

A Facile Synthesis of (2*RS*,3*S*,4*S*)-[3,4-²H₂]Homoserine Lactone

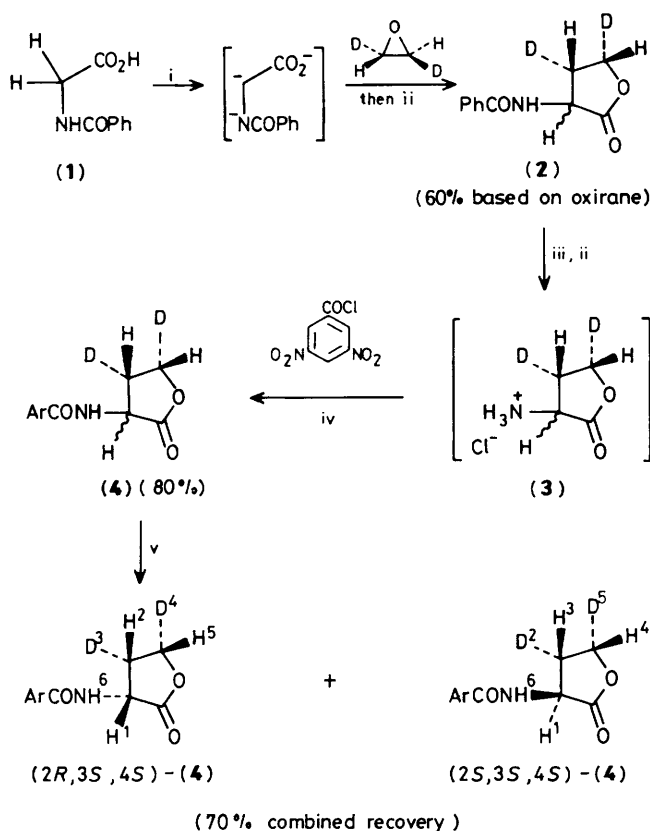
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Homoserine lactone chirally labelled with deuterium at C-3 and C-4 has been synthesized in two steps, starting from chirally labelled oxirane.

Homoserine is a substrate for a number of enzyme-catalysed reactions that result in bond-making and bond-breaking at carbons 3 and/or 4.^{1–5} To obtain mechanistic information, the stereochemical courses of several of these reactions have been determined,^{2,6–10} using chirally labelled homoserine samples obtained by rather lengthy syntheses.^{2,7,11,12} We now describe a new, especially facile synthesis of chirally labelled homoserine, made possible by the availability of the chiral labelling synthons (*R,R*)- and (*S,S*)-[2,3-²H₂]oxirane.¹³

Hippuric acid (**1**) was treated with three equivalents of lithium di-isopropylamide* (LDA) in hexamethylphosphoramide (HMPA)–tetramethylethylenediamine (TMEDA)–tetrahydrofuran (THF), and the resulting trianion¹⁴ was reacted with (*S,S*)-[2,3-²H₂]oxirane {generated from (2*R*,3*S*)-(triphenylsilyl)-[2,3-²H₂]oxirane}, Scheme 1.¹³ Acidification and heating of the alkylation reaction mixture gave *N*-benzoylhomoserine lactone (**2**), which after purification was hydrolysed with aqueous base. Reacidification and applica-



Scheme 1. Reagents and conditions: i, LDA (3 equiv.), HMPA-TMEDA-THF, -78°C ; ii, HCl (3 M), reflux, 15 min; iii, NaOH (6 M), reflux, 6 h; iv, NaHCO₃, H₂O; v, h.p.l.c. separation on D-naphthyl-alanine bonded-phase packing.

tion of heat provided racemic, chirally labelled homoserine lactone (3), which, for the purpose of demonstrating its configurational integrity, was converted directly to its *N*-(3,5-dinitrobenzoyl) derivative (4). The latter was resolved by h.p.l.c.¹⁵ Comparison with authentic standards showed that the (*S*) enantiomer elutes considerably ahead of the (*R*) enantiomer.

At 470 MHz, the ¹H n.m.r. resonances of the five lactone ring protons of *N*-(3,5-dinitrobenzoyl)homoserine lactone are clearly separated from one another (Figure 1), and assignments were made as follows. Single frequency ¹H decoupling experiments and COSY spectra had shown that the C-2 proton is at lowest field (δ 4.782) and the C-3 protons at highest field (δ 2.446 and 2.605). Apparent coupling constants were obtained by inspection and were confirmed by spectral simulation using the RACCOON computer program. Starting with the assumption that the bulky dinitrobenzamido substituent occupies a pseudoequatorial position on the lactone ring, couplings were analysed *via* the Karplus relationship, and signal assignments made as shown in Figure 2. Significantly, the axial protons resonate at higher field than do their equatorial counterparts. The relevant portions of the ¹H n.m.r. spectra of the chirally deuteriated 3,5-dinitrobenzamides (4) are also shown in Figure 1. As expected from the synthetic route, the stereochemical purities of the (2*R*) and (2*S*) compounds are quite high. The slight stereoisomeric impurity (3%, by n.m.r. integration) evident in (2*S*)-(4) is possibly the result of a slight lack of enantiomeric purity of the

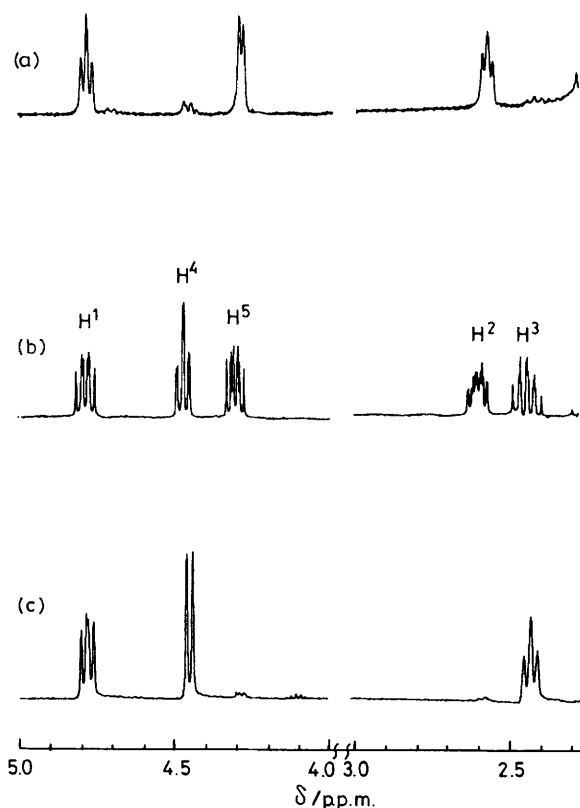


Figure 1. ¹H N.m.r. spectra (470 MHz; CD₃CN) of labelled [(a) (2*R*)-(4) and (c) (2*S*)-(4)] and (b) unlabelled *N*-(3,5-dinitrobenzoyl)homoserine lactone.

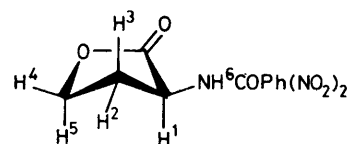


Figure 2. Perspective drawing of homoserine lactone. Coupling constants: $J_{1,6}$ 8.03, $J_{2,5}$ 6.50, $J_{1,2}$ 9.10, $J_{3,4}$ 9.08, $J_{1,3}$ 10.93, $J_{3,5}$ 10.63, $J_{2,3}$ 12.40, $J_{4,5}$ 9.06, $J_{2,4}$ 1.83 Hz.

di-isopropyl tartrate used in the chiral oxirane synthesis. {A recent reanalysis of a sample of (*S,S*)-[2,3-²H₂]oxirane indicated that it had an enantiomeric excess (e.e.) value of 94% (*cf.* ref. 14). This figure is consistent with the result shown in Figure 1(c) and the explanation that we have offered.} The relatively larger (*ca.* 7%) stereoisomeric impurity in (2*R*)-(4) is probably an artifact of the chromatographic resolution of the lactone enantiomers. The chiral h.p.l.c. column was severely overloaded, and both peaks tailed badly. Since the (2*R*) enantiomer was the second to elute, it became contaminated with the (2*S*) compound.

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