mol) of 85% MCPBA in 50 ml of CH_2Cl_2 was added dropwise. Following the addition, the reaction mixture was refluxed for 15 min and dried over $MgSO_4$. The removal of the solvent gave a dark oil which was dissolved in 50 ml of alcohol containing 0.38 g of KOH. After refluxing for 1.5 h the solution was diluted with water and extracted with CH_2Cl_2. The dried CH_2Cl_2 solution was analyzed as described above by GLC.

Oxidation of 1b. In a 250-ml three-necked flask equipped with dropping funnel and magnetic stirrer and containing 0.8 g of $NaCO_3$ in 50 ml of water was placed 1.43 g (0.0066 mol) of **1b** in 50 ml of CH_2Cl_2. The reaction mixture was cooled in an ice bath and 1.43 g (0.0066 mol) of 85% MCPBA in 50 ml of CH_2Cl_2 added dropwise. The solution was stirred for 15 min, washed with water, and dried over $MgSO_4$. Removal of the solvent gave 1.0 g (81%) of an oil identified as aniline (**4b**) by comparison of its ir and NMR spectra with those of an authentic sample.

Oxidation of 1b Followed by Reaction with 2,3-Dimethyl-1,3-butadiene. In a 250-ml three-necked flask equipped with dropping funnel and magnetic stirrer was placed 3.0 g (0.0131 mol) of **1b** in 50 ml of $CHCl_3$. The solution was cooled in an ice bath and 2.8 g (0.014 mol) of 85% MCPBA in 50 ml of $CHCl_3$ was added dropwise. After stirring for 15 min the precipitate was removed and 3 ml of 2,3-dimethyl-1,3-butadiene added. After stirring for 3 days the solution was washed with 10% $NaHCO_3$ solution and dried over $MgSO_4$. Removal of the solvent gave an oil which contained **4b** and **8** by TLC (silica gel). Removal of the aniline by molecular distillation gave a solid which was crystallized from cyclohexane to give 1.5 g (52%) of white crystals, mp 78–80 °C (lit.[15] mp 79–80 °C).

Registry No.—**1a,** 58241-34-2; **1b,** 13628-09-6; **1c,** 13616-64-3; **1d,** 13616-65-4; **1e,** 19552-05-7; **4a,** 104-94-9; **4b,** 62-53-3; **4c,** 106-40-1; **4d,** 106-47-8; **4e,** 100-01-6; **5a,** 58241-35-3; **5b,** 58241-36-4; **5c,** 58241-37-5; **5d,** 58267-78-0; **5e,** 58241-38-6; sulfur dichloride, 10545-99-0; piperidine-1-sulfenyl chloride, 16005-90-6.

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Thioasparagine and Derivatives for Peptide Synthesis. A Trifluoroacetic Acid Catalyzed Anisyl Transfer to Sulfur

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Thioamidation of *N*-*p*-methoxybenzyloxycarbonyl-L-β-cyanoalanine with H_2S–NH_3 furnished *N*-*p*-methoxybenzyloxy-L-thioasparagine. Deprotection of the latter with trifluoroacetic acid or HF yielded the asparagine analogue, thioasparagine (L-aspartic acid β-thioamide). *tert*-Butyloxycarbonyl-L-thioasparagine and benzyloxycarbonyl-L-thioasparagine were prepared from the corresponding β-cyanoalanine derivatives by similar treatment, and these likewise gave thioasparagine. During the deprotection of Meoz-L-thioasparagine in TFA a major side reaction (anisyl transfer) ensued that led to the formation of aspartic *p*-methoxybenzyl β-imidothiolic ester and, from this, β-*S*-*p*-methoxybenzyl aspartic thioester. This transfer reaction has been extended to the synthesis of a thioether, *S*-*p*-methoxybenzylcysteine. Anisyl transfer can therefore be a source of decreased yield in peptide synthesis when the *N*-p-methoxybenzyloxycarbonyl group is deprotected in the presence of a preformed or newly generated thiol group. *N*-Meoz-L-alanine and *p*-methoxybenzyl carbazate in TFA under very mild conditions are convenient alternate sources of the *p*-methoxybenzyl carbonium ion. *N*-*p*-Methoxybenzyl thioacetamide was synthesized for ^1H NMR reference.

Intramolecular hydrogen bonding between the carbonyl oxygen of specific carboxamide or peptide linkages and the amide hydrogen of other peptide or amide linkages in polypeptides is considered to be one of the key features in determining and stabilizing conformation. For example, in oxytocin and vasopressin[1] the asparagine residue participates in forming the characteristic "β turn" of the ring moiety by means of hydrogen bonding. These thoughts have led us to consider the effect on biological activity of subtle structural changes that might alter chiefly the degree of intramolecular hydrogen bonding. Replacement of a carboxamide by a thiocarboxamide might be expected to introduce electronic effects suitable for such a purpose.[2] Accordingly, the desire to replace asparagine in oxytocin with thioasparagine prompted synthesis of the latter. Thioasparagine had added interest as a possible antimetabolite of asparagine since asparagine holds an important position as a nutritional requirement in the metabolism of certain neoplastic cells lacking asparagine synthetase.[3] This report deals with the synthesis and chemical properties of thioasparagine and makes available a variety of derivatives expected to be suitable for its introduction into peptides. During the deprotection of *N*-*p*-methoxybenzyloxycarbonyl-L-thioasparagine to thioasparagine with trifluoroacetic acid an unexpected alkylating side reaction was encountered that led predominantly to the formation of the *S*-anisyl ester of β-thioaspartic acid. The reaction has preparative value also for the synthesis of an *S*-anisyl thioether, *S*-anisyl-L-cysteine. Possible implication of this side reaction for peptide synthesis has been pointed out.

General methods for the synthesis of thioamides include thiolysis of nitriles, amidines, or imidic esters with H_2S and the thionation of amides with phosphorus pentasulfide.[4] The base-catalyzed addition of H_2S to the nitrile was chosen for the synthesis of thioasparagine, with the amino nitrogen group being protected by the *p*-methoxybenzyloxy-

carbonyl group, inasmuch as Meoz-β-cyanoalanine was available in the laboratory from previous work.[5] In addition this route had recently proved suitable for preparing a series of amino acid thioamides corresponding to alanine, phenylalanine, dihydrophenylalanine, tyrosine, Dopa, and histidine from the *N*-Meoz-cyanoamine.[6]

Treatment of Meoz-β-cyanoalanine (**1a**) with H_2S and concentrated NH_3 furnished a solid product that when deprotected and examined on the amino acid analyzer showed incomplete thioamidation. Separation of Meoz-L-thioasparagine (**2a**) from the starting material was accomplished through crystallization of the dicyclohexylamine salt. After liberation from the DCHA salt, purified **2a** melted sharply some 37 °C below the corresponding asparagine derivative. Properties and yields are listed in Table I. Deprotection of **2a** with trifluoroacetic acid in the usual manner (TFA containing 3 equiv of anisole at 0 °C)[7] afforded crystalline thioasparagine (**3**). Thioasparagine showed the expected elemental analysis, infrared absorption, and chromatographic and electrophoretic behavior. However, the yield of **3** in the deprotection step was only 25%. Deprotection of **2a** in the presence of a large excess of anisole (43 equiv) raised the yield of **3** to 64%. Deprotection with HF in the absence and the presence of anisole gave **3** in 85 and 100% yield, respectively.

For comparison *tert*-butyloxycarbonyl (Boc) and benzyloxycarbonyl (Z) were also examined for suitability as N-protecting groups for the synthesis of thioasparagine. Boc-β-cyanoalanine (**1b**), known previously as its DCHA salt,[8] was obtained in the free state as a crude solid melting at 92–94 °C and was used directly. The thioamidation of **1b** and of **1c**[9] was accomplished with H_2S and NH_3 in the same manner as for **1a**. In each case a single recrystallization of the DCHA salt, with the precaution of not cooling below room temperature, was usually sufficient to remove the unreacted β-cyanoalanine derivative. Properties and yields of these derivatives of **3** are included in Table I. Deprotection of **2b** with TFA gave **3** in high yield in the presence or absence of anisole. Deprotection of **2c** with 30% HBr–acetic acid at 25 °C likewise was satisfactory. This latter reaction, however, tended to be susceptible to hydrolysis to give asparagine which was observed when a long reaction time or a large excess of reagent was used. In each of the three preparations of **3** the limiting step was thioamidation, the yields being 20–40%. The Meoz derivative is generally preferred on the basis of ease of recrystallization. For incorporating thioasparagine into peptides *N*-Boc-, *N*-Z-, and *N*-Meoz-L-thioasparagine should all be suitable providing appropriate care is taken in subsequent deprotection.

When the deprotection of **2a** was carried out in TFA in the presence of 2 equiv of anisole, in addition to **3**, 32% of another ninhydrin-positive compound was obtained that was less water soluble and higher melting. In the absence of anisole the yield of this by-product rose to 81%. The ^1H NMR spectrum of this material (two aromatic doublets) suggested the presence of an anisyl group. Strong absorption at 1675 cm^{-1} in the infrared is characteristic of thiolic esters.[10] Treatment with several equivalents of alkali at 25 °C or dilute acid at 100 °C afforded aspartic acid in close to quantitative yield. In the acid-treated material a nitroprusside-positive compound was present that was identified as anisyl mercaptan by oxidation to the well-characterized anisyl disulfide.[11] Alkaline hydrolysis yielded the disulfide directly. On this basis as well as elemental analysis the isolated by-product was considered to be β-*S*-anisyl aspartic thioester (**5**).

Alkylating side reactions involving a protecting group have been encountered occasionally before on deprotection

Table I. Syntheses and Properties of Thioasparagine and Derivatives

Starting compd RNHCHCOOH (1) $CH_2C{\equiv}N$	DCHA salt		Thioasparagine derivative[a] RNHCHCOOH (2) CH_2CSNH_2							Thioasparagine (3)	
			Carboxylic acid								
	Mp, °C	Crystn solvent	Mp, °C	Crystn solvent	Formula	Anal, %				Rotation $[\alpha]_D$, deg	Yield,[b] %
R = $CH_3OC_6H_4CH_2OCO$–, Meoz	155–165										
	174–176 (38)	EtOH	122–123 (87)	H_2O–EtOH	$C_{13}H_{16}N_2O_5S$	C, 50.0	H, 5.16	N, 8.97	S, 10.3	−36.6	68[c]
						50.2	5.17	8.77	10.2		
R = $C_6H_5CH_2OCO$–, Z	155–160		120–122								
	167–169 (39)	EtOH	123–125 (82)	H_2O	$C_{12}H_{14}N_2O_4S$	51.1	5.00	9.92	11.3	−42.9	90 + 4.3 asn
		Et_2O				50.9	5.01	9.80	11.2		+ 1.3 asp[d]
R = $(CH_3)_3COCO$–, Boc	174–178		65–68								
	183–184[e] (21)	EtOH	69–71 (70)	H_2O	$C_9H_{16}N_2O_4S{\cdot}H_2O$	40.6	6.81	10.5	12.1	−35.7	
						40.9	6.58	10.4	12.1		85 + 1.4 asn[f]

[a] Melting points of crude products are given first, those of purified products are below. Yields of **2** are given within parentheses. For analyses calculated values are given first; found values are below. Optical rotations are at 24–25 °C in methanol, *c* 1. [b] Deprotection yields as determined on the amino acid analyzer. [c] TFA and anisole for 20 min at 0 °C. [d] 8 equiv HBr–HOAc for 2 h at 25 °C; 4 equiv for 1 h gave 53% 3; 300 equiv for 35 min gave 19% 3 and 61% asn. [e] Anal. Calcd for $C_{21}H_{39}N_3O_4S$: C, 58.7; H, 9.15; N, 9.78; S, 7.45. Found: C, 59.0; H, 9.27; N, 9.68; S, 7.47. [f] TFA for 1 h at 25 °C.

Table II. ^{1}H NMR Chemical Shifts of Various *S*- and *N*-Anisyl and Benzyl Derivatives[a]

Registry no.	Compd	Solvent[b]	$ArCH_2$	$ArOCH_3$
2544-31-2	*S*-*p*-Methoxybenzyl-L-cysteine	1	3.4	3.55
16741-80-3	*S*-Benzyl-L-cysteine OMe·HCl	1	3.4	
		3	3.83	
17004-42-1	*p*-Methoxybenzyl disulfide	2	3.7	3.85
		3	3.69	3.72
6436-90-4	*N*-Benzylglycine OEt	1	4.18	
		3	3.73	
103-46-8	*S*-Benzylthioglycolic acid	1	3.39	
		3	3.82	
2393-23-9	*p*-Methoxybenzylamine	1	4.45	4.03
		3	3.62	3.70
6258-60-2	*p*-Methoxybenzyl mercaptan	1[c]	3.87	3.52
		3	3.70 (d)	3.72
58208-17-6	β-*S*-*p*-Methoxybenzyl aspartic thioester (5)	1	3.78	3.51
		3	3.68	3.76
		4[c]	3.55	3.76
58208-18-7	β-*p*-Methoxybenzyl aspartic imidothiolic ester (4a)	1	4.10	3.45
		3	3.71	3.73
58208-19-8	*N*-*p*-Methoxybenzyl thioacetamide[d]	1[c]	4.73	4.05
		2	4.73	3.83
		3	4.71 (d)	3.73

[a] Tetramethylsilane served as internal reference for spectra in Me_2SO-d_6. In other solvents Me_4Si generally served as external reference. [b] 1, TFA-*d*; 2, $CDCl_3$; 3, Me_2SO-d_6; 4, NaOD. [c] Some decomposition was apparent in this solvent. [d] Prepared from thioacetamide and *p*-methoxybenzylamine as described for *N*-benzyl thioacetamide [M. J. Schlatter, *J. Am. Chem. Soc.*, **64**, 2722 (1942)]; long needles, mp 82–84 °C. Anal. Calcd for $C_{10}H_{13}NOS$: C, 61.5; H, 6.71; N, 7.17; S, 16.4. Found: C, 61.7; H, 6.87; N, 7.19; S, 16.3.

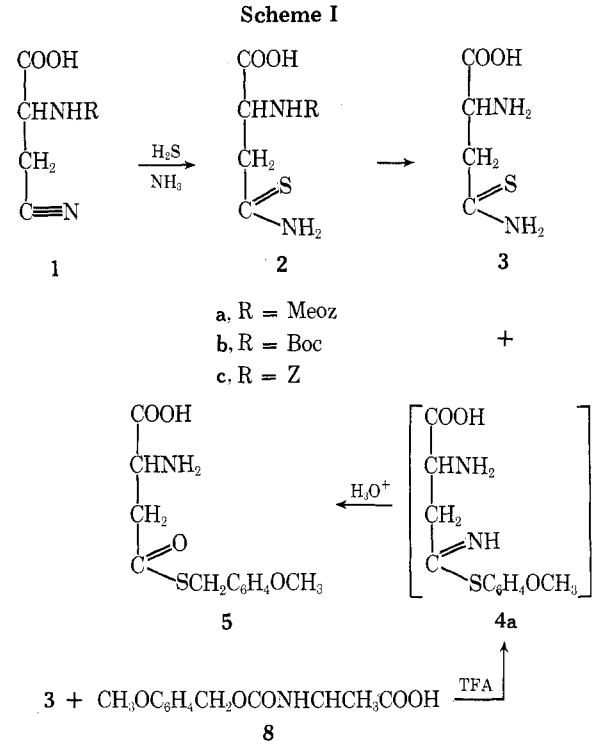
of certain amino acid derivatives under acidic conditions. Thus, C-alkylation of the tyrosine ring results when *O*-benzyltyrosine or *N*-carbobenzoxytyrosine is hydrolyzed in 6 N HCl at 100 °C[12] or solvolyzed with 50% TFA–CH_2Cl_2 or 50% HF–anisole,[13] or when *O*-benzyl 2-fluorophenyl ether is treated with TFA.[14] S-Alkylation to give *S*-benzylhomocysteine takes place when *N*-carbobenzoxy-L-methionine anilide is refluxed in concentrated HCl.[15] S-Alkylation of thioamides by alkyl halides is considered to be a very suitable preparative route to imidothiolic esters.[4] Therefore, it seemed likely that the thioasparagine β-thioamide had been alkylated by the *p*-methoxybenzyl (Meb) carbonium ion formed on deprotection of **1a** thereby giving the imidothiolic ester (**4a**). Consistent with an imidothiolic ester structure was the liberation of 0.7 mol of NH_3 after brief treatment of **4a** with dilute acid at 100 °C, as well as the formation of 0.2 mol of β-cyanoalanine and 0.4 mol each of aspartic acid and anisyl disulfide on treatment of **4a** with 1 N NaOH for 18 h at 25 °C.

Instances of N- and S-alkylation have been noted on treatment of thioamides with alcohols or halides under acidic conditions although N-alkylation appears to be less common.[16] That S-alkylation had resulted in forming the imidothiolic ester (**4a**) was suggested chiefly by the chemical shift of the anisyl methylene protons in Me_2SO-d_6 when ^{1}H NMR spectra were compared with those of various available *S*- and *N*-anisyl and benzyl compounds (Table II). ^{1}H NMR spectroscopy in TFA-*d* was less informative, the downfield shift of the *S*-anisyl methylene of **4a** probably being the result of protonation of the neighboring iminium nitrogen.

Conversion of imidothiolic esters to thiolic esters is known to be facile.[17] Owing to limited solubility crude **4a** had been recrystallized under mildly hydrolytic conditions during which presumably deimidation ensued to give the *S*-anisyl ester of β-thioaspartic acid (**5**). Consistent with this was the appearance of the carbonyl absorption band at 1675 cm^{-1} only after crude **4a** had been recrystallized. The likely sequence of reactions leading from **2a** to **5** is given in Scheme I.

The finding that the Meoz group of the *N*-Meoz thioamides of five monocarboxylic amino acids could be removed

Scheme I



with TFA with no detectable S-anisylation raises a question whether the S-anisylation observed with aspartic acid derivative **2a** is intramolecularly facilitated by the α-carboxyl group. That the sulfur is an important participant in the reaction is supported by the fact that under the same conditions *N*-Meoz-L-asparagine is deprotected to asparagine in over 90% yield. That the transfer of the anisyl group from the *N*-methoxybenzyloxycarbonyl group to the thioamide group of **2a** is probably intermolecular is indicated by the observation that **4a** results also when **3** and *N*-Meoz-alanine (**8**) are placed in TFA.

The facility with which the thioamide of **3** was alkylated by a *p*-methoxybenzyl cation generated on deprotection of an *N*-Meoz amino acid in TFA prompted us to examine the

reactivity of cysteinethiol under deprotection conditions in order to assess S-anisylation as a possible side reaction in the synthesis of cysteine containing peptides. An equimolar mixture of cysteine (**9**) and Meoz-alanine (**8**) in TFA at −20 °C afforded in 85% yield *S*-*p*-methoxybenzyl-L-cysteine (S-Meb-cys, **10**) that agreed in properties with a reference sample. Although the yield of **10** dropped to 6% when

$$\underset{\mathbf{9}}{HSCH_2CHNH_2COOH} + \mathbf{8} \xrightarrow[-20°C]{TFA} \underset{\mathbf{10}}{CH_3OC_6H_4CH_2SCH_2CHNH_2COOH}$$

the reaction was carried out in the presence of anisole S-anisylation merits consideration as a potential side reaction when in the course of peptide synthesis the *N*-Meoz group and an S-protecting group are to be removed simultaneously. In HF the transfer reaction to give **10** from **8** and **9** was not significant.

The commercially available *p*-methoxybenzyl carbazate ($CH_3OC_6H_4CH_2OCONHNH_2$) proved to be as effective as Meoz-ala as a source of the *p*-methoxybenzyl carbonium ion as it led to a 91% yield of **10** from **9**. Conversion of **9** to **10** with *p*-methoxybenzyl alcohol in acetic–sulfuric acids proceeded in 53% yield; treatment of **9** with *p*-methoxybenzyl chloride in liquid NH_3 gives a 78% yield of **10**.[18] The anisyl transfer reaction would therefore appear to offer an attractive alternative to the two latter routes to alkylate sulfur since yields are promising and conditions are milder, and it may be worth examination as a general route for anisylation of mercaptans and acylthiols. In addition, the possibility of extending the scope of this transfer reaction to the in situ generation and utilization of certain other carbonium ions remains to be examined.

Experimental Section

Elemental analyses were carried out by Micro-Tech Laboratories, Skokie, Ill. Proton magnetic resonance spectra were obtained on a Varian EM-360 spectrometer. Infrared spectra, melting points, and optical rotations were determined as described elsewhere.[19] Amino acid analyses were performed on a Beckman-Spinco automatic amino acid analyzer, Model 120.[20] System 1 refers to the 150-cm column, 30 °C, pH 3.25; system 2 to the 15-cm column, 30 °C, pH 4.26. *S*-*p*-Methoxybenzyl-L-cysteine was purchased from Peninsula Labs, San Carlos, Calif.; *p*-methoxybenzyl carbazate from the Protein Research Co., Institute for Protein Research, Osaka City, Japan; trifluoroacetic acid from Matheson Co., N.J.; *p*-methoxybenzylamine, *S*-benzylthioglycolic acid, and *N*-benzylglycine ethyl ester from Aldrich Chemical Co., Inc., Milwaukee, Wis.

Meoz-L-β-cyanoalanine (1a) was prepared essentially as described[5] except that a preparative scale (16 g of Meoz-L-asparagine in 110 ml of pyridine) and only a 4% excess of DCCI (11.5 g in 86 ml of pyridine) were used. Crude **1a** (13 g, mp 84–86 °C) was recrystallized from ether–benzene (50:150) with a few drops of petroleum ether added to induce turbidity. Cooling overnight at 0 °C furnished **1a** (12 g, 80%), mp 91–92 °C.

Meoz-L-thioasparagine (2a). A solution of **1a** (2.8 g, 10 mmol) in 280 ml of MeOH and 60 ml of concentrated NH_3 was saturated with a stream of H_2S at 0 °C. The solution was sealed and kept at room temperature for 4 days. It was then concentrated to one-sixth its volume, diluted with H_2O, and extracted with ether. The alkaline solution was cooled to 0 °C, adjusted to pH 2, and extracted with EtOAc. The organic extract was washed with H_2O and dried ($MgSO_4$) and the solvent was removed. The residue was taken up in 30 ml of EtOH and 9 ml of dicyclohexylamine was added. After 1 h at 0 °C the precipitated DCHA salt was filtered off (3.6 g), mp 155–156 °C. The DCHA salt was dissolved in 45 ml of boiling EtOH and the solution was allowed to stand at room temperature for 3.5 h to give **2a** DCHA salt (1.9 g, 38%), mp 174–176 °C. This material (4 g, 8.7 mmol) was dissolved in 150 ml of MeOH–H_2O (2:1) and stirred with Dowex 50 (H^+) resin (8% cross-link, 50–100 mesh, 80 g) for 2 h at 5–10 °C. The resin was filtered off and washed well with MeOH, and the solution was concentrated to incipient crystallization and then cooled to give **2a** (2.2 g, 87%), mp 122–123 °C. Recrystallization from EtOH–H_2O resulted in no change in melting point.

Boc-L-thioasparagine Hydrate (2b). Boc-L-β-CNala, like **1a** and **1c**, was prepared by carbodiimide dehydration of the asn derivative.[5,9] Prepared for reference, its DCHA salt melted at 166–168 °C (lit.[8] 166–167 °C). The crude **1b** melted at 92–94 °C and was converted to **2b** as described for **2a**. However, the DCHA salt of **2b** was prepared in EtOAc. Further, after treatment of the DCHA salt with the resin and evaporation of the methanol, the aqueous solution was diluted with a small amount of H_2O, saturated with NaCl, and extracted well with EtOAc. The extract was dried ($MgSO_4$) and concentrated. The residue crystallized on trituration with a little H_2O. After three recrystallizations the air-dried product analyzed as the hydrate.

Z-L-Thioasparagine (2c) was prepared as described for **2a** except that the aqueous MeOH solution obtained after treatment of the DCHA salt with resin was concentrated to turbidity, then diluted with H_2O and cooled to furnish the crystalline product.

Thioasparagine (3). A. A mixture of **2a** (3 g, 9.6 mmol) and anisole (45 ml, 414 mmol) was treated with TFA (150 ml) at 0 °C for 30 min. TFA was removed in vacuo and the residue was triturated with ether and collected on a filter. It was then treated with 2 ml of water, and the solution was adjusted to pH 5 with a few drops of pyridine. Any solid present was removed by filtration affording **4a** (0.2 g, 7%), mp 214–215 °C dec. The filtrate was taken to dryness and the solid was collected with the aid of EtOH affording **3** (920 mg, 64%), mp 195–198 °C dec. **3** was recrystallized in 93% yield by dissolution in the minimal volume of water with gentle warming and concentrating the solution to 0.3 ml and cooling: mp 195 °C dec; $[\alpha]^{26}D$ −49.8° (*c* 1, 1 N acetic acid); ir 3330–3030 (NH_3^+), 1625 (COO^-), 1470, 1208 (C=S), 869 cm^{-1}.

Anal. Calcd for $C_4H_8N_2O_2S$: C, 32.4; H, 5.44; N, 18.9; S, 21.6. Found: C, 32.3; H, 5.40; N, 19.1; S, 21.9.

On amino acid analysis in system 1, **3** eluted at 241 ml, 60 ml after asn and 64 ml before ala. Its ninhydrin color constant was 17.5 compared to 22.1 for leu. The 440/570 nm ninhydrin absorption ratio was 7.4. In 0.1 M solution at 25 °C for 24 h in 0.04 M NaOAc, pH 5.0, the stability of **3** was 97%; in 0.05 M Tris·HCl, pH 9.0, its stability was 86%.

B. A mixture of **2a** (0.5 g, 1.7 mmol), anisole (0.4 ml, 3.7 mmol), and TFA (5 ml) was treated as described under A furnishing **3** (60 mg, 25%), mp 195–198 °C, and **4a** that gave 32% **5** upon recrystallization in the manner described under **5** (A).

β-S-*p*-Methoxybenzyl Aspartic Thioester (5) and β-*p*-Methoxybenzyl Aspartic Imidothiolic Ester (4a). A. The crystalline **4a** appearing during the preparation of **3** was dissolved in hot H_2O by addition of a few drops of 3 N HCl. The solution was cooled directly and adjusted to pH 5 with pyridine to give **5** (140 mg, 32%), mp 213–215 °C dec. For analysis **5** was recrystallized three times: mp 214–215 °C dec; NMR (NaOD) δ 2.2–2.8 (2 H, m, β-CH_2), 3.2–3.6 (3 H, m, SCH_2 + α-CH), 3.77 (3 H, s, OCH_3), 6.75–7.40 (4 H, m, C_6H_4); ir 3030 (NH_3^+), 1686 (O=C–SR), 1620 cm^{-1} (COO^-).

Anal. Calcd for $C_{12}H_{15}NO_4S$: C, 53.5; H, 5.61; N, 5.20; S, 11.9. Found: C, 53.4; H, 5.63; N, 5.05; S, 11.9.

B. A mixture of **2a** (250 mg) and TFA (4 ml) was treated as described under **3** (A). All subsequent treatments were at 5 °C. The solid present after neutralization was collected by filtration and washed well with cold EtOH followed by Et_2O, furnishing **4a** (150 mg, 70%), mp 156–158 °C.

Anal. Calcd for $C_{12}H_{16}N_2O_3S$: C, 53.7; H, 6.01; N, 10.4; S, 11.9. Found: C, 53.6; H, 6.02; N, 10.1; S, 12.1.

The product (40 mg) was recrystallized as under A furnishing 90% **5**, mp 213–215 °C.

C. To a finely ground mixture of **3** (20 mg, 135 μmol) and Meoz ala (**8**, 34 mg, 140 μmol) at −20 °C was added with vigorous agitation TFA (1 ml) precooled to the same temperature. The colorless solution was allowed to warm to 0 °C. TFA was then evaporated, and the reaction was treated as described for **4a** under **3** (A), except that the temperature was maintained at 0 °C. After 30 min at 0 °C the precipitate was collected and air dried (18 mg, 49%), mp 151–155 °C. The product had the same ^1H NMR spectrum as **4a** and a mixture melting point with **4a** was undepressed. Recrystallization as described under **5** (A) gave 10 mg, mp 209–214 °C. This had the same ^1H NMR spectrum as **5** and showed no depression in a mixture melting point with **5**.

Hydrolyses of 5. A. To **5** (5.8 mg, 21.5 μmol) was added 0.2 ml of 1 N NaOH and the mixture was allowed to stand at room temperature. After 3 h the turbid solution gave a strong nitroprusside

reaction. After 3 days this reaction was negative and 92% asp was present on amino acid analysis. The crystalline deposit was collected by centrifugation, 1.24 mg, mp 98–100 °C. A mixture melting point was undepressed with a reference sample of 4-methoxybenzyl disulfide, mp 99–100 °C, synthesized from anisaldehyde and $(NH_4)_2S$.[11] Ir spectra likewise were identical.

B. A solution of **5** (0.1 g, 0.37 mmol) in 15 ml of 1.5 N HCl was heated under reflux for 1.5 h and yielded 95% asp. The mixture was cooled and extracted with EtOAc. The extract was dried ($MgSO_4$) and evaporated to furnish a yellow oil that was dissolved in 1 ml of EtOH and treated dropwise with a solution of I_2 in EtOH until a nitroprusside test became negative. Cooling led to crystallization of 4-methoxybenzyl disulfide (35 mg, 56%), mp 98–100 °C, that was identical with the reference material in the criteria given under A and also had the same ^1H NMR spectrum: ($CDCl_3$) δ 3.72 (4 H, m, SCH_2), 3.82 (6 H, s, OCH_3), 6.8–7.4 (8 H, aromatic).

***S*-*p*-Methoxybenzyl-L-cysteine (10). A.** To a solution of L-cysteine (500 mg, 4.1 mmol) in 5 ml of TFA cooled to −15 to −20 °C was added in portions over 30 min *p*-methoxybenzyl carbazate (810 mg, 4.1 mmol). The mixture was allowed to stand at 0 °C for 10 min and then was concentrated to dryness. The residue was taken up in H_2O and adjusted to pH 5. The precipitated **10** was collected and dried (920 mg, 92%). The dried precipitate was dissolved in H_2O at 50 °C with addition of a few drops of 3 N HCl and was reprecipitated by adjustment to pH 5. The product was filtered, washed with H_2O, then with EtOH, and dried (810 mg, 81%), mp 205–207 °C (placed in bath at 180 °C) (lit.[18] mp 198–199 °C). A mixture melting point with a reference sample of **10**, mp 206–208 °C, was undepressed. ^1H NMR spectra in TFA were likewise identical. The reaction in the presence of 2.6 equiv of anisole gave 6% **10**.

On amino acid analysis in system 2, **10** eluted at 114 ml as a single peak. Its ninhydrin color constant was 16.4.

B. To a finely ground mixture of **8** (104 mg, 0.41 mmol) and **9** (50 mg, 0.41 mmol) at −20 °C was added TFA (1 ml) precooled to −15 °C. After 7 min the resulting clear solution was allowed to warm to 0 °C where it was kept for 5 min. It was then concentrated and treated as described under **10** (A) to give a crude product (80 mg, 80%), mp 199–200 °C; after reprecipitation (75 mg, 75%), mp 204–206 °C. This was identical with **10** in ^1H NMR spectrum and amino acid analysis.

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Registry No.—**1a,** 31883-91-7; **1b,** 45159-34-0; **1c,** 3309-41-9; **2a,** 58208-20-1; **2a** DCHA salt, 58208-21-2; **2b,** 58208-22-3; **2b** DCHA salt, 58208-23-4; **2c,** 58208-24-5; **2c** DCHA salt, 58208-25-6; **3,** 58208-26-7; **8,** 16944-75-5; **9,** 52-90-4; TFA, 76-05-1; *p*-methoxybenzyl carbazate, 18912-37-3.

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Synthesis of *N*-Chloroacetyl Derivatives of Amino Acids and Their Use for the Conjugation of Polypeptides to Thiol-Carrying Compounds[1]

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The synthesis of the α-*N*-chloroacetyl derivatives of lysine *n*-butylamide (**4a**), lysine 2-methoxyethylamide (**4b**), phenylalanyllysine *n*-butylamide (**9a**), and phenylalanyllysine 2-methoxyethylamide (**9b**) is described. The free ϵ-NH_2 group of these compounds can initiate the growth of a polypeptide by polymerization of amino acid *N*-carboxyanhydrides, and this polypeptide can be coupled to thiol-carrying compounds via its *N*-chloroacetyl group, as illustrated by the use of **4a** to attach a polyalanine chain to the SH group of cysteine (11).

The compounds described in the present article were designed as coupling agents permitting the attachment of bulky substituent groups, such as artificial polypeptides, to the thiol groups of proteins.

The principle of their use is as follows. In a first step, the free ϵ-amino group of **4a, 4b, 9a,** or **9b** serves both as the initiator and the starting point for the growth of an artificial polypeptide obtained by polymerization of the *N*-carboxyanhydrides of the desired amino acids. This reaction is carried out either in organic media or in an aqueous buffer solution (Experimental Section). During a second step, the chlorine from the chloroacetyl group which now forms the head of the resulting polypeptide is allowed to react specifically with the free thiol group from the molecule to which conjugation is aimed. In principle a thiol group from a protein molecule (e.g., an antibody) would be used, but this will not be considered in the present article. Reported herein are the synthesis of **4a, 4b, 9a** and **9b,** as well as their re-