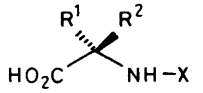
Convenient Synthesis of Stereospecifically Deuteriated Glycines from Glutamic Acid using a Combination of Enzymatic and Chemical Methods†

Enzo Santaniello,* Rosangela Casati, and Ada Manzocchi
Dipartimento di Chimica e Biochimica Medica, Università di Milano, Via Saldini, 50, 20133 Milano, Italy.

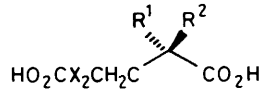
Chiral glycines (**1a**) and (**1b**) have been synthesized starting from stereospecifically deuteriated 4-aminobutanoic acids (**3a**) and (**3b**). Enzymatic decarboxylation of (2*S*)-glutamic acid (**2a**) in ^2H_2O and (2*R*,*S*)-[2,4,4-$^2H_3$]glutamic acid (**2b**) + (**2c**) in H_2O afforded (**3a**) and (**3b**), respectively. Chemical transformations of deuteriated pyrrolidinones (**4a**) and (**4b**) afforded stereospecifically labelled glycines (**1a**) and (**1b**) in good yields.

Several syntheses of the chiral [2-$^2H_1$]glycines (**1a**) and (**1b**) have been reported, and although they involve chemical sequences, one or two of the steps are mediated by an enzyme.[1–5] An entirely chemical approach for making optically enriched (but not chirally pure) monodeuteriated glycines relies upon the fact that the diastereotopic protons of glycine in cobalt(III) complexes are selectively exchanged by deuterium.[6,7] Alternatively, two chemical syntheses of chiral glycines starting from suitable chiral precursors have appeared in the recent literature. Thus, (*R*)- and (*S*)-[2-$^2H_1$] glycines can be prepared in a highly stereospecific manner starting from (*R*)- and (*S*)-*O*-benzylserine[8] or using D-glucose as a chiral template.[9] Apart from the biosynthetic applications of deuteriated or tritiated glycines,[10] stereospecifically labelled (**1a**) and (**1b**) also constitute a convenient starting material for the preparation of chiral acetic acids in 100—500 mg quantities.[11]



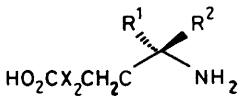
(**1**)

a; R^1 = H, R^2 = ^{2}H, X = H
b; R^1 = ^{2}H, R^2 = H, X = H
c; R^1 = H, R^2 = ^{2}H, X = CH_2Ph



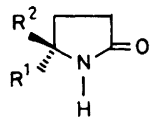
(**2**)

a; R^1 = NH_2, R^2 = H, X = H
b; R^1 = NH_2, R^2 = ^{2}H, X = ^{2}H
c; R^1 = ^{2}H, R^2 = NH_2, X = ^{2}H



(**3**)

a; R^1 = H, R^2 = ^{2}H, X = H
b; R^1 = ^{2}H, R^2 = H, X = ^{2}H



(**4**)

a; R^1 = ^{2}H, R^2 = H
b; R^1 = H, R^2 = ^{2}H

We present now our highly stereospecific synthesis of chiral glycines, starting from glutamic acids (**2a**) and (**2b**) + (**2c**). In this procedure, the carboxylic acid groups of (*S*)-(**2a**) and (**2b**) are stereospecifically replaced by deuterium or hydrogen *via* an enzymatic decarboxylation catalyzed by commercially available glutamate decarboxylase from *Escherichia coli* (Sigma, U.S.A.). It had been demonstrated by us[12] and others[13] that the reaction catalyzed by glutamate decarboxylase from *E. coli* proceeds with complete retention of configuration, in agreement with the general mechanism of the decarboxylation catalyzed by pyridoxal phosphate dependent enzymes.[14] By decarboxylation of (**2a**) in ^2H_2O in the presence of the enzyme, gram quantities of (4*R*)-[4 $^2H_1$]-4-aminobutanoic acid (**3a**) can be prepared with a high degree of stereospecificity. The above deuteriated γ-amino acid has been used as starting material for the synthesis of (2*S*,5*R*)-[5-$^2H_1$]proline[15] and stereospecifically deuteriated putrescine.[16]

Since it has previously been shown that chromic oxidation of pentane-1,5-diamine yields glycine directly, though in poor yields,[4] we first attempted direct oxidation of the γ-amino acid (**3**) to glycine (**1**). Several types of reagents for the chromic oxidation were tried at room temperature with no effect on 4-aminobutanoic acid (**3**), whereas under more drastic conditions, compound (**3**) yielded almost exclusively succinic acid, with less than 0.1% glycine.

We thought that since several manipulations were needed for the transformation of 4-aminobutanoic acid (**3**) into the α-amino acid (**1**), the cyclic form corresponding to (**3a**), namely (5*R*)-[5-$^2H_1$]pyrrolidin-2-one (**4a**), could be the most suitable starting material, as in the synthesis of stereospecifically deuteriated proline.[15] Thus, compound (**3a**) was only partially purified from the incubation mixture (see Experimental section) and directly cyclized to pyrrolidinone (**4a**)[17] (86% yield of isolated product).

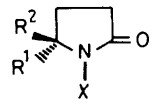
Protection of nitrogen seemed a mandatory step in the elaboration of the lactam (**4a**), *i.e.* preparation of *N*-protected pyrrol-2(5*H*)-one (**7**) from (**4a**) and cleavage to form *N*-protected glycine (**1c**). The crucial points of the overall transformation‡ were the choice of the method of protection of the lactamic nitrogen, the introduction of the double bond at positions 3 and 4, and the conditions for the cleavage of the double bond in the pyrrol-2(5*H*)-one (**7**).

At first we tried the protection of nitrogen with a benzoyl group, but introduction of phenylseleno or bromine§ groups onto (**5a**) was not satisfactory in our hands and this route was

† Presented as a preliminary communication at the Third European Symposium on Organic Synthesis, Canterbury, 1983.

‡ Single steps were modelled on unlabelled material and only after optimization of yields was the synthesis repeated on a stereospecifically labelled specimen.

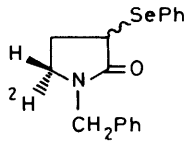
§ Photochemical α-bromination of *N*-benzoylpyrrolidin-2-one without solvent and on a 100 g scale has been described: H. Wamhoff and R. Lohmar, *Synthesis*, 1976, 331. In small scale or in solution in various solvents, yields of the monobromo derivative were low, the main by-product being the α,α′-dibrominated derivative. Furthermore, various methods for dehydrohalogenation of α-bromopyrrolidone afforded unsatisfactory yields of the desired pyrrol-2(5*H*)-one. On the other hand, selenylation of *N*-benzoylpyrrolidin-2-one afforded a mixture of mono- and di-selenylated derivatives of protected and deprotected lactams.



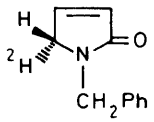
(5)

a; $R^1 = R^2 = H$, X = COPh

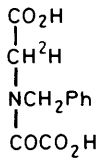
b; $R^1 = {}^2H$, $R^2 = H$, X = CH_2Ph



(6)



(7)



(8)

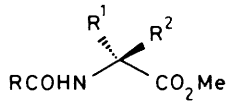
abandoned. We reasoned that the electron withdrawing properties of the benzoyl group enhanced too much the reactivity of the position 3 in the lactam ring, thus lowering the selectivity of the reactions required in order to introduce a double bond into the molecule.

For the above reasons, we chose as protecting group for the lactamic nitrogen a benzyl group, which could be efficiently introduced by a solid–liquid phase transfer reaction* (powdered potassium hydroxide, 18-crown-6, benzyl chloride or bromide in benzene). By this route, (5*R*)-[5-$^2H_1$]pyrrolidin-2-one (**5b**) could be prepared in 85% yield and introduction of a phenylseleno group at position 3 was achieved (LDA, −78 °C, PhSeCl),[18] affording (5*R*)-[5-$^2H_1$]-1-benzyl-3-phenylseleno-pyrrolidin-2-one (**6**) in 50—60% yields after flash chromatography.[19] Oxidation of the α-phenylseleno lactam (**6**) with ethanolic hydrogen peroxide[20] afforded in 80—85% yields (5*R*)-[5-$^2H_1$]pyrrol-2(5*H*)-one (**7**), which was stable enough to be purified by flash column chromatography. Cleavage of the C(3)–C(4) double bond of the pyrrol-2(5*H*)-one (**7**) was a crucial step, since partial oxidation of the benzyl group was observed for some experimental conditions in the ozonolysis–oxidation step employed for the purpose. Furthermore, in a few experiments with labelled material it was found that if alkaline hydrogen peroxide was used for oxidation of the ozonide formed, scrambling of the deuterium could occur and consequent racemization be observed. The best conditions for the formation of deuteriated *N*-benzylglycine (**1c**) from the pyrrol-2(5*H*)-one (**7**) were found to be ozonolysis (−78 °C, ethyl acetate) and oxidation with performic acid at room temperature (3 h). By this method however, only part of (**1c**) was directly formed and acid hydrolysis of the isolated oxalamide (**8**) was necessary. Overall yields of *N*-benzylglycine (**1c**) by the above procedure after purification by ion exchange column chromatography was 45—55%†.

Reductive cleavage of the benzyl group was achieved with Pd black in aqueous acetic acid and the desired chiral glycine (**1a**) was isolated after purification on an ion exchange column and characterized after crystallization from methanol–water.[8]

By the above synthetic procedure, starting from 90% deuteriated (4*R*)-[4-$^2H_1$]-(**3a**) prepared from (2*S*)-glutamic acid (**2a**) in ^2H_2O in the presence of glutamate decarboxylase from *E. coli*,‡ (2*R*)-2^2H_1-(**1a**) was prepared in 15—20% yield from 4-aminobutanoic acid (**3a**) with retention of practically all the label, as ascertained by ^1H n.m.r. and mass spectroscopy.

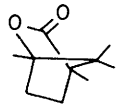
The percentage deuteriation and chiral purity of the final product was established by converting the deuteriated glycine into the camphanoylamide (**9a**) derived from (−)-(1*S*,4*R*)-ω-camphanic acid.[21] Mass spectrometric analysis of compound (**9a**) showed 90% of monodeuteriation and less than 0.1% of



(9)

a; $R^1 = {}^2H$, $R^2 = H$

b; $R^1 = H$, $R^2 = {}^2H$

R = 

dideuteriation. The chiral purity was established by examining the n.m.r. signals of compound (**9a**). The two diastereotopic protons are well separated in the presence of the shift reagent $Eu(dpm)_3$ in $CDCl_3$, the signals from the *pro-R* hydrogen atom occurring at higher field.[8] Integration of the peak areas of *pro-S* and *pro-R* hydrogens showed that the *pro-R* proton corresponded to the residual 10% of non-deuteriated compound, thus establishing that (*R*)-[2-$^2H_1$]glycine was virtually chirally pure. It was therefore concluded that the overall transformation proceeded with complete retention of the original stereochemistry.

For the preparation of (2*S*)-[2-$^2H_1$]glycine (**1b**), we started from commercially available (2*R*,*S*)-[2,4,4-$^2H_3$]-glutamic acid (**2b**) + (**2c**) (98% isotopically pure, MSD Isotopes, West Germany). Incubation of the racemic mixture of trideuteriated glutamic acid in H_2O in the presence of glutamate decarboxylase from *E. coli* afforded a mixture of (2*R*)-(**2b**) and (4*S*)-[2,2,4-$^2H_3$]-(**3b**), separable by ion exchange chromatography.[12] (4*S*)-[2,2,4-$^2H_3$]-4-Aminobutanoic acid (**3b**) was obtained with 98% deuteriation and further elaborated by the synthetic procedure described above. (2*S*)-Glycine (**1b**) could be obtained optically pure as established by the n.m.r. spectrum of its camphanoylamide (**9b**).

Experimental

All m.p.s are uncorrected. I.r. spectra were recorded for solutions in chloroform or for Nujol mulls. ^1H N.m.r. spectra were recorded on a Varian 360 L spectrometer for solutions in $CDCl_3$ using $SiMe_4$ as internal standard, and 100 MHz ^1H n.m.r. spectra on a Varian XL-100 instrument. Mass spectra were recorded on a LKB 2091 Gas Chromatograph–Mass Spectrometer by D.I.S. unless otherwise stated. The progress of all reactions and column chromatographies was monitored by t.l.c. on Merck silica gel HF_{254} plates and visualized by u.v. absorption or by exposing plates to iodine vapour or spraying with ninhydrin solution.[22] Distillations for analytical purposes were performed on a glass tube oven Buchi GKR-50.

(5R)-[5-$^2H_1$]*Pyrrolidin-2-one* (**4a**).—This compound was prepared essentially as described in our previous work on the

* The best yields were achieved by applying the reaction conditions found to be effective for *N*-alkylation of pyrroles and indoles: E. Santaniello, C. Farachi, and F. Ponti, *Synthesis*, 1979, 617.

† Some glycine was formed during this step by hydrolysis of minute amounts of *N*-benzoylglycine formed by oxidation of (**1c**), as revealed by t.l.c.

‡ Longer reaction times or use of less pure preparations of glutamate decarboxylase lowered the content of the monodeuteriated species and enhanced dideuteriation. This result could be due to contamination of the enzyme preparation with a transaminase. Transaminases are known to labilise α-hydrogen atoms of α-amino acids in a *pro-R* configuration; see ref. 1 and H. C. Dunathan, *Adv. Enzymol.*, 1971, **35**, 79.

synthesis of deuteriated proline,[15] the yields being improved by the use of small amounts of solvent and silylating agent. GAD SIGMA (type II) was used as the source of glutamate decarboxylase from *E. coli*, and incubation in ^2H_2O of (**2a**) (1.2 g, 8.16 mmol) as described[12] yielded (4*R*)-[4-$^2H_1$]-4-aminobutanoic acid (**3a**). At the end of the incubation, methanol was added and the crude mixture refluxed (20 min). After centrifugation (15 min at 1200 g), the supernatant liquid was evaporated to dryness and the crude residue of the enzymatic decarboxylation was dried (100 °C, 30 min) and then refluxed for 6 h in xylene (167 ml) containing hexamethyldisilazane (21.2 ml) and a few drops of dimethyldichlorosilane. At the end of the reaction, absolute ethanol (445 ml) was added and the solution evaporated. The crude residue was taken up with chloroform, filtered on a Celite pad, evaporated, and purified by silica gel column chromatography (Merck, 70—230 mesh). Fractions eluted with chloroform–methanol, 9:1, yielded the deuteriated pyrrolidone (**4a**) (607 mg, 86.4% yield); δ 1.90—2.50 (4 H, complex m, CH_2CH_2), 3.40 (1 H, br t, C^2HH), and 7.75 (1 H, m, exch.); *m/z* 86 (M^+), 57, 56, 44, 43 (100%), and 42. A sample was distilled for analytical purposes to afford pure (**4a**), b.p. 75 °C at 0.2 mmHg (Found: C, 56.05; H + D 9.45; N, 16.1. Calc. for C_4H_6DNO: C, 55.8; H + D, 9.3; N, 16.3%). Quantitative estimation of deuterium content of (4*R*)-[4-$^2H_1$]-4-aminobutanoic acid (**3a**) was performed by g.l.c.–mass spectrometry of its trifluoroacetamide butyl ester[23] using a column OV 17 (3 m, *t* 160 °C, R_t 5 min). For the analysis we considered peaks at *m/z* 182 ($M - 73$)$^+$ (unlabelled derivative), *m/z* 183 ($M - 73$)$^+$ [derivative of monodeuteriated (**3a**)], and *m/z* 184 ($M - 73$)$^+$, [derivative of dideuteriated (**3a**)]. Under the conditions of incubation previously described, the percentage of monodeuteriated species was 90.2%, along with 9.7% of unlabelled and <0.1% of dideuteriated species.

(5R)-[5-$^2H_1$]-1-*Benzylpyrrolidin*-2-*one* (**5b**).—A mixture of potassium hydroxide (484.5 mg, 8.65 mmol), 18-crown-6 (91.5 mg, 0.346 mmol), and deuteriated pyrrolidinone (**4a**) (491 mg, 5.70 mmol) was stirred at room temperature and then brought to reflux temperature. Then benzyl bromide (0.82 ml, 6.9 mmol) was added dropwise and the reaction mixture was refluxed for a further 1 h. At the end of the reaction (monitored by t.l.c., chloroform–methanol 9:1), the mixture was filtered and evaporated to dryness. The residue (1.23 g) was purified by silica gel column chromatography (Merck, 70—230 mesh). Elution with chloroform furnished the title compound, which was distilled at 182—185 °C at 14 mmHg to afford analytically pure (**5b**) (843 mg, 4.84 mmol, 85% yield) (Found: C, 74.85; H + D, 7.9; N, 7.9. $C_{11}H_{12}DNO$ requires C, 75.0; H + D 8.0; N, 7.95%); $\nu_{max.}$ 3 340, 2 850, 2 150, and 1 660 cm^{-1}; δ 1.70—2.75 (4 H, complex m, CH_2CH_2), 3.26 (1 H, br t, CH^2H), 4.47 (2 H, s, CH_2 benzylic), and 7.35 (5 H, aromatic complex); $[\alpha]_D = -0.44°$ (*c* 5.26 in $CHCl_3$); *m/z* 176 (M^+), 175, 147, 146, 105, 104, 91 (100%), and 85. Percentage of unlabelled and deuteriated species was established by gas chromatography–mass spectrometry (OV 17 3%, *t* 170 °C) focussing peaks at *m/z* 175 (M^+ of unlabelled compound, 9.7%), 176 (M^+ of monodeuteriated compound, 90.2%), and 177 (M^+ of dideuteriated compound, <0.1%).

(5R)-[5-$^2H_1$]-1-*Benzyl*-3-*phenylselenopyrrolidin*-2-*one* (**6**).—A solution of butyl-lithium in hexane (8 ml, 13.66 mmol), diisopropylamine (freshly distilled, 1.94 ml, 13.66 mmol) in dry THF (14 ml) was stirred at 0 °C under an argon atmosphere. After cooling at −78 °C, compound (**5b**) (1.2 g, 6.83 mmol) in dry THF (7 ml) was added. After 10 min benzeneselenenyl chloride (1.3 g, 6.83 mmol, recrystallized from hexane) in dry THF (8 ml) was slowly added. After 5 min, the reaction was allowed to warm to room temperature and then poured into 1M-M-formate buffer (pH 3; 180 ml) and extracted with ether. The extract was dried and evaporated and the oily residue (2.35 g) was purified by flash chromatography hexane–ethyl acetate, 7:3) to give the title compound (**6**) (1.28 g, 3.86 mmol, 56.5% yield); 1.90—2.60 (2 H, m, CH_2), 2.95 (1 H, m, CH^2H), 3.98 (1 H, m, CHSePh), 4.42 (2 H, s, CH_2 benzylic), and 7.00—7.85 (10 H, aromatic complex); *m/z*: 331, 175, 157, and 91 (100%). A sample was distilled for analytical purposes to afford pure (**6**), b.p. 240 °C at 0.2 mmHg (Found: C, 61.5; H + D 5.4; N, 4.1. $C_{17}H_{16}$ DNOSe requires C, 61.6; H, 5.5; N, 4.2%).

(5R)-[5-$^2H_1$]-1-*Benzyl*-3-*pyrrol*-2(5H)-*one* (**7**).—The above phenylselenopyrrolidinone (**6**) (782 mg, 2.36 mmol) was dissolved in ethanol (8.6 ml) and the solution was cooled at 0°C. 35% Hydrogen peroxide (1.4 ml) was then added dropwise and the mixture was stirred at 0 °C (the reaction was monitored by t.l.c., methylene chloride–acetone, 85:15). After 30 min, a solution of ferrous sulphate heptahydrate (4.12 g, 14.8 mmol) in water (20 ml) was added slowly. The mixture was diluted with water (40 ml), and extracted with methylene chloride. The organic layer was washed with aqueous sodium hydrogen carbonate and water, dried, and evaporated. The title pyrrolone (**7**) (344 mg, 1.98 mmol, 83.7% yield) was obtained after purification by flash chromatography (solvent system methylene chloride–acetone 87:13); $\nu_{max.}$ 3 000, 2 150, and 1 675br cm^{-1}; δ 3.85 (1 H, m, CH^2H), 4.61 (2 H, s, CH_2 benzylic), 6.20 (1 H, d, CH=, *J* 6 Hz), 7.02 (1 H, d, CH=, *J* 6 Hz), and 7.26 (5 H, aromatic complex); $[\alpha]_D = +1.48°$ (*c* 5.19 in $CHCl_3$); *m/z*: 174 (M^+), 173, 106, and 91 (100%). A sample was crystallized from di-isopropyl ether and had m.p. 60—61 °C (Found: C, 75.6; H + D, 6.8; N, 7.9. $C_{11}H_{10}DNO$ requires C, 75.8; H + D, 6.9; N, 8.0%). Percentage of deuteriated and unlabelled species were estimated on peaks 173 (M^+ of unlabelled, 10%), 174 (M^+ of monodeuteriated, 89.9%), and 175 (M^+ of dideuteriated, <0.1%).

(2R)-[2-$^2H_1$]-N-*Benzylglycine* (**1c**).—Ozone-enriched oxygen (*ca.* 5% enrichment) was bubbled into a solution of the pyrrolone (**7**) (174 mg, 1 mmol) in dry ethyl acetate (7 ml) at −80 °C for *ca.* 5 min. After evaporation of the solvent under argon, a solution of formic acid (56.56 μl, 1.5 mmol), 35% hydrogen peroxide (129 μl, 1.5 mmol) and water (0.415 ml) was added slowly at 0 °C. The mixture was stirred for 3 h at room temperature. The reaction was diluted with water (1 ml) and adjusted to pH 5 by addition of 2M sodium hydroxide. The precipitate was filtered off and worked up separately as indicated later. The filtrate was treated with KI (99.6 mg, 0.6 mmol), washed with methylene chloride (discarded), and evaporated. The oily residue was dissolved in 2M-HCl (0.8 ml) and heated at 100 °C in a sealed tube for 15 min. The solution was cooled, brought to pH 2 with a 2M aqueous NaOH and placed on an AG 50W × 2 column (22 ml, H^+ form after three cycles with 1M-HCl and aqueous 3% ammonia). The column was washed with water (260 ml) and eluted with aqueous 0.5% ammonia. Evaporation of the ammonia fractions yielded *N*-benzylglycine (**1c**) (4.3 mg). The oxalamide (**8**), obtained as the precipitate from the oxidation of the ozonide, and 2M-HCl (0.8 ml) were heated at 100 °C in a sealed tube for 15 min. The acid solution was cooled and purified by exchange column chromatography on an AG 50W × 2 column as previously described to yield *N*-benzylglycine (**1c**) (57 mg). All the fractions eluted with water from the two columns were evaporated and treated with 4M-HCl and heated at 100 °C in a sealed container for 30 min to yield (**1c**) (24.3 mg). The final yield of deuteriated *N*-benzylglycine (**1c**) was 85.6 mg (51.3%); δ (^2H_2O) 3.40 (1 H, br s, CH^2H), 4.05 (2 H, s, CH_2 benzylic), and 7.40 (5 H, aromatic complex).

(2R)-[2-$^2H_1$]*Glycine* (**1a**).—Pd black (10 mg) was added to a solution of *N*-benzylglycine (**1c**) (83 mg, 0.5 mmol) in acetic acid–water, 1:2 (1.5 ml). The resulting suspension was stirred under a hydrogen atmosphere at room temperature and pressure. The progress of the reaction was monitored by t.l.c. ($BuOH–H_2O–AcOH$, 6:2:2). In order to complete the hydrogenolysis, the catalyst was filtered off and replaced several times. When the reaction was complete, the catalyst was filtered off, and the aqueous solution extracted with toluene (discarded) and evaporated. The residue was dissolved in water, decolourised (charcoal), filtered, and evaporated. The residue consisting of deuterioglycine acetate was dissolved in water and placed on an ion exchange column, as described by Armarego *et al.*,[8] to yield (*R*)-glycine (34 mg, 0.45 mmol, 90%). After one crystallization (H_2O–MeOH) compound (**1a**) was analytically pure; m.p. 232—234 °C (decomp.) (Found: C, 31.75; H + D, 8.1; N, 18.3. Calc. for $C_2H_4DNO_3$: C, 31.6; H + D, 7.9; N, 18.4%).

(1S, 4R)-ω-*Camphanoylglycine Methyl Ester* (**9a**).—Deuteriated glycine (**1a**) obtained by the above method (30 mg, 0.39 mmol) was examined for deuterium content and chirality as its camphanoylamide methyl ester, prepared as described by Armarego.[8] The yield of compound (**9a**) after silica gel column chromatography (Merck, 70—230 mesh) was 75 mg (70%); *m/z* 270 (M^+), 223, 195, 188, 134, 107, and 83 (100%). Percentages of deuteriated and unlabelled species were estimated by g.l.c.–m.s. (SE 30 1%, *t* 150 °C) focussing peaks 269 (M^+ of unlabelled, 10%), 270 (M^+ of monodeuteriated, 89.9%), and 271 (M^+ of dideuteriated, <0.1%).

The 100 MHz ^1H n.m.r. spectrum recorded in the presence of $Eu(dpm)_3$ was in accord with data reported by Armarego *et al.*[8] and after integration of peaks the relative proportions of the two diastereotopic 2-protons at 4.03 and 4.13 p.p.m. showed that the sample of (*R*)-(**1a**) was optically pure.

(4S)-[2,4,4-$^2H_3$]-4-*Aminobutanoic Acid* (**3b**).—Enzymatic decarboxylation of (2*S*)-(**2c**) was carried out using the racemic mixture (2*R*,*S*)-[2,4,4-$^2H_3$]glutamic acid (**2b**) + (**2c**) (MSD Isotopes, West Germany). The enzymatic reaction was performed in H_2O as described[12] and the final mixture of deuteriated (2*R*)-(**2b**) and title (**3b**) were separated by ion exchange column chromatography.[12] A sample was recrystallized from ethanol–water, m.p. 202—205 °C (Found: C, 45.1; H + D 11.2; N, 13.0. Calc. for $C_4H_6D_3NO_2$: C, 45.3; H + D, 11.4; N, 13.2%). The product was lyophilized and cyclized to the corresponding pyrrolidinone (**4b**).

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