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Use of Anhydrous Hydrogen Fluoride in Peptide Synthesis. II. Procedures for the Syntheses of Simple Peptides

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Detailed conditions for handling simple peptide-derivatives with anhydrous hydrogen fluoride (HF) were presented. Since HF has a strong affinity for peptides, the removal of excess HF from the reaction products is rather difficult, but is essential to obtain the product as good crystals. Syntheses of the following peptides were carried out successfully as examples of the HF procedure: tosyl-L-phenylalanyl-L-phenylalanine, L-leucylglycine, L-arginylglycine, carbobenzoxy-L-prolyl-L-leucylglycine ethyl ester, L-aspartyl-L-phenylalanine amide, and BOC-β-alanyl-L-tryptophyl-L-methionyl-L-aspartyl-L-phenylalanine amide.

The possibility of removing the S-benzyl group by acidolysis was the first indication of the usefulness of anhydrous hydrogen fluoride (HF) for peptide synthesis;[1] this was confirmed by the formation of oxytocin from the fully-protected nonapeptide. The general properties of HF as a reagent for the removal of various protective groups have been discussed in our preceding papers.[2,3] In this study, detailed conditions for the synthesis of simple peptides were investigated to demonstrate how the HF-procedure can be applied in general peptide synthesis. The HF reaction was carried out in the apparatuses described in the preceding papers.[2,3] Although completely dry HF is desirable for accelerating the cleaving reaction, a further drying procedure was unnecessary for the synthesis of simple peptides when HF of more than 99% purity was used under mild conditions. Generally, the HF molecule has a strong affinity for peptides through the hydrogen bonds, and it takes a very long time to remove the excess HF from peptides, even *in vacuo*. The results shown in Fig. 1 demonstrate that 0.19, 1.57, and 1.81 equivalents of HF were still attached to acetyl-L-leucine, L-leucylglycine and L-leucylglycylglycine respectively, even when their HF-solution had been kept under a vacuum (2—3 mmHg) for 20 hr at room temperature. These results indicate that about 1.4 equivalents of HF are bound to an amino group and that about 0.2 equivalent of HF is attached to one peptide-bond under these conditions. Therefore, the difficulty in removing HF from peptides increases with the number of the peptide-bonds. Nevertheless, the complete removal

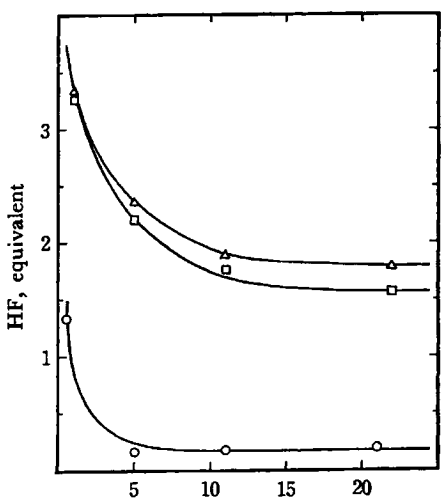

Fig. 1. Amount of absorbed HF in acetyl-amino acid or free peptides. Each material (0.1 g) was dissolved in HF (1 ml), and excess HF was removed using a plastic water pump (20—30 mmHg) for 30 min. Then, the residue was kept *in vacuo* (2—3 mmHg) at room temperature. Remaining HF was determined by titration with 0.1 N sodium hydroxide.
○ Acetyl-L-leucine
□ L-Leucylglycine
△ L-Leucylglycylglycine

of the bound HF in some way is essential if we are to obtain the product as good crystals.

First, we attempted to remove the isopropyl ester group from the tosyl-L-phenylalanyl-L-phenylalanine isopropyl ester. The reaction was carried out at 20°C for one hour in the presence of an equimolar amount of anisole. The excess HF was then removed by distillation *in vacuo*. In this case, the product is water-insoluble, and there is no free amino group in the molecule. Therefore,

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1) S. Sakakibara and T. Shimonishi, This Bulletin, **38**, 1412 (1965).
2) The 8th European Peptide Symposium, Sept. 18—23, Holland (1966).
3) S. Sakakibara, Y. Shimonishi, Y. Kishida, M. Okada and H. Sugihara, This Bulletin, **40**, 2164 (1967).

the small amount of HF remaining was easily removed by washing the product with water. When carbobenzoxy-L-leucylglycine was treated with HF, the product was absorbed once on a Dowex-50 column (H^+ form), and the excess HF was washed out thoroughly with water. Then the desired free peptide was recovered quantitatively by elution with ammonia. In another example, the bound HF was removed by passing the aqueous solution through a column of IR-45 (acetate form); the product, free L-leucylglycine, was thus obtained in about a 70% yield. This lower yield may be attributed to the partial absorption of the small peptide on the resin.

N^{α}-Carbobenzoxy-N^{G}-nitro-L-arginylglycine was treated with HF at 0°C for 30 min in the presence of two equivalents of anisole. Since the product is strongly basic, it was treated with IRA-400 (OH^- form) and then crystallized as acetate in a satisfactory yield. This procedure should be useful for the general synthesis of arginine-peptides.

In the case of the carbobenzoxypeptide ethyl ester, only the carbobenzoxy group is susceptible to HF; the ethyl ester group should stay intact during the reaction.[2,3] Thus, the carbobenzoxy-L-leucylglycine ethyl ester was treated with HF, and the product was directly combined with the carbobenzoxy-L-proline *p*-nitrophenyl ester to transform the product into a crystallizable compound. The excess HF was neutralized with triethylamine for the coupling reaction; carbobenzoxy-L-prolyl-L-leucylglycine ethyl ester was thus obtained in more than a 90% yield.

Next, carbobenzoxy-β-isopropyl-L-aspartyl-L-phenylalanine amide was treated with HF in the presence of three equivalents of anisole, after which the product was treated with IR-45 (acetate form) to eliminate the remaining HF. During the treatment, this material was also found to be absorbed onto the resin; the yield of L-aspartyl-L-phenylalanine amide was 67%. Paper electrophoresis of the reaction mixture showed no evidence of the formation of β-aspartyl peptide during the treatment, and the analytical data on the final product were identical with those reported by Davey *et al.*[4] Generally, aspartyl peptides are sensitive to acid; Dowex-50 treatment to remove the HF, followed by elution with ammonia, resulted in the partial decomposition of the product.

Finally, *t*-amyloxycarbonyl-L-tryptophyl-L-methionyl-L-aspartyl-L-phenylalanine amide was treated with HF. The product was then coupled with the *t*-butoxycarbonyl-β-alanine *p*-nitrophenyl ester to convert the product into *t*-butoxycarbonyl-β-alanyl-L-tryptophyl-L-methionyl-L-aspartyl-L-phenylalanine amide, which has been reported to be a biologically-active and crystallizable compound.[4] The yield and purity of the reaction product were satisfactory, as will be shown in the Experimental section; these results also indicate that aromatic amino acid and methionine residues suffered no serious side-reactions with HF during the procedure.

The application of the HF procedure to more complicated peptide derivatives is now in progress in our laboratory.

Experimental

HF-Reactions. Anhydrous hydrogen fluoride with a purity of 99.5% was obtained from the Matheson Chemical Co., U. S. A. It was used for the reactions without further drying. The HF reactions were carried out in the apparatus shown in Figs. 1 and 2 of the preceding paper,[3] and the reported procedure[3] was generally followed in handling HF. Although the minimum amount of HF which is required for completion of the reaction has not yet been determined, 5 to 10 *ml* of HF were sufficient for one gram of peptide. When a protective group which is slow to be removed is applied to the HF reaction, the use of more HF is recommended. After each reaction, the excess HF was removed using a plastic water-pump at 0°C and then the residue was kept under a vacuum (2—3 mmHg) for more than 5 hr, as has been described before.[3] Stirring was continued as long as possible during the evacuation procedure. Then, the residue was extracted with water, and some water-insoluble material was removed by extraction with chloroform or by passing the mixture through Hyflo Super-cel before the succeeding treatment. Used reaction cylinders were washed successively with water, methanol, and acetone, and then further cleaned with a mixture of conc. hydrochloric acid and conc. nitric acid (3 : 1 v/v). The outer diameters of the reaction vessels, which are made of Daiflon, gradually increase during repeated dryings at temperatures above 50°C. Therefore, they should be dried in a desiccator at room temperature after washing.

***p*-Toluenesulfonyl-L-phenylalanyl-L-phenylalanine Isopropyl Ester.** A solution of tosyl-L-phenylalanyl chloride[5] (4.63 g, 0.0137 mol) in tetrahydrofuran (30 *ml*) was slowly added to a suspension of L-phenylalanine isopropyl ester tosylate[3] (5.20 g, 0.0137 mol) and triethylamine (2 *ml*, 0.0143 mol) in tetrahydrofuran (40 *ml*) at −10°C. One more equivalent of triethylamine was then slowly stirred into the reaction mixture at the same temperature. After 2 hrs' stirring, the precipitate which had formed was filtered off, and then the filtrate was concentrated to a syrup, which was dissolved in ethyl acetate. The solution was washed with N hydrochloric acid, 5% sodium bicarbonate, and water, and dried over magnesium sulfate. The concentration of the dried solution gave crystals which were recrystallized from ethyl acetate and petroleum ether; yield, 5.20 g (75%); mp 125.5—126.5°C; $[\alpha]_D^{25}$ +15.7° (*c* 2, dimethylformamide). Found: C, 65.81; H, 6.25; N, 5.41%. Calcd for $C_{28}H_{32}O_5N_2S$: C, 66.13; H, 6.34; N, 5.51%.

***p*-Toluenesulfonyl-L-phenylalanyl-L-phenylalanine.** The tosyl-L-phenylalanyl-L-phenylalanine isopropyl ester (1.13 g, 2.2 mmol) and anisole (0.24 g, 2.2 mmol) were placed in a reaction cylinder together

4) J. M. Davey, A. H. Laird and J. S. Morley, *J. Chem. Soc.* (C), **1966**, 555.

5) E. A. Popenoe and V. du Vigneand, *J. Am. Chem. Soc.*, **76**, 6202 (1954).

with a Teflon-coated stirring bar. HF (10 ml) was added to it by distillation, and then the mixture was allowed to react at 20°C for one hr while being stirred. The excess HF was removed by distillation with a plastic water pump, and then the residue was kept under a vacuum for about 5 hr at room temperature. The residue was dissolved in ethyl acetate, and the desired product was extracted with a 5% sodium bicarbonate aqueous solution. The bicarbonate solution was acidified to pH 2 with 6 N hydrochloric acid, and the oily material which appeared was taken up in ethyl acetate. The ethyl acetate extract was dried over sodium sulfate, and then concentrated to a syrup which was crystallized by adding petroleum ether; yield, 0.88 g (85%); mp 197°C; $[\alpha]_D^{21}$ +22.8° (*c* 2, dimethylformamide). Found: C, 64.57; H, 5.84; N, 5.84%. Calcd for $C_{25}H_{26}O_5N_2S$: C, 64.37; H, 5.62; N, 6.01%.

L-Leucylglycine. Carbobenzoxy-L-leucylglycine[6] (1.0 g, 3.1 mmol) and anisole (0.33 ml, 3.1 mmol) were placed in a reaction cylinder[8] together with a Teflon-coated stirring bar. HF (about 10 ml) was added, and the mixture was stirred for 30 min at 0°C. The excess HF was removed under reduced pressure, and the residue was dried thoroughly for 10 hr *in vacuo* (3 mmHg), as has been described before. The dried residue was then dissolved in water, some insoluble material was removed with Hyflo Super-cel, and the solution was passed through a column of IR-45 (acetate form; about 7 ml of the resin were required). After washing the column with 5% acetic acid, the combined effluent was concentrated under reduced pressure, and the residue was dissolved in a minimum amount of water and crystallized by adding ethanol and ether. The crystals were dried *in vacuo* at 80°C for 24 hr; yield, 0.42 g (72%); $[\alpha]_D^{29}$ +85.0° (*c* 2, water). Value reported in the literature[6]: $[\alpha]_D$ +85.8° (*c* 2, water).

The dried reaction product of another run on the same scale was dissolved in water, and the solution was passed through a column of Dowex 50, X-8 (100—200 mesh; H^+ form; about 4 ml of the resin were used). The column was washed thoroughly with water until the washings became neutral, and then the product was eluted with 6 N ammonia. The concentration of the eluate under reduced pressure gave crystals which were recrystallized as has been described before. Yield of the dried crystals, 0.53 g (91%); $[\alpha]_D^{27}$ +85.6° (*c* 2, water).

L-Arginylglycine Acetate, Acetic Acid Solvate. N^{α}-Carbobenzoxy-N^{G}-nitro-L-arginylglycine[7] (1.5 g, 3.7 mmol) and anisole (0.8 ml, 7.4 mmol) were placed in an HF-reaction cylinder,[8] together with a Teflon-coated stirring bar, and HF (10 ml) was added to the mixture. The HF reaction was carried out at 0°C for 30 min with stirring, and then the excess HF was removed by distillation. The residue was dissolved in water, an insoluble material was extracted with chloroform, and the water solution was passed through a column of IRA-400 (OH^- form; 15 ml of resin were used). After adding 0.5 ml of acetic acid, the effluent was concentrated to a residue which was crystallized from glacial acetic acid and ethyl acetate. The crystals (mono-acetate, acetic acid solvate) were dried over phosphrous pentoxide at 80°C for 20 hr; yield, 1.14 g (89%); mp 167—170°C; $[\alpha]_D^{28}$ +38.3° (*c* 1, water). Value reported in the literature: mp 167—169°C;[7] $[\alpha]_D^{28}$ +38.9° (*c* 5.75, water);[7] $[\alpha]_D^{24-25}$ +37.6° (*c* 1, water).[8]

Found: C, 41.01; H, 7.28; N, 19.69%. Calcd for $C_{12}H_{15}O_7N_5$: C, 41.01; H, 7.19; N, 19.93%.

Carbobenzoxy-L-prolyl-L-leucylglycine Ethyl Ester. The carbobenzoxy-L-leucylglycine ethyl ester[6] (1.75 g, 5.0 mmol) and anisole (0.5 ml) were treated with HF (10 ml) as has been described previously. The excess HF was removed by distillation *in vacuo*. The dried product was dissolved in chloroform (30 ml), and the carbobenzoxy-L-proline *p*-nitrophenyl ester (1.85 g, 5.0 mmol) was added to the solution. The mixture was then neutralized with triethylamine (1 ml, 7 mmol) and with the *N*, *N*-diethylglycine ethyl ester[9] (2 ml), and allowed to react for 2 days at room temperature. The solution was then washed with N ammonia to remove the *p*-nitrophenol, and dried over magnesium sulfate. The concentration of the dried solution left crystals which were recrystallized from ethanol-water; yield, 2.1 g (94%); mp 150—151.5°C; $[\alpha]_D^{26.5}$ −80° (*c* 2.5, ethanol). Ressler and du Vigneaud reported a mp of 148—149°C and $[\alpha]_D$ −79.8° (*c* 2, ethanol).[10]

Bis-(Carbobenzoxy-L-aspartic Acid β-Isopropyl Ester) Piperazine Salt. β-Isopropyl-L-aspartate (29 g, 0.165 mol)[3] was treated with carbobenzoxy chloride (30 g, 0.17 mol) at room temperature by the Schotten-Baumann reaction using sodium bicarbonate (34 g, 0.4 mol). The reaction mixture was washed 3 times with ether, and then acidified to pH[2] with 6 N hydrochloric acid. The oil which separated was extracted with ether, and the ether extract was dried over sodium sulfate. Then the dried solution was concentrated to a syrup under reduced pressure. The residue was dissolved in methanol, and piperazine hexahydrate (16.1 g, 0.083 mol) was added to the solution. The mixture was concentrated to a residue under reduced pressure; this residue was flushed twice with toluene, and then recrystallized from 300 ml of methanol; yield 41.2 g (71%); mp 149—151°C: $[\alpha]_D^{26}$ −3.6° (*c* 2, dimethylformamide).

Found: C, 57.93; H, 6.89; N, 7.92%. Calcd for $C_{34}H_{48}O_{12}N_4$: C, 57.94; H, 6.86; N, 7.95%.

Carbobenzoxy-β-isopropyl-L-aspartyl-L-phenylalanine Amide. The carbobenzoxy-L-aspartic acid β-isopropyl ester (0.05 mol), which had been prepared from the piperazine salt (17.6 g) by shaking it with excess 2 N hydrochloric acid, and L-phenylalanine amide hydrochloride (10.0 g, 0.05 mol) were dissolved in dimethylformamide (50 ml), after which triethylamine (7 ml, 0.05 mol) was added to the mixture. Dicyclohexylcarbodiimide (10.3 g, 0.05 mol) was then stirred into the mixture at −10°C. These reagents were allowed to react for 30 min at the same temperature, and then for an additional 5 hr at room temperature. The urea formed was filtered off, and water (250 ml) was added to the filtrate to precipitate the product as crystals, which were collected by filtration and then recrystallized from hot ethanol (300 ml) and water

6) M. Bergmann, L. Zervas and J. Fruton, *J. Biol. Chem.*, **111**, 225 (1935).
7) K. Hofmann, W. D. Peckham and A. Rheiner, *J. Am. Chem. Soc.*, **78**, 238 (1956).
8) L. Zervas, T. T. Otani, M. Winitz and J. P. Greenstein, *ibid.*, **81**, 2878 (1959).
9) S. Sakakibara and M. Itoh, This Bulletin, **40**, 656 (1967).
10) C. Ressler and V. du Vigneaud, *J. Am. Chem. Soc.*, **76**, 3107 (1954).

(200 ml); yield, 17 g (74.6%); mp 171—174°C; $[\alpha]_D^{25}$ −27.5° (*c* 2.9, dimethylformamide).

Found: C, 63.11; H, 6.42; N, 9.22%. Calcd for $C_{24}H_{29}O_6N_3$: C, 63.28; H, 6.42; N, 9.23%.

L-Aspartyl-L-phenylalanine Amide. Carbobenzoxy-β-isopropyl-L-aspartyl-L-phenylalanine amide (1 g, 2.2 mmol) and anisole (0.71 ml, 6.6 mmol) were treated with HF (20 ml) at 0°C for 100 min. The excess HF was removed under reduced pressure, and the residue was dried thoroughly under a vacuum for more than 5 hr. The dried product was dissolved in water, and the solution was passed through a column of IR-45 (acetate form). The effluent was lyophilized, and the residue was crystallized from acetone and water and then dried over phosphorus pentoxide at room temperature; yield, 430 mg (66.6%); mp 189—190°C (decomp.); $[\alpha]_D^{23}$ +20.5° (*c* 1, dimethylformamide containing 1 eq. of N HCl).*2 Davey *et al.* reported mp 188—189°C (decomp.) and $[\alpha]_D$ +20.6° (*c* 1, dimethylformamide containing 1 eq. of N HCl).[4]

Found: C, 52.06; H, 6.62; N, 13.81%. Calcd for $C_{13}H_{17}O_4N_3 \cdot H_2O$: C, 52.51; H, 6.44; N, 14.13%.

***t*-Amyloxycarbonyl-L-tryptophyl-L-methionine Hydrazide.** Dicyclohexylcarbodiimide (2.1 g, 0.01 mol) was added to a solution of *t*-amyloxycarbonyl-L-tryptophan[11] (3.18 g, 0.01 mol) in tetrahydrofuran (60 ml) at −10°C. After about 10 min, a solution of L-methionine methyl ester hydrochloride (2.2 g, 0.011 mol) and triethylamine (1.54 ml, 0.011 mol) in chloroform (30 ml) was added to the reaction mixture. The whole mixture was kept 1 hr at 0°C, and then overnight at room temperature. The solvent was removed under reduced pressure, and the residue was dissolved in ethyl acetate (100 ml). Some insoluble materials were filtered off, and the filtrate was washed with 0.5 N hydrochloric acid, 5% sodium bicarbonate, and water, and dried over anhydrous sodium sulfate. The dried solution was concentrated to a syrup, which was then dissolved in ethanol (50 ml), and 90% hydrazine hydrate (5.56 g, 0.1 mol) was added to the solution. After 2 days at room temperature, the mixture was concentrated to a residue; this residue was flushed 3 times with toluene and then crystallized from ethanol by the addition of water. Yield of crude material, 3.50 g. This material was recrystallized from ethanol-water, and dried over phosphorus pentoxide *in vacuo* at 80°C for 10 hr; yield, 2.92 g (63.1%); mp 158—163°C; $[\alpha]_D^{24}$ −22.4° (*c* 1.08, dimethylformamide).

Found: C, 56.68; H, 7.30; N, 14.94%. Calcd for $C_{22}H_{33}O_4N_5S$: C, 57.00; H, 7.18; N, 15.11%.

***t*-Amyloxycarbonyl-L-tryptophyl-L-methionyl-L-aspartyl-L-phenylalanine Amide.** To a solution of *t*-amyloxycarbonyl-L-tryptophyl-L-methionine hydrazide (13.9 g, 0.03 mol) in dimethylformamide (30 ml), a 2.0 N hydrogen chloride-dioxane solution (30 ml, 0.06 mol) was added at −10°C, and then isoamyl nitrite (3.68 g, 0.0315 mol) was added slowly to the mixture at −20°C. After 30 min, a cold mixture of L-aspartyl-L-phenylalanine amide monohydrate (9.80 g, 0.033 mol) and triethylamine (15.6 ml, 0.11 mol) in dimethylformamide (75 ml) was added to the previous mixture, and the mixture was stirred at 0—3°C for 3 days. Then, cold water (300 ml) was added, and the pH of the mixture was adjusted to 2—3 with N hydrochlorci acid in order to precipitate the product. The precipitate was collected by filtration, and was washed with water and then ether. The crude product (19.4 g) obtained was recrystallized from ethanol; yield, 16.2 g (76.0%); mp 229—230°C (decomp.); $[\alpha]_D^{14}$ −31.3° (*c* 1, dimethylformamide).

Found: C, 59.04; H, 6.53; N, 11.67%. Calcd for $C_{35}H_{46}O_8N_6S$: C, 59.14; H, 6.52; N, 11.82%.

***t*-Butoxycarbonyl-β-alanine *p*-Nitrophenyl Ester.** Dicyclohexylcarbodiimide (2.50 g, 0.012 mol) was added to a solution of *t*-butoxycarbonyl-β-alanine (1.89 g, 0.01 mol)[12] and *p*-nitrophenol (1.67 g, 0.012 mol) in ethyl acetate (16 ml) at 0°C. The mixture was stirred for 1 hr at the same temperature, and then for an additional 24 hr at room temperature. The urea formed was removed by filtration, and the filtrate was concentrated to a residue under reduced pressure and crystallized by tritration with petroleum ether. The crude crystals were recrystallized from ethyl acetate-petroleum ether; yield, 1.53 g (49.3%); mp 59—61°C.

Found: C, 53.91; H, 5.82; N, 9.08%. Calcd for $C_{14}H_{18}O_6N_2$: C, 54.19; H, 5.85; N, 9.03%. The yield of the second crop of crystals was 0.36 g (11.6%).

***t*-Butoxycarbonyl-β-alanyl-L-tryptophyl-L-methionyl-L-aspartyl-L-phenylalanine Amide.** *t*-Amyloxycarbonyl-L-tryptophyl-L-methionyl-L-aspartyl-L-phenylalanine amide (294 mg, 0.41 mmol) and anisole (0.46 ml) were placed in a HF reaction cylinder, and then HF (about 5 ml) was distilled into the cylinder under a vacuum using a dry ice-methanol bath. The mixture was stirred for 30 min at 0°C, and then the excess HF was removed by distillation. After drying *in vacuo* for more than 10 hr at room temperature, the residue was extracted with water and some water-insoluble materials were removed with the aid of Hyflo Super-cel. The filtrate was lyophilized, the residue was suspended in dimethyl formamide (8 ml), and the *t*-butoxycarbonyl-β-alanine *p*-nitrophenyl ester (155 mg, 0.5 mmol) was added to the suspension together with triethylamine (0.2 ml, 1.50 mmol). The mixture was stirred at room temperature for 2 days, by which time the presence of free amino groups could no longer be recognized by thin-layer chromatography. Then, water was added to the mixture, and the pH was adjusted to 2 with 0.5 N hydrochloric acid. The mixture was kept for a while in a refrigerator, and then the crystalline product was collected by filtration. The crystals were washed with cold water and ether, and dried over phosphorus pentoxide *in vacuo*. The dried material was recrystallized from dimethylformamide-water, collected by filtration, washed with 50% aqueous dimethylformamide, water, and ether, and dried. After successive recrystallizations, the product was obtained as fine colorless crystals which were dried over phosphorus pentoxide *in vacuo* at room temperature for 24 hr; yield, 0.21 g (67%); mp 227—228°C (decomp.); $[\alpha]_D^{20}$ −28.8° (*c* 1, dimethylformamide). Values reported in the literature: mp 229—230°C (decomp.) and $[\alpha]_D^{20}$ −28.8° (*c* 1, dimethylformamide).[4]

Found: C, 57.80; H, 6.56; N, 12.51%. Calcd for $C_{37}H_{49}O_9N_7S$: C, 57.87; H, 6.43; N, 12.76%.

*2 This value was calculated from the dry weight.

11) S. Sakakibara, M. Shin, M. Fujino, Y. Shimonishi, S. Inouye and N. Inukai, This Bulletin, **38**, 1522 (1965); I. Honda, Y. Shimonishi and S. Sakakibara, *ibid.*, **40**, 2415 (1967).

12) P. H. Bentley, H. Gregory, A. H. Laird and J. S. Morley, *J. Chem. Soc.*, **1964**, 6130.