Tetrahedron Vol. 45, No. 19, pp. 6319 to 6330, 1989
Printed in Great Britain.

0040-4020/89 $3.00+.00

Non-Proteinogenic Amino Acid Synthesis: Synthesis of β,γ-Unsaturated α-Amino Acids from Aspartic Acid

Jack E. Baldwin*, Mark G. Moloney and Michael North

The Dyson Perrins Laboratory, University of Oxford,
South Parks Road, Oxford, OX1 3QY, U.K.

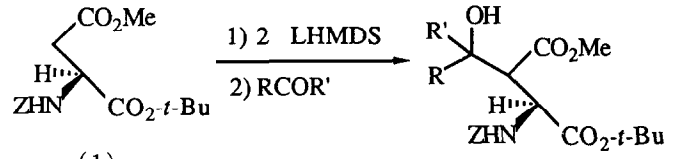
Key words: aspartic acid; unsaturated amino acids

(*Received in UK* 2 *June* 1989)

Abstract: A general, stereospecific, synthesis of β,γ-unsaturated α-amino acids using the β-anion derived from aspartic acid is described.

β,γ-Unsaturated amino acids have been found to be reversible or irreversible inhibitors of a number of enzymes,[1] and this has prompted a number of racemic syntheses of this class of amino acids.[1,2] Although a few members of this group of amino acids have been synthesised stereospecifically,[3] only two reports dealing with the general synthesis of optically active β,γ-unsaturated α-amino acids have so far been published,[4,5] and both make use of the Schollkopf bis-lactim ether. In this paper we report the synthesis of β,γ-unsaturated amino acids using the β-ester enolate derived from aspartic acid. This approach has also been used for the synthesis of other non-proteinogenic amino acids[6-8].

Diester (1) was reacted with two equivalents of lithium diisopropylamide (LDA), or two equivalents of lithium hexamethyldisilazide (LHMDS) under the conditions previously described,[6] followed by benzaldehyde or propanal, to give hydroxydiesters (2a,b) in



(1)

a, R=Ph, R'=H

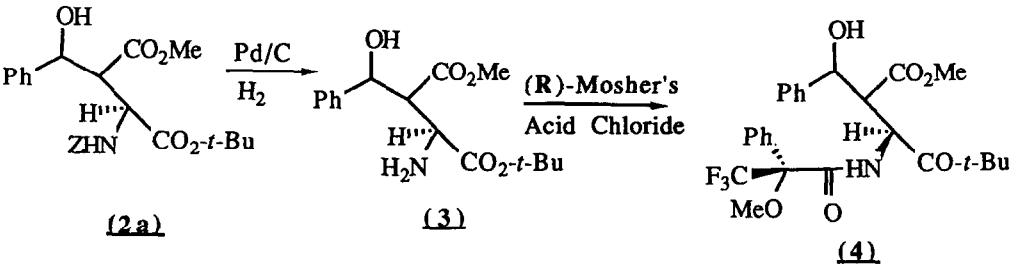
b, R=Et, R'=H

(2)

Scheme 1

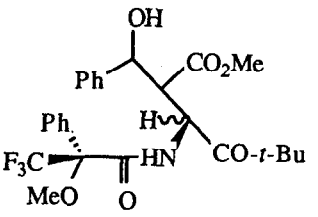
in 50-60% yield. Only two of the four possible diastereomers were obtained (as a 1:1 mixture), and these were identified as the stereoisomers with the hydroxyl and β-ester groups in a syn configuration as described later. Hydrogenolysis of hydroxydiester (2a) to amine (3) and subsequent conversion to the Mosher amide (4) allowed determination of the optical purity of the products. The corresponding amide (5), derived from racemic aspartic acid, was prepared similarly. The ^{19}F n.m.r spectrum of amide (5) showed

two peaks of equal intensity for each stereoisomer at C3/C4, showing that the enantiomers (at C2) were being resolved. The corresponding spectrum of amide (4) prepared using LDA as the base also showed two peaks for each stereoisomer and by integration the enantiomeric excess was calculated at 85%. The ^{19}F nmr spectrum of amide (4) prepared using LHMDS as the base, however, showed only one peak for each stereoisomer, implying that hydroxydiesters (2a) were obtained enantiomerically pure (within the detection limits of 250 MHz n.m.r).



(2a) (3) (4)

Scheme 2

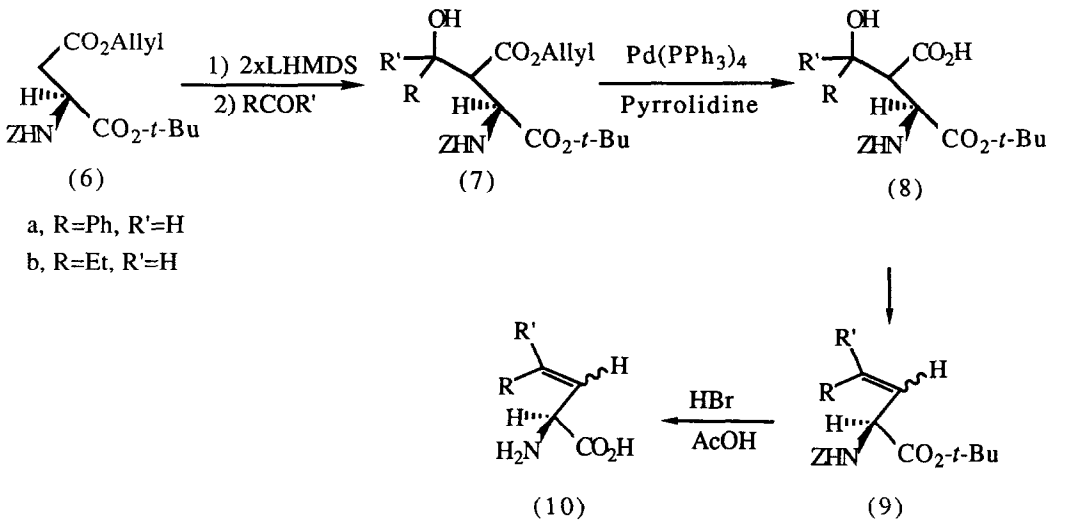


(5)

Saponification of diester (2b) with lithium hydroxide in aqueous methanol resulted in concomitant removal of both the β-methyl and α-t-butyl ester. Selective cleavage of the methyl ester under a variety of alternative conditions (e.g. lithium iodide in refluxing pyridine or DMF[9], DBN in refluxing o-xylene[9,10], sodium cyanide at 90% in HMPA[11], and "anhydrous hydroxide"[12]) all failed to yield the desired deprotected products.

In view of the failure of selective cleavage of the β-methyl ester, alternative β-ester protecting groups were sought. A suitable ester required formation under acidic conditions, stability to strong acid and to strong base, and cleavage without concomitant deprotection of benzyloxycarbonyl or t- butyl groups. The allyl ester was reported to be stable under all the required conditions[13] and could be cleaved with cuprates,[14] or by a palladium (0) complex and amine base.[15] In addition, there was ample literature precedent for the formation of enolates of allyl esters, since they have been used in the Ireland-Claisen rearrangement.[16]

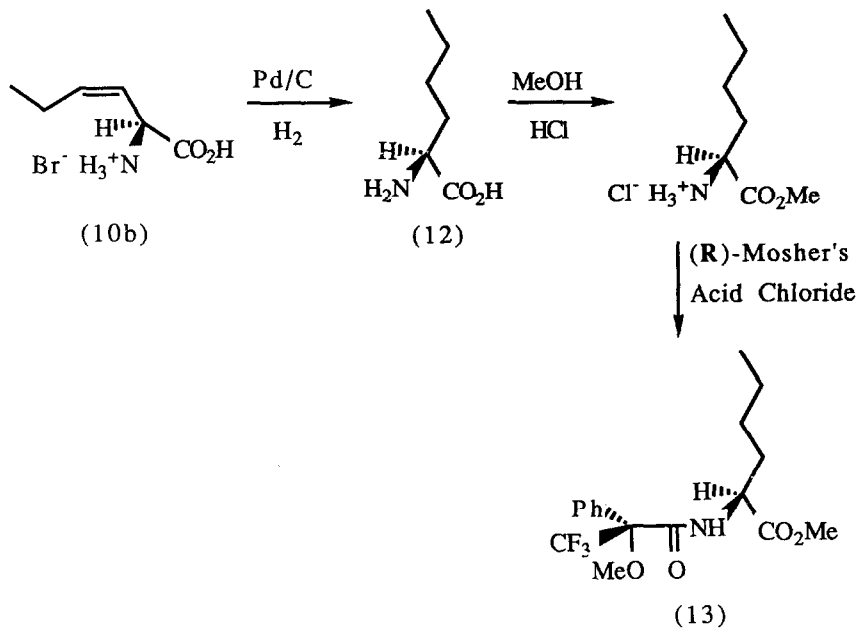
β-Allyl aspartate , prepared by treating (S)-aspartic acid with allyl alcohol under acidic conditions, was N-protected and esterified using the procedures developed for the corresponding β-methyl ester,[6] giving diester (6) as a white oil in 67% overall yield from (S)-aspartic acid (Scheme 3). The optical purity of (6) was demonstrated by cleavage of the β-allyl ester[17] to give t-butyl N-benzyloxycarbonyl-(S)-aspartate, which had been prepared previously[6]. Treatment of diester (6) with 2 equivalents of



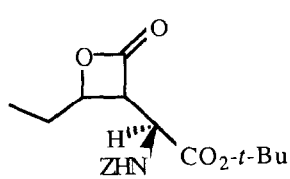
Scheme 3

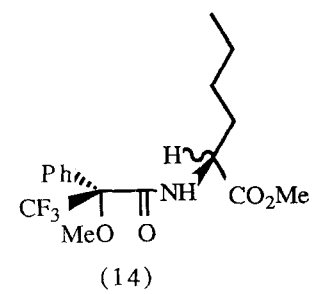
LHMDS followed by either benzaldehyde or propanal gave hydroxydiesters (7a, b) as a 1:1 mixture of two diastereomers. Cleavage of the allyl ester[17] gave hydroxyacids (8a, b) in 70-80% yield (Scheme 3). Attempted conversion of hydroxy acid (8a) to β,γ-unsaturated compound (9a) by treatment with either dimethylformamide-dimethylacetal (100°C, toluene)[18] or benzenesulphonyl chloride[19] gave only decomposition or low yields of the desired product. However, treatment with tribenzylphosphine /DEAD[21] gave alkene (9a) in 73% yield as a mixture of (E) and (Z)-isomers in 9:2 ratio. When hydroxyacid (8b) was reacted under these conditions (Z) alkene (9b) was formed in 60% yield as a single isomer. On reaction with benzenesulphonyl chloride, compound (8b) gave β-lactone (11) in 69% yield. Amino esters (9a, b) were deprotected with anhydrous HBr in glacial acetic acid, giving amino acids (10a, b) in good yield (Scheme 3).

The enantiomeric excess of the amino acid products was determined by hydrogenation of amino acid (10b) (5% Pd/C, 1 atm. H_2, EtOH) giving (S)-norleucine (12), which was re-esterified with methanolic HCl, and converted into (R)-Mosher amide (13) as shown in Scheme 4. Racemic norleucine was similarly converted into the analogous amide (14).



Scheme 4





The ^{19}F nmr spectrum of amide (14) showed two peaks of equal intensity, one for each stereoisomer at C2, whilst the ^{19}F nmr spectrum of amide (13) showed only one resonance, showing that the amino acids (10) were obtained enantiomerically pure within the detection limits of 250 MHz n.m.r.

Although β-lactam (11) was not useful for a synthesis of alkene (10b), it did allow the relative stereochemistry of the β-hydroxyacids and esters to be determined. Mulzer et al. had shown that for a wide range of β-lactones, the ^1H coupling constant across the lactone ring was 4.5 Hz if the protons were cis, and 6.0 Hz for a trans coupling.[20] The observed coupling constants for lactone (11) were 4.2, and 4.7 Hz, showing that both isomers of the β-lactone had a cis ring junction, and hence that the hydroxyacids and esters were formed with a syn arrangement of the hydroxyl and β-carboxyl groups. This was confirmed by the observation that hydroxyacid (8b) gave only the (Z)-isomer of alkene (9b) when treated with triphenylphosphine and DEAD, since this elimination has previously been shown to be a stereospecific trans elimination.[21]

In conclusion, a stereospecific synthesis of β,γ-unsaturated α-amino acids has been achieved. In addition it has been shown that the double bond can be reduced in high yield, giving a stereospecific synthesis of substituted amino acids.

EXPERIMENTAL

Optical rotations were recorded on a Perkin-Elmer 241 Polarimeter. Ir spectra were recorded on a Perkin-Elmer 681 spectrophotometer; only selected resonances are reported, and are reported as (s) strong, (m) medium, (w) weak, or (br) broad. ^1H nmr spectra were recorded on a Bruker WH 300 (300 MHz), or AM 250 (250 MHz) spectrometer. The residual solvent peak was used as an internal standard, and spectra were recorded in $CDCl_3$ unless otherwise stated. Multiplicities are reported as (br) broad), (s) singlet, (d) doublet, (t) triplet, (q) quartet, (m) multiplet, (dt) double triplet etc. ^{13}C nmr spectra were recorded at 62.85 MHz on a Bruker AM 250 spectrometer, using the solvent peak as an internal reference. When stated, the DEPT sequence[22] was used, but spectra are reported as if they had been recorded as off-resonance spectra. For ^1H, and ^{13}C nmr spectra, peaks are reported in ppm downfield of $SiMe_4$, and a * indicates that assignments may be interchanged. ^{19}F nmr spectra were recorded at 235.2 MHz on a Bruker AM 250 spectrometer. Peaks are reported in ppm downfield of $CFCl_3$, which was used as an external reference. Mass spectra were recorded on VG Analytical Ltd., ZAB1F, or MM30F mass spectrometers using the techniques of (DCI) ammonia desorption chemical impact, or (CI) ammonia chemical ionisation. Microanalyses were performed by Mrs. V. Lamburn, Dyson Perrins Laboratory, University of Oxford. All solvents were distilled before use. THF was distilled over sodium/benzophenone, diisopropylamine was distilled over calcium hydride and stored over 4A molecular sieves. n-BuLi was used as a solution in hexane,m and standardised with diphenylacetic acid prior to use.[23] Flash chromatography[24] was performed on silica gel (Merck Kieselgel 60 GF_{254}, 230-400 mesh). Reverse HPLC was performed on a Gilson Model 303 pump, Rheodyne 7125 injector, and Gilson Molochrome UV detector set at 254 nm. A flow rate of 4 ml min^{-1}, and a column (250 x 10.0 mm internal diameter) packed with Hypersil were used.

t-Butyl 2-benzyloxycarbonylamino-3-carbomethoxy-4-hydroxy-4-phenyl-(2S)-butanoate (2a).

To bis(trimethylsilyl)amine (12 ml, excess) in THF (100 ml) at 0°C under argon was added BuLi (0.9M; 25 ml), 32 mMol). After stirring at RT for 10 minutes, the solution

was cooled to -78°C and a solution of α-t-butyl β-methyl N-Z(S)-aspartate[6] (1) (5 g, 15 mMol) in THF (50 ml) was added. The resulting red solution was allowed to warm to -30°C, and stirred at between -30 and -40°C for 45 minutes. The solution was recooled to -78°C, and benzaldehyde (5 ml, excess) was added. The resulting yellow solution was stirred for 15 minutes at -78°C, and then poured into 1M hydrochloric acid (100 ml). The products were extracted with ether (3 x 100 ml), the combined organic phases were dried ($MgSO_4$) and evaporated in vacuo to give an orange oil which was subjected to flash chromatography (5% Et_2O/CH_2Cl_2) to give (2a) as a colourless oil. Yield 3.2 g (50%); (Found: C, 65.0; H, 66.6; N, 2.9. $C_{24}H_{29}NO_7$ requires C, 65.0; H, 6.5; N, 3.2%); ν_{max} (neat) 3408 m, 3038 w, 2980 m, and 1725 cm^{-1} s; δ_H 1.42 and 1.49 (2 x 9H, s, $OC(CH_3)_3$), 3.30 and 3.44 (2 x 1H, t, J 5.3 Hz, and dd, J 8.3, and 3.6 Hz, $CHCO_2Me$), 3.63 and 3.74 (2 x 3H, s, OCH_3), 4.17 and 4.57 (2 x 1H, dd, J 9.0 and 3.6 Hz, and 8.6 and 5.4 Hz, $NCHO_2$), 5.00 and 5.07 (2 x 1H, d, J 8.4, and 5.2 Hz, PhCHO), 5.11, and 5.13 (2 x 2H, s, CH_2Ph), 5.77 and 5.88 (2 x 1H, d, J 8.9 Hz, and 8.5 Hz, NH), 7.3-7.4 (10H, m, ArH); δ_C (DEPT) 27.68 and 27.86 (2 x q, $OC(\underline{C}H_3)_3$, 51.98 and 52.09 (2 x q, OCH_3), 53.50 and 53.59 (2 x d, $\underline{C}HCO_2Me^*$), 54.20 and 54.62 (2 x d, $N\underline{C}HCO_2^*$), 66.94 and 67.07 (2 x t, CH_2Ph), 72.07 and 72.56 (2 x d, PhCHO), 82.64 and 83.30 (2 x s, $OCMe_3$), 125.96, 126.52, 127.87, 128.03, 128.40, 128.61 and 129.96 (7 x d, ArCH), 136.01. 136.57, 140.37 and 140.80 (4 x s, ArC), 155.87 and 156.13 (2 x s, NCO_2), 169.01, 169.11, 171.78 and 172.67 (4 x s, CO_2); m/z (FD) 444 (MH^+).

t-Butyl 2-benzyloxycarbonylamino-3-carbomethoxy-4-hydroxy-(2S)-hexanoate (2b)

Compound (2b) was prepared in an identical manner to (2a) above, using diester (1)[6] (5.0 g, 15 mMol) and quenching the reaction with propanal (15 ml, excess). Flash chromatography (10% Et_2O/CH_2Cl_2) gave (2b) as a colourless oil. Yield 3.2 g (50%); (Found: C, 60.6; H, 7.3; N, 3.6. $C_{20}H_{29}NO_7$ requires: C, 60.8; H, 7.3; N, 3.5%); ν_{max} (neat) 3500 br, 3060 w, 2960 m, 1725 s, and 1152 cm^{-1} s; δ_H 0.97 (2 x 3H, t, J 7.4 Hz, $\underline{C}H_3CH_2$) 1.44 and 1.45 (2 x 9H, s, $OC(CH_3)_3$), 1.5-1.9 (2H, m, $MeCH_2$), 2.69 (1H, br, OH), 2.93 and 3.16 (2 x 1H, dd, J 6.2 and 2.9 Hz, and 5.7 and 4.2 Hz, $CHCO_2Me$), 3.7-3.9 (1H, m, CHO), 3.71 and 3.72 (2 x 3H, s, OCH_3), 4.58 and 4.72 (2 x 1H, dd, J 8.9 and 4.0 Hz, and 8.6 and 6.3 Hz, $NCHCO_2$), 5.18 and 5.19 (2 x 2H, s, CH_2Ph), 5.81 and 5.92 (2 x 1H, d, J 8.8 Hz, NH), 7.3-7.4 (5H, m, ArH) (The peak assignments were confirmed by a proton COSY experiment); δ_C (DEPT) 9.75 and 10.37 (2 x q, $\underline{C}H_3CH_2$), 27.78 and 27.83 (2 x q, $OC(\underline{C}H_3)_3$, 28.55 (t, $MeCH_2$), 51.25 and 51.69 (2 x d, $\underline{C}HCO_2Me^*$), 51.87 and 51.96 (2 x q, OCH_3), 53.87 and 55.06 (2 x d, $N\underline{C}HCO_2^*$), 66.86 and 67.12 (2 x t, CH_2Ph), 71.21 and 71.73 (2 x d, CHO), 82.76 and 83.05 (2 x s, $OCHMe_3$), 128.06, 128.17 and 128.50 (3 x d, ArCH), 136.18 (s, ArC), 156.41 (s, NCO_2), 169.33, 169.48, 171.95 and 172.60 (4 x s, CO_2); m/z (DCI) 413 ($M+,NH_4^+$, 18%), 396 (MH+, 100%), 386 (22), 340 (78), 322 (26), 296 (48), 91 (76).

t-Butyl 2-benzyloxycarbonylamino-3-carbomethoxy-4-hydroxy-4-phenyl-(2RS)-butanoate.

This compound was prepared according to the method described above for the corresponding (2S)-isomer (2a) using α-t-butyl β-methyl N-Z-(RS)-aspartate[6] (1 g, 3.0 mMol). This material was identical to hydroxydiester (3a) by t.l.c. and ^1H n.m.r. spectroscopy.

t-Butyl 2-amino-3-carbomethoxy-4-hydroxy-4-phenyl-(2S)-butanoate (3).

To hydroxydiester (2a) (400 mg, 0.9 mMol), dissolved in degassed ethanol (20 ml) was added 5% Pd/C (100 mg). The mixture was stirred under a hydrogen atmosphere for 2.5 hours. The reaction mixture was filtered through celite, and the ethanol evaporated in vacuo, to give (3) as a colourless oil. Yield 250 mg (90%); ν_{max} (neat) 3220 br, 2980 m, 1735 s, and 1155 cm^{-1} s; δ_H 1.45 and 1.52 (2 x s, 9H, $OC(CH_3)_3$), 3.2-3.4 (1H, m, $CHCO_2^*$), 3.56 (3H, s, OCH_3), 3.6-3.8 (1H, m, $NCHCO_2^*$), 4.23 (3H, br, NH_2 + OH), 5.1-5.4 (1H, m, PhCHO), 7.2-7.6 (5H, m, ArH); δ_C (DEPT) 27.60 and 27.69 (2 x q, $OC(\underline{C}H_3)_3$), 51.66 (q, OCH_3), 52.43, 52.97, 53.80 and 55.40 (4 x d, NCHCH), 70.74 and 73.06 (2 x d, PhCHO), 83.62 and 84.15 (2 x s, $OCMe_3$), 125.87, 126.01, 127.47, 127.65, 128.05 and 128.27 (6 x d, ArCH), 141.40 and 141.88 (2 x s, ArC), 169.12, 169.52, 171.21 and 171.29 (4 x s, CO_2); m/z (DCI) 310 (MH+, 100%), 254 (16), 204 (18), 148 (17) 102 (16).

t-Butyl 2-amino-3-carbomethoxy-4-hydroxy-4-phenyl-(2RS)butanoate.

This compound was prepared according to the method described above for amine (3), using racemic hydroxydiester (400 mg, 0.9 mmol). This material was identical to (S)-isomer (3) by ^{1}H n.m.r. spectroscopy.

t-Butyl N(2-methoxy-2-phenyl-3,3,3-trifluoro-(R)-propionoyl)-2-amino-3-carbomethoxy-4-hydroxy-4-phenyl-(2S)-butanoate (4).

To amine (3) (20 mg, 0.05 mMol) dissolved in $CDCl_3$ (0.5 ml), was added pyridine 1 drop), followed by (R)-Mosher's acid chloride[25] (20 mg, excess). The resulting solution was shaken, allowed to stand for 12 hours, then analysed without purification. δ_F -71.09 and -70.92; for material prepared using LDA as base, peaks at -70.79 and -70.49 of one sixth the intensity of the main peaks were also observed.

t-Butyl N(2-methoxy-2-phenyl-3,3,3-trifluoro-(R)-propionoyl)-2-amino-3-carbomethoxy-4-hydroxy-4-phenyl-(2RS)-butanoate (5).

This compound was prepared according to the method described for Mosher amide (4), using racemic amine. δ_F -71.09, -70.92, -70.79 and -70.49 ppm.

β-Allyl (S)-aspartate hydrochloride

Anhydrous hydrogen chloride(13.5 g,0.37mMol)was dissolved in allyl alcohol (125 ml) at RT. Anhydrous (S)-aspartic acid(16 g,0.12 mMol)was then added and the resulting suspension stirred at RT for 18 hours. The reaction mixture was poured into ether (500 ml), and the resulting precipitate filtered and dried under high vacuum, giving the product as a white solid. Yield 23 g (91%); m.p. 185-186°C (lit.,[26] 192°C); ν_{max} (nujol) 2910 br, 1740 s, and 1724 cm^{-1} s; δ_H (D_2O) 2.96 (1H, dd, J 18.1, and 5.1 Hz, CH_2CO_2), 3.03 (1H, dd, J 18.1, and 5.9 Hz, CH_2CO_2), 4.23 (1H, t, J 5.4 Hz, NCH), 4.51-4.53 (2H, m, OCH_2), 5.1-5.3 (2H, m, $=CH_2$), 5.7-5.9 (1H, m, $CH_2CH=$); m/z 174 MH+, 100%, 129 (26), 128 (25), 90 (20), 44 (39).

β-Allyl N-Z-(S)-aspartate.

To the above mono-ester (20 g, 0.1M) dissolved in water (400 ml), was added ether (150 ml), potassium carbonate (22.5 g, 0.16M) (caution; frothing), and benzyl chloroformate (27.5 g, 0.16 mMol). The solution was stirred at RT for 4 hours, the layers were separated, the aqueous layer was washed twice with ether (150 ml), and acidified to pH 1 with conc. hydrochloric acid. The resulting opaque solution was extracted with ether (3 x 100 ml), the combined organic phases were dried ($MgSO_4$) and evaporated in vacuo to give β-allyl N-Z(S)-aspartate as a colourless oil. Yield 22.6 g (77%); $[\alpha]_D^{20}$ + 15.3° (c 1.1 in $CHCl_3$); ν_{max} (neat) 3100 br, and 1730 cm^{-1} s; δ_H 2.92 (1H, dd, J 17.3 and 4.4 Hz, CH_2CO_2), 3.11 (1H, dd, J 17.3 and 4.3 Hz, CH_2CO_2), 4.59 (2H, d, J 5.6 Hz, $OCH_2CH=$), 4.70 (1H, d t, J 8.4 and 4.3 Hz, NCH), 5.13 (2H, s, OCH_2Ph), 5.2-5.4 (2H, m, $=CH_2$), 5.8-6.0 (2H, m, $CH_2CH=$+ NH), 7.3-7.5 (5H, m, ArH), 9.13 (1H, br, CO_2H); δ_C 36.26 (t, CH_2CO_2), 50.07 (d, NCH), 65.59 (t, OCH_2Ph*), 67.10 (t, OCH_2CH=*), 118.44 (t, $=CH_2$), 127.87, 127.99, and 128.31 (3 x d, ArCH), 131.43 (d, CH=), 135.83 (s, ArC), 156.14 (s, NCO_2), 170.51 and 174.10 (2 x s, CO_2); m/z (DCI) 325 (M+ NH_4^+, 100%), 308 (MH+, 24), 249 (14). 108 (8).

α-t-Butyl β-allyl N-Z-(S)-aspartate (6).

To β-allyl N-Z-(S)-aspartate (20 g, 65 mMol) dissolved in CH_2Cl_2 (200 ml) was added conc. sulphuric acid (2.0 ml) followed by isobutene (40 ml, excess). The solution was stirred at RT under an oil bubbler for 48 hours, then poured into saturated aqueous sodium bicarbonate solution (400 ml). The organic layer was separated, and the aqueous layer extracted with CH_2Cl_2 (3 x 200 ml). The combined organic phases were dried ($MgSO_4$), and evaporated in vacuo to give (6) as a white oil. An analytical sample was obtained by flash chromatography (CH_2Cl_2). Yield 22.5 g (95%); (Found: C, 62.6; H, 7.0;

N, 4.1. $C_{19}H_{25}NO_6$ requires: C, 62.8; H, 6.9; N, 3.9%); $[\alpha]_D^{25}$ + 18.2 (c 1.2 in $CHCl_3$); ν_{max} (neat) 3350 m, 2980 m, and 1730 cm^{-1} s; δ_H 1.45 (9H, s, $OC(CH_3)_3$), 2.84 (1H, dd, J 17.0 and 4.7 Hz, CH_3CO_2), 3.00 (1H, dd, J 17.0, and 4.3 Hz, CH_2CO_2), 4.5-4.6 (1H, m, NCH), 4.58 (2H, d, J 5.8 Hz, OCH_2CH=), 5.13 (2H, s, $PhCH_2O$), 5.2-5.4 (2H, m, $=CH_2$), 5.73 (1H, d, J 8.0 Hz, NH), 5.8-6.0 (1H, m, $CH=CH_2$), 7.3-7.4 (5H, m, ArH); δ_C 27.72 (q, $OC(CH_3)_3$, 36.75 (t, CH_2CO_2), 50.85 (d, NCH), 65.40 (t, OCH_2CH=*), 66.82 (t, OCH_2Ph*), 82.41 (s, $OCMe_3$), 118.49 (t, $=CH_2$), 127.93, 127.99 and 128.37 (3 x d, ArH), 131.64 (d, CH_2CH=), 136.19 (s, ArC), 155.82 (s, NCO_2), 169.39 and 170.28 (2 x s, CO_2); m/z (DCI) 381 (M+ NH_4+, 24%), 364 (MH+, 100%), 354 (52), 308 (82), 218 (80), 91 (66).

t-Butyl 2-benzyloxycarbonylamino-3-allyloxycarbonyl-4-hydroxy-4-phenyl-(2S)-butanoate (7a)

Compound (7a) was prepared in an identical manner to hydroxydiester (2a), using allyl ester (6) (5.0 g, 14 mMol), and benzaldehyde (5 ml, excess). Flash chromatography (5% Et_2O/CH_2Cl_2) gave (7a) as a colourless oil. Yield 2.9 g (45%); (Found: C, 66.2; H, 6.5; N. 2.8. $C_{26}H_{31}NO_7$ requires: C, 66.5; H, 6.6; N, 3.0%); ν_{max} (neat) 3406 m, 3038 w, 2980 m, 1725 s and 1500 cm^{-1} s; δ_H 1.41 and 1.50 (2 x 9H, s, $OC(CH_3)_3$), 3.04 (1H, d, J 4.5 Hz, NH), 3.35 and 3.46 (2 x 1H, t, and dd, J 5.2 Hz and 8.3, and 3.3 Hz, $CHCO_2$), 3.96 (1H, d, J 6.2 Hz, NH); 4.17 (1H, dd, J 8.9 and 3.4 Hz, NCH), 4.4-4.7 (3H, m, PhCHO+ NCH+OH), 4.9-5.4 (5H, m, $PhCH_2O$+ OCH_2CH=), 5.6-6.0 (2H, m, $=CH_2$), 7.3-7.4 (10H, m, ArH); δ_C (DEPT) 27.74 and 27.89 (2 x q, $OC(CH_3)_3$), 53.63 and 54.77 (2 x d, $CHCO_2$), 65.75 and 66.00 (2 x t, OCH_2Ph*), 66.91 and 67.03 (2 x t, OCH_2CH=*), 72.06 and 72.73 (2 x d, NCH), 82.65 and 83.29 (2 x d, PhCHO), 118.68 and 119.02 (2 x t, $=CH_2$), 125.96, 126.47, 126.58, 126.84, 127.79, 127,99, 128.07, 128.38 and 128.60 (9 x d, ArCH), 131.33 (d, $CH=CH_2$), 136.11, 136.35, 140.40 and 140.88 (4 x s, ArC), 155.90 and 155.94 (2 x s, NCO_2), 169.00, 169.10, 170.98 and 171.92 (4 x s, CO_2); m/z (DCI) 487 (M+ NH_4+, 19%), 470 (MH+, 75), 452 (30), 414 (38), 396 (83), 91 (100%).

t-Butyl 2-benzyloxycarbonylamino-3-allyloxycarbonyl-4-hydroxy-(2S)-hexanoate (7b).

Compound (7b) was prepared in an identical manner to hydroxydiester (2a), using allyl ester (6) (5.0 g, 14 mMol), and propanal (5 ml, excess). Flash chromatography (1:1 Et_2O/hexane) gave (7b) as a colourless oil. Yield 3.8 g (66%); (Found: C, 62.4; H, 7.6; N, 3.4. $C_{22}H_{31}NO_7$ requires C, 62.7; H, 7.4; N, 3,3%); ν_{max} (neat) 3405 m, 2978 m and 1725 cm^{-1} s; δ_H 0.98 (3H, t, J 7.3 Hz, CH_3CH_2), 1.44 and 1.46 (2 x 9H, s, $OC(CH_3)_3$, 1.5-1.8 (2H, m, $MeCH_2$), 2.61 (1H, d, J 6.3 Hz, NH), 2.98 and 3.19 (2 x 1H, dd, and t, J 5.9, and 2.8 Hz, and J 5.0 Hz, $CHCO_2$), 3.36 (1H, d, J 7.7 Hz, NH), 3.6-4.0 (1H, m, NCH), 4.5-4.8 (3H, m, OCH_2CH= + EtCHO), 5.13 and 5.14 (2 x 2H, s, $PhCH_2O$), 5.2-5.4 (2H, m, $=CH_2$), 5.7-6.0 (2H, m, $CH=CH_2$ + OH), 7.3-7.4 (5H, m, ArH); δ_C (DEPT) 9.78, and 10.36 (2 x q, CH_3CH_2), 27.83 (q, $OC(CH_3)_3$), 28.00 and 28.65 (2 x t, $MeCH_2$), 51.03 and 51.77 (2 x d, $CHCO_2$*), 53.90 and 55.20 (2 x d, NCH*), 65.72 (t, OCH_2Ph*), 67.05 and 67.12 (2 x t, OCH_2CH=*), 71.39 and 71.88 (2 x d, EtCHO), 82.76 and 82.98 (2 x s, $OCMe_3$), 118.81 and 118.97 (2 x t, $=CH_2$), 127.99, 128.09, 128.26 and 128.44 (4 x d, ArCH), 131.52 (d, $CH=CH_2$), 136.17 and 136.24 (2 x s, ArC), 156.31 and 156.41 (2 x s, NCO_2), 169.28, 169.37, 171.13 and 171.82 (4 x s, CO_2); m/z (DCI) 439 (M+ NH_4+, 18%), 422 (MH^+, 94), 366 (100), 322 (35), 108 (37), 91 (77).

t-Butyl 2-benzyloxycarbonylamino-3-carboxy-4-hydroxy-4-phenyl-(2S)-butanoate (8a).

To hydroxydiester (7a) (380 mg, 0.8 mMol) in degassed CH_2Cl_2 (3 ml) under argon, was added tetrakis(triphenylphosphine)-palladium(0) (26 mg, 0.02 mMol), triphenylphosphine (12 mg, 0.05 mMol), and pyrrolidine (0.1 ml, excess). The resulting yellow solution was stirred at RT for 10 minutes, washed with 1M hydrochloric acid, dried ($MgSO_4$), and evaporated in vacuo to give the crude product. Flash chromatography (30% Et_2O/ CH_2Cl_2) gave (8a) as a colourless oil. Yield 250 mg (72%) (neat) 3408 br, 3038 w, 2980 m and 1720 cm^{-1} s; δ_H 1.39 and 1.47 (2 x 9H, s, $OC(CH_3)_3$), 3.26 and 3.47 (2 x 1H, t, J 5.3 and 6.1 Hz, $CHCO_2$), 4.12, and 4.46 (2 x 1H, dd, J 9.1 and 3.6 Hz, and 7.9 and 5.6 Hz, NCH), 4.95 and 5.13 (2 x 1H, d, J 9.0 and 6.0 Hz, PhCHO), 5.07, and 5.09 (2 x 2H, s, OCH_2Ph), 5.88 and 5.99 (2 x 1H, d, J 9.3, and 8.0 Hz, NH), 6.00 (2H, br, OH + CO_2H), 7.3-7.4

(10H, m, ArH); δ_C (DEPT) 27.58 and 27.74 (2 x q, $OC(\underline{CH_3})_3$), 53.40, 54.27, 54.67 and 54.75 (4 x d, NCHCH), 67.09 and 67.28 (2 x t, $PhCH_2O$), 72.26 and 72.83 (2 x d, PhCHO), 83.04 and 83.75 (2 x s, $OCMe_3$), 126.55, 127.04, 128.08, 128.27, 128.66, 128.88 and 129.06 (7 x d, ArCH), 136.07, 136.62, 140.04 and 140.25 (4 x s, ArC), 158.55 (s, NCO_2), 169.35, 169.81, 174.86 and 175.50 (4 x s, CO_2); m/z (DCI) 447 ($M + NH_4^+$, 5%), 430 (MH +, 11), 391 (26), 374 (43), 356 (61), 222 (48), 108 (50), 91 (100%).

t-Butyl 2-benzyloxycarbonylamino-3-carboxy-4-hydroxy-(2S)-hexanoate (8b).

Compound (8b) was prepared in an identical manner to hydroxyacid (8a) above using hydroxydiester (14b) (2.9 g, 6.9 mMol). Flash chromatography (CH_2Cl_2) then 1:1 Et_2O/CH_2Cl_2) gave (8b) as a colourless oil. Yield 2.0 g (76%); ν_{max} (neat) 3700-2400 br, 1718 s, and 1684 cm^{-1} s; δ_H 0.98 and 0.99 (2 x 3H, t, J 7.3, and 7.4 Hz, $\underline{CH_3}CH_2$), 1.44 and 1.46 (2 x 9H, s, $OC(\underline{CH_3})_3$), 1.5-1.8 (2H, m, $MeCH_2$), 2.75 and 3.22 (2 x 1H, dd, J 8.1 and 6.2 Hz, and 5.9 and 3.5 Hz, $CHCO_2$), 3.7-4.0 (1H, m, CHO), 4.5-4.6 (1H, m, NCH), 5.14 and 5.15 (2 x 2H, s, $PHCH_2O$), 5.47 (2H, br, CO_2H + OH), 5.9-6.0 (1H, m, NH), 7.3-7.4 (5H, m, ArH); δ_C (DEPT) 9.51 and 10.26 (2 x q, $\underline{CH_3}CH_2$), 27.60 and 27.70 (2 x q, $OC(\underline{CH_3})_3$), 51.69, 52.99, 54.06 and 54.37 (4 x d, NCHCH), 67.29 and 67.59 (2 x t, $PhCH_2O$), 71.00 and 71.65 (2 x d, EtCHO), 83.12 and 83.99 (2 x s, $OCMe_3$), 127.96, 128.11, 128.29, 128.44 and 128.49 (5 x d, ArCH), 135.75 and 136.01 (2 x s, ArC), 156.79 and 157.11 (2 x s, NCO_2), 169.16, 169.28, 172,93 and 174.54 (4 x s, CO_2); m/z (CI) 399 ($M + NH_4^+$, 11%)(, 382 (MH^+, 35), 343 (58), 326 (100%), 308 (46), 91 (62).

α-t-Butyl N-Z-(S)-aspartate from α-t-butyl β-allyl N-Z-(S)-aspartate (6).

This compound was prepared in an identical manner to hydroxyacid (8a), above using diester (12) (800 mg, 2.2 mMol). Flash chromatography (30% Et_2O/CH_2Cl_2) gave (13) as a colourless oil. Yield 600 mg (84%); $[\alpha]_D^{25}$ + 12.0° (c 0.9 in $CHCl_3$); lit.,[27] -7.6° c 0.8 in $CHCl_3$). This material was identical to that prepared earlier[6].

α-t-Butyl β-methyl N-Z-(S)-aspartate (1) from α-t-butyl N-Z(S)-aspartate

To the above acid (50 mg, 0.15 mMol) dissolved in ether (10 ml) was added diazomethane dissolved in ether[28] (excess). The resulting yellow solution was stirred at RT for 1 hour, the solvents were evaporated in vacuo, and the residue subjected to flash chromatography (4% Et_2O/CH_2Cl_2), giving (1) as a colourless oil. Yield 36 mg (69%); $[\alpha]_D^{23}$ -18.7° (c 1.2 in EtOH), lit.,[29] -18.8° c 1.2 in EtOH); this material was identical to that reported earlier,[29] and indicated that the starting material(6) was optically pure.

2-Oxo-3-(1-t-butyloxycarbonyl-1-benzyloxycarbonylamino-(S-methyl)-4-ethyl-oxetane (11).

To hydroxyacid (8b) (500 mg, 1.4 mMol) dissolved in pyridine (13 ml) at 0°C was added benzenesulphonyl chloride (0.5 ml, excess). The resulting yellow solution was kept at 4°C for 18 hours, then poured into 1M hydrochloric acid (20 ml) and the products extracted with ether (3 x 20 ml). The combined organic phases were dried ($MgSO_4$) and evaporated in vacuo. Flash chromatography (CH_2Cl_2, then 3% Et_2O/CH_2Cl_2 gave (11) as a colourless oil. Yield 330 mg (69%); ν_{max} (neat) 3340 m, 2980 m, 1827 (s, β-lactone C=0) and 1722 cm^{-1} s; δ_H 1.01 (3H, d, J 7.3 Hz, $\underline{CH_3}CH_2$), 1.49 and 1.51 (2 x 9H, s, $OC(\underline{CH_3})_3$), 1.7-2.0 (2H, m, $MeCH_2$), 3.59 and 3.80 (2 x 1H, s, J 4.2, and 4.7 Hz, $CHCO_2$), 4.2-4.8 , (2H m, NCH + EtCHO), 5.13 and 5.14 (2 x 2H, s, $PhCH_2O$), 5.6-5.7 (1H, m, NH), 7.3-7.4 (5H, m, ArH); δ_C (DEPT) 8.75 and 8.87 (2 x q, $\underline{CH_3}CH_2$), 27.10 and 27.22 (2 x t, $MeCH_2$), 27.50 and 27.82 (2 x q, $OC(\underline{CH_3})_3$), 51.84 and 51.95 (2 x d, $CHCO_2$), 57.85 and 58.30 (2 x d, NCH), 67.41 (t, $PhCH_2O$), 75.83 (d, EtCHO), 84.20 and 84.40 (2 x s, $OCMe_3$), 128.09, 128.17, 128.31 and 128.55 (4 x d, ArCH), 135.93 (s, ArC), 155.58 and 156.14 (2 x s, NCO_2). 167.01, 167.19, 167.55 and 167.93 (4 x s, CO_2); m/z (CI) 381 ($M + NH_4^+$, 100%), 364 (MH^+, 24), 325 (51), 308 (12), 281 (24), 264 (10).

t-Butyl 2-benzyloxycarbonylamino-4-phenyl-(S)-3-but enoate (9a)

Method A.

Compound (9a) was prepared in an identical manner to β-lactone (11), using hydroxyacid (8a) (50 mg, 0.12 mMol). Flash chromatography (CH_2Cl_2 then 5% Et_2O/CH_2Cl_2) gave (9a) as a colourless oil. Yield 10 mg (23%), other data as reported below.

Method B

To hydroxyacid (8a) (400 mg, 0.9 mMol) dissolved in THF (7 ml) under argon was added triphenylphosphine (250 mg, 0.9 mmOl), and DEAD (added dropwise until solution turned permanently orange). The solution was stirred at RT for 20 minutes, the solvents were evaporated in vacuo, and the residue subjected to flash chromatography (5% Et_2O/ CH_2Cl_2) to give (9a) as a colourless oil. Yield 250 mg (73%); (Found: C, 71.6; H, 6.9; N. 3.5. $C_{22}H_{25}NO_4$ requires: C, 71.9; H, 6.8; N, 3.8%); ν_{max} (neat) 3340 m, 3034 w, 2980 m, 1720 s, and 1498 cm^{-1} s; δ_H 1.47 and 1.49 (2 x 9 H, s, $OC(CH_3)_3$), 4.98 (1H, t, J 6.5 Hz, NCH), 5.09 and 5.16 (2 x 2H, s, OCH_2Ph), 5.61 (1H, d, J 7.5 Hz, NH), 6.21 (1H, dd, J 15.9 Hz, and 6.0 Hz, PHCH=CH (E)-isomer), 6.67 (1H, d, J 15.9Hz, PhCh=(E)-isomer), 6.75 (1H, d, J 11.5 Hz, PhCH=(Z)-isomer); 7.2-7.5 (10H, m, ArH); δ_C 27.83 and 27.93 (2 x q, $OC(CH_3)_3$); 53.09 and 56.35 (2 x d, NCH), 66.79 and 67.00 (2 x t, OCH_2Ph), 82.70 (s, $OCMe_3$), 124.30 (d, PhCH=CH), 126.61, 127,61, 127.99, 128.10, 128.31, 128.47, 128.52 and 128.72 (8 x d, ArCH), 132.48, and 134.13 (2 x d, PhCH=), 136.08 and 136.26 (2 x s, ArC), 155.48 (s, NCO_2), 169.57 (s, CO_2); m/z (DCI) 385 ($M + NH_4^+$, 15%), 368 (MH^+), 329 (42), 312 (100%), 266 (47), 222 (32), 132 (25), 108 (23), 91 (70).

t-Butyl 2-benzyloxycarbonylamino-(S,Z)-3-hexenoate (9b).

Compound (9b) was prepared from hydroxyacid (8b) (500 mg, 1.3 mMol) using Method B as a colourless oil after flash chromatography (CH_2Cl_2). Yield 250 mg (60%); ν_{max} (neat) 3340 m, 2975 s, 1720 s and 1155 cm^{-1} s; δ_H 1.05 (3H, t, J 7.3 Hz, CH_3CH_2), 1.46 (9H, s, $OC(CH_3)_3$), 2.28 (2H, pentet, J 7.3 Hz, $MeCH_2$), 5.01 (1H, t, J 8.1 Hz, collapses to 1H, d, J 9.1Hz, on addition of D_2O, NCH), 5.12 (2H, s, OCH_2Ph), 5.25 (1H, tt, J 9.9 and 1.5 Hz, collapses to 1H, t, J 10.3 Hz, on irradiation to δ 2.3, NCHCH=), 5.58 (1H, d, J 7.0 Hz, exchanges with D_2O, NH), 5.69 (1H, dd, J 10.4 and 8.1 Hz, collapses to 1H, d, J 10.3 Hz, on irradiation at δ 2.3, EtCH=), 7.36 (5H, s, ArH); δ_C (DEPT) 13.74 (q, CH_3CH_2), 21.10 (t, $MeCH_2$), 27.70 (q, $OC(CH_3)_3$), 52.44 (d, NCH), 66.77 (t, OCH_2Ph), 82.08 (s, $OCMe_3$), 123.98 (d, CH=), 128.20 and 128.60 (2 x d, ArCH), 136.52 (s, ArC), 137.65 (d, CH=), 155.65 (s, NCO_2), 170.64 (s, CO_2); m/z (CI) 337 ($M + NH_4^+$, 78%), 320 (MH^+, 81), 310 (56), 281 (94), 264 (96), 220 (100%), 174 (72).

2-Amino-4-phenyl-(S)-3-butenoic acid (10a)

Alkene (9a) (277 mg, 0.7 mMol) was dissolved in a solution of 48% HBr in acetic acid, and the resulting solution stirred at RT for 45 minutes. The solvents were evaporated in vacuo as an azeotrope with toluene, giving an orange solid. The crude product was purified by reverse phase HPLC (10% MeOH/ H_2O), giving (10a) as a white solid. Yield 96 mg (72%); ν_{max} (KBr) 2980 br, 1740 s, and 1596 cm^{-1} m; δ_H (D_2O) 4.14 and 4.31 (2 x 1H, d, J 7.3 and 8.2 Hz, NCH), 6.19 (1H, dd, J 15.9 and 8.5 Hz, =CHCH 6.74 (1H, d, J 15.9 Hz, PhCH=, 6.92 (1H, d, J 12.5 Hz, PhCH=(Z)-isomer), 7.1-7.5 (5H, m, ArH); m/z (DCI) 178 (MH^+, 4%), 166 (10), 102 (100%), 86 (27).

2-Amino-(S,Z)-3-hexenoic acid hydrobromide (10b)

Compound (10b) was prepared in an identical manner to (10a) above using alkene (9b) (150 mg, 0.5 mMol). Amino acid (10b) was obtained as a white solid after the solvents were evaporated. Yield 96 mg (97%); ν_{max} 3600-2400 br, 1738 s, and 1210 cm^{-1} s; δ_H (D_2O) 0.86 3H, t, J 7.5 Hz, CH_3), 2.06 (2H, m, J 7.5 and 1.4 Hz, $MeCH_2$), 4.72 (1H, d, J 9.8 Hz, NCH), 5.26 (1H, tt, J 10.2 and 1.5 Hz, NCHCH=), 5.86 (1H, dt, J 10.5 and 7.5 Hz, EtCH=); m/z (DCI) 130 (MH^+, 100%),84 (21).

(S)-Norleucine (12).

Amino acid (10b) (90 mg, 0.4 mMol) was dissolved in degassed ethanol, and 5% Pd/C (100 mg) was added. The mixture was stirred under a hydrogen atmosphere for 5 hours. The reaction was filtered through celite, and evaporated to give (12) as a white solid. Yield 90 mg (99%); δ_H (D_2O) 0.82 (3H, t, J 7.4 Hz, CH_3), 1.1-1.4 (4H, m, $MeCH_2CH_2$), 1.6-1.9 (2H, m, $NCHCH_2$), 3.94 (1H, t, J 7.1 Hz, NCH); m/z (DCI) 132 (MH^+, 100%, 86) (49).

Methyl (S)-norleucine hydrochloride.

Amino acid (12) (90 mg, 0.4 mMol) was dissolved in HCl saturated methanol (5 ml), and the resulting solution stirred at RT for 18 hours. The solvents were evaporated in vacuo, to give methyl (S)-norleucine hydrochloride as a white solid. Yield 68 mg (95%); δ_H 0.92 (3H, t, J 7.5 Hz, CH_3CH_2), 1.2-1.7 (4H, m, $MeCH_2CH_2$), 1.9-2.2 (2H, m, $NCHCH_2$), 3.83 (3H, s, OCH_3), 4.0-4.2 (1H, m, NCH), 8.71 (3H, br, NH_3); m/z (DCI) 146 (MH^+, 100%), 86 (37).

Methyl (RS)-norleucine hydrochloride.

This compound was prepared according to the method described above for the (S)-isomer, using racemic norleucine[30] (5.0 g, 38 mMol). This material was identical to the (S)-isomer by ^1H n.m.r. spectroscopy.

Methyl N-(2-methoxy-2-phenyl-3,3,3-trifluoro-(R)-propionoyl)-(S)-norleucine (13).

This compound was prepared according to the method described above for Mosher amide (4), using methyl (S)-norleucine hydrochloride (20 mg, 0.04 mMol). δ_F -71.01.

Methyl N-(2-methoxy-2-phenyl-3,3,3-trifluoro-(R)-propionoyl)-(RS)-norleucine (14).

This compound was prepared according to the method described above for Mosher amide (4), using methyl (RS)-norleucine hydrochloride (20 mg, 0.04 mMol). σ_F -71.01, and -70.64.

Acknowledgements

The authors thank the S.E.R.C. for a quota award to M.N., Glaxo Group Research Ltd. (U.K.) for financial support to M.G.M., and Miss J. Bryant for typing this manuscript.

References

1. R.D. Allan, Aust.J.Chem., 1979, 32, 2507; M. Johnston, R. Raines, M. Chang, N. Esaki, K. Soda and C. Walsh, Biochemistry, 1981, 20, 4325; J.M. Girodeau, C. Agouridas, M. Masson, R. Pineau and F. Le Goffic, J.Med.Chem., 1986, 29, 1023.

2. J.W. Hines, Jr., E.G. Breitholle, M. Sato and C.H. Stammer, J.Org.Chem., 1976, 41, 1466; F. Heinzer and D. Bellus, Helv.Chim.Acta, 1981, 64, 2279.

3. D.D. Keith, J.A. Tortora, K. Ineichen and W. Leimgruber, Tetrahedron, 1975, 31, 2633; P.L. Beaulieu and P.W. Schiller, Tetrahedron Lett., 1988, 29, 2019.

4. U. Schollkopf, J. Nozulak and U. Groth, Tetrahedron, 1984, 40, 1409

5. U. Schollkopf, B. Hupfeld, S. Kuper, E. Egert and M. Dyrbusch, Angew.Chem.Int. Ed.Engl., 1988, 27, 433.

6. J.E. Baldwin, M.G. Moloney and M. North Tetrahedron, 1989, previous paper.

7. J.E. Baldwin, M. North, A. Flinn and M.G. Moloney, Tetrahedron, 1989, 1465.

8. J.E. Baldwin, M.G. Moloney and M. North, J.Chem.Soc., Perkin Trans 1, 1989, 833.

9. J.E. McMurray, 'Organic Reactions', Wiley, London, 1976, 24, Chapter 2; E. Haslam, Tetrahedron, 1980, 36, 2409.

10. D.H. Miles and E.J. Parish, Tetrahedron Lett., 1972, 13, 398718.

11. P. Muller and B. Siegfried, Helv.Chim.Acta, 1974, 57, 987.

12. P.G. Gassman and W.N. Schenk, J.Org.Chem., 1977, 42, 918

13. T.W. Greene, 'Protective Groups in Organic Synthesis', Wiley, Chichester, 1981, Chapter 5.

14. T.L. Ho, Synth.Commun., 1978, 8, 15.

15. P.D. Jeffrey and S.W. McCombie, J.Org.Chem., 1982, 47, 587

16. R.E. Ireland, R.H. Mueller and A.K. Willard, J.Am.Chem.Soc., 1976, 98, 2868.

17. R. Deziel, Tetrahedron Lett., 1987, 28, 4371.

18. S.Hara, H. Taguchi, H. Yamamoto and H. Nozaki, Tetrahedron Lett., 1975, 16 1545.

19. W. Adam, J. Baeza and J.C. Liu, J.Am.Chem.Soc., 1972, 94, 2000; A.P. Krapcho and E.G.E. Jahngen, Jr., J.Org.Chem., 1974, 39, 1322; A.P. Krapcho and E.G.E. Jahngen, Jr., J.Org.Chem., 1974, 39, 1650; S. Mageswaran and M.U.S. Sultanbawa, J.Chem.Soc., Perkin Trans 1, 1976, 884.

20. J. Mulzer, J. Segner and G. Bruntrup, Tetrahedron Lett., 1977, 18, 4651; J. Mulzer, M. Zippel, G. Bruntrup, J. Segner and J. Finke, Liebigs Ann.Chem., 1980, 1108

21. J. Mulzer and G. Bruntrup, Angew.Chem.,Int.Ed.Engl., 1977, 16, 255.

22. A.E. Derome, 'Modern NMR Techniques for Chemistry Research', Pergamon Press, Oxford, 1987, Chapter 6.

23. W.G. Kofron and L.M. Baclawski, J.Org.Chem., 1976, 41, 1879.

24. W.C. Still, M. Kahn and A. Mitra, J.Org.Chem., 1978, 43, 292

25. J.A. Dale, D.L. Dull and H.S. Mosher, J.Org.Chem., 1969, 34, 2543.

26. D. Wirth, D. Gilbert and A. Boutin, E.P.O. 149 582 A2/1985. (CA 104, 51112).

27. A. Vasella, R. Voeffray, J. Pless and R. Huguenin, Helv.Chim.Acta, 1983, 66, 1241.

28. T.H. Black, Aldrichimica Acta, 1983, 16, 3

29. I. Schon, T. Szirtes, T. Uberhardt, A. Rill, A. Csehi and B. Hegedus, Int.J. Pept.Protein.Res., 1983, 22 , 92.

30. Purchased from the Sigma Chemical Company.