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PEPTIDE SYNTHESIS VIA THE OXIDATION-REDUCTION CONDENSATION BY THE USE OF 2,2'-DIPYRIDYLDISULFIDE AS AN OXIDANT

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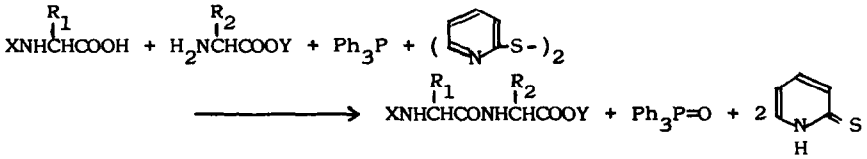
Some of thiols such as mercapto-pyridine, mercapto-purine, etc. which bear a mercapto-group α or γ with respect to a ring nitrogen are well known to exist in solution predominantly in the thione form[1]. We considered that the recently shown oxidation-reduction condensation[2] could be carried out in the absence of mercaptan scavenger when 2,2'-dipyridyldisulfide was used as the disulfide component. In the reaction, 2-mercaptopyridine, a hydrogenated product, produced along with peptide would isomerize to the stable thione form, which is unreactive with the other components.

We now describe an useful and practical method of peptide synthesis via the oxidation-reduction condensation shown in the following equation[4].

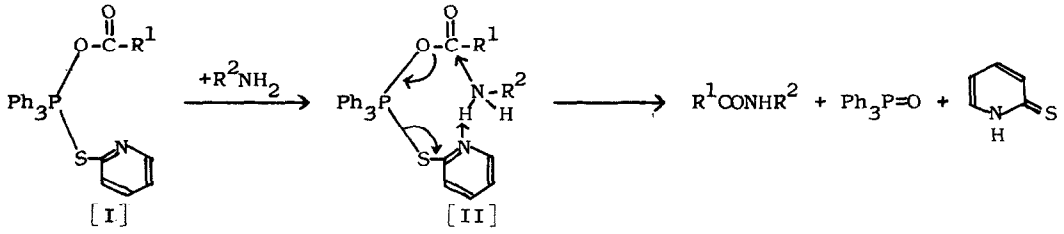


In a typical experiment carried out according to the Young test[3], ethyl glycinate (5 mmol) and triphenylphosphine (5 mmol) in methylene chloride were added at room temperature to a methylene chloride solution of equimolar amounts of N-benzoyl-L-leucine and 2,2'-dipyridyldisulfide. After stirring for 30 min, the solution was washed with dilute hydrochloric acid, aqueous sodium hydrogen carbonate, water, dried over sodium sulfate and the solvent was evaporated in vacuo. From the residue, Bz-L-Leu-Gly-OEt was obtained by chromatography on silica gel, 1.46g (91%), mp 148-52°, $[\alpha]_D^{20}$ -32.6° (c 3.1 EtOH), L isomer 96%[3]. Other disulfides also gave the favorable results except in the case of 4,4'-dipyridyldisulfide[5] : 5,5'-dinitro-2,2'-dipyridyldisulfide (93% yield, L isomer 95%) ; 2,2'-dibenzothiazolyldisulfide (87%, L isomer 89%) ; 2,2'-dibenzimidazolyldisulfide (72%, L isomer 88%) ; 4,4'-dipyridyldisulfide (70%, L isomer 60%).

On the other hand, diphenyl disulfide which affords thiophenol gave a poor result[6] (22% yield, L isomer 78%) in the absence of mercaptan scavenger.

The high reactivity with high optical purity in the case of 2,2'-dipyridyldisulfide can be explained by assuming initial formation of acyloxyphosphonium salt largely with pentacovalent character. This salt in turn reacts rapidly with the incoming amino component to afford peptide, phosphine oxide and the thione through a cyclic transition state[II] as depicted below.

Since such acceleration by the above mentioned concerted mechanism cannot lead to oxazolone formation, it is reasonable to consider that an oxazolone is not involved in this process and racemization is obviated.



[I] [II]

Next, we investigated the racemization in polar solvents or in the presence of chloride ion and the applicability of various kinds of amino acids to establish a broad application in peptide synthesis. The reaction takes place over at an adequate temperature range from -70° to boiling point of methylene chloride and no diminition in yield was observed by adding initially 1 equiv of pyrid-2-thione to the reaction system when amino acids were used as hydrates[7]. The reaction was further found not to be limited in the choice of solvent (benzene, dioxane, ethyl acetate, chloroform etc.) except in the cases of acetonitrile and N,N-dimethylformamide: but, favorable results by the Young test were obtained by adding initially 1 equiv of pyrid-2-thione at room temperature in acetonitrile (89% yield, L isomer 94%) and 2 equiv of pyrid-2-thione at -30° in N,N-dimethylformamide (88% yield, L isomer 94%). In the presence of chloride ion (use of glycine ester hydrochloride and 1 equiv of triethylamine in chloroform), addition of 2 equiv of pyrid-2-thione or 2 equiv of N-hydroxysuccinimide at room temperature gave favorable results : pyrid-2-thione (88%, L isomer 87%); N-hydroxysuccinimide[8] (91%, L isomer 96%).

A special practical merit of this method is that the peptides with side chains are produced directly in high yields as summarized in table 1.

No difficulties were encountered when methionine, cysteine, tryptophan were the sources of the carboxyl component and the hydroxyl groups in tyrosine, threonine and serine could be used without protection. Favorable result was obtained in the case of nitroarginine which is known to give a lactam[9] by ordinary methods and no nitrile formation was observed in the cases of glutamine

Table 1[a)]

Example	Peptide	Reaction[b)] Solvent	Yield[c)] %	mp[d)] °C	$[\alpha]_D^{T}$[d)] (c Solvent[b)])
1	Z-L-Met-Gly-OEt	CH_2Cl_2	92	94-6	$[\alpha]_D^{27}$ -19.8 (c 4.6 EtOH)
2	Z-L-Cys(SBzl)-Gly-OEt	"	91	97-9	$[\alpha]_D^{20}$ -27.0 (c 6 AcOH)
3	Z-L-Try-Gly-OEt	"	92	118-20	$[\alpha]_D^{20}$ -19.8 (c 2 EtOH)
4	Z-L-Tyr-L-Ileu-OMe	"	93	69-70	$[\alpha]_D^{23}$ -4.1 (c 2.6 DMF)
5	Z-L-Thr-Gly-OEt	"	83	105-7	$[\alpha]_D^{25}$ -13.8 (c 1 EtOH)
6	Z-L-Ser-Gly-OEt	"	84	98-100	$[\alpha]_D^{25}$ -5.5 (c 1 EtOH)
7	Z-L-Glu(NH$_2$)-Gly-OEt	"	86	168-70	$[\alpha]_D^{20}$ -7.3 (c 1 DMF)
8	Z-L-Asp(NH$_2$)-Gly-OEt	DMF+CH_2Cl_2	85	185-7	$[\alpha]_D^{20}$ -5.6 (c 1 ")
9	Z-NO_2-L-Arg-Gly-OEt	"	82	119-21	$[\alpha]_D^{22}$ -15.4 (c 2 MeOH)
10	Z-L-Phe-Gly-OEt	CH_2Cl_2	90	110-2	$[\alpha]_D^{20}$ -16.8 (c 2 EtOH)
11	Z-L-Val-Gly-OEt	Benzene	92	166-7	$[\alpha]_D^{22}$ -32.0 (c 1 MeOH)
12	Z-L-Ala-Gly|L-Leu-Gly-Gly-Gly-L-Leu-Gly-NHNHBOC[e)]	DMF	70	213-5	

a) Most experiments were carried out in 2-5 mmoles. Example 12 was in 0.45 mmoles at Dr. N. Yanaihara's Laboratory (Shizuoka College of Pharmacy, Oshika Shizuoka, Japan).
In many cases, peptides were separated from triphenylphosphine oxide and pyrid-2-thione by the addition of benzene or ether to the reaction mixture after evaporation of the reaction solvents. In some cases, peptides were separated by chromatography directly or after the removal of the thione by the following procedure; the thione was derived to the mercuric mercaptide by adding mercuric salt of acetoamide and was removed by washing with 0.5 N sulfuric acid.

b) DMF: N,N-dimethylformamide

c) Yield of peptide with given mp and $[\alpha]_D$ values.

d) Melting point and $[\alpha]_D$ values are good agreement with literature; satisfactory elemental analyses were obtained for new compounds.

e) Vertical line indicates the point of coupling. Amino acid ratio: Ala, 0.98; Gly, 4.99 and Leu, 2.02.

and asparagine and thus analytically pure peptides are made available by an extremely simple procedure. For example, 5 mmol of ethyl glycinate in methylene chloride was added at room temperature to a homogeneous methylene chloride solution of benzyloxycarbonyl-L-glutamine, triphenylphosphine and 2,2'-dipyridyl-

disulfide. After stirring for 3 hr, the precipitated peptide was filtered off, washed with dilute hydrochloric acid, aqueous sodium hydrogen carbonate, water, methylene chloride and petroleum ether. After drying in vacuo, Z-L-Glu(NH_2)-Gly-OEt was obtained in 86% yield, mp 168-70°, $[\alpha]_D^{20}$ -7.3°(c 1 N,N-dimethylformamide). Anal. Calcd for $C_{17}H_{23}O_6N_3$: C, 55.88; H, 6.35; N, 11.50. Found: C, 56.10; H, 6.11; N, 11.55. [lit. [10] mp 169-71°, $[\alpha]_D^{20}$ -7.2° (c 1 N,N-dimethylformamide)].

In conclusion, this method produces peptides in high yields with high optical purity with respect to various kinds of amino acids by simply mixing the reactants. There are few restrictions placed on the choice of solvent and the reaction proceeds over a broad temperature range allowing a wide choice of reaction conditions.

REFERENCES

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2) T. Mukaiyama, R. Matsueda, H. Maruyama and M. Ueki, J. Am. Chem. Soc., 91, 1554 (1969).
3) M. W. Williams and G. T. Young, J. Chem. Soc., 881 (1963).
The per cent of L isomer was caluculated from $[\alpha]_D^{20}$-34° as in Young's report.
4) Most of these results were reported at 7th Japanese Peptide Symposium held at Tokyo University, Tokyo, November, 1969.
5) This result may due to the non-participation of the sulfur component in the formation of a cyclic intermediate similar to [II].
6) According to Y. V. Mitin and G. P. Vlasov [Dokl. Acad. Nauk SSSR, 179, 353 (1968)], Z-Gly-Phe-Gly-OEt (Anderson test) was obtained in 40% yield with 30% racemate, in the absence of scavenger, by the use of triethylphosphite and iodomethylate of bis-(N,N-dimethyl-aminophenyl)disulfide.
7) Favorable result (90% yield, L isomer 94% by the Young test) was obtained even in the presence of a tenfold excess of water in dioxane.
8) Ethyl glycinate was added to the chloroform solution of the other components after the spot of phosphine disappeared (detected by tlc).
9) M. Bodanszky and J. T. Sheehan: Chemistry and Industry, 1268 (1960).
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