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(54) Titre : NOUVEAUX ANALOGUES DE NUCLEOSIDES ET D'OLIGONUCLEOTIDES
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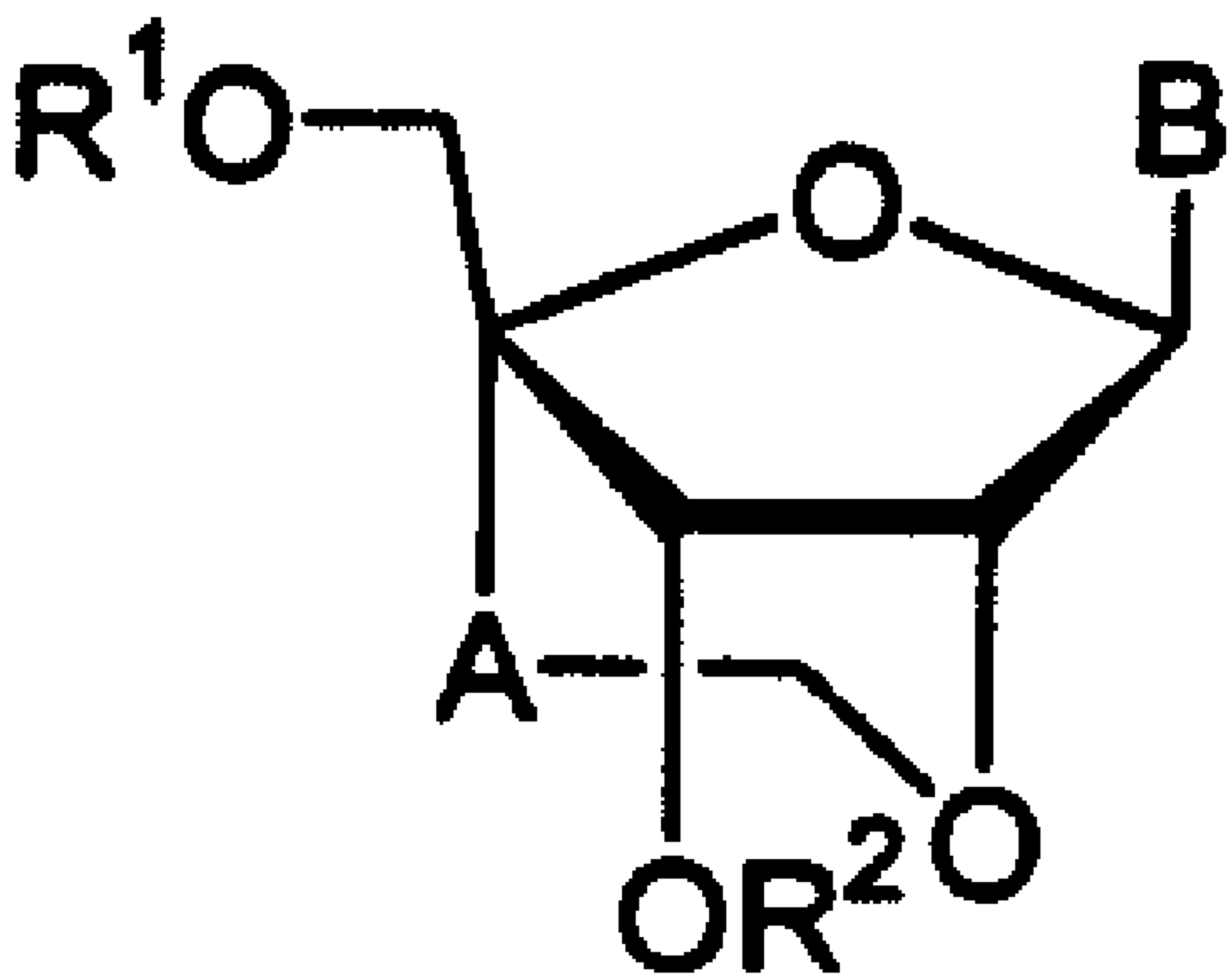


(1)

(57) Abrégé/Abstract:
[Subject] The object of the present invention is to provide novel oligonucleotide analogues, which exhibit antisense or antigene activity having excellent stability, or exhibit excellent activity as a detection agent (probe) for a specific gene or as a primer for

(57) Abrégé(suite)/Abstract(continued):

starting amplification, and to novel nucleoside analogues which are intermediates for their production. [Solution] A compound of the formula (1): (see formula 1) [wherein R^1 and R^2 are the same or different and represent a hydrogen atom, a hydroxyl protecting group, a phosphoric acid group, or -P(R^3)R^4 [wherein R^3 and R^4 are the same or different and represent a hydroxyl group, an amino group, an alkoxy group having from 1 to 4 carbon atoms, a cyanoalkoxy group having from 1 to 5 carbon atoms or an amino group substituted by an alkyl group having from 1 to 4 carbon atoms]; A represents an alkylene group having from 1 to 4 carbon atoms; and B represents a purin-9-yl group, a 2-oxo-pyrimidin-1-yl group or a substituted purin-9-yl group or a substituted 2-oxo-pyrimidin-1-yl group having a substituent selected from the following a group]; or the salt thereof; (α group) a hydroxyl group which may be protected, an alkoxy group having from 1 to 4 carbon atoms, a mercapto group which may be protected, an alkylthio group having from 1 to 4 carbon atoms, an alkoxy group having from 1 to 4 carbon atoms, an amino group which may be protected, a mono- or di-alkylamino group which may be substituted by an alkyl group having from 1 to 4 carbon atoms, an alkyl group having from 1 to 4 carbon atoms and a halogen atom. A compound of the formula (I), and the pharmacologically acceptable salts or derivatives thereof.

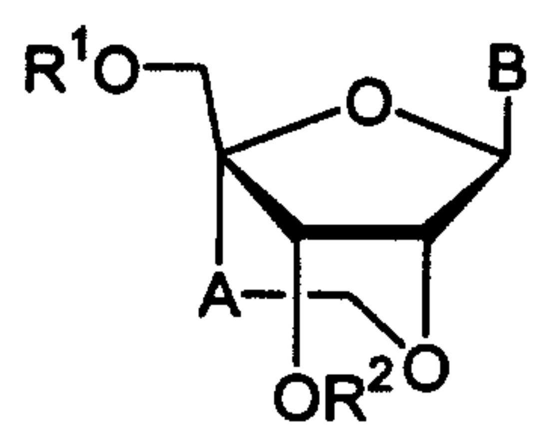
Abstract

[Subject]

The object of the present invention is to provide novel oligonucleotide analogues, which exhibit antisense or antigene activity having excellent stability, or exhibit excellent activity as a detection agent (probe) for a specific gene or as a primer for starting amplification, and to novel nucleoside analogues which are intermediates for their production.

[Solution]

A compound of the formula (1):



(1)

[wherein R^1 and R^2 are the same or different and represent a hydrogen atom, a hydroxyl protecting group, a phosphoric acid group, or $-P(R^3)R^4$ [wherein R^3 and R^4 are the same or different and represent a hydroxyl group, an amino group, an alkoxy group having from 1 to 4 carbon atoms, a cyanoalkoxy group having from 1 to 5 carbon atoms or an amino group substituted by an alkyl group having from 1 to 4 carbon atoms];

A represents an alkylene group having from 1 to 4 carbon atoms; and B represents a purin-9-yl group, a 2-oxo-pyrimidin-1-yl group or a substituted purin-9-yl group or a substituted 2-oxo-pyrimidin-1-yl group having a substituent selected from the following α group];

or the salt thereof;

(α group)

a hydroxyl group which may be protected, an alkoxy group having from 1 to 4 carbon atoms, a mercapto group which may be protected, an alkylthio group having from 1 to 4 carbon atoms, an alkoxy group having from 1 to 4 carbon atoms, an amino group which may be protected, a mono- or di-alkylamino group which may be substituted by an alkyl group having from 1 to 4 carbon atoms, an alkyl group having from 1 to 4 carbon atoms and a halogen atom. A compound of the formula (I), and the pharmacologically acceptable salts or derivatives thereof.

SPECIFICATION

NOVEL NUCLEOSIDE AND OLIGONUCLEOTIDE ANALOGUES

[Technical Field]

The present invention relates to novel oligonucleotide analogues, which exhibit antisense or antigene activity having excellent stability, or exhibit excellent activity as a detection agent (probe) for a specific gene or as a primer for starting amplification, and to novel nucleoside analogues which are intermediates for their production.

[Background Art]

Oligonucleotide analogues, which have excellent antisense or antigene activity and which are stable in the body are expected to be useful pharmaceuticals. In addition, oligonucleotide analogues having a high degree of stable complementary chain formation ability with DNA or mRNA are useful as detection agents for a specific gene or as primers for starting amplification.

In contrast, naturally-occurring oligonucleotides are known to be quickly decomposed by various nucleases present in the blood and cells. In some cases, naturally-occurring oligonucleotides may not have sufficient sensitivity for use as detection agents for specific genes or as primers for starting amplification due to limitations on their affinity with complementary base sequences.

In order to overcome these shortcomings, various non-naturally-occurring oligonucleotide analogues have been produced, and have been attempted to be developed for use as pharmaceuticals or detection agents for specific genes. Namely, known examples of such non-naturally-occurring oligonucleotide analogues include those in which an oxygen atom attached to a phosphorus atom in a phosphodiester bond of an oligonucleotide is replaced with a sulfur atom, those in which said oxygen atom is replaced with a methylene group, those in which said oxygen atom is replaced with a boron atom, and those in which a sugar moiety or base moiety of an oligonucleotide is chemically modified. For example, ISIS Corp. has developed thioate-type oligonucleotide ISIS2922 (Vitravene) as a therapeutic agent for human cytomegalovirus retinitis and ISIS2922 has been put on the open market in the United States.

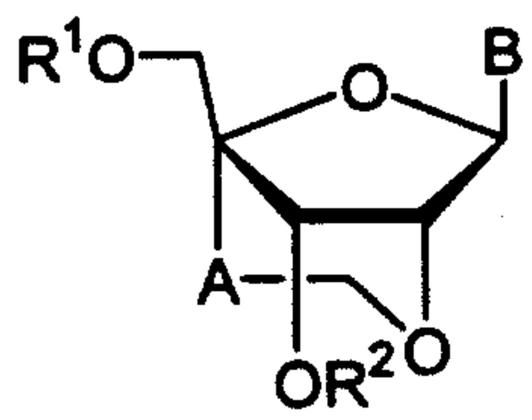
However, in consideration of the potency of the antisense or antigene activity in the above non-naturally-occurring oligonucleotide analogues, namely the ability to form a stable complementary chain with DNA or mRNA, stability with respect to various nucleases, and the manifestation of adverse side effects due to non-specific bonding with various proteins in the body, there has been a need for a non-naturally-occurring oligonucleotide analogue having even better stability in the body, a low incidence of adverse side effects and a high ability to form complementary chains.

[Disclosure of the Invention]

The inventors of the present invention conducted intensive research over a long period of time on non-naturally-occurring oligonucleotide analogues having excellent antisense or antigene activity, excellent stability in the body and a low incidence of adverse side effects. As a result of that research, they found that oligonucleotide analogues or nucleoside analogues having an ether bond in said molecules are useful as an antisense or antigene pharmaceutical having excellent stability, a detection agent (probe) for a specific gene, a primer for starting amplification or as intermediates for their production, and accomplished the present invention.

In the following, the present invention will be described in detail.

The novel nucleoside analogues of the present invention are compounds of the formula (1):



(1)

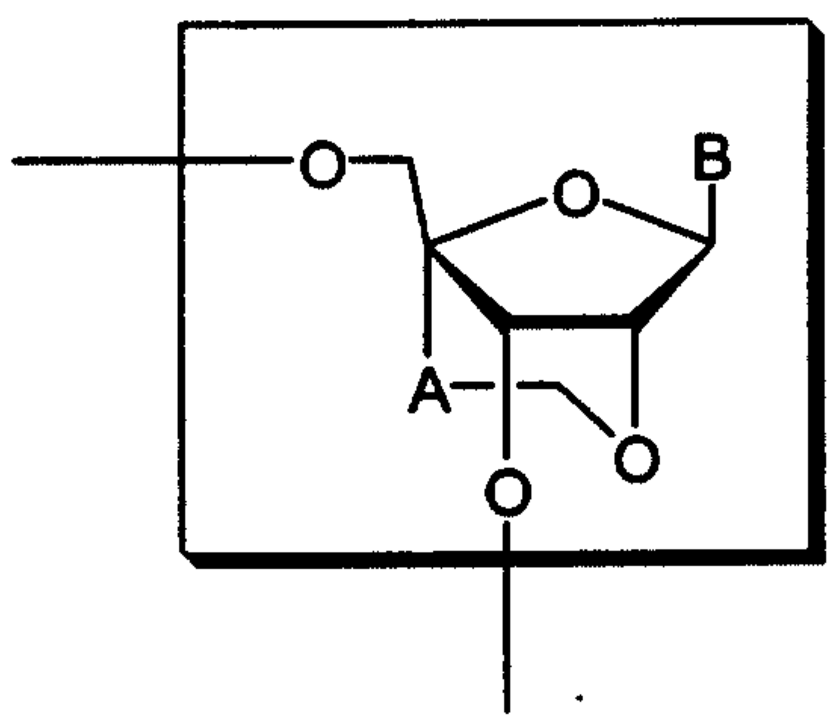
[wherein R^1 and R^2 are the same or different and represent a hydrogen atom, a hydroxyl protecting group, a phosphoric acid group, a protected phosphoric acid group or $-P(R^3)R^4$ [wherein R^3 and R^4 are the same or different and represent a hydroxyl group, a protected hydroxyl group, a mercapto group, a protected mercapto group, an amino group, an alkoxy group having from 1 to 4 carbon atoms, an alkylthio group having from 1 to 4 carbon atoms, a cyanoalkoxy group having from 1 to 5 carbon atoms or an amino group substituted by an alkyl group having from 1 to 4 carbon atoms];

A represents an alkylene group having from 1 to 4 carbon atoms; and

B represents a purin-9-yl group, a 2-oxo-pyrimidin-1-yl group or a substituted purin-9-yl group or a substituted 2-oxo-pyrimidin-1-yl group having a substituent selected from the following α group];

or salts thereof.

The oligonucleotide analogues of the present invention are oligonucleotide analogues having one or two or more structures of the formula (2) :



(2)

[wherein A represents an alkylene group having from 1 to 4 carbon atoms; and B represents a purin-9-yl group, a 2-oxo-pyrimidin-1-yl group or a substituted purin-9-yl group or a substituted 2-oxo-pyrimidin-1-yl group having a substituent selected from the following α group];

or a pharmacologically acceptable salt thereof.

(α group)

a hydroxyl group,

a protected hydroxyl group,

an alkoxy group having from 1 to 4 carbon atoms,

a mercapto group,

a protected mercapto group,

an alkylthio group having from 1 to 4 carbon atoms,

an amino group,

a protected amino group,

an amino group substituted by an alkyl group having from 1 to 4 carbon atoms,

an alkyl group having from 1 to 4 carbon atoms, and

a halogen atom.

"The alkylene group having from 1 to 4 carbon atoms" of A in the above formula (1) or (2) may include methylene, ethylene, trimethylene and tetramethylene groups, preferably a methylene group.

The protecting group of "the hydroxyl protecting group" of R^1 and R^2 and "the protected hydroxyl group" of R^3 and R^4 or the α group in the above formula (1) or (2) refers to a protecting group which can be cleaved by a chemical method such as hydrogenolysis, decomposition, hydrolysis, electrolysis and photolysis or a biological method such as hydrolysis in the human body, and such protecting groups may include "an aliphatic acyl group" such as an alkylcarbonyl group, e.g., formyl, acetyl, propionyl, butyryl, isobutyryl, pentanoyl, pivaloyl, valeryl, isovaleryl, octanoyl, nonanoyl, decanoyl, 3-methylnonanoyl, 8-methylnonanoyl, 3-ethyloctanoyl, 3,7-dimethyloctanoyl, undecanoyl, dodecanoyl, tridecanoyl, tetradecanoyl, pentadecanoyl, hexadecanoyl, 1-methylpentadecanoyl, 14-methylpentadecanoyl, 13,13-dimethyltetradecanoyl, heptadecanoyl, 15-methylhexadecanoyl, octadecanoyl, 1-methylheptadecanoyl, nonadecanoyl, eicosanoyl and heneicosanoyl, a carboxylated alkylcarbonyl group, e.g., succinoyl, glutaroyl and adipoyl, a halogeno lower alkylcarbonyl group, e.g., chloroacetyl, dichloroacetyl, trichloroacetyl and trifluoroacetyl, a lower alkoxy lower alkylcarbonyl group, e.g., methoxyacetyl, and an unsaturated alkylcarbonyl group, e.g., (E)-2-methyl-2-butenoyl;

"an aromatic acyl group" such as an arylcarbonyl group, e.g., benzoyl, α-naphthoyl and β-naphthoyl, a halogenoarylcarbonyl group, e.g., 2-bromobenzoyl and 4-chloro-benzoyl, a lower alkylated arylcarbonyl group, e.g., 2,4,6-trimethylbenzoyl and 4-toluoyl, a lower alkoxylated arylcarbonyl group, e.g., 4-anisoyl, a carboxylated arylcarbonyl group, e.g., 2-carboxybenzoyl, 3-carboxybenzoyl and 4-carboxybenzoyl, a nitrated arylcarbonyl group, e.g., 4-nitrobenzoyl and 2-nitrobenzoyl, a lower alkoxy carbonylated arylcarbonyl group, e.g., 2-(methoxycarbonyl)benzoyl and an arylated arylcarbonyl group, e.g., 4-phenylbenzoyl;

"a tetrahydropyranyl group or a tetrahydrothiopyranyl group" such as tetrahydropyran-2-yl, 3-bromotetrahydropyran-2-yl, 4-methoxytetrahydropyran-4-yl, tetrahydrothiopyran-2-yl and 4-methoxytetrahydrothiopyran-4-yl;

"a tetrahydrofuranyl group or a tetrahydrothiofuranyl group" such as tetrahydrofuran-2-yl and tetrahydrothiofuran-2-yl;

"a silyl group" such as a tri-lower alkylsilyl group, e.g., trimethylsilyl, triethylsilyl,

isopropyldimethylsilyl, t-butyldimethylsilyl, methyldiisopropylsilyl, methyldi-t-butylsilyl and triisopropylsilyl and a tri-lower alkylsilyl group substituted by one or two aryl groups, e.g., diphenylmethylsilyl, diphenylbutylsilyl, diphenylisopropylsilyl and phenyldiisopropylsilyl;
"a lower alkoxymethyl group" such as methoxymethyl, 1,1-dimethyl-1-methoxy-methyl, ethoxymethyl, propoxymethyl, isopropoxymethyl, butoxymethyl and t-butoxymethyl;
"a lower alkoxylated lower alkoxymethyl group" such as 2-methoxyethoxymethyl;
"a halogeno lower alkoxymethyl group" such as 2,2,2-trichloroethoxymethyl and bis(2-chloroethoxy)methyl;
"a lower alkoxylated ethyl group" such as 1-ethoxyethyl and 1-(isopropoxy)ethyl;
"a halogenated ethyl group" such as 2,2,2-trichloroethyl;
"a methyl group substituted by from 1 to 3 aryl groups" such as benzyl, α-naphthyl-methyl, β-naphthylmethyl, diphenylmethyl, triphenylmethyl, α-naphthyldiphenyl-methyl and 9-anthrylmethyl;
"a methyl group substituted by from 1 to 3 aryl groups wherein said aryl ring is substituted by a lower alkyl, lower alkoxy, halogen or cyano group" such as 4-methyl-benzyl, 2,4,6-trimethylbenzyl, 3,4,5-trimethylbenzyl, 4-methoxybenzyl, 4-methoxy-phenyldiphenylmethyl, 4,4'-dimethoxytriphenylmethyl, 2-nitrobenzyl, 4-nitrobenzyl, 4-chlorobenzyl, 4-bromobenzyl and 4-cyanobenzyl;
"a lower alkoxycarbonyl group" such as methoxycarbonyl, ethoxycarbonyl, t-butoxycarbonyl and isobutoxycarbonyl;
"a lower alkoxycarbonyl group substituted by halogen or a tri-lower alkylsilyl group" such as 2,2,2-trichloroethoxycarbonyl and 2-trimethylsilylethoxycarbonyl;
"an alkenyloxycarbonyl group" such as vinyloxycarbonyl and allyloxycarbonyl; and
"an aralkyloxycarbonyl group wherein said aryl ring may be substituted by one or two lower alkoxy or nitro groups" such as benzyloxycarbonyl, 4-methoxybenzyloxy-carbonyl, 3,4-dimethoxybenzyloxycarbonyl, 2-nitrobenzyloxy-carbonyl and 4-nitrobenzyloxycarbonyl.

"The hydroxyl protecting group" of R^1 and R^2 may referably include "the aliphatic acyl group", "the aromatic acyl group", "the methyl group substituted by from 1 to 3 aryl groups", "the methyl group substituted by from 1 to 3 aryl groups wherein said aryl ring is substituted by a lower alkyl, lower alkoxy, halogen or cyano group" or "the silyl group"; more preferably an acetyl group, a benzoyl group, a benzyl group, a p-methoxybenzoyl group, a dimethoxytrityl group, a monomethoxytrityl group or a tert-butyldiphenylsilyl group.

The protecting group of the "protected hydroxyl group" of R^3 and R^4 or the α group may preferably include "the aliphatic acyl group" or "the aromatic acyl group", more preferably a benzoyl group.

The protecting group of "the protected phosphoric acid group" of R^1 and R^2 in the above formula (1) represents a protecting group which can be cleaved by a chemical method such as hydrogenolysis, hydrolysis, electrolysis and photolysis and a biological method such as hydrolysis in the human body and such protecting groups may include "a lower alkyl group" such as methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl, s-butyl, tert-butyl, n-pentyl, isopentyl, 2-methylbutyl, neopentyl, 1-ethyl-propyl, n-hexyl, isohexyl, 4-methylpentyl, 3-methylpentyl, 2-methylpentyl, 1-methylpentyl, 3,3-dimethylbutyl, 2,2-dimethylbutyl, 1,1-dimethylbutyl, 1,2-dimethylbutyl, 1,3-dimethylbutyl, 2,3-dimethylbutyl and 2-ethylbutyl;
"a cyanated lower alkyl group" such as 2-cyanoethyl and 2-cyano-1,1-dimethylethyl;
"an ethyl group substituted by a silyl group" such as 2-methyldiphenylsilylethyl, 2-trimethylsilylethyl and 2-triphenylsilylethyl;
"a halogenated lower alkyl group" such as 2,2,2-trichloroethyl, 2,2,2-tribromoethyl, 2,2,2-trifluoroethyl and 2,2,2-trichloro-1,1-dimethylethyl;
"a lower alkenyl group" such as ethenyl, 1-propenyl, 2-propenyl, 1-methyl-2-propenyl, 1-methyl-1-propenyl, 2-methyl-1-propenyl, 2-methyl-2-propenyl, 2-ethyl-2-propenyl, 1-butenyl, 2-butenyl, 1-methyl-2-butenyl, 1-methyl-1-butenyl, 3-methyl-2-butenyl, 1-ethyl-2-butenyl, 3-butenyl, 1-methyl-3-butenyl, 2-methyl-3-butenyl, 1-ethyl-3-butenyl, 1-pentenyl, 2-pentenyl, 1-methyl-2-pentenyl, 2-methyl-2-pentenyl, 3-pentenyl, 1-methyl-3-pentenyl, 2-methyl-3-pentenyl, 4-pentenyl, 1-methyl-4-pentenyl, 2-methyl-4-pentenyl, 1-hexenyl, 2-hexenyl, 3-hexenyl, 4-hexenyl and 5-hexenyl,
"a cycloalkyl group" such as cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, norbornyl and adamantyl;
"a cyanated lower alkenyl group" such as 2-cyanobutenyl;
"an aralkyl group" such as benzyl, α-naphthylmethyl, β-naphthylmethyl,

indenylmethyl, phenanethrenylmethyl, anthracenylmethyl, diphenylmethyl, triphenylmethyl, 1-phenethyl, 2-phenethyl, 1-naphthylethyl, 2-naphthylethyl, 1-phenylpropyl, 2-phenylpropyl, 3-phenylpropyl, 1-naphthylpropyl, 2-naphthylpropyl, 3-naphthylpropyl, 1-phenylbutyl, 2-phenylbutyl, 3-phenylbutyl, 4-phenylbutyl, 1-naphthylbutyl, 2-naphthylbutyl, 3-naphthylbutyl, 4-naphthylbutyl, 1-phenylpentyl, 2-phenylpentyl, 3-phenylpentyl, 4-phenylpentyl, 5-phenylpentyl, 1-naphthylpentyl, 2-naphthylpentyl, 3-naphthylpentyl, 4-naphthylpentyl, 5-naphthylpentyl, 1-phenylhexyl, 2-phenylhexyl, 3-phenylhexyl, 4-phenylhexyl, 5-phenylhexyl, 6-phenylhexyl, 1-naphthylhexyl, 2-naphthylhexyl, 3-naphthylhexyl, 4-naphthylhexyl, 5-naphthylhexyl and 6-naphthylhexyl;

"an aralkyl group wherein said aryl ring is substituted by a nitro group or a halogen atom" such as 4-chlorobenzyl, 2-(4-nitrophenyl)ethyl, o-nitrobenzyl, 4-nitrobenzyl, 2,4-di-nitrobenzyl and 4-chloro-2-nitrobenzyl;

"an aryl group" such as phenyl, indenyl, naphthyl, phenanthrenyl and anthracenyl; and

"an aryl group substituted by a lower alkyl group, a halogen atom or a nitro group" such as 2-methylphenyl, 2,6-dimethylphenyl, 2-chlorophenyl, 4-chlorophenyl, 2,4-dichlorophenyl, 2,5-dichlorophenyl, 2-bromophenyl, 4-nitrophenyl and 4-chloro-2-nitrophenyl;

preferably "the lower alkyl group", "the lower alkyl group substituted by a cyano group", "the aralkyl group" or "the aralkyl group wherein said aryl ring is substituted by a nitro group or a halogen atom"; more preferably a 2-cyanoethyl group, a 2,2,2-trichloroethyl group or a benzyl group.

"The alkoxy group having from 1 to 4 carbon atoms" of R^3 and R^4 or the α group in the above formula (1) or (2) may include methoxy, ethoxy, n-propoxy, isopropoxy, n-butoxy, isobutoxy, s-butoxy or tert-butoxy, preferably a methoxy or ethoxy group.

The protecting group of "the protected mercapto group" of R^3 and R^4 or the α group in the above formula (1) or (2) may include, in addition to the hydroxyl protecting groups mentioned above, "a group which forms a disulfide" such as an alkylthio group, e.g., methylthio, ethylthio, tert-butylthio and an aralkylthio group such as benzylthio, preferably "the aliphatic acyl group" or "the aromatic acyl group", more preferably a benzoyl group.

"The alkylthio group having from 1 to 4 carbon atoms" of R^3 and R^4 or the α group in the above formula (1) or (2) may include methylthio, ethylthio, propylthio, isopropylthio, butylthio, isobutylthio, s-butylthio and tert-butylthio, preferably a methylthio or ethylthio group.

The protecting group of "the protected amino group" of the α group in the above formula (1) or (2) may include

"an aliphatic acyl group" such as an alkylcarbonyl group, e.g., formyl, acetyl, propionyl, butyryl, isobutyryl, pentanoyl, pivaloyl, valeryl, isovaleryl, octanoyl, nonanoyl, decanoyl, 3-methylnonanoyl, 8-methylnonanoyl, 3-ethyloctanoyl, 3,7-dimethyloctanoyl, undecanoyl, dodecanoyl, tridecanoyl, tetradecanoyl, pentadecanoyl, hexadecanoyl, 1-methylpentadecanoyl, 14-methylpentadecanoyl, 13,13-dimethyl-tetradecanoyl, heptadecanoyl, 15-methylhexadecanoyl, octadecanoyl, 1-methyl-heptadecanoyl, nonadecanoyl, eicosanoyl and heneicosanoyl, a carboxylated alkylcarbonyl group, e.g., succinoyl, glutaroyl and adipoyl, a halogeno lower alkylcarbonyl group, e.g., chloroacetyl, dichloroacetyl, trichloroacetyl and trifluoroacetyl, a lower alkoxy lower alkylcarbonyl group, e.g., methoxyacetyl, and an unsaturated alkylcarbonyl group, e.g., (E)-2-methyl-2-butenoyl;

"an aromatic acyl group" such as an arylcarbonyl group, e.g., benzoyl, α-naphthoyl and β-naphthoyl, a halogenoarylcarbonyl group, e.g., 2-bromobenzoyl and 4-chlorobenzoyl, a lower alkylated arylcarbonyl group, e.g., 2,4,6-trimethylbenzoyl and 4-toluoyl, a lower alkoxylated arylcarbonyl group, e.g., 4-anisoyl, a carboxylated arylcarbonyl group, e.g., 2-carboxybenzoyl, 3-carboxybenzoyl and 4-carboxybenzoyl, a nitrated arylcarbonyl group, e.g., 4-nitrobenzoyl and 2-nitrobenzoyl, a lower alkoxy carbonylated arylcarbonyl group, e.g., 2-(methoxycarbonyl)benzoyl and an arylated arylcarbonyl group, e.g., 4-phenylbenzoyl;

"a lower alkoxycarbonyl group" such as methoxycarbonyl, ethoxycarbonyl, t-butoxycarbonyl and isobutoxycarbonyl;

"a lower alkoxycarbonyl group substituted by halogen or a tri-lower alkylsilyl group" such as 2,2,2-trichloroethoxycarbonyl and 2-trimethylsilylethoxycarbonyl;

"an alkenyloxycarbonyl group" such as vinyloxycarbonyl and allyloxycarbonyl; and

"an aralkyloxycarbonyl group wherein said aryl ring may be substituted by a lower alkoxy or nitro group" such as benzyloxycarbonyl, 4-methoxybenzyloxycarbonyl, 3,4-dimethoxybenzyloxycarbonyl, 2-nitrobenzyloxycarbonyl, and 4-nitrobenzyloxycarbonyl, preferably "the aliphatic acyl group or "the aromatic acyl group", more preferably a benzoyl group.

"The amino group substituted by an alkyl group having from 1 to 4 carbon atoms" of R^3 and R^4 or the α group in the above formula (1) or (2) may include methylamino, ethylamino, propylamino, isopropylamino, butylamino, isobutylamino, s-butylamino, tert-butylamino, dimethylamino, diethylamino, dipropylamino, diisopropylamino, dibutylamino, diisobutylamino, di(s-butyl)amino and di(tert-butyl)amino, preferably methylamino, ethylamino, dimethylamino, diethylamino or diisopropylamino.

"The cyanoalkoxy group having from 1 to 5 carbon atoms" of R^3 and R^4 in the above formula (1) represents a group in which the above-described "the alkoxy group having from 1 to 4 carbon atoms" is substituted by a cyano group, and such a group may include cyanomethoxy, 2-cyanoethoxy, 3-cyanopropoxy, 4-cyanobutoxy, 3-cyano-2-methylpropoxy or 1-cyanomethyl-1,1-dimethylmethoxy, preferably a 2-cyanoethoxy group.

"The alkyl group having from 1 to 4 carbon atoms" of the α group in the above formula (1) or (2) may include methyl, ethyl, propyl, isopropyl, butyl, isobutyl, s-butyl and tert-butyl, preferably a methyl or ethyl group.

"The halogen atom" of the α group in the above formula (1) or (2) may include a fluorine atom, a chlorine atom, a bromine atom or an iodine atom, preferably a fluorine atom or a chlorine atom.

The preferred groups of "the purin-9-yl group" and "the substituted purin-9-yl group" of B in the above formula (1) or (2) may include, as a whole, 6-aminopurin-9-yl (i.e., adeninyl), 6-aminopurin-9-yl the amino group of which is protected, 2,6-diaminopurin-9-yl, 2-amino-6-chloropurin-9-yl, 2-amino-6-chloropurin-9-yl the amino group of which is protected, 2-amino-6-fluoropurin-9-yl, 2-amino-6-fluoropurin-9-yl the amino group of which is protected, 2-amino-6-bromopurin-9-yl, 2-amino-6-bromopurin-9-yl the amino group of which is protected, 2-amino-6-hydroxypurin-9-yl (i.e., guaninyl), 2-amino-6-hydroxypurin-9-yl the amino group of which is protected, 2-amino-6-hydroxypurin-9-yl the amino and hydroxyl groups of which are protected, 6-amino-2-methoxypurin-9-yl, 6-amino-2-chloropurin-9-yl, 6-amino-2-fluoropurin-9-yl, 2,6-dimethoxypurin-9-yl, 2,6-dichloropurin-9-yl or

6-mercaptopurin-9-yl, more preferably a 6-benzoylaminopurin-9-yl, adeninyl, 2-isobutyrylamino-6-hydroxypurin-9-yl or guaninyl group.

The preferred groups of "the 2-oxo-pyrimidin-1-yl group" and "the substituted 2-oxo-pyrimidin-1-yl group" of B in the above formula (1) or (2) may include, as a whole, 2-oxo-4-amino-pyrimidin-1-yl (i.e., cytosinyl), 2-oxo-4-amino-pyrimidin-1-yl the amino group of which is protected, 2-oxo-4-amino-5-fluoro-pyrimidin-1-yl, 2-oxo-4-amino-5-fluoro-pyrimidin-1-yl the amino group of which is protected, 4-amino-2-oxo-5-chloro-pyrimidin-1-yl, 2-oxo-4-methoxy-pyrimidin-1-yl, 2-oxo-4-mercapto-pyrimidin-1-yl, 2-oxo-4-hydroxy-pyrimidin-1-yl (i.e., uracinyl), 2-oxo-4-hydroxy-5-methylpyrimidin-1-yl (i.e., thyminyl) or 4-amino-5-methyl-2-oxo-pyrimidin-1-yl (i.e., 5-methylcytosinyl) group, more preferably 2-oxo-4-benzoylamino-pyrimidin-1-yl, cytosinyl, thyminyl, uracinyl, 2-oxo-4-benzoylamino-5-methyl-pyrimidin-1-yl or 5-methylcytosinyl group.

"The nucleoside analogue" refers to a non-natural type of "nucleoside" in which a purine or pyrimidine group is attached to sugar.

"The oligonucleotide analogue" refers to a non-natural type of "oligonucleotide" derivative in which from 2 to 50 "nucleosides", which may be the same or different, are bonded through a phosphoric acid diester bond and such analogues may preferably include sugar derivatives in which the sugar moiety is modified; thioate derivatives in which the phosphoric acid diester bond moiety is thioated; ester products in which a terminal phosphoric acid moiety is esterified; and amide products in which an amino group on a purine base is amidated, more preferably the sugar derivatives in which the sugar moiety is modified and the thioate derivatives in which the phosphoric acid diester moiety is thioated.

"The salt thereof" refers to salts of the compound (1) of the present invention since they can be converted to salts and such salts may preferably include inorganic salts for example metal salts such as alkali metal salts, e.g., sodium salts, potassium salts and lithium salts, alkaline earth metal salts, e.g., calcium salts and magnesium salts, aluminum salts, iron salts, zinc salts, copper salts, nickel salts and cobalt salts; amine salts such as inorganic salts, e.g., ammonium salts, organic salts, e.g., t-octylamine salts, dibenzylamine salts, morpholine salts, glucosamine salts, phenylglycine alkyl ester salts, ethylenediamine salts, N-methylglucamine salts, guanidine salts, diethylamine salts, triethylamine salts, dicyclohexylamine salts, N,N'-dibenzylethylenediamine salts, chloroprocaine salts, procaine salts, diethanol amine salts, N-benzyl-phenethylamine salts, piperazine salts, tetramethylammonium salts and a tris(hydroxymethyl)aminomethane salts; inorganic acid salts such as hydrohalogenic acid salts, e.g., hydrofluoric acid salts, hydrochloric acid salts, hydrobromic acid salts and hydroiodic acid salts, nitric acid salts, perchloric acid salts, sulfuric acid salts and phosphoric acid salts; organic acid salts such as lower alkanesulfonic acid salts, e.g., methanesulfonic acid salts, trifluoromethanesulfonic acid salts and ethanesulfonic acid salts, arylsulfonic acid salts, e.g., benzenesulfonic acid salts and p-toluenesulfonic acid salts, acetic acid salts, malic acid salts, fumaric acid salts, succinic acid salts, citric acid salts, tartaric acid salts, oxalic acid salts and maleic acid salts; and amino acid salts such as glycine salts, lysine salts, arginine salts, ornithine salts, glutamic acid salts and aspartic acid salts.

Since the modified oligonucleotides or the polynucleotide analogues of the present invention can be converted to a salt, "the pharmacologically acceptable salts thereof" refers to a salt thereof, and such salts may preferably include inorganic salts for example metal salts such as alkali metal salts, e.g., sodium salts, potassium salts lithium salts, alkaline earth metal salts, e.g., calcium salts and magnesium salts, aluminum salts, iron salts, zinc salts, copper salts, nickel salts and cobalt salts; amine salts such as inorganic salts, e.g., ammonium salts, organic salts, e.g., t-octylamine salts, dibenzylamine salts, morpholine salts, glucosamine salts, phenylglycine alkyl ester salts, ethylenediamine salts, N-methylglucamine salts, guanidine salts, diethylamine salts, triethylamine salts, dicyclohexylamine salts, N,N'-dibenzylethylenediamine salts, chloroprocaine salts, procaine salts, diethanolamine salts, N-benzyl-phenethylamine salts, piperazine salts, tetramethylammonium salts and tris(hydroxymethyl)aminomethane salts; inorganic acid salts such as hydrohalogenic acid salts, e.g., hydrofluoric acid salts, hydrochloric acid salts, hydrobromic acid salts and hydroiodic acid salts, nitric acid salts, perchloric acid salts, sulfuric acid salts and phosphoric acid salts; organic acid salts such as lower alkanesulfonic acid salts, e.g., methanesulfonic acid salts, trifluoromethanesulfonic acid salts and ethanesulfonic acid salts, arylsulfonic acid salts, e.g., benzenesulfonic acid salts and p-toluenesulfonic acid salts, acetic acid salts, malic acid salts, fumaric acid salts, succinic acid salts, citric acid salts, tartaric acid salts, oxalic acid salts and maleic acid salts; and amino acid salts such as glycine salts, lysine salts, arginine salts, ornithine salts, glutamic acid salts and aspartic acid salts.

Of the compounds (1) and the salts thereof of the present invention, preferred compounds may include

(1) compounds in which R^1 is a hydrogen atom, an aliphatic acyl group, an aromatic acyl group, a methyl group substituted by from 1 to 3 aryl groups, a methyl group substituted by from 1 to 3 aryl groups the aryl ring of which is substituted by a lower alkyl, lower alkoxy, halogen or cyano group, or a silyl group, and salts thereof;

(2) compounds in which R^1 is a hydrogen atom, an acetyl group, a benzoyl group, a benzyl group, a p-methoxybenzyl group, a dimethoxytrityl group, a mono-methoxytrityl group or a tert-butyldiphenylsilyl group, and salts thereof;

(3) compounds in which R^2 is a hydrogen atom, an aliphatic acyl group, an aromatic acyl group, a methyl group substituted by from 1 to 3 aryl groups, a methyl group substituted by from 1 to 3 aryl groups the aryl ring of which is substituted by a lower alkyl, lower alkoxy, halogen or cyano group, a silyl group, a phosphoramidite group, a phosphonyl group, a phosphoric acid group or a protected phosphoric acid group, and salts thereof;

(4) compounds in which R^2 is a hydrogen atom, an acetyl group, a benzoyl group, a benzyl group, a p-methoxybenzyl group, a tert-butyldiphenylsilyl group, $-P(OC_2H_4CN)(NCH(CH_3)_2)$, $-P(OCH_3)(NCH(CH_3)_2)$, a phosphonyl group or a 2-chlorophenyl or 4-chlorophenyl phosphoric acid group, and salts thereof;

(5) compounds in which A is a methylene group, and salts thereof;

(6) compounds in which B is a 6-aminopurin-9-yl (i.e., adeninyl), 6-aminopurin-9-yl the amino group of which is protected, 2,6-diaminopurin-9-yl, 2-amino-6-chloropurin-9-yl, 2-amino-6-chloropurin-9-yl the amino group of which is protected, 2-amino-6-fluoropurin-9-yl, 2-amino-6-fluoropurin-9-yl the amino group of which is protected, 2-amino-6-bromopurin-9-yl, 2-amino-6-bromopurin-9-yl the amino group of which is protected, 2-amino-6-hydroxypurin-9-yl (i.e., guaninyl), 2-amino-6-hydroxypurin-9-yl the amino group of which is protected, 2-amino-6-hydroxypurin-9-yl the amino group and hydroxyl group of which are protected, 6-amino-2-methoxypurin-9-yl, 6-amino-2-chloropurin-9-yl, 6-amino-2-fluoropurin-9-yl, 2,6-dimethoxypurin-9-yl, 2,6-dichloropurin-9-yl, 6-mercaptopurin-9-yl, 2-oxo-4-amino-pyrimidin-1-yl (i.e., cytosinyl), 2-oxo-4-amino-pyrimidin-1-yl the amino group of which is protected, 2-oxo-4-amino-5-fluoro-pyrimidin-1-yl, 2-oxo-4-amino-5-fluoro-pyrimidin-1-yl the amino group of which is protected, 4-amino-2-oxo-5-chloro-pyrimidin-1-yl, 2-oxo-4-methoxy-pyrimidin-1-yl, 2-oxo-4-mercapto-pyrimidin-1-yl, 2-oxo-4-hydroxy-pyrimidin-1-yl (i.e., uracinyl), 2-oxo-4-hydroxy-5-methylpyrimidin-1-yl (i.e.,

thyminyl), 4-amino-5-methyl-2-oxo-pyrimidin-1-yl (i.e., 5-methylcytosinyl) group or 4-amino-5-methyl-2-oxo-pyrimidin-1-yl group the amino of which group is protected, and salts thereof; and

(7) compounds in which B is a 6-benzoylaminopurin-9-yl, adeninyl, 2-isobutyrylamino-6-hydroxypurin-9-yl, guaninyl, 2-oxo-4-benzoylamino-pyrimidin-1-yl, cytosinyl, 2-oxo-5-methyl-4-benzoylamino-pyrimidin-1-yl, 5-methylcytosinyl, uracinyl or thyminyl group, and salts thereof.

The above (1) and (2), (3) and (4) or (6) and (7) indicate the more preferred compounds as the number becomes larger and in the formula (1), the compound obtained by optionally selecting R^1 from (1) and (2), optionally selecting R^2 from (3) and (4), optionally selecting A from (5) and optionally selecting B from (6) and (7) or by optionally combining them and the salts thereof are preferred and the compounds and the salts thereof selected from the following groups are particularly preferred.

(Group of compounds)

2'-O,4'-C-ethyleneguanosine,

2'-O,4'-C-ethyleneadenosine,

3',5'-di-O-benzyl-2'-O,4'-C-ethylene-6-N-benzoyladenosine,

3',5'-di-O-benzyl-2'-O,4'-C-ethylene-2-N-isobutyrylguanosine,

5'-O-dimethoxytrityl-2'-O,4'-C-ethylene-6-N-benzoyladenosine,

5'-O-dimethoxytrityl-2'-O,4'-C-ethylene-2-N-isobutyrylguanosine,

2'-O,4'-C-ethylene-2-N-isobutyrylguanosine,

2'-O,4'-C-ethylene-6-N-benzoyladenosine,

5'-O-dimethoxytrityl-2'-O,4'-C-ethylene-6-N-benzoyladenosine-3'-O-(2-cyanoethyl N,N-diisopropyl)phosphoramidite,

5'-O-dimethoxytrityl-2'-O,4'-C-ethylene-2-N-isobutyrylguanosine-3'-O-(2-cyanoethyl N,N-diisopropyl)phosphoramidite,

2'-O,4'-C-ethyleneuridine,

2'-O,4'-C-ethylene-5-methyluridine,

2'-O,4'-C-ethylenecytidine,

2'-O,4'-C-ethylene-5-methylcytidine,

3',5'-di-O-benzyl-2'-O,4'-C-ethyleneuridine,

5'-O-dimethoxytrityl-2'-O,4'-C-ethyleneuridine,

3',5'-di-O-benzyl-2'-O,4'-C-ethylene-5-methyluridine,

5'-O-dimethoxytrityl-2'-O,4'-C-ethylene-5-methyluridine,

3',5'-di-O-benzyl-2'-O,4'-C-ethylene-4-N-benzoylcytidine,
5'-O-dimethoxytrityl-2'-O,4'-C-ethylene-4-N-benzoylcytidine,
3',5'-di-O-benzyl-2'-O,4'-C-ethylene-4-N-benzoyl-5-methylcytidine,
5'-O-dimethoxytrityl-2'-O,4'-C-ethylene-4-N-benzoyl-5-methylcytidine,
2'-O,4'-C-ethylene-4-N-benzoylcytidine,
2'-O,4'-C-ethylene-4-N-benzoyl-5-methylcytidine,
5'-O-dimethoxytrityl-2'-O,4'-C-ethylene-uridine-3'-O-(2-cyanoethyl N,N-diisopropyl)phosphoramidite,
5'-O-dimethoxytrityl-2'-O,4'-C-ethylene-5-methyluridine-3'-O-(2-cyanoethyl N,N-diisopropyl)phosphoramidite,
5'-O-dimethoxytrityl-2'-O,4'-C-ethylene-4-N-benzoylcytidine-3'-O-(2-cyanoethyl N,N-diisopropyl)phosphoramidite, and
5'-O-dimethoxytrityl-2'-O,4'-C-ethylene-4-N-benzoyl-5-methylcytidine-3'-O-(2-cyanoethyl N,N-diisopropyl)phosphoramidite.

Of the oligonucleotide analogues containing one or two or more structures of the formula (2) and the pharmacologically acceptable salts thereof of the present invention, the preferred compounds may include

(8) oligonucleotide analogues in which A is a methylene group, and pharmacologically acceptable salts thereof;

(9) oligonucleotide analogues in which B is a 6-aminopurin-9-yl (i.e., adeninyl), 6-aminopurin-9-yl the amino group of which is protected, 2,6-diaminopurin-9-yl, 2-amino-6-chloropurin-9-yl, 2-amino-6-chloropurin-9-yl the amino group of which is protected, 2-amino-6-fluoropurin-9-yl, 2-amino-6-fluoropurin-9-yl the amino group of which is protected, 2-amino-6-bromopurin-9-yl, 2-amino-6-bromopurin-9-yl the amino group of which is protected, 2-amino-6-hydroxypurin-9-yl (i.e., guaninyl), 2-amino-6-hydroxypurin-9-yl the amino group of which is protected, 2-amino-6-hydroxypurin-9-yl the amino group and hydroxyl group of which are protected, 6-amino-2-methoxypurin-9-yl, 6-amino-2-chloropurin-9-yl, 6-amino-2-fluoropurin-9-yl, 2,6-dimethoxypurin-9-yl, 2,6-dichloropurin-9-yl, 6-mercaptopurin-9-yl, 2-oxo-4-amino-pyrimidin-1-yl (i.e., cytosinyl), 2-oxo-4-amino-pyrimidin-1-yl the amino group of which is protected, 2-oxo-4-amino-5-fluoro-pyrimidin-1-yl, 2-oxo-4-amino-5-fluoro-pyrimidin-1-yl the amino group of which is protected, 4-amino-2-oxo-5-chloro-pyrimidin-1-yl, 2-oxo-4-methoxy-pyrimidin-1-yl, 2-oxo-4-mercapto-pyrimidin-1-yl, 2-oxo-4-hydroxy-pyrimidin-1-yl (i.e., uracinyl), 2-oxo-4-hydroxy-5-

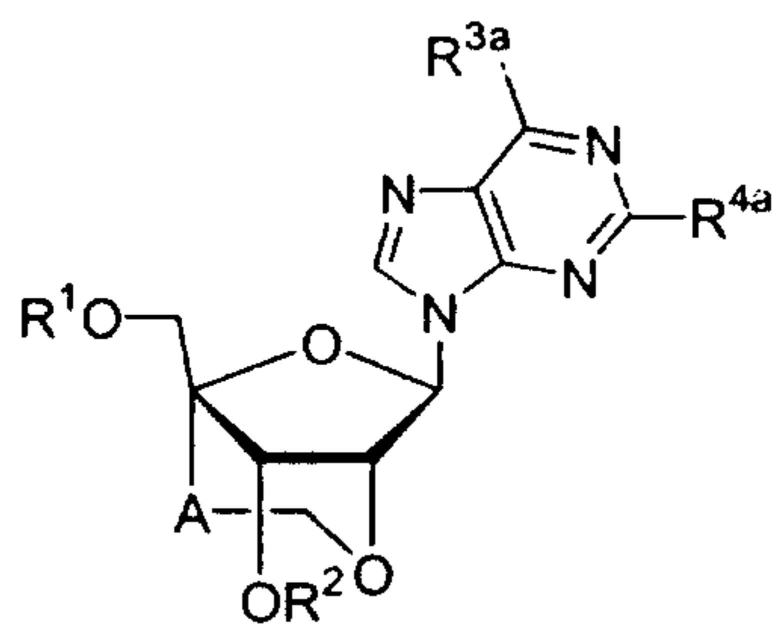
methylpyrimidin-1-yl (i.e., thyminyl), 4-amino-5-methyl-2-oxo-pyrimidin-1-yl (i.e., 5-methylcytosinyl) group or a 4-amino-5-methyl-2-oxo-pyrimidin-1-yl group the amino group of which is protected, and pharmacologically acceptable salts thereof; and

(10) oligonucleotide analogues in which B is a 6-benzoylaminopurin-9-yl, adeninyl, 2-isobutyrylamino-6-hydroxypurin-9-yl, guaninyl, 2-oxo-4-benzoylamino-pyrimidin-1-yl, cytosinyl, 2-oxo-5-methyl-4-benzoylamino-pyrimidin-1-yl, 5-methylcytosinyl, uracinyl or thyminyl group, and pharmacologically acceptable salts thereof.

The above (9) and (10) indicate the more preferred oligonucleotide analogues as the number becomes larger, and in the formula (2), the oligonucleotide analogues obtained by optionally selecting A from (8) and optionally selecting B from (9) and (10) or optionally combining these and the pharmacologically acceptable salts thereof are preferred.

The specific compounds included in the compound of the above formula (1) of the present invention are illustrated in Tables 1 and 2. However, the compounds of the present invention are not limited to those.

In Table 1 and Table 2 Exe. com. num. represents Exemplification compound number, Me represents a methyl group, Bn represents a benzyl group, Bz represents a benzoyl group, PMB represents a p-methoxybenzyl group, Tr represents a triphenylmethyl group, MMTr represents a 4-methoxytriphenylmethyl (monomethoxytrityl) group, DMTr represents a 4,4'-dimethoxytriphenylmethyl (dimethoxytrityl) group, TMTr represents a 4,4',4''-trimethoxytriphenylmethyl (trimethoxytrityl) group, TMS represents a trimethylsilyl group, TBDMS represents a tert-butyldimethylsilyl group, TBDPS represents a tert-butyldiphenylsilyl group and TIPS represents a triisopropylsilyl group.



(1')

[Table 1]

Exe. com. num.	A	R^1	R^2	R^{3a}	R^{4a}
1-1	CH_2	H	H	H	H
1-2	CH_2	H	H	H	NH_2
1-3	CH_2	H	H	H	OH
1-4	CH_2	H	H	OH	H
1-5	CH_2	H	H	OH	NH_2
1-6	CH_2	H	H	OH	OH
1-7	CH_2	H	H	NH_2	H
1-8	CH_2	H	H	NH_2	NH_2
1-9	CH_2	H	H	NH_2	Cl
1-10	CH_2	H	H	NH_2	F
1-11	CH_2	H	H	NH_2	Br
1-12	CH_2	H	H	NH_2	OH
1-13	CH_2	H	H	OMe	H
1-14	CH_2	H	H	OMe	OMe
1-15	CH_2	H	H	OMe	NH_2
1-16	CH_2	H	H	Cl	H
1-17	CH_2	H	H	Br	H
1-18	CH_2	H	H	F	H
1-19	CH_2	H	H	Cl	Cl
1-20	CH_2	H	H	SH	H

1-21	CH_2	Bn	H	NHBz	H
1-22	CH_2	Bn	H	OH	$NHCOCH(CH_3)_2$
1-23	CH_2	Bn	Bn	NHBz	H
1-24	CH_2	Bn	Bn	OH	$NHCOCH(CH_3)_2$
1-25	CH_2	PMB	H	NHBz	H
1-26	CH_2	PMB	H	OH	$NHCOCH(CH_3)_2$
1-27	CH_2	PMB	PMB	NHBz	H
1-28	CH_2	PMB	PMB	OH	$NHCOCH(CH_3)_2$
1-29	CH_2	Tr	H	NHBz	H
1-30	CH_2	MMTr	H	NHBz	H
1-31	CH_2	DMTr	H	NHBz	H
1-32	CH_2	TMTr	H	NHBz	H
1-33	CH_2	Tr	H	OH	$NHCOCH(CH_3)_2$
1-34	CH_2	MMTr	H	OH	$NHCOCH(CH_3)_2$
1-35	CH_2	DMTr	H	OH	$NHCOCH(CH_3)_2$
1-36	CH_2	TMTr	H	OH	$NHCOCH(CH_3)_2$
1-37	CH_2	TMS	H	NHBz	H
1-38	CH_2	TBDMS	H	NHBz	H
1-39	CH_2	TBDPS	H	NHBz	H
1-40	CH_2	TIPS	H	NHBz	H
1-41	CH_2	TMS	H	OH	$NHCOCH(CH_3)_2$
1-42	CH_2	TBDMS	H	OH	$NHCOCH(CH_3)_2$
1-43	CH_2	TBDPS	H	OH	$NHCOCH(CH_3)_2$
1-44	CH_2	TIPS	H	OH	$NHCOCH(CH_3)_2$
1-45	$(CH_2)_2$	H	H	H	H
1-46	$(CH_2)_2$	H	H	H	NH_2
1-47	$(CH_2)_2$	H	H	H	OH
1-48	$(CH_2)_2$	H	H	OH	H
1-49	$(CH_2)_2$	H	H	OH	NH_2
1-50	$(CH_2)_2$	H	H	OH	OH
1-51	$(CH_2)_2$	H	H	NH_2	H
1-52	$(CH_2)_2$	H	H	NH_2	NH_2

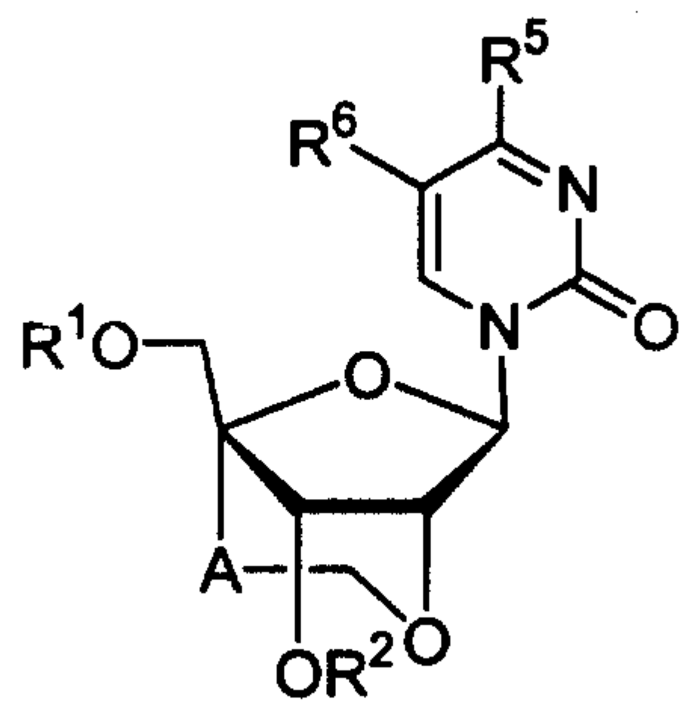
1-53	$(CH_2)_2$	H	H	NH_2	Cl
1-54	$(CH_2)_2$	H	H	NH_2	F
1-55	$(CH_2)_2$	H	H	NH_2	Br
1-56	$(CH_2)_2$	H	H	NH_2	OH
1-57	$(CH_2)_2$	H	H	OMe	H
1-58	$(CH_2)_2$	H	H	OMe	OMe
1-59	$(CH_2)_2$	H	H	OMe	NH_2
1-60	$(CH_2)_2$	H	H	Cl	H
1-61	$(CH_2)_2$	H	H	Br	H
1-62	$(CH_2)_2$	H	H	F	H
1-63	$(CH_2)_2$	H	H	Cl	Cl
1-64	$(CH_2)_2$	H	H	SH	H
1-65	$(CH_2)_2$	Bn	H	NHBz	H
1-66	$(CH_2)_2$	Bn	H	OH	$NHCOCH(CH_3)_2$
1-67	$(CH_2)_2$	Bn	Bn	NHBz	H
1-68	$(CH_2)_2$	Bn	Bn	OH	$NHCOCH(CH_3)_2$
1-69	$(CH_2)_2$	PMB	H	NHBz	H
1-70	$(CH_2)_2$	PMB	H	OH	$NHCOCH(CH_3)_2$
1-71	$(CH_2)_2$	PMB	PMB	NHBz	H
1-72	$(CH_2)_2$	PMB	PMB	OH	$NHCOCH(CH_3)_2$
1-73	$(CH_2)_2$	Tr	H	NHBz	H
1-74	$(CH_2)_2$	MMTr	H	NHBz	H
1-75	$(CH_2)_2$	DMTr	H	NHBz	H
1-76	$(CH_2)_2$	TMTr	H	NHBz	H
1-77	$(CH_2)_2$	Tr	H	OH	$NHCOCH(CH_3)_2$
1-78	$(CH_2)_2$	MMTr	H	OH	$NHCOCH(CH_3)_2$
1-79	$(CH_2)_2$	DMTr	H	OH	$NHCOCH(CH_3)_2$
1-80	$(CH_2)_2$	TMTr	H	OH	$NHCOCH(CH_3)_2$
1-81	$(CH_2)_2$	TMS	H	NHBz	H
1-82	$(CH_2)_2$	TBDMS	H	NHBz	H
1-83	$(CH_2)_2$	TBDPS	H	NHBz	H
1-84	$(CH_2)_2$	TIPS	H	NHBz	H

1-85	$(CH_2)_2$	TMS	H	OH	$NHCOCH(CH_3)_2$
1-86	$(CH_2)_2$	TBDMS	H	OH	$NHCOCH(CH_3)_2$
1-87	$(CH_2)_2$	TBDPS	H	OH	$NHCOCH(CH_3)_2$
1-88	$(CH_2)_2$	TIPS	H	OH	$NHCOCH(CH_3)_2$
1-89	$(CH_2)_3$	H	H	H	H
1-90	$(CH_2)_3$	H	H	H	NH_2
1-91	$(CH_2)_3$	H	H	H	OH
1-92	$(CH_2)_3$	H	H	OH	H
1-93	$(CH_2)_3$	H	H	OH	NH_2
1-94	$(CH_2)_3$	H	H	OH	OH
1-95	$(CH_2)_3$	H	H	NH_2	H
1-96	$(CH_2)_3$	H	H	NH_2	NH_2
1-97	$(CH_2)_3$	H	H	NH_2	Cl
1-98	$(CH_2)_3$	H	H	NH_2	F
1-99	$(CH_2)_3$	H	H	NH_2	Br
1-100	$(CH_2)_3$	H	H	NH_2	OH
1-101	$(CH_2)_3$	H	H	OMe	H
1-102	$(CH_2)_3$	H	H	OMe	OMe
1-103	$(CH_2)_3$	H	H	OMe	NH_2
1-104	$(CH_2)_3$	H	H	Cl	H
1-105	$(CH_2)_3$	H	H	Br	H
1-106	$(CH_2)_3$	H	H	F	H
1-107	$(CH_2)_3$	H	H	Cl	Cl
1-108	$(CH_2)_3$	H	H	SH	H
1-109	$(CH_2)_3$	Bn	H	NHBz	H
1-110	$(CH_2)_3$	Bn	H	OH	$NHCOCH(CH_3)_2$
1-111	$(CH_2)_3$	Bn	Bn	NHBz	H
1-112	$(CH_2)_3$	Bn	Bn	OH	$NHCOCH(CH_3)_2$
1-113	$(CH_2)_3$	PMB	H	NHBz	H
1-114	$(CH_2)_3$	PMB	H	OH	$NHCOCH(CH_3)_2$
1-115	$(CH_2)_3$	PMB	PMB	NHBz	H
1-116	$(CH_2)_3$	PMB	PMB	OH	$NHCOCH(CH_3)_2$

1-117	$(CH_2)_3$	Tr	H	NHBz	H
1-118	$(CH_2)_3$	MMTr	H	NHBz	H
1-119	$(CH_2)_3$	DMTr	H	NHBz	H
1-120	$(CH_2)_3$	TMTr	H	NHBz	H
1-121	$(CH_2)_3$	Tr	H	OH	$NHCOCH(CH_3)_2$
1-122	$(CH_2)_3$	MMTr	H	OH	$NHCOCH(CH_3)_2$
1-123	$(CH_2)_3$	DMTr	H	OH	$NHCOCH(CH_3)_2$
1-124	$(CH_2)_3$	TMTr	H	OH	$NHCOCH(CH_3)_2$
1-125	$(CH_2)_3$	TMS	H	NHBz	H
1-126	$(CH_2)_3$	TBDMS	H	NHBz	H
1-127	$(CH_2)_3$	TBDPS	H	NHBz	H
1-128	$(CH_2)_3$	TIPS	H	NHBz	H
1-129	$(CH_2)_3$	TMS	H	OH	$NHCOCH(CH_3)_2$
1-130	$(CH_2)_3$	TBDMS	H	OH	$NHCOCH(CH_3)_2$
1-131	$(CH_2)_3$	TBDPS	H	OH	$NHCOCH(CH_3)_2$
1-132	$(CH_2)_3$	TIPS	H	OH	$NHCOCH(CH_3)_2$
1-133	$(CH_2)_4$	H	H	H	H
1-134	$(CH_2)_4$	H	H	H	NH_2
1-135	$(CH_2)_4$	H	H	H	OH
1-136	$(CH_2)_4$	H	H	OH	H
1-137	$(CH_2)_4$	H	H	OH	NH_2
1-138	$(CH_2)_4$	H	H	OH	OH
1-139	$(CH_2)_4$	H	H	NH_2	H
1-140	$(CH_2)_4$	H	H	NH_2	NH_2
1-141	$(CH_2)_4$	H	H	NH_2	Cl
1-142	$(CH_2)_4$	H	H	NH_2	F
1-143	$(CH_2)_4$	H	H	NH_2	Br
1-144	$(CH_2)_4$	H	H	NH_2	OH
1-145	$(CH_2)_4$	H	H	OMe	H
1-146	$(CH_2)_4$	H	H	OMe	OMe
1-147	$(CH_2)_4$	H	H	OMe	NH_2
1-148	$(CH_2)_4$	H	H	Cl	H

1-149	$(CH_2)_4$	H	H	Br	H
1-150	$(CH_2)_4$	H	H	F	H
1-151	$(CH_2)_4$	H	H	Cl	Cl
1-152	$(CH_2)_4$	H	H	SH	H
1-153	$(CH_2)_4$	Bn	H	NHBz	H
1-154	$(CH_2)_4$	Bn	H	OH	$NHCOCH(CH_3)_2$
1-155	$(CH_2)_4$	Bn	Bn	NHBz	H
1-156	$(CH_2)_4$	Bn	Bn	OH	$NHCOCH(CH_3)_2$
1-157	$(CH_2)_4$	PMB	H	NHBz	H
1-158	$(CH_2)_4$	PMB	H	OH	$NHCOCH(CH_3)_2$
1-159	$(CH_2)_4$	PMB	PMB	NHBz	H
1-160	$(CH_2)_4$	PMB	PMB	OH	$NHCOCH(CH_3)_2$
1-161	$(CH_2)_4$	Tr	H	NHBz	H
1-162	$(CH_2)_4$	MMTr	H	NHBz	H
1-163	$(CH_2)_4$	DMTr	H	NHBz	H
1-164	$(CH_2)_4$	TMTr	H	NHBz	H
1-165	$(CH_2)_4$	Tr	H	OH	$NHCOCH(CH_3)_2$
1-166	$(CH_2)_4$	MMTr	H	OH	$NHCOCH(CH_3)_2$
1-167	$(CH_2)_4$	DMTr	H	OH	$NHCOCH(CH_3)_2$
1-168	$(CH_2)_4$	TMTr	H	OH	$NHCOCH(CH_3)_2$
1-169	$(CH_2)_4$	TMS	H	NHBz	H
1-170	$(CH_2)_4$	TBDMS	H	NHBz	H
1-171	$(CH_2)_4$	TBDPS	H	NHBz	H
1-172	$(CH_2)_4$	TIPS	H	NHBz	H
1-173	$(CH_2)_4$	TMS	H	OH	$NHCOCH(CH_3)_2$
1-174	$(CH_2)_4$	TBDMS	H	OH	$NHCOCH(CH_3)_2$
1-175	$(CH_2)_4$	TBDPS	H	OH	$NHCOCH(CH_3)_2$
1-176	$(CH_2)_4$	TIPS	H	OH	$NHCOCH(CH_3)_2$
1-177	CH_2	H	H	OH	$NHCOCH(CH_3)_2$
1-178	CH_2	H	H	NHBz	H
1-179	$(CH_2)_2$	H	H	OH	$NHCOCH(CH_3)_2$
1-180	$(CH_2)_2$	H	H	NHBz	H

1-181	$(CH_2)_3$	H	H	OH	$NHCOCH(CH_3)_2$
1-182	$(CH_2)_3$	H	H	NHBz	H
1-183	$(CH_2)_4$	H	H	OH	$NHCOCH(CH_3)_2$
1-184	$(CH_2)_4$	H	H	NHBz	H
1-185	CH_2	DMTr	$P(N(iPr)_2)(OC_2H_4CN)$	OH	$NHCOCH(CH_3)_2$
1-186	CH_2	DMTr	$P(N(iPr)_2)(OC_2H_4CN)$	NHBz	H
1-187	$(CH_2)_2$	DMTr	$P(N(iPr)_2)(OC_2H_4CN)$	OH	$NHCOCH(CH_3)_2$
1-188	$(CH_2)_2$	DMTr	$P(N(iPr)_2)(OC_2H_4CN)$	NHBz	H
1-189	$(CH_2)_3$	DMTr	$P(N(iPr)_2)(OC_2H_4CN)$	OH	$NHCOCH(CH_3)_2$
1-190	$(CH_2)_3$	DMTr	$P(N(iPr)_2)(OC_2H_4CN)$	NHBz	H
1-191	$(CH_2)_4$	DMTr	$P(N(iPr)_2)(OC_2H_4CN)$	OH	$NHCOCH(CH_3)_2$
1-192	$(CH_2)_4$	DMTr	$P(N(iPr)_2)(OC_2H_4CN)$	NHBz	H
1-193	CH_2	DMTr	$P(N(iPr)_2)(OCH_3)$	OH	$NHCOCH(CH_3)_2$
1-194	CH_2	DMTr	$P(N(iPr)_2)(OCH_3)$	NHBz	H
1-195	$(CH_2)_2$	DMTr	$P(N(iPr)_2)(OCH_3)$	OH	$NHCOCH(CH_3)_2$
1-196	$(CH_2)_2$	DMTr	$P(N(iPr)_2)(OCH_3)$	NHBz	H
1-197	$(CH_2)_3$	DMTr	$P(N(iPr)_2)(OCH_3)$	OH	$NHCOCH(CH_3)_2$
1-198	$(CH_2)_3$	DMTr	$P(N(iPr)_2)(OCH_3)$	NHBz	H
1-199	$(CH_2)_4$	DMTr	$P(N(iPr)_2)(OCH_3)$	OH	$NHCOCH(CH_3)_2$
1-200	$(CH_2)_4$	DMTr	$P(N(iPr)_2)(OCH_3)$	NHBz	H
1-201	CH_2	DMTr	P(O)(OH)H	OH	$NHCOCH(CH_3)_2$
1-202	CH_2	DMTr	P(O)(OH)H	NHBz	H
1-203	$(CH_2)_2$	DMTr	P(O)(OH)H	OH	$NHCOCH(CH_3)_2$
1-204	$(CH_2)_2$	DMTr	P(O)(OH)H	NHBz	H
1-205	$(CH_2)_3$	DMTr	P(O)(OH)H	OH	$NHCOCH(CH_3)_2$
1-206	$(CH_2)_3$	DMTr	P(O)(OH)H	NHBz	H
1-207	$(CH_2)_4$	DMTr	P(O)(OH)H	OH	$NHCOCH(CH_3)_2$
1-208	$(CH_2)_4$	DMTr	P(O)(OH)H	NHBz	H



(1")

[Table 2]

Exe. com. num.	A	R^1	R^2	R^5	R^6
2-1	CH_2	H	H	OH	H
2-2	CH_2	H	H	OH	CH_3
2-3	CH_2	H	H	NH_2	H
2-4	CH_2	H	H	NH_2	CH_3
2-5	CH_2	H	H	NH_2	F
2-6	CH_2	H	H	Cl	H
2-7	CH_2	H	H	OMe	H
2-8	CH_2	H	H	SH	H
2-9	CH_2	Bn	H	OH	H
2-10	CH_2	Bn	Bn	OH	H
2-11	CH_2	PMB	H	OH	H
2-12	CH_2	PMB	PMB	OH	H
2-13	CH_2	Tr	H	OH	H
2-14	CH_2	MMTr	H	OH	H
2-15	CH_2	DMTr	H	OH	H
2-16	CH_2	TMTr	H	OH	H
2-17	CH_2	TMS	H	OH	H
2-18	CH_2	TBDMS	H	OH	H
2-19	CH_2	TBDPS	H	OH	H

2-20	CH_2	TIPS	H	OH	H
2-21	CH_2	Bn	H	OH	CH_3
2-22	CH_2	Bn	Bn	OH	CH_3
2-23	CH_2	PMB	H	OH	CH_3
2-24	CH_2	PMB	PMB	OH	CH_3
2-25	CH_2	Tr	H	OH	CH_3
2-26	CH_2	MMTr	H	OH	CH_3
2-27	CH_2	DMTr	H	OH	CH_3
2-28	CH_2	TMTr	H	OH	CH_3
2-29	CH_2	TMS	H	OH	CH_3
2-30	CH_2	TBDMS	H	OH	CH_3
2-31	CH_2	TBDPS	H	OH	CH_3
2-32	CH_2	TIPS	H	OH	CH_3
2-33	CH_2	Bn	H	NHBz	H
2-34	CH_2	Bn	Bn	NHBz	H
2-35	CH_2	PMB	H	NHBz	H
2-36	CH_2	PMB	PMB	NHBz	H
2-37	CH_2	Tr	H	NHBz	H
2-38	CH_2	MMTr	H	NHBz	H
2-39	CH_2	DMTr	H	NHBz	H
2-40	CH_2	TMTr	H	NHBz	H
2-41	CH_2	TMS	H	NHBz	H
2-42	CH_2	TBDMS	H	NHBz	H
2-43	CH_2	TBDPS	H	NHBz	H
2-44	CH_2	TIPS	H	NHBz	H
2-45	CH_2	Bn	H	NHBz	CH_3
2-46	CH_2	Bn	Bn	NHBz	CH_3
2-47	CH_2	PMB	H	NHBz	CH_3
2-48	CH_2	PMB	PMB	NHBz	CH_3
2-49	CH_2	Tr	H	NHBz	CH_3
2-50	CH_2	MMTr	H	NHBz	CH_3
2-51	CH_2	DMTr	H	NHBz	CH_3

2-52	CH_2	TMTr	H	NHBz	CH_3
2-53	CH_2	TMS	H	NHBz	CH_3
2-54	CH_2	TBDMS	H	NHBz	CH_3
2-55	CH_2	TBDPS	H	NHBz	CH_3
2-56	CH_2	TIPS	H	NHBz	CH_3
2-57	$(CH_2)_2$	H	H	OH	H
2-58	$(CH_2)_2$	H	H	OH	CH_3
2-59	$(CH_2)_2$	H	H	NH_2	H
2-60	$(CH_2)_2$	H	H	NH_2	CH_3
2-61	$(CH_2)_2$	H	H	NH_2	F
2-62	$(CH_2)_2$	H	H	Cl	H
2-63	$(CH_2)_2$	H	H	OMe	H
2-64	$(CH_2)_2$	H	H	SH	H
2-65	$(CH_2)_2$	Bn	H	OH	H
2-66	$(CH_2)_2$	Bn	Bn	OH	H
2-67	$(CH_2)_2$	PMB	H	OH	H
2-68	$(CH_2)_2$	PMB	PMB	OH	H
2-69	$(CH_2)_2$	Tr	H	OH	H
2-70	$(CH_2)_2$	MMTr	H	OH	H
2-71	$(CH_2)_2$	DMTr	H	OH	H
2-72	$(CH_2)_2$	TMTr	H	OH	H
2-73	$(CH_2)_2$	TMS	H	OH	H
2-74	$(CH_2)_2$	TBDMS	H	OH	H
2-75	$(CH_2)_2$	TBDPS	H	OH	H
2-76	$(CH_2)_2$	TIPS	H	OH	H
2-77	$(CH_2)_2$	Bn	H	OH	CH_3
2-78	$(CH_2)_2$	Bn	Bn	OH	CH_3
2-79	$(CH_2)_2$	PMB	H	OH	CH_3
2-80	$(CH_2)_2$	PMB	PMB	OH	CH_3
2-81	$(CH_2)_2$	Tr	H	OH	CH_3
2-82	$(CH_2)_2$	MMTr	H	OH	CH_3
2-83	$(CH_2)_2$	DMTr	H	OH	CH_3

2-84	$(CH_2)_2$	TMTr	H	OH	CH_3
2-85	$(CH_2)_2$	TMS	H	OH	CH_3
2-86	$(CH_2)_2$	TBDMS	H	OH	CH_3
2-87	$(CH_2)_2$	TBDPS	H	OH	CH_3
2-88	$(CH_2)_2$	TIPS	H	OH	CH_3
2-89	$(CH_2)_2$	Bn	H	NHBz	H
2-90	$(CH_2)_2$	Bn	Bn	NHBz	H
2-91	$(CH_2)_2$	PMB	H	NHBz	H
2-92	$(CH_2)_2$	PMB	PMB	NHBz	H
2-93	$(CH_2)_2$	Tr	H	NHBz	H
2-94	$(CH_2)_2$	MMTr	H	NHBz	H
2-95	$(CH_2)_2$	DMTr	H	NHBz	H
2-96	$(CH_2)_2$	TMTr	H	NHBz	H
2-97	$(CH_2)_2$	TMS	H	NHBz	H
2-98	$(CH_2)_2$	TBDMS	H	NHBz	H
2-99	$(CH_2)_2$	TBDPS	H	NHBz	H
2-100	$(CH_2)_2$	TIPS	H	NHBz	H
2-101	$(CH_2)_2$	Bn	H	NHBz	CH_3
2-102	$(CH_2)_2$	Bn	Bn	NHBz	CH_3
2-103	$(CH_2)_2$	PMB	H	NHBz	CH_3
2-104	$(CH_2)_2$	PMB	PMB	NHBz	CH_3
2-105	$(CH_2)_2$	Tr	H	NHBz	CH_3
2-106	$(CH_2)_2$	MMTr	H	NHBz	CH_3
2-107	$(CH_2)_2$	DMTr	H	NHBz	CH_3
2-108	$(CH_2)_2$	TMTr	H	NHBz	CH_3
2-109	$(CH_2)_2$	TMS	H	NHBz	CH_3
2-110	$(CH_2)_2$	TBDMS	H	NHBz	CH_3
2-111	$(CH_2)_2$	TBDPS	H	NHBz	CH_3
2-112	$(CH_2)_2$	TIPS	H	NHBz	CH_3
2-113	$(CH_2)_3$	H	H	OH	H
2-114	$(CH_2)_3$	H	H	OH	CH_3
2-115	$(CH_2)_3$	H	H	NH_2	H

2-116	$(CH_2)_3$	H	H	NH_2	CH_3
2-117	$(CH_2)_3$	H	H	NH_2	F
2-118	$(CH_2)_3$	H	H	Cl	H
2-119	$(CH_2)_3$	H	H	OMe	H
2-120	$(CH_2)_3$	H	H	SH	H
2-121	$(CH_2)_3$	Bn	H	OH	H
2-122	$(CH_2)_3$	Bn	Bn	OH	H
2-123	$(CH_2)_3$	PMB	H	OH	H
2-124	$(CH_2)_3$	PMB	PMB	OH	H
2-125	$(CH_2)_3$	Tr	H	OH	H
2-126	$(CH_2)_3$	MMTr	H	OH	H
2-127	$(CH_2)_3$	DMTr	H	OH	H
2-128	$(CH_2)_3$	TMTr	H	OH	H
2-129	$(CH_2)_3$	TMS	H	OH	H
2-130	$(CH_2)_3$	TBDMS	H	OH	H
2-131	$(CH_2)_3$	TBDPS	H	OH	H
2-132	$(CH_2)_3$	TIPS	H	OH	H
2-133	$(CH_2)_3$	Bn	H	OH	CH_3
2-134	$(CH_2)_3$	Bn	Bn	OH	CH_3
2-135	$(CH_2)_3$	PMB	H	OH	CH_3
2-136	$(CH_2)_3$	PMB	PMB	OH	CH_3
2-137	$(CH_2)_3$	Tr	H	OH	CH_3
2-138	$(CH_2)_3$	MMTr	H	OH	CH_3
2-139	$(CH_2)_3$	DMTr	H	OH	CH_3
2-140	$(CH_2)_3$	TMTr	H	OH	CH_3
2-141	$(CH_2)_3$	TMS	H	OH	CH_3
2-142	$(CH_2)_3$	TBDMS	H	OH	CH_3
2-143	$(CH_2)_3$	TBDPS	H	OH	CH_3
2-144	$(CH_2)_3$	TIPS	H	OH	CH_3
2-145	$(CH_2)_3$	Bn	H	NHBz	H
2-146	$(CH_2)_3$	Bn	Bn	NHBz	H
2-147	$(CH_2)_3$	PMB	H	NHBz	H

2-148	$(CH_2)_3$	PMB	PMB	NHBz	H
2-149	$(CH_2)_3$	Tr	H	NHBz	H
2-150	$(CH_2)_3$	MMTr	H	NHBz	H
2-151	$(CH_2)_3$	DMTr	H	NHBz	H
2-152	$(CH_2)_3$	TMTr	H	NHBz	H
2-153	$(CH_2)_3$	TMS	H	NHBz	H
2-154	$(CH_2)_3$	TBDMS	H	NHBz	H
2-155	$(CH_2)_3$	TBDPS	H	NHBz	H
2-156	$(CH_2)_3$	TIPS	H	NHBz	H
2-157	$(CH_2)_3$	Bn	H	NHBz	CH_3
2-158	$(CH_2)_3$	Bn	Bn	NHBz	CH_3
2-159	$(CH_2)_3$	PMB	H	NHBz	CH_3
2-160	$(CH_2)_3$	PMB	PMB	NHBz	CH_3
2-161	$(CH_2)_3$	Tr	H	NHBz	CH_3
2-162	$(CH_2)_3$	MMTr	H	NHBz	CH_3
2-163	$(CH_2)_3$	DMTr	H	NHBz	CH_3
2-164	$(CH_2)_3$	TMTr	H	NHBz	CH_3
2-165	$(CH_2)_3$	TMS	H	NHBz	CH_3
2-166	$(CH_2)_3$	TBDMS	H	NHBz	CH_3
2-167	$(CH_2)_3$	TBDPS	H	NHBz	CH_3
2-168	$(CH_2)_3$	TIPS	H	NHBz	CH_3
2-169	$(CH_2)_4$	H	H	OH	H
2-170	$(CH_2)_4$	H	H	OH	CH_3
2-171	$(CH_2)_4$	H	H	NH_2	H
2-172	$(CH_2)_4$	H	H	NH_2	CH_3
2-173	$(CH_2)_4$	H	H	NH_2	F
2-174	$(CH_2)_4$	H	H	Cl	H
2-175	$(CH_2)_4$	H	H	OMe	H
2-176	$(CH_2)_4$	H	H	SH	H
2-177	$(CH_2)_4$	Bn	H	OH	H
2-178	$(CH_2)_4$	Bn	Bn	OH	H
2-179	$(CH_2)_4$	PMB	H	OH	H

2-180	$(CH_2)_4$	PMB	PMB	OH	H
2-181	$(CH_2)_4$	Tr	H	OH	H
2-182	$(CH_2)_4$	MMTr	H	OH	H
2-183	$(CH_2)_4$	DMTr	H	OH	H
2-184	$(CH_2)_4$	TMTr	H	OH	H
2-185	$(CH_2)_4$	TMS	H	OH	H
2-186	$(CH_2)_4$	TBDMS	H	OH	H
2-187	$(CH_2)_4$	TBDPS	H	OH	H
2-188	$(CH_2)_4$	TIPS	H	OH	H
2-189	$(CH_2)_4$	Bn	H	OH	CH_3
2-190	$(CH_2)_4$	Bn	Bn	OH	CH_3
2-191	$(CH_2)_4$	PMB	H	OH	CH_3
2-192	$(CH_2)_4$	PMB	PMB	OH	CH_3
2-193	$(CH_2)_4$	Tr	H	OH	CH_3
2-194	$(CH_2)_4$	MMTr	H	OH	CH_3
2-195	$(CH_2)_4$	DMTr	H	OH	CH_3
2-196	$(CH_2)_4$	TMTr	H	OH	CH_3
2-197	$(CH_2)_4$	TMS	H	OH	CH_3
2-198	$(CH_2)_4$	TBDMS	H	OH	CH_3
2-199	$(CH_2)_4$	TBDPS	H	OH	CH_3
2-200	$(CH_2)_4$	TIPS	H	OH	CH_3
2-201	$(CH_2)_4$	Bn	H	NHBz	H
2-202	$(CH_2)_4$	Bn	Bn	NHBz	H
2-203	$(CH_2)_4$	PMB	H	NHBz	H
2-204	$(CH_2)_4$	PMB	PMB	NHBz	H
2-205	$(CH_2)_4$	Tr	H	NHBz	H
2-206	$(CH_2)_4$	MMTr	H	NHBz	H
2-207	$(CH_2)_4$	DMTr	H	NHBz	H
2-208	$(CH_2)_4$	TMTr	H	NHBz	H
2-209	$(CH_2)_4$	TMS	H	NHBz	H
2-210	$(CH_2)_4$	TBDMS	H	NHBz	H
2-211	$(CH_2)_4$	TBDPS	H	NHBz	H

2-212	$(CH_2)_4$	TIPS	H	NHBz	H
2-213	$(CH_2)_4$	Bn	H	NHBz	CH_3
2-214	$(CH_2)_4$	Bn	Bn	NHBz	CH_3
2-215	$(CH_2)_4$	PMB	H	NHBz	CH_3
2-216	$(CH_2)_4$	PMB	PMB	NHBz	CH_3
2-217	$(CH_2)_4$	Tr	H	NHBz	CH_3
2-218	$(CH_2)_4$	MMTr	H	NHBz	CH_3
2-219	$(CH_2)_4$	DMTr	H	NHBz	CH_3
2-220	$(CH_2)_4$	TMTr	H	NHBz	CH_3
2-221	$(CH_2)_4$	TMS	H	NHBz	CH_3
2-222	$(CH_2)_4$	TBDMS	H	NHBz	CH_3
2-223	$(CH_2)_4$	TBDPS	H	NHBz	CH_3
2-224	$(CH_2)_4$	TIPS	H	NHBz	CH_3
2-225	CH_2	H	H	NHBz	H
2-226	CH_2	H	H	NHBz	CH_3
2-227	$(CH_2)_2$	H	H	NHBz	H
2-228	$(CH_2)_2$	H	H	NHBz	CH_3
2-229	$(CH_2)_3$	H	H	NHBz	H
2-230	$(CH_2)_3$	H	H	NHBz	CH_3
2-231	$(CH_2)_4$	H	H	NHBz	H
2-232	$(CH_2)_4$	H	H	NHBz	CH_3
2-233	CH_2	DMTr	$P(N(iPr)_2)(OC_2H_4CN)$	OH	H
2-234	CH_2	DMTr	$P(N(iPr)_2)(OC_2H_4CN)$	OH	CH_3
2-235	CH_2	DMTr	$P(N(iPr)_2)(OC_2H_4CN)$	NHBz	H
2-236	CH_2	DMTr	$P(N(iPr)_2)(OC_2H_4CN)$	NHBz	CH_3
2-237	$(CH_2)_2$	DMTr	$P(N(iPr)_2)(OC_2H_4CN)$	OH	H
2-238	$(CH_2)_2$	DMTr	$P(N(iPr)_2)(OC_2H_4CN)$	OH	CH_3
2-239	$(CH_2)_2$	DMTr	$P(N(iPr)_2)(OC_2H_4CN)$	NHBz	H
2-240	$(CH_2)_2$	DMTr	$P(N(iPr)_2)(OC_2H_4CN)$	NHBz	CH_3
2-241	$(CH_2)_3$	DMTr	$P(N(iPr)_2)(OC_2H_4CN)$	OH	H
2-242	$(CH_2)_3$	DMTr	$P(N(iPr)_2)(OC_2H_4CN)$	OH	CH_3
2-243	$(CH_2)_3$	DMTr	$P(N(iPr)_2)(OC_2H_4CN)$	NHBz	H

2-244	$(CH_2)_3$	DMTr	$P(N(iPr)_2)(OC_2H_4CN)$	NHBz	CH_3
2-245	$(CH_2)_4$	DMTr	$P(N(iPr)_2)(OC_2H_4CN)$	OH	H
2-246	$(CH_2)_4$	DMTr	$P(N(iPr)_2)(OC_2H_4CN)$	OH	CH_3
2-247	$(CH_2)_4$	DMTr	$P(N(iPr)_2)(OC_2H_4CN)$	NHBz	H
2-248	$(CH_2)_4$	DMTr	$P(N(iPr)_2)(OC_2H_4CN)$	NHBz	CH_3
2-249	CH_2	DMTr	$P(N(iPr)_2)(OCH_3)$	OH	H
2-250	CH_2	DMTr	$P(N(iPr)_2)(OCH_3)$	OH	CH_3
2-251	CH_2	DMTr	$P(N(iPr)_2)(OCH_3)$	NHBz	H
2-252	CH_2	DMTr	$P(N(iPr)_2)(OCH_3)$	NHBz	CH_3
2-253	$(CH_2)_2$	DMTr	$P(N(iPr)_2)(OCH_3)$	OH	H
2-254	$(CH_2)_2$	DMTr	$P(N(iPr)_2)(OCH_3)$	OH	CH_3
2-255	$(CH_2)_2$	DMTr	$P(N(iPr)_2)(OCH_3)$	NHBz	H
2-256	$(CH_2)_2$	DMTr	$P(N(iPr)_2)(OCH_3)$	NHBz	CH_3
2-257	$(CH_2)_3$	DMTr	$P(N(iPr)_2)(OCH_3)$	OH	H
2-258	$(CH_2)_3$	DMTr	$P(N(iPr)_2)(OCH_3)$	OH	CH_3
2-259	$(CH_2)_3$	DMTr	$P(N(iPr)_2)(OCH_3)$	NHBz	H
2-260	$(CH_2)_3$	DMTr	$P(N(iPr)_2)(OCH_3)$	NHBz	CH_3
2-261	$(CH_2)_4$	DMTr	$P(N(iPr)_2)(OCH_3)$	OH	H
2-262	$(CH_2)_4$	DMTr	$P(N(iPr)_2)(OCH_3)$	OH	CH_3
2-263	$(CH_2)_4$	DMTr	$P(N(iPr)_2)(OCH_3)$	NHBz	H
2-264	$(CH_2)_4$	DMTr	$P(N(iPr)_2)(OCH_3)$	NHBz	CH_3

In the above Table 1 and Table 2, preferred compounds include the compounds (1-5), (1-7), (1-23), (1-24), (1-31), (1-35), (1-39), (1-43), (1-49), (1-51), (1-67), (1-68), (1-75), (1-79), (1-83), (1-87), (1-93), (1-95), (1-111), (1-112), (1-119), (1-123), (1-127), (1-131), (1-137), (1-139), (1-155), (1-156), (1-163), (1-167), (1-171), (1-175), (1-177), (1-178), (1-185), (1-186), (1-193), (1-194), (1-201), (1-202), (2-1), (2-2), (2-3), (2-4), (2-10), (2-15), (2-19), (2-22), (2-27), (2-31), (2-34), (2-39), (2-43), (2-46), (2-51), (2-55), (2-57), (2-58), (2-59), (2-60), (2-66), (2-71), (2-75), (2-78), (2-83), (2-87), (2-90), (2-95), (2-99), (2-102), (2-107), (2-111), (2-113), (2-114), (2-115), (2-116), (2-122), (2-127), (2-131), (2-134), (2-139), (2-143), (2-146), (2-151), (2-155), (2-158), (2-163), (2-167), (2-169), (2-170), (2-171), (2-172), (2-178), (2-183), (2-187), (2-190), (2-195), (2-199), (2-202), (2-207), (2-211), (2-214), (2-219), (2-223), (2-225), (2-226), (2-233), (2-234), (2-235) or (2-236), more preferred compounds may include

2'-O,4'-C-ethyleneguanosine (1-5),

2'-O,4'-C-ethyleneadenosine (1-7),

3',5'-di-O-benzyl-2'-O,4'-C-ethylene-6-N-benzoyladenosine (1-23),

3',5'-di-O-benzyl-2'-O,4'-C-ethylene-2-N-isobutyrylguanosine (1-24),

5'-O-dimethoxytrityl-2'-O,4'-C-ethylene-6-N-benzoyladenosine (1-31),

5'-O-dimethoxytrityl-2'-O,4'-C-ethylene-2-N-isobutyrylguanosine (1-35),

2'-O,4'-C-ethylene-2-N-isobutyrylguanosine (1-177),

2'-O,4'-C-ethylene-6-N-benzoyladenosine (1-178),

5'-O-dimethoxytrityl-2'-O,4'-C-ethylene-2-N-isobutyrylguanosine-3'-O-(2-cyanoethyl N,N-diisopropyl)phosphoramidite (1-185),

5'-O-dimethoxytrityl-2'-O,4'-C-ethylene-6-N-benzoyladenosine-3'-O-(2-cyanoethyl N,N-diisopropyl)phosphoramidite (1-186),

2'-O,4'-C-ethyleneuridine (2-1),

2'-O,4'-C-ethylene-5-methyluridine (2-2),

2'-O,4'-C-ethylenecytidine (2-3),

2'-O,4'-C-ethylene-5-methylcytidine (2-4),

3',5'-di-O-benzyl-2'-O,4'-C-ethyleneuridine (2-10),

5'-O-dimethoxytrityl-2'-O,4'-C-ethyleneuridine (2-15),

3',5'-di-O-benzyl-2'-O,4'-C-ethylene-5-methyluridine (2-22),

5'-O-dimethoxytrityl-2'-O,4'-C-ethylene-5-methyluridine (2-27),

3',5'-di-O-benzyl-2'-O,4'-C-ethylene-4-N-benzoylcytidine (2-34),

5'-O-dimethoxytrityl-2'-O,4'-C-ethylene-4-N-benzoylcytidine (2-39),

3',5'-di-O-benzyl-2'-O,4'-C-ethylene-4-N-benzoyl-5-methylcytidine (2-46),

5'-O-dimethoxytrityl-2'-O,4'-C-ethylene-4-N-benzoyl-5-methylcytidine (2-51),

2'-O,4'-C-ethylene-4-N-benzoylcytidine (2-225),

2'-O,4'-C-ethylene-4-N-benzoyl-5-methylcytidine (2-226),

5'-O-dimethoxytrityl-2'-O,4'-C-ethylene-uridine-3'-O-(2-cyanoethyl N,N-diisopropyl)phosphoramidite (2-233),

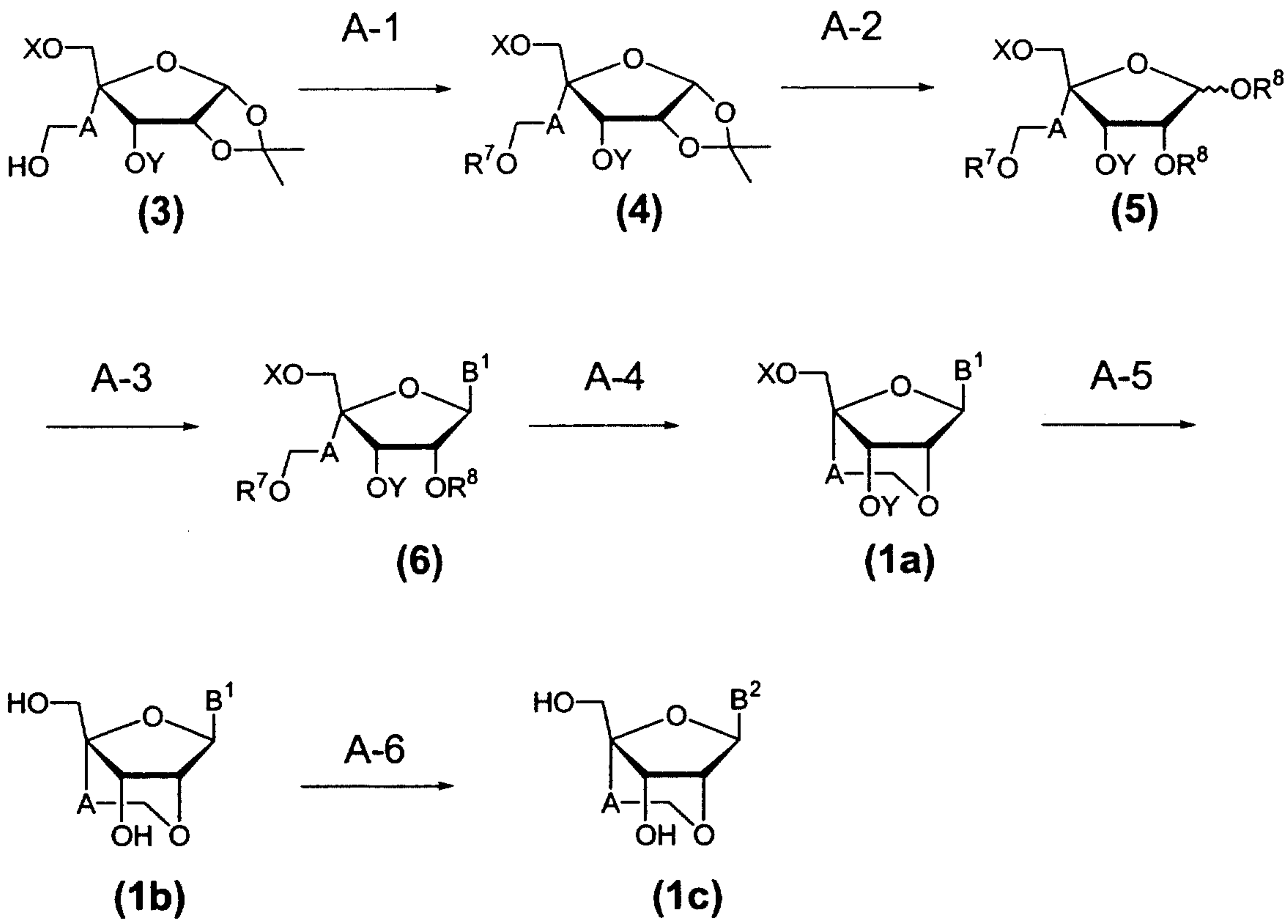
5'-O-dimethoxytrityl-2'-O,4'-C-ethylene-5-methyluridine-3'-O-(2-cyanoethyl N,N-diisopropyl)phosphoramidite (2-234),

5'-O-dimethoxytrityl-2'-O,4'-C-ethylene-4-N-benzoylcytidine-3'-O-(2-cyanoethyl N,N-diisopropyl)phosphoramidite (2-235), and

5'-O-dimethoxytrityl-2'-O,4'-C-ethylene-4-N-benzoyl-5-methylcytidine-3'-O-(2-cyanoethyl N,N-diisopropyl)phosphoramiditc (2-236).

The compound (1) of the present invention can be produced according to Process A described below.

Process A



In Process A, X represents a protecting group; Y represents a protecting group; A has the same meaning as defined above; while B^1 represents a purin-9-yl group, a substituted purin-9-yl group or a substituted 2-oxo-pyrimidin-1-yl group, said substituents being selected from the above substituents α but with the exclusion of an unprotected amino group of "an amino group which may be protected"; while B^2 represents a purin-9-yl group, a substituted purin-9-yl group or a substituted 2-oxo-pyrimidin-1-yl group, said substituents being selected from the above substituents α but with the exclusion of protected amino groups of "an amino group which may be protected"; R^7 represents a group which forms a leaving group; and R^8 represents an aliphatic acyl group having from 1 to 4 carbon atoms.

The protecting group of X is the same group as "the hydroxyl protecting group" in the above R^1.

The protecting group of Y is the same group as "the hydroxyl protecting group" in the above R^2.

"The group which forms a leaving group" of R^7 may include a lower alkylsulfonyl group such as methanesulfonyl and ethanesulfonyl; a halogen-substituted lower alkylsulfonyl group such as trifluoromethanesulfonyl; and an arylsulfonyl group such as p-toluenesulfonyl; preferably a methanesulfonyl group or a p-toluenesulfonyl group.

"The aliphatic acyl group having from 2 to 4 carbon atoms" of R^8 may include acetyl, propionyl, butyryl groups and the like, preferably an acetyl group.

In the following, each step of Process A will be described in detail.

(Step A-1)

The present step is to prepare a compound (4) by reacting a compound (3) which can be prepared by Methods B to D described later with a reagent for introducing a leaving group in the presence of a base catalyst in an inert solvent.

The solvent employable here may include aliphatic hydrocarbons such as hexane, heptane, ligroin and petroleum ether; aromatic hydrocarbons such as benzene, toluene and xylene; halogenated hydrocarbons such as methylene chloride, chloroform, carbon tetrachloride, dichloroethane, chlorobenzene and dichlorobenzene; esters such as ethyl formate, ethyl acetate, propyl acetate, butyl acetate and diethyl carbonate; ethers such as diethyl ether, diisopropyl ether, tetrahydrofuran, dioxane, dimethoxyethane and diethylene glycol dimethyl ether; ketones such as acetone, methyl ethyl ketone, methyl isobutyl ketone, isophorone and cyclohexanone; nitro compounds such as nitroethane and nitrobenzene; nitriles such as acetonitrile and isobutyronitrile; amides such as formamide, N,N-dimethylformamide, N,N-dimethylacetamide, N-methyl-2-pyrrolidone, N-methylpyrrolidinone and hexamethylphosphoric triamide; sulfoxides such as sulfolane; and pyridine derivatives; preferably pyridine.

The base catalyst employable here may preferably include a base such as triethylamine, pyridine and dimethylaminopyridine.

The reagent for introducing a leaving group may include alkylsulfonyl halides such as methanesulfonyl chloride and ethanesulfonyl bromide; and arylsulfonyl halides such as p-toluenesulfonyl chloride, preferably methanesulfonyl chloride and p-toluenesulfonyl chloride.

The reaction temperature varies depending on the starting material, the solvent, the reagent for introducing a leaving group and the base catalyst, but is usually from 0°C to 50°C, preferably from 10°C to 40°C.

The reaction time varies depending on the starting material, the solvent, the reagent for introducing a leaving group, the base catalyst and the reaction temperature, but is usually from 10 minutes to 24 hours, preferably from 1 to 10 hours.

After the reaction, the desired compound (4) of the present reaction is obtained, for example, by neutralizing the reaction solution, concentrating the reaction mixture, adding an organic solvent immiscible with water such as ethyl acetate, washing with water, separating an organic layer containing the desired compound, drying over anhydrous magnesium sulfate and distilling off the solvent.

The desired product thus obtained can be further purified, if necessary, by a conventional method, for example, recrystallization and silica gel column chromatography.

(Step A-2)

The present step is to prepare the compound (5) by reacting the compound (4) prepared in Step A-1 with an acid anhydride in the presence of an acid catalyst in a solvent.

The solvent employable here may include ethers such as diethyl ether, dioxane and tetrahydrofuran; nitriles such as acetonitrile and isobutyronitrile; amides such as formamide, N,N-dimethylformamide, N,N-dimethylacetamide, N-methyl-2-pyrrolidone, N-methylpyrrolidinone and hexamethylphosphororic triamide; and organic acids such as acetic acid; preferably acetic acid.

The acid catalyst employable here may include inorganic acids such as hydrochloric acid, sulfuric acid and nitric acid, preferably sulfuric acid (particularly concentrated sulfuric acid).

The acid anhydride employable here may include an anhydride of a lower aliphatic carboxylic acid such as acetic anhydride and propionic acid anhydride, preferably acetic anhydride.

The reaction temperature varies depending on the starting material, the solvent, the acid catalyst and the acid anhydride and is usually from 0°C to 50°C, preferably from 10°C to 40°C.

The reaction time varies depending on the starting material, the solvent, the acid catalyst, the acid anhydride and the reaction temperature, but is usually from 10 minutes to 12 hours, preferably from 30 minutes to 3 hours.

After the reaction, the desired compound (5) of the present reaction is obtained, for example, by concentrating the reaction mixture, adding an organic solvent immiscible with water such as ethyl acetate, washing with water, separating an organic layer containing the desired compound, drying over anhydrous magnesium sulfate and distilling off the solvent.

The desired product thus obtained can be further purified, if necessary, by a conventional method, for example, recrystallization, silica gel column chromatography and the like.

(Step A-3)

The present step is to prepare the compound (6) by reacting the compound (5) prepared in Step A-2 with a trimethylsilylated compound corresponding to the purine or pyrimidine which may have a desired substituent prepared according to a reference (H. Vorbrggen, K. Krolikiewicz and B. Bennua, Chem. Ber., 114, 1234-1255 (1981)) in the presence of an acid catalyst in an inert solvent.

The solvent employable here may include aromatic hydrocarbons such as benzene, toluene, xylene; halogenated hydrocarbons such as methylene chloride, chloroform, carbon tetrachloride, 1,2-dichloroethane, chlorobenzene and dichlorobenzene; nitriles such as acetonitrile and isobutyronitrile; amides such as formamide, N,N-dimethylformamide, N,N-dimethylacetamide, N-methyl-2-pyrrolidone, N-methylpyrrolidinone and hexamethylphosphoric triamide; carbon sulfide; preferably 1,2-dichloroethane.

The acid catalyst employable here may include Lewis acid catalysts such as $AlCl_3$, $SnCl_4$, $TiCl_4$, $ZnCl_2$, BF_3, trimethylsilyl trifluoromethanesulfonate; preferably trimethylsilyl trifluoromethanesulfonate.

The reaction temperature varies depending on the starting material, the solvent and the acid catalyst but is usually from 0°C to 100°C, preferably from 50°C to 80°C.

The reaction time varies depending on the starting material, the solvent, the acid catalyst and the reaction temperature but is usually from 1 hour to 24 hours, preferably from 1 hour to 8 hours.

After the reaction, the desired compound (6) of the present reaction is obtained, for example, by concentrating the reaction mixture, adding an organic solvent immiscible with water such as ethyl acetate, washing with water, separating an organic layer containing the desired compound, drying over anhydrous magnesium sulfate and distilling off the solvent.

The desired product thus obtained can be further purified, if necessary, by a conventional method, for example, recrystallization, silica gel column chromatography and the like.

(Step A-4)

The present step is to prepare the compound (1a) of the present invention by cyclization of the compound (6) prepared by Step A-3 in the presence of a base catalyst in an inert solvent.

The solvent employable here may include water; pyridine derivatives; acetonitriles such as acetonitrile and isobutyronitrile; amides such as formamide, N,N-dimethylformamide, N,N-dimethylacetamide, N-methyl-2-pyrrolidone, N-methylpyrrolidinone and hexamethylphosphoric triamide; and a mixture thereof, preferably a mixture of water and pyridine.

The base catalyst employable here may include alkali metal hydroxides such as sodium hydroxide and potassium hydroxide; alkali metal carbonates such as sodium carbonate and potassium carbonate; alkali metal alkoxides such as sodium methoxide and sodium ethoxide; and aqueous ammonia; preferably alkali metal hydroxides (particularly sodium hydroxide).

The reaction temperature varies depending on the starting material, the solvent and the base catalyst but is usually from 0°C to 50°C, preferably from 10°C to 30°C.

The reaction time varies depending on the starting material, the solvent, the acid catalyst and the reaction temperature but is usually from 1 minute to 5 hours, preferably from 1 minute to 30 minutes.

After the reaction, the desired compound (1a) of the present reaction is obtained, for example, by concentrating the reaction mixture, adding an organic solvent immiscible with water such as ethyl acetate, washing with water, separating an organic layer containing the desired compound, drying over anhydrous magnesium sulfate and distilling off the solvent.

The desired product thus obtained can be further purified, if necessary, by a conventional method, for example, recrystallization, silica gel column chromatography and the like.

(Step A-5)

The present step is to prepare the compound (1b) by reacting the compound (1a) obtained by Step A-4 with a deprotecting reagent in an inert solvent.

The deprotection method varies depending on the kind of protecting group and is not particularly limited unless it causes other side reactions and can be carried out, for example, by a method described in "Protective Groups in Organic Synthesis" (Theodora W. Greene and Peter G. M. Wuts, 1999, Published by A Wiley-Interscience Publication).

Particularly, the deprotection method can be carried out by the following methods in the case where the protecting group is (1) "an aliphatic acyl group or an aromatic acyl group", (2) "a methyl group substituted by from 1 to 3 aryl groups" or "a methyl group substituted by from 1 to 3 aryl groups the aryl ring of which is substituted by lower alkyl, lower alkoxy, halogen or cyano group" or (3) "a silyl group".

(1) In the case where the protecting group is an aliphatic acyl group or an aromatic acyl group, the deprotection reaction is usually carried out by treating it with a base in an inert solvent.

The solvent employable here is not particularly limited so long as it is easily mixed with water, does not inhibit the reaction and dissolves the starting material to some extent and may include aqueous or anhydrous amides such as dimethylformamide and dimethylacetamide; halogenated hydrocarbons such as methylene chloride, chloroform, 1,2-dichloroethane or carbon tetrachloride; and ethers such as tetrahydrofuran, diethyl ether and dioxane; preferably ethers, more preferably tetrahydrofuran.

The base employable here may include alkali metal hydroxides such as lithium hydroxide, potassium hydroxide and sodium hydroxide; alkali metal carbonates such as sodium carbonate and potassium carbonate; alkali metal alkoxides such as sodium methoxide and sodium ethoxide; and an ammonia solution such as aqueous ammonia and ammonia/methanol solution.

The reaction temperature is from 0°C to 60°C, preferably from 20°C to 40°C.

The reaction time is from 10 minutes to 24 hours, preferably from 1 hour to 3 hours.

After the reaction, the desired compound (1b) of the present reaction is obtained, for example, by concentrating the reaction mixture, adding an organic solvent immiscible with water such as ethyl acetate, washing with water, separating an organic layer containing the desired compound, drying over anhydrous magnesium sulfate and distilling off the solvent.

The desired product thus obtained can be further purified, if necessary, by a conventional method, for example, recrystallization, silica gel column chromatography and the like.

(2) In the case where the protecting group is "a methyl group substituted by from one to three aryl groups" or "a methyl group substituted by from one to three aryl groups the aryl ring of which is substituted by a lower alkyl, lower alkoxy, halogen or cyano group", the reaction is carried out in an inert solvent using a reducing agent.

The solvent employable here may preferably include alcohols such as methanol, ethanol and isopropanol; ethers such as diethyl ether, tetrahydrofuran and dioxane; aromatic hydrocarbons such as toluene, benzene and xylene; aliphatic hydrocarbons such as hexane and cyclohexane; esters such as ethyl acetate and propyl acetate; organic acids such as acetic acid; or a mixture of these organic solvents and water.

The reducing agent employable here is not particularly limited so long as it is usually used for a catalytic reduction and may preferably include palladium on carbon, Raney nickel, platinum oxide, platinum black, rhodium-aluminum oxide, triphenylphosphine-rhodium chloride and palladium-barium sulfate.

The pressure is not particularly limited but is usually from 1 to 10 atm.

The reaction temperature is from 0°C to 60°C, preferably from 20°C to 40°C.

The reaction time is from 10 minutes to 24 hours, preferably from one hour to three hours.

After the reaction, the desired compound (1b) of the present reaction is obtained, for example, by removing the reducing agent from the reaction mixture, adding an organic solvent immiscible with water such as ethyl acetate, washing with water, separating an organic layer containing the desired compound, drying over anhydrous magnesium sulfate and distilling off the solvent. The desired product thus obtained can be further purified, if necessary, by a conventional method, for example, recrystallization, silica gel column chromatography and the like.

In the case where the protecting group is "a methyl group substituted by three aryl groups", i.e., a trityl group, the deprotection reaction can be also carried out using an acid.

In this case, the solvent employable here may include aromatic hydrocarbons such as benzene, toluene and xylene; halogenated hydrocarbons such as methylene chloride, chloroform, carbon tetrachloride, 1,2-dichloroethane, chlorobenzene and dichlorobenzene; alcohols such as methanol, ethanol, isopropanol and tert-butanol; nitriles such as acetonitrile and isobutyronitrile; amides such as formamide, N,N-dimethylformamide, N,N-dimethylacetamide, N-methyl-2-pyrrolidone, N-methylpyrrolidinone and hexamethylphosphoric triamide; and organic acids such as acetic acid; preferably organic acids (particularly acetic acid) or alcohols (particularly tert-butanol).

The acid employable here may preferably include acetic acid or trifluoroacetic acid.

The reaction temperature is from 0°C to 60°C, preferably from 20°C to 40°C.

The reaction time is from 10 minutes to 24 hours, preferably from one 1 to 3 hours.

After the reaction, the desired compound (1b) of the present reaction is obtained, for example, by neutralizing the reaction mixture, adding an organic solvent immiscible with water such as ethyl acetate, washing with water, separating an organic layer containing the desired compound, drying over anhydrous magnesium sulfate and distilling off the solvent.

The desired product thus obtained can be further purified, if necessary, by a conventional method, for example, recrystallization, silica gel column chromatography and the like.

(3) In the case where the protecting group is "a silyl group", it can usually be removed by treating with a compound producing a fluorine anion such as tetrabutylammonium fluoride, hydrofluoric acid, hydrofluoric acid-pyridine and potassium fluoride, or organic acids such as acetic acid, methanesulfonic acid, para-toluenesulfonic acid, trifluoroacetic acid and trifluoromethanesulfonic acid, or inorganic acids such as hydrochloric acid.

In the case where the protecting group is removed by a fluorine anion, the reaction is sometimes promoted by adding organic acids such as formic acid, acetic acid and propionic acid thereto.

The solvent employable here is not particularly limited so long as it does not inhibit the reaction and dissolves the starting material to some extent and may preferably include ethers such as diethyl ether, diisopropyl ether, tetrahydrofuran, dioxane, dimethoxyethane and diethylene glycol dimethyl ether; nitriles such as acetonitrile and isobutyronitrile; water; organic acids such as acetic acid; and a mixture thereof.

The reaction temperature is from 0°C to 100°C, preferably from 20°C to 70°C.

The reaction time is from 5 minutes to 48 hours, preferably from one hour to 24 hours.

After the reaction, the desired compound (1b) of the present reaction is obtained, for example, by concentrating the reaction mixture, adding an organic solvent immiscible with water such as ethyl acetate, washing with water, separating an organic layer containing the desired compound, drying over anhydrous magnesium sulfate and distilling off the solvent. The desired product thus obtained can be further purified, if necessary, by a conventional method, for example, recrystallization, silica gel column chromatography and the like.

(Step A-6)

The present step is to prepare the compound (1c) of the present invention by reacting the compound (1b) obtained in Step A-5 with a deprotection reagent in an inert solvent.

The deprotection method varies depending on the kind of protecting group and is not particularly limited so long as it does not cause other side reactions and can be carried out, for example, by a method described in "Protective Groups in Organic Synthesis" (by Theodora W. Greene, 1981, published by A Wiley-Interscience Publication).

Particularly, the deprotection method can be carried out by the following method in the case where the protecting group is an aliphatic acyl group or an aromatic acyl group.

Namely, the deprotection method is usually carried out by reacting with a base in an inert solvent in the case where the protecting group is an aliphatic acyl group or an aromatic acyl group.

The solvent employable here is not particularly limited so long as it is easily mixed with water, does not inhibit the reaction and dissolves the starting material to some extent and may include aqueous or anhydrous alcohols such as methanol and ethanol; amides such as dimethylformamide and dimethylacetamide; halogenated hydrocarbons such as methylene chloride, chloroform, 1,2-dichloroethane or carbon tetrachloride; and ethers such as tetrahydrofuran, diethyl ether and dioxane; preferably alcohols; more preferably methanol.

The base employable here may include alkali metal hydroxides such as lithium hydroxide, potassium hydroxide and sodium hydroxide; alkali metal carbonates such as sodium carbonate and potassium carbonate; alkali metal alkoxides such as sodium methoxide and sodium ethoxide; and ammonia; preferably ammonia.

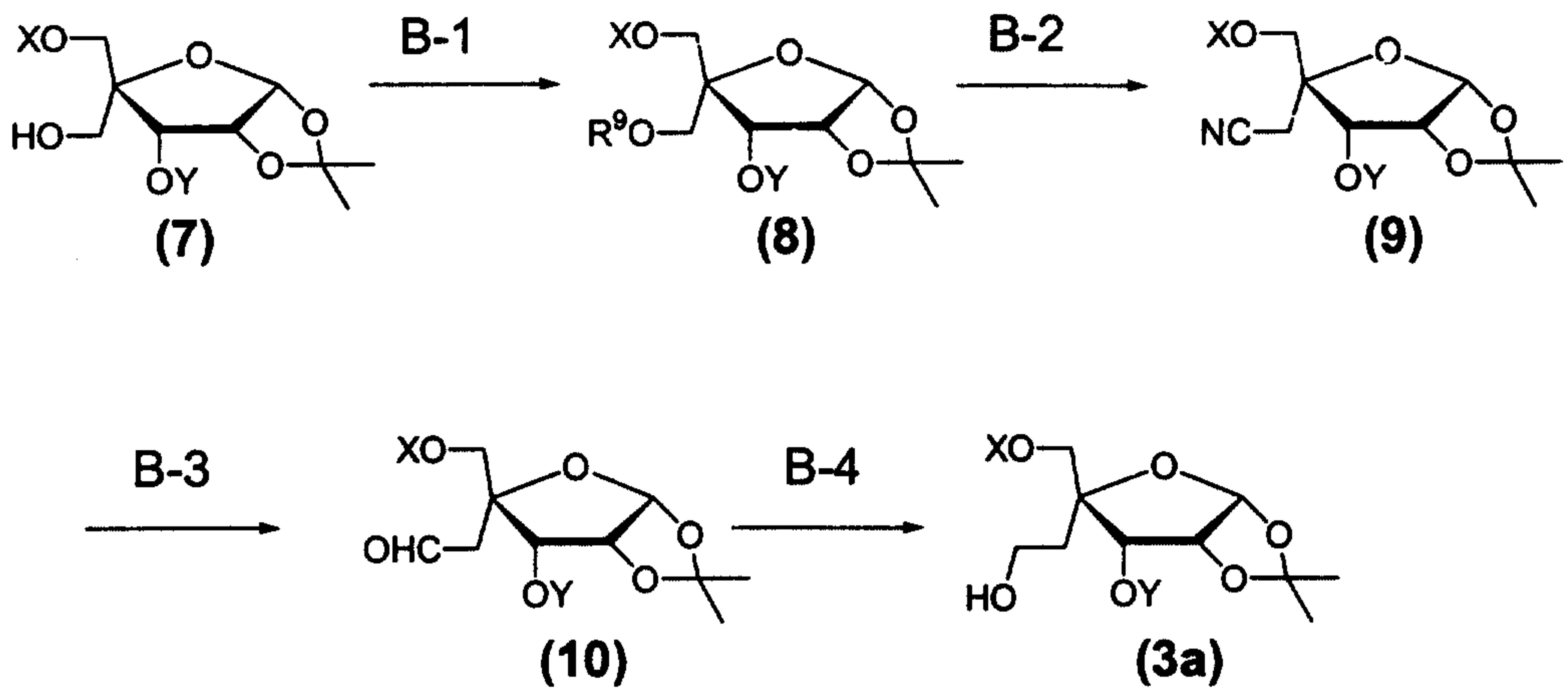
The reaction temperature is from 0°C to 50°C, preferably from 10°C to 40°C.

The reaction time is from 10 minutes to 24 hours, preferably from 10 minutes to 15 hours. After the reaction, the desired compound (1c) of the present reaction is obtained, for example, by concentrating the reaction mixture, adding an organic solvent immiscible with water such as ethyl acetate, washing with water, separating an organic layer containing the desired compound, drying over anhydrous magnesium sulfate and distilling off the solvent.

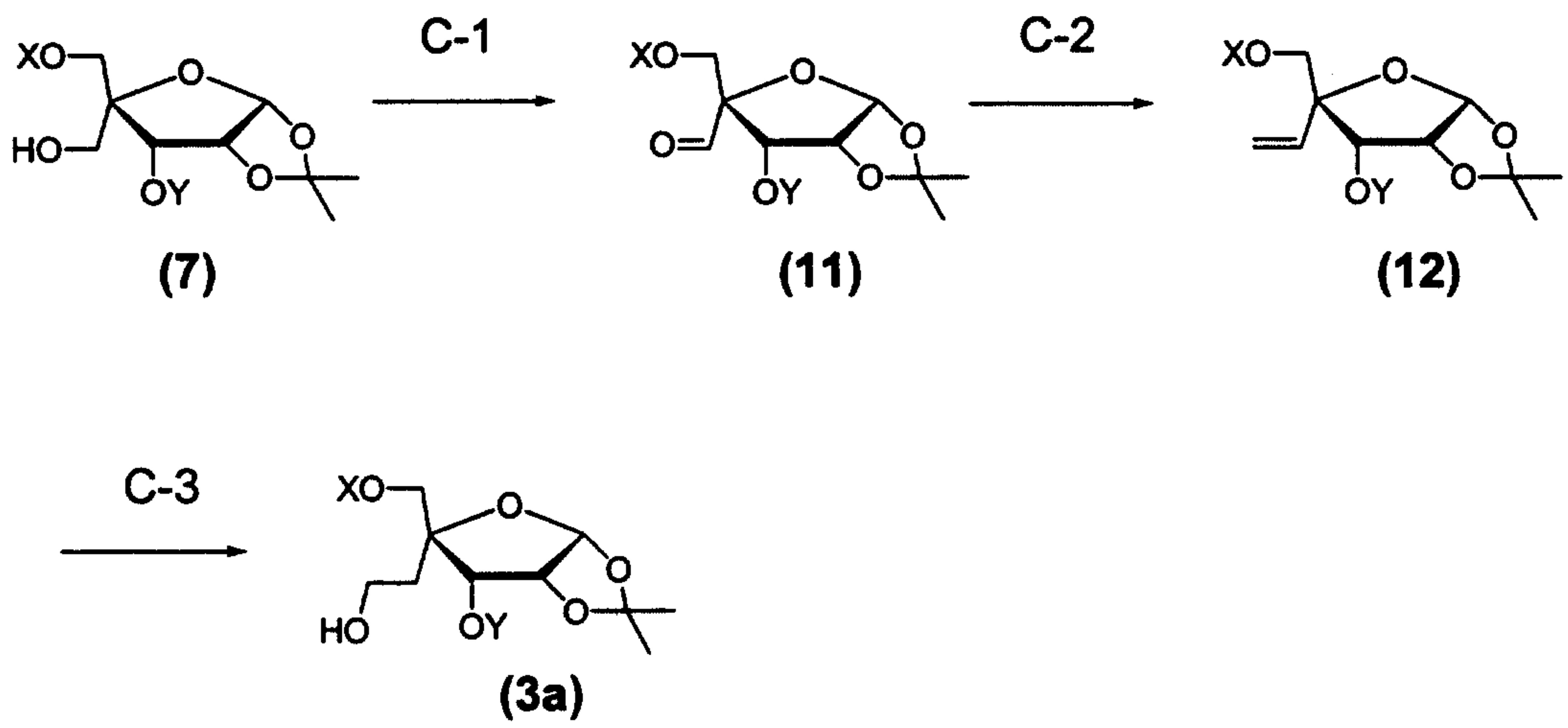
The desired product thus obtained can be further purified, if necessary, by a conventional method, for example, recrystallization, silica gel column chromatography and the like.

The intermediate (3) described above can be prepared by Processes B to D described below.

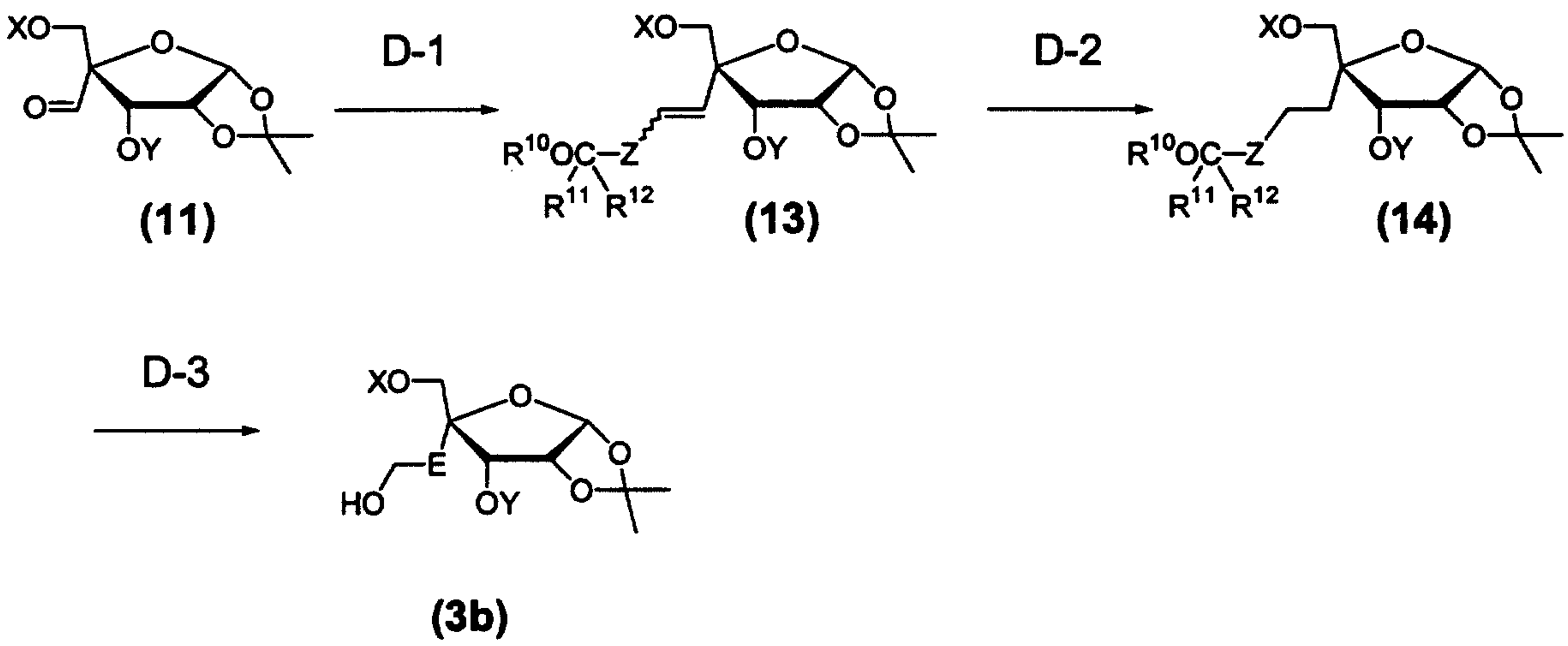
Process B



Process C



Process D



In Processes B to D, X and Y have the same meanings as defined above; R^9

represents a group which forms a leaving group; E represents an ethylene, trimethylene or tetramethylene group; and Z represents a single bond, a methylene or ethylene group.

The group which forms a leaving group of R^9 may include the group described in the above R^7, preferably a trifluoromethanesulfonyl group.

R^{11} and R^{12} are the same and represent a hydrogen atom or taken together form an oxygen atom.

In the case where R^{11} and R^{12} taken together form the oxygen atom, R^{10} represents an alkyl group having from 1 to 4 carbon atoms such as methyl, ethyl, propyl, isopropyl, butyl, isobutyl, s-butyl and tert-butyl, preferably a methyl group. In the case where R^{11} and R^{12} are the same and represent a hydrogen atom, R^{10} may include an aralkyl group such as a benzyl group; an alkoxyalkyl group such as a methoxymethyl group; an arylcarbonyloxymethyl group such as a benzoyloxymethyl group, an aralkyloxymethyl group such as a benzyloxymethyl group; an alkoxyalkoxyalkyl group such as a methoxyethoxymethyl group; a silyl group such as trimethylsilyl, t-butyldimethylsilyl, diphenylmethylsilyl, diphenylbutylsilyl, diphenylisopropylsilyl and phenyldiisopropylsilyl.

The compound (7), i.e., the starting material used in Process B or Process C can be prepared by the following method.

Namely, a compound corresponding to the compound (6) of which the "X" moiety is a hydrogen atom is prepared from 1,1,5,6-diisopropylidene D-glucose on public sale according to the method of the literature (R.D. Youssefyeh, J.P.H. Verheyden, J.G. Moffatt. J. Org. Chem., 44, 1301-1309 (1979)) and subsequently the compound (6) can be prepared according to the method of the literature (T. Waga, T. Nishizaki, I. Miyakawa, H. Ohrui, H. Meguro, Biosci. Biotechnol. Biochem., 57, 1433-1438 (1993)) (in the case of X = Bn).

(Process B)

(Step B-1)

The present step is to prepare the compound (8) by reacting the compound (7) prepared by the above method with a reagent for introducing a leaving group in the presence of a base catalyst in an inert solvent.

The solvent employable here may include amides such as dimethylformamide and dimethylacetamide; halogenated hydrocarbons such as methylene chloride, chloroform, 1,2-dichloroethane or carbon tetrachloride; and ethers such as tetrahydrofuran, diethyl ether and dioxane; preferably methylene chloride.

The base catalyst employable here may preferably include a base such as triethylamine, pyridine and dimethylaminopyridine.

The reagent employable for introducing a leaving group may preferably include trifluoromethanesulfonic acid chloride or trifluoromethanesulfonic anhydride.

The reaction temperature varies depending on the starting material, the solvent and the acid catalyst, but is usually from -100°C to -50°C, preferably from -100°C to -70°C.

The reaction time varies depending on the starting material, the solvent, the acid catalyst and the reaction temperature but is usually from 30 minutes to 12 hours, preferably from 30 minutes to 3 hours.

After the reaction, the desired compound (8) of the present reaction is obtained, for example, by concentrating the reaction mixture, adding an organic solvent immiscible with water such as ethyl acetate, washing with water, separating an organic layer containing the desired compound, drying over anhydrous magnesium sulfate and distilling off the solvent.

The desired product thus obtained can be further purified, if necessary, by a conventional method, for example, recrystallization, silica gel column chromatography and the like.

(Step B-2)

The present step is to prepare the compound (9) by reacting the compound (8) prepared by Step B-1 with a cyanating reagent in an inert solvent.

The solvent employable here may include amides such as dimethylformamide and dimethylacetamide; halogenated hydrocarbons such as methylene chloride, chloroform, 1,2-dichloroethane or carbon tetrachloride; ethers such as tetrahydrofuran, diethyl ether and dioxane; acetonitrile; dimethylsulfoxide and the like; preferably amides (dimethylformamide).

The cyanating reagent employable here may include KCN, NaCN and trimethylsilane cyanide, preferably NaCN.

The reaction temperature varies depending on the starting material, the solvent and the cyanating reagent but is usually from 0°C to 100°C, preferably from 30°C to 70°C.

The reaction time varies depending on the starting material, the solvent, the cyanating reagent and the reaction temperature but is usually from 30 minutes to 12 hours, preferably from one 1 to 3 hours.

After the reaction, the desired compound (9) of the present reaction is obtained, for example, by concentrating the reaction mixture, adding an organic solvent immiscible with water such as ethyl acetate, washing with water, separating an organic layer containing the desired compound, drying over anhydrous magnesium sulfate and distilling off the solvent.

The desired product thus obtained can be further purified, if necessary, by a conventional method, for example, recrystallization, silica gel column chromatography and the like.

(Step B-3)

The present step is to prepare the compound (10) by reacting the compound (9) prepared in Step B-2 with a reducing agent in an inert solvent.

The solvent employable here may include halogenated hydrocarbons such as methylene chloride, chloroform, 1,2-dichloroethane or carbon tetrachloride; aliphatic hydrocarbons such as hexane, heptane, ligroin and petroleum ether; aromatic hydrocarbons such as benzene, toluene and xylene; ethers such as diethyl ether, diisopropyl ether, tetrahydrofuran, dioxane, dimethoxyethane and diethylene glycol dimethyl ether; and ketones such as acetone, methyl ethyl ketone, methyl isobutyl ketone isophorone and cyclohexanone; preferably halogenated hydrocarbons (particularly methylene chloride).

The reducing agent employable here may include diisobutyl aluminum hydride and triethoxy aluminum hydride, preferably diisobutyl aluminum hydride.

The reaction temperature varies depending on the starting material, the solvent and the reducing agent but is usually from -100°C to -50°C, preferably from -90°C to -70°C.

The reaction time varies depending on the starting material, the solvent, the reducing agent and the reaction temperature but is usually from 30 minutes to 12 hours, preferably from 1 hour to 5 hours.

After the reaction, the desired compound (10) of the present reaction is obtained, for example, by concentrating the reaction mixture, adding an organic solvent immiscible with water such as ethyl acetate, washing with water, separating an organic layer containing the desired compound, drying over anhydrous magnesium sulfate and distilling off the solvent.

The desired product thus obtained can be further purified, if necessary, by a conventional method, for example, recrystallization, silica gel column chromatography and the like.

(Step B-4)

The present step is to prepare the compound (3a), one of the starting materials of Process A by reacting the compound (10) prepared in Step B-3 with a reducing agent in an inert solvent.

The solvent employable here may include alcohols such as methanol, ethanol, n-propanol, isopropanol, n-butanol, isobutanol, t-butanol, isoamyl alcohol, diethylene glycol, glycerine, octanol, cyclohexanol and methyl cellosolve; and acetic acid; preferably alcohols (particularly ethanol).

The reducing agent employable here may include alkali metal boron hydrides such as sodium boron hydride and lithium boron hydride; aluminum hydride compounds such as lithium aluminum hydride and lithium triethoxide aluminum hydride; and borane; preferably sodium boron hydride.

The reaction temperature varies depending on the starting material, the solvent and the reducing agent but is usually from 0°C to 50°C, preferably from 10°C to 40°C.

The reaction time varies depending on the starting material, the solvent, the reducing agent and the reaction temperature but is usually from 10 minutes to 12 hours, preferably from 30 minutes to 5 hours.

After the reaction, the desired compound (3a) of the present reaction is obtained, for example, by decomposing the reducing agent, concentrating the reaction mixture, adding an organic solvent immiscible with water such as ethyl acetate, washing with water, separating an organic layer containing the desired compound, drying over anhydrous magnesium sulfate and distilling off the solvent.

The desired product thus obtained can be further purified, if necessary, by a conventional method, for example, recrystallization, silica gel column chromatography and the like.

(Process C)

(Step C-1)

The present step is to prepare the compound (11) by reacting the compound (7) prepared in the above process with an oxidizing agent in an inert solvent.

The solvent employable here may include aliphatic hydrocarbons such as hexane, heptane, ligroin and petroleum ether; aromatic hydrocarbons such as benzene, toluene and xylene; halogenated hydrocarbons such as methylene chloride, chloroform, carbon tetrachloride, dichloroethane, chlorobenzene and dichlorobenzene; esters such as ethyl formate, ethyl acetate, propyl acetate, butyl acetate and diethyl carbonate; ethers such as diethyl ether, diisopropyl ether, tetrahydrofuran, dioxane, dimethoxyethane, diethylene glycol dimethyl ether; and ketones such as acetone, methyl ethyl ketone, methyl isobutyl ketone, isophorone and cyclohexanone; preferably halogenated hydrocarbons (particularly methylene chloride).

The oxidizing agent employable here may include the Swern reagent for oxidation, the Dess-Martin reagent for oxidation, a chromium trioxide complex such as pyridine hydrochloride/chromium trioxide complex (pyridinium chlorochromate and pyridinium dichromate), preferably the Swern reagent for oxidation (namely, dimethyl sulfoxide-oxalyl chloride).

The reaction temperature varies depending on the starting material, the solvent and the oxidizing agent but is usually from -100°C to -50°C, preferably from -100°C to -70°C.

The reaction time varies depending on the starting material, the solvent, the oxidizing agent and the reaction temperature but is usually from 30 minutes to 12 hours, preferably from 1 hour to 5 hours.

After the reaction, the desired compound (11) of the present reaction is obtained, for example, by decomposing the oxidizing agent, concentrating the reaction mixture, adding an organic solvent immiscible with water such as ethyl acetate, washing with water, separating an organic layer containing the desired compound, drying over anhydrous magnesium sulfate and distilling off the solvent.

The desired product thus obtained can be further purified, if necessary, by a conventional method, for example, recrystallization, silica gel column chromatography and the like.

(Step C-2)

The present step is to prepare the compound (12) by reacting the compound (11) prepared in Step C-1 with a carbon-increasing reagent in an inert solvent.

The solvent employable here may include aliphatic hydrocarbons such as hexane, heptane, ligroin and petroleum ether; aromatic hydrocarbons such as benzene, toluene and xylene; halogenated hydrocarbons such as methylene chloride, chloroform, carbon tetrachloride, dichloroethane, chlorobenzene and dichlorobenzene; esters such as ethyl formate, ethyl acetate, propyl acetate, butyl acetate and diethyl carbonate; ethers such as diethyl ether, diisopropyl ether, tetrahydrofuran, dioxane, dimethoxyethane, diethylene glycol dimethyl ether; and ketones such as acetone, methyl ethyl ketone, methyl isobutyl ketone, isophorone and cyclohexanone; preferably halogenated hydrocarbons (particularly methylene chloride).

The reagent employable here may include the Wittig reagent, Horner-Emmons reagent, Peterson reaction reagent, $TiCl_4$-CH_2Cl_2-Zn system reaction agent and Tebbe reagent, preferably the Wittig reagent, Horner-Emmons reagent and Tebbe reagent.

The reaction temperature varies depending on the starting material, the solvent and the carbon-increasing reagent but is usually from -20°C to 20°C, preferably 0°C.

The reaction time varies depending on the starting material, the solvent, the carbon-increasing reagent and the reaction temperature but is usually from 30 minutes to 12 hours, preferably from 1 hour to 5 hours.

After the reaction, the desired compound (12) of the present reaction is obtained, for example, by concentrating the reaction mixture, adding an organic solvent immiscible with water such as ethyl acetate, washing with water, separating an organic layer containing the desired compound, drying over anhydrous magnesium sulfate and distilling off the solvent.

The desired product thus obtained can be further purified, if necessary, by a conventional method, for example, recrystallization, silica gel column chromatography and the like.

(Step C-3)

The present step is to prepare the compound (3a) by selectively introducing a hydroxyl group to a terminal carbon of olefin of the compound (12) prepared in Step

C-2 in an inert solvent.

The solvent employable here may include aliphatic hydrocarbons such as hexane, heptane, ligroin and petroleum ether; aromatic hydrocarbons such as benzene, toluene and xylene; halogenated hydrocarbons such as methylene chloride, chloroform, carbon tetrachloride, dichloroethane, chlorobenzene and dichlorobenzene; esters such as ethyl formate, ethyl acetate, propyl acetate, butyl acetate and diethyl carbonate; ethers such as diethyl ether, diisopropyl ether, tetrahydrofuran, dioxane, dimethoxyethane and diethylene glycol dimethyl ether; and ketones such as acetone, methyl ethyl ketone, methyl isobutyl ketone, isophorone and cyclohexanone; preferably ethers (particularly tetrahydrofuran).

The reaction reagent employable here may include borane, disiamyl borane, thexyl borane, 9-BBN (9-borabicyclo[3.3.1]nonane), preferably the 9-BBN.

The reaction temperature varies depending on the starting material, the solvent and the reagent but is usually from 0°C to 50°C, preferably from 10°C to 40°C.

The reaction time varies depending on the starting material, the solvent, the reagent and the reaction temperature but is usually from 6 hours to 48 hours, preferably from 12 hours to 24 hours.

After the reaction, the desired compound (3a) of the present reaction is obtained, for example, by concentrating the reaction mixture, adding an organic solvent immiscible with water such as ethyl acetate, washing with water, separating an organic layer containing the desired compound, drying over anhydrous magnesium sulfate and distilling off the solvent.

The desired product thus obtained can be further purified, if necessary, by a conventional method, for example, recrystallization, silica gel column chromatography and the like.

(Process D)

(Step D-1)

The present step is to prepare the compound (13) by reacting the compound (11) prepared in Step C-1 with a carbon-increasing reagent in an inert solvent.

The solvent employable here may include aliphatic hydrocarbons such as hexane, heptane, ligroin and petroleum ether; aromatic hydrocarbons such as benzene, toluene and xylene; halogenated hydrocarbons such as methylene chloride, chloroform, carbon tetrachloride, dichloroethane, chlorobenzene and dichlorobenzene; esters such as ethyl formate, ethyl acetate, propyl acetate, butyl acetate and diethyl carbonate; ethers such as diethyl ether, diisopropyl ether, tetrahydrofuran, dioxane, dimethoxyethane and diethylene glycol dimethyl ether; and ketones such as acetone, methyl ethyl ketone, methyl isobutyl ketone, isophorone and cyclohexanone; preferably ethers (particularly tetrahydrofuran), more preferably halogenated hydrocarbons (particularly methylene chloride).

The carbon-increasing reagent employable here may include the Wittig reagent and Horner-Emmons reagent.

The reaction temperature varies depending on the starting material, the solvent and the reagent but is usually from -20°C to 40°C, preferably from 0°C to 20°C.

The reaction time varies depending on the starting material, the solvent, the reagent and the reaction temperature but is usually from 30 minutes to 12 hours, preferably from 1 hour to 5 hours.

After the reaction, the desired compound (13) of the present reaction is obtained, for example, by concentrating the reaction mixture, adding an organic solvent immiscible with water such as ethyl acetate, washing with water, separating an organic layer containing the desired compound, drying over anhydrous magnesium sulfate and distilling off the solvent.

The desired product thus obtained can be further purified, if necessary, by a conventional method, for example, recrystallization, silica gel column chromatography and the like.

(Step D-2)

The present step is to prepare the compound (14) by reacting the compound (13) prepared in Step D-1 with a reducing agent in an inert solvent.

The present step can be carried out according to (2) of Step A-5. In the case where R^{10} is an optionally substituted benzyl group and R^{11} and R^{12} are hydrogen atoms, the compound (3b) can be directly prepared in this step.

(Step D-3)

The present step is to prepare the compound (3b), one of the starting materials of Process A by reacting the compound (14) prepared in Step D-2 with a reducing agent.

(a) In the case where R^{11} and R^{12} taken together form an oxygen atom.

The solvent employable here may include alcohols such as methanol, ethanol, n-propanol, isopropanol, n-butanol, isobutanol, t-butanol, isoamyl alcohol, diethylene glycol, glycerine, octanol, cyclohexanol and methyl cellosolve; and acetic acid; preferably alcohols (particularly ethanol).

The reducing agent employable here may include alkali metal boron hydrides such as lithium boron hydride; aluminum hydride compounds such as lithium aluminum hydride and lithium triethoxide aluminum hydride; and borane; preferably borane and lithium aluminum hydride.

The reaction temperature varies depending on the starting material, the solvent and the reducing agent but is usually from 0°C to 50°C, preferably from 10°C to 40°C.

The reaction time varies depending on the starting material, the solvent, the reducing agent and the reaction temperature but is usually from 10 minutes to 12 hours, preferably from 30 minutes to 5 hours.

After the reaction, the desired compound (3b) of the present reaction is obtained, for example, by decomposing the reducing agent, concentrating the reaction mixture, adding an organic solvent immiscible with water such as ethyl acetate, washing with water, separating an organic layer containing the desired compound, drying over anhydrous magnesium sulfate and distilling off the solvent.

The desired product thus obtained can be further purified, if necessary, by a conventional method, for example, recrystallization, silica gel column chromatography and the like.

(b) In the case where R^{11} and R^{12} are hydrogen atoms and R^{10} is a group other than a benzyl group.

In the case where R^{10} is a silyl group, the present step can be carried out according to the method of (3) of Step A-5.

In the case where R^{10} is an aralkyl group such as a benzyl group; an alkoxyalkyl group such as a methoxymethyl group; an arylcarbonyloxymethyl such as a benzoyloxymethyl group or an aralkyloxymethyl group such as a benzyloxymethyl group; and an alkoxyalkoxyalkyl group such as a methoxyethoxymethyl group, an acid catalyst is used and the acid catalyst used in this case may include an organic acid such as p-toluenesulfonic acid, trifluoroacetic acid and dichloroacetic acid and a Lewis acid such as BF_3 and $AlCl_3$.

The solvent employable here may include aromatic hydrocarbons such as benzene, toluene and xylene; halogenated hydrocarbons such as methylene chloride, chloroform, carbon tetrachloride, 1,2-dichloroethane, chlorobenzene and dichlorobenzene; nitriles such as acetonitrile and isobutyronitrile; amides such as formamide, N,N-dimethylformamide, N,N-dimethylacetamide, N-methyl-2-pyrrolidone, N-methylpyrrolidinone and hexamethylphosphoric triamide; and carbon sulfide.

The reaction temperature varies depending on the starting material, the solvent and the acid catalyst but is usually from 0°C to 50°C, preferably from 10°C to 40°C.

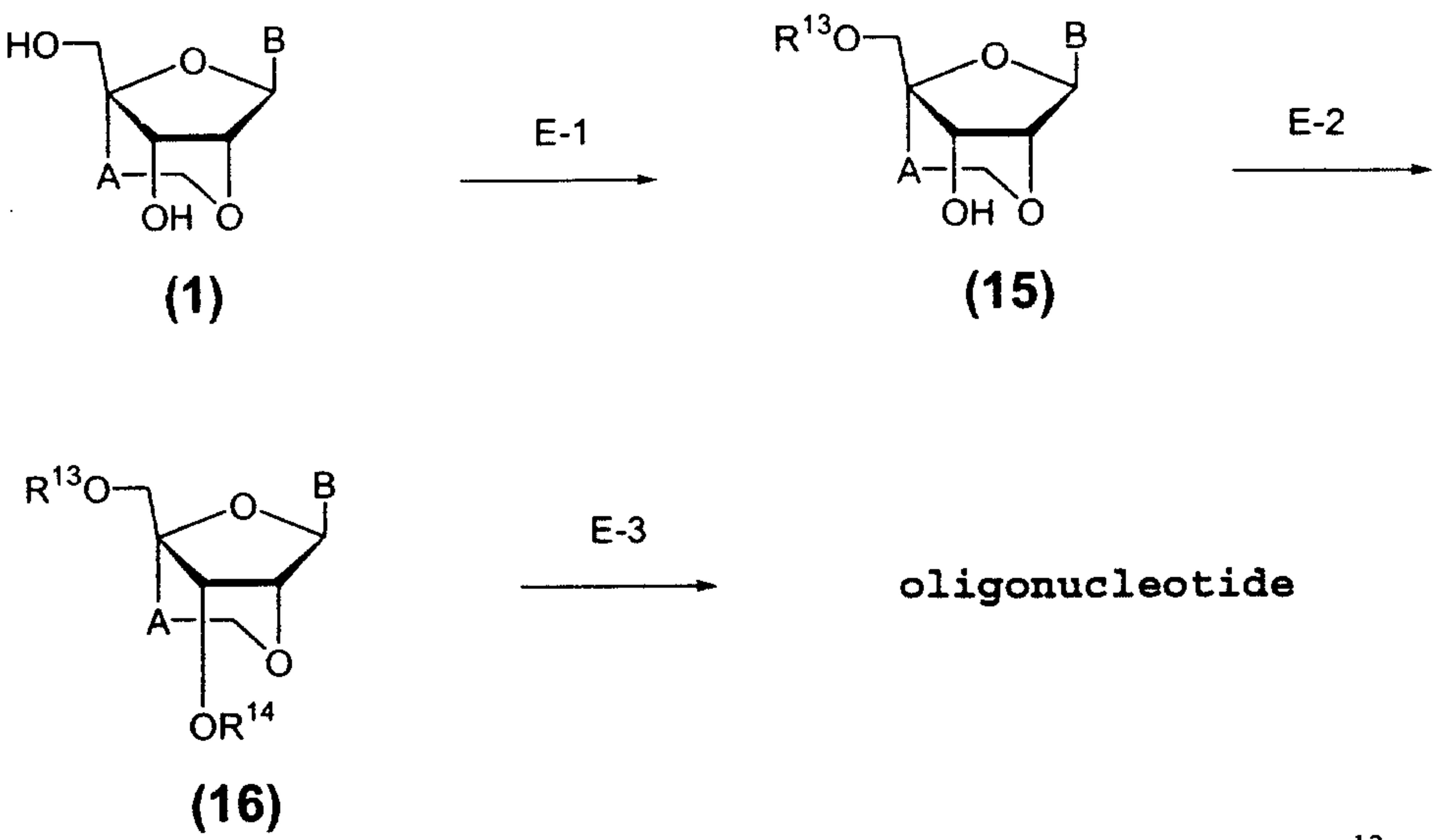
The reaction time varies depending on the starting material, the solvent, the acid catalyst and the reaction temperature and is usually from 10 minutes to 12 hours, preferably from 30 minutes to 5 hours.

After the reaction, the desired compound (3b) of the present reaction is obtained, for example, by neutralizing the reaction mixture, adding an organic solvent immiscible with water such as ethyl acetate, washing with water, separating an organic layer containing the desired compound, drying over anhydrous magnesium sulfate and distilling off the solvent.

The desired product thus obtained can be further purified, if necessary, by a conventional method, for example, recrystallization, silica gel column chromatography and the like.

Oligonucleotides containing a modified nucleoside or a thioate derivative thereof can be prepared by Process E described below using the compound (1) of the present invention.

<u>Process E</u>



In Process E, A and B have the same meaning as defined above; R^{13} represents a hydroxyl protecting group (particularly a trityl group which may be substituted by a methoxy group); R^{14} represents a phosphonyl group or a group formed by reacting mono-substituted chloro(alkoxy)phosphines or di-substituted alkoxyphosphines described later.

(Process E)

(Step E-1)

The present step is to prepare the compound (15) by reacting the compound (1) prepared in Process A with a protecting reagent in an inert solvent.

The solvent employable here may preferably include aromatic hydrocarbons such as benzene, toluene and xylene; halogenated hydrocarbons such as methylene chloride, chloroform, carbon tetrachloride, dichloroethane, chlorobenzene and dichlorobenzene; esters such as ethyl formate, ethyl acetate, propyl acetate, butyl acetate and diethyl carbonate; ethers such as diethyl ether, diisopropyl ether, tetrahydrofuran, dioxane, dimethoxyethane and diethylene glycol dimethyl ether; ketones such as acetone, methyl ethyl ketone, methyl isobutyl ketone, isophorone and cyclohexanone; nitrated compounds such as nitroethane and nitrobenzene; nitriles such as acetonitrile and isobutyronitrile; amides such as formamide, dimethylformamide (DMF), dimethylacetamide and hexamethylphosphoric triamide; sulfoxides such as dimethyl sulfoxide and sulfolane; aliphatic tertiary amines such as trimethylamine, triethylamine and N-methylmorpholine; and aromatic amines such as pyridine and picoline; more preferably halogenated hydrocarbons (particularly methylene chloride) and aromatic amines (particularly pyridine).

The protecting reagent employable here is not particularly limited so long as only the 5'-position can be selectively protected and it can be removed under acidic or neutral conditions but may preferably include triarylmethyl halides such as trityl chloride, monomethoxytrityl chloride and dimethoxytrityl chloride.

In the case where triarylmethyl halides are used as the protecting reagent, a base is usually used.

In such case, the base employable here may include heterocyclic amines such as pyridine, dimethylaminopyridine and pyrrolidinopyridine; and aliphatic tertiary amines such as trimethylamine and triethylamine; preferably pyridine, dimethylaminopyridine and pyrrolidinopyridine.

In the case where a liquid base is used as the solvent, since the base itself functions as an acid trapping agent, it is not necessary to add another base.

The reaction temperature varies depending on the starting material, the reagent and the solvent but is usually from 0°C to 150°C, preferably from 20°C to 100°C. The reaction time varies depending on the starting material, the solvent and the reaction temperature but is usually from 1 hour to 100 hours, preferably from 2 hours to 24 hours.

After the reaction, the desired compound (15) of the present reaction is obtained, for example, by concentrating the reaction mixture, adding an organic solvent immiscible with water such as ethyl acetate, washing with water, separating an organic layer containing the desired compound, drying over anhydrous magnesium sulfate and distilling off the solvent.

The desired product thus obtained can be further purified, if necessary, by a conventional method, for example, recrystallization, silica gel column chromatography and the like.

(Step E-2)

The present step is to prepare the compound (16) by reacting the compound (15) prepared in Step E-1 with mono-substituted chloro(alkoxy)phosphines or di-substituted alkoxyphosphines usually used for amiditation in an inert solvent.

The solvent employable here is not particularly limited so long as it does not affect the reaction and may preferably include ethers such as tetrahydrofuran, diethyl ether and dioxane; and halogenated hydrocarbons such as methylene chloride, chloroform, carbon tetrachloride, dichloroethane, chlorobenzene and dichlorobenzene.

The mono-substituted chloro(alkoxy)phosphines employable here may include phosphine drivatives such as chloro(morpholino)methoxyphosphine, chloro(morpholino)cyanoethoxyphosphine, chloro(dimethylamino)methoxyphosphine, chloro(dimethylamino)cyanoethoxyphosphine, chloro(diisopropylamino)methoxyphosphine and chloro(diisopropylamino)cyanoethoxyphosphine, preferably chloro(morpholino)methoxyphosphine, chloro(morpholino)cyanoethoxyphosphine, chloro(diisopropylamino)methoxyphosphine and chloro(diisopropylamino)cyanoethoxyphosphine.

In the case where the mono-substituted-chloro(alkoxy)phosphines are used, an acid trapping agent is used and in such case, the acid trapping agent employable here may include heterocyclic amines such as pyridine and dimethylaminopyridine; and aliphatic amines such as trimethylamine, triethylamine and diisopropylamine; preferably aliphatic amines (particularly diisopropylamine).

The di-substituted alkoxyphosphines employable here may include phosphine derivatives such as bis(diisopropylamino)cyanoethoxyphosphine, bis(diethylamino)methanesulfonylethoxyphosphine, bis(diisopropylamino)(2,2,2-trichloroethoxy)phosphine and bis(diisopropylamino)(4-chlorophenylmethoxy)phosphine, preferably bis(diisopropylamino)cyanoethoxyphosphine.

In the case where the di-substituted alkoxyphosphines are used, an acid is used, and in such case, the acid employable may preferably include tetrazole, acetic acid or p-toluenesulfonic acid.

The reaction temperature is not particularly limited but is usually from 0°C to 80°C, preferably room temperature.

The reaction time varies depending on the starting material, the reagent and the reaction temperature, but is usually from 5 minutes to 30 hours, preferably from 30 minutes to 10 hours in the case where the reaction is carried out at room temperature.

After the reaction, the desired compound (16) of the present reaction is obtained, for example, by appropriately neutralizing the reaction mixture, removing insolubles by filtration in the case where they exist, adding an organic solvent immiscible with water such as ethyl acetate, washing with water, separating an organic layer containing the desired compound, drying over anhydrous magnesium sulfate and distilling off the solvent. The desired product thus obtained can be further purified, if necessary, by a conventional method, for example, recrystallization, reprecipitation or chromatography and the like.

Alternatively, the present step is to prepare the compound (16) by reacting the compound (15) prepared in Step E-1 with tris-(1,2,4-triazolyl)phosphite in an inert solvent (preferably halogenated hydrocarbons such as methylene chloride), followed by the addition of water to effect H-phosphonation.

The reaction temperature is not particularly limited, but is usually from -20°C to 100°C, preferably from 10 to 40°C.

The reaction time varies depending on the starting material, the reagent and the reaction temperature and is usually from 5 minutes to 30 hours, preferably 30 minutes in the case where the reaction is carried out at room temperature.

After the reaction, the desired compound (16) of the present reaction is obtained, for example, by appropriately neutralizing the reaction mixture, removing insolubles by filtration in the case where they exist, adding an organic solvent immiscible with water such as ethyl acetate, washing with water, separating an organic layer containing the desired compound, drying over anhydrous magnesium sulfate and distilling off the solvent. The desired product thus obtained can be further purified, if necessary, by a conventional method, for example, recrystallization, reprecipitation or chromatography and the like.

(Step E-3)

In this step, the target oligonucleotide analogue is produced by an automated DNA synthesizer using at least one compound (16) prepared in step E-2 and commercially available phosphoramidite reagents required for producing an oligonucleotide analogue of a desired nucleotide sequence in accordance with conventional methods.

An oligonucleotide analogue having a desired nucleotide sequence can be synthesized by a DNA synthesizer such as the Perkin-Elmer Model 392 using the phosphoramidite method in accordance with the method described in the literature

(Nucleic Acids Research, 12, 4539 (1984)).

In addition, in the case of converting to a thioate as desired, a thioate derivative can be obtained in accordance with the method described in the literature (Tetrahedron Letters, 32, 3005 (1991), J. Am. Chem. Soc., 112, 1253 (1990)) using, besides sulfur, a reagent that forms a thioate by reacting with trivalent phosphoric acid such as tetraethylthiuram disulfide (TETD, Applied Biosystems Inc.) or Beaucage reagent (Millipore Corp.).

The resulting crude oligonucleotide analogue can be purified by OligoPak (reverse phase chromatocolumn) and the purity of the product can be confirmed by HPLC analysis.

The chain length of the resulting oligonucleotide analogue is normally 2 to 50 units, and preferably 10 to 30 units, in nucleoside units.

The complementary chain formation ability and nuclease enzyme resistance of the resulting oligonucleotide analogue can be determined according to the methods described below.

(Test Method 1)

The hybrid formation ability of the oligonucleotide analogue of the present invention with respect to complementary DNA and RNA can be determined by annealing the various resulting oligonucleotide analogues with an oligonucleotide analogue composed of naturally-occurring DNA or RNA having a complementary sequence and measuring the melting temperature (Tm value).

A sample solution containing equal amounts of oligonucleotide analogue and naturally-occurring complementary oligonucleotide in sodium phosphate buffer solution was put into a boiling water bath and then slowly cooled to room temperature over the course of time (annealing). The temperature of the solution was then raised little by little from 20°C to 90°C in the cell chamber of a spectrophotometer (e.g., Shimadzu UV-2100PC) followed by measurement of ultraviolet absorption at 260 nm.

(Test Method 2) Measurement of Nuclease Enzyme Resistance

To the oligonucleotide in a buffer solution was added a nuclease and the mixture was warmed. Examples of nucleases that are used include snake venom phosphodiesterase, endonuclease P1 and endonuclease S1. Although there are no particular restrictions on the buffer solution provided it is a buffer solution suitable for enzymes, Tris-HCl buffer is used in the case of snake venom phosphodiesterase, while sodium acetate buffer is used in the case of endonuclease P1. In addition, metal ions are added to the buffer solution as necessary. Examples of metal ions used include Mg^{2+} in the case of snake venom phosphodiesterase and Zn^{2+} in the case of endonuclease. The reaction temperature is preferably 0 to 100°C, and more preferably 30 to 50°C.

Ethylenediamine tetraacetic acid (EDTA) is added after a predetermined amount of time followed by heating at 100°C for 2 minutes in order to quench the reaction.

Examples of methods used to assay the amount of oligonucleotide remaining include a method in which the oligonucleotide is labelled with a radioisotope, etc. followed by assaying the cleavage reaction product with an image analyzer and so forth, a method in which the cleavage reaction product is assayed by reverse phase high-performance liquid chromatography (HPLC), and a method in which the cleavage reaction product is stained with a dye (such as ethidium bromide) and assayed by image processing using a computer.

Dosage forms of the oligonucleotide analogue having one, or two or more structures of the formula (2) of the present invention may be tablets, capsules, granules, powders or syrup for oral administration, or injections or suppositories for parenteral administration. These dosage forms are prepared by well-known methods using additives such as excipients (for example, organic excipients such as sugar derivatives, e.g. lactose, sucrose, glucose, mannitol and sorbitol; starch derivatives, e.g. corn starch, potato starch, α-starch and dextrin; cellulose derivatives, e.g. crystalline cellulose; gum arabic; dextran; and Pullulan; and inorganic excipients such as silicate derivatives, e.g. light silicic anhydride, synthesized aluminium silicate, calcium silicate and magnesium aluminate metasilicate; phosphates, e.g. calcium hydrogenphosphate; carbonates, e.g. calcium carbonate; and sulfates, e.g. calcium sulfate), lubricants (for example, stearic acid, stearic acid metal salts such as calcium stearate and magnesium stearate; talc; colloidal silica; waxes such as bee gum and spermaceti; boric acid; adipic acid; sulfates, e.g. sodium sulfate; glycol; fumaric acid; sodium benzoate; DL-leucine; fatty acid sodium salt; laurylsulfates such as sodium laurylsulfate and magnesium laurylsulfate; silicic acids such as silicic anhydride and silicic acid hydrate; and the above starch derivatives), binders (for example, hydroxypropyl cellulose, hydroxypropyl methyl cellulose, polyvinyl pyrrolidone, Macrogol and compounds similar to the above excipients), disintegrants (for example, cellulose derivatives, such as low-substituted hydroxypropyl cellulose, carboxymethyl cellulose, calcium carboxymethyl cellulose and internally bridged sodium carboxymethyl cellulose; and chemically modified starch-celluloses such as carboxymethyl starch, sodium carboxymethyl starch and bridged polyvinyl pyrrolidone), stabilizers (paraoxybenzoates such as methylparaben and propylparaben; alcohols such as chlorobutanol, benzyl alcohol and phenylethyl alcohol; benzalkonium chloride; phenol derivatives such as phenol and cresol; thimerosal; dehydroacetic acid; and sorbic acid), corrigents (for example, sweeteners, souring agents, flavors, etc. usually used), diluents, etc.

While the dose will vary depending on the condition of the disease, age of the patient, administration methods, etc., for example, in the case of oral administration, it is desirable to administer an active ingredient in an amount of from 0.01 mg/kg of body weight (preferably 0.1 mg/kg of body weight) to 1000 mg/kg of body weight (preferably 100 mg/kg of body weight) and in the case of intravenous administration, it is desirable to administer an active ingredient in an amount of from 0.001 mg/kg of body weight (preferably 0.01 mg/kg of body weight) to 100 mg/kg of body weight (preferably 10 mg/kg of body weight), as a single dose a day or in divided dose at several times for a day respectively.

[Example]

Example 1

3',5'-di-O-Benzyl-2'-O,4'-C-ethylene-4-N-benzoylcytidine

(exemplification compound number 2-34)

An aqueous 2N sodium hydroxide solution (68 ml) was added to a solution of the compound obtained in Reference example 11 (6.80 g, 8.86 mmol) in pyridine (136 ml) at 0°C and the mixture was stirred at room temperature for 1 hour. The reaction mixture was neutralized by dropwise addition of aqueous 20% acetic acid and extracted with chloroform. The chloroform layer was washed with saturated aqueous sodium chloride solution and concentrated in vacuo. The residue was purified by chromatography on a silica gel column (using dichloromethane : methanol = 100 : 3 as the eluant) to afford the title compound (3.3 g, 6.02 mmol, 68%).

^{1}H-NMR(400MHz, $CDCl_3$) : 8.64(2H, brs), 7.89(2H, d, 7.6Hz), 7.64-7.60(1H, m), 7.54-7.51(2H, m), 7.48-7.37(3H, m), 7.36-7.26(8H, m), 6.18(1H,s), 4.70(1H, d, 11Hz), 4.60(1H, d, 11Hz), 4.55(1H, d, 11Hz), 4.46(1H, d, 2.9Hz), 4.42(1H, d, 11Hz), 4.10-4.02(2H,m), 3.89(1H, d, 2.9Hz), 3.75(1H, d, 11Hz), 3.62(1H, d, 11Hz), 2.34-2.26(1H, m), 1.39-1.36(1H, m).

FAB-MAS(mNBA):554$(M+H)^+$

Example 2

2'-O,4'-C-ethylene-4-N-benzoylcytidine

(exemplification compound number 2-225)

A solution (31.7 ml) of 1.0 M trichloroborane in dichloromethane was added dropwise to a solution of the compound obtained in Example 1 (2.06 g, 3.72 mmol) in anhydrous methylenechloride (317 ml) at -78°C and the mixture was stirred at -78°C for 1 hour. The reaction mixture was slowly warmed to -20°C and the reaction vessel was placed into an ice-sodium chloride bath and the mixture was stirred at between -20°C and -10°C for 2 hours. Methanol (12 ml) was slowly added to the mixture and the mixture was stirred for 10 minutes. The pH of the reaction mixture was adjust to 7-8 by dropwise addition of saturated aqueous sodium hydrogencarbonate solution. The mixture was warmed to room temperature and concentrated in vacuo. The residue was purified by chromatography on a silica gel column (using dichloromethane : methanol = 100 : 5 as the eluant) to afford the title compound (1.21 g, 3.24 mmol, 87%) as a white solid.

^{1}H-NMR(500MHz, DMSO-d_6) : 11.23(1H,brs), 8.70(1H,d,7.2Hz), 8.00(2H,d,7.5Hz), 7.3-6(4H,m), 5.97(1H,s), 5.35(1H,dd,5 and 10Hz), 4.10(1H,dd,5 and 10Hz), 4.03(1H,d,3.2Hz), 3.95-3.85(2H,m) 3.83(1H,d,3.2Hz), 3.65-3.51(2H,m), 2.06-1.98(1H,m), 1.26(1).

FAB-MAS(mNBA):374$(M+H)^+$

Example 3

2'-O,4'-C-ethylene-cytidine

(exemplification compound number 2-3)

A solution of the compound obtained in Example 2 (0.1 g, 0.268 mmol) in methanol saturated with ammonia (12 ml) was allowed to stand overnight. The mixture was concentrated to dryness to afford the title compound (0.054 g, 75%) as a white solid.

^{1}H-NMR(500MHz, DMSO-d_6) : 8.18(1H, d, 7.4Hz), 7.10(2H, br), 5.84(1H, s), 5.69(1H, d, 7.6Hz), 5.27-5.24(2H, m), 3.86(1H, d, 3.2Hz), 3.90-3.78(2H, m), 3.76(1H, d, 3.2Hz), 3.56(1H, dd, 5.5 and 12Hz), 3.49(1H, dd, 5.5 and 12Hz), 2.01-1.93(1H,dt, 7.5 and 12Hz), 1.22(1H, dd, 3.6 and 13Hz).

FAB-MAS(mNBA):270$(M+H)^+$

Example 4

5'-O-Dimethoxytrityl-2'-O,4'-C-ethylene-4-N-benzoylcytidine

(exemplification compound number 2-39)

A solution of the compound obtained in Example 2 (1.29 g, 3.46 mmol) in anhydrous pyridine was azeotropically refluxed in order to remove water. The product was dissolved in anhydrous pyridine (26 ml) under nitrogen atmosphere and 4,4'-dimethoxytritylchloride (1.76 g, 5.18 mmol) was added to the solution and the mixture was stirred at room temperature overnight. A small amount of methanol was added to the reaction mixture and then the solvent was evaporated in vacuo. The residue was partitioned between water and chloroform and the organic layer was washed with saturated aqueous sodium hydrogencarbonate solution and saturated aqueous sodium chloride solution and concentrated in vacuo. The residue was purified by chromatography on a silica gel column (using dichloromethane : methanol = 100 : 5 as the eluant) to afford the title compound (2.10 g, 3.11 mmol, 90%) as a colorless amorphous solid.

^{1}H-NMR(270MHz, DMSO-d_6) : 11.27(1H,brs), 8.59(1H,m), 6.92-8.01(19H,m), 6.03(1H,s), 5.56(1H,m), 4.17(1H,m), 4.08(1H,m), 3.86(2H,m), 3.77(6H,s), 3.24(2H,m), 1.98(1H,m), 1.24(1H,m). FAB-MAS(mNBA):676$(M+H)^+$

Example 5

5'-O-Dimethoxytrityl-2'-O,4'-C-ethylene-4-N-benzoylcytidine-3'-O-(2-cyanoethyl N,N-diisopropyl)phosphoramidite

(exemplification compound number 2-235)

A solution of the compound obtained in Example 4 (6.53 g, 9.66 mmol) in anhydrous pyridine was azeotropically refluxed in order to remove water. The product was dissolved under nitrogen atmosphere in anhydrous dichloromethane (142 ml). N,N-diisopropylamine (2.80 ml, 16.1 mmol) was added to the solution and then

2-cyanoethyl N,N-diisopropylchlorophophoramidite (2.16 ml, 9.66 mmol) was added dropwise in an ice bath. The mixture was stirred at room temperature for 6 hours. The reaction mixture was washed with saturated aqueous sodium hydrogencarbonate solution and saturated aqueous sodium chloride solution and concentrated in vacuo. The residue was purified by chromatography on a silica gel column (using dichloromethane : triethylamine = 50 : 1 – dichloromethane : ethyl acetate : triethylamine = 60 : 30 : 1 as the eluant) to afford the title compound (7.10 g, 8.11 mmol, 84%) as a pale white compound.

^{1}H-NMR(400MHz, $CDCl_3$) : 1.1-1.2(12H,m), 1.35(1H,m), 2.11(1H,m), 2.3(2H,m), 3.35-3.7(6H,m), 3.8(6H,m), 3.9-4.1(2H,m), 4.33(1H,m), 4.45(1H,m), 6.23(1H,s), 6.9(4H,m), 7.3-7.9(15H,m), 8.7-8.8(1H,m).

Example 6

3',5'-Di-O-benzyl-2'-O,4'-C-ethylene-5-methyluridine

(exemplification compound number 2-22)

An aqueous 2N sodium hydroxide solution and mixture solution (5 ml), said mixture solution comprised of pyridine : methanol : water = 65 : 30 : 5, were added to the compound obtained in Reference example 10 (418 mg, 0.62 mmol) in pyridine : methanol : water = 65 : 30 : 5 (5 ml) at 0°C and the mixture was stirred at room temperature for 15 minutes. The reaction mixture was neutralized with 1N hydrochloric acid and extracted with ethyl acetate (about 30 ml). The organic layer was washed with saturated aqueous sodium hydrogencarbonate solution (about 30 ml) and saturated aqueous sodium chloride solution (about 30 ml), dried over anhydrous magnesium sulfate and then concentrated in vacuo. The residue was purified by chromatography on a silica gel column (using hexane : ethyl acetate = 1 : 1 as the eluant) to afford a colorless amorphous solid (228 mg, 0.49 mmol, 79%).

^{1}H-NMR (400MHz, $CDCl_3$) : 1.35(1H, d, 13Hz), 1.41(3H, s), 2.28(1H, dt, 9.4 and 13Hz), 3.60(1H, d, 11Hz), 3.76(1H, d, 11Hz), 3.94(1H, d, 3.0Hz), 4.10(1H, d, 7.0Hz), 4.14(1H, d, 7.0Hz), 4.31(1H, d, 3.0Hz), 4.51(1H, d, 12Hz), 4.54(1H, d, 12Hz), 4.58(1H, d, 12Hz), 4.75(1H, d, 12Hz), 6.06(1H, s), 7.3(10H, m), 7.91(1H, s), 8.42(1H, brs).

FAB-MAS(mNBA):465$(M+H)^+$

Example 7

2'-O,4'-C-ethylene-5-methyluridine

(exemplification compound number 2-2)

A solution of the compound obtained in Example 6 (195 mg, 0.42 mmol) in methanol (10 ml) was stirred under hydrogen atmosphere at atmospheric pressure in the presence of a hydrogenation catalyst for 5 hours. The reaction mixture was filtered in order to remove catalyst and the filtrate was concentrated in vacuo. The residue was purified by chromatography on a silica gel column (using dichloromethane : methanol = 10 : 1 as the eluant) to afford a colorless powder (76 mg, 0.268 mmol, 64%).

^{1}H-NMR (400MHz, CD_3OD) : 1.33(1H, dd, 3.8 and 13Hz), 1.86(3H, d, 0.9Hz), 1.94(1H, ddd, 7.5, 11.7 and 13Hz), 3.68(1H, d, 12Hz), 3.75(1H, d, 12Hz), 3.9-4.0(2H, m), 4.05(1H, d, 3.2Hz), 4.09(1H, d, 3.2Hz), 6.00(1H, s), 8.28(1H, d, 1.1Hz).

FAB-MAS(mNBA):285$(M+H)^+$

Example 8

5'-O-Dimethoxytrityl-2'-O,4'-C-ethylene-5-methyluridine

(exemplification compound number 2-27)

A solution of the compound obtained in Example 7 (1.45 g, 5.10 mmol) in anhydrous pyridine was azeotropically refluxed in order to remove water. The product was dissolved in anhydrous pyridine (44 ml) under nitrogen atmosphere and 4,4'-dimethoxytritylchloride (2.59 g, 7.65 mmol) was added to the solution and the mixture was stirred at room temperature overnight. A small amount of methanol was added to the reaction mixture and then the solvent was evaporated in vacuo. The residue was partitioned between water and chloroform and the organic layer was washed with saturated aqueous sodium hydrogencarbonate solution and saturated aqueous sodium chloride solution and concentrated in vacuo. The residue was purified by chromatography on a silica gel column (using dichloromethane : methanol = 100 : 10 as the eluant) to afford the title compound (2.42 g, 4.13 mmol, 81%) as colorless amorphous solid.

^{1}H-NMR(270MHz, DMSO-d_6) : 11.36(1H,s), 7.68(1H,s), 6.90-7.44(13H,m), 5.89(1H,s), 5.55(1H,d), 4.09(1H,m), 4.04(1H,d), 3.82(2H,m), 3.74(6H,s), 3.19(2H,m), 1.99(1H,m), 1.36(1H,m), 1.17(3H,s).

FAB-MAS(mNBA):587$(M+H)^+$

Example 9

5'-O-Dimethoxytrityl-2'-O,4'-C-ethylene-5-methyluridine-3'-O-(2-cyanoethyl N,N-diisopropyl)phosphoramidite

(exemplification compound number 2-234)

A solution of the compound obtained in Example 8 (4.72 g, 8.05 mmol) in anhydrous pyridine was azeotropically refluxed in order to remove water. The product was dissolved under nitrogen atmosphere in anhydrous dichloromethane (142 ml). N,N-diisopropylamine (2.80 ml, 16.1 mmol) was added to the solution and then 2-cyanoethyl N,N-diisopropylchlorophophoramidite (2.16 ml, 9.66 mmol) was added dropwise in an ice bath. The mixture was stirred at room temperature for 6 hours. The reaction mixture was washed with saturated aqueous sodium hydrogencarbonate solution and saturated aqueous sodium chloride solution and concentrated in vacuo. The residue was purified by chromatography on a silica gel column (using hexane : ethyl acetate : triethylamine = 50 : 50 : 1 – hexane : ethyl acetate : triethylamine = 30 : 60 : 1 as the eluant) to afford the title compound (5.64 g, 7.17 mmol, 89%) as a colorless amorphous solid.

^{1}H-NMR(400MHz, $CDCl_3$) :1.1-1.2(15H,m), 1.4(1H,m), 2.08(1H,m), 2.4(2H,m), 3.2-4.0(14H,m), 4.38(2H,m), 4.47(1H,m), 6.06(1H,s), 6.8-6.9(4H,m), 7.2-7.5(9H,m), 7.91(1H,m).

FAB-MAS(mNBA):787$(M+H)^+$

Example 10

3',5'-Di-O-benzyl-2'-O,4'-C-ethylene-6-N-benzoyladenosine

(exemplification compound number 1-23)

An aqueous 2N sodium hydroxide solution and mixture solution (5 ml), said mixture solution comprised of pyridine : methanol : water = 65 : 30 : 5, were added to compound obtained in Reference example 12 (238 mg, 0.30 mmol) in pyridine : methanol : water = 65 : 30 : 5 (5 ml) at 0°C and the mixture was stirred at room temperature for 15 minutes. The reaction mixture was neutralized with 1N hydrochloric acid and extracted with ethyl acetate (about 30 ml). The organic layer was washed with saturated aqueous sodium hydrogencarbonate solution (about 30 ml) and saturated aqueous sodium chloride solution (about 30 ml), dried over anhydrous magnesium sulfate and then concentrated in vacuo. The residue was purified by chromatography on a silica gel column (using dichloromethane : methanol = 50 : 1 as the eluant) to afford a colorless amorphous solid (133 mg, 0.23 mmol, 78%).

^{1}H-NMR (400MHz, $CDCl_3$) : 1.44(1H, d, 13Hz), 2.31(1H, dd, 13 and 19Hz), 3.56(1H, d, 11Hz), 3.70(1H, d, 11Hz), 4.10(2H, m), 4.24(1H, s), 4.45(1H, d, 12Hz), 4.53-4.67(4H, m), 6.52(1H, s), 7.3(10H, m), 7.53(2H, m), 7.62(1H, m), 8.03(2H, d, 7.6Hz), 8.66(1H, s), 8.78(1H, s), 9.00(1H, brs).

FAB-MAS(mNBA):578$(M+H)^+$

Example 11

2'-O,4'-C-Ethylene-6-N-benzoyladenosine

(exemplification compound number 1-178)

A 1M boron trichloride solution (1.5 ml, 1.5 mmol) in dichloromethane was slowly added dropwise to a solution of the compound obtained in Example 10 (116 mg, 0.20 mmol) in anhydrous methylenechloride (5 ml) at -78°C and the mixture was stirred at -78°C for 3 hours. To the reaction mixture was added a 1M boron trichloride solution (1.5 ml, 1.5 mmol) in dichloromethane and the mixture was stirred for 2 hours. The mixture was slowly warmed to room temperature and then quickly cooled to -78°C and then methanol (5 ml) was added to the mixture. The reaction mixture was slowly warmed to room temperature and concentrated in vacuo. The residue was purified by chromatography on a silica gel column (using dichloromethane : methanol = 9 : 1 as the eluant) to afford a white powder (49 mg, 0.17 mmol, 84%).

^{1}H-NMR (400MHz, CD_3OD) : 1.45(1H, dd, 4.3 and 13Hz), 2.12(1H, m), 3.72(1H, d, 12Hz), 3.79(1H, d, 12Hz), 4.04(1H, dd, 7.3 and 12Hz), 4.15(1H, dt, 4.3 and 9.4Hz), 4.36(1H, d, 3.2Hz), 4.43(1H, d, 3.2Hz), 6.57(1H, s), 7.57(2H, m), 7.66(1H, m), 8.09(2H, d, 8.0Hz), 8.72(1H, s), 8.85(1H, s).

FAB-MAS(mNBA):398$(M+H)^+$

Example 12

2'-O,4'-C-Ethyleneadenosine

(exemplification compound number 1-7)

A solution of the compound obtained in Example 11 (14 mg, 0.035 mmol) in methanol saturated with ammonia (1 ml) was allowed to stand overnight. The mixture was concentrated and the residue was purified by chromatography on a silica gel column (using dichloromethane : methanol = 10 : 1 as the eluant) to afford a white powder (10 mg, 0.034 mmol, 98%).

^{1}H-NMR (400MHz, CD_3OD) : 1.32(1H, dd, 4 and 13Hz), 2.04(1H, dt, 7.4 and 12Hz), 3.53(1H, dd, 5 and 12Hz), 3.61(1H, dd, 5.2 and 12Hz), 3.90(1H, dd, 7.4 and 12Hz), 3.97(1H, dt, 4 and 12Hz), 4.15(1H, d, 3.1Hz), 4.21(1H, d, 3.1Hz), 5.27(1H, t, 5.2Hz), 5.39(1H, d, 3.1Hz), 6.33(1H, s), 7.29(2H, s), 7.66(1H, m), 8.14(1H, s), 8.42(1H, s).

FAB-MAS(mNBA):294$(M+H)^+$

UV(λmax) : 260(pH7), 260(pH1), 258(pH13)

Example 13

5'-O-Dimethoxytrityl-2'-O,4'-C-ethylene-6-N-benzoyladenosine

(exemplification compound number 1-31)

A solution of the compound obtained in Example 11 (14 mg, 0.035 mmol) in anhydrous pyridine was azeotropically refluxed in order to remove water. The product was dissolved in anhydrous pyridine (1 ml) under nitrogen atmosphere and 4,4'-dimethoxytritylchloride (18 mg, 0.053 mmol) was added to the solution and the mixture was stirred at 40°C for 5 hours. A small amount of methanol was added to the reaction mixture and then the solvent was evaporated in vacuo. The residue was partitioned between water and chloroform and the organic layer was washed with saturated aqueous sodium hydrogencarbonate solution and saturated aqueous sodium chloride solution and concentrated in vacuo. The residue was purified by chromatography on a silica gel column (using dichloromethane : methanol = 100 : 5 as the eluant) to afford the title compound (18 mg, 0.026 mmol, 73%) as a colorless amorphous solid.

^{1}H-NMR (400MHz, $CDCl_3$) :1.63(1H,m), 2.14(1H,7.5,12,and 13Hz), 3.37(1H,d,11Hz), 3.41(1H,d,11Hz), 3.79(6H,s), 4.10(2H,m), 4.48(1H,d, 3.3Hz), 4.59(1H,d,3.3Hz), 6.54(1H,s), 6.85(4H,m), 7.2-7.6(12H,m), 8.02(2H,m), 8.45(1H,s), 8.82(1H,s), 9.02(1H,brs). FAB-MAS(mNBA):700$(M+H)^+$

Example 14

5'-O-Dimethoxytrityl-2'-O,4'-C-ethylene-6-N-benzoyladenosine-3'-O-(2-cyanoethyl N,N-diisopropyl)phosphoramidite

(exemplification compound number 1-186)

A solution of the compound obtained in Example 13 (16 mg, 0.023 mmol) in anhydrous pyridine was azeotropically refluxed in order to remove water. The product was dissolved under nitrogen atmosphere in anhydrous dichloromethane (0.5 ml). Tetrazole N,N-diisopropylamine salt (10 mg) was added to the solution and then 2-cyanoethyl N,N,N',N'-tetraisopropylphophoramidite (about 20 μl) was added dropwise in an ice bath. The mixture was stirred at room temperature overnight. The reaction mixture was washed with saturated aqueous sodium hydrogencarbonate solution and saturated aqueous sodium chloride solution and concentrated in vacuo. The residue was purified by chromatography on a silica gel column (using dichloromethane : ethyl acetate = 2 : 1 as the eluant) to afford the title compound (20 mg, 0.022 mmol, 97%) as a white solid.

^{1}H-NMR(400MHz, $CDCl_3$) :1.0-1.2(12H,m), 1.54(1H,m), 2.15(1H,m), 2.33(2H,m), 3.3-3.6(6H,m), 3.80(6H,s), 4.08(2H,m), 4.65(1H,m), 4.75(1H,m), 6.53(1H,s), 6.84(4H,m), 7.2-7.6(12H,m), 8.01(2H,m), 8.53(1H,s), 8.83(1H,s), 9.01(1H,brs).

FAB-MAS(mNBA):900$(M+H)^+$

Example 15

3',5'-Di-O-benzyl-2'-O,4'-C-ethyleneuridine

(exemplification compound number 2-10)

An aqueous 1N sodium hydroxide solution (2 ml) was added to a solution of the compound obtained in Reference example 13 (194 mg, 0.292 mmol) in pyridine (3 ml) at 0°C and the mixture was stirred at room temperature for 30 minutes. The reaction mixture was neutralized with 1N hydrochloric acid and extracted with ethyl acetate (10 ml). The organic layer was washed with saturated aqueous sodium hydrogencarbonate solution and saturated aqueous sodium chloride solution, dried over anhydrous magnesium sulfate and then concentrated in vacuo. The residue was purified by chromatography on a silica gel column (using dichloromethane : methanol = 100 : 3 as the eluant) to afford an colorless oil (105 mg, 0.233 mmol, 80%).

^{1}H-NMR (400MHz, $CDCl_3$) : 1.36(1H,m), 2.29(1H,m), 3.63(1H,d,11Hz), 3.74(1H,d, 11Hz), 3.87(1H,d, 2.9Hz), 4.03(2H,m), 4.29(1H,d,2.9Hz), 4.49(1H,d,12Hz), 4.50(1H,d,11Hz), 4.53(1H,d,11Hz), 4.73(1H,d,12Hz), 5.20(1H,dd, 2 and 8Hz), 6.04(1H,s), 7.2-7.4(10H,m), 8.13(1H,d,8.2Hz), 8.57(1H,brs).

FAB-MAS(mNBA):451$(M+H)^+$

Example 16

2'-O,4'-C-Ethyleneuridine

(exemplification compound number 2-1)

A solution of the compound obtained in Example 15 (100 mg, 0.222 mmol) in methanol (4 ml) was stirred under hydrogen atmosphere at atmospheric pressure in the presence of a hydrogenation catalyst for 5 hours. The reaction mixture was filtered in order to remove catalyst and the filtrate was concentrated in vacuo. The residue was purified by chromatography on a silica gel column (using dichloromethane : methanol = 10 : 1 as the eluant) to afford a colorless oil (45 mg, 0.167 mmol, 75%).

^{1}H-NMR (400MHz, CD_3OD) : 1.35(1H,dd,4 and 13Hz), 2.13(1H,ddd, 7,11 and 13Hz), 3.66(1H,d,12Hz), 3.73(1H,d,12Hz), 3.91-4.08(2H,m),4.01(1H,d,3.2Hz), 4.12(1H,d,3.2Hz), 5.66(1H,d,8.2Hz), 6.00(1H,s), 8.37(1H,d,8.2Hz).

FAB-MAS(mNBA):271$(M+H)^+$

Example 17

5'-O-Dimethoxytrityl-2'-O,4'-C-ethyleneuridine

(exemplification compound number 2-15)

A solution of the compound obtained in Example 16 (28 mg, 0.104 mmol) in anhydrous pyridine was azeotropically refluxed in order to remove water. The product was dissolved in anhydrous pyridine (3 ml) under nitrogen atmosphere and 4,4'-dimethoxytritylchloride (50 mg, 0.15 mmol) was added to the solution and the mixture was stirred at room temperature overnight. A small amount of methanol was added to the reaction mixture and then the solvent was evaporated in vacuo. The residue was partitioned between water and chloroform and the organic layer was washed with saturated aqueous sodium hydrogencarbonate solution and saturated aqueous sodium chloride solution and concentrated in vacuo. The residue was purified by chromatography on a silica gel column (using dichloromethane : methanol = 100 : 3 as the eluant) to afford the title compound (25 mg, 0.044 mmol, 42%) as a colorless oil.

^{1}H-NMR (400MHz, CD_3OD) : 1.35(1H,dd, 3 and 14Hz), 2.03(1H,ddd, 8,11 and 14Hz), 2.46(1H,d,8Hz), 3.36(1H,d,11Hz), 3.41(1H,d,11Hz), 3.80(3H,s), 3.81(3H,s), 3.97(2H,m), 4.21(1), 4.33(1H,brm), 5.31(1H,m), 6.10(1H,s), 6.86(4H,m), 7.2-7.5(9H,m), 8.27(1H,d,8.2Hz), 8.43(1H,brs).

FAB-MAS(mNBA):573$(M+H)^+$

Example 18

5'-O-Dimethoxytrityl-2'-O,4'-C-ethyleneuridine-3'-O-(2-cyanoethyl N,N-diisopropyl)phosphoramidite

(exemplification compound number 2-233)

A solution of the compound obtained in Example 17 (6 mg, 0.0105 mmol) in anhydrous pyridine was azeotropically refluxed in order to remove water. The product was dissolved under nitrogen atmosphere in anhydrous dichloromethane (0.5 ml). Tetrazole N,N-diisopropylamine salt (3 mg) was added to the solution and then 2-cyanoethyl N,N,N',N'-tetraisopropylphophoramidite (about 5 μl) was added dropwise in an ice bath. The mixture was stirred at room temperature overnight. The reaction mixture was washed with saturated aqueous sodium hydrogencarbonate solution and saturated aqueous sodium chloride solution and concentrated in vacuo. The residue was purified by chromatography on a silica gel column (using dichloromethane : ethyl acetate = 2 : 1 as the eluant) to afford the title compound (8 mg) as a white solid.

^{1}H-NMR(400MHz, $CDCl_3$) : 1.1-1.2(13H,m), 2.09(1H,m), 2.4 (2H,m), 3.3-3.6(6H,m), 3.81(6H,m), 3.94(2H,m), 4.35(1H,m), 4.47(1H,m), 5.18(1H,d,8.2Hz), 6.08(1H,s), 6.86(4H,m), 7.2-7.4(9H,m), 8.31(1H,d,8.2Hz).

FAB-MAS(mNBA):773$(M+H)^+$

Example 19

3',5'-Di-O-benzyl-2'-O,4'-C-ethylene-4-N-benzoyl-5-methylcytidine

(exemplification compound number 2-46)

An aqueous 1N sodium hydroxide solution (5 ml) was added to a solution of the compound obtained in Reference example 14 (310 mg, 0.396 mmol) in pyridine (5 ml) at 0°C and the mixture was stirred at room temperature for 20 minutes. The reaction mixture was neutralized by dropwise addition of aqueous 20% acetic acid and extracted with dichloromethane. The dichloromethane layer was washed with saturated aqueous sodium chloride solution and concentrated in vacuo. The residue was purified by chromatography on a silica gel column (using dichloromethane : methanol = 100 : 2 as the eluant) to afford the title compound (190 mg, 0.334 mmol, 84%).

^{1}H-NMR(400MHz, $CDCl_3$) : 1.37(1H,m), 1.58(3H,s), 2.30(1H,dt,10 and 13Hz), 3.64(1H,d,11Hz), 3.79(1H,d,11Hz), 3.95(1H,d,3.0Hz), 4.04(2H,dd,2.3 and 10Hz), 4.37(1H,d,3.0Hz), 4.50(1H,d,12Hz), 4.56(1H,d,11Hz), 4.61(1H,d,11Hz), 4.76(1H,d,12Hz), 6.11(1H,s), 7.2-7.5(13H,m), 8.09(1H,s), 8.29(2H,m).
FAB-MAS(mNBA):568$(M+H)^+$

Example 20

2'-O,4'-C-Ethylene-4-N-benzoyl-5-methylcytidine

(exemplification compound number 2-226)

A 1M boron trichloride solution (1.6 ml) in dichloromethane was added dropwise to a solution of the compound obtained in Example 19 (120 mg, 0.211 mmol) in anhydrous dichloromethane (5 ml) at -78°C and the mixture was stirred at -78°C for 4 hours. Methanol (1 ml) was slowly added dropwise to the mixture and the mixture was stirred for 10 minutes. The pH of the reaction mixture was adjusted to 7-8 by dropwise addition of saturated aqueous sodium hydrogencarbonate solution. The reaction mixture was warmed to room temperature and concentrated in vacuo. The residue was purified by chromatography on a silica gel column (using dichloromethane : methanol = 100 : 6 as the eluant) to afford the title compound (29 mg, 0.075 mmol, 36%) as a white solid.

^{1}H-NMR(400MHz, d-DMSO) : 1.24(1H,m), 2.01(3H,s), 2.0(1H,m), 3.54(1H,dd,5.4 and 12Hz), 3.64(1H,dd,5.4 and 12Hz), 3.88(3H,m), 4.10(1H,m), 5.36(1H,d,5.4Hz), 5.49(1H,t,5.0Hz), 5.95(1H,s), 7.4-7.6(3H,m), 8.21(2H,m), 8.49(1H,s), 13.17(1H,brs).
FAB-MAS(mNBA):388$(M+H)^+$

Example 21

5'-O-Dimethoxytrityl-2'-O,4'-C-ethylene-4-N-benzoyl-5-methylcytidine

(exemplification compound number 2-51)

A solution of the compound obtained in Example 20 (44 mg, 0.114 mmol) in anhydrous pyridine was azeotropically refluxed in order to remove water. The product was dissolved in anhydrous pyridine (1 ml) under nitrogen atmosphere and 4,4'-dimethoxytritylchloride (60 mg, 0.177 mmol) was added to the solution and the mixture was stirred at room temperature overnight. A small amount of methanol was added to the reaction mixture and then the solvent was evaporated in vacuo. The residue was partitioned between water and chloroform. The organic layer was washed with saturated aqueous sodium hydrogencarbonate solution and saturated aqueous sodium chloride solution and concentrated in vacuo. The residue was purified by chromatography on a silica gel column (using dichloromethane : methanol = 100 : 4 as the eluant) to afford the title compound (73 mg, 0.106 mmol, 93%) as a colorless oil.

^{1}H-NMR(400MHz, $CDCl_3$) : 1.46(1H,m), 1.49(3H,s), 2.06(1H,m), 2.59(1H,d, 8.6Hz), 3.36(1H,d,11Hz), 3.39(1H,d,11Hz), 3.80(3H, s), 3.81(3H, s), 3.99(2H,m), 4.30(1H,d, 3.3Hz), 4.39(1H,m), 6.12(1H,s), 6.85(4H,m), 7.2-7.5(12H,m), 8.03(1H,s), 8.28(2H,m).

FAB-MAS(mNBA):573$(M+H)^+$

Example 22

5'-O-Dimethoxytrityl-2'-O,4'-C-ethylene-4-N-benzoyl-5-methylcytidine-3'-O-(2-cyanoethyl N,N-diisopropyl)phosphoramidite

(exemplification compound number 2-236)

A solution of the compound obtained in Example 21 (35 mg, 0.0507 mmol) in anhydrous pyridine was azeotropically refluxed in order to remove water. The product was dissolved under nitrogen atmosphere in anhydrous dichloromethane (1 ml). Tetrazole N,N-diisopropylamine salt (17 mg) was added to the solution and then 2-cyanoethyl N,N,N',N'-tetraisopropylphophoramidite (32 μl, 0.1 mmol) was added dropwise in an ice bath. The mixture was stirred at room temperature overnight. The reaction mixture was washed with saturated aqueous sodium hydrogencarbonate solution and saturated aqueous sodium chloride solution and concentrated in vacuo. The residue was purified by chromatography on a silica gel column (using dichloromethane : ethyl acetate = 2 : 1 as the eluant) to afford the title compound (40 mg, 0.0445 mmol, 89%) as a white solid.

^{1}H-NMR(400MHz, $CDCl_3$) :1.1-1.2(12H,m), 1.36(3H,s), 1.37(1H,m), 2.10(1H,m), 2.36(2H,m), 3.3-3.6(6H,m), 3.81(6H,m), 3.98(2H,m), 4.42(1H,m), 4.49(1H,m), 6.11(1H,s), 6.88(4H,m), 7.2-7.5(12H,m), 8.14(1H,s), 8.28(2H,m).

FAB-MAS(mNBA):890$(M+H)^+$

Example 23

2'-O,4'-C-Ethylene-5-methylcytidine

(exemplification compound number 2-226)

A solution of the compound obtained in Example 20 (11.6 mg, 0.030 mmol) in methanol saturated with ammonia (2 ml) was allowed to stand overnight. The mixture was concentrated to afford a white solid (8.5 mg, 0.030 mmol).

^{1}H-NMR(400MHz, d-DMSO) : 1.20(1H,m), 1.82(3H,s), 1.97(1H,m), 3.49(1H,dd,5 and 12Hz), 3.58(1H,dd,5 and 12Hz), 3.85(2H,m), 5.23(1H,d,5Hz), 5.32(1H,t,5Hz), 5.84(1H,s), 6.7(1H,brs), 7.2(1H,brs), 8.08(1H,s).

FAB-MAS(mNBA):284$(M+H)^+$

UV(λmax) : 279(pH7), 289(pH1), 279(pH13)

Example 24

3',5'-Di-O-benzyl-2'-O,4'-C-ethylene-2-N-isobutyrylguanosine

(exemplification compound number 1-24)

An aqueous 1N sodium hydroxide solution (2 ml) was added to a solution of the compound obtained in Reference example 15 (about 200 mg) in pyridine (2 ml) and the mixture was stirred at room temperature for 15 minutes. The reaction mixture was neutralized with 1N hydrochloric acid and extracted with ethyl acetate. The organic layer was washed with saturated aqueous sodium hydrogencarbonate solution and saturated aqueous sodium chloride solution, dried over anhydrous magnesium sulfate and then concentrated in vacuo. The residue was purified by chromatography on a silica gel column (using dichloromethane : methanol = 50 : 1 as the eluant) to afford a colorless amorphous solid (20 mg, 0.036 mmol, 6%, 2 steps).

^{1}H-NMR (400MHz, $CDCl_3$) : 1.27(3H,s), 1.29(3H,s), 1.43(1H,dd,3 and 13Hz), 2.28(1H,m), 2.59(1H,qui,6.9Hz), 3.54(1H,d,11Hz), 3.68(1H,d,11Hz), 4.03(2H,m), 4.15(1H,d,3.0Hz), 4.31(1H,d,3.0Hz), 4.45(1H,d,12), 4.56(1H,d,12Hz), 4.61(1H,d,12Hz), 4.63(1H,d,12Hz), 6.18(1H,s), 7.2-7.4(10H,m), 8.19(1H,s), 11.93(1H,brs).

FAB-MAS(mNBA):560$(M+H)^+$

Example 25

2'-O,4'-C-Ethylene-2-N-isobutyrylguanosine

(exemplification compound number 1-177)

A solution of the compound obtained in Example 24 (10 mg, 0.018 mmol) in methanol (2 ml) was stirred under hydrogen atmosphere at atmospheric pressure in the presence of a hydrogenation catalyst for 5 hours. The reaction mixture was filtered in order to remove catalyst and the filtrate was concentrated in vacuo. The residue was purified by chromatography on a silica gel column (using dichloromethane : methanol = 10 : 2 as the eluant) to afford a colorless oil (5 mg, 0.013 mmol, 72%).

^{1}H-NMR (400MHz, CD_3OD) :1.21(3H,s), 1.22(3H,s), 1.41(1H,dd, 4 and 13Hz), 2.18(1H,m), 2.69(1H,qui,6.9Hz), 3.69(1H,d,12Hz), 3.76(1H,d,12Hz), 4.0(2H,m), 4.26(1H,d,3.2Hz), 4.30(1H,d,3.2Hz), 6.30(1H,s), 8.40(1H,s).

FAB-MAS(mNBA):380$(M+H)^+$

Example 26

5'-O-Dimethoxytrityl-2'-O,4'-C-ethylene-2-N-isobutyrylguanosine

(exemplification compound number 1-35)

A solution of the compound obtained in Example 25 (5 mg, 0.013 mmol) in anhydrous pyridine was azeotropically refluxed in order to remove water. The product was dissolved in anhydrous pyridine (1 ml) under nitrogen atmosphere and to 4,4'-dimethoxytritylchloride (14 mg, 0.04 mmol) was added to the solution and the mixture was stirred at 40°C for 3 hours. A small amount of methanol was added to the reaction mixture and then the solvent was evaporated in vacuo. The residue was purified by chromatography on a silica gel column (using dichloromethane : methanol = 100 : 6 as the eluant) to afford the title compound (4 mg, 0.0059 mmol, 45%) as colorless solid.

^{1}H-NMR (400MHz, $CDCl_3$) :1.26(3H,d,1.4Hz), 1.28(3H,d,1.4Hz), 1.66(1H,m), 2.15(1H,m), 2.59(1H,qui,6.9Hz), 3.65(1H,m), 3.78(1H,m), 4.06(2H,m), 4.35(1H,m), 4.38(1H,d,3.2Hz), 6.23(1H,s), 6.8(4H,m), 7.2-7.5(9H,m), 8.01(1H,s), 8.19(1H,brs).

FAB-MAS(mNBA):682$(M+H)^+$

Example 27

5'-O-Dimethoxytrityl-2'-O,4'-C-ethylene-2-N-isobutyrylguanosine-3'-O-(2-cyanoethyl N,N-diisopropyl)phosphoramidite

(exemplification compound number 1-185)

A solution of the compound obtained in Example 26 (4 mg, 0.0058 mmol) in anhydrous pyridine was azeotropically refluxed in order to remove water. The product was dissolved under nitrogen atmosphere in anhydrous dichloromethane (0.5 ml). Tetrazole N,N-diisopropylamine salt (5 mg) was added to the solution and then 2-cyanoethyl N,N,N',N'-tetraisopropylphophoramidite (9 μl, 0.03 mmol) was added dropwise in an ice bath. The mixture was stirred at room temperature for 1 hour. The reaction mixture was washed with saturated aqueous sodium hydrogencarbonate solution and saturated aqueous sodium chloride solution and concentrated in vacuo. The residue was purified by chromatography on a silica gel column (using dichloromethane : ethyl acetate = 2 : 1 as the eluant) to afford the title compound (4 mg) as a white solid.

^{1}H-NMR(400MHz, $CDCl_3$) :1.1-1.4(19H,m), 2.1(1H,m), 2.4(2H,m), 2.6(1H,m), 3.3-3.6(6H, m), 3.8(6H,s), 4.0-4.6(4H,m), 6.2(1H,s), 6.8(4H,m), 7.2-7.5(9H,m), 8.1(1H,s).

Example 28

2'-O,4'-C-Ethyleneguanosine

(exemplification compound number 1-5)

A solution of the compound obtained in Example 25 (0.5 mg) in methanol saturated with ammonia (0.5 ml) was allowed to stand at 60°C for 5 hours. The mixture was concentrated to afford a white powder (0.4 mg).

FAB-MAS(mNBA):310$(M+H)^+$ UV(λmax) : 255(pH7), 256(pH1), 258-266(pH13)

Example 29

Synthesis of oligonucleotide derivative

Synthesis of an oligonucleotide derivative was carried out using a mechanical nucleic acid synthesiser (ABI model392 DNA/RNA synthesiser: a product of Perkin-Elmer Corporation) on a scale of 1.0 μmole. The solvents, reagents and concentrations of phosphoramidite in every synthetic cycle are the same as those in the synthesis of natural oligonucleotides. Solvents, reagents and phosphoramidites of the natural type nucleosides are products of PE Biosystems Corporation. Every modified oligonucleotide derivative sequence was synthesized by repetition of condensation of the compound obtained in Example 9 or amidites containing the 4 species of nucleic acid base for nucleotide synthesis with 5'-hydroxythymidine produced by deprotection of the DMTr group of 5'-O-DMTr-thymidine (1.0 μmole) using trichloroacetic acid, the 3'-hydroxy group of the thymidine being attached to a CGP carrier. The synthetic cycle is as follows:

1) detritylation trichloroacetic acid/dichloromethane; 35sec

2) coupling phosphoramidite (about 20eq), tetrazole/acetonitrile; 25sec or 10min

3) capping 1-methylimidazole/tetrahydrofuran, acetic anhydride/pyridine/tetrahydrofuran; 15sec

4) oxidation iodine/water/pyridine/tetrahydrofuran; 15sec

In the above cycle 2) when the compound obtained in Example 9 was used the reaction time was 10 minutes and when phosphoramidites were used the reaction time was 25 seconds.

After synthesis of a desired oligonucleotide derivative sequence, the 5'-DTMr group was removed and then the carrier containing the desired product was conventionally treated with concentrated aqueous ammonia solution in order to detach the oligomer from the carrier and to deprotect the cyanoethyl group which is protecting the phosphoric group. The amino protecting group in adenine, guanine and cytosine was removed from the oligomer. The oligonucleotide derivative was purified by reverse-phase HPLC (HPLC: LC-VP: a product of Shimazu Corp.; column : Wakopak WS-DNA: a product of Wako Pure Chemical Industry Ltd.) to afford the desired oligonucleotide.

According to this synthetic method the following oligonucleotide sequence (which oligonucleotide is hereinafter refered to as "oligonucleotide 1") was obtained (0.23 μmol, yield 23%).

5'– gcgttttttgct –3' (exemplification of sequence number 2 in the sequence list) wherein the sugar moiety of the thymidines at base numbers 4 to 8 is 2'-O,4'-C ethylene.

Reference example 1

3,5-Di-O-benzyl-4-trifluoromethanesulfonyloxymethyl-1,2-O-isopropylidene-α-D-erythropentofuranose

Anhydrous pyridine (0.60 ml, 7.5 mmol) was added was added to a solution of 3,5-di-O-benzyl-4-hydroxymethyl-1,2-O-isopropylidene-α-D-erythropentofuranose (2000 mg, 5.0 mmol) in anhydrous dichloromethane (50 ml) and trifluoromethanesulfonic anhydride (1010 mg, 6.0 mmol) under nitrogen atmosphere at -78°C and the mixture was stirred for 40 minutes. The reaction mixture was partitioned between the methylenechloride and saturated aqueous sodium hydrogencarbonate solution (about 100 ml). The organic layer was washed with saturated aqueous sodium hydrogencarbonate solution (about 100 ml) and saturated aqueous sodium chloride solution (about 100 ml), dried over anhydrous magenesium sulfate and then concentrated in vacuo to give a white powder (2520 mg, 4.73 mmol, 95%) which was used in the next reaction without further purification.

^{1}H-NMR (400MHz, $CDCl_3$) : 1.34(3H, s), 1.63(3H, s), 3.48(1H, d, 10Hz), 3.53(1H, d, 10Hz), 4.21(1H, d, 5.0Hz), 4.5(4H, m), 4.74(1H, d, 12Hz), 4.80(1H, d, 12Hz), 5.01(1H, d, 12Hz), 5.73(1H, d, 4.6Hz), 7.3(10H, m).

Reference example 2

3,5-Di-O-benzyl-4-cyanomethyl-1,2-O-isopropylidene-α-D-erythropentofuranose

The compound obtained in Reference example 1 (2520 mg, 4.73 mmol) was dissolved in dimethylsulfoxide (50 ml) at 90°C. To the solution was added sodium cyanide (463 mg, 9.46 mmol) at room temperature and the mixture was stirred at 50°C for 3 hours. The reaction mixture was partitioned between water (about 100 ml) and ethyl acetate (about 100 ml). The organic layer was washed with saturated aqueous sodium chloride solution (about 100 ml), dried over anhydrous magnesium sulfate and then concentrated in vacuo. The residue was purified by chromatography on silica gel (using hexane : ethyl acetate = 4 : 1) to give a colorless oil (1590 mg, 3.89 mmol, 82%).

^{1}H-NMR (400MHz, $CDCl_3$) : 1.34(3H, s), 1.62(3H, s), 2.88(1H, d, 17Hz), 3.15(1H, d, 17Hz), 3.50(1H, d, 10Hz), 3.58(1H, d, 10Hz), 4.08(1H, d, 5.1Hz), 4.52(1H, d, 12Hz), 4.56(1H, d, 12Hz), 4.57(1H, m), 4.58(1H, d, 12Hz), 4.76(1H, d, 12Hz), 5.73(1H, d, 3.7Hz), 7.3(10H, m).

Reference example 3

3,5-Di-O-benzyl-4-formylmethyl-1,2-O-isopropylidene-α-D-erythropentofuranose

A 1.5M toluene solution of isobutylaluminium hydride (2 ml, 3.0 mmol) was slowly added dropwise to a solution of the compound obtained in Reference example

2 (610 mg, 1.49 mmol) in dichloromethane (10 ml) under nitrogen atmosphere at -78°C and the mixture was stirred for 1 hour at -78°C and then warmed to room temperature. To the reaction mixture was added methanol (5 ml) and saturated aqueous ammonium chloride solution (about 20 ml) and this mixture was stirred for 30 minutes. The reaction mixture was extracted with ethyl acetate (about 30 ml). The organic layer was washed with saturated aqueous sodium hydrogencarbonate solution (about 30 ml) and saturated aqueous sodium chloride solution (about 30 ml), dried over anhydrous magenesium sulfate and then concentrated in vacuo to give a product which was used in the next reaction without further purification.

Reference example 4

3,5-Di-O-benzyl-4-hydroxyethyl-1,2-O-isopropylidene-α-D-erythropentofuranose

$NaBH_4$ (7.6 mg, 0.2 mmol) was added to a solution of the compound obtained in Reference example 3 (154 mg, 0.377 mmol) in ethanol (5 ml) and the mixture was stirred at room temperature for 1 hour. The reaction mixture was partitioned between ethyl acetate (about 10 ml) and water (about 10 ml) and the organic layer was washed with saturated aqueous sodium chloride solution (about 10 ml), dried over anhydrous magenesium sulfate and then concentrated in vacuo. The residue was purified by chromatography on silica gel (using hexane : ethyl acetate = 2 : 1) to give a colorless oil (117 mg, 0.284 mmol, 75%).

^{1}H-NMR (400MHz, $CDCl_3$) : 1.33(3H, s), 1.66(3H, s), 1.78(1H, ddd, 4.0, 8.5, 15Hz), 2.51(1H, ddd, 3.4, 6.4, 15Hz), 3.31(1H, d, 10Hz), 3.54(1H, d, 10Hz), 3.80(2H, m), 4.13(1H, d, 5.3Hz), 4.43(1H, d, 12Hz), 4.52(1H, d, 12Hz), 4.55(1H, d, 12Hz), 4.65(1H, dd, 4.0, 5.3Hz), 4.77(1H, d, 12Hz), 5.77(1H, d, 4.0 Hz), 7.3 (10H, m).

FABMS(mNBA):415$(M+H)^+$, $[\alpha]_D$ +57.4°(0.91, methanol).

Reference example 5

3,5-Di-O-benzyl-4-formyl-1,2-O-isopropylidene-α-D-erythropentofuranose

Oxalyl chloride (6.02 ml, 69.0 mmol) was added to methylenechloride (200 ml) cooled at -78°C. A solution of dimethylsulfoxide (7.87 ml, 110 mmol) in anhydrous methylenechloride (100 ml) was added dropwise to this solution. After stirring for 20 minutes a solution of 3,5-di-O-benzyl-1,2-O-isopropylidene-α-D-erythropentofuranose (9210 mg, 23.02 mmol) in anhydrous dichloromethane (100 ml) was added dropwise to this mixture and the mixture was stirred for 30 minutes. Triethylamine (28 ml, 200 mmol) was added to this reaction mixture and the mixture was slowly warmed to room temperature. The reaction mixture was partitioned between the dichloromethane and water (about 300 ml). The organic layer was washed with water (about 300 ml) and saturated aqueous sodium chloride solution (about 300 ml), dried over anhydrous magnesium sulfate and then concentrated in vacuo. The residue was purified by chromatography on silica gel (using hexane : ethyl acetate = 5 : 1) to give a colorless oil (8310 mg, 20.88 mmol, 91%).

^{1}H-NMR (400MHz, $CDCl_3$) : 1.35(3H, s), 1.60(3H, s), 3.61(1H, d, 11Hz), 3.68(1H, d, 11Hz), 4.37(1H, d, 4.4Hz), 4.46(1H, d, 12Hz), 4.52(1H, d, 12Hz), 4.59(1H, d, 12Hz), 4.59(1H, dd, 3.4, 4.4Hz), 4.71(1H, d, 12Hz), 5.84(1H, d, 3.4Hz), 7.3 (10H, m), 9.91(1H, s). FABMS(mNBA):397$(M-H)^+$, 421$(M+Na)^+$,

$[\alpha]_D$ +27.4°(0.51, methanol).

Reference example 6

3,5-Di-O-benzyl-4-vinyl-1,2-O-isopropylidene-α-D-erythropentofuranose

A 0.5M toluene solution of Tebbe reagent (44 ml, 22 mmol) was added to a solution of the compound obtained in Reference example 5 (8310 mg, 20.88 mmol) in anhydrous tetrahydrofuran (300 ml) under nitrogen atmosphere at 0°C and the mixture was stirred at 0°C for 1 hour. Diethyl ether (300 ml) was added to the reaction mixture and then added 0.1N aqueous sodium hydroxide solution (20 ml) was slowly added. The mixture was filtrated through celite in order to remove precipitates and the precipitates were washed with diethyl ether (about 100 ml). The organic layer was dried over anhydrous magnesium sulfate and then concentrated in vacuo. The residue was purified by chromatography on basic alumina using dichloromethane to afford crude product which was further purified by chromatography on silica gel (using hexane : ethyl acetate = 8 : 1 – 5 : 1) to give a colorless oil (5600 mg, 14.14 mmol, 68%).

^{1}H-NMR (400MHz, $CDCl_3$) : 1.28(3H, s), 1.52(3H, s), 3.31(1H, d, 11Hz), 3.34(1H, d, 11Hz), 4.25(1H, d, 4.9Hz), 4.40(1H, d, 12Hz), 4.52(1H, d, 12Hz), 4.57(1H, dd, 3.9, 4.9Hz), 4.59(1H, d, 12Hz), 4.76(1H, d, 12Hz), 5.25(1H, dd, 1.8, 11Hz), 5.52(1H, dd, 1.8, 18Hz), 5.76(1H, d, 3.9Hz), 6.20(1H, dd, 11, 18Hz), 7.3 (10H, m).

FABMS(mNBA):419$(M+Na)^+$

Reference example 7

3,5-Di-O-benzyl-4-hydroxyethyl-1,2-O-isopropylidene-α-D-erythropentofuranose

A 0.5M tetrahydrofuran solution of 9-BBN (9-borabicyclo[3.3.1]nonane) (80 ml, 40 mmol) was added dropwise to a solution of the compound obtained in Reference example 6 (5500 mg, 13.89 mmol) in anhydrous tetrahydrofuran (200 ml) under nitrogen atmosphere and the mixture was stirred at room temperature overnight. Water was added to the reaction mixture until evolution of gas ceased, 3N aqueous sodium hydroxide solution (30 ml) was added and then slowly 30% aqueous hydrogen peroxide solution was added keeping between 30 and 50°C. This mixture was stirred for 30 minutes and partitioned between saturated aqueous sodium chloride solution (about 200 ml) and ethyl acetate (200 ml). The organic layer was washed with neutral phosphoric acid buffer solution (about 200 ml) and saturated aqueous sodium chloride solution (about 200 ml) and dried over anhydrous magnesium sulfate and then concentrated in vacuo. The residue was purified by chromatography on silica gel (using hexane : ethyl acetate = 2 : 1 – 1 : 1) to give a colorless oil (5370 mg, 12.97 mmol, 93%).

^{1}H-NMR (400MHz, $CDCl_3$) : 1.33(3H, s), 1.66(3H, s), 1.78(1H, ddd, 4.0, 8.5, 15Hz), 2.51(1H, ddd, 3.4, 6.4, 15Hz), 3.31(1H, d, 10Hz), 3.54(1H, d, 10Hz), 3.80(2H, m), 4.13(1H, d, 5.3Hz), 4.43(1H, d, 12Hz), 4.52(1H, d, 12Hz), 4.55(1H, d, 12Hz), 4.65(1H, dd, 4.0, 5.3Hz), 4.77(1H, d, 12Hz), 5.77(1H, d, 4.0 Hz), 7.3 (10H, m).
FABMS(mNBA):415$(M+H)^+$, $[\alpha]_D$ +57.4°(0.91, methanol).

Reference example 8

3,5-Di-O-benzyl-4-(p-toluenesulfonyloxyethyl)-1,2-O-isopropylidene-α-D-erythropentofuranose

Triethylamine (1.8 ml, 13 mmol), dimethylaminopyridine (30 mg, 0.25 mmol), and p-toluenesulfonyl chloride (858 mg, 4.5 mmol) were added to a solution of the compound obtained in Reference example 4 which was azeotropically refluxed with toluene (1035 mg, 2.5 mmol) in anhydrous dichloromethane (35 ml) under nitrogen atmosphere at 0°C and the mixture was stirred at room temperature overnight. The reaction mixture was partitioned between the dichloromethane and saturated aqueous sodium hydrogencarbonate solution (about 100 ml). The organic layer was washed with saturated aqueous sodium hydrogencarbonate solution (about 100 ml) and saturated aqueous sodium chloride solution (about 100 ml) and dried over anhydrous magnesium sulfate and then concentrated in vacuo. The residue was purified by chromatography on silica gel (using hexane : ethyl acetate = 3 : 1) to give a colorless oil (1340 mg, 2.6 mmol, 94%).

^{1}H-NMR (400MHz, $CDCl_3$) : 1.33(3H, s), 1.49(3H, s), 1.99(1H, dt, 7.6 and 15 Hz), 2.47(3H, s), 2.60(1H, ddd, 5.7, 7.6, 15Hz), 3.28(1H, d, 10Hz), 3.45(1H, d, 10Hz), 4.11(1H, d, 5.3Hz), 4.32(2H, m), 4.42(1H, d, 12Hz), 4.50(1H, d, 12Hz), 4.54(1H, d, 12Hz), 4.62(1H, dd, 4.0, 5.2Hz), 4.76(1H, d, 12Hz), 5.74(1H, d, 4.0 Hz), 7.3 (12H, m), 7.78(2H, d, 8.3Hz).

FAB-MAS(mNBA):569$(M+H)^+$

Reference example 9

1,2-Di-O-acetyl-3,5-di-O-benzyl-4-(p-toluenesulfonyloxyethyl)-α-D-erythropentofuranose

Acetic anhydride (1.88 ml, 20 mmol) and concentrated sulfuric acid (0.01 ml) were added to a solution of the compound obtained in Reference example 8 (1340 mg, 2.36 mmol) in acetic acid (15 ml) and the mixture was stirred at room temperature for 1 hour. The reaction mixture was poured into water (60 ml) in an ice-bath and stirred for 30 minutes and then partitioned between saturated aqueous sodium chloride solution (about 100 ml) and ethyl acetate (about 100 ml). The organic layer was washed with neutral phosphoric acid buffer solution, saturated aqueous sodium hydrogencarbonate solution and saturated aqueous sodium chloride solution and dried over anhydrous magnesium sulfate and then concentrated. The residue was purified by chromatography on silica gel (using hexane : ethyl acetate = 2 : 1) to give a colorless oil (1290 mg, 2.11 mmol, 89%, $\alpha : \beta = 1 : 5$).

^{1}H-NMR (400MHz, $CDCl_3$) : (β derivative) 1.86(3H, s), 2.05(3H, s), 2.08(1H, m), 2.18(1H, m), 2.42(3H, s), 3.30(1H, d, 10Hz), 3.33(1H, d, 10Hz), 4.23(1H, d, 5.1Hz), 4.24(2H, m), 4.42(2H, s), 4.45(1H, d, 12Hz), 4.55(1H, d, 12Hz), 5.28(1H, d, 5.1Hz), 6.01(1H, s), 7.3 (12H, m), 7.73(2H, d, 8.3Hz).

FAB-MAS(mNBA):613$(M+H)^+$

Reference example 10

2'-O-Acetyl-3',5'-di-O-benzyl-4'-p-toluenesulfonyloxyethyl-5-methyluridine

Trimethylsilylated thymine (500 mg, about 2 mmol), which was prepared according to a method of H.Vorbrggen, K. Krolikiewicz and B. Bennua (Chem.Ber., 114, 1234-1255 (1981)), was added to a solution of the compound obtained in Reference example 9 (650 mg, 1.06 mmol) in anhydrous 1,2-dichloroethane (15 ml) at room temperature under nitrogen atmosphere. Trimethylsilyl trifluoromethanesulfonate (0.36 ml, 2 mmol) was added dropwise to the mixture and the mixture was stirred at 50°C for 1 hour. Saturated aqueous sodium hydrogencarbonate solution (about 50 ml) was added to the reaction mixture and the mixture was filtered through celite. Dichloromethane (about 50 ml) was added to the filtrate. The organic layer was washed with saturated aqueous sodium hydrogencarbonate solution (about 50 ml) and saturated aqueous sodium chloride solution (about 50 ml) and dried over anhydrous magnesium sulfate and then concentrated in vacuo. The residue was purified by chromatography on silica gel (using hexane : ethyl acetate = 1.2 : 1) to give a colorless amorphous solid (432 mg, 0.64 mmol, 60%).

^{1}H-NMR (400MHz, $CDCl_3$) : 1.52(3H, d, 0.9Hz), 1.94(1H, dt, 7.5 and 15Hz), 2.06(3H, s), 2.23(1H, dt, 6.0 and 15Hz), 2.42(3H, s), 3.38(1H, d, 10Hz), 3.67(1H, d, 10Hz), 4.17(2H, m), 4.36(1H, d, 6.0Hz), 4.41(1H, d, 12Hz), 4.44(1H, d, 12Hz), 4.48(1H, d, 12Hz), 4.58(1H, d, 12Hz), 5.39(1H, dd, 5.1 and 6.0Hz), 6.04(1H, d, 5.1Hz), 7.3 (12H, m), 7.73(2H, dt, 1.8 and 8.3Hz), 8.18(1H, s).

FAB-MAS(mNBA):679$(M+H)^+$

Reference example 11

2'-O-Acetyl-3',5'-di-O-benzyl-4'-p-toluenesulfonyloxyethyl-4-N-benzoylcytidine

Trimethylsilylated benzoylcytosine (300 mg, about 1.0 mmol), which was prepared according to a method of H.Vorbrggen, K. Krolikiewicz and B. Bennua (Chem.Ber., 114, 1234-1255 (1981)), was added to a solution of the compound obtained in Reference example 9 (383 mg, 0.626 mmol) in anhydrous 1,2-dichloroethane (4 ml). Trimethylsilyl trifluoromethanesulfonate (0.18 ml, 0.995 mmol) at 0°C was added to the mixture and the mixture was stirred at 50°C for 1 hour. Saturated aqueous sodium hydrogencarbonate solution (about 10 ml) and methylenechloride (about 20 ml) was added to the mixture and then the mixture was stirred. The resulting white precipitates were filtered off through celite. The organic layer of the filtrate was washed with saturated aqueous sodium chloride solution (about 20 ml) and dried over anhydrous magnesium sulfate and then concentrated in vacuo to give a colorless amorphous solid (397 mg, 83%).

^{1}H-NMR(400MHz, $CDCl_3$) : 8.70(1H, br), 8.18(1H, d, 7.4Hz), 7.87(2H, d, 7.5Hz), 7.72(2H, d, 8.3Hz), 7.61-7.57(1H, m), 7.51-7.48(2H, m), 7.43-7.21(13H,m), 6.02(1H, d, 2.9Hz), 5.40(1H, dd, 5.8, 2.9Hz), 4.57(1H, d, 11Hz), 4.39(1H, d, 11Hz), 4.32-4.28(3H, m), 4.19-4.16(2H,m), 3.69(1H, d,11Hz), 3.31(1H, d, 11Hz), 2.40(3H, s), 2.30-2.23(1H, m), 2.06(3H, s), 1.95-1.89(1H, m)

FAB-MAS(mNBA):768$(M+H)^+$

Reference example 12

2'-O-Acetyl-3',5'-di-O-benzyl-4'-p-toluenesulfonyloxyethyl-6-N-benzoyladenosine

Trimethylsilylated benzoyladenosine (500 mg, about 2.0 mmol), which was prepared according to a method of H.Vorbrggen, K. Krolikiewicz and B. Bennua (Chem.Ber., 114, 1234-1255 (1981)), was added to a solution of the compound obtained in Reference example 9 (600 mg, 0.98 mmol) in anhydrous 1,2-dichloroethane (15 ml) at room temperature under nitrogen atmosphere. After dropwise addition of trimethylsilyl trifluoromethanesulfonate (0.36 ml, 2 mmol) to the mixture, the mixture was stirred at 50°C for 4 hour. Saturated aqueous sodium hydrogencarbonate solution (about 50 ml) and dichloromethane (50 ml) were added to the reaction mixture and the mixture was partitioned between these two layers. The organic layer was washed with saturated aqueous sodium hydrogencarbonate solution (about 50 ml) and saturated aqueous sodium chloride solution (about 50 ml) and dried over anhydrous magnesium sulfate and then concentrated in vacuo. The residue was purified by chromatography on silica gel (using dichloromethane : methanol = 50 : 1) to give a colorless amorphous solid (405 mg, 0.51 mmol, 52%).

^{1}H-NMR (400MHz, $CDCl_3$) : 2.0(1H, m), 2.06(3H, s), 2.32(1H, dt, 6.0 and 15Hz), 2.40(3H, s), 3.36(1H, d, 10Hz), 3.58(1H, d, 10Hz), 4.22(2H, m), 4.39(1H, d, 12Hz), 4.45(1H, d, 12Hz), 4.47(1H, d, 12Hz), 4.59(1H, d, 12Hz), 4.62(1H, d, 5.6Hz), 5.94(1H, dd, 4.5 and 5.6Hz), 6.21(1H, d, 4.5Hz), 7.2-7.3 (12H, m), 7.54(2H, m), 7.62(1H, dt, 1.2 and 6.2Hz), 7.72(2H, d, 8.3Hz), 8.02(2H, m), 8.21(1H, s), 8.75(1H, s), 8.97(1H, brs).

FAB-MAS(mNBA):792$(M+H)^+$

Reference example 13

2'-O-Acetyl-3',5'-di-O-benzyl-4'-p-toluenesulfonyloxyethyl-uridine

Trimethylsilylated uracil (200 mg, about 0.8 mmol), which was prepared according to a method of H.Vorbrggen, K. Krolikiewicz and B. Bennua (Chem.Ber., 114, 1234-1255 (1981)), was added to a solution of the compound obtained in Reference example 9 (200 mg, 0.327 mmol) in anhydrous 1,2-dichloroethane (8 ml) at room temperature under nitrogen atmosphere. After dropwise addition of trimethylsilyl trifluoromethanesulfonate (0.145 ml, 0.8 mmol) to the mixture, the mixture was stirred at 70°C for 1 hour. Saturated aqueous sodium hydrogencarbonate solution (about 10 ml) was added to the reaction mixture, the mixture was filtered through celite and dichloromethane (about 10 ml) was added to the filtrate. The organic layer was washed with saturated aqueous sodium hydrogencarbonate solution and saturated aqueous sodium chloride solution and dried over anhydrous magnesium sulfate and then concentrated in vacuo. The residue was purified by chromatography on silica gel (using dichloromethane : methanol = 100 : 2) to give a colorless oil (199 mg, 0.299 mmol, 92%).

^{1}H-NMR (400MHz, $CDCl_3$) : 1.94(1H,dt,7.4 and 15Hz), 2.07(3H,s), 2.23(1H,dt,5.9 and 15Hz), 2.43(3H,s), 3.36(1H,d,10Hz), 3.65(1H,d,10Hz), 4.17(2H,dd,6 and 7Hz), 4.31(1H,d, 5.9Hz), 4.38(1H,d,11Hz), 4.39(1H,d,11Hz), 4.40(1H,d,11Hz), 4.58(1H,d, 11Hz), 5.29(1H,dd,2.4 and 8.2Hz),5.33(1H,dd,4.5 and 6Hz), 6.00(1H,d,4.5Hz), 7.2-7.4(12H,m),7.61(1H,d,8.2Hz), 7.74(1H,d,8.3Hz), 8.14(1H,brs).

FAB-MAS(mNBA):665$(M+H)^+$

Reference example 14

2'-O-Acetyl-3',5'-di-O-benzyl-4'-p-toluenesulfonyloxyethyl-4-N-benzoyl-5-methylcytidine

Trimethylsilylated benzoyl 5-methylcytosine (400 mg, about 1.2 mmol), which was prepared according to a method of H.Vorbrggen, K. Krolikiewicz and B. Bennua (Chem.Ber., 114, 1234-1255 (1981)) was added to a solution of the compound obtained in Reference example 9 (400 mg, 0.653 mmol) in anhydrous 1,2-dichloroethane (6 ml). After addition of trimethylsilyl trifluoromethanesulfonate (0.180 µl, 1.0 mmol) to the mixture at 0°C, the mixture was stirred at 50°C for 1 hour.

The reaction mixture was warmed to room temperature. Saturated aqueous sodium hydrogencarbonate solution (about 5 ml) and methylenechloride (about 10 ml) were added to the reaction mixture and the mixture was stirred. The mixture was filtered through celite in order to remove white precipitates. The organic layer of the filtrate was washed with saturated aqueous sodium chloride solution and dried over anhydrous magnesium sulfate and then concentrated in vacuo to give a colorless amorphous solid (320 mg, 0.409 mmol, 63%).

^{1}H-NMR(400MHz, $CDCl_3$) : 1.68(3H,s), 1.95(1H,dt,7.3 and 15Hz), 2.07(3H,s), 2.25(1H,dt,6 and 15Hz), 2.43(3H,s), 3.40(1H,d,10Hz), 3.71(1H,d,10Hz), 4.18(2H,m), 4.37(1H,d,5.8Hz), 4.42(1H,d,12Hz), 4.46(1H,d,12Hz), 4.51(1H,d,12Hz), 4.61(1H,d, 12Hz), 5.42(1H,dd,4.9 and 5.8Hz), 6.07(1H,d,4.9Hz), 7.2-7.6(17H,m), 7.74(2H,d, 8.3Hz), 8.28(2H,d,7.0Hz).

FAB-MAS(mNBA):782$(M+H)^+$

Reference example 15

2'-O-Acetyl-3',5'-di-O-benzyl-4'-p-toluenesulfonyloxyethyl-2-N-isobutyrylguanosine

Trimethylsilylated isobutyrylguanosine (650 mg, about 1.5 mmol), which was prepared according to a method of H.Vorbrggen, K. Krolikiewicz and B. Bennua (Chem.Ber., 114, 1234-1255 (1981)), was added to a solution of the compound obtained in Reference example 9 (400 mg, 0.65 mmol) in anhydrous 1,2-dichloroethane (10 ml) at room temperature under nitrogen atmosphere. After addition of trimethylsilyl trifluoromethanesulfonate (0.2 ml, 1.2 mmol) to the mixture and the mixture was stirred at 50°C for 4 hour. Saturated aqueous sodium hydrogencarbonate solution (about 5 ml) was added to the reaction mixture and the organic layer was washed with saturated aqueous sodium hydrogencarbonate solution and saturated aqueous sodium chloride solution and dried over anhydrous magnesium sulfate and then concentrated in vacuo to give a product which was used in the next reaction without further purification.

(Test Example 1)

(Tm Measurement Test)

A sample solution (1000 μL) having a final concentration of NaCl of 100 mM, sodium phosphate buffer solution (pH 7.2) of 10 mM, oligonucleotide (1) of 4 μM, and complementary DNA (hereinafter referred to as oligonucleotide (2)), having a sequence indicated by its complementary chain (sequence: 5'-agcaaaaaacgc-3' (Sequence No. 1 of the Sequence List) or complementary RNA (hereinafter referred to as oligonucleotide (3)) having a sequence indicated by the sequence 5'-agcaaaaaacgc-3' (Sequence No. 1 of the Sequence List), of 4μM was warmed in a boiling water bath and slowly cooled to room temperature over the course of about two hours. The sample solution was then heated and measured using a spectrophotometer (UV-3100PC : a product of Shimadzu Corp.). The sample was heated in a cell (cell thickness: 1.0 cm, cylindrical jacket type) by circulating water heated with an incubator (Haake FE2 : a product of EKO Corp.), and the temperature was monitored using a digital thermometer (SATO SK1250MC). The temperature was raised from 20°C to 95°C and the intensity of ultraviolet absorbance at the maximum absorption wavelength in the vicinity of 260 nm was measured for each 1°C increase in temperature. Naturally-occurring DNA (hereinafter referred to as oligonucleotide (4)) having the sequence indicated by the sequence 5'-gcgttttttgct-3' (Sequence No. 2 of the Sequence List), which is the same sequence as oligonucleotide (1) (compound of Example 29), was used as the control, and the same procedure was performed.

The temperature at which the amount of change per 1°C reached a maximum was taken to be Tm (melting temperature), and the complementary chain formation ability of the oligonucleotide analogue was evaluated at this temperature.

The following shows the results of measuring the Tm values of oligonucleotide (4) (naturally-occurring DNA) and oligonucleotide (1) (Compound of Example 29) relative to oligonucleotide (2) (complementary DNA) and oligonucleotide (3) (complementary RNA).

[Table 3]

Tm (°C)		
Compound	Oligonucleotide (2)	Oligonucleotide (3)
Oligonucleotide (4)	48	44
Oligonucleotide (1)	61	75

As is clear from the above table, the oligonucleotide analogue of the present invention exhibited a remarkably higher Tm as well as remarkably higher complementary chain formation ability as compared with naturally-occurring DNA.

(Test Example 2)

(Measurement of Nuclease Enzyme Resistance)

Exonuclease or endonuclease was mixed into a buffer solution of oligonucleotide held at 37°C for 15 minutes. The mixed solution was then held at 37°C for a predetermined amount of time. Ethylenediamine tetraacetic acid (EDTA) was added to a portion of the mixed solution and the mixture was heated at 100°C for 2 minutes in order to stop the reaction. The amount of oligonucleotide remaining in the mixture was determined by reverse phase high-performance liquid column chromatography, and the time-based changes in the amount of oligonucleotide in the presence of nuclease were measured.

The oligonucleotide analogues of the present invention demonstrate remarkable nuclease resistance.

[Industrial Applicability]

The novel oligonucleotide analogue and nucleoside analogue of the present invention are useful as antisense or antigene pharmaceuticals having excellent stability, as detection agents (probes) of a specific gene, as primers for starting amplification or as intermediates for their production.

SEQUENCE LISTING

<110> Sankyo Company, Limited

<120> Novel Nucleoside and Nucleotide Derivatives

<130> FP200013

<140>
<141>

<150> JP HEI11-33863
<151> 1999-02-12

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<170> PatentIn Ver. 2.0

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<223> Description of Artificial Sequence: Synthesized oligonucleotide for testing Tm value

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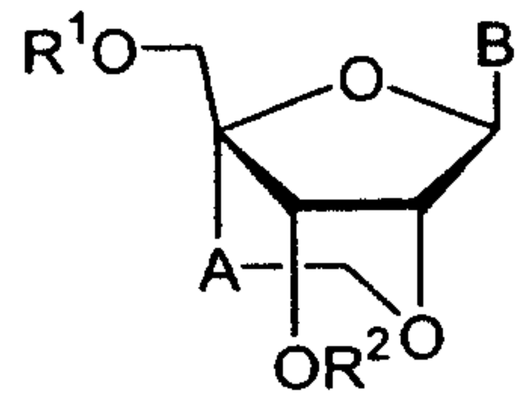
<223> Description of Artificial Sequence: Synthesized oligonucleotide for testing Tm value

<400> 2
gcgttttttg ct 12

The embodiments of the invention in which an exclusive property or privilege is claimed are defined as follows:

1. A compound of formula (1):



(1)

wherein:

R^1 and R^2 are the same or different and represent a hydrogen atom, a hydroxyl protecting group, a phosphoric acid group, a protected phosphoric acid group or $-P(R^3)R^4$ [wherein R^3 and R^4 are the same or different and represent a hydroxyl group, a protected hydroxyl group, a mercapto group, a protected mercapto group, an amino group, an alkoxy group having from 1 to 4 carbon atoms, an alkylthio group having from 1 to 4 carbon atoms, a cyanoalkoxy group having from 1 to 5 carbon atoms or an amino group substituted by an alkyl group having from 1 to 4 carbon atoms];

A represents an alkylene group having from 1 to 4 carbon atoms; and

B represents a purin-9-yl group, a 2-oxo-pyrimidin-1-yl group or a substituted purin-9-yl group or a substituted 2-oxo-pyrimidin-1-yl group having at least one substituent selected from the following α group;

or a salt thereof;

<u>α group</u>

a hydroxyl group,

a protected hydroxyl group,

an alkoxy group having from 1 to 4 carbon atoms,

a mercapto group,

a protected mercapto group,

an alkylthio group having from 1 to 4 carbon atoms,

an amino group,

a protected amino group,

an amino group substituted by an alkyl group having from 1 to 4 carbon atoms,

an alkyl group having from 1 to 4 carbon atoms, and a halogen atom.

2. The compound according to Claim 1 or a salt thereof, wherein R^1 is a hydrogen atom, an aliphatic acyl group, an aromatic acyl group, a methyl group substituted by from 1 to 3 aryl groups, a methyl group substituted by from 1 to 3 aryl groups the aryl ring of which is substituted by a lower alkyl, lower alkoxy, halogen or cyano group, or a silyl group.

3. The compound according to Claim 1 or a salt thereof, wherein R^1 is a hydrogen atom, an acetyl group, a benzoyl group, a benzyl group, a p-methoxybenzyl group, a dimethoxytrityl group, a mono-methoxytrityl group or a tert-butyldiphenylsilyl group.

4. The compound according to any one of Claims 1 to 3 or a salt thereof, wherein R^2 is a hydrogen atom, an aliphatic acyl group, an aromatic acyl group, a methyl group substituted by from 1 to 3 aryl groups, a methyl group substituted by from 1 to 3 aryl groups the aryl ring of which is substituted by a lower alkyl, lower alkoxy, halogen or cyano group, a silyl group, a phosphoramidite group, a phosphonyl group, a phosphoric acid group or a protected phosphoric acid group.

5. The compound according to any one of Claims 1 to 3 or a salt thereof, wherein R^2 is a hydrogen atom, an acetyl group, a benzoyl group, a benzyl group, a p-methoxy-benzyl group, a tert-butyldiphenylsilyl group, $-P(OC_2H_4CN)(NCH(CH_3)_2)$, $-P(OCH_3)(NCH(CH_3)_2)$, a phosphonyl group or a 2-chlorophenyl or 4-chlorophenyl phosphoric acid group.

6. The compound according to any one of Claims 1 to 5 or a salt thereof, wherein A is a methylene group.

7. The compound according to any one of Claims 1 to 6 or a salt thereof, wherein B is a 6-aminopurin-9-yl (i.e., adeninyl), 6-aminopurin-9-yl the amino group of which is protected, 2,6-diaminopurin-9-yl, 2-amino-6-chloropurin-9-yl, 2-amino-6-chloropurin-9-yl the amino group of which is protected, 2-amino-6-fluoropurin-9-yl, 2-amino-6-fluoropurin-9-yl the amino group of which is protected, 2-amino-6-bromopurin-9-yl, 2-amino-6-bromopurin-9-yl the amino group of which is protected,

2-amino-6-hydroxypurin-9-yl (i.e., guaninyl), 2-amino-6-hydroxypurin-9-yl the amino group of which is protected, 2-amino-6-hydroxypurin-9-yl the amino group and hydroxyl group of which are protected, 6-amino-2-methoxypurin-9-yl, 6-amino-2-chloropurin-9-yl, 6-amino-2-fluoropurin-9-yl, 2,6-dimethoxypurin-9-yl, 2,6-dichloropurin-9-yl, 6-mercaptopurin-9-yl, 2-oxo-4-amino-pyrimidin-1-yl (i.e., cytosinyl), 2-oxo-4-amino-pyrimidin-1-yl the amino group of which is protected, 2-oxo-4-amino-5-fluoro-pyrimidin-1-yl, 2-oxo-4-amino-5-fluoro-pyrimidin-1-yl the amino group of which is protected, 4-amino-2-oxo-5-chloro-pyrimidin-1-yl, 2-oxo-4-methoxy-pyrimidin-1-yl, 2-oxo-4-mercapto-pyrimidin-1-yl, 2-oxo-4-hydroxy-pyrimidin-1-yl (i.e., uracinyl), 2-oxo-4-hydroxy-5-methylpyrimidin-1-yl (i.e., thyminyl), 4-amino-5-methyl-2-oxo-pyrimidin-1-yl (i.e., methylcytosinyl) group or 4-amino-5-methyl-2-oxo-pyrimidin-1-yl group the amino group of which is protected.

8. The compound according to any one of Claims 1 to 6 or a salt thereof, wherein B is a 6-benzoylaminopurin-9-yl, adeninyl, 2-isobutyrylamino-6-hydroxypurin-9-yl, guaninyl, 2-oxo-4-benzoylamino-pyrimidin-1-yl, cytosinyl, 2-oxo-5-methyl-4-benzoylamino-pyrimidin-1-yl, 5-methylcytosinyl, uracinyl or thyminyl group.

9. A compound or a salt thereof selected from the following group:
2'-O,4'-C-ethyleneguanosine,
2'-O,4'-C-ethyleneadenosine,
3',5'-di-O-benzyl-2'-O,4'-C-ethylene-6-N-benzoyladenosine,
3',5'-di-O-benzyl-2'-O,4'-C-ethylene-2-N-isobutyrylguanosine,
5'-O-dimethoxytrityl-2'-O,4'-C-ethylene-6-N-benzoyladenosine,
5'-O-dimethoxytrityl-2'-O,4'-C-ethylene-2-N-isobutyrylguanosine,
2'-O,4'-C-ethylene-2-N-isobutyrylguanosine,
2'-O,4'-C-ethylene-6-N-benzoyladenosine,
5'-O-dimethoxytrityl-2'-O,4'-C-ethylene-6-N-benzoyladenosine-3'-O-(2-cyanoethyl N,N-diisopropyl)phosphoramidite,
5'-O-dimethoxytrityl-2'-O,4'-C-ethylene-2-N-isobutyrylguanosine-3'-O-(2-cyanoethyl N,N-diisopropyl)phosphoramidite,
2'-O,4'-C-ethyleneuridine,
2'-O,4'-C-ethylene-5-methyluridine,
2'-O,4'-C-ethylenecytidine,

2'-O,4'-C-ethylene-5-methylcytidine,

3',5'-di-O-benzyl-2'-O,4'-C-ethyleneuridine,

5'-O-dimethoxytrityl-2'-O,4'-C-ethyleneuridine,

3',5'-di-O-benzyl-2'-O,4'-C-ethylene-5-methyluridine,

5'-O-dimethoxytrityl-2'-O,4'-C-ethylene-5-methyluridine,

3',5'-di-O-benzyl-2'-O,4'-C-ethylene-4-N-benzoylcytidine,

5'-O-dimethoxytrityl-2'-O,4'-C-ethylene-4-N-benzoylcytidine,

3',5'-di-O-benzyl-2'-O,4'-C-ethylene-4-N-benzoyl-5-methylcytidine,

5'-O-dimethoxytrityl-2'-O,4'-C-ethylene-4-N-benzoyl-5-methylcytidine,

2'-O,4'-C-ethylene-4-N-benzoylcytidine,

2'-O,4'-C-ethylene-4-N-benzoyl-5-methylcytidine,

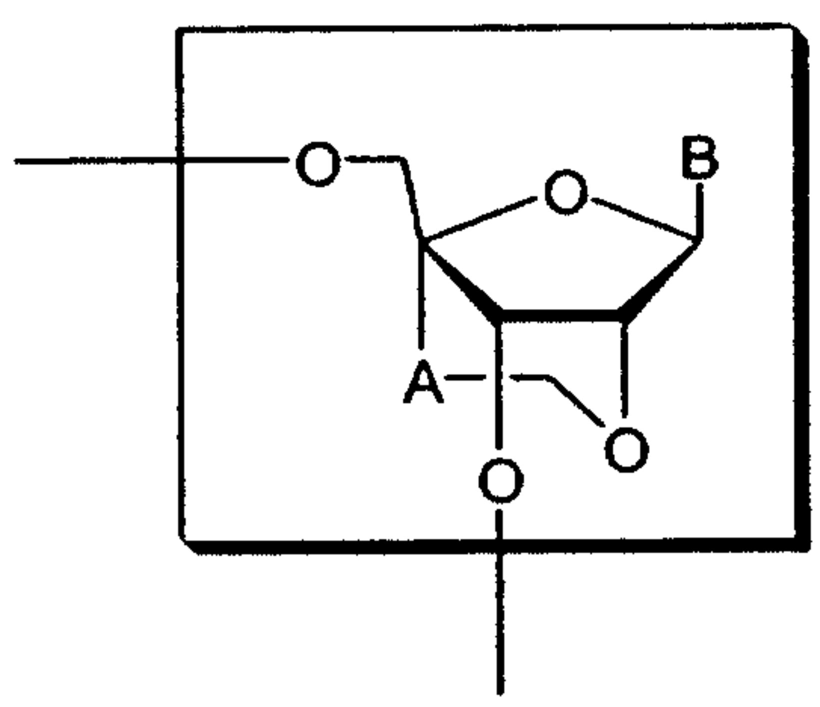
5'-O-dimethoxytrityl-2'-O,4'-C-ethylene-uridine-3'-O-(2-cyanoethyl N,N-diisopropyl)phosphoramidite,

5'-O-dimethoxytrityl-2'-O,4'-C-ethylene-5-methyluridine-3'-O-(2-cyanoethyl N,N-diisopropyl)phosphoramidite,

5'-O-dimethoxytrityl-2'-O,4'-C-ethylene-4-N-benzoylcytidine-3'-O-(2-cyanoethyl N,N-diisopropyl)phosphoramidite, and

5'-O-dimethoxytrityl-2'-O,4'-C-ethylene-4-N-benzoyl-5-methylcytidine-3'-O-(2-cyanoethyl N,N-diisopropyl)phosphoramidite.

10. An oligonucleotide analogue having one or two or more structures of formula (2):



(2)

wherein:

A represents an alkylene group having from 1 to 4 carbon atoms; and

B represents a purin-9-yl group, a 2-oxo-pyrimidin-1-yl group or a substituted purin-9-yl group or a substituted 2-oxo-pyrimidin-1-yl group having at least one substituent selected from the following α group;

or a pharmacologically acceptable salt thereof;

<u>α group</u>

a hydroxyl group,

a protected hydroxyl group,

an alkoxy group having from 1 to 4 carbon atoms,

a mercapto group,

a protected mercapto group,

an alkylthio group having from 1 to 4 carbon atoms,

an amino group,

a protected amino group,

an amino group substituted by an alkyl group having from 1 to 4 carbon atoms,

an alkyl group having from 1 to 4 carbon atoms, and

a halogen atom.

11. The oligonucleotide analogue according to Claim 10 or a pharmacologically acceptable salt thereof, wherein A is a methylene group.

12. The oligonucleotide analogue according to any one of Claims 10 and 11 or a pharmacologically acceptable salt thereof, wherein B is a 6-aminopurin-9-yl (i.e., adeninyl), 6-aminopurin-9-yl the amino group of which is protected, 2,6-diamino-purin-9-yl, 2-amino-6-chloropurin-9-yl, 2-amino-6-chloropurin-9-yl the amino group of which is protected, 2-amino-6-fluoropurin-9-yl, 2-amino-6-fluoropurin-9-yl the amino group of which is protected, 2-amino-6-bromopurin-9-yl, 2-amino-6-bromopurin-9-yl the amino group of which is protected, 2-amino-6-hydroxypurin-9-yl (i.e., guaninyl), 2-amino-6-hydroxypurin-9-yl the amino group of which is protected, 2-amino-6-hydroxypurin-9-yl the amino group and hydroxyl group of which are protected, 6-amino-2-methoxypurin-9-yl, 6-amino-2-chloropurin-9-yl, 6-amino-2-fluoropurin-9-yl, 2,6-dimethoxypurin-9-yl, 2,6-dichloro-purin-9-yl, 6-mercaptopurin-9-yl, 2-oxo-4-amino-pyrimidin-1-yl (i.e., cytosinyl), 2-oxo-4-amino-pyrimidin-1-yl

the amino group of which is protected, 2-oxo-4-amino-5-fluoro-pyrimidin-1-yl, 2-oxo-4-amino-5-fluoro-pyrimidin-1-yl the amino group of which is protected, 4-amino-2-oxo-5-chloro-pyrimidin-1-yl, 2-oxo-4-methoxy-pyrimidin-1-yl, 2-oxo-4-mercapto-pyrimidin-1-yl, 2-oxo-4-hydroxy-pyrimidin-1-yl (i.e., uracinyl), 2-oxo-4-hydroxy-5-methylpyrimidin-1-yl (i.e., thyminyl), 4-amino-5-methyl-2-oxo-pyrimidin-1-yl (i.e., methylcytosinyl) group or 4-amino-5-methyl-2-oxo-pyrimidin-1-yl group the amino group of which is protected.

13. The oligonucleotide analogue according to any one of Claims 10 and 11 or a pharmacologically acceptable salt thereof, wherein B is a 6-benzoylaminopurin-9-yl, adeninyl, 2-isobutyrylamino-6-hydroxypurin-9-yl, guaninyl, 2-oxo-4-benzoylamino-pyrimidin-1-yl, cytosinyl, 2-oxo-5-methyl-4-benzoylamino-pyrimidin-1-yl, 5-methylcytosinyl, uracinyl or thyminyl group.

14. A pharmaceutical composition comprising a pharmacologically active compound together with a carrier or diluent therefor, wherein said pharmacologically active compound is an oligonucleotide analogue according to any one of claims 10 to 13 or a pharmacologically acceptable salt thereof.

15. A probe for a gene comprising an oligonucleotide analogue according to any one of claims 10 to 13.

16. A primer for starting amplification comprising an oligonucleotide analogue according to any one of claims 10 to 13.

17. The use of an oligonucleotide analogue according to any one of claims 10 to 13 or a pharmacologically acceptable salt thereof in the manufacture of a medicament for the prevention or treatment of diseases preventable or treatable by the ability of said oligonucleotide analogue to exhibit pharmacologically useful antisense activity in the body of the patient after administration thereof.

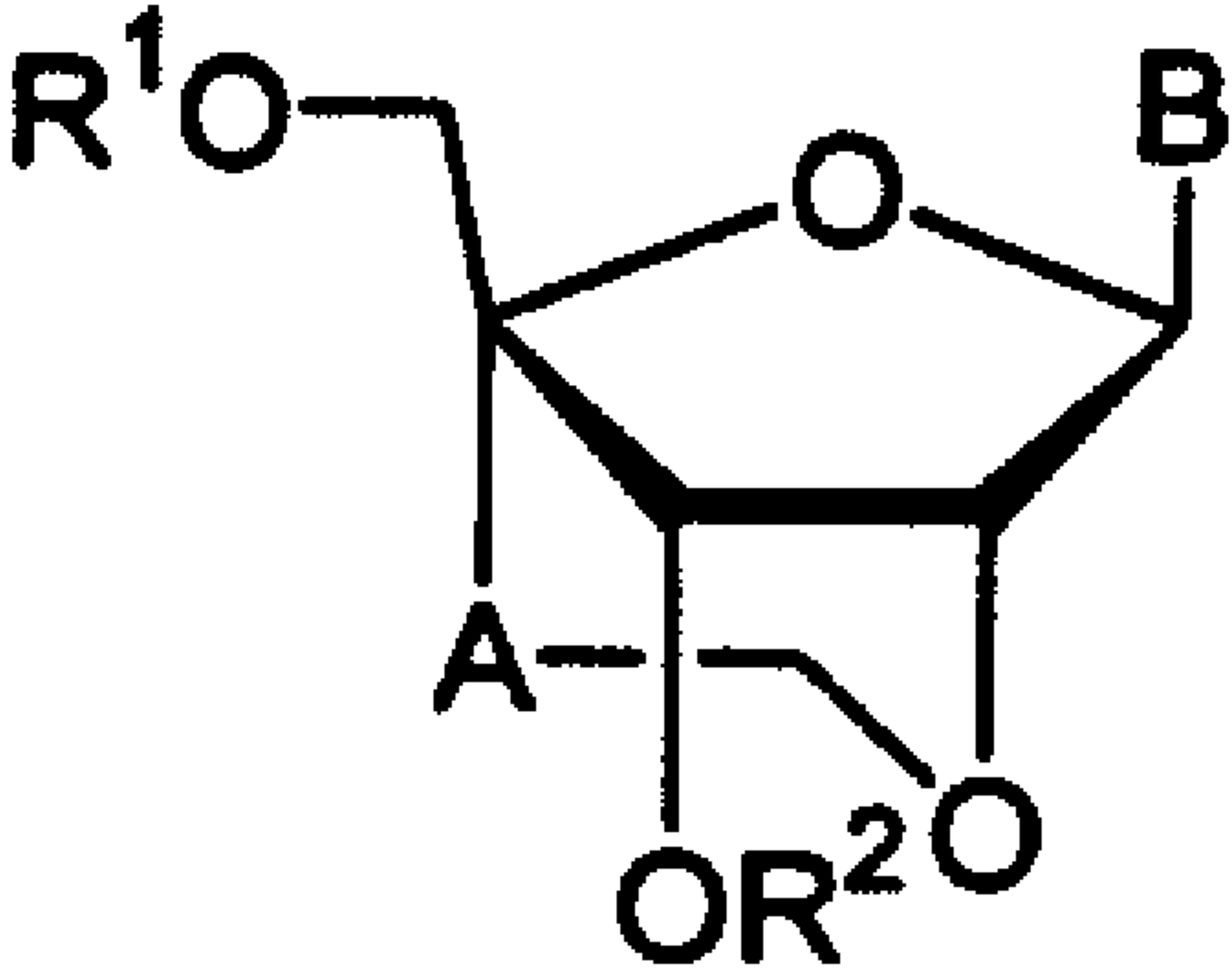
18. The use of an oligonucleotide analogue according to any one of claims 10 to 13 or a pharmacologically acceptable salt thereof in the manufacture of a medicament

for the prevention or treatment of diseases preventable or treatable by the ability of said oligonucleotide analogue to exhibit pharmacologically useful antigene activity in the body of the patient after administration thereof.

19. An oligonucleotide analogue according to any one of claims 10 to 13 or a pharmacologically acceptable salt thereof for use as a medicament.

20. An antisense agent containing an oligonucleotide analogue according to any one of claims 10 to 13 or a pharmacologically acceptable salt thereof.

21. An antigene agent containing an oligonucleotide analogue according to any one of claims 10 to 13 or a pharmacologically acceptable salt thereof.



(1)