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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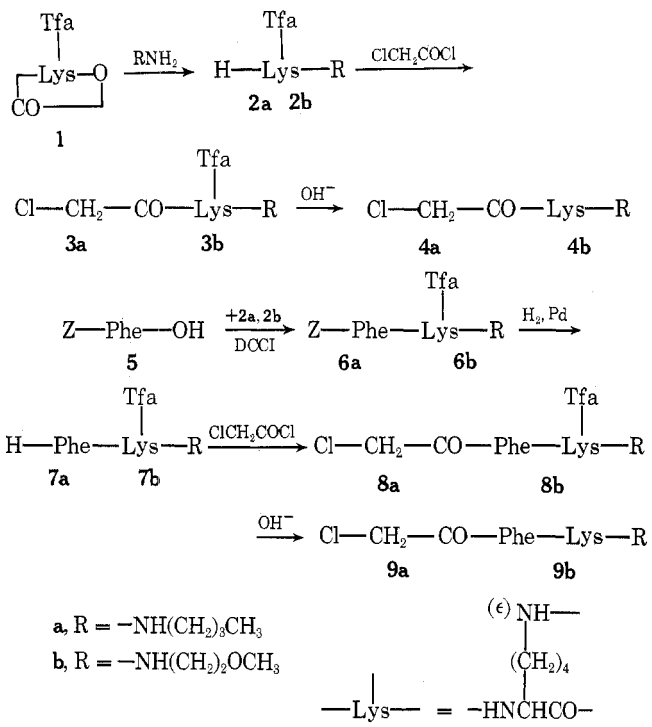
The synthesis of the α-*N*-chloroacetyl derivatives of lysine *n*-butylamide (**4a**), lysine 2-methoxyethylamide (**4b**), phenylalanyllysine *n*-butylamide (**9a**), and phenylalanyllysine 2-methoxyethylamide (**9b**) is described. The free ϵ-NH_2 group of these compounds can initiate the growth of 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-

Scheme I



activity toward *N*-carboxyanhydrides of amino acids and toward simple model compounds carrying thiol groups (Experimental Section).

The syntheses depicted in Scheme I do not involve any novel reactions, but some of the steps may require justification.

The use of the α-*N*-carboxyanhydride of ε-trifluoroacetyllysine has proven a convenient way to obtain **2a** and **2b** in nearly quantitative yields. The use of trifluoroacetyl and benzyloxycarbonyl groups has allowed selective nonsimultaneous cleavages while preserving the chloroacetyl group previously introduced.

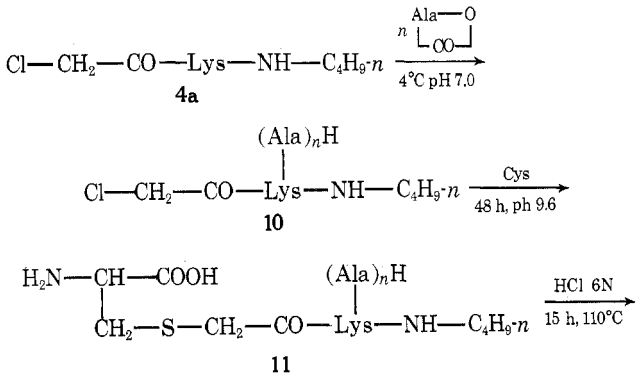
The removal of the protecting trifluoroacetyl group from the ε-amino group of **3a** or **3b** must be carried out within strict constraints of time and pH (Experimental Section) to avoid hydrolytic cleavage of the chlorine. The cleavage of the relevant amide bond proceeds faster with **3b** than with **3a**, probably owing to the influence of the methoxy group of the former. A similar difference was found between **8a** and **8b** during the removal of their protecting ε-*N*-trifluoroacetyl group.

The yields obtained by the formation of peptides **6a** and **6b** can appear rather low if we consider the values given in the Experimental Section. However, it must be pointed out that these are yields of very pure products. The constraints born upon difficult separations reduce those real yields to half.

All four compounds (**4a, 4b, 9a, 9b**) efficiently initiated the growth of peptide chains by polymerization of amino acid *N*-carboxyanhydrides. A typical reaction using the ε-NH_2 group of **4a** as the starting point for a polyalanine chain (**10**) is described in Scheme II and the Experimental Section.

Neither the true composition nor the yield of the reaction product can be determined from the 1:35 molar ratio for lysine vs. alanine that was found upon acid hydrolysis. This is because the initiator NH_2 from **4a** is not necessarily incorporated into the nascent polymer, so that some of the peptide chains must have been devoid of lysine. These uncertainties are, however, partially dissipated by the subsequent experiment in which the chloroacetyl group of **10**

Scheme II



(freed of residual **4a**) was used to conjugate this polymer to the thiol group of cysteine (Scheme II, below, and Experimental Section). This revealed that at least 40% of the polyalanine chains possessed a chloroacetyl group capable of reacting with the SH group from cysteine.

For the purpose set out in the introduction it was necessary that the active group intended as a reagent for thiol groups be unreactive toward other substituent groups found in proteins, particularly the NH_2 group. The *N*-chloroacetyl group appears to suit this need. Feinstein[2] and Hanson[3] have already reported upon the alkylation of the SH group of cysteine by the *N*-chloroacetyl derivatives of tyrosine and phenylalanine, but they did not specifically examine the nonreactivity of the NH_2 group of cysteine with these compounds. Here we show that the latter reaction does not occur under the conditions used since no self-conjugation of **4a** was observed, as testified by the absence of its expected reaction product, ε-*N*-carboxymethyllysine, from the acid hydrolysate. The same selectivity for thiol groups was observed with a different compound carrying both an SH and an NH_2 group, viz., γ-Glu-Cys-Gly (reduced glutathione).

We have also verified that none of the compounds **4a, 4b, 9a,** or **9b** reacted in detectable measure with *n*-butylamine, arginine, histidine, lysine, methionine, tryptophan, or tyrosine, at pH 8.2 or 9.6 (24 h, 25 °C). This was shown in two ways. Firstly, when reaction mixtures containing **4a, 4b, 9a,** or **9b** and one of these potential reaction partners were analyzed by high-voltage electrophoreses, the ninhydrin-positive spot of the latter compound persisted unchanged in intensity. Secondly, the subsequent addition of cysteine to such mixtures (24 h, 25 °C) caused the typical ninhydrin-positive spot of the *S*-alkyl derivative of cysteine to appear at the expected position and with an intensity similar to that seen in the absence of the potential competitor (Experimental Section). Amino acid analyses of the acid hydrolysates of the latter reaction mixtures confirmed that cysteine had been S-alkylated with unaltered yields (50–80%) despite prior exposure of the halogen compound to the potential competitor. This selectivity of the chloroacetyl group for thiols contrasts with the known reactivity, under similar conditions, of bromoacetyl or iodoacetyl groups toward amines and phenols.[4]

A further advantage of the chloroacetyl group compared to the iodoacetyl group is its relative stability, notably to light. Salts of these compounds can be stored for many months at 4 °C. In aqueous solution they are relatively stable up to pH 9.6. For example, a 10 mM aqueous solution of **4b** was found to have retained 20% of its cysteine-fixing capacity after 5 months storage at 25 °C, pH 9.6.

Compound **4b** is well soluble in water, compared to **4a,**

9a, or **9b.** The synthesis of **9a** and **9b** was undertaken with the aim of obtaining compounds having the same conjugating properties as **4a** and **4b** but with the added advantage of being themselves easily cleaved by the enzyme α-chymotrypsin which splits the peptide bond between phenylalanine and lysine (Experimental Section). Compounds **9a** and **9b** can therefore be used for the reversible attachment of substituent polypeptide chains to proteins resistant to this enzyme, as will be shown in a later publication.

Experimental Section

Amino acid analyses were carried out using a Beckman Unichrom apparatus. Infrared spectra were recorded on a Perkin-Elmer Model 457 grating infrared spectrophotometer. Elementary compositions were determined by A. Bernhardt Mikroanalytisches Laboratorium, Elbach, West Germany. Specific rotations were determined on a Perkin-Elmer Model 141 polarimeter with mercury lamp, spectral line 579.066 nm at 25 °C, concentration range 0.5–1%, in a 1:1 (v/v) mixture of water and methanol.

High-voltage electrophoresis (HVE) was performed on Whatman 3 MM paper at pH 1.9 and at 60 V/cm for 1 h. The migration rate of ε-DNP-Lys was used as the reference. The ratio of the migration rates *x*/*N*-ε-DNP-Lys was represented by high-voltage electrophoresis ratio of *x*.

Chromatographic Solvents (v/v). Solvent system (s.s.) 1, a 40:40:20 mixture of methanol, 2-propanol, and cyclohexane; s.s. 2, a 10:5:85 mixture of 2-propanol, ethyl acetate, and cyclohexane; s.s. 3, a 5:5:90 mixture of methanol, 2-propanol, and cyclohexane; s.s. 4, a 1:1 mixture of 2-propanol and cyclohexane; s.s. 5, a 30:30:40 mixture of methanol, 2-propanol, and cyclohexane; s.s. 6, a 35:35:30 mixture of methanol, 2-propanol, and cyclohexane; s.s. 7, a 20:10:70 mixture of 2-propanol, ethyl acetate, and cyclohexane.

ε-Trifluoroacetyl-L-lysine *N*-Carboxyanhydride (1). Dry $COCl_2$ was slowly bubbled through a suspension of 5 g (19 mmol) of ε-trifluoroacetyl-L-lysine[5] in 120 ml of dry dioxane for 4 h at 25 °C. After overnight stirring, dioxane, HCl, and $COCl_2$ were distilled off at 20 °C and 20 mmHg. The dry residue was taken up with dioxane, which was distilled off as before. During this step, 1 crystallized as a fine powder. Vacuum was maintained until all traces of HCl had been removed: mp 49–51 °C; ir (KBr) 3340, 1860, 1810, 1695, 1205, 1170 cm^{-1}.

Anal. Calcd for $C_9H_{11}N_2O_4F_3$: C, 40.30; H, 4.10. Found: C, 40.65; H, 4.25.

ε-Trifluoroacetyl-L-lysine *n*-Butylamide (2a). A solution of 5.36 g (20 mmol) of 1 in 120 ml of dioxane was rapidly mixed with a solution of 1.46 g (20 mmol) of *n*-butylamine in 50 ml of dioxane. After 15 min the solvent was evaporated at 0.1 mmHg. Silica gel thin layer chromatography using s.s. 1 revealed the absence of ε-trifluoroacetyllysine, less than 1% residual *n*-butylamine, and the presence of a ninhydrin-staining derivative of ε-trifluoroacetyllysine, indicating quantitative conversion of 1 into **2a.** Product **2a** was a white solid: mp 70–72 °C; ir (KBr) 3326, 1703, 1650, 1212, 1188, 1155 cm^{-1}.

Anal. Calcd for $C_{12}H_{22}N_3O_2F_3$: C, 48.48; H, 7.41. Found: C, 48.75; H, 7.65.

ε-Trifluoroacetyl-L-lysine 2-Methoxyethylamide (2b). The procedure was the same as for the conversion of 1 to **2a** except that a solution of 1.48 g (20 mmol) of 2-methoxyethylamine was used. The yield of **2b** from 1 was also quantitative. Product **2b** was a viscous oil: ir (KBr) 3310, 1710, 1655, 1211, 1189, 1155 cm^{-1}.

Anal. Calcd for $C_{11}H_{20}N_3O_3F_3$: C, 44.15; H, 6.69. Found: C, 44.35; H, 6.88.

α-*N*-Chloroacetyl-ε-trifluoroacetyl-L-lysine *n*-Butylamide (3a). To a solution of 3 g (10 mmol) of **2a** in 40 ml of dioxane were added slowly, simultaneously, under vigorous stirring, and with exclusion of atmospheric moisture, 1.13 g (10 mmol) of chloroacetyl chloride and 1 g (10 mmol) of triethylamine. After 30 min of stirring at 25 °C the precipitate of triethylamine hydrochloride was removed by filtration, the filtrate was taken to dryness at 20 mmHg, and the residue was purified by chromatography on a silica gel column (90 cm, diameter 30 mm) using as the eluent s.s. 2. This yielded 1.9 g (5 mmol) of **3a** (yield 50%) as a white solid: ninhydrin negative; mp 150–152 °C; ir (KBr) 3287, 1694, 1635, 1208, 1175 cm^{-1}.

Anal. Calcd for $C_{14}H_{23}N_3O_3ClF_3$: C, 44.97; H, 6.15; N, 11.24; Cl, 9.5. Found: C, 44.78; H, 6.01; N, 11.25; Cl, 9.8.

Mol wt. Calcd: 373. Found (Rast): 384.

α-*N*-Chloroacetyl-ε-trifluoroacetyl-L-lysine 2-Methoxyethylamide (3b). The procedure was the same as for the conversion of **2a** to **3a,** until the purification step, except that **2a** was replaced by 3 g (10 mmol) of **2b.** Product **3b** was purified by chromatography on a silica gel column (90 cm, diameter 30 mm) which was first eluted with 500 ml of s.s. 3. This eluate was discarded and product **3b** was then eluted with 2000 ml of s.s. 4. Evaporation of the solvent furnished 1.8 g (4.8 mmol) of **3b** as a white product (yield 48%): ninhydrin negative; mp 133–135 °C; ir (KBr) 3282, 1690, 1630, 1208, 1175 cm^{-1}.

Anal. Calcd for $C_{13}H_{21}N_3O_4ClF_3$: C, 41.54; H, 5.59; N, 11.18; Cl, 9.45. Found: C, 41.73; H, 5.70; N, 11.06; Cl, 9.72.

Mol wt. Calcd: 375. Found (Rast): 367.

α-*N*-Chloroacetyl-L-lysine *n*-Butylamide (4a). To 370 mg (1 mmol) of **3a** dissolved in 60 ml of methanol was added 60 ml of an aqueous solution (pH 9.6) of 0.63 g (6 mmol) of Na_2CO_3 and 1 g (12 mmol) of $NaHCO_3$. Alkaline hydrolysis of the trifluoroacetyl group was essentially complete after 3 days at 25 °C. The solution was then adjusted to pH 8.5 and evaporated to dryness (20 mmHg). The residue was dissolved in 2 ml of water and extracted with two 20-ml portions of chloroform. The organic solution was dried on Na_2SO_4, filtrated, and evaporated to give 240 mg (0.86 mmol) of **4a** as a white solid (yield 86%) soluble in water (2 mg/ml): HVE ratio of **4a** 1.67; mp 90–95 °C dec; ir (KBr) 3266, 2950, 2920, 1640 cm^{-1}; [M] +0.8°.

Anal. Calcd for $C_{12}H_{24}N_3O_2Cl$: C, 51.89; H, 8.64; N, 15.13; Cl, 12.79. Found: C, 51.73; H, 8.59; N, 15.07; Cl, 12.50.

α-*N*-Chloroacetyl-L-lysine 2-Methoxyethylamide (4b). The same procedure starting from 370 mg (1 mmol) of **3b** yielded 230 mg (0.82 mmol) of **4b** after 30 h of alkaline hydrolysis (yield 82%). Product **4b** was a white, water-soluble (10 mg/ml) solid: HVE ratio of **4b** 1.68; mp 50–60 °C dec; ir (KBr) 3270, 2920, 1640 cm^{-1}; [M] +1.3°.

Anal. Calcd for $C_{11}H_{22}N_3O_3Cl$: C, 47.22; H, 7.87; N, 15.02; Cl, 12.70. Found: C, 47.35; H, 7.82; N, 15.11; Cl, 12.89.

***N*-Benzyloxycarbonyl-L-phenylalanine-ε-trifluoroacetyl-L-lysine *n*-Butylamide (6a).** To a solution of 1.48 g (5 mmol) of **2a** and 1.5 g (5 mmol) of *N*-benzyloxycarbonyl-L-phenylalanine (**5**)[6] in 40 ml of chloroform was added 1.03 g (5 mmol) of dicyclohexylcarbodiimide, at 15 °C, under stirring. After 1 h at 25 °C the precipitate of dicyclohexylurea was removed by filtration and the filtrate was evaporated to dryness (20 mmHg), without an attempt being made to remove residual dicyclohexylurea and by-products.

Filtrate analysis carried out on silica gel thin layer developed in s.s. 1, revealed with ninhydrin, indicates the absence of **2a.** Longer reaction times (24 and 48 h) were not found useful: ir (KBr) 3310, 1700, 1640, 1260, 1210, 1180, 695 cm^{-1}.

Anal. Calcd for $C_{29}H_{37}N_4O_5F_3$: C, 60.21; H, 6.40. Found: C, 60.68; H, 6.62.

***N*-Benzyloxycarbonyl-L-phenylalanyl-ε-trifluoroacetyl-L-lysine 2-Methoxyethylamide (6b).** The same procedure was followed as for the conjugation of **2a** and **5** but starting from 1.49 g (5 mmol) of **2b:** ir (KBr) 3318, 1700, 1640, 1260, 1210, 1185, 695 cm^{-1}.

Anal. Calcd for $C_{28}H_{35}N_4O_6F_3$: C, 57.93; H, 6.03. Found: C, 58.26; H, 6.18.

L-Phenylalanyl-ε-trifluoroacetyl-L-lysine *n*-Butylamide (7a). Catalytic reduction with Pd black[7] was used to remove the benzyloxycarbonyl group from 2.5 mmol of crude **6a** (in methanol), and the catalyst was filtered off.

The filtrates were evaporated to dryness (20 mmHg) and **7a** was purified by chromatography on a silica gel column (80 cm, diameter 20 mm, flow rate 7 ml/min) with s.s. 4 as the eluent. The initial 400-ml fraction containing 300 mg of a mixture of substituted urea and **7a** was discarded. The next 500-ml fraction was evaporated (20 mmHg) and furnished 250 mg (0.56 mmol) of **7a** as a white solid (yield 22%). The product was homogeneous upon thin layer silica gel chromatography developed with s.s. 4. Different attempts to purify compounds **7** and **8** by crystallization appeared less practical and safe than purification by column chromatography.

Acid hydrolysis of **7a** produced lysine and phenylalanine in the expected equimolecular amounts: mp 122–124 °C; ir (KBr) 3330, 1710, 1630, 1210, 1185, 1160, 695 cm^{-1}.

Anal. Calcd for $C_{21}H_{31}N_4O_3F_3$: C, 56.75; H, 6.98; N, 12.61. Found: C, 56.52; H, 6.92; N, 12.68.

L-Phenylalanyl-ε-trifluoroacetyl-L-lysine 2-Methoxyethylamide (7b). The same procedure was used as for the conversion of **6a** to **7a** except for the final purification, which was carried out on 2 g of crude **6b.** The silica gel column was eluted with s.s. 5. The initial 750-ml fraction containing 450 mg of a mixture of substituted urea and **7b** was discarded. After evaporation the next 350-ml

fraction furnished 300 mg (0.67 mmol) of **7b** (yield 19%). This was a white solid which gave a single spot on a thin layer silica gel plate developed with s.s. 6. Acid hydrolysis of **7b** produced lysine and phenylalanine in equimolecular proportions: mp 88–90 °C; ir (KBr) 3330, 1710, 1630, 1210, 1186, 1163, 698 cm^{-1}.

Anal. Calcd for $C_{20}H_{29}N_4O_4F_3$: C, 53.81; H, 6.50; N, 12.55. Found: C, 53.95; H, 6.59; N, 12.48.

α-*N*-Chloroacetyl-L-phenylalanyl-ε-trifluoroacetyl-L-lysine *n*-Butylamide (8a). The chloroacetylation of 440 mg (1 mmol) of **7a** was carried out as described for the preparation of **3a** from **2a**. The product was purified by chromatography on a silica gel column (90 cm, diameter 20 mm, flow rate 7 ml/min) with s.s. 7 as the eluent (monitoring at 254 nm). The initial 250-ml fraction of eluate was discarded. The next 500-ml fraction was evaporated (20 mmHg) and furnished 170 mg (0.33 mmol) of **8a** as a white solid (yield 33%): mp 188–190 °C; ir (KBr) 3290, 1703, 1638, 1205, 1183, 1170, 692 cm^{-1}.

Anal. Calcd for $C_{23}H_{32}N_4O_4ClF_3$: C, 53.02; H, 6.14; N, 10.75; Cl, 6.82. Found: C, 52.87; H, 6.14; N, 10.40; Cl, 7.02.

Mol wt. Calcd: 520.5. Found (Rast): 535.

α-*N*-Chloroacetyl-L-phenylalanyl-ε-trifluoroacetyl-L-lysine 2-Methoxyethylamide (8b). The procedure was the same as for **8a**, but starting from 440 mg (1 mmol) of **7b**. The initial 400 ml of eluate was discarded and the next 800 ml furnished 180 mg (0.34 mmol) of **8b** as a white solid (yield 34%): mp 155–157 °C dec; ir (KBr) 3285, 1695, 1630, 1210, 1185, 700 cm^{-1}.

Anal. Calcd for $C_{22}H_{30}N_4O_5ClF_3$: C, 50.52; H, 5.74; N, 10.71; Cl, 6.79. Found: C, 50.74; H, 5.90; N, 10.61; Cl, 6.69.

Mol wt. Calcd: 522.5. Found (Rast): 508.

α-*N*-Chloroacetyl-L-phenylalanyl-L-lysine *n*-Butylamide (9a). The trifluoroacetyl group was removed from **8a** as described for the conversion of **3a** to **4a**, furnishing **9a** as a white solid insoluble in water (yield 78%). The product was homogeneous upon thin layer silica gel chromatography developed with s.s. 1 HVE ratio of **9a** 1.35. Acid hydrolysis of **9a** produced lysine and phenylalanine in the expected equimolecular amounts: mp 90 °C dec; ir (KBr) 3300, 2960, 2930, 1645, 695 cm^{-1}; [M] +15.9°.

Anal. Calcd for $C_{21}H_{33}N_4O_3Cl$: C, 59.36; H, 7.77; N, 13.19; Cl, 8.36. Found: C, 59.15; H, 7.82; N, 13.13; Cl, 8.15.

α-*N*-Chloroacetyl-L-phenylalanyl-L-lysine 2-Methoxyethylamide (9b). As for the conversion of **3b** to **4b**. Product **9b** was a white solid insoluble in water (yield 75%). The product was homogeneous upon thin layer silica gel chromatography (conditions as for **9a**). HVE ratio of **9b** 1.33. Acid hydrolysis of **9b** produced lysine and phenylalanine in the expected equimolecular amounts: mp 135 °C dec; ir (KBr) 3300, 2930, 1645, 698 cm^{-1}; [M] −68.3°.

Anal. Calcd for $C_{20}H_{31}N_4O_4Cl$: C, 56.27; H, 7.26; N, 13.13; Cl, 8.32. Found: C, 56.13; H, 7.20; N, 13.21; Cl, 8.20.

Synthesis of a Polymer of L-Alanine (10) with 4a as an Initiator of Polymerization. To 139 mg (0.5 mmol) of **4a** dissolved in 5 ml of dioxane and 10 ml of 0.05 M phosphate buffer, pH 7.0, was added, at 4 °C 1.15 g (10 mmol) of L-alanine *N*-carboxyanhydride dissolved in 5 ml of dioxane. After 24 h of stirring at 4 °C the solvent was evaporated at 20 mmHg. The dry residue was dissolved in 1 ml of water, filtered, and freed of excess **4a** by chromatography on Sephadex G-15 using 0.05 M NH_4HCO_3 as the eluent. The first fractions yielded 150 mg of **10** which by acid hydrolysis was identified as a polypeptide with an average lysine:alanine ratio of 1:35. The average molecular weight can be estimated from this ratio at a maximum value of 2500. On the other hand, the test for halogen detection is positive (Lassaigne–Beilstein), yield (from the *N*-carboxy anhydride) 20%. Note: similar results were obtained with compound **4b**.

Compounds **9a** and **9b** have been used successfully as initiator for *N*-carboxyanhydride of alanine, in an organic medium (dioxane).

Synthesis of 11 by Conjugation of the Thiol Group of Cysteine to the α-*N*-Chloroacetyl Group of Polypeptide 10. To 20 mg (about 0.02 mmol) of **10**, dissolved in 0.5 ml of 0.05 M $NaHCO_3$–Na_2CO_3 buffer, pH 9.6 (free of O_2 by saturation with N_2), was added 2.42 mg (0.02 mmol) of L-cysteine dissolved in 0.5 ml of the same buffer. The reaction was stopped after 2 days stirring at 25 °C under N_2. Acid hydrolysis (6 N HCl, 110 °C, 15 h) of the reaction product yielded *S*-carboxymethylcysteine, lysine, and alanine in a molar ratio of 0.4:1:35, besides some cysteine. The 40% yield indicated by this ratio is an underestimate because *S*-carboxymethylcysteine, the reaction product of the cysteine SH group with the α-*N*-chloroacetyl group of **4a**, is partially degraded under the hydrolysis conditions used.

Electrophoresis of Conjugation Products of Cysteine and 4a, 4b, 9a, 9b. HVE ratio of **4a** + Cys, 1.78; HVE ratio of **4b** + Cys, 1.81; HVE ratio of **9a** + Cys, 1.49; HVE ratio of **9b** + Cys, 1.54.

Enzymatic Cleavage by α-Chymotrypsin. To 4.2 mg (0.01 mmol) of **9a** dissolved in 1 ml of CH_3OH was added 3 mg (0.01 mmol) of reduced glutathione dissolved in 1 ml of 0.1 M $NaHCO_3$–Na_2CO_3 buffer, pH 9.6 (free of O_2). The reaction was stopped after 1 day stirring at 25°C under N_2, HVE ratio of conjugation product 1.27. The reaction mixture was evaporated under vacuum (20 mmHg) at 25 °C and dissolved in 1 ml of water. To this solution was added 3 mg of α-chymotrypsin dissolved in 0.1 ml of HCl (0.02 N) and pH adjusted to 8.5. The reaction of enzymatic cleavage was stopped after 18 h at 25 °C. Analysis carried out by high-voltage electrophoresis showed complete disappearance of the starting compound and formation of two new products: one, Lys-NH-C_4H_9, with a HVE ratio of 3.44, the other with a ratio of 0.64. This last compound after elution, acid hydrolysis, and amino acid analysis appeared to contain equimolar amounts of Glu, Gly, Phe, and CMCys.

Exactly similar results were obtained with compound **9b**.

Registry No.—**1**, 42267-27-6; **2a**, 58191-15-4; **2b**, 58191-16-5; **3a**, 58191-17-6; **3b**, 58191-18-7; **4a**, 58191-19-8; **4b**, 58191-20-1; **5**, 1161-13-3; **6a**, 58191-21-2; **6b**, 58191-22-3; **7a**, 58191-23-4; **7b**, 58191-24-5; **8a**, 58191-25-6; **8b**, 58191-26-7; **9a**, 58191-27-8; **9b**, 58191-28-9; $COCl_2$, 75-44-5; ε-trifluoroacetyl-L-lysine, 10009-20-8; *n*-butylamine, 109-73-9; 2-methoxyethylamine, 109-85-3; chloroacetyl chloride, 79-04-9; L-alanine *N*-carboxyanhydride, 2224-52-4; L-cysteine, 52-90-4; α-chymotrypsin, 9035-75-0.

References and Notes

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