TERT-LEUCINE AND ITS SIMPLE PEPTIDES* **

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N-Protected (benzyloxycarbonyl, tert-butyloxycarbonyl, and 2-nitrobenzenesulfenyl) derivatives of tert-leucine (Tle, 2-amino-3,3-dimethylbutanoic acid) were prepared and used in the synthesis of 13 dipeptides. From the condensation methods, activated esters appear of a limited applicability while N,N'-dicyclohexylcarbodiimide is the agent of choice. As model compounds for the synthesis of greater peptides, the tripeptides L-pyroglutamyl-L-histidyl-L-tert-leucine amide, and L- prolyl-L-tert-leucyl-glycine amide were prepared.

In this Laboratory, the relationship between structure and physical or biological properties is examined in several series of peptides[3-5]. In these investigations, the use of unnatural (or non-proteinogeneous) amino acids appears advisable since a suitable design can accentuate the specific effect of the side chain in the desired direction. Of a special importance are then derivatives of those amino acids in which a certain feature of the side chain (and thus the consequent effect of this feature on physical and biological properties of the whole molecule) has been maximized. Concerning the steric requirements of the hydrocarbon side chains (markedly affecting conformation of peptide chains, *cf.*[6]), such a maximization is represented by the tert-butyl group of tert-leucine (*I*, 2-amino-3,3-dimethylbutanoic acid); for a discussion see ref.[7]. In this paper, we wish to report preparation of some derivatives of the acid *I* useful in the synthesis of peptides and examples of their application.

The preparation of tert-leucine and its resolution into optical enantiomers has been reported[8-13]. In the present work, the intermediates for the preparation of DL-tert-leucine were obtained by the method of Abrash and Niemann[10]. The catalytic hydrogenation of the oxime of trimethylpyruvic acid was effected on Raney nickel. In the resolution step, use was made of the formyl derivative of DL-tert-leucine[8]. The reported applications of tert-leucine for the synthesis of peptides have been so far limited to the preparation of poly-DL-tert-leucine[14] and of some dipeptides by the asymmetric synthesis[12].

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** The symbol Tle is used throughout this paper for tert-leucine (2-amino-3,3-dimethylbutanoic acid) along with other recommended abbreviations for amino acids and peptides[1,2].

The amino group of tert-leucine was protected with benzyloxycarbonyl, tert-butyloxycarbonyl, and 2-nitrobenzenesulfenyl residues according to conventional procedures[15]. Similarly, a smooth reaction course accompanied the preparation of the methyl ester (with the use of diazomethane), *p*-nitrophenyl ester, and 1-hydroxysuccinimidyl ester (by the action of N,N'-dicyclohexylcarbodiimide). The steric effect of the tert-butyl group manifested itself in some cases of the peptide bond formation[7]. Thus, noteworthy is the markedly decreased reactivity of activated

TABLE I
Physical Data of Dipeptides and Analyses

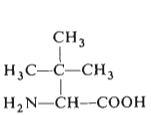
Dipeptide	Yield, % m.p., °C	$[\alpha]_D$ (*c*, dimethylformamide)	Calculated/Found % C	% H	% N
Nps-L-Tle-L-LeuOMe	78 152–154	−52·4°[a] (0·36)	55·45 55·83	7·10 7·30	10·21 10·45
Nps-D-Tle-L-LeuOMe	100 114–116	−20·7° (0·51)	55·45 55·70	7·10 7·19	10·21 10·10
Boc-L-Tle-L-PheOMe	81, 25 137–138	−6·4° (0·42)	64·43 64·26	8·18 8·29	7·10 7·33
Nps-L-Tle-L-PheOMe	94 oil	— —	— —	— —	— —
Nps-L-Tle-L-ValOMe	78 123–125	+9·2° (0·49)	54·57 54·74	6·82 6·92	10·53 10·78
Nps-D-Tle-L-ValOMe	100 101–102	−14·5° (0·49)	54·57 54·85	6·82 6·94	10·53 10·49
Nps-L-Tle-L-ProOMe	100 145–146	−176·9° (0·51)	54·66 54·40	6·37 6·37	10·63 10·78
Nps-D-Tle-L-ProOMe	100 115–117	+45·8° (0·49)	54·66 54·10	6·37 6·37	10·63 10·65
Nps-L-Tle-GlyOEt	100 148–150	−49·8° (0·5)	52·01 52·43	6·27 6·40	11·37 11·43
Z-L-Tle-GlyOEt	100 oil	— —	— —	— —	— —
Nps-L-Tle-L-TleOMe	90 95–96	−9·8° (0·5)	55·45 55·43	7·10 7·26	10·21 10·25
Nps-D-Tle-L-TleOMe	93 95–97	−14·7° (0·20)	55·45 55·28	7·10 6·98	10·21 9·97

[a] Acetic acid.

esters of tert-leucine. Condensations by the action of N,N'-dicyclohexylcarbodiimide proved to be of general applicability; best yields (80–100%, see Table I) in the syntheses of Tle-X dipeptides were obtained with the use of this agent.

The unusual stability of methyl esters with a C-terminal leucine under conditions of an alkaline saponification is a serious obstacle for further syntheses, *cf.*[9] However, it is possible to use the known[12] tert-butyl ester or to effect the reaction with free tert-leucine in the presence of sodium hydrogen carbonate according to Anderson and coworkers[16] as it has been now illustrated on the preparation of 2-nitrobenzenesulfenyl-L-prolyl-L-tert-leucine.

A more general approach to the synthesis of tert-leucine-containing peptides has been attempted on a synthesis of two tripeptides with a potential biological significance. Thus, the TRF analogue, L-pyroglutamyl-L-histidyl-L-tert-leucine amide (*II*), was obtained by condensation of L-pyroglutamyl-L-histidine hydrazide with L-tert-leucine amide according to the azide method[17]. The analogue was then purified on CM-Sephadex. The oxytocin (7–9)-tripeptide analogue, L-prolyl-L-tert-leucinyl-glycine amide (*III*), was prepared by the stepwise procedure. Condensation of glycine ethyl ester with 2-nitrobenzenesulfenyl-L-tert-leucine was effected with the use of N,N'-dicyclohexylcarbodiimide; after deblocking, 2-nitrobenzenesulfenyl-L-proline was attached according to the same method. The resulting protected tripeptide ester was subjected to ammonolysis to afford the corresponding amide from which the protecting group was removed with the formation of the tripeptide amide *III*.



I

L-□Glu-L-His-L-Tle-NH_2

II

L-Pro-L-Tle-Gly-NH_2

III

EXPERIMENTAL

Melting points are uncorrected. Analytical samples were dried at 0·01 Torr for 8 h. The purity of all substances was checked by thin-layer chromatography on silica gel and by electrophoresis.

DL-Tert-leucine

3,3-Dimethyl-2-oximinobutanoic acid[10] (74·4 g) was hydrogenated in methanol (800 ml) over Raney nickel (c. 7 g) at 80°C and 150 atm for 4 h. The catalyst was then filtered off and washed with water. The filtrate and washings were evaporated, the residue was dissolved in a minimum amount of water, the solution filtered, the filtrate evaporated, and the residue crystallised from water–acetone. Yield, 50·4 g (75·4%) of the electrophoretically homogeneous DL-tert-leucine, m.p. 252–254°C (decomposition) in accordance with the literature[10]. Optical rotation of L-tert-leucine: $[\alpha]_D$ −8·3° (*c* 0·53; water), +32·0° (*c* 0·44; acetic acid). As shown by analysis, the sample did not contain water when dried at 95°C/1 Torr for 12 h.

Benzyloxycarbonyl-L-tert-leucine

L-Tert-leucine (1 g) was added to a solution of sodium hydroxide (0·8 g) in water (20 ml). Benzyloxycarbonyl chloride (1·5 ml) was then introduced with stirring at room temperature, the whole stirred for 2 h, and washed with ether. The aqueous layer was acidified with dilute (1 : 1) hydrochloric acid and extracted with ethyl acetate. The extract was dried over anhydrous magnesium sulfate and evaporated under diminished pressure to afford 1·6 g (90%) of an oil, $[\alpha]_D$ +3·8° (*c* 0·39; dimethylformamide). For $C_{14}H_{19}NO_4$ (265·3) calculated: 63·38% C, 7·22% H, 5·28% N; found: 62·92% C, 7·13% H, 5·09% N.

Dicyclohexylammonium salt: M. p. 165–168°C (ethanol–ether), $[\alpha]_D$ −8·4° (*c* 0·59; methanol). For $C_{26}H_{42}N_2O_4$ (446·6) calculated: 69·92% C, 9·48% H, 6·27% N; found: 70·23% C, 9·42% H, 6·35% N.

Tert-butyloxycarbonyl-L-tert-leucine

Tert-butyloxycarbonyl azide (1·2 g) was added to a stirred suspension of L-tert-leucine (1 g) in dioxane (2·0 ml) and water (2·0 ml), the pH value being maintained at pH 9·8 by additions of 4M-NaOH (pH-stat). After 8 h, the mixture was washed with ether (10 ml), the aqueous layer acidified with citric acid (20% aqueous solution), and extracted with two 10 ml portions of ether and then ethyl acetate (5 ml). The organic layers were combined, dried over anhydrous magnesium sulfate, and evaporated under diminished pressure. The residue was kept under light petroleum overnight to deposit crystals. Yield, 1·4 g (88%); m.p. 100–101°C; $[\alpha]_D$ −4·8° (*c* 0·42; acetic acid); $[\alpha]_D$ −2·2° (0·49; methanol). For $C_{11}H_{21}NO_4$ (231·3) calculated: 57·12% C, 9·15% H, 6·06% N; found: 57·16% C, 8·99% H, 6·18% N.

2-Nitrobenzenesulfenyl-L-tert-leucine

2-Nitrobenzenesulfenyl chloride (1·4 g) was added portionwise over 25 min into a stirred solution of L-tert-leucine (1 g) in 2M-NaOH (3·3 ml) and dioxane (8·4 ml), the pH value being maintained at pH 8·8 by additions of 2M-NaOH. The stirring was continued for 30 min, the mixture diluted with water (70 ml), washed with ethyl acetate, and the aqueous layer acidified with 0·5M-H_2SO_4 to deposit a solid which was recrystallised from ether–light petroleum. Yield, 2·1 g (98%); m.p. 140–142°C; $[\alpha]_D$ −103·5° (*c* 0·34; dimethylformamide). For $C_{12}H_{16}N_2O_4S$ (284·3) calculated: 50·69% C, 5·67% H, 9·85% N; found: 50·70% C, 5·76% H, 9·61% N. The D-enantiomer was prepared analogously; $[\alpha]_D$ +98·8° (*c* 0·48; dimethylformamide).

L-Tert-leucine Methyl Ester

A stirred suspension of L-tert-leucine (2 g) in methanol (30 ml) was treated with ethereal diazomethane until the solid dissolved and the yellow colour persisted. The solvents were then evaporated and the residue distilled to afford 2 g (94·5%) of an oil, b.p. 68°C/20 Torr.

Hydrochloride: M.p. 176–178°C; $[\alpha]_D$ +17·1° (*c* 0·38; methanol). For $C_7H_{16}ClNO_2$ (181·7) calculated: 46·28% C, 8·88% H, 7·71% N; found: 46·62% C, 9·09% H, 7·93% N.

2-Nitrobenzenesulfenyl-L-tert-leucine 1-Hydroxysuccinimidyl Ester

1-Hydroxysuccinimide (0·23 g) and N,N′-dicyclohexylcarbodiimide (0·20 g) was added to a solution of 2-nitrobenzenesulfenyl-L-tert-leucine (0·28 g) in dichloromethane (8 ml), the whole stirred at room temperature for 10 h and kept overnight. The precipitate of N,N′-dicyclohexylurea

was filtered off and washed with dichloromethane. The filtrate and washings were combined, washed with water, dried over anhydrous magnesium sulfate, and evaporated under diminished pressure. The residue (m.p. 135—140°C) was recrystallised from dichloromethane–light petroleum. Yield, 0·32 g (84%); m.p. 146—148°C; $[\alpha]_D$ −80·5° (*c* 0·50; dimethylformamide). For $C_{16}H_{19}N_3O_6S$ (381·4) calculated: 50·38% C, 5·02% H, 11·02% N; found: 49·95% C, 4·95% H, 10·52% N.

Tert-butyloxycarbonyl-L-tert-leucine 1-Hydroxysuccinimidyl Ester

The procedure was analogous to the preceding case. Yield, quantitative; m.p. 140—142°C (ethanol); $[\alpha]_D$ −20·6° (*c* 0·52; dimethylformamide). For $C_{15}H_{24}N_2O_6$ (328·3) calculated: 54·84% C, 7·37% H, 8·53% N; found: 55·44% C, 7·39% H, 8·45% N.

Benzyloxycarbonyl-L-tert-leucine 4-Nitrophenyl Ester

4-Nitrophenol (0·28 g) and N,N′-dicyclohexylcarbodiimide (0·42 g) was added at −15°C to −20°C into a stirred solution of benzyloxycarbonyl-L-tert-leucine (0·54 g) in dichloromethane (10 ml) and the stirring was continued at the same temperature for 15 min. The mixture was then kept at 0°C for 4 days, the precipitate of N,N′-dicyclohexylurea filtered off, and the filtrate evaporated under diminished pressure. The residue was dissolved in ether and the ethereal solution filtered to remove the remaining N,N′-dicyclohexylurea. The filtrate was washed with 0·5M-$NaHCO_3$, dried over anhydrous magnesium sulfate, and evaporated under diminished pressure. The residue was dissolved in ether, the solution precipitated with light petroleum, and kept at 0°C overnight to deposit crystals. Yield, 0·39 g (50%); m.p. 91—92°C; $[\alpha]_D$ −18·2° (*c* 0·37 dimethylformamide). For $C_{20}H_{22}N_2O_6$ (386·4) calculated: 62·16% C, 5·73% H, 7·25% N; found: 61·80% C, 5·84% H, 7·58% N.

Protected Dipeptides

At −20°C, methyl ester of the appropriate amino acid (0·0011 mol) and N,N′-dicyclohexylcarbodiimide (0·001 mol) was added to a solution of the corresponding N-protected L- or D-tert-leucine (0·001 mol) in dichloromethane (10 ml). The mixture was stirred at −20°C for 15 min and then kept at 0°C for four days. The precipitate of N,N′-dicyclohexylurea was filtered off, the filtrate washed with an aqueous acid (0·5M-H_2SO_4 in the case of a 2-nitrobenzenesulfenyl group, 1M-HCl in the case of a benzyloxycarbonyl group, and 20% aqueous citric acid in the case of a tert-butyloxycarbonyl group) and then with 0·5M sodium hydrogen carbonate, dried over anhydrous magnesium sulfate, and evaporated under diminished pressure. The residue was crystallised from suitable solvents (Table I).

2-Nitrobenzenesulfenyl-L-prolyl-L-tert-leucine

A solution of 2-nitrobenzenesulfenyl-L-proline 1-hydroxysuccinimidyl ester (0·627 g) in dioxane (4·25 ml) was added to a solution of L-tert-leucine (0·25 g) and sodium hydrogen carbonate (0·3 g) in water (4·25 ml), the mixture stirred at room temperature for 1 h and then kept for four days. The dioxane was evaporated, the residue acidified at 20°C with 0·5M-H_2SO_4, and extracted with ethyl acetate. The extract was washed with two portions of saturated aqueous sodium chloride and water, dried over anhydrous magnesium sulfate, and evaporated under diminished pressure. The residue (m.p. 179—181°C) was crystallised from ether–light petroleum. Yield, 0·64 g (88·6%); $[\alpha]_D$ −11·7° (0·53; dimethylformamide). For $C_{17}H_{23}N_3O_5S$ (381·4) calculated: 53·52% C, 6·07% H, 11·01% N; found: 53·28% C, 6·10% H, 10·91% N.

L-Prolyl-L-tert-leucine Hydrochloride

A solution of 2-nitrobenzenesulfenyl-L-prolyl-L-tert-leucine (0·46 g) in ether (50 ml) was stirred with 3·8M-HCl (3 ml) for 3 min, the solid collected, washed with ether, and crystallised from methanol–ether. Yield, 0·3 g (94%); m.p. 155—157°C; $[\alpha]_D$ −43·1° (*c* 0·33; methanol). For $C_{11}H_{21}ClN_2O_3.H_2O$ (282·8) calculated: 46·72% C, 8·19% H, 9·91% N, 12·54% Cl; found: 47·02% C, 7·85% H, 10·06% N, 12·56% Cl.

N-Carboxy-L-tert-leucine Anhydride

Phosgene was introduced at 40°C into a suspension of L-tert-leucine (1 g) in dioxane (200 ml) until the solid dissolved (for 5 min). Excess phosgene was removed by a stream of nitrogen, the solution filtered, the filtrate evaporated, and the residue crystallised from chloroform–light petroleum. Yield, 1·2 g (100%); m.p. 122—125°C. For $C_7H_{11}NO_3$ (157·2) calculated: 53·49% C, 7·05% H, 8·91% N; found: 53·01% C, 7·08% H, 8·93% N.

L-Tert-leucine Amide

A solution of N-carboxy-L-tert-leucine anhydride (0·6 g) in dichloromethane (20 ml) was treated with excess of ammonia in chloroform and the mixture kept at 0°C overnight. The precipitate was collected and crystallised from methanol–ether. Yield, 0·4 g (80·5%); m.p. 97—99°C; $[\alpha]_D$ +50·6° (*c* 0·17; methanol). For $C_6H_{14}N_2O$ (130·2) calculated: 55·35% C, 10·84% H. 21·52% N; found: 55·22% C, 10·75% H, 21·32% N.

D-Tert-leucyl-L-phenylalanine Methyl Ester Hydrochloride

A solution of 2-nitrobenzenesulfenyl-D-tert-leucyl-L-phenylalanine methyl ester (1·37 g) in ether (20 ml) was stirred with 3·08M ethereal hydrogen chloride (10 ml) for 3 min to deposit a precipitate. The whole mixture was diluted with light petroleum, the solid collected, washed with light petroleum, and crystallised from methanol–ether. Yield, 0·75 g (87%); m.p. 266—268°C; $[\alpha]_D$ −49·3° (*c* 0·51; methanol). For $C_{16}H_{25}ClN_2O_3$ (280·8) calculated: 58·44% C, 7·66% H, 8·52% N; found: 58·83% C, 7·72% H, 8·74% N.

L-Tert-leucyl-L-phenylalanine Methyl Ester Trifluoroacetate

A solution of tert-butyloxycarbonyl-L-tert-leucyl-L-phenylalanine methyl ester (0·20 g) in trifluoroacetic acid (10 ml) was kept at room temperature for 10 min and evaporated. The residue was triturated with ether to deposit a solid which was crystallised from methanol–ether. Yield, 0·1 g (48·5%); m.p. 165—167°C; $[\alpha]_D$ +27·1° (*c* 0·24; methanol). For $C_{18}H_{25}F_3N_2O_5$ (406·4) calculated: 53·19% C, 6·20% H, 6·89% N; found: 53·10% C, 6·41% H, 6·93% N.

2-Nitrobenzenesulfenyl-L-prolyl-L-tert-leucyl-glycine Ethyl Ester

At −10°C, 2-nitrobenzenesulfenyl-L-proline dicyclohexylammonium salt (1·84 g) and N,N′-dicyclohexylcarbodiimide (0·85 g) was added into a solution of L-tert-leucyl-glycine methyl ester hydrochloride (1·05 g) in dichloromethane (35 ml), the whole stirred at −10°C for 30 min, and then kept at 0°C for 3 days. The N,N′-dicyclohexylurea was filtered off and washed with dichloromethane. The filtrate and washings were combined, washed with 0·5M-H_2SO_4 and 5·0M-$NaHCO_3$, dried over anhydrous magnesium sulfate, and evaporated under diminished pres-

sure. Yield, 1·3 g (67%); m.p. 146–147°C; $[\alpha]_D$ −19·9° (*c* 0·52; dimethylformamide). For $C_{21}H_{30}N_4O_6S$ (466·5) calculated: 54·06% C, 6·48% H, 12·01% N; found: 54·14% C, 6·47% H, 12·07% N.

2-Nitrobenzenesulfenyl-L-prolyl-L-tert-leucyl-glycine Amide

The tripeptide (1 g) from the preceding experiment was allowed to stand in methanolic ammonia (25 ml) for two days. The reaction was checked by thin-layer chromatography on silica gel (98 : 2 dichloromethane–ethanol). The reaction mixture was evaporated under diminished pressure and the residue crystallised from dichloromethane–light petroleum. Yield, 0·8 g (85%); m.p. 103–105°C; $[\alpha]_D$ −18·8° (*c* 0·50; dimethylformamide). For $C_{19}H_{27}N_5O_5S$ (437·5) calculated: 52·16% C, 6·22% H, 16·00% N; found: 52·50% C, 6·58% H, 15·36% N.

L-Prolyl-L-tert-leucyl-glycine Amide

The tripeptide amide (0·6 g) from the preceding experiment was dissolved in methanol (10 ml) and the solution was treated with 3M ethereal hydrogen chloride (3 ml). The removal of the 2-nitrobenzenesulfenyl group was checked by thin-layer chromatography on silica gel (benzene). The mixture was evaporated, the residue (0·4 g; 90%) dissolved in 50% aqueous methanol, and the solution applied to a column of Amberlite IR-410 (OH^-) ion exchange resin. The column was eluted with 50% aqueous methanol and the eluate freeze-dried. Yield, 0·20 g (56%), $[\alpha]_D$ −35·7 (0·22; water). For $C_{13}H_{24}N_4O_4.CH_3OH$ (316·4) calculated: 53·14% C, 8·92% H, 17·70% N; found: 52·99% C, 8·92% H, 17·32% N. Amino acid analysis: Pro 1·07, Tle 1, Gly 0·94.

L-Pyroglutamyl-L-histidyl-L-tert-leucine Amide

A suspension of L-pyroglutamyl-L-histidine hydrazide (0·32 g) in dimethyl sulfoxide (4·5 ml) and dimethylformamide (6·8 ml) was treated at 0°C with 6·4M hydrogen chloride in tetrahydrofuran (1·1 ml) and then at −30°C with butyl nitrite (0·23 ml), L-tert-leucine amide (0·150 g), and triethylamine (1 ml). The mixture was stirred at −20°C for 30 min and then kept at 0°C for three days. The triethylamine hydrochloride was filtered off and washed with dimethylformamide. The filtrate and washings were evaporated under diminished pressure, the residue triturated with ether (40 ml) and tetrahydrofuran (40 ml), the resulting oil dissolved in a minimum volume of methanol, and the solution precipitated with ether (40 ml) and tetrahydrofuran (40 ml). Since the precipitate could not be filtered, the material was dissolved in 2·5 : 10 : 1 000 acetic acid–pyridine–water (5 ml; pH 5·7) and the solution chromatographed on Sephadex CM (detection with the Pauli reagent). Elution with the above buffer solution yielded 0·035 g of a fraction containing (amino-acid analysis) glutamic acid and histidine only. Elution with 3% aqueous acetic acid afforded 0·4 g of an amorphous material which was dissolved in water and freeze-dried. Yield, 0·32 g (78%) of the L-pyroglutamyl-L-histidyl-L-tert-leucine amide, homogeneous on chromatography in 1 : 1 : 4 butanol–acetic acid–water or 30 : 20 : 6 : 12 butanol–pyridine–acetic acid–water; $[\alpha]_D$ −17·4 (*c* 0·56; acetic acid). Amino acid analysis: Glu 1·08, His 0·92, Tle 1·00. For $C_{17}H_{26}N_6O_4.CH_3COOH.H_2O$ (456·5) calculated: 49·99% C, 7·07% H, 18·41% N, found: 50·02% C, 6·82% H, 18·22% N.

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