

Description

Title of the Invention:

NUCLEIC ACID DERIVATIVE HAVING IMMUNOSTIMULATORY ACTIVITY

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Field of the Invention

[0001]

The present invention relates to a nucleic acid derivative having immunostimulatory activity. In more detail, it relates to a double-stranded oligonucleotide wherein the first strand is a CpG oligonucleotide, and the second strand binds to a lipid.

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Background Art

[0002]

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A vaccine which is a medicine for infectious disease or cancer utilizes an antigen-specific immune response. An adjuvant is a compound having an immunostimulatory activity which is used to enhance efficacy or durability of a vaccine, and various kinds of adjuvants such as aluminum salt, emulsion and liposome have been researched or developed (Non-patent Document 1 or the like).

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[0003]

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A single-strand oligodeoxynucleotide comprising a dinucleotide motif of unmethylated cytosine guanine (5'-CpG-3') (ssCpG ODN) is known as a one of the adjuvants. ssCpG ODNs are ligands of TLR9 (Toll-like receptor 9), and extremely efficient inducers of Th1 immunity or cytotoxic T-lymphocyte (CTL) responses thorough TLR9 to stimulate the immune system (Non-patent Document 1). However, there are problems related to in vivo stability, toxicity, pharmacokinetics or the like of ssCpG ODNs to use alone. A method that ssCpG ODN is encapsulated in a nanoparticle made of lipid bilayer (Non-patent Document 2), a method that lipid binds to 5' end of ssCpG ODN (Non-patent Document 3 or Patent Document 1) or the like is known as means for solving these problems.

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[0004]

Furthermore, it is known that the characteristicity as an adjuvant was

vanished when ssCpG ODN was administered as a double-stranded DNA (dsCpG ODN) by annealing the first strand and the second strand (Non-patent Document 4). Non-patent Document 5 discloses that only dsCpG ODN did not show the immunostimulatory activity, but when dsCpG ODN was encapsulated in a lipofectin particle, the dsCpG ODN comprising either CpG motif or GpC motif showed the immunostimulatory activity.

Prior Art Document

Patent Document

[0005]

Patent Document 1: WO2013/151771

Non-patent Document

[0006]

Non-patent Document 1: Trends in immunology, 2009, 30(1), 23-32

Non-patent Document 2: Advanced Drug Delivery Review, 2009, 61(3), 233-242

Non-patent Document 3: Nature, 2014, 507, 519-522

Non-patent Document 4: Eur. J. Immunol., 2003, 33, 1382-1392

Non-patent Document 5: BMB reports, 2010, 43(3), 164-169

Summary of the invention

Problems to be solved by the Invention

[0007]

The purpose of the present invention is to provide new nucleic acid derivatives having immunostimulatory activity which are useful as an adjuvant of a vaccine and/or a vaccine itself.

Means for Solving the Problem

[0008]

Non-patent Documents 4 and 5 disclose that administering dsCpG ODN did not show the immunostimulatory activity. Furthermore, Non-patent Document 5 discloses that when dsCpG ODN was encapsulated in a lipofectin particle, the dsCpG ODN comprising either CpG motif (ODN4531) or GpC motif (ODN4531GC) induced IL-8 and HLR-DRA expression, that is, by a method

which is independent of a CG sequence. It suggested based on the fact that dsCpG ODN was rapidly degraded in cells compared to ssCpG ODN that the reason why dsCpG ODN encapsulated in a lipofectin particle showed immunostimulatory activity is that encapsulation of dsCpG ODN may protect against rapid degradation (page 165, the right column, lines 14 to 16).
[0009]

The present inventors have intensively studied to synthesize a double-stranded oligonucleotide (a lipid binding double-stranded oligonucleotide of the present invention) wherein a first strand is a CpG oligonucleotide, and a second strand is an oligonucleotide comprising a sequence capable of hybridizing with the first strand and binds to a lipid. They found that the inductivity of antigen-specific CTL by a vaccine was enhanced and showed the anti-tumor effect by administering a lipid binding double-stranded oligonucleotide of the present invention as an adjuvant with a tumor antigen peptide (when a lipid binding double-stranded oligonucleotide of the present invention is used as an adjuvant, it is also referred as "an adjuvant of the present invention"). In addition, they found that there is a lipid binding double-stranded oligonucleotide in the present invention which shows strong anti-tumor effect by itself, that is, which is useful as a cancer vaccine (when a lipid binding double-stranded oligonucleotide of the present invention is used as a vaccine, it is also referred as "a vaccine of the present invention"). That is, a lipid binding double-stranded oligonucleotide of the present invention has the immunostimulatory activity. Furthermore, lipid binding double-stranded oligonucleotides of the present invention have high metabolic stability, water solubility and less toxicity to be safe enough as a medicine.

[0010]

For example, when an adjuvant of the present invention wherein the second strand is a DNA oligonucleotide, an oligonucleotide consisting of nucleoside derivatives having OMe at the 2' position of the sugar or an oligonucleotide consisting of nucleoside derivatives having F at the 2' position of the sugar was administered with a tumor antigen peptide, they showed the enhanced CTL inductivity or the strong anti-tumor effect compared to ssCpG ODN (e.g., Result 1-A, B, Result 7-A, B and Result 9-A, B in Example 3). On the other hand, the lipid binding double-stranded oligonucleotide wherein the

second strand is a RNA oligonucleotide (SEQ-40) did not show the anti-tumor effect (Result 7-B).

When an adjuvant of the present invention wherein a lipid comprising two of the acyl chains having 14 to 24 carbon atoms binds at 3' or 5' end of the second strand was administered with a tumor antigen peptide, it showed the strong anti-tumor effect compared to ssCpG ODN (e.g., Result 2-A, B in Example 3). On the other hand, when a double-stranded oligonucleotide wherein a lipid comprising two acyl chains having 10 carbon atoms binds at 5' end of the second strand (SEQ-12) was administered with a tumor antigen peptide, it did not show the enhanced anti-tumor effect compared to ssCpG ODN. In addition, when an adjuvant of the present invention wherein a lipid comprising two of the acyl chains having 12 to 20 carbon atoms binds at 3' or 5' end of the second strand, and furthermore a lipid binds at the other end was administered with a tumor antigen peptide, it showed the enhanced CTL inductivity or the strong anti-tumor effect compared to ssCpG ODN (e.g., Result 3-A, B in Example 3).

When an adjuvant of the present invention wherein the length of the second strand is 50 % to 100 % of that of the first strand (the CpG oligonucleotide) was administered with a tumor antigen peptide, it showed the enhanced CTL inductivity or the strong anti-tumor effect compared to ssCpG ODN (e.g., Result 4-A, B, Result 5-A, Result 8-A, B, and Result 9-A, B in Example 3).

When an adjuvant of the present invention wherein a lipid binds to a second strand through an oligonucleotide linker (e.g., dGdG, dTdT and dAdA) was administered with a tumor antigen peptide, it showed the further enhanced CTL inductivity or the stronger anti-tumor effect compared to an adjuvant of the present invention without a linker (e.g., Result 8-A, B in Example 3).

[0011]

In addition, lipid binding double-stranded oligonucleotides of the present invention have no systemic toxicity, and it suggested the high safety (Example 5).

[0012]

Furthermore, the result of immunization with an adjuvant of the

present invention and PCR γ protein derived from *Pseudomonas aeruginosa* which is a cause of an infectious disease also suggested the immunostimulatory activity as an adjuvant of a vaccine for an infectious disease (Example 6).

[0013]

5 When the adjuvant of the present invention wherein lipid binds to a second strand through an oligonucleotide linker (dGdGdGdGdG) (SEQ-121) was administered alone, it showed the strong anti-tumor effect compared to ssCpG ODN (Example 4).

[0014]

10 That is, the present invention is related to the followings.

(A1) A double-stranded oligonucleotide, wherein

a first strand is a CpG oligonucleotide consisting of 8 to 50 nucleotides, a second strand is an oligonucleotide consisting of 8 to 60 nucleotides and comprising a sequence capable of hybridizing with the first strand, but

15 excluding a RNA oligonucleotide,

the length of the second strand is 50 % or more of that of the first strand, and a lipid comprising C12 to C30 hydrocarbon chain(s) binds to the second strand through a linker.

(A2) The double-stranded oligonucleotide of (A1), wherein the oligonucleotide

20 of the second strand is an oligonucleotide consisting of DNA nucleosides and/or nucleoside derivatives.

(A3) The double-stranded oligonucleotide of (A2), wherein the nucleoside

25 derivative is a nucleoside having a substituent at the 2' position of the sugar and/or a nucleoside having a bridge structure between the 4' and 2' positions of the sugar.

(A4) The double-stranded oligonucleotide of (A3), wherein the bridge structure between the 4' and 2' positions of the sugar is 4'-(CH₂)_m-O-2', wherein m is an integer of 1 to 4.

30 (A5) The double-stranded oligonucleotide of any one of (A1) to (A4), wherein the lipid is a diacyl lipid.

(A6) The double-stranded oligonucleotide of (A1) to (A5), wherein the lipid binds at the 3' end and/or 5' end of the second strand.

(A7) The double-stranded oligonucleotide of any one of (A1) to (A6), wherein the linker is an oligonucleotide linker.

(A8) The double-stranded oligonucleotide of (A7), wherein the linker is $-(dX^1)_u-$, wherein X^1 is each independently, A, G, C or T, and u is an integer of 1 to 8.

(A9) The double-stranded oligonucleotide of (A1) selected from the group of consisting of SEQ-61, SEQ-119, SEQ-121, SEQ-170 and SEQ-192.

5 (A10) The double-stranded oligonucleotide of (A1) selected from the group of consisting of SEQ-59, SEQ-166, SEQ-168, SEQ-216, SEQ-272, SEQ-280, SEQ-290, SEQ-294, SEQ-310, SEQ-373 and SEQ-384.

(A11) A pharmaceutical composition comprising the double-stranded oligonucleotide of any one of (A1) to (A10).

10 (A12) The pharmaceutical composition of (A11), further comprising an antigen.

(A13) A method for treating or preventing a cancer or an infectious disease, comprising administering the double-stranded oligonucleotide of any one of (A1) to (A10).

(A14) Use of the double-stranded oligonucleotide of any one of (A1) to (A10) for
15 the manufacture of an agent for treating or preventing a cancer or an infectious disease.

(A15) The double-stranded oligonucleotide of any one of (A1) to (A10) for treating or preventing a cancer or an infectious disease.

[0015]

20 (A16) The pharmaceutical composition of (A12), wherein the antigen is a microbial antigen, a self-antigen or an addictive substance.

(A18) The pharmaceutical composition of (A16), wherein the microbial antigen is a bacterial antigen, a viral antigen or a parasitic antigen.

(A18) The pharmaceutical composition of (A16), wherein the self-antigen is a
25 tumor antigen, an antigen associated with Alzheimer's Disease, an antigen against a human antibody, or an antigen that is expressed from human endogenous retroviral elements.

(A19) The pharmaceutical composition of (A16), the addictive substance is nicotine or cocaine.

30 (A20) A method for increasing an immune response in a subject comprising administering to the subject an effective amount of the pharmaceutical composition of any one of (A12), (A16) to (A19).

(A21) The method of (A20), wherein the immune response is an increase in inductivity of specific cytotoxic T-lymphocyte compared to a control.

(A22) The method of (A20) or (A21), wherein the subject has a cancer or an infectious disease.

[0016]

In addition, the present invention includes the followings.

- 5 (B1) An adjuvant consisting of a double-stranded oligonucleotide, wherein a first strand is a CpG oligonucleotide consisting of 8 to 50 nucleotides, a second strand is an oligonucleotide consisting of 8 to 60 nucleotides and comprising a sequence capable of hybridizing with the first strand, and a lipid binds to the second strand through a linker.
- 10 (B2) The adjuvant of (B1), wherein the oligonucleotide of the second strand is an oligonucleotide consisting of DNA nucleosides and/or nucleoside derivatives.
- (B3) The adjuvant of (B2), wherein the nucleoside derivative is a nucleoside having a substituent at the 2' position of the sugar and/or a nucleoside having a bridge structure between the 4' and 2' positions of the sugar.
- 15 (B4) The adjuvant of (B3), wherein the substituent is OCH_3 .
- (B5) The adjuvant of any one of (B1) to (B4), wherein the lipid is a diacyl lipid.
- (B6) The adjuvant of (B5), wherein the acyl chain of the diacyl lipid has 14 to 30 carbon atoms.
- (B7) The adjuvant of (B5) or (B6), wherein the lipid binds at the 5' the end of
- 20 the second strand.
- (B8) The adjuvant of any one of (B1) to (B7), wherein the length of the second strand is 50 % or more of that of the first strand.
- (B9) The adjuvant of any one of (B1) to (B9), wherein the linker is an oligonucleotide linker.
- 25 (B10) The adjuvant of (B9), wherein the linker is dX^1dX^2 , wherein X^1 or X^2 is A, G, C or T.
- (B11) A vaccine composition comprising an antigen and the adjuvant of any one of (B1) to (B10).

[0017]

- 30 (B12) The vaccine composition of (B11) wherein the antigen is a microbial antigen, a self-antigen or an addictive substance.
- (B13) The vaccine composition of (B12) wherein the microbial antigen is a bacterial antigen, a viral antigen or a parasitic antigen.
- (B14) The vaccine composition of (B12) wherein the self-antigen is a tumor

antigen, an antigen associated with Alzheimer's Disease, an antigen against a human antibody, or an antigen that is expressed from human endogenous retroviral elements.

(B15) The vaccine composition of (B12) wherein the addictive substance is nicotine or cocaine.

(B16) A method of increasing an immune response in a subject comprising administering to the subject an effective amount of the vaccine composition of any one of (B11) to (B15).

(B17) The method of (B16), wherein the immune response is an increase in inductivity of induced specific cytotoxic T-lymphocyte compared to a control.

(B18) The method of (B16) or (B17), wherein the subject has a cancer or an infectious disease.

(B19) A method of treating a cancer or an infectious disease comprising administering to the subject an effective amount of the vaccine composition of any of (B11) to (B14) to reduce one or more symptoms of the cancer or infectious disease compared to a control.

Effect of the Invention

[0018]

Lipid binding double-stranded oligonucleotides of the present invention show the superior immunostimulatory activity against a target antigen. No systemic toxicity was detected, and therefore they are expected to apply to a medicine as an adjuvant of a vaccine and/or a vaccine itself.

Brief Description of the Drawings

[0019]

[Fig. 1] The anti-tumor effect when ssCpG ODN (ODN1826, SEQ-1), dsCpG ODN (SEQ-4) or the adjuvant of the present invention (SEQ-16) was administered with a tumor antigen peptide (TRP2 peptide).

[Fig. 2] The anti-tumor effect when ssCpG ODN (ODN1826, SEQ-1) or the adjuvant of the present invention having lipids at the both ends (SEQ-46 or SEQ-48) was administered with a tumor antigen peptide (TRP2 peptide).

[Fig. 3] The anti-tumor effect when ssCpG ODN (ODN1826, SEQ-1), ssCpG ODN introducing a lipid ligand (SEQ-2) or the adjuvant of the present

invention which the length of the complementary strand is different (SEQ-8 or SEQ-10) was administered with a tumor antigen peptide (TRP2 peptide).

[Fig. 4] The anti-tumor effect when ssCpG ODN (ODN1826, SEQ-1), ssCpG ODN introducing a lipid ligand (SEQ-2), the adjuvant of the present invention (SEQ-26) or Montanide was administered with a tumor antigen peptide (TRP2 peptide).

[Fig. 5] The anti-tumor effect when ssCpG ODN (ODN1826, SEQ-1), ssCpG ODN introducing a lipid ligand (SEQ-2) or the adjuvant of the present invention (SEQ-38) was administered with a tumor antigen peptide (TRP2 peptide).

[Fig. 6] The anti-tumor effect when ssCpG ODN (ODN2006, SEQ-49), the adjuvant of the present invention which the length of the complementary strand is different (SEQ-51 or SEQ-61) or the adjuvant of the present invention having a linker (SEQ-63 or SEQ-65) was administered with a tumor antigen peptide (TRP2 peptide).

[Fig. 7] The anti-tumor effect when ssCpG ODN (ODN2006, SEQ-49) or the adjuvant of the present invention wherein the nucleic monomer of the complementary strand is 2'-OMe-RNA (SEQ-67 or SEQ-69) was administered with a tumor antigen peptide (TRP2 peptide).

[Fig. 8] The anti-tumor effect when ssCpG ODN (ODN2006, SEQ-49) or the adjuvant of the present invention having an oligonucleotide linker (SEQ-119 or SEQ-192) was administered with a tumor antigen peptide (TRP2 peptide).

[Fig. 9] The anti-tumor effect when ssCpG ODN (ODN2006, SEQ-49) or the adjuvant of the present invention having an oligonucleotide linker (SEQ-121) was administered with a tumor antigen peptide (TRP2 peptide).

[Fig. 10] The anti-tumor effect when ssCpG ODN (ODN2006, SEQ-49) or the adjuvant of the present invention having an oligonucleotide linker (SEQ-152 or SEQ-158) was administered with a tumor antigen peptide (TRP2 peptide).

[Fig. 11] The anti-tumor effect when ssCpG ODN (ODN2006, SEQ-49) or the adjuvant of the present invention wherein a lipid binds at the 3' end and/or 5' end (SEQ-188, SEQ-192 or SEQ-194) was administered with a tumor antigen peptide (TRP2 peptide).

[Fig. 12] The anti-tumor effect when ssCpG ODN (ODN2006, SEQ-49) or the adjuvant of the present invention having an oligonucleotide linker (SEQ-164)

was administered with a tumor antigen peptide (TRP2 peptide).

[Fig. 13] The anti-tumor effect when ssCpG ODN (ODN2006, SEQ-49) or the adjuvant of the present invention wherein a lipid binds only at the 3' end (SEQ-170) was administered with a tumor antigen peptide (TRP2 peptide).

5 [Fig. 14] The anti-tumor effect of ssCpG ODN (ODN2006, SEQ-49) or the vaccine of the present invention (SEQ-121) under the absence of a tumor antigen peptide.

[Fig. 15] Antibody titer after subcutaneous administration of PCRV antigen vaccine with ssCpG ODN (ODN2006, SEQ-49), the adjuvant of the present
10 invention (SEQ-61 or SEQ-121) or Freund's adjuvant.

Embodiments for Carrying out the invention

[0020]

Terms used herein, unless otherwise indicated, are used in a sense
15 normally used in the art.

In the present invention, a genetic manipulation method which is well known in the art can be used. For example, it is a method described in Molecular Cloning, A Laboratory Manual, Fourth Edition, Cold Spring Harbor Laboratory Press (2012), or Current Protocols Essential Laboratory
20 Techniques, Current Protocols (2012).

[0021]

Terms used in this description are explained below. Each term, unless otherwise indicated, has the same meaning when it is used alone or together with other terms.

25 [0022]

In this description, "adjuvant" means a compound having the immunostimulatory activity which is utilized to enhance efficacy or durability of immune response of a vaccine antigen.

[0023]

30 A "nucleoside" means a compound that a nucleic acid base and a sugar are bonded by an N-glycoside bond.

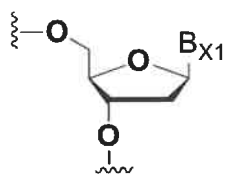
An "oligonucleotide" means nucleotides that some of same or different kinds of nucleotide are bonded by an internucleoside linkage such as a phosphodiester bond.

[0024]

A linkage between a sugar and a sugar in an oligonucleotide (internucleoside linkage) may be a linkage having a natural nucleic acid, phosphodiester (D-oligo), an artificially modified linkage or a linkage without phosphorus atom. Any linkage which is well-known in this field can be used. Examples of an artificially modified linkage are phosphorothioate (S-oligo), methylphosphonate (M-oligo) and boranophosphate. Furthermore, a linkage described in WO2013/022966, WO2011/005761, WO2014/012081, WO2015/125845 or the like can be used. An example of a linkage without phosphorus atom is a bivalent substituent deriving from non-aromatic carbocyclyl or the like substituted with alkyl, non-aromatic carbocyclyl, haloalkyl or halogen. Example is a bivalent substituent deriving from siloxane, sulfide, sulfoxide, sulfone, acetyl, acetyl formate, acetyl thioformate, acetyl methylene formate, acetyl thioformate, alkenyl, sulfamate, methyleneimino, methylenehydrazino, sulfonate, sulfonamide, amide or the like. In an oligonucleotide, linkages may be same or different.

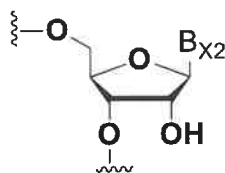
[0025]

In this description, a "DNA nucleoside" or "RNA nucleoside" means natural DNA nucleoside or natural RNA nucleoside, and a part of nucleotide, which is 1 unit for a component of an oligonucleotide. A "natural DNA nucleoside" is as below.



wherein B_{x1} is adenine, guanine, cytosine or thymine.

A "natural RNA nucleoside" is as below.



wherein B_{x2} is adenine, guanine, cytosine or uracil.

[0026]

A "DNA oligonucleotide" means an oligonucleotide which some DNA nucleosides are bounded, and an "RNA oligonucleotide" means an oligonucleotide which some RNA nucleosides are bounded.

[0027]

In this description, a "nucleoside derivative" means a nucleoside whose nucleic base and/or sugar part of DNA nucleoside or RNA nucleoside was artificially modified. Any well-known modification for a nucleoside in this field can be used.

[0028]

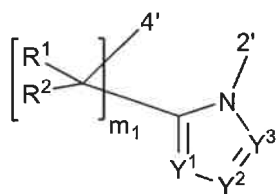
Examples of modification for a nucleic base are 5-methylcytosine, 5-hydroxy methylcytosine and 5-propynylcytosine.

[0029]

An example of modification for a sugar part is a substituent at the 2' position of a sugar. Examples are 2'-F, 2'-OCH₃ (2'-OMe) and 2'-OCH₂CH₂OCH₃ (2'-MOE).

The other example is the following bridge structure between the 4' and 2' positions of a sugar.

4'-(CR¹R²)_m-O-2', 4'-(CR¹R²)_m-S-2', 4'-(CR¹R²)_m-O-C(=O)-2', 4'-(CR¹R²)_m-NR³-O-(CR¹R²)_{m₁}-2', 4'-(CR¹R²)_{m₁}-C(=O)-NR³-2', 4'-(CR¹R²)_{m₂}-C(=O)-NR³-Y⁴-2', 4'-(CR¹R²)_{m₁}-SO₂-NR³-2', or



wherein

Y⁴ is O, S, NH or CH₂,

R¹ is each independently, hydrogen atom, halogen, cyano, substituted or unsubstituted alkyl, substituted or unsubstituted alkenyl or substituted or unsubstituted alkynyl,

R² is each independently, hydrogen atom, halogen, cyano, substituted or

unsubstituted alkyl, substituted or unsubstituted alkenyl or substituted or unsubstituted alkynyl,

R³ is hydrogen atom, substituted or unsubstituted alkyl, substituted or unsubstituted alkenyl, substituted or unsubstituted alkynyl, substituted or unsubstituted aromatic carbocyclyl, substituted or unsubstituted non-aromatic carbocyclyl, substituted or unsubstituted aromatic heterocyclyl, substituted or unsubstituted non-aromatic heterocyclyl, substituted or unsubstituted aromatic carbocyclalkyl, substituted or unsubstituted non-aromatic carbocyclalkyl, substituted or unsubstituted aromatic heterocyclalkyl or substituted or unsubstituted non-aromatic heterocyclalkyl,

Y¹ is CR⁴ or N,

Y² is CR⁵ or N,

Y³ is CR⁶ or N,

R⁴, R⁵ and R⁶ are each independently, hydrogen atom, halogen, cyano, substituted or unsubstituted alkyl, substituted or unsubstituted alkenyl, substituted or unsubstituted alkynyl, substituted or unsubstituted amino, substituted or unsubstituted alkyloxy, substituted or unsubstituted alkylcarbonylamino, substituted or unsubstituted alkenylcarbonylamino, substituted or unsubstituted alkynylcarbonylamino, substituted or unsubstituted alkylcarbamoyl, substituted or unsubstituted alkenylcarbamoyl, or substituted or unsubstituted alkynylcarbamoyl,

m is an integer of 1 to 4,

m₁ is an integer of 0 to 3, and

m₂ is 0 or 1.

[0030]

R¹ and R² is preferably hydrogen atom.

[0031]

R³ is preferably hydrogen atom, alkyl, alkenyl, alkynyl, aromatic carbocyclyl, non-aromatic carbocyclyl, aromatic heterocyclyl, non-aromatic heterocyclyl, aromatic carbocyclalkyl, non-aromatic carbocyclalkyl, aromatic heterocyclalkyl or non-aromatic heterocyclalkyl, and may have one or more substituent(s) selected from Group α.

Group α: a hydroxyl group, alkyl, alkyloxy, mercapto, alkylthio, amino, alkylamino and halogen.

[0032]

The bridge structure is preferably 4'-(CR¹ R²)_m-O-2' or 4'-(CR¹ R²)_{m1}-

C(=O)-NR³-2' (AmNA, Bridged nucleic acid),

wherein,

R¹ is each independently, hydrogen atom, halogen, cyano, substituted or unsubstituted alkyl, substituted or unsubstituted alkenyl, or substituted or unsubstituted alkynyl,

R² is each independently, hydrogen atom, halogen, cyano, substituted or unsubstituted alkyl, substituted or unsubstituted alkenyl, or substituted or unsubstituted alkynyl,

R³ is hydrogen atom, substituted or unsubstituted alkyl, substituted or unsubstituted alkenyl, or substituted or unsubstituted alkynyl,

m is an integer of 1 to 4, and

m₁ is an integer of 0 to 2.

[0033]

The bridge structure is more preferably 4'-(CH₂)_m-O-2', wherein m is an integer of 1 to 4, or 4'-C(=O)-NR³-2', wherein R³ is hydrogen atom or alkyl.

4'-(CH₂)_m-O-2', wherein m is an integer of 1 to 4, is more preferably 4'-CH₂-O-2' (LNA, Locked nucleic acid). Examples and the methods for preparation are described in WO98/39352, WO2003/068795, WO2005/021570 or the like.

4'-C(=O)-NR³-2', wherein R³ is hydrogen atom or alkyl, is more preferably 4'-C(=O)-NCH₃-2'. Examples and the methods for preparation are described in WO2011/052436.

[0034]

Examples of the well-known modification of nucleotide and the method for modification in this field are described in the following patent documents. WO98/39352, WO99/014226, WO2000/056748, WO2005/021570, WO2003/068795, WO2011/052436, WO2004/016749, WO2005/083124, WO2007/143315, WO2009/071680, WO2014/112463, WO2014/126229 and the like.

[0035]

"Halogen" includes a fluorine atom, a chlorine atom, a bromine atom and an iodine atom. A fluorine atom and a chlorine atom are especially preferable.

[0036]

“Alkyl” includes a C1 to C15, preferably a C1 to C10, more preferably a C1 to C6 and even more preferably a C1 to C4 linear or branched hydrocarbon group. Examples include methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl, sec-butyl, tert-butyl, n-pentyl, isopentyl, neopentyl, n-hexyl, isohexyl, n-heptyl, isoheptyl, n-octyl, isooctyl, n-nonyl and n-decyl.

A preferred embodiment of “alkyl” is methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl, sec-butyl, tert-butyl or n-pentyl. A more preferred embodiment is methyl, ethyl, n-propyl, isopropyl or tert-butyl.

[0037]

“Alkenyl” includes a C2 to C15, preferably a C2 to C10, more preferably a C2 to C6 and further preferably a C2 to C4 linear or branched hydrocarbon group having one or more double bond(s) at any position(s). Examples include vinyl, allyl, propenyl, isopropenyl, butenyl, isobutenyl, prenyl, butadienyl, pentenyl, isopentenyl, pentadienyl, hexenyl, isohexenyl, hexadienyl, heptenyl, octenyl, nonenyl, decenyl, undecenyl, dodecenyl, tridecenyl, tetradecenyl and pentadecenyl.

A preferred embodiment of “alkenyl” is vinyl, allyl, propenyl, isopropenyl or butenyl.

[0038]

“Alkynyl” includes a C2 to C10, preferably a C2 to C8, more preferably a C2 to C6 and further preferably a C2 to C4 linear or branched hydrocarbon group having one or more triple bond(s) at any position(s). Examples include ethynyl, propynyl, butynyl, pentynyl, hexynyl, heptynyl, octynyl, nonynyl and decynyl. Furthermore, it may have double bond(s) at any position(s).

A preferred embodiment of “alkynyl” is ethynyl, propynyl, butynyl or pentynyl.

[0039]

“Aromatic carbocyclyl” means a cyclic aromatic hydrocarbon group which is monocyclic or polycyclic having two or more rings. Examples include phenyl, naphthyl, anthryl and phenanthryl.

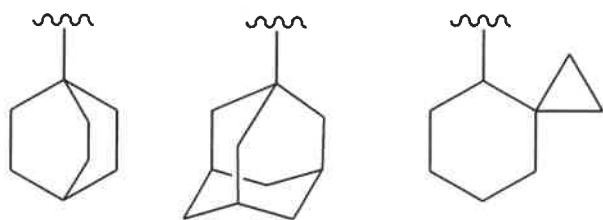
A preferred embodiment of “aromatic carbocyclyl” is phenyl.

[0040]

“Non-aromatic carbocyclyl” means a cyclic saturated hydrocarbon group or cyclic unsaturated non-aromatic hydrocarbon group, which is monocyclic or

polycyclic having two or more rings. Examples of the non-aromatic carbocyclyl, which is polycyclic having two or more rings, include a fused ring group wherein a non-aromatic carbocyclyl, which is monocyclic or polycyclic having two or more rings, is fused with a ring of the above "aromatic carbocyclyl".

In addition, examples of the "non-aromatic carbocyclyl" also include a group having a bridge and a group to form a spiro ring as follows:



The non-aromatic carbocyclyl, which is monocyclic, is preferably C3 to C16, more preferably C3 to C12 and further preferably C4 to C8 carbocyclyl. Examples include cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, cyclooctyl, cyclononyl, cyclodecyl, cyclopropenyl, cyclobutenyl, cyclopentenyl, cyclohexenyl, cycloheptenyl and cyclohexadienyl.

Examples of non-aromatic carbocyclyl, which is polycyclic having two or more rings, include indanyl, indenyl, acenaphthyl, tetrahydronaphthyl and fluorenyl.

[0041]

"Aromatic heterocyclyl" means an aromatic cyclyl, which is monocyclic or polycyclic having two or more rings, containing one or more of heteroatom(s) selected independently from O, S and N.

Examples of aromatic heterocyclyl, which is polycyclic having two or more rings, include a fused ring group wherein an aromatic heterocyclyl, which is monocyclic or polycyclic having two or more rings, is fused with a ring of the above "aromatic carbocyclyl" and/or "non-aromatic carbocyclyl".

The aromatic heterocyclyl, which is monocyclic, is preferably a 5- to 8-membered and more preferably 5- to 6- membered ring. Examples include pyrrolyl, imidazolyl, pyrazolyl, pyridyl, pyridazinyl, pyrimidinyl, pyrazinyl, triazolyl, triazinyl, tetrazolyl, furyl, thienyl, isoxazolyl, oxazolyl, oxadiazolyl, isothiazolyl, thiazolyl and thiadiazolyl.

Examples of aromatic heterocyclyl, which is bicyclic, include indolyl,

isoindolyl, indazolyl, indoliziny, quinoliny, isoquinoliny, cinnoliny,
phthalaziny, quinazoliny, naphthyridiny, quinoxaliny, puriny, pteridiny,
benzimidazolyl, benzisoxazolyl, benzoxazolyl, benzoxadiazolyl,
benzisothiazolyl, benzothiazolyl, benzothiadiazolyl, benzofuryl, isobenzofuryl,
5 benzothieryl, benzotriazolyl, imidazopyridyl, triazolopyridyl, imidazothiazolyl,
pyrazinopyridaziny, oxazolopyridyl and thiazolopyridyl.

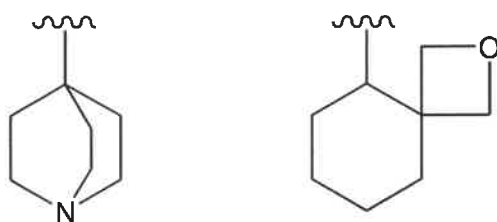
Examples of aromatic heterocyclyl, which is polycyclic having three or
more rings, include carbazolyl, acridiny, xanthenyl, phenothiaziny,
phenoxathiiny, phenoxaziny and dibenzofuryl.

[0042]

“Non-aromatic heterocyclyl” means a non-aromatic cyclyl, which is
monocyclic or polycyclic having two or more rings, containing one or more
heteroatom(s) selected independently from O, S and N.

Examples of non-aromatic heterocyclyl, which is polycyclic having two or
more rings, include a fused ring group wherein a non-aromatic heterocycle,
which is monocyclic or polycyclic having two or more rings, is fused with a ring
of “aromatic carbocyclyl”, “non-aromatic carbocyclyl” and/or “aromatic
heterocyclyl”.

In addition, examples of the “non-aromatic heterocyclyl” also include a
group having a bridge and a group to form a spiro ring as follows:



The non-aromatic heterocyclyl, which is monocyclic, is preferably a 3- to
8-membered and more preferably 5- to 6- membered ring. Examples include
dioxanyl, thiiranyl, oxiranyl, oxetanyl, oxathiolanyl, azetidiny, thianyl,
thiazolidiny, pyrrolidiny, pyrroliny, imidazolidiny, imidazoliny,
pyrazolidiny, pyrazoliny, piperidyl, piperaziny, morpholiny, morpholino,
thiomorpholiny, thiomorpholino, dihydropyridyl, tetrahydropyridyl,
tetrahydrofuryl, tetrahydropyranyl, dihydrothiazolyl, tetrahydrothiazolyl,
tetrahydroisothiazolyl, dihydrooxaziny, hexahydroazepiny,
tetrahydrodiazepiny, tetrahydropyridaziny, hexahydropyrimidiny,

dioxolanyl, dioxazinyl, aziridinyl, dioxolinyl, oxepanyl, thiolanyl, thiinyl and thiazinyl.

Examples of non-aromatic heterocyclyl, which is polycyclic having two or more rings, include indolinyl, isoindolinyl, chromanyl and isochromanyl.

5 [0043]

“Alkyloxy” means a group wherein “alkyl” binds to an oxygen atom. Examples include methyloxy, ethyloxy, n-propyloxy, isopropyloxy, n-butyloxy, tert-butyloxy, isobutyloxy, sec-butyloxy, pentyloxy, isopentyloxy and hexyloxy.

A preferred embodiment of “alkyloxy” is methyloxy, ethyloxy, n-
10 propyloxy, isopropyloxy or tert-butyloxy.

[0044]

“Haloalkyl” means a group wherein one or more “halogen” binds to “alkyl”. Examples include monofluoromethyl, monofluoroethyl, monofluoropropyl, 2,2,3,3,3-pentafluoropropyl, monochloromethyl,
15 trifluoromethyl, trichloromethyl, 2,2,2-trifluoroethyl, 2,2,2-trichloroethyl, 1,2-dibromoethyl, and 1,1,1-trifluoropropane-2-yl.

A preferred embodiment of “haloalkyl” is trifluoromethyl or trichloromethyl.

[0045]

20 “Alkylthio” means a group wherein “alkyl” binds to sulfur atom.

[0046]

“Alkylamino” includes monoalkylamino and dialkylamino.

“Monoalkylamino” means a group wherein a hydrogen atom attached to a nitrogen atom of an amino group is replaced with “alkyl”. Examples include
25 methylamino, ethylamino and isopropylamino. Preferably, it is methylamino or ethylamino.

“Dialkylamino” means a group wherein two hydrogen atoms attached to a nitrogen atom of an amino group are replaced with two “alkyl”. These two alkyl groups may be the same or different. Examples include dimethylamino, diethylamino, N, N-diisopropylamino, N-methyl-N-ethylamino and N-isopropyl-
30 N-ethylamino. Preferably, it is dimethylamino or diethylamino.

[0047]

“Alkylcarbonylamino” means a group wherein one or two hydrogen atoms attached to a nitrogen atom of an amino group are replaced with one or

two alkylcarbonyl. The two alkylcarbonyl groups may be the same or different. Examples include methylcarbonylamino, ethylcarbonylamino, propylcarbonylamino, isopropylcarbonylamino, tert-butylcarbonylamino, isobutylcarbonylamino, sec-butylcarbonylamino, dimethylcarbonylamino, diethylcarbonylamino and N, N-diisopropylcarbonylamino.

A preferred embodiment of “alkylcarbonylamino” is methylcarbonylamino and ethylcarbonylamino.

[0048]

“Alkenylcarbonylamino” means a group wherein one or two hydrogen atoms attached to a nitrogen atom of an amino group are replaced with one or two alkenylcarbonyl. The two alkenylcarbonyl groups may be the same or different. Examples include vinylcarbonylamino and propenylcarbonylamino.

[0049]

“Alkynylcarbonylamino” means a group wherein one or two hydrogen atoms attached to a nitrogen atom of an amino group are replaced with one or two alkynylcarbonyl. The two alkynylcarbonyl groups may be the same or different. Examples include ethynylcarbonylamino and propynylcarbonylamino.

[0050]

“Alkylcarbamoyl” means a group wherein one or two hydrogen atoms attached to a nitrogen atom of a carbamoyl group are replaced with one or two “alkyl”. These two alkyl groups may be the same or different. Examples include methylcarbamoyl, ethylcarbamoyl, dimethylcarbamoyl and diethylcarbamoyl.

[0051]

“Alkenylcarbamoyl” means a group wherein one or two hydrogen atoms attached to a nitrogen atom of a carbamoyl group are replaced with one or two “alkenyl”. These two alkenyl groups may be the same or different. Examples include vinylcarbamoyl and propenylcarbamoyl.

[0052]

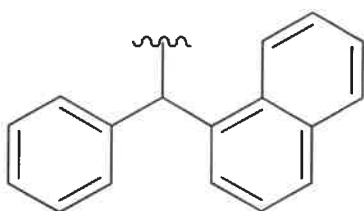
“Alkynylcarbamoyl” means a group wherein one or two hydrogen atoms attached to a nitrogen atom of a carbamoyl group are replaced with one or two “alkynyl”. These two alkynyl groups may be the same or different. Examples include ethynylcarbamoyl and propynylcarbamoyl.

[0053]

Alkyl part of “aromatic carbocyclalkyl”, “non-aromatic carbocyclalkyl”, “aromatic heterocyclalkyl” or “non-aromatic heterocyclalkyl” is the same as “alkyl”.

5 [0054]

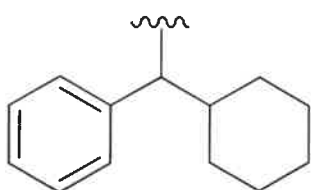
“Aromatic carbocyclalkyl” means an alkyl substituted with one or more “aromatic carbocycl”. Examples include benzyl, phenethyl, phenylpropyl, benzhydryl, trityl, naphthylmethyl and a group of the formula of



10 A preferred embodiment of “aromatic carbocyclalkyl” is benzyl, phenethyl or benzhydryl.

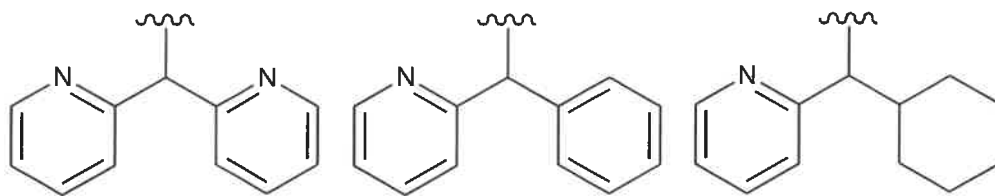
[0055]

“Non-aromatic carbocyclalkyl” means an alkyl substituted with one or more “non-aromatic carbocycl”. The “non-aromatic carbocyclalkyl” also includes “non-aromatic carbocyclalkyl” wherein the alkyl part is substituted with “aromatic carbocycl”. Examples include cyclopropylmethyl, cyclobutylmethyl, cyclopentylmethyl, cyclohexylmethyl and a group of the formula of



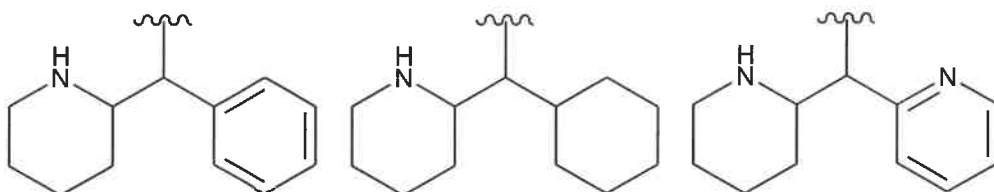
20 [0056]

“Aromatic heterocyclalkyl” means an alkyl substituted with one or more “aromatic heterocycl”. The “aromatic heterocyclalkyl” also includes “aromatic heterocyclalkyl” wherein the alkyl part is substituted with the above “aromatic carbocycl” and/or “non-aromatic carbocycl”. Examples include pyridylmethyl, furanylmethyl, imidazolylmethyl, indolylmethyl, benzothiophenylmethyl, oxazolylmethyl, isoxazolylmethyl, thiazolylmethyl, isothiazolylmethyl, pyrazolylmethyl, isopyrazolylmethyl, pyrrolidinylmethyl, benzoxazolylmethyl and groups of the formula of



[0057]

“Non-aromatic heterocyclalkyl” means an alkyl substituted with one or more “non-aromatic heterocyclalkyl”. The “non-aromatic heterocyclalkyl” also includes “non-aromatic heterocyclalkyl” wherein the alkyl part is substituted with “aromatic carbocycl”, “non-aromatic carbocycl” and/or “aromatic heterocycl”. Examples include tetrahydropyranylmethyl, morpholinylethyl, piperidinylmethyl, piperazinylmethyl and groups of the formula of



[0058]

Examples of the substituents for “substituted or unsubstituted alkyl”, “substituted or unsubstituted alkenyl”, “substituted or unsubstituted alkynyl”, “substituted or unsubstituted alkyloxy”, “substituted or unsubstituted alkylcarbonylamino”, “substituted or unsubstituted alkenylcarbonylamino”, “substituted or unsubstituted alkynylcarbonylamino”, “substituted or unsubstituted alkylcarbamoyl”, “substituted or unsubstituted alkenylcarbamoyl” or “substituted or unsubstituted alkynylcarbamoyl” include the following substituents. A carbon atom at any position(s) may be bonded to one or more group(s) selected from the following substituents.

Substituent: halogen, hydroxy, carboxy, amino, imino, hydroxyamino, hydroxyimino, formyl, formyloxy, carbamoyl, sulfamoyl, sulfanyl, sulfinio, sulfo, thioformyl, thiocarboxy, dithiocarboxy, thiocarbamoyl, cyano, nitro, nitroso, azide, hydrazino, ureide, amidino, guanidino, trialkylsilyl, alkyloxy, alkenyloxy, alkynyloxy, haloalkyloxy, alkylcarbonyl, alkenylcarbonyl, alkynylcarbonyl, monoalkylamino, dialkylamino, alkylsulfonyl, alkenylsulfonyl, alkynylsulfonyl, monoalkylcarbonylamino, dialkylcarbonylamino, monoalkylsulfonylamino, dialkylsulfonylamino, alkylimino, alkenylimino, alkynylimino, alkylcarbonylimino,

alkenylcarbonylimino, alkynylcarbonylimino, alkyloxyimino, alkenyloxyimino, alkynyloxyimino, alkylcarbonyloxy, alkenylcarbonyloxy, alkynylcarbonyloxy, alkyloxycarbonyl, alkenyloxycarbonyl, alkynyloxycarbonyl, alkylsulfanyl, alkenylsulfanyl, alkynylsulfanyl, alkylsulfinyl, alkenylsulfinyl, alkynylsulfinyl, monoalkylcarbamoyl, dialkylcarbamoyl, monoalkylsulfamoyl, dialkylsulfamoyl, aromatic carbocyclyl, non-aromatic carbocyclyl, aromatic heterocyclyl, non-aromatic heterocyclyl, aromatic carbocyclyloxy, non-aromatic carbocyclyloxy, aromatic heterocyclyloxy, non-aromatic heterocyclyloxy, aromatic carbocyclylcarbonyl, non-aromatic carbocyclylcarbonyl, aromatic heterocyclylcarbonyl, non-aromatic heterocyclylcarbonyl, aromatic carbocyclyloxycarbonyl, non-aromatic carbocyclyloxycarbonyl, aromatic heterocyclyloxycarbonyl, non-aromatic heterocyclyloxycarbonyl, aromatic carbocyclylalkyloxy, non-aromatic carbocyclylalkyloxy, aromatic heterocyclylalkyloxy, non-aromatic heterocyclylalkyloxy, aromatic carbocyclylalkyloxycarbonyl, non-aromatic carbocyclylalkyloxycarbonyl, aromatic heterocyclylalkyloxycarbonyl, non-aromatic heterocyclylalkyloxycarbonyl, aromatic carbocyclylalkylamino, non-aromatic carbocyclylalkylamino, aromatic heterocyclylalkylamino, non-aromatic heterocyclylalkylamino, aromatic carbocyclylsulfanyl, non-aromatic carbocyclylsulfanyl, aromatic heterocyclylsulfanyl, non-aromatic heterocyclylsulfanyl, non-aromatic carbocyclylsulfonyl, aromatic carbocyclylsulfonyl, aromatic heterocyclylsulfonyl, and non-aromatic heterocyclylsulfonyl. Furthermore, the substituent may have one or more substituent(s) selected from the above Group a.

[0059]

Examples of the substituents on the ring of “aromatic carbocyclyl”, “non-aromatic carbocyclyl”, “aromatic heterocyclyl” or “non-aromatic heterocyclyl” of “substituted or unsubstituted aromatic carbocyclyl”, “substituted or unsubstituted non-aromatic carbocyclyl”, “substituted or unsubstituted aromatic heterocyclyl”, and “substituted or unsubstituted non-aromatic heterocyclyl” include the following substituents. An atom at any position(s) on the ring may be bonded to one or more group(s) selected from the following substituents.

Substituent: halogen, hydroxy, carboxy, amino, imino, hydroxyamino,

hydroxyimino, formyl, formyloxy, carbamoyl, sulfamoyl, sulfanyl, sulfino, sulfo, thioformyl, thiocarboxy, dithiocarboxy, thiocarbamoyl, cyano, nitro, nitroso, azide, hydrazino, ureide, amidino, guanidino, trialkylsilyl, alkyl, alkenyl, alkynyl, haloalkyl, alkyloxy, alkenyloxy, alkynyloxy, haloalkyloxy,

5 alkyloxyalkyl, alkyloxyalkyloxy, alkylcarbonyl, alkenylcarbonyl, alkynylcarbonyl, monoalkylamino, dialkylamino, alkylsulfonyl, alkenylsulfonyl, alkynylsulfonyl, monoalkylcarbonylamino, dialkylcarbonylamino, monoalkylsulfonylamino, dialkylsulfonylamino, alkylimino, alkenylimino, alkynylimino, alkylcarbonylimino,

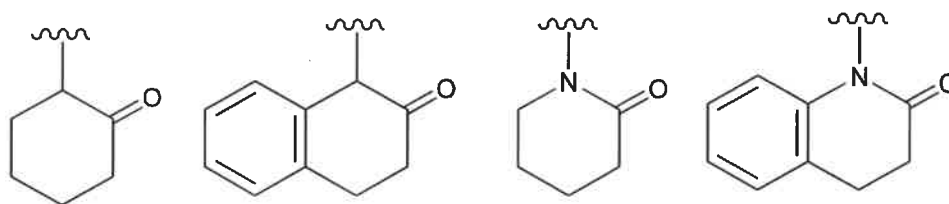
10 alkenylcarbonylimino, alkynylcarbonylimino, alkyloxyimino, alkenyloxyimino, alkynyloxyimino, alkylcarbonyloxy, alkenylcarbonyloxy, alkynylcarbonyloxy, alkyloxycarbonyl, alkenyloxycarbonyl, alkynyloxycarbonyl, alkylsulfanyl, alkenylsulfanyl, alkynylsulfanyl, alkylsulfinyl, alkenylsulfinyl, alkynylsulfinyl, monoalkylcarbamoyl, dialkylcarbamoyl, monoalkylsulfamoyl,

15 dialkylsulfamoyl, aromatic carbocyclyl, non-aromatic carbocyclyl, aromatic heterocyclyl, non-aromatic heterocyclyl, aromatic carbocycliloxy, non-aromatic carbocycliloxy, aromatic heterocycliloxy, non-aromatic heterocycliloxy, aromatic carbocyclylcarbonyl, non-aromatic carbocyclylcarbonyl, aromatic heterocyclylcarbonyl, non-aromatic heterocyclylcarbonyl, aromatic carbocycliloxyoxycarbonyl, non-aromatic carbocycliloxyoxycarbonyl, aromatic heterocycliloxyoxycarbonyl, non-aromatic heterocycliloxyoxycarbonyl, aromatic carbocyclylalkyl, non-aromatic carbocyclylalkyl, aromatic heterocyclylalkyl, non-aromatic heterocyclylalkyl, aromatic carbocyclylalkyloxy, non-aromatic carbocyclylalkyloxy, aromatic heterocyclylalkyloxy, non-aromatic heterocyclylalkyloxy, aromatic carbocyclylalkyloxycarbonyl, non-aromatic carbocyclylalkyloxycarbonyl, aromatic heterocyclylalkyloxycarbonyl, non-aromatic heterocyclylalkyloxycarbonyl, aromatic carbocyclylalkyloxyalkyl, non-aromatic carbocyclylalkyloxyalkyl, aromatic heterocyclylalkyloxyalkyl, non-aromatic heterocyclylalkyloxyalkyl, aromatic carbocyclylalkylamino, non-aromatic carbocyclylalkylamino, aromatic heterocyclylalkylamino, non-aromatic heterocyclylalkylamino, aromatic carbocyclylsulfanyl, non-aromatic carbocyclylsulfanyl, aromatic heterocyclylsulfanyl, non-aromatic heterocyclylsulfanyl, non-aromatic carbocyclylsulfonyl, aromatic carbocyclylsulfonyl, aromatic heterocyclylsulfonyl, and non-aromatic

heterocyclisulfonyl. Furthermore, the substituent may have one or more substituent(s) selected from the above Group a.

[0060]

Additionally, “substituted or unsubstituted non-aromatic carbocyclyl” and “substituted or unsubstituted non-aromatic heterocyclyl” may be substituted with “oxo”. In this case, it means a group wherein two hydrogen atoms on a carbon atom are substituted as below.



[0061]

Here, the present invention is explained in detail.

A lipid binding double-stranded oligonucleotide of the present invention consists of a double-stranded oligonucleotide, wherein a first strand is a CpG oligonucleotide consisting of 8 to 50 nucleotides, a second strand is an oligonucleotide consisting of 8 to 60 nucleotides and comprising a sequence capable of hybridizing with the first strand, and a lipid binds to the second strand through a linker.

More preferably, it is a double-stranded oligonucleotide, wherein a first strand is a CpG oligonucleotide consisting of 8 to 50 nucleotides, a second strand is an oligonucleotide consisting of 8 to 60 nucleotides and comprising a sequence capable of hybridizing with the first strand, but excluding a RNA oligonucleotide, the length of the second strand is 50 % or more of that of the first strand, and a lipid comprising C12 to C30 hydrocarbon chain(s) binds to the second strand through a linker.

[0062]

A first strand of a lipid binding double-stranded oligonucleotide of the present invention is a CpG oligonucleotide consisting of 8 to 50 nucleotides.

[0063]

The “CpG oligonucleotide (CpG ODN)” means a single-stranded oligonucleotide comprising dinucleotide of non-methylated cytosine guanine (5’-

CpG-3') motif (CpG motif) and it is well known that it can be useful as a vaccine adjuvant because it induces acquired immune response through TLR9 (Nat Rev Drug Discov, 2006, 5, 471-484, Expert Rev Vaccines., 2011, 10(4), 499-511). The CpG oligonucleotide used for the present invention comprises at least one CpG motif and may comprise several CpG motifs.

[0064]

The length of the CpG oligonucleotide used for the present invention is 8 to 50 nucleotides. For example, it is 8 to 50 nucleotides, 8 to 40 nucleotides, 8 to 30 nucleotides, 10 to 25 nucleotides, 15 to 25 nucleotides or 18 to 25 nucleotides.

[0065]

As "the CpG oligonucleotide", it is not especially limited to the CpG oligonucleotide, which is well known for the immunostimulatory activity in this field can be used. For example, the CpG oligonucleotide or the method for preparing is described in WO2006/065751, WO2007/092315, WO2008/068638, WO2010/067262, WO2010/125480, WO2014/047588, WO2014/134698, WO2015/041318, US2011/0300163 or the like. These CpG oligonucleotides can be synthesized in reference to methods described in the above documents.

The CpG oligonucleotides are classified based on the sequence, secondary structure and effect on human peripheral blood mononuclear cells (PBMC), into class A, class B, class C, class P or class S (Advanced drug delivery reviews, 2009, 61(3),195-204).

Class A: ODN1585, ODN2216, ODN2336 or the like;

Class B: ODNBW006, ODN D-SL01, ODN1668 (WO2005/063264), OND1826 (WO2007/030580), OND2006 (CpG7909, PF-3512676) (WO98/18810), ODN2007, ODN684 or the like; and

Class C: ODN D-SL03, ODN 2395, ODN M362 or the like. These can be purchased from InvivoGen as a research reagent. In addition,

CpG-28 (WO2000/056342),

CpG-685(GNKG-168) (Blood, 2010, 115(24), 5041),

CpG-ODN C274 (PLoS ONE, 2013, 8(4), e62373),

KSK-13 (KSK-CpG) (US7408050),

CpG ODN 10104 (CpG-10104) (Drug Data Rep, 2006, 28(3), 258),

CpG ODN-1585 (WO2001/022990),

ODN-5890 (WO2006/080946),
1018-ISS (WO2008/073661),
EMD-1201081 (HYB-2055, IMO-2055) (WO2005/009355),
D35-CpG, K3-CpG (GeneDesign, Inc.),

5 or the like. For the present invention, the CpG oligonucleotides of any class can be used. The CpG oligonucleotides of class A (e.g., ODN2216, ODN2336 and D35-CpG), the CpG oligonucleotides of class B (e.g., ODN1826, ODN2006, CpG-28, 1018-ISS, IMO2055, K3-CpG, ODN684 and D-LS01) or the CpG oligonucleotides of class C (e.g., D-LS03, ODN2395 and ODN M362) is
10 preferable. ODN1826 or ODN2006 is especially preferable.

[0066]

A second strand of a lipid binding double-stranded oligonucleotide of the present invention is an oligonucleotide consisting of 8 to 60 nucleotides and comprising a sequence capable of hybridizing with the CpG oligonucleotide
15 which is the first strand, but excluding a RNA oligonucleotide,

[0067]

Preferably, a second strand is an oligonucleotide consisting of 8 to 60 nucleotides and comprising a sequence capable of hybridizing with the CpG oligonucleotide which is a first strand under a stringent condition.

20 Any oligonucleotide can be used for a second strand as long as it can be hybridized with the CpG oligonucleotide under a stringent condition, and the one comprising 1 or several mismatch(es) in a part of hybridization can be exemplified.

For example, it is the oligonucleotide whose part for hybridization has at
25 least 70 % or more, preferably 80 % or more, more preferably 90 % or more, and most preferably 95 % or more homology to a complementary sequence of the CpG oligonucleotide of a first strand.

The homology shows the similarity as a score for example, by BLAST, a search program using algorithm discovered by Altschul et al. (The Journal of
30 Molecular Biology, 215, 403-410 (1990).)

[0068]

The "stringent conditions" mean conditions under which a base sequence forms hybrid (so-called specific hybrid) with a specific sequence but any base sequence without the equivalent function does not form hybrid (so-called non-

specific hybrid) with the specific sequences. People skilled in the art can easily select the conditions by changing a temperature during hybridization reaction or washing, salt concentration in hybridization or washing buffer, or the like. In detail, an example of the stringent conditions of this invention is, but not limited to the condition, which the oligonucleotide is hybridized in 6×SSC (0.9 M NaCl, 0.09 M trisodium citrate) or 6×SSPE (3 M NaCl, 0.2 M NaH₂PO₄, 20 mM EDTA·2Na, pH 7.4) at 42 °C and washed with 0.5×SSC at 42 °C. As a hybridization method, well-known methods in the art, for example, southern blot hybridization or the like can be used. In detail, it can be performed according to a method disclosed in Molecular Cloning: A Laboratory Manual, Second Edition (1989) (Cold Spring Harbor Laboratory Press), Current Protocols in Molecular Biology (1994) (Wiley-Interscience), DNA Cloning 1: Core Techniques, A Practical Approach, Second Edition (1995) (Oxford University Press) or the like.

[0069]

“1 or several mismatch(es)” means 1 to 5, preferably 1 to 3, and more preferably 1 or 2 mismatches.

[0070]

The length of a second strand of a lipid binding double-stranded oligonucleotide of the present invention is 8 to 60 nucleotides. For example, it is 8 to 60 nucleotides, 8 to 50 nucleotides, 8 to 40 nucleotides, 8 to 30 nucleotides, 10 to 25 nucleotides or 15 to 25 nucleotides. The length of the second strand can be same with that of the CpG oligonucleotide which is a first strand, and 1 or several nucleotide(s) shorter than that of the CpG oligonucleotide as long as it can be hybridized with the CpG oligonucleotide. Furthermore, the length of a second strand can be longer than that of the CpG oligonucleotide by adding 1 or several nucleotide(s) at one or both sides of a part hybridizing with the CpG oligonucleotide.

“1 or several nucleotide(s)” means 1 to 10, 1 to 5, 1 to 3, or 1 or 2 nucleotide(s).

A preferable length of a second strand depends on the length of the CpG oligonucleotide of a first strand. For example, it is a length of 50 % or more, 60 % or more, 70 % or more, 50 to 100 %, 60 to 100 % or 70 to 100 % of the length of a first strand. 50 to 100% of the length of a first strand is especially

preferable.

[0071]

The oligonucleotide of a second strand of a lipid binding double-stranded oligonucleotide of the present invention is an oligonucleotide which nucleosides
5 selected from the group consisting of DNA nucleosides, RNA nucleosides and nucleoside derivatives are bounded. All nucleosides can be same, or two or more kinds of nucleoside. However, an RNA oligonucleotide which all nucleosides are a RNA nucleoside is excluded. As a nucleoside comprised in a second strand, an oligonucleotide which DNA nucleosides and/or nucleoside
10 derivatives are bounded is preferable. All nucleosides can be a DNA nucleoside, nucleoside derivative, or both of them.

When an oligonucleotide comprises DNA nucleosides and nucleoside derivatives, example is an oligonucleotide comprising a center region and end regions at the both sides of the center region and comprising at least one
15 nucleoside derivative in the end region at the both sides. In detail, 5' and/or 3' end region(s) comprises 1 or more, preferably 1 to 5, and more preferably 2 to 3 nucleoside derivatives. The kind, number and position of modification(s) in one end region may be same or different from those in the other end region. In the other embodiment, it is an oligonucleotide randomly comprising
20 nucleoside derivatives.

[0072]

As a nucleoside derivative in a second strand of a lipid binding double-stranded oligonucleotide of the present invention, any modification(s) for a nucleoside which is well-known in this field such as the above examples can be
25 used.

A nucleoside having a substituent at the 2' position of sugar and/or a nucleoside having a bridge structure between at the 4' and 2' positions of sugar are preferable.

As a substituent at the 2' position of a sugar, F, OCH₃ or
30 OCH₂CH₂OCH₃ is preferable. OCH₃ is especially preferable.

As a bridge structure between the 4' and 2' positions of a sugar, 4'-(CH₂)_m-O-2', wherein m is an integer of 1 to 4, or 4'-C(=O)-NR³-2', wherein R³ is hydrogen atom or alkyl, is preferable.

[0073]

As an internucleoside linkage in the oligonucleotide of a second strand of a lipid binding double-stranded oligonucleotide of the present invention, any well-known internucleoside linkage such as the above examples in this field can be used. All internucleoside linkages can be same, or two or more kinds of linkage. D-oligo and/or S-oligo is preferable.

When 2 or more kinds of internucleoside linkages are comprised such as D-oligo and S-oligo, example is an oligonucleotide comprising a center region and end regions at the both sides of the center region, and comprising at least one unnatural internucleoside linkage (e.g., S-oligo) in the end region at the both sides and natural internucleoside linkage (e.g., D-oligo) in the center region. For example, 5' and/or 3' end region(s) comprises 1 or more, preferably 1 to 5, and more preferably 2 to 3 unnatural internucleoside linkages. The kind, number and position of modification(s) in one end region may be same or different from those in the other end region. In the other embodiment, it is an oligonucleotide randomly comprising unnatural internucleoside linkages.

[0074]

The CpG oligonucleotide of a first strand and oligonucleotide of a second strand in a lipid binding double-stranded oligonucleotide of the present invention can be synthesized according to the usual methods in this field. For example, they can be easily synthesized by an automated nucleic acid synthesizer which is commercially available (e.g., the synthesizer by AppliedBiosystems, Dainippon Seiki). A method for synthesizing is solid-phase synthesis using phosphoramidite, solid-phase synthesis using hydrogen phosphonate or the like. Examples are disclosed in the following Example 1, Tetrahedron Letters 22, 1859-1862 (1981) and the like.

[0075]

The synthesized first and second strands form a double-stranded oligonucleotide by hybridizing according to the well-known method. Examples are disclosed in the following Example 1, Example 1 in WO2013/089283 and the like.

[0076]

In the lipid binding double-stranded oligonucleotide of the present invention, a lipid binds to the second strand through a linker.

[0077]

“Lipid” means a hydrophobic compound. It is not limited as long as it is a well-known lipid, and may be a straight, branched or cyclic form. Examples are fatty acid with a C8 to C30 aliphatic chain(s) (e.g., farnesol), diacyl lipid, cholesterol, cholesterol derivatives, steroid acid (e.g., bile acid), lipid A, tocopherol and combination thereof. Examples of fatty acid with an aliphatic chain(s) are, but not limited to, unsaturated or saturated fatty acid in a straight or branched form, and fatty acid derivatives (e.g., fatty acid esters, fatty acid amides and fatty acid thioesters).

[0078]

As a lipid used for the present invention, a lipid comprising a hydrocarbon chain(s) is preferable. Preferably, it is a lipid comprising 1 or 2 hydrocarbon chain(s) or the like. Especially preferably, a lipid binding double-stranded oligonucleotide of the present invention has a diacyl lipid. When lipids bind at two or more positions and one lipid is a diacyl lipid, the other lipid can be a lipid except for a diacyl lipid.

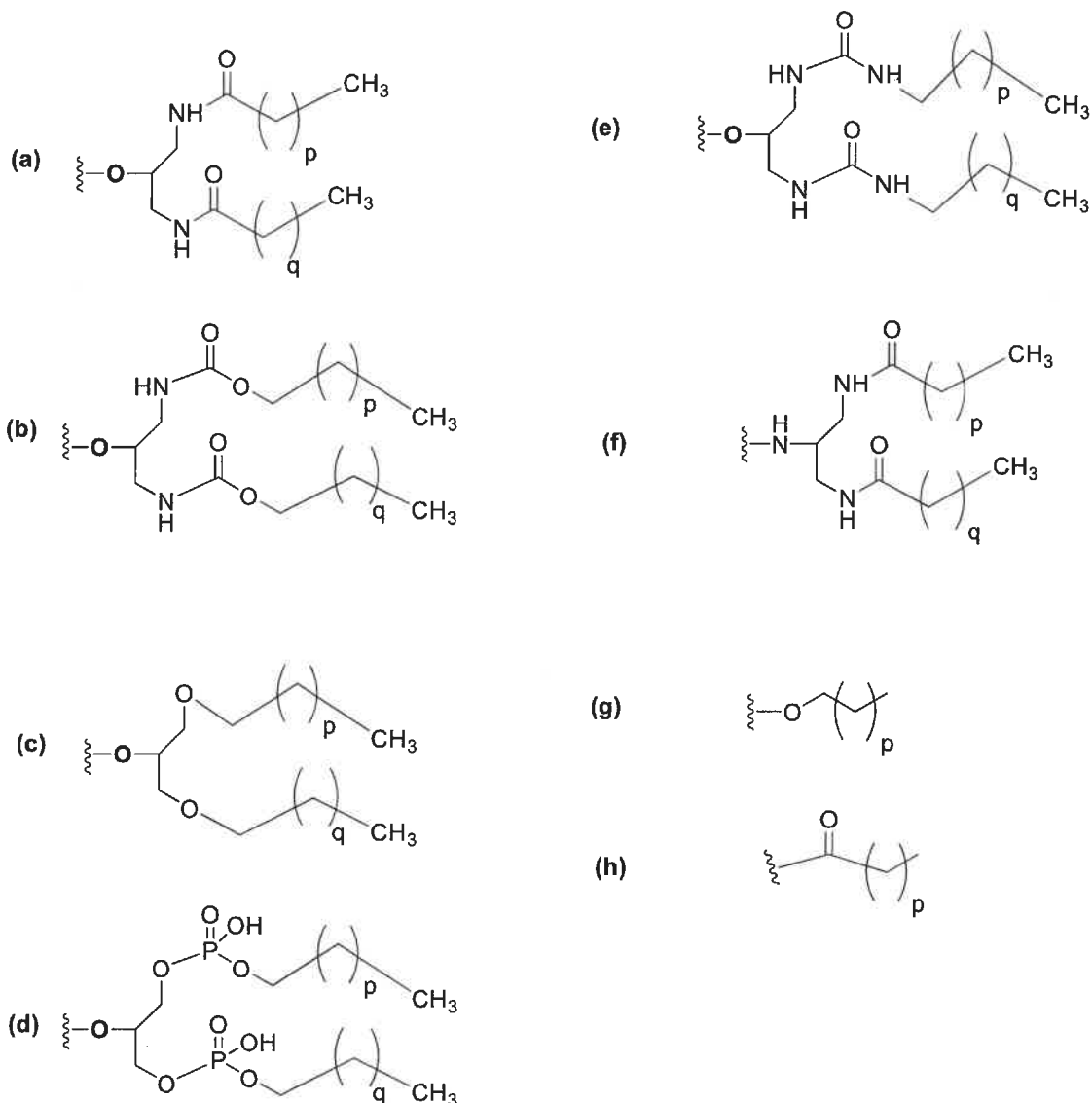
“Diacyl lipid” is phospholipid, glycolipid, sphingolipid or combination thereof, which comprising two hydrocarbon chains. Preferably, it is the group of the following (a) or (f).

Hydrocarbon chains in the lipid independently comprise about C8 to C30 carbon atoms. They can be saturated, unsaturated, or combination thereof, and branched. When the lipid has one chain, the length of the chain is preferably C8 to C30, and more preferably C8 to C20. When the lipid has two chains, the length of the chains is preferably C10 to C30, more preferably C12 to C30, and most preferably C14 to C24. When the lipid has two chains, the length of two chains may be same or different. Chains in a lipid bind a part comprising phosphoric acid, sugar or the like (a position binding with an oligonucleotide) through ester bond, amide bond, thioester bond, combination thereof or the like.

A lipid binding double-stranded oligonucleotide of the present invention has a lipid comprising a C12 to C30 hydrocarbon chain(s) in a second strand. When lipids bind at 2 or more positions and one lipid is a lipid comprising a C12 to C30 hydrocarbon chain(s), the number of carbon atoms of a hydrocarbon chain(s) in the other lipid can be less than 12.

[0079]

Examples of “lipid” are the followings.



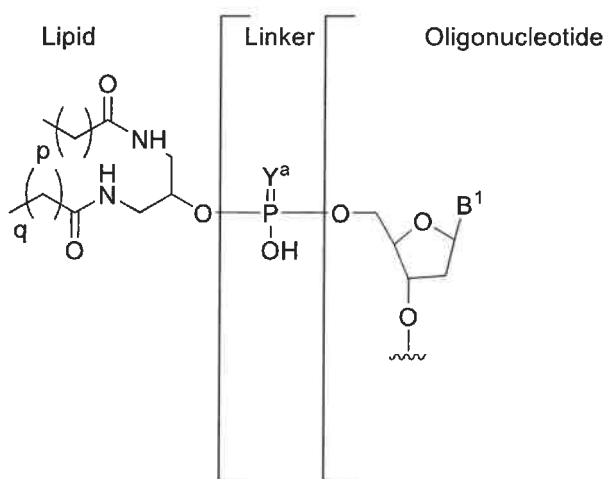
5 wherein p and q are each independently an integer of 6 to 28, preferably 8 to 28, more preferably 10 to 28, and most preferably 10 to 18.

[0080]

In a lipid binding double-stranded oligonucleotide of the present invention, a lipid can bind at any position of the second strand. It can bind at the 3' end, the 5' end or in the second strand.

[0081]

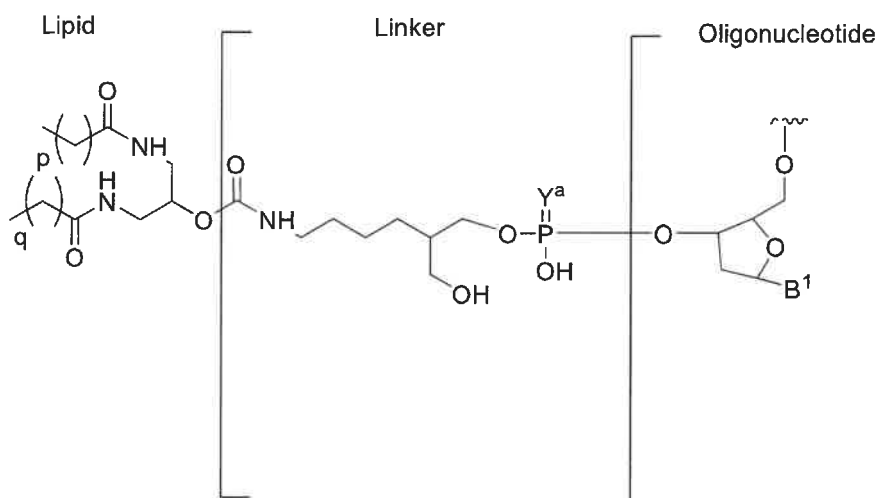
When the lipid binds at the 5' end, for example, it can bind as below.



wherein B^1 is a base at the 3' end of the second strand, Y^a is O or S, and p or q is each independently an integer of 6 to 28, and preferably 10 to 18.

5 [0082]

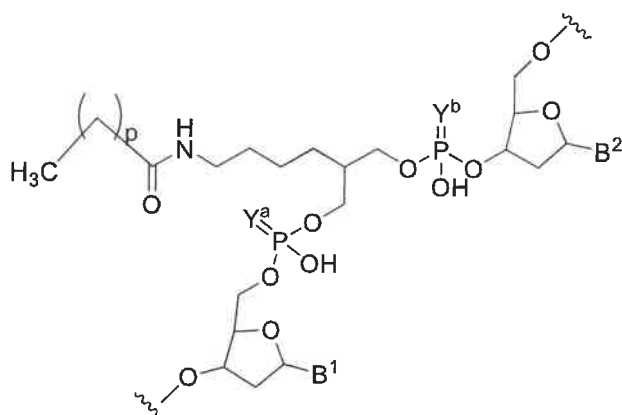
When the lipid binds at the 3' end, for example, it can bind as below.



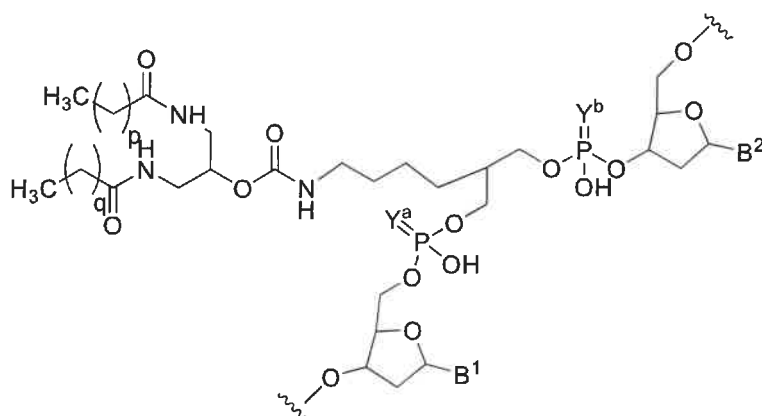
wherein B^1 is a base at the 5' end of the second strand, Y^a is O or S, and p or q is each independently an integer of 6 to 28, and preferably 10 to 18.

10 [0083]

When the lipid binds in a second strand, for example, it can bind as below.



or



5

wherein B¹ and B² are the neighboring bases in the second strand, Y^a or Y^b is each independently O or S, and p and q is each independently an integer of 6 to 28, and preferably 10 to 18.

Furthermore, the lipid preferably binds at one or two position(s) in the second strand. It preferably binds at 3' and/or 5' end. It more preferably binds at 5' end.

[0084]

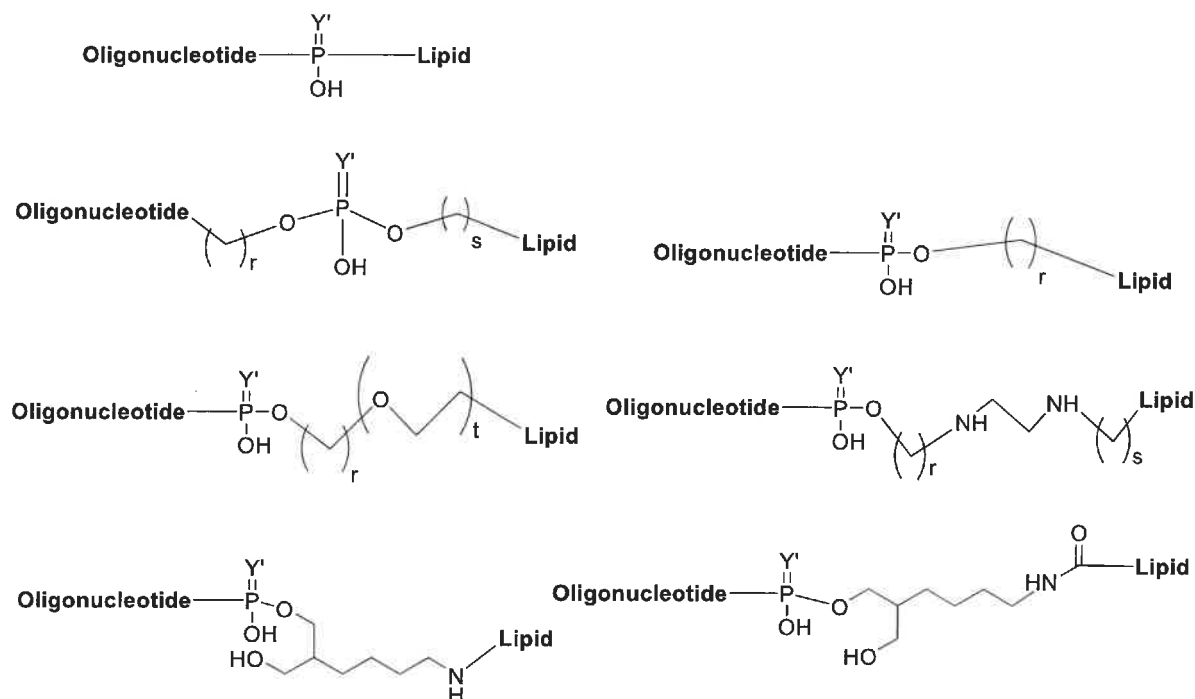
The lipid can be synthesized in reference to well-known methods in this field. Examples of the lipid or the preparation methods are disclosed in the following Example 1, Patent Document 1 and the like.

[0085]

In a lipid binding double-stranded oligonucleotide of the present invention, the lipid binds to a second strand through a linker. As a "linker", any linker used in this field can be used. Examples are polar linker, alkylene linker, ethylene glycol linker and ethylenediamine linker. When a lipid is a

phospholipid, it is a linker which is 4 to 26 atoms between oxygen atom of a second strand and phosphorus atom of a lipid. Examples are an oligonucleotide linker or the following linkers.

5 [0086]



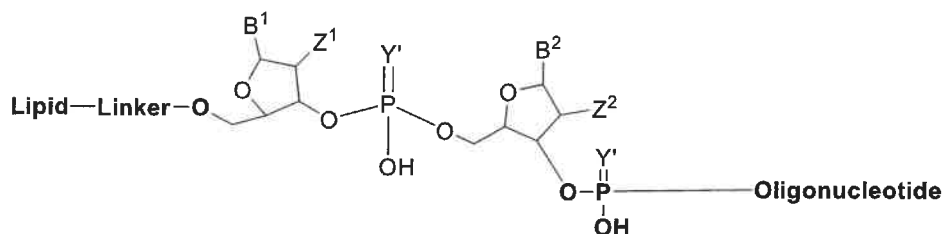
wherein Y' is O or S, and r or s is each independently an integer of 1 to 10, preferably 1 to 5, and more preferably 1 to 3. t is an integer of 1 to 4, preferably 1 to 3, and more preferably 2 or 3.

The linker can be synthesized in reference to the well-known method in this field. The oligonucleotide linker can be synthesized by the method similar to the method for synthesizing the oligonucleotides in the above example.

15 [0087]

The linker is preferably an oligonucleotide linker. The length of an oligonucleotide linker is 2 to 10, 2 to 5, 2, 3, 4 or 5 nucleotides. An example is the following.

[0088]



wherein B¹ or B² is adenine(A), guanine(G), cytosine (C), 5-methylcytosine (5-Me-C), thymine (T) or uracil(U). Y' is each independently O or S. Z¹ or Z² is each independently H or OH, and preferably H.

An example of an oligonucleotide linker is a DNA linker, that is, - (dX¹)_u-, wherein X¹ is each independently A, G, C or T, and u is an integer of 1 to 8. In detail, it is dG, dGdG, dGdGdGdG, dGdGdGdGdG, dT, dTdT, dTdTdTdT, dTdTdTdTdT or the like. dGdG, dGdGdGdGdG or dTdT is especially preferable. The internucleoside linkage in the DNA linker is preferably phosphorothioate linkage.

[0089]

3' end, 5' end or linker without binding lipid of a lipid binding double-stranded oligonucleotide of the present invention can be further modified. To be capable of tracking of the oligonucleotide, to improve pharmacokinetics or pharmacodynamics of the oligonucleotide, or to enhance the stability or binding affinity of the oligonucleotide, the well-known modified group in this field can be used. Examples are a protecting group of a hydroxyl group, reporter molecule, cholesterol, phospholipid, pigment, fluorescent molecule and the like.

Furthermore, 3' or 5' end without binding the lipid of a lipid binding double-stranded oligonucleotide of the present invention may comprise a phosphate ester moiety. The "phosphate ester moiety" means a phosphate group at the end comprising phosphate ester or modified phosphate ester. Although the phosphate ester moiety may be at the either end, it is preferably 5' end nucleoside. In detail, it is a group of the formula: -O-P(=O)(OH)OH or the modified group. That is, one or more of O or OH is optionally substituted with H, O, OR', S, N(R'), wherein R' is H, amino-protecting group, or substituted or unsubstituted alkyl, or alkyl. 5' or 3' end may each

independently comprise substituted or unsubstituted 1 to 3 phosphate ester moiety.

[0090]

The present invention encompasses a pharmaceutical composition (a
5 pharmaceutical composition of the present invention) or a vaccine composition
(a vaccine composition of the present invention) comprising a lipid binding
double-stranded oligonucleotide of the present invention (a vaccine or adjuvant
of the present invention of the present invention).

In addition, the present invention encompasses a pharmaceutical or
10 vaccine composition comprising an antigen and a lipid binding double-stranded
oligonucleotide of the present invention (an adjuvant of the present invention).

[0091]

An "antigen" is a molecule that is capable of inducing an immune
response. Examples include, but are not limited to, cells, cell extracts,
15 proteins, recombinant proteins, purified proteins, polypeptides, peptides,
polysaccharides, polysaccharide conjugates, peptide or non-peptide mimics of
polysaccharides, other molecules encoded by plasmid DNA, haptens, small
molecules, lipids, glycolipids, carbohydrates, whole killed pathogens, viruses,
viral extracts, live attenuated virus, viral vector, live attenuated bacteria,
20 bacterial vectors, multicellular organisms such as parasites and allergens.

The antigen may be provided as a single antigen or combination of
antigens. The antigen may be provided as a complex mixture of polypeptide
or oligonucleotide.

Antigens include, but are not limited to, microbial antigens, self-
25 antigens and addictive substances.

[0092]

A "microbial antigen" means an antigen of a microorganism, and the
microorganism includes, but is not limited to, a bacterium, virus, parasite and
fungus.

30 A "bacterium" is not especially limited as long as it is a bacterium which
causes a disease in human, pet, livestock or the like. In detail, it is
Streptococcus (Streptococcus pyogens, Streptococcus pneumoniae or the like),
Staphylococcus aureus (MSSA, MRSA or the like), Staphylococcus epidermidis,
Enterococcus, bacteria belonging to the genus Listeria, bacteria causing

meningitis, Gonococcus, pathogenic *Escherichia coli*, Friedländer bacilli, *Proteus* bacilli, *Bordetella pertussis*, *Pseudomonas aeruginosa*, *Serratia marcescens*, *Citrobacter*, *Acinetobacter*, *Enterobacter*, *Mycoplasma*, Clostridia, Tubercle bacilli, Cholera bacilli, *Yersinia pestis*, *Corynebacterium diphtheriae*,
5 Dysentery bacillus, *Bacillus anthracis*, *Treponema pallidum*, Tetanus bacillus, *Mycobacterium leprae*, *Legionella pneumophila*, *Leptospira*, *Borrelia*, *Francisella*, *Coxiella*, *Rickettsia*, *Chlamydia*, *Burkholderia mallei*, *Helicobacter pylori* or the like.

A “virus” is not especially limited as long as it is a virus which causes a
10 disease in human, pet, livestock or the like. Examples are influenza virus, respiratory syncytial virus (RSV), papilloma virus, hepatitis virus (type A, B, C, D, E, F, G, TT or the like), rhinovirus, variola virus, morbillivirus, rubella virus, poliovirus, varicella zoster virus, norovirus, Norwalk virus, sapovirus, Sapporo virus, mumps virus, adenovirus, enterovirus, rotavirus, human
15 immunodeficiency virus such as HIV-1 and HIV-2, rabies virus, T-lymphotropic virus, yellow fever virus, cytomegalovirus, SARS-CoV, coronavirus such as MERS-CoV, ebola virus, polyomavirus, JC virus, BK virus, herpes virus such as herpes simplex virus 1 (HSV1) and herpes simplex virus 2 (HSV2), lymphocryptovirus, roseolovirus, Japanese encephalitis virus, Coxsackie virus,
20 dengue virus, West Nile virus, coronavirus, parvovirus, Epstein-Barr virus, Marburg virus, hantavirus, Lassa virus, Chikungunya virus, Hantaan virus, louping ill virus, lymphocytic choriomeningitis virus, bornavirus, Rift Valley fever, Thogoto virus, Dhori virus, foot-and-mouth disease virus, Newcastle disease virus, bovine papular stomatitis virus, rinderpest virus, swine
25 vesicular disease virus, calicivirus, torovirus, African horse sickness virus, arterivirus, sheep pox virus, capripoxvirus, sheep-associated malignant catarrhal fever virus, viral hemorrhagic septicemia virus and vesicular stomatitis virus.

A “parasite” is not especially limited as long as it is a parasite which
30 causes a disease in human, pet, livestock or the like. Examples are *entamoeba histolytica*, malaria, toxoplasma, leishmania, cryptosporidium, trypanosoma, echinococcus, schistosoma japonicum, filaria, roundworm and diphyllbothrium.

A “Fungus” is not especially limited as long as it is a fungus which

causes a disease in human, pet, livestock or the like. In detail, it is aspergilloma, candida, cryptococcus, trichophyton, histoplasma, pneumocystis or the like.

[0093]

5 A “self-antigen” means a tumor antigen, an antigen associated with Alzheimer’s disease, an antigen against a human antibody, an antigen expressed from human endogenous retroviral elements or the like.

An example of a “tumor antigen” is an antigen specifically expressed in cancer cells. Examples include protein, peptide and fusion peptide comprising
10 them. Examples are peptides described in WO2006/090810, WO2007/145318, WO2008/047473, WO2008/102557, WO2009/025117, WO2009/025196, WO2009/1539992, WO2010/013485, WO2010/021112, WO2010/073551, WO2010/095428, WO2010/131452, WO2010/137295, WO2011/067920, WO2011/074236, WO2011/089921, WO2011/111392, WO2012/053200,
15 WO2012/053206, WO2012/169200, WO2013/024582, WO2013/061594, WO2014/041784, WO2014/087626 and the like.

An example of an antigen associated with Alzheimer's disease is tau, β -amyloid or the like.

An example of an antigen against a human antibody is IgE.

20 [0094]

An “addictive substance” means nicotine, cocaine or the like. An example of a nicotine antigen is a nicotine hapten conjugated to a carrier (e.g., diphtheria toxin).

[0095]

25 A pharmaceutical or vaccine composition of the present invention can further comprise a well-known adjuvant(s) as long as the effect of the lipid binding double-stranded oligonucleotide of the present invention is maintained. For example, it is cholera toxin, salmonella toxin, alum or an agonist for a Toll-like receptor (TLR) that is not TLR9. Examples of agonists
30 for TLR are an agonist for TLR3 such as stabilized poly(I:C); an agonist for TLR4 such as a derivative of lipopolysaccharide (LPS) (e.g., MPL and GLA); an agonist for TLR5 such as flagellin; an agonist for TLR7; and an agonist for TLR8. Examples include aluminum salt such as aluminum hydroxide, an immune stimulatory complex (ISCOM), oil-in-water or water-in-oil emulsion,

liposome and a delivery system such as nanoparticle and microparticle.

[0096]

As the following examples, a pharmaceutical or vaccine composition of the present invention comprising a tumor antigen has any or all of the following excellent characteristics:

- a) 1 % or more CTL inductivity.
- b) Inhibition on tumor implantation.
- c) Effect of tumor regression
- d) Good pharmacokinetics such as a high bioavailability, moderate clearance and the like. Especially, efficient delivery to lymph-nodes.
- e) High metabolic stability.
- f) No cytokine release syndrome.
- g) Weak local irritation.
- h) No mutagenicity.
- i) Low cardiovascular risk.
- j) Low acute toxicity risk.

[0097]

Any administration method and formulation for the pharmaceutical or vaccine composition of the present invention can be used if it is a well-known administration method and formulation in this field.

[0098]

A pharmaceutical or vaccine composition of the present invention may be administered in a number of ways depending upon whether local or systemic treatment is desired and upon the area to be treated. Examples of an administration method include topical (including ophthalmic, intravaginal, intrarectal, intranasal and transdermal), oral and parenteral. Examples of parenteral administration include intravenous injection or drip, subdermal, intraperitoneal or intramuscular injection, lung administration by aspiration or inhalation, intrathecal administration and intraventricular administration. Intravenous injection or subcutaneous administration is preferable.

[0099]

When the pharmaceutical or vaccine composition of the present invention is topically administered, a formulation such as a transdermal patch, ointment, lotion, cream, gel, drop, suppository, spray, liquid and powder can be

used.

Examples of the composition for oral administration include powder, granule, suspension or solution dissolved in water or non-aqueous vehicle, capsule, powder and tablet.

5 Examples of the composition for parenteral, intrathecal or intraventricular administration include sterile aqueous solutions which contain buffers, diluents and other suitable additives.

[0100]

10 A pharmaceutical or vaccine composition of the present invention can be obtained by mixing an effective amount with various pharmaceutical additives suitable for the administration form, such as excipients, binders, moistening agents, disintegrants, lubricants and diluents as needed. When the composition is an injection, it together with a suitable carrier can be sterilized to obtain a composition.

15 [0101]

Examples of the excipients include lactose, saccharose, glucose, starch, calcium carbonate and crystalline cellulose.

20 Examples of the binders include methylcellulose, carboxymethylcellulose, hydroxypropylcellulose, gelatin and polyvinylpyrrolidone.

Examples of the disintegrants include carboxymethylcellulose, sodium carboxymethylcellulose, starch, sodium alginate, agar and sodium lauryl sulfate.

25 Examples of the lubricants include talc, magnesium stearate and macrogol. Cacao oil, macrogol, methylcellulose or the like may be used as base materials of suppositories.

30 When the composition is prepared as solutions, emulsified injections or suspended injections, solubilizing agents, suspending agents, emulsifiers, stabilizers, preservatives, isotonic agents and the like which are usually used may be added as needed. For oral administration, sweetening agents, flavors or the like may be added.

[0102]

Dosing is dependent on severity and responsiveness of the disease state to be treated, with the course of treatment lasting from several days to several

months, or until a cure is effected or a diminution of the disease state is achieved. Optimal dosing schedules can be calculated from measurements of a pharmaceutical or vaccine composition accumulation in the body. Persons of ordinary skill in the art can determine optimal dosages, dosing methodologies and repetition rates.

[0103]

The content rate of a lipid binding double-stranded oligonucleotide of the present invention of a pharmaceutical or vaccine composition of the present invention is, but not especially limited to, usually about 0.01 to 99.99 wt% against 100 wt% of a pharmaceutical or vaccine composition.

When an antigen was comprised, considering the content rate of the antigen and an adjuvant of the present invention, the adjuvant is usually about 10 to 1000 parts by weight against 1 part by weight of the antigen.

The dosage of a pharmaceutical or vaccine composition is diversified depending on the desired degree of immune stimulation, age of the subject of administration, sex or the like, so it can be appropriately set, e.g., 0.001 to 10 mg/kg weight/day, to which it is not especially limited.

In addition, according to the degree of effectiveness of the desired pharmaceutical or vaccine composition, age of the subject of administration, sex or the like, a pharmaceutical or vaccine composition of the present invention can be administered to the same subject at several times, e.g., up to about 2 to 4 times. When a pharmaceutical or vaccine composition of the present invention is administered to the same subject at several times, the interval can be appropriately set, e.g., about 14 to 30 days.

[0104]

A pharmaceutical or vaccine composition of the present invention can be administered in combination of 1 or more kind(s) of medicines. The therapeutic agent known as a therapeutic agent for the target disease in this field can be used as the medicines. For example, when the disease is cancer, a pharmaceutical or vaccine composition of the present invention can be co-administered with a chemotherapeutic agent(s). When the disease is bacterial infections, a pharmaceutical or vaccine composition of the present invention can be co-administered with an antibiotic(s). A pharmaceutical or vaccine composition of the present invention may be simultaneously or

separately administered with the agent(s). In addition, a pharmaceutical or vaccine composition of the present invention and the therapeutic agent(s) may be administered as the different formulations independently comprising each one or a single formulation comprising the pharmaceutical or vaccine composition and the therapeutic agent(s). Furthermore, when the disease is cancer, a pharmaceutical or vaccine composition of the present invention can be administered in combination of the surgical treatment which is known in this field.

[0105]

Moreover, the present invention encompasses a method for increasing an immune response in a subject, comprising administering to the subject an effective amount of a pharmaceutical or vaccine composition of the present invention. An example of "immune response" is an increase in inductivity of specific cytotoxic T-lymphocyte (CTL) compared to a control. A specific method for measuring CTL inductivity is disclosed in, e.g., the following Example 3 and WO2013/024582.

[0106]

Furthermore, the present invention encompassed a method for treating a cancer or infectious disease, comprising administering to a subject an effective amount of a pharmaceutical or vaccine composition of the present invention to reduce one or more symptoms of the cancer or infectious disease compared to a control.

[0107]

"The subject" means any individual which is a target for a treatment with a pharmaceutical or vaccine composition of the present invention. Examples include mammals such as humans, laboratory animals (e.g., mice and rats), pets (e.g., dogs, cats, ferrets and birds) and livestock (e.g., cattle, pigs, chickens, goats, ostriches, sheep and horses).

[0108]

"An effective amount" means enough dosage to induce or enhance the immune response, provide the desired pharmacological and/or physiological effect, or provide a treatment for the disorder, disease or status. The exact dosage may be changeable depending on various factors such as the subject-dependent variable (e.g., age and health condition of immune system), disease,

stage of disease and the provided treatment.

[0109]

“Cancer” is not especially limited as long as it is a cancer which a human, pet, livestock or the like may contract. Examples are chronic myeloid leukemia (CML), acute myeloid leukemia (AML), lymphoma, Hodgkin's disease, Non-Hodgkin lymphoma, multiple myeloma, brain tumor, breast cancer, endometrial carcinoma, cervical cancer, ovarian cancer, esophageal cancer, stomach cancer such as diffuse gastric cancer, appendiceal cancer, colorectal cancer, liver cancer, hepatocellular carcinoma, gallbladder cancer, cholangiocarcinoma, pancreatic cancer, adrenal cancer, gastrointestinal stromal tumor, mesothelioma, head and neck carcinoma, laryngeal cancer, oral cancer, gingival cancer, tongue cancer, buccal mucosa cancer, salivary gland cancer, paranasal cancer, carcinoma of maxillary sinus, carcinoma of the frontal sinus, ethmoid sinus cancer, sphenoid sinus cancer, thyroid cancer, renal cancer, lung cancer such as Non-Small Cell Lung Cancer (NSCLC) and small cell lung cancer (SCLC), osteosarcoma, prostate cancer, testicular cancer, bladder cancer, rhabdomyosarcoma, skin cancer, anal carcinoma, chondrosarcoma, synovial sarcoma, endometriosis, soft tissue tumor and osteoblastoma.

[0110]

An “infectious disease” means acute or chronic infectious disease, especially viral infectious disease. Examples include, but not limited to, local or systemic viral infections such as immunodeficiency by HIV or the like, papilloma by HPV or the like, herpes by HSV or the like, encephalitis, influenza by human influenza virus A or the like and cold by human rhinovirus.

[0111]

In this description, meaning of each abbreviation is as follows:

Ac: acetyl

CPG: Controlled Pore Glass

DIEA: N,N-diisopropylethylamine

DMAP: 4-dimethylaminopyridine

DMTr: dimethoxytrityl

DMT-MM: 4-(4,6-dimethoxy-1,3,5-triazine-2-yl)-4-methylmorpholinium chloride

DMF: N,N'-dimethylformamide

Et: ethyl

Fmoc: 9-fluorenylmethyloxycarbonyl

HBTU: O-benzotriazole-N,N,N',N'-tetramethyluronium-hexafluoro-phosphate

5 Me: methyl

MMTr: 4-methoxyphenyldiphenylmethyl

MMTrCl: 4-methoxytrityl chloride

PBS: phosphate buffered saline

TBS: tert-butyldimethylsilyl

10 TBAF: tetrabutylammonium fluoride

TFA: trifluoroacetic acid

THF: tetrahydrofuran

Examples

15 [0112]

The present invention will be described in more detail with reference to, but not limited to, the following Examples, Reference Examples and Test Examples.

NMR analysis of each compound obtained in Examples was performed by
20 300 MHz or 400 MHz using CD₃OD, CDCl₃ or DMSO-d₆.

[0113]

UPLC analysis was performed under the following conditions.

Mobile phases: [A] is 0.1 % aqueous formic solution, [B] is acetonitrile solution containing 0.1 % aqueous formic acid

25 Gradient: linear gradient of 5 % to 100 % solvent [B] for 3.5 minutes was performed, and 100 % solvent [B] was maintained for 0.5 minute.

Column: ACQUITY UPLC (Registered trademark) BEH C18 (1.7 μm, i.d. 2.1 x 50 mm) (Waters)

Flow rate: 0.8 mL/min

30 PDA detection wavelength: 254 nm (Detection range 210-500 nm)

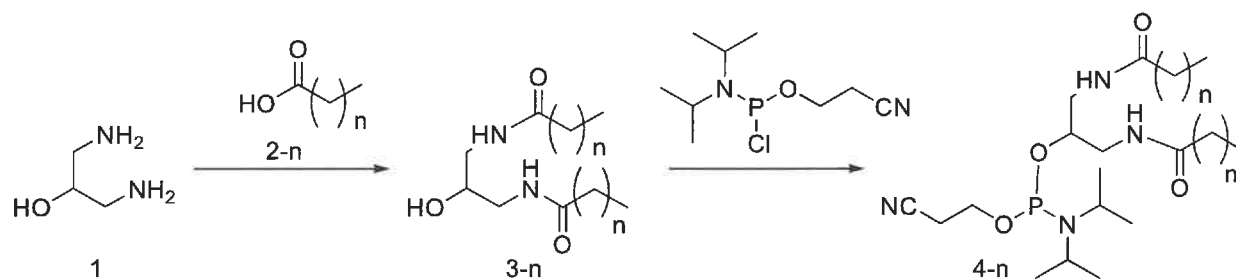
[0114]

Example 1 Synthesis of a lipid binding double-stranded oligonucleotide of the present invention

A) Synthesis of lipids

[0115]

1-1) Synthesis of 4-n



5 wherein n is an integer of 6 to 28.

[0116]

1-1-1) Synthesis of Compound 3-6

Step 1

10 Compound 2-6 (3.20 g, 22.2 mmol, Tokyo Chemical Industry Co., Ltd.) was dissolved in THF-DMF (5:1, 60 mL), and DIEA (4.84 mL, 27.7 mmol) and HBTU (8.84 g, 23.3 mmol) were added thereto. The mixture was stirred at room temperature for 30 minutes. Compound 1 (2.2 g, 24.41 mmol) in DMF solution (5 mL) was added dropwise over 10 minutes and stirred for 3 hours.

15 The amount of the reaction mixture was concentrated to the half under reduced pressure, and it was added dropwise to water (100 mL). The mixture was stirred for 10 minutes, and the resulting solid was collected by filtration. The solid was washed with water (50 mL) and acetonitrile (150 mL) to obtain Compound 3-6 (3.2 g, 9.34 mmol) as white solid.

20 ¹H-NMR (CDCl₃) δ: 6.45 (2H, t, J = 6.0 Hz), 4.39 (1H, s), 3.78-3.73 (1H, m), 3.33 (4H, t, J = 5.6 Hz), 2.22 (4H, t, J = 7.7 Hz), 1.67 (4H, dt, J = 31.0, 14.1 Hz), 1.30-1.27 (16H, m), 0.88 (6H, t, J = 6.8 Hz).

[0117]

1-1-2) Synthesis of Compound 4-8

Step 1

Compound 2-8 (7.65 g, 44.40 mmol, Wako Pure Chemical Industries, Ltd.) was dissolved in DMF (50.0 mL) and dichloromethane (100.0 mL). DIEA (8.72 mL, 66.6 mmol) and HBTU (18.52 g, 48.8 mmol) were added thereto, and the mixture was vigorously stirred at room temperature for 30 minutes. To

the resulting suspended solution, Compound 1 (2.0 g, 22.2 mmol) was added at room temperature, and the mixture was vigorously stirred for 3 hours. To the reaction mixture was added aqueous saturated sodium bicarbonate solution (20 mL), the resulting white solid was collected by filtration. The resulting solid
5 was washed with water (100 mL) and acetonitrile (100 mL) and dried to obtain Compound 3-8 (6.6 g, 16.6 mmol) as white solid.

$^1\text{H-NMR}(\text{CDCl}_3)\delta$: 6.29 (brs, 2H), 4.12 (s, 1H), 3.76 (dd, 1H, $J = 4.5, 4.5$ Hz), 3.42-3.35 (m, 2H), 3.31-3.25 (m, 2H), 2.22 (t, 4H, $J = 7.5$ Hz), 1.65-1.60 (m, 4H), 1.29-1.26 (m, 24H), 0.88 (t, 6H, $J = 6.5$ Hz).

10 ESI-MS(m/z): 340 ($M+1$).

Step 2

Compound 3-8 (2.88 g, 7.22 mmol) was suspended in dichloromethane (60 mL), and DIEA (5.30 mL, 30.3 mmol) was added thereto. Then, 2-
15 cyanoethyl N,N-diisopropylchlorophosphoramidite (3.23 mL, 14.5 mmol) was added at room temperature, and the mixture was heated under reflux for 2 hours. After cooling to room temperature, the reaction mixture was partitioned by the separatory funnel, and the organic layer diluted with dichloromethane (80 mL) was washed twice with aqueous saturated sodium
20 bicarbonate solution (20 mL), twice with water (20 mL), and once with brine (20 mL). After the resulting organic layer was dried over magnesium sulfate, the solvent was concentrated under reduced pressure. The resulting brown oil, Compound 4-8 (2.88 g, 4.81 mmol) was obtained as a crude product.

Formation of the compound was determined based on introduction of trivalent
25 phosphorus by $^{31}\text{P-NMR}$.

$^{31}\text{P-NMR}(\text{CDCl}_3)\delta$: 148.2 (s)

[0118]

1-1-3) Synthesis of Compound 4-10

Step 1

30 Compound 2-10 (9.78 g, 48.8 mmol, Tokyo Chemical Industry Co., Ltd.) was dissolved in DMF (150 mL) and dichloromethane (75 mL). DIEA (12.79 mL, 73.2 mmol) and HBTU (20.37 g, 53.7 mmol) were added thereto, and the mixture was vigorously stirred at room temperature for 45 minutes. To the resulting suspended solution, Compound 1 (2.2 g, 24.41 mmol) was added at

room temperature, and the mixture was vigorously stirred. Then, the mixture was heated to 80 °C, and then stirred for 4 hours. To the reaction mixture was added aqueous saturated sodium bicarbonate solution (200 mL) and water (50 mL), the resulting white solid was collected by filtration. The resulting solid was washed with water (200 mL) and acetonitrile (400 mL) to obtain Compound 3-10 (9.95 g, 21.88 mmol) as white solid.

¹H-NMR (CDCl₃)δ: 6.25 (2H, t, J = 5.8 Hz), 4.07 (1H, s), 3.75 (1H, s), 3.44-3.38 (2H, m), 3.29-3.23 (2H, m), 2.22 (4H, t, J = 7.6 Hz), 1.67-1.59 (4H, m), 1.30-1.25 (64H, m), 0.88 (6H, t, J = 6.8 Hz).

Step 2

Compound 3-10 (230 mg, 0.506 mmol) was suspended in dichloromethane (11 mL), and DIEA (0.353 mL, 2.023 mmol) was added thereto. Then, 2-cyanoethyl N,N-diisopropylchlorophosphoramidite (0.226mL, 1.012mmol) was added at room temperature, and the mixture was heated under reflux for 2 hours. After cooling to room temperature, the reaction mixture was partitioned by the separatory funnel, and the organic layer was washed twice with aqueous saturated sodium bicarbonate solution (10 mL), five times with water (10 mL) and once with brine (10 mL). After the resulting organic layer was dried over magnesium sulfate, the solvent was concentrated under reduced pressure. The resulting brown oil, Compound 4-10 (365 mg) was obtained as a crude product. Formation of the compound was determined based on introduction of trivalent phosphorus by ³¹P-NMR.

³¹P-NMR(CDCl₃)δ:148.2 (s)

[0119]

1-1-4) Synthesis of Compound 4-12

Step 1

Compound 2-12 (5.07 g, 22.19 mmol, Tokyo Chemical Industry Co., Ltd.) was dissolved in DMF (51.8 mL) and dichloromethane (28.6 mL). DIEA (5.81 mL, 33.3 mmol) and HBTU (9.26 g, 24.4 mmol) were added thereto, and the mixture was vigorously stirred at room temperature for 30 minutes. To the resulting suspended solution, Compound 1 (1.0 g, 11.1 mmol) was added at room temperature, and the mixture was vigorously stirred. Then, the mixture was heated to 40 °C, and then stirred for 2 hours. To the reaction mixture

was added aqueous saturated sodium bicarbonate solution (10 mL), the resulting white solid was collected by filtration. The resulting solid was washed with water (50 mL), acetonitrile (50 mL) and dichloromethane (50 mL) to obtain Compound 3-12 (4.8 g, 9.4 mmol) as white solid.

¹H-NMR(CDCl₃)δ: 6.20 (brs, 2H), 3.96 (d, 1H, J = 4.0 Hz, 1H), 3.75 (m, 1H), 3.40 (dd, 2H, J=4.0, 12.0 Hz), 3.25 (dd, 2H, J = 4.0, 12.0 Hz), 2.22 (t, 4H, J = 12.0 Hz, 2H), 1.62 (d, 4H, J = 8.0 Hz), 1.29-1.25 (m, 40H), 0.90-0.86 (m, 6H) ESI-MS(m/z): 512 (M+1).

Step 2

Compound 3-12 (5.10 g, 9.98 mmol) was suspended in dichloromethane (257 mL), and DIEA (6.97 mL, 39.9 mmol) was added thereto. Then, 2-cyanoethyl N,N-diisopropylchlorophosphoramidite (4.46 mL, 20.0 mmol) was added at room temperature, and the mixture was heated under reflux for 2 hours. After cooling to room temperature, the reaction mixture was partitioned by the separatory funnel, and the organic layer was washed twice with aqueous saturated sodium bicarbonate solution (100 mL), twice with water (100 mL) and once with brine (100 mL). After the resulting organic layer was dried over magnesium sulfate, the solvent was concentrated under reduced pressure. The resulting brown oil, Compound 4-12 (4.80 g, 6.75 mmol) was obtained as a crude product. Formation of the compound was determined based on introduction of trivalent phosphorus by ³¹P-NMR.

³¹P-NMR(CDCl₃)δ: 148.2 (s)

[0120]

1-1-5) Synthesis of Compound 4-14

Compound 2-14 (12.52 g, 48.8 mmol, Tokyo Chemical Industry Co., Ltd.) was dissolved in DMF (150 mL) and dichloromethane (75 mL). DIEA (12.79 mL, 73.2 mmol) and HBTU (20.37 g, 53.7 mmol) were added thereto, and the mixture was vigorously stirred at room temperature for 2.5 hours. To the resulting suspended solution, Compound 1 (2.2 g, 24.41 mmol) was added at room temperature, and the mixture was vigorously stirred. Then, the mixture was heated to 80 °C, and then stirred for 5 hours. To the reaction mixture was added aqueous saturated sodium bicarbonate solution (800 mL) and water (50 mL), the resulting white solid was collected by filtration. The resulting

solid was washed with water (500 mL), acetonitrile (300 mL) and dichloromethane (100 mL) to obtain Compound 3-14 (10.9 g, 19.23 mmol) as white solid.

¹H-NMR (CDCl₃)δ: 6.21 (2H, s), 3.97 (1H, d, J = 4.0 Hz), 3.77-3.74 (1H, m),
5 3.45-3.38 (2H, m), 3.28-3.22 (2H, m), 2.22 (4H, t, J = 7.6 Hz), 1.65-1.61 (4H, m),
1.29-1.25 (48H, m), 0.88 (6H, t, J = 6.8 Hz).

Step 2

Compound 3-14 (1.00 g, 1.76 mmol) was suspended in dichloromethane
10 (50 mL), and DIEA (0.924 mL, 5.29 mmol) was added thereto. Then, 2-
cyanoethyl N,N-diisopropylchlorophosphoramidite (0.870mL, 3.53mmol) was
added at room temperature, and the mixture was heated under reflux for 2
hours. After cooling to room temperature, the reaction mixture was diluted
with dichloromethane (50 mL). The solution was partitioned by the
15 separatory funnel, and then the organic layer was washed twice with aqueous
saturated sodium bicarbonate solution (100 mL), twice with water (100 mL)
and once with brine (100 mL). After the resulting organic layer was dried
over magnesium sulfate, the solvent was concentrated under reduced pressure.
The resulting white amorphous, Compound 4-14 (1.00 g, 1.30 mmol) was
20 obtained as a crude product. Formation of the compound was determined
based on introduction of trivalent phosphorus by ³¹P-NMR.

³¹P-NMR(CDCl₃)δ:148.2 (s)

[0121]

1-1-6) Synthesis of Compound 4-16

25 According to methods described in Non-patent Document 3, Compound 3-
16 was synthesized from Compound 2-16, and then Compound 4-16 was
synthesized.

Compound 3-16: ¹H-NMR(CDCl₃) δ:6.20 (brs, 2H), 3.95 (m, 1H), 3.76 (m, 1H),
3.40 (m, 2H), 3.25 (m, 2H), 2.24 (m, 4H), 1.68-1.20 (m, 60H), 0.88 (t, 6H, J=8.0
30 Hz)

Compound 4-16: ³¹P-NMR(CDCl₃) δ:148.2 (s)

[0122]

1-1-7) Synthesis of Compound 4-18

Step 1

Compound 2-18 (13.9 g, 44.4 mmol, Tokyo Chemical Industry Co., Ltd.) was dissolved in DMF (207 mL) and dichloromethane (214 mL). DIEA (16.3 mL, 93 mmol) and HBTU (18.5 g, 48.8 mmol) were added thereto, and the mixture was vigorously stirred at room temperature for 30 minutes. To the resulting suspended solution, Compound 1 (2.0 g, 22.2 mmol) was added at room temperature, and the mixture was vigorously stirred. Then, the mixture was heated to 40 °C, and then stirred for 2 hours. To the reaction mixture was added aqueous saturated sodium bicarbonate solution (10 mL), the resulting white solid was collected by filtration. The resulting solid was washed with water (100 mL), acetonitrile (100 mL) and dichloromethane (100 mL) to obtain Compound 3-18 (10.0 g, 14.7 mmol) as white solid.

¹H-NMR(CDCl₃)δ:6.20 (brs, 2H), 3.96 (m, 1H), 3.75 (m, 1H), 3.42 (m, 2H), 3.23 (m, 2H), 2.22 (m, 4H), 1.68-1.20 (m, 68H), 0.88 (m, 6H)

Step 2

Compound 3-18 (3.8 g, 5.60 mmol) was suspended in dichloromethane (230 mL), DIEA (5.86 mL, 33.6 mmol) was added thereto. Then, 2-cyanoethyl N,N-diisopropylchlorophosphoramidite (3.74mL, 16.79mmol) was added at room temperature, and the mixture was heated under reflux for 2 hours. After cooling to room temperature, the reaction mixture was partitioned by the separatory funnel, and the organic layer was washed twice with aqueous saturated sodium bicarbonate solution (100 mL), once with water (50 mL) and once with brine (50 mL). After the resulting organic layer was dried over magnesium sulfate, the solvent was concentrated under reduced pressure. After adding acetonitrile to the residue, white solid was collected by filtration. The resulting solid was washed with aqueous saturated sodium bicarbonate solution, water and acetonitrile to obtain Compound 4-18 (4.13 g, 4.70 mmol) as white solid. Formation of the compound was determined based on introduction of trivalent phosphorus by ³¹P-NMR.

³¹P-NMR(CDCl₃)δ:148.2 (s)
[0123]

1-1-8) Synthesis of Compound 4-20

Step 1

Compound 2-20 (10.2 g, 30.0 mmol, Tokyo Chemical Industry Co., Ltd.)

was dissolved in DMF (250 mL) and dichloromethane (250 mL). DIEA (7.86 mL, 45 mmol) and HBTU (12.52 g, 33.0 mmol) were added thereto, and the mixture was vigorously stirred at room temperature for 30 minutes. To the resulting suspended solution, Compound 1 (1.35 g, 15.0 mmol) was added at room temperature, and the mixture was vigorously stirred. Then, the mixture was heated to 40 °C, and then stirred for 2 hours. To the reaction mixture was added aqueous saturated sodium bicarbonate solution (50 mL), the resulting white solid was collected by filtration. The resulting solid was washed with water (50 mL), acetonitrile (50 mL) and dichloromethane (50 mL) to obtain Compound 3-20 (7.20 g, 9.79 mmol) as white solid.

¹H-NMR(CDCl₃)δ:6.18 (brs, 2H), 3.75 (m, 1H), 3.41 (m, 2H), 3.27 (m, 2H), 2.22 (m, 4H), 1.58-1.25 (m, 76H), 0.89-0.86 (m, 6H)

Step 2

Compound 3-20 (1.0 g, 1.36 mmol) was suspended in chloroform (50 mL), and DIEA (0.713 mL, 4.08 mmol) was added thereto. Then, 2-cyanoethyl N,N-diisopropylchlorophosphoramidite (0.607mL, 2.72mmol) was added at room temperature, and the mixture was heated under reflux for 2 hours. After cooling to room temperature, the reaction mixture was added dropwise to acetonitrile (300 mL) under vigorously stirring. The precipitated solid was collected by filtration, and the solid was washed twice with aqueous saturated sodium bicarbonate solution (20 mL), twice with water (20 mL) and twice with acetonitrile (20 mL). The resulting solid was dried under reduced pressure to obtain Compound 4-20 (843 mg, 0.901 mmol) as white solid. Formation of the compound was determined based on introduction of trivalent phosphorus by ³¹P-NMR.

³¹P-NMR(CDCl₃)δ:148.2 (s)

[0124]

1-1-9) Synthesis of Compound 4-22

Step 1

Compound 2-22 (8.79 g, 23.8 mmol, Wako Pure Chemical Industries, Ltd.) was dissolved in DMF (250 mL) and dichloromethane (250 mL). DIEA (6.25 mL, 35.8 mmol) and HBTU (9.95 g, 26.2 mmol) were added thereto, and the mixture was vigorously stirred at room temperature for 30 minutes. To the

resulting suspended solution, Compound 1 (1.07 g, 11.9 mmol) was added at room temperature, and the mixture was vigorously stirred. Then, the mixture was heated to 40 °C, and then stirred for 2 hours. To the reaction mixture was added aqueous saturated sodium bicarbonate solution (50 mL), the resulting white solid was collected by filtration. The resulting solid was washed with water (50 mL), acetonitrile (50 mL) and dichloromethane (50 mL) to obtain Compound 3-22 (8.10 g, 8.17 mmol) as white solid.

¹H-NMR(CDCl₃)δ:6.06 (brs, 2H), 3.73 (m, 1H), 3.36 (dd, 2H, J = 6.0, 14.4 Hz), 3.22 (dd, 2H, J = 5.2, 14.4 Hz), 2.17 (m, 4H), 1.61 (m, 4H), 1.59-1.24 (m, 80H), 0.87-0.84 (m, 6H)

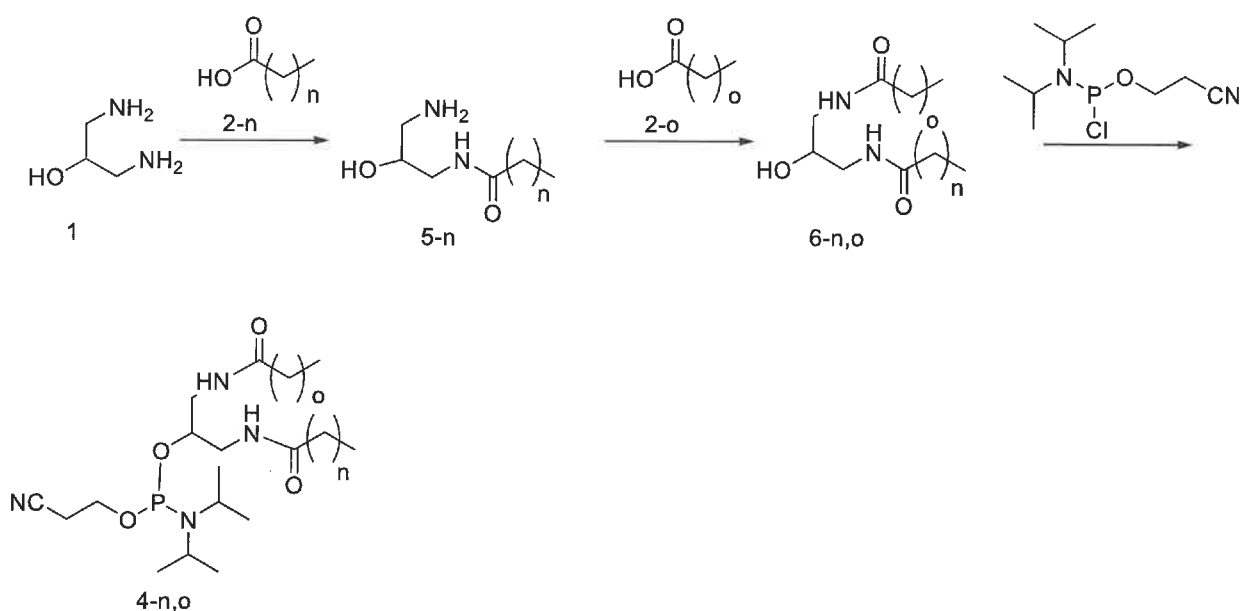
Step 2

Compound 3-22 (1.0 g, 1.36 mmol) was suspended in chloroform (50 mL), and DIEA (0.713 mL, 4.08 mmol) was added thereto. Then, 2-cyanoethyl N,N-diisopropylchlorophosphoramidite (0.607mL, 2.72mmol) was added at room temperature, and the mixture was heated under reflux for 2 hours. After cooling to room temperature, the reaction mixture was added dropwise to acetonitrile (300 mL) under vigorously stirring. The precipitated solid was collected by filtration, and the solid was washed twice with aqueous saturated sodium bicarbonate solution (20 mL), twice with water (20 mL) and twice with acetonitrile (20 mL). The resulting solid was dried under reduced pressure to obtain Compound 4-22 (814 mg, 0.821 mmol) as white solid. Formation of the compound was determined based on introduction of trivalent phosphorus by ³¹P-NMR.

³¹P-NMR(CDCl₃)δ:148.2 (s)

[0125]

1-2) Synthesis of Compound 4·n,o



wherein n or o is an integer of 6 to 28.

5 [0126]

1-2-1) Synthesis of Compound 4-8,18

Step 1

Compound 2-8 is dissolved in DMF and solution. DIEA and HBTU are added thereto, and the mixture is stirred at room temperature. To the resulting suspended solution, Compound 1 is added at room temperature, and the mixture was stirred. The activated solution of Compound 2-18 which is separately prepared [Compound 2-18 is dissolved in DMF and solution. DIEA and HBTU are added thereto, and the mixture is stirred at room temperature] was added to the reaction vessel, and the mixture is stirred at room temperature. The mixture is heated to 40 °C and then stirred. To the reaction mixture is added aqueous saturated sodium bicarbonate solution, the resulting solid is collected by filtration. The resulting solid is washed with water, acetonitrile and dichloromethane to obtain Compound 6-8,18.

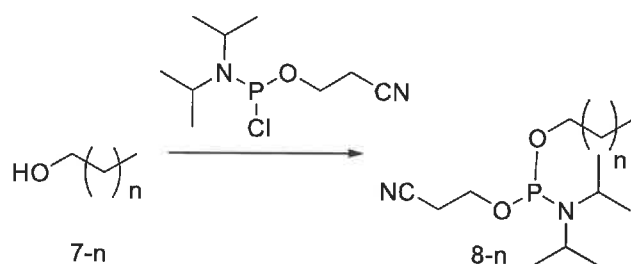
20

Step 2

Compound 6-8,18 is suspended in chloroform, and DIEA is added thereto. Then, 2-cyanoethyl N,N-diisopropylchlorophosphoramidite is added at room temperature, and the mixture is heated under reflux. After cooling to

room temperature, the reaction mixture is added dropwise to acetonitrile under stirring. The precipitated solid is collected by filtration, and the solid is washed twice with saturated sodium bicarbonate solution, twice with water and twice with acetonitrile. The resulting solid is dried under reduced pressure to obtain Compound 4-8,18. Formation of the compound is determined based on introduction of trivalent phosphorus by ^{31}P -NMR. [0127]

2) Synthesis of Compound 8-n



wherein n is an integer of 6 to 28.

[0128]

2-1) Synthesis of Compound 8-6

Compound 7-6 (1.00 g, 7.68 mmol, Tokyo Chemical Industry Co., Ltd.) was dissolved in dichloromethane (15 mL), and triethylamine (2.13 mL, 15.4 mmol) was added thereto. Then, 2-cyanoethyl N,N-diisopropylchlorophosphoramidite (1.71 mL, 7.68 mmol) was added at room temperature, and the mixture was stirred at room temperature for 3 hours. The reaction was stopped by aqueous saturated sodium bicarbonate solution, and the mixture was extracted twice with ethyl acetate (50 mL). The organic layer was washed once with aqueous saturated sodium bicarbonate solution (10 mL), three times with water (10 mL) and once with brine (10 mL), and then dried over magnesium sulfate. The organic layer was concentrated under reduced pressure to obtain brown oil, Compound 8-6 (2.60 g) as a crude product. Formation of the compound was determined based on introduction of trivalent phosphorus by ^{31}P -NMR.

^{31}P -NMR(CDCl_3) δ :147.2 (s)

[0129]

2-2) Synthesis of Compound 8-10

Compound 7-10 (1.00 g, 5.37 mmol, Tokyo Chemical Industry Co., Ltd.)

was dissolved in dichloromethane (15 mL), and triethylamine (1.49 mL, 10.7 mmol) was added thereto. Then, 2-cyanoethyl N,N-diisopropylchlorophosphoramidite (1.20 mL, 5.37 mmol) was added at room temperature, and the mixture was stirred at room temperature for 3 hours.

5 The reaction was stopped by aqueous saturated sodium bicarbonate solution, and the mixture was extracted twice with ethyl acetate (50 mL). The organic layer was washed once with aqueous saturated sodium bicarbonate solution (10 mL), three times with water (10 mL) and once with brine (10 mL), and then dried over magnesium sulfate. The organic layer was concentrated under
10 reduced pressure to obtain brown oil, Compound 8-10 (2.09 g) as a crude product. Formation of the compound was determined based on introduction of trivalent phosphorus by ^{31}P -NMR.

^{31}P -NMR(CDCl_3) δ :147.2 (s)

[0130]

15 2-3) Synthesis of Compound 8-12

Compound 7-12 (4.29 g, 20.0 mmol, Tokyo Chemical Industry Co., Ltd.) was dissolved in dichloromethane (52 mL), and DIEA (10.5 mL, 60.0 mmol) was added thereto. Then, 2-cyanoethyl N,N-diisopropylchlorophosphoramidite (5.36 mL, 24.00 mmol) was added at room temperature, and the mixture was
20 stirred at room temperature for 12 hours. The reaction was stopped by aqueous saturated sodium bicarbonate solution (20 mL), and then the mixture was partitioned by the separatory funnel. After the organic layer was washed once with water (100 mL), the organic layer was washed once with aqueous saturated sodium bicarbonate solution (100 mL) and twice with water
25 (100 mL), and then dried over magnesium sulfate. The organic layer was concentrated under reduced pressure to obtain brown oil, Compound 8-12 (4.80 g, 11.6 mmol) as a crude product. Formation of the compound was determined based on introduction of trivalent phosphorus by ^{31}P -NMR.

^{31}P -NMR(CDCl_3) δ :147.3 (s)

30 [0131]

2-4) Synthesis of Compound 8-14

Compound 7-14 (1.00 g, 4.12 mmol, NACALAI TESQUE, INC.) was dissolved in dichloromethane (15 mL), and triethylamine (1.14 mL, 8.25 mmol) was added thereto. Then, 2-cyanoethyl N,N-diisopropylchlorophosphoramidite

(0.92 mL, 4.12 mmol) was added at room temperature, and the mixture was stirred at room temperature for 3 hours. The reaction was stopped by aqueous saturated sodium bicarbonate solution, and the mixture was extracted twice with ethyl acetate (50 mL). The organic layer was washed once with aqueous saturated sodium bicarbonate solution (10 mL), three times with water (10 mL) and once with brine (10 mL), and then dried over magnesium sulfate. The organic layer was concentrated under reduced pressure to obtain brown oil, Compound 8-14 (1.87 g). Formation of the compound was determined based on introduction of trivalent phosphorus by ^{31}P -NMR.

^{31}P -NMR(CDCl_3) δ :147.2 (s)
[0132]

2-5) Synthesis of Compound 8-16

Compound 7-16 (5.41 g, 20.0 mmol, Tokyo Chemical Industry Co., Ltd.) was dissolved in dichloromethane (52 mL), and DIEA (10.5 mL, 60.0 mmol) was added thereto. Then, 2-cyanoethyl N,N-diisopropylchlorophosphoramidite (4.91 mL, 22.00 mmol) was added at room temperature, and the mixture was stirred at room temperature for 12 hours. The reaction was stopped with aqueous saturated sodium bicarbonate solution (20 mL), and then the mixture was partitioned by the separatory funnel. After the organic layer was washed with water (100 mL), the organic layer was washed once with aqueous saturated sodium bicarbonate solution (100 mL) and twice with water (100 mL), and then dried over magnesium sulfate. The organic layer was concentrated under reduced pressure to obtain brown oil, Compound 8-16 (4.60 g, 9.77 mmol) as a crude product. Formation of the compound was determined based on introduction of trivalent phosphorus by ^{31}P -NMR.

^{31}P -NMR(CDCl_3) δ :147.3 (s)
[0133]

2-6) Synthesis of Compound 8-18

Compound 7-18 (2.99 g, 10.0 mmol, Tokyo Chemical Industry Co., Ltd.) was dissolved in chloroform (81 mL), and DIEA (3.67 mL, 21.0 mmol) was added thereto. Then, 2-cyanoethyl N,N-diisopropylchlorophosphoramidite (2.34 mL, 10.5 mmol) was added at room temperature, and the mixture was stirred at room temperature for 12 hours. The reaction mixture was concentrated, and the residue was washed with acetonitrile (50 mL). The

precipitated solid was collected by filtration and washed with acetonitrile (50 mL). Then, yellow solid was dried under reduced pressure to obtain Compound 8-18 (1.22 g, 2.45 mmol). Formation of the compound was determined based on introduction of trivalent phosphorus by ^{31}P -NMR.

^{31}P -NMR(CDCl_3) δ :147.2 (s)

[0134]

2-7) Synthesis of Compound 8-20

Compound 7-20 (3.27 g, 10.0 mmol, Tokyo Chemical Industry Co., Ltd.) was dissolved in chloroform (81 mL), and DIEA (3.67 mL, 21.0 mmol) was added thereto. Then, 2-cyanoethyl N,N-diisopropylchlorophosphoramidite (2.34 mL, 10.5 mmol) was added at room temperature, and the mixture was stirred at room temperature for 12 hours. The reaction mixture was concentrated, and the residue was washed with acetonitrile (50 mL). The precipitated solid was collected by filtration and washed with acetonitrile (50 mL). Then, yellow solid was dried under reduced pressure to obtain Compound 8-20 (3.19 g, 6.06 mmol). Formation of the compound was determined based on introduction of trivalent phosphorus by ^{31}P -NMR.

^{31}P -NMR(CDCl_3) δ :147.2 (s)

[0135]

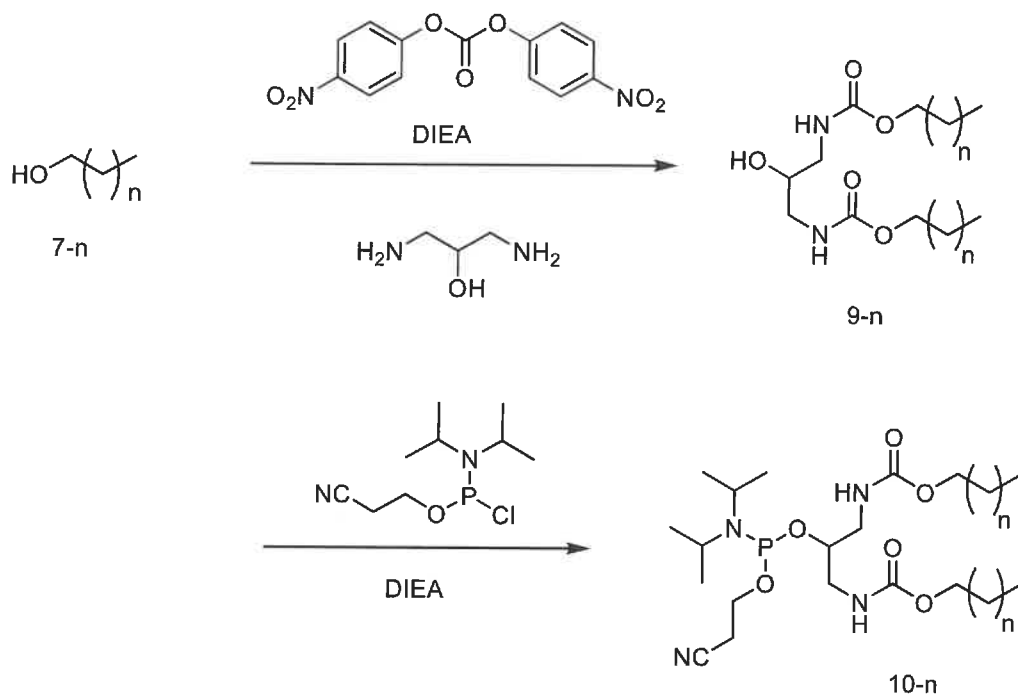
2-8) Synthesis of Compound 8-22

Compound 7-22 (3.55 g, 10.0 mmol, Tokyo Chemical Industry Co., Ltd.) was dissolved in chloroform (81 mL), and DIEA (3.67 mL, 21.0 mmol) was added thereto. Then, 2-cyanoethyl N,N-diisopropylchlorophosphoramidite (2.34 mL, 10.5 mmol) was added at room temperature, and the mixture was stirred at room temperature for 12 hours. The reaction mixture was concentrated, and the residue was washed with acetonitrile (50 mL). The precipitated solid was collected by filtration and washed with acetonitrile (50 mL). Then, yellow solid was dried under reduced pressure to obtain Compound 8-22 (4.97 g, 8.96 mmol). Formation of the compound was determined based on introduction of trivalent phosphorus by ^{31}P -NMR.

^{31}P -NMR(CDCl_3) δ :147.2 (s)

[0136]

3) Synthesis of Compound 10-n



wherein n is an integer of 6 to 28.

[0137]

5 3-1) Synthesis of Compound 10-16

Step 1

Under nitrogen atmosphere, to Compound 7-16 (12.1 g, 44.4 mmol) in DMF (104 mL)-dichloromethane (57.1 mL) solution, bis-(p-nitrophenyl) carbonate (13.5 g, 44.4 mmol) and DIEA (11.6 mL, 66.6 mmol) were added, and then the mixture was stirred at room temperature for 8 hours. Next, Compound 1 (2.0 g, 22.2 mmol) was added thereto, and the mixture was heated under reflux at 60 °C for 2 hours. The resulting solid was collected by filtration and washed with dichloromethane (100 mL), water (100 mL) and acetonitrile (100 mL), and then dried under reduced pressure to obtain Compound 9-16 (15.44 g, 22.6 mmol) as white solid.

¹H-NMR(CDCl₃) δ: 5.20 (brs, 2H), 4.05 (m, 4H), 3.79 (m, 1H), 3.24 (m, 4H), 1.55-1.21 (m, 68H), 0.88 (m, 6H)

Step 2

Under nitrogen atmosphere, to Compound 9-16 suspended in dichloromethane (582 mL), DIEA (15.8 mL, 90.0 mmol) and 2-cyanoethyl N,N-diisopropylchlorophosphoramidite (10.1 mL, 45.2 mmol) were added, and then

the mixture was heated under reflux at 50 °C for 2 hours. After cooling the reaction mixture to room temperature, the organic layer was washed twice with aqueous saturated sodium bicarbonate solution (300 mL), once with water (300 mL) and once with brine (300 mL). The organic layer was dried over magnesium sulfate, and then concentrated under reduced pressure. The residue was dissolved in dichloromethane (50 mL) and added dropwise to acetonitrile (150 mL) to powderize. The resulting solid was dried under reduced pressure to obtain Compound 10-16 (14.2 g, 16.1 mmol).

$^3\text{P-NMR}(\text{CDCl}_3)\delta:149.1\text{ (s)}$

[0138]

3-2) Synthesis of Compound 10-18

Step 1

Under nitrogen atmosphere, to Compound 7-18 (4 g, 13.4 mmol) in DMF (60 mL)-dichloromethane (40 mL) solution, bis-(p-nitrophenyl) carbonate (4.1 g, 13.4 mmol) and DIEA (3.5 mL, 20.1 mmol) were added, and then the mixture was stirred at room temperature for 5 hours. Next, Compound 1 (0.6 g, 6.7 mmol) in DMF solution (5 mL) was added thereto, and the mixture was stirred overnight. The resulting solid was collected by filtration and washed with dichloromethane, water and acetonitrile, and then dried under reduced pressure to obtain Compound 9-18 (4.1 g, 5.55 mmol) as white solid.

$^1\text{H-NMR}(\text{CDCl}_3)\delta:5.20\text{ (brs, 2H)}, 4.05\text{ (t, 4H, } J = 8\text{Hz)}, 3.79\text{ (s, 1H)}, 3.32\text{ (m, 2H)}, 3.23\text{ (m, 2H)}, 1.25\text{ (s, 72H)}, 0.88\text{ (t, 6H, } J = 8\text{Hz)}$

Step 2

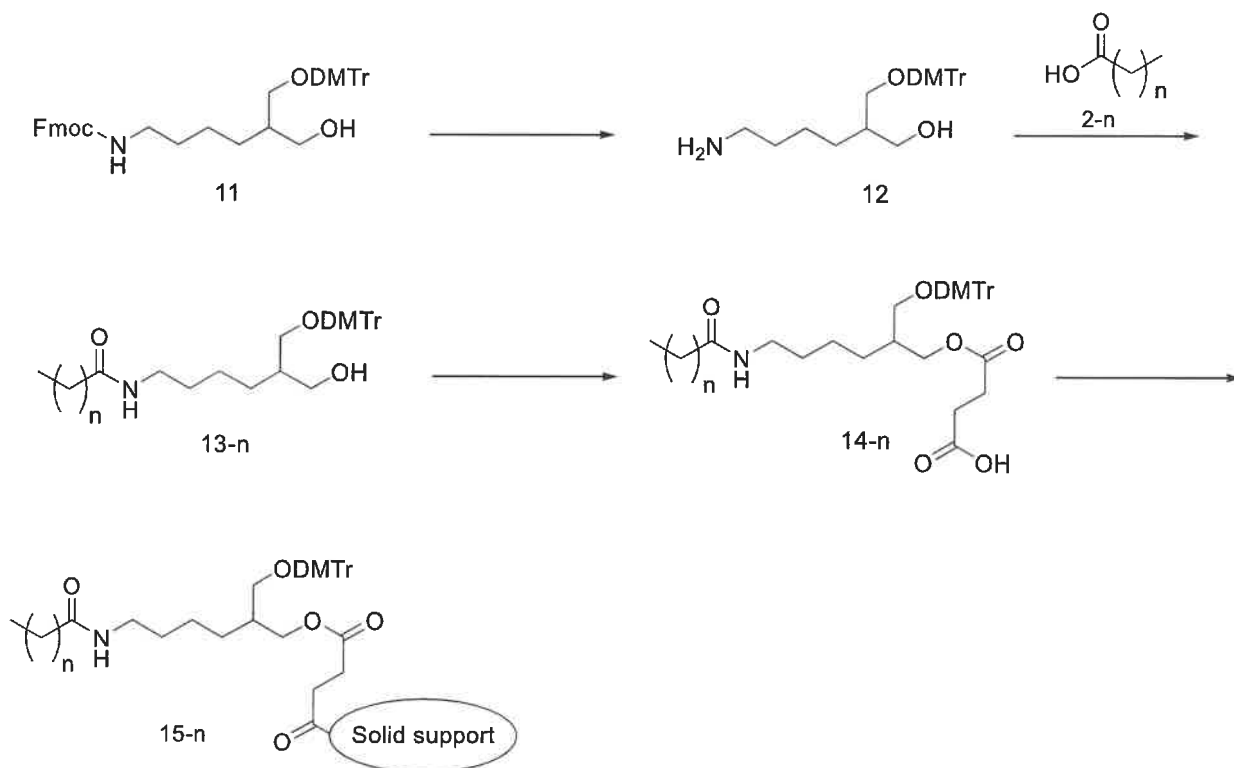
Under nitrogen atmosphere, to Compound 9-18 (1.0 g, 1.35 mmol) suspended in dichloromethane (60 mL), DIEA (1.2 mL, 6.8 mmol) and 2-cyanoethyl N,N-diisopropylchlorophosphoramidite (0.75 mL, 3.4 mmol) were added, and the mixture was stirred at 50°C for 1.5 hours. The reaction mixture was cooled to room temperature, diluted with dichloromethane (40 mL), and washed with aqueous saturated sodium bicarbonate solution (40 mL x 2), water (40 mL) and brine (40 mL). The organic layer was dried over magnesium sulfate, and then concentrated under reduced pressure. The residue was dissolved in dichloromethane (15 mL) and added dropwise to acetonitrile (150 mL) to powderize. The resulting solid was dried under

reduced pressure to obtain Compound 10-18 (0.98 g, 1.04 mmol) as white solid.

^{31}P -NMR(CDCl_3) δ :149.1 (s)

[0139]

5 4) Synthesis of Compound 15-n



wherein n is an integer of 6 to 28.

[0140]

10 4-1) Synthesis of Compound 15-6

Steps 1 and 2

To Compound 11 (US2014/0142253) (722 mg, 1.075 mmol) in dichloromethane (5.6 mL), diethylamine (1.4 mL, 13.40 mmol) was added, and the mixture was stirred at room temperature for 17 hours. After ethanol was added to the reaction mixture, and the mixture was stirred, the solvent was concentrated under reduced pressure. The resulting residue was coevaporated twice with ethanol to obtain the crude product of Compound 12.

To Compound 2-6 (239 mg, 1.505 mmol) in ethanol solution (5.0 mL), DMT-MM (476 mg, 1.720 mmol) was added, the mixture was stirred at room temperature for 15 minutes. The resulting reaction mixture was added to the crude product of Compound 12 in ethanol solution (2.5 mL), and the mixture

was stirred at room temperature for 4.5 hours. After the solvent was concentrated under reduced pressure, aqueous saturated sodium bicarbonate solution and water were added to the resulting residue, and extracted with ethyl acetate. The organic layer was washed with water and brine, and then dried over sodium sulfate. The solvent was concentrated under reduced pressure, and the resulting crude product was purified by silica gel column chromatography (n-hexane: ethyl acetate =70:30→20:80) to obtain Compound 13-6 (320 mg, Yield 52 %) as colorless oil.

¹H-NMR (CDCl₃)δ: 7.42-7.40 (2H, m), 7.32-7.26 (6H, m), 7.21 (1H, t, J = 7.2 Hz), 6.83 (4H, d, J = 8.8 Hz), 5.37 (1H, s), 3.79 (6H, s), 3.71-3.65 (1H, m), 3.63-3.57 (1H, m), 3.27 (1H, dd, J = 9.2, 4.0 Hz), 3.25-3.15 (2H, m), 3.07 (1H, dd, J = 9.2, 7.2 Hz), 2.45 (1H, t, J = 5.6 Hz), 2.12 (2H, t, J = 7.6 Hz), 1.78 (1H, s), 1.62-1.58 (2H, m), 1.47-1.40 (2H, m), 1.36-1.20 (12H, m), 0.87 (3H, t, J = 6.8 Hz).

Step 3

To Compound 13-6 (310 mg, 0.538 mmol) in dichloromethane (3.0 mL), DMAP (6.6 mg, 0.054 mmol), DIEA (0.282 mL, 1.614 mmol) and succinic anhydride (81 mg, 0.807 mmol) were added, and the mixture was stirred at room temperature for 2 days. The solvent was concentrated under reduced pressure, and then the resulting crude product was purified by silica gel column chromatography (chloroform: methanol =100:0→90:10) to obtain Compound 14-6 (360 mg, Yield 99 %) as colorless oil.

¹H-NMR (CDCl₃)δ: 7.42-7.40 (2H, m), 7.31-7.25 (6H, m), 7.19 (1H, t, J = 7.2 Hz), 6.82 (4H, d, J = 8.8 Hz), 5.74 (1H, t, J = 5.6 Hz), 4.24 (1H, dd, J = 10.8, 5.2 Hz), 4.12 (1H, dd, J = 10.8, 6.0 Hz), 3.79 (6H, s), 3.52-3.45 (1H, m), 3.24-2.98 (4H, m), 2.90 (1H, q, J = 7.2 Hz), 2.59-2.50 (3H, m), 2.14 (2H, t, J = 7.6 Hz), 1.90-1.85 (1H, m), 1.61-1.58 (2H, m), 1.48-1.18 (14H, m), 0.87 (3H, t, J = 6.8 Hz).

Step 4

To Compound 14-6 (216 mg, 0.320 mmol) in acetonitrile solution (42 mL), DIEA (0.186 mL, 1.065 mmol) and HBTU (89 mg, 0.234 mmol) were added, and the mixture was shaken at room temperature for 15 minutes. To the reaction mixture, Native Amino lcaa CPG 1000Å (ChemGenes Corporation)

(4.2 g) was added, and the mixture was shaken for 24 hours. After the reaction mixture was filtered, CPG resin was washed three times with acetonitrile and three times with diethyl ether, and dried under reduced pressure. To the dried CPG, a mixture of CapA (PROLIGO, L840045-06) and
5 CapB (PROLIGO, L850045-06) (1:1, 42 mL) was added, and the mixture was shaken for 1.5 hours. After filtrating the reaction mixture, CPG resin was washed twice with pyridine, twice with isopropanol and twice with diethyl ether, and dried under reduced pressure. The supported amount of Compound 14-6 was calculated by colorimetric assay of the DMTr cation, and Compound
10 15-6 whose supported amount is 53 $\mu\text{mol/g}$ was obtained.

[0141]

4-2) Synthesis of Compound 15-10

Steps 1 and 2

In a similar method to Step 1 of 4-1), the crude product of Compound 12
15 (392 mg) was obtained.

To Compound 2-10 (155 mg, 0.772 mmol) in ethanol solution (2.6 mL), DMT-MM (214 mg, 0.772 mmol) was added, the mixture was stirred at room temperature for 30 minutes. The resulting reaction mixture was added to the crude product of Compound 12 (392 mg) in ethanol solution (1.3 mL), and the
20 mixture was stirred at room temperature for 4 hours. To Compound 2-10 (71 mg, 0.356 mmol) in ethanol solution (1.3 mL), DMT-MM (99 mg, 0.356 mmol) was added, and the mixture was stirred at room temperature for 15 minutes. The mixture was combined with the other reaction mixture and stirred at room temperature for 1.5 hours. After the solvent was concentrated under reduced
25 pressure, aqueous saturated sodium bicarbonate solution and water were added to the resulting residue, and extracted with ethyl acetate. The organic layer was washed with water and brine, and dried over sodium sulfate. The solvent was concentrated under reduced pressure, and the resulting crude product was purified by silica gel column chromatography (n-hexane: ethyl
30 acetate =80:20→30:70) to obtain Compound 13-10 (165 mg, 44 %) as colorless oil.

$^1\text{H-NMR}$ (CDCl_3) δ : 7.41 (2H, d, $J = 8.0$ Hz), 7.31-7.26 (6H, m), 7.21 (1H, t, $J = 6.6$ Hz), 6.83 (4H, d, $J = 8.4$ Hz), 5.35 (1H, s), 3.79 (6H, s), 3.70-3.57 (2H, m), 3.28-3.16 (3H, m), 3.07 (1H, t, $J = 8.0$ Hz), 2.43 (1H, s), 2.12 (2H, t, $J = 7.2$ Hz),

1.78 (1H, s), 1.61-1.58 (2H, m), 1.46-1.39 (2H, m), 1.25 (20H, s), 0.87 (3H, t, J = 6.0 Hz).

Step 3

To Compound 13-10 (222 mg, 0.352 mmol) in dichloromethane (2.2 mL), DMAP (4.3 mg, 0.035 mmol), DIEA (0.184 mL, 1.055 mmol) and succinic anhydride (53 mg, 0.528 mmol) were added, and the mixture was stirred at room temperature for 2 days. The solvent was concentrated under reduced pressure, and then the resulting crude product was purified by silica gel column chromatography (chloroform: methanol =100:0→90:10) to obtain Compound 14-10 (243 mg, 94 %) as colorless oil.

¹H-NMR (CDCl₃)δ: 7.41 (2H, d, J = 6.0 Hz), 7.31-7.26 (6H, m), 7.20 (1H, d, J = 7.2 Hz), 6.82 (4H, d, J = 7.6 Hz), 5.59 (1H, s), 4.29 (1H, d, J = 10.4 Hz), 4.17-4.13 (1H, m), 3.79 (6H, s), 3.23-2.98 (4H, m), 2.58 (4H, s), 2.16 (2H, t, J = 8.0 Hz), 1.90 (1H, s), 1.60 (2H, s), 1.42-1.19 (22H, m), 0.88 (3H, t, J = 6.8 Hz).

Step 4

To Compound 14-10 (209 mg, 0.285 mmol) in acetonitrile solution (38 mL), DIEA (0.166 mL, 0.950 mmol) and HBTU (79 mg, 0.209 mmol) were added, and the mixture was shaken at room temperature for 15 minutes. To the reaction mixture, Native Amino lcaa CPG 1000Å (ChemGenes Corporation) (3.8 g) was added, and the mixture was shaken for 23 hours. After the reaction mixture was filtered, CPG resin was washed three times with acetonitrile and three times diethyl ether, and dried under reduced pressure. To the dried CPG, a mixture of CapA (PROLIGO, L840045-06) and CapB (PROLIGO, L850045-06) (1:1, 38 mL) was added, and the mixture was shaken for 1.5 hours. After filtrating the reaction mixture, CPG resin was washed twice with pyridine, twice with isopropanol and twice with diethyl ether, and dried under reduced pressure. The supported amount of Compound 14-10 was calculated by colorimetric assay of the DMTr cation, and Compound 15-10 whose supported amount is 40 μmol/g was obtained.

[0142]

4-3) Synthesis of Compound 15-14

Steps 1 and 2

In a similar method to Step 1 of 4-1), the crude product of Compound 12 (392 mg) was obtained.

To Compound 2-14 (345 mg, 1.346 mmol) in ethanol solution (4.0 mL), DMT-MM (372 mg, 1.346 mmol) was added, and the mixture was stirred at room temperature for 15 minutes. The resulting reaction mixture was added to crude product of Compound 12 in ethanol solution (2.0 mL), and the mixture was stirred at room temperature for 28 hours. After the solvent was concentrated under reduced pressure, aqueous saturated sodium bicarbonate solution and water were added to the resulting residue, and extracted with ethyl acetate. The organic layer was washed with water and brine, and dried over sodium sulfate. The solvent was concentrated under reduced pressure, and the resulting crude product was purified by silica gel column chromatography (n-hexane: ethyl acetate =70:30→20:80) to obtain Compound 13-14 (228 mg, Yield 37 %) as colorless oil.

¹H-NMR (CDCl₃)δ: 7.40 (2H, d, J = 6.8 Hz), 7.31-7.21 (7H, m), 6.83 (4H, d, J = 6.4 Hz), 5.35 (1H, s), 3.79 (6H, s), 3.67 (1H, s), 3.61 (1H, s), 3.28-3.19 (3H, m), 3.07 (1H, t, J = 7.2 Hz), 2.43 (1H, s), 2.12 (2H, t, J = 6.0 Hz), 1.77 (1H, s), 1.59 (2H, s), 1.43 (2H, s), 1.25 (28H, s), 0.88 (3H, s).

Step 3

To Compound 13-14 (224 mg, 0.326 mmol) in dichloromethane (2.2 mL), DMAP (4.0 mg, 0.033 mmol), DIEA (0.171 mL, 0.977 mmol) and succinic anhydride (49 mg, 0.488 mmol) were added, and the mixture was stirred at room temperature for 2 days. The solvent was concentrated under reduced pressure, and the resulting crude product was purified by silica gel column chromatography (chloroform: methanol =100:0→90:10) to obtain Compound 14-14 (163 mg, Yield 64 %) as colorless oil.

¹H-NMR (CDCl₃)δ: 7.40 (2H, d, J = 6.4 Hz), 7.30-7.26 (6H, m), 7.19 (1H, t, J = 7.2 Hz), 6.81 (4H, d, J = 7.2 Hz), 5.57 (1H, s), 4.29 (1H, d, J = 10.8 Hz), 4.17-4.13 (1H, m), 3.79 (6H, s), 3.23-2.98 (4H, m), 2.58 (4H, s), 2.15 (2H, t, J = 7.2 Hz), 1.90 (1H, s), 1.59 (4H, s), 1.46-1.25 (28H, m), 0.87 (3H, t, J = 5.6 Hz).

Step 4

To Compound 14-14 (162 mg, 0.206 mmol) in acetonitrile solution (28

mL), DIEA (0.122 mL, 0.700 mmol) and HBTU (58 mg, 0.154 mmol) were added, and the mixture was shaken at room temperature for 15 minutes. To the reaction mixture, Native Amino lcaa CPG 1000Å (ChemGenes Corporation) (2.8 g) was added, and the mixture was shaken for 24 hours. After the reaction mixture was filtered, CPG resin was washed three times with acetonitrile and three times with diethyl ether, and dried under reduced pressure. To the dried CPG, a mixture of CapA (PROLIGO, L840045-06) and CapB (PROLIGO, L850045-06) (1:1, 28 mL) was added, and the mixture was shaken for 1.5 hours. After filtrating the reaction mixture, CPG resin was washed twice with pyridine, twice with isopropanol and twice with diethyl ether, and dried under reduced pressure. The supported amount of Compound 14-14 was calculated by colorimetric assay of the DMTr cation, and Compound 15-14 whose supported amount is 42 µmol/g was obtained.

[0143]

4-4) Synthesis of Compound 15-18

Steps 1 and 2

In a similar method to Step 1 of 4-1), the crude product of Compound 12 (392 mg) was obtained.

To Compound 2-18 (466 mg, 1.490 mmol) in ethanol solution (5.0 mL), DMT-MM (471 mg, 1.702 mmol) was added, the mixture was stirred at room temperature for 15 minutes. The resulting reaction mixture was added to the crude product of Compound 12 in ethanol solution (2.5 mL), the mixture was stirred at room temperature for 4.5 hours. After the solvent was concentrated under reduced pressure, aqueous saturated sodium bicarbonate solution and water were added to the resulting residue, and extracted with ethyl acetate. The organic layer was washed with water and brine, and dried over sodium sulfate. The solvent was concentrated under reduced pressure, and the resulting crude product was purified by silica gel column chromatography (n-hexane: ethyl acetate =70:30→20:80) to obtain Compound 13-18 (494 mg, Yield 62 %) as colorless oil.

¹H-NMR (CDCl₃)δ: 7.41 (2H, d, J = 7.2 Hz), 7.32-7.26 (6H, m), 7.21 (1H, t, J = 7.2 Hz), 6.83 (4H, d, J = 8.8 Hz), 5.36 (1H, s), 3.79 (6H, s), 3.70-3.65 (1H, m), 3.63-3.57 (1H, m), 3.27 (1H, dd, J = 9.2, 4.0 Hz), 3.24-3.13 (2H, m), 3.07 (1H, dd, J = 9.2, 7.2 Hz), 2.45 (1H, t, J = 5.6 Hz), 2.12 (2H, t, J = 7.6 Hz), 1.78 (1H,

s), 1.63-1.25 (40H, m), 0.88 (3H, t, $J = 6.8$ Hz).

Step 3

To Compound 13-18 (352 mg, 0.473 mmol) in dichloromethane (3.5 mL),
5 DMAP (5.8 mg, 0.047 mmol), DIEA (0.248 mL, 1.419 mmol) and succinic
anhydride (71 mg, 0.709 mmol) were added, and the mixture was stirred at
room temperature for 2 days. The solvent was concentrated under reduced
pressure, and then the resulting crude product was purified by silica gel
column chromatography (chloroform: methanol =100:0→90:10) to obtain
10 Compound 14-18 (225 mg, 56 %) as colorless oil.

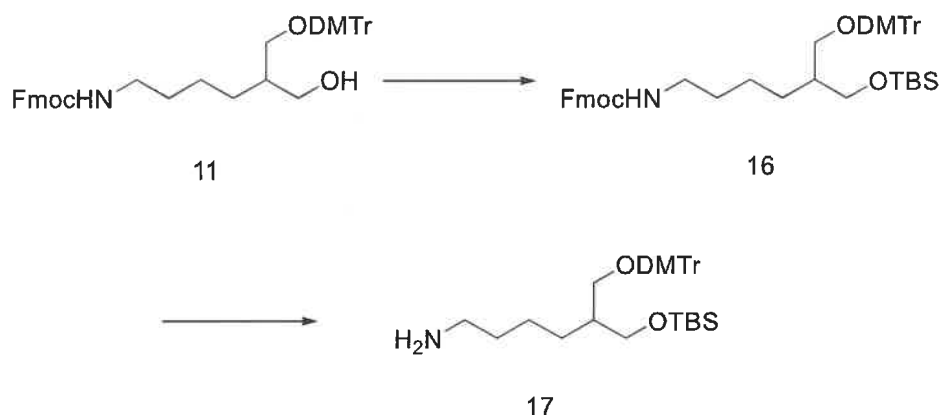
$^1\text{H-NMR}$ (CDCl_3) δ : 7.41 (2H, d, $J = 7.2$ Hz), 7.31-7.26 (6H, m), 7.20 (1H, t, $J =$
6.4 Hz), 6.82 (4H, d, $J = 7.6$ Hz), 5.57 (1H, s), 4.30 (1H, dd, $J = 10.4, 2.4$ Hz),
4.15 (1H, dd, $J = 10.4, 6.4$ Hz), 3.79 (6H, s), 3.24-2.98 (4H, m), 2.59 (4H, s),
2.16 (2H, t, $J = 7.6$ Hz), 1.90 (1H, s), 1.59 (4H, s), 1.44-1.21 (36H, m), 0.88 (3H,
15 t, $J = 5.6$ Hz).

Step 4

To Compound 14-18 (223 mg, 0.264 mmol) in acetonitrile solution (35
mL), DIEA (0.154 mL, 0.880 mmol) and HBTU (73 mg, 0.194 mmol) were
20 added, and the mixture was shaken at room temperature for 15 minutes. To
the reaction mixture, Native Amino lcaa CPG 1000Å (ChemGenes Corporation)
(3.5 g) was added, and the mixture was shaken for 24 hours. After the
reaction mixture was filtered, CPG resin was washed three times with
acetonitrile and three times diethyl ether, and dried under reduced pressure.
25 To the dried CPG, a mixture of CapA (PROLIGO, L840045-06) and CapB
(PROLIGO, L850045-06) (1:1, 35mL) was added, and the mixture was shaken
for 1.5 hours. After filtrating the reaction mixture, CPG resin was washed
twice with pyridine, twice with isopropanol and twice with diethyl ether, and
dried under reduced pressure. The supported amount of Compound 14-18 was
30 calculated by colorimetric assay of the DMTr cation, and Compound 15-18
whose supported amount is 56 $\mu\text{mol/g}$ was obtained.

[0144]

5) Synthesis of Compound 17



Steps 1 and 2

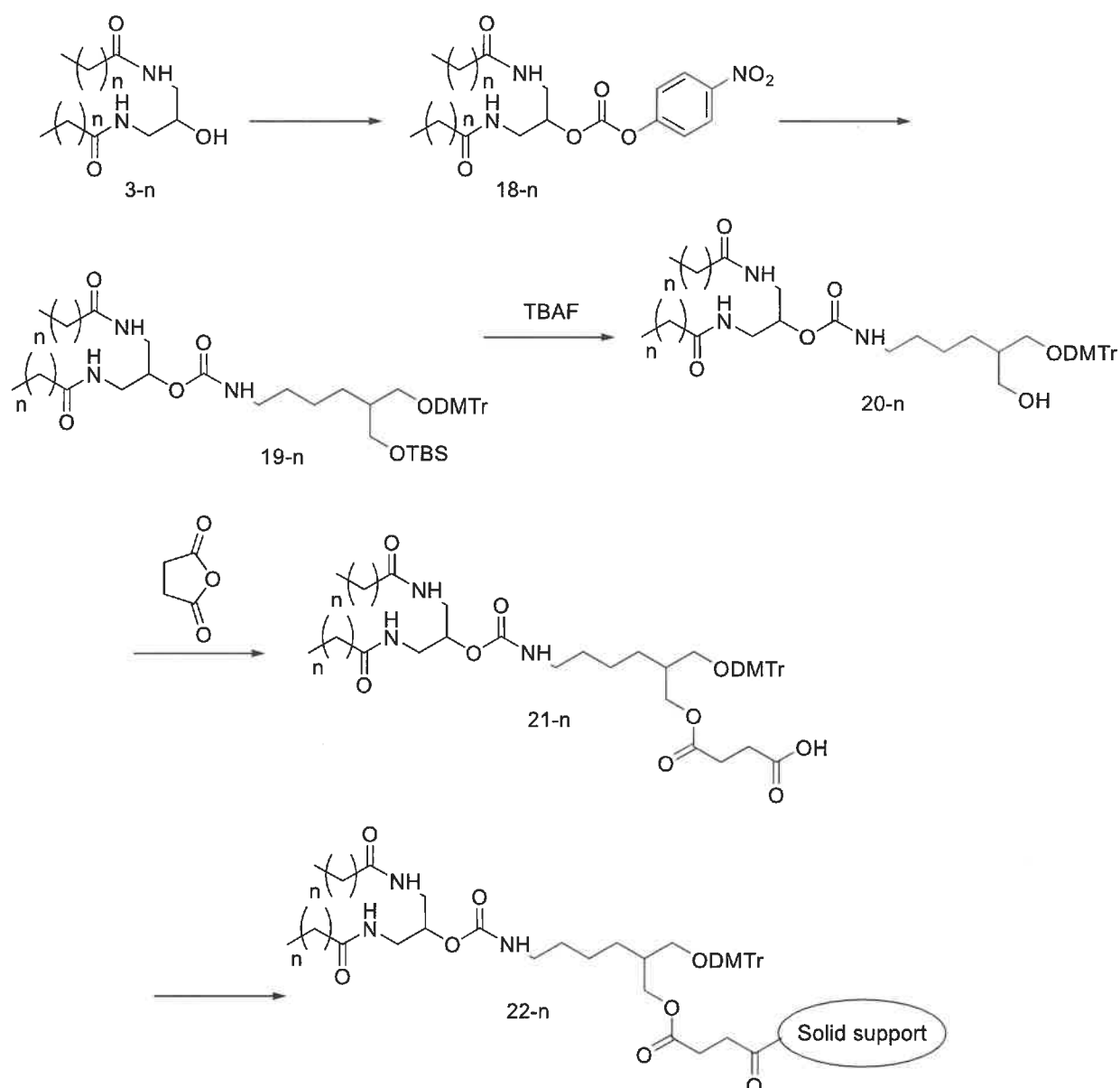
To Compound 11 (US2014/0142253, 292 mg, 0.435 mmol) in DMF solution (2.0 mL), imidazole (71 mg, 1.044 mmol) and t-butylchlorodimethylsilane (79 mg, 0.522 mmol) were added, and the mixture was stirred at room temperature for 16 hours. The reaction mixture was diluted with water and extracted with cyclopentyl methyl ether. The organic layer was washed with water and brine, and dried over sodium sulfate. The solvent was concentrated under reduced pressure to obtain the crude product of Compound 16 (352 mg).

To the crude product of Compound 16 (352 mg) in dichloromethane (2.4 mL), diethylamine (0.6 mL, 5.74 mmol) was added, and the mixture was stirred at room temperature for 16 hours. After ethanol was added to the reaction mixture, the solvent was concentrated under reduced pressure. The residue was coevaporated twice with ethanol, and the resulting crude product was purified by amino silica gel column chromatography (chloroform) to obtain Compound 17 (190 mg, 78 %) as colorless oil.

$^1\text{H-NMR}$ (CDCl_3) δ : 7.45-7.43 (2H, m), 7.32 (4H, d, $J = 8.8$ Hz), 7.29-7.25 (2H, m), 7.22-7.18 (1H, m), 6.81 (4H, d, $J = 8.8$ Hz), 3.79 (6H, s), 3.68-3.61 (2H, m), 3.08-3.02 (2H, m), 2.63 (2H, t, $J = 7.2$ Hz), 1.75-1.69 (1H, m), 1.41-1.30 (6H, m), 1.27-1.15 (2H, m), 0.84 (9H, s), 0.01 (6H, s).

[0145]

6) Synthesis of Compound 22-n



wherein n is an integer of 6 to 28.

[0146]

5 6-1) Synthesis of Compound 22-6

Step 1

To Compound 3-6 (1.0 g, 2.92 mmol) in THF (20 mL)-chloroform (20 mL) solution, DIEA (1.53 mL, 8.76 mmol), bis(nitrophenyl) carbonate (1.33 g, 4.38 mmol) and DMAP (178 mg, 1.46 mmol) were added, and the mixture was stirred at 60 °C for 1 hour. The reaction mixture was filtered. After the mother liquid was concentrated under reduced pressure, the resulting crude product was purified by silica gel column chromatography (hexane: ethyl acetate= 60:40→20:80) to obtain Compound 18-6 (982 mg, 66 %) as white solid.

¹H-NMR (CDCl₃) δ: 8.32-8.26 (2H, m), 7.42 (2H, dt, J = 9.9, 2.5 Hz), 6.36 (2H, t, J = 6.4 Hz), 4.80 (1H, ddd, J = 10.7, 5.6, 3.3 Hz), 3.65-3.50 (4H, m), 2.26 (4H, t, J = 7.6 Hz), 1.69-1.62 (4H, m), 1.28 (16H, dt, J = 19.1, 4.7 Hz), 0.87 (6H, t, J = 6.8 Hz).

5

Step 2

To Compound 17 (500 mg, 0.89 mmol) in dichloromethane (10.0 mL), Compound 18-6 (450 mg, 0.89 mmol) was added, and the mixture was stirred at room temperature for 2 hours. The reaction mixture was concentrated under reduced pressure and purified by amino silica gel column chromatography (hexane: ethyl acetate = 65:35 → 10:90) to obtain Compound 19-6 (625 mg, 76 %) as colorless oil.

10

¹H-NMR (CDCl₃) δ: 7.42 (2H, d, J = 7.4 Hz), 7.31 (4H, t, J = 6.2 Hz), 7.26 (3H, t, J = 3.9 Hz), 7.19 (1H, t, J = 7.2 Hz), 6.82 (4H, t, J = 6.0 Hz), 6.25 (2H, t, J = 5.8 Hz), 4.70 (2H, dd, J = 10.3, 5.3 Hz), 3.79 (6H, d, J = 4.4 Hz), 3.62 (2H, dd, J = 10.1, 5.1 Hz), 3.51 (2H, dd, J = 13.3, 6.4 Hz), 3.32-3.26 (2H, m), 3.08 (4H, dt, J = 20.2, 6.6 Hz), 2.19 (4H, t, J = 7.7 Hz), 1.70 (1H, t, J = 5.7 Hz), 1.61 (8H, t, J = 9.3 Hz), 1.42 (2H, t, J = 7.3 Hz), 1.26 (20H, tt, J = 26.0, 10.5 Hz), 0.88 (6H, dd, J = 12.0, 5.3 Hz), 0.83 (9H, s).

15

20

Step 3

To Compound 19-6 (625 mg, 0.67 mmol) in THF (10 mL), TBAF (1M THF, 1.34 mL, 1.34 mmol) was added, and the mixture was stirred at room temperature for 24 hours. The reaction mixture was concentrated under reduced pressure, and the resulting crude product was purified by diol silica gel column chromatography (hexane: ethyl acetate = 50:50 → 10:90) to obtain Compound 20-6 (541 mg, 99 %) as colorless liquid.

25

¹H-NMR (CDCl₃) δ: 7.41 (2H, t, J = 4.3 Hz), 7.26 (9H, ddt, J = 31.6, 12.0, 4.9 Hz), 6.83 (4H, d, J = 8.8 Hz), 6.38 (2H, q, J = 6.1 Hz), 4.88 (1H, t, J = 5.6 Hz), 4.67 (1H, t, J = 5.0 Hz), 3.79 (6H, t, J = 7.5 Hz), 3.69-3.61 (2H, m), 3.50-3.44 (2H, m), 3.30 (3H, tt, J = 20.6, 6.5 Hz), 3.15-3.06 (3H, m), 2.63 (1H, s), 2.21-2.17 (4H, m), 1.78 (1H, s), 1.62 (4H, t, J = 6.9 Hz), 1.43 (2H, t, J = 5.4 Hz), 1.30 (20H, dt, J = 29.2, 11.0 Hz), 0.87 (6H, t, J = 6.9 Hz).

30

[0147]

Step 4

To Compound 20-6 (541 mg, 0.66 mmol) in dichloromethane (2 mL), DIEA (0.35 mL, 1.98 mmol), DMAP (8.0 mg, 0.066 mmol) and succinic anhydride (132 mg, 1.32 mmol) were added, and the mixture was stirred at room temperature for 4 hours. The reaction mixture was concentrated under reduced pressure, and the resulting crude product was purified by silica gel column chromatography (chloroform: methanol =40:1→10:1) to obtain Compound 21-6 (591 mg, 97 %) as colorless liquid.

¹H-NMR (CDCl₃) δ: 7.41 (2H, d, J = 7.5 Hz), 7.31-7.25 (8H, m), 7.20 (1H, t, J = 7.2 Hz), 6.82 (4H, d, J = 8.5 Hz), 6.62 (1H, t, J = 6.3 Hz), 6.48 (1H, t, J = 6.5 Hz), 5.91 (1H, t, J = 5.5 Hz), 4.71 (1H, t, J = 5.3 Hz), 4.42 (1H, dd, J = 11.0, 3.2 Hz), 4.14 (1H, dd, J = 10.9, 5.9 Hz), 3.79 (6H, s), 3.40 (4H, tt, J = 20.4, 7.0 Hz), 3.08 (4H, dq, J = 33.3, 8.0 Hz), 2.69-2.49 (4H, m), 2.20 (4H, dd, J = 15.6, 8.2 Hz), 1.95 (1H, s), 1.61 (4H, d, J = 7.0 Hz), 1.27 (22H, d, J = 5.0 Hz), 0.87 (6H, dd, J = 6.8, 5.1 Hz).

Step 5

To Compound 21-6 (312 mg, 0.34 mmol) in a mixture of acetonitrile/dichloromethane (4:1, 25 mL), DIEA (0.30 mL, 1.70 mmol) and HBTU (142 mg, 0.37 mmol) were added, and the mixture was shaken at room temperature for 15 minutes. To the reaction mixture, HybridCPG amino form 2000Å (Prime Synthesis, Inc.) (2.8 g) was added, and the mixture was shaken for 24 hours. After the reaction mixture was filtered, HybridCPG resin was washed three times with acetonitrile and three times with diethyl ether, and dried under reduced pressure. To the dried HybridCPG, a mixture of THF/acetic anhydride/pyridine (8:1:1, 30 mL) was added, and the mixture was shaken for 3 hours. After the reaction mixture was filtered, HybridCPG resin was washed twice with pyridine, twice with isopropanol and twice with diethyl ether, and dried under reduced pressure. The supported amount of Compound 21-6 was calculated by colorimetric assay of the DMTr cation, and Compound 22-6 whose supported amount is 114 μmol/g was obtained.

[0148]

6-2) Synthesis of Compound 22-8

Step 1

To Compound 3-8 (1 g, 2.5 mmol) in THF (20 mL) and chloroform (20 mL), bis(nitrophenyl) carbonate (1.14 g, 3.76 mmol), DIEA (1.3 mL, 7.5 mmol) and DMAP (0.15 g, 1.25 mmol) were added, and the mixture was stirred at 60 °C for 1 hour. The reaction mixture was filtered. After the mother liquid was concentrated under reduced pressure, the resulting crude product was purified by silica gel column chromatography (hexane: ethyl acetate =60:40→20:80) to obtain Compound 18-8 (1.17 g, 83 %) as white solid.

¹H-NMR (CDCl₃) δ: 8.27 (2H, dt, J = 9.9, 2.5 Hz), 7.41 (2H, dt, J = 9.9, 2.5 Hz), 6.42 (2H, t, J = 6.5 Hz), 4.81-4.78 (1H, m), 3.65-3.50 (4H, m), 2.26 (4H, t, J = 7.6 Hz), 1.68-1.62 (4H, m), 1.28 (24H, t, J = 9.5 Hz), 0.87 (6H, t, J = 6.8 Hz).

Step 2

To Compound 17 (500 mg, 0.89 mmol) in dichloromethane (10.0 mL), Compound 18-8 (500 mg, 0.89 mmol) and DIEA (0.23 mL, 1.33 mmol) were added, and the mixture was stirred at room temperature for 2 hours. The reaction mixture was concentrated under reduced pressure, and then purified by amino silica gel column chromatography (hexane: ethyl acetate =50:50→10:90) to obtain Compound 19-8 (661 mg, 75 %) as pale yellow solid.

¹H-NMR (CDCl₃) δ: 7.42 (2H, d, J = 7.4 Hz), 7.31 (4H, t, J = 6.3 Hz), 7.26 (3H, t, J = 3.9 Hz), 7.19 (1H, t, J = 7.2 Hz), 6.81 (4H, d, J = 8.8 Hz), 6.25 (2H, t, J = 6.0 Hz), 4.70 (2H, q, J = 5.4 Hz), 3.79 (6H, s), 3.63 (2H, t, J = 5.3 Hz), 3.51 (2H, dd, J = 13.1, 6.5 Hz), 3.29 (2H, dd, J = 12.9, 7.0 Hz), 3.08 (4H, dt, J = 20.0, 6.5 Hz), 2.18 (4H, t, J = 7.7 Hz), 1.70 (1H, t, J = 5.8 Hz), 1.62 (6H, d, J = 11.2 Hz), 1.42 (2H, t, J = 7.2 Hz), 1.27 (28H, dt, J = 37.9, 14.6 Hz), 0.87 (6H, t, J = 6.8 Hz), 0.83 (9H, s).

Step 3

To Compound 19-8 (661 mg, 0.669 mmol) in THF (10 mL), TBAF (1 M THF, 1.34 mL, 1.34 mmol) was added, and the mixture was stirred at room temperature for 25 hours. The reaction mixture was concentrated under reduced pressure, and then purified by diol silica gel column chromatography (hexane: ethyl acetate= 50:50→10:90) to obtain Compound 20-8 (549 mg, 94 %) as colorless oil..

¹H-NMR (CDCl₃) δ: 7.41 (2H, t, J = 4.4 Hz), 7.32-7.19 (9H, m), 6.84 (4H, d, J =

8.8 Hz), 6.35 (2H, q, J = 6.1 Hz), 4.85 (1H, t, J = 5.8 Hz), 4.67 (1H, t, J = 5.1 Hz), 3.79 (6H, s), 3.64 (2H, dd, J = 14.3, 7.4 Hz), 3.47 (2H, dt, J = 14.1, 5.8 Hz), 3.30 (3H, tt, J = 19.5, 6.2 Hz), 3.15-3.06 (3H, m), 2.60 (1H, s), 2.21-2.17 (4H, m), 1.78 (1H, s), 1.62 (4H, s), 1.44 (2H, d, J = 5.1 Hz), 1.27 (28H, dd, J = 11.0, 3.7 Hz), 0.87 (6H, t, J = 6.8 Hz).

[0149]

Step 4

To Compound 20-8 (549 mg, 0.63 mmol) in dichloromethane (10 mL), DMAP (7.7 mg, 0.063 mmol), succinic anhydride (126 mg, 1.25 mmol) and DIEA (0.32 mL, 1.88 mmol) were added, and the mixture was stirred at room temperature for 4 hours. After the reaction mixture was concentrated under reduced pressure, the resulting crude product was purified by silica gel column chromatography (chloroform :methanol= 20:1→10:1) to obtain Compound 21-8 (582 mg, 95 %) as colorless liquid.

¹H-NMR (CDCl₃) δ: 7.41 (2H, d, J = 7.5 Hz), 7.31-7.25 (8H, m), 7.20 (1H, t, J = 7.2 Hz), 6.82 (4H, d, J = 8.7 Hz), 6.61 (1H, t, J = 6.2 Hz), 6.47 (1H, t, J = 6.4 Hz), 5.91 (1H, t, J = 5.6 Hz), 4.71 (1H, t, J = 5.1 Hz), 4.42 (1H, dd, J = 11.0, 3.2 Hz), 4.14 (1H, dd, J = 10.9, 5.8 Hz), 3.80 (6H, d, J = 6.1 Hz), 3.47-3.33 (4H, m), 3.08 (4H, ddd, J = 33.6, 15.6, 8.5 Hz), 2.69-2.49 (4H, m), 2.20 (4H, dd, J = 15.6, 8.2 Hz), 1.95 (1H, s), 1.55 (4H, dt, J = 34.3, 6.5 Hz), 1.27 (30H, t, J = 7.2 Hz), 0.87 (6H, t, J = 6.8 Hz).

Step 5

To Compound 21-8 (300 mg, 0.31 mmol) in a mixture of acetonitrile/dichloromethane (4:1, 25mL), DIEA (0.27 mL, 1.54 mmol) and HBTU (128 mg, 0.34 mmol) were added, and the mixture was shaken at room temperature for 15 minutes. HybridCPG amino form 2000Å (Prime Synthesis, Inc.)(2.5g) was added to the reaction mixture, and the mixture was shaken for 24 hours. After the reaction mixture was filtered, HybridCPG resin was washed three times with acetonitrile and three times with diethyl ether, and dried under reduced pressure. To the dried HybridCPG, a mixture of THF/acetic anhydride /pyridine (8:1:1, 30 mL) was added, and the mixture was shaken for 3 hours. After the reaction mixture was filtered, HybridCPG resin was washed twice with pyridine, twice with isopropanol, and twice with diethyl

ether, and dried under reduced pressure. The supported amount of Compound 21-8 was calculated by colorimetric assay of the DMTr cation, and Compound 22-8 whose supported amount is 107 $\mu\text{mol/g}$ was obtained.

[0150]

5 6-3) Synthesis of Compound 22-10

Step 1

To Compound 3-10 (2.0 g, 3.92 mmol) in THF (50 mL), pyridine (0.379 mL, 4.70 mmol) and 4-nitrophenyl chloroformate (947 mg, 4.70 mmol) were added, and the mixture was stirred at 60 °C for 1 hour. The reaction mixture
10 was filtered. After the mother liquid was concentrated under reduced pressure, the resulting crude product was purified by silica gel column chromatography (chloroform: methanol = 100:0 $\rightarrow$ 90:10) to obtain Compound 18-10 (1.1 g, 42 %) as white solid.

$^1\text{H-NMR}$ (CDCl_3) δ : 8.28 (2H, d, $J = 8.8$ Hz), 7.42 (2H, d, $J = 8.8$ Hz), 6.91 (2H, d, $J = 9.1$ Hz), 4.80 (1H, s), 3.57 (4H, m), 2.26 (4H, t, $J = 7.6$ Hz), 1.66 (4H, t, $J = 6.9$ Hz), 1.27 (40H, d, $J = 20.2$ Hz), 0.88 (6H, t, $J = 6.7$ Hz).

Step 2

To Compound 17 (41 mg, 0.074 mmol) in dichloromethane (5.0 mL),
20 Compound 18-10 (50 mg, 0.074 mmol) was added, and the mixture was stirred at room temperature for 1 hour. The reaction mixture was purified by silica gel column chromatography (chloroform: methanol = 100:0 $\rightarrow$ 90:10) to obtain Compound 19-10 (80 mg, 98 %) as yellow liquid.

$^1\text{H-NMR}$ (CDCl_3) δ : 7.42 (2H, d, $J = 7.5$ Hz), 7.31 (7H, t, $J = 6.4$ Hz), 6.81 (4H, d, $J = 8.8$ Hz), 6.23 (2H, d, $J = 5.5$ Hz), 4.68 (1H, s), 3.79 (6H, s), 3.63 (2H, t, $J = 5.4$ Hz), 3.52 (2H, t, $J = 6.8$ Hz), 3.28 (2H, t, $J = 7.3$ Hz), 3.11-3.04 (4H, m), 2.18 (4H, t, $J = 7.5$ Hz), 1.62 (6H, t, $J = 7.2$ Hz), 1.25 (40H, s), 0.88 (6H, t, $J = 6.8$ Hz), 0.84 (9H, s), 0.01 (6H, s)

30 Step 3

To Compound 19-10 (559.2 mg, 0.535 mmol) in THF (5 mL), triethylamine (4.4 mL, 7.21 mmol) and TBAF (1 M THF, 0.4 mL, 0.40 mmol) were added, and the mixture was stirred at room temperature for 12 hours. The reaction mixture was diluted with chloroform (10 mL), and then washed

with aqueous saturated sodium bicarbonate solution (10 mL). After the resulting organic layer was concentrated under reduced pressure, the resulting crude product was purified by silica gel column chromatography (chloroform :methanol =100:0→90:10) to obtain Compound 20-10 (384 mg, 77 %) as colorless liquid.

¹H-NMR (CDCl₃)δ: 7.41 (2H, t, J = 4.3 Hz), 7.31 (7H, d, J = 8.5 Hz), 6.84 (4H, d, J = 8.8 Hz), 6.32 (2H, t, J = 6.3 Hz), 4.83 (1H, t, J = 6.0 Hz), 4.67 (1H, s, J = 5.2 Hz), 3.79 (6H, s), 3.68-3.63 (2H, m), 3.47 (2H, dd, J = 12.9, 6.1 Hz), 3.34-3.24 (3H, m), 3.10 (3H, dt, J = 17.5, 5.6 Hz), 2.59 (1H, s), 2.21-2.17 (4H, m), 1.71 (1H, m), 1.62 (4H, t, J = 7.2 Hz), 1.25 (36H, s), 0.88 (6H, t, J = 6.8 Hz).

[0151]

Step 4

To Compound 20-10 (384 mg, 0.431 mmol) in dichloromethane (10.1 mL), DMAP (5.04 mg, 0.041 mmol) and succinic anhydride (62.0 mg, 0.619 mmol) were added, and the mixture was stirred at room temperature for 1 day. After the reaction mixture was concentrated under reduced pressure, the resulting crude product was purified by diol silica gel column chromatography (chloroform :methanol =100:0→90:10) to obtain Compound 21-10 (308 mg, 71 %) as colorless liquid.

¹H-NMR (CDCl₃)δ: 7.41 (2H, t, J = 4.3 Hz), 7.31 (6H, d, J = 8.5 Hz), 6.84 (4H, d, J = 8.8 Hz), 6.81 (1H, br s), 6.61 (1H, br s), 5.83 (1H, br s), 4.70 (1H, s), 4.38 (1H, m), 4.13 (1H, d, J = 10.4 Hz), 3.79 (6H, s), 3.78-3.41 (6H, m), 3.05 (6H, m), 2.89 (6H, m), 2.62-2.55 (6H, m), 2.20 (4H, d, J = 7.2 Hz), 1.94 (1H, s), 1.62 (4H, t, J = 7.2 Hz), 1.25 (36H, s), 0.88 (6H, t, J = 7.2 Hz).

Step 5

To Compound 21-10 (247 mg, 0.240 mmol) in a mixture of acetonitrile/dichloromethane (1:1, 20 mL), DIEA (0.168 mL, 0.982 mmol) and HBTU (100 mg, 0.264 mmol) were added, and the mixture was shaken at room temperature for 20 minutes. To the reaction mixture, Native Amino lcaa CPG 1000 Å (ChemGenes Corporation)(2.0 g) was added, and the mixture was shaken for 12 hours. After the reaction mixture was filtered, CPG resin was washed three times with dichloromethane and three times with diethyl ether, and dried under reduced pressure. To the dried HybridCPG, a mixture of

CapA (PROLIGO, L840045-06) and CapB (PROLIGO, L850045-06) (1:1, 20 mL) was added, and the mixture was shaken for 30 minutes. After the reaction mixture was filtered, CPG resin was washed twice with dichloromethane and twice with diethyl ether, and dried under reduced pressure. The supported amount of Compound 21-10 was calculated by colorimetric assay of the DMTr cation, and Compound 22-10 whose supported amount is 69 $\mu\text{mol/g}$ was obtained.

[0152]

6-4) Synthesis of Compound 22-12

Step 1

To Compound 3-12 (2.0 g, 3.92 mmol) in THF (50 mL), pyridine (0.379 mL, 4.70 mmol) and 4-nitrophenyl chloroformate (947 mg, 4.70 mmol) were added, and the mixture was stirred at 60 °C for 1 hour. The reaction mixture was filtered. After the mother liquid was concentrated under reduced pressure, the resulting crude product was purified by silica gel column chromatography (chloroform: methanol = 100:0 $\rightarrow$ 90:10) to obtain Compound 18-12 (1.1 g, 42 %) as white solid.

$^1\text{H-NMR}$ (CDCl_3) δ : 8.28 (2H, d, $J = 8.8$ Hz), 7.42 (2H, d, $J = 8.8$ Hz), 6.91 (2H, d, $J = 9.1$ Hz), 4.80 (1H, s), 3.57 (4H, m), 2.26 (4H, t, $J = 7.6$ Hz), 1.66 (4H, t, $J = 6.9$ Hz), 1.27 (40H, d, $J = 20.2$ Hz), 0.88 (6H, t, $J = 6.7$ Hz).

Step 2

To Compound 17 (41 mg, 0.074 mmol) in dichloromethane (5.0 mL), Compound 18-12 (50 mg, 0.074 mmol) was added, and the mixture was stirred at room temperature for 1 hour. The reaction mixture was purified by silica gel column chromatography (chloroform: methanol = 100:0 $\rightarrow$ 90:10) to obtain Compound 19-12 (80 mg, 98 %) as yellow liquid.

$^1\text{H-NMR}$ (CDCl_3) δ : 7.42 (2H, d, $J = 7.5$ Hz), 7.31 (6H, t, $J = 6.4$ Hz), 6.81 (4H, d, $J = 8.8$ Hz), 6.23 (2H, d, $J = 5.5$ Hz), 4.68 (1H, s), 3.79 (6H, s), 3.63 (2H, t, $J = 5.4$ Hz), 3.52 (2H, t, $J = 6.8$ Hz), 3.28 (2H, t, $J = 7.3$ Hz), 3.11-3.04 (4H, m), 2.18 (4H, t, $J = 7.5$ Hz), 1.62 (6H, t, $J = 7.2$ Hz), 1.25 (40H, s), 0.88 (6H, t, $J = 6.8$ Hz), 0.84 (6H, s), 0.01 (6H, s)

Step 3

To Compound 19-12 (90 mg, 0.082 mmol) in THF (5 mL), triethylamine (1.0 mL, 7.21 mmol) and TBAF (1 M THF, 0.4 mL, 0.40 mmol) were added, and the mixture was stirred at room temperature for 12 hours. The reaction mixture was diluted with chloroform (10 mL), and then washed with aqueous saturated sodium bicarbonate solution (10 mL). After the resulting organic layer was concentrated under reduced pressure, the resulting crude product was purified by silica gel column chromatography (chloroform: methanol =100:0→95:5) to obtain Compound 20-12 (84 mg, 100 %) as colorless liquid.

¹H-NMR (CDCl₃)δ: 7.41 (2H, t, J = 4.3 Hz), 7.31 (6H, d, J = 8.5 Hz), 6.84 (4H, d, J = 8.8 Hz), 6.34 (2H, t, J = 6.3 Hz), 4.83 (1H, s), 4.67 (1H, s), 3.79 (6H, s), 3.68-3.63 (2H, m), 3.47 (2H, dd, J = 12.9, 6.1 Hz), 3.34-3.24 (3H, m), 3.10 (3H, dt, J = 17.5, 5.6 Hz), 2.60 (1H, s), 2.21-2.17 (4H, m), 1.62 (4H, t, J = 6.9 Hz), 1.25 (40H, s), 0.88 (6H, t, J = 6.8 Hz).

[0153]

Step 4

To Compound 20-12 (60 mg, 0.061 mmol) in pyridine solution (2 mL), DMAP (0.7 mg, 0.006 mmol) and succinic anhydride (7.3 mg, 0.073 mmol) were added, and the mixture was stirred at room temperature for 4 days. After the reaction mixture was concentrated under reduced pressure, the resulting crude product was purified by silica gel column chromatography (chloroform: methanol =100:0→95:5) to obtain Compound 21-12 (56 mg, 85 %) as colorless liquid.

ESI-MS (m/z) : 1085 (M-H).

Step 5

To Compound 21-12 (52 mg, 0.048 mmol) in a mixture of acetonitrile/chloroform (1:1, 10 mL), DIEA (0.043 mL, 0.248 mmol) and HBTU (31 mg, 0.083 mmol) were added, and the mixture was shaken at room temperature for 15 minutes. To the reaction mixture, Native Amino lcaa CPG 1000Å (ChemGenes Corporation) (0.5 g) was added, and the mixture was shaken for 23 hours. After the reaction mixture was filtered, CPG resin was washed three times with acetonitrile and three times with diethyl ether, and dried under reduced pressure. To the dried CPG, a mixture of CapA (PROLIGO, L840045-06) and CapB (PROLIGO, L850045-06) (1:1, 5 mL) was

added, and the mixture was shaken for 1.5 hours. After the reaction mixture was filtered, CPG resin was washed twice with pyridine, twice with isopropanol and twice with diethyl ether, and then dried under reduced pressure. The supported amount of Compound 21-12 was calculated by colorimetric assay of the DMTr cation, and Compound 22-12 whose supported amount is 31 $\mu\text{mol/g}$ was obtained.

[0154]

6-5) Synthesis of Compound 22-14

Step 1

To Compound 3-14 (1.5 g, 3.92 mmol) in THF (60 mL), pyridine (0.320 mL, 3.97 mmol) and 4-nitrophenyl chloroformate (800 mg, 3.97 mmol) were added, and the mixture was heated under reflux for 3 hours. The reaction mixture was filtered. After the mother liquid was concentrated under reduced pressure, the resulting crude product was purified by silica gel column chromatography (hexane:ethyl acetate =66:34→45:55) to obtain Compound 18-14 (1.56 g, 81 %) as white solid.

$^1\text{H-NMR}$ (CDCl_3) δ : 8.29 (2H, d, $J = 10.0$ Hz), 7.43 (2H, d, $J = 10.0$ Hz), 6.31 (2H, t, $J = 6.4$ Hz), 4.81-4.76 (1H, m), 3.65-3.58 (2H, m), 3.55-3.48 (2H, m), 2.25 (4H, t, $J = 7.6$ Hz), 1.67-1.61 (4H, m), 1.25 (48H, s), 0.88 (6H, t, $J = 6.8$ Hz).

Step 2

To Compound 17 (1.06 g, 1.88 mmol) in dichloromethane (30 mL), Compound 18-14 (1.38 g, 1.88 mmol) was added, and the mixture was stirred at room temperature for 1 hour. After the solvent was concentrated under reduced pressure, the resulting crude product was purified by silica gel column chromatography (hexane:ethyl acetate =66:34→45:55) to obtain Compound 19-14 (1.47 g, 68 %) as white solid.

ESI-MS (m/z) : 1155 (M-H).

Step 3

To Compound 19-14 (1.47 g, 1.27 mmol) in THF (30 mL), TBAF (1 M THF, 3.81 mL, 3.81 mmol) was added, and the mixture was stirred at room temperature for 18 hours. After the reaction mixture was diluted with ethyl acetate, the solvent was concentrated under reduced pressure. The resulting

crude product was purified by silica gel column chromatography (hexane: ethyl acetate =50:50→5:95) to obtain Compound 20-14 (1.04 g, 79 %) as white solid.

¹H-NMR (CDCl₃) δ: 7.41 (2H, d, J = 7.6 Hz), 7.32-7.27 (6H, m), 7.21 (1H, t, J = 7.2 Hz), 6.83 (4H, d, J = 8.8 Hz), 6.37-6.35 (2H, m), 4.86 (1H, t, J = 5.6 Hz),
5 4.69-4.64 (1H, m), 3.79 (6H, s), 3.69-3.61 (2H, m), 3.50-3.24 (6H, m), 3.15-3.06 (3H, m), 2.61 (1H, s), 2.21-2.17 (4H, m), 1.25 (58H, s), 0.88 (6H, t, J = 6.8 Hz).
[0155]

Step 4

To Compound 20-14 (1.04 g, 0.998 mmol) in dichloromethane (20 mL),
10 DMAP (12 mg, 0.10 mmol), DIEA (0.523 mL, 2.99 mmol) and succinic anhydride (170 mg, 1.70 mmol) were added, and the mixture was stirred at room temperature for 16 hours. After the reaction mixture was concentrated under reduced pressure, the resulting crude product was purified by diol silica gel column chromatography (chloroform) to obtain Compound 21-14 (1.17 g) as
15 white solid.

ESI-MS (m/z) : 1141 (M-H).

Step 5

To Compound 21-14 (585 mg, 0.512 mmol) in a mixture of
20 acetonitrile/dichloromethane (1:1, 40 mL), DIEA (0.447 mL, 2.56 mmol) and HBTU (214 mg, 0.563 mmol) were added, and the mixture was shaken at room temperature for 15 minutes. To the reaction mixture, Native Amino lcaa CPG 1000Å (ChemGenes Corporation) (4.0 g) was added, and the mixture was shaken for 21 hours. After the reaction mixture was filtered, CPG resin was
25 washed with a mixture of acetonitrile/dichloromethane (1:1, 120 mL) and diethyl ether (60 mL), and then dried under reduced pressure. To the dried CPG, a mixture of CapA (PROLIGO, L840045-06) and CapB (PROLIGO, L850045-06) (1:1, 40 mL) was added, and the mixture was shaken for 1.5 hours. After the reaction mixture was filtered, CPG resin was washed with
30 pyridine (40 mL), isopropanol (60 mL) and diethyl ether (60 mL), and then dried under reduced pressure. The supported amount of Compound 21-14 was calculated by colorimetric assay of the DMTr cation, and Compound 22-14 whose supported amount is 40 μmol/g was obtained.

[0156]

6-6) Synthesis of Compound 22-18

Step 1

To Compound 3-18 (1.0 g, 1.47 mmol) in THF (40 mL), pyridine (0.143 mL, 1.76 mmol) and 4-nitrophenyl chloroformate (356 mg, 1.77 mmol) were added, and the mixture was stirred at 60 °C for 1 hour. After the reaction mixture was filtered, the mother liquid was concentrated under reduced pressure. The crude product of resulting solid was washed with ethyl acetate to obtain Compound 18-18 (508 mg, 41 %) as white solid.

¹H-NMR (CDCl₃)δ: 8.29 (2H, d, J = 8.8 Hz), 7.43 (2H, d, J = 9.1 Hz), 6.29 (2H, s), 3.60-3.50 (4H, m), 2.25 (4H, t, J = 7.6 Hz), 1.64 (4H, d, J = 6.6 Hz), 1.25 (64H, s), 0.88 (6H, t, J = 6.4 Hz).

Step 2

To Compound 17 (62 mg, 0.110 mmol) in dichloromethane (4.0 mL), Compound 18-18 (93 mg, 0.110 mmol) was added, and the mixture was stirred at room temperature for 1 hour. The reaction mixture was purified by silica gel column chromatography (chloroform: methanol =100:0→90:10) to obtain Compound 19-18 (113 mg, 81 %) as yellow liquid.

¹H-NMR (CDCl₃)δ: 7.42 (2H, d, J = 13.4 Hz), 7.31 (7H, d, J = 9.3 Hz), 6.81 (4H, d, J = 8.8 Hz), 6.22 (2H, s), 4.69 (1H, s), 3.78 (6H, s), 3.62 (2H, t, J = 5.6 Hz), 3.51 (2H, dd, J = 10.9, 6.1 Hz), 3.32-3.27 (2H, m), 3.11-3.04 (4H, m), 2.17 (4H, t, J = 3.7 Hz), 1.62 (6H, dd, J = 10.5, 4.7 Hz), 1.25 (64H, s), 0.88 (6H, t, J = 6.8 Hz), 0.83 (9H, s), 0.01 (6H, s).

Step 3

To Compound 19-18 (100 mg, 0.079 mmol) in THF (4 mL), triethylamine (0.1 mL, 0.79 mmol) and TBAF (1 M THF, 0.32 mL, 0.32 mmol) were added, and the mixture was stirred at room temperature for 24 hours. The reaction mixture was diluted with chloroform (10 mL), and then washed with aqueous saturated sodium bicarbonate solution (10 mL). After the resulting organic layer was dried over magnesium sulfate and concentrated under reduced pressure, the resulting crude product was purified by silica gel column chromatography (chloroform: methanol =100:0→95:5) to obtain Compound 20-18 (90 mg, 99 %) as colorless liquid.

¹H-NMR (CDCl₃)δ: 7.41 (2H, d, J = 8.6 Hz), 7.31 (7H, d, J = 8.8 Hz), 6.84 (4H, d, J = 8.6 Hz), 6.31 (2H, s), 4.81 (1H, s), 4.67 (1H, s), 3.79 (6H, s), 3.71-3.63 (2H, m), 3.49 (2H, dd, J = 14.7, 11.1 Hz), 3.30 (3H, tt, J = 18.8, 7.4 Hz), 3.13-3.07 (3H, m), 2.58 (1H, s), 2.18 (4H, d, J = 7.6 Hz), 1.60 (6H, dd, J = 9.2, 4.4 Hz) 1.25 (64H, s), 0.88 (6H, t, J = 6.3 Hz).

[0157]

Step 4

To Compound 20-18 (87 mg, 0.075 mmol) in pyridine solution (2 mL), DMAP (0.9 mg, 0.007 mmol) and succinic anhydride (15.8 mg, 0.151 mmol) were added, and the mixture was stirred at room temperature for 7 days. After the reaction mixture was concentrated under reduced pressure, the resulting crude product was purified by silica gel column chromatography (chloroform: methanol = 100:0 → 95:5) to obtain Compound 21-18 (85 mg, 90 %) as colorless liquid.

ESI-MS (m/z) : 1253 (M-H).

Step 5

To Compound 21-18 (85 mg, 0.068 mmol) in a mixture of acetonitrile/chloroform (1:1, 10 mL), DIEA (0.043 mL, 0.248 mmol) and HBTU (38 mg, 0.10 mmol) were added, and the mixture was stirred at room temperature for 15 minutes. To the reaction mixture, Native Amino lcaa CPG 1000Å (ChemGenes Corporation) (0.5 g) was added, and the mixture was shaken at room temperature for 23 hours. After the reaction mixture was filtered, CPG resin was washed three times with acetonitrile and three times with diethyl ether, and dried under reduced pressure. To the dried CPG, a mixture of CapA (PROLIGO, L840045-06) and CapB (PROLIGO, L850045-06) (1:1, 5 mL) was added, and the mixture was shaken for 1.5 hours. After the reaction mixture was filtered, CPG resin was washed twice with pyridine, twice with isopropanol and twice with diethyl ether, and dried under reduced pressure. The supported amount of Compound 21-18 was calculated by colorimetric assay of the DMTr cation, and Compound 22-18 whose supported amount is 15 μmol/g was obtained.

[0158]

6-7) Synthesis of Compound 22-20

Step 1

To Compound 3-20 (1.0 g, 1.36 mmol) in THF (35 mL), bis(4-nitrophenyl) carbonate (1.241 mL, 4.08 mmol) and DMAP (498 mg, 4.08 mmol) were added, and the mixture was stirred at 55 °C for 1 hour. The reaction mixture was concentrated under reduced pressure. To the residue, acetonitrile (50 mL) was added and the mixture was stirred until the solid was precipitated. To the suspended solution, water (20 mL) was added and the mixture was vigorously stirred. The resulting solid was collected by filtration and washed with water (50 mL) and acetonitrile (50 mL) in order to obtain Compound 18-20 (1.1 g, 92 %) as white solid.

¹H-NMR (CDCl₃)δ: 8.28 (2H, dd, J = 2.0, 6.8 Hz), 7.42 (2H, dd, J = 2.4, 7.2 Hz), 6.30 (2H, s), 4.79 (1H, s), 3.61 (2H, m), 3.52 (2H, m), 2.20 (4H, m), 1.66 (4H, m), 1.25 (72H, m), 0.88 (6H, t, J = 6.8 Hz).

Step 2

To Compound 17 (219 mg, 0.388 mmol) in THF (6.5 mL), Compound 18-20 (350 mg, 0.388 mmol) and DMAP (47.5 mg, 0.388 mmol) were added, and the mixture was stirred at 65 °C for 2 hours. To the reaction mixture, 10 % hydrous acetonitrile solution (70 mL) was added, and the mixture was stirred for a while. The precipitated solid was collected by filtration to obtain Compound 19-20 (420 mg, 82 %) as white solid.

¹H-NMR (CDCl₃)δ: 7.43 (2H, d, J = 7.6 Hz), 7.31 (7H, m), 6.81 (4H, d, J = 8.4 Hz), 6.26 (2H, br s), 4.73 (1H, br s), 4.69 (1H, br s), 3.79 (6H, s), 3.79 (6H, s), 3.63 (2H, m), 3.49 (2H, m), 3.29 (2H, d, J = 14.0 Hz), 3.10-3.06 (4H, m), 2.18 (4H, t, J = 7.6 Hz), 1.68 (4H, m), 1.42-1.25 (m, 72H), 0.88 (6H, t, J = 6.4 Hz), 0.83 (s, 9H), 0.00 (s, 6H).

Step 3

To Compound 19-20 (559 mg, 0.422 mmol) in THF (5 mL), TBAF (1 M THF, 0.506 mL, 0.506 mmol) was added, and the mixture was stirred at 50 °C for 5 hours. The reaction mixture was added dropwise to 10 % hydrous acetonitrile solution (100 mL), and then the precipitated solid was collected by filtration to obtain Compound 20-20 (344 mg, 67 %) as white solid.

¹H-NMR (CDCl₃)δ: 7.41 (2H, t, J = 4.3 Hz), 7.31 (7H, d, J = 8.5 Hz), 6.84 (4H, d,

$J = 8.8$ Hz), 6.35 (2H, br s), 4.85 (1H, s), 4.67 (1H, s), 3.79 (6H, s), 3.68 (2H, m), 3.47 (2H, dd, $J = 12.9, 6.1$ Hz), 3.34-3.24 (3H, m), 3.10 (3H, dt, $J = 17.5, 5.6$ Hz), 2.60 (1H, s), 2.21-2.17 (4H, m), 1.62 (4H, m), 1.60-1.45 (m, 4H), 1.25-1.01 (72H, m), 0.88 (6H, t, $J = 6.8$ Hz).

5 [0159]

Step 4

To Compound 20-20 (344 mg, 0.284 mmol) in dichloromethane (10 mL), DMAP (3.5 mg, 0.0284 mmol) and succinic anhydride (42.6 mg, 0.426 mmol) were added, and the mixture was stirred at room temperature overnight. The
10 reaction mixture was added dropwise to 10 % hydrous acetonitrile solution (100 mL), and the precipitated solid was collected by filtration to obtain Compound 21-20 (361 mg, 97 %) as white solid.

$^1\text{H-NMR}$ (CDCl_3) δ : 7.42 (2H, d, $J = 7.6$ Hz), 7.31-7.26 (7H, m), 6.83 (4H, t, $J = 8.4$ Hz), 6.56 (1H, br s), 6.40 (1H, br s), 5.89 (1H, br s), 4.71 (1H, m), 4.41 (1H, d, $J = 8.0$ Hz), 4.14 (1H, dd, $J = 6.0, 11.2$ Hz), 3.79 (6H, s), 3.65 (1H, m), 3.43-3.37
15 (4H, m), 3.05-3.02 (4H, m), 2.19-2.17 (4H, m), 1.61-1.25 (76H, m), 0.88 (6H, t, $J = 6.8$ Hz).

Step 5

20 To Compound 21-20 (190 mg, 0.145 mmol) in a mixture of acetonitrile/chloroform (1:3, 10 mL), DIEA (0.127 mL, 0.725 mmol) and HBTU (60.5 mg, 0.159 mmol) were added, and the mixture was shaken at 40 °C for 15 minutes. To the reaction mixture, Native Amino lcaa CPG 1000Å (ChemGenes Corporation) (3.0 g) was added, and the mixture was shaken for 2
25 hours. After the reaction mixture was filtered, CPG resin was washed three times with chloroform, once with ethanol and three times with acetonitrile, and dried under reduced pressure. To the dried CPG, a mixture of CapA (PROLIGO, L840045-06) and CapB (PROLIGO, L850045-06) (1:1, 20 mL) was added, and the mixture was shaken for 1.5 hours. After the reaction mixture
30 was filtered, CPG resin was washed three times with chloroform and three times with acetonitrile, and then dried under reduced pressure. The supported amount of Compound 21-20 was calculated by colorimetric assay of the DMTr cation, and Compound 22-20 whose supported amount is 48 $\mu\text{mol/g}$ was obtained.

[0160]

6-8) Synthesis of Compound 22-22

Step 1

To Compound 3-22 (2.0 g, 2.53 mmol) in THF (35 mL), bis-(p-
5 nitrophenyl) carbonate (1.54 g, 5.05 mmol) and DMAP (618 mg, 5.05 mmol)
were added, and the mixture was stirred at 65 °C for 2 hours. The reaction
mixture was concentrated under reduced pressure. To the residue,
acetonitrile (100 mL) was added, and the mixture was stirred until the solid
was precipitated. To the suspended solution, water (20 mL) was added, and
10 the mixture was vigorously stirred. The resulting solid was collected by
filtration and washed in order with water (100 mL) and acetonitrile (100 mL)
to obtain Compound 18-22 (2.27 g, 89 %) as white solid.
¹H-NMR (CDCl₃)δ: 8.28 (2H, d, J = 8.8 Hz) , 7.43 (2H, d, J = 8.82 Hz), 6.29 (2H,
br t, J = 6.4 Hz), 4.79 (1H, br s), 3.61 (2H, m), 3.52 (2H, m), 2.25 (4H, t, J = 7.6
15 Hz), 1.64 (4H, m), 1.26 (84H, m), 0.88 (6H, t, J = 6.0 Hz)

Step 2

To Compound 17 (666 mg, 1.81 mmol) in THF (10.0 mL), DMAP (144 mg,
1.81 mmol) and Compound 18-22 (1.13 g, 1.18 mmol) were added, and the
20 mixture was stirred at 65 °C for 2 hours. To the reaction mixture, acetonitrile
(150 mL) was added slowly. The resulting precipitate was collected by
filtration and washed three times with acetonitrile to obtain Compound 19-22
(1.5 g, 92 %) as white solid.
¹H-NMR (CDCl₃)δ: 7.42 (2H, d, J = 6.8 Hz), 7.32-7.20 (7H, m), 6.81 (4H, d, J =
25 6.8 Hz), 6.26 (2H, br m), 4.70 (2H, s), 3.63 (2H, m), 3.49 (2H, m), 3.31 (2H,
m), 3.09-3.04 (4H, m), 2.18 (4H, m), 1.60 (4H, m), 1.25 (84H, m), 0.86 (6H, t, J = 6.8
Hz), 0.84 (9H, s), 0.01 (6H, s)

Step 3

30 To Compound 19-22 (1.5 g, 1.07 mmol) in THF (8.9 mL), TBAF (1 M
THF, 1.64 mL, 1.69 mmol) was added, and the mixture was stirred at 65 °C for
2 hours. To the reaction mixture, acetonitrile (150 mL) was added slowly.
The resulting precipitate was collected by filtration and washed three times
with acetonitrile to obtain Compound 20-22 (1.2 g, 87 %) as white solid.

¹H-NMR (CDCl₃)δ: 7.41 (2H, t, J = 7.2 Hz), 7.31-7.21 (7H, m), 6.84 (4H, d, J = 8.0 Hz), 6.34 (2H, m), 4.83 (1H, s), 4.67 (1H, s), 3.79 (6H, s), 3.67 (2H, m), 3.46 (2H, m), 3.33-3.24 (3H, m), 3.10 (3H, m), 2.17 (4H, t, J = 7.6 Hz), 1.78 (4H, m), 1.44-1.25 (84H, m), 0.88 (6H, t, J = 6.0 Hz).

[0161]

Step 4

To Compound 20-22 (100 mg, 0.079 mmol) in dichloromethane (3 mL), DMAP (1 mg, 0.008 mmol) and succinic anhydride (11.9 mg, 0.118 mmol) were added, and the mixture was stirred at 45 °C for 4 hours. To the reaction mixture, acetonitrile (10 mL) was added dropwise, and the precipitated solid was collected by filtration. The resulting solid was washed three times with acetonitrile to obtain Compound 21-22 (361 mg, 97 %) as white solid.

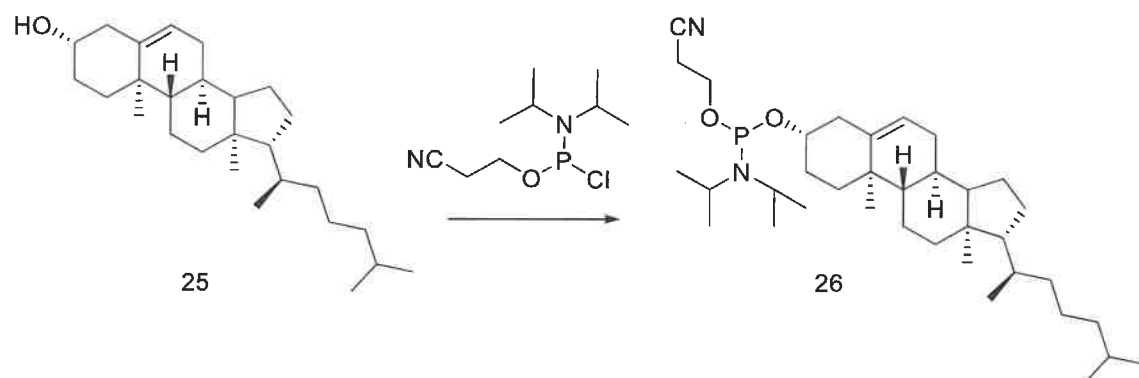
¹H-NMR (CDCl₃)δ: 7.42 (2H, d, J = 7.6 Hz), 7.31-7.26 (7H, m), 6.81 (4H, t, J = 8.8 Hz), 6.58 (1H, br m), 6.43 (1H, br m), 5.88 (1H, br m), 4.71 (1H, m), 4.40 (1H, d, J = 8.0 Hz), 4.14 (1H, dd, J = 6.0, 10.8 Hz), 3.79 (6H, s), 3.65 (1H, m), 3.43-3.37 (4H, m), 3.13-3.02 (4H, m), 2.63-2.53 (4H, m), 2.23-2.18 (4H, dd, J = 7.2, 14.8 Hz), 2.00-1.25 (4H, m), 1.25-1.11 (84H, m), 0.88 (6H, t, J = 6.8 Hz).

Step 5

To Compound 21-22 (92 mg, 0.067 mmol) in a mixture of acetonitrile/dichloromethane/chloroform (1:2:2, 10 mL), DIEA (0.059 mL, 0.336 mmol) and HBTU (28 mg, 0.074 mmol) were added, and the mixture was shaken at 40 °C for 15 minutes. To the reaction mixture, Native Amino lcaa CPG 1000Å (ChemGenes Corporation) (0.9 g) was added, and the mixture was shaken at 40 °C for 3 hours. After the reaction mixture was filtered, CPG resin was washed three times with chloroform, three times with acetonitrile and three times with ethanol, and dried under reduced pressure. To the dried CPG, a mixture of CapA (PROLIGO, L840045-06) and CapB (PROLIGO, L850045-06) (1:1, 60 mL) was added, and the mixture was shaken for 1.5 hours. After the reaction mixture was filtered, CPG resin was washed three times with acetonitrile, and dried under reduced pressure. The supported amount of Compound 21-22 was calculated by colorimetric assay of the DMTr cation, and Compound 22-22 whose supported amount is 47 μmol/g was obtained.

[0162]

8-1) Synthesis of Compound 26

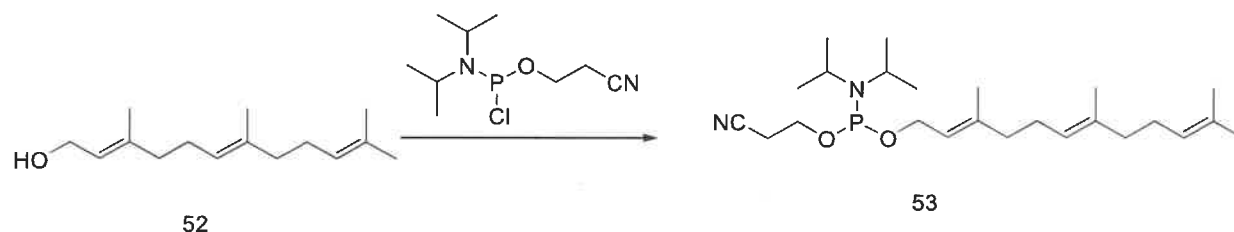


Under nitrogen atmosphere, to cholesterol (Compound 25, 1.00 g, 2.59 mmol, Wako Pure Chemical Industries, Ltd.) in dichloromethane (10 mL), DIEA (0.9 mL, 5.17 mmol) was added, and the mixture was cooled with ice-cold water. Methyl N,N-diisopropylchlorophosphoramidite (0.86 mL, 3.85 mmol) was added thereto and the mixture was stirred under ice cooling for 40 minutes. The reaction mixture was diluted with dichloromethane and ethyl acetate (100 mL), and then washed with aqueous saturated sodium bicarbonate solution (100 mL) and brine (100 mL). The organic layer was dried over magnesium sulfate, filtered, and then concentrated under reduced pressure. The resulting residue was purified by silica gel column chromatography (SiO₂:20 g, n-hexane: ethyl acetate =50:50→0:100) to obtain Compound 26 (1.45 g, Yield 95 %) as colorless foam. Formation of the compound was determined based on introduction of trivalent phosphorus by ³¹P-NMR.

³¹P-NMR(CDCl₃) δ:145.46 (d)

[0163]

8-2) Synthesis of Compound 5



Farnesol (Compound 52, 1.0 mL, 3.99 mmol, Junsei Chemical Co., Ltd.) was dissolved in dichloromethane (8.9 mL), and DIEA (1.53 mL, 8.78 mmol)

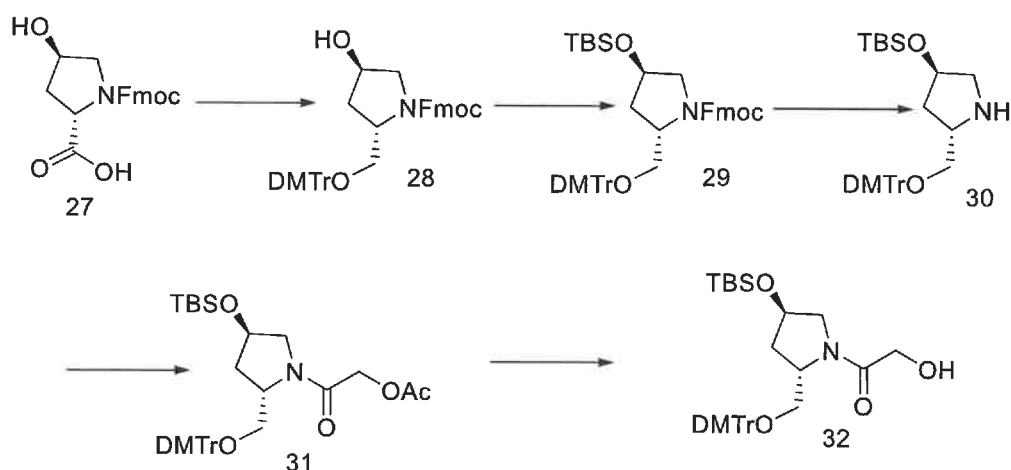
was added thereto. Then, 2-cyanoethyl N, N-diisopropylchlorophosphoramidite (0.98 mL, 4.39 mmol) was added thereto at room temperature, and the mixture was stirred at room temperature for 40 minutes. Dichloromethane (90 mL) and aqueous saturated sodium bicarbonate solution (100 mL) were added to the reaction solvent to stop the reaction, and then the mixture was partitioned by the separatory funnel. The organic layer was washed with brine (100 mL), and then dried over magnesium sulfate. The organic layer was concentrated under reduced pressure to obtain Compound 53 (1.20 g, 2.84 mmol) as pale yellow oil as a crude product. Formation of the compound was determined based on

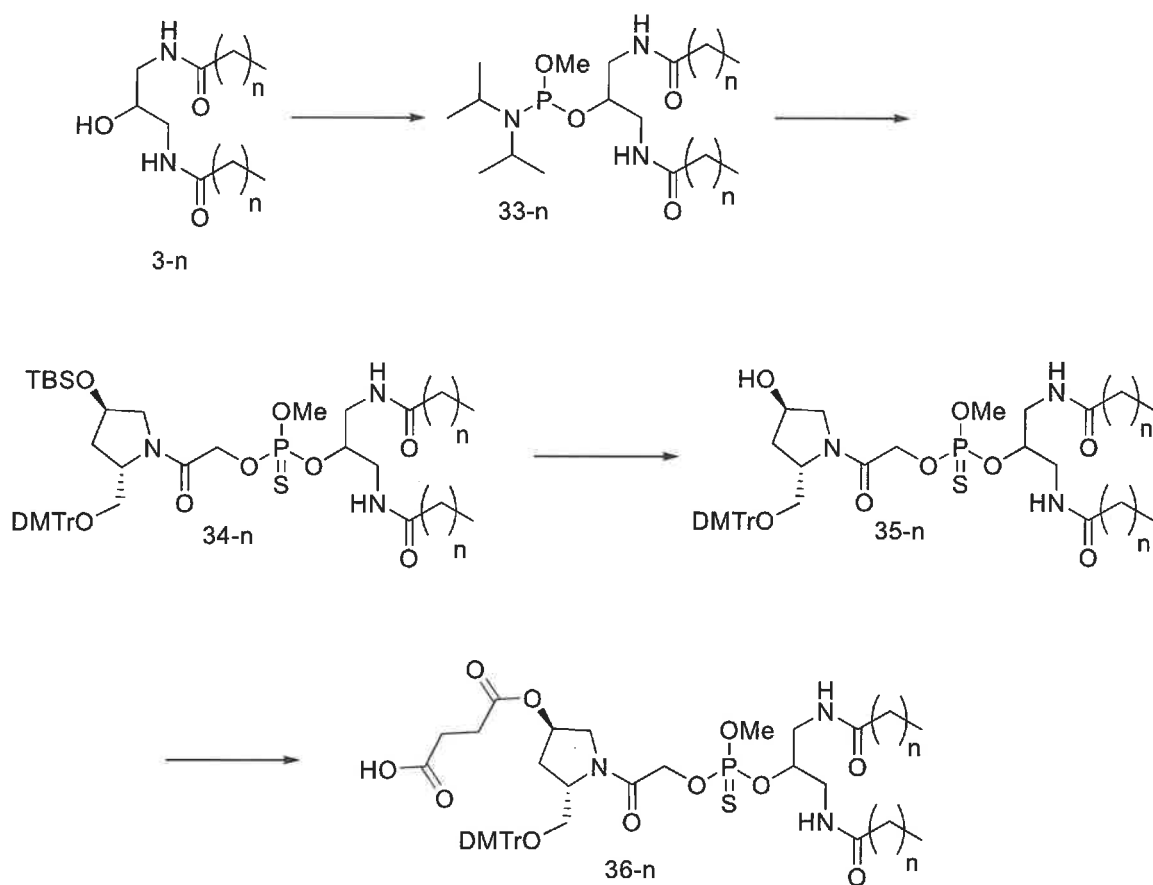
$^1\text{H-NMR}$ (CDCl_3) δ : 5.37 (1H, t, $J = 6.6$ Hz), 5.10-5.08 (2H, m), 4.19-4.13 (2H, m), 3.90-3.77 (2H, m), 3.66-3.54 (2H, m), 2.64 (2H, t, $J = 6.6$ Hz), 2.08-1.99 (8H, m), 1.68-1.67 (6H, m), 1.61-1.60 (6H, m), 1.20-1.17 (12H, m).

$^{31}\text{P-NMR}$ (CDCl_3) δ : 147.92 (1H, s).

[0164]

9) Synthesis of Compound 36-n





wherein n is an integer of 6 to 28.

[0165]

5 9-1) Synthesis of Compound 36-16

Step 1

According to methods described in Nucleic Acids Research, 42, 8796-8807 (2014), Compound 28 was synthesized from Compound 27 in two steps.

10 Step 2

To Compound 28 (5.0 g, 7.79 mmol) in DMF solution (20 mL), imidazole (690mg, 10.1mmol, 1.3eq.) and tert-butyldimethylsilyl chloride (1.29 g, 8.57 mmol, 1.1eq.) were added in order at room temperature, and the mixture was stirred at room temperature for 2 hours. The reaction mixture was diluted with ethyl acetate, washed with brine, and then dried over magnesium sulfate. The solvent was concentrated under reduced pressure. The resulting residue was purified by silica gel column chromatography (SiO_2 :120 g, n-hexane: ethyl acetate: triethylamine =90:10:1→65:35:1) to obtain Compound 29 (5.50 g, Yield 93 %) as colorless foam. By $^1\text{H-NMR}$, it was observed a mixture of rotamers,

which is 1:1.

ESI-MS (m/z) : 778 (M+H).

¹H-NMR(CDCl₃)δ: 7.68 (d, J=7.5Hz, 1H), 7.62 (d, J=7.5Hz, 1H), 7.58-7.51 (m, 1H), 7.40-7.05 (m, 14H), 6.74-6.63 (m, 5H), 4.61-4.49 (m, 1H), 4.33-4.15 (2m, 1H), 4.12-4.03 (m, 1H), 3.93-3.85 (m, 1H), 3.62 (s, 6H), 3.56 (dd, J=10.7, 5.4Hz, 0.5H), 3.41-3.31 (m, 1H), 3.16 (dd, J=9.0, 4.3Hz, 0.5H), 3.00 (m, 0.5H), 2.90 (m, 0.5H), 2.13-2.02 (m, 1H), 1.97-1.82 (m, 1H), 0.82 (s, 1.5H), 0.81 (s, 3H), 0.779 (s, 3H), 0.787 (s, 1.5H), 0.007 (s, 1.5H), 0.000 (s, 1.5H), -0.015 (s, 1.5H), -0.026 (s, 1.5H).

工程3

To Compound 29 (2.63 g, 3.48 mmol) in DMF solution (10 ml), piperidine (0.379 ml, 3.83 mmol, 1.1 eq.) was added. The mixture was stirred at room temperature for 2 hours, and then concentrated under reduced pressure. The residue was diluted with ethyl acetate, washed with brine, and then dried over magnesium sulfate. The solvent was concentrated under reduced pressure. Methanol (10 ml) was added thereto, and the resulting precipitate was filtered. The filtrate was concentrated to obtain the crude product of Compound 30 (2.20 g).

ESI-MS (m/z) : 524 (M+H). HPLC Peak RT = 3.29 min.

Step 4

To Compound 30 (2.20 g, crude) in dichloromethane (15 ml), triethylamine (3.65 ml, 36.9 mmol) and acetoxyacetyl chloride (3.65 ml, 36.9 mmol) were added in order at room temperature, and the mixture was stirred at room temperature for 2 hours. The reaction mixture was diluted with ethyl acetate, washed with brine, and then dried over magnesium sulfate. The solvent was concentrated under reduced pressure to obtain the crude product of Compound 31.

ESI-MS (m/z) : 634 (M+H). HPLC Peak RT = 3.40 min.

Step 5

After Compound 31 obtained in Step 4 was dissolved in methanol (10 ml), 28 % sodium methoxide-methanol solution (0.60ml) was added thereto,

and the mixture was stirred at room temperature for 1 hour. The reaction mixture was diluted with ethyl acetate, washed with brine, and then dried over magnesium sulfate. The solvent was concentrated under reduced pressure.

The resulting residue was purified by silica gel column chromatography

(SiO₂:80 g, n-hexane:ethyl acetate:triethylamine =80:20:1→65:35:1) to obtain Compound 32 (1.45 g, Yield from Compound 30: 71 %) as colorless foam. By ¹H-NMR, it was observed a mixture of rotamers, which is 78:22.

ESI-MS (m/z) : 592 (M+H). HPLC Peak RT = 3.36 min.

¹H-NMR(CDCl₃)δ: (Major) 7.39-7.32 (m, 2H), 7.32-7.18 (m, 7H), 6.86-6.79 (m, 4H), 4.73 (m, 1H), 4.35 (m, 1H), 4.06 (dd, J=15.1, 4.4Hz, 1H), 3.98 (dd, J=15.1, 4.4Hz, 1H), 3.79 (s, 6H), 3.57 (dd, J=10.0, 4.0Hz, 1H), 3.52 (dd, J=10.0, 6.0Hz, 1H), 3.47 (dd, J=4.4, 4.4Hz, 1H), 3.13 (dd, J=10.0, 2.5Hz, 1H), 3.08 (dd, J=10.0, 5.0Hz, 1H), 2.19 (m, 1H), 1.93 (ddd, J=13.0, 8.8, 6.0Hz, 1H), 0.87 (s, 9H), 0.08 (s, 3H), 0.07 (s, 3H). (Minor) 7.39-7.32 (m, 2H), 7.32-7.18 (m, 7H), 6.86-6.79 (m, 4H), 4.54 (m, 1H), 4.06-3.98 (m, 1H), 3.92 (dd, J=14.6, 4.3Hz, 1H), 3.79 (s, 6H), 3.68 (dd, J=12.0, 4.0Hz, 1H), 3.62-3.42 (m, 2H), 3.16-3.02 (m, 2H), 2.11 (ddd, J=13.0, 5.8, 4.0Hz, 1H), 2.02 (m, 1H), 0.86 (s, 9H), 0.051 (s, 3H), 0.049 (s, 3H).

[0166]

Step 6

To Compound 3-16 (2.00 g, 3.21 mmol), which was synthesized in a similar method to 1-1-6), suspended in dichloromethane (60 ml), DIEA (3.36 mL, 19.3 mmol, 4.0 eq.) was added, and the solution was added to methyl N,N-diisopropylchlorophosphoramidite (2.54 g, 12.8 mmol, 2.0 eq.) in dichloromethane (10 ml). The mixture was stirred at 45 °C for 10 minutes.

After cooling to room temperature, the mixture was poured to aqueous saturated sodium bicarbonate solution, extracted with chloroform, and then washed with brine. After drying over magnesium sulfate, the solvent was concentrated under reduced pressure. Over stirring, acetonitrile (20 ml) was added to the resulting residue. The precipitated object was collected by filtration to obtain Compound 33-16 (2.36 g, Yield 94 %) as white solid.

¹H-NMR(CDCl₃)δ: 6.39 (t, J = 5.5 Hz, 1H, -NH), 6.18 (t, J = 5.5 Hz, 1H, -NH), 3.95 (m, 0.5H), 3.67-3.51 (m, 4.5H), 3.42 (s, 1.5H), 3.39 (s, 1.5H), 3.19-3.11 (m, 1H), 3.05-2.97 (m, 1H), 2.30-2.15 (m, 4H), 1.70-1.57 (m, 4H), 1.36-1.23 (m, 56H), 1.23-1.14 (m, 12H), 0.88 (t, J=6.5Hz, 6H). ³¹P-NMR(CDCl₃)δ: 149.06

Step 7

To Compound 32 (700 mg, 1.18 mmol) and Compound 33-16 (1.87 g, 2.37 mmol, 2.0 eq.) dissolved in dichloromethane (14 ml), 1H-tetrazole (124 mg, 1.77 mmol, 1.5 eq.) was added, and the mixture was stirred at room temperature for 2 hours. 0.5 M DDTT solution ([dimethylamino-methylidene]amino]-3H-1,2,4-dithiazaolin-3-thion, 4.73 ml, dissolved in 3-picoline:acetonitrile =1:1) was added thereto, and the mixture was stirred at same temperature for 1 hour. The reaction mixture was diluted with ethyl acetate, washed with 10 % citric acid solution, aqueous saturated sodium bicarbonate solution and brine in order, and then dried over magnesium sulfate. The solvent was concentrated under reduced pressure, and the resulting residue was purified by silica gel column chromatography (SiO₂:45 g, n-hexane :ethyl acetate :triethylamine =75:25:1→50:50:1) to obtain Compound 34-16 (1.19 g, Yield 79 %)

¹H-NMR(CDCl₃)δ:7.40-7.11 (m, 9H), 6.87-6.78 (m, 4H), 4.94-4.84 (m, 1H), 4.82-4.72 (m, 1H), 4.58-4.43 (m, 2H), 4.38-4.31 (m, 1H), 3.86-3.53 (m, 4H), 3.80 (s, 3H), 3.79 (s, 6H), 3.33-2.99 (m, 4H), 2.30-1.88 (m, 6H), 1.65-1.55 (m, 4H), 1.35-1.20 (m, 56H), 0.91-0.84 (m, 15H), 0.08 (s, 3H), 0.01 (s, 3H).

Step 8

To Compound 34-16 (1.02 g, 0.780 mmol) in THF (10 ml), 1 mol/l TBAF-THF (0.937 ml, 1.2 eq.) was added at room temperature, and the mixture was stirred at room temperature for 1 hour. The reaction mixture was concentrated under reduced pressure. The resulting residue was purified by silica gel column chromatography (SiO₂:30 g, chloroform :methanol :triethylamine =97.5:2.5:1→90:10:1) to obtain Compound 35-16 (608 mg, Yield 65 %).

¹H-NMR(CDCl₃)δ:7.40-7.17 (m, 9H), 6.91 (m, 1H, -NH), 6.88-6.78 (m, 5H), 5.00-4.35 (m, 5H), 3.85-3.44 (m, 4H), 3.79 (s, 3H), 3.78 (s, 6H), 3.35-3.06 (m, 4H), 2.31-2.09 (m, 5H), 2.06-1.94 (m, 1H), 1.68-1.52 (m, 4H), 1.36-1.18 (m, 56H), 0.88 (t, J=7.0Hz, 6H).

Step 9

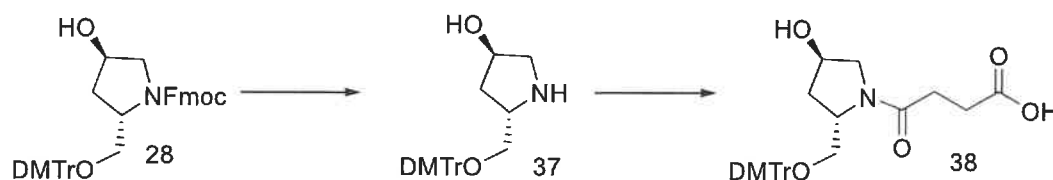
To Compound 35-16 (210 mg, 0.176 mmol) in dichloromethane (3 ml), triethylamine (0.073ml, 0.528mmol, 3.0eq.), succinic anhydride (35 mg, 0.352 mmol, 2.0 eq.) and DMAP (4 mg, 0.033 mmol) was added in order at room temperature, and the mixture was stirred at room temperature for 12 hours.

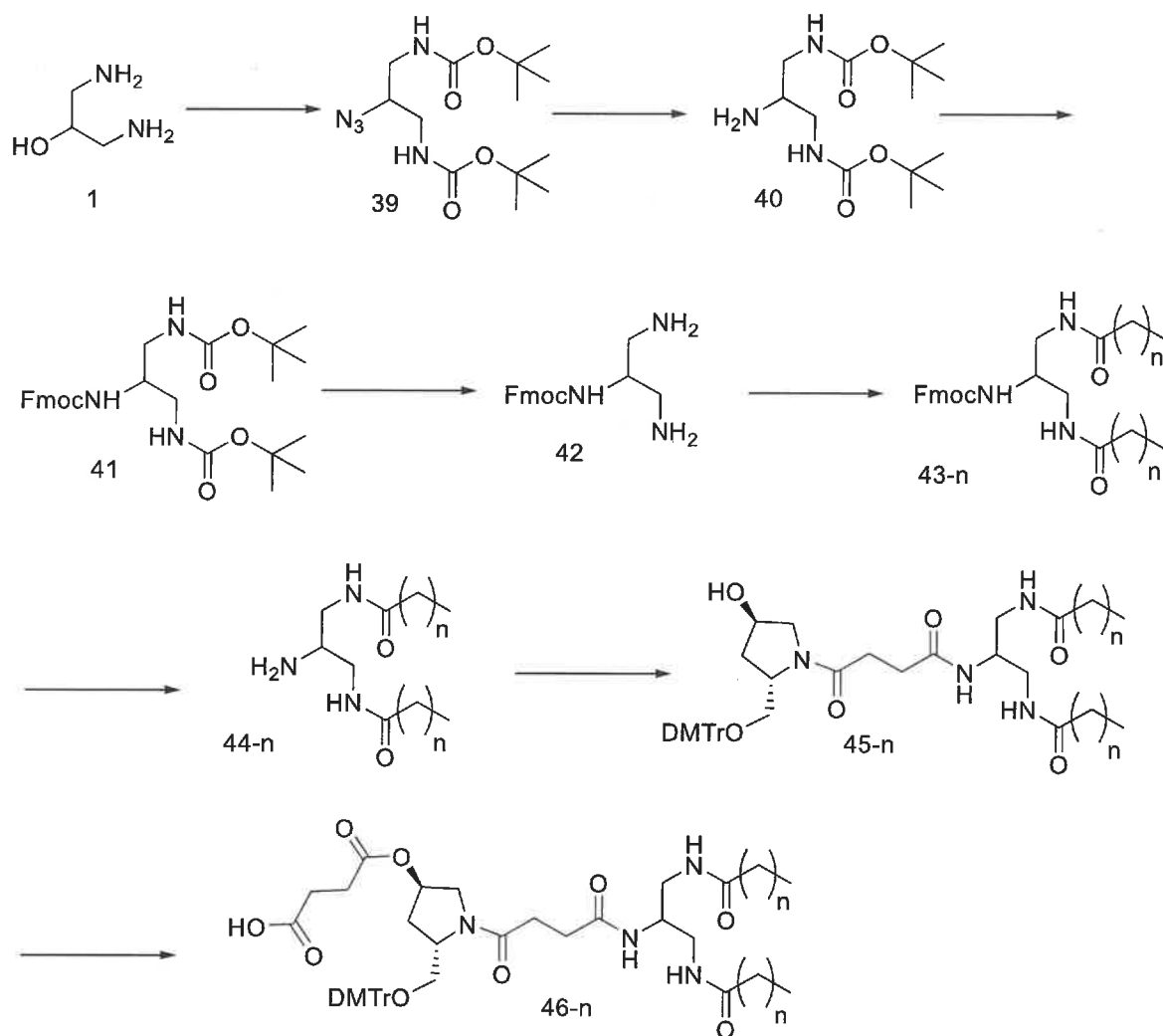
5 The reaction mixture was concentrated under reduced pressure. The resulting residue was purified by silica gel column chromatography (SiO₂:24 g, chloroform :methanol :triethylamine =100:0:1→95:5:1) to obtain Compound 36-16 (172 mg, Yield 76 %) as colorless solid.

¹H-NMR(CDCl₃)δ:7.38-7.32 (m, 2H), 7.38-7.32 (m, 7H), 6.90-6.76 (m, 4H), 5.42 (br.s, 1H), 5.04-4.39 (m, 4H), 3.87-3.55 (m, 4H), 3.79 (s, 3H), 3.78 (s, 6H), 3.31-3.07 (m, 4H), 2.65-2.43 (m, 4H), 2.38-2.28 (m, 1H), 2.28-2.08 (m, 5H), 1.67-1.53 (m, 4H), 1.35-1.19 (m, 56H), 0.88 (t, J=6.5Hz, 6H).

[0167]

10) Synthesis of Compound 46-n





wherein n is an integer of 6 to 28.

[0168]

5 10-1) Synthesis of Compound 46-16

Step 1

According to methods described in Nucleic Acids Research, 42, 8796-8807 (2014), Compound 37 was synthesized from Compound 28.

10 Step 2

To Compound 37 (3.00 g, 7.15 mmol) in dichloromethane (15 ml), triethylamine (1.98 ml, 14.3 mmol, 2.0 eq.) and succinic anhydride (751 mg, 7.51 mmol, 1.05 eq.) were added at room temperature, and the mixture was stirred at room temperature for 1 hour. The reaction mixture was concentrated under reduced pressure. The resulting residue was purified by silica gel column chromatography (SiO_2 :120g, chloroform: methanol:

triethylamine =95:5:1→75:25:1) to obtain Compound 38 (3.37 g, Yield 91 %) as colorless powder. By ¹H-NMR, it was observed a mixture of rotamers, which is 63:37.

ESI-MS (m/z) : 530 (M+H). HPLC Peak RT = 1.86 min.

5 ¹H-NMR(CDCl₃)δ:(Major) 7.39-7.33 (m, 2H), 7.30-7.22 (m, 6H), 7.22-7.16 (m, 1H), 6.85-6.77 (m, 4H), 4.50 (br.s, 1H), 4.41 (m, 1H), 3.88 (d, J=11.0Hz, 1H), 3.775 (s, 6H), 3.65 (dd, J=11.0, 4.0Hz, 1H), 3.43 (dd, J=9.2, 4.5Hz, 1H), 3.14 (dd, J=9.2, 2.7Hz, 1H), 2.85-1.97 (m, 6H). (Minor) 7.39-7.33 (m, 2H), 7.30-7.22 (m, 6H), 7.22-7.16 (m, 1H), 6.85-6.77 (m, 4H), 4.41 (m, 1H), 4.31 (br.s, 1H), 4.11 (d, 10 J=12.3Hz, 1H), 3.783 (s, 6H), 3.25 (dd, J=12.3, 3.5Hz, 1H), 3.18 (dd, J=9.5, 4.8Hz, 1H), 3.10 (dd, J=9.5, 4.8Hz, 1H), 2.85-1.97 (m, 6H).

Step 3

According to methods described in Journal of Medicinal Chemistry, 48, 15 7781 (2005), Compound 39 was synthesized from Compound 1.

Step 4

To Compound 39 (3.00 g, 7.15 mmol) in a mixture of THF-water (9:1) (30ml), triphenylphosphine (1.98 ml, 14.3 mmol, 2.0 eq.) was added at room 20 temperature, and the mixture was stirred at room temperature for 1 hour. The temperature was raised up to 70 °C, and the mixture was stirred for 4 hours. After cooling to room temperature, the reaction mixture was concentrated under reduced pressure to the crude product of obtain Compound 40 as colorless oil.

25

Step 5

To Compound 40 (7.15 mmol) in dichloromethane (30 ml), triethylamine (2.10 ml, 15.1 mmol, 1.2 eq.) and Fmoc-Cl (3.59 g, 13.9 mmol, 1.1 eq.) were added at room temperature, and the mixture was stirred at room temperature 30 for 1 hour. The reaction mixture was diluted with chloroform, washed with brine, and then dried over magnesium sulfate. The solvent was concentrated under reduced pressure. The resulting solid was washed with n-hexane and a little of chloroform, and then purified by silica gel column chromatography (SiO₂:120g, n-hexane :ethyl acetate =75:25→0:100) to obtain Compound 41

(4.18 g, Yield from Compound 39: 65 %) as colorless foam.

ESI-MS (m/z) : 512 (M+H). HPLC Peak RT = 2.71 min.

¹H-NMR(CDCl₃)δ:7.80 (d, J=7.5Hz, 2H), 7.60 (d, J=7.5Hz, 2H), 7.40 (dd, J=7.5, 7.5Hz, 2H), 7.30 (dd, J=7.5, 7.5Hz, 2H), 6.02 (br.s, 1H), 5.21 (br.s, 2H), 4.46-4.27 (m, 2H), 4.21 (m, 1H), 3.59 (m, 1H), 3.45-3.29 (m, 2H), 3.27-3.13 (m, 2H), 1.46 (s, 18H).

[0169]

Step 6

To Compound 41 (3.00 g, 7.15 mmol), TFA (30 ml) was added, and the mixture was stirred at room temperature for 1 hour. The mixture was concentrated under reduced pressure to obtain the crude product of Compound 42.

ESI-MS (m/z) : 312 (M+H). HPLC Peak RT = 1.00 min.

Step 7

To Compound 42 in dichloromethane (10 ml), triethylamine (1.17 ml, 8.44 mmol, 6.0 eq.) and stearoyl chloride (938 mg, 3.10 mmol, 2.2 eq.) was added at room temperature, and the mixture was stirred at room temperature for 1 hour. The resulting white precipitate was collected by filtration, washed with a little of chloroform, water and n-hexane, and then dried under reduced pressure to obtain Compound 43-16 (888 mg, Yield from Compound 40: 75 %) as colorless solid.

¹H-NMR(CDCl₃)δ:7.76 (d, J=7.5Hz, 2H), 7.60 (d, J=7.5Hz, 2H), 7.34 (d, J=7.5, 7.5Hz, 2H), 7.31 (d, J=7.5, 7.5Hz, 2H), 6.51 (br.s, 2H), 6.39 (br.s, 1H), 4.39-4.26 (m, 2H), 4.21 (m, 1H), 3.68-3.54 (m, 2H), 3.26-3.07 (m, 2H), 2.29-2.18 (m, 4H), 1.70-1.58 (m, 4H), 1.37-1.17 (m, 56H), 0.88 (t, J=7.0Hz, 6H).

Step 8

To Compound 43-16 (860 mg, 1.02 mmol) in DMF solution (5 ml), piperidine (0.111 ml, 1.12 mmol, 1.1 eq.) was added, and the mixture was stirred at 80 °C for 1.5 hours. After standing still at room temperature for 2 hours, the resulting precipitate was collected by filtration, washed with n-hexane, and dried under reduced pressure. The resulting solid was purified by silica gel column chromatography (SiO₂:24g, chloroform :methanol

=98:2→75:25) to obtain Compound 44-16 (198 mg, Yield 31 %) as colorless solid.

¹H-NMR(CDCl₃)δ:6.34 (br.s, 2H), 3.48-3.36 (m, 2H), 3.06-2.98 (m, 2H), 2.99 (s, 1H), 2.25-2.18 (m, 4H), 1.70-1.58 (m, 4H), 1.37-1.18 (m, 56H), 0.88 (t, J=7.0Hz, 6H).

Step 9

To Compound 38 (134 mg, 0.257 mmol) derived from Step 2 in DMF solution (2 mL), DIEA(225 μl, 1.29 mmol) and HBTU (127 mg, 0.334 mmol) were added at room temperature, and the mixture was stirred at room temperature for 10 minutes. The mixture was added to Compound 44-16 (218 mg, 17.1 mmol) in dichloromethane (3 mL) at 45 °C, and then the mixture was stirred for 30 minutes at 45 °C. The reaction mixture was washed with brine. After drying over magnesium sulfate, the solvent was concentrated under reduced pressure. The resulting residue was dissolved in ethyl acetate, and n-hexane was added thereto at 80 °C. The precipitated solid was collected by filtration to obtain Compound 45-16 (244 mg, Yield 85 %) as colorless solid. By ¹H-NMR, it was observed a mixture of rotamers, which is 63:37.

¹H-NMR(CDCl₃)δ:(Major) 7.35-7.32 (m, 2H), 7.31-7.16 (m, 7H), 6.85-6.77 (m, 4H), 6.46 (br.s, 1H, -NH), 4.38 (br.s, 1H), 3.781 (s, 6H), 3.72-3.58 (m, 2H), 3.51-3.36 (m, 2H), 3.28-3.08 (m, 3H), 2.78-2.60 (m, 2H), 2.48-1.98 (m, 7H), 1.64-1.56 (m, 4H), 1.35-1.19 (m, 56H), 0.88 (t, J=7.0Hz, 6H). (Minor) 7.35-7.32 (m, 2H), 7.31-7.16 (m, 7H), 6.85-6.77 (m, 4H), 6.46 (br.s, 1H, -NH), 4.53 (br.s, 1H), 3.783 (s, 6H), 3.72-3.58 (m, 2H), 3.51-3.36 (m, 2H), 3.28-3.08 (m, 3H), 2.48-1.98 (m, 7H), 1.64-1.56 (m, 4H), 1.35-1.19 (m, 56H), 0.88 (t, J=7.0Hz, 6H).

Step 10

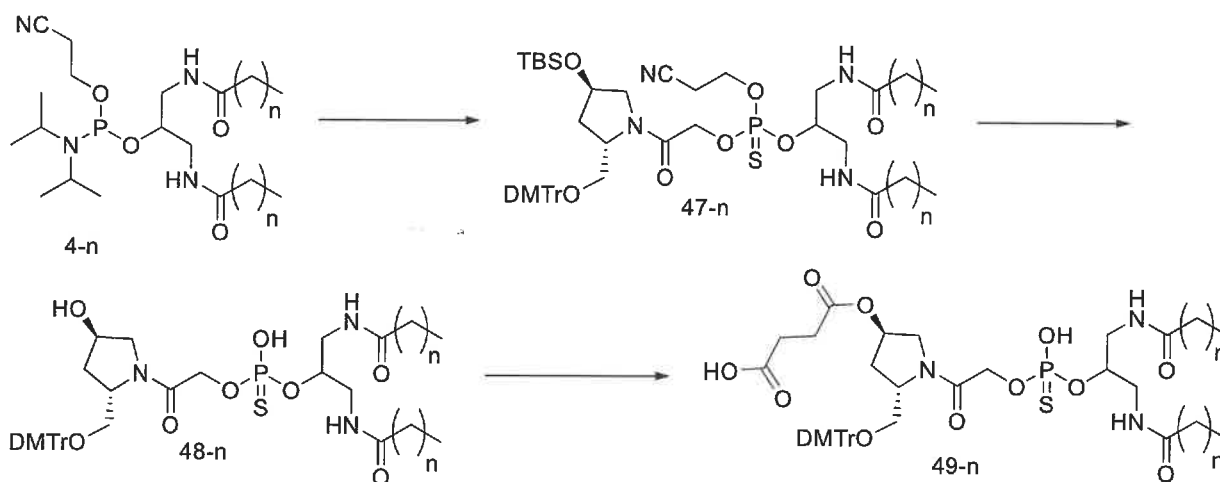
To Compound 45-16 (340 mg, 0.303 mmol) in dichloromethane (3 ml), triethylamine (0.237 ml, 1.71 mmol, 5.6 eq.), succinic anhydride (85 mg, 0.844 mmol, 2.8 eq.) and DMAP (4 mg) were added in order at room temperature. After the mixture was stirred at 45 °C for 2 hours, the reaction mixture was concentrated under reduced pressure. The resulting residue was purified by silica gel column chromatography (SiO₂:12 g, chloroform :methanol :triethylamine =100:0:1→80:20:1), and then washed with n-hexane to obtain

Compound 46-16 (246 mg, Yield 66 %) as colorless solid. By $^1\text{H-NMR}$, it was observed a mixture of rotamers, which is 77:23.

$^1\text{H-NMR}(\text{CDCl}_3)\delta$: (Major) 7.38-7.31 (m, 2H), 7.31-7.12 (m, 6H), 7.17-7.11 (m, 1H), 7.00 (br.s, 1H), 6.85-6.77 (m, 4H), 6.74 (br.s, 1H, -NH), 5.33 (br.s, 1H), 4.34 (br.s, 1H), 3.90-3.74 (m, 2H), 3.788 (s, 6H), 3.64-3.37 (m, 2H), 3.35-3.11 (m, 2H), 3.11-2.92 (m, 2H), 2.68-2.07 (m, 11H), 1.68-1.51 (m, 4H), 1.37-1.16 (m, 56H), 0.88 (t, $J=6.6\text{Hz}$, 6H). (Minor) 7.38-7.31 (m, 2H), 7.31-7.12 (m, 6H), 7.17-7.11 (m, 1H), 7.00 (br.s, 1H), 6.85-6.77 (m, 4H), 6.74 (br.s, 1H, -NH), 5.20 (br.s, 1H), 4.17 (br.s, 1H), 3.90-3.74 (m, 2H), 3.678 (s, 6H), 3.64-3.37 (m, 2H), 3.35-3.11 (m, 2H), 3.11-2.92 (m, 2H), 2.68-2.07 (m, 11H), 1.68-1.51 (m, 4H), 1.37-1.16 (m, 56H), 0.88 (t, $J=6.6\text{Hz}$, 6H).

[0170]

11) Synthesis of Compound 49-n



wherein n is an integer of 6 to 28.

[0171]

11-1) Synthesis of Compound 49-16

Step 1

In a similar method to synthesis of Compound 34-16 described in 9-1), Compound 47-16 was obtained as colorless foam (Yield 78 %).

$^1\text{H-NMR}(\text{CDCl}_3)\delta$: 7.34 (d, $J=8.0\text{Hz}$, 2H), 7.31-7.21 (m, 6H), 7.18 (m, 1H), 6.87-6.79 (m, 4H), 3.78 (s, 6H), 2.25-2.02 (m, 5H), 1.94-1.84 (m, 1H), 1.64-1.49 (m, 4H), 1.33-1.16 (m, 56H), 0.88 (t, $J=7.0\text{Hz}$, 6H), 0.87 (s, 9H), 0.06 (s, 6H).

Step 2

In a similar method to synthesis of Compound 35-16 described in 9-1), Compound 48-16 was obtained as colorless foam (Yield 61 %).

ESI-MS (m/z) : 1177 (M+). HPLC Peak RT = 3.64 min.

5 $^1\text{H-NMR}(\text{CDCl}_3)\delta$: 7.35 (d, J=7.8Hz, 2H), 7.31-7.16 (m, 7H), 6.87-6.78 (m, 4H), 4.98-4.49 (m, 3H), 4.48-4.31 (m, 2H), 3.91-3.44 (m, 5H), 3.78 (s, 6H), 3.37-3.08 (m, 4H), 2.27-2.11 (m, 5H), 2.08-1.94 (m, 1H), 1.66-1.52 (m, 4H), 1.34-1.21 (m, 56H), 0.88 (t, J=6.7Hz, 6H).

10 Step 3

To Compound 48-16 (624 mg, 0.529 mmol) in dichloromethane (5 ml), triethylamine (0.330 ml, 2.39 mmol, 4.5 eq.), succinic anhydride (106 mg, 0.159 mmol, 3.0 eq.) and DMAP (13 mg, 10.6 μmol , 0.2 eq.) were added in order at room temperature, and the mixture was stirred at room temperature for 12 hours. The reaction mixture was concentrated under reduced pressure. The resulting residue was purified by silica gel column chromatography (SiO_2 :24g, chloroform :methanol :triethylamine =97.5:2.5:1 $\rightarrow$ 80:20:1) to obtain Compound 49-16 (482 mg, Yield 71 %) as colorless solid.

[0172]

20 Although Compound 49-16 was a mixture of 2 kinds of isomers, a part of the mixture was separated by silica gel column chromatography and the various equipment data were measured.

Isomer 1 of Compound 49-16 (isomer which has higher Rf value based on TLC deploying with the solvent of chloroform : methanol = 5 : 1) : By $^1\text{H-NMR}$, it was observed a mixture of rotamers, which is 65:35.

ESI-MS (m/z) : 1277 (M+). HPLC Peak RT = 3.72 min.

25 $^1\text{H-NMR}(\text{CDCl}_3)\delta$: (Major) 7.34 (d, J=7.5Hz, 2H), 7.31-7.14 (m, 7H), 6.83 (d, J=8.3Hz, 4H), 5.45 (br.s, 1H), 4.75 (dd, J=14.1, 8.3Hz, 1H), 4.55-4.38 (m, 2H), 4.33 (m, 1H), 3.79 (s, 6H), 3.72-3.48 (m, 4H), 3.44-3.08 (m, 4H), 2.70-2.44 (m, 4H), 2.38-2.14 (m, 6H), 1.68-1.51 (m, 4H), 1.34-1.21 (m, 56H), 0.88 (t, J=6.7Hz, 6H). (Minor) 7.34 (d, J=7.5Hz, 2H), 7.31-7.14 (m, 7H), 6.83 (d, J=8.3Hz, 4H), 5.20 (br.s, 1H), 4.84 (m, 1H), 4.55-4.38 (m, 2H), 4.33 (m, 1H), 3.79 (s, 6H), 3.72-3.48 (m, 4H), 3.44-3.08 (m, 4H), 2.70-2.44 (m, 4H), 2.38-2.14 (m, 6H), 1.68-1.51

(m, 4H), 1.34-1.21 (m, 56H), 0.88 (t, J=6.7Hz, 6H). ^{31}P -NMR(CDCl_3) δ : 58.1

Isomer 2 of Compound 49-16 (isomer which has lower R_f value based on TLC deploying with the solvent of chloroform :methanol = 5 : 1)

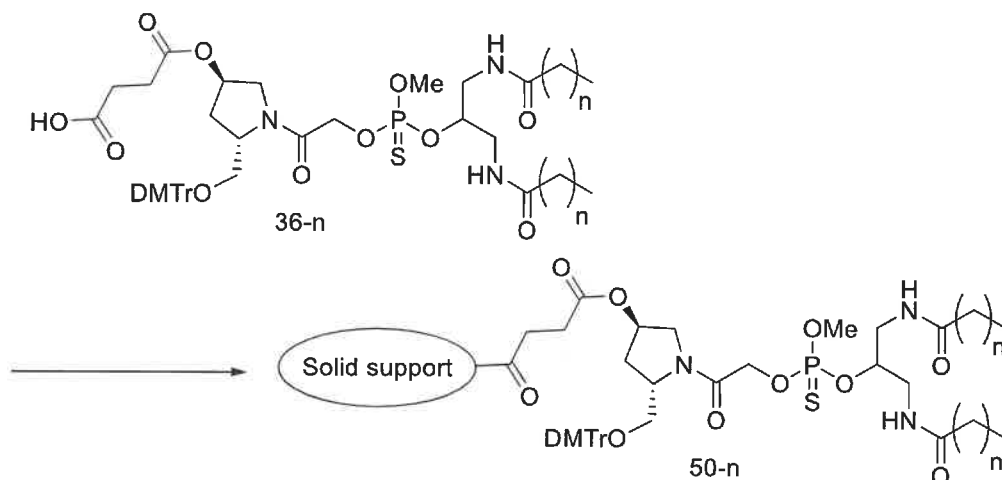
5 ESI-MS (m/z) : 1277 (M⁺). HPLC Peak RT = 3.72 min.

^1H -NMR(CDCl_3) δ : 7.83 (br.s, 1H), 7.37-7.10 (m, 9H), 6.74 (d, J=8.6Hz, 4H), 5.30 (br.s, 1H), 4.62 (dd, J=14.0, 9.2Hz, 1H), 4.42-4.33 (m, 2H), 4.30 (dd, J=14.0, 9.2Hz, 1H), 3.71 (s, 6H), 2.63-2.34 (m, 4H), 2.31-2.21 (m, 1H), 2.16-2.03 (m, 5H), 1.59-1.42 (m, 4H), 1.24-1.09 (m, 56H), 0.81 (t, J=6.7Hz, 6H). ^{31}P -

10 NMR(CDCl_3) δ : 57.3

[0173]

12) Synthesis of Compound 50-n



15 wherein n is an integer of 6 to 28.

[0174]

12-1) Synthesis of Compound 50-16

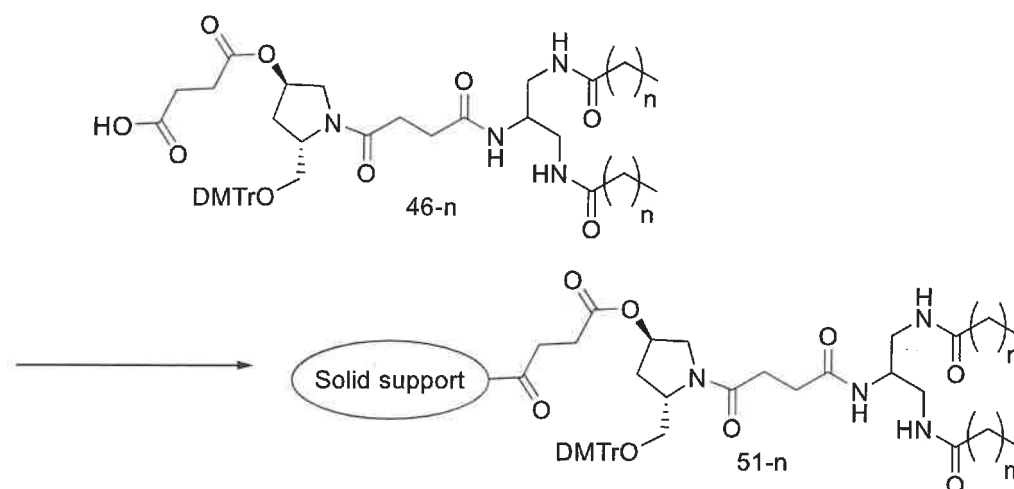
Compound 50-16 was obtained by subjecting Compound 36-16 obtained from 9-1) to the similar condition of a reaction to support on solid resin in 4).

20 The supported amount of Compound 36-16 was calculated by colorimetric assay of the DMTr cation, and Compound 50-16 whose supported amount is 32 $\mu\text{mol/g}$ was obtained.

In a similar method, Compound 49-n can be supported on solid resin.

[0175]

25 13) Synthesis of Compound 51-n



wherein n is an integer of 6 to 28.

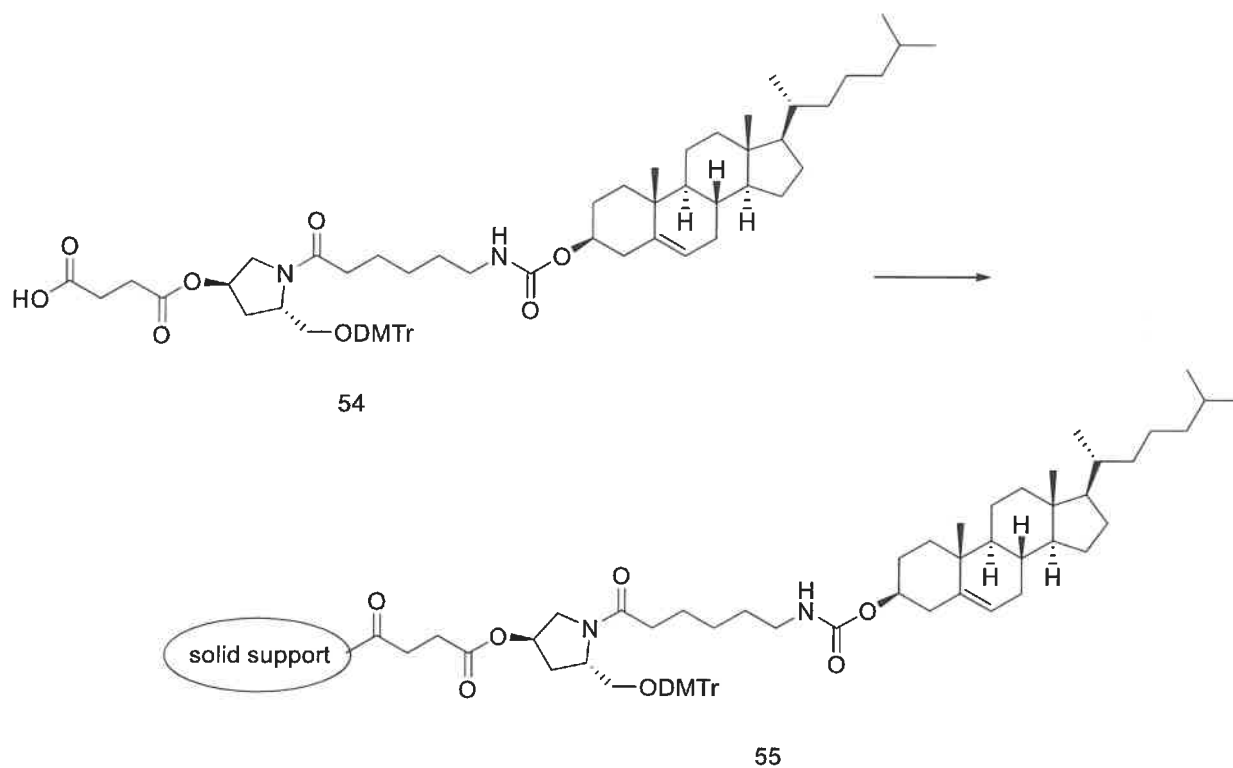
[0176]

5 13-1) Synthesis of compound 51-16

Compound 51-16 was obtained by subjecting Compound 46-16 obtained from 10-1) to the similar condition of a reaction to support on solid resin in 4). The supported amount of Compound 46-16 was calculated by colorimetric assay of the DMTr cation, and Compound 51-16 whose supported amount is 33
10 umol/g was obtained.

[0177]

14) Synthesis of compound 55



Compound 55 was obtained by subjecting Compound 54 described in US2008/0085869 to the similar condition of a reaction to support on solid resin in 4). The supported amount of Compound 54 was calculated by colorimetric assay of the DMTr cation, and Compound 55 whose supported amount is 90 $\mu\text{mol/g}$ was obtained.

[0178]

15) Synthesis of compound 63-n

(4.9 mL), DIEA (0.447 mL, 3.46 mmol) and HBTU (349 mg, 1.04 mmol) were added, and the mixture was stirred at room temperature for 30 minutes. Then, Compound 17 (0.488 g, 0.865 mmol) in DMF solution was added thereto, and the mixture was stirred overnight. After separating and extracting with
5 hexane/ethyl acetate =1:1 (2x50 mL) and aqueous saturated sodium bicarbonate solution (50 mL), the organic layers were combined, washed with brine (50 mL), and dried over magnesium sulfate. After filtrating, it was concentrated by rotary evaporator under reduced pressure, and purified by silica gel column chromatography (chloroform: methanol =100:0→70:30) to give
10 Compound 58 (0.837 g, 85 %) as colorless solid.

¹H-NMR (CDCl₃) δ: 7.75 (2H, d, J = 7.5 Hz), 7.60 (2H, d, J = 7.4 Hz), 7.42-7.38 (4H, m), 7.32-7.28 (7H, m), 7.24 (1H, s), 7.18 (1H, t, J = 7.3 Hz), 6.81 (4H, d, J = 8.9 Hz), 6.49 (1H, s), 6.08 (1H, s), 5.56 (1H, s), 4.39 (2H, d, J = 6.9 Hz), 4.21 (1H, t, J = 6.7 Hz), 3.79 (6H, s), 3.63-3.52 (15H, m), 3.34-3.28 (4H, m), 3.14
15 (2H, dd, J = 13.9, 6.4 Hz), 3.03 (2H, d, J = 5.6 Hz), 1.80-1.65 (5H, m), 1.63 (4H, s), 1.43-1.36 (2H, m), 1.31-1.26 (2H, m), 1.18-1.12 (2H, m), 0.84 (9H, s), 0.01 (6H, s).

Step 2

20 To Compound 58 (800 mg, 0.735 mmol) in DMF solution (8 mL), piperidine (80 uL) was added, and the mixture was stirred at room temperature for 2 hours. After separating and extracting with hexane/ethyl acetate =1:4 and water, it was dried over anhydrous sodium sulfate overnight. After filtrating, it was concentrated by rotary evaporator under reduced
25 pressure, and purified by amino silica gel column chromatography (chloroform: methanol =100:0→97:3) to give Compound 59 (0.178 g, 0.205 mmol) as colorless oil.

¹H-NMR (CDCl₃) δ: 7.37 (8H, dt, J = 50.4, 5.2 Hz), 7.26-7.18 (2H, m), 6.89 (1H, s), 6.82 (4H, t, J = 5.9 Hz), 6.25 (1H, t, J = 5.1 Hz), 3.81 (6H, s), 3.66-3.53 (15H, m), 3.35 (2H, q, J = 6.0 Hz), 3.16 (2H, dd, J = 13.9, 6.5 Hz), 3.04 (2H, d, J = 5.5 Hz), 2.80 (2H, t, J = 6.7 Hz), 1.80-1.17 (17H, m), 0.85 (9H, s), 0.03-0.01 (6H, m).

Step 3

After Compound 3-18 (140 mg, 0.205 mmol) and DMAP (63 mg, 0.514

mmol) were broken into small pieces in a flask, it dissolved in THF (1.8 mL). bis(4-nitrophenyl) carbonate (156 mg, 0.514 mmol) was added thereto, the mixture was stirred at 55 °C for 30 minutes. After cooling to room temperature, THF was concentrated under reduced pressure, and the residue was suspended in acetonitrile (10 mL) again. After heating to be almost solution, it cooled to room temperature, and the solid was precipitated. Then, the precipitation is promoted by ultrasonic breaking. The precipitated solid was collected by filtration, washed with acetonitrile (10 mL), water (10 mL) and acetonitrile (10 mL) in order, and then dried under vacuum to give Compound 18-18 (196 mg, 0.232 mmol) as pale yellow solid. Compound 18-18 (196 mg) was dissolved in THF (1.8 mL). Compound 59 (178 mg, 0.205 mmol) and DMAP (25 mg, 0.205 mmol) were added thereto, and the mixture was stirred at 55 °C for 2 hours. After cooling to room temperature, the solid was precipitated, and acetonitrile (18 mL) was added thereto. After heating and ultrasonic breaking, water (1.8 mL) was added thereto, and then the object, Compound 60-18 (251 mg, 0.160 mmol) as pale yellow solid, was obtained by filtration with a Kyriyama funnel.

[0180]

Step 4

After Compound 60-18 (246 mg, 0.157 mmol) in THF (4.9 mL) was cooled by ice-water bath, TBAF solution (1 M THF, 495 uL, 0.495 mmol) was added thereto, and the mixture was stirred and warmed to room temperature for 30 minutes. After the solvent was concentrated under reduced pressure, the resulting crude product was purified by amino silica gel column

chromatography (only chloroform) to give Compound 61-18 (192 mg, 0.132 mmol) as colorless solid.

¹H-NMR (CDCl₃) δ: 7.41 (2H, d, J = 7.3 Hz), 7.30 (5H, dd, J = 8.9, 2.1 Hz), 7.21 (1H, t, J = 7.2 Hz), 6.83 (5H, d, J = 8.7 Hz), 6.76 (1H, t, J = 6.2 Hz), 6.44 (1H, t, J = 6.6 Hz), 5.63 (1H, t, J = 5.7 Hz), 4.72-4.66 (1H, m), 3.79 (6H, s), 3.76-3.74 (1H, m), 3.64-3.48 (17H, m), 3.39-3.14 (6H, m), 3.06 (2H, dd, J = 9.0, 7.6 Hz), 2.76 (1H, t, J = 5.8 Hz), 2.50-2.46 (6H, m), 2.19 (6H, t, J = 7.6 Hz), 1.79-1.73 (7H, m), 1.49-1.42 (6H, m), 1.24 (68H, d, J = 11.8 Hz), 0.89-0.87 (6H, t, J = 6.3 Hz).

Step 5

To Compound 61-18 (191 mg, 0.131 mmol) in dichloromethane (1.9 mL), DIEA (0.069 mL, 0.393 mmol), DMAP (1.6 mg, 0.013 mmol) and succinic anhydride (20 mg, 0.197 mmol) were added, and the mixture was stirred under reflux for 3 hours. The solvent was concentrated under reduced pressure, and the residue was purified by diol silica gel column chromatography (only chloroform) to give Compound 62-18 (201 mg, 0.129 mmol) as colorless solid. ¹H-NMR (CDCl₃) δ: 7.42 (2H, d, J = 7.4 Hz), 7.30 (5H, d, J = 8.7 Hz), 7.19 (1H, t, J = 7.2 Hz), 6.81 (5H, d, J = 8.7 Hz), 6.74 (1H, t, J = 5.8 Hz), 6.26 (1H, t, J = 5.9 Hz), 5.55 (1H, t, J = 5.9 Hz), 4.72-4.67 (1H, m), 3.79 (6H, s), 3.58-3.49 (17H, m), 3.32-3.27 (6H, m), 3.14 (2H, q, J = 6.5 Hz), 3.04 (2H, d, J = 5.4 Hz), 2.49-2.46 (4H, m), 2.20 (5H, t, J = 7.5 Hz), 1.76-1.72 (4H, m), 1.65-1.62 (10H, m), 1.42-1.41 (3H, m), 1.25 (68H, s), 0.88 (6H, t, J = 6.6 Hz).

Step 6

After Compound 62-18 (153 mg, 0.098 mmol) was dissolved in a mixture of acetonitrile/dichloromethane (1:1, 10 mL), DIEA (0.067 mL, 0.392 mmol) and HBTU (41 mg, 0.108 mmol) were added, and the mixture was shaken at room temperature for 20 minutes. To the reaction mixture, Native Amino lcaa CPG 1000Å (ChemGenes Corporation) (1.0 g) was added, and the mixture was shaken for 24 hours. After the reaction mixture was filtered, CPG resin was washed twice with acetonitrile, twice with dichloromethane and twice with diethyl ether, and dried under reduced pressure. To the dried CPG, a mixture of CapA (PROLIGO, L840045-06) and CapB (PROLIGO, L850045-06) (1:1, 20mL) was added, and the mixture was shaken for 30 minutes. After the reaction mixture was filtered, CPG resin was washed twice with acetonitrile, twice with dichloromethane and twice with diethyl ether, and dried under reduced pressure. The supported amount of Compound 62-18 was calculated by colorimetric assay of the DMTr cation, and Compound 63-18 whose supported amount is 56 umol/g was obtained.

[0181]

B) Synthesis of oligonucleotides

Oligonucleotides used in examples of this description were synthesized using phosphoramidite method by AKTA Oligopilot10 (GE Healthcare), NS-8-I

(Dainippon Seiki co., ltd.) or NS-8-II (Dainippon Seiki co., ltd.). A monomer was prepared in 0.1 M acetonitrile solution using the amidite derived from the above amidite synthesis. The coupling time was 32 seconds to 10 minutes, and 8 to 10 equivalents of the amidite unit were used to condense with one monomer. 0.02 M Oxidizer (Sigma-Aldrich) and iodine/pyridine/water=12.7/9/1 (w/v/v) were used for PO oxidation. 50 mM DDTT ((dimethylamino-methylidene) amino-3H-1,2,4-dithiazoline-3-thion) in acetonitrile/3-picoline 1/1(v/v) or 1/4(v/v) and acetonitrile/pyridine 1/4 (v/v) solution were used for PS oxidation. ETT activator (5-ethylthio)-1H-tetrazole (Sigma-Aldrich) was used as an activator, CapA and CapB (Sigma-Aldrich) was used as a capping reagent. Deb (3 w/v% TCA CH₂ Cl₂ solution) (Wako Pure Chemical Industries, Ltd.) or Deb (3 w/v% Dichloroacetic acid, Toluene Solution) was used as a detritylation reagent.

SEQ-321, SEQ-323, SEQ-340, SEQ-343, SEQ-346, SEQ-349, SEQ-352, SEQ-355 and SEQ-358 were derived by providing Compound 22-18 to GeneDesign Inc. to consign nucleic synthesis and purification of oligonucleotides. DMT-butanediol phosphoramidite used for synthesis of SEQ-316 were purchased from ChemGenes Corporation.

[0182]

C) Synthesis of lipid-binding oligonucleotides

1) Synthesis from a synthesis amidite unit

A stirrer, Molecular Sieves 4A 1/16 and the amidite synthesized in the above A) (e.g., Compound 4-n, Compound 4-n,o, Compound 8-n and Compound 10-n. 10 to 100 equivalents of an oligonucleotide) were put in a microwave tube (2-5 ml, 10-20 ml) made by Biotage, and the solution was adjusted to 0.2 M with chloroform (added 2-methyl-2-butane as a stabilizer). After drying for 5 hours, a oligonucleotides supported to solid phase (CPG resin or polystyrene resin) and 0.25 M ETT activator, which is ((5-ethylthio)-1H-tetrazole) dichloromethane (the same amount of chloroform) were added, sealed and heated at 40 °C for 10 minutes to 1 hour. After cooling to room temperature, the reaction mixture was diluted twice with chloroform, and the resin was collected by filtration. The resulting resin was used in PS oxidization in NS-8-I (Dainippon Seiki co., ltd.) or NS-8-II (Dainippon Seiki co., ltd.). Then the dried resin was subject to the following deprotection condition I or II to

synthesize the target lipid-binding oligonucleotide.

2) Synthesis from lipid-supported resin

Using a resin supported lipid synthesized in the above A) (e.g., Compound 15-n, Compound 22-n, Compound 50-n, Compound 51-n, Compound 55 and Compound 63-n), the target lipid-binding oligonucleotides were synthesized in a similar method to the above B).

[0183]

D) Deprotection

1) Cleavage from the resin, and phosphate deprotection and base deprotection

For cutting out DNA oligonucleotide, 28 % ammonia water (SEQ-1, 3 or 49) or 28 % ammonia water/EtOH4/1(v/v) (Single strand oligonucleotides described in Examples except for SEQ-1, 3 and 49) was used and the solution was shaken at room temperature for 1 hour and at 55 °C for 5 hours. 1 ml, 5 ml or 10 ml of ammonia solution was used for 1 μmol, 5 μmol or 10 μmol synthesis, respectively, for cutting-out reaction. After the resin was washed with 50 % ethanol water, the filtrate was concentrated under reduced pressure to about 1 to 5 mL.

When the sequence contains RNA, the resulting solution was lyophilized to obtain white powder, and then the following deprotection reaction of the 2'-TBS group was performed.

[0184]

2) Deprotection of 2'-TBS group

To the resulting white powder, N-methylpyrrolidone/triethylamine/triethylamine trihydrofluoride=6/1/2(v/v) was added and the solution was stirred at 65 °C for 1.5 hours. To the reaction mixture was added the same amount of ethoxytrimethylsilane, and the solution was vigorously stirred at room temperature for 10 minutes to obtain the precipitate. After centrifugation at 2500 x g (2 minutes), the organic solvent layer was carefully removed. To the resulting precipitate, diethyl ether was added, and the solution was vigorously stirred. Then, in a similar way, the centrifugation was carried out and removed the organic solvent to obtain crude RNA unit (white solid).

[0185]

E) Purification

Oligonucleotides without a lipid ligand such as SEQ-1, 3 and 49 were purified by reversed phase HPLC in Condition 1.

Condition for reversed phase HPLC

5 Condition 1

Mobile phases

Buffer A: 100 mM TEAA (triethylammoniumacetate, pH 7.0) aqueous solution or 100 mM AcONa aqueous solution (pH5.4)

Buffer B: acetonitrile

10 B concentration gradient: 10-30 %

(Condition 1-1)

Column: Hydrosphere C18 (YMC co., ltd.) 100x20mm I.D., S-5 μ m, 12 nm

Flow rate: 10 mL/min

Column temperature: room temperature

15 Detection UV: 260 nm

(Condition 1-2)

Column: Hydrosphere C18 (YMC co., ltd.) 150x10mm I.D., S-5 μ m, 12 nm

Flow rate: 4 mL/min

20 Column temperature: room temperature

Detection UV: 260 nm

Single strand oligonucleotides with a lipid ligand(s) (Single strand oligonucleotides described in Examples except for SEQ-1, 3 and 49) was purified by reversed phase HPLC in Condition 2.

25 Condition 2

Condition for reversed phase HPLC

According to lipid solubility of the compound, B concentration at the beginning was adjusted from 20 % to 50 %.

30 Mobile phases

Buffer A: 100 mM TEAA (Triethylammonium acetate pH7.0) aqueous solution or 100 mM AcONa aqueous solution (pH5.4)

Buffer B: acetonitrile

B concentration gradient: 20-80 % (compounds having L₄₋₈ or L₄₋₁₀),

30-60 % (compounds having L_{Toc} or L_{chol}),
30-80 % (compounds having L_{4-12} , L_{4-14} , L_{4-16} , L_{4-18} , M_{22-12} or M_{51-16}),
40-80 % (compounds having L_{4-20} or L_{4-22}),
50-80 % (compounds having M_{22-18})

5

(Condition 2-1)

Column: YMC-Pack C4 (YMC co., ltd.) 100x20mm I.D., S-5 μ m, 12 nm

Flow rate: 10 mL/min

Column temperature: room temperature

10 Detection UV: 260 nm

(Condition 2-2)

Column: YMC-Pack C4 (YMC co., ltd.) 150x10mm I.D., S-5 μ m, 12 nm

Flow rate: 4 mL/min

15 Column temperature: room temperature

Detection UV: 260 nm

[0186]

F) Desalting and freeze-drying of the purified oligonucleotide

Using VivaSpin20 (MWCO 3000) (Sartorius) and Amicon Ultra-4

20 Centrifugal Filter Units-3K, ultrafiltration was repeated for the resulting oligonucleotide to remove salt component from the fraction. Then, it was lyophilized to obtain the target oligonucleotide as powder. For the oligonucleotides purified using TEAA solvent, the desalting procedure was carried out after transforming the salt form with 100 mM sodium acetate
25 solution (20 mL).

[0187]

G) Purity analysis of oligonucleotides

The resulting oligonucleotide was confirmed as the target sequence by matching the found molecular weights determined by UPLC/MS measurement
30 and the calculated molecular weights.

Condition 1 (SEQ-1, 3 or 49)

Xevo G2 ToF System (Waters)

Column: Aquity OST C18 (2.1x50 mm) (Waters)

Mobile phases

Buffer A: 200 mM 1,1,1,3,3,3-hexafluoro-2-propanol/8mM triethylamine
aqueous solution

Buffer B: methanol

B concentration gradient: 10-30 % (10 min)

5 Temperature: 50 °C

Flow rate: 0.2 mL/min

Condition 2 (Single strand oligonucleotides described in Examples except for
SEQ-1, 3 and 49)

10 Xevo G2 T of System (Waters)

Column: ACQUITY UPLC Protein BEH C4 Column, 300Å, 1.7µm,
2.1mm×100mm, 1/pkg (Waters)

Mobile phases

15 Buffer A: 200 mM 1,1,1,3,3,3-hexafluoro-2-propanol/8mM triethylamine
aqueous solution

Buffer B: methanol

B concentration gradient: 10-95 % (10 min)

Temperature: 50 °C

Flow rate: 0.2 mL/min

20 [0188]

The results were shown in Tables 1 to 3.

[0189]

[Table 1]

No.	Theoretical Mw [M-H]-	Found Mw [M]	No.	Theoretical Mw [M-H]	Found Mw [M]
SEQ-1	6363	6365	SEQ-76	5117	5116
SEQ-2	7064	7065	SEQ-78	5554	5554
SEQ-3	6254	6256	SEQ-80	5610	5610
SEQ-5	6955	6955	SEQ-82	5666	5666
SEQ-7	3842	3842	SEQ-84	6798	6799
SEQ-9	5367	5367	SEQ-86	6854	6855
SEQ-11	6731	6731	SEQ-88	6910	6911
SEQ-13	6843	6843	SEQ-90	8451	8452
SEQ-15	7012	7011	SEQ-92	8451	8452
SEQ-17	6698	6699	SEQ-94	8451	8452
SEQ-19	6369	6370	SEQ-96	8563	8564
SEQ-21	6040	6040	SEQ-98	8731	8733
SEQ-23	5736	5736	SEQ-100	8675	8677
SEQ-25	5423	5423	SEQ-102	8731	8733
SEQ-27	5133	5134	SEQ-104	8395	8396
SEQ-29	4829	4829	SEQ-106	8507	8508
SEQ-31	4500	4500	SEQ-108	8563	8564
SEQ-33	4211	4211	SEQ-110	6042	6042
SEQ-35	3898	3898	SEQ-112	6362	6363
SEQ-37	7570	7570	SEQ-114	7323	7323
SEQ-39	7291	7291	SEQ-116	6067	6067
SEQ-41	7330	7331	SEQ-118	6412	6413
SEQ-43	5844	5845	SEQ-120	7448	7447
SEQ-45	7363	7363	SEQ-122	7287	7287
SEQ-47	7419	7419	SEQ-124	7607	7607
SEQ-49	7697	7698	SEQ-126	8568	8569
SEQ-50	8211	8212	SEQ-128	7312	7312
SEQ-52	7569	7568	SEQ-130	7657	7658
SEQ-54	6967	6966	SEQ-132	8693	8693
SEQ-56	6348	6348	SEQ-134	8385	8386
SEQ-58	5722	5722	SEQ-136	5598	5599
SEQ-60	5409	5409	SEQ-138	8216	8218
SEQ-62	6049	6050	SEQ-140	5430	5431
SEQ-64	6099	6099	SEQ-142	8399	8398
SEQ-66	8932	8933	SEQ-143	9089	9090
SEQ-68	5859	5860	SEQ-144	8652	8651
SEQ-70	5816	5817	SEQ-145	5426	5427
SEQ-72	8451	8452	SEQ-147	5410	5410
SEQ-74	5161	5160	SEQ-149	5470	5470

[0190]

[Table 2]

No.	Theoretical Mw [M-H]-	Found Mw [M]	No.	Theoretical Mw [M-H]-	Found Mw [M]
SEQ-151	6758	6758	SEQ-229	5992	5992
SEQ-153	7102	7102	SEQ-231	6048	6049
SEQ-155	7793	7793	SEQ-233	5987	5987
SEQ-157	8139	8139	SEQ-235	8370	8370
SEQ-159	8484	8484	SEQ-237	6700	6701
SEQ-161	7761	7761	SEQ-239	7390	7392
SEQ-163	8074	8075	SEQ-241	6756	6757
SEQ-165	5912	5912	SEQ-243	7446	7448
SEQ-167	6602	6603	SEQ-245	6644	6645
SEQ-169	7638	7638	SEQ-247	7334	7336
SEQ-171	6380	6380	SEQ-249	6700	6701
SEQ-173	7368	7368	SEQ-251	7390	7391
SEQ-175	5782	5785	SEQ-253	6348	6349
SEQ-177	6408	6408	SEQ-254	5793	5792
SEQ-179	7509	7508	SEQ-256	6326	6328
SEQ-181	8481	8482	SEQ-257	5615	5615
SEQ-183	5694	5695	SEQ-259	5687	5687
SEQ-185	6073	6075	SEQ-261	5743	5743
SEQ-187	6764	6765	SEQ-263	5967	5968
SEQ-189	7800	7801	SEQ-265	6023	6024
SEQ-191	6129	6130	SEQ-267	5951	5952
SEQ-193	6820	6819	SEQ-269	6007	6010
SEQ-195	7856	7856	SEQ-271	6063	6064
SEQ-197	6017	6018	SEQ-273	6642	6642
SEQ-199	6707	6709	SEQ-275	6698	6699
SEQ-201	6073	6074	SEQ-277	6754	6754
SEQ-203	6764	6766	SEQ-279	6468	6469
SEQ-205	6007	6008	SEQ-281	6524	6525
SEQ-207	6063	6063	SEQ-283	6552	6553
SEQ-209	6119	6120	SEQ-285	7609	7608
SEQ-211	6698	6698	SEQ-287	7196	7196
SEQ-213	6754	6754	SEQ-289	8441	8442
SEQ-215	6810	6812	SEQ-291	7252	7252
SEQ-217	6119	6119	SEQ-293	8498	8499
SEQ-219	6175	6177	SEQ-295	7180	7184
SEQ-221	6810	6811	SEQ-297	7236	7240
SEQ-223	6867	6869	SEQ-299	7292	7296
SEQ-225	6192	6192	SEQ-301	7870	7873
SEQ-227	6028	6027	SEQ-303	7926	7929

[0191]

[Table 3]

No.	Theoretical Mw [M-H]-	Found Mw [M]	No.	Theoretical Mw [M-H]-	Found Mw [M]
SEQ-305	7983	7986	SEQ-346	5944	5942
SEQ-307	8425	8426	SEQ-348	9346	9345
SEQ-309	8537	8541	SEQ-349	5919	5916
SEQ-311	9115	9118	SEQ-351	7048	7046
SEQ-313	9171	9175	SEQ-352	5921	5920
SEQ-315	9227	9230	SEQ-354	8050	8049
SEQ-317	6247	6248	SEQ-355	5927	5926
SEQ-319	6213	6214	SEQ-357	6778	6777
SEQ-321	6109	6107	SEQ-358	5775	5771
SEQ-323	4248	4249	SEQ-360	5574	5575
SEQ-325	7236	7238	SEQ-362	5839	5840
SEQ-327	7292	7293	SEQ-364	5895	5897
SEQ-329	7348	7350	SEQ-366	5631	5631
SEQ-331	7926	7929	SEQ-368	5895	5896
SEQ-333	7983	7985	SEQ-370	5951	5953
SEQ-335	8039	8041	SEQ-372	8481	8483
SEQ-337	5619	5619	SEQ-374	8537	8539
SEQ-339	6433	6434	SEQ-376	8593	8595
SEQ-340	5775	5773	SEQ-378	9171	9173
SEQ-342	7342	7341	SEQ-380	7151	7154
SEQ-343	5936	5934	SEQ-381	5860	5861
SEQ-345	8341	8339	SEQ-383	8833	8835

5 [0192]

I) Preparation of the double-stranded oligonucleotide

Lipid binding double-stranded oligonucleotides of the present invention were prepared as below. After mixing the equimolecular amount of 100 μ M solution of each oligonucleotide, the solution was heated at 75 °C for 5 minutes, and naturally cooled to room temperature to obtain the double-stranded nucleic acids. Conformation of the double-stranded formation was carried out with size exclusion chromatography.

Column: YMC-PAC Diol-120 (4.6 x 300 mm) (YMC co., ltd.)

Mobile phases: 40 % acetonitrile in 1 x PBS solution

15 Flow rate: 0.5 mL/min

Temperature: room temperature

[0193]

The synthesized oligonucleotides are shown in Tables 4 to 27.

[0194]

[Table 4]

No.	ID (SEQ ID)	Sequence: CpG(5'⇒3') Complementary strand (3'⇒5')
SEQ-1	ODN1826 (1)	5'-t ⁺ c ⁺ c ⁺ a ⁺ t ⁺ g ⁺ a ⁺ c ⁺ g ⁺ t ⁺ t ⁺ c ⁺ c ⁺ t ⁺ g ⁺ a ⁺ c ⁺ g ⁺ t ⁺ t ⁺ -3'
SEQ-2	Amph1826 (1)	5'-L ₄₋₁₆ -t ⁺ c ⁺ c ⁺ a ⁺ t ⁺ g ⁺ a ⁺ c ⁺ g ⁺ t ⁺ t ⁺ c ⁺ c ⁺ t ⁺ g ⁺ a ⁺ c ⁺ g ⁺ t ⁺ t ⁺ -3'
SEQ-3	S-1 (2)	3'-a ⁺ g ⁺ gtactgcaaggactg ⁺ c ⁺ a ⁺ a ⁺ -5'
SEQ-4	ODN1826 (1) S-1 (2)	5'-t ⁺ c ⁺ c ⁺ a ⁺ t ⁺ g ⁺ a ⁺ c ⁺ g ⁺ t ⁺ t ⁺ c ⁺ c ⁺ t ⁺ g ⁺ a ⁺ c ⁺ g ⁺ t ⁺ t ⁺ -3' 3'-a ⁺ g ⁺ gtactgcaaggactg ⁺ c ⁺ a ⁺ a ⁺ -5'
SEQ-5	S-2 (2)	3'-a ⁺ g ⁺ gtactgcaaggactg ⁺ c ⁺ a ⁺ a ⁺ L ₄₋₁₆ -5'
SEQ-6	ODN1826 (1) S-2 (2)	5'-t ⁺ c ⁺ c ⁺ a ⁺ t ⁺ g ⁺ a ⁺ c ⁺ g ⁺ t ⁺ t ⁺ c ⁺ c ⁺ t ⁺ g ⁺ a ⁺ c ⁺ g ⁺ t ⁺ t ⁺ -3' 3'-a ⁺ g ⁺ gtactgcaaggactg ⁺ c ⁺ a ⁺ a ⁺ L ₄₋₁₆ -5'
SEQ-7	S-3 (3)	3'-a ⁺ g ⁺ gactg ⁺ c ⁺ a ⁺ a ⁺ L ₄₋₁₆ -5'
SEQ-8	ODN1826 (1) S-3 (3)	5'-t ⁺ c ⁺ c ⁺ a ⁺ t ⁺ g ⁺ a ⁺ c ⁺ g ⁺ t ⁺ t ⁺ c ⁺ c ⁺ t ⁺ g ⁺ a ⁺ c ⁺ g ⁺ t ⁺ t ⁺ -3' 3'-a ⁺ g ⁺ gactg ⁺ c ⁺ a ⁺ a ⁺ L ₄₋₁₆ -5'
SEQ-9	S-4 (4)	3'-c ⁺ t ⁺ gcaaggactg ⁺ c ⁺ a ⁺ a ⁺ L ₄₋₁₆ -5'
SEQ-10	ODN1826 (1) S-4 (4)	5'-t ⁺ c ⁺ c ⁺ a ⁺ t ⁺ g ⁺ a ⁺ c ⁺ g ⁺ t ⁺ t ⁺ c ⁺ c ⁺ t ⁺ g ⁺ a ⁺ c ⁺ g ⁺ t ⁺ t ⁺ -3' 3'-c ⁺ t ⁺ gcaaggactg ⁺ c ⁺ a ⁺ a ⁺ L ₄₋₁₆ -5'
SEQ-11	S-5 (2)	3'-a ⁺ g ⁺ gtactgcaaggactg ⁺ c ⁺ a ⁺ a ⁺ L ₄₋₈ -5'
SEQ-12	ODN1826 (1) S-5 (2)	5'-t ⁺ c ⁺ c ⁺ a ⁺ t ⁺ g ⁺ a ⁺ c ⁺ g ⁺ t ⁺ t ⁺ c ⁺ c ⁺ t ⁺ g ⁺ a ⁺ c ⁺ g ⁺ t ⁺ t ⁺ -3' 3'-a ⁺ g ⁺ gtactgcaaggactg ⁺ c ⁺ a ⁺ a ⁺ L ₄₋₈ -5'
SEQ-13	S-6 (2)	3'-a ⁺ g ⁺ gtactgcaaggactg ⁺ c ⁺ a ⁺ a ⁺ L ₄₋₁₂ -5'
SEQ-14	ODN1826(1) S-6 (2)	5'-t ⁺ c ⁺ c ⁺ a ⁺ t ⁺ g ⁺ a ⁺ c ⁺ g ⁺ t ⁺ t ⁺ c ⁺ c ⁺ t ⁺ g ⁺ a ⁺ c ⁺ g ⁺ t ⁺ t ⁺ -3' 3'-a ⁺ g ⁺ gtactgcaaggactg ⁺ c ⁺ a ⁺ a ⁺ L ₄₋₁₂ -5'
SEQ-15	S-7 (2)	3'-a ⁺ g ⁺ gtactgcaaggactg ⁺ c ⁺ a ⁺ a ⁺ L ₄₋₁₆ -5'
SEQ-16	ODN1826 (1) S-7 (2)	5'-t ⁺ c ⁺ c ⁺ a ⁺ t ⁺ g ⁺ a ⁺ c ⁺ g ⁺ t ⁺ t ⁺ c ⁺ c ⁺ t ⁺ g ⁺ a ⁺ c ⁺ g ⁺ t ⁺ t ⁺ -3' 3'-a ⁺ g ⁺ gtactgcaaggactg ⁺ c ⁺ a ⁺ a ⁺ L ₄₋₁₈ -5'
SEQ-17	S-8 (5)	3'-g ⁺ g ⁺ tactgcaaggactg ⁺ c ⁺ a ⁺ a ⁺ L ₄₋₁₈ -5'
SEQ-18	ODN1826 (1) S-8 (5)	5'-t ⁺ c ⁺ c ⁺ a ⁺ t ⁺ g ⁺ a ⁺ c ⁺ g ⁺ t ⁺ t ⁺ c ⁺ c ⁺ t ⁺ g ⁺ a ⁺ c ⁺ g ⁺ t ⁺ t ⁺ -3' 3'-g ⁺ g ⁺ tactgcaaggactg ⁺ c ⁺ a ⁺ a ⁺ L ₄₋₁₈ -5'
SEQ-19	S-9 (6)	3'-g ⁺ t ⁺ actgcaaggactg ⁺ c ⁺ a ⁺ a ⁺ L ₄₋₁₈ -5'
SEQ-20	ODN1826 (1) S-9 (6)	5'-t ⁺ c ⁺ c ⁺ a ⁺ t ⁺ g ⁺ a ⁺ c ⁺ g ⁺ t ⁺ t ⁺ c ⁺ c ⁺ t ⁺ g ⁺ a ⁺ c ⁺ g ⁺ t ⁺ t ⁺ -3' 3'-g ⁺ t ⁺ actgcaaggactg ⁺ c ⁺ a ⁺ a ⁺ L ₄₋₁₈ -5'
SEQ-21	S-10 (7)	3'-t ⁺ a ⁺ ctgcaaggactg ⁺ c ⁺ a ⁺ a ⁺ L ₄₋₁₈ -5'
SEQ-22	ODN1826 (1) S-10 (7)	5'-t ⁺ c ⁺ c ⁺ a ⁺ t ⁺ g ⁺ a ⁺ c ⁺ g ⁺ t ⁺ t ⁺ c ⁺ c ⁺ t ⁺ g ⁺ a ⁺ c ⁺ g ⁺ t ⁺ t ⁺ -3' 3'-t ⁺ a ⁺ ctgcaaggactg ⁺ c ⁺ a ⁺ a ⁺ L ₄₋₁₈ -5'
SEQ-23	S-11 (8)	3'-a ⁺ c ⁺ tgcaaggactg ⁺ c ⁺ a ⁺ a ⁺ L ₄₋₁₈ -5'
SEQ-24	ODN1826 (1) S-11 (8)	5'-t ⁺ c ⁺ c ⁺ a ⁺ t ⁺ g ⁺ a ⁺ c ⁺ g ⁺ t ⁺ t ⁺ c ⁺ c ⁺ t ⁺ g ⁺ a ⁺ c ⁺ g ⁺ t ⁺ t ⁺ -3' 3'-a ⁺ c ⁺ tgcaaggactg ⁺ c ⁺ a ⁺ a ⁺ L ₄₋₁₈ -5'
SEQ-25	S-12 (4)	3'-c ⁺ t ⁺ gcaaggactg ⁺ c ⁺ a ⁺ a ⁺ L ₄₋₁₈ -5'
SEQ-26	ODN1826 (1) S-12 (4)	5'-t ⁺ c ⁺ c ⁺ a ⁺ t ⁺ g ⁺ a ⁺ c ⁺ g ⁺ t ⁺ t ⁺ c ⁺ c ⁺ t ⁺ g ⁺ a ⁺ c ⁺ g ⁺ t ⁺ t ⁺ -3' 3'-c ⁺ t ⁺ gcaaggactg ⁺ c ⁺ a ⁺ a ⁺ L ₄₋₁₈ -5'
SEQ-27	S-13 (9)	3'-t ⁺ g ⁺ caaggactg ⁺ c ⁺ a ⁺ a ⁺ L ₄₋₁₈ -5'
SEQ-28	ODN1826 (1) S-13 (9)	5'-t ⁺ c ⁺ c ⁺ a ⁺ t ⁺ g ⁺ a ⁺ c ⁺ g ⁺ t ⁺ t ⁺ c ⁺ c ⁺ t ⁺ g ⁺ a ⁺ c ⁺ g ⁺ t ⁺ t ⁺ -3' 3'-t ⁺ g ⁺ caaggactg ⁺ c ⁺ a ⁺ a ⁺ L ₄₋₁₈ -5'

[0195]

[Table 5]

No.	ID (SEQ ID)	Sequence: CpG(5'⇒3') Complementary strand (3'⇒5')
SEQ-29	S-14(10)	3'-g [^] c [^] aaggactg [^] c [^] a [^] a [^] L ₄₋₁₈ -5'
SEQ-30	ODN1826(1) S-14(10)	5'-t [^] c [^] c [^] a [^] t [^] g [^] a [^] c [^] g [^] t [^] t [^] c [^] c [^] t [^] g [^] a [^] c [^] g [^] t [^] t-3' 3'-g [^] c [^] aaggactg [^] c [^] a [^] a [^] L ₄₋₁₈ -5'
SEQ-31	S-15(11)	3'-c [^] a [^] aggactg [^] c [^] a [^] a [^] L ₄₋₁₈ -5'
SEQ-32	ODN1826(1) S-15(11)	5'-t [^] c [^] c [^] a [^] t [^] g [^] a [^] c [^] g [^] t [^] t [^] c [^] c [^] t [^] g [^] a [^] c [^] g [^] t [^] t-3' 3'-c [^] a [^] aggactg [^] c [^] a [^] a [^] L ₄₋₁₈ -5'
SEQ-33	S-16(12)	3'-a [^] a [^] ggactg [^] c [^] a [^] a [^] L ₄₋₁₈ -5'
SEQ-34	ODN1826(1) S-16(12)	5'-t [^] c [^] c [^] a [^] t [^] g [^] a [^] c [^] g [^] t [^] t [^] c [^] c [^] t [^] g [^] a [^] c [^] g [^] t [^] t-3' 3'-a [^] a [^] ggactg [^] c [^] a [^] a [^] L ₄₋₁₈ -5'
SEQ-35	S-17(3)	3'-a [^] g [^] gactg [^] c [^] a [^] a [^] L ₄₋₁₈ -5'
SEQ-36	ODN1826(1) S-17(3)	5'-t [^] c [^] c [^] a [^] t [^] g [^] a [^] c [^] g [^] t [^] t [^] c [^] c [^] t [^] g [^] a [^] c [^] g [^] t [^] t-3' 3'-a [^] g [^] gactg [^] c [^] a [^] a [^] L ₄₋₁₈ -5'
SEQ-37	S-18(2)	3'-aOMe [^] gOMe [^] gOMe [^] uOMe [^] uOMe [^] aOMe [^] COMe [^] uOMe [^] uOMe [^] gOMe [^] COMe [^] aOMe [^] aOMe [^] gOMe [^] gOMe [^] aOMe [^] COMe [^] uOMe [^] uOMe [^] gOMe [^] COMe [^] aOMe [^] aOMe [^] L ₄₋₁₈ -5'
SEQ-38	ODN1826(1) S-18(2)	5'-t [^] c [^] c [^] a [^] t [^] g [^] a [^] c [^] g [^] t [^] t [^] c [^] c [^] t [^] g [^] a [^] c [^] g [^] t [^] t-3' 3'-aOMe [^] gOMe [^] gOMe [^] uOMe [^] uOMe [^] aOMe [^] COMe [^] uOMe [^] uOMe [^] gOMe [^] COMe [^] aOMe [^] aOMe [^] gOMe [^] gOMe [^] aOMe [^] COMe [^] uOMe [^] uOMe [^] gOMe [^] COMe [^] aOMe [^] aOMe [^] L ₄₋₁₈ -5'
SEQ-39	S-19(2)	3'-A [^] G [^] GTACTGCAAGGACTG [^] C [^] A [^] A [^] L ₄₋₁₈ -5'
SEQ-40	ODN1826(1) S-19(2)	5'-t [^] c [^] c [^] a [^] t [^] g [^] a [^] c [^] g [^] t [^] t [^] c [^] c [^] t [^] g [^] a [^] c [^] g [^] t [^] t-3' 3'-A [^] G [^] GTACTGCAAGGACTG [^] C [^] A [^] A [^] L ₄₋₁₈ -5'
SEQ-41	S-20(2)	3'-A _F [^] G _F [^] G _F [^] U _F A _F [^] C _F [^] U _F G _F [^] C _F [^] A _F [^] A _F [^] G _F [^] G _F [^] A _F [^] C _F [^] U _F G _F [^] C _F [^] A _F [^] A _F [^] L ₄₋₁₈ -5'
SEQ-42	ODN1826(1) S-20(2)	5'-t [^] c [^] c [^] a [^] t [^] g [^] a [^] c [^] g [^] t [^] t [^] c [^] c [^] t [^] g [^] a [^] c [^] g [^] t [^] t-3' 3'-A _F [^] G _F [^] G _F [^] U _F A _F [^] C _F [^] U _F G _F [^] C _F [^] A _F [^] A _F [^] G _F [^] G _F [^] A _F [^] C _F [^] U _F G _F [^] C _F [^] A _F [^] A _F [^] L ₄₋₁₈ -5'
SEQ-43	S-21(4)	3'-COMe [^] uOMe [^] uOMe [^] gOMe [^] COMe [^] aOMe [^] aOMe [^] gOMe [^] gOMe [^] aOMe [^] COMe [^] uOMe [^] uOMe [^] gOMe [^] COMe [^] aOMe [^] aOMe [^] L ₄₋₁₈ -5'
SEQ-44	ODN1826(1) S-21(4)	5'-t [^] c [^] c [^] a [^] t [^] g [^] a [^] c [^] g [^] t [^] t [^] c [^] c [^] t [^] g [^] a [^] c [^] g [^] t [^] t-3' 3'-COMe [^] uOMe [^] uOMe [^] gOMe [^] COMe [^] aOMe [^] aOMe [^] gOMe [^] gOMe [^] aOMe [^] COMe [^] uOMe [^] uOMe [^] gOMe [^] COMe [^] aOMe [^] aOMe [^] L ₄₋₁₈ -5'
SEQ-45	S-22(2)	3'-M ₁₅₋₆ [^] a [^] g [^] gtactgcaaggactg [^] c [^] a [^] a [^] L ₄₋₁₈ -5'
SEQ-46	ODN1826(1) S-22(2)	5'-t [^] c [^] c [^] a [^] t [^] g [^] a [^] c [^] g [^] t [^] t [^] c [^] c [^] t [^] g [^] a [^] c [^] g [^] t [^] t-3' 3'-M ₁₅₋₆ [^] a [^] g [^] gtactgcaaggactg [^] c [^] a [^] a [^] L ₄₋₁₈ -5'
SEQ-47	S-23(2)	3'-M ₁₅₋₁₀ [^] a [^] g [^] gtactgcaaggactg [^] c [^] a [^] a [^] L ₄₋₁₈ -5'
SEQ-48	ODN1826(1) S-23(2)	5'-t [^] c [^] c [^] a [^] t [^] g [^] a [^] c [^] g [^] t [^] t [^] c [^] c [^] t [^] g [^] a [^] c [^] g [^] t [^] t-3' 3'-M ₁₅₋₁₀ [^] a [^] g [^] gtactgcaaggactg [^] c [^] a [^] a [^] L ₄₋₁₈ -5'
SEQ-49	ODN2006(13)	5'-t [^] c [^] g [^] t [^] c [^] g [^] t [^] t [^] t [^] g [^] t [^] c [^] g [^] t [^] t [^] t [^] g [^] t [^] c [^] g [^] t [^] t-3'

[0196]

[Table 6]

No.	ID (SEQ ID)	Sequence: CpG(5'⇒3') Complementary strand (3'⇒5')
SEQ-50	S-24(14)	3'-a [^] g [^] cagcaaaacagcaaaacag [^] c [^] a [^] a [^] L ₄₋₁₈ -5'
SEQ-51	ODN2006(13) S-24(14)	5'-t [^] c [^] g [^] t [^] c [^] g [^] t [^] t [^] t [^] g [^] t [^] c [^] g [^] t [^] t [^] t [^] g [^] t [^] c [^] g [^] t [^] t-3' 3'-a [^] g [^] cagcaaaacagcaaaacag [^] c [^] a [^] a [^] L ₄₋₁₈ -5'
SEQ-52	S-25(15)	3'-c [^] a [^] gcaaaacagcaaaacag [^] c [^] a [^] a [^] L ₄₋₁₈ -5'
SEQ-53	ODN2006(13) S-25(15)	5'-t [^] c [^] g [^] t [^] c [^] g [^] t [^] t [^] t [^] g [^] t [^] c [^] g [^] t [^] t [^] t [^] g [^] t [^] c [^] g [^] t [^] t-3' 3'-c [^] a [^] gcaaaacagcaaaacag [^] c [^] a [^] a [^] L ₄₋₁₈ -5'
SEQ-54	S-26(16)	3'-g [^] c [^] aaaacagcaaaacag [^] c [^] a [^] a [^] L ₄₋₁₈ -5'
SEQ-55	ODN2006(13) S-26(16)	5'-t [^] c [^] g [^] t [^] c [^] g [^] t [^] t [^] t [^] g [^] t [^] c [^] g [^] t [^] t [^] t [^] g [^] t [^] c [^] g [^] t [^] t-3' 3'-g [^] c [^] aaaacagcaaaacag [^] c [^] a [^] a [^] L ₄₋₁₈ -5'
SEQ-56	S-27(17)	3'-a [^] a [^] aacagcaaaacag [^] c [^] a [^] a [^] L ₄₋₁₈ -5'
SEQ-57	ODN2006(13) S-27(17)	5'-t [^] c [^] g [^] t [^] c [^] g [^] t [^] t [^] t [^] g [^] t [^] c [^] g [^] t [^] t [^] t [^] g [^] t [^] c [^] g [^] t [^] t-3' 3'-a [^] a [^] aacagcaaaacag [^] c [^] a [^] a [^] L ₄₋₁₈ -5'
SEQ-58	S-28(18)	3'-a [^] a [^] cagcaaaacag [^] c [^] a [^] a [^] L ₄₋₁₈ -5'
SEQ-59	ODN2006(13) S-28(18)	5'-t [^] c [^] g [^] t [^] c [^] g [^] t [^] t [^] t [^] g [^] t [^] c [^] g [^] t [^] t [^] t [^] g [^] t [^] c [^] g [^] t [^] t-3' 3'-a [^] a [^] cagcaaaacag [^] c [^] a [^] a [^] L ₄₋₁₈ -5'
SEQ-60	S-29(19)	3'-a [^] c [^] agcaaaacag [^] c [^] a [^] a [^] L ₄₋₁₈ -5'
SEQ-61	ODN2006(13) S-29(19)	5'-t [^] c [^] g [^] t [^] c [^] g [^] t [^] t [^] t [^] g [^] t [^] c [^] g [^] t [^] t [^] t [^] g [^] t [^] c [^] g [^] t [^] t-3' 3'-a [^] c [^] agcaaaacag [^] c [^] a [^] a [^] L ₄₋₁₈ -5'
SEQ-62	S-30(19)	3'-a [^] c [^] agcaaaacag [^] c [^] a [^] a [^] t [^] t [^] L ₄₋₁₈ -5'
SEQ-63	ODN2006(13) S-30(19)	5'-t [^] c [^] g [^] t [^] c [^] g [^] t [^] t [^] t [^] g [^] t [^] c [^] g [^] t [^] t [^] t [^] g [^] t [^] c [^] g [^] t [^] t-3' 3'-a [^] c [^] agcaaaacag [^] c [^] a [^] a [^] t [^] t [^] L ₄₋₁₈ -5'
SEQ-64	S-31(19)	3'-a [^] c [^] agcaaaacag [^] c [^] a [^] a [^] g [^] g [^] L ₄₋₁₈ -5'
SEQ-65	ODN2006(13) S-31(19)	5'-t [^] c [^] g [^] t [^] c [^] g [^] t [^] t [^] t [^] g [^] t [^] c [^] g [^] t [^] t [^] t [^] g [^] t [^] c [^] g [^] t [^] t-3' 3'-a [^] c [^] agcaaaacag [^] c [^] a [^] a [^] g [^] g [^] L ₄₋₁₈ -5'
SEQ-66	S-32(14)	3'-aOMe [^] gOMe [^] cOMeaOMegOMeCOMeaOMeaOMeaOMeaOMeCOMeaOMe gOMeCOMeaOMeaOMeaOMeaOMeCOMeaOMegOMe [^] cOMe [^] aOMe [^] aOMe [^] L ₄₋₁₈ -5'
SEQ-67	ODN2006(13) S-32(14)	5'-t [^] c [^] g [^] t [^] c [^] g [^] t [^] t [^] t [^] g [^] t [^] c [^] g [^] t [^] t [^] t [^] g [^] t [^] c [^] g [^] t [^] t-3' 3'-aOMe [^] gOMe [^] cOMeaOMegOMeCOMeaOMeaOMeaOMeaOMeCOMeaOMe gOMeCOMeaOMeaOMeaOMeaOMeCOMeaOMegOMe [^] cOMe [^] aOMe [^] aOMe [^] L ₄₋₁₈ -5'

[0197]

[Table 7]

No.	ID (SEQ ID)	Sequence: CpG(5'⇒3') Complementary strand (3'⇒5')
SEQ-68	S-33(19)	3'-aOMe^cOMe^aOMeGOMeCOMe^aOMe^aOMe^aOMe^cOMe^aOMe^aOMe^L4-18-5'
SEQ-69	ODN2006(13) S-33(19)	5'-t^c^g^t^c^g^t^t^t^t^g^t^c^g^t^t^t^t^g^t^c^g^t^t-3' 3'-aOMe^cOMe^aOMeGOMeCOMe^aOMe^aOMe^aOMe^cOMe^aOMe^aOMe^L4-18-5'
SEQ-70	S-34(19)	3'-M ₁₅₋₁₀ ^a^c^agcaaaacag^c^a^a^L4-18-5'
SEQ-71	ODN2006(13) S-34(19)	5'-t^c^g^t^c^g^t^t^t^t^g^t^c^g^t^t^t^t^g^t^c^g^t^t-3' 3'-M ₁₅₋₁₀ ^a^c^agcaaaacag^c^a^a^L4-18-5'
SEQ-72	S-35(14)	3'-M ₁₅₋₁₀ ^a^g^cagcaaaacagcaaaacag^c^a^a^L4-12-5'
SEQ-73	ODN2006(13) S-35(14)	5'-t^c^g^t^c^g^t^t^t^t^g^t^c^g^t^t^t^t^g^t^c^g^t^t-3' 3'-M ₁₅₋₁₀ ^a^g^cagcaaaacagcaaaacag^c^a^a^L4-12-5'
SEQ-74	S-36(19)	3'-a^c^agcaaaacag^c^a^a^L _{Toc} -5'
SEQ-75	ODN2006(13) S-36(19)	5'-t^c^g^t^c^g^t^t^t^t^g^t^c^g^t^t^t^t^g^t^c^g^t^t-3' 3'-a^c^agcaaaacag^c^a^a^L _{Toc} -5'
SEQ-76	S-37(19)	3'-a^c^agcaaaacag^c^a^a^L _{Chol} -5'
SEQ-77	ODN2006(13) S-37(19)	5'-t^c^g^t^c^g^t^t^t^t^g^t^c^g^t^t^t^t^g^t^c^g^t^t-3' 3'-a^c^agcaaaacag^c^a^a^L _{Chol} -5'
SEQ-78	S-38(18)	3'-a^a^cagcaaaacag^c^a^a^L4-12-5'
SEQ-79	ODN2006(13) S-38(18)	5'-t^c^g^t^c^g^t^t^t^t^g^t^c^g^t^t^t^t^g^t^c^g^t^t-3' 3'-a^a^cagcaaaacag^c^a^a^L4-12-5'
SEQ-80	S-39(18)	3'-a^a^cagcaaaacag^c^a^a^L4-14-5'
SEQ-81	ODN2006(13) S-39(18)	5'-t^c^g^t^c^g^t^t^t^t^g^t^c^g^t^t^t^t^g^t^c^g^t^t-3' 3'-a^a^cagcaaaacag^c^a^a^L4-14-5'
SEQ-82	S-40(18)	3'-a^a^cagcaaaacag^c^a^a^L4-16-5'
SEQ-83	ODN2006(13) S-40(18)	5'-t^c^g^t^c^g^t^t^t^t^g^t^c^g^t^t^t^t^g^t^c^g^t^t-3' 3'-a^a^cagcaaaacag^c^a^a^L4-16-5'

[0198]

[Table 8]

No.	ID (SEQ ID)	Sequence: CpG(5'⇒3') Complementary strand (3'⇒5')
SEQ-84	S-41(16)	3'-g ⁺ c ⁺ aaaacagcaaaacag ⁺ c ⁺ a ⁺ a ⁺ L ₄₋₁₂ -5'
SEQ-85	ODN2006(13) S-41(16)	5'-t ⁺ c ⁺ g ⁺ t ⁺ c ⁺ g ⁺ t ⁺ t ⁺ t ⁺ g ⁺ t ⁺ c ⁺ g ⁺ t ⁺ t ⁺ t ⁺ g ⁺ t ⁺ c ⁺ g ⁺ t ⁺ t-3'
		3'-g ⁺ c ⁺ aaaacagcaaaacag ⁺ c ⁺ a ⁺ a ⁺ L ₄₋₁₂ -5'
SEQ-86	S-42(16)	3'-g ⁺ c ⁺ aaaacagcaaaacag ⁺ c ⁺ a ⁺ a ⁺ L ₄₋₁₄ -5'
SEQ-87	ODN2006(13) S-42(16)	5'-t ⁺ c ⁺ g ⁺ t ⁺ c ⁺ g ⁺ t ⁺ t ⁺ t ⁺ g ⁺ t ⁺ c ⁺ g ⁺ t ⁺ t ⁺ t ⁺ g ⁺ t ⁺ c ⁺ g ⁺ t ⁺ t-3'
		3'-g ⁺ c ⁺ aaaacagcaaaacag ⁺ c ⁺ a ⁺ a ⁺ L ₄₋₁₄ -5'
SEQ-88	S-43(16)	3'-g ⁺ c ⁺ aaaacagcaaaacag ⁺ c ⁺ a ⁺ a ⁺ L ₄₋₁₆ -5'
SEQ-89	ODN2006(13) S-43(16)	5'-t ⁺ c ⁺ g ⁺ t ⁺ c ⁺ g ⁺ t ⁺ t ⁺ t ⁺ g ⁺ t ⁺ c ⁺ g ⁺ t ⁺ t ⁺ t ⁺ g ⁺ t ⁺ c ⁺ g ⁺ t ⁺ t-3'
		3'-g ⁺ c ⁺ aaaacagcaaaacag ⁺ c ⁺ a ⁺ a ⁺ L ₄₋₁₆ -5'
SEQ-90	S-44(14)	3'-M ₁₅₋₆ ⁺ a ⁺ g ⁺ cagcaaaacagcaaaacag ⁺ c ⁺ a ⁺ a ⁺ L ₄₋₁₄ -5'
SEQ-91	ODN2006(13) S-44(14)	5'-t ⁺ c ⁺ g ⁺ t ⁺ c ⁺ g ⁺ t ⁺ t ⁺ t ⁺ g ⁺ t ⁺ c ⁺ g ⁺ t ⁺ t ⁺ t ⁺ g ⁺ t ⁺ c ⁺ g ⁺ t ⁺ t-3'
		3'-M ₁₅₋₆ ⁺ a ⁺ g ⁺ cagcaaaacagcaaaacag ⁺ c ⁺ a ⁺ a ⁺ L ₄₋₁₄ -5'
SEQ-92	S-45(14)	3'-M ₁₅₋₁₄ ⁺ a ⁺ g ⁺ cagcaaaacagcaaaacag ⁺ c ⁺ a ⁺ a ⁺ L ₄₋₁₀ -5'
SEQ-93	ODN2006(13) S-45(14)	5'-t ⁺ c ⁺ g ⁺ t ⁺ c ⁺ g ⁺ t ⁺ t ⁺ t ⁺ g ⁺ t ⁺ c ⁺ g ⁺ t ⁺ t ⁺ t ⁺ g ⁺ t ⁺ c ⁺ g ⁺ t ⁺ t-3'
		3'-M ₁₅₋₁₄ ⁺ a ⁺ g ⁺ cagcaaaacagcaaaacag ⁺ c ⁺ a ⁺ a ⁺ L ₄₋₁₀ -5'
SEQ-94	S-46(14)	3'-M ₁₅₋₁₈ ⁺ a ⁺ g ⁺ cagcaaaacagcaaaacag ⁺ c ⁺ a ⁺ a ⁺ L ₄₋₈ -5'
SEQ-95	ODN2006(13) S-46(14)	5'-t ⁺ c ⁺ g ⁺ t ⁺ c ⁺ g ⁺ t ⁺ t ⁺ t ⁺ g ⁺ t ⁺ c ⁺ g ⁺ t ⁺ t ⁺ t ⁺ g ⁺ t ⁺ c ⁺ g ⁺ t ⁺ t-3'
		3'-M ₁₅₋₁₈ ⁺ a ⁺ g ⁺ cagcaaaacagcaaaacag ⁺ c ⁺ a ⁺ a ⁺ L ₄₋₈ -5'
SEQ-96	S-47(14)	3'-M ₁₅₋₆ ⁺ a ⁺ g ⁺ cagcaaaacagcaaaacag ⁺ c ⁺ a ⁺ a ⁺ L ₄₋₁₈ -5'
SEQ-97	ODN2006(13) S-47(14)	5'-t ⁺ c ⁺ g ⁺ t ⁺ c ⁺ g ⁺ t ⁺ t ⁺ t ⁺ g ⁺ t ⁺ c ⁺ g ⁺ t ⁺ t ⁺ t ⁺ g ⁺ t ⁺ c ⁺ g ⁺ t ⁺ t-3'
		3'-M ₁₅₋₆ ⁺ a ⁺ g ⁺ cagcaaaacagcaaaacag ⁺ c ⁺ a ⁺ a ⁺ L ₄₋₁₈ -5'

[0199]

[Table 9]

No.	ID (SEQ ID)	Sequence: CpG(5'⇒3') Complementary strand (3'⇒5')
SEQ-98	S-48(14)	3'-M ₁₅₋₁₀ ⁺ a ⁺ g ⁺ cagcaaaacagcaaaacag ⁺ c ⁺ a ⁺ a ⁺ L ₄₋₁₈ -5'
SEQ-99	ODN2006(13) S-48(14)	5'-t ⁺ c ⁺ g ⁺ t ⁺ c ⁺ g ⁺ t ⁺ t ⁺ t ⁺ g ⁺ t ⁺ c ⁺ g ⁺ t ⁺ t ⁺ t ⁺ g ⁺ t ⁺ c ⁺ g ⁺ t ⁺ t-3'
		3'-M ₁₅₋₁₀ ⁺ a ⁺ g ⁺ cagcaaaacagcaaaacag ⁺ c ⁺ a ⁺ a ⁺ L ₄₋₁₈ -5'
SEQ-100	S-49(14)	3'-M ₁₅₋₁₄ ⁺ a ⁺ g ⁺ cagcaaaacagcaaaacag ⁺ c ⁺ a ⁺ a ⁺ L ₄₋₁₈ -5'
SEQ-101	ODN2006(13) S-49(14)	5'-t ⁺ c ⁺ g ⁺ t ⁺ c ⁺ g ⁺ t ⁺ t ⁺ t ⁺ g ⁺ t ⁺ c ⁺ g ⁺ t ⁺ t ⁺ t ⁺ g ⁺ t ⁺ c ⁺ g ⁺ t ⁺ t-3'
		3'-M ₁₅₋₁₄ ⁺ a ⁺ g ⁺ cagcaaaacagcaaaacag ⁺ c ⁺ a ⁺ a ⁺ L ₄₋₁₈ -5'
SEQ-102	S-50(14)	3'-M ₁₅₋₁₈ ⁺ a ⁺ g ⁺ cagcaaaacagcaaaacag ⁺ c ⁺ a ⁺ a ⁺ L ₄₋₁₈ -5'
SEQ-103	ODN2006(13) S-50(14)	5'-t ⁺ c ⁺ g ⁺ t ⁺ c ⁺ g ⁺ t ⁺ t ⁺ t ⁺ g ⁺ t ⁺ c ⁺ g ⁺ t ⁺ t ⁺ t ⁺ g ⁺ t ⁺ c ⁺ g ⁺ t ⁺ t-3'
		3'-M ₁₅₋₁₈ ⁺ a ⁺ g ⁺ cagcaaaacagcaaaacag ⁺ c ⁺ a ⁺ a ⁺ L ₄₋₁₈ -5'
SEQ-104	S-51(14)	3'-M ₁₅₋₆ ⁺ a ⁺ g ⁺ cagcaaaacagcaaaacag ⁺ c ⁺ a ⁺ a ⁺ L ₄₋₁₂ -5'
SEQ-105	ODN2006(13) S-51(14)	5'-t ⁺ c ⁺ g ⁺ t ⁺ c ⁺ g ⁺ t ⁺ t ⁺ t ⁺ g ⁺ t ⁺ c ⁺ g ⁺ t ⁺ t ⁺ t ⁺ g ⁺ t ⁺ c ⁺ g ⁺ t ⁺ t-3'
		3'-M ₁₅₋₆ ⁺ a ⁺ g ⁺ cagcaaaacagcaaaacag ⁺ c ⁺ a ⁺ a ⁺ L ₄₋₁₂ -5'
SEQ-106	S-52(14)	3'-M ₁₅₋₁₄ ⁺ a ⁺ g ⁺ cagcaaaacagcaaaacag ⁺ c ⁺ a ⁺ a ⁺ L ₄₋₁₂ -5'
SEQ-107	ODN2006(13) S-52(14)	5'-t ⁺ c ⁺ g ⁺ t ⁺ c ⁺ g ⁺ t ⁺ t ⁺ t ⁺ g ⁺ t ⁺ c ⁺ g ⁺ t ⁺ t ⁺ t ⁺ g ⁺ t ⁺ c ⁺ g ⁺ t ⁺ t-3'
		3'-M ₁₅₋₁₄ ⁺ a ⁺ g ⁺ cagcaaaacagcaaaacag ⁺ c ⁺ a ⁺ a ⁺ L ₄₋₁₂ -5'
SEQ-108	S-53(14)	3'-M ₁₅₋₁₈ ⁺ a ⁺ g ⁺ cagcaaaacagcaaaacag ⁺ c ⁺ a ⁺ a ⁺ L ₄₋₁₂ -5'
SEQ-109	ODN2006(13) S-53(14)	5'-t ⁺ c ⁺ g ⁺ t ⁺ c ⁺ g ⁺ t ⁺ t ⁺ t ⁺ g ⁺ t ⁺ c ⁺ g ⁺ t ⁺ t ⁺ t ⁺ g ⁺ t ⁺ c ⁺ g ⁺ t ⁺ t-3'
		3'-M ₁₅₋₁₈ ⁺ a ⁺ g ⁺ cagcaaaacagcaaaacag ⁺ c ⁺ a ⁺ a ⁺ L ₄₋₁₂ -5'
SEQ-110	S-54(18)	3'-a ⁺ a ⁺ cagcaaaacag ⁺ c ⁺ a ⁺ a ⁺ t ⁺ L ₄₋₁₈ -5'
SEQ-111	ODN2006(13) S-54(18)	5'-t ⁺ c ⁺ g ⁺ t ⁺ c ⁺ g ⁺ t ⁺ t ⁺ t ⁺ g ⁺ t ⁺ c ⁺ g ⁺ t ⁺ t ⁺ t ⁺ g ⁺ t ⁺ c ⁺ g ⁺ t ⁺ t-3'
		3'-a ⁺ a ⁺ cagcaaaacag ⁺ c ⁺ a ⁺ a ⁺ t ⁺ L ₄₋₁₈ -5'

[0200]

[Table 10]

No.	ID (SEQ ID)	Sequence: CpG(5'→3') Complementary strand (3'→5')
SEQ-112	S-55(18)	3'-a^a^cagcaaaacag^c^a^a^t^t^L ₄₋₁₈ -5'
SEQ-113	ODN2006(13)	5'-t^c^g^t^c^g^t^t^t^t^g^t^c^g^t^t^t^t^g^t^c^g^t^t-3'
	S-55(18)	3'-a^a^cagcaaaacag^c^a^a^t^t^L ₄₋₁₈ -5'
SEQ-114	S-56(18)	3'-a^a^cagcaaaacag^c^a^a^t^t^t^t^t^L ₄₋₁₈ -5'
SEQ-115	ODN2006(13)	5'-t^c^g^t^c^g^t^t^t^t^g^t^c^g^t^t^t^t^g^t^c^g^t^t-3'
	S-56(18)	3'-a^a^cagcaaaacag^c^a^a^t^t^t^t^t^L ₄₋₁₈ -5'
SEQ-116	S-57(18)	3'-a^a^cagcaaaacag^c^a^a^g^L ₄₋₁₈ -5'
SEQ-117	ODN2006(13)	5'-t^c^g^t^c^g^t^t^t^t^g^t^c^g^t^t^t^t^g^t^c^g^t^t-3'
	S-57(18)	3'-a^a^cagcaaaacag^c^a^a^g^L ₄₋₁₈ -5'
SEQ-118	S-58(18)	3'-a^a^cagcaaaacag^c^a^a^g^g^L ₄₋₁₈ -5'
SEQ-119	ODN2006(13)	5'-t^c^g^t^c^g^t^t^t^t^g^t^c^g^t^t^t^t^g^t^c^g^t^t-3'
	S-58(18)	3'-a^a^cagcaaaacag^c^a^a^g^g^L ₄₋₁₈ -5'
SEQ-120	S-59(18)	3'-a^a^cagcaaaacag^c^a^a^g^g^g^g^L ₄₋₁₈ -5'
SEQ-121	ODN2006(13)	5'-t^c^g^t^c^g^t^t^t^t^g^t^c^g^t^t^t^t^g^t^c^g^t^t-3'
	S-59(18)	3'-a^a^cagcaaaacag^c^a^a^g^g^g^g^L ₄₋₁₈ -5'
SEQ-122	S-60(16)	3'-g^c^aaaacagcaaaacag^c^a^a^t^L ₄₋₁₈ -5'
SEQ-123	ODN2006(13)	5'-t^c^g^t^c^g^t^t^t^t^g^t^c^g^t^t^t^t^g^t^c^g^t^t-3'
	S-60(16)	3'-g^c^aaaacagcaaaacag^c^a^a^t^L ₄₋₁₈ -5'
SEQ-124	S-61(16)	3'-g^c^aaaacagcaaaacag^c^a^a^t^t^L ₄₋₁₈ -5'
SEQ-125	ODN2006(13)	5'-t^c^g^t^c^g^t^t^t^t^g^t^c^g^t^t^t^t^g^t^c^g^t^t-3'
	S-61(16)	3'-g^c^aaaacagcaaaacag^c^a^a^t^t^L ₄₋₁₈ -5'
SEQ-126	S-62(16)	3'-g^c^aaaacagcaaaacag^c^a^a^t^t^t^t^t^L ₄₋₁₈ -5'
SEQ-127	ODN2006(13)	5'-t^c^g^t^c^g^t^t^t^t^g^t^c^g^t^t^t^t^g^t^c^g^t^t-3'
	S-62(16)	3'-g^c^aaaacagcaaaacag^c^a^a^t^t^t^t^t^L ₄₋₁₈ -5'
SEQ-128	S-63(16)	3'-g^c^aaaacagcaaaacag^c^a^a^g^L ₄₋₁₈ -5'
SEQ-129	ODN2006(13)	5'-t^c^g^t^c^g^t^t^t^t^g^t^c^g^t^t^t^t^g^t^c^g^t^t-3'
	S-63(16)	3'-g^c^aaaacagcaaaacag^c^a^a^g^L ₄₋₁₈ -5'

5 [0201]

[Table 11]

No.	ID (SEQ ID)	Sequence: CpG(5'→3') Complementary strand (3'→5')
SEQ-130	S-64(16)	3'-g ⁺ c ⁺ aaaacagcaaaacag ⁺ c ⁺ a ⁺ a ⁺ g ⁺ g ⁺ L ₄₋₁₈ -5'
SEQ-131	ODN2006(13)	5'-t ⁺ c ⁺ g ⁺ t ⁺ c ⁺ g ⁺ t ⁺ t ⁺ t ⁺ g ⁺ t ⁺ c ⁺ g ⁺ t ⁺ t ⁺ t ⁺ g ⁺ t ⁺ c ⁺ g ⁺ t ⁺ t ⁺ -3'
	S-64(16)	3'-g ⁺ c ⁺ aaaacagcaaaacag ⁺ c ⁺ a ⁺ a ⁺ g ⁺ g ⁺ L ₄₋₁₈ -5'
SEQ-132	S-65(16)	3'-g ⁺ c ⁺ aaaacagcaaaacag ⁺ c ⁺ a ⁺ a ⁺ g ⁺ g ⁺ g ⁺ g ⁺ L ₄₋₁₈ -5'
SEQ-133	ODN2006(13)	5'-t ⁺ c ⁺ g ⁺ t ⁺ c ⁺ g ⁺ t ⁺ t ⁺ t ⁺ g ⁺ t ⁺ c ⁺ g ⁺ t ⁺ t ⁺ t ⁺ g ⁺ t ⁺ c ⁺ g ⁺ t ⁺ t ⁺ -3 ,
	S-65(16)	3'-g ⁺ c ⁺ aaaacagcaaaacag ⁺ c ⁺ a ⁺ a ⁺ g ⁺ g ⁺ g ⁺ g ⁺ L ₄₋₁₈ -5'
SEQ-134	S-66(14)	3'-M ₂₂₋₁₈ ⁺ a ⁺ g ⁺ cagcaaaacagcaaaacag ⁺ c ⁺ a ⁺ a ⁺ -5'
SEQ-135	ODN2006(13)	5'-t ⁺ c ⁺ g ⁺ t ⁺ c ⁺ g ⁺ t ⁺ t ⁺ t ⁺ g ⁺ t ⁺ c ⁺ g ⁺ t ⁺ t ⁺ t ⁺ g ⁺ t ⁺ c ⁺ g ⁺ t ⁺ t ⁺ -3'
	S-66(14)	3'-M ₂₂₋₁₈ ⁺ a ⁺ g ⁺ cagcaaaacagcaaaacag ⁺ c ⁺ a ⁺ a ⁺ -5'
SEQ-136	S-67(20)	3'-M ₂₂₋₁₈ ⁺ a ⁺ g ⁺ cagcaaaaca ⁺ g ⁺ c ⁺ a ⁺ -5'
SEQ-137	ODN2006(13)	5'-t ⁺ c ⁺ g ⁺ t ⁺ c ⁺ g ⁺ t ⁺ t ⁺ t ⁺ g ⁺ t ⁺ c ⁺ g ⁺ t ⁺ t ⁺ t ⁺ g ⁺ t ⁺ c ⁺ g ⁺ t ⁺ t ⁺ -3'
	S-67(20)	3'-M ₂₂₋₁₈ ⁺ a ⁺ g ⁺ cagcaaaaca ⁺ g ⁺ c ⁺ a ⁺ -5'
SEQ-138	S-68(14)	3'-M ₂₂₋₁₂ ⁺ a ⁺ g ⁺ cagcaaaacagcaaaacag ⁺ c ⁺ a ⁺ a ⁺ -5'
SEQ-139	ODN2006(13)	5'-t ⁺ c ⁺ g ⁺ t ⁺ c ⁺ g ⁺ t ⁺ t ⁺ t ⁺ g ⁺ t ⁺ c ⁺ g ⁺ t ⁺ t ⁺ t ⁺ g ⁺ t ⁺ c ⁺ g ⁺ t ⁺ t ⁺ -3'
	S-68(14)	3'-M ₂₂₋₁₂ ⁺ a ⁺ g ⁺ cagcaaaacagcaaaacag ⁺ c ⁺ a ⁺ a ⁺ -5'
SEQ-140	S-69(20)	3'-M ₂₂₋₁₂ ⁺ a ⁺ g ⁺ cagcaaaaca ⁺ g ⁺ c ⁺ a ⁺ -5'
SEQ-141	ODN2006(13)	5'-t ⁺ c ⁺ g ⁺ t ⁺ c ⁺ g ⁺ t ⁺ t ⁺ t ⁺ g ⁺ t ⁺ c ⁺ g ⁺ t ⁺ t ⁺ t ⁺ g ⁺ t ⁺ c ⁺ g ⁺ t ⁺ t ⁺ -3'
	S-69(20)	3'-M ₂₂₋₁₂ ⁺ a ⁺ g ⁺ cagcaaaaca ⁺ g ⁺ c ⁺ a ⁺ -5'
SEQ-142	Amph2006(13)	5'-L ₄₋₁₆ ⁺ t ⁺ c ⁺ g ⁺ t ⁺ c ⁺ g ⁺ t ⁺ t ⁺ t ⁺ g ⁺ t ⁺ c ⁺ g ⁺ t ⁺ t ⁺ t ⁺ g ⁺ t ⁺ c ⁺ g ⁺ t ⁺ t ⁺ -3'
SEQ-143	Amph2006GG (13)	5'-L ₄₋₁₆ ⁺ g ⁺ g ⁺ t ⁺ c ⁺ g ⁺ t ⁺ c ⁺ g ⁺ t ⁺ t ⁺ t ⁺ g ⁺ t ⁺ c ⁺ g ⁺ t ⁺ t ⁺ t ⁺ g ⁺ t ⁺ c ⁺ g ⁺ t ⁺ t ⁺ -3'
SEQ-144	3'-Amph2006 (13)	5'-t ⁺ c ⁺ g ⁺ t ⁺ c ⁺ g ⁺ t ⁺ t ⁺ t ⁺ g ⁺ t ⁺ c ⁺ g ⁺ t ⁺ t ⁺ t ⁺ g ⁺ t ⁺ c ⁺ g ⁺ t ⁺ t ⁺ M ₅₁₋₁₆ -3'

[0202]

[Table 12]

No.	ID (SEQ ID)	Sequence: CpG(5'→3') Complementary strand (3'→5')
SEQ-145	S-70(20)	3'-a ⁺ g ⁺ cagcaaaaca ⁺ g ⁺ c ⁺ a ⁺ L ₄₋₁₈ -5'
SEQ-146	ODN2006(13)	5'-t ⁺ c ⁺ g ⁺ t ⁺ c ⁺ g ⁺ t ⁺ t ⁺ t ⁺ g ⁺ t ⁺ c ⁺ g ⁺ t ⁺ t ⁺ t ⁺ g ⁺ t ⁺ c ⁺ g ⁺ t ⁺ t ⁺ -3'
	S-70(20)	3'-a ⁺ g ⁺ cagcaaaaca ⁺ g ⁺ c ⁺ a ⁺ L ₄₋₁₈ -5'
SEQ-147	S-71(21)	3'-g ⁺ c ⁺ aaaacagcaaa ⁺ a ⁺ c ⁺ L ₄₋₁₈ -5'
SEQ-148	ODN2006(13)	5'-t ⁺ c ⁺ g ⁺ t ⁺ c ⁺ g ⁺ t ⁺ t ⁺ t ⁺ g ⁺ t ⁺ c ⁺ g ⁺ t ⁺ t ⁺ t ⁺ g ⁺ t ⁺ c ⁺ g ⁺ t ⁺ t ⁺ -3'
	S-71(21)	3'-g ⁺ c ⁺ aaaacagcaaa ⁺ a ⁺ c ⁺ L ₄₋₁₈ -5'
SEQ-149	S-72(19)	3'-a ⁺ c ⁺ agcaaaacag ⁺ c ⁺ a ⁺ a ⁺ L ₁₀₋₁₈ -5'
SEQ-150	ODN2006(13)	5'-t ⁺ c ⁺ g ⁺ t ⁺ c ⁺ g ⁺ t ⁺ t ⁺ t ⁺ g ⁺ t ⁺ c ⁺ g ⁺ t ⁺ t ⁺ t ⁺ g ⁺ t ⁺ c ⁺ g ⁺ t ⁺ t ⁺ -3'
	S-72(19)	3'-a ⁺ c ⁺ agcaaaacag ⁺ c ⁺ a ⁺ a ⁺ L ₁₀₋₁₈ -5'

[0203]

[Table 13]

No.	ID (SEQ ID)	Sequence: CpG(5'→3') Complementary strand (3'→5')
SEQ-151	S-73(18)	3'-a^a^cagcaaaacag^c^a^a^g^g^g^g^L4-18-5'
SEQ-152	ODN2006(13) S-73(18)	5'-t^c^g^t^c^g^t^t^t^t^g^t^c^g^t^t^t^t^g^t^c^g^t^t-3' 3'-a^a^cagcaaaacag^c^a^a^g^g^g^g^L4-18-5'
SEQ-153	S-74(18)	3'-a^a^cagcaaaacag^c^a^a^g^g^g^g^L4-18-5'
SEQ-154	ODN2006(13) S-74(18)	5'-t^c^g^t^c^g^t^t^t^t^g^t^c^g^t^t^t^t^g^t^c^g^t^t-3' 3'-a^a^cagcaaaacag^c^a^a^g^g^g^g^L4-18-5'
SEQ-155	S-75(18)	3'-a^a^cagcaaaacag^c^a^a^g^g^g^g^g^g^L4-18-5'
SEQ-156	ODN2006(13) S-75(18)	5'-t^c^g^t^c^g^t^t^t^t^g^t^c^g^t^t^t^t^g^t^c^g^t^t-3' 3'-a^a^cagcaaaacag^c^a^a^g^g^g^g^g^g^L4-18-5'
SEQ-157	S-76(18)	3'-a^a^cagcaaaacag^c^a^a^g^g^g^g^g^g^L4-18-5'
SEQ-158	ODN2006(13) S-76(18)	5'-t^c^g^t^c^g^t^t^t^t^g^t^c^g^t^t^t^t^g^t^c^g^t^t-3' 3'-a^a^cagcaaaacag^c^a^a^g^g^g^g^g^g^L4-18-5'

5 [0204]

[Table 14]

No.	ID (SEQ ID)	Sequence: CpG(5'→3') Complementary strand (3'→5')
SEQ-159	S-77(18)	3'-a^a^cagcaaaacag^c^a^a^g^g^g^g^g^g^g^g^L4-18-5'
SEQ-160	ODN2006(13) S-77(18)	5'-t^c^g^t^c^g^t^t^t^t^g^t^c^g^t^t^t^t^g^t^c^g^t^t-3' 3'-a^a^cagcaaaacag^c^a^a^g^g^g^g^g^g^g^g^L4-18-5'
SEQ-161	S-78(24)	3'-a^a^acagcaaaacag^c^a^a^g^g^g^g^g^g^L4-18-5'
SEQ-162	ODN2006(13) S-78(24)	5'-t^c^g^t^c^g^t^t^t^t^g^t^c^g^t^t^t^t^g^t^c^g^t^t-3' 3'-a^a^acagcaaaacag^c^a^a^g^g^g^g^g^g^L4-18-5'
SEQ-163	S-79(17)	3'-a^a^aacagcaaaacag^c^a^a^g^g^g^g^g^g^L4-18-5'
SEQ-164	ODN2006(13) S-79(17)	5'-t^c^g^t^c^g^t^t^t^t^g^t^c^g^t^t^t^t^g^t^c^g^t^t-3' 3'-a^a^aacagcaaaacag^c^a^a^g^g^g^g^g^g^L4-18-5'
SEQ-165	S-80(25)	3'-M22-18^a^g^cagcaaaacag^c^a^a-5'
SEQ-166	ODN2006(13) S-80(25)	5'-t^c^g^t^c^g^t^t^t^t^g^t^c^g^t^t^t^t^g^t^c^g^t^t-3' 3'-M22-18^a^g^cagcaaaacag^c^a^a-5'
SEQ-167	S-81(25)	3'-M22-18^g^g^a^g^cagcaaaacag^c^a^a-5'
SEQ-168	ODN2006(13) S-81(25)	5'-t^c^g^t^c^g^t^t^t^t^g^t^c^g^t^t^t^t^g^t^c^g^t^t-3' 3'-M22-18^g^g^a^g^cagcaaaacag^c^a^a-5'
SEQ-169	S-82(25)	3'-M22-18^g^g^g^g^g^a^g^cagcaaaacag^c^a^a-5'
SEQ-170	ODN2006(13) S-82(25)	5'-t^c^g^t^c^g^t^t^t^t^g^t^c^g^t^t^t^t^g^t^c^g^t^t-3' 3'-M22-18^g^g^g^g^g^a^g^cagcaaaacag^c^a^a-5'
SEQ-171	S-83(18)	3'-a^a^cagcaaaacag^c^a^a^a^a^a^L4-18-5'
SEQ-172	ODN2006(13) S-83(18)	5'-t^c^g^t^c^g^t^t^t^t^g^t^c^g^t^t^t^t^g^t^c^g^t^t-3' 3'-a^a^cagcaaaacag^c^a^a^a^a^a^L4-18-5'
SEQ-173	S-84(18)	3'-a^a^cagcaaaacag^c^a^a^a^a^a^a^L4-18-5'
SEQ-174	ODN2006(13) S-84(18)	5'-t^c^g^t^c^g^t^t^t^t^g^t^c^g^t^t^t^t^g^t^c^g^t^t-3' 3'-a^a^cagcaaaacag^c^a^a^a^a^a^a^L4-18-5'

[0205]

[Table 15]

No.	ID (SEQ ID)	Sequence: CpG(5'⇒3') Complementary strand (3'⇒5')
SEQ-175	S-85(25)	3'-a [^] g [^] cagcaaaacag [^] c [^] a [^] a [^] L ₁₀₋₁₈ -5'
SEQ-176	ODN2006(13)	5'-t [^] c [^] g [^] t [^] c [^] g [^] t [^] t [^] t [^] g [^] t [^] c [^] g [^] t [^] t [^] t [^] g [^] t [^] c [^] g [^] t [^] t-
	S-85(25)	3'-a [^] g [^] cagcaaaacag [^] c [^] a [^] a [^] L ₁₀₋₁₈ -5'
SEQ-177	S-86(17)	3'-a [^] a [^] aacagcaaaacag [^] c [^] a [^] a [^] L ₁₀₋₁₈ -5'
SEQ-178	ODN2006(13)	5'-t [^] c [^] g [^] t [^] c [^] g [^] t [^] t [^] t [^] g [^] t [^] c [^] g [^] t [^] t [^] t [^] g [^] t [^] c [^] g [^] t [^] t-
	S-86(17)	3'-a [^] a [^] aacagcaaaacag [^] c [^] a [^] a [^] L ₁₀₋₁₈ -5'
SEQ-179	S-87(25)	3'-a [^] g [^] cagcaaaacag [^] c [^] a [^] a [^] g [^] g [^] g [^] g [^] L ₁₀₋₁₈ -5'
SEQ-180	ODN2006(13)	5'-t [^] c [^] g [^] t [^] c [^] g [^] t [^] t [^] t [^] g [^] t [^] c [^] g [^] t [^] t [^] t [^] g [^] t [^] c [^] g [^] t [^] t-
	S-87(25)	3'-a [^] g [^] cagcaaaacag [^] c [^] a [^] a [^] g [^] g [^] g [^] g [^] L ₁₀₋₁₈ -5'
SEQ-181	S-88(14)	3'-M ₂₂₋₁₂ [^] a [^] g [^] cagcaaaacagcaaaacag [^] c [^] a [^] a [^] L ₈₋₁₀ -5'
SEQ-182	ODN2006(13)	5'-t [^] c [^] g [^] t [^] c [^] g [^] t [^] t [^] t [^] g [^] t [^] c [^] g [^] t [^] t [^] t [^] g [^] t [^] c [^] g [^] t [^] t-
	S-88(14)	3'-M ₂₂₋₁₂ [^] a [^] g [^] cagcaaaacagcaaaacag [^] c [^] a [^] a [^] L ₈₋₁₀ -5'
SEQ-183	S-89(19)	3'-M ₂₂₋₁₂ [^] a [^] c [^] agcaaaacag [^] c [^] a [^] a [^] L ₈₋₁₀ -5'
SEQ-184	ODN2006(13)	5'-t [^] c [^] g [^] t [^] c [^] g [^] t [^] t [^] t [^] g [^] t [^] c [^] g [^] t [^] t [^] t [^] g [^] t [^] c [^] g [^] t [^] t-
	S-89(19)	3'-M ₂₂₋₁₂ [^] a [^] c [^] agcaaaacag [^] c [^] a [^] a [^] L ₈₋₁₀ -5'
SEQ-185	S-90(18)	3'-M ₁₅₋₆ [^] a [^] a [^] cagcaaaacag [^] c [^] a [^] a [^] L ₄₋₁₈ -5'
SEQ-186	ODN2006(13)	5'-t [^] c [^] g [^] t [^] c [^] g [^] t [^] t [^] t [^] g [^] t [^] c [^] g [^] t [^] t [^] t [^] g [^] t [^] c [^] g [^] t [^] t-
	S-90(18)	3'-M ₁₅₋₆ [^] a [^] a [^] cagcaaaacag [^] c [^] a [^] a [^] L ₄₋₁₈ -5'
SEQ-187	S-91(18)	3'-M ₁₅₋₆ [^] a [^] a [^] cagcaaaacag [^] c [^] a [^] a [^] g [^] g [^] L ₄₋₁₈ -5'
SEQ-188	ODN2006(13)	5'-t [^] c [^] g [^] t [^] c [^] g [^] t [^] t [^] t [^] g [^] t [^] c [^] g [^] t [^] t [^] t [^] g [^] t [^] c [^] g [^] t [^] t-
	S-91(18)	3'-M ₁₅₋₆ [^] a [^] a [^] cagcaaaacag [^] c [^] a [^] a [^] g [^] g [^] L ₄₋₁₈ -5'
SEQ-189	S-92(18)	3'-M ₁₅₋₆ [^] a [^] a [^] cagcaaaacag [^] c [^] a [^] a [^] g [^] g [^] g [^] g [^] L ₄₋₁₈ -5'
SEQ-190	ODN2006(13)	5'-t [^] c [^] g [^] t [^] c [^] g [^] t [^] t [^] t [^] g [^] t [^] c [^] g [^] t [^] t [^] t [^] g [^] t [^] c [^] g [^] t [^] t-
	S-92(18)	3'-M ₁₅₋₆ [^] a [^] a [^] cagcaaaacag [^] c [^] a [^] a [^] g [^] g [^] g [^] g [^] L ₄₋₁₈ -5'

5 [0206]

[Table 16]

No.	ID (SEQ ID)	Sequence: CpG(5'⇒3') Complementary strand (3'⇒5')
SEQ-191	S-93(18)	3'-M ₁₅₋₁₀ ^a^a^cagcaaaacag^c^a^a^L ₄₋₁₈ -5'
SEQ-192	ODN2006(13) S-93(18)	5'-t^c^g^t^c^g^t^t^t^t^g^t^c^g^t^t^t^t^g^t^c^g^t^t-3' 3'-M ₁₅₋₁₀ ^a^a^cagcaaaacag^c^a^a^L ₄₋₁₈ -5'
SEQ-193	S-94(18)	3'-M ₁₅₋₁₀ ^a^a^cagcaaaacag^c^a^a^g^g^L ₄₋₁₈ -5'
SEQ-194	ODN2006(13) S-94(18)	5'-t^c^g^t^c^g^t^t^t^t^g^t^c^g^t^t^t^t^g^t^c^g^t^t-3' 3'-M ₁₅₋₁₀ ^a^a^cagcaaaacag^c^a^a^g^g^L ₄₋₁₈ -5'
SEQ-195	S-95(18)	3'-M ₁₅₋₁₀ ^a^a^cagcaaaacag^c^a^a^g^g^g^g^L ₄₋₁₈ -5'
SEQ-196	ODN2006(13) S-95(18)	5'-t^c^g^t^c^g^t^t^t^t^g^t^c^g^t^t^t^t^g^t^c^g^t^t-3' 3'-M ₁₅₋₁₀ ^a^a^cagcaaaacag^c^a^a^g^g^g^g^L ₄₋₁₈ -5'
SEQ-197	S-96(18)	3'-M ₁₅₋₆ ^a^a^cagcaaaacag^c^a^a^L ₄₋₁₆ -5'
SEQ-198	ODN2006(13) S-96(18)	5'-t^c^g^t^c^g^t^t^t^t^g^t^c^g^t^t^t^t^g^t^c^g^t^t-3' 3'-M ₁₅₋₆ ^a^a^cagcaaaacag^c^a^a^L ₄₋₁₆ -5'
SEQ-199	S-97(18)	3'-M ₁₅₋₆ ^a^a^cagcaaaacag^c^a^a^g^g^L ₄₋₁₆ -5'
SEQ-200	ODN2006(13) S-97(18)	5'-t^c^g^t^c^g^t^t^t^t^g^t^c^g^t^t^t^t^g^t^c^g^t^t-3' 3'-M ₁₅₋₆ ^a^a^cagcaaaacag^c^a^a^g^g^L ₄₋₁₆ -5'
SEQ-201	S-98(18)	3'-M ₁₅₋₁₀ ^a^a^cagcaaaacag^c^a^a^L ₄₋₁₆ -5'
SEQ-202	ODN2006(13) S-98(18)	5'-t^c^g^t^c^g^t^t^t^t^g^t^c^g^t^t^t^t^g^t^c^g^t^t-3' 3'-M ₁₅₋₁₀ ^a^a^cagcaaaacag^c^a^a^L ₄₋₁₆ -5'
SEQ-203	S-99(18)	3'-M ₁₅₋₁₀ ^a^a^cagcaaaacag^c^a^a^g^g^L ₄₋₁₆ -5'
SEQ-204	ODN2006(13) S-99(18)	5'-t^c^g^t^c^g^t^t^t^t^g^t^c^g^t^t^t^t^g^t^c^g^t^t-3' 3'-M ₁₅₋₁₀ ^a^a^cagcaaaacag^c^a^a^g^g^L ₄₋₁₆ -5'
SEQ-205	S-100(25)	3'-M ₂₂₋₁₄ ^a^g^cagcaaaacag^c^a^a^L ₈₋₆ -5'
SEQ-206	ODN2006(13) S-100(25)	5'-t^c^g^t^c^g^t^t^t^t^g^t^c^g^t^t^t^t^g^t^c^g^t^t-3' 3'-M ₂₂₋₁₄ ^a^g^cagcaaaacag^c^a^a^L ₈₋₆ -5'
SEQ-207	S-101(25)	3'-M ₂₂₋₁₄ ^a^g^cagcaaaacag^c^a^a^L ₈₋₁₀ -5'
SEQ-208	ODN2006(13) S-101(25)	5'-t^c^g^t^c^g^t^t^t^t^g^t^c^g^t^t^t^t^g^t^c^g^t^t-3' 3'-M ₂₂₋₁₄ ^a^g^cagcaaaacag^c^a^a^L ₈₋₁₀ -5'

[0207]

[Table 17]

No.	ID (SEQ ID)	Sequence: CpG(5'⇒3') Complementary strand (3'⇒5')
SEQ-209	S-102(25)	3'-M ₂₂₋₁₄ ^a^g^cagcaaaacag^c^a^a^L ₈₋₁₄ -5'
SEQ-210	ODN2006(13) S-102(25)	5'-t^c^g^t^c^g^t^t^t^t^g^t^c^g^t^t^t^t^g^t^c^g^t^t-3' 3'-M ₂₂₋₁₄ ^a^g^cagcaaaacag^c^a^a^L ₈₋₁₄ -5'
SEQ-211	S-103(25)	3'-M ₂₂₋₁₄ ^g^g^a^g^cagcaaaacag^c^a^a^L ₈₋₆ -5'
SEQ-212	ODN2006(13) S-103(25)	5'-t^c^g^t^c^g^t^t^t^t^g^t^c^g^t^t^t^t^g^t^c^g^t^t-3' 3'-M ₂₂₋₁₄ ^g^g^a^g^cagcaaaacag^c^a^a^L ₈₋₆ -5'
SEQ-213	S-104(25)	3'-M ₂₂₋₁₄ ^g^g^a^g^cagcaaaacag^c^a^a^L ₈₋₁₀ -5'
SEQ-214	ODN2006(13) S-104(25)	5'-t^c^g^t^c^g^t^t^t^t^g^t^c^g^t^t^t^t^g^t^c^g^t^t-3' 3'-M ₂₂₋₁₄ ^g^g^a^g^cagcaaaacag^c^a^a^L ₈₋₁₀ -5'
SEQ-215	S-105(25)	3'-M ₂₂₋₁₄ ^g^g^a^g^cagcaaaacag^c^a^a^L ₈₋₁₄ -5'
SEQ-216	ODN2006(13) S-105(25)	5'-t^c^g^t^c^g^t^t^t^t^g^t^c^g^t^t^t^t^g^t^c^g^t^t-3' 3'-M ₂₂₋₁₄ ^g^g^a^g^cagcaaaacag^c^a^a^L ₈₋₁₄ -5'
SEQ-217	S-106(25)	3'-M ₂₂₋₁₈ ^a^g^cagcaaaacag^c^a^a^L ₈₋₆ -5'
SEQ-218	ODN2006(13) S-106(25)	5'-t^c^g^t^c^g^t^t^t^t^g^t^c^g^t^t^t^t^g^t^c^g^t^t-3' 3'-M ₂₂₋₁₈ ^a^g^cagcaaaacag^c^a^a^L ₈₋₆ -5'
SEQ-219	S-107(25)	3'-M ₂₂₋₁₈ ^a^g^cagcaaaacag^c^a^a^L ₈₋₁₀ -5'
SEQ-220	ODN2006(13) S-107(25)	5'-t^c^g^t^c^g^t^t^t^t^g^t^c^g^t^t^t^t^g^t^c^g^t^t-3' 3'-M ₂₂₋₁₈ ^a^g^cagcaaaacag^c^a^a^L ₈₋₁₀ -5'
SEQ-221	S-108(25)	3'-M ₂₂₋₁₈ ^g^g^a^g^cagcaaaacag^c^a^a^L ₈₋₆ -5'
SEQ-222	ODN2006(13) S-108(25)	5'-t^c^g^t^c^g^t^t^t^t^g^t^c^g^t^t^t^t^g^t^c^g^t^t-3' 3'-M ₂₂₋₁₈ ^g^g^a^g^cagcaaaacag^c^a^a^L ₈₋₆ -5'
SEQ-223	S-109(25)	3'-M ₂₂₋₁₈ ^g^g^a^g^cagcaaaacag^c^a^a^L ₈₋₁₀ -5'
SEQ-224	ODN2006(13) S-109(25)	5'-t^c^g^t^c^g^t^t^t^t^g^t^c^g^t^t^t^t^g^t^c^g^t^t-3' 3'-M ₂₂₋₁₈ ^g^g^a^g^cagcaaaacag^c^a^a^L ₈₋₁₀ -5'
SEQ-225	S-110(18)	3'-M ₂₂₋₁₂ ^a^a^cagcaaaacag^c^a^a^L _{chol} -5'
SEQ-226	ODN2006(13) S-110(18)	5'-t^c^g^t^c^g^t^t^t^t^g^t^c^g^t^t^t^t^g^t^c^g^t^t-3' 3'-M ₂₂₋₁₂ ^a^a^cagcaaaacag^c^a^a^L _{chol} -5'
SEQ-227	S-111(18)	3'-M ₂₂₋₁₂ ^a^a^cagcaaaacag^c^a^a^L _{fari} -5'
SEQ-228	ODN2006(13) S-111(18)	5'-t^c^g^t^c^g^t^t^t^t^g^t^c^g^t^t^t^t^g^t^c^g^t^t-3' 3'-M ₂₂₋₁₂ ^a^a^cagcaaaacag^c^a^a^L _{fari} -5'

[0208]

[Table 18]

No.	ID (SEQ ID)	Sequence: CpG(5'⇒3') Complementary strand (3'⇒5')
SEQ-229	S-112(18)	3'-M ₂₂₋₁₂ ^a^a^cagcaaaacag^c^a^a^L ₈₋₁₀ -5'
SEQ-230	ODN2006(13) S-112(18)	5'-t^c^g^t^c^g^t^t^t^t^g^t^c^g^t^t^t^t^g^t^c^g^t^t-3' 3'-M ₂₂₋₁₂ ^a^a^cagcaaaacag^c^a^a^L ₈₋₁₀ -5'
SEQ-231	S-113(18)	3'-M ₂₂₋₁₂ ^a^a^cagcaaaacag^c^a^a^L ₈₋₁₄ -5'
SEQ-232	ODN2006(13) S-113(18)	5'-t^c^g^t^c^g^t^t^t^t^g^t^c^g^t^t^t^t^g^t^c^g^t^t-3' 3'-M ₂₂₋₁₂ ^a^a^cagcaaaacag^c^a^a^L ₈₋₁₄ -5'
SEQ-233	S-114(18)	3'-M _{chol} ^a^a^cagcaaaacag^c^a^a^L _{farl} -5'
SEQ-234	ODN2006(13) S-114(18)	5'-t^c^g^t^c^g^t^t^t^t^g^t^c^g^t^t^t^t^g^t^c^g^t^t-3' 3'-M _{chol} ^a^a^cagcaaaacag^c^a^a^L _{farl} -5'
SEQ-235	S-115(14)	3'-a^g^cagcaaaacaK ₂₂₋₁₈ gcaaaacag^c^a^a-5'
SEQ-236	ODN2006(13) S-115(14)	5'-t^c^g^t^c^g^t^t^t^t^g^t^c^g^t^t^t^t^g^t^c^g^t^t-3' 3'-a^g^cagcaaaacaK ₂₂₋₁₈ gcaaaacag^c^a^a-5'
SEQ-237	S-116(17)	3'-M ₁₅₋₆ ^a^a^aacagcaaaacag^c^a^a^L ₄₋₁₈ -5'
SEQ-238	ODN2006(13) S-116(17)	5'-t^c^g^t^c^g^t^t^t^t^g^t^c^g^t^t^t^t^g^t^c^g^t^t-3' 3'-M ₁₅₋₆ ^a^a^aacagcaaaacag^c^a^a^L ₄₋₁₈ -5'
SEQ-239	S-117(17)	3'-M ₁₅₋₆ ^a^a^aacagcaaaacag^c^a^a^g^g^L ₄₋₁₈ -5'
SEQ-240	ODN2006(13) S-117(17)	5'-t^c^g^t^c^g^t^t^t^t^g^t^c^g^t^t^t^t^g^t^c^g^t^t-3' 3'-M ₁₅₋₆ ^a^a^aacagcaaaacag^c^a^a^g^g^L ₄₋₁₈ -5'
SEQ-241	S-118(17)	3'-M ₁₅₋₁₀ ^a^a^aacagcaaaacag^c^a^a^L ₄₋₁₈ -5'
SEQ-242	ODN2006(13) S-118(17)	5'-t^c^g^t^c^g^t^t^t^t^g^t^c^g^t^t^t^t^g^t^c^g^t^t-3' 3'-M ₁₅₋₁₀ ^a^a^aacagcaaaacag^c^a^a^L ₄₋₁₈ -5'
SEQ-243	S-119(17)	3'-M ₁₅₋₁₀ ^a^a^aacagcaaaacag^c^a^a^g^g^L ₄₋₁₈ -5'
SEQ-244	ODN2006(13) S-119(17)	5'-t^c^g^t^c^g^t^t^t^t^g^t^c^g^t^t^t^t^g^t^c^g^t^t-3' 3'-M ₁₅₋₁₀ ^a^a^aacagcaaaacag^c^a^a^g^g^L ₄₋₁₈ -5'
SEQ-245	S-120(17)	3'-M ₁₅₋₆ ^a^a^aacagcaaaacag^c^a^a^L ₄₋₁₆ -5'
SEQ-246	ODN2006(13) S-120(17)	5'-t^c^g^t^c^g^t^t^t^t^g^t^c^g^t^t^t^t^g^t^c^g^t^t-3' 3'-M ₁₅₋₆ ^a^a^aacagcaaaacag^c^a^a^L ₄₋₁₆ -5'

[0209]

[Table 19]

No.	ID (SEQ ID)	Sequence: CpG(5'⇒3') Complementary strand (3'⇒5')
SEQ-247	S-121(17)	3'-M ₁₅₋₆ ^a^a^aacagcaaaacag^c^a^a^g^g^L ₄₋₁₆ -5'
SEQ-248	ODN2006(13) S-121(17)	5'-t^c^g^t^c^g^t^t^t^t^g^t^c^g^t^t^t^t^g^t^c^g^t^t-3' 3'-M ₁₅₋₆ ^a^a^aacagcaaaacag^c^a^a^g^g^L ₄₋₁₆ -5'
SEQ-249	S-122(17)	3'-M ₁₅₋₁₀ ^a^a^aacagcaaaacag^c^a^a^L ₄₋₁₆ -5'
SEQ-250	ODN2006(13) S-122(17)	5'-t^c^g^t^c^g^t^t^t^t^g^t^c^g^t^t^t^t^g^t^c^g^t^t-3' 3'-M ₁₅₋₁₀ ^a^a^aacagcaaaacag^c^a^a^L ₄₋₁₆ -5'
SEQ-251	S-123(17)	3'-M ₁₅₋₁₀ ^a^a^aacagcaaaacag^c^a^a^g^g^L ₄₋₁₆ -5'
SEQ-252	ODN2006(13) S-123(17)	5'-t^c^g^t^c^g^t^t^t^t^g^t^c^g^t^t^t^t^g^t^c^g^t^t-3' 3'-M ₁₅₋₁₀ ^a^a^aacagcaaaacag^c^a^a^g^g^L ₄₋₁₆ -5'
SEQ-253	K3-CpG(26)	5'-a^t^c^g^a^c^t^c^t^c^g^a^g^c^g^t^t^c^t^c-3'
SEQ-254	S-124(27)	3'-t^g^agagctcgcaa^g^a^g^L ₄₋₁₈ -5'
SEQ-255	K3-CpG(26) S-124(27)	5'-a^t^c^g^a^c^t^c^t^c^g^a^g^c^g^t^t^c^t^c-3' 3'-t^g^agagctcgcaa^g^a^g^L ₄₋₁₈ -5'
SEQ-256	D35-CpG(28)	5'-g^gtgcatcgatgcagggg^g^g-3'
SEQ-257	S-125(29)	3'-g^t^agctacgtccc^c^c^c^L ₄₋₁₈ -5'
SEQ-258	D35-CpG(28) S-125(29)	5'-g^gtgcatcgatgcagggg^g^g-3' 3'-g^t^agctacgtccc^c^c^c^L ₄₋₁₈ -5'
SEQ-259	S-126(25)	3'-M ₂₂₋₁₀ ^a^g^cagcaaaacag^c^a^a-5'
SEQ-260	ODN2006(13) S-126(25)	5'-t^c^g^t^c^g^t^t^t^t^g^t^c^g^t^t^t^t^g^t^c^g^t^t-3' 3'-M ₂₂₋₁₀ ^a^g^cagcaaaacag^c^a^a-5'
SEQ-261	S-127(25)	3'-M ₂₂₋₁₂ ^a^g^cagcaaaacag^c^a^a-5'
SEQ-262	ODN2006(13) S-127(25)	5'-t^c^g^t^c^g^t^t^t^t^g^t^c^g^t^t^t^t^g^t^c^g^t^t-3' 3'-M ₂₂₋₁₂ ^a^g^cagcaaaacag^c^a^a-5'
SEQ-261	S-127(25)	3'-M ₂₂₋₁₂ ^a^g^cagcaaaacag^c^a^a-5'
SEQ-262	ODN2006(13) S-127(25)	5'-t^c^g^t^c^g^t^t^t^t^g^t^c^g^t^t^t^t^g^t^c^g^t^t-3' 3'-M ₂₂₋₁₂ ^a^g^cagcaaaacag^c^a^a-5'

[0210]

[Table 20]

No.	ID (SEQ ID)	Sequence: CpG(5'⇒3') Complementary strand (3'⇒5')
SEQ-263	S-128(25)	3'-M ₂₂₋₂₀ ^a^g^cagcaaaacag^c^a^a-5'
SEQ-264	ODN2006(13) S-128(25)	5'-t^c^g^t^c^g^t^t^t^t^g^t^c^g^t^t^t^t^g^t^c^g^t^t-3' 3'-M ₂₂₋₂₀ ^a^g^cagcaaaacag^c^a^a-5'
SEQ-265	S-129(25)	3'-M ₂₂₋₂₂ ^a^g^cagcaaaacag^c^a^a-5'
SEQ-266	ODN2006(13) S-129(25)	5'-t^c^g^t^c^g^t^t^t^t^g^t^c^g^t^t^t^t^g^t^c^g^t^t-3' 3'-M ₂₂₋₂₂ ^a^g^cagcaaaacag^c^a^a-5'
SEQ-267	S-130(25)	3'-M ₂₂₋₁₂ ^a^g^cagcaaaacag^c^a^a^L ₈₋₆ -5'
SEQ-268	ODN2006(13) S-130(25)	5'-t^c^g^t^c^g^t^t^t^t^g^t^c^g^t^t^t^t^g^t^c^g^t^t-3' 3'-M ₂₂₋₁₂ ^a^g^cagcaaaacag^c^a^a^L ₈₋₆ -5'
SEQ-269	S-131(25)	3'-M ₂₂₋₁₂ ^a^g^cagcaaaacag^c^a^a^L ₈₋₁₀ -5'
SEQ-270	ODN2006(13) S-131(25)	5'-t^c^g^t^c^g^t^t^t^t^g^t^c^g^t^t^t^t^g^t^c^g^t^t-3' 3'-M ₂₂₋₁₂ ^a^g^cagcaaaacag^c^a^a^L ₈₋₁₀ -5'
SEQ-271	S-132(25)	3'-M ₂₂₋₁₂ ^a^g^cagcaaaacag^c^a^a^L ₈₋₁₄ -5'
SEQ-272	ODN2006(13) S-132(25)	5'-t^c^g^t^c^g^t^t^t^t^g^t^c^g^t^t^t^t^g^t^c^g^t^t-3' 3'-M ₂₂₋₁₂ ^a^g^cagcaaaacag^c^a^a^L ₈₋₁₄ -5'
SEQ-273	S-133(25)	3'-M ₂₂₋₁₂ ^g^g^a^g^cagcaaaacag^c^a^a^L ₈₋₆ -5'
SEQ-274	ODN2006(13) S-133(25)	5'-t^c^g^t^c^g^t^t^t^t^g^t^c^g^t^t^t^t^g^t^c^g^t^t-3' 3'-M ₂₂₋₁₂ ^g^g^a^g^cagcaaaacag^c^a^a^L ₈₋₆ -5'
SEQ-275	S-134(25)	3'-M ₂₂₋₁₂ ^a^g^cagcaaaacag^c^a^a^g^g^L ₈₋₁₀ -5'
SEQ-276	ODN2006(13) S-134(25)	5'-t^c^g^t^c^g^t^t^t^t^g^t^c^g^t^t^t^t^g^t^c^g^t^t-3' 3'-M ₂₂₋₁₂ ^g^g^a^g^cagcaaaacag^c^a^a^L ₈₋₁₀ -5'
SEQ-277	S-135(25)	3'-M ₂₂₋₁₂ ^g^g^a^g^cagcaaaacag^c^a^a^L ₈₋₁₄ -5'
SEQ-278	ODN2006(13) S-135(25)	5'-t^c^g^t^c^g^t^t^t^t^g^t^c^g^t^t^t^t^g^t^c^g^t^t-3' 3'-M ₂₂₋₁₂ ^g^g^a^g^cagcaaaacag^c^a^a^L ₈₋₁₄ -5'
SEQ-279	S-136(18)	3'-a^a^cagcaaaacag^c^ALNA^ALNA^-^g^g^L ₄₋₁₈ -5'
SEQ-280	ODN2006(13) S-136(18)	5'-t^c^g^t^c^g^t^t^t^t^g^t^c^g^t^t^t^t^g^t^c^g^t^t-3' 3'-a^a^cagcaaaacag^c^ALNA^ALNA^-^g^g^L ₄₋₁₈ -5'

[0211]

[Table 21]

No.	ID (SEQ ID)	Sequence: CpG(5'⇒3') Complementary strand (3'⇒5')
SEQ-281	S-137(18)	3'-a^a^cagcaALNAALNAacag^c^ALNA^ALNA^g^g^L ₄₋₁₈ -5'
SEQ-282	ODN2006(13) S-137(18)	5'-t^c^g^t^c^g^t^t^t^t^g^t^c^g^t^t^t^t^g^t^c^g^t^t-3' 3'-a^a^cagcaALNAALNAacag^c^ALNA^ALNA^g^g^L ₄₋₁₈ -5'
SEQ-283	S-138(18)	3'-a^ALNA^cagcaALNAALNAacag^c^ALNA^ALNA^g^g^L ₄₋₁₈ -5'
SEQ-284	ODN2006(13) S-138(18)	5'-t^c^g^t^c^g^t^t^t^t^g^t^c^g^t^t^t^t^g^t^c^g^t^t-3' 3'-a^ALNA^cagcaALNAALNAacag^c^ALNA^ALNA^g^g^L ₄₋₁₈ -5'
SEQ-285	S-139(18)	3'-a^a^c^a^g^c^a^a^a^a^c^a^g^c^a^a^g^g^g^g^g^L ₄₋₁₈ -5'
SEQ-286	ODN2006(13) S-139(18)	5'-t^c^g^t^c^g^t^t^t^t^g^t^c^g^t^t^t^t^g^t^c^g^t^t-3' 3'-a^a^c^a^g^c^a^a^a^a^c^a^g^c^a^a^g^g^g^g^g^L ₄₋₁₈ -5'
SEQ-287	S-140(30)	3'-M ₂₂₋₂₀ ^a^g^cagcaaaacagcaaa^a^c^a-5'
SEQ-288	ODN2006(13) S-140(30)	5'-t^c^g^t^c^g^t^t^t^t^g^t^c^g^t^t^t^t^g^t^c^g^t^t-3' 3'-M ₂₂₋₂₀ ^a^g^cagcaaaacagcaaa^a^c^a-5'
SEQ-289	S-141(14)	3'-M ₂₂₋₂₀ ^a^g^cagcaaaacagcaaaacag^c^a^a-5'
SEQ-290	ODN2006(13) S-141(14)	5'-t^c^g^t^c^g^t^t^t^t^g^t^c^g^t^t^t^t^g^t^c^g^t^t-3' 3'-M ₂₂₋₂₀ ^a^g^cagcaaaacagcaaaacag^c^a^a-5'
SEQ-291	S-142(30)	3'-M ₂₂₋₂₂ ^a^g^cagcaaaacagcaaa^a^c^a-5'
SEQ-292	ODN2006(13) S-142(30)	5'-t^c^g^t^c^g^t^t^t^t^g^t^c^g^t^t^t^t^g^t^c^g^t^t-3' 3'-M ₂₂₋₂₂ ^a^g^cagcaaaacagcaaa^a^c^a-5'
SEQ-293	S-143(14)	3'-M ₂₂₋₂₂ ^a^g^cagcaaaacagcaaaacag^c^a^a-5'
SEQ-294	ODN2006(13) S-143(14)	5'-t^c^g^t^c^g^t^t^t^t^g^t^c^g^t^t^t^t^g^t^c^g^t^t-3' 3'-M ₂₂₋₂₂ ^a^g^cagcaaaacagcaaaacag^c^a^a-5'
SEQ-295	S-144(30)	3'-M ₂₂₋₁₂ ^a^g^cagcaaaacagcaaa^a^c^a^L ₈₋₆ -5'
SEQ-296	ODN2006(13) S-144(30)	5'-t^c^g^t^c^g^t^t^t^t^g^t^c^g^t^t^t^t^g^t^c^g^t^t-3' 3'-M ₂₂₋₁₂ ^a^g^cagcaaaacagcaaa^a^c^a^L ₈₋₆ -5'

[0212]

[Table 22]

No.	ID (SEQ ID)	Sequence: CpG(5'⇒3') Complementary strand (3'⇒5')
SEQ-297	S-145(30)	3'-M ₂₂₋₁₂ [^] a [^] g [^] cagcaaaacagcaaa [^] a [^] c [^] a [^] L ₈₋₁₀ -5'
SEQ-298	ODN2006(13) S-145(30)	5'-t [^] c [^] g [^] t [^] c [^] g [^] t [^] t [^] t [^] g [^] t [^] c [^] g [^] t [^] t [^] t [^] g [^] t [^] c [^] g [^] t [^] t-3' 3'-M ₂₂₋₁₂ [^] a [^] g [^] cagcaaaacagcaaa [^] a [^] c [^] a [^] L ₈₋₁₀ -5'
SEQ-299	S-146(30)	3'-M ₂₂₋₁₂ [^] a [^] g [^] cagcaaaacagcaaa [^] a [^] c [^] a [^] L ₈₋₁₄ -5'
SEQ-300	ODN2006(13) S-146(30)	5'-t [^] c [^] g [^] t [^] c [^] g [^] t [^] t [^] t [^] g [^] t [^] c [^] g [^] t [^] t [^] t [^] g [^] t [^] c [^] g [^] t [^] t-3' 3'-M ₂₂₋₁₂ [^] a [^] g [^] cagcaaaacagcaaa [^] a [^] c [^] a [^] L ₈₋₁₄ -5'
SEQ-301	S-147(30)	3'-M ₂₂₋₁₂ [^] g [^] g [^] a [^] g [^] cagcaaaacagcaaa [^] a [^] c [^] a [^] L ₈₋₆ -5'
SEQ-302	ODN2006(13) S-147(30)	5'-t [^] c [^] g [^] t [^] c [^] g [^] t [^] t [^] t [^] g [^] t [^] c [^] g [^] t [^] t [^] t [^] g [^] t [^] c [^] g [^] t [^] t-3' 3'-M ₂₂₋₁₂ [^] g [^] g [^] a [^] g [^] cagcaaaacagcaaa [^] a [^] c [^] a [^] L ₈₋₆ -5'
SEQ-303	S-148(30)	3'-M ₂₂₋₁₂ [^] g [^] g [^] a [^] g [^] cagcaaaacagcaaa [^] a [^] c [^] a [^] L ₈₋₁₀ -5'
SEQ-304	ODN2006(13) S-148(30)	5'-t [^] c [^] g [^] t [^] c [^] g [^] t [^] t [^] t [^] g [^] t [^] c [^] g [^] t [^] t [^] t [^] g [^] t [^] c [^] g [^] t [^] t-3' 3'-M ₂₂₋₁₂ [^] g [^] g [^] a [^] g [^] cagcaaaacagcaaa [^] a [^] c [^] a [^] L ₈₋₁₀ -5'
SEQ-305	S-149(30)	3'-M ₂₂₋₁₂ [^] g [^] g [^] a [^] g [^] cagcaaaacagcaaa [^] a [^] c [^] a [^] L ₈₋₁₄ -5'
SEQ-306	ODN2006(13) S-149(30)	5'-t [^] c [^] g [^] t [^] c [^] g [^] t [^] t [^] t [^] g [^] t [^] c [^] g [^] t [^] t [^] t [^] g [^] t [^] c [^] g [^] t [^] t-3' 3'-M ₂₂₋₁₂ [^] g [^] g [^] a [^] g [^] cagcaaaacagcaaa [^] a [^] c [^] a [^] L ₈₋₁₄ -5'
SEQ-307	S-150(14)	3'-M ₂₂₋₁₂ [^] a [^] g [^] cagcaaaacagcaaaacag [^] c [^] a [^] a [^] L ₈₋₆ -5'
SEQ-308	ODN2006(13) S-150(14)	5'-t [^] c [^] g [^] t [^] c [^] g [^] t [^] t [^] t [^] g [^] t [^] c [^] g [^] t [^] t [^] t [^] g [^] t [^] c [^] g [^] t [^] t-3' 3'-M ₂₂₋₁₂ [^] a [^] g [^] cagcaaaacagcaaaacag [^] c [^] a [^] a [^] L ₈₋₆ -5'
SEQ-309	S-151(14)	3'-M ₂₂₋₁₂ [^] a [^] g [^] cagcaaaacagcaaaacag [^] c [^] a [^] a [^] L ₈₋₁₄ -5'
SEQ-310	ODN2006(13) S-151(14)	5'-t [^] c [^] g [^] t [^] c [^] g [^] t [^] t [^] t [^] g [^] t [^] c [^] g [^] t [^] t [^] t [^] g [^] t [^] c [^] g [^] t [^] t-3' 3'-M ₂₂₋₁₂ [^] a [^] g [^] cagcaaaacagcaaaacag [^] c [^] a [^] a [^] L ₈₋₁₄ -5'

[0213]

[Table 23]

No.	ID (SEQ ID)	Sequence: CpG(5'⇒3') Complementary strand (3'⇒5')
SEQ-311	S-152(14)	3'-M ₂₂₋₁₂ [^] g [^] g [^] a [^] g [^] cagcaaaacagcaaaacag [^] c [^] a [^] a [^] L ₈₋₆ -5'
SEQ-312	ODN2006(13) S-152(14)	5'-t [^] c [^] g [^] t [^] c [^] g [^] t [^] t [^] t [^] g [^] t [^] c [^] g [^] t [^] t [^] t [^] g [^] t [^] c [^] g [^] t [^] t-3' 3'-M ₂₂₋₁₂ [^] g [^] g [^] a [^] g [^] cagcaaaacagcaaaacag [^] c [^] a [^] a [^] L ₈₋₆ -5'
SEQ-313	S-153(14)	3'-M ₂₂₋₁₂ [^] g [^] g [^] a [^] g [^] cagcaaaacagcaaaacag [^] c [^] a [^] a [^] L ₈₋₁₀ -5'
SEQ-314	ODN2006(13) S-153(14)	5'-t [^] c [^] g [^] t [^] c [^] g [^] t [^] t [^] t [^] g [^] t [^] c [^] g [^] t [^] t [^] t [^] g [^] t [^] c [^] g [^] t [^] t-3' 3'-M ₂₂₋₁₂ [^] g [^] g [^] a [^] g [^] cagcaaaacagcaaaacag [^] c [^] a [^] a [^] L ₈₋₁₀ -5'
SEQ-315	S-154(14)	3'-M ₂₂₋₁₂ [^] g [^] g [^] a [^] g [^] cagcaaaacagcaaaacag [^] c [^] a [^] a [^] L ₈₋₁₄ -5'
SEQ-316	ODN2006(13) S-154(14)	5'-t [^] c [^] g [^] t [^] c [^] g [^] t [^] t [^] t [^] g [^] t [^] c [^] g [^] t [^] t [^] t [^] g [^] t [^] c [^] g [^] t [^] t-3' 3'-M ₂₂₋₁₂ [^] g [^] g [^] a [^] g [^] cagcaaaacagcaaaacag [^] c [^] a [^] a [^] L ₈₋₁₄ -5'
SEQ-317	S-155(18)	3'-M ₂₂₋₁₈ [^] Bu [^] Bu [^] a [^] a [^] cagcaaaacag [^] c [^] a [^] a [^] -5'
SEQ-318	ODN2006(13) S-155(18)	5'-t [^] c [^] g [^] t [^] c [^] g [^] t [^] t [^] t [^] g [^] t [^] c [^] g [^] t [^] t [^] t [^] g [^] t [^] c [^] g [^] t [^] t-3' 3'-M ₂₂₋₁₈ [^] Bu [^] Bu [^] a [^] a [^] cagcaaaacag [^] c [^] a [^] a [^] -5'
SEQ-319	S-156(18)	3'-M _{TEG-18} [^] a [^] a [^] cagcaaaacag [^] c [^] a [^] a [^] -5'
SEQ-320	ODN2006(13) S-156(18)	5'-t [^] c [^] g [^] t [^] c [^] g [^] t [^] t [^] t [^] g [^] t [^] c [^] g [^] t [^] t [^] t [^] g [^] t [^] c [^] g [^] t [^] t-3' 3'-M _{TEG-18} [^] a [^] a [^] cagcaaaacag [^] c [^] a [^] a [^] -5'
SEQ-321	S-157(25)	3'-M ₂₂₋₁₈ [^] ALNA [^] GLNA [^] 5mCLNAagcaaaacag [^] 5mCLNA [^] ALNA [^] ALNA [^] -5'
SEQ-322	ODN2006(13) S-157(25)	5'-t [^] c [^] g [^] t [^] c [^] g [^] t [^] t [^] t [^] g [^] t [^] c [^] g [^] t [^] t [^] t [^] g [^] t [^] c [^] g [^] t [^] t-3' 3'-M ₂₂₋₁₈ [^] ALNA [^] GLNA [^] 5mCLNAagcaaaacag [^] 5mCLNA [^] ALNA [^] ALNA [^] -5'
SEQ-323	S-158(31)	3'-M ₂₂₋₁₈ [^] ALNA [^] GLNA [^] 5mCLNAagca [^] ALNA [^] ALNA [^] ALNA [^] -5'
SEQ-324	ODN2006(13) S-158(31)	5'-t [^] c [^] g [^] t [^] c [^] g [^] t [^] t [^] t [^] g [^] t [^] c [^] g [^] t [^] t [^] t [^] g [^] t [^] c [^] g [^] t [^] t-3' 3'-M ₂₂₋₁₈ [^] ALNA [^] GLNA [^] 5mCLNAagca [^] ALNA [^] ALNA [^] ALNA [^] -5'

[0214]

[Table 24]

No.	ID (SEQ ID)	Sequence: CpG(5'⇒3') Complementary strand (3'⇒5')
SEQ-325	S-159(30)	3'-M ₂₂₋₁₄ ^a^g^cagcaaaacagcaaa^a^c^a^L ₈₋₆ -5'
SEQ-326	ODN2006(13) S-159(30)	5'-t^c^g^t^c^g^t^t^t^t^g^t^c^g^t^t^t^t^g^t^c^g^t^t-3' 3'-M ₂₂₋₁₄ ^a^g^cagcaaaacagcaaa^a^c^a^L ₈₋₆ -5'
SEQ-327	S-160(30)	3'-M ₂₂₋₁₄ ^a^g^cagcaaaacagcaaa^a^c^a^L ₈₋₁₀ -5'
SEQ-328	ODN2006(13) S-160(30)	5'-t^c^g^t^c^g^t^t^t^t^g^t^c^g^t^t^t^t^g^t^c^g^t^t-3' 3'-M ₂₂₋₁₄ ^a^g^cagcaaaacagcaaa^a^c^a^L ₈₋₁₀ -5'
SEQ-329	S-161(30)	3'-M ₂₂₋₁₄ ^a^g^cagcaaaacagcaaa^a^c^a^L ₈₋₁₄ -5'
SEQ-330	ODN2006(13) S-161(30)	5'-t^c^g^t^c^g^t^t^t^t^g^t^c^g^t^t^t^t^g^t^c^g^t^t-3' 3'-M ₂₂₋₁₄ ^a^g^cagcaaaacagcaaa^a^c^a^L ₈₋₁₄ -5'
SEQ-331	S-162(30)	3'-M ₂₂₋₁₄ ^g^g^a^g^cagcaaaacagcaaa^a^c^a^L ₈₋₆ -5'
SEQ-332	ODN2006(13) S-162(30)	5'-t^c^g^t^c^g^t^t^t^t^g^t^c^g^t^t^t^t^g^t^c^g^t^t-3' 3'-M ₂₂₋₁₄ ^g^g^a^g^cagcaaaacagcaaa^a^c^a^L ₈₋₆ -5'
SEQ-333	S-163(30)	3'-M ₂₂₋₁₄ ^g^g^a^g^cagcaaaacagcaaa^a^c^a^L ₈₋₁₀ -5'
SEQ-334	ODN2006(13) S-163(30)	5'-t^c^g^t^c^g^t^t^t^t^g^t^c^g^t^t^t^t^g^t^c^g^t^t-3' 3'-M ₂₂₋₁₄ ^g^g^a^g^cagcaaaacagcaaa^a^c^a^L ₈₋₁₀ -5'
SEQ-335	S-164(30)	3'-M ₂₂₋₁₄ ^g^g^a^g^cagcaaaacagcaaa^a^c^a^L ₈₋₁₄ -5'
SEQ-336	ODN2006(13) S-164(30)	5'-t^c^g^t^c^g^t^t^t^t^g^t^c^g^t^t^t^t^g^t^c^g^t^t-3' 3'-M ₂₂₋₁₄ ^g^g^a^g^cagcaaaacagcaaa^a^c^a^L ₈₋₁₄ -5'
SEQ-337	S-165(25)	3'-a^cOMe^agOMeCaOMeaaOMeacOMeagOMe^c^aOMe^a^L ₄₋₁₈ -5'
SEQ-338	ODN2006(13) S-165(25)	5'-t^c^g^t^c^g^t^t^t^t^g^t^c^g^t^t^t^t^g^t^c^g^t^t-3' 3'-a^cOMe^agOMeCaOMeaaOMeacOMeagOMe^c^aOMe^a^L ₄₋₁₈ -5'

5 [0215]

[Table 25]

No.	ID (SEQ ID)	Sequence: CpG(5'⇒3') Complementary strand (3'⇒5')
SEQ-339	ODN2216(32)	5'-g^g^gggacgatcg^g^g^g^g^g-3'
SEQ-340	S-166(33)	3'-M ₂₂₋₁₈ ^c^c^ccctgctagca^g^c^c-5'
SEQ-341	ODN2216(32) S-166(33)	5'-g^g^gggacgatcg^g^g^g^g^g-3' 3'-M ₂₂₋₁₈ ^c^c^ccctgctagca^g^c^c-5'
SEQ-342	ODN684(34)	5'-t^c^g^a^c^g^t^t^c^g^t^c^g^t^t^c^g^t^c^g^t^t^c-3'
SEQ-343	S-167(35)	3'-M ₂₂₋₁₈ ^a^g^ctgcaagcagc^a^a^g-5'
SEQ-344	ODN684(34) S-167(35)	5'-t^c^g^a^c^g^t^t^c^g^t^c^g^t^t^c^g^t^c^g^t^t^c-3' 3'-M ₂₂₋₁₈ ^a^g^ctgcaagcagc^a^a^g-5'
SEQ-345	D-LS01(36)	5'-t^c^g^c^g^a^c^g^t^t^c^g^c^c^g^a^c^g^t^t^c^g^g^t^a-3'
SEQ-346	S-168(37)	3'-M ₂₂₋₁₈ ^a^g^cgctgcaagcg^g^g^c-5'
SEQ-347	D-LS01(36) S-168(37)	5'-t^c^g^c^g^a^c^g^t^t^c^g^c^c^g^a^c^g^t^t^c^g^g^t^a-3' 3'-M ₂₂₋₁₈ ^a^g^cgctgcaagcg^g^g^c-5'
SEQ-348	D-LS03(38)	5'-t^c^g^c^g^a^a^c^g^t^t^c^g^c^c^g^c^g^t^t^c^g^a^a^c^g^c^g^g-3'
SEQ-349	S-169(39)	3'-M ₂₂₋₁₈ ^a^g^cgcttgcaagc^g^g^c-5'
SEQ-350	D-LS03(38) S-169(39)	5'-t^c^g^c^g^a^a^c^g^t^t^c^g^c^c^g^c^g^t^t^c^g^a^a^c^g^c^g^g-3' 3'-M ₂₂₋₁₈ ^a^g^cgcttgcaagc^g^g^c-5'
SEQ-351	ODN2395(40)	5'-t^c^g^t^c^g^t^t^t^t^c^g^g^c^g^c^g^c^g^c^c^g-3'
SEQ-352	S-170(41)	3'-M ₂₂₋₁₈ ^a^g^cagcaaaagcc^g^c^g-5'
SEQ-353	ODN2395(40) S-170(41)	5'-t^c^g^t^c^g^t^t^t^t^c^g^g^c^g^c^g^c^g^c^c^g-3' 3'-M ₂₂₋₁₈ ^a^g^cagcaaaagcc^g^c^g-5'
SEQ-354	ODNM362(42)	5'-t^c^g^t^c^g^t^c^g^t^t^c^g^a^a^c^g^a^c^g^t^t^g^a^t-3'
SEQ-355	S-171(43)	3'-M ₂₂₋₁₈ ^a^g^cagcagcaagc^t^t^g-5'
SEQ-356	ODNM362(42) S-171(43)	5'-t^c^g^t^c^g^t^c^g^t^t^c^g^a^a^c^g^a^c^g^t^t^g^a^t-3' 3'-M ₂₂₋₁₈ ^a^g^cagcagcaagc^t^t^g-5'
SEQ-357	ODN2336(44)	5'-g^g^ggacgacgtcg^g^g^g^g^g-3'
SEQ-358	S-172(45)	3'-M ₂₂₋₁₈ ^c^c^cctgctgcagc^a^c^c-5'
SEQ-359	ODN2336(44) S-172(45)	5'-g^g^ggacgacgtcg^g^g^g^g^g-3' 3'-M ₂₂₋₁₈ ^c^c^cctgctgcagc^a^c^c-5'

[0216]

[Table 26]

No.	ID (SEQ ID)	Sequence: CpG(5'⇒3') Complementary strand (3'⇒5')
SEQ-360	S-173(25)	3'-M ₂₂₋₆ ^a^g^cagcaaaacag^c^a^a-5'
SEQ-361	ODN2006(13) S-173(25)	5'-t^c^g^t^c^g^t^t^t^t^g^t^c^g^t^t^t^g^t^c^g^t^t-3' 3'-M ₂₂₋₆ ^a^g^cagcaaaacag^c^a^a-5'
SEQ-362	S-174(25)	3'-M ₂₂₋₆ ^a^g^cagcaaaacag^c^a^a^L ₈₋₁₀ -5'
SEQ-363	ODN2006(13) S-174(25)	5'-t^c^g^t^c^g^t^t^t^t^g^t^c^g^t^t^t^g^t^c^g^t^t-3' 3'-M ₂₂₋₆ ^a^g^cagcaaaacag^c^a^a^L ₈₋₁₀ -5'
SEQ-364	S-175(25)	3'-M ₂₂₋₆ ^a^g^cagcaaaacag^c^a^a^L ₈₋₁₄ -5'
SEQ-365	ODN2006(13) S-175(25)	5'-t^c^g^t^c^g^t^t^t^t^g^t^c^g^t^t^t^g^t^c^g^t^t-3' 3'-M ₂₂₋₆ ^a^g^cagcaaaacag^c^a^a^L ₈₋₁₄ -5'
SEQ-366	S-176(25)	3'-M ₂₂₋₈ ^a^g^cagcaaaacag^c^a^a-5'
SEQ-367	ODN2006(13) S-176(25)	5'-t^c^g^t^c^g^t^t^t^t^g^t^c^g^t^t^t^g^t^c^g^t^t-3' 3'-M ₂₂₋₈ ^a^g^cagcaaaacag^c^a^a-5'
SEQ-368	S-177(25)	3'-M ₂₂₋₈ ^a^g^cagcaaaacag^c^a^a^L ₈₋₁₀ -5'
SEQ-369	ODN2006(13) S-177(25)	5'-t^c^g^t^c^g^t^t^t^t^g^t^c^g^t^t^t^g^t^c^g^t^t-3' 3'-M ₂₂₋₈ ^a^g^cagcaaaacag^c^a^a^L ₈₋₁₀ -5'
SEQ-370	S-178(25)	3'-M ₂₂₋₈ ^a^g^cagcaaaacag^c^a^a^L ₈₋₁₄ -5'
SEQ-371	ODN2006(13) S-178(25)	5'-t^c^g^t^c^g^t^t^t^t^g^t^c^g^t^t^t^g^t^c^g^t^t-3' 3'-M ₂₂₋₈ ^a^g^cagcaaaacag^c^a^a^L ₈₋₁₄ -5'
SEQ-372	S-179(14)	3'-M ₂₂₋₁₄ ^a^g^cagcaaaacagcaaaacag^c^a^a^L ₈₋₆ -5'
SEQ-373	ODN2006(13) S-179(14)	5'-t^c^g^t^c^g^t^t^t^t^g^t^c^g^t^t^t^g^t^c^g^t^t-3' 3'-M ₂₂₋₁₄ ^a^g^cagcaaaacagcaaaacag^c^a^a^L ₈₋₆ -5'
SEQ-374	S-180(14)	3'-M ₂₂₋₁₄ ^a^g^cagcaaaacagcaaaacag^c^a^a^L ₈₋₁₀ -5'
SEQ-375	ODN2006(13) S-180(14)	5'-t^c^g^t^c^g^t^t^t^t^g^t^c^g^t^t^t^g^t^c^g^t^t-3' 3'-M ₂₂₋₁₄ ^a^g^cagcaaaacagcaaaacag^c^a^a^L ₈₋₁₀ -5'

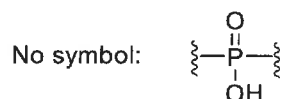
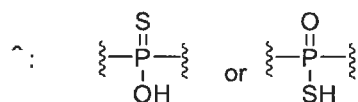
[0217]

[Table 27]

No.	ID (SEQ ID)	Sequence: CpG(5'⇒3') Complementary strand (3'⇒5')
SEQ-376	S-181(14)	3'-M ₂₂₋₁₄ ^a^g^cagcaaaacagcaaaacag^c^a^a^L ₈₋₁₄ -5'
SEQ-377	ODN2006(13) S-181(14)	5'-t^c^g^t^c^g^t^t^t^t^g^t^c^g^t^t^t^g^t^c^g^t^t-3' 3'-M ₂₂₋₁₄ ^a^g^cagcaaaacagcaaaacag^c^a^a^L ₈₋₁₄ -5'
SEQ-378	S-182(14)	3'-M ₂₂₋₁₄ ^g^g^a^g^cagcaaaacagcaaaacag^c^a^a^L ₈₋₆ -5'
SEQ-379	ODN2006(13) S-182(14)	5'-t^c^g^t^c^g^t^t^t^t^g^t^c^g^t^t^t^g^t^c^g^t^t-3' 3'-M ₂₂₋₁₄ ^g^g^a^g^cagcaaaacagcaaaacag^c^a^a^L ₈₋₆ -5'
SEQ-380	1018ISS(46)	5'-t^g^a^c^t^g^t^g^a^a^c^g^t^t^c^g^a^g^a^t^g^a-3'
SEQ-381	S-183(47)	3'-M ₂₂₋₁₈ ^a^c^tgacacttgca^a^g^c-5'
SEQ-382	1018ISS(46) S-183(47)	5'-t^g^a^c^t^g^t^g^a^a^c^g^t^t^c^g^a^g^a^t^g^a-3' 3'-M ₂₂₋₁₈ ^a^c^tgacacttgca^a^g^c-5'
SEQ-383	S-184(14)	3'-M ₂₂₋₂₂ ^Bu^Bu^a^g^cagcaaaacagcaaaacag^c^a^a-5'
SEQ-384	ODN2006(13) S-184(14)	5'-t^c^g^t^c^g^t^t^t^t^g^t^c^g^t^t^t^g^t^c^g^t^t-3' 3'-M ₂₂₋₂₂ ^Bu^Bu^a^g^cagcaaaacagcaaaacag^c^a^a-5'
SEQ-385	S-185(14)	3'-M ₂₂₋₂₂ ^g^g^g^g^g^a^g^cagcaaaacagcaaaacag^c^a^a-5'
SEQ-386	ODN2006(13) S-185(14)	5'-t^c^g^t^c^g^t^t^t^t^g^t^c^g^t^t^t^g^t^c^g^t^t-3' 3'-M ₂₂₋₂₂ ^g^g^g^g^g^a^g^cagcaaaacagcaaaacag^c^a^a-5'
SEQ-387	S-186(14)	3'-M ₂₂₋₂₂ ^Bu^Bu^g^g^g^g^g^a^g^cagcaaaacagcaaaacag^c^a^a-5'
SEQ-388	ODN2006(13) S-186(14)	5'-t^c^g^t^c^g^t^t^t^t^g^t^c^g^t^t^t^g^t^c^g^t^t-3' 3'-M ₂₂₋₂₂ ^Bu^Bu^g^g^g^g^g^a^g^cagcaaaacagcaaaacag^c^a^a-5'

[0218]

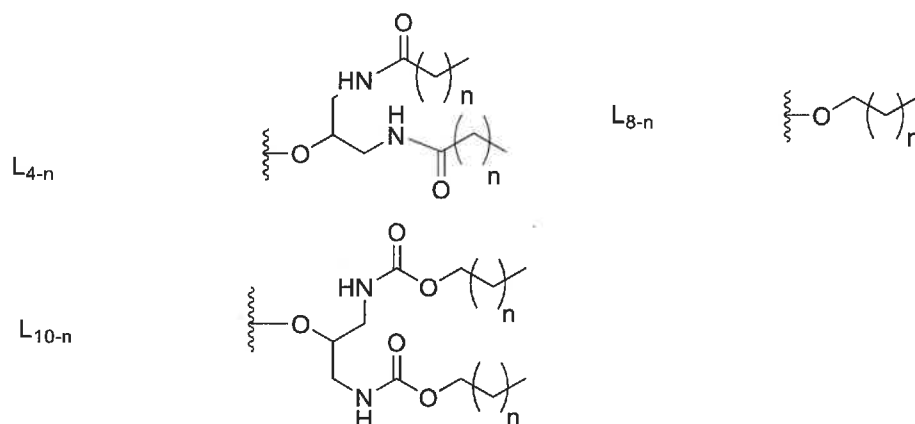
In Tables 4 to 27, n (small letter) is DNA, n_{OMe} is 2'-OMe-RNA, N (capital letter) is RNA, N_F is 2'-deoxy-2'-F-RNA, N_{LNA} is LNA and 5mC is 5-methylcytosine. ^ is -P(S)OH-, and the bond without any symbol is -P(O)OH-.



L is a compound introduced at the 5' end of oligonucleotide possibly comprising an oligonucleotide linker, and covalently binds with a hydroxyl group at the 5' end of oligonucleotide via the described bond. M is a compound introduced at the 3' end of oligonucleotide possibly comprising an oligonucleotide linker, and covalently binds with a hydroxyl group at the 3' end of oligonucleotide via the described bond. In L_{x-n}, x is a figure corresponding to the structure of Compound 4-n or Compound 10-n which is double strand or Compound 8-n which is single strand, and n is an integer of 6 to 28 corresponding to the carbon chain(s). In M_{x-n}, x is a figure corresponding to the structure of Compound 22-n, Compound 49-n or Compound 51-n which is double strand of Compound 15-n which is single strand, and n is an integer of 6 to 28 corresponding to the carbon chain(s).

[0219]

In detail, L_{x-n} is a group described below.

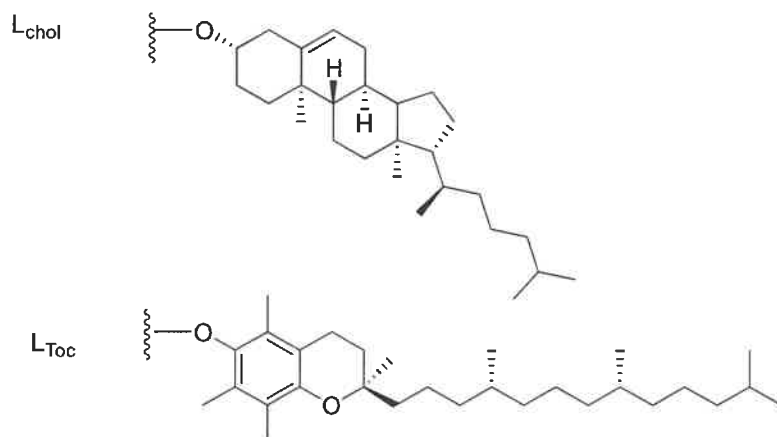


wherein n is an integer of 6 to 28.

$L_{4 \cdot n}$ is a group derived from Compound 4-n which is synthesized in 1-1) of the above A). $L_{8 \cdot n}$ is a group derived from Compound 8-n which is synthesized in 2) of the above A). $L_{10 \cdot n}$ is a group derived from Compound 10-n which is synthesized in 3) of the above A).

[0220]

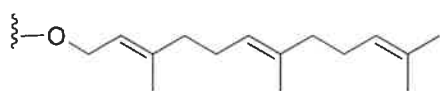
L_{chol} or L_{Toc} in Table is a group described below.



L_{chol} is a group derived from Compound 26 which is synthesized in 8-1) of the above A). L_{Toc} is a group derived from Compound 5'-Tocopherol-CE-Phosphoramidite which is purchased from Link Technologies Ltd.

[0221]

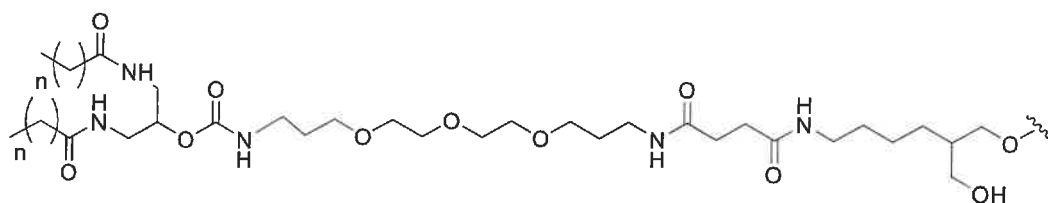
L_{farl} in Table is a group described below.



L_{farl} is a group deriving from Compound 53 synthesized in 8-2) of the above A).

[0222]

$M_{x \cdot n}$ is a group described below.

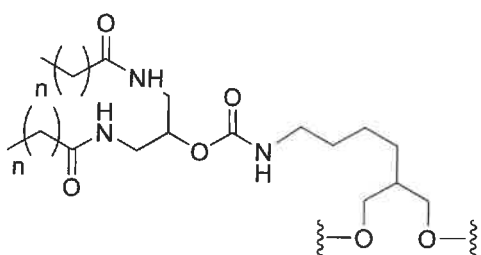


wherein n is an integer of 6 to 28.

$M_{TEG \cdot n}$ is a group deriving from Compound 63·n synthesized in 15) of the above A).

[0225]

$K_{22 \cdot n}$ in Table is a group described below.

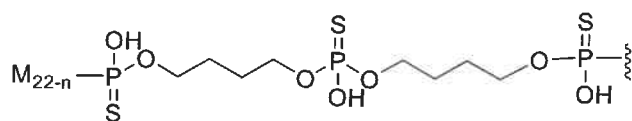


wherein n is an integer of 6 to 28.

$K_{22 \cdot n}$ is a group deriving from Compound 22·n synthesized in 6) of the above A).

[0226]

The following linker ($^{\wedge}Bu^{\wedge}Bu^{\wedge}$ in Table) is used for S-155, S-184 or S-186.



[0227]

Example 2 Activity evaluation of lipid binding double-stranded oligonucleotides of the present invention using the reporter assay (Materials and Methods)

HEK-BlueTM hTLR9 cells (Invivogen) used for the reporter assay were made by introducing human TLR9 gene and a reporter gene that secretory alkaline phosphatase gene bound to NF- κ B·AP-1 binding region sequence in HEK293 cells, and are the cells stably expressing the both genes. 3.6×10^4 of

the cells suspended in HEK-BlueTM Detection (Invitrogen) comprising substrates against the alkaline phosphatase were seeded on the 96 well plate, and 3 nM to 30 μ M of a lipid binding double-stranded oligonucleotide of the present invention, the known CpG oligonucleotides (ODN2006, SEQ-49), or
5 adjuvant based on design in Patent Document 1, i.e., ssCpG ODN introducing a lipid ligand (SEQ-142, 143 or 144) was added. After culturing in a 37 °C, 5 % CO₂ incubator for 16 hours, absorbency at 620 nm was measured using the culture supernatant developed a color. The result value was analyzed with TIBCO Spotfire software and calculated the 50 % effective concentration
10 (EC₅₀) of each oligonucleotide.

[0228]

(Result)

As previously described, it is known that the characteristicity as an adjuvant was vanished when ssCpG ODN was administered as a double-
15 stranded DNA (dsCpG ODN) by annealing the first and second strand (Non-patent Document 4). To examine that the adjuvant design of double-stranded nucleic acid shows an activity as a TLR9 agonist, activity evaluation of a double-stranded oligonucleotide was carried out by a reporter assay.

As shown in Table 28 and 29, ODN2006 (SEQ-49) which is a ssCpG ODN
20 showed the activity as a TLR9 agonist, and the EC₅₀ value was about 467 nM (Experiment 1, Table 28) and about 245 nM (Experiment 2, Table 29). On the other hand, a lipid binding double-stranded oligonucleotide of the present invention comprising ODN2006 as a CpG oligonucleotide showed TLR9 agonist activity at a range of several tens to one thousand and several hundreds nM.
25 Most adjuvants of the present invention described in Table 28 and 29 had a tendency to enhance the activity compared to ODN2006. In addition, 16 sequences of SEQ-57, SEQ-59, SEQ-61, SEQ-63, SEQ-65, SEQ-111, SEQ-117, SEQ-135, SEQ-146, SEQ-226, SEQ-282, SEQ-284, SEQ-294, SEQ-324, SEQ-361 and SEQ-363 had a tendency to slightly lower the activity compared to
30 ODN2006, but the difference was about 3 times. Therefore, it became clear that all lipid binding double-stranded oligonucleotides of the present invention have activity as a TLR9 agonist.

Furthermore, to examine that the modification form of SEQ-2 disclosed in Patent Document 1 applied for ODN2006 which is the human type CpG

oligonucleotide sequence shows an activity as TLR9 agonist, activity evaluation was carried out in the same manner (SEQ-141, 142 or 143). SEQ-142, 143 or 144 could not induce the activation of TLR9 in the measured concentration range, and it was impossible to calculate the EC₅₀ values (n.d. in Table 28). That is, it is suggested that design (ssCpG ODN introducing a lipid ligand) disclosed in Patent Document 1 cannot be useful as an adjuvant of vaccine and/or vaccine itself because it showed the activity for ODN1826 (mouse type) but did not for ODN2006 (human type) as described in the example.

In contrast, it is suggested that a lipid binding double-stranded oligonucleotide of the present invention can be useful as adjuvant of vaccine and/or vaccine itself regardless of the sequence of the CpG oligonucleotides because it showed the activity for ODN2006 as well as ODN1826.

[0229]

Experiment 1

[Table 28]

No.	EC50(nM)	No.	EC50(nM)	No.	EC50(nM)
SEQ-49	466.8	SEQ-89	300.9	SEQ-125	305.6
SEQ-51	405.9	SEQ-91	68.4	SEQ-127	299.9
SEQ-55	377.9	SEQ-93	106.4	SEQ-129	459.0
SEQ-57	976.8	SEQ-95	33.4	SEQ-131	312.2
SEQ-59	542.5	SEQ-97	308.9	SEQ-133	169.7
SEQ-61	608.1	SEQ-99	311.3	SEQ-135	509.8
SEQ-63	598.9	SEQ-101	33.5	SEQ-137	254.7
SEQ-65	500.9	SEQ-105	96.9	SEQ-139	58.4
SEQ-75	300.6	SEQ-111	490.1	SEQ-141	31.0
SEQ-77	296.8	SEQ-113	449.5	SEQ-146	1128.2
SEQ-79	162.9	SEQ-115	303.2	SEQ-148	376.6
SEQ-81	225.9	SEQ-117	616.5	SEQ-150	309.9
SEQ-83	324.9	SEQ-119	436.7	SEQ-142	n.d.
SEQ-85	72.7	SEQ-121	282.3	SEQ-143	n.d.
SEQ-87	102.8	SEQ-123	326.0	SEQ-144	n.d.

[0230]

Experiment 2

[Table 29]

No.	EC50(nM)	No.	EC50(nM)	No.	EC50(nM)
SEQ-49	245.3	SEQ-218	59.8	SEQ-292	58.9
SEQ-152	86.4	SEQ-220	57.2	SEQ-294	262.4
SEQ-154	94.5	SEQ-222	31.9	SEQ-296	10.1
SEQ-156	90.0	SEQ-224	12.4	SEQ-298	10.2
SEQ-158	56.6	SEQ-226	362.0	SEQ-300	11.4
SEQ-160	65.1	SEQ-228	21.0	SEQ-302	10.2
SEQ-162	89.7	SEQ-230	39.8	SEQ-304	11.6
SEQ-164	64.2	SEQ-232	72.7	SEQ-306	11.3
SEQ-166	90.4	SEQ-234	27.0	SEQ-308	8.4
SEQ-168	62.8	SEQ-236	203.1	SEQ-310	10.7
SEQ-170	46.0	SEQ-238	131.9	SEQ-312	19.1
SEQ-172	161.9	SEQ-240	62.0	SEQ-314	10.8
SEQ-174	101.0	SEQ-242	102.4	SEQ-316	10.9
SEQ-176	241.2	SEQ-244	101.8	SEQ-318	81.3
SEQ-178	210.1	SEQ-246	102.6	SEQ-320	68.4
SEQ-180	46.9	SEQ-248	108.3	SEQ-322	50.4
SEQ-182	11.2	SEQ-250	92.4	SEQ-324	261.1
SEQ-184	34.2	SEQ-252	87.4	SEQ-326	24.6
SEQ-186	127.7	SEQ-260	39.9	SEQ-328	24.1
SEQ-188	67.7	SEQ-262	25.7	SEQ-330	33.7
SEQ-190	46.9	SEQ-264	86.3	SEQ-332	24.8
SEQ-192	123.9	SEQ-266	58.1	SEQ-334	25.3
SEQ-194	99.8	SEQ-268	11.5	SEQ-336	31.4
SEQ-196	33.2	SEQ-270	16.3	SEQ-338	188.1
SEQ-198	114.9	SEQ-272	33.3	SEQ-361	771.8
SEQ-200	96.3	SEQ-274	29.7	SEQ-363	290.2
SEQ-202	89.5	SEQ-276	30.9	SEQ-365	177.4
SEQ-204	83.4	SEQ-278	33.3	SEQ-367	178.2
SEQ-206	40.7	SEQ-280	168.3	SEQ-369	135.9
SEQ-208	31.3	SEQ-282	275.1	SEQ-371	170.0
SEQ-210	56.7	SEQ-284	255.8	SEQ-373	21.5
SEQ-212	46.9	SEQ-286	45.0	SEQ-375	21.9
SEQ-214	47.4	SEQ-288	65.4	SEQ-377	29.8
SEQ-216	99.5	SEQ-290	151.7	SEQ-379	20.3

As shown in Table 30, even when the CpG oligonucleotides except for ODN2006 were used, most adjuvants of the present invention showed the tendency of activity enhancement compared to ssCpG ODN. It became clear that every lipid binding double-stranded oligonucleotide of the present invention shows activity as TLR9 agonist.

[0232]

[Table 30]

No.	EC50(nM)
SEQ-253 (K3-CpG)	2870.2
SEQ-255	173.4
SEQ-256 (D35-CpG)	3421046.2
SEQ-258	16328.8
SEQ-339 (ODN2216)	7244.7
SEQ-341	7915.8
SEQ-342 (ODN684)	2339.9
SEQ-344	111.4
SEQ-345 (D-LS01)	6280.7
SEQ-347	10.7
SEQ-351 (ODN2395)	11569.8
SEQ-353	1982.2
SEQ-354 (ODN M362)	16893838.1
SEQ-356	292.6

[0233]

Example 3 In vivo evaluation of adjuvants of the present invention

A) Evaluation of CTL inductivity

(Animals)

C57BL/6JJcl mice (6 to 8 weeks old) were purchased from CLEA Japan,

Inc.

(Components of Vaccine 1)

·OVA₂₅₇₋₂₆₄ peptide (SIINFEKL, SEQ ID NO: 22) which synthesized and purified by reversed phase HPLC and purchased from Sigma-Aldrich.

·an adjuvant of the present invention or the well-known CpG ODN

(Components of Vaccine 2)

- TRP₂₁₈₀₋₁₈₈ peptide (CSVYDFFVWL, SEQ ID NO: 23) which synthesized and purified by reversed phase HPLC and purchased from Sigma-Aldrich.
- an adjuvant of the present invention or the well-known CpG ODN

5

(Components of Vaccine 3)

- OVA₂₅₇₋₂₆₄ peptide or TRP₂₁₈₀₋₁₈₈ peptide
- Montanide ISA51 (SEPPIC)

[0234]

10 (Preparation of Vaccine 1)

The specific immunity was made by vaccinating a mouse twice at 7 days intervals. Each vaccine was that 100 µg of OVA peptide, and 1.57 or 4.71 nmol of an adjuvant of the present invention or well-known CpG ODN, were dissolved in 1×PBS. 100 µL of the vaccine was administered subcutaneous injection on the flank of a mouse.

15

(Preparation of Vaccine 2)

The specific immunity was induced by vaccinating a mouse twice at 7 days intervals. Each vaccine was that 100 µg of TRP2 peptide, and 1.57 or 4.71 nmol of an adjuvant of the present invention or well-known CpG ODN, were dissolved in 1×PBS. 100 µL of the vaccine was administered subcutaneous injection on the flank of a mouse.

20

(Preparation of Vaccine 3)

1:1 of 2 mg/ml of TRP2 peptide in PBS and Montanide (the above vaccine component 3) were mixed using a cylinder and pumping connector to form emulsion. 100 µL of the emulsion vaccine was administered subcutaneous injection on the flank of a mouse twice at 7 days intervals.

25

[0235]

30 (Tetramer staining)

On 7 days after second vaccination, the blood was collected, red blood cells were removed by BD Pharm Lyse (BD pharmingen), and the cells were suspended in FACS buffer (1% fetal bovine serum, 2 mM PBS with EDTA). Anti-mouse CD16/32 monoclonal antibody (BioXcell) was added thereto, and Fc

receptor blocking was carried out at 4 °C for 30 minutes. Next, PE labeled H-2Kb tetramer corresponding to the vaccine antigen (T-Select H-2Kb OVA Tetramer-SIINFEKL or T-Select H-2Kb TRP-2 Tetramer-SVYDFVWL; Both were purchased from Medical & Biological Laboratories Co., Ltd.) and Alexa 647 labeled anti-CD8 antibody (Medical & Biological Laboratories Co., Ltd.) were added thereto, and staining was carried out at room temperature for 45 minutes. Then, staining was carried out with DAPI (Invitrogen) and the cells were washed twice with FACS buffer. It was resuspended in FACS buffer and analyzed by FACS verse Flow Cytometer (BD bioscience) and FAC Suite software (BD bioscience). For analysis, after DAPI negative fraction was gated, white blood cells fraction was provided using forward and side-way scattering as indicators. In the white blood cells fraction, both positive cells of Alexa 647-CD8 and PE-H-2Kb Tetramer were defined as CTLs. CTL ratio in white blood cells was used for evaluation.

Calculation method of the indicator of CTL inductivity

After calculation of the average CTL inductivity of each group per experiment, the ratio against average value of CTL inductivity of ssCpG ODN, ODN1826 (SEQ-1) or ODN2006 (SEQ-49) as a control was calculated.

In the case of an adjuvant comprising ODN1826

$$\frac{\text{(Average of CTL inductivity of the adjuvant group)}}{\text{(Average of CTL inductivity of the ODN1826 group)}} = \text{the ratio against average value}$$

In the case of an adjuvant comprising ODN2006

$$\frac{\text{(Average of CTL inductivity of the adjuvant group)}}{\text{(Average of CTL inductivity of the ODN2006 group)}} = \text{the ratio against average value}$$

CTL inductivity of each adjuvant was shown when CTL inductivity of ssCpG ODN was taken as 1, so the bigger value means the higher CTL inductivity.

[0236]

B) Evaluation of anti-tumor effect of TRP2 peptide vaccine using an adjuvant of the present invention

After immunization by TRP2 peptide vaccine, B16F10 cells (ATTC) which is mouse melanoma expressing TRP2 protein was subcutaneously transplanted to evaluate anti-tumor effect. In a similar method to Example 3 A), on the next day that tetramer-positive ratio in peripheral blood of mouse vaccinated twice every 7 days was observed, B16F10 cells (1×10^5 cells/mouse) were subcutaneously transplanted in the right shoulder. The major and minor axes of the engrafted tumor were measured every 2 to 3 days, and the tumor volume was calculated by the formula:
(major axis $\times$ minor axis²)/2

Calculation method for indicators of anti-tumor effect

For the adjuvant comprising ODN1826

$100 - (\text{average tumor volume of adjuvant group}) / (\text{average tumor volume of ODN1826 group})$

For the adjuvant comprising ODN2006

$100 - (\text{average tumor volume of adjuvant group}) / (\text{average tumor volume of ODN2006 group})$

The compound with bigger value has the stronger anti-tumor activity.
[0237]

(Result using ODN1826 as a CpG oligonucleotide)

Result 1-A: Comparison of CTL inductivity between an adjuvant of the present invention and the known adjuvants

When mice were immunized with TRP2 peptide and ODN1826 (SEQ-1), TRP2-specific CTL was induced. When dsCpG ODN (SEQ-4) was used, TRP2-specific CTL inductivity was lowered at 0.46 times compared to ODN1826. That is, double strands led to lower the CTL inductivity compared to a single-strand adjuvant. This suggested that when the CpG oligonucleotide is just made as a double-stranded form, the immunostimulatory activity is lowered as disclosed in Non-patent Document 4 and the like. On the other hand, when an adjuvant of the present invention, i.e., lipid binding double-stranded oligonucleotide adjuvant (SEQ-16) was used for immunization, the CTL inductivity was enhanced at 1.46 times compared to ODN1826. Therefore, it

suggested that an adjuvant of the present invention has unexpected enhancement of activity. The results were shown in Table 31.

[0238]

[Table 31]

Peptide	Adjuvant	dose/head nmol	Ratio of average value of antigen-specific CTL
TRP2	SEQ-1	1.57	1.00
TRP2	SEQ-4	1.57	0.46
TRP2	SEQ-16	1.57	1.46

[0239]

Result 1-B: Comparison of anti-tumor effect between an adjuvant of the present invention and the known adjuvants

When mice were immunized with TRP2 peptide and ODN1826 (SEQ-1), they had little effect of inhibition on tumor progression of B16F10 cells. Furthermore, dsCpG ODN (SEQ-4) had little effect of inhibition on tumor progression of B16F10 cells. On the other hand, an adjuvant of the present invention (SEQ-16) showed the strong effect of inhibition on tumor progression of B16F10 cells, 43% compared to ODN1826 on Day 14 after transplantation. The results were shown in Figure 1.

[0240]

Result 2-A, B: CTL inductivity and anti-tumor effect of adjuvants of the present invention comprising a lipid ligand with different number of carbon atoms

The activity of an adjuvant of the present invention having a lipid comprising two acyl chains comprising 10 to 20 carbon atoms as a ligand was examined with OVA peptide. CTL inductivity and tumor inhibition rate of SEQ-12 (10 carbon atoms) was lowered compared to ODN1826 (SEQ-1). On the other hand, there is tendency to enhance CTL inductivity as the chain length is longer like SEQ-14 (14 carbon atoms), SEQ-6 (18 carbon atoms) and SEQ-16 (20 carbon atoms). In addition, tumor inhibition rate of SEQ-14 showed about 94 % inhibition of the tumor growth compared to ODN1826 on Day 14 after transplantation, and SEQ-6 and SEQ-16 perfectly inhibited the tumor growth on Day 14 after transplantation. The results were shown in Table 32.

[0241]

[Table 32]

Peptide	Adjuvant	dose/head nmol	Ratio of average value of antigen-specific CTL	Tumor inhibition rate (Day 14 after transplantation)
OVA	SEQ-1	1.57	1.00	0.00
OVA	SEQ-12	1.57	0.42	−62.88
OVA	SEQ-14	1.57	0.85	93.94
OVA	SEQ-6	1.57	0.77	100.00
OVA	SEQ-16	1.57	2.32	100.00

5 [0242]

Result 3-A: CTL inductivity of adjuvants of the present invention introduced lipids at the both ends

The activity of an adjuvant of the present invention with a lipid comprising two acyl chains comprising 20 carbon atoms as a ligand and further comprising a single-strand lipid comprising 8 or 12 carbon atoms at 3' end of the CpG oligonucleotide (SEQ-46 or SEQ-48) was examined with TRP2 peptide.
10 The both CTL inductivities were enhanced at about 2.8 times compared to ODN1826 (SEQ-1). The results were shown in Table 33.

[0243]

15 [Table 33]

Peptide	Adjuvant	dose/head nmol	Ratio of average value of antigen-specific CTL
TRP2	SEQ-1	1.57	1.00
TRP2	SEQ-46	1.57	2.81
TRP2	SEQ-48	1.57	2.84

[0244]

Result 3-B: Anticancer effect of adjuvants of the present invention introduced lipids at the both ends
20

When mice were immunized with TRP2 peptide and an adjuvant of the present invention, SEQ-46 or SEQ-48, it showed the strong effect of inhibition on tumor progression of B16F10 cells, respectively 52% or 43% on Day 13 after transplantation compared to ODN1826 (SEQ-1). The results were shown in
25 Figure 2.

[0245]

Result 4-A: CTL inductivity of adjuvants of the present invention with the different lengths of the complementary strand

In a double-stranded oligonucleotide adjuvant design, it is necessary to release the CpG oligonucleotide, which is an active ingredient, in the lymph nodes. The speed to dissociate a double strand is proportionate to the heat stability, and the more complementary sites in the double strand structure are, the more stably the double strand exists. Then, the activity of adjuvants of the present invention whose chain length of complementary strand is different was examined with TRP2 peptide.

The adjuvant whose complementary strand is 10 mer for ODN1826 (20 mer), i.e., length of the complementary strand for the CpG oligonucleotide is 50 % (SEQ-8) and the adjuvant whose complementary strand is 15 mer, i.e., length is 75 % (SEQ-10) were used. Both SEQ-8 and SEQ-10 dominantly enhanced CTL inductivity compared to ODN1826 (SEQ-1) at about 1.2 and 2.5 times, respectively. The results were shown in Table 34.

[0246]

[Table 34]

Peptide	Adjuvant	dose/head nmol	Ratio of average value of antigen-specific CTL
TRP2	SEQ-1	1.57	1.00
TRP2	SEQ-2	1.57	1.68
TRP2	SEQ-8	1.57	1.15
TRP2	SEQ-10	1.57	2.50

[0247]

Result 4-B: Anticancer effect of adjuvants of the present invention with the different lengths of the complementary strand

When mice were immunized with TRP2 peptide and ssCpG ODN introducing a lipid ligand in Patent Document 1 (SEQ-2), the tumor inhibition rate was about 3.5 % on Day 12 after transplantation compared to ODN1826 (SEQ-1). On the other hand, SEQ-8 which is an adjuvant of the present invention with 10 mer of complementary strand showed about 50 % of the tumor inhibition rate compared to ODN1826 on Day 12 after transplantation. Furthermore, SEQ-10 which has 15 mer of complementary strand showed the

strong effect of inhibition on tumor progression of B16F10 cells, about 76 % compared to ODN1826 on Day 12 after transplantation. The results were shown in Figure 3.

[0248]

- 5 Result 5-A: CTL inductivity of adjuvants of the present invention with the different chain length of complementary strand

The activity of an adjuvant of the present invention whose chain length of complementary strand is from 10 to 20 mer against ODN1826 (20 mer), i.e., complementary strand is 50 to 100 % against the CpG oligonucleotide was examined with TRP2 peptide. The adjuvant whose complementary strand is 14 mer (SEQ-28), 15 mer (SEQ-26), 17 mer (SEQ-22), 19 mer (SEQ-18) and 20 mer (SEQ-16) showed enhancement of CTL inductivity compared to ODN1826 (SEQ-1). Especially, adjuvant with 19 mer (SEQ-18), 17 mer (SEQ-22) and 15mer (SEQ-26) showed CTL inductivity at about 3 times compared to ODN1826 (SEQ-1). The results were shown in Table 35.

[0249]

[Table 35]

Peptide	Adjuvant	dose/head nmol	Ratio of average value of antigen-specific CTL
TRP2	SEQ-1	1.57	1.00
TRP2	SEQ-16	1.57	1.63
TRP2	SEQ-18	1.57	3.40
TRP2	SEQ-22	1.57	2.69
TRP2	SEQ-26	1.57	2.82
TRP2	SEQ-28	1.57	2.21
TRP2	SEQ-32	1.57	1.10
TRP2	SEQ-36	1.57	1.07

- 20 [0250]

Result 6-A: Comparison of CTL inductivity with Montanide

The activities of ODN1826 (SEQ-1), ssCpG ODN introducing a lipid ligand (SEQ-2), an adjuvant of the present invention (SEQ-26) and Montanide which is an adjuvant used a lot in the clinical trials as an adjuvant of a peptide vaccine were compared with TRP2 peptide as antigen. SEQ-2 and SEQ-26 showed higher CTL inductivity than ODN1826. On the other hand, CTL inductivity of Montanide was lowered to one-seventh compared to ODN1826.

The results were shown in Table 36.

[0251]

[Table 36]

Peptide	Adjuvant	dose/head nmol	Ratio of average value of antigen-specific CTL
TRP2	SEQ-1	1.57	1.00
TRP2	SEQ-2	1.57	3.23
TRP2	SEQ-26	1.57	2.82
TRP2	Montanide	100 μ L	0.13

5

[0252]

Result 6-B: Comparison of the anti-tumor effect against ssCpG ODN introducing a lipid ligand

When mice were immunized with TRP2 peptide and an adjuvant of the present invention, SEQ-26, it showed the very strong effect of inhibition on tumor progression of B16F10 cells, 76.8% on Day 12 after transplantation compared to ODN1826 (SEQ-1). The tumor inhibition rate of ssCpG ODN introducing a lipid ligand (SEQ-2) was 64.3% on Day 12 after transplantation compared to ODN1826. That is, an adjuvant of the present invention showed the stronger effect of inhibition on tumor progression of B16F10 cells than the adjuvant of SEQ-2. On the other hand, Montanide used as an adjuvant has no anti-tumor effect. The results were shown in Figure 4.

[0253]

Result 7-A: CTL inductivity of adjuvants of the present invention with the different kinds of nucleic acid monomer in a complementary strand

Activity of an adjuvant of the present invention whose nucleic acid monomers in the complementary strand were RNA, 2'-OMe-RNA or 2'-F-RNA was examined with TRP2 peptide. The adjuvant that all of the nucleic acid monomers were RNA (SEQ-40) or 2'-F-RNA (SEQ-42) did not show big enhancement of CTL inductivity compared to ODN1826 (SEQ-1). On the other hand, the adjuvant with 2'-OMe-RNA as a nucleic acid monomer (SEQ-38 and SEQ-44) showed enhancement of CTL inductivity at 2.9 or 2.4 times respectively compared to ODN1826. These results suggested that 2'-OMe-RNA is useful as nucleic acid monomer of an adjuvant of the present invention as well as DNA. The results were shown in Table 37.

[0254]

[Table 37]

Peptide	Adjuvant	dose/head nmol	Ratio of average value of antigen-specific CTL	Tumor inhibition rate (Day 14 after transplantation)
TRP2	SEQ-1	1.57	1.00	0.00
TRP2	SEQ-38	1.57	2.85	86.19
TRP2	SEQ-40	1.57	1.24	3.73
TRP2	SEQ-42	1.57	0.99	57.33
TRP2	SEQ-44	1.57	2.39	57.51
TRP2	SEQ-2	1.57	3.11	72.64

5 [0255]

Result 7-B: Anti-tumor effect of adjuvants of the present invention with the different kinds of nucleic acid monomer in a complementary strand

When mice were immunized with TRP2 peptide and an adjuvant of the present invention with 2'-OMe-RNA as nucleic acid monomers (SEQ-38), it showed the very strong effect of inhibition on tumor progression of B16F10 cells, about 86.2 % on Day 14 after transplantation compared to ODN1826 (SEQ-1). The tumor inhibition rate of ssCpG ODN introducing a lipid ligand (SEQ-2) was about 72.6 % on Day 14 after transplantation compared to ODN1826. That is, an adjuvant of the present invention showed the stronger effect of inhibition on tumor progression of B16F10 cells than the adjuvant of SEQ-2. In addition, the adjuvant whose chain length of SEQ-38 was shorten (SEQ-44) and the adjuvant with 2'-F-RNA as a nucleic acid monomer (SEQ-42) showed the effect of inhibition on tumor progression in B16F10 cells. On the other hand, the adjuvant with RNA as a nucleic acid monomer (SEQ-40) showed no effect of inhibition on tumor progression of B16F10 cells. The results were shown in Table 37 and Figure 5.

[0256]

(Result using ODN2006 as a CpG oligonucleotide)

Result 8-A: CTL inductivity of adjuvants of the present invention with the different chain length of the complementary strand or a linker

The activities of ODN2006 (SEQ-49, 24 mer) and adjuvants of the present invention were compared with TRP2 peptide as antigen. The adjuvant whose chain length of the complementary strand is 24 mer, i.e., the

length of the complementary strand against the CpG oligonucleotide is 100 % (SEQ-51) and the adjuvant whose chain length of the complementary strand is 15 mer, i.e., the length of the complementary strand against the CpG oligonucleotide is 62.5 % (SEQ-61) were used. Both of SEQ-51 and SEQ-61 showed high CTL inductivity at more than 5 times compared to ODN2006. Furthermore, the adjuvant with dTdT as a linker (SEQ-63) or dGdG (SEQ-65) was used. Both SEQ-63 and SEQ-65 showed very high CTL inductivity at about 10 times compared to ODN2006. The results were shown in Table 38. [0257]

[Table 38]

Peptide	Adjuvant	dose/head nmol	Ratio of average value of antigen-specific CTL
TRP2	SEQ-49	4.71	1.00
TRP2	SEQ-51	4.71	5.82
TRP2	SEQ-61	4.71	5.73
TRP2	SEQ-63	4.71	11.73
TRP2	SEQ-65	4.71	9.21

[0258]

Result 8-B: Anti-tumor effect of an adjuvant of the present invention with the different chain length of a complementary strand or a linker

When mice were immunized with TRP2 peptide and ODN2006 (SEQ-49), it showed little effect of inhibition on tumor progression of B16F10 cells. On the other hand, an adjuvant of the present invention whose length of the complementary strand is 100 % against the CpG oligonucleotide (SEQ-51) showed 20 % of the effect of inhibition on tumor progression of B16F10 cells on Day 10 after transplantation compared to ODN2006. In addition, each the adjuvant whose length of the complementary strand is 62.5 % against the CpG oligonucleotide (SEQ-61), and the adjuvant with dTdT as a linker (SEQ-63) or dGdG (SEQ-65) showed about 50 % of the effect of inhibition on tumor progression of B16F10 cells on Day 10 after transplantation compared to ODN2006. The results were shown in Figure 6.

[0259]

Result 9-A: CTL inductivity of adjuvants of the present invention whose nucleic acid monomer in the complementary strand is 2'-OMe-RNA

Activity of an adjuvant of the present invention whose nucleic acid monomer in the complementary strand is 2'-OMe-RNA was examined with TRP2. The chain length of the complementary strand is 24 mer, i.e., the adjuvant whose length of the complementary strand against the CpG oligonucleotides was 100 % (SEQ-67) and, the adjuvant whose length of the complementary strand is 15 mer, i.e., the adjuvant whose length of the complementary strand against the CpG oligonucleotide is 62.5 % (SEQ-69) were used. SEQ-67 and SEQ-69 showed CTL inductivity at 1.4 or 1.2 times respectively compared to ODN2006 (SEQ-49). The results were shown in Table 39.

[0260]

[Table 39]

Peptide	Adjuvant	dose/head nmol	Ratio of average value of antigen-specific CTL
TRP2	SEQ-49	4.71	1.00
TRP2	SEQ-67	4.71	1.39
TRP2	SEQ-69	4.71	1.21

[0261]

Result 9-B: Anti-tumor effect of adjuvants of the present invention whose nucleic acid monomer in the complementary strand is 2'-OMe-RNA

When mice were immunized with TRP2 peptide and an adjuvant of the present invention which has 2'-OMe-RNA as a nucleic acid monomer, adjuvant whose length of complementary strand against the CpG oligonucleotides is 100 % (SEQ-67) showed very strong effect of inhibition on tumor progression of B16F10 cells, about 75 % on Day 10 after transplantation compared to ODN2006 (SEQ-49). The adjuvant whose length of the complementary strand against CpG oligonucleotides is 62.5 % (SEQ-69) showed about 20 % of the effect of inhibition on tumor progression of B16F10 cells on Day 10 after transplantation compared to ODN2006. The results were shown in Figure 7.

These suggested that 2'-OMe-RNA was useful also for ODN2006 as nucleic acid monomer for an adjuvant of the present invention as well as DNA.

[0262]

In similar methods, CTL inductivity or anti-tumor effect of adjuvants of

the present invention was measured. Results are shown in Tables 40 to 68 and Figures 8 to 13 separately by experiments. For these experiments, TRP2 peptide was used and the dosage of the adjuvant of the present invention was 4.71 nmol. NT in tables means “not tested”.

5 [0263]

[Table 40]

Adjuvant	Ratio of average value of antigen-specific CTL
SEQ-49	1.00
SEQ-59	5.34
SEQ-57	4.28
SEQ-55	3.02
SEQ-53	2.86

[0264]

10 [Table 41]

Adjuvant	Ratio of average value of antigen-specific CTL	Tumor inhibition rate (Day 12 after transplantation)
SEQ-49	1.00	0.00
SEQ-119	2.05	55.1
SEQ-192	1.74	41.5

[0265]

[Table 42]

Adjuvant	Ratio of average value of antigen-specific CTL	Tumor inhibition rate (Day 12 after transplantation)
SEQ-49	1.00	0.00
SEQ-121	3.99	56.8

15

[0266]

[Table 43]

Adjuvant	Ratio of average value of antigen-specific CTL	Tumor inhibition rate (Day 14 after transplantation)
SEQ-49	1.00	0.00
SEQ-152	1.17	80.45
SEQ-154	2.47	NT
SEQ-158	3.06	100

[0267]

5 [Table 44]

Adjuvant	Ratio of average value of antigen-specific CTL
SEQ-49	1.00
SEQ-182	2.24
SEQ-184	2.38

[0268]

[Table 45]

Adjuvant	Ratio of average value of antigen-specific CTL	Tumor inhibition rate (Day 14 after transplantation)
SEQ-49	1.00	0.00
SEQ-186	2.37	NT
SEQ-188	1.49	51.03
SEQ-190	1.95	NT
SEQ-192	3.61	46.16
SEQ-194	2.48	55.02
SEQ-196	3.26	NT

10

[0269]

[Table 46]

Adjuvant	Ratio of average value of antigen-specific CTL	Tumor inhibition rate (Day 14 after transplantation)
SEQ-49	1.00	0.00
SEQ-156	1.70	NT
SEQ-160	3.65	NT
SEQ-162	3.44	NT
SEQ-164	4.88	53.69

[0270]

5 [Table 47]

Adjuvant	Ratio of average value of antigen-specific CTL
SEQ-49	1.00
SEQ-166	2.57
SEQ-168	1.96

[0271]

[Table 48]

Adjuvant	Ratio of average value of antigen-specific CTL	Tumor inhibition rate (Day 14 after transplantation)
SEQ-49	1.00	0.00
SEQ-170	3.10	79.09
SEQ-172	1.24	NT
SEQ-174	1.78	NT

10

[0272]

[Table 49]

Adjuvant	Ratio of average value of antigen-specific CTL
SEQ-49	1.00
SEQ-176	1.97
SEQ-178	5.44
SEQ-180	3.59
SEQ-206	1.07
SEQ-208	1.28
SEQ-214	1.38
SEQ-216	1.95

[0273]

5 [Table 50]

Adjuvant	Ratio of average value of antigen-specific CTL
SEQ-49	1.00
SEQ-198	3.12
SEQ-200	3.24
SEQ-204	3.98

[0274]

[Table 51]

Adjuvant	Ratio of average value of antigen-specific CTL
SEQ-49	1.00
SEQ-226	1.84
SEQ-228	2.10
SEQ-230	3.10
SEQ-232	1.28
SEQ-234	1.96

10

[0275]

[Table 52]

Adjuvant	Ratio of average value of antigen-specific CTL
SEQ-49	1.00
SEQ-218	2.87
SEQ-220	1.68
SEQ-222	3.11
SEQ-224	2.25

[0276]

5 [Table 53]

Adjuvant	Ratio of average value of antigen-specific CTL
SEQ-49	1.00
SEQ-236	2.66

[0277]

[Table 54]

Adjuvant	Ratio of average value of antigen-specific CTL
SEQ-49	1.00
SEQ-238	1.09
SEQ-240	1.45
SEQ-242	1.62
SEQ-244	3.24

10

[0278]

[Table 55]

Adjuvant	Ratio of average value of antigen-specific CTL
SEQ-49	1.00
SEQ-246	2.51
SEQ-248	1.67
SEQ-250	1.86
SEQ-252	1.14

15

[0279]

[Table 56]

Adjuvant	Ratio of average value of antigen-specific CTL
SEQ-256 (D35-CpG)	1.00
SEQ-258	2.46

5 [0280]

[Table 57]

Adjuvant	Ratio of average value of antigen-specific CTL
SEQ-49	1.00
SEQ-260	1.89
SEQ-262	1.45
SEQ-264	3.44
SEQ-266	2.51

[0281]

10 [Table 58]

Adjuvant	Ratio of average value of antigen-specific CTL
SEQ-49	1.00
SEQ-268	2.09
SEQ-270	2.31
SEQ-272	1.77
SEQ-274	4.03
SEQ-276	3.77
SEQ-278	2.58

[0282]

[Table 59]

Adjuvant	Ratio of average value of antigen-specific CTL
SEQ-49	1.00
SEQ-280	5.27
SEQ-282	2.82
SEQ-284	5.57
SEQ-286	2.40

[0283]

5 [Table 60]

Adjuvant	Ratio of average value of antigen-specific CTL
SEQ-49	1.00
SEQ-288	4.22
SEQ-290	2.80
SEQ-292	4.63
SEQ-294	5.30

[0284]

[Table 61]

Adjuvant	Ratio of average value of antigen-specific CTL
SEQ-49	1.00
SEQ-296	2.43
SEQ-298	2.08
SEQ-300	3.48
SEQ-302	2.58
SEQ-304	2.42
SEQ-306	3.04

10

[0285]

[Table 62]

Adjuvant	Ratio of average value of antigen-specific CTL
SEQ-49	1.00
SEQ-308	2.52
SEQ-310	4.91
SEQ-312	1.75
SEQ-314	2.58
SEQ-316	1.30

[0286]

5 [Table 63]

Adjuvant	Ratio of average value of antigen-specific CTL
SEQ-49	1.00
SEQ-318	4.19
SEQ-320	3.81
SEQ-322	2.15
SEQ-324	1.92

[0287]

[Table 64]

Adjuvant	Ratio of average value of antigen-specific CTL
SEQ-49	1.00
SEQ-326	4.27
SEQ-328	2.93
SEQ-330	2.53
SEQ-332	2.98
SEQ-334	1.66
SEQ-336	3.62
SEQ-338	1.72

10

[0288]

[Table 65]

Adjuvant	Ratio of average value of antigen-specific CTL
SEQ-339(ODN2216)	1.00
SEQ-341	1.25
SEQ-342(ODN684)	1.00
SEQ-344	1.20
SEQ-345(D-LS01)	1.00
SEQ-347	2.73

[0289]

5 [Table 66]

Adjuvant	Ratio of average value of antigen-specific CTL
SEQ-354(ODN M362)	1.00
SEQ-356	1.91

[0290]

[Table 67]

Adjuvant	Ratio of average value of antigen-specific CTL
SEQ-49	1.00
SEQ-373	2.21
SEQ-375	2.58
SEQ-377	1.57

10

[0291]

[Table 68]

Adjuvant	Ratio of average value of antigen-specific CTL
SEQ-49	1.00
SEQ-361	1.09
SEQ-365	2.04
SEQ-369	1.70
SEQ-371	1.11

15

[0292]

Example 4

Antigen-specific CTL inductivity and anti-tumor effect of ODN2006 (SEQ-49) and SEQ-121 in the absence of a tumor antigen peptide were evaluated. In similar methods of A) and B) of Example 3, the effect of vaccine without a tumor antigen peptide was verified. Both of the antigen-specific CTL values of SEQ-49 and SEQ-121 were undetectable. On the other hand, SEQ-121 showed 85.3 % of the effect of inhibition on tumor progression of B16F10 cells on Day 12 after transplantation compared to ODN2006. From these results, SEQ-121 was expected to apply to therapeutic vaccine with anti-tumor effect even it is a single agent (Table 69, Figure 14).

[0293]

[Table 69]

Adjuvant	Ratio of average value of antigen-specific CTL	Tumor inhibition rate (Day 12 after transplantation)
SEQ-49	Undetectable	0.00
SEQ-121	Undetectable	85.3

[0294]

Example 5 Irritation test in a single dose of adjuvants of the present invention

A) Subcutaneous irritation test in a single dose

Subcutaneous irritation of a compound of the present invention was evaluated by gross examination and histopathological examination about the skin at necropsy one day and one or four week(s) after subcutaneous administration in a single dose.

The compound of the present invention was subcutaneously administered to the center of back between the shoulder blades of rats. At necropsy one day, one or four week(s) after administration (isoflurane anesthesia, euthanasia by bleeding), skin at the administration site was collected, grossly observed and fixed in 10 % neutral buffered formalin solution. The fixed skin was cut out, embedded and put in a thin slice by the usual methods, and Hematoxylin-eosin (HE) stained sections were made. The pathological observers were observed the HE stained sections by an optical

microscope and recorded the histopathological findings. The changes in pathological findings were evaluated in four grades of Minimal; $\pm$, Mild; +, Moderate; 2+ and Marked; 3+. When any grade was not appropriate for the finding, it was recorded as Positive.

5 [0295]

(Result)

To verify the safety of adjuvants of the present invention as a medicine, the subcutaneous irritation was compared to that of Montanide used in clinical trials as an adjuvant, Amph1826 (SEQ-2) or ODN2006 (SEQ-49) which is the
10 well-known adjuvant.

Montanide is an adjuvant consisting of mineral oil and surfactant, and used in clinical trials as an adjuvant of peptide vaccine by preparation of emulsion with aqueous formulation. Although it is an adjuvant with excellent safety profile about systemic safety concerns, there is a problem about the
15 occurrence of skin induration caused by remaining emulsion in the administration site. In this experiment, the administration site reaction of the double-stranded oligonucleotide was compared to that of Montanide, and it was examined whether it could be an adjuvant with week local irritation.

Rat subcutaneous irritation tests in a single dose were carried out for
20 Montanide, SEQ-2, SEQ-49 or an adjuvant of the present invention (SEQ-61, SEQ-119, SEQ-121, SEQ-170, SEQ-192 or SEQ-216). Montanide was administered at 1 mL/site in emulsion of Montanide: saline (1:1). SEQ-2, SEQ-49 or an adjuvant of the present invention was dissolved in phosphate buffered saline and administered at 4.71, 15.7 or 47.1 nmol/1mL/site,
25 respectively.

As a result, it became clear that Montanide was not absorbed/decomposed/eliminated because white accumulation or white nodules was grossly observed under the skin and cyst-like structures was histologically confirmed one day, one and four week(s) after administration. Montanide
30 remaining under the skin continued eliciting inflammation from one day to four weeks after administration. One day after administration, neutrophil infiltration and edema were observed. One week after administration, neutrophil infiltration and edema continued, but the inflammation became slightly chronic to observe as granulomatous inflammation. Four weeks after

administration, thick fibrous capsule encapsulated Montanide was formed. Inflammation was slightly reduced compared to one week after administration, but even four weeks after administration, the animals with continuous neutrophil infiltration or edema were observed.

5 In the case of SEQ-2, inflammation cellular infiltration, edema and crust were observed one day and one week after administration. Even four weeks after administration, skin inflammation was not recovered, and granulomatous inflammation by continuous activation of monocyte/macrophage system was observed.

10 In the case of SEQ-49, and SEQ-61, SEQ-119, SEQ-121, SEQ-170, SEQ-192 and SEQ-216, which is an adjuvant of the present invention, acute inflammation, mainly neutrophil infiltration, was observed under the skin one day after administration. One week after administration, inflammation, mainly lymphocyte and macrophage, was observed from subcutaneous to
15 dermis tissue, and four weeks after administration, inflammation was almost recovered. Regarding SEQ-119, SEQ-121 and SEQ-170, subcutaneous irritation evaluation four weeks after administration was not carried out, but when their subcutaneous irritation one day and one week after administration were compared to those of SEQ-119, SEQ-121, SEQ-170 and SEQ-61, the
20 irritation were equal to or weaker than SEQ-61. These suggested that regarding SEQ-119, SEQ-121 and SEQ-170, inflammation was almost completely recovered four weeks after administration.

Tables 70 to 72 showed the results one day after administration, Tables 73 to 75 showed the results one week after administration, and Tables 76 and
25 77 showed the results four weeks after administration. The notes in the tables are as below.

1) Values after findings are numbers of the observed animals.

2) Values after findings are scores. The scores were calculated the total, provided that grades of each individual were put as Minimal; 1, Mild; 2 and
30 Moderate; 3.

[0296]

The above showed that compared between adjuvants of the present invention and Montanide, adjuvants of the present invention did not cause the accumulation of the compounds under the skin (low risk of induration),

necrosis or neutrophil infiltration was not occurred even one week after administration, and inflammation was almost completely recovered four weeks after administration. Therefore, it was concluded that subcutaneous irritation of the adjuvants of the present invention was weaker than that of Montanide. Also, compared between adjuvants of the present invention and Amph1826 (SEQ-2), inflammation caused by adjuvants of the present invention was almost completely recovered four weeks after administration, but on the other hand skin inflammation caused by SEQ-2 was not recovered, and granulomatous inflammation by continuous activation of monocyte/macrophage system was observed even four weeks after administration. Therefore, it was concluded that subcutaneous irritation of the adjuvants of the present invention was weaker than that of SEQ-2. Subcutaneous irritation of adjuvants of the present invention was no significant difference from that of ODN2006 (SEQ-49).

[0297]

[Table 70]

Compound name		Montanide	SEQ-49				SEQ-61			
Dose (nmol/site)		-	4.71	15.7	47.1	47.1	4.71	15.7	47.1	47.1
Number of animals		3	3	3	3	3	3	3	3	3
Number of deaths/imminent dissections		0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
Pathology	Gross ¹⁾	Subcutaneous:	3	0	0	0	0	0	0	0
		White accumulation								
		Subcutaneous:	0	0	0	1	0	0	0	0
		Red spots, Erythema								
	Histopathological	Subcutaneous:	3	6	6	6	5	6	6	6
		Neutrophilic infiltration ²⁾								
		Subcutaneous: Edema ²⁾	3	4	5	5	2	3	5	5
		Subcutaneous: Bleeding ²⁾	2	2	1	2	1	0	1	1
		Subcutaneous:	3	0	0	0	0	0	0	0
		Cyst-like structure ¹⁾								
		Muscle layer:	0	4	6	5	4	5	5	5
		Neutrophilic infiltration ²⁾								
		Muscle layer: Edema ²⁾	0	2	1	0	3	2	3	3

[0298]

[Table 71]

Compound name		SEQ-192					SEQ-216					SEQ-2		
Dose (nmol/site)		4.71	15.7	47.1	47.1	47.1	4.71	15.7	47.1	47.1	4.71	15.7	47.1	
Number of animals		3	3	3	3	3	3	3	3	3	3	3	3	
Number of deaths/imminent dissections		0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	
Pathology	Administration site	Histopathological ²⁾	Subcutaneous tissue	Inflammatory cell infiltration ²⁾	6	5	5	6	6	5	7	6	6	
				Edema ²⁾	7	6	4	5	7	4	3	5	6	
				Bleeding ²⁾	2	1	1	3	1	1	0	3	3	
				Necrosis ²⁾	1	0	1	1	0	0	1	1	1	
				Cyst-like structure ¹⁾	2	1	2	2	3	0	1	2	1	
		Dermis	Inflammatory cell infiltration ²⁾	6	6	6	7	7	6	5	9	6		
			Inflammatory cell infiltration ²⁾	2	3	4	3	6	2	4	7	6		
		Muscle layer	Edema ²⁾	1	2	3	2	2	1	1	3	4		
			Necrosis ²⁾	0	0	0	0	0	1	0	0	0		

[0299]

[Table 72]

Compound name		SEQ-121			SEQ-170			SEQ-119		
Dose (nmol/site)		4.71	15.7	47.1	4.71	15.7	47.1	4.71	15.7	47.1
Number of animals		3	3	3	3	3	3	3	3	3
Number of deaths/imminent dissections		0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
Pathology	Gross ¹⁾	Turbidity		0	0	0	1	1	0	2
		Erythema		0	0	0	1	2	0	0
	Histopathological ²⁾	Subcutaneous tissue	Inflammatory cell infiltration ²⁾ Edema ²⁾	5	5	6	3	6	8	6
				9	8	5	6	5	7	6
		Dermis	Inflammatory cell infiltration ²⁾	1	4	6	1	0	5	6
		Muscle layer	Inflammatory cell infiltration ²⁾	2	5	6	3	5	6	4

[0300]

[Table 73]

Compound name		Montanide	SEQ-49			SEQ-61		
Dose (nmol/site)		-	4.71	15.7	47.1	4.71	15.7	47.1
Number of animals		3	3	3	3	3	3	3
Number of deaths/imminent dissections		0/0	0/0	0/0	0/0	0/0	0/0	0/0
Pathology	Administration site	Gross ¹⁾	Subcutaneous:					
			White nodules					
			Epidermis : Crust					
			0	1	1	0	1	0
			2	0	0	0	0	0
			Subcutaneous : Necrosis ²⁾					
			Subcutaneous:					
			Neutrophilic infiltration ²⁾					
			Subcutaneous:					
			Monocyte infiltration ²⁾					
	Hepathological		0	3	6	8	2	3
			Subcutaneous:					
			Granulomatous inflammation ²⁾					
			7	0	0	0	0	0
			Subcutaneous : Edema ²⁾					
	Cyst-like structure ¹⁾		5	4	6	5	4	5
			3	0	0	0	0	0

[0301]

[Table 74]

Compound name		SEQ-192				SEQ-216				SEQ-2			
Dose (nmol/site)		4.71	15.7	47.1	47.1	4.71	15.7	47.1	47.1	4.71	15.7	47.1	47.1
Number of animals		3	3	3	3	3	3	3	3	3	3	3	3
Number of deaths/imminent dissections		0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
Pathology	Gross ¹⁾	White matter accumulation	0	0	0	0	0	0	0	0	0	0	0
		Dark red spots	0	0	0	0	0	0	1	0	0	0	0
		Edema	0	0	0	0	0	1	0	0	1	1	1
		Crust	0	1	1	1	0	1	2	0	0	0	0
	Histopathological ²⁾	Granulomatous inflammation ²⁾	0	0	0	0	0	0	0	0	0	0	0
		Inflammatory cell infiltration ²⁾	4	6	7	3	6	5		8	8	8	8
		Edema ²⁾	3	3	2	1	2	2	3	4	3	3	3
		Necrosis ²⁾	0	1	3	0	0	2	0	0	0	0	0
		Cyst-like structure ¹⁾	0	1	2	2	2	1	1	1	1	3	3
		Inflammatory cell infiltration ²⁾	2	5	7	0	5	8	4	6	9	9	9
		Muscle layer	0	5	3	0	4	4	0	4	6	6	6

[0302]

[Table 75]

Compound name		SEQ-121			SEQ-170			SEQ-119		
Dose (nmol/site)		4.71	15.7	47.1	4.71	15.7	47.1	4.71	15.7	47.1
Number of animals		3	3	3	3	3	3	3	3	3
Number of deaths/imminent dissections		0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
Pathology	Administration site	Gross ¹⁾	Turbidity		0	0	0	1	1	0
			Erythema		0	0	0	1	2	0
		Histopathological ²⁾	Subcutaneous	Inflammatory cell infiltration ²⁾	5	5	6	3	6	8
			Dermis	Inflammatory cell infiltration ²⁾	1	4	6	1	0	5
			Muscle layer	Inflammatory cell infiltration ²⁾	2	5	6	3	5	6
								1	2	4

[0303]

[Table 76]

Compound name		Montanide	SEQ-49				SEQ-61			
Dose (nmol/site)		–	4.71	15.7	47.1	47.1	4.71	15.7	47.1	47.1
Number of animals		3	3	3	3	3	3	3	3	3
Number of deaths/imminent dissections		0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
Pathology	Gross ¹⁾	Subcutaneous: White nodules	3	0	0	0	0	0	0	0
		Muscle layer: Monocyte infiltration ²⁾	0	0	0	0	0	0	0	1
	Administration site	Subcutaneous: Neutrophilic infiltration ²⁾	3	0	0	0	0	0	0	0
		Subcutaneous: Granulomatous infiltration ²⁾	6	0	0	0	0	0	0	0
		Histopathological	1	0	0	0	0	0	0	0
		Subcutaneous: Edema ²⁾	1	0	0	0	0	0	0	0
		Subcutaneous: Cyst-like structure accompanied with formation of fibrous capsule ¹⁾	3	0	0	0	0	0	0	0

[0304]

[Table 77]

Compound name		SEQ-192				SEQ-216				SEQ-2			
Dose (nmol/site)		4.71	15.7	47.1	47.1	4.71	15.7	47.1	47.1	4.71	15.7	47.1	47.1
Number of animals		3	3	3	3	3	3	3	3	3	3	3	3
Number of deaths/imminent dissections		0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
Pathology	Gross ¹⁾	White matter accumulation		0	0	0	0	0	0	0	0	0	0
		Crust		0	0	0	0	1	1	0	0	0	0
	Histopathological ²⁾	Subcutaneous	Granulomatous inflammation ²⁾	0	0	0	0	0	0	4	5	7	7
			Inflammatory cell infiltration ²⁾	0	0	0	0	0	0	0	0	0	0
			Edema ²⁾	0	0	0	0	0	0	0	0	0	0
		Dermis	Fibrosis ²⁾	0	0	0	0	1	0	0	0	0	0
			Cyst-like structure ¹⁾	0	0	0	0	0	0	0	0	0	0
	Administration site	Inflammatory cell infiltration ²⁾		0	1	0	0	1	0	0	4	7	7
	Pathology												

[0305]

B) Intradermal irritation test in a single dose

Subcutaneous irritation of adjuvants of the present invention of the
5 present invention is evaluated by gross examination and histopathological
examination about the skin at necropsy one day, one and four week(s) after
subcutaneous administration in a single dose.

An adjuvant of the present invention is subcutaneously administered to
skin of back of rats. The following procedure is carried out in a similar
10 method to A) of Example 5.

[0306]

Example 6 Evaluation of inducibility of antibody production of the adjuvant of
the present invention in immunization by *Pseudomonas aeruginosa* PCR
antigen vaccine

15 When antigen and adjuvant are administered as an infectious disease
vaccine and induced antibody production from B lymphocyte in the immunized
animal, effect of prevention and treatment for infectious disease is expected.
Therefore, in expectation of apply for an infectious disease vaccine of this
adjuvant, and antibody production from B lymphocyte was evaluated. PCR
20 antigen which is *Pseudomonas aeruginosa* was used for the examination as an
antigen. It has been already reported that *Pseudomonas aeruginosa* PCR
antigen have a vaccine effect (Moriyama et al., Infect. Immun., Sep. 2001, vol.
69, no. 9, 5908-5910), and therefore effect of inducibility of antibody production
by the adjuvant was evaluated compared to PCR antigen administration
25 group. Freund's adjuvant was used for a control group. Freund's adjuvant is
an adjuvant that tubercle bacillus killed by heating is mixed in oil consisting of
paraffin oil and mannide monooleate, and known as an adjuvant strongly
inducing antibody production in non-clinical studies. Freund's adjuvant
shows strong effect in non-clinical studies, but the use in clinical trials has
30 been prohibited because of concern of side effects (The European Agency for the
Evaluation of Medical Products Evaluation of Medicines for Human Use, 25
March 2004, Internet (URL:
http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/11/WC500015469.pdf)).

[0307]

Pcrv nucleotide sequence (SEQ ID NO: 48) derived from *Pseudomonas Aeruginosa* Strain PAO1 was cloned at NdeI-XhoI site in pET21a vector, and *Escherichia coli* strain BL21 (DE3) was transformed with the prepared
5 plasmid. Strain pcrV-BL21 (DE3) was cultured in 500 mL of LB/Ampicillin liquid medium at 37 °C, and 200 µL of 0.1 M IPTG was added when OD600 became 0.5 to induce expression of recombinant PCRv protein. After culturing at 16 °C for 18 hours, bacteria were separated by a centrifuge, and 15 ml of Buffer A (50 mM Tris-HCl (pH8.0), 0.2M NaCl) comprising 0.5% lysozyme (SIGMA Corporation) was added. After putting at 4 °C for 30 minutes,
10 ultrasonic treatment was carried out. After centrifugation at 12,000 rpm for 10 minutes, soluble fraction was obtained. 1 mL of Ni-NTA agarose gel (QIAGEN) was added to Polyprep column (BIO-RAD Laboratories, Inc) and equilibrated with 10mL of Buffer A. Soluble fraction was added to the column
15 and passed. The column was washed with 20 mL of Buffer A + 20 mM imidazole and eluted with 1 mL of Buffer A + 250 mM imidazole. Superdex 200 16/60 GL column was connected to AKTA Explorer (GE Healthcare), and equilibrated with 1.5 CV of Buffer A. The fraction eluted with imidazole was purified by gel filtration and recombinant PCRv protein C-end HIS (SEQ ID
20 NO: 49) was purified. The resulting recombinant PCRv protein was measured the concentration with BCA assay kit (PIERCE) and kept at -80°C.

[0308]

70 µg of recombinant PCRv protein and 33 nmol of an adjuvant of the present invention were mixed with saline to be 0.7 mL. To each 5 mice of 5
25 weeks old female A/J mice (Japan SLC, Inc.), 0.1 mL of immunogen was intradermally injected on Day 0, 7 and 27. As a control, only recombinant PCRv protein was used for immunization in a similar method. Regarding Freund's adjuvant, 100 µg of recombinant PCRv protein was mixed with saline to be 0.5 mL, and emulsion was prepared with the same amount of Complete
30 Freund's adjuvant (Difco Laboratories). 0.1 mL was intradermally injected on Day 7 as initial immunization. On Day 27, Incomplete Freund's adjuvant (Difco Laboratories) was used for immunization in a similar method. On Day 33, blood was collect using a heparin from tail vein of mice, and centrifuged at 6,000 rpm for 15 minutes. The supernatant was collected as an antiserum

and kept at -40 °C.

[0309]

Antibody titer was measured as blow. Recombinant PCRV protein was mixed with PBS (pH7.4) (Invitrogen) to be 1 µg/mL, and 20 µL/well of the solution was added to Nunc Maxisorp 384 well plate (Thermo). The plate was sealed with a plate seal and incubation was carried out at 4 °C over night. After washing twice with 90 µL/well of Washing Buffer (9 g/L NaCl, 0.5 g/L Proclin 150, 0.1 g/L Tween-20), 60 µL/well of 1×Assay Buffer (Invitrogen) was added. The plate was sealed with a plate seal and blocking was carried out at room temperature for 2 hours. After removing the liquid by tapping, antiserum was diluted with 1×Assay Buffer at 10^3 to 10^7 times and 20 µL/well of the solution was added. The plate was sealed with a plate seal and incubation was carried out at 4 °C over night. After washing three times with Washing Buffer, GOAT Anti-mouse IgG Fc-HRP (JacksonImmunoResearch) was diluted at 20,000 with 1×Assay Buffer and 20 µL/well of the solution was added. The plate was sealed with a plate seal and incubation was carried out at room temperature for 2 hours. After washing three times with Washing Buffer, 20 µL/well of TMB substrate (Dako) was added and incubation was carried out at room temperature for 30 minutes. 20 µL/well of 0.5 N sulfuric acid (Nacalai) was added, and absorbance at 450 nm was measured with a plate reader. The dilution ratio when absorbance at 450 nm is 1.0 was calculated and put as an antibody titer. The results were shown in Figure 15.

[0310]

The antibody titers were 3.0×10^6 for SEQ-121 which is the highest, 2.5×10^6 for Freund's adjuvant, 7.1×10^5 for SEQ-61 and 5.3×10^5 for ODN2006 (SEQ-49) in order. The antibody titer for only PCRV protein was 1.3×10^5 . These results suggested that the adjuvants of the present invention have strong immunostimulatory activity against B cells. Especially, antibody titer for SEQ-121 showed higher inducibility of antibody production than ODN2006 and almost same with antibody titer for Freund's adjuvant. Then, it was suggested that adjuvants of the present invention are excellent as an adjuvant for vaccine against infectious diseases. From these results, the adjuvants of the present invention are expected to apply for a vaccine against infectious diseases.

Industrial Applicability

[0311]

As it is clear from the above examples, lipid binding double-stranded
5 oligonucleotides of the present invention show the excellent
immunostimulatory activity. Therefore, they are very useful especially as an
adjuvant to enhance the effect of vaccine.

Sequence Listing

10

Claims

[Claim 1]

A pharmaceutical composition comprising a double-stranded

oligonucleotide, wherein

a first strand consists only of a CpG oligonucleotide consisting of 8 to 50 nucleotides,

a second strand is an oligonucleotide consisting of 8 to 60 nucleotides and comprising a sequence capable of hybridizing with the first strand, but

excluding an RNA oligonucleotide,

the length of the second strand is 50 % or more of that of the first strand, and

a lipid comprising C12 to C30 hydrocarbon chain(s) binds to the second strand through a linker.

[Claim 2]

The pharmaceutical composition comprising a double-stranded

oligonucleotide of claim 1, wherein the oligonucleotide of the second

strand is an oligonucleotide consisting of DNA nucleosides and/or

nucleoside derivatives.

[Claim 3]

The pharmaceutical composition comprising a double-stranded

oligonucleotide of claim 2, wherein the nucleoside derivative is a

nucleoside having a substituent at the 2' position of the sugar and/or a

nucleoside having a bridge structure between the 4' and 2' positions of the sugar.

[Claim 4]

The pharmaceutical composition comprising a double-stranded

oligonucleotide of claim 3, wherein the bridge structure between the 4'

and 2' positions of the sugar is 4'-(CH₂)_m-O-2', wherein m is an integer of

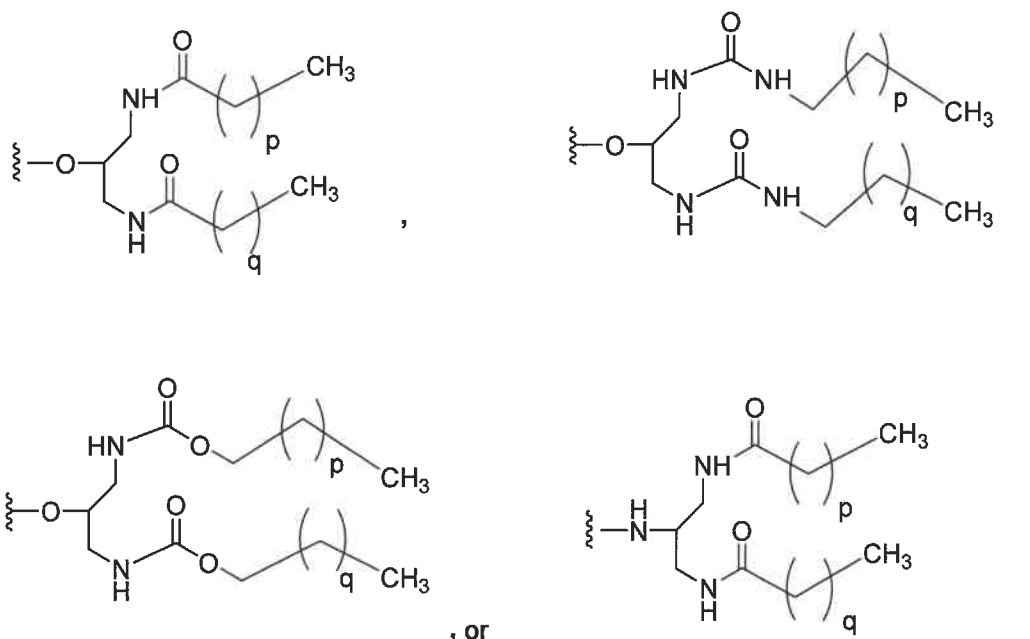
1 to 4.

[Claim 5]

The pharmaceutical composition comprising a double-stranded
 5 oligonucleotide of any one of claims 1 to 4, wherein the lipid is a diacyl
 lipid.

[Claim 6]

The pharmaceutical composition comprising a double-stranded
 10 oligonucleotide of claim 5, wherein the diacyl lipid is



wherein p and q are each independently an integer of 10 to 28.

15 [Claim 7]

The pharmaceutical composition comprising a double-stranded
 oligonucleotide of any one of claims 1 to 6, wherein the lipid binds at the
 3' end and/or 5' end of the second strand.

20 [Claim 8]

The pharmaceutical composition comprising a double-stranded

oligonucleotide of any one of claims 1 to 7, wherein the linker is an oligonucleotide linker.

[Claim 9]

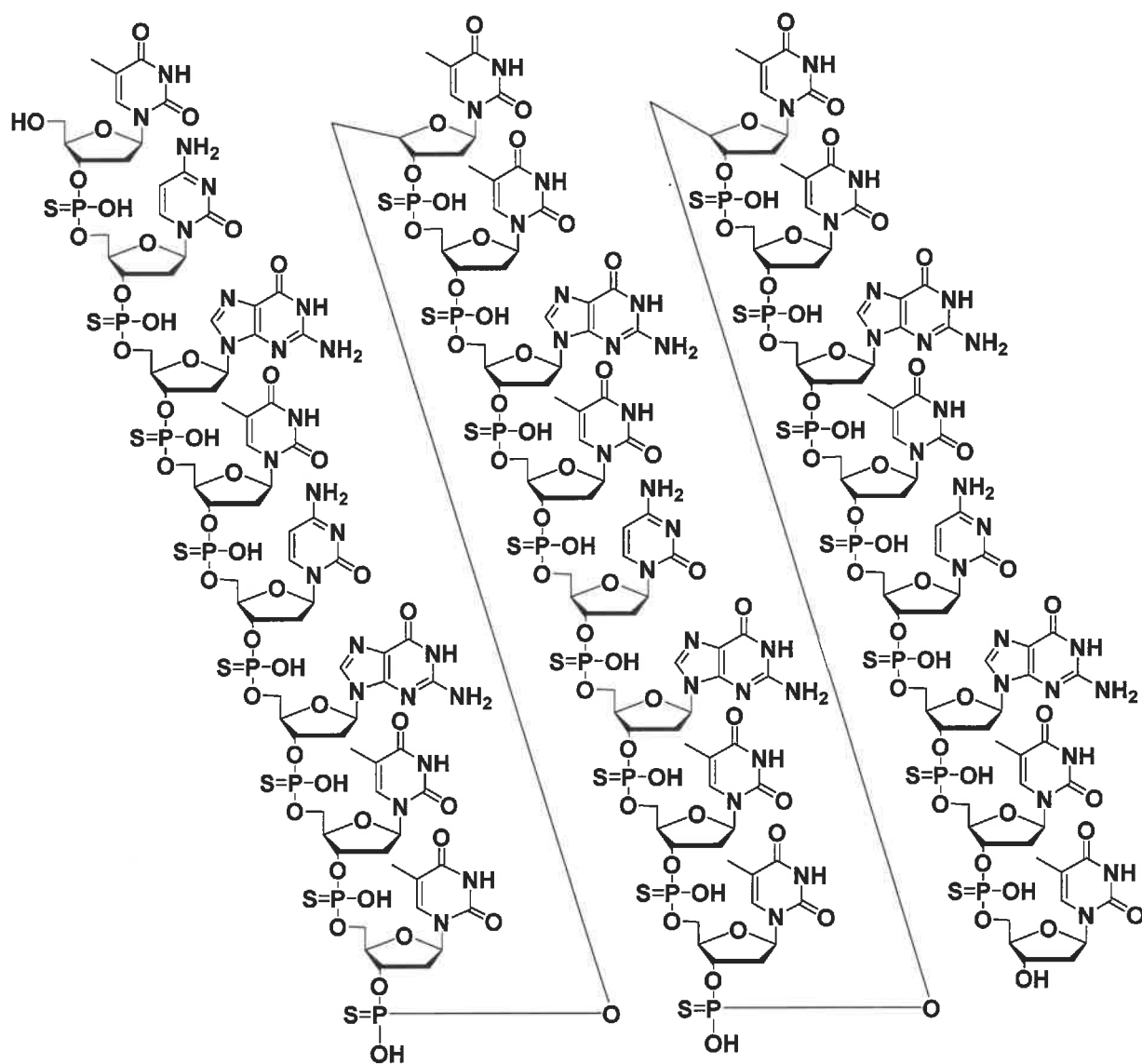
- 5 The pharmaceutical composition comprising a double-stranded oligonucleotide of claim 8, wherein the linker is $-(dX^1)_u-$, wherein X^1 is each independently, A, G, C or T, and u is an integer of 1 to 8.

[Claim 10]

- 10 The pharmaceutical composition comprising a double-stranded oligonucleotide of any one of claims 1 to 9, wherein the CpG oligonucleotide is an oligonucleotide consisting of the sequence of SEQ ID NO: 13.

- 15 [Claim 11]

A pharmaceutical composition comprising a double-stranded oligonucleotide consisting of a first strand and a second strand, wherein the first strand is a strand of the following formula:

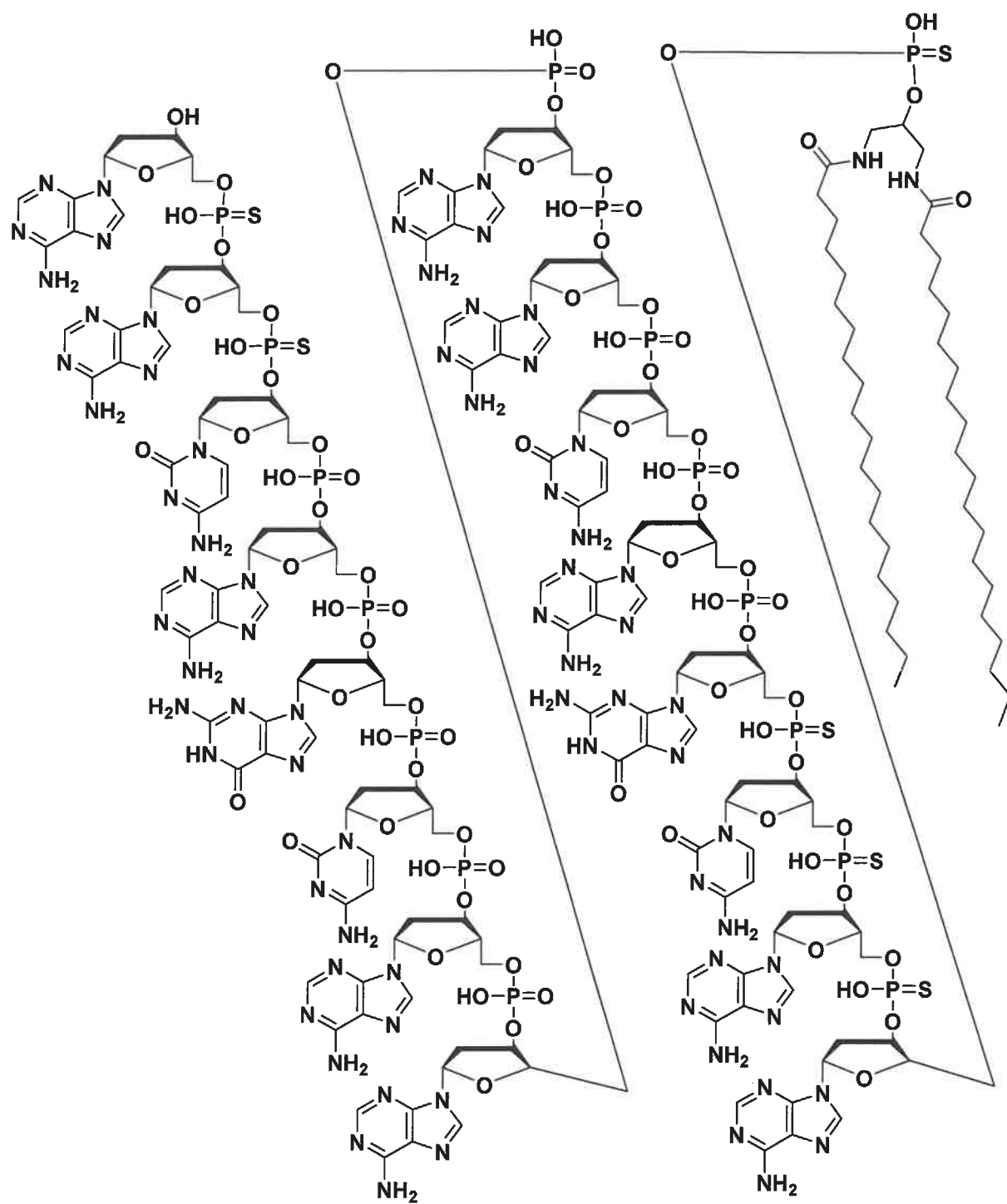


(ODN2006)

and the second strand is a strand represented by the formula selected

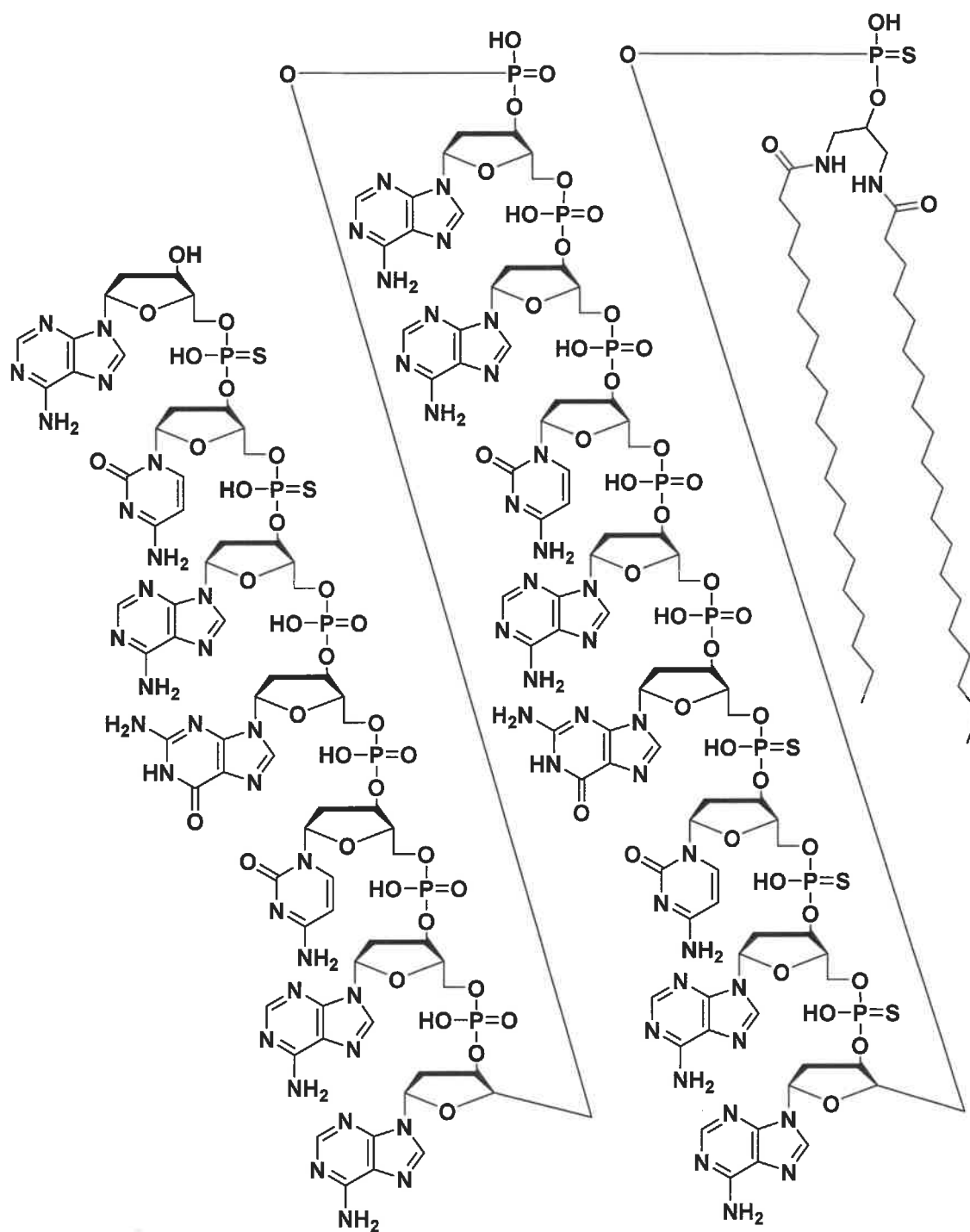
5 from the followings,

the formula :



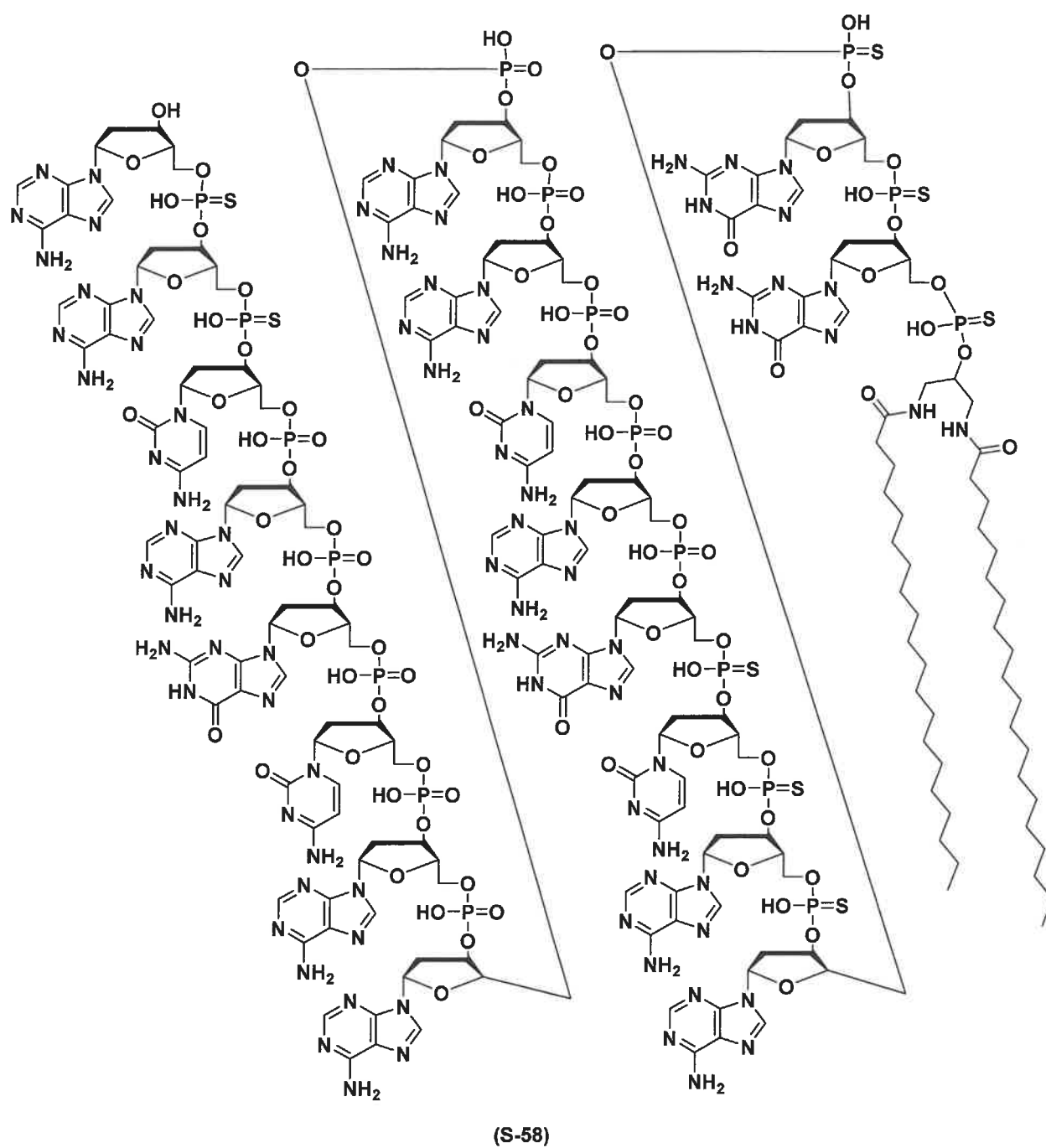
(S-28)

the formula :

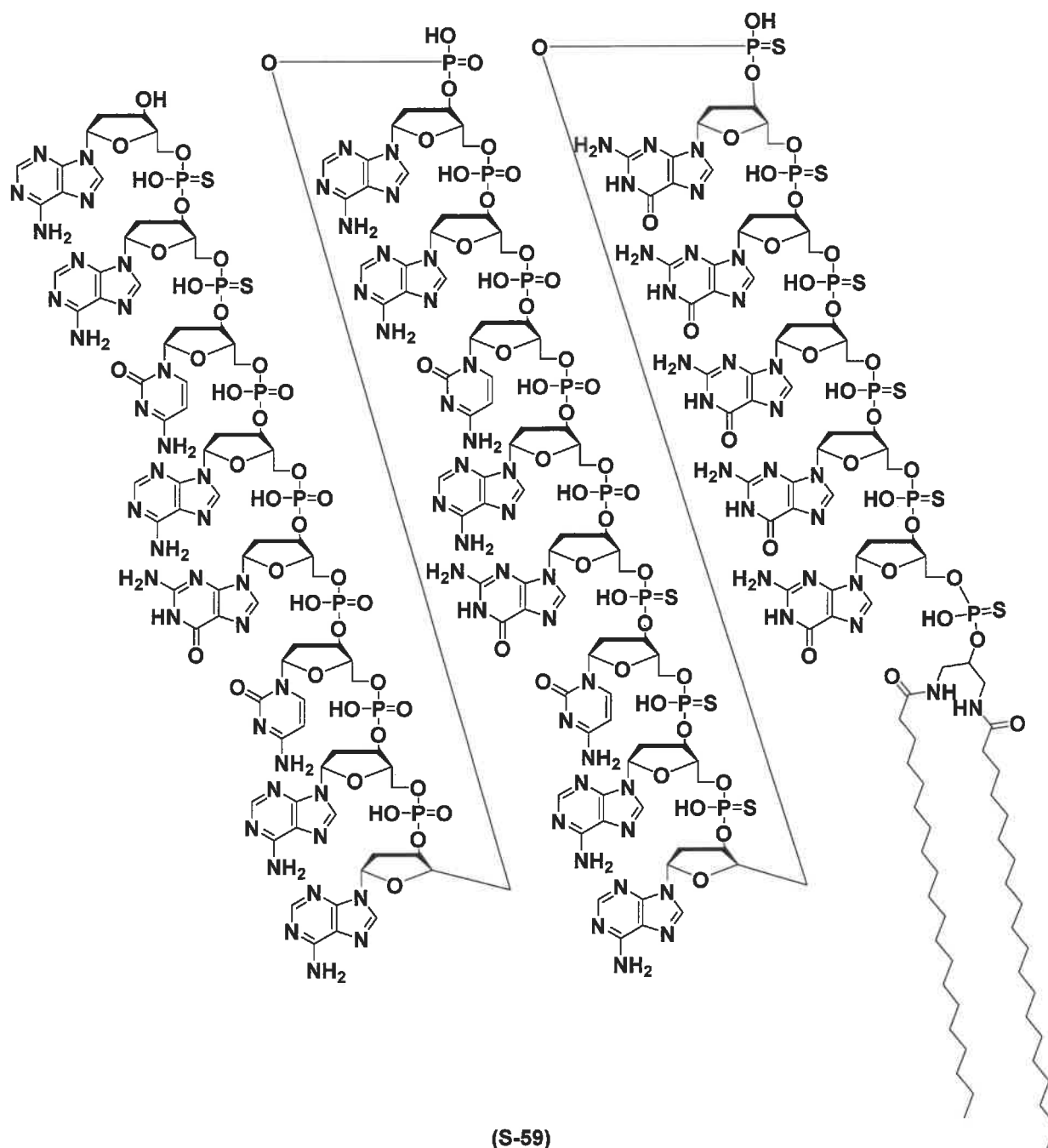


(S-29)

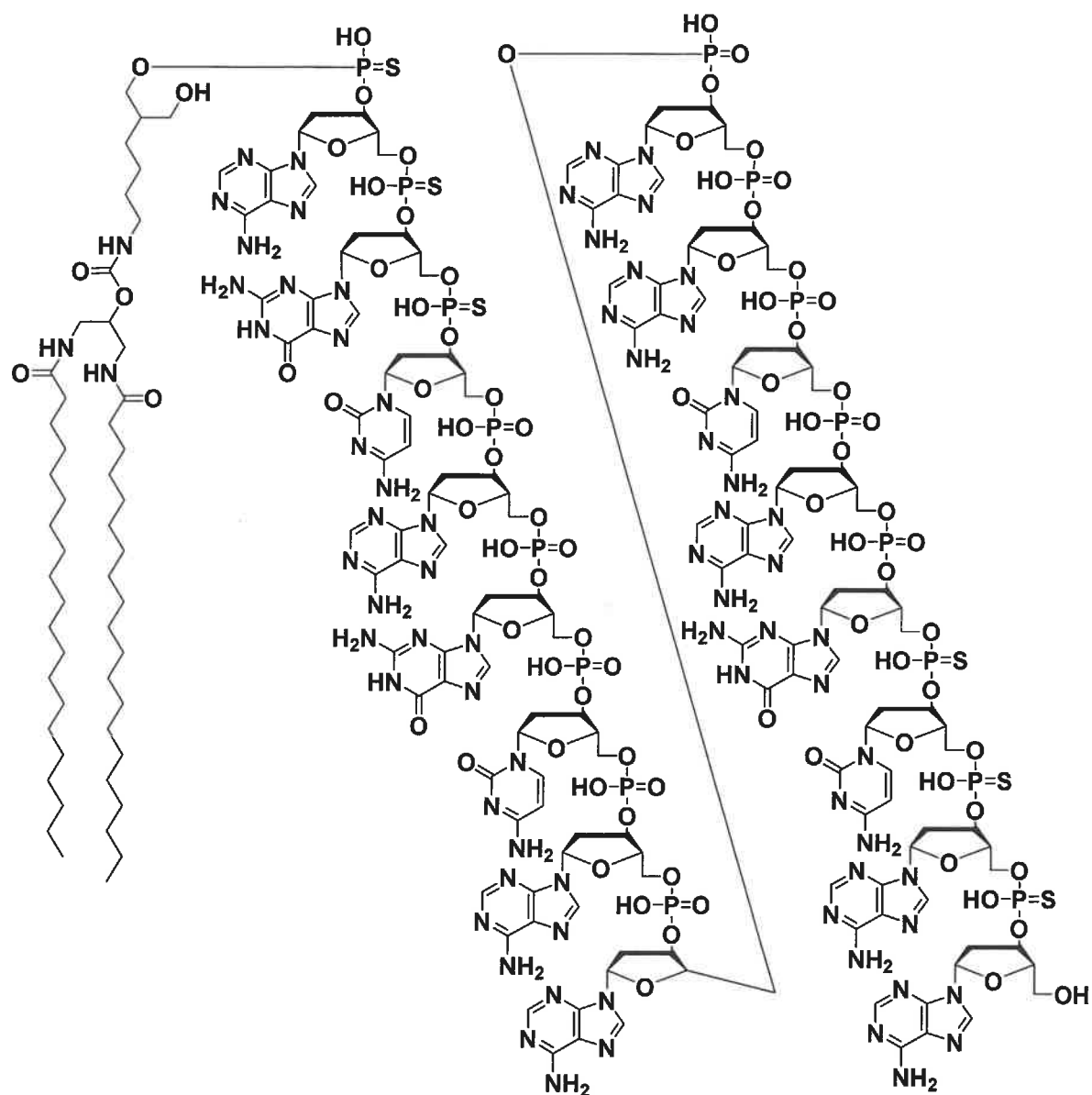
the formula :



the formula :



the formula :



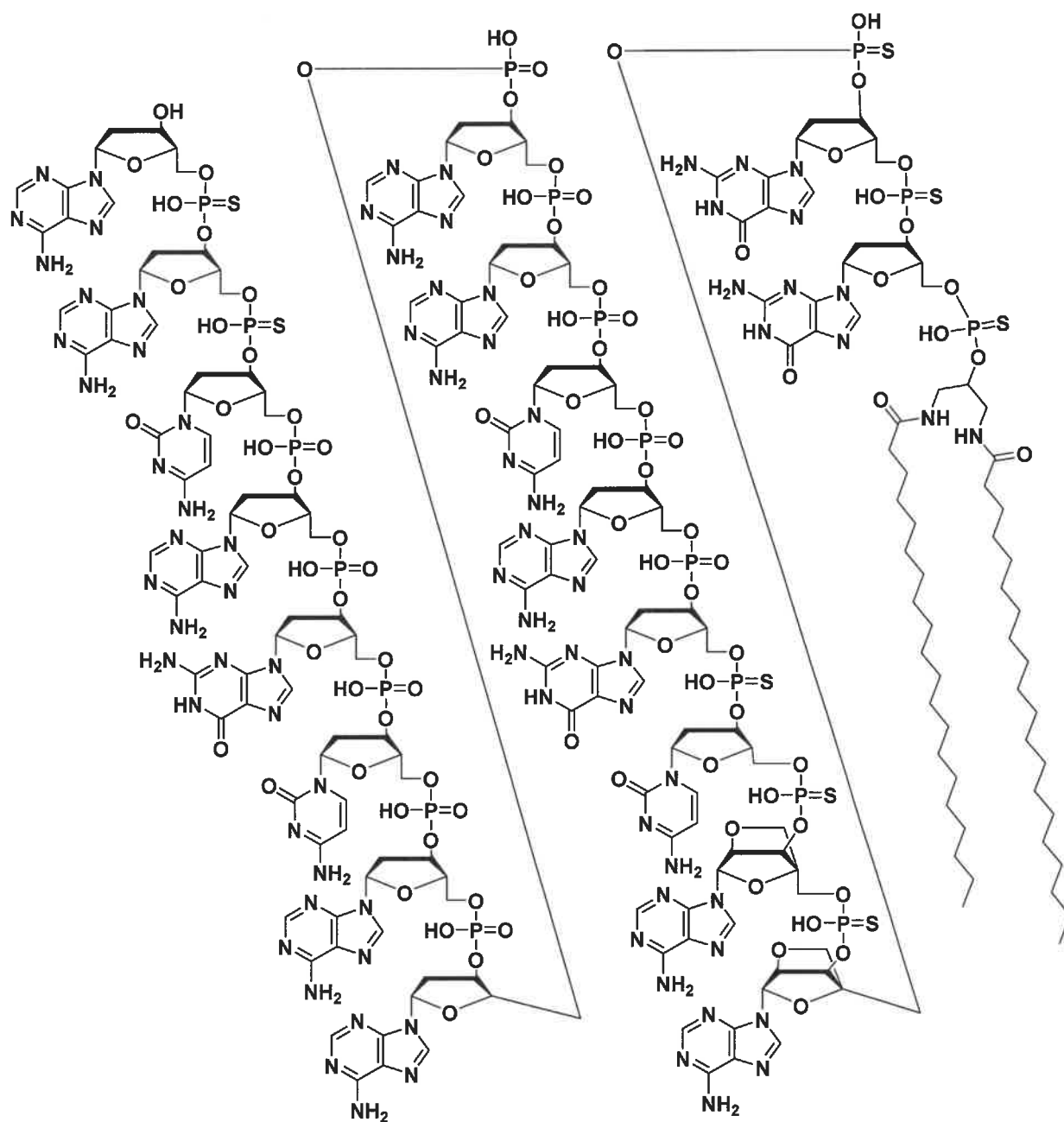
(S-80)

the formula :

(S-82)

the formula :

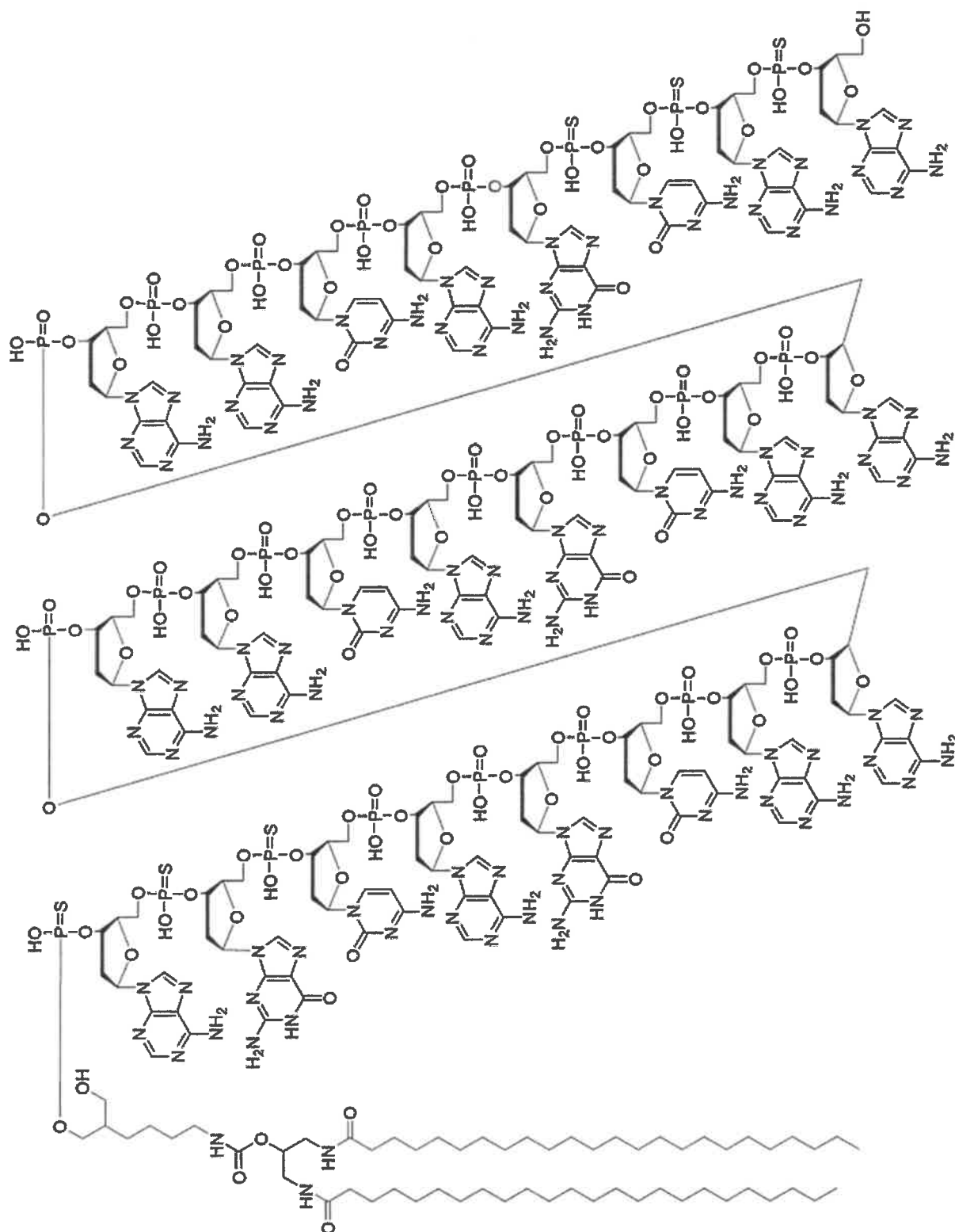




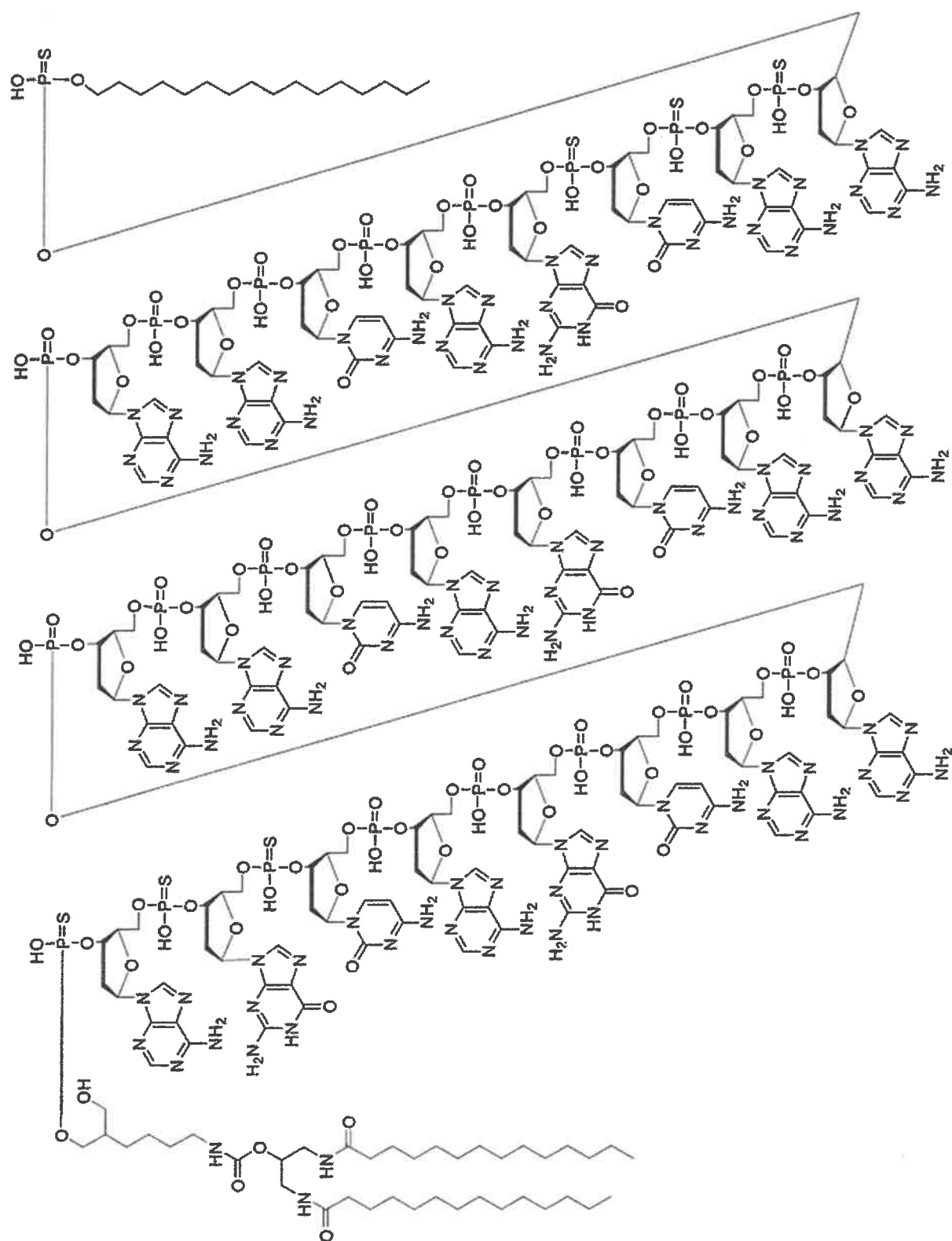
(S-136)

the formula :

188

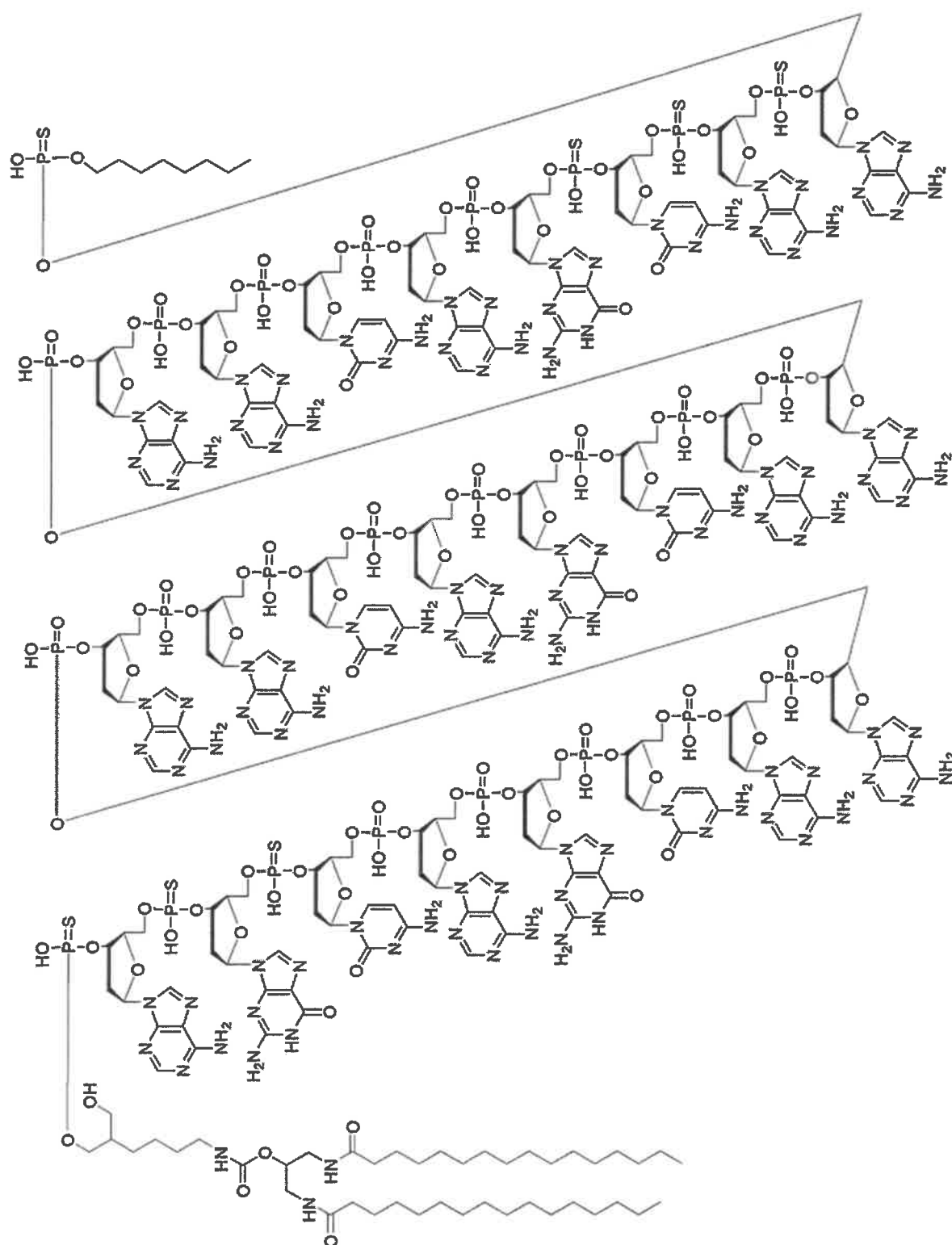


the formula :



(S-151)

and the formula :



[Claim 12]

The pharmaceutical composition of any one of claims 1 to 11, further
 5 comprising an antigen.

[Claim 13]

The pharmaceutical composition of claim 12, wherein the antigen is a microbial antigen, a self-antigen or an addictive substance.

5

[Claim 14]

The pharmaceutical composition of Claim 13, wherein the antigen is a microbial antigen, and the microbial antigen is a bacterial antigen, a viral antigen or a parasitic antigen.

10

[Claim 15]

The pharmaceutical composition of Claim 13, wherein the antigen is a self-antigen, and the self-antigen is a tumor antigen, an antigen associated with Alzheimer's Disease, an antigen against a human antibody, or an antigen that is expressed from human endogenous retroviral elements.

15

[Claim 16]

The pharmaceutical composition of Claim 13, wherein the antigen is an addictive substance, and the addictive substance is nicotine or cocaine.

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