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

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Synopsis

The synthesis of 18 co-oligopeptides of L-methionine and glycine is reported. A series of oligomers, Boc-Gly-Met$_n$-OMe (n = 1–6), and six hexamers containing five methionyl and one glycyl residue were synthesized using the mixed anhydride procedure. Polarimetric studies give evidence that oligomers in the Boc-Gly-Met$_n$-OMe series are essentially disordered in hexafluoroacetone sesquihydrate but begin forming secondary structures at $n > 4$ in trifluoroethanol. Differences in the molar rotation values found for the six hexamers in hexafluoroacetone sesquihydrate may indicate that these compounds, while primarily disordered, are present in slightly different conformations.

INTRODUCTION

In previous communications we have reported on the synthesis and conformational analysis of linear oligopeptides containing L-methionine.[1,2] We believe that a significant amount of information pertaining to the conformation of these oligomers can be learned using high-resolution nmr. A prerequisite to extracting stereochemically relevant information is the ability to assign each resonance in the NH and αCH region of the spectrum to a given residue in the oligomer. In order to aid our assignment of the NH resonances, we have prepared co-oligopeptides of glycine and methionine. Unlike the other naturally occurring α-amino acids, glycine contains two αCH protons. The NH adjacent to the glycine residue should, therefore, appear as a triplet. This will permit us to assign the NH of the glycine residue and, by comparison, the NH of the methionine residues. We also expect to learn about the influence of a glycine residue on the secondary structure of short peptides and about how this influence depends on the position of glycine in the chain.

In this article we report the synthesis and optical rotation studies of a series of peptides, Boc-Gly-Met$_n$-OMe (n = 1–6), and of six hexamers containing one glycine and five methionine residues.

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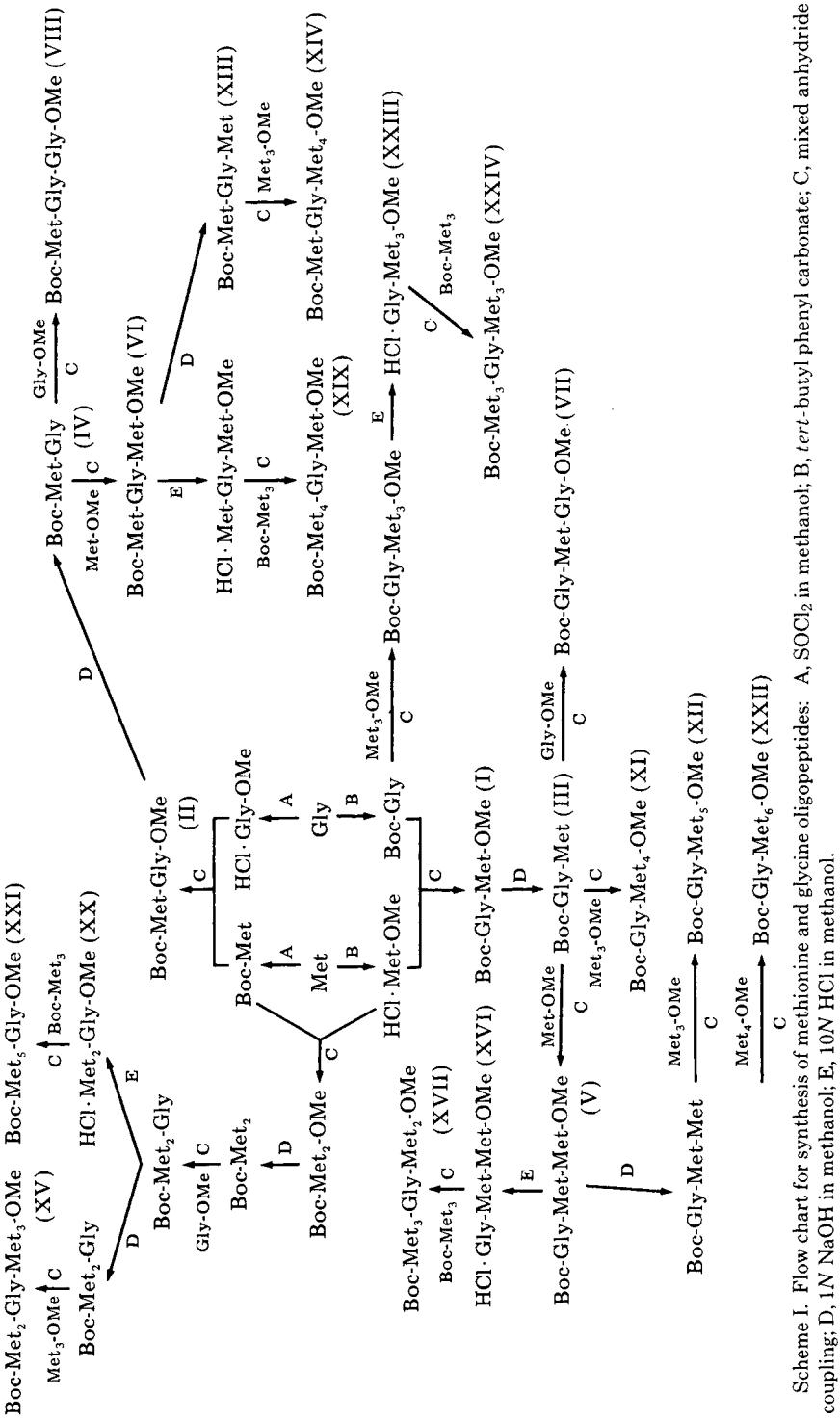
RESULTS AND DISCUSSION

The synthesis of the co-oligomers of methionine and glycine was carried out following the methods described in detail for the synthesis of the Boc-Val-Met$_n$-OMe series.[3] We commenced the synthesis by blocking the amine terminus of glycine and the carboxyl terminus of methionine with *tert*-butoxycarbonyl and methyl ester groups, respectively. The dipeptide, Boc-Gly-Met-OMe, was prepared from these fragments using isobutyl-chloroformate as the coupling agent.[4] All longer oligomers were prepared by selective removal of one of the protecting groups, followed by subsequent fragment coupling via the mixed anhydride procedure (Scheme I).

In general, the methods employed resulted in the formation of the desired product in high yield (70–95%). We did find slight differences in this synthesis as compared to the synthesis of the Boc-Val-Met$_n$-OMe series.[3] The valine-containing oligomers were either pure as recovered from the coupling reaction or could be purified by simply washing the crude product with several organic solvents. The glycine–methionine-containing peptides generally exhibited several minor impurities on silica thin layers and these impurities were not easily removed by recrystallization or repeated washings. It should be noted that these impurities were present even when analytically pure fragments and coupling reagents were used. Therefore we had to purify most oligomers longer than the tetrapeptide; this was accomplished by high-performance liquid chromatography on a silica gel column using chloroform/methanol (10:1) as eluent. After chromatography all peptides were homogeneous and gave the expected nitrogen and sulfur analyses (Table I).

We examined the optical purity of these peptides by measuring their optical rotation in both a structure-supporting [trifluoroethanol (TFE)] and a structure-breaking [hexafluoroacetone sesquihydrate (HFA)] solvent. The plot of molar rotation for the Boc-Gly-Met$_n$-OMe (n = 1–6) oligopeptides is linear in the latter solvent and deviates from linearity at n = 5 in the former (Fig. 1). These results suggest that the oligopeptides are predominantly disordered in HFA and free from significant amounts of racemization. This finding is in agreement with results of similar studies on other series of oligopeptides containing methionine[1] and alanine[5] which were also synthesized by the mixed anhydride procedure. The slope of the plot of $[\Phi]_M$ vs n gives the average contribution of an internal methionine residue to the molar rotation of the oligopeptide. The value of $[\Phi]_I$ found for the Boc-Gly-Met$_n$-OMe series is quite similar to that found for the Boc-Val-Met$_n$-OMe series[3] and the Boc-Met$_n$-OMe series.[1] This indicates that the methionine residues in the interior of these three oligopeptide series are in approximately equivalent environments. We should point out, however, that although these oligopeptides are primarily disordered in HFA, minor differences may exist between the exact conformation of the different molecules in solution. This is evidenced by the fact that there are variations in the molar rotations for the oligomers of methionine which contain one glycine residue at various positions in the hexapeptide. If one can calculate the molar rotation of a hexamer using Eq. (1) (see Ref. 6),



Scheme I. Flow chart for synthesis of methionine and glycine oligopeptides: A, $SOCl_2$ in methanol; B, *tert*-butyl phenyl carbonate; C, mixed anhydride coupling; D, 1*N* NaOH in methanol; E, 10*N* HCl in methanol.

TABLE I
Summary of Physical Properties of Co-Oligopeptides of Methionine and Glycine

Compound	mp[a] (°C)	$[\alpha]_D^{23}$ TFE[b,c]	$[\alpha]_D^{23}$ HFA[c,d]	Calcd. N	Calcd. S	Found[e] N	Found[e] S
Boc-Gly-Met-OMe	52–53	−7.82	−2.99	8.74	10.01	8.58	9.76
Boc-Met-Gly-OMe	87–88	−19.59	−13.30	8.74	10.01	8.67	10.15
Boc-Gly-Met-Met-OMe	71–75	−28.99	−19.00	9.31	14.20	9.24	14.29
Boc-Met-Gly-Met-OMe	oil	−13.07	−6.01	9.31	14.20	n.d.[f]	n.d.[f]
Boc-Met-Met-Gly-OMe	118–121	−45.86	−43.20	9.31	14.20	9.36	14.06
Boc-Gly-Gly-Met-OMe	90–91	n.d.[f]	−4.16	11.13	8.49	11.31	8.60
Boc-Gly-Met-Gly-OMe	103–104	−29.51	−21.61	11.13	8.49	11.07	8.37
Boc-Met-Gly-Gly-OMe	oil	−15.51	−3.41	11.13	8.49	n.d.[f]	n.d.[f]
Boc-Gly-$(Met)_3$-OMe	192–193	−48.52	−36.80	9.61	16.50	9.54	16.21
Boc-Gly-$(Met)_4$-OMe	210–211	−56.35	−52.30	9.81	17.96	9.77	17.78
Boc-Gly-$(Met)_5$-OMe	247 dec	−47.83	−54.00	9.94	18.96	9.83	18.70
Boc-Gly-$(Met)_6$-OMe	>300 dec	−44.38	−63.00	10.04	19.71	9.94	19.65
Boc-$(Met)_3$-Gly-$(Met)_3$-OMe	243–245 dec	−33.33[g]	−47.62[g]	10.04	19.71	9.89	19.63
Boc-Met-Gly-$(Met)_4$-OMe	226–227	−34.00	−52.81	9.94	18.96	9.80	18.73
Boc-$(Met)_2$-Gly-$(Met)_3$-OMe	229–230	−35.44	−44.10	9.94	18.96	10.01	18.70
Boc-$(Met)_3$-Gly-$(Met)_2$-OMe	229–231	−33.67	−48.67	9.94	18.96	9.83	18.78
Boc-$(Met)_4$-Gly-Met-OMe	217–219	−34.00	−51.31	9.94	18.96	9.78	18.72
Boc-$(Met)_5$-Gly-OMe	241–243 dec	−49.60	−68.73	9.94	18.96	10.11	18.80

[a] Melting points were measured using a Büchi capillary melting-point apparatus and are uncorrected.
[b] Trifluoroethanol.
[c] The concentrations used were approximately 0.5 mg/ml for tetrapeptides and lower oligomers and 0.3 mg/ml for all higher oligopeptides.
[d] Hexafluoroacetone sesquihydrate.
[e] Elemental analyses were performed by Galbraith Laboratories, Knoxville, Tenn.
[f] Not determined.
[g] The heptamer concentration was approximately 0.05 mg/ml.

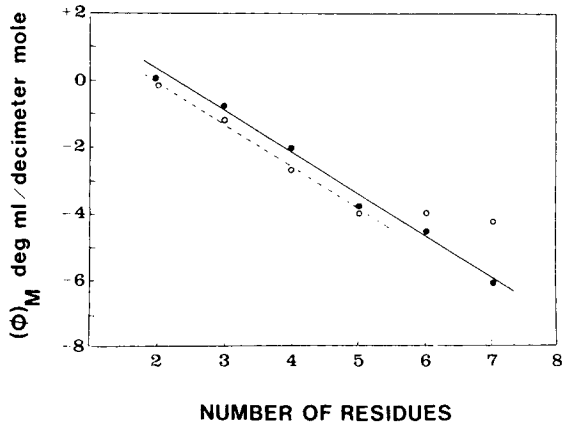


Fig. 1. Molar rotations of Boc-Gly-Met$_n$-OMe (n = 1–6) oligomers at the sodium D line: (●) HFA, (○) TFE. Oligomer concentration was approximately 5 mg/ml up to n = 3 and approximately 3 mg/ml for n = 4–6.

$$[\Phi]_{M_r} = [\Phi]_N + [\Phi]_C + n[\Phi]_I \quad (1)$$

where $[\Phi]_N$ is the contribution to molar rotation from the N terminus, $[\Phi]_C$ is the contribution to the molar rotation from the C terminus, and $[\Phi]_I$ is the contribution to the molar rotation from the internal residue, and assuming that chiral centers do not interact, then all of the hexamers with glycine at one of the four internal positions would have the same $[\Phi]_M$. The data in Table II show that, in fact, differences occur between the rotations of all hexamers, and in some cases these differences are rather pronounced. In addition there are significant deviations between $[\Phi]_M$ experimental and $[\Phi]_M$ calculated using compounds Boc-Met-Gly-Gly-OMe, Boc-Gly-Gly-Met-OMe, and Boc-Gly-Met-Gly-OMe as models for the N-terminal, C-terminal, and internal contribution to the molar rotation. These deviations suggest that either there are interactions between adjacent chiral centers

TABLE II
Molar Rotations of Hexapeptides Containing Methionine[a] and Glycine

Compound	M_r	$[\Phi]_M$ Found[b]	$[\Phi]_M$ Calcd.[c]
Boc-Gly-Met-Met-Met-Met-Met-OMe	845.14	−4.56	−3.41
Boc-Met-Gly-Met-Met-Met-Met-OMe	845.14	−4.46	−2.72
Boc-Met-Met-Gly-Met-Met-Met-OMe	845.14	−3.72	−2.72
Boc-Met-Met-Met-Gly-Met-Met-OMe	845.14	−4.12	−2.72
Boc-Met-Met-Met-Met-Gly-Met-OMe	845.14	−4.37	−2.72
Boc-Met-Met-Met-Met-Met-Gly-OMe	845.14	−5.80	−3.38

[a] All methionine residues are of the L-configuration.

[b] In HFA; the molar rotations are calculated using the equation $[\Phi]_M = ([\alpha]_D \times M_r)/10{,}000$.

[c] $[\Phi]_M^{calcd}$ was determined using Boc-Gly-Gly-Met-OMe, Boc-Gly-Met-Gly-OMe, and Boc-Met-Gly-Gly-OMe as models for the contribution of a methionyl residue in a C-terminal, internal, or N-terminal position of an oligopeptide.

in the molecule or that there are conformational differences between the hexapeptides in HFA. This latter possibility is strengthened by CD analysis of these compounds which will be reported elsewhere.

The molar rotations of the Boc-Gly-Met_n-OMe series in TFE deviate from linearity for $n > 4$, thus giving evidence for the onset of some secondary structure. Using optical rotation studies, it is not possible to unequivocally assign the nature of these conformations. CD studies give evidence that the heptamer Boc-Gly-Met_6-OMe may be somewhat helical in TFE, whereas it is primarily disordered in HFA.[7]

EXPERIMENTAL

L-Methionine was purchased from Bachem, Inc., Marina Del Rey, California. *N*-Methyl morpholine and tertiary butyl phenyl carbonate were purchased from Aldrich Chemical Co., New Jersey. 1,1,3,3-Tetramethylguanidine was obtained from Pfaltz and Bauer, New York. *t*-Butoxycarbonyl-glycyl-glycine was the generous gift of Dr. Israel Jacobson of the Weizmann Institute of Science, Israel. 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. Thin-layer chromatograms were run on 5 × 10 cm silica thin layers using one of the following developing systems: A, ethyl acetate; B, chloroform/methanol (2:1); C, chloroform/methanol (20:3); D, *n*-butanol/acetic acid/water (4:1:1); E, acetone.

PREPARATION OF COMPOUNDS

t-Butoxycarbonyl-Glycyl-L-Methionine Methyl Ester (*I*). *t*-Butoxycarbonyl-glycine[8] (8.75 g, 0.05 mol) was dissolved in 200 ml tetrahydrofuran in a round-bottomed flask containing a magnetic stirring bar. The solution was cooled to −20°C in a dry ice–carbon tetrachloride bath and *N*-methylmorpholine (5.0 g, 0.05 mol) added. Isobutylchloroformate (6.8 g, 0.05 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[9] (10.37 g, 0.052 mol) and *N*-methylmorpholine (5.25 g, 0.052 mol) in 10 ml dimethylformamide was added. The resulting mixture was allowed to warm slowly to room temperature over a 1-hr period and then diluted with 500 ml ethyl acetate. The mixture was extracted three times with 10% citric acid, three times with 5% sodium bicarbonate, and with saturated sodium chloride solution until neutral. The organic layer was dried over magnesium sulfate and the solvent removed under reduced pressure to give an oily residue. The oil was placed under petroleum ether and the product crystallized on standing overnight (15.8 g, 81%; mp 52–53°C): $[\alpha]_D^{23} - 7.82$ (c 0.55, TFE) and $[\alpha]_D^{23}$

− 2.99 (c 0.52, HFA). It gave one ninhydrin-negative, iodine-positive spot on thin layers of silica gel: $R_{fA} = 0.28$, $R_{fE} = 0.70$.

ANAL. Calcd. for $C_{13}H_{24}O_5N_2S$: N, 8.74; S, 10.01. Found: N, 8.58; S, 9.76.

t-Butoxycarbonyl-L-Methionyl-Glycine Methyl Ester (II). *t*-Butoxycarbonyl-L-methionine[8] was reacted with glycine methyl ester hydrochloride[10] as previously described for the preparation of *t*-butoxycarbonyl-glycyl-L-methionine methyl ester. The reaction yielded an oil that crystallized on standing. These crystals were placed over petroleum ether and allowed to stand overnight. Filtration yielded a product (76% yield; mp 87–88°C) that gave one ninhydrin-negative, iodine-positive spot on silica thin layers: $R_{fA} = 0.26$, $R_{fE} = 0.57$; $[\alpha]_D^{23}$ − 19.59 (c 0.485, TFE) and $[\alpha]_D^{23}$ − 13.30 (c 0.52, HFA).

ANAL. Calcd for $C_{13}H_{24}O_5N_2S$: N, 8.74; S, 10.01. Found: N, 8.67; S, 10.15.

t-Butoxycarbonyl-Glycyl-L-Methionine (III). *t*-Butoxycarbonyl-glycyl-L-methionine methyl ester (9.38 g, 0.0296 mol) was dissolved in 240 ml methanol and 1*N* sodium hydroxide (32.6 ml, 0.0325 mol) was added. The resulting solution was stirred at 25°C for 1 hr. At this time, all starting material had disappeared as judged by TLC. The solution was acidified with 10% citric acid to pH 4.0 and the solvent removed under reduced pressure. The remaining oil was diluted with saturated sodium chloride solution and extracted with ethyl acetate until no additional iodine-positive (silica gel) material was removed. The combined ethyl acetate (~750 ml) was extracted three times with 5% sodium bicarbonate solution. The aqueous portions were combined and acidified to pH 4.0 with 10% citric acid solution, causing the separation of an oil. The oil was extracted into ethyl acetate and the solvent dried over magnesium sulfate. The solvent was removed *in vacuo*, giving an oily solid (mp 93–100°C) that was homogeneous on silica thin layers: $R_{fB} = 0.31$; $[\alpha]_D^{23}$ − 2.69 (c 0.56, TFE) and $[\alpha]_D^{23}$ − 2.76 (c 0.55, HFA). This material was used without further purification.

t-Butoxycarbonyl-L-Methionyl-Glycine (IV). *t*-Butoxycarbonyl-L-methionyl-glycine methyl ester was saponified by the procedure described for the preparation of *t*-butoxycarbonyl-glycyl-L-methionine. After 1 hr of stirring, the product was worked up in a manner similar to Boc-Gly-Met(III), yielding an oil that when triturated with petroleum ether solidified: mp 118–120°C (73% yield), lit. mp 125–126 °C[11]; $[\alpha]_D^{23}$ −17.21 (c 0.50, TFE), lit. $[\alpha]_D$ − 12 (c 1.2, dimethylformamide). The product gave one ninhydrin-negative, iodine-positive spot on silica gel layers: $R_{fB} = 0.41$.

t-Butoxycarbonyl-Glycyl-L-Methionyl-L-Methionine Methyl Ester(V). *t*-Butoxycarbonyl-glycyl-*L*-methionine (6.35 g, 0.0210 mol) was dissolved in 100 ml tetrahydrofuran. The solution was then cooled to −20°C using a dry ice–carbon tetrachloride bath. *N*-Methyl morpholine (2.12 g, 0.0210 mol) was added, followed by the addition of isobutylchloroformate (2.86 g, 0.0210 mol), at which time a white precipitate formed. The mixture was allowed to stir at −20°C for 5 min, and then a solution of L-methionine

methyl ester hydrochloride[9] (4.25 g, 0.0213 mol) and *N*-methyl morpholine (2.15 g, 0.0213 mol) in dimethylformamide (18 ml) was added. The mixture was allowed to stir and warm to room temperature (1 hr), at which time ethyl acetate (250 ml) was added. The resulting organic layer was washed three times with 10% citric acid, three times with 5% sodium bicarbonate, three times with saturated sodium chloride solution, and then dried over magnesium sulfate. Concentration of the ethyl acetate *in vacuo* yielded an oil that solidified (8.46 g, 89%) when triturated with petroleum ether. Washing with hexane and hexane/ether (1:1) yielded a product (mp 71–75°C) that was homogeneous on thin layers of silica gel: $R_{f_A} = 0.23$, $R_{f_E} = 0.63$; $[\alpha]_D^{23} - 28.99$ (c 0.52, TFE) and $[\alpha]_D^{23} - 19.00$ (c 0.50, HFA).

ANAL. Calcd. for $C_{18}H_{33}O_6N_3S_2$: N, 9.31; S, 14.20. Found: N, 9.24; S, 14.29.

t-Butoxycarbonyl-L-Methionyl-Glycyl-L-Methionine Methyl Ester (VI). *t*-Butoxycarbonyl-L-methionyl-glycine was reacted with L-methionine methyl ester hydrochloride[9] as described for the preparation of *t*-butoxycarbonyl-glycyl-L-methionyl-L-methionine methyl ester. The reaction was run on a 0.0056-mol scale and an oil was obtained (80% yield). The crude product was purified using high-performance liquid chromatography on a prepacked silica column (E. M. Merck-Darmstadt). The oil was dissolved in a minimum volume of chloroform and introduced onto the column. The product was eluted with ethyl acetate/petroleum ether (1:1) and then ethyl acetate. Removal of the solvent gave an oil that resisted all attempts at crystallization. The oil gave one ninhydrin-negative, iodine-positive spot on thin layers of silica gel: $R_{f_A} = 0.33$; $[\alpha]_D^{23} - 13.07$ (c 0.68, TFE) and $[\alpha]_D^{23} - 6.01$ (c 0.92, HFA).

t-Butoxycarbonyl-Glycyl-L-Methionyl-Glycine Methyl Ester (VII). *t*-Butoxycarbonyl-glycyl-L-methionine and glycine methyl ester hydrochloride[10] were reacted in a procedure identical to that described for the preparation of *t*-butoxycarbonyl-glycyl-L-methionyl-L-methionine methyl ester. The crude oil was triturated in petroleum ether, yielding a white solid (73% yield). The solid was purified using high-performance liquid chromatography in a manner identical to that described for *t*-butoxycarbonyl-L-methionyl-glycyl-L-methionine methyl ester. Chromatography yielded an oil that solidified on trituration with petroleum ether. This solid (mp 103–104°C) was homogeneous on silica thin layers: $R_{f_A} = 0.32$, $R_{f_C} = 0.56$; $[\alpha]_D^{23} - 29.51$ (c 0.52, TFE) and $[\alpha]_D^{23} - 21.61$ (c 0.58, HFA).

ANAL. Calcd. for $C_{15}H_{27}O_6N_3S_1$: N, 11.13; S, 8.49. Found: N, 11.07; S, 8.37.

t-Butoxycarbonyl-L-Methionyl-Glycyl-Glycine Methyl Ester (VIII). *t*-Butoxycarbonyl-L-methionyl-glycine and glycine methyl ester hydrochloride[10] were coupled (80% yield) by the same procedure described for the synthesis of *t*-butoxycarbonyl-glycyl-L-methionyl-L-methionine methyl ester. The oil obtained in this preparation was purified by high-performance liquid chromatography by a method analogous to that used in the purification of *t*-butoxycarbonyl-L-methionyl-glycyl-L-methionine methyl ester. The oil obtained from the column could not be crystallized. TLC

on silica gel showed one ninhydrin-negative, iodine-positive spot: R_{fA} = 0.28, R_{fE} = 0.60, $[\alpha]_D^{23}$ − 15.51 (c 0.42, TFE) and $[\alpha]_D^{23}$ − 3.41 (c 0.67, HFA).

t-Butoxycarbonyl-Di-(Glycyl)-L-Methionine Methyl Ester (IX). *t*-Butoxycarbonyl-glycyl-glycine and methionine methyl ester hydrochloride[9] were coupled by the mixed anhydride procedure described in detail for the preparation of *t*-butoxycarbonyl-glycyl-L-methionyl-L-methionine methyl ester. The protected tripeptide product was crystallized (mp 90–91°C) from a small amount of ethyl acetate (50% yield). Chromatographic analysis on thin layers of silica showed one ninhydrin-negative, iodine-positive spot: R_{fA} = 0.21, R_{fE} = 0.49; $[\alpha]_D^{23}$ − 4.16 (c 0.39, HFA).

t-Butoxycarbonyl-Glycyl-Di-(L-Methionyl)-L-Methionine Methyl Ester (X). *t*-Butoxycarbonyl glycine[8] (0.61 g, 0.0035 mol) was dissolved in tetrahydrofuran and the solution cooled to −20°C in a dry ice–carbon tetrachloride bath. *N*-Methyl morpholine (0.354 g, 0.0035 mol) and then isobutyl chloroformate (0.476 g, 0.0035 mol) were added, producing a white precipitate. After stirring at −20°C for 3 min, di-(L-methionyl)-L-methionine methyl ester hydrochloride[12] (1.65 g, 0.00358 mol) and *N*-methyl morpholine (0.362 g, 0.00358 mol) dissolved in dimethylformamide were added. The resulting mixture was allowed to warm to room temperature (1 hr) and diluted with ethyl acetate. The organic layer was washed successively three times with 10% citric acid, three times with 5% sodium bicarbonate, and three times with saturated sodium chloride. The ethyl acetate solution was dried over magnesium sulfate and concentrated *in vacuo* to give a solid (1.58 g, 77%; mp 192–193°C). An analytical sample was obtained by recrystallization from ethyl acetate. The product gave one ninhydrin-negative, iodine-positive spot on silica thin layers: R_{fA} = 0.38, R_{fC} = 0.62, $[\alpha]_D^{23}$ − 48.52 (c 0.5, TFE) and $[\alpha]_D^{23}$ − 36.80 (c 0.5, HFA).

ANAL. Calcd. for $C_{23}H_{42}O_7N_4S_3$: N, 9.61; S, 16.50. Found: N, 9.54; S, 16.21.

t-Butoxycarbonyl-Glycyl-Tri-(L-Methionyl)-L-Methionine Methyl Ester (XI). *t*-Butoxycarbonyl-glycyl-L-methionine (1.30 g, 0.00430 mol) was dissolved in 50 ml tetrahydrofuran, and the resulting solution cooled to −20°C in a dry ice–carbon tetrachloride bath. *N*-Methyl morpholine (0.434 g, 0.00430 mol) was then added, followed by the addition of isobutylchloroformate (0.585 g, 0.0430 mol). After stirring at −20°C for 3 min, di-(L-methionyl)-L-methionine methyl ester hydrochloride[12] (2.09 g, 0.00453 mol) and *N*-methyl morpholine (0.458 g, 0.00453 mol) dissolved in 10 ml dimethylformamide were added to the reaction mixture. The resulting mixture was stirred at −20°C for 2 hr and then allowed to warm slowly to room temperature. The reaction was then diluted with ethyl acetate and washed three times each with 10% citric acid and 5% sodium bicarbonate. After the sodium bicarbonate wash, the product began crystallizing from the ethyl acetate solution. The crystals were collected by filtration and the filtrate washed with saturated sodium chloride solution. After the salt wash, more crystals precipitated and were collected

by filtration and combined (2.03 g) with the first crop. The filtrate was dried over magnesium sulfate and concentrated to a crystalline solid (0.44 g). Both fractions were identical on silica gel thin layers and were combined (80% yield): $R_{fA} = 0.22$, $R_{fC} = 0.65$. Recrystallization from methanol yielded a product (mp 210–211°C) that was homogeneous on silica thin layers: $R_{fA} = 0.22$, $R_{fC} = 0.65$; $[\alpha]_D^{23} - 56.35$ (c 0.35, TFE) and $[\alpha]_D^{23} - 52.30$ (c 0.30, HFA).

ANAL. Calcd. for $C_{28}H_{51}O_8N_5S_4$: N, 9.81; S, 17.96. Found: N, 9.77; S 17.78.

t-Butoxycarbonyl-Glycyl-Tetra-(L-Methionyl)-L-Methionine Methyl Ester (XII). *t*-Butoxycarbonyl-glycyl-L-methionyl-L-methionine[4] (0.875 g, 0.0020 mol) was dissolved in 25 ml tetrahydrofuran and the solution cooled to −20°C in a dry ice–carbon tetrachloride bath. *N*-Methyl morpholine (0.202 g, 0.0020 mol) followed by isobutylchloroformate (0.272 g, 0.0020 mol) were added to the solution. The resulting mixture was stirred for 5 min and then di-(L-methionyl)-L-methionine methyl ester hydrochloride[12] (0.922 g, 0.0022 mol) and *N*-methyl morpholine (0.222 g, 0.0022 mol) dissolved in 2.0 ml dimethylformamide were added to the stirring mixture. The reaction was allowed to stir at −20°C for 2 hr and then warmed slowly to room temperature. The reaction mixture was then diluted with chloroform (~300 ml) and washed three times each with 10% citric acid and 5% sodium bicarbonate, and two times with saturated sodium chloride sodium. The chloroform solution was dried over magnesium sulfate and concentrated *in vacuo* to a solid (1.69 g). This residue was dissolved in a minimum volume of chloroform and precipitated with ethyl acetate to give a compound (1.0 g, 59%; mp 247°C dec.) that was homogeneous on silica thin layers: $R_{fA} = 0.16$ and $R_{fC} = 0.68$; $[\alpha]_D^{23} - 47.83$ (c 0.30, TFE) and $[\alpha]_D^{23} - 54.00$ (c 0.30, HFA).

ANAL. Calcd. for $C_{33}H_{60}O_9N_6S_5$: N, 9.94; S, 18.96. Found: N, 9.83; S, 18.70.

t-Butoxycarbonyl-L-Methionyl-Glycyl-L-Methionine (XIII). *t*-Butoxycarbonyl-L-methionyl-glycyl-L-methionine methyl ester (3.26 g, 0.0072 mol) was dissolved in methanol (40 ml). Sodium hydroxide (1*N*) (7.9 ml, 0.0079 mol) was added and the resulting mixture stirred for 1 hr at 25°C. At this time the solution was acidified with 10% citric acid to pH 4.0. The methanol was then removed under reduced pressure, leaving an oil residue that was subsequently diluted with saturated sodium chloride solution. The oil was then extracted into ethyl acetate and washed with 10% citric acid solution. The organic phase was then extracted with three portions of 5% sodium bicarbonate. The combined sodium bicarbonate fractions were acidified to pH 4.0 with 10% citric acid, saturated with sodium chloride, and then extracted with ethyl acetate three times. The combined organic phase was washed with saturated sodium chloride solution until neutral and then dried over magnesium sulfate. The dried solvent was concentrated *in vacuo* to a solid (2.32 g, 74%; mp 133–135°C) which was homogeneous on thin layers of silica gel: $R_{fB} = 0.58$; $[\alpha]_D^{23} - 27.48$ (c 0.31, TFE). This material was used without further purification.

t-Butoxycarbonyl-L-Methionyl-Glycyl-Tri-(L-Methionyl)-L-Methionine Methyl Ester (XIV). *t*-Butoxycarbonyl-L-methionyl-glycyl-L-methionine and di-(L-methionyl)-L-methionine methyl ester hydrochloride[12] were coupled by the mixed anhydride procedure as described for the synthesis of *t*-butoxycarbonyl-glycyl-tetra-(L-methionyl)-L-methionine methyl ester. The solid obtained on removal of the chloroform (80% yield) was partially purified by washing with petroleum ether and then with ethyl acetate. The compound was further purified by high-performance liquid chromatography on a prepacked silica column (E. M. Merck-Darmstadt). The product was applied in chloroform and then eluted with chloroform, ethyl acetate/hexane (1:1), chloroform/methanol (20:1), and then chloroform/methanol (10:1). Removal of the solvent *in vacuo* yielded an analytically pure solid (mp 226–227°C) that was homogeneous on silica thin layers: $R_{fC} = 0.65$; $[\alpha]_D^{23} - 34.00$ (c 0.30, TFE) and $[\alpha]_D^{23} - 52.81$ (c 0.32, HFA).

ANAL. Calcd. for $C_{33}H_{60}O_9N_6S_5$: N, 9.94; S, 18.96. Found: N, 9.80; S, 18.73.

t-Butoxycarbonyl-Di-(L-Methionyl)-Glycyl-Di-(L-Methionyl)-L-Methionine Methyl Ester (XV). This compound was prepared from *t*-butoxycarbonyl-di-(L-methionyl)-glycine[4] and di-(L-methionyl)-L-methionine methyl ester hydrochloride[12] by the procedure described for the preparation of *t*-butoxycarbonyl-glycyl-tetra-(L-methionyl)-L-methionine methyl ester. The solid obtained in this manner was purified by precipitation from acetone with water (70% yield; mp 229–230°C). The product was homogeneous on silica thin layers: $R_{fC} = 0.62$; $[\alpha]_D^{23} - 35.44$ (c 0.30, TFE) and $[\alpha]_D^{23} - 44.1$ (c 0.30, HFA).

ANAL. Calcd. for $C_{33}H_{60}O_9N_6S_5$: N, 9.94; S, 18.96. Found: N, 10.01; S, 18.70.

Glycyl-L-Methionyl-L-Methionine Methyl Ester Hydrochloride (XVI). *t*-Butoxycarbonyl-glycyl-L-methionyl-L-methionine methyl ester (1.0 g, 0.0022 mol) was treated with 13.7 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 phosphorous pentoxide. The white solid (0.9 g, 70%, mp 50–54°C) was homogeneous on silica thin layers ($R_{fD} = 0.36$) using iodine or ninhydrin as the visualizing agent. This material was used without further purification.

t-Butoxycarbonyl-Tri-(L-Methionyl)-Glycyl-L-Methionyl-L-Methionine Methyl Ester (XVII). *t*-Butoxycarbonyl-di-(L-methionyl)-L-methionine[1] and glycyl-L-methionyl-L-methionine methyl ester were coupled by mixed anhydride procedure as described for the analogous hexapeptide *t*-butoxycarbonyl-glycyl-tetra-(L-methionyl)-L-methionine methyl ester. The crude product was purified using high-performance liquid chromatography on a prepacked silica column (E. M. Merck-Darmstadt). The material was applied onto the column in chloroform and then eluted with

chloroform and then chloroform/methanol (10:1). The pure solid product so obtained [mp 229–231°C; $[\alpha]_D^{23}$ − 33.67 (c 0.30, TFE) and $[\alpha]_D^{23}$ − 48.67 (c 0.30, HFA)] was homogeneous on silica thin layers (R_{fC} = 0.60).

ANAL. Calcd. for $C_{33}H_{60}O_9N_6S_5$: N, 9.94; S, 18.96. Found: N, 9.83; S, 18.78.

L-Methionyl-Glycyl-L-Methionine Methyl Ester Hydrochloride (XVIII). The *t*-butoxycarbonyl blocking group was cleaved from *t*-butoxycarbonyl-L-methionyl-glycyl-L-methionine methyl ester with 10*N* hydrochloric acid in methanol by a procedure identical to that used for the preparation of glycyl-L-methionyl-L-methionine methyl ester hydrochloride. The white solid (mp 60–65°C) was homogeneous on thin layers of silica (R_{fD} = 0.49) and was used without further purification.

t-Butoxycarbonyl-Tetra-(L-Methionyl)-Glycyl-L-Methionine Methyl Ester (XIX). This compound was prepared from *t*-butoxycarbonyl-di-(L-methionyl)-L-methionine[1] and L-methionyl-glycyl-L-methionine methyl ester hydrochloride by a procedure analogous to that used for the hexapeptide, *t*-butoxycarbonyl-glycyl-tetra-(L-methionyl)-L-methionine methyl ester. The crude product was purified using high-performance liquid chromatography on a prepacked silica column (E. M. Merck-Darmstadt), as described for compound XIV. The product (73% recovery, mp 217–219°C) was homogeneous on silica thin layers: R_{fC} = 0.57; $[\alpha]_D^{23}$ − 34.00 (c 0.30, TFE) and $[\alpha]_D^{23}$ − 51.31 (c 0.35, HFA).

ANAL. Calcd. for $C_{33}H_{60}O_9N_6S_5$: N, 9.94; S, 18.96. Found: N, 9.78; S, 18.72.

Di-(L-Methionyl)-Glycine Methyl Ester Hydrochloride (XX). *t*-Butoxycarbonyl-di-(L-methionyl)-glycine methyl ester was treated with 10*N* hydrochloric acid in methanol exactly as described for the synthesis of the analogous trimer, glycyl-L-methionyl-L-methionine methyl ester hydrochloride. The product (91% yield, mp 74–75°C) was homogeneous on silica thin layers (R_{fD} = 0.54). This material was used without further purification.

t-Butoxycarbonyl-Penta-(L-Methionyl)-Glycine Methyl Ester (XXI). *t*-Butoxycarbonyl-di-(L-methionyl)-L-methionine[13] and di-(L-methionyl)-glycine methyl ester hydrochloride were coupled by the same procedure used for the preparation of the analogous hexapeptide, *t*-butoxycarbonyl-glycyl-tetra-(L-methionyl)-L-methionine methyl ester. The crude product (92% yield) was purified using high-performance liquid chromatography on a prepacked silica column (E. M. Merck-Darmstadt). The sample (220 mg) was introduced in a solution of chloroform/methanol (1:1) and eluted with chloroform/methanol (10:1). The white solid [60 mg; mp 241–243°C dec.; $[\alpha]_D^{23}$ − 49.6 (c 0.31, TFE), and $[\alpha]_D^{23}$ − 68.73 (c 0.27, HFA)] was homogeneous on silica thin layers (R_{fC} = 0.62).

ANAL. Calcd. for $C_{33}H_{60}O_9N_6S_5$: N, 9.94; S, 18.96. Found: N, 10.11; S, 18.80.

t-Butoxycarbonyl-Glycyl-Penta-(L-Methionyl)-L-Methionine Methyl Ester (XXII). *t*-Butoxycarbonyl-glycyl-L-methionyl-L-methionine (0.652 g, 0.00149 mol) was dissolved in tetrahydrofuran (50 ml). The solution was

cooled to −20°C in a dry ice–carbon tetrachloride bath and *N*-methyl morpholine (0.150 g, 0.00149 mol) was added to the stirring solution, followed by isobutylchloroformate (0.203 g, 0.00149 mol). Addition of the latter caused the formation of a white precipitate. After stirring the reaction mixture for 3 min, a solution of tri-(L-methionyl)-L-methionine methyl ester hydrochloride[1] (0.951 g, 0.00160 mol) and *N*-methyl morpholine (0.162 g, 0.00160 mol) in dimethylformamide (~2.0 ml) was added. The resulting mixture was stirred for 3 hr at −20°C and then warmed to room temperature. Attempts to dilute with chloroform and wash with 10% citric acid were problematic due to emulsion formation. Instead, the solvent was removed *in vacuo* and the residue recovered on a sintered glass filter funnel, where it was washed successively with 10% citric acid, distilled water, 5% sodium bicarbonate, saturated sodium chloride solution, and distilled water. The residue was then dried *in vacuo* (72% yield) and further washed with hot ethyl acetate followed by cold methanol. The dried solid was then dissolved in acetone and precipitated with water. The product (mp >300°C dec.) was homogeneous on silica thin layers: R_{f_A} = 0.13, R_{f_C} = 0.70; $[\alpha]_D^{23}$ − 44.38 (c 0.30, TFE), and $[\alpha]_D^{23}$ − 63.00 (c 0.30, HFA).

ANAL. Calcd for $C_{38}H_{69}O_{10}N_7S_6$: N, 10.04; S, 19.71. Found: N, 9.94; S, 19.65.

Glycyl-Di-(L-Methionyl)-L-Methionine Methyl Ester Hydrochloride (XXIII). The *t*-butoxycarbonyl blocking group was cleaved from *t*-butoxycarbonyl-glycyl-di-(L-methionyl)-L-methionine methyl ester with 10*N* hydrochloride acid in methanol by a procedure identical to that used for the preparation of the analogous tripeptide, compound XVI. The white solid obtained (78% yield, mp 187–189°C) was homogeneous on thin layers of silica gel (R_{f_D} = 0.55). This compound was used without further purification.

t-Butoxycarbonyl-Tri-(L-Methionyl)-Glycyl-Di-(L-Methionyl)-L-Methionine Methyl Ester (XXIV). *t*-Butoxycarbonyl-di-(L-methionyl)-L-methionine[13] (0.766 g, 0.0015 mol) was dissolved in 30 ml tetrahydrofuran. The solution was cooled to −20°C in a dry ice–carbon tetrachloride bath and *N*-methyl morpholine (0.150 g, 0.0015 mol) followed by isobutylchloroformate (0.204 g, 0.0015 mol) was added. The resulting mixture was stirred for 3 min at −20°C and then a solution of *N*-methyl morpholine (0.150 g, 0.0015 mol) and glycyl-di-(L-methionyl)-L-methionine methyl ester hydrochloride (0.80 g, 0.00154 mol) in dimethylformamide (3 ml) was added. The reaction was allowed to stir at −20°C for 3 hr and then warmed to room temperature. Dilution with chloroform (400 ml) did not produce a clear solution and methanol was added until complete solution was achieved. On washing with citric acid solution, the product precipitated. Additional methanol was added to redissolve the precipitate, and the organic phase was washed three times with 10% citric acid, two times with 5% sodium bicarbonate, and once with saturated sodium chloride solution. The solvent was dried over magnesium sulfate and concentrated *in vacuo* to a small volume. The product was precipitated by the addition of ethyl

acetate followed by hexane (1.05 g, 72%; mp 243–245°C dec.). Chromatographic analysis on thin layers of silica gel indicated one ninhydrin-negative, iodine-positive spot: $R_{fC} = 0.66$; $[\alpha]_D^{23} - 33.33$ (c 0.05, TFE) and $[\alpha]_D^{23} - 47.62$ (c 0.074, HFA).

ANAL. Calcd. for $C_{38}H_{69}O_{10}N_7S_6$: N, 10.04; S, 19.71. Found: N, 9.89; S, 19.63.

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