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(54) PREPARATION OF RIBONUCLEOTIDE OLIGOMER

HERSTELLUNG EINES RIBONUKLEOTIDOLIGOMERS

PRÉPARATION D'OLIGOMÈRE RIBONUCLÉOTIDIQUE

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Description

TECHNICAL FIELD

[0001] The present invention relates to a method for preparing nucleotide oligomers. More specifically, the present invention relates to solid-phase synthesis of oligoribonucleotides.

BACKGROUND ART

[0002] There are known a variety of techniques for the preparation of nucleotide oligomers.

[0003] For example, methods of preparing the nucleotide oligomers can be found in the following references: Khorana et al., J. Molec. Biol. 72:209 (1972); Reese, Tetrahedron Lett. 34:3143 (1978); Beaucage and Caruthers, Tetrahedron Lett. 22:1859 (1981); US Patent No. 5,149,798; Agrawal and Goodchild, Tetrahedron Lett. 28:3539 (1987); Connolly et al. Biochemistry 23, 3443 (1984); Jager et al., Biochemistry 27:7237 (1988); Agrawal et al. Proc. Natl. Acad. Sci. USA 85:7079 (1988), e.g., Methods in Molecular Biology, Vol. 20, Protocols for Oligonucleotides and Analogs, p. 63-80 (S. Agrawal, Ed., Humana Press 1993); Methods in Molecular Biology, Vol. 26: Protocols for Oligonucleotide Conjugates (Agrawal, Ed., Humana Press, Totowa, N.J. 1994); Oligonucleotides and Analogues: A Practical Approach pp. 155-183 (Eckstein, Ed., IRL Press, Oxford 1991); Antisense Res. and Applns. pp. 375 (Crooke and Lebleu, Eds., CRC Press, Boca Raton, Fla. 1993); and Gene Regulation: Biology of Antisense RNA and DNA (Erickson and Izant, eds., Raven Press, New York, 1992).

[0004] Anti-sense RNA hybridizes to nucleic acid molecules to result in the inhibition of gene expression. Many researchers have reported the inhibition of expression of specific genes or therapeutic feasibility of particular diseases via the use of the antisense RNA (Barker et al. Proc. Natl. Acad. Sci. USA 93:514 (1996); Agrawal et al., Proc. Natl. Acad Sci. USA 85:7079 (1988); Letter et al., Proc. Natl. Acad Sci. USA 87:3420-3434 (1990); and Offensperger et al. EMBO J. 12:1257 (1993)).

[0005] Meanwhile, RNA-mediated interference (RNAi) is a phenomenon in which a 21-25-nucleotide small RNA fragment selectively binds to and degrades mRNA having a complementary sequence, thus resulting in the suppression of protein expression (Shen C, et al., FEBS Lett. 539 (1-3):111-4 (2003)). The RNAi phenomenon was first discovered in 1995 as a part of the gene-regulation mechanism in *Caenorphabditis elegans* and plants. In 1998, Dr. Andrew Fire of the Carnegie Institution of Washington and Dr. Craig Mello of the University of Massachusetts Medical School, and their team experimentally found that the expression of a specific gene can be significantly inhibited when double-stranded RNA (dsRNA) corresponding to a base sequence of the specific gene is *in-vivo* injected into C. *elegans* (Fire A, et al., Nature. 391 (6669):806-11 (1998)). The long-chain dsRNA injected into *C. elegans* is cleaved into a short double-stranded RNA fragment called small interfering RNA (siRNA) about 21-25 bp long, by the enzymatic action of Dicer belonging to a member of the RNase III family of nucleases which specifically cleave double-stranded RNAs. The resulting short dsRNA is then incorporated into the RNA-induced silencing complex (RISC) where the siRNA duplex is unwound into two strands. Thereafter, the siRNA separated into single-strands binds to a specific gene mRNA with a complementary sequence and makes it untranslatable, thus inhibiting the expression of the corresponding gene. Further, Elbashir and his colleagues have reported that the expression of a specific gene can be selectively inhibited by injection of short dsRNA (siRNA) consisting of 21 bases into cultured mammalian cells, this finding leading to significant increases in practical applicability of RNAi in mammalian cells (Elbashir, S.M. et al.. Nature 411 (6836):494-8 (2001)).

[0006] At present, siRNA-mediated gene expression inhibition techniques are widely used in functional understanding of various genes and a great deal of research has been actively focused on exploitation of such siRNAs for development of therapeutic agents for the treatment of intractable diseases such as cancers, infectious diseases, etc. (Mouldy Sioud. Therapeutic siRNAs. Trends in pharmacological Sciences 2004;22-28).

[0007] As discussed above, many attempts have been made to develop therapeutic agents or diagnostic agents using antisense RNAs and siRNAs. To this end, there is an urgent need for an efficient mass production scheme of oligoribonucleotides.

[0008] Synthesis of nucleotide oligomers is usually carried out by sequential coupling of monomer units on solid resins, using an automatic DNA/RNA (or oligonucleotide) synthesizer. DNA oligomers can be synthesized with a good yield. On the other hand, synthesis of RNA oligomers, *e.g.* ribonucleotide oligomers entails various disadvantages due to steric hindrance of a protecting group for a 2'-OH group, such as long synthesis period and low coupling efficiency resulting in low production yield, thus making it difficult to obtain high-purity RNA oligos.

DISCLOSURE OF THE INVENTION

TECHNICAL PROBLEM

[0009] As a result of a variety of extensive and intensive studies and experiments to solve the problems as described above and to find a method which is capable of achieving a convenient and high-purity production of oligomer species such as nucleotide oligomers, particularly ribonucleotide oligomers or small interfering RNAs (siRNAs), the inventors of the present ribonucleotide invention discovered that it is possible to achieve the production of ribonucleotide oligomers having significantly improved purity, through the use of a ribonucleotide dimer or ribonucleotide trimer as the first nucleotide synthon which will bind to solid supports. The present invention has been completed based on these findings.

[0010] Therefore, the object of the present invention is to provide a method for preparing ribonucleotide oligomers.

TECHNICAL SOLUTION

[0011] Impurities produced during the synthesis of nucleotide oligomers are composed mainly of short sequences having a less degree of coupling than full-length sequences (Nmers), and they are usually expressed as (N-1)mers, (N-2)mers, (N-x)mers, or the like. Impurity oligomers shorter than the full-length Nmers are mostly produced due to the incomplete capping in a capping step of the product following the coupling reaction, upon coupling of nucleotide units to solid supports.

[0012] Further, impurity species which are most difficult to separate during the purification of desired nucleotide oligomers are (N-1)mers that are eluted at a position close to that of the desired oligomers on chromatograms.

[0013] One study showed that (N-1)-mer and phosphodiester content could be reduced through the use of dimeric phosphoramidite synthons compared to the use of monomeric phosphoramidites (Krotz A.H. et al., Bioorganic Medicinal Chemistry Letters, 1997, Vol. 7, No.1, p.73-78).

[0014] A later study showed that (N-1)-mer content could be reduced through the use of trimeric phosphoramidite synthons compared to the use of monomeric phosphoramidites (Eleuteri A, et al., Nucleosides & Nucleotides, 1999, 18(3), p. 475-483).

[0015] However, according to the present invention using a dimer or trimer, not a monomer, in the first coupling reaction, the occurrences of (N-1)mers that are difficult to remove during the purification process are prevented and pure ribonucleotide oligomers are easily obtained with the formation of readily purifiable (N-2)mers or (N-3)mers. Particularly when it is desired to use the nucleotide oligomers as therapeutics, they are purified by chromatography techniques. In this respect, the Nmers and (N-1)mers are eluted at a very close time point, so it is difficult to satisfactorily accomplish the chromatographic separation of the (N-1)mers. However, when a ribonucleotide dimer or trimer is used as the first nucleotide block being coupled to solid supports, as disclosed in the present invention, the formation of (N-1)mer impurities is significantly decreased, thus leading to pronounced improvements of purification yields and consequently significant reductions of production costs.

[0016] As will be demonstrated in Examples which will follow hereinafter, the present invention enables reductions of the (N-1)mers that are mostly produced largely in the first coupling reaction, as well as overall decreases of (N-x)mer impurities. This is believed to be due to that when the coupling of a longer dimer or trimer instead of a monomer is made in the first coupling reaction on solid-supports, the next binding of a monomer to the coupled dimer or trimer is much more spatially advantageous than the binding of a next monomer to a non-capped site, which consequently lessens the formation of oligomers having a sequence length shorter than a desired oligomer.

[0017] Further, the present invention provides the following differences and excellent effects, as compared to the conventional art (WO 02/20543).

① Conventional art employs only dimers for the synthesis of nucleotide oligomers and therefore prepares the nucleotide oligomers of a dimer repeating sequence, whereas the present invention relates to the preparation of ribonucleotide oligomers, involving the use of a dimer or trimer unit only in the first coupling reaction on solid supports. That is, the conventional art requires various kinds of dimers of up to 10 kinds, when it is desired to prepare siRNA oligomers using dimer units. In other words, it is necessary to synthesize 10 kinds of dimers for this purpose, thus requiring long-term periods of synthesis and high production costs. In contrast, the present invention employs just one dimer or trimer species only in the first coupling step and then common inexpensive monomer units in the subsequent steps, which ribonucleotide enables the low-cost, high-purity production of the ribonucleotide oligomers.

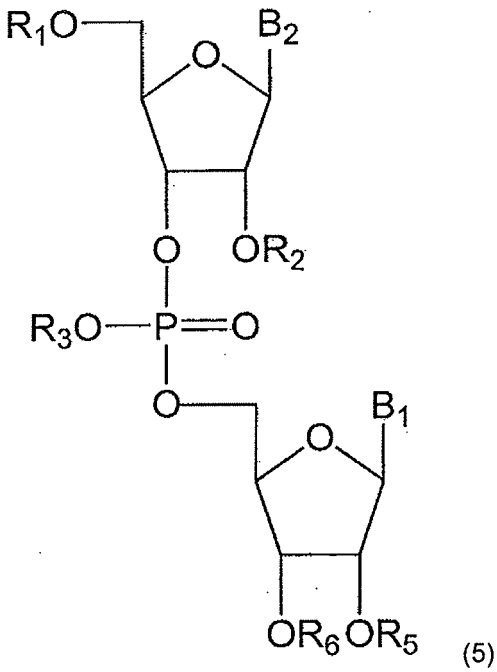
② Further, the present invention achieves a shorter synthesis time than the conventional art. Typically, a coupling reaction of RNA nucleotide oligomer synthesis takes a 10 times longer period of time than DNA nucleotide oligomer synthesis. In this connection, although the conventional art deals with a synthesis example of DNA nucleotide oligomers, it suggested that a coupling reaction of DNA dimers takes a period of 20 to 60 min. On the other hand,

according to the present invention, the coupling of the first dimer for the synthesis of RNA nucleotide oligomers takes 10 to 20 min and the subsequent monomer coupling takes 10 min, so the total synthesis time is much shorter than the conventional art. As a consequence, the present invention shortens production periods of products to thereby significantly reduce production costs, when the nucleotide oligomers are formulated into therapeutics.

[0018] The present invention provides a method for preparing ribonucleotide oligomers, comprising:

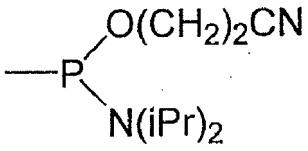
(a) coupling a ribonucleotide dimer or ribonucleotide trimer to a ribonucleoside attached to solid supports or to universal solid supports as a starting material;
(b) sequentially coupling ribonucleotide monomers to the resulting structures of Step (a) to prepare ribonucleotide oligomers; and
(c) removing the ribonucleotide oligomers from the solid supports;

wherein the ribonucleotide dimer in step (a) is represented by Formula 5:

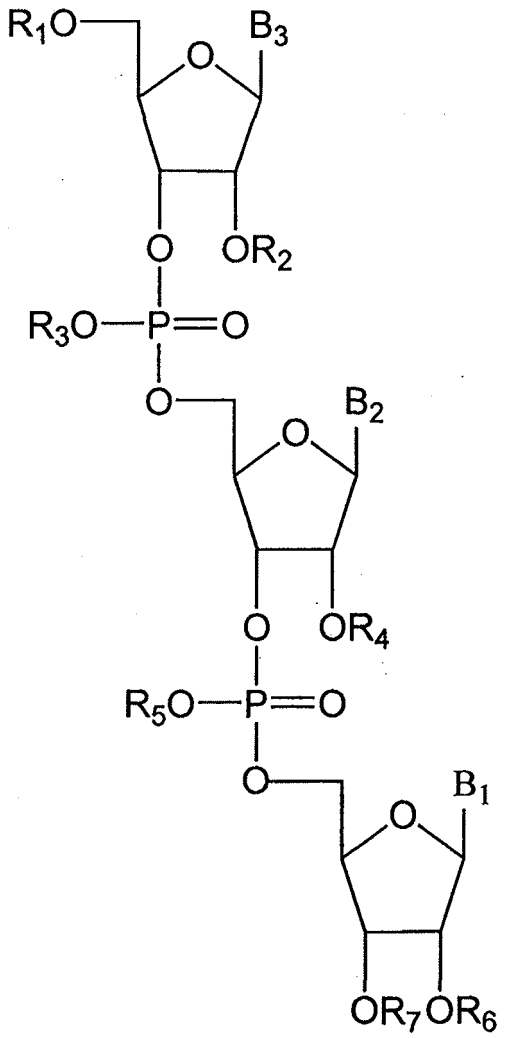


(5)

wherein R_1, R_2, R_3 and R_5 are each independently protecting groups, B_1 and B_2 are each independently nucleosidic bases, and R_6 is hydrogen or

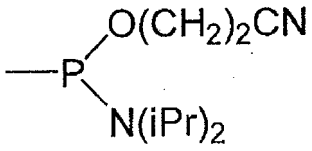


wherein iPr is isopropyl;
wherein the ribonucleotide trimer in step (a) is represented by Formula 6:



(6)

wherein R_1, R_2, R_3, R_4, R_5 and R_6 are each independently protecting groups, B_1, B_2 and B_3 are each independently nucleosidic bases, and R_7 is hydrogen or



wherein iPr is isopropyl.

[0019] In one embodiment of the present invention, the method for preparing ribonucleotide oligomers includes the steps of:

(a) coupling a ribonucleotide dimer according to Formula 5 above to a ribonucleoside attached to solid supports or to universal solid supports as a starting material;
(b) sequentially coupling ribonucleotide monomers to the resulting structures of Step (a) to prepare ribonucleotide oligomers; and
(c) removing the ribonucleotide oligomers from the solid supports.

[0020] In another embodiment of the present invention, the method for preparing ribonucleotide oligomers includes the steps of:

(a) coupling a ribonucleotide trimer according to Formula 6 above to a ribonucleoside attached to solid supports or to universal solid supports as a starting material;
(b) sequentially coupling ribonucleotide monomers to the resulting structures of Step (a) to prepare ribonucleotide oligomers; and
(c) removing the ribonucleotide oligomers from the solid supports.

[0021] As used herein, unless otherwise indicated, the term "nucleotide" is intended to encompass ribonucleotides, deoxyribonucleotides and derivatives thereof.

[0022] As used herein, the term "ribonucleotide" refers to a nucleotide that has no 2'-H of a carbon atom at position 2 of sugar, and is intended to encompass naturally-occurring ribonucleotides as well as analogues thereof. In the context of the present invention, for example, the term "ribonucleotide" also embraces derivatives of ribonucleotides where alkyl (for example, methyl or ethyl) is bonded to -OH on the C2 carbon of sugar or a halogen atom (for example, fluoro) or amino group instead of -OH is bonded to the C2 carbon of sugar.

[0023] The term "deoxyribonucleotide" refers to a nucleotide that contains 2'-H of sugar, and is intended to encompass naturally-occurring deoxyribonucleotides as well as analogues thereof.

[0024] Examples of nucleotide include backbone-modified nucleotides such as phosphorothioate DNA or RNA, phosphorodithioate DNA or RNA, and phosphoramidate DNA or RNA; sugar-modified nucleotides such as 2'-O-methyl RNA, 2'-O-ethyl RNA, 2'-O-methoxyethyl RNA, 2'-fluoro RNA, 2'-halogen RNA, 2'-amino RNA, 2'-O-alkyl RNA, 2'-O-alkoxy RNA, 2'-O-alkyl DNA, 2'-O-allyl DNA, 2'-O-alkynyl DNA, hexose DNA, pyranosyl RNA, anhydrohexitol DNA, and locked nucleic acid (LNA); and base-modified nucleotides comprising a base such as C-5 substituted pyrimidines (substituents include fluoro-, bromo-, chloro-, iodo-, methyl-, ethyl-, vinyl-, formyl-, ethynyl-, propynyl-, alkynyl-, thiazolyl-, imidazolyl-, and pyridyl-), 7-deazapurines with C-7 substituents (substituents include fluoro-, bromo-, chloro-, iodo-, methyl-, ethyl-, vinyl-, formyl-, alkynyl-, alkenyl-, thiazolyl-, imidazolyl-, and pyridyl-), inosine and diaminopurine.

[0025] The nucleotide of the present invention is a ribonucleotide, preferably a ribonucleoside phosphoramidite.

[0026] As described herein, the nucleotide oligomer may include various kinds of nucleotide oligomers, e.g. deoxyribonucleotide oligomers and their derivatives. As described and/or claimed herein, the nucleotide oligomer may be a naturally-occuring nucleotide oligomer as well as a modified nucleotide oligomer. For example, there may be mentioned backbone-modified nucleotide oligomers such as phosphorothioate DNA or RNA, phosphorodithioate DNA or RNA, and phosphoramidate DNA or RNA; sugar-modified nucleotide oligomers such as 2'-O-methyl RNA, 2'-O-ethyl RNA, 2'-O-methoxyethyl RNA, 2'-fluoro RNA, 2'-halogen RNA, 2'-amino RNA, 2'-O-alkyl RNA, 2'-O-alkoxy RNA, 2'-O-alkyl DNA, 2'-O-allyl DNA, 2'-O-alkynyl DNA, hexose DNA, pyranosyl RNA, anhydrohexitol DNA, and locked nucleic acid (LNA); and base-modified nucleotide oligomers such as C-5 substituted pyrimidines (substituents include fluoro-, bromo-, chloro-, iodo-, methyl-, ethyl-, vinyl-, formyl-, ethynyl-, propynyl-, alkynyl-, thiazolyl-, imidazolyl-, and pyridyl-), 7-deazapurine with C-7 substituents (substituents include fluoro-, bromo-, chloro-, iodo-, methyl-, ethyl-, vinyl-, formyl-, alkynyl-, alkenyl-, thiazolyl-, imidazolyl-, and pyridyl-), inosine and diamino purine.

[0027] The nucleotide oligomer of the present invention is a ribonucleotide oligomer.

[0028] Preferably, the ribonucleotide oligomer is one containing at least one ribonucleotide selected from 2'-O-halogen ribonucleotide, 2'-amino ribonucleotide, 2'-O-alkyl ribonucleotide and 2'-O-alkoxy ribonucleotide.

[0029] The present invention employs the ribonucleotide dimer or ribonucleotide trimer as the first coupling reactant that will be attached to solid supports. Depending on the kinds of ribonucleotides positioned at the 3'-terminus corresponding to the third carbon of sugar, *e.g.* the kinds of ribonucleotides bonded to the solid supports, the method of the present invention can be classified into 3 types as follows:

① The first one is a case where the ribonucleoside is positioned at the 3'-terminus. That is, the ribonucleotide dimer or ribonucleotide trimer as the first coupling reactant is coupled to solid supports on which a ribonucleoside monomer as a starting material of a synthesis process was preloaded, followed by sequential coupling of ribonucleotide monomers to the resulting structure to thereby prepare a ribonucleotide oligomer having a desired sequence.

② The second one is a case where the ribonucleotide dimer is positioned at the 3'-terminus. This case employs universal solid supports as a starting material. The universal solid supports as a starting material are employed in the first step of the synthesis process, and the ribonucleotide dimer is employed as the first coupling reactant. Thereafter, ribonucleotide monomers are sequentially coupled to the resulting structure to thereby prepare a ribonucleotide oligomer having a desired sequence.

③The third one is a case where the ribonucleotide trimer is positioned at the 3'-terminus. This case also employs the universal solid supports as a starting material. The universal solid supports as a starting material are employed in the first step of the synthesis process, and the ribonucleotide trimer is employed as the first coupling reactant. Thereafter, ribonucleotide monomers are sequentially coupled to the resulting structure to thereby prepare a ribo-

nucleotide oligomer having a desired sequence.

[0030] The most preferred one out of the above-mentioned three methods is a method where the solid supports to which one ribonucleoside was previously attached are employed as a starting material and the ribonucleotide dimer or trimer as the first coupling reactant is then coupled to the preloaded ribonucleoside.

[0031] As used herein, the term "universal solid supports" refers to solid supports that are free of a nucleoside or nucleotide oligomer covalently bonded thereto. Unlike the preloaded supports, the use of the universal solid supports enables the synthesis of any nucleotide oligomer regardless of the kinds of terminal sequences of the nucleotide oligomers. When the universal supports are employed, a terminal sequence of the final synthetic nucleotide oligomer is determined by a nucleotide synthon applied to the first coupling reaction of the nucleotide oligomer synthesis.

[0032] The present invention is practiced according to solid phase synthesis.

[0033] When the process of the present invention is carried out according to solid phase synthesis, a preferred embodiment of the present invention includes the following steps of:

(a) coupling a nucleotide dimer [$(NMP)_2$] or nucleotide trimer [$(NMP)_3$] to $C(NS)_1$] of a solid support-nucleoside [SS-$(NS)_1$] to prepare SS-$(NS)_1$-$(NMP)_2$ or SS-$(NS)_1$-$(NMP)_3$;
(b) sequentially coupling nucleotide monomers to the resulting structure of Step (a) to prepare an SS-$(NS)_1$-$(NMP)_2$-$(NMP)_{n-3}$ or SS-$(NS)_1$-$(NMP)_3$-$(NMP)_{n-4}$; and
(c) removing the solid supports (SS) from the SS-$(NS)_1$-$(NMP)_2$-$(NMP)_{n-3}$ or SS-$(NS)_1$- $(NMP)_3$-$(NMP)_{n-4}$ structure to obtain an $(NMP)_n$; wherein the nucleotide is a ribonucleotide, the nucleoside is a ribonucleoside and the nucleotide dimer and trimer are a ribonucleotide dimer or trimer according to Formula 5 or 6, respectively.

[0034] When the nucleotide dimer [$(NMP)_2$] or nucleotide trimer [$(NMP)_3$] is coupled in the first step to the solid supports to which a nucleoside monomer was previously attached, and nucleotide monomers are then sequentially coupled thereto, a nucleotide oligomer molecule can be prepared with significantly improved purity.

[0035] The solid support-nucleoside [SS-$(NS)_1$] is a structure where one ribonucleoside or deoxyribonucleoside molecule was attached to the solid supports. The solid supports may be any one that is used in the solid phase synthesis of nucleotide molecules. Alternatively, there may also be employed universal solid supports to which ribonucleoside or deoxyribonucleoside was not previously attached. Preferably, such solid supports should have the following properties: (i) substantially no solubility in the reagents used for the nucleotide oligomer synthesis, (ii) chemical stability against reagents used for nucleotide oligomer synthesis, (iii) feasibility of chemical modifications, (iv) loadability of desired nucleotide oligomers, (v) reasonable compression strength to withstand increasing pressure during the synthesis process, and (vi) desired particle size and distribution.

[0036] A material that can be used as the solid supports in the present invention may be preferably an inorganic polymer and include, for example, silica, porous glass, aluminum silicate, polystyrene, polyvinyl alcohol, polyvinyl acetate, borosilicate, metal oxide (such as alumina and nickel oxide) and clay. Most preferably, the solid supports for use in the present invention are controlled pore glass (CPG) and polystyrene.

[0037] The present invention employs an [SS-$(NS)_1$] where a nucleoside was previously attached to a surface of the solid supports, specifically an [SS-$(rNS)_1$] where a ribonucleoside was attached to a surface of the solid supports. The nucleoside is conventionally attached to the solid supports through a 3'-OH group of sugar.

[0038] The coupling of the ribonucleotide dimer or ribonucleotide trimer to the ribonucleoside may be carried out by various methods known in the art. For example, details of the coupling method can be found in the following literature: US Patent No. 4,458,066 and US Patent No. 4,415,732; Caruthers et al., Genetic Engineering, 4:1-17 (1982); and Users Manual Model 392 and 394 Polynucleotide Synthesizers, pages 6-1 through 6-22, Applied Biosystems, Part No. 901237 (1991).

[0039] Preferably, the coupling process is carried out according to a phosphoramidite method. For example, it may be performed as follows. A phosphoramidite derivative of the ribonucleotide dimer or ribonucleotide trimer is added to the ribonucleoside while simultaneously an activator, for example a weak acid (such as tetrazole, 5-ethylthiotetrazole, benzylthiotetrazole, etc.) is added. Most preferably, the usable activator is 5-ethylthiotetrazole. Addition of the weak acid leads to the formation of a reaction intermediate through protonation of phosphoramidite nitrogen. This is followed by the capping of the resulting product. The capping is preferably carried out with an acetic anhydride and 1-methylimidazole. Then, the capped product is oxidized using an oxidant such as iodine, so that an internucleotide linkage is converted into a more stable phosphodiester from labile phosphite. The order of capping and oxidation steps may be reversed. Following the oxidation step, a hydroxyl-protecting group is removed using a protic acid, for example, trichloroacetic acid or dichloroacetic acid.

[0040] The ribonucleotide dimer or ribonucleotide trimer of the present invention may have various kinds of linkages, preferably phosphodiester, phosphoramidate, alkylphosphoramidate, alkylphosphonate, phosphorothioate, alkylphosphotriester, or alkylphosphonothioate linkages, most preferably phosphodiester or phosphoramidate linkages.

[0041] Preferably, the ribonucleotide dimer and the ribonucleotide trimer of the present invention are the ribonucleotide dimer phosphoramidite and the ribonucleotide trimer phosphoramidite, respectively.

[0042] Therefore, the ribonucleotide oligomer of the present invention has a phosphodiester, phosphoramidate, alkylphosphoramidate, alkylphosphonate, phosphorothioate, alkylphosphotriester, or alkylphosphonothioate linkage, most preferably a phosphodiester or phosphoramidate linkage.

[0043] According to the present invention, the SS-$(NS)_{1}$-$(NMP)_{2}$-$(NMP)_{n-3}$ or SS-$(NS)_{1}$-$(NMP)_{3}$-$(NMP)_{n-4}$ having a desired sequence is finally prepared by sequential coupling of the ribonucleotide monomers to the ribonucleotide dimer or ribonucleotide trimer attached to the solid support-ribonucleoside [SS-$(NS)_{1}$].

[0044] When the ribonucleotide monomers are sequentially coupled, 5-ethylthiotetrazole is used as an activator.

[0045] Finally, the desired product $(NMP)_{n}$ is obtained by removal of the solid supports (SS) from the SS-$(NS)_{1}$-$(NMP)_{2}$-$(NMP)_{n-3}$ or SS-$(NS)_{1}$-$(NMP)_{3}$-$(NMP)_{n-4}$. When there is used the universal solid supports with no attachment of ribonucleoside the $(NMP)_{n}$ is obtained by removal of the solid supports (SS) from the SS-$(NMP)_{2}$-$(NMP)_{n-2}$ or SS-$(NMP)_{3}$-$(NMP)_{n-3}$.

[0046] Removal of the solid supports may be carried out by any conventional method known in the art. For example, the solid supports may be eliminated using ammonium hydroxide.

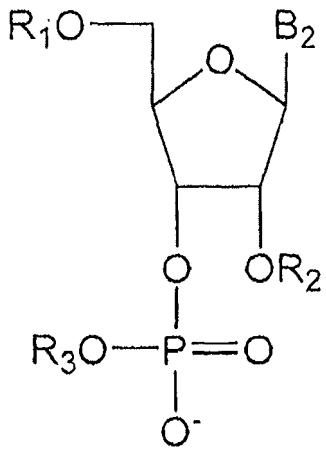
[0047] According to the preferred embodiment of the present invention, the method of the present invention may further include a step of removing the protecting groups attached to the ribonucleotide oligomer [$(NMP)_{n}$], before or after Step (c). Removal of the protecting groups may be carried out by any conventional method known in the art. For example, a phosphate protecting group may be removed with the treatment of thiophenol or ammonium hydroxide solution, whereas benzoyl and isobutyryl groups attached to the base may be removed by heating the ribonucleotide oligomer in an ammonium hydroxide solution.

[0048] There is no particular limit to a length of the ribonucleotide oligomer [$(NMP)_{n}$] prepared by the method of the present invention. Typically, the ribonucleotide oligomer is 10 to 50 nucleotides in length.

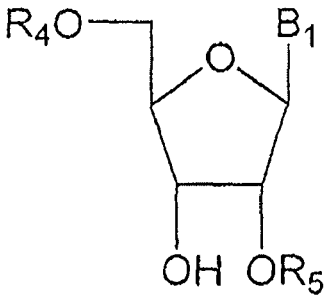
[0049] According to the method of the present invention, it is possible to efficiently synthesize a high-purity oligoribonucleotide within a shorter period of time. The method of the present invention provides a ribonucleotide oligomer having 15-20% higher purity than the conventional art.

[0050] In step (a) of the claimed method, it is preferred to employ the ribonucleotide dimer of Formula 5 wherein R_1 is dimethoxytrityl; R_2 and R_5 are each t-butyl-dimethylsilyl; and R_3 is halogen-substituted phenyl.

[0051] Further, the method of the present invention comprises the further step of preparing the ribonucleotide dimer, comprising coupling of a compound of Formula 1 and a compound of Formulae 2:



(1)



(2)

[0052] In Formulae 1 and 2, R_1, R_2, R_3, R_4 and R_5 are each independently protecting groups, and B_1 and B_2 are each independently nucleosidic bases.

[0053] Examples of the protecting groups R_1 and R_4 in Formulae 1 and 2 may independently include, but are not limited to, dimethoxytrityl, monomethoxytrityl, trityl, and 9-phenyl xanthen-9-yl (pixyl). Preferred examples of suitable groups for R_2 and R_5 may independently include, but are not limited to, t-butyl-dimetltylsilyl, tri-isopropyl silyloxymethyl (TOM), 1-(2-chloro ethoxy)ethyl (CEE), 2-cyanoethoxymethyl (CEM), bis(2-acetoxy)methyl (ACE), 1-(2-fluorophenyl)-4-methoxypiperidin-4-yl (Fpmp), 1-(4-chloro phenyl)-4-ethoxypiperidin-4-yl (Cpep), 1-[2-chloro-4-methyl)phenyl]-4-methoxy piperidin-4-yl (Ctmp), 4-nitrophenylethylsulfonyl (NPES), 4-chloro phenylethylsulfonyl (CPES), 1-(2-cyanoethoxy)ethyl (CNEE), trimethyl silylethoxymethyl (SEM), methoxyethoxymethyl (MEM), levulinyl, 4-nitropheylethyl (NPE), and 4-nitrophenylethyloxycarbonyl (NPEOC).

[0054] R_3 is preferably halogen-substituted phenyl or carbobenzoxyl, without being limited thereto. Each of B_1 and B_2 is independently adenine, cyrtosine, guanine, uracil or a derivative thereof.

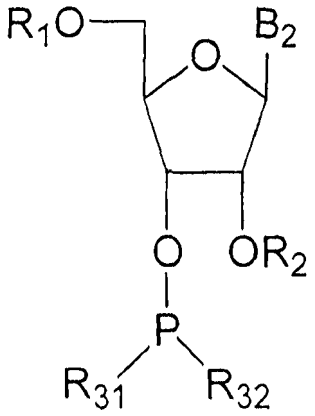
[0055] More preferably, in Formulae 1 and 2, R_1 and R_4 are dimethoxytrityl, R_2 and R_5 are t-butyl-dimethylsilyl, and R_3 is halogen-substituted phenyl (most preferably 2-chlorophenyl).

[0056] Each of B_1 and B_2 is a base to which a protecting group is attached or not. Examples of the base that can be positioned on B_1 and B_2 may include common bases such as adenine, cytosine, guanine and uracil, as well as their derivatives. Preferably, derivatives of the bases include xanthine, hypoxanthine, 2-aminoadenine, 6-methyl and other alkyl derivatives of adenine and guanine, 2-propyl and other alkyl derivatives of adenine and guanine, 5-halo uracil and cytosine, 6-azo uracil and cytosine, 5-uracil (pseudo uracil), 4-thiouracil, 8-halo, oxa, amino, thiol, thioalkyl and hydroxyl adenines and guanines, 5-trifluoro-methyl uracils and cytosines, and 7-methylguanine or inosine.

[0057] The protecting group may be attached to B_1 and B_2. Examples of the protecting group may include, but are not limited to, benzoyl or isobutyryl, acetyl, dimethylformamidine (DMF), phenoxyacetyl (PAC) and its derivative, and 4-t-butylphenoxyacetyl (TAC).

[0058] The reaction conditions for coupling of the compound of Formula 1 to the compound of Formula 2 are the same as those for coupling of the nucleotide dimer or trimer as described hereinbefore.

[0059] Further, the method of the present invention comprises the further step of preparing the rubonucleoide dimer [$(rNMP)_2$], comprising coupling a compound of Formula 3 and a compound of Formula 2:



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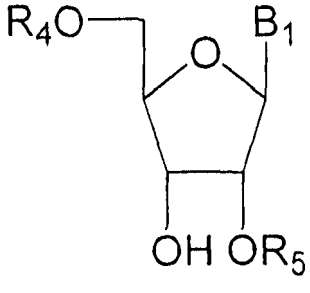


(2)

[0060] In Formulae 2 and 3, R_1, R_2, R_4 and R_5 are each independently protecting groups, and B_1 and B_2 are each independently nucleosidic bases.

[0061] Preferably, examples of the protecting groups R_1 and R_4 may independently include, but are not limited to, dimethoxytrityl, monomethoxytrityl, trityl, and 9-phenyl xanthen-9-yl (pixyl). Examples of suitable groups for R_2 and R_5 may include, but are not limited to, t-butyl-dimethylsilyl, tri-isopropyl silyloxymethyl (TOM), 1-(2-chloro ethoxy)ethyl (CEE), 2-cyanoethoxymethyl (CEM), bis(2-acetoxy)methyl (ACE), 1-(2-fluorophenyl)-4-methoxypiperidin-4-yl (Fpmp), 1-(4-chloro phenyl)-4-ethoxypiperidin-4-yl (Cpep), 1-[2-chloro-4-methyl)phenyl]-4-methoxy piperidin-4-yl (Ctmp), 4-nitrophenylethylsulfonyl (NPES), 4-chloro phenylethylsulfonyl (CPES), 1-(2-cyanoethoxy)ethyl (CNEE), trimethyl silylethoxymethyl (SEM), methoxyethoxymethyl (MEM), levulinyl, 4-nitropheylethyl (NPE), and 4-nitrophenylethyloxycarbonyl (NPEOC). Examples of suitable groups for R_{31} may include, but are not limited to, cyanoalkyloxy (such as cyanoethoxy and cyanomethoxy), 4-cyano-2-butenyloxy, and diphenylmethylsilylethoxy. Non-limiting examples of suitable groups for R_{32} may include dialkylamino. B_1 and B_2 are each independently adenine, cytosine, guanine, uracil or derivatives thereof.

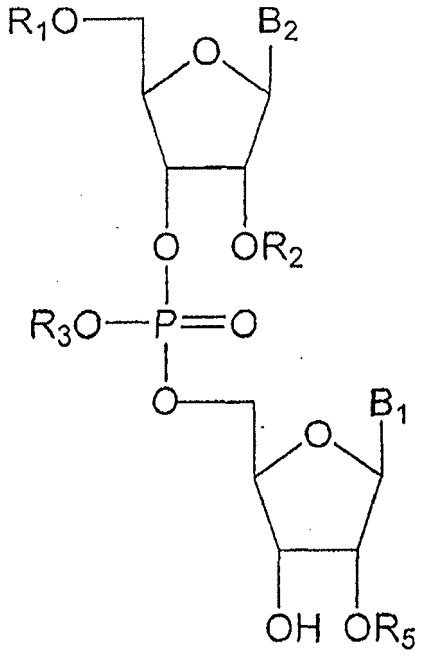
[0062] More preferably, in Formula 3, R_1 is dimethoxytrityl, R_2 is tert-butyl-dimethylsilyl, R_{31} is 2-cyanoethyloxy, and R_{32} is dialkylamino (most preferably diisopropylamino).

[0063] The coupling of the compound of Formula 2 to the compound of Formula 3 in the present invention may be carried out in the same manner as above, and a more preferred activator is 5-ethylthiotetrazole.

[0064] In step (a) of the claimed method, it is preferred to employ the ribonucleotide trimer of Formula 6 wherein R_1 is dimethoxytrityl; R_2, R_4 and R_6 are each t-butyl-dimethylsilyl; and R3 is 2-cyanoethyl and R5 is halogen substituted phenyl.

[0065] Further, the method of the present invention comprises the further step of preparing the ribonucleotide trimer, comprising the steps of:

(a) reacting a ribonucleotide dimer of Formula 4 with an acid to remove R_1 of Formula 4; and
(b) coupling the resulting product of Step (a) to a ribonucleoside 3'-phosphoramidite to prepare a ribonucleotide trimer.



(4)

[0066] In Formula 4, R_1, R_2, R_3 and R_5 are each independently protecting groups, and B_1 and B_2 are each independently nucleosidic bases.

[0067] In Formula 4, preferred examples of the protecting group R_1 may include, but are not limited to, dimethoxytrityl, monomethoxytrityl, trityl, and pixyl (9-phenyl xanthen-9-yl). Examples of suitable groups for R_2 and R_5 may include, but are not limited to, t-butyl-dimethylsilyl, tri-isopropyl silyloxymethyl (TOM), 1-(2-chloro ethoxy)ethyl (CEE), 2-cyanoethoxymethyl (CEM), bis(2-acetoxy)methyl (ACE), 1-(2-fluorophenyl)-4-methoxypiperidin-4-yl (Fpmp), 1-(4-chloro phenyl)-4-ethoxypiperidin-4-yl (Cpep), 1-[2-chloro-4-methyl)phenyl]-4-methoxy piperidin-4-yl (Ctmp), 4-nitrophenylethylsulfonyl (NPES), 4-chloro phenylethylsulfonyl (CPES), 1-(2.-cyanoethoxy)ethyl (CNEE), trimethyl silylethoxymethyl (SEM), methoxyethoxymethyl (MEM), levulinyl, 4-nitropheylethyl (NPE), and 4-nitrophenylethyloxycarbonyl (NPEOC). Examples of suitable groups for R_3 may include, but are not limited to, hydrogen, and halogen-substituted phenyl or carbobenzoxyl. B_1 and B_2 are each independently adenine, cytosine, guanine, uracil or derivatives thereof.

[0068] More preferably, in Formula 4, R_1 is dimethoxytrityl, R_2 is tert-butyl-dimethylsilyl, R_3 is hydrogen or halogen-

substituted phenyl (most preferably chlorophenyl), and R_5 is tert-butyl-dimethylsilyl.

[0069] Removal of R_1 from the ribonucleotide dimer of Formula 4 may be carried out by any conventional deprotection method known in the art, using a strong acid, for example, benzenesulfonic acid.

[0070] The coupling conditions of Step (b) in the above preparation method are the same as those conditions mentioned as above.

ADVANTAGEOUS EFFECTS

[0071] The present invention enables the efficient high-speed and high-purity synthesis of ribonucleotide oligomers. The method of the present invention provides a ribonucleotide oligomer having 15-20% higher purity than the conventional art.

MODE FOR INVENTION

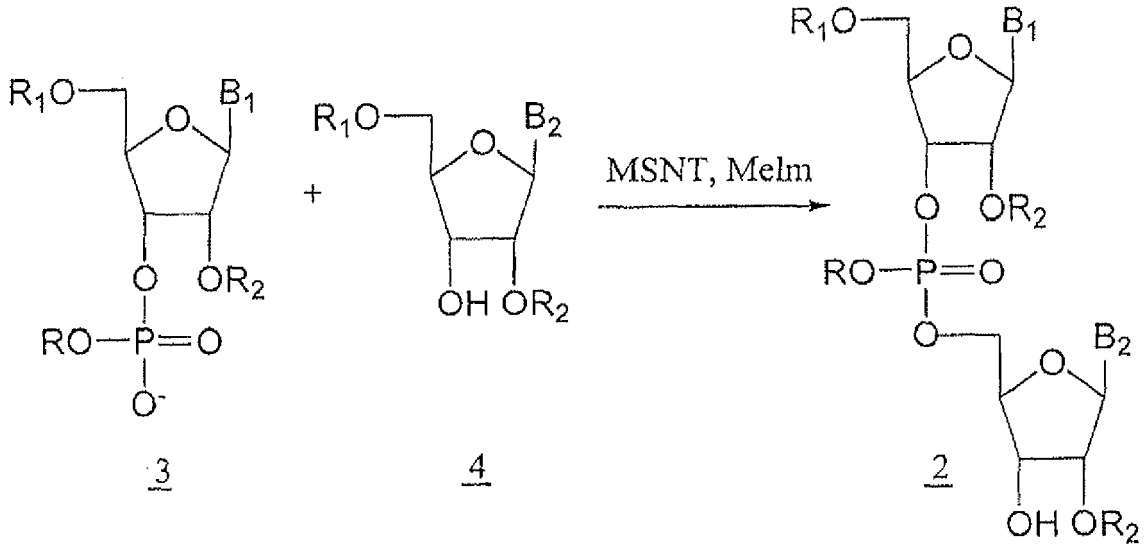
[0072] Now, the present invention will be described in more detail with reference to the following Examples.

[0073] Hereinafter, the measured ^{31}P-NMR values are values as measured using Varian Mercury Plus 300 MHz.

Example I: Synthesis of ribonucleotide dimers (phosphotriester method)

[0074]

[Reaction Scheme 1]



Synthesis of ribonucleotide dimers UpU, CpU and GpA (2a to 2c)

[0075] R_1 = DMTr (dimethoxytrityl), R_2 = TBDMS (tert-butyldimethylsilyl), R = o-chlorophenyl. 2a - B_1 = U, B_2 = U; 2b - B_1 = bzC, B_2 = U; 2c - B_1 = ibG, B_2 = bzA; 3a - B_1 = U; 3b - B_1 = bzC; 3c - B_1 = ibG; 4a - B_2 = U; 4b - B_2 = bzA. bz = benzoyl, ib = isobutyryl

Example 1: Synthesis of 5'-O-dimethoxytrityl-2'-O-t-butyldimethylsilyluridine-3'-O-(2-chlorophenylphosphate)-5'-O-2'-O-(t-butyldimethylsilyl)uridine (2a)

Step 1: Synthesis of 5'-O-dimethoxytrityl-2'-O-t-butyldimethylsilyluridine-3'-O-(2-chlorophenylphosphate) (triethylammonium salt) (3a)

[0076] Triazole (0.63 g, 9.24 mmol, Sigma Aldrich) and anhydrous triethylamine (1.3 mL, 9.15 mmol), Sigma Aldrich) were dissolved in dioxane (20 mL), and the solution was cooled to 5°C. A solution of O-chlorophenyl phosphodichloridate

(1.1 g, 4.53 mmol, Sigma Aldrich) in 5 mL of dioxane was added dropwise to the resulting solution. After one hour, the mixed solution was filtered and added to S'-O-dimethoxytrityl-2'-O-t-butyldimethylsilyluridine (2 g, 3.02 memol) in 10 mL of pyridine which had been cooled to -5°C. Then, 1-methylimidazole (0.38 mL, 4.6 mmol, Sigma Aldrich) was added thereto. After one hour, 0.1 M triethylammonium bicarbonate buffer (TEAB, 10 mL) was added to the above cooled solution which was then concentrated. The residue was dissolved in dichloromethane (50 mL) and washed with 0.1 M TEAB (50 mL), and the aqueous layer was extracted two times with 20 mL of dichloromethane. The organic layer was collected, washed with 0.1 M TEAB (100 mL), and dried over sodium sulfate. The residue was concentrated using a vacuum pump to give 2.78 g (yield: 97%) of the title compound.

Step 2: Synthesis of 5'-O-dimethoxytrityl-2'-O-(t-butyldimethylsilyl)uridine-3'-O-chlorophenylphosphate-5'-O-2'-O-(t-butyldimethylsilyl)uridine (2a)

[0077] 5'-O-dimethoxytrityl-2'-O-tert-butyldimethylsilyluridine-3'-O-(2-chlorophenylphosphate)triethylammonium salt (3a, 1.47 g, 1.54 mmol) prepared in Step 1 and 5'-O-dimethoxytrityl-2'-O-tert-butyldimethylsilyluridine (4a, 0.5 g, 1.4 mmol) were dissolved in 20 mL of pyridine and the solution was dried using a vacuum pump. 1-mesitylenesulfonyl-3-nitro-1,2,4-triazole (MSNT, 0.68 g, 2.31 mmol, Sigma Aldrich) in 5 mL of fresh pyridine was added to the dried product. The reaction solution was concentrated to about 3 mL, and 0.16 mL of 1-methylimidazole (1.89 mmol) was added thereto. After one hour, the reaction solution was cooled to 0 °C and 2 mL of water was then added thereto. The reaction solution was concentrated. The residue oil was dissolved in 15 mL of dichloromethane and washed with 15 mL of 0.1 M TEAB. The aqueous layer was washed with dichloromethane (3 x 5 mL). The organic layer was collected and dried over sodium sulfate. The residue was puritied by silica gel chromatography to afford the title compound (0.68 g, yield: 41 %).
^{31}P NMR (DMSO), δ_{ppm}: -6.38, -6.25

Example 2: Synthesis of 5'-O-dimethoxytrityl-N^4-benzoyl-2'-O-(t-butyldimethylsilyl)cytidine-3'-O-(2-chlorophenylphosphate)-5'-O-2'-O-(t-butyldimethylsilyl)uridine (2b)

Step 1: Synthesis of 5'-O-dimethoxytrityl-N^4-benzoyl-2'-O-(t-butyldimethylsilyl)cytidine-3'-O-(2-chlorophenylphosphate)(triethylammonium salt) (3b)

[0078] Triazole (0.69 g, 10 mmol) and anhydrous triethylamine (1.4 mL, 9.9 mmol) were dissolved in dioxane (20 mL) and the solution was cooled to 5°C . A solution of O-chlorophenyl phosphodichloridate (1.2 g, 4.90 mmol) in 5 mL of dioxane was added dropwise to the resulting solution. After one hour, the mixed solution was filtered and added to 5'-O-dimethoxytrityl-N^4-benzoyl-2'-O-(t-butyldimethylsilyl)cytidine (2.5 g, 3.27 mmol, Sigma Aldrich) in 10 mL of anhydrous pyridine which had been cooled to -5°C. Then, 1-methylimidazole (0.40 mL, 4.9 mmol, Sigma Aldrich) was added to the above solution. After one hour, 0.1 M TEAB (10 mL) was added to the cooled solution that was then concentrated. The residue was dissolved in dichloromethane (50 mL) and washed with 0.1 M TEAB (50 mL). The aqueous layer was extracted two times with 20 mL of dichloromethane. The organic layer was collected, washed with 0.1 M TEAB (100 mL), dried over sodium sulfate and concentrated using a vacuum pump to give 3.08 g (yield: 94%) of the title compound.

Step 2: Synthesis of 5'-O-dimethoxytrityl-N^4-benzoyl-2'-O-(t-butyldimethylsilyl)cytidine-3'-O-(2-chlorophenylphosphate-5'-O-2'-O-(t-butyldimethylsilyl)uridine (2b)

[0079] 5'-O-dimethoxytrityl-N^4-benzoyl-2'-O-t-butyldimethylsilyl)cytidine-3'-O-(2-chlorophenylphosphate) (triethylammonium salt) (3b, 3.24 g, 3.07 mmol) prepared in Step 1 and 5'-O-dimethoxytrityl-2'-O-tert-butyldimethylsilyluridine (4a, 1 g, 2.8 mmol) were dissolved in 20 mL of pyridine, and the solution was dried using a vacuum pump. MSNT (1.364 g, 4.61 mmol) in 10 mL of fresh pyridine was added to the dried product. The reaction solution was concentrated to about 3 mL and 0.25 mL of 1-methylimidazole (3.07 mmol) was added thereto. After one hour, the reaction solution was cooled to 0°C and 2 mL of water was added thereto. After the reaction solution was concentrated, the residue oil was dissolved in 15 mL of dichloromethane and washed with 15 mL of 0.1 M TEAB. The aqueous layer was washed with dichloromethane (3 x 5 mL), and the organic layer was collected and dried over sodium sulfate. The residue was purified by silica gel chromatography to afford the title compound (1.47 g, yield: 40 %).
^{31}P NMR (DMSO), δ_{ppm}: -6.42, -6.10

Example 3: Synthesis of 5'-O-dimethoxytrityl-N^2-isobutyryl-2'-O-(t-butyldimethylsilyl)guanosine-3'-O-chlorophenylphosphate-5'-O-N^4-benzoyl-2'-O-(t-butyldimethylsilyl)adenine (2c)

Step 1: Synthesis of 5'-O-dimethoxytrityl-N^2-isobutyryl-2'-O-(t-butyldimethylsilyl)guanosine-3'-O-(2-chlorophenylphosphate)(triethylammonium salt) (3c)

[0080] Triazole (1.37 g, 19.87 mmol) and anhydrous triethylamine (2.8 mL, 19.87 mmol) were dissolved in dioxane (20 mL) and the solution was cooled to 5°C. A solution of O-chlorophenyl phosphodichloridate (2.386 g, 9.74 mmol) in 5 mL of dioxane was added dropwise to the resulting solution. After one hour, the mixed solution was filtered and added to 5'-O-dimethoxytrityl-N^2-isobutyryl-2'-O-t-butyldimethylsilylguanosine (5 g, 6.5 mmol) in 10 mL of anhydrous pyridine which had been cooled to -5°C,'Then, 1-methylimidazole (0.80 mL, 9.74 mmol) was added thereto. After one hour, 0.1 M TEAB (10 mL) was added to the cooled solution which was then concentrated. The residue was dissolved in dichloromethane (50 mL) and washed with 0.1 M TEAB (50 mL), and the aqueous layer was extracted with dichloromethane (2 x 20 mL). The organic layer was collected, washed with 0.1 M TEAB (100 mL), dried over sodium sulfate and concentrated using a vacuum pump to give 6.55 g (yield: 95%) of the title compound.

Step 2: Synthesis of 5'-O-dimethoxytrityl-N^2-isobutyryl-2'-O-(t-butyldimethylsilyl)guanosine-3'-O-(2-chlorophenylphosphate)-5'-O-N^4-benzoyl-2'-O-(t-butyldmethylsilyl)adenine (2c)

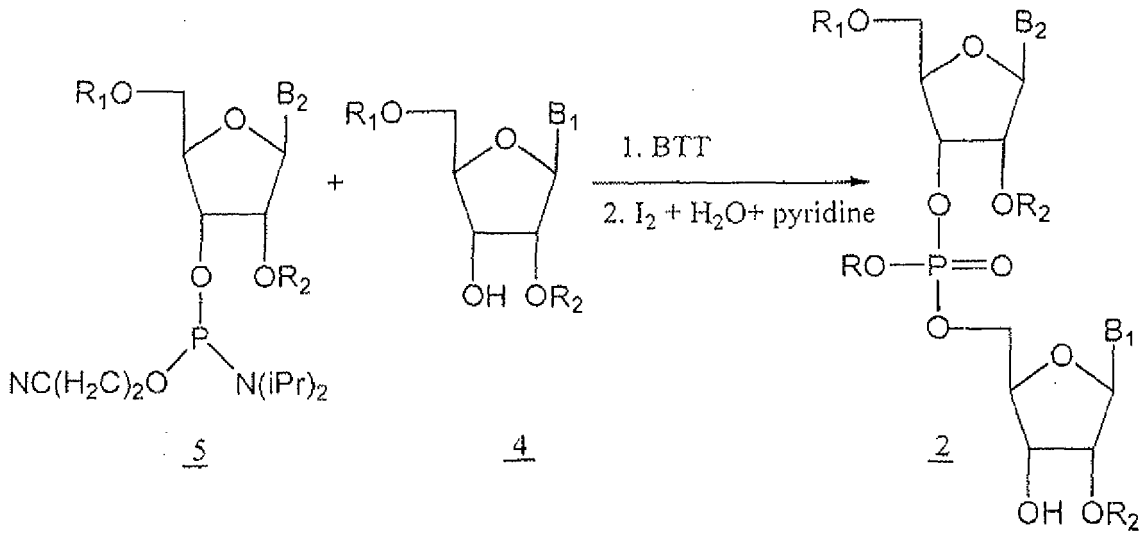
[0081] 5'-O-dimethoxytrityl-N^2-isobutyryl-2'-O-(t-butyldimethylsilyl)guanosine-3'-O-(2-chlorophenylphosphate)(triethylammonium salt) (3c, 1.34 g, 1.26 mmol) prepared in Step 1 and 5'-O-dimethoxytrityl-N^4-benzoyl-2'-O-tert-butyldimethylsilyladenine (4b, 1 g, 2.8 mmol) were dissolved in 20 mL of pyridine, and the solution was dried using a vacuum pump. MSNT (0.6 g, 1.89 mmol) dissolved in 10 mL of fresh pyridine was added to the dried product. The reaction solution was concentrated to about 3 mL and 0.16 mL of 1-methylimidazole (1.89 mmol) was added thereto. After 30 min, the reaction solution was cooled to 0°C and 2 mL of water was then added to the solution. The reaction solution was concentrated, and the residue oil was dissolved in 15 mL of dichloromethane and washed with 15 mL of 0.1 M TEAB. The aqueous layer was washed with dichloromethane (3 x 5 mL), and the organic layer was collected and dried over sodium sulfate. The residue was purified by silica gel chromatography to afford the title compound (1.265 g, yield: 84%).

^{31}P NMR (DMSO), δ_{ppm}: -6.33, -6.14

Example II: Synthesis of ribonucleotide dimer (phosphoramidite method)

[0082]

[Reaction Scheme 2]



Synthesis of ribonucleotide dimers UU, CU, GU and GA (2d to 2g).

[0083] R_1 = DMTr, R_2 = TBDMS, R = 2-cyanoethyl. 2d - B_1 = U, B_2 = U, 2e - B_1 = U, B_2 = bzC , 2f - B_1 = U, B_2 = ibG, 2g - B_1 = bzA, B_2 = ibG, 4a - B_1 = U, 4b - B_1 = bzA. 5a -B_2 = U, 5b-B_2 = bzC, 5c - B_2 = ibG.

Example 4: Synthesis of 5'-O-dimethoxytrityl-2'-O-(t-butyldimethylsilyl)uridyl-3'-O-[cyanoethoxyphosphino] (3'->5')-2'-O-t-butyldimethylsilyluridine (2d)

[0084] 5'-dimethoxytrityl-uridine-2'-O-t-butyldimethylsilyl-3'-[(2-cyanoethyl)-(N,N-diisopropyl)]phosphoramidite (5a, 1.085 g, 1.26 mmol) and 5'-O-dimethoxytrityl-2'-O-t-butyldimethylsilyluridine (4a, 0.3 g, 0.84 mmol) were dissolved in 10 mL of anhydrous acetonitrile, and the solution was concentrated until it became gum. 5-benzylthiotetrazole (0.483 g, 2.52 mmol, ChemGene) was dissolved in 20 mL of acetonitrile, and the solution was concentrated until crystals were formed. Two solutions were combined using 20 mL of acetonitrile and concentrated to 3 mL. After one hour, the combined solution was cooled to 0°C, and a 0.5 M iodine solution in 7.6 mL of THF:pyridine:water (7:1:2) was added thereto. The resulting solution was allowed to stand at room temperature for 5 min, and 3.8 mL of a 2 M $Na_2S_2O_3$ aqueous solution was then added thereto. After the solution was concentrated until it became gum, the residue was dissolved in 20 mL of dichloromethane and the aqueous layer was extracted with dichloromethane (3 x 5 mL). The organic layer was collected, washed with a 0.1 M TEAB aqueous solution (3 x 10 mL). and dried over sodium sulfate. The solution was concentrated and evaporated with toluene (2 x 10 mL) to remove the remaining pyridine. The residue was dissolved in dichloromethane and was purified by silica gel chromatography to afford the title compound (0.5 g, yield: 53%).
^{31}P NMR (DMSO), δ_{ppm}: -1.03, -0.71

Example 5: Synthesis of 5'-O-dimethoxytrityl-N^4-benzoyl-2'-O-(t-butyldemethylsilyl)cytidyl-3'-O-[cyano ethoxyphosphino] (3'->5')-2'-O-t-butyldimethylsilyluridine (2e)

[0085] 5'-dimethoxytrityl-N^4-benzoylcytidine-2'-O-t-butyldimethylsilyl-3'-[(2-cyanoethyl)-(N,N-diisopropyl)]phosphoramidite (5b, 1.928 g, 2.00 mmol) and 5'-O-dimethoxytrityl-2'-O-t-butyldimethylsilyluridine (4a, 0.358 g, 1.00 mmol) were dissolved in 10 mL of anhydrous acetonitrile, and the solution was concentrated until it became gum. 5-ethylthiotetrazole (0.528 g, 4 mmol, Sigma Aldrich) was dissolved in 20 mL of acetonitrile, and the solution was concentrated until crystals were formed. Two solutions were combined using 20 mL of acetonitrile. Thereafter, the combined solution was concentrated to 3 mL and cooled to 0°C after 4 hours, and a 0.5 M iodine solution in 12 mL of THF:pyridine:water (7:1:2) was then added thereto. This solution was allowed to stand at room temperature for 5 min and 6 mL of a 2 M $Na_2S_2O_3$ aqueous solution was added thereto. The solution was concentrated until it became gum. The residue was then dissolved in 20 mL of dichloromethane and the aqueous layer was extracted with dichloromethane (3 x 5 mL). The organic layer was collected, washed with a 0.1 M TEAB aqueous solution (3 x 10 mL), and dried over sodium sulfate. The solution was concentrated and evaporated with toluene (2 x 10 mL) to remove the remaining pyridine. The residue was dissolved in dichloromethane and purified by silica gel chromatography to afford the title compound (0.868 g, yield: 70%).
^{31}P NMR (DMSO), δ_{ppm}: -1.05, -0.74

Example 6: Synthesis of 5'-O-dimethoxytrityl-N^2-isobutyryl-2'-O-(t-butyldimethylsilyl)guanosyl-3'-O-[cyanoethoxyphosphino] (3'->5')-2'-O-t-butyldimethylsilyluridine (2f)

[0086] 5'-dimethoxytrityl-N^2-isobutyrylguanosine-2'-O-t-butyldimethylsilyl-3'-[(2-cyanoethyl)-(N,N-diisopropyl)]phosphoramidite (5c, 3.48 g, 3.59 mmol) and 5'-O-dimethoxytrityl-2'-O-t-butyldimethylsilyluridine (4a, 0.644 g, 1.8 mmol) were dissolved in 10 mL of anhydrous acetonitrile, and the solution was concentrated until it became gum. 5-benzylthiotetrazole (0.379 g, 7.18 mmol) was dissolved in 20 mL of acetonitrile and the solution was concentrated until crystals were formed. Two solutions were combined using 20 mL of acetonitrile. Thereafter, the combined solution was concentrated to 3 mL and cooled to 0°C after 1.5 hours, and a 0.5 M iodine solution in 22 mL of THF:pyridine:water (7:1:2) was then added thereto. This solution was allowed to stand at room temperature for 5 min and 11 mL of a 2 M $Na_2S_2O_3$ aqueous solution was added thereto. After the solution was concentrated until it became gum, the residue was dissolved in 20 mL of dichloromethane and the aqueous layer was extracted with dichloromethane (3 x 5 mL). The organic layer was collected, washed with a 0.1 M TEAB aqueous solution (3 x 10 mL), and dried over sodium sulfate. The solution was concentrated and evaporated with toluene (2x10 mL) to remove the remaining pyridine. The residue was dissolved in dichloromethane and purified by silica gel chromatography to afford the title compound (1.126 g, yield: 50%).
^{31}P NMR (DMSO), δ_{ppm}: -0.52, -0.68

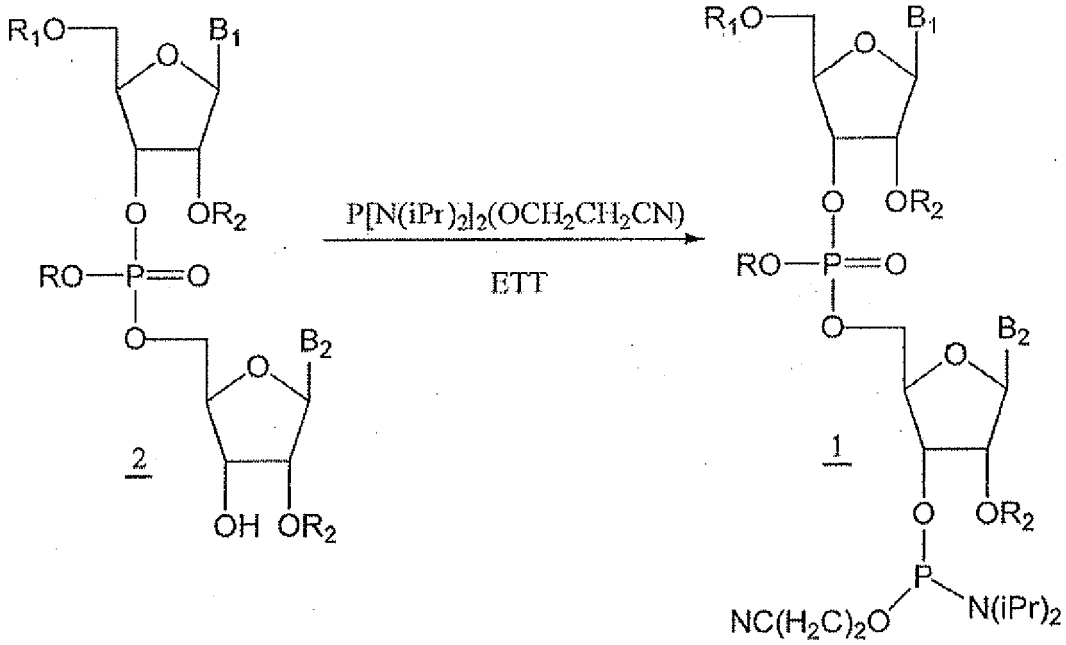
Example 7: Synthesis of 5'-O-dimethoxytrityl-N^2-isobutyryl-2'-O-(t-butyldimethylsilyl)guanosyl-3'-O-[cyanoethoxyphosphinol] (3'->5')-N^4-t-benzoyl-2'-O-t-butyldimethylsilyladenine (2g)

[0087] 5'-dimethoxytrityl-N^2-isobutyrylguanosine-2'-O-t-butyldimethylsilyl-3'-[(2-cyanoethyl)-(N,N-diisopropyl)]phosphoramidite (5c, 2.8 g, 2.88 mmol) and 5'-O-dimethoxytrityl-N^4-benzoyl-2'-O-t-butyldimethylsilyladenine (4b, 0.7 g, 1.:44 mmol) were dissolved in 10 mL of anhydrous acetonitrile, and the solution was concentrated until it became gum. 5-benzylthiotetrazole (1.075 g, 5.6 mmol) was dissolved in 20 mL of acetonitrile and the solution was concentrated until crystals were formed. Thereafter, two solutions were combined using 20 mL of acetonitrile. The combined solution was concentrated to 3 mL and cooled to 0°C after 1.5 hours, and a 0.5 M iodine solution in 18 mL of THF:pyridine:water (7:1:2) was then added thereto. This solution was allowed to stand at room temperature for 5 min and 9 mL of a 2 M $Na_2S_2O_3$ aqueous solution was added thereto. The solution was concentrated until it became gum. The residue was dissolved in 20 mL of dichloromethane and the aqueous layer was extracted with dichloromethane (3 x 5 mL). The organic layer was collected, washed with a 0.1 M TEAB aqueous solution (3 x 10 mL), and dried over sodium sulfate. The solution was concentrated and evaporated with toluene (2 x 10 mL) to remove the remaining pyridine. The residue was dissolved in dichloromethane and purified by silica gel chromatography to afford the title compound (2.182 g, yield: 96%).
^{31}P NMR (DMSO), δ_{ppm}: -0.69, -0.81

Example III: Synthesis of RNA dimer phosphoramidites

[0088]

[Reaction Scheme 3]



Synthesis of RNA ribonucleotide dimer phosphoramidites UU, CU, GU and GA (1a to 1d)

[0089] R_1 m DMTr, R_2 = TBDMS, R = 2-cyanoethyl. 1a - B_1 = U, B_2 = U, 1b - B_1 = bzC, B_2 = U, 1c - B_1 = ibG, B_2 = U, 1d - B_1 = ibG, B_2 = bzA, 2d - B_1 = U, B_2 = U, 2e - B_1 = bzC, B_2 = U, 2f - B_1 = ibG, B_2 = U, 2g - B_1 = ibG, B_2 = bzA.

Example 8: Synthesis of 5'-O-dimethoxytrityl-P-cyanoethylphosphoryl-2'-O-(t-butyldimethylsilyl)uridyl-3'-O-[(N,N-diisopropylamino)cyanoethoxyphosphino] (3'->5') 2'-O-t-butyldimethylsilyluridine (1a)

[0090] Compound 2d of Example 4 (0.503 g, 0.44 mmol) and 5-ethylthiotetrazole (0.074 g, 0.57 mmol) were dissolved in 10 mL of acetonitrile and the solution was concentrated. 10 mL of acetonitrile was placed in a reaction flask which

was then filled with argon, and bis-(diisopropylamino)-2-cyanoethoxy phosphine (0.17 mL, 0.57 mmol) was added dropwise thereto. The reaction solution was concentrated to about 1 mL, allowed to stand for 2 hours and then completely concentrated. The residue was dissolved in 10 mL of dichloromethane and saturated with a saturated $NaHCO_3$ aqueous solution. The organic layer was washed with a saturated $NaHCO_3$ aqueous solution (5 x 20 mL) and dried over sodium sulfate. The reaction solution was completely concentrated and water was added until the solution became turbid. Purification was carried out using a LiChroprep RP18 resin (Merck & Co., Inc., USA) to give the title compound (0.4 g, yield: 70%).
^{31}P NMR (DMSO), δ_{ppm}: ~ 138.4, ~ 148.9 ~ -1.03 ~ -0.73

Example 9: Synthesis of 5'-O-dimethoxytrityl-N^4-benzoyl-P-cyanoethylphosphoryl-2'-O-(t-butyldimethylsilyl)cytidyl-3'-O-[(N,N-diisopropylamino)cyanoethoxyphosphino] (3'->5')-2'-O-t-butyldimethylsilyluridine (1b)

[0091] Compound 2e of Example 5 (0.868 g, 0.70 mmol) and 5-ethylthiotetrazole (0.11 g, 0.84 mmol) were dissolved in 10 mL of acetonitrile and the solution was concentrated. 10 mL of acetonitrile was placed in a reaction flask which was then filled with argon, and bis-(diisopropylamino)-2-cyanoethoxy phosphine (0.17 mL, 0.57 mmol) was added dropwise thereto. The reaction solution was concentrated to about 1 mL, allowed to stand for 4 hours and then completely concentrated. The residue was dissolved in 10 mL of dichloromethane and saturated with a saturated $NaHCO_3$ aqueous solution. The organic layer was washed with a saturated $NaHCO_3$ aqueous solution (5 x 20 mL) and dried over sodium sulfate. The reaction solution was completely concentrated and water was added until the solution became turbid. Purification was carried out using a LiChroprep RP18 resin to give the title compound (0.58 g, yield: 60%).
^{31}P NMR (DMSO), δ_{ppm}: ~ 148.7, ~ 143.9, ~ -1.18, ~ -0.77

Example 10: Synthesis of 5'-O-dimethoxytrityl-N^2-isobutyryl-P-cyanoethylphosphoryl-2'-O-(t-butyldimethylsilyl)guanosyl-3'-O-[(N,N-diisopropylamino)cyanoethoxyphosphino] (3'->5')-2'-O-t-butyldimethylsilyluridine (1c)

[0092] Compound 2f of Example 6 (1.126 g, 0.09 mmol) and 5-ethylthiotetrazole (0.15 g, 1.17 mmol) were dissolved in 10 mL of acetonitrile and the solution was concentrated. 10 mL of acetonitrile was placed in a reaction flask which was then filled with argon, and bis-(diisopropylamino)-2-cyanoethoxy phosphine (0.35 mL, 1.17 mmol) was added dropwise thereto. The reaction solution was concentrated to about 1 mL, allowed to stand for 4 hours and then completely concentrated. The residue was dissolved in 10 mL of dichloromethane and saturated with a saturated $NaHCO_3$ aqueous solution. The organic layer was washed with a saturated $NaHCO_3$ aqueous solution (5 x 20 mL) and dried over sodium sulfate. The reaction solution was completely concentrated and water was added until the solution became turbid. Purification was carried out using a LiChroprep RP18 resin to give the title compound (0.75 g, yield: 57%).
^{31}P NMR (DMSO), δ_{ppm}: ~ 150, ~ 148.9, ~ -0.66, ~ -0.49

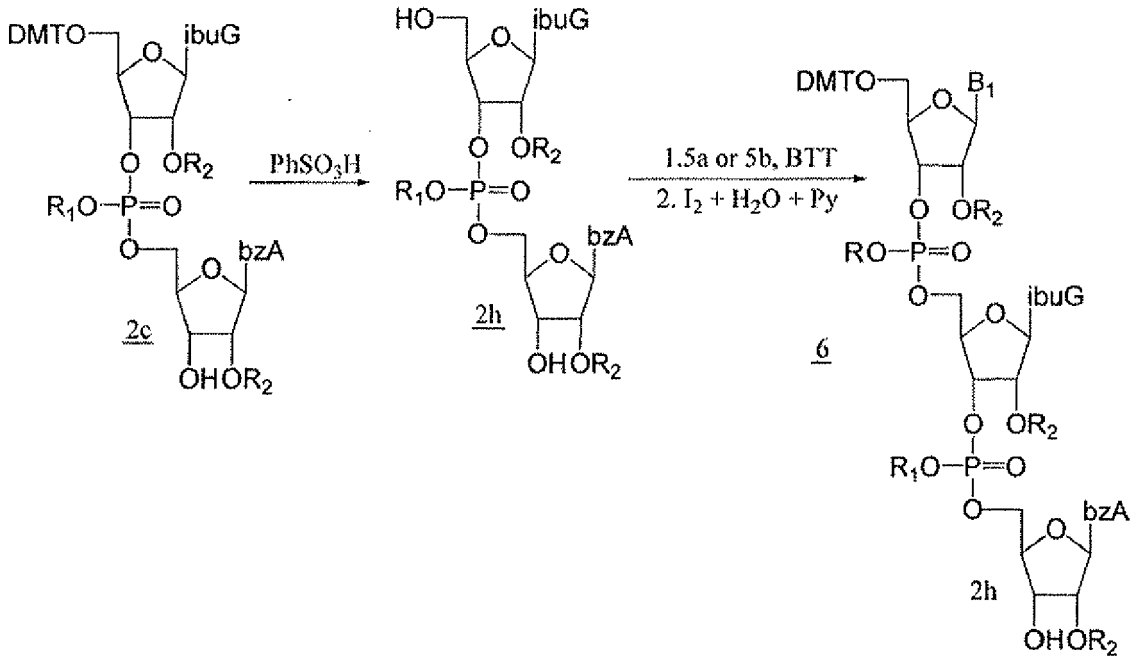
Example 11: Synthesis of 5'-O-dimethoxytrityl-N^2-isobutyryl-P-cyanoethylphosphoryl-2'-O-(t-butyldimethylsilyl)guanosyl-3'-O-[(N,N-diisopropylamino)cyanoethoxyphosphino] (3'->5')-N^4-benzoyl-2'-O-t-butyldimethylsilyladenine (1d)

[0093] Compound 2g of Example 7 (1.5 g, 1.09 mmol) and 5-ethylthiotetrazole (0.18 g, 1.42 mmol) were dissolved in 10 mL of acetonitrile and the solution was concentrated. 10 mL of acetonitrile was placed in a reaction flask which was then filled with argon, and bis-(diisopropylamino)-2-cyanoethoxy phosphine (0.43 mL, 1.42 mmol) was added dropwise thereto. The reaction solution was concentrated to about 1 mL, allowed to stand for 4 hours and then completely concentrated. The residue was dissolved in 10 mL of dichloromethane and saturated with a saturated $NaHCO_3$ aqueous solution. The organic layer was washed with a saturated $NaHCO_3$ aqueous solution (5 x 20 mL) and dried over sodium sulfate. The reaction solution was completely concentrated and water was added until the solution became turbid. Purification was carried out using a LiChroprep RP18 resin to give the title compound (1.081 g, yield: 63%).
^{31}P NMR (DMSO), δ_{ppm}: ~ 150, ~ 148.8, ~ -0.63, ~ -0.41

Example IV: Synthesis of RNA trinucleotides

[0094]

[Reaction Scheme 4]



Synthesis of RNA trinucleotides UGpA (6a) and CGpA (6b)

[0095] R_1= *o*-chlorophenyl, R = 2-cyanoethyl, R_2 = TBDMS. 6a - B_1 = U, 6b - B_1 = bzC

Example 12: Synthesis of 5'-O-dimethoxytrityl-2'-O-(t-butyldimethylsilyl)uridin-3'-yl chlorophenylphosphat-5'-yl N^2-isobutyryl-2'-O-(t-butyldimethylsilyl)guanosin-3'-yl N^4-benzoyl-2'-O-t-butyldimethylsilyladenin-5'-yl cyanoethylphosphate (6a)

Step 1: Synthesis of N^2-isobutyryl-2'-O-(t-butyldimethylsilyl)guanosin-3'-yl chlorophenyl phosphat-5'-yl N^4-benzoyl-2'-O-t-butyldimethylsilyladenine (2h)

[0096] 9 mL of 4% benzenesulfonic acid was added to a dichloromethane:methanol (7:3) solution which was then cooled to 0°C. The solution was added to Compound 2c (1.265 g, 0.88 mmol) dissolved in 9 mL of a dichloromethane:methanol (7:3) solution and allowed to stand at 0°C for 3 min. 25 mL of a saturated $NaHCO_3$ aqueous solution was added thereto, and the organic layer was washed with a saturated $NaHCO_3$ aqueous solution, dried over sodium sulfate and concentrated. The residue was purified by silica gel chromatography to afford the title compound (0.62 g, yield: 63%).

Step 2: Synthesis of 5'-O-dimethoxytrityl-2'-O-(t-butyldimethylsilyl)uridin-3'-yl chlorophenylphosphat-5'-yl N^2-isobutyryl-2'-O-(t-butyldimethylsilyl)guanosin-3'-yl N^4-benzoyl-2'-O-t-butyldimethylsilyladenin-5'-yl cyanoethylphosphate (6a)

[0097] U phosphoramidite (5a, 0.335 g, 0.39 mmol) and the compound of Step 1 (2h, 0.292 g, 0.26 mmol) were dissolved in anhydrous acetonitrile and the solution was concentrated. A reaction flask was filled with argon gas and 10 mL of anhydrous acetonitrile was added thereto. 5-benzylthiotetrazole (0.15g, 0.78 mmol) was dissolved in 10 mL of anhydrous acetonitrile and the solution was concentrated until crystals were formed, and then added to the nucleoside solution. The reaction solution was concentrated to about 3 mL and allowed to stand for 2 hours. The reaction solution was cooled to 0°C, and a 0.5M iodine solution in 2.4 mL of THF:pyridine:water (7:1:2) was added thereto. The resulting solution was allowed to stand at room temperature for 5 min, and 1.2 mL of a 2 M $Na_2S_2O_3$ aqueous solution was then added to the solution. After the solution was concentrated until it became gum, the residue was dissolved in 20 mL of dichloromethane, and the organic layer was washed with a 0.1 M TEAB aqueous solution. The aqueous layer was extracted with dichloromethane (3 x 5mL). The organic layer was collected, washed with a 0.1 M TEAB aqueous solution (3 x 10 mL), and dried over sodium sulfate. The solution was concentrated and evaporated with toluene (2 x 10 mL) to

remove the remaining pyridine. The residue was dissolved in dichloromethane and purified by silica gel chromatography to afford the title compound (0.413 g, yield: 84%).
^{31}P NMR (DMSO), δ_{ppm}: -6.3, -1.4

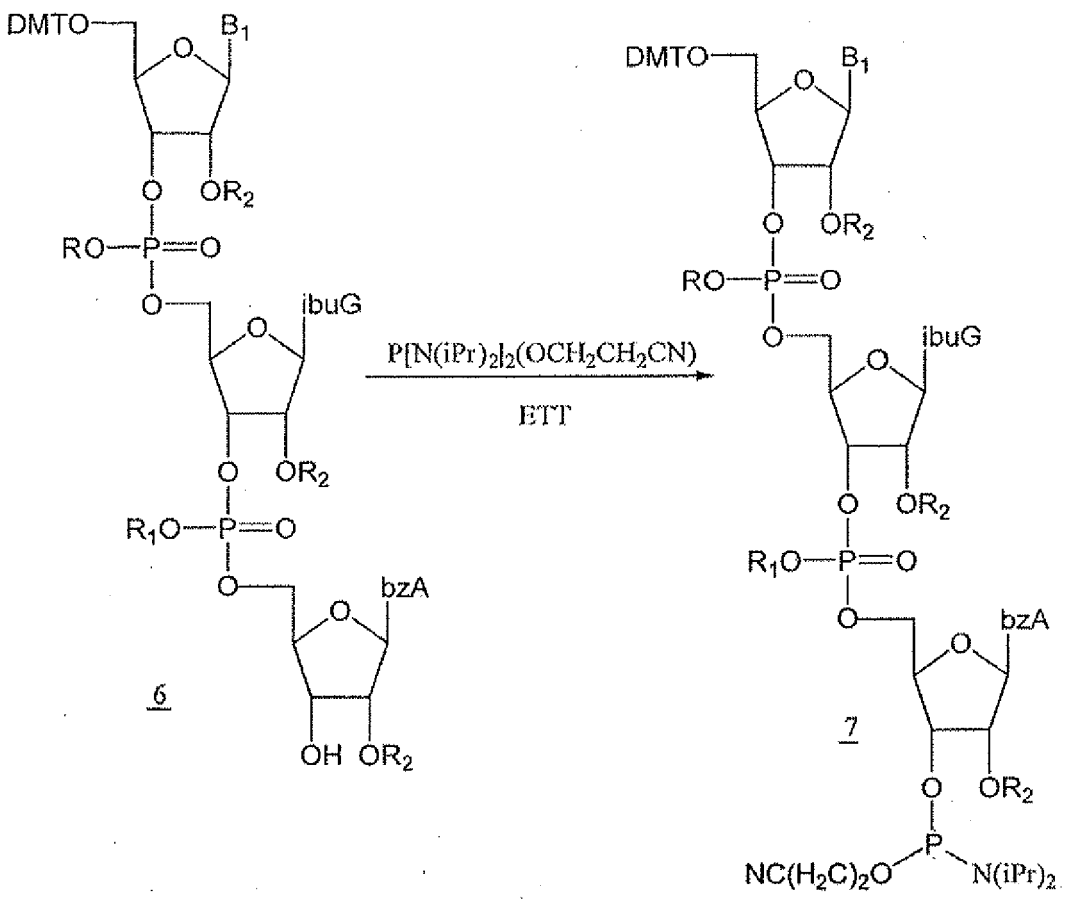
Example 13: Synthesis of 5'-O-dimethoxytrityl-N^4-benzoyl-2'-O-(t-butyldimethylsilyl)cytidin-3'-yl chlorophenyl-phosphat-5'-yl N^4-isobutyryl-2'-O-(t-butyldimethylsilyl)guanosin-3'-yl N^4-benzoyl-2'-O-t-butyldimethylsilyladenin-5'-yl cyanoethylphosphate (6b)

[0098] rC phosphoramidite (5b, 0.409 g, 0.42 mmol) and the compound of Step 1 of Example 12 (2h, 0.238 g, 0.21 mmol) were dissolved in anhydrous acetonitrile and concentrated. A reaction flask was filled with argon gas and 10 mL of anhydrous acetonitrile was added thereto. 5-benzylthiotetrazole (0.123 g, 0.64 mmol) was dissolved in 10 mL of anhydrous acetonitrile and the solution was concentrated until crystals were formed, and then added to the nucleoside solution. The reaction solution was concentrated to about 3 mL and allowed to stand for 3 hours. The reaction solution was cooled to 0°C, and a 0.5M iodine solution in 2.6 mL of THF:pyridine:water (7:1:2) was added thereto. The resulting solution was allowed to stand at room temperature for 5 min, and 1.3 mL) of a 2 M Na_2SO_3 aqueous solution was then added thereto. After the solution was concentrated until it became gum, the residue was dissolved in 20 mL of dichloromethane, and the organic layer was washed with a 0.1 M TEAB aqueous solution. The aqueous layer was extracted with dichloromethane (3 x 5 mL). The organic layer was collected, washed with a 0.1 M TEAB aqueous solution (3 x 10 mL), and dried over sodium sulfate. The solution was concentrated and evaporated with toluene (2 x 10 mL) to remove the remaining pyridine. The residue was dissolved in dichloromethane and purified by silica gel chromatography to afford the title compound (0.298 g, yield: 71%).
^{31}P NMR (DMSO), δ_{ppm}: -6.3, -1.2

Example V: Synthesis of ribonucleotide trimer phosphoramidites

[0099]

[Reaction Scheme 5]



Synthesis of ribonucleotide trimer phosphoramidites (7a and 7b)

[0100] R_1 = *o*-chlorophenyl, R = 2-cyanoethyl, R_2 = TBDMS. 6a, 7a - B_1=U, 6b, 7b - B_1=bzC

Example 14: Synthesis of 5'-O-dimethoxytrityl-2'-O-(t-butyldimethylsilyl)uridin-3'-yl chlorophenylphosphat-5'-yl N^2-isobutyryl-2'-O-(t-butyldimethylsilyl)guanosin-3'-yl N^4-benzoyl-2'-O-t-butyldimethylsilyadenin-5'-yl cyanoethylphosphate-3-O-(2-cyanoethyl)-N,N-diisopropylphosphoramidite (7a)

[0101] Compound 6a of Example 12 (0.41 g, 0.21 mmol) and 5-ethylthiotetrazole (0.037 g, 0.57 mmol) were dissolved in 10 mL of anhydrous acetonitrile and concentrated. 10 mL of anhydrous acetonitrile was placed in a reaction flask which was then filled with argon, and bis-(diisopropylamino)-2-cyanoethoxy phosphine (0.084 mL, 0.28 mmol) was added dropwise thereto. The reaction solution was concentrated to about 1 mL and allowed to stand for 4 hours, followed by complete concentration. The residue was dissolved in 10 mL of dichloromethane and saturated with a saturated $NaHCO_3$ aqueous solution. The organic layer was washed with a saturated $NaHCO_3$ aqueous solution (5 x 20 mL) and dried over sodium sulfate. The reaction solution was completely concentrated and water was added until the solution became turbid. Purification was carried out using a LiChroprep RP18 resin to give the title compound (0.319 g, yield: 72%). ^{31}P NMR (DMSO), δ_{ppm}: ~ 150.2, ~ 148.9, ~ -6, ~ -1.4

Example 15: Synthesis of 5'-O-dimethoxytrityl-N^4-benzoyl 2'-O-(t-butyldimethylsilyl)cytidin-3'-yl chlorophenylphosphat-5'-yl N^4-isobutyryl-2'-O-(t-butyldimethylsilyl)guanosin-3'-yl N^4-benzoyl-2'-O-t-butyldimethylsilyladenin-5'-yl cyanoethylphosphate-3-O-(2-cyanoethyl)-N,N-diisopropylphosphoramidite (7b)

[0102] Compound 6b of Example 13 (0.298 g, 0.15 mmol) and 5-ethylthiotetrazole (0.025 g, 0.20 mmol) were dissolved in 10 mL of anhydrous acetonitrile and concentrated. 10 mL of anhydrous acetonitrile was placed in a reaction flask

which was then filled with argon, and bis-(diisopropylamino)-2-cyanoethoxy phosphine (0.06 mL, 0.20 mmol) was added dropwise thereto. The reaction solution was concentrated to about 1 mL and allowed to stand for 4 hours, followed by complete concentration. The residue was dissolved in 10 mL of dichloromethane and saturated with a saturated $NaHCO_3$ aqueous solution. The organic layer was washed with a saturated $NaHCO_3$ aqueous solution (5 x 20 mL) and dried over sodium sulfate. The reaction solution was completely concentrated and water was added until the solution became turbid. Purification was carried out using a LiChroprep RP18 resin to give the title compound (0.180 g, yield: 60%).
^{31}P NMR (DMSO), δ_{ppm}: ~ 150.2, ~148.9, ~-6, ~ -1.0

Example VI: Synthesis of siRNAs using RNA dimer phosphoramidites

[0103] All of siRNAs were synthesized using a Polygen DNA/RNA synthesizer (Polygen) on a 0.8 μmol scale in a trityl-off mode. The 3'-terminus employed RNA CPG. The RNA CPG (Glen Research) was used in an amount of 30 μmol/g loading, and monomer bases were respectively rA^{tac}, rC^{tac}, rG^{tac} and U phosphoramidites (Proligo). The monomer and dimer phosphoramidites were each used in the form of a 0.05 M solution thereof in acetonitrile. Equivalents of the monomers and dimers were each 2.5 equivalents per cycle. An activator was 0.5 M 5-ethylthiotetrazole (in acetonitrile). Solid supports and protecting groups were deprotected by heating the reaction solution at 65°C for 2 hours using a mixture of aqueous ammonia and ethanol (3:1), and the solution was freeze-dried. The residue was dissolved in 0.4 mL of an N-methylpyridone:triethylamine:triethylamine trihydrofluoride (6:3:4) solution and heated at 65°C for 2 hours. 4 mL of *n*-butyl alcohol was added to the resulting solution which was then cooled in a refrigerator for 2 hours and centrifuged to obtain solid siRNAs, followed by freeze-drying. The yield of crude siRNAs was quantitatively analyzed using a UV spectrophotometer at 260 nm and the purity thereof was analyzed by reverse-phase HPLC. Extinction coefficients of naturally-occurring ribonucleotides for concentration calculation are as follows: rA, 15400: rC, 7200: U, 9900: and rG, 11500. A molecular weight of each siRNA was confirmed by mass analysis using MALDI-TOF (Bruker, Autoflex).

Example 16: Synthesis of GFP-sense siRNA using GU RNA dimer

[0104] GFP-sense siRNA had a sequence of 5'-GUU CAG CGU GUC CGG CGA GUU-3' (SEQ ID NO: 1). Synthesis of siRNA was carried out analogously to Example 15, and a coupling period of time for dimer GU and monomers was each 10 min. The dimer used for the first coupling step was GU, to which monomer units were then attached. Purity of the product was measured by reverse-phase chromatography, and an analyzer was an Agilent 1100 system. Chromatography buffer was a mixture of 100 mM TEAA (pH 7.0) and acetonitrile. Purity and yield of the siRNA product were compared with those of the GFP-sense siRNA which was obtained using the monomer instead of the dimer as the first ribonucleotide. The results are given in Table 1 below.

[Table 1]

First ribonucleotide	Purity of siRNA
Monomer	52%
Dimer	73%

Example 17: Synthesis of GFP-antisense siRNA using CU RNA dimer

[0105] GFP-antisense siRNA had a sequence of 5'-CUC GCC GGA CAC GCU GAA CUU-3' (SEQ ID NO: 2). Synthesis of siRNA was carried out analogously to Example 15, and a coupling period of time for dimer CU and monomers was each 10 min. The dimer used for the first coupling step was CU, to which monomer units were then attached. Purity of the product was measured by reverse-phase chromatography, and an analyzer was an Agilent 1100 system. Chromatography buffer was a mixture of 100 mM TEAA (pH 7.0) and acetonitrile. Purity and yield of the siRNA product were compared with those of the GFP-antisense siRNA which was obtained using the monomer instead of the dimer as the first ribonucleotide. The results are given in Table 2 below.

[Table 2]

First ribonucleotide	Purity of siRNA
Monomer	63%
Dimer	78%

Example 18: Synthesis of JNK-antisense siRNA using UU RNA dimer

[0106] JNK-antisense siRNA had a sequence of 5'-AGA AGG UAG GAC AUU CUU UUU-3' (SEQ ID NO: 3). Synthesis of siRNA was carried out analogously to Example 15, and a coupling period of time for dimer UU and monomers was each 10 min. The dimer used for the first coupling step was UU, to which monomer units were then attached. Purity of the product was measured by reverse-phase chromatography, and an analyzer was an Agilent 1100 system. Chromatography buffer was a mixture of 100 mM TEAA (pH 7.0) and acetonitrile. Purity and yield of the siRNA product were compared with those of the JNK-antisense siRNA which was obtained using the monomer instead of the dimer as the first ribonucleotide. The results are given in Table 3 below.

[Table 3]

First ribonucleotide	Purity of siRNA
Monomer	71%
Dimer	88%

Example 19: Synthesis of SEI-sense siRNA using GA RNA dimer

[0107] SEI-sense siRNA had a sequence of 5'-GGA AGG GUC UGA AGC GGA A-3' (SEQ ID NO: 4). Synthesis of siRNA was carried out analogously to Example 15, and a coupling period of time was 10 min and 15 min for monomers and dimer GA, respectively. The dimer used for the first coupling step was GA, to which monomer units were then attached. Purity of the product was measured by reverse-phase chromatography, and an analyzer was an Agilent 1100 system. Chromatography buffer was a mixture of 100 mM TEAA (pH 7.0) and acetonitrile. Purity and yield of the siRNA product were compared with those of the SEI-sense siRNA which was obtained using the monomer instead of the dimer as the first ribonucleotide. The results are given in Table 4 below.

[Table 4]

First ribonucleotide	Purity of siRNA
Monomer	65%
Dimer	78%

[0108] Although the preferred embodiments of the present invention have been disclosed for illustrative purposes, those skilled in the art will appreciate that various modifications, additions and substitutions are possible, without departing from the scope of the invention as disclosed in the accompanying claims.

INDUSTRIAL APPLICABILITY

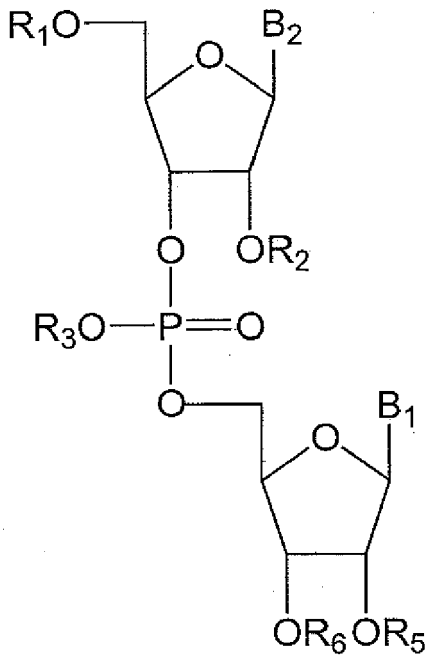
[0109] The present invention enables the efficient high-speed and high-purity synthesis of ribonucleotide oligomers. The method of the present invention provides a ribonucleotide oligomer having 15-20% higher purity than the conventional art.

Claims

1. A method for preparing ribonucleotide oligomers, comprising:

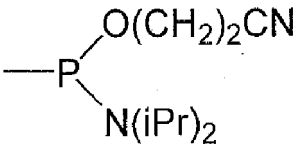
(a) coupling a ribonucleotide dimer or ribonucleotide trimer to a ribonucleoside attached to solid supports or to universal solid supports as a starting material;
(b) sequentially coupling ribonucleotide monomers to the resulting structures of Step (a) to prepare ribonucleotide oligomers; and
(c) removing the ribonucleotide oligomers from the solid supports;

wherein the ribonucleotide dimer in step (a) is represented by Formula 5:

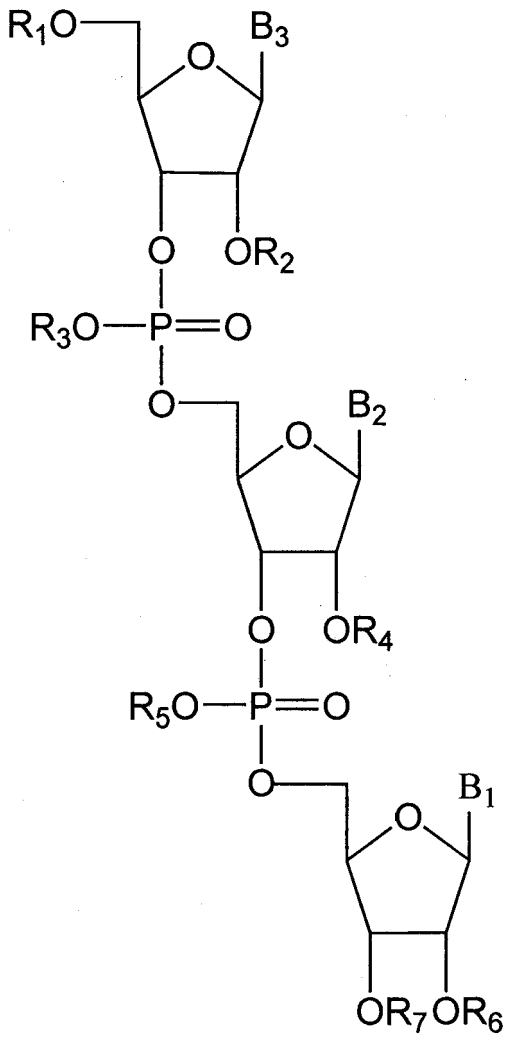


(5)

wherein R_1, R_2, R_3 and R_5 are each independently protecting groups, B_1 and B_2 are each independently nucleosidic bases, and R_6 is hydrogen or

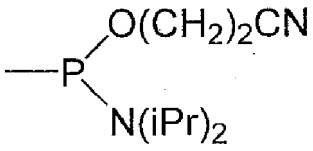


wherein iPr is isopropyl;
wherein the ribonucleotide trimer in step (a) is represented by Formula 6:



(6)

wherein R_1, R_2, R_3, R_4, R_5 and R_6 are each independently protecting groups, B_1, B_2 and B_3 are each independently nucleosidic bases, and R_7 is hydrogen or



wherein iPr is isopropyl.

2. The method of claim 1, wherein the method includes:

(a) coupling a ribonucleotide dimer according to claim 1 to a ribonucleoside attached to solid supports or to a universal solid supports as a starting material;
(b) sequentially coupling ribonucleotide monomers to the resulting structures of Step (a) to prepare ribonucleotide oligomers; and
(c) removing the ribonucleotide oligomers from the solid supports.

3. The method of claim 1, wherein the method includes:

(a) coupling a ribonucleotide trimer according to claim 1 to a ribonucleoside attached to solid supports or to a

universal solid supports as a starting material;
(b) sequentially coupling ribonucleotide monomers to the resulting structures of Step (a) to prepare ribonucleotide oligomers; and
(c) removing the ribonucleotide oligomers from the solid supports.

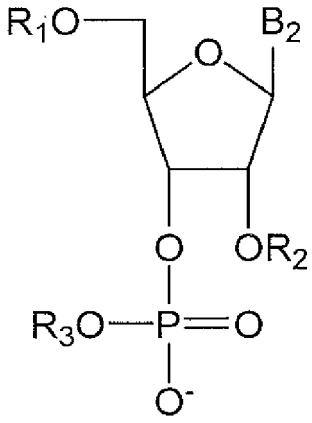
4. The method of any one of claims 1 to 3, wherein the ribonucleotide monomer is a ribonucleoside phosphoramidite.

5. The method of any one of claims 1 to 3, wherein the ribonucleotide oligomer is one containing at least one ribonucleotide selected from 2'-O-halogen ribonucleotide, 2'-amino ribonucleotide, 2'-O-alkyl ribonucleotide and 2'-O-alkoxy ribonucleotide.

6. The method of any one of claims 1 to 3, wherein the ribonucleotide oligomer has a phosphodiester, phosphoramidate, alkylphosphoramidate, alkylphosphonate, phosphorothioate, alkylphosphotriester, or alkylphosphonothioate linkage.

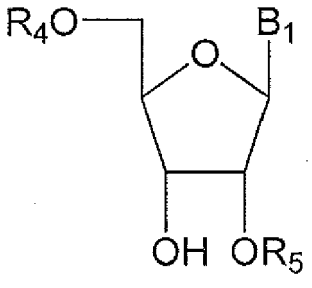
7. The method of claim 6, wherein the ribonucleotide oligomer has a phosphodiester or phosphoramidate linkage.

8. The method of claim 1 or 2, further comprising the step of preparing the ribonucleotide dimer of step (a) by a reaction including coupling of a compound of Formula 1 with a compound of Formula 2:



(1)

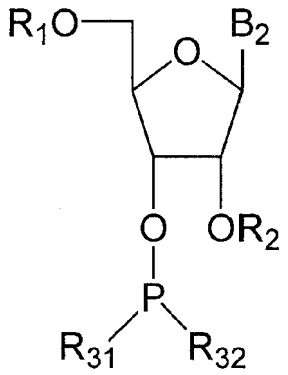
wherein R_1, R_2 and R_3 are each independently protecting groups, and B_2 is a nucleosidic base;



(2)

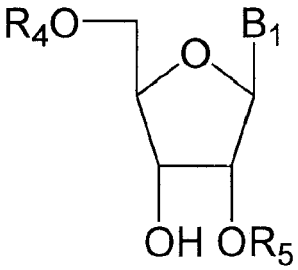
wherein R_4 and R_5 are each independently protecting groups, and B_1 is a nucleosidic base.

9. The method of claim 1 or 2, further comprising preparing the ribonucleotide dimer of step (a) by a reaction including coupling of a compound of Formula 3 with a compound of Formula 2:



(3)

wherein R_1 and R_2 are each independently protecting groups, R_{31} is 2-cyanoethoxy, cyanomethoxy, 4-cyano-2-butenyloxy or diphenylmethylsilylethoxy, R_{32} is dialkylamino, and B_2 is a nucleosidic base;

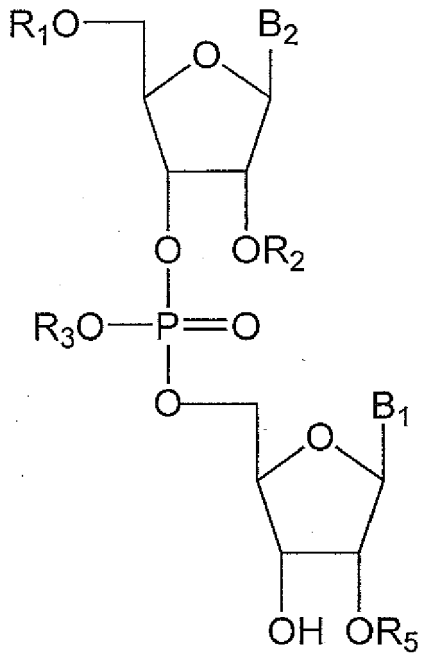


(2)

wherein R_4 and R_5 are each independently protecting groups, and B_1 is a nucleosidic base.

10. The method of claim 1 or 3, further comprising preparing the ribonucleotide trimer of step (a) by a reaction including:

(a) reacting a ribonucleotide dimer of Formula 4 with an acid to remove R_1 of Formula 4; and
(b) coupling the resulting product of Step (a) with a ribonucleoside 3'-phosphoramidite to prepare a ribonucleotide trimer :



(4)

wherein R_1, R_2, R_3 and R_5 are each independently protecting groups, and B_1 and B_2 are each independently nucleosidic bases.

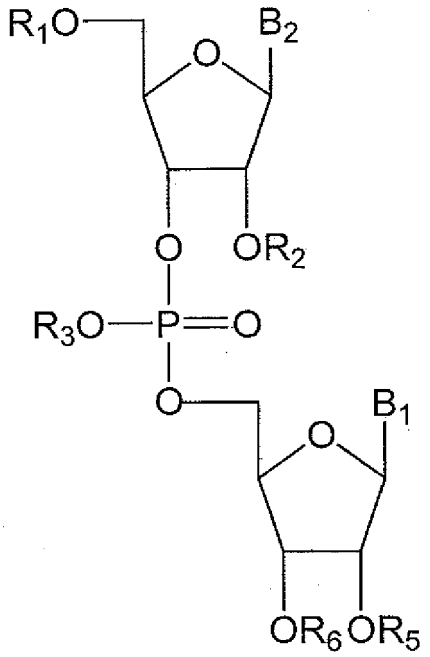
11. The method of claim 10, wherein the acid of Step (a) is benzenesulfonic acid.

Patentansprüche

1. Verfahren zum Herstellen von Ribonukleotidoligomeren, umfassend:

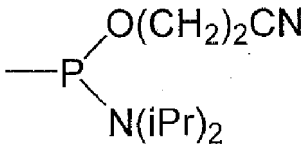
(a) Kuppeln eines Ribonukleotiddimers oder Ribonukleotidtrimers an ein Ribonukleosid, das an festen Trägern oder an universellen festen Trägern angebracht ist, als Ausgangsmaterial;
(b) sequenziell Kuppeln von Ribonukleotidmonomeren an die resultierenden Strukturen von Schritt (a) zur Herstellung von Ribonukleotidoligomeren; und
(c) Entfernen der Ribonukleotidoligomere von den festen Trägern;

wobei das Ribonukleotiddimer in Schritt (a) wiedergegeben ist durch Formel 5:

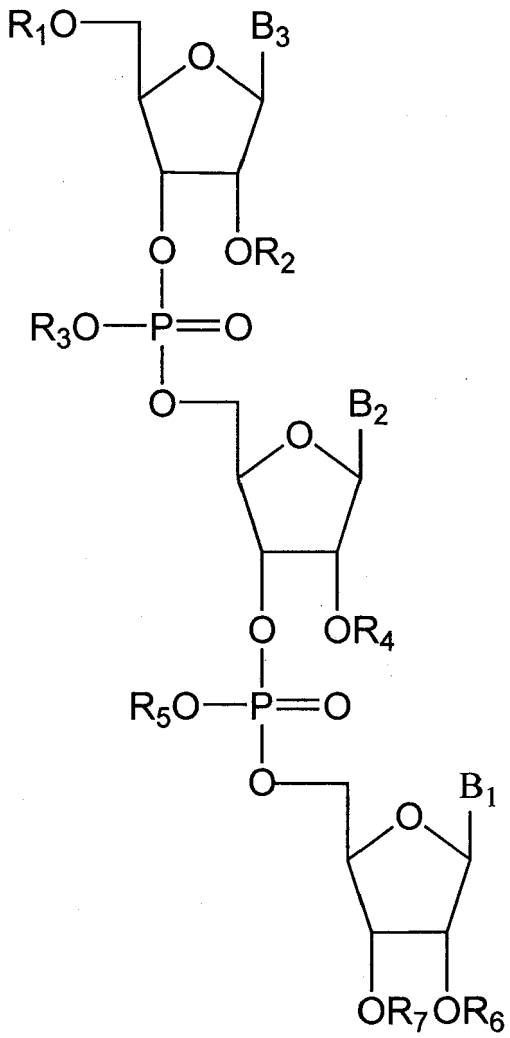


(5)

wobei R_1, R_2, R_3 und R_5 jeweils unabhängig für Schutzgruppen stehen, B_1 und B_2 jeweils unabhängig für nukleosidische Basen stehen und R_6 für Wasserstoff oder

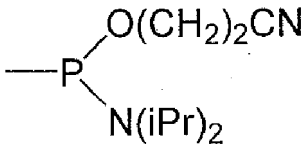


steht, wobei es sich bei iPr um Isopropyl handelt;
wobei das Ribonukleotidtrimer in Schritt (a) wiedergegeben ist durch Formel 6:



(6)

wobei R_1, R_2, R_3, R_4, R_5 und R_6 jeweils unabhängig für Schutzgruppen stehen, B_1, B_2 und B_3 jeweils unabhängig für nukleosidische Basen stehen und R_7 für Wasserstoff oder



steht, wobei es sich bei iPr um Isopropyl handelt.

2. Verfahren nach Anspruch 1, wobei das Verfahren Folgendes beinhaltet:

(a) Kuppeln eines Ribonukleotiddimers nach Anspruch 1 an ein Ribonukleosid, das an festen Trägern oder an universellen festen Trägern angebracht ist, als Ausgangsmaterial;
(b) sequenziell Kuppeln von Ribonukleotidmonomeren an die resultierenden Strukturen von Schritt (a) zur Herstellung von Ribonukleotidoligomeren; und
(c) Entfernen der Ribonukleotidoligomere von den festen Trägern.

3. Verfahren nach Anspruch 1, wobei das Verfahren Folgendes beinhaltet:

(a) Kuppeln eines Ribonukleotidtrimers nach Anspruch 1 an ein Ribonukleosid, das an festen Trägern oder an

universellen festen Trägern angebracht ist, als Ausgangsmaterial;
(b) sequenziell Kuppeln von Ribonukleotidmonomeren an die resultierenden Strukturen von Schritt (a) zur Herstellung von Ribonukleotidoligomeren; und
(c) Entfernen der Ribonukleotidoligomere von den festen Trägern.

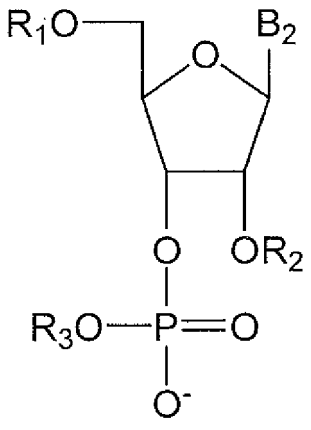
4. Verfahren nach einem der Ansprüche 1 bis 3, wobei es sich bei dem Ribonukleotidmonomer um ein Ribonukleosidphosphoramidit handelt.

5. Verfahren nach einem der Ansprüche 1 bis 3, wobei es sich bei dem Ribonukleotidoligomer um eines handelt, das mindestens ein Ribonukleotid enthält, das aus 2'-O-Halogenribonukleotid, 2'-Aminoribonukleotid, 2'-O-Alkylribonukleotid und 2'-O-Alkoxyribonukleotid ausgewählt ist.

6. Verfahren nach einem der Ansprüche 1 bis 3, wobei das Ribonukleotidoligomer über eine Phosphodiester-, Phosphoramidat-, Alkylphosphoramidat-, Alkylphosphonat-, Phosphorothioat-, Alkylphosphotriester- oder Alkylphosphonothioat-Verknüpfung verfügt.

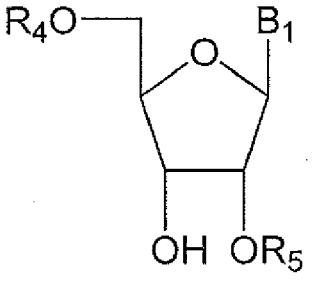
7. Verfahren nach Anspruch 6, wobei das Ribonukleotidoligomer über eine Phosphodiester- oder Phosphoramidat-Verknüpfung verfügt.

8. Verfahren nach Anspruch 1 oder 2, ferner umfassend den Schritt des Herstellens des Ribonukleotiddimers nach Schritt (a) durch eine Reaktion, welche das Kuppeln einer Verbindung der Formel 1 mit einer Verbindung der Formel 2 beinhaltet:



(1)

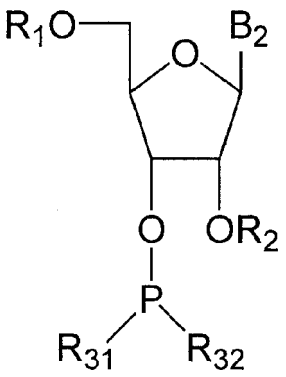
wobei R_i, R_2 und R_3 jeweils unabhängig für Schutzgruppen stehen und B_2 für eine nukleosidische Base steht;



(2)

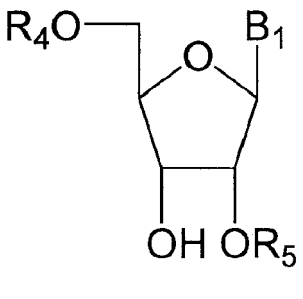
wobei R_4 und R_5 jeweils unabhängig für Schutzgruppen stehen und B_1 für eine nukleosidische Base steht.

9. Verfahren nach Anspruch 1 oder 2, ferner umfassend das Herstellen des Ribonukleotiddimers nach Schritt (a) durch eine Reaktion, welche das Kuppeln einer Verbindung der Formel 3 mit einer Verbindung der Formel 2 beinhaltet:



(3)

wobei R_1 und R_2 jeweils unabhängig für Schutzgruppen stehen, R_{31} für 2-Cyanoethoxy, Cyanomethoxy, 4-Cyano-2-butenyloxy oder Diphenylmethylsilylethoxy steht, R_{32} für Dialkylamino steht und B_2 für eine nukleosidische Base steht.

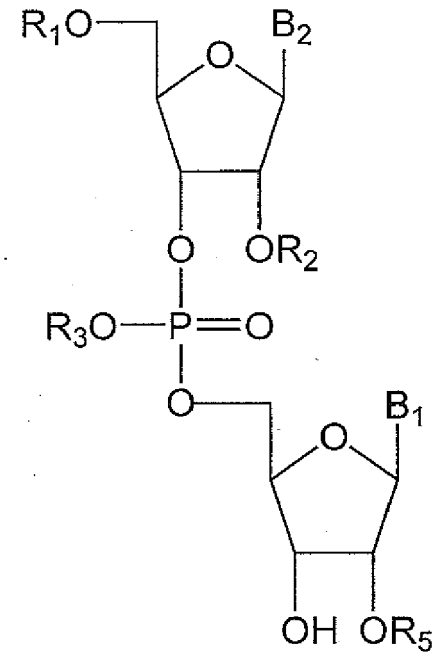


(2)

wobei R_4 und R_5 jeweils unabhängig für Schutzgruppen stehen und B_1 für eine nukleosidische Base steht.

10. Verfahren nach Anspruch 1 oder 3, ferner umfassend das Herstellen des Ribonukleotidtrimers nach Schritt (a) durch eine Reaktion, die Folgendes beinhaltet:

(a) Umsetzen eines Ribonukleotiddimers der Formel 4 mit einer Säure zum Entfernen von R_1 von Formel 4; und
(b) Kuppeln des resultierenden Produkts von Schritt (a) mit einem Ribonukleosid-3'-phosphoramidit zum Herstellen eines Ribonukleotidtrimers:



(4)

wobei R_1, R_2, R_3 und R_5 jeweils unabhängig für Schutzgruppen stehen und B_1 und B_2 jeweils unabhängig für nukleosidische Basen stehen.

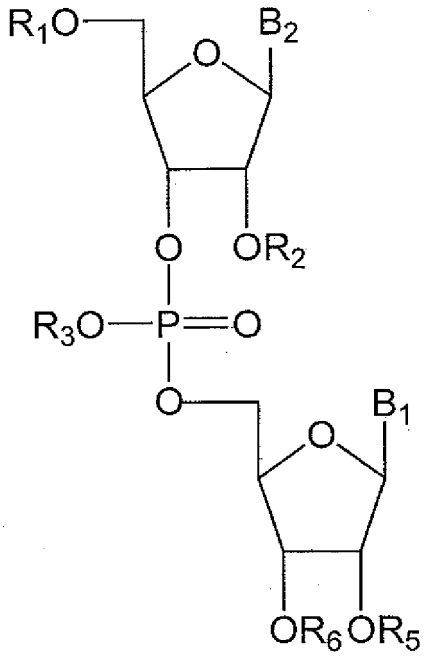
11. Verfahren nach Anspruch 10, wobei es sich bei der Säure von (a) um Benzolsulfonsäure handelt.

Revendications

1. Procédé de préparation d'oligomères ribonucléotidiques, comprenant :

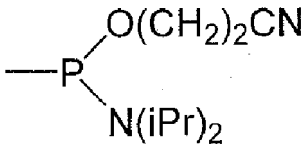
(a) le couplage d'un dimère ribonucléotidique ou d'un trimère ribonucléotidique à un ribonucléoside fixé à des supports solides ou à des supports solides universels en tant que matière première ;
(b) le couplage séquentiel des monomères ribonucléotidiques aux structures résultantes de l'étape (a) pour préparer des oligomères ribonucléotidiques ; et
(c) le retrait des oligomères ribonucléotidiques des supports solides ;

dans lequel le dimère ribonucléotidique de l'étape (a) est représenté par la formule 5 :

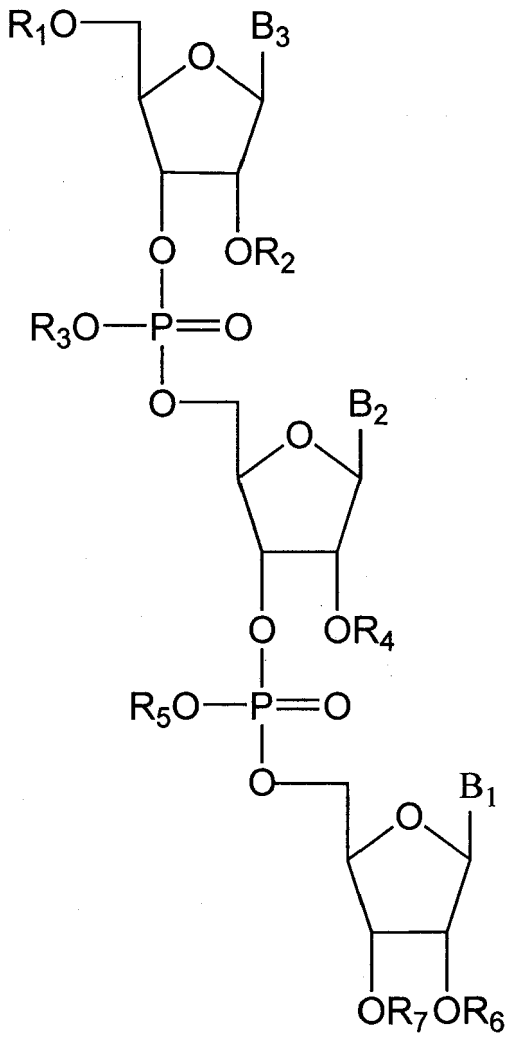


(5)

dans laquelle R_1, R_2, R_3 et R_5 sont chacun indépendamment des groupes de protection, B_1 et B_2 sont chacun indépendamment des bases nucléosidiques, et R_6 est un atome d'hydrogène ou

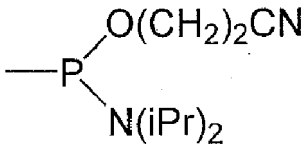


dans laquelle iPr est un groupe isopropyle ;
dans lequel le trimère ribonucléotidique de l'étape (a) est représenté par la formule 6 :



(6)

dans laquelle R_1, R_2, R_3, R_4, R_5 et R_6 sont chacun indépendamment des groupes de protection, B_1, B_2 et B_3 sont chacun indépendamment des bases nucléosidiques, et R_7 est un atome d'hydrogène ou



dans laquelle iPr est un groupe isopropyle.

2. Procédé selon la revendication 1, dans lequel le procédé comprend :

(a) le couplage d'un dimère ribonucléotidique selon la revendication 1 à un ribonucléoside fixé à des supports solides ou à des supports solides universels en tant que matière première ;
(b) le couplage séquentiel des monomères ribonucléotidiques aux structures résultantes de l'étape (a) pour préparer des oligomères ribonucléotidiques ; et
(c) le retrait des oligomères ribonucléotidiques des supports solides.

3. Procédé selon la revendication 1, dans lequel le procédé comprend :

(a) le couplage d'un trimère ribonucléotidique selon la revendication 1 à un ribonucléoside fixé à des supports

solides ou à des supports solides universels en tant que matière première ;
(b) le couplage séquentiel des monomères ribonucléotidiques aux structures résultantes de l'étape (a) pour préparer des oligomères ribonucléotidiques ; et
(c) le retrait des oligomères ribonucléotidiques des supports solides.

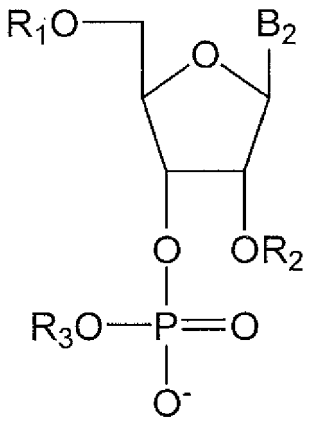
4. Procédé selon l'une quelconque des revendications 1 à 3, dans lequel le monomère ribonucléotidique est un phosphoramidite ribonucléosidique.

5. Procédé selon l'une quelconque des revendications 1 à 3, dans lequel l'oligomère ribonucléotidique est un oligomère contenant au moins un ribonucléotide sélectionné parmi un 2'-O-halogène ribonucléotide, un 2'-amino ribonucléotide, un 2'-O-alkyl ribonucléotide et un 2'-O-alcoxy ribonucléotide.

6. Procédé selon l'une quelconque des revendications 1 à 3, dans lequel l'oligomère ribonucléotidique a une liaison phosphodiester, phosphoramidate, alkylphosphoramidate, alkylphosphonate, phosphorothioate, alkylphosphotriester, ou alkylphosphonothioate.

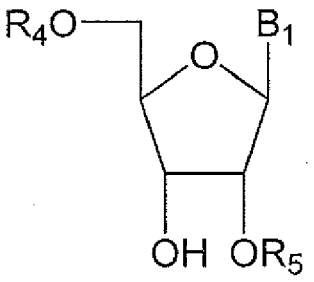
7. Procédé selon la revendication 6, dans lequel l'oligomère ribonucléotidique a une liaison phosphodiester ou phosphoramidate.

8. Procédé selon la revendication 1 ou 2, comprenant en outre l'étape de préparation d'un dimère ribonucléotidique de l'étape (a) par une réaction comprenant le couplage d'un composé de formule 1 avec un composé de formule 2 :



(1)

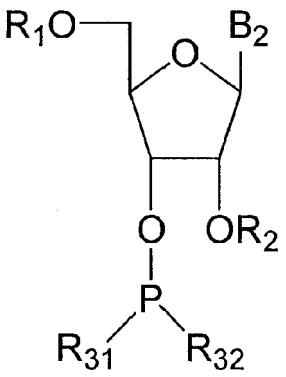
dans laquelle R_1, R_2 et R_3 sont chacun indépendamment des groupes de protection, et B_2 est une base nucléosidique ;



(2)

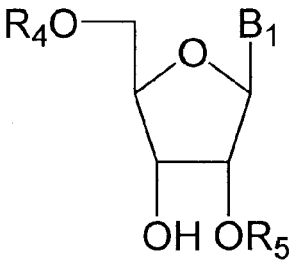
dans laquelle R_4 et R_5 sont chacun indépendamment des groupes de protection, et B_1 est une base nucléosidique.

9. Procédé selon la revendication 1 ou 2, comprenant en outre la préparation du dimère ribonucléotidique de l'étape (a) par une réaction comprenant le couplage d'un composé de formule 3 avec un composé de formule 2 :



(3)

dans laquelle R_1 et R_2 sont chacun indépendamment des groupes de protection, R_{31} est un groupe 2-cyanoéthoxy, cyanométhoxy, 4-cyano-2-butényloxy ou diphénylméthylsilyléthoxy, R_{32} est un groupe dialkylamino, et B_2 est une base nucléosidique ;

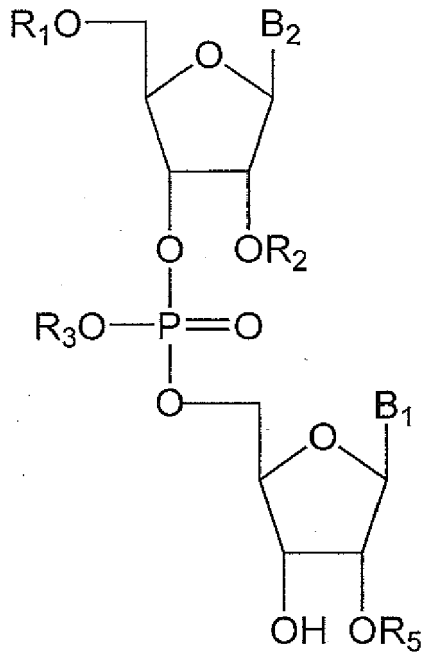


(2)

dans laquelle R_4 et R_5 sont chacun indépendamment des groupes de protection, et B_1 est une base nucléosidique.

10. Procédé selon la revendication 1 ou 3, comprenant en outre la préparation du trimère ribonucléotidique de l'étape (a) par une réaction comprenant :

(a) la réaction d'un dimère ribonucléotidique de formule 4 avec un acide pour éliminer R_1 de la formule 4 ; et
(b) le couplage du produit résultant de l'étape (a) avec un 3'-phosphoramidite ribonucléosidique pour préparer un trimère ribonucléotidique :



(4)

dans laquelle R_1, R_2, R_3 et R_5 sont chacun indépendamment des groupes de protection, et B_1 et B_2 sont chacun indépendamment des bases nucléosidiques.

11. Procédé selon la revendication 10, dans lequel l'acide de l'étape (a) est l'acide benzènesulfonique.

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This list of references cited by the applicant is for the reader's convenience only. It does not form part of the European patent document. Even though great care has been taken in compiling the references, errors or omissions cannot be excluded and the EPO disclaims all liability in this regard.

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