BIOORGANIC CHEMISTRY 3, 114–124 (1974)

Phenylalanine Oligopeptides
Synthesis, Polarimetric and Ultraviolet Absorption Studies

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Received May 22, 1973

A series of L-phenylalanine oligomers was synthesized by the dicyclohexylcarbodiimide and acylazide methods. The compounds prepared in this manner were chemically and optically pure and were obtained in good yield. A preliminary stereochemical analysis of these oligopeptides by means of polarimetry and ultraviolet absorption spectroscopy is reported. The vibrational fine structure observed in the ultraviolet spectra is also discussed.

INTRODUCTION

The analysis of the stabilization forces which maintain the various conformations in proteins has been the subject of a number of investigations and has hugely profited by the study of model compounds, in which the variety of conformations and hence of interactions are greatly reduced. Among all types of model compounds studied, linear homo-oligopeptides play a fundamental role. In fact, the peculiar type of information which one might gain from the investigation of homo-oligopeptides concerns the combined effect of chain length and nature of side chain on the stability of the various structures (*1–8*), allowing the evaluation of the contribution of particular bonds within a particular conformation and, consequently, shedding light on the nature of short and medium-range interactions (*9*), which are of relevant interest with the regard to the problem of the mechanism of protein folding.

In 1966 Auer and Doty (*10*) suggested that the class of α-helix-forming amino acids as proposed by Blout (*11*) should be further divided into two groups: a group with a single aliphatic substituent on the β-carbon (i.e., alanine) which forms unhindered helices, and a group which forms helices destabilized by steric interaction between bulky side chains and backbone (i.e., phenylalanine). In the latter case the rotation of the β-carbon of the benzylic side chain about the bond joining it to the α-carbon is severely restricted, and the rotation of the phenyl group in turn is also critically diminished. The correctness of such subclassification has been proved for polypeptides (*10, 12–17*).

However, quite recently, Goodman et al. (*1*) demonstrated that alanine oligomers may exist in α-helical or β-associated conformation, depending upon the solvent.

In this and in the accompanying paper (*18*) we report the extension of the oligopeptide approach to homo-oligopeptides derived from L-phenylalanine. In particular,

this paper will describe the synthesis of the homologous series BOC-L-(Phe)$_n$-OMe[1] ($n = 1$–9) and preliminary stereochemical investigations by means of polarimetry and uv absorption spectroscopy. In the accompanying paper a more detailed analysis of the stereochemistry of these oligopeptides by CD will be reported.

A study of the uv absorption and CD properties of the oligophenylalanines appears also to be of interest because the contributions of side chain chromophores both in the near and far-uv region and their dependence on conformation are not yet sufficiently understood. For this reason, the establishment of the conformation in which natural and synthetic aromatic oligopeptides exist in solution is difficult (*19–36*). An additional point of interest of this study concerns the possibility of comparing the vibrational fine structure in the near-uv CD and absorption spectra of the oligophenylalanines (*37–40*).

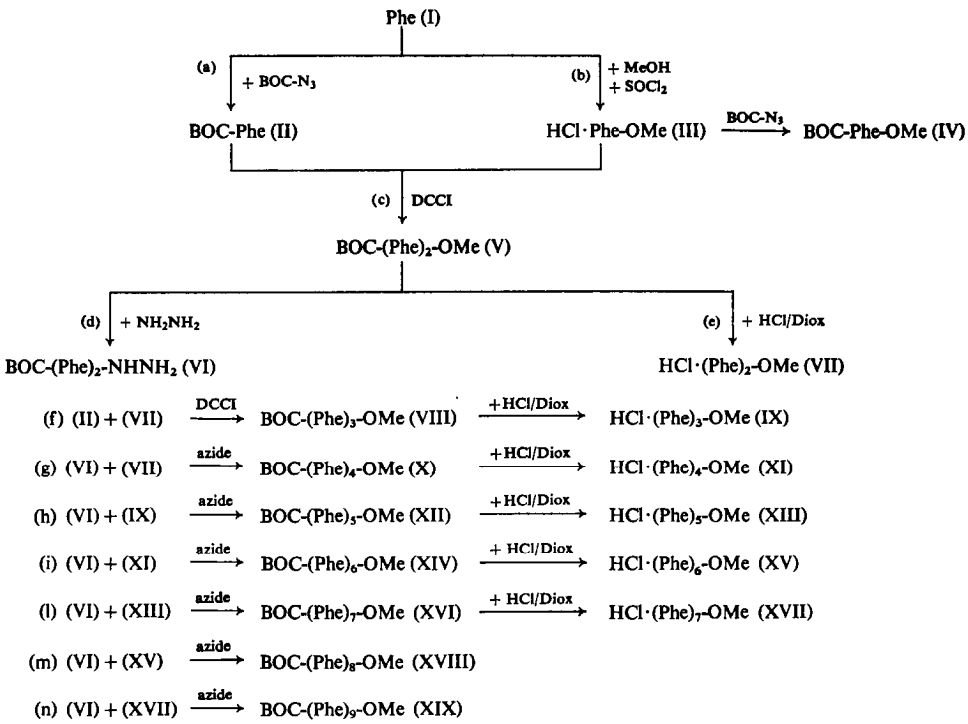
RESULTS AND DISCUSSION

Synthesis of Oligo-L-phenylalanines

The synthesis of the oligomer series was commenced by separately preparing N-terminal- and C-terminal-blocked L-phenylalanine (Scheme 1). *t*-Butyloxycarbonyl-L-phenylalanine (II) was prepared using the procedure described by Schnabel (*41*). The

SCHEME 1

PREPARATION OF BOC-L-(Phe)$_n$-OMe ($n = 1$–9)



[1] The following abbreviations are used: BOC, *t*-butyloxycarbonyl; OMe, methyl ester; Phe, phenyl alanine; TFA, trifluoroacetic acid; DCCI, dicyclohexylcarbodiimide; DMF, dimethylformamide; TFE, trifluoroethanol; HFIP, hexafluoroisopropanol; HFA, hexafluoroacetone sesquihydrate; TMP, trimethylphosphate; and tlc, thin layer chromatography.

carboxylic acid was protected by preparing the corresponding methyl ester hydrochloride according to the method of Boisonnas et al. (*42*). We have employed the methyl ester as the C-protecting group, since it can be easily transformed into the corresponding hydrazide [reaction (d) of Scheme 1], and it is stable under the conditions used to deblock the *t*-butyloxycarbonyl group (6 *N* hydrochloric acid in anhydrous dioxane, 60 min at room temperature). The dipeptide BOC-(L-Phe)$_2$-OMe (V) and

TABLE 1

SUMMARY OF PHYSICAL DATA OF OLIGOMERIC PEPTIDES DERIVED FROM L-PHENYLALANINE

Compound	*n*	Melting point (°C)	Recrystallization solvents	Yield (%)	R_{f_5}
IV	1	oil	ethyl ether/petroleum ether	70	0.85
V	2	121–122°	DMF/water	94	0.80
VIII	3	173–174°	DMF/water	70	0.75
X	4	196–197°	DMF/water	71	0.75
XII	5	213–214°	DMF/water	66	0.65
XIV	6	223–226°	DMF/water	60	0.65
XVI	7	>235°	DMF/water	69	0.65
XVIII	8	>235°	hot DMF/water	53	0.65
XIX	9	>235°	hot DMF/water	57	0.65

tripeptide BOC-(L-Phe)$_3$-OMe (VIII) were prepared using the dicyclohexylcarbodiimide coupling method (*43*) as described in the experimental section. Thus, the dicyclohexylcarbodiimide was used only at the stage of coupling the urethane blocked amino acid with peptide esters. The Scheme also demonstrates the use of the acylazide method (*44*) for synthesizing the higher homologs (X, XII, XIV, XVI, XVIII, and XIX). These compounds were prepared by adding the free amino group of H(L-Phe)$_n$-OMe ($n = 2$–7) to the *t*-butyloxycarbonyl-dipeptide hydrazide (VI) in the presence of *t*-butyl nitrite and dimethylformamide–ethyl acetate as the reaction solvent system. A summary of the melting points, recrystallization solvents, reaction yields and tlc R_f values of the oligomers prepared is presented in Table 1. The chemical purity of this series of oligopeptides was ascertained by ascending tlc in a number of solvent systems and by comparison of melting points, when available. The elemental analysis data of the oligomers are reported in Table 2.

Molar Rotation Studies

Optical purity is a necessary prerequisite for the formation of stable secondary structures by oligopeptides. Therefore, it is of paramount importance that all oligophenylalanines be optically pure in order for our conformational investigations to be meaningful. Goodman and coworkers (*45*) have demonstrated that the optical activity of oligopeptides is best treated by using units of molar rotation.[2] In the case of chemically and optically pure homo-oligomers, a plot of total molar rotation, ϕ, versus *n* (the number of

[2] $\phi = [\alpha] \times$ (molecular weight/10 000) $\times$ (degree-cm^2/mole).

TABLE 2

SUMMARY OF ELEMENTAL ANALYSIS DATA OF OLIGOMERIC PEPTIDES DERIVED FROM L-PHENYLALANINE

Compound	n	MW	C% Calcd Found	H% Calcd Found	N% Calcd Found
V	2	426.5	67.6	7.1	6.6
			67.2	7.1	6.6
VIII	3	573.7	69.1	6.8	7.3
			68.2	6.8	7.1
X	4	720.8	70.0	6.7	7.8
			69.3	6.6	7.6
XII	5	868.0	70.6	6.6	8.1
			69.6	6.6	8.1
XIV	6	1015.2	71.0	6.5	8.3
			70.1	6.4	8.0
XVI	7	1162.3	71.3	6.5	8.4
			70.2	6.4	8.1
XVIII	8	1309.5	71.5	6.5	8.5
			70.6	6.4	8.4
XIX	9	1456.7	71.6	6.4	8.6
			70.2	6.2	8.3

residues), should be linear in the absence of secondary structures. Figure 1 and Table 3 show that the ϕ_D values for the oligophenylalanines ($n = 1$–7) fall on a straight line in DMF within experimental error (the octamer and nonamer are not soluble in this solvent). Thus, we obtained a preliminary indication that little if any racemization occurred during the synthetic procedure. The optical purity of oligophenylalanines has been definitely confirmed by a polarimetric (589 nm) and circular dichroism (218 nm)

TABLE 3

OPTICAL ROTATIONS[a] OF *t*-BUTYLOXYCARBONYL OLIGOMERS DERIVED FROM L-PHENYLALANINE IN TFE AND DMF

	TFE		DMF	
n	$[\alpha]_D^{23}$	ϕ_D	$[\alpha]_D^{18}$	ϕ_D
1	+16.5	+0.46	− 2.2	−0.05
2	+ 0.0	+0.00	− 8.7	−0.35
3	−15.3	−0.88	−14.9	−0.85
4	−22.2	−1.60	−16.1	−1.15
5	−28.3	−2.45	−19.2	−1.65
6	−25.0	−2.54	−21.1	−2.15
7	not soluble		−22.1	−2.55

[a] Concn. 0.2%.

(*18*) investigation on their acid hydrolizates (6 *N*, HCl, 22 hr, 110°C). In all cases deviations from the values of the free L-phenylalanine reference solution are in the range 1–5%, i.e., within the range reported by Manning and Moore (*46*) for racemization of phenylalanine peptides during acid hydrolysis. These findings demonstrate that the dicyclohexylcarbodiimide and acylazide coupling methods as applied throughout this paper give oligomers of high optical purity. Figure 1 and Table 3 additionally show the

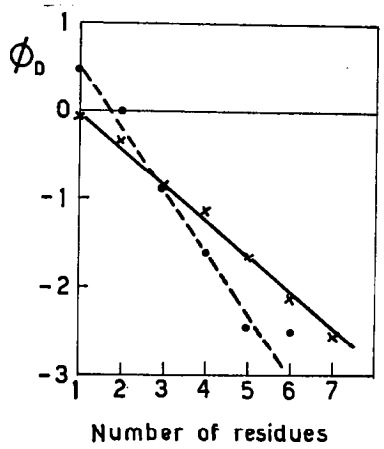


FIG. 1. Plot of total molar rotation at sodium *D* line of the *N*-*t*-butyloxycarbonyl-L-phenylalanine oligomers: (●) in TFE at 23°C; (×) in anhydrous DMF at 18°C.

ϕ_D values of oligo-L-phenylalanines in TFE. For most oligopeptides reported in the literature (*1–8*) TFE tends to support secondary structures. Our results show that the total molar rotations of the oligophenylalanines (up to $n = 5$) fall on a straight line in TFE, indicating that these peptides are in a nonperiodic conformation in this solvent. A positive deviation from a straight line dependence appears at the hexamer, suggesting the onset of an ordered structure in TFE at this stage.

Ultraviolet Absorption Studies

Absorption spectra of oligophenylalanines were measured between 280 and 200 nm (*47*). In this region, bands corresponding to $^1A_{1g} \rightarrow {}^1B_{2u}$ and $^1A_{1g} \rightarrow {}^1B_{1u}$ benzene transitions are observed.[3] The higher energy transition (*52*) appears as a band in the monomer, whereas as a pronounced shoulder in the higher homologs in TFE as a solvent (Fig. 2). This finding seems to be related to a substantial increase in intensity of an absorption band located below 200 nm (*51*, *52*). Furthermore, as expected, assuming phenylalanine peptides to be derivatives of phenylpropionic acid, the absorption shoulder at about 225–235 nm, characteristic of phenylacetic acid and its derivatives, appears to be absent (*53*). This experimental result shows unambiguously that the effect of the CO—X (X = NH, OMe) group on the spectroscopic properties of the toluene moiety is highly reduced when the units are separated by one methylene group.

[3] In the 200–230 nm region peptide, ester, and urethane chromophores also contribute, although at different degree, to the absorption spectrum (48–51).

As far as the $^1A_{1g} \rightarrow {}^1B_{2u}$ type transition is concerned, we have examined the phenylalanine oligomers in a number of solvent systems and at various temperatures. The results obtained (Figs. 3–5) can be summarized as follows:

(i) Figure 3 illustrates the effect of solvent polarity upon the position of the various bands; a red shift, associated with decrease in solvent polarity, stands out clearly (*37*, *39*). This effect is of interest since these hydrophobic chromophores (*54*) frequently are found in the low polarity region of proteins. In addition, the 267.5-nm and 247.5-nm bands present in the less polar *t*-amyl alcohol appear as shoulders in the more polar HFA.

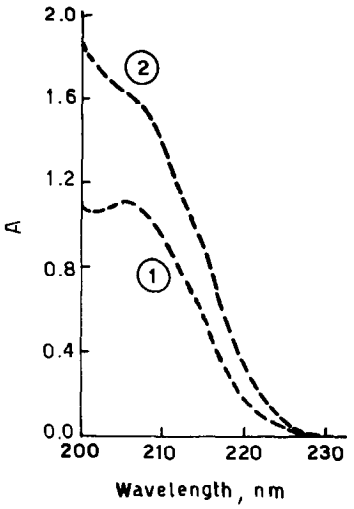


FIG. 2. Far-uv absorption spectra of BOC-L-Phe-OMe (1) and BOC-(L-Phe)$_2$-OMe (2) in TFE; 0.5 mm cell; concn 0.65 mg/ml.

(ii) The temperature effect is shown in Fig. 4 (*27*). At 296 K the absorption bands are better resolved than at 335 K and are also shifted to shorter wavelengths by about 0.3 nm. The areas under the two curves were the same, within our experimental accuracy.

(iii) In contrast to the results found in the case of the higher energy transition, the pattern of the lower energy band of the monomer does not change in going to dimer and higher oligomers. The effect of increasing oligomer chain length upon the total molar extinction coefficients is illustrated in Fig. 5. There is no evidence of hyper- or hypochromicity (*55*).

It is interesting to note that the vibrational structure of the 257-nm band of phenylalanine oligomers features the two series of bands characteristic of the substituted benzene nucleus (*56*, *57*). Both 0–0 and 0 + 520 cm^{-1} transitions give rise to progressions having the same spacing (930 cm^{-1}) (*40*, *53*, *55*). The two progressions observed exhibit differences in the intensities. The electric-dipole-allowed 0–0 subsystem (267.5 and 261 nm) is less intense than the vibrationally-induced 0 + 520 cm^{-1} subsystem (263.5 and 257.5 nm). At shorter wavelengths considerable overlapping of different transitions occurs.

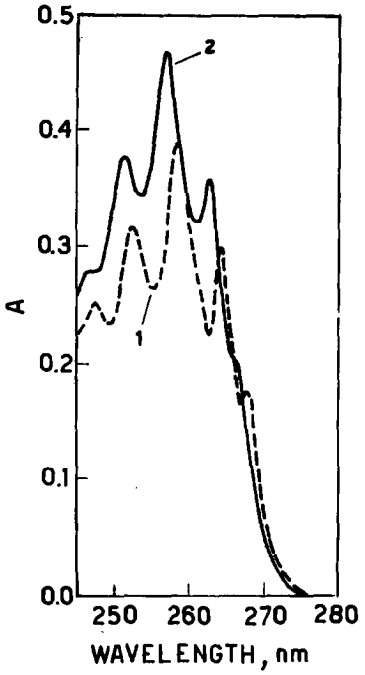


FIG. 3. Near-uv absorption spectra of BOC-(L-Phe)$_4$-OMe in *t*-amyl alcohol (1) and in HFA (2); 1 cm cell; concn 0.38 mg/ml (1), and 0.41 mg/ml (2).

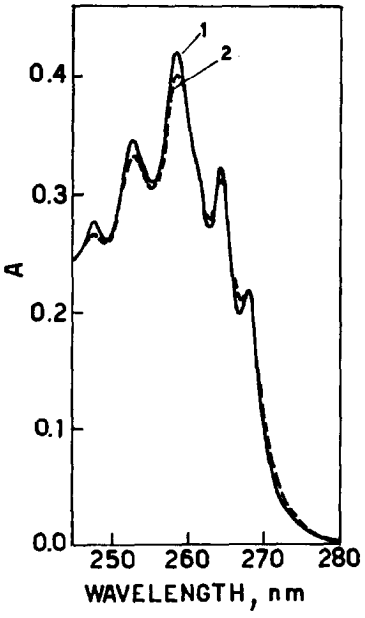


FIG. 4. Near-uv absorption spectra of BOC-(L-Phe)$_2$-OMe in TMP at 296 K (1) and 335 K (2); 1 cm cell; concn 0.41 mg/ml.

The relatively higher intensity of the 520 cm^{-1} progression decreases in going from benzene (where the 0–0 progression is absent) to phenylacetic, phenylpropionic, phenylbutyric, phenylvaleric acid derivatives and toluene. Thus, the vibronic structures of this band of phenylacetic acid and benzene, and phenylvaleric acid and toluene are very similar in shape. The relative intensities of these two progressions are reversed from phenylalanine oligomers (i.e., derivatives of phenylpropionic acid) to toluene. Since the 0–0 progression occurs in monosubstituted benzenes only because the side chain hyperconjugates with the aromatic ring, our results point out that phenylalanine compounds hyperconjugate less than toluene.

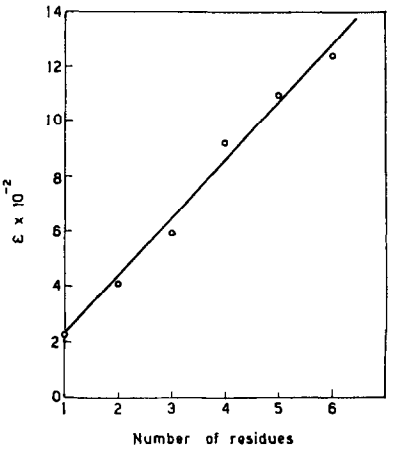


FIG. 5. Plot of total molar extinction coefficients at 257 nm of the *N-t*-butyloxycarbonyl-L-phenyl alanine oligomers in TFE.

EXPERIMENTAL

Reagents and Solvents

Trifluoroacetic acid, trifluoroethanol, hydrazine hydrate, L-phenylalanine, and *t*-butylnitrite were purchased from Fluka A.G., Basle. Dicyclohexylcarbodiimide and *t*-butylcarbazate were obtained from Schuchardt, Munchen. Triethylamine, ethyl acetate, chloroform, petroleum ether, thionyl chloride, and anhydrous sodium sulfate were obtained from Erba R.P. Reagent grade chemicals were used for most reactions without further purification. Ethyl ether was used after storage over sodium ribbon. Dimethylformamide, reagent grade, was vacuum distilled before use, and the middle fraction was used. Hexafluoroisopropanol and hexafluoroacetone sesquihydrate were purchased from Du Pont and Co., Wilmington, DL, trimethylphosphate from Aldrich Chem. Co.; methanol and dioxane from Merck A.G., Darmstadt. The above solvents were obtained in the purest form commercially available.

Preparation of Compounds

t-Butyloxycarbonyl-L-phenylalanine methyl ester (IV) (*58*). To a solution of L-phenylalanine methyl ester hydrochloride (III) (1.18 g; 5.5 mmole) and triethylamine (0.7 ml, 5.5 mmole) in 10 ml of dimethylformamide a solution of *t*-butylazidoformate (0.72 g,

5 mmole) in 5 ml of dimethylformamide was added at −10°C. The reaction was allowed to proceed for 1 hr at −10°C and for 3 days at −5°C under stirring. The solvent was removed under reduced pressure. The resulting oil was dissolved in ethyl acetate. The organic layer was washed with 0.5 M citric acid and water, dried over Na_2SO_4, filtered, and evaporated to dryness under reduced pressure. The yield of the resulting oil was 1.08 g (70%), from ethyl ether–petroleum ether: $[\alpha]_D^{20} = -3.1°$ ($c = 2$, methanol), $R_{f_5} = 0.85$, single ninhydrin-positive spot after exposure to concentrated HCl vapors.

Anal. Calcd for $C_{15}H_{21}NO_4$: C, 64.50; H, 7.55; N, 5.02. Found: C, 64.02; H, 6.81; N, 5.09.

t-Butyloxycarbonyl-L-phenylalanyl-L-phenylalanine methyl ester (*V*) (*59*). This compound has been prepared in 94% yield via diciclohexylcarbodiimide in anhydrous chloroform; mp 121–122°C; $[\alpha]_D^{21} = -14.2°$ ($c = 1$, methanol), $R_{f_4} = 0.75$ and $R_{f_3} = 0.9$, single ninhydrin-negative and chlorine-positive spot.

t-Butyloxycarbonyl-L-phenylalanyl-L-phenylalanine hydrazide (*VI*). *t*-Butyloxycarbonyl-L-phenylalanyl-L-phenylalanine methyl ester (V) (3.55 g, 8.3 mmole) was dissolved in 20 ml dimethylformamide, and hydrazine hydrate (10 ml) was added. The mixture was allowed to react for 3 days at room temperature and poured into 100 ml of water. The precipitate was collected, washed with water and petroleum ether, and dried over concentrated sulfuric acid. The yield was 3.25 g (90%) after recrystallization from dimethylformamide–water, mp 179–180°C, $[\alpha]_D^{20} = -22.5°$ ($c = 1$, dimethylformamide), $R_{f_4} = 0.75$, single chlorine- and picryl chloride/ammonia-positive spot.

Anal. Calcd for $C_{23}H_{30}N_4O_4$: C, 64.77; H, 7.08; N, 13.13. Found: C, 64.53; H, 6.66; N, 13.22.

t-Butyloxycarbonyl-di-(L-phenylalanyl)-L-phenylalanine methyl ester (*VIII*). *t*-Butyloxycarbonyl-L-phenylalanyl-L-phenylalanine methyl ester (V) (3.97 g, 9.3 mmole) was allowed to react with 10 ml of a 6 *N* HCl solution in anhydrous dioxane for 1 hr at room temperature in order to cleave the *t*-butyloxycarbonyl blocking group. The resulting L-phenylalanyl-L-phenylalanine methyl ester hydrochloride (VII) (*60*) (99% yield), mp 220°C, $[\alpha]_D^{25} = +12.4°$ ($c = 1$, water), $R_{f_4} = 0.85$, $R_{f_3} = 0.9$, single ninhydrin-positive spot, was coupled to *t*-butyloxycarbonyl-L-phenylalanine (II) (2.65 g, 10 mmole) using the dicyclohexylcarbodiimide method described for the corresponding dimer (V). The crude product was recrystallized from dimethylformamide–water to yield 3.71 g (70%) of a white powdery material, mp 173–174°C, $[\alpha]_D^{20} = -20.9°$ ($c = 1$, methanol), $R_{f_4} = 0.75$, single ninhydrin-negative and chlorine-positive spot.

t-Butyloxycarbonyl-tri-(L-phenylalanyl)-L-phenylalanine methyl ester (*X*). To a solution of *t*-butyloxycarbonyl-L-phenylalanyl-L-phenylalanine hydrazide (VI) (1.2 g, 2.8 mmole) in 30 ml of dimethylformamide, 6 *N* HCl in ethyl acetate (1.5 ml, 9 mmole) and *t*-butyl nitrite (0.4 ml, 3.1 mmole) were added with stirring at −30°C. The solution was held at −30°C for 30 min, cooled to −50°C, and the pH adjusted to 8 with triethylamine. L-Phenylalanyl-L-phenylalanine methyl ester hydrochloride (1.02 g, 2.8 mmole) in 20 ml of anhydrous dimethylformamide and triethylamine (0.5 ml, 3.1 mmole) were then added to the solution. The reaction was allowed to proceed at pH 8 for 3 days at −5°C and poured into 100 ml of water. The precipitate was collected, washed with ethyl ether, and dried over P_2O_5. The yield was 1.44 g (71%), after recrystallization from dimethylformamide–water, mp 196–197°C, $[\alpha]_D^{20} = -36.9°$ ($c = 0.4$, methanol), $R_{f_4} = 0.75$, single ninhydrin-negative and chlorine-positive spot.

The higher oligomers were prepared using the acylazide method (*44*) described above for the tetramer. The melting points, recrystallization solvents, yields, and R_{f_5} values are reported in Table 1. The elemental analysis are reported in Table 2. The optical rotations in trifluoroethanol and anhydrous dimethylformamide are shown in Table 3.

Measurements

The melting points were determined by a Tottoli apparatus and are not corrected. The optical rotations measurements were carried out on a Perkin-Elmer Model 141 polarimeter equipped with a thermostat. The values are expressed in terms of ϕ_D, total molar rotation at sodium *D* line.

Ultraviolet absorption measurements were carried out using a Cary Model 15 spectrophotometer equipped with a hydrogen light source and a thermostat. The values are expressed in terms of ε_T, total molar absorption coefficient.

Thin layer chromatography (SiO_2, Merck) was performed using the following solvent mixtures: (1) acetic acid–*n*-butanol–water (20:60:20), (2) ethyl acetate–benzene (2:1), (3) methanol–benzene (1:1), (4) methanol–benzene (1:2), and (5) methanol–benzene (1:5). The N-unprotected compounds were revealed by spraying the chromatograms with a 0.4% ninhydrin solution in acetone and the N-protected compounds by the hypochlorite–starch–iodide chromatic reaction. Hydrazides were revealed by spraying the chromatograms with 1% picryl chloride solution in 95% ethanol, followed by exposure to ammonia vapors.

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