Peptide Synthesis by Oxidation-Reduction Condensation. I. Use of NPS-Peptides as Amino Component

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(Received October 12, 1970)

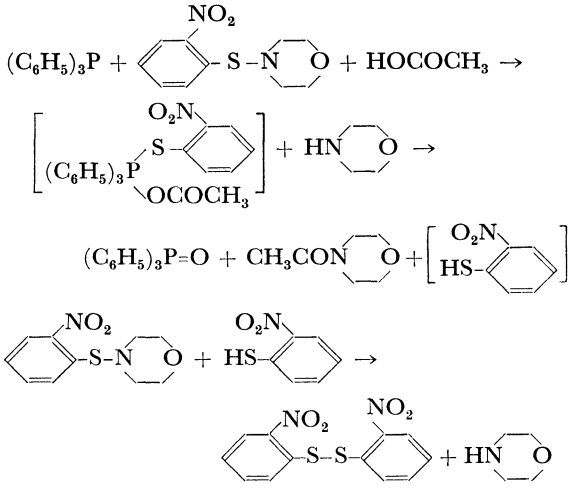
Sulfenamides were found to react readily with soft nucleophiles in the presence of active hydrogen compounds. A new method for the synthesis of carboxamide from metal carboxylate, sulfenamide and tertiary phosphine was developed. The effects of metal component were examined. Copper(II) salts gave the best result. In a similar way, peptides were synthesized by the reaction of copper(II) salts of acylamino acid, or free acylamino acid in the presence of cupric chloride and triethylamine, with NPS-amino acid derivatives and triphenylphosphine. When organo-mercuric compounds such as dianisylmercury were used in place of cupric chloride and triethylamine in the above reaction, almost complete retention of configuration was observed by the Young test.

In a previous communication,[1] a new amide (peptide) forming reaction by use of sulfenamide as an amino component was reported. The reaction is described more in detail in this paper.

Most sulfenic acid derivatives such as sulfenyl halide, sulfenate ester, disulfide, thiocyanate react very easily with phosphite triester. However, sulfenamide does not react with the ester because of the least polarizability of sulfur-nitrogen bond. On the other hand, sulfenamide is easily attacked by phosphite diester, which has a similar nucleophilicity as triester, to yield thiophosphate and amine.[2] The difference in reactivity between phosphite di- and tri-esters could be attributed to the presence or absence of active hydrogen. Mercaptan, which possesses a soft nucleophilic center and a proton in the molecule, also readily reacts with sulfenamide to give disulfide and amine. This reaction has been used for the removal of *o*-nitrophenylsulfenyl (NPS) protecting group introduced to α-amino function in peptide synthesis.[3] This shows that sulfenamides are activated by the protonation to yield active species, which are in turn easily attacked by soft nucleophile.

The reaction of sulfenamide and tertiary phosphine, a representative soft nucleophile, in the presence of active hydrogen compounds such as water and alcohol was tried first. When *p*-chlorobenzenesulfenanilide was allowed to react with triphenylphosphine in ether saturated with water, quantitative cleavage of sulfur-nitrogen bond was observed. On the other hand, no reaction occurred when the same reaction was tried in anhydrous methanol. However, quantitative cleavage of sulfur-nitrogen bond was observed by the addition of boron trifluoride etherate to the reaction system.

The same reaction of sulfenamide, phosphine and active hydrogen compound was tried by using carboxylic acid. After a short reaction period, acetomorpholide was obtained in a 63% yield from *o*-nitrobenzenesulfenic morpholide, triphenylphosphine and acetic acid. Formation of carboxamide is well explained by considering a subsequent acylation of the liberated amine with acyloxyphosphorane.



This reaction is not sufficient for the preparation of carboxamide because of the undesirable consumption of the starting sulfenamide by the subsequent reaction with mercaptan produced. This side reaction can be minimized by employing a mercaptan scavenger, which turns mercaptan into an inactive form. For example, when copper(II) hexanoate was allowed to react with *N*-*n*-butylbenzenesulfenamide and triphenylphosphine, a 95% yield of *N*-*n*-butylhexanamide was obtained, as shown in the following equation.

$$(R^1CO_2)_2Cu + 2R^2SNR^3R^4 + 2(C_6H_5)_3P \rightarrow 2R^1CONR^3R^4 + [(R^2S)_2Cu] + 2(C_6H_5)_3P{=}O$$

Since the copper(II) mercaptide produced does not react at all with sulfenamide in this reaction, yields of carboxamides are good as shown in Table 1. It is to be noted that mercury(II) and lead (II) salts of hexanoic acid also gave good results.

It is known that sulfenyl groups such as tritylsulfenyl and *o*-nitrophenylsulfenyl (NPS) can be used selectively as removable protecting groups in peptide synthesis. It can be expected that new peptide bond formation could be completed without removing the protecting group by the application of the above method to NPS-amino acid derivatives. *N*-Benzyl-*o*-nitrobenzenesulfenamide was chosen as a model compound and allowed to react with triphenylphosphine and benzoic acid. Contrary to expectation, the sulfenamide strongly resisted the

1) T. Mukaiyama, M. Ueki, H. Maruyama, and R. Matsueda, *J. Amer. Chem. Soc.*, **90**, 4490 (1968).

2) K. A. Petrov, N. K. Blinznyuk, and V. A. Savostenok, *Zh. Obshch. Khim.*, **31**, 1361 (1961); *Chem. Abstr.*, **55**, 23317e (1961).

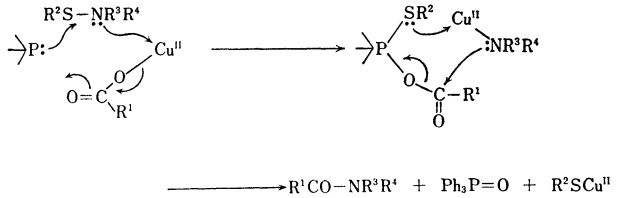
3) L. Zervas, D. Borovas, and E. Gazis, *J. Amer. Chem. Soc.*, **85**, 3660 (1963).

Table 1. Reactions of metal carboxylates, sulfenamides, and triphenylphosphine

Metal Carboxylate	Sulfenamide	Products: Carboxamide Structure	Carboxamide Yield (%)	$(C_6H_5)_3P=O$ Yield (%)
$(CH_3CO_2)_2Cu$	$C_6H_5S-N(C_2H_5)_2$	$CH_3CO-N(C_2H_5)_2$	60	quantitative
$(CH_3CO_2)_2Cu$	$C_6H_5S-NH(n-C_4H_9)$	$CH_3CO-NH(n-C_4H_9)$	quantitative	quantitative
$(CH_3CO_2)_2Cu\cdot H_2O$	$C_6H_5S-NH(n-C_4H_9)$	$CH_3CO-NH(n-C_4H_9)$	57	quantitative
$(n-C_5H_{11}CO_2)_2Cu$	$C_6H_5S-NH(n-C_4H_9)$	$n-C_5H_{11}CO-NH(n-C_4H_9)$	95	93
$(C_6H_5CO_2)_2Cu$	$C_6H_5S-NH(n-C_4H_9)$	$C_6H_5CONH(n-C_4H_9)$	62	quantitative
$(p-CH_3C_6H_4CO_2)_2Cu$	$C_6H_5S-NH(n-C_4H_9)$	$p-CH_3C_6H_4CONH(n-C_4H_9)$	80	90
$(n-C_5H_{11}CO_2)_2Hg$	$p-ClC_6H_4S-NH(n-C_4H_9)$	$n-C_5H_{11}CO-NH(n-C_4H_9)$	74	89
$(n-C_5H_{11}CO_2)_2Pb$	$p-ClC_6H_4S-NH(n-C_4H_9)$	$n-C_5H_{11}CO-NH(n-C_4H_9)$	68	84

reaction. This might be due to the lower electron density on nitrogen atom of this sulfenamide by conjugation with nitro group through sulfur *d* orbital and aromatic ring. However, it was found that addition of equimolar excess of triphenylphosphine gave rise to reaction. After the reaction period of 3 hr, *N*-benzylbenzamide was obtained in 90% yield by separating by column chromatography on silica gel. During the reaction about 1.5 equivalents of triphenylphosphine were oxidized even when reaction was carried out under nitrogen atmosphere, and copper mercaptide was obtained as a complex with triphenylphosphine. The detailed mechanism of the oxidation of the excess phosphine is not made clear as yet.

The above mentioned reaction of the preparation of carboxamide was modified to start from free carboxylic acid and sulfenamide. When *N*-*n*-butylbenzenesulfenamide was treated with hexanoic acid and triphenylphosphine in the presence of mercury(II) chloride and triethylamine. *N*-*n*-butylhexanamide was obtained in 79% yield. Various inorganic metallic compounds were examined through the reactions by using *N*-benzyl-*o*-nitrobenzenesulfenamide (see Table 2). The effcts of the metallic compounds in these reactions are explained by means of the following mechanism.



$$\longrightarrow R^1CO-NR^3R^4 + Ph_3P=O + R^2SCu^{II}$$

In the first stage of the reaction, metallic compound activates sulfur-nitrogen bond by accommodating the lone pair electrons on nitrogen atom. Thus a hard metal such as zinc is more effective in this stage. In the second stage, a metal component is used for mercaptide formation, which prevents a competitive thiolester forming reaction. Thus a soft metal such as mercury(II) is more effective. The presence of both zinc chloride and mercuric chloride in the reaction gave good results as expected.

The new method was applied to the peptide synthesis by using NPS-amino acid derivatives as amino component. First, synthesis of benzyloxycarbonyl (Z)-L-phenylalanylglycine ethyl ester, which is free from the problem of racemization, was tried. Copper(II) Z-L-phenylalaninate was prepared by treating sodium salt with aqueous copper(II) sulfate solution. To a stirred mixture of the copper salt in methylene chloride, NPS-Gly-OEt and 1 molar excess of triphenylphosphine were added. The copper salt dissolved soon and a clear reddish brown solution resulted. Z-L-Phe-Gly-OEt was obtained by ordinary treatment in 96% yield. Further, it was found that the reaction of free Z-L-Phe-OH with NPS-Gly-OEt and excess triphenylphosphine in the presence of copper(II) chloride and triethylamine also gave dipeptide in 90% yield.

Table 2. Reaction of benzoic acid, *N*-benzyl-*o*-nitrobenzenesulfenamide, and triphenylphosphine in the presence of metal chloride and triethylamine

Metal chloride	Conditions	Products: $C_6H_5CONHCH_2C_6H_5$ Yield (%)	$(C_6H_5)_3P=O$ Yield (%)
$CuCl_2$	RT 1 day	88	150
$HgCl_2$	RT 1 day	no reaction	—
$ZnCl_2$	RT 3 hr	71	100
$CuCl_2+ZnCl_2$	RT 1 day	75	100
$HgCl_2+ZnCl_2$	RT 1 day	80	92

2 equiv of triphenylphosphine was used.

The test for racemization in these two types of reactions was carried out by employing the Young method.[4] When copper(II) benzoyl(Bz)-L-leucinate, NPS-Gly-OEt and triphenylphosphine were allowed to react in methylene chloride at room temperature, Bz-Leu-Gly-OEt was obtaind in 77% yield. Its specific rotation was −19.0° (*c* 3.1, EtOH), which indicated that the L-isomer content was 57%. When Bz-L-Leu-OH, NPS-Gly-OEt and triphenylphosphine were allowed to react in the presence of copper(II) chloride and triethylamine in methylene chloride at room temperature for 15 hr, completely racemized Bz-Leu-Gly-OEt was obtained in 72% yield. No improvement was observed even when they were allowed to react at −70°C for 5 hr.

It is most probable that the racemization occurs

4) M. W. Williams and G. T. Young, *J. Chem. Soc.*, **1963**, 881.

through as oxazolone derived from the postulated acyloxyphosphorane intermediate. Thus, it is expected that basic species such as triethylamine and copper amide involved in the reaction would accelerate the formation of oxazolone from the intermediate.

It is known that the addition of acidic substances is very effective for the prevention of racemization in DCC[5] and mixed anhydride methods.[6] The effects of the acidic additives in this method were examined (Table 3). As expected, it was found that racemization was considerably suppressed by acidic additives according to their acidity except for *N*-hydroxysuccinimide. From its pK_a value, *N*-hydroxysuccinimide was more effective than expected. Pivalic acid, which showed no effect in the DCC method,[6] also showed some prevention. It was found that the reaction could be carried out without use of excess triphenylphosphine. Thus it is clear that acidic substances are useful not only for the prevention of racemization, but also for the activation of sulfenamides.

Table 3. Reaction of copper(II) Bz-L-leucinate, NPS-Gly-OEt, and triphenylphosphine in the presence of acidic additives

Additive	pK_a	Bz-L-Leu-Gly-OEt	
		Yield (%)	L-Isomer content (%)
none	—	77	57
C_6H_5OH	9.89	75	0
	9.40	64	84
p-$O_2NC_6H_4OH$	7.15	90	11
$(CH_3)_3CCO_2H$	5.01	57	39
2,4-$(O_2N)_2C_6H_3OH$	3.96	81	77
2,4,6-$(O_2N)_3C_6H_2OH$	0.38	48	80

From these findings basic substances such as triethylamine can be expected to be purged by employing the metallic salts derived from the above mentioned acidic compounds,mercaptan scavenger, in place of copper(II) chloride and triethylamine. This makes the reaction medium acidic and racemization would consequently be prevented. Mercury was found to be the best metal, since in such conditions metallic compounds are necessary only for complete trapping of mercaptan produced and not for the activation of sulfenamides. Mercury(II) bis-2,4-dinitrophenolate was prepared and the reaction of Bz-L-Leu-OH, NPS-Gly-OEt and triphenylphosphine was tried in the presence of the mercuric salt. As expected, Bz-L-Leu-Gly-OEt was obtained in 88% yield and its L-isomer content was 74%. When aryl mercuric compounds such as dianisyl mercury and anisyl mercuric bromide were used as a mercaptan scavenger, almost complete retention of configuration was observed as shown in Table 4.

Table 4. Reactions of Bz-L-Leu-OH, NPS-Gly-OEt, triphenylphosphine, and organomercuric compounds

Organomercuric compounds	Bz-L-Leu-Gly-OEt		$(C_6H_5)_3P=O$ Yield (%)
	Yield (%)	L-Isomer (%)	
$[2,4\text{-}(O_2N)_2C_6H_3O]_2Hg$	88	74	87
$(p\text{-}CH_3OC_6H_4)_2Hg$	93	87	99
p-$CH_3OC_6H_4HgBr$	92	93	97

In conclution, highly optically pure peptides can be prepared by the new method starting from the NPS-peptides as amino component by a one step procedure. Mild reaction condition starting with free carboxyl component is a merit of this new method as compared with the other method using NPS-peptides as an amino component.[7]

Experimental

Reagents. Sulfenamides were prepared from the corresponding chloride and amines. NPS-Gly-OEt was prepared by the method in literature.[3] Bz-L-Leu-OH, commercial product of Takara Kosan Co., was used without further purification.

Copper(II) salts of acylamino acids were prepared by treating the sodium salts of acylamino acids with copper (II) sulfate in aqueous solution. The copper salts precipitated were extracted with ethyl acetate, and the extracts were dried and evaporated *in vacuo*. The crude copper salts were purified by reprecipitation from chloroform-petroleum ether (bp 30—50°C).

(Z-L-Phe-O)$_2$Cu Found: C, 61.13; H, 5.04; N, 4.33%. Calcd for $C_{34}H_{32}N_2O_8Cu$; C, 61.86; H, 4.89; N, 4.24%.

(Bz-L-Leu-O)$_2$Cu Found: C, 57.87; H, 6.27; N, 4.97%. Calcd for $C_{26}H_{32}N_2O_6Cu$; C, 58.65; H, 6.02; N, 5.27%.

Mercury (II) bis-2,4-dinitrophenolate was prepared by the following procedure. A solution of 2,4-dinitrophenol and mercuric acetate in 1/1(v/v) aqueous ethanol was refluxed. Precipitates were collected by filtration and washed with water and acetone and dried.

Found: C, 30.51; H, 1.76; N, 5.61%. Calcd for $C_{12}H_8N_2O_6Hg$; C, 30.25; H, 1.68; N, 5.88%. *p*-Anisylmercuric bromide[8] and bis-*p*-anisylmercury[9] were prepared according to literature.

Reactions of Metal Carboxylates, Sulfenamides, and Triphenylphosphine(I). (General Procedure) Sulfenamide (10 mmol) in anhydrous methylene chloride (10 m*l*) was added drop by drop to a vigorously stirred mixture of metal carboxylate (5 mmol) and I (10 mmol) in methylene chloride (20 m*l*). The mixture was stirred for several hours and kept standing overnight. The precipitated copper mercaptide was removed by filtration, and carboxamide was obtained from the filtrate by distillation or chromatography on silica gel. Physical properties are as follows: $CH_3CON(C_2H_5)_2$, bp 93—100°C/40 mmHg; $CH_3CONH(n\text{-}C_4H_9)$, bp 110—112°C/22 mmHg; $n\text{-}C_5H_{11}CONH(n\text{-}C_4H_9)$, bp 95—98°C/0.05 mmHg; $C_6H_5CONH(n\text{-}C_4H_9)$, mp 68—70°C; $p\text{-}CH_3C_6H_4CONH(n\text{-}C_4H_9)$, mp 55—57°C: [Found: C, 75.60; H, 8.83; N, 7.30%. Calcd for $C_{12}H_{17}NO$; C, 75.75; H, 8.96; N, 7.32%.

5) E. Wünsch and F. Drees, *Chem. Ber.*, **99**, 110 (1966); J. E. Zimmerman and G. W. Anderson, *J. Amer. Chem. Soc.*, **89**, 7151 (1967).

6) G. W. Anderson, F. M. Callahan, and J. E. Zimmerman, *ibid.*, **89**, 178 (1967).

7) J. Savrda and D. H. Veyrat, *Tetrahedron Lett.*, **1968**, 6253; H. Faulstich, *Chimia*, **23**, 150 (1969).

8) F. Challenger and S. A. Miller, *J. Chem. Soc.*, **1938**, 894.

9) F. R. Preuss and I. Janshen, *Arch. Pharm.*, **293**, 933 (1960).

Reaction of Copper(II) Benzoate, N-Benzyl-o-nitrobenzenesulfenamide, and I. Reaction was carried out under the same conditions as described above except that I was used in one molar excess. The resulting solution was evaporated and reddish brown copper complex was precipitated from the residue by trituration with methanol. After filtration of the precipitate, the filtrate was condensed and chromatographed on silica gel. Copper complex and a small amount of disulfide were eluted by benzene. Elution with 1:1 mixture of benzene-methylene chloride gave *N*-benzylbenzamide (90%), mp 104—105°C. Further elution with 9:1 mixture of methylene chloride - methanol gave triphenylphosphine oxide (159%).

Reaction of Hexanoic, Acid, N-n-Butylbenzenesulfenamide, and I in the Presence of Mercury(II) Chloride and Triethylamine(TEA). Ten mmol of the sulfenamide in methylene chloride (10 m*l*) was added to a vigorously stirred mixture of hexanoic acid (10 mmol), I (10 mmol), mercuric chloride (5 mmol) and TEA (10 mmol) in methylene chloride (15 m*l*). After stirring for 3 hr at room temperature, mercury mercaptide precipitated was filtered off. The organic layer was dried and evaporated. From the residue, insoluble materials in petroleum ether (30—50°C) were removed by filtration. The filtrate was condensed and distilled to give *N-n*-butylhexanamide, (77%), bp 105—108°C/0.20 mmHg, which was purified by passing short column of silica gel.

Reactions of Benzoic Acid, N-Benzyl-o-nitrobenzenesulfenamide, and I in the Presence of Metal Chlorides and TEA. Reaction was carried out under similar conditions as mentioned above except that I was used in one molar excess when copper(II) chloride was employed. Isolation was carried out by chromatography on silica gel. The results are listed in Table 2.

Reaction of Copper(II) Z-L-phenylalaninate, NPS-Gly-OEt, and I. The copper salt (5 mmol), NPS-Gly-OEt (10 mmol), and I (20 mmol) were mixed in methylene chloride at room temperature. The resulting solution was stirred for 3 hr and kept standing overnight. After evaporation of the solvent, copper mercaptide-phosphine complex was precipitated by the addition of methanol and filtered off. The filtrate was condensed to about 5 m*l*, and 15 m*l* of ether and 25 m*l* of petroleum ether (30—50°C) were added to give Z-L-phe-Gly-OEt, 2.60 g (68%), mp 96—100°C. Additional 1.10 g (29%) of the peptide, mp 96—112°C, was obtained by chromatography on silica gel. The sample for analysis was recrystallized from ethyl acetate - petroleum ether, mp 109—111°C, $[\alpha]_D^{20}$ −17.0° (*c* 2, EtOH), lit,[10] mp 110—113°C, $[\alpha]_D^{20}$ −16.6° (*c* 2, EtOH).

Found: C, 65.59; H, 6.34; N, 7.02%. Calcd for $C_{21}H_{24}N_2O_5$; C, 65.61; H, 6.29; N, 7.29%.

10) R. W. Young, K. H. Wood, R. T. Joyce, and G. W. Anderson, *J. Amer. Chem. Soc.*, **78**. 2126 (1956).

Reaction of Z-L-Phe-OH, NPS-Gly-OEt, and I in the Presence of Copper(II) Chloride and TEA. NPS-Gly-OEt (10 mmol) in methylene chloride was added at room temperature to the stirred mixture of Z-L-Phe-OH (10 mmol), I (20 mmol), copper(II) chloride (5 mmol) and TEA (10mmol) in methylene chloride. The mixture was stirred for 1 day, washed with water and dried. The products were separated by chromatography on silica gel to give copper mercaptide-phosphine complex 3.12 g; Z-L-Phe-Gly-OEt 3.58 g, mp 109—112°C, $[\alpha]_D^{20}$ −17.3° (*c* 2, EtOH) and triphenylphosphine oxide, 4.28 g (154%).

Test for Racemization in This Type of Reaction by the Young Method.[4] *A) Reaction of Copper(II) Bz-L-leucinate, NPS-Gly-OEt, and I:* The reaction was carried out in the same manner as in the synthesis of Z-L-Phe-Gly-OEt. Hydrogen sulfide gas was introduced for 1 hr to the resulting solution. Cupric sulfide precipitated was filtered off and the filtrate was washed with 1N HCl, 5% $NaHCO_3$ solution and water, and dried. The products were then separated by preparative thin layer chromatography to give Bz-Leu-Gly-OEt 2.47 g (77%), mp 148—149°C. The crude material was subjected to measurement of optical purity without any purification to avoid fractionation, $[\alpha]_D^{20}$ −19.0° (*c* 3.1, EtOH), L-isomer content 56%.

B) Reaction of Bz-L-Leu-OH, NPS-Gly-OEt, and I in the Presence of Copper(II) Chloride and TEA: The reaction was carried out in the same manner as in the synthesis of Z-L-Phe-Gly-OEt starting from free carboxyl component. Hydrogen sulfide gas was introduced for 1 hr to the resulting solution. On filtering off the precipitated copper mercaptide, the filtrate was condensed and chromatographed on silica gel to give bis-*o*-nitrophenyl disulfide (quantitative), Bz-Leu-Gly-OEt 2.30 g (72%), mp 143—145°C, $[\alpha]_D^{20}$ 0°(*c* 3.1, EtOH) and triphenylphosphine oxide 3.34 g (120%).

Reaction of Copper(II) Bz-L-Leucinate, NPS-Gly-OEt, and I in the Presence of Acidic Additives. Copper(II) Bz-L-leucinate (5 mmol), NPS-Gly-OEt (10 mmol), I (10 mmol), and acidic substances listed in Table 3 (10 mmol) were mixed in methylene chloride at room temperature. The mixture was stirred for several hours and kept standing overnight. On filtering off copper mercaptide the filtrate was washed with 1N HCl, 5% $NaHCO_3$ solution and water and dried. On evaporation of the solvent, chromatographic separation gave crude Bz-Leu-Gly-OEt, which was subjected to the measurement of optical purity. The results are listed in Table 3.

Reactions of Bz-L-Leu-OH, NPS-Gly-OEt, I, and Organomercuric Compounds. The reaction was carried out by mixing equimolar amounts of Bz-L-Leu-OH, NPS-Gly-OEt, I, and bis(*p*-anisyl)mercury, mercury bis(2,4-dinitrophenolate) or *p*-anisylmercuric bromide in methylene chloride at room temperature. Work-up was done as above and results are listed in Table 4.