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DERIVATIVES OF 1-TOSYL-3-AMINO-2-PIPERIDONE: PREPARATION AND CONVERSION TO DERIVATIVES AND PEPTIDES OF ORNITHINE[1] *

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Abstract—The conversion of ditosylornithine (L-Ib) and N^{α}-benzyloxycarbonyl-N^{δ}-tosyl-ornithine (L-Ic and DL-Ic) to 1-tosyl-3-tosylamino-2-piperidone (L-IIb) and 1-tosyl-3-benzyloxycarbonylamino-2-piperidone (L-IIc and DL-IIc) by several methods is described. The utilization of these lactams for the synthesis of ornithine peptides is illustrated. Racemic and optically active 1-tosyl-3-aminopiperidone (IIa), prepared by selective liberation of the 3-amino function of IIc, was acylated with benzyloxycarbonylglycine and benzyloxycarbonyl-L-valine to yield Va and Vb, respectively. Removal of the benzyloxycarbonyl group followed by exposure to basic conditions converted Vb to the 2,5-piperazinedione derivative (VI). Aminolysis of the optically active 2-piperidone IIc or treatment of N^{α}-benzyloxycarbonyl-N^{δ}-tosyl-L-ornithine pentachlorophenyl ester (IVb) with ammonia afforded the amide IIIb which showed no evidence of optical activity when investigated spectrophotometrically. However, removal of either the benzyloxycarbonyl group or of all the protecting groups revealed that the amide IIIb had retained full chirality. Hydrazine and glycine ethyl ester also caused ring opening of the 1-tosyl-2-piperidone derivatives IIb and IIc. The resulting amino acid and peptide derivatives IIIa, b, VIIa, b and VIIIa–c, possessing blocking groups of differentiated reactivity, are suitable intermediates for further synthesis. The mode of detosylation of ditosylornithine with hydrogen bromide in acetic acid is compared with that of the lower homologue, ditosyl-α,γ-diaminobutyric acid.

AMINO acid derivatives with a tosylamino group in γ-position to a carboxyl group (tosylglutamic acid,[3] γ-tosylaminobutyric acid,[4] N^{γ}-tosyl-α,γ-diaminobutyric acid[4, 5]) readily cyclize to derivatives of N-tosylpyrrolidone when the carboxyl group is activated, e.g. as for peptide synthesis. Tosyl-α-aminoadipic acid similarly forms a 6-membered N-tosyllactam, although somewhat less readily.[6] The relatively reactive tosyllactams can serve as intermediates for the synthesis of esters and of amides, including peptides.[3–6] In the work to be reported here we have examined the con-

* Studies along the lines described in this paper were initiated independently at the Institute of Organic Chemistry and Biochemistry, Prague (cf.[2]), and at the Medical Research Center of the Brookhaven National Laboratory and the Department of Physiology of the Mount Sinai Medical and Graduate Schools of The City University of New York. Since the results were largely complementary joint publication was agreed upon.

version of derivatives of N^{δ}-tosylornithine (I) to derivatives of 3-amino-1-tosyl-2-piperidone (II) and the potentialities of the latter for the synthesis of derivatives and peptides of ornithine.

N^{α},N^{δ}-Ditosyl-L-ornithine (L-Ib), prepared by tosylation of the N^{α}-monotosyl derivative,[7] was cyclized to L-IIb* with thionyl chloride or preferably with α,α-dichlorodimethyl ether,[8,9] under reflux. These procedures are too drastic for use with N^{α}-benzyloxycarbonyl derivatives but N^{α}-benzyloxycarbonyl-N^{δ}-tosyl-L-ornithine[10] (L-Ic) could be converted to the lactam L-IIc by treatment with dicyclohexylcarbodiimide,[11] or, more suitably, by the mixed anhydride method[12–14] with isobutyl chloroformate[14] in the presence of base. Similarly, DL-Ic was cyclized to DL-IIc with 2-butyl chloroformate.[14] The IR spectra (taken in KBr) of the tosyllactam derivatives (Table 1) show generally a characteristic displacement, by about $+30\,cm^{-1}$ or more, of the lactam carbonyl stretching frequency as against piperidones unsubstituted on the nitrogen (e.g.[15,16]); a similar displacement has already been

TABLE 1. SOME IR BANDS IN THE SPECTRA OF PIPERIDONES

Compound	ν(C=O), cm^{-1}			NH stretch, cm^{-1} (position 3)
	amide I	urethane	Lactam	
L-IIa			1720	3300, 3700
L-IIa·HBr			1690	3220
DL-IIa·HBr·H_2O			1695	[a]
L-IIb			1725	3275
L-IIc		1695	1720, 1730	3350
DL-Va	1645	1695	1710	3360
L-Vb	1650	1695	1735	3300
3-tosylamino-2-piperidone			1658[b]	
2-piperidone			1652[c]	

[a] No distinct peak. [b] lit.[15] [c] lit.[16]

noted for acyclic N-tosylamides and for N-tosylpyrrolidones when the spectra were recorded in dioxan.[17]

An attempt to prepare the *p*-nitrophenyl ester L-IVa from the acid L-Ib and *p*-nitrophenol with dicyclohexylcarbodiimide once more led to the lactam L-IIc as the only isolated product. Bodanszky[18] has reported the successful preparation of L-IVa by essentially the same procedure; the difference in results is presumably due to the shorter reaction time used by the latter authors.[19] It has been noted[20] that the pentachlorophenyl ester[21] of N^{α}-benzyloxycarbonyl-N^{ω}-nitro-arginine shows less tendency to cyclize than the corresponding *p*-nitrophenyl ester. The pentachlorophenyl ester L-IVb was accordingly prepared from L-Ic and pentachlorophenol with dicyclohexylcarbodiimide and proved a stable, convenient intermediate.

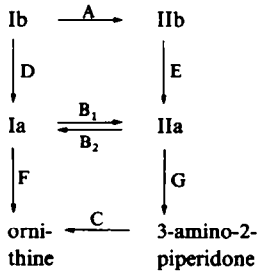
* The L, configurational designation is used in this paper for cyclic derivatives (2-piperidones, 2,5-piperazinediones) because of their obvious genetic relationship to the amino acids.

† The shift of the absorption to lower frequencies of the carbonyl group on passing from the amine to its salt cannot be due to the inductive effect of the ammonium group since this would act in the opposite direction (e.g.[22]). However, it may be ascribed to the increased strength of the C=O...H—N^{+} hydrogen bond.[23]

Removal of the benzyloxycarbonyl group from the 3-amino substituent of the tosyllactams L- and DL-IIc with hydrogen bromide in acetic acid afforded the 3-amino-1-tosyl-2-piperidones (L- and DL-IIa) as the hydrobromides. The racemic compound IIa crystallized as the monohydrate, which may account for the observed differences in the IR spectra (Table 1).† The hydrobromide of L-IIa was converted with an anion exchange resin to the free base which was obtained crystalline.

The lower homologue of IIa, i.e. 3-amino-1-tosyl-2-pyrrolidone, is obtained directly from ditosyl-α,γ-diaminobutyric acid on treatment with hydrogen bromide in acetic acid[5] by a reaction sequence involving cyclization and selective reduction of the tosyl group attached to the amino, as against amide, nitrogen. Under the same conditions ditosyl-L-ornithine (L-Ib) afforded a mixture of products identified by chromatography and electrophoresis as ornithine, N^{γ}-tosylornithine (Ia), 3-amino-1-tosyl-2-piperidone (IIa), and 3-amino-2-piperidone. In an effort to define the reactions leading to the various products (Scheme 1) we subjected these four compounds as well as 3-tosylamino-1-tosyl-2-piperidone (L-IIb) to the same conditions. The semiquantitative composition of the resulting reaction mixtures is given

SCHEME 1



in Table 2. Experiment 6 demonstrates that ornithine is not cyclized to the aminopiperidone under the conditions used and experiment 5 that the reverse reaction (C) is slow. Hence the aminopiperidone must arise by reaction G but since it did not accumulate in experiments 3 or 4 this reaction must also be slow; the bulk of the ornithine is evidently formed by reaction F. The results of experiments 3 and 4 establish the existence of the equilibrium B; the accumulation of tosylornithine (Ia) in experiment 3 indicates that reaction B_2 is comparable in rate with reaction

TABLE 2. REACTIONS OF ORNITHINE DERIVATIVES WITH HBr/AcOH

Experiment No.	Starting material	Products in reaction mixture			
		IIa	Ia	3-amino-2-piperidone	ornithine
1	IIb	++++	+++	++	++++
2	Ib	+++	+++	+	+++++
3	IIa	+++++	+++	++	+++
4	Ia	+++	+++	+	+++++
5	3-amino-2-piperidone	–	–	++++	++
6	ornithine	–	–	–	+++++

F, or faster, but the distinct if minor differences in the intensity patterns from experiments 3 and 4 show that the time required to establish equilibrium B is not negligible in comparison with the rate of reaction F (and possibly G). No direct evidence for the existence of equilibrium A can be derived from these experiments since only ninhydrin-positive compounds were detected; however, the difference in patterns between experiments 1 and 2 again shows that the rate at which any such equilibrium might be established cannot be very much greater than the rates of reactions D and E. It may be concluded that the difference in behaviour between the ditosyl derivatives of diaminobutyric acid and ornithine is not due to lesser stability of the tosyl group on the 6-membered as against the 5-membered ring toward the hydrogen bromide reagent but rather to a difference in the position of the equilibria between the N^{ω}-tosylated diamino acids and the corresponding N-tosyllactams (or, less probably, to differences in the rates at which these equilibria are established).

The racemic 3-amino-1-tosyl-2-piperidone (DL-IIa) was acylated with benzyloxycarbonylglycine using tetraethyl pyrophospite as the condensing agent[24] to give DL-Va; L-IIa was similarly acylated with benzyloxycarbonyl-L-valine by the mixed carbonic anhydride or dicyclohexylcarbodiimide procedures to afford L-Vb. Removal of the benzyloxycarbonyl group from L-Vb with hydrogen bromide and exposure of the product to an anion exchange resin gave a product identified as L-3-(2-propyl)-L-6-(3-tosylaminopropyl)-2,5-piperazinedione (L-VI), formed evidently by intramolecular aminolysis of the intermediate 1-tosyl-3-valylamino-2-piperidone (compare the analogous reaction of 3-glycylamino-1-tosyl-2-pyrrolidone).[25]

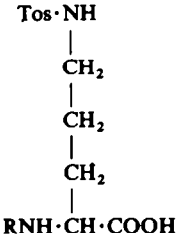
Ammonolysis of the lactam grouping of L-IIc afforded an amide IIIb which had zero optical rotation in three solvents in the visible region and showed no evidence of optical activity in methanol when the ORD and CD were examined in the wavelength range 350–210 mμ. The amide prepared from the pentachlorophenyl ester L-IVb and ammonia showed identical properties. However, removal of the benzyloxycarbonyl group from the amide IIIb prepared by either route afforded optically active N^{δ}-tosylornithine amide (IIIa) as the hydrobromide and simultaneous removal of all the blocking groups with aqueous hydrogen bromide gave L-ornithine with full optical activity. These conversions prove that the intermediate L-IIIb retains full chirality, its truly remarkable lack of optical activity notwithstanding. Its chiral properties do appear to affect its crystal structure since the racemic amide DL-IIIb prepared by ammonolysis of DL-IIc has a m.p. some 15° lower than L-IIIb.

On treatment with hydrazine the tosyllactams DL-IIc and DL-Va readily gave the hydrazides, DL-VIIa and DL-VIIb, respectively. Aminolysis of the lactam grouping in the ditosyl derivative L-IIb and the benzyloxycarbonyl derivatives L-IIc and DL-IIc with glycine ethyl ester in a small volume of nitromethane, or without solvent, afforded the corresponding derivatives of ornithylglycine (L-VIIIa and L- and DL-VIIIb) in good yield; similarly, the dipeptide lactam DL-Va gave the protected tripeptide DL-VIIIc. The ethyl ester of N^{α}-benzyloxycarbonyl-N^{δ}-tosyl-L-ornithylglycine (L-VIIIb) was also prepared by an alternate synthesis from the pentachlorophenyl ester IVb, while both the corresponding racemic DL-VIIIb and the tripeptide derivative DL-VIIIc were synthesized from the appropriate hydrazides by the azide method. In each case the products obtained by alternative routes showed identical properties.*

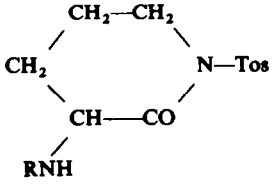
* L-VIIIa prepared by either route was dimorphous; the higher-melting form had the properties reported.[26]

The presence of three blocking groups of differentiated reactivity in the peptide derivatives VIIIb and VIIIc offers a number of possibilities for further synthesis. However, removal of the benzyloxycarbonyl, tosyl, and ethyl ester groups from the protected dipeptide DL-VIIIb was also accomplished in a single operation with hydrogen bromide in acetic acid. Analogously the tosyl and the ethyl ester groups were removed from L-VIIIa. Both DL- and L-ornithylglycine were isolated as the monohydrobromides in moderate yield.

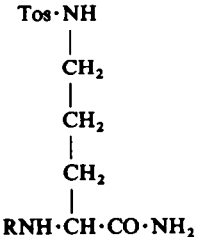
An electrophoretic analysis of the reaction mixture resulting from the treatment of VIIIa and b with hydrogen bromide after varying time intervals showed the expected order of reactivity of the protecting groups: the benzyloxycarbonyl group was cleaved rapidly, acidolysis of the ester grouping proceeded more slowly and reductive cleavage of the tosyl group required much longer reaction times. In the case of the ditosyl derivative L-VIIIa it was found that the N^{α}-tosyl group was eliminated more rapidly than the N^{δ}-tosyl group; appreciable amounts of N^{δ}-tosylornithylglycine were still present after 5 days' reaction at 40° (Table 3).



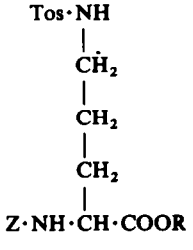
Ia: R = H
Ib: R = Tos
Ic: R = Z



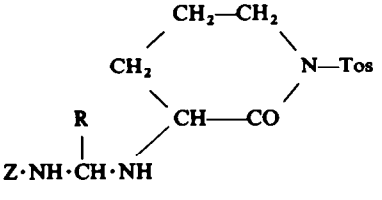
IIa: R = H
IIb: Tos
IIc: R = Z



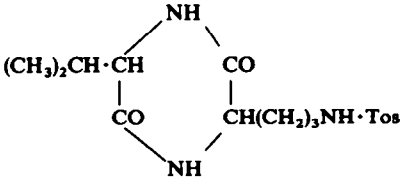
IIIa: R = H
IIIb: R = Z



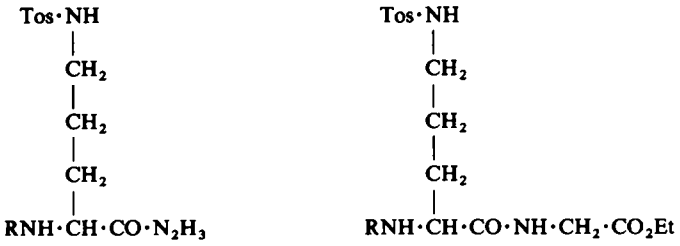
IVa: R = $C_6H_4NO_2$-*p*
IVb: R = C_6Cl_5



Va: R = H
Vb: R = $CH(CH_3)_2$



VI



VIIa: R = Z
VIIb: R = Z·NH·CH_2·CO

VIIIa: R = Tos
VIIIb: R = Z
VIIIc: R = Z·NH·CH_2·CO

TABLE 3. REMOVAL OF PROTECTING GROUPS FROM DERIVATIVES OF ORNITHYLGLYCINE WITH HBr/AcOH

Time hr	Products in reaction mixture from VIIIa at 90°					
	Orn(Tos)-Gly-OEt[a,b]	Orn(Tos-)-Gly[a,c]	Orn-Gly-OEt[d]	Orn	Gly	Orn-Gly
4	+++	++	±	−	−	±
8	++	++	+	−	−	+
24	+	+++	±	−	−	++
72	−	+++	−	−	−	++++
120	−	++	−	±	±	+++++
	Products in reaction mixture from VIIIb at 20°					
¼	+++++	+	±	−	−	±

[a] Not isolated; identity inferred from electrophoretic behaviour and formation from VIIIb.
[b] m 0·68 (E_1), 0·70 (E_2), 0·75 (E_3, E_4).
[c] m 0·70 (E_1), 0·43 (E_3), 0·46 (E_4).
[d] Not isolated; identity inferred from electrophoretic behaviour; m 1·2 (E_1, E_3, E_4).

EXPERIMENTAL

M.ps were determined in capillaries and corrected, unless otherwise stated. Optical rotations were determined with a Carl Zeiss photoelectric polarimeter 0·005°. IR spectra were recorded from pressed discs of KBr at 0·2–0·3% concentrations on a Perkin–Elmer Model 337 double-beam grating spectrophotometer. Elemental analyses were carried out at the Analytical Laboratory of the Prague Institute or by Galbraith Laboratories, Knoxville, Tennessee; mol wt determinations were carried out by Galbraith Laboratories.

Chromatography and electrophoresis. Paper chromatograms were run on Whatman No. 1 paper with descending solvent at 24° in the solvent systems 1-butanol–AcOH–H_2O, 4:1:1 (S_1), MeOH–cyclohexane, 4:1 (S_2), and MeOH–heptane, 1:1 (lower phase) (S_3) (all proportions by volume). TLC were run on silica gel (Kieselgel G, Merck). Paper electrophoresis was carried out in a moist-chamber apparatus at a potential gradient of about 20 V/cm on Whatman No. 3 paper during 30–60 min. The electrolytes were 1N AcOH (E_1), 5N AcOH (E_2), pyridine–AcOH buffer, pH 5·7 (E_3) and pyridine–HCOOH buffer, pH 4·4 (E_4). Ninhydrin-negative compounds were detected by the procedure of Zahn and Rexroth.[27] Electrophoretic mobilities (m) are given as ratios to the mobility of ornithine.

N^{δ}-*Tosyl*-L-*ornithine* (L-Ia)

L-Ornithine monohydrochloride (Nutritional Biochemicals Corporation, Cleveland, Ohio; $[\alpha]_D^{24}$ +

23·5°, 5·2% in 3N HCl) was converted to the N^{δ}-tosyl derivative by the procedure of Erlanger *et al.*[28] in 55% yield; m.p. 236–238° (dec), R_f 0·62 (S_1), m 0·43 (E_1), 0·55 (E_2), 0·44 (E_4), $[\alpha]_D^{24}$ + 21·6° (2% in 6N-HCl).*

N^{δ}-*Tosyl*-DL-*ornithine* (DL-Ia)

DL-Ornithine monohydrochloride (8·43 g) in water (30 ml) was heated with $CuCO_3$ (5·8 g) during 20 min and the cooled filtered soln was shaken for 45 min with TosCl (6·4 g) in ether (15 ml) and with 2N NaOH (31·5 ml) at room temp. The same amounts of sulphonyl chloride in ether and of 2N NaOH were then added, the mixture was shaken for an additional 2 hr, treated with 6N HCl and shaken until all the solid had dissolved. The aqueous layer was washed with more ether, treated with Na_2EDTA (19·0 g) and neutralized with 2N NaOH. After standing at 0° for 16 hr the product was collected, washed with water, and recrystallized from water; yield 10·5 g (73%), m.p. 223–229° (dec) [lit.[30] m.p. 229–232° (dec)], chromatographically homogeneous on TLC (S_1). (Found: C, 50·4; H, 6·3; N, 10·1; Calc. for $C_{12}H_{18}N_2O_4S$: C, 50·3; H, 6·3; N, 9·8%).

N^{α},N^{δ}-*Ditosyl*-L-*ornithine* (L-Ib)

N^{α}-Tosyl-L-ornithine[7] (5·72 g) in 2N NaOH (20 ml) was agitated with TosCl (5·0 g) in ether (33 ml) at room temp for 30 min and, after addition of more 2N NaOH (33 ml), for another 90 min. The aqueous layer was washed with ether and acidified; L-Ib separated as an oil which rapidly crystallized. The crystals were triturated with dil HCl, washed thoroughly with water, and dried; yield 7·0 g (80%), m.p. 186–187°. A sample for analysis was recrystallized from EtOH aq; m.p. 186·5–187·5°, $[\alpha]_D^{23}$ + 25·5° (c 1, in 95% EtOH). (Found: C, 51·7; H, 5·5; N, 6·6. $C_{19}H_{24}N_2O_6S_2$ requires: C, 51·8; H, 5·5; N, 6·4%).

N^{α}-*Benzyloxycarbonyl*-N^{δ}-*tosyl*-DL-*ornithine* (DL-Ic)

DL-Ia (6·95 g) in 2N NaOH (12 ml) was treated at 0° with stirring during 1½ hr with benzyl chloroformate (4·95 g) in ether (40 ml) and with more 2N NaOH (30·5 ml). The mixture was stirred at room temp for an additional 1½ hr, the aqueous layer was washed with ether, filtered (charcoal), and freed of ether with a stream of air. Acidification precipitated an oil which crystallized when agitated with the mother liquors during several hrs; yield 7·35 g, m.p. 133–136°. Recrystallization from EtOAc–ligroin afforded 6·10 g (60%) of DL-Ic, m.p. 138–139·5°. (Found: C, 57·1; H, 5·7; N, 6·8. $C_{20}H_{24}N_2O_6S$ requires: C, 57·1; H, 5·8; N, 6·9%).

1-*Tosyl*-L-3-*tosylamino*-2-*piperidone* (L-IIb)

Method A. L-Ib (0·88 g) was refluxed with $SOCl_2$ (3 ml) for 30 min, the soln was taken to dryness under reduced press and the residue freed from $SOCl_2$ by dissolution in benzene and evaporation. Crystallization from EtOH (2 ml) afforded 0·57 g (67%) of L-IIb, m.p. 144–145°; $[\alpha]_D^{23}$ −52·2° (0·2% in 98% AcOH). A sample for analysis was recrystallized from EtOH. (Found: C, 54·2; H, 5·3; N, 6·6. $C_{19}H_{22}N_2O_5S_2$ requires: C, 54·0; H, 5·2; N, 6·6%).

Method B. L-Ib (0·50 g) was refluxed with α,α-dichlorodimethyl ether (3 ml) until a clear soln was obtained and for 10 min more. Subsequently, volatile substances were evaporated under reduced press, finally at 80°, and the residue was crystallized by addition of a few drops of 2-propanol. The crude product was suspended in ether–ligroin, collected, and triturated successively with 5% $NaHCO_3$, H_2O, dil HCl, and H_2O; yield 0·31 g (75%), m.p. 143·5–145°. Recrystallization from EtOH gave 0·28 g, m.p. 144·5–145·5°, $[\alpha]_D^{25}$ − 57·1° (0·2% in 98% AcOH). (Found: C, 54·1; H, 5·3; N, 6·4%).

1-*Tosyl*-3-L-*benzyloxycarbonylamino*-2-*piperidone* (L-IIc)

Method A. L-Ic[10] (6·0 g) in EtOAc (55 ml) was treated, at 0°, with DCCI† (2·94 g) in EtOAc (10 ml). The reaction mixture was kept at 0° for 1–1½ hr more and at room temp for 2½ hr, the DCU† was filtered off, washed thoroughly with EtOAc, and the combined filtrate and washings were evaporated under reduced press. Recrystallization from EtOH afforded 3·0 g (52%) of L-IIc, m.p. 159–160°, $[\alpha]_D^{22}$ −68·6° (1% in DMFA† containing 1% AcOH). The same product, m.p. 160–161°, $[\alpha]_D^{25}$ −66·2°, R_f 0·93 (S_3), was obtained

* The literature records m.p. 210–215° (dec),[29] m.p. 236–238° (dec)[30], $[\alpha]_D^{20}$ +23·4° (1·85% in 3N HCl) m.p. 212° (dec) with $[\alpha]_D^{23}$ +20·8° (2% in 6N HCl),[28] m.p. 234–235·5° (dec) with $[\alpha]_D^{17}$ −20·0° (2·2% in 6N HCl) and m.p. 231–233° (dec) with $[\alpha]_D^{17}$ −20·3° (2·1% in 6N HCl).[31] The identity of optical rotations for the higher- and lower-melting forms of L-Ia makes it apparent that the compound is polymorphous.

† DCCI stands for dicyclohexylcarbodiimide, DCU for N,N'-dicyclohexylurea, DMFA for dimethylformamide.

when the reaction with DCCI was carried out under similar conditions (reaction time: 15 hr) in the presence of 1 mole of *p*-nitrophenol. (Found: C, 59·5; H, 5·5; N, 6·9; m.w.t., 405. $C_{20}H_{22}N_2O_5S$ requires: C, 59·7; H, 5·5; N, 7·0%; m.wt., 405).

Method B. L-Ic (6·7 g) in $CHCl_3$ (81 ml) containing NEt_3 (2·5 ml) was treated at 0° with isobutyl chloroformate (2·1 ml), the reaction mixture was allowed to come to room temp during 2½ hr under stirring, the solvent was evaporated under reduced press and the residue was recrystallized from EtOH; yield 5·4 g (84%), m.p. 159–160°, $[\alpha]_D^{25}$ −68·4° (2% in DMFA containing 1% AcOH).

1-*Tosyl*-3-DL-*benzyloxycarbonylamino*-2-*piperidone* (DL-IIc)

DL-Ic (16·6 g) in $CHCl_3$ (70 ml) containing N-ethylpiperidine (13·2 ml) was treated, at −15°, with 2-butyl chloroformate (5 ml), the reaction mixture was kept at −15° for 10 min, at room temp for 10 min more, warmed to 40° and diluted with ether (350 ml). After 12 hr at 5° the product was collected, washed with ether, H_2O, 5% $NaHCO_3$, H_2O, dil HCl, and H_2O and dried at 80°; yield 13·3 g (84%), m.p. 148–151°. A sample for analysis was recrystallized from EtOH; m.p. 151–152°. (Found: C, 59·6; H, 5·4; N, 6·9. $C_{20}H_{22}N_2O_5S$ requires: C, 59·7; H, 5·5; N, 7·0%).

N^{α}-*Benzyloxycarbonyl*-N^{δ}-*tosyl*-L-*ornithine pentachlorophenyl ester* (L-IVb)

L-Ic (5·0 g) and pentachlorophenol (3·48 g) in EtOAc (30 ml) were treated, at 0°, with DCCI (2·69 g). The reaction mixture, allowed to come to room temp during 3 hr, was treated with 1 drop of AcOH. After 15 min the DCU was filtered off, washed with EtOAc and the combined filtrate and washings were concentrated to crystallization; the crude product (7·5 g; m.p. 139–140°) was recrystallized from EtOAc to afford 6·8 g (85%) of pure L-IVb, m.p. 153–154° (unchanged by recrystallization from tetrahydrofuran-ether), $[\alpha]_D^{27}$ −4·35° (5% in $CHCl_3$). (Found: C, 47·0; H, 3·6; Cl, 26·0; N, 4·0. $C_{26}H_{23}Cl_5N_2O_6S$ requires: C, 47·2; H, 3·4; Cl, 26·2; N, 4·1%).

1-*Tosyl*-L-3-*amino*-2-*piperidone* (L-IIa)

L-IIc (5·0 g) was dissolved in 4N HBr/AcOH during 8 min with stirring, the soln was set aside for 30 min and diluted with ether (400 ml). The ppt was washed with more ether (3 × 400 ml) by decantation, collected, and recrystallized from MeOH-ether; yield 4·2 g (97%) of the hydrobromide, m.p. 233–235° (dec), $[\alpha]_D^{25}$ −33·4° (4% in MeOH). (Found: C, 41·2; H, 5·0; N, 7·81. $C_{12}H_{17}BrN_2O_3S$ requires: C, 41·3; H, 4·9; N, 8·02%). A soln of L-IIa·HBr (3·57 g) in MeOH (40 ml) was passed through a column of Rexyn RG 1(OH) anion exchange resin, the column was washed with MeOH (100 ml) and the combined eluates were evaporated under reduced press. The residue was dissolved in a small amount of EtOAc; dilution with ligroin induced crystallization of the L-IIa which was recrystallized from benzene; yield 2·76 g (99%), m.p. 105–106·5°, $[\alpha]_D^{26}$ +20·0° (4% in 95% EtOH), R_f 0·90 (S_2). (Found: C, 53·6; H, 6·2; N, 10·2. $C_{12}H_{16}N_2O_3S$ requires: C, 53·7; H, 6·0; N, 10·5%).

1-*Tosyl*-DL-3-*amino*-2-*piperidone hydrobromide* (DL-IIa·HBr)

DL-IIc (1·5 g) was treated with 4N HBr/AcOH (12 ml) and the product was isolated as described for the L-isomer above. Recrystallization from EtOH-ether afforded 1·18 g (86%) of the chromatographically (TLC, S_1) pure hydrobromide as the monohydrate, m.p. 215–217° (dec from 210°), m 0·72 (E_1), 0·73 (E_2), 0·86 (E_4). (Found: C, 39·1; H, 5·1; Br, 21·8; N, 7·8. $C_{12}H_{19}BrN_2O_4S$ requires: C, 39·2; H, 5·2; Br, 21·8; N, 7·6%).

DL-3-(*Benzyloxycarbonylglycylamino*)-1-*tosyl*-2-*piperidone* (DL-Va)

The hydrobromide of DL-IIc (1·10 g) was shaken with a saturated soln of NH_3 in $CHCl_3$ (15 ml) for 5 min at 5°, the suspension was filtered through a layer of charcoal and the filtrate was evaporated to dryness under reduced press. The residual oil was heated with benzyloxycarbonylglycine (0·69 g) and tetraethyl pyrophosphite (1·0 g) in diethyl phosphite (2·9 ml) at 100° for 45 min and the reaction mixture was poured into 4N HCl (50 ml). The oil which separated crystallized on shaking. The product was collected, washed with H_2O, triturated with 5% $NaHCO_3$, once more collected and washed with H_2O; recrystallization from dioxan-EtOH–H_2O afforded 1·12 g (81%) DL-Va, m.p. 162–163°. A sample for analysis was recrystallized from EtOH. (Found: C, 57·3; H, 5·5; N, 9·1; $C_{22}H_{25}N_3O_6S$ requires: C, 57·5; H, 5·5; N, 9·1%).

3-L-(*Benzyloxycarbonyl*-L-*valylamino*)-1-*tosyl*-2-*piperidone* (L-Vb)

Method A. Benzyloxycarbonyl-L-valine (2·0 g) in THF (15 ml) containing NEt_3 (1·1 ml) was treated, at

$-10°$, with isobutyl chloroformate (1·05 g). The mixture was stirred at $-10°$ for 10 min and L-IIa (2·3 g) in water (10 ml) containing NEt_3 (2 ml) was added; the stirred mixture was allowed to come to room temp during 1½ hr, the pH being maintained at 8. The solvents were removed under reduced press, the residue in EtOAc was washed with 5% $NaHCO_3$, H_2O, with 1N HCl until neutral, and again with H_2O. The organic layer was dried (Na_2SO_4), evaporated under reduced press and the residue was crystallized from EtOH; yield 2·2 g (76%), m.p. 144–145° (unchanged by recrystallization from EtOH–diisopropyl ether), $[\alpha]_D^{27}$ $-14·1°$ (1% in 95% EtOH), R_f 0·89 (S_2). (Found: C, 58·8; H, 6·5; N, 8·4. $C_{24}H_{31}N_3O_6S$ requires: C, 58·9; H, 6·7; N, 8·6%).

Method B. Benzyloxycarbonyl-L-valine (2·0 g) and L-IIa (2·13 g) in $CHCl_3$ (25 ml) were treated, at 0°, with DCCI (1·8 g). The mixture was stirred at room temp for 12 hr, treated with AcOH (2 drops) and after 15 min filtered from DCU. The filtrate was diluted with $CHCl_3$ (50 ml) and the product was isolated as in *Method* A; yield 3·69 g (95%), m.p. 144–146°, $[\alpha]_D^{27}$ $-12·8°$ (1% in 95% EtOH).

L-3-(2-*Propyl*)-L-6-(3-*tosylaminopropyl*)-2,5-*piperazinedione* (L-VI)

L-Vb (1·7 g) in AcOH (2 ml) was treated with 4N HBr/AcOH (6 ml) at room temp for 1 hr, the product was precipitated by dilution with ether (300 ml), collected, washed with more ether (600 ml) and dissolved in MeOH (30 ml). The soln was passed through a column of Rexyn RG1(OH), which was then washed with more MeOH. The combined eluates were evaporated to dryness and the residue was twice crystallized from EtOH; yield 1·15 g (90%), m.p. 233–235°, $[\alpha]_D^{24}$ $-39·6°$ (1% in DMFA), R_f 0·72 (S_2), ninhydrin-negative. (Found: C, 55·5; H, 6·8; N, 11·4. $C_{17}H_{25}N_3O_4S$ requires: C, 55·6; H, 6·9; N, 11·4%). The compound does not react with TosOH, with Ac_2O, or with benzaldehyde in pyridine at 80°. The IR spectrum shows bands at 1345 (*cis*-amide-III), 1675 (*cis*-amide-I), 3050, 3085 (N—H band "B"), and 3200 cm^{-1} (N—H band "A") regarded as characteristic of 2,5-piperazinediones[32–34] as well as a band at 3255 cm^{-1} (sulphonamide N—H), but lacks the absorption at about 1730 cm^{-1} associated with the lactam carbonyl group in 1-tosyl-2-piperidones (Table 1), the *trans*-amide-II band at 1550 cm^{-1} and the *trans*-amide N—H band at about 3300 cm^{-1} (cf.[32–34]).

N$^{\alpha}$-*Benzyloxycarbonyl*-N$^{\delta}$-*tosyl*-L-*ornithine amide* (L-IIIb)

Method A. Dry NH_3 was passed into a soln of L-IIc (1·3 g) in EtOH (200 ml) for 2 hr at 0°; the soln was set aside at room temp for 2 days and evaporated to dryness and the residue was crystallized from MeOH to yield 1·1 g (80%) of the amide, m.p. 149–150°, $[\alpha]_{546}^{25}$ and $[\alpha]_{578}^{25}$ 0° (5% in MeOH, DMFA, or 97% HCO_2H), R_f 0·72 (S_2). (Found: C, 57·1; H, 5·9; N, 10·2. $C_{20}H_{25}N_3O_5S$ requires: C, 57·3; H, 6·0; N, 10·0%). A 5% soln in MeOH showed zero rotation when examined spectropolarimetrically in the wavelength range 350–210 mμ (Jasco ORD/UV-5) and no detectable CD between 350–210 mμ (Jouan Dichrograph 185).

Method B. L-IVb (1·0 g) was added to an ice-cold sat soln of NH_3 in $CHCl_3$ (30 ml). After standing for 16 hr at room temp the mixture was filtered, the filtrate was evaporated to dryness and the residue was recrystallized from a small amount of MeOH at 4° (yield 0·7 g) and then twice from EtOH aq; yield 0·5 g (81%), m.p. 150–151°, $[\alpha]_D^{27}$ 0° (2% in MeOH), DMFA, or 97% HCO_2H).

Hydrolysis of L-IIIb. The amide L-IIIb prepared by *Method* A (419 mg) was refluxed with 59% HBr aq (5 ml), H_2O (1 ml), and phenol (100 mg) for 18 hr; the soln was diluted with water, extracted repeatedly with ether, and evaporated to dryness under reduced press. The residue was taken up in water, the soln was passed through a column of Dowex 50W × 4 (50–100 mesh), the column was washed with water and the ornithine was eluted with 0·5N HCl (250 ml). The eluates were evaporated to dryness, the residue was freed of excess HCl by repeated evaporation with water and crystallized twice from EtOH aq, the first time with the addition of pyridine. Yield 124 mg (74%) of ornithine monohydrochloride, $[\alpha]_D^{25}$ $+22·6°$ (0·5% in 5N HCl) [lit.* $[\alpha]_D$ $+22·5°$ (2% in 5N HCl)]. (Found: Cl, 20·8; N, 16·5; $C_5H_{13}ClN_2O_2$ requires: Cl, 21·0; N, 16·6%).

N$^{\alpha}$-*Benzyloxycarbonyl*-N$^{\delta}$-*tosyl*-DL-*ornithine amide* (DL-IIIb)

DL-IIc was converted to the amide as described for the L-isomer above; the product crystallized from MeOH aq and was recrystallized from the same solvent, m.p. 134–135·5°. (Found: C, 57·0; H, 5·8; N, 10·2; $C_{20}H_{25}N_3O_5S$ requires: C, 57·3; H, 6·0; N, 10·0%).

* Recalculated from the molar rotation for L-ornithine.[35]

N^{δ}-Tosyl-L-ornithine amide hydrobromide (L-IIIa)

L-IVb obtained by *Method* A (2·0 g) in AcOH (3 ml) was treated with 4N HBr/AcOH (5 ml) at room temp for 45 min. Separation of the product was completed by dilution with ether (100 ml), the hydrobromide was collected and recrystallized from MeOH–ether (1:1), yield 1·3 g (77%), m.p. 141–143°, $[\alpha]_D^{27}$ +14·3° (2% in MeOH). (Found: C, 39·1; H, 5·4; N, 11·3; $C_{12}H_{20}BrN_3O_3S$ requires: C, 39·4; H, 5·5; N, 11·5%). The protected amide prepared by *Method B* yielded an identical product, m.p. 140–142°, $[\alpha]_D^{27}$ +13·7° (2% in MeOH).

N^{α}-Benzyloxycarbonyl-N^{δ}-tosyl-DL-ornithine hydrazide (DL-VIIa)

DL-IIc (0·30 g) in MeOH (0·5 ml) and dioxan (0·5 ml) was set aside with 90% N_2H_4 (0·075 ml) at room temp for 3 hr; the mixture was evaporated to dryness under reduced press and the crystalline residue was triturated with a mixture of EtOH–ether and then collected; yield 0·32 g (99%), m.p. 145–146°. Recrystallization from EtOH–ether gave 0·27 g (84%) of DL-VIIa, m.p. 146°. (Found: C, 55·2; H, 6·0; N, 13·0; $C_{20}H_{26}N_4O_5S$ requires: C, 55·3; H, 6·0; N, 12·9%).

N^{α}-(Benzyloxycarbonylglycyl)-N^{δ}-tosyl-DL-ornithine hydrazide (DL-VIIb)

DL-Va (230 mg) in dioxan (1 ml) and 2-propanol (1 ml) was set aside with 40% $N_2H_4 \cdot H_2O$ (0·28 ml) at room temp for 24 hr. The mixture was diluted with 2-propanol (1 ml) and H_2O (4 ml) and seeded with crystals obtained in an earlier experiment. After 12 hr at 5° the product was collected and thoroughly washed with H_2O; yield 221 mg (92%), m.p. 156–158° (Kofler block). Recrystallization tended to lower the m.p. (Found: C, 53·9; H, 5·8; N, 14·2; $C_{22}H_{29}N_5O_6S$ requires: C, 53·8; H, 5·9; N, 14·2%).

Ditosyl-L-ornithylglycine ethyl ester (L-VIIIa)

L-IIb (2·93 g) was heated with glycine ethyl ester (2·17 g) and nitromethane (2·5 ml) in a stoppered flask at 97° for 2 hr and the cooled reaction mixture was diluted with 3N HCl. The crystalline product was triturated with 3N HCl, collected, washed with H_2O, $NaHCO_3$, and H_2O and recrystallized from EtOH aq to afford 3·34 g (91%) of L-VIIIa, m.p. 130–134°. A sample for analysis was recrystallized from EtOH aq; m.p. 133–134°. (Found: C, 52·7; H, 5·8; N, 8·2; $C_{23}H_{31}N_3O_7S_2$ requires: C, 52·6; H, 5·9; N, 8·0%).

N^{α}-Benzyloxycarbonyl-N^{δ}-tosyl-L-ornithylglycine ethyl ester (L-VIIIb)

Method A. L-IIc (3·0 g) was heated with glycine ethyl ester (1·2 g) at 90° for 2 hr. The cooled melt dissolved in $CHCl_3$ (30 ml) was washed with 1N HCl (2 × 30 ml), H_2O, 5% $NaHCO_3$, and H_2O, the soln was dried (Na_2SO_4) and evaporated to dryness. The residue (3·0 g) was repeatedly crystallized from EtOH to afford a product (2·2 g; 55%) m.p. 114–116°, $[\alpha]_D^{24}$ −1·71° (2% in 97% HCO_2H), $[\alpha]_D^{27}$ −5·5° (2% in 96% EtOH). The m.p. was raised to 133–134° on recrystallization from EtOH with seeding by the higher-melting form obtained by *Method* B. (lit.[26] m.p. 135–136°, $[\alpha]_D^{22}$ −6·5 ± 1° (2·1% in 96% EtOH).

Method B. Glycine ethyl ester hydrochloride (0·54 g) with NEt_3 (0·55 ml) in $CHCl_3$ (10 ml) was treated at 0° with L-IVb (2·0 g). The soln was set aside at room temp for 16 hr, evaporated to dryness and the residue, dissolved in EtOAc (50 ml), was washed with 1N HCl, H_2O, 5% $NaHCO_3$, and H_2O. The EtOAc soln was dried (Na_2SO_4), evaporated to dryness, and the residue was crystallized from EtOH to yield 1·3 g (87%) of L-VIIIb, m.p. 110–112°. A second crystallization raised the m.p. to 133–134° (yield 0·9 g); $[\alpha]_D^{27}$ −2·3° (2% in 97% HCO_2H), $[\alpha]_D^{27}$ −5·2° (2% in 96% EtOH).

N^{α}-Benzyloxycarbonyl-N^{δ}-tosyl-DL-ornithylglycine ethyl ester (DL-VIIIb)

Method A. DL-IIc (2·01 g) was heated with glycine ethyl ester (1·03 g) and nitromethane (2 ml) at 97° for 3½ hr in a stoppered flask, and the reaction mixture was poured into 3N HCl (40 ml). The oily product which separated crystallized after 2 hr' shaking; the crystalline material was triturated with more 3N HCl, collected, washed with H_2O, 5% $NaHCO_3$, and H_2O and recrystallized from EtOAc–ether; yield 2·26 g (89% on IIc), m.p. 100–102°, raised to 103–105° by further recrystallization from EtOH–ether. (Found: C, 57·3; H, 6·2; N, 8·1; $C_{24}H_{31}N_3O_7S$ requires: C, 57·0; H, 6·2; N, 8·3%).

Method B. A soln of DL-VIIb (0·60 g) in 60% AcOH aq (6 ml) and 0·86 ml 5N HCl under ether (6 ml) was stirred at −10° and treated with $NaNO_2$ (0·11 g) in H_2O (1 ml). The mixture was stirred at −10° for 5 min more, the ethereal layer was washed at the same temp with precooled 16·8% NaCl aq and briefly dried (Na_2SO_4). To this soln AcOH (0·2 ml), glycine ethyl ester (0·17 g), and dioxan (5 ml) were added and the mixture was set aside at 5° for 12 hr. The crystalline product was collected (0·20 g; m.p. 96–100°), the

filtrate was washed with H_2O and evaporated to dryness under reduced press. The residual oil crystallized from EtOH on addition of ether; yield 0·12 g, m.p. 96–100°. The combined crops were triturated with 5% $NaHCO_3$, collected, washed with H_2O, 1N HCl, and H_2O and dried; yield 0·32 g (45%), m.p. 101–103°, undepressed on admixture of a sample prepared by *Method* A. (Found: C, 57·2; H, 6·2; N, 8·2%).

N^{α}-(*Benzyloxycarbonylglycyl*)-N^{δ}-*tosyl*-DL-*ornithylglycine ethyl ester* (DL-VIIIc).

Method A. DL-VIIb (245 mg) in DMFA (4 ml) was treated, at −10°, with 2·4N HCl in THF (0·65 ml) and with butyl nitrite (110 mg). After being kept at −10° for 10 min the soln was neutralized with N-ethylpiperidine (pH 6·5 when spotted on moist indicator paper) and added to glycine ethyl ester (56 mg) in DMFA (4 ml). After standing at 5° for 12 hr the reaction mixture was poured into dil HCl; the oil which separated crystallized on being triturated with a small amount of acetone. After 12 hr at 5° the product was collected, washed with H_2O, 5% $NaHCO_3$, and H_2O and recrystallized twice from acetone–H_2O; yield 132 mg (47%), m.p. 143–145° (Kofler block), raised to 143–146° (Kofler block) by a further recrystallization from the same solvent. (Found: C, 55·4; H, 5·9; N, 9·8; $C_{26}H_{34}N_4O_8S$ requires: C, 55·5; H, 6·1; N, 10·0%).

Method B. DL-Va (2·03 g) was heated with glycine ethyl ester (0·91 g) and nitromethane (2 ml) in a stoppered flask at 100° for 2½ hr. The hot reaction mixture was diluted with 3N HCl until turbid and was shaken until the crystalline product separated. More 3N HCl was added and after 12 hr at 5° the product was collected, washed with H_2O, 5% $NaHCO_3$, and H_2O and recrystallized from acetone–H_2O to afford 2·07 g (83% on Va) of DL-VIIIc, m.p. 142–144°, undepressed on admixture of a sample prepared by *Method B*.

L-*Ornithylglycine hydrobromide*

L-VIIIa (525 mg) with phenol (500 mg) in 4N HBr/AcOH (5 ml) was kept in a stoppered flask at 90° for 5 days. The mixture was evaporated under reduced press, the syrupy residue was triturated with ether, the ether was decanted, the residual solid was washed twice more with ether by decantation, taken up in EtOH (2 ml) and treated with pyridine until no more ppt was formed. The suspension was diluted with 2-propanol (3 ml), the product was collected by centrifugation, washed with 2-propanol (2 × 3 ml) and dried *in vacuo* over NaOH. Electrophoresis (E_3) showed the presence of a neutral impurity (the mother liquors contained the basic dipetide and the byproduct in about equal amounts). The hydrobromide was taken up in a little H_2O, the soln was diluted with MeOH, filtered through a short column of charcoal and diluted with 2-propanol (about 30 ml); crystallization from the resulting emulsion was initiated by scratching. After standing at 5° for several days the product was collected and washed with 2-propanol; yield 66 mg (24%) of the monohydrate, m.p. 129–130° (unsharp; Kofler block), m 1·12 (E_1), 1·09 (E_2), 0·90 (E_3), 0·90 (E_4), $[\alpha]_D^{25}$ + 12·8° (0·36% in 5N HCl). (Found: C, 30·7; H, 6·3; Br, 29·9; N, 15·4; $C_7H_{16}BrN_3O_3$ requires: C, 31·1; H, 6·0; Br, 29·6; N, 15·5%).

DL-*Ornithylglycine hydrobromide*

DL-VIIIb (1·10 g) with phenol (0·2 g) in 4N HBr/AcOH (5·8 ml) was kept at 40° in a stoppered flask for 24 hr, with occasional shaking. The reaction mixture was worked up as in the preceding experiment; the crude product was dissolved in EtOH, treated with pyridine until no more precipitation occurred, the ppt was collected by centrifugation and dissolved in a minimum of H_2O. The soln was diluted with MeOH (3 ml), filtered through a small charcoal column, and further diluted with 2-propanol until turbid. After standing at 5° for several days the product crystallized; yield 0·133 g (22%), m.p. 212–213° (Kofler block; strongly dependent on the rate of heating). (Found: C, 31·4; H, 6·0; Br, 29·5; N, 15·3; $C_7H_{16}BrN_3O_3$ requires: C, 31·1; H, 6·0; Br, 29·6; N, 15·5%).

Reactions of orinithine derivatives with HBr/AcOH.

The appropriate derivative (0·1 mmole) was heated with about 4N HBr/AcOH (0·55 ml) and phenol (20 mg) in a stoppered vial at 70° for 2 or 3 hr. The mixture was taken to dryness under reduced press, the residue was washed 4 times with ether by decantation after centrifugation, dried *in vacuo* over NaOH for 12 hr, dissolved in H_2O (1 ml) and analysed by electrophoresis in electrolytes E_1 and E_4. The amounts of the products were judged visually from the ninhydrin colour and expressed on a scale of 1 to 5 crosses

(Table 2). The DL-3-amino-2-piperidone was prepared according to Fischer and Zemplén;[36] m 1·05 (E_1), 1·00 (E_2), 1·12 (E_4).

Removal of protecting groups from derivatives of ornithylglycine with HBr/AcOH.

The conditions were as above except that the reaction mixtures were kept at the temps and for the times indicated (Table 3) and the electrophoretic analyses were carried out in electrolytes E_2 and E_3.

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