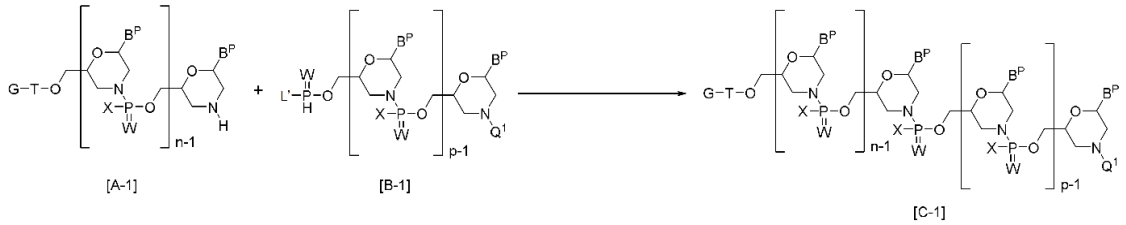
One embodiment of the invention is a method for obtaining a compound of the formula [C-1] by reacting a compound of the formula [A-1] with a compound of the formula [B-1]:



[A-1] [B-1] [C-1]

wherein

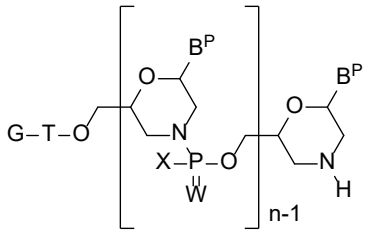
B^P, Q^1, W, X, G, T, n, L' and p are as defined above, and the method may comprise

Step (1): condensation; and

Step (2) oxidative amination.

[0026]

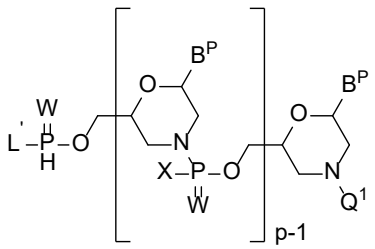
One embodiment of the invention is a method for obtaining a compound of the formula [C-1] comprising that a compound of the formula [A-1]:



[A-1]

wherein

B^P, W, X, G, T~~, n~~ and ~~p~~ n are as defined above, and a compound of the formula [B-1]:



[B-1]

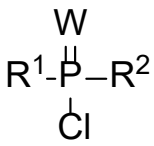
wherein

B^P, Q^1, W, X, L' and p are as defined above,

((benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate), PyClop (chlorotripyrrolidinophosphonium hexafluorophosphate), PyAOP ((7-azabenzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate), PyNTP (3-nitro-1,2,4-triazol-1-yl-tris(pyrrolidin-1-yl)phosphonium hexafluorophosphate), HATU (1-[bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxide hexafluorophosphate), COMU ((1-cyano-2-ethoxy-2-oxoethylideneaminooxy)dimethylamino-morpholino-carbenium hexafluorophosphate), HBTU (1-[bis(dimethylamino)methylene]-1H-benzotriazolium 3-oxide hexafluorophosphate), BOI (2-(benzotriazol-1-yl)oxy-1,3-dimethylimidazolidinium hexafluorophosphate), and DMINTP (1-(1',3'-dimethyl-1H-imidazol-2'-yl)-3-nitro-1,2,4-triazole hexafluorophosphate), preferably diphenyl chlorophosphate, bis(2,6-dimethylphenyl)chlorophosphate, PyBrop and BOI, and more preferably diphenyl chlorophosphate, bis(2,6-dimethylphenyl)chlorophosphate and PyBrop.

[0030]

In one embodiment of the invention, the Phosphorus Reagent 1 is a compound of the formula [P-2]:



wherein

W is an oxygen atom or a sulfur atom; and

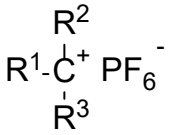
R^1 and R^2 are each the same or different and are each selected from the group consisting of an aromatic ring-O- optionally substituted and a heterocyclic ring optionally substituted, preferably phenoxy~~l~~, 2,6-dimethylphenoxy~~l~~ and 2-oxo-oxazolidide.

The reagent is preferably one selected from the group consisting of diphenyl chlorophosphate, bis(2,6-dimethylphenyl)chlorophosphate and bis(2-oxo-3-

preferably PyBrop.

[0032]

In one embodiment of the invention, the onium reagent is a compound of the formula [O]:



wherein

R^1, R^2, and R^3 ~~and R^4~~ are each the same or different and are each selected from the group consisting of halogen, C_{1-6} alkylamino, di(C_{1-6} alkyl)amino, cyclic C_{1-6} amino optionally substituted, aromatic amino optionally substituted, aromatic ring optionally substituted, hetero ring-O- optionally substituted and C_{1-6} arkylidene aminooxy optionally substituted; or

R^1 and R^2 are taken together with the carbon atom to which they are attached to form a ring structure, and R^3 is selected from the group consisting of halogen, C_{1-6} alkylamino, di(C_{1-6} alkyl)amino, cyclic C_{1-6} amino optionally substituted, aromatic amino optionally substituted, aromatic ring optionally substituted, and hetero ring-O- optionally substituted,

preferably, R^1 and R^2 are each the same or different and are each selected from the group consisting of di(C_{1-6} alkyl)amino and cyclic C_{1-6} amino optionally substituted, and R^3 is hetero ring-O- optionally substituted or C_{1-6} arkylidene aminooxy optionally substituted; or R^1 and R^2 are taken together with the carbon atom to which they are attached to form a ring structure, and R^3 is aromatic amino optionally substituted, and

more preferably, R^1 and R^2 are dimethylamino, and R^3 is O-(7-azabenzotriazol-1-yl) or O-(benzotriazol-1-yl); or R^1 is dimethylamino, R^2 is morpholino, and R^3 is 1-cyano-2-ethoxy-2-oxoethylidenaminooxy.

In one embodiment of the invention, the oxidizing agent is not limited so long as it can oxidize a phosphorus atom, and examples include iodine and carbon tetrachloride, preferably a solution of iodine in dichloromethane, a solution of iodine in chloroform, a solution of iodine in tetrahydrofuran, and more preferably a solution of iodine in tetrahydrofuran.

[0042]

<Amount of the oxidizing agent in Step (2)>

In one embodiment of the invention, the oxidizing ~~condensation~~ agent may be used in an amount, for example, within the range of 0.5 to 100 molar equivalent, preferably 1 to 50 molar equivalent, and more preferably 2 to 10 molar equivalent, with respect to the compound of the formula [B-1].

[0043]

<Organic amine in Step (2)>

In one embodiment of the invention, the organic amine is not limited and may be at least one selected from the group consisting of primary amines, secondary amines, tertiary amines, cyclic amines and aromatic amines, and examples include dimethylamine, diisopropylamine, piperidine, pyrrolidine, N-methylpiperazine and morpholine, preferably dimethylamine, diisopropylamine, piperidine or pyrrolidine, and more preferably dimethylamine .

[0044]

<Amount of the organic amine in Step (2)>

In one embodiment of the invention, the organic amine may be used in an amount, for example, within the range of 1 to 200 molar equivalent, preferably 2 to 100 molar equivalent, and more preferably 2 to 50 molar equivalent, with respect to the compound of the formula [B-1].

[0045]

<Reaction Solvent in Step (2)>

In one embodiment of the invention, the solvent used in Step (2) is not limited and may be at least one selected

from the group consisting of dichloromethane, chloroform, 1,2-dichloroethane, tetrahydrofuran, toluene, acetonitrile and ethyl acetate.

[0046]

<Reaction Temperature in Step (2)>

In one embodiment of the invention, the reaction temperature in Step (2) is not limited and may be -10 to 80°C, preferably 0 to 60°C, and more preferably 0 to 40°C.

[0047]

<Reaction Time in Step (2)>

In one embodiment of the invention, the reaction time in Step (~~1~~2) is not limited and may be 1 minute to 4 hours, preferably 2 minutes to 2 hours, and more preferably 2 minutes to 1 hours.

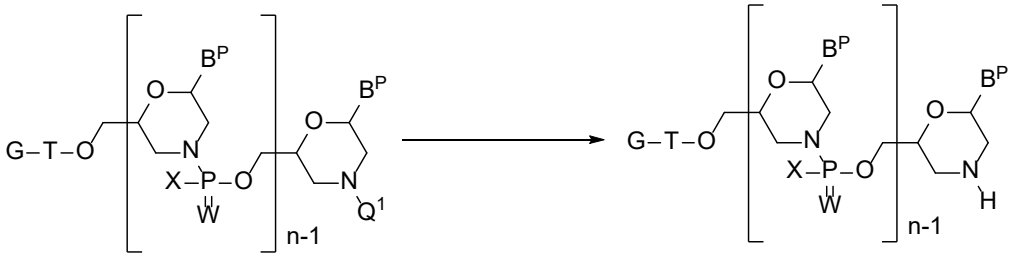
[0048]

<Compound of formula [A-1]>

[0049]

<Synthesis of compound of formula [A-1]>

One embodiment of the invention comprises a process to obtain a compound of the formula [A-1] from a compound of the formula [A-1-0]:



[A-1-0] [A-1]

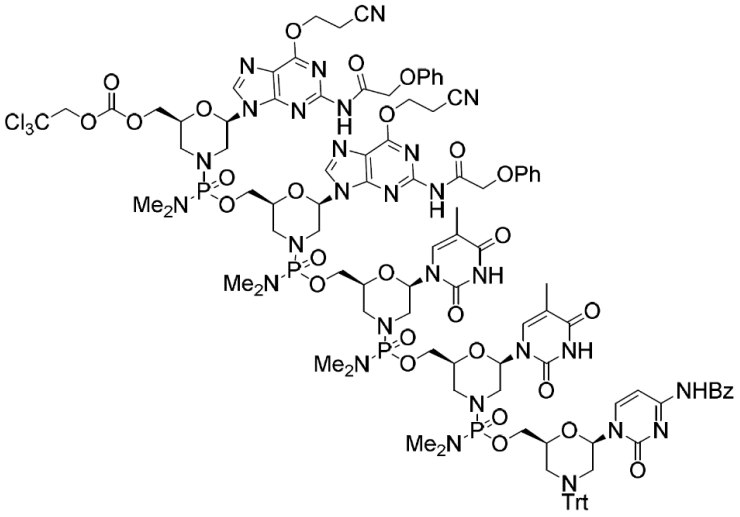
wherein

B^P, Q^1, W, X, G~~,~~ and T ~~and n~~ are as defined above, and

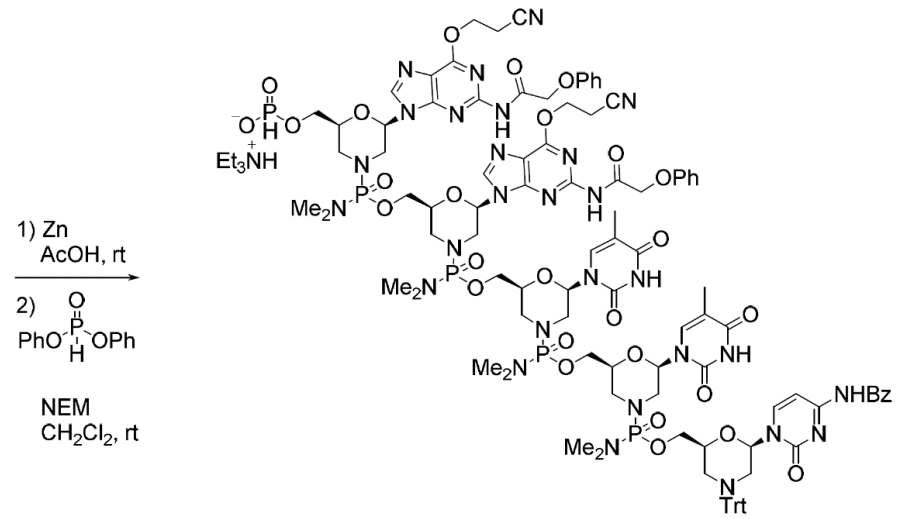
n is an integer from 1 to 50, preferably from 1 to 30, more preferably from 1 to 25, and most preferably from 1 to 15.

[0050]

The compound of the formula [A-1-0] can be prepared using

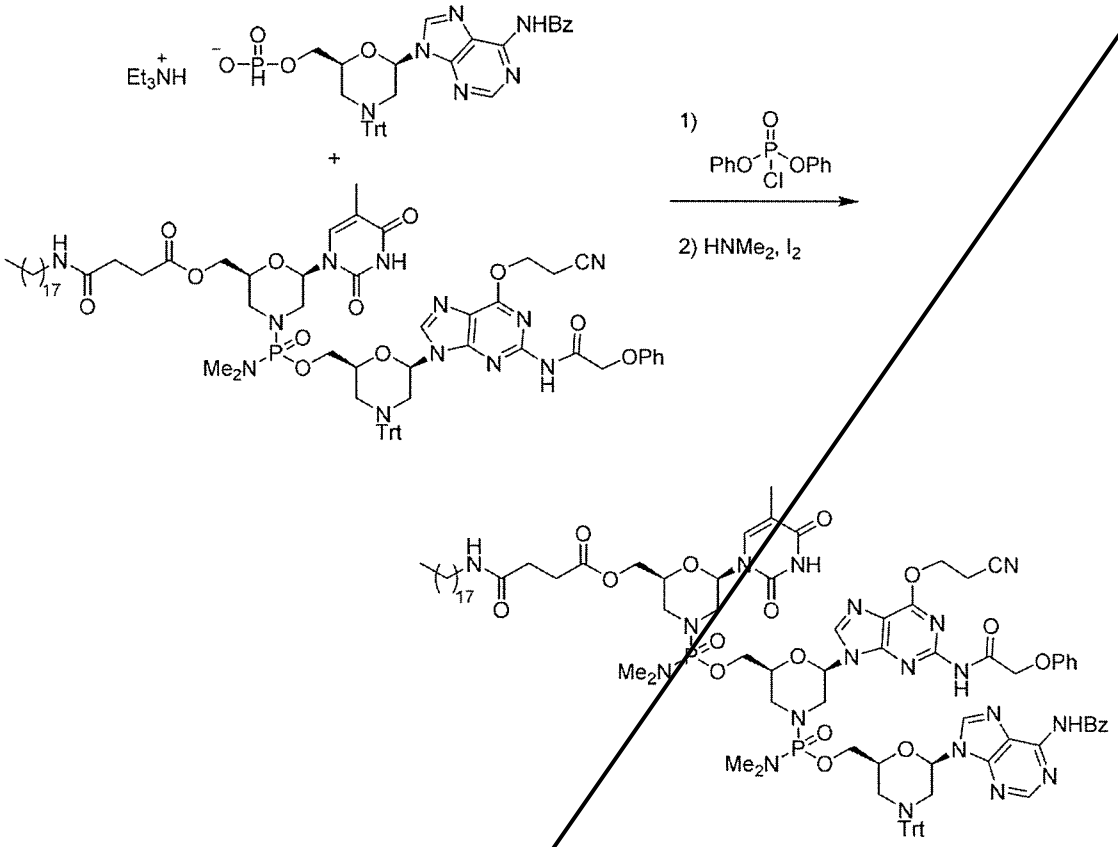


TrocO-PMO[$G^{CE,Pac}$-$G^{CE,Pac}$-T-T-C^{Bz}]-ON



TrocO-PMO[$G^{CE,Pac}$-$G^{CE,Pac}$-T-T-C^{Bz}]-ON (1 g, 0.40 mmol) was dissolved in 6.0 mL of tetrahydrofuran. To this solution, 230 µL (4.01 mmol) of acetic acid and 262 mg (4.01 mmol) of zinc powder were added and stirred at room temperature. After 1 hour, 115 µL (2.01 mmol) of acetic acid and 131 mg (~~4~~2.01 mmol) of zinc powder were added and stirred for 1 hour. After 1 hour, the reaction solution was filtered through Celite, and the solvent was removed from the filtrate. The resulting residue was purified by reversed-phase purification to afford HO-PMO[$G^{CE,Pac}$-$G^{CE,Pac}$-T-T-C^{Bz}]-ON (591.16 mg, 64%).

HO-PMO[$G^{CE,Pac}$-$G^{CE,Pac}$-T-T-C^{Bz}]-ON (441 mg, 0.19 mmol) and



G1-suc-PMO[T-G^{CE,Pac}]-OFF (17 mg, 0.0123 mmol) and H-phosphonate-PMO[A^{Bz}]-ON (triethylamine salt) (14 mg, 0.0185 mmol) and 20 μL (0.247 mmol) of N-methylimidazole were dissolved in 250 μL of dichloromethane. To this solution was added 25 μL (0.123 mmol) of diphenyl chlorophosphate, and the mixture was stirred at 40°C. After 10 minutes, 100 μL of the solution was mixed with 100 μL of an oxidizing solution (0.1 M iodine, 2 M dimethylamine/THF solution) and stirred for 10 minutes at room temperature. After completion of the reaction, a solution of 10% sodium thiosulfate in water was added to the reaction mixture, and the organic and aqueous layers were separated. The organic layer was diluted 20-fold with a solution of 80% acetonitrile in water and analyzed by HPLC (starting material Rt: 12.9 min, object product Rt: 18.4, 19.1 min, conversion yield 89.5%, ODS Condition 2). ESI-MS (+): 1819.9 (LC/MS condition 2)

The oligomers listed below were produced in a similar manner.

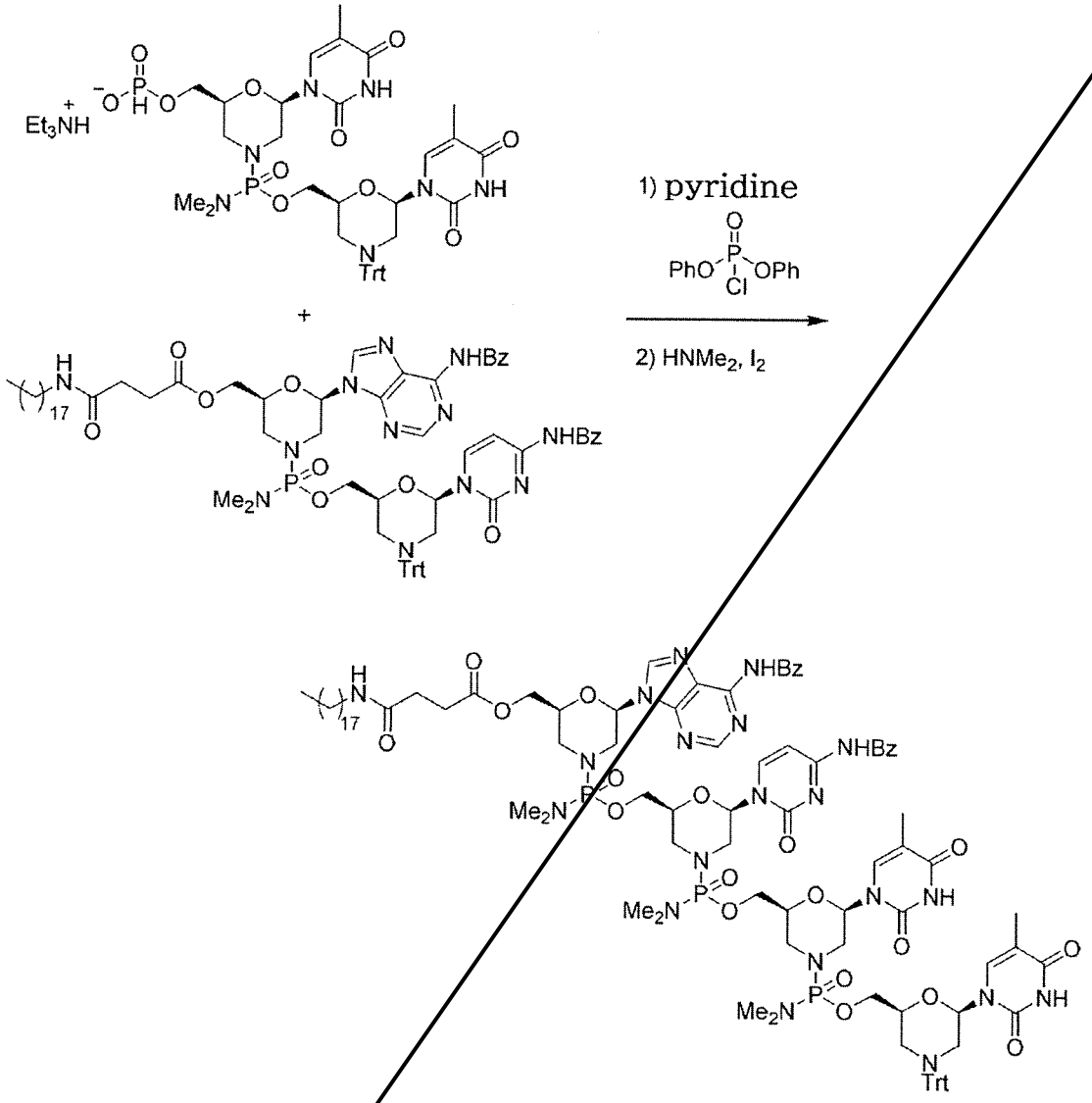
[Table 5]

G1-suc-PMO-OFF	H-Phosphonate	Activating Agent	Base	Conversion Yield (%)
T	A^{Bz}	diphenyl chlorophosphate	N-methyl imidazole	98.6
T	C^{Bz}	diphenyl chlorophosphate	pyridine	96.3
A^{Bz}	A	diphenyl chlorophosphate	pyridine	97.3
A^{Bz}	C^{Bz}	diphenyl chlorophosphate	pyridine	86.9
C^{Bz}	C^{Bz}	diphenyl chlorophosphate	pyridine	93.6
C^{Bz}	T	diphenyl chlorophosphate	pyridine	96.3
T-T	C^{Bz}	diphenyl chlorophosphate	pyridine	83.9
$A^{Bz}-C^{Bz}$	A^{Bz}	diphenyl chlorophosphate	pyridine	96.0
$A^{Bz}-C^{Bz}$	C^{Bz}	diphenyl chlorophosphate	pyridine	95.5
$A^{Bz}-G^{CE,Pac}$	C^{Bz}	diphenyl chlorophosphate	pyridine	81.3
$T-T-A^{Bz}$	A^{Bz}	diphenyl chlorophosphate	pyridine	95.1
$T-T-A^{Bz}$	C^{Bz}	diphenyl chlorophosphate	pyridine	98.0
$C^{Bz}-C^{Bz}-T-C^{Bz}-C^{Bz}$	T	bromotripyrolidino phosphonium= hexafluorophosphate	N-methyl imidazole	95.9
$T-G^{CE,Pac}-G^{CE,Pac}-G^{CE,Pac}-A^{Bz}T-C^{Bz}-C^{Bz}-A^{Bz}-G^{CE,Pac}$	A^{Bz}	diphenyl chlorophosphate	N-methyl imidazole	88.5

Example 4: Preparation of PMO Oligomer

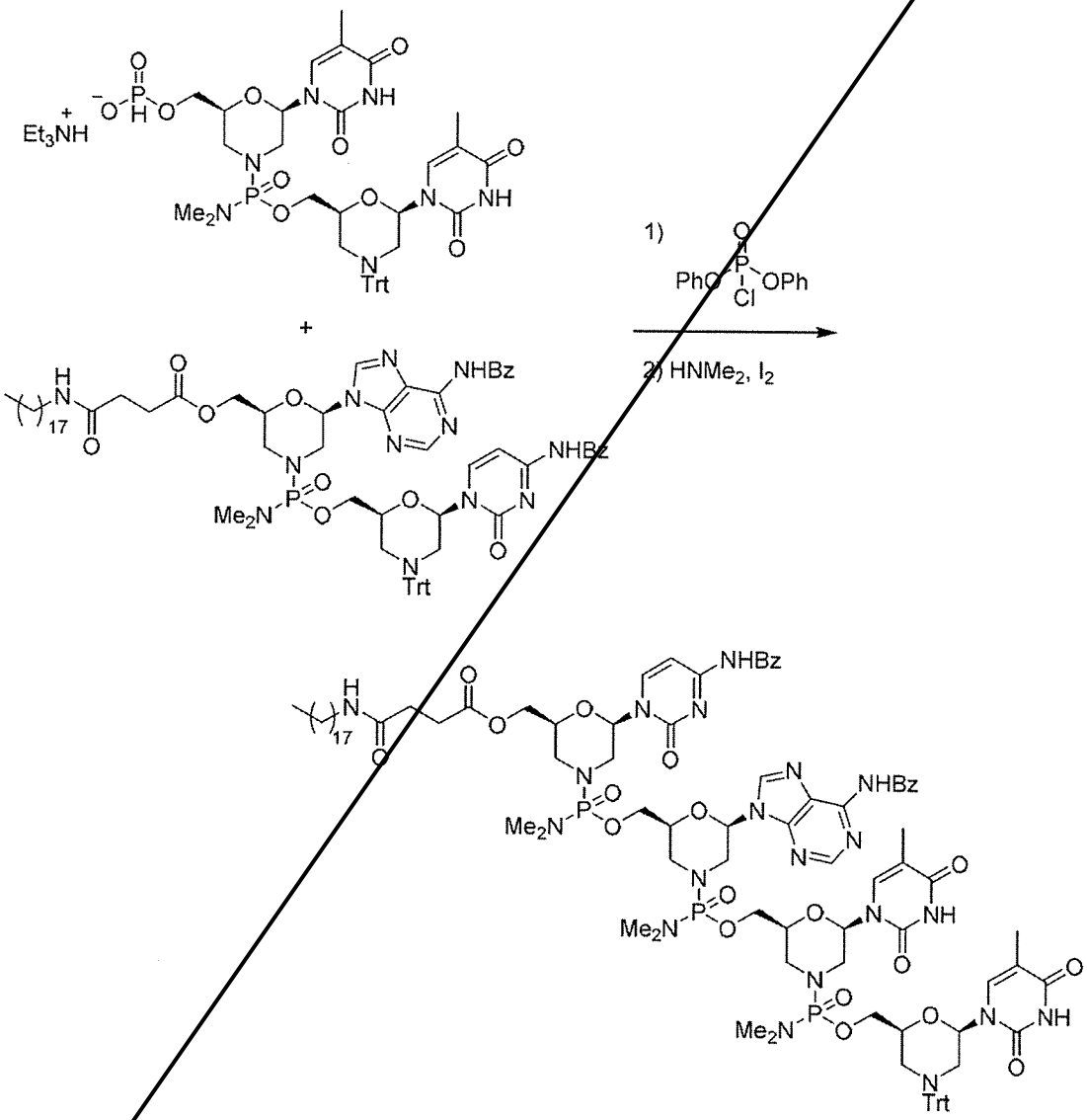
[0191]

Example 4-1: Preparation of G1-suc-PMO[A^{Bz}-C^{Bz}-T-T]-ON

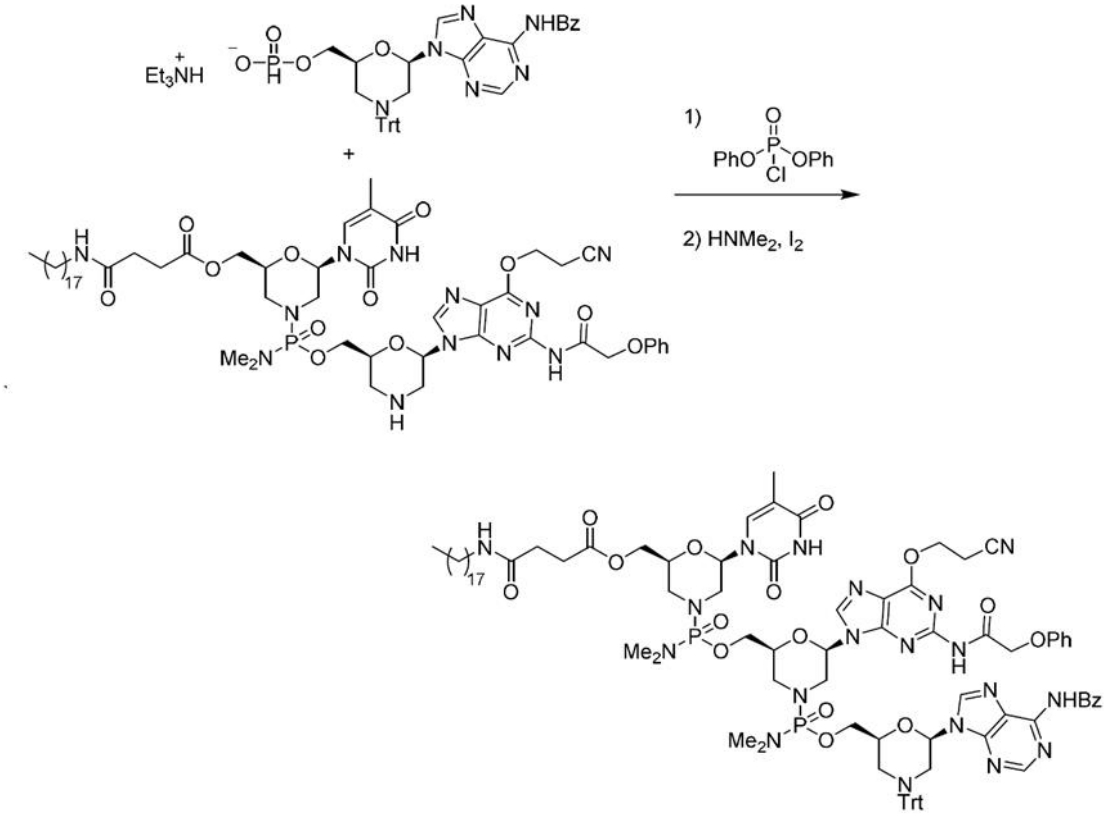


G1-suc-PMO[A^{Bz}-C^{Bz}]-OFF (28 mg, 0.025 mmol) and H-phosphonate-PMO[T-T]-ON (triethylamine salt) (37 mg, 0.0375 mmol) were dissolved in 500 µL of 20% pyridine/dichloromethane. To this solution was added 12 µL (0.0563 mmol) of diphenyl chlorophosphate, and the solution was stirred at room temperature. After 10 minutes, 100 µL of the solution was mixed with 100 µL of an oxidizing solution (0.1 M iodine, 2 M dimethylamine/THF solution) and stirred for 10 minutes at room temperature. After completion of the reaction, a solution of 10% sodium thiosulfate in water was added to the reaction mixture, and the organic and aqueous

layers were separated. The organic layer was diluted 20-fold with a solution of 80% acetonitrile in water and analyzed by HPLC (starting material Rt: 13.8 min, object product Rt: 15.3 min, conversion yield 97.2%, ODS Condition 1).
ESI-MS (+): 2026.9 (LC/MS condition 2)
[0192]
Example 4-2: Preparation of G1-suc-PMO[A^{Bz}-C^{Bz}-T-T]-ON



After mixing 2 mL of Liquid a (flow rate: 0.2 mL/min) with 2 mL of Liquid b (flow rate: 0.2 mL/min), the reaction was carried out in a 5 mL tube reactor at 60°C for 12.5 minutes. 100 μL of the resulting solution was mixed with 100



G1-suc-PMO[T-$G^{CE,Pac}$]-OFF (17 mg, 0.0123 mmol) and H-phosphonate-PMO[A^{Bz}]-ON (triethylamine salt) (14 mg, 0.0185 mmol) and 20 µL (0.247 mmol) of N-methylimidazole were dissolved in 250 µL of dichloromethane. To this solution was added 25 µL (0.123 mmol) of diphenyl chlorophosphate, and the mixture was stirred at 40°C. After 10 minutes, 100 µL of the solution was mixed with 100 µL of an oxidizing solution (0.1 M iodine, 2 M dimethylamine/THF solution) and stirred for 10 minutes at room temperature. After completion of the reaction, a solution of 10% sodium thiosulfate in water was added to the reaction mixture, and the organic and aqueous layers were separated. The organic layer was diluted 20-fold with a solution of 80% acetonitrile in water and analyzed by HPLC (starting material Rt: 12.9 min, object product Rt: 18.4, 19.1 min, conversion yield 89.5%, ODS Condition 2). ESI-MS (+): 1819.9 (LC/MS condition 2)

The oligomers listed below were produced in a similar manner.

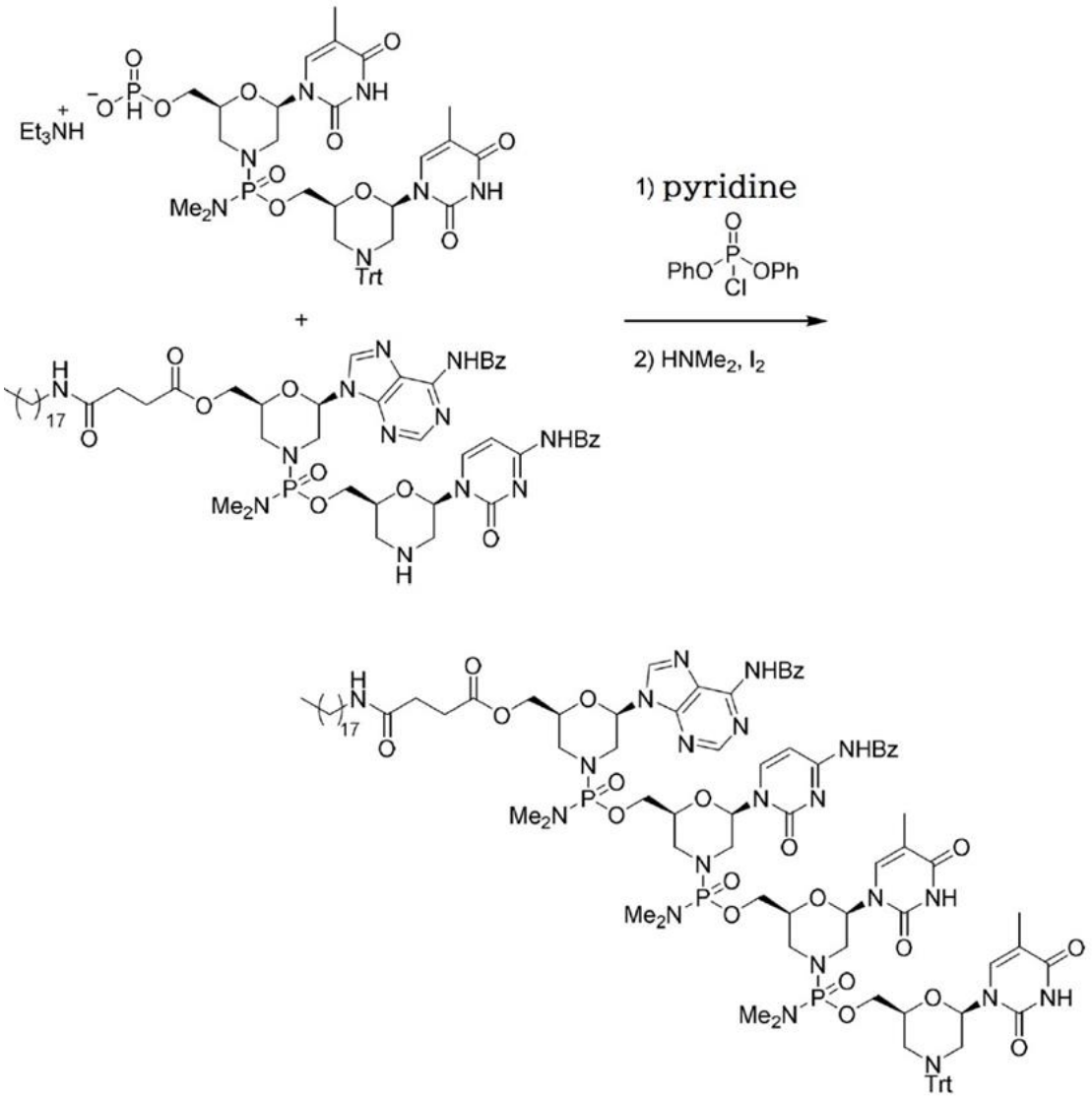
[Table 5]

G1−suc−PMO−OFF	H-Phosphonate	Activating Agent	Base	Conversion Yield (%)
T	A^{Bz}	diphenyl chlorophosphate	N-methyl imidazole	98.6
T	C^{Bz}	diphenyl chlorophosphate	pyridine	96.3
A^{Bz}	A^{Bz}	diphenyl chlorophosphate	pyridine	97.3
A^{Bz}	C^{Bz}	diphenyl chlorophosphate	pyridine	86.9
C^{Bz}	C^{Bz}	diphenyl chlorophosphate	pyridine	93.6
C^{Bz}	T	diphenyl chlorophosphate	pyridine	96.3
T−T	C^{Bz}	diphenyl chlorophosphate	pyridine	83.9
$A^{Bz}-C^{Bz}$	A^{Bz}	diphenyl chlorophosphate	pyridine	96.0
$A^{Bz}-C^{Bz}$	C^{Bz}	diphenyl chlorophosphate	pyridine	95.5
$A^{Bz}-G^{CE,Pac}$	C^{Bz}	diphenyl chlorophosphate	pyridine	81.3
$T-T-A^{Bz}$	A^{Bz}	diphenyl chlorophosphate	pyridine	95.1
$T-T-A^{Bz}$	C^{Bz}	diphenyl chlorophosphate	pyridine	98.0
$C^{Bz}-C^{Bz}-T-C^{Bz}-C^{Bz}$	T	bromotripyrrolidino phosphonium= hexafluorophosphate	N-methyl imidazole	95.9
$T-G^{CE,Pac}-G^{CE,Pac}-G^{CE,Pac}-A^{Bz}-T-C^{Bz}-C^{Bz}-A^{Bz}-G^{CE,Pac}$	A^{Bz}	diphenyl chlorophosphate	N-methyl imidazole	88.5

Example 4: Preparation of PMO Oligomer

[0191]

Example 4-1: Preparation of G1-suc-PMO[A^{Bz}-C^{Bz}-T-T]-ON



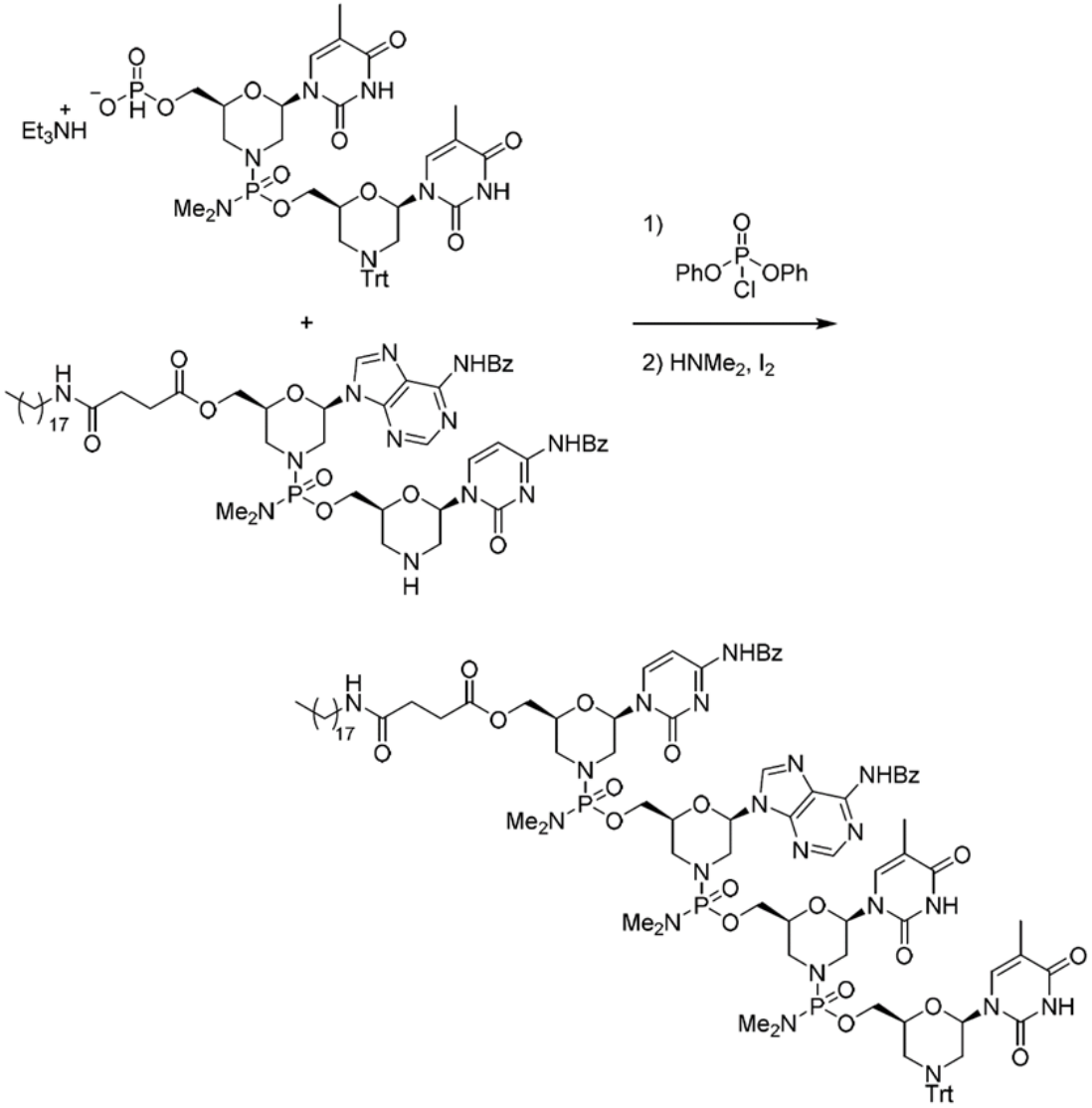
G1-suc-PMO[A^{Bz}-C^{Bz}]-OFF (28 mg, 0.025 mmol) and H-phosphonate-PMO[T-T]-ON (triethylamine salt) (37 mg, 0.0375 mmol) were dissolved in 500 µL of 20% pyridine/dichloromethane. To this solution was added 12 µL (0.0563 mmol) of diphenyl chlorophosphate, and the solution was stirred at room temperature. After 10 minutes, 100 µL of the solution was mixed with 100 µL of an oxidizing solution (0.1 M iodine, 2 M dimethylamine/THF solution) and stirred for 10 minutes at room temperature. After completion of the reaction, a solution of 10% sodium thiosulfate in water was added to the reaction mixture, and the organic and aqueous

layers were separated. The organic layer was diluted 20-fold with a solution of 80% acetonitrile in water and analyzed by HPLC (starting material Rt: 13.8 min, object product Rt: 15.3 min, conversion yield 97.2%, ODS Condition 1).

ESI-MS (+): 2026.9 (LC/MS condition 2)

[0192]

Example 4-2: Preparation of G1-suc-PMO[A^{Bz}-C^{Bz}-T-T]-ON



After mixing 2 mL of Liquid a (flow rate: 0.2 mL/min) with 2 mL of Liquid b (flow rate: 0.2 mL/min), the reaction was carried out in a 5 mL tube reactor at 60°C for 12.5 minutes. 100 μL of the resulting solution was mixed with 100