Third, the mixture t-AmONa–$Ni(OAc)_2$–M^1X_n was stirred for 2.5 h at 63 °C before adding the ketone.

Oxidation Procedure (Scheme II). Sodium alkoxides were prepared separately by reacting alcohols with NaH excess in refluxing THF for 3 h. These alkoxides, or the free alcohols, were then admitted to react with various combinations of NaH, t-AmONa, and $Ni(OAc)_2$ prepared as described above.

Catalytic Ketone Reductions (Table IV). Catalytic reductions carried out on 100 mM ketone were achieved in 50–60 mL of THF by the general procedure (vide supra) but using 210 mM initial NaH, instead of 50 mM, for the preparation of the reducing system.

Acknowledgments. This work was supported by DGRST (PROSCOM Committee No. 77.7.0770) and by the Centre National de la Recherche Scientifique (France) which are gratefully acknowledged. We are very grateful to Professors Robert O. Hutchins and Frederick D. Greene for their very helpful comments.

Registry No.—NaH, 7646-69-7; t-AmONa, 14593-46-5; 5-nonanol, 623-93-8; 2,4-dimethyl-3-pentanol, 600-36-2; 2,2,4,4-tetramethyl-3-pentanol, 14609-79-1; α-methylbenzenemethanol, 98-85-1; cyclohexanol, 108-93-0; cycloheptanol, 502-41-0; cyclooctanol, 696-71-9; cyclododecanol, 1724-39-6; *cis*-2-methylcyclohexanol, 7443-70-1; *trans*-2-methylcyclohexanol, 7443-52-9; *cis*-3,3,5-trimethylcyclohexanol, 933-48-2; *trans*-3,3,5-trimethylcyclohexanol, 767-54-4; 3,3,5,5-tetramethylcyclohexanol, 2650-40-0; *cis*-4-(1,1-dimethylethyl)cyclohexanol, 937-05-3; *trans*-4-(1,1-dimethylethyl)cyclohexanol, 21862-63-5; *endo*-bicyclo[2.2.1]heptan-2-ol, 497-36-9; *exo*-bicyclo[2.2.1]heptan-2-ol, 497-37-0; (1*R*-*endo*)-1,7,7-trimethylbicyclo[2.2.1]heptan-2-ol, 464-43-7; (1*R*-*exo*)-1,7,7-trimethylbicyclo[2.2.1]heptan-2-ol, 10334-13-1.

References and Notes

(1) For Part VIII, see ref 14.
(2) This work, together with ref 8 and 9, represents part of the research work of L.M. for her Ph.D. Thesis.
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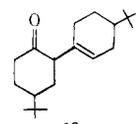
(20) When allowed to react with NaH–t-AmONa–$MgBr_2$ systems, ketone **12** afforded in near quantitative yield **19**, identified by comparison with an



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authentic sample prepared according to J. Reese, *Ber.*, **75**, 384 (1942).

9-(2-Sulfo)fluorenylmethyloxycarbonyl Chloride, a New Reagent for the Purification of Synthetic Peptides[1,2]

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A selective method was developed for the purification of synthetic peptides. It is based on the addition of the 9-(2-sulfo)fluorenylmethyloxycarbonyl (Sulfmoc) group to the free amine of the growing peptide chains at the end of a solid-phase synthesis. After cleavage from the support, the strongly acidic Sulfmoc-peptides are separated chromatographically from the nongrowing, terminated peptides. Finally, the Sulfmoc group is removed by mild base and the purified peptide is isolated. The reagent of choice for the derivatization was 9-(2-sulfo)fluorenylmethyloxycarbonyl chloride (Sulfmoc-Cl). It was synthesized from 9-fluorenylmethyloxycarbonyl chloride (Fmoc-Cl) by sulfonation with chlorosulfonic acid. The 4-nitrophenyl ester, Fmoc-ONp, was also prepared from Fmoc-Cl and then converted with $ClSO_3H$ to Sulfmoc-ONp. Both sulfonated reagents reacted readily with the free amino groups of peptide-resins in CH_2Cl_2 in the presence of pyridine or DIEA. The Sulfmoc group is stable to HF or HBr and to pyridine, but is readily removed by anhydrous bases such as morpholine or piperidine or by dilute aqueous Et_3N, Na_2CO_3, or NaOH. The efficacy of the technique was demonstrated by the purification of neutral, acidic and basic model peptides.

A selective purification procedure has been developed for the separation of synthetic peptide chains of the desired structure from unwanted terminated chains generated during a solid-phase synthesis.[3] It depends on the reaction of the free amino groups of those chains on the solid support that are still growing with a reversible protecting group bearing a strongly acidic function. After cleavage from the support, the two classes of peptide chains are separated chromatographically and the nonterminated peptides are regenerated to the free peptides. The 9-(2-sulfo)fluorenylmethyloxycarbonyl (Sulf-

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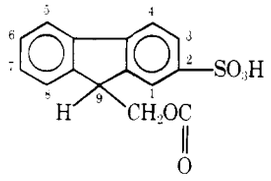
moc) group (1) fulfills the requirements for a reagent compatible with such a scheme.



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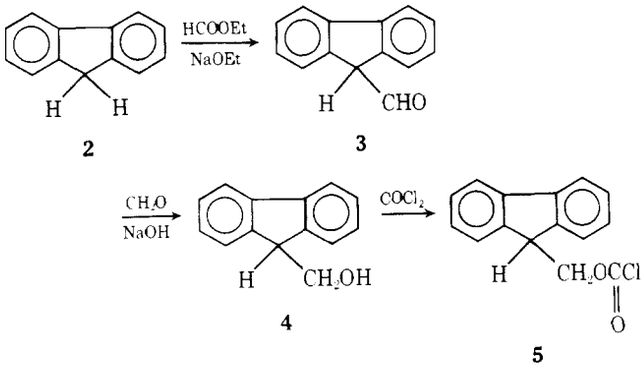
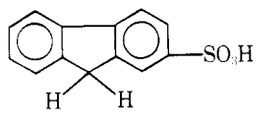
The Sulfmoc group is a modification of the 9-fluorenylmethyloxycarbonyl (Fmoc) amino-protecting group developed by Carpino and Han,[4] and is based on their finding that this group is stable toward acids, but is readily cleaved under mildly basic, nonhydrolytic conditions. Their amino acid and peptide derivatives were prepared by reaction of the amine with 9-fluorenylmethyl chloroformate or the corresponding azide. Fluorene **2** had been converted with ethyl formate and sodium ethoxide to 9-formylfluorene (**3**), which could then be reduced with formaldehyde in NaOH to 9-fluorenylcarbinol (**4**).[5] Carpino and Han[4] treated **4** with phosgene in dichloromethane to obtain the chloroformate **5**. This is a stable crys-



talline compound which can be stored without difficulty and is now commercially available.

For the present purpose it was necessary to introduce a strongly acidic functionality into the molecule to facilitate the ion-exchange separation of the derivatized peptides from the underivatized impurities. It had been shown by Chrzaszczewska and Machlanski[6] that treatment of fluorene in $CHCl_3$ with 1 equiv of chlorosulfonic acid gave fluorene-2-sulfonic acid (**6**), which could be crystallized as the potassium salt in



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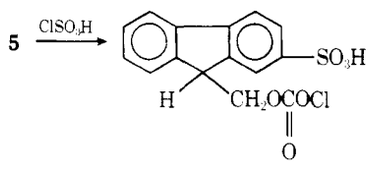
high yield. None of the acid chloride could be detected, even with excess $ClSO_3H$, but further sulfonation to fluorene-2,7-disulfonic acid then occurred. We have found that ring sulfonation of Fmoc-Cl (**5**) readily gives the required reagent, Sulfmoc-Cl (**7**).



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In recent years several methods for the purification of synthetic peptides have been developed that are based on the introduction of charged groups. These groups not only provide a charge difference but also enhance the water solubility of peptides. Young and his colleagues[7] protected the carboxyl terminus with a picolyl ester, and made use of the weakly basic group to purify intermediates by ion-exchange chromatography. Suzuki et al.[8] coupled a lysine residue to the amino terminus of the parent peptide and used it as a highly polar handle to facilitate purification. The temporary protecting group was then removed by an Edman degradation. Kunz[9] has developed the positively charged 2-(triphenylphosphonio)-ethoxycarbonyl (Peoc) group as a water solubilizing function removable by base. Kemp and Roberts[10] introduced the boronic acid group, *p*-(dihydroxyboryl)benzyloxycarbonyl (Dobz), as a handle for synthetic peptides. The Dobz-peptides are solubilized and purified by complexation with chromotropic acid, and finally deprotected by HBr or H_2/Pd. Very recently, Hubbuch et al.[11] designed a scheme involving cysteic acid for the purification of hydrophobic peptides by countercurrent distribution. N^{α}-protected cysteic acid was coupled by the mixed anhydride or active ester methods and, after purification of the cysteyl peptide, the group could be removed by an Edman reaction. Reagents bearing strongly acidic groups have also been applied in a different way to aid in purification. Thus, free peptide chains remaining after each coupling reaction were terminated by reaction with 3-nitrophthalic anhydride,[12] 2-sulfobenzoic acid anhydride,[13] or 3-sulfopropionic acid anhydride,[14] and the acidic properties of the resulting peptides were utilized in their removal from the desired final product at the end of the synthesis.

We recently devised a method for the affinity purification of synthetic peptides that depended, not on a charged group, but on a reactive sulfhydryl.[15] At the end of a stepwise solid-phase synthesis, residues of methionine and *S*-benzylcysteine were introduced at the N-terminus of the chain. After HF cleavage, the derivatized chains were selectively purified on an organomercury sepharose column and finally the product was regenerated by cyanogen bromide cleavage at the methionyl bond. This technique was very effective for the pufication of some large peptides, but is not readily applied to peptides containing other methionine or cysteine residues.

The general scheme for the application of the 9-(2-sulfo)-fluorenylmethyloxycarbonyl group to the purification of synthetic peptides is shown in Figure 1. The Sulfmoc group can be introduced by coupling the acid chloride or the nitrophenyl ester to the α-amino group of the synthetic peptide while it is still attached to the support used in the solid-phase synthesis. Since the Sulfmoc group is stable to HF or HBr it survives the deprotection and cleavage of the peptide from the support. The Sulfmoc group has the advantage that it will provide the only anion in the peptide at a pH below 2 because the α- and ω-carboxyl groups will be un-ionized at that low pH. Therefore, the Sulfmoc-peptide can be bound to weakly basic ion-exchange columns under acidic conditions in which underivatized peptides, even those with more than one carboxyl group, will not bind. After elution of terminated peptides and other unwanted contaminants, the Sulfmoc-peptide can be eluted at higher ionic strength (route a). Finally, the free peptide can be liberated by treatment with a mild base such as morpholine, ammonia, or aqueous tertiary amine. Alternatively, the Sulfmoc-peptides can be deprotected directly on the ion-exchange column by base, and the free peptide can then be eluted with acid (route b). It could be shown that contaminating terminated peptides are effectively removed by either route. The purpose of this paper is to describe the synthesis and characterization of reagents suitable for the introduction of the Sulfmoc group and to illustrate the purification method with examples of peptides containing neutral, acidic, or basic residues.

Results and Discussion

Synthesis of 9-(2-Sulfo)fluorenylmethyloxycarbonyl

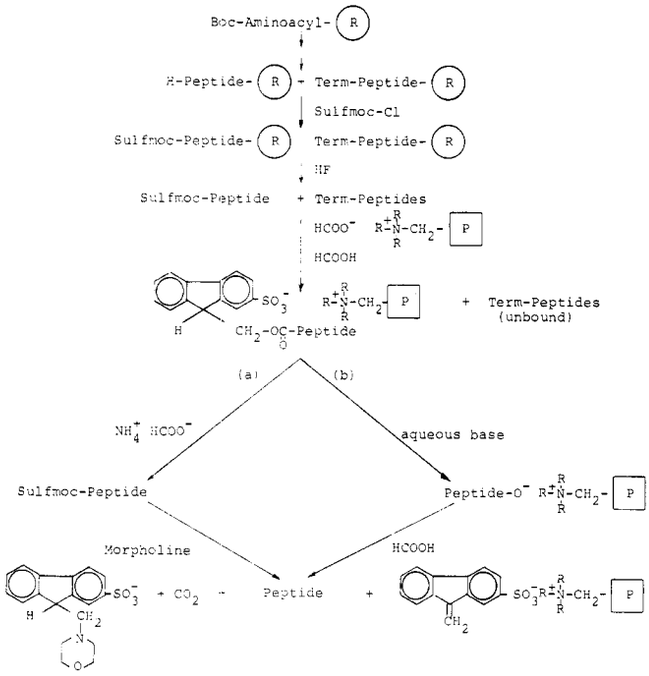
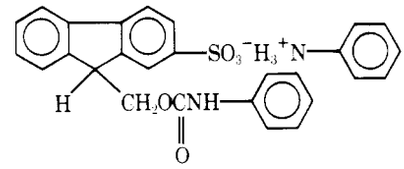


Figure 1. A general scheme for the application of the 9-(2-sulfo)fluorenylmethyloxycarbonyl group to the purification of synthetic peptides: Ⓡ is the resin support for solid-phase peptide synthesis; P (within box) is the ion-exchange polymer for the chromatographic purification; and Term-peptide represents the peptide chains that have been terminated at the α-amino group and are no longer growing.



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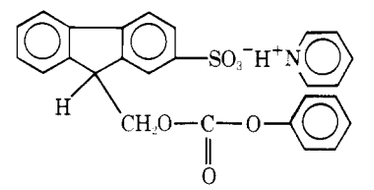

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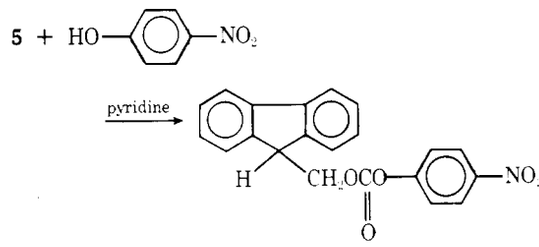

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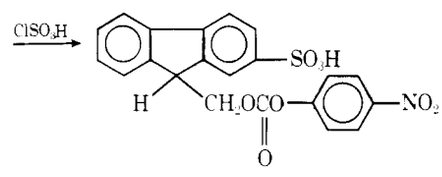

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Reagents. 9-(2-Sulfo)fluorenylmethyloxycarbonyl chloride (**7**) was prepared by treatment of 9-fluorenylmethyloxycarbonyl chloride (**5**) with 0.9 equiv of chlorosulfonic acid in dichloromethane at 0 °C for 2 h. Following extraction of unreacted **5**, the crystalline product **7** was obtained. It was homogeneous by thin-layer and ion-exchange chromatography. It had a sharp melting point, 138–140 °C, and was stable if kept dry, but was very hygroscopic. In electrophilic substitution reactions, fluorene is attacked at the 2 position.[16] This is true of nitration, bromination, sulfonation, and Friedel–Crafts reactions. In general, the nature of the substituent at position 9 (e.g., COOH, CH_3CONH–, or Br) has no effect on the orientation of the substitution and none was expected here. The position of the sulfo group of fluorene-2-sulfonic acid was established[17] by fusion with alkali. We have characterized the 9-(2-sulfo)fluorenylmethyloxycarbonyl chloride by elemental analysis and by UV, IR, and NMR spectroscopy. The data are compatible with structure **7**. Fluorene shows a complex NMR pattern,[18,19] with the proton pairs 2–3, 6–7, and 1–8 clustered between 7.1 and 7.5 ppm and H-4 and H-5 shifted farther down field between 7.6 and 7.8 ppm. Electron-withdrawing substituents at position 2, such as the nitro group, cause a marked downfield shift of H-1 to a singlet at 8.3 ppm and a shift of H-3 to a multiplet centered at 8.2 ppm, while the other resonances are only slightly affected.[18] We find that this characteristic downfield shift observed in the aromatic proton region of fluorene upon sulfonation is also observed upon sulfonation of 9-fluorenylmethyloxycarbonyl chloride. In both sulfonated derivatives a sharp resonance integrating for one proton appeared at 8.0–8.1 ppm, and is attributed to the C-1 proton. In addition, both compounds gave a narrow multiplet at 7.9 ppm corresponding to a downfield shift of H-3 into the region of H-4 and H-5.

The reagent **7** was further characterized by reaction with aniline and 8-hydroxyquinoline to give the crystalline anilide salt (**8**) and the hydroxyquinoline ester (**9**).

The Sulfmoc-4-nitrophenol active ester was best prepared by reaction of Fmoc-Cl (**5**) with 4-nitrophenol in pyridine to give Fmoc-ONp (**10**), followed by sulfonation to Sulfmoc-ONp (**11**).

Introduction of the Sulfmoc Group. The initial test of the Sulfmoc reagent involved the preparation of Sulfmoc-Gly-res, cleavage from the resin, chromatographic separation of the Sulfmoc-Gly from free Gly and terminated Gly, and deprotection of Sulfmoc-Gly by base to regenerate Gly. Boc-Gly-res was prepared by standard solid-phase procedures[20] and was deprotected with 50% TFA in CH_2Cl_2 and neutralized with 5% DIEA in CH_2Cl_2. The Gly-res was coupled for 2 h with Sulfmoc-Cl (2 equiv) in CH_2Cl_2 containing DIEA (3 equiv). A picrate titration[21] showed 2% of free amino groups remaining, indicating a good coupling yield from the Sulfmoc-Cl reaction. Similar results have been obtained with 10% pyridine–CH_2Cl_2 as the solvent and base. Sulfmoc-Gly-res was produced in 85% yield by reaction of Sulfmoc-ONp with Gly-res for 24 h in DMF containing DIEA.

Stability of the Sulfmoc Group toward Acid. Sulfmoc-Gly was cleaved from the resin in 75% yield by treatment with a 1:1 mixture of TFA and 30% HBr in HOAc[22] for 30 min at 25 °C. Electrophoresis (0.1 M pyridine acetate, pH 5.0) showed a major UV-positive, ninhydrin-negative spot at R_{asp} 0.95 corresponding to Sulfmoc-Gly and a small neutral spot for Gly. TLC on silica plates in 1-butanol–acetic acid–H_2O (4:1:1) showed R_f 0.51, and a trace of Gly, R_f 0.32. Amino acid analysis gave 6% of free glycine. An increase in the time of cleavage to 4 h did not increase the level of free glycine, indicating good stability of the Sulfmoc group to this strong acid. Cleavage of Sulfmoc-Gly-res with HF–10% anisole for 1 h at 0 °C gave very similar results. Treatment for 4 h did not increase the level of free glycine, showing that the Sulfmoc group was also stable to HF.

Table I. Paper Electrophoresis of Sulfmoc-Gly after Treatment with Base[a]

		R_{asp}[c]				
		0	0.3	0.54	0.74	0.95
no.	base[b]	(glycine)		(2-sulfodibenzofulvene)		(Sulfmoc-Gly)
1	H_2O	tr		0		+++
2	pyridine	tr		0		+++
3	1% Na_2CO_3	+++		+++		+
4	0.1 N NH_4OH	+++		+++		+
5	1% Et_3N/H_2O	+++		+++		0
6	10% DIEA/CH_2Cl_2	+++		+++		++
7	0.1 N NaOH	+++		+++	+	0
8	morpholine	+++	+++		+	+

[a] Paper electrophoresis in 0.1 M pyridine acetate, pH 5.0, 1500 V, 45 min. Observed under UV lamp and after ninhydrin spray. The + signs are qualitative estimates of the relative amounts. [b] Sulfmoc-Gly (2.6 μmol) treated with 0.1 mL of base reagent for 1 h, 25 °C. Samples 2, 4, 5, 6, and 8 were evaporated to dryness and dissolved in 0.2 mL of H_2O; sample 7 was diluted with 0.1 mL of 0.1 N HCl; samples 1 and 3 were diluted with 0.1 mL of H_2O. [c] R_f relative to aspartic acid, corrected for endosmotic flow. The R_{asp} 0 spot was ninhydrin-positive and UV-negative. All other spots were UV-positive and ninhydrin-negative. The R_{asp} 0.3 material streaked badly. It is presumably the 2-(sulfo)dibenzofulvene–morpholine adduct. The R_{asp} 0.74 material was not identified.

Lability of the Sulfmoc Group toward Base. The lability of the Sulfmoc group to various bases was tested in the following way. Sulfmoc-Gly-res was cleaved in HF, evaporated, and extracted with H_2O. Aliquots were evaporated to dryness and treated with the base. After 1 h at 25 °C the solution was either evaporated or neutralized and diluted with H_2O for analysis by paper electrophoresis. The paper was examined under the UV lamp and after ninhydrin spray. The results are summarized in Table I. It was concluded that Sulfmoc-Gly is stable in water and anhydrous pyridine, but is cleaved by anhydrous morpholine and by aqueous 1% Et_3N, 1% Na_2CO_3, 0.1 N NH_4OH, or 0.1 N NaOH. The pyridine and morpholine results agree with the findings of Carpino and Han.[4]

Rate constants for the release of the protecting groups from Sulfmoc-Gly-res and Fmoc-Gly-res were also determined by treating 10-mg samples at 25 °C with morpholine–dichloromethane, 1:9 (v/v). The filtrates and washing were diluted with CH_2Cl_2 and the absorbance was measured at 273 nm. The pseudo-first-order rate constants, k_1, in 10% morpholine–CH_2Cl_2 were 7.5×10^{-3} min^{-1} for Sulfmoc-Gly-res and 2.7×10^{-4} min^{-1} for Fmoc-Gly-res. Thus, the sulfonic acid group at position 2 of the ring increased the rate of proton abstraction at position 9 by a factor of about 30 in this solvent. The enhanced rate of deprotection by base may be advantageous in certain applications of these 9-(2-sulfo)fluorenylmethyloxycarbonyl compounds. It can be seen from Table II that replacement of CH_2Cl_2 with DMF increased the rate of removal of the Fmoc group by morpholine but had no effect on the removal of the Sulfmoc group. The rate of deprotection of both groups was greatly accelerated when the base in CH_2Cl_2 was changed from morpholine to piperidine.

Chromatographic Separation of Sulfmoc-Gly from Free Glycine and an N^{α}-Blocked Glycine. First, Sulfmoc-Gly-res (200 mg, 0.052 mmol) was cleaved in HF, and the product was dissolved in 1 M formic acid and applied to a 1 × 9-cm column of DEAE–cellulose. After elution with 15 mL of 1 M formic acid, a linear gradient was started consisting of 100 mL of 4 M $NH_4^+HCOO^-$, pH 3.2, flowing into 100 mL of 1 M formic acid. The effluent was monitored at 280 nm and by the ninhydrin reaction. A trace of UV material was found at the void volume (6 mL), together with 6.8% of free glycine. The remainder of the UV material, found between 84 and 105 mL and accounting for 97% of the amount applied to the column, was Sulfmoc-Gly (ϵ_{273} 2.07×10^4). After lyophilization it was treated with 5% aqueous Et_3N for 1 h. An amino acid analysis gave 94% recovery of glycine. Paper electrophoresis showed approximately 95% of the UV absorbing material at R_{asp} 0.54 (sulfodibenzofulvene), 5% at R_{asp} 0.74 (unknown), and no UV at R_{asp} 0.95 (Sulfmoc-Gly), indicating a complete deprotection.

A second chromatogram (Figure 2) was run in which Sulfmoc-Gly was mixed with 5 equiv of Gly and 3 equiv of Z-Gly (representing a terminated amino acid). The free amino acid, Gly, was not retained and appeared at the void volume (6 mL). The N^{α}-blocked Z-Gly, which contains an acidic carboxyl group, was only slightly retarded (12 mL). This was probably due to the aromatic Z group rather than to the essentially un-ionized carboxyl group. Finally, the Sulfmoc-Gly, containing the strong sulfonic acid group, eluted at 93 mL after the gradient started. Thus it was retained in 1 N formic acid and was not eluted until the concentration of formate ion was approximately 2 M (pH 2.7). It was also shown that the bound Sulfmoc-Gly could be decomposed by washing the DEAE–cellulose column with 1 M NH_4OH. The UV-absorbing 2-sulfodibenzofulvene eluted when the pH rose to 10.4.

Sulfmoc Purification of Neutral Peptides. A. Sulfmoc-Leu-Ala-Gly-Val-res. Having shown that Sulfmoc-Cl was an effective reagent for separating an amino acid from a terminated amino acid, we next applied it to a synthetic tetrapeptide-resin. The model, Leu-Ala-Gly-Val, was chosen

Table II. Effect of Solvent and Base on the Deprotection of Fmoc-Gly-res and Sulfmoc-Gly-res

compd	base	solvent	time, min	deprotection,[a] %
Fmoc-Gly-Res	10% morpholine	CH_2Cl_2	240	18
	10% morpholine	DMF	240	75
	10% piperidine	CH_2Cl_2	240	100
	50% morpholine	CH_2Cl_2	240	100
Sulfmoc-Gly-Res	10% morpholine	CH_2Cl_2	20	45
	10% morpholine	DMF	20	48
	10% piperidine	CH_2Cl_2	20	100
	50% morpholine	CH_2Cl_2	5	98

[a] Calculated from the measured absorbance at 273 nm of the filtrates.

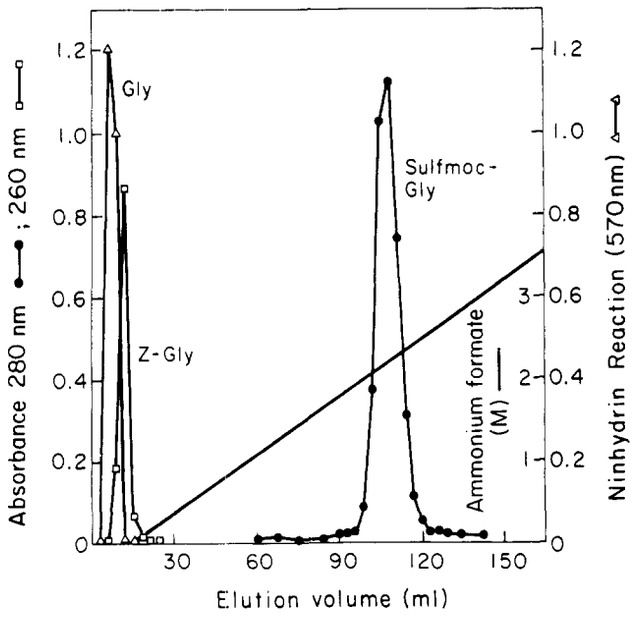
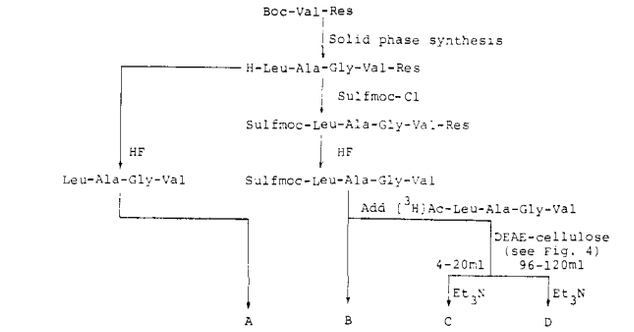


Figure 2. Chromatographic separation of Sulfmoc-Gly from Gly and Z-Gly. Column: 1 × 9-cm, DEAE–cellulose. Eluant: 1 M formic acid (15 mL), then a linear gradient of 4 M ammonium formate (100 mL) in 1 M formic acid flowing into 1 M formic acid (100 mL). Sample: mixture of Sulfmoc-Gly (0.026 mmol), Gly(0.13 mmol), and Z-Gly (0.078 mmol) in 1 M formic acid. Monitor Gly by ninhydrin reaction, Z-Gly at 260 nm, and Sulfmoc-Gly at 280 nm after a 1:25 dilution.



Component	Elution time (min)	Ion Exchange Chromatography on AA-15 Ninhydrin analysis (Mol %) A	B	C	D
V	99	0.18	0.26	0.24	0
AGV	207	0.43	0.51	0.52	0.09
LAGV	236	99.01	1.16	0.87	99.73
GV	266	0.18	0.23	0.18	0
LAV	316	0.12	0	0	0.12
LGV	369	0.08	0	0	0.06
LV	436	0	0	0	0

Figure 3. Solid-phase synthesis and purification of Leu-Ala-Gly-Val by the Sulfmoc method. Four samples of the tetrapeptide were obtained as shown in the upper part of the figure. These were each analyzed chromatographically for their peptide components as shown in the lower part of the figure. The lower limit of detection was approximately 0.03%.

because a chromatographic system is available to separate the tetrapeptide from all of the potential termination or deletion peptides.[23] If the new purification technique with Sulfmoc-Cl were completely successful we would expect to obtain the tetrapeptide product free of the terminated, nongrowing chains, but still containing any deletion peptides that may have been produced in the synthesis.

The protocol for such an experiment is shown in Figure 3. Boc-valyloxymethylphenylacetamidomethyl-copoly(styrene–1% divinylbenzene)-resin[24], Boc-Val-res, was prepared from our new aminomethyl-resin support.[25] The peptide chain was extended by three more residues using the standard solid-phase procedures[20] and then deprotected and neutralized. One portion was cleaved in HF (sample A). Part of it was hydrolyzed in 6 N HCl and amino acid ratios were determined to be Leu 1.00, Ala 1.00, Gly 0.99, Val 1.01. Another part of sample A was applied to a 0.9 × 58-cm column of Beckman AA-15 sulfonated polystyrene resin and eluted with pH 3.49 sodium citrate buffer as previously described for the analysis of the component peptides.[23] The remainder of the tetrapeptide-resin was treated with Sulfmoc-Cl (4 equiv) and diisopropylethylamine (DIEA) (15 equiv) in CH_2Cl_2 for 2 h. The Sulfmoc-Leu-Ala-Gly-Val-res was filtered, washed, and dried. A 100-mg sample was cleaved in HF–10% anisole for 1 h at 0 °C (sample B). Part of the resulting Sulfmoc-Leu-Ala-Gly-Val was applied directly to the AA-15 column. The remainder was mixed with an equimolar amount of synthetic [^{3}H]Ac-Leu-Ala-Gly-Val and applied to a 1 × 9-cm DEAE–cellulose column in 1 M formic acid. After elution with 32 mL of 1 M formic acid, a linear gradient consisting of 100 mL of 4 M ammonium formate/1 M formic acid running into 100 mL of 1 M formic acid was started. The tubes were monitored at 280 nm for Sulfmoc derivatives and ^{3}H was followed by scintillation counting. Since most terminated products would not be detected by the UV monitoring, the [^{3}H]acetylpeptide was added to show their position. It can be seen from Figure 4 that a small amount (0.6%) of UV-absorbing material eluted at the column volume, while the main Sulfmoc-peptide was fully retained in 1 M formic acid and did not elute until the ammonium

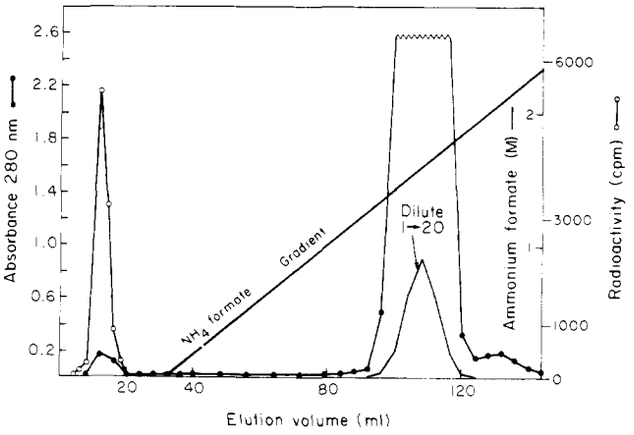


Figure 4. Chromatographic separation of Sulfmoc-Leu-Ala-Gly-Val from [^{3}H]Ac-Leu-Ala-Gly-Val and other terminated peptides. Column: 1 × 9-cm DEAE–cellulose equilibrated with 1 M formic acid. Sample: an equimolar mixture of synthetic Sulfmoc-Leu-Ala-Gly-Val and [^{3}H]Ac-Leu-Ala-Gly-Val after HF cleavage from their resin supports. Eluant: 1 M formic and (32 mL), then ammonium formate–formic acid gradient (200 mL). Monitor by absorbance at 280 nm and by scintillation counting.

formate concentration was 1.3–1.8 M. A small amount (1.1%) of a slower moving component was detected but not identified. Scintillation counting showed that the terminated, N^{α}-acetylpeptide was not retained on the tertiary amine column under these acidic conditions even though it contained a carboxyl group. In contrast, the sulfonic acid group ($pK_a \sim -1.3$) was fully ionized in 1 M formic acid and was retained on the column. Material from the two peaks of the DEAE–cellulose column was treated with 5% aqueous Et_3N to remove the Sulfmoc group and then was fractionated on the AA-15 column (samples C and D). The data of Figure 3 show that this synthesis produced, before purification (sample A), a product in which Leu-Ala-Gly-Val comprised 99.0% of the ninhydrin-positive peptides, but which was contaminated with terminated peptides Val, Gly-Val, and Ala-Gly-Val totaling 0.8% and deletion peptides Leu-Ala-Val and Leu-Gly-Val totaling 0.2%. Terminated peptides may have originated from

Table III. Purification of Sulfmoc-Phe-[^{3}H]Ala-Ser-Val on a DEAE-Cellulose column[a]

elution vol, mL	absorbance, A_{280} total	%	radioactivity cpm/0.1 mL total	%	mol ratio[b] ^{3}H/Sulfmoc	comment
7.5–20	0.51	0.4	5 101	4.5		terminated peptides (no Sulfmoc)
27.5–47.5	4.01	3.2	4 666	4.1	1.2	Fmoc-peptides
55–100	120.17	94.9	102 505	91.4	0.99	Sulfmoc-peptides
100–120	1.93	1.5	0	0		a fluorene derivative (no amino acids)

[a] See Figure 5 for details of the chromatography. [b] Calculated using a specific activity of 1.43×10^8 cpm/mmol, and ϵ_{280} 1.75×10^4 for Sulfmoc-peptides and ϵ_{280} 0.64×10^4 for Fmoc-peptides.

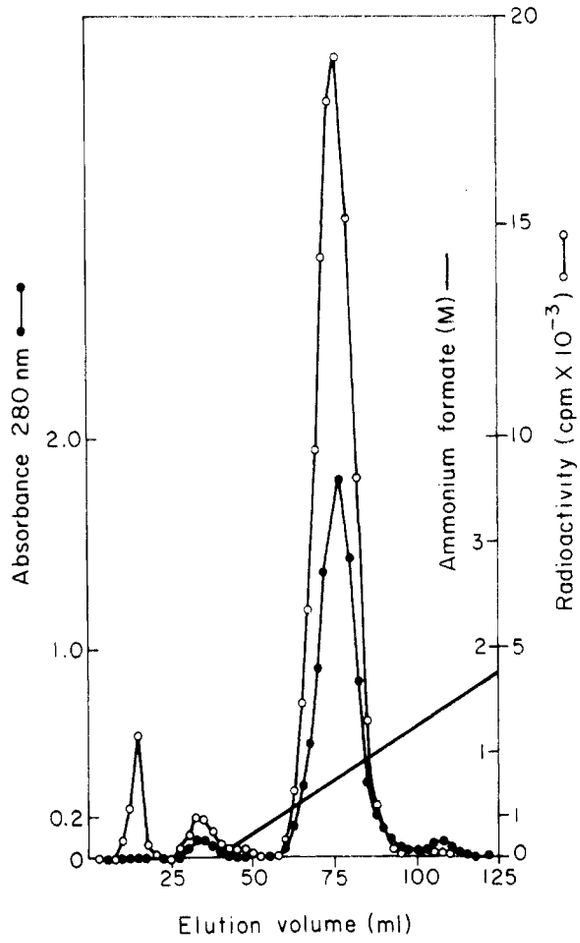


Figure 5. Chromatographic purification of Sulfmoc-Phe-[^{3}H]Ala-Ser-Val. Column: 1 × 9-cm DEAE-cellulose equilibrated with 1 M formic acid. Eluant: 1 M formic acid (37 mL), then ammonium formate–formic acid. Eluant: 1 M formic acid (37 mL), then ammonium formate–formic acid gradient (200 mL). Monitor by absorbance at 280 nm (dilute 1:50) and scintillation counting of 0.1-mL aliquots.

permanently or temporarily blocked chains or from sterically or kinetically unreactive chains. Sample B showed that 2.2% of free amino groups remained after treatment with Sulfmoc-Cl and cleavage with HF. The Sulfmoc-peptides were not detected. Sample C consisted of products not derivatized by Sulfmoc-Cl. Since the Sulfmoc group is stable to HF, these peaks are not due to loss of this protecting group. Sample D contained 98.3% of the UV-absorbing material eluted from the DEAE column. The AA-15 column showed no Val, Gly-Val, or Leu-Val and only 0.1% of Ala-Gly-Val, which means that essentially all of the terminated chains were effectively removed in this simple model synthesis. The deletion peptides Leu-Ala-Val and Leu-Gly-Val were not removed, and the 0.1% of remaining Ala-Gly-Val probably also represents a potential deletion peptide rather than a termination peptide. Thus, the levels of terminated peptides were reduced by an order of magnitude and the purity of the product was increased to 99.7% as a result of the Sulfmoc procedure.

B. Sulfmoc-Phe-[^{3}H]Ala-Ser-Val-res. In order to follow the ninhydrin-negative, UV-negative deletion peptides better, another model tetrapeptide was synthesized which contained a radiolabel. Thus, H-Phe-[^{3}H]Ala-Ser-Val-Res, containing 1.43×10^8 cpm/mmol, was synthesized by the standard methods described before.[26] Part of it (10 mg, 2 μmol) was cleaved in HF and run on the AA-15 column where 91.7% of the ninhydrin-positive material was found in the tetrapeptide peak at 426 min. Another portion (100 mg) of the tetrapeptide-resin was converted to the Sulfmoc derivative, cleaved, and run on the DEAE–cellulose column described before. The eluate was monitored by absorbance at 280 nm and by scintillation counting as shown in Figure 5. The results of the column are summarized in Table III. The bulk of the peptide was found in a large peak at 77 mL as the Sulfmoc-peptide and was well separated from radioactive or UV-absorbing material. About 4.5% of the counts were not retained, which were primarily terminated peptides but included any growing chains that did not react with the Sulfmoc-Cl. In this experiment a crude Sulfmoc-Cl preparation was used that also contained some unsulfonated Fmoc-Cl. The resulting Fmoc-peptides were slightly retarded in a 1 M formic acid and account for the peak at 35 mL. Finally, a small amount of a sulfonated fluorene product, probably 2-sulfodibenzofulvene, containing no ^{3}H and no amino acids after hydrolysis, appeared at 106 mL. The molar ratio of ^{3}H to Sulfmoc was 0.99 in the main peak as expected. The Sulfmoc-Phe-[^{3}H]Ala-Ser-Val from the main peak was deprotected with aqueous Et_3N and run on the AA-15 column at pH 3.49. Of the ninhydrin-positive material, 97.0% was now found in the tetrapeptide peak at 426 min.

Sulfmoc Purification of Acidic and Basic Peptides. It was important to know whether or not Sulfmoc peptides containing additional charged groups could be separated from the corresponding free peptides and terminated peptides. For that reason two hexapeptides were synthesized from the above tetrapeptide, one of which contained an additional carboxyl group and one an arginine residue. They were Boc-Leu-Glu(OBzl)-Phe-[^{3}H]Ala-Ser-Val-res and Boc-Leu-Arg-(Tos)-Phe-[^{3}H]Ala-Ser-Val-res.

Boc-Leu-Glu(OBzl)-Phe-[^{3}H]Ala-Ser(Bzl)-Val-res (100 mg, 200 μmol/g) was deprotected in 50% TFA, neutralized, and converted to the Sulfmoc derivative as before with Sulfmoc-Cl and DIEA in CH_2Cl_2. The Sulfmoc-peptide was cleaved from the resin in HF–10% anisole, washed with ether, extracted into 10% HOAc, and lyophilized. The product was dissolved in 1 mL of 1 N formic acid and applied to the 1 × 9-cm DEAE–cellulose column. It was eluted with 33 mL of 1 N formic acid and then with 200 mL of the 4 M ammonium formate–1 N formic acid gradient described before. About 15% of the radioactive peptide was recovered, unretained, and in the fractions between 5 and 10 mL of 1 N formic acid, and 85% was

recovered in the main fraction with a peak 28 mL from the start of the gradient. As a control, a sample of the free hexapeptide without the Sulfmoc group was run on the same column. As expected, this acidic peptide was not retained by the DEAE–cellulose when it was applied and eluted in 1 N formic acid, and therefore was readily and completely separated from the Sulfmoc-peptide. The purified Sulfmoc-Leu-Glu-Phe-[^{3}H]Ala-Ser-Val was deprotected in 5% aqueous triethylamine for 1 h and applied to the AA-15 column described earlier. It emerged in the pH 3.49 buffer at 259 min.

Boc-Leu-Arg(Tos)-Phe-[^{3}H]Ala-Ser(Bzl)-Val-res (100 mg, 200 μmol/g) was deprotected in 50% TFA, neutralized, and converted to the Sulfmoc derivative as before. The Sulfmoc group was removed from a sample (10 mg) by treatment with 1 mL of 50% morpholine–CH_2Cl_2 for 1 h at 25 °C, filtered, and washed. From the absorbance at 273 nm the resin was calculated to contain 197 μmol/g, showing an essentially quantitative addition and removal of the Sulfmoc group. The remaining 90 mg of Sulfmoc-peptide-resin was cleaved in HF–10% anisole, washed with ether, and extracted into 10 mL of glacial acetic acid. The recovery by ^{3}H counts and by UV absorbance was quantitative. The Sulfmoc-peptide was lyophilized and dissolved in 0.2 mL of HOAc plus 0.4 mL of 1 N formic acid and applied to a 1 × 40 cm column of Aminex AG 1-X2 quaternary amine ion-exchange resin. It was eluted successively with 40 mL of 1 N formic acid and 200 mL of a linear gradient of 1 N formic acid to 4 N formic acid to 40% acetic acid. About 2% of the UV-absorbing material and 18% of the ^{3}H counts were found to be unretained in the 1 N formic acid. The remaining material, accounting for 98% of the UV and 82% of ^{3}H counts, was found in a single sharp peak between 93 and 105 mL of the last gradient. This Sulfmoc-peptide could also be chromatographed on DEAE–cellulose in pyridine acetate buffer at pH 5, but the Aminex column was preferable. The main component from the Aminex column was lyophilized and deprotected in 5% aqueous triethylamine for 1 h. Acid hydrolysis and amino acid analysis showed $Leu_{1.05}$, $Arg_{0.99}$, $Phe_{0.92}$, $Ala_{1.03}$, $Ser_{0.94}$, $Val_{1.07}$. The hexapeptide was applied to the AA-15 column as before, but eluted with sodium citrate buffer at pH 6.4. Before the Sulfmoc procedure, the main peak was at 304 min and 8.7% of a contaminating peptide was present at 391 min. After the Sulfmoc procedure, the main peak was at 304 min, but the peak at 391 min was completely absent.

Alternative Purification Procedure. An alternative fractionation procedure (route b, Figure 1) was also shown to be suitable for the separation of growing peptide chains from terminated chains. To illustrate this route Sulfmoc-Leu-Glu(OBzl)-Phe-[^{3}H]Ala-Ser-Val-resin (100 mg) was prepared and cleaved as before. The Sulfmoc-peptide was then dissolved in 1 M formic acid and applied to a 1 × 9-cm column of AG 1-X2 resin. The column was washed with 1 M formic acid (50 mL) to remove terminated or underivatized peptides while retaining the Sulfmoc-peptide. Excess formic acid was removed by washing with 20 mL of water and then the Sulfmoc group was removed from the peptide by washing with 50 mL of 5% Et_3N in H_2O at a rate of 25 mL/h. The liberated 2-sulfodibenzofulvene and the free hexapeptide were both retained on the quaternary amine resin under these basic conditions. Excess Et_3N was removed by washing with 20 mL of water. Finally, the peptide was eluted with 1 M formic acid, while the strongly acidic sulfodibenzofulvene was still retained. In this instance 86% of the counts applied to the column were recovered at the breakthrough volume of the formic acid. Monitoring of the column eluates at 280 nm showed that the peptide fraction was not contaminated by the fulvene derivative. In many cases this simple procedure will be preferable to route a, in which the Sulfmoc-peptide is first isolated.

Conclusions

It is evident that not only neutral peptides but also peptides containing more than one carboxyl group or a strongly basic residue such as arginine can be derivatized with Sulfmoc-Cl and separated chromatographically from underivatized chains on the basis of the strongly acidic sulfonic acid function. At the low pH used, the free peptides or terminated peptides are not retained on the anion exchangers even if they contain excess carboxyl groups. The guanidine side chain does decrease the binding of the sulfonic acid derivative on the tertiary amine anion exchanger under these conditions, and the separation can be improved by the use of a quaternary amine resin. The facile cleavage of the Sulfmoc group under very mild, basic conditions allows the ready recovery of the purified peptide in quantitative yield. The Sulfmoc-peptide can also be deprotected by base while it is still attached to the ion-exchange column. This procedure allows the separation of the free peptide directly without isolation of the Sulfmoc-peptide intermediate.

Experimental Section

Materials and Methods. Boc-amino acids were from Beckman, Chemical Dynamics, and Bachem. Copoly(styrene–1% divinylbenzene), 200–400 mesh beads, was from Bio-Rad. Chlorosulfonic acid (Aldrich) was redistilled, bp 150 °C (uncorrected). Dichloromethane (Eastman) was distilled from $CaCO_3$. Other solvents and reagents were analytical reagent grade. Elemental analyses were by Mr. S. T. Bella or by the Schwarzkopf Microanalytical Laboratory and amino acid analyses were by Miss Marie LeDoux on a Beckman Model 121 analyzer. Scintillation counting was on a Beckman Model LS-350 and NMR spectra were on a Varian T-60 spectrometer. Melting points were taken on a Thomas-Hoover capillary apparatus and are uncorrected. Thin-layer chromatograms were on precoated 0.25 mm silica gel G plates from Analtech in 1-butanol–acetic acid–water (BAW) (4:1:1) or cyclohexane–chloroform (2:1). Spots were visualized under the short wavelength UV lamp or after spraying with a 2.0% solution of ninhydrin in acetone containing 0.2% acetic acid and 0.3% pyridine.

2-Sulfofluorene (6) was prepared according to Chrzaszczewska and Machlanski.[6] Fluorene (Aldrich, 1.0 g, 6.0 mmol) was dissolved in 4.5 mL of $CHCl_3$ and cooled to 0 °C. A solution of redistilled $ClSO_3H$ (0.44 mL, 6.6 mmol) in 0.9 mL of $CHCl_3$ was added dropwise with stirring. The solution turned dark green and a gray-green precipitate formed. The product was filtered, washed with $CHCl_3$, and dried at 65 °C in vacuo, yield 0.59 g. The solid was recrystallized from hot, dry EtOAc: NMR (D_3COD) δ 3.85 (s, 2, CH_2), 5.37 (s, 3, H_2O/RSO_3H), 7.2–7.9 (m, 6 aryl), 8.0 (s, 1, H-1 aryl); NMR (D_3CCN) δ 3.90 (s, 2, CH_2), 5.7 (s, H_2O/RSO_3H), 7.2–7.9 (m, 6, aryl), 8.0 (s, 1, H-1 aryl). Anal. Calcd for $C_{13}H_{10}SO_3 \cdot H_2O$: C, 59.00; H, 4.62. Found: C, 58.48; H, 4.60.

9-Fluorenylmethyloxycarbonyl Chloride (Fmoc-Cl, 5). Fmoc-Cl (Chemalog), 25 g, was dissolved in boiling ether and allowed to cool slowly at room temperature and then at 0 °C. Long prisms, 18 g, were obtained, mp 62–63 °C (lit.[4] 61.5–63 °C): TLC BAW, R_f 0.75; $CHCl_3$, R_f 0.75; cyclohexane–$CHCl_3$ (2:1), R_f 0.45; ϵ_{267}^{max} (EtOAc) 2.05×10^4. A slower moving impurity, R_f 0.27 ($CHCl_3$), was removed by the recrystallization: no ionic Cl^- by Volhard; 1.0 equiv of Cl^- by Volhard after heating in pyridine;[27] NMR (D_3COD) δ 4.0–4.6 (m, 3, $CHCH_2$), 7.1–7.8 (m, 8, aryl); NMR (D_3CCN) δ 4.0–4.3 (m, 1, CH), 4.67 (d, 2, J = 7 Hz, CH_2), 7.1–7.9 (m, 8, aryl).

9-(2-Sulfo)fluorenylmethyloxycarbonyl Chloride (Sulfmoc-Cl 7). Fmoc-Cl (5; 2.59 g, 10.0 mmol) was dissolved in 20 mL of CH_2Cl_2 and cooled to 0 °C. A solution of redistilled $ClSO_3H$ (0.56 mL, 9.0 mmol) in 10 mL of CH_2Cl_2 was added with stirring and cooling over a period of 15 min. The solution turned yellow and then light green, and after a total of 30 min a white precipitate formed and set to a solid. After another 90 min, 20 mL of cyclohexane was added to dissolve the unreacted Fmoc-Cl. The suspension was centrifuged and washed three times with 30 mL of 1:1 cyclohexane–CH_2Cl_2. The white solid was dried in vacuo at 25 °C for 48 h: yield, 2.26 g (74%); mp 138–148 °C; TLC BAW, R_f 0.60; $CHCl_3$, R_f 0.79; cyclohexane–$CHCl_3$ (2:1), R_f 0; ϵ_{273}^{max} (CH_2Cl_2) 2.07×10^4; ionic Cl^- (Volhard before pyridine) 0, nonionic Cl (Volhard after heating in pyridine)[27] 1.0 equiv; NMR (D_3COD) δ 4.2–4.5 (m, 3, $CHCH_2$), 5.4 (s, H_2O/RSO_3H), 7.1–7.9 (m, 6, aryl), 8.1 (s, 1, H-1 aryl); NMR (D_3CCN + 6 equiv of D_2O) δ 4.2–4.4 (m, 1, CH), 4.75 (d, 2, J = 5 Hz, CH_2), 5.4 (br s, HDO/RSO_3H), 7.3–7.9 (m, 6, aryl), 8.1 (s, 1, H-1 aryl).

Anal. Calcd for $C_{15}H_{11}O_5S$ Cl (338.77): C, 53.18; H, 3.27; S, 9.46; Cl, 10.47. Found: C, 53.11; H, 3.39; S, 9.39; Cl, 10.61.

Sulfmoc-Cl (0.25 mmol) was dissolved in 1 mL of 0.01 N HCl and applied to a 1 × 9-cm DEAE-cellulose column. It was retained on the column and was not eluted by washing with 50 mL of 0.01 N HCl. A linear gradient of HCl (0.01–1.0 N) was started and the Sulfmoc-Cl peak was found at 51 mL (~0.2 N HCl). Lyophilization gave a 75% yield of white solid: TLC in BAW, R_f 0.60.

Sulfmoc-Cl could be gradually extracted from water into ethyl acetate ($K \sim 0.2$), but was readily extracted from 1 N HCl into ethyl acetate ($K \sim 2$). The R_f in BAW was unchanged.

Anilinium 9-Fluorenylmethyloxycarbonylanilide-2-sulfonate (8). Sulfmoc-Cl, 135.6 mg (0.40 mmol), was dissolved in 0.40 mL of 9:1 CH_2Cl_2–EtOH and 0.1 mL (1.2 mmol, 3 equiv) of aniline was added. A white precipitate formed immediately. After 1 h at 25 °C and 18 h at 0 °C it was filtered and washed with CH_2Cl_2. The solid was evaporated to dryness, washed with water to remove aniline hydrochloride, and dried at 78 °C in vacuo, mp 248–249 °C. Anal. Calcd for $C_{27}H_{24}N_2SO_5$ (488.46): C, 66.39; H, 4.95; N, 5.73. Found: C, 66.39; H, 5.18; N, 5.71.

9-(2-Sulfo)fluorenylmethyl 8-Hydroxyquinoline Carbonate (Sulfmoc-OHQ, 9). A solution of Sulfmoc-Cl (169 mg, 0.50 mmol) in 1 mL of ethyl acetate was added slowly with stirring to a solution of recrystallized 8-hydroxyquinoline (218 mg, 1.5 mmol) in 1 mL of EtOAc. After 24 h, the yellow suspension was extracted three times with 5 mL of water. The remaining oil was separated from the EtAOc, dissolved in ethanol, and precipitated with ether. The yellow powder was triturated with ethanol and pumped dry: yield, 155 mg (69%); mp 240–250 °C dec.

Anal. Calcd for $C_{24}H_{17}NO_6S$ (447.45): C, 64.42; H, 3.83; N, 3.13. Found: C, 64.13; H, 3.91; N, 3.21.

9-Fluorenylmethyl 4-Nitrophenyl Carbonate (Fmoc-ONp, 10). Fmoc-Cl (1.0 g, 3.85 mmol) was dissolved in 5 mL of ether and cooled to 0 °C. A clear solution of 4-nitrophenol (535 mg, 3.85 mmol) and pyridine (1.5 mL) in 5 mL of ether at 0 °C was added with stirring. After 2 h, the yellow flocculent precipitate was centrifuged and washed with ether. The ether layers were washed with water and dried over $MgSO_4$. The product was precipitated with petroleum ether and recrystalized from ether: yield, 0.77 g (52%); mp 96–97 °C; TLC BAW, R_f 0.96; cyclohexane–$CHCl_3$ (2:1), R_f 0.20. Anal. Calcd for $C_{21}H_{15}NO_5$ (361.34): C, 69.80; H, 4.18; N, 3.88. Found: C, 69.93; H, 4.17; N, 3.93.

9-(2-Sulfo)fluorenylmethyl 4-Nitrophenyl Carbonate (Sulfmoc-ONp 11). A solution of redistilled chlorosulfonic acid (63 μL, 1 mmol) in 1 mL of CH_2Cl_2 was added to a solution of Fmoc-ONp (324 mg, 0.9 mmol) in 9 mL of CH_2Cl_2 at 0 °C. After stirring for 2 h at 0 °C the mixture was stirred for 6 h at 25 °C. TLC in BAW showed approximately equal amounts of Sulfmoc-ONp (R_f 0.70) and Fmoc-ONp (R_f 0.96). Another 63 μL of $ClSO_3H$ was added and after 18 h the ratio of Sulfmoc-ONp to Fmoc-ONp was about 9:1. Small amounts of brown oil and white solid were removed by centrifugation. The CH_2Cl_2 layer was extracted three times with 10 mL of water and the aqueous fraction was lyophilized. The glassy product was dissolved in 25 mL of CH_2Cl_2 and a small amount of oil was separated. The CH_2Cl_2 was extracted five times with 10 mL of H_2O. The CH_2Cl_2 layer contained about 3% each of Fmoc-ONp and Sulfmoc-ONp, while the aqueous phase showed only R_f 0.70 material. The aqueous fraction was lyophilized to a solid: yield 125 mg (40%); $\epsilon_{273}{}^{max}$ 1.79×10^4. Sulfmoc-ONp was stable to 0.5% DIEA in CH_2Cl_2 for 24 h, but was approximately 50% decomposed by 5% DIEA in CH_2Cl_2 in 24 h.

The Sulfmoc-ONp was dissolved in 0.01 N HCl and applied to a 1 × 9.5-cm column of DEAE-cellulose. It was eluted with 30 mL of 0.01 N HCl and then with 200 mL of a linear gradient of 0.01 N HCl to 1 N HCl. A trace of UV-absorbing material (unsulfonated) appeared at the column volume and a small peak (2% of total) appeared at 82 mL (~0.3 N HCl). The main peak was centered at 148 mL (0.65 N HCl). It was lyophilized to give a white solid, R_f 0.70 (BAW). Anal. Calcd for $C_{21}H_{15}O_8NS \cdot H_2O$ (459.42): C, 54.90; H, 3.70; N, 3.05. Found: C, 54.9; H, 4.0; N, 3.1.

9-(2-Sulfo)fluorenylmethyloxycarbonylglycine-resin (Sulfmoc-Gly-Res). Chloromethyl-copoly(styrene–1% divinylbenzene) 200–400 mesh beads (Pierce, 20 g, 15 mmol of Cl) was suspended in 80 mL of DMF and placed in a 50 °C bath. Boc-Gly (2.1 g, 12 mmol) and cesium carbonate (1.9 g, 6 mmol) were suspended in 10 mL of DMF and added to the resin.[28] After mixing with an overhead paddle stirrer for 18 h at 50 °C the resin was filtered and washed several times with DMF, DMF–H_2O (9:1), DMF, and CH_2Cl_2. The dried resin contained 0.23 mmol of glycine per gram. A 1.0-g sample was deprotected with 50% TFA–CH_2Cl_2 for 30 min, washed, neutralized with 5% DIEA–CH_2Cl_2 for 10 min, and washed with CH_2Cl_2.

A. Reaction with Sulfmoc-Cl and DIEA. Sulfmoc-Cl (155 mg, 0.46 mmol) was dissolved in 0.2 mL of EtOAc and 5 mL of CH_2Cl_2 and added to 1 g of Gly-res (0.23 mmol) that had been suspended in 5 mL of CH_2Cl_2 containing 0.12 mL (0.69 mmol) of DIEA. After shaking for 2 h the resin was filtered and washed with CH_2Cl_2 several times and dried. A picrate titration for uncoupled Gly-res showed 0.005 mmol/g (2%). Fluorescamine also gave a faintly positive test. Sulfmoc-Gly was cleaved from the resin by the following. (1) The resin (200 mg) was suspended in 1 mL of TFA and 1 mL of 30% HBr in HOAc was added. After 30 min at 25 °C in a closed vessel, the resin was filtered, washed with TFA, and evaporated, and the product was extracted into 5 mL of HOAc. Lyophilization gave 14.7 mg (81%). (2) A 200-mg sample was treated for 1 h at 0 °C in 4.5 mL of HF and 0.5 mL of anisole. After evaporation to dryness the Sulfmoc-Gly was extracted three times with 5 mL of water and the water was extracted with ether and lyophilized, yield 49%.

B. Reaction with Sulfmoc-Cl and Pyridine. Sulfmoc-Cl (155 mg, 0.46 mmol) was dissolved in 0.2 mL of EtOAc and 5 mL of CH_2Cl_2 and added to 1 g of Gly-res (0.23 mmol) suspended in 4 mL of CH_2Cl_2 and 1 mL of pyridine. The solution turned yellow and a white precipitate formed, which redissolved after 15 min. After 2 h of shaking, the resin was filtered and washed with $CHCl_3$ and CH_2Cl_2 and dried. A picrate titration showed <0.3% of free amine. A 12.0-mg sample was treated with 0.2 mL of morpholine in 0.2 mL of CH_2Cl_2 for 2 h at 25 °C. From the absorbance of the filtrate at 273 nm, 0.21 mmol/g of Sulfmoc group was cleaved (91%). A picrate titration of the resin after the morpholine treatment showed 0.20 mmol/g of free amino groups, in agreement with the morpholine data.

C. Reaction with Sulfmoc-ONp and DIEA. Sulfmoc-ONp (8.42 mg, 0.019 mmol) was dissolved in 0.4 mL of DMF and 0.1 mL of a 5% solution of DIEA in DMF and was added to 50.6 mg of Gly-res (0.0115 mmol of Gly) suspended in 0.5 mL of DMF. After shaking for 24 h the resin was filtered and washed with 0.5% DIEA in CH_2Cl_2. The absorbance at 280 nm showed that 0.010 mmol of Sulfmoc-ONp had been consumed, and absorbance at 400 nm showed that 0.0097 mmol of $^-$ONp had been formed, indicating a yield of Sulfmoc-Gly-res of ~85%.

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Registry No.—**5,** 28920-43-6; **6,** 52525-94-7; **7,** 67827-06-9; **8,** 67827-08-1; **9,** 67827-09-2; **10,** 67827-10-5; **11,** 67827-11-6; Sulfmoc-Gly, 67827-12-7; fluorene, 86-73-7; aniline, 62-53-3; 8-hydroxyquinoline, 148-24-3; 4-nitrophenol, 100-02-7; Boc-Gly, 1138-80-3; Sulfmoc-Leu-Ala-Gly-Val, 67827-13-8; Sulfmoc-Phe-[^{3}H]Ala-Ser-Val, 67827-14-9; Sulfmoc-Leu-Phe-[^{3}H]Ala-Ser-Val, 67827-15-0; Sulfmoc-Leu-Arg-Phe-[^{3}H]Ala-Ser-Val, 67827-16-1.

References and Notes

(1) Supported in part by Grant AM 01260 from the U.S. Public Health Service and by a grant from the Hoffmann-LaRoche Foundation.
(2) The peptide nomenclature follows the Tentative Rules of the IUPAC-IUB Commission [*J. Biol. Chem.*, **241**, 2491 (1966); **247**, 977 (1972)]. Other abbreviations are: Sulfmoc = 9-(2-sulfo)fluorenylmethyloxycarbonyl; Fmoc = 9-fluorenylmethyloxycarbonyl; ONp = 4-nitrophenyl ester; OHQ = 8-hydroxyquinoline ester; TFA = trifluoroacetic acid; DIEA = diisopropyl ethyl amine; R_{asp} = mobility relative to aspartic acid; res = resin; DEAE = diethylaminoethyl; DMF = dimethylformamide.
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Thermal Decomposition of 1,2,3,4-Thiatriazoles. On the Question of Thioacyl Azide and Thioacylnitrene Intermediates

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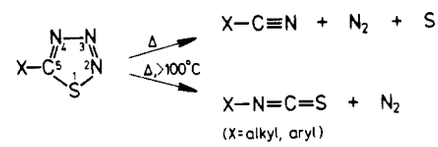
The kinetics of decomposition of 5-phenyl-1,2,3,4-thiatriazole to benzonitrile, sulfur, and nitrogen were investigated in bromobenzene at 52.2–61.1 °C and found to be first order with E_a = 118.0 kJ·mol^{-1} and $\Delta S^{\ddagger}$ = 11.7 J·mol^{-1} K^{-1}. A linear Hammett correlation was obtained with substituted phenylthiatriazoles ($\rho \approx 0$). Kinetic isotope effects with 4-^{15}N- and 2-^{15}N-labeled phenylthiatriazole were observed to be ~4 and 0%, respectively. The effect of Lewis acids and of dipolarophiles on the decomposition was investigated. The overall observations are interpreted to imply a three-step mechanism via (*E*)-thiobenzoyl azide. There is no evidence for a thiobenzoylnitrene intermediate. Phenyl isothiocyanate, which is formed from phenylthiatriazole above ~100 °C, is suggested to result from a concerted rearrangement from (*Z*)-thiobenzoyl azide.

Thiatriazoles (5-substituted 1,2,3,4-thiatriazoles) decompose at room temperature or on slight heating, giving rise to sulfur, nitrogen, and an organic compound, generally in high yields (Scheme I).[1] Alkyl isothiocyanates are reported to be formed when the decomposition of alkylthiatriazoles is carried out at elevated temperature (Scheme I), while at room temperature only alkanenitriles are formed.[2] The thermal decomposition of thiatriazoles has attracted attention because of the synthetic possibilities of this reaction[1] and because of the possible intermediacy of hitherto unknown types of compounds: thioacylnitrenes, RC(=S)N:; thiazirines, RC=NS; and thioacyl azides, R(C=S)N_3.[3]

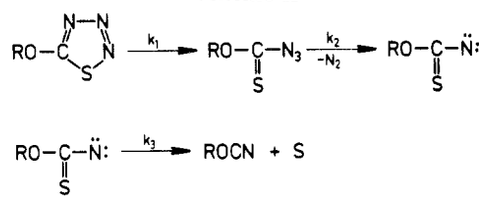
Jensen and Holm investigated the thermal decomposition of 2-^{15}N-labeled 5-isobutoxy-1,2,3,4-thiatriazole and found that the nitrogen lost belonged almost exclusively (98.9%) to the N(2) and N(3) positions.[4] No specific conclusions about the existence of intermediates can be drawn from this result.[4]

Jensen et al. studied the kinetics of the thermal decomposition of 5-alkoxy-1,2,3,4-thiatriazoles in dibutyl phthalate at 15–40 °C by manometric measurement of the nitrogen evolved.[5] The reaction was found to be first order with activation energies for different 5-alkoxythiatriazoles of 101.7–104.2 kJ·mol^{-1} and activation entropies of 13.4–19.7 J·mol^{-1} K^{-1}. It was pointed out that the activation energies for the different alkoxythiatriazoles differ very little, in agreement with the assumption that the reaction proceeds via the same mechanism in all cases (Scheme II). The decomposition of 5-alkoxythiatriazoles was compared with the thermal decomposition of alkylthiatriazoles mentioned[2] (Scheme I), and it was assumed that the formation of isothiocyanates in the latter case may be interpreted to mean that thioacylnitrenes, RC(=S)N:, are intermediates. These were believed to either thiobenzoyl chloride and tetrabutylammonium azide were

Scheme I



Scheme II



lose sulfur and form nitrile or rearrange to isothiocyanate. An analogous byproduct, RO–NCS, was not observed in the thermal degradation of alkoxythiatriazoles.

A scheme was suggested for the decomposition of alkoxythiatriazoles involving opening of the ring with formation of a thioacyl azide as the first step (Scheme II).[5]

Jensen et al. state that if this mechanism is correct, the thioacyl azide must decompose rapidly because the characteristic azide band at approximately 2130 cm^{-1} is not observed in the infrared spectrum of a decomposing thiatriazole.

Pilgram et al.[6] tried to intercept the suggested thioacyl azide intermediate with trivalent phosphorus. With phosphorus triamides, 1:1 reaction products are formed, formulated as 2,2-dihydro-1,3,4,5,2-thiatriazaphosphorines. However, it does not seem possible to decide whether these compounds are formed by attack of the reagent directly on the heterocyclic ring or by attack on a preformed thioacyl azide.

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