

1

3,701,772

PRODUCTION OF NUCLEOTIDE ANHYDRIDES
 Kiminori Tamura, Manami Morozumi, Yutaka Noda,
 Morio Suzuki, and Hiroshi Yoshino, Choshi-shi, Japan,
 assignors to Yamasa Shoyu Kabushiki Kaisha, Arai,
 Choshi-shi, Chiba-ken, Japan
 No Drawing. Filed June 1, 1970, Ser. No. 42,579
 Claims priority, application Japan, June 12, 1969,
 44/45,786

Int. Cl. C07d 51/50

U.S. Cl. 260—211.5 R

4 Claims

ABSTRACT OF THE DISCLOSURE

Nucleotide anhydrides can be synthesized, in each case, by a single-step process by causing a nucleoside-5'-monophosphate and diphenyl phosphorochloridate or tetraphenyl pyrophosphate to react in a suitable solvent and in the presence of an alkylamine and then adding to the resulting process materials alkylammonium salt of an acid dissolved in pyridine or one of its derivatives.

BACKGROUND OF THE INVENTION

This invention relates generally to nucleotide anhydrides and production thereof and more particularly to a new and advanced process for producing nucleotide anhydrides by a one-step synthesis in a short time and in an economical manner.

The term "nucleotide anhydride" is herein used to designate an anhydride of nucleoside-5'-monophosphate and an acid whose acidity is weaker than diphenylphosphate. The anhydrides can be represented by the following general formula and consist of the compounds indicated in Table 1.

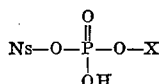


TABLE 1

[Ns: nucleoside]

Residue (—X)	Group of compounds	Examples
$\left(\begin{array}{c} \text{O} \\ \parallel \\ \text{—P—O—H} \\ \\ \text{OH} \end{array} \right)_n$	Nucleoside-5'-diphosphates. Nucleoside-5'-triphosphates.	ADP, CDP, UDP, etc. UTP, GTP, TTP, etc.
$\begin{array}{c} \text{O} \\ \parallel \\ \text{—P—O—(sugar)} \\ \\ \text{OH} \end{array}$	Sugar nucleotides.....	UDPG, GDPM, ADPG, UDPGal, etc.
$\begin{array}{c} \text{O} \\ \parallel \\ \text{—S—OH} \\ \\ \text{O} \end{array}$	Nucleoside sulfatophosphates.	Active sulfate, etc.
$\begin{array}{c} \text{—C—R} \\ \parallel \\ \text{O} \end{array}$	Nucleotide acylanhydrides. Nucleotide aminoacylanhydrides.	Acetyladenylate. Aminoacyl adenylate.
$\begin{array}{c} \text{O} \\ \parallel \\ \text{—P—OR} \\ \\ \text{OH} \end{array}$	Coenzymes.....	CoA, FAD, DCP—choline, NADP, etc.

These nucleotide anhydrides are very important substances for the metabolism within living organisms, and pharmaceutical and biochemical demand thereof is recently increasing.

For example, CDP-choline is effective for treatment of external head injuries, coma due to external injury

2

to the brain, etc. Trisodium inosine-5'-triphosphate (ITP-Na₃) and trisodium thymidine-5'-triphosphate (TTP-Na₃) are present within muscles and are valuable reagents for research on metabolism within living organisms. Adenosine triphosphate (ATP) is used for the circulation, particularly peripheral vascular disturbances, edema, beri-beri, and muscular fatigue and for the treatment of myositis (myitis, sarcitis), myasthenia, rheumatosis, arthritis, neuralgia, and other similar ailments.

Flavine adenine dinucleotide (FAD) is used as a medicine in the treatment of hepatic disturbances, trophopathy in pregnant women and lactating (breast-feeding) women, and toxicosis (toxinos, toxipathy) such as alcohol damage and nicotinism. In addition, disodium uridine-5'-diphosphoglucose (UDPG-Na₂) is of physiological value, and CDP-ethanolamine can be used as one kind of nucleotide coenzyme. Moreover, uridylyl sulfate, adenosine-5'-2,4-dinitrophenyl phosphate, adenylyl-5'-yl-carbobenzy oxyglycine, and other nucleotide anhydrides are highly promising for future development and application as medicines and biochemical reagents.

Among various methods so far established for synthesizing these nucleotide anhydrides, the anion-exchange method is considered at present to be the most advantageous. The anion-exchange method comprises the following two steps. The first step is reaction of a nucleoside-5'-monophosphate with tetraphenyl pyrophosphate or diphenyl phosphorochloridate in the presence of a tertiary base in dioxane or a mixture of dioxane and dimethyl formamide.

The reaction product is P¹-nucleoside-5'-P²-diphenyl pyrophosphate. The second step is reaction of purified P¹-nucleoside-5'-P²-diphenyl pyrophosphate with an acid (whose acidity is weaker than diphenyl phosphate) to produce a nucleotide anhydride. The second reaction is based on the transition effect of pyridine. Various nucleoside-5'-monophosphates and acids can be combined arbitrarily by the anion exchange method to prepare various nucleotide anhydrides.

In this case, the purification of the intermediate product obtained by the first reaction is accomplished by concentrating the reaction mixture under reduced pressure to remove the solvent, adding ethyl ether to the resulting residue to precipitate the compound, letting the process material stand at 0° C. for 30 to 60 minutes, and removing the ether by decantation. However, since this intermediate material is unstable when exposed to moisture and heat, it is necessary to carry out the concentration under an anhydrous condition, at a low temperature, and in a high vacuum. Furthermore, an extremely large quantity of ethyl ether is required for precipitation, giving rise to great danger for a large scale operation. Thus the method is industrially disadvantageous. Moreover, in the process for isolation of the intermediate material, its significant loss is unavoidable.

Furthermore, water or ethyl ether admixed with the intermediate material obtained by precipitation markedly inhibits the second reaction and lowers the yield. Accordingly, it is disadvantageously necessary to resort to a process step of dissolving the precipitate in dioxane and concentrating the resulting solution under reduced pressure to remove these liquids by azeotropy.

Thus, the above described known method requires two stages of reaction, and the operation for isolating the intermediate material, moreover, is complicated and requires a long time and complicated production equipment. Furthermore, a large quantity of the solvent is necessary, and a considerable amount of the intermediate material is lost.

On account of these difficulties this method is not suitable for industrial production.

SUMMARY OF THE INVENTION

It is an object of the present invention to overcome the above described difficulties by utilizing certain findings we have made and thereby to provide a new advanced process for synthesizing nucleotide anhydrides whereby the reaction can be completed in a short time in one step by a simple operation without isolation of the intermediate materials.

We have found that a nucleotide anhydride can be synthesized in a single operation by causing a nucleoside-5'-monophosphate and diphenyl phosphochloridate or tetraphenyl pyrophosphate to react in a suitable solvent and in the presence of an amine and then adding thereto an acid which is weaker than diphenyl phosphate dissolved in pyridine or one of its derivatives.

According to the present invention, briefly summarized, there is provided a process for synthesizing nucleotide anhydrides wherein diphenyl phosphochloridate or tetraphenyl pyrophosphate is added to an alkyl-ammonium salt of a nucleoside-5'-monophosphate in dioxane, N,N-dimethylformamide or N,N-dimethylacetamide and in the presence of an alkylamine, and, to the resulting process materials, a solution in pyridine of an alkylammonium salt of an acid to be condensed with the 5'-phosphate part of the desired nucleotide.

The nature, details, and utility of the invention will be more clearly apparent from the following detailed description beginning with general considerations and concluding with specific examples of practice illustrating preferred embodiments of the invention.

DETAILED DESCRIPTION

A nucleoside-5'-monophosphate is generally used in the form of an alkylammonium salt in order to facilitate dissolution thereof in a reaction solvent. Particularly in consideration of solubility in the solvent, use of a salt of a trialkylamine or quaternary ammonium hydroxide (for example, tri-n-butylamine, tri-n-octylamine, methyl-tri-n-butylammonium hydroxide, or methyl-tri-n-octylammonium hydroxide) is recommended.

Depending on the molar ratio between the nucleoside-5'-monophosphate and alkylamine base in condensation, the mono (alkylammonium) salt and the di alkylammonium salt may be obtained.

While either kind of salt can be used, the mono (alkylammonium) salt is principally used because of its reactivity.

In the present invention, the following nucleotides can be employed as one of starting materials:

Natural nucleotides such as 5'-cytidylic acid, 5'-deoxyadenylic acid, 5'-deoxyinosinic acid, 5'-deoxyguanylic acid, 5'-deoxycytidylic acid, 5'-thymidylic acid, and adenosine (2',3'-cyclic phosphate) 5'-phosphate and unnatural nucleotides such as 2-methyl-thio-5'-inosinic acid etc.

Any (reaction) solvent, which dissolves alkylammonium salts of nucleoside-5'-phosphate and does not inhibit the reaction, can be employed in this invention. Examples of such reaction solvents we have found to be suitable are N,N-dimethyl-formamide, N,N-dimethylacetamide, and dioxane. While the minimum quantity of the solvent to be used is 5 times (in moles) that of the nucleoside-5'-phosphate, a preferable range is from 10 to 50 times (in moles).

The above three solvents can be used not only alone, respectively, but also as a mixture of two or three thereof. In addition, in order to increase the solubility of the reaction reagents, tetrahydrofuran, n-hexane, benzene, toluene, ethyl acetate, acetone, or chloroform can be added to the solvent to an extent that the reaction is not inhibited. The limit to the quantity be added is 2 to 3 times by volume that of the reaction solvent, and equal volume or less is preferable.

Diphenyl phosphochloridate or tetraphenylpyrophos-

phate should be used so that its molar ratio to nucleoside-5'-phosphate is 1-2, preferentially 1-1.5.

Examples of alkylamine which is added as a reaction stabilizer are tri-n-ethylamine, tri-n-ethylamine, tri-n-octylamine, N-methylpiperidine, and diethylaniline. The stabilizer should be employed in an amount more than 0.5 mole, preferentially 1.2-2 moles, per one molar of the nucleoside-5'-phosphate.

After the reaction of nucleoside-5'-phosphate with diphenyl phosphochloridate or tetraphenylpyrophosphate, a desired acid dissolved in pyridine or its derivative is directly added to the reaction mixture, where the reaction forming nucleotide anhydride from the nucleoside-5'-monophosphate and the acid can be observed. The added acid should be a weaker acid than diphenyl phosphate. An acid whose acidity is stronger than that of diphenyl phosphate failed to form an acid anhydride with nucleoside-5'-phosphate.

Examples of an acid weaker than diphenyl phosphate are: Anhydrous sulfuric acid, phosphates such as orthophosphate, pyrophosphate, and triphosphate; sugar phosphates such as glucose-1-phosphate, mannose-1-phosphate, and galactose-1-phosphate; carboxylic acids such as acetic acid, and propionic acid, carbobenzyloxy amines; peptides; phenols such as 2,4-dinitrophenol; nucleosides; and acid constituents of coenzymes (for example, panthethin-4', 4'-bisphosphate, flavine mononucleotide, phenyl phosphate, choline phosphate, and nicotinamide phosphate).

In consideration of the solubility of the acid in an organic solvent, the acid is ordinarily added in the form of a salt of an alkylamine as, for example, tri-n-butylamine or tri-n-octylamine.

The acid should be used in an amount of more than one mole, preferentially 1-2 moles, per one molar of the nucleoside-5'-phosphate.

Substances which dissolve in pyridine with difficulty, such as flavine nucleotide, is dissolved in another solvent such as a formamide before mixing with pyridine.

It is essential to add pyridine or its derivative together with the acid simultaneously: The reaction of the acid with P¹-nucleoside-5'-P²-diphenyl pyrophosphate is regarded as an anion exchange reaction where diphenyl phosphate (a strong acid) moiety of the latter compound is replaced by the weak acid. Pyridine or its derivatives are essential for this anion exchange reaction.

This is the reason why pyridine or its derivative should be added together with the acid.

In addition to pyridine that is used satisfactorily, several pyridine derivatives such as picoline, lutidine, and cholidine can also be used. 2-50 moles, particularly 5-30 moles, of pyridine or its derivative per 1 mole of the nucleotide gives a good yield.

The reactions in the process according to the invention are generally completed in a relatively short time, although the time depends on the kind and quantity of the solvent used and the temperature.

P¹-nucleoside-5'-P²-diphenyl pyrophosphate is usually synthesized almost immediately after mixing nucleoside-5'-phosphate and diphenyl phosphochloridate or tetraphenyl pyrophosphate under the conditions described previously. Therefore, the acid dissolved in pyridine or its derivative can be added to the reaction mixture almost immediately after mixing nucleoside-5'-phosphate and diphenylphosphochloridate or tetraphenyl pyrophosphate. The anion exchange reaction is completed in a few minutes to a few hours, in a high yield, with formation of only small amounts of by-products.

The anion exchange reaction can also be carried out a few minutes to a few hours after mixing nucleoside-5'-phosphate and diphenyl phosphochloridate or tetraphenyl pyrophosphate.

It is surprising that the nucleotide anhydride can be synthesized directly in one vessel containing a complicated solvent system where several materials are added

successively. Furthermore, the synthesis of the nucleotide anhydride proceeds rapidly in a high yield, with formation of only small amounts of by-products.

The inventors discovered this surprising fact for the first time. The present invention, based on this new discovery, thus establishes an economical process for synthesizing nucleotide anhydrides which is a superior, as will be indicated hereinafter, to the known method mentioned hereinbefore.

The nucleotide anhydrides produced by the process of the invention are purified by a combination of processes such as ion exchange resin column chromatography, and precipitation process with an organic solvent and/or other precipitants. The purified products were identified with respective nucleotide anhydrides by means of paper chromatography with several solvents, and physical, chemical and enzymatic analyses.

In order to indicate still more fully the nature and utility of the present invention, the following specific examples of practice constituting preferred embodiments of the invention and results are set forth, it being understood that these examples are presented as illustrative only and that they are not intended to limit the scope of the invention.

Example 1.—Trisodium cytidine-5'-diphosphate (CDP Na_3)

To one mole of mono (methyl tri-n-butylammonium)-5'-cytidylate, 1,500 mol. of N,N-dimethylacetamide was added, and then 250 ml. of diphenyl phosphorochloridate was added.

The mixture was agitated for 5 minutes to dissolve the materials. 500 ml. of tri-n-butylamine and 2 l. of dioxane were then added to the batch.

After stirring for 5 minutes, 2 l. of pyridine containing 2 moles of mono (tri-n-butylammonium) phosphate was added to the batch.

After agitation, the mixture was left standing for one hour.

Upon completion of the reaction, the reaction mixture was concentrated in vacuo, added with 7 l. of water, then extracted with one litre of benzene.

The water layer is diluted to 50 l., and applied to an anion exchange resin Duolite A101D (Cl type, 10 l.) column. Cytidine-5'-diphosphate was eluted from the column with 0.1 N hydrochloride, and crystallized as trisodium salt (387 grammes (g.) (11.0 percent moisture)). Yield 73.5 percent.

Molar ratio of the components is indicated in Table 2:

TABLE 2

	Molar ratio		
	Cytidine	Total P	Acid labile P
Analytical value.....	1.00	2.02	0.99
Theoretical value.....	1.00	2.00	1.00

Example 2.—Trisodium inosine-5'-triphosphate (ITP Na_3)

To 200 millimoles of mono (tri-n-butylammonium) 5'-inosinate, 120 ml. of N,N-dimethyl acetamide and mixture of 250 millimoles of tetraphenylpyrophosphate, 100 ml. of tri-n-butylamine, and 600 ml. of dioxane were added successively.

After agitating 200 ml. of pyridine containing 400 millimoles of di (tri-n-butylammonium) pyrophosphate was then added to the batch.

The mixture was then left standing for 40 minutes synthesized inosine 5'-triphosphate was isolated from the reaction mixture by means of anion-exchange resin Duolite A101D (Cl type, 10 litres) column chromatography, and obtained amorphous powder as trisodium salt. (107.9 g. (10.3 percent moisture content)) Yield, 84.3 percent.

Molar ratio of the components is indicated in Table 3.

TABLE 3

	Molar ratio	
	Inosine	Total P
Analytical value.....	1.00	2.88
Theoretical value.....	1.00	3.00

Example 3.—Trisodium thymidine-5'-triphosphate (TTP Na_3)

To 10 millimoles of mono (tri-n-butylammonium)-5'-thymidylate, 5 ml. of N,N-dimethylformamide and mixture of 2.3 ml. of diphenylphosphorochloridate, 5 ml. of tri-n-butylamine, and 20 ml. of dioxane were added successively, after agitating 100 ml. of pyridine containing 20 millimoles of di (tri-n-butylammonium) pyrophosphate was added to the batch. Then, after 30 minutes, thymidine-5'-triphosphate was isolated from the reaction mixture as usual. The yield was 79.7 percent.

Example 4.—Disodium uridine-5'-diphosphoglucose (UDPG Na_2)

10 millimoles of mono (tri-n-butylammonium) 5'-uridylate was dissolved in 5 ml. of N,N-dimethylformamine, and 5 ml. of dioxane, 6 ml. of tri-n-butylamine, and 2.3 ml. of diphenyl phosphorochloridate were added to the resulting solution.

After 10 minutes 15 ml. of pyridine containing 12 millimoles of mono (tri-n-butylammonium) glucose-1-phosphate was added to the mixture.

After allowing to stand for two hours, the solvent was removed under reduced pressure.

The remaining substances were dissolved in water and applied to anion exchange resin Dowex 1 X8 (Cl-) column (100 ml.). Uridine-5'-diphosphoglucose was eluted from the column as usual, and obtained amorphous powder as disodium salt (4.12 g.). Yield, 67.5 percent.

Molar ratio of the components is indicated in Table 4.

TABLE 4

	Molar ratio			Acid labile P
	Uridine	Glucose	Total P	
Analytical value.....	1.00	0.98	1.99	1.02
Theoretical value.....	1.00	1.00	2.00	1.00

In addition 98.6 percent of the preparation was dehydrogenized by the action of UDPG dehydrogenase.

Example 5.—Adenosine-5'-diphosphoglucose (ADPG)

To one millimole of mono (methyl-tri-n-butylammonium) 5'-adenylate, 1 ml. of N,N-dimethyl acetamide, 0.22 ml., of diphenyl phosphorochloridate 1 ml. of dioxane, and 0.5 ml. of tri-n-butylamine were added successively.

After 30 minutes, 3 ml. of pyridine containing 1.5 millimoles of mono (tri-n-butylammonium) glucose-1-phosphate was added to the mixture. During one hour' reaction, adenosine-5'-diphosphoglucose was synthesized in a yield of 81.5 percent.

Example 6.—Flavine-adenine-dinucleotide (FAD)

One millimole of mono (tri-n-octylammonium) 5'-adenylate was dissolved in 1 ml. of N,N-dimethyl acetamide, and to the resulting solution, 0.25 ml. of diphenyl phosphorochloridate 2 ml. of dioxane, and 0.5 ml. of tri-n-butylamine were added.

Then 2 ml. of formamide containing 1.8 millimoles of a mono (methyl-tri-n-butylammonium) salt of flavine mononucleotide and 4 ml. of pyridine was added to the mixture. During 1.5 hours' reaction, flavine-adenine-dinucleotide was synthesized in a yield of 78.3 percent.

Example 7.—CDP-ethanolamine

Two millimoles of mono (methyl-tri-n-octylammonium) 5'-cytidylate was dissolved in 2 ml. of N,N-dimeth-

7

yl formamide, and to resulting solution, 0.5 ml. of diphenyl phosphorochloridate, 1 ml. of tri-n-butylamine, and 15 ml. of dioxane were added, the resulting solution and then being mixed.

5 ml. of pyridine containing 3 millimoles of mono (methyl-tri-n-butylammonium) salt of o-phosphoethanolamine was then added to the mixture. During 30 minutes' reaction, CDP ethanolamine, was synthesized in a yield of 52.7 percent.

Example 8.—Uridyl sulfate

10 millimoles of mono (tri-n-octylammonium) 5'-uridylylate was dissolved in 20 ml. of dioxane, and to the resulting solution, 2.5 ml. of diphenyl phosphorochloridate and 2.1 ml. of triethylamine were added.

After the resulting batch was left standing for 5 minutes at room temperature, 20 millimoles of tri-n-butylammonium sulfate and 5 ml. of pyridine were added to the mixture.

During one hours' reaction uridylyl sulfate was synthesized in a yield of 40 percent.

Example 9.—Adenosine-5'-2,4-dinitrophenyl-phosphate

To 1 millimole of mono (methyl-tri-n-butylammonium) 5'-adenylate, 2 ml. of N,N-dimethyl acetamide was added, and then 0.25 ml. of diphenyl phosphorochloridate and 0.6 ml. of tri-n-butylamine were added after allowing it to stand at room temperature for one hour, 2 ml. of pyridine containing a solution of 2 millimoles of 2,4-dinitrophenol was added to the mixture. During 2 hours' reaction at 0° C., adenosine-5'-2,4-dinitrophenyl phosphate synthesized in a yield of 91 percent.

Example 10.—Adenyl-5'-yl-carbobenzyl oxyglycine

To 1 millimole of mono (tri-n-octylammonium) 5'-adenylate, 2 ml. of N,N-dimethyl acetamide was added, after agitation a mixture of 0.25 ml. of diphenyl phosphorochloridate, 4 ml. of dioxane, and 0.5 ml. of tri-n-butylamine were added to the solution, which was then thoroughly agitated for dissolution.

2 ml. of pyridine containing 250 mg. of carbobenzyl oxyglycine was then added to the reaction mixture. During 3 hours' reaction, adenyl-5'-yl-carbobenzyl oxyglycine was synthesized in a yield of 32 percent.

What is claimed is:

1. A one-stage process for producing a nucleotide anhydride which comprises adding a member selected from the group consisting of diphenyl phosphorochloridate and tetraphenyl pyrophosphate to an alkylammonium salt of a nucleoside-5'-monophosphate in a reaction solvent selected from the group consisting of dioxane, dimethylformamide, and dimethylacetamide and in the presence of an alkylamine and adding to the resulting mixture an alkylammonium salt of an acid dissolved in pyridine or one of its derivatives whereby said anhydride is produced in a single stage without isolation of an intermediate.

2. A process for producing a nucleotide anhydride anhydride as claimed in claim 1 in which the quantities of the principal substances used therein in terms of multiples of the quantity in moles of said nucleoside-5'-monophosphate are 10 to 50 times of said dioxane, dimethylformamide or dimethylacetamide, 1 to 1.5 times of said diphenyl phosphorochloridate, 2 to 10 times of said acid, and 5 to 30 times of said pyridine solvent, and the process is carried out at a reaction temperature of from 0 to 30° C.

3. A process for producing nucleotide anhydride as claimed in claim 2 in which:

said alkyl-ammonium salt of a nucleoside-5'-monophosphate is selected from

mono(methyl-tri-n-butylammonium)-5'-cytidylate,
mono(tri-n-butylammonium)5'-inosinate,
mono(tri-n-butylammonium)-5'-thymidylate,

8

mono(tri-n-butylammonium)-5'-uridylylate,
mono(methyl-tri-n-butylammonium)-5'-adenylate,
mono(tri-n-octylammonium)-5'-adenylate,
mono(methyl-tri-n-octylammonium)-5'-cytidylate,
mono(tri-n-octylammonium)-5'-uridylylate,
mono(methyl-tri-n-butylammonium)-5'-adenylate, and
mono(tri-n-octylammonium)-5'-adenylate;

and said alkyl-ammonium salt of an acid is selected from

mono(tri-n-butylammonium) phosphate,
di(tri-n-butylammonium)pyrophosphate,
mono(tri-n-butylammonium)glucose-1-phosphate,
mono(methyl-tri-n-butylammonium)salt of flavine mononucleotide,
mono(methyl-tri-n-butylammonium)salt of flavine mononucleotide,
mono(methyl-tri-n-butylammonium)salt of o-phosphoethanolamine,
tri-n-butylammonium sulfate,
2,4-dinitrophenol, and
carbobenzyloxyglycine,

to produce, respectively, one of the compounds selected from

trisodium cytidine-5'-diphosphate (CDP Na₃),
trisodium inosine-5'-triphosphate (ITP Na₃),
trisodium thymidine-5'-triphosphate (TTP Na₃),
disodium uridine - 5' - diphosphoglucose (UDPG Na₂),
adenosine-5'-diphosphoglucose (ADPG),
flavine-adenine-dinucleotide (FAD),
CDP-ethanolamine,
uridylyl sulfate,
adenosine-5'-2,4-dinitrophenylphosphate, and
adenyl-5'-yl-carbobenzyl oxyglycine.

4. In a process for the preparation of a nucleotide anhydride by the steps of reacting a nucleoside-5'-monophosphate with a reagent selected from a member of the group consisting of tetraphenyl pyrophosphate and diphenyl phosphorochloridate in the presence of a tertiary base and in a solvent, isolating and purifying the resultant P¹-nucleoside-5'-P²-diphenyl pyrophosphate, and then reacting said P¹-nucleoside-5'-P²-diphenyl pyrophosphate with an acid having a weaker acidity than diphenyl phosphate to produce said anhydride, the improvement which comprises carrying out said preparation in a single stage without isolation and purification of said P¹-nucleoside-5'-P²-diphenyl pyrophosphate by mixing an alkylammonium salt of said nucleoside-5'-monophosphate with said reagent in a solvent which does not inhibit the reaction in the presence of an alkylamine and then adding directly to the resultant mixture an alkylammonium salt of said acid dissolved in pyridine or a pyridine derivative to form said anhydride.

References Cited

UNITED STATES PATENTS

3,089,869	5/1963	Mauvernay	260—211.5 R
3,299,043	1/1967	Schramm et al.	260—211.5 R
3,321,146	5/1967	Moffatt	260—211.5 R
3,321,463	5/1967	Moffatt	260—211.5 R

LEWIS GOTTS, Primary Examiner
J. R. BROWN, Assistant Examiner

U.S. Cl. X.R.

260—999