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Versatile Synthesis of L-α-Amino Acids Stereospecifically Labelled on the β-Carbon Atom

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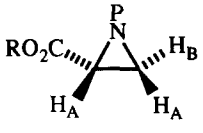
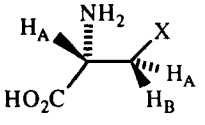
Abstract : Inversion of the stereochemistry at the α-centre of stereospecifically labelled samples of (2S)-isoserine (**7**) using a sequence involving an intramolecular substitution reaction has allowed synthesis of the stereospecifically labelled aziridines (**19**) to be completed. These are synthons for a very versatile preparation of L-amino acids which are stereospecifically labelled at the β-carbon atom.

Elucidation of the mechanism of action of the many enzymes which metabolise amino acids has attracted much attention over the years. An important aspect of these studies has been the discovery of the stereochemical outcome of the reactions catalysed by these enzymes.[1] In most naturally-occurring amino acids, the β-carbon atom is prochiral and so the stereochemical consequences of reactions at this centre require that stereospecifically labelled substrates and products be synthesised. Many different syntheses have been devised[1] and recently, because of our interest in the enzymes which metabolise D-amino acids and are, therefore, targets for anti-bacterial drugs, we have devised an extremely versatile approach to the synthesis of samples of D-amino acids which are stereospecifically labelled at the β-carbon atom with deuterium.[2] This involved synthesis of the stereospecifically labelled aziridines (**1, H_A = ^2H**) and (**1, H_B = ^2H**)) which have served as the common intermediates in the preparation of a large number of different labelled amino acids (**2**).[2-7] We have used these labelled compounds in studies on enzyme stereochemistry.[3-7] We have also extended the use of 2-carboxyaziridines for the synthesis of α-amino acids by discovering conditions under which these compounds may be ring-opened regio- and stereo-specifically by carbon nucleophiles.[3,8]



1



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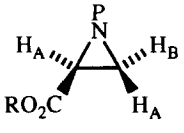

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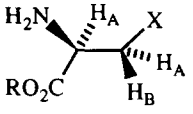
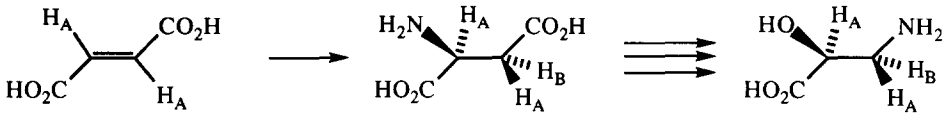


4

1a, P = CPh_3, R = Me

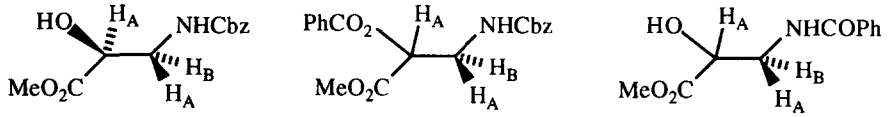
Because L-amino acids (**4**) are more common in nature than the corresponding D-analogues (**2**), an equally versatile synthesis of stereospecifically labelled L-amino acids (**4**) would be invaluable for studies of enzyme mechanism and so we have adapted our synthesis to prepare the protected labelled aziridines (**3**) of the L-series. This will allow the ring opening methodology which we have already developed to be used to synthesise a very wide variety of stereospecifically labelled L-amino acids (**4**). The simplest solution to this problem would be to invert the stereochemistry at the α-centre of some intermediate in our synthesis of the D-aziridines (**1**). The key intermediates in this synthesis are the stereospecifically labelled samples of (2S)-isoserine (**7**, $\mathbf{H_A} = {}^2\mathbf{H}$) and (**7**, $\mathbf{H_B} = {}^2\mathbf{H}$), prepared by a chemico-enzymatic route[2] from the fumaric acids (**5**) and (**5**, $\mathbf{H_A} = {}^2\mathbf{H}$) as shown in Scheme 1 below.



5 **6** **7**

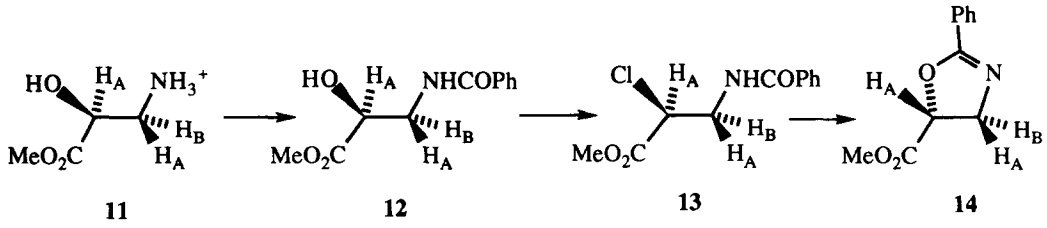
Scheme 1

Initial attempts to achieve inversion at the α-centre of derivatives of (2S)-isoserine (**7**) by use of the Mitsunobu reaction gave complex mixtures of products. Mesylation of the carbobenzyloxy methyl ester (**8**) followed by reaction with caesium benzoate in HMPA gave a good yield of the benzoate (**9**) which, when deprotected by hydrogenolysis, underwent migration of the benzoyl group to nitrogen yielding the benzoyl amide (**10**). This was shown to be a 2:1 mixture of diastereoisomers by preparation of the Mosher's ester and examination of the ^{1}H- and ^{19}F-NMR spectra. Comparison of the NMR spectra of the Mosher's ester with those of an authentic sample prepared from methyl N-benzoyl-(2S)-isoserinate (**12**) showed that the major diastereoisomer had resulted from inversion of stereochemistry at C-2.



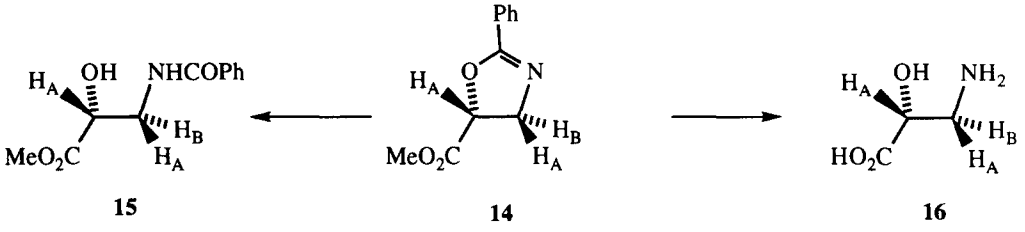
8 **9** **10**

The use of intramolecular nucleophilic substitution at the α-centre from an amide on the adjacent nitrogen to achieve inversion was an appealing possibility and so we prepared methyl N-benzoyl-(2S)-isoserinate (**12**) in 80 % yield by reaction of the methyl ester (**11**)[2] with benzoyl chloride under Schotten Baumann conditions in aqueous sodium bicarbonate at 0 °C for four hours. When the amide (**12**) was reacted with thionyl chloride in chloroform at temperatures less than 5 °C, the chloride (**13**) was obtained after 24 hours in 85 % yield. Reaction for shorter times led to mixtures of the oxazoline (**14**) and the chloride (**13**), implying that the chloride (**13**) was obtained *via* the oxazoline (**14**) by substitution by chloride anion. Heating the chloride (**13**) in toluene for 36 hours gave the oxazoline (**14**) in 85 % yield. The oxazoline (**14**) could also be obtained directly in good yield from the amide (**12**) either by reaction with methanesulfonic anhydride or with trifluoromethanesulfonic anhydride at room temperature for 24 hours in dichloromethane containing three equivalents of pyridine.



Scheme 2

Having obtained the oxazoline (**14**), the next step was hydrolysis to leave the new stereochemical centre at C-2 intact. This was achieved by heating the oxazoline to reflux in 1N aqueous hydrochloric acid in methanol which led to the benzoyl amide (**15**) which had a rotation of opposite sign to that of the amide (**12**) and which, on conversion to the Mosher's ester, was shown by ^{19}F- and ^{1}H- NMR spectroscopy to have an enantiomeric excess of >95 %. Heating the oxazoline (**14**) to reflux with 5N aqueous hydrochloric acid for 5 hours gave (2R)-isoserine (**16**) in 70 % yield. The optical purity was assessed by converting this to the benzoyl methyl ester (**15**) by the route used to prepare the enantiomeric compound (**12**). Conversion to the Mosher's ester by reaction with (R)(-)-α-methoxy-α-trifluoromethylphenylacetyl chloride followed by NMR studies showed an enantiomeric excess of > 95 %. Heating the oxazoline (**14**) for longer periods than 5 hours was shown to reduce the enantiomeric excess slightly.

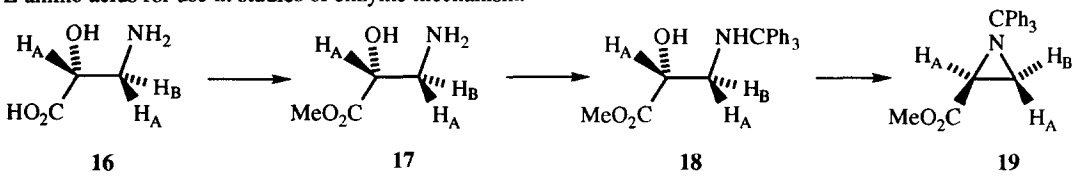


Scheme 3

Having succeeded in inverting the centre C-2, we were now able to use the same reaction sequence to convert the labelled samples of (2S)-isoserine (**7, H_A = ^2H**) and (**7, H_B = ^2H**) to the corresponding samples of (2R)-isoserine (**16, H_A = ^2H**) and (**16, H_B = ^2H**) as described in detail in the experimental section. The ^1H- and ^2H-NMR spectra of all pairs of diastereotopically labelled compounds in the reaction sequence confirmed the stereospecificity of the sequence and the inversion at C-2 was confirmed by comparison of the spectra of the labelled samples of (2S)- and (2R)-isoserine.

Conversion of the labelled samples (**16**) of (2R)-isoserine to the labelled aziridines (**19**) was carried out as shown in Scheme 4. The samples were first converted to the methyl esters (**17**) in 95 % yield, using thionyl chloride in methanol and the esters were protected as the trityl derivatives (**18**) by reacting with trityl chloride and triethylamine in chloroform at 0 °C for 4 hours. The resulting products were then converted to the aziridines (**19**) by first reacting with *para*-toluenesulfonyl chloride in pyridine and then cyclising by heating the tosylate with triethylamine in tetrahydrofuran. The ^1H-NMR spectra of the aziridines (**19**), (**19, H_A = ^2H**) and (**19, H_B = ^2H**) are shown in Figure 1 and are in keeping with the spectra[2] shown in Figure 2 of the diastereoisomerically labelled aziridines (**1**) from which we have synthesised a variety of stereospecifically labelled D-amino acids. The

availability of the labelled aziridines **(19)** now affords an equally versatile synthesis of the corresponding labelled L-amino acids for use in studies of enzyme mechanism.



Scheme 4

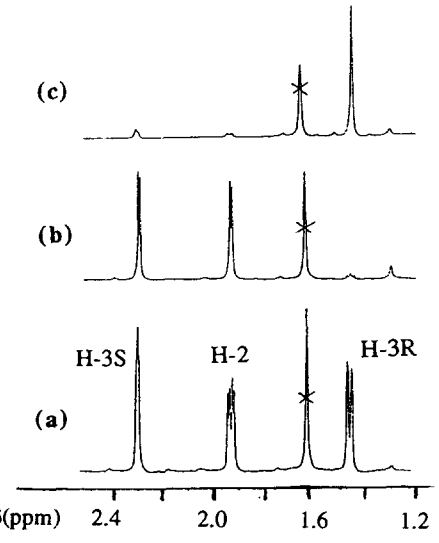


Figure 1: Part of the 360 MHz ^1H NMR spectra in C^2HCl_3 of: **(a)** unlabelled methyl (2S)-N-tritylaziridine-2-carboxylate **(19)**; **(b)** methyl (2S,3R)-N-trityl-[3-$^2H_1$]-aziridine-2-carboxylate **(19, H_B = ^2H)**; **(c)** methyl (2S,3S)-N-trityl-[2,3-$^2H_2$]-aziridine-2-carboxylate **(19, H_A = ^2H)**

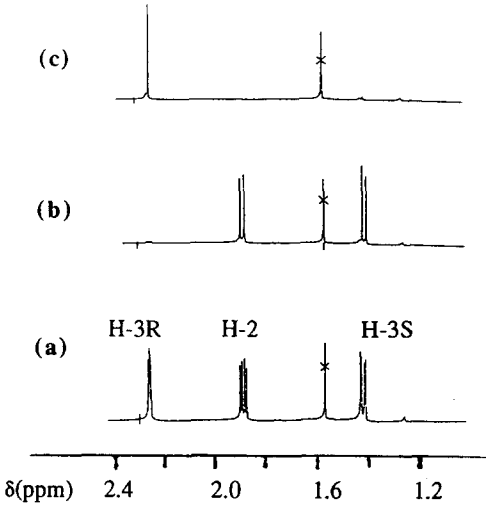


Figure 2: Part of the 360 MHz ^1H NMR spectra in C^2HCl_3 of: **(a)** unlabelled methyl (2R)-N-tritylaziridine-2-carboxylate **(1a)**; **(b)** methyl (2R,3R)-N-trityl-[3-$^2H_1$]-aziridine-2-carboxylate **(1a, H_B = ^2H)**; **(c)** methyl (2R,3S)-N-trityl-[2,3-$^2H_2$]-aziridine-2-carboxylate **(1a, H_A = ^2H)**

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 PE241 polarimeter using a 1 dm pathlength cell. IR spectra were recorded on a Perkin-Elmer 1720 Fourier-transform instrument. Mass spectra were recorded by Dr. A. Sada on a Kratos MS-80 instrument. 3-NBA refers to 3-nitrobenzyl alcohol. All ^1H-NMR spectra were recorded on a Bruker WM360 instrument (360 MHz); ^{13}C-NMR spectra (^1H-decoupled) on a Bruker A-C 250SY (62.9 MHz) instrument by Mr. C. M. Dadswell or a Bruker AMX 500 (125.8 MHz) instrument by Dr. A. G. Avent; ^{19}F-NMR spectra on a Jeol GS270 (254 MHz) instrument by Ms S. Fairhurst; and ^2H-NMR spectra on a Bruker A-C 250SY instrument (38.4 MHz) by Mr. C. M. Dadswell. J values are given in Hz. Residual solvent peaks were used to reference all NMR spectra. TLC was carried out on Merck Kieselgel

60 F_{254} precoated silica gel plates thickness 0.2 mm (ART 5719). Column chromatography was performed using Merck Kieselgel 60 (230-400 mesh —ART 9385). Ion-exchange resins were purchased in the chloride form from Aldrich and converted into the required ionic form by passage of at least a five-fold excess of the relevant ion through a column of the resin, followed by washing with distilled water. Microanalyses were carried out at Wellcome Research Laboratories, Beckenham.

Methyl N-benzoyl-(2S)-isoserinate **(12)** — Benzoyl chloride (2.1 cm^3, 18.09 mmol) was added to a vigorously stirred solution of methyl (2S)-isoserinate hydrochloride **(11)**[2] (2.52 g, 16.2 mmol) and sodium bicarbonate (4.54 g, 53.99 mmol) in water (40 cm^3) at 0 °C. The mixture was allowed to warm to room temperature and was stirred for 2 hours and extracted with ethyl acetate (4 x 30 cm^3). The combined organic extracts were washed with saturated brine and dried ($MgSO_4$). Removal of the solvent *in vacuo* followed by flash chromatography on silica gel using ethyl acetate - petroleum spirit (7 : 3) as eluent afforded *methyl N-benzoyl-(2S)-isoserinate* **(12)** as a white solid (2.25 g, 62 %); m.p. 83 - 85 °C; $[\alpha]_D^{28}$ + 5.9 ° (*c* 1, $CHCl_3$); (Found: C, 58.9; H, 5.9; N, 6.3. $C_{11}H_{13}NO_4$ requires C, 59.2; H, 5.8; N, 6.3 %); m/z [FAB$^+$ (3-NBA)] 224 ([M + H]$^+$); ν_{max} (KBr) / cm^{-1} 3427 (OH), 3308 (NH), 1744 (ester) and 1640 (amide); δ_H (C^2HCl_3) 3.58 (1H, brs, OH), 3.78 (3H, s, OCH_3), 3.78-3.90 (2H, m, 3-CH_2), 4.38 (1H, t, J 4.6, H-2), 6.81 (1H, m, NH) and 7.38-7.74 (5H, m, aromatics); δ_C (C^2HCl_3) 43.2 (C-3), 52.7 (OCH_3), 70.1 (C-2), 127.0, 128.5 and 131.7 (aromatic C-H), 133.8 (ipso aromatics), 168.3 (NC=O) and 173.3 (OC=O).

The optical purity of the product was shown to be > 95 % by stirring the amide **(12)** (50 mg, 0.224 mmol) with (R)(-)-α-methoxy-α-trifluoromethylphenylacetyl chloride (50μl, 0.269 mmol) in dichloromethane (1 ml) containing pyridine (54 μl, 0. 67 mmol) overnight at room temperature. Excess dichloromethane was added and the solution was washed with water, saturated aqueous $CuSO_4$, water and brine and dried ($MgSO_4$). Removal of the solvent *in vacuo* gave the Mosher's ester; m/z [FAB$^+$ (NBA)] 440 ([M + H]$^+$); δ_H (360 MHz, C^2HCl_3) 3.65 (3H, s OCH_3), 3.8 (1H, m, H-3), 3.83 (3H, s, OCH_3), 4.08 (1H, m, H-3), 5.45 (1H, m, H-2), 6.23 (br t, NH) and 7.25 - 7.6 (10H, m, aromatics); δ_F (254 MHz, C^2HCl_3) -71.968. The spectra of the diastereoisomeric Mosher's ester of amide **(15)** are described below.

Methyl N-benzoyl-(2S,3R)-[3-$^2H_1$]-isoserinate **(12, H_B = ^2H)** was prepared as above using methyl (2S,3R)-[3-$^2H_1$]-isoserinate hydrochloride **(11, H_B = ^2H)**[2] (2.50 g, 16.1 mmol). The product (2.46 g, 68 %) was a white solid; m.p. 83 - 85 °C; $[\alpha]_D^{25}$ + 5.7 ° (*c* 1, $CHCl_3$); m/z [FAB$^+$ (3-NBA)] 225 ([M + H]$^+$); ν_{max} (KBr) / cm^{-1} 3514 (OH), 3306 (NH), 1738 (ester) and 1638 (amide); δ_H (C^2HCl_3) 3.61 (1H, brs, OH), 3.79 (3H, s, OCH_3), 3.82 (1H, m, H-3S), 4.39 (1H, m, H-2), 6.72 (1H, brs, NH) and 7.38 - 7.75 (5H, m, aromatics); δ_C (C^2HCl_3) 42.9 (t, C-3), 52.9 (OCH_3), 70.1 (C-2), 127.0, 128.6 and 131.7 (aromatic C-H), 133.9 (ipso aromatics), 168.2 (NC=O) and 173.4 (OC=O).

Methyl N-benzoyl-(2S,3S)-[2,3-$^2H_2$]-isoserinate **(12, H_A = ^2H)** was prepared as above using methyl (2S,3S)-[2,3-$^2H_2$]-isoserinate hydrochloride **(11, H_A = ^2H)**[2] (175 mg, 1.12 mmol). The product (190 mg, 76 %) was a white solid; m.p. 87 - 89 °C; $[\alpha]_D^{18}$ + 7.0 ° (*c* 1, $CHCl_3$); m/z [FAB$^+$ (3-NBA)] 226 ([M + H]$^+$); ν_{max} (KBr) / cm^{-1} 3516 (OH), 3307 (NH), 1733 (ester) and 1638 (amide); δ_H (C^2HCl_3) 1.65 (1H, brs, OH), 3.79 (3H, s, OCH_3), 3.83 (1H, m, H-3R), 6.55 (1H, brs, NH) and 7.39-7.47 (5H, m, aromatics); δ_C (C^2HCl_3) 42.8 (t, C-3), 53.1 (OCH_3), 69.7 (t, C-2), 127.0, 128.5 and 131.9 (aromatic C-H), 133.9 (ipso aromatics), 168.5 (NC=O) and 173.5 (OC=O).

Methyl (2S)-2-chloro-3-benzoylaminopropanoate **(13)** — Thionyl chloride (1.0 cm^3, 13.71 mmol) was added to a solution of methyl N-benzoyl-(2S)-isoserinate **(12)** (0.160 g, 0.717 mmol) in chloroform (1.3 cm^3) at 0 °C. The reaction was left in a refrigerator (< 5°C) for 24 hours. The solvent was removed *in vacuo* and the residue was dissolved in chloroform (20 cm^3). The solution was washed with 10 % aqueous sodium carbonate (10 cm^3) and saturated brine (10 cm^3). The organic layer was dried ($MgSO_4$) and the solvent was removed *in vacuo*. Flash chromatography on silica gel using diethyl ether - petroleum spirit (3 : 2) as eluent afforded *methyl (2S)-2-chloro-3-benzoylaminopropanoate* **(13)** (0.147 g, 85 %) as a white solid; m.p. 69 - 71 °C; $[\alpha]_D^{23}$ - 23.2 ° (*c* 1, $CHCl_3$); (Found: C, 54.4; H, 5.0; N, 5.7; Cl, 15.0. $C_{11}H_{12}NO_3Cl$ requires C, 54.7; H, 5.0; N, 5.8; Cl, 14.7 %); m/z [FAB$^+$ (3-NBA)] 244 and 242 (ratio 1 : 3, $[M + H]^+$); ν_{max} (KBr) / cm^{-1} 3294 (NH), 1761 (ester) and 1638 (amide); δ_H (C^2HCl_3) 3.81 (3H, s, OCH_3), 3.86-4.05 (2H, m, 3-CH_2), 4.57 (1H, dd, J 6, J 6, H-2), 6.73 (1H, brs, NH) and 7.24-7.76 (5H, m, aromatics); δ_C (C^2HCl_3) 42.9 (C-3), 53.3 (OCH_3), 54.3 (C-2), 127.0, 128.6 and 131.9 (aromatic C-H), 133.7 (ipso aromatics), 167.6 (NC=O) and 169.0 (OC=O).

(5R)-2-Phenyl-5-methoxycarbonyloxazoline **(14)** — *Method A.* Thionyl chloride (2.0 cm^3, 27.42 mmol), was added dropwise to a solution of methyl N-benzoyl-(2S)-isoserinate **(12)** (140 mg, 0.628 mmol) in chloroform (1.0 cm^3) at 0 °C. The reaction was allowed to warm to 4 °C and was maintained at this temperature for 12 hours (refrigerator). The solvent was removed *in vacuo* and the residue was dissolved in chloroform (15 cm^3). The solution was washed with 10 % aqueous sodium carbonate (5 cm^3) and saturated brine (5 cm^3) and dried ($MgSO_4$) and the solvent was removed *in vacuo*. The residue was dissolved in toluene (10 cm^3) and heated to reflux for 36 hours. Removal of the solvent *in vacuo* followed by flash chromatography on silica gel using diethyl ether - petroleum spirit (3 : 2) as eluent afforded *(5R)-2-phenyl-5-methoxycarbonyloxazoline* **(14)** as a white solid (109 mg, 85 %); m.p. 35 - 38 °C; $[\alpha]_D^{28}$ +166.1 ° (*c* 1, $CHCl_3$); (Found: C, 64.3; H, 5.5; N, 6.9. $C_{11}H_{11}NO_3$ requires C, 64.4; H, 5.4; N, 6.8 %); m/z [FAB$^+$ (3-NBA)] 206 ($[M + H]^+$); ν_{max} (KBr) / cm^{-1} 1724 (ester) and 1655 (C=N); δ_H (C^2HCl_3) 3.79 (3H, s, OCH_3), 4.15 (1H, ABX, $J_{4R,4S}$ 15, $J_{4R,5}$ 7, H-4R) 4.45 (1H, ABX, $J_{4S,4R}$ 15, $J_{4S,5}$ 11, H-4S), 5.08 (1H, dd, $J_{5,4R}$ 7, $J_{5,4S}$ 11, H-5) and 7.24-7.94 (5H, m, aromatics); δ_C (C^2HCl_3) 52.6 (CH_3O), 59.4 (C-4), 75.8 (C-5), 126.9 (ipso aromatics), 128.4 and 131.7 (aromatic C-H), 164.0 (C-2) and 171.1 (OC=O).

Method B. A solution of methanesulfonic anhydride (2.12 g, 12.17 mmol) in dichloromethane (15 cm^3) was added to a solution of methyl N-benzoyl-(2S)-isoserinate **12** (2.10 g, 9.417 mmol) and pyridine (2.31 cm^3, 28.561 mmol) in dichloromethane (58 cm^3) at 0 °C. After 30 min. the reaction was allowed to warm to room temperature and stirred overnight. The solution was washed with water (15 cm^3), saturated aqueous copper sulphate (2 x 15 cm^3), water (15 cm^3) and saturated brine (15 cm^3). The organic extracts were dried ($MgSO_4$) and the solvent was removed *in vacuo*. Flash chromatography on silica gel using diethyl ether - petroleum spirit (3 : 2) as eluent afforded *(5R)-2-phenyl-5-methoxycarbonyloxazoline* **(14)** as a white solid (1.63 g, 84 %); m.p. 36 -38 °C; $[\alpha]_D^{28}$ + 166.8 ° (*c* 1, $CHCl_3$). Spectra were identical to those for the product prepared using method A above.

Method C. Trifluoromethanesulfonic anhydride (0.098 cm^3, 0.583 mmol) was added to a solution of methyl N-benzoyl-(2S)-isoserinate **(12)** (0.100 g, 0.448 mmol) and pyridine (0.109 cm^3, 1.344 mmol) in dichloromethane (3 cm^3) at 0 °C. After 30 min. the reaction was allowed to warm to room temperature and stirred overnight. The solution was washed with water (1 cm^3), saturated aqueous copper sulphate (2 x 1 cm^3), water (1 cm^3) and saturated brine (1 cm^3). The organic extracts were dried ($MgSO_4$) and the solvent was removed *in vacuo*. Flash chromatography on silica gel using diethyl ether - petroleum spirit (3 : 2) as eluent afforded *(5R)-2-phenyl-5-methoxycarbonyloxazoline* **(14)** as a white solid (0.069 g, 75 %); m.p. 35 - 38 °C; $[\alpha]_D^{28}$ + 166.3 ° (*c* 1, $CHCl_3$). Spectra were identical to those for the product prepared using method A above.

(4R,5R)-2-Phenyl-5-methoxycarbonyl-[4-$^2H_1$]-oxazoline **(14, H_B = ^2H)** was prepared from methyl N-benzoyl-(2S,3R)-[3-$^2H_1$]-isoserinate **(12, H_B = ^2H)** (5.50 g, 24.55 mmol) using method B as above. The product was a white solid (4.30 g, 86 %); m.p. 35 - 38 °C; $[\alpha]_D^{21}$ + 165.4 ° (*c* 1, $CHCl_3$); m/z [FAB$^+$ (3-NBA)] 207 ([M + H]$^+$); ν_{max} (KBr) / cm^{-1} 1724 (ester) and 1655 (C=N); δ_H (C^2HCl_3) 3.77 (3H, s, OCH_3), 4.32 (1H, d, $J_{4S,5}$ 10.9, H-4S), 5.06 (1H, d, $J_{5,4S}$ 10.9, H-5) and 7.37-7.97 (5H, m, aromatics); δ_C (C^2HCl_3) 52.6 (OCH_3), 59.1 (t, C-4), 75.7 (C-5), 126.9 (ipso aromatics), 128.4 and 131.6 (aromatic C-H), 164.0 (C-2) and 171.1 (OC=O).

(4S,5R)-2-Phenyl-5-methoxycarbonyl-[4,5-$^2H_2$]-oxazoline **(14, H_A = ^2H)** was prepared from N-benzoyl methyl (2S,3S)-[2,3-$^2H_2$]-isoserinate **(12, H_A = ^2H)** (1.19 g, 5.289 mmol) using method B as above. The product (0.924 g, 85 %) was a white solid; m.p. 34 - 36 °C; $[\alpha]_D^{28}$ + 162.9 ° (*c* 1, $CHCl_3$); m/z [FAB$^+$ (3-NBA)] 208 ([M + H]$^+$); ν_{max} (KBr) / cm^{-1} 1725 (ester) and 1654 (C=N); δ_H (C^2HCl_3) 3.79 (3H, s, OCH_3), 4.12 (1H, s, H-4R) and 7.24-7.97 (5H, m, aromatics); δ_C (C^2HCl_3) 52.6 (OCH_3), 59.0 (t, C-4), 75.4 (t, C-5), 127.0 (ipso aromatics), 128.4 and 131.7 (aromatic C-H), 164.0 (C-2) and 171.1 (OC=O).

Methyl N-benzoyl-(2R)-isoserinate **(15)** — A solution of (5R)-2-phenyl-5-methyloxycarbonyloxazoline **(14)** (0.5 g , 2.439 mmol) in methanol (10.9 cm^3) was heated to reflux with 1N aqueous hydrochloric acid (4.3 cm^3). The reaction was cooled and the solvent was removed *in vacuo*. The resulting white solid was dissolved in water (15 cm^3) and 10% aqueous sodium carbonate was added until pH 11 was reached. The aqueous solution was extracted with ethyl acetate (3 x 15 cm^3). The organic extracts were dried ($MgSO_4$) and the solvent was removed *in vacuo*. Flash chromatography on silica gel using ethyl acetate - petroleum spirit (7 : 3) as eluent afforded *methyl N-benzoyl-(2R)-isoserinate* **(15)** as a white solid (0.38 g, 70 %); m.p. 86 - 88 °C; $[\alpha]_D^{23}$ - 7.6 ° (*c* 1, $CHCl_3$); (Found: C, 59.35; H, 6.1; N, 6.0. $C_{11}H_{13}NO_4$ requires C, 59.2; H, 5.8; N, 6.3 %). Spectra were identical to those for the enantiomeric compound **(12)**.

The optical purity of the product was shown to be > 95 % by stirring the amide **(15)** (50 mg, 0.224 mmol) with (R)(-)-α-methoxy-α-trifluoromethylphenylacetyl chloride (50μl, 0.269 mmol) in dichloromethane (1 ml) containing pyridine (54 μl, 0. 67 mmol) overnight at room temperature. Excess dichloromethane was added and the solution was washed with water, saturated aqueous $CuSO_4$, water and brine and dried ($MgSO_4$). Removal of the solvent *in vacuo* gave the Mosher's ester; m/z [FAB$^+$ (NBA)] 440 ([M + H]$^+$); δ_H (360 MHz, C^2HCl_3) 3.53 (3H, s OCH_3), 3.81 (1H, m, H-3), 3.81 (3H, s, OCH_3), 4.01 (1H, m, H-3), 5.42 (1H, m, H-2), 6.5 (br t, NH) and 7.3 - 7.7 (10H, m, aromatics); δ_F (254 MHz, C^2HCl_3) -72.274. The spectra of the diastereoisomeric Mosher's ester of amide **(12)** are described above.

Methyl N-benzoyl-(2R,3R)-[3-$^2H_1$]-isoserinate **(15, H_B = ^2H)** was prepared as above using (4R,5R)-2-phenyl-5-methoxycarbonyl-[4-$^2H_1$]-oxazoline **(14, H_B = ^2H)** (4.00 g, 19.61 mmol). The product was a white solid (2.94 g, 67 %); m.p. 86 - 88 °C; $[\alpha]_D^{23}$ - 7.4 ° (*c* 1, $CHCl_3$); m/z [FAB$^+$ (3-NBA)] 225 ([M + H]$^+$); ν_{max} (KBr) / cm^{-1} 3516 (OH), 3300 (NH), 1733 (ester) and 1637 (amide); δ_H (C^2HCl_3) 3.61 (1H, brs, OH), 3.79 (3H, s, OCH_3), 3.82 (1H, m, H-3S), 4.39 (1H, m, H-2), 6.61 (1H, brs, NH) and 7.38-7.75 (5H, m, aromatics); δ_C (C^2HCl_3) 42.9 (t, C-3), 52.9 (OCH_3), 70.1 (C-2), 127.0, 128.6 and 131.7 (aromatic C-H), 133.9 (ipso aromatics), 168.2 (NC=O) and 173.4 (OC=O).

Methyl N-benzoyl-(2R,3S)-[2,3-$^2H_2$]-isoserinate **(15, H_A = ^2H)** was prepared as above using (4S,5R)-2-phenyl-5-methoxycarbonyl-[4,5-$^2H_2$]-oxazoline **(14, H_A = ^2H)** (70 mg, 0.338 mmol). The product (60 mg, 79 %) was a white solid; m.p. 86 - 88 °C; $[\alpha]_D^{23}$ - 7.8 ° (*c* 1, $CHCl_3$); m/z [FAB$^+$ (3-NBA)] 226 ([M + H]$^+$); ν_{max} (KBr) / cm^{-1} 3420 (OH), 3305 (NH), 1732 (ester) and 1637 (amide); δ_H (C^2HCl_3) 3.67 (1H, brs, OH), 3.76 (1H, s, H-3R), 3.79 (3H, s, OCH_3), 6.64 (1H, brs, NH) and 7.24-7.87 (5H, m, aromatics); δ_C (C^2HCl_3) 42.9 (t, C-3),

52.7 (OCH_3), 69.7 (t, C-2), 127.0 and 128.5 and 131.6 (aromatic C-H), 133.8 (ipso aromatics), 168.4 (NC=O) and 173.3 (OC=O).

(2R)-Isoserine **(16)** — (5R)-2-Phenyl-5-methoxycarbonyloxazoline **(14)** (1.5 g , 7.317 mmol) was heated to reflux with 5N aqueous hydrochloric acid (30 cm^3) for 5 hours. The mixture was allowed to cool, resulting in precipitation of benzoic acid, and it was washed with diethyl ether (2 x 10 cm^3). The aqueous layer was concentrated *in vacuo* and the resulting white solid was dissolved in water (5 cm^3) and applied to a Dowex 1 X 2-200 (OH^-) ion-exchange column. The column was eluted with water and 5% aqueous acetic acid. The ninhydrin positive fractions by TLC (2 : 1 : 1, n-butanol : acetic acid : water) were combined and the solvent was removed *in vacuo* to afford *(2R)-isoserine* **(16)** (0.54 g, 70 %) as a white solid; m.p. 199 - 201 °C; $[\alpha]_D^{23}$ + 28.6 ° (*c* 1, H_2O) {lit.,[9] + 32.4 °}; m/z [FAB$^+$ (glycerol)] 106 ([M + H]$^+$); ν_{max} (KBr) / cm^{-1} 3246 (OH), 3200 (NH), 3000-2500 (COOH) and 1657 (acid); δ_H (^2H_2O) 2.85 (1H, dd $J_{3R,2}$ 8.4, $J_{3R,3S}$ 13.1, H-3R), 3.08 (1H, dd, $J_{3S,2}$ 3.5, $J_{3S,3R}$ 13.1, H-3S) and 3.97 (1H, dd, $J_{2,3R}$ 8.4, $J_{2,3S}$ 3.5, H-2).

(2R,3R)-[3-$^2H_1$]-Isoserine **(16, H_B = ^2H)** was prepared as above using (4R,5R)-2-phenyl-5-methoxycarbonyl-[4-$^2H_1$]-oxazoline **(14, H_B = ^2H)** (1.10 g, 5.340 mmol). The product (0.429 g, 75 %) was a white solid; m.p. 199-201 °C; $[\alpha]_D^{23}$ + 30.8 ° (*c* 1, H_2O); m/z [FAB$^+$ (glycerol)] 107 ([M + H]$^+$); ν_{max} (KBr) / cm^{-1} 3246 (OH), 3200 (NH), 3000-2500 (COOH) and 1657 (acid); δ_H (^2H_2O) 3.08 (1H, d, $J_{3S,2}$ 3.8, H-3S) and 3.98 (1H, d, $J_{2,3S}$ 3.8, H-2).

(2R,3S)-[2,3-$^2H_2$]-Isoserine **(16, H_A = ^2H)** was prepared as above using (4S,5R)-2-phenyl-5-methoxycarbonyl-[4, 5-$^2H_2$]-oxazoline **(14 H_A = ^2H)** (0.638 g, 3.082 mmol). The product (0.263 g, 80 %) was a white solid; m.p. 199-201 °C; $[\alpha]_D^{23}$ + 29.6 ° (*c* 1, H_2O); m/z [FAB$^+$ (glycerol)] 108 ([M + H]$^+$); ν_{max} (KBr) / cm^{-1} 3246 (OH), 3200 (NH), 3000-2500 (COOH) and 1613 (acid); δ_H (^2H_2O) 2.85 (1H, s, H-3R).

Methyl (2R)-isoserinate hydrochloride **(17)** —Thionyl chloride (0.5 cm^3, 6.855 mmol) was added dropwise with care to methanol (3 cm^3) at 0 °C. The resulting solution was added to (2R)-isoserine **(16)** (200 mg, 1.90 mmol) at 0 °C. The reaction was maintained at 0 °C until the solid dissolved and was allowed to warm to room temperature and stirred overnight. Removal of the solvent *in vacuo* afforded methyl (2R)-isoserinate hydrochloride **(17)** (209 mg, 70 %) as a yellow solid; m.p. 104-105 °C; $[\alpha]_D^{23}$ + 18.2 ° (*c* 1, H_2O); m/z [FAB$^+$ (glycerol)] 120 ([M+ H]$^+$); ν_{max} (KBr) / cm^{-1} 3537 (OH), 3059 (NH) and 1742 (ester); δ_H (^2H_2O) 3.01 (1H, dd, $J_{3R,2}$ 8.4, $J_{3R,3S}$ 13.3, H-3R), 3.21 (1H, dd, $J_{3S,2}$ 4.1, $J_{3S,3R}$ 13.3, H-3S), 3.57 (3H, s, OCH_3) and 4.34 (1H, dd, $J_{2,3R}$ 8.4, $J_{2,3S}$ 4.1, H-2).

Methyl (2R,3R)-[3-$^2H_1$]-isoserinate hydrochloride **(17, H_B = ^2H)** was prepared as above using (2R,3R)-[3-$^2H_1$]-isoserine **(16, H_B = ^2H)** (6.80 g, 64.15 mmol). The product (9.24 g, 92 %) was a yellow solid; m.p. 104-105 °C; $[\alpha]_D^{23}$ + 18.9 ° (*c* 1, H_2O); m/z [FAB$^+$ (glycerol)] 121 ([M + H]$^+$); ν_{max} (KBr) / cm^{-1} 1736 (ester); δ_H (^2H_2O) 3.18 (1H, d, $J_{3S,2}$ 3.2, H-3S), 3.54 (3H, s, OCH_3) and 4.33 (1H, d, $J_{2,3S}$ 3.2, H-2).

Methyl (2R,3S)-[2,3-$^2H_2$]-isoserinate hydrochloride **(17, H_A = ^2H)** was prepared as above using (2R,3S)-[2, 3-$^2H_1$]-isoserine **(16, H_A = ^2H)** (0.35 g, 3.3 mmol). The product (0.485 g, 94 %) was a yellow solid; m.p. 104-105 °C; $[\alpha]_D^{23}$ + 18.6 ° (*c* 1, H_2O); m/z [FAB$^+$ (glycerol)] 122 ([M + H]$^+$); ν_{max} (KBr) / cm^{-1} 3512 (OH), 3038 (NH) and 1746 (ester); δ_H (^2H_2O) 2.96 (1H, brs, H-3R) and 3.56 (3H, s, OCH_3).

Methyl (2R)-N-tritylisoserinate **(18)** — A solution of triphenylmethyl chloride (0.512g, 1.837 mmol) in chloroform (2 cm^3) was added dropwise to a solution of methyl (2R)-isoserinate hydrochloride **(16)** (0.250g,

1.61 mmol) and triethylamine (0.6 cm^3, 4.305 mmol) in chloroform (2 cm^3) at 0 °C. After 24 hours, the solution was washed with 10% aqueous citric acid (2 cm^3), water (2 cm^3) and saturated brine (2 cm^3). The organic layer was dried ($MgSO_4$) and the solvent was removed *in vacuo*. Flash chromatography on silica gel using ethyl acetate - petroleum spirit (3 : 7) as eluent afforded *methyl (2R)-N-tritylisoserinate* **(17)** as a white solid (0.501g, 86 %); m.p. 94-96 °C, $[\alpha]_D^{25}$ -16.9 ° (*c* 1, $CHCl_3$); (Found: C, 76.2; H, 6.5; N, 3.85. $C_{23}H_{23}NO_3$ requires C, 76.45; H, 6.4; N, 3.9 %); m/z [FAB$^+$ (3-NBA)] 362 ([M + H]$^+$); ν_{max} (KBr) / cm^{-1} 3459 (OH), 3300 (NH) and 1738 (ester); δ_H (C^2HCl_3) 2.24 (1H, brs, OH), 2.54 (1H, ABX, $J_{3S,3R}$ 12, $J_{3S,2}$ 3, H-3S), 2.60 (1H, ABX, $J_{3R,3S}$ 12, $J_{3R,2}$ 4.5, H-3R), 3.22 (1H, brs, NH), 3.89 (3H, s, OCH_3), 4.35 (1H, dd, $J_{3R,2}$ 4.5, $J_{3S,2}$ 3, H-2) and 7.21-7.68 (15H, m, aromatics); δ_C (C^2HCl_3) 46.2 (C-3), 52.4 (OCH_3), 70.2 (CPh), 70.4 (C-2), 126.3, 127.8 and 128.5 (aromatic C-H), 145.5 (ipso aromatics) and 174.6 (OC=O).

Methyl (2R,3R)-[3-$^2H_1$]-N-tritylisoserinate **(18, H_B = ^2H)** was prepared as above using methyl (2R,3R)-[3-$^2H_1$]-isoserinate hydrochloride **(17, H_B = ^2H)** (0.717 g, 4.6 mmol). The product (1.490 g, 89 %) was a white solid; m.p. 94-96 °C; $[\alpha]_D^{25}$ - 16.4 ° (*c* 1, $CHCl_3$); m/z [FAB$^+$ (3-NBA)] 363 ([M + H]$^+$); ν_{max} (KBr) / cm^{-1} 3485 (OH), 3318 (NH) and 1713(ester); δ_H (C^2HCl_3) 2.13 (1H, brs, OH), 2.48 (1H, d, $J_{3S,2}$ 3.5, H-3S), 3.12 (1H, brs, NH), 3.88 (3H, s, OCH_3), 4.31 (1H, d, $J_{2,3S}$ 3.5, H-2) and 7.20-7.60 (15H, m, aromatics); δ_C (C^2HCl_3) 45.9 (t, C-3), 52.5 (OCH_3), 70.2 (CPh), 70.4 (C-2), 126.4, 127.8 and 128.5 (aromatic C-H), 145.6 (ipso aromatics) and 174.7 (OC=O).

Methyl (2R,3S)-[2,3-$^2H_2$]-N-tritylisoserinate **(18, H_A = ^2H)** was prepared as above using methyl (2R,3S)-[2,3-$^2H_1$]-isoserinate hydrochloride **(17, H_A = ^2H)** (0.463 g, 2.940 mmol). The product (0.830 g, 78 %) was a white solid; m.p. 94-96 °C; $[\alpha]_D^{25}$ - 17.0 ° (*c* 1, $CHCl_3$); m/z [FAB$^+$ (3-NBA)] 364 ([M + H]$^+$); ν_{max} (KBr) / cm^{-1} 3486 (OH), 3318 (NH) and 1714 (ester); δ_H (C^2HCl_3) 2.13 (1H, brs, OH), 2.52 (1H, s, H-3R), 3.07 (1H, brs, NH), 3.88 (3H, s, OCH_3) and 7.17-7.46 (15H, m, aromatics); δ_C (C^2HCl_3) 45.8 (t, C-3), 52.5 (OCH_3), 70.2 (CPh), 70.4 (t, C-2), 126.3, 127.8 and 128.5 (aromatic C-H), 145.5 (ipso aromatics) and 174.7 (OC=O).

Methyl (2S)-N-tritylaziridine-2-carboxylate **(19)** — *p*-Toluenesulfonyl chloride (0.866 g, 4.541 mmol) was added in portions to a solution of methyl (2R)-N-tritylisoserinate **(18)** (0.500 g, 1.385 mmol) in pyridine (1.7 cm^3) at -15 °C. The reaction was allowed to warm to 0 °C and stirred for 18 hours. After removal of the solvent *in vacuo*, the residue was partitioned between ethyl acetate (40 cm^3) and water (20 cm^3). The organic layer was separated and washed with 10% aqueous citric acid (4 x 10 cm^3) and dried ($MgSO_4$). Removal of the solvent *in vacuo* afforded a white solid. A solution of this solid and triethylamine (0.5 cm^3, 3.587 mmol) in tetrahydrofuran (3 cm^3) was heated at reflux for 22 hours. The solvent was removed *in vacuo*, and the residue was dissolved in ethyl acetate (40 cm^3). The solution was washed with 10 % aqueous citric acid (2 x 10 cm^3) and 1M aqueous sodium hydrogen carbonate (2 x 10 cm^3) and dried ($MgSO_4$). Removal of the solvent *in vacuo* afforded a brown solid which was subjected to flash chromatography on silica gel using chloroform - petroleum spirit (3 : 2) followed by chloroform as eluent. The product, *methyl (2S)-N-tritylaziridine-2-carboxylate* **(19)** was obtained as colourless crystals after recrystallisation from methanol (0.285 g, 60 %); m.p. 127-129 °C; $[\alpha]_D^{23}$ - 87.2 ° (*c* 0.7, $CHCl_3$); (Found: C, 80.2; H, 6.2; N, 4.2. $C_{23}H_{21}NO_2$ requires C, 80.5; H, 6.1; N, 4.1 %); m/z [FAB$^+$ (3-NBA)] 266 ([M - Ph]$^+$); ν_{max} (KBr) / cm^{-1} 1743 (ester); δ_H (C^2HCl_3) 1.41 (1H, dd, $J_{3R,2}$ 6.2, $J_{3R,3S}$ 1.6, H-3R), 1.89 (1H, dd, $J_{3R,2}$ 6.2, $J_{3S,2}$ 2.7, H-2), 2.25 (1H, dd, $J_{3S,2}$ 2.7, $J_{3S,3R}$ 1.6, H-3S), 3.76 (3H, s, OCH_3) and 7.24-7.51 (15H, m, aromatics); δ_C (C^2HCl_3) 28.7 (C-3), 31.6 (C-2), 52.1 (OCH_3), 74.3 (CPh_3), 126.9, 127.6 and 129.3 (aromatic CH), 143.6 (ipso aromatic) and 171.9 (OC=O).

Methyl (2S,3R)-N-trityl-[3-$^2H_1$]aziridine-2-carboxylate **(19, H_B = ^2H)** was prepared as above using methyl (2R,3R)-[3-$^2H_1$]-N-tritylisoserinate **(17, H_B = ^2H)** (0.528 g, 1.459 mmol). The product (0.305 g, 61 %) was

colourless crystals; m.p. 126-128 °C; $[\alpha]_D^{25}$ - 86.8 ° (*c* 0.7, $CHCl_3$); m/z [FAB$^+$ (3-NBA)] 267 ([[M - Ph]$^+$); ν_{max} (KBr) / cm^{-1} 1744 (ester); δ_H (C^2HCl_3) 1.90 (1H, d, $J_{2,3S}$ 2.7, H-2), 2.26 (1H, d, $J_{3S,2}$ 2.7, H-3S), 3.78 (3H, s, OCH_3), and 7.22-7.53 (15H, m, aromatics); δ_D (38.4 MHz; $CHCl_3$) 1.42 (1^2H, brs, ^2H-3R); δ_C (C^2HCl_3) 28.4 (t, C-3); 31.6 (C-2), 52.1 (OCH_3), 74.4 (CPh), 126.9, 127.6 and 129.2 (aromatic C-H), 143.6 (ipso aromatics) and 171.9 (OC=O).

Methyl (2S,3S)-N-trityl-[2,3-$^2H_2$]aziridine-2-carboxylate (**19, H_A = ^2H**) was prepared as above using methyl (2R,3S)-[2,3-$^2H_2$]-N-tritylisoserinate (**17, H_A = ^2H**) (0.745 g, 2.052 mmol). The product (0.368 g, 52 %) was colourless crystals; m.p. 127-129 °C; $[\alpha]_D^{25}$ - 86.3 ° (*c* 0.7, $CHCl_3$); m/z [FAB$^+$ (3-NBA)] 268 ([M - Ph]$^+$); ν_{max} (KBr) / cm^{-1} 1743 (ester); δ_H (C^2HCl_3) 1.45 (1H, s, H-3R), 3.78 (3H, s, OCH_3) and 7.22-7.53 (15H, m, aromatics); δ_D (38.4 MHz; $CHCl_3$) 1.92 (1^2H, brs, ^2H-3S) and 2.28 (1^2H, brs, ^2H-2); δ_C (C^2HCl_3) 28.2 (t, C-3); 31.3 (t, C-2), 52.0 (OCH_3), 74.3 (CPh), 126.9, 127.6 and 129.1 (aromatic C-H), 143.5 (ipso aromatics) and 171.9 (OC=O).

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