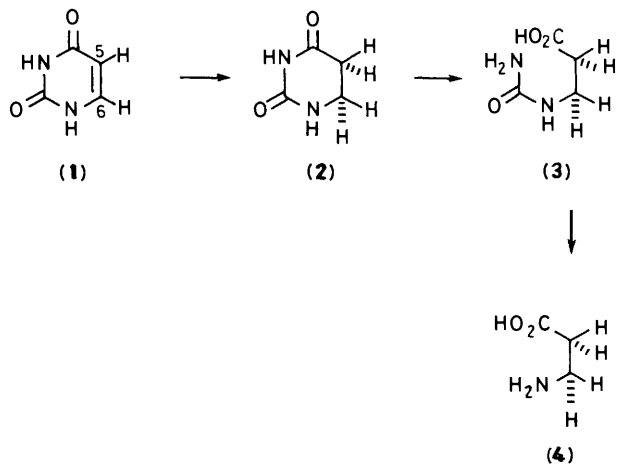
Stereochemistry of Catabolism of the RNA Base Uracil[1]

David Gani and Douglas W. Young*
School of Chemistry and Molecular Sciences, University of Sussex, Falmer, Brighton BN1 9QJ

A mammalian enzyme system has been used to study the stereochemistry of the catabolism of the pyrimidine uracil (**1**) to the amino acid β-alanine (**4**). Use of [5-^{2}H]- and [6-^{2}H]-uracils and of ^2H_2O in the incubations yielded stereospecifically deuteriated samples of β-alanine. Assays, involving total synthesis of samples of β-alanine unambiguously labelled with deuterium in each of the four C–H bonds have shown that, in the first step in the catabolic process, uracil is reduced by dihydrouracil dehydrogenase with overall *anti* addition of hydrogen, at the *si* face at C-6 and the *si* face at C-5.

The RNA base uracil (**1**) is catabolised to β-alanine (**4**) in a variety of organisms by the process shown in Scheme 1. In the first and rate-limiting step in this process, the pyrimidine is reduced to 5,6-dihydrouracil (**2**) by the enzyme dihydrouracil dehydrogenase [now known as dihydrothymine dehydrogenase (EC 1.3.1.2)]. Subsequent hydrolysis of the reduced pyrimidine (**2**) to β-ureidopropionic acid (**3**) and decarbamoylation to yield β-alanine (**4**), carbon dioxide, and ammonia completes the process. The end product β-alanine (**4**) may be further degraded *via* malonic semialdehyde to malonyl CoA.

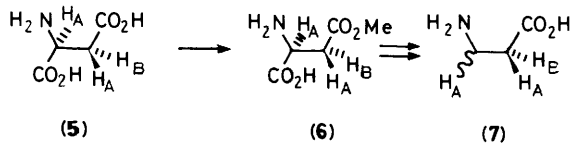


Scheme 1.

The degradation of the RNA base uracil (**1**) is a key metabolic process and the rate-limiting enzyme, dihydrothymine dehydrogenase has been shown to have decreased activity in cancer cells.[2] In order to investigate aspects of the interaction of the substrate with the active site of this enzyme, we decided to assess the stereochemistry of the process with respect to both C-5 and C-6 of the pyrimidine. The enzyme from rat liver has recently been purified to homogeneity by Weber[3] who showed that it contained 4 moles of FAD. This would imply flavin intermediacy in the overall reduction of the pyrimidine by NADPH and so should allow the reduction process to be accompanied by labelling if the incubation were conducted in ^2H_2O. Since it is known[4] that the enzyme is also present in bovine liver which was more readily available to us in quantity, we applied Weber's purification sequence to bovine liver from a freshly slaughtered animal. We found that much of the enzymic activity was lost in the final steps of the purification in our hands and so we opted to use a partially purified enzyme preparation in our studies. This meant that the product which we isolated was not 5,6-dihydrouracil (**2**) but β-alanine (**4**).

Our intention was now to use this enzyme system to metabolize [5-^{2}H]- and [6-^{2}H]-uracils in H_2O and unlabelled uracils in ^2H_2O and to isolate the resultant labelled samples of β-alanine. An assay for the absolute stereochemistry of the label in these samples would be required and so we decided to prepare samples of β-alanine which were stereospecifically labelled in each of the four C–H bonds in an unambiguous manner. These would, we hoped, allow us to develop a ^{1}H or ^{2}H n.m.r. spectral assay for the absolute stereochemistry of the label in our metabolic products.

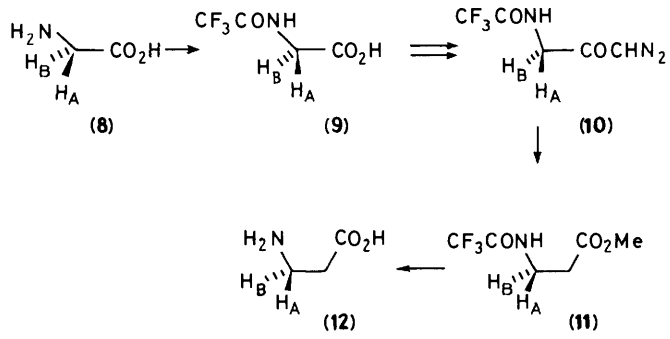
We have already prepared (2*S*,3*R*)-[3-$^2H_1$]- and (2*S*,3*S*)-[2,3-$^2H_2$]-aspartic acids, (**5**; $H_B = {}^2H$) and (**5**; $H_A = {}^2H$),[5] and have



Scheme 2.

used these to prepare other labelled compounds of biological interest.[5–7] Some of these compounds have been used in metabolic studies.[8] These labelled aspartic acids have been converted into the corresponding β-methyl esters (**6**; $H_B = {}^2H$) and (**6**; $H_A = {}^2H$)[7] and so if decarboxylation and hydrolysis could be performed without loss of chirality at the β-centre, we would have a synthesis of samples of β-alanine stereospecifically labelled with deuterium at C-2 in an unambiguous manner. Decarboxylation of the esters (**6**) by heating in the melt with *para*-methoxyacetophenone followed by hydrolysis with 6M-hydrochloric acid at reflux yielded samples of the deuteriated β-alanines, (**7**; $H_B = {}^2H$) and (**7**; $H_A = {}^2H$).

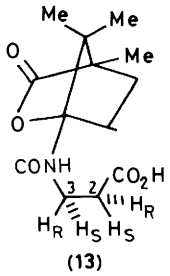
For synthesis of the C-3 deuteriated β-alanines we used (2*R*)- and (2*S*)-[2-$^2H_1$]-glycines, (**8**; $H_A = {}^2H$) and (**8**; $H_B = {}^2H$) respectively, which we had prepared[8] by exchange of the 2 *pro-R* hydrogen in unlabelled glycine with ^2H_2O and in [2,2-$^2H_2$]glycine with H_2O respectively. These were treated with trifluoroacetic anhydride to give the corresponding trifluoroacetamides (**9**) which were converted into the crude acid chlorides with thionyl chloride. Reaction of the acid chlorides with diazomethane gave the labelled diazo ketones (**10**; $H_A = {}^2H$) and (**10**; $H_B = {}^2H$). The diazo ketones (**10**) were now subjected to Wolff rearrangement by photolysis in methanol to yield the methyl esters (**11**) which were hydrolysed directly to the corresponding [3-$^2H_1$]-β-alanines. Since the Wolff rearrangement is expected[9] to occur with retention of configuration even at a migrating primary chiral centre,[5] it was



Scheme 3.

expected that the β-alanine obtained from (2*R*)-[2-$^2H_1$]glycine (**8**; $H_A = {}^2H$) would be (3*R*)-[3-$^2H_1$]-β-alanine (**12**; $H_A = {}^2H$) and that obtained from (2*S*)-[2-$^2H_1$]-glycine would be (3*S*)-[3-$^2H_1$]-β-alanine (**12**; $H_B = {}^2H$).

Having now synthesized four samples of β-alanine which should be stereospecifically labelled in an unambiguous manner, it was now necessary to assess the stereochemical integrity of these samples and to provide an assay for our metabolic studies. Both of these aims were readily achieved for [3-$^2H_1$]-β-alanines since, when the camphanoyl amide (**13**) of β-alanine was prepared, the now diastereotopic protons on C-3 could be differentiated in the 360 MHz ^1H n.m.r. spectrum (Figure 1a). The corresponding region of the ^1H n.m.r. spectra of the amides (**13**) from (3*R*)- and (3*S*)-[3-$^2H_1$]-β-alanines (Figure 1b and 1c respectively) showed that these compounds were optically pure. Our assignment of absolute stereochemistry to these compounds still relied on the assumption that the Wolff rearrangement proceeded with retention of stereochemistry at the migrating centre. Although this was well-founded, a check was carried out using the trifluoroacetamide (**14**; $H_B = {}^2H$)



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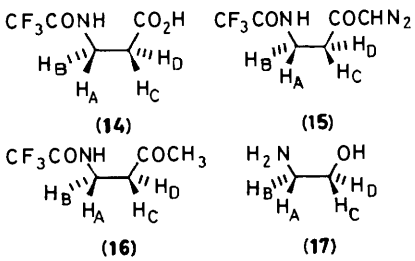


obtained by photolysis of the diazo ketone (**10**; $H_B = {}^2H$) in water. Reaction of this with thionyl chloride gave the acid chloride which, on treatment with diazomethane, gave the diazo ketone (**15**; $H_B = {}^2H$). Reduction with 55% aqueous hydrogen iodide in chloroform afforded the methyl ketone (**16**; $H_B = {}^2H$) which on Baeyer-Villiger oxidation with trifluoroperacetic acid followed by hydrolysis yielded (2*S*)-[2-$^2H_1$]-2-aminoethanol (**17**; $H_B = {}^2H$). The *N*,*O*-dicamphanoyl derivative of this compound had a ^1H n.m.r. spectrum in keeping with that of an authentic sample.[8] Since the stereospecifically labelled centre is not affected in any of the reactions in this sequence, our assignment of absolute stereochemistry to (3*R*)-and (3*S*)-[3-$^2H_1$]-β-alanines, (**12**; $H_A = {}^2H$) and (**12**; $H_B = {}^2H$) respectively, is confirmed.

Assessment of the chiral purity of our samples of (2*R*)- and (2*S*)-[2-$^2H_1$]-β-alanine unfortunately proved less facile. The 360 MHz ^1H n.m.r. spectrum of the amide (**13**) did not allow us

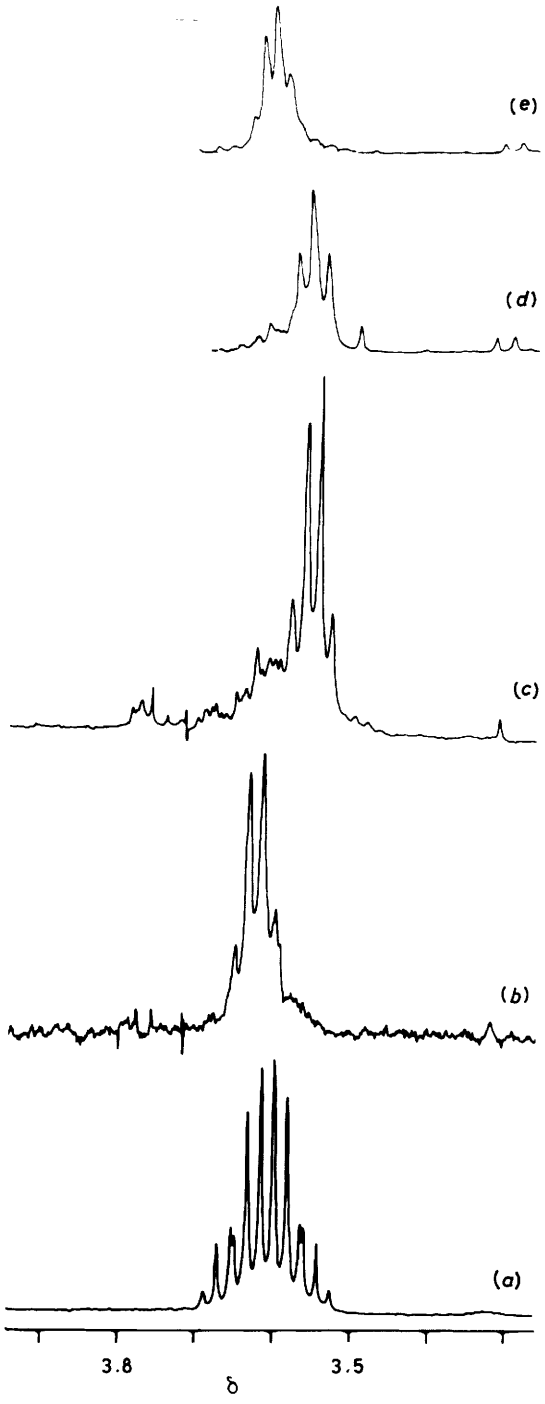
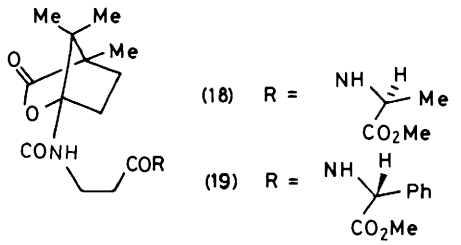


Figure 1. 360 MHz ^1H N.m.r. spectra (C^2HCl_3) of the β-alanine C-3 protons in (a) the amide (**13**); (b) synthetic (3*R*)-[3-$^2H_1$]-(**13**) derived from (2*R*)-[2-$^2H_1$]-glycine; (c) synthetic (3*S*)-[3-$^2H_1$]-(**13**) derived from (2*S*)-[2-$^2H_1$]-glycine; (d) [2,3-$^2H_2$]-(**13**) from catabolism of uracil in ^2H_2O; (e) [3-$^2H_1$]-(**13**) from catabolism of [6-^2H]-uracil in H_2O.

to differentiate between the 2-*pro*-*R*- and 2-*pro*-*S*-hydrogens which were more distant from the chiral derivatising group than the more amenable C-3 hydrogens. Attempts to improve the situation by introduction of chirality at the carboxyl end of the molecule using the amides (**18**) and (**19**) failed, even when lanthanide shift reagents were employed. We therefore decided to convert the C-2 labelled β-alanines (**7**; $H_B = {}^2H$) and (**7**; $H_A = {}^2H$) into (1*R*)- and (1*S*)-[1-$^2H_1$]-2-aminoethanols for



which we had an excellent ^1H n.m.r. spectral assay.[8,10] This was achieved by first preparing the trifluoroacetamides (**14**; H_D = ^2H) and (**14**; H_C = ^2H) from these compounds. The amides (**14**) were then converted *via* the acid chlorides into the diazoketones (**15**; H_D = ^2H) and (**15**; H_C = ^2H) and thence to the methyl ketones (**16**; H_D = ^2H), and (**16**; H_C = ^2H) using the route used above in the conversion of the trifluoroacetamide (**14**, H_B = ^2H) into the corresponding methylketone (**16**; H_B = ^2H). Baeyer-Villiger oxidation followed by hydrolysis, as before, gave samples of 2-aminoethanol which were converted into the *N,O*-dicamphanoyl derivatives. These had ^1H n.m.r. spectra (Figure 2d and e respectively) which, although slightly obscured by unlabelled or racemic material, were in keeping with assignment[8,10] of the compounds as derivatives of (1*R*)-[1-$^2H_1$]-2-aminoethanol and (1*S*,2*RS*) [1,2-$^2H_2$]-2-aminoethanol, (**17**; H_D = ^2H) and (**17**; H_C = ^2H) respectively. This was the expected result and confirmed that the Baeyer-Villiger step in the above sequence had proceeded with retention of configuration at the migrating centre. The sequence could, therefore, be used as an assay for C-2 labelled β-alanines from the metabolic studies.

We were now in a position to examine the stereochemical outcome of the catabolism of uracil to β-alanine. In a preliminary experiment using the disappearance of $\lambda_{max.}$ 340 nm due to NADPH to assess the rates of catabolism in H_2O and in ^2H_2O, we obtained an approximate value, $k_H/k_{^2H}$ = 4.4, for the process. Experiments in ^2H_2O were therefore left longer than the corresponding experiments in H_2O and yields were lower in these experiments, due presumably in part to the further catabolism of the β-alanine produced when the longer reaction time was used. In all experiments the catabolism was coupled to regeneration of NADPH using glucose-6-phosphate and glucose-6-phosphate dehydrogenase. Dialysis of ammonium salts from the bovine liver enzyme system was necessary to avoid inhibition of glucose-6-phosphate dehydrogenase.

The ^1H n.m.r. spectra of the *N*-camphanoyl derivatives (**13**; 3-H_S = 2-H_S = ^2H) and (**13**; 3-H_R = ^2H) derived from the samples of β-alanine obtained by catabolism of unlabelled uracil in ^2H_2O and [6-^2H]-uracil[11] in H_2O respectively are shown in Figures 1d and 1e respectively. These clearly show that the former sample is deuteriated in the 3-*pro-S* hydrogen whilst the latter is deuteriated in the 3-*pro-R* hydrogen. Thus hydrogen has been added at the *si* face of uracil at C-6.

Catabolism of [5-$^2H_1$]uracil[11] gave a sample of β-alanine which was converted by our assay procedure into (1*R*)-[1-$^2H_1$]-2-aminoethanol (**17**; H_D = ^2H) as assessed by the ^1H n.m.r. spectrum of its *N,O*-dicamphanoyl derivative (Figure 2f). In spite of the small loss in stereospecificity introduced in the conversion of β-alanine into 2-aminoethanol, it is evident that [5-$^2H_1$]uracil must have been metabolised to (2*R*)-[2-$^2H_1$]-β-alanine. The problems caused by the isotope effect in the ^2H_2O incubation unfortunately gave an insufficient yield of the [2,3-$^2H_2$]-β-alanine for the degradation sequence to yield sufficient *N,O*-dicamphanoyl-2-aminoethanol for purification so that an unambiguous assay for the stereochemistry of the label at C-2 of the β-alanine might be obtained. It is evident from the experiment using [5-$^2H_1$]uracil, however, that reduction has occurred from the *si* face at C-5 of uracil.

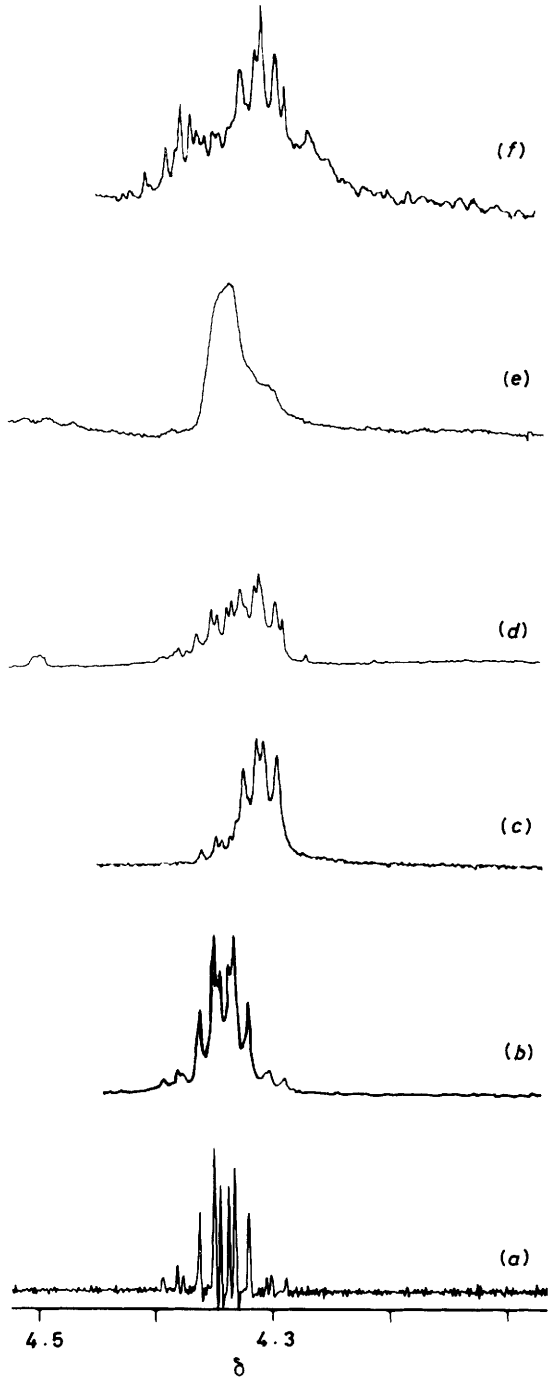


Figure 2. 360 MHz ^1H N.m.r. spectra (C^2HCl_3) of the 2-aminoethanol C-1 protons of *N,O*-dicamphanoyl-2-aminoethanol in (a) the unlabelled compound with line-narrowing programme; (b) the unlabelled compound without line-narrowing; (c) authentic[8] (1*R*)-[1-$^2H_1$]-compound; (d) the compound obtained by degradation of synthetic (2*R*)-[2-$^2H_1$]-β-alanine; (e) the compound obtained from synthetic (2*S*,3*RS*)-[2,3-$^2H_2$]-β-alanine; (f) the compound obtained by degradation of the β-alanine obtained by catabolism of [5-^2H]-uracil in H_2O.

The results show that, in the catabolism of uracil, the pyrimidine ring is reduced by dihydrouracil dehydrogenase with overall *anti* addition of hydrogen. In this respect the reaction is a typical flavin-dependent redox reaction where *anti*

addition or removal of hydrogen is the rule.[12] The absolute stereochemistry of the process requires that hydrogen be added at the *si* face at C-6 and the *si* face at C-5 of the pyrimidine.

Experimental

M.p.s were determined on a Kofler hot-stage apparatus. I.r. spectra were recorded on Perkin-Elmer 257, 457, and 477 instruments and ^{1}H n.m.r. spectra on Perkin-Elmer R12 (60 MHz) and R32 (90 MHz) and Bruker WH360 (360 MHz) instruments. Mass spectra were recorded on Kratos MS25 and MS80 instruments and enzyme assays were obtained on a Pye Unicam SP800 spectrophotometer modified to drive an external Kipp and Zonen DB8 chart recorder. Trifluoroperacetic acid was prepared from 87% H_2O_2 and trifluoroacetic anhydride using the method of Arigoni and co-workers,[13] and ethereal diazomethane was prepared[14] from Diazald.

β-Alanine (**4**) *by Decarboxylation of* β-*Methyl Aspartate* (**6**).—β-Methyl aspartate hydrochloride[7] (130 mg, 0.71 mmol) was intimately ground with *p*-methoxyacetophenone (200 mg, 1.33 mmol) in a mortar. The mixture was heated rapidly to 160 °C and then more slowly, over 90 min, to 180 °C under nitrogen, with occasional shaking. When evolution of CO_2 was judged to be complete, the amber flux was allowed to cool and was heated to reflux with aqueous 6M-HCl (5 ml) for *ca.* 30 min. The cooled, deep-red reaction mixture was extracted with chloroform (4 × 10 ml) and the aqueous phase was diluted to 10 ml and filtered through a small pad of glass wool; the filtrate was lyophilized to a pale brown gum. The gum was dissolved in water (20 ml) and was applied to an Amberlite IR45 weakly basic ion-exchange column (10 × 2 cm) with water as eluant; 100 ml of effluent was collected and the solvent was removed from this under reduced pressure to yield a colourless glass which crystallised within several hours (39 mg, 62%), m.p. 200—204 °C (decomp.), (lit.,[15] 207 °C), δ (5% ^{2}HCl in ^2H_2O, external $SiMe_4$ at 0 p.p.m.) 3.17 (2 H, t, *J* 7 Hz, 3-CH_2) and 2.56 (2 H, t, *J* 7 Hz, 2-CH_2) identical with the spectrum of authentic material.

(2R)-[2-$^2H_1$]-β-*Alanine* (**7**; H_B = ^{2}H). This compound was prepared as above using β-methyl (2*S*,3*R*)-[3-$^2H_1$]aspartate[7] (**6**; H_B = ^{2}H). The product, m.p. 201—203 °C (decomp.), had δ (5% ^{2}HCl in ^2H_2O, external $SiMe_4$ at 0 p.p.m.) 3.25 (2 H, d, *J* 7 Hz, 3-CH_2) and 2.75 (1 H, br t, J 7 Hz, 2-CH)

(2S,3RS)-[2,3-$^2H_2$]-β-*Alanine* (**7**; H_A = ^{2}H). This compound was prepared as above using β-methyl (2*S*,3*S*)-[2,3-$^2H_2$]-aspartate[7] (**6**; H_A = ^{2}H). The product, m.p. 199—200 °C (decomp.), had δ (5% ^{2}HCl in ^2H_2O, external Me_4Si at 0 p.p.m.), 3.21 (1 H, br d, *J* 7 Hz, 3-CH), and 2.58 (1 H, br d, *J* 7 Hz, 2-CH).

N-*Trifluoroacetylglycine* (**9**).—Glycine (200 mg, 2.7 mmol) was suspended in dry tetrahydrofuran (5 ml) and trifluoroacetic anhydride (1 g, 4.7 mmol) was added dropwise at 0 °C. The pale yellow solution was allowed to warm to room temperature over 45 min and the solvents were reduced to 0.5 ml under reduced pressure. Water (0.5 ml) was added and the reaction mixture was lyophilized to a cream coloured amorphous solid (452 mg, 98%), m.p. 115—117 °C. A small amount was recrystallised from $CHCl_3$–light petroleum, (b.p. 40—60 °C). The crystals took several days to form, m.p. 117—118 °C (Found: C, 28.2; H, 2.4; N, 8.2. $C_4H_4F_3NO_3$ requires C, 28.1; H, 2.4; N, 8.2%); $\nu_{max.}$ (Nujol) 3 318 (NH) and 1 720 cm^{-1} (br, CONH and CO_2H); δ (C^2HCl_3, $CHCl_3$ at 7.24 p.p.m.), 9.45 (1 H, br m, COOH), 7.70 (1 H, br s, NH) and 3.86 (2 H, d, *J* 6 Hz, CH_2, after ^2H_2O shake this became a singlet).

1-*Diazo*-3-*trifluoroacetylaminopropan*-2-*one* (**10**).—*N*-Trifluoroacetylglycine (240 mg, 1.4 mmol) was heated to reflux in thionyl chloride (5 ml) for 70 min. The solvent was removed under reduced pressure to yield an amber coloured oil which was dried under high vacuum at low temperature for 30 min to give the acid chloride (251 mg, 94%), $\nu_{max.}$(Nujol) 3 340 (NH), 1 805 (COCl), and 1 730 cm^{-1} (CONH). The acid chloride was dissolved in dichloromethane–diethyl ether (4 ml, 1 : 1) and was added dropwise to a cold, stirred excess of ethereal diazomethane. The solution was allowed to warm to room temperature over 2 h and the excess of diazomethane was removed in a stream of nitrogen. The solvents were removed under reduced pressure and the residual dark gum was recrystallised from $CHCl_3$–Et_2O–light petroleum (b.p. 40—60 °C) to give the crude product. The diazo ketone was again recrystallised (yield 109 mg, 40%), m.p. 93—44 °C (Found: C, 32.0; H, 2.6; N, 18.9. $C_5H_4F_3N_3O_2$ requires C, 30.8; H, 2.1; N, 21.5%); $\nu_{max.}$(Nujol) 3 290 (NH), 2 120 (CH–$\overset{+}{N}$≡N) 1 750sh and 1 718 cm^{-1} (COCH and CONH); δ (C^2HCl_3, external $SiMe_4$ at 0 p.p.m.) 5.43 (1 H, s, CHN_2) and 4.09 (2 H, d, *J* 5 Hz, 3-CH_2).

(3R)-[3-$^2H_1$]-1-*Diazo*-3-*trifluoroacetylaminopropan*-2-*one* (**10**; H_A = ^{2}H). This compound was prepared as above starting from (2*R*)-[2-$^2H_1$]glycine (**8**; H_A = ^{2}H).[8] The intermediate *N*-trifluoroacetylglycine melted at 112—115 °C and the acid chloride had $\nu_{max.}$(Nujol) 3 280 (NH), 1 815 (COCl), and 1 710 cm^{-1} (CONH). The crude diazo ketone was obtained in *ca.* 45% yield from labelled glycine, m.p. 90—93 °C (after one recryst.), $\nu_{max.}$(Nujol) 3 320 (NH), 2 120 ($\overset{+}{N}$≡N), 1 786sh and 1 720 cm^{-1} (COCH and CONH); δ (C^2HCl_3, $SiMe_4$ at 0 p.p.m.), 7.35 (1 H, br s, NH), 5.52 (1 H, s, CHN_2), and 4.20 (1 H, d, *J* 5 Hz, 3-CH) together with other absorptions.

(3S)-[3-$^2H_1$]-1-*Diazo*-3-*trifluoroacetylaminopropan*-2-*one* (**10**; H_B = ^{2}H). This compound was prepared in similar manner using (2*S*)-[2-$^2H_1$]glycine (**8**; H_B = ^{2}H).[8] The *N*-trifluoroacetylglycine melted at 113—116 °C and the acid chloride had $\nu_{max.}$(Nujol) 3 315 (NH), 1 823 (COCl), and 1 708 cm^{-1} (CONH). The crude diazo ketone was obtained in an overall yield of *ca.* 80% from labelled glycine after one recrystallisation, m.p. 91—94 °C, $\nu_{max.}$(Nujol) 3 300 (NH), 2 120 ($\overset{+}{N}$≡N), and 1 715 cm^{-1} (COCH and CONH); δ (C^2HCl_3, $CHCl_3$ at 7.24 p.p.m.), 7.51 (1 H, br s, NH) and 5.40 (1 H, s, CHN_2). The absorption for 3-CH was obscured by peaks arising from impurities.

Methyl N-*Trifluoroacetyl*-β-*alaninate* (**11**).—1-Diazo-3-trifluoroacetylaminopropan-2-one (**10**) (195 mg, 1 mmol) was dissolved in dry methanol (180 ml) and the solution was purged with nitrogen for 20 min. The solution was irradiated under a reduced nitrogen flow, with an internal 125 W medium-pressure u.v. lamp fitted with a quartz filter. After 2 h the solvent was removed under reduced pressure to yield a yellow oil (196 mg, 98%; *ca.* 75% pure). This had spectra in keeping with those of an authentic sample of methyl *N*-trifluoroacetyl-β-alanine prepared from the acid chloride of *N*-trifluoroacetyl -β-alanine (**14**) and methanol. Preparation of the acid chloride is discussed later in this section and the methyl ester (**11**) was purified by distillation (b.p. 130 °C at 10 mmHg) (Found: C, 35.5; H, 4.1; N, 6.8. $C_6H_8F_3NO_3$ requires C, 36.2; H, 4.0; N, 7.0%), *m/z* 200 (M + 1)$^+$ and 199 (M^+); $\nu_{max.}$(Nujol) 3 345 (NH) and 1 740br cm^{-1} (CO_2Me and CONH); δ (C^2HCl_3, external $SiMe_4$ at 0 p.p.m.) 7.50 (1 H, br s, NH), 3.80 (3 H, s, OCH_3), 3.68 (2 H, q, *J* 7 Hz, 3-CH_2), and 2.68 (2 H, t, *J* 7 Hz, 2-CH_2).

Methyl (3R)-[3-$^2H_1$]-N-*Trifluoroacetyl*-β-*alaninate* (**11**; H_A = ^{2}H). This compound was prepared as above using (3*R*)-[3-$^2H_1$]-1-diazo-3-trifluoroacetylaminopropan-2-one (**10**; H_A = ^{2}H) in 96% crude yield, δ (C^2HCl_3, $SiMe_4$ at 0 p.p.m.), 7.22 (1 H, br s, NH), 3.85 (3 H, s, OCH_3), 3.7 (1 H, m, 3-CH_2), and 2.73 (2 H, d, *J* 7 Hz, 2-CH_2).

Methyl (3S)-[3-$^2H_1$]-N-*Trifluoroacetyl*-β-*alaninate* (**11**;

H_B = ^2H). This compound was prepared as above in 92% yield using the (3*R*)-[3-$^2H_1$]-diazo ketone (**10**; H_B = ^2H), δ (C^2HCl_3, external $SiMe_4$ at 0 p.p.m.), 7.30 (1 H, br s, NH), 3.72 (3 H, s, OCH_3), 3.63 (1 H, m, 3-CH), and 2.63 (2 H, d, *J* 7 Hz, 2-CH_2).

(3S)-[3-$^2H_1$]-N-*Trifluoroacetyl*-β-*alanine* (**14**; H_B = ^2H). This compound was prepared in 96% yield by conducting the above photolysis in tetrahydrofuran–water (100:1) on the same scale. The product had δ (C^2HCl_3:2% $^2H_6$-DMSO, external $SiMe_4$ at 0 p.p.m.), 8.47 (1 H, br s, CO_2H), 7.56 (1 H, br s, NH), 3.68 (1 H, m, 3-CH and some impurities), and 2.58 (2 H, d, *J* 7 Hz, 2-CH_2).

β-*Alanine Hydrochloride* (**4**) *from the Ester* (**11**).—Methyl *N*-trifluoroacetyl-β-alanine (**11**) (42 mg, 0.21 mmol) was heated to reflux with 6M-HCl (3 ml) for 2 h. The solution was cooled and lyophilized to yield a dark gum. The gum was dissolved in water (10 ml) and eluted through a small (5 cm) column (1 × 5 cm) of Amberlite IR45 weakly basic ion-exchange resin. The effluent collected (30 ml) was acidified to pH 2 and lyophilized to a pale yellow amorphous solid (23 mg, 87%) with the expected spectra.

(3R)-[3-$^2H_1$]-β-*Alanine Hydrochloride* (**12**; H_A = ^2H). This compound was prepared from the (3*R*)-[3-$^2H_1$] ester (**11**; H_A = ^2H) as above δ (^2H_2O, external $SiMe_4$ at 0 p.p.m.), 3.16 (1 H, t, *J* 7 Hz, 3-CH), and 2.53 (2 H, d, *J* 7 Hz, 2-CH_2).

(3S)-[3-$^2H_1$]-β-*Alanine hydrochloride* (**12**; H_B = ^2H). This compound was prepared from the (3*S*)-[3-$^2H_1$] ester (**11**; H_B = ^2H) as above and converted directly into the *N*-camphanoyl amide (**13**).

N-*Camphanoyl*-β-*alanine* (**13**).—β-Alanine (200 mg, 2.25 mmol) was dissolved in 2M-NaOH (2.5 ml, 5 mmol) and the solution was shaken with a solution of (−)-camphanoyl chloride (350 mg, 1.62 mmol) in toluene (4 ml). After 2.5 h, the toluene was separated and the aqueous phase was extracted with chloroform (2 × 3 ml). 6M-HCl was added dropwise until the solution became turbid (*ca.* pH 3). The solution was again extracted with chloroform (3 × 10 ml). The organic phase was dried (Na_2SO_4) and the solvent was removed under reduced pressure to yield a colourless glass which crystallised with time (328 mg, 75%). A small sample was recrystallised from $CHCl_3$–light petroleum (b.p. 40—60 °C), m.p. 128—30 °C (Found: C, 57.4; H, 7.2; N, 5.4. $C_{13}H_{19}NO_5$ requires C, 58.0; H, 7.1; N, 5.2%); *m/z* (EI) 269 (M^+) and 223 ([M − HCO_2H]$^+$); δ (360.13 MHz, C^2HCl_3, internal $SiMe_4$ at 0 p.p.m.), 7.00 (1 H, br t, NH), 5.91 (1 H, br s, COOH), 3.60 (2 H, m, 10 lines, 3-CH_2), 2.64 (2 H, t, *J* 6.1 Hz, 2-CH_2), 2.53 (1 H, m), 1.92 (2 H, m) and 1.70 (1 H, m) (CH_2 groups of camphanoyl), 1.10 (6 H, s, 2 × CH_3 of camphanoyl), and 0.89 (3 H, s, CH_3 of camphanoyl). Addition of [Eu(dpm)$_3$] did not cause the triplet at 2.64 to resolve into the diastereotopic protons. Only line broadening was observed; δ (360.13 MHz, 10% NaO^2H, HO^2H at 4.6 p.p.m.) (β-alanine portion only), 3.28 (2 H, m, 10 lines, 3-CH_2) and 2.22 (2 H, t, *J* 7 Hz, 2-CH_2).

(3R)-N-*Camphanoyl*-[3-$^2H_1$]-β-*alanine* (**13**; 3-H_R = ^2H).—This compound was prepared using (−)-camphanoyl chloride and (3*R*)-[3-$^2H_1$]-β-alanine (**12**; H_A = ^2H) using the method described above, *m/z* (EI), 270 (M^+); δ (360.13 MHz, C^2HCl_3, $SiMe_4$ at 0 p.p.m.) (β-alanine portion only, see Figure 1b), 3.63 (1 H, m, 4 lines, 3-H_s) and 2.64 (2 H, d, *J* 6.1 Hz, 2-CH_2).

(3S)-N-*Camphanoyl*-[3-$^2H_1$]-β-*alanine* (**13**; 3-H_S = ^2H).—This compound was prepared as above from (3*S*)-[3-$^2H_1$]-β-alanine (**12**; H_B = ^2H), *m/z* (EI) 270 (M^+); δ (360.13 MHz, C^2HCl_3, $CHCl_3$ at 7.2 p.p.m.) (β-alanine portion only, see Figure 1c) 3.57 (1 H, m, 4 lines, 3-H_R) and 2.64 (2 H, d, *J* 6.1 Hz, 2-CH_2).

The Amide (**18**).—*N*-Camphanoyl-β-alanine (**13**) (50 mg, 0.19 mmol) was heated to reflux in thionyl chloride (5 ml) for 1 h. The solvent was removed under reduced pressure to yield the acid chloride as a colourless oil, $\nu_{max.}$(Nujol) 3 340 (NH) and 1 800—1 765 cm^{-1} (COCl and lactone). The oil was dissolved in dry chloroform (3 ml) at 0 °C with stirring and a solution of methyl L-alaninate hydrochloride (50 mg, 0.398 mmol) was added followed by triethylamine (80 mg, 0.79 mmol) in chloroform (0.5 ml) dropwise during 2 min. The solution was stirred overnight (10 h) and then diluted to 15 ml and washed with aqueous 2M-HCl (2 × 5 ml) and then water (5 ml). After drying ($MgSO_4$) the solvent was removed under reduced pressure to yield a colourless oil (50.2 mg, 75%) which was crystallised from $CHCl_3$–Et_2O to yield the product (41 mg), m.p. 142 °C (Found: C, 57.4; H, 7.5; N, 7.8. $C_{17}H_{26}N_2O_6$ requires C, 57.6; H, 7.3; N, 7.9%), *m/z* (EI) 354 (M^+), 295 [(M − CO_2Me)$^+$], and 252 [(M − $NHCH(CH_3)CO_2Me$)$^+$]; δ (360.13 MHz, C^2HCl_3, $SiMe_4$ at 0 p.p.m.) 7.05 (1 H, br t, *H*NCH_2), 6.26 (1 H, br d, CHN*H*), 4.59 (1 H, m, 5 lines, *J* 7.3Hz, L-alanine CH), 3.76 (3 H, s, CO_2CH_3), 3.61 (2 H, m, six lines, CH_2N), 2.48 (2 H, t, *J* 6.2 Hz, CH_2CO), 2.50, 1.91, and 1.68 (4 H total, 2 × CH_2 of camphanoyl), 1.41 (3 H, d, *J* 7.2 Hz, CH_3 of L-alanine) and 1.10, 1.09, and 0.89 (3 H, each, s, 3 × C*H*$_3$ of camphanoyl).

The Amide (**19**).—*N*-Camphanoyl-β-alanine (**13**) (135 mg, 0.5 mmol) was converted into the acid chloride as described above. A solution of the chloride (142 mg, 0.49 mmol) in dry chloroform (10 ml) was cooled to 0°C and stirred and methyl D-phenylglycinate hydrochloride (120 mg, 0.6 mmol) was added followed by triethylamine (153 mg, 1.5 mmol) in chloroform (2 ml), dropwise over 2—3 min. The solution was allowed to warm to room temperature and stirring was continued for 2 h. The solution was then washed with 2M-hydrochloric acid (2 ml), saturated aqueous sodium hydrogen carbonate (2 ml), and water (1 ml). The chloroform solution was dried (Na_2SO_4) and the solvent removed under reduced pressure to yield the crude product as a slightly yellow oil (183 mg, 88%). The oil was dissolved in chloroform and filtered through a small pad of silica to yield an almost colourless oil (162 mg, 78%) which refused to crystallise, *m/z* (positive CI, ammonia carrier), 417 [(M + H)$^+$], *m/z* (EI), 416.206 0 (M^+, $C_{22}H_{28}N_2O_6$ requires 416.194 7); δ (360 MHz, C^2HCl_3, $CHCl_3$ at 7.24 (p.p.m.), 7.36—7.30 (5 H, m, Ph protons), 7.00 (1 H, br t, β-ala NH), 6.58 (1 H, br d, phenylglycine NH), 5.55 (1 H, d, *J* 7.1 Hz, α-H of phenylglycine), 3.72 (3 H, s, OCH_3), 3.58 (2 H, m, 3-CH_2 of β-ala), 2.51—2.43 (3 H, m, 2-CH_2 of β-ala and camphanoyl CH), 1.98—1.83 (2 H, m, CH_2 of camphanoyl), 1.64 (1 H, m, camphanoyl CH), and 1.06, 1.03, and 0.75 (3 H, each s, camphanoyl CH_3). Other peaks were present in the spectrum. The use of shift reagents and extensive decoupling experiments did not allow unambiguous stereochemical assignment of the C-3 protons of the β-alanine portion. Synthesis of the (3*R*)- and (3*S*)-[3-$^2H_1$]-β-alanyl compounds did not clarify the situation even when the protons at C-2 were irradiated.

N-*Trifluoroacetyl*-β-*alanine* (**14**).—β-Alanine (375 mg, 4.21 mmol) was suspended in dry tetrahydrofuran (3.5 ml) with stirring at −5 °C and trifluoroacetic anhydride (1 g, 4.76 mmol) was added dropwise. The colourless solution was allowed to warm to room temperature over 1 h and the solvent was reduced to *ca.* 1 ml under reduced pressure. Water (0.5 ml) was added and the solution was lyophilized to yield a white foam. (775 mg, 99%). The ^1H n.m.r. spectrum showed that the product was pure. A small sample was recrystallised from $CHCl_3$–light petroleum (b.p. 60—80 °C to yield white needles, m.p. 118—120 °C (Found: C, 32.5; H, 3.3; N, 7.5. Calc. for $C_5H_6F_3NO_3$: C, 32.4; H, 3.2; N, 7.6%); *m/z* (EI), 185 (M^+), 167 [(M − H_2O)$^+$] and 139 [(M − HCO_2H)$^+$]; $\nu_{max.}$ (Nujol) 3 600—2 600 (H bonded NH, OH) and 1 705 cm^{-1} (br, CONH and CO_2H); δ (C^2HCl_3–[$^2H_6$]-DMSO, external $SiMe_4$ at 0 p.p.m.) 9.00 (1 H,

br s, CO_2H), 7.91 (1 H, br s, NH), 3.61 (2 H, q, *J* 7 Hz, 3-CH_2), and 2.60 (2 H, t, *J* 7 Hz, 2-CH_2).

(2R)-N-*Trifluoroacetyl* [2-$^2H_1$]-β-*alanine* (**14**; H_D = ^2H). This compound was prepared as above from (2*R*)-[2-$^2H_1$]-β-alanine (**7**; H_B = ^2H) (92%), m.p. 110—112 °C, $\nu_{max.}$(Nujol) 3 600—2 600 (H bonded NH,OH) and 1 705 cm^{-1} (br, CONH and CO_2H), δ (C^2HCl_3–2% [$^2H_6$]-DMSO, internal $SiMe_4$ at 0 p.p.m.) 3.54 (2 H, t, *J* 7 Hz, 3-CH_2), and 2.53 (1 H, br m, 2-CH).

(2S,3RS)-N-*Trifluoroacetyl*-[2,3-$^2H_2$]-β-*alanine* (**14**; H_C = ^2H). This was prepared as above from (2*S*,3*RS*)-[2,3-$^2H_2$]-β-alanine (**7**; H_A = ^2H) (91%), m.p. 114—115 °C, $\nu_{max.}$ (Nujol) 3 600—2 600 (H bonded NH, OH) and 1 708 cm^{-1} (br, CONH and CO_2H); δ (C^2HCl_3–2% [$^2H_6$]-DMSO, external $SiMe_4$ at 0 p.p.m.) 3.51 (1 H, br t, 3-CH), and 2.56 (1 H, br m, 2-CH).

1-*Diazo*-4-*trifluoroacetylaminobutan*-2-*one* (**15**).—*N*-Trifluoroacetyl-β-alanine (**14**) (200 mg, 1.08 mmol) was dissolved in thionyl chloride (2.5 ml) and the solution was heated at reflux for 70 min. The solvent was removed under reduced pressure to yield the acid chloride as a pale yellow oil which was dried at 5 °C under high vacuum (218 mg, 99%) $\nu_{max.}$(Nujol) 3 305 (NH), 1 792 (COCl), 1 705 cm^{-1} (CONH). The acid chloride (208 mg, 1.02 mmol) was dissolved in ether (1.5 ml) and dichloromethane (2.5 ml) and was added dropwise to a cold stirred solution of ethereal diazomethane (*ca.* four-fold excess, 15 ml). After the addition was complete, the solution was allowed to warm to room temperature over 90 min. The solvents and excess of CH_2N_2 were removed in a stream of nitrogen and then under reduced pressure to yield a sticky yellow oil which crystallised on scratching (211 mg, 99%), m.p. 36—37 °C. A small amount was recrystallised from $CHCl_3$–light petroleum (b.p. 60—80 °C) to yield waxy yellow crystals, m.p. 39—40 °C (Found: C, 34.9; H, 3.3; N, 17.2. $C_6H_6F_3N_3O_2$ requires C, 34.5; H, 2.9; N, 20.1%), *m/z* (EI), 168 ([*M* − CH_2]$^+$); $\nu_{max.}$(Nujol) 3 310 (NH), 2 125 (CHN$^+$≡N), and 1 712 cm^{-1} (CO of ketone and amide); δ (C^2HCl_3, external $SiMe_4$ at 0 p.p.m.) 7.45 (1 H, br s, NH), 5.31 (1 H, s, CHN_2), 3.62 (2 H, q, *J* 6.5 Hz, 4-CH_2), and 2.63 (2 H, t, *J* 6.5 Hz, 3-CH_2).

(3R)-[3-$^2H_1$]-1-*Diazo*-4-*trifluoroacetylaminobutan*-2-*one* (**15**; H_D = ^2H). This compound was prepared from N-trifluoroacetyl-(2*R*)-[2-$^2H_1$]-β-alanine (**14**; H_D = ^2H) as above in 93% yield m.p. 36—37 °C. The intermediate acid chloride had $\nu_{max.}$ (Nujol) 3 305 (NH), 1 793 (COCl), and 1 712 cm^{-1} (CONH). The final product had *m/z*, 169 [(*M* − CHN_2)$^+$]; $\nu_{max.}$ (Nujol) 3 600—3 100br (NH), 2 123 (CHN$^+$≡N) and 1 703 cm^{-1} (CO of ketone and amide); δ (C^2HCl_3, $CHCl_3$ at 7.24 p.p.m.) 7.41 (1 H, br s, NH), 5.33 (1 H, s, CHN_2), 3.62 (2 H, m, 4-CH_2 and some impurity), and 2.61 (1 H, br t, 3-CH).

(3S,4RS)-[3,4-$^2H_2$]-1-*Diazo*-4-*trifluoroacetylaminobutan*-2-*one* (**15**; H_C = ^2H). This compound was prepared from (2*S*,3RS)-*N*-trifluoroacetyl [2,3-$^2H_2$]-β-alanine (**14**; H_C = ^2H) as above in 94% yield, m.p. 35—37 °C. The intermediate acid chloride had $\nu_{max.}$(Nujol) 3 300 (NH), 1 796 (COCl), and 1 705 cm^{-1} (CONH). The final product had *m/z*, 170 ([*M* − CHN_2]$^+$); $\nu_{max.}$(Nujol) 3 600—3 200 (NH), 2 125 (CHN≡N) and *ca.* 1 700 cm^{-1} (CO) (the spectrum was weak); δ (C^2HCl_3, $CHCl_3$ at 7.24 p.p.m.) 5.33 (1 H, s, CHN_2) 3.62 (1 H, m, 4-CH and impurities, and 2.61 (1 H, br d, 3-CH).

4-*Trifluoroacetylaminobutan*-2-*one* (**16**).—The diazoketone (**15**) (185 mg, 0.89 mmol) was dissolved in chloroform (8 ml) and HI (55% aqueous, *ca.* 0.5 ml) was slowly added dropwise with swirling, in a separating funnel. When the evolution of nitrogen had ceased, additional HI (55% equivalent in volume to the first addition, *ca.* 0.5 ml) was added and the solution was allowed to stand for 10 min. Water (1 ml) was added and the organic layer was separated and washed with water (2 ml), dilute aqueous $Na_2S_2O_3$ until colourless, and water (1 ml). The chloroform solution was finally dried (Na_2SO_4) and the solvent was removed under reduced pressure to give a pale yellow oil (147 mg, 90%); *m/z* (EI), 183 (*M*$^+$), 168 [(*M* − CH_3)$^+$], 140 [(*M* − CH_3CO)$^+$], and 126 [(*M* − CH_3COCH_2)$^+$]; $\nu_{max.}$ (Nujol) 3 335 (NH) and 1 714 cm^{-1} (br, CONH and COMe); δ (C^2HCl_3, external $SiMe_4$ at 0 p.p.m.) 7.31 (1 H, br s, NH), 3.53 (2 H, q, *J* 6.5 Hz, 4-CH_2), 2.71 (2 H, t, *J* 6.5 Hz, 3-CH_2), and 2.13 (3 H, s, CH_3CO). A small amount of the ketone was converted into its semicarbazide, m.p. 193—194 °C (Found: C, 35.1; H, 4.7; N, 23.2. $C_7H_{11}F_3N_4O_2$ requires C, 35.0; H, 4.6; N, 23.3%), *m/z* (EI), 240 (*M*$^+$), 223 [(*M* − NH_3)$^+$] and 181 [(*M* − $NHCONH_2$)$^+$].

(4S) [4-$^2H_1$]-4-*Trifluoroacetylaminobutan*-2-*one* (**16**; H_B = $^2H_2$). This compound was prepared as described above, using (3*S*)-[3-$^2H_1$]-*N*-trifluoroacetyl-β-alanine (**14**; H_B = ^2H) *via* the acid chloride and diazo ketone. The intermediate acid chloride had $\nu_{max.}$(Nujol) 3 320 (NH), 1 798 (COCl), and 1 725 cm^{-1} (CONH) and the intermediate diazo ketone $\nu_{max.}$(Nujol) 3 620—2 800 (NH), 2 118 (CHN$^+$≡N) and 1 718 cm^{-1} (COCH and CONH). The ketone (**16**; H_B = ^2H) had *m/z* 169 [(M − CH_3)$^+$]; δ (C^2HCl_3, $SiMe_4$ at 0 p.p.m.), 7.30 (1 H, br s, NH), *ca.* 3.5 (1 H, under impurities 4-H), 2.72 (2 H, d, *J* 7 Hz, 3-CH_2), and 2.12 (3 H, s, CH_3CO).

(3R)-[3-$^2H_1$]-4-*Trifluoroacetylaminobutan*-2-*one* (**16**; H_D = ^2H). This compound was prepared (77%) from the (3*R*)-[3-$^2H_1$]diazo ketone (**15**; H_D = ^2H) as described above. The product had *m/z*, 169 [(*M* − CH_3)$^+$], 141 [(*M* − CH_3CO)$^+$], and 126 [(*M* − CH_3COCH^2H)$^+$]; $\nu_{max.}$(Nujol) 3 400 (NH) and 1 705 cm^{-1} ($COCH_3$ and CONH); δ (C^2HCl_3, $CHCl_3$ at 7.24 p.p.m.), 3.52 (2 H, m, 4-CH_2), 2.71 (1 H, t, *J* 6.5 Hz, 3-CH_2), and 2.11 (3 H, s, CH_3CO).

(3S,4RS)-[3,4-$^2H_2$]-4-*Trifluoroacetylaminobutan*-2-*one* (**16**; H_C = ^2H). This compound was prepared (75%) from the (3*S*,4*RS*)-[3,4-$^2H_2$]diazoketone (**15**; H_C = ^2H) as described above. The product, *m/z* 169, 170 [(*M* − CH_3)$^+$] and 127 [(*M* − CH^2HCOCH_3)$^+$], was evidently not fully deuteriated at C-4, $\nu_{max.}$ (Nujol) 3 620—3 140 (NH) and 1 710 cm^{-1} (CONH and $COCH_3$); δ (C^2HCl_3, $CHCl_3$ at 7.24 p.p.m.), 3.54 (1 H, br d, 4-CH), 2.71 (1 H, br d, *J* 7 Hz, 3 CH), and 2.13 (3 H, s, $COCH_3$).

2-*Aminoethanol* (**17**).—4-Trifluoroacetylaminobutan-2-one (**16**) (200 mg, 1.09 mmol) was dissolved in a solution of trifluoroperacetic acid in dichloromethane (2.2 M; 7 ml) freshly prepared[13] from 87% H_2O_2. The solution was refluxed for 2.5 h. The cooled solution was reduced to *ca.* 1 ml under reduced pressure and then dissolved in CH_2Cl_2 (20 ml). The organic phase was washed with saturated aqueous $NaHCO_3$ and water (2 ml). The solution was dried (Na_2SO_4) and the solvent was removed under reduced pressure to yield a colourless oil (192 mg, 88%). g.c. (2 m of 3% OV-101 at 100 °C and 0.5 ml s^{-1}) showed two peaks, one, R_t 121 s, accounted for 75% of the product and the other, R_t 165 s accounted for 25% of the product.

The mixture (150 mg, 0.75 mmol) was heated to reflux with 6M-HCl (10 ml) for 90 min. The cooled dark brown solution was lyophilized to yield a dark sticky residue (75 mg). T.l.c. on cellulose in isopropyl alcohol–ammonia (*d* 0.880)–H_2O (25:6:5) showed that the ratio of 2-aminoethanol: β-alanine was 3:1. The residue was dissolved in water (10 ml) and eluted through a small column of Dowex 1 × 8 (6 ml, pre-converted to the OH form). The column was washed with water (20 ml) and the effluent was acidified to pH 2 and lyophilized to a colourless dry gum (72% yield; 63% from the ketone). The ^1H n.m.r. spectrum was identical with that of an authentic sample of 2-aminoethanol hydrochloride.

(1R)-[1-$^2H_1$]-2-*Aminoethanol Hydrochloride* (**17**; H_D = ^2H).

This compound was prepared as above from the ketone (**16**; $H_D = {}^2H$). The product (57% yield from ketone) had a ^1H n.m.r. spectrum similar to that of an authentic sample.[8]

(1S,2RS)-[1,2-$^2H_2$]-2-*Aminoethanol Hydrochloride* (**17**; $H_C = {}^2H$). This compound was prepared as above from the ketone (**16**; $H_C = {}^2H$). The product (57%) had a ^1H n.m.r. spectrum similar to that of an authentic sample.[8]

(2S)-N,O-*Dicamphanoyl*-[2-$^2H_1$]-2-*aminoethanol.*—(2*S*)-[2-$^2H_1$]-2-Aminoethanol (**17**; $H_B = {}^2H$) was prepared as above from the (4*S*)-[4-$^2H_1$]-ketone (**16**; $H_B = {}^2H$). This was converted into the *N,O*-dicamphanoyl derivative by a known[8] method. The 360.13 MHz ^1H n.m.r. spectrum (C^2HCl_3, internal $SiMe_4$ at 0 p.p.m.), had ethanolamine signals at δ 4.38 (2 H, m, 1-CH_2), 3.58 (1 H, m, 2-H_R) and clearly indicated the 2*S* configuration.[8] The Wolff rearrangement had, therefore, occurred with retention of stereochemistry at the primary migrating centre.

(1R)-N,O-*Dicamphanoyl*-[1-$^2H_1$]-2-*aminoethanol.* This compound was prepared by a known[8] method from (1*R*)-[1-$^2H_1$]-2-aminoethanol (**17**; $H_D = {}^2H$) prepared as described above. The 360.13 MHz ^1H n.m.r. spectrum (C^2HCl_3, $CHCl_3$ at 7.24 p.p.m.) is shown in Figure 2d and was similar to that prepared previously[8] (see Figure 2c) but deuterium incorporation was slightly lower and some racemization was evident, *m/z* (EI) 422 (M^+) and 375 [$(M - HCO_2H)^+$]—*ca.* 85% $^2H_1$ and 15% $^2H_0$.

(1S,2RS)-N,O-*Dicamphanoyl*-[1,2-$^2H_2$]-2-*aminoethanol.* This compound was prepared by a known[8] method from the (1*S*,2*RS*)-[1,2-$^2H_2$]-2-aminoethanol (**17**; $H_C = {}^2H$) prepared above. The product had a ^1H n.m.r. spectrum, part of which is shown in Figure 2e. This was in keeping with that[8] of an authentic sample.[8]

Preparation of the Uracil-metabolizing Enzyme System in H_2O.—Fresh bovine liver (250 g, less than 3 h old) obtained from Fitter and Sons Ltd., Norlington Lane, Ringmer, East Sussex, was cut into 24 pieces, perfused in 0.15M-KCl (500 ml) for 10 min, washed with more 0.15M-KCl and immediately rinsed with a buffer (2 × 500 ml) containing sucrose (0.25 M), potassium phosphate (35 mM, pH 7.4), mercaptoethanol (5 mM), and $MgCl_2.6H_2O$ (2.5 mM). These operations were conducted at 2 °C. The liver was blotted dry with tissue paper and homogenised in the above buffer (1 300 ml) for 1 min at high speed in a Waring blender. The cell debris was removed at 38 000 g for 75 min at 2 °C in one run. The supernatant clear amber solution was carefully removed using spinal needles to avoid disrupting the sediment. The pH was adjusted to 4.85 by the dropwise addition of 5% AnalaR acetic acid with slow stirring at 2 °C. Stirring was continued for 15 min, when the precipitated protein was removed by centrifugation at 38 000 g for 10 min at 2 °C.

The supernatant solution was immediately adjusted to pH 7.4 by dropwise addition of 0.5M-KOH at 2 °C. The entire solution (1 120 ml) was treated slowly with pre-crushed ammonium sulphate (235.2 g, 210 g l^{-1} to 33% saturation with continued stirring. After 30 min of complete dissolution, the precipitated protein was removed by centrifugation at 25 000 g for 10 min. The sediment was discarded.

The supernatant solution was treated with pre-crushed ammonium sulphate (162.4 g, 145 g l^{-1} to 55% saturation. After 30 min of stirring at 2 °C, the precipitated protein was collected by centrifugation at 35 000 g for 10 min. The pale grey-brown sediment was rapidly rinsed with the above buffer solution from which the sucrose had been omitted (100 ml total) and the excess of fluid from the centrifuge tube was removed immediately by syringe without disturbing the sediment. The protein was dissolved in this same buffer (110 ml) and dialysed against this buffer (4 l) at 2 °C for 90 min in Visking tubing.

Preparation of the Uracil-metabolizing Enzyme System in ^2H_2O.—Solutions of the enzyme in deuterium oxide were prepared as described above except that the dialysis was carried out using buffers made up in ^2H_2O, the final protein solution in ^2H_2O/buffer (110 ml) being dialysed against two changes of ^2H_2O/buffer (220 ml each) for 1 h each.

Enzyme assays. Those were conducted at 37 °C by monitoring Δ(O.D.) at 340 nm for NADPH→NADP. Assay mixtures contained: potassium phosphate (35 mM, pH 7.4), NADPH (0.24 mM) and uracil (20 μM) in a total volume of 3 ml. Reactions were started by the addition of enzyme. Blanks contained no uracil. The rate was monitored by measuring the difference, Δ(O.D.), between blank and assay mixtures over the first 15 min. 1 Unit (0.278 nKat) is defined as the amount of enzyme which catalyses the reduction of 1 μmol thymine or 1.25 μmol uracil per hour under the conditions of the assay. Protein solutions prepared as above routinely yielded 500—600 units (139—167 nKat) of dihydrothymine dehydrogenase.

Determination of the Deuterium Isotope Effect.—Assays were carried out using uracil exactly as above but in 99.8% ^2H_2O solution. Kinetic data were not accurately reproducible due to low rates and the presence of other NADPH oxidising enzymes. The kinetic isotope effect (k_H/k_D) was calculated to be 4.4 ± 1.2.

Metabolism of Uracil and Isolation of β-*Alanines.*—(1) *Unlabelled uracil in* H_2O. The incubation mixture contained uracil (50 mg, 0.45 mmol), NADPH (10 mg, 1.25×10^{-2} mmol), glucose -6-phosphate monosodium salt (125 mg, 0.44 mmol), glucose-6-phosphate dehydrogenase (1 unit, 16.7 nKat) and the above liver enzyme system (6 ml) containing dihydrothymine dehydrogenase (26 units, 7.2 nKat) and an excess of the other enzymes in a total volume of 60 ml of a buffer pH 7.4, containing potassium phosphate (35 mM), mercaptoethanol (5 mM), and $MgCl_2.6H_2O$ (2.5 mM). This was incubated at 37 °C for 16 h. The protein was denatured by heating to 95 °C for 2 min and ethanol (10 ml) was added. The precipitated protein was removed by centrifugation at 15 000 g for 20 min. The supernatant solution was treated with propanol (1 ml) to prevent frothing and the volume of the solution was reduced to *ca.* 5 ml under reduced pressure. Ethanol (5 ml) was added and insoluble material was removed by centrifugation. The supernatant solution showed the presence of β-alanine when chromatographed on cellulose plates using Pr^iOH–NH_3(*d* 0.880)–H_2O (25:6:5) as eluant and developed with ninhydrin. Some traces of other ninhydrin positive material were also detected. The solution was applied to three 20 × 20 cm × 1 mm cellulose plates and developed using the same system. β-Alanine was removed from the plates by suspending the cellulose in H_2O–EtOH (1:1) and stirring for 10 min. The cellulose was filtered off through a small pad of Celite and the filtrate was lyophilized to yield *ca.* 10 mg of crude β-alanine. The 90 MHz ^1H n.m.r. spectrum in ^2H_2O showed two triplets identical with those of authentic material.

(2) [6-^2H]*Uracil in* H_2O. The incubation was performed as described above except that [6-^2H]uracil[11] (50 mg, 0.45 mmol) was used and the amount of glucose-6-phosphate dehydrogenase was increased to 2 units (33.4 nKat). After 31 h at 37 °C, the protein was denatured and the β-alanine was isolated as described above to give *ca.* 10 mg of crude [3-$^2H_1$]-β-alanine. The compound showed identical R_F mobility (0.42) to authentic material, and had a ^1H n.m.r. spectrum in keeping with that of the synthetic control.

The compound was converted into its *N*-(−)-camphanoyl derivative as described elsewhere in this work. The 360 MHz ^1H

n.m.r. spectrum (Figure 1e) shows that the β-carbon is (*R*) by comparison with the synthetic sample (Figure 1b); *m/z* (EI) 270 (M^+) and 224 ($[M - HCO_2H]^+$)—*ca.* 90% $^2H_1$.

(3) [5-^{2}H]*Uracil in* H_2O. The incubation was performed as described above except that [5-^{2}H]uracil[11] (50 mg, 0.45 mmol) was used. After 31 h at 37 °C the protein was denatured and the reaction was worked up as described above to yield *ca.* 10 mg of crude [2-$^2H_1$]-β-alanine, R_F 0.42 on cellulose in the usual system. The ^{1}H n.m.r. spectrum was in keeping with that of the synthetic control. A sample of the *N*-camphanoyl derivative was prepared using *ca.* 0.5 mg of this material, *m/z* (EI), 270 (M^+) and 224 $[(M - HCO_2H)^+]$—*ca.* 76% $^2H_1$.

The remainder of the β-alanine was converted into [1-$^2H_1$]-2-aminoethanol by the method developed for the synthetic material as described above (*i.e.* trifluoroacetylation, preparation of the acid chloride and diazoketone, reduction to the methyl ketone, Baeyer-Villiger oxidation and hydrolysis of the protecting groups) without purification of the intermediates. The 2-aminoethanol (*ca.* 1 mg) (identified by ninhydrin development of cellulose plates) was purified on one 20 × 20 × 1 mm cellulose plate to yield a pale brown smear which was converted into the *N,O*-dicamphanoyl derivative in the usual way. The 360.13 MHz ^{1}H n.m.r. spectrum, part of which is shown in Figure 2f, showed unambiguously that the product had the (1*R*) configuration by comparison with the spectrum of the authentic compound[8] (Figure 2c and d); *m/z* (EI) 422 (M^+), 394 $[(M - CO)^+]$, 376 $[(M - HCO_2H)^+]$, and 361 $[(M - HCO_2H - CH_3)^+]$, showing *ca.* 71% $^2H_1$.

(4) *Uracil in* ^2H_2O. The incubation was carried out on twice the scale as described above in 120 ml of ^2H_2O/buffer at pH 7.4 and 37 °C. Twice as much dialysed liver enzyme extract was used (24 ml, 104 units, 28.8 nKat of dihydrothymine dehydrogenase) and the incubation was worked up after 88 h instead of 31 h. β-Alanine was isolated in the usual way to yield *ca.* 8 mg of crude compound showing an identical R_F value to the authentic compound, and having the expected ^{1}H n.m.r. spectrum.

Roughly 25% of the β-alanine was converted into its *N*-camphanoyl derivative as described above. The ^{1}H n.m.r. spectrum of this compound is shown in Figure 1d. The stereochemistry at C-3 was shown to be indisputably (3*S*) by comparison with the spectrum of the synthetic specimen (Figure 1c); *m/z* (EI), 271 (M^+) and 225 $[(M - HCO_2H)^+]$, *ca.* 80% $^2H_2$, 20% $^2H_1$, and 0% $^2H_0$.

The remaining 75% of the product was converted into 2-aminoethanol and purified as described above. The *N,O*-dicamphanoyl derivative was prepared in the usual way, but insufficient material was obtained for a useful ^{1}H n.m.r. spectral chirality assay; *m/z* (EI), 423 (M^+), 395 $[(M - CO)^+]$, 377 $[(M - HCO_2H)^+]$ and 362 $[(M - HCO_2H - CH_3)^+]$—*ca.* 65% $^2H_2$, 35% $^2H_1$ (±10%).

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