Table I
Chymotryptic Reactivities of Some Methyl Esters[a]

Compd	Expts[b]	10^3 S_0,[c] M	10^7 E_0,[c] M	k_c/K_m, M^{-1} sec^{-1}
L-1	13	5.0–28.0	2.11	2060 ± 85
L-2	15	1.0–6.80	2.11	12,572 ± 1400
L-3	14	1.5–15.0	4.05	5265 ± 438
L-5	24	2.0–25.0	2.10	10,095 ± 684

[a] At 25°, pH 7.0 in 15% v/v acetonitrile–water. Ionic strength 0.1 M (NaCl). [b] Number of points in Lineweaver–Burk plot. [c] S_0 and E_0 are initial substrate concentration ranges and enzyme concentrations, respectively.

unreactive in slurries of DL-4 in water or 15% v/v acetonitrile–water and the DL mixture was used for kinetics studies.

Chymotryptic reactivities of L-1, L-2, and L-3 in solution were measured by the specificity constant k_c/K_m. This ratio is the most accurate reflection of α-chymotrypsin specificity for a substrate.[3] It has the units of a second-order rate constant. Values of k_c/K_m, listed in Table I, are compared with that found for N-acetyl-L-tyrosine methyl ester (L-5), a specific α-chymotrypsin substrate. α-Chymotrypsin exhibited no reactivity toward 5×10^{-3} M solutions of the D isomers of 1–3 and DL-4, even at enzyme concentrations of $\sim 10^{-4}$ M.

Because the reactivities of the four esters for which specificity constants were measured fall within a sixfold range, it may be concluded that introduction of a methoxy substituent into the meta position of L-5 has little effect on chymotryptic reactivity. In contrast, the enzyme is sensitive to small changes in substrate structure at the para position, since DL-4 is unreactive but L-3 is nearly as reactive as L-5. The inability of α-chymotrypsin to hydrolyze DL-4 is consistent with the report that the enzyme is inert toward para-substituted N-acyl-L-tyrosines.[4] This low reactivity is thought to be due to unfavorable steric interactions of the substrate aromatic ring with the enzyme.[4] With L-3 these interactions evidently are diminished enough to permit efficient hydrolysis to occur. Of the dopa precursors tested here, however, DL-1 is more suitable than DL-2 or DL-3 for large-scale resolutions, primarily because of its higher solubility in water.

Registry No.—L-1, 51703-90-3; D-1, 51703-91-4; DL-1, 16024-50-3; L-2, 51593-50-1; D-2, 51593-51-2; DL-2, 51593-52-3; L-3, 51593-53-4; D-3, 51593-54-5; DL-3, 51703-92-5; L-5, 2440-79-1; 6,[5] 51593-55-6; 7,[5] 51593-56-7; 10,[5] 51593-57-8; 11,[5] 51593-58-9; 14,[5] 51593-59-0; 15,[5] 30037-41-3; 16,[5] 28104-71-4; α-chymotrypsin, 9004-07-3; 4-benzyloxy-3-methoxybenzaldehyde, 2426-87-1; acetylglycine, 543-24-8; acetic anhydride, 108-24-7; N-acetyl-L-tyrosine, 537-55-3.

Supplementary Material Available. Details of the syntheses, resolutions, and kinetic procedures, including elemental analyses and ir and nmr spectral data, will appear following these pages in the microfilm edition of this volume of the journal. Photocopies of the supplementary material from this paper only or microfiche (105 × 148 mm, 24× reduction, negatives) containing all of the supplementary material for the papers in this issue may be obtained from the Journals Department, American Chemical Society, 1155 16th St., N.W., Washington, D. C. 20036. Remit check or money order for $3.00 for photocopy or $2.00 for microfiche, referring to code number JOC-74-2291.

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Mild Cleavage of a Peptide Bond through the Assistance of the Neighboring Phenylazo Moiety

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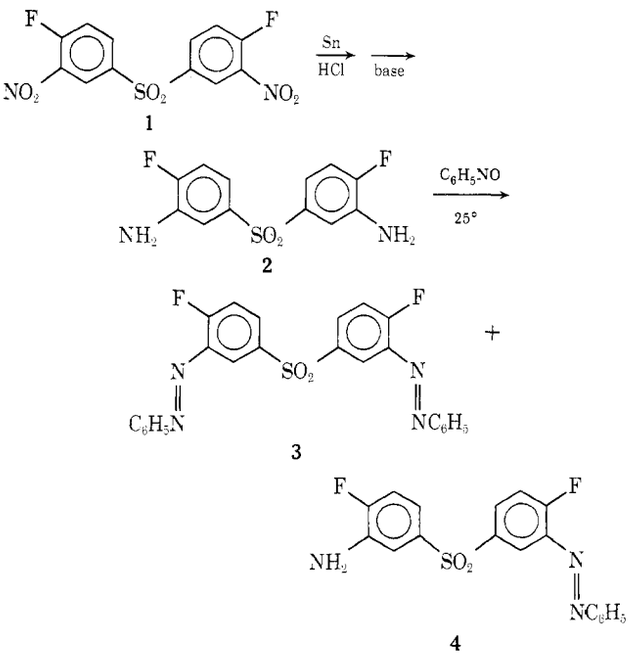
The facile removal of an amino-protecting group through the participation of an adjacent o-phenylazophenoxyacetyl moiety was recently reported from our laboratory.[1] The work involved the cleavage of a neighboring amide bond after reduction of the azo group. The success of this procedure led us to propose that the phenylazo moiety might be useful in another research program, the object of which is the development of a new procedure for the stepwise degradation of peptide chains. We report here the successful use of the phenylazo group as an effective participant in the rupture of the peptide bond of glycylglycine.

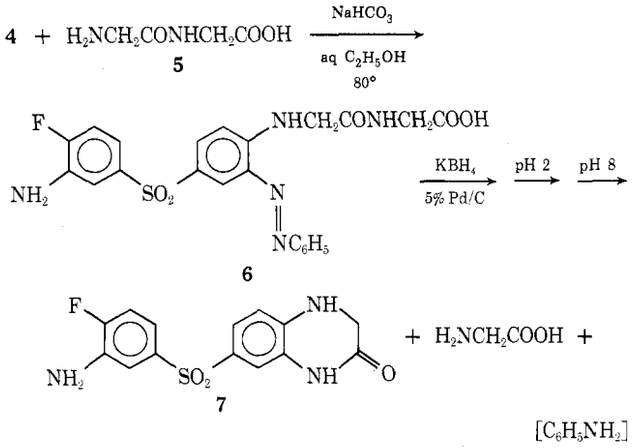
The commercially available[2] protein cross-linking[3] reagent, bis(4-fluoro-3-nitrophenyl) sulfone (1), was used as the starting material. It was easily reduced to the corresponding bis(aminofluorophenyl) sulfone, 2, which was, in turn, condensed with nitrosobenzene to provide two phenylazo products, 3 and 4, in 28 and 36% yields, respectively



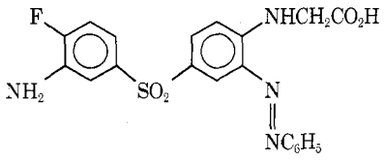
(2.2 mol of nitrosobenzene per mole of 2). Decreasing the relative amount of nitrosobenzene increased the yield of the monophenylazo product, 4, to a high of 40%. However, using a greater excess of nitrosobenzene (up to 3.7 mol per mole of 2) never resulted in a larger yield of the bisphenylazo product, 3.

3-Amino-4,4′-difluoro-3′-phenylazophenyl sulfone (4) was used in the remainder of this work because, first, it was always obtained in greater quantity than was 3 and, second, adequate purification of sufficient amounts of 3 was much more tedious and generally required high-pressure liquid chromatography even after 3 was partially separated from 4.

Intermediate 4 was easily condensed with the dipeptide, glycylglycine (5), in a nucleophilic aromatic substitution which afforded 4-(4′-fluoro-3′-aminophenylsulfonyl)-2-phenylazo-N-phenylglycylglycine (6). Reduction of the azo group in 6 with potassium borohydride at room tempera-



ture followed by acidification and neutralization resulted in the cleavage of the peptide linkage and the formation of 7-(4′-fluoro-3′-aminophenylsulfonyl)-3,4-dihydro-2(1*H*)-quinoxalone (**7**), glycine, and aniline. The glycine obtained from this reaction mixture was also treated with reagent **4** to produce 4-(4′-fluoro-3′-aminophenylsulfonyl)-2-phenylazo-*N*-phenylglycine (**8**). Reduction of this intermediate in a similar manner also led to the formation of **7**.



The reduction of the azo moiety in **6** with potassium borohydride and palladium on carbon probably proceeds in two stages: first to hydrazo group, and then, with scission, to the amine. Cyclization to the quinoxalone **7** could conceivably occur after either of these stages; however, earlier work with this neighboring group[1] and with the hydroxylamino moiety[4,5] has provided some evidence that nucleophilic cyclizations of this type occur most rapidly under acidic conditions. The ring-forming reaction, therefore, probably proceeds after the completion of the reduction and cleavage of the azo group, since any excess reducing agent would be destroyed at a pH of 2.0.

The present work demonstrates that the neighboring phenylazo moiety was an effective participant in the mild cleavage of a peptide bond.

Experimental Section

The thin layer chromatograms (tlc) were run on microscope slides (75 × 25 mm) which were coated with a 0.25-mm layer of silica gel (J. T. Baker, 7GF) and activated at 110° for 1 hr. Spotting was performed using 0.5–1.0 μl of a 1% solution. The zones were detected as yellow areas on a purple background after spraying with a 0.5% $KMnO_4$ solution sometimes followed with heating, or by irradiation with ultraviolet light (254 mμ). Melting points were determined in capillary tubes in a stirred oil bath and are corrected. Infrared spectra were run on a Perkin-Elmer Infracord Model 137 or a Perkin-Elmer spectrophotometer Model 521. Microanalyses were carried out by Alfred Bernhardt Mikroanalytisches Laboratorium, 5251 Elbach über Engelskirchen, West Germany.

Bis(3-amino-4-fluorophenyl) Sulfone Dihydrochloride (2 2HCl). Bis(4-fluoro-3-nitrophenyl) sulfone (**1**, 1.032 g, 3.0 mmol), 3.5 g (0.029 g-atom) of mossy tin, and 23 ml of concentrated hydrochloric acid were stirred together for 1.5 hr. The reaction mixture was made alkaline (pH 10.0) with concentrated aqueous NaOH and the resulting mixture was extracted with CH_2Cl_2. The organic layer was separated and the solvent was removed under reduced pressure. The residue (**2**) was dissolved in MeOH and the solution was treated with HCl gas until it was saturated. Addition of ether to the methanolic solution caused the separation of 988 mg (92.2%) of a white solid (dihydrochloride of **2**): mp 178.5–180.0°; ir (Nujol) 3500 (NH), 1325, 1145 cm^{-1} (SO_2).

Anal. Calcd for $C_{12}H_{12}Cl_2F_2N_2O_2S$ (dihydrochloride of **2**): C, 40.40; H, 3.38; Cl, 19.90; F, 10.62; N, 7.85; S, 8.96. Found: C, 40.05; H, 3.19; Cl, 19.58; F, 10.45; N, 7.60; S, 8.97.

Bis(4-fluoro-3-phenylazophenyl) Sulfone (3) and 3-Amino-4-4′-difluoro-3′-phenylazophenyl Sulfone (4). A mixture of 1.686 g (4.72 mmol) of the dihydrochloride of **2** in equal amounts of water and CH_2Cl_2 was adjusted to pH 9.5. The organic layer was concentrated under reduced pressure to afford the solid free base form of **2** (1.189 g, 4.18 mmol). This was combined with 963 mg (8.99 mmol) of nitrosobenzene and 15 ml of glacial HOAc and the resultant mixture was stirred at room temperature for 7 days. The reaction solution was concentrated to a small volume by distillation under reduced pressure and the thick residue was placed on the top of an open column of silicic acid (100 mesh). Chromatography into several colored bands was effected using benzene solvent, but only the two orange bands were collected and used.

The first orange fraction was concentrated to a solid (532 mg) of the same color which showed two zones on tlc plates (R_f 0.6 and 0.4, 99:1 C_6H_6–HOAc). High-pressure liquid chromatography of this mixture provided an analytically pure sample of **3** (Waters Associates Model ALC-100, 0.375 in. × 6 ft Porasil column, 50:50 C_6H_6–CCl_4): tlc R_f 0.4 (99:1 C_6H_6–HOAc); ir (Nujol) 1580 (N=N), 1325, and 1145 cm^{-1} (SO_2); mp 170.0–172.0°.

Anal. Calcd for $C_{24}H_{16}F_2N_4O_2S$ (**3**): C, 62.35; H, 3.46; N, 12.11. Found: C, 62.42; H, 3.28; N, 11.62, 11.73.

The second orange band afforded an orange solid which weighed 567 mg (**4**): tlc R_f 0.3 and 0.1 (99:1 C_6H_6–HOAc); mp 184.0–189.0°; ir (Nujol) 3560, 3450 (NH_2) 1575 (N=N), 1310, and 1135 cm^{-1} (SO_2). The faster tlc zone represented a relatively small amount of an impurity (probably **3**) which could not be easily removed, but did not interfere in further chemical transformations of this product (see below).

4-(4′-Fluoro-3′-aminophenylsulfonyl)-2-phenylazo-*N*-phenylglycylglycine (6). A mixture of 373 mg (1.0 mmol) of **4** (as prepared above), 264 mg (2.0 mmol) of glycylglycine, 252 mg of $NaHCO_3$, 6 ml of H_2O, and 24 ml of absolute EtOH was heated to the reflux temperature for 13 hr. The EtOH was removed by distillation at reduced pressure and the aqueous residue was extracted with CH_2Cl_2 at pH 2.0. The organic layer was dried and then the solvent was removed at reduced pressure. The solid residue (140 mg) was recrystallized from aqueous EtOH to afford 100 mg (21%) of pure **6**: mp 173.5–174.3°; ir (Nujol) 3500, 3390 (NH), 1730 (carboxyl carbonyl), 1650 (amide carbonyl), 1550 (N=N), 1300, and 1150 cm^{-1} (SO_2).

Anal. Calcd for $C_{22}H_{20}FN_5O_5S$ (**6**): C, 54.45; H, 4.12; F, 3.92; N, 14.42; S, 6.46. Found: C, 54.35; H, 4.30; F, 3.88; N, 14.24; S, 6.60.

Reduction of 6 and Subsequent Cleavage of the Peptide Bond in the Glycylglycine Side Chain. Preparation of 7-(4′-Fluoro-3′-aminophenylsulfonyl)-3,4-dihydro-2(1*H*)-quinoxalone (7). A mixture of 244 mg (0.5 mmol) of **6**, 24 mg of 5% Pd/C, 108 mg (2.0 mmol) of KBH_4, and 40 ml of water was stirred at room temperature for 1 hr. Tlc of the reaction mixture showed a zone for **6** which indicated that reduction was incomplete. More reducing reagents were added until a total of 8.0 mml of KBH_4 and 48 mg of 5% Pd/C were in the mixture. After an additional 2 hr of stirring, the aqueous mixture was filtered through Celite and the filtrate was adjusted to pH 2.0. It was stored for 3 hr, the pH was changed to 8.5, and the resultant mixture was stored for 16 hr. A tlc analysis (solvent 90% ether, 10% MeOH, and 2 drops of HOAc) at this point showed a multicomponent mixture, the fastest zone of which had the same R_f as that of aniline. A precipitate was separated from the weakly basic mixture and was collected by filtration. The filtrate was extracted with CH_2Cl_2 in order to isolate a second fraction of a crystalline product. Both fractions were combined [67 mg (42%) of **7**] and recrystallized from MeOH and then from MeOH–C_6H_6: darkens at 200°, decomposes at 210°; ir (Nujol) 3500, 3440 (NH and NH_2), 1685 (lactam carbonyl), 1310, and 1140 cm^{-1} (SO_2). The ir and tlc of this product were identical with those of the product obtained from the reductive cyclization of 4-(4′-fluoro-3′-aminophenylsulfonyl)-2-phenylazo-*N*-phenylglycine (**8**, see below) and for which satisfactory elemental analysis was obtained.

The aqueous filtrate that remained after the isolation of **7** above was concentrated under reduced pressure almost to dryness. To the residue was added 187 mg (0.5 mmol) of **4**, 84 mg (1.0 mmol) of $NaHCO_3$, 3 ml of H_2O, and 10 ml of EtOH. The mixture was heated to the reflux temperature for 14 hr. The sol-

vents were removed by distillation under reduced pressure and the residue was distributed between water and CH_2Cl_2. The pH was adjusted to 2.0 and several extractions were made with CH_2Cl_2. The organic layer was dried and the solvent was removed *in vacuo*. The residual solid weighed 30 mg and its tlc zone had the same R_f as that of **8**, the preparation of which is described below. The overall yield of **8** from **6** was 14%.

4-(4′-Fluoro-3′-aminophenylsulfonyl)-2-phenylazo-*N*-phenylglycine (8). A mixture of 373 mg (1.0 mmol) of **4**, 168 mg (2.0 mmol) of $NaHCO_3$, 75 mg (1.0 mmol) of glycine, 18 ml of MeOH, and 5 ml of H_2O was heated to the reflux temperature for 10 hr. The solvents were removed by distillation under reduced pressure and the residue was mixed with water and extracted with CH_2Cl_2 at pH 2.0. The organic layer was dried and concentrated *in vacuo* to 170 mg (40%) of **8**. Recrystallization from aqueous EtOH afforded an analytically pure sample: mp 169.0–170.0°; ir (Nujol) 3590, 3470 (NH and NH_2), 1740 (carboxyl carbonyl), 1600 (N=N), 1310, and 1130 cm^{-1} (SO_2).

Anal. Calcd for $C_{20}H_{17}FN_4O_4S$ (**8**): C 56.10; H, 3.97; F, 4.44; N, 13.05; S, 7.47. Found: C, 56.25; H, 4.11; F, 4.31; N, 12.94; S, 7.32.

Reductive Cyclization of 8. Preparation of 7-(4′-Fluoro-3′-aminophenylsulfonyl)-3,4-dihydro-2(1*H*)-quinoxalone (7). A mixture of 227 mg (0.53 mmol) of **8**, 500 mg (9.27 mmol) of KBH_4, 60 mg of 5% Pd/C, and 125 ml of H_2O was stirred at room temperature for 3 hr. More KBH_4 (500 mg) and 5% Pd/C (40 mg) were added to the reaction mixture and stirring was continued for another 1 hr. The catalyst was removed by filtration through a layer of Celite and the pH of the filtrate was adjusted to 2.0. A precipitate separated during a 1-hr storage period. The pH was changed to 8.9 and the mixture was filtered. The solid was recrystallized from C_6H_6-MeOH to afford 120 mg (71%) of **7** which decomposes beginning at 170°. The ir and tlc of this product were identical with those determined on the product from the reduction and subsequent reactions of **6** (see above).

Anal. Calcd for $3C_{14}H_{12}FN_3O_3S \cdot 2C_6H_6$ (benzene solvate of **7**): C, 57.90; H, 4.32; F, 5.09; N, 11.25; S, 8.59. Found: C, 57.76; H, 4.17; F, 4.86; N, 11.36; S, 8.44.

Acknowledgment. Support of this research by the National Science Foundation (Grant GP-33582) is gratefully acknowledged.

Registry No.—**1**, 312-30-1; **2** dihydrochloride, 51472-56-1; **3**, 51472-57-2; **4**, 51472-58-3; **5**, 556-50-3; **6**, 51472-59-4; **7**, 51472-60-7; **8**, 51472-61-8.

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Nucleophilic Cleavage of the 1,2,5-Thia- and -Selenadiazole Rings[1]

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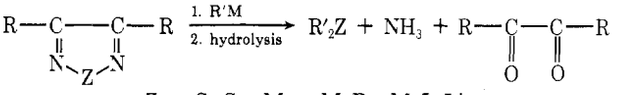
Some years ago we showed that 1,2,5-thiadiazole and its monosubstituted derivatives, but not 1,2,5-selenadiazole,[2] undergo deprotonation with incorporation of deuterium[3,4] when treated by deuterioxide in heavy water solution. As a matter of fact, both 1,2,5-thia- and -selenadiazole rings unsubstituted, or alkyl and aryl mono- and disubstituted, are rather stable when treated with aqueous or alcoholic hydroxide,[2,3,5] but reaction occurs with Grignard reagents and lithium alkyls.[1,4,6] The present work is concerned with the reaction of some alkyl and aryl disubstituted 1,2,5-thia- and -selenadiazoles towards such strong nucleophiles.

Both 1,2,5-thiadiazoles and 1,2,5-selenadiazoles react with Grignard reagents or lithium alkyls at temperatures as low as −70° to yield, after hydrolysis, a thio- or selenoether, ammonia, and 1,2-dicarbonyl compounds (Scheme I). The reaction often yields also small amounts of nitrogen-containing heterocycles such as imidazoles or pyrazines (Table I).

Scheme I



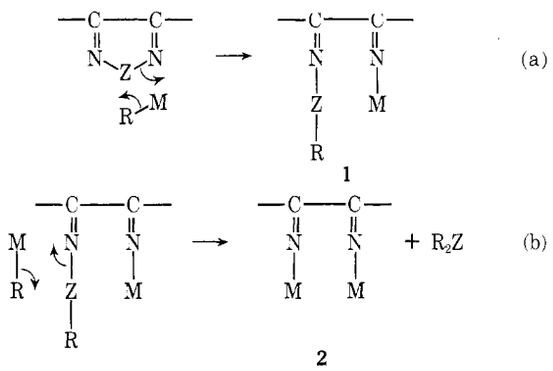
Z = S, Se; M = MgBr, MgI, Li

The reaction of 3,4-diphenyl-1,2,5-thiadiazole and *n*-butylmagnesium bromide give rise to thioalcohol and olefin in place of the thioether. The products derived from the heterocyclic substrate were unchanged.

The formation of thio- or selenoether or thiol suggests the sulfur or selenium atoms as the centers of the nucleophilic attack. Examples of nucleophilic attack at the sulfur atom of an S–N bond are known; the nearest analogy to this study may be found in the reaction between isothiazoles and *n*-butyllithium,[7,8] or between a Grignard reagent and sulfur nitride S_4N_4,[9] where the NSN angle and N–S bond distances[10] are similar to those of the 1,2,5-thiadiazole ring.[3]

We suggest that the first step in the process is insertion of the Grignard reagent or lithium alkyl (R–M) on a S–N or Se–N bond, followed by cleavage of the ring and formation of the intermediate **1** (Scheme IIa). Reaction of **1**

Scheme II



Z = S, Se; M = MgBr, MgI, Li

with a second molecule of organometallic reagent forms thio- or selenoether and the final product **2**, which contains the same —N=C—C=N— system as the starting diazole (Scheme IIb). Production of thiol and olefin with *n*-butyl Grignard reagent is consistent with reduction of intermediate **1** rather than nucleophilic substitution. This reaction course does not affect the formation of **2**, but it requires a further molecule of the organometallic compound to transform the thiol into its magnesium salt. Hydrolysis of the diimines **2** finally leads to the dicarbonyl compound and ammonia.

Formation of 2,3,5,6-tetraphenylpyrazine from 3,4-diphenyl-1,2,5-thia- and -selenadiazole, which involves a redox reaction, may take place during hydrolysis. It is significant that the same compound is also formed by reaction between benzil and ammonia.[11] 2,4,5-Triphenylimidazole (**5**) and the easily hydrolyzed 1-benzoyl-2,4,5-triphenylimidazole (**4**) can arise from the 2-isoimidazole (**3**) during the hydrolysis reaction[12] (Scheme III).