New synthetic routes to α-amino acids and γ-oxygenated α-amino acids. Reductive denitration and oxidative transformations of γ-nitro-α-amino acids

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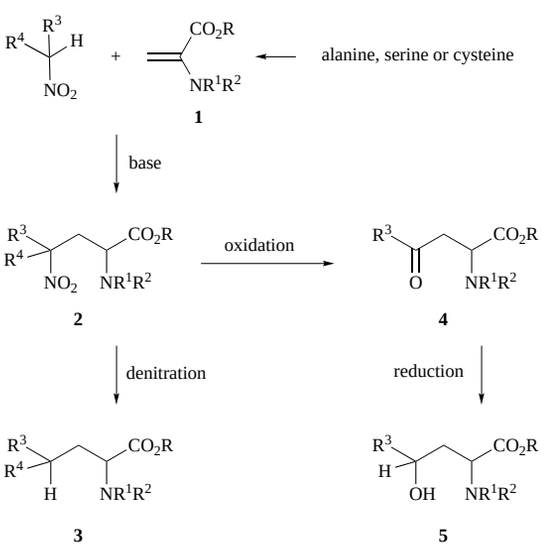
Transformation of γ-nitro-α-amino acid derivatives into α-amino acids by reductive denitration, into the γ-oxo-α-amino acids by ozonolysis of the corresponding amino acid ester nitronate derivatives, and into γ-hydroxy-α-amino acid derivatives by subsequent reduction of the oxo functionality, can be achieved in good yields. As the γ-nitro-α-amino acid derivatives are prepared from *N*,*O*-protected dehydroalanines derivable from the corresponding alanine, serine and cysteine derivatives by specific routes, the overall procedures provide a means for selective conversion of these simple α-amino acids into more complex ones.

In the preceding paper, we reported that conjugate addition to a dehydroalanine **1** of a nitroalkane provides a general synthesis of γ-nitro-α-amino acids **2** (Scheme 1).[1] The nitro group of a nitroalkane can be transformed into a variety of other functional groups[2] and so these γ-nitro-α-amino acids **2** have the potential to be precursors of a variety of other γ-substituted α-amino acids.

Nitro groups that are attached to tertiary carbon atoms or to secondary carbon atoms that are activated by a vicinal electron-withdrawing group can be reductively denitrated under radical conditions.[3–5] Thus, sequential application of conjugate addition of a nitroalkane to a dehydroalanine **1** (available from alanine, serine and cysteine, appropriately protected) and reductive denitration, followed by deprotection steps, affords a means of selectively converting simple amino acids into more complex α-amino acids **3** (Scheme 1). We have previously communicated



Scheme 1

an example of this synthetic sequence in the conversion of an L-alanine derivative into the corresponding L-leucine.[6]

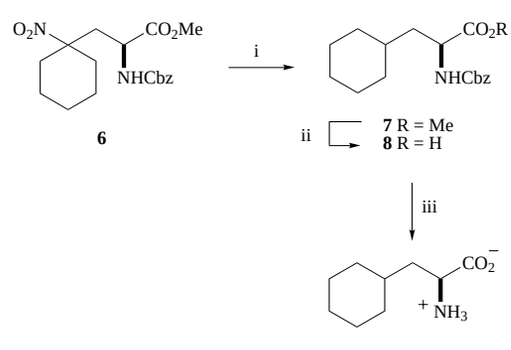
Modification of the nitro group of γ-nitro-α-amino acids **2** into oxo and hydroxy functionalities also provides a new route to γ-oxygenated α-amino acids **4** and **5** (Scheme 1). A number of high-yielding, mild procedures have been reported for the conversion of nitro groups into carbonyls.[7–11] These methods include a reductive procedure using aqueous $TiCl_3$,[8] the ozonolysis of nitronates,[9] and oxidative procedures using permanganates.[10] Application of this chemistry to γ-nitro-α-amino acids **2** would give γ-oxo-α-amino acid derivatives **4** which can be reduced to the protected γ-hydroxy-α-amino acid **5** and subsequently deprotected to give the corresponding amino acids. A preliminary example of this route was reported in our synthesis of γ-lactam analogues of β-lactam antibiotics.[12]

Results and discussion

Reductive denitration of γ-nitro-α-amino acid derivatives

3-Cyclohexylalanine **9**, *N*-Cbz-leucine **12**, *N*-Cbz-glutamic acid **15** and *N*-phthaloylglutamic acid **18** have been prepared in good yields by reductive denitration of the corresponding *N*-protected γ-nitro-α-amino acid esters **2**, followed by deprotection steps.

The synthesis of 3-cyclohexylalanine **9**, the saturated-ring analogue of phenylalanine, which uses the reductive denitration strategy, is outlined in Scheme 2.† Methyl 2-(benzyloxycarbonylamino)-3-(1′-nitrocyclohexyl)propanoate[1] **6** was treated with tributylstannane and 2,2′-azobis-(2-methylpropionitrile)

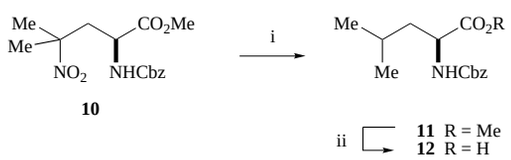


Scheme 2 *Reagents and conditions*: i, tributylstannane, AIBN, C_6H_6, heat, 4 h; ii, NaOH (10%), room temp., 16 h; iii, cyclohexene, 10% Pd/C, EtOH, heat, 20 min

† All the amino acid derivatives in this work are racemic although for simplicity only one of the enantiomers is shown in the Schemes.

(AIBN) in refluxing benzene[3,4] for 4 h which gave methyl 2-(benzyloxycarbonylamino)-3-cyclohexylpropanoate **7** in 65% yield after the tin compounds were removed by chromatography. Compound **7** was saponified with 10% aqueous sodium hydroxide and the resultant *N*-protected cyclohexylalanine derivative **8** was hydrogenolysed under catalytic hydrogen transfer conditions to give racemic 3-cyclohexylalanine **9** in 80% overall yield from the doubly protected compound **7**. Previous syntheses of 3-cyclohexylalanine **9**, and compounds containing this amino acid residue, have involved catalytic reduction of a phenylalanine derivative.[13–16] Unlike these routes, our new route to 3-cyclohexylalanine **9** should be easily modified to provide an entry to derivatives with additional ring substitution by use of substituted nitrocyclohexanes in the initial conjugate addition to a dehydroalanine derivative.

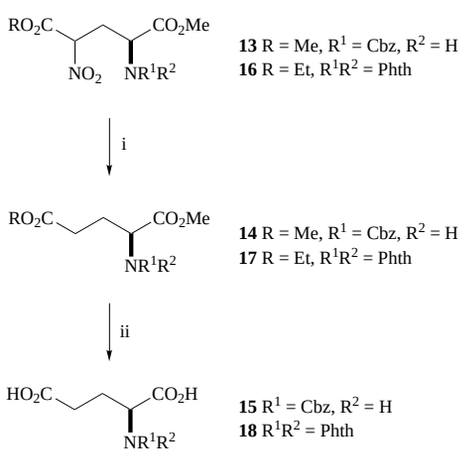
A new route to *N*-Cbz-leucine **12** was also developed. Treatment of methyl 2-(benzyloxycarbonylamino)-4-methyl-4-nitropentanoate[1] **10** under reductive denitration conditions gave *N*-Cbz-leucine methyl ester **11** in 67% yield (Scheme 3). Saponifi-



Scheme 3 *Reagents and conditions*: i, tributylstannane, AIBN, C_6H_6, heat, 4 h; ii, NaOH (10%), room temp., 16 h

cation of the ester **11** afforded a 91% yield of *N*-Cbz-leucine[17] **12**. The carbamate protecting group can be removed under a variety of mild conditions.[18] This synthesis was the foundation on which our recent stereoselective conversion of an L-alanine derivative into the corresponding L-leucine derivative was based.[6] Previous syntheses of leucine have centred on the introduction of the functional groups by reactions on isobutyl halides, 3-methylbutyraldehyde and 4-methylpentanoic acid derivatives.[19] The present method differs in approach in that the amino acid functional groups are already in place and it is the side-chain which is elaborated in the procedure.

The synthesis of glutamic acid derivatives follows a similar approach to that outlined above. Reductive denitration of dimethyl 2-(benzyloxycarbonylamino)-4-nitropentane-1,5-dioates[1] **13** with tributylstannane and AIBN gave the denitrated diester **14** in 65% yield (Scheme 4). Base hydrolysis of the



Scheme 4 *Reagents and conditions*: i, tributylstannane, AIBN, C_6H_6, heat, 4 h; ii, NaOH (10%), room temp., overnight

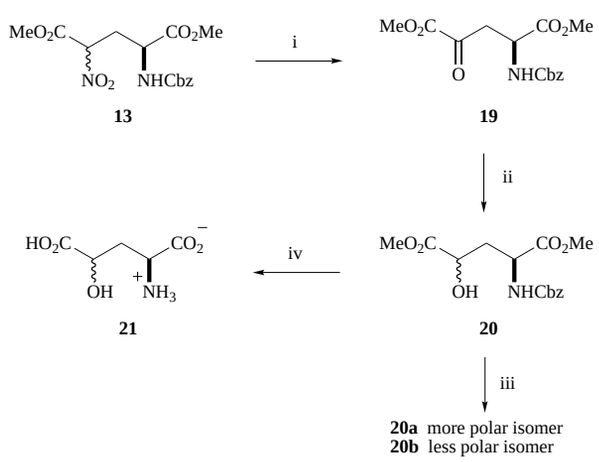
diester **14** gave *N*-Cbz-glutamic acid **15** in 71% yield after recrystallisation. The *N*-phthaloylglutamic acid, **18**, was synthesised in a similar way (Scheme 4). The mixture of diastereomeric nitro diesters[1] **16** was reductively denitrated as above to give 5-ethyl 1-methyl 2-phthalimidopentane-1,5-dioate **17** in 58% yield after column chromatography. Both ester functionalities were successfully hydrolysed by treatment with base to give *N*-phthaloylglutamic acid[20] **18** in 75% yield.

While the reductive denitration methodology is restricted to tertiary or activated secondary nitroalkanes, the utility of the approach outlined in this paper could be extended to include removal of nitro groups from unactivated secondary positions by initial conversion of the nitro group into a keto or hydroxy functionality, both of which can be reductively removed by selective processes, thereby allowing synthesis of amino acids with long side chains. Reductive deoxygenation of the secondary alcohol[21–23] of the corresponding γ-hydroxy-α-amino acid **5** is one such approach. While we have not yet investigated reductive deoxygenation of such compounds, we now report the efficient conversion of γ-nitro-α-amino acid derivatives **2** into the corresponding γ-hydroxy-α-amino acid derivatives **5** (Scheme 1) as is required in the preliminary step.

A new synthesis of γ-oxygenated α-amino acids

This methodology is illustrated by syntheses of the 4-oxoglutamic acid derivative **19**, the 4-hydroxyglutamic acids **21**, the 4-oxo- and 4-hydroxy-norvaline derivatives **23**–**27**, the protected homoserine aldehyde **29** and the aspartic acid derivative **31**.

The route to 4-hydroxyglutamic acids **21** is outlined in Scheme 5 and involves oxidation of dimethyl 2-(benzyloxycarb-



Scheme 5 *Reagents and conditions*: i, *N*-Benzyltrimethylammonium hydroxide, MeOH, stirred for 10 min, cooled to −78 °C and treated with O_3–O_2, Me_2S, warmed to room temp. and stirred overnight; ii, $NaBH_4$, MeOH, −20 °C, stirred for 30 min, then HCl–MeOH; iii, HPLC (μ-Porasil column); iv, H_2, 10% Pd/C, HCl–MeOH, for 1 h, then LiOH, stirred at 70 °C overnight

onylamino)-4-nitropentane-1,5-dioates **13** which are readily available by conjugate addition of methyl nitroacetate to methyl 2-(benzyloxycarbonylamino)acrylate.[1] With a minor modification, the McMurry, Melton and Padgett method of formation of carbonyls by the ozonolysis of nitronate anions[9] has proved to be most useful in conversion of the nitro adducts to the corresponding ketone. *N*-Benzyltrimethylammonium hydroxide was used as the base to generate the nitronate in place of the recommended sodium methoxide, and the yield of pure dimethyl 2-(benzyloxycarbonylamino)-4-oxopentane-1,5-dioate **19** from nitro compound **13** was 69%. When sodium methoxide was substituted as the base, prolonged reaction times were required in larger scale preparations, and this often resulted in the formation of substantial amounts of hydrolysed by-products. The ozonolysis method was also found best in our related studies leading to the synthesis of amino acids containing an azetidine ring.[24]

Treatment of the ketone **19** with $NaBH_4$ in anhydrous methanol at −20 °C, followed by decomposition of the excess

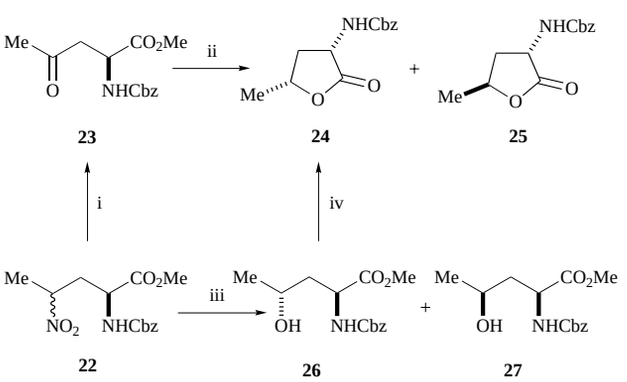
borohydride with methanolic HCl, gave the alcohols **20a** and **20b** as a 1:1 diastereomeric mixture in 93% yield (Scheme 5). Addition of methanolic HCl to decompose the excess reagent and use of a low reaction temperature were essential for high yields. Substantial hydrolysis of the products was observed when these conditions were not employed. The two diastereomers were readily separated by normal phase HPLC but the absolute stereochemistry [(2*RS*,4*RS*) or (2*RS*,4*SR*)] of each could not be assigned directly. As is illustrated in the following synthesis of 4-hydroxynorvaline derivatives, an indirect method for assigning relative stereochemistry is available by conversion of the compounds to the corresponding γ-butyrolactones; in this case the *erythro*-(2*RS*,4*SR*)-isomer would give the *trans*-disubstituted butyrolactone while the *threo*-(2*RS*,4*RS*)-isomer would give the *cis*-disubstituted isomer.

Deprotection of the 2-(benzyloxycarbonylamino)-4-hydroxypentane-1,5-dioic acid diesters **20** was carried out in high yield by hydrogenolysis over palladium on carbon, under acid catalysis, to give dimethyl 4-hydroxyglutamate hydrochloride which was then warmed overnight with aqueous lithium hydroxide and carefully acidified to pH 3 to afford 4-hydroxyglutamic acid **21**. This amino acid was obtained as a 1:1 mixture of diastereomers in 92% yield after purification of the product by ion-exchange chromatography on Dowex-1-acetate.[25,26] The alternate deprotection sequence proved to be unsatisfactory as base hydrolysis of the dimethyl esters **20** with aqueous sodium or lithium hydroxide, followed by acidification, gave a mixture of the diacid **21** and the corresponding lactones.

4-Hydroxyglutamic acid has been isolated from the green parts of the plants, *Phlox decussa*[27] and *Linaria vulgaris*[28] and has been synthesised in a low yield as a diastereomeric mixture by Benoiten and Bouthillier[29] and Hanafusa and co-workers[30] using malonic ester methodology.

The transformation of a dehydroalanine into 4-hydroxyglutamic acids **21** establishes a new route for construction of the 4-hydroxy-2-amino acid skeleton. Incorporation of stereocontrol into the sequence by asymmetric reduction of the prochiral ketone functionality and through the use of an enantiomerically pure asymmetric dehydroalanine as starting material would greatly improve the usefulness of the methodology and is addressed in other work.[6,31]

The synthetic route to 4-hydroxynorvaline derivatives **24**–**27** follows that used to synthesise 4-hydroxyglutamic acid **21** and is outlined in Scheme 6. Methyl 2-(benzyloxycarbonylamino)-4-nitropentanoates **22**, readily available by the addition of nitroethane to methyl 2-(benzyloxycarbonylamino)acrylate,[1] were converted by ozonolysis of the corresponding nitronate into the 4-oxo derivative **23** in 84% yield.



Scheme 6 *Reagents and conditions*: i, *N*-Benzyltrimethylammonium hydroxide, MeOH, stirred for 10 min, cooled to −78 °C and treated with O_3–O_2, Me_2S, warmed to room temp. and stirred overnight; ii, $NaBH_4$, MeOH, −20 °C, stirred for 1 h, cooled to −40 °C, HCl–MeOH; iii, *N*-benzyltrimethylammonium hydroxide, MeOH, stirred for 10 min, cooled to −78 °C and treated with O_3–O_2, then $NaBH_4$ at −78 °C, at 4 °C for 10 min, cooled to −78 °C and HCl–MeOH added until pH < 3; iv, C_6H_6, heat

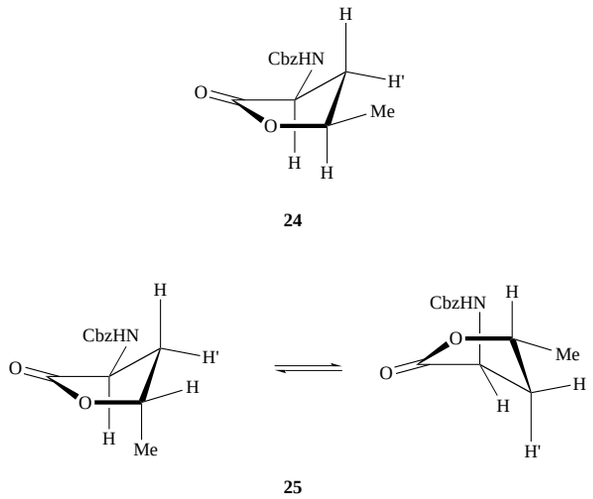


Fig. 1 Conformations of the lactones **24** and **25**

Treatment of methyl 2-(benzyloxycarbonylamino)-4-oxopentanoate **23** with $NaBH_4$ in anhydrous methanol at −20 °C, followed by decomposition of the excess borohydride with methanolic HCl, gave the corresponding *cis*- and *trans*-4-hydroxyamino acid lactones, **24** and **25** respectively, as a 1:1 mixture in quantitative yield. Gelin and Chignac have reported the preparation of γ-lactones by similar reduction of 4-oxocarboxylic acid esters.[32] From a synthetic viewpoint, formation of lactone products instead of the hydroxy esters is of little consequence as hydrolysis of the lactones **24** and **25** would give the same acids as those arising from hydrolysis of the corresponding 4-hydroxy esters **26** and **27**. Lactone formation has a practical value, however, as it allows assignment of the relative stereochemistry of the products and, by interconversion, the related acyclic 4-hydroxy esters.

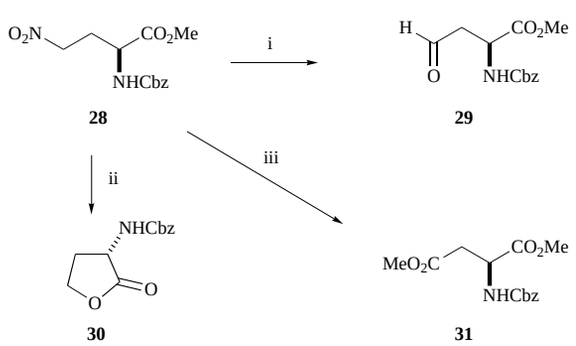
The two lactone isomers were separated by normal phase HPLC. The structures of the lactones **24** and **25** were assigned unequivocally by use of ^{1}H NMR spectroscopy on the basis of the chemical shifts of the two geminal protons, 3- and 3′-H on the 2,4-disubstituted-γ-butyrolactones, and on the magnitude of the vicinal coupling constants of these ring protons.[33] The more polar isomer (3-H resonance at δ 2.86, 3′-H resonance at δ 1.79; $J_{2,3}$ 12.2, $J_{3,4}$ 10.8 Hz) has the *cis*-stereochemistry **24**. The less polar compound (3-H resonance at δ 2.47, 3′-H resonance at δ 2.33) has the *trans*-stereochemistry **25**. In lactone **24** and related *cis*-2,4-disubstituted-γ-butyrolactones the coupling constants $J_{2,3}$ and $J_{3,4}$ are larger than in the *trans*-2,4-disubstituted-γ-butyrolactones, and are characteristic of *quasi*-axial–*quasi*-axial interactions indicating that the conformation shown in Fig. 1, in which both the C-2 and C-4 substituents are in *quasi*-equatorial positions, is strongly preferred. The proton 3-H is thus in a *quasi*-axial position and, as expected, it resonates 1.07 ppm downfield of the *quasi*-equatorial proton 3′-H in the ^{1}H NMR spectrum. For the *trans*-2,4-disubstituted-γ-butyrolactone **25**, the difference in the chemical shifts of the protons, 3- and 3′-H is small (0.14 ppm) and the vicinal coupling constants to these protons are similar in magnitude, observations that are consistent with there being two accessible conformers (Fig. 1) that are in fast exchange on the NMR timescale so that 3- and 3′-H have similar magnetic environments.

Incomplete lactonisation occurred in several runs of this reaction to give a small amount (0–20%) of the *erythro*-4-hydroxy-2-amino acid ester **27** in addition to the lactones; in these cases the yield of the *trans*-isomer **25** was reduced by an equivalent amount. The corresponding *threo*-isomer **26** was never detected under these reaction conditions presumably because cyclisation of the *threo*-alcohol **26** to form the *cis*-lactone **24** is more favourable as both the C-2 and C-4 substituents in **24** can occupy *quasi*-equatorial positions.

The 4-hydroxy acid esters, **26** and **27**, were prepared in good yield by direct reduction, $NaBH_4$ at 4 °C, of the intermediate ozonide obtained by ozonolysis of the nitronate derived from **22** (Scheme 6). The isomers were separated easily by flash column chromatography to afford the *threo*-isomer **26** in 39% yield and the *erythro*-isomer **27** in 31% yield, together with 17% yield of the corresponding *trans*-lactone **25**; the structures of the acyclic 4-hydroxy acid esters were assigned by conversion of the less polar isomer into the corresponding *cis*-lactone **24** upon heating in refluxing benzene, thereby establishing that it was the *threo*-isomer **26**.

No further investigation was undertaken into the deprotection of either the lactones **24** and **25** or esters **26** and **27** as they were prepared primarily as precursors for the synthesis of cyclic hydroxamates of biological interest as will be described separately.[34] The free 4-hydroxynorvalines should be accessible under conditions similar to those used for the deprotection of the esters **20** leading to the free 4-hydroxyglutamic acids **21**.

Homoserine aldehyde (2-amino-4-oxobutanoic acid) is a synthetically useful precursor of a range of other 4-hydroxy-2-amino acids through reactions that involve addition to the formyl group.[35] The methodology that we have introduced in this paper allows a new synthesis of this compound in protected form **29** as is shown in Scheme 7.



Scheme 7 *Reagents and conditions*: i, *N*-Benzyltrimethylammonium hydroxide, MeOH, stirred for 10 min, pyridine, cooled to −78 °C, treated with O_3–O_2 for 15 min, Me_2S at −78 °C, left to stand overnight at room temp.; ii, *N*-benzyltrimethylammonium hydroxide, MeOH, stirred for 10 min, cooled to −78 °C, treated with O_3–O_2 for 30 min, $NaBH_4$ at −78 °C, raised temp. to 0 °C, HCl–MeOH; iii, *N*-benzyltrimethylammonium hydroxide, MeOH, stirred for 10 min, cooled to −78 °C and treated with O_3–O_2, $NaBH_4$ at −78 °C, after 1 h raised temp. to 0 °C, HCl–MeOH

Conversion of methyl 2-(benzyloxycarbonylamino)-4-nitrobutanoate[1] **28** to the aldehyde **29** requires oxidative cleavage of the nitrogen–carbon bond. Two relatively straightforward methods have been reported for the conversion of primary nitroalkanes into aldehydes. Kornblum and co-workers have reported the conversion of 1-nitrodecane into decanal by potassium permanganate oxidation in 92% yield.[10] With the ozonolysis method, McMurry and co-workers reported the conversion of 1-nitrooctane into octanal in 65% yield.[9] In order to avoid over-reaction in the latter case, however, it was found necessary to control the amount of ozone introduced into the reaction. Optimum yields of octanal were obtained when exactly 1 equiv. of ozone was used.

Treatment of the nitro adduct **28** with sodium *tert*-butoxide followed by potassium permanganate and boric acid, conditions similar to those employed in the preparation of decanal,[10] gave less than 10% of the desired aldehyde **29**. Application of McMurry's ozonolysis method[9] for the oxidation also proved to be unsatisfactory. Treatment of the intermediate ozonide, derived by treatment of the nitronate obtained from **28** with ozone at −78 °C, with $NaBH_4$ and allowing the reaction mixture to rise immediately to room temperature gave a complex mixture from which 2-(benzyloxycarbonylamino)-γ-butyrolactone **30** was isolated in 12% yield. When the reaction mixture was held at −78 °C for 1 h after addition of the $NaBH_4$, and worked up in methanolic HCl, the major product proved to be the aspartic acid derivative, dimethyl 2-(benzyloxycarbonylamino)butane-1,4-dioate **31**, which was obtained in 30% yield (Scheme 7). The problems in the conversion of primary nitroalkanes into aldehydes arise as a result of over-oxidation of the reaction intermediates. This was overcome by applying pyridine as an additive to the reaction. The use of pyridine in ozonolysis has been reported to favour the formation of aldehydes rather than ozonides or peroxides.[36,37] Thus treatment of the nitronate of **28**, generated by the addition of *N*-benzyltrimethylammonium hydroxide to a methanolic solution of the nitro intermediate, with ozone in the presence of 1 equiv. of pyridine, followed by reduction with dimethyl sulfide, gave the protected homoserine aldehyde **29** in 50% yield (Scheme 7).

Conclusions

A number of studies have shown the value of dehydroalanines in the synthesis of more complex α-amino acids,[1,6,12,20,38–48] and the work described in this paper is a further example of this fact. Conjugate addition of nitroalkanes to a dehydroalanine derivative followed by reductive denitration of the resultant γ-nitro-α-amino acid derivative provides a new route for synthesis of proteinogenic (leucine and glutamic acid derivatives) and non-proteinogenic α-amino acids. A new method for the synthesis of γ-oxygenated α-amino acids has also been established. The method involves transformation of γ-nitro-α-amino acid derivatives into the corresponding γ-oxo-α-amino acids and γ-hydroxy-α-amino acids, the γ-oxo-α-amino acids being obtained by ozonolysis of the corresponding amino acid nitronate derivatives, and the γ-hydroxy derivatives by subsequent reduction of the oxo functionality. As dehydroalanines can be prepared in specific routes from alanine,[49] serine[50–52] or cysteine[53,54] the route allows the conversion of these simple amino acids into more complex amino acids. In an extension of this work, convenient routes to γ-lactams and azetidines of biological interest have been established that involve other transformations of γ-nitro-α-amino acid derivatives[12,24] and stereocontrol which avoids the formation of racemic α-amino acids has been achieved through the use of a chiral dehydroalanine precursor.[6,31]

Experimental

General experimental details have been reported previously.[1]

Infrared spectra were recorded either on a Perkin-Elmer 1600 FTIR, a Digilab FTS-50 or a Perkin-Elmer 710B spectrophotometer from neat liquid films (sodium chloride plates) or as chloroform solutions as indicated. ^{1}H NMR spectra were recorded on a Varian EM-390 (90 MHz), a Varian XL-100 (100 MHz), a Bruker AC-200F (200 MHz), a Bruker AMX-400 (400 MHz) or a Bruker AMX-600 (600 MHz) spectrometer.

Analytical high performance liquid chromatography (HPLC) was carried out on a system consisting of a Waters model 6000A pump, U6K injector, a Waters model 440 ultraviolet detector (set at a wavelength of 254 nm) and a Waters model R401 refractive index detector. Preparative HPLC was performed with a Waters model 510EF pump, U6K injector, a Waters model 481 ultraviolet detector (set at a wavelength of 254 nm) and a Waters model R403 refractive index detector. The columns used were Whatman Partisil 5 (analytical) 4.6 mm ID × 25 cm, Brownlee LiChrosorb Si-100 (5 μm) (analytical) 4.6 mm ID × 25 cm, Waters μ-Porasil (semi-preparative) 7.8 mm ID × 30 cm, Whatman Partisil M9 10/50 (semi-preparative) 9.4 mm ID × 50 cm and Whatman Partisil 10 (preparative) 22.0 mm ID × 50 cm.

Ozone was generated from industrial grade oxygen with a Wallace and Tiernan laboratory ozonator, model BA-023.

Methyl 2-(benzyloxycarbonylamino)-3-cyclohexylpropanoate 7

A solution of methyl 2-(benzyloxycarbonylamino)-3-(1′-nitrocyclohexyl)propanoate[1] **6** (348 mg, 0.96 mmol) and tributylstannane (0.32 cm^3, 350 mg, 1.2 mmol) in dry benzene (12 cm^3) was treated with 2,2′-azobis-(2-methylpropionitrile) (AIBN) (32.8 mg, 0.20 mmol) and heated at reflux for 4 h, allowed to cool and concentrated under reduced pressure to a yellow oil. Chromatography over silica (light petroleum) eluted the organotin compounds, and further elution with ether–light petroleum (3:1) gave *methyl* 2-(*benzyloxycarbonylamino*)-3-*cyclohexylpropanoate* **7** (185 mg, 65%) as an oil (Found: M^+, 319.1775. $C_{18}H_{25}NO_4$ requires *M*, 319.1783); ν_{max}(film)/cm^{-1} 3300 (NH) and 1710br (C=O ester and urethane); δ_H(90 MHz; $CDCl_3$) 0.70–1.90 (13 H, m, cyclohexyl H, 3-H_a and H_b), 3.70 (3 H, s, OCH_3), 4.15–4.50 (1 H, m, 2-H), 5.12 (3 H, br s, NH and CH_2Ar) and 7.32 (5 H, s, ArH); *m*/*z* (EI) 319 (M^+, 3%), 260 (59) and 184 (11).

2-(Benzyloxycarbonylamino)-3-cyclohexylpropanoic acid (*N*-Cbz-3-cyclohexylalanine) 8

Methyl 2-(benzyloxycarbonylamino)-3-cyclohexylpropanoate **7** (380 mg, 1.2 mmol) was treated with aqueous sodium hydroxide (10%, 20 cm^3) and warmed gently until the reaction mixture became homogeneous. The reaction mixture was allowed to stand at room temperature for 16 h and was then acidified with aqueous hydrochloric acid (3 mol dm^{-3}) and extracted with ether (3 × 30 cm^3). The combined ethereal extracts were washed with water (30 cm^3), dried and concentrated under reduced pressure to leave a yellow solid. Two recrystallisations from hexane–ethyl acetate gave 2-(benzyloxycarbonylamino)-3-cyclohexylpropanoic acid **8** (293 mg, 81%) as prisms; mp 115–116.5 °C (lit.,[39] mp 114–116 °C); ν_{max}(film)/cm^{-1} 3600–2500br (OH), 3450 (NH) and 1710br (C=O acid and urethane); δ_H(100 MHz; $CDCl_3$) 0.78–2.16 (13 H, m, cyclohexyl H, 3-H_a and H_b), 4.22–4.62 (1 H, br m, 2-H), 5.14 (2 H, s, CH_2Ar), 5.08–5.52 (1 H, br s, NH), 7.38 (5 H, s, ArH) and 7.42–8.08 (1 H, br s, CO_2H); *m*/*z* (CI-CH_4) 306 ($M^+ + 1$, 12%), 304 ($M^+ - 1$, 5), 262 ($M^+ - CO_2$, 46) and 119 (100).

3-Cyclohexylalanine 9

A solution of 2-(benzyloxycarbonylamino)-3-cyclohexylpropanoic acid **8** (120 mg, 0.39 mmol) in ethanol was treated with cyclohexene (5 cm^3), 10% palladium on charcoal (100 mg) and heated at reflux for 20 min. The reaction mixture was then diluted with ethanol (20 cm^3) and filtered through a pad of Celite. The solid residues were washed thoroughly with hot ethanol and the combined filtrate was concentrated under reduced pressure to leave a white solid (67 mg), which was recrystallised from water to give 3-cyclohexylalanine **9** (64 mg, 96%) as needles; mp 237–238 °C (lit.,[13] mp 229–230 °C); ν_{max}(Nujol)/cm^{-1} 3600–2000br (NH_3^+), 1620, 1590 (CO_2^-) and 1510 (NH_3^+ bend); δ_H(100 MHz; CD_3OD–D_2O) 0.74–1.94 (13 H, m, cyclohexyl H, 3-H_a and H_b) and 3.68 (1 H, dd, *J* 9.0 and 6.0, 2-H).

Methyl 2-(benzyloxycarbonylamino)-4-methylpentanoate (*N*-Cbz-leucine methyl ester) 11

A solution of methyl 2-(benzyloxycarbonylamino)-4-methyl-4-nitropentanoate[1] **10** (600 mg, 1.85 mmol) and tributylstannane (0.64 cm^3, 700 mg, 1.85 mmol) in dry benzene (15 cm^3) was treated with AIBN (30 mg, 0.18 mmol) and heated at reflux for 4 h, allowed to cool and concentrated under reduced pressure to a colourless oil. Chromatography over silica (hexane) eluted the organotin compounds, and further elution with ether gave methyl 2-(benzyloxycarbonylamino)-4-methylpentanoate **11** (350 mg, 67%) as an oil (lit.,[17] oil); ν_{max}(film)/cm^{-1} 3350 (NH) and 1760–1705br (C=O ester and urethane); δ_H(100 MHz; $CDCl_3$) 0.91 [6 H, d, *J* 6.0, C$(CH_3)_2$], 1.40–1.70 (3 H, m, 4-H, 3-H_a and H_b), 3.70 (3 H, s, OCH_3), 4.22–4.50 (1 H, m, 2-H), 5.10 (3 H, s over br m, NH and CH_2Ar) and 7.34 (5 H, s, ArH). This material was further characterised as the acid **12**.

2-(Benzyloxycarbonylamino)-4-methylpentanoic acid (*N*-Cbz-leucine) 12

Methyl 2-(benzyloxycarbonylamino)-4-methylpentanoate **11** (250 mg, 0.90 mmol) was treated with aqueous sodium hydroxide (10%; 20 cm^3) and warmed gently until the reaction mixture became homogeneous. The reaction mixture was allowed to stand at room temperature for 16 h and was then acidified with aqueous hydrochloric acid (3 mol dm^{-3}) and extracted with ether (3 × 30 cm^3). The combined ethereal extracts were washed with water, dried and concentrated under reduced pressure to leave a yellow oil (217 mg, 91%) which was recrystallised from ether–light petroleum to give 2-(benzyloxycarbonylamino)-4-methylpentanoic acid **12** as needles; mp 54–55 °C (lit.,[17] mp 52–55 °C); δ_H(90 MHz; $CDCl_3$) 0.87 [6 H, d, *J* 6.0, C$(CH_3)_2$], 1.30–1.93 (3 H, m, 4-H, 3-H_a and H_b), 3.80–4.10 (1 H, br s, OH), 4.42–4.70 (1 H, m, 2-H), 5.10 (2 H, s, CH_2Ar), 5.16–5.41 (1 H, br s, NH) and 7.25 (5 H, s, ArH).

Dimethyl 2-(benzyloxycarbonylamino)pentane-1,5-dioate (*N*-Cbz-glutamic acid dimethyl ester) 14

Dimethyl 2-(benzyloxycarbonylamino)-4-nitropentane-1,5-dioates[1] **13** (367 mg, 1.04 mmol) and tributylstannane (332 mg, 1.04 mmol, 1.1 equiv.) in dry benzene (20 cm^3) were treated with AIBN (32.8 mg, 0.20 mmol) and heated at reflux for 4 h, allowed to cool and concentrated under reduced pressure to an orange oil. The crude residue was purified by flash column chromatography over silica (light petroleum) to remove the tin compounds. Further elution with ethyl acetate–light petroleum (2:3) gave *dimethyl* 2-(*benzyloxycarbonylamino*)*pentane*-1,5-dioate **14** (207 mg, 65%) as a colourless viscous oil [R_f 0.48 analytical TLC, silica 60, 0.25 mm layer, ethyl acetate–light petroleum (2:3)]. A small sample was further purified for elemental analysis by semi-preparative normal phase HPLC [Whatman Partisil M9 10/50, ethyl acetate–light petroleum (1:3), flow rate 3.0 cm^3 min^{-1}] (Found: C, 58.1; H, 6.3; N, 4.7. $C_{15}H_{19}NO_6$ requires C, 58.2; H, 6.2; N, 4.5%); ν_{max}(liquid film)/cm^{-1} 3340 (NH), 3029 (aromatic CH), 2954 (CH), 2848 (CH) and 1740–1700br (C=O ester); δ_H(600 MHz; $CDCl_3$) 1.95–2.03 (1 H, m, 3-H_a), 2.19–2.26 (1 H, m, 3-H_b), 2.35–2.47 (2 H, m, 4-H), 3.68 (3 H, s, OCH_3), 3.76 (3 H, s, OCH_3), 4.39–4.44 (1 H, m, 2-H), 5.12 (2 H, s, CH_2Ar), 5.20–5.28 (1 H, m, NH) and 7.30–7.44 (5 H, m, ArH); δ_C(50 MHz; $CDCl_3$) 27.62 (CH_2), 29.89 (CH_2), 51.79 (CH_2), 52.52 (OCH_3), 53.28 (CH), 67.06 (CH_2Ar), 128.09 (ArC), 128.19 (ArC), 128.51 (ArC), 136.11 (*ipso* ArC), 155.88 ($NHCO_2$), 172.26 (CO) and 173.08 (CO); *m*/*z* (EI) 309 (M^+, 1.3%), 206 (10), 108 (19), 107 (10), 92 (11) and 91 (100).

2-(Benzyloxycarbonylamino)pentane-1,5-dioic acid (*N*-Cbz-glutamic acid) 15

Dimethyl 2-(benzyloxycarbonylamino)pentane-1,5-dioate **14** (240 mg, 0.77 mmol) was treated with aqueous sodium hydroxide (10%; 2 cm^3) and warmed gently until the reaction mixture became homogeneous. The reaction was allowed to stir at room temperature overnight and was then acidified with aqueous hydrochloric acid (3 mol dm^{-3}) and extracted with ether (3 × 30 cm^3). The combined ethereal extracts were washed with water (3 × 20 cm^3), dried and concentrated under reduced pressure to leave a yellow oil. The oil was recrystallised from ether–light petroleum to give 2-(benzyloxycarbonylamino)pentane-1,5-dioic acid **15** (155 mg, 71%) as a white powder; mp 121–122 °C (lit.,[40] mp 120 °C); δ_H(200 MHz; $CDCl_3$–[$^2H_6$]DMSO) 1.92–2.52 (4 H, m, 3- and 4-H), 4.28–4.44 (1 H, m, 2-H), 5.12 (2 H, s, CH_2Ar), 5.64–5.76 (1 H, br d, *J* 8.0, NH) and 7.32–7.40 (5 H, m, ArH).

5-Ethyl 1-methyl 2-phthalimidopentane-1,5-dioate 17

5-Ethyl 1-methyl 4-nitro-2-phthalimidopentane-1,5-dioates[1] **16** (353 mg, 0.97 mmol) and tributylstannane (310 mg, 1.07 mmol, 1.1 equiv.) in dry benzene (20 cm^3) were treated with AIBN

(35.0 mg, 0.213 mmol) and heated at reflux for 4 h, allowed to cool and concentrated under reduced pressure to an orange oil. The crude residue was purified by flash column chromatography over silica (ethyl acetate–light petroleum; 2:3, R_f 0.55) to give 5-*ethyl* 1-*methyl* 2-*phthalimidopentane*-1,5-*dioate* **17** (180 mg, 58%) as a colourless viscous oil. A small sample was further purified for elemental analysis by preparative normal phase HPLC [Whatman Partisil 10, ethyl acetate–light petroleum (1:3), flow rate 13.5 cm^3 min^{-1}, t_R 43.2 min] (Found: C, 60.3; H, 5.6; N, 4.5. $C_{16}H_{17}NO_6$ requires C, 60.2; H, 5.4; N, 4.4%); ν_{max}(liquid film)/cm^{-1} 2978 (CH), 2950 (CH) and 1778–1688br (C=O phthalimide, ester); δ_H(400 MHz; $CDCl_3$) 1.21 (3 H, t, *J* 7.0, CH_3), 2.35–2.41 (2 H, m, 4-H_a and H_b), 2.44–2.55 (1 H, m, 3-H_a), 2.63–2.68 (1 H, m, 3-H_b), 3.76 (3 H, s, OCH_3), 4.02–4.13 (2 H, m, OCH_2), 4.94 (1 H, dd, *J* 10.0 and 5.0, 2-H), 7.73–7.78 (2 H, m, ArH) and 7.85–7.90 (2 H, m, ArH); δ_C(50 MHz; $CDCl_3$) 14.06 (CH_3), 24.18 (CH_2), 30.82 (CH_2), 51.12 (OCH_3), 52.78 (CH), 60.60 (OCH_2), 123.55 (ArC), 131.68 (*ipso* ArC), 134.24 (ArC), 167.46 (CO), 169.23 (CO) and 172.11 (CO); *m*/*z* (EI) 319 (M^+, 0.4%), 287 (19), 260 (12), 214 (15), 187 (16), 186 (100), 163 (60), 104 (19) and 76 (13).

2-Phthalimidopentane-1,5-dioic acid (*N*-phthaloylglutamic acid) 18

5-Ethyl 1-methyl 2-phthalimidopentane-1,5-dioate **17** (40 mg, 0.125 mmol) was treated with aqueous sodium hydroxide (10%; 2 cm^3) and warmed gently until the reaction mixture became homogeneous. The reaction was allowed to stir at room temperature overnight and was then acidified with aqueous hydrochloric acid (3 mol dm^{-3}) and extracted with ether (3 × 30 cm^3). The combined ethereal extracts were washed with water (3 × 20 cm^3), dried and concentrated under reduced pressure to leave a yellow oil. The residue was recrystallised from ether–light petroleum to give 2-phthalimidopentane-1,5-dioic acid **18** (26 mg, 75%) as a white solid; mp 158–160 °C (lit.,[20] mp 160–161 °C); δ_H(200 MHz; $CDCl_3$) 2.04–2.64 (4 H, m, 3- and 4-H), 4.68–4.76 (1 H, m, 2-H), 4.60–5.80 (2 H, br s, OH) and 7.72–8.00 (4 H, m, ArH).

Dimethyl 2-(benzyloxycarbonylamino)-4-oxopentane-1,5-dioate 19

N-Benzyltrimethylammonium hydroxide in methanol (40% w/v; 2.57 cm^3, 6.12 mmol) was added to a 1:1 diastereomeric mixture of dimethyl 2-(benzyloxycarbonylamino)-4-nitropentane-1,5-dioates[1] **13** (2.09 g, 5.90 mmol) in anhydrous methanol (20 cm^3) and stirred for 10 min to generate the nitronate anion. The methanolic solution was cooled to −78 °C, then treated with a stream of ozone–oxygen until the reaction mixture turned light blue. Dimethyl sulfide (1.53 cm^3) was then added to the mixture at −78 °C and the mixture was allowed to warm to room temperature. The reaction mixture was left to stand overnight and then evaporated to dryness. The residue was acidified with aqueous hydrochloric acid (3 mol dm^{-3}) and then extracted three times with chloroform. The combined chloroform extracts were washed five times with water, dried, then evaporated to dryness to give the product as a yellow oil (1.86 g). The crude product was purified by flash chromatography over silica (light petroleum–ethyl acetate; 11:9) to give *dimethyl* 2-(*benzyloxycarbonylamino*)-4-*oxopentane*-1,5-*dioate* **19** (1.31 g, 69%) as a colourless oil (Found: C, 55.7; H, 5.7; N, 4.1. $C_{15}H_{17}NO_7$ requires C, 55.7; H, 5.3; N, 4.3%); ν_{max}($CHCl_3$)/cm^{-1} 3433w, 2958w, 1734s, 1701m and 1507m; δ_H(400 MHz; $CDCl_3$) 3.46–3.49 (2 H, m, 3- and 3′-H), 3.75 (3 H, s, OCH_3), 3.88 (3 H, s, OCH_3), 4.70–4.78 (1 H, m, 2-H), 5.11 (2 H, s, CH_2Ar), 5.68 (1 H, br d, *J* 8.1, NH) and 7.30–7.40 (5 H, m, ArH); *m*/*z* (CI) 324 (M^+ + 1, 88%), 306 (2), 292 (3), 280 (59), 238 (3), 220 (6), 181 (15), 178 (13) and 91 (100); *m*/*z* (EI) 264 (0.6%), 236 (0.3), 220 (<1), 217 (9), 115 (7), 108 (8) and 91 (100).

Dimethyl 2-(benzyloxycarbonylamino)-4-hydroxypentane-1,5-dioates 20a and 20b

Sodium borohydride (300 mg, 8.10 mmol) was added to a solution of dimethyl 2-(benzyloxycarbonylamino)-4-oxopentane-1,5-dioate **19** (620 mg, 1.90 mmol) in anhydrous methanol (30 cm^3) at −20 °C. The reaction mixture was stirred at −20 °C for 30 min, after which time it was acidified with hydrochloric acid (9 mol dm^{-3}) in methanol at that temperature. The volatile components of the solution were removed under vacuum, and the residue was extracted three times with chloroform. The combined extracts were filtered *via* a short silica gel column (ethyl acetate), then evaporated to dryness under vacuum to give a 1:1 diastereomeric mixture of *dimethyl* 2-(*benzyloxycarbonylamino*)-4-*hydroxypentane*-1,5-*dioates* **20** (580 mg, 93%) as a colourless oil (Found: C, 55.4; H, 5.7; N, 4.3. $C_{15}H_{19}NO_7$ requires C, 55.4; H, 5.9; N, 4.3%).

The two diastereomers **20** were further separated and purified by HPLC [μ-Porasil column, light petroleum–ethyl acetate (3:2), flow rate 3.0 cm^3 min^{-1}]. The *more polar isomer* **20** (t_R 15.6 min) was obtained as a colourless oil (Found: M^+, 325.1161. $C_{15}H_{19}NO_7$ requires *M*, 325.1161); ν_{max}($CHCl_3$)/cm^{-1} 3517w, 3417w, 2983w, 2950w, 1740s, 1735s and 1507m; δ_H(400 MHz; $CDCl_3$) 2.17–2.29 (2 H, m, 3- and 3′-H), 3.36–3.45 (1 H, br s, OH), 3.75 (3 H, s, OCH_3), 3.77 (3 H, s, OCH_3), 4.23–4.30 (1 H, m, 4-H), 4.54–4.64 (1 H, m, 2-H), 5.12 (2 H, s, CH_2Ar), 5.71 (1 H, br d, *J* 7.3, NH) and 7.30–7.39 (5 H, m, ArH); *m*/*z* (EI) 325 (M^+, 1%), 293 (0.8), 266 (5), 222 (6), 108 (30), 107 (11), 91 (100) and 79 (10).

The *less polar isomer* **20b** (t_R 14.8 min) was also obtained as a colourless oil (Found: M^+, 325.1153. $C_{15}H_{19}NO_7$ requires *M*, 325.1161); ν_{max}($CHCl_3$)/cm^{-1} 3530w, 3427w, 2957w, 2929w, 1740s, 1735s and 1507s; δ_H(400 MHz; $CDCl_3$) 2.16 (1 H, ddd, *J* 14.3, 7.4 and 7.4, 3-H), 2.45 (1 H, ddd, *J* 14.3, 4.9 and 4.9, 3′-H), 3.15–3.23 (1 H, br s, OH), 3.74 (6 H, s, 2 × OCH_3), 4.30–4.37 (1 H, m, 4-H), 4.61 (1 H, ddd, *J* 7.6, 7.4 and 4.9, 2-H), 5.12 (2 H, s, CH_2Ar), 5.61 (1 H, br d, *J* 7.6, NH) and 7.30–7.39 (5 H, m, ArH); *m*/*z* (EI) 325 (M^+, 0.5%), 293 (1), 266 (2), 222 (3), 108 (25), 107 (11), 91 (100) and 79 (10).

4-Hydroxyglutamic acid 21

To a solution of a 1:1 diastereomeric mixture of dimethyl 2-(benzyloxycarbonylamino)-4-hydroxypentane-1,5-dioates **20** (99 mg, 0.305 mmol) in anhydrous methanol (7 cm^3), 10% palladium on carbon (100 mg) was added, followed by hydrochloric acid (8 mol dm^{-3}) in methanol (0.1 cm^3). The reaction mixture was stirred under hydrogen for 1 h, after which the volatile components were removed under vacuum. Dimethyl 4-hydroxyglutamate hydrogen chloride was obtained as a colourless solid (65 mg) in quantitative yield. Aqueous lithium hydroxide (0.1064 mol dm^{-3}; 9.68 cm^3) was added to dimethyl 4-hydroxyglutamate hydrogen chloride (65 mg, 0.34 mmol), and the mixture was stirred at 70 °C overnight. It was then allowed to cool and acidified to pH 3 with aqueous hydrochloric acid (3 mol dm^{-3}). The aqueous solution was placed on a 1 × 20 cm column of Dowex-1-acetate[25,26] (10–50 mesh), previously washed with water. Water (100 cm^3) was initially used as the eluent and the amino acid was then eluted with aqueous acetic acid (0.5 mol dm^{-3}). Evaporation of the eluate afforded a 1:1 diastereomeric mixture of 4-hydroxyglutamic acid **21** (54 mg, 92%), mp 164–165 °C, solidified and remelted mp 171–173 °C [lit.,[29] mp 166 °C, solidified and remelted mp 172–173 °C].

Methyl 2-(benzyloxycarbonylamino)-4-oxopentanoate 23

A 1:1 diastereomeric mixture of methyl 2-(benzyloxycarbonylamino)-4-nitropentanoates[1] **22** (2.22 g, 7.16 mmol) in anhydrous methanol (20 cm^3) was treated with *N*-benzyltrimethylammonium hydroxide in methanol (40% w/v; 3.38 cm^3, 8.07 mmol) and stirred for 10 min to generate the nitronate anion. The mixture was cooled to −78 °C, then treated with a stream of ozone–oxygen until the mixture turned light blue.

Dimethyl sulfide (2 cm^3) was added to the solution at −78 °C and the mixture allowed slowly to come to room temperature. It was then left to stand overnight. The volatile components were removed under vacuum and the residue acidified with aqueous hydrochloric acid (3 mol dm^{-3}), then extracted three times with chloroform. The combined chloroform extracts were washed five times with water, dried, then evaporated to dryness to give the crude product as a yellow oil (2.20 g). The crude product was purified by flash chromatography over silica (light petroleum–ethyl acetate; 11:9) to give *methyl* 2-(*benzyloxycarbonylamino*)-4-*oxopentanoate* **23** (1.68 g, 84%) as a pale yellow oil (Found: M^+, 279.1108. $C_{14}H_{17}NO_5$ requires *M*, 279.1107); $\nu_{max}(CHCl_3)/cm^{-1}$ 3436w, 2958w, 1779w, 1719s, 1508s, 1455w and 1439w; δ_H(400 MHz; $CDCl_3$) 2.15 [3 H, s, C(O)CH_3], 2.98 (1 H, dd, *J* 18.3 and 4.1, 3-H), 3.21 (1 H, dd, *J* 18.3 and 4.3, 3′-H), 3.72 (3 H, s, OCH_3), 4.56 (1 H, ddd, *J* 8.6, 4.3 and 4.1, 2-H), 5.11 (2 H, s, CH_2Ar), 5.77 (1 H, br d, *J* 8.6, NH) and 7.29–7.39 (5 H, m, ArH); *m*/*z* (EI) 279 (M^+, 5%), 249 (1), 220 (6), 176 (10), 108 (40), 107 (16), 92 (15), 91 (100) and 65 (13).

2-(Benzyloxycarbonylamino)-4-methyl-γ-butyrolactones 24 and 25

Sodium borohydride (50 mg, 1.35 mmol) was added to a solution of methyl 2-(benzyloxycarbonylamino)-4-oxopentanoate **23** (130 mg, 0.465 mmol) in anhydrous methanol (5 cm^3) at −20 °C. The solution was stirred at −20 °C for 1 h and then allowed to rise to 0 °C. The reaction mixture was cooled to −40 °C, methanolic hydrochloric acid (9 mol dm^{-3}) was then added to decompose the excess hydride. The volatile components were removed under vacuum and the residue extracted several times with chloroform. The combined extracts were dried, then filtered *via* a short silica gel column. After removal of solvent under vacuum, a 1:1 diastereomeric mixture of 2-(benzyloxycarbonylamino)-4-methyl-γ-butyrolactones **24** and **25** (108 mg, 93%) was obtained as a colourless oil (Found: C, 62.4; H, 6.0; N, 5.6. $C_{13}H_{15}NO_4$ requires C, 62.6; H, 6.1; N, 5.6%).

The *cis*- and *trans*-isomers were further separated and purified by HPLC [μ-Porasil column, light petroleum–ethyl acetate (3:1), flow rate 3.0 cm^3 min^{-1}]. The first band to be eluted was evaporated to dryness to yield the *trans*-isomer **25** as a colourless oil. The purity of **25** was checked by analytical HPLC [Brownlee LiChrosorb Si-100 column, light petroleum–ethyl acetate (7:3), flow rate 1.0 cm^3 min^{-1}, t_R 9.0 min]; $\nu_{max}(CHCl_3)/cm^{-1}$ 3430w, 2988w, 2936w, 1781s, 1725s and 1513m; δ_H(400 MHz; $CDCl_3$) 1.42 (3 H, d, *J* 6.6, CH_3), 2.33 (1 H, ddd, *J* 12.8, 10.6 and 8.3, 3′-H) {coupling constants resolved by the use of tris[3-(2,2,2-trifluorohydroxyethylidene)-(−)-camphorato]-europium(III) shift reagent in a separate experiment}, 2.47 (1 H, ddd, *J* 12.8, 7.2 and 4.0, 3-H), 4.47–4.53 (1 H, m, 2-H), 4.75–4.86 (1 H, m, 4-H), 5.13 (2 H, s, CH_2Ar), 5.23–5.34 (1 H, br, NH) and 7.29–7.39 (5 H, m, ArH); *m*/*z* (EI) 249 (M^+, 11%), 108 (53), 91 (100) and 65 (15).

The *cis*-isomer **24** was also obtained as a colourless oil, its purity was also checked by analytical HPLC [Brownlee LiChrosorb Si-100 column, light petroleum–ethyl acetate (7:3), flow rate 1.0 cm^3 min^{-1}, t_R 9.1 min]; $\nu_{max}(CHCl_3)/cm^{-1}$ 3429w, 2988w, 2936w, 1781s, 1724s and 1513s; δ_H(400 MHz; $CDCl_3$) 1.46 (3 H, d, *J* 5.9, CH_3), 1.79 (1 H, ddd, *J* 12.2, 12.2 and 10.8, 3′-H), 2.79–2.92 (1 H, m, 3-H), 4.45–4.59 (1 H, m, 2-H), 4.54 (1 H, ddq, *J* 11.0, 10.8 and 5.9, 4-H), 5.12 (2 H, s, CH_2Ar), 5.33–5.45 (1 H, br s, NH) and 7.27–7.40 (5 H, m, ArH); *m*/*z* (EI) 249 (M^+, 13%), 108 (58), 91 (100) and 65 (10).

Methyl 2-(benzyloxycarbonylamino)-4-hydroxypentanoates 26 and 27

A 1:1 diastereomeric mixture of methyl 2-(benzyloxycarbonylamino)-4-nitropentanoates[1] **22** (2.33 g, 7.53 mmol) in anhydrous methanol (20 cm^3) was treated with *N*-benzyltrimethylammonium hydroxide in methanol (40% w/v; 3.20 cm^3, 7.61 mmol) and stirred for 10 min to generate the nitronate anion. The mixture was cooled to −78 °C, then treated with a stream of ozone–oxygen until the mixture turned light blue. Sodium borohydride (362 mg, 9.8 mmol) was added to the reaction mixture at −78 °C. The temperature of the resulting solution was then raised to 4 °C and kept at that temperature for 10 min. It was then cooled to −78 °C and methanolic hydrochloric acid (9 mol dm^{-3}) was added until the solution had a pH < 3. Volatile components of the mixture were removed under vacuum. The residue was re-dissolved in chloroform, washed twice with water, then dried and the solvent removed under vacuum. Purification of the crude product, immediately after work-up, by flash chromatography over silica (light petroleum–ethyl acetate; 1:1) gave in order of elution after work-up:– *trans*-2-(benzyloxycarbonylamino)-4-methyl-γ-butyrolactone **25** (328 mg, 17%) as a colourless oil; the *threo*-isomer *methyl* (2RS,4SR)-2-(*benzyloxycarbonylamino*)-4-*hydroxypentanoate* **26** (759 mg, 39%) as a colourless oil (Found: C, 60.2; H, 11.0; N, 5.2. $C_{14}H_{19}NO_5$ requires C, 59.8; H, 11.4; N, 5.0%); δ_H(400 MHz; $CDCl_3$) 1.21 [3 H, d, *J* 6.4, -CH(OH)CH_3], 1.65 (1 H, ddd, *J* 13.9, 11.0 and 3.2, 3-H), 1.89 (1 H, ddd, *J* 13.9, 10.7 and 3.4, 3′-H), 3.39 (1 H, br s, OH), 3.76 (3 H, s, OCH_3), 3.79–3.89 (1 H, m, 4-H), 4.59 (1 H, ddd, *J* 10.7, 7.8 and 3.2, 2-H), 5.13 (2 H, s, CH_2Ar), 5.75 (1 H, br d, *J* 7.8, NH) and 7.29–7.41 (5 H, m, ArH); and the *erythro*-isomer *methyl* (2RS,4RS)-2-(*benzyloxycarbonylamino*)-4-*hydroxypentanoate* **27** (659 mg, 31%) as a colourless oil (Found: C, 60.0; H, 11.1; N, 5.0. $C_{14}H_{19}NO_5$ requires C, 59.8; H, 11.4; N, 5.0%); δ_H(400 MHz; $CDCl_3$) 1.24 [3 H, d, *J* 6.4, CH(OH)CH_3], 1.71–1.79 (1 H, br s, OH), 1.85–1.95 (1 H, m, 3-H), 1.95–2.05 (1 H, m, 3′-H), 3.76 (3 H, s, OCH_3), 3.92–4.03 (1 H, m, 4-H), 4.41–4.50 (1 H, m, 2-H), 5.12 (2 H, s, CH_2Ar), 5.73 (1 H, br d, *J* 7.8, NH) and 7.30–7.41 (5 H, m, ArH).

Conversion of the *threo*-alcohol 26 into the *cis*-lactone 24

A solution of *threo*-methyl 2-(benzyloxycarbonylamino)-4-hydroxypentanoate **26** (32 mg, 0.11 mmol) in benzene (30 cm^3) was slowly distilled to dryness to leave the *cis*-lactone **24** (28 mg, 99%) as a colourless oil, which co-chromatographed with and had an identical ^{1}H NMR to that of **24** obtained by sodium borohydride reduction of the ketone **23**.

Methyl 2-(benzyloxycarbonylamino)-4-oxobutanoate 29

Methyl 2-(benzyloxycarbonylamino)-4-nitrobutanoate[1] **28** (112 mg, 0.399 mmol) in anhydrous methanol (5 cm^3) was treated with *N*-benzyltrimethylammonium hydroxide in methanol (40% w/v; 0.17 cm^3, 0.40 mmol) and stirred for 10 min to generate the nitronate anion. Pyridine (32 mg, 0.41 mmol) was added to the mixture, which was then cooled to −78 °C. It was treated with a stream of ozone–oxygen for 15 min after which the solution turned pale blue. Dimethyl sulfide (0.10 cm^3) was then added to the mixture at −78 °C and the mixture was allowed to rise slowly to room temperature. The solution was left to stand overnight after which the volatile components were removed under vacuum. The residue was acidified with aqueous hydrochloric acid (3 mol dm^{-3}) and then extracted three times with chloroform. The combined chloroform extracts were washed five times with water, dried, filtered, then evaporated to dryness. The crude product was purified by flash chromatography over silica (light petroleum–ethyl acetate; 1:1) to give *methyl* 2-(*benzyloxycarbonylamino*)-4-*oxobutanoate* **29** (52 mg, 50%) as a colourless oil (Found: M^+, 265.0950. $C_{13}H_{15}NO_5$ requires *M*, 265.0950); $\nu_{max}(CHCl_3)/cm^{-1}$ 3431w, 2957w, 1788m, 1722s, 1510s, 1456m and 1439m; δ_H(400 MHz; $CDCl_3$) 3.00–3.16 (2 H, m, 3- and 3′-H), 3.74 (3 H, s, OCH_3), 4.50–4.59 (1 H, m, 2-H), 5.11 (2 H, s, CH_2Ar), 5.72 (1 H, br d, *J* 8.2, NH), 7.30–7.39 (5 H, m, ArH) and 9.71 (1 H, s, CHO); *m*/*z* (EI) 265 (M^+, 1.7%), 237 (<1), 206 (3), 162 (4), 108 (30), 107 (13), 91 (100), 87 (10), 86 (18), 85 (19), 84 (24) and 83 (27).

2-(Benzyloxycarbonylamino)-γ-butyrolactone 30

Methyl 2-(benzyloxycarbonylamino)-4-nitrobutanoate[1] **28** (144 mg, 0.50 mmol) in anhydrous methanol (5 cm^3) was treated with *N*-benzyltrimethylammonium hydroxide in methanol (40% w/v; 0.21 cm^3, 0.50 mmol) and stirred for 10 min to generate the nitronate anion. The mixture was cooled to −78 °C, then treated with a stream of ozone–oxygen for 30 min. Sodium borohydride (55 mg, 1.5 mmol) was added to the solution at −78 °C. The temperature of the mixture was allowed to rise to 0 °C and hydrochloric acid (8 mol dm^{-3}) in methanol was then added to decompose the excess reagent. The volatile components were removed under vacuum and the residue extracted several times with chloroform. The combined extracts were washed once with water, dried, filtered and the solvent removed under vacuum. The residue was purified by flash chromatography over silica (light petroleum–ethyl acetate; 9:11), followed by HPLC [μ-Porasil column, light petroleum–ethyl acetate (3:2), flow rate 3.0 cm^3 min^{-1}] to give 2-(*benzyloxycarbonylamino*)-*γ-butyrolactone* **30** (14 mg, 12%) as a colourless oil (Found: M^+, 235.0842. $C_{12}H_{13}NO_4$ requires *M*, 235.0844); $\nu_{max}(CHCl_3)/cm^{-1}$ 3429w, 2927w, 1784s, 1723s and 1512m; δ_H(400 MHz; $CDCl_3$; 318 K) 2.14–2.27 (1 H, m, 3-H), 2.68–2.82 (1 H, m, 3′-H), 4.19–4.30 (1 H, m, 2-H), 4.40–4.44 (2 H, m, 4-H), 5.13 (2 H, s, C*H*$_2$Ar), 5.27–5.39 (1 H, br, NH) and 7.27–7.38 (5 H, m, ArH); *m/z* (EI) 235 (M^+, 11%), 108 (65), 107 (16), 92 (9), 91 (100), 86 (10), 65 (11) and 57 (12).

Dimethyl 2-(benzyloxycarbonylamino)butane-1,4-dioate 31

Methyl 2-(benzyloxycarbonylamino)-4-nitrobutanoate[1] **28** (301 mg, 1.07 mmol) in anhydrous methanol (10 cm^3) was treated with *N*-benzyltrimethylammonium hydroxide in methanol (40% w/v; 0.45 cm^3, 1.07 mmol) and stirred for 10 min to generate the nitronate anion. The mixture was cooled to −78 °C, then treated with a stream of ozone–oxygen for 30 min. Sodium borohydride (118 mg, 3.21 mmol) was added to the solution at −78 °C. The temperature of the mixture was held at −78 °C for 1 h, allowed to rise to 0 °C and hydrochloric acid (8 mol dm^{-3}) in methanol was then added to decompose the excess reagent. The volatile components were removed under vacuum and the residue extracted several times with chloroform. The combined extracts were washed once with water, dried, filtered and the solvent removed under vacuum. The residue was purified by flash chromatography over silica (light petroleum–ethyl acetate; 11:9) and HPLC [μ-Porasil column, light petroleum–ethyl acetate (4:1), flow rate 3.0 cm^3 min^{-1}] to give *dimethyl* 2-(*benzyloxycarbonylamino*)*butane*-1,4-*dioate* **31** (94 mg, 30%) as a colourless oil (Found: M^+, 295.1041. $C_{14}H_{17}NO_6$ requires *M*, 295.1056); $\nu_{max}(CHCl_3)/cm^{-1}$ 3435w, 2957w, 1730s, 1509s and 1440m; δ_H(100 MHz; $CDCl_3$) 2.08–2.32 and 3.02–3.15 (1 H each, m, 3- and 3′-H), 3.67 (3 H, s, OCH_3), 3.74 (3 H, s, OCH_3), 4.56–4.76 (1 H, m, 2-H), 5.14 (2 H, s, C*H*$_2$Ar), 5.60–5.95 (1 H, m, NH) and 7.20–7.40 (5 H, m, ArH); *m/z* (EI) 295 (M^+, 2%), 236 (2), 108 (24), 107 (10) and 91 (100).

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