AN ALTERNATIVE ROUTE TO N^{α}-METHYLAMINO ACID DERIVATIVES: SYNTHESIS AND CONFORMATION OF SOME N^{α}-ACETYL-N^{α}-METHYLAMINO ACID METHYLAMIDES*,**

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The methylation of tert-butyl- or isopropyl- esters of N^{α}-4-toluenesulfonyl-amino acids by dimethylsulfate in aqueous alkaline solution in the presence of a detergent gives the corresponding N^{α}-methylderivatives. Using this synthetic route the N^{α}-acetylderivatives of MeAla, MeLeu, MeOrn and MePhe methylamides have been prepared and the solution conformation of these dipeptide units has been investigated. ^{1}H and ^{13}C NMR spectra show that, while in dimethylsulfoxide the tertiary amide group is mostly in *cis*-conformation, in protic solvents the equilibrium is shifted towards *trans*-conformation. Circular dichroism spectra reveal some specific features (e.g. high band intensities in protic solvents) which cannot be explained solely on the basis of the increased rigidity caused by the N^{α}-methyl group. We tried to explain these effects supposing that the tertiary amide group is deviated from the planar arrangement (due to the interaction between the methyl groups, a substituent on C^{α} and a solvent molecule attached to the oxygen atom) and contributes to the observed circular dichroism as the inherently chiral chromophore.

N^{α}-Methylamino acids are useful for studies of the effect of structural modifications on biological and physical properties of peptides. The N^{α}-methyl group limits flexibility of the peptide backbone and diminishes the number of accessible conformers. Thus, such a modification influences the interactions of the given peptide with receptor and, consequently, also markedly changes biological activities with respect to non-methylated precursors. Simultaneously the peptide analogs containing N^{α}-methylamino acids are more resistant to degradation by proteolytic enzymes. For the above reasons the N^{α}-methylamino acids are also frequently used as a suitable tool for conformational studies of

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** The nomenclature and symbols follow published recommendations[1]. Additional abbreviations include: DCHA, dicyclohexylamine; ACN, acetonitrile; TFE, 2,2,2-trifluoroethanol; HFP, 1,1,1,3,3,3-hexafluoro-2-propanol; DMSO, dimethylsulfoxide.

amino acid and peptide derivatives and an adequate attention is paid to their synthesis.

The original N^{α}-methylation of N^{α}-Tos-amino acids[2] has been gradually modified[3-8] in order to employ milder reaction conditions. This means especially a methylation of Boc- or Z-amino acids by CH_3I and NaH or Ag_2O at room or slightly elevated temperature. However, applying these procedures we often encountered a problem of product inhomogeneity which neccessitated further chromatographic purification of the resulting N^{α}-methylated derivatives.

In this paper we describe a modification of the above procedures leading to pure N^{α}-methylamino acid derivatives which can be utilized in further synthesis of peptides. At mild reaction conditions tert-butyl[9] or isopropylesters[10] of N^{α}-Tos-amino acids[11] were methylated by dimethylsulfate in alkaline aqueous medium. The mixing of organic and aqueous phases was facilitated by a detergent. In this way, after extraction with ethyl acetate, the N^{α}-methylderivatives *Ia–e* were obtained in almost quantitative yields. The traces of non-methylated material either remained in the aqueous phase of the reaction mixture or were removed by washing the ethyl acetate solution with 4 M-NaOH. The usefulness of this method as a simple and easy preparation of N^{α}-methylamino acid derivatives is demonstrated by a synthesis of methylamides of N^{α}-acetyl-N^{α}-methylamino acids *IVa–e*.

The compounds with free carboxyl group which afforded crystalline salts *IIa–e* with dicyclohexylamine were obtained from the protected derivatives *Ia–e* either by a reaction with trifluoroacetic acid (*Ia–c*) or by refluxing with a mixture of hydrochloric acid and dioxane (*Id, Ie*). The N^{α}-amino group of the MeOrn derivative was protected with tertiary butyl group prior to formation of the DCHA salt[12]. The condensation reaction of *IIa–e* with methylamine hydrochloride by dicyclohexylcarbodiimide and 1-hydroxybenzotriazole afforded methylamides *IIIa–e*. The N^{α}-Tos groups were removed by calcium in liquid ammonia and the resulting N^{α}-methylaminoderivatives were acetylated in the presence of triethylamine (the compounds *IVa–e*). The N^{α}-Boc group of *IVe* was removed by trifluoroacetic acid (compound *IVg*). For the comparison of $[\alpha]_D$ values of the corresponding N^{α}-methylamino acids the esters *Ia–e* were hydrolysed by hydrobromic acid in the presence of phenol. The N^{α}-methylamino acids were liberated by ion-exchange chromatography.

The so-called dipeptide units, methylamides of N-acetylamino acids, represent the most simple fragment of a peptide chain which still involves all the decisive interactions between a side chain of the coded amino acid residue and the neighbouring amide groups. The investigation of these models provides fundamental information for conformational studies of larger peptides and proteins. However, these compounds are rather flexible and conclusions on their conformation are often ambiguous (cf. e.g.[13]). On the other hand, the

N^{α}-methylderivatives of dipeptide units (compounds *IVa–d, IVg*) should be less flexible especially as regards torsion around the N^{α}–C^{α} bond. At the same time there are new unfavorable steric interactions within the tertiary amide group. The group possesses only limited ability to interact with the carboxy terminal secondary amide group and/or with the solvent. In addition, different electronic structure of the tertiary amide group influences spectral properties. (The amide π–π^*transitions are no more degenerate). Hence, the N^{α}-methylderivatives of dipeptide units appear to be interesting model compounds for conformational investigation in solution using NMR spectroscopy and circular dichroism. Two compounds from this series (*IVa, IVd*) have been already investigated by Ivanov et al., also by CD[14] and NMR[15]. In this work we present a more complete set of compounds exhibiting varying steric requirements and structure of side chains. The experimental conditions are also extended including further solvents and a dependence of spectra on temperature. The set of diamides under study includes also methylamides of N^{α}-acetylornithine (*IVh*) and N^{α}-acetylphenylalanine (*IVi*).

Acetylphenylalanine methylamide (*IVi*) was prepared according to the published procedure[17]. Acetylornithine methylamide (*IVh*) was synthesized from N^{α}-Tos-ornithine[18] by successive N^{δ}-Boc protection[12], esterification according to Brenner (*If*), reaction with methylamine (*IIIf*), N^{α}-deprotection by calcium in liquid ammonia, acetylation in the presence of triethylamine (*IVf*) and N^{δ}-deprotection by trifluoroacetic acid (*IVh*).

Tos-X-OR		Tos-X-OH.DCHA		Tos-X-$NHCH_3$		Ac-X-$NHCH_3$	
I		*II*		*III*		*IV*	
Ia–IVa,	X = MeLeu;	R = Bu^t		*IIe–IVe*,	X = MeOrn(Boc)		
Ib–IVb,	X = MeVal;	R = Bu^t		*IIIf–IVf*,	X = Orn(Boc)		
Ic–IVc,	X = MePhe;	R = Bu^t		*IVg*,	X = MeOrn		
Id–IVd,	X = MeAla;	R = Pr^i		*IVh*,	X = Orn		
Ie,	X = MeOrn(Ac);	R = Pr^i		*IVi*,	X = Phe		
If,	X = Orn(Boc);	R = CH_3					

EXPERIMENTAL

Analytical samples were dried over phosphorus pentoxide in vacuo at room temperature. Melting points were determined on a Kofler block and are uncorrected. Thin-layer chromatography was performed on Silufol plates (Kavalier, Czechoslovakia) in the following solvent systems: 2-butanol–98% formic acid–water (75:13.5:11.5) (S1); 2-butanol–25% aqueous ammonia–water (85:7.5:7.5) (S2); 1-butanol–acetic acid–water (4:1:1) (S3) and 1-butanol–pyridine–acetic acid–water

(15:10:3:6) (S4). Electrophoreses were carried out on Whatman 3MM paper (moist chamber, 20V/cm, 1h) in 1 M acetic acid (pH 2.4) and in a pyridine–acetate buffer (pH 5.7). The detection was with ninhydrin or by chlorination method. Optical rotations were determined on a Perkin Elmer 141 MCA polarimeter.

Esters of N^{α}-4-Toluenesulfonyl-N^{α}-methylamino Acids (*Ia–e*, Table I)

To the isopropyl- or tert-butylester of N^{α}-4-toluenesulfonylamino acid (50 mmol) in 4 M-NaOH (33 ml) a detergent (1.5 ml) and dimethylsulfate (23 ml, 250 mmol) were added at 0°C under stirring. The mixture was stirred at room temperature for 4 h while 4 M-NaOH was being added in order to maintain the alkaline pH (3 × 3.3 ml). Then the mixture was extracted with ethyl acetate (3 × 100 ml), the extract was washed with 4 M-NaOH (2 × 100 ml), saturated sodium sulfate solution (3 × 100 ml), dried by anhydrous sodium sulfate and taken down, yielding chromatographically uniform oily products (with the exception of *Ib*). The products did not show NH vibrations (3345 cm^{-1}). The structure of compounds *Ia–e* was confirmed by mass spectra.

Dicyclohexylammonium Salts of N^{α}-4-Toluenesulfonyl-N^{α}-methylamino Acids (*IIa–e*, Table II)

Hydrolysis of esters Ia–e : The tert-butylesters *Ia–c* (50 mmol) were dissolved in trifluoroacetic acid (20 ml) and after 2 h standing the mixtures were taken down. The isopropylester *Id* (30 g, 98

TABLE I
Esters of N^{α}-4-toluenesulfonyl-N^{α}-methylamino acids[a] *Ia–e*

Compound (Yield, %)	M.p., °C	$[\alpha]_D^{22}$ [b]	Formula (M.w.)	Calculated/Found			
				%C	%H	%N	%S
Ia	c. 20[c]	−31.9°	$C_{18}H_{29}NO_4S$	60.82	8.22	3.94	9.02
(85)			(355.5)	61.10	8.24	3.79	8.62
Ib	62–63[d]	−33.1°	$C_{17}H_{27}NO_4S$	59.79	7.97	4.10	9.39
(97)			(341.5)	59.62	8.07	3.99	9.71
Ic	oil	–	$C_{21}H_{27}NO_4S$	64.76	6.99	3.60	8.23
(99)			(389.5)	65.08	7.12	3.53	8.11
Id	oil	−32.2°	$C_{14}H_{21}NO_4S$	56.16	7.07	4.68	10.71
(98)			(299.4)	55.93	7.14	4.87	10.35
Ie	oil	−19.9°	$C_{18}H_{28}N_2O_5S$	56.23	7.34	7.29	8.34
(94)			(384.5)	55.97	7.38	7.02	8.63

[a] The N^{α}-methylamino acids obtained by the acidic hydrolysis of esters *Ia–e* exhibited $[\alpha]_D$ values (measured in 6M-HCl, *c* 1.0) comparable to the published data: MeLeu +30.9°, MeVal +32.3°, MePhe +26.8°, MeAla +11.2° (ref.[9]), MeOrn +20.3° (ref.[3]); [b] measured in dimethylformamide; [c] the compound crystallized upon cooling the oily product at 0°C; [d] light petroleum.

mmol) was refluxed for 2 h with 4 M solution of hydrochloric acid in dioxane (680 ml) and the mixture was taken down. The isopropylester *Ie* (50 g, 131 mmol) was refluxed in 4 M hydrochloric acid for 4 h (850 ml), the product was after cooling extracted with ethyl acetate (2 × 200 ml) and the aqueous solution was taken down. In all the above cases the oily residues were evaporated three times with water and three times with benzene. The oily derivatives of leucine, valine and phenylalanine were directly converted to dicyclohexylammonium salts.

N^{α}-4-*Toluenesulfonyl*-N^{α}-*methylalanine* was obtained as a crystalline compound, m.p. 117–122°C. Recrystallization from ethyl acetate–benzene afforded the pure product, m.p. 121–123°C, yield 87 %. $[\alpha]_D^{23}$ +11.1° (*c* 0.5, dimethylformamide). For $C_{11}H_{15}NO_4S$ (257.3) calculated: 51.34% C, 5.87% H, 5.44% N, 12.46% S; found: 51.28% C, 5.94% H, 5.29% N, 12.22% S.

N^{α}-4-*Toluenesulfonyl*-N^{α}-*methylornithine* was obtained, after pH adjustement to 6.5 with ammonia of the aqueous solution of the hydrolysis product, as a crystalline compound, m.p. 175–182°C. Recrystallization from aqueous ethanol afforded 71% of the pure product, m.p. 180–182°C. TLC R_F: 0.19 (S1), 0.53 (S2), 0.48 (S3), 0.59 (S4). Electrophoresis: $E_{2.4}^{Gly}$ 0.96; $E_{5.7}^{His}$ 0.18. $[\alpha]_D^{22}$ −50.5° (*c* 0.4, dimethylformamide). For $C_{13}H_{20}N_2O_4S$ (300.4) calculated: 51.98% C, 6.71% H, 9.33% N, 10.67% S; found: 51.47% C, 6.69% H, 9.36% N, 10.47% S.

N^{α}-4-*Toluenesulfonyl*-N^{α}-*methyl*-N^{δ}-*tert-butyloxycarbonylornithine* was prepared according to Schnabel[12] from N^{α}-4-toluenesulfonyl-N^{α}-methylornithine (15g, 50 mmol) and tert-butyloxycarbonylazide (7.8 g, 55 mmol) in dioxane (10 ml) and water (10 ml) at pH 9.8. The pH of the mixture was maintained for 5 h by gradual additions of 4 M-NaOH (23 ml). The obtained sirup was chromatographically uniform, TLC R_F: 0.31 (S1), 0.76 (S2), 0.81 (S3), 0.74 (S4). $[\alpha]_D^{22}$ −8.9° (*c* 0.6, dimethylformamide). For $C_{18}H_{28}N_2O_6S$ (400.5) calculated: 53.98% C, 7.05% H, 6.99% N, 8.01% S; found: 54.12% C, 6.81% H, 6.84% N, 8.34% S.

TABLE II
Dicyclohexylammonium salts of N^{α}-4-toluenesulfonyl-N^{α}-methylamino acids *IIa–e*

Compound (Yield, %)	M.p., °C	$[\alpha]_D^{22}$ [a]	Formula (M.w.)	Calculated/Found			
				%C	%H	%N	%S
IIa	147–148[b]	−18.3°	$C_{26}H_{44}N_2O_4S$	64.97	9.23	5.83	6.63
(81)			(480.7)	65.41	9.28	5.71	6.24
IIb	162–164[c]	−18.6°	$C_{25}H_{42}N_2O_4S$	64.37	9.07	6.00	6.87
(94)			(466.7)	64.22	9.12	6.23	6.81
IIc	154–155[b]	−16.1°	$C_{29}H_{42}N_2O_4S$	67.68	8.23	5.44	6.23
(91)			(514.7)	67.71	8.05	5.67	5.98
IId	159–161[c]	−1.0°	$C_{23}H_{38}N_2O_4S$	62.98	8.73	6.38	7.30
(70)			(438.6)	63.12	8.59	6.49	7.51
IIe	71–73[b]	−13.6°	$C_{30}H_{51}N_3O_6S$	61.94	8.84	7.22	5.51
(88)			(581.8)	61.85	8.72	7.50	5.23

[a] Measured in dimethylformamide; [b] ethyl acetate–ether; [c] ethanol–ether.

Dicyclohexylamine (14.5 ml) was added dropwise to solutions of the above N^{α}-methylamino acid derivatives (80 mmol) in ethanol (25 ml) and diethylether (80 ml) at 0°C. After the precipitation started, 250 ml of ether was added and after 2 h at 0°C the product was filtered, washed with ether and recrystallized from ethanol–diethylether or ethyl acetate–diethylether.

Methylamides of N^{α}-4-Toluenesulfonyl-N^{α}-methylamino Acids (*IIIa–e*, Table III)

1-Hydroxybenzotriazole (1.5 g, 11 mmol), dicyclohexylcarbodiimide (2.3 g, 11 mmol) and the compound *II*(*a–e*) (10 mmol) were added to methylamine hydrochloride (1.1 g, 15 mmol) in dimethylformamide (50 ml) at −12°C under stirring. The mixture was stirred at −12°C for 30 min, for 2 h at 0°C and left for 18 h at +5°C. After filtration and evaporation of the solvent the oily residue was taken up in ethyl acetate, washed with 5% $NaHCO_3$ and water, dried with Na_2SO_4 and taken down. The residue crystallized after the addition of light petroleum. The product was filtered off, washed with light petroleum and recrystallized.

Methylamides of N^{α}-Acetyl-N^{α}-methylamino Acids (*IVa–e*, Table IV)

The methylamides *III*(*a–c*) (14 mmol) were reduced by calcium (1.6 g) in liquid ammonia (300 ml) till the permanent blue coloration of the reaction mixture. After 1 min the excess calcium was removed by methanol (0.4 ml, added dropwise) and the ammonia was distilled off. The residue was dissolved in iced CO_2 saturated water (250 ml), the solution was further saturated with CO_2 and filtered. The filtrate was washed with ether, concentrated at a temperature below 30°C to about 40

TABLE III
Methylamides of N^{α}-4-toluenesulfonyl-N^{α}-methylamino acids *IIIa–e*

Compound (Yield, %)	M.p., °C	$[\alpha]_D^{22}$ [a]	Formula (M.w.)	Calculated/Found			
				%C	%H	%N	%S
IIIa	92–94[b]	+7.2°	$C_{15}H_{24}N_2O_3S$	57.67	7.74	8.97	10.26
(80)			(312.4)	58.03	7.81	8.96	10.12
IIIb	177–179[b]	+33.6°	$C_{14}H_{22}N_2O_3S$	56.35	7.43	9.39	10.75
(90)			(298.4)	56.90	7.48	9.61	10.82
IIIc	103–105[b]	−21.9°	$C_{18}H_{22}N_2O_3S$	62.40	6.40	8.09	9.26
(64)			(346.5)	62.64	6.60	8.23	9.02
IIId	82–84[d]	+4.8°	$C_{12}H_{18}N_2O_3S$	53.31	6.71	10.36	11.86
(73)			(270.4)	53.60	6.74	10.38	11.65
IIIe	114–116[c]	−42.3°[e]	$C_{19}H_{31}N_3O_2S$	55.19	7.56	10.16	7.75
(73)			(413.5)	55.29	7.75	10.50	7.52

[a] Measured in dimethylformamide; [b] ethyl acetate–light petroleum; [c] aqueous ethanol; [d] benzene–light petroleum; [e] measured in chloroform.

ml and the second precipitated portion of $CaCO_3$ was filtered off through a layer of silica. The aqueous solution was taken down and the residue dissolved in dioxane (10 ml) and ether (10 ml). Triethylamine (1.7 g, 12 mmol) was added at 0°C and after dropwise addition of acetylchloride (0.9 ml, 12 mmol) the mixture was stirred at room temperature for 30 min and taken down. The residue was extracted with ethyl acetate (3 × 100 ml) and after evaporation the residue was dissolved in 30% aqueous ethanol. The solution was filtered through columns of anion exchanger Dowex 1X2 (60 ml) and cation exchanger Dowex 50X2 (60 ml). The eluate was taken down and dried in a dessicator over phosphorus pentoxide. Ether or benzene–light petroleum were added and the product which precipitated at +5°C was filtered off and washed with ether. The structure of compounds *IV*(*a*–*e*) was confirmed by mass spectra.

Methylamide of N^{τ}-Acetyl-N^{τ}-methylornithine (*IVg*)

A solution of *IVe* (0.7 g, 2.4 mmol) in trifluoroacetic acid (7.5 ml) was taken down after 10 min standing at room temperature. The residue was extracted with ether (5 × 30 ml) and boiling ethyl acetate (300 ml). The combined extracts were taken down and the residue dried over phosphorus pentoxide (0.7 g, 90%). The trifluoroacetate dissolved in water was poured on a column of Amberlite IR4B (12 ml). The aqueous eluent (50 ml) was taken down affording *IVg* as a foam (0.43 g). $E_{2.4}^{Gly}$ 1.3; $E_{5.7}^{His}$ 1.0. TLC R_F: 0.14 (S1), 0.12 (S3), 0.38 (S4). $[\alpha]_D^{23}$ −32° (*c* 0.5, dimethylformamide). For $C_9H_{19}N_3O_2$ (201.3) calculated: 53.71% C, 9.53% H, 20.88% N; found: 53.92% C, 9.41% H, 21.01% N.

TABLE IV
Methylamides of N^{τ}-acetyl-N^{τ}-methylamino acids *IVa*–*e*

Compound (Yield, %)	M.p., °C	$[\alpha]_D^{22}$ [a]	Formula (M.w.)	Calculated/Found		
				%C	%H	%N
IVa	76–79[b]	−232.7°	$C_{10}H_{20}N_2O_4$	59.97	10.07	13.99
(60)			(200.3)	60.01	10.33	14.10
IVb	76–78[c]	−282°	$C_9H_{18}N_2O_2$	58.04	9.74	15.08
(61)			(186.3)	57.61	9.91	14.73
IVc	51–52[b]	−135.2°	$C_{13}H_{18}N_2O_2$	66.64	7.74	11.95
(45)			(234.3)	66.85	7.89	11.88
IVd	67–68[c]	−285°	$C_7H_{14}N_2O_2$	53.14	8.97	17.70
(45)			(158.2)	52.86	8.99	18.00
IVe	foam	−41.4°[e]	$C_{14}H_{27}N_3O_4$	55.80	9.02	13.94
(32[d])			(301.4)	55.50	9.29	14.04

[a] Measured in chloroform; [b] benzene–light petroleum; [c] ether; [d] R_F: 0.50 (S1), 0.54 (S2), 0.57 (S3), 0.66 (S4); [e] measured in dimethylformamide.

Hydrolysis of Esters *Ia–e*

The esters *I(a–e)* were boiled with 42% hydrobromic acid (18 ml) and phenol (3.6 g) for 30 min (in the case of *Ie* 10 h). The aqueous layer was separated, washed with ether and taken down. The evaporation with water was repeated three times. The hydrobromide was dissolved in water and poured on a column of Dowex 50WX2. Bromide ions were washed away with water and the N^{γ}-methylamino acid was eluted with 10% pyridine (200 ml). Optical rotations of these N^{γ}-methylamino acids are compared with published data in a footnote to Table I.

N^{γ}-Methylornithine was liberated from the hydrobromide using the ion exchanger IRA 410 and water elution. After evaporation ethanol was added and the crystals of N^{α}-methylamino acid were filtered off and recrystallized from aqueous ethanol.

Acetylphenylalanine Methylamide (*IVi*)

Acetylchloride (4.0 ml, 50 mmol) and triethylamine (7.0 ml, 50 mmol) were added within 15 min (under stirring at −40°C) to a solution of phenylalanine methylester hydrochloride (10.8 g, 50 mmol) and triethylamine (7.0 ml, 50 mmol) in chloroform (70 ml). The mixture was stirred at room temperature for 30 min, taken down and extracted with ethyl acetate (3 × 50 ml). Evaporation of the combined extracts yielded 10.8 g of acetylphenylalanine methylester, m.p. 85–89°C, after recrystallization from ethyl acetate–light petroleum 10.3 g (87%), m.p. 89–91°C; $[\alpha]_D^{22}$ +16.8°(*c* 0.5, chloroform).

To a solution of the methylester (2.4 g, 10 mmol) in methanol (2 ml) 10% solution of methylamine in methanol (5 ml) was added and the mixture was allowed to stand for 12 h at 0°C. The solvent was taken down and the residue twice evaporated with methanol. The methylamide *IVi* crystallized in the refrigerator from methanol–ether. Yield 2.1 g, m.p. 197–203°C, after recrystallization 1.9 g, m.p. 205–207°C, $[\alpha]_D^{23}$ −9.9° (*c* 0.5, chloroform). For $C_{12}H_{16}N_2O_6$ (220.3) calculated: 65.43% C, 7.32 % H, 12.72% N; found: 65.31% C, 7.44% H, 12.57% N.

Methylester of N^{γ}-4-Toluenesulfonyl-N^{δ}-tert-butyloxycarbonylornithine (*If*)

N^{γ}-4-Toluenesulfonyl-N^{α}-tert-butyloxycarbonylornithine (19.3 g, 50 mmol), prepared according to Schnabel[12], was dissolved in dioxane (100 ml) and converted to the methylester *If* (19.0 g, m.p. 91–94°C) by a reaction with diazomethane in ether. Crystallization from ethyl acetate–light petroleum afforded 18.8 g (94%), m.p. 93–95°C, $[\alpha]_D^{23}$ −13.7° (*c* 0.5, dimethylformamide). For $C_{18}H_{28}N_2O_6S$ (708.5) calculated: 53.98% C, 7.05% H, 7.00% N, 8.01% S; found: 53.71% C, 7.22% H, 7.12% N, 7.74% S.

Methylamide of N^{γ}-4-Toluenesulfonyl-N^{δ}-tert-butyloxycarbonylornithine (*IIIf*)

A reaction of the methylester *If* (16 g, 40 mmol) with 10% methanolic methylamine (20 ml) in methanol (10 ml) afforded the methylamide *IIIf* (13.2 g, m.p. 154–159°C). Crystallization from methanol–ether afforded 11.8 g (74%), m.p. 159–161 C, $[\alpha]_D^{23}$ +16.6° (*c* 0.5, dimethylformamide). For $C_{18}H_{29}N_3O_5S$ (399.5) calculated: 54.11% C, 7.31% H, 10.51% N, 8.02% S; found: 53.92% C, 7.58% H, 10.57% N, 8.24% S.

Methylamide of N^{γ}-Acetyl-N^{δ}-tert-butyloxycarbonylornithine (*IVf*)

The N^{γ}-4-toluenesulfonyl group of the amide *IIIf* (3.99 g, 10 mmol) was removed using the same procedure as for the compounds *IVa–e* (2.24 g, 85%). $E_{2,4}^{Hip}$ 0.72; $E_{5,7}^{Hip}$ 0.93. TLC R_F: 0.48 (S1), 0.48

(S2), 0.60 (S3), 0.68 (S4). The free base (3.2 g, 12 mmol) was acetylated (as in the case of *IVa–e*) to give 2.9 g of the product, m.p. 105–110°C. Precipitation from ethanol–ether gave 1.8 g (52%) of pure *IVf*, m.p. 108–111°C. TLC R_F: 0.59 (S1), 0.70 (S2), 0.71 (S3), 0.78 (S4). $[\alpha]_D^{23}$ +1.7° (*c* 0.5, chloroform). For $C_{13}H_{25}N_3O_4 . 1/2\ H_2O$ (296.4) calculated: 52.68% C, 8.84% H, 14.17% N; found: 52.69% C, 8.75% H, 14.14% N.

Acetylornithine Methylamide (*IVh*)

The procedure described for the preparation of *IVg* was used to remove the N^{α}-tert-butyloxycarbonyl group from *IVf* (0.83 g, 2.8 mmol) which gave the trifluoroacetate of *Vf* (0.69 g, 81%). $E_{2.4}^{His}$ 0.84; $E_{5.7}^{His}$ 1.00. $[\alpha]_D^{23}$ −23.4° (*c* 0.2, dimethylformamide). For $C_8H_{17}N_3O_2 . CF_3CO_2H$ (301.3) calculated: 39.87% C, 6.02% H, 13.95% N; found: 40.08% C, 6.57% H, 13.66% N. The methylamide *IVh* was liberated from the trifluoroacetate (0.45 g) similarly as in the case of *IVg* affording 0.28 g of the foam like product. TLC R_F: 0.19 (S1), 0.14 (S3), 0.45 (S4). For $C_8H_{17}N_3O_2$ (187.2) calculated: 51.32% C, 9.15% H, 22.44% N; found: 51.08% C, 9.12% H, 22.61% N.

Spectroscopic Measurements

^1H and ^{13}C NMR spectra were measured on a FT NMR spectrometer Varian XL-200 (^1H at 200 MHz, ^{13}C at 50.31 MHz) in deuteriochloroform, 1,1,1,3,3,3-hexafluoro-2-propanol and hexadeuteriodimethylsulfoxide with tetramethylsilane as an internal reference.

CD Spectra were measured on a Roussel-Jouan Dichrographe CD 185/II and a Jobin Yvon Autodichrographe Mark V. The measurements were performed in quartz cells with the optical path length of 0.01 – 1 cm either at room temperature or as a function of temperature using a Jobin Yvon Variocryostat. The concentration of solutions was about 1.5×10^{-3} mol l^{-1} and the solvents included 0.01 M phosphate buffer, 2,2,2-trifluoroethanol, 1,1,1,3,3,3-hexafluoro-2-propanol, acetonitrile and dimethylsulfoxide. The measurements in dimethylsulfoxide were taken in a cell of 0.001 cm path length at a concentration of about 7×10^{-3} mol l^{-1}. The spectral data are given as molar ellipticities [Θ] (deg $cm^2 dmol^{-1}$).

RESULTS AND DISCUSSION

NMR *Spectra*

Both ^1H and ^{13}C NMR spectra of the three investigated diamides (*IVa, IVb, IVd* – all with aliphatic side chains) revealed an equilibrium between the *cis*- and *trans*- conformers of the tertiary amide group (Tables V, VI, cf. also Ivanov et al.[15]). For all three compounds the *cis*- conformer population is high in dimethylsulfoxide (up to 35%) and does not depend markedly on the bulkiness of the side chain. On the other hand, the *cis*- conformer population is low in both protic solvents (especially in hexafluoropropanol) and decreases slightly with increasing substituent volume. The cause of this conformational transition consists in close contacts between the neighbouring methyl groups. The difference in population of the *cis*- conformer in dimethylsulfoxide and hexafluoropropanol (or chloroform) is probably related to hydrogen bonding of the protic solvent to oxygen atoms of the carbonyl groups. In a protic solvent

TABLE V

Proton NMR data of compounds CH_3 CO $N(CH_3)$ CHR CO NH CH_3

Solvent	Isomer (%)	CH_3CO	NCH_3	$C^{\alpha}H$	$J(\alpha,\beta)$	$C^{\beta}H$	$J(\beta,\beta)$	$J(\beta,\gamma)$	$C^{\gamma}H$	$J(\gamma,\delta)$	$C^{\delta}H$	CONH	J(NH, CH_3)	CH_3
						Ala (R = CH_3)								
$CDCl_3$	*trans* (87%)	2.13	2.94	5.15	7.15	1.32	–	–	–	–	–	6.55	4.8	2.77
	cis (13%)	2.14	2.80	4.42	7.0	1.46	–	–	–	–	–	6.97	4.8	2.84
$(CF_3)_2CHOH$	*trans* (90%)	2.02	2.83	4.89	7.15	1.26	–	–	–	–	–	6.32	5.0	2.70
	cis (10%)	2.04	2.75	*a*	7.0	1.34	–	–	–	–	–	6.01	5.0	2.74
$(CD_3)_2SO$	*trans* (70%)	2.01	2.82	4.93	6.8	1.18	–	–	–	–	–	7.60	4.5	2.56
	cis (30%)	2.01	2.64	4.39	6.8	1.27	–	–	–	–	–	7.85	4.6	2.60
						Leu (R = CH_2 $CH(CH_3)_2$)								
$CDCl_3$	*trans* (92%)	2.12	2.93	5.08	8.8; 6.6	1.73; 1.62	14.0	8.4; 5.4	1.45	6.5; 6.5	0.94; 0.90	6.38	4.8	2.76
	cis (8%)	2.16	2.80	4.29	10.2; 4.6	*b* *b*	*b*	*b* *b*	*b*	*b* *b*	*b* *b*	6.61	4.8	2.83
$(CF_3)_2CHOH$	*trans* (94%)	2.07	2.89	5.06	9.6; 6.0	1.71; 1.56	14.0	8.1; 5.2	1.36	6.5; 6.4	0.94; 0.90	6.48	4.9	2.73
	cis (6%)	2.13	2.81	*a*	*a* *a*	*b* *b*	*b*	*b* *b*	*b*	6.6; 6.6	0.97; 0.94	6.06	4.8	2.78

$(CD_3)_2SO$	*trans* (79%)	2.02	2.82	4.99	9.6; 6.1	1.60; 1.51	14.0	8.2; 4.8	1.37	6.6; 6.3	0.88; 0.83	7.64	4.5	2.55
	cis (21%)	2.03	2.69	4.22	8.8; 6.1	*c* *c*	*c*	*c* *c*	*c*	6.6; 6.4	0.91; 0.89	7.94	4.6	2.60
						Val (R = $CH(CH_3)_2$)								
$CDCl_3$	*trans* (97%)	2.12	3.00	4.53	11.3	2.30	–	6.4; 6.7	0.95; 0.84	–	–	6.45	4.9	2.76
	cis (3%)	2.20	2.89	3.71	10.2	*b*	–	6.5; 6.8	1.03; 0.89	–	–	*b*	4.7	2.82
$(CF_3)_2CHOH$	*trans* (97%)	2.06	2.97	*a*	11.2	2.22	–	6.5; 6.5	0.93; 0.82	–	–	6.70	5.0	2.71
	cis (3%)	2.12	2.93	*a*	*b*	*b*	–	6.9; 6.9	0.94; 0.86	–	–	*b*	4.8	2.75
$(CD_3)_2SO$	*trans* (67%)	2.01	2.93	4.54	11.0	2.07	–	6.5; 6.6	0.83; 0.74	–	–	7.81	4.6	2.54
	cis (33%)	2.07	2.75	3.69	10.5	2.16	–	6.5; 6.7	0.85; 0.79	–	–	8.04	4.6	2.60

The value of parameter could not be determined due to: [a] overlap by strong signal of solvent; [b] very low intensity; [c] overlap by major isomer.

the *trans–cis* conformational transition would not bring an energy profit because in that case the unfavorable interactions between methyl groups would be replaced by interactions of the N^{α}-methyl group with oxygen atom having its effective volume increased by the attached solvent molecule.

Circular Dichroism Spectra

When compared to analogous compounds not having the N^{α}-methyl group[13,14] the CD spectra of diamides *IVa*, *IVb* and *IVd* (with aliphatic side chain) (Figs 1 – 5, Tables VII and VIII) are less sensitive to changes of a side-chain structure, solvent nature and/or temperature. The long wavelength amide $n-\pi^*$ band is always negative and higher in intensity tham the amide $\pi-\pi^*$ bands. In protic solvents always only one positive band is observed in the $\pi-\pi^*$ region, while in acetonitrile (and also heptane[14]) a pair of bisigned $\pi-\pi^*$ bands is detected. Of these, the long wavelength band is negative and as a shoulder or even as a maximum (Fig.1). Intensity of the $n-\pi^*$ band increases with increasing solvent polarity and with the increasing bulkiness of the side chain (however in the latter

TABLE VI
Carbon-13 NMR data of compounds CH_3 CO $N(CH_3)$ CHR CO NH CH_3 in hexadeuteriodimethylsufoxide

Isomer (%)	CH_3	CO	NCH_3	C^{α}	C^{β}	C^{γ}	C^{δ}	CONH	CH_3
					Ala (R = CH_3)				
trans (70%)	22.07	170.38	31.48	51.31	14.51	–	–	171.32	25.80
cis (30%)	21.65	169.98	28.19	55.98	15.38	–	–	170.71	25.90
					Leu (R = CH_2 $CH(CH_3)_2$)				
trans (79%)	22.05	170.54	31.35	53.56	37.15	24.53	21.60; 23.26	171.07	25.76
cis (21%)	22.14	170.04	28.43	58.59	38.10	24.53	21.71; 22.89	170.38	25.76
					Val (R = $CH(CH_3)_2$)				
trans (67%)	22.01	170.38	31.28	60.87	26.55	19.54; 18.88	–	170.64	25.48
cis (33%)	22.10	169.84	28.24	66.38	27.14	19.32; 18.62	–	170.14	25.48

case the band maximum is gradually shifted towards longer wavelength) (Table VII). In the π–π^* transition region the increasing volume of the side-chain is

TABLE VII
CD data of compounds *IVa*, *IVb*, *IVd*, *IVg*, and *IVh* in solvents of various polarity

Solvent	λ [a], nm ($[\Theta] \times 10^{-3}$, deg cm^2 dmol^{-1})	
	n–π^* band	π–π^* band
	N^{α}-Acetyl-N^{α}-methylalanine methylamide (*IVd*)	
DMSO	223.5 (−13.8)	not measured
ACN	222.5 (−17.5)	s 208 (−12.6) 190[b] (+10.3)
Water	211.5 (−19.3)	185[b] (+10.0)
TFE	219 (−30.4)	s 202 (−10.0) 186 (+14.3)
HFP	218 (−28.0)	187 (+5.0)
	N^{α}-Acetyl-N^{α}-methylleucine methylamide (*IVa*)	
DMSO	228.5 (−19.0)	not measured
ACN	225 (−25.1)	s 206 (−16) 187[b] (+20.0)
Water	217 (−23.6)	185[b] (+10.0)
TFE	221 (−41.8)	s 201 (−3.3) 188 (+14.5)
HFP	220 (−53.5)	189.5(+18.0)
	N^{α}-Acetyl-N^{α}-methylvaline methylamide (*IVb*)	
DMSO	227.5 (−44.5)	not measured
ACN	226.5 (−47.2)	s 206 (−1.1) 190.5(+24.3)
Water	221 (−57.0)	195 (+28.4)
TFE	221.5 (−67.6)	192.5(+26.0)
HFP	221 (−88.8)	192 (+43.5)
	N^{α}-Acetyl-N^{α}-methylornithine methylamide (*IVg*)	
Water	217 (−19.1)	190.5(+11.7)
TFE	219 (−26.7)	191 (+19.5)
	N^{α}-Acetylornithine methylamide (*IVh*)	
Water		190.5(+11.7)
TFE	211 (−9.6)	185[b] (+15.0)

[a] s, shoulder; [b] end value.

manifested acetonitrile by a decreaseof the negative band intensity (Fig. 1), while in protic solvents also by the intensity increase of the positive band and by a bathochromic shift of its maximum (Fig. 3).

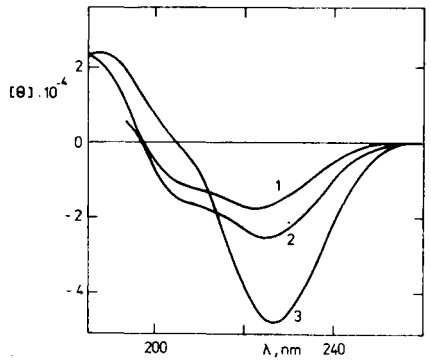

FIG. 1

CD spectra in acetonitrile of *1* N^{α}-acetyl-N^{α}--methylalanine methylamide (*IVd*), *2* N^{α}-acetyl-N^{α}-methylleucine methylamide (*IVa*) and *3* N^{α}-acetyl-N^{α}-methylvaline methylamide (*IVb*)

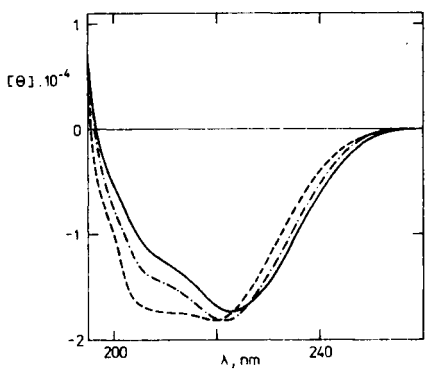

FIG. 2

Temperature dependence of CD spectra of N^{α}-acetyl-N^{α}-methylalanine methylamide (*IVd*) in acetonitrile. Temperatures: (·····) +40 C, (-·-·-·-) 0 C, (- - - -) −40 C

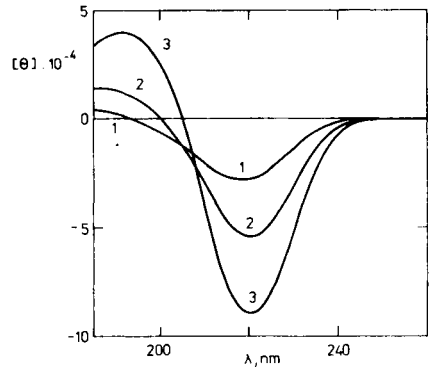

FIG. 3

CD spectra in 1,1,1,3,3,3-hexafluoro-2-propanol of *1* N^{α}-acetyl-N^{α}-methylalanine methylamide (*IVd*), *2* N^{α}-acetyl-N^{α}-methylleucine methylamide (*IVa*) and *3* N^{α}-acetyl-N^{α}--methylvaline methylamide (*IVb*)

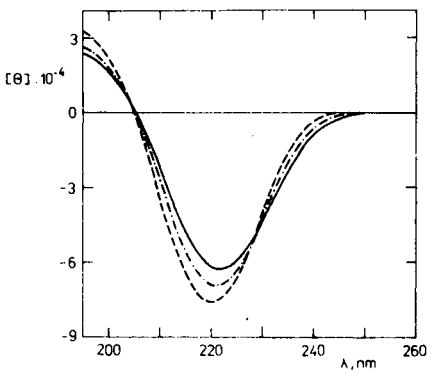

FIG. 4

Temperature dependence of CD spectra of N^{α}-acetyl-N^{α}-methylvaline methylamide (*IVb*) in 2,2,2-trifluoroethanol. Temperatures: (·····) +40 C, (-·-·-·-) 0 C, (- - - - -) −40 C

The interpretation of these spectroscopic effects in terms of conformation of dipeptide units is based mainly on a comparison with the previous study of

TABLE VIII
Temperature dependence of CD of compounds *IVd* and *IVb*

Solvent	Temperature C	λ [a], nm ($[\Theta]$) × 10^{-3}, deg cm^2 dmol^{-1}	
		n-π^* band	end value
		N^{α}-Acetyl-N^{α}-methylalanine methylamide (*IVd*)	
TFE	+40	220 (−30.4)	195 (+5.1)
	0	218 (−30.4)	195 (+5.7)
	−40	217 (−29.5)	195 (−4.0)
ACN	+40	223 (−17.5) s 211 (−12.8)	195 (+7.5)
	0	221 (−18.3) s 210 (−14.4)	195 (+7.5)
	−40	219.5(−18.0) s 208 (−17.2)	195 (+7.5)
		N^{α}-Acetyl-N^{α}-methylvaline methylamide (*IVb*)	
TFE	+40	222 (−62.5)	195 (+23.0)
	0	221 (−69.5)	195 (+26.0)
	−40	220 (−76.6)	195 (+33.0)
ACN	+40	227 (−47.5)	195 (+16.0)
	0	226.5(−54.2)	195 (+12.0)
	−40	225.5(−61.8)	195 (+9.0)

[a] s, shoulder.

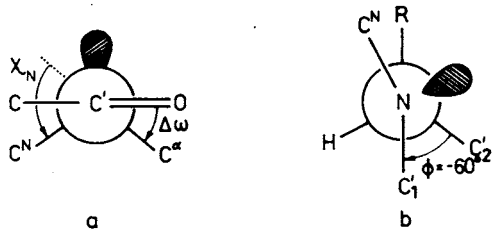

FIG. 5
The non-planar tertiary *trans*-amide group in *R* configuration; a viewed along C′—N^{α} bond; b the view along N^{α}—C^{α} bond of a diamide unit in α_R conformation ($\Phi = -60°$) showing the pyramidal arrangement of bonds on the N^{α}-atom (from a) in relation to the substituent at C^{α}

non-N^{α}-methylated diamides from this laboratory (see Maloň et al.[13]) as well as with the results of older studies by Ivanov et al. [14]and Madison and Kopple[16]. Within our set of N^{α}-methylated diamides we observe only two types of CD spectra. These can be ascribed, qualitatively, to the A and B spectral types as identified in the above cited paper[13] dealing with spectra of the more simple non-N^{α}-methylated compounds. In this work the type A spectrum is most closely approximated by the spectrum of the methylvaline derivative *IVb* in hexafluoropropanol (Fig. 3, Table VII), while the best approximation for the type B is the spectrum of the methylalanine diamide *IVd* in acetonitrile (Fig. 1, Table VII). CD spectrum of the type B (i.e. having a negative n–π^* band and a negative π–π^* couplet) has been ascribed to an equilibrium mixture of conformers involving mainly folded conformations C_7^{eq} and a 3_{10}-helix[13]. Ivanov et al.[14] assign a similar spectrum (N^{α}-methylated diamides *IVb* and *IVd* in heptane) to a mixture of C_7^{eq} and extended γ-conformations. However, the latter assignment does not respect sufficiently the strong negative $\pi-\pi^*$ band which, according to theoretical calculations[14,21], cannot correspond to C_7^{eq} conformation. Upon cooling the acetonitrile solution of *IVd* (Fig. 2, Table VIII) the intensity of the long wavelength negative band (between 205 and 210 nm, i.e. belonging to the π–π^* transition within the tertiary amide group) significantly increases, while the n–π^* band intensity does not change. Evidently, the conformation characterized by a negative π–π^* couplet is gradually stabilized. Most probably, it is a folded C_7^{ax} conformation which, according to Popov et al.[20], is markedly preferred for N^{α}-methylated diamides. Although the calculation of optical rotatory strength[21] predicts a positive n–π^* band for this conformation it is necessary to take into account that: (i) the combined conformational energy and rotational strength map (for n–π^* transition calculated for *IVd* (ref.[14]) shows the C_7^{ax} energy minimum in the close proximity of a nodal plane (i.e. a low intenzity band is to expected) and (ii) the calculation of optical activity does not include n–π^* transition perturbation induced by a side chain. Within both C_7 conformations this contribution should be negative for the C-terminal secondary amide group (which is closer in space to the substituent at C^{α}) as follows from the simple amide quadrant rule[22]. This conformational situation can be approximated using monosubstituted 2,5-piperazinediones as models (e.g. cyclo(L-Leu-Gly)). In solution these compounds assume a boat conformation with the substituent in pseudoaxial orientation ($\Phi>0$, $\Psi<0$) and their CD spectra reveal a negative n–π^* band together with a negative couplet of π–π^* bands[23].

Another distinct feature of the type B spectra of N^{α}-methylated derivatives is a much higher intensity of the n–π^* band when compared with non-N^{α}-methylated compounds. This increase can only in part be explained by a limited flexibility. The N^{α}-methylation results also in a marked red shift of the

n–π^* band maximum (4.5 nm for *IVd*, 10.5 nm for *IVb* – see data in ref.[13]). Such a shift can be explained in the best way as a consequence of superposition with a positive band lying at higher wavelength than the maximum of the negative $\pi-\pi^*$ band. This situation is most discernible in the spectrum of the methylvaline derivative *IVb* (Fig. 1). A tentative deconvolution of this spectrum to single Gaussian bands suggests that, besides the negative couplet, an intense positive band is present with the maximum above 205 nm. A significant intensity increase of this band, which also corresponds to π–π^* transition of the tertiary amide group, is related to a large intensity increase of the negative n–π^* band. The dependence on temperature of the CD of methylvaline derivative *IVb* in acetonitrile (Table VIII) is similar to the analogous dependence in trifluoroethanol (Fig. 4), i.e. with decreasing temperature the intensity of both the negative n–π^* band and the positive π–π^* band increases. Hence the conformation which is stabilized at low temperature contributes by a negative n–π^* band and by a positive bathochromically shifted π–π^* band. This conformation differs from the one preferred at the same conditions for *IVd*.

The type A spectrum which exhibits, besides the negative maximum of the n–π^* transition, only one apparent maximum in the region of π–π^* transitions has been ascribed to conformations having oxygen atoms accessible to solvation[13,16] (extended C_5 and/or distorted α_R, the latter being more probable due to high band intensities). Maloň et al.[13] observed that steric bulkiness when present either in the diamide side chain or in the solvent molecule (e.g. 1,1,1,3,3,3-hexafluoro-2-propanol) leads to a preference of this spectral type. Ivanov et al.[14,15] ascribed these spectra (*IVb* and *IVd* in water) to the extended (γ) conformation.

Band intensities in the A type spectrum of the methylvaline derivative *IVb* in hexafluoropropanol (Table VII, Fig. 3) are extraordinarily high. When compared to spectra of the analogous valine compound in the same solvent[13] the intensity is about six times higher for the n–π^* band and ten times higher for the π–π^* band. Hence it is substantially higher than values usually found for not only linear peptides[24], but also for conformationally stabilized amides (e.g. dioxopiperazines[23-25]) or regular, e.g. α-helical polypeptides[26]. Maximum of the n–π^* is observed at high wavelength (by 12 nm higher than for the non-methylated compound), despite the strongly polar solvent. This is again an indication that an additional intense positive band is present at the short wavelenght side on the n–π^* band. Deconvolution of the A type spectrum into single Gaussian components which is of course only orientational due to ambiguities inherent to this procedure reveals that a positive band is present above 205 nm if the actual position of the n–π^* band maximum is assumed to be 215 nm (cf. refs[27,18]). Other bands in the short wavelength region form probably a couplet of negative and positive bands (as in the spectrum of *IVb* in

acetonitrile – Fig. 1). Upon cooling the solution of *IVb* in trifluoroethanol (Table VIII, Fig. 4) the intensities of both the n–π^* and π–π^* bands increase. The increase is slower for the π–π^* band. Maximum of the negative band is gradually shifted towards shorter wavelengths. Hence, the type A spectrum contains a characteristic component having two intense bands: a negative n–π^* band and a less intense positive π–π^* band. The latter band corresponds, according to its position, to the tertiary amide group. Participation of this component is maximal for the methylvaline derivative *IVb* in hexafluoropropanol (type A spectrum) and decreases with (i) decreasing volume of the side chain (Figs 1 and 3), with (ii) decreasing polarity or proton donating capability of the solvent (Table VII) and with (iii) increasing temperature (Fig. 4, Table VIII).

According to our opinion the above described properties of N^{α}-methylated diamides *IVa*, *IVb* and *IVd* cannot be explained either by increased rigidity of the molecules under study or by self association of molecules in solution. The latter possibility seems particularly improbable because: (i) the N^{α}-methylation should decrease the tendency of diamides to associate; (ii) the biggest spectral effects are observed in strongly polar solvents which prevent efficiently the formation of hydrogen bonds between solute molecules; (iii) the used diamide concentrations are low; (iv) the temperature dependent CD does not reveal any fundamental change within the temperature range of 80 °C. On the other hand, the extraordinarily high band intensities can be caused by a potential non-planarity (i.e. inherent chirality) of the tertiary amide group. The non-planar deformation could be stabilized in the rigid molecule of the N^{α}-methylated diamide. In general, theoretical calculations show that the inherently chiral amide group should contribute to the overall rotational strength by a bisigned pair of n–π^* and π–π^* bands[29]. The n–π^* band should be more intense. When the type A spectrum is compared with CD spectra of the chiral amide group with defined rigid geometry (embedded in the polycyclic skeleton)[27,28] it is clear that there is a striking similarity of absolute band intensities and also of the n–π^*/π–π^* intensity ratio.

As shown by NMR spectroscopy the unfavorable steric interactions within the tertiary amide group of these diamides result, under certain conditions, in a transition towards *cis*-conformation. Another way to decrease these interactions could be a deviation of this group from planar arrangement. This could be a dominant mechanism in such situations where a transition towards *cis*-conformation cannot be realised. The main feature of such distortion is a formation of pyramidal arrangement of bonds to the amide nitrogen atom[30,31]. In accord with this idea we observe the most intense CD bands of the tertiary amide group in hexafluoropropanol where, due to its proton donating capability, the population of the *cis*-tertiary amide group is strongly suppressed (Tables V and VI). At these conditions the contribution of

the non-planar amide group appears to be maximized either by the largest deformation or by the highest population of the distorted chromophore. In order to explain the sharp increase of CD band intensities with increasing volume of the substituent on C^{α} we assume that there are significant close contacts between the N^{α}-methyl group and the amino acid side chain within the diamide conformation preferred in the protic solvent (i.e. the torsion angle Φ is close to -60). These requirements appear to be satisfied by e.g. the α_R conformation[13,16] or a similar one. Also the above mentioned contact could be diminished by non-planar deformation of the tertiary amide group. The magnitude or probability of the occurrence of his deformation would increase with increasing steric requirements of the amino acid side chain. CD spectra of the type A cannot serve as a proof of the existence of α_R conformation by themselves because the large contribution of the inherently chiral chromophore masks smaller contributions arising from the interaction of amide chromophores.

The signs of the observed CD bands of the non-planar amide group allow to estimate its chirality. As follows from the CD of model lactams[27,28] and from theoretical calculations[29–32] the negative n–π^* and the positive π–π^* bands belong to the transoid amide group characterised by torsion angles $\Delta\omega > 0$ and $\chi_V < 0$ (having the type *R* absolute configuration according to a suggestion of Bláha and Maloň[31]). In Fig. 5a this situation is depicted in Newman projection. (It is the so-called regular pyramidal arrangement, characterized by a relation $-2\Delta\omega = \chi_N$). In Fig. 5b a relation between the N^{α}-methyl group and the amino acid side chain is shown for the α_R conformation ($\Phi = -60$). The deviation of the amide group from planarity as described in Fig. 5 is capable to decrease non bonded interactions of the N^{α}-methyl group simultaneously with the acetyl group and the amino acid side chain if such interactions exist within the preferred diamide conformation.

CD spectra of ornithine diamides *IVh* and *IVg* (Fig. 6, Table V) ressemble closely the spectra of analogous diamides derived from alanine (see Table V and ref.[13]). The spectrum of acetylornithine methylamide (*IVh*) in water belongs according to Maloň et al.[13] to the 3_{10}-helical conformation, while the spectrum in trifluoroethanol is ascribed to the α_R conformation or to its mixture with the extended C_5 conformation. Fig. 6 demonstrates clearly the effect of N^{α}-methyl substitution involving the above analysed intensity increase and bathochromic shift of dichroic bands. In general, methylamide of N^{α}-acetyl-N^{α}-methylornithine (*IVg*) adopts in protic solvents the same conformation as its methylalanine analog *IVd*, i.e. probably the α_R conformation in which the tertiary amide group is, at least to some extent, non-planar. According to similar intensities of dichroic bands it follows (Table V) that steric requirements of alanine and ornithine side chains are not markedly different.

The diamides *IVi* and *IVc* derived from phenylalanine exhibit CD spectra (Fig. 7, Table IX) with significant participation of bands belonging to electronic

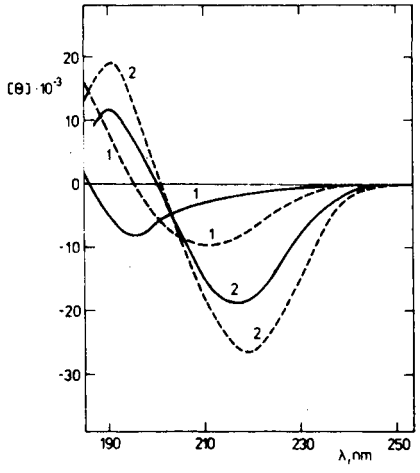

FIG. 6
CD spectra of *1* acetylornithine methylamide (*IVh*) and *2* N^{α}-acetyl-N^{α}-methylornithine methylamide (*IVg*) in water (———) and in trifluoroethanol (– – – –)

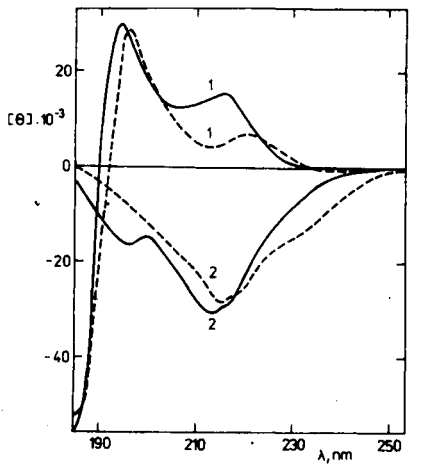

FIG. 7
CD spectra of *1* acetylphenylalanine methylamide (*IVi*) and *2* N^{α}-acetyl-N^{α}-methylphenylalanine methylamide (*IVc*) in water (———) and in acetonitrile (– – – –)

TABLE IX
CD data of compounds *IVi* and *IVc* in water and acetonitrile

Solvent	λ [a], nm ([Θ] × 10^{-3}, deg cm^2 dmol^{-1})				
	Acetylphenylalanine methylamide (*IVi*)				
Water		216(+15.8)	m 206 (+12.8)	194.5(+30.4)	185[b] (−55.2)
ACN	239(−0.5)	221 (+6.9)	m 212.5(+4.6)	196 (+28.7)	185[b] (−52.3)
	N^{α}-Acetyl-N^{α}-methylphenylalanine methylamide (*IVc*)				
Water		213[c] (−30.6)	m 200.5(−14.5)	196 (−16.9)	185[b] (−3.5)
ACN	s 228(−17.0)	215[d] (−28.4)	s 210 (−21.7)		185[b] (0.0)

[a] m Minimum, s shoulder; [b] end value; [c] additional shoulder at 216 nm; [d] additional shoulder at 218 nm.

transitions within the aromatic chromophore. These bands overlap in part with those of the amide transitions. Acetylphenylalanine methylamide (*IVi*) (cf. also Matsuura et al.[17]) exhibits positive maxima at 216 nm in water and at 221 nm in acetonitrile. These bands correspond to the B_{1u} aromatic transition. The amide n–π^* band is also probably positive in water (similarly to the acetylleucine methylamide[13], the side chain of which appears to be comparable to that of phenylalanine as far as steric requirements are concerned) and both these bands are superposed. In acetonitrile the n–π^* band possesses a negative sign (cf. also the leucine derivative[13]) and is responsible for the minimum at 212.5 nm and for the weak negative maximum (the long wavelength tail) at 229 nm. The pattern observed is a consequence of a superposition of the wider amide n–π^* band with the more narrow positive aromatic band. The sharp positive band at 194 – 196 nm belongs to the aromatic E_{1u} transition. The negative band observed at the shortest wavelengths can be assigned to amide π–π^* transition. Hence, the phenylalanine diamide *IVi* exhibits amide bands analogous to those of the leucine diamide[13] and, consequently, it possesses also similar conformations, i.e. the 3_{10}-helical conformation in aqueous solution and the same conformation in a mixture with the C_7^{eq} conformation in acetonitrile.

The intense negative bands of the methylamide of N^{α}-acetyl-N^{α}-methylphenylalanine (*IVc*) (Fig.7, Table IX) result from a superposition of the negative aromatic B_{1u} band (maximum at 213 nm, shoulder at 216 nm in water, 215 and 218 nm in acetonitrile) with the negative amide n–π^* band (shoulder at 228 nm in acetonitrile). In the short wavelength region the spectra of *IVc* are rather featureless, probably due to mutual compensation of oppositely signed bands. It is only possible to state that the negative CD dominates the 200 – 205 nm region (the π–π^* transition of the tertiary amide group) and that the short wavelength aromatic band is of lower intensity and probably also of opposite sign when compared with *IVi*.

According to circular dichroism the conformation of N^{α}-methylated diamide *IVc* is approximately the same in both solvents. Because the long wavelength amide π–π^* band has a negative sign it could be most probably the folded C conformation which is a preferred one for the N^{α}-methylated diamides. Moreover this conformation can be further stabilized by the intramolecular interaction between the aromatic ring and the amide groups in a pseudocyclic arrangement. This type of conformation and intramolecular interaction can be simulated using e.g. cyclo(L-Phe-Gly)[33] as a model. This compound also exhibits a negative n–π^* band[23]. For both compounds the decisive source of negative rotational strength of the n–π^* transition consists in its perturbation by the side chain. The intensity amplification of the negative n–π^* band as well as the compensation of the negative amide π–π^* band is probably contributed by chiral non-planar amide group as described for the previous cases.

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