Synthesis of Stereospecifically Labelled D-Prop-2-ynylglycine and Investigation of the Action of D-Amino Acid Oxidase

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Stereospecifically deuteriated samples of D-prop-2-ynylglycine **1** are synthesised by reaction of the labelled aziridines **13** with a carbon nucleophile followed by deprotection; incubation of these samples with D-amino acid oxidase indicates that, in formation of the lactone **5**, deprotonation at C-3 is non-stereospecific, strongly supporting non-enzymatic deprotonation as a key step in the formation of this compound.

D-Prop-2-ynylglycine **1** and the corresponding L-isomer are substrates for D-amino acid oxidase (EC 1.4.3.3) and L-amino acid oxidase (EC 1.4.3.2) respectively.[1] D-Amino acid oxidase is inhibited by D-propynylglycine after a large number of catalytic oxidations whereas L-propynylglycine acts only as a substrate.[1] The species which accumulates on incubation of the appropriate propynylglycine with the appropriate enzyme has been shown[2] to be the γ-lactone **5** and the mechanism shown in (Scheme 1) has been suggested to account for its formation.[2]

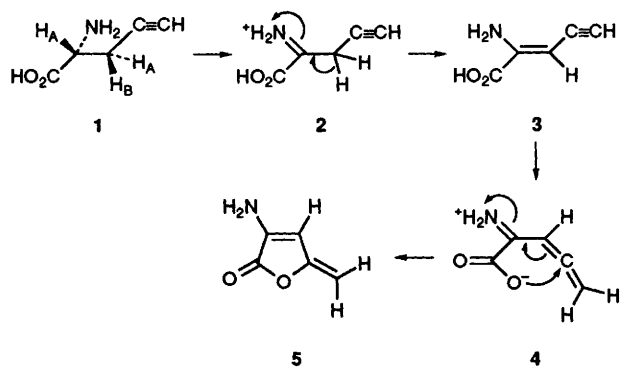
In this mechanism, initial oxidation to the iminium species **2** would be followed by tautomerism to the enamine **3** and rearrangement to the allene **4**. The allene **4** would then cyclise to the lactone **5**. Proton loss at C-3 might either be enzyme catalysed or non-enzymatic and Walsh[2] has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

We chose the stereospecifically labelled aziridines **6**, R = CPh_3, H_B = ^2H, and **6**, R = CPh_3, H_A = ^2H, as the starting point for our synthesis, having previously prepared these compounds by a chemico-enzymatic synthesis.[3] We had shown these to be opened regiospecifically at C-3 with a variety of heteroatom nucleophiles[3] and ring opening with an appropriate carbon nucleophile might be expected to yield the target compounds. 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.[4–6]

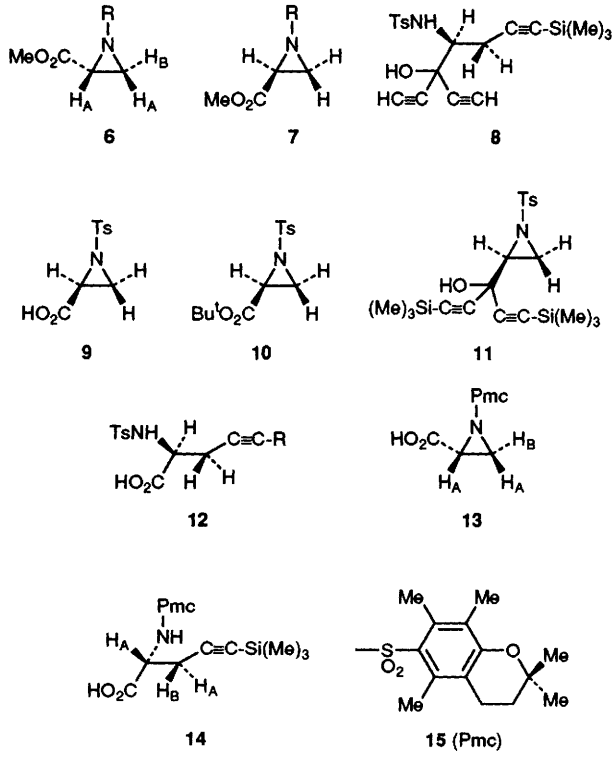
In initial experiments, we used the more readily available L-isomer of the aziridine, **7**, R = CPh_3, and converted it into the tosylate **7**, R = Ts,† $[\alpha]_D$ + 50.2 (*c* 1),† in 70% yield by deprotection with trifluoroacetic acid and reaction of the unstable product **7**, R = H, with toluene-*p*-sulfonyl chloride in pyridine. Reaction of the tosylate **7**, R = Ts, with lithium trimethylsilylacetylide led to reaction at both ester and aziridine groups, yielding the monotrimethylsilyltris-adduct **8**,† $[\alpha]_D$ +49.6 (*c* 0.28)†, in 50% yield. Hydrolysis of the *N*-tosyl methyl ester **7**, R = Ts, to the corresponding acid **9**,† $[\alpha]_D$ −52.3 (*c* 1)†, in 100% yield using sodium hydroxide allowed the *tert*-butyl ester **10** (mp 97–100 °C),† $[\alpha]_D$ −55.3 (*c* 0.67)†, to be prepared in 72% yield using Bu^{t}OH–DCC–DMAP. However reaction of this with lithium trimethylsilylacetylide led unaccountably to reaction only at the ester group, the aziridine remaining untouched and the product being the diacetylene **11**,† $[\alpha]_D$ −7.1 (*c* 0.9)†, in 88% yield. A recent report[6] has also found that Grignard and organolithium reagents attack the ester function in such aziridines.

Reasoning that carboxylate attack might be prevented and regiospecificity in the ring opening reaction might be enforced if the anion of the acid **9** were used as the electrophile, the acid **9** was reacted with excess lithium trimethylsilylacetylide and the products **12** were obtained. Although a mixture of the free acetylene **12**, R = H,† (30%) and the trimethylsilylacetylene **12**, R = Me_3Si† (49%), was obtained, the carboxylate had directed attack entirely to C-3.

Unfortunately, attempts to convert the *N*-tosyl derivative into the free amino acid were not successful and so we investigated the possibility of finding a substituent which would have the electron withdrawing properties of tosyl for the ring opening reaction but which would be more easily removed. Ramage's arginine protecting group 2,2,5,7,8-pentamethylchroman-6-sulfonyl (Pmc, **15**)[7] seemed appropriate for this purpose and we prepared the labelled *N*-Pmc esters, **6**, R = Pmc, H_B = ^2H,† and **6**, R = Pmc, H_A = ^2H,† $[\alpha]_D$ +42.6 (*c* 0.4),‡ from the *N*-tritylaziridines **6**, R = CPh_3, in 30% yield



Scheme 1



Ts = *p*-$MeC_6H_4SO_2$

by deprotection to the aziridines **6**, R = H, and reaction with Pmc chloride under Schotten–Baumann conditions. Saponification led to the labelled acids **13**,† mp 121–124 °C, $[\alpha]_D$ + 38.3 (*c* 0.4),‡ in quantitative yield and reaction with lithium trimethylsilylacetylide gave the clean protected amino acids **14** (mp 112–115 °C),† $[\alpha]_D$ −29.2 (*c* 1.5),‡ in 30% yield. Deprotection was achieved using refluxing trifluoroacetic acid, giving the target compounds (2*R*,3*R*)-[3-$^2H_1$]- and (2*R*,3*S*)-[2,3-$^2H_2$]-propynylglycine, **1**, H_B = ^2H, and **1**, H_A = ^2H, respectively in quantitative yield. The ^1H NMR spectra of these samples showed them to be stereospecifically labelled.

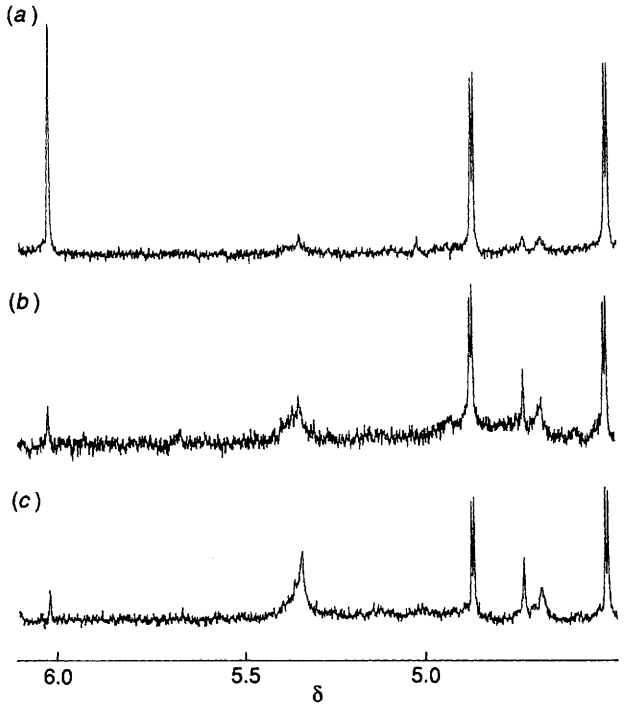


Fig. 1 ^1H NMR spectra (360 MHz; C^2HCl_3) of the lactones **5** formed when (*a*) (2*R*)-propynylglycine; (*b*) (2*R*,3*R*)-[3-$^2H_1$]-propynylglycine; and (*c*) (2*R*,3*S*)-[2,3-$^2H_2$]-propynylglycine were incubated with D-amino acid oxidase

When unlabelled D-propynylglycine **1** was incubated with 20% m/m D-amino acid oxidase and 2% m/m catalase in 0.02 mol dm^{-3} HEPES buffer at pH 8.03 and 20 °C for 3 h and the solution was extracted with C^2HCl_3, the ^1H NMR spectrum [Fig. 1(*a*)] was that of the lactone **5** as reported by Walsh.[2] When either of the labelled samples of D-propynylglycine **1** was used as substrate then, from integration of the ^1H NMR spectra [Fig 1(*b*) and 1(*c*), respectively], in each case the hydrogen on the endocyclic double bond was 80–90% deuteriated and 10–20% protium was observed. 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 the result is in keeping with the second step in the mechanism being a non-enzyme catalysed process.

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Footnotes

† This compound had satisfactory spectroscopic and analytical properties. $[\alpha]_D$ values were all determined in $CHCl_3$ solution.
‡ This value refers to the unlabelled compound.

References

1 P. Marcotte and C. Walsh, *Biochemistry*, 1976, **15**, 3070.
2 P. Marcotte and C. Walsh, *Biochemistry*, 1978, **17**, 5613.
3 B. S. Axelsson, K. J. O'Toole, P. A. Spencer and D. W. Young, *J. Chem. Soc., Chem. Commun.*, 1991, 1085; B. S. Axelsson, K. J. O'Toole, P. A. Spencer and D. W. Young, *J. Chem. Soc., Perkin Trans. 1*, 1994, 807.
4 J. E. Baldwin, R. M. Adlington and N. G. Robinson, *J. Chem. Soc., Chem. Commun.*, 1987, 153.
5 J. E. Baldwin, R. M. Adlington, I. A. O'Neil, C. Schofield, A. C. Spivey and J. B. Sweeney, *J. Chem. Soc., Chem. Commun.*, 1989, 1852.
6 J. E. Baldwin, A. C. Spivey, C. J. Schofield and J. B. Sweeney, *Tetrahedron*, 1993, **49**, 6309.
7 R. Ramage, J. Green and A. J. Blake, *Tetrahedron*, 1991, **47**, 6353.