THE CONFORMATIONAL STATES OF CYCLOPEPTIDE SYSTEMS. VIII. SYNTHESIS OF CYCLOHEXAPEPTIDES CONTAINING RESIDUES OF L-VALINE, L-NORVALINE, L-LEUCINE, AND GLYCINE

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We have previously investigated the conformational states of cyclic peptides constructed from residues of L(D)-alanine and glycine [1-7]. It was shown that such compounds take part in a complex conformational equilibrium which is displaced according to the primary structure of the cyclopeptide and the external conditions. A common feature of all the preferred conformations found is the presence in them of two intramolecular NH···CO hydrogen bonds of the 4 → 1 type formed by oppositely located amino acid residues and serving as a distinguishing feature of the "pleated sheet" structure. In nonpolar media, two additional hydrogen bonds are formed. However, the large number of structures participating in the equilibrium greatly complicates the interpretation of the spectral characteristics. In order to obtain a clearer picture it appeared desirable to study cyclohexapeptides with limited conformational mobility.

For investigation we selected a series of cyclopeptides which contained, in place of the methyl side groups of alanine residues, more voluminous radicals: propyl (compounds 2, 5, and 7), isobutyl (compound 3), and isopropyl (compounds 1, 4, and 6).†

Cyclo (-L-Val-Gly-L-Val-Gly-L-Val-Gly-) (1)
Cyclo (-L-Nva-Gly-L-Nva-Gly-L-Nva-Gly-) (2)
Cyclo (-L-Leu-Gly-L-Leu-Gly-L-Leu-Gly-) (3)
Cyclo (-L-Val-Gly-Gly-L-Val-Gly-Gly-) (4)
Cyclo (-L-Nva-Gly-Gly-L-Nva-Gly-Gly-) (5)
Cyclo (-L-Val-L-Val-Gly-L-Val-L-Val-Gly-) (6)
Cyclo (-L-Nva-L-Nva-Gly-L-Nva-L-Nva-Gly-). (7)

The present paper describes the synthesis of the cyclopeptides listed. Compounds (1-3) were obtained by Scheme 1 using the usual methods of peptide synthesis; the linear hexapeptides (30), (31), and (32) were prepared by the growth of the chains of the corresponding dipeptides by the mixed-anhydride method. The carboxyl functions were blocked by tert-butyl groups; the N-terminal amino acids, by benzyloxycarbonyl protective groups. Cyclization was performed with fairly high yields (38-60%, Table 1) by the p-nitrophenyl ester method. The

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†To denote the amino acid residues we used the nomenclature recommended by the International Union of Pure and Applied Chemistry [8].

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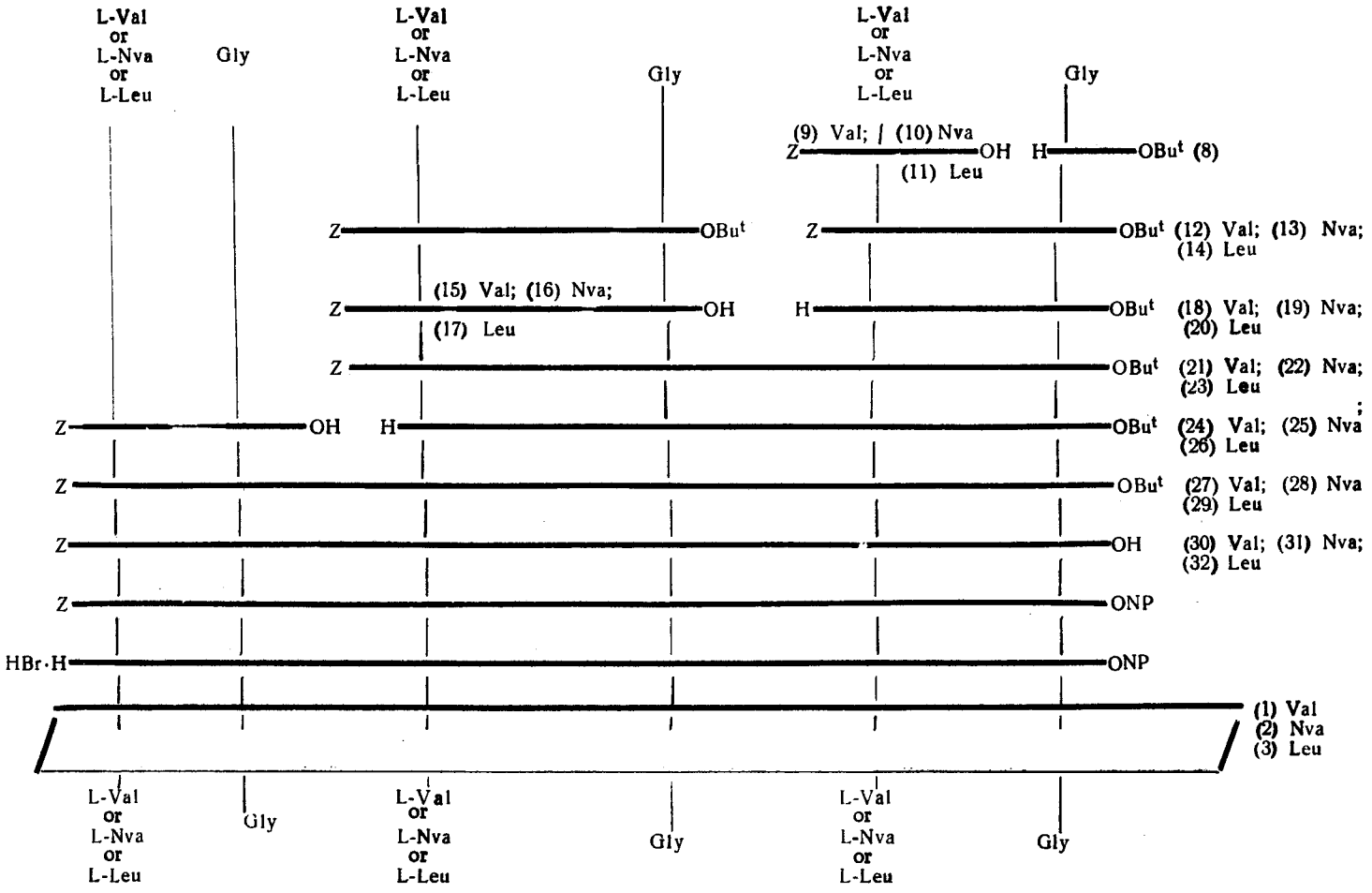
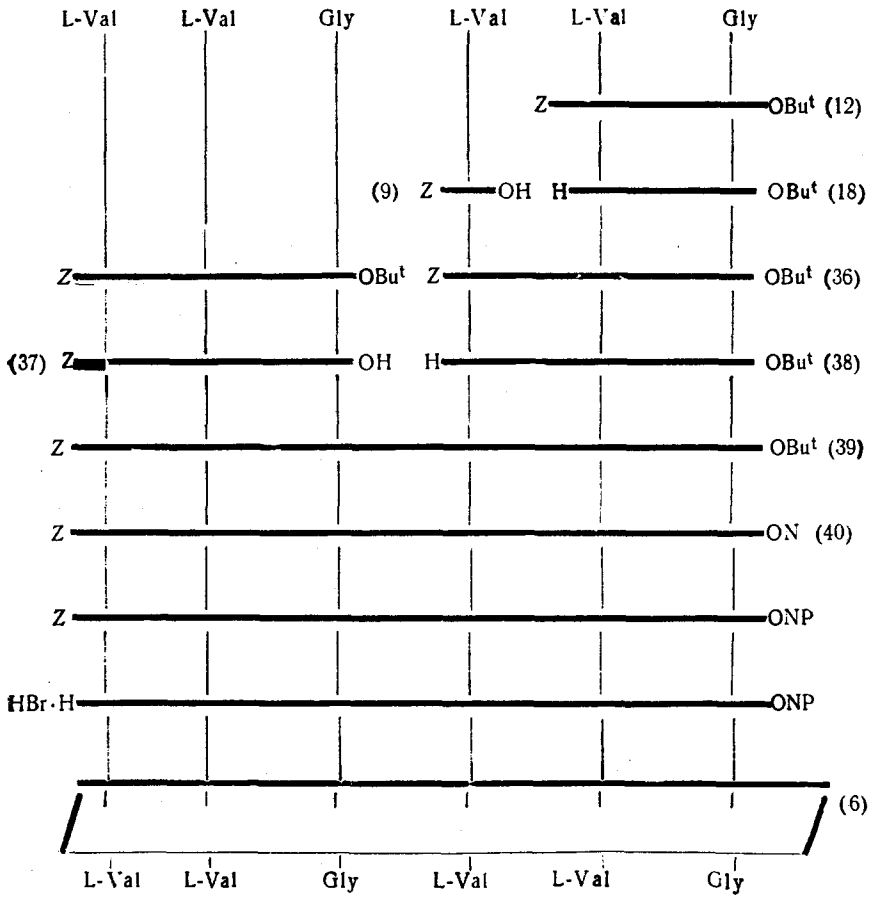
SCHEME 1


L-Val or L-Nva or L-Leu
Gly
L-Val or L-Nva or L-Leu
Gly
L-Val or L-Nva or L-Leu
Gly
(9) Val; / (10) Nva
(11) Leu
Z OH
H OBut (8)
Z OBut
Z OBut (12) Val; (13) Nva; (14) Leu
(15) Val; (16) Nva;
(17) Leu
Z OH
H OBut (18) Val; (19) Nva; (20) Leu
Z OBut (21) Val; (22) Nva; (23) Leu
Z OH
H OBut (24) Val; (25) Nva; (26) Leu
Z OBut (27) Val; (28) Nva (29) Leu
Z OH (30) Val; (31) Nva; (32) Leu
Z ONP
HBr·H ONP
(1) Val (2) Nva (3) Leu
L-Val or L-Nva or L-Leu
Gly
L-Val or L-Nva or L-Leu
Gly
L-Val or L-Nva or L-Leu
Gly

SCHEME 2



cyclopeptide (6) was obtained similarly but with the building up of the chain by the 3 + 3 method (Scheme 2).

To synthesize compounds (4), (5), and (7), we also used the so-called double reaction (Scheme 3).

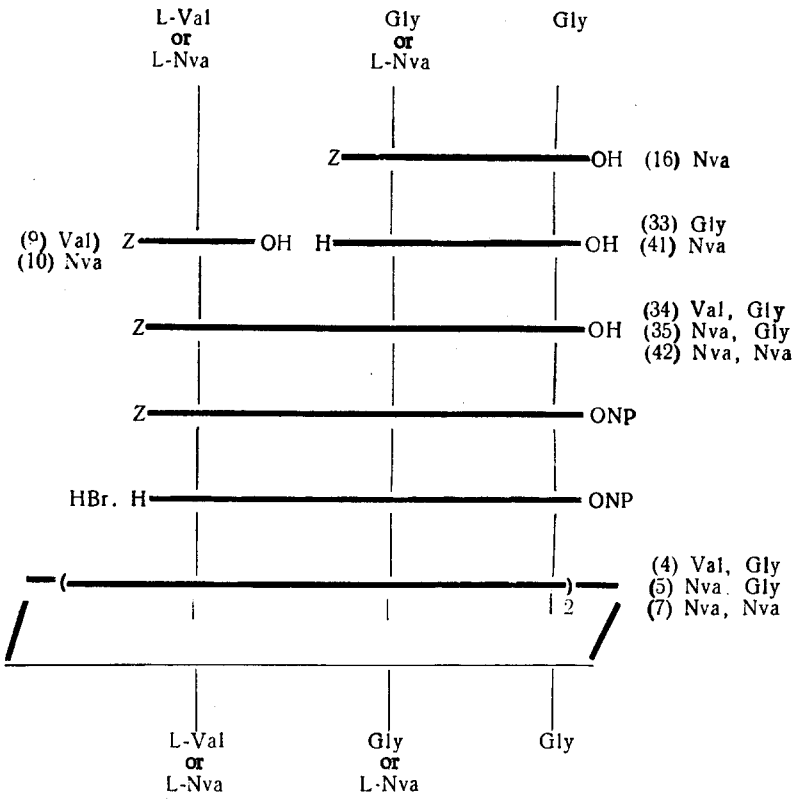
In addition to the results of thin-layer chromatography, elementary analysis, and mass spectroscopy, the structure and purity of all the cyclopeptides were confirmed by their IR and NMR spectra, which will be discussed in subsequent communications.

EXPERIMENTAL

All the melting points are uncorrected. The individuality of all the compounds obtained was checked by chromatography in alumina (activity grade II) and on Eastman silica gel plates. For all the compounds the results of elementary analysis agreed satisfactorily with the calculated figures for the C, H, and N contents. The constants and yields of the cyclopeptides are given in Table 1 and the constants of the intermediate compounds in Table 2.

1. The tert-Butyl Esters of the Benzyloxycarbonylpeptides (12)-(14). At −10°C with stirring, 12.0 ml (90 mmole) of isobutyl chloroformate was added to a solution of 90 mmole of a benzyloxycarbonylamino acid (9), (10), or (11) and 12.6 ml (90 mmole) of triethylamine in 100 ml of anhydrous chloroform. After 10 min, a suspension of 15 g (90 mmole) of the phosphite of the tert-butyl ester of glycine [9] and 12.6 ml (90 mmole) of triethylamine in 20 ml of chloroform was added to the reaction mixture. The mixture was stirred at 0°C for 5 h and at room temperature for 12 h. Then it was treated with 10% citric acid, water, and a saturated solution of $NaHCO_3$ and was dried with $MgSO_4$. After evaporation and recrystallization from a mixture of methanol and water, the yield was 60-97%.

SCHEME 3



2. The Benzyloxycarbonyldipeptides (15)-(17). A solution of 10 mmole of the tert-butyl ester of a benzyloxycarbonyldipeptide (12)-(14) in 30 ml of anhydrous CF_3COOH was kept at room temperature for 30 min and was then evaporated.

The residual oil was dissolved in absolute benzene and the solution was again evaporated. The residue was dissolved in ethyl acetate and extracted with a solution of $NaHCO_3$, the bicarbonate extracts were acidified with concentrated HCl and extracted with ethyl acetate, and the ethyl acetate extract was dried with $MgSO_4$ and evaporated. Yield 75-95%.

3. tert-Butyl Esters of the Benzyloxycarbonyltetra- and -hexapeptides (21)-(23) and (27)-(29). A solution of 10 mmole of the tert-butyl ester of a benzyloxycarbonyldi- or -tetrapeptide (12)-(14) or (21)-(23) in 60 ml of methanol was hydrogenated in the presence of palladium black until the initial substance had disappeared (monitored chromatographically). The catalyst was filtered off and the filtrate was evaporated in vacuum. The yield of the amino ester (18)-(20) or (24)-(26) was 95-100%.

At −10°C with stirring, 1.35 ml (10 mmole) of isobutyl chloroformate was added to a solution of 10 mmole of a benzyloxycarbonyldipeptide (15), (16), or (17) and 1.4 ml (10 mmole) of triethylamine in 25 ml of dry chloroform. After 10 min, a solution of an amino ester (18)-(20) or (24)-(26) in 20 ml of chloroform was added to the mixed anhydride so obtained. The subsequent working up was performed as in experiment 1. Yield 65-80%.

4. The Benzyloxycarbonyltripeptides (34) and (35). At −10°C with stirring, 6.7 ml (50 mmole) of isobutyl chloroformate was added to a solution of 50 mmole of a benzyloxycarbonyl·(amino acid) (9) or (10) and 6.95 ml (50 mmole) of triethylamine and 50 ml of absolute tetrahydrofuran. After 15 min, a solution of 6.6 g (50 mmole) of glycylglycine (33) and 6.3 g (75 mmole) of sodium bicarbonate in 30 ml of water cooled to 0°C was added to the mixed anhydride formed. The reaction mixture was stirred at 0°C for 3 h, acidified with 6 N HCl, and extracted with ethyl acetate (3 × 100 ml), and the organic layer was evaporated. The residue was treated with dry ether and the precipitate was filtered off, and it was recrystallized from a mixture of dioxane and ethyl acetate; yield 55-70%.

TABLE 1. Physicochemical Properties of the Cyclohexapeptides (1)-(7)

Cpd. No.	Compound	mp, °C	$[\alpha]_D^{20}$, deg c (in CF_3COOH)	mol. wt. (mass spectrum)	Yield on cyclization
(1)	Cyclo [-(L-Val-Gly)$_3$-]	335 (decomp.)	−2,5; 1	468	60
(2)	Cyclo [-(L-Nva-Gly)$_3$-]	340 (decomp.)	+3,3; 1	468	55
(3)	Cyclo [-(L-Leu-Gly)$_3$-]	325 (decomp.)	−26,6; 1	510	39
(4)	Cyclo [-(L-Val-Gly-Gly)$_2$-]	330 (decomp.)	−5,0; 1	426	36
(5)	Cyclo [-(L-Nva-Gly-Gly)$_2$-]	335 (decomp.)	+4,4; 1	426	40
(6)	Cyclo [-(L-Val-L-Val-Gly)$_2$-]	350 (decomp.)	−152,0; 0,5	510	29
(7)	Cyclo [-(L-Nva-L-Nva-Gly)$_2$-]	350 (decomp.)	−87,2; 0,5	510	20

TABLE 2. Physicochemical Constants of the Linear Intermediate Compounds

Cpd. No.	mp, °C	$[\alpha]_D^{20}$, deg	c	Solvent
(12)	145	−26,6	1	EtOH
(13)	81	−21,0	1	EtOH
(14)	63—65	−23,0	1	EtOH
(15)	145—146	−22,8	1	EtOH
(16)	98	−17,0	1	EtOH
(17)	114—115	−27,0	1	EtOH
(21)	198—200	−5,0	1	DMFA
(22)	150	−17,0	1	EtOH
(23)	145—148	−23,7	1	EtOH
(27)	263—265	−4,1	1	DMFA
(28)	230 (decomp.)	−14,0	0,2	EtOH
(29)	230—233	−21,8	1	DMFA
(30)	300	−1,2	1	DMFA
(31)	234	−10	0,2	EtOH
(32)	340	−10,2	1	DMFA
(34)	194—196	−7,3	1	DMFA
(35)	173	−6,0	1	EtOH
(36)	168—170	−1,0	1	DMFA
(37)	218	+5,3	1	DMFA
(39)	249—250	−3,8	1	DMFA
(40)	234	−0,8	1	DMFA
(42)	171	−36,0	1	EtOH

5. Benzyloxycarbonyl-L-norvalyl-L-norvalylglycine (42). A solution of 35 mmole of benzyloxycarbonyl-L-norvalylglycine (16) in 60 ml of 35% HBr in CH_3COOH was kept for 40 min and was then evaporated and the residue was treated with absolute ether. The crystals were filtered off, washed with absolute ether, and dried in vacuum. The yield of the hydrobromide of L-norvalylglycine (41) was 90%.

By the mixed-anhydride method under the conditions of experiment 4, 30 mmole of benzyloxycarbonyl-L-norvaline (10) and 30 mmole of the hydrobromide of (41) formed the tripeptide (42) with a yield of 65%.

6. tert-Butyl Ester of Benzyloxycarbonyl-L-valyl-L-valylglycine (36). By the method of mixed anhydrides in a similar manner to experiment 1, 4.19 g (17 mmole) of benzyloxycarbonyl-L-valine (9) and 3.9 g (17 mmole) of the tert-butyl ester of L-valylglycine (18) [the product of the hydrogenolysis of (12) under the conditions of experiment 3] in absolute tetrahydrofuran gave the ester (36) with a yield of 70%.

7. tert-Butyl Ester of the Benzyloxycarbonylhexapeptide (39). By the method of mixed anhydrides in absolute DMFA in a similar manner to experiment 1, 18.9 g (46 mmole) of benzyloxycarbonyl-L-valyl-L-valylglycine (37) [from the ester (36) under the conditions of experiment 2] and 15.3 g (46 mmole) of the tert-butyl ester of L-valyl-L-valylglycine (38) [the product of the hydrogenation of (36) under the conditions of experiment 3] gave the hexapeptide (39) with a yield of 63%.

8. The Benzyloxycarbonylhexapeptides (30)-(32) and (40). A solution of 10 mmole of a tert-butyl ester of a benzyloxycarbonylhexapeptide (27)-(29) or (39) in 30 ml of trifluoroacetic acid was kept at room temperature for 40 min. Then the solution was evaporated in vacuum, and the residue was treated with dry ether, filtered off, and dried over KOH in vacuum. Yield 93-98%.

9. Cyclohexapeptide (1)-(7). To a solution of 7 mmole of a benzyloxycarbonylhexa- or -tripeptide (30)-(32), (34), (35), (40), or (42) in 50 ml of absolute pyridine was added 3.9 g (12 mmole) of di(p-nitrophenyl) sulfite, and the mixture was stirred at room temperature for 48 h. Then it was evaporated in vacuum, 50 ml of a mixture of diethyl ether and petroleum ether (1:1) was added, and the precipitate was filtered off. It was washed on the filter with a mixture of diethyl ether and petroleum ether (1:1) until the filtrate no longer gave a yellow coloration when it was shaken with a solution of $NaHCO_3$. The product obtained, containing 85-90% of the p-nitrophenyl ester of a benzyloxycarbonyhexa- or -tripeptide was dissolved in 3 ml of glacial acetic acid, and 30 ml of a 35% solution of HBr in

glacial acetic acid was added. The reaction mixture was kept at room temperature for 40 min and was evaporated in vacuum, and the residue was treated with absolute ether. The precipitate that deposited was filtered off, dried, and dissolved in 35 ml of absolute DMFA to which 2 ml of glacial acetic acid had been added. The resulting solution was added with stirring over 6 h to 600 ml of absolute pyridine heated to 65°C. After the addition of the whole of the solution, the reaction mixture was stirred at 65°C for 72 h and was then evaporated in vacuum, the residue was dissolved in 50 ml of a mixture of ethanol and water (1:1), and the solution was filtered and was passed successively through the ion-exchange resins Dowex 1×1 (HCO_3^- form) and Dowex 50×1 (H^+ form). The columns were washed with 500 ml of a mixture of methanol and water (1:1), and the eluate was evaporated and was chromatographed on neutral alumina (activity grade II) and on silica gel in ethyl acetate–ethanol systems (gradient elution). The yields of the cyclopeptides (1) and (2) were ≈60%, of (3), (4), and (5) ≈ 40%, and of (6) and (7) ≈ 25%.

SUMMARY

The synthesis of seven cyclic hexapeptides containing the residues of L-valine, L-norvaline, L-leucine, and glycine has been effected.

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