (2 × CH_3), 21.4 (*C*-3′), 24.6 (*C*-3), 26.6 [C-(CH_3)$_2$], 32.6 (*C*-4′), 53.9 (*C*-2), 74.1 [*C*-(CH_3)$_2$], 89.0 (*C*≡CSiMe$_3$), 100.1 (C≡*C*SiMe$_3$), 118.4, 124.7, 128.5, 136.5, 136.7 and 154.8 (quaternary Ar-*C*) and 174.9 (CO_2H).

(2*R*)-*N*-(2,2,5,7,8-Pentamethylchroman-6-ylsulfonyl)-2-{(1*R*)-[1-$^2H_1$]-3-trimethylsilylprop-2-ynyl}glycine 23, H_B = ^2H

This was prepared as described above using (2*R*,3*R*)-*N*-2,2,5,7,8-pentamethylchroman-6-ylsulfonyl)[3-$^2H_1$]aziridine-2-carboxylate **22**, H_B = ^2H (0.5 g, 1.4 mmol) and was a solid (0.2 g, 30%); mp 99–102 °C; $[\alpha]_D^{25}$ −28.0 (*c* 0.7, $CHCl_3$); *m*/*z* [+ve FAB (3-nba)] 475 ([M + Na]$^+$) and 453 ([M + H]$^+$); ν_{max}(KBr)/cm^{-1} 2176 (C≡C) and 1721 (CO_2H); δ_H(360 MHz, $C^2H_3O^2H$) 0.1 [9H, s, Si(CH_3)$_3$], 1.4 (6H, s, 2 × CH_3), 1.9 (2H, t, *J* 6.8, CH_2), 2.2 (3H, s, CH_3), 2.5 (1H, obscured, *H*-3*S*), 2.6 [2 × 3H, 2 × s, C-(CH_3)$_2$], 2.7 (2H, t, *J* 6.8, CH_2) and 3.8 (1H, d, $J_{2,3S}$ 5.9, *H*-2); δ_C(125.8 MHz, $C^2H_3O^2H$) 0.2 [Si(CH_3)$_3$], 12.2 (CH_3), 17.3 and 18.3 (2 × CH_3), 21.4 (*C*-3′), 24.4 (t, *C*-3), 26.6 [C-(CH_3)$_2$], 32.6 (*C*-4′), 53.7 (*C*-2), 74.1 [*C*-(CH_3)$_2$], 87.1 (*C*≡CSiMe$_3$), 105.8 (C≡*C*SiMe$_3$), 118.4, 124.7, 128.5, 136.5, 136.7 and 154.5 (quaternary Ar-*C*) and 174.9 (CO_2H).

(2*R*)-*N*-(2,2,5,7,8-Pentamethylchroman-6-ylsulfonyl)-2-{(1*S*)-[1-$^2H_1$]-3-trimethylsilylprop-2-ynyl}[2-$^2H_1$]glycine 23, H_A = ^2H

This was prepared as outlined above from (2*R*,3*S*)-*N*-(2,2,5,7,8-pentamethylchroman-6-ylsulfonyl)[2,3-$^2H_2$]aziridine-2-carboxylate **22**, H_A = ^2H (0.3 g, 0.8 mmol) and was a solid (0.1 g, 28%); mp 99–102 °C; $[\alpha]_D^{28}$ −34.3 (*c* 0.4, $CHCl_3$); *m*/*z* [+ve FAB (3-nba)] 454 ([M + H]$^+$); ν_{max}(KBr)/cm^{-1} 2178 (C≡C) and 1718 (CO_2); δ_H(360 MHz, C^2HCl_3) 0.1 [9H, s, Si(CH_3)$_3$], 1.4 (6H, s, 2 × CH_3), 1.8 (2H, t, *J* 6.8, CH_2), 2.2 (3H, s, CH_3), 2.6 (1H, s, *H*-3*R*), 2.6 [2 × 3H, 2 × s, C-(CH_3)$_2$] and 2.7 (2H, t, *J* 6.7, CH_2); δ_C(125.8 MHz, C^2HCl_3) 0.1 [Si(CH_3)$_3$], 12.2 (CH_3), 17.3 and 18.3 (2 × CH_3), 21.5 (*C*-3′), 24.3 (t, *C*-3), 26.6 [C-(CH_3)$_2$], 32.6 (*C*-4′), 53.3 (t, *C*-2), 74.1 [*C*-(CH_3)$_2$], 87.1 [*C*≡CSi(CH_3)$_3$], 105.8 [C≡*C*Si(CH_3)$_3$], 118.5, 124.8, 128.5, 136.5, 136.7 and 154.9 (quaternary Ar-*C*) and 174.0 (CO_2H).

(2*S*)-2-Propynylglycine *ent*-1

(2*S*)-*N*-(2,2,5,7,8-Pentamethylchroman-6-ylsulfonyl)-2-(3-trimethylsilylprop-2-ynyl)glycine *ent*-**23** (15 mg, 0.03 mmol) was dissolved in TFA (1 ml) and heated to reflux under argon for 18 hours. The solvent was removed *in vacuo*, and the remaining TFA was azeotroped with diethyl ether (3 × 20 ml). The resultant brown oil was partitioned between ethyl acetate (5 ml) and water (5 ml), and the organic layer was extracted with water (3 × 5 ml). The solvent was removed *in vacuo* from the combined aqueous fractions to yield (2*S*)-2-propynylglycine *ent*-**1** as an off-white solid containing a small amount of the methyl ketone **19** (4 mg, 100%); mp 215 °C (decomp.); $[\alpha]_D^{22}$ −28.7 (*c* 0.9, H_2O); δ_H(360 MHz, $C^2H_3O^2H$), 2.3 (1H, t, $J_{5,3R}$ 2.6, *H*-5), 2.5 (1H, ddd, $J_{3R,5}$ 2.6, $J_{3R,2}$ 7.4, $J_{3R,3S}$ 16.8, *H*-3*R*), 2.6 (1H, ddd, $J_{3S,5}$ 2.6, $J_{3S,2}$ 4.5, $J_{3S,3R}$ 16.7, *H*-3*S*) and 3.6 (2H, m, *H*-2). A second set of peaks in the spectrum was present for the methyl ketone **19**, δ_H(360 MHz, $C^2H_3O^2H$) 2.2 (3H, exch. s, $COCH_3$), 2.7 (1H, dd, $J_{3A,2}$ 8.4, $J_{3A,3B}$ 17.4, *H*-3A), 2.9 (1H, dd, $J_{3B,2}$ 4.0, $J_{3A,3B}$ 17.4, *H*-3B), *H*-2 obscured.

(2*R*)-2-Propynylglycine 1

This was prepared as described above using (2*R*)-*N*-(2,2,5,7,8-pentamethylchroman-6-ylsulfonyl)-2-(3-trimethylsilylprop-2-ynyl)glycine **23** (20 mg, 0.04 mmol) and was a solid (5 mg, 100%); mp 221 °C (decomp.); $[\alpha]_D^{25}$ +25.7 (*c* 0.8, H_2O); (*m*/*z* on $[M - CO_2H]^+$ found 68.051 04; C_4H_6N requires 68.050 02); *m*/*z* [electrospray] 114 ($[M + H]^+$); ν_{max}(KBr)/cm^{-1} 1686 (CO_2H); δ_H(360 MHz, $C^2H_3O^2H$) 2.6 (1H, t, $J_{5,3S}$ $J_{5,3R}$ 2.6, *H*-5), 2.8 (1H, ddd, $J_{3S,5}$ 2.6, $J_{3S,2}$ 7.4, $J_{3S,3R}$ 16.8, *H*-3*S*), 2.9 (1H, ddd, $J_{3R,5}$ 2.6, $J_{3R,2}$ 4.6, $J_{3R,3S}$ 16.8, *H*-3*R*) and 4.1 (1H, dd, $J_{2,3S}$ 4.6, $J_{2,3R}$ 7.4, *H*-2). A second set of peaks in the spectrum was present for the methyl ketone **19** δ_H(360 MHz, $C^2H_3O^2H$) 2.2 (3H, exch. s, $COCH_3$), 3.0 (1H, dd, $J_{3A,2}$ 8.4, $J_{3B,3A}$ 17.5, *H*-3A), 3.3 (1H, dd, $J_{3B,2}$ 4.0, $J_{3B,3A}$ 17.5, *H*-3B), *H*-2 obscured.

(2*R*)-2-{(1*R*)-[1-$^2H_1$]Propynyl}glycine 1, $H_B = {}^2H$

This was prepared as described above using (2*R*)-*N*-(2,2,5,7,8-pentamethylchroman-6-ylsulfonyl)-2-{(1*R*)-[1-$^2H_1$]-3-trimethylsilylprop-2-ynyl}glycine **23**, $H_B = {}^2H$ (135 mg, 0.3 mmol), and was a solid (35 mg, 100%); mp 225 °C (decomp.); $[\alpha]_D^{28}$ +22.6 (*c* 0.6, H_2O); *m*/*z* [+ve FAB (3-nba)] 69 ($[M - CO_2H]^+$); ν_{max}(KBr)/cm^{-1} 1700 (CO_2H); δ_H(360 MHz, $C^2H_3O^2H$) 2.6 (1H, d, $J_{5,3S}$ 2.5, *H*-5), 2.9 (1H, br, *H*-3*S*) and 4.1 (1H, d, $J_{2,3S}$ 5.7, *H*-2); δ_C(125.8 MHz, $C^2H_3O^2H$) 21.7 (t, *C*-3), 53.4 (*C*-2), 75.2 (*C*≡CH), 78.0 (C≡*C*H) and 171.2 (CO_2H). The presence of ketone **19** was again noted.

(2*R*)-2-{(1*S*)-[1-$^2H_1$]Propynyl}[2-$^2H_1$]glycine 1, $H_A = {}^2H$

This was prepared as described above using (2*R*)-*N*-(2,2,5,7,8-pentamethylchroman-6-ylsulfonyl)-2-{(1*S*)-[1-$^2H_1$]-3-trimethylsilylprop-2-ynyl}glycine **23**, $H_A = {}^2H$ (80 mg, 0.2 mmol) and was a solid (25 mg, 100%); mp 217 °C (decomp.); $[\alpha]_D^{28}$ +22.6 (*c* 0.7, H_2O); *m*/*z* [+ve FAB (3-nba)] 116 ($[M + H]^+$); ν_{max}(KBr)/cm^{-1} 1700 (CO_2H); δ_H(360 MHz, $C^2H_3O^2H$) 2.6 (1H, d, $J_{5,3R}$ 2.5, *H*-5) and 2.8 (1H, br, *H*-3*R*); δ_C(125.8 MHz, $C^2H_3O^2H$) 21.4 (t, *C*-3), 53.0 (t, *C*-2), 75.3 (*C*≡CH), 77.8 (C≡*C*H) and 171.0 (CO_2H). The spectrum again indicated contamination by ketone **19**.

Incubation of (2*R*)-2-propynylglycine 1 with D-amino acid oxidase

(2*R*)-2-Propynylglycine **1** (80 mg, 0.71 mmol) was dissolved in 0.02 M HEPES buffer (12 ml) at pH 8.03 and 25 °C. D-Amino acid oxidase (EC 1.4.3.3) (16 mg, 20% w/w) was added followed by catalyse (EC 1.11.1.6) (1.6 mg, 2% w/w) and the mixture was incubated at room temperature (25 °C) for 3 hours and filtered through Celite. The aqueous mixture was extracted with C^2HCl_3 (3 × 10 ml). The combined organic layers were dried (Na_2SO_4) and the solvent was concentrated to *ca*. 0.5 ml under a stream of argon. The ^1H NMR spectrum of the residue was run immediately, δ_H(360 MHz, C^2HCl_3) 4.2 (2H, br s, NH_2), 4.5 (1H, d, *J* 2.4, *H*-5), 4.8 (1H, d, *J* 2.4, *H*-5) and 6.0 (1H, s, *H*-3); λ_{max}(MeOH)/nm 316 (ε 2183).

Incubation of (2*R*)-2-{(1*R*)-[1-$^2H_1$]propynylglycine 1, $H_B = {}^2H$ with D-amino acid oxidase

(2*R*)-2-{(1*R*)-[1-$^2H_1$]Propynylglycine **1**, $H_B = {}^2H$ (80 mg, 0.7 mmol) was dissolved in 0.02 M HEPES buffer (12 ml) at pH 8.02 at 25 °C. D-Amino acid oxidase (16.6 mg, 20% w/w) was added followed by catalase (1.7 mg, 2% w/w) and the mixture was incubated at room temperature (25 °C) for 3 hours and filtered through Celite. The aqueous mixture was extracted with C^2HCl_3 (3 × 10 ml). The combined organic layers were dried (Na_2SO_4) and the solvent was concentrated to *ca*. 0.5 ml under a stream of argon. The ^1H NMR spectrum was run immediately, δ_H(360 MHz, C^2HCl_3) 4.5 (1H, d, *J* 2.4, *H*-5), 4.9 (1H, d, *J* 2.4, *H*-5) and 6.0 (1H, s, integration indicates 17% protium at *H*-3).

Incubation of (2*R*)-2-{(1*S*)-[1-$^2H_1$]propynyl}[2-$^2H_1$]glycine 1, $H_A = {}^2H$ with D-amino acid oxidase

(2*R*)-2-{(1*S*)-[1-$^2H_1$]Propynyl}[2-$^2H_1$]glycine **1**, $H_A = {}^2H$ (80 mg, 0.7 mmol) was dissolved in 0.02 M HEPES buffer (10 ml) at pH 8.02 at 25 °C. D-Amino acid oxidase (16 mg, 20% w/w) was added followed by catalase (1.7 mg, 2% w/w) and the mixture was incubated at 25 °C for 3 hours and filtered through Celite. The aqueous mixture was extracted with C^2HCl_3 (3 × 10 ml). The combined organic layers were dried (Na_2SO_4) and the solvent was concentrated to *ca*. 0.5 ml under a stream of argon. The ^1H NMR spectrum was run immediately, δ_H(360 MHz, C^2HCl_3) 4.6 (1H, d, *J* 2.4, *H*-5), 4.8 (1H, d, *J* 2.4, *H*-5) and 6.0 (1H, s, integration indicates 20% protium at *H*-3).

Incubation of (2*R*)-2-propynylglycine 1 with D-amino acid oxidase in deuteriated buffer

(2*R*)-2-Propynylglycine **1** (65 mg, 0.6 mmol) was dissolved in 0.02 M HEPES buffer (10 ml) which had been prepared by dissolving 4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid (0.95 mg) in ^2H_2O (20 ml) with adjustment of the pH to 8.03 with 20% NaO^2H in ^2H_2O. D-Amino acid oxidase (13 mg, 20% w/w) was added followed by catalase (1.3 mg, 2% w/w) and the mixture was incubated at room temperature (25 °C) for 3 hours and filtered through Celite. The aqueous mixture was extracted with C^2HCl_3 (3 × 10 ml). The combined organic layers were dried (Na_2SO_4) and the solvent was concentrated to *ca*. 0.5 ml under argon. The ^1H NMR spectrum was run immediately, δ_H(360 MHz, C^2HCl_3) 4.5 (1H, s, integration indicated 71% deuteriation at *H*-5), 4.9 (1H, s, integration indicated 92% deuteriation at *H*-5) and 6.0 (1H, s, *H*-3).

Incubation of (2*R*)-2-propynylglycine 1 with D-amino acid oxidase after exchange of the acetylenic proton with deuterium

(2*R*)-2-Propynylglycine **1** (10 mg, 0.09 mmol) was left standing in $C^2H_3O^2H$ for 7 days. The ^1H NMR spectrum indicated the disappearance of the acetylenic proton at δ 2.6, and the solvent was removed *in vacuo*. The resultant solid was dissolved in 0.02 M HEPES buffer (6 ml) at pH 8.03 and 25 °C. D-Amino acid oxidase (2 mg, 20% w/w) was added, followed by catalase (0.2 mg, 2% w/w), and the mixture was incubated at room temperature (25 °C) for 3 hours and filtered through Celite. The aqueous mixture was extracted with C^2HCl_3 (3 × 5 ml). The combined organic layers were dried (Na_2SO_4) and the solvent was concentrated to *ca*. 0.5 ml under a stream of argon. The ^1H NMR spectrum was run immediately, δ_H(360 MHz, C^2HCl_3) 4.6 (1H, d, *J* 2.5, *H*-5), 4.9 (1H, d, *J* 2.5, *H*-5) and 6.0 (1H, s, *H*-3). Integration showed 100% protonation at C-5.

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