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(54) **3'-END NUCLEOSIDE UNIT COMPRISING PHOSPHORAMIDITE**

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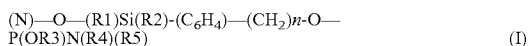
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(57) **ABSTRACT**

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The purpose of the present invention is therefore to provide a method for binding a 3'-end nucleoside unit comprising any base to a hydroxyl group on a solid-phase support under completely the same condition as in DNA chain elongation reaction. The present invention relates to a 3'-end nucleoside unit comprising phosphoramidite that is a compound represented by the following formula:

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wherein (N) represents any nucleoside or its derivative, each of R1, R2, R4 and R5 is an alkyl or aryl group, R3 is a phosphate-protecting group, and n is an integer of from 1 to 5; a solid-phase support having said 3'-end nucleoside unit; and a method for the synthesis of a nucleic acid oligomer with the use of said solid-phase support.

(30) **Foreign Application Priority Data**

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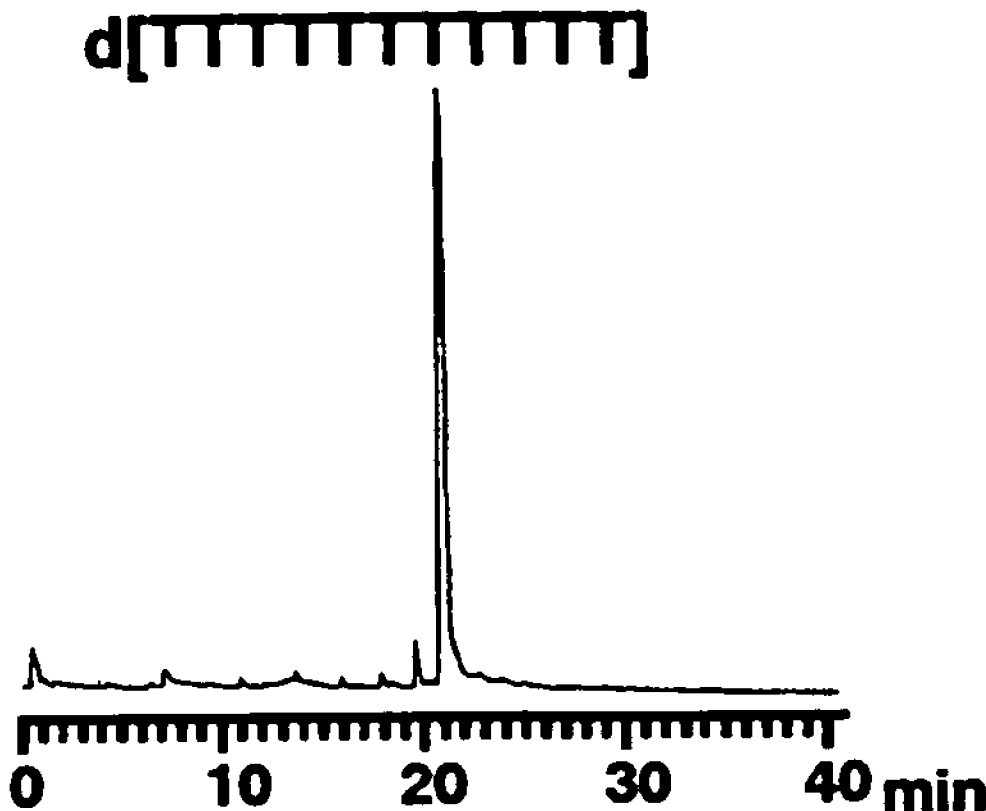
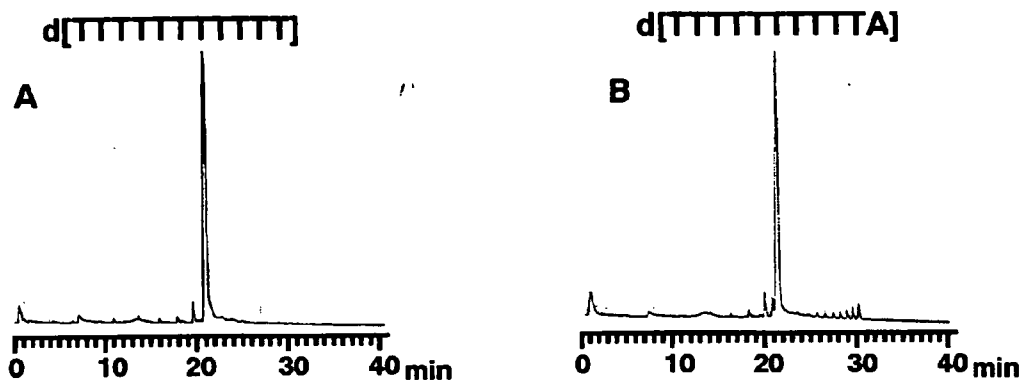


FIG. 1



### 3'-END NUCLEOSIDE UNIT COMPRISING PHOSPHORAMIDITE

#### FIELD OF THE INVENTION

[0001] The invention relates to a 3'-end nucleoside unit that is be advantageously used in a phosphoramidite method without protecting a base moiety, which was developed by the present inventors.

#### BACKGROUND ART

[0002] In conventional DNA synthesis methods, the introduction of a 3'-end nucleoside unit on a solid-phase support was done by the formation of amide bond with an amino group on the solid-phase support using a linker such as a succinate linker or silyl linkers for the 3'-end nucleoside.

[0003] For example, a benzoic acid-type compound:  $iP_2Si-C_6H_4-C(O)-$  type that was developed by one of the present inventors, SEKINE Mitsuo, is known as a silyl linker that can be cut out under a neutral condition (Non-Patent Document 1). However, since such silyl linker will be introduced into amino groups on the solid-phase support by acylation, the amino groups contained in dA, dC and dG have to be protected in advance with an appropriate protecting group such as DMTr.

[0004] Furthermore, as the DMTr protecting group in the base moiety of dC is relatively stable, treatment with 5% trifluoroacetic acid- $CH_2Cl_2$  solution for 30 min would be required to completely remove said protecting group. However, SiO bonds contained in the silyl linker and those formed between the silyl linker and a synthesized DNA oligomer would likely be cleaved under such a very acidic condition as in the above treatment.

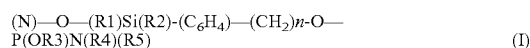
[0005] Non-Patent Document 1: Wada, T.; Mochizuki, A.; Sato, T.; Seike, M.; M., Tetrahedron Letters, 1998, 39, 5593-5596

#### SUMMARY OF THE INVENTION

##### Problems to be Solved by the Invention

[0006] The purpose of the present invention is therefore to provide a method for binding a 3'-end nucleoside unit comprising any base to a hydroxyl group on a solid-phase support under completely the same condition as in DNA chain elongation reaction. Thus, as the DNA chain elongation reaction can be carried out with almost 100% reaction efficiency, the present inventors have studied hard in order to enable the introduction reaction of the 3'-end nucleoside unit on the solid-phase support under the same condition. Finally, the present inventors have solved the above problems by introducing a silyl linker and a phosphoramidite group into the 3'-end nucleoside unit and have completed the present invention.

[0007] The present invention relates to a 3'-end nucleoside unit comprising phosphoramidite that is a compound represented by the following formula:



wherein (N) represents any nucleoside or its derivative, each of R1, R2, R4 and R5 is an alkyl or aryl group, R3 is a phosphate-protecting group, and n is an integer of from 1 to 5.

[0008] The present invention further relates to a solid-phase support having said 3'-end nucleoside unit, for example, at a ratio of 20-30  $\mu\text{mol/g}$ ; to a method for the synthesis of a nucleic acid oligomer with the use of said solid-phase support, especially, to a phosphoramidite method with the use of an activating agent comprising an alcohol-type compound, or a mixture of the alcohol-type compound and an acid catalyst.

#### Advantages of the Invention

[0009] The solid-phase supports having hydroxyl groups on their surfaces area are now available by using the 3'-end nucleoside unit comprising the phosphoramidite according to the present invention. DNA synthesized with the use of the above phosphoramidite unit would be hardly cut out even under a basic condition such as with ammonia in contrast to the conventional methods. Furthermore, if the phosphoramidite unit comprising the silyl linker according to the present invention is used in the phosphoramidite method without the protecting base moiety, which was developed by the present inventors, no protecting group for the base moiety of the nucleic acids will be necessary in a process of the introduction of the nucleoside on the solid-phase support.

#### BRIEF DESCRIPTION OF DRAWING

[0010] FIG. 1 shows a chart in an anion-exchange HPLC of DNA oligomer.

#### BEST MODE FOR CARRYING OUT THE INVENTION

[0011] The silyl group may have any substituents of R1 and R2 known for those skilled in the art, such as, for example, an alkyl group having 1 to 5 carbon atoms or an aryl group such as benzyl, phenyl and naphthyl group, which may have a substituent of the above alkyl, nitro, cyano, halogeno or alkoxy group at any position.

[0012] Any phosphate-protecting group known for those skilled in the art may be used, 2-cyanoethyl, 4-nitrophenylethyl, N-(trifluoroacetyl)aminobutyl, or 4-[N-methyl-N-(2,2,2-trifluoroacetyl)amino]butyl group being preferable.

[0013] R4 and R5 in the above formula are an alkyl having 1 to 4 carbon atoms, or aryl such as benzyl, phenyl and naphthyl group, an isopropyl group being preferable.

[0014] Furthermore, the benzene ring structure of the present compound may have any substituent known for those skilled in the art, which, for example, is selected from the group consisting of alkyl having 1 to 4 carbon atoms, halogeno, nitro, cyano and methoxy groups. The groups of "—CONH—" and "Si" are bound to the benzene ring in a para-position.

[0015] The compound of the present invention may be easily synthesized by those skilled in the art with reference to the following examples. Conditions that are not specifically described in the present specification may be optionally selected by those skilled in the art.

#### EXAMPLES

[0016] The present invention will be explained more in detail in line with the examples, which should not be construed to impose any limitations on the scope of the present invention.

## 4-diisopropylsilylbenzoic acid methyl ester (2)

[0017] 4-diisopropylsilylbenzoic acid (9 g, 38 mmol) was dissolved in methanol (300 mL), and conc. H<sub>2</sub>SO<sub>4</sub> (15 mL) was added dropwise to the solution cooled on ice. After being heated to reflux for 2 hours, the reaction solution was dissolved in chloroform (500 mL). The solution was then extracted two times with water (300 mL) and three times with 5 wt % aqueous solution (300 mL) of sodium hydrogen carbonate. An organic layer was collected and dehydrated with anhydrous sodium sulfate and filtered so that the resulting solvent was distilled out under a reduced pressure. The resulting crude product was then purified by silica gel column chromatography. After eluted with hexane having 0-5% ethyl acetate gradient, the solvent was distilled out to give a desired product (8.8 g, 93%). Its NMR data are as follows:

[0018] <sup>1</sup>H NMR (CDCl<sub>3</sub>): 0.93-1.06 (m, 12H), 1.18-1.27 (m, 2H), 3.90 (s, 3H), 3.96 (t, 1H, J=3.2 Hz), 7.58 (d, 2H, J=8.1 Hz), 7.98 (d, 2H, J=8.1 Hz).

[0019] <sup>13</sup>C NMR (CDCl<sub>3</sub>): 10.6, 18.5, 18.6, 52.2, 128.1, 128.2, 128.3, 130.5, 140.6, 167.1.

## 4-(hydroxymethyl)phenyl-diisopropylsilane (3)

[0020] LiAlH<sub>4</sub> (1.2 g, 32 mmol) was dissolved in anhydrous THF (80 mL), and to this was slowly added dropwise the anhydrous THF solution (80 mL) 4-diisopropylsilylbenzoic acid methyl ester (2) (8 g, 32 mmol). The resulting mixture was then stirred for 10 min and ethyl acetate (20 mL) was added slowly to it. The reaction mixture was diluted with dichloromethane (500 mL), and then extracted three times with 0.2 N hydrochloric acid aqueous solution (400 mL). An organic layer was collected and dehydrated with anhydrous sodium sulfate and filtered so that the resulting solvent was distilled out under a reduced pressure to give a desired product (7.2 g, quant). Its NMR data are as follows:

[0021] <sup>1</sup>H NMR (CDCl<sub>3</sub>): 1.02 (2d, 12H, J=7.3 Hz), 1.17-1.23 (m, 2H), 3.09 (brs, 1H), 3.94 (t, 1H, J=3.2 Hz), 4.58 (s, 2H), 7.29 (d, 2H, J=7.6 Hz), 7.48 (d, 2H, J=7.6 Hz).

[0022] <sup>13</sup>C NMR (CDCl<sub>3</sub>): 10.7, 18.4, 18.6, 64.8, 126.0, 132.9, 135.4, 141.6.

## 4-(acetoxymethyl)phenyl-diisopropylsilane (4)

[0023] Acetic anhydride (3.1 mL, 33 mmol) and 4-N,N-dimethylaminopyridine (7.3 mg, 6 mmol) were added under argon atmosphere to pyridine (100 mL) dissolving 4-(hydroxymethyl)phenyl-diisopropylsilane (3) (4.9 g, 22 mmol). The resulting mixture was then stirred for 2 hours at a room temperature and methanol (20 mL) was added to it. The reaction mixture was diluted with ethyl acetate (400 mL), and then extracted three times with saturated saline solution (300 mL). An organic layer was collected and dehydrated with anhydrous sodium sulfate and filtered so that the resulting solvent was distilled out under a reduced pressure to give a desired product (5.4 g, 93%). Its NMR data are as follows:

[0024] <sup>1</sup>H NMR (CDCl<sub>3</sub>): 1.03 (2d, 12H, J=7.0 Hz), 1.20-1.24 (m, 2H), 2.09 (s, 3H), 3.96 (t, 1H, J=3.1 Hz), 5.10 (s, 2H), 7.32 (d, 2H, J=8.1 Hz), 7.51 (d, 2H, J=8.1 Hz).

[0025] <sup>13</sup>C NMR (CDCl<sub>3</sub>): 10.7, 18.4, 18.6, 20.9, 66.1, 127.1, 134.0, 135.5, 136.5, 170.4.

## 5'-[O-(4,4'-dimethoxytrityl)], 3'-[O-4-(acetoxymethyl)phenyl-diisopropylsilyl]thymidine (5t)

[0026] 1,3-dichloro-4,4-dimethylhydantoin (761 mg, 3.9 mmol) was added to anhydrous CH<sub>2</sub>Cl<sub>2</sub> solution (10 mL) of 4-(acetoxymethyl)phenyl-diisopropylsilane (4) (508 mg, 1.9 mmol). The resulting mixture was then stirred for 30 min at a room temperature and added to anhydrous CH<sub>2</sub>Cl<sub>2</sub> solution (10 mL) dissolving 5'-O-(4,4'-dimethoxytrityl)thymidine (954 mg, 1.8 mmol) and imidazole (595 mg, 8.8 mmol). The reaction mixture was stirred for 30 min at a room temperature and mixed with water (5 mL). After 5 min, the reaction mixture was diluted with chloroform (100 mL) and extracted three times with 5 wt % aqueous solution (100 mL) of sodium hydrogen carbonate. An organic layer was collected and dehydrated with anhydrous sodium sulfate and filtered so that the resulting solvent was distilled out under a reduced pressure. The resulting crude product was then purified by silica gel column chromatography (1% pyridine). After eluted with hexane having 50-100% chloroform gradient and chloroform having 0-3% methanol gradient, the solvent was distilled out to give a desired product (1.1 g, 75%). Its NMR data are as follows:

[0027] <sup>1</sup>H NMR (CDCl<sub>3</sub>): 0.95-1.07 (m, 12H), 1.18-1.26 (m, 2H), 1.53 (s, 3H), 2.09 (s, 3H), 2.27-2.31 (m, 1H), 2.48-2.56 (m, 1H), 3.39 (d, 1H, J=8.1 Hz), 3.50 (d, 1H, J=8.6 Hz), 3.75 (s, 6H), 4.16 (d, 1H, J=2.4 Hz), 4.67 (d, 1H, J=5.7 Hz), 5.11 (s, 2H), 6.51 (t, 1H, J=4.1 Hz), 6.82 (dd, 4H, J=2.4 Hz, J=8.9 Hz), 7.18-7.67 (m, 14H), 10.3 (brs, 1H).

[0028] <sup>13</sup>C NMR (CDCl<sub>3</sub>): 11.7, 11.8, 11.9, 12.4, 16.8, 17.1, 17.16, 17.19, 17.21, 20.7, 41.6, 54.9, 63.1, 65.8, 73.1, 77.2, 84.7, 86.6, 86.8, 110.8, 112.9, 123.4, 124.9, 126.7, 126.9, 127.1, 127.6, 127.7, 127.8, 129.7, 133.3, 134.1, 134.4, 134.97, 135.01, 135.2, 135.7, 136.5, 143.9, 149.1, 150.3, 158.3, 163.9, 170.4.

[0029] MS m/z calcd for M+Na; 829.3496. Found; 829.3452.

## 5'-[O-(4,4'-dimethoxytrityl)], 3'-[O-4-(acetoxymethyl)phenyl-diisopropylsilyl], 2-deoxyadenosine (5a)

[0030] 4-(acetoxymethyl)phenyl-diisopropylsilane (4) (420 mg, 1.6 mmol) was dissolved in anhydrous CH<sub>2</sub>Cl<sub>2</sub> solution (8 mL), and 1,3-dichloro-4,4-dimethylhydantoin (629 mg, 3.2 mmol) was added to it. The resulting mixture was then stirred for 30 min at a room temperature and added to anhydrous CH<sub>2</sub>Cl<sub>2</sub> solution (8 mL) dissolving 5'-O-(4,4'-dimethoxytrityl)-2'-deoxyadenosine (796 mg, 1.4 mmol) and imidazole (489 mg, 7.2 mmol). The reaction mixture was stirred for 30 min at a room temperature and mixed with water (5 mL). After 5 min, the reaction mixture was diluted with chloroform (100 mL) and extracted three times with 5 wt % aqueous solution (100 mL) of sodium hydrogen carbonate. An organic layer was collected and dehydrated with anhydrous sodium sulfate and filtered so that the resulting solvent was distilled out under a reduced pressure. The resulting crude product was then purified by silica gel column chromatography (1% pyridine). After eluted with hexane having 50-100% chloroform gradient and chloroform having 0-3% methanol gradient, the solvent was distilled out to give a desired product (850 mg, 72%). Its NMR data are as follows:

[0031]  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ): 0.98-1.07 (m, 12H), 1.22-1.31 (m, 2H), 2.11 (s, 3H), 2.48-2.55 (m, 1H), 2.75-2.89 (m, 1H), 3.31 (d, 1H,  $J=4.6$  Hz), 3.38 (d, 1H,  $J=4.6$  Hz), 3.76 (s, 6H), 4.28 (d, 1H,  $J=2.4$  Hz), 4.67 (t, 1H,  $J=2.6$  Hz), 5.10 (s, 2H), 6.09 (s, 1H), 6.50 (dd, 1H,  $J=5.9$  Hz,  $J=7.3$  Hz), 6.76 (d, 4H,  $J=8.6$  Hz), 7.17-7.38 (m, 11H), 7.50 (d, 2H,  $J=7.3$  Hz), 7.99 (s, 1H), 8.28 (s, 1H).

[0032]  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ ): 12.1, 12.2, 17.4, 21.0, 40.9, 55.2, 63.5, 66.1, 73.5, 84.5, 86.4, 87.1, 112.9, 113.0, 119.9, 126.7, 127.2, 127.7, 128.0, 129.9, 133.7, 134.6, 135.48, 135.51, 137.0, 138.8, 144.3, 149.4, 152.6, 155.3, 158.3, 170.6

[0033] MS  $m/z$  calcd for  $\text{M}+\text{H}$ , 816.3793. Found; 816.3711.

5'-[O-(4,4'-dimethoxytrityl)], 3'-[O-4-(hydroxymethyl)phenyl-diisopropylsilyl]thymidine (6t)

[0034] 5'-[O-(4,4'-dimethoxytrityl)], 3'-[O-4-(acetoxymethyl)phenyl-diisopropylsilyl]thymidine (5t) (925 mg, 1.2 mmol) was treated with  $\text{tBuNH}_2\text{-MeOH}$  (1:4, v/v, 20 mL) for 3 hours at a room temperature. The reaction mixture was diluted with chloroform (100 mL), and then extracted three times with saturated saline solution (100 mL). An organic layer was collected and dehydrated with anhydrous sodium sulfate and filtered so that the resulting solvent was distilled out under a reduced pressure. The resulting crude product was then purified by silica gel column chromatography (1% pyridine). After eluted with hexane having 50-100% chloroform gradient and chloroform having 0-3% methanol gradient, the solvent was distilled out to give a desired product (781 mg, 89%). Its NMR data are as follows:

[0035]  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ): 0.92-1.00 (m, 12H), 1.17-1.25 (m, 2H), 1.56 (s, 3H), 2.15-2.38 (m, 1H), 2.53-2.68 (m, 1H), 3.31 (dd, 1H,  $J=2.7$  Hz,  $J=10.5$  Hz), 3.43 (dd, 1H,  $J=2.7$  Hz,  $J=10.5$  Hz), 3.77 (s, 6H), 4.12 (d, 1H,  $J=2.4$  Hz), 4.63 (t, 1H,  $J=2.7$  Hz), 4.67 (d, 1H,  $J=5.7$  Hz), 6.44 (dd, 1H,  $J=5.9$  Hz,  $J=7.3$  Hz), 6.77 (dd, 4H,  $J=2.4$  Hz,  $J=8.9$  Hz), 7.19-7.35 (m, 11H), 7.44 (d, 2H,  $J=7.8$  Hz), 7.61 (s, 1H), 8.15 (brs, 1H).

[0036]  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ ): 12.0, 17.4, 41.8, 55.2, 63.3, 64.9, 73.3, 84.8, 86.8, 87.1, 111.0, 113.1, 126.1, 126.9, 127.8, 129.8, 129.9, 132.4, 134.5, 135.0, 135.2, 135.5, 142.2, 144.1, 150.3, 158.4, 163.9.

[0037] MS  $m/z$  calcd for  $\text{M}+\text{H}$ , 787.3391. Found; 787.3413.

5'-[O-(4,4'-dimethoxytrityl)], 3'-[O-4-(hydroxymethyl)phenyl-diisopropylsilyl], 2-deoxyadenosine (6a)

[0038] 5'-[O-(4,4'-dimethoxytrityl)], 3'-[O-4-(acetoxymethyl)phenyl-diisopropylsilyl]2-deoxyadenosine (5a) (610 mg, 0.75 mmol) was treated with  $\text{tBuNH}_2\text{-MeOH}$  (1:4, v/v, 15 mL) for 3 hours at a room temperature. The reaction mixture was diluted with chloroform (100 mL), and then extracted three times with saturated saline solution (100 mL). An organic layer was collected and dehydrated with anhydrous sodium sulfate and filtered so that the resulting solvent was distilled out under a reduced pressure. The resulting crude product was then purified by silica gel column chromatography (1% pyridine). After eluted with hexane having 50-100% chloroform gradient and chloroform having 0-3% methanol gradient, the solvent was distilled out to give a desired product (530 mg, 92%). Its NMR data are as follows:

[0039]  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ): 0.93-1.03 (m, 12H), 1.20-1.29 (m, 2H), 2.48-2.55 (m, 1H), 2.75-2.89 (m, 1H), 3.22 (dd, 1H,  $J=4.1$  Hz,  $J=10.3$  Hz), 3.39 (dd, 1H,  $J=4.1$  Hz,  $J=10.3$  Hz), 3.73 (s, 6H), 4.21 (d, 1H,  $J=3.8$  Hz), 4.69 (s, 3H), 6.01 (s, 2H), 6.50 (t, 1H,  $J=6.2$  Hz), 6.74 (d, 4H,  $J=8.9$  Hz), 7.13-7.33 (m, 11H), 7.50 (d, 2H,  $J=8.1$  Hz), 7.81 (s, 1H), 8.26 (s, 1H).

[0040]  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ ): 12.2, 12.3, 17.46, 17.51, 17.55, 17.6, 40.8, 55.2, 63.2, 64.9, 73.0, 77.2, 84.2, 86.4, 86.7, 113.0, 119.8, 123.6, 126.3, 126.7, 127.7, 128.0, 128.1, 128.9, 129.87, 129.9, 132.6, 134.7, 135.5, 135.6, 135.8, 138.7, 142.5, 144.4, 149.5, 149.6, 152.8, 155.3, 158.3.

[0041] MS  $m/z$  calcd for  $\text{M}+\text{H}$ , 774.3687. Found; 774.3747.

5'-[O-(4,4'-dimethoxytrityl)], 3'-O-[O-4-(2-cyanoethyl  $\text{N,N}$ -diisopropylphosphoramidite) benzyl-diisopropylsilyl]thymidine (7t)

[0042] 5'-[O-(4,4'-dimethoxytrityl)], 3'-O-[4-O-(hydroxymethyl)phenyl-diisopropylsilyl]thymidine (6t) (770 mg, 1.0 mmol) was subjected to azeotropic distillation sequentially with pyridine, toluene and dichloromethane to be dehydrated and dissolved in anhydrous THF (10 mL). To the resulting solution was added diisopropylethylamine (242  $\mu\text{L}$ , 1.1 mmol) and (2-cyanoethyl) ( $\text{N,N}$ -diisopropylamino)chlorophosphine (242  $\mu\text{L}$ , 1.5 mmol). After being stirred for 30 min, the reaction solution was poured into water (20 mL) and diluted with chloroform (200 mL), and then extracted three times with saturated saline solution (200 mL). An organic layer was collected and dehydrated with anhydrous sodium sulfate and filtered so that the resulting solvent was distilled out under a reduced pressure. The resulting crude product was then purified by silica gel column chromatography (1% triethylamine). After eluted with hexane having 50-100% chloroform gradient and chloroform having 0-3% methanol gradient, the solvent was distilled out to give desired white solid (850 mg, 88%). Its NMR data are as follows:

[0043]  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ): 0.94-1.06 (m, 12H), 1.17-1.29 (m, 15H), 1.50 (s, 3H), 2.13-2.30 (m, 1H), 2.35-2.48 (m, 1H), 2.60 (t, 2H,  $J=6.3$  Hz), 3.27 (dd, 1H,  $J=2.7$  Hz,  $J=10.5$  Hz), 3.45 (dd, 1H,  $J=2.7$  Hz,  $J=10.5$  Hz), 3.61-3.87 (m, 10H), 4.14 (d, 1H,  $J=2.1$  Hz), 4.65-4.76 (m, 3H), 6.48 (dd, 1H,  $J=5.7$  Hz,  $J=7.8$  Hz), 6.80 (dd, 4H,  $J=2.4$  Hz,  $J=8.9$  Hz), 7.21-7.37 (m, 11H), 7.46 (d, 2H,  $J=7.6$  Hz), 7.63 (s, 1H), 9.45 (brs, 1H).

[0044]  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ ): 11.8, 11.9, 12.0, 12.4, 16.9, 17.1, 17.27, 17.32, 17.4, 20.3, 20.4, 22.8, 22.90, 22.94, 24.47, 24.55, 24.57, 24.7, 41.7, 43.0, 43.2, 45.2, 45.3, 55.1, 58.3, 58.5, 63.3, 65.0, 65.3, 67.8, 73.2, 77.2, 84.8, 86.7, 87.0, 110.9, 113.01, 113.04, 117.4, 126.0, 126.1, 126.8, 127.7, 127.8, 129.76, 129.80, 132.3, 134.0, 134.3, 135.0, 135.2, 135.4, 140.2, 140.3, 144.0, 150.2, 158.4, 163.8.

[0045]  $^{31}\text{P}$  NMR ( $\text{CDCl}_3$ ): 149.3

5'-[O-(4,4'-dimethoxytrityl)], 3'-O-[4-O-(2-cyanoethyl  $\text{N,N}$ -diisopropylphosphoramidite)benzyl-diisopropylsilyl]2'-deoxyadenosine (7a)

[0046] 5'-[O-(4,4'-dimethoxytrityl)], 3'-[O-4-(hydroxymethyl)phenyl-diisopropylsilyl]2'-deoxyadenosine (6a) (450

mg, 0.58 mmol) was subjected to azeotropic distillation sequentially with pyridine, toluene and dichloromethane to be dehydrated and dissolved in anhydrous THF (6 mL). To the resulting solution was added diisopropylethylamine (144  $\mu$ L, 0.64 mmol). The resulting solution was cooled to  $-78^{\circ}$  C., mixed with (2-cyanoethyl) (N,N-diisopropylamino)chlorophosphine (141  $\mu$ L, 0.87 mmol) and then gradually brought back to a room temperature. After being stirred for 30 min, the reaction solution was poured into water (20 mL) and diluted with chloroform (200 mL), and then extracted three times with saturated saline solution (200 mL). An organic layer was collected and dehydrated with anhydrous sodium sulfate and filtered so that the resulting solvent was distilled out under a reduced pressure. The resulting crude product was then purified by silica gel column chromatography (1% triethylamine). After eluted with hexane having 50-100% chloroform gradient and chloroform having 0-3%

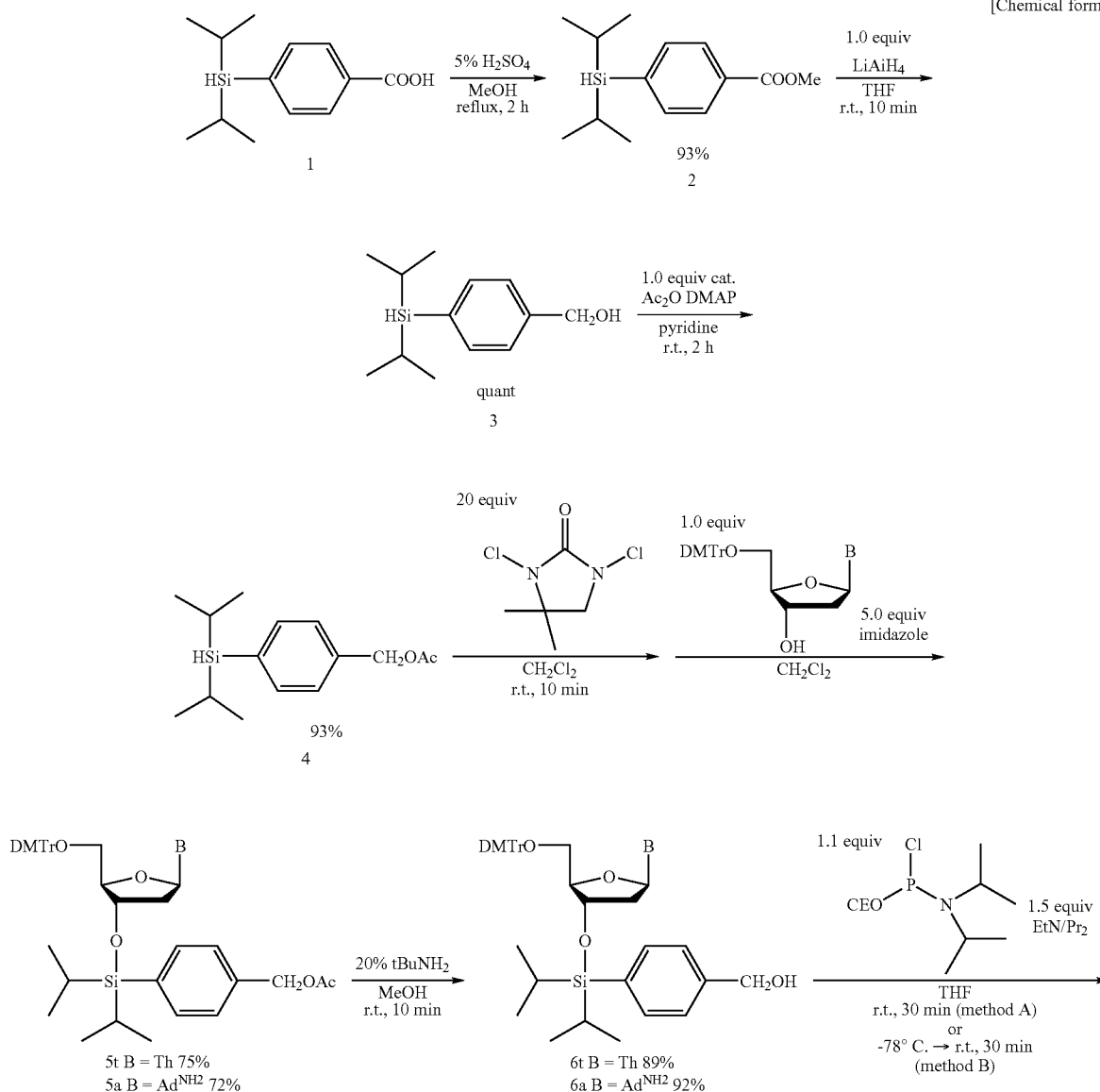
methanol gradient, the solvent was distilled out to give desired white solid (500 mg, 87%). Its NMR data are as follows:

**[0047]**  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ): 0.98-1.05 (m, 12H), 1.16-1.29 (m, 15H), 2.48-2.69 (m, 3H), 2.72-2.87 (m, 1H), 3.31 (dd, 1H,  $J=4.1$  Hz,  $J=10.3$  Hz), 3.39 (dd, 1H,  $J=4.1$  Hz,  $J=10.3$  Hz), 3.60-3.86 (m, 10H), 4.28 (d, 1H,  $J=2.4$  Hz), 4.67-4.78 (m, 3H), 6.06 (s, 2H), 6.51 (t, 1H,  $J=6.4$  Hz), 6.77 (d, 4H,  $J=8.6$  Hz), 7.18-7.38 (m, 11H), 7.49 (d, 2H,  $J=7.0$  Hz), 7.98 (s, 1H), 8.28 (s, 1H).

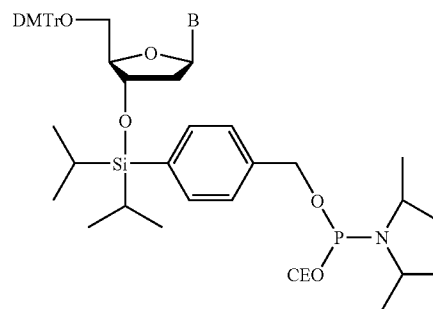
**[0048]**  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ ): 12.2, 12.3, 17.46, 17.51, 17.55, 17.6, 40.8, 55.2, 63.2, 64.9, 73.0, 77.2, 84.2, 86.4, 86.7, 113.0, 119.8, 123.6, 126.3, 126.7, 127.7, 128.0, 128.1, 128.9, 129.87, 129.9, 132.6, 134.7, 135.5, 135.6, 135.8, 138.7, 142.5, 144.4, 149.5, 149.6, 152.8, 155.3, 158.3.

**[0049]**  $^{31}\text{P}$  NMR ( $\text{CDCl}_3$ ): 149.3.

[Chemical formula 1]



-continued



7t B = Th 88% (method A)  
7a B = Ad<sup>NH2</sup> 87% (method B)

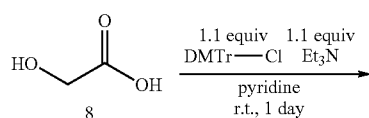
#### Triethylammonium, O-(4,4'-dimethoxytrityl)acetic acid (9)

[0050] 4,4'-dimethoxytrityl chloride was added to pyridine solution (100 mL) dissolving hydroxyacetic acid (760 mg, 10 mmol) and triethylamine (1.45 mL, 11 mmol). Stirring for 24 hours at a room temperature gave 20 mL of ethanol, which was diluted with chloroform (500 mL) and extracted three times with 0.5 M triethylammonium carbonate buffer (300 mL). An organic layer was collected and dehydrated with anhydrous sodium sulfate and filtered so that the resulting solvent was distilled out under a reduced pressure. The resulting crude product was then purified by silica gel column chromatography. After eluted with chloroform having 0-3% methanol gradient, the solvent was distilled out to give a desired product (3.5 g, 73%). Its NMR data are as follows:

[0051] <sup>1</sup>H NMR (CDCl<sub>3</sub>): 1.15 (t, 9H, J=7.3 Hz), 2.97 (dd, 6H, J=7.0 Hz, J=14.9 Hz), 3.55 (s, 2H), 3.64 (s, 6H), 6.77 (dd, 4H, J=2.4 Hz, J=7.0 Hz), 7.06-7.17 (m, 3H), 7.39 (dd, 4H, J=2.0 Hz, J=7.4 Hz), 7.43 (d, 2H, J=1.4 Hz).

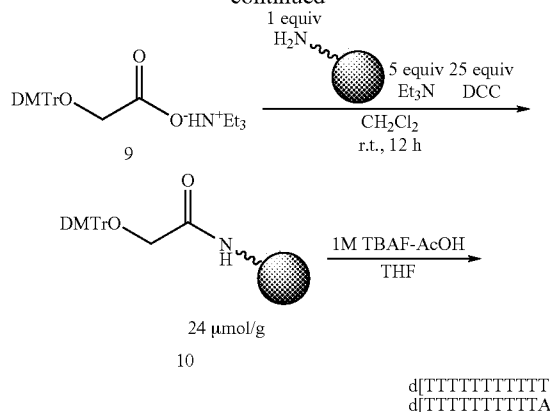
#### Preparation of a Solid-Phase Support (10)

[0052] Solid-phase support (highly cross-linked polystyrene: HCP) sufficiently dried (500 mg, 17 μmol), triethylammonium, O-(4,4'-dimethoxytrityl)acetic acid 3-18 (260 μmol) and DCC (268 mg 1.3 mmol) were dissolved into dichloromethane (5 mL) and stirred for 12 hours at a room temperature. After the completion of the reaction, the solid-phase support was filtered, washed with acetonitrile, dried and added to pyridine solution (4.5 mL) of acetic anhydride (0.5 ml) and DMAP (5 mg). After being stirred for 3 hours, the solid-phase support was filtered again and washed with acetonitrile. The introduction ratio of the compound was measured by colorimetric determination of the trityl group (24 μmol/g).



[Chemical formula 2]

-continued



#### DNA Synthesis with the Use of the Silyl Linker

[0053] The synthesis of d[TTTTTTTTTTT] and d[TTTTTTTTTTTA] was carried out with the use of the HCP solid-phase support (1 μmol, 24 μmol/g) and the phosphoramidite unit (7t) or (7a) comprising the silyl linker, or thymidine 3' phosphoramidite unit by means of DNA/RNA Synthesizer 392 (Applied Biosystem Inc.: ABI). Each elongation cycle of the oligomer was shown in TABLE 1 below.

TABLE 1

step	operation	Reagent(s)	time, (min)
1	washing	CH <sub>3</sub> CN	0.2
2	detritylation	3% Cl <sub>3</sub> CCOOH/CH <sub>2</sub> Cl <sub>2</sub>	1.5
3	washing	CH <sub>3</sub> CN	0.4
4	coupling	0.1M amidite + 0.2M HO <sup>t</sup> Bt in CH <sub>3</sub> CN—NMP (15:1, v/v)	1.0
5	washing	CH <sub>3</sub> CN	0.2
6	coupling	0.1M amidite + 0.2M HO <sup>t</sup> Bt in CH <sub>3</sub> CN—NMP (15:1, v/v)	1.0
7	washing	CH <sub>3</sub> CN	0.2
8	oxidation	0.1M I <sub>2</sub> in Py-H <sub>2</sub> O-THF (20:2:78, v/v/v)	0.5
9	washing	CH <sub>3</sub> CN	0.4

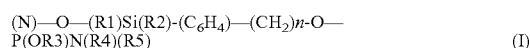
[0054] The DMTr group was then removed by the treatment with 3% trichloroacetic acid in CH<sub>2</sub>Cl<sub>2</sub> (2 mL) for one minute, and the solid-phase support was washed with

CH<sub>2</sub>Cl<sub>2</sub> (1 mL×3) and CH<sub>3</sub>CN (1 mL×3). The cyanoethyl group was then removed by the treatment with 10% DBU in CH<sub>3</sub>CN (500 μL). After being washed with CH<sub>3</sub>CN (1 mL×3), the solid-phase support was treated with anhydrous THF solution (500 μL) dissolving TBAF (131 mg, 0.5 mmol) and acetic acid (24 μL, 0.5 mmol) for one hour in order to cut out the DNA oligomer. The resulting mixture solution was desalted with Sep-Pak C18 cartridge to give a desired product.

#### INDUSTRIAL APPLICABILITY

[0055] Various solid-phase material may be selected by using the 3'-end nucleoside unit comprising phosphoramidite according to the present invention, making it possible to synthesize a high through-put DNA chip wherein the solid-phase may be directly used as the chip.

1. 3'-end nucleoside unit comprising phosphoramidite that is a compound represented by the following formula:



wherein (N) represents any nucleoside or its derivative, each of R1, R2, R4 and R5 is an alkyl or aryl group, R3 is a phosphate-protecting group, and n is an integer of from 1 to 5.

2. The compound according to claim 1 wherein R1 and R2 are an alkyl group having 1 to 5 carbon atoms.

3. The compound according to claim 1 wherein the aryl group of R1 and R2 has a substituent of alkyl, nitro, cyano, halogeno or methoxy group.

4. The compound according to any one of claims 1 to 3 wherein the phosphate-protecting group is 2-cyanoethyl, 4-nitrophenylethyl, N-(trifluoroacetyl)aminobutyl, or 4-[N-methyl-N-(2,2,2-trifluoroacetyl)amino]butyl group.

5. The compound according to claim 4 wherein the phosphate-protecting group is 2-cyanoethyl.

6. The compound according to claim 1 wherein R4 and R5 are an alkyl having 1 to 4 carbon atoms, benzyl, phenyl, or naphthyl group.

7. The compound according to claim 6 wherein R4 and R5 are an isopropyl group.

8. The compound according to claim 1 wherein a benzene ring structure has a substituent.

9. The compound according to claim 8 wherein the substituent of the benzene ring structure is selected from the group consisting of alkyl having 1 to 4 carbon atoms, halogeno, nitro, cyano and methoxy groups.

10. The compound according to claim 1, which is 5'-[O-(4,4'-dimethoxytrityl)], 3'-O-[4-O-(2-cyanoethyl N,N-diisopropyl phosphoramidite) benzyl-diisopropylsilyl]thymidine.

11. The compound according to claim 1, which is 5'-[O-(4,4'-dimethoxytrityl)], 3'-O-[4-O-(2-cyanoethyl N,N-diisopropyl phosphoramidite) benzyl-diisopropylsilyl]2'-deoxyadenosine.

12. A solid-phase support having the 3'-end nucleoside unit according to claim 1 introduced thereon.

13. The solid-phase support according to claim 12 having the 3'-end nucleoside unit at a ratio of 20-30 μmol/g.

14. The solid-phase support according to claim 12, which is HCP solid-phase support.

15. A method for the synthesis of a nucleic acid oligomer with the use of the solid-phase support according to claim 12.

16. The method according to claim 15, which is a phosphoramidite one with the use of an activating agent comprising an alcohol-type compound, or a mixture of the alcohol-type compound and an acid catalyst.

\* \* \* \* \*