Stereospecific Synthesis of *N*-Protected Statine and Its Analogues *via* Chiral Tetramic Acid

Patrick Jouin * and Bertrand Castro
CCIPE, rue de la Cardonille, BP 5055, 34033 Montpellier Cedex
Dino Nisato *
Sanofi Recherche, rue de Professeur J. Blayac, 34082 Montpellier Cedex

The condensation of a chiral *N*-protected amino acid with Meldrum's acid in the presence of isopropenyl chloroformate (IPCF) and of 4-*N*,*N*-dimethylaminopyridine (DMAP) has been examined. The cyclisation of the reaction product, by heating in an organic solvent, gave the *N*-protected tetramic acid derivatives (**4a**—i) which, after reduction, afforded the corresponding *threo*-4-hydroxypyrrolidin-2-ones (**5a**—i). The regioselective alkaline or acid hydrolysis of the *N*-protected pyrrolidin-2-ones led to enantiomeric pure *N*-Boc-statine (**6a**) and its analogues (**6b**—i) in 40—60% yield from the *N*-protected amino acids. The corresponding statine methyl esters (**7a**—i) could also be synthesized in high yield.

Statine, (3*S*,4*S*)-4-amino-3-hydroxy-6-methylheptanoic acid is an unusual γ-amino acid present in both pepstatine,[1] a natural acid protease inhibitor, and also a new class of antiviral cytotoxic depsipeptides.[2] This novel amino acid was also used, as a transition state analogue,[3] for the synthesis of renin inhibitors,[4a,c] a key enzyme, in the renin–angiotensin system.[5]

Several syntheses of statine and its analogues have been published. The most commonly used among them are based on the aldol condensation of an *N*-protected L-amino aldehyde (as the source of C-3 and C-4) and a metallated acetic acid derivative[6a,d] which afford a separable mixture of *N*-protected (3*S*,4*S*) and (3*R*,4*S*) diastereoisomers. More recently, Woo[6e] reported a chiral synthesis of statine, starting from an *N*-protected L-amino aldehyde, in which he used as the source of C-2 and C-1 the chiral boron enolate derivative of (*S*)-4-H-isopropyl-3-(methylthioacetyl)oxazolidin-2-one according to the methodology of Evans. As several authors have noted, racemisation of the aldehyde is a ready process,[6b] and in the extrapolation to a large-scale this was very critical.[7] An alternative approach avoiding use of the aldehyde intermediate was *via* the *N*-protected statone, [(4*S*)-4-amino-6-methyl-3-oxopentanoic acid].[8a,c]

Unfortunately, until now, a totally stereoselective reduction of the β-oxo ester to the *threo* isomer has remained elusive; the only published route, involved laborious column chromatography to achieve diastereoisomer separation.[9]

A totally enantiomeric induction at C-3 of a statone precursor was reasonably possible if the 3-oxo function was part of a chiral cyclic structure. Effectively, a specific *cis*-reduction was observed by Katsuki *et al.*[8c] during the hydrogenolysis of the racemic 4-hydroxy-5-isobutylpyrrolin-2(5*H*)-one in an attempt to synthesize statine *via* the Dieckmann route. This approach seemed to us not only very promising but also interesting since it offered the possibility of studying, at the same time, the formation of a chiral tetramic acid.[10a–d]

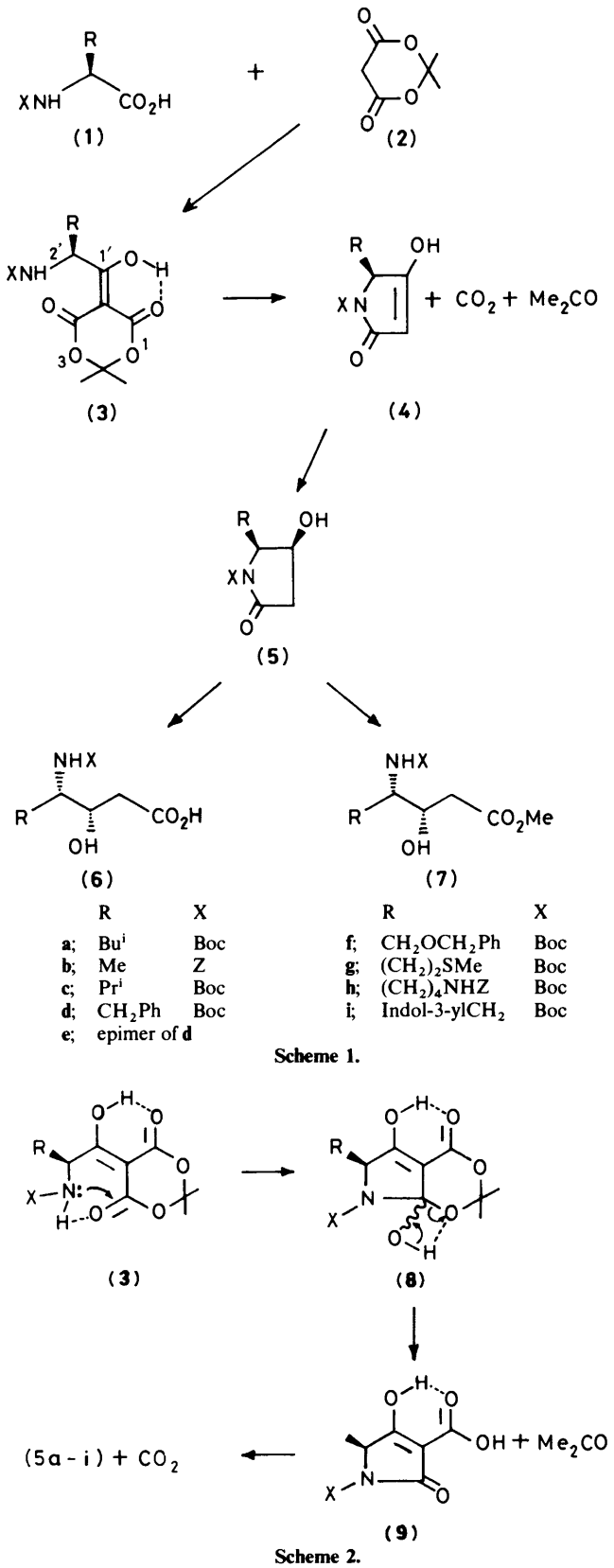
We report herein a stereocontrolled four-step synthesis of the *N*-protected statine and its analogues, in both enantiomeric purity and high yield from *N*-protected L-amino acids; this synthesis led, of course, also to the synthesis of compounds with the opposite configuration.

Results and Discussion

The stereocontrolled reduction of chiral tetramic acids (**4a**—i) gave the 4-hydroxy derivatives (**5a**—i) which were good precursors for the synthesis of the *N*-protected statine, and several statine analogues (**6b**—i), and their corresponding esters (**7a**—i). The tetramic acid derivatives (**4a**—i) could be easily obtained in high yield by a new process, from Meldrum's acid and an *N*-protected chiral amino acid activated with isopropenyl chloroformate (IPCF). The acylation of Meldrum's acid is well established as a synthesis for β-oxo esters.[11] Moreover, the problem encountered in the acylation with *N*-protected amino acids was principally to find out some suitable non-racemizable activating agent for the carboxylic function. Classical mixed or symmetrical anhydrides or acyl chlorides proved to be unsuitable; among the activating agents tested dicyclohexylcarbodi-imide (DCC), and the usual chloroformates gave compounds (**3**) in unsatisfactory yield (in particular with chloroformate, the corresponding ester was formed[12]). Also Williard *et al.*[10d] were unable to obtain any reaction from Meldrum's acid and *N*-phthaloylvaline acyl chloride. By contrast, the *in situ* activation of *N*-protected amino acids (**1a**—i) with isopropenyl chloroformate gave, as usual, an intermediate anhydride, which, in the presence of 4-*N*,*N*-dimethylaminopyridine, readily afforded compounds (**3a**—i) by reaction with the Meldrum's acid. Among the amines tested (*e.g.* pyridine, triethylamine, or di-isopropylamine) only DMAP was satisfactory; thus this reaction was readily performed by the slow addition of IPCF to a solution at −5 °C of the *N*-protected amino acid, Meldrum's acid, and 2 equiv. of DMAP in dichloromethane as solvent. These reaction conditions were very stringent: any change in the procedure (e.g. stoicheiometry of the amine, reversal addition of the reagents) leading to a lower yield. In general, any effort to purify oily products (**3a**—i) (Scheme 1) by column chromatography were unsuccessful.

The cyclization to give a tetramic acid was performed unexpectedly by refluxing the intermediate products (**3a**—i) in acetonitrile or ethyl acetate for 15—20 min; in methanol too we obtained quantitatively the cyclic product instead of the expected β-oxo ester.† A tentative mechanism for this cyclisation is indicated in Scheme 2, where a particular stabilized conformation of the intermediates (**3**) is shown. We postulate first a five-membered ring closure *via* a four-centre nucleophilic attack of the nitrogen lone pair; the latter is favoured by NH hydrogen bonding to one of the carbonyl functions, the other carbonyl group being hydrogen-bonded to form a particularly stabilized conformation. The unstable gem acetal monoester (**8**) so formed then gives the tetramic acid precursor (**9**) which is immediately

† Formation of the corresponding β-oxo ester was also observed in ethanol.



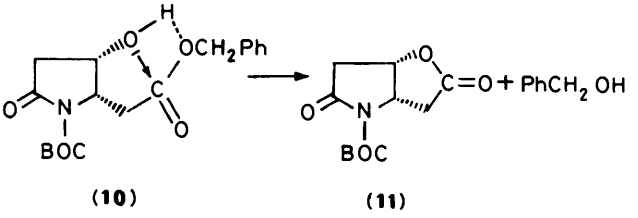
	R	X		R	X
a;	Bu^i	Boc	**f**;	CH_2OCH_2Ph	Boc
b;	Me	Z	**g**;	$(CH_2)_2SMe$	Boc
c;	Pr^i	Boc	**h**;	$(CH_2)_4NHZ$	Boc
d;	CH_2Ph	Boc	**i**;	Indol-3-ylCH_2	Boc
e;	epimer of **d**				

Scheme 1.

Scheme 2.

decarboxylated. The ring closure was not only facilitated but also stabilized by the hydrogen bonding of the migrating hydrogen atom to the ester function (see Scheme 2). The driving force for this reaction was thought to be the formation of acetone as a very good leaving group. The tetramic acid derivatives (**4a**—i) were purified by double extraction in hydrogen carbonate solution or were utilized as crude products for the next step.

The reduction of tetramic acid derivatives (**4a**—i) was performed according to the methodology of Katsuki *et al.*,[8c] with minor modifications this earlier work having demonstrated that catalytic or chemical hydrogenation of 5-isobutylpyrrolidine-2,4-dione gave the corresponding *threo*-4-hydroxy compound in high enantiomeric purity. Thus the cyclic statines (**5a**—i) were smoothly obtained by chemical reduction ($NaBH_4$) at acid pH or by catalytic hydrogenation at room temperature in ethyl acetate as solvent under 20 atm. Catalytic hydrogenation was suitable only for compounds which had no labile protecting group such as benzyloxycarbonyl (Z) or with amino acids such as phenylalanine. The n.m.r. spectra of cyclic statines at 360 MHz confirmed the *cis* reduction by the absence of characteristic signals for the *erythro* form; the coupling constant $J_{3,4}$ for compound (**5b**) was 6.5 Hz, it being of a *threo* configuration.[13] Further chemical evidence of the totally stereoinduced *cis* reduction was provided by formation of compound (**11**) (Scheme 3) from the tetramic acid analogue



(**10**) (**11**)

Scheme 3.

obtained from *N*-Boc-β-benzyl-L-aspartic acid; the 4-hydroxypyrrolidin-2-one (**10**) gave, spontaneously, compound (**11**) which was almost the only isolable product (see Experimental section). The regioselective hydrolysis of *N*-acylpyrrolidin-2-ones (**5a**—i) with 1M NaOH in acetone by a literature method[14] gave the *N*-Boc statine and its analogues in excellent yields (Table). The corresponding methyl esters could also be easily synthesized (Table) by methanolysis with 2M sodium methoxide. This procedure was unsatisfactory for ring opening of the *N*-benzyloxycarbonyl-lactam (**5b**) because of concomitant hydrolysis of the carbamate function.[15] The acid hydrolysis of (**5b**), in 2M hydrochloric acid at a reflux temperature for 2 h in dioxane as solvent, gave the corresponding *N*-benzyloxycarbonylstatine (**6b**). When the solvent was replaced by methanol, the methyl (3*S*,4*S*)-4-benzyloxycarbonylamino-3-hydroxypentanoate was obtained (93%).

Experimental

All amino acid derivatives were purchased from BACHEM (Switzerland); 4-*N*,*N*-dimethylaminopyridine (DMAP) as well as Meldrum's acid were recrystallized before use; isopropenyl chloroformate (IPCF) was provided by S.N.P.E. (Société Nationale des Poudres et Explosifs). T.l.c. were performed on Kieselgel F254 (Merck) pre-coated plates and spots were visualized by charring (sulphuric acid–ammonium sulphate). The R_F values were determined with the following solvent systems: A, methanol–ethyl acetate–acetic acid (3:95:2); B, methanol–dichloromethane–acetic acid (5:93:2); C, hexane–ethyl acetate (25:75) or D, hexane–ethyl acetate (50:50). Preparative liquid chromatography (gravity flow) was performed on Merck Kieselgel 60 (70—230 mesh). M.p.s were determined on a Büchi capillary melting point apparatus and are uncorrected. Optical rotations were run on a Perkin-Elmer 241 MC polarimeter in a 10 cm cell, at molar concentration in methanol or dimethylformamide. ^1H and ^{13}C N.m.r. spectra

Table. Physicochemical properties and analytical and spectroscopic data for *N*-protected statines and their methyl esters

Compd.	Yield (%)	Solvent	M.p. (°C)	$[\alpha]_D^{20}$ (*c* 1 in MeOH)	R_F (eluant B)	R_F (eluant D)	Formula	Found (%) (Calc.) C	H	N	δ_H
(6a)	90	Acetone–hexane	117—119[a]	−41	0.43		$C_{13}H_{25}NO_5$	56.4 (56.71)	9.15 (9.15)	5.05 (5.09)	−8.05 (6 H, dd, CH_3), 1.26 (2 H, m, CH_2), 1.36 (9 H, s, Boc), 1.55 (1 H, m, CH), 2.22 (2 H, dk, 2-H), 3.50 (1 H, m, 4-H), 3.81 (1 H, m, 3-H), 6.22 (1 H, d, NH)
(7a)	90	Hexane	57—58	−40		0.77	$C_{14}H_{27}NO_5$	57.95 (58.11)	9.6 (9.40)	4.90 (4.84)	0.84 (6 H, dd, CH_3), 1.25 (2 H, m, CH_2), 1.37 (9 H, s, Boc), 1.54 (1 H, m, CH), 2.30 (2 H, qd, 2-H), 3.51 (1 H, m, 4-H), 3.57 (3.57 (3 H, s, OCH_3), 3.83 (1 H, m, 3-H), 4.78 (1 H, d, 0-H), 6.28 (1 H, d, NH)
(6b)	83	—	Oil	−15	0.27						−1.22 (3 H, d, 4-CH_3), 2.53 (2 H, qd, 2-H), 4.16 (1 H, m, 4-H), 4.32 (1 H, m, 3-H), 5.22 (2 H, q, CH_2Ph), 5.36 (1 H, s, NH), 7.40 (5 H, s, Ph)
(7b)	93	Hexane–ether	90—92	−17.5		0.44	$C_{14}H_{19}NO_5$	59.45 (59.78)	6.75 (6.81)	5.0 (4.98)	−1.03 (3 H, d, 4-CH_3), 2.35 (2 H, qd, 2-H), 3.58 (3 H, s, OCH_3), 3.62 (1 H, m, 4-H), 3.90 (1 H, m, 3-H), 4.81 (1 H, s, OH), 5.02 (2 H, s, CH_2Ph), 7.35 (5 H, m, Ph)
(6c)	85	—	Oil	−43	0.45						−0.82 (3 H, d, 6-CH_3), 0.89 (3 H, d, 7-CH_3), 1.37 (9 H, s, Boc), 1.71 (1 H, m, 5-CH), 2.22 (2 H, qd, 2-H), 3.04 (1 H, m, 4-H), 4.02 (1 H, m, 3-H)
(7c)	85	—	Oil	−40[c]		0.74					−0.82 (3 H, d, 6-CH_3), 0.89 (3 H, d, 7-CH_3), 1.38 (9 H, s, Boc), 1.72 (1 H, m, 5-CH), 2.32 (2 H, qd, 2-H), 3.04 (1 H, m, 4-H), 3.57 (3 H, s, OCH_3), 4.05 (1 H, m, 3-H), 4.64 (1 H, d, OH), 6.10 (1 H, d, NH)
(6d)	87	Ether	148—150[b]	−37	0.44		$C_{16}H_{23}NO_5$	62.05 (62.11)	7.45 (7.49)	4.5 (4.53)	−1.29 (9 H, s, Boc), 2.7 (2 H, qd, CH_2Ph), 2.29 (2 H, qd, 2-H), 3.66 (1 H, m, 4-H), 3.88 (1 H, m, 3-H), 6.49 (1 H, s, NH), 7.2 (5 H, m, Ph)
(7d)	90	Hexane–ether	97—98	−36		0.66	$C_{17}H_{25}NO_5$	63.55 (63.14)	7.68 (7.79)	4.37 (4.33)	−1.30 (9 H, s, Boc), 2.37 (2 H, qd, 2-H), 2.70 (2 H, qd, CH_2Ph), 3.57 (3 H, s, OCH_3), 3.67 (1 H, m, 4-H), 3.91 (1 H, m, 3-H), 4.99 (1 H, d, OH), 7.21 (5 H, m, Ph)
(6e)		Ether	146—148	+37							
(6f)	88	—	Oil	−3.5	0.47						−1.38 (9 H, s, Boc), 2.30 (2 H, qd, 2-H), 3.44 (2 H, qd, CH_2O), 3.70 (1 H, m, 4-H), 4.05 (1 H, m, 3-H), 4.46 (2 H, s, CH_2Ph), 6.34 (1 H, d, NH), 7.32 (5 H, m, Ph)
(7f)	87	—	Oil	−5		0.69					−1.37 (9 H, s, Boc), 2.37 (2 H, qd, 2-H), 3.43 (2 H, qd, CH_2O), 3.58 (3 H, s, OCH_3), 3.69 (1 H, m, 4-H), 4.06 (1 H, m, 3-H), 4.46 (2 H, s, CH_2Ph), 4.88 (1 H, d, OH), 6.38 (1 H, d, NH), 7.32 (5 H, m, Ph)
(6g)	92	Hexane–ether	107—108	−30.6	0.40		$C_{12}H_{23}NO_5S$	48.8 (49.13)	7.95 (7.90)	4.7 (4.77)	−1.37 (9 H, s, Boc), 1.6 (2 H, m, 5-CH_2), 2.02 (3 H, s, SCH_3), 2.24 (2 H, qd, 2-H), 2.43 (2 H, m, 6-CH_2), 3.51 (1 H, m, 4-H), 3.86 (1 H, m, 3-H), 6.39 (1 H, d, NH)
(7g)	89	—	Oil	−31		0.56					−1.36 (9 H, s, Boc), 1.6 (2 H, m, 5-CH_2), 2.02 (3 H, s, SCH_3), 2.30 (2 H, qd, 2-H), 2.41 (2 H, m, 6-CH_2), 3.51 (1 H, m, 4-H), 3.57 (3 H, s, OCH_3), 3.89 (1 H, m, 3 H), 4.88 (1 H, d, OH), 6.45 (1 H, d, NH)
(6h)	92	Hexane	97—100	−19.5	0.40		$C_{21}H_{32}N_2O_7$	59.4 (59.42)	7.5 (7.60)	6.5 (6.60)	−1.26 (4 H, m, CH_2–CH_2), 1.37 (9 H, s, Boc), 1.44 (2 H, m, 7-CH_2), 2.24 (2 H, qd, 2-H), 2.98 (2 H, m, 8-CH_2), 3.38 (1 H, m, 4-H), 3.86 (1 H, m, 3-H), 5.0 (2 H, s, CH_2Ph), 6.26 (1 H, d, NH), 7.2 (1 H, 8-NH), 7.3 (5 H, m, Ph)
(7h)	92	—	Oil	−19		0.35					−1.25 (4 H, m, CH_2–CH_2), 1.36 (9 H, s, Boc), 1.45 (2 H, m, 7-CH_2), 2.30 (2 H qd, 2-H), 2.98 (2 H, m, OCH_2), 3.37 (1 H, m, 4-H), 3.58 (3 H, s, OCH_3), 3.88 (1 H, m, 3-H), 4.83 (1 H, d, OH), 5.0 (2 H, s, CH_2Ph), 6.31 (1 H, d, NHBoc), 7.21 (1 H, b, NHCO), 7.32 (5 H, m, Ph)
(6i)	75	Ether	162—164	−39	0.32		$C_{18}H_{24}N_2O_5$	62.15 (62.05)	6.85 (6.94)	7.9 (8.04)	−1.37 (9 H, s, Boc), 2.31 (2 H, qd, 2-H), 2.84 (2 H, qd, 5-CH_2), 3.75 (1 H, m, 4-H), 3.97 (1 H, m, 3-H), 6.44 (1 H, d, Boc–NH), 6.98—7.56 (5 H, m, Ph), 10.75 (1 H, s, NH)
(7i)	88	—	Oil	−31		0.42					−1.36 (9 H, s, Boc), 2.41 (2 H, qd, 2-H), 2.85 (2 H, qd, 5-CH_2), 3.56 (3 H, s, OCH_3), 3.77 (1 H, s, 4-H), 3.99 (1 H, s, 3-H), 4.99 (1 H, d, OH), 6.45 (1 H, d, Boc–NH), 6.98—7.59 (5 H, m, Ph)

[a] Lit., m.p. 117—118 °C, $[\alpha]_D^{24}$ −39.6° (*c* 0.3, MeOH); D. H. Rich, E. T. Sun, and A. S. Boparai, *J. Org. Chem.*, 1978, **43**, 3624. [b] Lit., m.p. 148—148.5 °C, $[\alpha]_D^{24}$ −37° (*c* 1.1, MeOH); D. H. Rich and E. T. Sun, *J. Med. Chem.*, 1980, **23**, 27. [c] *c* 0.5 in MeOH.

were recorded on a Bruker 360 MHz instrument; the chemical shifts are reported in p.p.m. downfield from tetramethylsilane as internal standard in hexadeuteriodimethyl sulphoxide. The epimeric products obtained exhibited identical physicochemical properties (R_F, ^1H n.m.r., m.p.) as the corresponding described compounds.

(2'S)-5-[(1-*Hydroxy*-4-*methyl*-2-*t*-*butoxycarbonylamino*)-*pentylidene*]-2,2-*dimethyl*-1,3-*dioxane*-4,6-*dione* (**3a**).—To a solution of the dried amino acid derivative (**1a**) (4.62 g, 20 mmol) in dichloromethane (100 ml), Meldrum's acid (3 g, 21 mmol) and DMAP (5.6 g, 46.1 mmol) were added. The mixture was cooled at −5 °C, and a solution of IPCF (2.6 ml, 21.5 mmol) in dichloromethane (10 ml) was added dropwise with stirring during 0.5 h. After 2 h, cold 5% aqueous potassium hydrogen sulphate was added with vigorous stirring. The separated organic layer was washed with brine, dried, and evaporated to dryness to give the crude product (**3a**), R_F 0.60 (eluant A), which was used without further purification.

(2'S)-5-[(1-*Hydroxy*-3-*phenyl*-2-*t*-*butoxycarbonylamino*)-*propylidene*]-2,2-*dimethyl*-1,3-*dioxane*-4,6-*dione* (**3d**).—*Chloroformate activation.* A solution of IPCF (1.3 ml) in anhydrous dichloromethane (10 ml) was slowly added over a period of 1 h to a stirred mixture of Boc-L-PheOH (**1d**) (2.8 g), DMAP (5.6 g), and Meldrum's acid (1.6 g) in dichloromethane (30 ml) at −5 °C. The mixture was stirred for a further 2 h at this temperature, after which it was washed with 5% aqueous potassium hydrogen sulphate and water, dried, and evaporated under reduced pressure to give a white solid. This was recrystallized to afford (**3d**) (3.4 g, 85%), m.p. 120—121 °C; $[\alpha]_D^{20}$ +88° (*c* in DMF); δ_C 26 (q, 2-CH_3), 27 (q, Boc), 47 (t, CH_2Ph), 55 (d, 2'-CH), 78 (s, OBu^t), 90 (s, 5-C), 105 (s, 1-C), 126—137 (5-C, Ph), 155 (s, NCO), 165 (s, 6-C), and 195 (s, 3-C); δ_H 1.29 (9 H, Boc), 1.66 (6 H, s, 2-Me), 2.88 (2 H, m, CH_2Ph), 5.57 (1 H, m, 2'-H), 7.20—7.36 (5 H, m, Ph), 7.42 (1 H, d, NH), and 11.66 (1 H, s, OH), (Found: C, 61.6; H, 6.55; N, 3.4. $C_{20}H_{25}NO_7$ requires C, 61.37; H, 6.44; N, 3.58%).

DCC activation. DCC (1.2 g) was added to a solution of the protected amino acid (**1d**) (1.33 g), Meldrum's acid (750 mg), and DMAP (900 mg) in dichloromethane (20 ml) at room temperature. The mixture was stirred for 3 h after which it was filtered and the filter washed with dichloromethane (10 ml). The organic phase was washed with 50% aqueous potassium hydrogen sulphate, water, and brine, dried, and evaporated to dryness. The residual solid recrystallized from methanol–diethyl ether (1:1) to yield compound (**3d**) (300 mg, 15%), as white crystals, identical with the product prepared above.

The epimeric product of (**3d**) was prepared in the same manner; m.p. 118—120 °C (from methanol–diethyl ether); $[\alpha]_D^{20}$ −87.5° (*c* 1 in DMF).

(5S)-4-*Hydroxy*-5-*isobutyl*-1-*t*-*butoxycarbonylpyrrol*-2(5H)-*one* (**4a**).—The crude product (**3a**) obtained above was dissolved in ethyl acetate (100 ml), and the solution was maintained at reflux temperature for 0.5 h, the cyclization being monitored by t.l.c. using eluant A. The cooled solution was then extracted with 5% aqueous sodium hydrogen carbonate and the aqueous phase acidified to pH 2 with powdered citric acid; it was then extracted with ethyl acetate. This extract was washed with water, dried, and evaporated to dryness to provide the title compound (**4a**) as an uncoloured oil [4.5 g, 88% from (**1a**)]; R_F 0.38 (eluant A); $[\alpha]_D^{20}$ +101° (*c* 1 MeOH); δ_H 0.79 (6 H, q, CH_3), 1.40 (9 H, s, Boc), 1.60 (1 H, m, CH), 1.70 (2 H, m, CH_2), 4.33 (1 H, q, 5-H), 4.85 (1 H, s, 3-H), and 13 (1 H, s, OH). This material could be neither crystallized nor further purified by chromatography.

The following 5-alkylpyrrol-2(5*H*)-ones (**4**) were prepared *via* the same procedure. (5S)-1-*Benzyloxycarbonyl*-4-*hydroxy*-5-*methylpyrrol*-2(5H)-*one* (**4b**). A colourless oil [89% from (**1b**)]; R_F 0.33 (eluant A); $[\alpha]_D^{20}$ +60.5° (*c* 1 in MeOH); δ_H 1.39 (3 H, d, 5-Me), 4.41 (1 H, q, 5-H), 4.88 (1 H, s, 3-H), 5.22 (2 H, q, CH_2Ph), 7.4 (5 H, m, Ph), and 12.38 (1 H, s, OH).

(5S)-4-*Hydroxy*-5-*isopropyl*-1-*t*-*butoxycarbonylpyrrol*-2(5H)-*one* (**4c**). White crystals [91% yield from Boc-L-ValOH (**1c**)], m.p. 120—121 °C; R_F 0.42 (eluant A); $[\alpha]_D^{20}$ +123° (*c* 1 in MeOH); δ_H 0.76 (3 H, d, 7-Me) 1.02 (3 H, d, 6-Me), 1.42 (9 H, s, Boc), 2.33 (1 H, m, 5-CH), 4.25 (1 H, d, 5-H), 4.85 (1 H, s, 3-H), and 6.19 (1 H, s, OH) (Found: C, 59.45; H, 7.65; N, 5.78. $C_{12}H_{19}NO_4$ requires C, 59.73; H, 7.94; N, 5.80%).

(5S)-5-*Benzyl*-4-*hydroxy*-1-*t*-*butoxycarbonylpyrrol*-2(5H)-*one* (**4d**). Compound (**3d**) (800 mg) in methanol (20 ml) was warmed at reflux temperature for 20 min after which time the solution was cooled and evaporated; the title compound provided white crystals from diethyl ether–hexane (1:2) (570 mg, 95%), m.p. 141—142 °C; R_F 0.48 (eluant A); $[\alpha]_D^{20}$ +230° (*c* 1 in MeOH); δ_H 1.5 (9 H, s, Boc), 3.21 (2 H, m, CH_2Ph), 4.60 (1 H, q, 5-H), 4.66 (1 H, s, 3-H), and 7.1 (5 H, m, Ph) (Found: C, 66.1; H, 6.5; N, 4.5. $C_{16}H_{19}NO_4$ requires: C, 66.42; H, 6.62; N, 4.84%).

(5R)-5-*Benzyl*-4-*hydroxy*-1-*t*-*butoxycarbonylpyrrol*-2(5H)-*one* (**4e**). This compound had m.p. 148—149 °C (from diethyl ether); $[\alpha]_D^{20}$ −230° (*c* 1 in MeOH) (Found: C, 66.4; H, 6.6; N, 4.85. $C_{16}H_{19}NO_4$ requires C, 66.42; H, 6.62; N, 4.84%).

(5S)-5-*Benzyloxymethyl*-4-*hydroxy*-1-*t*-*butoxycarbonyl*-*aminopyrrol*-2(5H)-*one* (**4f**). The title compound was isolated as its sodium salt [80% yield from Boc-L-Ser(OBzl)OH (**1f**)]; R_F 0.46 (solvent A); $[\alpha]_D^{20}$ +55° (*c* 1 in MeOH); δ_H 1.36 (9 H, s, Boc), 3.65 (1 H, q, 5-H), 3.78 (2 H, m, CH_2O), 3.89 (1 H, s, 3-H), 4.43 (2 H, q, CH_2Ph), and 7.27 (5 H, m, Ph) (Found: C, 56.55; H, 5.8; N, 3.85. $C_{17}H_{20}NNaO_5$ requires C, 59.82; H, 5.91; N, 4.10%).

(5S)-4-*Hydroxy*-5-(2-*methylthioethyl*)-1-*t*-*butoxycarbonyl*-*aminopyrrol*-2(5H)-*one* (**4g**). The material isolated (85%) after a one-pot reaction from (**1g**) contained unidentified impurities that could not be removed; R_F 0.44 (eluant A); δ_H 1.45 (9 H, s, Boc), 1.99 (3 H, s, SMe), 2.0 (2 H, m, 5-CH_2), 2.28 (2 H, m, 6-CH_2), 4.46 (1 H, q, 5-H), 4.88 (1 H, s, 3-H), and 12.38 (1 H, s, OH).

(5S)-5-(4-*Benzyloxycarbonylaminobutyl*)-4-*hydroxy*-1-*t*-*butoxycarbonylaminopyrrol*-2(5H)-*one* (**4h**). The title compound [82% yield from Boc-L-Lys(Z)OH (**1h**)] had R_F 0.28 (solvent A); $[\alpha]_D^{20}$ +72° (*c* 1 in MeOH); δ_H 1.37 (4 H, m, CH_2CH_2), 1.43 (9 H, s, Boc), 1.87 (2 H, m, CH_2), 2.95 (2 H, m, CH_2NH), 4.38 (1 H, q, 5-H), 4.97 (1 H, s, 3-H), 4.98 (2 H, s, OCH_2), 7.20 (1 H, t, NH), 7.32 (5 H, m, Ph), and 12.26 (1 H, s, OH).

(5S)-4-*Hydroxy*-5-*indol*-3-*ylmethyl*-1-*t*-*butoxycarbonyl*-*aminopyrrol*-2(5H)-*one* (**4i**). This compound [85% yield from Boc-L-TryOH (**1i**)] had m.p. 96—98 °C (from diethyl ether), R_F 0.42 (solvent A); $[\alpha]_D^{20}$ +229° (*c* 1 in MeOH); δ_H 1.50 (9 H, s, Boc), 3.40 (2 H, m, CH_2), 4.60 (1 H, s, 3-H), 4.63 (1 H, q, 5-H), 6.89—7.50 (5 H, m, indole), 10.8 (1 H, s, NH), and 12.25 (1 H, s, OH) (Found: C, 66.05; H, 6.5; N, 8.7. $C_{18}H_{20}N_2O_4$ requires C, 65.84; H, 6.14; N, 8.53%).

(4S,5S)-4-*Hydroxy*-5-*isobutyl*-1-*t*-*butoxycarbonylpyrrolidin*-2-*one* (**5a**).—(i) *Hydride reduction.* A solution of the crude pyrrol-2(5*H*)-one (**4a**) dissolved in a mixture of dichloromethane (100 ml) and acetic acid (10 ml) was cooled in an ice-bath and stirred vigorously whilst being treated portionwise with sodium borohydride (1.4 g) during 1 h; the mixture was then maintained for a further 4 h at the same temperature. It was then poured into cold water and the organic layer was washed with water, dried (Na_2SO_4), and evaporated to dryness. Chromatography of the residue on silica gel with eluant D yielded the pyrrolidin-2-one (**5a**) [3.6 g, 70% from (**1a**)], m.p. 90—91 °C (from hexane); R_F 0.58 (eluant C); $[\alpha]_D^{20}$ +63° (*c* 1 in

MeOH); δ_H 0.90 (6 H, dd, Me), 1.45 (9 H, s, Boc), 1.70 (2 H, m, CH_2), 1.75 (1 H, m, CH), 2.45 (2 H, qd, 3-H), 4.30 (1 H, q, 4-H), and 5.20 (1 H, br, OH) (Found: C, 60.3; H, 9.2; N, 5.5. $C_{13}H_{23}NO_4$ requires C, 60.68; H, 9.01; N, 5.44%).

(ii) *Catalytic hydrogenation.* The crude product (**4a**) was dissolved in ethyl acetate (100 ml), and was hydrogenated in the presence of Adam's catalyst (400 mg) in a Parr reactor under 20 atm at room temperature for 18 h. The mixture was then filtered and the filtrate evaporated to dryness. Chromatography of the residue on silica gel with eluant D then yielded (**5a**) (55%).

The corresponding 5-alkyl-4-hydroxypyrrolidin-2-ones (**5b**—i) were prepared *via* method (i) as described for the synthesis of (**5a**).

(4S,5S)-1-*Benzyloxycarbonyl*-4-*hydroxy*-5-*methylpyrrolidin*-2-*one* (**5b**). The compound was obtained as oil from (**1b**) (58%); R_F 0.36 (eluant C); $[\alpha]_D^{20}$ +50° (*c* 1 in MeOH), δ_H 1.2 (3 H, d, Me), 2.54 (2 H, qd, 3-H), 4.15 (1 H, m, $J_{4,5}$ 6.6 Hz, 5-H), 4.82 (1 H, q, 4-H), 5.21 (2 H, q, Me), 5.35 (1 H, br, OH), and 7.4 (5 H, m, Ph).

(4S,5S)-4-*Hydroxy*-5-*isopropyl*-1-*t*-*butoxycarbonylpyrrolidin*-2-*one* (**5c**). This was prepared from Boc-L-ValOH (**3c**) (57%), m.p. 99—101 °C; R_F 0.55 (eluant C); $[\alpha]_D^{20}$ +60° (*c* 1 in MeOH); δ_H 0.95 (6 H, dd, Me), 1.43 (9 H, s, Boc), 2.22 (1 H, m, CH), 2.43 (2 H, qd, 3-H), 3.90 (1 H, q, 5-H), 4.46 (1 H, q, 4-H), and 5.35 (1 H, br, OH) (Found: C, 59.0; H, 8.65; N, 5.8. $C_{12}H_{21}NO_4$ requires C, 59.21; H, 8.70; N, 5.76%).

(4S,5S)-5-*Benzyl*-4-*hydroxy*-1-*t*-*butoxycarbonylpyrrolidin*-2-*one* (**5d**). This was synthesized from (**3d**) (60%), m.p. 120—124 °C (from diethyl ether); R_F 0.54 (eluant C), $[\alpha]_D^{20}$ +43° (*c* 1 in MeOH); δ_H 1.35 (9 H, s, Boc), 2.37 (2 H, qd, 3-H), 3.0 (2 H, qd, CH_2Ph), 4.25 (1 H, m, 5 H), 4.30 (1 H, m, 4-H), 5.5 (1 H, d, OH), and 7.25 (5 H, m, Ph) (Found: C, 66.0; H, 7.2; N, 4.85. $C_{16}H_{21}NO_4$ requires C, 65.96; H, 7.27; N, 4.81%).

(4R,5R)-5-*Benzyl*-4-*hydroxy*-1-*t*-*butoxycarbonylpyrrolidin*-2-*one* (**5e**). This had m.p. 119—120 °C (from diethyl ether); $[\alpha]_D^{20}$ −43.5° (*c* 1 in MeOH) (Found: C, 65.95; H, 6.25; N, 4.8. $C_{16}H_{21}NO_4$ requires C, 65.96; H, 7.27; N, 4.81%).

(4S,5S)-5-*Benzyloxymethyl*-4-*hydroxy*-1-*t*-*butoxycarbonyl*-*pyrrolidin*-2-*one* (**5f**). The compound was obtained as white crystals [78% yield from Boc-L-Ser(OBz1)OH (**1f**)], m.p., 100—101 °C (from acetone–hexane); R_F 0.52 (eluant C); $[\alpha]_D^{20}$ +59° (*c* 1 in MeOH); δ_H 1.40 (9 H, s, Boc), 2.50 (2 H, qd, 3-H), 3.77 (2 H, qd, CH_2O), 4.10 (1 H, m, 5-H), 4.45 (1 H, m, 4-H), 4.48 (2 H, q, CH_2Ph), 7.30 (5 H, m, Ph) (Found: C, 62.2; H, 7.1; N, 4.9. $C_{17}H_{23}NO_5$ requires C, 63.54; H, 7.21; N, 4.36%).

(4S,5S)-4-*Hydroxy*-5-(2-*methylthioethyl*)-1-*t*-*butoxy*-*carbonylpyrrolidin*-2-*one* (**5g**). The compound was obtained as an oil [68% yield from Boc-L-MetOH (**1g**)]; R_F 0.51 (eluant C); δ_H 1.44 (9 H, s, Boc), 2.0 (2 H, m, CH_2), 2.05 (3 H, s, Me), 2.48 (2 H, qd, 3-H), 2.51 (2 H, m, CH_2S), 4.04 (1 H, m, 5-H), 4.33 (1 H, m, 4-H), and 5.38 (1 H, d, OH).

(4S,5S)-5-(4-*Benzyloxycarbonylaminobutyl*)-4-*hydroxy*-1-*t*-*butoxycarbonylpyrrolidin*-2-*one* (**5h**). The compound was obtained as oil [60% yield from (**1h**)], R_F 0.31 (eluant C); $[\alpha]_D^{20}$ +26° (*c* 1 in MeOH); δ_H 1.33 (4 H, m, CH_2CH_2), 1.43 (9 H, s, Boc), 1.66 (2 H, m, CH_2), 2.46 (2 H, qd, 3-H), 3.0 (2 H, m, CH_2NH), 3.92 (1 H, m, 5-H), 4.31 (1 H, m, 4-H), 5.0 (2 H, s, OCH_2), 5.30 (1 H, d, OH), 7.20 (1 H, t, NH), and 7.3 (5 H, m, Ph).

(4S,5S)-4-*Hydroxy*-5-*indol*-3-*ylmethyl*-1-*t*-*butoxycarbonyl*-*pyrrolidin*-2-*one* (**5i**). The compound was obtained as oil [70% yield from Boc-L-TryOH (**1i**)]; R_F 0.38 (eluant C); $[\alpha]_D^{20}$ +7° (*c* 1 in MeOH); δ_H 1.35 (9 H, s, Boc), 2.40 (2 H, qd, 3-H), 3.15 (2 H, qd, CH_2), 4.30 (1 H, m, 4-H), 4.30 (1 H, m, 5-H), 5.49 (1 H, d, OH), 6.98—7.60 (5 H, m, indole), and 7.57 (1 H, s, NH) (Found: C, 65.35; H, 6.75; N, 8.25. $C_{18}H_{22}N_2O_4$ requires C, 65.44; H, 6.71; N, 8.48%).

(3S,4S)-3-*Hydroxy*-6-*methyl*-4-*t*-*butoxycarbonylamino*-*heptanoic Acid* (**6a**). A solution of the pyrrolidin-2-one (**5a**) (3.6 g) in acetone (15 ml) was treated dropwise with NaOH (1M; 4 ml) at room temperature during 2 min. The hydrolysis was complete within 5 min (t.l.c. eluant D) when the solution was carefully acidified (pH 3—4) with dilute HCl and extracted with ethyl acetate (200 ml). The extract was washed with water, dried, and evaporated to dryness to yield (**6a**).

(3S,4S)-4-*Benzyloxycarbonylamino*-3-*hydroxypentanoic acid* (**6b**). A solution of the pyrrolidin-2-one (**5b**) (1.25 g) in dioxane (20 ml) and aqueous HCl (1M; 4 ml) was heated to reflux for 2 h and then cooled. Ethyl acetate (100 ml) was added to the solution and the separated organic layer was extracted with 5% aqueous sodium hydrogen carbonate. The extract was acidified to pH 3—4 with solid citric acid and the mixture was extracted with ethyl acetate. The extract was then washed with water and brine and evaporated to dryness to yield the acid (**6b**) as a colourless amorphous solid (1.1 g, 83%).

(3S,4S)-*Methyl* 3-*Hydroxy*-6-*methyl*-4-*t*-*butoxycarbonyl*-*aminoheptanoate* (**7a**). Sodium methoxide (2M; 0.6 ml) was slowly added to a solution of the pyrrolidin-2-one (**5a**) (280 mg) in methanol (5 ml) at room temperature. After 10 min the solution was concentrated and the sticky solid residue was treated with 5% aqueous potassium hydrogen sulphate (50 ml); the resulting mixture was then extracted with ethyl acetate and worked up as usual. Pure methyl ester (**7a**) (260 mg, 90%), m.p. 57—58 °C (from hexane) was obtained by flash chromatography on silica gel with ether.

The other statine methyl ester analogues [except (**7f**); Table] were synthesized by the same procedure.

(3S,4S)-*Methyl* 4-*Benzyloxycarbonylamino*-3-*hydroxypent*-*anoate* (**7b**).—A solution of the pyrrolidin-2-one (**5b**) (500 mg) in methanol (5 ml) and methanolic hydrochloric acid (2M; 1.5 ml) was heated to reflux for 20 min. The solvent was removed under reduced pressure and the residue after repeated evaporations in the presence of methanol, was chromatographed on silica gel with diethyl ether to give the ester (**7b**) (525 mg, 93%), m.p. 90—92 °C (from hexane–ether, 4:1).

(3aS,6aS)-4-*t*-*Butoxycarbonyl*-cis-*dihydrofuro*[3,2-b]*pyrrole*-2,5-*dione* (**12**).—The chemical reduction of the (4S,5S)-5-benzyloxycarbonylmethyl-4-hydroxy-1-t-butoxycarbonylpyrrolidin-2-one, obtained from Boc-Asp(OBz)OH as described for the compound (**4a**), gave two products. Although the more polar compound (R_F 0.39, eluant D) evolved spontaneously with time to the less polar one (R_F 0.43), the conversion was more achieved by heating in dichloromethane. Column chromatography on silica gel (eluant D) gave the cyclic lactone (**11**) (1.3 g, 50%), m.p. 163—164 °C (from acetone–ether): $[\alpha]_D^{20}$ +61.7° (*c* 1 in DMF); δ_H 1.35 (9 H, s, Boc), 2.80 (2 H, qd, 3-H), 2.90 (2 H, qd, CH_2CO), 4.78 (1 H, m, 5-H), and 5.06 (1 H, m, $J_{4,5}$ 6.2 Hz, 4-H); (Found: C, 54.85; H, 6.3; N, 5.85. $C_{11}H_{15}NO_5$ requires C, 54.77; H, 6.27; N, 5.81%); the more polar compound, separated by chromatography, but not isolated in a solid form, was identified as the expected (4*S*,5*S*)-5-benzyloxycarbonylmethyl-4-hydroxy-1-t-butoxycarbonylpyrrolidin-2-one from its ^1H n.m.r. spectra; δ_H 1.42 (9 H, s, Boc), 2.55 (2 H, qd, 3-H), 2.76 (2 H, qd, CH_2CO), 4.41 (1 H, m, 5-H), 4.47 (1 H, m, 4-H), 5.55 (1 H, d, OH), 5.10 (2 H, q, CH_2Ph), and 7.40 (5 H, m, Ph).

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