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Synthesis of the Four Stereoisomeric Forms of α,β-Diaminobutyric Acid and Some Derivatives Suitable for Peptide Synthesis

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Dedicated to Professor Dr. Theodor Wieland on his 60th Birthday

Summary: A synthetic route to the four stereoisomers of α,β-diaminobutyric acid (α,β-A_2bu) has been developed. The L-*threo*, D-*threo*, L-*erythro* and D-*erythro* isomers were prepared from the corresponding threonine and *allo*-threonine isomers. *N*-Tosylation under Schotten-Baumann conditions was followed by esterification with diazomethane and *O*-tosylation in pyridine to give *N,O*-ditosylthreonine methyl ester (IV). Successive treatments with ammonia-saturated methanol and 6N HCl afforded, after several recrystallizations from water, N^{α}-tosyl-α,β-diaminobutyric acid (V) of the same configuration as the starting threonine along with racemic mixtures. Presumably, the sulfonamide moiety influenced the steric course of the reaction, causing a double inversion of the C^{β} asymmetry center *via* intermediate aziridine formation. Simultaneous α,β-elimination produced the accompanying racemic mixtures *via* 1-tosylaminocrotonic acid methyl ester. Removal of the tosyl group by the action of sodium in liquid ammonia gave α,β-diaminobutyric acid (VI). The configurations of the four isomers were established by NMR and ORD spectra and by conversion of N^{α}-tosyl-L-*threo*-α,β-diaminobutyric acid (L-*threo*-V) to *N*-tosyl-L-threonine (L-*threo*-II) through treatment with nitrous acid. The *threo* and *erythro* isomers of α,β-A_2bu (VI) and N^{α}-tosyl-α,β-A_2bu (V) were further characterized by their elution behavior on ion-exchange column chromatography (amino acid analyzer) and by their ninhydrin color constants (C_{HW}). The *erythro* isomers of α,β-A_2bu gave higher color constants (20.6) than the *threo* isomers (5.6 to 5.8). In the case of N^{α}-tosyl-α,β-A_2bu, however, the *threo* isomers produced higher ninhydrin color values (11.3–11.4) than the *erythro* isomers (1.7 to 1.8). The NMR spectra (in DCl) of N^{α}-tosyl-L-*threo*-α,β-A_2bu (L-*threo*-V) exhibited an H_{α}-H_{β} coupling constant (6.6 Hz) which is identical with that of the *erythro* isomers of the free amino acid (α,β-A_2bu). Several derivatives of L-*threo*-α,β-diaminobutyric acid (L-*threo*-VI) suitable for use in peptide synthesis were prepared, including N^{α}-Tos-N^{β}-Boc-α,β-A_2bu, N^{β}-Boc-α,β-A_2bu, N^{α}-Z-N^{β}-Boc-α,β-A_2bu, N^{α}-Z-N^{β}-Boc-α,β-A_2bu-OMe, and N^{α}-Z-α,β-A_2bu-OMe × HCl.

Synthese der vier Stereoisomeren von α,β-Diaminobuttersäure und einiger für Peptidsynthesen geeigneter Derivate

Zusammenfassung: Ein Syntheseweg zur Darstellung der vier stereoisomeren α,β-Diaminobuttersäuren (α,β-A_2bu) wurde ausgearbeitet. Die Isomeren der Konfiguration L-*threo*, D-*threo*, L-*erythro*,

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Abbreviations: the recommendations of the IUPAC-IUB Commission on Biochemical Nomenclature in *Biochemistry* **5**, 1445–1453; 2485–2489 (1966); *Biochemistry* **6**, 362–364 (1967); *Biochemistry* **11**, 1726–1732 (1972); *J. Biol. Chem.* **241**, 2491–2495, (1966) are followed. The diastereoisomers of threonine and α,β-diaminobutyric acid (α,β-A_2bu) and derivatives are designated by L-*threo*, D-*threo* L-*allo* or L-*erythro* and D-*allo* or D-*erythro* following their respective roman numerals. Boc, *tert*-butyloxycarbonyl; OMe, methyl ester; Tos, *p*-toluenesulfonyl; Z, benzyloxycarbonyl.

und D-*erythro* wurden von den entsprechenden Isomeren des Threonins und *allo*-Threonins ausgehend hergestellt. *N*-Tosylierung nach Schotten-Baumann, Veresterung mit Diazomethan und *O*-Tosylierung in Pyridin lieferte *N*,*O*-Ditosylthreonin-methylester (IV). Stufenweise Behandlung mit gesättigtem methanolischem Ammoniak und 6N HCl ergab, neben racemischen Gemischen, nach mehrmaligem Umkristallisieren aus Wasser, die N^{α}-Tosyl-α,β-diaminobuttersäure (V) mit derselben Konfiguration wie das Threonin, von dem man ausgegangen war. Vermutlich wird der sterische Verlauf der Reaktion von der Sulfonamidgruppe dahingehend beeinflußt, daß eine doppelte Walden-Umkehr des asymmetrischen β-Kohlenstoffatoms über eine Aziridin-Zwischenstufe stattfindet. Gleichzeitig bilden sich racemische Gemische infolge α,β-Eliminierung und Bildung von intermediärem α-Tosylamino-crotonsäure-methylester. Die Abspaltung der Tosylgruppe mit Natrium in flüssigem Ammoniak lieferte α,β-Diaminobuttersäure (VI). Die Konfigurationen der vier Stereoisomeren wurden durch Kernmagnetische Resonanz und Optische Rotationsdispersionsspektroskopie ermittelt und darüber hinaus durch Umwandlung von N^{α}-Tosyl-L-*threo*-α,β-diaminobuttersäure (L-*threo*-*V*) in das bekannte *N*-Tosylthreonin mittels salpetriger Säure. Die *threo*- und *erythro*-Isomeren der α,β-A_2bu (VI) und N^{α}-Tosyl-α,β-A_2bu (V) wurden weiterhin durch ihre Retentionszeiten bei der Ionenaustauschchromatographie im Aminosäure-Analysator und durch die Ninhydrin-Konstanten (C_{HW}) charakterisiert. Der *erythro*-Isomeren der α,β-A_2bu besaßen höhere Farbkonstanten (20,6) als die *threo*-Isomeren (5,6 − 5,8). Im Falle der *N*-Tosyl-α,β-A_2bu erzeugten jedoch die *threo*-Isomeren höhere Werte (11,3 − 11,4) als die *erythro*-Isomeren (1,7 − 1,8). Die Kernmagnetischen Resonanzspektren (in DCl) der N^{α}-Tosyl-L-*threo*-α,β-A_2bu (L-*threo*-V) zeigten eine H_{α}-H_{β}-Kupplungskonstante (6,6 Hz), die mit derjenigen der *erythro*-Isomeren der freien Diaminobuttersäure identisch ist. Folgende, für Peptidsynthesen brauchbare, geschützte Derivate wurden dargestellt: N^{α}-Tos-N^{β}-Boc-α,β-A_2bu, N^{β}-Boc-α,β-A_2bu, N^{α}-Z-N^{β}-Boc-α,β-A_2bu, N^{α}-Z-N^{β}-Boc-α,β-A_2bu-OMe und N^{α}-Z-α,β-A_2bu-OMe × HCl.

The incidence of α,β-diaminobutyric acid in nature has only recently been established, in contrast to the wider distribution of α,γ-diaminobutyric acid[1,2]. The amphomycin group of peptide antibiotics was a source of isolation of α,β-diaminobutyric acid[3]. This basic amino acid possesses two asymmetric carbon atoms (C^{α} and C^{β}) which give rise to four stereoisomeric forms. Aspartocin yielded L-*threo*- and D-*erythro*-α,β-diaminobutyric acid on hydrolysis[4]. These two forms have also been isolated and identified from hydrolysates of amphomycin[5] and other antibiotics[3]. The configurations were established by nuclear magnetic resonance (NMR) spectroscopy[4]. We were interested in synthesizing a peptide analog of the antitumor agent actinomycin D[6,7] in which the L-threonine residues would be replaced by isosteric L-*threo*-α,β-diaminobutyric acid residues. This bis-lactam analog in which the lactone oxygens of actinomycin D have been replaced by -NH- groups[8,9] is part of a series to be prepared for assessing the contribution which the peptide moieties make to the antitumor activity of actinomycin. Since no synthetic route to the individual optically active isomers of α,β-diaminobutyric acid has been recorded, we have developed a scheme of preparation starting from the appropriate threonine isomers. We report herein the preparation of all four stereoisomers, describing in detail that of L-*threo*-α,β-diaminobutyric acid (L-*threo*-VI)*. Several derivatives of L-*threo*-α,β-diaminobutyric acid suitable for use in peptide synthesis were prepared, including N^{α}-Tos-N^{β}-Boc-

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α,β-A_2bu, N^{β}-Boc-α,β-A_2bu, N^{α}-Z-N^{β}-Boc-α,β-A_2bu, N^{α}-Z-N^{β}-Boc-α,β-A_2bu-OMe, and N^{α}-Z-α,β-A_2bu-OMe × HCl.

Experimental procedures

Melting points were determined on a Fisher-Johns apparatus and are corrected. Optical rotations were determined on a Rudolph Model 200 manual spectropolarimeter. ORD curves were obtained on a Cary model 60 spectropolarimeter; NMR spectroscopy was performed with a Varian A60 instrument. Amino acid analyses were done on the short column (5.5 cm high) of a Beckman Model 121 auto-analyzer containing spherical polystyrene-divinylbenzene-based strong-sulfonic acid cation-exchange resin, PA 35, and using 0.35N sodium citrate buffer, pH 5.26, at a flow rate of 105 ml/h. Elemental analyses were performed by Werby Laboratories, Inc., Boston, Massachusetts.

Threonine (I)

L-Threonine (L-*threo*-I) and D-threonine (D-*threo*-I) were commericial products (Ajinomoto Company of New York). L-*allo*-Threonine (L-*erythro*-I) and D-*allo*-threonine (D-*erythro*-I) were prepared by the method of Elliott[11]. L-*erythro*-I: m. p. 265–266°C $[\alpha]_D^{20}$: +8.9° (*c*=3.3 in water). Lit.[11]: m. p. 273–274°C, $[\alpha]_D^{24}$: +9.3° (*c*=3.3 in water). D-*erythro*-I: m. p. 265–268°C, $[\alpha]_D^{20}$: −9.2° (*c*=3.9 in water). Lit.[11] m. p. 272–273°C $[\alpha]_D^{25}$: −9.1° (*c*=3.9 in water).

N-Tosylthreonine (II)

Prepared by the method of Theodoropoulos and Craig [12], Table 1.

N-Tosylthreonine methyl ester (III)

To a cooled (0°C) stirred solution of II (136.5 g, 0.5 mol) in dioxane (350–400 ml), diazomethane in ether was added dropwise until the yellow color persisted. The mixture was stirred for 1.5 h, excess diazomethane was destroyed by addition of acetic acid, and the mixture evaporated. The residual oil was dissolved in ethyl acetate (800 ml) and washed with 1M $NaHCO_3$ (three times), saturated NaCl (two times), dried ($MgSO_4$), and evaporated. The product crystallized and was generally obtained in over 85% yield and used in this form for the *O*-tosylation. Recrystallization and physical data, see Table 1.

N,O-Ditosylthreonine methyl ester (IV)

To a stirred solution of III (144 g, 0.5 mol) in pyridine (700 ml) at 0°C to −5°C, *p*-toluenesulfonyl chloride (190.6 g, 1 mol) in pyridine (350 ml) was added dropwise. The solution, which darkened slightly, was allowed to warm to room temperature and was stirred for 13 h. Evaporation of the pyridine gave an oil which was partitioned between water (300 ml) and ethyl acetate (300 ml). The aqueous phase was extracted with ethyl acetate (3 × 100 ml); the organic phases were combined, washed with 25% aqueous pyridine (three times), water (three times), 1N HCl (several times until the wash was acidic), saturated NaCl and dried ($MgSO_4$). On evaporation the product started to crystallize. Recrystallization solvent and experimental data, see Table 1.

Direct *N,O*-ditosylation of threonine methyl ester affords IV possessing the same physical characteristics, but in generally lower overall yield.

N^{α}-Tosyl-α,β-diaminobutyric acid (V)

IV (95 g, 0.22 mol) was treated with ammonia-saturated methanol (1000 ml) in a sealed round bottom flask for 48 h at room temperature. The methanol was evaporated and traces of ammonia removed under high vacuum. The residual solid was then hydrolyzed in refluxing 6N HCl (800 ml) for 3 h. After cooling, the solution was evaporated to dryness and flushed with water several times. The solid was dissolved in the minimum amount of boiling water, filtered and adjusted to pH 7 with ammonium hydroxide. On cooling, a crude crystalline product formed which was recrystallized from water. It yielded a mixture which was washed after filtration with cold ethanol. A third recrystallization afforded pure material. Data, see Tables 1 and 2. The mother liquors contained optically inactive material. Thin-layer chromatography (Silica gel; sec-butanol/formic acid/water 150:27:23) showed the presence of V (racemic, R_F=0.55) and of 1-tosylaminocrotonic acid (R_F=0.35).

α,β-*Diaminobutyric acid* (VI)

To a stirred solution of V (1.5 g, 5.5 mmol) in liquid ammonia (750 ml) at the boiling point (ca. −33°C) was added sodium (257 mg) in small portions over 20 min until the blue color of the solution persisted for 30 sec. Ammonium chloride (600 mg) was added and the ammonia allowed to evaporate; final traces were removed *in vacuo*. The residue was suspended in boiling ethanol and water added until solution, and the mixture acidified with hydrochloric acid. On filtration and neutralization with pyridine, crystals of the monohydrochloride monohydrate formed. Data, see Tables 1 and 2.

Conversion of N^{α}-tosyl-L-threo-α,β-diaminobutyric acid (L-threo-V) to N^{α}-tosyl-L-threonine

Compound L-*threo*-V (0.5 g, 1.8 mmol) was dissolved in water (10 ml) and sodium nitrite (152 mg) added. Glacial acetic acid was added to attain pH 3.5 and the mixture was stirred for 15 h. On acidification to pH 1, a solid formed which was extracted into ethyl acetate.

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Table 1. Properties of stereoisomers of α,β-diaminobutyric acid (VI), N^{α}-tosyl-α,β-diaminobutyric acid (V) and several threonine derivatives.

Compound	Diastereo-isomer	Yield [%]	Recrystallization Solvent	$[\alpha]_D^{20}$ [°]	Melting Point [°C]	Formula [m. w.]	Elemental Analysis (Calculated) Found C	H	N
						$C_{11}H_{15}N_1O_5S$ (273.3)	(48.3)	(5.53)	(5.15)
Tos-Thr-OH II	L-*threo*[a]	70	H_2O	+15.0[b]	80–82		–	–	–
	D-*threo*	68		−14.5[b]	79–83		–	–	–
	L-*erythro*	63		+20.3[c]	156–157		48.1	4.88	56.1
	D-*erythro*	30	ethylacetate/hexane	−21.8[d]	166–168		48.3	5.82	4.94
						$C_{12}H_{17}N_1O_5S$ (287.3)	(50.2)	(5.96)	(4.87)
Tos-Thr-OMe III	L-*threo*[e]	100	chloroform/ether/hexane	− 7.8[b]	98–98.5		–	–	–
	D-*threo*	97		+ 8.0[b]	97–98		–	–	–
	L-*erythro*	85		−23.8[f]	92–94		50.4	6.18	.64
	D-*erythro*	96		+24.7[f]	97–98		49.6	6.21	4.50
Tos | Tos-Thr-OMe IV	L-*threo*	54	methanol[h]	+20.5[d]	146–149	$C_{19}H_{23}N_1O_7S_2$ (441.5)	(51.7)	(5.25)	(3.17)
	D-*threo*	61		−19.0[d]	144–146		51.8	5.32	3.04[g]
	L-*erythro*	64		−11.0[f]	163–165		–	–	–
	D-*erythro*	68		+11.8[f]	162–163		51.8	5.14	3.17
							51.9	5.25	3.04
Tos-α,β-A_2bu-OH V	L-*threo*	20	H_2O	+28,2[i]	233–235	$C_{11}H_{16}N_2O_4S_1 \times 0.5\ H_2O$ (281.2)	(47.0)	(6.05)	(9.96)
	D-*threo*	17.5		−28.1[i]	231–232		47.4	6.54	9.88
							–	–	–
						$C_{11}H_{16}N_2O_4S_1$ (272.3)	(48.5)	(5.92)	(10.31)
	L-*erythro*	25	ammonium hydroxide (10%)	+47.9[i]	275–276		48.5	5.95	9.83
	D-*erythro*	33		−49.4[i]	271–272		48.1	6.10	10.0
						$C_4H_{10}N_2O_2 \times HCl \times H_2O$[j] (172.6)	(27.8)	(7.59)	(16.2)
H-α,β-A_2bu-OH VI	L-*threo*	54	ethanol/water	+39.3[i]	239–240		27.4	7.94	16.0[k]
	D-*threo*	53		−38.1[i]	225–226		27.5	7.86	16.1[l]
	L-*erythro*	28		+10.3[i]	202–204		27.5	7.85	16.2[m]
	D-*erythro*	32		−13.2[i]	202–207		27.6	7.70	16.3[n]

[a] Lit.[13,13a] $[\alpha]_D^{20}$: +14.8° (*c*=2, in methanol); m. p. 136–137°C.
[b] (*c*=2, in methanol).
[c] (*c*=1.23, in methanol).
[d] (*c*=1, in methanol).
[e] Lit.[13a] $[\alpha]_D^{16}$: −8.0° (*c*=2, in methanol); m. p. 100–101°C.
[f] (*c*=1, in dimethylformamide).
[g] S, Calcd: 14.5; found: 14.3.
[h] Methanol, acidified with a few drops of glacial acetic acid.
[i] (*c*=1, in 6N HCl).
[j] Samples for analysis were dried (P_2O_5/KOH) at room temperature *in vacuo* for 15 h.
[k] Cl, Calcd: 20.5; found: 21.1.
[l] Cl, Found: 20.8.
[m] Cl, Found: 21.1.
[n] Cl, Found: 21.0.

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The organic phase was dried ($MgSO_4$) and evaporated to yield 0.36 g (72%) of an oil. Crystallization from ether/hexane gave N^{α}-tosyl-L-threonine, m. p. 123 to 124°C; $[\alpha]_D^{20}$: +12.3° (c=1.26, in methanol).

Action of diethylamine on N,O-ditosyl-L-threonine methyl ester

A. Formation of L-*erythro*-*N*-tosyl-2-methyloxycarbonyl-3-methylaziridine (VII): Compound L-*threo*-IV (2.2 g, 5 mmol) was dissolved in tetrahydrofuran (20 ml) and freshly distilled diethylamine (0.52 ml, 5 mmol) was added. The solution was kept between 30–40°C for 6 h and then diluted with ethyl ether. The diethylamine salt of *p*-toluenesulfonic acid crystallized and and filtered off. The mother liquor was evaporated and the residual oil crystallized from ether to give 0.9 g (67%); m. p. 123–125°C; $[\alpha]_D^{20}$: −83.1° (c=1, in methanol); IR (KCl) = 895 cm^{-1} (aziridine).

$C_{12}H_{15}NO_4S$ (269.3) Calcd.: C 53.5 H 5.61 N 5.20
Found: C 53.4 H 6.01 N 5.03

B. Formation of 1-tosylaminocrotonic acid methyl ester (VIII): Treatment of compound L-*threo*-V (13.2 g, 30 mmol) in tetrahydrofuran (90 ml) with diethylamine (3.1 ml, 30 mmol) under the conditions described in A gave 4.5 g (56%) of VIII; m. p. 118–120°C; $[\alpha]_D^{20}$: 0° (c=1, CH_3OH); NMR ($CDCl_3$) δ 2.0 doublet (J=7.2 Hz) (CH_3-CH=); 2.42 singlet (CH_3-C_6H_4-); 3.47 singlet (-CO_2CH_3); 6.0 complex (-SO_2-NH-CH=); 7.05 quadruplet (CH_3-CH=); 7.5 quadruplet (CH-C_6H_4-SO_2-).

$C_{12}H_{15}NO_4S$ (269.3) Calcd.: C 53.5 H 5.61 N 5.20
Found: C 53.9 H 5.82 N 5.18

N^{α}-Tosyl-N^{β}-tert-butyloxycarbonyl-L-threo-α,β-diaminobutyric acid (IX)

N^{α}-Tosyl-L-*threo*-α,β-diaminobutyric acid (11.4 g, 42 mmol) was dissolved in 1N NaOH (42 ml) and dioxane (15 ml). *tert*-Butyloxycarbonyl azide (24 ml, 0.17 mol) was added in dioxane (10 ml) and the mixture stirred while the pH was kept constant at 9.5 by pH-stat-controlled addition of 4N NaOH. After stirring at room temperature for 64 h, the mixture was poured into water and extracted twice with ethyl acetate. The aqueous phase was acidified with 1M citric acid. The oil formed was extracted into ethyl acetate. After washing the organic phase with saturated NaCl (twice), it was dried ($MgSO_4$) and evaporated. The residual oil was crystallized from methanol by the addition of water to yield 12.3 g of IX (79%); m. p. 153–154°C; $[\alpha]_D^{20}$: +40.3° (c=1, in methanol).

$C_{16}H_{24}N_2O_6S_1$ (372.4) Calcd: C 51.6 H 6.49 N 7.52
Found: C 51.4 H 6.50 N 7.31

N^{β}-tert-Butyloxycarbonyl-L-threo-α,β-diaminobutyric acid (X)

A solution of IX (4 g, 67 mmol) in liquid ammonia (1200 ml) was reduced by addition of sodium as described for VI. Sodium consumption was 496 mg. The mixture was neutralized by the addition of NH_4Cl (1.15 g) and the ammonia evaporated. Last traces of ammonia were removed *in vacuo* and the product crystallized from ethanol/water after adjusting the pH to 6.5 to provide 1.8 g of X (79%); m. p. 251–252°C; $[\alpha]_D^{20}$: +19.9° (c=0.9, in glacial acetic acid).

$C_9H_{18}N_2O_4$ (218.3) Calcd.: C 49.5 H 8.31 N 12.8
Found: C 49.6 H 8.65 N 12.8

N^{α}-Benzyloxycarbonyl-N^{β}-tert-butyloxycarbonyl-L-threo-α,β-diaminobutyric acid (XI)

To an ice cold solution of X (1 g, 4.6 mmol) in 1N NaOH (4.6 ml) was added benzyloxycarbonyl chloride (0.78 ml, 5.5 mmol) in ether (10 ml) dropwise over a period of 40 min. The reaction was pH-stat-controlled at pH 10.5 by the addition of 1N NaOH. After 1 h the reaction was complete and 4.6 ml of 1N NaOH had been consumed. The mixture was poured into water and extracted twice with ether. The aqueous phase was acidified with 1M citric acid and the oil formed extracted into ethyl acetate. After washing the ethyl acetate phase with saturated NaCl (twice), it was dried ($MgSO_4$) and evaporated. The residual oil was crystallized from methanol/water giving 1.35 g (83.5%); m. p. 108 to 110°C; $[\alpha]_D^{20}$: −36.4° (c=1, in methanol).

$C_{17}H_{24}N_2O_6$ (352.4) Calcd.: C 57.9 H 6.86 N 7.95
Found: C 57.7 H 7.02 N 8.02

N^{α}-Benzyloxycarbonyl-N^{β}-tert-butyloxycarbonyl-L-threo-α,β-diaminobutyric acid methyl ester (XII)

To an ice cold stirred ether solution of XI (0.8 g, 2.3 mmol) diazomethane in ether was added dropwise until the yellow color persisted. After stirring for approximately 10 min, the excess diazomethane was destroyed by the addition of a few drops of glacial acetic acid and the solution washed with 1M $NaHCO_3$ (twice), H_2O (twice), dried ($MgSO_4$) and evaporated. The oil obtained was crystallized twice from methanol/water, giving 0.6 g (72%); m. p. 80–81°C; $[\alpha]_D^{20}$: +40° (c=1, in methanol).

$C_{18}H_{26}N_2O_6$ (366.4) Calcd.: C 59.0 H 7.15 N 7.64
Found: C 59.0 H 7.16 N 7.57

N^{α}-Benzyloxycarbonyl-L-threo-α,β-diaminobutyric acid methyl ester hydrochloride (XIII)

An ice cold solution of XII (0.86 g, 2.3 mmol) in freshly distilled trifluoroacetic acid (10 ml) was stirred for 1 h. The trifluoroacetic acid was removed *in vacuo* and the residual oil dissolved in a small volume of ether.

Excess 1.4N hydrogen chloride in ether was added with stirring. The product precipitated and was triturated several times with dry ether before filtering and drying to yield 0.67 g (94%). Crystallization from methanol/ether gave 0.45 g fine needles, of m. p. 197–199°C; $[\alpha]_D^{20}$: −14.3° (c=1, in dimethylformamide).

$C_{13}H_{19}N_2O_4Cl$ (302.8)

Calcd.:	C 51.6	H 6.33	N 9.25	Cl 11.7
Found:	C 51.7	H 6.75	N 9.41	Cl 11.8

Results and Discussion

Treatment of L-threonine with *p*-toluenesulfonyl chloride[12] in 1N NaOH gave the *N*-tosyl derivative (L-*threo*-II), which was esterified with diazomethane. *O*-Tosylation was attained in pyridine to afford *N*,*O*-ditosyl-L-threonine methyl ester (L-*threo*-IV). Ammonolysis for 48 h in ammonia-saturated methanol followed directly by hydrolysis in refluxing 6N hydrochloric acid gave, after several recrystallizations from water, pure N^{α}-tosyl-L-*threo*-α,β-diaminobutyric acid (L-*threo*-V). Scission of the N^{α}-tosyl group was accomplished by the action of sodium in liquid ammonia[14] to yield L-*threo*-α,β-diaminobutyric acid monohydrochloride monohydrate (L-*threo*-VI).

The configuration of the isomer was established by NMR spectroscopy in 2N deuterium chloride[4],

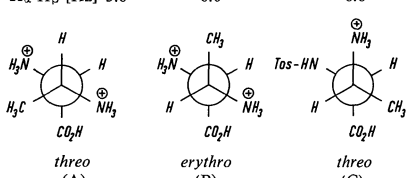

Fig. 1. Newman projections of L-*threo*-α,β-diaminobutyric acid (A); L-*erythro*-α,β-diaminobutyric acid (B); and N^{α}-tosyl-L-*threo*-α,β-diaminobutyric acid (C) in acidic solution (2N DCl).

In the free amino acid (VI) isomers the charged amino groups are *trans* to each other (A, B) and, therefore, the α and β protons are *gauche* in the *threo* form (A) (H_{α}-H_{β} coupling constant of 3.6 Hz) and *trans* in the *erythro* form (B) (H_{α}-H_{β} coupling constant of 6.6 Hz). The H_{α}-H_{β} coupling constant of 6.6 Hz for the N^{α}-tosyl derivative of L-*threo*-α,β-diaminobutyric acid indicates that the charged β-amino group is located closest to the sulfonamide group (C).

Table 2. In this solvent the charged amino groups are *trans* to each other, and therefore, the α and β protons are *gauche* in the *threo* from and *trans* in the *erythro* form (Fig. 1A, B). Consequently, the *threo* isomer is characterized by a smaller H_{α}-H_{β} coupling constant (3.6 Hz) than that (6.6 Hz) of the *erythro* isomer[15]. The synthetic diaminobutyric acid has an H_{α}-H_{β} coupling constant of 3.6 Hz, and a positive Cotton effect was observed in optical rotatory dispersion studies[4,16] thus confirming the L-*threo* configuration of compounds V and VI.

It is somewhat surprising that the ammonolysis reaction did not proceed by a normal S_N2 type mechanism, which was expected, to give the *erythro* derivative from *N*,*O*-ditosyl-L-threonine methyl ester. The ammonolysis reaction afforded, however, N^{α}-tosyl-L-*threo*-α,β-diaminobutyric acid as the sole optically active product, in recoveries ranging form 17% to 33%. The remaining material was optically inactive. Thin-layer chromatography (Silica gel, *sec*-butanol/formic acid/water 150:27:23) showed that it contained N^{α}-tosyl-α,β-diaminobutyric acid (R_F=0.55), presumably racemic mixtures such as L,D-*threo* or L,D-*erythro* forms and some 1-tosylaminocrotonic acid (R_F=0.35). In the absence of kinetic data, discussions of possible mechanisms remain entirely hypothetical. Since no L-*erythro*-diastereoisomer could be detected in the ammonolysis reaction mixture by amino acid analyzer tests, an S_N1 mechanism might be somewhat unlikely. An explanation of the observed phenomena is that two processes are operative during the reaction, *i. e.* (a) double inversion *via* aziridine formation and (b) α,β-elimination leading to racemic mixtures.

The sulfonamide moiety might exert a neighboring group influence* (a) on the stereochemical course,

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* A neighboring group influence of the sulfonamide moiety on this type of reaction was observed when N^{α}-tosylamino-L-*erythro*-β-chlorobutyric acid m. p. 169 to 170°C; $[\alpha]_D^{25}$: +28.4° (c=1, in methanol); correct elemental analysis (CH, N) for $C_{11}H_{14}NO_4SCl$ (291.7)] reacted with conc. ammonia to give N^{α}-tosyl-α,β-diaminobutyric acid, among other products, while N^{α}-benzyloxycarbonylamino-L-*erythro*-β-chlorobutyric acid [m. p. 114–116°C; $[\alpha]_D^{25}$: +3.5° (c=1, in methanol); correct elemental analysis (CH, N) for $C_{12}H_{14}NO_4Cl$

Table 2.: NMR spectroscopic, ORD, and ninhydrin color values and positions in amino acid analysis of α,β-diaminobutyric acid (VI) stereoisomers and their N^{α}-tosyl derivatives (V).

Compound	Diastereo-isomer	Ninhydrin color C_H^W (1 μmol)	Elution[a] [min before NH_3]	ORD: Molar rotation, Cotton effect, Max 224 nm	NMR (in 2N DCl) (δ values)
α,β-Diaminobutyric acid VI	L-*threo*	5.8	9	$+1841^0$	doublet 1.5 ($J = 6.6$ Hz) (CH_3-CH-); complex 4.1 (CH_3-CH-CH-);
	D-*threo*	5.6	9	-1851^0	doublet 4.6 ($J = 3.6$ Hz) (CH_3-CH-CH-)
	L-*erythro*	20.6	7	$+1021^0$	doublet 1.5 ($J = 6.7$ Hz) (CH_3-CH); complex 4.2 (CH_3-CH-CH-);
	D-*erythro*	20.6	7	-1112^0	doublet 4.5 ($J = 6.2$ Hz) (CH_3-CH-CH-)
N^{α}-Tosyl-α,β-diaminobutyric acid V	L-*threo*	11.3	23		doublet 1.4 ($J = 6.6$ Hz) (CH_3-CH-); singlet 2.4 (CH_3-C_6H_4-);
	D-*threo*	11.4	23		complex 3.7 (CH_3-CH-CH); doublet 4.2 ($J = 6.6$ Hz) (CH_3-CH-CH-); quadruplet 7.3 – 7.9 (CH_3-C_6H_4-)
	L-*erythro*	1.7	24		doublet 1.24 ($J = 6.7$ Hz) (CH_3-CH-); singlet 2.4 (CH_3-C_6H_4-);
	D-*erythro*	1.8	24		complex 3.9 (CH_3-CH-CH-); doublet 4.45 ($J = 3.6$ Hz) (CH_3-CH-CH-); quadruplet 7.3 – 7.9 (CH_3-C_6H_4-)

[a] Determined on a Beckman Amino Acid Analyzer, Model 121; short column (5.5 cm), PA 35 resin, 0.35N sodium citrate buffer, pH 5.26, flow rate 105 ml/h.

presumably through intermediate aziridine formation** which is accompanied by inversion at the β carbon atom. Ammonolysis of the aziridine proceeds *via* a second inversion resulting in a product with the same (L-*threo*) configuration as the starting material. Attempts to isolate the aziridine from *N,O*-ditosyl-L-threonine methyl ester (L-*threo*-IV) by the action of diethylamine[17] gave ambigous results. In some experiments the aziridine, *N*-tosyl-2-methyloxycarbonyl-3-methyl-aziridine (VII), could be isolated and in others 1-tosylaminocrotonic acid methyl ester (VIII), resulting from α,β-elimination, was the sole product. α,β-Elimination (b) also takes place during the ammonolysis reaction, since varying amounts of racemic mixtures were isolated besides L-*threo*-V.

The NMR spectra of N^{α}-tosyl-L-*threo*-α,β-diaminobutyric acid, in 2N deuterium chloride revealed an H_{α}-H_{β} coupling constant of 6.6 Hz (see Table 2), which is consistent with that of the *erythro* form of the free diaminobutyric acid. This is probably due to the conformation adopted by the N^{α}-tosyl derivative in acidic media. The NMR spectrum indicates that the conformation is as shown in Fig. 1C, in which the charged β-amino group is closest to the sulfonamide group. In the *threo*-isomer this renders the α and β protons *trans* to each other and gives rise to the larger coupling constant observed. The configuration of L-*threo*-V was confirmed by conversion to *N*-tosyl-L-threonine

(271.7)] remained unchanged even after prolonged treatment with conc. ammonia.

These observations were part of Dr. R. P. Patel's unpublished work on preparation of L-α,β-diaminobutyric acid stereoisomers in a series of reactions starting from L-threonine methyl ester, *via* L-*erythro* α-amino-β-chlorobutyric acid methyl ester [m. p. 185 to 187°C; $[\alpha]_D^{24}$: -17.2^0 ($c = 1$, in water)], N^{α}-tosyl-amino-L-*erythro*-β-chlorobutyric acid methyl ester [m. p. 92 – 93°C; $[\alpha]_D^{23}$: $+11.2^0$ ($c = 1$, CH_3OH)], the corresponding acid (see above), and N^{α}-tosyl-α,β-diaminobutyric acid (mixture of isomers), followed by detosylation with sodium in liquid ammonia. This route was abandoned since the ensuing mixtures of isomers were difficult to separate.

** Attempts to obtain aziridine formation by ammonolysis of N^{α}-benzyloxycarbonyl-*O*-tosyl-L-threonine methyl ester in NH_3 saturated methanol for 48 h at room temperature repeatedly failed.

[17] Okawa, K., Kinutani, T. & Sakai, K. (1968) *Bull. Chem. Soc. Jap.* **41**, 1353 – 1355.

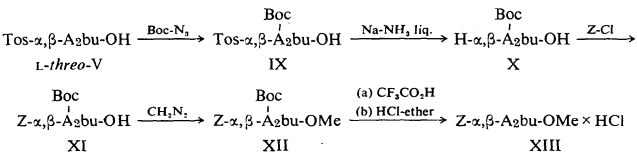

Fig. 2. Preparation of derivatives of L-*threo*-α,β-diaminobutyric acid suitable for use in peptide synthesis.

through the action of nitrous acid, which is known to proceed with retention of configuration[18].

The three other isomers were prepared by the same route starting from D-threonine, L-*allo*-threonine (L-*erythro*-I) and D-*allo*threonine (D-*erythro*-I)[11] giving D-*threo*-, L-*erythro*- and D-*erythro*-α,β-diaminobutyric acid, respectively. In each instance, the NMR spectra of the N^{α}-tosyl derivatives (V) revealed the same phenomena discussed above, *i. e.* the H_{α}-H_{β} coupling constant in the *erythro* series exhibited a value corresponding to the free *threo*-α,β-diaminobutyric acid, see Table 2. The ORD patterns of the free acids (VI) were as expected for the respective isomers, *i. e.* a negative Cotton effect for the D-*threo* and D-*erythro* compounds and a positive Cotton effect for the L-*erythro* compound.

The *threo* and *erythro* isomers were further characterized by their behavior on ion-exchange chromatography. The positions of elution from the short column of the Beckman model 121 automatic amino acid analyzer were recorded and the color constants for the ninhydrin reaction determined for each isomer[19,20]. As previously observed[4,5], the *erythro* compounds gave higher color constants than the *threo* compounds (*threo*, 5.8; *erythro*, 20.6), see Table 2. The N^{α}-tosyl compounds (V) were also analyzed on the short column and in this series of derivatives the *threo* compounds gave higher ninhydrin values than the *erythro* isomers (N^{α}-tosyl *threo*, 11.4; N^{α}-tosyl *erythro*, 1.7)*. Indeed, N^{α}-tosyl-L-*threo*-α,β-diaminobutyric acid gave a higher color constant than the unprotected diamino acid L-*threo*-VI) see Table 2.

Several derivatives of L-*threo*-α,β-diaminobutyric acid suitable for use in peptide synthesis were also prepared as illustrated in Fig. 2. Treatment of N^{α}-tosyl-L-*threo*-α,β-diaminobutyric acid with *tert*-butyloxycarbonyl azide[21–23] under controlled pH conditions[24] gave the N^{β}-*tert*-butyloxycarbonyl derivative IX. Removal of the N^{α}-tosyl group by the action of sodium in liquid ammonia[14] gave N^{β}-*tert*-butyloxycarbonyl-L-*threo*-diaminobutyric acid (X). Reaction of X with benzyloxycarbonyl chloride[25] under controlled pH conditions gave N^{α}-benzyloxycarbonyl-N^{β}-*tert*-butyloxycarbonyl-L-*threo*-diaminobutyric acid (XI) which, upon esterification with diazomethane, yielded the methyl ester (XII). Acidolytic cleavage of the N^{β}-*tert*-butyloxycarbonyl group was achieved by the action of trifluoroacetic acid[26]. The product was converted to the hydrochloride (XIII) by the action of hydrogen chloride in ether. All reactions proceeded in good yield and crystalline products were obtained at each stage.

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* This cross-correlation appears to be consistent with that observed with NMR spectra of free and N^{α}-tosyl derivatives of the respective *threo* and *erythro* isomers, discussed above.

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