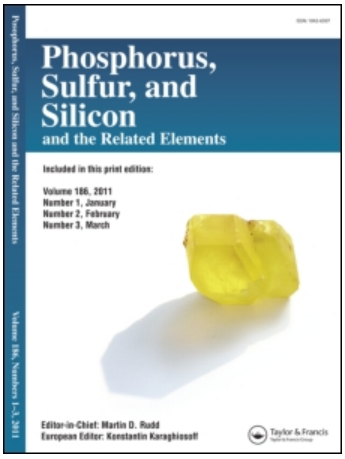

Phosphorus, Sulfur, and Silicon and the Related Elements
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713618290

AN APPLICATION OF ORGANIC PHOSPHORUS COMPOUNDS IN THE PEPTIDE AND NUCLEOTIDE SYNTHESIS

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To cite this Article Mukaiyama, Teruaki(1976) 'AN APPLICATION OF ORGANIC PHOSPHORUS COMPOUNDS IN THE PEPTIDE AND NUCLEOTIDE SYNTHESIS', Phosphorus, Sulfur, and Silicon and the Related Elements, 1: 2, 371 — 387
To link to this Article: DOI: 10.1080/03086647608073345
URL: http://dx.doi.org/10.1080/03086647608073345

Phosphorus and Sulfur
1976, Vol. 1, pp. 371-387

AN APPLICATION OF ORGANIC PHOSPHORUS COMPOUNDS IN THE PEPTIDE AND NUCLEOTIDE SYNTHESIS †

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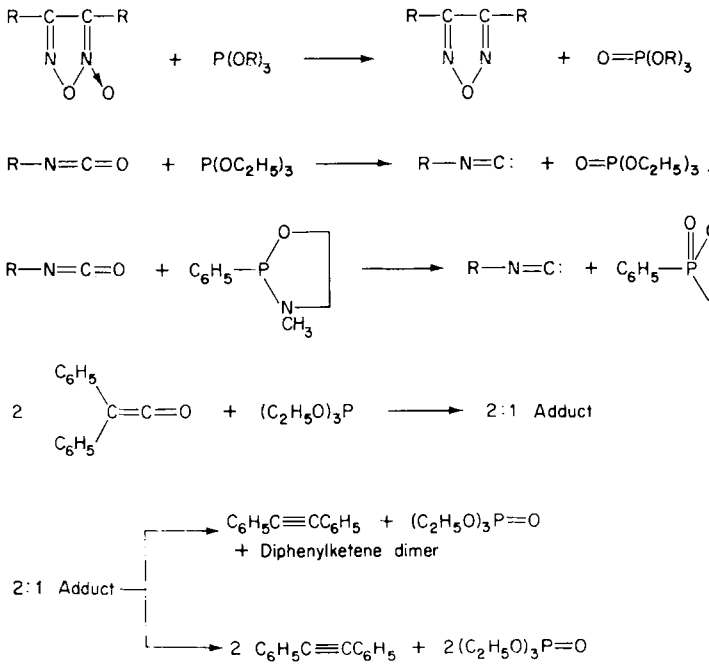
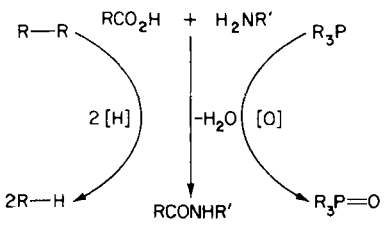
Organic trivalent phosphorus compounds, such as trialkyl phosphites and trialkyl- or triarylphosphines, are well known to be powerful deoxygenation reagents and have been used in various reduction reactions; for example, as illustrated in the following reactions developed in our laboratory.[1-3] However it is rather a new aspect to make use of their deoxygenative property for the generation of active acyl groups. Since the discovery of the reaction of mercuric acetate with trivalent phosphorus compounds to yield acetic anhydride,



our attention has been focused on the generations of acyl groups, and several novel reactions have been found which are applicable to the preparation of acid anhydride, peptide, and nucleotide linkages. In considering these reactions described herein, it is necessary to choose appropriate oxidation reagents together with reducing reagents (trivalent phosphorus compounds), because these dehydration reactions essentially proceed *via* an oxidation-reduction process as depicted in the following scheme.



† Plenary Lecture. The Vth International Conference of Organic Phosphorus Chemistry, Gdansk, Poland, September 1974.

It is well known that mercuric compounds behave as oxidizing reagents. Concerning their reactions with trivalent phosphorus compounds, we found that mercuric or mercurous carboxylates oxideize triethyl phosphite or tributylphosphine to yield the corresponding acid anhydrides along with triethyl phosphate or tributylphosphine oxide.[4]

$$(RCO_2)_2Hg + R'_3P \longrightarrow (RCO_2)_2O + Hg + R'_3P{=}O$$

We also found that phenylmercuric acetate behaves similarly toward tributylphosphine to form acetic anhydride, diphenylmercury, and mercury[5] as shown in the following equation.

$$2C_6H_5Hg{-}OCOCH_3 \xrightarrow{(C_4H_9)_3P} (CH_3CO)_2O + (C_6H_5)_2Hg + Hg + (C_4H_9)_3P{=}O$$

On the other hand, arylmercuric carboxylates are readily available from the reaction of diarylmercury with carboxylic acids.[6] Thus the combination of these two reactions seems to make it possible to prepare acid anhydrides from two molecules of the corresponding free carboxylic acids by way of exchange and oxidation-reduction reactions.

$$Ar_2Hg + RCO_2H \longrightarrow ArHgOCOR + ArH$$

$$2\,ArHgOCOR + R'_3P \longrightarrow Ar_2Hg + (RCO)_2O + Hg + R'_3P{=}O$$

Indeed, treatment of diphenyl- or di-*p*-anisylmercury with tributylphosphine, in the presence of two equivalents of carboxylic acid, leads to the formation of the corresponding acid anhydrides along with mercury and tributylphosphine oxide in high yields.[5]

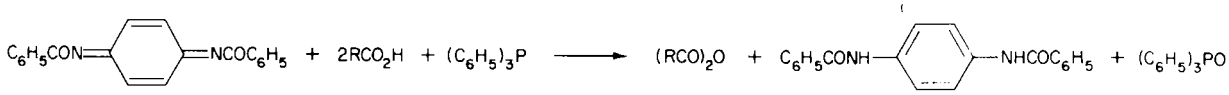
$$Ar_2Hg + (C_4H_9)_3P + 2RCO_2H \longrightarrow (RCO)_2O + Hg + 2ArH + (C_4H_9)_3P{=}O$$

$Ar{=}C_6H_5$, p-$CH_3O{-}C_6H_4$

In this reaction, the acid anhydrides are directly prepared through dehydration-condensation from free carboxylic acids by the use of a hydrogen acceptor (diarylmercury) and an oxygen acceptor (tributylphosphine). Similar dehydration-condensation reactions can be carried out successfully by the use of a trialkylphosphine and conjugated dicarbonyl compounds such as dibenzoylethylene.[7]

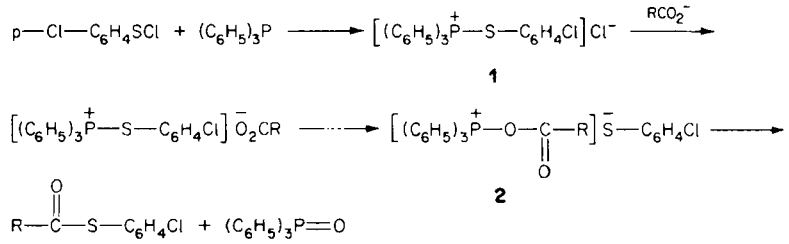
$$C_6H_5COCH{=}CHCOC_6H_5 + (C_4H_9)_3P + 2RCO_2H \longrightarrow (RCO)_2O + C_6H_5COCH_2CH_2COC_6H_5 + (C_4H_9)_3P{=}O$$

p-Quinone dibenzimide is found to be more effective as a hydrogen acceptor, and treatment with triphenylphosphine in the presence of a carboxylic acid leads to the formation of the corresponding acid anhydride in high yield along with triphenylphosphine oxide and *p*-phenylenedibenzamide.[8] In all of these reactions acyloxyphosphonium salt is considered to be formed as an intermediate and to act as a reactive acylating reagent.



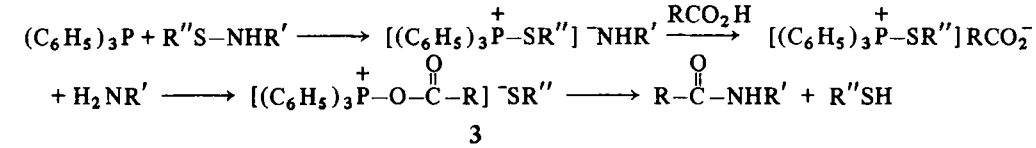
Furthermore treatment of the phosphonium salt (1), produced from a sulfenyl chloride and triphenylphosphine, with sodium or triethylammonium salts of carboxylic acids is found to result in the formation of the corresponding thiol esters in high yields. This result can also be explained by assuming the formation of acyl-oxyphosphonium thiolates (2), which decompose into thiol esters as shown in the following scheme.[9]

In this reaction the sulfenyl chloride is shown to play a role as an oxidizing agent through a process in which the RS-moiety, introduced initially as a cation for the formation of the phosphonium salt, has changed into the thiolate anion. Similar results are also observed in the reaction of disulfides with tertiary phosphine in the presence of free carboxylic acid. The corresponding thiol esters are produced in high yields along with thiols and phosphine oxides.[10]



$$RCOOH + RSSR + R_3P \longrightarrow RCOSR + RSH + R_3P{=}O$$

This has been further extended to the preparation of the amide linkage. The corresponding carboxamides can be obtained by treating sulfenamides with carboxylic acids in the presence of triphenylphosphine.[11] The key step of the reaction in both cases is the formation of an acyloxyphosphonium thiolate (3) resulting from an exchange reaction of the sulfenyl group with a carboxylate anion.



Unfortunately, this reaction is not applicable to the ideal preparation of carboxamides because the thiol liberated in the first step reacts further with the sulfenamide to give undesirable side-products: disulfides and amines. However after various studies, it was established that this side reaction can be practically avoided when the carboxylic acids are used as their cupric salts, in which case the thiolate anions are captured as cupric thiolates.[12] Thus carboxamides can be obtained in high yields.

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

In this way, for example, *N*-butylcapronamide is produced in 95% yield by treating cupric capronate with two moles each of triphenylphosphine and *N*-*n*-butylbenzenesulfenamide at room temperature. Sometimes an excess of triphenylphosphine must be used to complete the reaction because it is consumed through coordination to the cupric thiolates. Thus four equivalents of triphenylphosphine are necessary in the reaction of *N*-benzyl-*o*-nitrobenzenesulfenamide and cupric benzoate.[12] Sulfenamide derivatives of amino acids are readily available from the reaction of amino acids with sulfenyl chlorides, and the sylfenyl groups are generally employed as a useful protecting group for the amino groups in peptide synthesis. Based on these facts, peptide-linkages can easily be constructed and the reaction of $(Z{-}L{-}Phe)_2Cu$ with Nps–Gly–OEt in the presence of triphenylphosphine gives Z–L–Phe–Gly–OEt in high yield.[12]

$$(Z{-}L{-}Phe)_2Cu + 2Nps{-}Gly{-}OEt + 4(C_6H_5)_3P \longrightarrow 2Z{-}L{-}Phe{-}Gly{-}OEt + Cu(S{-}C_6H_4{-}NO_2{-}o)_2 \cdot 2P(C_6H_5)_3 + 2(C_6H_5)_3P{=}O$$

An unsatisfactory result is obtained with respect to racemization during this reaction with the Young test, probably because of the generation of amino acid ester anions as intermediates. However the situation can be improved by adding an acidic substance to keep the reaction medium neutral or acidic.[12] As a thiol scavenger, other kinds of metal chlorides can also be used in place of cupric carboxylates in the above reaction. Accordingly, free carboxylic acids can be used as shown in the following equation.

$$2R^1CO_2H + 2R^3S{-}NHR^2 + 2(C_6H_5)_3P + MCl_2 + 2Et_3N \longrightarrow 2R^1CONHR^2 + 2(C_6H_5)_3P{=}O + (R^3S)_2M + 2Et_3N{\cdot}HCl$$

The concept of preparing acyloxyphosphonium salts from arylthiophosphonium salts can be further extended to the synthesis of peptides starting from the free *N*-protected amino acids and free amino esters by

utilizing the reaction of triphenylphosphine with diaryl disulfides. Thus, Z–L–Phe–Gly–OEt is obtained in high yield by treating Z–L–Phe–OH with H–Gly–OEt in the presence of *bis*(*o*-nitrophenyl) disulfide, mercuric chloride, triphenylphosphine and triethylamine.

$$Z\text{–}L\text{–}Phe\text{–}OH + H\text{–}Gly\text{–}OEt + HgCl_2 + (o\text{-}O_2NC_6H_4S)_2 + Ph_3P + 2Et_3N \longrightarrow$$
$$Z\text{–}L\text{–}Phe\text{–}Gly\text{–}OEt + Hg(S\text{–}C_6H_4\text{–}NO_2\text{–}o)_2 + Ph_3P{=}O + 2Et_3NHCl$$

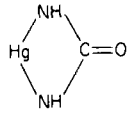
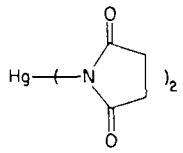
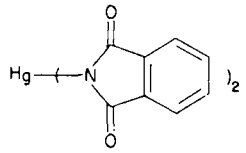
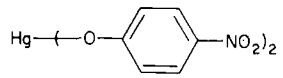
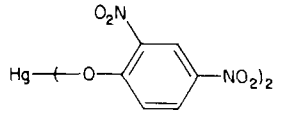
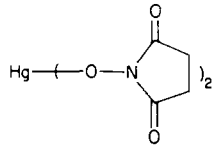
Various kinds of metals can also be employed in this type of reaction, but soft acids such as mercuric, silver and cupric salts are usually found to yield better results. This method makes it possible to synthesize peptides starting from the free, *N*-protected amino acids and amino acid esters, but it gives rise to racemization when amines are used to neutralize hydrogen chloride. In order to eliminate bases from the reaction system, various kinds of metal compounds have been examined which would yield thiolates. These compounds can be classified into two categories: (a) mercuric salts of urea, succinimide, *p*-nitrophenol, etc., which afford thiolates on treatment with triphenylphosphine and disulfides, probably through direct attack by the thiolate anion on the phosphonium salts. In this case, the stabilities of the X^- anions involved in the salts are expected to have a great effect on racemization and, in accordance with this, the optical purity of the dipeptides is found to increase as the X^- anion stability increases, except in the case of *N*-hydroxy succinimide.

$$(R^3S)_2 \xrightarrow{Ph_3P} [Ph_3\overset{+}{P}\text{–}SR^3]R^3S^- \xrightarrow{MX_2} [Ph_3\overset{+}{P}\text{–}SR^3]X^- + XMSR^3 \xrightarrow[-(R^3S)_2M,-HX]{R^1CO_2H}$$
$$[Ph_3\overset{+}{P}\text{–}O\text{–}\overset{O}{\overset{\|}{C}}\text{–}R^1]X^- \xrightarrow{H_2NR^2} R^1CONHR^2 + Ph_3P{=}O + HX$$

4,4′-Dichlorodiphenyl disulfide was used as a disulfide component (Table I); (b) di-*p*-anisylmercury and *p*-anisylmercuric bromide which can react only with thiols to yield mercuric thiolates and anisole, but cannot react with the phosphonium thiolates.

TABLE I

The effects of added mercuric salts (type a)

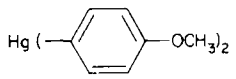
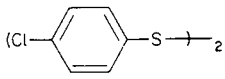
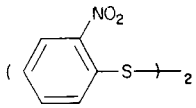
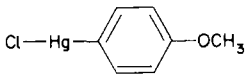
MX_2	Bz–L–Leu–Gly–OEt Yield %	L-Isomer %
	81	23
	91	46
	85	47
	82	51
	92	73
	89	59

$$(R^3S)_2 \xrightarrow{R^2_3P} [R^2_3\overset{+}{P}-SR^3]\,^-SR^3 \xrightarrow{R^1CO_2H} [R^2_3\overset{+}{P}-SR^3]\,^- O-\overset{O}{\overset{\|}{C}}-R^1 + R^3SH$$

$$R^3SH + HgX_2 \longrightarrow HX + R^3S-HgX$$

By the use of the type (b) compounds, it is found that satisfactory results are obtained as shown in Table II.[13]

TABLE II
The effects of added mercuric compounds (type b)

MX_2	$(RS)_2$	Yield %	L-Isomer %
		88	90
"		92	95
	"	92	94

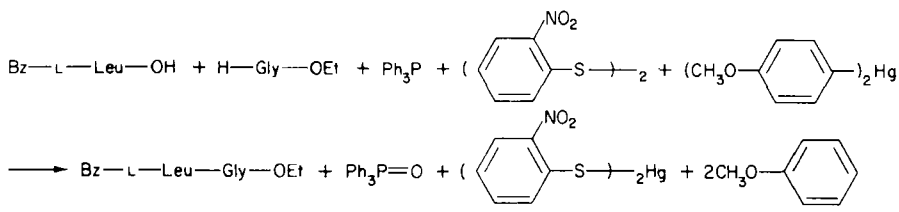
The high optical purity in these cases may be attributable to the absence of oxazolone formation. In this reaction system the intermediate acyloxyphosphonium salts are attacked only by H–Gly–OEt to produce the peptides and thiols which yield mercuric thiolates and anisole through the reaction with mercuric compounds. Since anisole is produced directly through protonation of the mercuric compounds by thiols, the anisyl anion is absent during thiolate formation and oxazolone formation can be prevented. Further, a wide variety of solvents can be used in this reaction and satisfactory results are obtained both in yields and optical purities except in the cases of reactions in *N,N*-dimethylformamide and acetonitrile (Table III).[13]

In addition to anisylmercuric compounds, other kinds of reagents which are reactive enough to capture thiols have also been shown to participate in this type of reaction. For example sulfenic acid esters, which are known to react with thiols to yield disulfides, can be effectively used as scavengers.

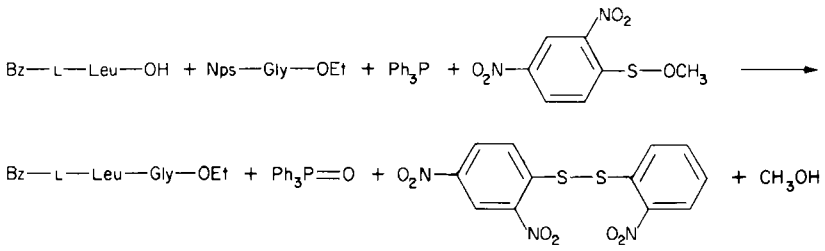
$$R^1S-OR^2 + R^3SH \longrightarrow R^1-S-S-R^3 + R^2OH$$

The reaction of Bz–L–Leu–OH with Nps–Gly–OEt in the presence of triphenylphosphine and methyl 2,4-dinitrobenzenesulfenate gives Bz–L–Leu–Gly–OEt in high yield.[14]

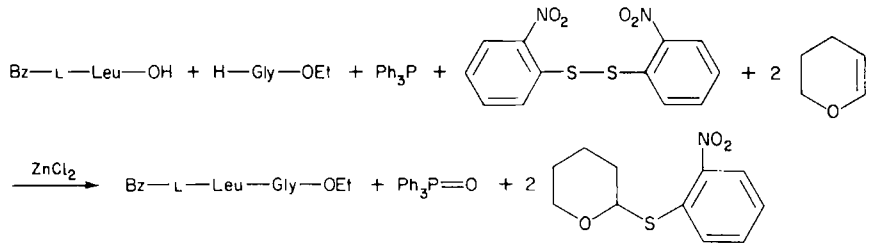
TABLE III
Solvent effects of the reaction



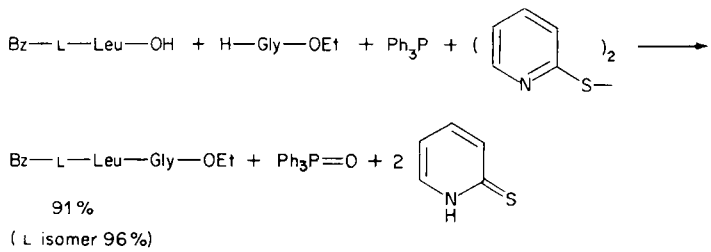
Solvent	Yield %	L-isomer %
Benzene	91	95
Dioxane	89	89
Chloroform	96	96
Ethyl acetate	94	90
THF	91	89
Methylene chloride	92	95
Ethylene chloride	94	94
DMF	86	37
Acetonitrile	88	66



Further, vinyl ethers can also act as thiol scavengers in a similar reaction as depicted in the following equation.[14]



Dipeptides obtained in both of these reactions undergo little racemization, and highly optically active products are usually obtained. Some thiols such as 2(1*H*)-pyridinethione, which bear a sulfhydryl group at the α- or γ-position, are well known to exist predominantly in the thione form in solution.[15] Accordingly, peptide synthesis by means of the phosphine-disulfide system appears to take place successfully in the absence of thiol scavengers when the above mentioned disulfides are used. In this reaction, 2(1*H*)-pyridinethione, a hydrogenated product produced along with peptides, is expected to isomerize to the stable thione form which is unreactive toward the other components. Thus treatment of Bz–L–Leu–OH with H–Gly–OEt in the presence of triphenylphosphine and 2,2′-dithiodipyridine is shown to lead to the formation of Bz–L–Leu–Gly–OEt in high yield.[14]



According to the Young test, racemization is highly depressed in this reaction system and dipeptides with high optical purity are obtained. For example, synthetic Bz–L–Leu–Gly–OEt was shown to be 96% pure L-isomer by the Young test. Other disulfides which similarly isomerize to the thione forms after accepting hydrogen also give satisfactory results for the formation of the peptide linkage, except in the case of 4,4′-dithiodipyridine.[16]

TABLE IV

Effects of disulfide on the reaction

$$\text{Bz–L–Leu–OH} + \text{H–Gly–OEt} + \text{Ph}_3\text{P} \xrightarrow{\text{RSSR}} \text{Bz–L–Leu–Gly–OEt} + \text{Ph}_3\text{P=O} + 2\text{RSH}$$

	Bz–L–Leu–Gly–OEt	
Disulfide	Yield (%)	L-isomer (%)
2,2′-dithiodipyridine	91	96
2,2′-dithio*bis*(5-nitropyridine)	93	95
2,2′-dithio*bis*benzothiazole	87	89
2,2′-dithio*bis*benzimideazole	72	88
4,4′-dithiodipyridine	70	60
diphenyl disulfide	22	78

This reaction is found to possess some significant advantages in peptide synthesis; the reaction can be carried out in a wide variety of solvent systems although some modifications are necessary when it is tried in acetonitrile or *N,N*-dimethylformamide. (Satisfactory results by the Young test have been obtained by adding initially one equivalent of 2(1*H*)-pyridinethione at room temperature in acetonitrile (89% yield, L-isomer 94%) and two equivalents of 2(1*H*)-pyridinethione at −30° in *N,N*-dimethylformamide (88% yield, L-isomer 94%). In the presence of chloride ion (use of glycine ester hydrochloride and one equivalent of triethylamine in chloroform), addition of two equivalents of 2(1*H*)-pyridinethione or two equivalents of *N*-hydroxysuccinimide at room temperature gives favorable results as measured by the Young test; 2(1*H*)-pyridinethione (88%, L-isomer 87%); *N*-hydroxysuccinimide (91%, L-isomer 96%). The presence of water in the reaction system has little effect on the reaction as indicated by results obtained by adding initially one equivalent of 2(1*H*)-pyridinethione in the presence of a tenfold excess of water in dioxane (90% yield, L-isomer 94% by the Young test). Further, the reaction can be carried out over a wide range of temperatures from −70° to the boiling point of methylene chloride. The fact that the solvent can be freely chosen makes it possible to employ the solid phase method. A special practical merit of this method lies in its compatibility with most of the other functional groups: no difficulties are found when methionine, cysteine, and tryptophan are the source of the carbonyl components; and the hydroxyl groups in tyrosine, threonine and serine can be used without protection. Favorable results are obtained in the case of nitroarginine, which is known to give a lactam by ordinary methods,[17] and no nitrile formation is observed in the cases of glutamine and asparagine. For example, while undesirable dehydration reactions frequently accompany peptide synthesis by the use of DCC, e.g. the formation of anhydro derivatives of asparagine or glutamine, the present method excludes such formation because the reagents [$\text{Ph}_3\text{P–(PyS)}_2$] are inert toward substrates of low acidity such as the amide linkages, as illustrated below.[18]

$$\text{Z–L–Asp(NH}_2\text{)–OH} + \text{H–L–Cys(Bzl)–OMe} \longrightarrow \text{Z–L–Asp(NH}_2\text{)–L–Cys(Bzl)–OMe} + \text{Anhydro compound}$$

DCC	35%	28%
Oxid-Red. Cond.	73%	1%

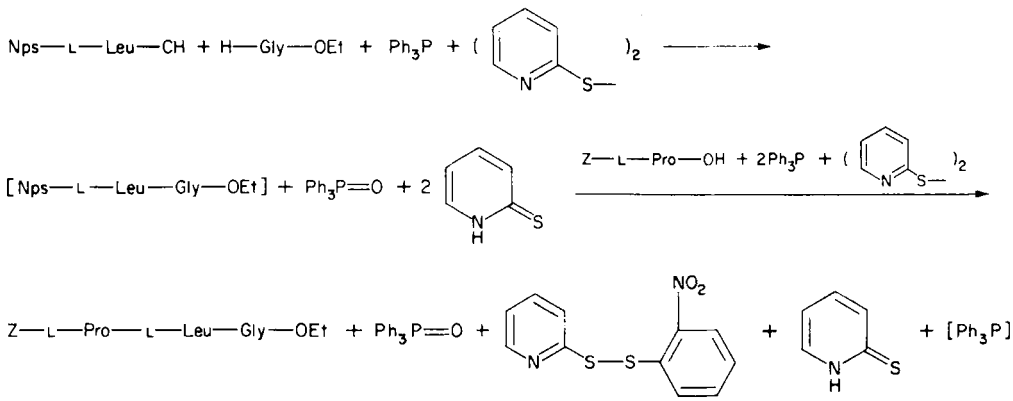
Thus analytically pure peptides are made available by an extremely simple procedure. Peptides with side chains prepared by this procedure are listed in Table V.

TABLE V
Peptides synthesized by oxidation-reduction condensation

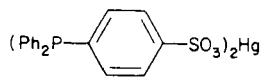
Peptides	Solvent	Yield %	mp °C	$[\alpha]_D$	(*c*, solvent)
Z–Cys(Bzl)–Gly–OEt	CH_2Cl_2	91%	97-99	–27.0	(6, AcOH)
Z–Trp–Gly–OEt	CH_2Cl_2	92	118-120	–19.8	(2, EtOH)
Z–Trp–Ile–OMe	CH_2Cl_2	93	69-70	–14.1	(2.6, DMF)
Z–Thr–Gly–OEt	CH_2Cl_2	83	105-107	–13.8	(1, EtOH)
Z–Ser–Gly–OEt	CH_2Cl_2	84	98-100	– 5.5	(1, EtOH)
Z–Gln–Gly–OEt	CH_2Cl_2	86	168-170	– 7.3	(1, DMF)
Z–Asn–Gly–OEt	DMF–CH_2Cl_2	85	185-187	– 5.6	(1, DMF)
Z–Arg(NO_2)–Gly–OEt	DMF–CH_2Cl_2		119-121	–15.4	(2, MeOH)
Z–Cys(Bzl)–Pro–Leu–Gly–NH_2	DMF	86	169-170	–59.4	(2.5, DMF)
Z–Cys(Bzl)–Tyr–Ile–Gln–Asn–Cys(Bzl)–Pro–Leu–Gly–NH_2	DMF	91	242.4 (dec.)	–44.1	(2, DMF)
Z–Ala–Gly–Leu–Gly_3–Leu–Gly–NHNHBoc	DMF	70	213.5°		

All optically active amino acids were of the L-configuration.

A very useful application of the Ph_3P–$(PyS)_2$ reagent is the elongation by two peptide bonds in a single procedure. For example, Nps–L–Leu–OH and Gly–OEt were coupled by Ph_3P–$(PyS)_2$ and, after the usual washing and drying, the reaction mixture containing Nps–L–Leu–Gly–OEt was further treated with Z–L–Pro–OH and Ph_3P–$(PyS)_2$ to afford Z–L–Pro–L–Leu–Gly–OEt in 78% yield. Z–L–He–L–Gln–L–Asn–OBu^t was also obtained in 72% yield by coupling Z–L–He–OH with Nps–L–Gln–L–Asn–OBu^t. Pyroglutamine formation was not observed; therefore this method would be useful for the synthesis of glutamyl peptides.



Acylamino acids and peptidylic acid active esters can also be prepared by the oxidation-reduction condensation.[18] Some results are shown in Table VI. Since active esters of Gln, Asn, and Arg(NO_2) precipitated from the reaction mixture, analytically pure crystalline products could be obtained by simple filtration and washing. More soluble active esters were prepared using a modified phosphine,



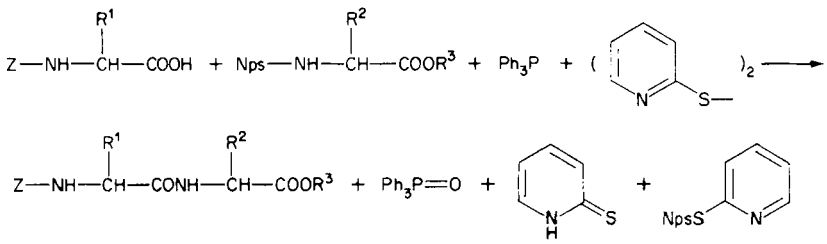
Using this modification, the corresponding phosphine oxides were removed by washing with water, and pure active esters were obtained after one recrystallization.

TABLE VI

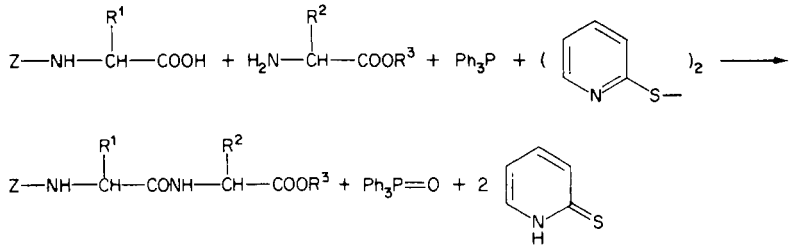
Active Esters	Yield %	mp°C	$[\alpha]_D$	(*c*, solvent)
Z–L–Glu(NH_2)–OPep	72	182-184.5	−17.3°	(1, DMF)
Nps–L–Glu(NH_2)–ONSu	74	146-147	−59.8°	(2, DMF)
Z–L–Asp–(NH_2)–OPep	74	167-168.5	−26.7°	(1, DMF)
Z–L–Arg(NO_2)–OPep	71	109-111	−14.0°	(1, DMF)
Bz–L–Leu–ONSu	59	168-169		
Z–L–Phe–ONSu	75	136-137.5	−54.2°	(1, DMF)
Z–L–Val–ONSu	61	116-117.5	−25.8°	(2, Dioxane)
Z–L–Phe–OPep	86	157.5-158	−51.4°	(1, DMF)
Z–L–Val–OPep	82	140	−22.6°	(1, DMF)
Z–L–Phe–OQu	70	138.5-139.5	−70.6°	(2, DMF)
Z–L–Ala–OQu	72	101-102	−67.7°	(1, DMF)

The above-mentioned peptide synthesis by the oxidation-reduction condensation with Ph_3P–$(PyS)_2$ can be summarized by the following three equations.

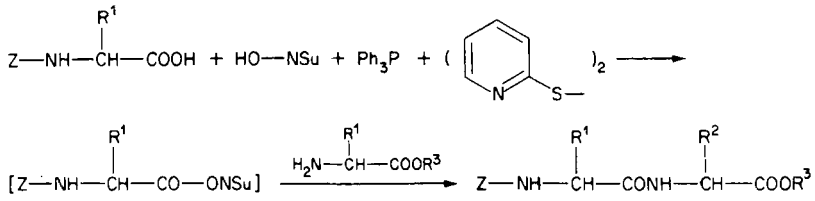
Type A



Type B



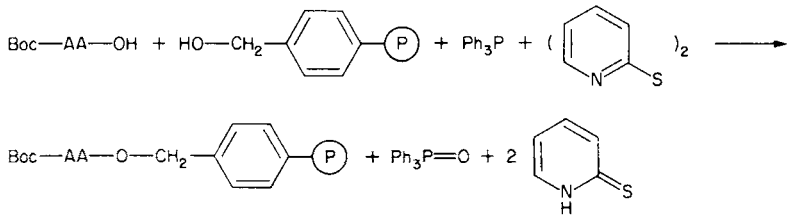
Type C



Utilization of these reactions has led to the successful formation of bioactive oxytocin. The protected nonapeptide amide was obtained in 91% yield by a type C reaction using a 50 per cent excess of reagents.

After HF treatment and air oxidation, the synthetic oxytocin (in solution) showed avian depressor activity of 104 unit/mg.[19] Next, an application of the oxidation-reduction condensation method to solid phase peptide synthesis was investigated. This study was focused on developing a general procedure for introducing the first C-terminal amino acid to the polymer support followed by succeeding couplings.

In the solid-phase peptide synthesis with *N,N'*-dicyclohexylcarbodiimide, each peptide linkage is formed under mild conditions. However, the first amino acid is introduced into the resin by treating an amino acid and the resin at 80° for 48 hours. It seems desirable that both the attachment of the first amino acid to the resin and the subsequent couplings can be carried out under mild conditions using the same procedure. For this purpose, we studied the attachment of amino acids to the resin and chainlengthening of the resulting aminoacyl resin with subsequent amino acids by the oxidation-reduction condensation. First, the usual 2% crosslinked chloromethyl resin (Scwarz-Mann, New York, Cl content 2.0 mmol/g) was converted into a hydroxymethyl resin by a literature procedure[20] and esterifications of this resin with various amino acids and peptide fragments were studied.



In a typical experiment, 2.0 g of the hydroxymethyl resin in 20 ml of CH_2Cl_2 was shaken for 30 minutes in a vessel for solid phase synthesis and the resin was washed with CH_2Cl_2. After addition of 10 ml of the CH_2Cl_2 containing three equivalents (12 mmol) each of Boc–L–Phe–OH and 2,2'-dithiodipyridine, the suspension was shaken for a few minutes, and 5 ml of CH_2Cl_2 containing three equivalents (12 mmol) of triphenylphosphine was added at room temperature. The mixture was shaken for 24 hours at room temperature and was washed with CH_2Cl_2, EtOH and DMF. From the washing solvents, 3.76 mmol of Boc–L–Phe–OH (47% of Boc–L–Phe–OH used) was recovered. The remaining free hydroxy groups on the resin were protected by acetylation with ten equivalents (40 mmol) of acetic anhydride and triethylamine in DMF for 2 hours as described in the literature.[20] The resin was washed with DMF, EtOH and CH_2Cl_2, filtered and dried *in vacuo*. A 50 mg sample was cleaved with anhydrous HF. The Phenylalanine liberated was found by an amino acid analyzer to be 0.38 mmol/g or 6.3% yield based on Boc–L–Phe–OH used and 19% yield by hydroxyl content. By this procedure the esterification of various amino acids and peptide fragments was studied and favorable results were obtained as summarized in Table VII. Amino acid hydrates and peptide fragments were easily attached to the resin by this procedure. No nitrile formation or transesterification was observed in the case of Asp and Glu. Favorable results were also obtained with Arg, Cys and Trp which have never been reported to be linked.

TABLE VII
Attachment of amino acids to hydroxymethyl resin

Amino acid	Solvent	Content (mmol/g)	
Boc–Asn–OH	DMF	0.19	(Asn)
Boc–Gln–OH	DMF	0.21	(Gln)
Boc–Arg(NO2)–OH	DMF	0.12	
Boc–Trp–OH	DMF	0.12	
Boc–Cys(Bzl)–OH	CH_2Cl_2	0.18	
Boc–Met–OH	CH_2Cl_2	0.15	

Bzl Boc–His–OH	DMF	0.44	Bzl (His)
Boc–Gly–OH	CH_2Cl_2	0.44	
Boc–Ala–OH·1/2H_2O	CH_2Cl_2	0.25	
Boc–Phe–OH	CH_2Cl_2	0.38	
Boc–Pro–OH	CH_2Cl_2	0.16	
Boc–Val–OH	CH_2Cl_2	0.12	
Boc–Ile–OH·1/2H_2O	CH_2Cl_2	0.11	
Boc–Ala–Ala–OH·H_2O	CH_2Cl_2	0.11	(Ala–Ala)
Z Boc–Lys–Phe–OH	CH_2Cl_2	0.26	(Lys, Phe)

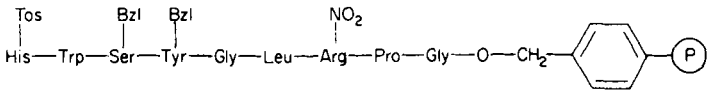
Boc–Met–OH and Boc–His(Bzl)–OH were successfully attached by this procedure. This method has the following special merits in solid phase peptide synthesis: the procedure permits esterification of all the Boc-amino acids and peptides tested (see Table VII), and it allows an attachment of a desired amount of these amino acids and peptides in a rather homogeneous environment on the resin. According to the present esterification procedure, the reaction proceeds under milder conditions and in shorter reaction time than those carried out by the ordinary method. Moreover, the reaction conditions and the operations are the same as for subsequent chain lengthening acylations and, consequently, incorporation of the esterification step in the automated process is possible. Utilization of this method has led to the successful synthesis of bio-active LH–RH and ACTH.

SYNTHESIS OF LH–RH

Pure LH–RH is synthesized by fragment condensation on solid support *via* oxidation-reduction condensation by employing two types of chain elongation: that from C-terminal amino acid (A type elongation) and that from N-terminal amino acid to C-terminal amino acid (B type elongation).[21]

i) A type elongation.

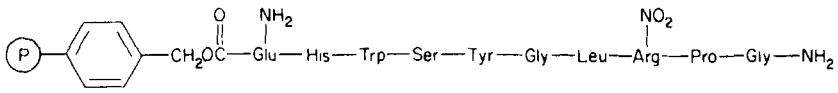
Synthesis of nonapeptide (2-10 sequence) of LH–RH is successfully achieved by the solid phase method as shown in the next scheme.



The nonapeptide was liberated by ammonolysis and was converted into LH–RH by coupling with Glu–OPcp.

ii) B type elongation.

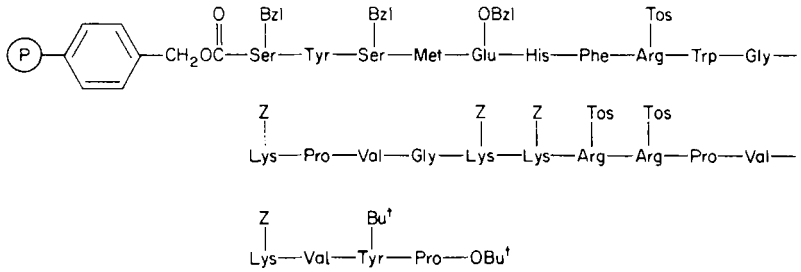
The building of decapeptide is carried out by employing three fragments of 1, 2-6 and 7-10 sequences of LH–RH as shown in the next scheme.



The decapeptide amide is liberated with HF and converted into LH–RH by cyclization of the glutaminyl residue. Fully active LH–RH is obtained in 51% yield calculated from the first resin-glutamine stage. In this manner chain elongation can be carried out with minimum protection of side chains and excess amino components can be recovered unchanged.

SYNTHESIS OF ACTH (1-24) BY B TYPE ELONGATION

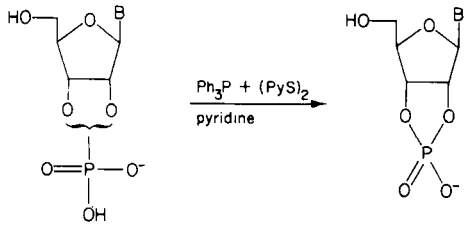
ACTH (1-24) is chain lengthened on solid support by oxidation-reduction condensation as shown in the next scheme.[22]



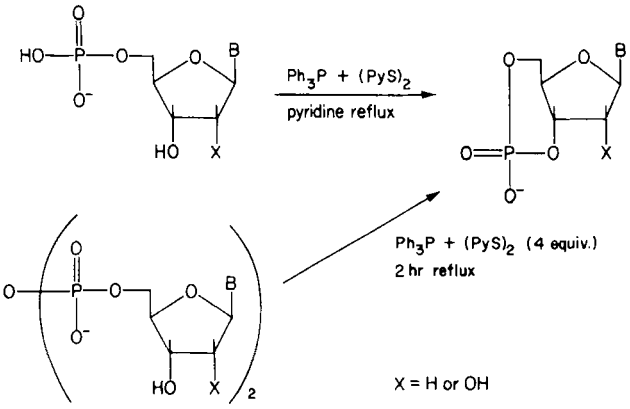
Pure ACTH (1-24) was obtained in 25% yield from the initial resin-Ser-Tyr after HF treatment and purification with Sephadex G-25 and CMC. The peptide was characterized as ACTH (1-24) in 7 AcOH·9H_2O and the $[\alpha]_D$ value was in good agreement with the literature value. Amino acid analyses of acid hydrolysate and enzymatic digestion gave similar results, and the activity *in vivo* assay was equal to Ciba's tetracosactide. The oxidation-reduction condensation reaction has also been shown to be applicable to the synthesis of nucleotides.

PREPARATION OF (2′, 3′- and 3′, 5′-) CYCLIC NUCLEOTIDES

Nucleoside 2′, 3′- and 3′, 5′-cyclic phosphates are synthesized by the oxidation-reduction condensation as shown in the following scheme. The results are summarized in Table VIII.[23]



B: uracil, adenine, cytosine, guanine.



Synthesis of adenosine 2′,3′-cyclic phosphate 5′-phosphoromorpholidate 5

TABLE VIII
Synthesis of (2′, 3′- and 3′, 5′-) cyclic nucleotides

Nucleotide	Yield (%)	Nucleotide	Yield (%)
2′, 3′-cyclic AMP	quant.	3′, 5′-cyclic AMP	85
2′, 3′-cyclic CMP	quant.	3′, 5′-cyclic CMP	56
2′, 3′-cyclic GMP	quant.	3′, 5′-cyclic GMP	85
2′, 3′-cyclic UMP	quant.	3′, 5′-cyclic UMP	80
4′, 5′-cyclic FMN[a]	quant.	3′, 5′-cyclic TMP	70

[a] FMN refers to riboflavine 5′-phosphate.

SYNTHESIS OF OLIGONUCLEOTIDES

Next it was established that $C_{3'\to5'}$-linked deoxyribooligonucleotides were successfully synthesized by the present method. Various trinucleoside diphosphates can be prepared in high yields as shown in Table IX.[23]

TABLE IX
Synthesis of trinucleoside diphosphates

Nucleotide	Yield (%)	Nucleotide	Yield (%)	Nucleotide	Yield (%)
d-TpTpT	65	d-TpApC	50	d-ApApA	60
d-TpTpA	55	d-TpCpC	52	d-ApApC	55
d-TpTpC	58	d-TpCpT	63	d-CpApT	55
d-TpApT	60	d-ApTpT	61	d-TpGpT	63
d-TpApA	58	d-ApTpA	55	d-TpGpG	50

Further, TpTpTpT was obtained in 50% yield from TpTpT and three equivalents of 3′-O-acetylthymidine 5′-phosphate.

SYNTHESIS OF OLIGONUCLEOTIDES BY THE USE OF PHOSPHORODIANILIDATES

Nucleoside 5′-phosphorodianilidates were prepared as shown in the next scheme. The results are summarized in Table X.[24]

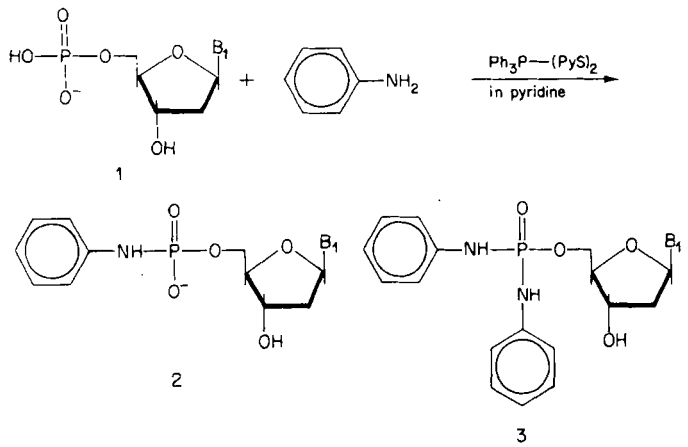
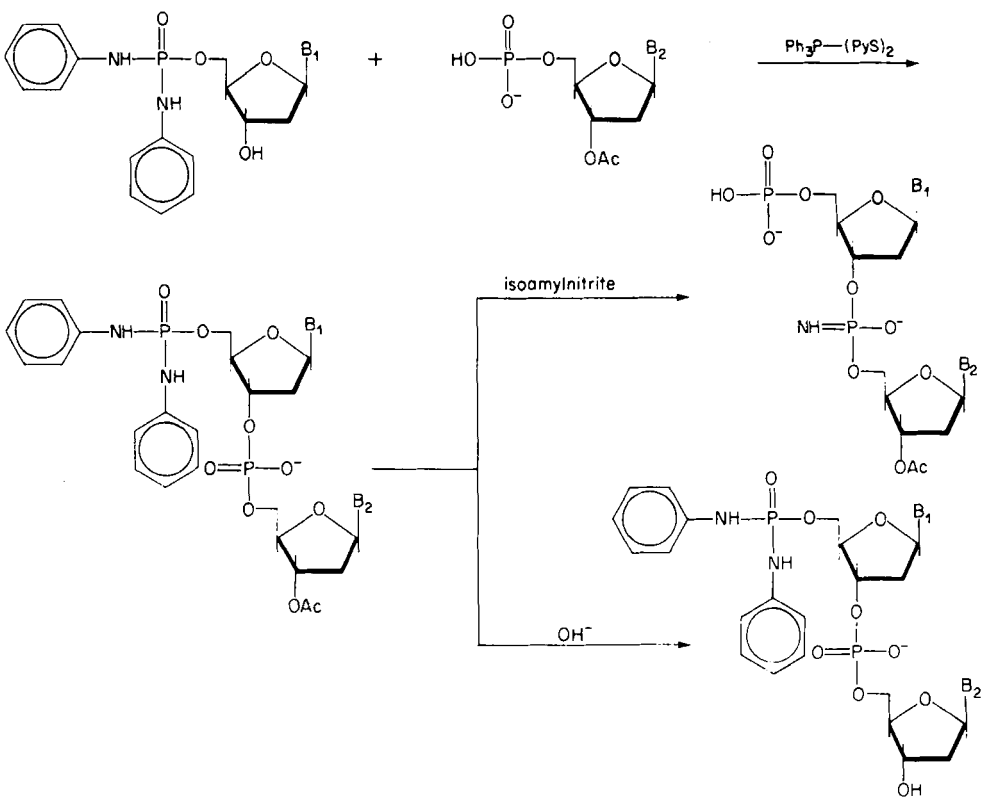


TABLE X
Synthesis of nucleotide 5′-phosphorodiamidate[a]

B_1	Amine	Reaction time (day)	Yields (%) Phosphoro-monoamidate (2)	Phosphoro-diamidate (3)
thymine	aniline	2	35	65
thymine	morpholine	5 (hr)	82.5	17.5
		1	57	43
		3	17	64[b]
N^6-benzoyl adenine	aniline	2	35	65
N^4-anisoyl cytosine	aniline	2	41	59
N^2-benzoyl guanine	aniline	2	40	60

[a] Ten equivalents each of $Ph_3P-(PyS)_2$ and ten equivalents of amine were used on parent nucleoside 5′-phosphate.
[b] TppT was also produced in 17% yield.

Protected dinucleotides were prepared by the condensation of the phosphorodianilidates with suitably protected mononucleotides according to the following scheme. The results are summarized in Table XI.[24]



Similarly various other dinucleotides d-pA^{Bz}pT, d-pA^{Bz}pC^{An}, d-pTpT, d-pTpA^{Bz}, d-pTpC^{An} were also prepared in good yields.

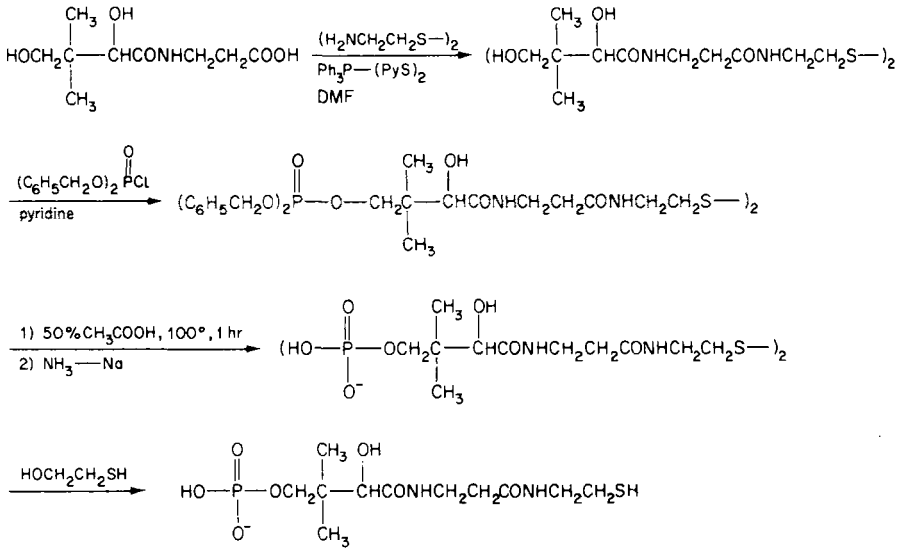
TABLE XI
Synthesis of protected dinucleotides bearing 5′-phosphate groups

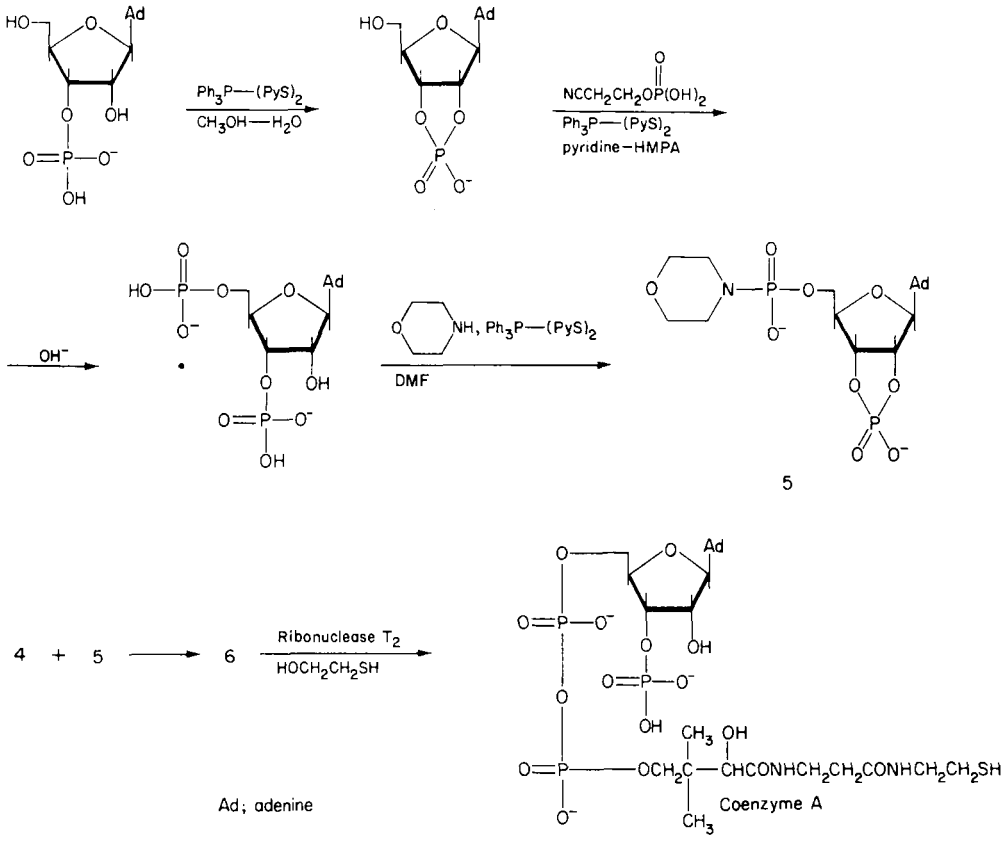
Dinucleotide	3′–OH[b] containing component	5′-phosphate containing component	Amount (mmol)	$Ph^3P–(PyS)_2$ (mmol)	Yield (%)[a]
d-pTpTOAc	d-pT	d-pTOAc	0.1	0.5	70
d-pTpC^{An}OAc	d-pT	d-pC^{An}OAc	0.07	0.5	50
d-pTpA^{Bz}OAc	d-pT	d-pA^{Bz}OAc	0.07	0.5	51
d-pA^{Bz}pTOAc	d-pA^{Bz}	d-pTOAc	0.1	1	75
d-pA^{Bz}pA^{Bz}OAc	d-pA^{Bz}	d-pA^{Bz}OAc	0.1	1	70
d-pA^{Bz}pC^{An}OAc	d-pA^{Bz}	d-pC^{An}OAc	0.1	1	70

[a] Yields were determined spectrophotometrically in water.
[b] In each case 0.05 mmol amount of component was used.

TOTAL SYNTHESIS OF COENZYME A

The total synthesis of coenzyme A by utilization of oxidation-reduction condensation in each important step was carried out according to the following scheme.[25]





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