Synthesis of β-Amino-acid Peptides. Part 3.[1] Preparation of Racemic and Chiral 3-Aminobutyric Acid Derivatives and Peptides Using Dihydro-oxazin-6-ones and Conventional Coupling Reagents

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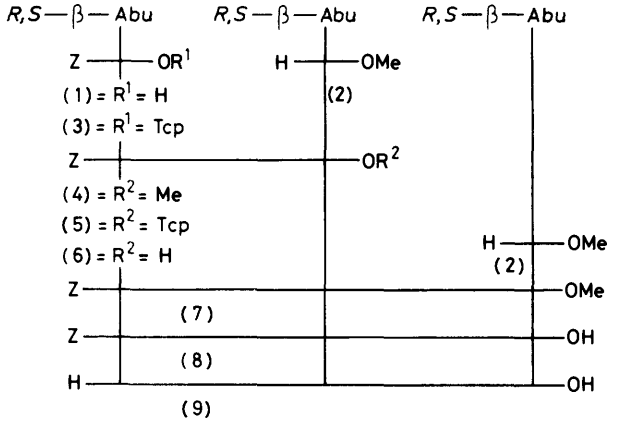
Peptides of *R,S* and *S*-3-aminobutyric acid have been prepared by conventional methods and also by using chiral oxazin-6-ones, the latter method proving to be superior because of the absence of the side-reactions. *S*-3-Aminobutyric acid derivatives were prepared by Arndt–Eistert homologation.

We report progress made in our studies on the synthesis of β-amino-acid peptides and in the use of oxazin-6-ones to effect peptide coupling. Some of our findings with *R,S*-3-aminobutyric acid [2] and *S*-3-aminobutyric acid [3] have already been published.[4]

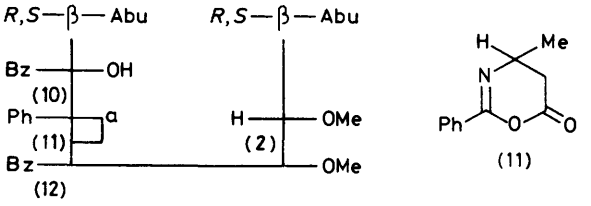
In preliminary work racemic derivatives were used to conserve the *S*-amino-acid. Attempted preparations of di- and tri-peptides proved unsatisfactory † when using dicyclohexylcarbodi-imide (DCCI), and 1-hydroxybenzotriazole [5](HBT), isobutyl chloroformate [6-8] (CMA) and also the pivaloyl mixed anhydride [9] for extension to the tripeptide (7). Preparation of *R,S* and *S*-peptides by aminolysis of trichlorophenyl active esters in the presence of hexamethylenephosphoramide proved to be the method of choice. When using the solvent the reaction was accompanied by a marked exotherm suggesting that a more highly activated intermediate may be formed.[10] Scheme 1 details the route.



Tcp = $C_6H_2Cl_3$-2,4,5
Z = $PhCH_2OCO$

Scheme 1

Re-investigation of the reaction of the *R,S*-benzoylamino-acid [11] (10) with acetic anhydride [11,12] provided the crystalline anhydride (13) in addition to the *R,S*-oxazin-6-one (11), not previously reported, and the transacylated *R,S*-3-acetylaminobutyric acid.[9] Cyclisation of compound (10) using the CMA method provided an excellent yield of the *R,S*-oxazin-6-one (11). Additionally good yields of the *R,S*-anilide (14) [11] and *R,S*-dipeptide (12) were recorded on reaction with aniline and the amino-ester (2) respectively (Scheme 2). Extension to the tripeptide was not undertaken since it was felt that the reactions could now be applied to the *S*-isomer.



a Represents dihydro-oxazinone system

R,S - $[Bz{\cdot}NHCH(Me){\cdot}CH_2CO]_2O$
(13)
R,S - $Bz{\cdot}NHCHMe{\cdot}CH_2{\cdot}CONHPh$
(14)

Scheme 2

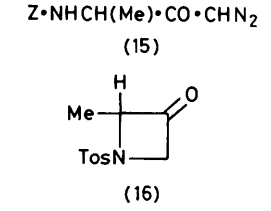
S-3-Aminobutyric acid [3] required for preparation of chiral peptides may be prepared by Arndt–Eistert homologation.[13] Both benzyloxycarbonyl [14] and tosyl-L-alanine [15] after conversion into their respective diazoketones were subject to rearrangement, providing protected *S*-3-aminobutyric acid derivatives. Of the two routes, that employing the benzyloxycarbonyl derivative was by far the superior method. It was possible to isolate *S*-3-benzyloxycarbonylaminobutyric acid (26) directly in 82%, or the methyl ester (27) in 88% yield *via* the diazoketone (15). By deprotection of (27) the derivatives (26) and (19) required for subsequent peptide coupling reactions were made available.

Attempts to retrieve *S*-3-tosylaminobutyric acid by a direct procedure failed and (+)-*N*-tosyl-2-methylazetidin-3-one (16) could be recovered. Thus cyclisation to the azetidin-3-one (16) took precedence over rearrangement of the intermediate diazoketone (15).[16]

Cyclisation of *S*-3-benzoylaminobutyric acid (17) using

† Side-reactions were predominant thus, with DCCI the major product was the *N*-acylurea (NAU) (71%); DCCI/Hbt gave NAU (12%) and (4) (37%); CMA gave (4) (13—15%).

CMA activation at −10 °C in the presence of *N*-methylmorpholine furnished the chiral oxazinone [17] (18) in very high yield, paralleling studies with the racemic derivatives. If the reaction was allowed to proceed at 0 °C, isobutyl *S*-3-benzoylaminobutyrate became the major product (95%) whilst only 0.5% of the corresponding oxazinone (18) could be isolated. It may be conjectured that isobutyl alcohol arising from cyclisation of the CMA of (17) underwent reaction with the optically active oxazine (18) at the rather higher temperature. Acylation of the *S*-isomer (19) with the optically active oxazinone (18) at room temperature (Scheme 3) proceeded with a marked exotherm and provided the (+)-dipeptide (20) in 97% yield. Hydrolysis of (20) and reactivation of the (−)-dipeptide acid (21) similarly led to the (+)-rotary peptide oxazinone (22) in 82% yield. *C*-Terminal elongation *via* (22) proceeded smoothly giving the (+)-tripeptide (23) in good yield. Finally

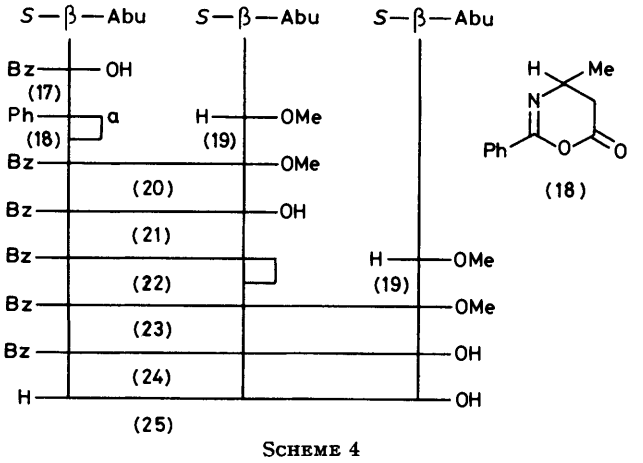


Tos = Toluene-*p*-sulphonyl

SCHEME 3

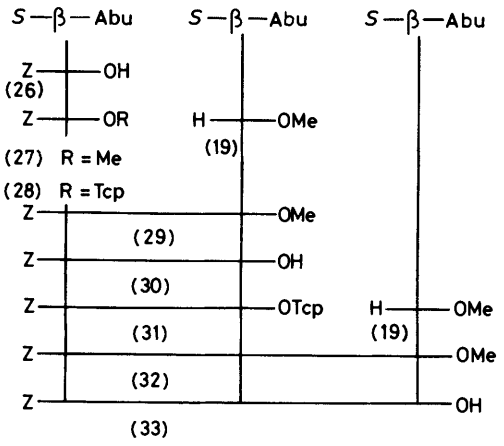
deprotection of the (+)-tripeptide (23) at the C-terminus by saponification and at the *N*-terminus by electrolytic reduction [18] gave the required free (−)-tripeptide (25).

Based on experience with the racemic amino-acid, the trichlorophenyl ester/HMPA route was the method of



SCHEME 4

choice for peptide synthesis with *N*-benzyloxycarbonyl protected derivatives. In practice the yield of (−)- and (+)-rotary peptides (29) and (32) were markedly better than for their racemic counterparts. Scheme 4 details the sequence of reactions which proceeded unexceptionally to give the required protected tripeptide (32). Saponification of compound (32), followed by catalytic hydrogen transfer [19] furnished the deprotected (−)-tripeptide (25) identical in all respects with that obtained by the oxazinone route.



SCHEME 5

Retrospective comparison of conventional and oxazinone coupling methods demonstrated that the latter gave higher yields, was not accompanied by side-reactions and proved to be a superior method from a manipulative standpoint.

EXPERIMENTAL

M.p.s were determined either on Gallenkamp or Electrothermal Apparatus. I.r. spectra were recorded on liquid films and Nujol mulls using a Perkin-Elmer 137 or 577 spectrophotometer. ^{1}H N.m.r. spectra (60 MHz) were recorded using a Varian A60 or Perkin–Elmer R12B instrument, with tetramethylsilane as internal standard. Mass spectra were obtained with an AE1 MS30 instrument or using the PCMU Service. Optical rotations were measured on a Bellingham & Stanley polarimeter. Solvents were purified and dried before use according to published methods.[20] In general, neutral products were isolated by washing solutions in ethyl acetate successively with hydrochloric acid (0.5M), sodium hydroxide (0.5M), and water; this was followed by drying, using anhydrous magnesium sulphate, and evaporation under reduced pressure using a rotary evaporator. Standard procedures for esterification, hydrolyses, and other routine preparations are described once in the Experimental section. 10% Palladium–charcoal was used in all hydrogenations. T.l.c. on Merck Kieselgel G (0.25 mm) employed the following solvent systems (v/v): (A) chloroform–ethyl acetate (96 : 4), (B) ethyl acetate–acetone (5 : 1), (C) n-butanol–acetic acid–water (4 : 1 : 1), (D) benzene–ethyl acetate (21 : 4), (E) ethyl acetate, (F) benzene–ethyl acetate (23 : 2), (G) chloroform, and (H) chloroform–cyclohexane–acetic acid (8 : 2 : 1). Light petroleum refers to that fraction with b.p. 40—60 °C; ether refers to diethyl ether throughout.

R,S-*Derivatives and Peptides of β-Aminobutyric Acid. The Synthesis of β-Aminobutyryl-β-aminobutyryl-β-aminobutyric Acid* (9).*—*Preparation of methyl-β-aminobutyrate*

* Symbols *R*, *S* have been omitted for clarity, for all other racemic compounds.

(2) *hydrochloride*. Thionyl chloride (11.9 g, 0.1 mol) was added dropwise with stirring to ice–salt cooled (−10 °C) dry methanol (50 ml); this was followed by addition of β-aminobutyric acid [2] (10 g, 0.097 mol). The reaction was heated under reflux for 3 h after which the excess of thionyl chloride was evaporated to leave an oily residue. Toluene (3 × 25 ml) was added and the solvent evaporated. The *ester* (2) *hydrochloride* (15.36 g, 69.0%) crystallised from ethyl acetate, m.p. 121—125 °C, R_{FA} 0.49 (Found: C, 39.2; H, 7.8; Cl, 23.4; N, 9.3. $C_5H_{12}ClNO_2$ requires C, 39.1; H, 7.8; Cl, 23.1; N, 9.1%).

Preparation of 2,4,5-Trichlorophenyl-β-benzyloxycarbonyl-aminobutyrate (3).—A solution of DCCI (6.3 g, 30.39 mmol) in pyridine (10 ml) was added gradually to a stirred and cooled (−5 °C) solution of *N*-protected amino-acid [21] (1) (6 g, 25.3 mmol) and 2,4,5-trichlorophenol (5.97 g, 30.39 mmol) in pyridine (12 ml). The reaction was stirred for 3 h at −5 °C, set aside for 30 h at 0 °C, and then diluted with ethyl acetate (120 ml) and left for 1 h at 0 °C. DCU (4.65 g, 68.9%, m.p. 226—228 °C) was filtered off and the filtrate evaporated to dryness. The oily residue was dissolved in ether and more DCU (1.9 g, 28.3%, m.p. 226—228 °C) recovered. After evaporation, the residue, an oil, was triturated with light petroleum to yield the *ester* (3) (9.8 g, 93.0%), m.p. 64—67 °C raised to 71—72 °C from light petroleum (Found: C, 51.7; H, 4.05; N, 3.3. $C_{18}H_{16}Cl_3NO_4$ requires C, 51.9; H, 3.9; N, 3.4%).

Preparation of Methyl β-Benzyloxycarbonylaminobutyryl-β-aminobutyrate (4).—A solution of the amino-ester (2) hydrochloride (1.22 g, 7.92 mmol) and triethylamine (0.8 g, 1.1 ml, 7.92 mmol) in ethyl acetate (10 ml) was added to a stirred solution of the active ester (3) (3 g, 7.2 mmol) in HMPA (10 ml) at room temperature. There was an exothermic reaction (10—15 min) and the mixture solidified. The precipitate was filtered off to give the fully protected *dipeptide* (4) (1.6 g, 66.6%), m.p. 106—108 °C (Found: C, 60.6; H, 7.00; N, 8.3. $C_{17}H_{24}N_2O_5$ requires C, 60.7; H, 7.2; N, 8.3%).

Preparation of β-Benzyloxycarbonylaminobutyryl-β-aminobutyric Acid (6).—A solution of the fully protected dipeptide (4) (5 g, 14.88 mmol) in aqueous methanol (30%; 25 ml) was cooled (0 °C) and aqueous sodium hydroxide (1N; 15 ml) gradually added with stirring. The reaction was stirred overnight. Methanol was then evaporated, the aqueous solution extracted with ethyl acetate, acidified with hydrochloric acid (5N) to Congo Red, and set aside at room temperature for 1 h. The precipitate was filtered off, and crystallised from ethyl acetate to yield the N-*protected dipeptide acid* (6) (3.52 g, 73.4%), m.p. 186—190 °C raised to 191—193 °C on recrystallisation (Found: C, 59.5; H, 6.6; N, 9.0. $C_{16}H_{22}N_2O_5$ requires C, 59.6; H, 6.9; N, 8.7%).

Preparation of Methyl β-benzyloxycarbonylaminobutyryl-β-aminobutyryl-β-aminobutyrate (7) via *the Active Ester Route in HMPA*.—The experimental details are as described for the preparation of the dipeptide (4) *via* the active ester (3). The free amino dipeptide ester from (4) (6.5 g, 34.4 mmol) (prepared by hydrogenation of (4) over 10% palladium on charcoal and used without purification) in HMPA (6 ml) was added to a stirred solution of the *N*-protected amino-acid active ester (3) (12.0 g, 28.8 mmol) in HMPA (15 ml) at room temperature. There was an exothermic reaction and the mixture solidified in 20—30 min. The desired product was isolated according to the procedure detailed above. Recrystallisation from methanol–ether yielded the fully protected tripeptide (7) (7.7 g, 63.6%), m.p. 185—187 °C, R_{FB} 0.35 (Found: C, 59.5; H, 7.3; N, 9.7. $C_{21}H_{31}N_3O_6$ requires C, 59.8; H, 7.4; N, 10.0%).

Preparation of β-Benzyloxycarbonylaminobutyryl-β-aminobutyryl-β-aminobutyric Acid (8).—A solution of the fully protected tripeptide (7) (4.0 g, 9.49 mmol) in aqueous methanol (85 ml, 20%) was cooled (5 °C) and treated with sodium hydroxide solution (1N; 10.5 ml) added in three portions during 6 min. The mixture was stirred at room temperature for 24 h and the product isolated as for (6). Recrystallisation from methanol–ether gave the N-*protected tripeptide acid* (8) (2.65 g, 68.8%), m.p. 210—213 °C, raised to 230—232 °C (Found: C, 59.0; H, 7.5; N, 10.4. $C_{20}H_{29}N_3O_6$ requires C, 59.00; H, 7.2; N, 10.3%).

Preparation of β-Aminobutyryl-β-aminobutyryl-β-aminobutyric Acid (9).—The foregoing *N*-protected tripeptide acid (2.4 g, 5.88 mmol) was dissolved in methanol (130 ml) and hydrogenolysed over 10% palladium–charcoal (0.3 g) during 6 h. Filtration and evaporation yielded a mixture R_{FC} 0.32 (ninhydrin positive) 0.66 (ninhydrin positive). Spraying with Bromocresol Green and hydroxylamine–ferric chloride solutions showed that the faster moving spot represented free amino-tripeptide methyl ester. *Free tripeptide* (9) (0.5 g, 32%), m.p. 240—241 °C was isolated by recrystallisation from methanol (Found: C, 53.0; H, 8.3; N, 15.4. $C_{12}H_{23}N_3O_4$ requires C, 52.7; H, 8.5; N, 15.4%).

Attempted Preparation of R,S-*4,5-Dihydro-4-methyl-2-phenyl-1,3-oxazin-6-one* (11) *by Cyclisation of β-Benzamidobutyric Acid* (10).—Acetic anhydride (22 g, 20 ml, 215 mmol) and β-benzamidobutyric acid [11] (2 g, 9.7 mmol) were kept at 100 °C for 20 min. Removal of acetic anhydride at water-pump vacuum afforded an oil. The oil was taken up in ethyl acetate and examined by t.l.c., R_{FD} 0.05, 0.38, and 0.85; all three spots gave a reaction on being sprayed with hydroxylamine and ferric chloride solutions.

Isolation of β-Benzamidobutyryl Symmetrical Anhydride (13).—Oil from the foregoing experiment was triturated several times with light petroleum and the combined petroleum washes preserved for further work-up. The residual material crystallised from ether–light petroleum to afford the *symmetrical anhydride* (13) * (1.29 g, 64.5%), m.p. 86—89 °C, raised to 110—112 °C on crystallisation from ethyl acetate–light petroleum, R_{FD} 0.05; R_{FE} 0.35 (Found: C, 66.5; H, 6.3; N, 7.3. $C_{22}H_{24}N_2O_5$ requires C, 66.7; H, 6.1; N, 7.1%).

Isolation of Minor Components: 4,5-*Dihydro-4-methyl-2-phenyl-1,3-oxazin-6-one* (11).—A silica gel column (50 g) loaded with 500 mg of the residue obtained by evaporation of the above mother-liquids (R_{FD} 0.38, 0.85) was eluted progressively with benzene containing 8, 16, and 32% ethyl acetate. The first component eluted from the column (R_{FF} 0.68, R_{FD} 0.85) was a mobile liquid, the *oxazinone* (11) (108 mg, 5.4%), $\nu_{max.}$ (film): 1 795, 1 675, 1 230, 1 140, 1 040, 1 020, and 700 cm^{-1} (Found: C, 70.1; H, 6.2; N, 7.3%; $M^{+\cdot}$, 189. $C_{11}H_{11}NO_2$ requires C, 69.8; H, 5.9; N, 7.4%; $M^{+\cdot}$, 189).

Ethyl β-Benzamidobutyrate.—The second minor component eluted from the column (R_{FD} 0.38) crystallised from light petroleum, m.p. 42—44 °C. T.l.c. behaviour (ability to react with hydroxylamine and ferric chloride spraying reagents) and spectroscopic examination led to assignment of the compound as ethyl *R,S*-β-benzamidobutyrate (75 mg, 3.8%) confirmed by comparison with an authentic sample.

* Previously reported as an oil, ref. 11.

Synthesis of 4,5-*Dihydro*-4-*methyl*-2-*phenyl*-1,3-*oxazin*-6-*one* (11).—Isobutyl chloroformate (1.59 g, 1.52 ml, 11.64 mmol) in dichloromethane (2 ml) was added gradually to β-benzamidobutyric acid [11] (10) (2 g, 9.7 mmol) and triethylamine (1.2 g, 1.66 ml, 11.64 mmol) in dichloromethane at −15 °C to −10 °C. The reaction was stirred at this temperature for 15 min, at 0 °C for 10 min, and then at room temperature for another 15 min. Dichloromethane was evaporated and the solvent changed to petroleum, thus allowing insoluble triethylamine hydrochloride to separate and be filtered off. Evaporation of the filtrate yielded the pure oxazinone (11) (1.66 g, 91.6%) identical in all respects with the previously obtained samples.

Preparation of β-*Benzamidobutyranilide* [11] (14) *using Oxazinone* (11).—A solution of aniline (35.2 mg, 0.38 mmol) in dry ether (5 ml) was added to a solution of the dihydrooxazinone derivative (11) (60 mg, 0.32 mmol) in dry ether (8 ml). The mixture was hand-swirled and then allowed to stand at room temperature whereupon the anilide [11] (14) separated (68 mg, 76.2%), m.p. 181—183 °C, raised to 185—186 °C from ethyl acetate, R_{FD} 0.27.

Preparation of Methyl β-*Benzamidobutyryl*-β-*aminobutyrate* (12) via *the Oxazinone* (11).—A solution of methyl β-aminobutyrate (0.68 g, 5.8 mmol) in dichloromethane (4 ml) was added to the (±)-oxazinone derivative (11) (1.1 g, 5.8 mmol) in ether (4 ml) at room temperature. There was a noticeable rise in temperature of the mixture during hand-swirling, quickly followed by separation of the product. Solvent was evaporated and the *dipeptide* (12) (1.18 g, 65.6%), m.p. 126—129 °C, recrystallised from ethyl acetate–petroleum, m.p. 133—135 °C, R_{FF} 0.35 (Found: C, 62.9; H, 7.0; N, 9.4. $C_{16}H_{22}N_2O_4$ requires C, 62.7; H, 7.2; N, 9.1%).

Derivatives and Peptides of S-β-*Aminobutyric Acid.** *The Synthesis of* β-*Aminobutyryl*-β-*Aminobutyryl*-β-*Aminobutyric Acid* (25).—*Preparation of* N-*benzyloxycarbonyl*-L-*alanyl diazoketone* † (15) via *the CMA route*.[22] Isobutyl chloroformate (15.02 g, 15 ml, 0.11 mol) was added to a stirred and cooled (solid CO_2–acetone bath at −15 to −5 °C) solution of *N*-benyloxycarbonyl-L-alanine [14] (22.4 g, 0.1 mol) and *N*-methylmorpholine (11.1 g, 12.3 ml, 0.11 mol) in dichloromethane (100 ml). The precipitate, *N*-methylmorpholine hydrochloride (11.6, 84.4%), was filtered off, and to the filtrate a dried ethereal solution of excess diazomethane (prepared from 40 g of *N*-methyl-*N*-nitrosurea) was added. The reaction system was stirred for 30 min at −5 to 0 °C and subsequently kept in the cold (0 °C) for 24 h. The solvent was removed at reduced pressure and the residue crystallised from methanol and ether to yield the *diazoketone* (15) (20.6 g, 83.2%), m.p. 74—77 °C, raised to 89—91 °C on recrystallisation from ether–petroleum, $[\alpha]_D^{20}$ −16.1° (*c* 1 in ethyl acetate) (Found: C, 58.4; H, 5.6; N, 16.7. $C_{12}H_{13}N_3O_3$ requires C, 58.3; H, 5.3; N, 17.0%).

Preparation of Methyl β-*Benzyloxycarbonylaminobutyrate* (27).—Benzyloxycarbonyl-L-alanyl diazoketone (15) (20 g, 80.9 mmol) prepared by the foregoing reaction was dissolved in dry methanol (140 ml). Several drops of silver benzoate (1 g) dissolved in triethylamine [24] (9.1 g, 11.6 ml) were added to the stirred solution at room temperature. There was an appreciable exotherm and evolution of nitrogen took place. A few more drops of catalyst were added to the stirred reaction followed by addition of decolourizing charcoal and then filtration through a Hyflo filterbed under suction. The filtrate was evaporated to dryness and the residue taken up in ethyl acetate and worked up as for a neutral compound; it was then dried and evaporated to dryness. The residue crystallised on trituration with petroleum to yield the N-*protected amino-acid ester* (27) (17.9 g, 88.2%), m.p. 46—49 °C, raised to 55—56 °C on crystallisation from boiling petroleum, $[\alpha]_D^{20}$ −50° (*c* 1 in methanol) (Found: C, 62.4; H, 6.9; N, 5.3. $C_{13}H_{17}NO_4$ requires C, 62.1; H, 6.8; N, 5.6%).

Isolation of β-*Benzylozycarbonylaminobutyric Acid* (26).—A solution of the aforementioned ester (27) (5 g, 19.9 mmol) in aqueous methanol (30%; 40 ml) was cooled to 0 °C and a solution of sodium hydroxide (2N; 10.9 ml) added gradually; the mixture was then stirred for 6 h at room temperature. The product was isolated as for compound (6) and recrystallised from ether–petroleum to give the N-*protected acid* (26) (3.6 g, 77.8%), m.p. 105—107 °C, raised to 109—110 °C, $[\alpha]_D^{20}$ −26.0° (*c* 1 in chloroform) (Found: C, 60.8; H, 6.4; N, 5.8. $C_{12}H_{15}NO_4$ requires, C, 60.75; H, 6.4; N, 5.9).

Preparation of β-*Benzyloxycarbonylaminobutyric Acid* (26) *by a Direct Route from Diazoketone* (15).—Benzyloxycarbonyl-L-alanyl diazoketone (15) (16 g, 64.7 mmol) was dissolved in aqueous tetrahydrofuran (41 ml, 35%) with stirring at room temperature. Silver benzoate [24] (1 g) dissolved in triethylamine (11.6 ml) was then added dropwise to the gently stirred solution of diazoketone. After 12 min, a considerable exotherm coupled with effervescence occurred. When the reaction cooled to room temperature, more catalyst was added and the reaction stirred vigorously. The solution was filtered with the addition of Hyflo filter aid under suction and the filter-cake washed with boiling ethyl acetate (80 ml). The organic solvents were removed under reduced pressure and the aqueous solution neutralised (2N NaOH; 40 ml), after which decolouring charcoal was added. The clear solution obtained on filtration was made acid (conc. hydrochloric acid) to Congo Red, extracted with ethyl acetate, and the organic phase treated as in the foregoing experiment. The recrystallised residue yielded β-benzyloxycarbonylaminobutyric acid (26) (12.2 g, 79.7%), m.p. 108—110 °C, $[\alpha]_D^{20}$ −26° (*c* 1 in chloroform). The synthesis was also carried out using aqueous acetone as solvent and gave a yield of 82%.

Isolation of 4-*Methyl*-1-*tosylazetidin*-3-*one* (16)‡.—Crude *N*-tosyl-L-alanyl chloride (12 g, 45.9 mmol) (prepared from *N*-tosyl-L-alanine [15] and thionyl chloride) in absolute ether (150 ml) was added to a stirred and cooled (−5 °C) ethereal solution of diazomethane (*ca.* 5 fold excess, prepared from *N*-methyl-*N*-nitrosourea) and kept for 30 min at −5 °C and for 2 h at 0 °C. Evaporation gave a yellow oil (10 g) which was dissolved in tetrahydrofuran (40 ml) to which was added aqueous silver nitrate (10%; 10 ml). After being heated under reflux overnight the solution was evaporated and passed through a column of silica gel in chloroform. A fraction (R_{FG} 0.62) was evaporated and recrystallised from light petroleum to yield compound (16) (0.84 g), m.p. 78—79 °C, $[\alpha]_D^{20}$ +80° (*c* 1 in chloroform) (Found: C, 55.2; H, 5.3; N, 5.7; S, 13.4%; $M^{+\bullet}$ +1 240; $C_{11}H_{13}NO_3S$ requires, C, 55.2; H, 5.5; N, 5.85: S, 13.4%; $M^{+\bullet}$ +1 240), $\nu_{max.}$ (Nujol) 1 828 cm^{-1}.[25]

The Synthesis of β-*Aminobutyryl*-β-*aminobutyryl*-β-*aminobutyric Acid* (25) *with* (+)- *and* (−)-*Oxazinone Derivatives.—Preparation of* β-*benzoylaminobutyric acid* (17). β-

* Symbol *S* has been omitted for clarity for all other optically active derivatives.

† Previously isolated as an oil, ref. 23.

‡ The compound (16) gives a 2,4-dinitrophenylhydrazone and is not subject to hydrolysis.

Aminobutyric acid [3] (6 g, 0.058 mol) was treated with benzoyl chloride (8.9 g, 7.4 ml, 0.064 mol) according to the Schotten–Baumann procedure. The product was recrystallised from ethyl acetate and petroleum to yield the *benzoyl derivative* (17) (8.6 g, 68.2%), m.p. 143—145 °C, raised to 144—146 °C, $[\alpha]_D^{20}$ +24.2° (*c* 3 in acetone), R_{FH} 0.40 (Found: C, 63.7; H, 6.3; N, 6.9. $C_{11}H_{13}NO_3$ requires C, 63.75; H, 6.3; N, 6.8%).

Preparation of (+)-4,5-*dihydro*-4-*methyl*-2-*phenyl*-1,3-*oxazin*-6-*one* [17] (18): *isolation of isobutyl* β-*benzoylaminobutyrate*. Isobutyl chloroformate (0.82 g, 0.79 ml, 6.0 mmol) in dichloromethane (5 ml) was added gradually to a solution of β-benzoylaminobutyric acid (17) (1 g, 4.58 mmol) and triethylamine (0.6 g, 0.43 ml, 5.8 mmol) in dichloromethane (20 ml) at 0 °C. The reaction was stirred for 15 min at 0 °C and for a further 15 min at room temperature. Dichloromethane was evaporated and replaced by ether. The precipitated triethylamine hydrochloride was filtered off and the filtrate evaporated to give an oil which crystallised from boiling petroleum to afford the *isobutyl ester* (1.2 g, 93.3%), m.p. 65—68 °C, raised to 73—74 °C, $[\alpha]_D^{20}$ +24.2° (*c* 1 in chloroform) (Found: C, 68.3; H, 8.0; N, 5.4. $C_{15}H_{21}NO_3$ requires C, 68.4; H, 8.0; N, 5.3%).

Preparation of methyl β-*benzoylaminobutyryl*-β-*aminobutyrate* (20). Methyl β-aminobutyrate * (19) (0.7 g, 6.0 mmol) in diethyl ether (10 ml) was added to a solution of the oxazinone derivative [17] (18) (1 g, 5.3 mmol) in diethyl ether (10 ml) at room temperature; there was a noticeable and immediate exotherm. Crystalline material separated from the solution and this was filtered off to yield the *fully protected dipeptide* (20) (1.55 g, 97%), m.p. 171—174 °C, raised to 172—174 °C from ethyl acetate, $[\alpha]_D^{20}$ +6.65° (*c* 1 in methanol) (Found: C, 62.6; H, 7.0; N, 9.3. $C_{16}H_{22}N_2O_4$ requires C, 62.7; H, 7.2; N, 9.1%).

Isolation of β-*benzoylaminobutyryl*-β-*aminobutyric acid* (21). The dipeptide ester (20) (3.5 g, 11.4 mmol) from the foregoing reaction was taken up in aqueous methanol (25%; 65 ml) and saponified with sodium hydroxide (2N; 6.3 ml) in the usual way. N-*Protected dipeptide acid* (21) was isolated in a yield of 2.6 g (78.8%), m.p. 192—194 °C, raised to 193—195 °C by recrystallisation from methanol–ether, $[\alpha]_D^{20}$ −7.1° (*c* 1 in dimethylformamide), R_{FH} 0.35 (Found: C, 61.5; H, 6.7; N, 9.8. $C_{15}H_{20}N_2O_4$ requires C, 61.6; H, 6.9; N, 9.6%).

Preparation of (−)-2-(2-*benzoylamino*-2-*methylethyl*)-4,5-*dihydro*-4-*methyl*-1,3-*oxazin*-6-*one* (22). Isobutyl chloroformate (0.614 g, 0.6 ml, 4.5 mmol) in acetonitrile (10 ml) was added in two portions to a stirred and cooled (−15 to −10 °C) solution of *N*-benzoyl dipeptide acid (21) (1.2 g, 4.10 mmol) and triethylamine (0.45 g, 0.6 ml, 4.5 mmol) in acetonitrile (45 ml). The reaction was stirred for 15 min at room temperature. Acetonitrile was evaporated off and replaced by ether and the precipitate filtered off. The filtrate was evaporated to yield an oil, which crystallised *in vacuo* over phosphorus pentoxide to give compound (22) (0.92 g, 82.1%), m.p. 179—181 °C, raised to 183—185 °C from acetonitrile, $[\alpha]_D^{20}$ +7.6° (*c* 1 in acetone) (Found: C, 65.9; H, 6.8; N, 10.05%; $M^{+\cdot}$ 274; $C_{15}H_{18}N_2O_3$ requires C, 65.7; H, 6.6; N, 10.2%; $M^{+\cdot}$ 274).

Preparation of methyl β-*benzoylaminobutyryl*-β-*aminobutyryl*-β-*aminobutyrate* (23) via *the peptide oxazinone* (22). A solution of methyl β-aminobutyrate (19) (0.28 g, 2.4 mmol) in acetonitrile (5 ml) was added to a solution of the peptide oxazinone (22) (0.6 g, 2.18 mmol) in acetonitrile (20 ml) at room temperature. The reaction was heated under reflux for 20 min and then allowed to cool to room temperature. Solvent was evaporated off and the solid residue triturated with ether and filtered off to yield the *fully protected tripeptide* (23) (0.62 g, 72.0%), m.p. 233—235 °C, raised to 237—239 °C from methanol, $[\alpha]_D^{20}$ +14.0° (*c* 0.5 in dimethylformamide) (Found: C, 61.3: H, 7.5: N, 10.7. $C_{20}H_{29}N_3O_5$ required C, 61.4; H, 7.5; N, 10.7%).

Isolation of β-*benzoylaminobutyryl*-β-*aminobutyryl*-β-*aminobutyric acid* (24). Sodium hydroxide (2N; 1.12 ml) was added during 5 min to a stirred and cooled (5 °C) solution of fully protected tripeptide (23) (0.8 g, 2.04 mmol) in aqueous pyridine (40 ml, 20%). The solution was stirred for 10 h at room temperature. The aqueous phase was treated in the usual way as for (6) to give the N-*protected tripeptide acid* (24) (0.58 g, 75.3%), m.p. 339 °C, $[\alpha]_D^{20}$ −5.4° (*c* 0.4 in dimethylformamide) (Found: C, 60.2; H, 7.2; N, 10.8. $C_{19}H_{27}N_3O_5$ requires C, 60.5; H, 7.2; N, 11.1%).

Isolation of β-*aminobutyryl*-β-*aminobutyryl*-β-*aminobutyric acid* (25). A solution of the foregoing benzoyl derivative (24) (0.180 g, 0.47 mmol) and tetramethylammonium bromide (536 mg, 3.5 mmol) in methanol (25 ml) was electrolysed between a mercury cathode and a shielded platinum anode.[18] Electrolysis was carried for 45—60 min at constant current (0.08 A) and variable potential using a Potentiostat Type TR 40/3A. The end-point for the reaction was detected by t.l.c. and by liberation of hydrogen at the cathode. The reaction solution was then evaporated to dryness, the residue taken up in water, and the insoluble material, which was shown to be the blocked tripeptide (24) (30 mg, 17%), filtered off. The aqueous solution was desalted by electrodialysis, using cation- and anion-exchange membranes on the Shandon Electrodialyser and Desalter after Wood.[26] The process was monitored by a drop of the current to a steady-state value (0.6—0.08 A). The experiment was terminated after 2 h. Evaporation of the solution to dryness yielded the free *linear tripeptide* (25) (54 mg, 41.4%), m.p. 263—265 °C, $[\alpha]_D^{20}$ −9.0° (*c* 1 in water), R_{FG} 0.32 (Found: C, 52.6; H, 8.3; N, 15.35%; $M^{+\cdot}$, 274. $C_{12}H_{23}N_3O_4$ requires C, 52.7; H, 8.5; N, 15.4%; $M^{+\cdot}$, 274).

Preparation of 2,4,5-*Trichlorophenyl*-β-*benzyloxycarbonylaminobutyrate* (28).—The *trichlorophenyl derivative* (28) was isolated as for compound (3) (38.6 g, 91.6%), m.p. 77—80 °C, raised to 92—94 °C from boiling petroleum, $[\alpha]_D^{20}$ −17.0 °C (*c* 1 in chloroform) (Found: C, 52.2; H, 3.6; Cl, 25.3; N, 3.5. $C_{18}H_{16}Cl_3NO_4$ requires C, 51.9; H, 3.9; Cl, 25.5; N, 3.4%).

Preparation of Methyl Benzyloxycarbonylaminobutyryl-β-*aminobutyric* (29).—The *fully protected peptide* (29) was recovered as for compound (4) (10.0 g, 70.8%), m.p. 139—141 °C, raised to 141—145 °C from ethyl acetate–petroleum, R_{FC} 0.65, $[\alpha]_D^{20}$ −5.6° (*c* 5 in acetone) (Found: C, 60.8; H, 7.3; N, 8.3. $C_{17}H_{24}N_2O_5$ requires C, 60.7; H, 7.2; N, 8.3%).

Isolation of β-*Benzyloxycarbonylaminobutyryl*-β-*aminobutyric Acid* (30).—Details of the procedure are as for compound (6). The *dipeptide acid* (30) (8.6 g, 90.5%) recrystallised from acetone–ethyl acetate had m.p. 170—173 °C, raised to 174—176 °C, $[\alpha]_D^{20}$ +10.4° (*c* 2 in dimethylformamide), R_{FH} 0.2 (Found: C, 59.6; H, 6.9; N, 8.9. $C_{16}H_{22}N_2O_5$ requires C, 59.6; H, 6.9; N, 8.7%).

Preparation of 2,4,5-*Trichlorophenyl* β-*Benzoyloxycarbonylaminobutyryl*-β-*aminobutyrate* (31).—DCCl (5.6 g,

* Prepared by hydrogenolysis of (27) over palladium charcoal and used without further purification.

27.0 mmol) was added to a stirred solution of *N*-protected dipeptide acid (30) (8 g, 25 mmol) and 2,4,5-trichlorophenol (5.2 g, 27.0 mmol) in pyridine (180 ml) at −10 to −5 °C. The mixture was treated as for compound (3). The organic solution was evaporated to dryness and the residue dissolved in ethyl acetate under reflux. The solution crystallised to yield the crude *2,4,5-trichlorophenyl derivative* (31) (11.4 g, 84.4%), m.p. 155—158 °C, raised to 163—165 °C, $[\alpha]_D^{20}$ −20° (*c* 0.25 in methanol), R_{FA} 0.43 (Found: C, 52.7; H, 4.6; Cl, 21.4; N, 5.8. $C_{22}H_{23}Cl_3N_2O_5$ requires C, 52.6; H, 4.6; Cl, 21.2; N, 5.6%).

Preparation of Methyl β-Benzyloxycarbonylaminobutyryl-β-aminobutyryl-β-aminobutyrate (32).—Methyl β-aminobutyrate (19) (2.6 g, 22 mmol) dissolved in HMPA (5 ml) was added to a stirred solution of the trichlorophenyl derivative (31) (10 g, 19.9 mmol) in HMPA (25 ml). The reaction was stirred at room temperature; within 25 min it had crystallised. Ethyl acetate (25 ml) was added to the reaction mixture which was warmed and kept at 80—90 °C for 1 h, then allowed to cool to room temperature; more ethyl acetate (50 ml) was added and the solution filtered. The separated solid was washed with ice-cold ethyl acetate and then recrystallised from methanol to give the *fully protected tripeptide* (32) (5.2 g, 62%), m.p. 203—205 °C, raised to 204—205 °C, $[\alpha]_D^{20}$ +26.6° (*c* 0.3 in methanol), R_{FB} 0.46. The mother liquors were re-extracted, providing additional tripeptide (32) (0.6 g), overall yield 69.2% (Found: C, 59.8; H, 7.4; N, 9.8. $C_{21}H_{31}N_3O_6$ requires C, 59.8; H, 7.4; N, 10.0%).

Isolation of β-Benzyloxycarbonylaminobutyryl-β-aminobutyryl-β-aminobutyric Acid (33).—The procedure used for compound (8) gave the *N-protected tripeptide acid* (33) (2.6 g, 72.2%), m.p. 222—224 °C, raised to 223—225 °C from methanol–ether, $[\alpha]_D^{20}$ +5.2° (*c* 2 in dimethylformamide) (Found: C, 58.9; H, 7.0; N, 10.5. $C_{20}H_{29}N_3O_6$ requires C, 59.0; H, 7.2; N, 10.3%).

Isolation of β-Aminobutyryl-β-aminobutyryl-β-aminobutyric Acid (25) via *Catalytic Transfer Hydrogenation*.[19]—A solution of *N*-protected tripeptide acid (33) (2.0 g, 4.9 mmol) from the foregoing reaction was dissolved in methanol (180 ml) and cyclohexene (0.75 g, 0.9 ml, 9.2 mmol) to which was added 10% palladium on charcoal catalyst (0.5 g). The mixture was gently heated under reflux for 20 min, filtered, the catalyst washed with boiling methanol (100 ml), and the filtrate concentrated to give the free linear tripeptide (25) (1.1 g, 84.6%), m.p. 268—270 °C, identical in all respects with that previously isolated, $[\alpha]_D^{20}$ −9.0° (*c* in 1 in water), R_{FC} 0.32 (Found: C, 52.6; H, 8.3; N, 15.35. Calc. for $C_{12}H_{23}N_3O_4$: C, 52.7; H, 8.5; N, 15.4%).

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