The Effect of Homologous Amino Acid Replacement on the Conformation of Oligopeptides. I. Synthesis of Co-Oligopeptides Containing Methionine and Valine

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Synopsis

The preparation of a series of co-oligopeptides Boc-Val-Met_n-OMe (n = 1–6), as well as Boc-Met_3-Val-Met_2-OMe and Boc-Met_3-Val-Met_3-OMe, is described. The synthesis was carried out by a classic method employing the mixed anhydride procedure (isobutylchloroformate) for all coupling reactions. All oligopeptides after purification were homogeneous on silica thin layers and gave correct elemental analysis. They were judged to be optically pure using molar rotation studies at the sodium D line.

INTRODUCTION

Homologous series of oligopeptides have been extensively employed as model compounds to study many chemical and physical factors which affect protein structure. Goodman and collaborators examined peptides based on γ-methyl- and γ-ethyl-L-glutamate, β-methyl-L-aspartate, and L-alanine.[1–6] They found that a critical chain length existed before the onset of helicity in series of such oligopeptides.[7–9] Toniolo and coworkers[10–14] have extended these studies to include amino acids such as valine, isoleucine, phenylalanine, and leucine, which apparently assume β-associated structures in solvents such as trifluorethanol, ethylene glycol, and mixtures of these with water.

We recently reported the synthesis and conformational analysis of a series of methionine-containing oligopeptides (Boc-Met_n-OMe, n = 2–7,9).[15,16] We found that these peptides begin forming helices at the heptamer in TFE, but remain disordered up to the nonamer in hexafluoroacetone sesquihydrate. Bonora and Toniolo[17] found that the same series of peptides existed in a β-conformation for $n = 5$ in ethylene glycol and $n \geq 5$ in TFE/water mixtures.

We believe that studies on carefully prepared series of cooligopeptides where guest residues are judiciously placed in a parent compound can be very valuable in answering several of the remaining questions in the conformational analysis of linear peptides. A study of the Boc-Met_n-OMe

Biopolymers, Vol. 17, 2189–2197 (1978)

0006-3525/78/0017-2189$01.00

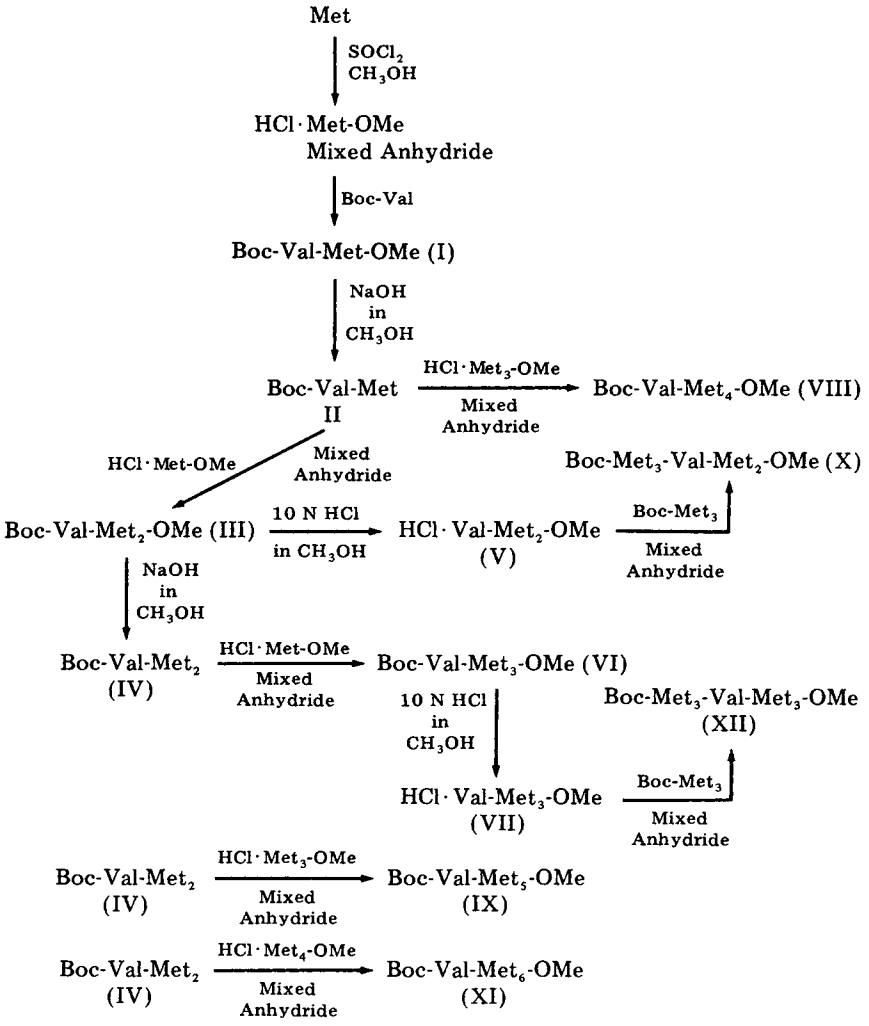
oligomers by 220 MHz nmr has revealed that, up to the hexamer, each individual NH is resolvable.[18] In an attempt to assign these NH's to individual residues of the hexapeptide, we have embarked on a program to replace systematically one methionine residue by another amino acid. We believe that information gained from such studies can also be applied to the way in which various amino acids interact to determine the eventual secondary structure of short peptide chains.

The present communication reports the synthesis of cooligopeptides where one valine has replaced a methionine residue at the amine terminal and center position of certain Boc-$(Met)_n$-OMe oligomers: Boc-Val-Met_n-OMe (n = 1–6) and of Boc-Met_3-Val-Met_2-OMe and Boc-Met_3-Val-Met_3-OMe.

RESULTS AND DISCUSSION

The synthesis of the Boc-Val-Met_n-OMe oligomers was commenced by separately blocking the amine and carboxyl termini of valine and methionine with *tert*-butyloxycarbonyl and methyl ester blocking groups, respectively. Boc-Val-Met-OMe was prepared by coupling Boc-Val to methionine methyl ester using the mixed anhydride procedure of Anderson et al.[19] The extension of the chain length was carried out by removing the methyl ester blocking group using saponification in aqueous methanol and adding the appropriate nucleophile as shown in Scheme I. The removal of the Boc group was accomplished using 10*N* hydrochloric acid in methanol as described previously.[20] This procedure resulted in almost quantitative recovery of the corresponding peptide methyl ester hydrochlorides. We were unable to saponify the methyl ester from Boc-Val-Met_3-OMe or any of the higher analogs. This finding is consistent with results reported for homo-oligomers of L-alanine and L-methionine.[6,15] Recently, Luisi and coworkers[21] reported the saponification of the methyl ester from co-oligopeptides of glycine and tryptophan. It is possible that the differences observed between the present study and that of Luisi and coworkers[21] is a consequence of the different solubilities of the various peptide esters in the saponification medium. The co-oligomers of valine and methionine above the tripeptide are very insoluble in methanol. The preparation of the higher oligomers, therefore, was accomplished by coupling the appropriate peptide methyl ester hydrochloride to either Boc-Val-Met or Boc-Val-Met-Met.

The coupling reaction generally resulted in yields of 65–85%. Minimal purification was required in most cases in order to obtain chemically homogeneous products. In several of the higher peptides, the crude product showed trace impurities on silica thin layers. These impurities were removed by washing the solid product with cold ethyl acetate or methanol. In the case of Boc-Val-Met_6-OMe, the product was purified using high-performance liquid chromatography on a silica gel column with chloroform/methanol (11:1) as the eluent. After purification, all peptides were



Scheme I
Preparation of Co-oligomers of Valine and Methionine

homogeneous on silica thin layers using at least two solvent systems and gave the expected nitrogen and sulfur analyses (see Table I).

The mixed anhydride procedure has been shown in several cases to give peptides which are essentially free from racemization. In order to examine the optical purity of the Boc-Val-Met$_n$-OMe series, we measured the total molar rotation in a solvent known to support secondary structure (trifluoroethanol) and one which does not (hexafluoracetone sesquihydrate). Total molar rotations ($[\phi]_M$) were calculated using the equation developed by Goodman et al.[8,22]:

$$[\phi]_M = \frac{[\alpha]_D \times M_r}{10{,}000}$$

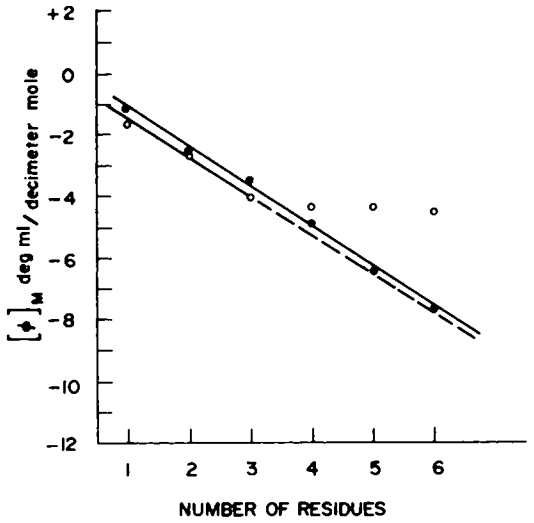


Fig. 1. Molar rotations of Boc-Val-Met$_n$-OMe (n = 1–6) oligomers at the sodium D line: (●) hexafluoroacetone sesquihydrate, (○) trifluoroethanol.

where M_r is the molecular weight of the oligopeptide.

As expected, the plot of $[\phi]_M$ vs n is linear in hexafluoroacetone sesquihydrate solvent but deviates from linearity in trifluoroethanol. These results suggest that the Boc-Val-Met$_n$-OMe oligomers are optically pure and begin forming secondary structures in trifluoroethanol by the heptamer. We have also examined the susceptibility of an unblocked derivative of one of these peptides to be hydrolyzed by cell extracts from *E. coli*.[23] Using high-voltage paper electrophoresis, we found that L-Val-L-Met-L-Met was hydrolyzed completely at a rate comparable to that of L-Met-L-Met-L-Met. Since the extract of this strain was unable to hydrolyze L-Met-D-Met-L-Met under identical conditions, these experiments provide additional evidence that very little racemization occurs during the peptide coupling reaction.

The results of CD studies on the Boc-Val-Met$_n$-OMe oligomers suggest that these compounds begin forming helices in trifluoroethanol at the heptamer. We have synthesized additional oligomers where one methionine is replaced by a glycine residue.[24] The detailed conformational analysis of these compounds, as well as the oligomers whose synthesis is reported herein, is discussed in the third paper of this series.[25]

EXPERIMENTAL

L-Methionine was purchased from Bachem Inc., Marina Del Rey, California. *N*-Methyl morpholine and *tert*-butyloxycarbonylazide were obtained from the Aldrich Chemical Co., New Jersey. Boc-L-valine was purchased from Bachem, Inc. It was homogeneous in silica thin layers $CHCl_3$/MeOH (2:1) and had an $[\alpha]_D^{23} = -6.0$ (c 1.0 acetic acid), lit. $[\alpha]_D^{20} =$

TABLE I
Summary of Physical Properties of Val-Met Co-Oligopeptides

Peptide	mp[a] (°C)	$[\alpha]_D^{23}$ [b]	R_{f_I}[c]	$R_{f_{II}}$[c]	$R_{f_{III}}$[c]	$R_{f_{IV}}$[c]	Calcd. N	Calcd. S	Found[e] N	Found[e] S
Boc-Val-Met-OMe	110–111	−46.3 (C 0.59)	0.61	0.35	0.70	0.72	7.73	8.85	7.63	8.96
Boc-Val-Met_2-OMe	136.5–137.5	−52.9 (C 0.50)	0.56	0.16	0.68	0.72	8.51	12.99	8.35	13.14
Boc-Val-Met_3-OMe	184–185	−65.0 (C 0.49)	0.49	0.05	0.63	0.71	8.97	15.39	8.90	15.60
Boc-Val-Met_4-OMe	225–227	−58.2 (C 0.56)	—	—	0.57	0.70	9.26	16.96	9.42	17.10
Boc-Val-Met_5-OMe	>240 dec	−49.2 (C 0.55)	—	—	0.55	0.70[d]	9.47	18.07	9.46	18.25
Boc-Val-Met_6-OMe	>250 dec	−44.6 (C 0.13)	—	—	0.52	0.70[d]	9.63	18.89	9.59	18.62
Boc-Met_3-Val-Met_2-OMe	>240 dec	−62.2 (C 0.33	—	—	0.50	0.70[d]	9.47	18.07	9.61	18.36
Boc-Met_3-Val-Met_3-OMe	>250 dec	−53.3 (C 0.33)	—	—	0.47	0.71[d]	9.63	18.89	9.70	19.04

[a] Melting points were measured using a Buchi melting-point apparatus and are uncorrected.

[b] In trifluoroethanol.

[c] The solvent systems used were: (I) ethyl acetate, (II) ethyl acetate/hexane (1:1), (III) chloroform/methanol (2:1), (IV) acetone.

[d] Significant tailing observed.

[e] Analyses were carried out by Galbraith Laboratories, Knoxville, Tennessee.

−5.8 (c 1.2 acetic acid).[26] It was used without further purification. All other reagents and solvents were of the highest purity available.

The optical rotation measurements were carried out on a Perkin-Elmer model 141 polarimeter equipped with a thermostat. Elemental analyses were performed by Galbraith Laboratories, Knoxville, Tennessee. The thin-layer chromatograms were run on 5 × 10 cm silica thin layers using one of the following developing systems: I, ethyl acetate; II, ethyl acetate/hexane (1:1); III, chloroform/methanol (10:1); IV, acetone; V, chloroform/methanol (2:1).

Preparation of Compounds

t-Butoxycarbonyl-L-Valyl-L-Methionine-Methyl Ester (*I*). *t*-Butoxycarbonyl-L-valine (13.02 g, 0.06 mol) was dissolved in 150 ml reagent-grade ethyl acetate in a 500-ml round-bottomed flask. The solution was cooled to −20°C in a dry ice–carbon tetrachloride bath and *N*-methyl morpholine (6.06 g, 0.06 mol) was added. Isobutylchloroformate (7.16 g, 0.06 mol) was then added, causing the immediate precipitation of a white solid. The mixture was stirred for 5 min at −20°C, at which time a solution of L-methionine methyl ester hydrochloride[27] (13.2 g, 0.066 mol) and *N*-methyl morpholine (6.7 g, 0.066 mol) in dimethyl formamide was added.

The resulting mixture was stirred at −20°C for 1 hr and allowed to warm to room temperature. Ethyl acetate (250 ml) was added and the organic layer was extracted three times with 10% citric acid (80 ml), three times with 5% sodium bicarbonate (80 ml), and with saturated sodium chloride solution until neutral. The organic layer was dried over anhydrous $MgSO_4$ and removed under pressure to give a white powder (18.5 g, 85%). Recrystallization from hexane resulted in the formation of colorless needles. The product has a mp 110–111°C; $[\alpha]_D^{23}$ − 32.4 (c 0.52 HFA). It gave one ninhydrin-negative, iodine-positive spot on thin layers of silica: $R_{f_I} = 0.61$, $R_{f_{II}} = 0.35$, $R_{f_{III}} = 0.7$, $R_{f_{IV}} = 0.72$.

ANAL. Calcd. for $C_{16}H_{30}N_2O_5S$: N, 7.73; S, 8.85. Found: N, 7.63; S, 8.96.

t-Butoxycarbonyl-L-Valyl-L-Methionine (II). *t*-Butoxycarbonyl-L-valyl-L-methionine methyl ester (15.9 g, 0.044 mol) was dissolved in 300 ml methanol and 1*N* sodium hydroxide (48.5 ml, 0.0485 mol) was added. The resulting solution was stirred at 25°C for 1½ hr. At this time the reaction was judged by thin-layer monitoring to be virtually complete. The solution was acidified with 10% citric acid to pH 4 and concentrated under reduced pressure. The remaining oil was diluted with 20 ml saturated sodium chloride solution and extracted with ethyl acetate until no additional iodine positive material could be removed from the aqueous layer. The combined ethyl acetate layers (~300 ml) were extracted with three 50-ml portions of 5% sodium bicarbonate solution. The combined aqueous extracts were acidified with 10% citric acid, causing the precipitation of an oil. The oil was extracted into ethyl acetate (≃300 ml). The ethyl acetate was dried over anhydrous $MgSO_4$ and the solvent removed *in vacuo*. The crude white product was recrystallized from ethyl acetate (12.3 g, 80%): mp 98–102°C; $[\alpha]_D^{23}$ −38.9 (c 0.59 TFE). It was homogeneous on thin layers of silica: $R_{f_{III}} = 0.2$, $R_{f_V} = 0.43$.

ANAL. Calcd. for $C_{15}H_{28}N_2O_5S$: N, 8.04; S, 9.20. Found: N, 7.89; S, 8.95.

t-Butoxycarbonyl-L-Valyl-L-Methionyl-L-Methionine Methyl Ester (III). *t*-Butoxycarbonyl-L-valyl-L-methionine (6.96 g, 0.02 mol) was reacted with methionine methyl ester (4.40 g, 0.022 mol) using a procedure identical to that described for the analogous dipeptide. The crude product was recrystallized from ethyl acetate to give (7.4 g, 75%) of a white crystalline powder: mp 136.5–137.5°C; $[\alpha]_D^{23}$−51.28 (c 0.56 HFA). The product gave one iodine-positive, ninhydrin-negative spot on silica thin layers; $R_{f_I} = 0.56$, $R_{f_{II}} = 0.16$, $R_{f_{III}} = 0.68$, $R_{f_{IV}} = 0.72$.

ANAL. Calcd. for $C_{21}H_{39}N_3O_6S_2$: N, 8.51; S, 12.99. Found: N, 8.35; S, 13.14.

t-Butoxycarbonyl-L-Valyl-L-Methionyl-L-Methionine (IV). The methyl ester was cleaved from *t*-butoxycarbonyl-L-valyl-L-methionyl-L-methionine methyl ester (5.9 g, 0.012 mol) using 13.2 ml of 1*N* sodium hydroxide in 125 ml methanol. The condition of the reaction and the workup were identical to those employed for the corresponding dimer (II). The product was recrystallized from ethyl acetate to give a white powdery

material (4.5 g), which was homogeneous on silica thin layers [R_{fIII} = 0.1 (broad); R_{fV} = 0.43] using iodine vapor as the developing agent: mp 146–147°C, $[\alpha]_D^{23}$ −54.5 (c 0.56 TFE).

ANAL. Calcd. for $C_{20}H_{37}N_3O_6S_2$: N, 8.76; S, 13.37. Found: N, 8.65; S, 13.26.

L-Valyl-L-Methionyl-L-Methionine-Methyl Ester Hydrochloride (V). Boc-L-valyl-L-methionyl-L-methionine-methyl ester (0.90 g) was dissolved in 9 ml of freshly prepared 10*N* hydrochloric acid in methanol at 0°C. The reaction was allowed to proceed for 2 min after complete dissolution of the peptide. The product was then precipitated by rapidly pouring the methanolic solution into a large excess of stirred anhydrous ether. The precipitate was filtered, washed several times with anhydrous ether, and dried *in vacuo* over P_2O_5. A yellow-white solid (0.74 g, 94%; mp 199–201°C dec), which was homogeneous on silica thin layers (R_{fV} = 0.58) using iodine or ninhydrin as the developing agent, was recovered. This material was used directly in coupling reactions.

t-Butoxycarbonyl-L-Valyl-di-(L-Methionyl)-L-Methionine Methyl Ester (VI). *t*-Butoxycarbonyl-L-valyl-L-methionyl-L-methionine (1.92 g, 0.004 mol) was dissolved in 30 ml tetrahydrofuran and the solution was cooled to −20°C in a dry ice–carbon tetrachloride bath. *N*-Methyl morpholine (0.404 g, 0.004 mol) was added to the stirred solution, followed by isobutyl chloroformate (0.544 g, 0.004 mol). Addition of the latter caused the formation of a white precipitate. After stirring the resulting mixture for 3 min, a solution of methionine methyl ester hydrochloride (0.88 g, 0.0044 mol) and *N*-methyl morpholine (0.44 g, 0.0044 mol) in 3 ml of dry dimethyl formamide was added. The resulting mixture was allowed to slowly warm to room temperature and worked up as described for the analogous dipeptide. The product was recrystallized from hot ethyl acetate. A white powdery material (1.7 g, 70%; mp 184–185°C) was recovered. It was homogeneous on silica thin layers (R_{fI} = 0.49, R_{fII} = 0.05, R_{fIII} = 0.63, R_{fIV} = 0.71) using iodine vapor as the developing agent: $[\alpha]_D^{23}$ = −54.29 (c 0.52 HFA).

ANAL. Calcd. for $C_{26}H_{48}N_4O_7S_3$: N, 8.97; s, 15.39. Found: N, 8.90; S; 15.60.

L-Valyl-Di(L-Methionyl)-L-Methionine Methyl Ester Hydrochloride (VII). Boc-L-valyl-di-(L-methionyl)-L-methionine-methyl ester was treated with 1*N* HCl in methanol exactly as described for the analogous trimer. The solid hydrochloride (mp 226°C dec) was recovered in 99% yield. It was homogeneous on silica thin layers (R_{fV} = 0.55) and was used without further purification.

t-Butoxycarbonyl-L-Valyl-Tri-(L-Methionyl)-L-Methionine Methyl Ester (VIII). *t*-Butoxycarbonyl-L-valyl-L-methionine was coupled to di-(L-methionyl)-L-methionine methyl ester[20] using the mixed anhydride procedure. The reaction was carried out in tetrahydrofuran on a 0.002-mol scale. Isolation of the product was via a procedure identical to that described for the analogous dipeptide. The product (73% yield) gave a single iodine-positive, ninhydrin-negative spot on silica thin layers (R_{fIII} = 0.57, R_{fIV} = 0.70 tailing): mp 225–227°C; $[\alpha]_D^{23}$ = −64.5 (c 0.55 HFA).

ANAL. Calcd. for $C_{41}H_{57}N_5O_8S_4$: N, 9.26; S, 16.96. Found: N, 9.42; S, 17.10.

t-Butoxycarbonyl-L-Valyl-Tetra-(L-Methionyl)-L-Methionine Methyl Ester (IX). *t*-Butoxycarbonyl-L-valyl-L-methionyl-L-methionine was reacted with di-L-methionyl-L-methionine methyl ester[20] using the mixed anhydride procedure. The coupling was carried out in tetrahydrofuran on a 0.0013-mol scale. The product was isolated by diluting the crude reaction mixture in chloroform followed by extractions with 5% sodium bicarbonate, 10% citric acid, and saturated sodium chloride until neutral. The chloroform layer was dried over $MgSO_4$, evaporated almost to dryness in a rotary evaporator, and the product was precipitated with hexane. The white solid which was recovered (750 mg, 65%; mp > 240°C dec) was homogeneous on silica thin layers using iodine as the developing agent: $R_{f\,III} = 0.55$, $R_{f\,IV} = 0.70$ tailing; $[\alpha]_D^{23}$ −72.7 (c 0.33 HFA).

ANAL. Calcd. for $C_{36}H_{66}N_6O_9S_5$: N, 9.47; S, 18.07. Found: N, 9.46; S, 18.25.

t-Butoxycarbonyl-Tri-(L-Methionyl)-L-Valyl-L-Methionyl-Methionine Methyl Ester (X). *t*-Butoxycarbonyl-di-(L-methionyl)-L-methionine[15] was coupled to L-valyl-L-methionyl-L-methionine methyl ester using a procedure identical to that described for the preparation of Boc-Val-Met_5-OMe. The product (70% yield; mp > 240°C dec) gave one iodine-positive, ninhydrin-negative spot on thin layers of silica: $R_{f\,III} = 0.5$, $R_{IV} = 0.70$ tailing; $[\alpha]_D^{23} = 81.90$ (c 0.32 HFA).

ANAL. Calcd. for $C_{36}H_{66}N_6O_9S_5$: N, 9.47; S, 18.07. Found: N, 9.61; S, 18.36.

t-Butoxycarbonyl-L-Valyl-Penta-(L-Methionyl)-L-Methionine Methyl Ester (XI). *t*-Butoxycarbonyl-L-valyl-L-methionyl-L-methionine and tri-(L-methionyl)-L-methionine methyl ester[15] were coupled using the mixed anhydride procedure as described previously for the analogous hexamer. The crude product contained one minor purity and was purified using high-performance liquid chromatography on a prepacked silica column (E. M. Merck-Darmstadt). The product was eluted using chloroform/methanol (11:1). The white powder recovered after chromatography (80% yield; mp > 250°C dec) was homogeneous on silica thin layers: $R_{f\,III} = 0.52$, $R_{f\,IV} = 0.70$ tailing; $[\alpha]_D^{23}$ −75.6 (c 0.10 HFA).

ANAL. Calcd. for $C_{41}H_{75}N_7O_{10}S_6$: N, 9.63; S, 18.89. Found: N, 9.59; S, 18.62.

t-Butoxycarbonyl-Tri-(L-Methionyl)-L-Valyl-Di-(L-Methionyl)-L-Methionine Methyl Ester (XII). *t*-Butoxycarbonyl-di-L-methionyl-L-methionine[15] was reacted with L-valyl-di-(L-methionyl)-L-methionine methyl ester using the mixed anhydride procedure. The reaction was run on a 0.0012-mol scale and the conditions of reaction and isolation were identical to those described for Boc-Val-Met_5-OMe. The crude heptamer contained two minor trailing impurities on silica thin layers. These impurities were removed by washing the solid with methanol, ethyl acetate, and finally ether. The product thus obtained (70% yield; mp > 250°C) was homogenous on silica thin layers: $R_{f\,III} = 0.47$, $R_{f\,IV} = 0.7$ tailing; $[\alpha]_D^{23}$ −83.11 (c 0.26 HFA).

ANAL. Calcd. for $C_{41}H_{75}N_7O_{10}S_6$: N, 9.63; S, 18.89. Found: N, 9.70; S, 19.04.

The authors wish to acknowledge support of grants from the National Institutes of Health (GM-22086 and 22087). F.N. and J.M.B. are Research Career Development Awardees of the United States Public Health Service (GM-00025 and 00094).

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Received August 1, 1977
Accepted January 18, 1978